Metabolomic Profiles of Current Cigarette Smokers (MS# MC-16-0012)

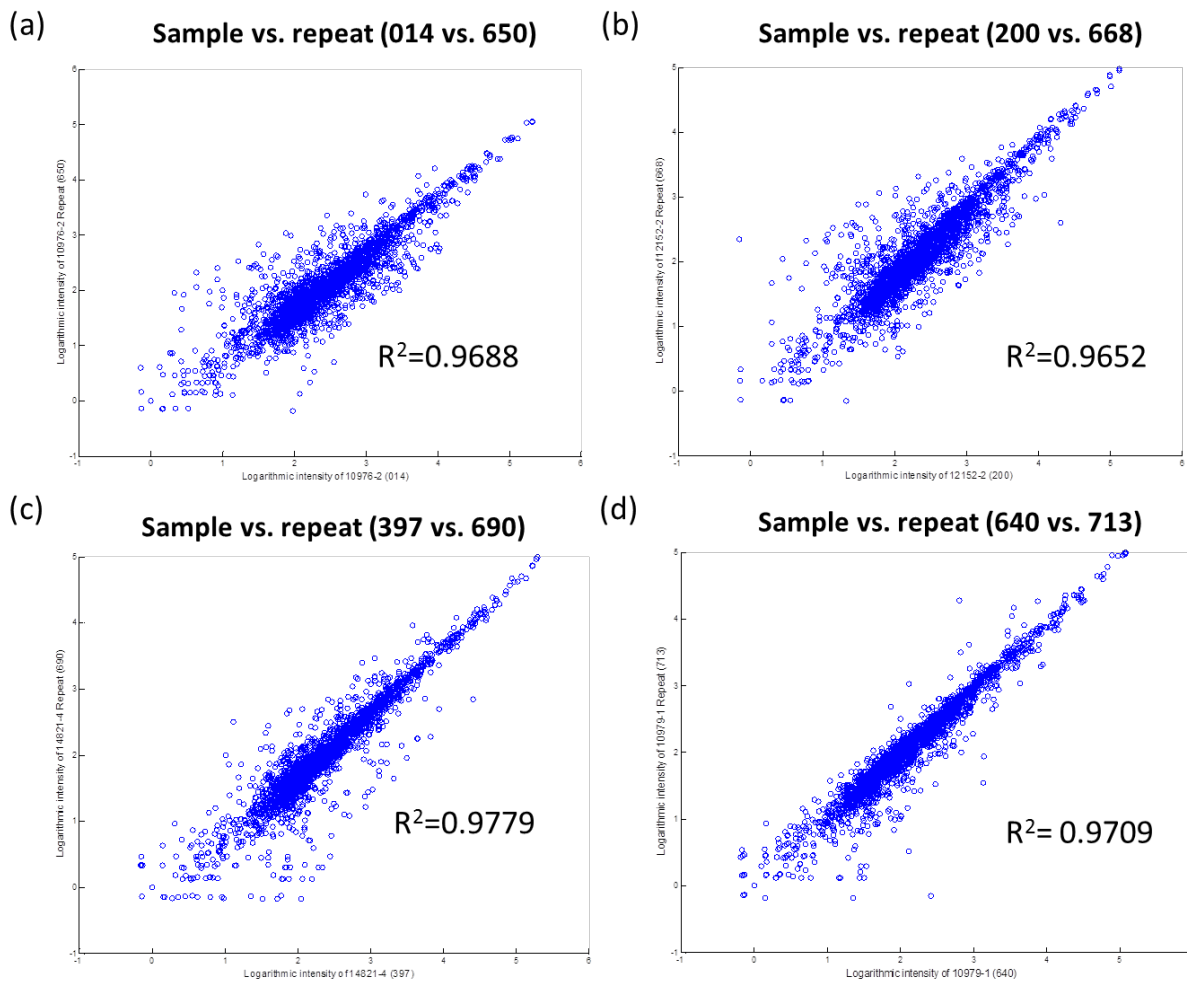**Hsu et al., Supplemental information.**

