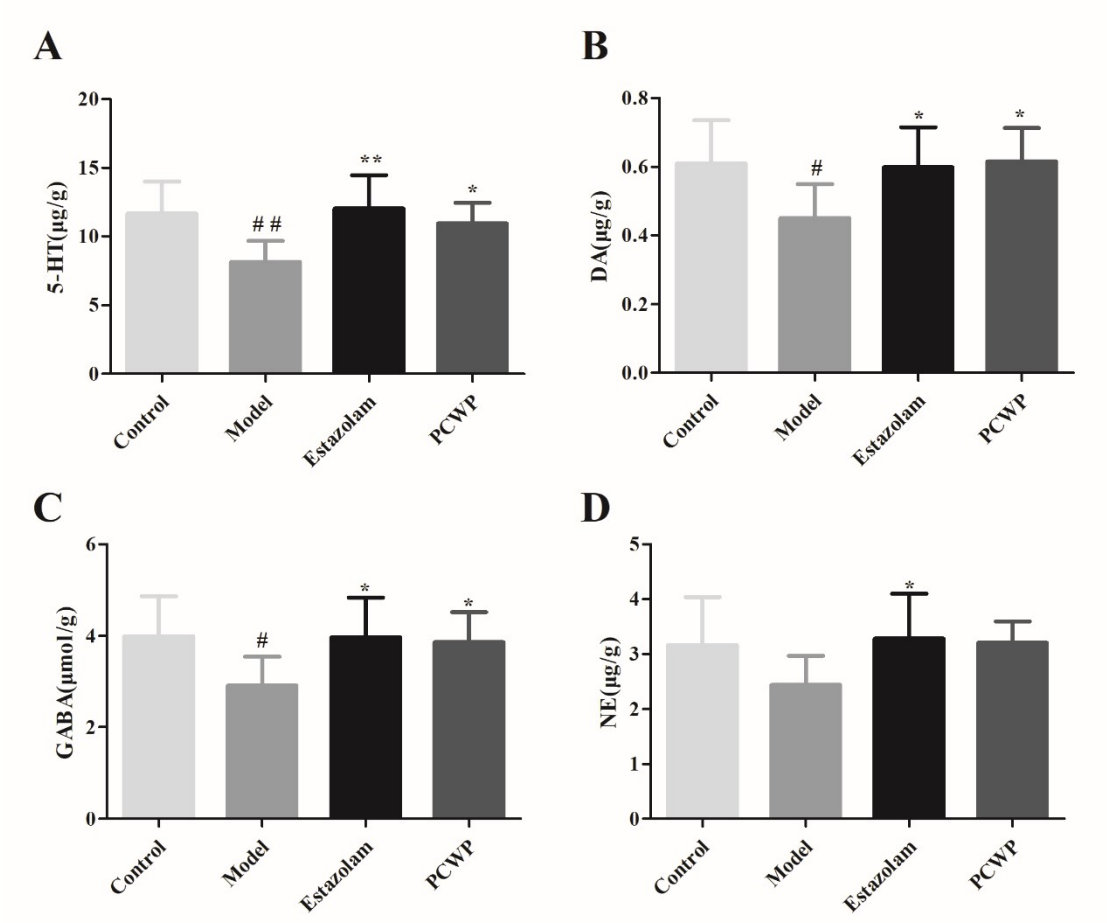
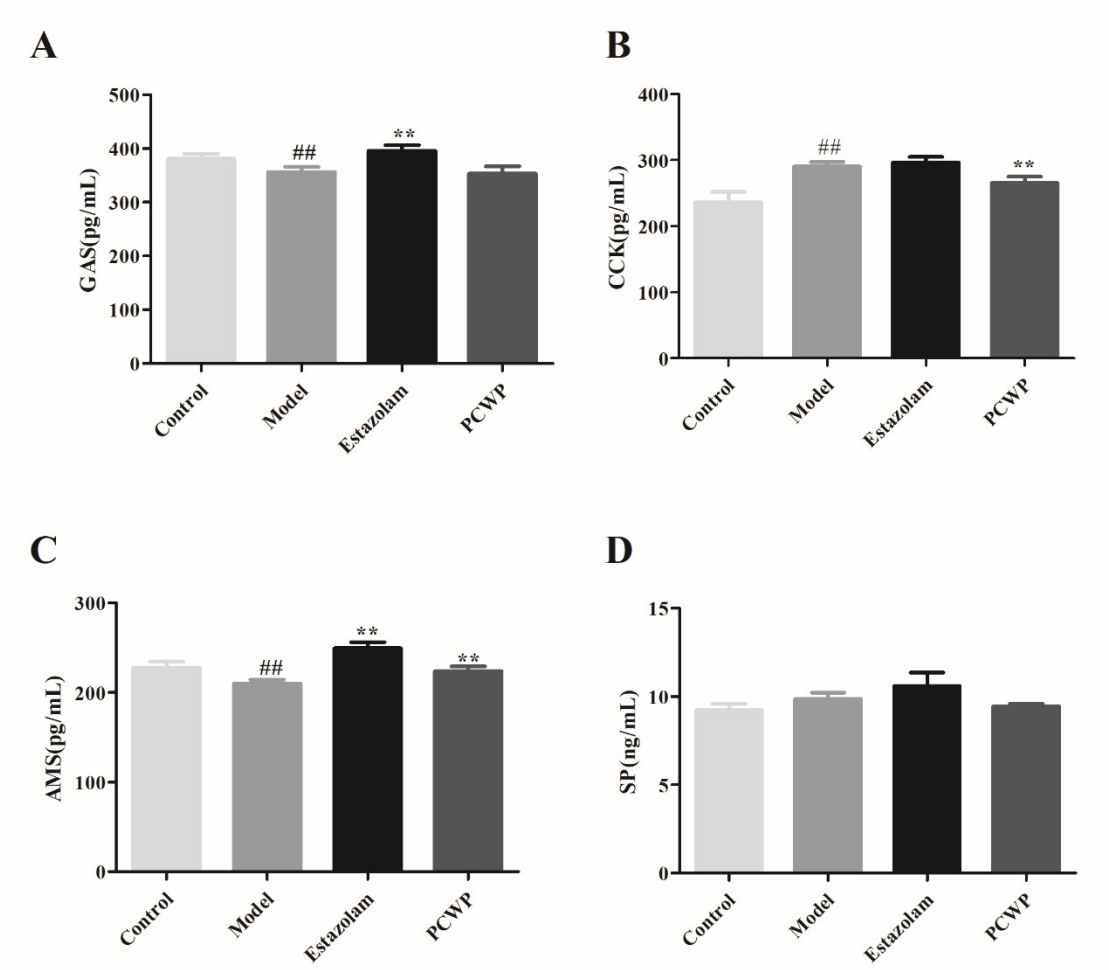
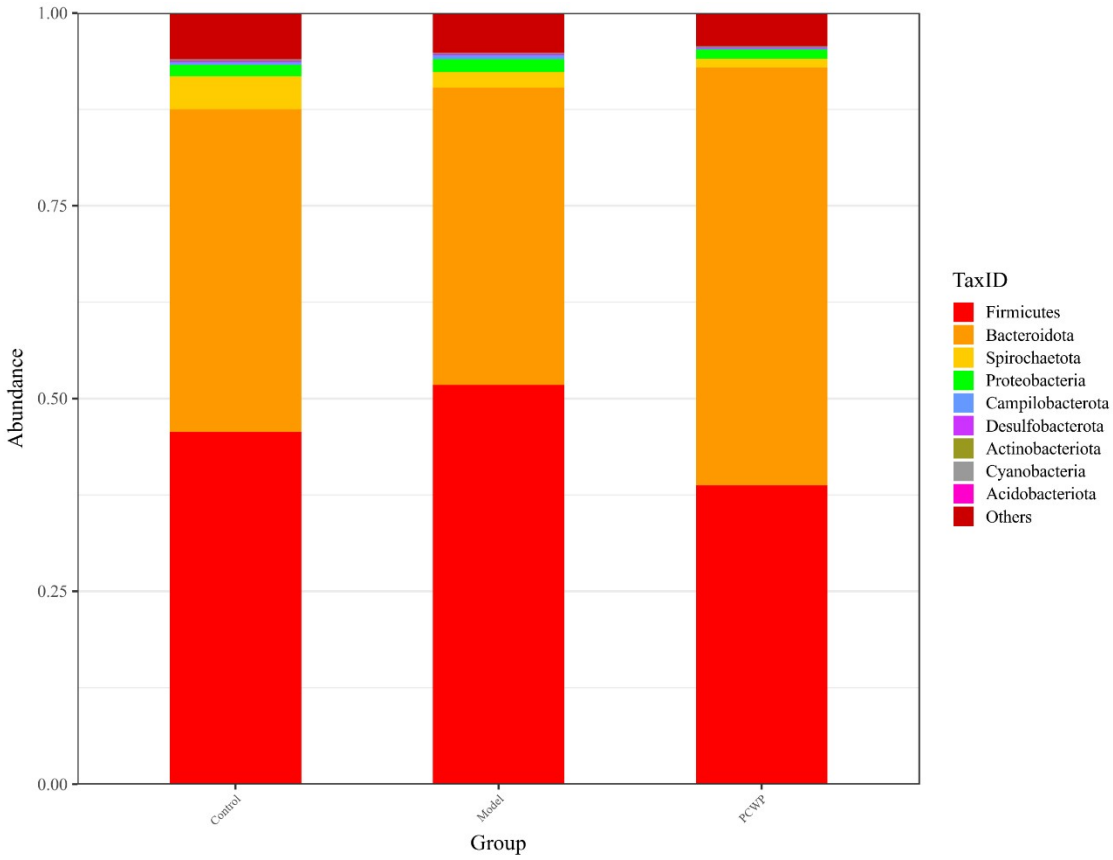
Supplementary materials



