Electronic Supplementary Material (ESI) for Food & Function.

Supplementary information

Supplementary Tables

Score	Weight loss (%)	Stool consistency	Occult blood or gross bleeding
0	None	Normal	Negative
1	1-5	Loose stool	Negative
2	5-10	Loose stool	Hemoccult positive
3	10-15	Diarrhea	Hemoccult positive
4	＞15	Diarrhea	Gross bleeding

Table S1 Criteria for scoring disease activity index (DAI)

Normal stools = well-formed pellets; loose stools = pasty stool that does not stick to the anus; and diarrhea = liquid stools that sticks to the anus.

Table S2 Histology scoring criteria

Score	Crypt architecture	Tissue damage	Goblet cell loss	Inflammatory cell infiltration
0	Normal	No damage	Normal	Occasional infiltration
1	Irregular	Discrete lesions	10-25% loss	Increasing leukocytes in lamina propria
2	Moderate crypt loss (10-50%)	Mucosal erosions	25-50% loss	The confluence of leukocytes extending to the submucosa
3	Severe crypt loss (50-90%)	Extensive mucosal damage	Above 50% loss	Transmural extension of inflammatory infiltrates
4	Small/medium-sized ulcers (<10 crypt widths)			
5	Large ulcers (>10 crypt widths)			

Table S3. The primary antibodies and the secondary antibodies used in immunofluorescence staining

Antibody	Dilution	Product code	Manufacturers	Application
ZO-1	1 : 500	Ab307799	Abcam	IF
Occludin	1 : 500	27260-1-AP	Proteintech	IF
Mucin2	1 : 500	27675-1-AP	Proteintech	IF
Cy3 Goat Anti-Rabbit IgG (H+L)	1 : 1000	A0516	Beyotime	IF
Alexa Fluor® 488 Goat Anti-Rabbit lgG H&L	1 : 1000	Ab150077	Abcam	IF

Table S4. Primer sequences used for RT-qPCR analysis.

Gene	Forward primer(5'-3')	Reverse primer(5'-3')

β-actin	TCAGCAAGCAGGAGTACGATG	AACGCAGCTCAGTAACAGTCC
Occludin	ATGTCCGGCCGATGCTCTC	TTTGGCTGCTCTTGGGTCTGTAT
Zo-1	TTTTTGACAGGGGGAGTGG	TGCTGCAGAGGTCAAAGTTCAAG
Tnf-α	GACGTGGAACTGGCAGAAGAG	TTGGTGGTTTGTGAGTGTGAG
Tlr4	ATGGCATGGCTTACACCACC	GAGGCCAATTTTGTCTCCACA
Myd88	TCATGTTCTCCATACCCTTGGT	AAACTGCGAGTGGGGTCAG

Supplementary Figures

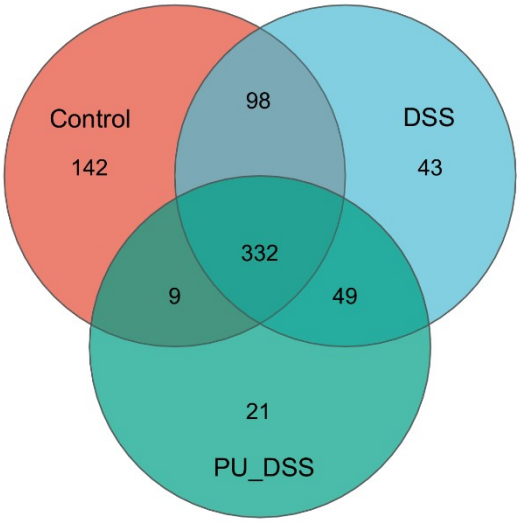

Fig. S1 The Venn diagram at OTU level.

