MULTI-OMICS TO ILLUMINATE SIGNALING MOLECULES OF THE GUT MICROBIOTA-BRAIN AXIS

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ABSTRACT

Yunjia Lai: Multi-Omics to Illuminate Signaling Molecules of the Gut Microbiota-Brain Axis
(Under the direction of Kun Lu)

The human gastrointestinal (GI) tract harbors hundreds of trillions of microorganisms, collectively referred to as the gut microbiota. Complex, dynamic, and metabolically active by nature, these commensal microbes carry critical functional roles in mediating host physiology and health, with a perturbed or dysbiotic state linked to a long list of diseases. Recent studies support that gut microbiota, in close proximity to numerous local neurons and immune cells, also actively interacts with the brain, fueling enormous research interest as recently encapsulated as the "microbiota-gut-brain axis." Although the neuronal gut-brain pathway (through the vagus nerve) is not disputable, the molecular underpinnings of a humoral route (through blood / lymphatic circulation) by which innumerable gut microbial molecular cues translocate from gut to circulating blood with potentials to cross the blood-brain barrier, remain largely unclear. From an environmental health perspective, although studies have been reported on xenobiotic-induced gut microbial perturbations and associated disease risks, whether and how a humoral gut microbiota-brain axis is involved in neurotoxicant exposure-induced effects remains unexplored. There is thus an urgent need to probe the neuroactive potential of microbiota for biomedical and public health causes.

Recent upgrade of omics approaches, especially mass spectrometry-based metabolomics and associated cheminformatic algorithms for compound identification, shows promise for enabling high-coverage and untargeted metabolite profiling with much improved accuracy. This dissertation thus aims to integrate these cutting-edge tools and rules for metabolome-wide analysis

to probe the metabolome-wide patterns while identifying high-impact molecular cues of the humoral microbiota-brain axis.

In Chapter 1, we first conducted a comprehensive review of existing studies to evaluate the functional roles of reported gut microbial molecules (including small molecules and peptides) in lieu of the gut-brain axis and associated neurologic / mental disorders. We call for massive multi-omics mining efforts in resolving the complexity of the gut microbiota-metabolome network while highlighting related chances and challenges for biomedical, nutritional, and environmental causes.

In Chapter 2, for a preliminary check, we developed a targeted metabolomics assay to quantitatively determine literature-reported neurotransmitters, tryptophan metabolites, and indole derivatives (n=50) in specific pathogen-free C57BL/6 mice. We validated the assay, evaluated distribution *in vivo* for these molecules, and collected information as needed for method development of global metabolomics analysis applied to later chapters.

To probe the microbiota-brain axis, for a proof-of-concept, we combine high-resolution mass spectrometry profiling, targeted and untargeted compound annotation procedures, and 16S rRNA gene sequencing (as needed) to comparatively profile feces, blood sera, and cerebral cortical brain tissues of C57BL/6 mice under steady state (germ-free vs. conventionally raised; Chapter 3), under neurotoxicant exposure (perfluorooctanoic acid-treated vs. control; Chapter 4), and under dietary treatment of reportedly neuroprotective components (black raspberry-based diet vs. standard AIN-76A diet; Chapter 5). In all three scenarios, we identified distinct metabolome-wide (and microbial) patterns owing to the presence of or perturbed gut microbiota, with biochemical details in support of an active role of the humoral microbiota-brain axis. Together, the massive, novel, and highly informative datasets generated in this dissertation may provide fundamental insights into how resident microbiota interacts with host bodies via the gut-brain axis while laying

the foundation for incorporating the role of microbiota into neurotoxicity assessment and for the development of biomarkers and countermeasures against risks induced by the ever-prevailing environmental chemical exposures.

To my parents

"If you think you can, you can." *~ Prof. Sherry X. Zhang*
in her master class Advanced Level Bilingual Translation & Interpretation
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always look up to him for his encyclopedic knowledge and enduring tenacity for making things happen. I also want to thank Dr. Gang-yu Liu, for her eye-opening reminders and life-changing suggestions while I was at Davis. Lastly, I extend my appreciation to Dr. Jenny Ting, for support, for sharing her life lessons and advice, for deciding to team up with Kun and let me to be a part of her inspiring microbiota-radiation project. I learned a lot working closely with her then-postdoc Dr. Hao Guo and the amazing experience of keeping stepping up from sporadic, unexpected preliminary data towards fruition of good and solid science and eventually to the acceptance of our manuscript is the best "class" I have taken at UNC.

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3-HK	3-hydroxykynurenine
5-HIAA	5-hydroxyindoleacetic acid
5-HT	serotonin or 5-hydroxytryptamine
5-HTP	5-hydroxy-L-tryptophan
AA	anthranilic acid
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
Acc	accuracy
ACh	acetylcholine
ACN	acetonitrile
ACS	American Society of Chemistry
AD	Alzheimer's Disease
AGC	automatic gain control
AgRP	hypothalamic agouti-related protein
AHLs	N-acylhomoserine lactones
AhR	aryl hydrocarbon receptor
AIN	American Institute of Nutrition
AKG	α-ketoglutarate
Ala	alanine
ANOVA	analysis of variance
ARC	hippocampal arcuate nucleus
Arg	arginine

ASD	analytical chemical standard
ASVs	amplicon sequence variants
Aβ	β-amyloid
BAs	bile acids
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
BEH	ethylene bridged hybrid
BH_4	tetrahydrobiopterin
BMI	body mass index
BRB	black raspberry
CA	cholic acid
CDCA	chenodeoxycholic acid
CGRP	calcitonin gene-related peptide
ChemRICH	chemical similarity enrichment analysis
CNS	central nervous system
CoA	coenzyme A
COMT	catechol-O-methyltransferase
CONV-R	conventionally raised
CYP2D6	Cytochrome P450 2D6
CYP450	Cytochrome P450
DCA	deoxycholic acid
DHA	docosahexaenoic acid

DHICA	5,6-dihydroxyindole-2-carboxylic acid
DMSO	dimethyl sulfoxide
DOPAC	3,4-dihydroxyphenylacetic acid
DOPEG	3,4-dihydroxyphenylglycol O-sulfate
Drd1	Dopamine receptor D1 gene
ECs	enterochromaffin cells
ENS	enteric nervous system
EPA	eicosapentaenoic acid
ERK	extracellular signal-regulated kinase
ESI	electrospray ionization
FAs	fatty acids
Ffar2	free fatty acid receptor 2
FGF15	fibroblast growth factor 15
FGFP	the Flemish Gut Flora Project
FGFR1	fibroblast growth factor receptor 1
FWHM	full width at half maximum
FXR	farnesoid X receptor
GABA	γ-aminobutyric acid
GABA-T	4-aminobutyrate transaminase
GAD	L-glutamic acid decarboxylase
GadB	glutamate decarboxylase β
GBM	gut-brain module

GF	germ-free
GI tract	gastrointestinal tract
GlcNAc	Nα-acetyl-L-glutamine
Gln	glutamine
GLP-1	glucagon-like peptide 1
Glu	glutamate
Gly	glycine
GNPS	The Global Natural Product Social Molecular Networking
GPCR	G-protein coupled receptor pathway
GPR43	G Protein-Coupled Receptor 43
GS/GOGAT cycle	glutamine synthase/glutamate:2-oxoglutarate aminotransferase cycle
GSH	glutathione, reduced
GSIS	glucose-stimulated insulin secretion
GSSG	glutathione disulfide
HDCA	hyodeoxycholic acid
HESI	heated electrospray ionization
HETE	5-hydroxyeicosatetraenoic acid
HFD	high fat diet
HILIC	hydrophilic interaction chromatography
HMDB	Human Metabolome Database
HODE	hydroxyoctadecadienoic acid
HPA	hypothalamic-pituitary-adrenal axis

HPLA	hydroxyphenyllactic acid
HRAM	high resolution accurate mass
HRMS	high resolution mass spectrometry
HSD	Tukey's honestly significant difference
HSS	high strength silica
I3A	indole-3-carboxyladehyde
I3S	indoxyl sulfate
IAA	indole-3-acetic acid
IAcrA	indole-3-acrylic acid
IACUC	The Institutional Animal Care & Use Committee
IDO	indoleamine 2,3-dioxygenases
IEB	intestinal epithelial barrier
IgA	Immunoglobulin A
IgM	Immunoglobulin M
IL-10	Interleukin 10
ILA	indole-3-lactate
IPA	indole-3-propionate
ISDs	internal chemical standard
KEGG	Kyoto Encyclopedia of Genes and Genomes
KP	kynurenine pathway
Kyn	kynurenine
Kyna	kynurenic acid

L-DOPA	L-3,4-dihydroxyphenylalanine
LAT	large neutral amino acid transporters
LC	liquid chromatography
LCA	lithocholic acid
LDR	linear dynamic range
LLOQ	lower limit of quantitation
LPS	lipopolysaccharides
LTQ	linear ion trap
MAMP	microorganism-associated molecular pattern
MAO	monoamine oxidase
MCA	muricholic acid
MeOH	methanol
Met	methionine
MIA	maternal immune activation
MoNA	MassBank of North America
MOPEG	3-methoxy-4-hydroxyphenylglycol
MS	mass spectrometry OR multiple sclerosis
MS/MS	tandem mass spectra
MSEA	metabolite set enrichment analysis
MSI	Metabolomics Standard Initiative
NAD	nicotinamide adenine dinucleotide
NAFLD	non-alcoholic fatty liver disease

NAS	N-acetylserotonin
NCE	normalized collision energy
NGS	next-generation sequencing
NLRs	nucleotide-binding and leucine-rich repeat-containing receptors
NMDARs	*N*-methyl-D-aspartate receptor
Nod1	Nucleotide Binding Oligomerization Domain Containing 1
Nod2	Nucleotide Binding Oligomerization Domain Containing 2
NodDKO	Nod1/Nod2 double knockout
NPY	neuropeptide tyrosine
PAG	phenylacetyl L-glutamine OR N-(2-phenylacetyl)glycine
PCA	principal component analysis
PCoA	principal coordinate analysis
PD	Parkinson's Disease
PFAS	per- and polyfluoroalkyl substances
PFOA	perfluorooctanoic acid
PGNs	peptidoglycans
Phe	phenylalanine
PKA	protein kinase
PLA	3-phenyllactate
PNGs	peptidoglycans
POMC	pro-opiomelanocortin
PPAR-α	peroxisome proliferator activated receptor alpha

PRM	parallel reaction monitoring
Pro	proline
PRRs	pattern recognition receptors
PS	phosphatidylserine
PYY	peptide YY
QA/QC	quality assurance / quality control
QIIME	Quantitative Insights Into Microbial Ecology
QoL	Quality of Life
QqQ	triple quadrupole
Quin	quinolinic acid
ROS	reactive oxidative species
RSD	relative standard deviation
SAH	S-(5'-adenosyl)-L-homocysteine
SCFAs	short-chain fatty acids
SIL	staple-isotope labeling
SMPDB	The Small Molecule Pathway Database
SPF	Specific Pathogen Free
SRM	selected reaction monitoring
SULT	sulfotransferase
TCA cycle	tricarboxylic acid cycle
TCDCA	tauro-chenodeoxycholic acid
TDO	tryptophan 2,3-dioxygenase

TGR5	Takeda G-protein-coupled bile acid receptor
TIC	total ion chromatogram
TLRs	toll-like receptors
TMAO	trimethylamine *N*-oxide
TNF-α	tumor necrosis factor-α
Tph1	tryptophan hydrolase 1 gene
TriHOME	trihydroxyoctadecenoic acid
Trp	tryptophan
Tyr	tyrosine
TαMCA	tauro-α-muricholic acid
TβMCA	tauro-β-muricholic acid
UHPLC	ultra-high performance liquid chromatography
UPLC	ultra-performance liquid chromatography
VIP	variable importance plot
XICs	extracted ion chromatograms
α7nAChRs	α7-nicotinic acetylcholine receptors
αMCA	α-muricholic acid
αMSH	α-melanocyte-stimulating hormone
αSyn	α-synuclein
βMCA	β-muricholic acid

CHAPTER 1: MICROBIOTA AND THE HUMORAL GUT-BRAIN AXIS: MOLECULES AND MECHANISMS[1]

1.1 Introduction

The advance of next-generation sequencing (NGS) technologies over the past decade has led to explosive growth in the knowledge of the trillions of microbes inhabiting our gastrointestinal (GI) tract, collectively known as gut microbiota (P. J. Turnbaugh et al., 2007). The gut microbiota has been discovered to mediate a range of host physiological processes, including but not limited to energy metabolism (Peter J. Turnbaugh et al., 2006), immune cell development(Hooper, Littman, & Macpherson, 2012), epithelial homeostasis (Wells, Rossi, Meijerink, & van Baarlen, 2011), and importantly, the gut-brain axis, a classical concept that has been recently reviving thanks to the NGS efforts (Foster & McVey Neufeld, 2013). Indeed, accumulative research, mostly based on models *in vivo*, has explored the role of microbiota in the neuronal, immune, and endocrine aspects of the bidirectional gut-brain communication (Dinan & Cryan, 2017; Fung, Olson, & Hsiao, 2017; N. Powell, M. M. Walker, & N. J. Talley, 2017). For example, Sudo and colleagues first reported that commensal microbes could perturb murine stress responses through the hypothalamic-pituitary-adrenal (HPA) axis (Sudo et al., 2004). Compared to conventionally colonized controls, germ-free (GF) mice displayed heightened stress responses and lower levels of the neuroprotective brain-derived neurotrophic factor (BDNF) in cortex and hippocampus. These pathophenotypes were dramatically reversed through select microbial reconstitution but only under a critical

[1] Chapter 1 was reproduced from my manuscript - Lai Y., Lu K.*: Towards deciphering the microbiome-gut-brain axis in health and disease: a comprehensive molecular review. ***Front. Immun.*** (In Preparation).

developmental window, indicating a causal but conditional role of gut microbiota in regulating stress responses. Likewise, mice regularly fed *Lactobacillus rhamnosus* exhibited improved emotional behavior that is likely to be explained by altered neurotransmission in the central GABAergic system mediating through the vagus nerve (Bravo et al., 2011). More recently, using a maternal immune activation (MIA) mouse model, Hsiao and colleagues probed gut bacterial effects on autism spectrum disorder (ASD) pathogenesis (Hsiao et al., 2013). The authors observed in MIA offspring ASD-mimicking gastrointestinal (GI) barrier defects and behavioral abnormalities, which were substantially restored through exposing the human commensal *Bacteroidetes fragilis*, likely owing to effects of gut bacterial products on host metabolomes. All three pioneering attempts indicate an active role of gut microbiota in brain development and function, opening doors for new research and treatment for mental disorders as well as CNS-mediated metabolic diseases.

While the idea of manipulating resident microbes as therapeutic targets for improving mental health outcomes is tempting, knowledge gaps and research challenges persist. These include, but not limit to, incomplete understanding of microbe-host crosstalk, ill-informed human relevance from animal models, and technical challenges in methodology standardization, confounder adjustment, and data interpretation (Hooks, Konsman, & O'Malley, 2018). Controversies prevail on to what extent the microbiome-gut-brain axis can be defined and determined for human populations. It was not until recently, however, that the first population-scale evidence linking microbiota to mental health has been published(Valles-Colomer et al., 2019), moving beyond limitations of mainstream evidence mostly derived from animal models. Through correlating fecal metagenomic features of over 1,000 individuals of the Flemish Gut Flora Project (FGFP) cohort with indicators of quality of life (QoL) and depression, this study associated

butyrate producing *Faecalibacterium* and *Coprococcus* with higher QoL scores and identified significantly lower *Dialister* and *Coprococcus* spp. in patients that had been diagnosed with depression. Gut-brain module analyses further singled out the microbial synthetic potential of 3,4-dihydroxyphenylacetic acid (DOPAC), a dopamine metabolite, as positively correlated with QoL scores alongside altered GABA pathways in depression. Another recent study examined metagenome-wide association of gut microbiome features for schizophrenia in a human cohort. The study identified functional potentials of short-chain fatty acid (SCFA) synthesis, tryptophan metabolism, and synthesis/degradation of neurotransmitters in the pathogenesis of schizophrenia (Zhu et al., 2020). Further transplantation of *Streptococcus vestibularis*, a schizophrenia-enriched bacterium, was discovered to induce deficits in social behavior and alter peripheral neurotransmitter profiles in mice. Although these emerging findings are by nature mostly descriptive and require more in-depth causal validation, they strengthen the notion that the gut microbiota harbors important neuroactive potentials that can be harvested to modulate gut-brain signaling and improve mental health outcomes.

It is generally accepted that gut-brain communication occurs via at least two routes, i.e. (1) the vagus nerve (neuronal) that connects central nervous system (CNS) and enteric nervous system (ENS, also known as the "second brain"), and (2) the circulatory system (humoral) which encompasses both blood and lymphatic circulation (de la Fuente-Nunez, Meneguetti, Franco, & Lu, 2018; Dinan & Cryan, 2017) (**Figure 1.1**). Microbiota residing in the GI tract likely impacts gut-brain signaling through both, but the mechanistic underpinnings remain largely unknown. This review will focus on humoral pathways, for which gut microbial products such as metabolites and neuropeptides likely act as means of trade in the microbiome-gut-brain communication due to their small size and/or ease of interorgan transportation, in parallel with signaling/regulatory potentials

(Donia & Fischbach, 2015; Sharon et al., 2014). Indeed, increasing studies showed that gut microbe-related compounds could modulate host homeostasis (Nicholson et al., 2012; Wikoff et al., 2009), alter barrier permeability (Kelly et al., 2015), and induce neuroimmune responses (Veiga-Fernandes & Pachnis, 2017), with multifaceted functional roles as neurotransmitters, as reactive oxygen species (ROS) scavengers, or as ligands for activating immune signal transduction, to name a few. Surprisingly, research in this this regard remain largely unexamined, calling for concerted efforts for mapping out the much uncharted biochemical pathways that drive or mediate the microbiome-gut-brain signaling (Rogers et al., 2016).

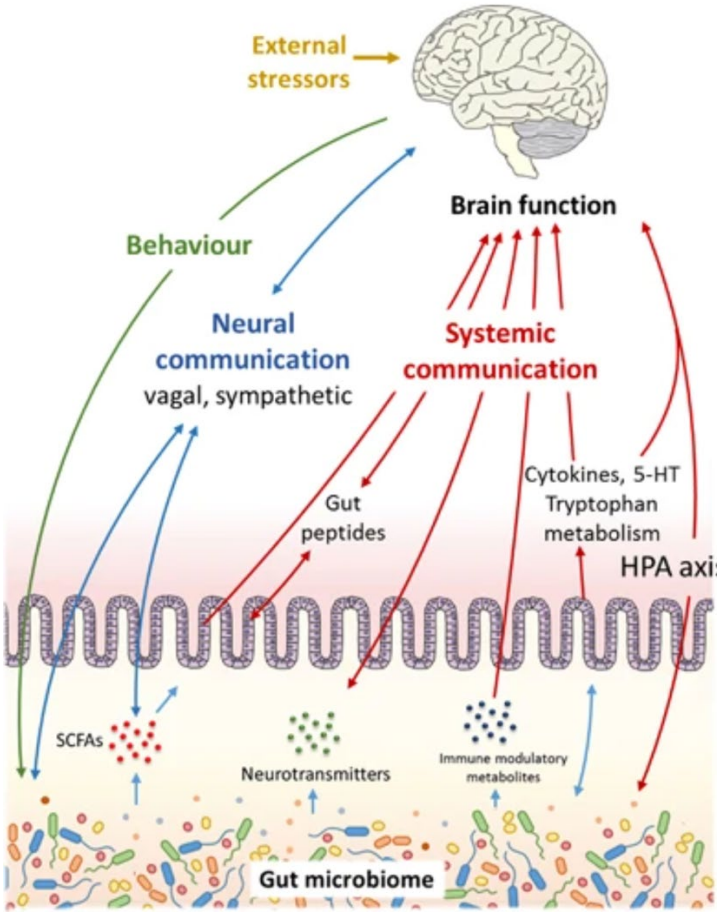

Figure 1.1 Communication pathways linking the gut microbiome with brain function include both neuronal and humoral routes, through vagus nerve and blood/lymphatic circulation, respectively (Rogers et al., 2016).

The objective of this chapter is to conduct a comprehensive and up-to-date molecular perspective to the fledging field of microbiome-gut-brain axis. Here, we collect a complete list of gut microbiota-related compounds, spanning from small molecules (e.g., short-chain fatty acids, bile acids, neurotransmitters, and tryptophan catabolites) to the larger neuropeptides (e.g., microorganism-associated molecular patterns, α-synuclein). We first summarize by chemical class the most recent advances regarding their fate and activities *in vivo* under normal and/or environmentally challenged conditions. Then, we turn to seminal disease models of mental disorders to discuss specific mechanistic pathways revolving around these molecules to inform how commensal microorganisms can signal mammalian host CNS and influence health outcomes. Finally, we discuss the implications of existing studies while underscoring the vast opportunities and challenges for future gut microbiome research in biomedical, nutritional, and public health sciences.

1.2 Short-chain fatty acids (SCFAs)

SCFAs are saturated fatty acids consisting of one to six carbon atoms. In mammalian colon, acetate, propionate, and butyrate are produced in large quantities through microbial fermentation of indigestible dietary fibers, comprising over 95% of the total SCFAs with a typical molar ratio of 6:2:2 (den Besten et al., 2013). The biosynthesis, uptake, and distribution of the three SCFAs *in vivo* has been well summarized recently (Flint, Duncan, Scott, & Louis, 2015; Koh, De Vadder, Kovatcheva-Datchary, & Backhed, 2016; Morrison & Preston, 2016). Briefly, acetate can be broadly transformed by gut bacteria from acetyl-CoA that is derived from pyruvate, an important metabolic intermediate of glycolysis-citric acid cycle (den Besten et al., 2013). More substrate-specific and species-conserved, propionate is synthesized through multi-step reduction either of

lactate, or of succinate, or depends on substrates of deoxyhexose sugars (e.g., fucose and rhamnose)(Morrison & Preston, 2016), whereas butyrate can be formed either from downstream acetyl-CoA products including butyryl-CoA and butyryl-phosphate, from lactate and acetate by specific sets of gut bacteria (e.g. *Eubacterium hallii* and *Anaerostipes caccae*), or from proteins through lysine pathway, as recent metagenomics data suggested (Vital, Howe, & Tiedje, 2014). The concentration profile of SCFAs varies along the GI tract but typically peaks at cecum and proximal colon, followed by a decline towards distal colon (Koh et al., 2016). Such decreasing luminal SCFA levels can be partially explained by transport of acetate and propionate into circulation for metabolism in other organs as well as the local consumption of butyrate by colonocytes (Koh et al., 2016).

SCFAs have been intensely studied as key bacterial metabolites conferring a multitude of benefits for host health. A range of bacterial strains have been confirmed as SCFA producers, including *Bacteroides* spp., *Clostridium* spp., and *Streptococcus* spp. (Koh et al., 2016). Since SCFAs act as an importance energy source while exhibiting signaling activities, a large proportion of existing studies targeted SCFAs for linking gut microbiota to metabolic syndromes such as diabetes and obesity. Interestingly, although SCFAs are associated with many metabolic benefits such as improved glucose tolerance (Bjursell et al., 2011) and increased thermogenesis (Kovatcheva-Datchary et al., 2015), fecal SCFAs levels in obese patients are higher than in their lean controls, warranting future elucidation (Schwiertz et al., 2010). SCFAs also contribute to gut integrity and immune homeostasis; inside gut lumen, SCFAs modulate pH, balance redox equivalent production, and fortify gut lining (den Besten et al., 2013; Morrison & Preston, 2016; Ríos-Covián et al., 2016). Importantly, from an immunological perspective, SCFAs activate G protein-coupled receptors (GPRs) while inhibiting histone deacetylases (HDACs) (Koh et al.,

2016), control regulatory T cells (T_{regs}) expansion (Smith et al., 2013), and regulate local synthesis while responsible for the release of important endocrine agents such as glucagon-like peptide 1 (GLP-1) (den Besten et al., 2013) and serotonin (Reigstad et al., 2015).

Accumulating studies show that gut microbiota-derived SCFAs also have profound effects on host brain function or CNS-mediated pathogenesis through, for example, regulating neurodevelopment (e.g. microglia maturation) (Erny et al., 2015), modulating BBB permeability (Braniste et al., 2014), and acting on ENS for appetite control (Frost et al., 2014). Erny and colleagues showed that eradicating a complex host microbiota, either constitutionally (as found in GF mice) or through an induced fashion (e.g., by antibiotics, or abx), led to severe defects in the density, morphology, and maturation of microglia, the brain's resident macrophages (Erny et al., 2015). In turn, a four-week administration of SCFAs, a cocktail of acetate, propionate, and butyrate, markedly restored the defective microglia in GF mice. In this work, interestingly, while the *Ffar2*$^{-/-}$ mice (i.e., double knockout of genes encoding the free fatty acid receptor 2) displayed massive compromises of microglia, the FFAR2 protein (also known as the GPR43) were not detected in CNS tissues, leaving how SCFAs communicated to the CNS incompletely elucidated. Moreover, Sampson and the team demonstrated in a mouse model of Parkinson's disease (PD) that the gut microbiota are required for the hallmark motor and GI dysfunction of PD (Sampson et al., 2016). One established mechanism is through postnatal gut-brain signaling by microbial SCFAs to mediate neuroinflammatory responses and the aggregation of α-synuclein (αSyn) protein. In addition, Perry and colleagues identified acetate as a microbiome-brain-β-cell axis mediator for promoting metabolic syndrome (Perry et al., 2016). Using whole-body turnover rate determination and stable-isotope tracer analysis *in vivo*, the authors observed for HFD-fed rats marked increase in acetate turnover, for which gut microbiome was confirmed as arguably the main source.

Strikingly, such increase in bacteria-produced acetate was further discovered to promote glucose-stimulated insulin secretion (GSIS) via the activation of the parasympathetic nervous system, resulting in hyperphagia, obesity, and related sequelae, all of which prevented by severing the vagus nerve. Beyond these three seminal studies on SCFA-brain interaction, accumulating research also showed that SCFAs can modulate BBB (Al-Asmakh & Hedin, 2015), regulate satiety (den Besten et al., 2013), and interact extensively with ENS, where the SCFA receptor GPR41 is highly expressed (Koh et al., 2016; Nøhr et al., 2013).

1.3 Bile acids (BAs)

Bile acids, or BAs, are steroid derivatives synthesized *de novo* from cholesterol in liver and are further conjugated with taurine or glycine, collectively known as primary BAs (Hofmann, 1999). Upon dietary intake, primary BAs are discharged from gallbladder into gut lumen, where they emulsify dietary fats and experience massive microbial transformation into bioactive products called secondary BAs (Hofmann, 1999). With amphipathic properties, BAs have long been considered as detergent molecules to facilitate lipid uptake, transport, and metabolism, with enterohepatic circulatory pool cycles occurring with a frequency dependent on patterns of food intake. However, more recent work revealed that BAs also serve as signaling agents that activate the nuclear receptor farnesoid X receptor (FXR) and Takeda G-protein-coupled bile acid receptor (TGR5), both of which, although working seemingly independently, have been demonstrated as potent regulators for BA synthesis, energy metabolism, and immune cell homeostasis (Kuipers, Bloks, & Groen, 2014; Sun & Chang, 2014; Wahlstrom, Sayin, Marschall, & Backhed, 2016).

Understanding how gut microbiota is involved in BA synthesis and signaling will benefit the fundamental design of therapeutic solutions to a range of diseases, e.g. diabetes(Lefebvre,

Cariou, Lien, Kuipers, & Staels, 2009), non-alcoholic fatty liver disease (NAFLD) (Arab, Karpen, Dawson, Arrese, & Trauner, 2017; Schroeder & Backhed, 2016), and colon cancer (Bernstein, Bernstein, Payne, Dvorakova, & Garewal, 2005). On one hand, BAs are known to curtail gut bacterial growth to maintain a balanced ecological state for gut epithelial homeostasis. In turn, bacteria harbor versatile enzymatic potentials for transforming secreted primary BAs into secondary BAs through deconjugation and dehydroxylation, typically leading to increased chemical structural diversity and signaling potency. Targeted metabolomic profiling of BAs in GF-, abx-treated, and conventionally raised rats showed that the absence of host microbiota, as found in both GF and abx-treated mice, led to less diverse BA profiles that were instead dominated by taurine-conjugated BAs in extrahepatic sites including kidney, heart, and blood relative to their conventional counterparts (J. R. Swann et al., 2011). Furthermore, Sayin and colleagues identified in conventionally raised C57BL/6 mice decreased levels of muricholic acid (MCA) but not cholic acid (CA) in the enterohepatic system, as compared with GF individuals (Sayin et al., 2013). Using *Fxr*-knockout murine strains, the study discerned that gut microbiota governed the expression of ileum fibroblast growth factor 15 (FGF15) and liver 7α-cholesterol hydrolase in an FXR-dependent manner, specifically by reducing tauro-β-muricholic acid (TβMCA), a natural FXR antagonist.

To date, only a few emerging studies linked BAs to the evolving definition of the microbiome-gut-brain axis. Liu and colleagues recently demonstrated that BAs could induce antidiabetic effects through signaling the FGF receptor 1 (FGFR1) in hypothalamic agouti-related protein (AgRP)-producing neurons (S. Liu et al., 2018). Specifically, oral gavage of taurocholic acid markedly improved glucose tolerance in *ob/ob* obese mice by upregulating ileum expression of FGF15 (the murine counterpart of FGF19 in human) likely through an FXR-dependent pathway.

Several other studies suggest that bacteria-derived BAs may also trigger the gut-liver-brain axis for satiety control (Parséus et al., 2017), improved glucose metabolism (as found for bariatric surgery) (Dixon, Lambert, & Lambert, 2015; J. V. Li et al., 2011), and the remedy of alcohol-associated liver injury even in the presence of cirrhosis and alcoholic hepatitis (J. S. Bajaj, 2019). Beyond those, BAs are linked to altered brain function and behaviors as well. One study using rodent models of ASD associated decreased abundances of bile-metabolizing gut bacterial species, such as *Bifidobacterium* and *Blautia* spp., with increased GI dysfunction and autism-like behavioral scores (Golubeva et al., 2017). Another study revealed that a gut-based bariatric surgery led to larger bile acid pools and attenuated cocaine-induced increase of dopamine levels in nucleus accumbens as well as reduced addiction-related behaviors; using knockout mouse models, the study identified TGR5 as a key mediator (Reddy et al., 2018).

For Alzheimer's disease (AD), two recent large human cohort studies (n>1,400) associated altered serum BA profiles with markers of cognitive decline and AD pathophysiology, supporting the hypothesis that circulating BAs contribute to AD pathogenesis in which gut microbiota plays a role (MahmoudianDehkordi et al., 2019; Nho et al., 2019). Of note, significantly lower levels of circulating CA (a primary BA) and higher levels of deoxycholic acid (or DCA, a secondary BA converted from CA by bacteria), alongside their glycine and/or taurine conjugates, were observed in AD patients as compared to their age-matched normal controls. A strong positive association was identified (and further validated) between the surging DCA-to-CA ratios and markers of cognitive impairment, indicating gut-bacterial 7α-dehydroxylation of CA as one potential mechanism of AD pathogenesis. In future studies, it would be of value to explore whether BAs act locally on TGR5-expressing ENS to signal the CNS (Kuipers et al., 2014) or cross the BBB from peripheral systems in their nonpolar unconjugated form to reach the CNS directly, as indicated by

previous data (Higashi et al., 2017; Kamp & Hamilton, 1993; Mertens, Kalsbeek, Soeters, & Eggink, 2017; Parry et al., 2010; Quinn & DeMorrow, 2012). Moreover, the role of BAs in AD pathogenesis could be gender-specific, as suggested in a more recent study that used targeted multi-compartmental metabolomics to compare 28 BAs in enterohepatic circulation of AD transgenic mice (PP/PS1 induced) with conventional controls. Results showed that compared with conventional controls, female AD transgenic mice had decreased levels of taurine primary BAs and increased levels of secondary BAs in plasma and liver, while male counterparts showed an opposite trend (Wu et al., 2020).

1.4 Tryptophan metabolites

L-Tryptophan (Trp) is an essential aromatic amino acid exclusively obtained from diet such as eggs, fish, and milk, with a required daily intake of 3.5 mg/kg of body weight (Cervenka, Agudelo, & Ruas, 2017). Trp is actively involved in protein biosynthesis and cell growth while serving as substrates for producing metabolites crucial for host physiological processes spanning immune cell homeostasis, energy balance, and neurotransmission. In mammalian systems, except across the BBB to reach the CNS directly, Trp is transported throughout the body in albumin-bound forms by large neutral amino acid transporters (LAT) (Ruddick et al., 2006). The degradation of Trp (only in its free forms) (McMenamy & Oncley, 1958), mostly occurs inside the GI tract through three distinct pathways, namely kynurenine pathway (KP), serotonin pathway, and production of indoles. In human body, over 95% of Trp is metabolized through KP, generating a range of kynurenine (Kyn) derivatives of neuroactivity including the end-product nicotinamide adenine dinucleotide (NAD^+), a cofactor essential for cellular energy metabolism (Bender, 1983; Cervenka et al., 2017). The Trp-Kyn pathway has been detailed in recent two review articles from

evolutionary and drug development perspectives (Cervenka et al., 2017; Platten, Nollen, Rohrig, Fallarino, & Opitz, 2019). Smaller portions of Trp are converted into serotonin (5-HT) and many other downstream neurotransmitters, such as N-acetylserotonin (NAS), melatonin, and 5-hydroxyindoleacetate (5-HIAA) that are suggested to mediate the microbiome-gut-brain axis (Clarke et al., 2013; Y. Li, Hao, Fan, & Zhang, 2018). In addition, gut microbiota also transforms Trp into indole and/or indolyl derivatives, of which many have been identified as key regulators of inflammation and mucosal homeostasis (Arora, Sharma, & Bae, 2015; Perdew, Murray, & Hubbard, 2015).

The crucial role of all three Trp catabolic fluxes in orchestrating the gut-brain signaling has been increasingly identified in the recent years, particularly as it relates to a range of neurological pathology and an active regulatory role of gut microbiota (Agus, Planchais, & Sokol, 2018; Roager & Licht, 2018). Such notion has been recently buttressed by the aforementioned FGFP cohort study (Valles-Colomer et al., 2019), from which gut-brain module (GBM) analyses successfully identified the presence of animal-like GBM (5-HT synthesis I) rather than the plant-like GBM (5-HT synthesis II) in around 20% of gut-associated genomes from the Integrated Microbial Genomes (IMG); microbial genera such as *Akkermansia* and *Alistipes* were discovered as potential 5-HT producers. This opens doors of targeting gut microbiome for regulating Trp pathways towards improved mental health outcomes.

1.4.1 Serotonin

Perhaps the best-known biochemical details on microbiota-Trp interaction as it relates to the gut-brain axis revolve around serotonin (or 5-hydroxytryptamine, 5-HT), a monoamine neurotransmitter with multifaceted physiological functions spanning mood control, appetites and

cognitive behaviors in brain, in parallel with its regulation of GI motility (Gershon & Tack, 2007) and hemostasis (Mercado et al., 2013) in the GI tract. Strikingly, over 90% of 5-HT are synthesized *de novo* in gut by endocrine cells such as enterochromaffin cells (ECs) and myenteric neurons, where commensal microbes are heavily involved. Novel signaling cascades by which gut microbiota acts on local 5-HT biosynthesis was recently reported; one is through signaling colonic ECs directly to promote the gene expression for tryptophan hydrolase 1 (TPH1) (Yano et al., 2015). In a more recent study, gut microbiota was discovered to constantly regulate the maturation of ENS even till adulthood via an enteric 5-HT network (De Vadder et al., 2018). Note that gut-derived 5-HT cannot cross the BBB, thus, 5-HT levels in brain would solely depend on its CNS synthesis *in situ* (Rogers et al., 2016); how microbe-mediated 5-HT metabolism in ENS (and the extended circulatory systems) can impact CNS and lead to neuro-related changes will be a critical direction of future research. Aside from 5-HT, many of its derivatives are worth exploring, e.g., 5-HIAA, tryptamine and melatonin, alongside many other types of neurochemicals as summarized below.

1.4.2 Kynurenines

Kyn and its downstream catabolites, collectively termed kynurenines, are increasingly recognized to regulate brain health. In the CNS, 40% of the Kyn pool are synthesized *de novo*, whereas the rest 60% are transported over from peripheral sites through BBB; for Trp-Kyn pathway in peripheral tissues where enzyme levels are much higher than in brain, the first step is the rate-limiting one, of which both indoleamine 2,3-dioxygenases (IDOs) (at extrahepatic sites) and tryptophan 2,3-dioxygenase (TDO) (in liver) catalyzes and converts Trp to *N*-formylkynurenine, the immediate precursor of Kyn (Kanai et al., 2009). Kyn metabolism inside

the brain yields several sets of metabolites with distinct effects on CNS and in a cell-specific manner. One is microglial generation of 3-hydroxykynurenine (3-HK) and its immediate derivative quinolinic acid (Quin), with the former linked to oxidative stress and apoptosis inside the CNS and the latter exhibiting neuronal excitotoxicity through activating the *N*-methyl-D-aspartate receptor (NMDARs) (Amori, Guidetti, Pellicciari, Kajii, & Schwarcz, 2009; Guillemin, Smythe, Takikawa, & Brew, 2005; Müller & Schwarz, 2007). The other is kynurenic acid (Kyna), a Kyn derivative produced by CNS astrocytes that exerts neuroprotective effects by serving as an antagonist to both NMDARs and α7-nicotinic acetylcholine receptors (α7nAChRs) (Albuquerque & Schwarcz, 2013) while being an agonist for aryl hydrocarbon receptor (AhR) (DiNatale et al., 2010), an important transcription factor of xenobiotic metabolism and a potent immunological regulator. Monitoring Quin-to-Kyna ratios in CNS, therefore, will help inform the status of CNS excitation and neuroinflammation (Cervenka et al., 2017; O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). Overall, CNS accumulation of Kyn due to peripheral inflammation has been generally associated with mental disorders such as depression and schizophrenia (Y. Chen & Guillemin, 2009). It would be beneficial for future studies to dissect how gut microbes are involved in controlling peripheral Trp-Kyn availability, especially to identify specific chemical species that can cross the BBB to reach the CNS and associated transporters.

1.4.3 Indoles

Bacterial Trp products, notably indole and a number of downstream indolyl derivatives, have been identified as potent regulators for sustaining mucosal homeostasis and eliciting proper immune responses(J. Gao et al., 2018). One striking example is indole-3-propionic acid (IPA), an indolyl metabolite that is exclusively produced by bacteria of the Clostridiales family including

Clostridium sporogenes (Wikoff et al., 2009). IPA is an active ROS scavenger itself (i.e. an antioxidant) and can activate the AhR receptor for promoting the release of anti-inflammatory cytokines (such as interleukin-10, i.e. IL-10) while suppressing the expression of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) (Madhukumar Venkatesh et al., 2014). Likewise, indole-3-acrylic acid (IAcrA) produced by *Peptostreptococcus russellii* (a mucin user) is also an AhR agonist that suppresses inflammation. IAcrA also binds to pregnane X receptor (PXR) to promote gene expression of *muc2* that encodes mucin 2 for enhancing the integrity of intestinal epithelial barrier (IEB) (Wlodarska et al., 2017). As for indole-CNS interactions, a recent *in vivo* study of multiple sclerosis (MS) demonstrated that bacterial Trp-derived indoles, including indole, indole-3-sulfate (I3S), IPA, and indole-3-carboxaldehyde (I3A), can directly modulate CNS inflammation through activation of AhR receptors that are expressed in astrocytes (Rothhammer et al., 2016). A recent human cohort study has also correlated Trp metabolites including indole, indole-3-acetic acid, and skatole with quantitative metrics of functional and anatomical connectivity of the extended central reward network that incorporates amygdala, nucleus accumbens, and anterior insula; additional association was also found between these Trp metabolites with body mass index (BMI), food addiction, and anxiety symptoms (Osadchiy et al., 2018). Note that microbiota-derived indole compounds can easily enter the mammalian circulatory system and reshape host blood metabolomes (J. Gao et al., 2018; Wikoff et al., 2009), in this light, more research should be conducted to identify and track peripheral indole species that are able to cross BBB and reach the brain and to examine how they mediate CNS-resident immune homeostasis and neurological functions.

1.5 Small-molecule neurotransmitters

Neurotransmitters are presynaptic compounds that are released to evoke postsynaptic electrical signaling (through binding to receptors *in situ*) and fulfill neurophysiological functions, and typically have a complete and fast cycle of synthesis, release, and re-uptake (Purves, 2012). The interesting fact that a realm of neurotransmitters, e.g. GABA, 5-HT, and histamine, can be synthesized in large quantities by gut bacteria on a daily basis has led to increasing inquiries on their potential role in the microbiome-gut-brain crosstalk and in turn, how these compounds are used by microbes and/or host in regulating local microbial growth and ecology in GI tract per se (Dinan & Cryan, 2017; Mittal et al., 2017; Strandwitz, 2018). Indeed, in recent years, theories of microbial endocrinology are continuously developing to specifically deal with neurotransmitters that have evolutionarily shared synthetic pathways, receptor families, and neuroendocrine effects between mammals and microbes (Mark Lyte, 2004; M. Lyte, 2011; Villageliu, Rasmussen, & Lyte, 2018). Such interkingdom crosstalk in the language of neurotransmitter has been recently justified by the landmark FGFP cohort study (Valles-Colomer et al., 2019). The results specifically identified perturbed GABA synthetic pathways, 5-HT metabolism, and dopamine metabolites as associated with quality of life (QoL) proxies and indicators of depression. For small-molecule neurotransmitters, we will focus on amino acids (e.g., glutamate, GABA, and 5-HT) and biogenic amines (e.g., dopamine, norepinephrine, and epinephrine).

1.5.1 Glutamine and γ-aminobutyric acid

L-Glutamate (Glu) and γ-aminobutyric acid (GABA) are respectively the major excitatory and inhibitory neurotransmitters in human CNS and interestingly, are biochemically interconvertible via the two-way (glutamine-)Glu-GABA cycle (Hertz, 2013; Purves, 2012). Both

glutamatergic and GABAergic loops are complex systems incorporating the fate of Glu/GABA, neurotransmission (through activating receptors widespread in host), and inactivation by high-affinity transport systems in glial cells (mainly astrocytes), with the machineries detailed elsewhere (Mazzoli & Pessione, 2016). For Glu-GABA metabolism, it is generally agreed that Glu cannot pass the BBB (Hawkins, 2009; Janeczko, Stoll, Chang, Guan, & Burrin, 2007) and therefore, CNS Glu only comes from synthesis *in situ*, either relying on glutamine (Gln) as precursor or through transamination of 2-oxoglutarate from citric acid cycle (Cherlyn et al., 2010; Mazzoli & Pessione, 2016); on the other hand, it is supported that GABA is able to cross the BBB (Boonstra et al., 2015; Kakee et al., 2001). In the CNS, GABA can be derived from glycolysis, or from Glu by L-glutamic acid decarboxylase (GAD), an enzyme almost exclusively present in GABAergic neurons. In parallel, numerous bacterial strains have been identified to produce Glu (e.g. *Bifidobacterium* spp., *Lactobacillus* spp., *Corynebacterium glutamycum*) and GABA (e.g. *Lactobacillus* spp., *Escherichia coli*, and *Pseudomonas* spp.) in large quantities for functional purposes such as in interspecies interaction (Mazzoli & Pessione, 2016). Studies revealed that both Glu and GABA can serve as energy substrates for gut microbes, balance luminal pH, alter pathogen virulence, and regulate gut motility, etc (Mazzoli & Pessione, 2016; Strandwitz, 2018). In this light, microbiome-host interaction through glutamatergic and/or GABAergic systems could be viable pathways to explore.

Studies linking gut bacteria derived Glu and GABA to neurological health phenotypes are only emerging. Perhaps the most cited evidence in this regard will be the landmark probiotic intervention study mentioned earlier (Bravo et al., 2011). Specifically, the authors discovered that *Lactobacillus rhamnosus*, a lactic acid bacterium, exerted a direct effect on murine CNS GABA receptor expression while conferring anxiolytic benefits in mice, as demonstrated in both elevated

plus maze (EPM) behavioral test and stress hormone analyses. Through vagomization trials, the authors further identified vagus nerve as a constitutive pathway of communication between gut-resident microbes and brain. More recently, Pokusaeva and colleagues showed in a rat model that oral administration of *Bifidobacterium breve* NCIMB8807 pESHgadB, an engineered GABA-producing strain with overexpression of *GadB* genes that encodes GAB, led to reduced sensitivity to visceral pain as compared with wild-type strains (Pokusaeva et al., 2017). In a more recent study, through combining fecal transplant trials, Zheng and colleagues revealed that gut microbiota from schizophrenia patients could modulate the Gln-Glu-GABA cycle and induce schizophrenia-relevant behaviors in mice (Zheng et al., 2019).

1.5.2 Catecholamines: dopamine, norepinephrine, and epinephrine

Catecholamines, including dopamine, norepinephrine, and epinephrine, are biogenic amines neurotransmitters that carry crucial roles in a wide range of neurophysiological and behavioral processes, spanning from CNS homeostasis, attention, emotional control, to coordination of body movement (Purves, 2012; Volkow, Wise, & Baler, 2017). In the CNS, dopamine is produced in the cytoplasm of presynaptic terminals through decarboxylation of its immediate precursor, L-3,4-dihydroxyphenylalanine (L-DOPA) that is derived from tyrosine (Tyr) as the rate-limiting step. In downstream steps, dopamine serves as substrates for producing norepinephrine and epinephrine(Purves, 2012). Although the three compounds are biochemically interconvertible, they carry distinct activities and functions in the CNS. More specifically, dopamine, mainly located in the corpus striatum (an area for motor control), has essential roles in motivation, reinforcement, reward, and hedonistic regulation (Fetissov, 2017). Many substances of abuse (e.g., cocaine, amphetamine) work by altering dopaminergic synapses in the CNS.

Moreover, drug design for psychotherapeutic use typically mimics dopamine's structure to fulfill anti-anxiety, anti-depressant, and antipsychotic benefits. Norepinephrine is known for its regulation of attention, sleep/wakefulness, and feeding behavior, whereas epinephrine, at much lower CNS levels, may have an impact on memory and learning (Purves, 2012).

The microorganisms have long been recognized as capable of producing catecholamines; for example, *Bacillus*, *Serratia* and *Proteus* spp. synthesize dopamine, while *Escherichia*, *Bacillus*, *Saccharomyces* spp. have been found to produce norepinephrine (M. Lyte, 2011; Mittal et al., 2017; Roshchina, 2010). Although it is yet to be confirmed whether gut commensal bacteria contribute to catecholamine metabolism of the host, accumulating evidence suggest that they do (Diaz Heijtz et al., 2011; Kiraly et al., 2016; W. H. Liu et al., 2016; Moya-Perez, Perez-Villalba, Benitez-Paez, Campillo, & Sanz, 2017). Heijtz et al. discovered contrasting neurophenotypes between GF and specific-pathogen-free (SPF) conventional mice based on behavioral test scores, neurochemical concentration profiles, and patterns of gene expression. Increased turnover rates of dopamine and norepinephrine were found in GF mice, suggesting a role of microbiota (Diaz Heijtz et al., 2011). Further, Kiraly and colleagues revealed in a mouse model that antibiotics-knockdown of gut microbiota, without affecting cocaine metabolism and behavior-modulating stress hormones, resulted in altered cocaine-mediated behaviors, specifically with heightened sensitivity to cocaine reward in conjunction with changes in the dopaminergic system such as elevated activity of the CNS D1 dopamine receptor *Drd1* (Kiraly et al., 2016). More recently, emerging human cohort studies associated intestinal microbial compositions and functional genes with altered dopaminergic systems in disease models such as depression and PD (Bedarf et al., 2017; Valles-Colomer et al., 2019). However, because it is generally believed that all three polar catecholamines

cannot cross BBB (Purves, 2012), how bacteria-mediated catecholamine metabolism relates to the gut-brain crosstalk remains elusive and warrants elucidation.

1.5.3 Other small-molecule neurotransmitters

Aside from 5-HT, glutamate/GABA, and catecholamines, many other neurotransmitters also exhibit potentials to mediate the microbiota-brain axis and alter paths of associated psychopathologies, although current evidence and understanding are only emerging or at best suggestive. These include but not limit to histamine, trace amines (β-phenylethylamine, tyramine, and tryptamine), glycine, acetylcholine (ACh), serine, and taurine etc, with confirmed microbial sources and suggested roles in gut-brain signaling. For instance, histamine is an organic nitrogenous mediator of inflammatory responses and itching and contributes to tissue swelling during inflammation through increasing capillary permeability to white blood cells and proteins. One recent study showed that the probiotic strain *Lactobacillus reuteri*, carrying histidine decarboxylase, converted histidine to histamine, which was further demonstrated to suppress TNF through modulation of protein kinase (PKA) and extracellular signal-regulated kinase (ERK) signaling (Thomas et al., 2012). Likewise, trace amines such as tryptamine and β-phenylethylamine are known to play significant roles in appetite control, attention, and emotional processing. In parallel, a range of lactic acid bacteria have been confirmed as their synthesizer, with *Lactobacillus bulgaricus* and *Clostridium sporogenes* for producing tryptamine while *Enterococcus faecalis* and *Leuconostoc* strains for the synthesis of both β-phenylethylamine and tyramine (Pessione et al., 2009; Williams et al., 2014). Future studies are warranted on the neurotransmitters for which the kinetics of microbial synthesis in the GI tract are better modeled,

gut microbial proportion to the CNS levels differentiated, and their functional roles in modulating the gut-brain signaling delineated.

1.6 Neuropeptides

1.6.1 Microorganism-associated molecular patterns

On both molecular and ecological levels, microorganisms are in constant flux of biochemical signaling for intra- and/or inter-species communication, inhabitant assessment, trigger of immune responses, and formation of symbiotic biofilm, etc. At center are the microorganism-associated molecular patterns (MAMPs), i.e., microbial protein or peptide molecular motifs that are highly class-specific and evolutionally conserved through which microbial species identifies one another. As evidenced, MAMPs, of which many are pathogens to mammals, can translocate from gut to remote peripheral blood system to induce immune responses, raising the possibility that they are involved in the microbiome-gut-brain axis. Two examples of note are lipopolysaccharides (LPS) and bacterial peptidoglycans (PNGs) that are derived from cell walls and/or membranes of either Gram-negative or Gram-positive bacteria of microbiota.

1.6.2 Lipopolysaccharides

Lipopolysaccharides (LPS), also known as endotoxin and a major component from Gram-negative bacteria, can translocate from the GI tract to systemic circulation through leaky mucosal linings. Once sensed by pattern recognition receptors (PRRs) (e.g., toll-like receptor 4, or TLR4) of the host innate immune system, cytokines are produced that can either signal directly to the CNS or excite vagal and spinal afferent neurons, given that PRRs of LPS (e.g., TLRs) are prevalently expressed in GI epithelial cells, ENS neurons, and the rest anatomical makeup of the

gut-brain axis. There have been multiple studies linking LPS as a molecular cue of intestinal flora to the brain. For example, Maes and colleagues reported for patients with both chronic fatigue syndrome (Maes, Kubera, Leunis, & Berk, 2012) and chronic depression (Michael Maes et al., 2012) increased circulating levels of immunoglobulins such as IgA and IgM in response against LPS propagated from gut bacteria, suggesting a role of LPS in the microbe-brain interplay. In addition, administration of LPS, either through injection or oral gavage, can lead to exacerbated neurological pathophenotypes in mice such as depression-mimicking behaviors (O'Connor et al., 2009) and cognitive impairment (Zhao et al., 2019). These collectively demonstrate for LPS as a potent molecular cue of microbiota to influence host brain function and behaviors.

1.6.3 Peptidoglycans

Peptidoglycans (PGNs) constitute another type of peptides with confirmed microbial sources (Gram-positive) and effects on brain function. A seminal study by Arentsen and colleagues reported serum levels of PGNs in GF mice to be extremely low or below detection limits even using ultra-sensitive mass spectrometry platforms, as in contrast to conventional normal mice, indicating gut microbiota as a major source for peripheral PGNs (Arentsen et al., 2017). Microbial PGNs, once relocated to circulating blood (including under normal physiological conditions) to be recognized by cytosolic nucleotide-binding and leucine-rich repeat-containing receptors (NLRs), a central immunological regulator that encompasses an array of innate immune sensors and receptors (e.g., NOD1 and NOD2), can trigger downstream immune responses and regulate CNS health. Indeed, recent evidence supports such link between microbial PGNs and CNS health. On one hand, the NLR family, as the major PRRs of PGNs, have recently been shown to play an active role in shaping and controlling gut microbiota, with notable examples of NLRP6 and NLRP12 that

have been demonstrated to regulate intestinal inflammation in parallel with gut microbial composition and metabolism (Guo, Gibson, & Ting, 2020). Importantly, Pusceddu and colleagues for the first time revealed a novel role of NLRs in gut-brain signaling (Pusceddu et al., 2019). The authors observed for knockout mouse models (NodDKO) deficient in both *Nod1* and *Nod2* exacerbated stress-induced behaviors, impaired cerebral serotonergic system, a decline in hippocampal neurogenesis, and increased GI permeability. Strikingly, administration of fluoxetine, a selective 5-HT reuptake inhibitor, corrected these behavior deficits and restored 5-HT signaling. On the part of PGNs per se, the MIA model of ASD was established because maternal immune activation itself, through infecting pregnant mice with proinflammatory MAMPs (such as PGNs), induces ASD-like behaviors in the mouse offspring. Such effects of PGNs at the maternal-fetal interface suggest a critical role of maternal microbiota in shaping fetal brain health, which was backed by several latest studies examining placental mobility of PGN-teichoic acid complex, a cell wall component of pathogen *Streptococcus pneumoniae*, in parallel with fetal outcomes of neuro-proliferation and cognitive development (Humann et al., 2016); the details are reviewed elsewhere with critical questions asked (Gonzalez-Santana & Diaz Heijtz, 2020).

1.6.4 Neuropeptides in appetite control

The GI tract, with tubular tissue lining of the ENS all over, is home to a range of neuroendocrine cells and their neuropeptide products including neuropeptide tyrosine (NPY), calcitonin gene-related peptide (CGRP), peptide YY (PYY), and GLP-1. NPY and CGRP are expressed at all levels throughout the gut-brain axis, while PYY and GLP-1 are exclusively produced in distal ileum and colon by local enteroendocrine L cells. Due to their proximity to intestinal mucosa, where microbiota resides, question arises as to whether gut microbiota interacts

with these neuropeptides to further modulate the gut-brain signaling. One major area of note is about gut microbial regulation of appetite control. As a proof-of-principle, recent studies have demonstrated a role of SCFAs in appetite regulation. One study, through *in vivo* and *in vitro* experiments, discovered that colonic propionate derived from microbiota stimulated local release of both PYY and GLP-1 from L-cells in wild-type mice, but not in in *Ffa2*$^{-/-}$ knockout mice which were deficient in expression of FFA2, the receptor for SCFAs. The results suggest that gut microbiota harbors the great potential to modulate neuropeptide profiles, and associated satiety perception and food intake behaviors (Psichas et al., 2015).

Another review article, from a meta-analysis perspective, also underscores a potential role of microbiota in appetite control (Fetissov, 2017). Specifically, GABAergic and glutamatergic neurons, both belonging to the hippocampal arcuate nucleus (ARC) family and closely related to the two-way Glu/GABA cycle, are key regulators of appetite status. GABAergic neurons express the orexigenic NPY and agouti-related protein (AgRP), whereas glutamatergic neurons express pro-opiomelanocortin (POMC), a precursor of α-melanocyte-stimulating hormone (αMSH) and an anorexigenic neuropeptide. Often, these NPY, AgRP, and POMC hormone populations are viewed as the 'first-order' neurons in satiety and hunger pathways. Interestingly, recent studies together suggest a causal role of gut bacteria to influence host energy metabolism and feed behaviors, with colonic bacterial growth profiles found to be much overlapping with satiety hormone release (e.g., GLP-1, PYY) and satiety perception, especially during first 20 minutes upon meal induction (Fetissov, 2017). The author also speculated bacterial metabolites (e.g., quorum sensing acylhomoserine lactones) that are excreted to regulate growth dynamics, if detected in portal circulation, might act directly on hypothalamic neurons.

1.6.5 Peptides of neurodegenerative hallmark: α-synuclein and β-amyloid

The relationship between gut microbiota and pathogenesis of neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD), remains an intriguing research topic to explore. Aggregated CNS levels of β-amyloid (Aβ) and α-synuclein (α-syn) are the two main pathological hallmark proteins associated respectively with AD and PD. Recent studies showed that gut microbiota is capable of producing extracellular amyloid-like proteins, which in turn can exacerbate the pathogenic course of α-syn in aged rats; these suggested a multifaceted role of microbiota in mediating the course of neurodegenerative diseases (Friedland, 2015). For specific pathogenesis, note that α-syn, once turned the pathologic state, has a prion-like activity to propagate and translocate through the vagus nerve of gut-brain axis and leads to impaired CNS physiology and behavioral deficits (Cryan, O'Riordan, Sandhu, Peterson, & Dinan, 2020). This hypothesis was supported by a recent report by Sangjune and colleagues (Kim et al., 2019). The authors discovered that through gut injection of α-syn fibrils, murine endogenous α-syn was converted to a pathologic species that spread from gut to the brain, resulting in pathological traits of PD such as degeneration of dopamine neurons and motor (and non-motor) deficits. Strikingly, these pathological traits were prevented via truncal vagotomy or α-syn deficiency (through knockout of the *Snca* gene), suggesting vagus nerve as an important conduit for α-syn pathology in PD. The neuronal routes of vagus nerve in the course of PD was further supported by epidemiological studies (one Danish cohort (Svensson et al., 2015) and one Swedish cohort (B. Liu et al., 2017)) aiming at dissecting the role of truncal vagotomy procedures. Because of the vicinity of mucosa-residing microbiota to the ENS, whether and how gut microbiota contributes to such α-syn pathogenesis in PD through the route of vagus nerve remains to be resolved.

1.7 Implications

1.7.1 Discovering novel molecules of microbiota

Gut microbiota is complex, dynamic, and metabolically active, harboring numerous novel compounds to be discovered and neuroactive potentials to be mined. Identifying novel compounds through which our commensal microbiota mediates the CNS function thus presents a pressing issue to resolve. For example, bacteria excrete quorum sensing molecules such as N-acylhomoserine lactones (AHLs) for interspecies communication to regulate local population growth and nutrient use. Previous studies showed that gut-derived AHLs could alter how neurons function (D. T. Hughes & Sperandio, 2008), but how they reach humoral circulation remains poorly characterized. Besides, gut microbiota could also be a source of steroid hormones, with cortisol being a typical example in which the gut bacterial strain *Clostridium scindens* performs such glucocorticoid-to-androgen conversion (Ridlon et al., 2013). Moreover, some bacteria-derived compounds, although at extremely low levels, might be implicated in a wide range of host neurophysiological processes. One example is tetrahydrobiopterin (BH_4), a cofactor essential for normal CNS dopamine synthesis and function. Recently, a study confirmed the gut resident *Adlercreutzia equolifaciens* and *Microbacterium schleiferi* as BH_4 producers, which could be a potential source to the CNS BH_4 (Belik et al., 2017). Another example is vitamin B6, which can also be bacterially derived in gut (Kau, Ahern, Griffin, Goodman, & Gordon, 2011). Vitamin B6 is the precursor of pyridoxal phosphate, a cofactor essential for GAD enzymatic conversion of glutamate to GABA. The lack of vitamin B6 could result in massive decrease in CNS GABA levels, the subsequent loss of synaptic inhibition, and seizures (Purves, 2012). Also, amines with confirmed bacterial sources, such putrescine, spermidine, spermine and cadaverine, have been shown to be implicated in CNS responses to stress (Bienenstock & Collins, 2010).

1.7.2 Unravelling inter-class interaction

As the field of microbiome-gut-brain axis is experiencing a paradigm shift of asking what they are to what they do and eventually, to how we could leverage the gut microbial capacity for health causes, further measures should be taken to go beyond curating these neurochemicals or dissecting their role in a reductionist manner and to instead gain an integrated perspective of them all. In other words, it is in principle important to identify potential interactions between seemingly distinct compound classes and biochemical pathways for systems biology-level insights of the interspecies and interorgan communication. Indeed, emerging studies support such interclass interactions. For example, SCFAs produced by *Clostridium* spp. have been shown to modulate microbial 5-HT biosynthesis in gut enterochromaffin cells, specifically through promoting gene expression of *Tph1* (Reigstad et al., 2015). Another recent study has suggested that cortisol mediates metabolism of portal 5-HT levels (Mudd, Berding, Wang, Donovan, & Dilger, 2017). Moreover, factors associated with altered gut permeability or worsening of the gut-brain axis structure (e.g., leaky gut and leaky BBB) may converge as a systemic process, where many compounds spanning disparate chemical pools are likely to be involved (Kelly et al., 2015; Madhukumar Venkatesh et al., 2014).

1.7.3 Opportunities and challenges

Recent evidence from human cohort studies and animal models collectively support that our commensal microbiota is actively modulating our neurophysiological state, emotions, and behaviors on a day-to-day basis. Targeting gut microbiota, especially through discovery and use of their neuroactive molecules, appeal to health scientists and medical practitioners as one promising avenue of research for improved health outcomes. In this light, frameworks of drug

development are transformed to incorporate NGS-assisted data mining efforts of the microbiome for novel discovery, compound screening, and the design of synthetic biology approaches, with goals to counteract neurological disorders (Donia & Fischbach, 2015; Williams et al., 2014) or bacteria-mediated side effects of drugs (Bhatt, Redinbo, & Bultman, 2017; Wallace et al., 2010). In addition, nutritional research and the food industries have started leveraging the power of gut-resident microbes to design novel diets (Valdes, Walter, Segal, & Spector, 2018). Such application ranges from prebiotics and probiotics that carry neuroprotective potentials (together coined as *psychobiotics*) for achieving improved psychological states even in healthy normal individuals (Dinan & Cryan, 2017), to the development of ketogenic diet for anti-seizure benefits (Olson et al., 2018). Moreover, environmental toxicological research for public health causes may target gut microbiota as a functional entity or 'organ' in mediating the fate and neurotoxic effects of widely applied environmental toxicants, such as pesticides (B. Gao, Bian, Mahbub, & Lu, 2017). In a nutshell, targeting microbiota-neurochemical network represents a crucial venue of research for neurological and/or psychological health causes.

Challenges will be encountered for advancing the field of microbiome-gut-brain axis. To date, much of the molecular mechanism underlying such crosstalk remains incompletely elucidated, as hampered by methodological limitations in standardization of neuro-phenotyping (Hooks et al., 2018), development of relevant *in vivo* models (J. M. Lyte, Proctor, Phillips, Lyte, & Wannemuehler, 2019), microbiota characterization (Hamady & Knight, 2009), microbial source apportionment (for example, from host or direct food intake) (Zimmermann, Zimmermann-Kogadeeva, Wegmann, & Goodman, 2019), confounder adjustment(Valles-Colomer et al., 2019), and neurochemistry analysis (Vernocchi, Del Chierico, & Putignani, 2016), etc. Since the gut-brain axis is by nature bidirectional, dynamic, and elastic, defining microbiota's neuroactive

potential in quantitative proxies is inherently challenging. For this, the 56 gut-brain modules curated in the recent FGFP cohort study (Valles-Colomer et al., 2019), each referring to a biochemical step (either for the production or degradation) of a single neurochemical, may be a valuable resource to start with. In addition, because current evidence is mostly based on animal models, data extrapolation to human populations can be problematic; more large-scale epidemiological data are needed, especially those in coupling with cutting-edge omics approaches (e.g. metabolomics, metagenomics) (Vernocchi et al., 2016). Furthermore, even for compounds of validated neuroactive benefits (e.g., SCFAs), cautions should be taken regarding dose levels and routes of delivery for application to clinical treatment to ensure both safety and efficacy. This entails knowledge of pharmacokinetics modeling, critical time window of dosage, individual disease susceptibility and comorbidity, as well as intra- and interindividual variability of gut microbial dynamics, etc.

1.8 Conclusion

In this review, we compiled a realm of gut microbiota-derived molecular cues, spanning from small molecules to the larger neuropeptides, to conduct a comprehensive literature review linking their neuroactive potential to the gut-brain axis. We first summarized their biosynthesis, uptake, activities, and functions in general. We then turned to specific disease models to carefully evaluate their roles in lieu of microbiota-gut-brain axis as evidenced in human cohort studies, animal trials, and/or *in vitro* data. With the up-to-date information collected, future direction of research may revolve around the discovery and development of novel microbial molecules of gut-brain health, which entails massive multi-omics mining efforts of gut microbiota in parallel with mechanistic exploration of inter-class compound interaction. Finally, aside from the vast promises

and opportunities the field holds for biomedical, nutritional, and environmental sciences, we also discussed challenges to tackle for the many years to come.

CHAPTER 2: TARGETED METABOLOMICS OF 50 NEUROTRANSMITTERS AND TRYPTOPHAN METABOLITES OF FECES, BLOOD SERA, AND BRAIN TISSUES IN C57BL/6 MICE[2]

2.1 Introduction

Recent next-generation sequencing (NGS) efforts have led to explosive growth in knowledge associating the trillions of the commensal microorganisms to human health (Kau et al., 2011). One important direction of research is to gauge the neuroactive potentials gut microbiota might hold for modulating the gut-brain axis, either through neuronal or humoral routes, and to discover actionable therapeutic targets towards improved mental health (Dinan & Cryan, 2017; Groen, de Clercq, Nieuwdorp, Hoenders, & Groen, 2018). Such advances require that the much uncharted biochemical underpinnings of the microbiome-gut-brain axis be deciphered in the first place. Previous evidence derived from animal models (J. Gao et al., 2018; Platten et al., 2019) and human cohort studies (Valles-Colomer et al., 2019) indicates that the gut bacteria-tryptophan/neurotransmitter metabolic network might be a key in orchestrating the gut-brain signaling in response to internal and external stimulus.

L-Tryptophan (Trp) is an essential aromatic amino acid with an indole scaffold by structure. Trp is exclusively obtained from diet and once administrated, it undergoes three downstream catabolic pathways, including kynurenine (Kyn) pathway, serotonin (5-HT) biosynthesis, and microbial generation of indole derivatives (Cervenka et al., 2017). Emerging research showed that

[2] Chapter 2 was reproduced from my published work - Lai Y., Liu C.-W., Chi L., Ru H., Lu K.*: High-resolution metabolomics of 50 neurotransmitters and tryptophan metabolites in feces, serum, and brain tissues using UHPLC-ESI-Q Exactive mass spectrometry, ***ACS Omega***, **6(12)**:8094-8103, doi:10.1021/acsomega.0c05789, 2021.

the gut microbiota actively regulates these metabolic fluxes to act on local enteric nervous system (ENS) or to the remote central nervous system (CNS) (Dinan & Cryan, 2017). For example, a recent landmark study has identified a signaling cascade through which the gut bacteria can act on local ENS synthesis of 5-HT, a well-studied monoamine neurotransmitter with multifaceted biological function in mammals (Yano et al., 2015). Another study further demonstrated that the gut microbiota constantly regulates the maturation of ENS until adulthood through an enteric 5-HT network (De Vadder et al., 2018). Importantly, gut bacteria-derived indole compounds have been discovered to not only mitigate enteric inflammation in gut, but impact CNS homeostasis and activity. A recent *in vivo* study of multiple sclerosis showed that indole, indole-3-propionic acid (IPA), and indole-3-carboxaldehyde (I3A) can directly modulate CNS inflammation by activating the aryl hydrogen receptor (AhR) that is expressed in astrocytes (Rothhammer et al., 2016). A more recent human cohort study associated indole, indole-3-acetic acid (IAA), and skatole with anatomical and functional measures of extended central reward network and measures of diet behaviors and anxiety symptoms (Osadchiy et al., 2018). The dominating Kyn pathway (accounting for ~95% of the total Trp catabolic fluxes) with multifaceted CNS effects can also be affected by gut bacteria due to their control over peripheral Trp-Kyn availability (Cervenka et al., 2017). In parallel with Trp pathways, numerous bacterial strains such as *Lactobacillus rhamnosus* and *Bifidobacterium breve* are known to be able to produce and use neurotransmitters such as γ-aminobutyric acid (GABA) in large quantities *in situ* and beyond, with demonstrated effects on host neurophysiology (Bravo et al., 2011).

Hypothesis testing revolving around how gut microbes modulate brain function via the Trp-neurotransmitter network requires reliable, sensitive, and accurate analytical assays. Trp metabolites and neurotransmitters are structurally diverse, with wide-ranging ionization constants

(i.e., pKa values) and contrasting endogenous concentration levels. In recent years, liquid chromatography tandem mass spectrometry methods have been developed to determine Trp derivatives (G. Y. Chen, Zhong, Zhou, & Zhang, 2018; Henykova et al., 2016; Lefevre et al., 2019; Whiley et al., 2019).

However, most of the existing methods only cover 20 or fewer Trp metabolites while leaving classical neurotransmitters largely unmeasured and, none of the assays tested all relevant samples matrices (i.e., blood, feces, and brain) for advancing the field of the microbiome-gut-brain axis. Importantly, data collected by triple-quadrupole (QqQ) tandem mass spectrometry in selected reaction monitoring (SRM) mode, although sensitive and robust, renders only unit-mass resolution and offers little ion fragmentation details of the transition monitored. This drawback inherently defies novel discovery and may induce potential quantitation biases due to interferences from isobars (with close retention time, similar structure, and fragmentation pattern) when analyzing complex sample matrices. By contrast, high-resolution MS/MS analysis, specifically using quadrupole-orbitrap mass spectrometry in parallel reaction monitoring (PRM) mode, not only records full accurate-mass spectral profile of all fragmentation ion products (<5ppm) that enables novel discovery and structural elucidation in addition to unambiguous quantitation, but confers comparable or better instrumental sensitivity compared with unit-mass SRM analysis owing to the high resolving power (e.g., up to 140,000 full width at half maximum (FWHM) for 200 Da using the most basic Q Exactive instrumental model) (Ronsein et al., 2015). These benefits prompt us to develop a comprehensive, accurate, and sensitive assay using high-resolution mass spectrometry for quantifying Trp catabolites, neurotransmitters, and bacterial indole derivatives with potential neuroactive effects on mammalian hosts. The assay will be validated and applied to targeted

metabolomic analysis of mouse serum, feces, and brain, and may be a useful analytical resource for advancing the field of microbiome-gut-brain axis.

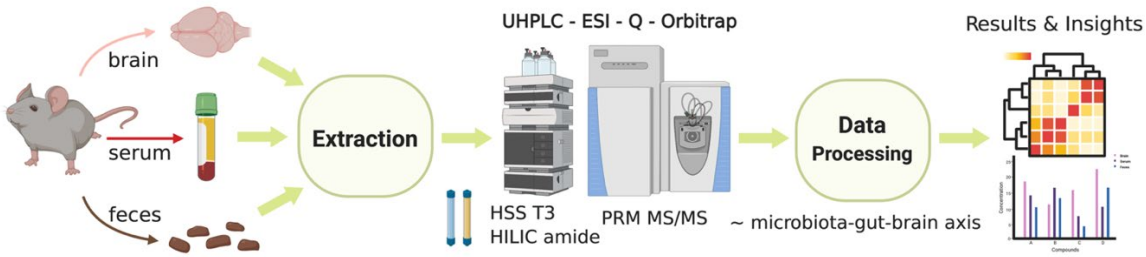

Figure 2.1 Technical schematic illustration of high-resolution LC-MS/MS quantitation of 50 neurotransmitters and tryptophan metabolites of significance to the mammalian microbiome-gut-brain axis.

2.2 Materials and Methods

2.2.1 Chemicals and reagents

LC/MS-grade (Optima) solvents and reagents, including water, acetonitrile (ACN), methanol (MeOH) and formic acid, were purchased from Thermo Fisher Scientific (Waltham, WA, USA). Ammonium formate salt of trace metal purity and dimethyl sulfoxide (DMSO) of ACS reagent grade (>99.9%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). A total of 50 authentic analytical standards, spanning from classical neurotransmitters, tryptophan metabolites to bacterial indole derivatives, were procured from Sigma-Aldrich (St. Louis, MO, USA) and Cayman Chemical (Ann Arbor, MI, USA). Stable-isotope labelled (SIL) internal standards were purchased from Cambridge Isotopes Laboratories Inc. (Tewksbury, MA, USA) and CDN Isotopes (Pointe-Claire, Quebec, Canada).

2.2.2 Stock solution preparation

All chemical standards, including the 50 analytical standards (ASDs) and 8 SIL internal standards (ISDs), were in granular powder forms at delivery to the lab and stored at -80 ºC while not in use. Stock solutions of individual standards were made by weighing the original powder to be exact using disposable antistatic microspatula (USA Scientific, Ocala, FL, USA) on a Fisher Scientific analytical balance at 0.1 mg precision (Hampton, NH, USA) and dissolving in MeOH, water, or DMSO to achieve a concentration level of 1.0 mg/mL or less, depending on analyte solubility specified on the Material Safety Data Sheet (MSDS). Molar concentration was converted from mass concentration using molecular weight that was directly obtained from MSDS distributed by the chemical manufacturers.

2.2.3 Animal rearing and sample harvest

Male specific-pathogen-free-grade C57BL/6 mice of ~7-week-old were purchased from Jackson Laboratories (Bar harbor, ME, USA) and housed at the University of Georgia animal facility under the following condition: 22 ˚C, 40-70% humidity, and a 12:12 hour light:dark cycle. Standard pelleted rodent diets and tap water were supplied *ad libitum*. All mice were observed for one week before experimental use. For sample harvest, mice were CO_2-sacrificed and at time of euthanasia, and serum, feces, and brain samples were collected, snap-frozen, and stored at -80 ºC prior to analysis. The animals were treated humanely, with all animal procedures approved by the University of Georgia Institutional Animal Care and Use Committee.

2.2.4 Sample preparation

Serum, feces, and cerebral cortical brain tissues are selected for targeted analysis due to their relevance to the microbiome-gut-brain axis. The extraction procedures were kept as simple as can be, shunning intricate and selective procedures for considerations of analyte coverage and throughput of analysis.

Blood sera: Thawed on ice, an exact aliquot of 40 µL serum sample was used, to which 360 µL ice-cold ISD-containing MeOH was added, vortexed, and incubated at -20 °C for 30 min. Then, the samples were centrifuged at 15,000×*g* for 10 min to precipitate protein and particulates. Aliquoted supernatants were subsequently dried in a CentriVap vacuum concentrator (Labconco, MO, USA).

Feces: Thawed on ice, ~20 mg of fecal matter was aliquoted for each sample to a 1.5 mL Eppendorf tube (Hamburg, Germany), which was further filled with ~30 mg acid-washed glass beads (Sigma-Aldrich, St. Louis, MO). To every 25 mg fecal matter a total of 600 µL ice-cold ISD-containing MeOH:water (50:50, *v/v*) solution was added for extraction. The sample was homogenized on a TissueLyzer (Qiagen, Hilden, Germany) for 10 min at 50 Hz and centrifuged at 12,000×*g* for 10 min. Supernatant aliquots were collected and dried in a CentriVap vacuum concentrator (Labconco, MO, USA).

Cerebral cortical brain tissues: Thawed on ice, ~20 mg thawed brain tissue of the cortical-hippocampal region was sliced off and placed in a 2-mL screw cap microcentrifuge tubes (VWR, Radnor, PA) containing a 5-mm i.d. clean stainless-steel beads (Qiagen, Hilden, Germany). To every 20 mg brain tissue 400 µL ice-cold ISD-spiked MeOH was added. The samples were homogenized on a TissueLyzer (Qiagen, Hilden, Germany) at 50 Hz for 2 min and incubated at -

20 °C for 1 h prior to centrifugation at 18,000×*g* for 10 min. Supernatant aliquots of the brain extracts were transferred to a CentriVap vacuum evaporator (Labconco, MO, USA) for dryness.

Upon instrumental analysis, all dried extract aliquots were reconstituted in 98:2 water/acetonitrile and 5:95 water/acetonitrile, respectively for HSS T3 (reverse phase, C18) and HILIC amide chromatographic analysis. Details of sample extraction, supernatant aliquoting and solvent resuspension are summarized in **Supplementary Table 2.1**.

2.2.5 Instrumental analysis

High-resolution LC-MS/MS analysis was performed using a Thermo Scientific Vanquish UHPLC system coupled to a Q-Exactive mass spectrometer interfaced with a heated electrospray ionization (HESI) source and a hybrid quadrupole-orbitrap mass analyzer. The mass spectrometer was operated at 70,000 mass resolution (FWHM for 200 Da) for fullscan analysis (for method development) and 13,500 in parallel reaction monitoring (PRM) mode (for targeted analysis) (Waltham, WA, USA). The instrument was calibrated at least once a week in both ESI positive and negative modes using Thermo Scientific Pierce^{TM} LTQ Velos ESI ion calibration solutions (Waltham, WA, USA), to ensure optimal and robust instrumental performances throughout the analysis regarding mass accuracy (<5ppm), ion transfer, and ion isolation, and instrumental sensitivity.

Two complimentary chromatographic columns were used, including Waters Acquity UPLC HSS T3 (reverse phase C18, 100Å, 1.8 µm, 2.1 mm×100 mm) and Waters Acquity BEH amide (hydrophilic interaction chromatography, i.e., HILIC, 130Å, 1.7 µm, 2.1 mm×150 mm) (Milford, MA, USA). For HSS T3, the mobiles phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in ACN (B), with a 15-min gradient: 2% B at 0-1 min; 2% B to 15% B, 1-3

min; 15% B to 50% B, 3-6 min; 50% B to 98% B, 6-7.5 min; 98% B, held at 7.5-11.5 min; 98% B to 2% B, 11.5-11.6 min; 2% B at 11.6-15 min. As of BEH amide, the mobile phases comprised 50:50 (*v/v*) ACN:water (A) and 15:5:80 (*v/v/v*) water:MeOH:ACN (B) with both added with 10 mM ammonium formate, and a 11-min gradient was used: 95% B at 0 min; 95% B to 50% B, 0-3.5 min; 50% B to 5% B, 3.5-5.5 min; 5% B, 5.5-6.5 min; 5% B to 95% B, 6.5-6.7 min; 95% B, 6.7-11 min.

The analyses were conducted in electrospray ionization (ESI) positive mode with sheath gas flow rate set as 60 L/min, aux gas flow rate at 10 L/min; sweep gas flow rate at 1 L/min, spray voltage at 2.75 kV, capillary temperature at 325 ºC, and aux gas heater temperature at 400 ºC. The mass spectral data for quantitation were acquired in PRM mode, with AGC target set as 2e5, maximum injection time (IT) as 50 ms, and an isolation window of 1.2 *m/z* for ion fragmentation. The PRM was performed according to an inclusion list containing the elemental composition, (ESI) species, charge numbers, (ionization) polarity, range of retention time for fragmentation, and normalized collision energy (NCE) of higher-energy C-trap dissociation (HCD) that have had optimized for each individual compound.

2.2.6 Data processing and quantitation

The *.RAW data acquired in both fullscan and PRM modes can be manually inspected in XCalibur Qual browser 4.1.31.9 (Waltham, WA, USA) and automatic batch peak integration was performed in TraceFinder 4.1 (Waltham, WA, USA). Twelve-point calibration standard solutions of 0.5 nmol/L to 10 μmol/L were made, and calibration curves with at least five consecutive points were generated for each compound, with ASD-to-ISD quant ion peak area ratio being y-axis and

ASD-to-ISD amount ratio x-axis. Results of peak area auto-integration in TraceFinder was manually inspected in XCalibur and corrected whenever necessary.

2.2.7 Method validation

The method validation was carried out following the guidelines in "*Bioanalytical method validation: Guidance for industry*" by the U.S. Food and Drug Administration (FDA) (FDA, 2018).

Linear dynamic range (LDR): the LDR was determined by linear regression fitting (R^2 > 0.99) between ASD-to-ISD quant ion peak area ratios (*y*-axis) and ASD-to-ISD amount ratios (*x*-axis), with the lower end (i.e., lower limit of quantitation, LLOQ) defined as the lowest point tested that had an analytical precision lower than 20%.

Intra- and inter-day accuracy and precision: to assess assay robustness, calibration standard solutions at three different concentration levels (including LLOQ) were used to determine intra- and inter-day accuracy and precision of analysis. The intraday tests were conducted using three replicate injections within the same day (n=3), whereas the interday tests were based on three separate days, each with three replicate injections (n=12). Precision represented by percent relative standard deviation (RSD%) of ASD-to-ISD peak area ratio replicates was calculated in Microsoft Excel (Redmond, WA, USA) as: RSD% = 100%*σ/μ, where μ is the mean and σ is the standard deviation; the accepted precision of analysis was set at 20%. While accuracy (Acc) was calculated by Acc = 100%*experimental value/actual value.

Analytical recovery and matrix effect: the analyte recovery was evaluated by analyzing all spiked SIL internal standards in the three sample matrices.

Carryover: potential carryover of analysis was assessed by injecting resuspension solvent blanks every ten samples or calibration standard solutions during analysis. Same set of columns were used for all three biological matrices, which were conducted separately to fully evaluate sample-specific carryover.

2.3 Results and discussion

2.3.1 Method development and optimization

A technical schematic of the high-resolution LC-MS/MS assay is given in **Figure 2.1**, which was created by the first author with BioRender.com under a paid subscription (invoice no. 458B55C1-0003). A list of targets was first obtained from careful literature review and the authentic chemical standards were purchased as commercially available. Based on preliminary tests of authentic standards, two complementary chromatographic techniques (i.e., HSS T3 and BEH amide) were applied considering the structural diversity and lipophilicity of the 50 compounds and to minimize ion co-elution, with PRM extraction ion chromatograms (XICs) containing at least five points for accurate quantitation. Fullscan analysis of authentic standards was first conducted, to survey for each compound the chromatographic retention time, ESI polarity, major ion species (selected as precursor ion), and the corresponding accurate *m/z*. For SIL chemicals, the extent of hydrogen-deuterium exchange was further examined to assess isotope-labeling/chemical purity and the suitability-of-use as internal standards for LC-MS analysis. Then, retention time scheduled PRM MS/MS analysis of select precursor ions was conducted to determine optimal NCE for each compound and to identify major product ions, which were further confirmed by accurate mass (<5ppm) querying experimental mass spectral databases including mzCloud (www.mzcloud.org, Waltham, MA, USA) and metlin (www.metlin.scripps.edu, La Jolla,

CA, USA), MoNA (www.mona.fiehnlab.ucdavis.edu, Davis, CA, USA) and hmdb (www.hmdb.ca, Edmonton, Alberta, Canada). PRM transition with the most dominant and stable product ion (relatively independent of NCE, as indicated by ion breakdown curves in mzCloud) was chosen for quantitation, whereas others were kept for confirmatory purposes. **Table 2.1** summarizes the resultant parameters optimized for the 50 analytes and select SIL internal standards. In addition, Figure 2 shows optimized PRM XICs and total ion chromatograms (TICs) of the 50 compounds.

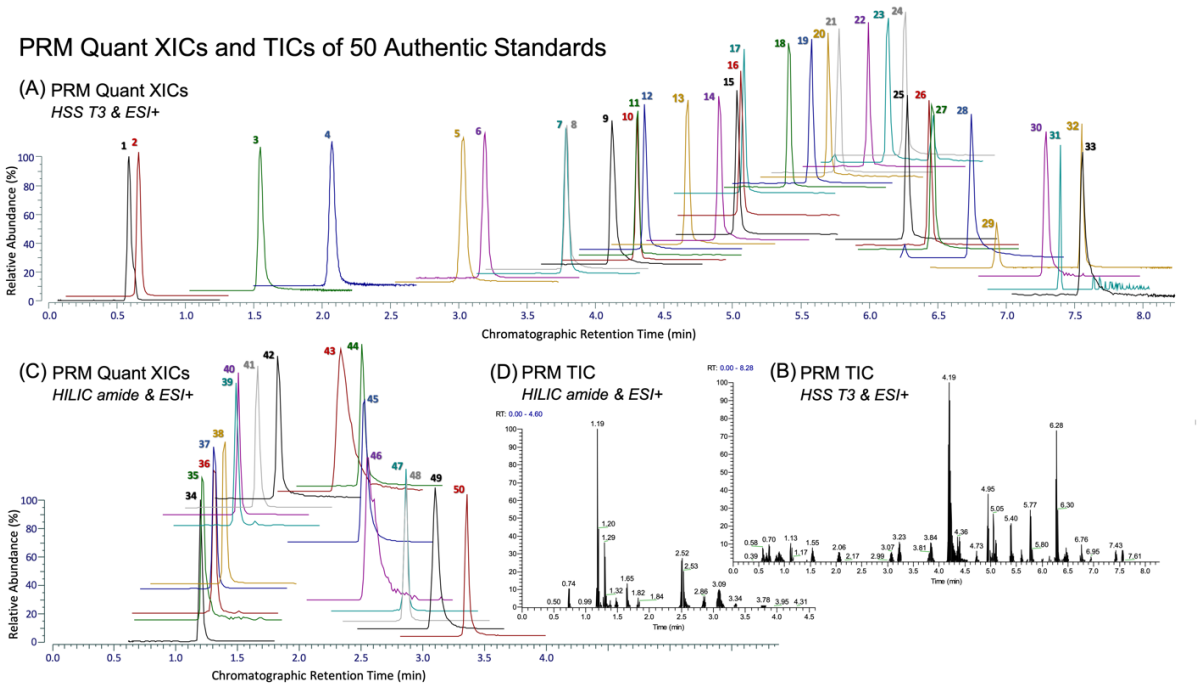

Figure 2.2 PRM XICs of quant ions (A, C) and TICs (B, D) of 50 authentic chemical standards (each 5 picomoles on column) under two complementary chromatographic conditions. Compounds of analysis: 1, L-arginine (Arg); 2, L-glutamate (Glu); 3, 2-pyrrolidinone; 4, nudifloramide; 5, kynurenine (Kyn); 6, L-phenylalanine (Phe); 7, 3-hydroxyanthranilate (3-Ohaa); 8, 2-phenethylamine (PEA); 9, N-methylphenethylamine (NMPEA); 10, L-tryptophan (Trp); 11, indole-3-acrylate (IAcrA); 12, xanthurenate (XA); 13, tryptamine; 14, N-methyltryptamine; 15, N-acetylserotonin (NAS); 16, phenylacetyl L-glutamine (PAG); 17, hippurate; 18, N-(2-phenylacetyl)glycine (PAA); 19, anthranilate (2AA); 20, p-coumarate; 21, N-[3-[2-(formylamino)-5-methoxyphenyl]-3-oxypropyl]-acetamide (AMFK); 22, indole-3-lactate; 23, indole-3-carboxylate; 24, indole-3-carboxaldehyde (I3A); 25, melatonin; 26, indole-3-acetate (IAA); 27, indole-3-ethanol (IEt); 28, coumarin; 29, indole-3-propionate (IPA); 30, indole-3-acetonitrile (IAN); 31, 4-methoxyindole; 32, methyl indole-3-acetate (meIAA); 33, indole; 34, acetylcholine (ACh); 35, 3-Aminopiperidine-2,6-dione; 36, 5-hydroxyindole-3-acetate (5-HIAA); 37, 5-hydroxy-Nω-methyltryptamine (N-methyl-5HT); 38, tyramine; 39, kynurenate (Kyna); 40, serotonin (5-HT); 41, choline; 42, nicotinate; 43, L-methionine (Met); 44, L-proline (Pro); 45, histamine; 46, L-tyrosine (Tyr); 47, L-pyroglutamate (PCA); 48, Nα-acetyl-L-glutamine (GlcNAc); 49, trimethylamine N-oxide (TMAO); 50, γ-aminobutyrate (GABA).

2.3.2 Method validation

Table 2.2 summarizes the linearity and intra- and interday precision and accuracy for this assay. Each calibration curve contained at least five consecutive points, and the linearity was determined as linear correlation coefficient > 0.99. Except for N-methyl-5HT, all other 49 compounds achieved a linear range with $R^2 > 0.995$. Since orbitrap mass spectrometry operates in such a high resolution, extraction ion chromatogram of the quant ion typically ends up with no noise ions spotted in the background. This means that detection limits could not be defined based on signal-to-noise ratio that is commonly applied to assessing unit-mass tandem mass spec performances. Instead, LLOQ, or the lower end of linear dynamic range (LDR), was defined as both intra- and interday precision < 20%. As shown in **Table 2.2**, the LDR typically spanned three orders of magnitude, with LLOQ ranging from 0.5 nmol/L to 100 nmol/L (injected 1 μL on column) for all 50 chemicals. The detections limits and linear ranges were comparable or better in comparison with previously reported assays using tandem mass spectrometry (G. Y. Chen et al., 2018; Lefevre et al., 2019; Marcos et al., 2016). At higher end of concentration levels, we did not observe any pattern that indicated compound interactions (e.g., competition/saturation) amid the chemical mixtures during our analyses. For method accuracy and precision, a rule-of-thumb criteria are 80-120% for accuracy and precision of < 20%. We noted that all 50 compounds had excellent inter-day precision of analysis (< 20%, n=12), with 32 compounds less than 10% at the LLOQ and other 18 less than 20%; for interday accuracy, except for L-methionine (128.0%), indole-3-lactic acid (122.9%) and kynurenic acid (120.2%), other 47 compounds ranged from 82.3% to 116.1%, indicating overall excellent method accuracy. The analyte recovery rate was assessed by spiked SIL internal standards at start of sample extraction with results are summarized in **Supplementary Table 2.2** and **Supplementary Figure 2.2**. Of note, analyte recovery can be

different depending on the biological matrix, as represented by d4-5HT and d2-GABA. This should be carefully addressed in studies aiming at interorgan comparison. In addition, it should be noted that unlike most other compound and/or matrices, d4-5HT reached a mean recovery of 128.6% in brain, exceeding 100%. This indicated that detection of 5-HT may involve matrix effect, meriting internal standard calibration using its own SIL chemical standards. Carryover was evaluated by examining inserted resuspension solvent blank injections with every ten injections of samples or standard solutions. Except for occasional cases of Phe (0.027%) and Trp (0.033%) of the previous injection, no observable carryover was found for all other compounds throughout the analysis.

2.3.3 Sample analysis

Comprehensive multi-compartmental analysis of Trp metabolites and neuroactive chemicals *in vivo* is crucial to the discovery and validation of gut bacterial mediation of gut-brain interactions. In this study, the validated assay was used to determine the 50 Trp metabolites and neurotransmitters in serum, feces, and brain of conventional SPF C57BL/6 mice, as a preliminary screening of molecules related to the microbiome-gut-brain axis.

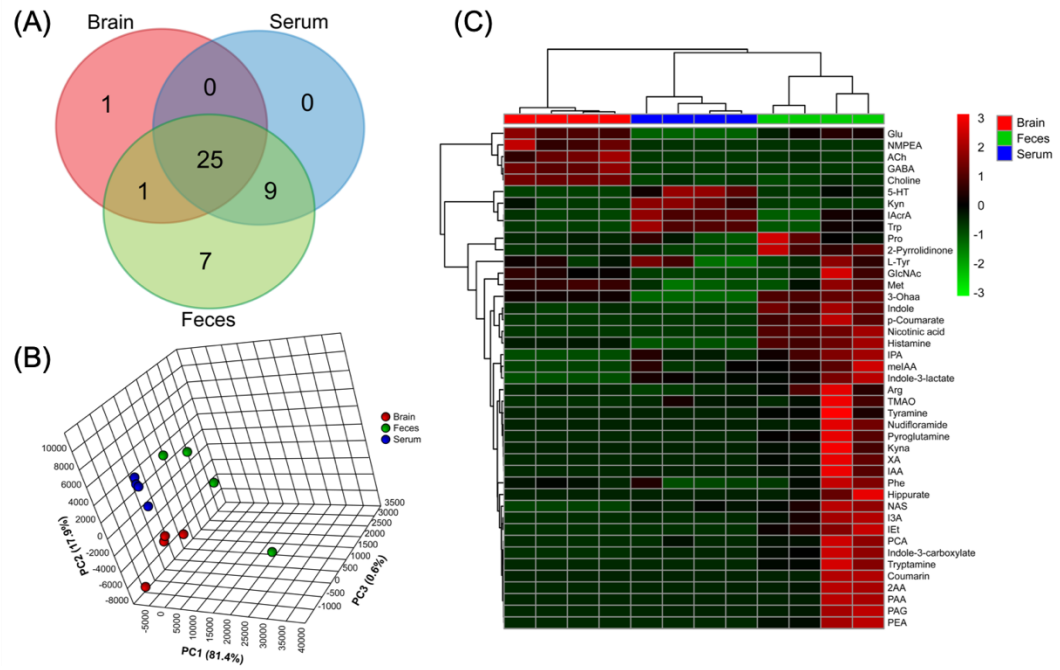

Figure 2.3 Tissue-specific distribution of the 50 neurotransmitters and tryptophan metabolites in conventional SPF C57BL/6 mice. (A) Venn diagram of detected compounds; (B) 3D PCA score plot (biological replicates n=4 for each matrix); (C) Heatmap of metabolite abundance created from sample concentration levels with unit: brain (nmol/g), feces (nmol/g), and serum (μmol/mL).

As shown in **Figure 2.3A**, a total of 43 compounds were detected and successfully quantified in samples. Among these, 25 metabolites were detected in all three matrices, including an array of gut bacteria-derived indole compounds (i.e., indole, IAA, IAcrA, I3A, and indole-3-lactate) that have been demonstrated as potent inflammation mitigators (e.g., as reactive oxygen species (ROS) scavengers or AhR ligands) (Rothhammer et al., 2016; Wlodarska et al., 2017). Such prevailing distribution of indole derivatives in the gut-blood-brain system suggests, as important proof-of-concept, that the gut microbiota may affect the brain function through humoral pathways. Moreover, metabolites of the glutamine-glutamate/GABA cycle such as GABA, Glu, GlcNAc and 2-pyrrolidinone, were extensively detected in all three organ compartments. This indicates gut bacterial GABAergic pathways as a potential mechanistic route by which our

commensal microbiota modulates the gut-brain signaling (Baj et al., 2019). The result is consistent with a recently published large cohort study providing the first human population-based evidence associating altered GABA pathways with depression (Valles-Colomer et al., 2019). In addition, choline and its derivatives ACh and TMAO, were detected in all three samples, so were nicotinate (or niacin) and its nicotinamide derivative nudifloramide. For more general interests, amino acid or derivatives of neuroactive potential were found to prevail in the gut-blood-brain systems in large quantities. This includes, but not limit to the aromatic Trp, Phe, Tyr, hippurate along with non-aromatic ones such as Met, Pro, Arg, histamine. By and large, our data indicate several interesting directions of research in the budding field of microbiome-gut-brain axis, and further studies are warranted, for example, SIL flux analysis, transporter identification, and mono-association microbiome analysis.

Some bacterial compounds seem be strictly compartmentalized *in vivo*. For example, IPA, methyl IAA, and indole-3-carboxylate were found in serum and feces but not in brain, likely due to lack of transporters across the brain-blood barrier (BBB). Such tissue-specificity or organ compartmentalization can also be seen in Figure 3B, where principal component analysis (PCA) identified a distinct separation among the three sample matrices with PC1 of 81.4% and PC2 of 17.9%. Future studies may consider pooling multiple samples as a quality control (QC) sample to inject intermittently to better delineate natural biological variance and analytical precision in real analysis. The heatmap in **Figure 2.3C** illustrates the relative abundances of detected compounds in individual sample matrices, further showing the tissue-specific distribution of these neuroactive molecules.

2.4 Conclusion

An accurate, precise, and sensitive high-resolution accurate-mass UHPLC-ESI-MS/MS assay was developed and validated for targeted analysis of 50 neurotransmitters and tryptophan metabolites in mouse serum, feces, and brain. Using high-resolution accurate-mass PRM transitions, the 50 compounds were unambiguously quantified. Sample preparation procedures were kept as simple as can be, for consideration of compound coverage and throughput of analysis. Two complementary chromatographic methods were applied, to address the wide-ranging pKa, lipophilicity, and structural diversity of the Trp family molecules and neurotransmitters. Future studies aiming for more extended measurement or discovery of the Trp pathways and bacterial neuroactive molecules may find these chromatographic setting useful as a starting point. With wide linearity, analytical robustness and sensitivity, this assay is suitable for routine analysis of Trp catabolites and neurotransmitters *in vivo*, which shall benefit the elucidation of mechanistic routes through which the gut microbiota mediates mental health.

Table 2.1 List of compounds of analysis with chromatographic retention time and PRM transitions. *

Peak #	Compound	Abbrev.	Formula	Chromat.	RT (min)	SIL ISD	Polarity	Precursor	PrecursorMZ	NCE	Quant MZ	Confirm MZ
1	L-Arginine	Arg	C6H14N4O2	HSS T3	0.59	d5-Gln	Pos	[M+H]+	175.1190	50	70.0651	116.0706; 60.0556; 130.0975
2	L-Glutamic acid	Glu	C5H9NO4	HSS T3	0.66	d5-Gln	Pos	[M+H]+	148.0604	40	84.0444	130.04987; 102.0550
3	2-Pyrrolidinone		C4H7NO	HSS T3	1.55	d3-Trp	Pos	[M+H]+	86.0600	40	86.0600	69.03362; 58.06537
4	Nudifloramide		C7H8N2O2	HSS T3	2.07	d4-Kyn	Pos	[M+H]+	153.0659	100	110.0600	135.0551; 80.0493
5	Kynurenine	Kyn	C10H12N2O3	HSS T3	3.03	d4-Kyn	Pos	[M+H]+	209.0921	30	94.0647	192.0655; 74.0233
6	L-Phenylalanine	Phe	C9H11NO2	HSS T3	3.19	d3-Trp	Pos	[M+H]+	166.0863	50	120.0808	131.0491; 149.0597
7	3-Hydroxyanthranilic acid	3-Ohaa	C7H7NO3	HSS T3	3.78	d4-Kyn	Pos	[M+H]+	154.0499	70	80.0492	108.0444; 136.0395
8	2-Phenethylamine	PEA	C8H11N	HSS T3	3.79	d3-Trp	Pos	[M+H]+	122.0963	100	105.0699	79.0542; 103.0542
9	N-Methylphenethylamine	NMPEA	C9H13N	HSS T3	4.12	d3-Trp	Pos	[M+H]+	136.1121	50	105.0697	103.0541; 79.0541; 53.0388
10	L-Tryptophan	Trp	C11H12N2O2	HSS T3	4.30	d3-Trp	Pos	[M+H]+	205.0970	40	146.0599	188.0705
11	Indole-3-acrylic acid	IAcrA	C11H9NO2	HSS T3	4.31	d3-Trp	Pos	[M+H]+	188.0708	50	118.0646	146.0596; 170.0597
12	Xanthurenic acid	XA	C10H7NO4	HSS T3	4.36	d3-Trp	Pos	[M+H]+	206.0449	60	178.0497	132.0441
13	Tryptamine		C10H12N2	HSS T3	4.67	d3-Trp	Pos	[M+H]+	161.1073	40	144.0808	117.0699; 143.0730; 115.0542
14	N-Methyltryptamine		C11H14N2	HSS T3	4.91	d3-Trp	Pos	[M+H]+	175.1231	90	144.0807	143.0729; 117.0697
15	N-Acetylserotonin	NAS	C12H14N2O2	HSS T3	5.03	d3-Trp	Pos	[M+H]+	219.1128	80	115.0539	160.0756
16	Phenylacetyl L-glutamine	PAG	C13H16N2O4	HSS T3	5.08	d3-Trp	Pos	[M+H]+	265.1183	40	130.0499	84.0444; 91.0542
17	Hippuric acid		C9H9NO3	HSS T3	5.06	d3-Trp	Pos	[M+H]+	180.0655	50	105.0335	95.0489; 77.0382
18	N-(2-Phenylacetyl)glycine	PAA	C10H11NO3	HSS T3	5.41	d2-IAA	Pos	[M+H]+	194.0812	50	91.0542	76.0393
19	Anthranilic acid	2AA	C7H7NO2	HSS T3	5.58	d3-Trp	Pos	[M+H]+	138.0550	50	120.0440	92.0495; 65.0386
20	p-Coumaric Acid		C9H8O3	HSS T3	5.74	d2-IAA	Pos	[M+H]+	165.0546	50	119.0491	147.0441; 91.0542
21	N-[3-[2-(Formylamino)-5-methoxyphenyl]-3-oxypropyl]-acetamide	AFMK	C13H16N2O4	HSS T3	5.78	d2-IAA	Pos	[M+H]+	265.1183	35	178.0862	136.0757; 114.0550; 188.0706
22	Indole-3-lactic acid		C11H11NO3	HSS T3	5.99	d2-IAA	Pos	[M+H]+	206.0812	50	118.0650	130.0654; 146.0598; 170.0600
23	Indole-3-carboxylic acid		C9H7NO2	HSS T3	6.14	d2-IAA	Pos	[M+H]+	162.0550	80	116.0493	144.0442; 118.0649; 117.0570
24	Indole-3-carboxaldehyde	I3A	C9H7NO	HSS T3	6.26	d2-IAA	Pos	[M+H]+	146.0596	80	91.0540	118.0648; 117.0570
25	Melatonin		C13H16N2O2	HSS T3	6.29	d2-IAA	Pos	[M+H]+	233.1287	65	159.0679	174.0914; 131.0730
26	Indole-3-acetic acid	IAA	C10H9NO2	HSS T3	6.44	d2-IAA	Pos	[M+H]+	176.0708	90	130.0649	103.0539
27	Indole-3-ethanol	IEt	C10H11NO	HSS T3	6.45	d2-IAA	Pos	[M+H]+	162.0919	50	144.0813	135.0810; 130.0657; 116.0500
28	Coumarin		C9H6O2	HSS T3	6.77	d2-IPA	Pos	[M+H]+	147.0441	90	91.0542	103.0542; 65.0386
29	Indole-3-propionic acid	IPA	C11H11NO2	HSS T3	6.93	d2-IPA	Pos	[M+H]+	190.0863	80	130.0646	55.0183
30	Indole-3-acetonitrile	IAN	C10H8N2	HSS T3	7.33	d2-IPA	Pos	[M+H]+	157.0760	80	117.0573	130.0651; 90.0464; 89.0386
31	4-Methoxyindole		C9H9NO	HSS T3	7.40	d2-IPA	Pos	[M+H]+	148.0757	100	105.0579	133.0525; 117.0573; 104.0495
32	Methyl indole-3-acetic acid	meIAA	C11H11NO2	HSS T3	7.55	d2-IPA	Pos	[M+H]+	190.0862	80	130.0652	103.0545
33	Indole		C8H7N	HSS T3	7.56	d2-IPA	Pos	[M+H]+	118.0648	110	91.0537	117.0568; 65.0381
34	Acetylcholine	ACh	C7H16NO2	HILIC amide	1.20	d13-ACh	Pos	[M+H]+	146.1176	50	87.0441	60.0808
35	3-Aminopiperidine-2,6-dione		C5H8N2O2	HILIC amide	1.21	d4-5HT	Pos	[M+H]+	129.0659	40	84.0444	56.0498

36	5-Hydroxyindole-3-acetic acid	5-HIAA	C10H9NO3	HILIC amide	1.30	d3-Trp	Pos	[M+H]+	192.0655	80	117.0573	118.0647; 91.0537
37	5-Hydroxy-Nω-methyltryptamine	me5HT	C11H14N2O	HILIC amide	1.30	d4-5HT	Pos	[M+H]+	191.1179	40	160.0758	148.0758; 132.0807
38	Tyramine		C8H11NO	HILIC amide	1.40	d4-5HT	Pos	[M+H]+	138.0913	80	103.0542	121.0648; 91.0542; 93.0699; 95.0491
39	Kynurenic acid	Kyna	C10H7NO3	HILIC amide	1.49	d4-5HT	Pos	[M+H]+	190.0499	90	116.0490	162.0547; 89.0381
40	Serotonin	5-HT	C10H12N2O	HILIC amide	1.50	d4-5HT	Pos	[M+H]+	177.1022	90	115.0540	117.0570; 130.0649
41	Choline		C5H14NO	HILIC amide	1.66	d13-ACh	Pos	[M+H]+	104.1070	110	60.0802	58.0645
42	Nicotinic acid	niacin	C6H5NO2	HILIC amide	1.82	d2-GABA	Pos	[M+H]+	124.0393	110	96.0444	80.0495; 78.0338; 53.0386
43	L-Methionine	Met	C5H11NO2S	HILIC amide	2.34	d3-GABA	Pos	[M+H]+	150.0589	20	104.0528	56.0495; 133.0318; 61.0106; 102.0550
44	L-Proline	Pro	C5H9NO2	HILIC amide	2.50	d2-GABA	Pos	[M+H]+	116.0706	50	70.0651	68.0495; 116.0706
45	Histamine		C5H9N3	HILIC amide	2.53	d3-Trp	Pos	[M+H]+	112.0869	100	95.0604	68.0495; 81.0447; 54.0338; 67.0417
46	L-Tyrosine	Tyr	C9H11NO3	HILIC amide	2.57	d3-Trp	Pos	[M+H]+	182.0812	60	91.0542	119.0491; 123.0441
47	L-Pyroglutamic acid	PCA	C5H7NO3	HILIC amide	2.87	d3-Trp	Pos	[M+H]+	130.0497	50	84.0439	102.0546
48	Nα-Acetyl-L-glutamine	GlcNAc	C7H12N2O4	HILIC amide	2.88	d2-GABA	Pos	[M+H]+	189.0870	40	130.0498	129.0659; 172.0603
49	Trimethylamine N-oxide	TMAO	C3H9NO	HILIC amide	3.10	d2-GABA	Pos	[M+H]+	76.0757	35	58.0654	59.0732
50	γ-Aminobutyric acid	GABA	C4H9NO2	HILIC amide	3.36	d2-GABA	Pos	[M+H]+	104.0703	40	87.0437	86.0597
ISD	Acetylcholine-d13, (N,N,N-trimethyl-d9; 1,1,2,2-d4)	d13-ACh	C7H3D13NO2	HILIC amide	1.20	-	Pos	[M+H]+	159.1992	50	91.0694	91.06938; 69.13753
ISD	d2-γ-Aminobutyric acid	d2-GABA	C4H7D2NO2	HILIC amide	3.35	-	Pos	[M+H]+	106.0832	40	89.0565	89.05647; 88.07254
ISD	L-Glutamine-2,3,3,4,4-d5	d5-Gln	C5H5D5N2O3	HSS T3	0.67	-	Pos	[M+H]+	152.1078	40	135.0810	135.08097; 89.0756; 106.1022
ISD	L-Tryptophan-2,3,3-d3	d3-Trp	C11H9D3N2O2	HILIC amide	2.06	-	Pos	[M+H]+	208.1106	40	147.0661	147.06608; 191.08896; 84.95950
ISD	Serotonin-α,α,β,β-d4	d4-5-HT	C10H8D4N2O	HILIC amide	1.52	-	Pos	[M+H]+	181.1274	90	118.0732	118.07319; 121.08283; 134.09101
ISD	L-Glutamine-2,3,3,4,4-d5	d5-Gln	C5H5D5N2O3	HILIC amide	3.81	-	Pos	[M+H]+	152.1078	40	135.0810	135.08097; 89.0756; 106.1022
ISD	L-Kynurenine (Ring-D4, 3,3-D2)	d4-Kyna	C10H8D4N2O3	HSS T3	2.97	-	Pos	[M+H]+	213.1172	30	98.0900	98.09003; 198.10228
ISD	L-Tryptophan-2,3,3-d3	d3-Trp	C11H9D3N2O2	HSS T3	4.34	-	Pos	[M+H]+	208.1106	40	147.0661	147.06608; 191.08896; 84.95950
ISD	Indole-3-acetic-2,2-d2 acid	d2-IAA	C10H7D2NO2	HSS T3	6.49	-	Pos	[M+H]+	178.0832	90	132.0775	132.07747; 105.06665
ISD	Indole-3-propionic-2,2-d2 acid	d2-IPA	C11H9D2NO2	HSS T3	6.94	-	Pos	[M+H]+	192.0988	110	130.0650	130.06495; 56.02437

***** Peak #: peak numbering in **Figure 2**; Chromat.: Chromatography method; RT: retention time; NCE: normalized collision energy (NCE) of higher-energy C-trap dissociation (HCD); ISD: internal standards.

Table 2.2 Method Validation: Linearity, Intra-, and Interday Precision and Accuracy. *

Name	Calibration curve*	R^2	Linear range (nmol/L)	CV-Interday** (%)	Accuracy-Interday (%)	CV-day1 (%)	Accuracy-day1 (%)	CV-day2 (%)	Accuracy-day2 (%)	CV-day3 (%)	Accuracy-day3 (%)
PEA	y = 0.0009x - 0.015	0.9999	5 - 10000	14.7	103.7	4.5	103.1	8.3	124.1	2.3	87.5
2-Pyrrolidinone	y = 0.0014x + 0.02	0.9995	10 - 1000	8.0	105.4	12.1	97.7	12.9	116.4	13.7	107.6
Pyroglutamine	y = 0.0002x + 0.0187	0.9992	100 - 10000	12.2	100.5	7.1	92.9	8.6	91.2	3.6	117.9
3-Ohaa	y = 0.0038x - 1.0377	0.9988	100 - 10000	3.7	101.3	4.7	99.3	0.6	107.0	0.9	99.1
4-Methoxyindole	y = 0.0004x + 0.0019	0.9998	50 - 10000	2.8	101.8	11.1	98.7	2.8	105.0	4.3	103.3
N-methyl-5HT	y = 0.0005x + 0.0126	0.9923	20 - 1000	21.5	93.3	5.9	95.6	5.3	92.5	6.0	85.1
5-HIAA	y = 0.196x + 0.8928	0.9992	0.5 - 1000	5.4	93.6	4.6	90.3	4.4	89.0	14.8	95.1
ACh	y = 0.0026x + 0.156	0.9982	0.5 - 10000	18.1	116.0	2.6	106.1	10.3	146.7	11.4	111.0
AMFK	y = 0.0042x + 0.3512	0.9966	5 - 10000	17.4	116.1	10.6	145.2	8.6	114.2	4.9	105.1
2AA	y = 0.0009x - 0.0063	0.9999	2 - 10000	11.8	114.7	11.6	124.4	7.8	106.5	5.4	128.0
Choline	y = 0.0004x + 0.0031	0.9984	2 - 10000	10.1	108.6	11.2	123.9	8.6	101.4	4.1	109.2
Coumarin	y = 0.0024x - 0.0941	0.9999	20 - 10000	8.7	113.4	19.7	123.6	19.7	113.9	9.9	116.3
Hippuric acid	y = 0.0003x - 0.0042	0.9996	5 - 10000	7.5	107.8	10.5	102.8	13.8	110.9	4.8	117.7
Histamine	y = 0.097x - 1.7507	0.9986	10 - 500	18.7	82.3	7.7	90.2	7.1	67.6	3.2	71.4
Indole	y = 0.0004x - 0.0103	0.9985	100 - 5000	8.0	97.3	6.2	106.1	2.6	95.4	1.5	87.6
IAA	y = 0.0008x + 0.0419	0.9995	10 - 10000	4.8	95.0	6.7	88.0	16.0	96.5	4.6	100.6
IAN	y = 0.00004x - 0.0025	0.9996	100 - 10000	6.6	101.3	3.9	102.9	1.8	109.2	10.8	93.0
IAcrA	y = 0.0005x - 0.0063	0.9997	5 - 10000	14.0	103.9	7.6	86.7	0.6	121.4	4.9	107.7
I3A	y = 0.0051x + 0.0802	0.9994	2 - 10000	2.0	102.8	11.4	104.3	1.8	104.3	9.2	102.4
Indole-3-carboxylate	y = 0.0001x - 0.0106	0.9996	100 - 10000	10.0	96.8	2.0	83.0	15.4	98.9	14.0	105.5
IEt	y = 0.0042x + 0.065	0.9999	5 - 10000	15.3	108.8	8.6	133.7	12.0	102.3	4.1	99.3
Indole-3-lactic acid	y = 0.0003x - 0.0174	0.9994	20 - 10000	13.0	122.9	14.9	136.7	16.0	126.3	18.0	128.8
IPA	y = 0.0011x + 0.0041	0.9997	20 - 10000	5.2	106.7	3.6	104.6	16.1	109.6	2.0	112.6
Kyna	y = 0.0025x + 0.0448	0.9994	50 - 10000	12.4	120.2	7.1	119.3	9.4	135.2	11.2	126.1
Kyn	y = 0.0041x - 0.005	0.9997	10 - 10000	2.6	96.5	13.7	96.6	5.8	95.0	6.0	94.3
Arg	y = 0.003x - 0.1939	0.9989	5 - 10000	14.6	100.7	8.4	87.7	6.2	121.4	13.1	93.7
Glu	y = 0.0014x + 0.1077	0.9985	5 - 10000	8.1	93.2	3.5	85.7	11.5	99.4	12.1	87.8
Met	y = 0.00006x - 0.0034	0.9984	100 - 10000	14.9	128.0	5.6	135.2	8.6	134.5	5.5	142.4
Phe	y = 0.002x - 0.0058	0.9999	5 - 10000	4.8	94.0	2.0	91.7	6.3	93.4	3.8	97.1
Pro	y = 0.0064x + 0.2074	0.9994	5 - 5000	3.5	99.7	16.9	94.9	4.6	100.3	3.1	103.4
PCA	y = 0.0005x - 0.0163	0.9994	50 - 10000	7.1	96.6	7.7	100.5	13.2	86.4	17.6	99.7
Trp	y = 0.0012x + 0.0038	0.9998	2 - 10000	3.0	102.5	7.3	100.2	9.6	106.6	19.8	103.0
Tyr	y = 0.0001x + 0.0164	0.9977	500 - 10000	5.3	92.7	8.9	90.2	18.8	90.8	2.6	89.6
Melatonin	y = 0.0184x + 0.0187	0.9995	1 - 1000	7.1	96.2	3.6	100.3	7.8	98.4	6.5	86.0

melAA	y = 0.003x - 0.0259	0.9999	1 - 10000	8.3	112.9	15.3	118.4	8.7	111.9	4.7	121.2
PAA	y = 0.0016x - 0.0188	0.9999	5 - 10000	6.9	107.8	9.1	114.7	14.9	113.6	11.0	103.0
AFMK	y = 0.0006x - 0.0009	1	2 - 500	8.7	92.4	0.8	90.7	7.6	97.1	7.2	81.8
NAS	y = 0.023x - 0.6326	0.9994	2 - 5000	8.7	100.7	2.4	111.8	2.7	90.3	3.9	100.6
NMPEA	y = 0.0015x + 0.041	0.9991	5 - 10000	15.5	113.5	7.7	139.1	4.8	109.9	10.7	105.1
Nicotinic acid	y = 0.00006x + 0.0003	0.9984	50 - 10000	16.1	88.8	9.6	98.1	10.6	68.8	11.9	88.4
niacin	y = 0.0021x - 0.0145	0.9987	10 - 1000	4.8	99.9	2.0	100.6	7.0	105.5	6.3	93.7
Nα-Acetyl-L-glutamine	y = 0.0007x - 0.0281	0.9998	20 - 10000	4.8	104.9	11.4	108.0	3.6	101.2	1.6	110.2
GlcNAc	y = 0.0001x - 0.0217	0.9961	100 - 10000	18.7	106.7	4.2	81.4	5.4	122.9	6.1	122.5
Phenylacetylglutamine	y = 0.0001x - 0.0217	0.9961	100 - 10000	2.2	101.2	4.2	101.4	5.4	104.2	6.1	99.1
PAG	y = 0.0022x + 0.1629	0.999	20 - 10000	7.9	110.9	15.1	119.9	3.9	107.9	16.0	115.7
5-HT	y = 0.0002x - 0.00003	0.999	1 - 500	5.6	103.6	8.0	100.0	5.9	102.3	6.1	112.1
TMAO	y = 0.001x - 0.0053	0.9997	5 - 500	13.2	97.7	2.8	108.9	4.2	102.7	8.5	79.2
Tyramine	y = 0.0049x + 0.6964	0.9959	5 - 10000	8.8	91.3	10.1	82.1	15.0	95.7	12.8	87.6
XA	y = 0.0012x - 0.011	0.9998	5 - 10000	4.8	94.6	6.3	92.7	9.0	89.3	6.2	96.2
GABA	y = 0.0002x + 0.0037	0.9998	20 - 10000	6.2	91.6	1.4	89.6	7.3	87.8	8.7	88.8

* The calibration curve listed here are those obtained by plotting ASD-to-ISD peak area ratio (y-axis) over absolute concentration of targeted analytes (x-axis). ** Intra- and interday precision and accuracy at the LLOQ level.

CHAPTER 3: HIGH-COVERAGE METABOLOMICS UNCOVERS MICROBIOTA-DRIVEN BIOCHEMICAL LANDSCAPE OF INTERORGAN TRANSPORT AND GUT-BRAIN COMMUNICATION IN MICE[3]

3.1 Introduction

The mammalian body and particularly the gastrointestinal (GI) tract is inhabited by hundreds of trillions of microbes, collectively termed the microbiota (P. J. Turnbaugh et al., 2007). Complex, dynamic, and metabolically active by nature, these commensal microbes have been discovered to constantly interact with the host as a crucial mediator for physiological processes spanning energy harvest (Peter J. Turnbaugh et al., 2006), immune cell development (Hooper et al., 2012), and gut epithelial homeostasis (Wells et al., 2011). Interestingly, recent studies support that microbiota also harbors novel neuroactive potential with links to neurological and/or psychiatric disorders, as further encapsulated as the "microbiota-gut-brain axis" (Cryan et al., 2020). For example, using maternal immune activation (MIA) mouse, a model of autism spectrum disorder (ASD), Hsiao and colleagues discovered (Hsiao et al., 2013) that both ASD-mimicking GI barrier defects and behavioral abnormalities MIA offspring exhibited were restored through colonizing human commensal *Bacteroidetes fragilis*, supporting a gut-microbiota-brain connection for autism. In a recent work by Valles-Colomer and colleagues (Valles-Colomer et al., 2019), analyses of a large human cohort correlating fecal metagenomic features with indicators of quality of life and depression identified microbial strains, pathways, and metabolites pertaining to

[3] Chapter 3 was reproduced from my accepted work - Lai Y., Liu C.-W., Yang Y., Hsiao Y.-C., Ru H., Lu K.*: High-coverage metabolomics uncovers microbiota-driven biochemical landscape of interorgan transport and gut-brain communication in mice, ***Nat. Commun.***, 2021 (Accepted).

mental health and gut-brain interaction, providing the first population-scale evidence linking microbiota to mental health outcomes.

Despite the emerging data, whether and how microbiota controls brain function remains largely undefined. It has been postulated that at least two routes are involved (de la Fuente-Nunez et al., 2018), namely (i) the vagus nerves (neuronal) that connects the central nervous system (CNS) and enteric nervous system (ENS, the "second brain") and (ii) the circulatory system (humoral) encompassing blood and lymphatic circulation. Gut microbes, in close proximity to numerous local neurons and immune cells, may either act on the ENS *in situ* to signal the CNS remotely, or more likely, they synthesize or transform molecular cues that can translocate from gut lumen to systemic circulation, and possibly cross the blood-brain barrier (BBB) and affect CNS directly (Needham, Kaddurah-Daouk, & Mazmanian, 2020). Although there have been sporadic studies targeting a few microbial molecules or chemical classes in this regard, represented by α-synuclein (Kim et al., 2019), 3,4-dihydroxyphenylacetate (Valles-Colomer et al., 2019), and bile acids (MahmoudianDehkordi et al., 2019), many of these still have yet to be validated as gut-brain mediators.

In this work, we use high coverage comparative metabolomics analyses combining targeted and untargeted annotation strategies to address this (**Supplementary Figure 3.1**) (Konjevod et al., 2021; Hemi Luan, Wang, & Cai, 2019). We profile fecal, blood sera, and cerebral cortical brain tissues of eight-week-old germ-free (GF) C57BL/6 mice and their age-matched conventionally raised (CONV-R) specific-pathogen-free counterparts using high-resolution mass spectrometry (HRMS). We assess group patterns using univariate and multivariate statistics, annotate chemical structures of all distinct ion features through an integrated cheminformatic approach, and leverage a suite of statistical and data visualization tools for systematic comparisons across sample

compartments. Here, we report 701 unique metabolites of differentiated GF/CONV-R profiles; potential links to the humoral microbiota-gut-brain axis are examined through enrichment analyses, random forest classification, and metabolomic network analysis. This work presents a unique multi-metabolome perspective and datasets for probing microbial influences on mammalian interorgan transport and gut-blood-brain interaction.

3.2 Materials and Method

3.2.1 Chemicals and reagents

LC/MS-grade (Optima^{TM}) solvents including methanol (MeOH; catalog #A456-4), acetonitrile (ACN; catalog #A955-4), water (catalog #W6-4) and formic acid (catalog #A117-50), were obtained from Fisher Scientific (Waltham, WA, USA). Stable-isotope-labeling internal standards for a range of classical neurotransmitters and tryptophan catabolites, including acetylcholine-d13 (N,N,N-trimethyl-d9; 1,1,2,2-d4) (d13-ACh; catalog #D-1780), γ-aminobutyric acid-d2 (d2-GABA; catalog #D-1731), L-glutamine-2,3,3,4,4-d5 (d5-Gln; catalog #D-2532), L-tryptophan-2,3,3-d3 (d3-Trp; catalog #D-7419), serotonin-α,α,β,β-d4 (d4-5HT; catalog #D-1550), L-kynurenine (ring-d4, 3,3-d2) (d6-Kyn; catalog #DLM-7842), indole-3-acetic-2,2-d2 acid (d2-IAA; catalog #D-1709) and indole-3-propionic-2,2-d2 acid (d2-IPA; catalog #D-7686) were obtained from CDN Isotopes (Pointe-Claire, Quebec, Canada) and Cambridge Isotopes (Tewksbury, MA, USA). For quality control, these internal standards were spiked in sample pretreatment for monitoring sample recovery and analytical variability.

3.2.2 Animals

All animal procedures were approved by the Institutional Animal Care & Use Committee (IACUC) at The University of North Carolina at Chapel Hill (UNC). Conventionally raised (CONV-R) wild-type C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and housed under specific-pathogen-free (SPF) conditions at the UNC animal facility for multiple generations; germ-free (GF) mice were generated and housed in stringently germ-free chambers for generations at the National Gnotobiotic Rodent Resource Center of UNC in Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited facilities (Chapel Hill, NC, USA). C57BL/6 mouse littermates of ~7 weeks old raised under GF and conventional conditions were age-matched upon selection for sacrifice and analyses, resulting in a final total of 24 mice, including 12 GF mice and 12 SPF CONV-R mice with 6 males and 6 females included in each. The animals were raised under the following conditions: 22ºC, 40-70% humidity, and a daily 12:12 hour light-dark cycle. For dietary administration, we consistently fed all mice the same purified (and sterile) standard Prolab RHM 3000 pelleted rodent diets (St. Louis, MO, USA) and provided tap water ad libitum. Prior to sacrifice and sample harvest, all mice were observed under their original housing conditions for one week; animals were not considered if they exhibited significant signs of serious injury or morbidity (e.g., malocclusion or fight wounds). Upon euthanization in CO_2 chambers, feces, brain, and blood sera were harvested, snap-frozen, and stored in -80 ºC freezer before analysis.

3.2.3 Sample extraction

Fecal matter, blood sera, and brain tissues (of the cerebral cortical region) were aliquoted and extracted for metabolome analyses. To maximize coverage and throughput, extraction

procedures were kept as simple and non-selective as can be. For feces, ~20 mg thawed samples were aliquoted to a 1.5-mL Eppendorf tube (Hamburg, Germany) with ~30 mg acid-washed glass beads added (Sigma-Aldrich, St. Louis, MO). For every 10 mg feces aliquots, 300 µL of ice-cold MeOH:water (50:50, *v/v*) with spiked ISDs were added for extraction. The samples were homogenized on a TissueLyzer (Qiagen, Hilden, Germany) at 50 Hz for 10 min and centrifuged at 12,000×*g* for 10 min (Eppendorf, Hamburg, Germany). A 100-µL aliquot of the supernatant was transferred and dried in a CentriVap vacuum evaporator (Labconco, MO, USA). For serum, 20 µL was aliquoted from each sample in a 1.5-mL Eppendorf tube (Hamburg, Germany). A total of 180 µL ice-cold ISD-containing MeOH was added, vortexed, and incubated at -20 ºC freezer for 30 min. The extracts were centrifuged at 15,000×*g* for 10 min for precipitation of particulates and proteins. Aliquoted 100-µL of supernatants were SpeedVac-dried as described above. For cerebral cortical brain tissues, ~20 mg was aliquoted from each sample to a 2-mL screw cap microcentrifuge tubes (VWR, Radnor, PA, USA) that contained clean stainless-steel beads of 5-mm i.d. To every 20 mg tissue, 400 µL of the ice-cold ISD-containing MeOH was added for extraction. The samples were homogenized on a TissueLyzer (Qiagen, Hilden, Germany) at 50 Hz for 2 min and further incubated at -20ºC for 1 h prior to centrifugation at 18,000×*g* for 10 min. One hundred-µL of the extractant supernatants were SpeedVac-dried as described before. Upon instrumental analysis, all dried extracts, including feces, sera, and brain tissue, were resuspended in 50 µL ACN:water (2:98, *v/v*).

