

Supplementary Material

Effect of short-term use of FFP2 (N95) mask on salivary metabolome of young healthy volunteers: A pilot study.

Sk Ramiz Islam, Debasish Prusty, Subhadip Maiti, Raju Dutta, Partha Chattopadhyay and
Soumen Kanti Manna

Table S1: Metabolite quantitation method and calibration

Table S2: Results of permutation test for the PLS-DA analysis

Table S3: Metabolites of interest

Figure S1: PCA with pooled QC samples

Figure S2: PCA with pre- and post-mask samples

Figure S3: Heatmaps showing sample clustering pattern

Figure S4: Internal standard-normalized changes in metabolite abundance

Figure S5: Sum-normalized changes in metabolite abundance

Figure S6: myo-Inositol-normalized changes in metabolite abundance

Figure S7: Correlation between metabolite abundances

Table S1: Quantifier, qualifier ions, R² and linearity range for quantitation of metabolites.

Compound identity	Quantifier (m/z)	Qualifiers (m/z)	R ²	Linear range
L-Fucose	117	160, 277	0.995	24.4nM – 6.25 µM
5-Aminovaleric acid	174	200, 318	0.998	24.4nM – 25.0 µM
Putrescine	174	200, 214	0.987	24.4nM – 6.25 µM
Phloretic acid	179	192, 310	0.999	24.4nM – 25.0 µM
Citric acid	273	347, 363	0.977	24.4nM – 12.5 µM
Benzenepropionic acid	104	207, 222	0.999	24.4nM – 6.25 µM

Table S2: Results of leave-one-out cross validation and permutation tests for PLS-DA model.

	R2	Q2	P value (100 permutations)
Internal-standard normalized	0.43	-1.56	0.11
Sum-normalized	0.51	-1.38	0.83
<i>myo</i> -Inositol normalized	0.50	-1.16	0.80

Table S3: Metabolites showing change in abundance between paired samples upon use of FFP2 mask for 30 minutes.

Compound identity	Retention time (min)	NIST match factor	Level of identification ^a	Fold change ^b	P value ^b
L-Fucose (conformer 1)	27.67	> 850	Level 1	1.62	0.0045
L-Fucose (conformer 2)	27.87	> 700	Level 1	1.53	0.0067
5-Aminovaleric acid	25.03	> 900	Level 1	1.81	0.0118
Benzenepropionic acid	19.74	> 500	Level 1	1.64	0.0024
Citric acid	29.53	> 750	Level 1	-1.5	0.0214
Phloretic acid	27.90	> 500	Level 1	1.83	0.0077
Putrescine	27.47	> 850	Level 1	1.91	0.0149
Aminomalonic acid	21.43	> 800	Level 2	1.80	0.0020
Compound 354 (sugar related)	43.93		Level 3	2.22	0.0032
Unidentified X23a	25.22		Level 4	1.88	0.0087

^a Level of identification as per Metabolomics Standards Initiative.

^b Fold change and P values were obtained from the volcano plot of internal standard-normalized data using two-tailed paired t-test.

Supplementary Figure Legends

Figure S1: 2D PCA score plot of (A) sum-normalized, (B) internal standard-normalized and (C) myo-inositol-normalized salivary metabolomic data including pooled QC samples. Red and green indicates pooled samples and saliva samples, respectively.

Figure S2: 2D PCA scores plot of (A) sum-normalized, (B) internal standard-normalized and (C) myo-inositol-normalized salivary metabolomic data. Green and red indicates saliva samples collected before and after mask use, respectively.

Figure S3: Heatmap analysis of (A) sum-normalized, (B) internal standard-normalized and (C) myo-inositol-normalized data showing highly conserved individual metabotypes irrespective of mask use. Alphabets (A, B, ...I, J) represents individual volunteers. Samples collected before and after use of FFP2 masks are indicated by 1 and 2, respectively.