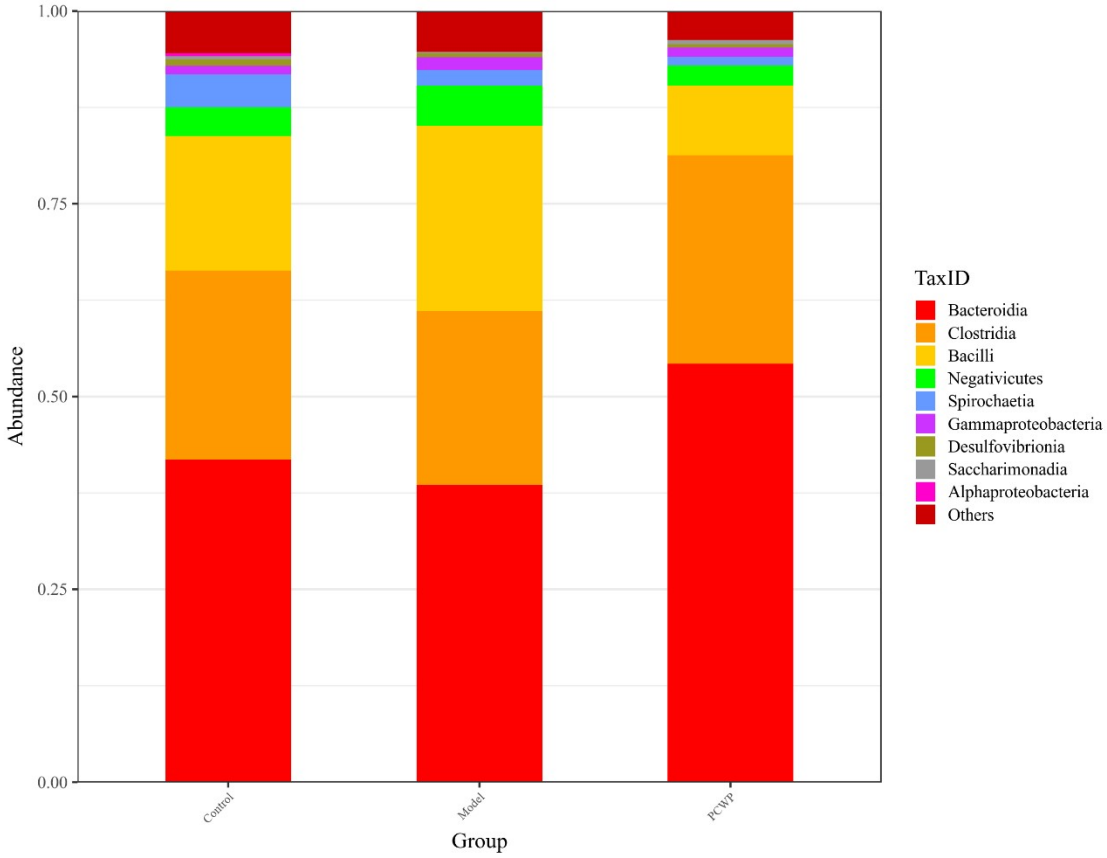
Supplementary Figure 1. Effect of PCWP on the neurotransmitters in the hypothalamus in rats with anxiety disorder. 5-HT (A), DA (B), GABA (C), NE (D). Control: control group; Model: sleep deprivation–induced anxiety disorder group; PCWP: PCWP 100 mg/kg treatment. Results are expressed as mean ± standard deviation (SD) ($\bar{x} \pm s$, $n = 6$); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, *vs*. the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, *vs*. the model group.



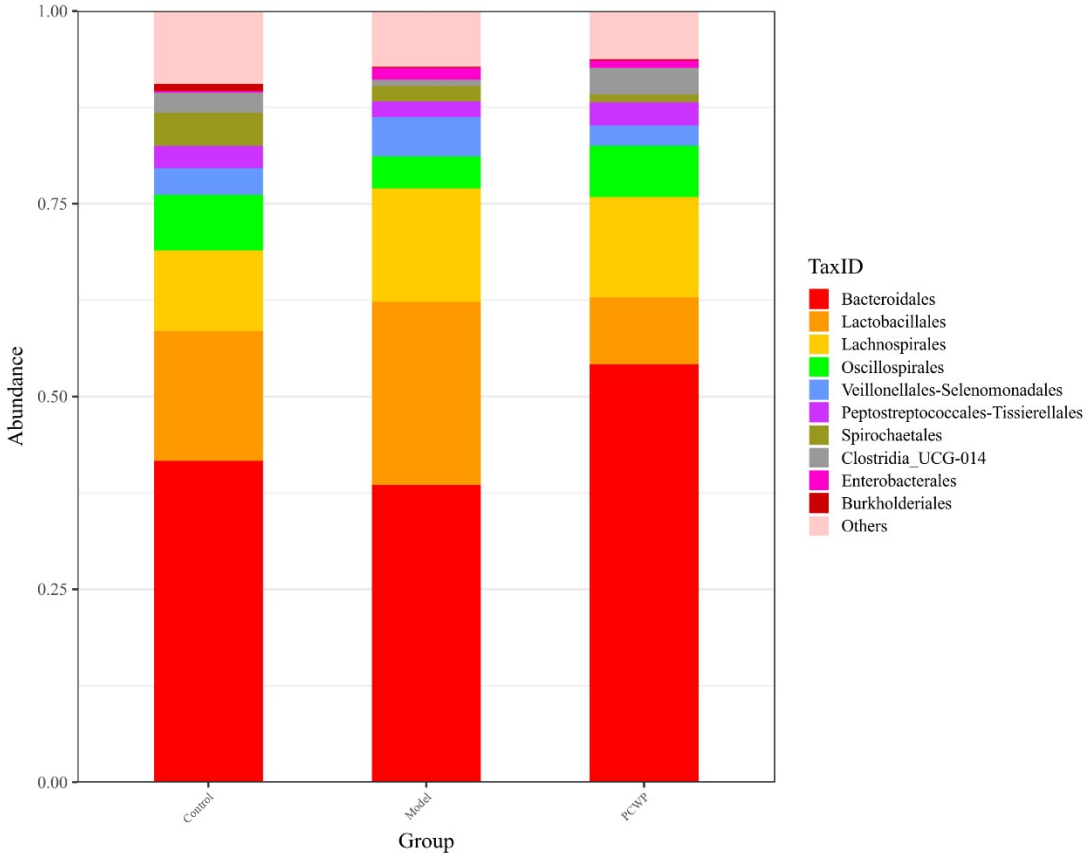
Supplementary Figure 2. Effect of PCWP on serum biochemical parameters GAS (A), CCK(B), AMS(C), and SP(D), in rats with sleep deprivation–induced anxiety disorder. Control: control group; Model: sleep deprivation–induced anxiety disorder group; PCWP: PCWP 100 mg/kg treatment. Results are expressed as mean ± standard deviation (SD) ($\bar{x} \pm s$, $n = 6$); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, *vs*. the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, *vs*. the model group.



