Electronic Supplementary Material (ESI) for Food & Function.

Stored white tea ameliorates DSS-induced ulcerative colitis in mice by modulating the composition of the gut microbiota and intestinal metabolites

Supplementary Methods

1. LC-MS analytical conditions

The chromatographic column was an HSS T3 column (100 mm × 2.1 mm, 1.8 μm, Waters, Manchester, UK), and the column temperature was set as 40 ℃. The binary mobile phase was applied at a flow rate of 0.4 mL·min^{-1}. Phase A was deionized water containing 0.1% formic acid (v/v), and phase B was acetonitrile containing 0.1% formic acid (v/v). The gradient elution program was set as follows: 0–0.5 min, 2% phase B; 0.5–10 min, 2%–15% phase B; 10–18 min, 15%–40% phase B; 18–20 min, 40%–90% phase B; 20–20.9 min, 90% phase B; 20.9–21 min, 90%–2% phase B; and 21–25 min, 2% phase B. The injection volume was 3 μL. The mass spectrometer was operated in positive electrospray ionization (ESI) mode; the ionization voltage was set at 3.5 kV; the capillary temperature was 300 ℃; the S-lens RF level was 50; the sheath gas flow rate was 45 L·min^{-1}; the temperature and flow rate of the auxiliary gas were set at 350 ℃ and 10 L·min^{-1}, respectively; and the MS scanning range was set as m/z 80–1200.

2. Gut microbiota analysis

2.1. Library preparation and sequencing

Sequencing libraries were generated using NEB Next® Ultra™DNA Library Prep Kit for Illumina (New England Biolabs, Massachusetts, MA, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Fisher, San Jose, CA, USA) and Agilent Bioanalyzer 2100 system (Agilent, Santa Clara, CA, USA). At last, the library was sequenced on an Illumina NovaSeq 600 platform (Illumina, San Diego, CA, USA) and 250bp paired-end reads were generated.

2.2. Paired-end reads assemblies

Paired-end reads from the original DNA fragments were merged using FLASH (version 1.2.7), a very fast and accurate analysis tool, which was designed to merge paired-end reads when at least some of the reads overlap the read generated from the opposite end of the same DNA fragment.

Paired-end reads was assigned to each sample according to the unique barcodes.

2.3. OTU cluster and Species annotation

Sequences analysis was performed by UPARSE software package using the UPARSE-OTU and UPARSE-OTUref algorithms. Sequences with ≥97% similarity were assigned to the same OTUs. We pick a representative sequence for each OTU and use the RDP classifier to annotate taxonomic information for each representative sequence. The level of classification is the phylum, class, order, family, genus and species. Structural changes in species composition represented by cumulative bar charts of relative abundance.

2.4. α-Diversity and β-diversity analysis

UPARSE software in-house Perl scripts were used to analyze alpha (within samples) and beta (among samples) diversity. The α-diversity analysis was shown by Observed_species index, PD_whole_tree index, Chao1 index and Ace index. The β-diversity analysis was performed by principal coordinate analysis (PCoA) based on unifrac distance, where QIIME software was used to calculate weighted and unweighted unifrac distance.

2.5. Bacterial differential analysis

Linear discriminant analysis Effect Size (LEfSe) was used for the quantitative analysis of biomarkers within different groups. Linear discriminant analysis (LDA) was used to assess the significance of the differing species. LDA scores >3 was identified as differential species. LEfSe analyses were performed via the Galaxy online website (http://huttenhower.sph.harvard.edu/galaxy/).

Supplementary Tables

Table S1. Identifications of chemical components and its relative content in 2021WP and 2011WP extracts