Figure S4: Changes in internal standard-normalized abundance of 5-aminovaleric acid, L-fucose isomers, putrescine, phloretic acid, aminomalonic acid, benzenepropionic acid, citric acid, compound 354 and X23a upon wearing FFP2 mask for 30 minutes. P values (Wilcoxon signed-rank test) are indicated in each panel.

Figure S5: Changes in sum-normalized abundance of 5-aminovaleric acid, L-fucose isomers, putrescine, phloretic acid, aminomalonic acid, benzenepropionic acid, citric acid, compound 354 and X23a upon wearing FFP2 mask for 30 minutes. P values (Wilcoxon signed-rank test) are indicated in each panel.

Figure S6: Changes in *myo*-inositol-normalized abundance of 5-aminovaleric acid, L-fucose isomers, putrescine, phloretic acid, aminomalonic acid, benzenepropionic acid, citric acid,

compound 354 and X23a upon wearing FFP2 mask for 30 minutes. P values (Wilcoxon signed-rank test) are indicated in each panel.

Figure S7: Pearson correlation between abundances of 5-aminovaleric acid, L-fucose isomers, putrescine, phloretic acid, aminomalonic acid, benzenepropionic acid, citric acid, unidentified compound 354 and X23 in (A) internal standard-normalized, (B) *myo*-inositol-normalized and (C) sum-normalized data. A correlation coefficient cut-off of 0.6 was used for these plots.

