Supplementary materials

METHODS

The GC-MS method for measuring butyric acid content

For pretreatment, the collected supernatant assay samples were acidified. The supernatant was placed in a 5 mL polyethylene centrifuge tube with 0.4 mL of 50% sulfuric acid and ether, incubated in a shaker at room temperature and 200 rpm for 45 min, then centrifuged at 3000 rpm for 5 min, and the supernatant was removed in another sterile centrifuge tube and dehydrated by adding anhydrous calcium chloride, after which the supernatant was further used for GC-MS analysis.

GC-MS conditions: column Agilent 123-7032 DB-WAX quartz capillary column (30 m x 320 μm x 0.25 μm); ramp-up procedure: column temperature starting at 60 °C, hold for 2 min, ramp up to 220 °C at 10 °C/min, hold for 20 min, helium as carrier gas, flow rate of 1 mL/min. Mass spectrometry conditions: EI ion source 70 eV, ion source temperature 230 °C, quadrupole temperature 150 °C, solvent delay time 2 min, scan mass range m/z 20-150.

Hepatic tissue hematoxylin eosin and oil red O staining

Hematoxylin eosin staining was performed and the isolated liver tissues were taken and cut into 2×3 mm size tissue blocks, after which they were fixed in 4% formalin. The fixed tissues were washed three times with PBS and processed according to the following procedure. Gradient dehydration treatment, 70% ethanol 0.5-1 h, 80% ethanol 0.5 h, 90% ethanol 0.5 h, 95% ethanol 0.5 h, 95% ethanol 0.5 h, anhydrous ethanol 1 h, anhydrous ethanol 0.5 h, anhydrous ethanol 0.5 h. Tissues were transparent and immersed in wax, 100% xylene I for 15 min and 100% xylene II for 15 min, and transferred to melted paraffin I for 1 h and paraffin II for 1 h after transparency. Tissue sections were dewaxed and rehydrated after staining. Hematoxylin staining: Hematoxylin staining for 3-5 min, using 1% hydrochloric acid alcohol fractionation, the color gradually returned to blue, water washing. Eosin staining: the sections were sequentially stained with 50% ethanol for 1 min, 70% ethanol for 10 s, 80% ethanol for 10 s, 95% ethanol for 2 min, anhydrous ethanol for 2

min, anhydrous ethanol for 2 min, and eosin re-staining for 4 min. Dehydration blocking: anhydrous ethanol, xylene (1:1) mixture for 2 min, xylene I for 5 min, xylene II for 5 min. microscopic examination was used and images were acquired for analysis.

The specific steps of Oil Red O staining are as follows. Fresh frozen sections were fixed by rewarming and drying frozen sections, fixed in fixative for 15 min, and then washed with tap water and dried. For oil red staining, sections were stained by immersion in oil red staining solution for 8-10 min. For background differentiation, sections were removed and left for 3 s and then sequentially submerged in 60% isopropanol for 3 s and 5 s each. Sections were sequentially submerged in 2 dips of pure water for 10 s each. For hematoxylin staining, the sections were removed, left for 3 s and then immersed in hematoxylin for 3-5 min, 3 times pure water immersion wash for 5 s, 10 s and 30 s in sequence. differentiation solution (60% alcohol as solvent) for 2-8 s, 2 times distilled water immersion wash for 10 s in sequence, return blue solution for 1 s. The sections were gently immersed in tap water for 2 times immersion wash for 5 s and 10 s each, and the staining effect was examined microscopically. To seal the tablets, glycerin gelatin sealer was used to seal the tablets. Microscopic microscopy, image acquisition and analysis.

SUPPLEMENTAL TABLES

Table S1 Strains and plasmids used in this study

Strain or plasmid	Characteristics	Source
Strains		
E. coli		
Trans-DH5α	F-φ80 lac ZΔM15 *Δ*(lacZYA-arg F) U169 endA1 recA1 hsdR17 (rk-,mk+) supE44λ- thi -1 gyrA96 relA1 phoA	TransGen Biotech
B. subtilis		
SCK6	ErmR, 1A751 derivate, *lacA*::P*xylA-comK*	MiaoLing Plasmid Sharing Platform
RS06551	ErmR, 1A751 derivate, *lacA*::P*xylA-comK*, *ΔskfA ΔsdpC ΔacdA ΔackA* :: butyryl-CoA: acetate: CoA transferase	This work
Plasmids		
pCas	*repA*101(Ts) *kan* P_{cas}-cas9 P_{araB}-*Red lacI*^{q} P_{trc}-*sgRNA-pMB1*	(1)
pTargetF	Harboring sgRNAs, with or without donor DNAs	(1)
pTargetF-P1	*pMB1 aadA* sgRNA-*skfA*	This work
PTargetF-P2	*pMB1 aadA* sgRNA-*sdpC*	This work
pTargetF-P3	*pMB1 aadA sgRNA-acdA*	This work
pTargetF-P4	*pMB1 aadA sgRNA-ackA*	This work
PTargetF-P5	*pMB1 aadA* sgRNA- butyryl-CoA: acetate: CoA transferase	This work

