

SUPPORTING INFORMATION

SUPPORTING FIGURES

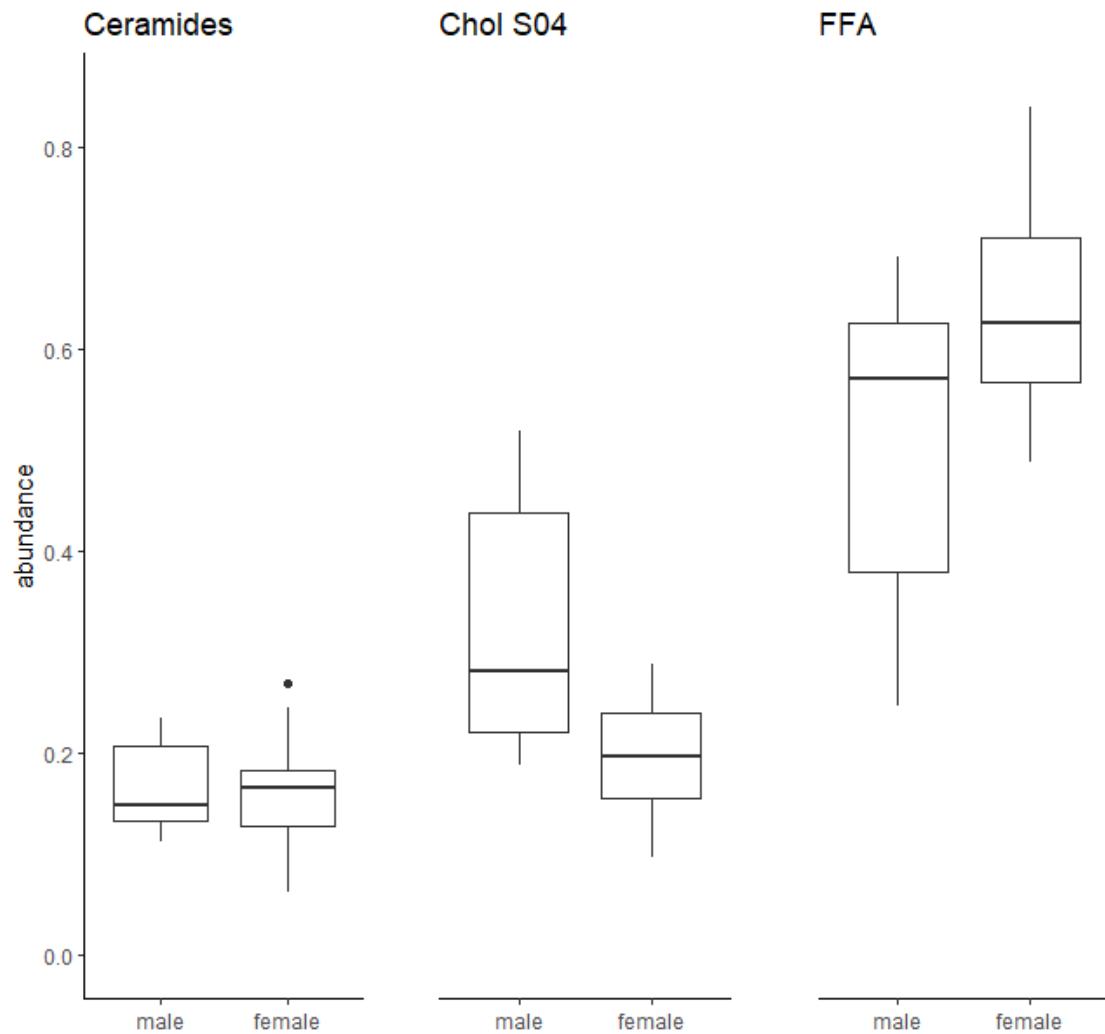


Figure S1: Impact of sex on total abundance of ceramides, cholesterol and free fatty acids.

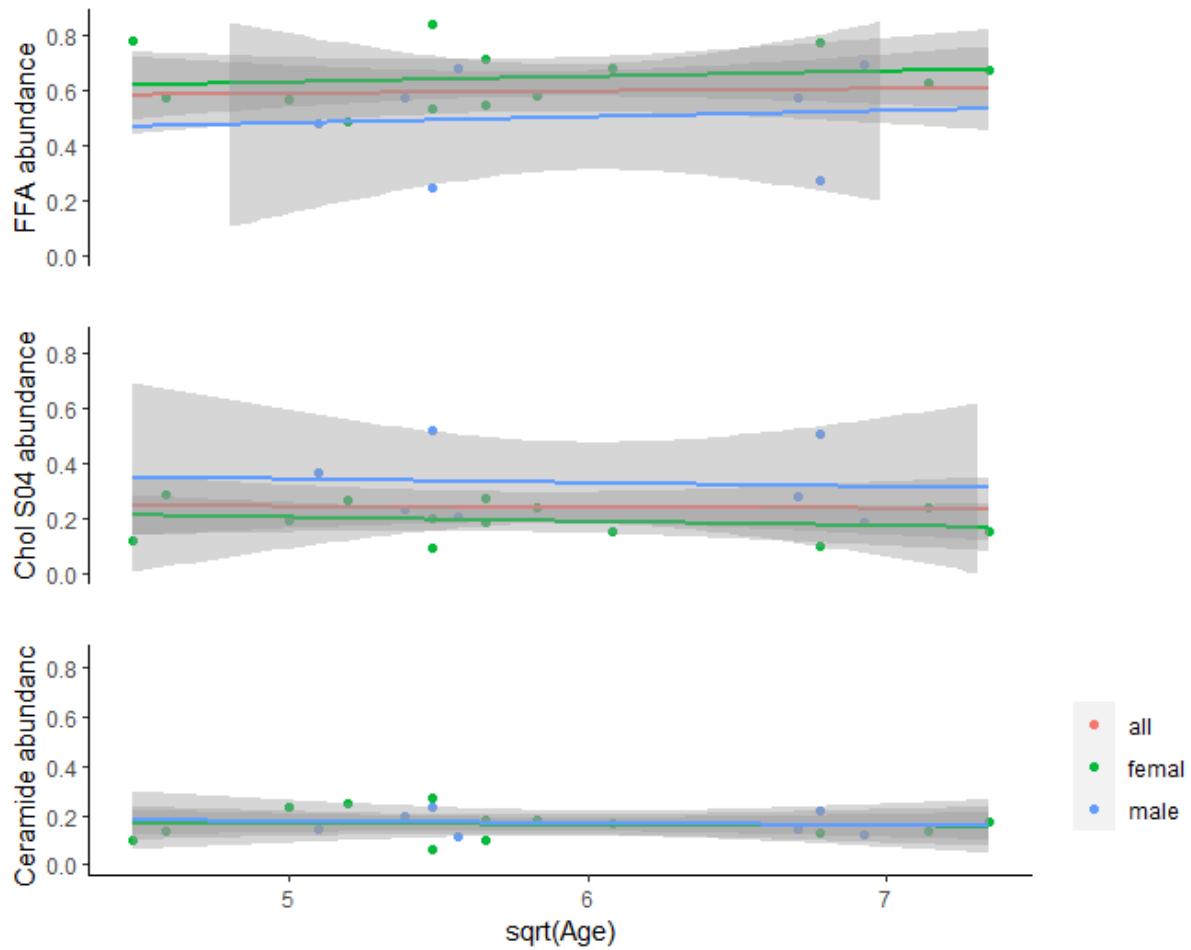


Figure S2: Correlation of age with total abundance of ceramides, cholesterol and free fatty acids.