Supplementary Figures

Figure S1

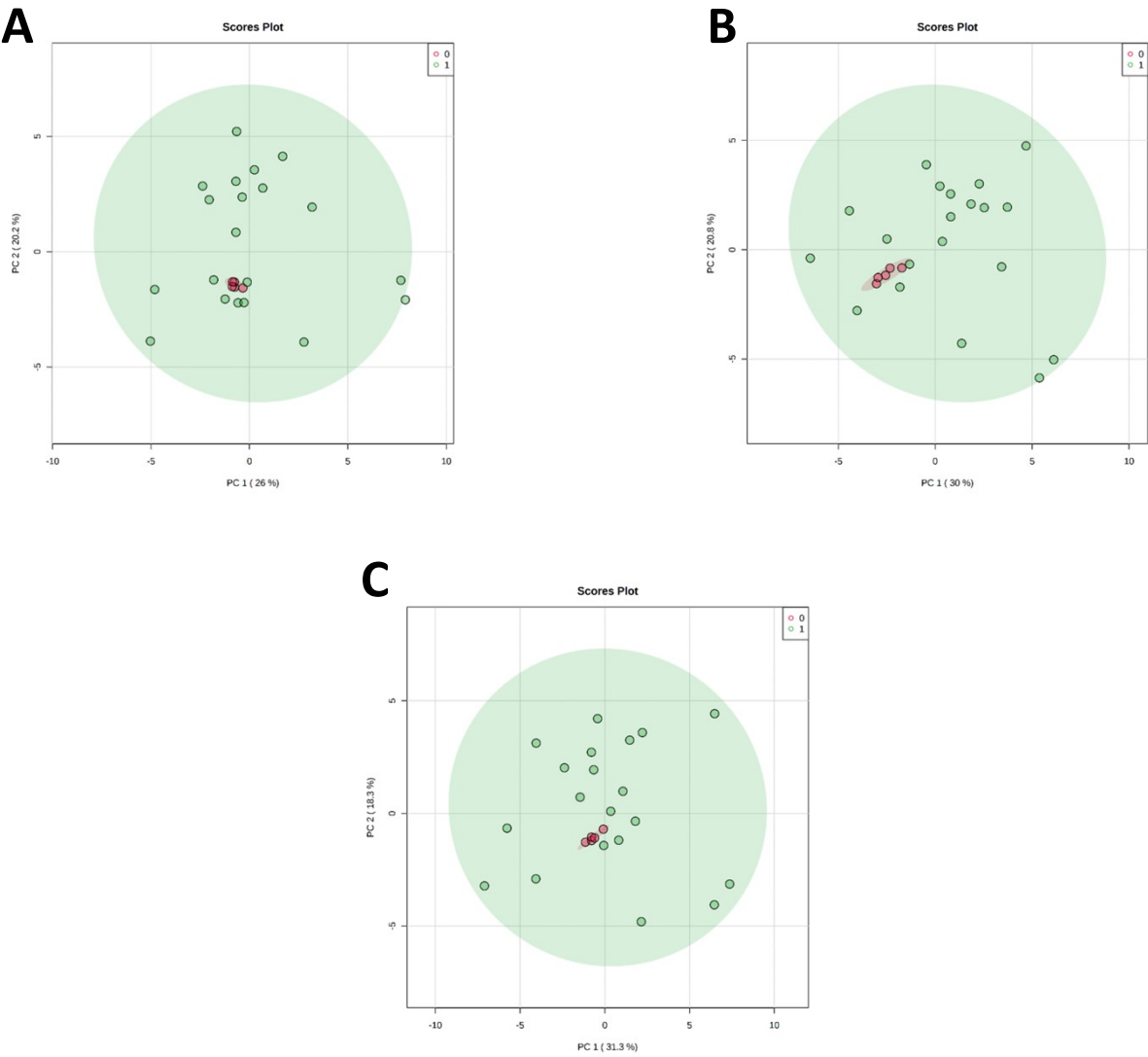


Figure S2