Table S2 Primers and N20 sequence used in this study

Name	Sequence (5'-3')
Primer	
ΔskfA-up-S	ACAGCGCTTGCTCTGTTTCCG
ΔskfA-up-A	TTCCCATTCTTTTTGGTTTCTTTTCATAAG
ΔskfA-down-S	CATCCTGCTATGAGAGCTATTTAACATTTGAG
ΔskfA-down-A	TTAAACCCTTTGATCAGCTTTGCTTCAC
ΔsdpC-up-S	AATGAATCTTTCACAAAGAAGACAGTATTG
ΔsdpC-up-A	CAATAGCCTAAGTAATTTACTTTTCAAATTATTATACC
ΔsdpC-down-S	GCAAAAACCCTAAAATTGATTCATCAATAATC
ΔsdpC-down-A	CAGGAAATAAACCGGCTGCAATTGTATC
ΔacdA-up-S	CATCAATAATATCTTCTTCGATCTCAAGATCATC
ΔacdA-up-A	CTGGTCATCTCCAGAATGCTCGCG
ΔacdA-down-S	CTCATGTTCTTCTGATAATGAAAAATTCATTGC
ΔacdA- down-A	CACGCCGCGCCGCCTC
ΔackA-up-S	GATTTCTCTGACCACATGCGCAAGTTC
ΔackA-up-A	GCGCGCGACGTTGTTCGTTTAG
ΔackA-down-S	TCCTGCGTTAATTGCAATAATTTTGGAC
ΔackA- down-S	CAAGCTGAAACAGTTTTTCAAAGATAAGGTTC
BCoAT-S	ATGGATTTTACGGAATTGTATGCGCAG

BCoAT-A	TCAGCGCTTGTTGCTCCTGCG
N20	
skfA edition	CTGCTGGCTGCATGGGCTGT
sdpC edition	TTCATTAGTAGGACTCTCTA
acdA edition	TAGAATTCATGGGCGTATAC
ackA edition	CACTCAAGAAATTCAAAGAC
BCoAT edition	CATAATGAACGCAAAAAAAC
gRNA sequence	
gRNA scaffold	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTT ATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTG

Table S3

Results of multiplex analysis of species and gene functions at different levels

Table S4

168 annotated compounds peak intensity table

Table S5

Enrichment analysis pathway annotation results

Table S6

Functional meta-analysis pathway annotation results

Table S7

Data set for Spearman correlation analysis

References

1. Jiang Y, Chen B, Duan C, Sun B, Yang J, Yang S. 2015. Multigene Editing in the Escherichia coli Genome via the CRISPR-Cas9 System. Applied and Environmental Microbiology 81:2506-2514.

SUPPLEMENTAL FIGURES

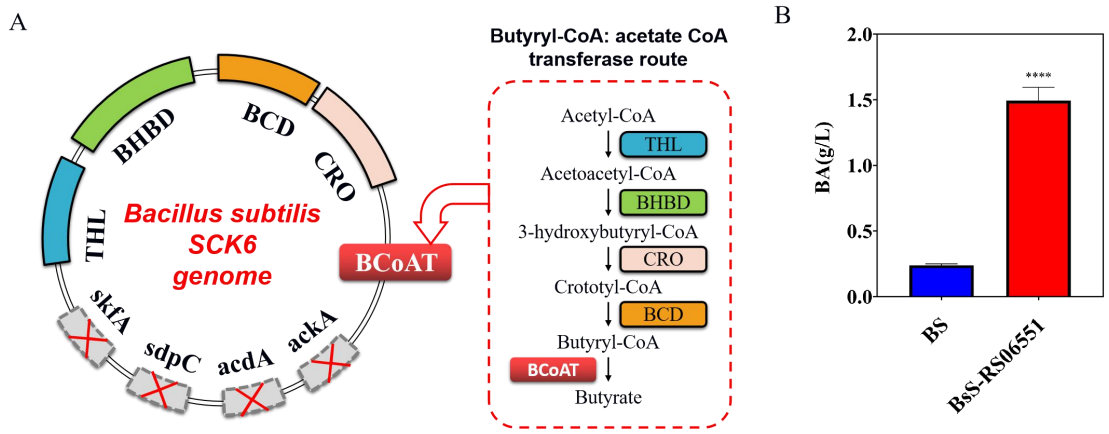


FIG S1

A.Construction strategy of butyrate-producing bacteria. B. Butyric acid production of each strain of engineering bacteria. Abbreviations: THL, thiolase; BHBD, β-hydroxybutyl-CoA dehydrongenase; BCD, butyryl-CoA dehydrogenase; CRO, crotonase; BCoAT, Butyryl-CoA:acetate CoA transferase; skfA, sporulation killing factor; sdpC, sporulation delaying protein C; acdA, acyl-CoA dehydrogenase; acetate kinase; BS, *B. subtilis* SCK6.