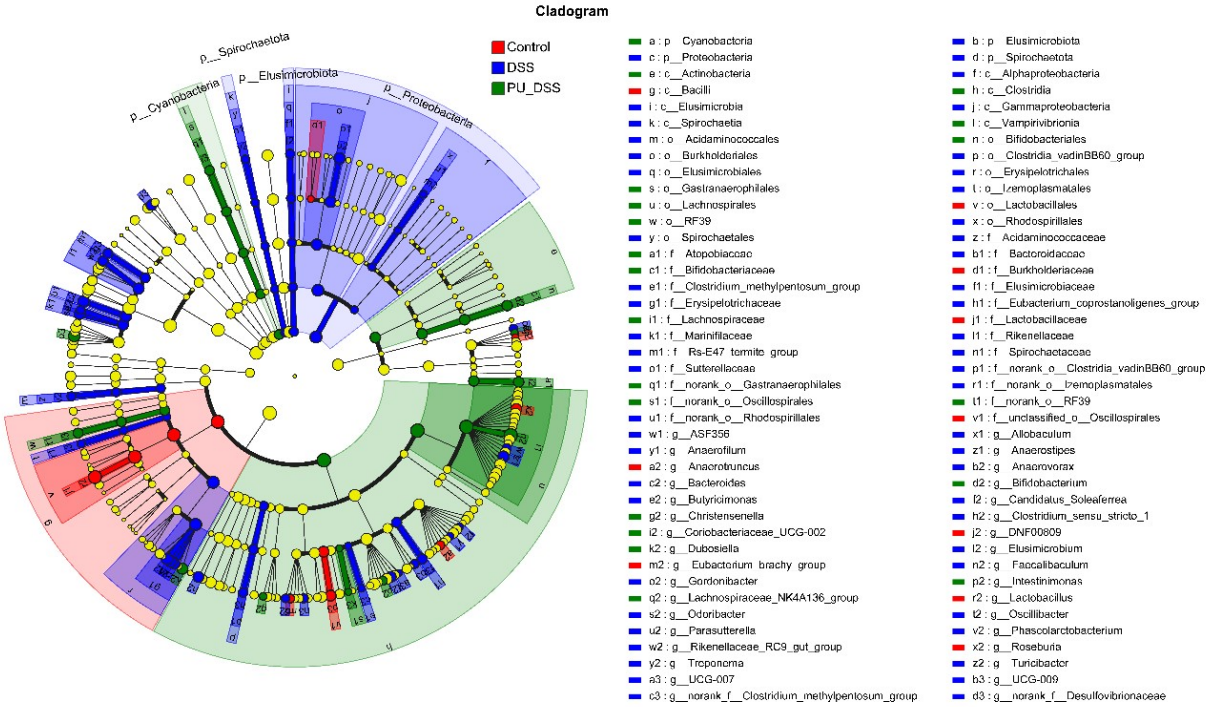

Fig. S2 The linear discriminant analysis effect size.

