SUPPLEMENTARY METHODOLOGIES:

1. Methodologies of gut microbiota by 16S rRNA amplicon sequencing

Sample preparation[1]**:** Fecal samples were freshly collected at week 16 and immediately stored at -80°C. Total fecal DNA was extracted using the CTAB/SDS method. The V4 region of the 16S rRNA was amplified using the universal primers 515F and 806R. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs), and the PCR products were purified with GeneJET Gel Extraction Kit (Thermo Scientific). Sequencing libraries were generated using Ion Plus Fragment Library Kit (Thermo Scientific) following manufacturer's recommendations. The library quality was assessed on the Qubit 2.0 Fluorometer (Thermo Scientific). At last, the library was sequenced on an Ion S5™ XL platform (Thermo Scientific) and 400 bp/600 bp single end reads were generated.

Data acquisition: Paired-end reads were merged using FLASH (V1.2.7), and the Raw fastq files were processed by QIIME (version 1.7.0). Sequence analysis was performed by Uparse software (v7.0.1001), and Sequences with ≥ 97% identity were assigned to the same Operational Taxonomic Units (OTUs).

2. Methodologies of non-targeted metabolome analysis

Metabolites extraction from colon contents [2,3]**:** The colonic content was mixed with ddH_2O (4°C) and 100 mg of the sample was extracted with 1000 μL of pre-cooled methanol (-20°C). After centrifugation, the supernatant was evaporated and the residue was dissolved in 400 μL methanol aqueous solution (1:1, 4°C). For the quality control (QC) samples, 20 μL of the extract was taken from each sample. These QC

samples were used to monitor the deviations of the analytical results and to compare them with the errors caused by the analytical instrument itself. The rest of the samples were used for LC-MS detection.

UPLC Conditions[4]: Chromatographic separation was accomplished in an Acquity UPLC system equipped with an ACQUITY UPLC® HSS T3 (150 × 2.1 mm, 1.8 μm, Waters) column maintained at 4°C. The temperature of the auto sampler was 4°C. Gradient elution of analytes was carried out with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 0.25 mL/min. Injection of sample (5 μL) was done after equilibration. An increasing linear gradient of solvent B (v/v) was used as follows: 0~1 min, 2% B; 1~9.5 min, 2%~50% B; 9.5~14 min, 50%~98% B; 14~15 min, 98% B; 15~15.5 min, 98%~2% B; 15.5~17 min, 2%.

Mass spectrometry conditions[5]: ESI-MS^n experiments were executed on the Thermo LTQ Orbitrap XL mass spectrometer with the spray voltage of 4.8 kV and -4.5 kV in positive and negative modes, respectively. Sheath gas and auxiliary gas were set at 45 and 15 arbitrary units, respectively. The capillary temperature was 325°C. The voltages of capillary and tube were 35 V and 50 V, -15 V and -50 V in positive and negative modes, respectively. The Orbitrap analyzer scanned over a mass range of m/z 89-1000 for full scan at a mass resolution of 60000. Data dependent acquisition (DDA) MS/MS experiments were performed with CID scan. The normalized collision energy was 30 eV. Dynamic exclusion was implemented with a repeat count of 2, and exclusion duration of 15 s.

Data processing[6]: UPLC-QTOF-MS raw data were analyzed with MarkerLynx

Application Manager 4.1 (Waters Corp.). The matrix from UPLC-QTOF-MS was introduced into SIMCA-P 11.0 software (Umetrics) and standardized to a mean of 0 and variance of 1, according to the formula [X - mean(X)]/ std (X), for multivariate statistical analysis. The t test with false discovery rate correction was used to measure the significance of each metabolite. Orthogonal partial least-squared discriminant analysis (OPLS-DA) were conducted to identify the metabolite discrimination between the two group samples. Differential metabolites were defined with variable importance in the projection (VIP) > 1.0 obtained from OPLS-DA and *P* values less than 0.05 obtained from t test. Differential metabolites were tentatively identified by database matching, i.e., Human Metabolome Database (HMDB) (http://www.hmdb.ca), Metlin (http://metlin.scripps.edu), massbank (http://www.massbank.jp/), LipidMaps (http://www.lipidmaps.org), mzclound (https://www.mzcloud.org).