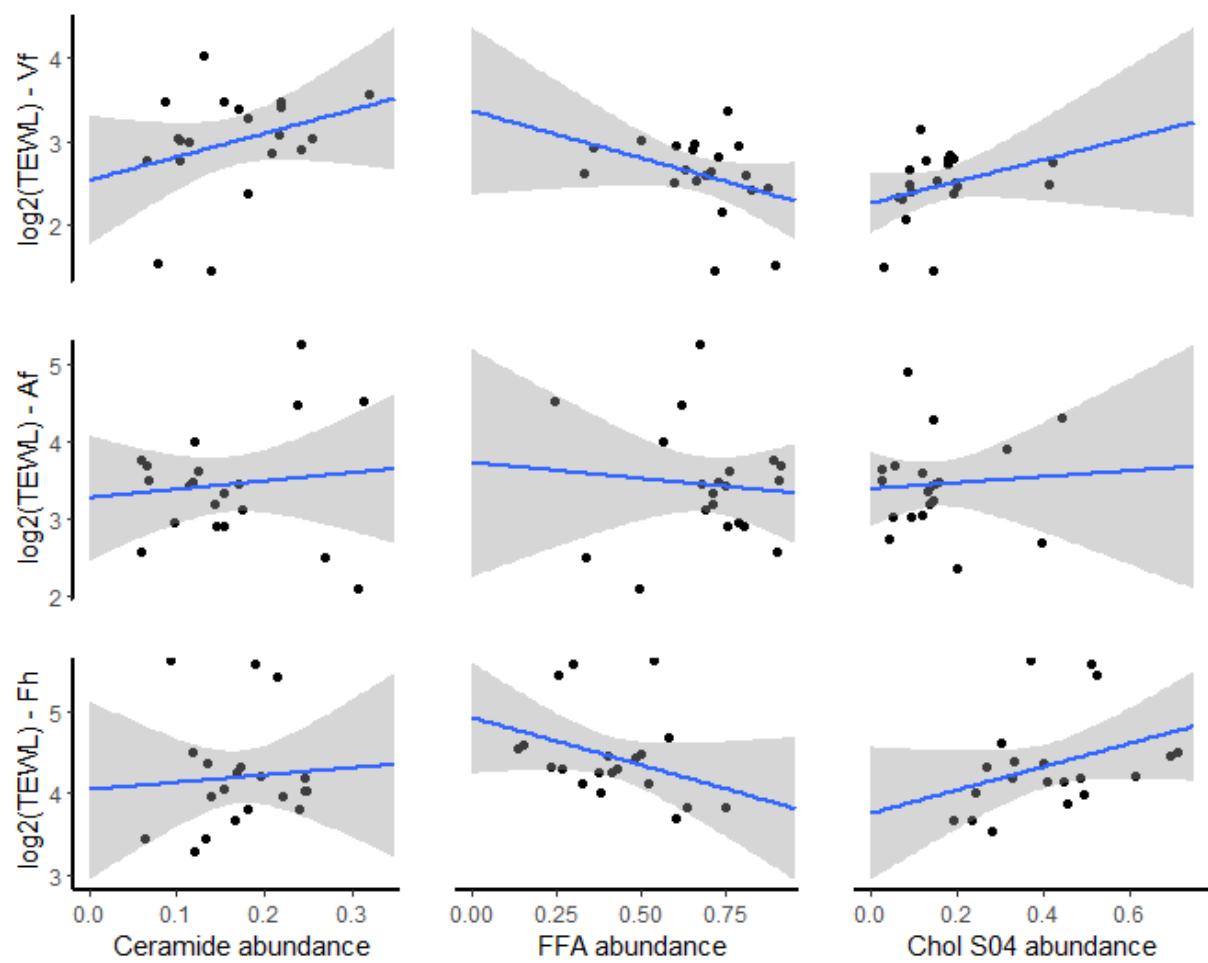


Figure S3: Correlation of TEWL with total abundance of ceramides, cholesterol and free fatty acids.

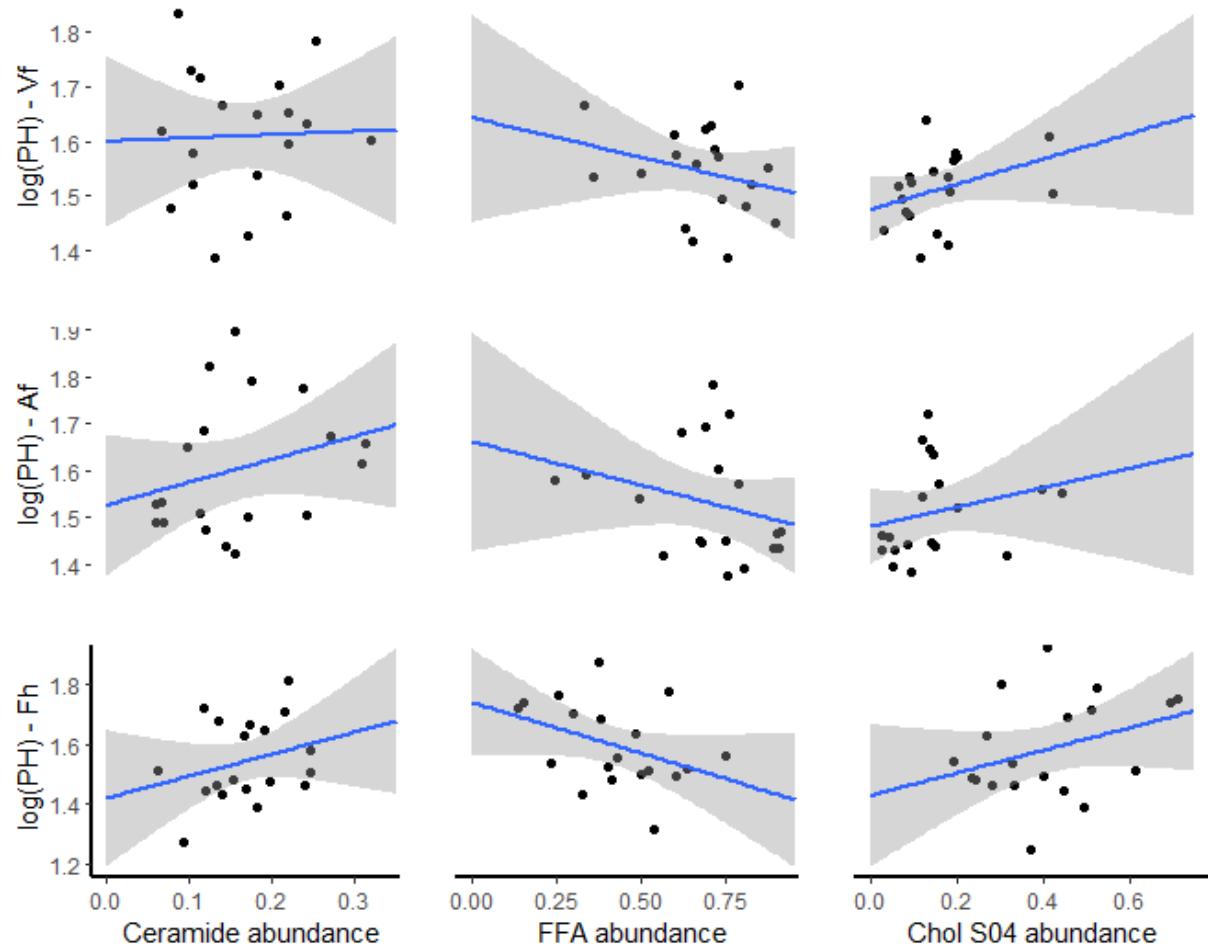


Figure S4: Correlation of pH with total abundance of ceramides, cholesterol and free fatty acids.