3.2.4 Instrumental analysis

Global metabolome profiling was performed on a Thermo Fisher Scientific Vanquish UHPLC coupling to a high-resolution accurate-mass (HRAM) Q-Exactive mass spectrometer

interfacing with a heated electrospray ionization (HESI) source and a hybrid quadrupole-orbitrap mass analyzer (Waltham, MA, USA). For chromatographic separation, a Waters Acquity UPLC HSS T3 column (100Å, 1.8 μm, 2.1 mm × 100 mm) was used (Milford, MA, USA). The mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in ACN (B), flowing at a rate of 0.4 mL/min under 40 ºC with a 15-min gradient: 2% B, 0-1 min; 2% to 15% B, 1-3 min; 15% to 50% B, 3-6 min; 50% to 98% B, 6-7.5 min; 98% B, 7.5-11.5 min; 98% to 2% B, 11.5-11.6 min; 2% B, 11.6-15 min. HESI positive and negative ionization were both performed, with following ion source setting: sheath gas 60 L/min, aux gas 10 L/min, sweep gas 1 L/min, spray voltage 2.75 kV, capillary 325 ºC and aux gas heater 400 ºC. All mass spectral data (in *.RAW format) were acquired through the Thermo XCalibur program (version 4.1) (Waltham, MA, USA). For MS1 full scan profiling (Q not used) to examine global metabolomic profiles and to screen for distinct ion features, the mass spectrometer was operated scanning mass range of 80-1,000 Da with a mass resolution of 70,000 full width at half height (FWHM) at 200 Da. For MS/MS spectra acquisition in later unknown structural annotation stages (with both Q and orbitrap running), the mass spectrometer was operated scanning for 80-1,000 Da in fullscan MS1 mode (FWHM 70,000; AGC target 3e6; max IT 200 ms) alternating with parallel reaction monitoring (PRM) MS/MS mode with normalized collision energy averaging 10, 50 and 100 (FWHM 17 500; AGC target 2e5; max IT 50 ms).

3.2.5 Data processing, statistics, and visualization

MS1 fullscan *.RAW data were converted to *.mzXML files in ProteoWizards MS Convert (Palo Alto, CA, USA) and uploaded to Scripps XCMS (Tautenhahn, Patti, Rinehart, & Siuzdak, 2012) (La Jolla, CA, USA) for data processing, resulting in master peak tables aligning

all samples separately from three sample types acquired under two HESI polarity modes. The dataset was analyzed in R by Welch's *t*-test with adjusted p-values (i.e., q-values) calculated based on the Benjamin-Hochberg procedure to compare GF and CONV-R groups. To further determine the effect of gender, two-way analysis of variance (two-way ANOVA) was conducted. Raw peak areas for each ion feature were first pareto-scaled and log-transformed to meet the test assumptions of ANOVA (e.g., no significant outliers, normality, and homogeneity of variance). Then, two-way ANOVA and post-hoc Tukey's (honestly significant difference) HSD test were performed to determine main effects of microbiota and gender and to test for within-group statistical difference, respectively. Plots and analyses including Box and Whisker plot, barplot, principal component analysis score and scree plot, variable importance plot, PieDonut chart, as well as heatmaps were generated in R (version 4.0.1) (Vienna, Austria) using packages including readxl (version 1.3.1), dplyr (version 1.0.6), tidyverse (version 1.3.1), ggplot2 (version 3.3.3), ggpubr (version 0.4.0), ggthemes (version 4.2.4), ggsignif (version 0.6.1), RColorBrewer (version 1.1.2), dendextend (version 1.15.1), randomForest (version 4.6.14), FactoMineR (version 2.4), factoextra (version 1.0.7), pheatmap (version 1.0.12), wesanderson (version 0.3.6) and webr (version 0.1.5) (Team, 2010). Specifically for principal component analysis, we performed Pareto scaling first and included all ion features from the alignment table for the given sample matrix under specific heated ESI mode; the PCA score plots involved 17,386 ion features for feces (HESI+) (**Figure 2a-b**) and 10,012 features in total for blood sera (HESI+) (**Figure 5a-b**). Random forest classification was conducted using the randomForest (version 4.6.14) and rfPermute (version 2.2) packages of R (Vienna, Austria) to generate variable importance plots to rank individual metabolites, with hyperparameters and permutation results provided (**Addition Information, Appendix for Chapter 3**). Metabolite networks were constructed using a MetaMapp approach (Dinesh K.

Barupal et al., 2012) (web-based portal, version 2020) that calculated biological pathways relevance (KEGG reactant pairs) and chemical structural similarity (Tanimoto coefficient>0.7) which were further visualized in CytoScape with version 2.7.2 for Window 10 OS and version 3.8.0 for a macOS (Seattle, WA, USA). Chemical similarity enrichment analysis (ChemRICH) plots were constructed to visualize changing metabolites based on chemical classes (Davis, CA, USA) (Dinesh Kumar Barupal & Fiehn, 2017). Quantitative metabolite set enrichment analysis (qMSEA) was conducted on altered metabolites in MetaboAnalyst 4.0 (Montreal, QC, Canada) using their PubChem CID identifiers and log-transformed peak area pair as data input to compute based on 99 *a priori* defined sets of metabolites in pathway-associated metabolites sets (SMPDB); metabolite sets that contained at least two compounds were used.

3.2.6 Compound identification

Since the millennium, untargeted/global metabolomics has been fast evolving with significant development in gas/liquid chromatography-mass spectrometry technologies, featuring the rise of accurate-mass measurement that has since opened doors of confident de novo determination of elemental composition and chemical structure in a high-throughput, sensitive, and non-selective manner (Junot, Fenaille, Colsch, & Bécher, 2014). The conceptual differences between untargeted analysis, targeted analysis, and the like in metabolomics necessitates clear elucidation. The untargeted/global metabolomics analysis aims to detect as many features as possible in a single run and after a differential statistical comparison, the significant ones are sent to compound identification for hypothesis generation; annotation strategies can be either knowledge-based/targeted (matching against a pre-determined in-house spectral library or experimental databases) or data-driven/untargeted (inferring directly from data, e.g., using

streamlined in silico cheminformatic algorithms and scoring). While, in the case of targeted metabolomics, one has previous information of what he/she will look for, such as precursor/fragment transitions, and usually seeks a quantitative analysis to determine absolute concentration levels using commercial labeled standards. In this study, we conducted profiled all ion features, performed statistics for those aligned, and leveraged both targeted (with known reference) and untargeted (infer from data directly) cheminformatic strategies for annotations with goals to identify/annotate all ion features with statistical significance (**Supplementary Figure 3.1**). We refer to our approach as "high-coverage annotation," since both knowledge-based/targeted and data-driven/untargeted annotation strategies were performed on all significant ion features in a streamlined manner, allowing for integrated scoring, cross-validation, and post retention time prediction and filtering that helped achieve highest metabolome coverage possible.

Specifically, we collected a total of 20,939 tandem mass spectra of distinct ion features (pertaining to the microbiota-gut-brain connection) for structural annotation. These included 16,001 for feces, 3977 for sera, and 961 for cerebral tissues combining HESI positive and negative modes. The *.RAW data were converted into *.abf format using the Reifycs Abf Converter (https://www.reifycs.com/AbfConverter/), uploaded to MS-DIAL 4.16 (Riken, Japan) (Tsugawa et al., 2015) to obtain *.MAT files that contained MS1 accurate mass, MS1 isotopic abundances, MS/MS spectra, and retention time for each ion feature with an acquired tandem mass spectrum. The targeted annotation procedure exploits an in-house experimental library established from 423 authentic chemical standards of canonical pathways, neurotransmitters, and literature-reported microbial metabolites; the local library stores chromatographic retention time, accurate mass, and characteristic MS2 fragment ions. For untargeted analyses, a cutting-edge cheminformatic pipeline was used integrating machine learning-based retention time predication (Retip) (Bonini, Kind,

Tsugawa, Barupal, & Fiehn, 2020), embedded experimental mass spectral database search using GNPS (M. Wang et al., 2016), MassBank (Horai et al., 2010), and ReSpect (Sawada et al., 2012), totaling 28,293 spectra), and rule-based in silico cheminformatic analysis (MS-FINDER, version 3.30) (Tsugawa et al., 2016) which integrates a number of cheminformatic algorithms while querying major biomolecule chemical databases such as HMDB, LipidMAPS and PubChem Biomolecules adding up to over 100 million structures. The detailed settings of MS-FINDER are provided in Supplementary Information. Importantly, elemental composition was predicted (based on MS1 accurate mass, MS1 isotopic ratios and MS/MS product ions) and filtered based on the Seven Golden Rules (Kind & Fiehn, 2007), which refers to an algorithm consisting of seven heuristic rules, i.e. (i) restriction for element numbers, (ii) LEWIS and SENIOR rules, (iii) isotopic patterns, (iv) H/C ratios, (v) element ratios of N, O, P, S versus C, (vi) elemental ratio probabilities, and (vii) presence of trimethylsilylation (for GC/derivatization platforms if applicable). Afterwards, top five candidates were sent to structural dereplication, and chromatographic retention times were predicted afterwards for these structures using the Retip (version 0.5.4) and h2o (version 3.32.1.3) package of R (Vienna, Austria) and compared with true values for lipophilicity validation and annotation cleanup. The resultant top-score structures were manually assessed and curated carefully to boost annotation confidence; the post-curation strategies included, but not limited to, chemical inference, biological relevance, comparison of targeted and untargeted results, and the use of additional experimental mass spectral database search including Metlin (La Jolla, CA, USA), MoNA (Davis, CA, USA), HMDB (Edmonton, AB, Canada), and mzCloud (Waltham, MA, USA).

The application of specific quality criteria to filter out low-quality MS/MS data and annotations is not only common but essential in untargeted metabolomics practices. In this study,

however, we did not exclude any ion features of low abundance (under MS1 fullscan) for MS/MS acquisition, nor did we apply thresholds of minimum/noise of ion intensity for filtering out low quality/noisy tandem MS/MS. This is because (i) we aimed for untargeted annotation in an unbiased manner for all ion features regardless of low abundances, and (ii) our integrated and streamlined scoring strategy carried a penalty mechanism itself (**Supplementary Figure 3.1c**), meaning, if the tandem mass spectrum per se is too noisy or the quality is poor, the resultant scores will be correspondingly low, and the annotations will be naturally filtered out in the final annotation list (**Supplementary Figure 3.1d**). For the finalized tables of changed fecal metabolome (**Supplementary Table 3.2**), serum metabolome (**Supplementary Table 3.3**), and brain metabolome (**Supplementary Table 3.4**), we only included identification/annotation results of highest confidence, i.e. level 1 confirmed structure and level 2 probable structure according to Metabolomics Standard Initiative (MSI) to best inform future studies on microbiota in health and diseases (Schrimpe-Rutledge, Codreanu, Sherrod, & McLean, 2016; Schymanski et al., 2014; Sumner et al., 2007).

3.3 Results

3.3.1 High-coverage metabolomics of GF vs. CONV-R mice

To uncover metabolites underlying microbiota-host interaction in light of gut-brain signaling, we conducted metabolomics, targeted and untargeted, on fecal matter, blood sera, and brain tissues (cerebral cortex slices) for 12 GF and 12 CONV-R C57BL/6 mice (8-week-old) (**Figure 3.1a**). Using UHPLC-HESI-HRMS, we detected and aligned ion features into master peak tables for each sample type based on MS1 fullscan data, generating total ion feature numbers ranging from highest 17,386 for feces (heated ESI+) to lowest 6,334 for brain tissues (heated ESI+)

(**Figure 3.1b**). We statistically assessed these tables to screen for features of GF/CONV-R difference (fold change≥1.5, p-value<0.01, Welch's *t*-test) (**Figure 3.1b-c**), yielding a total of 20,939 significant ion features including 16,001 for feces, 3,977 for sera, and 961 for brain tissues. Tandem mass spectra were acquired for all these features, and highest metabolome coverage possible was achieved through a cheminformatics pipeline incorporating targeted and untargeted annotation procedures (**Figure 3.1a**; **Supplementary Figure 3.1**). The targeted procedure matched the unknown against an in-house spectral library containing retention time, accurate mass, and MS2 characteristic fragments for 423 authentic chemical standards of canonical pathway metabolites and 46 literature-reported microbial by-products including neurotransmitters (**Supplementary Table 3.1**). Meanwhile, untargeted analyses entailed validated rules and tools for formula generation (Kind & Fiehn, 2007), structural dereplication (Tsugawa et al., 2016), and machine learning-based retention time prediction (Bonini et al., 2020). We manually curated and combined identification results from both procedures, yielding confidently annotated 533 fecal metabolites, 231 serum metabolites, and 58 metabolites of either level 1 confirmed structure or level 2 probable structure (Schymanski et al., 2014) (**Figure 3.1d**; **Supplementary Table 3.2-3.4**).

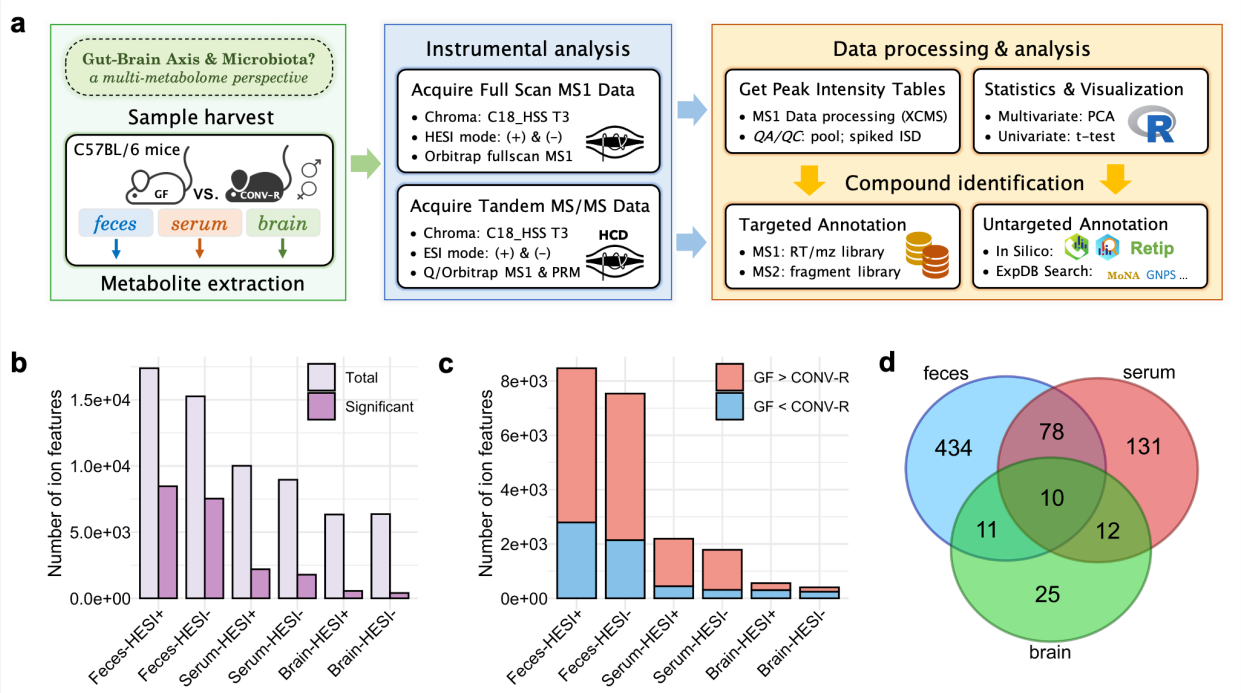

Figure 3.1 Overview of experimental approach and multi-metabolomics analyses. (**a**) Experimental workflow started with sample harvest and metabolite extraction of feces, blood sera and cerebral cortical brain tissues from 8-week age-matched germ-free (GF, N=12) and conventionally raised (CONV-R) specific-pathogen-free C57BL/6 mice (N=12). A novel high coverage metabolomics approach was used featuring high-resolution orbitrap mass spectrometry, targeted screening based on an in-house mass spectral library, untargeted profiling based on a streamlined cheminformatic pipeline for de novo structural dereplication, univariate and multivariate statistics, and versatile visualization approaches. (**b**) Number of total and significant ion features (p-value<0.01, fold change≥1.5, two-sided Welch's *t*-test) detected for feces, sera, and brain tissues under HESI positive and negative modes of analysis. (**c**) Trend distribution of significantly altered ion features. (**d**) Venn diagram of all identified metabolites of GF/CONV-R difference among the three sample matrices. Abbreviations: Chroma, chromatography; HESI, heated electrospray ionization; PRM, parallel reaction monitoring; QA/QC, quality assurance/quality control; ISD, internal standard; PCA, principal component analysis; RT-mz library, retention time and mass-to-charge ratio pair library; ExpDB, experimental database; MoNA, MassBank of North America; GNPS, The Global Natural Product Social Molecular Networking.

3.3.2 Microbiota as a master regulator of gastrointestinal metabolism

Because gut is the largest niche for commensal microbes and houses most critical sites of digestion, homeostasis, and immunity, gut lumen metabolomes represent a unique avenue for probing microbiota-host interaction (Nick Powell, Marjorie M. Walker, & Nicholas J. Talley, 2017). Our metabolomics revealed distinct fecal patterns due to microbiota, as illustrated in

principal component analysis (PCA) (**Figure 3.2a-b**) and metabolomic cloudplot (**Figure 3.2c**). Note that the 16,001 distinct fecal features accounted for 49.0% of the total detected. High coverage cheminformatics successfully resolved 533 fecal metabolites from these features.

To gain a "landscape" view, chemical similarity enrichment analysis (ChemRICH) (Dinesh Kumar Barupal & Fiehn, 2017) was performed. Seventy chemical classes were clustered, covering a wide lipophilicity range with varied compound numbers (node size) and overall trends of change (node color); for the color spectrum of node, red indicated that among the metabolites of GF/CONV-R difference, GF-enriched ones outnumbered those increased in CONV-R and/or overall embraced larger fold changes (**Figure 3.2d**; **Supplementary Table 3.5-3.6**). Most significantly enriched were cholic acids, oligopeptides, glutamates, indoles, and nucleosides, followed by other amino acids (e.g., sulfur, aromatic), lipids (e.g., acylcarnitines, fatty acids), and neurotransmitter families including taurine and catecholamines. To interpret in the context of biologic pathways, we conducted quantitative metabolite sets enrichment analysis (qMSEA) based on 99 a priori defined sets of metabolites in the Small Molecule Pathway Database (SMPDB) (Chong et al., 2018; Frolkis et al., 2010). We identified 71 microbiota-perturbed pathways in the GI tract (adjusted $p<0.05$) (**Supplementary Table 3.7**) with top 50 plotted (**Figure 3.2e**). Our analyses enriched purines, tryptophan metabolism, bile acid biosynthesis, porphyrin metabolism and fatty acid metabolism, alongside numerous other amino acids (e.g., arginine, proline, tyrosine, phenylalanine, aspartate, glycine, serine, lysine, and branched-chain amino acids), lipid metabolism (e.g., phospholipids, carnitines, and sex steroid hormones), cofactors (e.g., vitamin K, vitamin B_5, biotin, vitamin B_3 and vitamin B_6), and polyamine metabolism (e.g., spermidine) etc. Both analyses confirmed for microbiota as a master regulator of gut metabolism, in protein metabolism (over 100 oligopeptides altered), energy harvest (e.g., fatty acid β-oxidation, carnitine

shuttle, citric acid cycle, and gluconeogenesis), and numerous signaling pathways that involved amino acids and lipids as substrates or ligands.

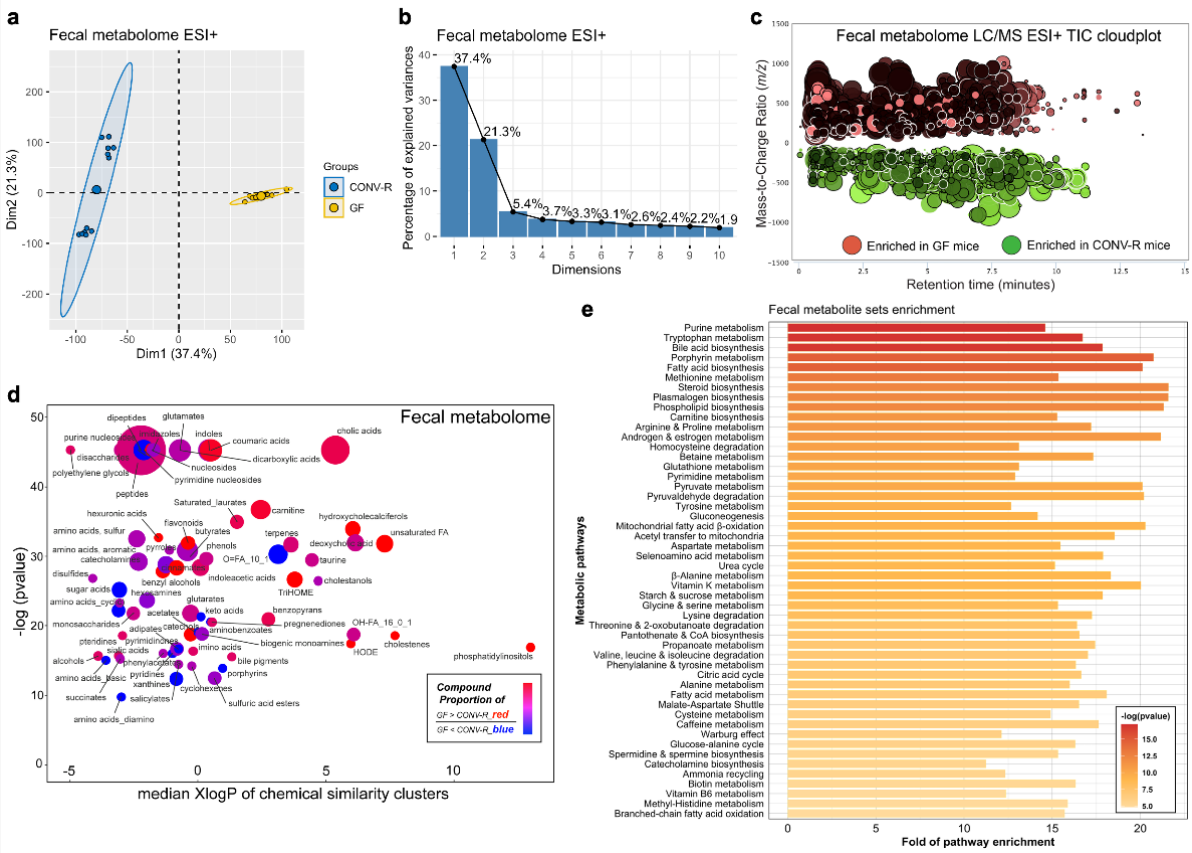

Figure 3.2 Distinct fecal metabolome profiles owing to the presence of microbiota. (**a, b**) Principal component analysis score plot (a) and scree plot (b) for assessing fecal metabolomic data comparing GF (N=12) and CONV-R mice (N=12) under heated ESI+ mode. (**c**) Metabolomic total ion chromatogram cloudplot of significant ion features in feces between groups (p-value<0.01, fold change≥1.5, two-sided Welch's *t*-test) using heated ESI+ data as an example; larger circle size indicated larger fold change values ranging from 1.5-5,300.6. (**d**) Chemical similarity enrichment analysis (ChemRICH) clustering of 533 identified altered fecal metabolites by chemical similarity with *x*-axis of mediation logarithmic additive octanol-water partition coefficients (XlogP) and *y*-axis for sets statistical significance based on the Kolmogorov–Smirnov test; the node size depicted total compound numbers for each cluster set and node color scale the proportion of GF-enriched vs. CONV-R enriched metabolites. (**e**) Quantitative metabolite set enrichment analysis (qMSEA) based on 99 a priori defined sets of metabolites identified a total of 71 significantly perturbed fecal metabolic pathways (adjusted p<0.05) with top 50 shown. Abbreviations: Dim, dimension; LC/MS, liquid chromatography-mass spectrometry; TIC, total ion chromatogram; TriHOME, trihydroxyoctadecenoic acid; FA, fatty acid; HODE, hydroxyoctadecadienoic acid; CoA, coenzyme A.

We turned to individual fecal metabolites and select pathways for in-depth analyses. Using random forest model, altered fecal metabolites were ranked by their relative contribution to group separation with top 50 shown in a variable importance plot (VIP) (**Figure 3.3a**). Top ranked members were structurally diverse, indicating a multifaceted and complex nature of the microbiome-metabolome network. We specifically focused on aromatic amino acid pathways because they have confirmed microbial involvement and neuromodulatory activities (Cervenka et al., 2017; Dodd et al., 2017; Platten et al., 2019), enriched with tryptophan metabolism being the second most significant (**Figure 3.2e**), represented major compound classes perturbed (in addition to those for energy harvest and protein metabolism) (**Figure 3.2d**), and included multiple metabolite members highly ranked in the random forest classification model, including kynurenine (4^{th}), shikimate (12^{th}), and serotonin (21^{st}) (**Figure 3.3a**). In the integrated view (**Figure 3.3b**), all three tryptophan catabolic fluxes were enhanced when microbiota was present, as characterized by (i) reduced tryptophan pools (1.4-fold), (ii) decrease levels of kynurenine, 5-hydroxy-L-tryptophan and indole, and (iii) increased levels of serotonin (5-HT) (5.7-fold), kynurenate (4.1-fold), and indoles of 1.8-36.3-fold changes including indole-3-propionate (IPA), indole-3-acetate (IAA), indole-3-lactate (ILA) and indole-3-carboxaldehyde (I3A) (**Figure 3.3b-c**). We also observed distinct bile acid patterns with fold changes as large as three orders of magnitude (**Figure 3.3d**; **Supplementary Table 3.2**). Note that free-form primary bile acids such as chenodeoxycholate, cholate, muricholates (αMCA+βMCA) were markedly higher in CONV-R feces, while their taurine- or glycine- conjugates had an opposite trend. Correspondingly, secondary bile acids were found to be much enriched in CONV-R feces than in GF's (**Figure 3.3d**; **Supplementary Table 3.2**).

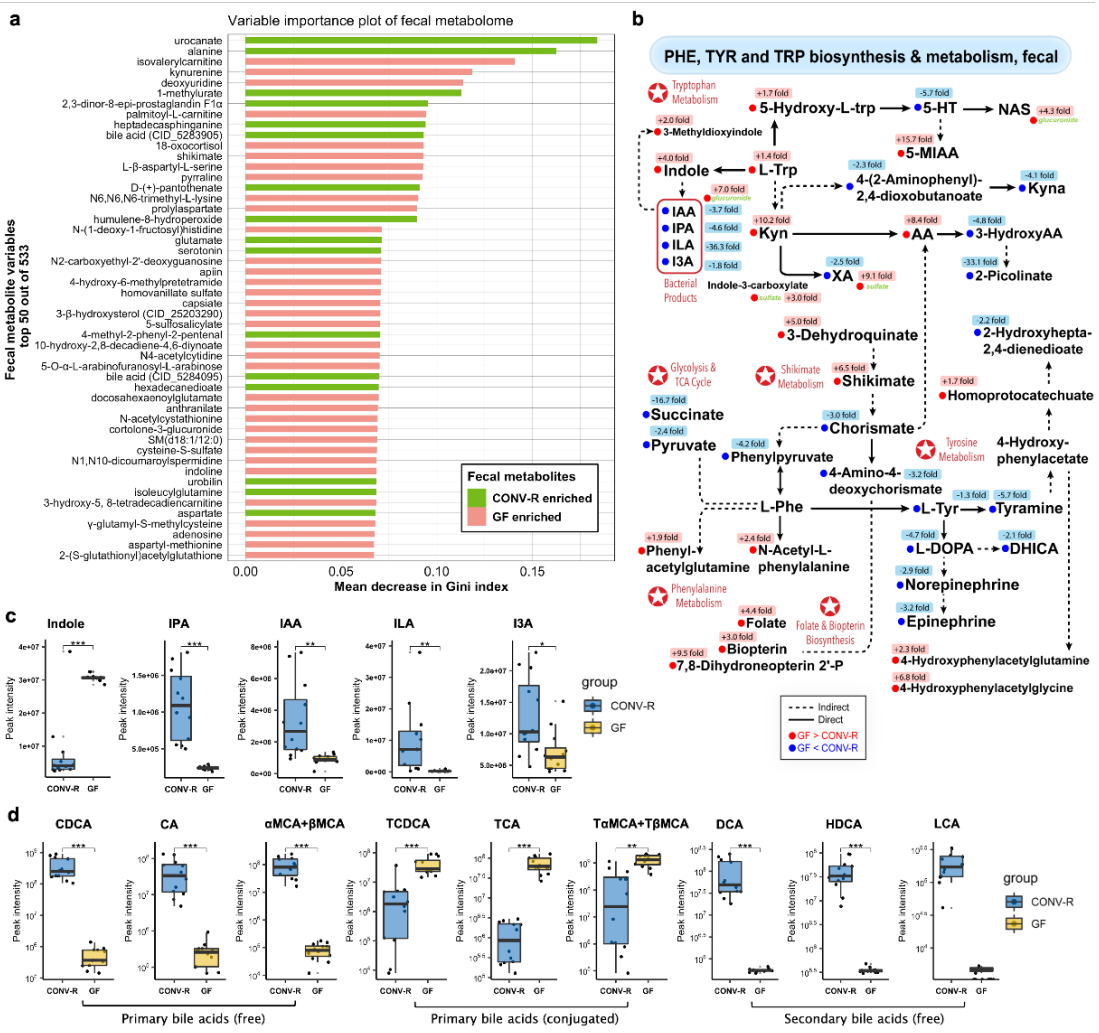

Figure 3.3 Select high-impact gut metabolites and pathways as regulated by microbiota. (**a**) Variable importance plot of top 50 fecal metabolites (y-axis) ranked by contribution to mean decrease accuracy of Gini coefficient (x-axis) in the random forest model for discerning group difference. (**b**) Diagram summary of perturbed fecal pathways of phenylalanine, tyrosine, and tryptophan biosynthesis and metabolism, with significantly altered metabolites labeled in red (GF>CONV-R) or blue (GF<CONV-R) (two-sided Welch's *t*-test, fold change≥1.5, p<0.01). (c, d) Box and Whisker plots of fecal indoles (**c**) and bile acid profiles (**d**) as synthesized or mediated by microbiota, with the box ranging from the first quartile to the third while the whiskers going from each quartile to the minimum or maximum (GF, N=12; CONV-R, N=12), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, two-sided Welch's *t*-test; exact p-values and adjusted p-values (i.e., q-values) are provided in Supplementary Table 2. Abbreviations: SM, sphingomyelin; Phe, L-phenylalanine; Tyr, L-tyrosine; Trp, L-tryptophan; 5-HT, 5-hydroxytryptamine; NAS, N-acetylserotonin; 5-MIAA, 5-methoxyindole-3-acetate; Kyna, kynurenate; Kyn, L-kynurenine; AA, anthranilate; XA, xanthurenate; IAA, indole-3-acetate; IPA, indole-3-propionate; ILA, indole-3-lactate; I3A, indole-3-carboxaldehyde; L-DOPA, L-3,4-dihydroxyphenylalanine; DHICA, 5,6-dihydroxyindole-2-carboxylate; CDCA, chenodeoxycholate; CA, cholate; αMCA, α-muricholate; TCDCA, taurochenodeoxycholate; TCA, taurocholate; TαMCA, tauro α-muricholate; DCA, deoxycholate; HDCA, hyodeoxycholate; LCA, lithocholate.

3.3.3 Microbiota extensively mediates gut neurotransmitter production and transformation

The discovery that our commensal microbiota comprises innumerable neurotransmitter producers has recently led to exciting hypotheses questioning their effects on host neurotransmission, gut-brain signaling, and mental health outcomes (Mittal et al., 2017; Strandwitz, 2018). Here, we offer a complete analysis of fecal neurotransmitter profiles comparing GF and CONV-R mice. As selectively shown (**Figure 3.4a-d**), we found for a number of neurotransmitters much elevated levels in the presence of microbiota, spanning Class A (Rhodopsin-like) G-protein coupled receptor pathway (GPCR) amines including acetylcholine, epinephrine, histamine, serotonin and tyramine (**Figure 3.4a**), Class C metabotropic neurotransmitter L-glutamate and GABA (**Figure 3.4b**) to neurotransmitters (or their precursors) of other channels or receptors, e.g., taurine, L-homocysteate, L-3,4-dihydrophenylalanine (L-DOPA) (**Figure 3.4c**). Many neurotransmitters were virtually absent in GF feces, as indicated by peak intensities as low as detection limits even on ultra-sensitive mass spectrometry assays of ours. This suggests an essential role of resident microbiota for neurotransmitter production. Several neuroendocrine signaling molecules, namely glycine, cortisol, adenosine and 2-aminoadipate embraced an opposite trend (**Figure 3.4d**), suggesting endogenous sources other than influences from microbiota.

We took turns to specifically examine two major neurotransmitter pathways, namely catecholamine biosynthesis (**Figure 3.4e**) and the glutamine/glutamate-GABA cycle (**Figure 3.4f**), since both have been suggested recently as potential routes for microbes to modulate host neurotransmission extending beyond the GI tract to the entire body. Catecholamines, namely dopamine, norepinephrine, and epinephrine, are biogenic amine neurotransmitters with crucial functions in motivation, reward, and hedonistic regulation (Volkow et al., 2017). Consistent with

previous data (Asano et al., 2012), our results showed markedly higher fecal levels of norepinephrine (2.9-fold), epinephrine (3.2-fold) as well as two dopamine precursors tyramine (5.7-fold) and L-DOPA (4.7-fold). Though fecal dopamine levels manifested no statistical difference, we found two related sulfate-conjugates (dopamine-3-O-sulfate and dopamine-4-sulfate) substantially elevated when microbiota was present (**Figure 3.4e**), warranting future analyses. Glutamine/glutamate-GABA metabolism, coupling with much altered tricarboxylic acid (TCA) cycle, was also perturbed in gut, represented by decreased L-glutamine (2.3-fold) and elevated L-glutamate (2.5-fold) and GABA (6.8-fold) with the presence of microbiota (**Figure 3.4f**).

Unexpectedly, we observed a realm of conjugated compounds altered owing to microbiota, involving glucuronides and sulfates of neurotransmitter (**Figure 3.4g**). We constructed a heatmap of them, with 28 enriched in CONV-R feces and 5 with opposite trends (**Figure 3.4g**). Based on extensive literature search, we highlighted those with neuromodulatory properties in bold texts, including 11 compounds much diminished (e.g., N-acetylserotonin glucuronide) and 3 enriched (e.g., dopamine 4-sulfate) when microbiota was present, alongside other conjugates of diet-derived flavonoids (e.g., genistein 4'-O-glucuronide), bile acids (e.g., taurocholate 3-sulfate) and indole derivatives (e.g., indole-3-carboxylate-O-sulfate, indole-3-acetate O-glucuronide) (**Figure 3.4g**). These data together support that the present microbiota plays an integral role in neurotransmitter metabolism in local gut lumen in terms of production, transformation, and bioavailability, warranting future efforts to delineate microbial species-level contributions in a time- and space-specific manner.

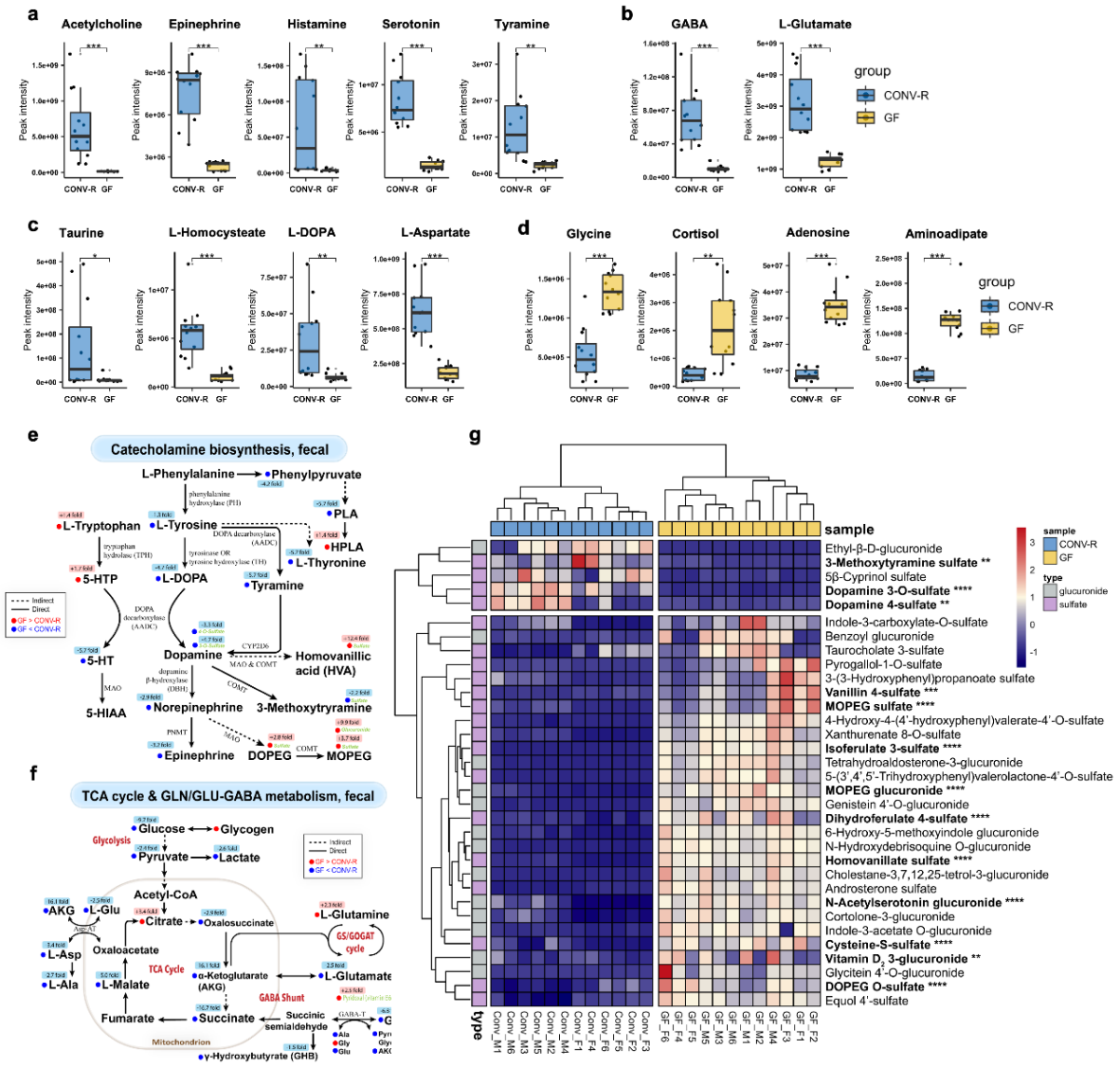

Figure 3.4 Microbiota extensively mediates neurotransmitter production, deconjugation and transformation in the GI tract. (**a-d**) Box and Whisker plots of fecal Class A (Rhodopsin-like) GPCR amine neurotransmitters (a), fecal Class C metabotropic neurotransmitter L-glutamate and GABA (b), CONV-R enriched neurotransmitters and related molecules (c) and GF enriched neurotransmitters and related molecules (d), with the box ranging from the first quartile to the third while the whiskers going from each quartile to the minimum or maximum (GF, N=12; CONV-R, N=12), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, two-sided Welch's *t*-test; exact p-values and adjusted p-values (i.e., q-values) are provided in Supplementary Table 2. (**e**) Diagram summary of altered fecal catecholamine biosynthetic pathways due to microbiota, with significantly altered metabolites labeled in red (GF>CONV-R) or blue (GF<CONV-R) (two-sided Welch's *t*-test, fold change≥1.5, p<0.01). (**f**) Diagram summary of altered fecal (glutamine-)glutamate-GABA metabolism coupling with citric acid cycle due to microbiota, with significantly altered metabolites labeled in red (GF>CONV-R) or blue (GF<CONV-R) (Welch's *t*-test, fold change≥1.5, p<0.01). (**g**) Heatmap clustering of distinct fecal metabolites from Phase II reaction; glucuronides or sulfates of neurotransmitters or related compounds were labeled by bold texts and asterisks showing statistical significance.

3.3.4 Microbial impacts on circulating blood metabolism and fecal-blood exchange

With detailing of microbiota's control over gut metabolism and neurotransmitter profiles, question arises as to whether these local effects will propagate from gut to peripheral organs. To investigate this, we profiled the circulating blood sera of GF and CONV-R mice. We observed systemic perturbations, though not as striking as fecal data, for serum metabolome due to microbiota (**Figure 3.5a-c**), with 3,977 significant ion features detected that accounted for 21.0% of total aligned features. We successfully resolved 231 unique structures from these altered features (**Supplementary Table 3.3**).

To infer, we performed chemical similarity enrichment and quantitative pathway enrichment analyses. ChemRICH plot clustered the serum metabolites into 27 chemical classes, spanning carnitines, dipeptides, indoles alongside lipid species, amino acids, and organic acids (**Figure 3.5d**; **Supplementary Table 3.8-3.9**). We discovered that 175 out of the total 231 metabolites (75.8%) was downregulated in the presence of microbiota, also indicated by the prevailing purple-to-red colors (**Figure 3.5d**). qMSEA analysis revealed for circulating blood 61 significantly enriched pathways (adjusted $p<0.05$) (**Supplementary Table 3.10**), with top 50 shown (**Figure 3.5e**). In line with fecal data, top enriched serum pathways spanned from porphyrin metabolism, ketone body metabolism, bile acid biosynthesis to numerous amino acid metabolism including the aromatic amino acid family (**Figure 3.5e**). The results show that microbiota has systemic effects and can modulate peripheral blood circulation, raising possibilities of microbiota-gut-brain axis through humoral transport of molecular cues in vivo.

Similar to fecal data, we focused on individual serum metabolites for in-depth analyses to delineate potential gut-blood network and neuroendocrine pathways. Random forest analyses ranked all altered 231 serum metabolites by contribution to group separation, with top 50 shown

(**Figure 3.6a**). In addition to metabolites for energy processes (e.g., acylcarnitines, oligopeptides, ketone bodies), we noted a realm of microbial products in sera. For example, the top-ranked 4-ethylphenol, is a known tyrosine metabolite synthesized by bacteria (Bone, Tamm, & Hill, 1976; Nicholson et al., 2012). Phenyl sulfate ranked third with surging levels in CONV-R sera (173.33-fold), is a confirmed gut microbiota-derived uremic toxin contributing to albuminuria in diabetic kidney disease (Kikuchi et al., 2019) (**Figure 3.6a**; **Supplementary Table 3.3**). Another phenolic derivative N-(2-phenylacetyl)glycine (PAG), with 10.5-fold elevation with microbiota, was ranked the fifth place. In the VIP chart, we also observed several tryptophan indole derivatives enriched in CONV-R serum. These included indoxyl sulfate (4,351.6-fold), IPA (7.3-fold), methyl indole-3-acetate (4.3-fold) alongside other indoles (**Supplementary Figure 3.2a**). The results together support that microbiota's systemic effects on host involve humoral transport of microbial molecular cues.

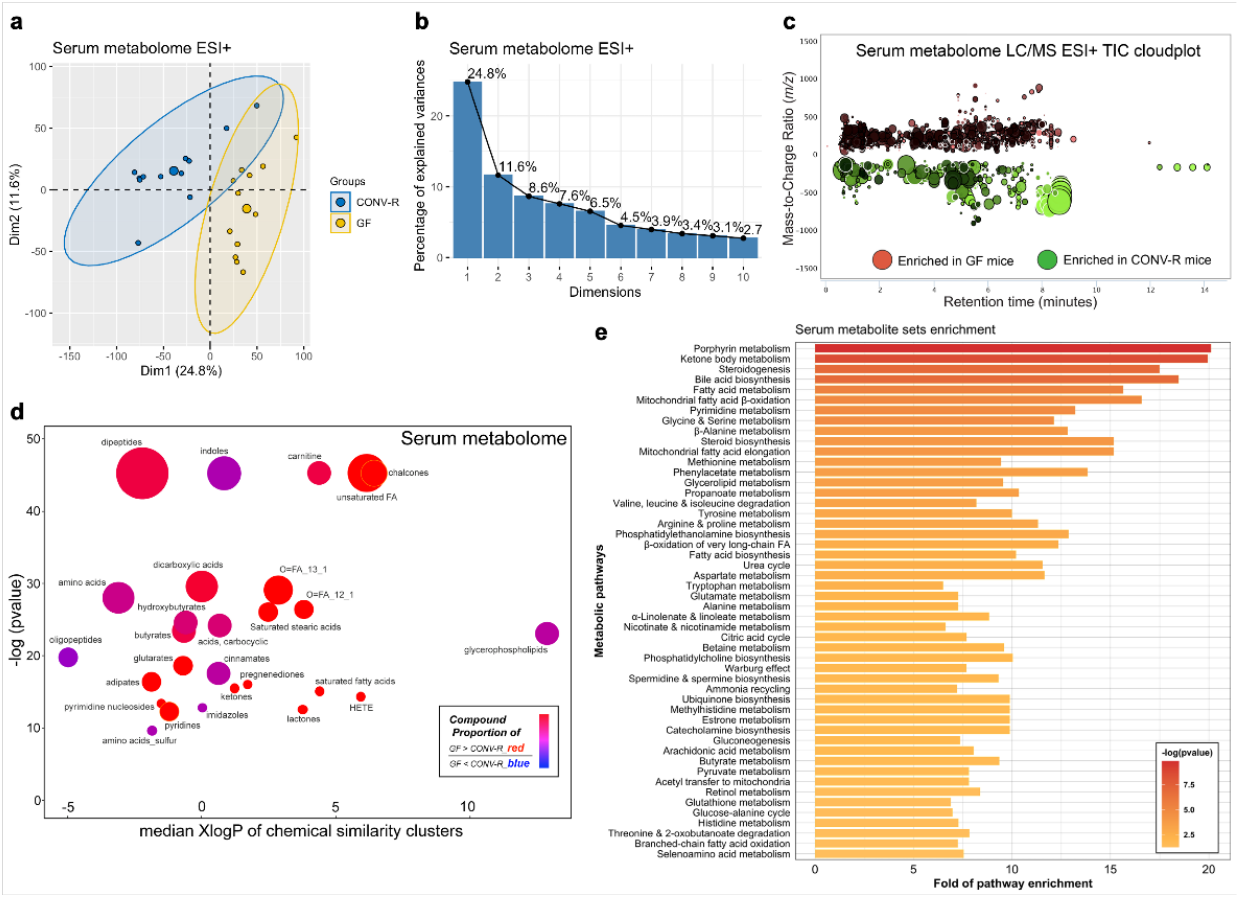

Figure 3.5 Global effects of microbiota on host circulating blood metabolism. (**a, b**) Principal component analysis score plot (a) and scree loading plot (b) for assessing serum metabolomic data comparing GF (N=12) and CONV-R mice (N=12) under heated ESI+ mode. (**c**) Metabolomic total ion chromatogram cloudplot of significant features in serum between groups (p-value<0.01, fold change≥1.5, two-sided Welch's *t*-test) using heated ESI+ as an example; larger circle size indicated larger fold change values ranging from 1.5-1,347.1. (**d**) Chemical similarity enrichment analysis (ChemRICH) clustering of 231 identified altered serum metabolites by chemical similarity with *x*-axis of mediation logarithmic additive octanol-water partition coefficients (XlogP) and *y*-axis for sets statistical significance based on the Kolmogorov–Smirnov test; the node size depicted total compound numbers for each cluster set and node color scale the proportion of GF-enriched vs. CONV-R enriched metabolites. (**e**) Quantitative metabolite set enrichment analysis (qMSEA) based on 99 *a priori* defined sets of metabolites identified a total of 57 significantly perturbed serum metabolic pathways (adjusted p<0.05) with top 50 shown. Abbreviations: LC/MS, liquid chromatography-mass spectrometry; TIC, total ion chromatogram; HETE, 5-hydroxyeicosatetraenoic acid; FA, fatty acid.

To detail the microbiota-serum metabolome network, we focused on aromatic amino acid pathways (**Figure 3.6b**) with multiple top-ranked phenolics and indoles on the VIP chart (**Figure 3.6a**). Compared with fecal data (**Figure 3.3b**), we observed for CONV-R sera overall a consistent

pattern, such as enhanced fluxes of tryptophan catabolism featuring decreased kynurenine and enriched indoles. That said, interestingly, we noted that serum serotonin levels, though at high peak intensity (10^8), were not statistically different between CONV-R and GF mice, which was in conflict with studies illuminating microbiota's regulation of peripheral serotonin levels (Yano et al., 2015). We re-evaluated our data and discovered that serum serotonin levels were markedly higher in male CONV-R mice than in male GF mice ($p = 0.0022$, pairwise Wilcoxon rank-sum test, with CONV-R/GF fold change of 1.6), while female mice exhibited no statistical difference ($p = 0.18$) (**Supplementary Figure 3.2b**), suggesting gender as a potential factor in serotonin pathways. We also examined serum neurotransmitter and neuromodulatory compounds (**Supplementary Figure 3.2c**) and noted that glycine, cortisol, and 2-aminoadipate shared a similar trend with fecal data (decreased in CONV-R mice). Whereas histamine and GABA embraced an opposite trend with markedly lower levels in CONV-R than in GF sera. Surprisingly, CONV-R-gut-enriched neurotransmitters such as acetylcholine, epinephrine, and taurine were either below detection levels in serum or exhibited no statistical difference between groups. The results together show that microbiota's modulation of peripheral circulating neurotransmitters is strictly compartmentalized.

To further delineate gut-blood exchange, we focused on metabolites of GF/CONV-R difference shared by fecal and serum matrices. In total, 88 metabolite pairs are shown in MetaMapp network graphs (Dinesh K. Barupal et al., 2012) for a side-by-side comparison (**Figure 3.6c-d**). The network analyses generated nine compound clusters, including fatty acids, amino acids, and carnitines/choline. The overall change in serum was smaller than in feces, as indicated by generally smaller node sizes and larger adjusted p-values of Welch's *t*-test (**Figure 3.6c-d**). We noted that fatty acid pairs including microbiota-derived adipate (Karlsson, Mapelli, & Olsson, 2017) and

sebacate (Visconti et al., 2019) embraced a contrasting gut-blood pattern, with 20 elevated in feces but lowered in serum for microbiota-harboring mice. Conversely, phenolics such as hydroxyphenyllactate and 4-hydroxy-3-methoxycinnamate were lower in feces but higher in blood sera for CONV-R mice. Apart from these two, other compound classes showed similar fecal-blood patterns of change, spanning amino acid derivatives (including indoles), nucleotides, steroids, and carnitines/choline. The results support that microbiota's impacts on local gut epithelial homeostasis, energy harvest and signaling can translocate and propagate to peripheral blood circulation, raising further possibilities of humoral transport and effects on host CNS.

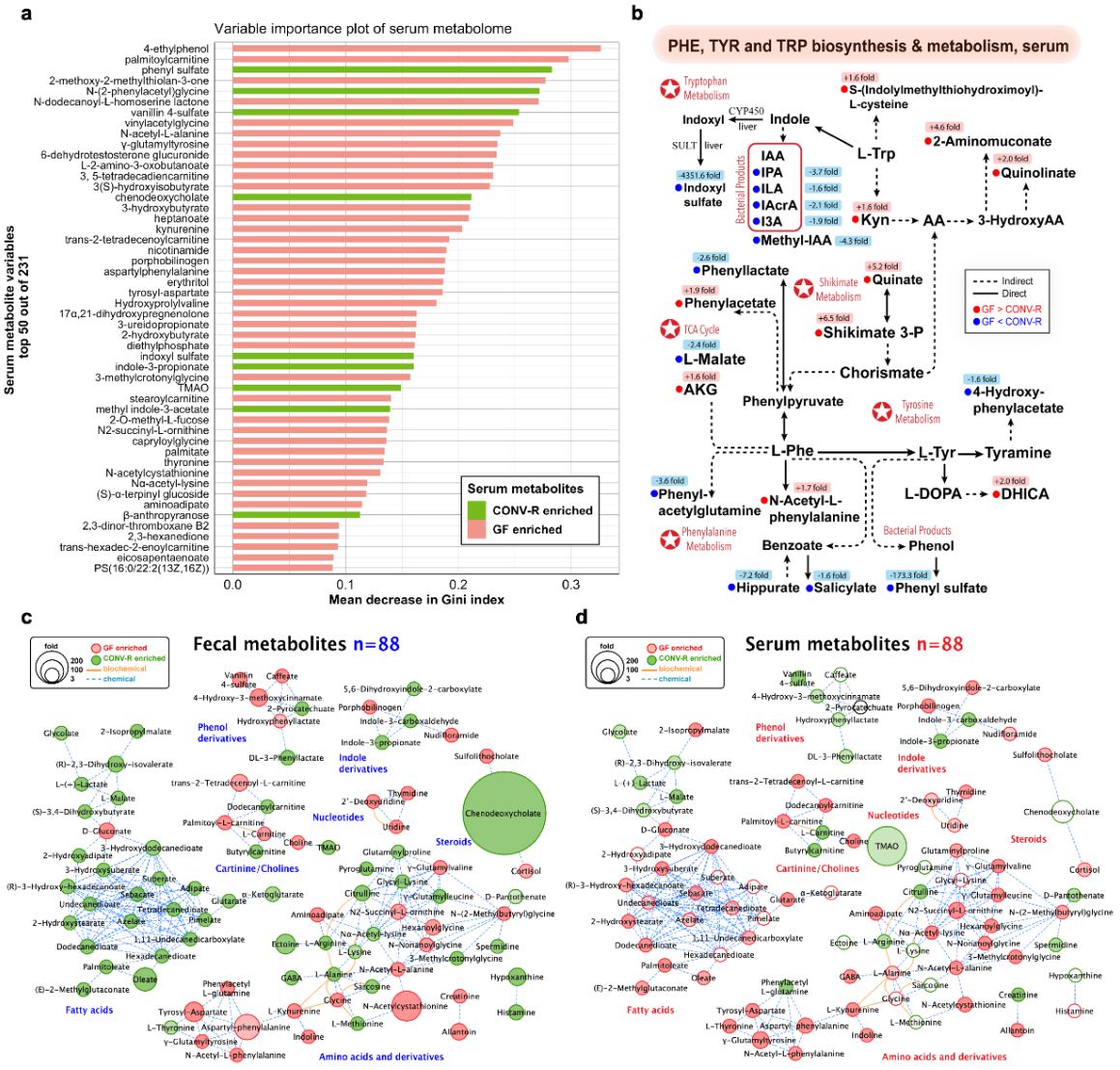

Figure 3.6 Select high-impact metabolites and pathways in circulating blood and their enterohepatic transport. (**a**) Variable importance plot of top 50 serum metabolites (*y*-axis) ranked by contribution to mean decrease accuracy of Gini coefficient (*x*-axis) in the random forest model for discerning group difference. (**b**) Diagram summary of perturbed serum pathways of phenylalanine, tyrosine and tryptophan biosynthesis and metabolism. (**c, d**) MetaMapp metabolomic networks of 88 metabolite pairs altered in feces (c) and serum (d), with nodes representing individual metabolites, edges for biochemical (KEGG reactant pairs) and chemical (Tanimoto coefficient>0.7) relationships, and lower transparency for lower adjusted p-values (<0.05, two-sided Welch's *t*-test). Abbreviations: TMAO, trimethylamine N-oxide; PS, phosphatidylserine; Phe, L-phenylalanine; Tyr, L-tyrosine; Trp, L-tryptophan; Kyn, L-kynurenine; AA, anthranilate; IAA, indole-3-acetate; IPA, indole-3-propionate; ILA, indole-3-lactate; IArcA, indole-3-acrylate; I3A, indole-3-carboxaldehyde; Shikimate 3-P, shikimate 3-phosphate; DHICA, 5,6-dihydroxyindole-2-carboxylate; AKG, α-ketoglutarate; L-DOPA, L-3,4-dihydroxyphenylalanine; SULT, sulfotransferase; CYP450, cytochrome 450; TCA cycle, tricarboxylic acid cycle.

3.3.5 Integrated analyses of cerebral cortical brain, feces, and blood serum metabolomes

To further determine whether commensal microbiota modulates brain biochemistry, we profiled the metabolome of cerebral cortical brain tissues comparing GF and CONV-R mice. We focused on the cerebral cortex region because it is the largest site of neural integration (occupying over two-thirds of mammalian brain mass), carries pivotal roles in brain functions such as thinking, memory, language and consciousness (Fernández, Llinares-Benadero, & Borrell, 2016; Friedland, 2015), and importantly, cerebral cortical injuries are often linked to onset of mental disorders such as depression (Pandya, Altinay, Malone, & Anand, 2012) for which many also are common comorbidities to gut dysbiosis. In total, we identified 58 altered metabolites in cerebral cortical brain tissues (**Supplementary Table 3.4**). To interpret these, we constructed a network graph of all metabolites and ranked them based on random forest model (**Figure 3.7a**). The network showed changes in diverse compounds, represented by indoles, amino acids, nucleotides, and fatty acids. On the VIP chart, we discovered an array of markers and pathways related to oxidative stress and reactive oxygen species (ROS) reaction. For example, the methionine/glutathione transsulfuration pathway was extensively perturbed in brain, as characterized by elevated levels of L-methionine (1.47-fold), S-(5'-adenosyl)-L-homocysteine (SAH, 1.30-fold), L-glutathione (GSH, 15.1-fold), and 5'-deoxy-5'-methylthioadenosine (1.62-fold) when microbiota was present (**Supplementary Figure 3.3**). We also noted for CONV-R brain elevated levels of two eicosanoids prostaglandin B2 (1.8-fold) and E2 (1.7-fold), α-ketoglutarate (AKG, 1.4-fold) (a sensitive oxidative stress indicator) as well as the antioxidative ketone body 3-hydroxybutyrate (2.0-fold). In addition to generic markers, we discovered a range of microbiota-derived metabolites implicated in redox homeostasis. These spanned short-chain fatty acids (e.g., butyrate, caproate), indoles (e.g., indoxyl sulfate, indole-3-lactate), trimethylamine-*N*-oxide (TMAO), shikimate and phenylalanine

derivatives (e.g., hydroxyphenyllactate, PAG), all elevated in CONV-R brain tissues compared with GF mice (**Figure 3.7a**). The results support that commensal microbiota can largely mediate, directly or indirectly, redox homeostasis, energy metabolism as well as neuronal signaling inside the mammalian CNS.

To further interpret in light of the gut-brain axis, we compared multiple matrices for an integrated analysis, with examples shown in boxplots (**Figure 3.7b**). We noted under heated ESI- analysis mode, ion feature *m/z* 212.002 at retention time of 4.9 minutes was much elevated in CONV-R mice while virtually absent in GF mice, with highest fold changes in both sera (4,351.6-fold) and brain tissues (26.8-fold) (**Figure 3.7b**). In silico formula analyses determined the elemental composition of this ion feature to be $C_8H_6NO_4S$ ([M-H]-), based on which the neutral structure was dereplicated and confirmed as indoxyl sulfate (**Figure 3.7c**). Indoxyl sulfate was a known uremic toxin and cardiotoxin originated from host-microbiota metabolism of dietary tryptophan. Our results indicated that microbiota-derived indoxyl sulfate may cross the BBB and reach the CNS, which was consistent with two emerging studies (Chu et al., 2019; Jonathan R. Swann, Spitzer, & Diaz Heijtz, 2020). Similar to indoxyl sulfate that has microbial relevance and shared trends across compartmental matrices, we also noted two other aromatic amino acid derivatives indole-3-lactate and PAG that were enriched in CONV-R mice. Other potential humoral pathways indicated in this study were exemplified for CONV-R mice by elevated levels of TMAO, methionine, vitamin B_5 in all three matrices and decreased levels of allantoin and corticosterone in serum and brain tissues (**Figure 3.7b**; **Supplementary Table 3.1**).

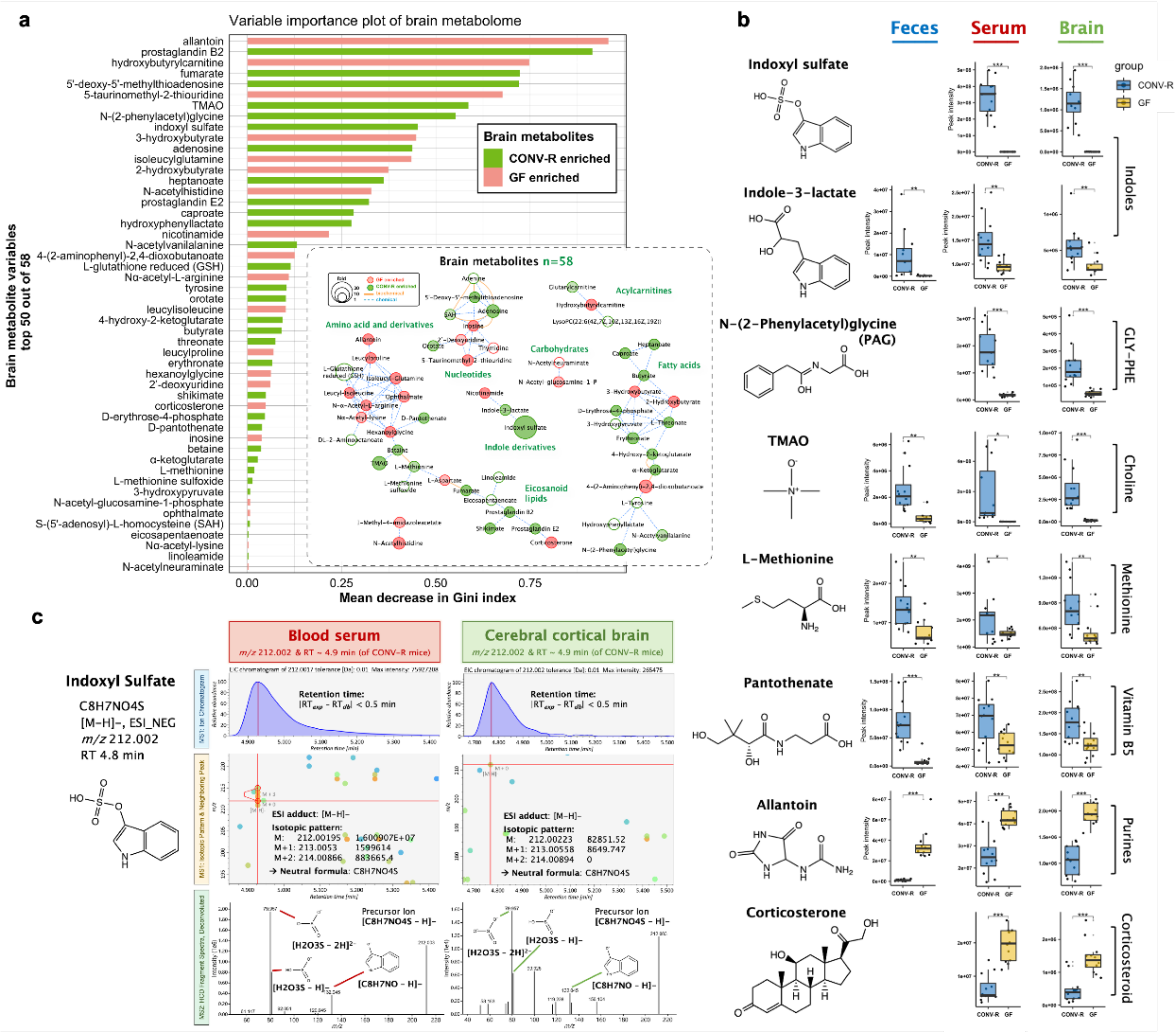

Figure 3.7 Metabolites of microbial neuroactive potential: integrated analyses of cerebral cortical brain, fecal and blood serum metabolomes. (**a**) Variable importance plot of top 50 brain metabolites (*y*-axis) ranked by contribution to mean decrease accuracy of Gini coefficient (*x*-axis) in the random forest model for discerning group difference; embedded was a MetaMapp network view of all 58 metabolites altered in cerebral cortical brain tissues owing to microbiota, with nodes representing individual metabolites, edges for biochemical (Kyoto Encyclopedia of Genes and Genomes, i.e., KEGG reactant pairs) and chemical (Tanimoto coefficient>0.7) relationships and lower transparency for lower p-values (<0.05, two-sided Welch's *t*-test). (**b**) Box and Whisker plots of select metabolites exhibiting systemic alterations across feces, blood sera and cerebral cortical brain tissues as mediated by microbiota, with the box ranging from the first quartile to the third while the whiskers going from each quartile to the minimum or maximum (n=24), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, two-sided Welch's *t*-test; exact p-values and adjusted p-values (i.e., q-values) are provided in Supplementary Table 4 . (**c**) Structural annotation of ion feature *m/z* 212.002 at retention time of 4.9 minutes as indoxyl sulfate that was highly enriched in CONV-R blood sera (4,351.6-fold) and cerebral cortical brain tissues (26.8-fold) compared with GF mice. Abbreviations: PAG, N-(2-phenylacetyl)glycine; TMAO, trimethylamine N-oxide; Gly-Phe, glycine-phenylalanine dipeptide; HCD, higher-energy C-trap dissociation; EIC, extracted ion chromatogram; *m/z*, mass-to-charge ratios; RT_{exp}, experimental retention time (from data); RT_{db}, reference retention time (from chemical standard).

3.3.6 Microbial rewiring of host biochemistry has gender-specific characteristics.

Sex dimorphism has been increasingly identified in host-microbiota interaction (Santos-Marcos et al., 2019). Here, to assess the gender-specificity of microbiota-metabolome signatures in light of the humoral gut-brain axis, we revisited the master peak tables and performed two-way analysis of variance (ANOVA) for all ion features considering both variables of microbiota (GF/COVN-R) and gender (male/female). For features with significant main effect of microbiota, a considerable proportion also exhibited microbiota×gender interaction in feces (32.6% out of 63.0%, i.e., 51.7%, HESI+), while less for sera (36.5%, HESI+) and least for cortical brain (10%, HESI+) (adjusted $p<0.05$) (**Figure 3.8a**); for a more general check, a main effect of gender has been identified for a number of features in all three matrices, among which fecal features embraced the largest proportion with microbiota×gender interaction (82.8%, HESI+), while less for sera (51.3%, HESI+) and least for brain tissues (11.0%, HESI+) (**Figure 3.8b**). Grouping data by gender, post-hoc Tukey's honestly significant difference (HSD) test was conducted comparing GF and CONV-R (adjusted $p<0.05$) to examine whether individual features of significant main effects of microbiota exhibited sex-specificity (i.e., only of GF/CONV-R difference either in male or female) with the gender-specific distribution summarized in Venn diagrams (**Supplementary Figure 3.4**) and pie charts (**Figure 3.8c-e**). In parallel, we revisited the annotation tables to examine gender specificity of the altered metabolites, yielding PieDonut distributions (**Figure 3.8f-h**) that embraced a consistent trend with ion feature pie chart (i.e., feces < sera ≈ brain) in terms of the proportion of sex-specific molecular signatures. Beyond GF/CONV-R comparison and for a more general check, we also performed Tukey's HSD test on GF/CONV-R grouped data to compare male and female within each, with ion feature distribution summarized in Venn diagrams and pie charts (**Supplementary Figure 3.5**).

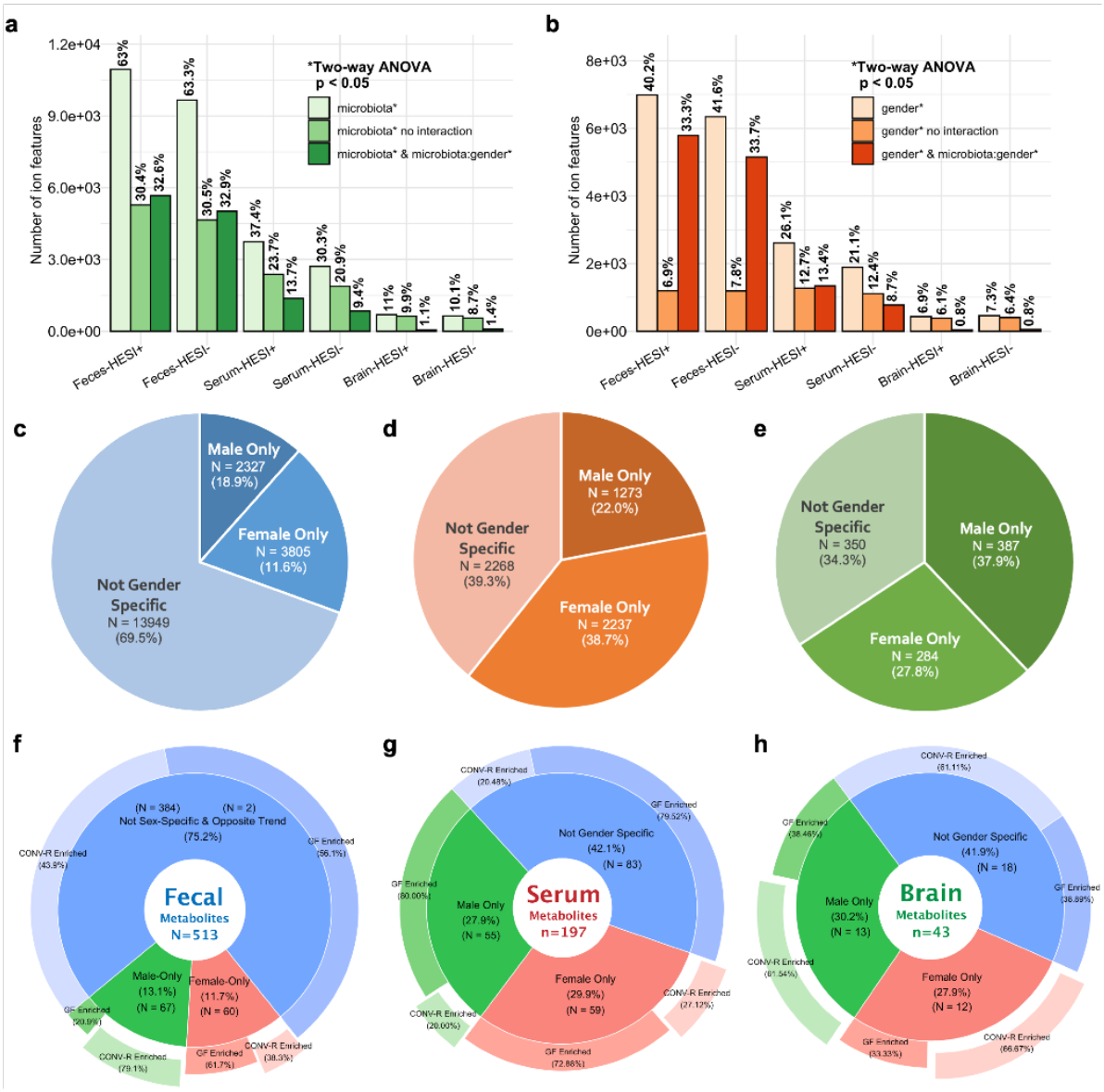

Figure 3.8 Microbial rewiring of host metabolism and gut-brain axis exhibits gender-specific characteristics. (**a**) Proportions of ion features with significant main effects of microbiota as determined by two-way analysis of variance (ANOVA) (adjusted p<0.05). (**b**) Proportions of ion features with significant main effects of gender as determined by two-way ANOVA (adjusted p<0.05). (**c-e**) Pie charts for gender-specific distribution of ion features in feces (c), sera (d), and cerebral cortical brain (e) combining both HESI positive and negative modes that had main effects of microbiota (two-way ANOVA, adjusted p<0.05) while exhibiting statistical GF/CONV-R difference in at least one gender (post-hoc Tukey's HSD test, adjusted p<0.05). (**f-h**) PieDonut charts for gender-specific distribution of the identified compounds in feces (f), sera (g), and cerebral cortical brain (h) as determined by two-way ANOVA (adjusted p<0.05) and post-hoc Tukey's HSD test (adjusted p<0.05).

To examine sex-specific biochemical details, MetaMapp network graphs (**Supplementary Figure 3.6-3.8**) and MSEA charts (**Supplementary Figure 3.9**) were generated from sex-specific metabolites of GF/CONV-R difference (**Supplementary Table 3.11**). We noted for fecal metabolomes amino acids, oligopeptides, oxylipins, bile acids, and nucleobases that were either only altered in males (67 compounds) or in females (60 compounds) (**Supplementary Figure 3.6**). Specifically, tryptophan catabolites kynurenate, xanthurenate, indole-3-carboxaldehyde and 4-(2-aminophenyl)-2,4-dioxobutanoate were elevated exclusively in male CONV-R feces compared to GF males (**Supplementary Figure 3.6a**). Known microbial products also exhibited alterations selective to gender, as represented by sebacate and adipate only for male feces (**Supplementary Figure 3.6a**) and by 2,3-butadione, a known bacterial toxin linked to health conditions such as inflammatory bowel diseases (Ahmed, Greenwood, Costello, Ratcliffe, & Probert, 2016), cystic fibrosis (Whiteson et al., 2014), and nonalcoholic fatty liver disease (Raman et al., 2013), that was enriched only in female fecal metabolomes while microbiota was present (**Supplementary Figure 3.6b**). For sera, we observed a realm of lipids, organic acids, and amino acids that exhibited gender-specific qualities (**Supplementary Figure 3.6**). Specifically, for male blood sera multiple acylcarnitines had differentiated levels between GF and CONV-R (**Supplementary Figure 3.7a**), while female sera involved an array of phospholipids, oxylipins, and long-chain fatty acids that were all lower in CONV-R than in GF (**Supplementary Figure 3.7b**). We also noted several aromatic amino acid derivatives that were selectively altered in females but not in males, including tryptophan catabolites (e.g. indole-3-carboxaldehyde, indole-3-lactate) and ferulate (a neuroprotectant) (Z. Liu et al., 2019). As for metabolites in cortical brain, we discovered 13 altered specific to male mice and 9 to females, with multiple compounds involved in redox homeostasis and energy metabolism (**Supplementary Figure 3.8**). Of note, prostaglandins (B2 and E2), GSH,

AKG, and nicotinamide were only altered in male cortical brains (**Supplementary Figure 3.8a**), while SAH, indole-3-lactate, caproate, 2-hydroxybutyrate, and eicosapentaenoate (EPA) underwent changes only in females (**Supplementary Figure 3.8b**). Taken together, microbiota-metabolome-brain axis exhibited gender-specific characteristics that need to be considered in future studies of microbiota's functional roles in the given pathophenotypes.

3.4 Discussion

Recent data support that commensal microbiota regulates mammalian brain function, opening doors of targeting microbiota for neuroprotective causes. However, biochemical underpinnings for such microbial effects in light of the gut-brain axis remains largely unclear. Metabolomics can address this but often encounters low compound identification rates due to limited references and tools such as commercially available chemical standards and mass spectral databases. To expand metabolome coverages, recent upgrade of accurate-mass measurement and cheminformatic tools shows promise, enabling in silico prediction of elemental composition, molecular structures, and chromatographic retention time with integrated and validated procedures. In this light, we sought to apply a complete suite of these rules and tools (untargeted procedures), alongside the conventional use of an in-house library based on hundreds of reference chemical standards (targeted procedures), to conduct high coverage mapping for the biochemical landscape of the "microbiota-gut-brain axis." Fecal matter, blood sera and cerebral cortical brain tissues of GF C57BL/6 and CONV-R mice of 8-week-old were harvested, extracted, and analyzed using high-resolution LC-HESI-HRMS. Using statistics and cheminformatics, we screened 20,939 distinct ion features combining both HESI modes and identified hundreds of altered metabolites including 533 for fecal, 231 for serum, and 58 for brain tissues. To our knowledge, these datasets

represent the first high-coverage metabolome characterization for microbiota's effects on host biochemistry in light of humoral route for interorgan transport and gut-brain communication.

We gained a "landscape" view of these microbiota-mediated biochemical changes through both chemical and pathway enrichment analyses. For metabolism in gut lumen where trillions of microbes reside and constant host-microbiota interaction occurs and converges, a total of 70 chemical class clusters and 71 pathways were significantly perturbed, represented by 14 amino acid pathways (aromatic amino acids in particular), purines, bile acids, porphyrins, vitamins (e.g., B and K) and polyamines alongside massive protein catabolism (over 100 oligopeptides altered) and energy harvest events (e.g., TCA cycle, acylcarnitine shuttle). In compliance with past studies (Cervenka et al., 2017; Dodd et al., 2017; Platten et al., 2019), fecal aromatic amino acids, especially the tryptophan and tyrosine metabolism, stood out with high statistical significance and 50 multiple top-ranked metabolite members in random forest classification. We offered an integrated view of fecal aromatic amino acid changes and observed for microbiota-harboring mice (i) enhanced fluxes of tryptophan pathways featuring decreased tryptophan and enriched indoles, serotonin, xanthurenate and kynurenate, (ii) altered shikimate pathway with lowered shikimate and increased levels of chorismate derivatives, (iii) suppressed folate biosynthesis and (iv) enriched tyrosine and downstream by-products including catecholamine neurotransmitters. Of significance, we also identified over 30 altered fecal bile acids with the free form bile acids (primary and secondary) much elevated while conjugated primary bile acids significantly lowered in the presence of microbiota, including those associated with neurodegenerative disease outcomes such as Alzheimer's disease (MahmoudianDehkordi et al., 2019; Nho et al., 2019).

Systematic effects of microbiota on blood circulation were characterized, as represented by 231 altered metabolites of 27 chemical classes and 61 significantly perturbed pathways

spanning porphyrin metabolism, ketone bodies, bile acid biosynthesis and a range of amino acids, etc. Note that many of the serum pathways were also perturbed in gut, with the latter generally embracing larger number of altered metabolites in the given pathway in parallel with compartment-specific details to note. For instance, similar to intestinal changes, microbiota-harboring mice exhibited distinct serum aromatic amino acids featuring (i) enriched levels of microbiota-derived indoles such as indoxyl sulfate and IPA (an important ligand to Pregnane X receptors (Alexeev et al., 2018; M. Venkatesh et al., 2014)), (ii) altered shikimate pathway with decreased levels in shikimate-3-phosphate and quinate, (iii) enriched phenolic metabolites phenyllactae, hippurate, salicylate and phenyl sulfate, while no differences were detected for tyrosine and associated catecholamine products as observed in gut. Notably, the shikimate pathway, which mammalian cells lack but heavily involved in microorganisms, were detected to be perturbed by microbiota with effects on other aromatic amino acids and associated neurotransmitter generation (e.g., serotonin, tyramine). This suggests a need to evaluate the microbiota-shikimate-host network and associated health risks, for example, of using shikimate pathway-inhibiting neurotoxic herbicides (e.g. glyphosate) that were long believed to be innocuous for mammals (Mesnage et al., 2019). On the molecular level, for instance, random forest classification ranked 4-ethylphenol, a microbial metabolite of tyrosine (Bone et al., 1976; Nicholson et al., 2012) and the precursor of the microbial 4-ethylphenylsulfate (Hsiao et al., 2013), as the top metabolite variable to CONV-R/GF group separation. Together, these microbiota-driven systemic changes coupling to gut metabolic alteration may serve as a valuable reference for future studies probing microbial influences on host physiology and health, warranting efforts to further delineate genes, enzymes and transporters involved as well as specific microbial members in play.

We also applied high-coverage metabolomics searching for brain signatures owing to microbiota (**Supplementary Figure 3.1**). Targeting the cerebral cortical brain region, we sought to find proof-of-principle of the microbiota-brain network. In total, 58 altered brain metabolites were identified, spanning oxidative stress makers from methionine/glutathione metabolites, eicosanoid prostaglandins, ketone bodies, to microbiota-derived metabolites such as butyrate, indoxyl sulfate, TMAO and shikimate/phenylalanine derivatives hydroxyphenyllactate and PAG. Overall, the results were consistent with recently emerging studies, featuring specific metabolites (e.g. 2-hydroxybutyrate, TMAO, glutathione, and acetylneuraminate) (Konjevod et al., 2021; Matsumoto et al., 2013) and pathways (e.g. oxidative stress, transsulfuration) (Hertel et al., 2019; Konjevod et al., 2021; H. Luan et al., 2015), while complementing well with others that touched upon short-chain fatty acids (Erny et al., 2015), microbe-associated molecular patterns (e.g. lipopolysaccharides) (O'Connor et al., 2009) and phenolics (Valdés et al., 2015). Our new data support microbiota's control over physiological conditions of the CNS, particularly in transsulfuration, redox homeostasis and neuroinflammation.

One central theme of the microbiome field is to probe the neuro-modulatory activities of resident microbiota in light of gut-brain signaling, given their large capacity to produce neurotransmitters and associations with many mental and/or neurologic disease outcomes such as depression (Valles-Colomer et al., 2019), autism spectrum disorder (De Angelis et al., 2013; Hsiao et al., 2013), Alzheimer's disease (Nho et al., 2019) and Parkinson disease (Kim et al., 2019). However, only a few molecular cues underlying such microbiota-gut-brain axis have been reported to date, leaving the mechanistic underpinnings largely unclear (Konjevod et al., 2021). Our high-coverage approach incorporating targeted and untargeted procedures allowed us to address this; the metabolomes of GF/CONV-R difference, either with common or opposite trends among

sample matrices, indicate possibilities of direct or indirect humoral pathways, as partially supported by reported knowledge concerning interorgan transport of gut microbial metabolites and in turn, their effects on permeabilities of the BBB (Angelino et al., 2019; Braniste et al., 2014; Cervenka et al., 2017).

We discovered for gut microbiota as a master regulator of neurotransmitter production in the GI tract, as demonstrated in (i) massive depletion of over 15 "classical" neurotransmitters without microbiota, (ii) integrated mapping of changes in catecholamine biosynthesis and glutamine/glutamate-GABA metabolism, as well as (iii) tryptophan pathway-derived neurotransmitters (e.g., serotonin, kynurenate). Further, we found extensive control of microbiota over their transformation as well, particularly through deconjugation. The results complement well recent studies confirming gut bacteria as neurotransmitter producers while supporting local gut microbial capacities to reactivate phase II metabolites from their conjugated forms (e.g. glucuronides, sulfates), likely through hydrolytic enzymes such as β-glucuronidases (GUSs) and/or β-glucosidases (Bhatt et al., 2017; Biernat, Li, & Redinbo, 2018). Interestingly, except for histamine, glycine, aminoadipate and cortisol, the majority of neurotransmitters with distinct fecal patterns were either not detected, exhibited no statistical difference, or showed opposite trends (e.g., GABA, histamine) in the serum metabolome; of note, serum serotonin levels were significantly elevated in male CONV-R mice (compared with male GF) but were not significantly different between female groups. These together show that despite the large metabolic activities of the microbe-neurotransmitter network in the intestine, humoral transport and/or systemic effects of these microbiota-derived neurotransmitters are relatively confined in gut and can be affected by gender. Such stringent organ compartmentation and gender-specific characteristics of microbial neurotransmitters need to be studied in the future. That said, we nevertheless discovered a range

of compounds potentially involved in interorgan transport and gut-brain communication, for which the presence of microbiota contributed surging levels extending from gut and/or blood to cerebral cortex. These spanned indoles (e.g., indoxyl sulfate, indole-3-lactate), phenolics (e.g., 4-ethylphenol, phenyl sulfate, PAG), choline derivatives (e.g., TMAO), vitamin B (e.g., pantothenate), butyrate, and methionine, with some virtually absent in GF mice. Of note, indoxyl sulfate, a known uremic toxin and an oxidative stress marker originated from gut bacterial indoxyl (Evenepoel, Meijers, Bammens, & Verbeke, 2009; Wikoff et al., 2009), embraced the highest fold changes of elevation in both circulating blood (4,351.6-fold) and brain tissues (26.8-fold) with the presence of microbiota. Likewise, TMAO was ranked the third for highest fold changes in both serum (267.3-fold) and brain (7.32-fold) alongside elevated levels in gut lumen (5.90-fold) owing to microbiota. TMAO, also a uremic toxin and an oxidative stress marker, is an oxidation product of gut bacterial trimethylamine through host hepatic flavin monooxygenase with demonstrated adverse effects in cardiovascular diseases (Z. Wang et al., 2011). On the contrary, antioxidant agents such as pantothenate and *Bifidobacterium longum* product indole-3-lactate (Meng et al., 2020), were also much elevated in both serum and brain when microbiota was present. Together, these findings buttress the notion that commensal microbiota can mediate the CNS (especially redox homeostasis and neuroinflammation) through direct humoral pathways, but the effects may vary depending on multiple factors such as specific microbiota composition and their metabolites, as well as gender, age, and the health status of mammalian hosts (Nicholson et al., 2012).

The strength of this study lies in the use of germ-free mouse model, the sensitive and high-coverage metabolomics approach and a suite of statistics and visualization tools that allows complete profiling and mining of microbiota-specific molecular signatures as well as their multicompartmental comparison for microbiota-gut-brain interpretation. Cautions and limitations

of the present study include: (i) our metabolomics results are by nature descriptive, necessitating more focused efforts testing specific hypotheses of humoral interorgan connections (through, for example, fecal transplantation experiments and stable isotope tracer-assisted flux analysis); (ii) the metabolome changes observed in this study should be considered as molecular phenotypic representations of host-microbiota interaction rather than microbial effects alone (Visconti et al., 2019); (iii) use of peak area for statistics instead of absolute molar concentrations essential for inferring actual metabolic activities at given organ matrices; (iv) level 2 annotations confirmed with accurate-mass MS/MS analyses and machine learning-predicted retention time in this study may still fall short for an unambiguous stereoisomeric assignment; (v) biochemical pathways were interpreted for breadth and mostly from a reductionist angle, warranting studies to further delineate at bacteria- and/or pathway-specific levels and to investigate interaction between seemingly distinct chemical classes. In summary, we present a high-coverage metabolomics approach for characterizing microbiota's effects on host metabolome in light of interorgan transport and gut-brain crosstalk in mice; the novel findings and insights from this study may prove valuable for future microbiome research.

Data availability: The relevant metabolomics raw data and master alignment ion feature tables generated for this study have been deposited in the National Metabolomics Data Repository (accession IDs: ST001756, ST001757, and ST001758) with Project No. PR001126 (http://doi.org/10.21228/M8569X). All source data for figures and tables of this work are available online with the publication and/or at GitHub (https://github.com/darciliz/natcommun_hcm_code); requests for additional information or data can be addressed to the corresponding author upon reasonable demand. Experimental and in silico mass spectral databases used for post

curation/validation purposes included MassBank of North America (MoNA) (https://mona.fiehnlab.ucdavis.edu/), Metlin (https://metlin.scripps.edu/), HMDB (https://hmdb.ca/), and mzCloud (https://www.mzcloud.org/).

Code availability: R Codes and associated data for reproducing part of the figures in this study have been deposited at both GitHub (https://github.com/darciliz/natcommun_hcm_code) and Zenodo (doi:10.5281/zenodo.5016278). Unless noted otherwise, all other analyses such as ChemRICH and MetaMapp were done using web-based portal and visualized in standalone GUI software such as Adobe Illustrator (version 2020; 2021) and CytoScape (version 2.7.2 for Window 10 OS and version 3.8.0 for a macOS).

Conflicts of interest: The authors declare no competing interests.

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Author contributions: K.L., Y.L., and H.R. conceived the study. Y.L. and C.-W.L. performed instrumental analyses. Y.L. conducted statistical analyses, compound identification, and data visualization. Y.Y., Y.-C.H. and Y.L. raised animals and harvested samples. Y.L. wrote the original manuscript; Y.L. and K.L. revised the manuscript. All contributing authors have agreed to the submission of this manuscript for publication.

CHAPTER 4: CHARACTERIZING MOLECULAR SIGNATURES OF HUMORAL MICROBIOTA-GUT-BRAIN AXIS OWING TO SUBCHRONIC PERFLUOROOCTANOIC ACID EXPOSURE IN MICE: AN INTEGRATED METABOLOMICS AND METAGENOMICS STUDY[4]

4.1 Introduction

The human gastrointestinal (GI) tract harbors a dense, complex, and dynamic microbial community, collectively referred to as gut microbiota (P. J. Turnbaugh et al., 2007). These resident microbes carry critical functional roles in mediating host physiology and health, with a perturbed or dysbiotic status linked to a range of metabolic syndromes or immunological conditions such as diabetes (Z. Wang et al., 2011), cirrhosis (Jasmohan S. Bajaj et al., 2014), and inflammatory bowel disease (Nishida et al., 2018). Interestingly, recent work, mostly animal model-based, support that gut microbiota also actively interacts with the brain (Cryan et al., 2020; Dinan & Cryan, 2017; Sherwin, Bordenstein, Quinn, Dinan, & Cryan, 2019). The most seminal work identified gut microbiota as a crucial mediator in stress responses (Sudo et al., 2004), brain development (Diaz Heijtz et al., 2011), brain immune homeostasis (Erny et al., 2015), and host serotonin biosynthesis (Yano et al., 2015), with broad implications for microbiota's control over the pathogenesis of neurologic / mental disorders such as autism. More important, a few recent large human cohort studies, through correlating fecal metagenomic features with mental health status and disease

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phenotypes, provided the first population-scale evidence linking microbial neuroactive potentials to neurologic / mental health (Valles-Colomer et al., 2019; Zheng et al., 2019).

Surprisingly, to date, the molecular underpinnings for microbiota's control over the brain function remains largely unclear. Although neuronal pathways through the vagus nerve are not disputable, increasing hypotheses have been postulated that a humoral route is involved, through which gut microbes, with their promiscuous enzymatic activities, synthesize or transform a realm of neuroactive or signaling metabolites that can translocate from gut to circulating blood, with further possibilities to cross the blood-brain barrier (Morais, Schreiber, & Mazmanian, 2021). Meanwhile, humans, especially these highly susceptible individuals, are exposed to numerous chemical exposures on a daily basis. We have demonstrated profound perturbations of gut microbiota induced by a range of xenobiotic exposures in terms of microbial composition, functional genes, and metabolites, with some model neurotoxicants tested (Bian et al., 2017; B. Gao et al., 2017; Lu et al., 2014). We also elucidated that gut microbiota mediates exposure-induced disease risks, as exemplified by arsenic exposure-related cardiovascular risks (Chi et al., 2019). However, how gut microbiota interacts with neurotoxicant exposures, whether a humoral microbiota-gut-brain axis is involved, as well as the implications for neurologic health effects, are all important questions that necessitates clear elucidation.

To advance our understanding of microbial modulation on neurotoxicant-induced effects, in this work, for a proof-of-concept, we examine in mice whether and how a humoral microbiota-brain axis is involved owing to perfluorooctanoic acid (PFOA) exposure, a kind of per- and polyfluoroalkyl substances (PFASs) that have been used for decades as additives in manufacturing fluoropolymer coatings and consumer products (Lau et al., 2007). Teeming with the strongest carbon-fluorine bonds, PFASs are persistent and bioaccumulative, and are increasingly detected

in ambient environments, wildlife, and humans in the U.S. and worldwide (Lau et al., 2007). PFAS exposure has been associated with fetal weight loss, impaired neurodevelopment, and cognitive decline (Melzer, Rice, Depledge, Henley, & Galloway, 2010; Steenland, Fletcher, & Savitz, 2010); specific toxicological effects have been identified in multiple studies *in vivo* spanning neuroendocrine disruption, liver damage, and carcinogenesis, etc. We specifically focus on PFOA as our model PFAS neurotoxicant for the following reasons: (i) most prevalent in the environment and among the most dominant species in human PFAS exposure, (ii) water soluble and thus highly transportable in terms of exposure routes (e.g., drinking water) and toxicokinetics (due to short chain length, C8), with demonstrated ability to transcend placenta, (iii) once ingested, has relatively higher rates of elimination *in vivo* compared to PFASs with longer chains and expected to be more readily observable for biochemical changes/toxicological effects, and (iv) exhibits high affinity for binding to protein such as β-lipoproteins, albumin, and liver fatty acid binding protein (L-FABP) with observable neurotoxic effects *in vivo* and *in vitro* (Post, Cohn, & Cooper, 2012).

Here in Chapter 4, we perform a subchronic PFOA exposure scheme on C57BL/6 mice. We combine high-coverage multicompartmental metabolomics and fecal 16S rRNA gene sequencing to characterize PFOA-induced microbial and metabolome changes, with specific aims to examine neuroactive biochemical alterations in both host bodies and microbiota and to probe a potential role of humoral microbiota-brain axis. This work may yield critical knowledge of microbiota-gut-brain crosstalk in environmental risks while forming new basis of toxicity testing, risk assessment, and public health intervention for exposures to per- and polyfluoroalkyl substances and many other numerous other neurotoxicants beyond.

4.2 Materials and Methods

4.2.1 Chemicals and reagents

Optima™ LC/MS-grade solvents and reagents acetonitrile (ACN), methanol (MeOH), water and formic acid were obtained from Fisher Scientific (Waltham, WA, USA). Stable-isotope-labeling (SIL) standards of common neurotransmitters and tryptophan metabolites including L-tryptophan-2,3,3-d3 (d3-Trp), γ-aminobutyric acid-d2 (d2-GABA), L-glutamine-2,3,3,4,4-d5 (d5-Gln), acetylcholine-d13 (N,N,N-trimethyl-d9; 1,1,2,2-d4) (d13-ACh), serotonin-α,α,β,β-d4 (d4-5HT), indole-3-propionic-2,2-d2 acid (d2-IPA), and indole-3-acetic-2,2-d2 acid (d2-IAA) were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada); L-kynurenine (ring-d4, 3,3-d2) (d6-Kyn) was purchased from Cambridge Isotopes (Tewksbury, MS, USA).

4.2.2 Animals, exposure, and sample harvest

The workflow of this study is illustrated in **Figure 4.1**. Specifically, specific-pathogen-free (SPF) C57BL/6 mice were acquired from Jackson Laboratory (Bar harbor, ME, USA) and housed at the University of North Carolina (UNC) animal facility feeding *ad libitum* on tap water and standard Prolab RHM 3000 pelleted diet (St. Louis, MO, USA). For the 13-week exposure scheme, twenty age-matched littermates of 8 weeks old were randomly assigned to control group (clean water) and exposure group (1 ppm PFOA-spiked water), each containing 5 males and 5 females. The environmental conditions were kept at 22 ºC and 40-70% humidity with a daily 12:12-hour light:dark cycle. At week 13, all mice were carbon dioxide-euthanized and necropsied for sample collection. Whole blood was withdrawn into blood collection tubes (BD Microtainer®, NJ, USA), kept for an hour, and centrifuged at 10,000×*g* for 1 min at room temperature; the supernatant layer (sera) was collected. Meanwhile, feces and bulk brain were snap-frozen and stored at -80 ºC prior

to analysis. All animals were treated humanely following protocols approved by the UNC Institutional Animal Care & Use Committee (IACUC).

4.2.3 16S rRNA gene sequencing

Whole DNA was extracted from fecal pellets using the DNeasy PowerSoil® kit (Qiagen, Hilden, Germany). For 16S amplicon sequencing, DNA was amplified using the 515F and 806R primers to target the variable region 4 (V4) of 16S rDNA gene (Caporaso et al., 2012). The amplified DNA was determined on a NanoDrop™ Lite Spectrophotometer (Waltham, MA, USA) for normalization. Resultant DNA was barcoded, pooled, and sequenced on an Illumina MiSeq system (San Diego, CA, USA). Raw *.fastq data in 300-bp format were processed using the QIIME2 pipeline (Bolyen et al., 2019), where paired-end reads were demultiplexed and denoised by the q2-deblur plugin (Phred quality score cutoff < 20) to generate the feature table of amplicon sequence variants (ASVs). The ASVs were taxonomically assigned based on the Greengenes database with a pretrained naïve Bayes qiime2 classifier (i.e., gg-13-8-99-515-806-nb-classifier.qza). Biological pathways were predicted from these amplicon genes using the q2-picrust2 plugin (Douglas et al., 2020). The 16S rRNA sequencing data were further analyzed and visualized in R (R Core Team, 2020) using *ade4*, *qiime2R*, and *microbiotaProcess* packages.

4.2.4 Metabolite extraction

For multicompartmental comparative metabolome profiling of humoral gut-blood-brain exchanges, metabolites were extracted from relevant samples collected at necropsy, including aliquots of ~20 mg feces, 20 µL sera, and ~20 mg cerebral cortical brain slices. Fecal aliquots were

extracted with 300 µL of ice-cold SIL standard-spiked MeOH:water (50:50, *v/v*) assisted with ~10 mg acid-washed glass beads (Sigma-Aldrich, St. Louis, MO). The mixture was homogenized on a TissueLyzer (Qiagen, Hilden, Germany) for 10 min at 50 Hz, centrifuged at 12,000×*g* for 10 min (Eppendorf, Hamburg, Germany); 100 µL supernatant was transferred and dried in a CentriVap vacuum evaporator (Labconco, MO, USA). For 20 µL sera, 180 µL ice-cold ISD-spiked MeOH was added, vortexed, and incubated at -20 ºC for 30 min. Serum samples were centrifuged at 15,000×*g* for 10 min for particulate and protein precipitation, and 100 µL supernatant were transferred to a CentriVap vacuum concentrator for dryness. For 20 mg brain tissues, 400 µL ice-cold ISD-spiked MeOH and a clean stainless-steel bead (5 mm i.d.) were added; samples were homogenized on TissueLyzer for 2 min at 50 Hz, incubated at -20ºC for 1 h, and centrifuged at 18,000×*g* for 10 min; 100 µL supernatant was concentrated to dryness. Upon instrumental analysis, all dried extracts were resuspended in ACN:water (2:98, *v/v*) for injection.

4.2.5 Instrumental analysis

Global metabolomic profiling was conducted using a Thermo Fisher Scientific Vanquish UHPLC coupling to high-resolution *Q Exactive* mass spectrometer featuring a heated electrospray ionization (HESI) source and hybrid quadrupole-orbitrap mass analyzer (Waltham, MA, USA). Chromatographic separation was achieved using a Waters Acquity UPLC HSS T3 column (100Å, 1.8 µm, 2.1 mm × 100 mm) (Milford, MA, USA), using mobile phases (A) 0.1% formic acid in water and (B) 0.1% formic acid in ACN with a flowrate of 0.4 mL/min under 40 ºC for a 15-min LC gradient: 2% B, 0-1 min; 2% to 15% B, 1-3 min; 15% to 50% B, 3-6 min; 50% to 98% B, 6-7.5 min; 98% B, 7.5-11.5 min; 98% to 2% B, 11.5-11.6 min; 2% B, 11.6-15 min. Both positive and negative HESI data were acquired under the following ion source condition: sheath gas 60

L/min, sweep gas 1 L/min, auxiliary gas 10 L/min, capillary 325 ºC, auxiliary gas heater 400 ºC, and spray voltage 2.75 kV. For MS1 fullscan profiling (orbitrap running only), the mass spectrometer was operated with mass resolution 70,000 full width at half height (FWHM) scanning a mass range of 80-1,000 Da. For tandem mass spectral analysis (quadrupole-orbitrap running), the instrument was operated in hybrid fullscan MS1 (FWHM 70,000; AGC target 3e6; max IT 200 ms) alternating with parallel reaction monitoring (PRM) MS/MS with stepped normalized collision energies set at 10, 50 and 100 (FWHM 17,500; AGC target 2e5; max IT 50 ms). Stringent quality assurance/quality control (*QA/QC*) were performed, including timely mass calibration, solvent blank injection, method blank injection, sample randomization, intermittent QC (pooled sample extracts), and regular monitoring of ISD variations across samples.

4.2.6 Metabolomic data processing, statistics, and visualization

MS1 *.raw data were uploaded to XCMS online (La Jolla, CA, USA) for data processing, yielding six master ion feature tables aligning all 20 samples from three sample type (i.e., fecal pellets, blood sera, and brain) under two HESI modes (positive and negative). Group differentiation was inspected through principal component analysis and Welch's *t*-test in R (R Core Team, 2020), and data visualization was further conducted using *ggplot2*, *ggpubr*, *FactoMineR* and *pheatmap* packages of R (R Core Team, 2020); random forest classification was performed to rank individual metabolites/features based on phenotypic discrimination using the *randomForest* package of R (R Core Team, 2020). Quantitative metabolite set enrichment analysis (qMSEA) was performed in MetaboAnalyst 5.0 (Edmonton, Alberta, Canada) based on 99 *a priori* defined metabolite sets from the Small Molecule Pathway Database (SMPDB). Chemical similarity enrichment analysis (ChemRICH) plots were generated based on Kolmogorov-Smirnov

test to cluster altered metabolites into chemical class modules (Davis, CA, USA). MetaMapp metabolomic networks were constructed through computing reactant pairs from Kyoto Encyclopedia of Genes and Genomes (KEGG) (for biological pathway relevance) and Tanimoto coefficients (>0.7, for chemical structural similarity) which were visualized in CytoScape 3.8.0 (Seattle, WA, USA).

4.2.7 High-coverage compound annotation

To achieve highest metabolome coverage and identification confidence possible, we set out to annotate all ion features of group difference through a streamlined cheminformatics pipeline that combined conventional reference mass spectral search and novel *in silico* cheminformatic prediction featuring an integrated post curation strategy. Specifically, reference search exploited an in-house spectral library of over 420 authentic chemicals standards of canonical pathway metabolites, neurotransmitters, and literature-cataloged microbial byproducts, against which the unknown spectral data was matched by known MS1 accurate mass, MS2 characteristic ion fragments, and chromatographic retention time. Meanwhile, untargeted *in silico* prediction, from formula determination to structural dereplication, was implemented in MS-FINDER 3.42, using accurate mass, isotopic ratios, and tandem mass spectra as input with settings given elsewhere (**Supplementary Information for Chapter 3**, see additional information). Top ranked candidates were sent to post curation procedures based on the overall identification score (cutoff > 3.5), mass accuracy confirmation, and retention time predicted from random forest modeling of chromatographic retention-lipophilicity relationships. All resultant annotations were manually inspected through strategies including but not limited to extensive search of publicly available experimental spectral databases (e.g., Metlin, MoNA, GNPS, MassBank, and mzCloud), chemical

inferences, and biological plausibility and relevance. Confidence level was assigned to each annotation according to the reporting standard set by the Metabolomics Standard Initiative (MSI); only level 1 & 2 annotations were kept for analysis and interpretation.

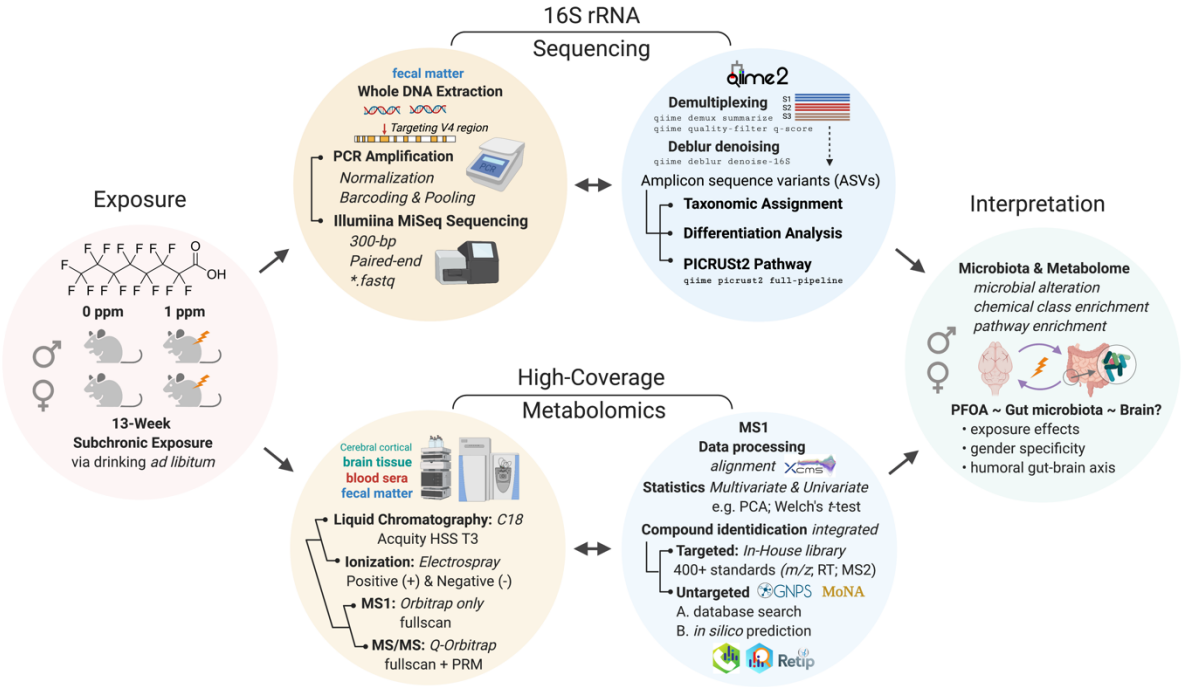


Figure 4.1 Schematic workflow of using 16S rRNA metagenomic gene sequencing and high-coverage multicompartmental metabolomics for probing effects of perfluorooctanoic acid exposure and potential humoral microbiota-gut-brain axis on the molecular level. *Exposure*: a subchronic exposure scheme of 13 weeks was performed on male and female C57BL/6 mice through feeding ad libitum on PFOA-spiked water vs. clean tap water. *High-coverage metabolomics*: fecal, blood serum, and cortical brain tissue extracts were analyzed using liquid chromatography coupled to hybrid quadrupole-orbitrap mass spectrometry; statistical analyses were conducted to assess the overall metabolomic pattern while screening for ion features of statistical difference in each sample matrix; an integrated cheminformatic pipeline featuring an in-house reference spectral library, *in silico* prediction of formula and chemical structure, extensive search against experimental spectral databases, and retention time prediction based on random forest modeling of chromatographic retention-lipophilicity relationships. *16S rRNA gene sequencing*: fecal DNA was extracted and normalized for barcoding and Illumina MiSeq sequencing; *.fastq raw data were demultiplexed and denoised to generate feature tables of amplicon sequence variants (ASVs); taxonomic assignment, differential analysis, and PICRUSt pathway prediction were performed on ASV feature tables to explore exposure-induced microbial changes and pathways in the GI tract. Abbreviations: PCA, principal component analysis; PRM, parallel reaction monitoring; *m/z*, mass-to-charge ratio; MS2, tandem mass spectra; GNPS, the Global Natural Product Social Molecular Networking; MoNA, MassBank of North America.

4.3 Results and Discussion

4.3.1 PFOA exposure-induced metabolome-wide signatures: a global view

We performed high-resolution LC-MS analysis for global metabolomic profiling of feces, sera, and cerebral cortical brain tissues of mice to probe effects of subchronic perfluorooctanoic acid exposure (**Figure 4.1**). XCMS data processing procedures yielded six master tables (three sample matrices, two HESI modes) aligning all ion features for assessing group pattern and screening of significant signatures for downstream structural annotation, analysis, and interpretation. **Figure 4.2** shows the distribution of ion features of control group (0 ppm) and exposure group (1 ppm). Based on two-sided Welch's *t*-test, we detected 5,513 fecal HESI+ ion features (out of 19,275), 3,795 fecal HESI- ion features (out of 17,607), 3,329 serum HESI+ ion features (out of 16,618), 2,753 serum HESI- ion features (out of 11,914), 1,810 brain HESI+ ion features (out of 7,315), and 1,857 brain HESI- ion features (out of 7,486) that are statistically significant (fold change≥1.2 and $p<0.05$) (**Figure 4.2A-B**); false discovery rate correction or *q*-values were calculated for each ion feature but was not used in screening of significant features towards annotation and analysis, considering the interactive and complex nature of the metabolic network *in vivo* in which even weakly significantly altered members may actively play multifaceted functional roles in multiple pathways.

Tandem mass spectra were acquired in MS1-MS2 hybrid mode targeting all these significant ion features (totaling 19,057). These spectra were batched computed in MS-FINDER 3.42 (Riken, Japan) and cleaned up using an integrated approach as detailed in (**Supplementary Figure 3.1**). In parallel, reference in-house mass spectral library was used against which unknown mass spectra data was matched for hits. Only Level 1 and Level 2 annotations were kept for downstream analysis and interpretation, resulting in a total of 269 fecal metabolites (236 with fold

change≥1.5 and *q*-value<0.05) (**Supplementary Table 4.1**), 253 serum metabolites (201 with fold change≥1.5 and *q*-value<0.05) (**Supplementary Table 4.2**), and 161 cerebral cortical brain metabolites (125 with fold change≥1.5 and *q*-value<0.05) (**Supplementary Table 4.3**) of differentiated levels between control (0 ppm) and exposure (1 ppm) groups, as shown in a Venn diagram (**Figure 4.2C**).

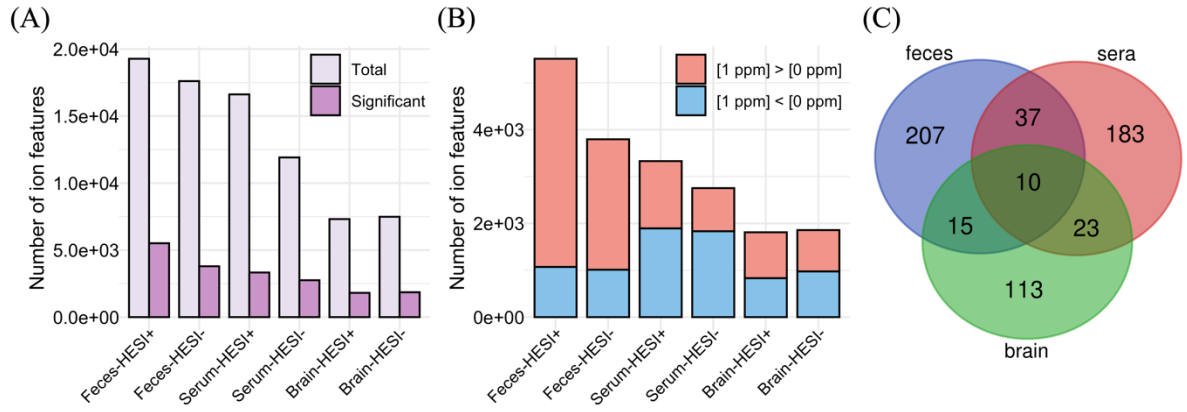


Figure 4.2 Distribution of ion features and annotated altered metabolites among feces, blood sera, and cerebral cortical brain tissues. (**A**) Number of total and significant ion features (p-value<0.05, fold change≥1.2, two-sided Welch's t-test) detected for feces, sera, and brain tissues under HESI positive and negative modes of analysis. (**B**) Trend distribution of significantly altered ion features. (**C**) Venn diagram of all identified metabolites of differentiated levels owing to perfluorooctanoic acid exposure.

4.3.2 PFOA exposure-induced gut microbiome changes

Gut microbial differences owing to subchronic perfluorooctanoic acid exposure can be readily discerned through multivariate statistical analyses including hierarchical clustering of samples (**Figure 4.3A**) and principal coordinate analysis (PCoA) plot (**Figure 4.3B**) based on the Bray-Curtis dissimilarity measures of β-diversity. **Figure 4.3C** shows identified gut bacteria at varied taxonomic levels, with relative abundances, trend of change, and effect sizes detailed in **Figure 4.3D**. At the phylum level, Firmicutes bacteria were decreased under perfluorooctanoic acid exposure with largest abundances and effect size, among which the most decreased included

the *Clostridium* spp. (e.g., Ruminococcaceae family), important probiotic bacterial members capable of converting dietary components to neuroprotective indoles and short-chain fatty acids. While, though in lower abundances, multiple bacteria members of phylum Actinobacteria and Verrucomicrobiota were increased under exposure; notable members at the genus level included *Akkermansia* spp. (e.g., *Akkermansia muciniphila*) and *Bifidobacterium* spp., with reported health benefits against obesity, diabetes, and inflammation (Depommier et al., 2019; Ejtahed et al., 2011; Schneeberger et al., 2015).

Microbial functional pathways were further predicted based on the PICRUst2 algorithm, with most significant ones (fold change≥5, q<0.05; n=63) plotted on a heatmap (**Supplementary Figure 4.1**). Among these predicted microbial functional pathways that were highly differentiated between groups, surprisingly, the majority (50/63) were downregulated in gut microbiota of perfluorooctanoic acid-treated mice than those of control. In line with taxonomic data that identified *Clostridium* spp., as the most diminished (in highest abundance and effect size), the downregulated pathways involved aromatic amino acid metabolism, such as L-tyrosine degradation, phenylacetate degradation, meta cleavage pathways of aromatic compounds, and folate transformation. We also noted multiple pathways of neurotransmitters downregulated in exposure-treated group, including phenylethylamine, L-arginine, and taurine, as opposed to a few upregulated ones such as β-alanine and glycine betaine degradation (**Supplementary Figure 4.1**).

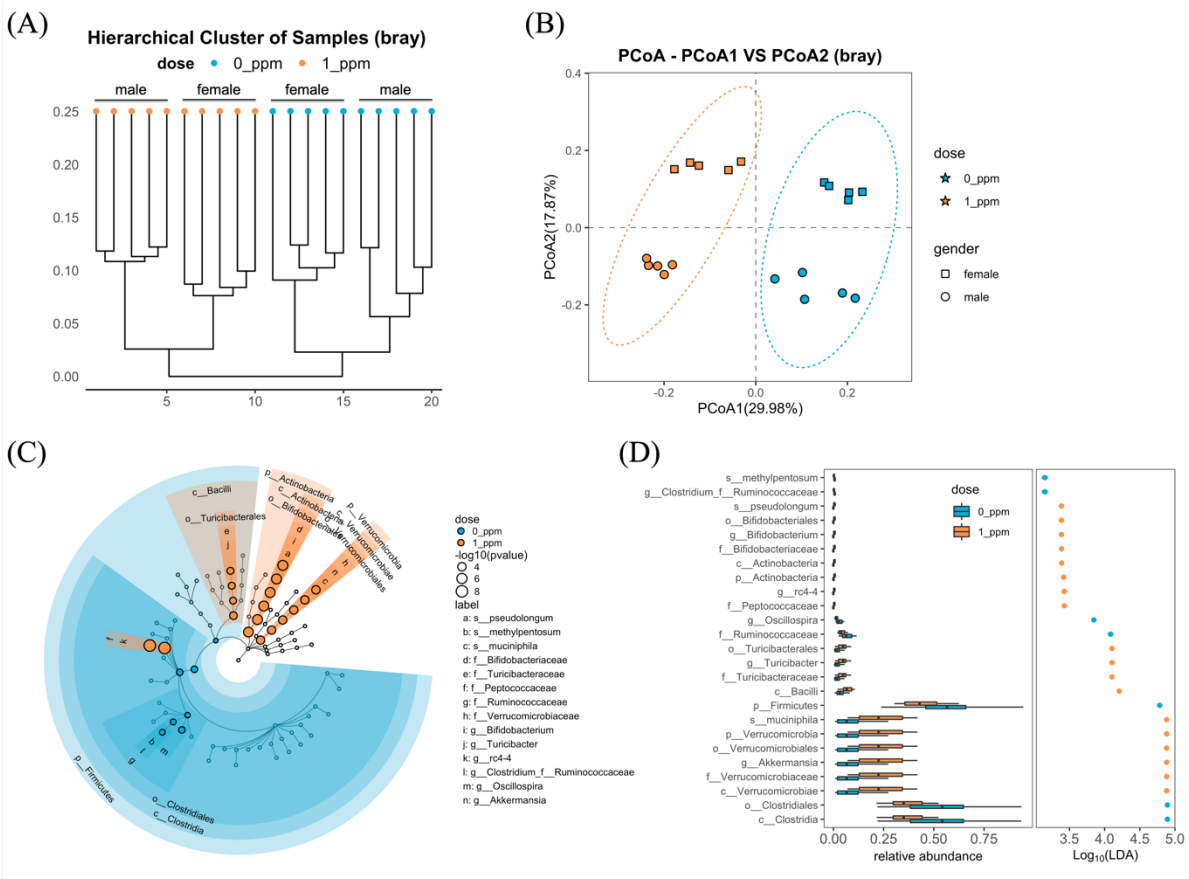


Figure 4.3 Gut microbiome compositional profiles of mice in the control (0 ppm) vs. perfluorooctanoic acid (1 ppm)-treated mice as revealed by 16S rRNA gene sequencing. (**A**) Hierarchical clustering of sequenced fecal gene amplicons based on Bray-Curtis dissimilarity. (**B**) Principal coordinate analysis (PCoA) plot of samples based on Bray-Curtis dissimilarity. (**C**) Circular clade tree of microbial taxonomic features. (**D**) boxplot of microbial taxonomically assigned features and corresponding effect size as determined by linear discriminant analysis (LDA).

4.3.3 Effects of PFOA exposure on fecal metabolome

Given the gut microbial composition changes, we conducted gut lumen metabolome profiling through LC-MS analysis of feces samples, to further probe the impact of perfluorooctanoic acid on gut microbial functional roles and host-microbe interaction. Using heated ESI positive data as an example, multivariate principal component analysis revealed a distinct pattern between control (0 ppm) and exposure (1 ppm) groups from peak intensity profiles of all aligned ion features (*n*=19,275) (**Figure 4.4A**). From significantly altered ion

features, we annotated 269 fecal metabolites of group differentiated levels. Chemical similarity enrichment analysis (ChemRICH) clustered these metabolites into 34 chemical classes (adjusted $p<0.05$), spanning a wide lipophilicity range (*x*-axis) with varied number of metabolites in each (node size) and overall trends of change (node color) (**Figure 4.4B**; **Supplementary Table 4.5**). The most significantly enriched was dipeptides that were upregulated in feces of exposure-treated mice, in line with multiple lipid chemical sets (e.g., fatty acids, acylcarnitines), suggesting together enhanced catabolic processes and shifted energy metabolic status under perfluorooctanoic acid exposure. In parallel, multiple microbiota-mediated metabolite clusters, including indoles, phenols, butyrates, porphyrins and cinnamates, were enriched as well.

To assess fecal metabolome changes in biological contexts, we performed quantitative pathway sets enrichment analysis (qMSEA) based on 99 *a priori* metabolite sets in MetaboAnalyst 5.0 (Montreal, QC, Canada) of the 269 altered fecal metabolites. In total, 58 biological pathways were significantly enriched (adjusted p-value<0.05) (**Supplementary Table 4.6**), with top 25 shown in **Figure 4.4C**. On this chart, multiple pathways were reported to be extensively regulated by microbiota, including tryptophan metabolism (top-ranked), glutamate metabolism (4^{th} ranked), bile acid biosynthesis (7^{th} ranked), phenylacetate metabolism, phenylalanine & tyrosine metabolism, and pantothenate and coenzyme A (CoA) biosynthesis. On individual metabolite level, we discovered an array of gut bacterial derivatives of aromatic amino acids (i.e., tryptophan, phenylalanine, and tyrosine) that were markedly decreased in feces of perfluorooctanoic acid exposed mice compared with the control group (**Figure 4.5A-B**). These spanned indoles including indole-3-ethanol (-1.75-fold), indole-3-acetate (-1.75-fold), indole-3-propionate (-1.96-fold), and indole-3-acetylaldehyde (-2.58-

fold), as well as phenylalanine derivatives such as phenyl sulfate (-3.19-fold), phenylacetyl L-glutamine (-4.17-fold), and hippurate (-16.98-fold). Meanwhile, a range of neurotransmitters and related neuroactive compounds were extensively altered, with notable examples shown in **Figure 4.5C-D**. These observations were consistent with our 16S rRNA gene sequencing data identifying massive decreases of *Clostridium* spp., a major bacterial synthesizer converting tryptophan into indole derivatives or transforming tyrosine into tyramine.

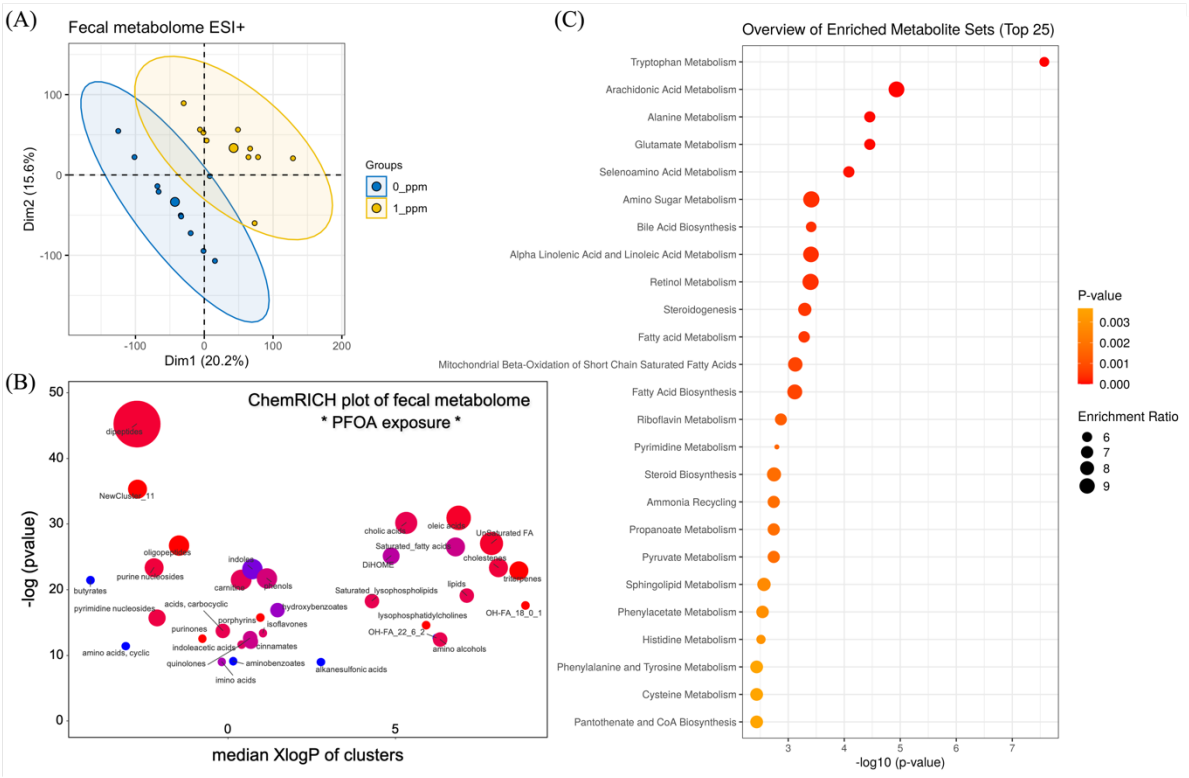


Figure 4.4 Fecal metabolomic pattern owing to perfluorooctanoic acid exposure. (**A**) Principal component analysis score plot for assessing fecal metabolomic data under heated ESI+ mode (totaling 19,275 ion features) comparing 0 ppm (n=10) and 1 ppm (n=10). (**B**) Chemical similarity enrichment analysis (ChemRICH) clustering of 269 identified altered fecal metabolites by chemical similarity with x-axis of mediation logarithmic additive octanol-water partition coefficients (XlogP) and y-axis for sets statistical significance based on the Kolmogorov–Smirnov test; the node size depicted total compound numbers for each cluster set and node color scale the proportion of exposure-upregulated metabolites (1 ppm>0 ppm, red) vs. exposure-downregulated metabolites (1 ppm<0 ppm, blue). (**C**) Quantitative metabolite set enrichment analysis (qMSEA) based on 99 *a priori* defined sets of metabolites identified a total of 58 significantly perturbed fecal metabolic pathways (adjusted p<0.05) with top 25 shown.

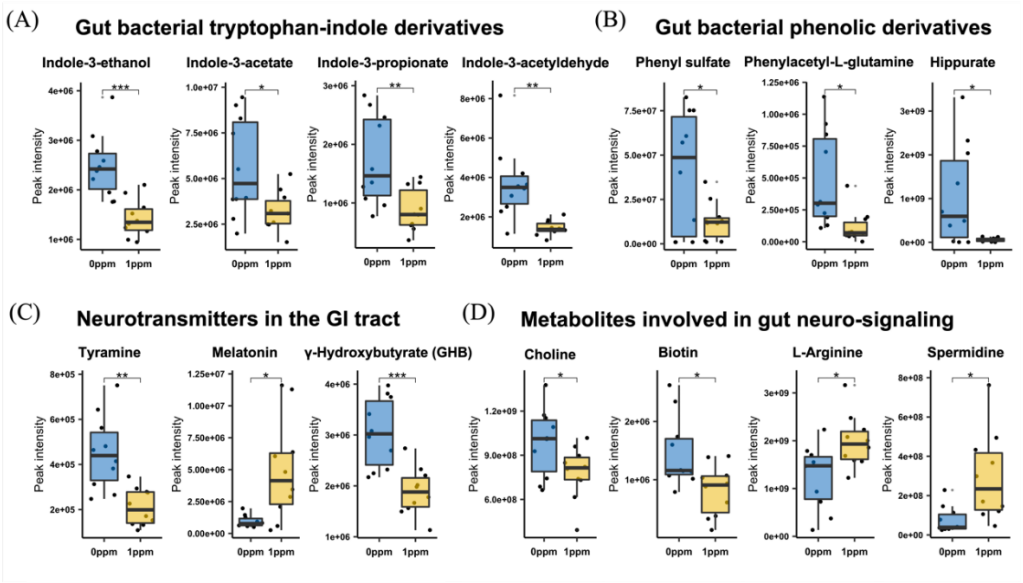


Figure 4.5 Box and Whisker plots of fecal indoles (**A**), fecal phenolics (**B**), fecal neurotransmitters (**C**), and other neuroactive compounds (**D**) as synthesized or mediated by gut microbiota, with the box ranging from the first quartile to the third while the whiskers going from each quartile to the minimum or maximum (control 0 ppm, n=10 vs. exposure 1 ppm, n=10), *p<0.05, **p<0.01, ***p<0.001, two-sided Welch's t-test.

4.3.4 Effects of PFOA exposure on serum metabolism and gut-blood transport

The circulating blood serum metabolome represents a unique avenue for probing whole body status, exposure-induced systemic changes, and potential humoral interorgan transport (e.g., the gut-blood barrier, blood-brain barrier). Here, our high-coverage serum metabolomics pipeline successfully resolved 253 altered serum metabolites at Level 1 and 2 confidence level owing to perfluorooctanoic exposure. As shown in **Figure 4.6A**, ChemRICH analysis identified 34 chemical sets (adjusted p<0.05) of a wide lipophilicity range (x-axis) with differing metabolite numbers for each (node size) and overall trends of change (node color) (**Supplementary Table 4.7**). Of note, indoles, phenols, butyrates that were linked to altered gut bacterial changes were also highly enriched in serum metabolome, with virtually all tryptophan catabolites downregulated (**Figure 4.6B**); this suggests humoral gut-blood exchange of intestinal flora-derived metabolites,

including those involved in neuroactive signaling and neuroinflammation (Cervenka et al., 2017; J. Gao et al., 2018).