Reference

1 Zhang, X.; Zhao, Y.; Xu, J.; Xue, Z. S.; Zhang, M. H.; Pang, X, Y.; Zhang, X. J.; Zhao, L. P. Modulation of gut microbiota by berberine and metformin during the treatment of high-fat diet-induced obesity in rats. *Sci Rep*. **2015**, *5,* 14405.

2 Ponnusamy, K.; Choi, J. N.; Kim, J.; Lee, S. Y.; Lee, C. H. Microbial community and metabolomic comparison of irritable bowel syndrome faeces, *J. Med. Microbiol.*, **2011**, *60*, 817-827.

3 Ng, J. S. Y.; Ryan, U.; Trengove, R. D.; Maker, G. L. Development of an untargeted metabolomics method for the analysis of human faecal samples using

Cryptosporidium-infected samples, *Mol. Biochem. Parasitol.*, **2012**, *185*, 145-150.

4 Sangster, T.; Major, H.; Plumb, R.; Wilson A. J.; Wilson, I. D. A pragmatic and readily implemented quality control strategy for HPLC-MS and GC-MS-based metabonomic analysis, *Analyst*, **2006**, *131*, 1075-1078.

5 Liu, R.; Hong, J.; Xu, X.; Feng, Q.; Zhang, D.; Gu, Y.; Shi, J., et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention, *Nat. Med.*, **2017**, *23*, 859-868.

6 Wang, Q.; Garrity, G. M.; Tiedje J. M.; Cole, J. R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy, *Appl. Environ. Microbiol.*, **2007**, *73*, 5261-5267.

Supplementary Table. Designed Primer Sets for qRT-PCR

Gene	Primer	5'- 3'	Size（bp）
Mus-Fxr	sense	TGGGCTAGGCAAAAGATGTGA	160
	antisense	ACTGCTGCTTCTTGAGTGCT	
Mus-Shp	sense	CCAGTAGAGTGGTAGCCCG	360
	antisense	CTCCCCTCCGTACAGGTCAT	
Mus-Fgf15	sense	AGTACCTGTACTCCGCTGGT	148
	antisense	ACGTCCTTGATGGCAATCGT	
Mus-Spt1	sense	CACCGAGCACTATGGGATCA	303
	antisense	AGGGACTCTCCCACCACTTT	
Mus-Spt2	sense	TCACCTCCATGAAGTGCATC	109
	antisense	CAGGCGTCTCCTGAAATACC	
Mus-Spt3	sense	ACACAATCCTAAGACCCAGCA	141
	antisense	AGACTGGCTTATCCTCAGCATA	
Mus-Cer2	sense	AAGTGGGAAACGGAGTAGCG	131
	antisense	ACAGGCAGCCATAGTCGTTC	
Mus-Cer4	sense	GGATTAGCTGATCTCCGCAC	197
	antisense	CCAGTATGTCTCCTGCCACA	
Mus-Cer5	sense	CTTCTCCGTGAGGATGCTGT	113
	antisense	GTGTCATTGGGTTCCACCTT	
Mus-β-actin	sense	GTCCCTCACCCTCCCAAAAG	266
	antisense	GCTGCCTCAACACCTCAACCC	
Homo-Fxr	sense	AACCATACTCGCAATACAGCAA	185
	antisense	ACAGCTCATCCCCTTTGATCC	
Homo-Shp	sense	CCCCAAGGAATATGCCTGCC	104
	antisense	TAGGGCGAAAGAAGAGGTCCC	
Homo-Fgf15	sense	CCAGAAGACAGGCAGTAGT	135
	antisense	CTGGAGGGATTTGGGAAGG	
Homo-Spt1	sense	CTCCTCCCAGAGGAAGAACTGG	163
	antisense	TTGCTCTCTTTCAGGCCACT	
Homo-Spt2	sense	TCACCTCCTGTAGTGGAGCA	138
	antisense	GCCCATCTCTTTCAGGCGTC	
Homo-Spt3	sense	GGAGTTGGAGGACCTTGTGG	152
	antisense	GCACAAGCGATGTGTGGTTT	
Homo-Cer2	sense	TCTTGATGCCCTCCCCTTTG	110
	antisense	TCTAGATCGGCCCAGGTCAA	
Homo-Cer4	sense	TGCAGCTGCTCCGGGTA	253
	antisense	TGGACAGCATTCTCTGCTGG	
Homo-Cer5	sense	TGATTTTGCAAGTCTGAGATGGG	323
	antisense	ACAGGTCACCTTTCCCCTGA	
Homo-β-actin	sense	CTCCATCCTGGCCTCGCTGT	268
	antisense	GCTGTCACCTTCACCGTTCC	