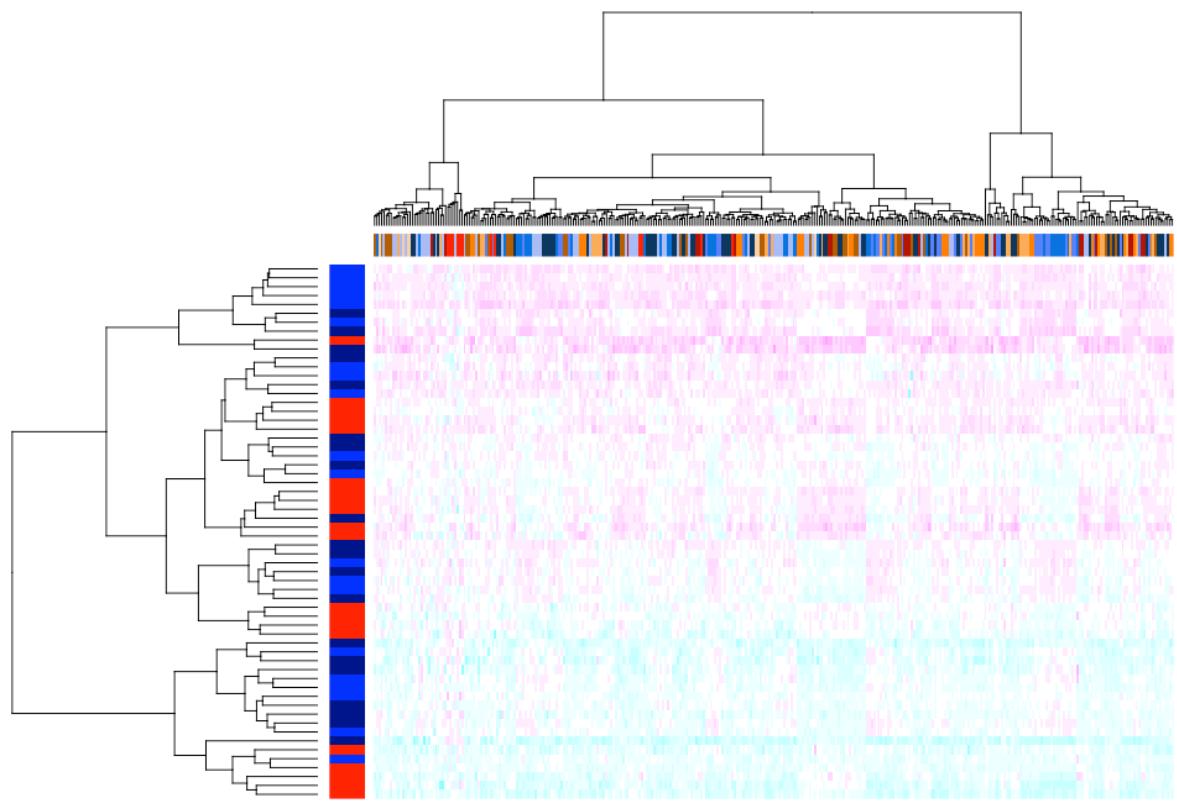
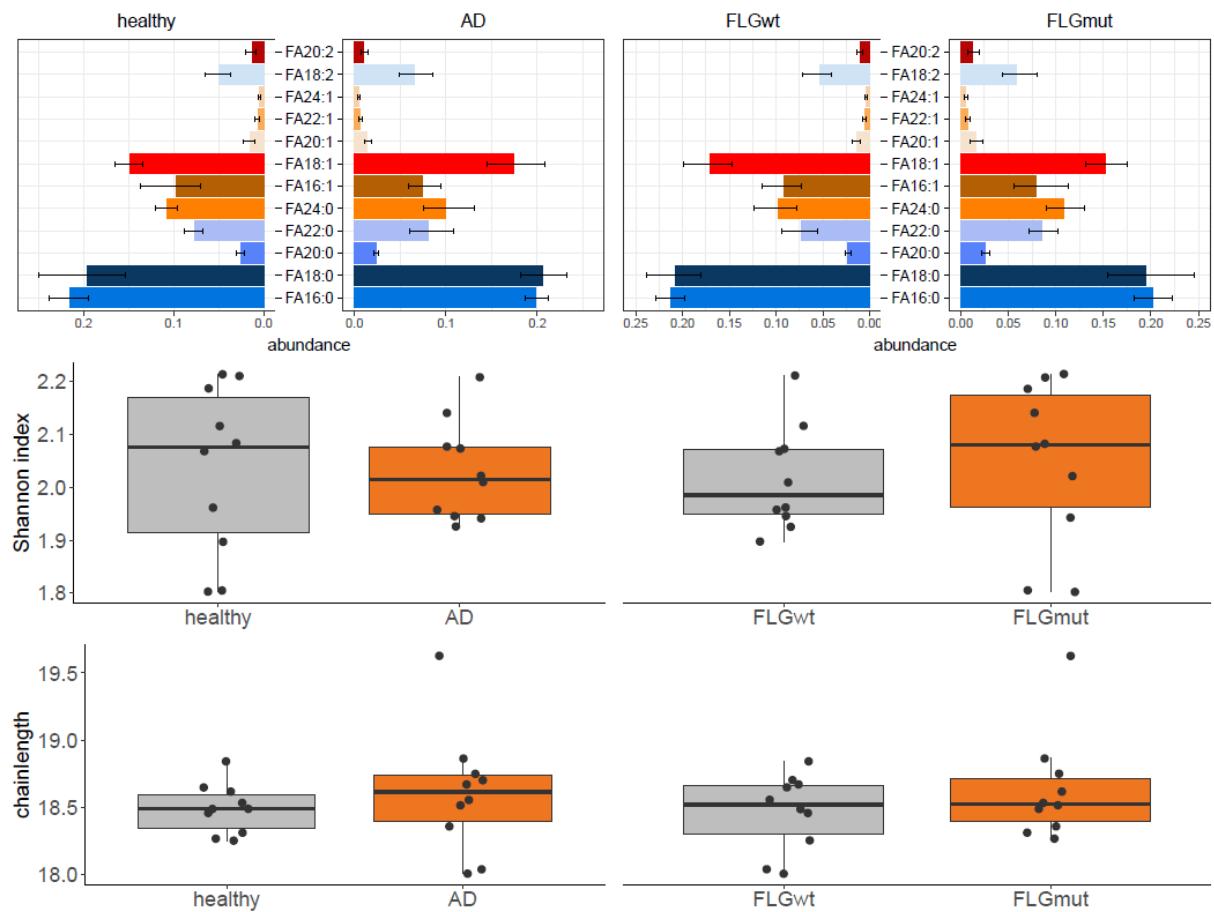


Figure S5: Heatmap of ceramides in healthy individuals and patients with AD. The colored bar at the top indicates ceramide subclass composition. The colored bar at the side indicates sample site: Forehead (red), forearm (blue), antecubital fossa (darkblue).