Identification	Retention time (min)	Theoretical *m/z*	Detected *m/z*	Mass error (ppm)	Peak area in 2021WP extracts	Peak area in 2011WP extracts	P-value[b]
Flavanols							
GC[a]	4.7	307.0812	307.0810	0.76	2.00e8±4.51e6	1.60e8±1.45e7	＜0.001
EGC[a]	7.3	307.0812	307.0809	0.75	1.24e9±1.62e7	1.25e9±5.68e7	0.822
C[a]	7.7	291.0864	291.0862	0.58	1.85e8±4.20e6	8.80e7±6.20e6	＜0.001
EC[a]	9.9	291.0864	291.0862	0.45	1.09e9±1.37e7	7.75e8±4.23e7	＜0.001
EGCG[a]	10.1	459.0923	459.0918	0.96	3.10e9±3.56e7	2.84e9±9.40e7	0.001
GCG[a]	11.0	459.0923	459.0918	0.96	4.38e8±6.68e6	2.47e8±1.28e7	＜0.001
EGCG-3''-O-Me[a]	12.3	473.1079	473.1068	2.27	1.77e6±4.92e4	2.56e6±1.59e5	＜0.001
EGC 3,5-digallate	12.9	611.1023	611.1023	＜0.01	2.09e7±8.55e5	9.05e6±1.03e6	＜0.001
ECG[a]	12.9	443.0973	443.0968	1.02	1.49e9±1.35e7	1.21e9±5.44e7	＜0.001
Epiafzelechin 3-gallate[a]	14.3	427.1022	427.1019	0.75	8.12e7±1.34e6	5.12e7±2.95e6	＜0.001
Quercetin[a]	16.5	303.0499	303.0498	0.54	2.89e7±7.71e5	2.35e7±1.67e6	＜0.001
Kaempferol[a]	18.1	287.0550	287.0549	0.45	1.22e8±4.51e6	3.73e7±2.01e6	＜0.001
Phenolic acids							
Gallic acid[a]	2.3	171.0288	171.0288	0.02	3.91e7±1.81e6	4.26e7±7.00e6	0.264
Theogallin[a]	3.0	345.0815	345.0811	1.19	3.70e9±6.99e7	4.09e9±1.64e8	＜0.001
Chlorogenic acid[a]	8.0	355.1021	355.1020	0.31	1.04e8±4.30e6	8.77e7±3.57e6	＜0.001
Strictinin[a]	8.5	635.0874	635.0870	0.74	4.93e8±1.31e8	4.90e8±1.47e8	0.973
Ellagic acid[a]	13.0	303.0135	303.0134	0.55	1.65e7±9.61e5	7.32e6±9.84e5	＜0.001
Dimeric catechins							

Procyanidin B3	7.1	579.1491	579.1494	0.52	1.99e7±7.33e5	6.29e6±8.60e5	＜0.001
Procyanidin B2[a]	8.7	579.1496	579.1493	0.59	3.36e8±5.67e6	1.74e8±1.15e6	＜0.001
Procyanidin C1	9.6	867.2124	867.2124	0.06	1.31e7±2.26e5	1.08e7±2.56e5	＜0.001
Theasinensin A	9.6	915.1611	915.1609	0.23	4.49e7±1.48e6	1.60e7±1.19e6	＜0.001
EC-(4beta->8)-EGCG	9.9	747.1550	747.1546	0.54	2.10e7±8.86e5	1.45e7±3.49e5	＜0.001
EC-(4alpha->8)-ECG	10.6	731.1607	731.1597	1.29	6.56e6±1.01e5	4.36e6±1.70e5	＜0.001
Theasinensin F	11.3	899.1661	899.1657	0.50	2.86e7±5.93e5	2.38e7±3.83e5	＜0.001
Theaflavin[a]	16.2	565.1341	565.1336	0.81	3.75e7±2.27e6	1.37e7±7.70e5	＜0.001
Theaflavin-3-gallate[a]	16.6	717.1447	717.1441	0.77	1.06e7±5.78e5	3.27e6±1.99e5	＜0.001
Theaflavin 3,3’-digallate[a]	16.8	869.1554	869.1552	0.30	8.33e6±3.99e5	1.71e6±4.56e4	＜0.001
Theaflavin-3’-gallate[a]	16.8	717.1447	717.1443	0.53	7.26e6±3.58e5	1.41e6±4.73e4	＜0.001
Amnio acids							
Arginine[a]	0.6	175.1189	175.1190	0.25	1.50e9±5.31e7	9.28e8±2.09e7	＜0.001
Histidine[a]	0.6	156.0767	156.0768	0.47	6.27e7±1.64e6	2.85e7±2.18e6	＜0.001
Serine[a]	0.6	106.0499	106.0499	0.63	2.30e8±5.70e6	1.36e8±1.70e7	＜0.001
Aspartic acid[a]	0.7	134.0449	134.0448	0.45	1.35e8±4.79e6	1.71e8±8.08e6	＜0.001
Gamma-Aminobutyric acid[a]	0.7	104.0706	104.0713	6.74	1.60e8±1.02e6	1.39e8±1.95e6	＜0.001
Glutamic acid[a]	0.7	130.0500	130.0499	0.62	6.52e8±5.60e6	4.37e8±4.58e6	＜0.001
Proline[a]	0.7	116.0706	116.0709	2.34	2.51e9±8.51e7	1.04e9±1.28e9	0.037
Threonine[a]	0.7	120.0655	120.0657	1.47	7.79e8±1.62e7	6.48e8±1.31e7	＜0.001
Valine[a]	0.7	118.0865	118.0866	0.76	3.73e9±3.13e8	3.08e9±5.13e7	0.001
Theanine[a]	1.0	175.1078	175.1078	0.23	2.56e10±1.28e8	1.79e10±3.06e8	＜0.001
Pyroglutamic acid[a]	1.3	130.0500	130.0500	0.23	5.86e8±1.13e7	2.11e9±9.20e7	＜0.001
Leucine[a]	1.5	132.1019	132.1021	1.30	3.36e9±7.64e7	2.24e9±5.95e7	＜0.001
Phenylalanine[a]	3.2	166.0862	166.0863	0.07	6.44e9±1.78e8	4.05e9±2.14e8	＜0.001
Tryptophan[a]	5.5	205.0973	205.0972	0.78	4.64e9±3.27e7	2.63e9±1.72e8	＜0.001