Figures

Fig S1. Evaluation of the reproducibility on samples and their repeats. Scatter plots of logarithmic intensities of samples and their matching repeats shown in (a-d) represent high correlations (Pearson Correlation > 0.9), indicating high resemblances between samples and the repeated injections.
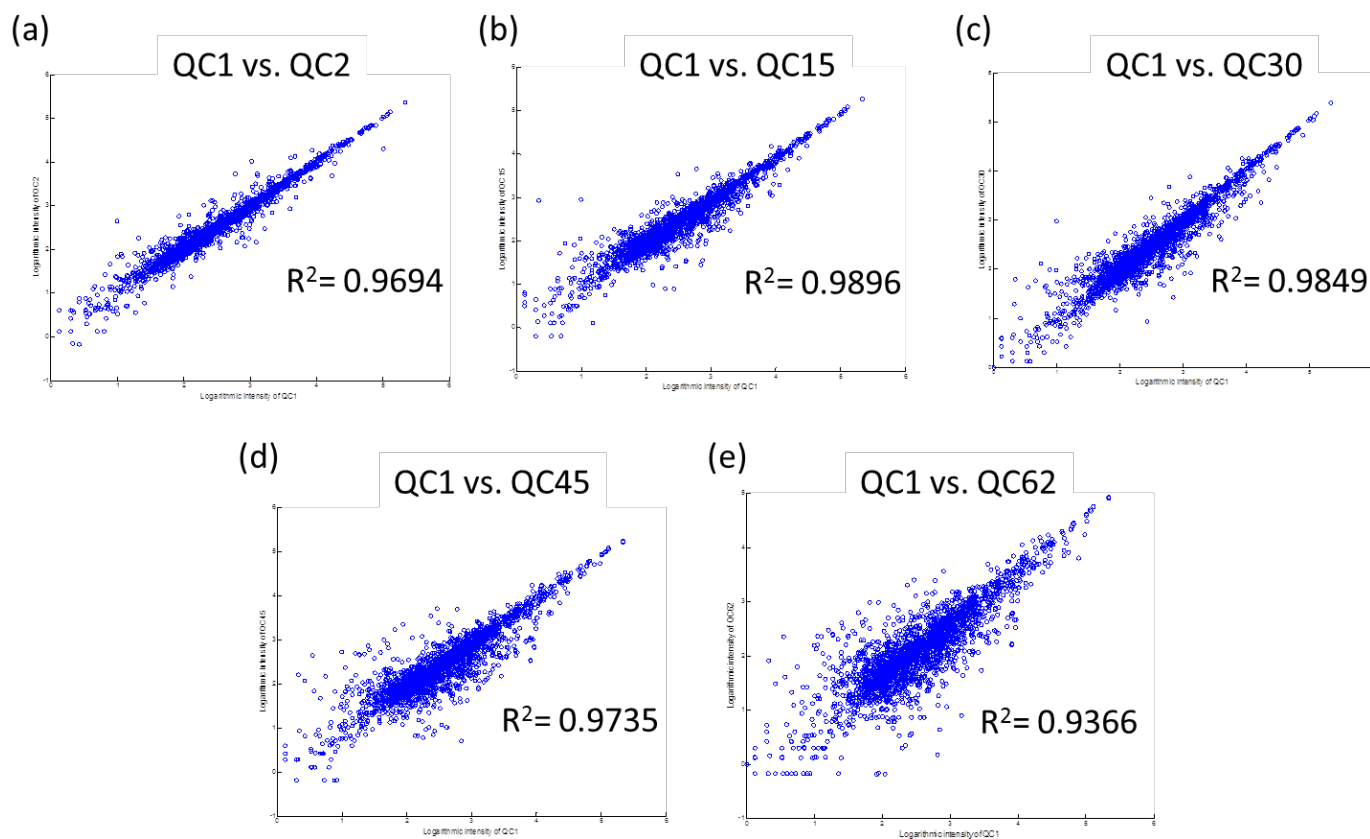
Fig S2. Evaluation of the reproducibility on QC samples. Scatter plots of logarithmic intensities of (a) QC1 vs. QC2; (b) QC1 vs. QC15; (c) QC1 vs. QC30; (d) QC1 vs. QC45; and (e) QC1 vs. QC62 exhibit high correlations (Pearson Correlation > 0.9), indicating a high resemblance among QC samples.
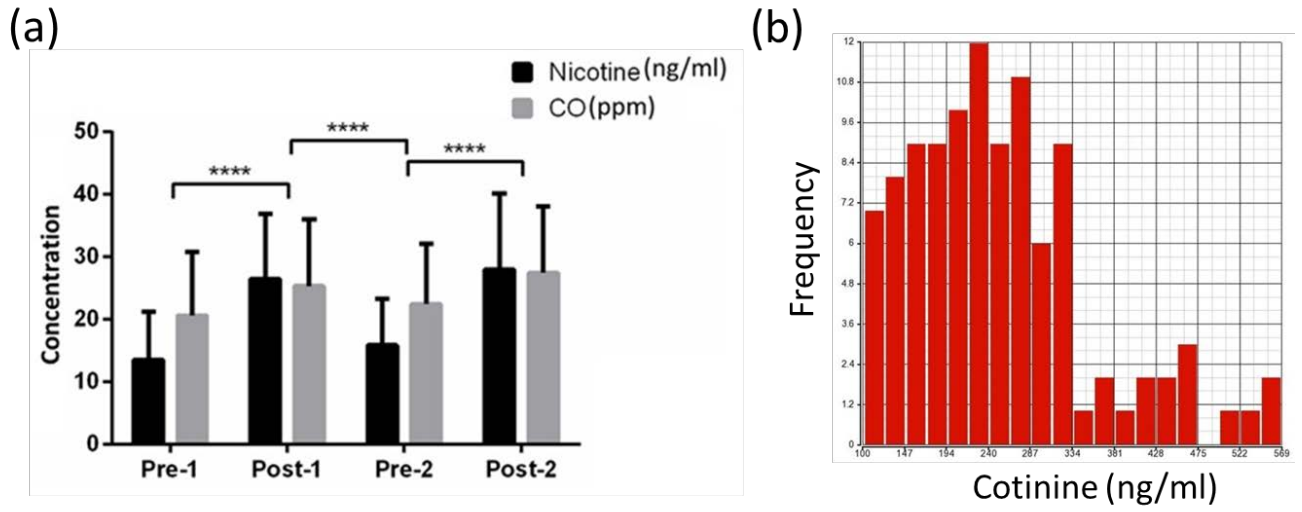
(a)

(b)

Fig. S3. (a) measurements of smoke exposure showed significant increase of nicotine and CO level after 1st and 2nd cigarette smoking, and decrease levels during the one hour interval, and (b) cotinine levels across subjects.

