Supplementary Information

Metabolomics Method to Comprehensively Analyze Amino Acids in Different Domains

Haiwei Gu[1, 2*], Jianhai Du[3], Fausto Carnevale Neto[1, 4], Patrick Andrew Carroll[5], Sally Turner[3], E. Gabriela Chiorean[6, 7], Robert N. Eisenman[5], and Daniel Raftery[1, 8*]

[1] Northwest Metabolomics Research Center, Department of Anesthesiology and Pain Medicine, University of Washington, 850 Republican St., Seattle, WA 98109, USA

[2] Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China Institute of Technology, 418 Guanglan Ave., Nanchang, Jiangxi Province 330013, China

[3] Department of Biochemistry, University of Washington, 750 Republican St., Seattle, WA 98109, USA

[4] Department of Organic Chemistry, Institute of Chemistry, Sao Paulo State University, Rua Francisco Degni 55, Araraquara, Sao Paulo 14800-900, Brazil

[5] Division of Basic Sciences, Fred Hutchinson Cancer Research Center, MS A2-025, P.O. Box 19024, Seattle, WA, 98109, USA

[6] Department of Medicine, University of Washington, 825 Eastlake Ave East, Seattle, WA 98109, USA

[7] Indiana University Melvin and Bren Simon Cancer Center, 535 Barnhill Dr, Indianapolis, IN, 46202, USA

[8] Public Health Sciences Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., Seattle, WA 98109, USA

* Corresponding Authors:
Haiwei Gu, PhD
Department of Anesthesiology and Pain Medicine
University of Washington
850 Republican St.
Seattle, WA 98109
Tel: 206-685-4753
Fax: 206-616-4819
Email: haiwei@uw.edu

Professor Daniel Raftery, PhD
Department of Anesthesiology and Pain Medicine
University of Washington
850 Republican St.
Seattle, WA 98109
Tel: 206-543-9709
Fax: 206-616-4819
Email: draftery@uw.edu

Table S1. The optimized MS parameters to measure amino acids in this study.

Amino Acid	Precursor Ion	Product Ion	CE	Fragmentor	Accelerator voltage
isoleucine/leucine[a]	132.1	86.1	10	80	1
valine	118.2	72.2	10	80	1
glutamine[b]	147.1	83.8	20	80	1
glutamic acid	148.0	84.2	15	80	1
tryptophan	205.1	118.0	25	80	5
proline	116.1	70.2	15	80	1
threonine	120.1	74.2	10	80	1
histidine	156.1	110.0	10	80	5
alanine	90.1	43.9	10	60	1
serine	105.9	60.1	10	60	1
aspartic acid	133.9	74.0	15	80	1
tyrosine	182.1	136.1	10	80	3
methionine	150.0	104.1	10	80	1
cysteine[c]	121.8	75.9	15	140	1
lysine[b]	147.0	84.1	15	80	7
phenylalanine	166.1	120.1	10	80	5
arginine	175.1	70.2	25	80	1
asparagine	132.9	74.0	15	80	1
glycine	76.2	29.9	10	60	1

[a]Isoleucine and leucine have the same optimized MS parameters.

[b]Glutamine and lysine have different but very similar optimized MS parameters. In this study, they were measured separately, but they were combined for data analysis.

[c]We could not obtain a good sensitivity or peak shape for cysteine; therefore, it was excluded from analysis in this study.