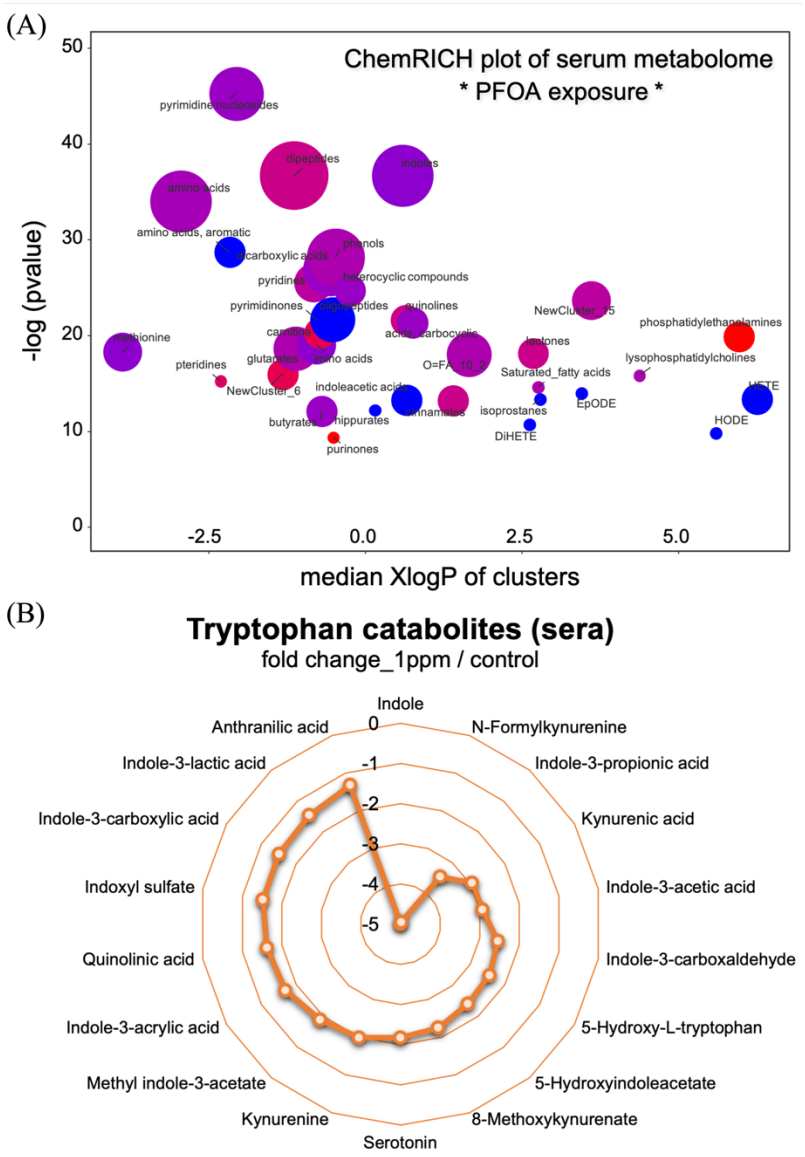


Figure 4.6 Serum metabolome changes owing to subchronic perfluorooctanoic acid exposure. (**A**) ChemRICH clustering of 253 identified altered serum metabolites by chemical similarity with *x*-axis of mediation logarithmic additive octanol-water partition coefficients (XlogP) and *y*-axis for sets statistical significance based on the Kolmogorov–Smirnov test; the node size depicted total compound numbers for each cluster set and node color scale the proportion of exposure-upregulated metabolites (1 ppm>0 ppm, red) vs. exposure-downregulated metabolites (1 ppm<0 ppm, blue). (**B**) Radar plot of altered tryptophan metabolites in circulating blood owing to perfluorooctanoic acid exposure.

To further explore gut-blood exchange, we focused on the 47 metabolites that were altered in both fecal and the blood serum matrices. For a side-by-side comparison, a MetaMapp approach was applied to both sample matrices to constructing metabolomic network graphs connecting metabolites based on biochemical (KEGG reactant pair) relationship and chemical similarity (Tanimoto coefficient>0.7) (**Figure 4.7**). The network analyses generated seven metabolite clusters, including three amino acid groups (i.e., aromatic tryptophan and phenylalanine, and other non-aromatics), three lipid groups (i.e., bile acids, acylcarnitines, and fatty acids), and nucleotide derivatives. Of note, same trend of change (same node color) was discovered for gut microbiota-derived aromatic amino acids and bile acids spanning indole-3-propionate, indole-3-acetate, 2,8-quinolinediol, and 7-sulfocholic acid, supporting a humoral route through which PFOA-induced gut bacteria-metabolome shifts can translocate across from local gut lumen to blood circulation with propagated health effects.

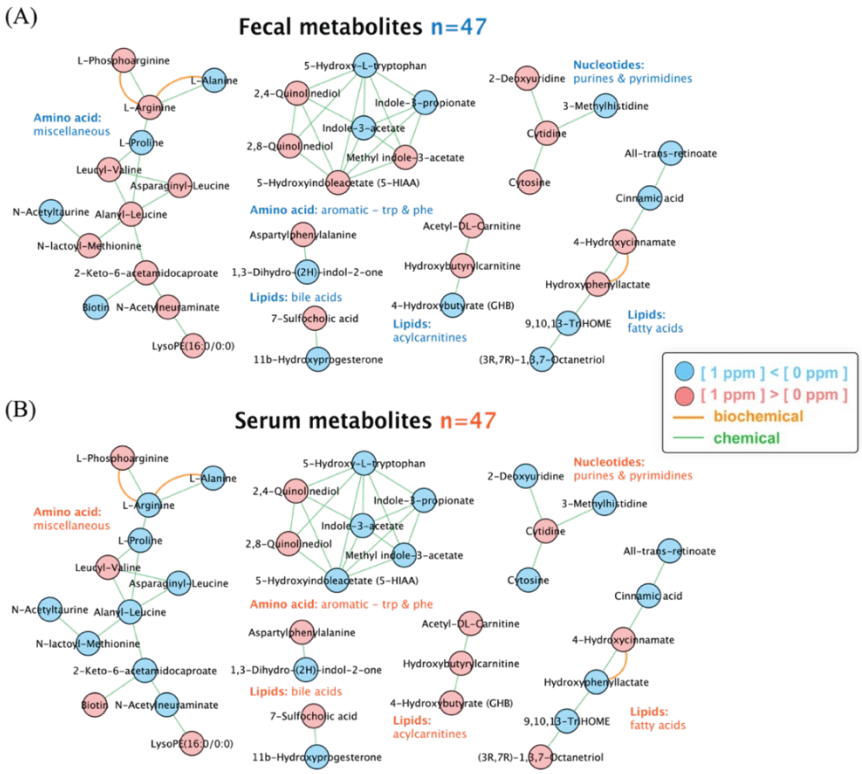


Figure 4.7 MetaMapp metabolomic networks of metabolite pairs (*n*=47) altered in both feces (**A**) and blood sera (**B**), with nodes representing individual metabolites, edges for biochemical (KEGG reactant pairs) and chemical (Tanimoto coefficient>0.7) relationships.

4.3.5 Effects of PFOA exposure on cortical brain metabolism

To determine the effect of perfluorooctanoic acid on brain biochemical profiles, we profiled the metabolome of cerebral cortical brain tissues comparing control (0 ppm) and the exposure (1 ppm) group. We focused on the cerebral cortical region, the largest site of mammalian neutral integration with critical brain functions (e.g., memory, language, and thinking), because injuries of this region are often linked to the onset of mental disorders such as depression (Pandya et al., 2012) for which many also are common comorbidities to gut dysbiosis (Rogers et al., 2016). Through the untargeted high-coverage metabolomics pipeline, we identified 161 metabolites in cerebral cortical brain tissues of differentiated levels owing to PFOA exposure (125 with fold change≥1.5 and *q*-value<0.05). To interpret these, we performed qMSEA analysis on all altered

metabolites to delineate specific biological pathways in response to perfluorooctanoic acid exposure. In total, 89 out of the 99 *a priori* pathway sets were significantly enriched (adjusted $p<0.05$) (**Supplementary Table 4.8**), with top 50 pathways shown in **Figure 4.8A**. Among pathways of top enrichment fold values, altered energy metabolism (e.g., citric acid cycle, carnitine synthesis), oxidative stress (e.g., phytanic acid peroxisomal oxidation), and neuroactive signaling pathways (e.g., taurine and hypotaurine metabolism, spermidine and spermine biosynthesis, catecholamine biosynthesis, glycine & serine metabolism, and pterin biosynthesis) were involved. Such massive scale and neurobiochemical relevance of pathway perturbations in cerebral cortex represented direct impacts of perfluorooctanoic acid on the brain, meriting further investigations for delineating the neurotoxic effects and potential functional roles of gut microbiota.

On the metabolite level, random forest classification ranked the individual metabolites by their relative contribution to mean decrease in Gini index, with top 50 shown in the variable importance plot (**Figure 4.8B**). These highly ranked metabolites were diverse by structure but can be largely categorized into amino acids and/or derivatives (e.g., cysteine, methionine, threonate, and aspartate, symmetric dimethylarginine, and 4-(2-aminophenyl)-2,4-dioxobutanoate), organic acids (e.g., oxalacetate, isocitrate, glycerate, glycolate, malonate, quinate, azelate), lipids (12-KETE, pimelylcarnitine), and vitamins (e.g., pyridoxal, pyridoxine, pyridoxal 5-phosphate, and ascorbate). Of note, altered metabolites of largest fold changes involved oxidative stress (i.e., L-glutathione reduced, GSH +320.4-fold), pterin biosynthesis (i.e., dihydroneopterin phosphate, -19.6-fold), and tryptophan metabolism (4-(2-aminophenyl)-2,4-dioxobutanoate, +18.9-fold), and catecholamine metabolism (e.g., norepinephrine, +7.48-fold; dopamine, +7.02-fold). Other altered neurotransmitters of lower fold changes included acetylcholine (+1.4-fold), tyramine (-2.31-fold), adenosine (-3.46-fold), and L-glutamate (+1.25-fold). In parallel, we spotted for exposure-treated

mice increased cortical brain levels of diacetyl, a neurotoxic agent associated with β-amyloid clumping in the brain (More, Vartak, & Vince, 2012). All these observations showed that ingestions of PFOA leads massive changes of biochemical profiles and associated neuronal signaling in mouse cortical brain, supporting previous work linking per- and polyfluoroalkyl substances to impaired neurodevelopment and cognitive decline.

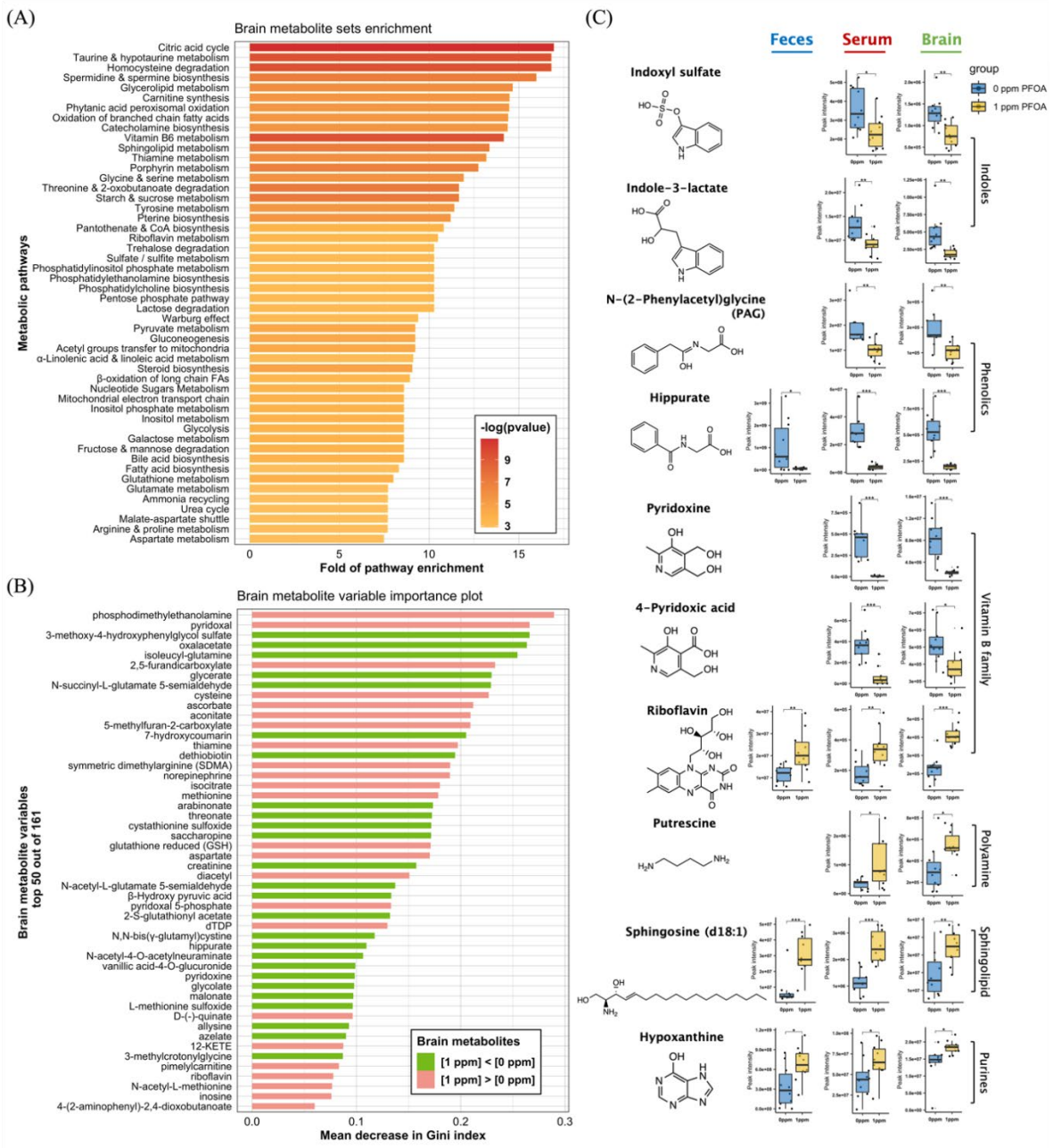


Figure 4.8 Cortical brain metabolome alterations and inter-organ comparison. (**A**) Quantitative metabolite set enrichment analysis (qMSEA) based on *a priori* defined sets of metabolites identified a total of 89 significantly perturbed cortical brain metabolic pathways (adjusted $p<0.05$) with top 50 shown. (**B**) Variable importance plot of top 50 brain metabolites (*y*-axis) as ranked by relative contribution to mean decrease accuracy of Gini coefficient (*x*-axis) in the random forest model for discerning group difference. (**C**) Box and Whisker plots of select metabolites exhibiting systemic alterations across feces, blood sera and cerebral cortical brain tissues owing to subchronic exposure to perfluorooctanoic acid exposure, with the box ranging from the first quartile to the third while the whiskers going from each quartile to the minimum or maximum ($n=20$), *$p<0.05$, **$p<0.01$, ***$p<0.001$, two-sided Welch's *t*-test. Abbreviations: PFOA, perfluorooctanoic acid; dTDP, deoxythymidine 5'-diphosphate.

4.3.6 Integrated multi-metabolome analysis and humoral gut-brain transport

To further probe whether blood-brain exchanges or more specifically, a humoral microbiota-brain exchange was involved in effects of PFOA exposure, we focused on the 33 metabolites shared by blood sera and cerebral cortical brain (**Figure 4.2C**). We compared to check for their cross-organ concordance and to interpret potential gut microbial relevance. As shown in **Figure 4.8C**, we discovered multiple gut bacteria-derived aromatic metabolites, including indoxyl sulfate, indole-3-lactate, N-(2-phenylacetyl)glycine (PAG), and hippurate, all at decreased levels in blood sera and cortical brain of mice treated with perfluorooctanoic acid compared with the control group. Indoxyl sulfate is a known uremic toxin and cardiotoxin originated from host-microbiota metabolism of dietary tryptophan (gut bacteria-synthesized indole hepatically converted to indoxyl sulfate) (Wikoff et al., 2009). Indole-3-lactate was another bacterial indole derivative that has been shown to exhibit anti-inflammatory benefits in local gut immunity (Meng et al., 2020); their potential roles in the central nervous system, however, remain underappreciated. These were in line with our 16S rRNA sequencing data identifying markedly lower abundances of *Clostridium* spp. (for productions of indoles in general) and *Bifidobacterium* spp. (for indole-3-lactate) as the major gut microbiome composition changes induced by PFOA exposure. Likewise, N-(2-phenylacetyl)glycine, or phenylacetylglycine (PAG), is a glycine conjugate of phenylacetic acid, a phenolic metabolite of microbiota that has been confirmed in germ-free conventionalization in rats (Nicholls, Mortishire-Smith, & Nicholson, 2003). Hippurate was a host-microbe phenolic co-metabolite that has been shown to correlate with gut microbiome diversity (Pallister et al., 2017) and metabolic health status. Unexpected, we found for indoxyl sulfate, indole-3-lactate, and PAG same trends of change in blood and cortical brain of germ-free mice (compared with conventionally raised controls) in Chapter 3. This further supports that the elimination or

perturbation of gut bacteria, either constitutional (germ-free mice) or induced (e.g., by PFOA exposure), can lead to diminished gut luminal profiles of neuroactive metabolites with direct impacts on the central nervous system through active humoral gut-blood-brain exchanges. Other potential humoral microbiota-brain pathways involved in health effects of PFOA spanned a diverse panel of host-microbe co-metabolites, as exemplified by downregulated serum and brain levels of vitamin B_6 (including pyridoxine and 4-pyridoxic acid), in parallel with elevated levels of vitamin B_2 (riboflavin), polyamines (e.g., putrescine), sphingolipids (e.g., sphingosine (d18:1)), and purine nucleoside derivatives (e.g., hypoxanthine) (**Figure 4.8C**). Note that one recent work (Shi et al., 2020), in much consistency with our profiling observations, has reported that exposure to PFOA induced cognitive deficits, altered gut microbial composition, and caused inflammation in gut and brain. Our high-coverage metabolome-wide multicompartmental characterization advanced beyond these toxicological phenotypic observations by delineating the molecular cues of gut bacterial influences through a humoral gut-brain route.

4.4 Conclusion

In conclusion, through our multi-omics profiling and cross-compartmental comparison, we yielded the first evidence in support of a shifted humoral microbiota-brain crosstalk in mice as induced by PFOA exposure. The massive, novel, and highly informative datasets generated in this work may provide fundamental insights into how gut microbiota interacts with host bodies via the gut-brain axis and lay the foundation for incorporating microbiota into neurotoxicity assessment and for development of biomarkers and countermeasures against risks induced by environmental chemical exposures.

CHAPTER 5: DISCOVERING NEUROACTIVE METABOLOME CHANGES IN MICE OWING TO DIETARY ADMINISTRATION OF BLACK RASPBERRY AND POTENTIAL ROLES OF MICROBIOTA-BRAIN AXIS[5]

5.1 Introduction

Given the multi-omics discoveries in Chapter 3 and 4 that supported a humoral route of the microbiota-brain axis, further questions point to opportunities for harnessing such host-microbe interaction for improved neurologic and/or mental health outcomes. Currently, however, simple, straightforward, and selective approaches to such intervention are underdeveloped and/or not adequately assessed, partially due to lack of understanding of the molecular details underlying the microbiota-gut-brain axis. In line with Chapter 3 (GF vs. CONV-R mice, at steady states) and Chapter 4 (CONV-R mice, under PFOA exposure), we provided high-coverage metabolome-wide data that first indicated microbial influences through humoral gut-brain routes at both steady states and under neurotoxicant-challenged conditions. Here in Chapter 5, we take turns to probe likely metabolome changes owing to neuroprotective intervention approaches.

In a recent seminal study, Bravo and colleagues demonstrated that administrating with the *Lactobacillus rhamnosus* strain improved emotional behaviors in mice, likely through altering the neurotransmission in the central GABAergic system via the vagus nerve (Bravo et al., 2011). This not only sheds lights on previously reported health benefits of probiotics (e.g., yogurt) that contains high abundances of the *Lactobacillus* family bacteria but points to a potential prospect of using

[5] Chapter 5 was reproduced from my manuscript - Lai Y., Tu P., Yang Y., Ru H., Lu K.*: Discovering metabolome changes of neuroprotection from dietary administration of black raspberry in mice, ***J. Agric. Food Chem.*** (in preparation).

dietary intervention for inducing neuroprotective effects from gut microbiota. One notable dietary intervention option is the polyphenol-rich berry fruits (e.g., black raspberry) with demonstrated antioxidant activities spanning free radical scavenging, anti-glycation, and anti-β-amyloid aggregation (Bieri, 1980; Ma et al., 2018; Oghumu et al., 2017). We have recently tested black raspberry (BRB)-rich diet in mouse models to examine diet-microbiota interaction, detailing BRB-rich diet-driven gut microbial compositional and functional changes including enrichment of beneficial gut bacteria (e.g., *Akkermansia muciniphila*), altered vitamin biosynthesis and aromatic amino acid metabolism, as well as ameliorated oxidative stress (Tu et al., 2018). However, whether and how a BRB-reshaped microbiota harbors neuroprotective potential that extends from gut to the brain remains unexplored.

In Chapter 5, we use the global metabolomics methods and annotation pipelines developed to perform a multicompartmental examination of metabolome changes in mice fed with BRB-rich diet, with a secondary, derivative goal to compare and probe a potential role of humoral microbiota-brain axis. The rationale of this chapter is that the implementation of this aim will provide novel proof-of-principle for leveraging dietary intervention towards desired gut microbial changes that harbor neuroprotective potentials while yielding novel datasets that inform future use of berry fruits for improved health outcomes.

5.2. Materials and Methods

5.2.1 Chemicals and reagents

LC/MS-grade solvents and reagents (Optima^{TM}) including methanol (MeOH), acetonitrile (ACN), formic acid, and water were purchased from Fisher Scientific (Waltham, WA, USA). For quality control purposes, eight stable-isotope-labeling (SIL) chemical standards of common

tryptophan catabolites and neurotransmitters were procured and spiked into extracting solvents for tracking of the whole metabolite extraction procedures. These standards included L-kynurenine (ring-d4, 3,3-d2) (d6-Kyn) which was purchased from Cambridge Isotopes (Tewksbury, MS, USA), as well as acetylcholine-d13 (N,N,N-trimethyl-d9; 1,1,2,2-d4) (d13-ACh), γ-aminobutyric acid-d2 (d2-GABA), L-glutamine-2,3,3,4,4-d5 (d5-Gln), indole-3-acetic-2,2-d2 acid (d2-IAA), indole-3-propionic-2,2-d2 acid (d2-IPA), serotonin-α,α,β,β-d4 (d4-5HT), and L-tryptophan-2,3,3-d3 (d3-Trp) that were obtained from the CDN Isotopes Company (Pointe-Claire, Quebec, Canada).

5.2.2 Animals and BRB diet preparation

Specific-pathogen-free (SPF) C57BL/6 mice of ~8 weeks old were procured from the Jackson Laboratory (Bar harbor, ME, USA) and housed for one week at the animal facility of the University of North Carolina (UNC) feeding *ad libitum* on tap water and standard Prolab RHM 3000 pelleted diet (St. Louis, MO, USA) for acclimation purposes before the dietary administration scheme started. Procedures for preparing the BRB diet were based on a previous work with slight modifications (Oghumu et al., 2017). In brief, whole ripe black raspberry fruits (*Rubus occidentalis*) of the Jewel Variety were freeze-dried, grounded into powder, and stored at -20 ºC until use. Upon use, the BRB powder were homogenized with the standard custom purified American Institute of Nutrition (AIN)-76A animal diet pellets (Dyets, Inc., Bethlehem, PA) (protein, 20.8 kcal %; carbohydrates, 67.7 kcal %; and fat, 11.5 kcal %) (Bieri, 1980) by a ratio of 1:9 (*w/w*) at the expense of cornstarch to make the BRB-rich diet; the prepared BRB diet was stored at 4 ºC before feeding to the mice. At week 9, a total of 20 acclimated female SPF C57BL/6 mice were randomly assigned into two groups (n=10 per group) feeding on the standard AIN-76A diet and BRB-rich diet for 7 seven weeks, respectively. During the dietary treatment, all mice were housed with tap

water provided *ad libitum* under the condition of 22 ºC, 40-70% humidity, and a daily 12:12 hour light-dark cycle. At the end of the 7-week dietary administration, samples including feces, blood sera and whole brain were collected, snap-frozen, and stored under -80 ºC for further analyses. Throughout the experiment, we treated all animals humanely and followed protocols approved by the UNC Institutional Animal Care & Use Committee (IACUC).

5.2.3 Sample preparation

Metabolites were extracted from fecal pellets, blood sera, and cerebral cortical brain slices for acquiring a multi-compartmental snapshot of novel metabolome changes owing to BRB dietary treatment. In brief, ~20 mg feces, 20 µL sera, and ~20 mg cerebral cortical brain tissues were aliquoted for use. The feces, mixed with ~10 mg acid-washed glass beads (Sigma-Aldrich, St Louis, MO), were extracted by adding 300 µL of ice-cold SIL standard-spiked MeOH:water (50:50, *v/v*) and homogenization with a TissueLyzer (Qiagen, Hilden, Germany) at 50 Hz for 10 min. The samples were then centrifuged at 12,000×*g* for 10 min (Eppendorf, Hamburg, Germany) and 100 µL supernatant was transferred and dried in a CentriVap vacuum evaporator (Labconco, MO, USA). The 20 µL sera was added with 180 µL ice-cold SIL ISD-spiked MeOH, briefly vortexed, and incubated at -20 ºC for 30 min to allow for sufficient protein precipitation. The serum samples were centrifuged at 15,000×*g* for 10 min to precipitate particulates and proteins, and 100 µL supernatant were transferred to a CentriVap vacuum concentrator for dryness. For 20 mg brain tissue aliquots, 400 µL ice-cold ISD-spiked MeOH and a clean stainless-steel bead (5 mm i.d., Qiagen, Hilden, Germany) were added. The brain samples were homogenized on a TissueLyzer for 2 min at 50 Hz, incubated at -20 ºC for one hour, and centrifuged at 18,000×*g* for 10 min; 100 µL pf the resultant supernatant was concentrated to dryness. All dried extracts were cap-closed

and stored under -80 ºC before use. Upon instrumental analysis, all dried extracts were reconstituted in ACN:water (2:98, *v/v*) for injection.

5.2.4 Instrumental analysis

High-resolution accurate-mass (HRAM) liquid chromatography-mass spectrometry (LC-MS) analysis was performed using a Thermo Fisher Scientific Vanquish UHPLC coupled to a *Q Exactive* mass spectrometer equipped with a heated electrospray ionization (HESI) source and a hybrid quadrupole-orbitrap mass analyzer (Waltham, MA, USA). The injected sample extracts were first chromatographically separated on a Waters Acquity UPLC HSS T3 column (100Å, 1.8 μm, 2.1 mm × 100 mm) (Milford, MA, USA); the mobile phases consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid, flowing at 0.4 mL/min, 40 ºC with the following LC gradient: 2% B, 0-1 min; 2% to 15% B, 1-3 min; 15% to 50% B, 3-6 min; 50% to 98% B, 6-7.5 min; 98% B, 7.5-11.5 min; 98% to 2% B, 11.5-11.6 min; 2% B, 11.6-15 min.

Both positive and negative HESI data were acquired using the Thermo XCalibur 4.0 software (Waltham, WA, USA) under the following ion source condition: sheath gas 60 L/min, sweep gas 1 L/min, auxiliary gas 10 L/min, capillary 325 ºC, auxiliary gas heater 400 ºC, and spray voltage 2.75 kV. Our metabolomics analyses consisted of two stages, including (i) MS1 fullscan profiling (orbitrap only) to assess group difference and screening of significant ion features and (ii) tandem mass spectral analysis for structural elucidation. For MS1 data, the mass spectrometer was operated with mass resolution 70,000 full width at half height (FWHM) scanning a mass range of 80-1,000 Da. For MS/MS analysis (quadrupole-orbitrap running), the instrument was running in hybrid fullscan MS1 (FWHM 70,000; AGC target 3e6; max IT 200 ms) alternating with parallel reaction monitoring (PRM) MS/MS modes; stepped normalized collision energies were set at 10,

50 and 100 (FWHM 17,500; AGC target 2e5; max IT 50 ms). Throughout the analysis, stringent quality assurance/quality control (*QA/QC*) were performed, including timely mass calibration, solvent blank injection, method blank injection, sample randomization, intermittent QC (pooled extracts), and regular monitor of ISD variations across samples and injections.

5.2.5 Metabolomics data processing and statistical analyses

Raw MS1 fullscan profile data in *.raw format was sequentially converted to *.mzXML files and *.abf files using the ProteoWizard MS Convert Program (Palo Alto, CA, USA) and Reifycs Abf Converter (Riken, Yokohama, Japan), respectively. The *.abf files were processed in MS-DIAL 4.60 Program (Riken, Yokohama, Japan) using the following key settings: (1) Mode: ddMSMS; (2) Data collection parameters: Retention Range: 0-15 min; Mass Range: 0-1,000 Da; (3) Centroid parameters: MS1 tolerance, 0.01, MS2 tolerance 0.025 (set but not applied in this case); (4) Isotope recognition: Maximum charged number, 2; (5) Peak detection parameters: Smoothing method, linear weighted moving average; Smoothing level, 3; Minimum peak width, 5; Minimum peak height, 1,000. (6) Peak spotting parameters: Mass slice width, 0.05; (7) Alignment: Retention time tolerance, 0.05; MS1 tolerance, 0.015; Retention factor, 0.5; MS1 factor, 0.5; Peak counter filter, 17; N% detected in at least one group, 80. As a result, six master ion feature alignment tables were yielded from the combinations of three sample types (feces, sera, and brain) and two HESI modes (positive & negative).

Statistical analyses were performed in R (R Core Team, 2020) to assess group metabolomic pattern, screen for significant ion features, and to interpret annotated metabolites in chemical and biological pathway contexts. Specifically, principal component analysis was performed with score and scree plots generated using the *ggpubr*, *ggplot2*, *FactoMineR, factoextra* packages of R.

Significant ion features were determined based on two-sided Welch's *t*-test using R packages including *tidyverse*, *purrr*, and *qvalues*. MetaMapp metabolomic networks were constructed through computing reactant pairs from Kyoto Encyclopedia of Genes and Genomes (KEGG) (biological pathway relevance) and Tanimoto coefficients (>0.7, chemical structural similarity) which were further visualized in CytoScape 3.8.0 (Seattle, WA, USA). Chemical similarity enrichment (ChemRICH) plots were generated using the Kolmogorov-Smirnov test to cluster altered metabolites by chemical class (Davis, CA, USA). Quantitative metabolite set enrichment analysis (qMSEA) in MetaboAnalyst 5.0 (Edmonton, Alberta, Canada) using 99 *a priori* defined metabolites sets from the Small Molecule Pathway Database (SMPDB). Variable importance plots were created to rank individual metabolites using the *randomForest* package of R.

5.2.6 Compound annotation

Compound annotation was conducted using an integrated cheminformatic pipeline combining conventional reference mass spectral search and *in silico* de novo cheminformatic prediction. The reference search used an in-house spectral library of 423 authentic chemicals standards of canonical pathway metabolites, neurotransmitters, and literature-cataloged microbial byproducts, against which the unknown data was matched by known MS1 accurate mass, MS2 characteristic ion fragments, and chromatographic retention time. Meanwhile, untargeted *in silico* prediction, from formula determination to structural dereplication, was implemented in MS-FINDER 3.42, using accurate mass, isotopic ratios, and tandem mass spectra as the input. Top ranked candidates were sent to post curation procedures based on the overall identification score (>3.5), mass accuracy confirmation (<10ppm), and retention time predicted from random forest modeling of chromatographic retention-lipophilicity relationships. All resultant annotations were

manually inspected through integrated strategies including but not limited to extensive search of publicly available experimental spectral databases such as Metlin (https://metlin.scripps.edu/), MassBank of North America (https://mona.fiehnlab.ucdavis.edu/), GNPS (https://gnps.ucsd.edu/), MassBank (http://www.massbank.jp/), and mzCloud (https://www.mzcloud.org/), as well as chemical inferences and biological plausibility/relevance. Confidence levels were assigned to each individual annotated compound according to the reporting standard set by the Metabolomics Standard Initiative; only level 1 & 2 annotations were kept for further analysis and interpretation.

5.3 Results and Discussion

5.3.1 Global metabolomics of feces, blood sera, and cerebral cortical brain: an overview

For this study, data processing procedures through MS-DIAL yielded six master alignment tables of ion features that allowed for statistical screening of ion features of BRB/AIN-76A difference. Based on two-sided Welch's *t*-test, we detected 5,032 fecal HESI+ ion features (out of 17,908), 4,799 fecal HESI- ion features (out of 17,558), 1,511 serum HESI+ ion features (out of 13,677), 2,296 serum HESI- ion features (out of 14,240), 2,238 brain HESI+ ion features (out of 8,524), and 608 brain HESI- ion features (out of 10,300) that are statistically significant (fold change$\geq$1.5 and $p<0.01$). Since metabolites commonly interacts in network fluxes in which each may not be treated as independent variables, we loosened up the significance threshold (fold change$\geq$1.2 and $p<0.05$) and collected tandem mass spectra in MS1-MS2 hybrid mode targeting them all for each of the three sample types and ESI modes, totaling 20,535 spectra in *.mat format through the MS-DIAL 4.60 computational end. These spectra were batched processed in MS-FINDER 3.42 and cleaned up using an integrated approach detailed in (**Supplementary Figure 3.1**, Chapter 3). In parallel, reference mass spectral library was used against which unknown mass

spectra data was matched for hits. Only Level 1 and Level 2 annotations were kept for downstream analysis and interpretation, resulting in a total of 285 fecal metabolites (**Supplementary Table 5.1**), 257 serum metabolites (**Supplementary Table 5.2**), and 73 cortical brain metabolites (**Supplementary Table 5.3**) of AIN-76A/BRB difference.

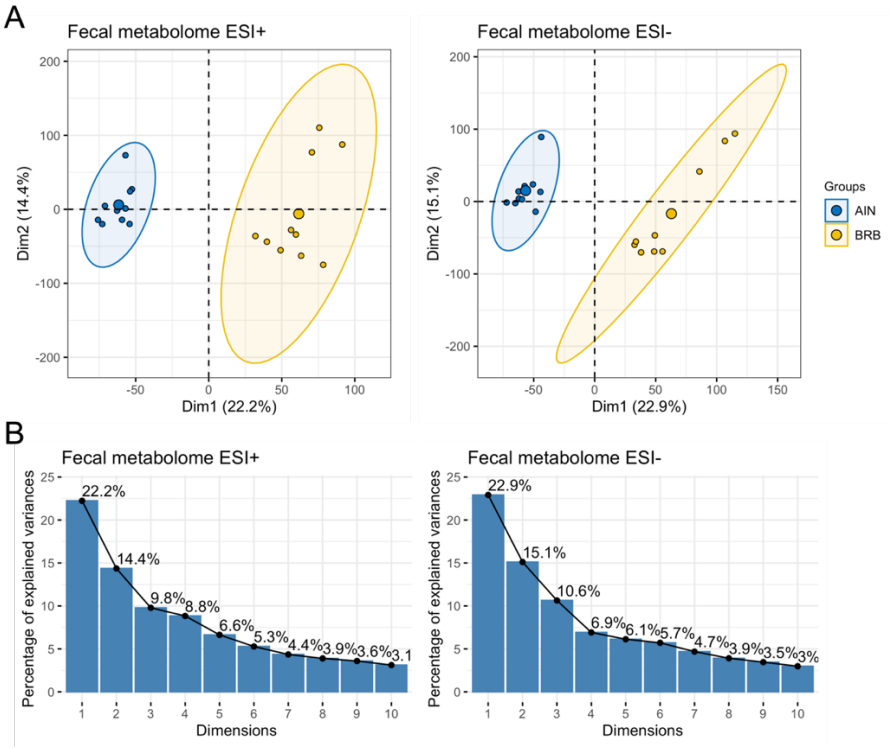


Figure 5.1 Distinct fecal metabolomic pattern of BRB-rich diet (*n*=10) vs. AIN-76A diet (*n*=10), as revealed by (**A**) score plots and (**B**) scree plots from principal component analysis (PCA) of peak area profiles of all 17,908 and 17,558 ion features aligned respectively from HESI+ and HESI- mass spectral data of fecal extracts.

5.3.2 Effects of BRB-rich diet on fecal metabolome *in vivo*

As shown in **Figure 5.1**, principal component analyses of all aligned ion features for fecal data, either HESI+ or HESI-, revealed distinct fecal metabolomic patterns between BRB-rich diet and the standard AIN-76A diet (**Figure 5.1A**) with scree components detailed in **Figure 5.1B**. **Figure 5.2** illustrates the chemical similarity enrichment analysis (ChemRICH) for clustering of the 285 annotated fecal metabolites (**Supplementary Table 5.4**). Thirty-seven chemical sets were

enriched (adjusted $p<0.05$), covering a wide lipophilicity range with varied compound numbers (node size) and overall trends of change (node color). The most significantly enriched were catecholamines, dipeptides, dicarboxylic acids, cinnamates, phenols, indoles, flavonoids, and hippurates, followed by amino acids (e.g., aromatic) and lipids (e.g., cholic acid, carnitines, prostaglandins). The highly enriched catecholamines and flavonoids suggested that BRB-rich diet effectively modulated gut local metabolism of neurotransmitters and polyphenol compositional profiles, while the significance of indoles, phenols, and other aromatic amino acid derivatives suggested an altered microbiota-metabolome network.

To interpret the altered fecal metabolome in biological contexts, we moved beyond chemical class modules to conduct pathway sets enrichment analysis (qMSEA) based on 99 *a priori* metabolite sets in MetaboAnalyst 5.0 (Montreal, QC, Canada). A total of 57 pathways were significantly perturbed (adjusted p-value<0.05) (**Supplementary Table 5.5**), with top 25 shown in **Figure 5.3**. Most enriched pathways involved nicotinate and nicotinamide metabolism, aromatic amino acid metabolism (e.g., tyrosine metabolism, phenylacetate metabolism, tryptophan metabolism), inositol (phosphate) metabolism, and catecholamine biosynthesis. Overall, canonical pathways of sugars, lipids, and amino acids were extensively perturbed, with multiple pathways of neurotransmitters.

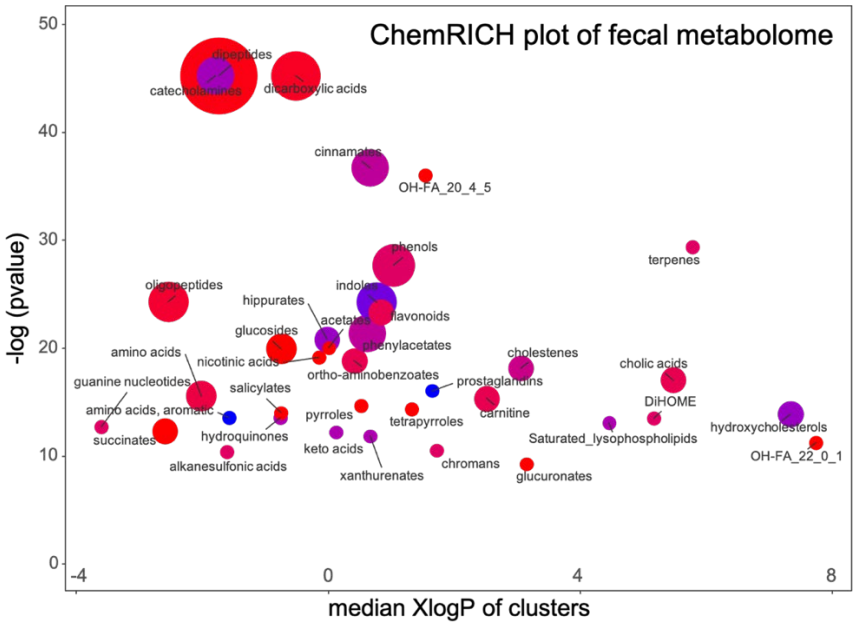


Figure 5.2 Chemical similarity enrichment analysis (ChemRICH) clustering of 285 altered fecal metabolites by structural similarity with *x*-axis of median logarithmic additive octanol-water partition coefficients (XlogP) and *y*-axis for sets statistical significance based on the Kolmogorov-Smirnov test; the node size depicted total compound numbers for each cluster set and node color continuously scale the relative proportion of BRB enriched (red) vs. AIN-76A enriched (blue) metabolites.

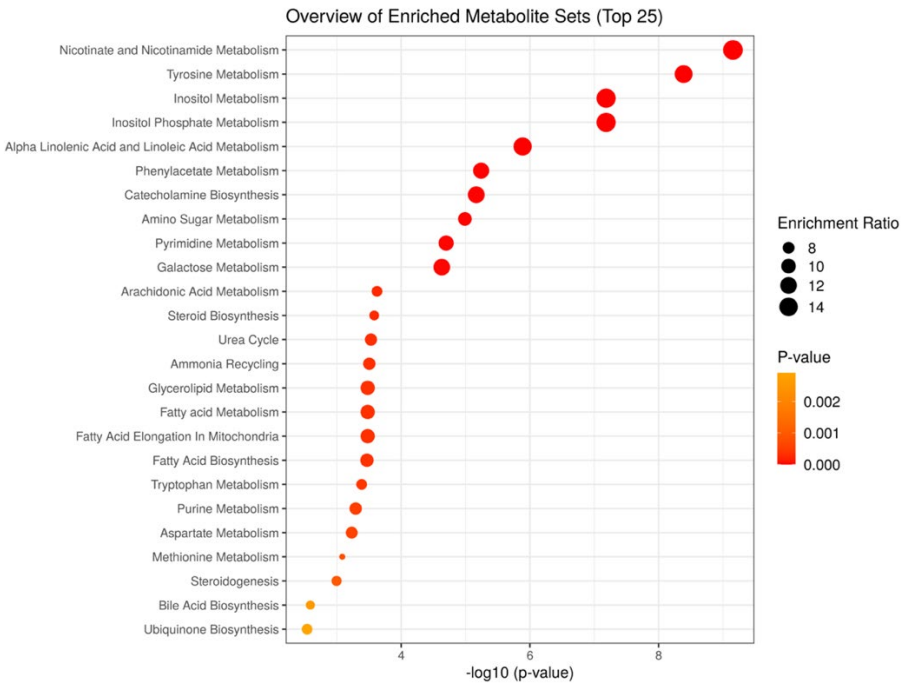


Figure 5.3 Altered fecal pathways (with top 25 shown) as determined by quantitative metabolite set enrichment analysis (qMSEA) based on 99 *a priori* defined sets of metabolites of the SPMDB databases in MetaboAnalyst 5.0 (Montreal, QC, Canada) (adjusted p<0.05).

We turned to individual fecal metabolites and select pathways for in-depth analyses and interpretation. Random forest classification was performed on all altered individual fecal metabolites (*n*=285) which were ranked by their relative contribution to group separation, with top 50 shown in the variable importance plot (VIP) (**Figure 5.4**). Most of the top ranked members were BRB diet-enriched (35/50) and structurally diverse, suggesting a complex and multifaceted nature of fecal metabolome changes induced by the BRB-rich diet compared with the standard AIN-76A diet. Specifically, these involved an array of neurotransmitters or neuroactive compounds including cyclic melatonin (ranked 2nd), (R)-N-methylsalsolinol (ranked 7th), norepinephrine sulfate (ranked 8th), dihydroneopterin phosphate (i.e., 7,8-dihydroneopterin 3'-phosphate), and 5-hydroxyindoleacetic acid (5-HIAA, ranked 21st). Meanwhile, multiple microbiota-related metabolites were spotted in the VIP plot, including *p*-cresol (phenylalanine and

tyrosine catabolites by anaerobic gut bacteria), lithocholate 3-O-glucuronide (secondary bile acid), and 5β-cyprinol sulfate (a bile acid intermediate). Ellagic acid, ranked 12th, is a polyphenol flavonoid (Häkkinen, Kärenlampi, Mykkänen, Heinonen, & Törrönen, 2000) with elevated levels in BRB than in the AIN-76A diet group; differentiated profiles of polyphenols are shown in a heatmap (**Figure 5.5A**), suggesting food-derived polyphenols as a potential player in the neuroactive modulation of local homeostasis and signaling in the GI tract. To move beyond pathways and sources, we conducted extensive literature search for individual neuroactive markers of BRB/AIN-76A difference to probe specific health effects of the BRB-rich diet. Among the unexpectedly discovered, catecholamine metabolites stood out, with increased levels of L-3,4-dihydrophenylalanine **(**L-DOPA) and dopamine (**Figure 5.5B**) in BRB diet and decreased levels of neurotoxic catecholamine derivatives including normetanephrine (a tumor biomarker) (Eisenhofer et al., 2005), (R)-N-methylsalsolinol (a dopaminergic neurotoxin) (Maruyama, Abe, Tohgi, Dostert, & Naoi, 1996), and homovanilic acid, a potentially toxic compound with cerebrospinal fluid levels associated with neurodegenerative conditions such as schizophrenia (Kasa et al., 1982) (**Figure 5.5C**). Such alterations in catecholamine metabolism suggested beneficial effects of BRB-rich diet for neuroprotection.

Together, the fecal metabolomic data indicated that, at least locally in the GI tract, dietary intake of BRB components effectively modulated the microbiota-metabolome network with altered neuroactive signaling profiles and potential health impacts which merit in-depth characterization and investigation.

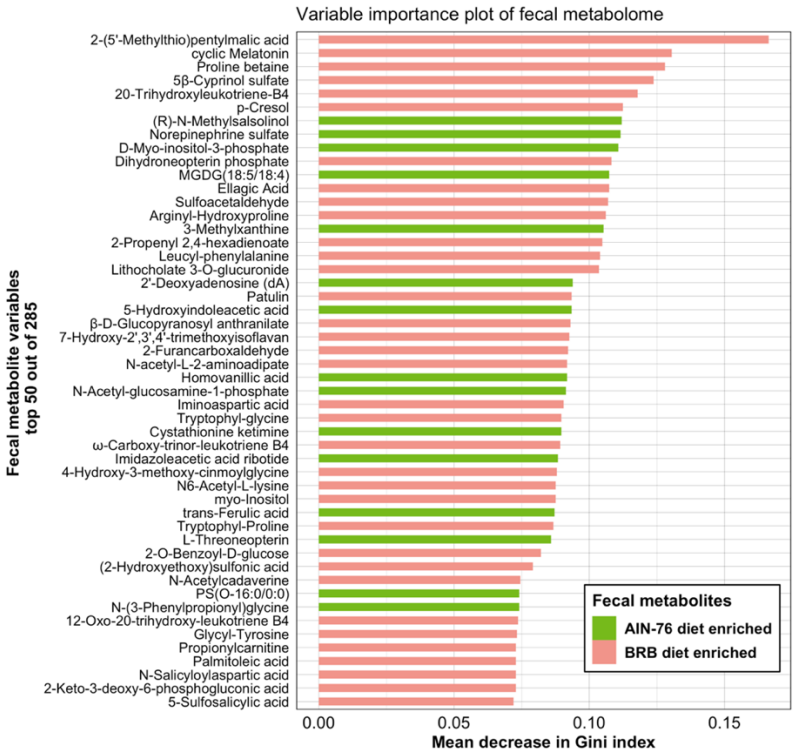


Figure 5.4 Variable importance plot based on random forest classification of 285 annotated fecal metabolites as ranked by relative contribution to mean decrease accuracy of Gini coefficient (*x*-axis) with top 50 fecal metabolites selectively shown (*y*-axis).

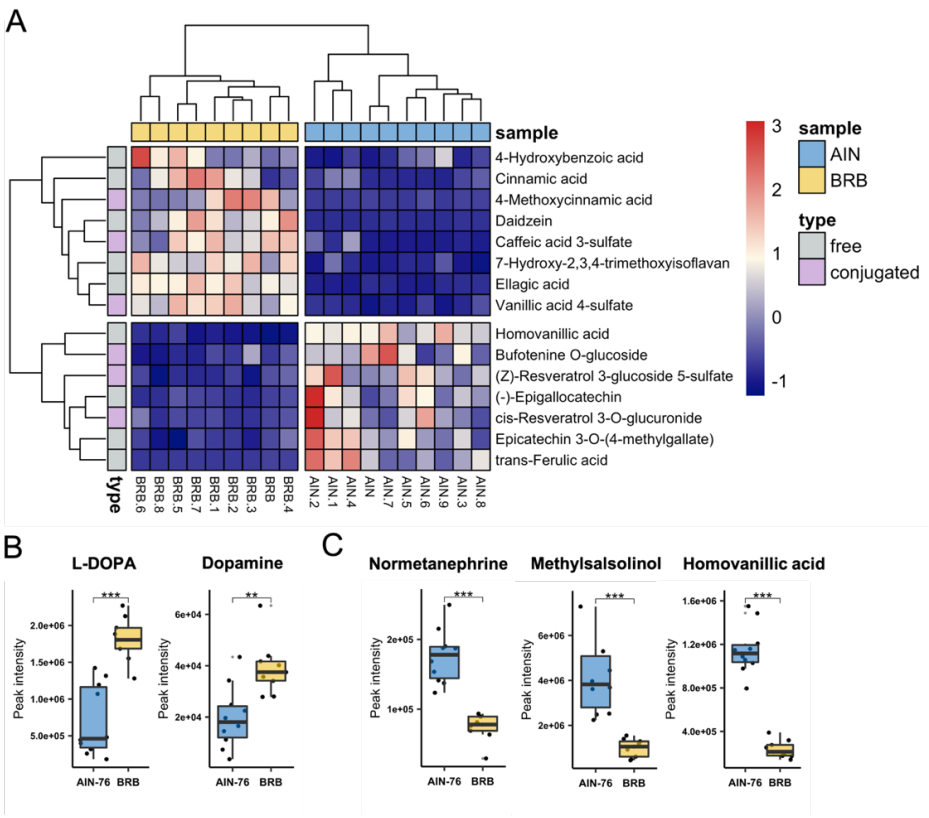


Figure 5.5 Altered fecal profiles of neuroactive compounds as induced by BRB-rich diet administration: a selective view. (**A**) heatmap of common food-derived polyphenol components with documented antioxidant properties; **(B-C)** Box and whisker's plot of altered catecholamine metabolites, including BRB diet-enriched neuroprotective L-3,4-dihydrophenylalanine (L-DOPA) and dopamine (B), and downstream dopamine derivatives with potential harmful effects that were decreased in the BRB diet group as compared with the standard AIN-76A diet (C), including normetanephrine (a tumor biomarker), (R)-N-methylsalsolinol (a dopaminergic neurotoxin), and homovanilic acid, a potentially toxic compound that has been associated with neurodegenerative conditions such as schizophrenia and narcolepsy; the box ranged from the first quartile to the third while the whiskers spanned each quartile to the minimum or maximum (n=20), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, two-sided Welch's t-test.

5.3.3. Effects of BRB-rich diet on serum metabolome *in vivo*

Given the effects of BRB diet on fecal metabolome, neurotransmitters, and microbiota-mediated aromatic amino acids in particular, question arises as to whether administrating BRB-rich diet exerts systemic effects, specifically, on circulating blood metabolome. In total, we resolved structures for 257 serum metabolites of BRB/AIN-76A difference (fold change≥1.2 and p<0.05) level 1 and level 2 confidence of identification. Through qMSEA analysis of these altered

metabolites, a total of 51 biological pathways were significantly enriched (adjusted p-value<0.05) (**Figure 5.6**; **Supplementary Table 5.6**). The most significantly enriched pathways involved cellular reduction-oxidation (redox) homeostasis (e.g., pentose phosphate pathway), energy metabolism (e.g., α-linolenic acid & linoleic acid metabolism, mitochondrial fatty acid β-oxidation, carnitine synthesis), as well as aromatic amino acid metabolism (e.g., tryptophan metabolism, tyrosine metabolism).

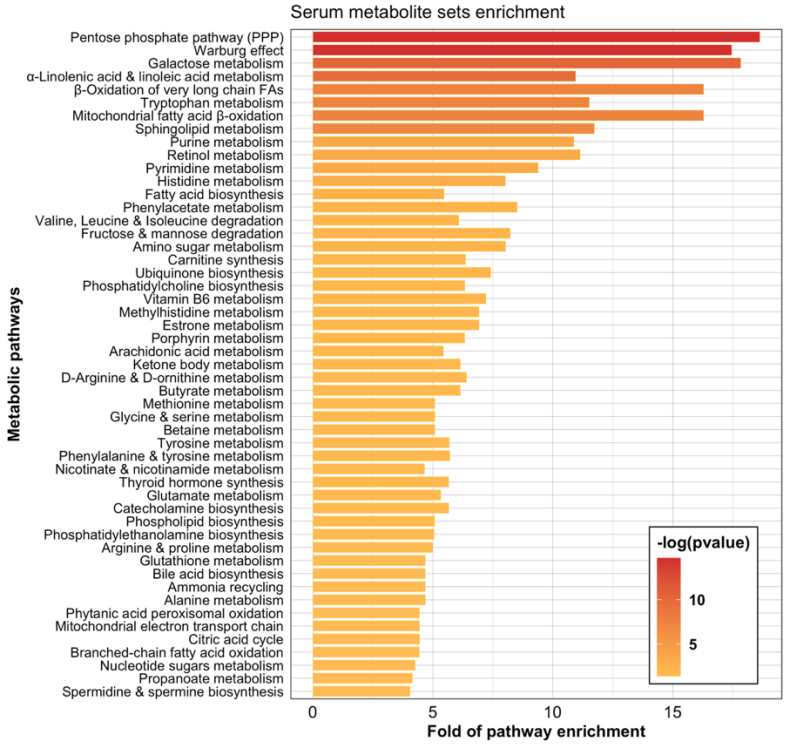


Figure 5.6 Altered serum pathways (with top 50 shown) as determined by quantitative metabolite set enrichment analysis (qMSEA) based on 99 *a priori* defined sets of metabolites of the SPMDB databases in MetaboAnalyst 5.0 (Montreal, QC, Canada) (adjusted p<0.05).

On the individual metabolite level, random forest classification was performed, with top 50 ranked metabolite variables plotted in the VIP graph (**Figure 5.7**). Similar to fecal metabolites, most of the top-ranked members were BRB diet-enriched (39/50), with chemical classes spanning phenols, indoles, fatty acids, and nucleotides. Specifically, top-ranked phenolic compounds

spanning O-methoxycatechol-*O*-sulfate (ranked 1st), 4-ethylphenol (ranked 2nd), 4-hydroxybenzoic acid (ranked 17th), and vanillic acid 4-sulfate (ranked 19th), and pyrocatechol sulfate (ranked 50th) were discovered to all embrace increased levels in BRB-treated groups as compared with AIN-76A controls, suggesting systemic effects of dietary intake of polyphenol-rich food (in this case, BRB-rich diet) on blood circulation. Of note, 4-ethylphenol not only has its dietary sources, but also is a known tyrosine metabolite synthesized by bacteria (Bone et al., 1976; Nicholson et al., 2012). In parallel, multiple tryptophan catabolites that were linked to microbiota while exhibiting neuroactive potentials were highly ranked as well. These included anthranilic acid, hippuric acid, and indole-3-acrylic acid. Meanwhile, we also spotted a range of lipid molecules, spanning phospholipids (e.g., LysoPC(20:5), LysoPC(22:6), LysoPC(P-16:0), and LysoPC(20:3)), fatty acids including the short-chain caproic acid and long-chain polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), as well as oxylipins (e.g., 13(*S*)-HODE, 9(*S*)-HODE). Some of these lipid molecules are essential (e.g., DHA and EPA) to the host body with demonstrated protective roles as an antioxidant / anti-inflammatory agent or as potent ligands for triggering profound anti-inflammatory activities through binding to, for example, the peroxisome proliferator activated receptor alpha (PPAR-α) (Yum, Na, & Surh, 2016; Zúñiga et al., 2011). While others (e.g., 13(*S*)-HODE, 9(*S*)-HODE) are known markers for elevated level of oxidative stress (Vangaveti, Baune, & Kennedy, 2010). Overall, the serum metabolomics results served as proof-of-principle that BRB dietary intake can exert systemic effects on blood circulation but with multi-faceted health effects.

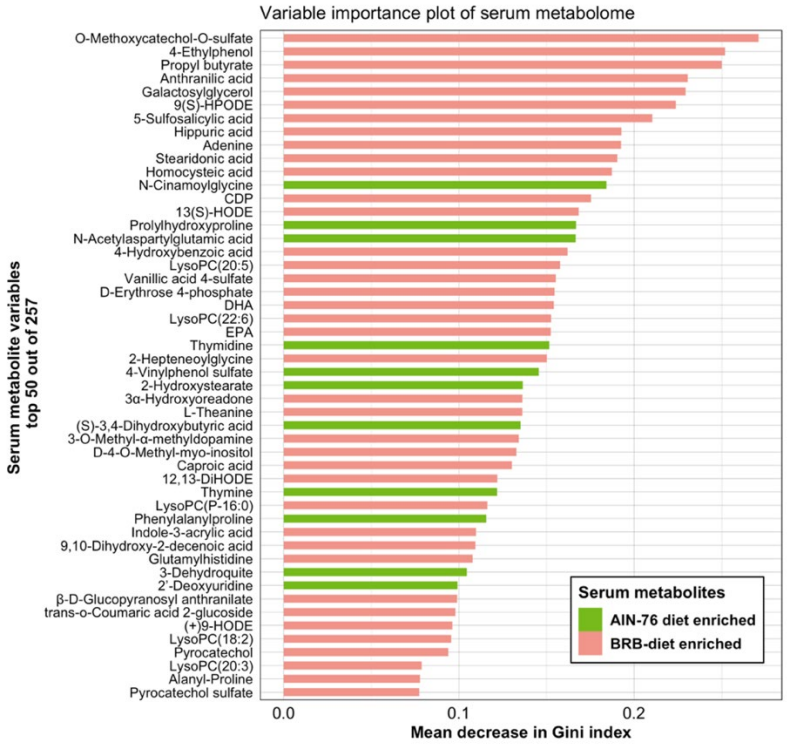


Figure 5.7 Variable importance plot from random forest classification of 257 annotated serum metabolites as ranked by relative contribution to mean decrease accuracy of Gini coefficient (*x*-axis) with top 50 serum metabolites selectively shown (*y*-axis).

5.3.4. Comparative view of altered metabolites shared by fecal and serum metabolomes

With the detailing of fecal and serum metabolome changes owing to BRB dietary intake, question remains to what extent their local effects in the GI tract, especially those related to gut microbiota, can propagate from gut to blood circulation and peripheral organs. Here, we comparatively examined altered fecal and serum metabolomes for overlapping pathways and metabolites. Venn diagrams showed 35 overlapping pathways (**Figure 5.8A**; **Supplementary Table 5.7**) and 36 overlapping metabolites (**Figure 5.8B**; **Supplementary Table 5.8**) that were altered in both feces and serum. Notable shared pathways with relevance to neuroactive potential and gut microbial mediation included aromatic amino acid metabolism (e.g., tryptophan metabolism, phenylalanine & tyrosine metabolism), catecholamine biosynthesis, phenylacetate

metabolism, glutamate metabolism, and arginine and proline metabolism, etc. (**Supplementary Table 5.8**). From the 36 overlapping metabolites (**Supplementary Table 5.8**), trends of change (i.e., AIN-76A>BRB or the opposite) are in concordance for 21 between both matrices while the rest 15 showed an opposite trend. Strikingly, we discovered for tryptophan metabolism similar pattern in feces and sera for both kynurenine (e.g., anthranilic acid) and serotonin (e.g., 5-hydroxyindoleacetic acid) branches but interestingly, not the gut microbiota-mediated indole biosynthesis (**Figure 5.8C**). Both indole-3-propionic acid (IPA) and indole-3-carboxyladehyde (I3A) had decreased levels in feces of BRB-fed mice than in the standard AIN-76A fed mice, while embracing an increased trend in blood sera (**Figure 5.8C**). Note that IPA and I3A, both gut bacteria-derived (from *Clostridium* spp. and *Lactobacillus* spp., respectively), are potent ligands to the aryl hydrocarbon receptors and antioxidants with neuroprotective effects (Alexeev et al., 2018; Swimm et al., 2018). Like tryptophan-indole metabolism, same opposite trends of change between feces and serum were also observed for phenylalanine metabolism, for which L-phenylalanine and its bacterial metabolites hydrophenyllactic acid, a natural antioxidant that can decrease reactive oxidative species (ROS) (Beloborodova et al., 2012), were decreased in feces of mice fed with BRB-rich diet while both increased in blood sera (**Figure 5.8D**). In line with increased serum levels of oxylipins (e.g., 13(S)-HODE, 9(S)-HODE) and ω-3 fatty acids (e.g., DHA and EPA), the seemingly "active transport" of gut bacterial indoles and phenolic derivatives of neuroprotection from gut into circulating blood indicated an active role of gut microbiota in modulating systemic oxidative stress, although the molecular underpinnings require much elucidation in future work.

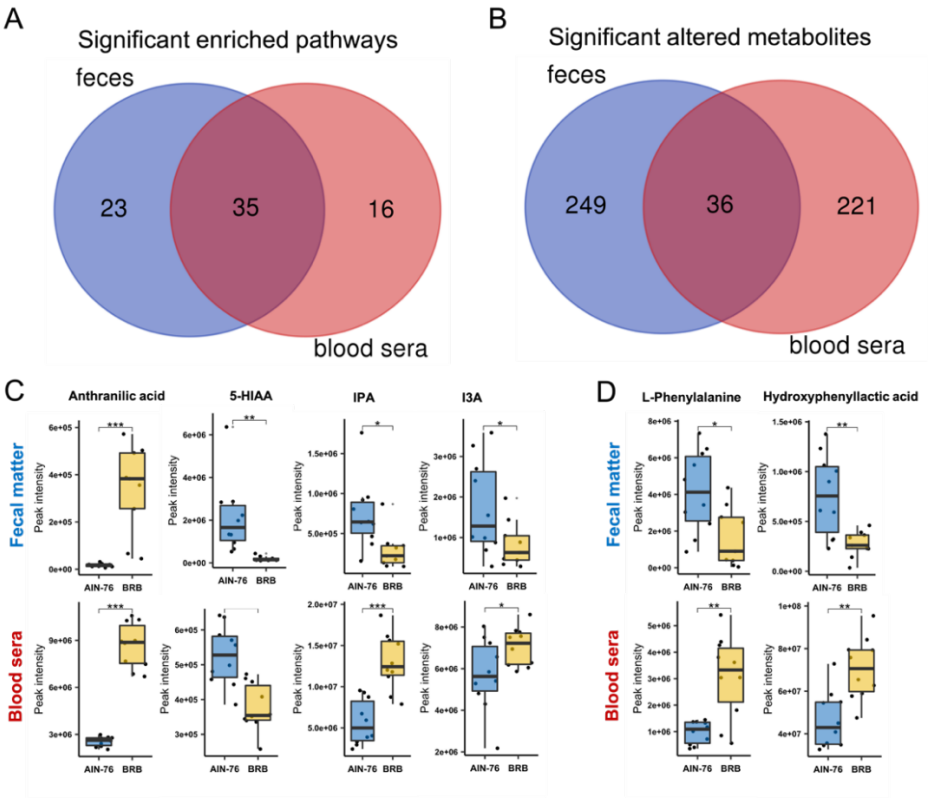


Figure 5.8 Comparative view of shared altered compounds in feces and blood sera owing to BRB-rich dietary administration. (**A**) Venn diagram of significantly enriched pathways in feces and blood sera; (**B**) Venn diagram of significantly altered metabolites in feces and blood sera; (**C-D**) Box and whisker plots of aromatic phenolic compounds altered in both matrices including tryptophan metabolites (C) or phenylalanine metabolites (D), with the box ranging from the first quartile to the third while the whiskers spanning each quartile to the minimum or maximum (n=20), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, two-sided Welch's t-test. Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; IPA, indole-3-propionic acid; I3A, indole-3-carboxaldehyde.

5.3.5. Effects of BRB-rich diet on cerebral cortical brain metabolome

In total, we resolved 73 metabolites of BRB/AIN-76A difference in cerebral brain tissues at annotation confidence level 1 & 2. To gain a network view, a MetaMapp approach was used connecting all metabolites into clusters based on biological pathways (Kyoto Encyclopedia of Genes and Genomes, KEGG reactant pairs) and chemical structural similarity (Tanimoto coefficient>0.7) (**Figure 5.9A**). In total, 10 clusters were generated, spanning lipids (e.g., acylcarnitines, phospholipids, and fatty acids), tryptophan metabolites, and polyamines, etc.

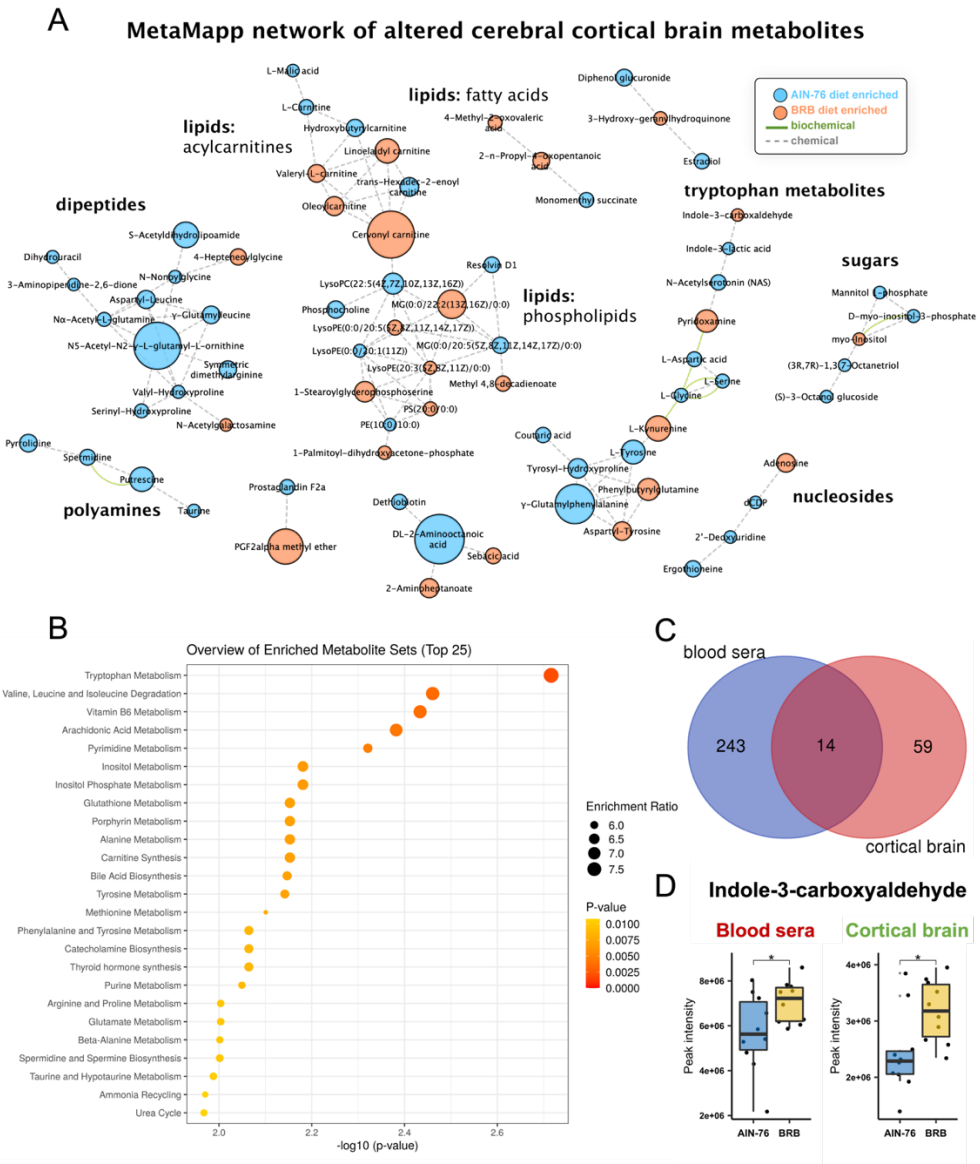


Figure 5.9 Altered cortical brain metabolites and a comparative view of shared altered compounds in blood sera and brain tissues owing to BRB-rich dietary administration. (**A**) MetaMapp metabolomic network of 73 altered metabolites in cerebral cortical brain tissues comparing BRB-rich diet vs. standard AIN-76A diet, with nodes representing individual metabolites (larger size for larger fold changes), edges for biochemical (Kyoto Encyclopedia of Genes and Genomes, KEGG reactant pairs) and chemical (Tanimoto coefficient>0.7) relationships, and lower transparency for lower adjusted p-values (two-sided Welch's *t*-test). (**B**) Altered fecal pathways (with top 25 shown) as determined by quantitative metabolite set enrichment analysis (qMSEA) based on 99 *a priori* defined sets of metabolites of the SPMDB databases in MetaboAnalyst 5.0 (Montreal, QC, Canada) (adjusted p<0.05). **(C)** Venn diagram of altered metabolites in sera and brain tissues. (**D**) Box and whisker plots of indole-3-carboxaldehyde altered in blood sera and brain tissues, with the box ranging from the first quartile to the third while the whiskers spanning each quartile to the minimum or maximum (*n*=20), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, two-sided Welch's *t*-test. Abbreviations: I3A, indole-3-carboxaldehyde.

Further quantitative pathway sets enrichment analysis identified 35 significantly altered pathways in cortical brain, with top 25 shown (**Figure 5.9B**; **Supplementary Table 5.9**). By enrichment fold and significance, tryptophan metabolism topped the chart, followed by branch-chain amino acids (i.e., valine, leucine, and isoleucine degradation), vitamin B6 metabolism, arachidonic acid metabolism, pyridine metabolism, and inositol & inositol phosphate metabolism, etc. (**Figure 5.9B**). Remarkably, among the top 25 enriched, we spotted biological pathways involved in neurotransmitter metabolism and signaling, spanning catecholamine biosynthesis, thyroid hormone synthesis, arginine and proline metabolism, glutamate metabolism, β-alanine metabolism, spermidine & spermine biosynthesis, and taurine & hypotaurine metabolism. These together indicated the potential of using BRB-rich diet for modulating neurobiochemical profiles in the central nervous system but meanwhile presented the complexity of diet-brain interaction and associated neuro-signaling events.

To probe whether microbiota was involved, specifically through a humoral pathway, we focused on the 14 altered metabolites shared by blood sera and cortical brain (**Figure 5.9C**). We compared across matrices in search of compounds of gut microbial relevance while checking for potential cross-organ concordance. We found that indole-3-carboxaldehyde (I3A) were enriched in both blood sera (+1.23-fold) and cortical brain tissues (+1.31-fold) of mice fed with BRB-rich diet compared with the AIN-76A control group. I3A is a metabolite converted from dietary tryptophan by human gut bacteria (e.g., *Lactobacillus* spp.), and a potent agonist to the aryl hydrocarbon receptor (AhR), a key cytosolic transcription factor in regulating immunological activities (Perdew et al., 2015). As anti-inflammatory benefits have been extensively reported for I3A, its elevated concentration levels in both blood sera and brain of BRB-fed mice may serve as one proof-of-concept for using berry fruit-based dietary intervention to modulate the humoral

microbiota-gut-brain axis towards improved neuroactive states in both gut microbiota and host bodies.

5.4 Conclusion

Taken together, for a proof-of-concept, we fed C57BL/6 mice with BRB-rich diet and standard AIN-76A diet and conducted the first high-coverage metabolome profiling *in vivo* of feces, blood sera, and cerebral brain tissues to probe systemic effects of dietary intake of BRB components and to explore potential involvement of a humoral microbiota-brain axis. Our results showed that BRB components effectively modulated the microbiota-metabolome network with altered neuroactive signaling profiles in local gut lumen environments, as exemplified by altered tryptophan metabolism and catecholamine biosynthetic pathways. Of note, administration of BRB components led to higher fecal levels of L-DOPA and dopamine and lower levels of neurotoxic derivatives in gut lumen including normetanephrine (a tumor biomarker), (R)-N-methylsalsolinol (a dopaminergic neurotoxin), and homovanilic acid (associated with neurodegenerative conditions). We also discovered massive alterations of oxidative stress markers and neurobiochemical changes of these aromatic metabolites (e.g., indoles, phenolics) in blood sera alongside neurotransmitters such as acetylcholine and γ-aminobutyrate. Similar to feces and sera, BRB-induced cortical brain metabolome changes also extensively involved neurotransmitter metabolism and signaling, such as tryptophan metabolism, catecholamine biosynthesis, and taurine & hypotaurine metabolism. With the complex and multifaceted metabolome datasets of multicompartment at hand, we not only discovered extensive changes in neurochemical profiles but discovered humoral exchanges of gut bacteria-derived metabolites, as exemplified by massive detection of indoles and phenolics in systemic blood circulation and importantly, the concordance

of indole-3-carboxaldehyde (an anti-inflammatory agent derived from gut bacteria) enriched in both sera and cortical brain. The massive, novel metabolomics datasets generated in this work may represent a valuable resource for using berry-based dietary intervention to reshape gut microbiota and/or induce neuroprotective effects *in vivo*.

CHAPTER 6: SUMMARY AND FUTURE WORK

Current knowledge of gut microbiota's influences on the gut-brain axis and associated disease risks are limited and progressing slow - only a few microbial molecular cues have been recognized and targeted for gut-brain axis, leaving the overall biochemical landscape and molecular underpinnings largely uncharted. In real world scenarios, further, whether and how such microbiota-gut-brain axis is involved in effects of neurotoxicant exposure and/or neuroprotective benefits of dietary intervention approaches remains unexplored. Systems omics profiling, particularly metabolomics, can address this given its data-driven and unbiased nature for hypothesis generation and prioritization. In complement with upstream omics, metabolome profiling is in principle advantageous because it directly captures molecular representation of biological and/or pathological phenotypes (Fiehn, 2002).

The overarching goal of this work therefore was to illuminate, on the metabolome-wide level, the neuroactive potential of gut microbiota in host bodies for neuroprotective benefits. We combine a highly innovative metabolomics approach and 16S rRNA gene sequencing (as needed) to achieve this goal. The high-coverage metabolomic profiling integrates state-of-the-art tools, algorithms, and post-curation strategies. In pursuit of this goal, our overall objectives are 1) to identify novel molecular pathways that are driven by microbiota in light of humoral interorgan transport and gut-brain communication and 2) to develop microbiota-based biomarkers and strategies to reduce health risks induced by neurotoxic xenobiotic exposures. Our central hypothesis is that gut microbiota is a major factor that drives the biochemical landscape of gut-

brain crosstalk under steady states as well as neurotoxic-challenged conditions and exhibit novel neuroactive potential that can be harnessed for improved mental health.

In Chapter 1, we first conducted a comprehensive review of microbial molecules that have been discovered to exhibit neuroactive effects in the host bodies. In the review, we discussed both small molecules and the larger neuropeptides. For each class of these molecules, we introduced their biosynthesis, uptake, activities, and functions in general, and then turned to specific disease models to carefully evaluate their roles in lieu of microbiota-gut-brain axis. With the research progress summarized up to date, future directions of research were discussed revolving around the discovery and development of novel microbial molecules of gut-brain health. We call for massive multi-omics mining efforts of gut microbiota in parallel with mechanistic exploration of inter-class compound interaction. Beyond the vast promises and opportunities the field holds for biomedical, nutritional, and environmental sciences, we also discussed challenges that need to be addressed.

In Chapter 2, as a preliminary attempt to probe humoral distribution of microbial metabolites in host bodies, we developed a targeted metabolomics assay based on high-resolution UHPLC-ESI-MS/MS to quantify in normal specific pathogen-free C57BL/6 mouse feces, blood sera, and cerebral cortical brain tissues literature-reported neurotransmitters, tryptophan metabolites, and indole derivatives (n=50) that have been proposed, hypothetically, to act on the gut-brain axis. For method validation, we evaluated the limit of detection, reproducibility, analyte recovery, and carryover of analysis. We successfully measured the concentration profiles in mice and collect useful analytical and biological information that were helpful with analytical method development of the untargeted metabolomics analysis applied in later chapters.

In Chapter 3 (*Specific Aim 1*), we used high-coverage metabolomics to comparatively profile feces, blood sera, and cerebral cortical brain tissues of germ-free C57BL/6 mice and their

age-matched conventionally raised counterparts. Results revealed for all three matrices metabolomic signatures owing to microbiota, yielding hundreds of identified metabolites including 533 altered for feces, 231 for sera, and 58 for brain with numerous significantly enriched pathways involving aromatic amino acids and neurotransmitters. Multicompartmental comparative analyses single out microbiota-derived metabolites potentially implicated in interorgan transport and the gut-brain axis, as exemplified by indoxyl sulfate and trimethylamine-N-oxide. Gender-specific characteristics of these landscapes are discussed. Our findings may be valuable for future research probing microbial influences on host metabolism and gut-brain communication.

In Chapter 4 (*Specific Aim 2*), we perform a 13-week subchronic PFOA exposure scheme on C57BL/6 mice. We combined high-coverage multicompartmental metabolomics and fecal 16S rRNA gene sequencing to characterize PFOA-induced metabolome (and microbial) changes, with specific aims to examine neuroactive biochemical alteration and a potential role of the humoral microbiota-brain axis. We not only identified PFOA-perturbed microbial members (e.g., *Clostridium* spp., *Bifidobacterium* spp.) but confirmed in the high-coverage metabolomics data the massive alterations in the specific metabolite profiles in the GI tract (e.g., tryptophan metabolites, neurotransmitters). Through interorgan comparison of fecal, blood, and cortical brain metabolomes, we discovered an array of bacterial metabolites or related signaling molecules that showed the same trends of change amongst all or part of the three compartments. Remarkably, some of these metabolites shared the same trend in results of Chapter 3 where GF mice also exhibited a decreasing trend across the three sample matrices, as exemplified by indoxyl sulfate, indole-3-lactate, and N-(2-phenylacetyl)glycine. We believe this work has yielded critical knowledge of a humoral microbiota-gut-brain crosstalk in environmental exposure induced risks

that need to be considered in future efforts in toxicity testing, risk assessment, and public health intervention dealing with PFAS chemical exposures or other neurotoxicants in general.

In Chapter 5 (*Specific Aim 3*), we fed C57BL/6 mice with BRB-rich diet and standard AIN-76A diet and conducted the first high-coverage metabolome profiling *in vivo* of feces, blood sera, and cerebral brain tissues to probe both local gut and systemic effects of dietary intake of BRB components and to explore potential role of the humoral microbiota-brain axis. In line with the previous work in the lab, our results showed that BRB components effectively modulated the microbiota-metabolome network with altered neuroactive signaling profiles in all three sample compartments, particularly in tryptophan metabolism and signaling neurotransmitters (e.g., catecholamine biosynthesis). Through cross-organ comparison, we discovered a range of gut bacteria-derived indoles and phenolics in systemic blood circulation, including indole-3-carboxaldehyde (I3A), an AhR agonist with anti-inflammatory activities, enriched in both sera and cortical brain when treated with BRB-rich diet. The massive, novel metabolomics datasets generated in this work may represent a valuable resource for using berry-based dietary intervention to reshape gut microbiota and/or induce neuroprotective effects *in vivo*.

In summary, through novel multi-omics analyses, this dissertation work aims to further advance our understanding of host-microbe interaction featuring the first attempt to probe whether and how a humoral microbiota-brain axis is involved *in vivo* using a truly untargeted metabolomics approach. For a proof-of-concept, data has been yielded for mice under steady states (i.e., GF vs. CONV-R), under neurotoxicant exposure (i.e., PFOA-treated vs. control), and under dietary treatment of reportedly neuroprotective components (i.e., BRB-rich diet vs. standard AIN-76A diet) that not only confirmed gut microbial influences through a humoral route (from gut to blood, and potentially further to brain), but delineated the metabolome-wide details with high coverage

and accuracy that we believe will benefit the field of gut microbiota, neuroscience, and public health in the long run. Future work, either mechanistic or translational, may focus on a specific set / class of molecules or pathways identified in this dissertation through a more targeted approach, for example, through SIL flux analysis, transporter identification, and mono-association microbiome analysis to validate discoveries of this dissertation work and to resolve the complex interplay of the microbiota-metabolome network and neuroactive signaling of the mammalian host.

APPENDIX A: SUPPORTING INFOTMATION FOR CHAPTER 2

***Topic**: Targeted metabolomics of 50 neurotransmitters and tryptophan metabolites in feces, blood sera, and cerebral cortical brain tissues in mice - method development and preliminary proof-of-principle*

Supplementary Figures

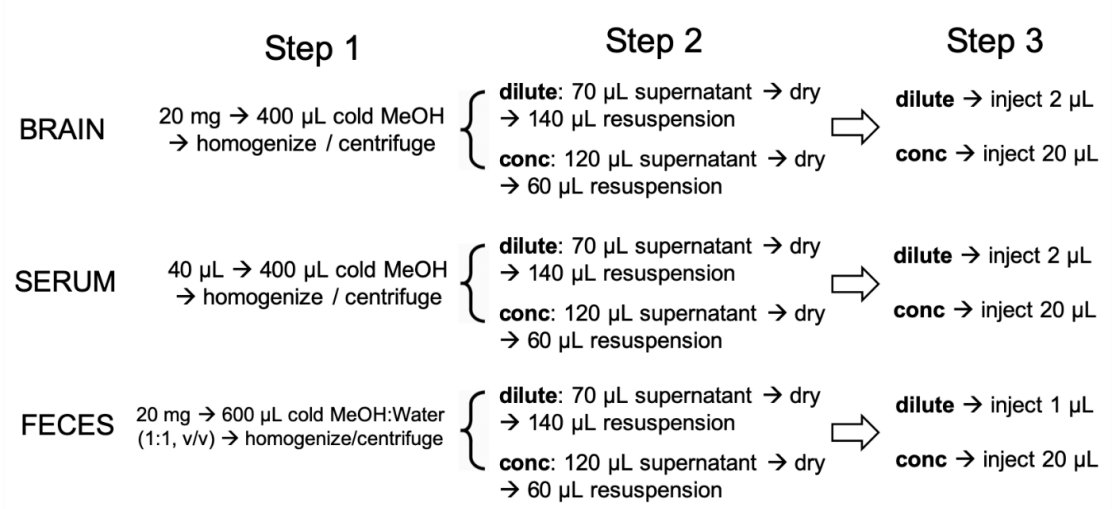
Supplementary Figure 2.1. Overview of sample processing for addressing wide dynamic ranges of compounds

Supplementary Figure 2.2. Barplots of analytical recovery in sample matrices as indicated by SIL standards

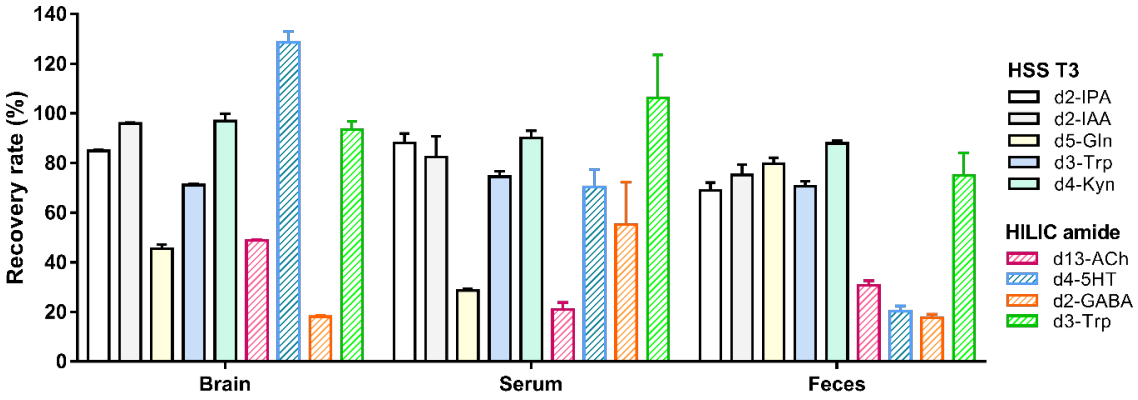
Supplementary Tables

Supplementary Table 2.1. Sample pretreatment procedures for individual compounds

Supplementary Table 2.2. Analyte recovery rates in sample matrices



Supplementary Figure 2.1. Overview of sample processing procedures. 'conc' stands for 'concentration'.



Supplementary Figure 2.2. Barplots of analytical recovery in the three sample matrices (Mean ± SEM, *n*=4).

Supplementary Table 2.1. Sample adaptation to maintain within linearity of assay.

	Compound Name	Brain	Serum	Feces
1	3-Aminopiperidine-2,6-dione	*conc	conc	conc
2	5-HIAA	conc	conc	conc
3	5-Hydroxy-Nω-methyltryptamine	conc	conc	conc
4	Acetylcholine	*dilute	dilute	dilute
5	Choline	dilute	dilute	dilute
6	Histamine	conc	conc	conc
7	Kynurenic acid	conc	conc	conc
8	L-Methionine	dilute	dilute	dilute
9	L-Proline	conc	dilute	conc
10	L-Pyroglutamic acid	dilute	dilute	conc
11	L-Tyrosine	dilute	dilute	conc
12	Nicotinic acid	conc	conc	dilute
13	Nα-Acetyl-L-glutamine	conc	conc	conc
14	Serotonin	conc	dilute	conc
15	Trimethylamine N-oxide	dilute	dilute	dilute
16	Tyramine	conc	conc	conc
17	γ-Aminobutyric acid	dilute	conc	conc
18	2-Phenethylamine	conc	conc	conc
19	2-Pyrrolidinone	dilute	dilute	dilute
20	3-Hydroxyanthranilic acid	dilute	dilute	conc
21	4-Methoxyindole	conc	conc	conc
22	AMFK	conc	conc	conc
23	Anthranilic acid	conc	dilute	conc
24	Coumarin	conc	conc	conc
25	Hippuric acid	conc	conc	conc
26	Indole	conc	conc	conc
27	Indole-3-acetic acid	conc	conc	conc
28	Indole-3-acetonitrile	conc	conc	conc
29	Indole-3-acrylic acid	dilute	dilute	dilute
30	Indole-3-carboxaldehyde	conc	dilute	dilute
31	Indole-3-carboxylic acid	conc	conc	conc
32	Indole-3-ethanol	conc	conc	conc
33	Indole-3-lactic acid	conc	dilute	dilute
34	Indole-3-propionic acid	conc	dilute	conc
35	Kynurenine	conc	dilute	conc
36	L-Arginine	dilute	dilute	dilute
37	L-Glutamic acid	dilute	dilute	dilute
38	L-Phenylalanine	dilute	dilute	dilute
39	L-Tryptophan	dilute	dilute	dilute
40	Melatonin	conc	conc	conc
41	Methyl IAA	conc	conc	conc
42	N-(2-Phenylacetyl)glycine	conc	dilute	dilute
43	N-Acetylserotonin	conc	conc	conc
44	N-Methylphenethylamine	dilute	conc	conc
45	N-Methyltryptamine	conc	conc	conc
46	Nudifloramide	conc	dilute	dilute
47	p-Coumaric acid	conc	conc	conc
48	Phenylacetyl L-glutamine	conc	conc	conc
49	Tryptamine	conc	conc	conc
50	Xanthurenic acid	conc	conc	conc

* Specific procedures of '*dilute*' and '*conc*' (short for 'concentration') can be found in **Supplementary Figure 2.1**. These adaptations in sample preparation are conducted to address the vast dynamic range of endogenous levels so that the analytes could be quantified within the linearity of the assay.

Supplementary Table 2.2. Analyte recovery in the three sample matrices based on spiked SIL internal standards.

% Recovery	HILIC amide				HSS T3				
Sample	**d13-ACh**	**d4-5HT**	**d2-GABA**	**d3-Trp**	**d2-IPA**	**d2-IAA**	**d5-Gln**	**d3-Trp**	**d4-Kyn**
Brain_1	48.96	131.68	18.83	96.49	84.77	96.51	49.96	70.07	98.14
Brain_2	47.28	116.06	16.85	89.49	84.62	95.22	45.84	70.55	91.37
Brain_3	48.36	137.19	17.11	86.33	84.36	95.59	41.86	72.34	93.2
Brain_4	50.02	129.4	19.62	101.32	85.94	96.59	44.29	71.72	105.04
Serum_1	22.9	60.42	13.12	94.68	91.37	100.2	27.46	77.06	93.79
Serum_2	12.59	67.86	45.63	85.35	93.94	80.39	30.86	77.04	81.46
Serum_3	23.42	91.35	95.11	157.96	90.16	60.49	27.81	75.78	92.43
Serum_4	24.99	61.12	66.56	86.72	76.85	88.34	28.1	68.32	92.77
Feces_1	33.07	15.01	14.02	48.97	69.63	82.14	78.2	70.56	91.44
Feces_2	25.11	18.49	19.89	79.34	77.65	82.81	84.1	75.08	85.19
Feces_3	31.59	25.85	20.3	91.04	63.74	66.78	83.02	65.07	88.29
Feces_4	32.94	21.32	16.24	80.55	65.32	69.02	73.89	71.78	86.66

APPENDIX B: SUPPORTING INFOTMATION FOR CHAPTER 3

***Topic**: High coverage multicompartmental metabolomics profiling of germ-free mice vs. conventionally raised counterparts for probing the humoral microbiota-gut-brain axis*

Supplementary Figures

Supplementary Figure 3.1
Schematic illustration of compound identification procedures.

Supplementary Figure 3.2
Box and Whisker plots of select serum compounds: indoles and neurotransmitters.

Supplementary Figure 3.3
Schematic illustration of altered methionine/glutathione transsulfuration pathway in cerebral cortical brain comparing GF and CONV-R mice.

Supplementary Figure 3.4
Distribution of ion features of GF/CONV-R difference considering gender (male/female) as the moderator variable: Venn diagrams for each matrix-HESI combination.

Supplementary Figure 3.5
Distribution of ion features of male/female difference considering microbiota (GF/CONV-R) as the moderator variable: Venn diagrams and pie charts.

Supplementary Figure 3.6
MetaMapp network of sex-specific metabolites in feces comparing GF and CONV-R mice.

Supplementary Figure 3.7
MetaMapp network of sex-specific metabolites in blood sera comparing GF and CONV-R mice.

Supplementary Figure 3.8
MetaMapp network of sex-specific metabolites in brain comparing GF and CONV-R mice.

Supplementary Figure 3.9
Metabolite set enrichment analysis (MSEA) of gender-specific metabolites.

Additional information:
MS-FINDER settings; Random Forest classification for generating the variable importance plots (VIPs).

Supplementary Tables

Supplementary Table 3.1
List of microbial products, neurotransmitters, and canonical metabolites (n=423 compounds): an in-house library and detected GF/CONV-R difference in feces, blood sera, and cortical brain tissues.

Supplementary Table 3.2
Unique list of 533 fecal metabolites altered in feces comparing GF and CONV-R mice.

Supplementary Table 3.3
Unique list of 231 serum metabolites altered in feces comparing GF and CONV-R mice.

Supplementary Table 3.4
Unique list of 58 brain metabolites altered in feces comparing GF and CONV-R mice

Supplementary Table 3.5
ChemRICH results for fecal metabolome changes on compound class level.

Supplementary Table 3.6
ChemRICH results for fecal metabolome changes for individual metabolite assignment.

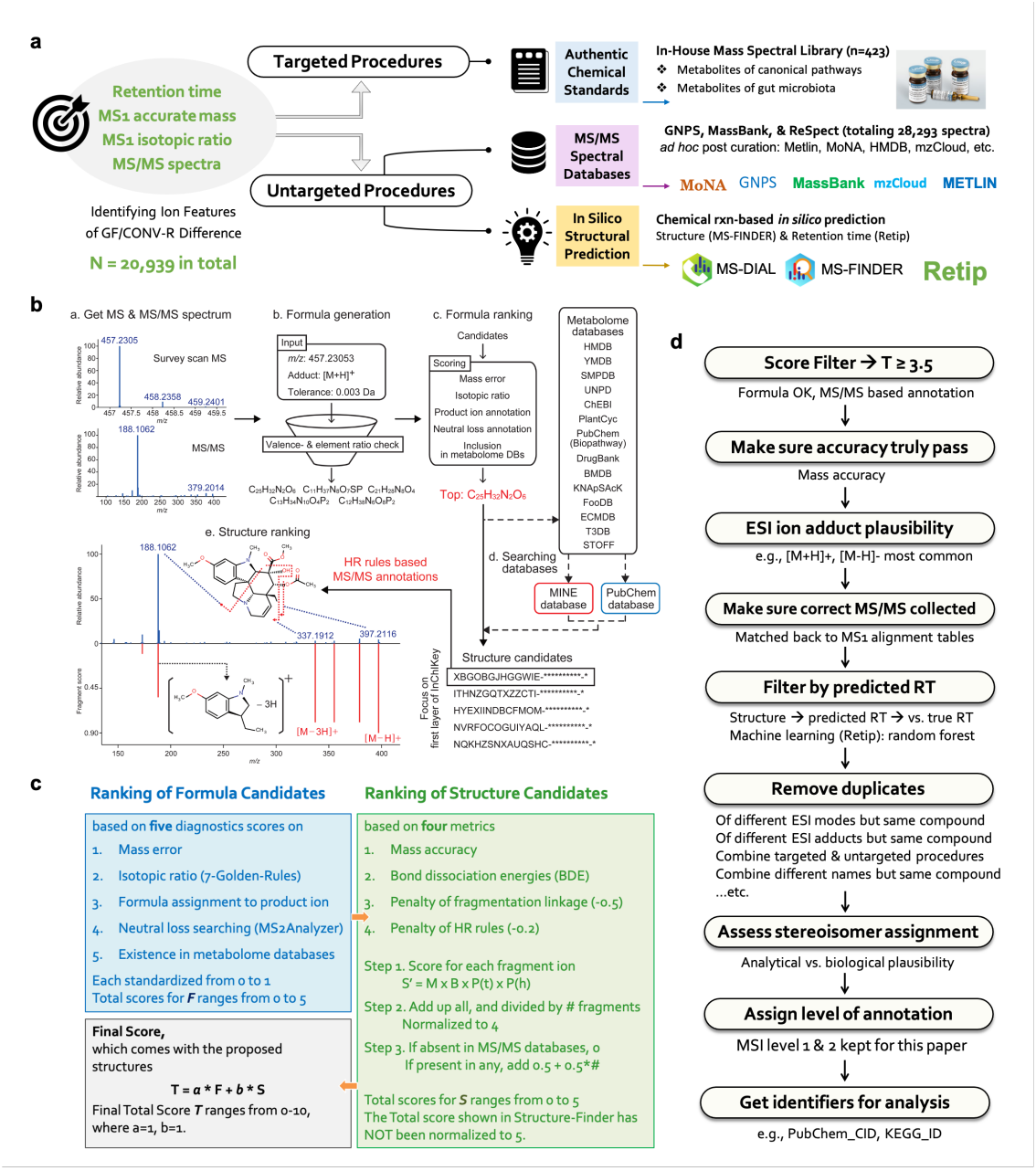
Supplementary Table 3.7
Quantitative pathway enrichment analysis (qMSEA) results for fecal metabolome changes.

Supplementary Table 3.8
ChemRICH results for serum metabolome changes on compound class level.

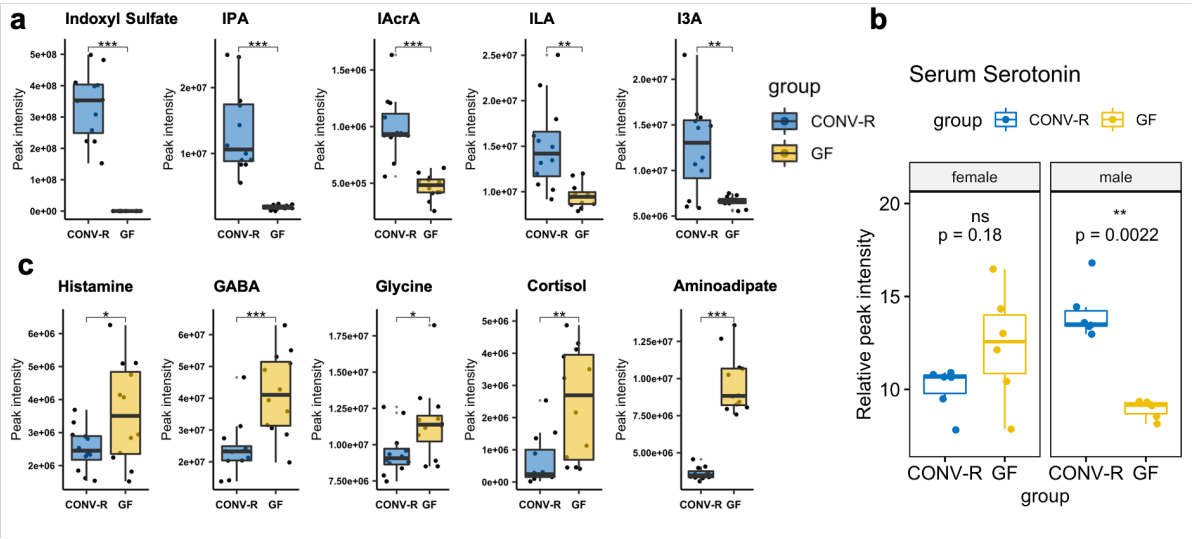
Supplementary Table 3.9
ChemRICH results for serum metabolome changes for individual metabolite assignment.

Supplementary Table 3.10
Quantitative pathway enrichment analysis (qMSEA) results for serum metabolome changes.

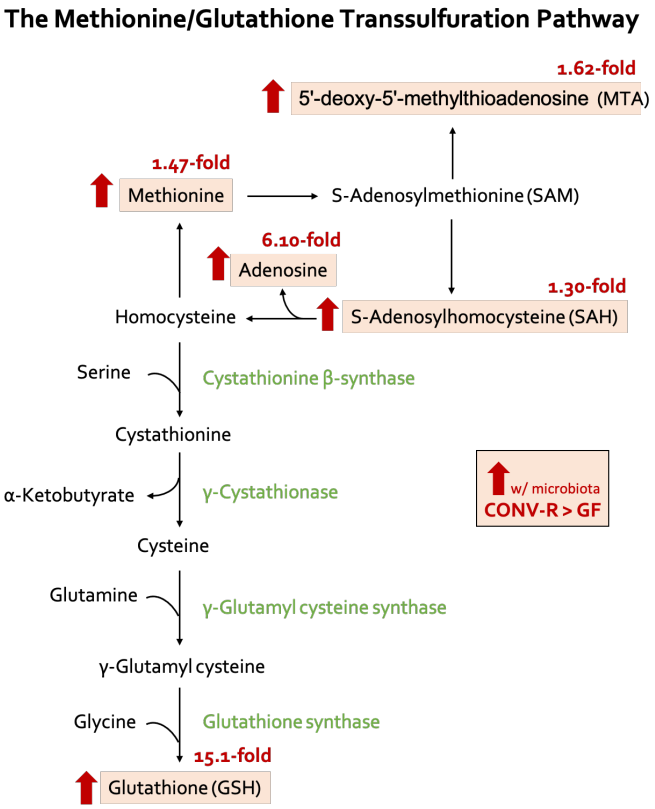
Supplementary Table 3.11
Gender-specific metabolites of GF/CONV-R difference (two-way ANOVA; Tukey's HSD test).



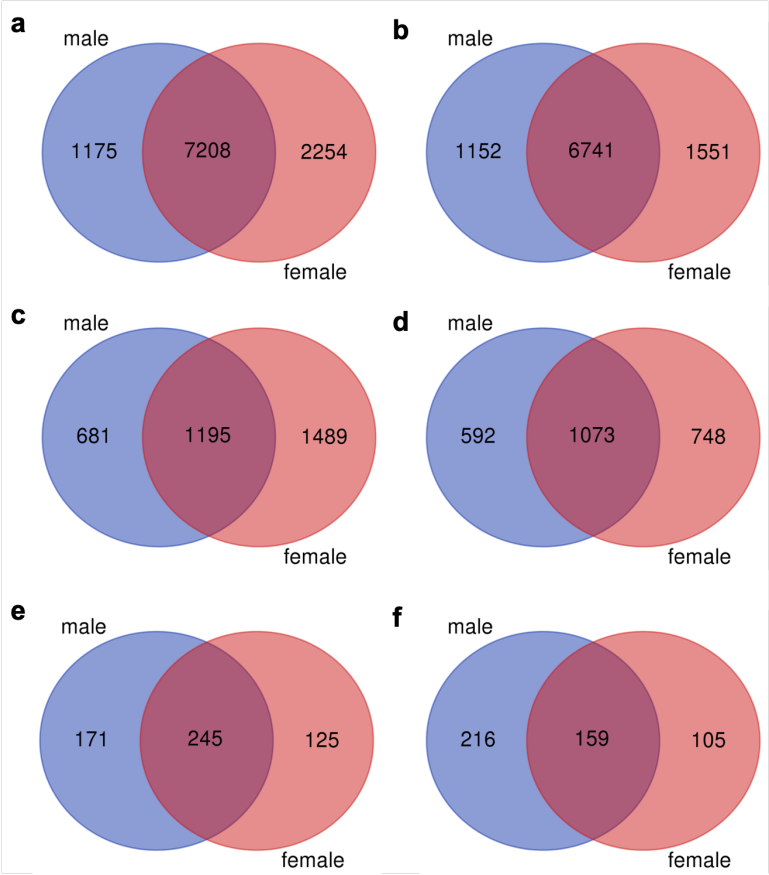
Supplementary Figure 3.1. Schematic illustration of compound identification: (**a**) Workflow combining targeted and untargeted annotation procedures; License-wise, the symbols of lightbulb and target/arrow were purchased from Iconfinder.com (transaction No. 0003685707, 0003685712), symbols of library and database were drawn by the first author of this article using Adobe Illustrator 2021, while the logos of MS-DIAL and MS-FINDER were distributed by CC-BY-SA 4.0 license and could be used for publication purposes; (**b**) *in silico* prediction of chemical formula and structure were based on chemical bond energies, hydrogen-rearrangement rules, and heuristic rules, as detailed in MS-FINDER(Tsugawa et al., 2016); (**c**) a streamlined scoring strategy for steps of (**b**); (**d**) strategies and procedures for post-curation of *in silico* annotations in this work.



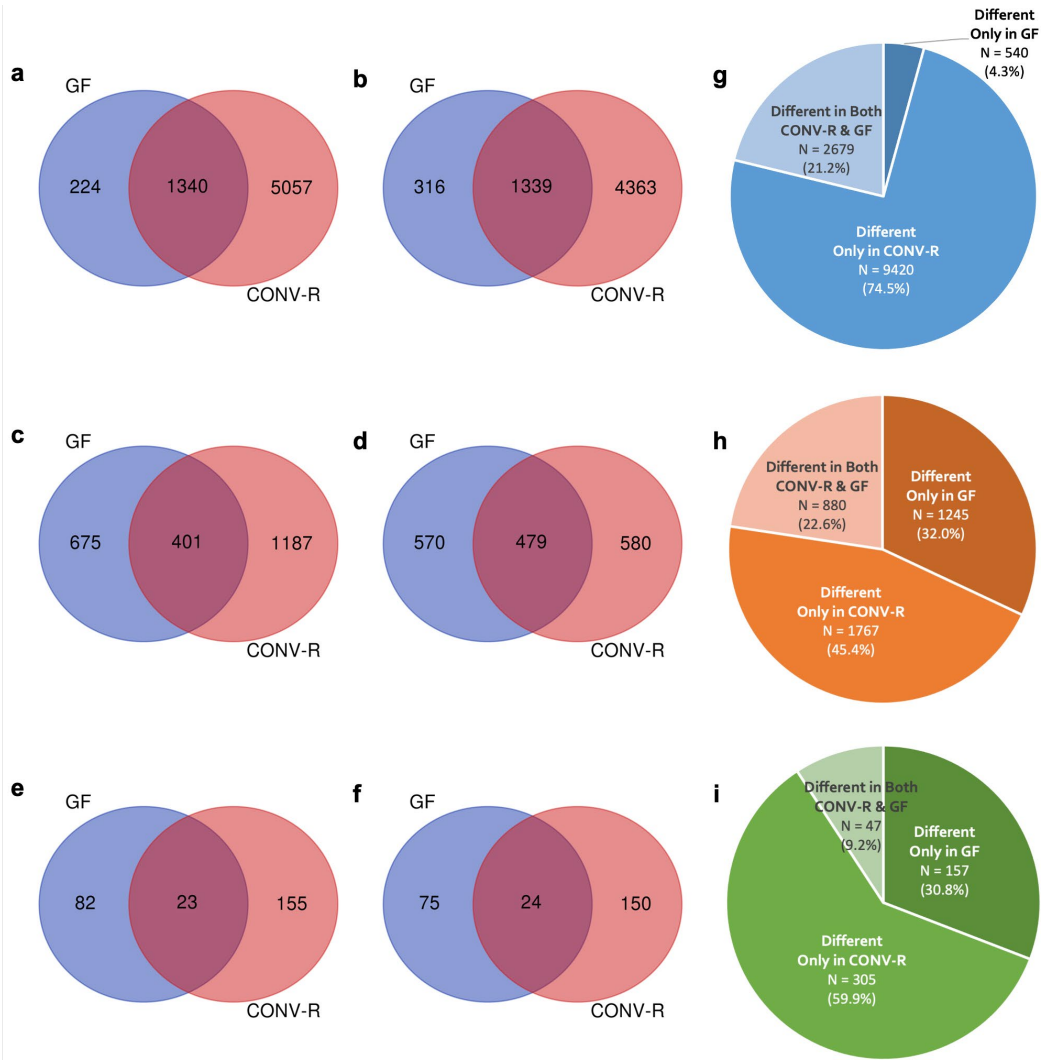
Supplementary Figure 3.2. Box and Whisker plots of select serum compounds: (**a**) indoles comparing CONV-R (N=12) and GF mice (N=12), with the box ranging from the first quartile to the third while the whiskers going from each quartile to the minimum or maximum, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, two-sided Welch's *t*-test; exact p-values and adjusted p-values are provided in Supplementary Table 3; (**b**) serotonin levels examined in a gender-specific manner with the box ranging from the first quartile to the third while the whiskers going from each quartile to the minimum or maximum (N=6 for each group), ns: p≥0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, multigroup pairwise Wilcoxon rank-sum test; (**c**) neurotransmitters and neuromodulatory molecules comparing CONV-R (N=12) and GF mice (N=12), with the box ranging from the first quartile to the third while the whiskers going from each quartile to the minimum or maximum, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, two-sided Welch's *t*-test; exact p-values and adjusted p-values are provided in Supplementary Table 3.



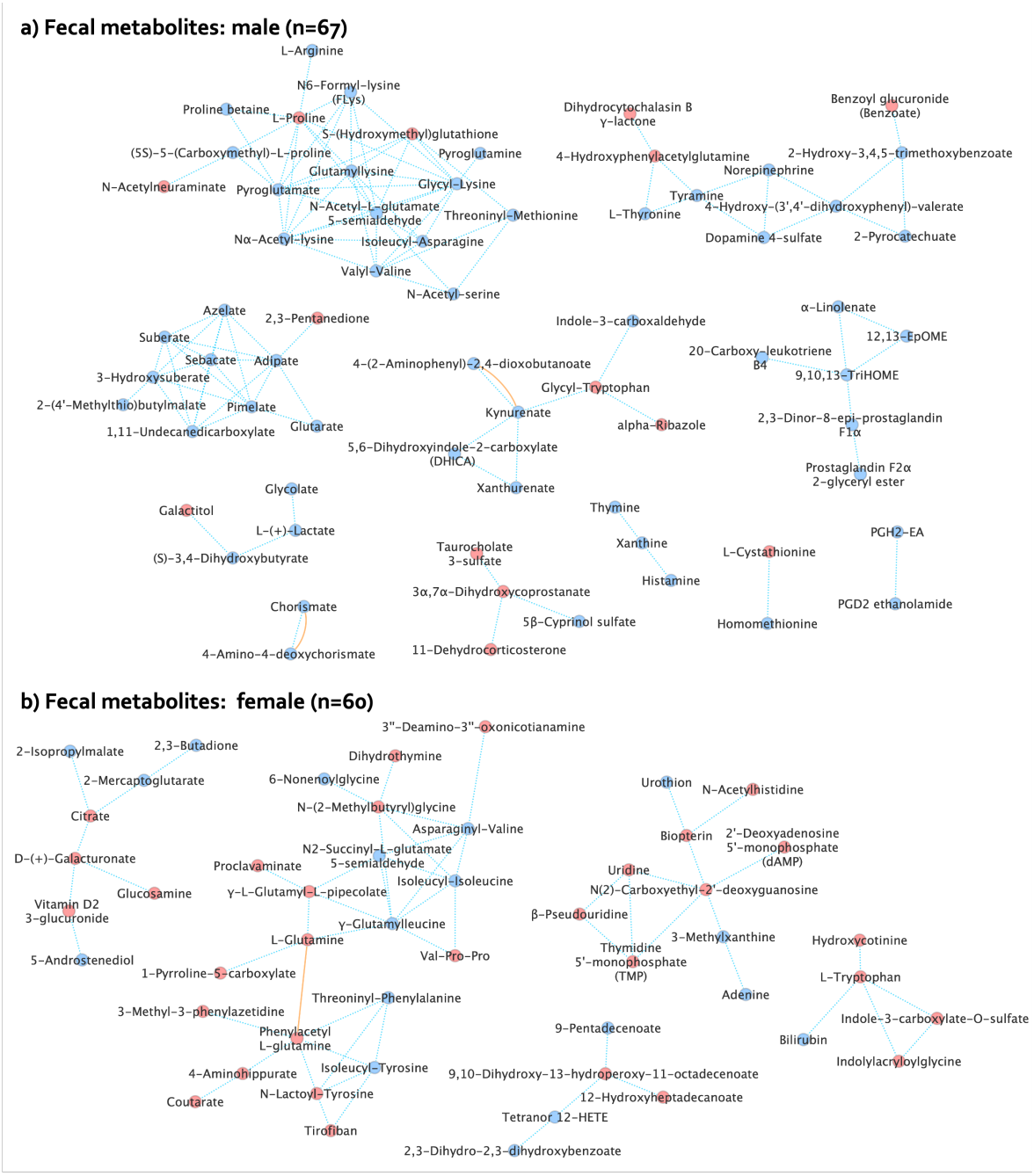
Supplementary Fig 3.3. Schematic illustration of altered methionine/glutathione transsulfuration pathway in cerebral cortical brain comparing GF and CONV-R mice(Belalcázar, Ball, Frost, Valentovic, & Wilkinson, 2014; A. N. Hughes & Oxford, 2014).



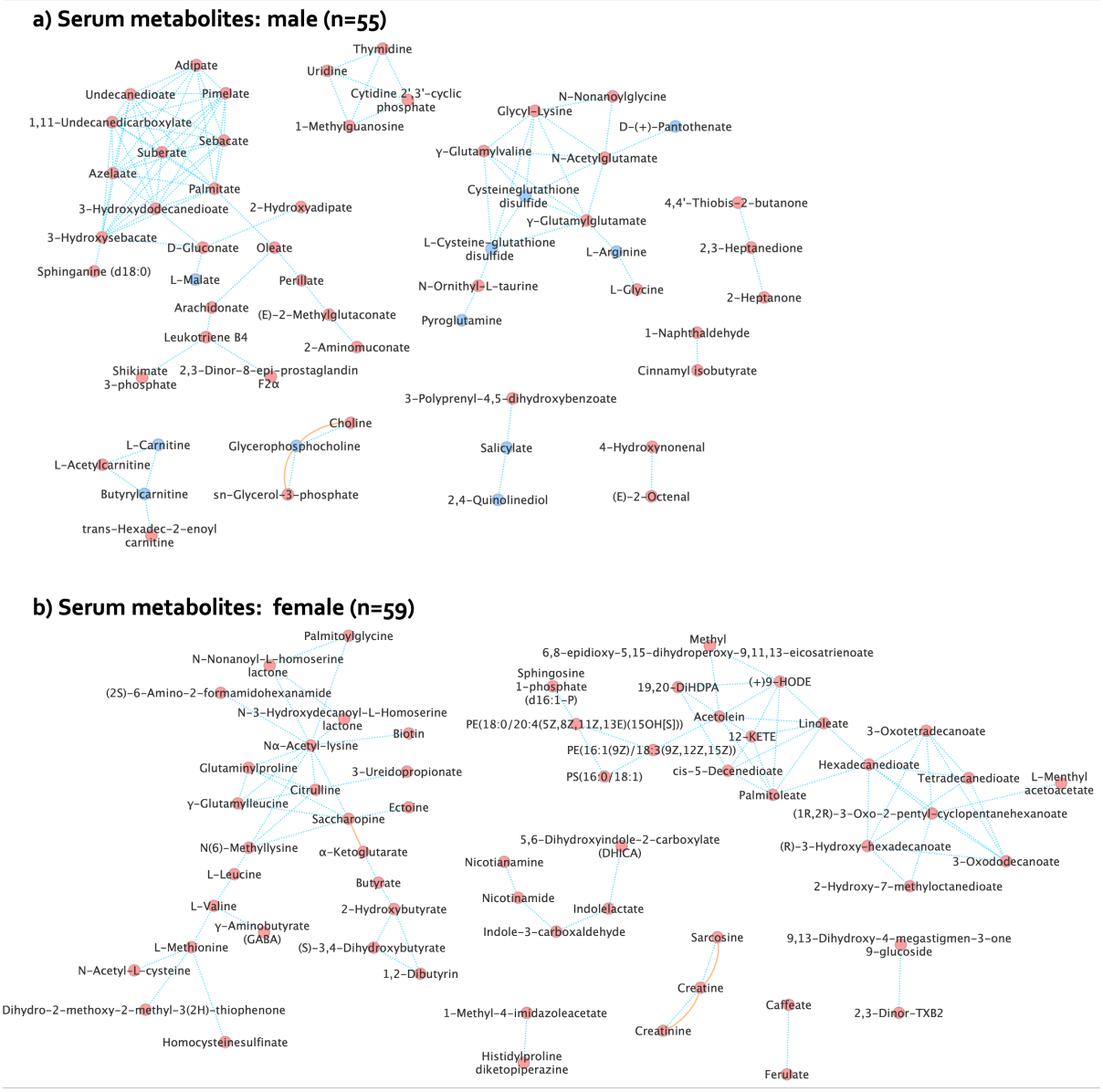
Supplementary Figure 3.4. Distribution of ion features of GF/CONV-R difference (focal variable considering gender (male/female, moderator variable) as determined by two-way ANOVA (adjusted $p<0.05$) and *post hoc* Tukey's HSD test (adjusted $p<0.05$). (**a-f**) Venn diagram illustrating ion features of each matrix-HESI combination that had significant main effects of microbiota (two-way ANOVA, adjusted $p<0.05$) while exhibiting significant GF/CONV-R difference either in male or female or both (Tukey's HSD test, adjusted $p<0.05$): (a) feces, HESI+; (b) feces, HESI-; (c) serum, HESI+; (d) serum, HESI-; (e) cerebral cortical brain tissues, HESI+; (f) cerebral cortical brain tissues, HESI-.



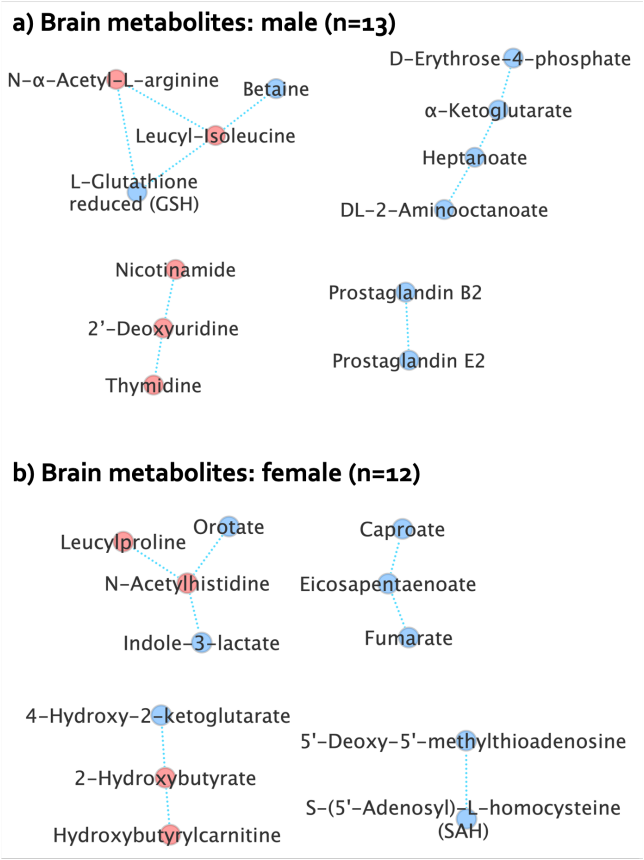
Supplementary Figure 3.5. Distribution of ion features of male/female difference (focal variable) considering microbiota (GF/CONV-R) as the moderator variable as determined by two-way ANOVA (adjusted $p<0.05$) and *post hoc* Tukey's HSD test (adjusted $p<0.05$). (**a-f**) Venn diagram illustrating ion features of each matrix-HESI combination that had significant main effects of gender (two-way ANOVA, adjusted $p<0.05$) while exhibiting significant male/female difference either in GF, CONV-R, or both (Tukey's HSD test, adjusted $p<0.05$): (a) feces, ESI+; (b) feces, ESI-; (c) serum, ESI+; (d) serum, ESI-; (e) cerebral cortical brain tissues, ESI+; (f) cerebral cortical brain tissues, ESI-. (**g-i**) Pie charts illustrating the distribution of ion features (combining both HESI modes for each sample matrix) that had main effects of gender (adjusted $p<0.05$) while exhibiting significant male/female difference either in GF, CONV-R, or both based on *post hoc* Tukey's HSD test (adjusted p-value<0.05).



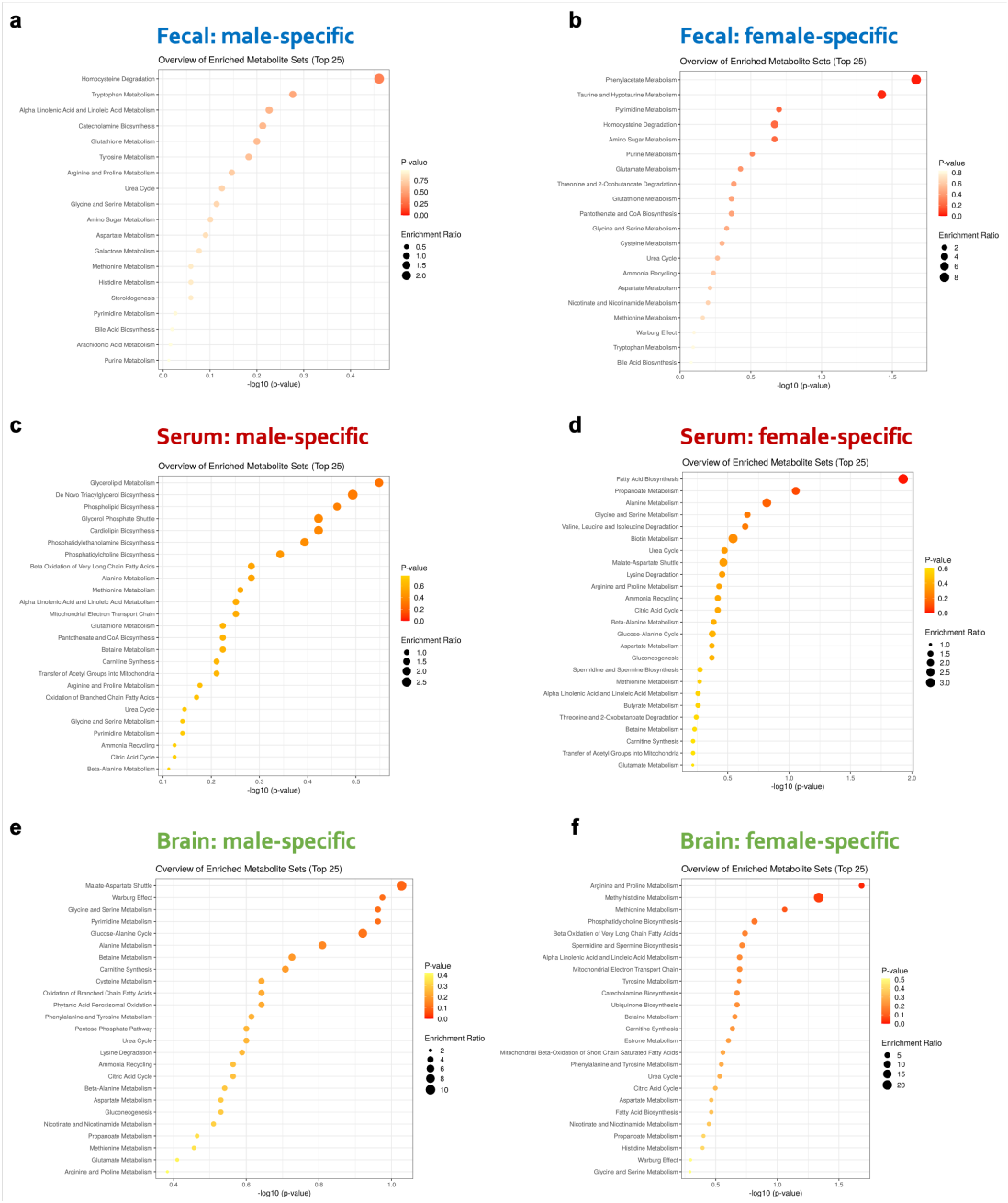
Supplementary Figure 3.6. MetaMapp network view of sex-specific metabolites in feces comparing GF and CONV-R mice as determined by two-way ANOVA (adjusted p<0.05) and *post hoc* Tukey's HSD test (adjusted p<0.05): (**a**) fecal metabolites altered in male but not female; (**b**) fecal metabolites altered in female but not male. Node colors: blue (CONV-R>GF); red (CONV-R<GF). Edges: blue dotted lines indicate chemical structural similarity (Tanimoto coefficient>0.7), while orange solid lines indicate biochemical relevance (KEGG reactant pair).



Supplementary Figure 3.7. MetaMapp network view of sex-specific metabolites in blood sera comparing GF and CONV-R mice as determined by two-way ANOVA (adjusted $p<0.05$) and *post hoc* Tukey's HSD test (adjusted $p<0.05$): (**a**) serum metabolites altered in male but not female; (**b**) serum metabolites altered in female but not male. For node colors, blue means CONV-R>GF, red means CONV-R<GF; for edges, blue dotted lines indicate chemical structural similarity (Tanimoto coefficient>0.7), and orange solid lines indicate biochemical relevance (KEGG reactant pair).



Supplementary Figure 3.8. MetaMapp network view of sex-specific metabolites in cerebral cortical brain tissues comparing GF and CONV-R mice as determined by two-way ANOVA (adjusted $p<0.05$) and *post hoc* Tukey's HSD test (adjusted $p<0.05$): (**a**) brain metabolites altered in male but not female; (**b**) brain metabolites altered in female but not male. For node colors, blue means CONV-R>GF, red means CONV-R<GF; for edges, blue dotted lines indicate chemical structural similarity (Tanimoto coefficient > 0.7), and orange solid lines indicate biochemical relevance (KEGG reactant pair).



Supplementary Figure 3.9. Metabolite set enrichment analysis (MSEA) of gender-specific metabolites for different sample matrices based on 99 *a priori* defined sets of metabolites (adjusted p-value<0.05): (**a**) male, fecal; (**b**) female, fecal; (**c**) male, sera; (**d**) female, sera; (**e**) male, cerebral cortical brain tissues; (**f**) female, cerebral cortical brain tissues.

Additional information

Settings of MS-FINDER 3.3 (running with Windows 10 OS)
for *in silico* compound annotation of ion features with an acquired tandem mass spectrum

Module	Parameter item / options	Value
Method	Spectral database search	yes
	… by in silico fragmentor	yes
	Spectral database option	
	Use internal experimental library	MassBank, GNPS, ReSpect
	Use in silico spectra of LipidBlast	Solvent type: HCOONH4
	User-defined DB	NULL
	Precursor oriented option	“Precursor oriented spectral search”
Mass spectrum	Mass tolerance type	“ppm”
	Mass tolerance (MS1)	10
	Mass tolerance (MS2)	10
	Relative abundance cutoff	1%
	Mass range	40-1000
Formula finder	LEWIS and SENIOR check	yes
	Isotopic ratio tolerance	5%
	Element ratio check	Common range (99.7%) covering
	Element selection	O, N, P, S
Structure finder	In silico MS/MS, tree depth	2
Data source	Local databases	HMDB (Human), Urine (Human), Saliva (Human), Feces (Human), Serum (Human), CSF (Human), SPMDB (Human), LipidMAPS (Lipids), ECMDB (E. coli), PubChem (Biomolecules)
	MINEs setting	Never use it.
	PubChem Online setting	Never use it.

Random Forest Models for Generating Variable Importance Plots
(for Figure 3.3a, Figure 3.6a & Figure 3.7a):
Hyperparameters, OOB estimate error rate, and confusion matrices
using R packages *randomForest* (ver. 4.16.4) and *rfPermute* (ver. 2.2)

Feces (533 metabolites)

```
library("randomForest", "rfPermute")
set.seed(2020)
rfPermute(as.factor(Group) ~ ., data = vip_fecCID, importance = TRUE, nrep = 1000)
Call:
 randomForest(formula = as.factor(Group) ~ ., data = vip_fecCID, importance = TRUE)
               Type of random forest: classification
                     Number of trees: 500
No. of variables tried at each split: 23
        OOB estimate of  error rate: 0%
Confusion matrix:
                  CONV-R     GF     class.error
CONV-R            12          0           0
GF                 0          12          0
```

Serum (231 metabolites)

```
library("randomForest", "rfPermute")
set.seed(2020)
rfPermute(as.factor(Group) ~ ., data = vip_serCID, importance = TRUE, nrep = 1000)
Call:
 randomForest(formula = as.factor(Group) ~ ., data = vip_serCID, importance = TRUE)
               Type of random forest: classification
                     Number of trees: 500
No. of variables tried at each split: 15
        OOB estimate of  error rate: 0%
Confusion matrix:
                  CONV-R     GF     class.error
CONV-R            12          0           0
GF                 0          12          0
```

Brain (58 metabolites)

```
library("randomForest", "rfPermute")
set.seed(2020)
rfPermute(as.factor(Group) ~ ., data = vip_brnCID, importance = TRUE, nrep = 1000)
Call:
 randomForest(formula = as.factor(Group) ~ ., data = vip_brnCID, importance = TRUE)
               Type of random forest: classification
                     Number of trees: 500
No. of variables tried at each split: 7
        OOB estimate of  error rate: 0%
Confusion matrix:
                  CONV-R     GF     class.error
CONV-R            12          0           0
GF                 0          12          0
```

Random Forests typically do not overfit. But hyperparameter knobs may need fine tuning to best avoid overfitting dealing with noisy data. In this work, (1) **`nodesize.`** the minimum size for terminal nodes (leaves in the tree) was set as the default value of 1 (for classification); (2) **`maxnodes.`** the maximum number of nodes in each decision tree was set as default with no limit specified, the largest tree was to be fit subject to the constraints of **`nodesize`**. (Bruce, Bruce, & Gedeck, 2020).