Alkaloids							
Theobromine[a]	4.8	181.0721	181.0721	0.17	5.90e8±1.44e7	6.86e8±3.66e7	＜0.001
Caffeine[a]	8.2	195.0878	195.0877	0.46	6.06e10±9.98e8	5.99e10±1.52e9	0.354
EPSFs							
8-C S-EGC-cThea	10.5	418.1491	418.1493	0.41	2.23e6±4.96e4	6.16e7±1.98e6	＜0.001
8-C R-EGC-cThea	11.4	418.1491	418.1493	0.53	2.56e6±1.18e5	7.23e7±2.16e6	＜0.001
8-C S-EC-cThea	12.6	402.1541	402.1541	0.12	6.30e6±5.87e5	7.39e7±1.86e6	＜0.001
8-C S-EGCG-cThea[a]	13.0	570.1598	570.1602	0.56	5.34e6±2.25e5	9.46e7±5.43e6	＜0.001
8-C R-EC-cThea	13.2	402.1541	402.1541	0.10	7.11e6±1.36e5	9.42e7±2.97e6	＜0.001
8-C R-EGCG-cThea[a]	13.2	570.1598	570.1601	0.49	9.92e6±4.68e5	2.75e8±1.39e7	＜0.001
8-C S-ECG-cThea	14.4	554.1650	554.1653	0.43	1.58e6±9.75e4	4.09e7±1.97e6	＜0.001
8-C R-ECG-cThea	14.6	554.1650	554.1653	0.43	3.00e6±9.86e4	5.42e7±2.19e6	＜0.001
Flavonoid glycosides							
Apigenin 6,8-C-diglucoside[a]	10.7	595.1656	595.1653	0.44	7.44e7±2.22e6	7.24e7±4.90e6	0.391
Quercetin triglucoside	11.5	789.2078	789.2076	0.22	3.19e7±5.06e6	2.62e7±4.38e6	0.062
Myricetin 3-O-galactoside[a]	11.9	481.0978	481.0974	0.81	1.80e8±1.21e7	1.44e8±8.90e6	＜0.001
Quercetin diglucoside	11.9	627.1551	627.1548	0.46	4.92e7±1.41e6	4.51e7±2.47e6	0.006
Apigenin 6-C-glucosyl-8-C-arabinoside	12.1	565.1552	565.1547	0.85	1.52e8±5.82e6	1.40e8±3.50e6	0.002
Myricetin 3-O-glucoside[a]	12.1	481.0979	481.0974	0.89	2.34e8±4.17e6	2.05e8±7.96e6	＜0.001
Quercetin 3-glucosylrutinoside	12.7	773.2127	773.2124	0.41	7.85e8±4.93e7	7.25e8±3.22e7	0.033
Vitexin[a]	13.0	433.1132	433.1126	1.36	4.78e7±1.05e6	4.94e7±1.98e6	0.106
Quercetin 3-O-rutinoside	13.1	611.1602	611.1596	0.97	2.93e8±1.51e7	3.17e8±1.62e7	0.022
Kaempferol 3-galactosylrutinoside	13.1	757.2179	757.2174	0.66	2.91e8±1.07e7	2.09e8±1.15e7	＜0.001
Kaempferitrin[a]	13.2	579.1715	579.1702	2.26	6.75e7±6.10e5	9.10e7±2.69e6	＜0.001
Quercetin 3-O-galactoside[a]	13.2	465.1028	465.1024	0.75	2.79e8±1.31e7	2.22e8±1.08e7	＜0.001
Isovitexin[a]	13.2	433.1131	433.1126	1.34	3.54e7±5.64e6	4.14e7±1.61e6	0.047