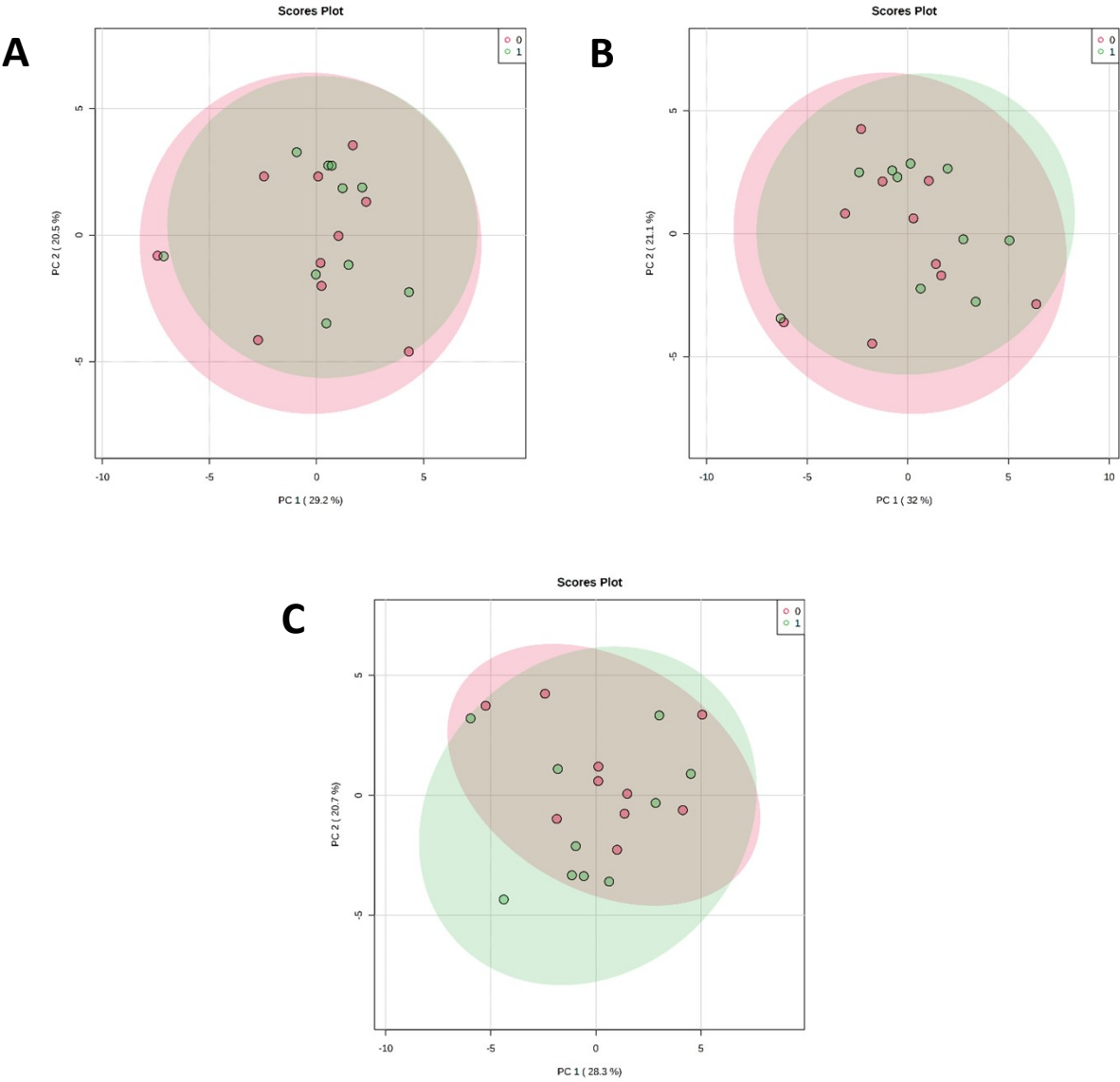
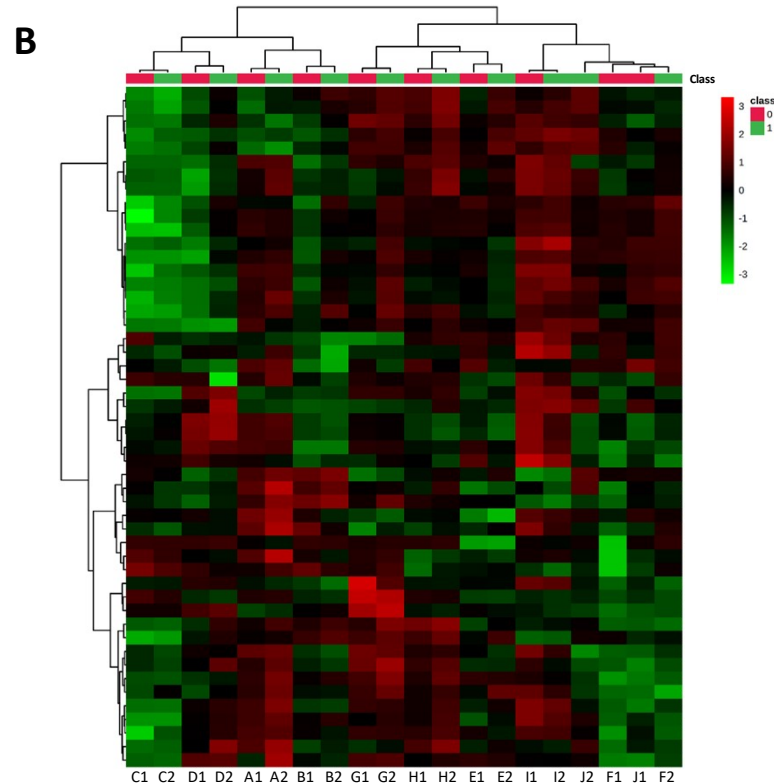
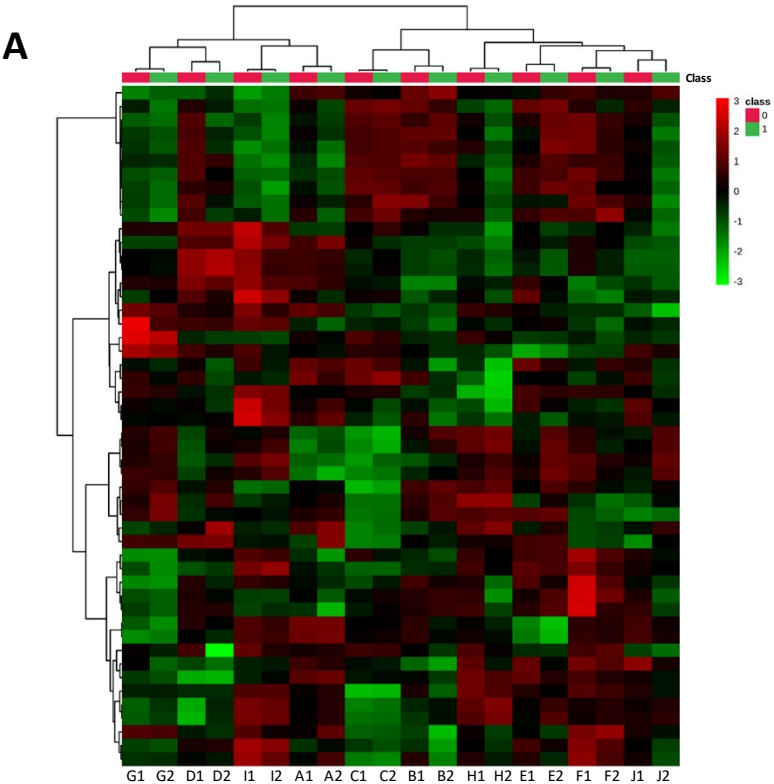