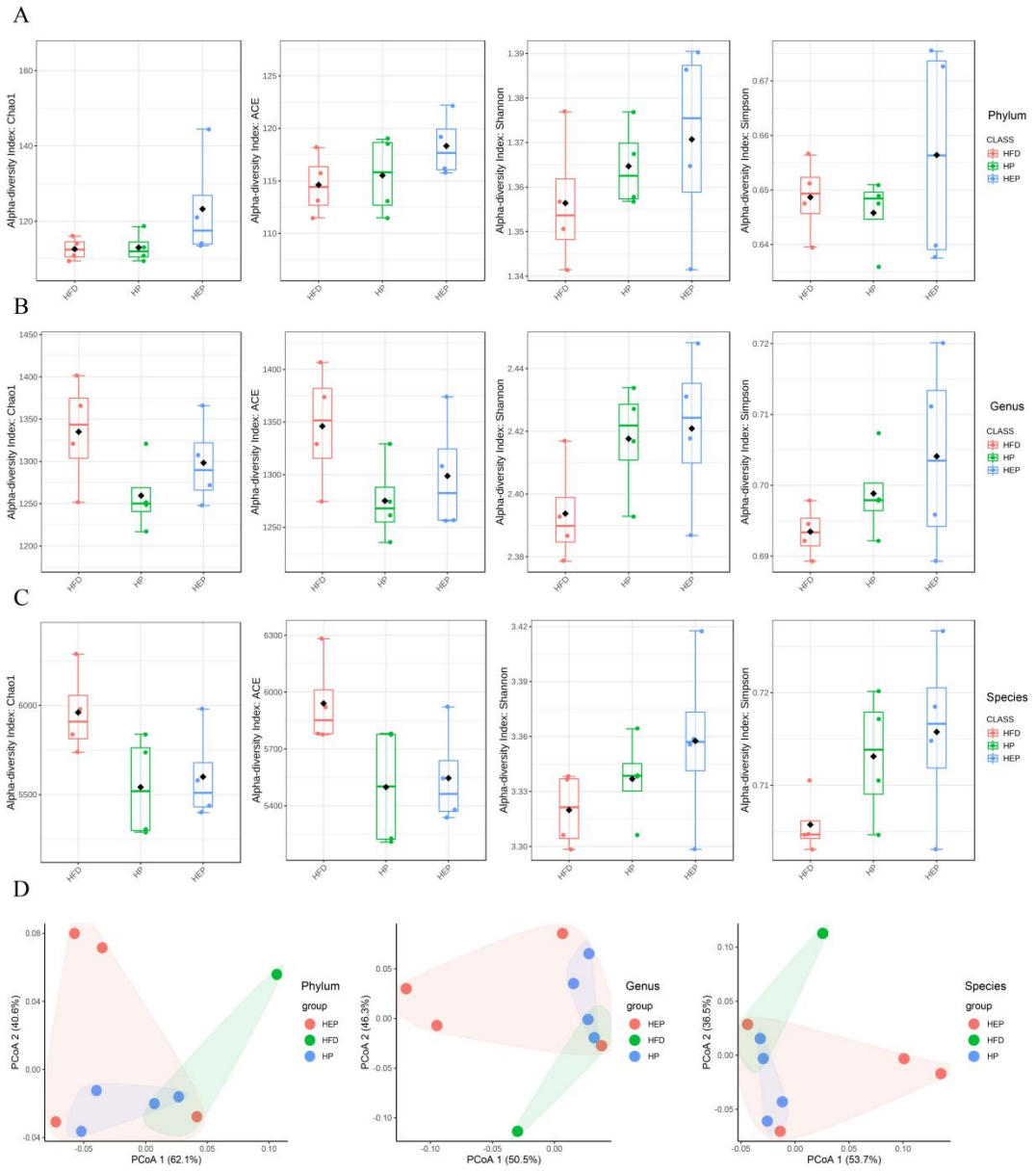


FIG S2

The shift of gut microbiota in different groups of mice according to macrogenomic data. A. Results of alpha diversity analysis at the phylum level. B. Results of alpha diversity analysis at the genus level. C. Results of alpha diversity analysis at the species level. D. PCoA analysis based on the relative abundance of species at the phylum, genus and species levels, respectively.

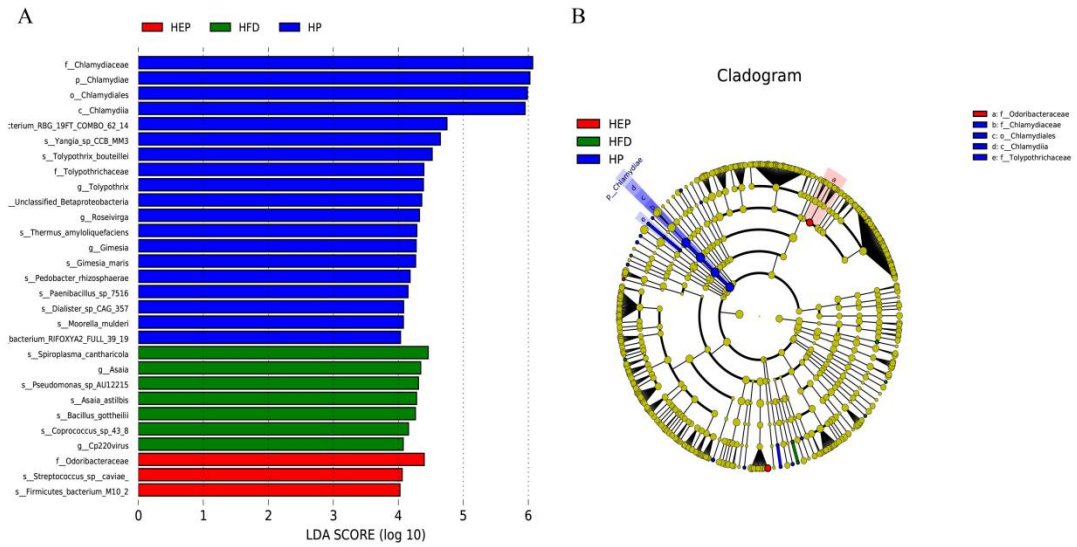


FIG S3

LEfSe analysis of different species between groups. The linear discriminant analysis (LDA) distribution diagram analysis (LAD score >4.0) showed a clear alteration of the microbiota in different individuals. A. Distribution map of LDA values of different species. B. Branch map of different species.