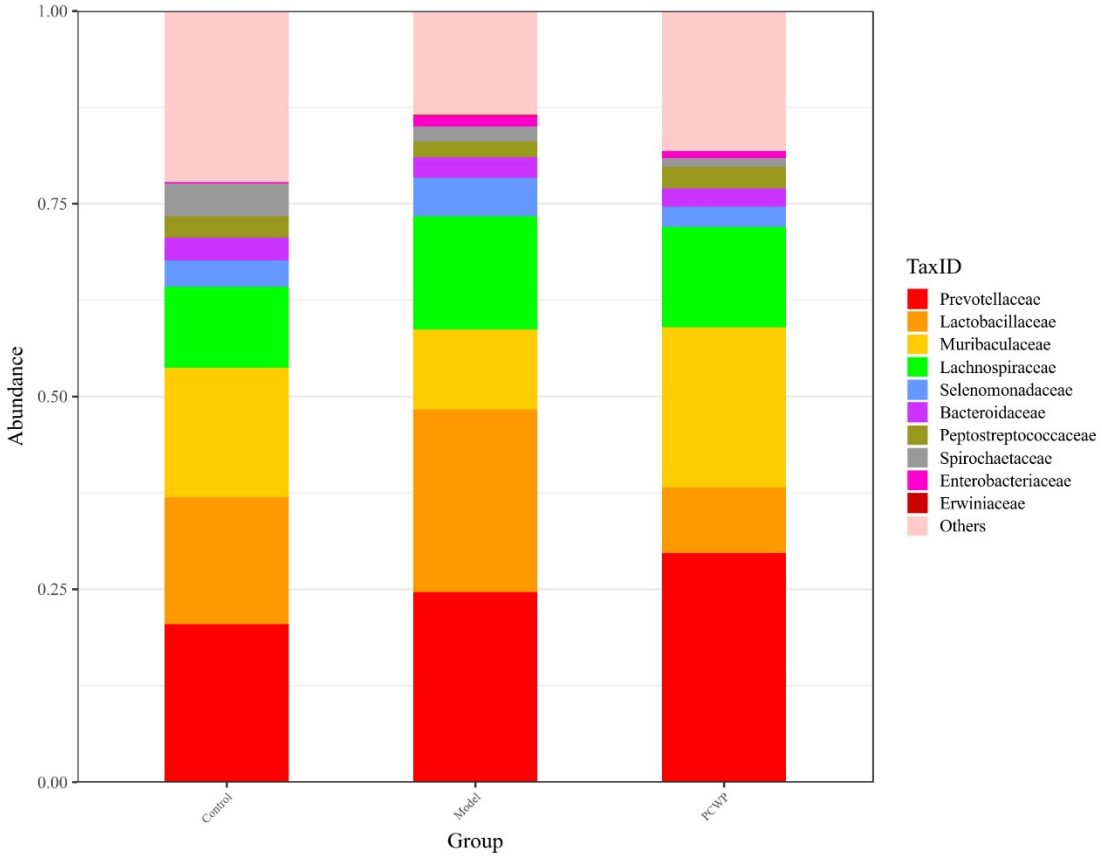
Supplementary Figure 3. Phylum level information of the samples.



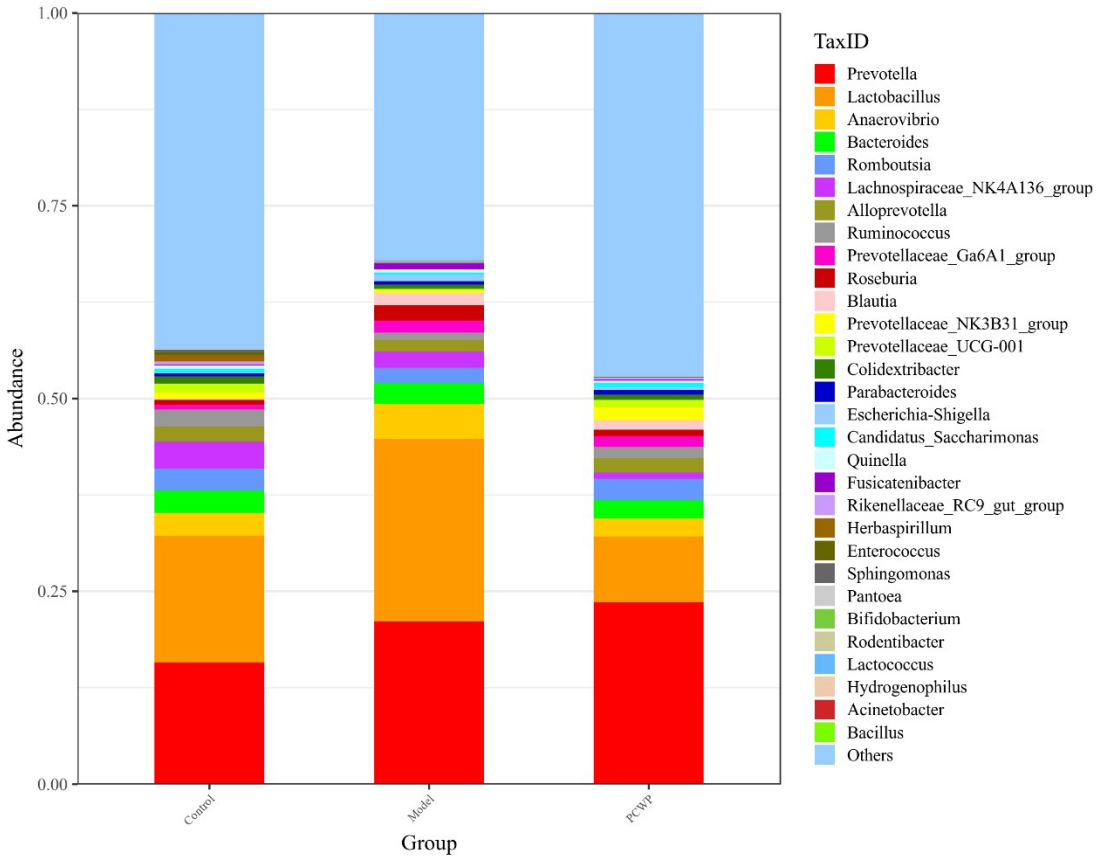
Supplementary Figure 4. Class level information of the samples.