Sources of Variation
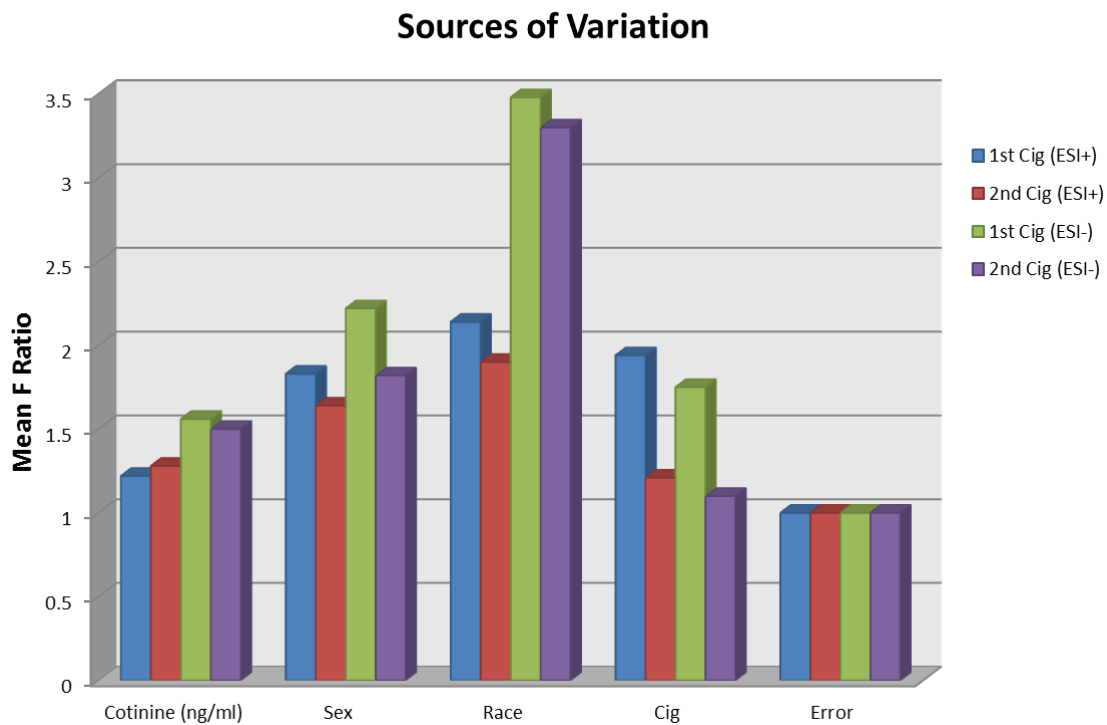
Fig S4. Sources of Variation plot. All the factors including cotinine, sex, race, and pre- & post-cigarette (cig) in the ANCOVA model are listed on the X-axis (including random error). The Y-axis represents the Mean F-ratio (variability in the data not explained by the other factors) of all the metabolites.
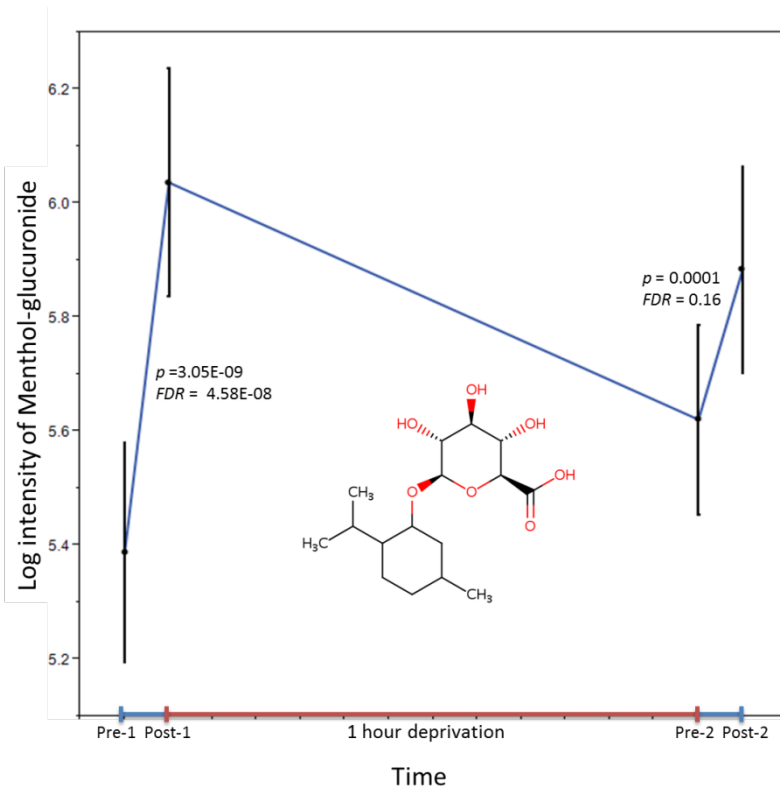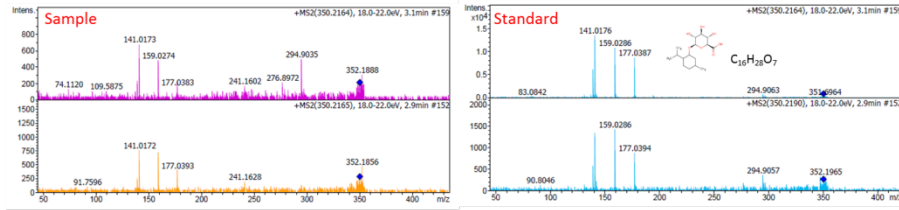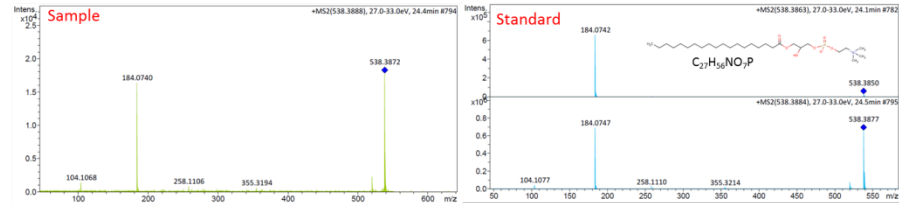
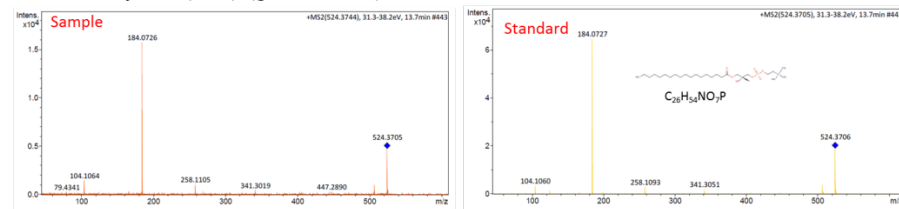Fig. S5. Dynamic changes of the menthol-glucuronide from cigarette smoking.
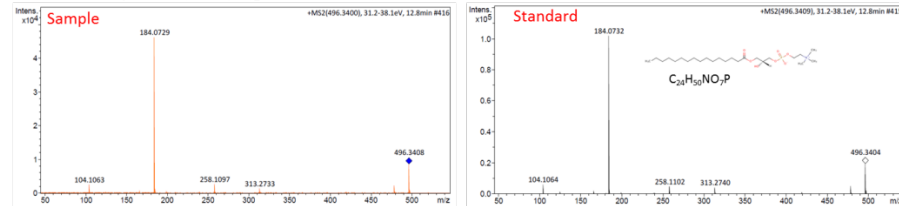
(a)