Quercetin 3-O-glucoside[a]	13.4	465.1028	465.1024	0.75	2.68e8±5.32e7	2.45e8±8.76e6	0.316
Kaempferol 7-(6''-galloylglucoside)	13.4	601.1186	601.1182	0.70	1.53e7±5.32e5	1.21e7±4.66e5	＜0.001
Kaempferol 3-glucosylrutinoside	13.6	757.2179	757.2175	0.52	1.18e9±4.02e7	9.23e8±3.03e7	＜0.001
Apigenin 6-C-arabinoside-8-C-glucoside[a]	13.7	565.1546	565.1552	1.12	5.25e5±1.65e4	6.62e5±2.82e4	＜0.001
Kaempferol 3-O-galactoside[a]	13.9	449.1080	449.1074	1.22	2.14e8±5.78e6	1.29e8±3.43e6	＜0.001
Kaempferol-3-rutinoside[a]	14.0	595.1656	595.1652	0.62	2.79e8±8.87e6	2.01e8±9.69e6	＜0.001
Kaempferol 3-(6''-galloylglucoside)	14.0	601.1188	601.1182	0.97	9.63e6±1.22e6	8.26e6±1.44e6	0.104
Kaempferol 3-O-glucoside[a]	14.2	449.1079	449.1074	1.05	3.47e8±7.40e6	2.32e8±5.98e6	＜0.001
Kaempferol 3-(6-acetylgalactoside)	15.5	491.1186	491.1180	1.24	7.68e5±9.11e4	1.08e6±2.73e5	0.037
Quercetin 3-(3-p-coumaroylglucoside)	16.1	611.1395	611.1384	1.78	4.70e6±2.49e5	6.94e6±7.88e5	＜0.001
2''-O-trans-p-Coumaroylastragalin	17.3	595.1444	595.1441	0.57	1.58e6±5.47e4	1.90e6±7.38e4	＜0.001
Kaempferol 3-dicoumarylglucoside	19.5	741.1810	741.1809	0.22	1.13e6±1.52e5	1.00e6±4.51e4	0.1
Others							
Sucrose	0.8	343.1232	343.1231	0.44	5.92e8±2.54e8	4.67e8±2.31e8	0.392
Theanine glucoside[a]	1.2	337.1605	337.1603	0.71	2.00e9±6.85e7	2.73e9±3.82e8	0.005
Pyrogallol[a]	2.0	127.0390	127.0392	1.94	2.71e7±5.06e5	1.54e7±2.20e6	＜0.001
1-Ethyl-5-hydroxy-2-pyrrolidinone[a]	2.6	130.0862	130.0865	1.72	3.80e9±2.67e7	2.48e9±8.92e7	＜0.001
Cinchonain Ia	14.5	453.1180	453.1177	0.73	2.93e6±9.21e4	1.04e6±4.06e4	＜0.001

[a] Confirmed with authentic standards.

[b] *P*-values were determined by independent sample t-test.

Table S2. Identifications of intestinal metabolites in mice and its relative content in the CK, DSS, DSS+5-ASA, DSS+2021WP, and DSS+2011WP groups.