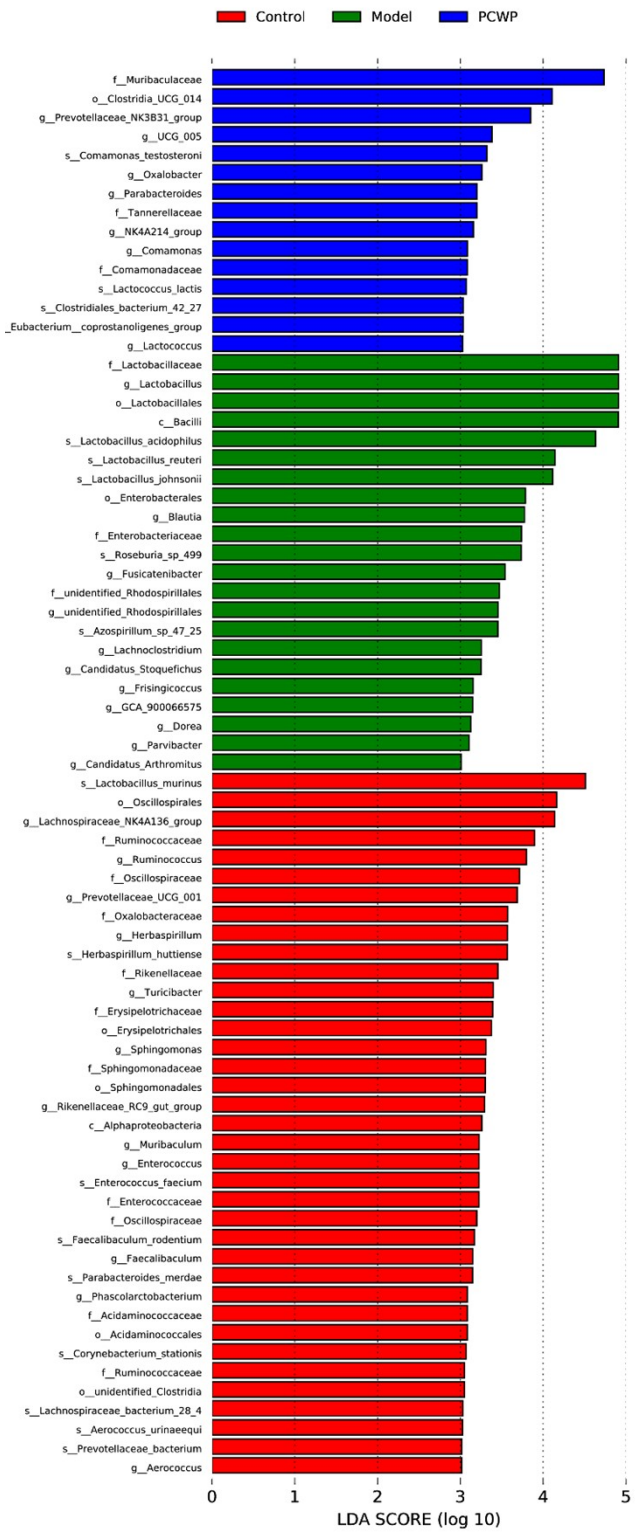
Supplementary Figure 5. Order level information of the samples.



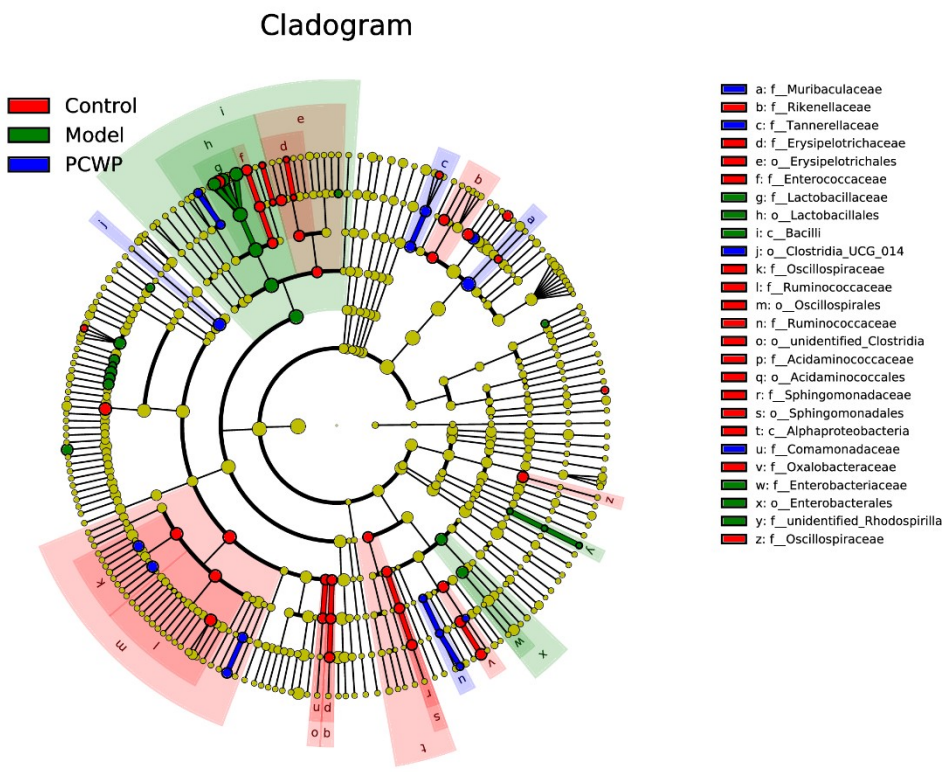
Supplementary Figure 6. Family level information of the samples.



Supplementary Figure 7. Genus level information of the samples.



Supplementary Figure 8. Histogram showing the LDA scores, where it indicates the effective size and ranking of each differentially abundant taxon (LDA>3).



Supplementary Figure 9. Cladogram depicting the output of the LEfSe algorithm; significantly distinct taxonomic nodes are colored and branch areas are shaded according to the effect size of the taxa.