Supplementary Table 3.1. List of microbial products, neurotransmitters, and canonical metabolites (n=423 compounds): an in-house library and detected GF/CONV-R difference in feces, blood sera, and cortical brain tissues (check symbol).

	Name_reference standard	Formula	HESI mode	Exact mass	ESI adduct	Accurate *m/z*	RT (min)	Feces	Sera	Brain
1	(+)-Equol 4'-sulfate	C15H14O6S	Neg	322.0511	proton	321.0432814	6.42	✔		
2	(+)-Warfarin	C19H16O4	Neg	308.1049	proton	307.0970286	7.57			
3	(+)13-HODE	C18H32O3	Neg	296.2351	proton	295.2273072	8.65			
4	(+)9-HODE	C18H32O3	Neg	296.2351	proton	295.2273072	8.6		✔	
5	(±)-2-Hydroxyoctanoic acid	C8H16O3	Neg	160.1099	proton	159.1021136	7.04			
6	(±)-Epinephrine	C9H13NO3	Neg	183.0895	proton	182.0817138	0.82			
7	(±)-Sodium 2,3-dihydroxyisovalerate h	C5H10O4	Neg	134.0579	proton	133.050081	1.23	✔		
8	(±)-Sodium 3-hydroxybutyrate	C4H8O3	Neg	104.0473	proton	103.0395168	1.46			
9	(R)-3-Hydroxybutyric acid	C4H8O3	Neg	104.0473	proton	103.0395168	1.46		✔	✔
10	(S)-2-Hydroxybutyric acid	C4H8O3	Neg	104.0473	proton	103.0395168	1.84	✔		
11	1,11-Undecanedicarboxylic acid	C13H24O4	Neg	244.1675	proton	243.1596254	7.8	✔	✔	
12	1,3,7-Trimethyluric acid	C8H10N4O3	Neg	210.0753	proton	209.067462	4.52			
13	1,7-Dimethyluric acid	C7H8N4O3	Neg	196.0596	proton	195.0518128	4.12			
14	12-HETE	C20H32O3	Neg	320.2351	proton	319.2273072	8.77		✔	
15	15(S)-HETE	C20H32O3	Neg	320.2351	proton	319.2273072	8.79			
16	2-Deoxyribose-5-p-Na+	C5H11O7P	Neg	214.0242	proton	213.0164136	0.73			
17	2-Hydroxy Decanoic acid	C10H20O3	Neg	188.1412	proton	187.133412	7.92			
18	2-Hydroxy stearic acid	C18H36O3	Neg	300.2664	proton	299.2586056	10	✔	✔	
19	2-Hydroxy-3-methylbutyric acid	C5H10O3	Neg	118.063	proton	117.055166	3.86	✔		
20	2-Hydroxy-3-methylvaleric acid	C6H12O3	Neg	132.0786	proton	131.0708152	5.3			
21	2-Hydroxybutyric acid sodium salt	C4H8O3	Neg	104.0473	proton	103.0395168	1.79		✔	✔
22	2-Hydroxyisocaproic acid	C6H12O3	Neg	132.0786	proton	131.0708152	5.35	✔		
23	2-Phenoxyethanol	C8H10O2	Neg	138.0681	proton	137.060251	6.1			
24	2,3-dihydroxybenzoic acid	C7H6O4	Neg	154.0266	proton	153.0187826	5.17		✔	
25	2,3-pyridinedicarboxylic acid	C7H5NO4	Neg	167.0219	proton	166.014032	1.3		✔	
26	2,4-Dichlorophenoxyacetic acid	C8H6Cl2O3	Neg	219.9694	proton	218.9615736	7.66			
27	2,4-Quinolinediol	C9H7NO2	Neg	161.0477	proton	160.0398512	5.72		✔	
28	2,5-Dihydroxybenzoic acid	C7H6O4	Neg	154.0266	proton	153.0187826	4.72			
29	2,8-Quinolinediol	C9H7NO2	Neg	161.0477	proton	160.0398512	5.33			
30	2'-Deoxyadenosine 5'-monophosphate	C10H14N5O6P	Neg	331.0682	proton	330.0603424	1.1	✔		

31	2'-Deoxycytidine (dC)	C9H13N3O4	Neg	227.0906	proton	226.0827768	0.98	✔		
32	2'-Deoxyguanosine 5'-monophosphate	C10H14N5O7P	Neg	347.0631	proton	346.0552574	1.21			
33	2’-deoxyuridine	C9H12N2O5	Neg	228.0746	proton	227.0667932	1.73		✔	✔
34	3-Chloro-L-tyrosine	C9H10ClNO3	Neg	215.0349	proton	214.027093	3.28			
35	3-Hydroxy anthranilic acid	C7H7NO3	Neg	153.0426	proton	152.0347662	3.72	✔		
36	3-Hydroxy Decanoic acid	C10H20O3	Neg	188.1412	proton	187.133412	7.41			
37	3-Indolylacetonitrile	C10H8N2	Neg	156.0687	proton	155.0609198	7.25			
38	3-Methyl-2-oxobutanoic acid	C5H8O3	Neg	116.0473	proton	115.0395168	2.52	✔		
39	3-Methylcrotonyl glycine	C7H11NO3	Neg	157.0739	proton	156.0660646	4.45		✔	
40	3-Methylxanthine	C6H6N4O2	Neg	166.0491	proton	165.0412486	3.03	✔		
41	3-Nitro-L-tyrosine	C9H10N2O5	Neg	226.059	proton	225.051144	3.71	✔		
42	3-Ureidopropionic acid	C4H8N2O3	Neg	132.0535	proton	131.0456648	0.95		✔	
43	3,4-Dihydroxy-L-phenylalanine (L-DOPA	C9H11NO4	Neg	197.0688	proton	196.0609796	1.1	✔		
44	3,7-Dimethyluric acid	C7H8N4O3	Neg	196.0596	proton	195.0518128	0.67			
45	4-Hydroxybutyric acid (GHB)	C4H8O3	Neg	104.0473	proton	103.0395168	1.24			
46	4-Methyl-2-oxovaleric acid	C6H10O3	Neg	130.063	proton	129.055166	4.9	✔		
47	5-Hydroxy Indole-3-acetic acid	C10H9NO3	Neg	191.0582	proton	190.0504154	4.86			
48	5-Methyltetrahydrofolic acid	C20H25N7O6	Neg	459.1866	proton	458.178798	3.91			
49	5'-Deoxy-5'-methylthioadenosine	C11H15N5O3S	Neg	297.0896	proton	296.081731	4.29			✔
50	5(S)-HETE	C20H32O3	Neg	320.2351	proton	319.2273072	8.82			
51	5β-CHOLANIC ACID-3-ONE	C24H38O3	Neg	374.2821	proton	373.2742548	9.17			
52	6-phospho-D-gluconate	C6H13O10P	Neg	276.0246	proton	275.0168078	0.66	✔		
53	9-Methyluric acid	C6H6N4O3	Neg	182.044	proton	181.0361636	1.38			
54	Acesulfame K	C4H5NO4S	Neg	162.9939	proton	161.986104	2.41			
55	Acetaminophen	C8H9NO2	Neg	151.0633	proton	150.0555004	3.95			
56	Acetaminophen sulfate	C8H9NO5S	Neg	231.0201	proton	230.0123174	3.6			
57	Adenine	C5H5N5	Neg	135.0545	proton	134.046668	0.9	✔		✔
58	Adenosine	C10H13N5O4	Neg	267.0967	proton	266.0889248	1.66			✔
59	Adenosine 5'-monophosphate	C10H14N5O7P	Neg	347.0631	proton	346.0552574	0.94			
60	Adipic acid	C6H10O4	Neg	146.0579	proton	145.050081	4.05	✔	✔	

Supplementary Table 3.1. Cont’d.

61	AFMK, i.e. N-[3-[2-(formylamino)-5-me	C13H16N2O4	Neg	264.111	proton	263.1031766	5.78			
62	Allantoin	C4H6N4O3	Neg	158.044	proton	157.0361636	0.7	✔	✔	✔
63	Anthranilic acid	C7H7NO2	Neg	137.0477	proton	136.0398512	5.53			
64	Argininosuccinic acid disodium salt hyd	C10H18N4O6	Neg	290.1226	proton	289.1148038	0.66			
65	Atorvastatin	C33H35FN2O5	Neg	558.253	proton	557.2451622	8.14			
66	Azelaic acid	C9H16O4	Neg	188.1049	proton	187.0970286	6.22	✔	✔	
67	Bilirubin	C33H36N4O6	Neg	584.2635	proton	583.2556466	11.14	✔		
68	Biotin	C10H16N2O3S	Neg	244.0882	proton	243.0803336	5.24		✔	
69	Boc-Arg-OH	C11H22N4O4	Neg	274.1641	proton	273.1562722	4.89			
70	Caffeic acid	C9H8O4	Neg	180.0423	proton	179.0344318	5.12	✔	✔	
71	CDP	C9H15N3O11P2	Neg	403.0182	proton	402.010357	0.86			
72	Celecoxib	C17H14F3N3O2S	Neg	381.0759	proton	380.068053	8.33			
73	Chenodeoxycholic acid	C24H40O4	Neg	392.2926	proton	391.284819	8.42	✔	✔	
74	Chlorpyrifos	C9H11Cl3NO3PS	Neg	348.9263	proton	347.9184586	9.11			
75	Cholic acid	C24H40O5	Neg	408.2876	proton	407.279734	7.89	✔		
76	Cimetidine	C10H16N6S	Neg	252.1157	proton	251.1078846	1.02			
77	cis-Aconitic acid	C6H6O6	Neg	174.0164	proton	173.0086126	1.31		✔	
78	Citraconic acid	C5H6O4	Neg	130.0266	proton	129.0187826	2.05			
79	Citric acid	C6H8O7	Neg	192.027	proton	191.0191768	1.1	✔		
80	CMPF	C12H16O5	Neg	240.0998	proton	239.0919436	7.46			
81	Cortisone	C21H28O5	Neg	360.1937	proton	359.1858388	6.9			
82	Creatine	C4H9N3O2	Neg	131.0695	proton	130.0616484	0.69		✔	
83	Cytidine	C9H13N3O5	Neg	243.0855	proton	242.0776918	0.89	✔		
84	D-(-)-Quinic acid	C7H12O6	Neg	192.0634	proton	191.0555602	0.73		✔	
85	D-(+)-Cellobiose	C12H22O11	Neg	342.1162	proton	341.1083812	0.68	✔		
86	D-Arabinonic acid sodium salt	C5H10O6	Neg	166.0477	proton	165.039911	0.67	✔		
87	D-Erythrose-4-P, Na	C4H9O7P	Neg	200.0086	proton	199.0007644	0.68			✔
88	D-Gluconic acid	C6H12O7	Neg	196.0583	proton	195.0504752	0.67	✔	✔	
89	D-myo-inositol-3-phosphate	C6H13O9P	Neg	260.0297	proton	259.0218928	0.65			
90	D-Pantothenic acid hemicalcium salt	C9H17NO5	Neg	219.1107	proton	218.1028422	3.68	✔	✔	✔

Supplementary Table 3.1. Cont'd.

91	D-ribose-5-P-2Na+	C5H11O8P	Neg	230.0192	proton	229.0113286	0.68			
92	dCDP	C9H15N3O10P2	Neg	387.0233	proton	386.015442	0.98			
93	Deoxycholic acid	C24H40O4	Neg	392.2926	proton	391.284819	8.5	✔		
94	dGDP	C10H15N5O10P2	Neg	427.0294	proton	426.02159	3.5			
95	Dihydrofolic acid	C19H21N7O6	Neg	443.1553	proton	442.1474996	4.58			
96	Disodium (2RS)-2-hydroxyadipate	C6H10O5	Neg	162.0528	proton	161.044996	1.69		✔	
97	DL-3-Hydroxybutyric acid	C4H8O3	Neg	104.0473	proton	103.0395168	1.47			
98	DL-3-Phenyllactic acid	C9H10O3	Neg	166.063	proton	165.055166	5.76	✔	✔	
99	DL-p-Hydroxyphenyllactic acid	C9H10O4	Neg	182.0579	proton	181.050081	4.34	✔	✔	✔
100	Dodecanedioic acid	C12H22O4	Neg	230.1518	proton	229.1439762	7.52	✔	✔	
101	Dopamine	C8H11NO2	Neg	153.079	proton	152.0711496	1.12			
102	Doxycycline hyclate	C22H24N2O8	Neg	444.1533	proton	443.1454334	6.18			
103	Ectoine	C6H10N2O2	Neg	142.0742	proton	141.066399	0.73			
104	Ellagic Acid	C14H6O8	Neg	302.0063	proton	300.9984426	5.7			
105	Erythromycin, Cell Culture Grade	C37H67NO13	Neg	733.4612	proton	732.4533922	6.62			
106	Ethyl-β-D-glucuronide	C8H14O7	Neg	222.0739	proton	221.0661244	1.29	✔		
107	FAD	C27H33N9O15P2	Neg	785.1571	proton	784.1493038	5.09			
108	Famotidine	C8H15N7O2S3	Neg	337.0449	proton	336.037108	3.85			
109	Ferulic acid	C10H10O4	Neg	194.0579	proton	193.050081	5.87			
110	Fexofenadine	C32H39NO4	Neg	501.2879	proton	500.2800684	7.07			
111	Folic acid	C19H19N7O6	Neg	441.1397	proton	440.1318504	4.64	✔		
112	Fumaric acid	C4H4O4	Neg	116.011	proton	115.0031334	1.31			✔
113	Furosemide	C12H11ClN2O5S	Neg	330.0077	proton	328.9998936	7.11			
114	GDP	C10H15N5O11P2	Neg	443.0243	proton	442.016505	2.77			
115	Gemfibrozil	C15H22O3	Neg	250.1569	proton	249.1490612	8.6			
116	Genistein	C15H10O5	Neg	270.0528	proton	269.044996	7.2			
117	Glucose-1-phosphate	C6H13O9P	Neg	260.0297	proton	259.0218928	0.67			
118	glucose-6-phosphate	C6H13O9P	Neg	260.0297	proton	259.0218928	0.67			
119	Glutaric acid	C5H8O4	Neg	132.0423	proton	131.0344318	2.42	✔	✔	
120	Gly-Gly	C4H8N2O3	Neg	132.0535	proton	131.0456648	0.6			

Supplementary Table 3.1. Cont'd.

121	Glycochenodeoxycholic Acid	C26H43NO5	Neg	449.3141	proton	448.3062818	7.91			
122	GLYCOCHOLIC ACID HYDRATE	C26H43NO6	Neg	465.309	proton	464.3011968	7.42			
123	GLYCODEOXYCHOLIC ACID	C26H43NO5	Neg	449.3141	proton	448.3062818	7.99	✔		
124	glycolic acid	C2H4O3	Neg	76.01604	proton	75.0082184	0.76	✔	✔	
125	GLYCOLITHOCHOLIC ACID	C26H43NO4	Neg	433.3192	proton	432.3113668	8.5			
126	GLYCOURSODEOXYCHOLIC ACID	C26H43NO5	Neg	449.3141	proton	448.3062818	7.44			
127	Glyphosate	C3H8NO5P	Neg	169.014	proton	168.0061838	0.65			
128	Guanidineacetic acid	C3H7N3O2	Neg	117.0538	proton	116.0459992	0.65			
129	Guanine	C5H5N5O	Neg	151.0494	proton	150.041583	0.93	✔		
130	Guanosine	C10H13N5O5	Neg	283.0917	proton	282.0838398	1.95	✔		
131	Guanosine 5'-monophosphate	C10H14N5O8P	Neg	363.058	proton	362.0501724	0.97			
132	Hexadecanedioic acid	C16H30O4	Neg	286.2144	proton	285.206573	8.4	✔	✔	
133	Hexanoyl glycine	C8H15NO3	Neg	173.1052	proton	172.097363	5.79	✔	✔	✔
134	Hippuric acid	C9H9NO3	Neg	179.0582	proton	178.0504154	5.03		✔	
135	Homocysteic acid	C4H9NO5S	Neg	183.0201	proton	182.0123174	0.65	✔		
136	Hydrochlorothiazide	C7H8ClN3O4S2	Neg	296.9645	proton	295.9566508	4.94			
137	Hydroxy-Proline	C5H9NO3	Neg	131.0582	proton	130.0504154	0.64			
138	Hyodeoxycholic acid	C24H40O4	Neg	392.2926	proton	391.284819	7.96	✔		
139	hypoxanthine	C5H4N4O	Neg	136.0385	proton	135.0306844	1.11	✔	✔	
140	Indole-3-carboxaldehyde	C9H7NO	Neg	145.0528	proton	144.0449362	6.24	✔	✔	
141	Indole-3-carboxylic acid	C9H7NO2	Neg	161.0477	proton	160.0398512	6.11			
142	Indoleacrylic acid	C11H9NO2	Neg	187.0633	proton	186.0555004	6.7		✔	
143	Indolelactic acid	C11H11NO3	Neg	205.0739	proton	204.0660646	5.97	✔	✔	✔
144	Indomethacin	C19H16ClNO4	Neg	357.0768	proton	356.0689556	8.06			
145	Indoxyl Sulfate	C8H7NO4S	Neg	213.0096	proton	212.0017532	5.34		✔	✔
146	Inosine	C10H12N4O5	Neg	268.0808	proton	267.0729412	1.97			
147	Inosine 5'-monophosphate (IMP)	C10H13N4O8P	Neg	348.0471	proton	347.0392738	0.98			
148	Isocitric acid	C6H8O7	Neg	192.027	proton	191.0191768	0.87			
149	Isovaleroyglycine	C7H13NO3	Neg	159.0895	proton	158.0817138	4.49			
150	Kynurenic acid	C10H7NO3	Neg	189.0426	proton	188.0347662	4.68	✔		

Supplementary Table 3.1. Cont'd.

151	Kynurenine	C10H12N2O3	Neg	208.0848	proton	207.0769632	2.83		✓	
152	L-(-)-Malic acid	C4H6O5	Neg	134.0215	proton	133.0136976	0.86	✓		
153	L-(-)-Norepinephrine	C8H11NO3	Neg	169.0739	proton	168.0660646	0.7			
154	L-(+)-Lactate	C3H6O3	Neg	90.03169	proton	89.0238676	1.03		✓	
155	L-2-Aminoadipic acid	C6H11NO4	Neg	161.0688	proton	160.0609796	0.73	✓		
156	L-Alanine	C3H7NO2	Neg	89.04768	proton	88.0398512	0.63	✓	✓	
157	L-Arginine	C6H14N4O2	Neg	174.1117	proton	173.1038454	0.58	✓	✓	
158	L-Ascorbic acid	C6H8O6	Neg	176.0321	proton	175.0242618	0.89			
159	L-Aspartic acid	C4H7NO4	Neg	133.0375	proton	132.0296812	0.64	✓		✓
160	L-Aspartyl-l-phenylalanine methyl este	C14H18N2O5	Neg	294.1216	proton	293.1137408	5.31			
161	L-Cystathionine	C7H14N2O4S	Neg	222.0674	proton	221.0595994	0.6			
162	L-Cysteine-glutathione disulfide	C13H22N4O8S2	Neg	426.0879	proton	425.0800762	0.7		✓	
163	L-Glutamic acid	C5H9NO4	Neg	147.0532	proton	146.0453304	0.65	✓		
164	L-Glutamine	C5H10N2O3	Neg	146.0691	proton	145.061314	0.64	✓		
165	L-Glutathione oxidized	C20H32N6O12S2	Neg	612.152	proton	611.1441302	1.21		✓	
166	L-Glutathione reduced (GSH)	C10H17N3O6S	Neg	307.0838	proton	306.0759772	1.03			✓
167	L-Glycine	C2H5NO2	Neg	75.03203	proton	74.024202	0.61	✓		
168	L-Histidine	C6H9N3O2	Neg	155.0695	proton	154.0616484	0.57	✓		
169	L-Isoleucine	C6H13NO2	Neg	131.0946	proton	130.0867988	1.4			
170	L-Leucine	C6H13NO2	Neg	131.0946	proton	130.0867988	1.4		✓	
171	L-Lysine	C6H14N2O2	Neg	146.1055	proton	145.0976974	0.57	✓	✓	
172	L-Methionine	C5H11NO2S	Neg	149.051	proton	148.0432216	1.03	✓	✓	✓
173	L-Methionine sulfoxide	C5H11NO3S	Neg	165.046	proton	164.0381366	0.66			✓
174	L-Phenylalanine	C9H11NO2	Neg	165.079	proton	164.0711496	3			
175	L-Proline	C5H9NO2	Neg	115.0633	proton	114.0555004	0.72	✓		
176	L-Pyroglutamic acid	C5H7NO3	Neg	129.0426	proton	128.0347662	1.17			
177	L-Serine	C3H7NO3	Neg	105.0426	proton	104.0347662	0.62			
178	L-Threonic acid	C4H8O5	Neg	136.0372	proton	135.0293468	0.69	✓		✓
179	L-Threonine	C4H9NO3	Neg	119.0582	proton	118.0504154	0.64	✓		
180	L-Tryptophan	C11H12N2O2	Neg	204.0899	proton	203.0820482	4.23	✓		

Supplementary Table 3.1. Cont'd.

181	L-Tyrosine	C9H11NO3	Neg	181.0739	proton	180.0660646	1.35	✔		✔
182	L-Valine	C5H11NO2	Neg	117.079	proton	116.0711496	0.88		✔	
183	Lansoprazole	C16H14F3N3O2S	Neg	369.0759	proton	368.068053	1.48			
184	Lithocholic acid	C24H40O3	Neg	376.2977	proton	375.289904	9.11	✔		
185	Malonic acid	C3H4O4	Neg	104.011	proton	103.0031334	0.91	✔		
186	Methyl 4-hydroxybenzoate	C8H8O3	Neg	152.0473	proton	151.0395168	6.44			
187	Methylmalonic acid	C4H6O4	Neg	118.0266	proton	117.0187826	1.61			
188	Myo-Inositol	C6H12O6	Neg	180.0634	proton	179.0555602	0.62			
189	N-(2-Methylbutyryl)glycine	C7H13NO3	Neg	159.0895	proton	158.0817138	4.35	✔	✔	
190	N-(2-Phenylacetyl)glycine	C10H11NO3	Neg	193.0739	proton	192.0660646	5.38		✔	✔
191	N-(3-Phenylpropionyl)glycine	C11H13NO3	Neg	207.0895	proton	206.0817138	5.99			
192	N-Acetyl-glucosamine	C8H15NO6	Neg	221.0899	proton	220.082108	0.72			
193	N-acetyl-glucosamine-1-phosphate	C8H16NO9P	Neg	301.0563	proton	300.0484406	0.69			✔
194	N-Acetyl-L-alanine	C5H9NO3	Neg	131.0582	proton	130.0504154	1.45	✔	✔	
195	N-Acetyl-L-cysteine	C5H9NO3S	Neg	163.0303	proton	162.0224874	2.1		✔	
196	N-Acetyl-L-methionine	C7H13NO3S	Neg	191.0616	proton	190.0537858	4.45	✔		
197	N-Acetyl-L-phenylalanine	C11H13NO3	Neg	207.0895	proton	206.0817138	5.8	✔	✔	
198	N-Acetyl-L-tyrosine	C11H13NO4	Neg	223.0845	proton	222.0766288	4.52			
199	N-Acetyl-serine	C5H9NO4	Neg	147.0532	proton	146.0453304	0.88	✔		
200	N-Acetylneuraminic acid	C11H19NO9	Neg	309.106	proton	308.0981514	0.71	✔		✔
201	N-Acetylserotonin	C12H14N2O2	Neg	218.1055	proton	217.0976974	5.05			
202	N-Butyrylglycine	C6H11NO3	Neg	145.0739	proton	144.0660646	3.11		✔	
203	N-Cinnamoylglycine	C11H11NO3	Neg	205.0739	proton	204.0660646	6.16			
204	N-epsilon-Formyl-lysine	C7H14N2O3	Neg	174.1004	proton	173.0926124	0.78	✔		
205	N-Formyl-L-methionine	C6H11NO3S	Neg	177.046	proton	176.0381366	4.06			
206	N-Isobutylrylglycine	C6H11NO3	Neg	145.0739	proton	144.0660646	2.85			
207	Na-Acetyl-lysine	C8H16N2O3	Neg	188.1161	proton	187.1082616	0.74	✔	✔	✔
208	Naringenin	C15H12O5	Neg	272.0685	proton	271.0606452	7.14			
209	Naringin	C27H32O14	Neg	580.1792	proton	579.1713722	5.91			
210	Nicotinic acid	C6H5NO2	Neg	123.032	proton	122.024202	1.02	✔		

Supplementary Table 3.1. Cont'd.

211	Nα-Acetyl-L-glutamine	C7H12N2O4	Neg	188.0797	proton	187.0718782	0.97			
212	Nα-Acetyl-L-ornithine	C7H14N2O3	Neg	174.1004	proton	173.0926124	0.66			
213	O-Phosphorylethanolamine	C2H8NO4P	Neg	141.0191	proton	140.0112688	0.61			
214	Omeprazole	C17H19N3O3S	Neg	345.1147	proton	344.1068814	5.96			
215	Orotic acid	C5H4N2O4	Neg	156.0171	proton	155.0092814	0.86			✔
216	Oxipurinol	C5H4N4O2	Neg	152.0334	proton	151.0255994	1.4			
217	p-Coumaric Acid	C9H8O3	Neg	164.0473	proton	163.0395168	5.7	✔		
218	Palmitic acid	C16H32O2	Neg	256.2402	proton	255.2323922	8.25		✔	
219	Pantoprazole	C16H15F2N3O4S	Neg	383.0751	proton	382.0673044	6.42			
220	Paracetamol β-D-glucuronide	C14H17NO8	Neg	327.0954	proton	326.0875872	1.12			
221	Phenobarbital (CRM)	C12H12N2O3	Neg	232.0848	proton	231.0769632	1.43			
222	Phenylacetyl L-glutamine	C13H16N2O4	Neg	264.111	proton	263.1031766	5.05	✔	✔	
223	phosphoenolpyruvate	C3H5O6P	Neg	167.9824	proton	166.974551	0.95			
224	Pimelic acid	C7H12O4	Neg	160.0736	proton	159.0657302	5	✔	✔	
225	Pioglitazone	C19H20N2O3S	Neg	356.1195	proton	355.111632	1.23			
226	Purine	C5H4N4	Neg	120.0436	proton	119.0357694	1.3			
227	Pyridoxine	C8H11NO3	Neg	169.0739	proton	168.0660646	1.15			
228	Pyruvic Acid	C3H4O3	Neg	88.01604	proton	87.0082184	0.85	✔		
229	Ranitidine	C13H22N4O3S	Neg	314.1413	proton	313.1334292	4.04			
230	Riboflavin	C17H20N4O6	Neg	376.1383	proton	375.130453	5.16			
231	Rosuvastatin	C22H28FN3O6S	Neg	481.1683	proton	480.160451	7.64			
232	S-(5′-Adenosyl)-L-homocysteine	C14H20N6O5S	Neg	384.1216	proton	383.113758	1.24			✔
233	S-Methyl-L-cysteine	C4H9NO2S	Neg	135.0354	proton	134.0275724	0.77			
234	Saccharin	C7H5NO3S	Neg	182.999	proton	181.991189	4.05			
235	Salicylic Acid	C7H6O3	Neg	138.0317	proton	137.0238676	6.5		✔	
236	Sebacic acid	C10H18O4	Neg	202.1205	proton	201.1126778	6.7	✔	✔	
237	Shikimate	C7H10O5	Neg	174.0528	proton	173.044996	0.86	✔		✔
238	sn-Glycerol-3-phosphate	C3H9O6P	Neg	172.0137	proton	171.0058494	0.65			
239	Sodium taurodeoxycholate hydrate(xH	C26H45NO6S	Neg	499.2967	proton	498.288918	7.74			
240	Suberic acid	C8H14O4	Neg	174.0892	proton	173.0813794	5.67	✔	✔	

Supplementary Table 3.1. Cont'd.

241	Succinic acid	C4H6O4	Neg	118.0266	proton	117.0187826	1.38	✔		
242	Taurine	C2H7NO3S	Neg	125.0147	proton	124.0068382	0.64	✔		
243	TAURO α-MURICHOLIC ACID SODIUM S	C26H45NO7S	Neg	515.2917	proton	514.283833	6.5	✔		
244	TAURO β-MURICHOLIC ACID SODIUM S	C26H45NO7S	Neg	515.2917	proton	514.283833	6.55			
245	TAURO ω-MURICHOLIC ACID SODIUM S	C26H45NO7S	Neg	515.2917	proton	514.283833	6.44			
246	Taurochenodeoxycholic acid	C26H45NO6S	Neg	499.2967	proton	498.288918	7.6	✔		
247	Taurocholic acid sodium salt hydrate	C26H45NO7S	Neg	515.2917	proton	514.283833	7.05	✔		
248	TAURODEHYDROCHOLIC ACID	C26H39NO7S	Neg	509.2447	proton	508.2368854	7.59			
249	Taurohyodeoxycholic acid	C26H45NO6S	Neg	499.2967	proton	498.288918	7.03			
250	Taurolithocholic acid 3-sulfate	C26H45NO8S2	Neg	563.2586	proton	562.25082	7.5			
251	Tauroursodeoxycholic Acid, Sodium Sal	C26H45NO6S	Neg	499.2967	proton	498.288918	7.01	✔		
252	Tetradecanedioic acid	C14H26O4	Neg	258.1831	proton	257.1752746	8.01	✔	✔	
253	Tetrahydrobiopterin	C9H15N5O3	Neg	241.1175	proton	240.109659	0.74			
254	Theophylline	C7H8N4O2	Neg	180.0647	proton	179.0568978	4.31			
255	Thymidine	C10H14N2O5	Neg	242.0903	proton	241.0824424	3.44	✔	✔	✔
256	Thymidine 5'-monophosphate disodiur	C10H15N2O8P	Neg	322.0566	proton	321.048775	1.31	✔		
257	Thymine	C5H6N2O2	Neg	126.0429	proton	125.0351006	1.76	✔		
258	Topiramate	C12H21NO8S	Neg	339.0988	proton	338.0909576	1.76			
259	trans-Urocanic acid	C6H6N2O2	Neg	138.0429	proton	137.0351006	1	✔		
260	Undecanedioic acid	C11H20O4	Neg	216.1362	proton	215.128327	7.15	✔	✔	
261	Uric acid	C5H4N4O3	Neg	168.0283	proton	167.0205144	1.06	✔		
262	uridine	C9H12N2O6	Neg	244.0695	proton	243.0617082	1.27	✔	✔	
263	Uridine 5'-monophosphate (UMP)	C9H13N2O9P	Neg	324.0359	proton	323.0280408	0.86			
264	Ursodeoxycholic acid	C24H40O4	Neg	392.2926	proton	391.284819	7.95			
265	Xanthurenic acid	C10H7NO4	Neg	205.0375	proton	204.0296812	4.41	✔		
266	α-Hydroxyglutaric acid	C5H8O5	Neg	148.0372	proton	147.0293468	1.07			
267	α-ketoglutaric acid	C5H6O5	Neg	146.0215	proton	145.0136976	0.97	✔	✔	✔
268	α-MURICHOLIC ACID	C24H40O5	Neg	408.2876	proton	407.279734	7.51	✔		
269	β-Glycerophosphate	C3H9O6P	Neg	172.0137	proton	171.0058494	0.66			
270	β-Hydroxy pyruvic acid	C3H4O4	Neg	104.011	proton	103.0031334	0.66			✔

Supplementary Table 3.1. Cont'd.

271	β-MURICHOLIC ACID	C24H40O5	Neg	408.2876	proton	407.279734	7.61			
272	β-Nicotinamide adenine dinucleotide h	C21H27N7O14P2	Neg	663.1091	proton	662.1012932	0.98			
273	β-Pseudouridine	C9H12N2O6	Neg	244.0695	proton	243.0617082	0.96	✔		
274	ω-MURICHOLIC ACID	C24H40O5	Neg	408.2876	proton	407.279734	7.43			
275	(-)-Cotinine	C10H12N2O	Pos	176.095	proton	177.1027832	1.25			
276	(+)-Equol 4'-sulfate	C15H14O6S	Pos	322.0511	proton	323.0589314	6.42			
277	(+)-Ibuprofen	C13H18O2	Pos	206.1307	proton	207.1384978	8.35			
278	(+)-Warfarin	C19H16O4	Pos	308.1049	proton	309.1126786	7.57			
279	(±)-Epinephrine	C9H13NO3	Pos	183.0895	proton	184.0973638	0.82	✔		
280	(1S,2S)-(+)-Pseudoephedrine	C10H15NO	Pos	165.1154	proton	166.123183	4.15			
281	(R)-(+)-Atenolol	C14H22N2O3	Pos	266.163	proton	267.1708592	3.98			
282	(S)-Naproxen	C14H14O3	Pos	230.0943	proton	231.1021144	6.38			
283	1-Methyl-4-imidazoleacetic acid	C6H8N2O2	Pos	140.0586	proton	141.0663998	0.77		✔	✔
284	1-Methyl-L-histidine	C7H11N3O2	Pos	169.0851	proton	170.0929476	0.59		✔	
285	1,11-Undecanedicarboxylic acid	C13H24O4	Pos	244.1675	proton	245.1752754	7.8		✔	
286	1,3,7-Trimethyluric acid	C8H10N4O3	Pos	210.0753	proton	211.083112	4.52			
287	1,7-Dimethyluric acid	C7H8N4O3	Pos	196.0596	proton	197.0674628	4.12			
288	1,7-Dimethylxanthine	C7H8N4O2	Pos	180.0647	proton	181.0725478	4.28			
289	17α-Hydroxyprogesterone	C21H30O3	Pos	330.2195	proton	331.227308	8.12			
290	2-nonyl-3-hydroxy-4-Quinolone	C18H25NO2	Pos	287.1885	proton	288.196344	8.5			
291	2-Phenethylamine	C8H11N	Pos	121.0891	proton	122.0969696	3.65			
292	2-Phenoxyethanol	C8H10O2	Pos	138.0681	proton	139.075901	6.1			
293	2-Phenylglycine	C8H9NO2	Pos	151.0633	proton	152.0711504	1.08			
294	2-Picolinic acid	C6H5NO2	Pos	123.032	proton	124.039852	1	✔		
295	2-Pyrrolidinone	C4H7NO	Pos	85.05276	proton	86.0605862	1.5			
296	2,3-pyridinedicarboxylic acid	C7H5NO4	Pos	167.0219	proton	168.029682	1.3			
297	2,4-Quinolinediol	C9H7NO2	Pos	161.0477	proton	162.0555012	5.72		✔	
298	2,8-Quinolinediol	C9H7NO2	Pos	161.0477	proton	162.0555012	5.33	✔		
299	2'-Deoxyadenosine (dA)	C10H13N5O3	Pos	251.1018	proton	252.1096598	1.89			
300	2'-Deoxyadenosine 5'-monophosphate	C10H14N5O6P	Pos	331.0682	proton	332.0759924	1.1			

Supplementary Table 3.1. Cont'd.

301	2'-Deoxycytidine (dC)	C9H13N3O4	Pos	227.0906	proton	228.0984268	0.98	✔		
302	2'-Deoxyguanosine 5'-monophosphate	C10H14N5O7P	Pos	347.0631	proton	348.0709074	1.21			
303	2’-deoxyuridine	C9H12N2O5	Pos	228.0746	proton	229.0824432	1.73		✔	
304	3-Aminophenol	C6H7NO	Pos	109.0528	proton	110.0605862	0.99	✔		
305	3-Aminopiperidine-2,6-dione	C5H8N2O2	Pos	128.0586	proton	129.0663998	0.6			
306	3-Chloro-L-tyrosine	C9H10ClNO3	Pos	215.0349	proton	216.042743	3.28			
307	3-Hydroxy anthranilic acid	C7H7NO3	Pos	153.0426	proton	154.0504162	3.72			
308	3-Indoleethanol	C10H11NO	Pos	161.0841	proton	162.0918846	6.44			
309	3-Indolepropionic acid	C11H11NO2	Pos	189.079	proton	190.0867996	6.9	✔	✔	
310	3-Indolylacetonitrile	C10H8N2	Pos	156.0687	proton	157.0765698	7.25			
311	3-Methyl-Histidine	C7H11N3O2	Pos	169.0851	proton	170.0929476	0.59			
312	3-Methylcrotonyl glycine	C7H11NO3	Pos	157.0739	proton	158.0817146	4.45			
313	3-Methylxanthine	C6H6N4O2	Pos	166.0491	proton	167.0568986	3.03			
314	3-Nitro-L-tyrosine	C9H10N2O5	Pos	226.059	proton	227.066794	3.71			
315	3-Ureidopropionic acid	C4H8N2O3	Pos	132.0535	proton	133.0613148	0.95		✔	
316	3,4-Dihydroxy-L-phenylalanine (L-DOP/	C9H11NO4	Pos	197.0688	proton	198.0766296	1.1	✔		
317	4-Aminobenzoic acid	C7H7NO2	Pos	137.0477	proton	138.0555012	3.69			
318	4-Hydroxybutyric acid (GHB)	C4H8O3	Pos	104.0473	proton	105.0551668	1.24			
319	4-Methoxyindole	C9H9NO	Pos	147.0684	proton	148.0762354	7.38			
320	5-Hydroxy Indole-3-acetic acid	C10H9NO3	Pos	191.0582	proton	192.0660654	4.86			
321	5-hydroxy-Nω-methyl tryptamine (oxal	C11H14N2O	Pos	190.1106	proton	191.1184324	3.03			
322	5-Methyltetrahydrofolic acid	C20H25N7O6	Pos	459.1866	proton	460.194448	3.91			
323	5'-Deoxy-5'-methylthioadenosine	C11H15N5O3S	Pos	297.0896	proton	298.097381	4.29			✔
324	6-hydroxy melatonin	C13H16N2O3	Pos	248.1161	proton	249.1239116	5.37			
325	7-Methylxanthine	C6H6N4O2	Pos	166.0491	proton	167.0568986	2.47			
326	7,8-dihydro-L-Biopterin	C9H13N5O3	Pos	239.1018	proton	240.1096598	1.26			
327	9-Methyluric acid	C6H6N4O3	Pos	182.044	proton	183.0518136	1.38			
328	Acetaminophen	C8H9NO2	Pos	151.0633	proton	152.0711504	3.95			
329	Acetaminophen sulfate	C8H9NO5S	Pos	231.0201	proton	232.0279674	3.6			
330	Acetyl-DL-Carnitine	C9H18NO4, +	Pos	204.1236	M+	204.1235768	0.98			

Supplementary Table 3.1. Cont’d.

331	Acetylcholine chloride	C7H16NO2, +	Pos	146.1181	M+	146.1180976	0.81	✔		
332	Adenine	C5H5N5	Pos	135.0545	proton	136.062318	0.9	✔		✔
333	Adenosine	C10H13N5O4	Pos	267.0967	proton	268.1045748	1.66			✔
334	Adenosine 5'-monophosphate	C10H14N5O7P	Pos	347.0631	proton	348.0709074	0.94			
335	AFMK, i.e. N-[3-[2-(formylamino)-5-me	C13H16N2O4	Pos	264.111	proton	265.1188266	5.78			
336	All-trans Retinoic acid	C20H28O2	Pos	300.2089	proton	301.2167438	9.54			
337	Amitriptyline hydrochloride	C20H23N	Pos	277.183	proton	278.1908648	7.1			
338	Anthranilic acid	C7H7NO2	Pos	137.0477	proton	138.0555012	5.53			
339	Argininosuccinic acid disodium salt hyd	C10H18N4O6	Pos	290.1226	proton	291.1304538	0.66			
340	Atorvastatin	C33H35FN2O5	Pos	558.253	proton	559.2608122	8.14			
341	Atrazine	C8H14ClN5	Pos	215.0938	proton	216.1015924	7.65			
342	Betaine	C5H11NO2	Pos	117.079	proton	118.0867996	0.68			✔
343	Bilirubin	C33H36N4O6	Pos	584.2635	proton	585.2712966	11.14	✔		
344	Biotin	C10H16N2O3S	Pos	244.0882	proton	245.0959836	5.24			
345	Boc-Arg-OH	C11H22N4O4	Pos	274.1641	proton	275.1719222	4.89			
346	Butyryl-L-carnitine	C11H21NO4	Pos	231.1471	proton	232.1548756	3.9	✔	✔	
347	Caffeic acid	C9H8O4	Pos	180.0423	proton	181.0500818	5.12			
348	Caffeine	C8H10N4O2	Pos	194.0804	proton	195.088197	4.94			
349	Carbamazepine	C15H12N2O	Pos	236.095	proton	237.1027832	7.19			
350	Carbaryl	C12H11NO2	Pos	201.079	proton	202.0867996	7.59			
351	CDP	C9H15N3O11P2	Pos	403.0182	proton	404.026007	0.86			
352	Celecoxib	C17H14F3N3O2S	Pos	381.0759	proton	382.083703	8.33			
353	Cholesteryl oleate	C45H78O2	Pos	650.6001	proton	651.6079738	9.5			
354	Choline	C5H14NO, +	Pos	104.1075	M+	104.1075334	0.63	✔	✔	
355	Cimetidine	C10H16N6S	Pos	252.1157	proton	253.1235346	1.02			
356	Cinnabarinic acid	C14H8N2O6	Pos	300.0382	proton	301.0460598	6.53			
357	Clotrimazole	C22H17ClN2	Pos	344.108	proton	345.1158442	7.37			
358	Corticosterone	C21H30O4	Pos	346.2144	proton	347.222223	7.44		✔	✔
359	Cortisone	C21H28O5	Pos	360.1937	proton	361.2014888	6.9			
360	Coumarin	C9H6O2	Pos	146.0368	proton	147.0446026	6.72			

Supplementary Table 3.1. Cont'd.

361	Creatine	C4H9N3O2	Pos	131.0695	proton	132.0772984	0.69		✔	
362	Creatinine	C4H7N3O	Pos	113.0589	proton	114.0667342	0.66	✔	✔	
363	Cytidine	C9H13N3O5	Pos	243.0855	proton	244.0933418	0.89	✔		
364	Cytosine	C4H5N3O	Pos	111.0433	proton	112.051085	0.66	✔		
365	D-(-)-Quinic acid	C7H12O6	Pos	192.0634	proton	193.0712102	0.73			
366	D-Pantothenic acid hemicalcium salt	C9H17NO5	Pos	219.1107	proton	220.1184922	3.68	✔	✔	✔
367	D,L-O-Desmethyl Venlafaxine	C16H25NO2	Pos	263.1885	proton	264.196344	5.39			
368	dCDP	C9H15N3O10P2	Pos	387.0233	proton	388.031092	0.98			
369	Dexamethasone	C22H29FO5	Pos	392.1999	proton	393.2077166	1.38			
370	dGDP	C10H15N5O10P2	Pos	427.0294	proton	428.03724	3.5			
371	Diltiazem	C22H26N2O4S	Pos	414.1613	proton	415.1691446	6.74			
372	Dodecanedioic acid	C12H22O4	Pos	230.1518	proton	231.1596262	7.52		✔	
373	Dopamine	C8H11NO2	Pos	153.079	proton	154.0867996	1.12			
374	Doxycycline hyclate	C22H24N2O8	Pos	444.1533	proton	445.1610834	6.18			
375	Doxylamine	C17H22N2O	Pos	270.1732	proton	271.1810292	4.94			
376	Ectoine	C6H10N2O2	Pos	142.0742	proton	143.082049	0.73	✔	✔	
377	Erythromycin, Cell Culture Grade	C37H67NO13	Pos	733.4612	proton	734.4690422	6.62			
378	Escitalopram	C20H21FN2O	Pos	324.1638	proton	325.1716078	6.65			
379	Ethanolamine Hydrochloride	C2H7NO	Pos	61.05276	proton	62.0605862	0.59			
380	Famotidine	C8H15N7O2S3	Pos	337.0449	proton	338.052758	3.85			
381	Ferulic acid	C10H10O4	Pos	194.0579	proton	195.065731	5.87		✔	
382	Fexofenadine	C32H39NO4	Pos	501.2879	proton	502.2957184	7.07			
383	Flavone	C15H10O2	Pos	222.0681	proton	223.075901	8.1			
384	Fluoxetine	C17H18NOF3	Pos	309.134	proton	310.1418664	7.13			
385	Folic acid	C19H19N7O6	Pos	441.1397	proton	442.1475004	4.64			
386	Gabapentin	C9H17NO2	Pos	171.1259	proton	172.1337472	4.33			
387	GDP	C10H15N5O11P2	Pos	443.0243	proton	444.032155	2.77			
388	Genistein	C15H10O5	Pos	270.0528	proton	271.060646	7.2			
389	Glucose-1-phosphate	C6H13O9P	Pos	260.0297	proton	261.0375428	0.67			
390	Gly-Gly	C4H8N2O3	Pos	132.0535	proton	133.0613148	0.6			

Supplementary Table 3.1. Cont'd.

391	Guanidineacetic acid	C3H7N3O2	Pos	117.0538	proton	118.0616492	0.65			
392	Guanine	C5H5N5O	Pos	151.0494	proton	152.057233	0.93			
393	Guanosine	C10H13N5O5	Pos	283.0917	proton	284.0994898	1.95	✔		
394	Guanosine 5'-monophosphate	C10H14N5O8P	Pos	363.058	proton	364.0658224	0.97			
395	Hexadecanedioic acid	C16H30O4	Pos	286.2144	proton	287.222223	8.4		✔	
396	Hexanoyl glycine	C8H15NO3	Pos	173.1052	proton	174.113013	5.79		✔	
397	Hippuric acid	C9H9NO3	Pos	179.0582	proton	180.0660654	5.03		✔	
398	Histamine	C5H9N3	Pos	111.0796	proton	112.0874684	0.54	✔	✔	
399	Homocysteic acid	C4H9NO5S	Pos	183.0201	proton	184.0279674	0.65			
400	Hydrocortisone / Cortisol	C21H30O5	Pos	362.2093	proton	363.217138	6.85	✔	✔	
401	Hydroxy-Proline	C5H9NO3	Pos	131.0582	proton	132.0660654	0.64			
402	hypoxanthine	C5H4N4O	Pos	136.0385	proton	137.0463344	1.11		✔	
403	Imidazole	C3H4N2	Pos	68.03745	proton	69.0452714	0.63			
404	Indigo	C16H10N2O2	Pos	262.0742	proton	263.082049	8.2			
405	Indirubin	C16H10N2O2	Pos	262.0742	proton	263.082049	8.19			
406	Indole	C8H7N	Pos	117.0578	proton	118.0656712	7.52	✔		
407	Indole-3-acetic acid	C10H9NO2	Pos	175.0633	proton	176.0711504	6.39	✔		
408	Indole-3-carboxaldehyde	C9H7NO	Pos	145.0528	proton	146.0605862	6.24	✔	✔	
409	Indole-3-carboxylic acid	C9H7NO2	Pos	161.0477	proton	162.0555012	6.11			
410	Indoleacrylic acid	C11H9NO2	Pos	187.0633	proton	188.0711504	6.7		✔	
411	Indolelactic acid	C11H11NO3	Pos	205.0739	proton	206.0817146	5.97		✔	
412	Indomethacin	C19H16ClNO4	Pos	357.0768	proton	358.0846056	8.06			
413	Inosine	C10H12N4O5	Pos	268.0808	proton	269.0885912	1.97			✔
414	Inosine 5'-monophosphate (IMP)	C10H13N4O8P	Pos	348.0471	proton	349.0549238	0.98			
415	Isobutyryl-L-carnitine	C11H21NO4	Pos	231.1471	proton	232.1548756	3.78			
416	Isovaleroyglycine	C7H13NO3	Pos	159.0895	proton	160.0973638	4.49			
417	Isovaleryl-L-carnitine	C12H23NO4	Pos	245.1627	proton	246.1705248	4.9	✔		
418	Kynurenic acid	C10H7NO3	Pos	189.0426	proton	190.0504162	4.68	✔		
419	Kynurenine	C10H12N2O3	Pos	208.0848	proton	209.0926132	2.83	✔	✔	
420	L-(-)-Norepinephrine	C8H11NO3	Pos	169.0739	proton	170.0817146	0.7	✔		

Supplementary Table 3.1. Cont'd.

421	L-2-Aminoadipic acid	C6H11NO4	Pos	161.0688	proton	162.0766296	0.73	✓		
422	L-Acetylcarnitine	C9H18NO4, +	Pos	204.1236	M+	204.1235768	0.99		✓	
423	L-Alanine	C3H7NO2	Pos	89.04768	proton	90.0555012	0.63	✓		
424	L-Arginine	C6H14N4O2	Pos	174.1117	proton	175.1194954	0.58	✓	✓	
425	L-Aspartic acid	C4H7NO4	Pos	133.0375	proton	134.0453312	0.64			✓
426	L-Aspartyl-l-phenylalanine methyl este	C14H18N2O5	Pos	294.1216	proton	295.1293908	5.31			
427	L-Carnitine	C7H16NO3, +	Pos	162.113	M+	162.1130126	0.65	✓	✓	
428	L-Cystathionine	C7H14N2O4S	Pos	222.0674	proton	223.0752494	0.6	✓		
429	L-Cysteine	C3H7NO2S	Pos	121.0197	proton	122.0275732	0.61			
430	L-Cysteine-glutathione disulfide	C13H22N4O8S2	Pos	426.0879	proton	427.0957262	0.7		✓	
431	L-Glutamic acid	C5H9NO4	Pos	147.0532	proton	148.0609804	0.65	✓		
432	L-Glutamine	C5H10N2O3	Pos	146.0691	proton	147.076964	0.64	✓		
433	L-Glutathione oxidized	C20H32N6O12S2	Pos	612.152	proton	613.1597802	1.21		✓	
434	L-Glutathione reduced (GSH)	C10H17N3O6S	Pos	307.0838	proton	308.0916272	1.03			✓
435	L-Glycine	C2H5NO2	Pos	75.03203	proton	76.039852	0.61		✓	
436	L-Histidine	C6H9N3O2	Pos	155.0695	proton	156.0772984	0.57	✓		
437	L-Homocysteine	C4H9NO3	Pos	119.0582	proton	120.0660654	0.78	✓		
438	L-Isoleucine	C6H13NO2	Pos	131.0946	proton	132.1024488	1.4			
439	L-Leucine	C6H13NO2	Pos	131.0946	proton	132.1024488	1.4		✓	
440	L-Lysine	C6H14N2O2	Pos	146.1055	proton	147.1133474	0.57	✓	✓	
441	L-Methionine	C5H11NO2S	Pos	149.051	proton	150.0588716	1.03	✓	✓	✓
442	L-Methionine sulfoxide	C5H11NO3S	Pos	165.046	proton	166.0537866	0.66			✓
443	L-Phenylalanine	C9H11NO2	Pos	165.079	proton	166.0867996	3			
444	L-Pipecolic acid	C6H11NO2	Pos	129.079	proton	130.0867996	0.92			
445	L-Proline	C5H9NO2	Pos	115.0633	proton	116.0711504	0.72	✓		
446	L-Pyroglutamic acid	C5H7NO3	Pos	129.0426	proton	130.0504162	1.17			
447	L-Serine	C3H7NO3	Pos	105.0426	proton	106.0504162	0.62			
448	L-Threonine	C4H9NO3	Pos	119.0582	proton	120.0660654	0.64	✓		
449	L-Tryptophan	C11H12N2O2	Pos	204.0899	proton	205.0976982	4.23	✓		
450	L-Tyrosine	C9H11NO3	Pos	181.0739	proton	182.0817146	1.35	✓		✓

Supplementary Table 3.1. Cont'd.

451	L-Valine	C5H11NO2	Pos	117.079	proton	118.0867996	0.88		✔	
452	Lansoprazole	C16H14F3N3O2S	Pos	369.0759	proton	370.083703	1.48			
453	Lidocaine	C14H22N2O	Pos	234.1732	proton	235.1810292	1.21			
454	Linoleamide	C18H33NO	Pos	279.2562	proton	280.2640258	9.28	✔		✔
455	Malathion	C10H19O6PS2	Pos	330.0361	proton	331.0438894	8.37			
456	Melatonin	C13H16N2O2	Pos	232.1212	proton	233.1289966	6.27			
457	Metformin	C4H11N5	Pos	129.1014	proton	130.1092656	1			
458	Methyl 4-hydroxybenzoate	C8H8O3	Pos	152.0473	proton	153.0551668	6.44			
459	Methyl indole-3-acetate	C11H11NO2	Pos	189.079	proton	190.0867996	7.51		✔	
460	Metoprolol	C15H25NO3	Pos	267.1834	proton	268.191259	5.63			
461	N-(2-Methylbutyryl)glycine	C7H13NO3	Pos	159.0895	proton	160.0973638	4.35	✔	✔	
462	N-(2-Phenylacetyl)glycine	C10H11NO3	Pos	193.0739	proton	194.0817146	5.38		✔	
463	N-(3-Aminopropyl)-2-pyrrolidinone	C7H14N2O	Pos	142.1106	proton	143.1184324	1.07		✔	
464	N-(3-hydroxy-7-cis tetradecenoyl)-L-Hc	C18H31NO4	Pos	325.2253	proton	326.2331216	8.39			
465	N-(3-Oxododecanoyl-L-homoserine lac	C16H27NO4	Pos	297.194	proton	298.2018232	8.31			
466	N-(3-Phenylpropionyl)glycine	C11H13NO3	Pos	207.0895	proton	208.0973638	5.99			
467	N-(β-ketocaproyl)-L-homoserine lacton	C10H15NO4	Pos	213.1001	proton	214.107928	5.04			
468	N-3-hydroxydecanoyl-L-Homoserine la	C14H25NO4	Pos	271.1783	proton	272.186174	7.55		✔	
469	N-3-hydroxyoctanoyl-L-Homoserine lac	C12H21NO4	Pos	243.1471	proton	244.1548756	6.45			
470	N-3-oxo-hexadec-11(Z)-enoyl-L-Homos	C20H33NO4	Pos	351.2409	proton	352.2487708	8.86			
471	N-3-oxo-hexadecanoyl-L-Homoserine l	C20H35NO4	Pos	353.2566	proton	354.26442	9.18			
472	N-3-oxo-tetradec-7(Z)-enoyl-L-Homose	C18H29NO4	Pos	323.2096	proton	324.2174724	8.6			
473	N-3-oxo-tetradecanoyl-L-homoserine l	C18H31NO4	Pos	325.2253	proton	326.2331216	8.75			
474	N-3-oxodecanoyl-L-homoserine lactone	C14H23NO4	Pos	269.1627	proton	270.1705248	7.8			
475	N-3-oxooctanoyl-L-homoserine lactone	C12H19NO4	Pos	241.1314	proton	242.1392264	6.78			
476	N-Acetyl-D-galactosamine	C8H15NO6	Pos	221.0899	proton	222.097758	0.71	✔		
477	N-Acetyl-glucosamine	C8H15NO6	Pos	221.0899	proton	222.097758	0.72			
478	N-Acetyl-L-cysteine	C5H9NO3S	Pos	163.0303	proton	164.0381374	2.1			
479	N-Acetyl-L-methionine	C7H13NO3S	Pos	191.0616	proton	192.0694358	4.45	✔		
480	N-Acetyl-L-phenylalanine	C11H13NO3	Pos	207.0895	proton	208.0973638	5.8	✔	✔	

Supplementary Table 3.1. Cont'd.

481	N-Acetyl-L-tyrosine	C11H13NO4	Pos	223.0845	proton	224.0922788	4.52			
482	N-Acetyl-serine	C5H9NO4	Pos	147.0532	proton	148.0609804	0.88			
483	N-Acetylneuraminic acid	C11H19NO9	Pos	309.106	proton	310.1138014	0.71	✔		✔
484	N-Acetylserotonin	C12H14N2O2	Pos	218.1055	proton	219.1133474	5.05			
485	N-Butyryl-L-homoserine lactone	C8H13NO3	Pos	171.0895	proton	172.0973638	4.05			
486	N-Butyrylglycine	C6H11NO3	Pos	145.0739	proton	146.0817146	3.11		✔	
487	N-Cinnamoylglycine	C11H11NO3	Pos	205.0739	proton	206.0817146	6.16			
488	N-cis-octadec-9Z-enoyl-L-Homoserine l	C22H39NO3	Pos	365.293	proton	366.3008034	9.75			
489	N-cis-tetradec-9Z-enoyl-L-Homoserine	C18H31NO3	Pos	309.2304	proton	310.2382066	8.79			
490	N-decanoyl-L-homoserine lactone	C14H25NO3	Pos	255.1834	proton	256.191259	8.21			
491	N-dodecanoyl-L-homoserine lactone	C16H29NO3	Pos	283.2147	proton	284.2225574	8.68		✔	
492	N-epsilon-Formyl-lysine	C7H14N2O3	Pos	174.1004	proton	175.1082624	0.78			
493	N-Formyl-L-methionine	C6H11NO3S	Pos	177.046	proton	178.0537866	4.06			
494	N-heptanoyl-L-homoserine lactone	C11H19NO3	Pos	213.1365	proton	214.1443114	7.06			
495	N-hexadecanoyl-L-Homoserine lactone	C20H37NO3	Pos	339.2773	proton	340.2851542	9.64			
496	N-hexanoyl-L-homoserine lactone	C10H17NO3	Pos	199.1208	proton	200.1286622	6.33			
497	N-Isobutylrylglycine	C6H11NO3	Pos	145.0739	proton	146.0817146	2.85			
498	N-methyl tryptamine	C11H14N2	Pos	174.1157	proton	175.1235174	4.85			
499	N-Methylphenethylamine	C9H13N	Pos	135.1048	proton	136.1126188	4			
500	N-nonanoyl-L-Homoserine lactone	C13H23NO3	Pos	241.1678	proton	242.1756098	7.95		✔	
501	N-octadecanoyl-L-Homoserine lactone	C22H41NO3	Pos	367.3086	proton	368.3164526	10.32			
502	N-octanoyl-L-homoserine lactone	C12H21NO3	Pos	227.1521	proton	228.1599606	7.74			
503	N-pentadecanoyl-L-Homoserine lacton	C19H35NO3	Pos	325.2617	proton	326.269505	9.37			
504	N-tetradecanoyl-L-homoserine lactone	C18H33NO3	Pos	311.246	proton	312.2538558	8.8			
505	N-tridecanoyl-L-Homoserine lactone	C17H31NO3	Pos	297.2304	proton	298.2382066	8.9			
506	N-Undecanoyl-L-Homoserine lactone	C15H27NO3	Pos	269.1991	proton	270.2069082	8.45			
507	N,N-Dimethylglycine	C4H9NO2	Pos	103.0633	proton	104.0711504	0.65			
508	Na-Acetyl-lysine	C8H16N2O3	Pos	188.1161	proton	189.1239116	0.74		✔	
509	NADH	C21H28N7O14P2, +	Pos	664.1169	M+	664.1169428	0.99			
510	Naringenin	C15H12O5	Pos	272.0685	proton	273.0762952	7.14			

Supplementary Table 3.1. Cont'd.

511	Naringin	C27H32O14	Pos	580.1792	proton	581.1870222	5.91			
512	Nicotinamide	C6H6N2O	Pos	122.048	proton	123.0558356	1.06		✔	✔
513	Nicotinic acid	C6H5NO2	Pos	123.032	proton	124.039852	1.02	✔		
514	Norfluoxetine	C16H16F3NO	Pos	295.1184	proton	296.1262172	7.11			
515	Nudifloramide	C7H8N2O2	Pos	152.0586	proton	153.0663998	2	✔	✔	
516	Nα-Acetyl-L-glutamine	C7H12N2O4	Pos	188.0797	proton	189.0875282	0.97			
517	Nα-Acetyl-L-ornithine	C7H14N2O3	Pos	174.1004	proton	175.1082624	0.66			
518	O-Methyl-DL-serine	C4H9NO3	Pos	119.0582	proton	120.0660654	0.68		✔	
519	O-Phosphorylethanolamine	C2H8NO4P	Pos	141.0191	proton	142.0269188	0.61			
520	Octanoyl-L-carnitine	C15H29NO4	Pos	287.2096	proton	288.2174724	6.66	✔		
521	Oleamide	C18H35NO	Pos	281.2719	proton	282.279675	9.68			
522	Oleoyl Ethanolamide	C20H39NO2	Pos	325.2981	proton	326.3058884	9.5			
523	Omeprazole	C17H19N3O3S	Pos	345.1147	proton	346.1225314	5.96			
524	p-Coumaric Acid	C9H8O3	Pos	164.0473	proton	165.0551668	5.7			
525	Palmitoyl Ethanolamide	C18H37NO2	Pos	299.2824	proton	300.2902392	9.38	✔		
526	Palmitoyl-L-carnitine	C23H45NO4	Pos	399.3348	proton	400.342666	8.26	✔	✔	
527	Pantoprazole	C16H15F2N3O4S	Pos	383.0751	proton	384.0829544	6.42			
528	Paracetamol β-D-glucuronide	C14H17NO8	Pos	327.0954	proton	328.1032372	1.12			
529	Paroxetine	C19H20FNO3	Pos	329.1427	proton	330.1505392	1.27			
530	Phenobarbital (CRM)	C12H12N2O3	Pos	232.0848	proton	233.0926132	1.43			
531	Phenylacetyl L-glutamine	C13H16N2O4	Pos	264.111	proton	265.1188266	5.05			
532	Phosphocholine	C5H15NO4P, +	Pos	184.0739	M+	184.073866	0.65			
533	Pioglitazone	C19H20N2O3S	Pos	356.1195	proton	357.127282	1.23			
534	Pipecolinic acid	C6H11NO2	Pos	129.079	proton	130.0867996	0.91	✔		
535	Piperine	C17H19NO3	Pos	285.1365	proton	286.1443114	8.11			
536	Purine	C5H4N4	Pos	120.0436	proton	121.0514194	1.3			
537	Putrescine	C4H12N2	Pos	88.10004	proton	89.1078682	0.52			
538	Pyridoxamine dihydrochloride	C8H12N2O2	Pos	168.0899	proton	169.0976982	0.61			
539	Pyridoxine	C8H11NO3	Pos	169.0739	proton	170.0817146	1.15	✔		
540	Quinine	C20H24O2N2	Pos	324.1838	proton	325.1915934	1.06			

Supplementary Table 3.1. Cont'd.

541	Ranitidine	C13H22N4O3S	Pos	314.1413	proton	315.1490792	4.04			
542	Riboflavin	C17H20N4O6	Pos	376.1383	proton	377.146103	5.16		✔	
543	Rosuvastatin	C22H28FN3O6S	Pos	481.1683	proton	482.176101	7.64			
544	S-(5'-Adenosyl)-L-homocysteine	C14H20N6O5S	Pos	384.1216	proton	385.129408	1.24		✔	✔
545	S-Methyl-L-cysteine	C4H9NO2S	Pos	135.0354	proton	136.0432224	0.77			
546	Sarcosine	C3H7NO2	Pos	89.04768	proton	90.0555012	0.63	✔	✔	
547	Serotonin	C10H12N2O	Pos	176.095	proton	177.1027832	2.42	✔		
548	sn-Glycero-3-phosphocholine	C8H20NO6P	Pos	257.1028	proton	258.110644	0.64		✔	
549	sn-Glycerol-3-phosphate	C3H9O6P	Pos	172.0137	proton	173.0214994	0.65		✔	
550	Spermidine	C7H19N3	Pos	145.1579	proton	146.1657144	0.49	✔	✔	
551	Spermine	C10H26N4	Pos	202.2157	proton	203.2235606	0.56			
552	Sphinganine (d18:0)	C18H39NO2	Pos	301.2981	proton	302.3058884	8.03		✔	
553	Sphingosine (d18:1)	C18H37NO2	Pos	299.2824	proton	300.2902392	7.96			
554	Stearamide	C18H37NO	Pos	283.2875	proton	284.2953242	10.33			
555	Taurine	C2H7NO3S	Pos	125.0147	proton	126.0224882	0.64	✔		
556	Testosterone	C19H28O2	Pos	288.2089	proton	289.2167438	7.91			
557	Tetradecanedioic acid	C14H26O4	Pos	258.1831	proton	259.1909246	8.01		✔	
558	Tetrahydrobiopterin	C9H15N5O3	Pos	241.1175	proton	242.125309	0.74			
559	Theobromine	C7H8N4O2	Pos	180.0647	proton	181.0725478	3.87			
560	Theophylline	C7H8N4O2	Pos	180.0647	proton	181.0725478	4.31			
561	Thiamine	C12H17N4OS, +	Pos	265.1123	M+	265.1123012	0.63	✔		
562	Thiamine pyrophosphate	C12H19N4O7P2S, +	Pos	425.045	M+	425.0449664	0.66			
563	Thymidine	C10H14N2O5	Pos	242.0903	proton	243.0980924	3.44	✔	✔	✔
564	Thymidine 5'-monophosphate disodiur	C10H15N2O8P	Pos	322.0566	proton	323.064425	1.31			
565	Thymine	C5H6N2O2	Pos	126.0429	proton	127.0507506	1.76	✔		
566	Tigloyl-L-carnitine	C12H21NO4	Pos	243.1471	proton	244.1548756	4.57			
567	Topiramate	C12H21NO8S	Pos	339.0988	proton	340.1066076	1.76			
568	trans-2-Tetradecenoyl-L-carnitine	C21H39NO4	Pos	369.2879	proton	370.2957184	7.98	✔	✔	
569	trans-3'-Hydroxycotinine	C10H12N2O2	Pos	192.0899	proton	193.0976982	0.97			
570	trans-Urocanic acid	C6H6N2O2	Pos	138.0429	proton	139.0507506	1	✔		

Supplementary Table 3.1. Cont'd.

571	Triamterene	C12H11N7	Pos	253.1076	proton	254.1154136	1.07			
572	Trimethylamine N-oxide	C3H9NO	Pos	75.06841	proton	76.0762354	0.65	✔	✔	✔
573	Tryptamine hydrochloride	C10H12N2	Pos	160.1	proton	161.1078682	4.61			
574	Tyramine	C8H11NO	Pos	137.0841	proton	138.0918846	1.51	✔		
575	Undecanedioic acid	C11H20O4	Pos	216.1362	proton	217.143977	7.15		✔	
576	Urea	NH2CONH2	Pos	60.03236	proton	61.0401864	0.67			
577	uridine	C9H12N2O6	Pos	244.0695	proton	245.0773582	1.27	✔	✔	
578	Uridine 5'-monophosphate (UMP)	C9H13N2O9P	Pos	324.0359	proton	325.0436908	0.86			
579	Valeryl-L-carnitine	C12H23NO4	Pos	245.1627	proton	246.1705248	4.99			
580	Venlafaxine	C17H27NO2	Pos	277.2042	proton	278.2119932	6.16			
581	Xanthine	C5H4N4O2	Pos	152.0334	proton	153.0412494	2.41	✔		
582	Xanthurenic acid	C10H7NO4	Pos	205.0375	proton	206.0453312	4.41	✔		
583	α-(Methylamino)isobutyric acid	C5H11NO2	Pos	117.079	proton	118.0867996	0.76			
584	β-Alanine	C3H7NO2	Pos	89.04768	proton	90.0555012	0.61			
585	β-Glycerophosphate	C3H9O6P	Pos	172.0137	proton	173.0214994	0.66			
586	β-Pseudouridine	C9H12N2O6	Pos	244.0695	proton	245.0773582	0.96			
587	γ-Aminobutyric acid	C4H9NO2	Pos	103.0633	proton	104.0711504	0.65	✔	✔	
588	γ-CEHC	C15H20O4	Pos	264.1362	proton	265.143977	7.36			

Supplementary Table 3.1. Cont'd.

Supplementary Table 3.2. Unique list of 533 fecal metabolites altered in feces comparing GF and CONV-R mice (Welch's t-test, fold change≥1.5, p<0.01).

	ESI mode	Platform	RT (min)	PRECURSOR MZ	PRECURSOR	PubChem_CID	Compound name (feces)	fold	updown (GF/C	pvalue	qvalue	MSI level
1	Neg	untargeted	4.95	273.007	[M-H]-	71749556	Isoferulate 3-sulfate	2176.5	UP	6.98983E-11	2.3991E-10	2
2	Neg	targeted	7.43	407.279734	[M-H]-	5283852	α-Muricholate + β-Muricholate (αMCA+βMCA)	1177.0	DOWN	0.000543353	0.00041082	1
3	Pos	untargeted	7.77	284.6559	[M+H]2+	121893	Protoporphyrinogen IX	1109.7	DOWN	0.003831344	0.0025081	2
4	Neg	untargeted	6.71	329.1031	[M-H]-	53481022	Aflatoxin B1 dialcohol	958.8	DOWN	0.005081651	0.00309875	2
5	Neg	targeted	8.42	391.284819	[M-H]-	10133	Chenodeoxycholate	723.4	DOWN	0.000789239	0.0005787	1
6	Neg	untargeted	8.06	591.3524	[M+FA-H]-	10940565	16-Glutaryloxy-1α,25-dihydroxyvitamin D3	705.9	UP	1.54267E-05	1.5466E-05	2
7	Neg	untargeted	7.8	405.2645	[M-H2O-H]-	53481262	3α,6β,7β,12α-Tetrahydroxy-5β-cholanoate	669.6	DOWN	5.2442E-05	4.7967E-05	2
8	Neg	untargeted	8.41	389.2701	[M-H]-	53477693	Nutriacholate	563.5	DOWN	5.12429E-06	5.5745E-06	2
9	Neg	untargeted	9.18	403.2848	[M-H]-	53481453	11'-Carboxy-γ-chromanol	537.3	DOWN	0.001459124	0.00101225	2
10	Neg	untargeted	4.73	333.0281	[M-H]-	124202100	5-(3',4',5'-Trihydroxyphenyl)-γ-valerolactone-O-methyl-O-sulfate	512.0	UP	5.20517E-12	2.6157E-11	2
11	Pos	untargeted	5.28	565.1537	[M+H]+	5840046	Apiin	477.1	UP	1.74039E-11	8.7209E-11	2
12	Pos	untargeted	7.94	615.3127	[M+H]+	193825	Harderoporphyrinogen	408.5	DOWN	0.009725621	0.00564121	2
13	Neg	untargeted	6.04	505.0977	[M+FA-H]-	124202362	Glycitein 4'-O-glucuronide	385.8	UP	0.000338048	0.00026659	2
14	Neg	untargeted	8.41	873.5441	[M-H]-	52927610	PI(15:1(9Z)/22:2(13Z,16Z))	333.2	DOWN	0.000532443	0.00040332	2
15	Neg	untargeted	3.53	340.1152	[M-H]-	53478751	Phosphatidylcholine 40:6	318.4	UP	9.82984E-07	1.2089E-06	2
16	Neg	targeted	7.99	448.3062818	[M-H]-	22833539	Glycodeoxycholate	316.8	DOWN	0.002955112	0.00191432	1
17	Pos	untargeted	8.99	265.2519	[M+H]+	5367327	(9Z,12Z,15Z)-Octadecatrien-1-ol	276.3	DOWN	0.005222551	0.00328357	2
18	Neg	untargeted	8.5	611.3798	[M-H]-	42622727	Cholestane-3,7,12,25-tetrol-3-glucuronide	245.4	UP	3.15846E-08	5.2237E-08	2
19	Neg	untargeted	7.92	593.3356	[M-H]-	440785	L-Urobilin	215.1	DOWN	0.000509451	0.00038741	2
20	Pos (& Neg)	untargeted	7.38	391.2838	[M+H-H2O]+	5283869	3α,7α,12β-Trihydroxy-5β-cholanoate	212.6	DOWN	0.002473222	0.00170956	2
21	Neg	untargeted	8.54	629.3332	[M+FA-H]-	52928632	PI(P-18:0/0:0)	193.4	UP	2.63938E-10	7.6984E-10	2
22	Pos	untargeted	6.96	326.2323	[M+H]+	131753085	N-[[3-Hydroxy-2-(2-pentenyl)cyclopentyl]acetyl]isoleucine	187.3	UP	3.04969E-11	1.3856E-10	2
23	Neg	targeted	7.89	407.279734	[M-H]-	5460314	Cholate	175.6	DOWN	0.003352823	0.00214175	1
24	Pos	untargeted	8.33	413.3767	[M+H]+	50990081	4,4-Dimethyl-5α-cholesta-8,24-dien-3-β-ol	175.1	UP	1.61304E-08	3.4486E-08	2
25	Pos	untargeted	7.19	407.2784	[M+H]+	188292	7-Ketodeoxycholate	174.2	DOWN	0.000133846	0.00012682	2
26	Pos	untargeted	1.83	265.0849	[M+H]+	152314	N-Acetylcystathionine	171.5	UP	1.00491E-11	5.4586E-11	2
27	Neg	targeted	8.5	391.284819	[M-H]-	222528	Deoxycholate	169.4	DOWN	0.00291414	0.0018909	1
28	Pos	untargeted	8.09	339.2681	[M+H-H2O]+	6439582	Tetracosahexaenoate	163.3	DOWN	0.001580895	0.00114833	2
29	Pos	untargeted	7.81	355.2622	[M+H-H2O]+	5284174	3β-Hydroxychola-5,7-dien-24-oate	158.6	DOWN	0.000904415	0.00070151	2
30	Neg	untargeted	7.56	835.5343	[M-H]-	5771758	PI(16:0/18:1(9Z))	151.6	DOWN	0.003582978	0.00227224	2
31	Pos (& Neg)	untargeted	4.36	281.1125	[M+H]+	93078	Aspartyl-phenylalanine	124.5	UP	8.03801E-14	1.0494E-12	2
32	Neg	untargeted	8.62	637.3958	[M+Cl]-	52929659	PA(P-16:0/14:1(9Z))	122.0	UP	3.33145E-09	7.0571E-09	2
33	Pos	untargeted	8.19	389.2673	[M+H]+	5284144	3α,12α-Dihydroxy-5β-chola-7,9(11)-dien-24-oate	120.3	DOWN	0.000131422	0.00012474	2
34	Neg	targeted	7.96	391.284819	[M-H]-	9963687	Hyodeoxycholate	119.7	DOWN	0.000346141	0.00027241	1
35	Pos	untargeted	6.84	400.2694	[M+H]+	71464551	(5Z)-13-Carboxytridec-5-enoylcarnitine	119.1	UP	7.75156E-09	1.8019E-08	2
36	Neg	untargeted	4.99	324.1561	[M+Cl]-	131801513	3-Hydroxyhexanoylcarnitine	118.7	UP	2.60236E-13	2.3631E-12	2
37	Pos	untargeted	9.2	249.2574	[M+H]+	21629432	6Z,9Z,12Z-Octadecatriene	111.0	DOWN	0.008650118	0.00509725	2
38	Pos	untargeted	6.51	441.2014	[M+H]+	5321825	Diferuloylputrescine	109.2	UP	1.12552E-10	4.3075E-10	2
39	Pos	untargeted	8.56	373.2723	[M+H]+	5284076	3α-Hydroxy-5β-chola-8(14),11-dien-24-oate	101.6	DOWN	0.004183032	0.00270854	2
40	Pos	untargeted	9.29	283.2624	[M+H]+	445639	Oleate	100.5	DOWN	0.004985368	0.00315317	2
41	Neg	untargeted	1.33	199.0719	[M-H]-	194618	Alanylclavam	93.2	UP	8.34777E-12	3.9061E-11	2
42	Neg	untargeted	7.53	521.2404	[M-H2O-H]-	167918	Tetrahydroaldosterone-3-glucuronide	91.5	UP	4.05653E-09	8.393E-09	2
43	Pos (& Neg)	untargeted	1.85	380.1122	[M+H]+	440018	S-Lactoylglutathione	80.9	UP	2.21609E-11	1.0635E-10	2
44	Neg	untargeted	1.35	257.0781	[M-H]-	440569	Imidazoleacetate riboside	79.0	UP	1.57112E-11	6.6582E-11	2
45	Neg	untargeted	4.79	303.0171	[M-H]-	124202075	5-(3',4',5'-Trihydroxyphenyl)-γ-valerolactone-4'-O-sulfate	71.4	UP	7.32809E-09	1.4173E-08	2

46	Pos	targeted	5.33	162.0555012	[M+H]+	97250	2,8-Quinolinediol	70.0	DOWN	0.024842341	0.012756	1
47	Pos	untargeted	6.99	371.2579	[M+H-H2O]+	5284095	3α,12α-Dihydroxy-5β-chola-7,14-dien-24-oate	67.8	DOWN	0.000337055	0.00028921	2
48	Pos	targeted	0.73	143.082049	[M+H]+	126041	Ectoine	65.0	DOWN	0.038250024	0.01862512	1
49	Neg	targeted	9.11	375.289904	[M-H]-	9903	Lithocholate	64.9	DOWN	1.88856E-05	1.865E-05	1
50	Pos	untargeted	3.06	297.1071	[M+H]+	19816752	Tyrosyl-Aspartate	63.2	UP	1.5099E-14	2.8299E-13	2
51	Neg	untargeted	1.28	229.083	[M-H]-	3838871	Prolyl-Aspartate	61.8	UP	1.62492E-12	9.9627E-12	2
52	Pos	untargeted	5.8	447.0912	[M+H]+	45782816	Genistein 4'-O-glucuronide	61.2	UP	1.99856E-09	5.3305E-09	2
53	Pos	untargeted	6.64	407.2777	[M+H-2H2O]+	54676866	Ascorbylstearate	57.1	DOWN	0.009810948	0.00568365	2
54	Neg	untargeted	2.17	263.0706	[M-H]-	21488123	Aspartyl-Methionine	55.7	UP	7.5131E-12	3.5733E-11	2
55	Pos (& Neg)	targeted & u	6.97	533.3254	[M+NH4]+	6675	Taurocholate	55.6	UP	1.13505E-05	1.3375E-05	1
56	Pos	untargeted	3.07	489.1174	[M+H]+	13804	Citicoline	53.9	UP	6.08294E-08	1.1453E-07	2
57	Pos	untargeted	5.3	497.1328	[2M+H]+	274178	L-β-Aspartyl-L-aspartate	52.5	UP	5.12001E-07	7.9894E-07	2
58	Pos	untargeted	8.09	357.2788	[M+H]+	17756764	(1S)-1-Hydroxy-23,24-didehydro-25,26,27-trinorcalciol	52.0	DOWN	0.001131178	0.00085431	2
59	Neg	untargeted	2.12	288.1202	[M-H]-	14586819	Isotabtoxin	49.9	UP	2.78444E-13	2.4981E-12	2
60	Pos (& Neg)	untargeted	6.04	438.2372	[M+H]+	15270795	N1,N10-Dicoumaroylspermidine	48.4	UP	3.35487E-11	1.5107E-10	2
61	Pos	untargeted	1.41	145.0495	[M+H]+	1551553	3-Methylglutaconate	48.3	DOWN	0.004413212	0.00283794	2
62	Pos	untargeted	6.87	314.2318	[M+H]+	11953821	O-[(4Z)-Decenoyl]carnitine	47.7	UP	0.0003571	0.00030445	2
63	Pos	targeted	0.81	146.1180976	M+	187	Acetylcholine	46.2	DOWN	0.000893651	0.00069419	1
64	Neg	targeted & u	1.08	135.0309	[M-H]-	790	Hypoxanthine	42.9	DOWN	5.98245E-05	5.4141E-05	1
65	Pos	untargeted	6.8	667.2341	[M+H]+	439177	Glycogen	41.7	UP	2.40918E-13	2.4128E-12	2
66	Neg	untargeted	4.95	193.0506	[M-H]-	445858	4-Hydroxy-3-methoxycinnamate	41.3	UP	4.22826E-11	1.5466E-10	2
67	Pos	targeted	0.63	265.1123012	M+	1130	Thiamine	38.3	DOWN	0.005669581	0.00352559	1
68	Pos	untargeted	2.68	260.1607	[M+H]+	68578696	Lysyl-Hydroxyproline	37.4	DOWN	0.001077814	0.00081865	2
69	Neg	untargeted	4.38	224.056	[M-H]-	3083688	Vanilloylglycine	37.1	UP	1.11941E-05	1.1481E-05	2
70	Neg	targeted	5.97	204.0660646	[M-H]-	676157	Indole-3-lactate	36.3	DOWN	0.008774618	0.00503774	1
71	Pos	targeted	7.98	370.2957184	[M+H]+	53481699	trans-2-Tetradecenoyl-L-carnitine	35.4	UP	2.26399E-07	3.7905E-07	1
72	Neg	untargeted	3.53	302.136	[M-H]-	9882882	Nicotianamine	35.4	UP	4.13599E-05	3.8497E-05	2
73	Pos (& Neg)	targeted & u	0.71	310.1138014	[M+H]+	445063	N-Acetylneuraminate	35.4	UP	0.000243583	0.00021665	1
74	Neg	untargeted	2.99	295.0933	[M-H]-	19365650	Aspartyl-tyrosine	34.2	UP	0.008100227	0.00469691	2
75	Neg	targeted & u	0.85	165.0398	[M-H]-	122045	Arabinonate	33.8	UP	9.9305E-12	4.5127E-11	1
76	Neg	untargeted	0.7	699.1659	[M+FA-H]-	11954072	2-(S-Glutathionyl)acetyl glutathione	33.6	UP	9.39293E-12	4.3103E-11	2
77	Pos	targeted	1	124.039852	[M+H]+	1018	2-Picolinate	33.1	DOWN	1.17458E-05	1.3793E-05	1
78	Neg	targeted	0.93	150.041583	[M-H]-	135398634	Guanine	32.5	DOWN	0.006689885	0.00396675	1
79	Neg	targeted	0.7	157.0361636	[M-H]-	204	Allantoin	31.7	UP	7.15997E-06	7.5826E-06	1
80	Neg	untargeted	1.86	318.1302	[M-H]-	45480617	γ-D-Glutamyl-meso-diaminopimelate	31.6	UP	1.13469E-11	5.0451E-11	2
81	Pos	untargeted	5.05	340.1499	[M+H]+	71464583	Lysine tyrosylquinone	28.9	UP	8.99281E-14	1.1417E-12	2
82	Pos	untargeted	1.6	217.1548	[M+CH3OH+H]+	129397	N-(3-Acetamidopropyl)pyrrolidin-2-one	28.0	DOWN	0.004279346	0.00276291	2
83	Neg	targeted & u	1.47	133.0504	[M-H]-	440279	(R)-2,3-Dihydroxy-isovalerate	27.8	DOWN	0.002687559	0.00176015	1
84	Pos	untargeted	6.51	177.054	[M+H]+	131751124	10-Hydroxy-2,8-decadiene-4,6-diynoate	27.0	UP	6.88694E-12	3.9037E-11	2
85	Pos	untargeted	7.28	425.289	[M+H]+	5283905	3α,7α,12α,19-Tetrahydroxy-5β-cholanoate	25.0	DOWN	0.000292793	0.00025506	2
86	Pos	untargeted	0.62	221.0766	[M+H]+	53480673	L-β-Aspartyl-L-serine	25.0	UP	1.54543E-13	1.6982E-12	2
87	Neg	targeted	0.67	195.0504752	[M-H]-	10690	D-Gluconate	24.2	UP	1.05604E-12	7.1145E-12	1
88	Neg	untargeted	0.63	174.0879	[M-H]-	9750	Citrulline	23.3	DOWN	4.17568E-06	4.6158E-06	2
89	Pos (& Neg)	untargeted	0.73	263.0877	[M+H]+	25207301	L-β-Aspartyl-L-glutamate	23.1	UP	2.35367E-14	4.0576E-13	2
90	Pos	untargeted	2.31	391.1824	[M+H]+	18744842	Serylvalylglycylglutamate	22.2	UP	1.22951E-06	1.7816E-06	2

Supplementary Table 3.2. Cont'd.

91	Pos	untargeted	6.97	480.2758	[M+Na]+	52922058	N-[(4Z,7Z,10Z,13Z,16Z,19Z)-Docosahexaenoyl]-L-glutamate	21.9	UP	3.71521E-06	4.8456E-06	2
92	Neg	untargeted	5.36	258.0382	[M-H]-	439217	Glucosamine 6-phosphate	21.7	DOWN	0.008838845	0.00507028	2
93	Neg	targeted	5.79	172.097363	[M-H]-	99463	Hexanoylglycine	20.7	UP	0.022907475	0.01168769	1
94	Neg	targeted	1.29	221.0661244	[M-H]-	18392195	Ethyl-β-D-glucuronide	20.4	DOWN	1.85586E-05	1.8351E-05	1
95	Neg	untargeted	4.2	341.0961	[M-H]-	84723	4-Nitrophenyl N-acetyl-β-D-galactosaminide (GlcNAc-PNP)	20.3	UP	5.41633E-11	1.9093E-10	2
96	Neg	untargeted	3.54	216.9813	[M-H]-	7322	5-Sulfosalicylate	20.2	UP	0.000196759	0.00016187	2
97	Pos (& Neg)	untargeted	0.92	262.1393	[M+H]+	6427003	Aspartyl-lysine	19.7	UP	4.88498E-14	7.0508E-13	2
98	Neg	untargeted	1.61	231.0983	[M-H]-	127370	N2-Succinyl-L-ornithine	19.4	UP	2.6823E-13	2.4228E-12	2
99	Neg	untargeted	1.8	155.0824	[M-H2O-H]-	439232	N-Acetylornithine	19.3	UP	8.66374E-12	4.0293E-11	2
100	Neg	untargeted	3.2	384.0936	[M-H]-	131751604	(S)-2,3-Dihydro-3,5-dihydroxy-2-oxo-3-indoleacetate 5-glucoside	18.8	UP	2.18315E-07	3.028E-07	2
101	Neg	untargeted	2.24	298.0794	[M-H]-	135407175	8-Hydroxyguanosine	18.5	UP	7.37652E-11	2.519E-10	2
102	Pos	untargeted	8.44	391.2838	[M+Na]+	6436905	Octadecylfumarate	18.1	DOWN	1.04232E-05	1.2382E-05	2
103	Pos (& Neg)	untargeted	1.21	113.0345	[M+H]+	1174	Uracil	17.8	DOWN	6.99053E-05	7.022E-05	2
104	Pos	untargeted	5.36	316.2227	[M+H]+	235009	Val-Val-Val	17.6	DOWN	0.002724126	0.00186076	2
105	Pos	untargeted	5.65	339.1082	[M+H]+	9945785	3-O-p-Coumaroylquinate	17.3	UP	2.35367E-14	4.0576E-13	2
106	Pos	targeted	0.54	112.0874684	[M+H]+	774	Histamine	17.1	DOWN	0.009044857	0.00529878	1
107	Neg	untargeted	1.2	188.0563	[M-H]-	70914	N-Acetylglutamate	16.9	DOWN	0.000693029	0.00051316	2
108	Neg	targeted & u	1.3	117.0191	[M-H]-	1738118	Succinate	16.7	DOWN	0.000664459	0.00049378	1
109	Neg	targeted	0.97	145.0136976	[M-H]-	51	α-Ketoglutarate	16.1	DOWN	0.007599146	0.00444121	1
110	Pos (& Neg)	targeted	1	139.0507506	[M+H]+	1178	trans-Urocanate	16.1	DOWN	2.05674E-06	2.8398E-06	1
111	Neg	targeted & u	4.82	129.0556	[M-H]-	47	3-Methyl-2-oxovalerate	15.9	DOWN	0.00046792	0.00035836	1
112	Neg	untargeted	6.14	245.1393	[M-H]-	16663321	3-Hydroxydodecanedioate	15.7	DOWN	0.009126881	0.00521553	2
113	Pos	untargeted	5.35	206.081	[M+H]+	18986	5-Methoxyindoleacetate	15.7	UP	2.85194E-12	1.908E-11	2
114	Pos	untargeted	1.36	260.1605	[M+H]+	22935676	Isoleucyl-Glutamine	15.4	DOWN	0.000113099	0.00010882	2
115	Pos	targeted & u	6.65	288.216	[M+H]+	11953814	L-Octanoylcarnitine	15.3	UP	1.57858E-08	3.3825E-08	1
116	Neg	untargeted	5.74	366.2029	[M+Cl]-	121454166	3-Hydroxydecanoylcarnitine	15.2	DOWN	0.000967399	0.000697	2
117	Neg	untargeted	1.3	73.02898	[M-H]-	1032	Propionate	14.8	DOWN	0.000594279	0.00044582	2
118	Pos	untargeted	7.38	405.2626	[M+CH3OH+H]+	53481537	9'-Carboxy-γ-tocotrienol	14.6	DOWN	0.001159867	0.00087331	2
119	Neg	untargeted	5.03	369.1769	[M-H]-	159663	Androsterone sulfate	14.0	UP	2.89762E-11	1.1185E-10	2
120	Neg	untargeted	1.61	213.0878	[M-H2O-H]-	69041809	Threoninyl-Hydroxyproline	13.9	UP	2.22045E-14	3.2431E-13	2
121	Neg	untargeted	1.25	343.1249	[M-H]-	4454759	1-O-α-D-Glucopyranosyl-D-mannitol	13.8	UP	1.02585E-13	1.1177E-12	2
122	Neg	targeted	2.52	115.0395168	[M-H]-	49	α-Ketoisovalerate	13.5	DOWN	0.000818297	0.00059828	1
123	Pos (& Neg)	targeted	0.64	126.0224882	[M+H]+	1123	Taurine	13.4	DOWN	0.028120105	0.01421465	1
124	Pos	untargeted	0.65	262.1036	[M+H]+	87275314	Glutaminylaspartate	13.4	UP	8.62266E-12	4.7701E-11	2
125	Neg	targeted	5.76	165.055166	[M-H]-	3848	DL-3-Phenyllactate	13.1	DOWN	0.005610781	0.00338557	1
126	Pos	untargeted	5.66	260.1859	[M+H]+	3246938	Hexanoylcarnitine	13.0	UP	1.20492E-09	3.4054E-09	2
127	Neg	untargeted	7.89	449.326	[M-H]-	122312	Coprocholate	12.7	DOWN	0.000148222	0.00012472	2
128	Neg	targeted & u	7.44	229.1445	[M-H]-	12736	Dodecanedioate	12.5	DOWN	0.009125924	0.00521504	1
129	Pos	untargeted	7.38	313.2156	[M+Na]+	10634	5-Androstenediol	12.4	DOWN	0.001768083	0.00127045	2
130	Pos	untargeted	0.63	191.0661	[M+H]+	302430	L-β-Aspartyl-L-glycine	12.4	UP	2.22045E-16	9.7489E-15	2
131	Neg	untargeted	3.74	261.0075	[M-H]-	29981063	Homovanillate sulfate	12.4	UP	2.22045E-16	8.1557E-15	2
132	Neg	untargeted	3.97	366.1299	[M-H]-	70678657	N-Hydroxydebrisoquine O-glucuronide	12.1	UP	8.39329E-14	9.5754E-13	2
133	Pos	untargeted	8.16	441.3355	[M+H]+	24779630	24α,24β-Dihomo-9,10-secocholesta-5,7,10(19),24α-tetraen-1α,3,25	12.0	DOWN	2.76962E-08	5.6423E-08	2
134	Neg	untargeted	1.81	245.0594	[M-H2O-H]-	13894650	γ-Glutamyl-S-methylcysteine	11.8	UP	1.35669E-13	1.3739E-12	2
135	Neg	untargeted	4.22	486.2192	[M-H]-	65938	Ac-Ser-Asp-Lys-Pro-OH	11.8	UP	1.77636E-15	4.3193E-14	2

Supplementary Table 3.2. Cont'd.

136	Neg	untargeted	5.29	172.0976	[M-H]-	70912	N-Acetylleucine	11.8	UP	4.32779E-09	8.8933E-09	2
137	Neg	untargeted	0.67	351.0562	[M-H]-	3390357	α-L-Threo-4-Hex-4-enopyranuronosyl-D-galacturonate	11.3	UP	8.13536E-07	1.0152E-06	2
138	Neg	untargeted	5.41	338.0884	[M-H]-	196513	6-Hydroxy-5-methoxyindole glucuronide	11.3	UP	6.66134E-16	2.0065E-14	2
139	Pos	untargeted	8.19	371.2576	[M+H]+	5283992	3-Oxo-4,6-choladienoate	11.2	DOWN	0.008479832	0.00501027	2
140	Pos	untargeted	0.94	318.1291	[M+CH3OH+H]+	107461	N4-Acetylcytidine	11.1	UP	1.23013E-13	1.4271E-12	2
141	Pos	untargeted	7.95	288.2892	[M+H]+	3247037	C17 Sphinganine	11.0	DOWN	0.000495497	0.00040907	2
142	Neg	untargeted	9.14	271.2281	[M-H]-	15569776	(R)-3-Hydroxy-hexadecanoate	10.8	DOWN	2.37702E-07	3.2715E-07	2
143	Neg	untargeted	5.46	365.1963	[M-H]-	5280877	20-Carboxy-leukotriene B4	10.7	DOWN	0.006932003	0.00409443	2
144	Pos (& Neg)	targeted & u	1.02	122.024202	[M-H]-	937	Nicotinate	10.7	DOWN	0.00011271	9.6896E-05	1
145	Neg	untargeted	1.58	256.0934	[M-H]-	11010621	Pyro-L-glutaminyl-L-glutamine	10.7	UP	5.96206E-07	7.6097E-07	2
146	Pos	targeted	4.9	246.1705248	[M+H]+	6426851	Isovaleryl-L-carnitine	10.5	UP	1.44987E-05	1.6638E-05	1
147	Pos	targeted & u	2.84	209.092	[M+H]+	161166	L-Kynurenine	10.2	UP	4.91385E-13	4.3483E-12	1
148	Pos	untargeted	7.05	307.1896	[M+H]+	9839519	Capsiate	10.2	UP	1.37015E-11	7.1132E-11	2
149	Neg	untargeted	3.41	233.0126	[M-H]-	131834405	(4-Ethyl-2,6-dihydroxyphenyl)oxidanesulfonate	10.0	UP	0.000364313	0.00028538	2
150	Pos	untargeted	1.24	344.1344	[M+H]+	131752248	N-(1-Deoxy-1-fructosyl)tyrosine	10.0	UP	1.68914E-09	4.5842E-09	2
151	Neg	untargeted	4.06	359.0983	[M-H]-	22833525	3-Methoxy-4-hydroxyphenylglycol glucuronide	9.9	UP	4.87403E-11	1.7463E-10	2
152	Pos	untargeted	1.36	259.0922	[M+H]+	445408	Ribothymidine	9.8	UP	0	0	2
153	Neg	untargeted	0.87	161.0456	[M-H2O-H]-	64689	D-Glucose	9.7	DOWN	0.004265762	0.00265577	2
154	Pos	untargeted	7.22	384.2732	[M+H]+	53481683	3-Hydroxy-5, 8-tetradecadiencarnitine	9.6	UP	2.95089E-07	4.8272E-07	2
155	Pos	untargeted	5.81	207.0653	[M+CH3OH+H]+	12582097	1-Oxo-1H-2-benzopyran-3-carboxaldehyde	9.6	UP	0.005343389	0.00334952	2
156	Neg	untargeted	0.69	105.0191	[M-H]-	439194	Glycerate	9.6	DOWN	0.00288373	0.00187347	2
157	Neg	untargeted	5.31	332.0386	[M-H]-	136032516	7,8-Dihydroneopterin 2'-phosphate	9.5	UP	0.002222437	0.00148424	2
158	Neg	untargeted	4.12	230.9965	[M-H]-	20822599	Vanillin 4-sulfate	9.3	UP	0.000509666	0.00038756	2
159	Neg	untargeted	5.1	227.0574	[M-H]-	289793	2-Hydroxy-3,4,5-trimethoxybenzoate	9.3	DOWN	0.008053293	0.00467313	2
160	Neg	untargeted	4.26	283.9869	[M-H]-	11522049	Xanthurenate 8-O-sulfate	9.1	UP	6.86488E-09	1.3368E-08	2
161	Pos (& Neg)	targeted & u	0.6	162.0761	[M+H]+	469	L-2-Aminoadipate	9.0	UP	9.53527E-08	1.7225E-07	1
162	Neg	untargeted	0.6	175.072	[M+FA-H]-	273022	D-1-Amino-2-pyrrolidinecarboxylate	9.0	DOWN	1.56628E-05	1.5684E-05	2
163	Pos	targeted	8.26	400.342666	[M+H]+	11953816	Palmitoyl-L-carnitine	9.0	UP	2.70397E-06	3.6359E-06	1
164	Pos	untargeted	7.38	247.1684	[M+Na]+	5312409	5,8-Tetradecadienoate	8.8	DOWN	8.41173E-05	8.2948E-05	2
165	Pos (& Neg)	targeted & u	3.81	220.1177	[M+H]+	6613	D-(+)-Pantothenate	8.8	DOWN	7.1216E-05	7.1401E-05	1
166	Pos	untargeted	8.59	429.3716	[M+H]+	10025615	1-Hydroxyvitamin D5	8.6	DOWN	0.000312709	0.00027051	2
167	Pos	untargeted	7.21	426.3197	[M+CH3OH+H]+	53477457	Prostaglandin H2-ethanolamide (PGH2-EA)	8.6	DOWN	0.002766381	0.00188629	2
168	Neg	targeted	7.6	498.288918	[M-H]-	387316	Taurochenodeoxycholate	8.6	UP	0.000633103	0.00047246	1
169	Pos (& Neg)	targeted	11.14	585.2712966	[M+H]+	5280352	Bilirubin	8.5	DOWN	0.00568908	0.00353603	1
170	Neg	untargeted	0.97	174.0406	[M-H]-	65065	N-Acetyl-L-aspartate	8.5	DOWN	0.000181593	0.00015035	2
171	Pos	untargeted	5.29	181.0857	[M+H]+	1549095	Coniferyl alcohol	8.5	UP	9.6756E-10	2.8315E-09	2
172	Pos	untargeted	4.44	138.0548	[M+H]+	227	Anthranilate	8.4	UP	0.001064577	0.00080974	2
173	Pos	untargeted	4.39	235.1076	[M+H]+	119507	S-4-Hydroxymephenytoin	8.3	UP	0	0	2
174	Pos	untargeted	0.82	274.0923	[M+Na]+	13730	Deoxyadenosine (dA)	8.3	UP	2.62013E-14	4.4054E-13	2
175	Neg	untargeted	5.74	455.2509	[M-H]-	10253562	Sulfolithocholate	8.3	UP	0	0	2
176	Pos (& Neg)	untargeted	3.03	328.1385	[M+H]+	101039148	N-(1-Deoxy-1-fructosyl)phenylalanine	8.2	UP	1.40233E-09	3.8809E-09	2
177	Neg	untargeted	4.73	236.0557	[M-H]-	45782784	Methyl 2,3-dihydro-3,5-dihydroxy-2-oxo-3-indoleacetate	8.1	UP	0.006182732	0.00369605	2
178	Neg	untargeted	5.26	289.0388	[M-H]-	124202068	4-Hydroxy-5-(4'-hydroxyphenyl)-valerate-4'-O-sulfate	8.0	UP	2.6681E-08	4.4738E-08	2
179	Neg	untargeted	4.4	225.0763	[M-H]-	52920332	4-Hydroxy-(3',4'-dihydroxyphenyl)-valerate	7.7	DOWN	0.00919357	0.005249	2
180	Neg	untargeted	4.43	365.1357	[M-H]-	10981970	2'-α-Mannosyl-L-tryptophan	7.7	UP	1.06833E-10	3.4963E-10	2

Supplementary Table 3.2. Cont'd.

181	Pos	untargeted	0.67	235.0921	[M+H]+	53480674	L-β-Aspartyl-L-threonine	7.6	UP	9.30367E-14	1.1707E-12	2
182	Neg	targeted	3.03	165.0412486	[M-H]-	70639	3-Methylxanthine	7.6	DOWN	0.045440938	0.02127853	1
183	Pos	untargeted	9.21	429.3715	[M+H]+	25203290	4α-Hydroxymethyl-4β-methyl-5α-cholesta-8,24-dien-3β-ol	7.6	UP	0.000730546	0.00058066	2
184	Neg	untargeted	3.95	253.1196	[M-H]-	14274616	Pyrraline	7.6	UP	3.5245E-06	3.9434E-06	2
185	Neg	untargeted	4.53	328.1519	[M-H]-	56928070	Valclavam	7.5	UP	2.44249E-15	5.6419E-14	2
186	Neg	untargeted	6.74	505.2669	[M-H]-	20979920	(3β,9R)-5-Megastigmene-3,9-diol 9-[apiosyl-(1->6)-glucoside]	7.5	UP	4.05262E-11	1.4909E-10	2
187	Neg	untargeted	5.66	264.0879	[M-H]-	124202120	2-(2-Phenylacetoxy)propionylglycine	7.5	UP	1.18203E-11	5.2277E-11	2
188	Pos	untargeted	7.82	319.19	[M+H]+	5280346	Ubiquinone-2	7.2	DOWN	0.001931076	0.00137467	2
189	Neg	untargeted	4.78	246.098	[M-H]-	91825606	Malonylcarnitine	7.1	UP	4.96936E-13	3.9999E-12	2
190	Pos	untargeted	7.41	387.2519	[M+H-H2O]+	5283996	7α,12α-Dihydroxy-3-oxo-4-cholenoate	7.1	DOWN	0.001596987	0.00115893	2
191	Neg	untargeted	8.76	321.2423	[M-H]-	5283145	15(S)-HETE	7.1	DOWN	0.000310714	0.00024677	2
192	Pos	untargeted	1.61	300.1186	[M+H-H2O]+	131752243	N-(1-Deoxy-1-fructosyl)histidine	7.1	UP	2.11298E-12	1.4911E-11	2
193	Pos	untargeted	3.92	237.1235	[M+H]+	96814	Alanyl-Phenylalanine	7.0	UP	2.94622E-06	3.9302E-06	2
194	Neg	targeted	6.7	201.1126778	[M-H]-	5192	Sebacate	7.0	DOWN	0.010648809	0.00597586	1
195	Pos (& Neg)	untargeted	2.7	247.1286	[M+H]+	7015683	γ-Glutamylvaline	7.0	UP	3.55271E-15	9.079E-14	2
196	Pos (& Neg)	untargeted	4.36	352.1011	[M+H]+	124202110	Indole-3-acetate O-glucuronide	7.0	UP	7.98266E-07	1.1985E-06	2
197	Pos (& Neg)	untargeted	4.01	360.2129	[M+H]+	71464664	Glu-Ile-Val	6.9	DOWN	0.002298792	0.00160286	2
198	Pos (& Neg)	targeted & u	0.89	136.0617	[M+H]+	190	Adenine	6.9	DOWN	0.003626845	0.00239058	1
199	Pos (& Neg)	targeted & u	0.65	104.0711504	[M+H]+	119	γ-Aminobutyrate (GABA)	6.8	DOWN	4.59883E-05	4.8105E-05	1
200	Pos (& Neg)	targeted & u	0.86	244.0925	[M+H]+	6175	Cytidine	6.8	UP	1.24098E-08	2.7335E-08	1
201	Pos	untargeted	3.8	227.1022	[M+NH4]+	440732	4-Hydroxyphenylacetylglycine	6.8	UP	8.88178E-16	3.0109E-14	2
202	Neg	untargeted	2.43	181.0367	[M-H]-	69726	1-Methylurate	6.7	DOWN	0.000564726	0.00042544	2
203	Neg	untargeted	4.39	229.1553	[M-H]-	435949	Isoleucyl-Valine	6.7	DOWN	1.2021E-07	1.7569E-07	2
204	Pos	untargeted	8.14	413.2648	[M+Na]+	53477692	7-Hydroxy-3-oxocholanoate	6.7	DOWN	0.000926926	0.00071673	2
205	Pos	untargeted	7.22	429.2808	[M+H]+	91666454	Prostaglandin F2α 2-glyceryl ester	6.6	DOWN	0.008086712	0.00480768	2
206	Neg (& Pos)	targeted & u	4.45	190.0537858	[M-H]-	448580	N-Acetyl-L-methionine	6.6	UP	1.25011E-13	1.2946E-12	1
207	Pos	untargeted	6.53	267.1585	[M+H]+	213591	Ipomeamaronol	6.6	UP	0	0	2
208	Neg	targeted & u	1.02	167.021	[M-H]-	1175	Urate	6.6	UP	1.21558E-09	2.8975E-09	1
209	Neg	targeted	0.86	173.044996	[M-H]-	8742	Shikimate	6.5	UP	1.04361E-14	1.75E-13	1
210	Pos	untargeted	7.68	353.2297	[M+Na]+	9858729	9,12,13-TriHOME	6.4	DOWN	0.00135628	0.00100242	2
211	Pos	targeted	2	153.0663998	[M+H]+	69698	Nudifloramide	6.4	UP	0.004753221	0.00302587	1
212	Neg	untargeted	2.07	147.0662	[M-H]-	439230	Mevalonate	6.3	DOWN	0.000470221	0.00035998	2
213	Pos	untargeted	7.56	351.2141	[M+Na]+	9548882	2,3-Dinor-8-epi-prostaglandin F1α	6.3	DOWN	0.001128804	0.00085273	2
214	Pos	untargeted	7.14	317.2465	[M+H]+	8955	Pregnenolone	6.2	UP	0.001444264	0.00105967	2
215	Pos	untargeted	1.38	175.1079	[M+NH4]+	66141	N-Acetylproline	6.1	DOWN	6.19E-05	6.2931E-05	2
216	Pos	untargeted	8.41	237.1842	[M+H]+	131752029	Humulene-8-hydroperoxide	6.0	DOWN	0.000495771	0.00040927	2
217	Pos	untargeted	6.74	207.065	[M+H]+	11127419	Vinyl caffeate	5.9	UP	9.351E-06	1.1225E-05	2
218	Pos	targeted	0.65	76.0762354	[M+H]+	1145	Trimethylamine N-oxide (TMAO)	5.9	DOWN	0.001218949	0.00091217	1
219	Pos	untargeted	2.94	219.134	[M+H]+	7015694	Serylleucine	5.9	DOWN	0.000504086	0.00041542	2
220	Pos	untargeted	3.9	255.134	[M+H]+	57357510	Aldosine	5.8	UP	2.33379E-06	3.1836E-06	2
221	Pos	untargeted	7.38	173.132	[M+H]+	121677	1,2-Dihydro-1,1,6-trimethylnaphthalene	5.8	DOWN	0.002296988	0.00160176	2
222	Neg	untargeted	1.36	218.0662	[M-H]-	46878420	O-Succinyl-L-homoserine	5.8	UP	3.34621E-13	2.8954E-12	2
223	Pos	untargeted	1.4	219.1335	[M+H]+	3325403	L-Lysopine	5.8	DOWN	0.001444371	0.00105974	2
224	Pos (& Neg)	targeted	0.98	228.0984268	[M+H]+	13711	Deoxycytidine (dC)	5.7	UP	8.97133E-07	1.3334E-06	1
225	Pos (& Neg)	untargeted	3.6	233.1496	[M+H]+	18218220	Isoleucyl-Threonine	5.7	DOWN	7.37906E-06	9.049E-06	2

Supplementary Table 3.2. Cont'd.

226	Pos	untargeted	3.16	203.1393	[M+H]+	5246009	Isoleucyl-Alanine	5.7	DOWN	6.73482E-05	6.7903E-05	2
227	Pos	untargeted	0.72	321.0585	[M+Na]+	115145	Benzoyl glucuronide (Benzoate)	5.7	UP	0.001486704	0.00108699	2
228	Pos	untargeted	8.09	375.2885	[M+H]+	92997	3β-Hydroxy-5-cholenoate	5.7	DOWN	0.0014532	0.00106544	2
229	Pos	targeted	2.42	177.1027832	[M+H]+	5202	Serotonin	5.7	DOWN	1.89753E-06	2.6384E-06	1
230	Neg	targeted	6.44	514.283833	[M-H]-	101657566	Tauro α-muricholate + Tauro β-muricholate	5.6	UP	4.77818E-05	4.4001E-05	1
231	Neg	untargeted	1.15	286.1041	[M-H]-	126923	N-Ribosylhistidine	5.6	UP	3.15733E-07	4.2336E-07	2
232	Neg	untargeted	7.37	403.2484	[M+FA-H]-	131769828	24,25,26,27-Tetranor-23-oxo-hydroxyvitamin D3	5.6	DOWN	0.000193731	0.00015957	2
233	Pos (& Neg)	untargeted	0.75	173.0923	[M+H-H2O]+	439283	Diaminopimelate	5.6	UP	6.84902E-08	1.2764E-07	2
234	Pos	targeted	1.51	138.0918846	[M+H]+	5610	Tyramine	5.6	DOWN	0.00207983	0.0014681	1
235	Neg	untargeted	4.85	275.0233	[M-H]-	187489	Dihydroferulate 4-sulfate	5.6	UP	2.07164E-06	2.4049E-06	2
236	Pos	untargeted	4.39	231.1701	[M+H]+	352038	Leucyl-Valine	5.4	DOWN	2.52074E-06	3.4109E-06	2
237	Pos	untargeted	8.42	434.3258	[M+H]+	115245	Glycolithocholate	5.4	DOWN	0.004891944	0.00310177	2
238	Neg	targeted	1.45	130.0504154	[M-H]-	88064	N-Acetyl-L-alanine	5.4	UP	1.22347E-13	1.2742E-12	1
239	Pos	untargeted	7.31	361.2836	[M+H-2H2O]+	52922080	N-Oleoyl asparagine	5.4	UP	1.04398E-07	1.8688E-07	2
240	Neg	targeted & u	7.12	498.2873	[M-H]-	12443252	Tauroursodeoxycholate	5.3	UP	0.000548781	0.00041454	1
241	Pos (& Neg)	targeted & u	1.83	284.0991	[M+H]+	135398635	Guanosine	5.3	UP	2.97988E-07	4.871E-07	1
242	Pos	untargeted	3.29	283.104	[M+H]+	65095	1-Methylinosine	5.3	UP	0.003218446	0.00215543	2
243	Pos (& Neg)	untargeted	1.58	258.1087	[M+H]+	92918	5-Methylcytidine	5.2	UP	2.98174E-09	7.6493E-09	2
244	Neg	targeted	0.69	135.0293468	[M-H]-	5460407	L-Threonate	5.2	UP	2.4038E-10	7.085E-10	1
245	Pos	untargeted	4.18	223.1073	[M+H]+	97415	Glycyl-Phenylalanine	5.2	DOWN	1.08312E-05	1.2821E-05	2
246	Pos	targeted & u	7	363.2165	[M+H]+	5754	Cortisol	5.2	UP	0.001068637	0.00081248	1
247	Neg	targeted	0.65	182.0123174	[M-H]-	177491	Homocysteate	5.1	DOWN	0.000131621	0.00011172	1
248	Neg	untargeted	0.72	203.0673	[M-H]-	4677380	Aspartyl-Alanine	5.1	UP	1.06807E-06	1.3065E-06	2
249	Pos (& Neg)	untargeted	1.5	352.1177	[M+H]+	5460887	S-(2-Hydroxyethyl)glutathione	5.1	UP	7.63179E-10	2.305E-09	2
250	Neg	untargeted	2.14	220.0282	[M-H]-	54097042	S-(3-Oxo-3-carboxy-n-propyl)cysteine	5.1	DOWN	0.002330626	0.00154939	2
251	Neg (& Pos)	targeted & u	0.81	133.0141	[M-H]-	222656	L-Malate	5.0	DOWN	7.97029E-05	7.0414E-05	1
252	Neg	untargeted	1.27	189.0404	[M-H]-	439351	3-Dehydroquinate	5.0	UP	3.06545E-06	3.4594E-06	2
253	Pos	untargeted	3.64	195.1228	[M+NH4]+	27919	N'-Nitrosonornicotine	5.0	DOWN	5.14435E-09	1.2395E-08	2
254	Pos	untargeted	7.87	413.3039	[M+NH4]+	5283120	PGD2 ethanolamide	5.0	DOWN	0.005163259	0.00325107	2
255	Neg	untargeted	1.29	141.0669	[M-H2O-H]-	439925	N(5)-Methyl-L-glutamine	5.0	UP	1.4988E-13	1.4906E-12	2
256	Pos	targeted	0.49	146.1657144	[M+H]+	1102	Spermidine	5.0	DOWN	0.000178867	0.00016454	1
257	Pos	untargeted	8.63	445.3669	[M+CH3OH+H]+	22833566	25-Hydroxyvitamin D2	4.9	UP	3.45873E-05	3.7011E-05	2
258	Neg	untargeted	0.98	193.0352	[M-H]-	444791	D-Glucuronate	4.9	UP	4.44089E-16	1.4109E-14	2
259	Pos	untargeted	2.78	164.0738	[M+H]+	10329619	Homomethionine	4.9	DOWN	0.004441912	0.00285395	2
260	Pos	untargeted	5.01	481.179	[2M+H]+	18618152	D-Gycero-L-galacto-octulose	4.9	UP	0.000212161	0.00019153	2
261	Neg	untargeted	0.75	193.0353	[M-H]-	441476	D-(+)-Galacturonate	4.9	UP	1.37446E-13	1.388E-12	2
262	Neg	targeted & u	3.41	152.035	[M-H]-	86	3-Hydroxyanthranilate	4.8	DOWN	0.000178388	0.0001479	1
263	Neg	untargeted	0.61	215.0327	[M-H]-	443198	2-C-Methyl-D-erythritol 4-phosphate	4.8	DOWN	0.000372968	0.00029152	2
264	Neg	targeted	5.35	131.0708152	[M-H]-	92779	2-Hydroxyisocaproate	4.8	DOWN	0.001116619	0.00079472	1
265	Neg	untargeted	0.91	144.0777	[M-H]-	500	4-Guanidinobutanoate	4.7	UP	1.9717E-07	2.7614E-07	2
266	Pos (& Neg)	targeted	1.1	198.0766296	[M+H]+	6047	3,4-Dihydroxy-L-phenylalanine (L-DOPA)	4.7	DOWN	0.007113662	0.00429524	1
267	Neg	untargeted	3.36	232.0282	[M-H]-	122136	Dopamine 3-O-sulfate	4.7	DOWN	8.89773E-06	9.2736E-06	2
268	Pos	targeted	6.9	190.0867996	[M+H]+	3744	Indole-3-propionate	4.6	DOWN	6.33915E-05	6.4287E-05	1
269	Neg	untargeted	3.7	215.0497	[M-H]-	86492	Bisnorbiotin	4.6	DOWN	2.2513E-05	2.1923E-05	2
270	Neg	untargeted	6.54	145.0292	[M-H]-	323	Coumarin	4.5	DOWN	2.57929E-08	4.3407E-08	2

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271	Neg	targeted	2.42	131.0344318	[M-H]-	743	Glutarate	4.5	DOWN	0.026336644	0.01321078	1
272	Neg	untargeted	3.63	204.9812	[M-H]-	54110629	Pyrogallol-1-O-sulfate	4.5	UP	0.00095916	0.00069162	2
273	Neg	untargeted	8.86	257.2117	[M-H]-	182089	3-Hydroxypentadecanoate	4.4	DOWN	4.22006E-05	3.9223E-05	2
274	Pos	untargeted	8.02	330.2626	[M+NH4]+	6439847	9(S)-HPODE	4.4	UP	9.57574E-09	2.175E-08	2
275	Pos	untargeted	9.08	573.3751	[M+H]+	6443809	Vitamin D2 3-glucuronide	4.4	UP	0.002031483	0.00143791	2
276	Pos (& Neg)	untargeted	0.64	201.9839	[M+H]+	115015	Cysteine-S-sulfate	4.4	UP	4.93352E-09	1.1943E-08	2
277	Neg	untargeted	1.1	350.1097	[M-H]-	123962	N-Acetyl-9-O-acetylneuraminate	4.4	DOWN	0.00527512	0.00320286	2
278	Neg	targeted	3.86	117.055166	[M-H]-	99823	2-Hydroxy-3-methylbutyrate	4.4	DOWN	8.395E-05	7.3898E-05	1
279	Neg	targeted	4.64	440.1318504	[M-H]-	6037	Folate	4.4	UP	2.60314E-07	3.5509E-07	1
280	Pos	untargeted	3.65	160.1332	[M+H]+	69522	DL-2-Aminooctanoate	4.3	DOWN	0.002582344	0.00177576	2
281	Pos	untargeted	4.47	476.2141	[2M+NH4]+	5351619	Ergothioneine	4.3	UP	3.86961E-06	5.0268E-06	2
282	Neg	untargeted	4.79	429.1037	[M+Cl]-	45479483	N-Acetylserotonin glucuronide	4.3	UP	4.65766E-08	7.4576E-08	2
283	Neg	untargeted	0.9	206.9969	[M+FA-H]-	21398693	2-Mercaptoglutarate	4.3	DOWN	0.009860346	0.00558395	2
284	Pos	untargeted	3.2	281.149	[M+H]+	7009554	Valyl-Tyrosine	4.3	UP	1.32236E-05	1.5332E-05	2
285	Pos (& Neg)	targeted	4.35	160.0973638	[M+H]+	193872	N-(2-Methylbutyryl)glycine	4.3	UP	0.000480925	0.00039824	1
286	Pos	untargeted	4.75	151.0751	[M+H]+	5280535	4-Coumaryl alcohol	4.2	UP	2.07858E-09	5.5203E-09	2
287	Neg	untargeted	0.79	281.0871	[M-H]-	74539967	5-O-α-L-Arabinofuranosyl-L-arabinose	4.2	UP	1.02141E-14	1.7165E-13	2
288	Pos	untargeted	6.45	615.313	[M+Na]+	26818	Mesobilirubinogen	4.2	DOWN	9.63112E-05	9.3951E-05	2
289	Pos	untargeted	5.55	182.0809	[M+NH4]+	997	Phenylpyruvate	4.2	DOWN	4.23864E-05	4.4641E-05	2
290	Neg	targeted	0.66	275.0168078	[M-H]-	91493	6-Phospho-D-gluconate	4.2	DOWN	0.006524103	0.00387875	1
291	Pos	untargeted	0.58	189.16	[M+H]+	440120	N6,N6,N6-Trimethyl-L-lysine	4.2	UP	1.9642E-09	5.2485E-09	2
292	Neg	untargeted	4.26	293.15	[M-H]-	342468	Isoleucyl-Tyrosine	4.2	DOWN	0.005736976	0.00345448	2
293	Neg	untargeted	4.97	243.1715	[M-H]-	435718	Leucyl-Isoleucine	4.2	DOWN	0.000132776	0.00011263	2
294	Neg	untargeted	9	391.2851	[M-H]-	5283835	3β,12α-Dihydroxy-5α-cholanoate	4.2	DOWN	0.006329594	0.0037749	2
295	Neg	untargeted	8.7	313.2386	[M-H]-	25320870	12,13-DHOME	4.1	DOWN	5.89967E-07	7.5358E-07	2
296	Neg	untargeted	1.44	187.1083	[M-H]-	92843	Glycylleucine	4.1	DOWN	0.002504878	0.00165307	2
297	Neg	targeted	10	299.2586056	[M-H]-	439887	2-Hydroxystearate	4.1	DOWN	0.00369359	0.00233528	1
298	Pos	untargeted	4.2	330.1077	[M+Na]+	134128	Carbocysteine-lysine	4.1	UP	6.73982E-07	1.0273E-06	2
299	Pos (& Neg)	targeted	4.68	190.0504162	[M+H]+	3845	Kynurenate	4.1	DOWN	0.032585861	0.01618462	1
300	Pos	untargeted	2.23	268.1035	[M+H]+	60961	Adenosine	4.1	UP	1.68633E-08	3.5905E-08	2
301	Neg	untargeted	7.37	531.2989	[M-H]-	53477904	5β-Cyprinol sulfate	4.1	DOWN	0.000154571	0.00012963	2
302	Neg	untargeted	7.76	329.2331	[M-H]-	14968868	9,10,13-TriHOME	4.1	DOWN	0.004811292	0.0029528	2
303	Pos (& Neg)	targeted & u	1.98	153.0408	[M+H]+	1188	Xanthine	4.1	DOWN	3.76011E-06	4.8985E-06	1
304	Neg	untargeted	5.4	212.0924	[M-H]-	688505	N-(3-Oxohexanoyl)homoserine lactone	4.1	UP	9.8441E-08	1.4609E-07	2
305	Neg	targeted & u	0.67	341.1083	[M-H]-	5988	Sucrose (or Cellobiose)	4.0	UP	1.73666E-09	3.9758E-09	1
306	Neg	untargeted	4.76	245.012	[M-H]-	187488	3-(3-Hydroxyphenyl)propanoate sulfate	4.0	UP	0.00476426	0.00292723	2
307	Pos	targeted	7.52	118.0656712	[M+H]+	798	Indole	4.0	UP	7.28266E-06	8.9418E-06	1
308	Pos	untargeted	6.38	216.1588	[M+H]+	10176752	N-Nonanoylglycine	4.0	UP	8.32522E-10	2.4874E-09	2
309	Pos	untargeted	3.92	263.1391	[M+H]+	7020641	Phenylalanylproline	3.9	UP	0.000193541	0.00017644	2
310	Neg	untargeted	1.28	185.0567	[M-H]-	20977730	Pyroglutamylglycine	3.9	UP	0.003041027	0.00196334	2
311	Pos	untargeted	4.9	233.1647	[M+H]+	115282	2,6-Pipecoloxylidide	3.9	UP	2.49992E-07	4.1506E-07	2
312	Pos	untargeted	1.14	211.0718	[M+H-H2O]+	13712	Deoxyuridine (dU)	3.8	UP	5.85132E-12	3.4025E-11	2
313	Neg	untargeted	3.12	345.082	[M+FA-H]-	4596190	Salicylate β-D-glucoside	3.8	UP	0.000156421	0.00013105	2
314	Pos (& Neg)	targeted & u	0.6	156.0767	[M+H]+	6274	L-Histidine	3.8	UP	5.93798E-08	1.1202E-07	1
315	Pos	targeted	6.39	176.0711504	[M+H]+	802	Indole-3-acetate	3.7	DOWN	0.004356327	0.00280613	1

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316	Neg	untargeted	1.46	263.0227	[M-H]-	3035420	3-Methoxy-4-Hydroxyphenylglycol sulfate	3.7	UP	0.000314546	0.00024957	2
317	Pos	untargeted	6.64	514.283	[M+H]+	72222	Sulfolithocholylglycine	3.7	UP	0.002023612	0.00143298	2
318	Pos (& Neg)	untargeted	8.82	438.2974	[M+H]+	42607469	PE(P-16:0e/0:0)	3.7	UP	0.000398548	0.00033619	2
319	Neg	targeted	0.91	103.0031334	[M-H]-	867	Malonate	3.7	DOWN	0.012435801	0.00684054	1
320	Neg	untargeted	5.53	366.1494	[M-H]-	14475583	Tyrosyl-Tryptophan	3.7	UP	9.64061E-07	1.1873E-06	2
321	Pos	untargeted	3.56	180.0632	[M+Na]+	169485	3-Methylcrotonylglycine	3.7	DOWN	0.000826476	0.00064806	2
322	Neg	untargeted	5.65	406.2334	[M-H]-	53481585	Neurotensin 11-13	3.6	DOWN	8.42902E-05	7.4176E-05	2
323	Neg	targeted & u	5.6	163.0401	[M-H]-	637542	4-Coumarate	3.6	DOWN	0.003639144	0.0023043	1
324	Neg	untargeted	0.75	380.0772	[M-H]-	441106	4-Hydroxy-6-methylpretetramide	3.5	UP	1.84643E-06	2.1624E-06	2
325	Pos	untargeted	1.08	231.0976	[M+H-2H2O]+	9816781	5'-Amino-5'-deoxyadenosine	3.5	UP	1.03613E-11	5.606E-11	2
326	Pos (& Neg)	untargeted	0.75	232.129	[M+H]+	18218188	Asparaginyl-Valine	3.5	DOWN	0.001602928	0.00116284	2
327	Pos	untargeted	8.09	647.5089	[M+H]+	44260123	SM(d18:1/12:0)	3.4	UP	3.83766E-06	4.9894E-06	2
328	Pos (& Neg)	untargeted	7.35	543.2794	[M+H]+	53480447	Cortolone-3-glucuronide	3.4	UP	9.94408E-10	2.8982E-09	2
329	Neg	targeted & u	0.81	132.0302	[M-H]-	5960	L-Aspartate	3.4	DOWN	3.85605E-06	4.2878E-06	1
330	Pos	untargeted	2.23	290.086	[M+Na]+	135398592	Deoxyguanosine (dG)	3.4	UP	2.59792E-13	2.572E-12	2
331	Neg	targeted	1.1	191.0191768	[M-H]-	31348	Citrate	3.4	UP	0.001192927	0.00084378	1
332	Neg	untargeted	6.38	379.1664	[M+Cl]-	13783449	11-Dehydrocorticosterone	3.3	UP	8.39757E-08	1.2638E-07	2
333	Neg	untargeted	0.69	209.066	[M-H]-	245622	D-Altro-D-manno-Heptose	3.3	UP	2.28133E-11	9.1376E-11	2
334	Neg	untargeted	9.03	342.2646	[M-H]-	168381	Dodecanoylcarnitine	3.3	DOWN	0.000161292	0.00013479	2
335	Neg	untargeted	1.12	159.0294	[M-H2O-H]-	194024	2-Keto-3-deoxy-D-gluconate	3.3	DOWN	0.001746567	0.00119222	2
336	Pos	untargeted	5.38	231.1699	[M+NH4]+	131802990	6-Nonenoylglycine	3.3	DOWN	0.000134845	0.00012767	2
337	Pos	untargeted	3.36	234.0431	[M+H]+	123932	Dopamine 4-sulfate	3.3	DOWN	0.00822336	0.00487838	2
338	Pos	untargeted	7.48	394.2244	[M+NH4]+	44263344	18-Oxocortisol	3.3	UP	1.37439E-07	2.4014E-07	2
339	Pos (& Neg)	untargeted	4.25	267.1334	[M+H]+	7010579	Threoninyl-Phenylalanine	3.3	DOWN	4.00502E-05	4.2369E-05	2
340	Neg	untargeted	1.97	187.1086	[M-H]-	259613	Glycyl-Isoleucine	3.3	DOWN	1.36467E-05	1.3809E-05	2
341	Neg	targeted & u	5.05	179.0347	[M-H]-	689043	Caffeate	3.3	UP	0.00015105	0.00012691	1
342	Neg	untargeted	4.95	487.2415	[M-H]-	459070	7-Sulfocholate	3.3	DOWN	5.07109E-05	4.65E-05	2
343	Pos (& Neg)	untargeted	3.21	224.0914	[M+H-2H2O]+	53481630	Fumarycarnitine	3.2	DOWN	0.000113744	0.00010938	2
344	Pos	untargeted	5.95	302.1747	[M+H]+	36811	Dobutamine	3.2	UP	7.70335E-07	1.1605E-06	2
345	Pos	untargeted	5.33	279.1698	[M+H]+	7010565	Phenylalanyl-isoleucine	3.2	DOWN	0.003807893	0.00249462	2
346	Neg	untargeted	3.66	224.056	[M-H]-	45266632	4-Amino-4-deoxychorismate	3.2	DOWN	0.009676525	0.00549154	2
347	Neg	untargeted	1.13	172.0614	[M-H]-	447989	(5S)-5-(Carboxymethyl)-L-proline	3.2	DOWN	0.004302087	0.00267579	2
348	Pos	targeted	0.82	184.0973638	[M+H]+	5816	Epinephrine	3.2	DOWN	1.63198E-06	2.3E-06	1
349	Neg	untargeted	4.15	338.111	[M-H]-	135564621	N(2)-Carboxyethyl-2'-deoxyguanosine	3.2	UP	9.64091E-09	1.8038E-08	2
350	Pos	untargeted	0.7	237.134	[M+H-H2O]+	20849429	Homoanserine	3.2	UP	9.46329E-08	1.7109E-07	2
351	Pos (& Neg)	untargeted	2.64	217.1548	[M+H]+	107475	Valyl-Valine	3.2	DOWN	0.000735825	0.00058442	2
352	Pos	untargeted	0.71	85.02834	[M+H-H2O]+	58	2-Ketobutyrate	3.1	UP	1.83325E-08	3.8719E-08	2
353	Neg	targeted	6.22	187.0970286	[M-H]-	2266	Azelate	3.1	DOWN	0.022692959	0.01159198	1
354	Pos	untargeted	0.94	217.0822	[M+H]+	6438678	N(3)-(4-Methoxyfumaroyl)-2,3-diaminopropionate	3.1	UP	0.002295062	0.00160058	2
355	Pos	untargeted	3.5	294.1815	[M+H-H2O]+	24978507	Val-Pro-Pro	3.1	UP	1.51473E-05	1.7329E-05	2
356	Pos	untargeted	4.98	245.1858	[M+H]+	13817313	Isoleucyl-Leucine	3.1	DOWN	1.64317E-06	2.314E-06	2
357	Neg	targeted & u	7.93	257.1754	[M-H]-	13185	Tetradecanedioate	3.1	DOWN	0.000121585	0.00010383	1
358	Pos	untargeted	0.69	203.1501	[M+H]+	123831	Asymmetric dimethylarginine	3.1	UP	1.95768E-09	5.2329E-09	2
359	Pos	untargeted	7.38	181.1222	[M+CH3OH+H]+	11469151	4-Phenyl-3-buten-2-ol	3.1	DOWN	5.23803E-05	5.4146E-05	2
360	Pos	untargeted	4.45	195.1125	[M+H]+	136927122	Laccarin	3.1	UP	1.16904E-06	1.7012E-06	2

Supplementary Table 3.2. Cont'd.