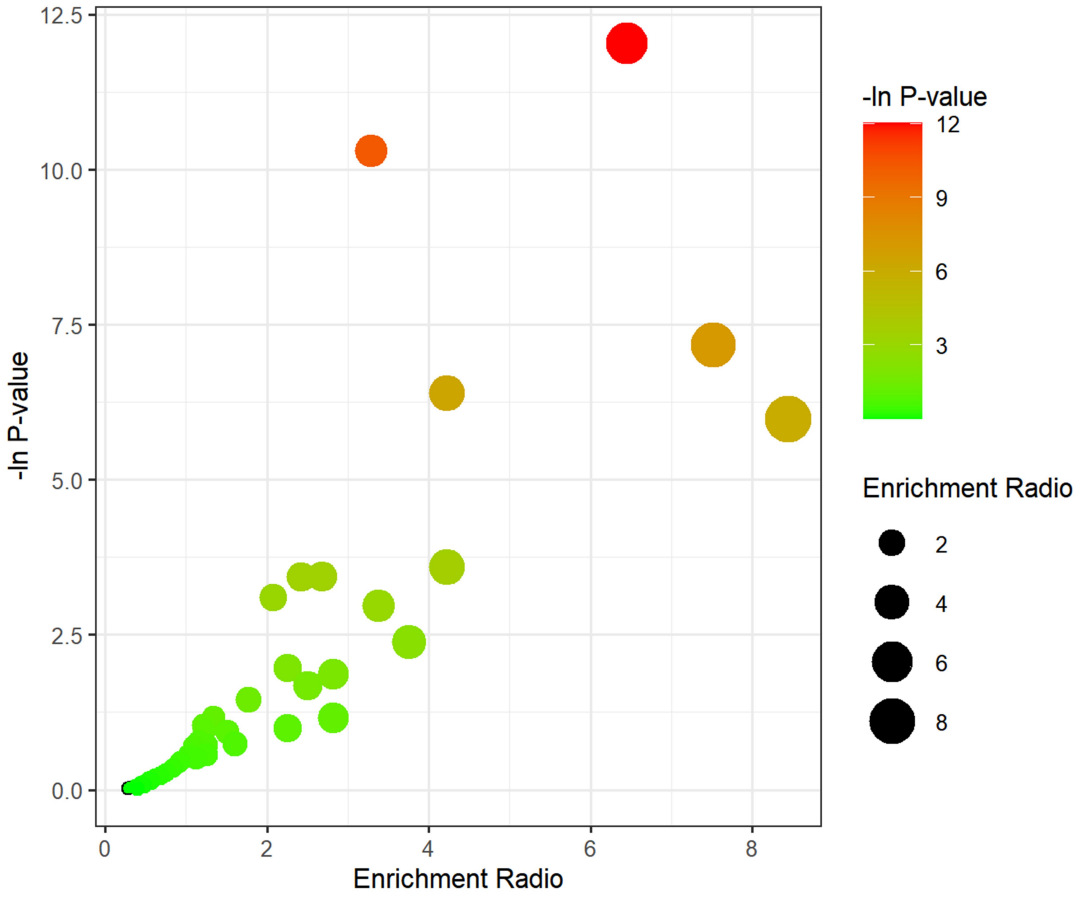


FIG S4

Enrichment analysis pathway annotation results. The plot of functional enrichment analysis for differential metabolites.

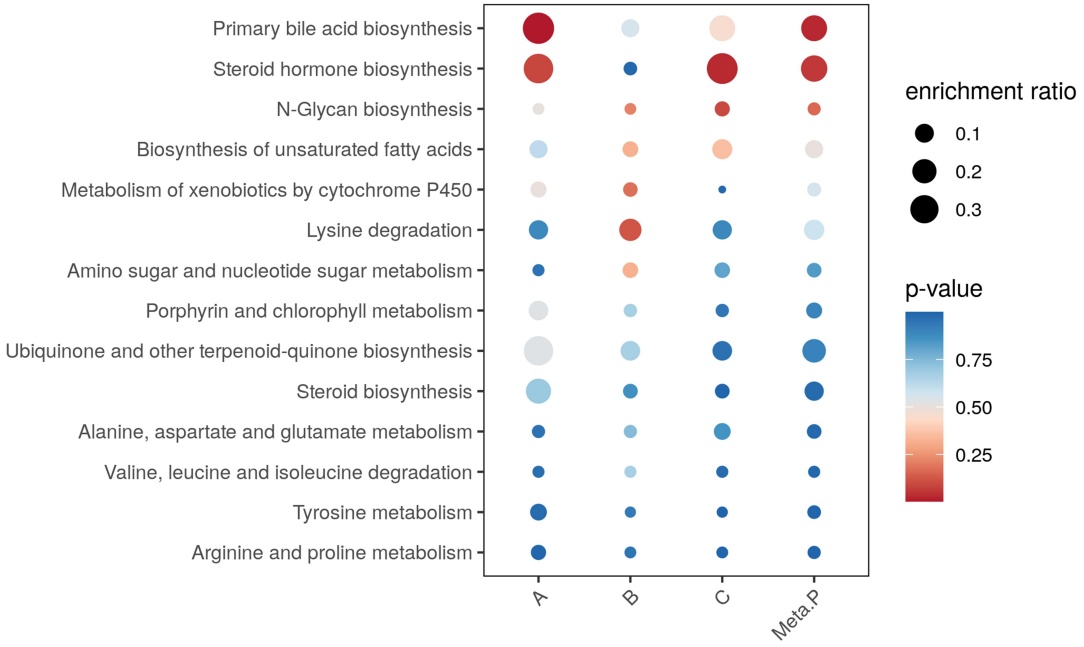
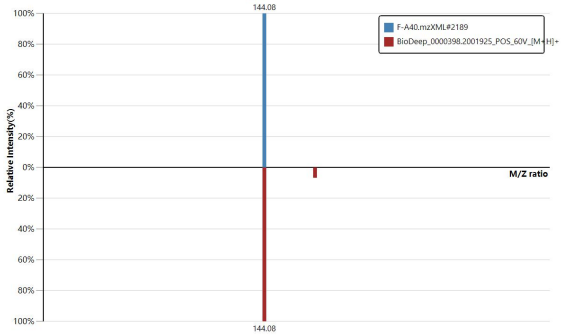