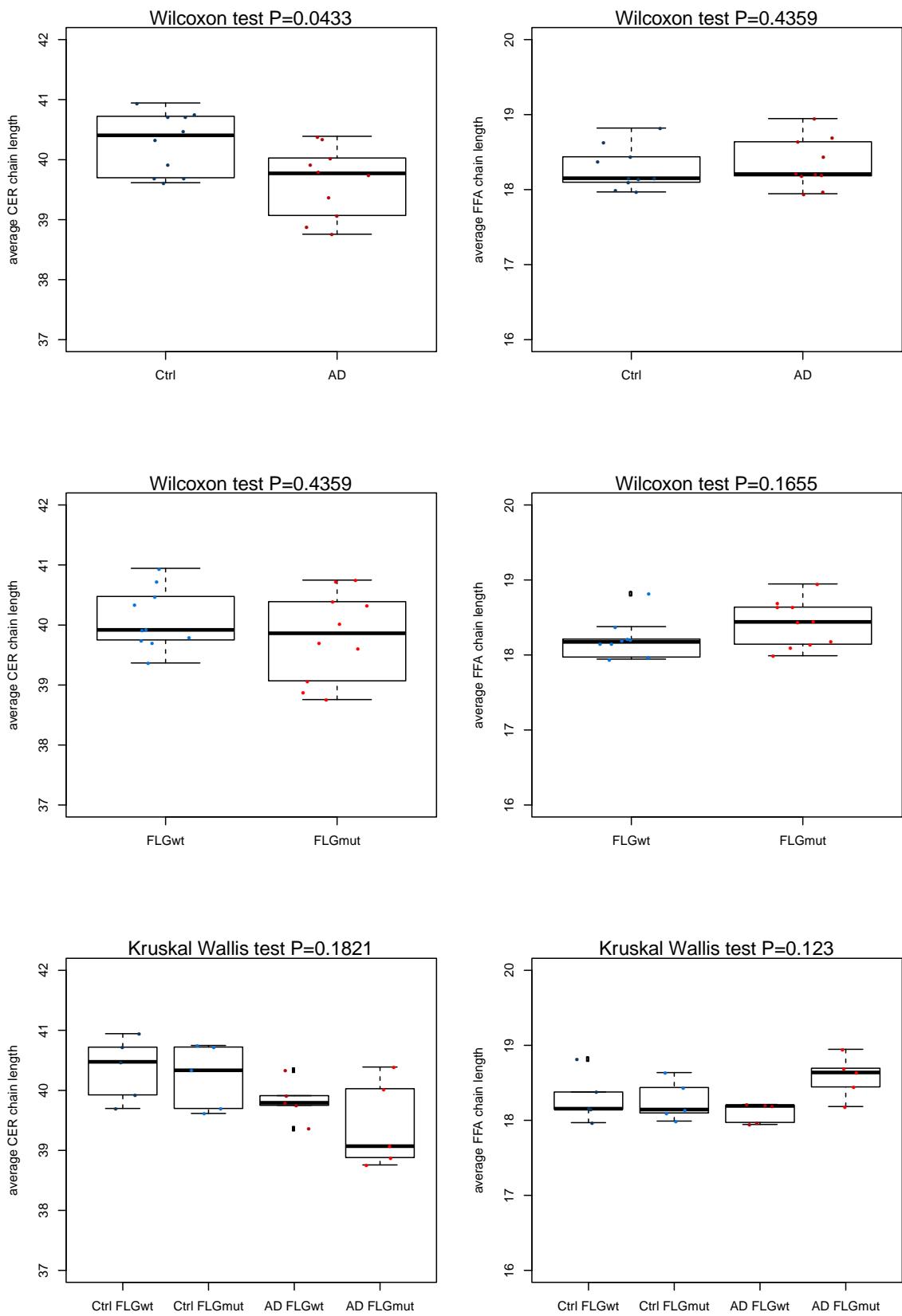


Figure S7: Average chain length of CER (left) and FFA (right) at the forehead. Comparison between Ctrl and AD (upper row), FLGwt and FLGmut (middle row) and all 4 study groups (lower row).

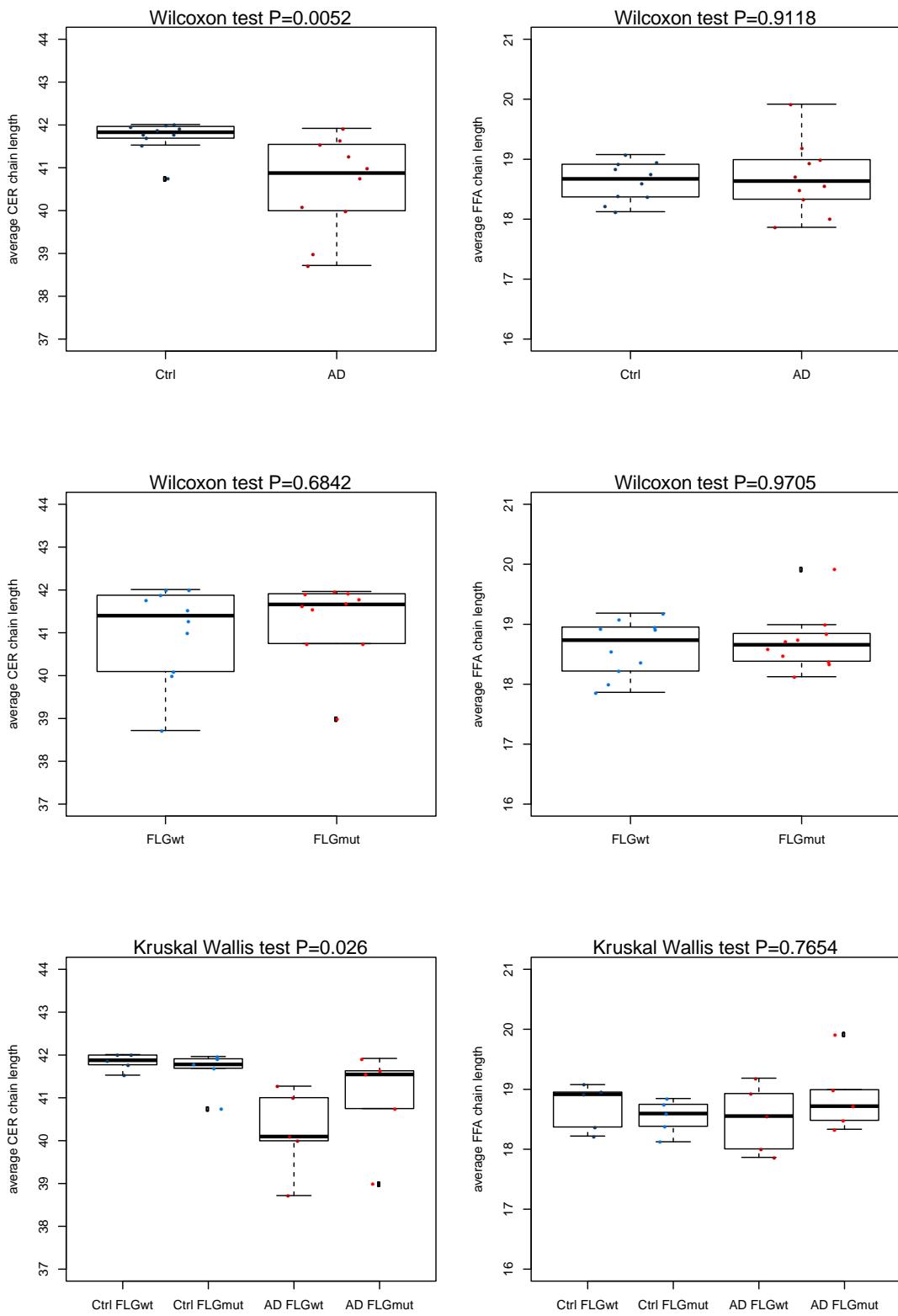


Figure S8: Average chain length of CER (left) and FFA (right) at the cubital fossa. Comparison between Ctrl and AD (upper row), FLGwt and FLGmut (middle row) and all 4 study groups (lower row).