Menthol-glucuronide (q = 4.58E-08)

Sample — +MS2(350.2164), 18.0-22.0eV, 3.1min #159

74.1120 109.5875 141.0173 159.0274 177.0383 241.1602 276.8972 294.9035 352.1888

Standard — +MS2(350.2164), 18.0-22.0eV, 3.1min #159

141.0176 159.0286 177.0387 83.0842 294.9063 35?.?964 $C_{16}H_{28}O_7$

+MS2(350.2165), 18.0-22.0eV, 2.9min #152

91.7596 141.0172 177.0393 241.1628 352.1856

+MS2(350.2190), 18.0-22.0eV, 2.9min #152

90.8046 159.0286 177.0394 294.9057 352.1965

(b)

LysoPC(19:0) (q = 1.29E-06)

Sample — +MS2(538.3888), 27.0-33.0eV, 24.4min #794

184.0740 538.3872 104.1068 258.1106 355.3194

Standard — +MS2(538.3863), 27.0-33.0eV, 24.1min #782

184.0742 $C_{27}H_{56}NO_7P$ 538.3850 104.1077 258.1110 355.3214

+MS2(538.3884), 27.0-33.0eV, 24.5min #795

184.0747 538.3877

(c)

LysoPC(18:0) (q = 6.64E-06)

Sample — +MS2(524.3744), 31.3-38.2eV, 13.7min #443

184.0726 524.3705 79.4341 104.1064 258.1105 341.3019 447.2890

Standard — +MS2(524.3705), 31.3-38.2eV, 13.7min #443

184.0727 $C_{26}H_{54}NO_7P$ 524.3706 104.1060 258.1093 341.3051

(d)

LysoPC(16:0) (q = 6.64E-06)

Sample — +MS2(496.3400), 31.2-38.1eV, 12.8min #416

184.0729 496.3408 104.1063 258.1097 313.2733

Standard — +MS2(496.3409), 31.2-38.1eV, 12.8min #415

184.0732 $C_{24}H_{50}NO_7P$ 496.3404 104.1064 258.1102 313.2740

(e)

LysoPC(14:0) (q = 6.64E-06)

Sample — +MS2(468.3078), 26.3-32.1eV, 19.6min #635

184.0740 99.9684 268.2634 363.6408 468.3050 580.8008

Standard — +MS2(468.3059), 26.3-32.1eV, 19.8min #643

184.0732 $C_{22}H_{46}NO_7P$ 468.3055 104.1067 285.2411

+MS2(468.3097), 26.3-32.1eV, 19.9min #647

184.0743 104.1062 211.1027 286.2488 368.2755 468.3094 547.7764

(f)

LysoPE(18:0/0:0) (q = 9.22E-06)

Sample — +MS2(482.3229), 26.6-32.5eV, 23.6min #767

341.3064 155.0116 216.0649 267.2695 310.3118 421.2740

Standard — +MS2(482.3259), 26.6-32.5eV, 23.6min #767

341.3058 $C_{23}H_{48}NO_7P$ 155.0111 216.0642 267.2691 310.3114 421.2719

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Fig S6. Structures and MS/MS spectra of representative metabolites and the authentic standard compounds. (a) menthol-glucuronide, (b) lysoPC(19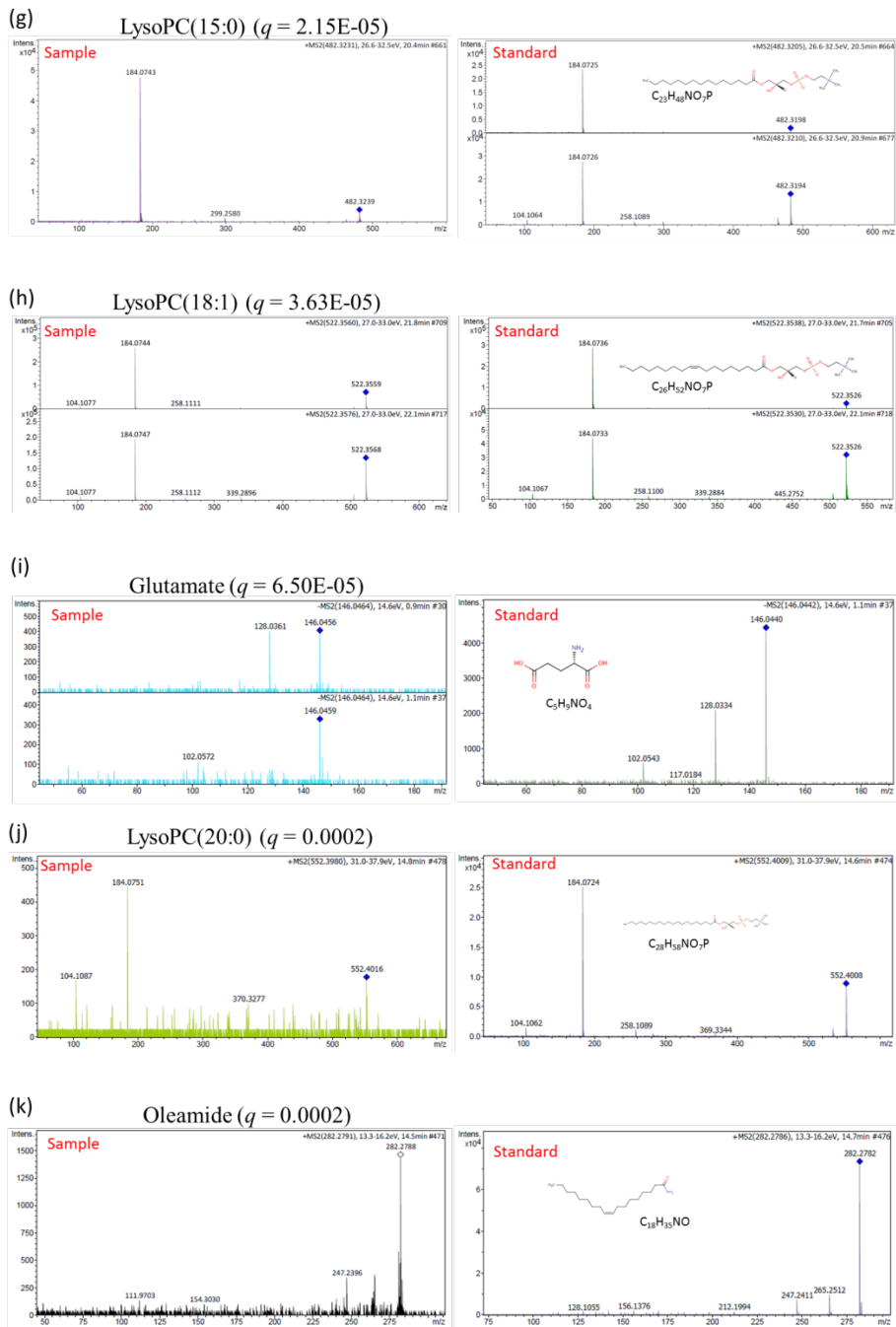:0), (c) lysoPC(18:0), (d) lysoPC(16:0), (e) lysoPC(14:0), (f) lysoPE(18:0/0:0), (g) lysoPC(15:0), (h) lysoPC(18:1), (i) glutamate, (j) lysoPC(20:0) and (k) oleamide.