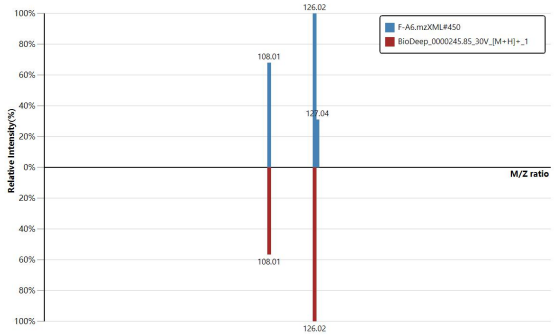
FIG S5

Functional meta-analysis pathway annotation results. The plot of functional meta-analysis for differential metabolites in pairs of 3 groups. A, HFD and HP; B, HFD and HEP; C, HP and HEP.

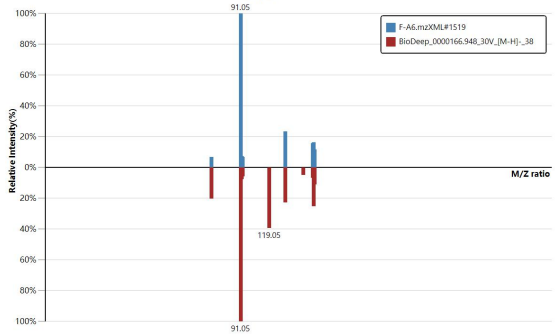
Tryptamine



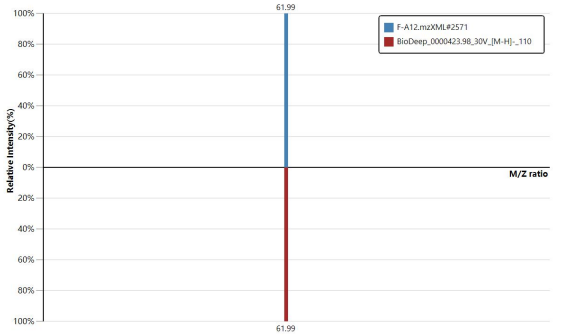
Taurine



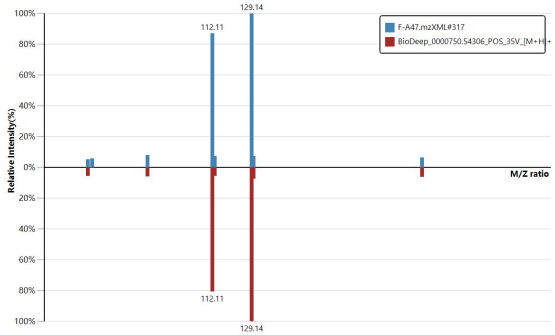
Phenylpyruvic acid



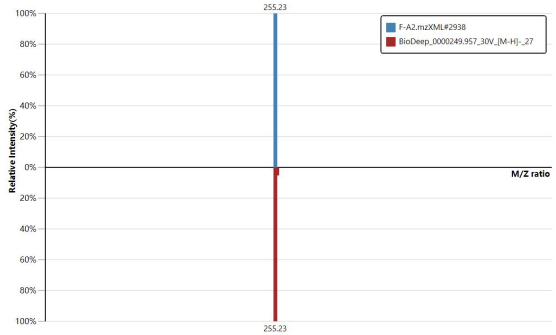
trans-Cinnamic acid



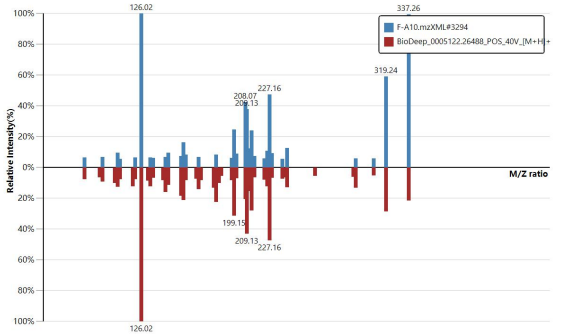
Spermine



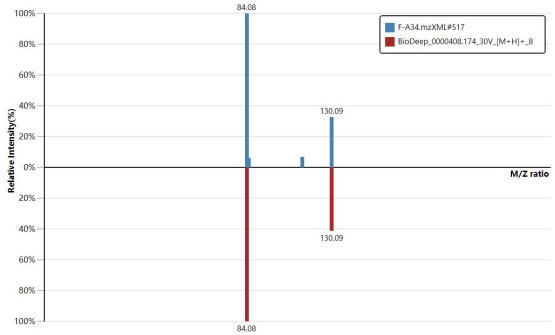