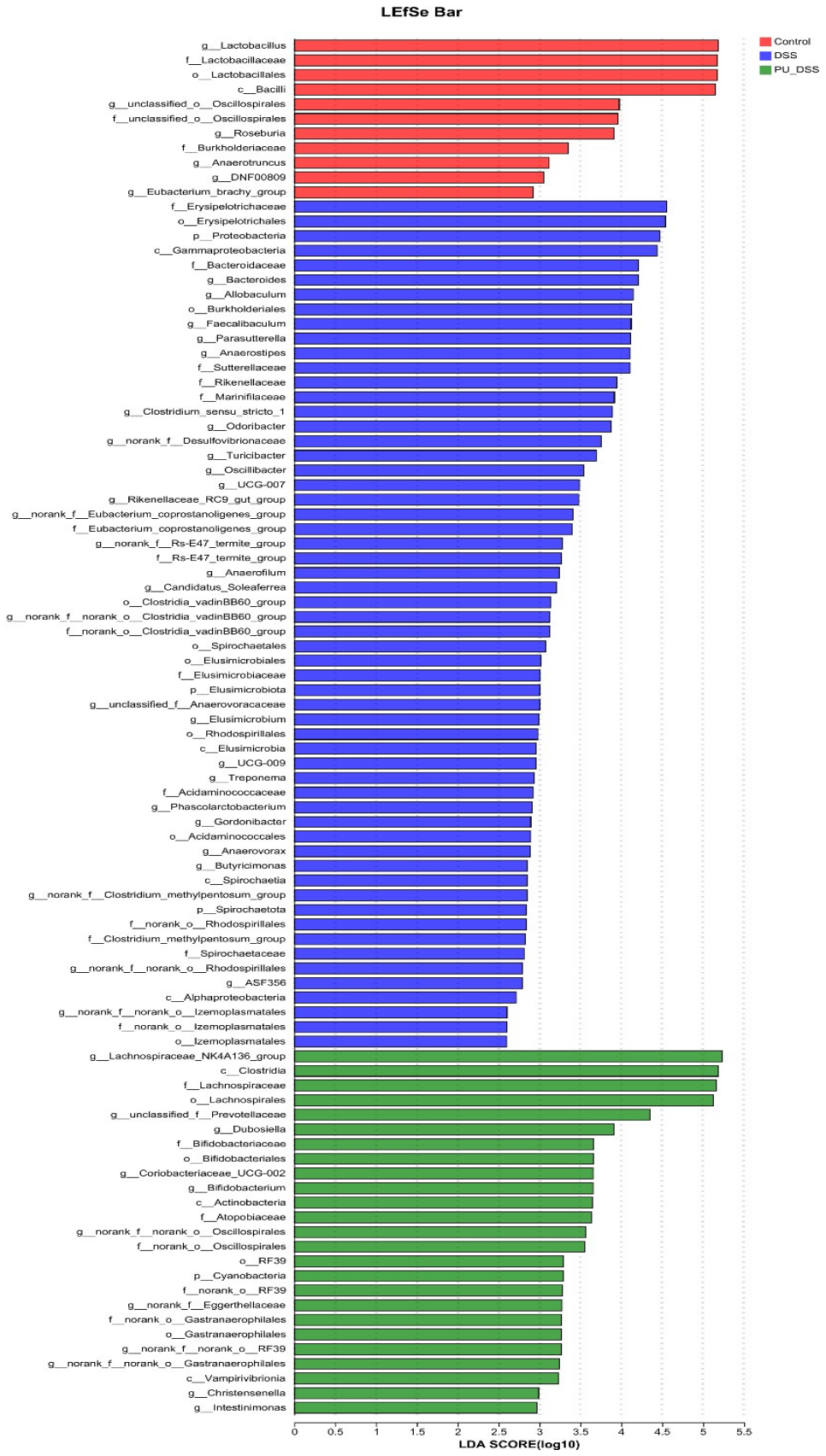

Fig. S3 Identification of differentially enriched taxa by linear discriminant analysis (LDA) effect size.

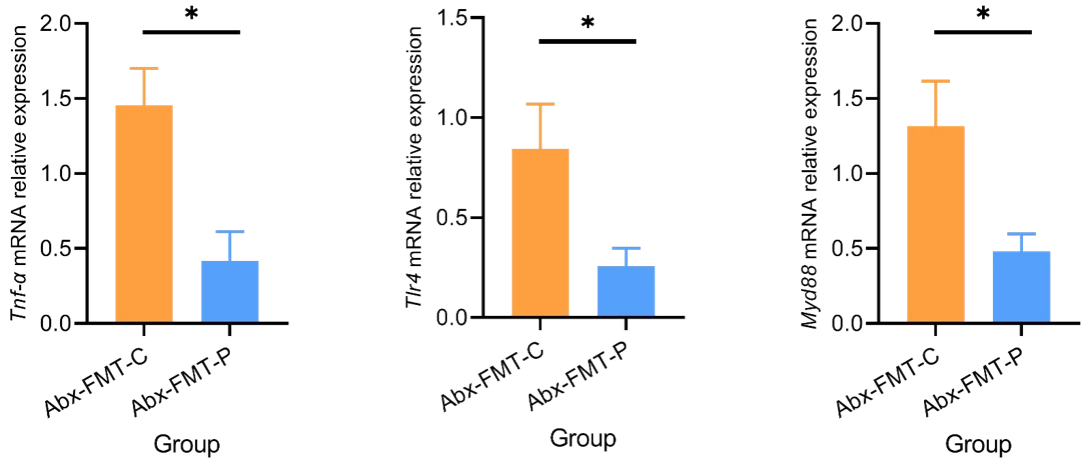

Fig. S4 The mRNA level of *Tnf-α*, *Tlr4*, and *Myd88* in the colon.