Figure S3



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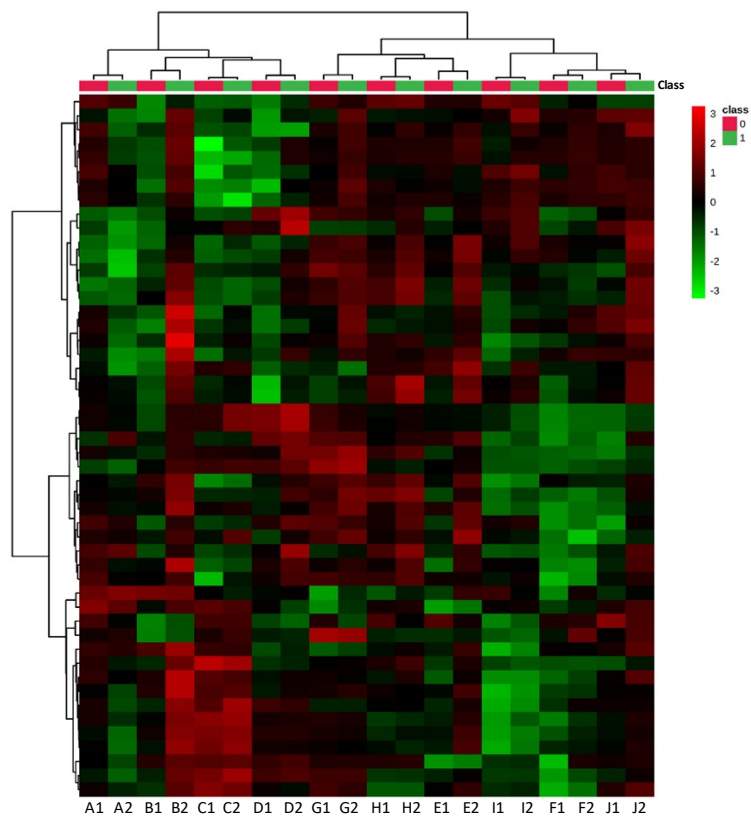