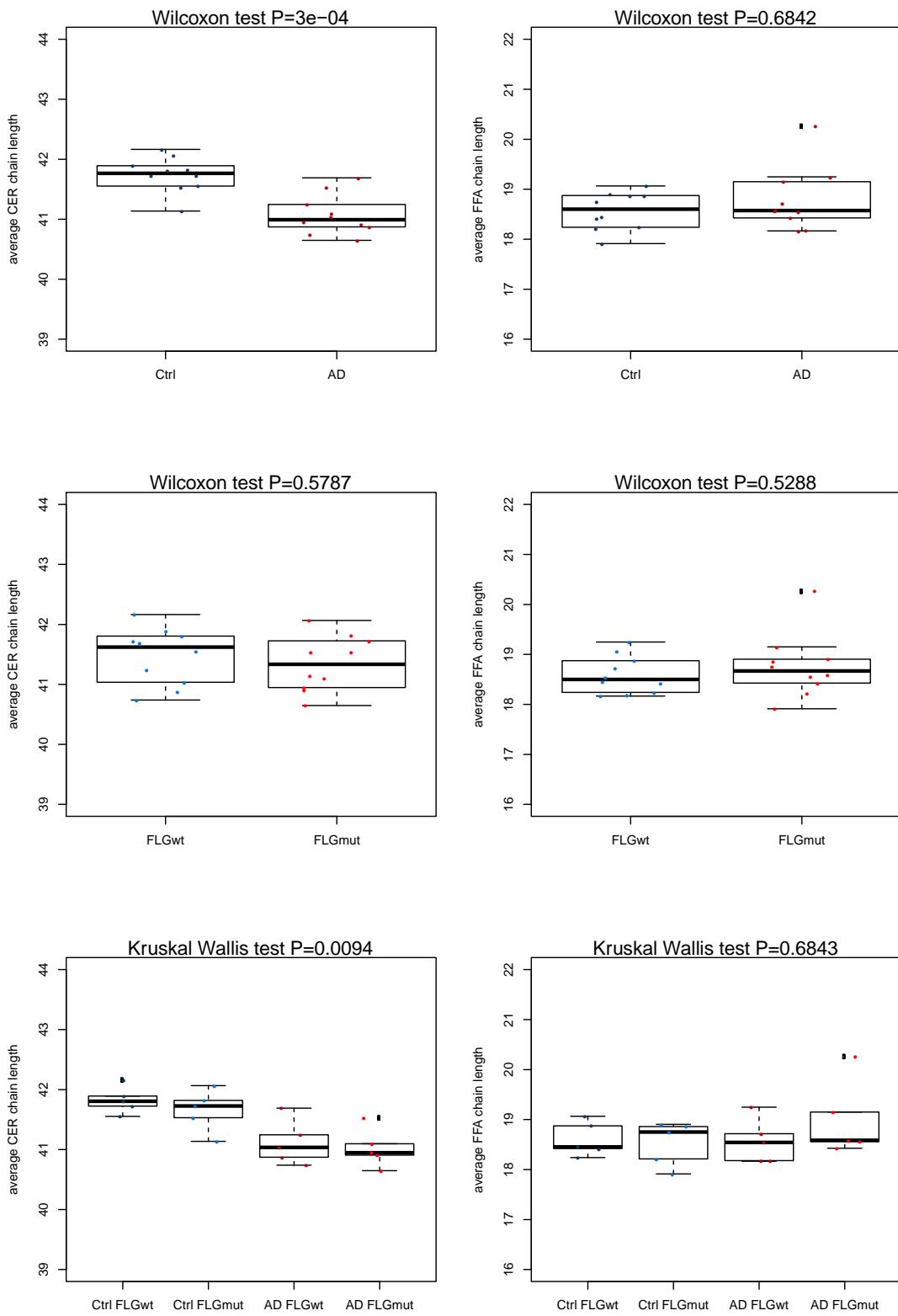


Figure S9: Average chain length of CER (left) and FFA (right) at the proximal lower arm. Comparison between Ctrl and AD (upper row), FLGwt and FLGmut (middle row) and all 4 study groups (lower row).

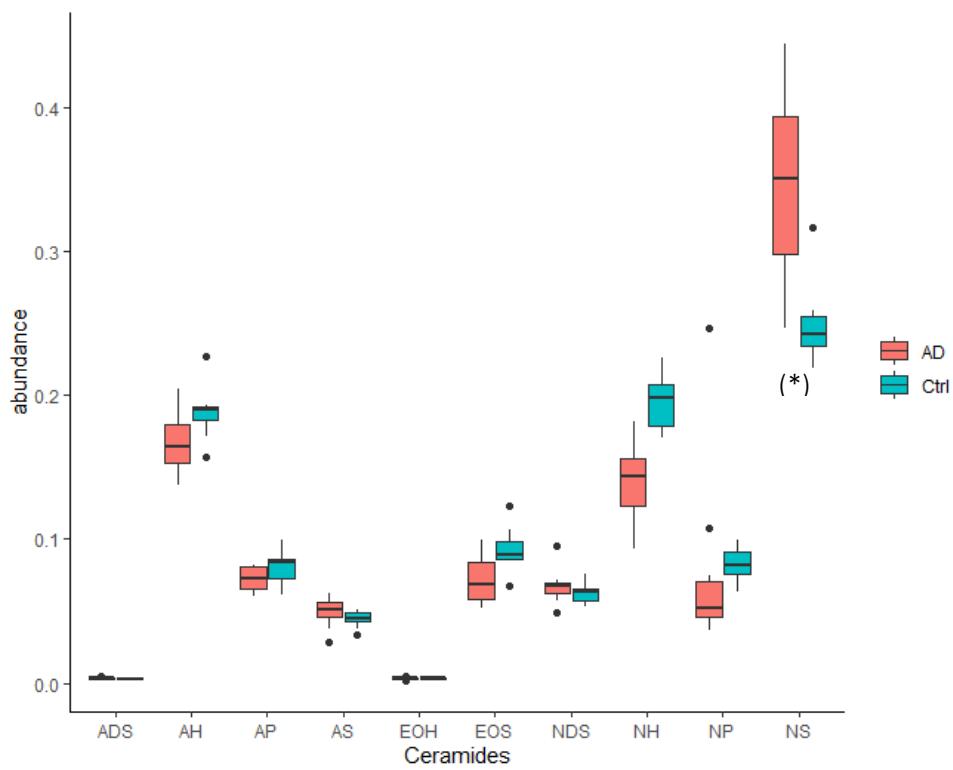
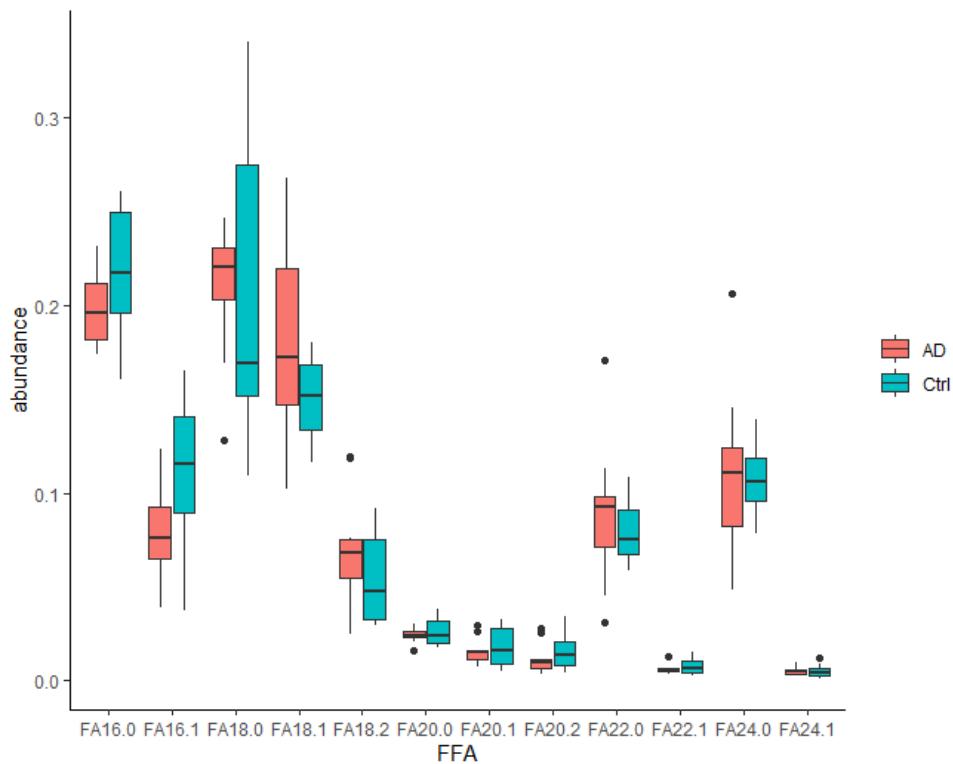
A**B**

Figure S10: Ceramide (A) and FFA (B) abundance in AD patients vs healthy individuals. (A) and in AD patients with *FLG* mutation carrier vs. non-carrier (B). Significant differences are indicated by (*).