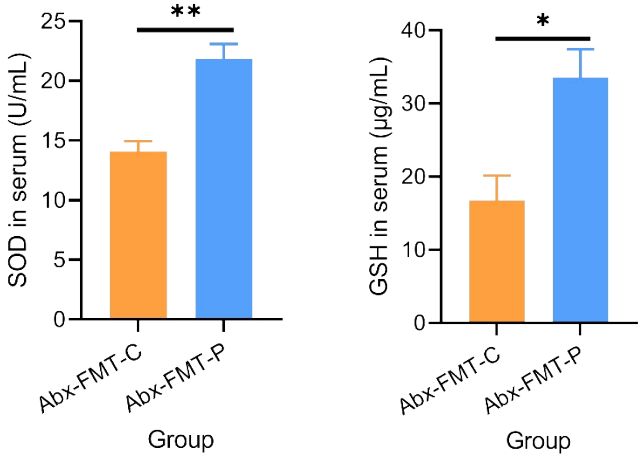

Fig. S5 The levels of SOD and GSH in serum of Abx-FMT-C and Abx-FMT-P group mice.

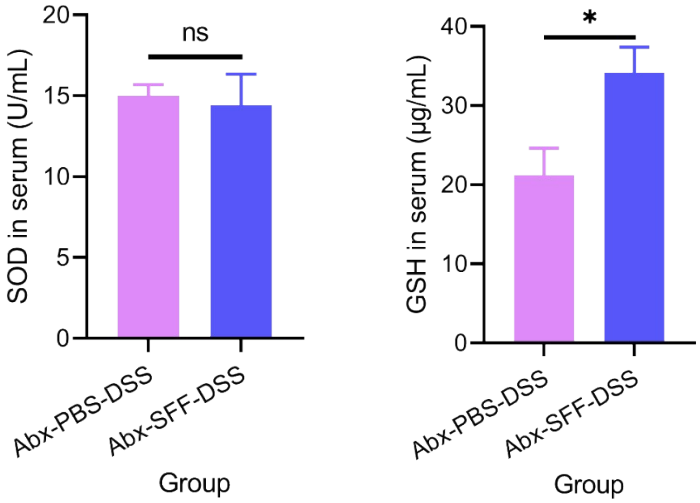

Fig. S6 The levels of SOD and GSH in serum of Abx-PBS-DSS and Abx-SFF-DSS group mice.

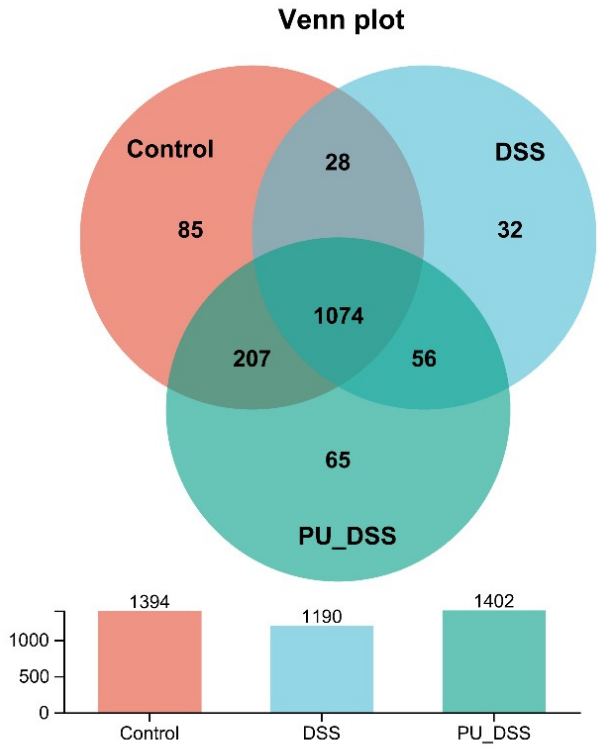

Fig. S7 Venn diagram of metabolites detected in each group (Control, DSS, and PU + DSS).

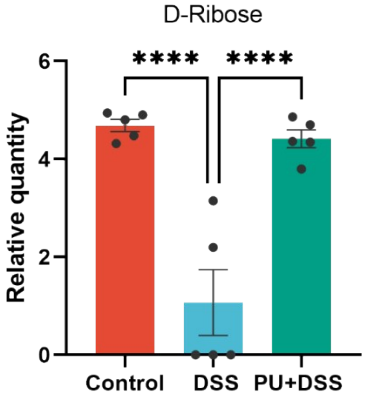

Fig. S8 The relative content of D-ribose in the stool of mice in each group.