Palmitic acid



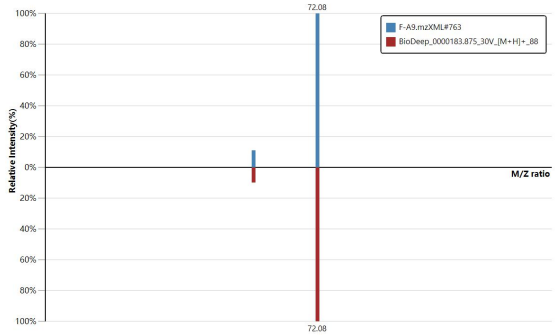
Taurocholic acid



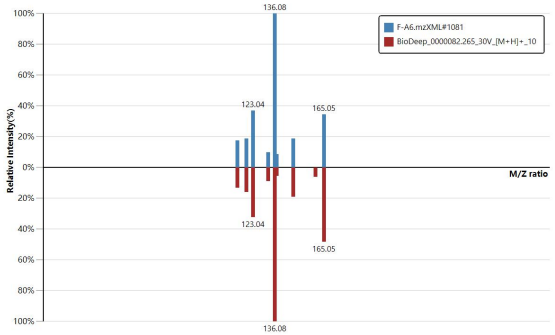
Pipecolic acid



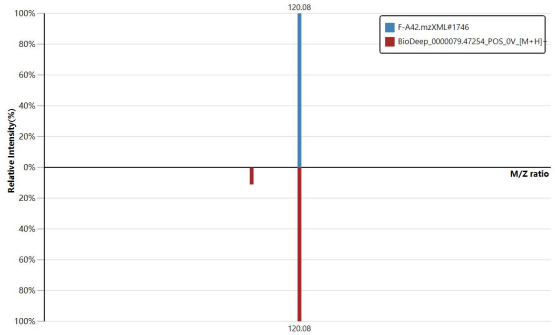
L-Valine



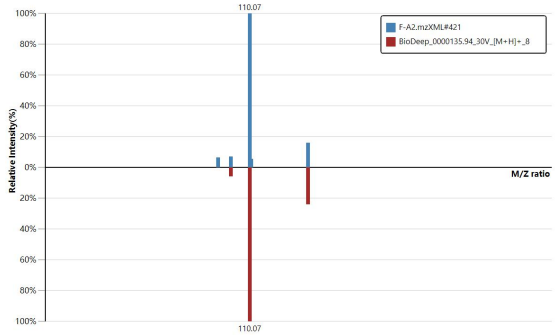
L-Tyrosine



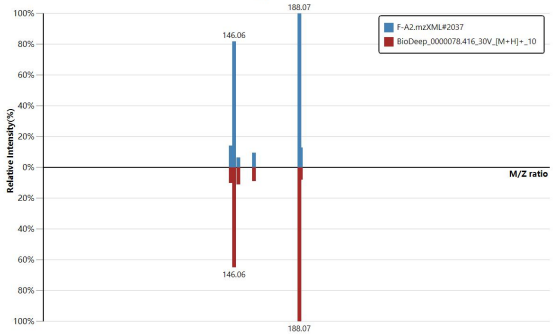
L-Phenylalanine



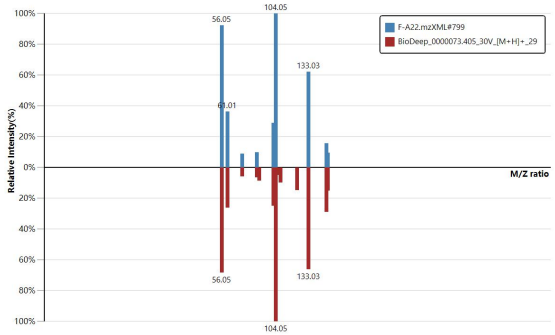
L-Histidine



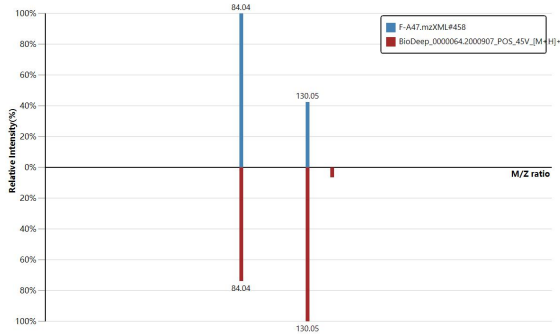
L-Tryptophan



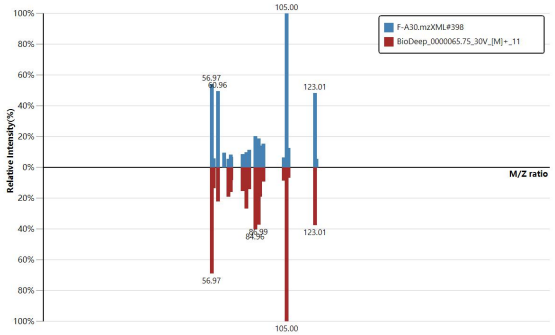
L-Methionine



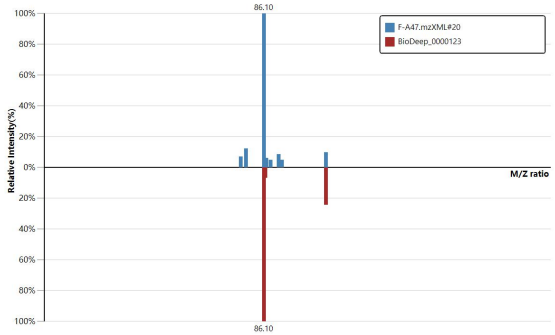
L-Glutamine



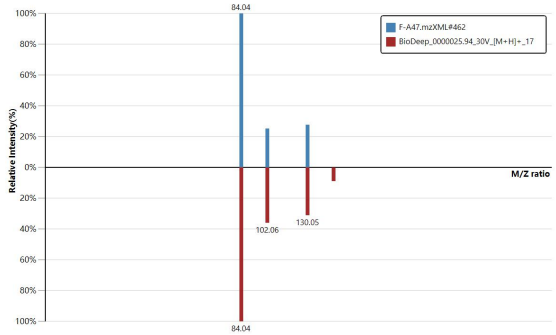