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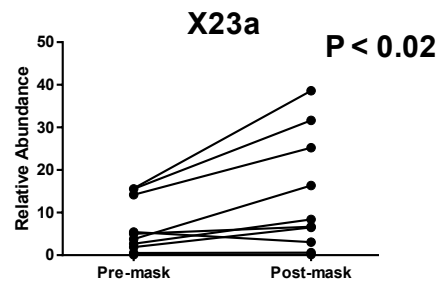
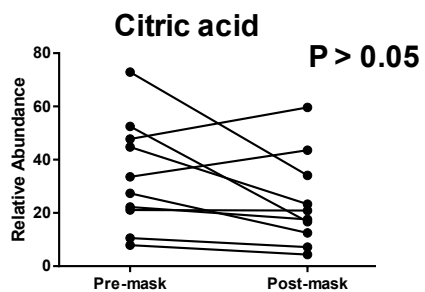
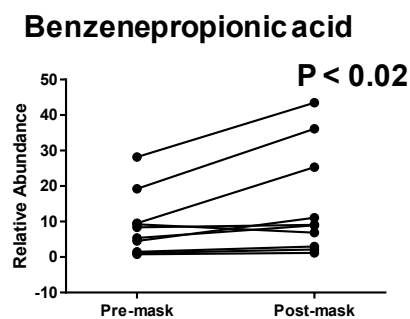
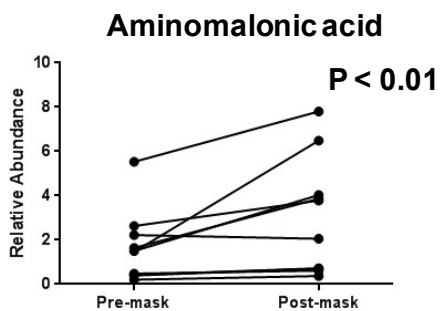
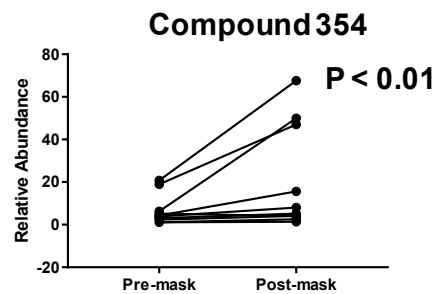
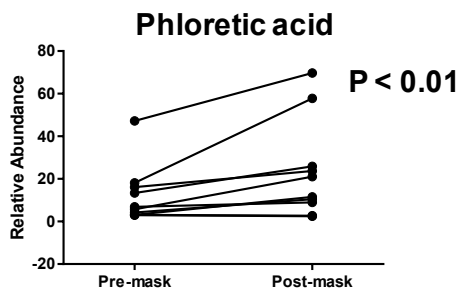
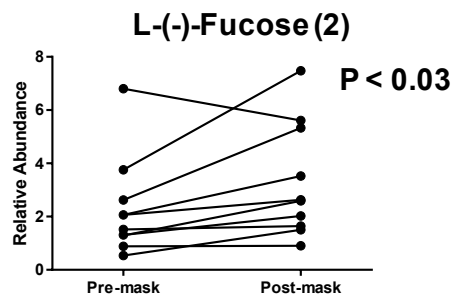
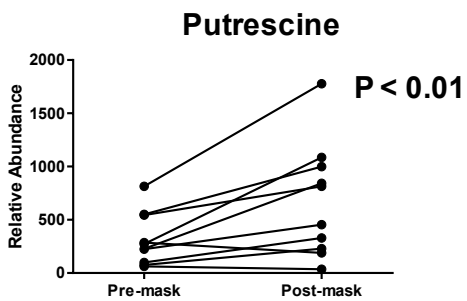
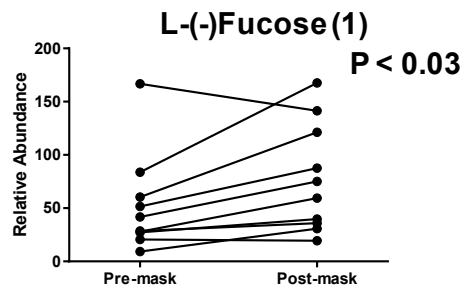
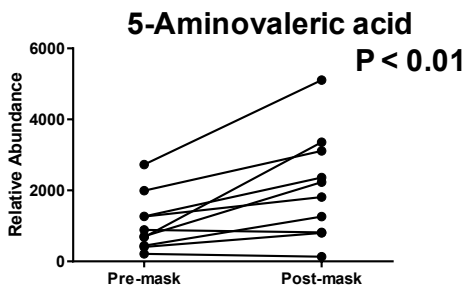