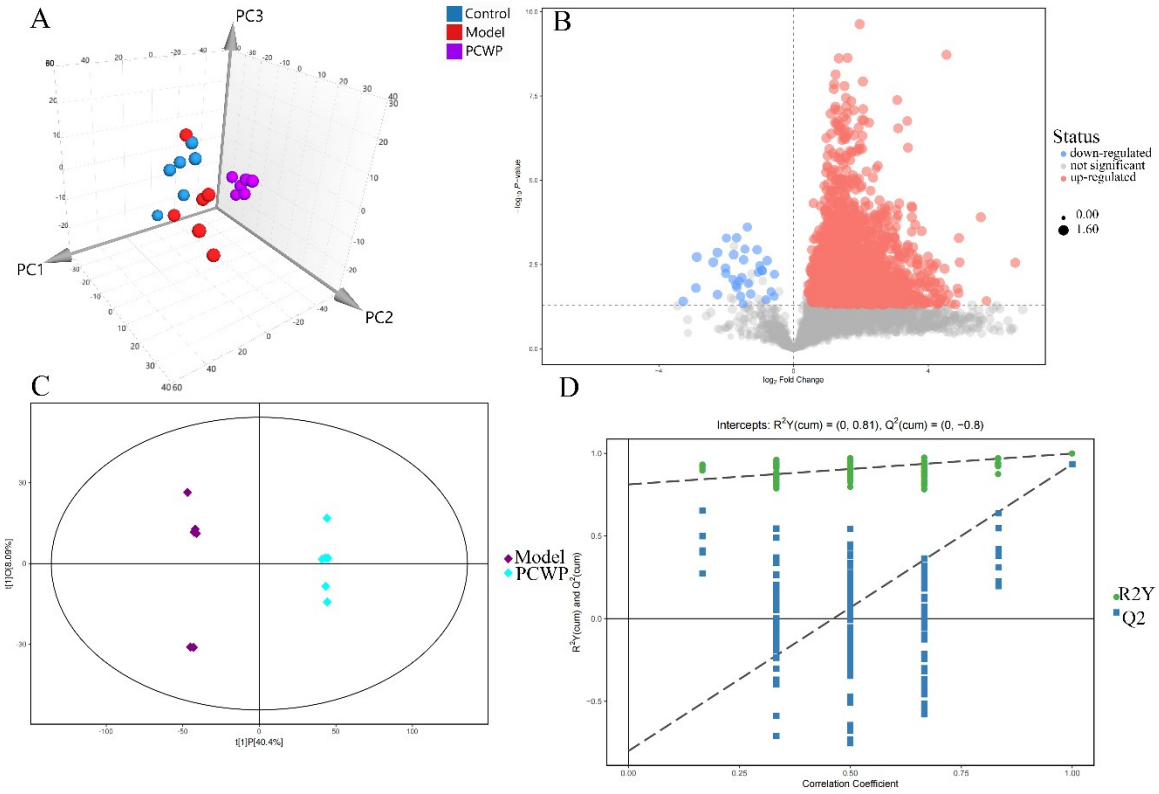
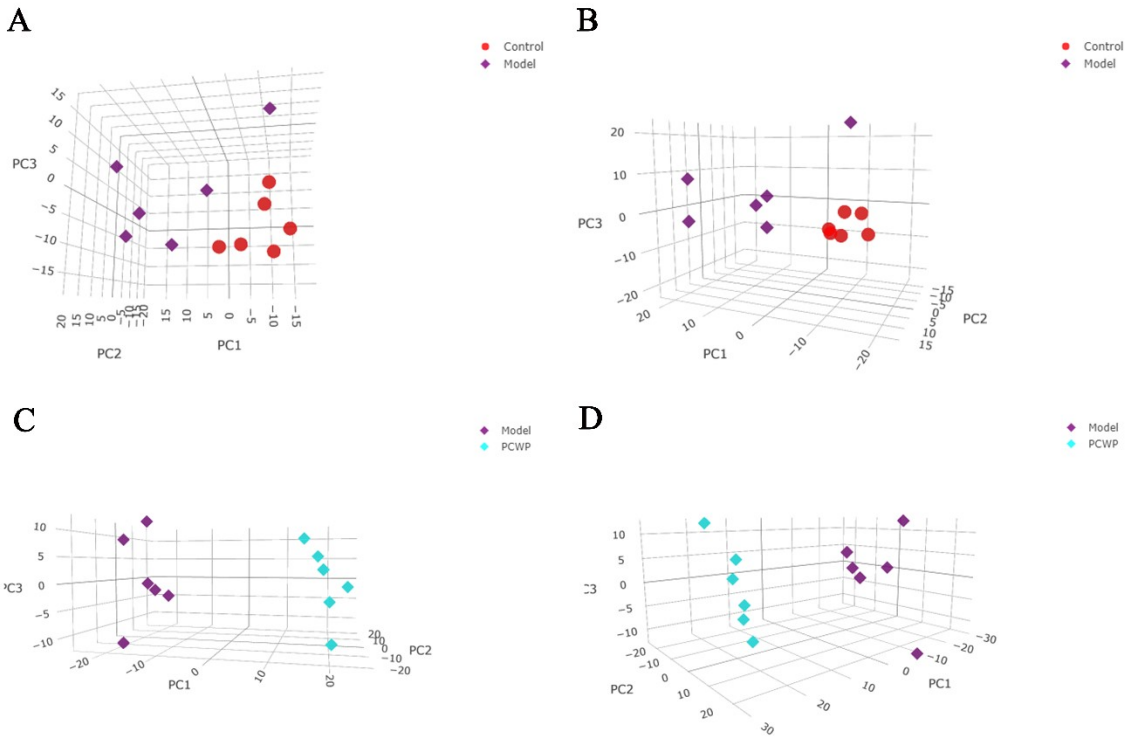
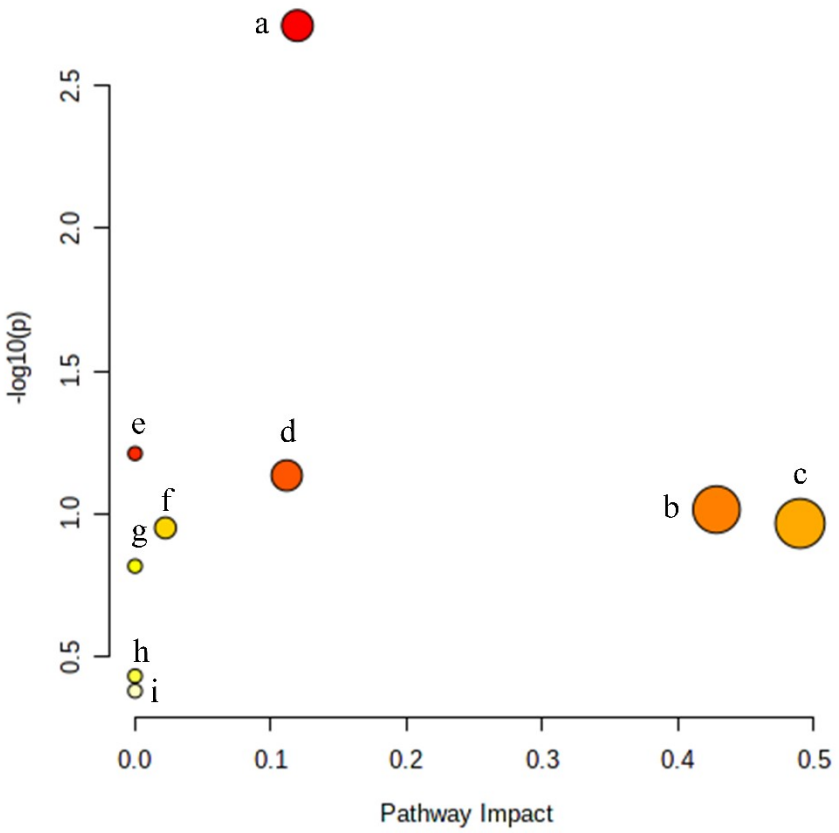


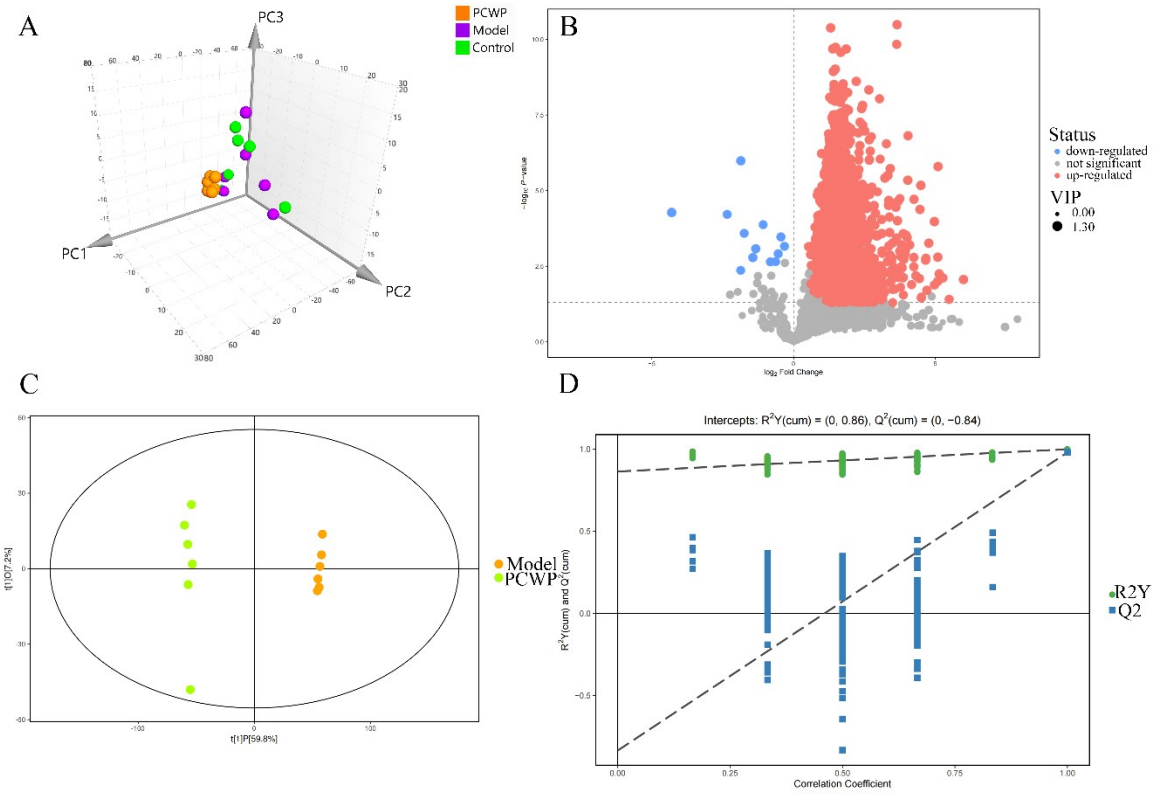
Figure 10. Scatter plots of the PCA score scatter 3D plot (A). Volcano plot of differential fecal metabolites between the model and PCWP (B). OPLS-DA score plot (C). Corresponding validation plot from the OPLS-DA model (D) in the negative-ion mode.