(a) Race

(b) Gender

Fig S7. **Race and gender differences evaluated by Random Forests classification**. The forest accuracies were calculated and the sample classifications were presented by multi-dimensional scaling (MDS) plots. In the plots, (a) Blacks (red) vs. White (blue), and (b) females (red) vs. males (blue) were separated with % accuracy in the classifications.

Fig S8. 3D scatter plot of PCA score and loadings on the significant metabolites by (a) race and; (b) gender.

Fig S9. **Levels of lipid metabolites versus QC samples in this study.** Data significant based on two sample t-test with Benjamini-Hochberg correction (FDR < 0.05).

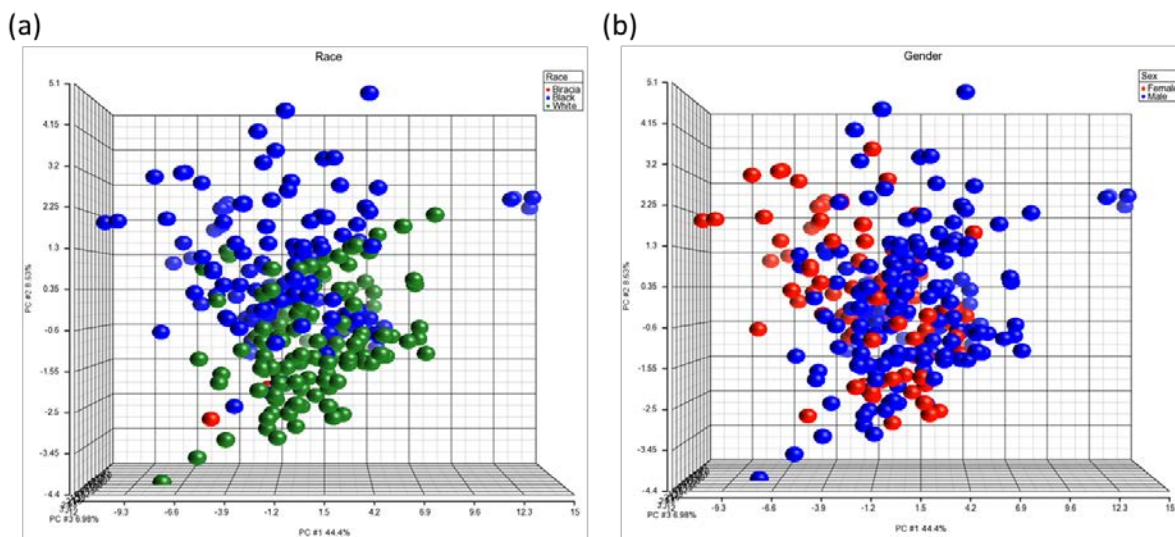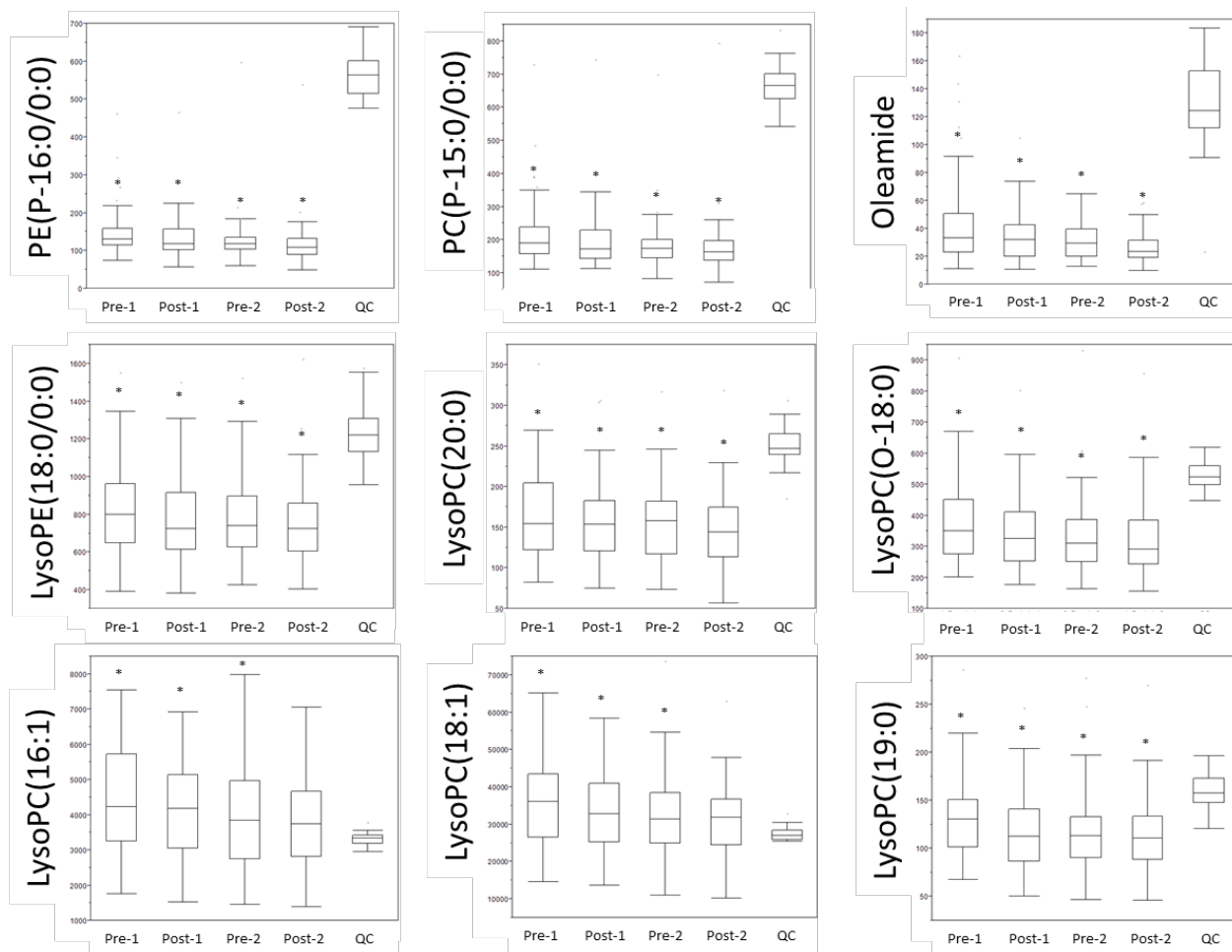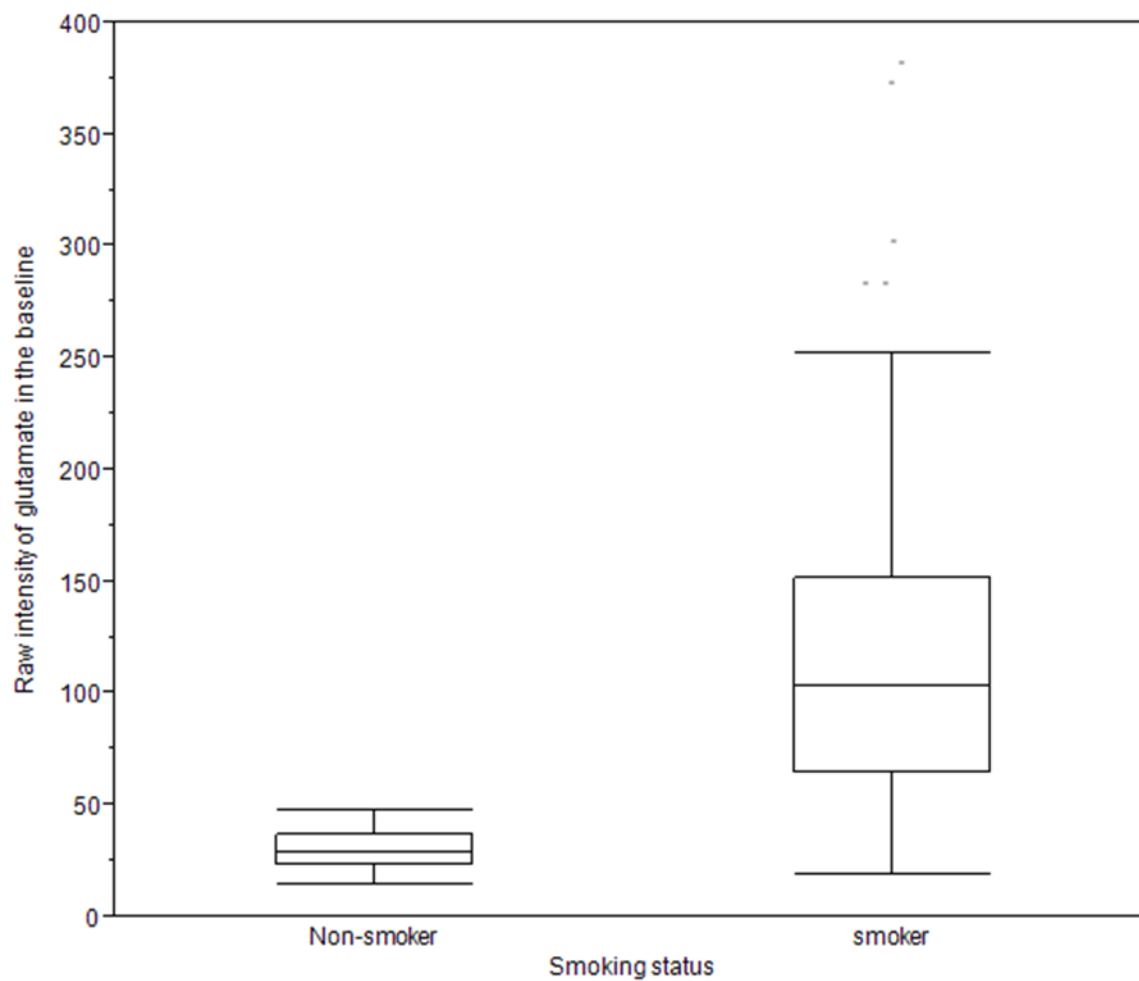**Fig S10.** Box plots of glutamate levels among non-smokers from QC samples and smokers in the baseline.

Tables

Table S1. Significant metabolites after cigarette smoking.