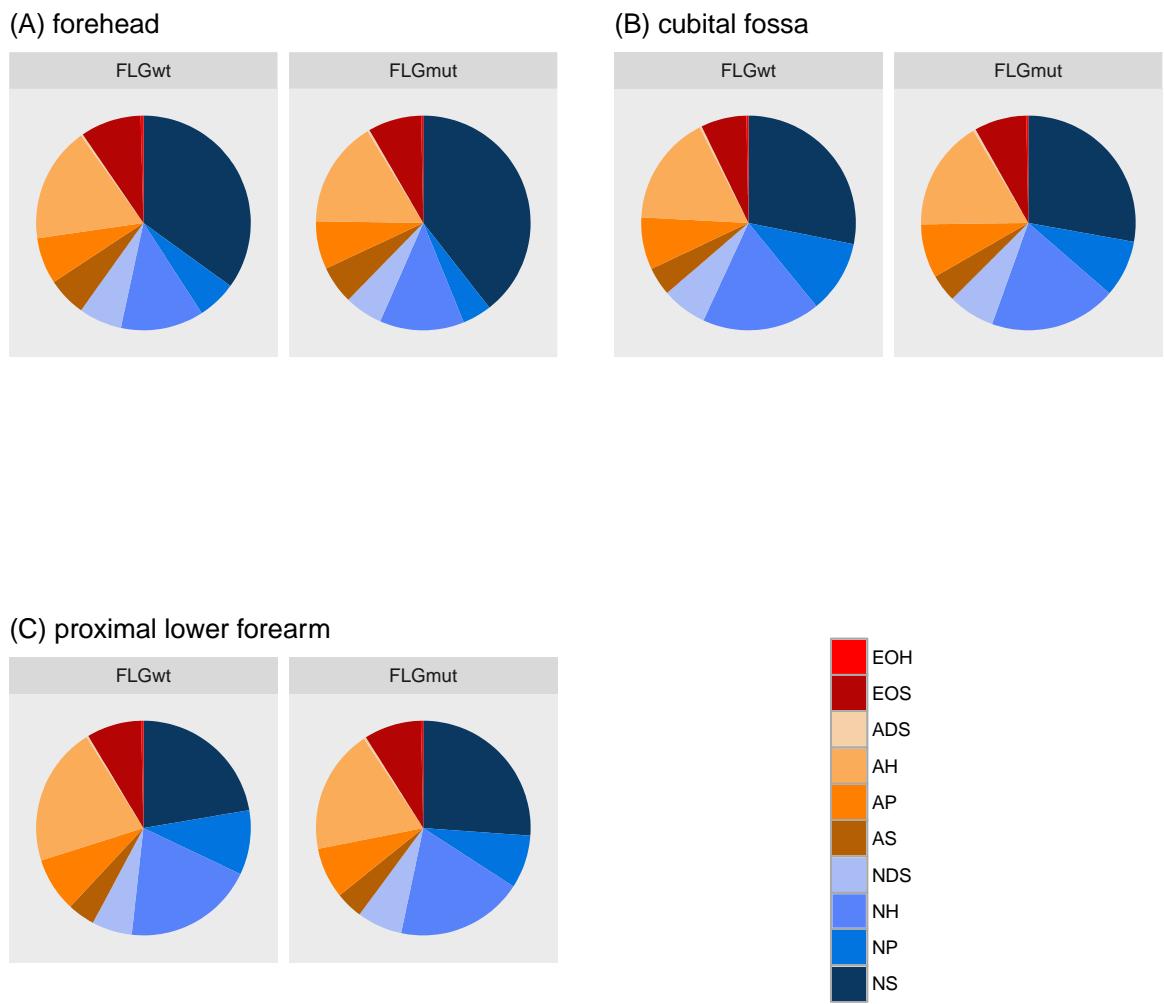


Figure S11: Ceramide abundance in *FLG* mutation carrier vs. non-carrier at three different sites (A) forehead, (B) cubital fossa and (C) proximal lower arm. Blue=α-hydroxy CERs, red=nonhydroxy CERs and brown/orange=esterified ω-hydroxy CERs.

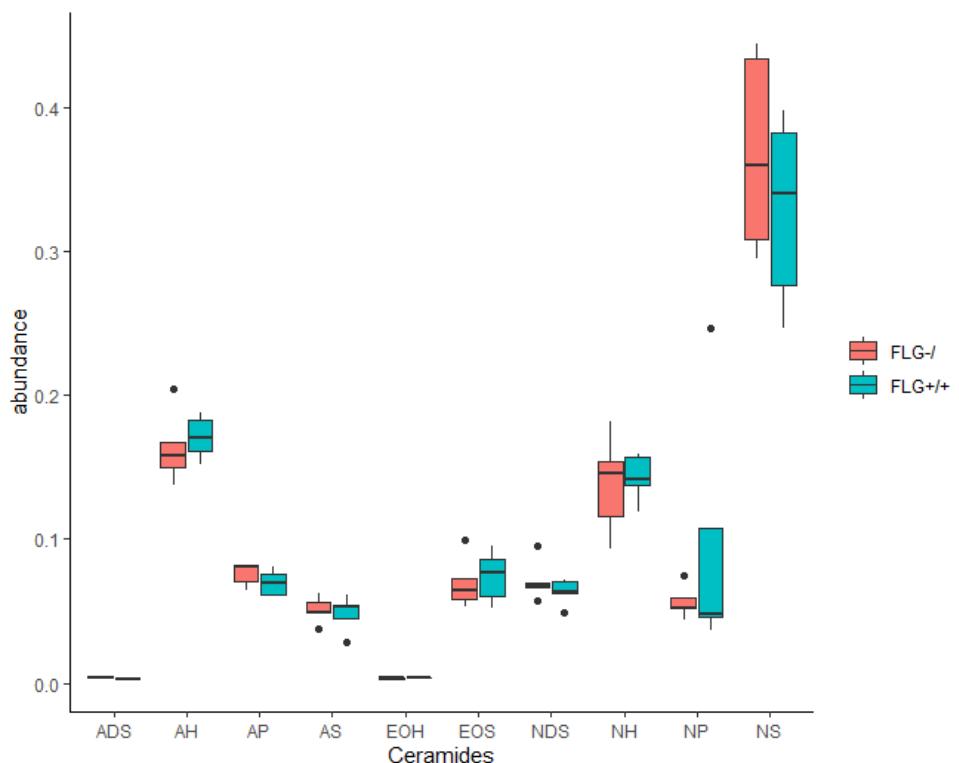
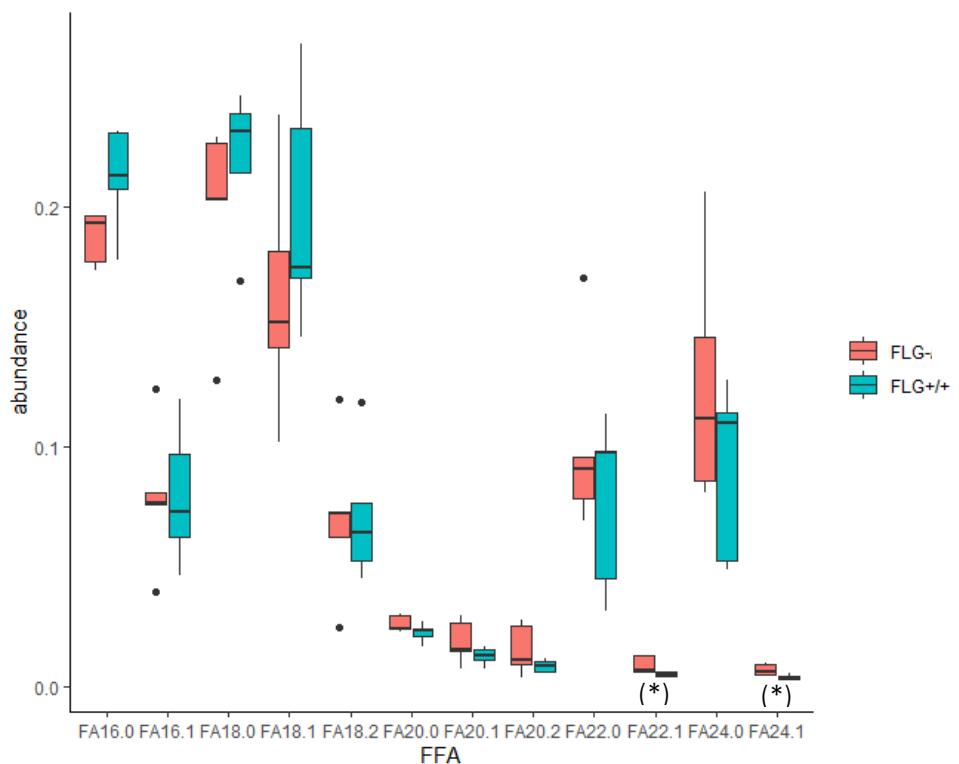
A**B**

Figure S12: Ceramide (A) and FFA (B) abundance in AD patients comparing *FLG* mutation carrier vs. non-carrier. Significant differences are indicated by (*).