L-Serine



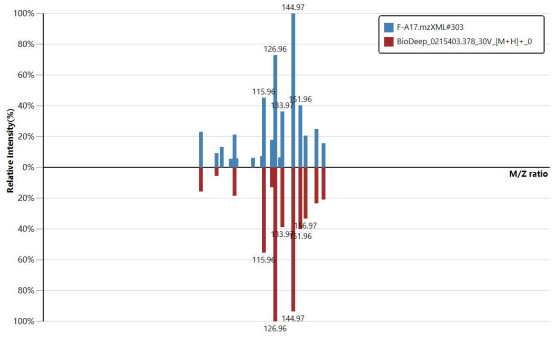
L-Leucine



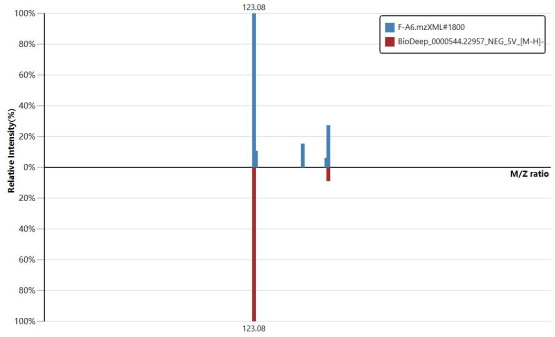
L-Glutamic acid



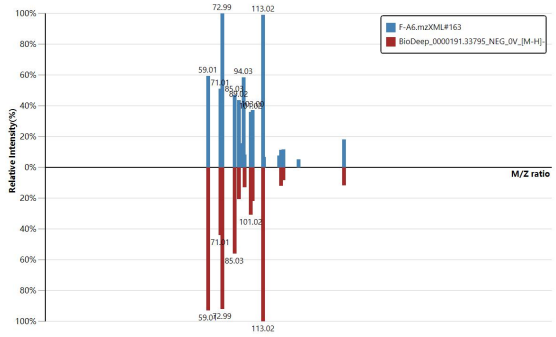
L-Dopa



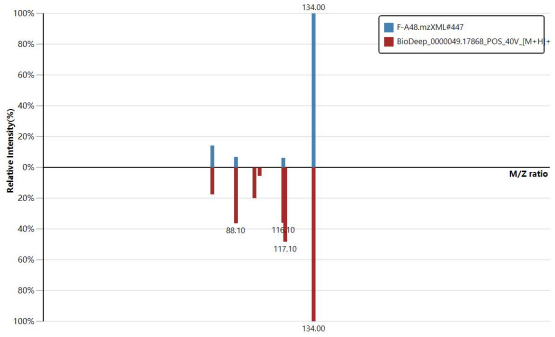
Homogentisic acid



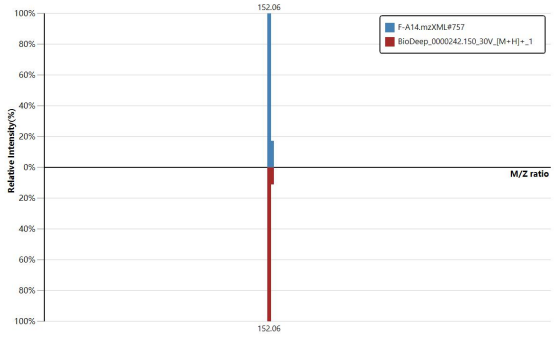
Glucuronic acid



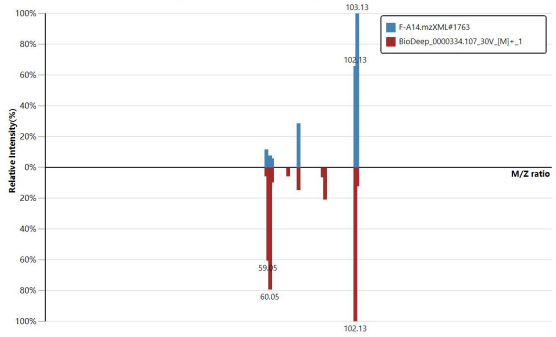
L-Aspartic acid



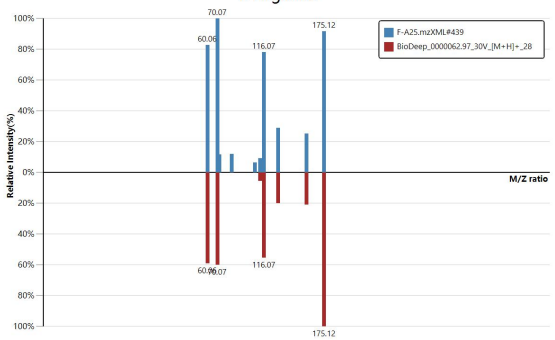
Guanine



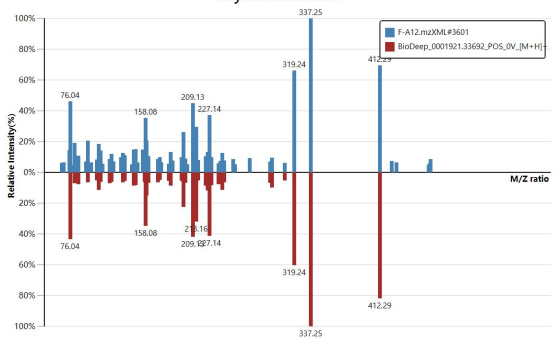
gamma-Aminobutyric acid



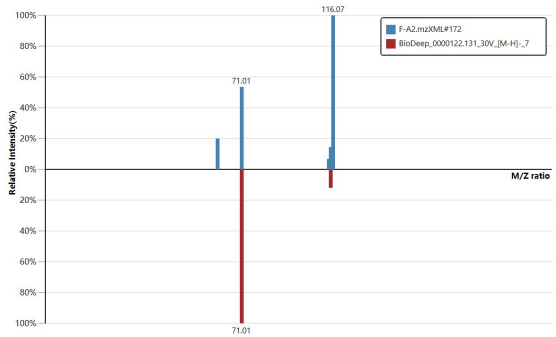
L-Arginine



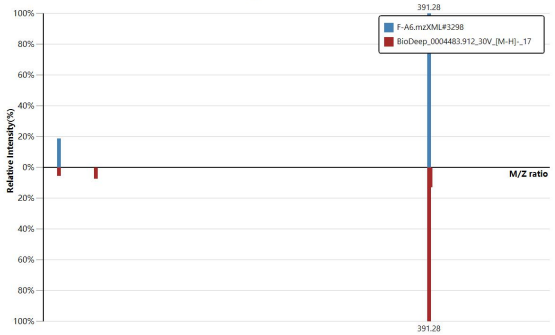