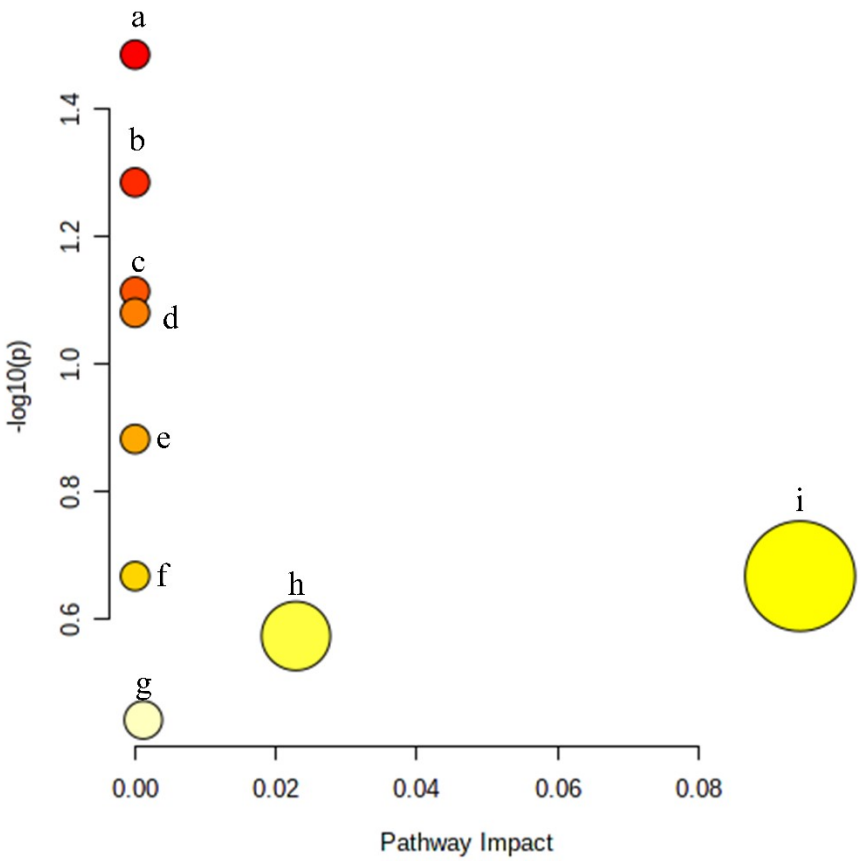
Supplementary Figure 11. Scatter plots of the PCA score scatter 3D plot between control and model (A: positive ion mode, B: negative ion mode), Scatter plots of the PCA score scatter 3D plot between model and PCWP (A: positive ion mode, B: negative ion mode).



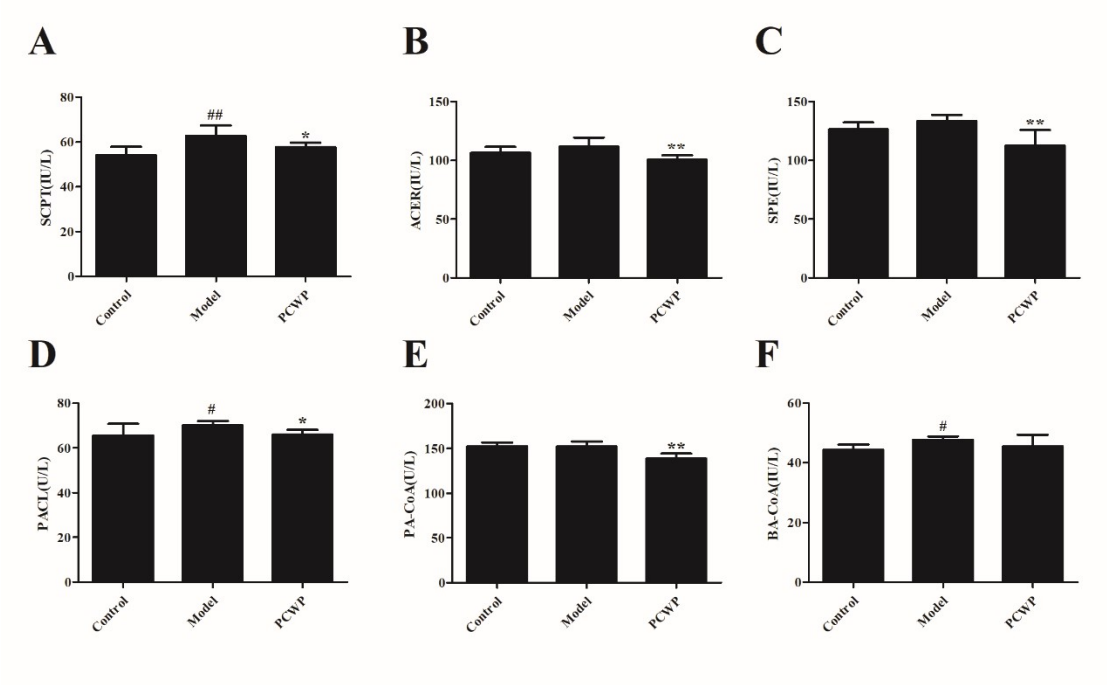
Supplementary Figure 12. Prediction of the metabolic pathway impact in the feces using the KEGG online database. Sphingolipid metabolism (a), taurine and hypotaurine metabolism (b), vitamin B6 metabolism (c), glycerophospholipid metabolism (d), linoleic acid metabolism (e), primary bile acid biosynthesis (f), α-linolenic acid metabolism (g), arachidonic acid metabolism (h), and tyrosine metabolism (i). The –ln(P) values from the pathway enrichment analysis are indicated on the X-axis and impact values on the Y-axis.