361	Pos	untargeted	4.91	254.1021	[M+H]+	57329379	N-Lactoyl-Tyrosine	3.1	UP	0.00037089	0.00031509	2
362	Pos	untargeted	1.51	145.1332	[M+H]+	189087	N-Acetylcadaverine	3.0	DOWN	0.000306171	0.00026546	2
363	Neg	untargeted	5.11	225.0404	[M-H]-	12039	Chorismate	3.0	DOWN	0.003355036	0.00214301	2
364	Pos	untargeted	5.97	181.0856	[M+H]+	15572	2-Phenyl-1,3-dioxolane-4-methanol	3.0	UP	3.80904E-08	7.5186E-08	2
365	Neg	untargeted	3.86	236.0788	[M-H]-	135449517	Biopterin	3.0	UP	2.89182E-06	3.2754E-06	2
366	Neg	untargeted	4.91	239.9969	[M-H]-	124202109	Indole-3-carboxylate-O-sulfate	3.0	UP	0.002621098	0.00172138	2
367	Neg	untargeted	7.45	331.2482	[M+FA-H]-	15110021	12-Hydroxyheptadecanoate	3.0	UP	0.009183906	0.00524415	2
368	Neg	targeted	6.42	321.0432814	[M-H]-	29979373	(+)-Equol 4'-sulfate	3.0	UP	1.53275E-10	4.7837E-10	1
369	Pos	targeted & u	0.66	114.0665	[M+H]+	588	Creatinine	3.0	UP	3.01014E-05	3.2586E-05	1
370	Neg	untargeted	3.52	271.0937	[M+FA-H]-	1021	Porphobilinogen	3.0	UP	4.57172E-06	5.0196E-06	2
371	Pos	untargeted	7.09	364.269	[M+NH4]+	5282957	9,10-Dihydroxy-13-hydroperoxy-11-octadecenoate	3.0	UP	0.0003603	0.00030693	2
372	Neg	untargeted	0.75	85.02908	[M-H]-	650	2,3-Butadione	3.0	DOWN	0.006535392	0.00388475	2
373	Pos	untargeted	7.64	464.2819	[M+H-H2O]+	6257035	Dihydrocytochalasin B γ-lactone	3.0	UP	0.007779174	0.00464743	2
374	Neg	targeted & u	7.07	215.1281	[M-H]-	15816	Undecanedioate	3.0	DOWN	0.003002916	0.00194154	1
375	Neg	untargeted	0.59	189.0045	[M-H]-	972	Oxalosuccinate	2.9	DOWN	3.89306E-06	4.3259E-06	2
376	Pos (& Neg)	untargeted	3.87	231.17	[M+H]+	7010531	Valyl-Isoleucine	2.9	DOWN	1.36681E-05	1.5787E-05	2
377	Pos (& Neg)	untargeted	3.37	233.1497	[M+H]+	7021827	Threoninyl-Leucine	2.9	DOWN	1.93842E-06	2.6904E-06	2
378	Pos	untargeted	4.37	262.1187	[M+H]+	102763	Glycyl-Tryptophan	2.9	UP	0.000425779	0.00035669	2
379	Neg	targeted	0.78	173.0926124	[M-H]-	70923	N6-Formyl-lysine (FLys)	2.9	DOWN	0.002609569	0.00171463	1
380	Neg	untargeted	0.75	166.0176	[M+FA-H]-	5862	L-Cysteine	2.9	DOWN	0.003021583	0.0019522	2
381	Pos	untargeted	1.69	251.1059	[M+H]+	7020164	Threoninyl-Methionine	2.9	DOWN	0.002482787	0.00171539	2
382	Neg	targeted & u	8.33	285.2075	[M-H]-	10459	Hexadecanedioate	2.9	DOWN	0.00156887	0.00108162	1
383	Pos	targeted	0.7	170.0817146	[M+H]+	439260	Norepinephrine	2.9	DOWN	0.004031394	0.00262236	1
384	Neg	untargeted	1.16	228.0987	[M-H]-	14354813	Asparaginyl-Proline	2.8	UP	0.000523513	0.00039715	2
385	Pos	untargeted	8.89	297.2418	[M+H]+	5356421	12,13-EpOME	2.8	DOWN	0.002791416	0.00190143	2
386	Pos	untargeted	1.73	259.1284	[M+H]+	69247902	γ-L-Glutamyl-L-pipecolate	2.8	UP	0.000477813	0.00039592	2
387	Pos	untargeted	4.81	279.1335	[M+H]+	440780	alpha-Ribazole	2.8	UP	1.83563E-06	2.5593E-06	2
388	Pos	untargeted	1.94	261.1444	[M+H]+	151023	γ-Glutamylleucine	2.8	DOWN	0.000645477	0.00051962	2
389	Pos (& Neg)	targeted & u	0.68	222.097	[M+H]+	84265	N-Acetylgalactosamine (GalNAc)	2.8	DOWN	2.35323E-07	3.9273E-07	1
390	Pos	untargeted	7.59	655.2742	[M+H]+	114935	Coproporphyrin III	2.8	DOWN	0.000173032	0.00015975	2
391	Pos	targeted	9.38	300.2902392	[M+H]+	4671	Palmitoyl ethanolamide	2.8	DOWN	0.028896447	0.01455972	1
392	Pos	untargeted	0.71	157.1082	[M+NH4]+	777	4-Amino-5-hydroxymethyl-2-methylpyrimidine	2.8	UP	2.83638E-05	3.0851E-05	2
393	Neg	untargeted	1.02	249.0069	[M-H]-	22833570	3,4-Dihydroxyphenylglycol O-sulfate	2.8	UP	1.94907E-06	2.2731E-06	2
394	Neg	untargeted	4.86	289.003	[M-H]-	131834358	3-[3-Methoxy-4-(sulfooxy)phenyl]oxirane-2-carboxylate	2.8	UP	2.15035E-08	3.6986E-08	2
395	Pos (& Neg)	targeted	0.61	90.0555012	[M+H]+	5950	L-Alanine	2.7	DOWN	9.14258E-07	1.3566E-06	1
396	Pos	untargeted	5.81	220.0969	[M+NH4]+	8422	(2-Naphthalenyloxy)acetate	2.7	DOWN	0.001743137	0.00125433	2
397	Pos	untargeted	0.7	196.0601	[M+H-2H2O]+	440848	N2-Succinyl-L-glutamate 5-semialdehyde	2.7	DOWN	0.006201579	0.00381014	2
398	Pos	untargeted	4.36	120.0808	[M+H]+	10328	Indoline	2.7	UP	4.30767E-14	6.3779E-13	2
399	Pos	untargeted	1.62	187.1075	[M+H]+	83525	Alanyl-Proline	2.7	UP	0.000243259	0.00021639	2
400	Pos	untargeted	7.38	149.132	[M+H]+	519195	1-Methyl-4-(1-methylpropyl)-benzene	2.7	DOWN	0.000364313	0.00031002	2
401	Pos	untargeted	5.8	630.3246	[2M+NH4]+	9839580	Fructoselysine	2.7	UP	7.09276E-05	7.1142E-05	2
402	Pos	targeted	0.66	112.051085	[M+H]+	597	Cytosine	2.7	DOWN	0.015448787	0.0084298	1
403	Neg	untargeted	3.74	260.0235	[M-H]-	514186	O(4')-Sulfo-L-tyrosine	2.7	UP	1.36904E-05	1.385E-05	2
404	Neg	targeted & u	1.02	187.1089	[M-H]-	92832	Nα-Acetyl-lysine	2.7	DOWN	3.36972E-05	3.1858E-05	1
405	Neg	untargeted	4.96	133.0293	[M-H]-	14090	Phenylglyoxal	2.7	UP	1.80474E-07	2.5506E-07	2

Supplementary Table 3.2. Cont'd.

406	Pos	untargeted	1.88	247.1285	[M+H]+	3549397	L-β-Aspartyl-L-leucine	2.7	DOWN	9.56526E-05	9.3361E-05	2
407	Pos	untargeted	6.39	618.2375	[M+Na]+	53477754	Taurocholate 3-sulfate	2.7	UP	0.009937106	0.00574625	2
408	Neg	untargeted	1.03	382.1359	[M-H]-	5288905	Lacto-N-biose I	2.7	UP	7.13377E-07	8.9917E-07	2
409	Pos (& Neg)	targeted & u	0.63	90.0555012	[M+H]+	1088	Sarcosine	2.6	DOWN	4.72546E-06	6.0276E-06	1
410	Neg	targeted	0.88	146.0453304	[M-H]-	65249	N-Acetyl-serine	2.6	DOWN	0.001837623	0.00124805	1
411	Pos (& Neg)	targeted & u	1.76	127.0507506	[M+H]+	1135	Thymine	2.6	DOWN	0.009927136	0.00574131	1
412	Neg	untargeted	3.32	215.0556	[M-H]-	25244228	cis-Trihomoaconitate	2.6	DOWN	0.000227251	0.0001847	2
413	Pos	untargeted	8.58	279.2316	[M+H]+	5280934	α-Linolenate	2.6	DOWN	0.002518999	0.00173742	2
414	Pos (& Neg)	targeted & u	0.64	120.0660654	[M+H]+	6288	L-Threonine	2.6	UP	5.29212E-11	2.2558E-10	1
415	Neg	untargeted	0.65	89.02407	[M-H]-	107689	L-(+)-Lactate	2.6	DOWN	3.9138E-05	3.6569E-05	2
416	Pos (& Neg)	untargeted	2.09	203.139	[M+H]+	96801	Alanyl-Leucine	2.6	DOWN	0.005055301	0.00319164	2
417	Pos	targeted	0.78	120.0660654	[M+H]+	91552	L-Homocysteine	2.6	UP	1.05083E-09	3.0367E-09	1
418	Neg	untargeted	3.52	309.109	[M-H]-	94340	γ-Glutamyltyrosine	2.6	UP	4.55105E-06	4.9986E-06	2
419	Pos	untargeted	7.63	192.1376	[M+NH4]+	5363896	4-Methyl-2-phenyl-2-pentenal	2.5	DOWN	9.87124E-07	1.4551E-06	2
420	Pos (& Neg)	targeted & u	3.31	243.0976	[M+H]+	5789	Thymidine	2.5	UP	2.49584E-07	4.1444E-07	1
421	Pos	untargeted	5.17	222.0758	[M+H]+	14033762	Methyl dioxindole-3-acetate	2.5	UP	0.002521782	0.00173911	2
422	Neg	targeted	3.71	225.051144	[M-H]-	65124	3-Nitro-L-tyrosine	2.5	UP	8.46813E-05	7.4495E-05	1
423	Pos (& Neg)	targeted	4.41	206.0453312	[M+H]+	5699	Xanthurenate	2.5	DOWN	0.0443359	0.02119813	1
424	Neg	untargeted	2.01	166.0506	[M-H]-	1050	Pyridoxal	2.5	UP	9.39355E-07	1.1594E-06	2
425	Neg	targeted & u	0.72	75.00826	[M-H]-	757	Glycolate	2.5	DOWN	0.000215014	0.0001756	1
426	Neg	untargeted	1.2	144.0663	[M-H]-	18189	4-Acetamidobutanoate	2.5	DOWN	0.001075253	0.00076787	2
427	Neg	targeted & u	4.24	181.0504	[M-H]-	9378	DL-p-Hydroxyphenyllactate	2.5	UP	1.49968E-05	1.5069E-05	1
428	Neg	untargeted	1.55	161.0452	[M-H]-	193530	2-Hydroxyadipate	2.5	DOWN	2.32243E-05	2.2559E-05	2
429	Pos (& Neg)	targeted & u	0.63	148.0601	[M+H]+	33032	L-Glutamate	2.5	DOWN	2.26674E-05	2.5134E-05	1
430	Neg	targeted	5.67	173.0813794	[M-H]-	10457	Suberate	2.5	DOWN	0.015041267	0.0080685	1
431	Neg	targeted & u	0.6	74.02414	[M-H]-	750	Glycine	2.5	UP	8.30725E-07	1.0351E-06	2
432	Pos	untargeted	4.54	217.1337	[M+H]+	14219281	Nβ-Methyltetrahydroharmol	2.5	UP	6.355E-10	1.9592E-09	2
433	Pos	untargeted	1.45	193.0969	[M+H]+	10219774	Hydroxycotinine	2.5	UP	6.13175E-06	7.6481E-06	2
434	Neg	untargeted	2.01	143.0348	[M-H]-	6368126	(E)-2-Methylglutaconate	2.5	DOWN	0.002036216	0.00137071	2
435	Neg	targeted	0.96	243.0617082	[M-H]-	15047	β-Pseudouridine	2.5	UP	0.00366709	0.00232021	1
436	Pos	untargeted	0.62	74.06004	[M+H]+	215	Aminoacetone	2.5	UP	8.14984E-11	3.2682E-10	2
437	Neg	untargeted	3.57	333.1303	[M-H]-	56927739	Pseudaminate	2.4	UP	2.15292E-05	2.1039E-05	2
438	Pos	untargeted	7.05	175.0752	[M+H]+	6259976	3-Propylidene-1(3H)-isobenzofuranone	2.4	UP	4.85198E-06	6.1747E-06	2
439	Pos (& Neg)	targeted & u	0.68	114.0559	[M-H]-	145742	L-Proline	2.4	UP	0.001512472	0.00104606	1
440	Pos	untargeted	1.11	217.118	[M+H]+	9834371	Threoninyl-Proline	2.4	UP	1.02532E-08	2.3095E-08	2
441	Neg	targeted & u	0.85	87.0082184	[M-H]-	1060	Pyruvate	2.4	DOWN	4.14604E-05	3.8584E-05	1
442	Pos	untargeted	4.39	276.1335	[M+CH3OH+H]+	12897225	N-(2-Methoxy-4-pyridyl)-N'-phenylurea	2.4	UP	1.68152E-05	1.9099E-05	2
443	Neg	untargeted	0.69	100.0403	[M-H]-	535	1-Aminocyclopropane-1-carboxylate	2.4	UP	0.000425774	0.00032852	2
444	Pos	untargeted	2.3	276.1554	[M+CH3OH+H]+	11736661	Glutaminylproline	2.4	DOWN	1.24617E-05	1.4543E-05	2
445	Neg (& Pos)	targeted	5.8	206.0817138	[M-H]-	74839	N-Acetyl-L-phenylalanine	2.4	UP	0.002466259	0.00163022	1
446	Pos	untargeted	3.82	230.0422	[M+Na]+	472	4-(2-Aminophenyl)-2,4-dioxobutanoate	2.3	DOWN	0.003902828	0.00254909	2
447	Pos	untargeted	8.67	255.2311	[M+H]+	445638	Palmitoleate	2.3	DOWN	0.000608118	0.00049254	2
448	Pos	untargeted	5.46	446.2281	[M+Na]+	53480920	LysoPE(0:0/14:1(9Z))	2.3	UP	0.000210595	0.00019026	2
449	Pos	untargeted	3.65	229.1547	[M+H]+	80817	Leucylproline	2.3	UP	4.96742E-06	6.3082E-06	2
450	Pos (& Neg)	targeted & u	0.62	147.0765	[M+H]+	5961	L-Glutamine	2.3	UP	2.50504E-07	4.1583E-07	1

Supplementary Table 3.2. Cont'd.

451	Pos	untargeted	4.97	282.0974	[M+H]+	61152160	4-Hydroxyphenylacetylglutamine	2.3	UP	3.10818E-10	1.0413E-09	2
452	Pos	untargeted	0.54	204.1345	[M+H]+	306144	Glycyl-Lysine	2.3	DOWN	0.008402004	0.00497036	2
453	Pos	untargeted	7.3	441.2455	[M+H]+	60947	Tirofiban	2.3	UP	0.00021236	0.00019169	2
454	Neg	untargeted	1.85	274.141	[M-H]-	7015704	Glutamyllysine	2.3	DOWN	0.002189244	0.00146413	2
455	Neg	untargeted	4.54	221.0456	[M-H]-	5459922	O-Succinylbenzoate	2.3	UP	7.37753E-06	7.7952E-06	2
456	Pos	untargeted	2.3	253.0926	[M+H]+	135398593	Deoxyinosine (dI)	2.3	UP	1.08781E-07	1.941E-07	2
457	Pos	untargeted	3.96	148.1119	[M+H]+	22249	3-Methyl-3-phenylazetidine	2.2	UP	0.000358106	0.00030523	2
458	Pos	untargeted	3.92	248.0586	[M+H]+	54166459	3-Methoxytyramine sulfate	2.2	DOWN	0.002644443	0.00181313	2
459	Neg	untargeted	6.66	272.0921	[M-H]-	5461103	L-Thyronine	2.2	DOWN	0.005110595	0.00311437	2
460	Pos	untargeted	3.27	350.2068	[M+H]+	449034	Coutarate	2.2	UP	0.001485467	0.0010862	2
461	Neg	untargeted	1.64	301.1039	[M-H]-	11954188	3''-Deamino-3''-oxonicotianamine	2.2	UP	9.55233E-05	8.327E-05	2
462	Pos	targeted	3.78	232.1548756	[M+H]+	213144	Butyrylcarnitine	2.2	DOWN	0.010393729	0.00597634	1
463	Pos	untargeted	1.8	218.1387	[2M+NH4]+	11747	2,3-Pentanedione	2.2	UP	0.000376747	0.00031958	2
464	Pos	untargeted	4.92	245.1858	[M+H]+	13879965	Isoleucyl-Isoleucine	2.2	DOWN	0.000503627	0.00041508	2
465	Pos	untargeted	4.37	326.0379	[M+H]+	135565297	Urothion	2.2	DOWN	0.002046089	0.00144705	2
466	Pos	untargeted	1.13	174.0764	[M+H]+	192878	N-Acetyl-L-glutamate 5-semialdehyde	2.2	DOWN	0.002754132	0.00187887	2
467	Neg	untargeted	2.36	171.0298	[M-H]-	5280801	2-Hydroxyhepta-2,4-dienedioate	2.2	DOWN	0.00400904	0.00251313	2
468	Pos (& Neg)	targeted & u	0.7	147.1127	[M+H]+	5962	L-Lysine	2.2	DOWN	2.48243E-05	2.7314E-05	1
469	Pos	untargeted	5.12	338.105	[M+H]+	447123	S-(Hydroxymethyl)glutathione	2.2	UP	6.6702E-09	1.5729E-08	2
470	Neg	untargeted	1.11	260.0777	[M-H]-	77879498	Dihydromaleimide β-D-glucoside	2.2	UP	0.000362488	0.00028409	2
471	Neg	targeted	5	159.0657302	[M-H]-	385	Pimelate	2.2	DOWN	0.016768241	0.00888278	1
472	Neg	untargeted	1.2	200.056	[M-H]-	13943174	N-Acetyl-L-2-aminoadipate	2.1	UP	6.22932E-05	5.6176E-05	2
473	Neg	untargeted	0.67	119.0347	[M-H]-	150929	(S)-3,4-Dihydroxybutyrate	2.1	DOWN	0.001579869	0.00108853	2
474	Neg	untargeted	5.36	223.0248	[M-H]-	9920917	Dehydrochorismate	2.1	DOWN	0.000784091	0.00057522	2
475	Pos (& Neg)	untargeted	8.39	417.3362	[M+H-H2O]+	5284239	3α,7α-Dihydroxycoprostanate	2.1	UP	0.001898377	0.00135392	2
476	Pos	targeted	0.6	223.0752494	[M+H]+	439258	L-Cystathionine	2.1	UP	1.59879E-08	3.4209E-08	1
477	Neg	untargeted	5.34	367.1046	[M-H]-	131752769	3-O-Caffeoyl-4-O-methylquinate	2.1	UP	0.00345047	0.00219714	2
478	Pos	untargeted	0.67	221.113	[M+H]+	439454	N-Acetyl-β-glucosaminylamine	2.1	DOWN	2.68734E-07	4.4325E-07	2
479	Pos	untargeted	4.79	225.0758	[M+H]+	637775	Sinapate	2.1	UP	3.59317E-05	3.8325E-05	2
480	Pos (& Neg)	targeted	1.27	245.0773582	[M+H]+	6029	Uridine	2.1	UP	0.001930064	0.00137403	1
481	Pos	untargeted	6.35	165.0912	[M+H]+	3018936	p-Menthα-2,4(8)-dien-9,3-olide	2.1	UP	1.02614E-05	1.2207E-05	2
482	Neg	untargeted	4.05	238.0358	[M+FA-H]-	119405	5,6-Dihydroxyindole-2-carboxylate (DHICA)	2.1	DOWN	0.001615828	0.00111106	2
483	Neg	untargeted	1.11	128.0353	[M-H]-	7405	Pyroglutamate	2.1	DOWN	0.009513918	0.00540954	2
484	Pos	targeted & u	0.54	130.0862	[M+H]+	849	Pipecolate	2.0	DOWN	9.03596E-05	8.8596E-05	1
485	Pos	untargeted	1.69	230.1135	[M+CH3OH+H]+	75619	N-Acetylhistidine	2.0	UP	0.000115424	0.00011086	2
486	Pos	untargeted	6.23	209.0808	[M+H]+	45093080	5-(3',4'-Dihydroxyphenyl)-γ-valerolactone	2.0	UP	0.000146944	0.0001379	2
487	Neg	untargeted	4.33	162.0556	[M-H]-	151066	3-Methyldioxyindole	2.0	UP	7.10596E-05	6.3289E-05	2
488	Neg	untargeted	0.94	131.0348	[M-H]-	10103208	2-Hydroxy-4-oxopentanoate	2.0	DOWN	0.001317978	0.00092292	2
489	Neg	untargeted	3.75	153.019	[M-H]-	19	2-Pyrocatechuate	2.0	DOWN	0.00182405	0.00123976	2
490	Neg	untargeted	2.13	199.0609	[M-H]-	22394751	(Z)-3-(1-Formyl-1-propenyl)pentanedioate	2.0	UP	2.10279E-05	2.0589E-05	2
491	Pos	untargeted	3.2	182.1176	[M+H]+	85575714	2-(5-Methyl-2-furanyl)-3-piperidinol	1.9	DOWN	2.41203E-05	2.6606E-05	2
492	Neg	targeted	1.1	330.0603424	[M-H]-	12599	2'-Deoxyadenosine 5'-monophosphate (dAMP)	1.9	UP	0.014557653	0.00783972	1
493	Neg	untargeted	1.02	129.0191	[M-H]-	523	2,5-Dioxopentanoate	1.9	UP	0.00079169	0.00058036	2
494	Neg	untargeted	4.57	235.0648	[M-H]-	44237181	2-(4'-Methylthio)butylmalate	1.9	DOWN	0.007355557	0.00431545	2
495	Pos	untargeted	1.7	144.1018	[M+H]+	115244	Proline betaine	1.9	DOWN	0.007976373	0.00475037	2

Supplementary Table 3.2. Cont'd.

496	Neg	untargeted	1.58	175.0612	[M-H]-	5280523	2-Isopropylmalate	1.9	DOWN	0.000232977	0.00018892	2
497	Neg	targeted	5.05	263.1031766	[M-H]-	92258	Phenylacetyl L-glutamine	1.9	UP	0.000468277	0.00035861	1
498	Neg (& Pos)	targeted & u	0.99	148.0436	[M-H]-	6137	L-Methionine	1.9	DOWN	0.002505867	0.00165365	1
499	Neg	untargeted	0.59	178.0718	[M-H]-	439213	Glucosamine	1.8	UP	0.005500155	0.00332533	2
500	Pos	targeted	0.63	104.1075334	M+	305	Choline	1.8	UP	2.56511E-06	3.465E-06	1
501	Pos (& Neg)	targeted	6.24	146.0605862	[M+H]+	10256	Indole-3-carboxaldehyde	1.8	DOWN	0.010399843	0.00597941	1
502	Neg	untargeted	1.38	245.1141	[M-H]-	332962	Aspartyl-Leucine	1.8	DOWN	0.003079803	0.00198549	2
503	Pos	untargeted	1.63	195.0764	[M+H]+	2148	4-Aminohippurate	1.8	UP	0.000135187	0.00012796	2
504	Neg	targeted	1.31	321.048775	[M-H]-	9700	Thymidine 5'-monophosphate (TMP)	1.8	UP	0.016063611	0.00855013	1
505	Pos	untargeted	0.64	183.0861	[M+H]+	11850	Galactitol	1.8	UP	0.005409267	0.00338531	2
506	Pos	targeted & u	0.97	162.1123	[M]+	10917	L-Carnitine	1.8	UP	0.004375418	0.00281682	1
507	Pos	untargeted	8.19	297.2415	[M+H]+	6443013	13(S)-HODE	1.8	UP	0.002963841	0.00200501	2
508	Neg	untargeted	1.69	244.1295	[M-H]-	4414300	Isoleucyl-Asparagine	1.8	DOWN	0.008210624	0.00475271	2
509	Neg	untargeted	2.59	155.0348	[M-H]-	9964159	2,3-Dihydro-2,3-dihydroxybenzoate	1.8	DOWN	0.008676877	0.00498812	2
510	Neg	untargeted	3.76	167.0348	[M-H]-	547	3,4-Dihydroxybenzeneacetate (Homoprotocatechuate)	1.7	UP	0.00294432	0.00190815	2
511	Pos	untargeted	3.22	221.0919	[M+H]+	439280	5-Hydroxy-L-tryptophan	1.7	UP	0.00129549	0.00096239	2
512	Pos	targeted	1.15	170.0817146	[M+H]+	1054	Pyridoxine	1.7	DOWN	0.016069098	0.00872228	1
513	Neg	untargeted	4.21	189.0768	[M-H]-	22328017	3-Hydroxysuberate	1.7	DOWN	0.00535621	0.00324667	2
514	Neg	targeted	7.8	243.1596254	[M-H]-	10458	1,11-Undecanedicarboxylate	1.7	DOWN	0.001258356	0.00088536	1
515	Neg	untargeted	1.75	156.0662	[M-H2O-H]-	79198664	Hydroxyvalerylglycine	1.7	UP	3.35356E-05	3.1718E-05	2
516	Pos	untargeted	4.4	245.0924	[M+H]+	5370648	Indolylacryloylglycine	1.7	UP	0.000528282	0.00043355	2
517	Pos	untargeted	2.06	219.1337	[M+H]+	14426033	Isoleucyl-Serine	1.7	DOWN	0.004482673	0.00287665	2
518	Neg	untargeted	0.76	112.0402	[M-H]-	1196	1-Pyrroline-5-carboxylate	1.7	UP	3.42428E-05	3.2333E-05	2
519	Pos	targeted	0.6	129.0663998	[M+H]+	11984188	Pyroglutamine	1.6	DOWN	0.022611049	0.01174895	1
520	Neg	untargeted	5.39	209.1295	[M-H]-	7074739	L,L-Cyclo(leucylprolyl)	1.6	DOWN	1.52536E-06	1.8134E-06	2
521	Pos	untargeted	0.61	257.1245	[2M+H]+	676414	Dihydrothymine	1.6	UP	9.94887E-05	9.6788E-05	2
522	Pos	untargeted	3.95	318.1663	[M+CH3OH+H]+	11778669	Glycylprolylhydroxyproline	1.6	UP	0.000598712	0.00048567	2
523	Neg (& Pos)	targeted & u	0.59	173.1043	[M-H]-	6322	L-Arginine	1.6	DOWN	0.006655138	0.00394834	1
524	Neg	targeted & u	3.96	145.0503	[M-H]-	196	Adipate	1.6	DOWN	0.003845064	0.002421	1
525	Neg	untargeted	1.65	201.088	[M-H]-	25203549	Proclavaminate	1.6	UP	0.003523838	0.00223854	2
526	Pos	targeted	0.99	110.0605862	[M+H]+	11568	3-Aminophenol	1.6	DOWN	0.00060593	0.00049094	1
527	Pos	untargeted	8.29	241.2156	[M+H]+	6365140	9-Pentadecenoate	1.6	DOWN	0.001601646	0.001162	2
528	Pos	untargeted	8.67	249.1845	[M+H-H2O]+	15730832	Tetranor 12-HETE	1.5	DOWN	0.000260601	0.00023004	2
529	Pos	untargeted	1.72	246.1447	[M+NH4]+	11902892	Prolylhydroxyproline	1.5	DOWN	0.006432848	0.0039324	2
530	Neg	targeted	1.79	103.0395168	[M-H]-	440864	2-Hydroxybutyrate	1.5	DOWN	0.045826734	0.02143656	1
531	Pos (& Neg)	targeted	4.23	205.0976982	[M+H]+	6305	L-Tryptophan	1.4	UP	0.007529363	0.00451611	1
532	Pos	targeted	9.28	280.2640258	[M+H]+	6435901	Linoleamide	1.4	DOWN	0.032625816	0.01620196	1
533	Pos (& Neg)	targeted	1.35	182.0817146	[M+H]+	6057	L-Tyrosine	1.3	DOWN	0.015436533	0.008424	1

Supplementary Table 3.2. Cont'd.

Supplementary Table 3.3. Unique list of 231 serum metabolites altered in sera comparing GF and CONV-R mice.

	ESI mode	Platform	RT (min)	PRECURSOR	PRECURSOR	PubChem_CID	Compound name (serum)	fold	updown (GF/CONV-R)	pvalue	qvalue	MSI level
1	Neg	targeted & untarget	4.86	212.0020	[M-H]-	10258	Indoxyl sulfate	4351.6	DOWN	3.2495E-07	8.601E-06	2
2	Pos	untargeted	9.07	458.3453	[M+NH4]+	108335	Acetolein	267.9	DOWN	0.00766567	0.0129739	2
3	Pos	targeted	0.65	76.0762	[M+H]+	1145	Trimethylamine N-oxide (TMAO)	267.3	DOWN	0.01047009	0.0160589	1
4	Neg	untargeted	4.25	172.9910	[M-H]-	74426	Phenyl sulfate	173.3	DOWN	0.00216083	0.0068446	2
5	Neg	targeted	8.42	391.2848	[M-H]-	10133	Chenodeoxycholate	92.1	DOWN	0.02610647	0.0437199	1
6	Pos	untargeted	1.29	311.1215	[M+H]+	94340	γ-Glutamyltyrosine	34.6	UP	6.6956E-07	1.108E-05	2
7	Pos	untargeted	8.37	712.4926	[M+H]+	52924229	PE(16:1(9Z)/18:3(9Z,12Z,15Z))	33.0	DOWN	0.00989824	0.0154776	2
8	Pos	untargeted	1.26	233.1129	[M+H]+	127370	N2-Succinyl-L-ornithine	28.7	UP	1.3798E-06	1.817E-05	2
9	Pos	untargeted	4.4	281.1130	[M+H]+	93078	Aspartylphenylalanine	20.7	UP	2.5501E-08	1.019E-06	2
10	Pos	untargeted	0.6	179.0901	[M+H]+	5179954	2-O-Methyl-L-fucose	19.9	UP	0.00071512	0.0021418	2
11	Pos	untargeted	8.66	748.5228	[M+H-2H2O]+	52929784	PE(18:0/20:4(5Z,8Z,11Z,13E)(15OH[S]))	17.5	DOWN	0.00893115	0.0144413	2
12	Pos	untargeted	1.84	265.0845	[M+H]+	152314	N-Acetylcystathionine	14.5	UP	1.3064E-07	3.325E-06	2
13	Pos (& Neg)	targeted	5.38	194.0817	[M+H]+	68144	N-(2-Phenylacetyl)glycine	10.5	DOWN	7.3979E-06	6.323E-05	1
14	Neg	targeted	5.17	153.0188	[M-H]-	19	2,3-Dihydroxybenzoate	10.0	DOWN	0.04581166	0.0679186	1
15	Neg	untargeted	4.15	230.9968	[M-H]-	20822599	Vanillin 4-sulfate	9.7	DOWN	0.00013512	0.0007605	2
16	Pos	targeted	6.9	190.0868	[M+H]+	3744	Indole-3-propionate	7.3	DOWN	7.0359E-05	0.0003603	1
17	Pos (& Neg)	targeted	5.03	180.0661	[M+H]+	464	Hippurate	7.2	DOWN	0.02744174	0.0306081	1
18	Neg	targeted	1.46	103.0395	[M-H]-	92135	3-Hydroxybutyrate	7.2	UP	2.6482E-06	3.706E-05	1
19	Pos	untargeted	3.75	248.1125	[M+H-2H2O]+	9903897	Histidinyl-Glutamine	7.1	UP	0.0026093	0.0058145	2
20	Pos	untargeted	3.55	249.0846	[M+Na]+	1021	Porphobilinogen	7.0	UP	5.382E-07	9.54E-06	2
21	Neg	targeted	5.12	179.0344	[M-H]-	689043	Caffeate	6.9	DOWN	0.02987525	0.0486779	1
22	Pos	untargeted	0.81	176.1028	[M+H]+	9750	Citrulline	6.9	DOWN	0.00014328	0.0006091	2
23	Pos	targeted	0.77	141.0664	[M+H]+	75810	1-Methyl-4-imidazoleacetate	6.8	DOWN	0.01383129	0.0191391	1
24	Pos	targeted	8.68	284.2226	[M+H]+	10221437	N-Dodecanoyl-L-homoserine lactone	6.8	UP	0.01527588	0.0203789	1
25	Neg	untargeted	6.82	461.2153	[M-H]-	5460852	6-Dehydrotestosterone glucuronide	6.4	UP	2.6583E-06	3.716E-05	2
26	Neg	untargeted	4.4	177.0223	[M-H]-	8096	3,3'-Thiobispropanoate	6.0	DOWN	0.00130451	0.0046017	2
27	Pos	untargeted	1.18	304.1504	[M+H]+	12313328	Nicotianamine	5.7	UP	6.0503E-06	5.444E-05	2
28	Pos	untargeted	3.06	297.1081	[M+H]+	19816752	Tyrosyl-Aspartate	5.5	UP	2.7252E-08	1.065E-06	2
29	Pos	untargeted	4.9	274.1100	[M+H]+	5461103	L-Thyronine	5.3	UP	1.719E-08	7.71E-07	2
30	Neg	targeted	0.73	191.0556	[M-H]-	6508	D-(-)-Quinate	5.2	UP	3.3988E-06	4.433E-05	1
31	Neg	untargeted	0.95	116.0351	[M-H]-	440033	L-2-Amino-3-oxobutanoate	5.1	UP	7.0384E-06	7.543E-05	2
32	Pos	untargeted	1.39	105.0545	[M+H]+	440873	(S)-3-Hydroxyisobutyrate	5.1	UP	3.9843E-06	3.964E-05	2
33	Neg	untargeted	2.6	142.0507	[M-H]-	53477718	Vinylacetylglycine	4.7	UP	1.1619E-08	9.123E-07	2
34	Neg	untargeted	1.09	156.0298	[M-H]-	5280499	2-Aminomuconate	4.6	UP	0.00791697	0.0180621	2
35	Pos	untargeted	6.83	349.2371	[M+H]+	192735	17α,21-Dihydroxypregnenolone	4.6	UP	1.5066E-07	3.712E-06	2
36	Neg	untargeted	5.3	170.0819	[M-H]-	10130163	N-Butyryl-L-homoserine lactone	4.3	UP	4.4702E-06	5.322E-05	2
37	Pos	targeted	7.51	190.0868	[M+H]+	74706	Methyl indole-3-acetate	4.3	DOWN	0.00232595	0.0053353	1
38	Pos	targeted	1.07	143.1184	[M+H]+	82111	N-(3-Aminopropyl)-2-pyrrolidinone	4.2	UP	4.4385E-05	0.0002524	1
39	Neg	untargeted	8.24	239.1653	[M-H]-	43045	L-Menthyl acetoacetate	4.2	UP	0.00061115	0.0024884	2
40	Pos	untargeted	1.19	87.0439	[M+H-2H2O]+	222285	Erythritol	4.1	UP	3.6675E-06	3.733E-05	2
41	Neg	untargeted	8.5	760.5115	[M-H]-	5283499	PS(16:0/18:1)	4.1	UP	0.00054367	0.0022505	2
42	Pos (& Neg)	targeted & untarget	5.79	174.1130	[M+H]+	99463	Hexanoylglycine	4.0	UP	7.5069E-05	0.0003785	1
43	Pos	targeted	0.73	143.0820	[M+H]+	126041	Ectoine	4.0	DOWN	0.01836542	0.0230108	1
44	Neg	untargeted	7.84	228.1603	[M-H]-	1712391	N-Decanoylglycine	3.9	UP	0.00148908	0.0050989	2
45	Neg (& Pos)	targeted & untarget	6.85	361.2021	[M-H]-	5754	Cortisol	3.8	UP	0.0048119	0.0123979	1
46	Neg	targeted	4.45	156.0661	[M-H]-	169485	3-Methylcrotonyl glycine	3.7	UP	1.8021E-06	2.793E-05	1
47	Pos	untargeted	6.56	131.1065	[M+H]+	8094	Heptanoate	3.7	UP	1.3489E-08	6.575E-07	2
48	Neg	untargeted	7.82	289.1417	[M-H]-	46173940	3-Polyprenyl-4,5-dihydroxybenzoate	3.7	UP	0.00389475	0.0106046	2
49	Neg	targeted	5.05	263.1032	[M-H]-	92258	Phenylacetyl L-glutamine	3.6	DOWN	0.00067618	0.0027106	1
50	Pos (& Neg)	untargeted	6.84	202.1434	[M+H]+	84290	Capryloylglycine	3.6	UP	7.449E-07	1.188E-05	2

51	Neg	untargeted	8.41	241.1804	[M-H]-	454064	3-Oxotetradecanoate	3.4	UP	0.00117266	0.0042283	2
52	Pos (& Neg)	targeted	0.95	133.0613	[M+H]+	111	3-Ureidopropionate	3.3	UP	4.6468E-06	4.46E-05	1
53	Neg (& Pos)	targeted & untarget	8.34	285.2068	[M-H]-	10459	Hexadecanedioate	3.3	UP	0.00839145	0.0188492	1
54	Neg (& Pos)	targeted & untarget	2.31	131.0347	[M-H]-	743	Glutarate	3.2	UP	0.00012165	0.0007013	1
55	Pos	targeted	0.49	146.1657	[M+H]+	1102	Spermidine	3.2	DOWN	0.00482991	0.009259	1
56	Neg	targeted	2.1	162.0225	[M-H]-	12035	N-Acetyl-L-cysteine	3.1	DOWN	0.00680765	0.0161334	1
57	Pos (& Neg)	targeted	8.01	259.1909	[M+H]+	13185	Tetradecanedioate	3.1	UP	0.00642255	0.0114506	1
58	Pos	targeted	7.44	347.2222	[M+H]+	5753	Corticosterone	3.0	UP	4.7484E-07	8.716E-06	1
59	Neg	untargeted	8.71	814.5569	[M-H]-	52925310	PS(16:0/22:2(13Z,16Z))	2.9	UP	0.00016496	0.000882	2
60	Neg	untargeted	8.71	301.2167	[M-H]-	446284	Eicosapentaenoate	2.9	UP	3.4307E-05	0.0002668	2
61	Pos	targeted	7.95	242.1756	[M+H]+	56662027	N-Nonanoyl-L-homoserine lactone	2.9	UP	0.00339434	0.0070979	1
62	Pos	untargeted	7.59	317.1952	[M+H]+	13325862	(S)-α-Terpinyl glucoside	2.8	UP	5.8194E-09	3.718E-07	2
63	Neg	untargeted	3.41	229.1185	[M-H]-	61158071	Hydroxyprolyl-Valine	2.8	UP	2.5226E-09	4.101E-07	2
64	Pos	untargeted	8.19	424.3404	[M+H]+	53477834	Linoelaidyl carnitine	2.8	UP	1.6269E-05	0.000115	2
65	Pos	untargeted	7.73	368.2790	[M+H]+	53481681	3, 5-Tetradecadiencarnitine	2.8	UP	2.0647E-07	4.746E-06	2
66	Pos (& Neg)	targeted	0.57	147.1133	[M+H]+	5962	L-Lysine	2.8	DOWN	0.02663989	0.0299454	1
67	Pos	untargeted	1.5	174.1238	[M+H]+	25244460	(2S)-6-Amino-2-formamidohexanamide	2.8	UP	7.8502E-05	0.0003916	2
68	Pos	targeted	1.24	385.1294	[M+H]+	439155	S-(5'-Adenosyl)-L-homocysteine (SAH)	2.8	UP	0.00063093	0.0019407	1
69	Pos (& Neg)	untargeted	4.57	173.0808	[M+H]+	11805205	cis-4-Octenedioate	2.7	UP	5.7658E-07	1.001E-05	2
70	Neg	untargeted	8.59	267.1961	[M-H]-	16061079	(1R,2R)-3-Oxo-2-pentyl-cyclopentanehexanoate	2.7	UP	0.00654636	0.0156578	2
71	Neg	targeted	1.79	103.0395	[M-H]-	11266	2-Hydroxybutyrate	2.7	UP	3.073E-05	0.0002448	1
72	Neg	untargeted	1.14	160.0614	[M-H]-	469	Aminoadipate	2.7	UP	2.1066E-07	6.272E-06	2
73	Neg	untargeted	7.72	350.2108	[M-H]-	52931110	Sphingosine 1-phosphate (d16:1-P)	2.6	DOWN	0.00903342	0.0198762	2
74	Pos	untargeted	8.42	319.2259	[M+H]+	5280383	Leukotriene A4	2.6	UP	0.00011581	0.0005209	2
75	Neg	targeted & untarget	8.71	319.2273	[M-H]-	5312983	12-HETE	2.6	UP	3.3608E-05	0.0002625	1
76	Pos	untargeted	1.78	164.0737	[M+NH4]+	526189	Dihydro-2-methoxy-2-methyl-3(2H)-thiophenone	2.6	UP	1.8324E-06	2.215E-05	2
77	Pos	untargeted	8.53	428.3716	[M+H]+	52922056	Stearoylcarnitine	2.6	UP	6.3763E-06	5.665E-05	2
78	Neg	targeted	5.76	165.0552	[M-H]-	3848	DL-3-Phenyllactate	2.6	DOWN	0.00646673	0.0155111	1
79	Neg	untargeted	7.98	213.1492	[M-H]-	439717	3-Oxododecanoate	2.5	UP	0.00135457	0.0047392	2
80	Pos	targeted	8.26	400.3427	[M+H]+	11953816	Palmitoyl-L-carnitine	2.5	UP	1.3963E-05	0.0001023	1
81	Pos	untargeted	5.7	474.2887	[M+NH4]+	10253562	Sulfolithocholate	2.5	UP	0.00604644	0.0109601	2
82	Pos	untargeted	4.59	123.0804	[M+H]+	31242	4-Ethylphenol	2.5	UP	6.1768E-08	1.856E-06	2
83	Neg	untargeted	5.64	242.1398	[M-H]-	91825636	Tiglylcarnitine	2.4	UP	0.00153217	0.0052139	2
84	Pos	untargeted	5.65	201.1120	[2M+H]+	10931	Senecioate	2.4	UP	0.00917768	0.0147121	2
85	Pos	untargeted	1.01	135.0275	[M+H]+	222656	L-Malate	2.4	DOWN	0.00159212	0.0039823	2
86	Neg	untargeted	8.12	253.2174	[M-H]-	445638	Palmitoleate	2.3	UP	0.0002138	0.0010665	2
87	Pos	untargeted	4.89	175.0785	[M+H]+	61989	4,4'-Thiobis-2-butanone	2.3	UP	1.2362E-06	1.68E-05	2
88	Neg	untargeted	4.82	230.1030	[M-H]-	6453952	Suberylglycine	2.3	UP	5.1791E-06	5.941E-05	2
89	Pos (& Neg)	targeted	5.72	162.0555	[M+H]+	54680871	2,4-Quinolinediol	2.3	DOWN	0.01349276	0.0188403	1
90	Neg	untargeted	8.24	361.2382	[M-H]-	16061148	19,20-DiHDPA	2.3	UP	0.00107567	0.0039521	2
91	Pos	untargeted	8.12	398.3254	[M+H]+	53477817	trans-Hexadec-2-enoyl carnitine	2.2	UP	5.571E-05	0.0003008	2
92	Neg	untargeted	3.85	276.1453	[M-H]-	50906608	β-Anthropyranose	2.2	DOWN	4.8927E-05	0.0003516	2
93	Pos (& Neg)	untargeted	8.81	305.2460	[M+H]+	444899	Arachidonate	2.2	UP	0.0097759	0.0153503	2
94	Pos	untargeted	8.4	452.3706	[M+H]+	71464509	(11Z,14Z)-Eicosadienoylcarnitine	2.2	UP	0.00022594	0.0008698	2
95	Pos	untargeted	6.43	161.0595	[M+H]+	7092	6-Methylcoumarin	2.1	UP	0.00011295	0.0005115	2
96	Neg	targeted	5.24	243.0803	[M-H]-	171548	Biotin	2.1	UP	0.00874846	0.0194255	1
97	Pos	untargeted	7.26	343.2107	[M+H]+	5283138	2,3-Dinor-TXB2	2.1	UP	0.00184496	0.0044563	2
98	Pos (& Neg)	targeted	6.7	188.0712	[M+H]+	5375048	Indole-3-acrylate	2.1	DOWN	2.2736E-05	0.0001493	1
99	Pos	targeted	7.98	370.2957	[M+H]+	53481699	trans-2-Tetradecenoylcarnitine	2.1	UP	6.6225E-06	5.829E-05	1
100	Neg	targeted & untarget	6.64	201.1125	[M-H]-	5192	Sebacate	2.0	UP	0.00156135	0.005291	1

Supplementary Table 3.3. Cont'd.

101	Neg	untargeted	8.71	317.2122	[M-H]-	5283162	12-KETE	2.0	UP	0.00140901	0.0048863	2
102	Neg	untargeted	5.98	141.0918	[M-H]-	104896	4-Ene-valproate	2.0	UP	0.00245514	0.0075444	2
103	Neg	untargeted	3.46	143.0345	[M-H]-	6368126	(E)-2-Methylglutaconate	2.0	UP	0.00215758	0.0068366	2
104	Neg (& Pos)	targeted & untarget	7.46	229.1441	[M-H]-	12736	Dodecanedioate	2.0	UP	0.00040861	0.0017975	1
105	Pos	targeted	5.87	195.0657	[M+H]+	445858	Ferulate	2.0	DOWN	0.0100342	0.0156178	1
106	Pos	untargeted	6.18	509.2337	[2M+Na]+	11736661	Glutaminylproline	2.0	UP	0.00246159	0.0055674	2
107	Neg	targeted	1.3	166.0140	[M-H]-	1066	Quinolinate	2.0	UP	0.04667637	0.0688995	1
108	Pos	untargeted	7.76	344.2790	[M+H]+	168381	Dodecanoylcarnitine	2.0	UP	0.00017259	0.0007039	2
109	Neg	untargeted	6.64	491.2238	[M-H]-	131769835	rac-5,6-Epoxy-retinoyl-β-D-glucuronide	2.0	UP	0.00261246	0.0079016	2
110	Neg	untargeted	2.5	175.0607	[M-H]-	5280523	2-Isopropylmalate	2.0	UP	7.4862E-05	0.0004861	2
111	Neg	untargeted	4.47	192.0305	[M-H]-	119405	5,6-Dihydroxyindole-2-carboxylate (DHICA)	2.0	UP	0.0009764	0.0036628	2
112	Neg	untargeted	9.59	279.2328	[M-H]-	5280450	Linoleate	1.9	UP	0.00264165	0.0079666	2
113	Neg	untargeted	6.59	245.1387	[M-H]-	16663321	3-Hydroxydodecanedioate	1.9	UP	4.4645E-05	0.0003271	2
114	Neg	targeted	5	159.0657	[M-H]-	385	Pimelate	1.9	UP	0.02474458	0.0419696	1
115	Neg	untargeted	3.6	113.0607	[M-H]-	19707	2,3-Hexanedione	1.9	UP	6.4474E-07	1.389E-05	2
116	Pos	untargeted	4.65	155.0701	[M+H]+	61166	2-Furanylmethyl propanoate	1.9	UP	7.9699E-06	6.67E-05	2
117	Neg	targeted & untarget	8.3	255.2331	[M-H]-	985	Palmitate	1.9	UP	3.918E-07	9.887E-06	1
118	Neg	untargeted	4.1	125.0240	[M-H]-	74710	5-Methylfuran-2-carboxylate	1.9	UP	0.00100776	0.0037555	2
119	Pos (& Neg)	untargeted	5.82	219.1226	[M+H]+	3017884	3-Hydroxysebacate	1.9	UP	0.00172304	0.0042222	2
120	Neg	targeted & untarget	0.68	425.0789	[M-H]-	53477713	Cysteineglutathione disulfide	1.9	DOWN	9.6279E-05	0.0005874	1
121	Pos (& Neg)	targeted & untarget	6.17	146.0599	[M+H]+	10256	Indole-3-carboxaldehyde	1.9	DOWN	0.00182079	0.0044096	1
122	Pos	untargeted	0.6	296.0652	[M+H-H2O]+	440417	Phosphoribosylformylglycineamidine	1.9	DOWN	0.00096481	0.002697	2
123	Neg	untargeted	4.65	127.0763	[M-H]-	60983	2,3-Heptanedione	1.9	UP	1.4935E-05	0.0001379	2
124	Pos	untargeted	4.63	137.0597	[M+H]+	999	Phenylacetate	1.9	UP	1.1011E-05	8.512E-05	2
125	Neg	targeted	4.05	145.0501	[M-H]-	196	Adipate	1.9	UP	0.0165803	0.0309281	1
126	Pos (& Neg)	targeted	1.21	613.1598	[M+H]+	65359	L-Glutathione oxidized (GSSG)	1.9	DOWN	0.00713266	0.0123342	1
127	Neg	untargeted	7.8	401.2177	[M-H]-	52921873	5S-HETE di-endoperoxide	1.9	UP	3.7163E-06	4.714E-05	2
128	Pos (& Neg)	targeted	1.11	137.0463	[M+H]+	790	Hypoxanthine	1.9	DOWN	0.02000112	0.0243442	1
129	Pos	targeted	0.7	427.0957	[M+H]+	25203382	L-Cysteine-glutathione disulfide	1.9	DOWN	0.00010224	0.0004749	1
130	Neg	targeted & untarget	8.59	295.2274	[M-H]-	5312830	(+)9-HODE	1.9	UP	0.00092735	0.0035153	1
131	Neg	untargeted	9.26	312.2541	[M-H]-	151008	Palmitoylglycine	1.8	UP	0.00653491	0.0156368	2
132	Pos	targeted & untarget	0.62	258.1097	[M+H]+	71920	Glycerophosphocholine	1.8	DOWN	0.00067203	0.0020402	1
133	Pos	untargeted	5.72	233.1383	[M+H]+	5327007	1,2-Dibutyrin	1.8	UP	0.00030626	0.0011025	2
134	Pos (& Neg)	untargeted	3.87	213.0732	[M+Na]+	22328017	3-Hydroxysuberate	1.8	UP	0.00024582	0.0009293	2
135	Neg	untargeted	0.97	238.0847	[M-H]-	14257919	N-Ornithyl-L-taurine	1.8	UP	6.6622E-05	0.000445	2
136	Neg	untargeted	7.87	413.2179	[M-H]-	5283151	Methyl 6,8-epidioxy-5,15-dihydroperoxy-9,11,13-eicosatrienoate	1.8	UP	0.00029085	0.0013703	2
137	Neg	targeted & untarget	10	299.2586	[M-H]-	439887	2-Hydroxystearate	1.8	UP	0.00019512	0.0009961	1
138	Pos	targeted & untarget	3.85	232.1539	[M+H]+	213144	Butyrylcarnitine	1.8	DOWN	0.00013712	0.0005884	1
139	Pos	untargeted	7.27	157.1220	[M+H]+	5283344	4-Hydroxynonenal	1.8	UP	0.00223289	0.0051722	2
140	Pos	targeted & untarget	1.03	123.0553	[M+H]+	936	Nicotinamide	1.8	UP	1.522E-09	1.444E-07	1
141	Neg	targeted	4.34	181.0501	[M-H]-	9378	DL-p-Hydroxyphenyllactate	1.8	DOWN	0.00756921	0.0174709	1
142	Pos	untargeted	6.03	201.1120	[M+H]+	7563148	cis-5-Decenedioate	1.8	UP	0.00216312	0.0050478	2
143	Neg	targeted	5.67	173.0814	[M-H]-	10457	Suberate	1.8	UP	0.01568558	0.0297398	1
144	Neg	untargeted	1.18	202.1192	[M-H]-	306144	Glycyl-Lysine	1.8	UP	0.00839808	0.01886	2
145	Neg (& Pos)	targeted & untarget	7.08	215.1285	[M-H]-	15816	Undecanedioate	1.8	UP	0.0067146	0.015965	1
146	Pos	untargeted	3.86	177.1121	[2M+H]+	264	Butyrate	1.8	UP	0.00046605	0.001537	2
147	Neg (& Pos)	targeted & untarget	1.49	130.0870	[M-H]-	6106	L-Leucine	1.8	UP	8.3024E-05	0.0005247	1
148	Pos (& Neg)	targeted & untarget	5.73	208.0966	[M+H]+	74839	N-Acetyl-L-phenylalanine	1.7	UP	0.00086797	0.0024901	1
149	Neg	targeted	0.67	195.0505	[M-H]-	10690	D-Gluconate	1.7	UP	0.00024047	0.0011741	1
150	Neg (& Pos)	targeted	4.35	158.0817	[M-H]-	193872	N-(2-Methylbutyryl)glycine	1.7	UP	0.00187175	0.0061139	1

Supplementary Table 3.3. Cont'd.

151	Pos	untargeted	6.97	189.0906	[M+CH3OH+H]+	6195	1-Naphthaldehyde	1.7	UP	0.0002801	0.0010296	2
152	Pos	targeted	0.65	104.0712	[M+H]+	119	γ-Aminobutyrate (GABA)	1.7	UP	0.00089016	0.0025385	1
153	Pos	untargeted	8.42	389.2157	[M+H]+	72791584	9,13-Dihydroxy-4-megastigmen-3-one 9-glucoside	1.7	UP	0.00082477	0.0023942	2
154	Pos	targeted	7.55	272.1862	[M+H]+	71353010	N-3-Hydroxydecanoyl-L-Homoserine lactone	1.7	UP	0.01365735	0.0189863	1
155	Neg	untargeted	9.14	506.3260	[M-H]-	52925139	LysoPE(20:1(11Z)/0:0)	1.7	DOWN	0.00585054	0.0143564	2
156	Neg	untargeted	8.35	281.2487	[M-H]-	445639	Oleate	1.7	UP	0.00255285	0.0077676	2
157	Pos	untargeted	3.19	298.1144	[M+H]+	96373	1-Methylguanosine	1.7	UP	3.9571E-06	3.945E-05	2
158	Neg (& Pos)	targeted & untarget	7.73	243.1595	[M-H]-	10458	1,11-Undecanedicarboxylate	1.7	UP	0.00361016	0.0100077	1
159	Pos	untargeted	5.42	141.0907	[M+H]+	6441809	2-Propyl-2,4-pentadienoate	1.7	UP	0.00018708	0.0007498	2
160	Neg	untargeted	0.63	253.0094	[M-H]-	121947	Shikimate 3-phosphate	1.7	UP	0.00131275	0.0046245	2
161	Pos	untargeted	7.97	327.2155	[M+H]+	9548881	2,3-Dinor-8-epi-prostaglandin F2α	1.7	UP	0.00310231	0.006629	2
162	Pos	untargeted	6.83	205.1218	[M+H]+	24884264	Cinnamyl isobutyrate	1.7	UP	2.0738E-05	0.0001388	2
163	Neg	untargeted	0.94	153.0307	[M-H]-	654	Diethylphosphate	1.7	UP	5.4877E-08	2.521E-06	2
164	Pos	untargeted	5.42	221.0598	[M+H-2H2O]+	176907	Dihydrodaidzein	1.7	UP	9.3569E-05	0.0004459	2
165	Neg	untargeted	1.21	188.0560	[M-H]-	185	N-Acetylglutamate	1.7	UP	0.00395665	0.010732	2
166	Neg	targeted	1.45	130.0504	[M-H]-	88064	N-Acetyl-L-alanine	1.7	UP	9.8925E-12	5.085E-09	1
167	Pos	untargeted	5.72	277.1025	[M+H]+	92865	γ-Glutamylglutamate	1.7	UP	0.00142154	0.0036547	2
168	Neg	untargeted	4.6	247.1182	[M-H]-	65137	Histidylproline diketopiperazine	1.7	UP	0.00059379	0.0024279	2
169	Neg	untargeted	9.13	566.3448	[M-H]-	52926294	PS(21:0/0:0)	1.7	DOWN	0.00529565	0.0132809	2
170	Neg	untargeted	5.62	275.1494	[M-H]-	5280691	4-Hydroxycinnamoylagmatine	1.6	UP	0.00272085	0.0081412	2
171	Pos (& Neg)	targeted	2.83	209.0926	[M+H]+	161166	Kynurenine	1.6	UP	2.1666E-06	2.514E-05	1
172	Neg	untargeted	5.43	203.0923	[M-H]-	131839802	2-Hydroxy-7-methyloctanedioate	1.6	UP	0.00412103	0.0110657	2
173	Pos	targeted	8.03	302.3059	[M+H]+	91486	Sphinganine (d18:0)	1.6	UP	0.02694175	0.0301961	1
174	Neg	untargeted	8.34	335.2225	[M-H]-	5283128	Leukotriene B4	1.6	UP	0.00837175	0.018817	2
175	Neg	targeted & untarget	1.07	173.0088	[M-H]-	643757	cis-Aconitate	1.6	UP	0.00287632	0.0084856	1
176	Pos	untargeted	1.14	162.0759	[M+CH3OH+H]+	1059	Pyrroline hydroxycarboxylate	1.6	UP	4.333E-05	0.0002479	2
177	Pos	untargeted	3.85	147.0439	[M+H]2+	25245902	S-(Indolylmethylthiohydroximoyl)-L-cysteine	1.6	UP	9.9533E-05	0.000466	2
178	Neg	targeted	6.5	137.0239	[M-H]-	10253	Salicylate	1.6	DOWN	0.01246851	0.0251374	1
179	Neg	untargeted	1.35	304.0337	[M-H]-	53481030	Cytidine 2',3'-cyclic phosphate	1.6	UP	0.00015579	0.000846	2
180	Neg	untargeted	9.16	271.2275	[M-H]-	15569776	(R)-3-Hydroxy-hexadecanoate	1.6	UP	0.00134783	0.0047208	2
181	Neg	untargeted	0.76	166.0174	[M-H]-	161712	Homocysteinesulfinate	1.6	UP	3.7041E-05	0.0002829	2
182	Neg	untargeted	1.03	547.0406	[M-H]-	440219	UDP-4-dehydro-6-deoxy-D-glucose	1.6	UP	0.00563458	0.0139401	2
183	Pos	untargeted	5.73	120.0807	[M+H]+	10328	Indoline	1.6	UP	0.00100853	0.0027894	2
184	Pos	untargeted	7.54	309.1670	[M+CH3OH+H]+	160556	Saccharopine	1.6	UP	0.00021241	0.0008289	2
185	Neg	targeted & untarget	0.67	157.0366	[M-H]-	204	Allantoin	1.6	UP	0.00031636	0.0014675	1
186	Pos (& Neg)	targeted	0.74	189.1239	[M+H]+	92832	Nα-Acetyl-lysine	1.6	UP	1.5879E-06	2.001E-05	1
187	Neg	untargeted	4.88	131.0709	[M-H]-	10796774	2-Hydroxy-3-methylpentanoate	1.6	UP	0.00078978	0.0030847	2
188	Neg	targeted & untarget	0.94	145.0141	[M-H]-	51	α-Ketoglutarate	1.6	UP	0.00955333	0.0206776	1
189	Pos	targeted	2	153.0664	[M+H]+	69698	Nudifloramide	1.6	UP	0.01039909	0.0159879	1
190	Neg	targeted	1.23	133.0501	[M-H]-	440279	2,3-Dihydroxyisovalerate	1.6	DOWN	0.0054391	0.0135606	1
191	Pos (& Neg)	targeted & untarget	5.97	206.0817	[M+H]+	92904	Indolelactate	1.6	DOWN	0.00207615	0.0048901	1
192	Neg	untargeted	4.15	259.1291	[M-H]-	151023	γ-Glutamylleucine	1.6	UP	0.00010439	0.0006243	2
193	Neg	untargeted	4.22	151.0399	[M-H]-	127	4-Hydroxyphenylacetate	1.6	DOWN	5.1813E-05	0.0003677	2
194	Neg	untargeted	6.59	159.1024	[M-H]-	134459	3-Hydroxyvalproate	1.6	UP	0.00087164	0.0033441	2
195	Neg	untargeted	0.77	119.0348	[M-H]-	150929	(S)-3,4-Dihydroxybutyrate	1.5	DOWN	0.00730598	0.0170166	2
196	Pos	untargeted	5.64	184.1330	[M+NH4]+	1256	Perillate	1.5	UP	0.00059281	0.0018463	2
197	Pos (& Neg)	untargeted	6.25	216.1595	[M+H]+	10176752	N-Nonanoylglycine	1.5	UP	0.00034313	0.0012039	2
198	Pos	untargeted	0.61	161.1284	[M+H]+	164795	N(6)-Methyllysine	1.5	UP	0.0001903	0.00076	2
199	Neg	untargeted	3.81	357.0821	[M-H]-	49844972	Dihydrocaffeate 3-O-glucuronide	1.5	UP	0.00865849	0.0192815	2
200	Pos	untargeted	6.96	295.1515	[M+H]+	170295	Mono-(2-ethyl-5-hydroxyhexyl) phthalate	1.5	UP	0.00789989	0.0132555	2

Supplementary Table 3.3. Cont'd.

201	Pos	untargeted	6.18	197.1174	[M+CH3OH+H]+	3314	Eugenol	1.5	UP	0.00130036	0.0034107	2
202	Pos	untargeted	2.77	87.0804	[M+H-H2O]+	8109	3-Ethoxy-1-propanol	1.5	UP	0.00810334	0.0134965	2
203	Pos	untargeted	6.16	97.1011	[M+H-H2O]+	8051	2-Heptanone	1.5	UP	0.00570655	0.0105024	2
204	Neg	untargeted	6.16	125.0970	[M-H]-	16900	(E)-2-Octenal	1.5	UP	0.00810234	0.0183725	2
205	Neg	targeted	0.63	88.0399	[M-H]-	5950	L-Alanine	1.5	UP	0.00680454	0.0161278	1
206	Pos	untargeted	2.73	247.1285	[M+H]+	7015683	γ-Glutamylvaline	1.5	UP	1.2332E-05	9.279E-05	2
207	Neg (& Pos)	targeted & untarget	6.16	187.0972	[M-H]-	2266	Azelaate	1.5	UP	0.006805	0.0161286	1
208	Pos	targeted	0.68	120.0661	[M+H]+	97963	O-Methyl-DL-serine	1.5	DOWN	0.02874842	0.0316806	1
209	Pos	targeted	0.65	173.0215	[M+H]+	439162	sn-Glycerol-3-phosphate	1.5	UP	0.04211752	0.0420868	1
210	Neg	targeted	1.69	161.0450	[M-H]-	193530	2-Hydroxyadipate	1.5	UP	0.01195896	0.0243934	1
211	Pos (& Neg)	targeted	0.88	118.0868	[M+H]+	6287	L-Valine	1.5	UP	0.00639687	0.0114176	1
212	Pos (& Neg)	targeted	3.11	146.0817	[M+H]+	88412	N-Butyrylglycine	1.4	UP	0.04519238	0.0444281	1
213	Pos	targeted	0.54	112.0875	[M+H]+	774	Histamine	1.4	UP	0.0377389	0.0387158	1
214	Pos	targeted	0.63	104.1075	M+	305	Choline	1.4	UP	4.8074E-05	0.0002684	1
215	Neg (& Pos)	targeted & untarget	0.69	130.0616	[M-H]-	586	Creatine	1.4	UP	0.00092516	0.0035087	1
216	Pos (& Neg)	targeted	3.44	243.0981	[M+H]+	5789	Thymidine	1.4	UP	0.00158438	0.0039678	1
217	Pos	targeted	5.16	377.1461	[M+H]+	493570	Riboflavin	1.4	UP	0.02845872	0.0314445	1
218	Pos	targeted & untarget	0.65	162.1130	M+	10917	L-Carnitine	1.4	DOWN	0.00031518	0.0011266	1
219	Pos (& Neg)	targeted	1.03	150.0589	[M+H]+	6137	L-Methionine	1.4	DOWN	0.04007275	0.0405199	1
220	Pos	targeted	0.59	170.0929	[M+H]+	92105	1-Methyl-L-histidine	1.3	DOWN	0.04115114	0.0413508	1
221	Pos (& Neg)	targeted	1.73	229.0824	[M+H]+	13712	2'-Deoxyuridine	1.3	UP	0.01026069	0.0158486	1
222	Pos	targeted	0.63	90.0555	[M+H]+	1088	Sarcosine	1.3	DOWN	0.03953169	0.0401051	1
223	Pos (& Neg)	targeted	3.68	220.1185	[M+H]+	6613	D-(+)-Pantothenate	1.3	DOWN	0.00606492	0.0109846	1
224	Pos (& Neg)	targeted	1.27	245.0774	[M+H]+	6029	Uridine	1.3	UP	0.01136834	0.0169283	1
225	Pos	targeted	0.99	204.1236	M+	7045767	L-Acetylcarnitine	1.3	UP	7.7846E-05	0.0003891	1
226	Pos	targeted	0.66	114.0667	[M+H]+	588	Creatinine	1.2	DOWN	9.75E-05	0.0004592	1
227	Pos (& Neg)	targeted	0.58	175.1195	[M+H]+	6322	L-Arginine	1.2	DOWN	0.00652869	0.0115862	1
228	Pos	targeted	0.6	129.0664	[M+H]+	11984188	Pyroglutamine	1.2	DOWN	0.02381109	0.0276273	1
229	Neg	targeted	1.03	89.0239	[M-H]-	107689	L-(+)-Lactate	1.2	DOWN	0.00216145	0.0068461	1
230	Pos	targeted	0.61	76.0399	[M+H]+	750	L-Glycine	1.2	UP	0.03006599	0.0327553	1
231	Neg	targeted	0.76	75.0082	[M-H]-	757	Glycolate	1.2	DOWN	0.00650241	0.015577	1

Supplementary Table 3.3. Cont'd.

Supplementary Table 3.4. Unique list of 58 cortical brain metabolites altered in sera comparing GF and CONV-R mice.

	ESI mode	Platform	RT (min)	PRECURSOR MZ	PRECURSOR	PubChem_CID	Compound name (brain)	fold	updown (GF/Conv)	pvalue	qvalue	MSI level
1	Neg	targeted & untargeted	4.84	212.0022	[M-H]-	10258	Indoxyl sulfate	26.8	DOWN	2.8977E-06	0.00028	1
2	Pos (& Neg)	targeted	1.03	308.0916	[M+H]+	124886	L-Glutathione reduced (GSH)	15.1	DOWN	0.04281753	0.2253	1
3	Pos	targeted	0.65	76.0762	[M+H]+	1145	Trimethylamine N-oxide (TMAO)	7.3	DOWN	0.00115121	0.01483	1
4	Pos (& Neg)	targeted & untargeted	1.59	268.1035	[M+H]+	60961	Adenosine	6.1	DOWN	6.8386E-05	0.00178	1
5	Neg	untargeted	4.82	206.0452	[M-H]-	472	4-(2-Aminophenyl)-2,4-dioxobutanoate	5.9	UP	0.0006178	0.01416	2
6	Neg	targeted	5.38	192.0661	[M-H]-	68144	N-(2-Phenylacetyl)glycine (PAG)	4.0	DOWN	0.00054903	0.01303	1
7	Neg	untargeted	0.75	196.0743	[M-H]-	273260	N-Acetylhistidine	4.0	UP	6.6416E-07	9E-05	2
8	Neg	targeted	4.34	181.0501	[M-H]-	9378	Hydroxyphenyllactate	3.0	DOWN	0.0370364	0.24955	1
9	Neg	untargeted	3.51	258.1452	[M-H]-	22935676	Isoleucyl-Glutamine	2.9	UP	5.8746E-06	0.00047	2
10	Neg	untargeted	0.94	117.0192	[M-H2O-H]-	2781043	Erythronate	2.9	DOWN	0.00048954	0.01203	2
11	Neg	targeted	1.79	103.0395	[M-H]-	11266	2-Hydroxybutyrate	2.8	UP	4.8064E-06	0.00041	1
12	Pos	targeted	7.44	347.2222	[M+H]+	5753	Corticosterone	2.8	UP	6.0938E-05	0.00163	1
13	Pos (& Neg)	untargeted	4.73	254.1021	[M+H]+	12681285	N-Acetylvanilalanine	2.7	DOWN	0.01411771	0.10863	2
14	Neg	targeted	0.86	173.0450	[M-H]-	8742	Shikimate	2.4	DOWN	0.00017799	0.00549	1
15	Neg	untargeted	2.4	396.0535	[M-H]-	53481004	5-Taurinomethyl-2-thiouridine	2.4	UP	7.4818E-09	1E-05	2
16	Pos	untargeted	1.24	248.1491	[M+H]+	53481617	Hydroxybutyrylcarnitine	2.4	UP	0.00014313	0.00314	2
17	Pos	untargeted	6.03	117.0909	[M+H]+	8892	Caproate	2.1	DOWN	3.3102E-07	1.8E-05	2
18	Neg	targeted & untargeted	5.72	172.0976	[M-H]-	99463	Hexanoylglycine	2.1	UP	6.4968E-05	0.00267	1
19	Neg	targeted	1.46	103.0395	[M-H]-	92135	3-Hydroxybutyrate	2.0	UP	0.0001617	0.00511	1
20	Neg	untargeted	1.1	288.1203	[M-H]-	7018721	Ophthalmate	2.0	UP	0.00439925	0.05517	2
21	Neg	targeted	5.97	204.0661	[M-H]-	92904	Indole-3-lactate	1.9	DOWN	0.00617745	0.07127	1
22	Pos	untargeted	1.83	276.1434	[M+H]+	71317118	Glutarylcarnitine	1.9	DOWN	0.01563668	0.11687	2
23	Neg	targeted	0.7	157.0362	[M-H]-	204	Allantoin	1.8	UP	2.3941E-08	1.2E-05	1
24	Pos	untargeted	7.88	317.2105	[M+H-H2O]+	5288144	Prostaglandin B2	1.8	DOWN	1.8917E-07	1.2E-05	2
25	Pos	untargeted	5.11	160.1332	[M+H]+	69522	DL-2-Aminooctanoate	1.8	DOWN	0.04813004	0.24181	2
26	Pos	untargeted	7.88	375.2144	[M+Na]+	5280360	Prostaglandin E2	1.7	DOWN	0.00160458	0.01902	2
27	Pos (& Neg)	targeted	1.35	182.0817	[M+H]+	6057	L-Tyrosine	1.7	DOWN	0.03741765	0.20747	1
28	Pos	untargeted	6.03	163.1328	[M+CH3OH+H]	8094	Heptanoate	1.7	DOWN	1.6423E-07	1.1E-05	2
29	Neg	untargeted	3.98	227.1404	[M-H]-	44369311	Leucylproline	1.7	UP	0.00027771	0.0078	2
30	Pos	untargeted	5	245.1856	[M+H]+	435718	Leucyl-Isoleucine	1.6	UP	0.00108358	0.01418	2
31	Pos (& Neg)	targeted & untargeted	4.25	298.0959	[M+H]+	439176	5'-Deoxy-5'-methylthioadenosine	1.6	DOWN	3.0374E-06	0.00013	1
32	Neg	untargeted	0.67	196.9852	[M+Cl]-	599	4-Hydroxy-2-ketoglutarate	1.6	DOWN	5.9715E-05	0.00255	2
33	Pos	untargeted	4.09	89.0597	[M+H]+	264	Butyrate	1.6	DOWN	0.00017162	0.00362	2
34	Pos	untargeted	8.76	303.2309	[M+H]+	446284	Eicosapentaenoate	1.5	DOWN	0.02866303	0.17421	2
35	Neg	targeted	1.31	115.0031	[M-H]-	444972	Fumarate	1.5	DOWN	1.656E-08	1.2E-05	1
36	Pos	untargeted	8.38	568.3379	[M+H]+	10415542	LysoPC(22:6(4Z,7Z,10Z,13Z,16Z,19Z))	1.5	DOWN	0.04841496	0.24267	2
37	Neg	untargeted	0.93	215.1147	[M-H]-	67427	N-α-Acetyl-L-arginine	1.5	UP	0.00054026	0.01289	2
38	Neg	targeted	0.68	199.0008	[M-H]-	122357	D-Erythrose-4-phosphate	1.5	DOWN	0.00026402	0.0075	1
39	Pos (& Neg)	targeted	1.03	150.0589	[M+H]+	6137	L-Methionine	1.5	DOWN	0.01153513	0.09344	1
40	Pos	targeted	0.77	141.0664	[M+H]+	75810	1-Methyl-4-imidazoleacetate	1.4	UP	0.01031922	0.08574	1

41	Pos (& Neg)	targeted	0.66	166.0538	[M+H]+	847	L-Methionine sulfoxide	1.4	DOWN	0.03938332	0.21416	1
42	Pos (& Neg)	targeted	3.68	220.1185	[M+H]+	6613	D-Pantothenate	1.4	DOWN	0.00362976	0.03718	1
43	Neg	targeted	0.97	145.0137	[M-H]-	51	α-Ketoglutarate	1.4	DOWN	0.00102884	0.02054	1
44	Pos (& Neg)	targeted	1.24	385.1294	[M+H]+	439155	S-(5'-Adenosyl)-L-homocysteine (SAH)	1.3	DOWN	0.01617816	0.11969	1
45	Neg	targeted	0.86	155.0093	[M-H]-	967	Orotate	1.3	DOWN	0.00860869	0.0916	1
46	Neg	targeted	0.69	135.0293	[M-H]-	151152	L-Threonate	1.3	DOWN	3.7549E-05	0.00193	1
47	Pos	targeted	1.97	269.0886	[M+H]+	135398641	Inosine	1.3	UP	0.00086395	0.01212	1
48	Pos (& Neg)	targeted	3.44	243.0981	[M+H]+	5789	Thymidine	1.2	UP	0.02923947	0.1766	1
49	Pos	targeted	0.68	118.0868	[M+H]+	247	Betaine	1.2	DOWN	0.00391192	0.03942	1
50	Pos	targeted	9.28	280.2640	[M+H]+	6435901	Linoleamide	1.2	DOWN	0.04389476	0.22868	1
51	Pos (& Neg)	targeted	0.71	310.1138	[M+H]+	445063	N-Acetylneuraminate	1.2	UP	0.02067119	0.14125	1
52	Neg	targeted	1.73	227.0668	[M-H]-	13712	2'-Deoxyuridine	1.2	UP	0.01626756	0.14603	1
53	Pos (& Neg)	targeted	0.64	134.0453	[M+H]+	5960	L-Aspartate	1.2	UP	0.01208569	0.09681	1
54	Neg	targeted	0.69	300.0484	[M-H]-	440272	N-Acetyl-glucosamine-1-phosphate	1.2	UP	0.01186251	0.11646	1
55	Neg	targeted	0.66	103.0031	[M-H]-	964	3-Hydroxypyruvate	1.2	DOWN	0.0412593	0.26752	1
56	Pos (& Neg)	targeted	0.74	189.1239	[M+H]+	92832	Nα-Acetyl-lysine	1.1	UP	0.01148931	0.09316	1
57	Pos (& Neg)	targeted	0.9	136.0623	[M+H]+	190	Adenine	1.1	DOWN	0.0424354	0.22409	1
58	Pos	targeted	1.06	123.0558	[M+H]+	936	Nicotinamide	1.1	UP	4.8746E-06	0.0002	1

Supplementary Table 3.4. Cont'd.

Supplementary Table 3.5. ChemRICH results for fecal metabolome changes on compound class level (Kolmogorov–Smirnov test).

	Cluster name	Cluster size	p-values	FDR	Key compound	Altered metabolites	Increased	Decreased	Increased ratio	Altered Ratio
1	cholic acids	21	2.2E-20	1.1E-19	Sulfolithocholate	21	16	5	0.8	1
2	coumaric acids	11	2.2E-20	1.1E-19	Homovanillate sulfate	11	10	1	0.9	1
3	dicarboxylic acids	11	2.2E-20	1.1E-19	Diaminopimelate	11	4	7	0.4	1
4	dipeptides	81	2.2E-20	1.1E-19	S-4-Hydroxymephenytoin	81	47	34	0.6	1
5	disaccharides	4	2.2E-20	1.1E-19	5-O-α-L-Arabinofuranosyl-L-arabinose	4	2	2	0.5	1
6	glutamates	8	2.2E-20	1.1E-19	N(5)-Methyl-L-glutamine	8	3	5	0.4	1
7	imidazoles	4	2.2E-20	1.1E-19	N-(1-Deoxy-1-fructosyl)histidine	4	1	3	0.2	1
8	indoles	11	2.2E-20	1.1E-19	6-Hydroxy-5-methoxyindole glucuronide	11	4	7	0.4	1
9	nucleosides	4	2.2E-20	1.1E-19	Ribothymidine	4	4	0	1	1
10	oligopeptides	18	2.2E-20	1.1E-19	Hydroxyphenylacetylglycine	18	13	5	0.7	1
11	peptides	3	2.2E-20	1.1E-19	N-Acetyl-L-methionine	3	1	2	0.3	1
12	polyethylene glycols	3	2.2E-20	1.1E-19	S-Lactoylglutathione	3	2	1	0.7	1
13	purine nucleosides	9	2.2E-20	1.1E-19	Deoxyadenosine (dA)	9	5	4	0.6	1
14	pyrimidine nucleosides	9	2.2E-20	1.1E-19	N4-Acetylcytidine	9	0	9	0	1
15	carnitine	8	1.1E-16	5.2E-16	Malonylcarnitine	8	7	1	0.9	1
16	Saturated_laurates	4	6.7E-16	2.9E-15	3-Hydroxyhexanoylcarnitine	4	3	1	0.8	1
17	hydroxycholecalciferols	5	1.9E-15	7.8E-15	24α,24β-Dihomo-9,10-secocholesta-5,7,10(19),24α-tetraen-1α,3,25-triol	5	5	0	1	1
18	hexuronic acids	3	6.4E-15	2.5E-14	D-Glucuronate	3	3	0	1	1
19	amino acids, sulfur	6	7.7E-15	2.8E-14	L-Homocysteine	6	2	4	0.3	1
20	flavonoids	4	1.3E-14	4.6E-14	Apiin	4	4	0	1	1
21	deoxycholic acid	6	1.4E-14	4.6E-14	Nutriacholate	6	3	3	0.5	1
22	UnSaturated FA	6	1.5E-14	4.9E-14	5,8-Tetradecadienoate	6	6	0	1	1
23	terpenes	5	1.7E-14	5.3E-14	Ipomeamaronol	5	3	2	0.6	1
24	pyrroles	3	4E-14	1.1E-13	Pyrraline	3	1	2	0.3	1
25	butyrates	10	4.1E-14	1.1E-13	10-Hydroxy-2,8-decadiene-4,6-diynoate	10	3	7	0.3	1
26	O=FA_10_1	8	7E-14	1.9E-13	Tetradecanedioate	8	0	8	0	1
27	phenols	4	1.3E-13	3.5E-13	Coniferyl alcohol	4	2	2	0.5	1
28	taurine	4	1.6E-13	4E-13	Taurocholate	4	2	2	0.5	1
29	amino acids, aromatic	7	2.1E-13	5E-13	O(4')-Sulfo-L-tyrosine	7	2	5	0.3	1
30	catecholamines	6	3.1E-13	7.2E-13	Dobutamine	6	1	5	0.2	1
31	indoleacetic acids	6	4.6E-13	1E-12	5-Methoxyindoleacetate	6	4	2	0.7	1
32	cinnamates	5	4.9E-13	1.1E-12	3-O-p-Coumaroylquinate	5	5	0	1	1
33	benzyl alcohols	4	8.5E-13	1.8E-12	3,4-Dihydroxyphenylglycol O-sulfate	4	4	0	1	1
34	disulfides	3	2.3E-12	4.7E-12	2-(S-Glutathionyl)acetyl glutathione	3	1	2	0.3	1
35	TriHOME	5	2.6E-12	5.2E-12	12,13-DHOME	5	5	0	1	1

36	cholestanols	3	3.3E-12	6.3E-12	Tetrahydroaldosterone-3-glucuronide	3	1	2	0.3	1
37	sugar acids	5	1.2E-11	2.2E-11	D-Gluconate	5	0	5	0	1
38	hexosamines	5	5.4E-11	9.9E-11	N-Acetylgalactosamine (GalNAc)	5	1	4	0.2	1
39	amino acids, cyclic	3	7.7E-11	1.4E-10	L-Histidine	3	1	2	0.3	1
40	amino acids	4	2.3E-10	4.1E-10	L-Kynurenine	4	0	4	0	1
41	monosaccharides	4	3.3E-10	5.6E-10	D-Altro-D-manno-Heptose	4	2	2	0.5	1
42	glutarates	6	3.3E-10	5.6E-10	2,5-Dioxopentanoate	6	3	3	0.5	1
43	keto acids	3	5.5E-10	8.9E-10	Pyruvate	3	0	3	0	1
44	benzopyrans	4	8.2E-10	1.3E-09	Coumarin	4	3	1	0.8	1
45	aminobenzoates	3	1.2E-09	1.9E-09	4-Aminohippurate	3	0	3	0	1
46	pregnenediones	3	1.2E-09	1.9E-09	11-Dehydrocorticosterone	3	2	1	0.7	1
47	acetates	3	5.3E-09	7.9E-09	Glycolate	3	0	3	0	1
48	biogenic monoamines	4	6.7E-09	9.8E-09	Nβ-Methyltetrahydroharmol	4	1	3	0.2	1
49	OH-FA_16_0_1	4	7.1E-09	0.00000001	(R)-3-Hydroxy-hexadecanoate	4	2	2	0.5	1
50	catechols	4	7.1E-09	0.00000001	5-(3',4',5'-Trihydroxyphenyl)-γ-valerolactone-O-methyl-O-sulfate	4	4	0	1	1
51	cholestenes	3	8.4E-09	1.2E-08	25-Hydroxyvitamin D2	3	3	0	1	1
52	pteridines	3	8.6E-09	1.2E-08	Folate	3	2	1	0.7	1
53	HODE	3	2.6E-08	3.4E-08	9(S)-HPODE	3	3	0	1	1
54	phosphatidylinositols	3	4.6E-08	0.00000006	PI(P-18:0/0:0)	3	3	0	1	1
55	adipates	3	5.7E-08	7.1E-08	L-2-Aminoadipate	3	0	3	0	1
56	pyrimidinones	4	5.7E-08	7.1E-08	Uracil	4	1	3	0.2	1
57	imino acids	3	8E-08	9.8E-08	Val-Pro-Pro	3	2	1	0.7	1
58	pyridines	3	1.1E-07	0.00000013	2-Picolinate	3	0	3	0	1
59	phenylacetates	3	1.1E-07	0.00000013	4-Hydroxy-5-(4'-hydroxyphenyl)-valerate-4'-O-sulfate	3	1	2	0.3	1
60	sialic acids	3	1.5E-07	0.00000017	Pseudaminate	3	2	1	0.7	1
61	alcohols	3	1.6E-07	0.00000018	1-O-α-D-Glucopyranosyl-D-mannitol	3	2	1	0.7	1
62	bile pigments	3	1.8E-07	0.00000021	Mesobilirubinogen	3	2	1	0.7	1
63	succinates	3	2.4E-07	0.00000027	N2-Succinyl-L-ornithine	3	1	2	0.3	1
64	amino acids, basic	3	2.9E-07	0.00000032	Asymmetric dimethylarginine	3	0	3	0	1
65	salicylates	3	5.2E-07	0.00000056	Salicylate β-D-glucoside	3	1	2	0.3	1
66	cyclohexenes	3	6.5E-07	0.00000069	Shikimate	3	1	2	0.3	1
67	porphyrins	3	9.2E-07	0.00000096	Coproporphyrin III	3	0	3	0	1
68	sulfuric acid esters	4	3.9E-06	0.000004	Xanthurenate 8-O-sulfate	4	1	3	0.2	1
69	xanthines	4	4.3E-06	0.0000043	Urate	4	0	4	0	1
70	amino acids, diamino	3	0.000056	0.000056	N6,N6,N6-Trimethyl-L-lysine	3	0	3	0	1

Supplementary Table 3.5. Cont'd.

Supplementary Table 3.6. ChemRICH results for fecal metabolome changes for individual metabolite assignment (Kolmogorov–Smirnov test).

	Compound_Name	Pubchem_CID	pvalue	foldchange	ClusterN	xlogp	ClusterLabel	TreeLabels	FDR
1	Sulfolithocholate	10253562	0	5.6	43	6.966	cholic acids		0
2	S-4-Hydroxymephenytoin	119507	0	0.53	3	0.368	dipeptides	Q	0
3	Ribothymidine	445408	0	10	36	-1.936	nucleosides	t	0
4	Ipomeamaronol	213591	0	0.014	39	1.016	terpenes		0
5	Homovanillate sulfate	29981063	2.2E-16	2.3	26	-1.757	coumaric acids		1.2E-13
6	L-β-Aspartyl-L-glycine	302430	2.2E-16	0.031	38	-4.535	dipeptides	R	1.2E-13
7	D-Glucuronate	444791	4.4E-16	20	34	-1.54	hexuronic acids	Z	2.3E-13
8	6-Hydroxy-5-methoxyindole glucuronide	196513	6.7E-16	0.06	44	-0.046	indoles		3.5E-13
9	Hydroxyphenylacetylglycine	440732	8.9E-16	0.077	3	-0.711	oligopeptides	Q	4.7E-13
10	Ac-Ser-Asp-Lys-Pro-OH	65938	1.8E-15	0.24	21	-5.369	oligopeptides		9.5E-13
11	Valclavam	56928070	2.4E-15	12	89	-4.135	monobactams		1.3E-12
12	γ-Glutamylvaline	7015683	3.6E-15	2.2	35	-2.996	dipeptides		1.9E-12
13	Shikimate	8742	1E-14	0.12	94	-1.094	cyclohexenes	F	5.2E-12
14	5-O-α-L-Arabinofuranosyl-L-arabinose	74539967	1E-14	2.8	24	-2.748	disaccharides	w	5.2E-12
15	Tyrosyl-Aspartate	19816752	1.5E-14	12	101	-3.896	dipeptides		7.8E-12
16	Threoninyl-Hydroxyproline	69041809	2.2E-14	4	21	-4.603	oligopeptides		1.1E-11
17	3-O-p-Coumaroylquinate	9945785	2.4E-14	120	77	-0.837	cinnamates		1.2E-11
18	L-β-Aspartyl-L-glutamate	25207301	2.4E-14	22	30	-4.534	dipeptides		1.2E-11
19	Deoxyadenosine (dA)	13730	2.6E-14	0.31	22	-1.766	purine nucleosides	s	1.3E-11
20	Indoline	10328	4.3E-14	0.00085	142	0.799	indoles	J	2.2E-11
21	Aspartyllysine	6427003	4.9E-14	2.5	76	-4.134	dipeptides		2.5E-11
22	Aspartylphenylalanine	93078	8E-14	0.45	5	-2.76	dipeptides		4.1E-11
23	N-Hydroxydebrisoquine O-glucuronide	70678657	8.4E-14	11	53	0.355			4.3E-11
24	Lysine tyrosylquinone	71464583	9E-14	2.2	147	-3.92	quinones		4.6E-11
25	L-β-Aspartyl-L-threonine	53480674	9.3E-14	4.9	74	-4.681	dipeptides	C	4.8E-11
26	1-O-α-D-Glucopyranosyl-D-mannitol	4454759	1E-13	27	24	-5.004	alcohols	w	5.1E-11
27	N-Acetyl-L-alanine	88064	1.2E-13	0.62	127	-0.382	dipeptides	S	6.1E-11
28	N4-Acetylcytidine	107461	1.2E-13	0.12	20	-2.122	pyrimidine nucleosides	A	6.1E-11
29	N-Acetyl-L-methionine	448580	1.3E-13	2.7	46	0.589	peptides		6.6E-11
30	γ-Glutamyl-S-methylcysteine	13894650	1.4E-13	2.6	11	-3.038	dipeptides	Y	7.1E-11
31	D-(+)-Galacturonate	441476	1.4E-13	510	34	-1.54	hexuronic acids	Z	7.1E-11
32	L-β-Aspartyl-L-serine	53480673	1.5E-13	5.7	74	-5.141	dipeptides	C	7.6E-11
33	N(5)-Methyl-L-glutamine	439925	1.5E-13	0.25	52	-3.559	glutamates		7.6E-11
34	Glycogen	439177	2.4E-13	0.0046	24	-6.737	glucans	w	1.2E-10
35	Deoxyguanosine (dG)	135398592	2.6E-13	3.3	10	-1.671	nucleosides		1.3E-10
36	3-Hydroxyhexanoylcarnitine	131801513	2.6E-13	4.2	109	-0.493	Saturated_laurates		1.3E-10
37	Isotabtoxin	14586819	2.8E-13	8.2	21	-4.175	dipeptides		1.4E-10
38	N2-Succinyl-L-ornithine	127370	2.7E-13	0.35	122	-3.661	succinates		1.4E-10
39	O-Succinyl-L-homoserine	46878420	3.3E-13	2.8	133	-1.75		v	1.6E-10
40	L-Kynurenine	161166	4.9E-13	0.51	117	-2.868	amino acids	g	2.4E-10

41	Malonylcarnitine	91825606	5E-13	3	27	-1.22	carnitine	L	2.5E-10
42	D-Gluconate	10690	1.1E-12	0.21	13	-3.739	sugar acids		5.4E-10
43	Prolyl-Aspartate	3838871	1.6E-12	5.2	76	-1.37	dipeptides		7.9E-10
44	N-(1-Deoxy-1-fructosyl)histidine	131752243	2.1E-12	2.5	82	-2.114	imidazoles		1E-09
45	5-Methoxyindoleacetate	18986	2.9E-12	0.33	16	0.834	indoleacetic acids		1.4E-09
46	5-(3',4',5'-Trihydroxyphenyl)-γ-valerolacton	124202100	5.2E-12	2.7	112	-0.757	catechols		2.6E-09
47	Deoxyuridine (dU)	13712	5.9E-12	0.4	100	-1.516	pyrimidine nucleosides	x	2.9E-09
48	10-Hydroxy-2,8-decadiene-4,6-diynoate	131751124	6.9E-12	6.4	151	1.08	butyrates		3.4E-09
49	Aspartyl-Methionine	21488123	7.5E-12	5.4	46	-3.038	dipeptides		3.7E-09
50	Alanylclavam	194618	8.3E-12	0.48	89	-3.816	clavulanic acids		4E-09
51	Glutaminylaspartate	87275314	8.6E-12	32	76	-5.262	dipeptides		4.2E-09
52	N-Acetylornithine	439232	8.7E-12	0.0009	122	-3.031	succinates		4.2E-09
53	2-(S-Glutathionyl)acetyl glutathione	11954072	9.4E-12	1.7	11	-6.979	disulfides	Y	4.5E-09
54	N-Acetylcystathionine	152314	1E-11	0.15	46	-2.933	peptides		4.8E-09
55	5'-Amino-5'-deoxyadenosine	9816781	1E-11	7	22	-2.434	purine nucleosides	s	4.8E-09
56	Arabinonate	122045	9.9E-12	0.49	13	-3.067	sugar acids		4.8E-09
57	γ-D-Glutamyl-meso-diaminopimelate	45480617	1.1E-11	12	47	-4.291	oligopeptides		5.3E-09
58	2-(2-Phenylacetoxy)propionylglycine	124202120	1.2E-11	8.1	5	0.949	dipeptides		5.7E-09
59	Capsiate	9839519	1.4E-11	5.8	26	4.246	coumaric acids		6.7E-09
60	Imidazoleacetate riboside	440569	1.6E-11	0.22	82	-1.367	imidazoles		7.6E-09
61	Apiin	5840046	1.7E-11	2.8	39	-1.505	flavonoids		8.1E-09
62	S-Lactoylglutathione	440018	2.2E-11	0.4	11	-4.987	polyethylene glycols	Y	1E-08
63	D-Altro-D-manno-Heptose	245622	2.3E-11	0.15	25	-2.369	monosaccharides	M	1.1E-08
64	N-[[3-Hydroxy-2-(2-pentenyl)cyclopentyl]ac	131753085	3E-11	9.1	55	3.153			1.4E-08
65	Androsterone sulfate	159663	2.9E-11	0.35	43	3.003	cholic acids		1.4E-08
66	N1,N10-Dicoumaroylspermidine	15270795	3.4E-11	3.1	90	1.56	coumaric acids		1.6E-08
67	(3β,9R)-5-Megastigmene-3,9-diol 9-[apiosyl	20979920	4.1E-11	35	24	-0.864		w	1.9E-08
68	4-Hydroxy-3-methoxycinnamate	445858	4.2E-11	3.2	75	0.512	coumaric acids		2E-08
69	3-Methoxy-4-hydroxyphenylglycol glucuron	22833525	4.9E-11	1.9	77	-1.904	cinnamates		2.3E-08
70	L-Threonine	6288	5.3E-11	0.055	95	-3.496	amino acids	h	2.5E-08
71	4-Nitrophenyl N-acetyl-β-D-galactosaminid	84723	5.4E-11	0.62	152	-0.145	disaccharides	X	2.5E-08
72	Isoferulate 3-sulfate	71749556	7E-11	2.4	75	-1.334	coumaric acids		3.3E-08
73	8-Hydroxyguanosine	135407175	7.4E-11	390	10	-2.862	nucleosides		3.4E-08
74	Aminoacetone	215	8.1E-11	0.18	93	-0.919			3.8E-08
75	Diferuloylputrescine	5321825	1.1E-10	18	90	1.378	coumaric acids		5.1E-08
76	2'-α-Mannosyl-L-tryptophan	10981970	1.1E-10	4.4	44	-3.813	indoleacetic acids		5.1E-08
77	(+)-Equol 4'-sulfate	29979373	1.5E-10	2.4	28	-0.664	flavonoids		6.9E-08
78	L-Threonate	5460407	2.4E-10	3.8	14	-2.395	butyrates		1.1E-07
79	PI(P-18:0/0:0)	52928632	2.6E-10	9.9	148	5.659	phosphatidylinositols		1.2E-07
80	4-Hydroxyphenylacetylglutamine	61152160	3.1E-10	3.8	3	-0.71	dipeptides	Q	1.4E-07

Supplementary Table 3.6. Cont'd.

81	Nβ-Methyltetrahydroharmol	14219281	6.4E-10	23	16	1.219	biogenic monoamines		2.9E-07
82	S-(2-Hydroxyethyl)glutathione	5460887	7.6E-10	5.3	11	-4.64	polyethylene glycols	Y	3.5E-07
83	N-Nonanoylglycine	10176752	8.3E-10	3	70	2.759	stearic acids		3.8E-07
84	Coniferyl alcohol	1549095	9.7E-10	14	118	0.416	phenols		4.4E-07
85	Cortolone-3-glucuronide	53480447	9.9E-10	34	40	0.922	pregnanes		4.5E-07
86	L-Homocysteine	91552	1.1E-09	0.24	110	-2.217	amino acids, sulfur	u	5E-07
87	Hexanoylcarnitine	3246938	1.2E-09	7.6	109	0.959	Saturated_laurates		5.4E-07
88	Urate	1175	1.2E-09	0.24	9	-1.191	xanthines		5.4E-07
89	N-(1-Deoxy-1-fructosyl)phenylalanine	101039148	1.4E-09	1.6	53	-0.26	oligopeptides		6.3E-07
90	Sucrose (or Cellobiose)	5988	1.7E-09	0.17	24	-3.277	disaccharides	w	7.6E-07
91	N-(1-Deoxy-1-fructosyl)tyrosine	131752248	1.7E-09	8.3	53	-1.396	oligopeptides		7.6E-07
92	Asymmetric dimethylarginine	123831	0.000000002	0.2	119	-2.559	amino acids, basic	B	8.9E-07
93	N6,N6,N6-Trimethyl-L-lysine	440120	0.000000002	0.28	85	-2.992	amino acids, diamino	n	8.9E-07
94	Genistein 4'-O-glucuronide	45782816	0.000000002	7	39	-0.106	flavonoids		8.9E-07
95	4-Coumaryl alcohol	5280535	2.1E-09	48	125	0.655	phenols		9.3E-07
96	5-Methylcytidine	92918	0.000000003	0.2	20	-2.012	pyrimidine nucleosides	A	1.3E-06
97	PA(P-16:0/14:1(9Z))	52929659	3.3E-09	2.1	63	12.555	lysophospholipids		1.5E-06
98	Tetrahydroaldosterone-3-glucuronide	167918	4.1E-09	0.022	40	0.954	cholestanols		1.8E-06
99	N-Acetylleucine	70912	4.3E-09	0.57	4	1.053	oligopeptides	U	1.9E-06
100	Cysteine-S-sulfate	115015	4.9E-09	0.28	129	-4.104	disulfides		2.1E-06
101	N'-Nitrosonornicotine	27919	5.1E-09	0.11	136	0.165	nitrosamines		2.2E-06
102	S-(Hydroxymethyl)glutathione	447123	6.7E-09	2.4	11	-4.998	polyethylene glycols	Y	2.9E-06
103	Xanthurenate 8-O-sulfate	11522049	6.9E-09	1.6	23	-1.044	sulfuric acid esters		0.000003
104	5-(3',4',5'-Trihydroxyphenyl)-γ-valerolacton	124202075	7.3E-09	3	112	-0.626	catechols		3.2E-06
105	(5Z)-13-Carboxytridec-5-enoylcarnitine	71464551	7.8E-09	7.7	81	3.121	carnitine		3.4E-06
106	9(S)-HPODE	6439847	9.6E-09	34	62	5.979	HODE		4.1E-06
107	N(2)-Carboxyethyl-2'-deoxyguanosine	135564621	9.6E-09	2.1	10	-1.3	purine nucleosides		4.1E-06
108	Threoninyl-Proline	9834371	0.00000001	2.7	21	-3.573	oligopeptides		4.3E-06
109	Cytidine	6175	0.000000012	0.3	20	-2.193	pyrimidine nucleosides	A	5.1E-06
110	L-Cystathionine	439258	0.000000016	0.58	115	-3.209	amino acids, sulfur		6.8E-06
111	L-Octanoylcarnitine	11953814	0.000000016	12	18	2.097	Saturated_laurates		6.8E-06
112	4,4-Dimethyl-5α-cholesta-8,24-dien-3-β-ol	50990081	0.000000016	1.8	69	9.798	terpenes	K	6.8E-06
113	Adenosine	60961	0.000000017	0.14	22	-2.367	purine nucleosides	s	7.2E-06
114	2-Ketobutyrate	58	0.000000018	0.64	49	-0.173	butyrates	a	7.6E-06
115	3-[3-Methoxy-4-(sulfooxy)phenyl]oxirane-2	131834358	0.000000022	3.5	26	-2.098	coumaric acids		9.3E-06
116	Coumarin	323	0.000000026	0.0036	116	1.022	benzopyrans		0.000011
117	4-Hydroxy-5-(4'-hydroxyphenyl)-valerate-4'	124202068	0.000000027	4	51	-1.874	phenylacetates	H	0.000011
118	24α,24β-Dihomo-9,10-secocholesta-5,7,10(	24779630	0.000000028	11	12	6.057	hydroxycholecalciferols		0.000012
119	Cholestane-3,7,12,25-tetrol-3-glucuronide	42622727	0.000000032	63	40	4.697	cholestanols		0.000013
120	2-Phenyl-1,3-dioxolane-4-methanol	15572	0.000000038	0.29	91	0.28			0.000016

Supplementary Table 3.6. Cont'd.

121	N-Acetylserotonin glucuronide	45479483	0.000000047	32	44	-0.507	indoles		0.00002
122	L-Histidine	6274	0.000000059	0.0018	37	-3.429	amino acids, cyclic		0.000024
123	Citicoline	13804	0.000000061	0.13	20	-4.899	pyrimidine nucleosides	A	0.000025
124	Diaminopimelate	439283	0.000000068	0.38	45	-3.464	dicarboxylic acids	f	0.000028
125	11-Dehydrocorticosterone	13783449	0.000000084	3.8	121	1.291	pregnenediones	I	0.000035
126	L-2-Aminoadipate	469	0.000000095	0.036	45	-2.991	adipates	f	0.000039
127	Homoanserine	20849429	0.000000095	2.4	37	-3.437	dipeptides		0.000039
128	N-(3-Oxohexanoyl)homoserine lactone	688505	0.000000098	710	21	1.39			0.00004
129	N-Oleoyl asparagine	52922080	0.0000001	19	59	6.279	glutamates		0.000041
130	Deoxyinosine (dI)	135398593	0.00000011	110	10	-1.346	purine nucleosides		0.000045
131	Isoleucyl-Valine	435949	0.00000012	0.24	4	-1.247	dipeptides	U	0.000049
132	18-Oxocortisol	44263344	0.00000014	3.1	108	-0.043	pregnenediones		0.000057
133	Phenylglyoxal	14090	0.00000018	0.3	113	0.677	glyoxal		0.000073
134	4-Guanidinobutanoate	500	0.0000002	0.59	139	-0.946	guanidines	d	0.000081
135	(S)-2,3-Dihydro-3,5-dihydroxy-2-oxo-3-indo	131751604	0.00000022	2.1	48	-1.603	indoles		0.000088
136	trans-2-Tetradecenoyl-L-carnitine	53481699	0.00000023	7	81	6.314	carnitine		0.000092
137	N-Acetylgalactosamine (GalNAc)	84265	0.00000024	0.39	42	-1.488	hexosamines		0.000096
138	(R)-3-Hydroxy-hexadecanoate	15569776	0.00000024	4.1	102	6.118	OH-FA_16_0_1		0.000096
139	2,6-Pipecoloxylidide	115282	0.00000025	0.31	142	1.221	acetanilides	J	0.000099
140	L-Glutamine	5961	0.00000025	0.0032	52	-4.077	amino acids, basic		0.000099
141	Thymidine	5789	0.00000025	0.079	36	-1.335	pyrimidine nucleosides	t	0.000099
142	Folate	6037	0.00000026	0.74	96	-3.972	pteridines	y	0.0001
143	N-Acetyl-β-glucosaminylamine	439454	0.00000027	0.056	42	-1.984	hexosamines		0.00011
144	3-Hydroxy-5, 8-tetradecadiencarnitine	53481683	0.0000003	42	81	3.594			0.00012
145	Guanosine	135398635	0.0000003	61	10	-2.272	purine nucleosides		0.00012
146	N-Ribosylhistidine	126923	0.00000032	0.34	82	-4.195	imidazoles		0.00013
147	L-β-Aspartyl-L-aspartate	274178	0.00000051	0.36	38	-4.892	dipeptides	R	0.0002
148	Pyro-L-glutaminyl-L-glutamine	11010621	0.0000006	2.7	111	-2.382	dipeptides	E	0.00023
149	12,13-DHOME	25320870	0.00000059	14	19	4.866	TriHOME		0.00023
150	Carbocysteine-lysine	134128	0.00000067	0.057	11	-3.521	dipeptides	Y	0.00026
151	Lacto-N-biose I	5288905	0.00000071	3.5	42	-3.454	disaccharides		0.00027
152	Dobutamine	36811	0.00000077	0.3	71	2.005	catecholamines		0.0003
153	α-L-Threo-4-Hex-4-enopyranuronosyl-D-gal	3390357	0.00000081	14	94	-2.537		F	0.00031
154	Indole-3-acetate O-glucuronide	124202110	0.0000008	1.4	44	-0.344	indoleacetic acids		0.00031
155	Glycine	750	0.00000083	0.093	93	-3.35	amino acids		0.00032
156	Deoxycytidine (dC)	13711	0.0000009	0.27	20	-1.592	pyrimidine nucleosides	A	0.00034
157	L-Alanine	5950	0.00000091	0.3	95	-2.824	peptides	h	0.00035
158	Tyrosyl-Tryptophan	14475583	0.00000096	7.1	16	-2.212	dipeptides		0.00036
159	Pyridoxal	1050	0.00000094	0.0083	124	-0.754	vitamin b 6	r	0.00036
160	4-Methyl-2-phenyl-2-pentenal	5363896	0.00000099	6.8	113	3.41	aldehydes		0.00037

Supplementary Table 6. Cont'd.

161	Phosphatidylcholine 40:6	53478751	0.00000098	2.5	132	16.064	phosphatidylcholines		0.00037
162	Aspartyl-Alanine	4677380	0.0000011	6.6	38	-4.009	dipeptides	R	0.00041
163	Laccarin	136927122	0.0000012	190	59	1.13			0.00045
164	Serylvalylglycylglutamate	18744842	0.0000012	2.1	68	-5.258	oligopeptides		0.00045
165	L,L-Cyclo(leucylprolyl)	7074739	0.0000015	71	141	1.485	dipeptides		0.00056
166	Epinephrine	5816	0.0000016	0.0059	71	-0.324	catecholamines		0.00059
167	Isoleucyl-Leucine	13817313	0.0000016	2.6	4	-0.467	dipeptides	U	0.00059
168	4-Hydroxy-6-methylpretetramide	441106	0.0000018	1.8	87	2.72			0.00066
169	N1-(α-D-Ribosyl)-5,6-dimethyl-benzimidazo	440780	0.0000018	2.5	7	0.465	nucleosides	m	0.00066
170	3,4-Dihydroxyphenylglycol O-sulfate	22833570	0.0000019	4.5	84	-2.493	benzyl alcohols		0.0007
171	Serotonin	5202	0.0000019	0.32	16	0.366	biogenic monoamines		0.0007
172	Threoninyl-Leucine	7021827	0.0000019	2.3	68	-0.144	dipeptides		0.0007
173	trans-Urocanate	1178	0.0000021	0.15	146	-0.138	acrylates	l	0.00076
174	Dihydroferulate 4-sulfate	187489	0.0000021	0.24	26	-1.588	coumaric acids		0.00076
175	Aldosine	57357510	0.0000023	2.4	147	-0.08	piperidines		0.00083
176	Leucyl-Valine	352038	0.0000025	0.55	4	-1.036	dipeptides	U	0.0009
177	Choline	305	0.0000026	0.019	150	-0.837	ethanolamines		0.00094
178	Palmitoyl-L-carnitine	11953816	0.0000027	6.8	18	6.649	carnitine		0.00097
179	Alanyl-Phenylalanine	96814	0.0000029	0.38	5	-1.877	dipeptides		0.001
180	Biopterin	135449517	0.0000029	2200	96	-2.938	pteridines	y	0.001
181	3-Dehydroquinate	439351	0.0000031	0.062	13	-2.122			0.0011
182	Pyrraline	14274616	0.0000035	12	134	-2.352	pyrroles	q	0.0012
183	N-[(4Z,7Z,10Z,13Z,16Z,19Z)-Docosahexaenc	52922058	0.0000037	3	59	8.006	glutamates		0.0013
184	SM(d18:1/12:0)	44260123	0.0000038	7.5	55	10.62	sphingomyelins		0.0013
185	Xanthine	1188	0.0000038	0.0057	9	-0.654	xanthines		0.0013
186	Oxalosuccinate	972	0.0000039	0.066	56	-1.314		o	0.0014
187	L-Aspartate	5960	0.0000039	0.31	133	-3.707	amino acids, acidic	v	0.0014
188	Ergothioneine	5351619	0.0000039	4.1	37	-0.319	amino acids, cyclic		0.0014
189	Citrulline	9750	0.0000042	0.32	52	-3.909	amino acids, diamino		0.0015
190	γ-Glutamyltyrosine	94340	0.0000046	0.75	3	-3.538	dipeptides	Q	0.0016
191	Sarcosine	1088	0.0000047	0.0047	93	-0.794	n-substituted glycines		0.0016
192	Porphobilinogen	1021	0.0000046	0.089	7	-1.116	pyrroles	m	0.0016
193	3-Propylidene-1(3H)-isobenzofuranone	6259976	0.0000049	9.8	128	2.171	benzylidene compounds		0.0017
194	Nutriacholate	53477693	0.0000051	4.2	61	5.765	deoxycholic acid		0.0017
195	Leucylproline	80817	0.000005	0.24	107	-1.466	dipeptides		0.0017
196	Hydroxycotinine	10219774	0.0000061	4.4	92	-0.8	dipeptides		0.0021
197	Allantoin	204	0.0000072	0.24	97	-1.169	hydantoins		0.0024
198	O-Succinylbenzoate	5459922	0.0000074	3.4	128	-1.026	biphenyl compounds		0.0025
199	Isoleucyl-Threonine	18218220	0.0000074	4.2	103	-2.574	dipeptides		0.0025
200	Indole	798	0.0000073	0.46	136	1.423	indoles		0.0025

Supplementary Table 3.6. Cont'd.

201	Dopamine 3-O-sulfate	122136	0.0000089	0.61	32	-1.796	catecholamines		0.003
202	Vinyl caffeate	11127419	0.0000094	13	105	1.394	cinnamates		0.0031
203	Octadecyl fumarate	6436905	0.00001	2.4	151	9.221	O=FA_22_2		0.0033
204	p-Menthα-2,4(8)-dien-9,3-olide	3018936	0.00001	2.1	60	1.872	terpenes	N	0.0033
205	Vanilloylglycine	3083688	0.000011	8	90	-0.588			0.0037
206	Glycyl-Phenylalanine	97415	0.000011	0.37	5	-2.403	dipeptides		0.0037
207	Taurocholate	6675	0.000011	0.003	17	2.771	taurine	D	0.0037
208	Glutaminylproline	11736661	0.000012	2.9	106	-4.154	dipeptides		0.0039
209	2-Picolinate	1018	0.000012	0.021	134	-0.102	pyridines	q	0.0039
210	Valyl-Tyrosine	7009554	0.000013	3.1	3	-0.139	oligopeptides	Q	0.0043
211	O(4')-Sulfo-L-tyrosine	514186	0.000014	120	41	-4.557	amino acids, aromatic	z	0.0046
212	Isovaleryl-L-carnitine	6426851	0.000014	2.1	27	0.329	carnitine	L	0.0046
213	Glycyl-Isoleucine	259613	0.000014	0.001	4	-2.428	dipeptides	U	0.0046
214	Valyl-Isoleucine	7010531	0.000014	4	104	0.972	dipeptides		0.0046
215	16-Glutaryloxy-1α,25-dihydroxyvitamin D3	10940565	0.000015	4.3	12	4.917	hydroxycholecalciferols		0.0048
216	Val-Pro-Pro	24978507	0.000015	7.6	86	-0.581	imino acids		0.0048
217	DL-p-Hydroxyphenyllactate	9378	0.000015	0.4	51	-0.478	phenols	H	0.0048
218	D-1-Amino-2-pyrrolidinecarboxylate	273022	0.000016	0.12	86	-0.965	nitrosamines		0.0051
219	N-(2-Methoxy-4-pyridyl)-N'-phenylurea	12897225	0.000017	2.3	16	0.314			0.0054
220	Lithocholate	9903	0.000019	0.66	1	8.263	cholic acids		0.006
221	Ethyl-β-D-glucuronide	18392195	0.000019	170	34	-1.027	hexuronic acids	Z	0.006
222	(Z)-3-(1-Formyl-1-propenyl)pentanedioate	22394751	0.000021	5.8	29	-0.222			0.0066
223	Pseudaminate	56927739	0.000022	4.3	33	-3.094	sialic acids		0.0069
224	Bisnorbiotin	86492	0.000023	0.36	11	-0.253		Y	0.0072
225	2-Hydroxyadipate	193530	0.000023	0.046	13	-0.758	adipates		0.0072
226	L-Glutamate	33032	0.000023	0.21	52	-3.349	glutamates		0.0072
227	2-(5-Methyl-2-furanyl)-3-piperidinol	85575714	0.000024	9.5	152	0.833		X	0.0074
228	L-Lysine	5962	0.000025	0.35	45	-2.949	dicarboxylic acids	f	0.0077
229	4-Amino-5-hydroxymethyl-2-methylpyrimid	777	0.000028	0.23	50	-1.267	pyrimidines	i	0.0086
230	Creatinine	588	0.00003	0.14	97	-0.126	imidazoles		0.0092
231	Nα-Acetyl-lysine	92832	0.000034	0.46	79	-2.673	dipeptides	V	0.01
232	Hydroxyvalerylglycine	79198664	0.000034	16	30	-1.071	glutamates		0.01
233	1-Pyrroline-5-carboxylate	1196	0.000034	0.31	78	-0.233	pyrroles		0.01
234	25-Hydroxyvitamin D2	22833566	0.000035	62	12	7.704	cholestenes		0.011
235	Sinapate	637775	0.000036	3.3	75	0.752	coumaric acids		0.011
236	L-(+)-Lactate	107689	0.000039	0.16	31	-0.591	butyrates	k	0.012
237	Nicotianamine	9882882	0.000041	20	80	-3.946	dipeptides		0.012
238	Pyruvate	1060	0.000041	0.04	49	-0.426	keto acids	a	0.012
239	3-Hydroxypentadecanoate	182089	0.000042	0.34	102	5.549	OH-FA_16_0_1		0.012
240	Threoninyl-Phenylalanine	7010579	0.00004	2.8	5	-0.33	oligopeptides		0.012

Supplementary Table 3.6. Cont'd.

241	Phenylpyruvate	997	0.000042	0.23	123	0.907	phenylpyruvic acids	p	0.012
242	γ-Aminobutyrate (GABA)	119	0.000046	0.39	139	-0.668	butyrates	d	0.014
243	Tauro ω-muricholate	21124703	0.000048	3.1	17	4.044	taurine	D	0.014
244	4-Phenyl-3-buten-2-ol	11469151	0.000052	2.5	54	2.317		P	0.015
245	7-Sulfocholate	459070	0.000051	7.5	43	2.796	cholic acids		0.015
246	3α,6β,7β,12α-Tetrahydroxy-5β-cholanoate	53481262	0.000052	4.9	33	2.822	gangliosides		0.015
247	Hypoxanthine	790	0.00006	0.4	9	0.556	hypoxanthines		0.017
248	N-Acetylproline	66141	0.000062	0.43	126	-0.157	dipeptides	T	0.018
249	N-Acetyl-L-2-aminoadipate(2-)	13943174	0.000062	29	47	-0.549	dipeptides		0.018
250	Indole-3-propionate	3744	0.000063	0.16	7	0.974	indoles	m	0.018
251	Isoleucyl-Alanine	5246009	0.000067	11	104	0.317	dipeptides		0.019
252	D-(+)-Pantothenate	6613	0.000071	0.2	74	-1.343	beta-alanine	C	0.02
253	3-Methyldioxyindole	151066	0.000071	0.074	48	0.449	indoles		0.02
254	Fructoselysine	9839580	0.000071	2.8	135	-5.899	monosaccharides		0.02
255	Uracil	1174	0.00007	0.081	50	-0.875	pyrimidinones	i	0.02
256	L-Malate	222656	0.00008	0.13	14	-1.474	dicarboxylic acids		0.022
257	3-Nitro-L-tyrosine	65124	0.000085	0.4	58	-1.636	azides	c	0.024
258	2-Hydroxy-3-methylbutyrate	99823	0.000084	0.33	31	0.064	butyrates	k	0.024
259	Neurotensin 11-13	53481585	0.000084	1.8	3	-0.656	oligopeptides	Q	0.024
260	5,8-Tetradecadienoate	5312409	0.000084	5.2	8	5.589	UnSaturated FA		0.024
261	Pipecolate	849	0.00009	0.24	85	0.173	dipeptides	n	0.025
262	Mesobilirubinogen	26818	0.000096	0.043	15	1.319	bile pigments		0.026
263	3''-Deamino-3''-oxonicotianamine	11954188	0.000096	50	80	-1.653	dipeptides		0.026
264	L-β-Aspartyl-L-leucine	3549397	0.000096	250	72	-2.574	oligopeptides		0.026
265	Dihydrothymine	676414	0.000099	1.8	97	-0.867	pyrimidinones		0.027
266	Fumarycarnitine	53481630	0.00011	1.8	27	-1.337		L	0.03
267	Isoleucyl-Glutamine	22935676	0.00011	3.9	35	-3.155	dipeptides		0.03
268	Nicotinate	937	0.00011	0.23	92	-0.989	pyridines		0.03
269	N-Acetylhistidine	75619	0.00012	0.18	37	-0.987	dipeptides		0.032
270	Tetradecanedioate	13185	0.00012	0.063	2	4.558	O=FA_10_1	e	0.032
271	6-Nonenoylglycine	131802990	0.00013	41	59	2.243	arachidonic acids		0.035
272	3α,12α-Dihydroxy-5β-chola-7,9(11)-dien-24	5284144	0.00013	4.4	6	4.418	cholestenes	G	0.035
273	7-Ketodeoxycholate	188292	0.00013	0.11	61	3.68	cholic acids		0.035
274	Leucyl-Isoleucine	435718	0.00013	0.22	4	-0.467	dipeptides	U	0.035
275	Homocysteate	177491	0.00013	0.17	129	-4.001	disulfides		0.035
276	4-Aminohippurate	2148	0.00014	0.14	145	-3.137	aminobenzoates		0.037
277	5-(3',4'-Dihydroxyphenyl)-γ-valerolactone	45093080	0.00015	4.3	73	0.872	catechols		0.039
278	Coprocholate	122312	0.00015	0.42	1	5.524	cholestanols		0.039
279	5β-Cyprinol sulfate	53477904	0.00015	93	43	3.208	cholic acids		0.039
280	Caffeate	689043	0.00015	3.3	105	0.62	cinnamates		0.039

Supplementary Table 3.6. Cont'd.

281	Salicylate β-D-glucoside	4596190	0.00016	13	91	-0.827	salicylates		0.041
282	Dodecanoylcarnitine	168381	0.00016	0.091	18	4.373	Saturated_laurates		0.041
283	Coproporphyrin III	114935	0.00017	0.38	15	0.964	porphyrins		0.043
284	3-Hydroxyanthranilate	86	0.00018	0.21	58	0.634	aminobenzoates	c	0.046
285	N-Acetyl-L-aspartate	65065	0.00018	0.44	38	-1.265	dipeptides	R	0.046
286	Spermidine	1102	0.00018	0.0098	99	-0.654	putrescine		0.046
287	Phenylalanylproline	7020641	0.00019	4.7	5	0.567	dipeptides		0.048
288	24,25,26,27-Tetranor-23-oxo-hydroxyvitam	131769828	0.00019	9.6	12	5.035	hydroxycholecalciferols		0.048
289	5-Sulfosalicylate	7322	0.0002	0.0066	137	-0.754	salicylates		0.05
290	LysoPE(0:0/14:1(9Z))	53480920	0.00021	3.3	132	3.37			0.052
291	Tirofiban	60947	0.00021	0.015	3	2.682	amino acids, aromatic	Q	0.052
292	D-Gycero-L-galacto-octulose	18618152	0.00021	2	25	-2.655	monosaccharides	M	0.052
293	Glycolate	757	0.00022	0.18	31	-1.117	acetates	k	0.054
294	cis-Trihomoaconitate	25244228	0.00023	81	8	-1.381			0.056
295	2-Isopropylmalate	5280523	0.00023	7.1	56	-0.709	dicarboxylic acids	o	0.056
296	Alanyl-Proline	83525	0.00024	0.15	107	-2.901	dipeptides		0.058
297	N-Acetylneuraminate	445063	0.00024	8.4	33	-3.525	sialic acids		0.058
298	Tetranor 12-HETE	15730832	0.00026	5	131	4.275	OH-FA_16_3_1		0.062
299	3α,7α,12α,19-Tetrahydroxy-5β-cholanoate	5283905	0.00029	8.6	1	2.539	cholic acids		0.069
300	N-Acetylcadaverine	189087	0.00031	0.1	143	0.008	acetates		0.073
301	3-Methoxy-4-Hydroxyphenylglycol sulfate	3035420	0.00031	180	118	-2.052	benzyl alcohols		0.073
302	15(S)-HETE	5283145	0.00031	3.4	62	6.74	HODE		0.073
303	1-Hydroxyvitamin D5	10025615	0.00031	4.1	12	9.787	hydroxycholecalciferols		0.073
304	3α,12α-Dihydroxy-5β-chola-7,14-dien-24-o	5284095	0.00034	91	6	4.152	cholic acids	G	0.079
305	Glycitein 4'-O-glucuronide	124202362	0.00034	2.5	39	-0.053	flavonoids		0.079
306	Hyodeoxycholate	9963687	0.00035	34	1	6.389	deoxycholic acid		0.08
307	3-Methyl-3-phenylazetidine	22249	0.00036	0.38	54	1.357		P	0.082
308	Dihydromaleimide β-D-glucoside	77879498	0.00036	1.7	42	-1.8			0.082
309	(4-Ethyl-2,6-dihydroxyphenyl)oxidanesulfor	131834405	0.00036	480	84	0.173	benzyl alcohols		0.082
310	O-[(4Z)-Decenoyl]carnitine	11953821	0.00036	1.7	18	3.235	carnitine		0.082
311	1-Methyl-4-(1-methylpropyl)-benzene	519195	0.00036	2	54	4.403	styrenes	P	0.082
312	9,10-Dihydroxy-13-hydroperoxy-11-octade	5282957	0.00036	320	19	3.785	TriHOME		0.082
313	N-Lactoyl-Tyrosine	57329379	0.00037	2.3	3	-0.78	dipeptides	Q	0.083
314	2-C-Methyl-D-erythritol 4-phosphate	443198	0.00037	2	25	-3.364	sugar phosphates	M	0.083
315	2,3-Pentanedione	11747	0.00038	0.3	120	0.089	pentanones	j	0.084
316	PE(P-16:0e/0:0)	42607469	0.0004	35	148	5.562			0.088
317	1-Aminocyclopropane-1-carboxylate	535	0.00043	0.24	95	-3.054	amino acids, cyclic	h	0.094
318	Glycyl-Tryptophan	102763	0.00043	0.059	7	-2.851	dipeptides	m	0.094
319	Phenylacetyl L-glutamine	92258	0.00047	0.18	5	-0.302	dipeptides		0.1
320	N-(2-Methylbutyryl)glycine	193872	0.00048	0.23	138	0.207	dipeptides	O	0.1

Supplementary Table 3.6. Cont'd.