Glycocholic acid



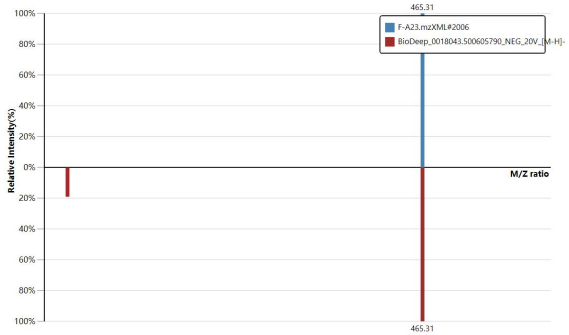
Fumaric acid



Deoxycholic acid



Cholesterol sulfate



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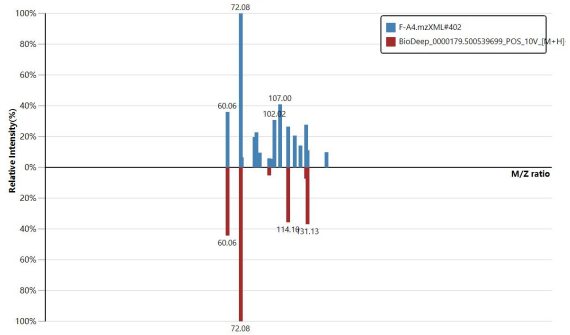
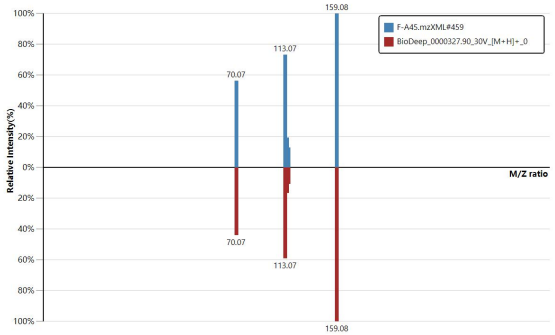
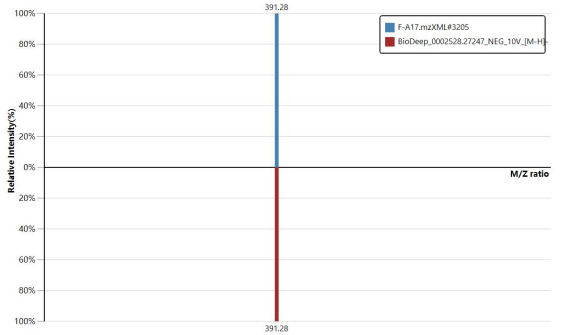


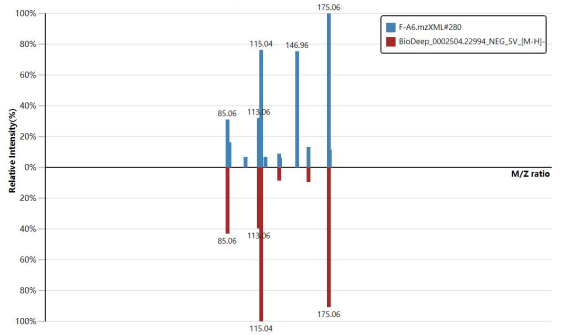
Citrulline



Chenodeoxycholic acid



2-Isopropylmalic acid



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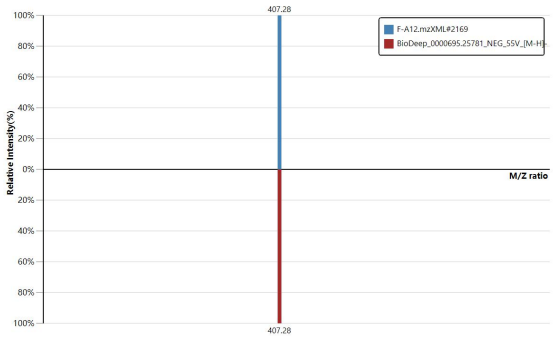
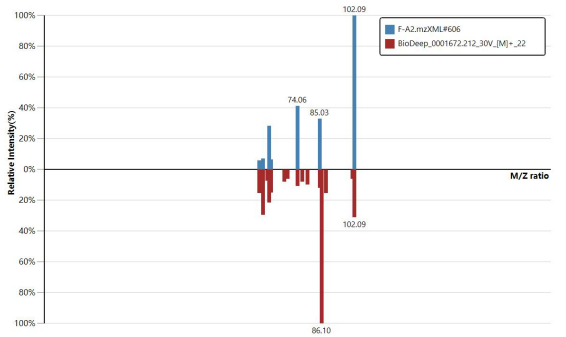


Cadaverine



Urocanic acid