Identification	Retention time (min)	Theoretical *m/z*	Detected *m/z*	Mass error (ppm)	VIP-Value	*P*-Value [b]	Peak Area				
							CK	DSS	DSS+5-ASA	DSS+2021WP	DSS+2011WP
Short chain fatty acids											
Propionic acid[a]	0.7	73.0295	73.0284	15.47	1.2	0.059	1.16e8±3.76e7	4.51e7±2.32e7	8.91e7±3.36e7	7.50e7±1.05e7	8.15e7±3.78e7
Butyric acid[a]	0.7	87.0452	87.0440	13.32	1.3	0.001	1.00e8±2.66e7	1.00e7±5.80e6	3.90e7±1.68e7	3.37e7±1.18e7	3.25e7±2.30e7
Isobutyric acid[a]	0.9	87.0452	87.0439	14.47	1.3	0.002	1.25e8±3.34e7	1.42e7±8.46e6	4.94e7±2.26e7	4.35e7±1.83e7	4.56e7±3.21e7
Valeric acid[a]	1.1	101.0608	101.0598	10.48	0.4	/	1.22e7±7.22e6	3.09e6±2.73e6	5.16e6±3.84e6	1.13e7±1.85e7	3.05e6±2.25e6
Isovaleric acid[a]	1.3	101.0608	101.0598	10.29	1.0	0.007	1.35e7±5.17e6	1.88e6±7.89e5	6.82e6±4.32e6	6.88e6±5.78e6	4.39e6±4.01e6
Bile acids											
Alpha-MCA[a]	8.8	407.2803	407.2804	0.21	1.3	0.016	1.57e8±5.63e7	4.25e7±2.87e7	3.41e7±2.14e7	3.62e7±2.13e7	4.81e7±2.45e7
Beta-MCA[a]	8.8	407.2803	407.2804	0.21	1.2	/	4.24e7±2.18e7	6.70e7±6.34e7	2.55e7±1.53e7	3.55e7±1.61e7	3.78e7±2.03e7
T-beta-MCA[a]	9.5	514.2844	514.2848	0.67	0.5	/	5.03e6±3.66e6	8.60e6±7.83e6	4.18e6±1.28e6	1.09e7±9.00e6	5.94e6±2.03e6
T-alpha-MCA[a]	9.6	514.2844	514.2848	0.71	0.4	/	4.56e6±2.96e6	1.75e6±1.36e6	6.72e5±8.41e4	5.09e6±6.99e6	2.25e6±1.15e6
UDCA[a]	10.6	391.2854	391.2860	1.43	1.2	0.001	1.25e7±6.41e6	7.06e5±4.81e5	1.66e6±1.11e6	2.27e6±8.85e5	3.27e6±1.19e6
CA[a]	11.4	407.2803	407.2804	0.21	0.6	/	6.12e6±5.43e6	1.83e6±1.37e6	4.76e6±5.10e6	2.98e6±2.03e6	2.14e6±1.15e6
TCA[a]	11.9	514.2844	514.2848	0.67	0.9	/	3.69e6±2.60e6	7.40e5±3.91e5	2.55e6±2.10e6	1.96e6±1.05e6	1.58e6±3.98e5
CDCA[a]	12.4	391.2854	391.2859	1.38	1.0	0.015	1.53e6±1.09e6	1.14e5±7.90e4	2.65e5±1.26e5	3.41e5±4.87e5	3.79e5±2.62e5
DCA[a]	12.5	391.2854	391.2859	1.38	1.2	0.003	1.63e8±1.04e8	3.92e6±4.12e6	1.30e7±6.52e6	1.21e7±8.57e6	1.35e7±5.17e6
TCDCA[a]	12.5	498.2895	498.2904	1.83	0.8	/	8.24e5±5.72e5	1.82e5±4.88e4	2.85e5±1.01e5	3.95e5±3.61e5	4.50e5±2.44e5
TDCA[a]	12.6	498.2895	498.2901	1.13	1.0	0.048	2.01e6±1.88e6	5.54e4±2.54e4	8.33e4±5.11e4	8.21e4±5.85e4	1.34e5±6.37e4
LCA[a]	13.0	375.2905	375.2908	0.95	1.2	0.003	2.39e7±1.10e7	9.37e5±7.86e5	2.89e6±2.71e6	2.01e6±1.70e6	5.61e6±2.58e6
HDCA[a]	10.6	391.2854	/	/	/	/	Not Found	Not Found	Not Found	Not Found	Not Found
GLCA[a]	12.9	432.3119	/	/	/	/	Not Found	Not Found	Not Found	Not Found	Not Found