321	γ-L-Glutamyl-L-pipecolate	69247902	0.00048	1.6	47	-3.068	dipeptides		0.1
322	Mevalonate	439230	0.00047	0.2	56	-0.856	hydroxy acids	o	0.1
323	3-Methyl-2-oxovalerate	47	0.00047	0.17	49	0.689	keto acids	a	0.1
324	Humulene-8-hydroperoxide	131752029	0.0005	1.6	98	3.733			0.11
325	L-Urobilin	440785	0.00051	3	15	0.906	bile pigments		0.11
326	C17 Sphinganine	3247037	0.0005	4.4	83	5.699	ceramides		0.11
327	α-Muricholate	5283852	0.00054	3.7	1	5.366	cholic acids		0.11
328	Vanillin 4-sulfate	20822599	0.00051	6.6	26	-1.525	coumaric acids		0.11
329	Indolylacryloylglycine	5370648	0.00053	5.7	7	0.4	dipeptides	m	0.11
330	Serylleucine	7015694	0.0005	5	68	-0.604	dipeptides		0.11
331	Isoleucyl-Isoleucine	13879965	0.0005	2.4	4	-0.678	dipeptides	U	0.11
332	Asparaginyl-Proline	14354813	0.00052	25	106	-4.512	dipeptides		0.11
333	PI(15:1(9Z)/22:2(13Z,16Z))	52927610	0.00053	2.2	63	13.859	phosphatidylinositols		0.11
334	Tauroursodeoxycholate	12443252	0.00055	2.1	17	4.856	taurine	D	0.11
335	1-Methylurate	69726	0.00056	0.2	9	-1.049	xanthines		0.11
336	Glycylprolylhydroxyproline	11778669	0.0006	21	67	-4.534	dipeptides		0.12
337	3-Aminophenol	11568	0.00061	0.66	58	0.243	phenols	c	0.12
338	Propionate	1032	0.00059	0.015	140	0.173	propionates		0.12
339	Taurochenodeoxycholate	387316	0.00063	0.028	17	4.856	taurine	D	0.12
340	Palmitoleate	445638	0.00061	8.5	8	7.054	UnSaturated FA		0.12
341	γ-Glutamylleucine	151023	0.00065	0.062	35	-2.216	dipeptides		0.13
342	N-Acetylglutamate	70914	0.00069	0.065	30	-0.907	glutamates		0.13
343	Succinate	1738118	0.00066	120	135	-3.116	hexosamines		0.13
344	Valyl-Valine	107475	0.00074	0.46	66	-1.816	dipeptides		0.14
345	4α-Hydroxymethyl-4β-methyl-5α-cholesta-8	25203290	0.00073	5.1	69	9.095	terpenes	K	0.14
346	3-Methylcrotonylglycine	169485	0.00083	0.2	149	-0.03			0.15
347	Chenodeoxycholate	10133	0.00079	0.34	1	6.178	deoxycholic acid		0.15
348	2,5-Dioxopentanoate	523	0.00079	0.0019	57	-0.642	glutarates	b	0.15
349	Dehydrochorismate	9920917	0.00078	56	87	0.321	hydroxybenzoate ethers		0.15
350	α-Ketoisovalerate	49	0.00082	0.11	49	0.12	keto acids	a	0.15
351	Acetylcholine	187	0.00089	0.47	150	-0.097	biogenic amines		0.16
352	3β-Hydroxycholα-5,7-dien-24-oate	5284174	0.0009	5.3	6	5.995	cholic acids	G	0.16
353	Pyrogallol-1-O-sulfate	54110629	0.00096	10	84	-0.683	benzyl alcohols		0.17
354	3-Hydroxydecanoylcarnitine	121454166	0.00097	2.5	18	1.783	carnitine		0.17
355	7-Hydroxy-3-oxocholanoate	53477692	0.00093	20	61	5.769	cholic acids		0.17
356	Anthranilate	227	0.0011	0.009	58	0.497	aminobenzoates	c	0.2
357	2-Hydroxyisocaproate	92779	0.0011	0.4	31	0.844	butyrates	k	0.2
358	Lysyl-Hydroxyproline	68578696	0.0011	6.5	67	-4.056	dipeptides		0.2
359	4-Acetamidobutanoate	18189	0.0011	0.22	138	-0.392	glutamates	O	0.2
360	(1S)-1-Hydroxy-23,24-didehydro-25,26,27-t	17756764	0.0011	190	12	6.698	hydroxycholecalciferols		0.2

Supplementary Table 3.6. Cont'd.

361	Cortisol	5754	0.0011	0.0057	108	0.553	pregnenediones		0.2
362	2,3-Dinor-8-epi-prostaglandin F1α	9548882	0.0011	12	88	2.581	prostaglandins f		0.2
363	9'-Carboxy-γ-tocotrienol	53481537	0.0012	2.2	28	4.456	benzopyrans		0.21
364	Citrate	31348	0.0012	0.23	56	-3.69	dicarboxylic acids	o	0.21
365	Trimethylamine N-oxide (TMAO)	1145	0.0012	0.11	99	-0.618	methylamines		0.21
366	2-Hydroxy-4-oxopentanoate	10103208	0.0013	3.4	14	-1.209			0.22
367	5-Hydroxy-L-tryptophan	439280	0.0013	0.026	16	-2.315	amino acids, aromatic		0.22
368	1,11-Undecanedicarboxylate	10458	0.0013	0.47	2	3.989	O=FA_10_1	e	0.22
369	L-Lysopine	3325403	0.0014	3	85	-2.651	dipeptides	n	0.23
370	Pregnenolone	8955	0.0014	0.14	6	4.833	pregnenes	G	0.23
371	9,12,13-TriHOME	9858729	0.0014	5.1	19	3.735	TriHOME		0.23
372	Coutarate	449034	0.0015	37	145	0.164			0.24
373	Benzoyl glucuronide (Benzoate)	115145	0.0015	0.37	91	-0.167	benzoates		0.24
374	11'-Carboxy-γ-chromanol	53481453	0.0015	4.9	28	6.994	benzopyrans		0.24
375	3β-Hydroxy-5-cholenoate	92997	0.0015	0.54	6	6.998	cholic acids	G	0.24
376	L-Proline	145742	0.0015	0.34	86	-0.185	imino acids		0.24
377	(S)-3,4-Dihydroxybutyrate	150929	0.0016	0.32	14	-1.631	butyrates		0.25
378	7α,12α-Dihydroxy-3-oxo-4-cholenoate	5283996	0.0016	2.7	6	2.821	cholic acids	G	0.25
379	5,6-Dihydroxyindole-2-carboxylate	119405	0.0016	0.49	23	0.839	indoles		0.25
380	Hexadecanedioate	10459	0.0016	0.52	2	5.696	O=FA_10_1	e	0.25
381	Asparaginyl-Valine	18218188	0.0016	19	66	-4.082	oligopeptides		0.25
382	9-Pentadecenoate	6365140	0.0016	2.5	8	6.485	UnSaturated FA		0.25
383	Tetracosahexaenoate	6439582	0.0016	7.5	29	9.791	UnSaturated FA		0.25
384	(2-Naphthalenyloxy)acetate	8422	0.0017	0.15	28	1.791	acetates		0.26
385	2-Keto-3-deoxy-D-gluconate	194024	0.0017	0.36	13	-2.649	sugar acids		0.26
386	N-Acetyl-serine	65249	0.0018	0.31	127	-1.514	dipeptides	S	0.27
387	2-Pyrocatechuate	19	0.0018	0.37	137	0.777	hydroxybenzoates		0.27
388	5-Androstenediol	10634	0.0018	0.57	69	3.642	terpenes	K	0.27
389	3α,7α-Dihydroxycoprostanate	5284239	0.0019	1.9	1	7.609	cholic acids		0.28
390	Uridine	6029	0.0019	0.0084	100	-2.117	pyrimidine nucleosides	x	0.28
391	Ubiquinone-2	5280346	0.0019	15	60	3.878	ubiquinone	N	0.28
392	Tyramine	5610	0.0021	0.45	32	-0.03	biogenic monoamines		0.29
393	Vitamin D2 3-glucuronide	6443809	0.002	3.9	40	8.929	cholestenes		0.29
394	Sulfolithocholylglycine	72222	0.002	0.32	65	6.138	deoxycholic acid	W	0.29
395	(E)-2-Methylglutaconate	6368126	0.002	1.8	29	-0.093	glutarates		0.29
396	Urothion	135565297	0.002	5.9	96	-1.977	pteridines	y	0.29
397	Glutamyllysine	7015704	0.0022	9.6	47	-3.776	dipeptides		0.3
398	7,8-Dihydroneopterin 2'-phosphate	136032516	0.0022	3	10	-4.673	purine nucleosides		0.3
399	S-(3-Oxo-3-carboxy-n-propyl)cysteine	54097042	0.0023	2.7	115	-3.082	amino acids, sulfur		0.31
400	N(3)-(4-Methoxyfumaroyl)-2,3-diaminopro	6438678	0.0023	1.7	149	-3.762	dicarboxylic acids		0.31

Supplementary Table 3.6. Cont'd.

401	1,2-Dihydro-1,1,6-trimethylnaphthalene	121677	0.0023	0.66	54	4.611	naphthalenes	P	0.31
402	Glu-Ile-Val	71464664	0.0023	2	35	-2.074	oligopeptides		0.31
403	Prostaglandin H2-ethanolamide (PGH2-EA)	53477457	0.0028	17	55	4.918			0.33
404	L-Methionine	6137	0.0025	0.16	78	-1.853	amino acids, sulfur		0.33
405	(R)-2,3-Dihydroxy-isovalerate	440279	0.0027	0.32	14	-0.846	butyrates		0.33
406	3-Methoxytyramine sulfate	54166459	0.0026	5.2	32	-1.904	catecholamines		0.33
407	3α,7α,12β-Trihydroxy-5β-cholanoate	5283869	0.0025	5.6	1	4.093	cholic acids		0.33
408	DL-2-Aminooctanoate	69522	0.0026	0.19	45	-0.19	dicarboxylic acids	f	0.33
409	N6-Formyl-lysine (FLys)	70923	0.0026	0.45	79	-2.909	dipeptides	V	0.33
410	N-Acetyl-L-phenylalanine	74839	0.0025	0.59	5	0.867	dipeptides		0.33
411	Glycylleucine	92843	0.0025	0.17	4	-2.217	dipeptides	U	0.33
412	Threoninyl-Methionine	7020164	0.0025	2.5	46	-0.608	dipeptides		0.33
413	N-Acetyl-L-glutamate 5-semialdehyde	192878	0.0028	0.049	30	-0.493	glutamates		0.33
414	Indole-3-carboxylate-O-sulfate	124202109	0.0026	3.1	130	-0.032	indoleacetic acids		0.33
415	Methyl dioxindole-3-acetate	14033762	0.0025	53	48	0.316	indoles		0.33
416	Val-Val-Val	235009	0.0027	0.49	66	-1.463	oligopeptides		0.33
417	12,13-EpOME	5356421	0.0028	54	19	5.884	TriHOME		0.33
418	α-Linolenate	5280934	0.0025	3.7	8	7.538	UnSaturated FA		0.33
419	L-Cysteine	5862	0.003	0.08	110	-2.575	amino acids, sulfur	u	0.34
420	Deoxycholate	222528	0.0029	0.03	1	5.756	cholic acids		0.34
421	Glycodeoxycholate	22833539	0.003	3.9	65	4.928	deoxycholic acid	W	0.34
422	Pyroglutamylglycine	20977730	0.003	19	144	-4.005	dipeptides		0.34
423	13(S)-HODE	6443013	0.003	1.9	62	5.929	HODE		0.34
424	Undecanedioate	15816	0.003	0.55	2	2.851	O=FA_10_1	e	0.34
425	Aspartyl-Leucine	332962	0.0031	0.023	72	-2.574	oligopeptides		0.34
426	3,4-Dihydroxybenzeneacetate	547	0.0029	0.35	73	0.197	phenylacetates		0.34
427	1-Methylinosine	65095	0.0032	0.22	10	-1.805	purine nucleosides		0.34
428	Glycerate	439194	0.0029	0.24	14	-1.723	sugar acids		0.34
429	Proclavaminate	25203549	0.0035	2.2	89	-2.08	aza compounds		0.36
430	Cholate	5460314	0.0034	2.3	1	3.612	cholic acids		0.36
431	3-O-Caffeoyl-4-O-methylquinate	131752769	0.0035	6.6	77	-1.076	cinnamates		0.36
432	Chorismate	12039	0.0034	0.064	60	0.144	cyclohexenes	N	0.36
433	Adipate	196	0.0038	0.35	2	0.006	adipates	e	0.37
434	4-(2-Aminophenyl)-2,4-dioxobutanoate	472	0.0039	0.092	117	0.194	amino acids	g	0.37
435	Norepinephrine	439260	0.004	0.18	71	-0.714	catecholamines		0.37
436	4-Coumarate	637542	0.0036	27	125	0.751	coumaric acids		0.37
437	Phenylalanyl-isoleucine	7010565	0.0038	2.8	5	1.566	dipeptides		0.37
438	2-Hydroxyhepta-2,4-dienedioate	5280801	0.004	120	29	-0.083	glutarates		0.37
439	2-Hydroxystearate	439887	0.0037	0.46	114	7.733	OH-FA_16_0_1		0.37
440	PI(16:0/18:1(9Z))	5771758	0.0036	3.2	63	12.995	phosphatidylinositols		0.37

Supplementary Table 3.6. Cont'd.

441	Protoporphyrinogen IX	121893	0.0038	0.067	15	3.402	porphyrins		0.37
442	Adenine	190	0.0036	0.31	9	-1.287	purines		0.37
443	β-Pseudouridine	15047	0.0037	0.39	36	-2.477	pyrimidine nucleosides	t	0.37
444	3α-Hydroxy-5β-chola-8(14),11-dien-24-oate	5284076	0.0042	5.3	6	6.656	cholic acids	G	0.38
445	Hexanoylglycine	99463	0.023	0.34	70	1.052			0.39
446	PGD2 ethanolamide	5283120	0.0052	6.2	55	1.985			0.39
447	Dihydrocytochalasin B γ-lactone	6257035	0.0078	11	53	4.652			0.39
448	2-Mercaptoglutarate	21398693	0.0099	4	57	-1.13		b	0.39
449	6Z,9Z,12Z-Octadecatriene	21629432	0.0087	5.6	98	9.412			0.39
450	4-Amino-4-deoxychorismate	45266632	0.0097	9.3	60	-0.351		N	0.39
451	Aflatoxin B1 dialcohol	53481022	0.0051	24	28	-1.459			0.39
452	Prostaglandin F2α 2-glyceryl ester	91666454	0.0081	3	88	3.337			0.39
453	Galactitol	11850	0.0054	0.21	25	-3.896	alcohols	M	0.39
454	(9Z,12Z,15Z)-Octadecatrien-1-ol	5367327	0.0052	8.3	8	7.647	alcohols		0.39
455	Taurine	1123	0.028	0.24	99	-1.678	alkanesulfonic acids		0.39
456	3,4-Dihydroxy-L-phenylalanine (L-DOPA)	6047	0.0071	0.015	41	-2.631	amino acids, aromatic	z	0.39
457	L-Tyrosine	6057	0.015	0.18	41	-2.711	amino acids, aromatic	z	0.39
458	L-Tryptophan	6305	0.0075	0.01	7	-2.023	amino acids, aromatic	m	0.39
459	L-Thyronine	5461103	0.0051	79	41	-1.786	amino acids, aromatic	z	0.39
460	L-Arginine	6322	0.0067	0.43	119	-3.585	amino acids, basic	B	0.39
461	Ectoine	126041	0.038	0.074	80	-0.187	amino acids, diamino		0.39
462	Homomethionine	10329619	0.0044	5.6	78	-1.495	amino acids, sulfur		0.39
463	1-Oxo-1H-2-benzopyran-3-carboxaldehyde	12582097	0.0053	2.3	116	1.058	benzopyrans		0.39
464	Bilirubin	5280352	0.0057	2.3	15	2.167	bile pigments		0.39
465	Histamine	774	0.009	0.083	146	-0.748	biogenic monoamines	l	0.39
466	2,3-Butadione	650	0.0065	0.37	120	-0.164	butanones	j	0.39
467	2-Hydroxybutyrate	440864	0.046	3.7	31	-0.233	butyrates	k	0.39
468	Butyrylcarnitine	213144	0.01	0.15	27	-0.179	carnitine	L	0.39
469	Dopamine 4-sulfate	123932	0.0082	0.38	32	-1.796	catecholamines		0.39
470	4-Hydroxy-(3',4'-dihydroxyphenyl)-valerate	52920332	0.0092	1.8	73	0.052	catechols		0.39
471	3β,12α-Dihydroxy-5α-cholanoate	5283835	0.0063	8.3	1	5.756	cholic acids		0.39
472	3-Oxo-4,6-choladienoate	5283992	0.0085	56	6	6.873	cholic acids	G	0.39
473	Taurocholate 3-sulfate	53477754	0.0099	11	17	1.474	cholic acids	D	0.39
474	2,3-Dihydro-2,3-dihydroxybenzoate	9964159	0.0087	3.1	94	-0.242	cyclohexenes	F	0.39
475	Lithocholate glycine conjugate	115245	0.0049	0.36	65	7.435	deoxycholic acid	W	0.39
476	Pimelate	385	0.017	0.4	2	0.575	dicarboxylic acids	e	0.39
477	Malonate	867	0.012	0.53	140	-0.299	dicarboxylic acids		0.39
478	3-Hydroxydodecanedioate	16663321	0.0091	22	64	1.968	dicarboxylic acids		0.39
479	3-Hydroxysuberate	22328017	0.0054	2.7	64	-0.308	dicarboxylic acids		0.39
480	Pyroglutamate	7405	0.0095	0.46	111	-0.827	dipeptides	E	0.39

Supplementary Table 3.6. Cont'd.

481	Alanyl-Leucine	96801	0.0051	0.027	4	-1.691	dipeptides	U	0.39
482	Proline betaine	115244	0.008	0.34	126	-0.618	dipeptides	T	0.39
483	Glycyl-Lysine	306144	0.0084	0.0024	79	-3.777	dipeptides	V	0.39
484	Isoleucyl-Tyrosine	342468	0.0057	0.27	3	-1.789	dipeptides	Q	0.39
485	Prolylhydroxyproline	11902892	0.0064	3.2	67	-1.661	dipeptides		0.39
486	Pyroglutamine	11984188	0.023	9	141	-1.014	dipeptides		0.39
487	Isoleucyl-Serine	14426033	0.0045	7.6	103	-3.034	dipeptides		0.39
488	Aspartyl-tyrosine	19365650	0.0081	5.4	101	-3.896	dipeptides		0.39
489	Ascorbylstearate	54676866	0.0098	35	88	9.35	Epoxy FA_24		0.39
490	Palmitoyl ethanolamide	4671	0.029	0.62	70	6.651	ethanolamines		0.39
491	α-Ketoglutarate	51	0.0076	0.51	57	-1.056	glutarates	b	0.39
492	Glutarate	743	0.026	0.16	57	-0.352	glutarates	b	0.39
493	3-Methylglutaconate	1551553	0.0044	4.9	29	-0.212	glutarates		0.39
494	Glucosamine	439213	0.0055	0.036	83	-1.764	hexosamines		0.39
495	Glucosamine 6-phosphate	439217	0.0088	0.093	83	-3.422	hexosamines		0.39
496	Guanine	135398634	0.0067	2.5	9	-0.763	hypoxanthines		0.39
497	(5S)-5-(Carboxymethyl)-L-proline	447989	0.0043	48	144	-0.179	imino acids		0.39
498	Indole-3-acetate	802	0.0044	0.21	7	0.805	indoleacetic acids	m	0.39
499	Indole-3-lactate	676157	0.0088	3	7	0.21	indoleacetic acids	m	0.39
500	Indole-3-carboxaldehyde	10256	0.01	0.38	130	1.466	indoles		0.39
501	Methyl 2,3-dihydro-3,5-dihydroxy-2-oxo-3-i	45782784	0.0062	2.6	48	0.024	indoles		0.39
502	DL-3-Phenyllactate	3848	0.0056	0.64	123	0.658	lactates	p	0.39
503	Linoleamide	6435901	0.033	10	98	7.137	linoleic acids		0.39
504	D-Glucose	64689	0.0043	0.42	25	-1.697	monosaccharides	M	0.39
505	Azelate	2266	0.023	0.25	2	1.713	O=FA_10_1	e	0.39
506	Sebacate	5192	0.011	0.017	2	2.282	O=FA_10_1	e	0.39
507	Suberate	10457	0.015	0.31	2	1.144	O=FA_10_1	e	0.39
508	Dodecanedioate	12736	0.0091	0.45	2	3.42	O=FA_10_1	e	0.39
509	2-(4'-Methylthio)butylmalate	44237181	0.0074	3.7	64	-0.639	O=FA_9_1		0.39
510	12-Hydroxyheptadecanoate	15110021	0.0092	4.3	114	6.054	OH-FA_16_0_1		0.39
511	Isoleucyl-Asparagine	4414300	0.0082	3.4	72	-3.513	oligopeptides		0.39
512	20-Carboxy-leukotriene B4	5280877	0.0069	9	131	2.625	oxo-ETE		0.39
513	3-(3-Hydroxyphenyl)propanoate sulfate	187488	0.0048	0.43	51	-1.349	phenylacetates	H	0.39
514	Harderoporphyrinogen	193825	0.0097	0.1	15	-0.534	porphyrins		0.39
515	2'-Deoxyadenosine 5'-monophosphate (dAI	12599	0.015	0.58	22	-3.424	purine nucleosides	s	0.39
516	Nudifloramide	69698	0.0048	0.19	92	-1.166	pyridines		0.39
517	Cytosine	597	0.015	0.12	50	-0.784	pyrimidinones	i	0.39
518	Thymine	1135	0.0099	0.0063	50	-0.694	pyrimidinones	i	0.39
519	N-(3-Acetamidopropyl)pyrrolidin-2-one	129397	0.0043	0.31	143	-0.312	pyrrolidinones		0.39
520	2-Hydroxy-3,4,5-trimethoxybenzoate	289793	0.0081	0.37	87	1.149	salicylates		0.39

Supplementary Table 3.6. Cont'd.

521	N-Acetyl-9-O-acetylneuraminate	123962	0.0053	0.17	33	-2.785	sialic acids		0.39
522	N2-Succinyl-L-glutamate 5-semialdehyde	440848	0.0062	1.7	30	-1.123	succinates		0.39
523	6-Phospho-D-gluconate	91493	0.0065	0.34	13	-4.968	sugar acids		0.39
524	Kynurenate	3845	0.033	0.069	23	0.665	sulfuric acid esters		0.39
525	Xanthurenate	5699	0.044	0.0014	23	0.802	sulfuric acid esters		0.39
526	2,8-Quinolinediol	97250	0.025	0.27	23	0.628	sulfuric acid esters		0.39
527	Thiamine	1130	0.0057	0.18	22	-0.461	thiazoles	s	0.39
528	Thymidine 5'-monophosphate (dTMP)	9700	0.016	0.0061	36	-2.993	thymine nucleotides	t	0.39
529	9,10,13-TriHOME	14968868	0.0048	10	19	3.735	TriHOME		0.39
530	L-Carnitine	10917	0.0044	0.5	27	-1.741	trimethyl ammonium compounds	L	0.39
531	Oleate	445639	0.005	2.2	8	8.192	UnSaturated FA		0.39
532	Pyridoxine	1054	0.016	0.0015	124	-1.224	vitamin b 6	r	0.39
533	3-Methylxanthine	70639	0.045	0.18	9	-0.512	xanthines		0.39

Supplementary Table 3.6. Cont'd.

Supplementary Table 3.7. Quantitative pathway enrichment analysis (qMSEA) results for fecal metabolome changes.

	Pathway (feces)	Total Cmpd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR	Enrichment fold
1	Purine Metabolism	74	11	63.468	4.3478	8.80E-20	6.25E-18	4.91E-18	14.59772759
2	Tryptophan Metabolism	60	13	72.7	4.3478	1.41E-19	9.90E-18	4.91E-18	16.72110033
3	Bile Acid Biosynthesis	65	8	77.616	4.3478	2.07E-19	1.43E-17	4.91E-18	17.85178711
4	Porphyrin Metabolism	40	3	90.187	4.3478	2.46E-17	1.67E-15	4.27E-16	20.74313446
5	Fatty Acid Biosynthesis	35	2	87.485	4.3478	3.01E-17	2.01E-15	4.27E-16	20.12167073
6	Methionine Metabolism	43	9	66.745	4.3478	1.03E-15	6.77E-14	1.21E-14	15.35144211
7	Steroid Biosynthesis	48	1	93.89	4.3478	7.67E-15	4.99E-13	7.34E-14	21.59482957
8	Plasmalogen Synthesis	26	1	93.848	4.3478	8.27E-15	5.29E-13	7.34E-14	21.58516951
9	Phospholipid Biosynthesis	29	2	92.682	4.3478	1.10E-14	6.92E-13	8.66E-14	21.3169879
10	Carnitine Synthesis	22	4	66.407	4.3478	8.12E-14	5.04E-12	5.77E-13	15.27370164
11	Arginine and Proline Metabolism	53	7	74.801	4.3478	1.05E-13	6.42E-12	6.79E-13	17.20433323
12	Androgen and Estrogen Metabolism	33	1	91.994	4.3478	1.51E-13	9.08E-12	8.96E-13	21.15874695
13	Homocysteine Degradation	9	3	56.988	4.3478	4.56E-13	2.69E-11	2.49E-12	13.10731864
14	Betaine Metabolism	21	3	75.298	4.3478	6.01E-13	3.49E-11	3.05E-12	17.31864391
15	Glutathione Metabolism	21	5	56.993	4.3478	1.04E-12	5.94E-11	4.93E-12	13.10846865
16	Pyrimidine Metabolism	59	8	56.062	4.3478	1.67E-12	9.35E-11	7.41E-12	12.89433737
17	Pyruvate Metabolism	48	3	87.458	4.3478	2.08E-12	1.14E-10	8.69E-12	20.11546069
18	Pyruvaldehyde Degradation	10	2	87.819	4.3478	5.12E-12	2.77E-10	2.02E-11	20.19849119
19	Tyrosine Metabolism	72	11	55.066	4.3478	6.12E-12	3.24E-10	2.29E-11	12.66525599
20	Gluconeogenesis	35	3	61.642	4.3478	9.54E-12	4.96E-10	3.38E-11	14.17774507
21	Mitochondrial Beta-Oxidation of Short Chain Satur	27	1	88.209	4.3478	1.09E-11	5.56E-10	3.69E-11	20.28819173
22	Transfer of Acetyl Groups into Mitochondria	22	2	80.58	4.3478	1.46E-11	7.28E-10	4.70E-11	18.5335112
23	Aspartate Metabolism	35	8	67.231	4.3478	1.83E-11	8.98E-10	5.66E-11	15.46322278
24	Selenoamino Acid Metabolism	28	3	77.751	4.3478	2.33E-11	1.12E-09	6.89E-11	17.8828373
25	Urea Cycle	29	8	65.861	4.3478	2.64E-11	1.24E-09	7.50E-11	15.14812089
26	Beta-Alanine Metabolism	34	6	79.685	4.3478	2.82E-11	1.30E-09	7.70E-11	18.32765997
27	Vitamin K Metabolism	14	1	87.072	4.3478	3.02E-11	1.36E-09	7.89E-11	20.02668016
28	Starch and Sucrose Metabolism	31	2	77.644	4.3478	3.11E-11	1.37E-09	7.89E-11	17.85822715
29	Glycine and Serine Metabolism	59	14	66.6	4.3478	1.19E-10	5.10E-09	2.90E-10	15.31809191
30	Lysine Degradation	30	4	74.999	4.3478	1.43E-10	6.02E-09	3.39E-10	17.2498735
31	Threonine and 2-Oxobutanoate Degradation	20	2	71.322	4.3478	6.55E-10	2.69E-08	1.46E-09	16.40415842
32	Pantothenate and CoA Biosynthesis	21	2	71.88	4.3478	6.57E-10	2.69E-08	1.46E-09	16.53249919
33	Propanoate Metabolism	42	4	75.761	4.3478	2.34E-09	9.11E-08	5.03E-09	17.42513455
34	Phenylalanine and Tyrosine Metabolism	28	4	71.038	4.3478	2.70E-09	1.03E-07	5.55E-09	16.33883803
35	Citric Acid Cycle	32	3	72.332	4.3478	2.73E-09	1.03E-07	5.55E-09	16.63645982

36	Valine, Leucine and Isoleucine Degradation	60	5	74.021	4.3478	2.87E-09	1.03E-07	5.65E-09	17.02493215
37	Alanine Metabolism	17	5	69.494	4.3478	3.52E-09	1.23E-07	6.76E-09	15.9837159
38	Fatty acid Metabolism	43	1	78.617	4.3478	8.02E-09	2.73E-07	1.50E-08	18.08201849
39	Malate-Aspartate Shuttle	10	3	71.809	4.3478	1.38E-08	4.56E-07	2.52E-08	16.5161691
40	Caffeine Metabolism	24	1	76.664	4.3478	2.12E-08	6.79E-07	3.76E-08	17.6328258
41	Cysteine Metabolism	26	4	64.815	4.3478	2.17E-08	6.79E-07	3.76E-08	14.90753945
42	Warburg Effect	58	6	52.728	4.3478	2.36E-08	7.08E-07	3.99E-08	12.12751277
43	Glucose-Alanine Cycle	13	4	70.884	4.3478	4.44E-08	1.29E-06	7.33E-08	16.30341782
44	Catecholamine Biosynthesis	20	4	48.834	4.3478	9.30E-08	2.60E-06	1.50E-07	11.23188739
45	Spermidine and Spermine Biosynthesis	18	2	66.619	4.3478	9.51E-08	2.60E-06	1.50E-07	15.32246193
46	Ammonia Recycling	32	7	53.637	4.3478	9.76E-08	2.60E-06	1.51E-07	12.33658402
47	Biotin Metabolism	8	1	70.98	4.3478	2.41E-07	6.04E-06	3.65E-07	16.32549795
48	Vitamin B6 Metabolism	20	2	53.754	4.3478	5.01E-07	1.20E-05	7.30E-07	12.36349418
49	Methylhistidine Metabolism	4	1	69.01	4.3478	5.03E-07	1.20E-05	7.30E-07	15.87239523
50	Oxidation of Branched Chain Fatty Acids	26	1	68.273	4.3478	6.55E-07	1.44E-05	9.12E-07	15.70288422
51	Phytanic Acid Peroxisomal Oxidation	26	1	68.273	4.3478	6.55E-07	1.44E-05	9.12E-07	15.70288422
52	Glycerolipid Metabolism	25	1	67.737	4.3478	7.91E-07	1.58E-05	1.08E-06	15.57960348
53	Folate Metabolism	29	2	60.165	4.3478	1.10E-06	2.08E-05	1.47E-06	13.83803303
54	Glutamate Metabolism	49	9	41.571	4.3478	1.75E-06	3.15E-05	2.30E-06	9.561387368
55	Steroidogenesis	43	1	65.048	4.3478	1.94E-06	3.30E-05	2.51E-06	14.96112977
56	Phosphatidylcholine Biosynthesis	14	1	64.079	4.3478	2.64E-06	4.22E-05	3.29E-06	14.73825843
57	Phosphatidylethanolamine Biosynthesis	12	1	64.079	4.3478	2.64E-06	4.22E-05	3.29E-06	14.73825843
58	Glycolysis	25	2	54.02	4.3478	1.04E-05	0.0001451	1.27E-05	12.42467455
59	Pterine Biosynthesis	29	1	57.026	4.3478	2.00E-05	0.0002596	2.40E-05	13.1160587
60	Pentose Phosphate Pathway	29	1	56.469	4.3478	2.31E-05	0.0002772	2.73E-05	12.98794793
61	Thiamine Metabolism	9	1	55.83	4.3478	2.73E-05	0.0002998	3.13E-05	12.84097705
62	Histidine Metabolism	43	3	51.062	4.3478	2.73E-05	0.0002998	3.13E-05	11.74433047
63	Trehalose Degradation	11	1	51.13	4.3478	8.61E-05	0.0007748	9.70E-05	11.75997056
64	Galactose Metabolism	38	2	48.994	4.3478	9.18E-05	0.0007748	0.000102	11.26868761
65	Arachidonic Acid Metabolism	69	2	46.086	4.3478	0.0001533	0.0010732	0.000167	10.5998436
66	Nicotinate and Nicotinamide Metabolism	37	3	34.309	4.3478	0.0002595	0.0015573	0.000279	7.891117347
67	Alpha Linolenic Acid and Linoleic Acid Metabolism	19	1	45.641	4.3478	0.0002915	0.0015573	0.000309	10.49749298
68	Amino Sugar Metabolism	33	6	27.249	4.3478	0.0004755	0.0019021	0.000496	6.267307604
69	Phenylacetate Metabolism	9	2	23.566	4.3478	0.0064567	0.01937	0.006644	5.420212521
70	Taurine and Hypotaurine Metabolism	12	2	27.931	4.3478	0.0066785	0.01937	0.006774	6.424168545
71	Thyroid hormone synthesis	13	1	23.443	4.3478	0.01651	0.01937	0.01651	5.391922352

Supplementary Table 3.7. Cont'd.

Supplementary Table 3.8. ChemRICH results for serum metabolome changes on compound class level (Kolmogorov–Smirnov test).

	Cluster name	Cluster size	p-values	FDR	Key compound	Altered m	Increased	Decreased	Increased rati	Altered Ratio
1	carnitine	5	2.2E-20	1.2E-19	Stearoylcarnitine	5	4	1	0.8	1
2	chalcones	6	2.2E-20	1.2E-19	3, 5-Tetradecadiencarnitine	6	6	0	1	1
3	dipeptides	21	2.2E-20	1.2E-19	N-Acetyl-L-alanine	21	17	4	0.8	1
4	indoles	9	2.2E-20	1.2E-19	Indoxyl sulfate	9	3	6	0.3	1
5	UnSaturated FA	11	2.2E-20	1.2E-19	cis-4-Octenedioate	11	11	0	1	1
6	dicarboxylic acids	8	1.4E-13	6.3E-13	N2-Succinyl-L-ornithine	8	7	1	0.9	1
7	O=FA_13_1	7	2.3E-13	9E-13	Sebacate	7	7	0	1	1
8	amino acids	8	6.7E-13	2.3E-12	Kynurenine	8	4	4	0.5	1
9	O=FA_12_1	4	3.4E-12	1E-11	3-Hydroxydodecanedioate	4	4	0	1	1
10	Saturated_stearic acids	4	4.9E-12	1.3E-11	Capryloylglycine	4	4	0	1	1
11	hydroxybutyrates	5	2.1E-11	5.1E-11	3-Hydroxybutyrate	5	3	2	0.6	1
12	acids, carbocyclic	5	3.1E-11	6.9E-11	D-(-)-Quinate	5	3	2	0.6	1
13	butyrates	5	6.5E-11	1.4E-10	Homocysteinesulfinate	5	4	1	0.8	1
14	glycerophospholipids	5	9.5E-11	1.8E-10	PS(16:0/22:2(13Z,16Z))	5	2	3	0.4	1
15	oligopeptides	4	2.6E-09	4.7E-09	Cysteineglutathione disulfide	4	1	3	0.2	1
16	glutarates	4	8.3E-09	0.000000014	Glutarate	4	4	0	1	1
17	cinnamates	5	2.4E-08	0.000000038	Cinnamyl isobutyrate	5	2	3	0.4	1
18	adipates	4	7.6E-08	0.00000011	Aminoadipate	4	4	0	1	1
19	pregnenediones	3	1.1E-07	0.00000016	17α,21-Dihydroxypregnenolone	3	3	0	1	1
20	ketones	3	1.9E-07	0.00000025	2,3-Hexanedione	3	3	0	1	1
21	Saturated_fatty acids	3	2.8E-07	0.00000036	Heptanoate	3	3	0	1	1
22	HETE	3	5.9E-07	0.00000072	12-HETE	3	3	0	1	1
23	pyrimidine nucleosides	3	1.5E-06	0.0000017	Thymidine	3	3	0	1	1
24	imidazoles	3	2.6E-06	0.000003	Creatinine	3	1	2	0.3	1
25	lactones	3	3.6E-06	0.0000038	N-Butyryl-L-homoserine lactone	3	3	0	1	1
26	pyridines	4	4.7E-06	0.0000049	Nicotinamide	4	4	0	1	1
27	amino acids, sulfur	3	6.4E-05	0.000064	N-Acetylcystathionine	3	1	2	0.3	1

Supplementary Table 3.9. ChemRICH results for serum metabolome changes for individual metabolite assignment (Kolmogorov–Smirnov test).

	Compound.Name	Pubchem.ID	pvalue	foldchange	CID	ClusterNumber	xlogp	ClusterLabel	TreeLabels	FDR
1	Glycolate	757	0.0065	0.86	757	18	-1.117	glycolates		0.4
2	L-(+)-Lactate	107689	0.0022	0.82	107689	18	-0.591	hydroxybutyrates		0.2
3	Pyroglutamine	11984188	0.024	0.81	11984188	44	-1.014	dipeptides	a	0.43
4	L-Arginine	6322	0.0065	0.8	6322	6	-3.585	amino acids	b	0.4
5	Creatinine	588	0.000097	0.8	588	64	-0.126	imidazoles	c	0.015
6	D-(+)-Pantothenate	6613	0.0061	0.79	6613	59	-1.343	beta-alanine	d	0.39
7	Sarcosine	1088	0.04	0.75	1088	40	-0.794	n-substituted glycines		0.43
8	1-Methyl-L-histidine	92105	0.041	0.74	92105	13	-2.811	methylhistidines		0.43
9	L-Methionine	6137	0.04	0.72	6137	56	-1.853	amino acids, sulfur		0.43
10	L-Carnitine	10917	0.00032	0.72	10917	25	-1.741	trimethyl ammonium compounds	e	0.043
11	O-Methyl-DL-serine	97963	0.029	0.67	97963	7	-3.437	amino acids		0.43
12	(S)-3,4-Dihydroxybutyrate	150929	0.0073	0.65	150929	10	-1.631	hydroxybutyrates	f	0.4
13	p-Hydroxyphenylacetate	127	0.000052	0.64	127	20	0.117	acids, carbocyclic	g	0.0088
14	Indolelactate	92904	0.0021	0.64	92904	9	0.21	indoles	h	0.2
15	2,3-Dihydroxyisovalerate	440279	0.0054	0.64	440279	10	-0.846	butyrates	f	0.37
16	Salicylate	10253	0.012	0.62	10253	19	0.509	hippurates		0.4
17	PS(21:0/0:0)	52926294	0.0053	0.6	52926294	2	5.188	lysophospholipids		0.37
18	LysoPE(20:1(11Z)/0:0)	52925139	0.0059	0.58	52925139	2	6.784	glycerophospholipids		0.39
19	DL-p-Hydroxyphenyllactate	9378	0.0076	0.56	9378	20	-0.478	phenols	g	0.4
20	Butyrylcarnitine	213144	0.00014	0.55	213144	25	-0.179	carnitine	e	0.021
21	Glycerophosphocholine	71920	0.00067	0.55	71920	63	-2.928	lecithins	i	0.084
22	L-Cysteine-glutathione disulfide	25203382	0.0001	0.54	25203382	29	-3.176	oligopeptides		0.016
23	Hypoxanthine	790	0.02	0.54	790	41	0.556	hypoxanthines	j	0.4
24	L-Glutathione oxidized (GSSG)	65359	0.0071	0.53	65359	29	-6.622	oligopeptides		0.4
25	Phosphoribosylformylglycineamidine	440417	0.00096	0.53	440417	46	-4.323			0.11
26	Indole-3-carboxaldehyde	10256	0.0018	0.53	10256	23	1.466	indoles	k	0.17
27	Cysteineglutathione disulfide	53477713	0.000096	0.53	53477713	29	-4.967	oligopeptides		0.015
28	Ferulate	445858	0.01	0.5	445858	11	0.512	cinnamates		0.4
29	Indole-3-acrylate	5375048	0.000023	0.47	5375048	23	1.228	indoles	k	0.0041
30	β-Anthropyranose	50906608	0.000049	0.46	50906608	46	-0.697	amino sugars		0.0084
31	2,4-Quinolinediol	54680871	0.013	0.43	54680871	57	0.41	quinolones		0.4
32	L-Malate	222656	0.0016	0.42	222656	10	-1.474	dicarboxylic acids	f	0.16
33	DL-3-Phenyllactate	3848	0.0065	0.39	3848	12	0.658	acids, carbocyclic	l	0.4
34	Sphingosine 1-phosphate (d16:1-P)	52931110	0.009	0.38	52931110	2	3.651	lysophospholipids		0.4
35	L-Lysine	5962	0.027	0.36	5962	6	-2.949	protein hydrolysates	b	0.43
36	N-Acetyl-L-cysteine	12035	0.0068	0.32	12035	36	-0.133	amino acids, sulfur		0.4
37	Spermidine	1102	0.0048	0.32	1102	65	-0.654	putrescine		0.35
38	Phenylacetyl L-glutamine	92258	0.00068	0.28	92258	4	-0.302	dipeptides	m	0.084
39	Ectoine	126041	0.018	0.25	126041	32	-0.187	amino acids		0.4
40	Methyl indole-3-acetate	74706	0.0023	0.23	74706	9	1.126	indoles	h	0.2

41	3,3'-Thiobispropanoate	8096	0.0013	0.17	8096	45	0.261	propionates		0.14
42	1-Methyl-4-imidazoleacetate	75810	0.014	0.15	75810	13	0.017	imidazoles		0.4
43	Citrulline	9750	0.00014	0.15	9750	6	-3.909	amino acids	b	0.021
44	Caffeate	689043	0.03	0.14	689043	11	0.62	cinnamates		0.43
45	Hippurate	464	0.027	0.14	464	4	0.154	dipeptides	m	0.43
46	Indole-3-propionate	3744	0.00007	0.14	3744	9	0.974	indoles	h	0.012
47	Vanillin 4-sulfate	20822599	0.00014	0.1	20822599	11	-1.525	cinnamates		0.021
48	2,3-Dihydroxybenzoate	19	0.046	0.1	19	50	0.777	hydroxybenzoates		0.43
49	N-(2-Phenylacetyl)glycine	68144	0.0000074	0.095	68144	4	0.425	dipeptides	m	0.0014
50	PE(18:0/20:4(5Z,8Z,11Z,13E)(15OH[S]))	52929784	0.0089	0.057	52929784	2	13.395	glycerophospholipids		0.4
51	PE(16:1(9Z)/18:3(9Z,12Z,15Z))	52924229	0.0099	0.03	52924229	2	12.866	glycerophospholipids		0.4
52	Chenodeoxycholate	10133	0.026	0.011	10133	35	6.178	deoxycholic acid		0.43
53	Phenol sulfate	74426	0.0022	0.0058	74426	20	-0.9	sulfuric acid esters	g	0.2
54	Trimethylamine N-oxide (TMAO)	1145	0.01	0.0037	1145	0	-0.618	methylamines		0.4
55	Acetolein	108335	0.0077	0.0037	108335	37	8.612	triglycerides		0.4
56	Indoxyl sulfate	10258	0.00000032	0.00023	10258	23	-0.075	indoles	k	6.9E-05
57	L-Glycine	750	0.03	1.2	750	40	-3.35	amino acids		0.43
58	L-Acetylcarnitine	7045767	0.000078	1.3	7045767	25	-1.001	carnitine	e	0.013
59	Uridine	6029	0.011	1.3	6029	8	-2.117	pyrimidine nucleosides		0.4
60	2'-Deoxyuridine	13712	0.01	1.3	13712	8	-1.516	pyrimidine nucleosides		0.4
61	Riboflavin	493570	0.028	1.4	493570	9	-2.049	flavins	h	0.43
62	Thymidine	5789	0.0016	1.4	5789	8	-1.335	pyrimidine nucleosides		0.16
63	Creatine	586	0.00093	1.4	586	40	-2.404	guanidines		0.11
64	Choline	305	0.000048	1.4	305	63	-0.837	ethanolamines	i	0.0083
65	Histamine	774	0.038	1.4	774	13	-0.748	biogenic monoamines		0.43
66	N-Butyrylglycine	88412	0.045	1.4	88412	32	-0.086	dipeptides		0.43
67	L-Valine	6287	0.0064	1.5	6287	7	-2.169	amino acids		0.4
68	2-Hydroxyadipate	193530	0.012	1.5	193530	34	-0.758	adipates		0.4
69	sn-Glycerol-3-phosphate	439162	0.042	1.5	439162	24	-3.109	glycerophosphates	n	0.43
70	Azelaate	2266	0.0068	1.5	2266	1	1.713	O=FA_13_1		0.4
71	γ-Glutamylvaline	7015683	0.000012	1.5	7015683	3	-2.996	dipeptides		0.0022
72	L-Alanine	5950	0.0068	1.5	5950	7	-2.824	peptides		0.4
73	(E)-2-Octenal	16900	0.0081	1.5	16900	61	2.988	aldehydes		0.4
74	2-Heptanone	8051	0.0057	1.5	8051	30	2.145	ketones		0.38
75	3-Ethoxy-1-propanol	8109	0.0081	1.5	8109	24	0.092	propylene glycols	n	0.4
76	Eugenol	3314	0.0013	1.5	3314	11	1.955	cinnamates		0.14
77	Mono-(2-ethyl-5-hydroxyhexyl) phthalate	170295	0.0079	1.5	170295	12	2.316	acids, carbocyclic	l	0.4
78	Dihydrocaffeate 3-O-glucuronide	49844972	0.0087	1.5	49844972	11	-1.212			0.4
79	N(6)-Methyllysine	164795	0.00019	1.5	164795	6	-2.559	dipeptides	b	0.028
80	N-Nonanoylglycine	10176752	0.00034	1.5	10176752	5	2.759	Saturated_stearic acids	o	0.045

Supplementary Table 3.9. Cont'd.

81	Perillate	1256	0.00059	1.5	1256	62	2.274	cyclohexenes		0.076
82	3-Hydroxyvalproate	134459	0.00087	1.6	134459	39	1.29	OH-FA_8_0_1	p	0.1
83	γ-Glutamylleucine	151023	0.0001	1.6	151023	3	-2.216	dipeptides		0.016
84	Nudifloramide	69698	0.01	1.6	69698	47	-1.166	pyridines		0.4
85	α-Ketoglutarate	51	0.0096	1.6	51	38	-1.056	glutarates		0.4
86	2-Hydroxy-3-methylpentanoate	10796774	0.00079	1.6	10796774	18	0.633	pentanoic acids		0.096
87	Nα-Acetyl-lysine	92832	0.0000016	1.6	92832	33	-2.673	dipeptides	q	0.00033
88	Allantoin	204	0.00032	1.6	204	64	-1.169	hydantoins	c	0.043
89	Saccharopine	160556	0.00021	1.6	160556	3	-3.176	dipeptides		0.03
90	Indoline	10328	0.001	1.6	10328	4	0.799	indoles	m	0.11
91	UDP-4-dehydro-6-deoxy-D-glucose	440219	0.0056	1.6	440219	8	-5.286	uridine diphosphate sugars		0.38
92	Homocysteinesulfinate	161712	0.000037	1.6	161712	56	-4.877	butyrates		0.0065
93	(R)-3-Hydroxy-hexadecanoate	15569776	0.0013	1.6	15569776	14	6.118	dicarboxylic acids		0.14
94	Cytidine 2',3'-cyclic phosphate	53481030	0.00016	1.6	53481030	8	-3.262	deoxycytosine nucleotides		0.024
95	S-(Indolylmethylthiohydroximoyl)-L-cystei	25245902	0.0001	1.6	25245902	23	-1.693	indoles	k	0.016
96	Pyrroline hydroxycarboxylate	1059	0.000043	1.6	1059	6	-0.997	pyrroles	b	0.0075
97	cis-Aconitate	643757	0.0029	1.6	643757	22	-1.034	glutarates	r	0.23
98	Leukotriene B4	5283128	0.0084	1.6	5283128	21	4.499	HETE		0.4
99	Sphinganine (d18:0)	91486	0.027	1.6	91486	2	6.268	ceramides		0.43
100	2-Hydroxy-7-methyloctanedioate	131839802	0.0041	1.6	131839802	49	0.462	O=FA_9_1		0.3
101	Kynurenine	161166	0.0000022	1.6	161166	4	-2.868	amino acids	m	0.00045
102	4-Hydroxycinnamoylagmatine	5280691	0.0027	1.6	5280691	19	0.203			0.22
103	Histidylproline diketopiperazine	65137	0.00059	1.7	65137	13	-0.197	peptides, cyclic		0.076
104	γ-Glutamylglutamate	92865	0.0014	1.7	92865	3	-4.176	dipeptides		0.15
105	N-Acetyl-L-alanine	88064	9.9E-12	1.7	88064	36	-0.382	dipeptides		2.3E-09
106	N-Acetylglutamate	185	0.004	1.7	185	3	-0.907	dipeptides		0.3
107	Dihydrodaidzein	176907	0.000094	1.7	176907	43	0.548	isoflavones	s	0.015
108	Diethylphosphate	654	5.5E-08	1.7	654	24	-0.363	organophosphates	n	1.2E-05
109	Cinnamyl isobutyrate	24884264	0.000021	1.7	24884264	12	3.077	cinnamates	l	0.0038
110	2,3-Dinor-8-epi-prostaglandin F2α	9548881	0.0031	1.7	9548881	60	2.254	f2-isoprostanes	t	0.24
111	Shikimate 3-phosphate	121947	0.0013	1.7	121947	62	-2.752			0.14
112	2-Propyl-2,4-pentadienoate	6441809	0.00019	1.7	6441809	53	2.12	UnSaturated FA		0.028
113	1,11-Undecanedicarboxylate	10458	0.0036	1.7	10458	1	3.989	O=FA_13_1		0.27
114	1-Methylguanosine	96373	0.000004	1.7	96373	41	-2.13	dideoxynucleosides	j	0.00079
115	Oleate	445639	0.0026	1.7	445639	26	8.192	UnSaturated FA		0.22
116	N-3-Hydroxydecanoyl-L-Homoserine lacto	71353010	0.014	1.7	71353010	16	3.306	depsipeptides		0.4
117	9,13-Dihydroxy-4-megastigmen-3-one 9-g	72791584	0.00082	1.7	72791584	58	-0.248	norisoprenoids		0.099
118	γ-Aminobutyrate	119	0.00089	1.7	119	7	-0.668	butyrates		0.11
119	1-Naphthaldehyde	6195	0.00028	1.7	6195	12	2.205	naphthalenes	l	0.039
120	N-(2-Methylbutyryl)glycine	193872	0.0019	1.7	193872	32	0.207	dipeptides		0.18

Supplementary Table 3.9. Cont'd.

121	D-Gluconate	10690	0.00024	1.7	10690	34	-3.739	adipates		0.034
122	N-Acetyl-L-phenylalanine	74839	0.00087	1.7	74839	4	0.867	dipeptides	m	0.1
123	L-Leucine	6106	0.000083	1.8	6106	7	-1.389	amino acids		0.013
124	Butyrate	264	0.00047	1.8	264	38	0.742	butyrates		0.062
125	Undecanedioate	15816	0.0067	1.8	15816	1	2.851	O=FA_13_1		0.4
126	Glycyl-Lysine	306144	0.0084	1.8	306144	33	-3.777	dipeptides	q	0.4
127	Suberate	10457	0.016	1.8	10457	1	1.144	O=FA_13_1		0.4
128	cis-5-Decenedioate	7563148	0.0022	1.8	7563148	48	1.766	UnSaturated FA		0.2
129	Nicotinamide	936	1.5E-09	1.8	936	47	-1.236	pyridines		3.4E-07
130	4-Hydroxynonenal	5283344	0.0022	1.8	5283344	61	2.16	UnSaturated FA		0.2
131	2-Hydroxystearate	439887	0.0002	1.8	439887	49	7.733	OH-FA_18_0_1		0.029
132	Methyl 6,8-epidioxy-5,15-dihydroperoxy-9	5283151	0.00029	1.8	5283151	37	3.887	Epoxy FA_21		0.04
133	N-Ornithyl-L-taurine	14257919	0.000067	1.8	14257919	44	-2.463		a	0.011
134	3-Hydroxysuberate	22328017	0.00025	1.8	22328017	14	-0.308	dicarboxylic acids		0.035
135	1,2-Dibutyrin	5327007	0.00031	1.8	5327007	10	1.244	glycerides	f	0.042
136	Palmitoylglycine	151008	0.0065	1.8	151008	5	6.742	Saturated_fatty acids	o	0.4
137	(+)9-HODE	5312830	0.00093	1.9	5312830	21	5.929	HETE		0.11
138	5S-HETE di-endoperoxide	52921873	0.0000037	1.9	52921873	37	3.016			0.00074
139	Adipate	196	0.017	1.9	196	1	0.006	adipates		0.4
140	Phenylacetate	999	0.000011	1.9	999	12	1.253	acids, carbocyclic	l	0.002
141	2,3-Heptanedione	60983	0.000015	1.9	60983	30	1.227	ketones		0.0027
142	3-Hydroxysebacate	3017884	0.0017	1.9	3017884	14	0.83	dicarboxylic acids		0.16
143	5-Methylfuran-2-carboxylate	74710	0.001	1.9	74710	51	0.573	furans	u	0.11
144	Palmitate	985	0.00000039	1.9	985	1	7.57	Saturated FA		8.4E-05
145	2-Furanylmethyl propanoate	61166	0.000008	1.9	61166	51	0.901	methacrylates	u	0.0015
146	2,3-Hexanedione	19707	0.00000064	1.9	19707	30	0.658	ketones		0.00014
147	Pimelate	385	0.025	1.9	385	1	0.575	dicarboxylic acids		0.43
148	3-Hydroxydodecanedioate	16663321	0.000045	1.9	16663321	14	1.968	O=FA_12_1		0.0078
149	Linoleate	5280450	0.0026	1.9	5280450	26	7.865	UnSaturated FA		0.22
150	5,6-Dihydroxyindole-2-carboxylate	119405	0.00098	2	119405	57	0.839	indoles		0.11
151	2-Isopropylmalate	5280523	0.000075	2	5280523	10	-0.709	dicarboxylic acids	f	0.012
152	rac-5,6-Epoxy-retinoyl-β-D-glucuronide	131769835	0.0026	2	131769835	55	3.25	Epoxy FA_26		0.22
153	Dodecanoylcarnitine	168381	0.00017	2	168381	27	4.373	carnitine		0.025
154	Quinolinate	1066	0.047	2	1066	52	-0.569	pyridines		0.43
155	Glutaminylproline	11736661	0.0025	2	11736661	3	-4.154	dipeptides		0.22
156	Dodecanedioate	12736	0.00041	2	12736	1	3.42	O=FA_12_1		0.054
157	(E)-2-Methylglutaconate	6368126	0.0022	2	6368126	22	-0.093	glutarates	r	0.2
158	4-Ene-valproate	104896	0.0025	2	104896	17	2.376	UnSaturated FA		0.22
159	12-KETE	5283162	0.0014	2	5283162	53	6.14	UnSaturated FA		0.15
160	Sebacate	5192	0.0016	2	5192	1	2.282	O=FA_13_1		0.16

Supplementary Table 3.9. Cont'd.

161	trans-2-Tetradecenoylcarnitine	53481699	0.0000066	2.1	53481699	15	6.314	chalcones		0.0013
162	2,3-Dinor-TXB2	5283138	0.0018	2.1	5283138	60	2.276	EpETrE	t	0.17
163	Biotin	171548	0.0087	2.1	171548	33	0.463	imidazoles	q	0.4
164	6-Methylcoumarin	7092	0.00011	2.1	7092	43	1.414	coumarins	s	0.017
165	(11Z,14Z)-Eicosadienoylcarnitine	71464509	0.00023	2.2	71464509	15	8.082	chalcones		0.032
166	Arachidonate	444899	0.0098	2.2	444899	17	8.349	UnSaturated FA		0.4
167	trans-Hexadec-2-enoyl carnitine	53477817	0.000056	2.2	53477817	54	6.538	chalcones		0.0095
168	19,20-DiHDPA	16061148	0.0011	2.3	16061148	17	5.834	DiHDPE		0.12
169	Suberylglycine	6453952	0.0000052	2.3	6453952	5	0.316	dicarboxylic acids	o	0.001
170	4,4'-Thiobis-2-butanone	61989	0.0000012	2.3	61989	45	0.791			0.00025
171	Palmitoleate	445638	0.00021	2.3	445638	26	7.054	UnSaturated FA		0.03
172	Senecioate	10931	0.0092	2.4	10931	22	0.798	butyrates	r	0.4
173	Tiglylcarnitine	91825636	0.0015	2.4	91825636	54	-0.004	chalcones		0.15
174	4-Ethylphenol	31242	6.2E-08	2.5	31242	20	1.802	phenols	g	1.4E-05
175	Sulfolithocholate	10253562	0.006	2.5	10253562	35	6.966			0.39
176	Palmitoyl-L-carnitine	11953816	0.000014	2.5	11953816	27	6.649	carnitine		0.0026
177	3-Oxododecanoate	439717	0.0014	2.5	439717	28	4.202	O=FA_12_1	v	0.15
178	Stearoylcarnitine	52922056	0.0000064	2.6	52922056	27	7.787	carnitine		0.0012
179	Dihydro-2-methoxy-2-methyl-3(2H)-thiopl	526189	0.0000018	2.6	526189	45	0.113			0.00037
180	12-HETE	5312983	0.000034	2.6	5312983	21	6.224	HETE		0.0061
181	Leukotriene A4	5280383	0.00012	2.6	5280383	21	6.284	EpODE		0.018
182	Aminoadipate	469	0.00000021	2.7	469	6	-2.991	adipates	b	4.6E-05
183	2-Hydroxybutyrate	11266	0.000031	2.7	11266	18	-0.233	hydroxybutyrates		0.0055
184	(1R,2R)-3-Oxo-2-pentyl-cyclopentanehexa	16061079	0.0065	2.7	16061079	28	4.399	Saturated_fatty acids	v	0.4
185	cis-4-Octenedioate	11805205	0.00000058	2.7	11805205	48	0.628	UnSaturated FA		0.00012
186	S-(5'-Adenosyl)-L-homocysteine	439155	0.00063	2.8	439155	41	-4.215	adenosine	j	0.079
187	(2S)-6-Amino-2-formamidohexanamide	25244460	0.000079	2.8	25244460	44	-1.471		a	0.013
188	3, 5-Tetradecadiencarnitine	53481681	0.00000021	2.8	53481681	15	5.235	chalcones		4.6E-05
189	Linoelaidyl carnitine	53477834	0.000016	2.8	53477834	15	6.944	chalcones		0.0029
190	Hydroxyprolyl-Valine	61158071	2.5E-09	2.8	61158071	59	-0.433		d	5.7E-07
191	(S)-α-Terpinyl glucoside	13325862	5.8E-09	2.8	13325862	58	1.261	monoterpenes		1.3E-06
192	N-Nonanoyl-L-homoserine lactone	56662027	0.0034	2.9	56662027	16	3.76	lactones		0.26
193	Eicosapentaenoate	446284	0.000034	2.9	446284	17	8.022	UnSaturated FA		0.0061
194	PS(16:0/22:2(13Z,16Z))	52925310	0.00016	2.9	52925310	2	13.304	glycerophospholipids		0.024
195	Corticosterone	5753	0.00000047	3	5753	31	1.704	pregnenediones		0.0001
196	Tetradecanedioate	13185	0.0064	3.1	13185	1	4.558	O=FA_13_1		0.4
197	Glutarate	743	0.00012	3.2	743	38	-0.352	glutarates		0.018
198	Hexadecanedioate	10459	0.0084	3.3	10459	1	5.696	O=FA_13_1		0.4
199	3-Ureidopropionate	111	0.0000046	3.3	111	42	-1.628			0.00089
200	3-Oxotetradecanoate	454064	0.0012	3.4	454064	28	5.34	O=FA_12_1	v	0.13

Supplementary Table 3.9. Cont'd.

201	Capryloylglycine	84290	0.00000074	3.6	84290	5	2.19	Saturated_stearic acids	o	0.00015
202	3-Polyprenyl-4,5-dihydroxybenzoate	46173940	0.0039	3.7	46173940	50	3.793	benzoates		0.29
203	Heptanoate	8094	1.3E-08	3.7	8094	1	2.449	Saturated_fatty acids		2.9E-06
204	3-Methylcrotonyl glycine	169485	0.0000018	3.7	169485	42	-0.03			0.00037
205	Cortisol	5754	0.0048	3.8	5754	31	0.553	pregnenediones		0.35
206	N-Decanoylglycine	1712391	0.0015	3.9	1712391	5	3.328	Saturated_stearic acids	o	0.15
207	Hexanoylglycine	99463	0.000075	4	99463	5	1.052	Saturated_stearic acids	o	0.012
208	PS(16:0/18:1)	5283499	0.00054	4.1	5283499	2	11.355	glycerophospholipids		0.07
209	Erythritol	222285	0.0000037	4.1	222285	24	-2.552	sugar alcohols	n	0.00074
210	L-Menthyl acetoacetate	43045	0.00061	4.2	43045	35	3.997			0.077
211	N-(3-Aminopropyl)-2-pyrrolidinone	82111	0.000044	4.2	82111	65	-0.588	pyrrolidinones		0.0077
212	N-Butyryl-L-homoserine lactone	10130163	0.0000045	4.3	10130163	16	0.915	lactones		0.00088
213	17α,21-Dihydroxypregnenolone	192735	0.00000015	4.6	192735	31	2.434	pregnenediones		3.3E-05
214	2-Aminomuconate	5280499	0.0079	4.6	5280499	22	-2.802		r	0.4
215	Vinylacetylglycine	53477718	1.2E-08	4.7	53477718	42	-0.263	dipeptides		2.7E-06
216	(S)-3-Hydroxyisobutyrate	440873	0.000004	5.1	440873	39	-0.666	hydroxybutyrates	p	0.00079
217	L-2-Amino-3-oxobutanoate	440033	0.000007	5.1	440033	7	-3.258	keto acids		0.0013
218	D-(-)-Quinate	6508	0.0000034	5.2	6508	34	-1.979	acids, carbocyclic		0.00068
219	L-Thyronine	5461103	1.7E-08	5.3	5461103	43	-1.786	thyroxine	s	3.8E-06
220	Tyrosyl-Aspartate	19816752	2.7E-08	5.5	19816752	19	-3.896	dipeptides		6E-06
221	Nicotianamine	12313328	0.0000061	5.7	12313328	52	-3.13	pyridines		0.0012
222	6-Dehydrotestosterone glucuronide	5460852	0.0000027	6.4	5460852	55	2.566			0.00054
223	N-Dodecanoyl-L-homoserine lactone	10221437	0.015	6.8	10221437	16	5.467	lactones		0.4
224	Porphobilinogen	1021	0.00000054	7	1021	9	-1.116	pyrroles	h	0.00012
225	Histidinyl-Glutamine	9903897	0.0026	7.1	9903897	13	-4.984	oligopeptides		0.22
226	3-Hydroxybutyrate	92135	0.0000026	7.2	92135	39	-0.499	hydroxybutyrates	p	0.00053
227	N-Acetylcystathionine	152314	0.00000013	15	152314	36	-2.933	amino acids, sulfur		2.9E-05
228	2-O-Methyl-L-fucose	5179954	0.00072	20	5179954	46	-1.333	hexoses		0.089
229	Aspartylphenylalanine	93078	2.6E-08	21	93078	4	-2.76	dipeptides	m	5.8E-06
230	N2-Succinyl-L-ornithine	127370	0.0000014	29	127370	3	-3.661	dicarboxylic acids		0.00029
231	γ-Glutamyltyrosine	94340	0.00000067	35	94340	19	-3.538	dipeptides		0.00014

Supplementary Table 3.9. Cont'd.

Supplementary Table 3.10. Quantitative pathway enrichment analysis (qMSEA) results for serum metabolome changes (adjusted p-value<0.05).

	Pathway (Sera)	Total Cmpd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR	Enrichment fold
1	Porphyrin Metabolism	40	2	87.445	4.3478	2.08E-12	1.38E-10	1.375E-10	20.11247067
2	Ketone Body Metabolism	13	1	86.715	4.3478	4.08E-11	2.65E-09	1.3474E-09	19.94456967
3	Steroidogenesis	43	2	76.047	4.3478	1.75E-09	1.12E-07	3.3029E-08	17.49091495
4	Bile Acid Biosynthesis	65	3	80.312	4.3478	2E-09	1.26E-07	3.3029E-08	18.47187083
5	Fatty acid Metabolism	43	2	68.03	4.3478	5.04E-08	3.13E-06	6.654E-07	15.64699388
6	Mitochondrial Beta-Oxidation of Long Chain Sa	28	1	72.133	4.3478	1.53E-07	9.36E-06	1.6877E-06	16.59068954
7	Pyrimidine Metabolism	59	4	57.458	4.3478	2.23E-07	1.34E-05	2.0647E-06	13.21541929
8	Glycine and Serine Metabolism	59	9	52.734	4.3478	2.5E-07	1.48E-05	2.0647E-06	12.12889277
9	Steroid Biosynthesis	48	1	65.975	4.3478	1.44E-06	8.33E-05	8.6747E-06	15.17434105
10	Fatty Acid Elongation In Mitochondria	35	1	65.975	4.3478	1.44E-06	8.33E-05	8.6747E-06	15.17434105
11	Beta-Alanine Metabolism	34	3	55.778	4.3478	1.45E-06	8.33E-05	8.6747E-06	12.82901697
12	Methionine Metabolism	43	6	41.096	4.3478	3.26E-06	0.000179	0.000017945	9.452136713
13	Phenylacetate Metabolism	9	2	60.179	4.3478	4.48E-06	0.000242	0.000022432	13.84125305
14	Glycerolipid Metabolism	25	2	41.529	4.3478	4.76E-06	0.000252	0.000022432	9.55172731
15	Propanoate Metabolism	42	4	45.008	4.3478	5.54E-06	0.000288	0.000024366	10.35190211
16	Valine, Leucine and Isoleucine Degradation	60	4	35.64	4.3478	9.43E-06	0.000481	0.000038891	8.197249183
17	Tyrosine Metabolism	72	4	43.474	4.3478	1.85E-05	0.000926	0.000070613	9.999079994
18	Arginine and Proline Metabolism	53	7	49.251	4.3478	1.93E-05	0.000944	0.000070613	11.32779797
19	Phosphatidylethanolamine Biosynthesis	12	1	55.975	4.3478	2.63E-05	0.00126	0.000091201	12.87432725
20	Beta Oxidation of Very Long Chain Fatty Acids	17	1	53.71	4.3478	4.64E-05	0.002181	0.00015316	12.35337412
21	Fatty Acid Biosynthesis	35	6	44.377	4.3478	5.48E-05	0.00252	0.00017217	10.20677124
22	Urea Cycle	29	4	50.245	4.3478	6.45E-05	0.002902	0.00019347	11.55641934
23	Aspartate Metabolism	35	3	50.659	4.3478	6.75E-05	0.002969	0.00019365	11.65163991
24	Tryptophan Metabolism	60	5	28.251	4.3478	0.000101	0.004332	0.00027706	6.497768987
25	Alanine Metabolism	17	4	31.571	4.3478	0.000177	0.007443	0.00044985	7.261373568
26	Glutamate Metabolism	49	4	31.571	4.3478	0.000177	0.007443	0.00044985	7.261373568
27	Alpha Linolenic Acid and Linoleic Acid Metaboli	19	3	38.44	4.3478	0.000215	0.0086	0.00052328	8.841253048
28	Nicotinate and Nicotinamide Metabolism	37	4	28.791	4.3478	0.000222	0.008658	0.00052328	6.621969732
29	Citric Acid Cycle	32	4	33.464	4.3478	0.000263	0.010004	0.00059914	7.696766181
30	Betaine Metabolism	21	3	41.738	4.3478	0.00029	0.010729	0.00063795	9.599797599

31	Phosphatidylcholine Biosynthesis	14	2	43.674	4.3478	0.000337	0.012116	0.00071656	10.04508027
32	Spermidine and Spermine Biosynthesis	18	2	40.53	4.3478	0.000409	0.014316	0.00082854	9.321955932
33	Ammonia Recycling	32	3	31.337	4.3478	0.000422	0.014346	0.00082854	7.207553245
34	Warburg Effect	58	3	33.419	4.3478	0.000427	0.014346	0.00082854	7.686416118
35	Catecholamine Biosynthesis	20	1	42.968	4.3478	0.000507	0.016223	0.00088052	9.882699296
36	Ubiquinone Biosynthesis	20	1	42.968	4.3478	0.000507	0.016223	0.00088052	9.882699296
37	Methylhistidine Metabolism	4	1	42.968	4.3478	0.000507	0.016223	0.00088052	9.882699296
38	Estrone Metabolism	24	1	42.968	4.3478	0.000507	0.016223	0.00088052	9.882699296
39	Gluconeogenesis	35	2	31.999	4.3478	0.000592	0.016574	0.0010017	7.359814159
40	Arachidonic Acid Metabolism	69	4	35.026	4.3478	0.000657	0.017752	0.0010849	8.056028336
41	Butyrate Metabolism	19	1	40.686	4.3478	0.000799	0.020764	0.0012856	9.357836147
42	Pyruvate Metabolism	48	2	33.963	4.3478	0.000918	0.022948	0.0014089	7.811536869
43	Transfer of Acetyl Groups into Mitochondria	22	2	33.963	4.3478	0.000918	0.022948	0.0014089	7.811536869
44	Retinol Metabolism	37	1	36.45	4.3478	0.001785	0.041064	0.0026687	8.383550301
45	Glucose-Alanine Cycle	13	2	30.363	4.3478	0.00182	0.041064	0.0026687	6.983531901
46	Glutathione Metabolism	21	2	29.982	4.3478	0.001907	0.041064	0.0027356	6.895901375
47	Histidine Metabolism	43	4	31.635	4.3478	0.002066	0.041326	0.0029016	7.276093657
48	Threonine and 2-Oxobutanoate Degradation	20	1	34.055	4.3478	0.002758	0.052397	0.0037919	7.832696996
49	Oxidation of Branched Chain Fatty Acids	26	2	31.508	4.3478	0.003017	0.054309	0.0040639	7.246883481
50	Selenoamino Acid Metabolism	28	1	32.831	4.3478	0.003427	0.058259	0.0045236	7.551175307
51	Phospholipid Biosynthesis	29	2	26.073	4.3478	0.005668	0.090693	0.0073354	5.996825981
52	Phenylalanine and Tyrosine Metabolism	28	1	29.207	4.3478	0.006403	0.096043	0.0076835	6.717650306
53	Cysteine Metabolism	26	1	29.207	4.3478	0.006403	0.096043	0.0076835	6.717650306
54	Malate-Aspartate Shuttle	10	1	29.207	4.3478	0.006403	0.096043	0.0076835	6.717650306
55	Phytanic Acid Peroxisomal Oxidation	26	1	29.207	4.3478	0.006403	0.096043	0.0076835	6.717650306
56	Pantothenate and CoA Biosynthesis	21	1	28.766	4.3478	0.006897	0.096043	0.0081285	6.616219697
57	Sphingolipid Metabolism	40	1	28.21	4.3478	0.007572	0.096043	0.0087676	6.48833893
58	Carnitine Synthesis	22	4	20.523	4.3478	0.010804	0.097238	0.012294	4.720318322
59	Riboflavin Metabolism	20	1	23.068	4.3478	0.017529	0.14023	0.019329	5.305671834
60	Lysine Degradation	30	4	20.298	4.3478	0.017572	0.14023	0.019329	4.668568011
61	Purine Metabolism	74	3	16.452	4.3478	0.025029	0.15018	0.027081	3.783982704
62	Glycerol Phosphate Shuttle	11	1	15.765	4.3478	0.054712	0.27356	0.055437	3.625971756
63	Mitochondrial Electron Transport Chain	19	1	15.765	4.3478	0.054712	0.27356	0.055437	3.625971756
64	De Novo Triacylglycerol Biosynthesis	9	1	15.765	4.3478	0.054712	0.27356	0.055437	3.625971756
65	Cardiolipin Biosynthesis	11	1	15.765	4.3478	0.054712	0.27356	0.055437	3.625971756
66	Biotin Metabolism	8	2	14.812	4.3478	0.055437	0.27356	0.055437	3.406780441

Supplementary Table 3.10. Cont'd.

Supplementary Table 3.11. Gender-specific metabolites of GF/CONV-R difference (two-way ANOVA, adjusted p<0.05; Tukey's HSD test, adjusted p<0.05).

(1) feces_male only (n=67)

PubChem_CID	Compound name (feces)	MSI level	male (tukey_hsd)	female (tukey_hsd)	male-fold (GF/CONV-R)	updown (GF/	male (tukey_hsd) adj. pvalue
19	2-Pyrocatechuate	2	**	ns	0.340263703	DOWN	0.00901
196	Adipate	1	*	ns	0.439556707	DOWN	0.0156
385	Pimelate	1	****	ns	0.275369778	DOWN	0.00000143
472	4-(2-Aminophenyl)-2,4-dioxobutanoate	2	***	ns	0.396365344	DOWN	0.000643
743	Glutarate	1	***	ns	0.103308312	DOWN	0.000243
757	Glycolate	1	**	ns	0.292927232	DOWN	0.00175
774	Histamine	1	****	ns	0.03404674	DOWN	0.00000187
1135	Thymine	1	*	ns	0.371360534	DOWN	0.0145
1188	Xanthine	1	****	ns	0.208288535	DOWN	5.39E-10
2266	Azelate	1	****	ns	0.20245791	DOWN	0.00000117
3845	Kynurenate	1	***	ns	0.105034248	DOWN	0.000906
5192	Sebacate	1	****	ns	0.083259906	DOWN	0.00000196
5610	Tyramine	1	****	ns	0.133765767	DOWN	0.000000661
5699	Xanthurenate	1	**	ns	0.27197639	DOWN	0.00143
6322	L-Arginine	1	**	ns	0.362904579	DOWN	0.00996
7405	Pyroglutamate	2	**	ns	0.288845595	DOWN	0.00975
10256	Indole-3-carboxaldehyde	1	*	ns	0.45878112	DOWN	0.0449
10457	Suberate	1	****	ns	0.259828644	DOWN	0.00000954
10458	1,11-Undecanedicarboxylate	1	****	ns	0.427463692	DOWN	0.00000591
11747	2,3-Pentanedione	2	**	ns	2.681107212	UP	0.00502
11850	Galactitol	2	*	ns	6.613067283	UP	0.0173
12039	Chorismate	2	****	ns	0.207776376	DOWN	0.000000112
65249	N-Acetyl-serine	1	**	ns	0.239452395	DOWN	0.00466
70923	N6-Formyl-lysine (FLys)	1	**	ns	0.240368856	DOWN	0.00404
92832	Nα-Acetyl-lysine	1	****	ns	0.326492363	DOWN	2.33E-08
102763	Glycyl-Tryptophan	2	**	ns	13.3760737	UP	0.0015
107475	Valyl-Valine	2	*	ns	0.157119496	DOWN	0.0224
107689	L-(+)-Lactate	2	**	ns	0.216350255	DOWN	0.00757
115145	Benzoyl glucuronide (Benzoate)	2	****	ns	7.877435964	UP	0.000000193
115244	Proline betaine	2	****	ns	0.354138022	DOWN	1.78E-08
119405	5,6-Dihydroxyindole-2-carboxylate (DHI	2	****	ns	0.502544133	DOWN	0.0000314
123932	Dopamine 4-sulfate	2	****	ns	0.154984299	DOWN	0.00000934

145742	L-Proline	1	**	ns	1.729403078	UP	0.00705
150929	(S)-3,4-Dihydroxybutyrate	2	*	ns	0.346023826	DOWN	0.0121
192878	N-Acetyl-L-glutamate 5-semialdehyde	2	****	ns	0.300499669	DOWN	0.0000492
289793	2-Hydroxy-3,4,5-trimethoxybenzoate	2	***	ns	0.049212918	DOWN	0.00027
306144	Glycyl-Lysine	2	*	ns	0.28234073	DOWN	0.0139
439258	L-Cystathionine	1	**	ns	2.098036118	UP	0.00205
439260	Norepinephrine	1	****	ns	0.567742044	DOWN	0.00000896
440780	alpha-Ribazole	2	****	ns	2.900291999	UP	0.00000623
445063	N-Acetylneuraminate	1	*	ns	1.339265429	UP	0.044
447123	S-(Hydroxymethyl)glutathione	2	***	ns	2.10654773	UP	0.000118
447989	(5S)-5-(Carboxymethyl)-L-proline	2	****	ns	0.192785273	DOWN	2.77E-09
4414300	Isoleucyl-Asparagine	2	*	ns	0.44642731	DOWN	0.0409
5280877	20-Carboxy-leukotriene B4	2	****	ns	0.075048146	DOWN	1.33E-08
5280934	α-Linolenate	2	*	ns	0.236765093	DOWN	0.0123
5283120	PGD2 ethanolamide	2	****	ns	0.339215682	DOWN	0.000077
5284239	3α,7α-Dihydroxycoprostanate	2	*	ns	4.8904502	UP	0.0106
5356421	12,13-EpOME	2	***	ns	0.236238998	DOWN	0.000106
5461103	L-Thyronine	2	****	ns	0.347416288	DOWN	0.000000315
6257035	Dihydrocytochalasin B γ-lactone	2	*	ns	4.544391079	UP	0.0105
7015704	Glutamyllysine	2	**	ns	0.219343414	DOWN	0.00321
7020164	Threoninyl-Methionine	2	**	ns	0.284277257	DOWN	0.00715
9548882	2,3-Dinor-8-epi-prostaglandin F1α	2	**	ns	0.085942504	DOWN	0.0013
10329619	Homomethionine	2	**	ns	0.103236137	DOWN	0.00148
11984188	Pyroglutamine	1	***	ns	0.375462487	DOWN	0.000127
13783449	11-Dehydrocorticosterone	2	****	ns	3.293448239	UP	0.00000454
14968868	9,10,13-TriHOME	2	**	ns	0.147554821	DOWN	0.00157
22328017	3-Hydroxysuberate	2	***	ns	0.44742984	DOWN	0.000287
44237181	2-(4'-Methylthio)butylmalate	2	****	ns	0.407688689	DOWN	0.0000639
45266632	4-Amino-4-deoxychorismate	2	*	ns	0.183899627	DOWN	0.0272
52920332	4-Hydroxy-(3',4'-dihydroxyphenyl)-valer	2	****	ns	0.077215573	DOWN	1.08E-08
53477457	Prostaglandin H2-ethanolamide (PGH2-E	2	****	ns	0.066120763	DOWN	0.000000181
53477754	Taurocholate 3-sulfate	2	****	ns	55.85662939	UP	0.0000854
53477904	5β-Cyprinol sulfate	2	****	ns	0.274537561	DOWN	0.00000467
61152160	4-Hydroxyphenylacetylglutamine	2	****	ns	2.273495012	UP	0.00000108
91666454	Prostaglandin F2α 2-glyceryl ester	2	****	ns	0.095460838	DOWN	0.0000357

Supplementary Table 3.11. Cont'd.

(2) feces_female only (n=60)

PubChem_CID	Compound name (feces)	MSI level	male (tukey_hsd)	female (tukey_hsd)	female-fold (GF/CONV-R)	updown	female (tukey_hsd) adj. pvalue
190	Adenine	1	ns	****	0.104814858	DOWN	0.00000137
535	1-Aminocyclopropane-1-carboxylate	2	ns	**	7.655798817	UP	0.00571
597	Cytosine	1	ns	****	0.243196833	DOWN	0.00000135
650	2,3-Butadione	2	ns	****	0.221773574	DOWN	0.00000158
777	4-Amino-5-hydroxymethyl-2-methylpyri	2	ns	***	6.820226241	UP	0.001
1123	Taurine	1	ns	***	0.053375223	DOWN	0.000108
1196	1-Pyrroline-5-carboxylate	2	ns	***	1.344022753	UP	0.000278
2148	4-Aminohippurate	2	ns	*	3.229138765	UP	0.0128
5862	L-Cysteine	2	ns	*	0.262832709	DOWN	0.0157
5961	L-Glutamine	1	ns	****	2.583105389	UP	0.0000021
6029	Uridine	1	ns	*	5.146930294	UP	0.0457
6288	L-Threonine	1	ns	****	3.177985196	UP	0.0000642
6305	L-Tryptophan	1	ns	**	1.731203875	UP	0.00171
8422	(2-Naphthalenyloxy)acetate	2	ns	***	0.251724374	DOWN	0.00025
9700	Thymidine 5'-monophosphate (TMP)	1	ns	*	2.667413273	UP	0.0183
10634	5-Androstenediol	2	ns	****	0.057441997	DOWN	0.00000473
12599	2'-Deoxyadenosine 5'-monophosphate (	1	ns	***	2.172309142	UP	0.000288
15047	β-Pseudouridine	1	ns	****	12.5932759	UP	6.42E-10
22249	3-Methyl-3-phenylazetidine	2	ns	***	6.967416449	UP	0.000262
31348	Citrate	1	ns	**	10.33572399	UP	0.00173
60947	Tirofiban	2	ns	*	3.831255496	UP	0.0128
70639	3-Methylxanthine	1	ns	****	0.072295396	DOWN	0.0000101
75619	N-Acetylhistidine	2	ns	**	5.262404688	UP	0.01
92258	Phenylacetyl L-glutamine	1	ns	*	3.331103335	UP	0.0302
115015	Cysteine-S-sulfate	2	ns	****	8.203780745	UP	0.0000013
151023	γ-Glutamylleucine	2	ns	***	0.508839058	DOWN	0.000562
193872	N-(2-Methylbutyryl)glycine	1	ns	****	5.800030332	UP	0.0000974
342468	Isoleucyl-Tyrosine	2	ns	****	0.193228681	DOWN	0.0000129
439213	Glucosamine	2	ns	***	7.165342232	UP	0.000732

Supplementary Table 3.11. Cont'd.

440848	N2-Succinyl-L-glutamate 5-semialdehyd	2	ns	****	0.228910968	DOWN	6.33E-08
441476	D-(+)-Galacturonate	2	ns	****	7.499661081	UP	0.0000012
449034	Coutarate	2	ns	****	24.57316879	UP	0.0000274
676414	Dihydrothymine	2	ns	****	1.725564852	UP	0.0000766
689043	Caffeate	1	ns	*	5.936636753	UP	0.0178
5280352	Bilirubin	1	ns	**	0.072892305	DOWN	0.00172
5280523	2-Isopropylmalate	2	ns	****	0.363552013	DOWN	0.000000325
5282957	9,10-Dihydroxy-13-hydroperoxy-11-octa	2	ns	***	17.87947289	UP	0.00033
5370648	Indolylacryloylglycine	2	ns	**	1.790616632	UP	0.00183
6365140	9-Pentadecenoate	2	ns	****	0.542702216	DOWN	0.00000397
6443809	Vitamin D2 3-glucuronide	2	ns	*	4.523967799	UP	0.0106
7010579	Threoninyl-Phenylalanine	2	ns	****	0.261999093	DOWN	0.0000967
9920917	Dehydrochorismate	2	ns	****	0.425774285	DOWN	0.000000374
9964159	2,3-Dihydro-2,3-dihydroxybenzoate	2	ns	****	0.388945238	DOWN	7.29E-09
10219774	Hydroxycotinine	2	ns	****	3.0389106	UP	0.000015
11954188	3''-Deamino-3''-oxonicotianamine	2	ns	****	2.538721772	UP	0.00000119
13879965	Isoleucyl-Isoleucine	2	ns	**	0.466176683	DOWN	0.00491
15110021	12-Hydroxyheptadecanoate	2	ns	***	34.70099988	UP	0.000276
15730832	Tetranor 12-HETE	2	ns	**	0.63673754	DOWN	0.00812
18218188	Asparaginyl-Valine	2	ns	****	0.219179574	DOWN	3.21E-08
21398693	2-Mercaptoglutarate	2	ns	****	0.134896433	DOWN	0.00000131
24978507	Val-Pro-Pro	2	ns	***	9.611792223	UP	0.000313
25203549	Proclavaminate	2	ns	*	3.108548867	UP	0.0177
57329379	N-Lactoyl-Tyrosine	2	ns	***	3.686367311	UP	0.000144
69247902	γ-L-Glutamyl-L-pipecolate	2	ns	****	29.91352968	UP	0.00000206
124202109	Indole-3-carboxylate-O-sulfate	2	ns	***	20.4772479	UP	0.000107
131752769	3-O-Caffeoyl-4-O-methylquinate	2	ns	***	15.36585932	UP	0.000749
131802990	6-Nonenoylglycine	2	ns	****	0.328285021	DOWN	0.00000766
135449517	Biopterin	2	ns	****	4.470152673	UP	0.000000175
135564621	N(2)-Carboxyethyl-2'-deoxyguanosine	2	ns	****	6.043172714	UP	5.42E-08
135565297	Urothion	2	ns	**	0.305291796	DOWN	0.00303

Supplementary Table 3.11. Cont'd.

(3) serum_male only (n=55)							
PubChem_CID	**Compound name (serum)**	**MSI level**	**male (tukey_hsd)**	**female (tukey_hsd)**	**male-fold (GF/CONV-R)**	**updown (GF,**	**male (tukey_hsd) adj. pvalue**
185	N-Acetylglutamate	2	**	ns	1.920894749	UP	0.00499
196	Adipate	1	***	ns	2.099014746	UP	0.00044
305	Choline	1	****	ns	1.523812711	UP	0.00008
385	Pimelate	1	**	ns	2.203571672	UP	0.00119
750	L-Glycine	1	*	ns	1.203558985	UP	0.0108
985	Palmitate	1	**	ns	1.765393724	UP	0.00339
1256	Perillate	2	*	ns	1.442424465	UP	0.016
2266	Azelaate	1	**	ns	1.489280199	UP	0.00368
5192	Sebacate	1	***	ns	2.049392372	UP	0.000864
5789	Thymidine	1	*	ns	1.285884987	UP	0.0219
6029	Uridine	1	*	ns	1.285284017	UP	0.0238
6195	1-Naphthaldehyde	2	***	ns	1.685160857	UP	0.000872
6322	L-Arginine	1	**	ns	0.742211552	DOWN	0.00939
6613	D-(+)-Pantothenate	1	***	ns	0.609645899	DOWN	0.000574
8051	2-Heptanone	2	**	ns	1.487417296	UP	0.0021
10253	Salicylate	1	*	ns	0.57896255	DOWN	0.0328
10457	Suberate	1	**	ns	1.860301032	UP	0.00208
10458	1,11-Undecanedicarboxylate	1	**	ns	1.904389081	UP	0.00284
10690	D-Gluconate	1	**	ns	1.72795849	UP	0.00337
10917	L-Carnitine	1	**	ns	0.762235285	DOWN	0.00115
15816	Undecanedioate	1	**	ns	1.88067247	UP	0.00236
16900	(E)-2-Octenal	2	**	ns	1.498894017	UP	0.0044
60983	2,3-Heptanedione	2	**	*	1.649721531	UP	0.0016
61989	4,4'-Thiobis-2-butanone	2	**	ns	1.868694267	UP	0.00463
71920	Glycerophosphocholine	1	*	ns	0.708870368	DOWN	0.0483
91486	Sphinganine (d18:0)	1	*	ns	1.641143249	UP	0.0341
92865	γ-Glutamylglutamate	2	*	ns	1.425612594	UP	0.0144
96373	1-Methylguanosine	2	**	ns	1.544863411	UP	0.0037
121947	Shikimate 3-phosphate	2	**	ns	1.708359861	UP	0.00571

Supplementary Table 3.11. Cont'd.

193530	2-Hydroxyadipate	1	*	ns	1.426100968	UP	0.0258
213144	Butyrylcarnitine	1	**	ns	0.588789956	DOWN	0.0033
222656	L-Malate	2	*	ns	0.440328021	DOWN	0.0393
306144	Glycyl-Lysine	2	*	ns	3.477090436	UP	0.0229
439162	sn-Glycerol-3-phosphate	1	**	ns	1.717233937	UP	0.00827
444899	Arachidonate	2	**	ns	1.730891198	UP	0.00655
445639	Oleate	2	*	ns	2.301924894	UP	0.0226
3017884	3-Hydroxysebacate	2	*	ns	1.982490217	UP	0.0109
5280499	2-Aminomuconate	2	**	ns	6.581395219	UP	0.00764
5283128	Leukotriene B4	2	**	ns	1.464205053	UP	0.0035
5283344	4-Hydroxynonenal	2	*	ns	1.605631878	UP	0.0148
6368126	(E)-2-Methylglutaconate	2	*	ns	1.803572434	UP	0.0139
7015683	γ-Glutamylvaline	2	*	ns	1.952528876	UP	0.0293
7045767	L-Acetylcarnitine	1	**	ns	1.219254636	UP	0.00761
9548881	2,3-Dinor-8-epi-prostaglandin F2α	2	*	ns	1.960817898	UP	0.0112
10176752	N-Nonanoylglycine	2	**	ns	1.608910496	UP	0.00694
11984188	Pyroglutamine	1	***	ns	0.722958384	DOWN	0.000269
14257919	N-Ornithyl-L-taurine	2	*	ns	1.714229927	UP	0.0156
16663321	3-Hydroxydodecanedioate	2	**	ns	2.027146258	UP	0.00113
24884264	Cinnamyl isobutyrate	2	**	ns	2.337918259	UP	0.00388
25203382	L-Cysteine-glutathione disulfide	1	****	ns	0.585845599	DOWN	0.0000279
46173940	3-Polyprenyl-4,5-dihydroxybenzoate	2	****	ns	5.698007075	UP	0.00000134
53477713	Cysteineglutathione disulfide	1	****	ns	0.575532409	DOWN	0.000037
53477817	trans-Hexadec-2-enoyl carnitine	2	**	ns	1.674557697	UP	0.00944
53481030	Cytidine 2',3'-cyclic phosphate	2	**	ns	1.646418355	UP	0.00117
54680871	2,4-Quinolinediol	1	**	ns	0.258760554	DOWN	0.00258

Supplementary Table 3.11. Cont'd.

(4) serum_female only (n=59)

PubChem_CID	Compound name (serum)	MSI level	male (tukey_hsd)	female (tukey_hsd)	female-fold (GF/CONV-R)	updown (GF,	female (tukey_hsd) adj. pvalue
51	α-Ketoglutarate	1	ns	**	2.221320699	UP	0.00291
111	3-Ureidopropionate	1	ns	****	3.2043933	UP	0.0000339
119	γ-Aminobutyrate (GABA)	1	ns	**	1.846058409	UP	0.00135
264	Butyrate	2	ns	**	2.004955813	UP	0.00224
586	Creatine	1	ns	*	1.811632348	UP	0.032
588	Creatinine	1	ns	**	0.829967137	DOWN	0.00727
936	Nicotinamide	1	ns	*	1.859351865	UP	0.0243
1088	Sarcosine	1	ns	*	0.719415448	DOWN	0.0473
6106	L-Leucine	1	ns	**	1.528253281	UP	0.00712
6137	L-Methionine	1	ns	*	0.611793727	DOWN	0.0136
6287	L-Valine	1	ns	*	1.479651784	UP	0.024
9750	Citrulline	2	ns	****	0.01153337	DOWN	0.000000016
10256	Indole-3-carboxaldehyde	1	ns	*	0.442688589	DOWN	0.0202
10459	Hexadecanedioate	1	ns	****	3.919658143	UP	0.000012
11266	2-Hydroxybutyrate	1	ns	****	3.028544423	UP	0.000000427
12035	N-Acetyl-L-cysteine	1	ns	***	0.211916948	DOWN	0.000698
13185	Tetradecanedioate	1	ns	****	4.376175634	UP	0.000000332
43045	L-Menthyl acetoacetate	2	ns	**	15.50772068	UP	0.00281
65137	Histidylproline diketopiperazine	2	ns	*	2.312695088	UP	0.0381
75810	1-Methyl-4-imidazoleacetate	1	ns	***	0.112584231	DOWN	0.000712
92832	Nα-Acetyl-lysine	1	ns	****	1.772799963	UP	0.0000154
92904	Indolelactate	1	ns	**	0.613666538	DOWN	0.007
108335	Acetolein	2	ns	****	0.00215763	DOWN	1.34E-10
119405	5,6-Dihydroxyindole-2-carboxylate (DHI(	2	ns	*	1.569254815	UP	0.0109
126041	Ectoine	1	ns	**	0.294567797	DOWN	0.00154
150929	(S)-3,4-Dihydroxybutyrate	2	ns	*	0.396906072	DOWN	0.047
151008	Palmitoylglycine	2	ns	*	3.256494446	UP	0.0219
151023	γ-Glutamylleucine	2	ns	*	1.273274442	UP	0.0418

Supplementary Table 3.11. Cont'd.

160556	Saccharopine	2	ns	*	1.836460647	UP	0.0237
161712	Homocysteinesulfinate	2	ns	*	2.181337155	UP	0.0331
164795	N(6)-Methyllysine	2	ns	**	1.33011896	UP	0.00667
171548	Biotin	1	ns	****	3.525349062	UP	0.00000155
439717	3-Oxododecanoate	2	ns	*	5.756464752	UP	0.0107
445638	Palmitoleate	2	ns	*	3.273594462	UP	0.0397
445858	Ferulate	1	ns	**	0.431961476	DOWN	0.00538
454064	3-Oxotetradecanoate	2	ns	*	7.602430157	UP	0.0117
526189	Dihydro-2-methoxy-2-methyl-3(2H)-thio	2	ns	****	3.921762797	UP	0.0000776
689043	Caffeate	1	ns	****	0.346277054	DOWN	0.000000363
5280450	Linoleate	2	ns	*	4.149273581	UP	0.0155
5283138	2,3-Dinor-TXB2	2	ns	**	2.245406485	UP	0.00146
5283151	Methyl 6,8-epidioxy-5,15-dihydroperoxy	2	ns	*	2.729045784	UP	0.0257
5283162	12-KETE	2	ns	*	5.469118032	UP	0.0223
5283499	PS(16:0/18:1)	2	ns	***	18.39878889	UP	0.000653
5312830	(+)9-HODE	1	ns	*	3.329851093	UP	0.0146
5327007	1,2-Dibutyrin	2	ns	*	2.794923087	UP	0.039
7563148	cis-5-Decenedioate	2	ns	*	2.31327727	UP	0.0456
11736661	Glutaminylproline	2	ns	*	3.466884472	UP	0.0405
12313328	Nicotianamine	2	ns	****	417.6786043	UP	0.000000437
15569776	(R)-3-Hydroxy-hexadecanoate	2	ns	*	2.377818575	UP	0.0245
16061079	(1R,2R)-3-Oxo-2-pentyl-cyclopentaneher	2	ns	**	10.38917381	UP	0.00512
16061148	19,20-DiHDPA	2	ns	*	3.322795393	UP	0.0373
25244460	(2S)-6-Amino-2-formamidohexanamide	2	ns	**	4.070658099	UP	0.00678
52924229	PE(16:1(9Z)/18:3(9Z,12Z,15Z))	2	ns	****	0.016838634	DOWN	8.32E-08
52929784	PE(18:0/20:4(5Z,8Z,11Z,13E)(15OH[S]))	2	ns	****	0.032119771	DOWN	0.00000222
52931110	Sphingosine 1-phosphate (d16:1-P)	2	ns	*	0.209033397	DOWN	0.0236
56662027	N-Nonanoyl-L-homoserine lactone	1	ns	*	5.696973357	UP	0.0112
71353010	N-3-Hydroxydecanoyl-L-Homoserine lact	1	ns	*	2.300231682	UP	0.0358
72791584	9,13-Dihydroxy-4-megastigmen-3-one 9	2	ns	*	2.562561009	UP	0.0209
131839802	2-Hydroxy-7-methyloctanedioate	2	ns	*	2.592072169	UP	0.0195

Supplementary Table 3.11. Cont'd.

(5) brain_male only (n=13)

PubChem_CID	Compound name (brain)	MSI level	male (tukey_hsd)	female (tukey_hsd)	male-fold (GF/CONV-R)	updown (GF,	male (tukey_hsd) adj. pvalue
51	α-Ketoglutarate	1	**	ns	0.705543688	DOWN	0.00196
247	Betaine	1	*	ns	0.817546503	DOWN	0.0243
936	Nicotinamide	1	***	ns	1.098087208	UP	0.000489
5789	Thymidine	1	*	ns	1.256104231	UP	0.033
8094	Heptanoate	2	**	ns	0.703395149	DOWN	0.00243
13712	2'-Deoxyuridine	1	*	ns	1.212705141	UP	0.039
67427	N-α-Acetyl-L-arginine	2	*	ns	1.421268451	UP	0.0118
69522	DL-2-Aminooctanoate	2	****	ns	0.298409499	DOWN	0.00000311
122357	D-Erythrose-4-phosphate	1	*	ns	0.680065773	DOWN	0.0143
124886	L-Glutathione reduced (GSH)	1	****	ns	0.00698639	DOWN	0.0000581
435718	Leucyl-Isoleucine	2	***	ns	1.780385419	UP	0.000403
5280360	Prostaglandin E2	2	**	ns	0.60651291	DOWN	0.00127
5288144	Prostaglandin B2	2	***	ns	0.589843509	DOWN	0.000113

(6) brain_female only (n=12)

PubChem_CID	Compound name (brain)	MSI level	male (tukey_hsd)	female (tukey_hsd)	female-fold (GF/CONV-R)	updown (GF,	female (tukey_hsd) adj. pvalue
599	4-Hydroxy-2-ketoglutarate	2	ns	**	0.571285368	DOWN	0.00172
967	Orotate	1	ns	**	0.666214258	DOWN	0.00592
8892	Caproate	2	ns	*	0.492791789	DOWN	0.0406
11266	2-Hydroxybutyrate	1	ns	***	3.284979129	UP	0.000295
92904	Indole-3-lactate	1	ns	**	0.5113475	DOWN	0.00866
273260	N-Acetylhistidine	2	ns	****	4.319364425	UP	0.000000792
439155	S-(5'-Adenosyl)-L-homocysteine (SAH)	1	ns	**	0.630594503	DOWN	0.00312
439176	5'-Deoxy-5'-methylthioadenosine	1	ns	**	0.598169232	DOWN	0.00128
444972	Fumarate	1	ns	***	0.666942276	DOWN	0.000478
446284	Eicosapentaenoate	2	ns	*	0.592491801	DOWN	0.0149
44369311	Leucylproline	2	ns	**	1.559927608	UP	0.00265
53481617	Hydroxybutyrylcarnitine	2	ns	***	3.739229257	UP	0.00068

Supplementary Table 3.11. Cont'd.

APPENDIX C: SUPPORTING INFOTMATION FOR CHAPTER 4

***Topic**: Probing molecular signatures of humoral microbiota-gut-brain axis owing to subchronic perfluorooctanoic acid exposure in mice – an integrated metabolomics and metagenomics study*

Supplementary Figures

Supplementary Figure 4.1.
Heatmap view of picrust2-predicted fecal metagenomic pathways with fold change≥3 & adjusted p-value<0.01.

Supplementary Tables

Supplementary Table 4.1
Unique list of 269 fecal metabolites altered in feces of PFOA exposure group (1 ppm) vs. control (0 ppm).

Supplementary Table 4.2
Unique list of 253 serum metabolites altered in blood sera of PFOA exposure group (1 ppm) vs. control (0 ppm).

Supplementary Table 4.3
Unique list of 161 serum metabolites altered in cortical brain tissues of PFOA exposure group (1 ppm) vs. control (0 ppm).

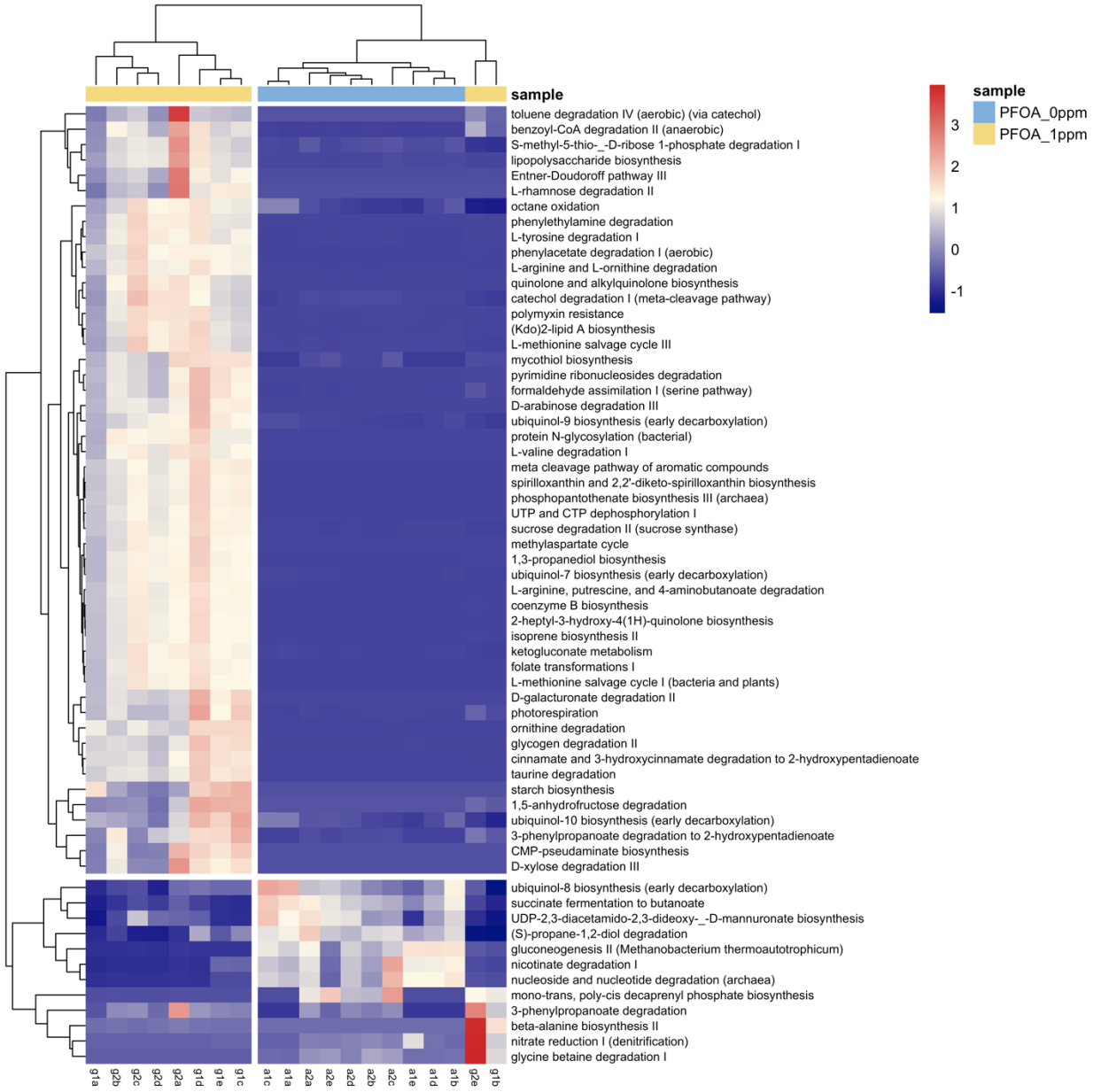
Supplementary Table 4.4
PICRUSt2 predicted metagenomic pathways (fold change ≥ 3, q-value < 0.01).

Supplementary Table 4.5
ChemRICH results for fecal metabolome changes on compound class level (adjusted p-value<0.05).

Supplementary Table 4.6
Quantitative pathway enrichment analysis (qMSEA) results for fecal metabolome changes.

Supplementary Table 4.7
ChemRICH results for fecal metabolome changes for individual metabolite assignment.

Supplementary Table 4.8
Quantitative pathway enrichment analysis (qMSEA) results for cortical brain metabolome changes.



Supplementary Figure 4.1. Heatmap view of picrust2-predicted fecal metagenomic pathways with fold change≥3 and adjusted p-value<0.01 comparing 0 ppm and 1 ppm of PFOA dosage.

Supplementary Table 4.1 Unique list of 269 fecal metabolites altered in feces of PFOA exposure group (1 ppm) vs. control (0 ppm).

	ESI mode	Platform	RT (min)	PRECURSORMZ	PRECURSORTYPE	PubChem_CID	Compound Name	fold	updown (1/0)	pvalue	qvalue	MSI level
1	Positive & Negative	targeted	5.03	180.0660654	[M+H]+	464	Hippuric acid	16.98	DOWN	0.022689903	0.018379196	1
2	Positive	untargeted	6.45	606.3507	[M+H]+	53481557	Dynorphin B (6-9)	15.42	UP	0.00103549	0.002378855	2
3	Positive	untargeted	6.31	394.2952	[M+H]+	53477457	PGH2-EA	14.12	DOWN	0.002856937	0.004424299	2
4	Positive	untargeted	7.31	754.5413	[M+H]+	24778634	PC(14:0/20:4(5Z,8Z,11Z,14Z))	11.73	UP	0.004185333	0.005766568	2
5	Positive	untargeted	2.96	219.1333	[M+H]+	7015695	Serylleucine	10.69	UP	0.000885572	0.00218751	2
6	Positive	untargeted	0.72	277.1024	[M+H]+	92865	gamma-Glutamylglutamic acid	10.42	UP	0.000124589	0.000825415	2
7	Positive & Negative	untargeted	5.86	358.2694	[M+H]+	259327	Leu-Leu-Leu	9.54	UP	0.001995311	0.003526651	2
8	Negative	untargeted	8.66	375.255	[M-H]-	11660820	MG(0:0/20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	9.36	UP	0.008376416	0.016994484	2
9	Negative	untargeted	9.62	433.3335	[M-H]-	53477686	3a,7a-Dihydroxycoprostanic acid	9.19	UP	0.000592554	0.003413518	2
10	Positive	untargeted	3.22	203.1394	[M+H]+	96801	Alanyl-Leucine	9.17	UP	0.006722172	0.007960855	2
11	Positive & Negative	untargeted	2.93	276.1557	[M+H]+	7015684	Epsilon-(gamma-Glutamyl)-lysine	9.17	UP	0.000236809	0.001097634	2
12	Positive	untargeted	3.58	203.1391	[M+H]+	7408079	Alanyl-Isoleucine	8.95	UP	0.049058484	0.033480841	2
13	Negative	untargeted	10.02	281.249	[M-H]-	445639	Oleic acid	8.90	UP	0.000396747	0.002673924	2
14	Negative	targeted	9.11	375.289904	[M-H]-	11740284	Lithocholic acid	8.84	UP	0.00531698	0.012693648	1
15	Positive & Negative	untargeted	8.31	440.2775	[M+H]+	52925150	LysoPE(15:0/0:0)	8.81	UP	7.98903E-05	0.00065662	2
16	Positive	untargeted	4.07	304.1773	[M+H]+	53481675	Pimelylcarnitine	8.78	UP	0.002832972	0.004399436	2
17	Positive	untargeted	4.21	260.1602	[M+H]+	7020102	Isoleucyl-Glutamine	8.28	UP	0.00342305	0.005005975	2
18	Positive & Negative	untargeted	8.15	426.2603	[M+H]+	9547070	LysoPE(14:0/0:0)	8.09	UP	5.70894E-05	0.000562059	2
19	Positive	untargeted	5.6	318.1802	[M+H]+	323786	Tryptophyl-Leucine	7.55	UP	0.000188279	0.000993055	2
20	Positive	untargeted	8.45	568.3397	[M+H]+	10415542	LysoPC(22:6(4Z,7Z,10Z,13Z,16Z,19Z))	7.16	UP	0.007662489	0.008683118	2
21	Negative	untargeted	9.26	277.2182	[M-H]-	5280934	Alpha-Linolenic acid	7.13	UP	0.006662288	0.014752737	2
22	Positive	untargeted	3.64	233.1496	[M+H]+	57281443	Threoninyl-Isoleucine	7.12	UP	0.000888859	0.00218751	2
23	Positive & Negative	untargeted	3.39	233.1498	[M+H]+	18218222	Leucyl-Threonine	7.06	UP	0.000480073	0.001564583	2
24	Negative	untargeted	9.18	405.302	[M-H]-	53480969	MG(0:0/22:4(7Z,10Z,13Z,16Z)/0:0)	6.95	UP	0.000119275	0.001551943	2
25	Positive	untargeted	1.39	219.1341	[M+H]+	7020902	Threoninyl-Valine	6.88	UP	0.002490081	0.004064849	2
26	Positive	untargeted	6.56	596.2559	[M+H]+	53477754	Taurocholic acid 3-sulfate	6.82	DOWN	0.000179404	0.000970369	2
27	Negative	untargeted	8.82	377.271	[M-H]-	5282280	2-Arachidonylglycerol	6.80	UP	0.002536252	0.007949203	2
28	Positive	untargeted	4.7	316.2228	[M+H]+	235009	Val-Val-Val	6.74	UP	0.000632761	0.001821907	2
29	Positive	untargeted	7.42	259.0959	[M+H]+	40424401	cis-4-Hydroxyequol	6.48	UP	0.040971553	0.029048875	2
30	Positive	untargeted	10.7	415.3565	[M+H]+	25202625	4Alpha-hydroxymethyl-5alpha-cholesta-8,24	6.33	UP	0.007526282	0.008587084	2
31	Positive & Negative	untargeted	4.15	231.1701	[M+H]+	352038	Leucyl-Valine	6.33	UP	0.000105096	0.000745773	2
32	Negative	untargeted	2.57	215.1407	[M-H]-	409682	Valyl-Valine	6.22	UP	0.002771224	0.008374194	2
33	Negative	untargeted	9.58	279.2333	[M-H]-	5280450	Linoleic acid	6.00	UP	0.002060734	0.007077275	2
34	Positive	untargeted	4.15	502.2516	[M+H]+	24779246	PC(8:2(2E,4E)/8:2(2E,4E))	5.94	UP	0.002345202	0.003903525	2
35	Positive	untargeted	3.54	260.1606	[M+H]+	23561640	Lysyl-Hydroxyproline	5.86	UP	0.006675199	0.007933379	2
36	Positive	untargeted	9.59	563.2634	[M+H]+	4971	Protoporphyrin IX	5.84	UP	0.001628936	0.003117492	2
37	Negative	untargeted	4.12	229.1564	[M-H]-	7010532	Valyl-Isoleucine	5.70	UP	0.004588176	0.011546443	2
38	Positive	untargeted	7.39	314.2679	[M+H]+	151008	Palmitoylglycine	5.65	DOWN	0.006671858	0.007933379	2
39	Positive & Negative	untargeted	8.65	454.291	[M+H]+	9547069	LysoPE(16:0/0:0)	5.63	UP	0.000152692	0.000900308	2
40	Positive	untargeted	11.14	429.3717	[M+H]+	25201774	4alpha-Formyl-4beta-methyl-5alpha-cholest	5.61	UP	0.002902594	0.004472177	2

41	Negative	untargeted	5.16	204.0304	[M-H]-	440752	4,6-Dihydroxy-2-quinolinecarboxylic acid	5.58	DOWN	0.020527958	0.030239514	2
42	Positive	untargeted	7.1	589.299	[M+H]+	6276321	D-Urobilin	5.43	UP	0.003397227	0.004984569	2
43	Negative	targeted	10	299.2586056	[M-H]-	439887	2-Hydroxy stearic acid	5.41	UP	0.002799721	0.008441426	1
44	Negative	targeted & untargeted	1.06	167.0205144	[M-H]-	1175	Uric acid	5.29	UP	0.002071536	0.007090757	1
45	Positive & Negative	untargeted	3.38	189.1236	[M+H]+	92832	N6-Acetyl-L-lysine	5.28	UP	0.011087144	0.011255548	2
46	Positive	untargeted	10.07	413.3402	[M+H]+	22833566	25-Hydroxyvitamin D2	5.25	UP	0.000253874	0.001141747	2
47	Positive	untargeted	4.15	233.1286	[M+H]+	896	Melatonin	5.23	UP	0.011535469	0.011526033	2
48	Negative	untargeted	8.96	391.2855	[M-H]-	5283835	3b,12a-Dihydroxy-5a-cholanoic acid	5.18	UP	0.002164872	0.007284056	2
49	Positive	untargeted	7.6	655.2747	[M+H]+	114935	Coproporphyrin III	5.13	UP	0.005183995	0.006651435	2
50	Positive	targeted	8.26	400.342666	[M+H]+	11953816	Palmitoyl-L-carnitine	5.08	UP	0.016455261	0.014655889	1
51	Positive	untargeted	1.29	248.1493	[M+H]+	53481617	Hydroxybutyrylcarnitine	5.04	UP	0.032706614	0.024315156	2
52	Positive	untargeted	8.6	457.3676	[M+H]+	10494	Oleanolic acid	4.89	UP	0.021172175	0.017480757	2
53	Negative	untargeted	5.71	121.066	[M-H]-	7409	1-Phenylethanol	4.85	UP	5.24319E-05	0.001114759	2
54	Positive	untargeted	5	528.3038	[M+H]+	53480944	LysoPE(0:0/22:5(7Z,10Z,13Z,16Z,19Z))	4.84	UP	0.001974918	0.003504418	2
55	Negative	untargeted	4.94	132.0457	[M-H]-	321710	1,3-Dihydro-(2H)-indol-2-one	4.81	DOWN	0.018323449	0.028327516	2
56	Positive	untargeted	8.03	328.3202	[M+H]+	27902	Stearoylethanolamide	4.79	UP	1.7404E-05	0.000331895	2
57	Positive	untargeted	8.54	452.2751	[M+H]+	131751724	Cytochalasin Opho	4.79	UP	0.003675095	0.005254832	2
58	Negative	untargeted	9.33	271.2285	[M-H]-	15569776	(R)-3-Hydroxy-hexadecanoic acid	4.73	UP	0.001418899	0.005676229	2
59	Negative	untargeted	10.65	327.2911	[M-H]-	5225199	2(R)-Hydroxyicosanoic acid	4.72	UP	0.002164164	0.007284056	2
60	Negative	untargeted	7.6	471.2435	[M-H]-	21252312	Chenodeoxycholic acid 3-sulfate	4.68	DOWN	0.006569745	0.01460016	2
61	Positive	targeted	0.98	204.1235768	M+	7045767	Acetyl-DL-Carnitine	4.67	UP	0.042216388	0.545124357	1
62	Positive & Negative	untargeted	4.23	310.1137	[M+H]+	445063	N-Acetylneuraminic acid	4.63	UP	0.001694409	0.003183951	2
63	Positive	untargeted	7.88	256.2635	[M+H]+	69421	Palmitic amide	4.57	UP	0.000801739	0.002072402	2
64	Negative	untargeted	11.77	355.3223	[M-H]-	193484	2(R)-Hydroxydocosanoic acid	4.56	UP	0.001562364	0.005977928	2
65	Positive	untargeted	5.17	442.2654	[M+H]+	6440643	Leukotriene E3	4.49	UP	1.44914E-06	0.000106253	2
66	Negative	untargeted	4.99	243.1722	[M-H]-	435718	Leucyl-Isoleucine	4.27	UP	0.001448847	0.005751672	2
67	Negative	targeted	5.05	263.1031766	[M-H]-	92258	Phenylacetyl L-glutamine	4.17	DOWN	0.013620413	0.023224239	1
68	Positive	untargeted	9.36	329.2465	[M+H]+	445580	Docosahexaenoic acid (DHA)	4.16	UP	4.90335E-05	0.000517841	2
69	Negative	targeted	7.05	514.283833	[M-H]-	6675	Taurocholic acid	4.12	DOWN	0.014815113	0.024563681	1
70	Positive	targeted & untargeted	7.96	300.2902392	[M+H]+	5280335	Sphingosine (d18:1)	4.11	UP	0.000231877	0.00109372	1
71	Positive & Negative	untargeted	5.47	279.17	[M+H]+	6992310	Leucyl-phenylalanine	4.09	UP	0.006720912	0.007960855	2
72	Positive & Negative	untargeted	5.26	585.3239	[M+H]+	21252309	Cholic acid glucuronide	3.92	UP	0.000593938	0.001752548	2
73	Positive	targeted	10.33	284.2953242	[M+H]+	31292	Stearamide	3.91	UP	4.22401E-05	0.000484421	1
74	Positive	untargeted	11.19	451.3549	[M+H]+	5280483	Vitamin K1	3.87	UP	0.005429183	0.006866576	2
75	Positive	targeted & untargeted	0.49	146.1657144	[M+H]+	1102	Spermidine	3.86	UP	0.013445384	0.012749687	1
76	Negative	untargeted	9.71	313.239	[M-H]-	10236635	12,13-DHOME	3.86	UP	0.002374443	0.00767567	2
77	Negative	untargeted	8.49	317.2123	[M-H]-	5283162	12-KETE	3.83	DOWN	0.001018783	0.004662788	2
78	Negative	untargeted	3.84	244.1307	[M-H]-	18218182	Asparaginyl-Leucine	3.82	UP	0.000319181	0.002448316	2
79	Negative	untargeted	2.28	253.0697	[M-H]-	440551	L-Phosphoarginine	3.79	UP	0.034798909	0.043693552	2
80	Positive	untargeted	5.57	542.3188	[M+H]+	11757087	LysoPC(20:5(5Z,8Z,11Z,14Z,17Z))	3.77	UP	0.002120109	0.003652748	2

Supplementary Table 4.1. Cont'd.

81	Positive	untargeted	9.48	305.2468	[M+H]+	444899	Arachidonic acid	3.73	UP	0.001602845	0.003086627	2
82	Positive	untargeted	7.19	331.0802	[M+H]+	5280417	3,7-Dimethylquercetin	3.72	UP	0.002691919	0.004261378	2
83	Positive	untargeted	5	245.1856	[M+H]+	13817313	Isoleucyl-Leucine	3.68	UP	0.000216096	0.001065243	2
84	Positive	targeted	0.89	244.0933418	[M+H]+	6253	Cytidine	3.67	UP	0.042086975	0.545124357	1
85	Positive	untargeted	10.03	808.5827	[M+H]+	24778948	PC(18:1(9Z)/20:4(5Z,8Z,11Z,14Z))	3.66	UP	0.001218977	0.002615795	2
86	Positive	untargeted	1.42	170.0926	[M+H]+	64969	3-Methylhistidine	3.66	DOWN	0.022149915	0.018051332	2
87	Negative	untargeted	4.88	135.0454	[M-H]-	440113	4-Hydroxyphenylacetaldehyde	3.65	UP	0.008278012	0.016852864	2
88	Positive	targeted	9.28	280.2640258	[M+H]+	6435901	Linoleamide	3.62	UP	0.010568763	0.010889684	1
89	Positive	targeted & untargeted	0.96	245.0773582	[M+H]+	15047	beta-Pseudouridine	3.57	DOWN	0.011503927	0.011504851	1
90	Negative	untargeted	3.51	293.1147	[M-H]-	111299	gamma-Glutamylphenylalanine	3.56	UP	0.0021651	0.007284056	2
91	Positive	untargeted	0.8	187.1077	[M+H]+	83525	Alanyl-Proline	3.52	UP	0.000554396	0.001683189	2
92	Positive	untargeted	3.19	298.1148	[M+H]+	96373	1-Methylguanosine	3.50	UP	0.011426745	0.011455125	2
93	Positive	untargeted	2.04	189.1235	[M+H]+	92843	Glycylleucine	3.47	UP	2.6969E-05	0.000394749	2
94	Negative	untargeted	0.63	199.9691	[M-H]-	115015	Cysteine S-sulfate	3.47	DOWN	0.000778986	0.004041563	2
95	Positive & Negative	targeted & untargeted	0.66	166.0537866	[M+H]+	158980	L-Methionine sulfoxide	3.44	DOWN	0.000635327	0.001821907	1
96	Negative	untargeted	6.11	165.056	[M-H]-	2762430	3,4-Dihydroxyphenylacetone	3.32	UP	6.60459E-05	0.001205382	2
97	Positive & Negative	targeted	7.2	271.060646	[M+H]+	5280961	Genistein	3.31	UP	0.011461471	0.011478642	1
98	Positive	untargeted	8.4	383.2191	[M+H]+	9547180	1-Tetradecanoyl-sn-glycero-3-phosphate	3.26	DOWN	0.010280777	0.545124357	2
99	Negative	untargeted	9.04	297.2441	[M-H]-	643684	Ricinoleic acid	3.25	UP	0.001995313	0.006980466	2
100	Positive	untargeted	3.54	295.1286	[M+H]+	69040603	Tyrosyl-Hydroxyproline	3.22	UP	0.00241377	0.003987703	2
101	Positive	untargeted	10.37	401.3402	[M+H]+	123743	7a-Hydroxy-cholestene-3-one	3.21	UP	0.000789009	0.002063225	2
102	Positive	untargeted	8.99	372.2891	[M+H]+	5283451	Docosahexaenoyl Ethanolamide	3.20	UP	0.003398635	0.004984569	2
103	Negative	untargeted	5.71	119.0503	[M-H]-	998	Phenylacetaldehyde	3.20	UP	0.000172808	0.001759932	2
104	Positive & Negative	untargeted	9.2	303.2312	[M+H]+	446284	Eicosapentaenoic acid	3.19	UP	0.000827215	0.002098914	2
105	Negative	untargeted	5.5	172.9917	[M-H]-	74426	Phenyl sulfate	3.19	DOWN	0.030454921	0.039688398	2
106	Negative	targeted	5.76	165.055166	[M-H]-	3848	DL-3-Phenyllactic acid	3.16	UP	0.00011139	0.001501592	1
107	Positive	untargeted	9.06	431.3151	[M+H]+	3081085	7alpha-Hydroxy-3-oxo-4-cholestenoate	3.12	UP	0.020292929	0.016998731	2
108	Positive	untargeted	9.63	439.3574	[M+H]+	131752052	α,γ-onoceradienedione	3.12	UP	0.006438358	0.007728967	2
109	Negative	untargeted	0.73	236.0776	[M-H]-	3081391	Fructoseglycine	3.10	DOWN	0.005714556	0.01330166	2
110	Negative	untargeted	1.99	187.1093	[M-H]-	259613	Glycyl-Isoleucine	3.09	UP	0.000757612	0.003976805	2
111	Negative	untargeted	9.07	368.2809	[M-H]-	22833575	cis-5-Tetradecenoylcarnitine	3.08	UP	0.003943383	0.010520746	2
112	Positive	targeted & untargeted	0.66	112.051085	[M+H]+	597	Cytosine	3.08	UP	0.035887827	0.545124357	1
113	Positive	untargeted	2	487.2155	[M+H]+	122304	Glucosylgalactosyl hydroxylysine	3.06	UP	0.003755638	0.005335875	2
114	Positive	untargeted	9.31	207.174	[M+H]+	8814	4-(1,1,3,3-Tetramethylbutyl)-phenol	3.05	UP	0.008198028	0.009107529	2
115	Positive	untargeted	4.52	156.102	[M+H]+	2230	Arecoline	3.05	DOWN	6.79344E-05	0.000606137	2
116	Negative	untargeted	4.86	152.0355	[M-H]-	86	3-Hydroxyanthranilic acid	3.00	DOWN	0.047345216	0.054667353	2
117	Negative	untargeted	4.84	431.0832	[M-H]-	66430	17-Beta-Estradiol-3,17-beta-sulfate	2.99	DOWN	0.008603637	0.017378547	2
118	Positive	untargeted	9.12	254.2473	[M+H]+	56936054	Palmitoleamide	2.96	UP	0.003721871	0.005301272	2
119	Negative	untargeted	1.37	245.1145	[M-H]-	332962	Aspartyl-Leucine	2.95	UP	4.28272E-05	0.001011888	2
120	Negative	untargeted	8.63	343.2279	[M-H]-	6439179	17-HDoHE	2.94	DOWN	0.007007433	0.015227239	2

Supplementary Table 4.1. Cont'd.

121	Positive & Negative	untargeted	4.58	263.1393	[M+H]+	7020641	Phenylalanylproline	2.91	UP	0.001385026	0.002833969	2
122	Negative	untargeted	0.93	217.1197	[M-H]-	3621685	Leucyl-Serine	2.90	UP	0.00015253	0.001653187	2
123	Positive	untargeted	5.76	514.285	[M+H]+	53477756	Sulfolithocholylglycine	2.89	DOWN	0.045291151	0.545124357	2
124	Positive & Negative	untargeted	1.47	233.149	[M+H]+	7021828	Threoninyl-Leucine	2.88	UP	0.00279338	0.004365827	2
125	Negative	untargeted	7.17	329.0666	[M-H]-	23670	Aflatoxin G2	2.86	UP	0.009342624	0.018228503	2
126	Positive	targeted	5.72	162.0555012	[M+H]+	54680871	2,4-Quinolinediol	2.85	UP	0.000802316	0.002072402	1
127	Positive	untargeted	3.89	127.039	[M+H]+	1057	1,2,3-Trihydroxybenzene	2.85	UP	0.044711467	0.03118373	2
128	Negative	untargeted	4.52	227.057	[M-H]-	289793	2-Hydroxy-3,4,5-trimethoxybenzoic acid	2.79	UP	7.10486E-05	0.001247024	2
129	Negative	targeted & untargeted	8.77	319.2273072	[M-H]-	5312983	12-HETE	2.78	DOWN	0.002293579	0.007536199	1
130	Positive	untargeted	6.04	117.0909	[M+H]+	8892	Caproic acid	2.77	DOWN	1.59191E-06	0.000106253	2
131	Positive	untargeted	8.39	328.32	[M+H]+	5282309	N,N-Dimethylsphingosine	2.76	UP	0.000935403	0.002250679	2
132	Positive & Negative	targeted	5.33	162.0555012	[M+H]+	97250	2,8-Quinolinediol	2.74	UP	0.006632665	0.007894073	1
133	Positive	untargeted	4.52	165.055	[M+H]+	637542	4-Hydroxycinnamic acid	2.69	UP	6.33449E-05	0.000587219	2
134	Positive	untargeted	10.33	310.3092	[M+H]+	247220	N-Hexadecanoylpyrrolidine	2.69	UP	0.00069147	0.001919817	2
135	Positive	untargeted	3.73	223.1079	[M+H]+	97415	Glycyl-Phenylalanine	2.68	UP	0.000930874	0.002243014	2
136	Positive & Negative	untargeted	1.74	261.144	[M+H]+	151023	gamma-Glutamylleucine	2.67	UP	0.002590375	0.004166377	2
137	Positive	untargeted	5.57	345.1419	[M+H]+	5057430	Tyrosyl-Tyrosine	2.67	DOWN	0.026490266	0.020737771	2
138	Positive	untargeted	0.63	262.1399	[M+H]+	6427003	Aspartyllysine	2.66	UP	0.00199921	0.003526651	2
139	Positive	untargeted	5.58	278.1751	[M+H]+	6442578	Dinorcapsaicin	2.66	DOWN	0.01020863	0.010632275	2
140	Positive	untargeted	8.8	427.3563	[M+H]+	25200700	4alpha-Formyl-4beta-methyl-5alpha-cholest	2.63	UP	0.001364109	0.002798031	2
141	Positive	targeted & untargeted	8.03	302.3058884	[M+H]+	91486	Sphinganine (d18:0)	2.62	UP	0.002779594	0.004353004	1
142	Positive	untargeted	0.66	235.0926	[M+H]+	7016067	Threoninyl-Aspartate	2.61	UP	0.00025538	0.001142456	2
143	Positive	untargeted	7.97	318.2993	[M+H]+	122121	Phytosphingosine	2.60	UP	0.001804374	0.003310434	2
144	Positive	untargeted	1.39	247.1289	[M+H]+	14304554	Isoleucyl-Aspartate	2.60	UP	0.002375658	0.003935257	2
145	Positive	untargeted	3.09	160.076	[M+H]+	800	Indole-3-acetaldehyde	2.58	DOWN	0.004245996	0.005812152	2
146	Negative	untargeted	0.86	190.036	[M-H]-	40772	gamma-Carboxyglutamic acid	2.58	DOWN	0.034001504	0.04305935	2
147	Positive	untargeted	7.25	163.0386	[M+H]+	5281426	Umbelliferone	2.57	DOWN	0.00048924	0.001586113	2
148	Negative	untargeted	8.76	321.2433	[M-H]-	5283145	15(S)-Hydroxyeicosatrienoic acid	2.57	DOWN	0.002855062	0.008564459	2
149	Positive	untargeted	4.23	127.0388	[M+H]+	237332	5-Hydroxymethyl-2-furancarboxaldehyde	2.56	UP	0.007861214	0.008842258	2
150	Positive & Negative	targeted & untargeted	1.95	284.0994898	[M+H]+	6802	Guanosine	2.54	UP	0.006951138	0.008165368	1
151	Positive	untargeted	0.71	263.0873	[M+H]+	25207301	L-beta-aspartyl-L-glutamic acid	2.52	UP	8.74356E-05	0.000677045	2
152	Negative	untargeted	1.8	427.0066	[M-H]-	135398651	IDP	2.51	UP	0.006220394	0.014042442	2
153	Negative	untargeted	3.63	171.0779	[M-H]-	79101	Glycylproline	2.50	DOWN	0.003794617	0.010249815	2
154	Positive	targeted	9.5	326.3058884	[M+H]+	5283454	Oleoyl Ethanolamide	2.47	UP	0.045193633	0.031441165	1
155	Positive	untargeted	7.02	406.2947	[M+H]+	53477513	12-HETE-GABA	2.47	UP	0.02338245	0.018807786	2
156	Negative	untargeted	4.52	181.051	[M-H]-	9378	Hydroxyphenyllactic acid	2.46	UP	0.000591468	0.003412024	2
157	Negative	untargeted	2.43	217.1199	[M-H]-	24443	Pantothenamide	2.46	UP	0.00102916	0.004664752	2
158	Positive & Negative	untargeted	1.98	222.0795	[M+H]+	131770418	N-lactoyl-Methionine	2.41	UP	0.008343076	0.009213461	2
159	Negative	untargeted	0.66	152.0024	[M-H]-	1549098	3-Sulfinoalanine	2.39	DOWN	1.66088E-05	0.000772527	2
160	Positive	untargeted	9	381.181	[M+H]+	44241258	Di-4-coumaroylputrescine	2.39	UP	0.009781578	0.010265329	2

Supplementary Table 4.1. Cont'd.