Figure S5

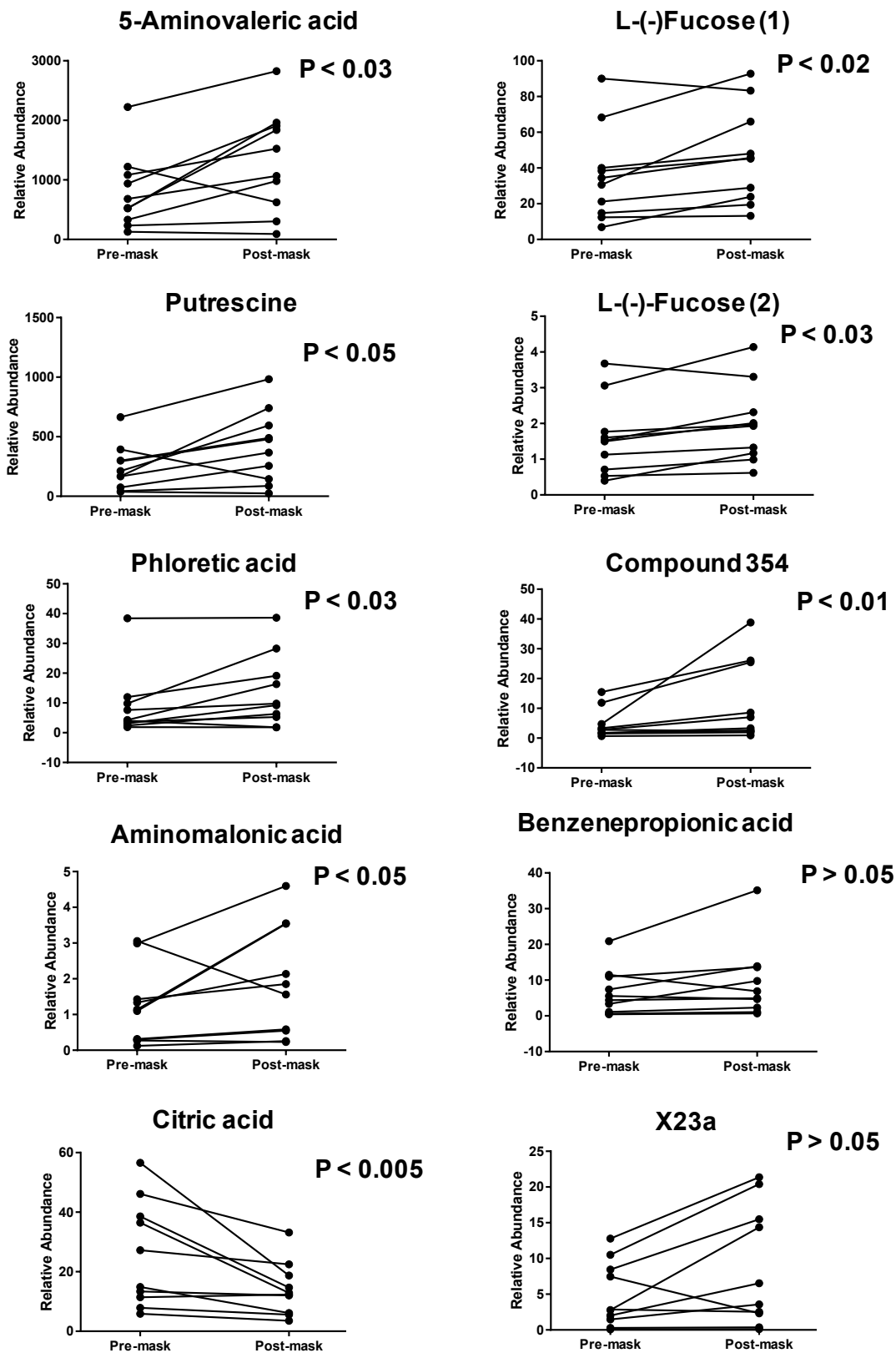