GDCA[a]	12.5	448.3068	/	/	/	/	Not Found	Not Found	Not Found	Not Found	Not Found
TLCA[a]	13.0	482.2946	/	/	/	/	Not Found	Not Found	Not Found	Not Found	Not Found
TDCA[a]	12.6	498.2895	/	/	/	/	Not Found	Not Found	Not Found	Not Found	Not Found
Amino acids											
Glutamic acid[a]	0.6	146.0459	146.0447	8.13	0.9	/	9.53e7±3.39e7	4.72e7±9.97e6	6.68e7±1.55e7	4.63e7±2.41e7	4.14e7±2.09e7
Aspartic acid[a]	0.6	132.0302	132.0291	8.62	1.1	0.073	1.32e7±3.67e6	7.84e6±6.76e6	6.43e6±1.77e6	7.21e6±3.67e6	4.93e6±3.80e6
Arginine[a]	0.7	173.1044	173.1036	4.48	1.0	0.095	1.36e7±1.02e7	1.64e6±1.66e6	3.40e6±1.90e6	2.49e6±1.60e6	3.22e6±1.57e6
Valine[a]	0.7	116.0717	116.0707	8.6	0.6	/	1.80e9±2.17e8	2.39e9±7.15e8	2.03e9±2.65e8	2.28e9±4.17e8	2.51e9±4.00e8
Proline[a]	0.7	114.0561	114.0553	6.56	0.7	/	1.76e8±9.80e7	1.89e8±7.32e7	1.59e8±2.58e7	1.78e8±5.33e7	1.31e8±4.91e7
Tyrosine[a]	0.9	180.0666	180.0657	5.13	1.2	0.097	2.69e6±1.10e6	5.20e6±5.32e6	2.62e6±1.36e6	2.88e6±2.78e6	9.87e5±4.40e5
Threonine[a]	1.0	118.051	118.0499	9.09	1.0	0.023	5.45e6±3.41e6	1.09e6±5.75e5	2.44e6±5.56e5	2.41e6±8.30e5	2.74e6±8.11e5
Leucine[a]	1.0	130.0874	130.0864	7.68	0.2	/	1.21e8±3.94e7	1.46e8±8.74e7	1.65e8±1.23e8	1.69e8±2.47e8	6.28e7±3.65e7
Others											
Taurine	0.7	124.0074	124.0062	9.63	1.1	0.245	1.85e8±1.18e8	2.00e8±4.25e7	1.32e8±7.56e7	1.04e8±5.07e7	1.45e8±6.65e7

[a] Confirmed with authentic standards.

[b] *P*-values were determined by Kruskal-Wallis test.

Supplementary Figure

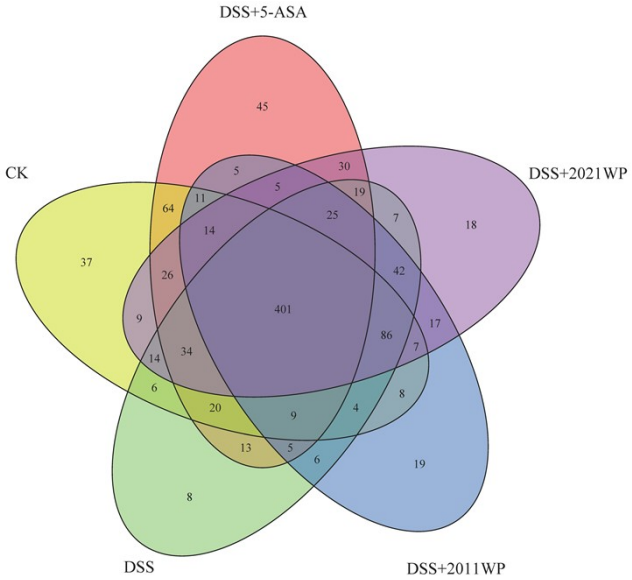

Figure S1. Venn diagram of OTU levels among the CK, DSS, DSS+5-ASA, DSS+2021WP, and DSS+2011WP groups.