161	Negative	untargeted	3.55	245.1149	[M-H]-	7015683	gamma-Glutamylvaline	2.39	UP	0.0027322	0.008335333	2
162	Negative	untargeted	5.83	323.1622	[M-H]-	71448979	2,3-Epoxymenaquinone	2.39	DOWN	0.023211118	0.032910875	2
163	Positive	untargeted	0.67	262.103	[M+H]+	13655621	Glutamylasparagine	2.38	UP	0.00025421	0.001141747	2
164	Positive	untargeted	9.14	425.3421	[M+H]+	5282347	Alpha-Tocotrienol	2.37	UP	0.01496913	0.013727435	2
165	Positive	untargeted	4.36	198.1236	[M+H]+	3083620	L-Histidine trimethylbetaine	2.36	DOWN	0.001330864	0.002758482	2
166	Positive	targeted & untargeted	9.68	282.279675	[M+H]+	5283387	Oleamide	2.36	UP	0.004411047	0.005957445	1
167	Negative	untargeted	7.26	487.2376	[M-H]-	459070	7-Sulfocholic acid	2.33	UP	0.022907268	0.032586247	2
168	Positive	untargeted	2.26	152.0568	[M+H]+	135398634	Guanine	2.33	UP	0.015181155	0.013859459	2
169	Positive	untargeted	6.63	330.2639	[M+H]+	53477801	4,8 Dimethylnonanoyl carnitine	2.32	DOWN	0.017228014	0.015142086	2
170	Positive	untargeted	2.26	268.1035	[M+H]+	135398592	Deoxyguanosine	2.31	UP	0.020288229	0.016998731	2
171	Positive	untargeted	8.41	378.2629	[M+H]+	53477510	12-HETE-Gly	2.29	DOWN	0.003240246	0.004821772	2
172	Positive	untargeted	9.31	383.33	[M+H]+	440558	7-Dehydrodesmosterol	2.28	UP	0.002485071	0.004061384	2
173	Negative	untargeted	8.46	337.2388	[M-H]-	5283146	11,12-DiHETrE	2.27	DOWN	0.004906777	0.012104509	2
174	Positive	untargeted	7.68	396.2744	[M+H]+	5283120	PGD2 ethanolamide	2.25	DOWN	0.001941805	0.003472843	2
175	Positive & Negative	untargeted	0.72	249.1083	[M+H]+	53861142	gamma-Glutamylthreonine	2.24	UP	0.00327434	0.004860922	2
176	Negative	untargeted	8.59	363.2156	[M-H]-	5866	Tetrahydrocortisone	2.23	DOWN	0.002762905	0.008374194	2
177	Positive	untargeted	9.92	455.3516	[M+H]+	5951616	Masticadienonic acid	2.22	UP	0.017462307	0.015295642	2
178	Negative	untargeted	0.94	343.1263	[M-H]-	4454759	1-O-alpha-D-Glucopyranosyl-D-mannitol	2.21	DOWN	0.002420617	0.007769802	2
179	Positive & Negative	targeted	5.12	181.0500818	[M+H]+	689043	Caffeic acid	2.21	UP	0.048469284	0.545124357	1
180	Positive	untargeted	8.43	301.2155	[M+H]+	444795	All-trans-retinoic acid	2.21	DOWN	0.000890141	0.00218751	2
181	Negative	untargeted	3.96	389.0733	[M-H]-	85208222	Dimethyl 2-galloylgalactarate	2.21	DOWN	0.004275128	0.011041581	2
182	Negative	untargeted	8.73	345.2441	[M-H]-	5317600	Ginkgoic acid	2.18	DOWN	0.014599265	0.024365327	2
183	Negative	untargeted	8.38	293.2126	[M-H]-	16061062	15(16)-EpODE	2.14	DOWN	0.003059813	0.008938143	2
184	Negative	untargeted	8.38	359.2235	[M-H]-	16061139	Resolvin D5	2.14	DOWN	0.014548012	0.024293707	2
185	Positive & Negative	untargeted	6.57	286.2369	[M+H]+	72348	Myristoylglycine	2.14	DOWN	0.011969893	0.011811574	2
186	Negative	untargeted	5.55	136.077	[M-H]-	5610	Tyramine	2.13	DOWN	0.001114408	0.004931912	2
187	Negative	untargeted	8.25	311.2234	[M-H]-	16061067	12,13-DiHODE	2.12	DOWN	0.006526912	0.014557225	2
188	Positive	targeted	9.38	300.2902392	[M+H]+	4671	Palmitoyl Ethanolamide	2.12	UP	0.030658592	0.023137933	1
189	Positive & Negative	targeted & untargeted	1.97	269.0885912	[M+H]+	135398641	Inosine	2.08	UP	0.015565638	0.014112501	1
190	Negative	untargeted	0.63	209.0308	[M-H]-	3037582	Galactaric acid	2.07	DOWN	0.029429155	0.038663639	2
191	Positive	untargeted	8.8	322.273	[M+H]+	5283449	Alpha-Linolenoyl ethanolamide	2.06	UP	0.018743753	0.016095205	2
192	Negative	targeted & untargeted	1.73	227.0667932	[M-H]-	13712	2-Deoxyuridine	2.06	UP	0.017301559	0.027268898	1
193	Negative	untargeted	8.24	389.2709	[M-H]-	53477693	Nutriacholic acid	2.04	UP	0.02128254	0.030996832	2
194	Positive	untargeted	1.84	137.0457	[M+H]+	790	Hypoxanthine	2.04	UP	0.014196935	0.013247668	2
195	Negative	untargeted	7.27	329.2338	[M-H]-	14968868	9,10,13-TriHOME	2.04	DOWN	0.004017918	0.010664419	2
196	Positive	targeted	4.9	246.1705248	[M+H]+	6426851	Isovaleryl-L-carnitine	2.03	DOWN	0.022209869	0.018091356	1
197	Positive	targeted	4.99	246.1705248	[M+H]+	16226475	Valeryl-L-carnitine	2.02	UP	0.046084936	0.545124357	1
198	Positive	untargeted	8.02	546.3079	[M+H]+	5855402	Americine	2.00	UP	0.015978608	0.014338504	2
199	Positive	untargeted	4.63	300.0959	[M+H]+	135407175	8-Hydroxyguanosine	1.99	DOWN	0.00135082	0.002780529	2
200	Negative	untargeted	2.88	227.1406	[M-H]-	80817	Leucylproline	1.99	DOWN	0.019719391	0.029555082	2

Supplementary Table 4.1. Cont'd.

201	Positive	targeted & untargeted	6.9	190.0867996	[M+H]+	3744	Indole-3-propionic acid	1.96	DOWN	0.007635507	0.008667256	1
202	Positive	untargeted	0.67	191.1028	[M+H]+	439283	Diaminopimelic acid	1.95	UP	0.00661789	0.007884924	2
203	Negative	untargeted	5.69	147.0452	[M-H]-	5372954	Cinnamic acid	1.95	DOWN	0.007842679	0.01630459	2
204	Negative	untargeted	2.87	265.1197	[M-H]-	7010579	Threoninyl-Phenylalanine	1.93	UP	0.021933016	0.031612655	2
205	Negative	untargeted	3.43	211.0617	[M-H]-	4947	Propyl gallate	1.92	DOWN	0.003143752	0.009099563	2
206	Positive	untargeted	2.89	294.1452	[M+H]+	22935675	Phenylalanyl-Glutamine	1.90	UP	0.005459736	0.006896519	2
207	Positive	untargeted	7.42	503.3362	[M+H]+	436072	Medicagenic acid	1.90	UP	0.021899242	0.545124357	2
208	Negative	untargeted	0.64	203.0673	[M-H]-	6427052	Glutamylglycine	1.89	UP	0.001754733	0.00642885	2
209	Positive	untargeted	8.76	480.307	[M+H]+	53480949	LysoPE 18:1	1.88	UP	0.005888256	0.007282278	2
210	Positive	untargeted	2.25	290.0854	[M+H]+	440349	N-Succinyl-2-amino-6-ketopimelate	1.88	UP	0.014290136	0.013299711	2
211	Positive	untargeted	0.63	285.119	[M+H]+	7017195	gamma-Glutamylhistidine	1.88	UP	0.00155149	0.003027104	2
212	Positive	targeted & untargeted	5.16	377.146103	[M+H]+	493570	Riboflavin	1.87	UP	0.008546866	0.009351863	1
213	Positive	untargeted	6.07	163.133	[M+H]+	85279741	(3R,7R)-1,3,7-Octanetriol	1.87	DOWN	7.69246E-07	7.6824E-05	2
214	Negative	untargeted	4.87	183.0302	[M-H]-	85782	3,4-Dihydroxymandelic acid	1.87	UP	0.001382551	0.005590726	2
215	Negative	untargeted	0.63	124.9918	[M-H]-	7866	2-Hydroxyethanesulfonate	1.84	DOWN	0.049572627	0.056436581	2
216	Positive	untargeted	8.26	129.1272	[M+H]+	5364959	(E)-2-Octen-1-ol	1.80	UP	0.000138081	0.000858233	2
217	Negative	untargeted	0.69	100.0406	[M-H]-	535	1-Aminocyclopropanecarboxylic acid	1.80	DOWN	0.002091244	0.007132475	2
218	Positive	targeted	5.24	245.0959836	[M+H]+	171548	Biotin	1.79	DOWN	0.014515452	0.545124357	1
219	Negative	untargeted	0.59	241.0925	[M-H]-	6992830	Histidinyl-Serine	1.76	UP	0.004985542	0.012171536	2
220	Positive & Negative	untargeted	0.93	276.1187	[M+H]+	150914	N2-gamma-Glutamylglutamine	1.75	UP	0.004534474	0.006079853	2
221	Positive	targeted	6.39	176.0711504	[M+H]+	802	Indole-3-acetic acid	1.75	DOWN	0.02361991	0.018971352	1
222	Positive	targeted	6.44	162.0918846	[M+H]+	10685	3-Indoleethanol	1.75	DOWN	0.000496953	0.001593147	1
223	Positive & Negative	targeted	3.44	243.0980924	[M+H]+	5789	Thymidine	1.74	UP	0.042998737	0.030236646	1
224	Positive & Negative	targeted	1.27	245.0773582	[M+H]+	6029	Uridine	1.73	UP	0.013903619	0.013059416	1
225	Negative	untargeted	4.63	138.0561	[M-H]-	255670	Ethyl 2-pyrrolecarboxylate	1.73	DOWN	2.91087E-05	0.000931479	2
226	Positive & Negative	untargeted	1.81	221.0924	[M+H]+	439280	5-Hydroxy-L-tryptophan	1.72	DOWN	0.005501456	0.006930855	2
227	Positive	untargeted	4.15	195.0654	[M+H]+	445858	trans-Ferulic acid	1.71	DOWN	3.46713E-06	0.000161076	2
228	Positive	untargeted	0.73	295.096	[M+H]+	13894653	gamma-L-Glutamyl-L-methionine sulfoxide	1.70	DOWN	0.007844339	0.008826251	2
229	Positive	untargeted	0.61	188.1758	[M+H]+	496	N1-Acetylspermidine	1.69	UP	0.029182063	0.022316308	2
230	Negative	untargeted	4.28	208.062	[M-H]-	3025746	3-Carbamoyl-2-phenylpropionic acid	1.69	DOWN	0.031997693	0.041076652	2
231	Positive	untargeted	1.88	190.1075	[M+H]+	87772907	1-Hydroxyhexanoylglycine	1.67	DOWN	0.048969662	0.033440738	2
232	Negative	targeted	3.71	225.051144	[M-H]-	65124	3-Nitro-L-tyrosine	1.67	DOWN	0.025835336	0.035274462	1
233	Negative	targeted & untargeted	0.72	114.0555004	[M-H]-	145742	L-Proline	1.64	DOWN	0.015197073	0.024983104	1
234	Positive	targeted & untargeted	0.94	348.0709074	[M+H]+	6083	Adenosine 5'-monophosphate	1.64	DOWN	0.004751656	0.545124357	1
235	Negative	untargeted	1.12	166.0181	[M-H]-	159864	N-Acetyltaurine	1.63	DOWN	0.022484282	0.03213437	2
236	Positive	untargeted	3.89	279.1335	[M+H]+	13055260	Tyrosyl-Proline	1.62	UP	0.013934119	0.013077022	2
237	Negative	targeted	1.24	103.0395168	[M-H]-	10413	4-Hydroxybutyric acid (GHB)	1.61	DOWN	0.000422658	0.002789305	1
238	Positive	untargeted	7.67	386.289	[M+H]+	53481679	3-Hydroxy-cis-5-tetradecenoylcarnitine	1.61	DOWN	0.03143511	0.023564103	2
239	Positive	targeted & untargeted	0.58	175.1194954	[M+H]+	6322	L-Arginine	1.60	UP	0.014075375	0.013166437	1
240	Negative	untargeted	5.93	200.1292	[M-H]-	84290	Capryloylglycine	1.60	DOWN	0.00016434	0.001724801	2

Supplementary Table 4.1. Cont'd.

241	Positive	untargeted	9.17	340.2845	[M+H]+	6436908	Oleoyl glycine	1.59	UP	0.026194373	0.020554348	2
242	Positive	untargeted	1.61	117.0546	[M+H]+	49	Alpha-ketoisovaleric acid	1.58	UP	0.02767965	0.021422637	2
243	Negative	untargeted	4.77	186.0775	[M-H]-	194080	2-Keto-6-acetamidocaproate	1.58	UP	0.009420834	0.018303176	2
244	Negative	untargeted	8.81	269.2128	[M-H]-	5282997	3-Oxohexadecanoic acid	1.56	UP	0.028386204	0.037768081	2
245	Positive	targeted	7.51	190.0867996	[M+H]+	74706	Methyl indole-3-acetate	1.56	UP	0.021022673	0.017404687	1
246	Positive	untargeted	8.53	255.2317	[M+H]+	445638	Palmitoleic acid	1.54	UP	0.005764018	0.007169613	2
247	Positive	untargeted	4.37	281.1134	[M+H]+	93078	Aspartylphenylalanine	1.54	UP	0.044406489	0.031042387	2
248	Negative	untargeted	0.65	168.9816	[M-H]-	443233	(S)-3-Sulfonatolactate	1.51	DOWN	0.0059629	0.775109253	2
249	Positive	untargeted	8.07	333.2029	[M+H]+	44263342	11b-Hydroxyprogesterone	1.51	DOWN	0.003362214	0.545124357	2
250	Positive	untargeted	3.84	208.0607	[M+H]+	14033762	Dioxindole-3-acetic acid	1.50	DOWN	0.027451129	0.021280319	2
251	Negative	untargeted	3.66	162.0562	[M-H]-	151066	3-Methyldioxyindole	1.49	DOWN	0.036492641	0.045106194	2
252	Negative	targeted	0.63	88.0398512	[M-H]-	5950	L-Alanine	1.49	DOWN	0.011160624	0.020341877	1
253	Negative	targeted	7.41	187.133412	[M-H]-	5312798	3-Hydroxydecanoic acid	1.49	UP	0.006623555	0.01469599	1
254	Positive	untargeted	0.6	213.0749	[M+H]+	160637	Vanillactic acid	1.47	UP	0.027418917	0.021260287	2
255	Positive	untargeted	1.38	140.0343	[M+H]+	980	4-Nitrophenol	1.46	DOWN	0.027489981	0.021305489	2
256	Positive	untargeted	0.63	74.05995	[M+H]+	215	Aminoacetone	1.46	DOWN	0.023695707	0.545124357	2
257	Positive	untargeted	2.3	195.0766	[M+H]+	2148	4-Aminohippuric acid	1.45	DOWN	0.019455837	0.016550852	2
258	Positive	untargeted	3.22	125.0708	[M+H]+	193545	Methylimidazole acetaldehyde	1.44	DOWN	0.039016865	0.027972017	2
259	Negative	untargeted	0.85	178.018	[M-H]-	1080	S-Carboxymethyl-L-cysteine	1.43	DOWN	0.018840478	0.028856434	2
260	Positive	untargeted	7.89	295.2266	[M+H]+	10469728	13-HOTE	1.39	UP	0.035153098	0.545124357	2
261	Positive	untargeted	3.97	237.1236	[M+H]+	96814	Alanyl-Phenylalanine	1.35	DOWN	0.0470047	0.032437403	2
262	Positive	untargeted	8.7	157.1223	[M+H]+	5283344	4-Hydroxynonenal	1.34	UP	0.049346472	0.033646991	2
263	Positive	untargeted	2.82	279.1017	[M+H]+	7009567	gamma-Glutamylmethionine	1.34	UP	0.024933061	0.01981622	2
264	Positive	targeted	8.79	310.2382066	[M+H]+	35028743	C14:1-Δ9-cis-(L)-HSL	1.32	UP	0.044600855	0.0311261	1
265	Negative	untargeted	2.41	123.0453	[M-H]-	9958	4-Methylcatechol	1.31	DOWN	0.034718177	0.043641767	2
266	Positive	untargeted	2.43	192.0654	[M+H]+	1826	5-Hydroxyindoleacetic acid	1.30	UP	0.042361031	0.029856413	2
267	Negative	untargeted	4.2	110.0248	[M-H]-	12473	Pyrrole-2-carboxylic acid	1.26	DOWN	0.023737469	0.033473077	2
268	Positive	targeted	0.63	104.1075334	M+	305	Choline	1.26	DOWN	0.04488159	0.031269707	1
269	Negative	untargeted	0.74	177.041	[M-H]-	439373	L-Gulonolactone	1.23	DOWN	0.033688318	0.042780948	2

Supplementary Table 4.1. Cont'd.

Supplementary Table 4.2 Unique list of 253 serum metabolites altered in blood sera of PFOA exposure group (1 ppm) vs. control (0 ppm).

	ESI mode	Platform	RT (min)	PRECURSORMZ	PRECURSORTYPE	PubChem_CID	Compound Name (serum)	fold	updown (1/0)	pvalue	qvalue	MSI level
1	Positive	untargeted	1.01	231.1702	[M+H]+	352038	Leucyl-Valine	254.43	UP	0.001782609	0.004724752	2
2	Negative	targeted & untargeted	0.72	114.0555	[M-H]-	145742	L-Proline	141.29	DOWN	0.00597477	0.028886671	1
3	Negative	untargeted	1.25	378.0977	[M-H]-	440018	S-Lactoylglutathione	130.96	DOWN	4.68084E-07	3.33805E-05	2
4	Positive	untargeted	7.59	578.3454	[M+H]+	52926292	PS(22:2(13Z,16Z)/0:0)	82.96	DOWN	0.003844193	0.008515883	2
5	Negative	untargeted	7.8	644.3203	[M-H]-	53481595	S-(11-hydroxy-9-deoxy-delta12-PGD2)-glutathione	79.84	DOWN	6.96558E-05	0.001278463	2
6	Negative	untargeted	4.62	168.0668	[M-H]-	1054	Pyridoxine	69.61	DOWN	0.000180086	0.002438727	2
7	Negative	untargeted	1.01	215.1150	[M-H]-	67427	N-a-Acetyl-L-arginine	61.65	UP	0.001155139	0.008966135	2
8	Positive	untargeted	8.77	363.2190	[M+H]+	657311	Cortisol	58.36	DOWN	0.000750184	0.00243616	2
9	Negative	untargeted	1.26	184.0002	[M-H]-	68841	Phosphoserine	57.77	DOWN	0.008328615	0.036320912	2
10	Negative	untargeted	8.14	351.2174	[M-H]-	5283116	Prostaglandin E2	45.05	DOWN	0.011678294	0.045692961	2
11	Negative	targeted	0.65	182.0123	[M-H]-	177491	Homocysteic acid	39.24	UP	9.53892E-10	5.44199E-07	1
12	Negative	untargeted	6	223.0980	[M-H]-	240452	5-(4-Hydroxy-3-methoxyphenyl)pentanoic acid	37.29	DOWN	0.000685154	0.006131495	2
13	Positive	untargeted	4.79	370.1979	[M+H]+	24779486	PC(7:0/0:0)	34.61	DOWN	0.000566792	0.001979111	2
14	Negative	untargeted	4.28	168.0782	[M-H]-	64969	3-Methylhistidine	33.72	DOWN	0.030211087	0.086574453	2
15	Negative	untargeted	3.22	204.9812	[M-H]-	54110629	Pyrogallol-1-O-sulfate	26.79	UP	0.001105942	0.008704072	2
16	Negative	untargeted	7.9	347.2235	[M-H]-	7835	11b,21-Dihydroxy-5b-pregnane-3,20-dione	26.60	DOWN	0.001778002	0.012099678	2
17	Negative	untargeted	7.81	375.2188	[M-H]-	44251266	Resolvin D1	26.53	DOWN	0.001081693	0.00855712	2
18	Negative	untargeted	6.98	365.1969	[M-H]-	5280877	20-Carboxy-leukotriene B4	25.62	DOWN	0.02804529	0.0817018	2
19	Positive	untargeted	5.95	878.1263	[M+H]+	135398697	S-(5-Hydroxy-2-furoyl)-CoA	23.90	UP	4.13225E-10	6.9396E-08	2
20	Positive	untargeted	7.69	558.3176	[M+H]+	24779232	PC(6:2(3E,5E)/14:2(11E,13E))	22.69	DOWN	0.005095159	0.010392747	2
21	Negative	untargeted	6.19	196.0978	[M-H]-	21100	Metanephrine	21.68	DOWN	0.001673305	0.011618172	2
22	Negative	untargeted	7.25	508.2690	[M-H]-	52926300	PS(17:1(9Z)/0:0)	18.97	DOWN	0.007750643	0.03465789	2
23	Negative	untargeted	1.1	220.0648	[M-H]-	131770418	N-lactoyl-Methionine	17.54	DOWN	0.003508551	0.019853385	2
24	Positive	untargeted	7.38	570.2812	[M+H]+	52926281	PS(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	15.77	DOWN	0.003442981	0.007831713	2
25	Positive	untargeted	1.64	149.0448	[M+H]+	439391	D-2-Hydroxyglutaric acid	15.22	UP	5.39711E-05	0.000310505	2
26	Negative	untargeted	5.93	449.1090	[M-H]-	9889961	Phloretin 2'-O-glucuronide	13.39	UP	0.008207687	0.035973218	2
27	Positive	untargeted	3.41	282.1199	[M+H]+	27476	1-Methyladenosine	13.06	DOWN	8.12565E-06	7.13151E-05	2
28	Positive	untargeted	4.96	264.0534	[M+H]+	3035453	Epinephrine sulfate	12.29	UP	4.28769E-06	4.50041E-05	2
29	Negative	untargeted	7.21	487.2386	[M-H]-	459070	7-Sulfocholic acid	12.10	UP	0.000566494	0.005362716	2
30	Negative	untargeted	5.42	529.2639	[M-H]-	131751607	Cinncassiol D2 glucoside	11.86	DOWN	0.000590898	0.005504308	2
31	Negative	untargeted	5.3	155.0714	[M-H]-	5312943	5-oxo-7-octenoic acid	11.54	DOWN	0.000119251	0.001861948	2
32	Positive	untargeted	5.07	250.1436	[M+H]+	13890535	2-Pentanamido-3-phenylpropanoic acid	11.52	DOWN	0.001621861	0.00442367	2
33	Negative	untargeted	1.25	342.1193	[M-H]-	131752248	N-(1-Deoxy-1-fructosyl)tyrosine	10.57	DOWN	8.33611E-09	2.28277E-06	2
34	Negative	targeted	0.57	145.0977	[M-H]-	5962	L-Lysine	10.51	DOWN	0.018902819	0.063373895	1
35	Positive	untargeted	5.54	201.1120	[M+H]+	9543671	cis-4-Decenedioic acid	10.49	UP	3.6337E-07	7.04117E-06	2
36	Positive	untargeted	9.19	340.2848	[M+H]+	6436908	Oleoyl glycine	10.47	DOWN	0.00365194	0.008177314	2
37	Negative	untargeted	3.99	252.0512	[M-H]-	4520077	N-Salicyloylaspartic acid	9.31	DOWN	0.000229346	0.002938529	2
38	Positive	targeted	0.64	132.0661	[M+H]+	5810	Hydroxy-Proline	9.11	DOWN	0.022102885	0.029706586	1
39	Positive	untargeted	1.56	276.1441	[M+H]+	71317118	Glutarylcarnitine	8.62	DOWN	1.08617E-06	1.63004E-05	2
40	Negative	untargeted	6.81	195.1026	[M-H]-	7733	Isobutyl 2-furanpropionate	8.56	DOWN	0.000510204	0.004996962	2

41	Negative	untargeted	7.83	353.2333	[M-H]-	5283078	Prostaglandin F2a	8.51	DOWN	0.000426887	0.004388122	2
42	Positive	untargeted	1.56	294.1548	[M+H]+	131752725	N-(1-Deoxy-1-fructosyl)isoleucine	8.36	DOWN	4.53453E-06	4.7035E-05	2
43	Positive	untargeted	1.02	234.1332	[M+H]+	53481613	Hydroxypropionylcarnitine	8.30	DOWN	1.08795E-05	8.98565E-05	2
44	Positive & Negative	targeted & untargeted	5.03	180.0661	[M+H]+	464	Hippuric acid	7.82	DOWN	0.000113752	0.000569031	1
45	Positive	untargeted	3.44	291.0975	[M+H]+	4656408	L-N-(1H-Indol-3-ylacetyl)aspartic acid	7.82	DOWN	1.07839E-09	1.22682E-07	2
46	Negative	untargeted	2.52	175.0614	[M-H]-	5280523	2-Isopropylmalic acid	7.74	DOWN	7.39116E-06	0.000233181	2
47	Positive & Negative	untargeted	1.96	326.0873	[M+H]+	124202061	Dihyroxy-1H-indole glucuronide I	7.66	UP	0.002464326	0.006052172	2
48	Positive & Negative	untargeted	3.25	328.1396	[M+H]+	101039148	N-(1-Deoxy-1-fructosyl)phenylalanine	7.39	DOWN	3.77581E-14	3.32903E-11	2
49	Negative	untargeted	3.87	206.0458	[M-H]-	472	4-(2-Aminophenyl)-2,4-dioxobutanoic acid	7.04	DOWN	6.17136E-06	0.000205242	2
50	Positive	untargeted	1.09	312.1102	[M+H]+	10859892	N-(1-Deoxy-1-fructosyl)methionine	6.85	DOWN	2.28866E-05	0.000162167	2
51	Negative	untargeted	3.89	189.0767	[M-H]-	22328017	3-Hydroxysuberic acid	6.26	DOWN	3.90304E-06	0.000153566	2
52	Positive	untargeted	2.42	398.0678	[M+H]+	53481004	5-Taurinomethyl-2-thiouridine	6.18	DOWN	0.003317213	0.00764627	2
53	Positive	untargeted	8.72	295.2262	[M+H]+	13917187	(9S,10E,12Z,15Z)-9-Hydroxy-10,12,15-octadecatrien	6.03	DOWN	0.009118648	0.015998385	2
54	Positive	untargeted	6.65	131.1067	[M+H]+	8094	Heptanoic acid	5.91	UP	0.000177596	0.000797355	2
55	Negative	untargeted	1.22	182.0464	[M-H]-	6723	4-Pyridoxic acid	5.67	DOWN	8.23711E-05	0.001449655	2
56	Negative	untargeted	7.1	369.2281	[M-H]-	5283137	Thromboxane B2	5.51	DOWN	0.001388514	0.01009111	2
57	Negative	untargeted	3.24	236.0932	[M-H]-	11075454	N-lactoyl-Phenylalanine	5.31	DOWN	4.50845E-07	3.28352E-05	2
58	Negative	untargeted	8.2	333.2072	[M-H]-	5288144	Prostaglandin B2	5.16	DOWN	0.001090196	0.008608455	2
59	Negative	untargeted	5.6	116.0507	[M-H]-	798	Indole	4.99	DOWN	0.000240426	0.003005409	2
60	Positive	untargeted	1.72	237.0870	[M+H]+	910	N'-Formylkynurenine	4.95	DOWN	0.004597611	0.009697584	2
61	Negative	untargeted	8.14	349.2034	[M-H]-	5280937	Prostaglandin E3	4.92	DOWN	0.002903617	0.017118417	2
62	Negative	untargeted	7.36	166.0876	[M-H]-	1669	3-Methoxytyramine	4.68	DOWN	0.002064595	0.013538603	2
63	Negative	untargeted	8.4	293.2125	[M-H]-	9839084	9-OxoODE	4.66	DOWN	0.008030772	0.035509549	2
64	Negative	targeted & untargeted	8.65	295.2273	[M-H]-	6443013	(+)13-HODE	4.60	DOWN	0.009837339	0.040603542	1
65	Negative	untargeted	4.15	275.1041	[M-H]-	131770415	N-lactoyl-Tryptophan	4.58	DOWN	0.000117186	0.001850919	2
66	Negative	untargeted	8.75	293.2123	[M-H]-	16061060	9(10)-EpODE	4.26	DOWN	0.014346872	0.052795944	2
67	Positive	untargeted	7.83	319.2263	[M+H]+	5280383	Leukotriene A4	4.22	DOWN	0.001419615	0.003998838	2
68	Negative	targeted	0.9	134.0467	[M-H]-	190	Adenine	4.20	DOWN	0.000100628	0.001656023	1
69	Positive	untargeted	1.03	314.1234	[M+H]+	193088	Tyramine glucuronide	4.02	DOWN	0.000149568	0.000700507	2
70	Negative	untargeted	7.8	367.2125	[M-H]-	53477781	11-Dehydro-thromboxane B2	4.02	DOWN	0.00939324	0.039236272	2
71	Positive	untargeted	6.58	101.0596	[M+H]+	1088	Senecioic acid	3.96	DOWN	8.37993E-14	5.91069E-11	2
72	Positive	untargeted	4.4	177.0913	[M+H]+	5284656	Ethyl cinnamate	3.92	DOWN	0.002103755	0.005368522	2
73	Negative	untargeted	4.67	269.0670	[M-H]-	119239	Phenylglucuronide	3.90	DOWN	0.036923603	0.099207432	2
74	Negative	untargeted	4.19	229.1559	[M-H]-	435949	Isoleucyl-Valine	3.85	UP	5.91573E-10	4.04993E-07	2
75	Negative	targeted	0.91	103.0031	[M-H]-	867	Malonic acid	3.70	UP	2.8984E-06	0.000126386	1
76	Positive	untargeted	8.67	327.2316	[M+H]+	131769928	2-Hydroxydesogestrel	3.69	DOWN	0.001015177	0.003060015	2
77	Positive & Negative	untargeted	1.86	258.1198	[M+H]+	135476770	4a-Hydroxytetrahydrobiopterin	3.54	DOWN	0.001576376	0.004329747	2
78	Negative	targeted	5.7	163.0395	[M-H]-	637542	p-Coumaric Acid	3.54	UP	0.001487038	0.010560501	1
79	Positive	untargeted	6.05	243.1019	[M+H]+	91469	Equol	3.48	UP	0.007290529	0.01359675	2
80	Positive	targeted	6.9	190.0868	[M+H]+	3744	Indole-3-propionic acid	3.46	DOWN	7.92653E-06	7.02373E-05	1

Supplementary Table 4.2. Cont'd.

81	Positive	targeted	0.63	265.1123	[M+H]+	1130	Thiamine	3.44	UP	0.000160359	0.000738148	1
82	Positive & Negative	targeted	0.86	404.0260	[M+H]+	6132	CDP	3.41	DOWN	6.51269E-06	6.03659E-05	1
83	Positive	untargeted	3.6	216.1234	[M+H]+	53481611	Propenoylcarnitine	3.35	DOWN	0.002342088	0.005831773	2
84	Positive	untargeted	2.75	159.0655	[M+H]+	5312	Succinylacetone	3.33	DOWN	0.000797114	0.002557941	2
85	Positive	untargeted	0.66	337.1717	[M+H]+	75046985	N2-Fructopyranosylarginine	3.26	DOWN	1.80653E-06	2.37726E-05	2
86	Positive	targeted	7.74	228.1600	[M+H]+	6914579	N-Octanoyl-L-homoserine lactone	3.23	DOWN	0.010669717	0.017925124	1
87	Positive	untargeted	8.61	504.3080	[M+H]+	53480934	LysoPE(0:0/20:3(5Z,8Z,11Z))	3.20	UP	0.001142876	0.003364418	2
88	Positive	targeted	0.52	89.1079	[M+H]+	1045	Putrescine	3.19	UP	0.021577484	0.02924564	1
89	Positive	targeted	1.26	240.1097	[M+H]+	135402011	7,8-Dihydro-L-Biopterin	3.17	UP	0.000373757	0.001425001	1
90	Negative & Positive	targeted & untargeted	0.66	164.0381	[M-H]-	158980	L-Methionine sulfoxide	3.02	DOWN	0.014252904	0.052544954	1
91	Negative	untargeted	1.01	189.0047	[M-H]-	972	Oxalosuccinic acid	2.99	UP	0.003224577	0.018584187	2
92	Positive	untargeted	1.24	248.1486	[M+H]+	53481617	Hydroxybutyrylcarnitine	2.96	UP	0.001536487	0.004246644	2
93	Positive	untargeted	1.13	259.0919	[M+H]+	445408	Ribothymidine	2.94	DOWN	6.47928E-06	6.03659E-05	2
94	Negative	untargeted	5.6	188.0354	[M-H]-	3845	Kynurenic acid	2.94	DOWN	0.001200762	0.009193252	2
95	Positive	untargeted	3.08	100.0757	[M+H]+	239	2-Piperidinone	2.93	DOWN	0.001873837	0.004931677	2
96	Negative	untargeted	6	174.0562	[M-H]-	802	Indole-3-acetic acid	2.93	DOWN	0.005945402	0.028832942	2
97	Positive & Negative	untargeted	1.27	238.0937	[M+H]+	135449517	Biopterin	2.92	DOWN	0.00437685	0.009343708	2
98	Positive	untargeted	8.08	333.2046	[M+H]+	44263342	11b-Hydroxyprogesterone	2.88	DOWN	0.006850819	0.013056227	2
99	Negative	untargeted	7.21	365.2332	[M-H]-	12444612	3b-Allotetrahydrocortisol	2.85	DOWN	0.00619498	0.029762171	2
100	Positive	untargeted	3.62	203.1388	[M+H]+	7408078	Alanyl-Isoleucine	2.83	UP	0.001001715	0.003032394	2
101	Negative	untargeted	6.6	134.0612	[M-H]-	904	N-Acetylarylamine	2.75	DOWN	0.002853147	0.016926139	2
102	Negative	untargeted	8.35	335.2224	[M-H]-	5283128	Leukotriene B4	2.74	DOWN	0.000428761	0.004397489	2
103	Positive	untargeted	1.64	167.0553	[M+H]+	80220	1-Methylxanthine	2.72	UP	1.76093E-05	0.000131852	2
104	Positive	untargeted	4.46	216.1596	[M+H]+	10176752	N-Nonanoylglycine	2.71	DOWN	0.000875085	0.002750586	2
105	Positive	untargeted	8.39	526.2924	[M+H]+	52925132	LysoPE(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	2.68	UP	0.00130628	0.003745404	2
106	Positive	untargeted	4.42	185.0813	[M+H]+	10805	Vanylglycol	2.60	UP	7.03184E-06	6.42465E-05	2
107	Negative & Positive	targeted & untargeted	6.24	144.0449	[M-H]-	10256	Indole-3-carboxaldehyde	2.54	DOWN	1.2222E-05	0.000343512	1
108	Positive	untargeted	4.11	89.0597	[M+H]+	264	Butyric acid	2.53	DOWN	2.2693E-07	5.21573E-06	2
109	Negative	untargeted	8.42	500.2788	[M-H]-	42607465	LysoPE(20:4(5Z,8Z,11Z,14Z)/0:0)	2.50	UP	8.42447E-05	0.001460109	2
110	Positive & Negative	untargeted	1.3	221.0919	[M+H]+	439280	5-Hydroxy-L-tryptophan	2.44	DOWN	8.64097E-05	0.000458946	2
111	Negative	targeted	4.86	190.0504	[M-H]-	1826	5-Hydroxyindoleacetic acid	2.40	DOWN	5.34292E-05	0.001045082	1
112	Positive & Negative	targeted	5.24	245.0960	[M+H]+	171548	Biotin	2.39	UP	0.001717229	0.004598436	1
113	Negative	untargeted	4.51	355.0681	[M-H]-	25171992	Caffeic acid 3-O-glucuronide	2.37	UP	0.013494343	0.050537944	2
114	Positive	targeted	7.95	242.1756	[M+H]+	56662027	N-Nonanoyl-L-Homoserine lactone	2.36	DOWN	0.00168295	0.004531665	1
115	Positive	untargeted	1.06	188.0914	[M+H]+	194080	2-Keto-6-acetamidocaproate	2.36	DOWN	0.000570623	0.001988551	2
116	Positive & Negative	untargeted	8.43	478.2923	[M+H]+	52925130	LysoPE(18:2(9Z,12Z)/0:0)	2.35	UP	0.006076834	0.011926058	2
117	Negative	targeted & untargeted	0.61	74.0242	[M-H]-	750	L-Glycine	2.34	UP	0.002274601	0.014418534	1
118	Negative	targeted	0.71	308.0982	[M-H]-	445063	N-Acetylneuraminic acid	2.32	DOWN	6.17581E-06	0.000205242	1
119	Negative & Positive	targeted	5.33	160.0399	[M-H]-	97250	2,8-Quinolinediol	2.30	UP	0.002063753	0.013538603	1
120	Negative	untargeted	3.57	149.0244	[M-H]-	11915	Phenylglyoxylic acid	2.29	DOWN	3.28858E-05	0.000723916	2

Supplementary Table 4.2. Cont'd.

121	Negative	untargeted	7.42	228.1605	[M-H]-	1712391	N-Decanoylglycine	2.27	DOWN	0.007646607	0.034327208	2
122	Positive	untargeted	4.19	276.1811	[M+H]+	53481624	Hydroxyhexanoycarnitine	2.27	UP	0.000286767	0.001154837	2
123	Negative	untargeted	3.89	127.0765	[M-H]-	60983	2,3-Heptanedione	2.26	DOWN	3.34771E-06	0.000137237	2
124	Positive	untargeted	8.19	500.2769	[M+H]+	52925146	LysoPE(20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	2.26	UP	0.006910915	0.013124756	2
125	Negative	untargeted	6	218.0462	[M-H]-	76230	8-Methoxykynurenate	2.25	DOWN	0.004478737	0.023713553	2
126	Positive	untargeted	0.96	200.1284	[M+H]+	131802981	2-Octenoylglycine	2.18	UP	0.000622006	0.002115355	2
127	Positive	targeted	2.42	177.1028	[M+H]+	408	Serotonin	2.17	UP	0.008992955	0.015844928	1
128	Positive	targeted & untargeted	7.96	300.2902	[M+H]+	5280335	Sphingosine (d18:1)	2.17	UP	2.94091E-05	0.00019984	1
129	Positive	targeted & untargeted	0.64	258.1106	[M+H]+	71920	sn-Glycero-3-phosphocholine	2.14	DOWN	1.31389E-07	3.30979E-06	1
130	Negative	targeted & untargeted	0.85	87.0082	[M-H]-	1060	Pyruvic Acid	2.13	UP	2.5742E-06	0.000116709	1
131	Positive	targeted	4.29	298.0974	[M+H]+	439176	5'-Deoxy-5'-methylthioadenosine	2.12	DOWN	0.000200346	0.000878805	1
132	Positive	targeted & untargeted	4.99	246.1705	[M+H]+	16226475	Valeryl-L-carnitine	2.11	UP	0.005075092	0.010375331	1
133	Positive	untargeted	6.14	475.0861	[M+H]+	5281764	Chicoric acid	2.10	UP	0.036581785	0.041765203	2
134	Negative	untargeted	4.13	230.9971	[M-H]-	20822599	Vanillin 4-sulfate	2.07	DOWN	0.000941206	0.007744638	2
135	Negative	untargeted	1.24	71.0135	[M-H]-	880	Pyruvaldehyde	2.06	DOWN	0.010944843	0.043690295	2
136	Positive	untargeted	7.09	351.2140	[M+H]+	25063347	Resolvin E1	2.05	DOWN	0.007978874	0.014497184	2
137	Negative & Positive	targeted & untargeted	5.72	160.0399	[M-H]-	54680871	2,4-Quinolinediol	2.04	UP	2.03276E-06	9.77511E-05	1
138	Negative	untargeted	7.72	180.1033	[M-H]-	197139	3-O-Methyl-a-methyldopamine	2.04	DOWN	0.001711711	0.011785207	2
139	Positive	untargeted	8.78	480.3079	[M+H]+	52924041	LysoPE(18:1(9Z)/0:0)	2.02	UP	0.000269016	0.001098074	2
140	Negative	targeted & untargeted	8.82	319.2273	[M-H]-	5280733	5(S)-HETE	2.01	DOWN	0.002317178	0.014607272	1
141	Positive	untargeted	4.7	173.0809	[M+H]+	11805205	cis-4-Octenedioic acid	2.00	UP	0.003822435	0.008478336	2
142	Negative	untargeted	8.34	297.2074	[M-H]-	9543673	8E-Heptadecenedioic acid	1.99	DOWN	0.049340445	0.118813492	2
143	Positive	targeted	2.83	209.0926	[M+H]+	161166	Kynurenine	1.99	DOWN	0.000276334	0.001122845	1
144	Negative	untargeted	0.62	122.9757	[M-H]-	160226	Sulfoacetaldehyde	1.96	DOWN	0.000693679	0.006162782	2
145	Negative	targeted	1.1	191.0192	[M-H]-	311	Citric acid	1.95	DOWN	0.009323044	0.03906117	1
146	Positive	untargeted	8.04	301.2153	[M+H]+	444795	All-trans-retinoic acid	1.94	DOWN	0.030158009	0.036526141	2
147	Negative	untargeted	4.6	203.0926	[M-H]-	131839802	2-hydroxy-7-methyl-Octanedioic acid	1.93	DOWN	0.000591345	0.005504308	2
148	Positive	untargeted	4.41	281.1129	[M+H]+	93078	Aspartylphenylalanine	1.92	UP	0.001574933	0.004329155	2
149	Negative	untargeted	5.35	163.0403	[M-H]-	997	Phenylpyruvic acid	1.91	UP	0.004815443	0.024899339	2
150	Positive	targeted & untargeted	5.16	377.1461	[M+H]+	493570	Riboflavin	1.89	UP	0.006159785	0.012048624	1
151	Positive	targeted	7.51	190.0868	[M+H]+	74706	Methyl indole-3-acetate	1.89	DOWN	0.035995125	0.041228887	1
152	Positive	untargeted	4.59	205.0974	[M+H]+	6305	L-Tryptophan	1.88	DOWN	0.000763172	0.002471508	2
153	Positive	untargeted	4.6	167.1066	[M+H]+	1256	Perillic acid	1.88	DOWN	0.002762305	0.006618071	2
154	Positive	untargeted	5.27	203.1392	[M+H]+	96801	Alanyl-Leucine	1.87	DOWN	0.001784458	0.004724752	2
155	Negative	untargeted	8.64	520.2681	[M-H]-	85036846	1-Oleoylglycerophosphoserine	1.86	UP	0.008715125	0.037453929	2
156	Positive & Negative	targeted	5.38	194.0817	[M+H]+	68144	N-(2-Phenylacetyl)glycine (PAG)	1.83	DOWN	0.001795509	0.004745632	1
157	Positive & Negative	targeted	1.73	229.0824	[M+H]+	13712	2'-Deoxyuridine	1.82	DOWN	2.90708E-07	5.96068E-06	1
158	Negative	untargeted	4.6	141.0924	[M-H]-	104896	4-ene-Valproic acid	1.82	DOWN	0.00028306	0.003306896	2
159	Negative	untargeted	5.25	236.0570	[M-H]-	95664	N-Benzoylaspartic acid	1.82	DOWN	0.016997744	0.059340779	2
160	Negative	untargeted	7.97	329.2324	[M-H]-	14968868	9,10,13-TriHOME	1.81	DOWN	0.037700322	0.10026856	2

Supplementary Table 4.2. Cont'd.

161	Positive	untargeted	5.83	497.1686	[M+H]+	10028772	Mycophenolic acid O-acyl-glucuronide	1.81	UP	0.036849271	0.04192777	2
162	Positive	untargeted	1.17	238.0706	[M+H]+	45782784	Methyl 2,3-dihydro-3,5-dihydroxy-2-oxo-3-indoleac	1.81	DOWN	0.005485878	0.010986375	2
163	Positive & Negative	targeted & untargeted	1.17	130.0504	[M+H]+	7405	L-Pyroglutamic acid	1.81	DOWN	2.04484E-06	2.57555E-05	1
164	Positive & Negative	untargeted	5.87	145.1223	[M+H]+	379	Caprylic acid	1.80	UP	0.00521574	0.010547197	2
165	Positive & Negative	targeted	0.88	118.0868	[M+H]+	6287	L-Valine	1.80	UP	0.000286852	0.001154837	1
166	Negative	untargeted	9.2	301.2170	[M-H]-	446284	Eicosapentaenoic acid	1.80	DOWN	0.006429797	0.030483835	2
167	Positive	untargeted	6.11	163.1329	[M+H]+	85279741	(3R,7R)-1,3,7-Octanetriol	1.78	UP	0.004458147	0.009465691	2
168	Negative	untargeted	5.43	253.0696	[M-H]-	440551	L-Phosphoarginine	1.75	UP	0.001031547	0.008259665	2
169	Negative	untargeted	7.26	157.0872	[M-H]-	173719	3-Oxovalproic acid	1.75	UP	5.27431E-05	0.001038491	2
170	Positive	untargeted	5.61	137.0598	[M+H]+	999	Phenylacetic acid	1.73	DOWN	0.003551381	0.007992744	2
171	Negative	untargeted	6.24	197.0820	[M-H]-	124202116	2,3-Methylene suberic acid	1.73	DOWN	0.008312126	0.036320912	2
172	Positive	targeted	7.8	245.1753	[M+H]+	10458	1,11-Undecanedicarboxylic acid	1.72	UP	0.041958793	0.045926682	1
173	Positive	untargeted	1.37	246.1448	[M+H]+	18218182	Asparaginyl-Leucine	1.72	DOWN	0.00754027	0.013929919	2
174	Negative	targeted & untargeted	0.89	242.0777	[M-H]-	6253	Cytidine	1.72	UP	0.004798606	0.024831035	1
175	Positive	untargeted	1.07	185.0304	[M+H]+	250388	5-Hydroxyisourate	1.72	UP	0.003183207	0.007419823	2
176	Negative	targeted & untargeted	0.97	145.0137	[M-H]-	51	α-Ketoglutaric acid	1.71	UP	0.044595298	0.111627526	1
177	Positive	untargeted	5.45	153.0909	[M+H]+	6435833	2-Propenyl 2,4-hexadienoate	1.71	UP	0.000722102	0.002380524	2
178	Positive	untargeted	3.59	311.1231	[M+H]+	94340	gamma-Glutamyltyrosine	1.71	DOWN	0.046420519	0.049323394	2
179	Positive	targeted	0.61	142.0269	[M+H]+	1015	O-Phosphorylethanolamine	1.70	UP	0.002841237	0.006770386	1
180	Negative	untargeted	1.22	143.0352	[M-H]-	1551553	3-Methylglutaconic acid	1.69	DOWN	0.000722857	0.006312126	2
181	Positive & Negative	untargeted	8.65	454.2911	[M+H]+	9547069	LysoPE(16:0/0:0)	1.69	UP	0.004049277	0.008836977	2
182	Positive	untargeted	5.83	219.1226	[M+H]+	3017884	3-Hydroxysebacic acid	1.69	UP	0.012885473	0.020519659	2
183	Negative	targeted	6.7	186.0555	[M-H]-	5375048	Indole-3-acrylic acid	1.69	DOWN	0.011571019	0.045447894	1
184	Negative	untargeted	8.52	317.2123	[M-H]-	6439678	5-HEPE	1.69	DOWN	0.035155748	0.095941708	2
185	Negative	untargeted	4.92	155.0714	[M-H]-	54042244	8-Hydroxy-5,6-octadienoic acid	1.69	DOWN	0.001941258	0.012977842	2
186	Positive	targeted & untargeted	1.02	124.0399	[M+H]+	938	Nicotinic acid	1.68	DOWN	3.90042E-05	0.000246516	1
187	Positive	untargeted	8.73	285.2211	[M+H]+	6436079	Retinal	1.67	DOWN	0.01618133	0.024038154	2
188	Negative	untargeted	4.43	283.0808	[M-H]-	154035	p-Cresol glucuronide	1.66	UP	0.00037243	0.003952979	2
189	Positive	untargeted	1.13	255.0286	[M+H]+	121947	Shikimic acid 3-phosphate (S3P)	1.66	DOWN	1.00614E-05	8.50919E-05	2
190	Positive & Negative	targeted & untargeted	0.99	204.1236	[M+H]+	7045767	L-Acetylcarnitine	1.65	UP	3.24683E-06	3.68185E-05	1
191	Positive	untargeted	1.01	72.0808	[M+H]+	31268	Pyrrolidine	1.64	UP	1.89179E-05	0.000139285	2
192	Positive	untargeted	7.39	328.2477	[M+H]+	11645581	(9E)-9-nitrooctadecenoic Acid	1.63	DOWN	0.02684188	0.033747971	2
193	Negative	untargeted	1.03	307.0349	[M-H]-	65063	dUMP	1.63	UP	0.001680472	0.011642638	2
194	Positive	untargeted	4.73	254.1026	[M+H]+	57329379	N-lactoyl-Tyrosine	1.63	DOWN	0.004717203	0.009861367	2
195	Negative & Positive	targeted & untargeted	3.44	241.0824	[M-H]-	5789	Thymidine	1.62	DOWN	1.55038E-06	8.22786E-05	1
196	Negative	targeted & untargeted	1.3	166.0140	[M-H]-	1066	Quinolinic acid	1.62	UP	0.006061734	0.029204002	1
197	Positive	untargeted	0.79	248.1126	[M+H]+	22833583	Malonylcarnitine	1.61	UP	0.018453177	0.026156225	2
198	Negative	untargeted	1.47	351.1208	[M-H]-	439179	Cotinine glucuronide	1.61	DOWN	0.007222995	0.032965949	2
199	Positive	untargeted	4.78	147.0441	[M+H]+	323	Coumarin	1.61	UP	2.73018E-05	0.000189165	2
200	Positive & Negative	targeted & untargeted	4.52	224.0923	[M+H]+	68310	N-Acetyl-L-tyrosine	1.60	DOWN	0.010168098	0.017382335	1

Supplementary Table 4.2. Cont'd.

201	Positive	untargeted	6.65	152.1071	[M+H]+	4715025	2-(2-Furanyl)piperidine	1.60	UP	0.000141166	0.000671863	2
202	Negative	targeted	0.73	160.0610	[M-H]-	469	L-2-Aminoadipic acid	1.59	DOWN	0.034277772	0.094585677	1
203	Positive	targeted	0.98	228.0984	[M+H]+	13711	2'-Deoxycytidine (dC)	1.59	DOWN	0.00485676	0.010069546	1
204	Negative	targeted	5.3	131.0708	[M-H]-	10796774	2-Hydroxy-3-methylvaleric acid	1.59	DOWN	0.041943007	0.107392551	1
205	Positive	untargeted	4.84	134.0599	[M+H]+	321710	1,3-Dihydro-(2H)-indol-2-one	1.59	DOWN	0.003449541	0.007833529	2
206	Negative	untargeted	3.46	158.0826	[M-H]-	6991982	3-Dehydrocarnitine	1.57	DOWN	0.001064583	0.008445172	2
207	Negative	untargeted	4.74	252.0882	[M-H]-	12681285	N-Acetylvanilalanine	1.56	DOWN	0.005659293	0.02788186	2
208	Negative	targeted & untargeted	0.7	157.0362	[M-H]-	204	Allantoin	1.56	DOWN	0.001238342	0.00938842	1
209	Negative	targeted & untargeted	8.77	319.2273	[M-H]-	5312983	12-HETE	1.56	DOWN	0.017370519	0.060365126	1
210	Positive	targeted & untargeted	1.06	123.0558	[M+H]+	936	Nicotinamide	1.55	UP	0.000306005	0.001216669	1
211	Positive	untargeted	3.35	127.0503	[M+H]+	1135	Thymine	1.53	DOWN	3.51391E-06	3.93276E-05	2
212	Negative	targeted	1.38	181.0362	[M-H]-	108714	9-Methyluric acid	1.52	UP	0.012311619	0.047421397	1
213	Negative	targeted & untargeted	4.83	212.0022	[M-H]-	10258	3-Indoxyl sulfate	1.52	DOWN	0.016426073	0.058266106	1
214	Negative	untargeted	3.16	243.1354	[M-H]-	61158802	Isoleucyl-Hydroxyproline	1.51	DOWN	0.00308711	0.017971498	2
215	Positive	targeted & untargeted	6.11	162.0555	[M+H]+	69867	Indole-3-carboxylic acid	1.51	DOWN	9.06188E-08	2.55668E-06	1
216	Positive	untargeted	5.65	153.0546	[M+H]+	127	p-Hydroxyphenylacetic acid	1.51	DOWN	0.004731552	0.009885504	2
217	Positive	untargeted	4.66	155.0702	[M+H]+	61166	2-Furanylmethyl propanoate	1.50	UP	0.002196408	0.005536851	2
218	Negative	targeted & untargeted	1.11	135.0307	[M-H]-	790	Hypoxanthine	1.50	UP	0.043965117	0.110413438	1
219	Negative	untargeted	6.31	183.1027	[M-H]-	5312883	5-oxo-7E-decenoic acid	1.49	DOWN	0.000319329	0.003596813	2
220	Negative	untargeted	8.72	387.2166	[M-H]-	4616	3-keto-Digoxigenin	1.49	DOWN	0.011878158	0.046151175	2
221	Positive	untargeted	1.85	218.1384	[M+H]+	107738	Propionylcarnitine	1.48	UP	0.006233271	0.012138504	2
222	Negative	untargeted	5.7	147.0454	[M-H]-	5372954	Cinnamic acid	1.47	DOWN	0.009255671	0.038926704	2
223	Positive	targeted & untargeted	5.97	206.0817	[M+H]+	92904	Indole-3-lactic acid	1.46	DOWN	0.007059731	0.013314172	1
224	Positive	targeted	6.45	244.1549	[M+H]+	66769741	N-3-hydroxyoctanoyl-L-Homoserine lactone	1.45	UP	0.005118905	0.010411076	1
225	Positive	untargeted	5.83	201.1122	[M+H]+	131751116	alpha-Carboxy-delta-nonalactone	1.45	UP	0.01192531	0.019447775	2
226	Negative	targeted & untargeted	1.31	115.0031	[M-H]-	444972	Fumaric acid	1.44	DOWN	0.012708191	0.04854956	1
227	Negative	targeted	4.34	181.0501	[M-H]-	9378	DL-p-Hydroxyphenyllactic acid	1.42	DOWN	0.043725139	0.110093466	1
228	Negative	untargeted	0.8	166.0181	[M-H]-	159864	N-Acetyltaurine	1.42	DOWN	0.010727554	0.042973249	2
229	Positive	targeted & untargeted	0.58	175.1195	[M+H]+	6322	L-Arginine	1.42	DOWN	0.000170058	0.000772453	1
230	Positive	targeted	4.05	172.0974	[M+H]+	10130163	N-Butyryl-L-homoserine lactone	1.41	UP	0.002616234	0.006328294	1
231	Positive	targeted	0.6	129.0664	[M+H]+	11984188	3-Aminopiperidine-2,6-dione	1.40	UP	0.000349435	0.001349783	1
232	Negative	targeted	1.07	147.0293	[M-H]-	439939	α-Hydroxyglutaric acid	1.40	DOWN	0.01859268	0.062836242	1
233	Positive	targeted	0.66	112.0511	[M+H]+	597	Cytosine	1.39	DOWN	0.000724124	0.002384465	1
234	Positive	untargeted	4	242.0107	[M+H]+	124202109	Indole-3-carboxilic acid-O-sulfate	1.38	UP	0.023817911	0.031054204	2
235	Negative	targeted & untargeted	0.76	75.0082	[M-H]-	757	Glycolic acid	1.37	UP	0.013252865	0.050126888	1
236	Positive & Negative	targeted & untargeted	1.35	182.0817	[M+H]+	6057	L-Tyrosine	1.36	DOWN	0.000422456	0.001574918	1
237	Positive	untargeted	0.77	144.1019	[M+H]+	115244	Proline betaine	1.36	UP	0.003471757	0.007877761	2
238	Negative	targeted	0.67	165.0399	[M-H]-	122045	D-Arabinonic acid	1.35	DOWN	0.026050178	0.07781004	1
239	Negative	targeted	1.4	130.0868	[M-H]-	6106	L-Leucine	1.35	DOWN	0.033230697	0.092667513	1
240	Negative	targeted	0.69	135.0293	[M-H]-	5460407	L-Threonic acid	1.34	DOWN	0.04824438	0.117454853	1

Supplementary Table 4.2. Cont'd.

241	Positive	targeted	0.68	118.0868	[M+H]+	247	Betaine	1.33	UP	0.02261039	0.030101886	1
242	Positive	targeted	5.53	138.0555	[M+H]+	227	Anthranilic acid	1.32	DOWN	0.013129326	0.020733599	1
243	Negative	targeted & untargeted	4.06	176.0381	[M-H]-	439750	N-Formyl-L-methionine	1.32	DOWN	0.025565958	0.077035924	1
244	Negative	untargeted	8.3	380.2567	[M-H]-	644260	Sphinganine 1-phosphate	1.30	UP	0.008620419	0.037116825	2
245	Positive	untargeted	1.61	113.0345	[M+H]+	1174	Uracil	1.29	DOWN	4.05577E-05	0.000253607	2
246	Negative	targeted	0.63	88.0399	[M-H]-	5950	L-Alanine	1.27	DOWN	0.018028058	0.061710422	1
247	Negative	untargeted	1.19	253.0936	[M-H]-	656504	Galactosylglycerol	1.27	DOWN	0.025474354	0.076838791	2
248	Negative	untargeted	0.67	174.0885	[M-H]-	9750	Citrulline	1.26	DOWN	0.011780948	0.046008478	2
249	Negative	targeted	1.24	103.0395	[M-H]-	10413	4-Hydroxybutyric acid (GHB)	1.26	UP	0.002997861	0.01752646	1
250	Positive	untargeted	1.06	149.0267	[M+H]+	473	2-Oxo-4-methylthiobutanoic acid	1.25	UP	0.035549415	0.040917709	2
251	Positive	targeted	0.74	189.1239	[M+H]+	192590	Na-Acetyl-lysine	1.24	DOWN	0.000501571	0.001794001	1
252	Positive & Negative	targeted & untargeted	0.69	132.0773	[M+H]+	586	Creatine	1.23	DOWN	0.021801475	0.029436103	1
253	Positive	untargeted	3.88	155.0703	[M+H]+	82755	Hydroxytyrosol	1.23	DOWN	0.026418383	0.033382127	2

Supplementary Table 4.2. Cont'd.

Supplementary Table 4.3 Unique list of 161 serum metabolites altered in cortical brain tissues of PFOA exposure group (1 ppm) vs. control (0 ppm).

	ESI mode	Platform	RT (min)	PRECURSORMZ	PRECURSORTYPE	PubChem_CID	Compound Name (brain)	fold	updown (1ppm/0	pvalue	qvalue	MSI level
1	Positive & Negative	targeted	1.03	308.0916	[M+H]+	124886	L-Glutathione reduced (GSH)	320.40	UP	0.004284655	0.018127112	1
2	Positive & Negative	untargeted	3.06	499.1167	[M+H]+	440072	N,N'-Bis(gamma-glutamyl)cystine	31.68	DOWN	0.000724572	0.004842537	2
3	Positive	untargeted	1.47	336.0732	[M+H]+	24771767	Dihydroneopterin phosphate	19.64	DOWN	5.57155E-05	0.000677032	2
4	Negative	untargeted	4.82	206.0459	[M-H]-	472	4-(2-Aminophenyl)-2,4-dioxobutanoic acid	18.90	UP	0.000118708	0.001119711	2
5	Positive	untargeted	5.37	215.1390	[M+H]+	445027	Dethiobiotin	16.49	DOWN	0.000165612	0.001599736	2
6	Positive	untargeted	4.34	239.0701	[M+H]+	171386	Cystathionine sulfoxide	12.00	DOWN	8.29904E-07	3.41812E-05	2
7	Negative	untargeted	0.91	132.0303	[M-H]-	5960	L-Aspartic acid	10.63	UP	0.004018696	0.017221199	2
8	Negative & Positive	targeted & untargeted	0.89	175.0243	[M-H]-	54670067	L-Ascorbic acid	10.38	UP	1.02776E-05	0.000182031	1
9	Negative	untargeted	0.72	143.0462	[M-H]-	24141	N-Nitrosoproline	9.12	DOWN	0.020512246	0.06253728	2
10	Negative & Positive	untargeted	3.57	275.1248	[M-H]-	160556	Saccharopine	8.83	DOWN	0.001041955	0.005801001	2
11	Positive	untargeted	1.07	248.0315	[M+H]+	1051	Pyridoxal 5'-phosphate	8.43	UP	1.48658E-07	1.19464E-05	2
12	Negative	targeted & untargeted	1.31	173.0086	[M-H]-	643757	cis-Aconitic acid	8.27	UP	4.1082E-08	3.81657E-06	1
13	Positive	untargeted	9.6	563.2673	[M+H]+	4971	Protoporphyrin IX	7.60	UP	0.003103127	0.014095267	2
14	Negative	targeted	0.7	168.0661	[M-H]-	439260	L-(-)-Norepinephrine	7.48	UP	9.90213E-07	3.93193E-05	1
15	Positive	untargeted	4.39	344.1155	[M+H]+	74346580	p-Coumaroyl 3-hydroxytyrosine	7.34	DOWN	0.011766468	0.040808803	2
16	Negative	untargeted	1.06	154.9985	[M-H]-	76720	2,5-Furandicarboxylic acid	7.34	UP	1.52996E-06	5.26796E-05	2
17	Positive & Negative	untargeted	0.86	232.0814	[M+H]+	440848	N2-Succinyl-L-glutamic acid 5-semialdehyde	7.17	DOWN	0.001535309	0.008371741	2
18	Positive	targeted	1.12	154.0868	[M+H]+	681	Dopamine	7.02	UP	0.001474967	0.008126355	1
19	Positive	untargeted	9.5	447.3099	[M+H]+	73946406	24-Oxo-1alpha,23,25-trihydroxyvitamin D3	7.00	UP	0.00129447	0.007350232	2
20	Negative	untargeted	8.77	317.2123	[M-H]-	5283162	12-KETE	6.49	UP	9.93867E-05	0.000992523	2
21	Positive & Negative	targeted & untargeted	0.66	166.0538	[M+H]+	158980	L-Methionine sulfoxide	6.35	DOWN	3.57961E-05	0.00049272	1
22	Negative	untargeted	1.23	301.0559	[M-H]-	122553541	Pyrogallol-2-O-glucuronide	5.89	DOWN	0.003374389	0.014941308	2
23	Positive	untargeted	5.85	217.1545	[M+H]+	409682	Valyl-Valine	5.53	DOWN	0.000633549	0.004406948	2
24	Negative	untargeted	0.74	558.0646	[M-H]-	192	Adenosine diphosphate ribose	5.31	UP	0.014961921	0.048702037	2
25	Negative	untargeted	4.88	258.1460	[M-H]-	22935676	Isoleucyl-Glutamine	4.93	DOWN	0.000109568	0.001069378	2
26	Negative	untargeted	0.9	168.0431	[M-H]-	151438	Phosphodimethylethanolamine	4.79	UP	2.25541E-08	2.46781E-06	2
27	Negative	untargeted	0.7	105.0192	[M-H]-	439194	Glyceric acid	4.65	DOWN	7.88809E-06	0.000149382	2
28	Positive	untargeted	9.47	362.2678	[M+H]+	5283389	N-Arachidonoyl glycine	4.40	UP	0.001897539	0.009796082	2
29	Positive	untargeted	9.64	303.2319	[M+H]+	446284	Eicosapentaenoic acid	4.38	UP	0.002646704	0.012551204	2
30	Positive & Negative	targeted & untargeted	1.03	150.0589	[M+H]+	6137	L-Methionine	4.05	UP	5.6773E-08	6.38141E-06	1
31	Negative	untargeted	0.74	144.0665	[M-H]-	207	Allysine	3.98	DOWN	1.06439E-07	8.06282E-06	2
32	Positive & Negative	untargeted	3.65	170.0812	[M+H]+	1054	Pyridoxine	3.90	DOWN	0.000515295	0.003775441	2
33	Negative & Positive	targeted & untargeted	4.45	190.0538	[M-H]-	6180	N-Acetyl-L-methionine	3.89	UP	2.77968E-06	7.47244E-05	1
34	Negative	untargeted	1.49	120.0122	[M-H]-	5862	L-Cysteine	3.86	UP	4.35926E-07	2.10431E-05	2
35	Negative	targeted	0.73	191.0556	[M-H]-	6508	D-(-)-Quinic acid	3.82	UP	0.000120825	0.001135335	1
36	Negative	untargeted	1.6	411.0115	[M-H]-	439488	dIDP	3.77	DOWN	0.004624552	0.01918303	2
37	Positive	untargeted	4.74	361.0892	[M+H]+	5281792	Rosmarinic acid	3.76	DOWN	0.005572385	0.022189464	2
38	Positive	untargeted	1.13	338.0656	[M+H]+	53477721	Nicotinic acid mononucleotide	3.67	DOWN	0.007371573	0.027808959	2
39	Negative	untargeted	3.49	341.0852	[M-H]-	101579718	Vanillic acid-4-O-glucuronide	3.64	DOWN	0.001164509	0.00637087	2
40	Negative	untargeted	0.65	263.0215	[M-H]-	3035420	3-Methoxy-4-Hydroxyphenylglycol sulfate	3.48	DOWN	9.09169E-07	3.69962E-05	2

41	Negative	untargeted	1.37	172.0616	[M-H]-	192878	N-Acetyl-L-glutamate 5-semialdehyde	3.46	DOWN	0.000828226	0.00486635	2
42	Positive & Negative	targeted & untargeted	1.66	268.1046	[M+H]+	60961	Adenosine	3.46	DOWN	0.013265022	0.045233148	1
43	Negative	untargeted	3.07	125.0241	[M-H]-	74710	5-Methylfuran-2-carboxylic acid	3.44	UP	1.835E-06	5.7709E-05	2
44	Positive	untargeted	9.53	331.2830	[M+H]+	123409	MG(0:0/16:0/0:0)	3.38	UP	8.59526E-05	0.0009384	2
45	Positive	untargeted	9.07	324.2891	[M+H]+	5283446	Linoleoyl ethanolamide	3.37	UP	0.000769303	0.005034625	2
46	Positive	untargeted	0.74	230.0957	[M+H]+	5351619	Ergothioneine	3.29	UP	0.006936575	0.02657334	2
47	Negative	untargeted	1.09	364.0812	[M-H]-	11954071	2-S-Glutathionyl acetate	3.27	DOWN	0.002979399	0.013385252	2
48	Negative	untargeted	3.39	203.0923	[M-H]-	131839802	2-Hydroxy-7-methyl-octanedioic acid	3.16	DOWN	0.008190121	0.029787073	2
49	Positive	untargeted	0.56	126.1026	[M+H]+	3614	1-Methylhistamine	3.13	UP	0.002335169	0.011466293	2
50	Positive	untargeted	4.22	334.1399	[M+H]+	100094	Glutamyltryptophan	3.11	UP	0.00426821	0.018073	2
51	Negative	untargeted	4.75	170.0820	[M-H]-	13999	N-Butyryl-L-homoserine lactone	3.09	DOWN	0.001634257	0.008366969	2
52	Negative	untargeted	0.76	85.0293	[M-H]-	650	Diacetyl	3.00	UP	2.18244E-08	2.46781E-06	2
53	Negative	untargeted	3.01	157.0507	[M-H]-	5280533	Isopropylmaleate	2.96	DOWN	0.002210674	0.01054735	2
54	Positive	targeted	0.77	141.0664	[M+H]+	75810	1-Methyl-4-imidazoleacetic acid	2.85	UP	7.09106E-05	0.00080807	1
55	Negative	untargeted	7.39	351.2175	[M-H]-	5280360	Prostaglandin E2	2.84	UP	2.979E-06	7.82152E-05	2
56	Negative	untargeted	0.63	174.0403	[M-H]-	65065	N-Acetyl-L-aspartic acid	2.80	UP	0.01706834	0.05390679	2
57	Positive & Negative	untargeted	1.54	202.0709	[M+H]+	13943174	N-acetyl-L-2-aminoadipate(2-)	2.76	DOWN	0.001419435	0.007913164	2
58	Negative	targeted & untargeted	0.76	75.0082	[M-H]-	757	Glycolic acid	2.75	DOWN	2.79243E-06	7.47244E-05	1
59	Positive	targeted & untargeted	9.5	326.3059	[M+H]+	5283454	Oleoyl ethanolamide	2.73	UP	0.002534744	0.012182771	1
60	Negative	targeted	5.03	178.0504	[M-H]-	464	Hippuric acid	2.73	DOWN	0.000126917	0.001172438	1
61	Positive	untargeted	10.04	340.2841	[M+H]+	6436908	Oleoyl glycine	2.73	UP	0.012926601	0.04432377	2
62	Positive	untargeted	3.38	282.1192	[M+H]+	27476	1-Methyladenosine	2.70	DOWN	0.000460075	0.003446643	2
63	Positive	untargeted	8.18	424.3425	[M+H]+	53477834	Linoelaidyl carnitine	2.69	UP	0.001614406	0.008697556	2
64	Positive	untargeted	9.92	244.1898	[M+H]+	454092	N-Undecanoylglycine	2.67	UP	0.010166126	0.03617162	2
65	Positive & Negative	untargeted	9.47	305.2468	[M+H]+	444899	Arachidonic acid	2.65	UP	0.000109218	0.001139577	2
66	Positive	untargeted	8.17	448.3419	[M+H]+	6710116	N-Stearoyl tyrosine	2.64	UP	0.000304377	0.002534268	2
67	Positive	targeted	5.97	206.0817	[M+H]+	92904	Indolelactic acid	2.64	DOWN	0.005104757	0.020710885	1
68	Negative	targeted	0.66	103.0031	[M-H]-	964	β-Hydroxy pyruvic acid	2.61	DOWN	5.10285E-09	8.10493E-07	1
69	Positive	untargeted	10.06	385.3454	[M+H]+	5280795	Vitamin D3	2.61	UP	0.001439493	0.007990226	2
70	Positive	untargeted	7.75	344.2791	[M+H]+	168381	Dodecanoylcarnitine	2.53	UP	0.00342074	0.015159438	2
71	Negative	untargeted	2.88	156.0665	[M-H]-	169485	3-Methylcrotonylglycine	2.51	DOWN	0.000672553	0.004088687	2
72	Negative	targeted & untargeted	0.67	165.0399	[M-H]-	122045	D-Arabinonic acid	2.44	DOWN	2.66847E-08	2.8563E-06	1
73	Positive	targeted	0.63	265.1123	[M+H]+	1130	Thiamine	2.42	UP	3.03619E-07	1.87701E-05	1
74	Negative	targeted	0.91	103.0031	[M-H]-	867	Malonic acid	2.35	DOWN	0.004654155	0.019257157	1
75	Positive	untargeted	8.33	426.3584	[M+H]+	46907933	Oleoylcarnitine	2.32	UP	0.000281483	0.00237286	2
76	Positive	targeted	1.51	138.0919	[M+H]+	5610	Tyramine	2.31	DOWN	0.005419829	0.02165171	1
77	Positive	untargeted	0.65	198.0891	[M+H]+	75619	N-Acetylhistidine	2.30	DOWN	0.021590286	0.066436759	2
78	Negative	untargeted	2.24	147.0660	[M-H]-	449	Mevalonic acid	2.29	DOWN	0.001882615	0.009297469	2
79	Positive	untargeted	10.34	319.2624	[M+H]+	92786	Allopregnolone	2.27	UP	0.005390037	0.021553557	2
80	Positive	untargeted	0.96	168.0653	[M+H]+	1050	Pyridoxal	2.26	UP	7.15092E-08	7.52474E-06	2

Supplementary Table 4.3. Cont'd.

81	Positive	untargeted	6.3	161.0596	[M+H]+	7092	6-Methylcoumarin	2.24	DOWN	0.006307573	0.024505366	2
82	Positive	targeted & untargeted	0.49	146.1657	[M+H]+	1102	Spermidine	2.24	UP	0.002959601	0.013679705	1
83	Positive	untargeted	1.12	188.0556	[M+H]+	133503	1-(Malonylamino)cyclopropanecarboxylic acid	2.22	DOWN	0.000542902	0.003914033	2
84	Negative	untargeted	1.15	127.0037	[M-H]-	4161539	5-Hydroxy-2-furoic acid	2.20	UP	0.043834994	0.112686354	2
85	Positive	targeted & untargeted	8.26	400.3427	[M+H]+	11953816	Palmitoyl-L-carnitine	2.18	UP	0.000992871	0.006015621	1
86	Negative	untargeted	3	183.0299	[M-H]-	85782	3,4-Dihydroxymandelic acid	2.15	DOWN	0.001809506	0.009017816	2
87	Positive	targeted & untargeted	0.66	114.0667	[M+H]+	588	Creatinine	2.13	DOWN	1.51195E-07	1.19464E-05	1
88	Negative	untargeted	6.01	245.1391	[M-H]-	16663321	3-Hydroxydodecanedioic acid	2.08	DOWN	0.006927663	0.026029438	2
89	Negative	untargeted	2.96	627.1205	[M-H]-	3289289	CMP-N-glycoloyl-beta-neuramite(2-)	2.05	UP	0.003627775	0.015891774	2
90	Negative	untargeted	8.64	289.1805	[M-H]-	5355130	Octyl 4-methoxycinmic acid	2.05	UP	0.004843983	0.01989215	2
91	Positive	untargeted	8.86	518.3207	[M+H]+	52924045	LysoPC(18:3(6Z,9Z,12Z))	2.04	UP	0.004184344	0.017763509	2
92	Negative	untargeted	0.71	130.9986	[M-H]-	970	Oxalacetic acid	2.02	DOWN	0.000226685	0.001797336	2
93	Positive	untargeted	10.34	310.3093	[M+H]+	247220	N-Hexadecanoylpyrrolidine	2.00	UP	0.001668542	0.008950215	2
94	Positive	untargeted	8.67	496.3383	[M+H]+	460602	LysoPC(16:0)	1.98	UP	0.004521199	0.018869595	2
95	Positive	untargeted	3.17	655.1731	[M+H]+	11954072	2-(S-Glutathionyl)acetyl glutathione	1.98	DOWN	0.000313638	0.002585266	2
96	Negative	untargeted	0.76	426.0225	[M-H]-	6022	ADP	1.95	DOWN	0.000774133	0.00460345	2
97	Positive & Negative	untargeted	9.36	329.2461	[M+H]+	445580	Docosahexaenoic acid	1.94	UP	0.002492449	0.012019727	2
98	Positive	untargeted	7.25	163.0392	[M+H]+	5281426	7-Hydroxycoumarin	1.92	DOWN	7.01652E-05	0.000804389	2
99	Positive & Negative	untargeted	5.86	145.1222	[M+H]+	379	Caprylic acid	1.91	DOWN	0.001861408	0.009663772	2
100	Positive	untargeted	1.33	261.1438	[M+H]+	4524287	gamma-Glutamylleucine	1.89	DOWN	0.037761393	0.103753504	2
101	Positive	untargeted	1.83	232.1294	[M+H]+	18218188	Asparaginyl-Valine	1.89	UP	0.002167746	0.010829301	2
102	Positive	untargeted	1.8	218.1388	[M+H]+	107738	Propionylcarnitine	1.87	DOWN	0.004352966	0.018259914	2
103	Positive	untargeted	7.67	250.1778	[M+H]+	3451347	N,O-Didesmethylvenlafaxine	1.87	UP	0.044530289	0.118023853	2
104	Negative	targeted	0.87	191.0192	[M-H]-	1198	Isocitric acid	1.86	UP	5.0355E-09	8.10493E-07	1
105	Negative	untargeted	5.33	240.1357	[M-H]-	131057	Leuhistine	1.86	DOWN	0.036637356	0.09852947	2
106	Positive	untargeted	0.69	203.1502	[M+H]+	169148	Symmetric dimethylarginine	1.86	UP	9.50535E-06	0.00017943	2
107	Positive & Negative	untargeted	1.12	259.0926	[M+H]+	445408	Ribothymidine	1.84	DOWN	0.026397259	0.078913746	2
108	Negative	untargeted	5.58	187.0973	[M-H]-	2266	Azelaic acid	1.84	DOWN	0.005956933	0.023186249	2
109	Negative	targeted	5.38	192.0661	[M-H]-	68144	N-(2-Phenylacetyl)glycine (PAG)	1.83	DOWN	0.003919428	0.016913571	1
110	Positive	targeted	7.96	300.2902	[M+H]+	5280335	Sphingosine (d18:1)	1.82	UP	0.005365213	0.021517808	1
111	Negative	untargeted	4.56	171.0662	[M-H]-	11805205	cis-4-Octenedioic acid	1.82	DOWN	0.004212642	0.017835	2
112	Positive	targeted	5.16	377.1461	[M+H]+	493570	Riboflavin	1.80	UP	4.73713E-06	0.000111464	1
113	Negative	targeted	0.69	135.0293	[M-H]-	5460407	L-Threonic acid	1.80	DOWN	3.40573E-06	8.38453E-05	1
114	Negative	untargeted	1.22	143.0347	[M-H]-	1551553	3-Methylglutaconic acid	1.79	DOWN	0.006733206	0.025529809	2
115	Negative	targeted & untargeted	7.52	229.1440	[M-H]-	12736	Dodecanedioic acid	1.79	DOWN	0.004877482	0.020013025	1
116	Positive	untargeted	8.29	289.1798	[M+H]+	5756	Estriol	1.78	UP	0.001765974	0.009281583	2
117	Positive	targeted	9.38	300.2902	[M+H]+	4671	Palmitoyl Ethanolamide	1.78	UP	0.005160811	0.020904006	1
118	Positive	untargeted	8.51	520.3391	[M+H]+	11005824	LysoPC(18:2(9Z,12Z))	1.77	UP	0.005166697	0.020910726	2
119	Positive & Negative	untargeted	8.84	438.2980	[M+H]+	42607469	PE(P-16:0e/0:0)	1.76	UP	0.007888307	0.029465386	2
120	Positive	untargeted	8.91	572.3724	[M+H]+	52924039	LysoPC(22:4(7Z,10Z,13Z,16Z))	1.75	UP	0.010710229	0.037727579	2

Supplementary Table 4.3. Cont'd.

121	Negative	targeted	0.86	173.0450	[M-H]-	8742	Shikimate	1.75	DOWN	0.027294787	0.078317844	1
122	Negative	untargeted	1.26	231.0145	[M-H]-	54454045	Acetyl citrate	1.73	DOWN	0.03489629	0.095086618	2
123	Negative	untargeted	5.28	209.0456	[M-H]-	14124	Vanilpyruvic acid	1.73	UP	3.33807E-05	0.000431388	2
124	Positive	targeted	0.52	89.1079	[M+H]+	1045	Putrescine	1.70	UP	0.01263549	0.043487314	1
125	Negative	targeted & untargeted	8.77	319.2273	[M-H]-	5312983	12-HETE	1.68	UP	0.009822435	0.03471893	1
126	Negative	untargeted	1.57	298.0695	[M-H]-	41635	D-4'-Phosphopantothete	1.67	UP	0.005159395	0.02079123	2
127	Negative	untargeted	4.85	212.0020	[M-H]-	10258	Indoxyl sulfate	1.66	DOWN	0.001960999	0.009588399	2
128	Negative & Positive	targeted & untargeted	1.17	128.0348	[M-H]-	7405	L-Pyroglutamic acid	1.66	DOWN	0.00235094	0.01113029	1
129	Negative	untargeted	0.78	119.0349	[M-H]-	150929	(S)-3,4-Dihydroxybutyric acid	1.65	DOWN	0.005546064	0.021933798	2
130	Positive	untargeted	8.41	544.3392	[M+H]+	24779476	LysoPC(20:4(5Z,8Z,11Z,14Z))	1.65	UP	0.027259704	0.080621146	2
131	Positive	targeted	9.28	280.2640	[M+H]+	6435901	Linoleamide	1.65	UP	0.012183612	0.041990538	1
132	Negative	untargeted	1.13	226.0118	[M-H]-	193475	L-Glutamic acid 5-phosphate	1.65	DOWN	0.001186581	0.006420286	2
133	Positive	untargeted	5.36	253.0951	[M+H]+	65058	Deoxyinosine	1.63	DOWN	0.02116581	0.065465684	2
134	Positive	untargeted	7.89	304.1752	[M+H]+	53481699	Pimelylcarnitine	1.62	UP	8.93196E-08	8.65107E-06	2
135	Negative	untargeted	7.9	333.2074	[M-H]-	5280885	Delta-12-Prostaglandin J2	1.61	DOWN	0.021852007	0.066130541	2
136	Negative	untargeted	4.72	252.0874	[M-H]-	57329379	N-Lactoyl-Tyrosine	1.58	DOWN	0.019604569	0.060439588	2
137	Negative	untargeted	4.06	340.1162	[M-H]-	12114857	Phosphatidylcholine 40:6	1.58	UP	0.02272585	0.067952863	2
138	Positive	untargeted	0.99	403.0269	[M+H]+	164628	dTDP	1.58	UP	1.15143E-06	4.01029E-05	2
139	Positive	untargeted	1.11	352.1240	[M+H]+	440197	N-Acetyl-4-O-acetylneuraminic acid	1.55	DOWN	0.000213876	0.001905883	2
140	Negative	untargeted	5.43	224.0564	[M-H]-	3083688	Vanilloylglycine	1.54	DOWN	0.015870333	0.050940005	2
141	Positive	targeted	0.99	110.0606	[M+H]+	11568	3-Aminophenol	1.53	DOWN	0.012861777	0.044173901	1
142	Positive	untargeted	8.33	494.3231	[M+H]+	24779461	LysoPC(16:1(9Z))	1.47	UP	0.030085644	0.087116158	2
143	Negative	targeted	0.98	662.1013	[M-H]-	5893	β-Nicotinamide adenine dinucleotide (NAD)	1.46	UP	0.022986412	0.068510863	1
144	Negative & Positive	targeted & untargeted	0.86	323.0280	[M-H]-	6030	Uridine 5'-monophosphate (UMP)	1.45	DOWN	0.039519257	0.103722803	1
145	Negative	targeted	10	299.2586	[M-H]-	439887	2-Hydroxy stearic acid	1.44	UP	0.007571357	0.027945778	1
146	Positive	targeted	0.66	291.1305	[M+H]+	16950	Argininosuccinic acid	1.44	DOWN	0.015843079	0.052063142	1
147	Positive	targeted & untargeted	0.98	349.0549	[M+H]+	8582	Inosine 5'-monophosphate (IMP)	1.44	DOWN	0.035474776	0.098676828	1
148	Positive	targeted	0.78	175.1083	[M+H]+	70923	N-epsilon-Formyl-lysine	1.43	UP	0.000465125	0.003464406	1
149	Positive	targeted	0.81	146.1181	M+	187	Acetylcholine	1.41	UP	0.002014363	0.010281137	1
150	Negative & Positive	targeted & untargeted	1.97	267.0729	[M-H]-	6021	Inosine	1.41	UP	0.000626893	0.003877741	1
151	Negative	untargeted	0.73	236.0235	[M-H]-	169591	S-Cysteinosuccinic acid	1.39	DOWN	0.019302364	0.059698819	2
152	Positive	targeted	3.44	243.0981	[M+H]+	5789	Thymidine	1.38	DOWN	0.047027258	0.123366958	1
153	Positive & Negative	targeted	1.21	613.1598	[M+H]+	65359	L-Glutathione oxidized (GSSG)	1.35	DOWN	0.034253869	0.096132766	1
154	Negative	untargeted	5.83	236.0924	[M-H]-	11075454	N-Lactoyl-Phenylalanine	1.34	DOWN	0.04960218	0.123473222	2
155	Positive	targeted	0.65	118.0616	[M+H]+	763	Guanidineacetic acid	1.34	DOWN	0.006255222	0.02437858	1
156	Positive	targeted	4.99	246.1705	[M+H]+	16226475	Valeryl-L-carnitine	1.33	DOWN	0.027584486	0.081156715	1
157	Negative	untargeted	4.65	182.0457	[M-H]-	6723	4-Pyridoxic acid	1.33	DOWN	0.014049601	0.046458757	2
158	Negative	untargeted	1.84	135.0313	[M-H]-	790	Hypoxanthine	1.32	UP	0.023348401	0.069254398	2
159	Negative	targeted	0.65	146.0453	[M-H]-	33032	L-Glutamic acid	1.25	UP	0.004104806	0.017485148	1
160	Positive	targeted	0.88	148.0610	[M+H]+	65249	N-Acetyl-serine	1.23	UP	0.0408152	0.110065159	1
161	Negative	targeted	0.9	134.0467	[M-H]-	190	Adenine	1.22	UP	0.003647058	0.015933714	1

Supplementary Table 4.3. Cont'd.

Supplementary Table 4.4 PICRUSt2 predicted metagenomic pathways (fold change ≥ 3, q-value < 0.01).

#OTU ID	Source	Pathway	fold change	updown (1ppm	pvalue	qvalues
P241-PWY	MetaCyc	coenzyme B biosynthesis	Unlimited	UP	7.11851E-06	5.21196E-05
PWY-2201	MetaCyc	folate transformations I	Unlimited	UP	0.003164511	0.002114756
PWY-2221	MetaCyc	Entner-Doudoroff pathway III	Unlimited	UP	0.001792973	0.001420264
PWY-3941	MetaCyc	β-alanine biosynthesis II	Unlimited	UP	0.001772502	0.00141198
PWY-5519	MetaCyc	D-arabinose degradation III	Unlimited	UP	0.003164511	0.002114756
PWY-6143	MetaCyc	CMP-pseudaminate biosynthesis	Unlimited	UP	0.003152591	0.002114756
PWY-6486	MetaCyc	D-galacturonate degradation II	Unlimited	UP	0.003164517	0.002114756
PWY-6581	MetaCyc	spirilloxanthin and 2,2'-diketo-spirilloxanthin biosynthesis	Unlimited	UP	0.003164664	0.002114756
PWY-6654	MetaCyc	phosphopantothenate biosynthesis III (archaea)	Unlimited	UP	0.003163777	0.002114756
PWY-6660	MetaCyc	2-heptyl-3-hydroxy-4(1H)-quinolone biosynthesis	Unlimited	UP	0.003161542	0.002114756
PWY-6662	MetaCyc	quinolone and alkylquinolone biosynthesis	Unlimited	UP	0.003163138	0.002114756
PWY-6713	MetaCyc	L-rhamnose degradation II	Unlimited	UP	0.015604225	0.009054223
PWY-6728	MetaCyc	methylaspartate cycle	Unlimited	UP	0.014932007	0.008736076
PWY-6760	MetaCyc	D-xylose degradation III	Unlimited	UP	0.003164197	0.002114756
PWY-6992	MetaCyc	1,5-anhydrofructose degradation	Unlimited	UP	0.001042721	0.000875133
PWY-7209	MetaCyc	pyrimidine ribonucleosides degradation	Unlimited	UP	0.003162133	0.002114756
PWY-7385	MetaCyc	1,3-propanediol biosynthesis	Unlimited	UP	0.002696702	0.001939957
PWY-7528	MetaCyc	L-methionine salvage cycle I (bacteria and plants)	Unlimited	UP	0.003118101	0.002114756
PWY-5178	MetaCyc	toluene degradation IV (aerobic) (via catechol)	384.7827008	UP	0.002242248	0.001708942
PWY-7031	MetaCyc	protein N-glycosylation (bacterial)	147.9582577	UP	0.000358848	0.000346556
CENTBENZCOA-PWY	MetaCyc	benzoyl-CoA degradation II (anaerobic)	33.94163155	UP	0.003428481	0.002258932
PWY-5430	MetaCyc	meta cleavage pathway of aromatic compounds	7.90110429	UP	0.002009078	0.001565069
PWY0-321	MetaCyc	phenylacetate degradation I (aerobic)	7.317927784	UP	0.003702452	0.002372914
HCAMHPDEG-PWY	MetaCyc	3-phenylpropanoate degradation to 2-hydroxypentadienoate	6.601691614	UP	0.000116011	0.000151458
PWY-6690	MetaCyc	cinnamate and 3-hydroxycinnamate degradation to 2-hydroxypentadienoate	6.601691614	UP	0.000116011	0.000151458
PWY-7391	MetaCyc	isoprene biosynthesis II	6.584419792	UP	0.00604152	0.003785986
DENITRIFICATION-PWY	MetaCyc	nitrate reduction I (denitrification)	6.254711139	UP	0.008709897	0.005270749
PWY-6071	MetaCyc	phenylethylamine degradation	6.1453306	UP	0.00258866	0.001881432
PWY0-1277	MetaCyc	3-phenylpropanoate degradation	5.831606019	UP	8.13667E-05	0.000127464
PWY-1541	MetaCyc	taurine degradation	5.687968627	DOWN	6.87138E-05	0.000121107
PWY-3661	MetaCyc	glycine betaine degradation I	5.68504817	UP	0.008625829	0.005242375
KETOGLUCONMET-PWY	MetaCyc	ketogluconate metabolism	4.91412148	DOWN	6.72676E-05	0.000121107
PWY-6383	MetaCyc	mono-trans, poly-cis decaprenyl phosphate biosynthesis	4.806578652	UP	0.002887095	0.002045612
PWY1G-0	MetaCyc	mycothiol biosynthesis	4.368564342	UP	0.003579187	0.002335432
PWY-181	MetaCyc	photorespiration	4.143722278	UP	0.008326046	0.005126472

PWY-5941	MetaCyc	glycogen degradation II	4.006597884	DOWN	4.68252E-05	0.000111251
PWY-1622	MetaCyc	formaldehyde assimilation I (serine pathway)	3.909363769	UP	0.009329986	0.005597942
VALDEG-PWY	MetaCyc	L-valine degradation I	3.643055333	DOWN	0.004287171	0.002735229
ARGDEG-PWY	MetaCyc	L-arginine, putrescine, and 4-aminobutanoate degradation	3.632263964	DOWN	0.000155742	0.000189306
ORNARGDEG-PWY	MetaCyc	L-arginine and L-ornithine degradation	3.632263964	DOWN	0.000155742	0.000189306
ORNDEG-PWY	MetaCyc	ornithine degradation	3.625396883	DOWN	0.000171964	0.000200386
KDO-NAGLIPASYN-PWY	MetaCyc	(Kdo)2-lipid A biosynthesis	3.620521145	DOWN	0.000164429	0.000196477
PWY-4361	MetaCyc	S-methyl-5-thio-α-D-ribose 1-phosphate degradation I	3.569901797	DOWN	1.03777E-08	3.00442E-07
PWY-7527	MetaCyc	L-methionine salvage cycle III	3.552723397	DOWN	1.22699E-08	3.00442E-07
PWY-722	MetaCyc	nicotinate degradation I	3.497982283	DOWN	9.65736E-05	0.000136272
PWY-5532	MetaCyc	nucleoside and nucleotide degradation (archaea)	3.495875916	DOWN	0.000127077	0.000164383
TYRFUMCAT-PWY	MetaCyc	L-tyrosine degradation I	3.447565391	UP	0.00093084	0.000795438
PWY-5677	MetaCyc	succinate fermentation to butanoate	3.391856738	DOWN	3.03982E-06	3.297E-05
LPSSYN-PWY	MetaCyc	lipopolysaccharide biosynthesis	3.384787544	DOWN	0.000486019	0.000442117
PWY-7185	MetaCyc	UTP and CTP dephosphorylation I	3.287246273	DOWN	7.43312E-05	0.000124099
PWY-622	MetaCyc	starch biosynthesis	3.285459956	DOWN	7.47433E-05	0.000124099
PWY0-1338	MetaCyc	polymyxin resistance	3.281269234	DOWN	0.003700885	0.002372914
PWY-3801	MetaCyc	sucrose degradation II (sucrose synthase)	3.23878471	DOWN	7.06008E-05	0.000121398
P221-PWY	MetaCyc	octane oxidation	3.236018199	DOWN	0.000260682	0.00026443
PWY-5415	MetaCyc	catechol degradation I (meta-cleavage pathway)	3.135761343	DOWN	6.62988E-06	5.21196E-05
UBISYN-PWY	MetaCyc	ubiquinol-8 biosynthesis (early decarboxylation)	3.120679776	DOWN	0.000248667	0.000259717
PWY-5855	MetaCyc	ubiquinol-7 biosynthesis (early decarboxylation)	3.106360048	DOWN	0.000238181	0.000250621
PWY-5856	MetaCyc	ubiquinol-9 biosynthesis (early decarboxylation)	3.106360048	DOWN	0.000238181	0.000250621
PWY-5857	MetaCyc	ubiquinol-10 biosynthesis (early decarboxylation)	3.106360048	DOWN	0.000238181	0.000250621
PWY-6708	MetaCyc	ubiquinol-8 biosynthesis (early decarboxylation)	3.106360048	DOWN	0.000238181	0.000250621
PWY-7090	MetaCyc	UDP-2,3-diacetamido-2,3-dideoxy-α-D-mannuronate biosynthesis	3.081971956	DOWN	3.74207E-05	0.000107312
PWY-6142	MetaCyc	gluconeogenesis II (Methanobacterium thermoautotrophicum)	3.071546365	DOWN	1.42572E-05	6.70082E-05
PWY-7013	MetaCyc	(S)-propane-1,2-diol degradation	3.020237933	DOWN	1.68154E-09	1.18548E-07

Supplementary Table 4.4. Cont'd.

Supplementary Table 4.5 ChemRICH results for fecal metabolome changes on compound class level (adjusted p-value<0.05).

	Cluster name	Cluster size	p-values	FDR	Key compound	Altered metabolites	Increased	Decreased	Increased ratio	Altered Ratio
1	dipeptides	44	2.2E-20	7.5E-19	Glycylleucine	44	38	6	0.9	1
2	NewCluster_11	6	4.4E-16	7.5E-15	Leucyl-Serine	6	6	0	1	1
3	oleic acids	10	3.6E-14	4E-13	12-HETE-Gly	10	9	1	0.9	1
4	cholic acids	8	7.6E-14	6.4E-13	Taurocholic acid 3-sulfate	8	6	2	0.8	1
5	UnSaturated FA	9	1.7E-12	1.2E-11	Docosahexaenoic acid (DHA)	9	8	1	0.9	1
6	oligopeptides	7	2.4E-12	1.4E-11	Aspartyl-Leucine	7	7	0	1	1
7	Saturated_fatty acids	6	2.9E-12	1.4E-11	Stearoylethanolamide	6	3	3	0.5	1
8	DiHOME	5	1.2E-11	5E-11	Ricinoleic acid	5	2	3	0.4	1
9	purine nucleosides	6	7E-11	2.4E-10	8-Hydroxyguanosine	6	5	1	0.8	1
10	cholestenes	6	7E-11	2.4E-10	25-Hydroxyvitamin D2	6	5	1	0.8	1
11	indoles	7	8.6E-11	2.7E-10	3-Indoleethanol	7	1	6	0.1	1
12	triterpenes	6	1.1E-10	3.1E-10	7-Dehydrodesmosterol	6	6	0	1	1
13	phenols	7	3.6E-10	9.3E-10	Hydroxyphenyllactic acid	7	4	3	0.6	1
14	carnitine	7	4.4E-10	1.1E-09	cis-5-Tetradecenoylcarnitine	7	5	2	0.7	1
15	butyrates	3	4.7E-10	1.1E-09	3-Sulfinoalanine	3	0	3	0	1
16	lipids	4	4.9E-09	0.00000001	MG(0:0/22:4(7Z,10Z,13Z,16Z)/0:0)	4	3	1	0.8	1
17	Saturated_lysophospholipids	4	0.000000011	0.000000022	LysoPE(14:0/0:0)	4	3	1	0.8	1
18	OH-FA_18_0_1	3	0.000000022	0.000000041	2(R)-Hydroxydocosanoic acid	3	3	0	1	1
19	hydroxybenzoates	4	0.000000045	0.000000081	2-Hydroxy-3,4,5-trimethoxybenzoic acid	4	1	3	0.2	1
20	porphyrins	3	0.00000014	0.00000024	Protoporphyrin IX	3	3	0	1	1
21	pyrimidine nucleosides	5	0.00000015	0.00000024	?-Pseudouridine	5	4	1	0.8	1
22	lysophosphatidylcholines	3	0.00000045	0.0000007	LysoPC(20:5(5Z,8Z,11Z,14Z,17Z))	3	3	0	1	1
23	acids, carbocyclic	4	0.000001	0.0000015	3,4-Dihydroxyphenylacetone	4	3	1	0.8	1
24	isoflavones	3	0.0000015	0.0000021	Umbelliferone	3	2	1	0.7	1
25	OH-FA_22_6_2	3	0.0000031	0.0000042	12-HETE	3	0	3	0	1
26	indoleacetic acids	4	0.0000032	0.0000042	5-Hydroxy-L-tryptophan	4	2	2	0.5	1
27	purinones	3	0.0000035	0.0000044	Uric acid	3	3	0	1	1
28	amino alcohols	4	0.0000041	0.0000049	Sphingosine (d18:1)	4	3	1	0.8	1
29	cinnamates	4	0.0000055	0.0000065	trans-Ferulic acid	4	2	2	0.5	1
30	quinolones	3	0.0000087	0.0000098	2,4-Quinolinediol	3	2	1	0.7	1
31	amino acids, cyclic	3	0.000011	0.000012	L-Histidine trimethylbetaine	3	0	3	0	1
32	aminobenzoates	3	0.00011	0.00011	4-Aminohippuric acid	3	0	3	0	1
33	imino acids	3	0.00012	0.00012	N6-Acetyl-L-lysine	3	1	2	0.3	1
34	alkanesulfonic acids	3	0.00012	0.00012	Taurocholic acid	3	0	3	0	1

Supplementary Table 4.6 Quantitative pathway enrichment analysis (qMSEA) results for fecal metabolome changes.

	Pathways (feces)	Total Cmpd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR	Enrichment fold
1	Tryptophan Metabolism	60	6	31.2	5.2632	2.6796E-08	1.5542E-06	1.5542E-06	5.927952576
2	Arachidonic Acid Metabolism	69	4	49.286	5.2632	0.00001168	0.00066577	0.00033872	9.364265086
3	Alanine Metabolism	17	3	33.841	5.2632	0.000035019	0.0019611	0.00050778	6.429738562
4	Glutamate Metabolism	49	3	33.841	5.2632	0.000035019	0.0019611	0.00050778	6.429738562
5	Selenoamino Acid Metabolism	28	2	34.254	5.2632	0.000082845	0.0044736	0.000961	6.508207934
6	Amino Sugar Metabolism	33	1	51.236	5.2632	0.00038684	0.020503	0.0025737	9.734762122
7	Bile Acid Biosynthesis	65	5	32.654	5.2632	0.00038882	0.020503	0.0025737	6.204210366
8	Alpha Linolenic Acid and Linoleic Acid Metabolism	19	5	48.672	5.2632	0.00039248	0.020503	0.0025737	9.247606019
9	Retinol Metabolism	37	1	51.071	5.2632	0.00039937	0.020503	0.0025737	9.703412373
10	Steroidogenesis	43	2	39.87	5.2632	0.00050613	0.0248	0.0027402	7.575239398
11	Fatty acid Metabolism	43	2	34.675	5.2632	0.00051969	0.024945	0.0027402	6.588197294
12	Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	27	2	43.889	5.2632	0.00074789	0.035151	0.0034086	8.338843289
13	Fatty Acid Biosynthesis	35	5	45.77	5.2632	0.00076399	0.035151	0.0034086	8.69623043
14	Riboflavin Metabolism	20	2	35.99	5.2632	0.001351	0.060793	0.0055363	6.838045296
15	Pyrimidine Metabolism	59	4	26.519	5.2632	0.0015948	0.070171	0.0055363	5.038569691
16	Steroid Biosynthesis	48	1	42.662	5.2632	0.0017927	0.077086	0.0055363	8.105715154
17	Ammonia Recycling	32	2	36.75	5.2632	0.0018136	0.077086	0.0055363	6.98244414
18	Propanoate Metabolism	42	2	36.75	5.2632	0.0018136	0.077086	0.0055363	6.98244414
19	Pyruvate Metabolism	48	2	36.75	5.2632	0.0018136	0.077086	0.0055363	6.98244414
20	Sphingolipid Metabolism	40	1	40.13	5.2632	0.0027117	0.10575	0.0066364	7.624639003
21	Phenylacetate Metabolism	9	2	37.647	5.2632	0.0028754	0.10926	0.0066364	7.152872777
22	Histidine Metabolism	43	3	30.145	5.2632	0.0030649	0.1134	0.0066364	5.72750418
23	Phenylalanine and Tyrosine Metabolism	28	1	38.233	5.2632	0.0036615	0.13181	0.0066364	7.264211886
24	Cysteine Metabolism	26	1	38.233	5.2632	0.0036615	0.13181	0.0066364	7.264211886
25	Pantothenate and CoA Biosynthesis	21	1	38.233	5.2632	0.0036615	0.13181	0.0066364	7.264211886
26	Pentose Phosphate Pathway	29	1	38.233	5.2632	0.0036615	0.13181	0.0066364	7.264211886
27	Nicotinate and Nicotinamide Metabolism	37	1	38.233	5.2632	0.0036615	0.13181	0.0066364	7.264211886
28	Butyrate Metabolism	19	1	38.233	5.2632	0.0036615	0.13181	0.0066364	7.264211886
29	Thiamine Metabolism	9	1	38.233	5.2632	0.0036615	0.13181	0.0066364	7.264211886
30	Ethanol Degradation	19	1	38.233	5.2632	0.0036615	0.13181	0.0066364	7.264211886
31	Mitochondrial Beta-Oxidation of Medium Chain Saturated Fatty Acids	27	1	38.233	5.2632	0.0036615	0.13181	0.0066364	7.264211886
32	Mitochondrial Beta-Oxidation of Long Chain Saturated Fatty Acids	28	1	38.233	5.2632	0.0036615	0.13181	0.0066364	7.264211886
33	Purine Metabolism	74	4	32.329	5.2632	0.0041014	0.13181	0.0072086	6.14246086
34	Vitamin K Metabolism	14	1	36.184	5.2632	0.0050204	0.13181	0.0085642	6.874905001
35	Methionine Metabolism	43	3	26.344	5.2632	0.0054406	0.13181	0.0090159	5.005319957

36	Spermidine and Spermine Biosynthesis	18	1	32.731	5.2632	0.0083931	0.19304	0.013522	6.218840249
37	Glutathione Metabolism	21	1	30.895	5.2632	0.010939	0.24065	0.015865	5.87000304
38	Glucose-Alanine Cycle	13	1	30.895	5.2632	0.010939	0.24065	0.015865	5.87000304
39	Glycine and Serine Metabolism	59	4	29.253	5.2632	0.012549	0.25098	0.015865	5.558025536
40	Urea Cycle	29	3	29.329	5.2632	0.013261	0.25196	0.015865	5.57246542
41	Beta-Alanine Metabolism	34	1	29.482	5.2632	0.013363	0.25196	0.015865	5.601535188
42	Methylhistidine Metabolism	4	1	29.482	5.2632	0.013363	0.25196	0.015865	5.601535188
43	Arginine and Proline Metabolism	53	3	29.321	5.2632	0.013408	0.25196	0.015865	5.570945432
44	Aspartate Metabolism	35	2	29.321	5.2632	0.013517	0.25196	0.015865	5.570945432
45	Citric Acid Cycle	32	1	29.317	5.2632	0.013676	0.25196	0.015865	5.570185439
46	Biotin Metabolism	8	1	29.317	5.2632	0.013676	0.25196	0.015865	5.570185439
47	Gluconeogenesis	35	1	29.317	5.2632	0.013676	0.25196	0.015865	5.570185439
48	Threonine and 2-Oxobutanoate Degradation	20	1	29.317	5.2632	0.013676	0.25196	0.015865	5.570185439
49	Transfer of Acetyl Groups into Mitochondria	22	1	29.317	5.2632	0.013676	0.25196	0.015865	5.570185439
50	Warburg Effect	58	1	29.317	5.2632	0.013676	0.25196	0.015865	5.570185439
51	Valine, Leucine and Isoleucine Degradation	60	2	25.42	5.2632	0.0168	0.25196	0.019105	4.829761362
52	Phospholipid Biosynthesis	29	2	24.572	5.2632	0.018948	0.25196	0.021135	4.668642651
53	Tyrosine Metabolism	72	3	24.211	5.2632	0.025499	0.25196	0.027904	4.6000532
54	Oxidation of Branched Chain Fatty Acids	26	2	23.491	5.2632	0.02987	0.25196	0.031853	4.463254294
55	Beta Oxidation of Very Long Chain Fatty Acids	17	2	23.504	5.2632	0.030206	0.25196	0.031853	4.465724274
56	Betaine Metabolism	21	1	20.672	5.2632	0.044004	0.25196	0.044004	3.927648579
57	Phosphatidylcholine Biosynthesis	14	1	20.672	5.2632	0.044004	0.25196	0.044004	3.927648579
58	Phosphatidylethanolamine Biosynthesis	12	1	20.672	5.2632	0.044004	0.25196	0.044004	3.927648579

Supplementary Table 4.6. Cont'd.

Supplementary Table 4.7 ChemRICH results for fecal metabolome changes for individual metabolite assignment.

	Cluster name	Cluster size	p-values	FDR	Key compound	Altered metabolites	Increased	Decreased	Increased ratio	Altered Ratio
1	pyrimidine nucleosides	8	2.2E-20	7.7E-19	2'-Deoxyuridine	8	2	6	0.2	1
2	dipeptides	12	1.1E-16	1.3E-15	Isoleucyl-Valine	12	6	6	0.5	1
3	indoles	10	1.1E-16	1.3E-15	Indole-3-carboxylic acid	10	2	8	0.2	1
4	amino acids	10	1.7E-15	1.5E-14	4-(2-Aminophenyl)-2,4-dioxobutanoic acid	10	3	7	0.3	1
5	amino acids, aromatic	4	3.4E-13	2.4E-12	5-Hydroxy-L-tryptophan	4	0	4	0	1
6	phenols	9	5.8E-13	3.4E-12	Vanylglycol	9	3	6	0.3	1
7	dicarboxylic acids	8	1.9E-12	9.5E-12	Malonic acid	8	2	6	0.2	1
8	pyridines	5	8.2E-12	3.6E-11	Nicotinic acid	5	2	3	0.4	1
9	heterocyclic compounds	4	1.8E-11	7.1E-11	Epinephrine sulfate	4	1	3	0.2	1
10	NewCluster_15	5	5E-11	1.8E-10	PS(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	5	2	3	0.4	1
11	oligopeptides	6	3.7E-10	1.1E-09	N-(1-Deoxy-1-fructosyl)phenylalanine	6	0	6	0	1
12	pyrimidinones	3	3.8E-10	1.1E-09	Thymine	3	0	3	0	1
13	quinolines	4	4E-10	1.1E-09	2,4-Quinolinediol	4	2	2	0.5	1
14	acids, carbocyclic	4	5.4E-10	1.3E-09	Phenylglyoxylic acid	4	1	3	0.2	1
15	carnitine	4	1.5E-09	3.5E-09	L-Acetylcarnitine	4	3	1	0.8	1
16	phosphatidylethanolamines	4	2.3E-09	0.000000005	LysoPE(20:4(5Z,8Z,11Z,14Z)/0:0)	4	4	0	1	1
17	imino acids	5	5.3E-09	0.000000011	L-Pyroglutamic acid	5	1	4	0.2	1
18	glutarates	6	7.9E-09	0.000000015	Glutarylcarnitine	6	2	4	0.3	1
19	methionine	5	0.000000011	0.00000002	Homocysteic acid	5	1	4	0.2	1
20	lactones	4	0.000000013	0.000000023	N-Nonanoyl-L-Homoserine lactone	4	2	2	0.5	1
21	O=FA_10_2	6	0.000000014	0.000000024	cis-4-Decenedioic acid	6	2	4	0.3	1
22	NewCluster_6	4	0.00000012	0.00000018	Hydroxypropionylcarnitine	4	3	1	0.8	1
23	lysophosphatidylcholines	3	0.00000013	0.0000002	LysoPE(18:1(9Z)/0:0)	3	1	2	0.3	1
24	pteridines	3	0.00000023	0.00000034	7,8-Dihydro-L-Biopterin	3	2	1	0.7	1
25	Saturated_fatty acids	3	0.00000045	0.00000063	Heptanoic acid	3	1	2	0.3	1
26	EpODE	3	0.00000083	0.0000011	Thromboxane B2	3	0	3	0	1
27	HETE	4	0.0000015	0.000002	Resolvin D1	4	0	4	0	1
28	isoprostanes	3	0.0000016	0.000002	Prostaglandin F2a	3	0	3	0	1
29	indoleacetic acids	4	0.0000017	0.000002	L-N-(1H-Indol-3-ylacetyl)aspartic acid	4	0	4	0	1
30	cinnamates	4	0.0000018	0.0000021	p-Coumaric Acid	4	2	2	0.5	1
31	hippurates	3	0.0000049	0.0000055	Hippuric acid	3	0	3	0	1
32	butyrates	4	0.0000054	0.0000059	Senecioic acid	4	1	3	0.2	1
33	DiHETE	3	0.000022	0.000023	Leukotriene B4	3	0	3	0	1
34	HODE	3	0.000054	0.000055	(9S,10E,12Z,15Z)-9-Hydroxy-10,12,15-octadecatrienoic acid	3	0	3	0	1
35	purinones	3	0.000085	0.000085	1-Methylxanthine	3	3	0	1	1

Supplementary Table 4.8 Quantitative pathway enrichment analysis (qMSEA) results for cortical brain metabolome changes.

	Pathway (brain)	Total Cr	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR	Enrichment
1	Citric Acid Cycle	32	5	89.234	5.2632	1.4391E-13	1.2808E-11	1.2808E-11	16.95432437
2	Vitamin B6 Metabolism	20	4	74.548	5.2632	9.1967E-12	8.0931E-10	4.0925E-10	14.16400669
3	Taurine and Hypotaurine Metabolism	12	2	88.506	5.2632	1.8559E-11	1.6146E-09	4.1294E-10	16.81600547
4	Homocysteine Degradation	9	2	88.506	5.2632	1.8559E-11	1.6146E-09	4.1294E-10	16.81600547
5	Starch and Sucrose Metabolism	31	3	61.39	5.2632	4.0457E-09	3.4388E-07	6.0011E-08	11.66400669
6	Threonine and 2-Oxobutanoate Degradation	20	3	61.39	5.2632	4.0457E-09	3.4388E-07	6.0011E-08	11.66400669
7	Spermidine and Spermine Biosynthesis	18	4	84.098	5.2632	6.3562E-09	5.2756E-07	8.0814E-08	15.97849217
8	Sphingolipid Metabolism	40	2	70.329	5.2632	7.3974E-09	6.0658E-07	8.2296E-08	13.3624031
9	Porphyrin Metabolism	40	2	67.088	5.2632	1.5435E-08	1.2503E-06	1.5264E-07	12.74661803
10	Glycerolipid Metabolism	25	4	77.169	5.2632	9.6329E-08	7.7064E-06	8.5733E-07	14.6619927
11	Thiamine Metabolism	9	2	69.424	5.2632	1.3589E-07	0.000010736	1.0995E-06	13.19045448
12	Glycine and Serine Metabolism	59	9	62.778	5.2632	2.1334E-07	0.000016641	1.5823E-06	11.92772458
13	Catecholamine Biosynthesis	20	4	75.676	5.2632	3.4071E-07	0.000026235	2.3325E-06	14.37832497
14	Pterine Biosynthesis	29	2	58.946	5.2632	4.2078E-07	0.000031979	0.000002513	11.1996504
15	Oxidation of Branched Chain Fatty Acids	26	3	75.818	5.2632	4.6415E-07	0.000034812	0.000002513	14.40530476
16	Carnitine Synthesis	22	3	76.242	5.2632	4.7133E-07	0.000034879	0.000002513	14.48586411
17	Phytanic Acid Peroxisomal Oxidation	26	3	76.12	5.2632	4.8001E-07	0.000035041	0.000002513	14.4626843
18	Tyrosine Metabolism	72	11	60.028	5.2632	5.586E-07	0.000040219	0.000002762	11.40522876
19	Steroid Biosynthesis	48	3	47.706	5.2632	3.9502E-06	0.00028047	0.000018504	9.064067487
20	Pyruvate Metabolism	48	3	48.569	5.2632	8.9987E-06	0.00062991	0.000036404	9.228036176
21	Gluconeogenesis	35	3	48.569	5.2632	8.9987E-06	0.00062991	0.000036404	9.228036176
22	Transfer of Acetyl Groups into Mitochondria	22	3	48.569	5.2632	8.9987E-06	0.00062991	0.000036404	9.228036176
23	Glutathione Metabolism	21	4	42.144	5.2632	0.000029088	0.0019489	0.00011256	8.007295942
24	Nucleotide Sugars Metabolism	20	2	45.233	5.2632	0.000048129	0.0031765	0.00013818	8.594201246
25	Inositol Metabolism	33	2	45.233	5.2632	0.000048129	0.0031765	0.00013818	8.594201246
26	Bile Acid Biosynthesis	65	2	45.233	5.2632	0.000048129	0.0031765	0.00013818	8.594201246
27	Glycolysis	25	2	45.233	5.2632	0.000048129	0.0031765	0.00013818	8.594201246
28	Galactose Metabolism	38	2	45.233	5.2632	0.000048129	0.0031765	0.00013818	8.594201246
29	Fructose and Mannose Degradation	32	2	45.233	5.2632	0.000048129	0.0031765	0.00013818	8.594201246
30	Mitochondrial Electron Transport Chain	19	2	45.233	5.2632	0.000048129	0.0031765	0.00013818	8.594201246
31	Inositol Phosphate Metabolism	26	2	45.233	5.2632	0.000048129	0.0031765	0.00013818	8.594201246
32	Pantothenate and CoA Biosynthesis	21	2	56.913	5.2632	0.000065482	0.003798	0.00018212	10.81338349
33	Beta Oxidation of Very Long Chain Fatty Acids	17	2	47.009	5.2632	0.00011885	0.0067744	0.00032053	8.931638547
34	Riboflavin Metabolism	20	2	55.211	5.2632	0.00012256	0.0068632	0.00032081	10.49000608
35	Warburg Effect	58	5	49.411	5.2632	0.00020978	0.011538	0.00046468	9.388014896

36	Pentose Phosphate Pathway	29	1	54.101	5.2632	0.00021929	0.011842	0.00046468	10.27910777
37	Sulfate/Sulfite Metabolism	22	1	54.101	5.2632	0.00021929	0.011842	0.00046468	10.27910777
38	Lactose Degradation	9	1	54.101	5.2632	0.00021929	0.011842	0.00046468	10.27910777
39	Phosphatidylinositol Phosphate Metabolism	17	1	54.101	5.2632	0.00021929	0.011842	0.00046468	10.27910777
40	Trehalose Degradation	11	1	54.101	5.2632	0.00021929	0.011842	0.00046468	10.27910777
41	Phosphatidylcholine Biosynthesis	14	1	54.101	5.2632	0.00021929	0.011842	0.00046468	10.27910777
42	Phosphatidylethanolamine Biosynthesis	12	1	54.101	5.2632	0.00021929	0.011842	0.00046468	10.27910777
43	Alpha Linolenic Acid and Linoleic Acid Metabolism	19	3	47.972	5.2632	0.00029906	0.014056	0.00061899	9.114607083
44	Aspartate Metabolism	35	8	39.318	5.2632	0.00050196	0.02309	0.0010153	7.470360237
45	Arginine and Proline Metabolism	53	8	40.463	5.2632	0.00067875	0.030544	0.0013271	7.687908497
46	Urea Cycle	29	7	40.5	5.2632	0.00071709	0.031552	0.0013271	7.69493844
47	Glutamate Metabolism	49	7	40.566	5.2632	0.00071747	0.031552	0.0013271	7.70747834
48	Fatty Acid Biosynthesis	35	2	43.751	5.2632	0.00072212	0.031552	0.0013271	8.312623499
49	Ammonia Recycling	32	5	40.505	5.2632	0.00073169	0.031552	0.0013271	7.695888433
50	Malate-Aspartate Shuttle	10	5	40.482	5.2632	0.00075076	0.031552	0.0013271	7.691518468
51	Beta-Alanine Metabolism	34	4	40.443	5.2632	0.00076048	0.031552	0.0013271	7.684108527
52	Fatty acid Metabolism	43	2	45.644	5.2632	0.00084943	0.032278	0.0014538	8.672290622
53	Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	27	2	41.114	5.2632	0.00089653	0.033171	0.0015055	7.811597507
54	Estrone Metabolism	24	2	36.727	5.2632	0.00095385	0.034339	0.0015721	6.978074175
55	Purine Metabolism	74	10	34.924	5.2632	0.0012135	0.042471	0.0019636	6.635506916
56	Histidine Metabolism	43	6	40.263	5.2632	0.0020323	0.069097	0.0032298	7.649908801
57	Methionine Metabolism	43	7	38.013	5.2632	0.0020793	0.069097	0.0032467	7.222412221
58	Betaine Metabolism	21	3	37.974	5.2632	0.0021257	0.069097	0.0032619	7.21500228
59	Valine, Leucine and Isoleucine Degradation	60	5	40.357	5.2632	0.002327	0.072136	0.0035102	7.667768658
60	Alanine Metabolism	17	4	39.299	5.2632	0.0029481	0.088442	0.0040693	7.466750266
61	Cysteine Metabolism	26	5	39.24	5.2632	0.0029507	0.088442	0.0040693	7.455540356
62	Propanoate Metabolism	42	4	39.21	5.2632	0.0029726	0.088442	0.0040693	7.449840401
63	Folate Metabolism	29	4	39.21	5.2632	0.0029726	0.088442	0.0040693	7.449840401
64	Lysine Degradation	30	5	39.215	5.2632	0.0029972	0.088442	0.0040693	7.450790394
65	Tryptophan Metabolism	60	4	39.145	5.2632	0.0030725	0.088442	0.0040693	7.4374905
66	Glucose-Alanine Cycle	13	3	39.122	5.2632	0.0030922	0.088442	0.0040693	7.433120535
67	Phenylalanine and Tyrosine Metabolism	28	2	39.158	5.2632	0.0031112	0.088442	0.0040693	7.43996048
68	Nicotinate and Nicotinamide Metabolism	37	4	38.963	5.2632	0.0031287	0.088442	0.0040693	7.402910777
69	Amino Sugar Metabolism	33	2	38.999	5.2632	0.0031651	0.088442	0.0040693	7.409750722
70	Arachidonic Acid Metabolism	69	4	38.969	5.2632	0.0032006	0.088442	0.0040693	7.404050768

Supplementary Table 4.8. Cont'd.

71	Phospholipid Biosynthesis	29	2	36.979	5.2632	0.004366	0.088442	0.0054729	7.025953792
72	Selenoamino Acid Metabolism	28	4	31.777	5.2632	0.0095782	0.17241	0.01184	6.037581699
73	Pyrimidine Metabolism	59	4	27.65	5.2632	0.011764	0.19999	0.014343	5.253457972
74	Lactose Synthesis	20	2	25.407	5.2632	0.019459	0.31135	0.021932	4.827291382
75	Caffeine Metabolism	24	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
76	Fatty Acid Elongation In Mitochondria	35	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
77	Androgen and Estrogen Metabolism	33	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
78	Ketone Body Metabolism	13	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
79	Butyrate Metabolism	19	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
80	Retinol Metabolism	37	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
81	Glycerol Phosphate Shuttle	11	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
82	Steroidogenesis	43	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
83	Ethanol Degradation	19	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
84	Plasmalogen Synthesis	26	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
85	Mitochondrial Beta-Oxidation of Medium Chain Saturated Fatty Acids	27	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
86	Mitochondrial Beta-Oxidation of Long Chain Saturated Fatty Acids	28	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
87	De Novo Triacylglycerol Biosynthesis	9	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
88	Cardiolipin Biosynthesis	11	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635
89	Androstenedione Metabolism	24	1	25.898	5.2632	0.021932	0.32898	0.021932	4.920580635

Supplementary Table 4.8. Cont'd.

APPENDIX D: SUPPORTING INFOTMATION FOR CHAPTER 5

***Topic**: Discovering neuroactive metabolome-wide changes owing to black raspberry administration in mice and potential role of humoral microbiota-brain axis*

Supplementary Tables

Supplementary Table 5.1
Unique list of 285 fecal metabolites altered in feces of BRB-rich diet treated group vs. standard AIN-76A diet treated group.

Supplementary Table 5.2
Unique list of 257 serum metabolites altered in feces of BRB-rich diet treated group vs. standard AIN-76A diet treated group.

Supplementary Table 5.3
Unique list of 73 cortical brain metabolites altered in feces of BRB-rich diet treated group vs. standard AIN-76A diet treated group.

Supplementary Table 5.4
ChemRICH results for fecal metabolome changes on compound class level (adjusted p-value<0.05).

Supplementary Table 5.5
Quantitative pathway enrichment analysis (qMSEA) results for fecal metabolome changes (adjusted p-value<0.05).

Supplementary Table 5.6
Quantitative pathway enrichment analysis (qMSEA) results for serum metabolome changes (adjusted p-value<0.05).

Supplementary Table 5.7
Overlapping pathways (n=35) between feces and sera from qMSEA (adjusted p-value<0.05).

Supplementary Table 5.8
Overlapping metabolites (n=36) between feces and sera (fold change ≥ 1.2 & $p < 0.05$).

Supplementary Table 5.9
Quantitative pathway enrichment analysis (qMSEA) results for cerebral cortical brain metabolome changes (adjusted p-value<0.05).

Supplementary Table 5.1 Unique list of 285 fecal metabolites altered in feces of BRB-rich diet treated group vs. standard AIN-76A diet treated group.