Metabolite ID	Observed m/z	RT	Chemical taxonomy	Adduct	Compound MW (Da)	Mass difference (Delta)	Post-cig 1 vs. Pre-cig 1			Post-cig 2 vs. Pre-cig 2		
							p	q	Trend	p	q	Trend
Menthol-glucuronide	331.18	3.07	Prenol Lipids	M-H	332.18	0.00045	3.05E-09	4.58E-08	↑	0.0001	0.16	↑
1α,25-dihydroxy-11-(4-hydroxymethylphenyl)-9,11-didehydrovitamin D3	543.34	5.52	Sterol Lipids	M+Na	520.36	0.00626	6.40E-09	6.02E-07	↓	0.0070	0.48	↓
LysoPC(19:0)	538.39	5.87	Glycerophospholipids	M+H	537.38	0.00130	5.14E-08	1.29E-06	↓	0.0280	0.59	↓
229.05-	229.05	2.71	Unknown				8.64E-08	6.48E-07	↑	0.0061	0.57	↑
104.10+	104.10	4.87	Unknown				1.33E-07	2.66E-06	↓	0.0156	0.54	↓
LysoPC(18:0)	524.37	5.51	Glycerophospholipids	M+H	523.36	0.00108	4.59E-07	6.64E-06	↓	0.0224	0.55	↓
LysoPC(16:0)	496.34	4.87	Glycerophospholipids	M+H	495.33	0.00087	6.25E-07	6.64E-06	↓	0.0039	0.42	↓
LysoPC(14:0)	468.31	4.31	Glycerophospholipids	M+H	467.30	0.00068	7.32E-07	6.64E-06	↓	0.0211	0.54	↓
LysoPC(16:1)	494.32	4.47	Glycerophospholipids	M+H	493.32	0.00077	7.97E-07	6.64E-06	↓	0.0022	0.35	↓
LysoPE(18:0/0:0)	482.32	5.50	Glycerophospholipids	M+H	481.32	0.00085	1.38E-06	9.22E-06	↓	0.0195	0.54	↓
2-Octenoylcarnitine	286.20	2.41	Fatty Acid Esters	M+H	285.19	0.00052	4.46E-06	2.03E-05	↓	0.0355	0.64	↓
LysoPC(15:0)	482.32	4.58	Glycerophospholipids	M+H	481.32	0.00063	5.16E-06	2.15E-05	↓	0.0070	0.48	↓
LysoPC(18:1)	522.36	5.03	Glycerophospholipids	M+H	521.35	0.00122	1.14E-05	3.63E-05	↓	0.0177	0.54	↓
698.2+	698.29	2.15	Unknown				1.81E-05	5.17E-05	↑	0.0006	0.26	↑
763.98+	763.98	4.87	Unknown				2.12E-05	5.90E-05	↓	0.0141	0.54	↓
Glutamate	146.05	0.33	Amino Acids, Peptides, and Analogues	M-H	147.05	0.00075	2.67E-05	6.50E-05	↓	0.0114	0.70	↓
184.07+	184.07	5.38	Unknown				5.42E-05	0.0001	↓	0.0242	0.57	↓
LysoPC(20:2)	548.37	5.20	Glycerophospholipids	M+H	547.36	0.00002	6.22E-05	0.0001	↓	0.0280	0.59	↓
Chenodeoxycholic acid sulfate	507.22	4.17	Sterol Lipids	M+Cl	472.25	0.00533	8.00E-05	0.0001	↓	0.0074	0.59	↓
508.34+	508.34	4.73	Unknown				8.30E-05	0.0002	↓	0.0208	0.54	↓
LysoPC(20:0)	552.40	6.22	Glycerophospholipids	M+H	551.40	0.00081	9.50E-05	0.0002	↓	0.0025	0.35	↓
272.66+	272.66	5.52	Unknown				0.0001	0.0002	↓	0.0396	0.65	↓
Oleamide	282.28	4.36	Fatty Amides	M+H	281.27	0.00044	0.0001	0.0002	↓	0.0004	0.24	↓
512.36+	512.36	5.18	Unknown				0.0002	0.0003	↓	0.0220	0.55	↓
543.84+	543.84	5.52	Unknown				0.0003	0.0005	↓	0.0010	0.28	↓
LysoPC(O-18:0)	510.39	5.72	Glycerophospholipids	M+H	509.38	0.00073	0.0003	0.0005	↓	0.0158	0.54	↓
483.36+	483.36	5.04	Unknown				0.0006	0.0008	↓	0.0230	0.56	↓
PC(P-15:0/0:0)	466.33	5.69	Glycerophospholipids	M+H	427.37	0.00059	0.0006	0.0008	↓	0.0013	0.31	↓
531.41+	531.41	8.19	Unknown				0.0007	0.0008	↑	0.0110	0.53	↑
772.96+	772.96	4.87	Unknown				0.0008	0.0009	↓	0.0109	0.53	↓
PE(P-16:0/0:0)	438.30	5.01	Glycerophospholipids	M+H	437.29	0.00044	0.0013	0.0015	↓	0.0011	0.28	↓