Supplementary Figure Captions

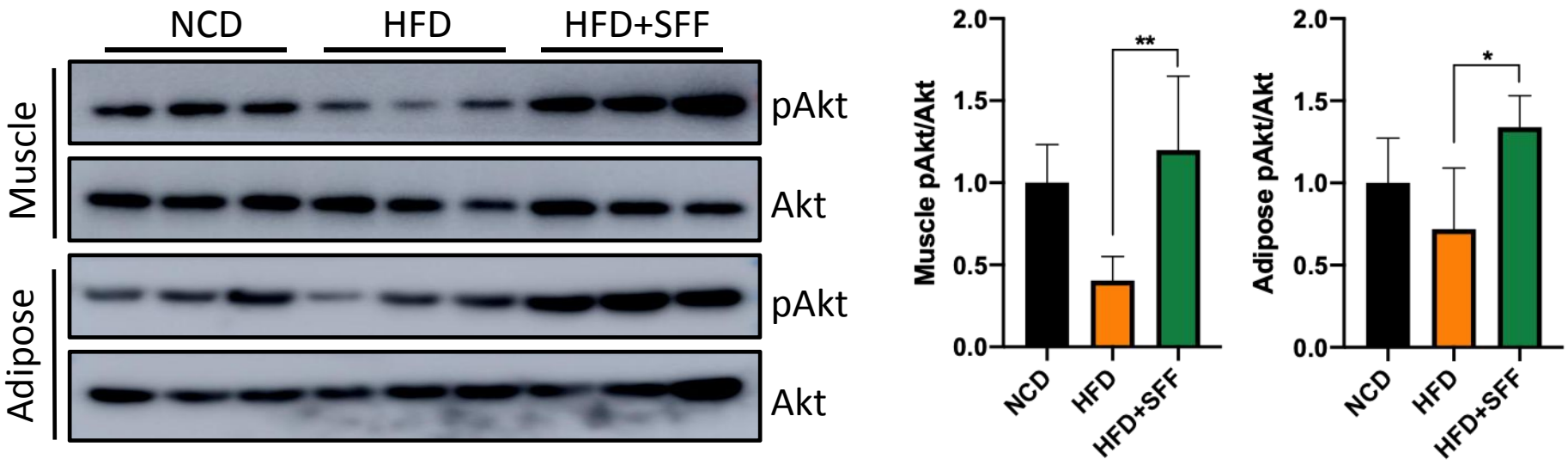
Supplementary figure 1. SFF alleviates the inhibition of insulin signaling in peripheral tissues of DOI mice. Western blot of Akt and phosphorylated Akt in muscle and adipose tissue; quantitative analysis of the protein level was performed by densitometry using ImageJ software. Data are expressed as mean ± SD. **P* < 0.05 and ***P* < 0.01 .

Supplementary figure 2. Analysis of differential metabolites. OPLS-DA score plot (a). Each data point represents one mouse sample, and the distance between two points in the plot indicates the similarity between the two samples. The number of up/down regulated metabolites caused by SFF treatment (b).

Supplementary figure 3. Ceramide positively correlates with metabolic disorders in DOI mice. Correlation analysis between ceramide and various parameters of metabolic disorders, including BMI, FBG, body weight and HOMA-IR.