SUPPORTING TABLES

Variable	Contr FLG+/+	Contr FLG-/	AD FLG+/+	AD FLG-/
FLG	0 (0%)	5 (100%)	0 (0%)	5 (100%)
Sex male	2 (40%)	1 (20%)	2 (40%)	2 (40%)
Rhinitis	1 (20%)	1 (20%)	3 (60%)	5 (100%)
Asthma	0 (0%)	0 (0%)	4 (80%)	3 (60%)
Age mean (sd) median (min-max)	36.2 (10.43) 31 (29-54)	33.8 (7.29) 32 (27-46)	33 (13) 26 (20-48)	35.8 (12.07) 32 (21-51)
Age-Onset median (min-max)	-	-	1 (0-4)	0 (0-6)
Onset-months mean (sd) median (min-max)	-	-	16.8 (17.7) 12 (6-48)	19.8 (30.83) 1 (1-72)
oSCORAD mean (sd) median (min-max)	-	-	29.8 (4.76) 31 (22-34)	37.6 (6.66) 37 (29-46)
Palmar hyperlinearity	none: N=5 weak: N=0 moderate: N=0 strong: N=0	none: N=0 weak: N=0 moderate: N=2 strong: N=3	none: N=3 weak: N=1 moderate: N=1 strong: N=0	none: N=0 weak: N=0 moderate: N=0 strong: N=5
Sebostasis	none: N=2 weak: N=3 moderate: N=0 strong: N=0	none: N=0 weak: N=2 moderate: N=1 strong: N=2	none: N=1 weak: N=4 moderate: N=0 strong: N=0	none: N=0 weak: N=1 moderate: N=2 strong: N=2

Table S1: Proband characteristics.

	Ceramide	MLP, N=5	
		mean	95% CI
nonhydroxy-CER	NS	18.73	(17.88-19.58)
	NP	8.87	(7.35-10.38)
	NH	21.48	(19.27-23.69)
	NDS	6.02	(5.41-6.64)
α -hydroxy-CER	AS	4.09	(3.34-4.85)
	AP	8.96	(6.81-11.11)
	AH	22.47	(20.73-24.22)
	ADS	0.31	(0.27-0.35)
esterified ω -hydroxy CER	EOS	8.71	(6.04-11.39)
	EOP	-	-
	EOH	0.35	(0.27-0.43)

Table S2: Ceramide composition in healthy volunteers without *FLG* mutations measured by MLP=Metabolon Lipid Panel.

		Ceramide		FFA		Chol S04	
		β/r	P	β/r	P	β/r	P
Fh	age	-0.00	0.6441	0.00	0.6688	-0.00	0.7523
	sex	0.02	0.3385	0.13	0.0896	-0.16	0.0219
Af	age	-0.00	0.6682	0.00	0.4935	-0.00	0.4202
	sex	-0.04	0.3265	0.19	0.0240	-0.15	0.0035
Vf	age	-0.00	0.8803	0.00	0.7057	-0.00	0.6349
	sex	-0.01	0.8414	0.12	0.1102	-0.11	0.0236
all sites	age	-0.00	0.6850	0.00	0.5795	-0.00	0.5835
	sex	-0.01	0.8104	0.15	0.0410	-0.14	0.0080

Table S3: Correlation of age and sex on lipid composition

	TEWL								pH						
	All	AD	healthy	P	FLG-/	FLG+/+	P	All	AD	healthy	P	FLG-/	FLG+/+	P	
Fh	18.4±1.6	19.7±1.6	17.1±1.6	0.5064	19.2±1.6	17.6±1.6	0.6977	4.7±1.1	4.9±1.1	4.5±1.2	0.1141	5.0±1.1	4.4±1.1	0.0505	
Fa	9.7±1.5	10.4±1.4	9.1±1.6	0.4654	8.6±1.4	11.1±1.5	0.1641	5.0±1.1	5.2±1.1	4.8±1.1	0.2008	5.3±1.1	4.7±1.1	0.0674	
Paired t-test															
Fh vs. Fa	2.9x10 ⁻⁵	0.0053	0.0039		0.0005	0.0206		0.0113	0.0463	0.0871		0.2328	0.0080		

Table S4: TEWL and pH measurements in healthy individuals and patients with AD as well as *FLG* competent and *FLG* deficient individuals. Fh: forehead, Fa: forearm.

		Ceramide		FFA		Chol S04	
		r	P	r	P	r	P
Fh	TEWL	0.068	0.7768	-0.394	0.0856	0.460	0.0412
	pH	0.221	0.3631	-0.400	0.0897	0.296	0.2177
Af	TEWL	-0.047	0.8453	-0.099	0.6772	0.017	0.9448
	pH	0.283	0.2396	-0.290	0.2291	0.350	0.1417
Vf	TEWL	0.313	0.1794	-0.424	0.0624	0.433	0.0565
	pH	0.047	0.8473	-0.263	0.2764	0.435	0.0626

Table S5: Correlation of TEWL and pH on lipid composition

Class	Subclass	Ceramide	Forehead			Cubital Fossa			Proximal lower forearm		
			FC	95% CI	p	FC	95% CI	p	FC	95% CI	P
nonhydroxy-CER	NS	S(C17)24:0	2.00	(1.05-3.81)	0.0368	2.23 (1.29-3.84)	0.0064		1.38	(0.75-2.57)	0.2825
		S(C18)16:0	2.92 (1.34-6.36)	0.0096		4.87 (2.09-11.35)	0.0012		2.51	(1.2-5.24)	0.0175
		S(C18)18:0	1.98	(1.16-3.4)	0.0158	2.30 (1.42-3.71)	0.0024		1.45	(1.02-2.06)	0.0419
		S(C18)21:0	2.30	(1.18-4.48)	0.0170	3.02 (1.49-6.14)	0.0051		1.97	(0.99-3.92)	0.0524
		S(C18)22:0	2.52	(1.23-5.18)	0.0149	3.44 (1.61-7.35)	0.0039		2.03	(1.07-3.85)	0.0322
		S(C18)23:0	2.23	(1.23-4.07)	0.0117	2.55 (1.36-4.77)	0.0068		1.44	(0.83-2.51)	0.1834
		S(C18)24:0	2.87 (1.41-5.85)	0.0060		2.76 (1.44-5.3)	0.0046		1.93	(1-3.7)	0.0491
	NP	P(C16)24:0	0.95	(0.58-1.56)	0.8222	0.35 (0.2-0.62)	0.0012		0.45 (0.3-0.67)	5.76x10⁻⁴	
		P(C16)25:0	0.95	(0.58-1.55)	0.8208	0.28 (0.15-0.52)	0.0005		0.38 (0.24-0.59)	2.72x10⁻⁴	
		P(C16)26:0	0.90	(0.55-1.49)	0.6655	0.31 (0.16-0.58)	0.0015		0.36 (0.24-0.52)	4.65x10⁻⁵	
		P(C16)27:0	1.11	(0.77-1.61)	0.5444	0.37 (0.22-0.63)	0.0011		0.46 (0.31-0.7)	0.0013	
		P(C17)24:0	1.15	(0.73-1.82)	0.5213	0.42 (0.24-0.72)	0.0034		0.49 (0.32-0.75)	0.0025	
		P(C17)25:0	0.93	(0.57-1.49)	0.7376	0.32 (0.18-0.56)	5.1x10⁻⁴		0.40 (0.25-0.65)	0.0011	
		P(C17)26:0	1.20	(0.79-1.82)	0.3694	0.35 (0.2-0.59)	5.7x10⁻⁴		0.44 (0.28-0.69)	0.0017	
	NH	P(C18)25:0	1.12	(0.74-1.7)	0.5548	0.47 (0.27-0.8)	0.0078		0.59	(0.39-0.89)	0.0159
		H(C16)24:0	1.15	(0.7-1.88)	0.5591	0.45 (0.28-0.73)	0.0025		0.45 (0.29-0.7)	0.0015	
		H(C16)25:0	0.99	(0.67-1.48)	0.9750	0.33 (0.19-0.57)	5.6x10⁻⁴		0.37 (0.24-0.58)	2.4x10⁻⁴	
		H(C16)26:0	1.02	(0.66-1.59)	0.9142	0.39 (0.23-0.65)	0.0012		0.39 (0.26-0.61)	3.4x10⁻⁴	
		H(C16)27:0	1.06	(0.68-1.65)	0.7928	0.68	(0.4-1.16)	0.1470	0.55 (0.39-0.8)	0.0031	
		H(C16)28:0	1.16	(0.8-1.67)	0.4183	0.65	(0.41-1.03)	0.0639	0.58 (0.41-0.84)	0.0062	
		H(C17)25:0	1.14	(0.71-1.84)	0.5608	0.51 (0.32-0.8)	0.0062		0.46 (0.31-0.7)	0.0010	
		H(C17)26:0	1.06	(0.7-1.61)	0.7730	0.44 (0.26-0.74)	0.0044		0.50 (0.32-0.77)	0.0040	
		H(C17)27:0	1.20	(0.74-1.95)	0.4276	0.49 (0.32-0.76)	0.0030		0.54 (0.38-0.78)	0.0022	
		H(C19)25:0	1.10	(0.71-1.71)	0.6536	0.71	(0.44-1.15)	0.1558	0.59 (0.41-0.85)	0.0066	
	NDS	H(C19)26:0	1.24	(0.84-1.81)	0.2554	0.69	(0.42-1.15)	0.1475	0.60 (0.41-0.87)	0.0097	
		H(C19)27:0	1.39	(0.9-2.16)	0.1287	0.78	(0.45-1.36)	0.3556	0.63 (0.46-0.87)	0.0074	
α -hydroxy-CER	AS	DS(C23)18:0	1.59	(0.94-2.66)	0.0781	2.42 (1.43-4.08)	0.0024		1.23	(0.76-2.01)	0.3785
		S(C18)a20:0	2.67 (1.38-5.2)	0.0066		2.80	(1.19-6.55)	0.0220	1.56	(0.73-3.32)	0.2301
		S(C18)a26:0	1.96	(1.09-3.53)	0.0270	2.24 (1.33-3.77)	0.0053		1.62	(0.9-2.94)	0.1037
	AH	S(C19)a26:0	2.17 (1.35-3.5)	0.0032		1.65	(0.95-2.84)	0.0711	1.18	(0.69-1.99)	0.5263