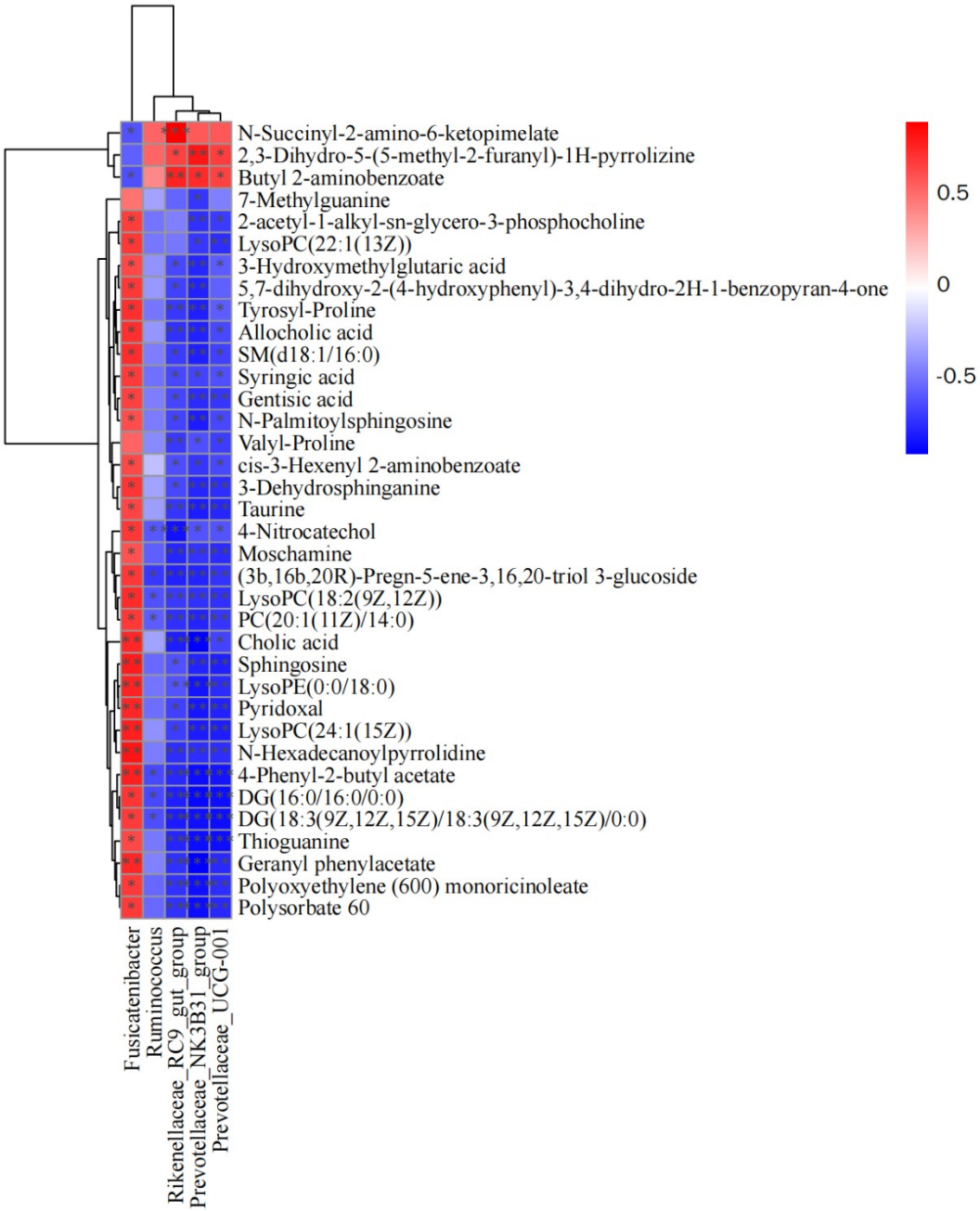
Supplementary Figure 13. Scatter plots of the PCA score scatter 3D plot (A). Volcano plot of differential serum metabolites between the model and PCWP (B). OPLS-DA score plot (C). Corresponding validation plot from the OPLS-DA model (D) in the negative-ion mode.



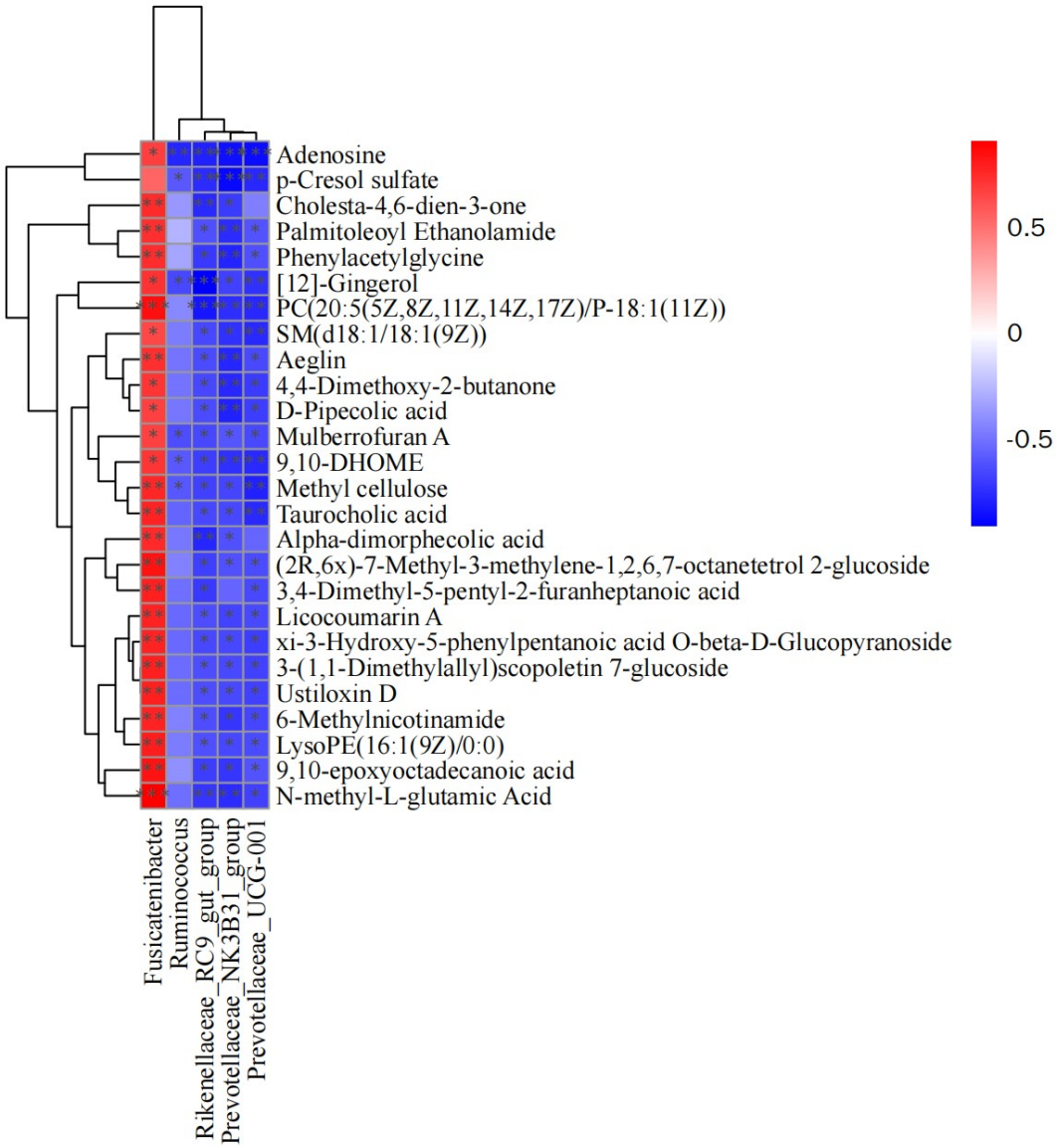
Supplementary Figure 14. Prediction of the metabolic pathway impact in the serum using the KEGG online database. Linoleic acid metabolism (a), taurine and hypotaurine metabolism (b), phenylalanine metabolism (c), α-linolenic acid metabolism (d), sphingolipid metabolism (e), arachidonic acid metabolism (f), purine metabolism (g), primary bile acid biosynthesis (h), and glycerophospholipid metabolism (i). The –ln(P) values from the pathway enrichment analysis are indicated on the X-axis and the impact values on the Y-axis.