Figure S6

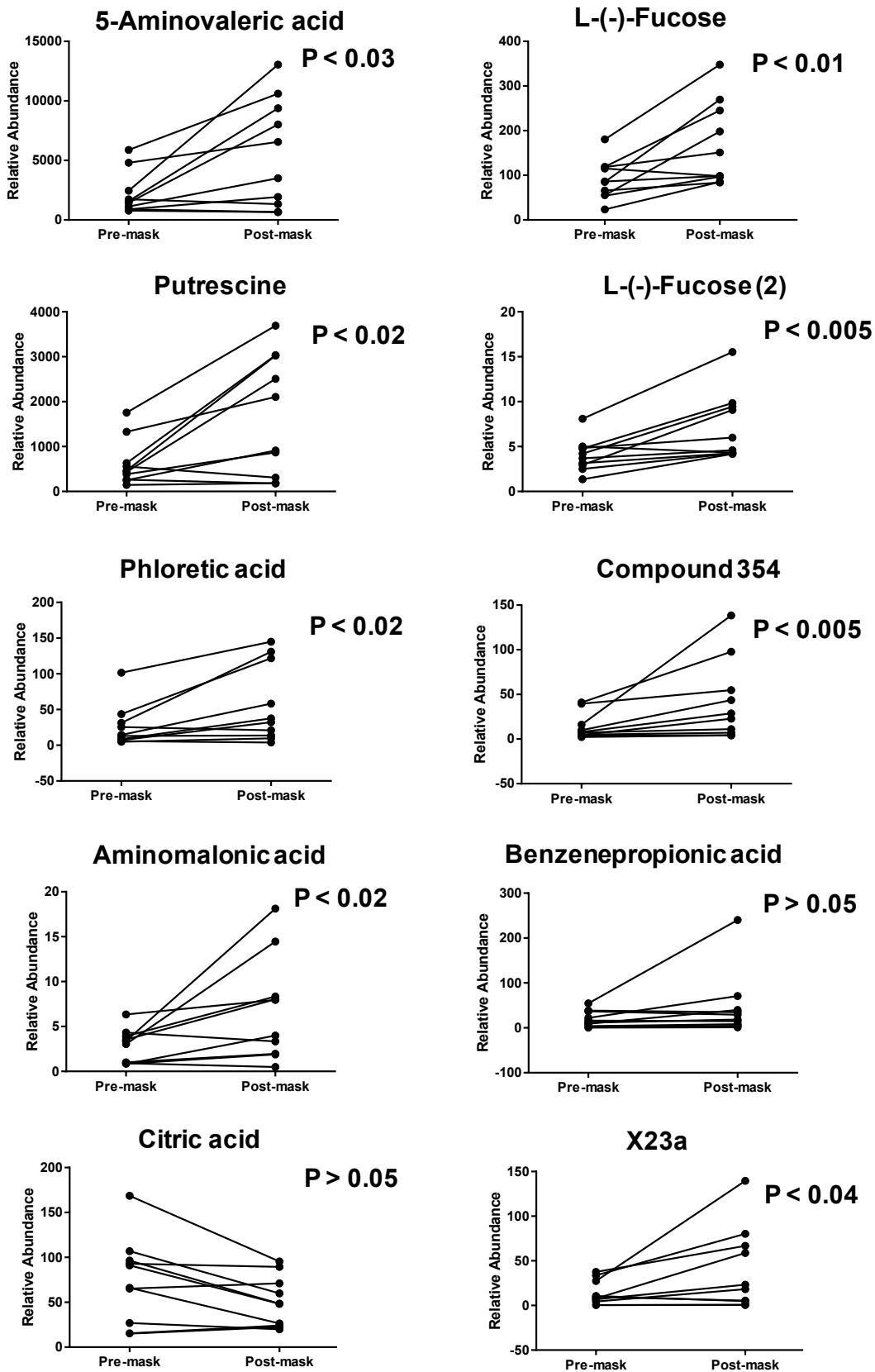


Figure S7