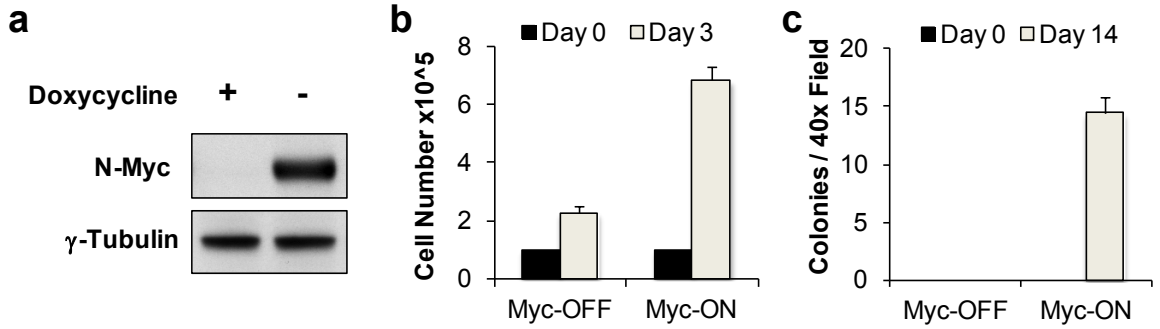

Fig. S1. The characterization of Myc-On and Myc-Off cells. a) Western blot showing that Tet21N cells can express a doxycycline-repressible *N*-Myc construct which allows for inducible *N*-Myc expression in the presence/absence of doxycycline (Myc-Off/Myc-On), b) ectopic *N*-Myc induces hyperproliferation, and c) *N*-Myc induces anchorage-independent growth in soft agar, an indicator of malignant transformation.

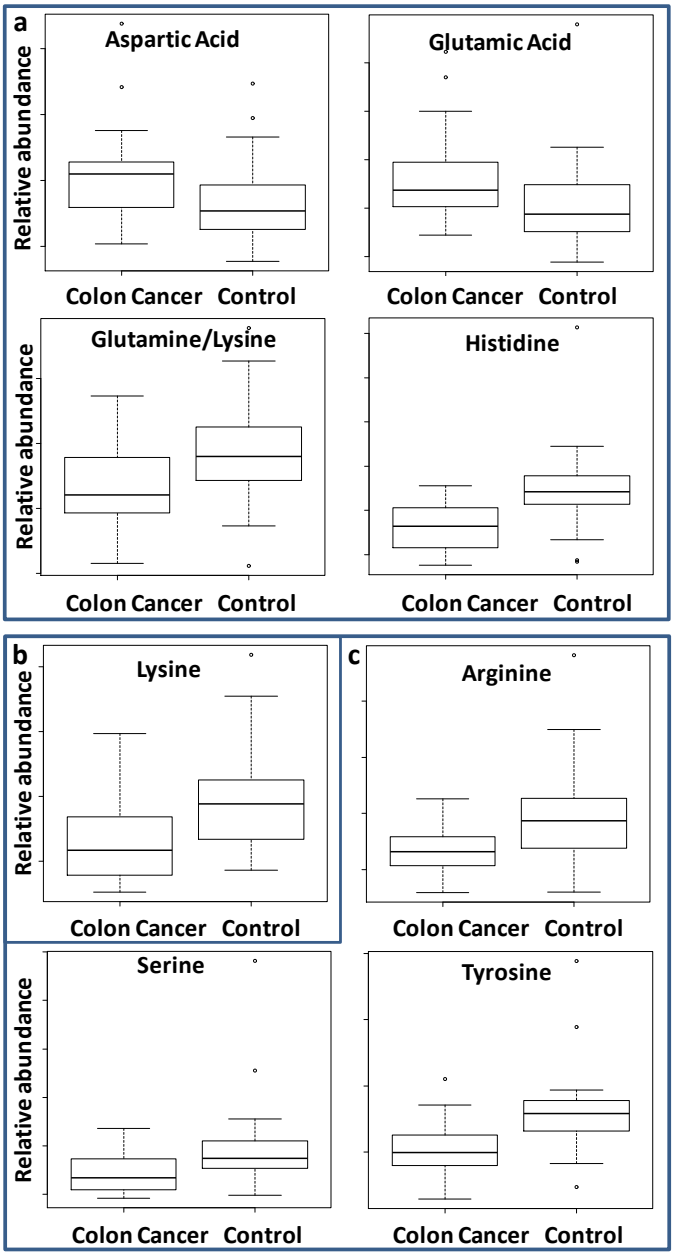

Fig. S2. Box-and-whisker plots for the amino acid markers in constructing the model in Fig. 5d.: a) aspartic acid, glutamic acid, glutamine/lysine, and histidine from FAAs, b) lysine from FSPAAs, and c) arginine, serine, and tyrosine from IPAAs.

Separate Excel File: The integrated areas and BCA values for cell and serum samples.