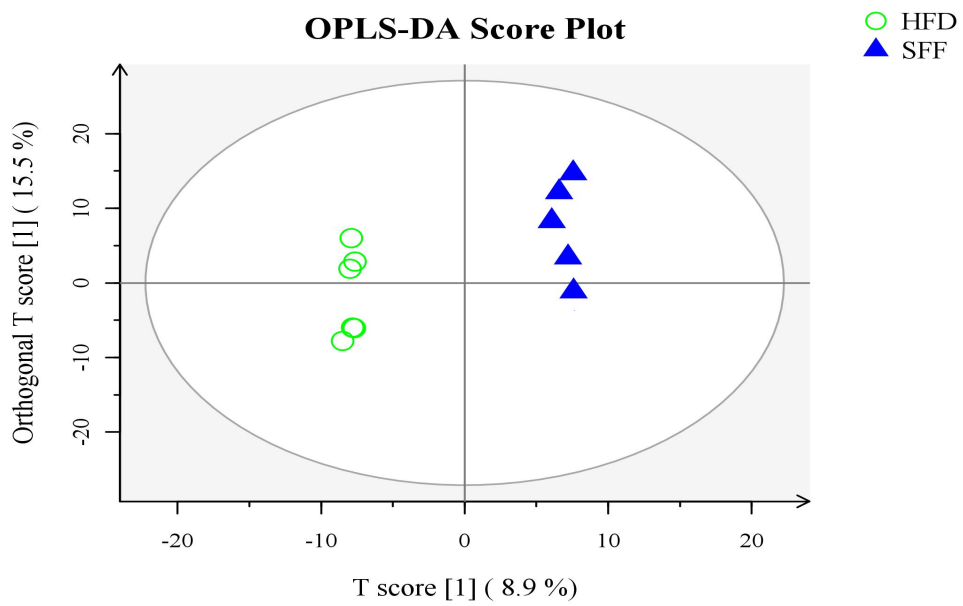
Supplementary figure 4. SFF is unable to alleviate hyperlipidaemia in pseudo-sterile mice. Total-CHO and TG in serum (a) and liver (b) are shown. Data are presented as mean ± SD. '*', '**' and '***' indicate statistical significance at the $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Supplementary Fig. 1