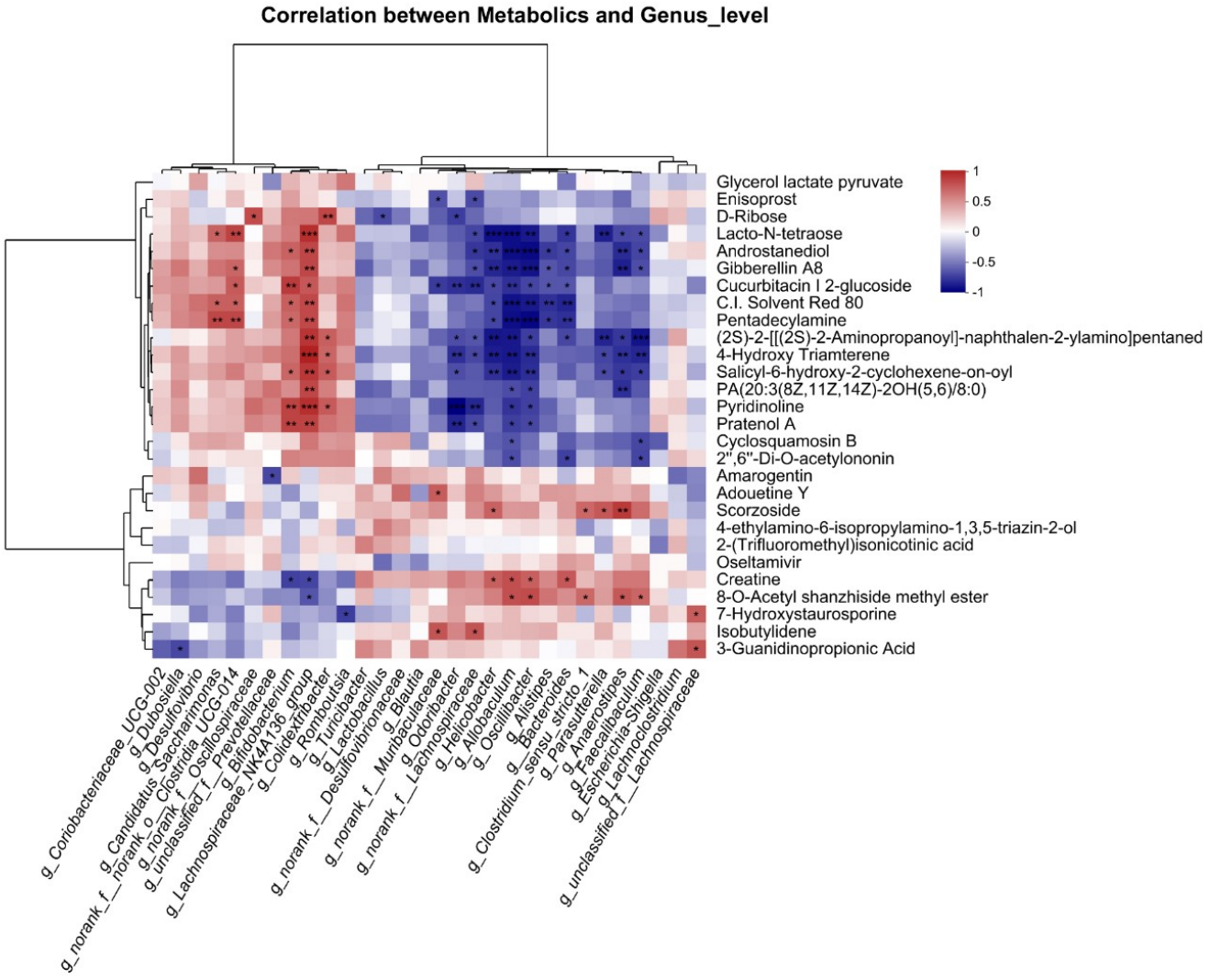

Fig. S9 Correlation between gut microbiota at the genus level and the top 30 metabolites with substantial changes between the DSS and PU + DSS groups.

Supplementary Materials and methods

The animal experiment was approved by the Dalian Polytechnic University Animal Experiment Ethics Committee (Approval No. DLPU2022029).