Metabolite ID	All smokers		Menthol		Non-menthol	
	r^1	p^2	r	p	r	p
272.66+	-0.33	**0.006**	-0.12	0.455	-0.60	**0.001**
104.10+	-0.33	**0.006**	-0.13	0.405	-0.56	**0.003**
1α,25-dihydroxy-11-(4-hydroxymethylphenyl)-9,11-didehydrovitamin D3	-0.33	**0.007**	-0.24	0.133	-0.43	**0.027**
184.07+	-0.29	**0.015**	-0.14	0.395	-0.50	**0.008**
LysoPC (19:0)	-0.28	**0.022**	-0.14	0.371	-0.37	0.055
Menthol-glucuronide	0.20	**0.043**	0.31	**0.008**	0.11	0.524
Oleamide	-0.23	0.061	-0.19	0.233	-0.32	0.104
543.84+	-0.22	0.072	-0.14	0.381	-0.31	0.112
508.34+	-0.21	0.080	-0.15	0.339	-0.29	0.135
LysoPC(16:1)	-0.21	0.085	-0.09	0.590	-0.40	**0.040**
LysoPC(20:0)	-0.20	0.095	-0.08	0.598	-0.36	0.067
LysoPC(18:0)	-0.19	0.118	-0.12	0.463	-0.30	0.132
512.36+	-0.18	0.135	-0.11	0.486	-0.29	0.138
772.96+	-0.18	0.146	-0.19	0.233	-0.18	0.365
763.98+	-0.17	0.156	-0.15	0.360	-0.22	0.268
LysoPC(18:1)	-0.17	0.165	-0.15	0.353	-0.18	0.369
LysoPC(16:0)	-0.16	0.186	-0.07	0.642	-0.25	0.213
483.36+	-0.15	0.213	0.03	0.830	-0.39	**0.047**
Chenodeoxycholic acid sulfate	-0.10	0.289	-0.05	0.671	-0.10	0.577
LysoPC(15:0)	-0.13	0.291	0.02	0.919	-0.23	0.249
229.05-	0.10	0.302	-0.03	0.799	0.43	**0.011**
LysoPC(20:2)	-0.11	0.393	0.00	0.998	-0.23	0.252
LysoPE(18:0/0:0)	-0.09	0.454	0.01	0.960	-0.25	0.208
2-Octenoylcarnitine	0.08	0.499	0.04	0.815	0.14	0.471
PE(P-16:0/0:0)	0.08	0.512	0.10	0.523	0.04	0.844
LysoPC(14:0)	-0.08	0.536	0.06	0.716	-0.23	0.243
LysoPC(O-18:0)	-0.05	0.664	0.05	0.749	-0.27	0.177
698.29+	-0.05	0.670	-0.01	0.940	-0.23	0.248
PC(P-15:0/0:0)	-0.05	0.677	-0.03	0.850	-0.05	0.815
531.41+	0.01	0.913	0.03	0.834	-0.05	0.812
Glutamate	-0.01	0.941	0.01	0.934	-0.08	0.660

[*] Data significant at $p < 0.05$.

[1,2] r and p-value (correlation) were computed based on Spearman's rank correlation coefficient.

Table S3. Correlation of menthol-glucuronide in the baseline to other metabolites.

Metabolites	r^1	p^2
229.05-	0.47	**3.95E-07**
2-Octenoylcarnitine	0.37	**0.0018**
Glutamate	0.30	**0.0020**
Oleamide	-0.36	**0.0026**
LysoPC(16:1)	-0.31	**0.0096**
508.34+	-0.28	**0.0224**
Chenodeoxycholic acid sulfate	0.19	0.0543
LysoPC(20:2)	-0.19	0.1261
104.10+	-0.17	0.1633
763.98+	-0.16	0.1855
LysoPC(18:1)	-0.14	0.2703
698.29+	-0.12	0.3171
LysoPC(18:0)	0.12	0.3463
LysoPC(15:0)	-0.10	0.3963
PE(P-16:0/0:0)	0.10	0.4012
PC(P-15:0/0:0)	0.10	0.4361
LysoPC(14:0)	-0.09	0.4477
543.84+	0.09	0.4746
772.96+	-0.08	0.5216
531.41+	0.08	0.5318
LysoPC(19:0)	-0.06	0.6177
512.36+	-0.06	0.6444
272.66+	-0.04	0.7248
LysoPC(16:0)	-0.04	0.7693
LysoPC(O-18:0)	-0.02	0.8904
483.36+	0.01	0.9051
1α,25-dihydroxy-11-(4-hydroxymethylphenyl)-9,11-didehydrovitamin D3	-0.01	0.9134
LysoPE(18:0/0:0)	0.01	0.9402
184.07+	0.00	0.9850
LysoPC(20:0)	0.00	0.9978

[*] Data significant at $p < 0.05$.

[1,2] r and p-value (correlation) were computed based on Spearman's rank correlation coefficient.

Metabolite ID	Nicotine boost		CO boost	
	r^1	p^2	r	p
19:0 Lyso PC	0.369	**0.005**	0.164	0.204
LysoPC(20:2)	0.323	**0.014**	0.064	0.623
272.66+	0.280	**0.035**	0.194	0.131
Menthol-glucuronide	0.264	**0.042**	0.227	**0.035**
LysoPC(14:0)	0.213	0.111	0.129	0.319
LysoPC(15:0)	0.212	0.113	0.001	0.993
184.07+	0.192	0.153	0.108	0.403
LysoPC(20:0)	0.186	0.167	0.110	0.393
543.84+	0.174	0.196	0.155	0.229
LysoPC(18:0)	0.167	0.215	0.243	0.057
512.36+	0.166	0.218	-0.007	0.959
PE(P-16:0/0:0)	0.156	0.247	0.015	0.908
Chenodeoxycholic acid sulfate	-0.147	0.261	0.048	0.660
508.34+	0.151	0.263	-0.035	0.786
763.98+	0.144	0.285	-0.012	0.926
104.10+	0.143	0.288	0.014	0.914
Oleamide	0.138	0.306	0.293	**0.021**
531.41+	0.111	0.411	0.383	**0.002**
LysoPC(16:0)	0.104	0.441	0.167	0.194
LysoPC(16:1)	0.103	0.448	0.095	0.462
Glutamate	-0.089	0.500	0.068	0.533
LysoPC(18:1)	0.085	0.527	0.200	0.119
229.05-	0.083	0.528	0.089	0.410
LysoPC(O-18:0)	0.067	0.618	-0.035	0.787
483.36+	0.057	0.672	0.288	**0.023**
698.29+	0.021	0.875	-0.091	0.482
1?,25-dihydroxy-11-(4-hydroxymethylphenyl)-9,11-didehydrovitamin D3	0.020	0.884	0.143	0.268
772.96+	-0.014	0.917	-0.219	0.087
2-Octenoylcarnitine	-0.013	0.922	-0.013	0.917
LysoPE(18:0/0:0)	-0.012	0.930	0.152	0.237
PC(P-15:0/0:0)	-0.005	0.972	0.000	0.997

[*] Data significant at $p < 0.05$.

[1,2] r and p-value (correlation) were computed based on Spearman's rank correlation coefficient.