	ESI mode	Platform	RT (min)	PRECURSORMZ	PRECURSORTYPE	PubChem_CID	Metabolite me	Fold change	updown (BRB,	pvalue	qvalue	MSI level
1	Positive	untargeted	7.78	786.5173	[M+NH4]+	52922089	MGDG(18:5(3Z,6Z,9Z,12Z,15Z)/18:4(6Z,9Z,12Z,15Z))	unlimited	DOWN	0.00279	0.005944	2
2	Negative	untargeted	7.86	813.468	[M-H]-	52926720	PG(18:4(6Z,9Z,12Z,15Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	unlimited	UP	4.6E-06	8.46E-05	2
3	Negative	untargeted	4.19	288.0732	[M-H]-	440349	N-Succinyl-2-amino-6-ketopimelate	3858.35	UP	0.00956	0.016972	2
4	Negative	untargeted	5.62	551.3242	[M-H]-	126511	Lithocholate 3-O-glucuronide	879.92	UP	5.8E-06	9.84E-05	2
5	Negative	untargeted	4.51	381.1924	[M-H]-	53481460	12-Oxo-20-trihydroxy-leukotriene B4	729.33	UP	5.9E-06	0.0001	2
6	Negative	untargeted	1.79	152.0356	[M-H]-	86	3-Hydroxyanthranilic acid	679.78	UP	0.00321	0.007967	2
7	Positive	untargeted	5	300.1073	[M+H]+	75595895	beta-D-Glucopyranosyl anthranilate	528.83	UP	2.3E-05	0.000187	2
8	Negative	untargeted	5.15	299.0773	[M-H]-	4596190	Salicylic acid beta-D-glucoside	363.68	UP	0.00034	0.001661	2
9	Positive	untargeted	9.24	567.2968	[M+H]+	72422	Mesoporphyrin IX	287.05	UP	0.00039	0.001538	2
10	Negative	untargeted	4.27	383.2086	[M-H]-	53481513	20-Trihydroxy-leukotriene-B4	281.31	UP	2.3E-06	5.59E-05	2
11	Negative	untargeted	3.54	249.0808	[M-H]-	44237334	2-(5'-Methylthio)pentylmalic acid	279.07	UP	0.00014	0.000875	2
12	Negative	untargeted	3.97	153.0196	[M-H]-	4696	Patulin	251.08	UP	0.00245	0.006602	2
13	Negative	untargeted	1.24	95.01381	[M-H]-	7362	2-Furancarboxaldehyde	250.96	UP	0.00035	0.001695	2
14	Negative	untargeted	2.96	147.0123	[M-H]-	473	2-Oxo-4-methylthiobutanoic acid	215.73	DOWN	0.00774	0.014603	2
15	Positive	untargeted	1.51	310.1484	[M+]+	69045664	Arginyl-Hydroxyproline	205.80	UP	0.00937	0.013609	2
16	Negative	untargeted	0.88	503.1623	[M-H]-	439586	Maltotriose	194.83	UP	0.00062	0.002514	2
17	Positive	untargeted	7.82	255.0644	[M+H]+	5281708	Daidzein	155.15	UP	1.7E-05	0.000152	2
18	Negative	untargeted	3.53	232.0288	[M-H]-	122136	Dopamine 3-O-sulfate	123.98	DOWN	0.00144	0.004564	2
19	Negative	untargeted	3.64	230.9972	[M-H]-	10657183	2,4-Dihydroxyacetophenone 5-sulfate	123.58	UP	0.00015	0.000932	2
20	Positive	untargeted	6.39	484.2432	[M+H]+	131751431	Caffeoylferuloylspermidine	123.24	DOWN	3.6E-05	0.000262	2
21	Positive	untargeted	4.92	252.0863	[M+H]+	5280527	4-Hydroxy-3-methoxy-cinmoylglycine	99.85	UP	1.6E-05	0.000145	2
22	Negative	untargeted	5.13	107.0504	[M-H]-	2879	p-Cresol	84.34	UP	0.00013	0.000864	2
23	Positive	untargeted	6.97	505.3505	[M+H]+	6710742	6beta-Hydroxyasiatic acid	67.56	UP	7.1E-08	3.61E-06	2
24	Negative	untargeted	4.2	225.0406	[M-H]-	12039	Chorismate	62.80	UP	3.3E-06	6.88E-05	2
25	Positive	untargeted	6.48	417.1913	[M+H]+	53461967	Armillaric acid	58.06	DOWN	3.4E-05	0.000252	2
26	Negative	untargeted	1.99	235.0644	[M-H]-	44237182	2-(4'-Methylthio)butylmalic acid	51.09	UP	0.00017	0.001027	2
27	Negative	untargeted	0.94	186.0771	[M-H]-	194080	2-Keto-6-acetamidocaproate	48.04	DOWN	0.03229	0.039302	2
28	Negative	untargeted	5.66	283.083	[M-H]-	14704603	2-O-Benzoyl-D-glucose	45.90	UP	1.1E-09	5.59E-07	2
29	Negative	untargeted	5.19	151.0765	[M-H]-	6435833	2-Propenyl 2,4-hexadienoate	37.89	UP	1.9E-05	0.000217	2
30	Negative	untargeted	6.96	441.1625	[M-H]-	102172	Cortisol 21-sulfate	36.78	DOWN	0.00208	0.005908	2
31	Negative	untargeted	3.62	216.9816	[M-H]-	7322	5-Sulfosalicylic acid	35.43	UP	0.00468	0.010318	2
32	Positive	untargeted	1.16	254.0888	[M+H]+	135565113	L-Threoneopterin	33.16	DOWN	1.6E-06	2.69E-05	2
33	Positive	untargeted	4.29	168.1017	[M+H]+	82413815	2-(2-Furanyl)-3-piperidinol	31.46	DOWN	0.02349	0.025882	2
34	Negative	untargeted	4.41	177.0557	[M-H]-	699414	4-Methoxycinnamic acid	27.83	UP	0.00278	0.00723	2
35	Positive	untargeted	11.02	567.419	[M+H]+	131752157	Nigroxanthin	27.01	UP	4.3E-05	0.000299	2
36	Negative	targeted	0.91	182.08191	[M-H]-	5816	Epinephrine	26.58	DOWN	0.00023	0.001242	1
37	Negative	untargeted	5.89	193.0508	[M-H]-	445858	trans-Ferulic acid	25.01	DOWN	0.00199	0.005737	2
38	Positive	untargeted	7.89	298.2738	[M+NH4]+	5280450	Linoleic acid	23.34	UP	2.7E-05	0.000218	2
39	Positive	untargeted	1.22	276.0503	[M+NH4]+	3080745	2-Keto-3-deoxy-6-phosphogluconic acid	22.90	UP	7.4E-05	0.000456	2
40	Negative	untargeted	4.72	142.0874	[M-H]-	7016563	Proline betaine	21.97	UP	0.02857	0.036171	2

41	Negative	untargeted	3.5	202.018	[M-H]-	135905	Cystathionine ketimine	21.68	DOWN	0.00588	0.012066	2
42	Negative	untargeted	6.82	531.2988	[M-H]-	53477904	5b-Cyprinol sulfate	20.92	UP	2.8E-05	0.000281	2
43	Positive	targeted	1.854	252.10834	[M+H]+	13730	2'-Deoxyadenosine (dA)	20.84	DOWN	0.00357	0.007054	1
44	Negative	targeted	5.352	136.04012	[M-H]-	227	Anthranilic acid	19.60	UP	0.00081	0.00306	1
45	Negative	untargeted	0.72	110.0248	[M-H]-	12473	Pyrrole-2-carboxylic acid	19.16	UP	0.00747	0.014258	2
46	Positive	untargeted	7.31	267.1951	[M+H]+	15730832	Tetranor 12-HETE	18.80	UP	3.5E-06	4.72E-05	2
47	Positive	untargeted	7.5	430.3159	[M+H]+	566787	Hexadecanedioic acid mono-L-carnitine ester	18.06	DOWN	0.0216	0.024384	2
48	Negative	untargeted	3.73	109.0295	[M-H]-	289	Pyrocatechol	17.44	UP	0.04606	0.050143	2
49	Positive	targeted	6.425	162.09108	[M+H]+	10685	Indole-3-ethanol	17.40	UP	0.00833	0.012537	1
50	Positive	targeted	1.143	348.06909	[M+H]+	135398597	2'-Deoxyguanosine 5'-monophosphate (dGMP)	16.49	UP	0.0143	0.018329	1
51	Negative	targeted & ur	5.656	300.99915	[M-H]-	5281855	Ellagic Acid	15.72	UP	7.9E-08	8.33E-06	1
52	Negative	targeted	0.641	300.03922	[M-H]-	7115	N-Acetyl-glucosamine-1-phosphate	14.61	DOWN	0.00033	0.001602	1
53	Negative	untargeted	1.38	337.0447	[M-H]-	16019958	Imidazoleacetic acid ribotide	13.91	DOWN	0.0149	0.02312	2
54	Negative	untargeted	0.59	199.9694	[M-H]-	115015	Cysteine-S-sulfate	13.14	DOWN	0.01137	0.019228	2
55	Negative	targeted	0.596	259.01236	[M-H]-	440194	D-Myo-inositol-3-phosphate	12.51	DOWN	4.2E-06	7.92E-05	1
56	Negative	untargeted	1.85	313.0621	[M-H]-	5280528	Geranyl-PP	11.99	UP	0.03234	0.039341	2
57	Negative	untargeted	4.06	187.0069	[M-H]-	4615423	p-Cresol sulfate	11.13	UP	0.00013	0.000858	2
58	Positive	untargeted	0.72	247.1034	[M+H]+	18218176	Asparaginyl-Asparagine	11.07	UP	0.02453	0.026695	2
59	Negative	untargeted	5.68	121.0659	[M-H]-	31242	4-Ethylphenol	10.79	UP	0.00012	0.000807	2
60	Positive	untargeted	5.32	184.0966	[M+H]+	149048	3,4,5,6-Tetrahydrohippuric acid	10.76	DOWN	8.4E-07	1.68E-05	2
61	Positive	untargeted	10.56	425.3781	[M+]+	22833530	Campestanol	10.73	UP	1.2E-05	0.000113	2
62	Negative	untargeted	5.41	190.0512	[M-H]-	1826	5-Hydroxyindoleacetic acid (5-HIAA)	10.67	DOWN	0.00649	0.012937	2
63	Negative	untargeted	3.87	163.0402	[M-H]-	997	Phenylpyruvic acid	10.51	DOWN	0.01973	0.028071	2
64	Negative	untargeted	0.78	193.0717	[M-H]-	445238	Methyl beta-D-glucopyranoside	10.28	UP	0.00112	0.003853	2
65	Negative	untargeted	0.81	130.0146	[M-H]-	796	Iminoaspartic acid	10.27	UP	0.00044	0.001996	2
66	Negative	untargeted	3.33	244.1307	[M-H]-	18218182	Asparaginyl-Leucine	10.10	UP	0.00102	0.003604	2
67	Positive	untargeted	0.89	146.0924	[M+H]+	500	4-Guanidinobutanoic acid	9.89	UP	0.03572	0.034602	2
68	Negative	untargeted	3.36	195.0667	[M-H]-	77539036	3-(3-Hydroxyphenyl)-2-methyllactic acid	9.77	DOWN	0.0002	0.001132	2
69	Positive	untargeted	0.61	190.1073	[M+H]+	131802903	3-hydroxyhexanoylglycine	9.58	DOWN	0.01096	0.01518	2
70	Positive	untargeted	8.16	401.3415	[M+H]+	5283731	Calcidiol	9.52	UP	0.00385	0.00741	2
71	Positive	untargeted	6.98	329.0649	[M+]+	72277	(-)-Epigallocatechin	9.45	DOWN	0.00291	0.006124	2
72	Negative	untargeted	0.68	103.0401	[M-H]-	92135	(R)-3-Hydroxybutyric acid	9.41	UP	9.1E-05	0.000656	2
73	Negative	untargeted	0.6	122.9757	[M-H]-	160226	Sulfoacetaldehyde	9.31	UP	0.02131	0.029605	2
74	Negative	targeted & ur	5.747	165.05518	[M-H]-	3848	DL-3-Phenyllactic acid	9.25	UP	3.4E-05	0.000319	1
75	Positive	untargeted	5.7	261.008	[M+H]+	102261219	Caffeic acid 3-sulfate	9.16	UP	8.8E-06	9.07E-05	2
76	Negative	untargeted	0.6	140.9863	[M-H]-	17841823	(2-Hydroxyethoxy)sulfonic acid	9.09	UP	0.02967	0.037142	2
77	Positive	untargeted	6.2	484.3009	[M+H]+	52926303	PS(O-16:0/0:0)	9.08	DOWN	1.6E-08	1.53E-06	2
78	Negative	untargeted	5.3	216.124	[M-H]-	107738	Propionylcarnitine	9.03	UP	5E-08	6.32E-06	2
79	Negative	untargeted	5.13	197.0455	[M-H]-	736172	Vanillylmandelic acid	9.01	UP	0.00037	0.00175	2
80	Positive	untargeted	6.42	186.1121	[M+H]+	131802910	4-Hepteneoylglycine	8.90	DOWN	3.5E-05	0.000257	2

Supplementary Table 5.1. Cont'd.

81	Negative	untargeted	0.73	87.00865	[M-H]-	1060	Pyruvic acid	8.51	DOWN	0.00527	0.011221	2
82	Positive	untargeted	6.32	468.3066	[M+H]+	42607332	N-Arachidonoyl tyrosine	8.46	DOWN	0.00107	0.00313	2
83	Negative	untargeted	0.67	179.0562	[M-H]-	892	myo-Inositol	8.42	UP	0.01849	0.026894	2
84	Negative	targeted & ur	1.207	321.0484	[M-H]-	9700	Thymidine 5'-monophosphate (dTMP)	8.34	UP	0.01023	0.017838	1
85	Positive	untargeted	3.2	246.1444	[M+H]+	56991460	Asparaginyl-Isoleucine	8.26	UP	0.00376	0.007306	2
86	Positive	untargeted	8.47	273.2416	[M+H]+	3013895	11-Hydroxyhexadecanoic acid	8.14	UP	0.02222	0.024888	2
87	Positive	untargeted	2.08	232.1287	[M+H]+	18218188	Asparaginyl-Valine	8.06	UP	0.0082	0.012429	2
88	Negative	untargeted	2.98	262.0392	[M-H]-	3035453	Epinephrine sulfate	7.98	DOWN	0.00113	0.003886	2
89	Positive	untargeted	6.76	321.1689	[M+NH4]+	4831	Pipemidic acid	7.88	UP	0.00066	0.00225	2
90	Negative	untargeted	4.59	289.1661	[M-H]-	73817234	1-Octen-3-yl glucoside	7.83	UP	7.6E-06	0.000117	2
91	Negative	untargeted	1.88	248.0235	[M-H]-	123747	Norepinephrine sulfate	7.73	DOWN	0.00012	0.000818	2
92	Positive	targeted	0.925	130.08609	[M+H]+	439227	L-Pipecolic acid	7.69	DOWN	0.04942	0.042986	1
93	Positive	untargeted	8.64	277.2163	[M+]+	5312427	Palmitoleic acid	7.50	UP	0.00101	0.003025	2
94	Negative	untargeted	3.93	192.0669	[M-H]-	21350391	3-Carbamoyl-2-phenylpropioldehyde	7.45	UP	0.00012	0.000818	2
95	Positive	targeted	6.253	208.09619	[M+H]+	152323	N-(3-Phenylpropionyl)glycine	7.35	DOWN	0.0017	0.004251	1
96	Positive	untargeted	1.88	254.0654	[M+H]+	4520077	N-Salicyloylaspartic acid	7.20	UP	4.3E-07	1.13E-05	2
97	Negative	untargeted	5.99	315.124	[M-H]-	54100766	7-Hydroxy-2',3',4'-trimethoxyisoflavan	7.18	UP	2.3E-06	5.66E-05	2
98	Negative	targeted	5.583	206.08179	[M-H]-	74839	N-Acetyl-L-phenylalanine	7.06	UP	0.00089	0.003247	1
99	Positive	untargeted	5.97	160.0757	[M+H]+	800	Indole-3-acetaldehyde	6.95	DOWN	0.03323	0.032873	2
100	Negative	untargeted	4.99	300.136	[M-H]-	14409732	Tryptophyl-Proline	6.80	UP	0.00324	0.008014	2
101	Negative	untargeted	1.09	145.0618	[M-H]-	5961	L-Glutamine	6.72	UP	0.00078	0.002968	2
102	Positive	untargeted	6.72	295.2264	[M+H]+	16061060	9(10)-EpODE	6.64	UP	1.3E-10	6.09E-08	2
103	Negative	targeted	3.245	165.04143	[M-H]-	70639	3-Methylxanthine	6.48	DOWN	0.00128	0.004246	1
104	Positive	untargeted	8.42	487.3407	[M+]+	14153905	2-Deoxybrassinolide	6.33	UP	3E-06	4.21E-05	2
105	Positive	targeted	1.019	123.04715	[M+H]+	936	Niacinamide	6.28	UP	9.2E-09	1.02E-06	1
106	Positive	untargeted	4.82	295.1651	[M+H]+	342468	Isoleucyl-Tyrosine	6.24	UP	5E-05	0.000337	2
107	Positive	untargeted	0.77	287.0754	[M+H]+	124202060	Diphenol glucuronide	6.14	UP	0.00025	0.001138	2
108	Positive	untargeted	7.83	302.2328	[M+H]+	53481660	Nonoylcarnitine	6.11	UP	3.7E-05	0.000268	2
109	Negative	untargeted	5.43	237.0769	[M-H]-	735755	3,4,5-Trimethoxycinnamic acid	5.97	DOWN	0.00299	0.007604	2
110	Positive	untargeted	5.16	304.1653	[M+H]+	19816756	Tryptophyl-Valine	5.91	UP	0.00317	0.0065	2
111	Negative	untargeted	5.29	246.992	[M-H]-	382946	Vanillic acid 4-sulfate	5.90	UP	4.2E-06	7.92E-05	2
112	Negative	untargeted	5.41	277.1559	[M-H]-	7010566	Phenylalanyl-Isoleucine	5.86	UP	5.2E-05	0.000435	2
113	Positive	untargeted	0.9	70.06523	[M+H]+	79803	1-Pyrroline	5.73	UP	0.0069	0.011051	2
114	Negative	targeted	0.619	195.05083	[M-H]-	10690	D-Gluconic acid	5.71	UP	0.03639	0.042403	1
115	Negative	targeted	8.404	295.2276	[M-H]-	5312830	(+)9-HODE	5.69	UP	0.01783	0.026248	1
116	Negative	untargeted	0.99	229.1196	[M-H]-	10242944	Valyl-Hydroxyproline	5.64	UP	0.0021	0.005937	2
117	Negative	untargeted	4.38	260.1038	[M-H]-	263471	Tryptophyl-glycine	5.64	UP	0.00128	0.004237	2
118	Positive	untargeted	3.57	341.1197	[M+]+	14717809	Tyrosyl-Histidine	5.63	DOWN	0.00106	0.003095	2
119	Positive	untargeted	1.06	128.0705	[M+H]+	24771808	(S)-2,3,4,5-Tetrahydropiperidine-2-carboxylate	5.51	DOWN	0.03123	0.031581	2
120	Negative	untargeted	4.47	499.1295	[M-H]-	74083657	Lyoniresinol 9'-sulfate	5.50	DOWN	0.00254	0.006774	2

Supplementary Table 5.1. Cont'd.

121	Positive	untargeted	5.42	279.1702	[M+H]+	6992310	Leucyl-phenylalanine	5.30	UP	0.00143	0.003813	2
122	Positive	untargeted	4.96	245.1855	[M+H]+	13879965	Isoleucyl-Isoleucine	5.27	UP	0.00177	0.004361	2
123	Positive	untargeted	0.94	158.0447	[M+H]+	5280499	2-Aminomuconic acid	5.04	UP	4.8E-06	5.94E-05	2
124	Positive	untargeted	4.61	200.0919	[M+NH4]+	1738	Homovanillic acid	5.00	DOWN	9.1E-08	4.3E-06	2
125	Negative	untargeted	3.6	231.1356	[M-H]-	7021828	Threoninyl-Leucine	4.98	UP	2.7E-05	0.000278	2
126	Negative	untargeted	2.55	237.0882	[M-H]-	92829	Glycyl-Tyrosine	4.96	UP	0.00037	0.00176	2
127	Negative	untargeted	3.6	334.0582	[M-H]-	135398608	Dihydroneopterin phosphate	4.89	UP	2.4E-05	0.000248	2
128	Negative	untargeted	1.02	200.0565	[M-H]-	13943174	N-acetyl-L-2-aminoadipate(2-)	4.88	UP	6.2E-09	1.87E-06	2
129	Negative	untargeted	3.67	241.0835	[M-H]-	5789	Thymidine	4.88	UP	0.00049	0.002125	2
130	Positive	untargeted	3.71	367.1893	[M+H]+	74039123	Bufotenine O-glucoside	4.82	DOWN	0.00278	0.005929	2
131	Negative	targeted	2.329	115.03998	[M-H]-	49	3-Methyl-2-oxobutanoic acid	4.70	DOWN	0.01697	0.025371	1
132	Negative	untargeted	3.35	143.1191	[M-H]-	189087	N-Acetylcadaverine	4.67	UP	0.00126	0.004193	2
133	Positive	untargeted	3.76	158.0813	[M+H]+	66141	N-Acetylproline	4.66	UP	0.02033	0.023377	2
134	Negative	untargeted	4.98	229.0984	[M-H]-	129373222	cyclic Melatonin	4.63	UP	1.4E-07	1.18E-05	2
135	Negative	untargeted	9.67	313.2383	[M-H]-	10236635	12,13-DHOME	4.60	UP	0.00218	0.006108	2
136	Positive	untargeted	0.63	255.1454	[M+H]+	7408625	Valyl-Histidine	4.55	UP	0.03176	0.031893	2
137	Negative	untargeted	5.64	147.0452	[M-H]-	5372954	Cinnamic acid	4.53	UP	0.00516	0.011038	2
138	Positive	untargeted	0.89	279.1074	[M+H]+	137383	Isovalerylglucuronide	4.44	UP	0.04549	0.040654	2
139	Positive	untargeted	3.85	333.1554	[M+H]+	131750788	Tryptophyl-γ-glutamate	4.37	UP	0.03712	0.035464	2
140	Negative	untargeted	4.89	145.0871	[M-H]-	62908	Methyl DL-Leucate	4.37	DOWN	0.03113	0.038269	2
141	Positive	untargeted	2.92	297.1084	[M+H]+	19365650	Aspartyl-Tyrosine	4.35	UP	0.02747	0.028809	2
142	Negative	targeted	0.62	165.0399	[M-H]-	122045	D-Arabinonic acid	4.33	UP	8.3E-05	0.000614	1
143	Positive	untargeted	4.79	232.1176	[M+H]+	6453952	Suberylglycine	4.32	UP	6.4E-08	3.39E-06	2
144	Negative	untargeted	0.59	162.0408	[M-H]-	435401	L-N-Carboxymethylserine	4.25	UP	0.01064	0.018347	2
145	Positive	untargeted	8.89	457.3681	[M+H]+	10494	Oleanolic acid	4.24	UP	5.6E-05	0.000368	2
146	Positive	untargeted	3.38	231.1339	[M+H]+	61158071	Hydroxyprolyl-Valine	4.20	UP	0.00832	0.012537	2
147	Negative	untargeted	3.55	195.066	[M-H]-	21563790	3-(3,4-Dihydroxyphenyl)-2-methylpropionic acid	4.20	DOWN	0.00135	0.004367	2
148	Positive	untargeted	2.18	203.1392	[M+H]+	96801	Alanyl-Leucine	4.18	UP	0.00629	0.010341	2
149	Positive	untargeted	6.04	455.3227	[M+NH4]+	42607469	PE(P-16:0e/0:0)	4.18	UP	1.5E-06	2.54E-05	2
150	Negative	untargeted	0.65	351.0577	[M-H]-	3390357	a-L-threo-4-Hex-4-enopyranuronosyl-D-galacturonic ac	4.17	UP	0.01091	0.018673	2
151	Negative	untargeted	5.85	403.1031	[M-H]-	29986850	cis-Resveratrol 3-O-glucuronide	4.15	DOWN	0.01087	0.018621	2
152	Positive	untargeted	6.22	194.1171	[M+H]+	12989303	(R)-N-Methylsalsolinol	4.11	DOWN	0.00012	0.00065	2
153	Negative	untargeted	8.22	438.2633	[M-H]-	52925150	LysoPE(15:0/0:0)	4.08	UP	0.00388	0.009038	2
154	Positive	untargeted	0.54	146.1652	[M+H]+	1102	Spermidine	4.07	DOWN	0.00956	0.013824	2
155	Negative	untargeted	1.4	187.1087	[M-H]-	92832	N6-Acetyl-L-lysine	3.92	UP	0.00151	0.004713	2
156	Negative	untargeted	1.21	488.1093	[M-H]-	5289009	Chondroitin 6'-sulfate	3.91	UP	0.01047	0.018126	2
157	Positive	untargeted	4.29	263.1419	[M+H]+	17863274	Isoleucyl-Methionine	3.91	UP	0.0004	0.001568	2
158	Negative	targeted & ur	0.711	75.00841	[M-H]-	757	Glycolic acid	3.91	UP	0.00024	0.001306	1
159	Negative	untargeted	0.78	269.0881	[M-H]-	14717808	Aspartyl-Histidine	3.91	UP	0.00039	0.001828	2
160	Positive	untargeted	6.72	339.1797	[M+H]+	53481589	Omega-Carboxy-trinor-leukotriene B4	3.88	UP	3.8E-11	3.15E-08	2

Supplementary Table 5.1. Cont'd.

161	Negative	targeted	4.652	188.03853	[M-H]-	3845	Kynurenic acid	3.81	DOWN	0.00039	0.001831	1
162	Positive	untargeted	3.3	157.086	[M+H]+	54042244	8-Hydroxy-5,6-octadienoic acid	3.73	DOWN	0.01765	0.021181	2
163	Negative	untargeted	1.39	231.0989	[M-H]-	127370	N2-Succinyl-L-ornithine	3.71	UP	0.00263	0.006961	2
164	Negative	untargeted	1.27	73.02917	[M-H]-	1032	Propionic acid	3.64	UP	0.04674	0.050624	2
165	Negative	untargeted	8	333.2073	[M-H]-	5288144	Prostaglandin B2	3.61	DOWN	7.3E-05	0.000556	2
166	Positive	targeted	6.279	200.12753	[M+H]+	10058590	N-hexanoyl-L-homoserine lactone	3.57	DOWN	0.01069	0.014897	1
167	Negative	targeted	1.341	117.01897	[M-H]-	1110	Succinic acid	3.51	UP	0.04578	0.049956	1
168	Positive	targeted	0.862	136.04248	[M+H]+	24417	S-Methyl-L-cysteine	3.49	UP	0.03754	0.03569	1
169	Positive	untargeted	3.33	137.0597	[M+H]+	999	Phenylacetic acid	3.48	DOWN	5.6E-05	0.000368	2
170	Positive	targeted	7.043	214.14317	[M+H]+	443437	N-Heptanoyl-L-homoserine lactone	3.48	DOWN	0.02484	0.026885	1
171	Positive	untargeted	6.94	253.1432	[M+]+	12736	Dodecanedioic acid	3.47	DOWN	0.02152	0.024335	2
172	Negative	untargeted	5.68	119.0503	[M-H]-	998	Phenylacetaldehyde	3.45	DOWN	0.01784	0.026249	2
173	Negative	targeted & ur	0.856	103.00368	[M-H]-	867	Malonic acid	3.42	UP	0.00189	0.00554	1
174	Positive	untargeted	4.22	233.1491	[M+H]+	18218220	Isoleucyl-Threonine	3.41	UP	1.8E-05	0.000157	2
175	Positive	untargeted	3.82	168.0655	[M+H]+	1050	Pyridoxal	3.39	UP	0.03468	0.033886	2
176	Negative	targeted	0.765	308.09872	[M-H]-	445063	N-Acetylneuraminic acid	3.38	UP	9.3E-05	0.000663	1
177	Positive	untargeted	4.74	134.0601	[M+H]+	321710	1,3-Dihydro-(2H)-indol-2-one	3.37	DOWN	0.00777	0.01197	2
178	Negative	untargeted	1.18	100.0403	[M-H]-	535	1-Aminocyclopropanecarboxylic acid	3.36	DOWN	0.03071	0.037961	2
179	Negative	targeted & ur	1.03	147.02977	[M-H]-	43	α-Hydroxyglutaric acid	3.34	UP	0.01425	0.022396	1
180	Positive	targeted	0.648	162.11208	M+	10917	L-Carnitine	3.30	DOWN	0.00615	0.01017	1
181	Negative	untargeted	1.2	258.1462	[M-H]-	131750767	Hydroxyprolyl-Lysine	3.28	UP	0.01512	0.02339	2
182	Positive	untargeted	2.59	193.0975	[M+H]+	107963	Hydroxycotinine	3.25	UP	0.00044	0.001704	2
183	Positive	untargeted	3.66	247.1289	[M+H]+	332962	Aspartyl-Leucine	3.21	UP	0.00335	0.006757	2
184	Negative	untargeted	4.71	236.0563	[M-H]-	95664	N-Benzoylaspartic acid	3.21	DOWN	0.00549	0.0115	2
185	Positive	untargeted	2.65	129.0659	[M+H]+	93556	Dihydrothymine	3.16	UP	0.0002	0.000973	2
186	Negative	targeted & ur	0.68	289.1153	[M-H]-	16950	Argininosuccinic acid	3.15	UP	0.03631	0.042343	1
187	Positive	untargeted	8.71	425.3409	[M+]+	121948	24-Hydroxycholesterol	3.10	DOWN	0.0008	0.002556	2
188	Negative	untargeted	4.52	137.0607	[M-H]-	31236	2-Phenoxyethanol	3.10	DOWN	0.00513	0.010997	2
189	Positive	untargeted	0.57	243.0951	[M+]+	439454	N-Acetyl-b-glucosaminylamine	3.09	DOWN	0.03482	0.033972	2
190	Positive	untargeted	4.4	231.1703	[M+H]+	352038	Leucyl-Valine	3.09	UP	0.02016	0.023249	2
191	Positive	untargeted	1.16	190.0711	[M+H]+	70914	N-Acetylglutamic acid	3.09	UP	0.00024	0.00108	2
192	Negative	untargeted	4.93	469.0818	[M-H]-	131752139	(Z)-Resveratrol 3-glucoside 5-sulfate	3.09	DOWN	0.00051	0.002195	2
193	Negative	untargeted	3.38	116.0717	[M-H]-	138	5-Aminopentanoic acid	3.05	UP	0.02293	0.031103	2
194	Positive	untargeted	0.69	113.0711	[M+NH4]+	8871	2-Hydroxypyridine	3.04	UP	0.04337	0.039367	2
195	Positive	untargeted	4.39	276.1337	[M+H]+	85362	Alanyltryptophan	3.04	UP	0.04654	0.041304	2
196	Negative	untargeted	1.81	154.0145	[M-H]-	3505109	4-Nitrocatechol	3.01	DOWN	0.03602	0.042176	2
197	Negative	untargeted	1.22	219.0778	[M-H]-	439280	5-Hydroxy-L-tryptophan	2.99	DOWN	0.00463	0.010259	2
198	Negative	untargeted	0.93	174.0406	[M-H]-	65065	N-Acetyl-L-aspartic acid	2.99	UP	0.00382	0.008938	2
199	Negative	untargeted	6.43	589.3024	[M-H]-	440784	D-Urobilinogen	2.98	UP	0.00828	0.01532	2
200	Positive	untargeted	4.66	217.0973	[M+H]+	98285	L-1,2,3,4-Tetrahydro-beta-carboline-3-carboxylic acid	2.95	DOWN	0.02667	0.028172	2

Supplementary Table 5.1. Cont'd.

201	Positive	untargeted	7.48	482.3284	[M+H]+	9547068	LysoPE(18:0/0:0)	2.95	DOWN	0.01277	0.016937	2
202	Positive	untargeted	1.07	140.0344	[M+H]+	72924	6-Hydroxynicotinic acid	2.91	UP	0.00168	0.004212	2
203	Negative	untargeted	5.08	137.0245	[M-H]-	135	4-Hydroxybenzoic acid	2.90	UP	0.00602	0.012293	2
204	Negative	untargeted	2.07	220.0291	[M-H]-	54097042	S-(3-Oxo-3-carboxy-n-propyl)cysteine	2.90	DOWN	5.8E-05	0.000468	2
205	Positive	untargeted	6.78	328.2483	[M+H]+	11645581	(9E)-9-Nitrooctadecenoic acid	2.89	UP	0.00044	0.001692	2
206	Negative	untargeted	2.04	143.0351	[M-H]-	1551553	3-Methylglutaconic acid	2.87	UP	0.00498	0.010799	2
207	Positive	untargeted	6.08	466.2898	[M+H]+	24779456	LysoPC(14:1(9Z))	2.87	DOWN	0.00021	0.001006	2
208	Positive	untargeted	4.72	220.0604	[M+H]+	76230	8-Methoxykynurenate	2.85	UP	0.00111	0.0032	2
209	Negative	untargeted	8.04	337.2384	[M-H]-	5283144	8,9-DiHETrE	2.82	DOWN	0.01103	0.018842	2
210	Negative	untargeted	4.51	181.0509	[M-H]-	9378	Hydroxyphenyllactic acid	2.81	DOWN	0.00402	0.009252	2
211	Negative	untargeted	8.44	241.1813	[M-H]-	454064	3-Oxotetradecanoic acid	2.81	DOWN	0.00741	0.014167	2
212	Positive	untargeted	7.87	346.2594	[M+H]+	131770390	3-hydroxyundecanoyl carnitine	2.81	UP	8.7E-05	0.000519	2
213	Positive	targeted & ur	6.403	176.07045	[M+H]+	802	Indole-3-acetic acid	2.75	DOWN	0.00697	0.011114	1
214	Negative	untargeted	0.88	125.0358	[M-H]-	1135	Thymine	2.73	DOWN	0.00508	0.010936	2
215	Positive	untargeted	9.2	415.2843	[M+H]+	53481452	11'-Carboxy-alpha-tocotrienol	2.71	UP	0.02986	0.030583	2
216	Positive	targeted & ur	0.629	126.02174	[M+H]+	1123	Taurine	2.69	DOWN	0.00512	0.008971	1
217	Negative	untargeted	7.27	363.2185	[M-H]-	5866	Tetrahydrocortisone	2.67	DOWN	0.0042	0.009545	2
218	Positive	untargeted	8.2	441.3352	[M+]+	11954197	7-a,25-Dihydroxycholesterol	2.65	DOWN	0.00142	0.00379	2
219	Positive	untargeted	1.72	251.1063	[M+H]+	7020165	Threoninyl-Methionine	2.65	UP	0.00055	0.001988	2
220	Negative	untargeted	4.27	293.1513	[M-H]-	4466134	Tyrosyl-Leucine	2.59	UP	0.00545	0.011457	2
221	Positive	untargeted	5.46	318.1805	[M+H]+	9797038	Isoleucyl-Tryptophan	2.58	UP	0.00064	0.002214	2
222	Positive	untargeted	4.64	180.0657	[M+NH4]+	5281426	Umbelliferone	2.57	UP	5.5E-06	6.63E-05	2
223	Negative	untargeted	4.62	196.0614	[M-H]-	6047	L-Dopa	2.57	UP	1.9E-05	0.000221	2
224	Positive	untargeted	1.52	207.0796	[M+H]+	259185	Methionyl-glycine	2.57	UP	0.00195	0.004669	2
225	Positive	untargeted	1.23	269.1606	[M+H]+	9900065	Histidinyl-Isoleucine	2.55	UP	0.00357	0.007054	2
226	Positive	untargeted	6.41	593.3311	[M+H]+	26818	Mesobilirubinogen	2.52	UP	0.00838	0.012594	2
227	Negative	untargeted	4.53	194.046	[M-H]-	439316	Dopaquinone	2.51	UP	6.5E-05	0.000509	2
228	Positive	targeted	6.896	190.08539	[M+H]+	3744	Indole-3-propionic acid	2.51	DOWN	0.01356	0.017679	1
229	Positive	untargeted	0.63	166.0864	[M+H]+	6140	L-Phenylalanine	2.45	DOWN	0.01092	0.015149	2
230	Negative	untargeted	1.38	172.0614	[M-H]-	192878	N-Acetyl-L-glutamate 5-semialdehyde	2.45	UP	0.00252	0.006747	2
231	Negative	untargeted	7.75	471.2429	[M-H]-	21252312	Chenodeoxycholic acid 3-sulfate	2.45	DOWN	0.00537	0.011339	2
232	Positive	untargeted	9.14	455.3337	[M+H]+	196302	27-Norcholestanehexol	2.44	DOWN	0.00407	0.007687	2
233	Positive	untargeted	4.72	86.09643	[M+H]+	8082	Piperidine	2.43	UP	0.02637	0.02795	2
234	Negative	untargeted	3.6	171.0777	[M-H]-	79101	Glycylproline	2.43	UP	0.01535	0.023639	2
235	Positive	targeted	5.707	162.05475	[M+H]+	54680871	2,4-Quinolinediol	2.39	DOWN	7.8E-07	1.61E-05	1
236	Positive	untargeted	4.78	216.1229	[M+H]+	131802912	1-Hydroxyoct-2-enoylglycine	2.38	UP	0.03462	0.033848	2
237	Negative	untargeted	4.24	182.0822	[M-H]-	1237	Normetanephrine	2.36	DOWN	4.6E-06	8.45E-05	2
238	Negative	untargeted	5.85	369.2289	[M-H]-	5280888	6-Keto-prostaglandin F1a	2.34	DOWN	0.00354	0.008468	2
239	Negative	untargeted	11.74	355.3215	[M-H]-	193484	2(R)-hydroxydocosanoic acid	2.32	UP	0.0079	0.014791	2
240	Negative	targeted	5.839	263.09586	[M-H]-	171161	AFMK, i.e. N-[3-[2-(formylamino)-5-methoxyphenyl]-3-	2.27	DOWN	7.8E-06	0.00012	1

Supplementary Table 5.1. Cont'd.

241	Positive	untargeted	7.43	480.3126	[M+H]+	9547071	LysoPE(18:1(9Z)/0:0)	2.27	UP	0.00482	0.008619	2
242	Negative	untargeted	9.17	433.3324	[M-H]-	53477686	3a,7a-Dihydroxycoprostanic acid	2.24	UP	0.01401	0.02213	2
243	Negative	untargeted	2.11	159.0665	[M-H]-	6999745	3-Methyladipic acid	2.23	UP	0.00056	0.002336	2
244	Negative	untargeted	3.62	149.0246	[M-H]-	11915	Phenylglyoxylic acid	2.23	DOWN	0.04712	0.050918	2
245	Negative	untargeted	1.79	138.0196	[M-H]-	980	4-Nitrophenol	2.16	UP	0.00085	0.003144	2
246	Negative	untargeted	1.66	235.0729	[M-H]-	910	N'-Formylkynurenine	2.16	DOWN	0.0006	0.00245	2
247	Negative	untargeted	3.65	347.0391	[M-H]-	135398640	Inosine monophosphate (IMP)	2.16	DOWN	0.00082	0.003069	2
248	Negative	targeted	6.377	137.02425	[M-H]-	338	Salicylic Acid	2.14	UP	0.00935	0.016696	1
249	Negative	untargeted	1.85	129.0558	[M-H]-	47	3-Methyl-2-oxovaleric acid	2.12	UP	0.00322	0.007976	2
250	Positive	untargeted	7.38	427.2651	[M+H]+	53481917	Prostaglandin D2-1-glyceryl ester	2.11	UP	0.00032	0.001347	2
251	Negative	untargeted	3.46	191.0204	[M-H]-	311	Citric acid	2.10	DOWN	0.00123	0.004128	2
252	Negative	untargeted	5.05	455.1023	[M-H]-	21146794	Epicatechin 3-O-(4-methylgallate)	2.09	DOWN	0.00083	0.003093	2
253	Negative	targeted	6.264	144.04529	[M-H]-	10256	Indole-3-carboxaldehyde	2.08	DOWN	0.04796	0.051589	1
254	Positive	untargeted	0.72	168.0326	[M+H]+	159864	N-Acetyltaurine	2.06	UP	0.00573	0.009742	2
255	Negative	untargeted	1.65	188.0933	[M-H]-	131802904	4-hydroxyhexanoylglycine	2.05	UP	0.0164	0.024707	2
256	Negative	untargeted	3.78	131.0714	[M-H]-	10796774	2-Hydroxy-3-methylpentanoic acid	2.02	DOWN	0.0024	0.006523	2
257	Positive	untargeted	9.18	417.337	[M+H]+	53481412	7 alpha,26-Dihydroxy-4-cholesten-3-one	2.02	UP	0.00062	0.002152	2
258	Positive	untargeted	7.72	493.3524	[M+]+	10850	TG(8:0/8:0/8:0)	2.00	DOWN	0.02788	0.029123	2
259	Positive	untargeted	5.02	306.0473	[M+H]+	53481030	Cytidine 2',3'-cyclic phosphate	2.00	DOWN	0.00153	0.003974	2
260	Negative	untargeted	1.88	152.0715	[M-H]-	681	Dopamine	1.98	UP	0.00177	0.005308	2
261	Negative	untargeted	8.61	361.239	[M-H]-	22665100	(Z)-2,4-Dihydroxy-6-(8-pentadecenyl)benzoic acid	1.94	UP	0.00352	0.008448	2
262	Positive	untargeted	3.71	206.0449	[M+H]+	440752	4,6-Dihydroxy-2-quinolinecarboxylic acid	1.94	DOWN	0.0192	0.022529	2
263	Negative	untargeted	5.21	195.03	[M-H]-	66886955	4-Hydroxy-benzenepropanedioate	1.93	UP	0.01849	0.026894	2
264	Positive	untargeted	7.4	398.2897	[M+H]+	53481911	PGF2a ethanolamide	1.93	DOWN	0.01038	0.014628	2
265	Positive	targeted	9.498	326.3038	[M+H]+	5283454	Oleoyl ethanolamide	1.88	UP	0.00369	0.007213	1
266	Negative	untargeted	5.65	315.182	[M-H]-	124202108	Menthol-glucoronide	1.87	UP	0.00052	0.002226	2
267	Positive	untargeted	0.69	219.0977	[M+H]+	440103	γ-Glutamylalanine	1.87	UP	0.00386	0.007415	2
268	Negative	untargeted	6.86	454.2267	[M-H]-	53481511	20-Hydroxy-leukotriene E4	1.86	DOWN	0.0015	0.004713	2
269	Negative	untargeted	2.7	222.0409	[M-H]-	440741	4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoic acid	1.84	DOWN	0.009	0.01625	2
270	Negative	untargeted	5.54	313.0937	[M-H]-	131752916	2-O-Acetylarbutin	1.84	UP	0.00356	0.008512	2
271	Negative	untargeted	9.63	299.2589	[M-H]-	439887	2-Hydroxystearate	1.83	UP	0.02342	0.031551	2
272	Positive	untargeted	9.68	412.2845	[M+NH4]+	52929772	LysoPA(P-16:0e/0:0)	1.82	DOWN	0.00056	0.002016	2
273	Positive	targeted & ur	7.918	289.2157	[M+H]+	6013	Testosterone	1.80	UP	0.01065	0.014863	1
274	Positive	untargeted	6.99	333.2059	[M+H]+	44263342	11b-Hydroxyprogesterone	1.78	DOWN	0.00867	0.012908	2
275	Positive	untargeted	9.53	393.275	[M+H]+	87443544	3-Hydroxy-10'-apo-b,γ-carotel	1.74	UP	0.01367	0.017765	2
276	Negative	untargeted	3.58	130.0874	[M-H]-	6306	L-Isoleucine	1.73	UP	0.02985	0.037235	2
277	Negative	untargeted	2.95	265.1197	[M-H]-	7010579	Threoninyl-Phenylalanine	1.71	UP	0.04773	0.05142	2
278	Negative	targeted & ur	8.537	295.22742	[M-H]-	6443013	(+)13-HODE	1.68	UP	0.03427	0.040872	1
279	Negative	untargeted	0.72	209.0669	[M-H]-	245622	D-altro-D-manno-Heptose	1.64	UP	0.00813	0.0151	2
280	Negative	untargeted	3.81	138.0561	[M-H]-	255670	Ethyl 2-pyrrolecarboxylate	1.61	UP	4E-05	0.000355	2

Supplementary Table 5.1. Cont'd.

281	Negative	untargeted	6.56	341.197	[M-H]-	53477747	2,3-Dinor-6-keto-prostaglandin F1 a	1.61	DOWN	0.00471	0.010376	2
282	Negative	untargeted	0.6	124.9914	[M-H]-	7866	2-Hydroxyethanesulfote	1.59	UP	0.0311	0.038266	2
283	Positive	untargeted	0.59	142.0476	[M+]+	6288	L-Threonine	1.51	DOWN	0.04428	0.039914	2
284	Positive	untargeted	8.42	279.2312	[M+]+	985	Palmitic acid	1.45	DOWN	0.00015	0.000781	2
285	Positive	untargeted	6.13	433.3308	[M+H]+	6446280	24-Hydroxycalcitriol	1.40	DOWN	0.03089	0.03133	2

Supplementary Table 5.1. Cont'd.

Supplementary Table 5.2 Unique list of 257 serum metabolites altered in feces of BRB-rich diet treated group vs. standard AIN-76A diet treated group.

	ESI mode	Platform	RT (min)	PRECURSORMZ	PRECURSORTYPE	PubChem_CID	Metabolite me	Fold	updown (BRB/AIN)	pvalue	qvalue	MSI level
1	Negative	untargeted	0.95	283.1031	[M-H]-	9921875	Glutamylhistidine	10611.09	UP	1.07E-07	1.414E-05	2
2	Positive	untargeted	0.8	233.0423	[M+CH3OH+H]+	122357	D-Erythrose 4-phosphate	3595.76	UP	2.32E-10	1.181E-07	2
3	Positive	targeted & untargeted	0.894	404.02481	[M+H]+	6132	CDP	2387.11	UP	7.06E-09	1.519E-06	1
4	Negative	untargeted	0.95	193.0716	[M-H]-	5320294	D-4-O-Methyl-myo-inositol	1239.31	UP	9.78E-11	1.13E-07	2
5	Negative	untargeted	4.21	216.9809	[M-H]-	7322	5-Sulfosalicylic acid	299.25	UP	1.51E-06	0.0001053	2
6	Positive	untargeted	4.99	300.1078	[M+H]+	75595895	beta-D-Glucopyranosyl anthranilate	175.06	UP	0.00078	0.0228925	2
7	Positive	untargeted	5.39	249.0079	[M+H]+	382946	Vanillic acid 4-sulfate	81.69	UP	0.000145	0.0059178	2
8	Negative	untargeted	5.69	121.0658	[M-H]-	31242	4-Ethylphenol	72.70	UP	7.33E-07	6.241E-05	2
9	Positive	untargeted	8.47	277.2154	[M+H]+	5312508	Stearidonic acid	56.54	UP	1.49E-07	1.814E-05	2
10	Negative	untargeted	3.64	188.9864	[M-H]-	3083879	Pyrocatechol sulfate	27.08	UP	8.46E-06	0.000378	2
11	Positive	untargeted	8.14	564.3041	[M+]+	11757087	LysoPC(20:5(5Z,8Z,11Z,14Z,17Z))	21.51	UP	9.25E-06	0.0005672	2
12	Negative	untargeted	4.82	325.0927	[M-H]-	5280759	trans-o-Coumaric acid 2-glucoside	19.25	UP	5.38E-08	8.129E-06	2
13	Negative	untargeted	5.9	199.0071	[M-H]-	6426766	4-Vinylphenol sulfate	12.82	DOWN	0.009837	0.0679736	2
14	Negative	untargeted	1.13	253.0932	[M-H]-	656504	Galactosylglycerol	12.65	UP	8.56E-09	2.472E-06	2
15	Positive	targeted	0.861	184.03049	[M+H]+	177491	Homocysteic acid	12.22	UP	1.45E-09	4.856E-07	1
16	Negative	untargeted	3.64	109.0295	[M-H]-	289	Pyrocatechol	11.46	UP	3.09E-05	0.0010423	2
17	Negative	untargeted	7.14	129.092	[M-H]-	7770	Propyl butyrate	8.03	UP	7.75E-07	6.458E-05	2
18	Positive	untargeted	8.03	313.2368	[M+H]+	6439847	9(S)-HPODE	8.01	UP	4.98E-07	4.689E-05	2
19	Negative	targeted	6.091	204.06648	[M-H]-	709625	N-Cinamoylglycine	7.33	DOWN	1.62E-05	0.0006202	1
20	Negative	untargeted	5.18	203.0018	[M-H]-	22473	O-Methoxycatechol-O-sulfate	6.96	UP	0.000197	0.0044706	2
21	Negative	untargeted	7.82	337.2382	[M-H]-	5283144	8,9-DiHETrE	6.70	DOWN	0.004464	0.0399652	2
22	Negative	untargeted	5.57	184.0976	[M-H]-	10932172	2-Hepteneoylglycine	6.05	UP	0.000127	0.0032287	2
23	Negative	untargeted	4.73	424.0035	[M-H2O-H]-	135398619	Guanosine diphosphate	4.99	UP	0.029328	0.1246911	2
24	Negative	untargeted	9.48	633.4896	[M-H]-	52929605	PA(O-20:0/12:0)	4.98	UP	0.000315	0.0063858	2
25	Positive & Negative	untargeted	3.72	248.1133	[M+H]+	22833583	Malonylcarnitine	4.86	DOWN	0.02927	0.2263684	2
26	Positive	untargeted	5.37	225.11	[M+]+	25141159	9,10-Dihydroxy-2-decenoic acid	4.80	UP	2.42E-09	6.506E-07	2
27	Positive	untargeted	8.15	542.3233	[M+]+	11005824	LysoPC(18:2(9Z,12Z))	4.26	UP	4.86E-07	4.665E-05	2
28	Negative	untargeted	8.45	241.1804	[M-H]-	454064	3-Oxotetradecanoic acid	4.08	DOWN	0.019901	0.100771	2
29	Negative	untargeted	9.14	301.2167	[M-H]-	446284	Eicosapentaenoic acid	3.91	UP	1.96E-08	4.31E-06	2
30	Positive & Negative	targeted	0.947	136.06177	[M+H]+	190	Adenine	3.90	UP	0.000341	0.0120674	1
31	Positive	untargeted	10.96	423.3463	[M+]+	131779656	MG(0:0/i-21:0/0:0)	3.84	UP	0.000808	0.0235049	2
32	Negative	targeted	9.057	373.27487	[M-H]-	5283906	5β-Cholanic acid-3-one	3.43	DOWN	0.003028	0.0307655	1
33	Positive & Negative	targeted	5.559	138.05489	[M+H]+	227	Anthranilic acid	3.40	UP	1.5E-07	1.814E-05	1
34	Positive	targeted	8.05	286.14587	[M+H]+	638024	Piperine	3.35	UP	0.00102	0.0282145	1
35	Positive	untargeted	9.54	725.5555	[M+]+	53481781	SM(d18:0/16:1(9Z))	3.31	UP	0.034029	0.2410904	2
36	Positive	targeted	3.182	166.08611	[M+H]+	6140	L-Phenylalanine	3.18	UP	0.002023	0.0472038	1
37	Positive	targeted	1.113	137.04549	[M+H]+	790	Hypoxanthine	3.01	UP	0.018459	0.183349	1
38	Negative	untargeted & targeted	4.73	425.012	[2M-H]-	10258	Indoxyl sulfate	2.98	UP	0.007214	0.0560945	2
39	Positive	untargeted	6.8	500.3036	[M+H]+	12443252	Tauroursodeoxycholic acid	2.94	UP	0.005115	0.090243	2
40	Positive	untargeted	8.18	313.2371	[M+H]+	16061067	12,13-DiHODE	2.88	UP	5.36E-06	0.0003507	2

41	Positive & Negative	targeted & untargeted	5.033	180.06541	[M+H]+	464	Hippuric acid	2.85	UP	9.6E-07	8.015E-05	1
42	Negative	untargeted	7.78	183.1389	[M-H]-	7714	xi-γ-Undecalactone	2.81	DOWN	0.033568	0.1351919	2
43	Positive	untargeted	4.74	134.06	[M+H]+	321710	1,3-Dihydro-(2H)-indol-2-one	2.78	UP	0.01024	0.1353922	2
44	Negative	untargeted	5.57	163.0397	[M-H]-	997	Phenylpyruvic acid	2.77	UP	0.000227	0.004992	2
45	Positive	untargeted	10.71	546.3536	[M+H]+	53480467	LysoPC(20:3(5Z,8Z,11Z))	2.73	UP	0.000917	0.0260532	2
46	Negative	untargeted	7.97	213.1492	[M-H]-	439717	3-Oxododecanoic acid	2.71	DOWN	0.034508	0.1371923	2
47	Negative	untargeted	6.59	197.1552	[M-H]-	5282729	trans-Dodec-2-enoic acid	2.68	DOWN	0.022763	0.1082778	2
48	Positive	untargeted	10.71	538.3865	[M+H]+	24779321	PC(O-18:0/1:0)	2.64	UP	0.003679	0.0716922	2
49	Positive	untargeted	7.33	253.1434	[M+H]+	131751906	3alpha-Hydroxyoreadone	2.58	UP	4.23E-06	0.0002803	2
50	Positive	untargeted	0.72	175.1077	[M+H]+	439378	L-Theanine	2.56	UP	2.13E-08	3.887E-06	2
51	Positive	untargeted	8.91	331.2618	[M+H]+	6441454	Docosapentaenoic acid (22n-6)	2.51	DOWN	0.030155	0.2288959	2
52	Negative	untargeted	8.54	343.2275	[M-H]-	6439179	17-HDoHE	2.46	UP	0.001088	0.0155482	2
53	Negative	untargeted	8.44	303.1963	[M-H]-	102030	16a-Hydroxydehydroisoandrosterone	2.44	UP	0.014936	0.0848794	2
54	Negative	untargeted	8.21	732.4813	[M-H]-	52925317	PS(16:1(9Z)/16:0)	2.43	DOWN	0.014926	0.0848794	2
55	Positive	untargeted	9.62	331.2632	[M+H]+	5497182	Docosapentaenoic acid	2.34	DOWN	0.00016	0.0064177	2
56	Negative	untargeted	8.17	215.1651	[M-H]-	5312805	3-Hydroxydodecanoic acid	2.33	DOWN	0.021982	0.1066957	2
57	Negative & Positive	targeted & untargeted	6.592	186.05617	[M-H]-	5375048	Indole-3-acrylic acid	2.32	UP	3.52E-06	0.0001939	1
58	Positive	untargeted	0.96	141.0658	[M+NH4]+	938	Nicotinic acid	2.31	DOWN	0.022441	0.2008859	2
59	Positive	untargeted	8.57	570.3538	[M+H]+	53480473	LysoPC(22:5(4Z,7Z,10Z,13Z,16Z))	2.29	UP	1.11E-06	8.851E-05	2
60	Positive	targeted & untargeted	6.903	190.08569	[M+H]+	3744	Indole-3-propionic acid	2.29	UP	5.41E-05	0.0025932	1
61	Negative	targeted & untargeted	0.876	74.02428	[M-H]-	750	L-Glycine	2.25	DOWN	0.036861	0.1420071	1
62	Positive & Negative	targeted	0.681	166.05267	[M+H]+	158980	L-Methionine sulfoxide	2.23	UP	0.002716	0.0592375	1
63	Positive	untargeted	1.87	326.0871	[M+H]+	124202061	Dihyroxy-1H-indole glucuronide I	2.22	UP	0.047875	0.2770504	2
64	Negative	untargeted	4.26	350.0876	[M-H]-	124202110	Indole-3-acetic-acid-O-glucuronide	2.21	UP	0.012693	0.0775838	2
65	Positive	untargeted	1.38	353.1348	[M+H]+	439179	Cotinine glucuronide	2.21	DOWN	0.035213	0.2437226	2
66	Negative & Positive	untargeted	5.69	228.1239	[M-H]-	53481615	Butenylcarnitine	2.20	DOWN	0.011983	0.075153	2
67	Negative	untargeted	7.99	181.1231	[M-H]-	131751613	Methyl 4,8-decadienoate	2.20	UP	0.010291	0.0695811	2
68	Negative	untargeted	4.62	269.0663	[M-H]-	119239	Phenyl glucuronide	2.20	UP	0.020951	0.1037476	2
69	Negative	targeted	5.206	153.01917	[M-H]-	19	2,3-dihydroxybenzoic acid	2.19	UP	0.000358	0.007016	1
70	Positive	untargeted	10.01	828.5476	[M+H]+	52923373	PC(20:5(5Z,8Z,11Z,14Z,17Z)/20:4(5Z,8Z,11Z,14Z))	2.17	UP	0.002079	0.0479358	2
71	Positive	untargeted	8.58	279.2316	[M+H]+	5280934	Alpha-Linolenic acid	2.17	UP	0.00286	0.0612792	2
72	Positive	untargeted	9.31	546.3528	[M+]+	497299	LysoPC(18:0)	2.12	UP	0.015216	0.1666859	2
73	Positive & Negative	targeted & untargeted	1.217	385.12784	[M+H]+	439155	S-(5′-Adenosyl)-L-homocysteine	2.11	UP	0.005397	0.0928428	1
74	Positive & Negative	untargeted	0.73	229.1184	[M+H]+	11902892	Prolylhydroxyproline	2.10	DOWN	7.08E-07	6.352E-05	2
75	Negative	untargeted	4.07	230.9965	[M-H]-	10657183	2,4-Dihydroxyacetophenone 5-sulfate	2.10	DOWN	0.000775	0.0121928	2
76	Negative	untargeted	5.67	115.0765	[M-H]-	8892	Caproic acid	2.06	UP	1.32E-07	1.667E-05	2
77	Negative	untargeted	4.8	274.1296	[M-H]-	71317118	Glutarylcarnitine	2.06	DOWN	0.037702	0.1435659	2
78	Positive	untargeted	0.95	231.17	[M+H]+	352038	Leucyl-Valine	2.05	DOWN	0.011114	0.1421996	2
79	Negative	untargeted	4.63	137.0242	[M-H]-	135	4-Hydroxybenzoic acid	2.05	UP	2.66E-07	2.984E-05	2
80	Negative	untargeted	2.53	340.1406	[M-H]-	7009650	Histidinyl-Tryptophan	2.04	DOWN	0.021453	0.1052665	2

Supplementary Table 5.2. Cont'd.

81	Negative	untargeted	3.31	248.0782	[M-H]-	1076	S-Acetyldihydrolipoamide	2.04	UP	0.010151	0.0691479	2
82	Positive	untargeted	7.86	253.1432	[M+H]+	5280344	Ubiquinol	2.04	UP	2.18E-06	0.0001587	2
83	Positive	untargeted	5.91	181.0861	[M+H]+	23378291	3-(3-Hydroxyphenyl)-2-methylpropionic acid	2.03	DOWN	0.005558	0.0946633	2
84	Negative	untargeted	5.77	217.1081	[M-H]-	128458	2-Hydroxydecanedioic acid	2.02	DOWN	0.010411	0.0700965	2
85	Negative	untargeted	9.56	476.3164	[M-H]-	42607471	PE(P-19:1(12Z)/0:0)	2.01	DOWN	0.009402	0.0661116	2
86	Negative	untargeted	5.22	447.1552	[2M-H]-	89	Hydroxykynurenine	2.00	UP	0.000103	0.0028054	2
87	Negative	untargeted	8.64	243.1965	[M-H]-	5288266	(R)-3-Hydroxy-tetradecanoic acid	1.99	DOWN	0.030878	0.129044	2
88	Negative	untargeted	8.19	375.2171	[M-H]-	44251266	Resolvin D1	1.98	UP	0.003051	0.0308743	2
89	Positive	untargeted	7.41	600.3287	[M+]+	52926292	PS(22:2(13Z,16Z)/0:0)	1.98	UP	0.000105	0.0045306	2
90	Negative	untargeted	0.95	178.0181	[M-H]-	1080	S-Carboxymethyl-L-cysteine	1.97	UP	0.004196	0.038293	2
91	Negative	untargeted	6.11	201.0221	[M-H]-	20822574	4-Ethylphenyl sulfate	1.96	UP	0.033744	0.135452	2
92	Negative	untargeted	9.31	464.3146	[M-H]-	42607470	PE(P-18:0/0:0)	1.95	UP	0.006282	0.0504971	2
93	Negative	untargeted	8.74	343.2275	[M-H]-	5995	Testosterone propiote	1.91	UP	0.000341	0.0067654	2
94	Negative	untargeted	7.61	187.1341	[M-H]-	15241411	2,6-Dimethyl-7-octene-2,3,6-triol	1.90	DOWN	0.018388	0.0963034	2
95	Negative	untargeted	6.77	130.0659	[M-H]-	6736	3-Methylindole	1.89	DOWN	0.00525	0.0446395	2
96	Positive & Negative	targeted & untargeted	3.453	243.09721	[M+H]+	5789	Thymidine	1.89	DOWN	0.001743	0.0419935	1
97	Negative	untargeted	8.55	790.5601	[M-H]-	9547096	PS(18:0/18:0)	1.88	UP	0.000225	0.0049717	2
98	Negative	untargeted	0.77	101.0243	[M-H]-	96	Acetoacetic acid	1.85	DOWN	0.010747	0.0711398	2
99	Negative	untargeted	6.84	365.196	[M-H]-	5280877	20-Carboxy-leukotriene B4	1.85	UP	0.033611	0.1351919	2
100	Negative & Positive	untargeted	7.22	329.2336	[M-H]-	14968868	9,10,13-TriHOME	1.85	UP	0.02167	0.1058822	2
101	Positive & Negative	untargeted	9.31	329.2469	[M+H]+	445580	Docosahexaenoic acid	1.84	UP	6.67E-05	0.0030782	2
102	Positive	untargeted	10.71	780.5492	[M+CH3OH+H]+	5283497	PE(O-16:1(1Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	1.84	UP	0.022731	0.2017787	2
103	Positive	untargeted	3.54	289.033	[M+H]+	46878379	Sedoheptulose 7-phosphate(2-)	1.84	UP	0.03793	0.253186	2
104	Negative	untargeted	8.42	295.2281	[M-H]-	6443013	13S-hydroxyoctadecadienoic acid	1.84	UP	3.99E-05	0.0012788	2
105	Negative	untargeted	5.32	336.0726	[M-H]-	53481014	2,8-Dihydroxyquinoline-beta-D-glucuronide	1.83	DOWN	0.016006	0.0886546	2
106	Positive	untargeted	9.68	378.2624	[M+H]+	53477509	15-HETE-Gly	1.83	UP	0.006327	0.1021383	2
107	Negative & Positive	targeted & untargeted	1.672	227.06683	[M-H]-	13712	2'-Deoxyuridine	1.80	DOWN	0.000419	0.0079575	1
108	Negative	targeted	8.545	295.22748	[M-H]-	5312830	(+)9-HODE	1.79	UP	9.45E-06	0.0004132	1
109	Positive	untargeted	4.16	474.1734	[M+H]+	135403648	Folinic acid	1.79	DOWN	0.006361	0.1025086	2
110	Negative	untargeted	5.95	337.1429	[M-H]-	3035848	Nicotine glucuronide	1.79	DOWN	0.043074	0.1535801	2
111	Positive & Negative	untargeted	4.06	190.1075	[M+H]+	131802903	3-Hydroxyhexanoylglycine	1.78	DOWN	0.035267	0.2437226	2
112	Negative	untargeted	6.65	213.1128	[M-H]-	131751112	alpha-Carboxy-delta-decalactone	1.78	DOWN	0.028561	0.1235377	2
113	Negative	untargeted	9.93	447.3479	[M-H]-	13475120	2-Deoxycastasterone	1.76	UP	0.000353	0.006945	2
114	Negative	untargeted	3.96	153.0558	[M-H]-	82755	Hydroxytyrosol	1.74	UP	0.049478	0.1618026	2
115	Negative	untargeted	2.57	164.0354	[M-H]-	101399	Formylanthranilic acid	1.74	UP	0.01021	0.06944	2
116	Negative	targeted	0.632	130.05095	[M-H]-	5810	Hydroxy-Proline	1.72	DOWN	0.012359	0.0763468	1
117	Positive	untargeted	2.83	159.0288	[M+H]+	5280500	2-Maleylacetate	1.71	DOWN	0.018323	0.1827723	2
118	Negative	targeted	1.347	130.08711	[M-H]-	6106	L-Leucine	1.70	UP	0.006253	0.050466	1
119	Negative	targeted	4.846	295.96371	[M-H]-	3639	Hydrochlorothiazide	1.69	UP	0.002972	0.0303873	1
120	Positive	untargeted	10.41	432.3465	[M+H]+	6710071	N-Stearoyl phenylalanine	1.68	UP	0.011822	0.1462249	2

Supplementary Table 5.2. Cont'd.

121	Positive & Negative	targeted	5.676	165.05441	[M+H]+	637542	p-Coumaric Acid	1.68	UP	0.000772	0.0228109	1
122	Positive	untargeted	0.88	162.1122	[M+CH3OH+H]+	849	Pipecolic acid	1.67	UP	0.000597	0.0184796	2
123	Positive	untargeted	3.33	127.0502	[M+H]+	1135	Thymine	1.67	DOWN	0.007155	0.1103565	2
124	Positive	untargeted	1.38	126.0549	[M+H]+	51090994	3,6-Dihydronicotinic acid	1.66	DOWN	0.027444	0.2204672	2
125	Negative	untargeted	8.9	299.2589	[M-H]-	21993177	2-Hydroxystearate	1.66	DOWN	3.66E-05	0.0012019	2
126	Negative	untargeted	0.97	119.0348	[M-H]-	150929	(S)-3,4-Dihydroxybutyric acid	1.65	DOWN	0.001915	0.0229469	2
127	Positive	untargeted	9.54	398.2527	[M+NH4]+	5317587	[6]-Gingerdiol 3,5-diacetate	1.65	UP	0.01962	0.1889031	2
128	Positive	untargeted	8.33	568.3402	[M+H]+	10415542	LysoPC(22:6(4Z,7Z,10Z,13Z,16Z,19Z))	1.64	UP	8.49E-05	0.0038404	2
129	Positive & Negative	untargeted	7.98	370.2588	[M+NH4]+	5283116	Prostaglandin E2	1.64	UP	0.021188	0.1954573	2
130	Positive & Negative	untargeted	1.82	258.1199	[M+H]+	135476770	4a-Hydroxytetrahydrobiopterin	1.63	UP	0.00402	0.0772643	2
131	Negative	untargeted	7.68	180.1027	[M-H]-	197139	3-O-Methyl-a-methyldopamine	1.63	UP	5.58E-05	0.0016579	2
132	Negative	untargeted	3.26	238.0869	[M-H2O-H]-	71920	Glycerophosphocholine	1.63	UP	0.000273	0.0058037	2
133	Negative	targeted & untargeted	0.657	157.03651	[M-H]-	204	Allantoin	1.63	DOWN	0.028568	0.1235377	1
134	Negative	untargeted	3.21	188.0925	[M-H]-	87772907	1-Hydroxyhexanoylglycine	1.63	DOWN	0.015588	0.0872828	2
135	Negative	untargeted	4.2	151.0399	[M-H2O-H]-	91528	3,4-Dihydroxyphenylglycol	1.62	DOWN	0.000536	0.0094711	2
136	Positive	untargeted	1.63	225.0868	[M+H]+	12313328	L-Nicotianine	1.60	DOWN	0.00377	0.073037	2
137	Positive	untargeted	1.68	289.0248	[M+CH3OH+H]+	54676864	Ascorbic acid-2-sulfate	1.60	UP	0.020108	0.1909366	2
138	Negative	untargeted	5.24	155.0714	[M-H]-	54042244	8-Hydroxy-5,6-octadienoic acid	1.59	UP	0.002436	0.0271301	2
139	Positive	targeted	4.651	206.04477	[M+H]+	5699	Xanthurenic acid	1.59	DOWN	0.002769	0.0601327	1
140	Positive	untargeted	8.02	558.3192	[M+H]+	24779232	PC(6:2(3E,5E)/14:2(11E,13E))	1.58	UP	0.007475	0.1139085	2
141	Negative	untargeted	2.47	303.0831	[M-H]-	188803	N-Acetylaspartylglutamic acid	1.58	DOWN	0.010683	0.0710112	2
142	Negative	untargeted	6.32	135.0814	[M-H]-	6943	2-Isopropylphenol	1.57	UP	0.005483	0.0461653	2
143	Positive	untargeted	2.37	103.0754	[M+H]+	10430	Isovaleric acid	1.57	DOWN	0.026003	0.2173035	2
144	Positive & Negative	targeted	0.967	245.07666	[M+H]+	15047	β-Pseudouridine	1.56	DOWN	0.005371	0.0925582	1
145	Positive	untargeted	4.55	127.1117	[M+H]+	87170471	(3E,5Z)-3,5-Octadien-1-ol	1.56	DOWN	0.016535	0.1725827	2
146	Positive	untargeted	5.91	199.0965	[M+H]+	124202116	2,3-Methylene suberic acid	1.55	DOWN	0.004856	0.0877415	2
147	Positive	targeted	0.875	146.11732	M+	6060	Acetylcholine	1.55	UP	0.010838	0.1405284	1
148	Positive	untargeted	8.1	490.2906	[M+]+	460604	LysoPC(14:0)	1.54	UP	0.010711	0.1398147	2
149	Positive	untargeted	9.72	382.3312	[M+H]+	15221000	N-Stearoyl proline	1.54	UP	0.046582	0.275418	2
150	Positive	untargeted	9.19	696.4981	[M+H]+	52925054	PE(P-16:0/18:4(6Z,9Z,12Z,15Z))	1.53	UP	0.016855	0.1746011	2
151	Positive	untargeted	8.54	991.6699	[2M+H]+	460602	LysoPC(16:0)	1.53	UP	0.021972	0.1985219	2
152	Positive	untargeted	6.35	335.2214	[M+H]+	5280885	Delta-12-Prostaglandin J2	1.53	DOWN	0.047865	0.2770504	2
153	Negative	targeted & untargeted	4.342	181.05052	[M-H]-	9378	DL-p-Hydroxyphenyllactic acid	1.52	UP	0.001116	0.0158554	1
154	Negative & Positive	targeted & untargeted	1.013	148.04352	[M-H]-	6137	L-Methionine	1.52	UP	0.04262	0.1528366	1
155	Positive	untargeted	6.92	253.1798	[M+H]+	51551	Dihydromyoporone	1.51	DOWN	0.012829	0.1524412	2
156	Positive	untargeted	1.05	187.1079	[M+H]+	83525	Alanyl-Proline	1.50	UP	1.02E-05	0.000616	2
157	Negative	untargeted	6.55	134.061	[M-H]-	904	N-Acetylarylamine	1.50	UP	0.001162	0.0162048	2
158	Negative	untargeted	3.83	206.0456	[M-H]-	472	4-(2-Aminophenyl)-2,4-dioxobutanoic acid	1.50	UP	0.008138	0.0598817	2
159	Negative	untargeted	1.96	182.0457	[M-H]-	6723	4-Pyridoxic acid	1.49	DOWN	0.004868	0.0423449	2
160	Positive	targeted & untargeted	1.07	152.05316	[M+H]+	135398634	Guanine	1.49	UP	0.011188	0.1427736	1

Supplementary Table 5.2. Cont'd.

161	Negative	untargeted	5.01	179.0714	[M-H]-	66336	3-Methoxybenzenepropanoic acid	1.48	UP	0.023374	0.1098902	2
162	Negative	untargeted	6.77	171.1025	[M-H]-	75704	9-Oxo-nonoic acid	1.48	DOWN	0.004749	0.0417183	2
163	Negative	untargeted	0.76	228.0814	[M-H]-	5351619	Ergothioneine	1.48	UP	0.005216	0.0444443	2
164	Positive	untargeted	8.42	880.6029	[M+H]+	131820880	PE-NMe(11D5/11M5)	1.48	DOWN	0.011495	0.144031	2
165	Negative	untargeted	4.25	135.045	[M-H]-	999	Phenylacetic acid	1.47	UP	0.001244	0.0170875	2
166	Positive	targeted	0.71	104.0703	[M+H]+	223130	γ-Aminobutyric acid (GABA)	1.47	DOWN	0.023225	0.2043962	1
167	Negative	untargeted	4.85	131.0713	[M-H]-	439960	D-Leucic acid	1.47	DOWN	0.000824	0.0126897	2
168	Negative	untargeted	10.02	639.5368	[2M-H]-	3032822	Pregnediol	1.47	DOWN	0.038941	0.1463285	2
169	Positive	untargeted	0.97	74.02362	[M+H]+	3080609	Dehydroglycine	1.46	UP	0.011942	0.1467844	2
170	Negative	targeted	0.848	116.07169	[M-H]-	6287	L-Valine	1.46	UP	0.039428	0.1470132	1
171	Positive	untargeted	8.74	510.3549	[M+H]+	24779463	LysoPC(17:0)	1.46	UP	0.003156	0.0640806	2
172	Negative	untargeted	9.22	297.2437	[M-H]-	643684	Ricinoleic acid	1.46	DOWN	0.035221	0.1382601	2
173	Positive	untargeted	9.43	362.2682	[M+H]+	5283389	N-Arachidonoyl glycine	1.45	UP	0.027188	0.2199934	2
174	Positive	untargeted	0.72	128.0705	[M+H]+	4479247	alpha-(Methylenecyclopropyl)glycine	1.45	UP	0.042637	0.2653918	2
175	Positive	untargeted	8.04	351.2164	[M+H]+	5280937	Prostaglandin E3	1.45	UP	0.042124	0.2653918	2
176	Positive	untargeted	5.67	174.1123	[M+H]+	70912	N-Acetylleucine	1.44	DOWN	0.008684	0.1238675	2
177	Negative	untargeted	9.81	305.2481	[M-H]-	5280581	8,11,14-Eicosatrienoic acid	1.44	DOWN	0.046693	0.1573673	2
178	Negative	untargeted	3.53	167.0348	[M-H]-	547	3,4-Dihydroxybenzeneacetic acid	1.43	UP	0.044122	0.1550507	2
179	Positive & Negative	targeted	5.774	174.11241	[M+H]+	99463	Hexanoyl glycine	1.42	DOWN	0.030434	0.2299334	1
180	Positive	untargeted	0.69	212.1004	[M+]+	65072	Homocitrulline	1.42	DOWN	0.043678	0.2684295	2
181	Positive	untargeted	0.72	174.0193	[M+]+	82142	S-Methyl-L-cysteine sulfoxide	1.42	DOWN	0.017137	0.1764724	2
182	Negative	untargeted	8.59	319.2275	[M-H]-	5280733	5-HETE	1.41	UP	0.015677	0.08755	2
183	Negative	untargeted	3.21	326.125	[M-H]-	101039148	N-(1-Deoxy-1-fructosyl)phenylalanine	1.40	DOWN	0.023082	0.1090912	2
184	Negative	untargeted	5.32	143.0715	[M-H]-	151138	4-Hydroxycyclohexylcarboxylic acid	1.40	UP	0.000191	0.0043988	2
185	Positive	untargeted	8.5	504.3072	[M+H]+	53480951	LysoPE(20:3(5Z,8Z,11Z)/0:0)	1.40	DOWN	0.045225	0.2718939	2
186	Negative	untargeted	5.21	260.1143	[M-H]-	53481628	Methylmalonylcarnitine	1.39	DOWN	0.0073	0.0563283	2
187	Negative & Positive	targeted & untargeted	4.878	190.05072	[M-H]-	1826	5-Hydroxy Indole-3-acetic acid	1.39	DOWN	0.000508	0.0091285	1
188	Negative	untargeted	3.62	168.0667	[M-H]-	1054	Pyridoxine	1.39	UP	0.002427	0.0270672	2
189	Negative	untargeted	8.27	524.2772	[M-H]-	52925132	LysoPE(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	1.39	UP	0.011229	0.0724696	2
190	Negative	untargeted	9.94	311.2586	[M-H2O-H]-	3084463	MG(16:0/0:0/0:0)	1.38	DOWN	0.041783	0.1515853	2
191	Positive	untargeted	8.79	385.3053	[M+H]+	52922071	N-Palmitoyl glutamine	1.38	UP	0.033358	0.2398587	2
192	Positive & Negative	untargeted	1.21	191.0551	[M+H]+	439351	3-Dehydroquite	1.38	DOWN	0.005602	0.0946845	2
193	Negative	untargeted	0.95	246.0054	[M+Cl]-	587	Phosphocreatine	1.38	DOWN	0.048097	0.1595962	2
194	Positive	untargeted	8.56	518.321	[M+H]+	52924045	LysoPC(18:3(6Z,9Z,12Z))	1.38	UP	0.009439	0.1295031	2
195	Negative	untargeted	5.7	242.1396	[M-H]-	91825636	Tiglylcarnitine	1.37	DOWN	0.01047	0.0701103	2
196	Negative	untargeted	8.07	227.2012	[M-H]-	11005	Myristic acid	1.37	DOWN	0.041492	0.151091	2
197	Positive	targeted	5.038	246.16928	[M+H]+	53481619	Valeryl-L-carnitine	1.37	UP	0.029844	0.2283231	1
198	Negative	untargeted	3.32	281.0887	[M-H]-	65095	1-Methylinosine	1.37	DOWN	0.008158	0.0599726	2
199	Negative	untargeted	3.87	230.1395	[M-H]-	439829	Butyrylcarnitine	1.36	UP	0.03316	0.1344281	2
200	Positive	targeted	1.958	153.06557	[M+H]+	69698	Nudifloramide (2-Py)	1.36	DOWN	0.040883	0.2628231	1

Supplementary Table 5.2. Cont'd.

201	Negative	untargeted	5.15	85.06574	[M-H]-	11552	Iso-Valeraldehyde	1.36	DOWN	0.003227	0.0320173	2
202	Positive	untargeted	5.39	86.09631	[M+H]+	8082	Piperidine	1.36	UP	0.004934	0.0881733	2
203	Positive	targeted	0.787	141.0657	[M+H]+	75810	1-Methyl-4-imidazoleacetic acid	1.35	DOWN	0.001063	0.0289945	1
204	Negative	untargeted	7.13	141.0922	[M-H]-	104896	4-ene-Valproic acid	1.35	UP	0.013698	0.081449	2
205	Positive	untargeted	8.36	566.3211	[M+]+	24779476	LysoPC(20:4(5Z,8Z,11Z,14Z))	1.35	UP	0.034478	0.2423389	2
206	Negative	untargeted	6.21	217.1081	[M-H]-	3017884	3-Hydroxysebacic acid	1.35	UP	0.012054	0.0752711	2
207	Positive	untargeted	8.69	480.3432	[M+H]+	10917802	LysoPC(P-16:0)	1.35	UP	0.001187	0.0314933	2
208	Negative	untargeted	1.07	183.0157	[M-H]-	101957720	5-Hydroxyisourate	1.34	UP	0.009534	0.0666959	2
209	Positive	untargeted	0.79	174.0874	[M+H]+	558	2-Oxoarginine	1.34	UP	0.007617	0.1149665	2
210	Positive & Negative	targeted	6.107	162.0544	[M+H]+	69867	Indole-3-carboxylic acid	1.33	DOWN	0.034912	0.2436178	1
211	Positive & Negative	targeted & untargeted	4.324	158.08104	[M+H]+	169485	3-Methylcrotonyl glycine	1.33	DOWN	0.018665	0.1846622	1
212	Positive	untargeted	6.49	200.1282	[M+H]+	131802983	4-Octenoylglycine	1.33	UP	0.01765	0.1792046	2
213	Positive	targeted	4.302	160.09663	[M+H]+	193872	N-(2-Methylbutyryl)glycine	1.33	DOWN	0.006601	0.1051152	1
214	Positive	untargeted	3.37	282.1195	[M+H]+	27476	1-Methyladenosine	1.33	DOWN	0.025519	0.214925	2
215	Negative	untargeted	8.74	293.2122	[M-H]-	9839084	9-OxoODE	1.33	DOWN	0.015128	0.08579	2
216	Positive & Negative	targeted & untargeted	0.723	116.07052	[M+H]+	145742	L-Proline	1.33	UP	0.02134	0.1956845	1
217	Positive	untargeted	8.96	667.4762	[M+]+	70699022	PE-Cer(d14:2(4E,6E)/19:0)	1.33	UP	0.041149	0.2635401	2
218	Positive	targeted & untargeted	0.718	114.06601	[M+H]+	588	Creatinine	1.32	DOWN	0.034737	0.2434662	1
219	Positive	untargeted	0.75	198.1237	[M+H]+	3083620	L-Histidine trimethylbetaine	1.32	UP	0.015265	0.1666859	2
220	Negative	untargeted	5.14	216.1238	[M-H]-	107738	Propionylcarnitine	1.31	DOWN	0.042778	0.1532014	2
221	Positive & Negative	targeted & untargeted	5.782	208.09656	[M+H]+	74839	N-Acetyl-L-phenylalanine	1.31	UP	0.000499	0.015959	1
222	Negative	targeted	0.657	300.03897	[M-H]-	7115	N-acetyl-glucosamine-1-phosphate	1.31	DOWN	0.002306	0.0261218	1
223	Positive & Negative	untargeted	0.74	203.1503	[M+H]+	169148	Symmetric dimethylarginine	1.31	DOWN	0.001543	0.0387244	2
224	Negative	untargeted	4.6	168.0665	[M-H2O-H]-	443435	N-(3-(S)-Hydroxybutyryl)homoserine lactone	1.31	UP	0.048877	0.1608996	2
225	Negative	untargeted	4.62	151.0399	[M-H]-	127	p-Hydroxyphenylacetic acid	1.30	UP	0.007392	0.0567164	2
226	Negative	untargeted	5.24	131.0713	[M-H]-	10796774	2-Hydroxy-3-methylpentanoic acid	1.29	DOWN	0.002568	0.027943	2
227	Positive	untargeted	0.68	70.06519	[M+H]+	79803	1-Pyrroline	1.29	UP	0.041505	0.265347	2
228	Negative	untargeted	8.27	315.2536	[M-H]-	12235230	(9S,10S)-9,10-Dihydroxyoctadecanoate	1.29	UP	0.013268	0.0798347	2
229	Negative	targeted	0.886	155.00961	[M-H]-	967	Orotic acid	1.28	UP	0.007999	0.059183	1
230	Negative	untargeted	7.56	185.1183	[M-H]-	5282982	3-Oxodecanoic acid	1.28	UP	0.020977	0.103815	2
231	Positive & Negative	untargeted	4.6	262.165	[M+H]+	57357187	Hydroxyisovaleroyl carnitine	1.27	DOWN	0.019198	0.1872004	2
232	Negative	untargeted	3.77	99.04511	[M-H2O-H]-	69362	3-Hydroxyisovaleric acid	1.27	DOWN	0.01582	0.0880268	2
233	Negative	untargeted	4.06	126.056	[M-H]-	24771808	(S)-2,3,4,5-Tetrahydropiperidine-2-carboxylate	1.27	UP	0.013019	0.0788045	2
234	Positive	untargeted	5.59	452.2767	[M+H]+	53480923	LysoPE(0:0/16:1(9Z))	1.27	UP	0.016254	0.1716636	2
235	Positive	untargeted	4.62	295.1657	[M+CH3OH+H]+	7020641	Phenylalanylproline	1.27	DOWN	0.000433	0.0144685	2
236	Positive	untargeted	1.06	229.1546	[M+H]+	80817	Leucylproline	1.27	DOWN	0.022995	0.2035467	2
237	Positive	untargeted	9.55	281.2473	[M+H]+	5280450	Linoleic acid	1.26	UP	0.013389	0.1549391	2
238	Positive	untargeted	0.97	189.1232	[M+H]+	92832	N6-Acetyl-L-lysine	1.26	DOWN	0.00229	0.052316	2
239	Negative	untargeted	0.68	117.0192	[M-H]-	1110	Succinic acid	1.26	UP	0.03092	0.129143	2
240	Negative	untargeted	4	231.0872	[M-H]-	20111767	Glycerol 1-propanoate diacetate	1.25	UP	0.044521	0.1553196	2

Supplementary Table 5.2. Cont'd.

241	Negative	targeted	4.431	158.08221	[M-H]-	546304	Isovaleroyglycine	1.25	UP	0.034996	0.1379129	1
242	Positive	targeted	1.372	198.07642	[M+H]+	6047	3,4-Dihydroxy-L-phenylalanine (L-DOPA)	1.25	DOWN	0.039114	0.2572899	1
243	Negative	untargeted	6.06	157.0871	[M-H]-	3731686	2-n-Propyl-4-oxopentanoic acid	1.25	UP	0.037292	0.1429385	2
244	Positive	targeted	0.905	142.0296	[M+H]+	1015	O-Phosphorylethanolamine	1.24	DOWN	0.026822	0.2186942	1
245	Negative	targeted	1.373	130.05086	[M-H]-	88064	N-Acetyl-L-alanine	1.24	DOWN	0.000618	0.0104844	1
246	Negative	untargeted	3.78	163.0609	[M-H]-	25310	L-Fucose	1.24	DOWN	0.001736	0.0215552	2
247	Negative	untargeted	0.68	256.0593	[M+Cl]-	84265	N-Acetylgalactosamine	1.23	DOWN	0.005532	0.0464065	2
248	Negative	targeted & untargeted	6.165	144.04527	[M-H]-	10256	Indole-3-carboxaldehyde	1.23	UP	0.049969	0.1626091	1
249	Positive	targeted	1.031	228.09721	[M+H]+	13711	2'-Deoxycytidine (dC)	1.23	DOWN	0.015536	0.1682243	1
250	Positive & Negative	targeted	1.416	182.08086	[M+H]+	6057	L-Tyrosine	1.22	UP	0.013868	0.1581233	1
251	Positive	untargeted	5.79	192.102	[M+H]+	12835	5-Methoxytryptophol	1.22	UP	0.025481	0.214925	2
252	Positive	targeted & untargeted	0.699	76.07561	[M+H]+	1145	Trimethylamine N-oxide	1.22	DOWN	0.017358	0.1776586	1
253	Positive	untargeted	8.2	454.3293	[M+H]+	15298292	1-Tetradecyl-sn-glycero-3-phosphocholine	1.22	UP	0.00113	0.0303948	2
254	Negative	targeted	1.543	103.04011	[M-H]-	441	DL-3-Hydroxybutyric acid	1.22	DOWN	0.033797	0.1355643	1
255	Negative	untargeted	4.56	293.1241	[M-H]-	76463850	Ethyl (S)-3-hydroxybutyrate glucoside	1.21	UP	0.009259	0.0655566	2
256	Positive	targeted	4.97	246.1693	[M+H]+	169235	Isovaleryl-L-carnitine	1.21	UP	0.029697	0.2278795	1
257	Positive	untargeted	3.53	227.1023	[M+H]+	1021	Porphobilinogen	1.20	DOWN	0.009418	0.1295031	2

Supplementary Table 5.2. Cont'd.

Supplementary Table 5.3 Unique list of 73 cortical brain metabolites altered in feces of BRB-rich diet treated group vs. standard AIN-76A diet treated group.

	ESI mode	Platform	RT (min)	PRECURSORMZ	PRECURSORTYP	PubChem_CID	Metabolite name	Fold	updown (BRB/AIN)	pvalue	qvalue	MSI level
1	Positive	untargeted	5.12	160.1336	[M+H]+	69522	DL-2-Aminooctanoic acid	9.09	DOWN	0.008695	0.03597782	2
2	Positive	untargeted	1.56	304.1497	[M+H]+	131752643	N5-Acetyl-N2-γ-L-glutamyl-L-ornithine	8.60	DOWN	0.000618	0.03451233	2
3	Positive	untargeted	8.56	472.3414	[M+H]+	53477842	Cervonyl carnitine	8.59	UP	0.022288	0.04214726	2
4	Positive	untargeted	3.57	295.1296	[M+H]+	111299	γ-Glutamylphenylalanine	7.03	DOWN	0.005606	0.03523052	2
5	Positive	untargeted	8.34	372.3116	[M+NH4]+	5283074	PGF2alpha methyl ether	6.07	UP	0.003267	0.03522256	2
6	Positive	untargeted	9.39	428.3739	[M+NH4]+	53480968	MG(0:0/22:2(13Z,16Z)/0:0)	4.70	UP	0.008026	0.03594	2
7	Positive	untargeted	3.35	250.0934	[M+H]+	1076	S-Acetyldihydrolipoamide	4.12	DOWN	0.002556	0.03522256	2
8	Positive	untargeted	2.95	209.0925	[M+H]+	161166	L-Kynurenine	3.97	UP	0.002214	0.03522256	2
9	Positive	untargeted	8.51	424.3426	[M+H]+	53477834	Linoelaidyl carnitine	3.81	UP	0.017155	0.03923313	2
10	Positive	targeted & untargeted	0.527	89.10717	[M+H]+	1045	Putrescine	3.79	DOWN	0.029624	0.04687835	1
11	Positive	targeted & untargeted	1.38	182.08086	[M+H]+	6057	L-Tyrosine	3.68	DOWN	0.008664	0.03594	1
12	Positive	untargeted	1.45	169.0976	[M+H]+	1052	Pyridoxamine	3.41	UP	0.00403	0.03522256	2
13	Positive	untargeted	4.2	310.1762	[M+NH4]+	9943917	Phenylbutyrylglutamine	3.26	UP	0.004845	0.03523052	2
14	Positive	untargeted	8.56	570.354	[M+H]+	53480473	LysoPC(22:5(4Z,7Z,10Z,13Z,16Z))	3.05	DOWN	0.005579	0.03523052	2
15	Positive	untargeted	8.5	524.2985	[M+H]+	74819135	1-Stearoylglycerophosphoserine	2.98	UP	0.003845	0.03522256	2
16	Positive	untargeted	4.67	295.1286	[M+H]+	69040603	Tyrosyl-Hydroxyproline	2.78	DOWN	0.019877	0.04059069	2
17	Positive	untargeted	4.04	146.1177	[M+H]+	227939	2-Aminoheptanoate	2.72	UP	0.003648	0.03522256	2
18	Positive	untargeted	8.79	426.3583	[M+H]+	46907933	Oleoylcarnitine	2.68	UP	0.037507	0.05175425	2
19	Positive	targeted & untargeted	1.796	268.10333	[M+H]+	60961	Adenosine	2.64	UP	0.014045	0.03735934	1
20	Positive	untargeted	8.5	436.2822	[M+K]+	53477817	trans-Hexadec-2-enoyl carnitine	2.61	DOWN	0.045759	0.0571566	2
21	Positive	untargeted	3.35	297.1091	[M+H]+	19365650	Aspartyl-Tyrosine	2.59	UP	0.007014	0.03553096	2
22	Positive	untargeted	3.84	247.1291	[M+H]+	332962	Aspartyl-Leucine	2.52	DOWN	0.019375	0.04038962	2
23	Positive	untargeted	0.88	72.08085	[M+H]+	31268	Pyrrolidine	2.43	DOWN	0.046328	0.05754632	2
24	Positive	targeted	5.098	246.16959	[M+H]+	53481619	Valeryl-L-carnitine	2.43	UP	0.01209	0.03707214	1
25	Positive	untargeted	4.31	261.1447	[M+H]+	4524287	γ-Glutamylleucine	2.40	DOWN	0.00763	0.03581861	2
26	Negative	untargeted	0.69	201.1364	[M-H]-	169148	Symmetric dimethylarginine	2.32	DOWN	0.007786	0.17063503	2
27	Positive	targeted	0.602	184.07269	[M+H]+	1014	Phosphocholine	2.31	DOWN	0.005552	0.03523052	1
28	Positive	untargeted	1.13	248.1492	[M+H]+	53481617	Hydroxybutyrylcarnitine	2.26	DOWN	0.040384	0.05367262	2
29	Positive	untargeted	3.26	287.0761	[M+H]+	124202060	Diphenol glucuronide	2.22	DOWN	0.015526	0.03837316	2
30	Positive	untargeted	6.03	141.0911	[M+H-H2O]+	3731686	2-n-Propyl-4-oxopentanoic acid	2.16	UP	0.007476	0.03577431	2
31	Positive	untargeted	2.91	186.1129	[M+H]+	131802910	4-Hepteneoylglycine	2.07	UP	0.039443	0.05308935	2
32	Negative	untargeted	9.6	375.2549	[M-H]-	11660820	MG(0:0/20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	2.07	DOWN	0.001586	0.11572258	2
33	Positive	untargeted	3.91	230.0962	[M+H]+	5351619	Ergothioneine	2.06	DOWN	0.013747	0.03735934	2
34	Positive	targeted	0.548	146.16502	[M+H]+	1102	Spermidine	2.05	DOWN	0.011889	0.03707214	1
35	Negative	untargeted	5.4	201.1134	[M-H]-	5192	Sebacic acid	2.04	UP	5.37E-06	0.00936217	2
36	Positive	untargeted	8.11	359.2221	[M+H-H2O]+	44251266	Resolvin D1	2.02	DOWN	0.000151	0.02758632	2
37	Positive	untargeted	4.86	295.1657	[M+]+	5757	Estradiol	1.95	DOWN	0.022847	0.04240063	2
38	Positive	untargeted	8.04	183.1382	[M+H]+	131751613	Methyl 4,8-decadienoate	1.91	UP	0.001722	0.03522256	2
39	Positive	untargeted	5.71	350.2077	[M+H]+	449034	Coutaric acid	1.90	DOWN	0.01057	0.03675833	2
40	Positive	untargeted	4.11	219.1119	[M+H]+	903	N-Acetylserotonin (NAS)	1.86	DOWN	0.00827	0.03594	2

41	Positive	untargeted	4.82	429.271	[2M+H]+	445027	Dethiobiotin	1.85	DOWN	0.006524	0.03523052	2
42	Positive	untargeted	8.81	279.1586	[M+]+	10199004	Monomenthyl succinate	1.85	DOWN	0.036216	0.05099796	2
43	Positive & Nega	targeted & untargeted	0.609	134.04448	[M+H]+	5960	L-Aspartic acid	1.82	DOWN	0.017802	0.0393943	1
44	Positive	untargeted	8.38	337.2378	[M+H-H2O]+	5283078	Prostaglandin F2a	1.79	DOWN	0.006648	0.03523052	2
45	Positive	untargeted	8.07	500.2768	[M+H]+	53480938	LysoPE(0:0/20:5(5Z,8Z,11Z,14Z,17Z))	1.79	UP	0.002079	0.03522256	2
46	Positive	untargeted	3.15	162.1133	[M+H]+	10917	L-Carnitine	1.78	DOWN	0.026392	0.04447451	2
47	Positive	untargeted	7.88	554.3456	[M+H]+	52926280	PS(20:0/0:0)	1.65	UP	0.019561	0.04049546	2
48	Negative	targeted	0.939	187.07202	[M-H]-	182230	Nα-Acetyl-L-glutamine	1.63	DOWN	0.004291	0.14732233	1
49	Positive	targeted	0.602	76.03915	[M+H]+	750	L-Glycine	1.63	DOWN	0.00938	0.03609615	1
50	Positive	targeted	0.606	106.04951	[M+H]+	5951	L-Serine	1.62	DOWN	0.029074	0.04643249	1
51	Positive	untargeted	0.96	257.0535	[M+K]+	69043006	Serinyl-Hydroxyproline	1.60	DOWN	0.016936	0.03917969	2
52	Negative	targeted & untargeted	4.796	129.05563	[M-H]-	70	4-Methyl-2-oxovaleric acid	1.58	UP	9.46E-05	0.05161655	1
53	Positive	untargeted	7.99	426.2622	[M+NH4]+	167650	1-Palmitoyl-dihydroxyacetone-phosphate	1.51	UP	0.019004	0.04003135	2
54	Positive	untargeted	8.37	263.1644	[M+H]+	25244947	3-Hydroxy-geranylhydroquinone	1.48	UP	0.044905	0.05673247	2
55	Positive	untargeted	0.91	115.0505	[M+H]+	649	Dihydrouracil	1.43	DOWN	0.013473	0.03717818	2
56	Positive	untargeted	3.48	269.0886	[M+K]+	10242944	Valyl-Hydroxyproline	1.43	DOWN	0.012208	0.03707214	2
57	Negative	untargeted	10.09	506.3263	[M-H]-	53480931	LysoPE(0:0/20:1(11Z))	1.43	DOWN	0.043884	0.24696888	2
58	Positive	targeted	0.61	126.02172	[M+H]+	1123	Taurine	1.42	DOWN	0.015174	0.03805372	1
59	Positive	untargeted	8.45	504.3072	[M+H]+	53480951	LysoPE(20:3(5Z,8Z,11Z)/0:0)	1.39	UP	0.021678	0.04187098	2
60	Positive	untargeted	6.32	238.1413	[M+]+	10176752	N-Nonoylglycine	1.38	DOWN	0.016224	0.03854013	2
61	Negative	targeted	0.644	259.02148	[M-H]-	440194	D-myo-inositol-3-phosphate	1.36	DOWN	0.005909	0.15667049	1
62	Positive	untargeted	6.03	163.1334	[M+H]+	85279741	(3R,7R)-1,3,7-Octanetriol	1.34	DOWN	0.012024	0.03707214	2
63	Negative	untargeted	0.59	115.0038	[M-H2O-H]-	222656	L-Malic acid	1.34	DOWN	0.031178	0.22740603	2
64	Positive	targeted	0.657	129.0657	[M+H]+	134508	3-Aminopiperidine-2,6-dione	1.33	DOWN	0.040116	0.05349725	1
65	Positive	targeted	6.138	146.05966	[M+H]+	10256	Indole-3-carboxaldehyde	1.31	UP	0.017641	0.03938918	1
66	Positive	untargeted	0.57	219.027	[M+K]+	892	myo-Inositol	1.31	UP	0.000804	0.03451233	2
67	Negative	targeted & untargeted	1.077	386.00717	[M-H]-	150855	dCDP	1.29	DOWN	0.036015	0.23295293	1
68	Negative	untargeted	0.72	243.028	[M-H2O-H]-	130418	Mannitol 1-phosphate	1.27	DOWN	0.021999	0.20961683	2
69	Negative	untargeted	8.78	504.3092	[M-H2O-H]-	9546803	PE(10:0/10:0)	1.27	DOWN	0.033631	0.23131456	2
70	Positive	untargeted	0.64	244.0792	[M+]+	84265	N-Acetylgalactosamine	1.26	UP	0.027778	0.04541918	2
71	Positive	untargeted	6.78	293.1963	[M+H]+	22269604	(S)-3-Octanol glucoside	1.26	DOWN	0.010713	0.03693359	2
72	Negative	targeted & untargeted	1.672	227.06693	[M-H]-	13712	2'-Deoxyuridine	1.25	DOWN	1.64E-05	0.01633336	1
73	Negative	targeted & untargeted	5.91	204.06609	[M-H]-	676157	Indole-3-lactic acid	1.21	DOWN	0.028574	0.222468	1

Supplementary Table 5.3. Cont'd.

Supplementary Table 5.4 ChemRICH results for fecal metabolome changes on compound class level (adjusted p-value<0.05).

	Cluster name	Cluster size	p-values	FDR	Key compound	Altered metabolites	Increased	Decreased	Increased ratio	Altered Ratio
1	catecholamines	7	2.2E-20	2.7E-19	Normetanephrine	7	2	5	0.3	1
2	dicarboxylic acids	12	2.2E-20	2.7E-19	Suberylglycine	12	11	1	0.9	1
3	dipeptides	32	2.2E-20	2.7E-19	N-acetyl-L-2-aminoadipate(2-)	32	31	1	1	1
4	cinnamates	7	1.1E-16	1E-15	Caffeic acid 3-sulfate	7	3	4	0.4	1
5	OH-FA_20_4_5	3	2.2E-16	1.6E-15	Omega-Carboxy-trinor-leukotriene B4	3	3	0	1	1
6	terpenes	3	1.7E-13	1.1E-12	6beta-Hydroxyasiatic acid	3	2	1	0.7	1
7	phenols	9	9.3E-13	4.9E-12	4-Ethylphenol	9	6	3	0.7	1
8	oligopeptides	8	2.7E-11	1.2E-10	(9E)-9-Nitrooctadecenoic acid	8	7	1	0.9	1
9	indoles	8	2.8E-11	1.2E-10	5-Hydroxyindoleacetic acid	8	1	7	0.1	1
10	flavonoids	4	7.2E-11	2.7E-10	Umbelliferone	4	3	1	0.8	1
11	phenylacetates	7	5.2E-10	1.7E-09	Homovanillic acid	7	3	4	0.4	1
12	hippurates	4	9.1E-10	2.8E-09	N-Salicyloylaspartic acid	4	1	3	0.2	1
13	acetates	3	0.000000002	5.7E-09	Glycolic acid	3	3	0	1	1
14	glucosides	5	2.2E-09	5.7E-09	2-O-Benzoyl-D-glucose	5	5	0	1	1
15	nicotinic acids	3	4.7E-09	0.000000012	Niacinamide	3	3	0	1	1
16	ortho-aminobenzoates	4	6.6E-09	0.000000015	beta-D-Glucopyranosyl anthranilate	4	3	1	0.8	1
17	cholestenes	4	0.000000013	0.000000028	7 alpha,26-Dihydroxy-4-cholesten-3-one	4	2	2	0.5	1
18	cholic acids	4	0.000000039	0.000000079	2-Deoxybrassinolide	4	3	1	0.8	1
19	prostaglandins	3	0.0000001	0.0000002	Prostaglandin B2	3	0	3	0	1
20	amino acids	5	0.00000017	0.00000031	L-Glutamine	5	4	1	0.8	1
21	carnitine	4	0.00000022	0.00000038	Propionylcarnitine	4	3	1	0.8	1
22	pyrroles	3	0.00000042	0.0000007	Ethyl 2-pyrrolecarboxylate	3	3	0	1	1
23	tetrapyrroles	3	0.00000059	0.00000095	Mesoporphyrin IX	3	3	0	1	1
24	salicylates	3	0.00000082	0.0000013	Salicylic acid beta-D-glucoside	3	3	0	1	1
25	hydroxycholesterols	4	0.00000091	0.0000013	24-Hydroxycholesterol	4	1	3	0.2	1
26	hydroquinones	3	0.0000013	0.0000018	Diphenol glucuronide	3	1	2	0.3	1
27	amino acids, aromatic	3	0.0000013	0.0000018	Bufotenine O-glucoside	3	0	3	0	1
28	DiHOME	3	0.0000013	0.0000018	9(10)-EpODE	3	2	1	0.7	1
29	Saturated_lysophospholipids	3	0.0000021	0.0000027	PS(O-16:0/0:0)	3	1	2	0.3	1
30	guanine nucleotides	3	0.0000029	0.0000036	Dihydroneopterin phosphate	3	2	1	0.7	1
31	succinates	4	0.0000044	0.0000052	N2-Succinyl-L-ornithine	4	4	0	1	1
32	keto acids	3	0.0000049	0.0000057	3-Methyl-2-oxovaleric acid	3	1	2	0.3	1
33	xanthurenates	3	0.0000071	0.0000079	Kynurenic acid	3	1	2	0.3	1
34	OH-FA_22_0_1	3	0.000013	0.000014	2(R)-hydroxydocosanoic acid	3	3	0	1	1
35	chromans	3	0.000027	0.000028	7-Hydroxy-2',3',4'-trimethoxyisoflavan	3	2	1	0.7	1
36	alkanesulfonic acids	3	0.00003	0.000031	Taurine	3	2	1	0.7	1
37	glucuronates	3	0.000094	0.000094	Lithocholate 3-O-glucuronide	3	3	0	1	1

Supplementary Table 5.5 Quantitative pathway enrichment analysis (qMSEA) results for fecal metabolome changes (adjusted p-value<0.05).

	Pathway (feces)	Total Cmpd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR	Enrichment folc
1	Nicotinate and Nicotinamide Metabolism	37	2	85.775	5.5556	6.986E-10	4.0519E-08	4.0519E-08	15.43937648
2	Tyrosine Metabolism	72	7	73.607	5.5556	4.088E-09	2.3301E-07	1.1855E-07	13.24915401
3	Inositol Metabolism	33	1	82.813	5.5556	6.5445E-08	3.6649E-06	9.4896E-07	14.90622075
4	Inositol Phosphate Metabolism	26	1	82.813	5.5556	6.5445E-08	3.6649E-06	9.4896E-07	14.90622075
5	Alpha Linolenic Acid and Linoleic Acid Metabolism	19	1	75.687	5.5556	1.2984E-06	0.000070116	0.000015062	13.62355101
6	Phenylacetate Metabolism	9	2	63.001	5.5556	5.7367E-06	0.00030404	0.000055454	11.34008928
7	Catecholamine Biosynthesis	20	3	66.825	5.5556	6.8306E-06	0.00035519	0.000056596	12.02840377
8	Amino Sugar Metabolism	33	4	51.313	5.5556	0.000010249	0.00052272	0.000074308	9.23626611
9	Pyrimidine Metabolism	59	5	58.222	5.5556	0.00002006	0.001003	0.00012928	10.47987616
10	Galactose Metabolism	38	1	66.059	5.5556	0.000023462	0.0011497	0.00013608	11.89052488
11	Arachidonic Acid Metabolism	69	3	41.509	5.5556	0.00023825	0.011436	0.0010996	7.471560228
12	Steroid Biosynthesis	48	2	39.286	5.5556	0.00026311	0.012366	0.0010996	7.071423429
13	Urea Cycle	29	3	45.867	5.5556	0.00029574	0.013604	0.0010996	8.255993952
14	Ammonia Recycling	32	2	46.263	5.5556	0.00031372	0.014118	0.0010996	8.327273382
15	Glycerolipid Metabolism	25	1	54.094	5.5556	0.00033257	0.014633	0.0010996	9.736842105
16	Fatty acid Metabolism	43	1	54.094	5.5556	0.00033257	0.014633	0.0010996	9.736842105
17	Fatty Acid Elongation In Mitochondria	35	1	54.094	5.5556	0.00033257	0.014633	0.0010996	9.736842105
18	Fatty Acid Biosynthesis	35	3	50.206	5.5556	0.00034126	0.014633	0.0010996	9.037007704
19	Tryptophan Metabolism	60	9	41.432	5.5556	0.00041274	0.01651	0.00126	7.457700338
20	Purine Metabolism	74	2	46.732	5.5556	0.00051105	0.019931	0.001482	8.411692706
21	Aspartate Metabolism	35	4	44.905	5.5556	0.00058851	0.022363	0.0016254	8.082835337
22	Methionine Metabolism	43	2	35.485	5.5556	0.00082581	0.030555	0.0021771	6.387248902
23	Steroidogenesis	43	2	40.179	5.5556	0.0010123	0.036444	0.0025529	7.232162143
24	Bile Acid Biosynthesis	65	7	37.992	5.5556	0.0025852	0.090481	0.0055234	6.838505292
25	Ubiquinone Biosynthesis	20	1	41.511	5.5556	0.0029061	0.098807	0.0055234	7.471920225
26	Transfer of Acetyl Groups into Mitochondria	22	2	40.987	5.5556	0.0030121	0.099401	0.0055234	7.377600979
27	Cysteine Metabolism	26	1	40.875	5.5556	0.003206	0.10259	0.0055234	7.35744114
28	Glycolysis	25	1	40.875	5.5556	0.003206	0.10259	0.0055234	7.35744114
29	Alanine Metabolism	17	1	40.875	5.5556	0.003206	0.10259	0.0055234	7.35744114
30	Pyruvate Metabolism	48	1	40.875	5.5556	0.003206	0.10259	0.0055234	7.35744114
31	Glucose-Alanine Cycle	13	1	40.875	5.5556	0.003206	0.10259	0.0055234	7.35744114
32	Gluconeogenesis	35	1	40.875	5.5556	0.003206	0.10259	0.0055234	7.35744114
33	Pyruvaldehyde Degradation	10	1	40.875	5.5556	0.003206	0.10259	0.0055234	7.35744114
34	Glycine and Serine Metabolism	59	2	39.808	5.5556	0.0032379	0.10259	0.0055234	7.165382677
35	Androgen and Estrogen Metabolism	33	1	39.325	5.5556	0.0040571	0.10259	0.0065365	7.078443372

36	Androstenedione Metabolism	24	1	39.325	5.5556	0.0040571	0.10259	0.0065365	7.078443372
37	Porphyrin Metabolism	40	1	37.429	5.5556	0.0053745	0.11824	0.0084249	6.737166103
38	Glutathione Metabolism	21	1	36.197	5.5556	0.0064277	0.13498	0.0098107	6.515407877
39	Taurine and Hypotaurine Metabolism	12	1	35.182	5.5556	0.0074329	0.14866	0.011054	6.332709338
40	Phenylalanine and Tyrosine Metabolism	28	2	30.753	5.5556	0.0090959	0.17282	0.013189	5.535495716
41	Valine, Leucine and Isoleucine Degradation	60	5	26.687	5.5556	0.010062	0.18111	0.014234	4.803621571
42	Spermidine and Spermine Biosynthesis	18	1	32.391	5.5556	0.010983	0.18672	0.015168	5.830333357
43	Warburg Effect	58	4	27.332	5.5556	0.016141	0.25825	0.021381	4.919720642
44	Glutamate Metabolism	49	3	27.315	5.5556	0.01622	0.25825	0.021381	4.916660667
45	Vitamin B6 Metabolism	20	1	28.776	5.5556	0.017895	0.25825	0.023065	5.179638563
46	Citric Acid Cycle	32	3	26.494	5.5556	0.019675	0.25825	0.024808	4.768881849
47	Starch and Sucrose Metabolism	31	1	26.305	5.5556	0.024732	0.29678	0.029173	4.734862121
48	Ketone Body Metabolism	13	2	25.485	5.5556	0.027369	0.30106	0.029173	4.587263302
49	Arginine and Proline Metabolism	53	2	25.397	5.5556	0.027637	0.30106	0.029173	4.571423429
50	Oxidation of Branched Chain Fatty Acids	26	2	25.423	5.5556	0.027692	0.30106	0.029173	4.576103391
51	Butyrate Metabolism	19	1	25.39	5.5556	0.027829	0.30106	0.029173	4.570163439
52	Mitochondrial Electron Transport Chain	19	1	25.39	5.5556	0.027829	0.30106	0.029173	4.570163439
53	Phytanic Acid Peroxisomal Oxidation	26	1	25.39	5.5556	0.027829	0.30106	0.029173	4.570163439
54	Carnitine Synthesis	22	1	25.39	5.5556	0.027829	0.30106	0.029173	4.570163439
55	Propanoate Metabolism	42	1	25.296	5.5556	0.028167	0.30106	0.029173	4.553243574
56	Vitamin K Metabolism	14	1	25.296	5.5556	0.028167	0.30106	0.029173	4.553243574
57	Lysine Degradation	30	1	20.954	5.5556	0.04875	0.30106	0.049605	3.771689826
58	Threonine and 2-Oxobutanoate Degradation	20	1	19.219	5.5556	0.060449	0.30106	0.060449	3.459392325

Supplementary Table 5.5. Cont'd.

Supplementary Table 5.6 Quantitative pathway enrichment analysis (qMSEA) results for serum metabolome changes (adjusted p-value<0.05).

	Pathway (sera)	Total Cmpd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR	Enrichment fold
1	Pentose Phosphate Pathway	29	1	98.069	5.2632	6.996E-17	3.5679E-15	2.1348E-15	18.63296094
2	Warburg Effect	58	2	91.881	5.2632	8.3717E-17	4.1859E-15	2.1348E-15	17.45725034
3	Galactose Metabolism	38	1	93.864	5.2632	2.3524E-12	1.1527E-10	3.9991E-11	17.83401733
4	Alpha Linolenic Acid and Linoleic Acid Metabolism	19	8	57.653	5.2632	1.7695E-11	8.4935E-10	2.2561E-10	10.95398237
5	Tryptophan Metabolism	60	6	60.637	5.2632	4.0939E-09	1.9241E-07	3.5197E-08	11.52093783
6	Beta Oxidation of Very Long Chain Fatty Acids	17	1	85.744	5.2632	4.8309E-09	2.2222E-07	3.5197E-08	16.29122967
7	Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	27	1	85.744	5.2632	4.8309E-09	2.2222E-07	3.5197E-08	16.29122967
8	Sphingolipid Metabolism	40	2	61.762	5.2632	1.8678E-08	8.2185E-07	1.1907E-07	11.73468612
9	Purine Metabolism	74	3	57.278	5.2632	0.000056006	0.0024083	0.00031737	10.88273294
10	Retinol Metabolism	37	1	58.643	5.2632	0.000082952	0.003484	0.00042305	11.14208086
11	Pyrimidine Metabolism	59	6	49.435	5.2632	0.000099512	0.00408	0.00046137	9.392574859
12	Histidine Metabolism	43	2	42.251	5.2632	0.00020508	0.0082031	0.00087158	8.027625779
13	Fatty Acid Biosynthesis	35	9	28.792	5.2632	0.0005688	0.022183	0.0022314	5.470436237
14	Phenylacetate Metabolism	9	1	44.827	5.2632	0.0012425	0.047215	0.0045262	8.517061864
15	Valine, Leucine and Isoleucine Degradation	60	4	32.028	5.2632	0.0015297	0.056598	0.0051141	6.085271318
16	Fructose and Mannose Degradation	32	1	43.325	5.2632	0.0016044	0.057759	0.0051141	8.231684147
17	Amino Sugar Metabolism	33	1	42.29	5.2632	0.0019071	0.066749	0.0057213	8.03503572
18	Carnitine Synthesis	22	3	33.509	5.2632	0.0022221	0.07555	0.0062959	6.366659067
19	Ubiquinone Biosynthesis	20	2	39.043	5.2632	0.0025844	0.085286	0.0069371	7.418110655
20	Phosphatidylcholine Biosynthesis	14	2	33.371	5.2632	0.0028391	0.09085	0.0072396	6.340439276
21	Vitamin B6 Metabolism	20	2	37.979	5.2632	0.0032237	0.099936	0.0078291	7.215952272
22	Methylhistidine Metabolism	4	1	36.468	5.2632	0.0048083	0.14425	0.010662	6.928864569
23	Estrone Metabolism	24	1	36.468	5.2632	0.0048083	0.14425	0.010662	6.928864569
24	Porphyrin Metabolism	40	2	33.343	5.2632	0.0061019	0.17085	0.012843	6.335119319
25	Arachidonic Acid Metabolism	69	6	28.66	5.2632	0.0062953	0.17085	0.012843	5.445356437
26	D-Arginine and D-Ornithine Metabolism	11	1	33.706	5.2632	0.0072754	0.18916	0.013539	6.404088767
27	Ketone Body Metabolism	13	2	32.368	5.2632	0.0074333	0.18916	0.013539	6.149870801
28	Butyrate Metabolism	19	2	32.368	5.2632	0.0074333	0.18916	0.013539	6.149870801
29	Glycine and Serine Metabolism	59	3	26.774	5.2632	0.0088626	0.20384	0.014896	5.087019304
30	Methionine Metabolism	43	3	26.774	5.2632	0.0088626	0.20384	0.014896	5.087019304
31	Betaine Metabolism	21	2	26.796	5.2632	0.0090546	0.20384	0.014896	5.09119927
32	Phenylalanine and Tyrosine Metabolism	28	4	30.074	5.2632	0.011393	0.22786	0.017415	5.714014288
33	Nicotinate and Nicotinamide Metabolism	37	3	24.508	5.2632	0.011583	0.22786	0.017415	4.656482748
34	Tyrosine Metabolism	72	7	29.927	5.2632	0.01161	0.22786	0.017415	5.686084511
35	Catecholamine Biosynthesis	20	3	29.848	5.2632	0.012469	0.22786	0.017539	5.671074631

36	Glutamate Metabolism	49	3	28.055	5.2632	0.012626	0.22786	0.017539	5.330407357
37	Thyroid hormone synthesis	13	1	29.83	5.2632	0.012724	0.22786	0.017539	5.667654659
38	Phospholipid Biosynthesis	29	2	26.715	5.2632	0.01543	0.22786	0.020709	5.075809394
39	Phosphatidylethanolamine Biosynthesis	12	1	26.602	5.2632	0.019924	0.25901	0.026054	5.054339565
40	Arginine and Proline Metabolism	53	5	26.309	5.2632	0.020456	0.25901	0.026081	4.998670011
41	Ammonia Recycling	32	1	24.664	5.2632	0.025916	0.28507	0.030039	4.686122511
42	Glutathione Metabolism	21	1	24.664	5.2632	0.025916	0.28507	0.030039	4.686122511
43	Bile Acid Biosynthesis	65	1	24.664	5.2632	0.025916	0.28507	0.030039	4.686122511
44	Alanine Metabolism	17	1	24.664	5.2632	0.025916	0.28507	0.030039	4.686122511
45	Oxidation of Branched Chain Fatty Acids	26	2	23.388	5.2632	0.029593	0.28507	0.032534	4.443684451
46	Citric Acid Cycle	32	1	23.419	5.2632	0.03062	0.28507	0.032534	4.449574403
47	Mitochondrial Electron Transport Chain	19	1	23.419	5.2632	0.03062	0.28507	0.032534	4.449574403
48	Phytanic Acid Peroxisomal Oxidation	26	1	23.419	5.2632	0.03062	0.28507	0.032534	4.449574403
49	Nucleotide Sugars Metabolism	20	1	22.461	5.2632	0.034774	0.28507	0.036193	4.26755586
50	Propanoate Metabolism	42	1	21.828	5.2632	0.037805	0.28507	0.038561	4.147286822
51	Spermidine and Spermine Biosynthesis	18	1	21.314	5.2632	0.040452	0.28507	0.040452	4.049627603

Supplementary Table 5.6. Cont'd.

Supplementary Table 5.7 Overlapping pathways (n=35) between feces and sera from qMSEA (adjusted p-value<0.05).

	Name of pathway
1	Ketone Body Metabolism
2	Phenylacetate Metabolism
3	Propanoate Metabolism
4	Tryptophan Metabolism
5	Methionine Metabolism
6	Glutathione Metabolism
7	Glutamate Metabolism
8	Catecholamine Biosynthesis
9	Phenylalanine and Tyrosine Metabolism
10	Oxidation of Branched Chain Fatty Acids
11	Tyrosine Metabolism
12	Mitochondrial Electron Transport Chain
13	Ubiquinone Biosynthesis
14	Warburg Effect
15	Pyrimidine Metabolism
16	Ammonia Recycling
17	Butyrate Metabolism
18	Spermidine and Spermine Biosynthesis
19	Porphyrin Metabolism
20	Amino Sugar Metabolism
21	Fatty Acid Biosynthesis
22	Nicotinate and Nicotinamide Metabolism
23	Alanine Metabolism
24	Valine, Leucine and Isoleucine Degradation
25	Citric Acid Cycle
26	Galactose Metabolism
27	Arachidonic Acid Metabolism
28	Bile Acid Biosynthesis
29	Purine Metabolism
30	Glycine and Serine Metabolism
31	Phytanic Acid Peroxisomal Oxidation
32	Alpha Linolenic Acid and Linoleic Acid Metabolism
33	Carnitine Synthesis
34	Vitamin B6 Metabolism
35	Arginine and Proline Metabolism

Supplementary Table 5.8 Overlapping metabolites (n=36) between feces and sera (fold change ≥ 1.2 & p < 0.05).

	PubChem_CID	Name	Comment	Fold_feces	updown (BRB/AIN)_feces	Fold_sera	updown (BRB/AIN)_sera	Concordance
1	997	Phenylpyruvic acid	Phenylalanine metabolism	10.51	DOWN	2.77	UP	no
2	999	Phenylacetic acid	phenylacetate, phenylalanine metabolism	3.48	DOWN	1.47	UP	no
3	3744	Indole-3-propionic acid	aa: tryptophan metabolites_indole	2.51	DOWN	2.29	UP	no
4	5789	Thymidine	nucleotide	4.88	UP	1.89	DOWN	no
5	6047	3,4-Dihydroxy-L-phenylalanine (L-DOPA)	neurotransmitter	2.57	UP	1.25	DOWN	no
6	6140	L-Phenylalanine	aa: phenylalanine derivative	2.45	DOWN	3.18	UP	no
7	9378	DL-p-Hydroxyphenyllactic acid	aa: tyrosine metabolite, HPLA	2.81	DOWN	1.52	UP	no
8	10256	Indole-3-carboxaldehyde	aa: tryptophan metabolites	2.08	DOWN	1.23	UP	no
9	92832	N6-Acetyl-L-lysine	aa: acetyl amino acids	3.92	UP	1.26	DOWN	no
10	107738	Propionylcarnitine	lipid: acylcarnitines	9.03	UP	1.31	DOWN	no
11	321710	1,3-Dihydro-(2H)-indol-2-one	indolines	3.37	DOWN	2.78	UP	no
12	352038	Leucyl-Valine	aa: peptides	3.09	UP	2.05	DOWN	no
13	10657183	2,4-Dihydroxyacetophenone 5-sulfate		123.58	UP	2.10	DOWN	no
14	24771808	(S)-2,3,4,5-Tetrahydropiperidine-2-carboxylate		5.51	DOWN	1.27	UP	no
15	54042244	8-Hydroxy-5,6-octadienoic acid	lipids: fatty acids	3.73	DOWN	1.59	UP	no
16	135	4-Hydroxybenzoic acid	polyphenol	2.90	UP	2.05	UP	yes
17	227	Anthranilic acid	aa: tryptophan metabolites	19.60	UP	3.40	UP	yes
18	289	Pyrocatechol		17.44	UP	11.46	UP	yes
19	1110	Succinic acid	TCA cycle	3.51	UP	1.26	UP	yes
20	1135	Thymine	nucleotide	2.73	DOWN	1.67	DOWN	yes
21	1826	5-Hydroxy Indole-3-acetic acid	aa: tryptophan metabolites	10.67	DOWN	1.39	DOWN	yes
22	7115	N-acetyl-glucosamine-1-phosphate		14.61	DOWN	1.31	DOWN	yes
23	7322	5-Sulfosalicylic acid	salicylic acid derivative	35.43	UP	299.25	UP	yes
24	8082	Piperidine		2.43	UP	1.36	UP	yes
25	31242	4-Ethylphenol	phenols - bacteria	10.79	UP	72.70	UP	yes
26	74839	N-Acetyl-L-phenylalanine	aa: acetyl amino acids	7.06	UP	1.31	UP	yes
27	79803	1-Pyrroline		5.73	UP	1.29	UP	yes
28	382946	Vanillic acid 4-sulfate	sulfate	5.90	UP	81.69	UP	yes
29	5280450	Linoleic acid	lipids: fatty acids	23.34	UP	1.26	UP	yes
30	5283144	8,9-DiHETrE	lipids: oxylipins	2.82	DOWN	6.70	DOWN	yes
31	5312830	(+)9-HODE	lipids: oxylipins	5.69	UP	1.79	UP	yes
32	6443013	13S-hydroxyoctadecadienoic acid	lipids: fatty acids, oxylipins	1.68	UP	1.84	UP	yes
33	10796774	2-Hydroxy-3-methylpentanoic acid	lipids: fatty acids	2.02	DOWN	1.29	DOWN	yes
34	75595895	beta-D-Glucopyranosyl anthranilate		528.83	UP	175.06	UP	yes
35	131802903	3-Hydroxyhexanoylglycine		9.58	DOWN	1.78	DOWN	yes
36	454064	3-Oxotetradecanoic acid	lipids: fatty acids	2.81	DOWN	4.08	DOWN	yes

Supplementary Table 5.9 Quantitative pathway enrichment analysis (qMSEA) results for cerebral cortical brain metabolome changes (adjusted p-value<0.05).

	Pathway (brain)	Total Cmpd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR	Enrichment fold
1	Tryptophan Metabolism	60	2	41.609	5.2632	0.0019231	0.075002	0.015566	7.905646755
2	Valine, Leucine and Isoleucine Degradation	60	1	38.588	5.2632	0.0034634	0.13161	0.015566	7.331661347
3	Vitamin B6 Metabolism	20	1	38.191	5.2632	0.0036854	0.13636	0.015566	7.25623195
4	Arachidonic Acid Metabolism	69	1	37.426	5.2632	0.0041504	0.14942	0.015566	7.110883113
5	Pyrimidine Metabolism	59	3	32.734	5.2632	0.0047773	0.1672	0.015566	6.219410245
6	Inositol Metabolism	33	1	34.37	5.2632	0.0065941	0.2242	0.015566	6.530247758
7	Inositol Phosphate Metabolism	26	1	34.37	5.2632	0.0065941	0.2242	0.015566	6.530247758
8	Glutathione Metabolism	21	1	33.934	5.2632	0.0070342	0.22509	0.015566	6.447408421
9	Porphyrin Metabolism	40	1	33.934	5.2632	0.0070342	0.22509	0.015566	6.447408421
10	Alanine Metabolism	17	1	33.934	5.2632	0.0070342	0.22509	0.015566	6.447408421
11	Carnitine Synthesis	22	1	33.934	5.2632	0.0070342	0.22509	0.015566	6.447408421
12	Bile Acid Biosynthesis	65	2	32.571	5.2632	0.0071326	0.22509	0.015566	6.188440492
13	Tyrosine Metabolism	72	2	32.428	5.2632	0.0072139	0.22509	0.015566	6.16127071
14	Methionine Metabolism	43	5	30.329	5.2632	0.0079292	0.22509	0.015566	5.7624639
15	Phenylalanine and Tyrosine Metabolism	28	1	32.545	5.2632	0.008624	0.22509	0.015566	6.183500532
16	Catecholamine Biosynthesis	20	1	32.545	5.2632	0.008624	0.22509	0.015566	6.183500532
17	Thyroid hormone synthesis	13	1	32.545	5.2632	0.008624	0.22509	0.015566	6.183500532
18	Purine Metabolism	74	3	31.143	5.2632	0.0089253	0.22509	0.015566	5.917122663
19	Arginine and Proline Metabolism	53	2	31.258	5.2632	0.0099171	0.22509	0.015566	5.938972488
20	Glutamate Metabolism	49	2	31.258	5.2632	0.0099171	0.22509	0.015566	5.938972488
21	Beta-Alanine Metabolism	34	2	31.081	5.2632	0.009962	0.22509	0.015566	5.905342757
22	Spermidine and Spermine Biosynthesis	18	2	31.458	5.2632	0.0099634	0.22509	0.015566	5.976972184
23	Taurine and Hypotaurine Metabolism	12	1	31.327	5.2632	0.010283	0.22509	0.015566	5.952082383
24	Ammonia Recycling	32	3	30.635	5.2632	0.01071	0.22509	0.015566	5.820603435
25	Urea Cycle	29	1	30.999	5.2632	0.010776	0.22509	0.015566	5.889762882
26	Aspartate Metabolism	35	1	30.999	5.2632	0.010776	0.22509	0.015566	5.889762882
27	Malate-Aspartate Shuttle	10	1	30.999	5.2632	0.010776	0.22509	0.015566	5.889762882
28	Selenoamino Acid Metabolism	28	2	27.289	5.2632	0.012268	0.22509	0.016727	5.184868521
29	Glycine and Serine Metabolism	59	2	29.081	5.2632	0.012647	0.22509	0.016727	5.525345797
30	Betaine Metabolism	21	1	29.751	5.2632	0.012867	0.22509	0.016727	5.652644779
31	Androgen and Estrogen Metabolism	33	1	27.153	5.2632	0.018473	0.22509	0.022514	5.159028728
32	Estrone Metabolism	24	1	27.153	5.2632	0.018473	0.22509	0.022514	5.159028728
33	Sphingolipid Metabolism	40	1	25.667	5.2632	0.022633	0.22509	0.025219	4.876690986
34	Homocysteine Degradation	9	1	25.667	5.2632	0.022633	0.22509	0.025219	4.876690986
35	Phosphatidylethanolamine Biosynthesis	12	1	25.667	5.2632	0.022633	0.22509	0.025219	4.876690986
36	Citric Acid Cycle	32	1	9.8463	5.2632	0.17789	0.71157	0.17789	1.870782034
37	Pyruvate Metabolism	48	1	9.8463	5.2632	0.17789	0.71157	0.17789	1.870782034
38	Transfer of Acetyl Groups into Mitochondria	22	1	9.8463	5.2632	0.17789	0.71157	0.17789	1.870782034
39	Warburg Effect	58	1	9.8463	5.2632	0.17789	0.71157	0.17789	1.870782034

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