16S rRNA gene sequencing and analysis

The V3-V4 regions of the bacterial 16S rRNA gene were amplified using primers 338F (5' -CCTACGGGRSGCAGCAG- 3') and 806R (5' -GGACTACVSGGGTATCTAAT- 3'). MiSeq sequencing results in double-ended sequence data. According to the overlapping relationship between the PE reads, the paired reads are merged into a sequence, and the quality of the reads and the effect of the merge are quality-controlled filtered. Differentiate the sample according to the barcode and primer sequences at the beginning sequence's beginning and end and the end of the sequence to obtain the effective sequence and correct the sequence direction. The reads were quality-filtered by using software: FLASH and Trimmomatic. The acquired high-quality sequences were then clustered into Operational Taxonomic Units (OTUs), a term used to classify groups of bacteria, at a cut-off similarity value of 97% using USEARCH (Edgar, 2013) software. The data were analyzed on the free online platform of Majorbio Cloud Platform (www.majorbio.com).

The conditions of LC-MS/MS analysis

LC conditions: Column: ACQUITY HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm; Waters, USA); Column temperature: 40°C; Injection volume: 3 μL. Mobile phases: consisted of 0.1% formic acid in water: acetonitrile (95: 5, v/v) (solvent A) and 0.1% formic acid in acetonitrile: isopropanol: water (47.5: 47.5: 5, v/v/v) (solvent B). Positive

ion mode separation gradient: 0-3 min, mobile phase B was increased from 0% to 20%; 3-4.5 min, mobile phase B was increased from 20% to 35%; 4.5-5 min, mobile phase B was increased from 35% to 100%; 5-6.3 min, mobile phase B was maintained at 100%; 6.3-6.4 min, mobile phase B was decreased from 100% to 0%; 6.4-8 min, mobile phase B was maintained at 0%. Separation gradient in negative ion mode: 0-1.5 min, mobile phase B rises from 0 to 5%; 1.5-2 min, mobile phase B rises from 5% to 10%; 2-4.5 min, mobile phase B rises from 10% to 30%; 4.5-5 min, mobile phase B rises from 30% to 100%; 5-6.3 min, mobile phase B linearly maintains 100%; 6.3-6.4 min, the mobile phase B decreased from 100% to 0%; 6.4-8 min, the mobile phase B was linearly maintained at 0%. Flow rate: 0.4 mL/min.

MS conditions: Source temperature: 425°C; Sheath gas flow rate: 50 arb; Aux gas flow rate: 13 arb; Ion-spray voltage floating (ISVF): -3500V in negative mode and 3500 V in positive mode, respectively; Normalized collision energy: 20-40-60 V rolling for MS/MS. Full MS resolution: 60000; MS/MS resolution: 7500. Data acquisition was performed with the Data Dependent Acquisition (DDA) mode. The detection was carried out over a mass range of 70-1050 m/z.

The data were analyzed on the free online platform of Majorbio Cloud Platform. The metabolites with VIP > 1, $p < 0.05$ were determined as significantly different metabolites based on the variable importance in the project (VIP) obtained by the OPLS-DA model and the p-value generated by Student's t test.

ELISA assays

Cells were grown until 80 to 90% confluence in 12-well plates and treated with D-

Ribose (20 μM) for 24 h, followed by the treatments with or without LPS (1 μg/mL serum-free culture medium). After 6 h incubation and centrifugation (4°C, 1000 × g for 20 min), cell supernatants were collected for ELISA assays (ml063159 for IL-6 and ml002095-C for TNFα).

Nitric oxide (NO) production assay

Briefly, the cell culture medium was withdrawn at the end of the treatment and the appropriate volume of diluted DAF-FM DA (5 μM) was added. The diluted DAF-FM DA (200 μL) was added to the 24-well plates. The DAF-FM DA that had not penetrated the cells was adequately eliminated by three PBS washes, after 20 min of incubation. Imaging was performed using a fluorescence microscope (RVL-100, ECHO, Singapore).

ROS production assay

The ROS was determined in RAW264.7 cells by fluorescence of DCFH-DA. Briefly, after two washes with PBS (pH = 7.4), cells were treated with DCFH-DA (10 μM, diluted in serum-free medium) for 20 min at 37°C. Imaging was performed using a fluorescence microscope (RVL-100, ECHO, Singapore).

JC-1 assay

Briefly, after treatment, RAW264.7 cells were incubated with JC-1 (10 μM, diluted in JC-1 staining buffer) for 20 min at 37°C, and rinsed off the free probe using JC-1 staining buffer. A fluorescence microscope (RVL-100, ECHO, Singapore) and Image Pro Plus software were used to measure the intensity of fluorescence.