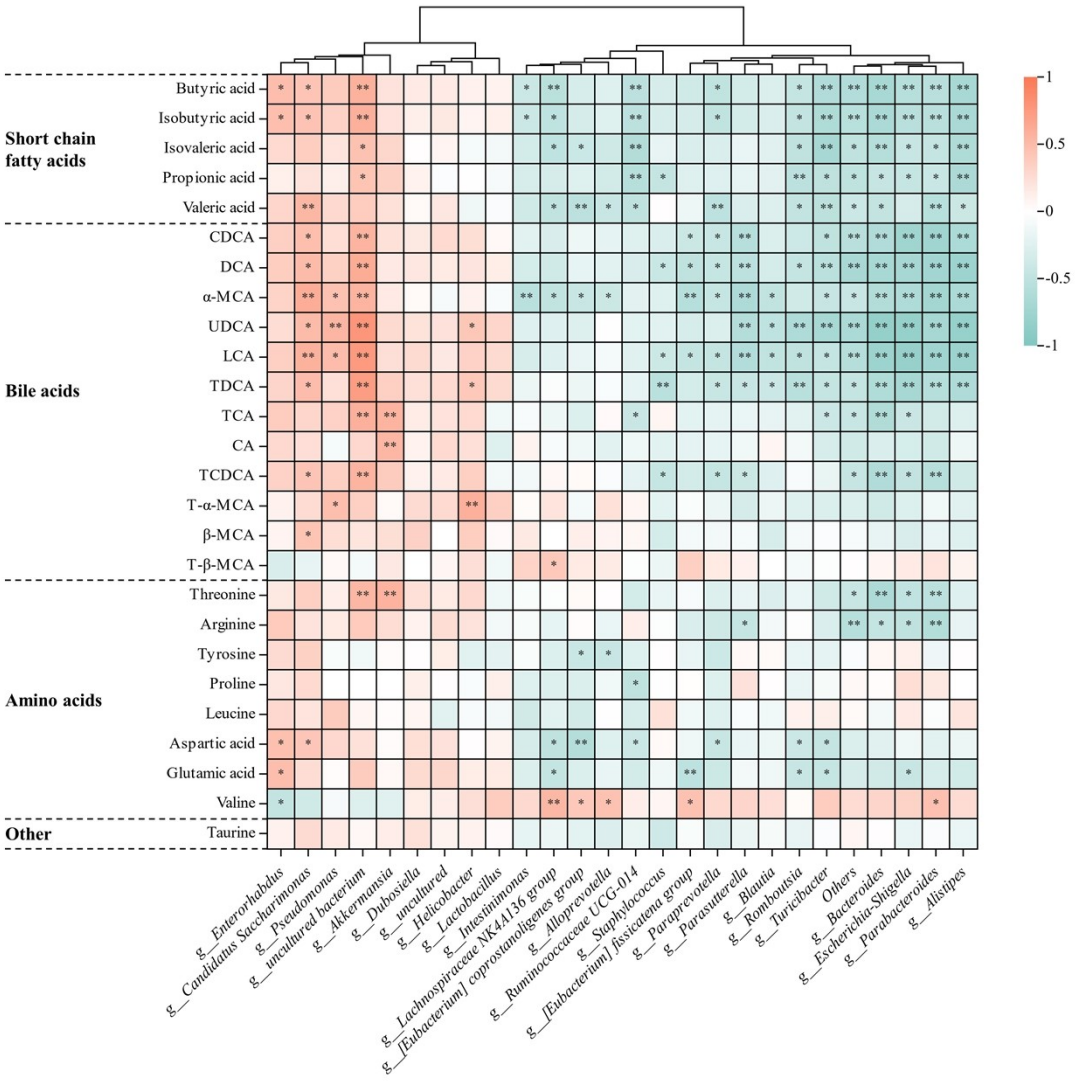

Figure S2. The heatmap of Spearman's correlation analysis between 26 intestinal metabolites and gut microbiota. Different colors represent the value of correlation coefficient: red indicates positive correlation and blue indicated negative correlation. *P < 0.05, **P < 0.01.