Supplementary Fig. 2

a



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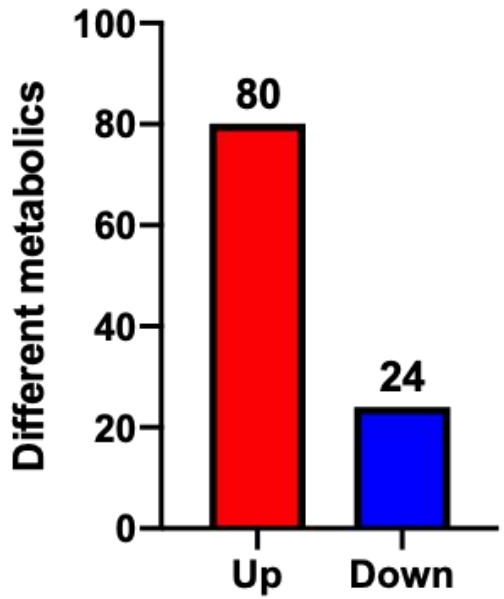
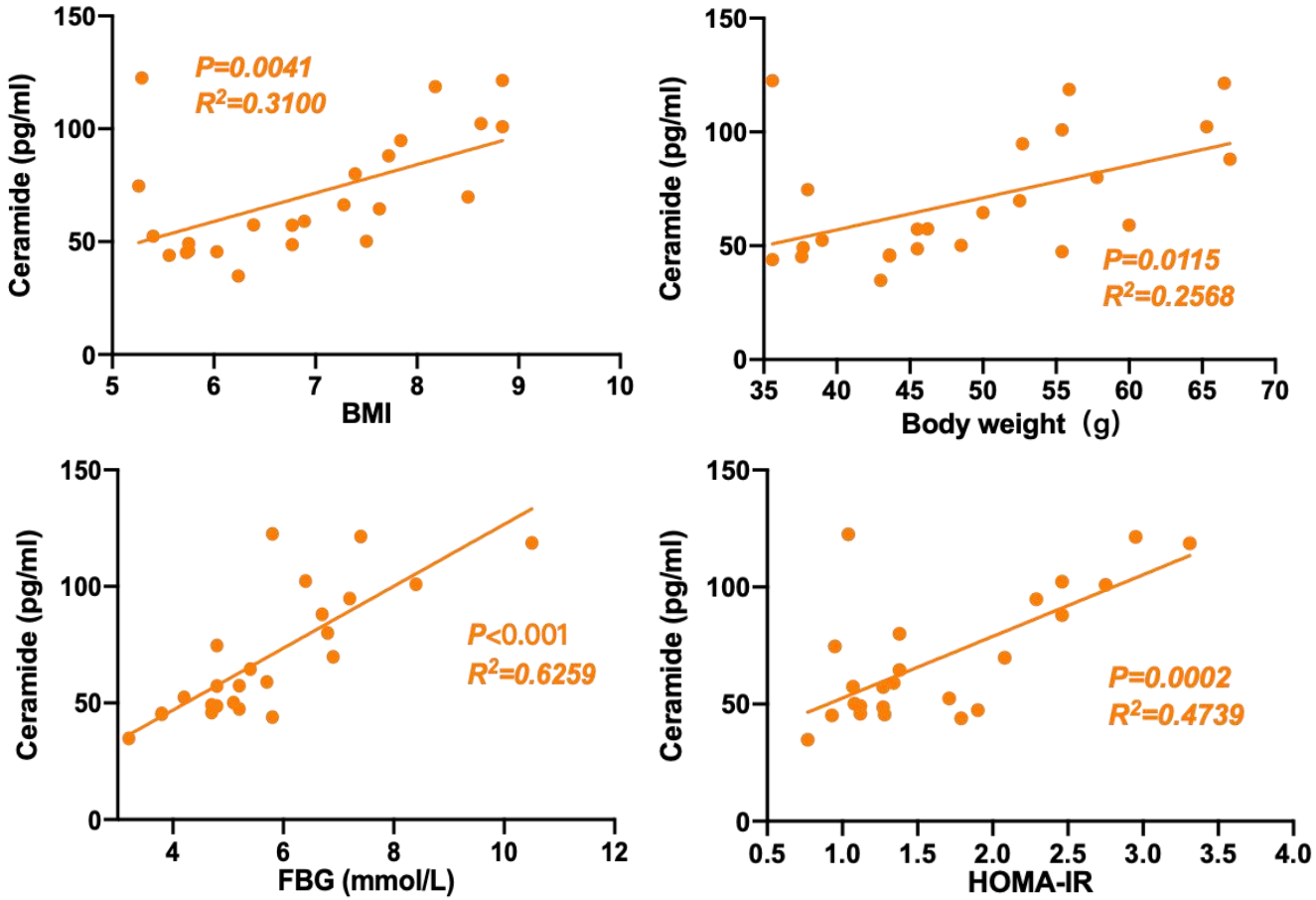
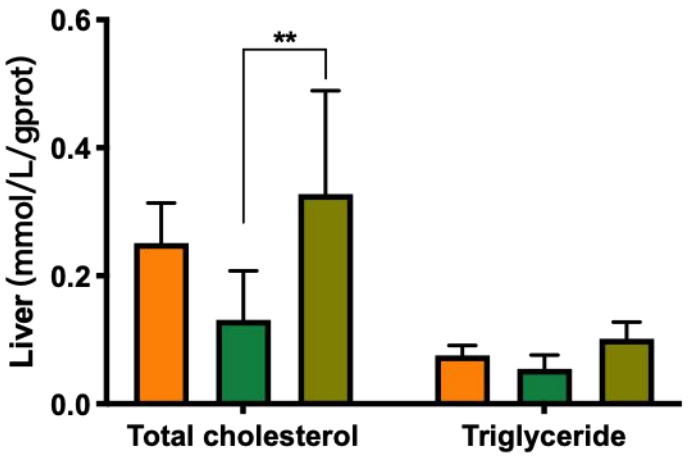


Supplementary Fig. 3



Supplementary Fig. 4

a



b