Supplementary Figure 15. Activity of the metabolism–related enzymes in the serum



Supplementary Figure 16. Heat maps showing the Spearman's correlation analysis between the gut flora and fecal metabolic profile reversed by PCWP.



Supplementary Figure 17. Heat maps showing the Spearman's correlation analysis between gut flora and serum metabolic profile reversed by PCWP.

Supplementary Table 1. Potential fecal biomarkers from different groups identified in the positive-ion mode.

MS2 name	RT (s)	Mass(m/z)	Chang trend		VIP (PCWP/ Model)	P-value (PCWP/ Model)
			Model/ Control	PCWP/ Model		
Butyl 2-aminobenzoate	380.330	194.1178	↓	↑	1.146	0.002
3-Dehydrosphinganine	161.836	300.2891	↑	↓	1.207	0.003
6-Chloro-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine	279.816	188.0708	↑	↓	1.337	0.002
N-Hexadecanoylpyrrolidine	80.171	310.3105	↑	↓	1.082	0.002
LysoPE(0:0/18:0)	219.310	482.3234	↑	↓	1.264	0.001
Geranyl phenylacetate	237.634	273.1845	↑	↓	1.302	0.025
2,3-Dihydro-5-(5-methyl-2-furanyl)-1H-pyrrolizine	241.686	188.1069	↓	↑	1.285	0.005
Moschamine	47.751	353.1493	↑	↓	1.262	0.004
PC(20:1(11Z)/14:0)	163.667	760.5829	↑	↓	1.295	0.016
DG(18:3(9Z,12Z,15Z)/18:3(9Z,12Z,15Z)/0:0)	30.934	613.4792	↑	↓	1.342	0.000
(3b,16b,20R)-Pregn-5-ene-3,16,20-triol 3-glucoside	262.815	497.3036	↑	↓	1.359	0.005
DG(16:0/16:0/0:0)	30.815	591.4978	↑	↓	1.323	0.001
N-Succinyl-2-amino-6-ketopimelate	411.092	290.0861	↓	↑	1.091	0.022
SM(d18:1/16:0)	202.564	703.5751	↑	↓	1.426	0.000
Valyl-Proline	353.507	215.1391	↑	↓	1.066	0.012
Tyrosyl-Proline	290.487	279.1334	↑	↓	1.426	0.000
N-Palmitoylsphingosine	33.479	538.5194	↑	↓	1.421	0.000
Polysorbate 60	265.929	435.2963	↑	↓	1.357	0.002
LysoPC(22:1(13Z))	209.897	578.4184	↑	↓	1.234	0.014
LysoPC(24:1(15Z))	207.527	606.4489	↑	↓	1.433	0.001
LysoPC(18:2(9Z,12Z))	216.452	520.3395	↑	↓	1.423	0.001

cis-3-Hexenyl 2-aminobenzoate	53.792	220.1330	↑	↓	1.179	0.013
Polyoxyethylene (600) monoricinoleate	219.365	341.3051	↑	↓	1.254	0.000
4-Phenyl-2-butyl acetate	237.298	193.1226	↑	↓	1.492	0.000
2-acetyl-1-alkyl-sn-glycero-3-phosphocholine	213.883	524.3709	↑	↓	1.231	0.001
Sphingosine	85.716	300.2891	↑	↓	1.243	0.000

Supplementary Table 2. Potential fecal biomarkers from different groups identified in the negative ion mode.

MS2 name	RT (s)	Mass(m/z)	Chang trend		VIP (PCWP/ Model)	P-Value (PCWP/ Model)
			Model/ Control	PCWP/ Model		
Thioguanine	163.402	166.0178	↑	↓	1.170	0.013
Syringic acid	177.075	197.0458	↑	↓	1.373	0.020
7-Methylguanine	197.001	164.0575	↑	↓	1.265	0.007
4-Nitrocatechol	106.69	154.0143	↑	↓	1.426	0.003
Gentisic acid	60.429	153.0191	↑	↓	1.338	0.000
Pyridoxal	134.233	166.0507	↑	↓	1.416	0.004
Allocholic acid	195.326	407.2802	↑	↓	1.364	0.020
Genistein	41.2047	269.0459	↑	↓	1.222	0.009
Cholic acid	232.161	407.2802	↑	↓	1.401	0.015
5,7-dihydroxy-2-(4-hydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one	37.96765	271.0612	↑	↓	1.267	0.001
3-Hydroxymethylglutaric acid	379.4005	161.0452	↑	↓	1.315	0.011
Taurine	310.037	124.0067	↑	↓	1.235	0.027

Supplementary Table 3. Potential serum biomarkers from different groups identified in the positive-ion mode.

MS2 name	RT(s)	Mass(m/z)	Chang trend		VIP (PCWP/ Model)	P-Value (PCWP/ Model)
			Model/ Control	PCWP/ Model		
(2R,6x)-7-Methyl-3-methylene-1,2,6,7-octanetetrol 2-glucoside	64.290	367.1956	↑	↓	1.158	0.002
Taurocholic acid	78.094	516.3015	↑	↓	1.146	0.003
Ustiloxin D	277.650	495.2415	↑	↓	1.153	0.003
Aeglin	277.748	511.2146	↑	↓	1.150	0.003
6-Methylnicotinamide	57.482	151.0964	↑	↓	1.181	0.001
SM(d18:1/18:1(9Z))	201.102	729.5889	↑	↓	1.055	0.001
Adenosine	178.446	268.1038	↑	↓	1.179	0.013
4,4-Dimethoxy-2-butanone	56.762	133.0859	↑	↓	1.184	0.001
Methyl cellulose	70.848	455.2476	↑	↓	1.145	0.007
Cholesta-4,6-dien-3-one	32.022	383.3304	↑	↓	1.127	0.000
Licocoumarin A	276.069	407.1885	↑	↓	1.167	0.003
D-Pipecolic acid	57.578	130.0863	↑	↓	1.188	0.001
Palmitoleoyl Ethanolamide	86.557	298.2738	↑	↓	1.111	0.000
3-(1,1-Dimethylallyl)scopoletin 7-glucoside	275.992	423.1622	↑	↓	1.158	0.004
xi-3-Hydroxy-5-phenylpentanoic acid O-beta-D-Glucopyranoside	274.269	379.1367	↑	↓	1.148	0.005
LysoPE(16:1(9Z)/0:0)	202.240	452.2768	↑	↓	1.116	0.022
Alpha-dimorphecolic acid	87.402	279.2313	↑	↓	1.166	0.003
Phenylacetylglycine	205.989	194.0807	↑	↓	1.012	0.006
Mulberrofuran A	74.494	393.2096	↑	↓	1.053	0.001
PC(20:5(5Z,8Z,11Z,14Z,17Z)/P-18:1(11Z))	153.323	790.5741	↑	↓	1.010	0.013
3,4-Dimethyl-5-pentyl-2-furanheptanoic acid	49.620	295.2262	↑	↓	1.093	0.003

Supplementary Table 4. Potential serum biomarkers from different groups identified in the negative-ion mode.

MS2 name	RT (s)	Mass(m/z)	Chang trend		VIP (PCWP/ Model)	P-Value (PCWP / Model)
			Model/ Control	PCWP/ Model		

N-methyl-L-glutamic Acid	12.185	160.0613	↑	↓	1.149	0.008
p-Cresol sulfate	24.351	187.0067	↑	↓	1.011	0.004
[12]-Gingerol	152.455	377.27	↑	↓	1.228	0.010
9,10-epoxyoctadecanoic acid	50.376	297.2441	↑	↓	1.225	0.001
9,10-DHOME	91.089	313.2394	↑	↓	1.252	0.001