		H(C16)a25:0	1.21 (0.71-2.07)	0.4616	0.44 (0.26-0.73)	0.0037	0.53 (0.37-0.77)	0.0021
ADS	DS(C20)a24:0	1.92 (1.22-3.01)	0.0073	1.87 (1.15-3.03)	0.0147	1.70 (1.2-2.42)	0.0051	

Table S6: Ceramide species (pmol/disk) significantly different at a threshold of 0.01 between AD patients and controls in at least one of the three sites: forehead, cubital fossa, proximal lower arm. FC=fold change, CI=confidence interval.

Ceramides						
	Co	AD	p-value	FLGwt	FLGmut	p-value
forehead	0.817	0.786	0.0753	0.811	0.793	0.2176
Cubital fossa	0.852	0.807	0.0021	0.846	0.843	0.9118
Proximal lower forearm	0.841	0.832	0.0185	0.836	0.840	0.8534
Free Fatty Acids						
forehead	0.839	0.843	0.6305	0.828	0.868	0.1051
Cubital fossa	0.813	0.778	0.4813	0.787	0.781	0.7394
Proximal lower forearm	0.801	0.782	0.3930	0.787	0.790	0.9118

Table S7: Balance of ceramides and free fatty acids measured by the Shannon Index.

Ceramide class	AD	Healthy	P-value (t-test)	P-value (wilcox)
NS	mean: 9.1±11.5 median: 9.0 (4.9,17.4)	mean: 13.1±6.0 median: 14.1 (9.2,17.1)	0.3434	0.4359
NH	mean: -4.5±3.4 median: -5.2 (-6.7,-2.4)	mean: -8.2±2.5 median: -8.5 (-9.9,-6.5)	0.01397	0.02881

Table S8: Differential analysis of CER[NS] and CER[NH] in healthy individuals and patients with AD.

	Forehead				Cubital Fossa				Proximal Lower Forearm			
	FLGwt		FLGmut		FLGwt		FLGmut		FLGwt		FLGmut	
Ceramide	mean	95%CI	mean	95%CI	mean	95%CI	mean	95%CI	mean	95%CI	mean	95%CI
NS	34.97	31.6-38.35	39.45	32.93-45.98	28.18	19.89-36.47	27.82	21.35-34.28	22.33	18.87-25.78	26.13	22.12-30.15
NP	5.88	3.85-7.92	4.39	3.54-5.24	10.93	5.27-16.59	8.55	6.77-10.33	9.73	6.12-13.34	8.01	6.7-9.32
NH	12.51	11.25-13.78	12.73	10.41-15.04	17.78	14.01-21.55	19.1	15.89-22.32	19.7	17.95-21.44	19.16	16.16-22.16
NDS	6.5	5.61-7.4	5.74	5.3-6.17	6.8	5.94-7.66	7.04	6.1-7.98	6.05	5.64-6.46	6.82	5.75-7.88
AS	5.85	5.43-6.28	5.71	5.05-6.37	4.27	3.57-4.97	4.17	3.55-4.79	4.14	3.49-4.78	4.1	3.47-4.74
AP	6.99	6.58-7.39	7.23	6.36-8.1	7.86	6.55-9.18	8.1	7.09-9.12	8.18	6.94-9.42	7.67	6.98-8.35
AH	17.39	15.75-19.02	16.04	13.28-18.81	16.64	13.92-19.36	16.66	14.83-18.49	20.98	19.45-22.52	18.78	17.8-19.75
ADS	0.33	0.31-0.35	0.35	0.26-0.44	0.34	0.3-0.39	0.38	0.32-0.43	0.32	0.28-0.35	0.35	0.28-0.42
EOS	9.15	7.27-11.03	8.04	6.83-9.24	6.88	5.35-8.4	7.86	6.35-9.37	8.24	6.7-9.79	8.63	7.2-10.06
EOH	0.42	0.33-0.51	0.32	0.26-0.38	0.31	0.26-0.35	0.31	0.27-0.36	0.34	0.3-0.39	0.35	0.32-0.38

Table S9: Ceramide proportions (relative abundance) and their 95% CIs in FLG wildtype (FLGwt) and FLG mutation carriers (FLGmut).

Ceramide			
class	Analyte	Fatty Acid Class	Sphingoid Base
AH	H(C16)a24:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C16)a25:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C16)a26:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C16)a27:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C16)a28:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C17)a24:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C17)a25:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C17)a26:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C17)a27:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C17)a29:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C18)a20:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C18)a22:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C18)a24:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C18)a25:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C18)a26:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C18)a27:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C18)a28:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C19)a24:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C19)a25:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C19)a26:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C20)a24:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C20)a25:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C20)a26:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C20)a28:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C21)a24:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C21)a25:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C21)a26:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C22)a16:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C22)a24:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C22)a25:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AH	H(C22)a26:0	α-hydroxy fatty acids	6-hydroxy sphingosine
AS	S(C16)a24:0	α-hydroxy fatty acids	sphingosine
AS	S(C16)a25:0	α-hydroxy fatty acids	sphingosine
AS	S(C16)a26:0	α-hydroxy fatty acids	sphingosine
AS	S(C16)a27:0	α-hydroxy fatty acids	sphingosine
AS	S(C16)a28:0	α-hydroxy fatty acids	sphingosine
AS	S(C17)a24:0	α-hydroxy fatty acids	sphingosine
AS	S(C17)a25:0	α-hydroxy fatty acids	sphingosine
AS	S(C17)a26:0	α-hydroxy fatty acids	sphingosine
AS	S(C17)a27:0	α-hydroxy fatty acids	sphingosine
AS	S(C17)a28:0	α-hydroxy fatty acids	sphingosine
AS	S(C18)a17:0	α-hydroxy fatty acids	sphingosine
AS	S(C18)a18:0	α-hydroxy fatty acids	sphingosine

NP	P(C18)25:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C18)26:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C18)27:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C18)28:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C18)29:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C19)24:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C19)25:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C19)26:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C20)23:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C20)24:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C20)25:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C20)26:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C20)27:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C20)28:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C21)24:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C21)25:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C21)26:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C22)22:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C22)24:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C22)25:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C22)26:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C22)28:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C24)24:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C24)25:0	non-hydroxy fatty acids	phytosphingosine
NP	P(C24)26:0	non-hydroxy fatty acids	phytosphingosine

Table S10: Ceramide analytes of the Metabolon Lipid Panel.