SUPPORTING INFORMATION

Hawk tea prevents high-fat diet-induced obesity in mice by activating the AMPK/ACC/SREBP1c signaling pathways and regulating the gut microbiota

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The composition and energy content for each diet are described in Table S1.

Table S1. Compositions of experimental diets

Ingredient (%)	**10% low-fat control diet (TP23302)**	**60% high-fat model diet (TP23300)**
Protein (Casein, Cystine)	194	276
Cornstarch	497	0
Dextrin, Sucrose	176	250
Soy oil (Added with TBHQ[1])	24	34
Lard	16	307
Cellulose	48	68
Mineral mixture, Vitamin mixture, Choline citrate	45	65
Total	1000	1000
Caloric density (Kcal/g)	3.6	5.1
Protein	19.4%	19.4%
Carbohydrate	70.6%	20.6%
Fat	10%	60%

Flavonoid Components and Contents of Hawk tea extract

Material

Hyperoside, Isoquercitrin and Astragalin were purchased from Chengdu Push Bio-technology Co., Ltd. (Sichuan, China) (Item Number: PS0453, PS0400 and PS1099, Separately). Quercetin was purchased from Sichuan Weikeqi Biological Technology Co., Ltd. (Sichuan, China) (Item Number: WKQ-0000265). Kaempferol was purchased from Chengdu Pufei De Biotech Co., Ltd. (Sichuan, China) (Item Number: JOT-10060).

Chromatographic grade methanol and acetonitrile were purchased from Alltech Scientific (Beijing, China).

Chromatographic conditions

The method is same as our previous article.[2] Flavonoids of Hawk tea extract were tested by HPLC (LC-20AD, Shimadzu) with a photodiode array detector (DAD; Shimadzu). A Hypersil ODS2 column (5μm, 4.6 mm×250 mm i.d., SinoChrom, Dalian, China) with the mobile phase, aqueous 0.1% formic acid solution (A) and acetonitrile (B) and a flow rate of 1 mL/min with a 10μL injection volume were used, and the column oven temperature was set to 35◦C. For better isolation, the gradient program was set as follows: 0–38 min, 11–20% B; 38–66 min, 20–40% B; 66–66.01 min, 40–11% B; and 66.01–70 min, 11% B. The detection wavelength was set to 350 nm.

For this operation, a chromatogram of Hawk tea extract (Figure S1) was obtained at 350 nm.

Method Validation

The relative standard deviation (RSD) of the repeatability (retention time, RT; and retention peak area, RPA) was 0.83% to 1.33% among the five peaks. The RSD of the precision was no more than 2.00%, and the stability was less than 1.50%. The limits of detection (LOD) and limits of quantification (LOQ) of the five analytes, and the calibration curves are shown below in Table S2.

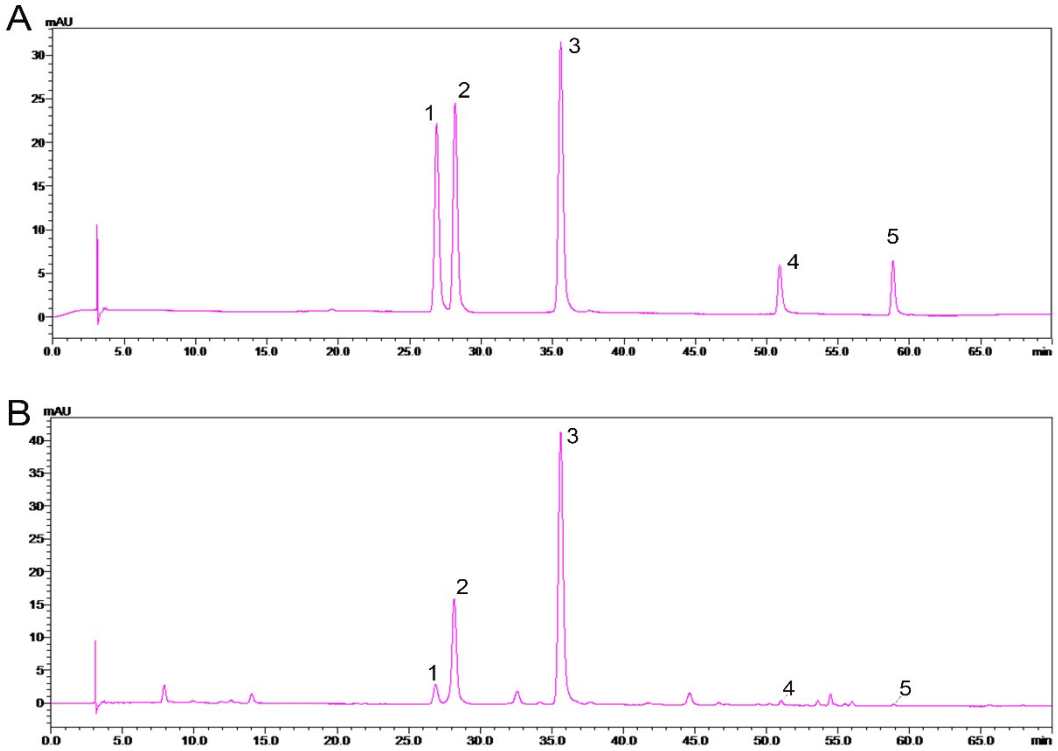


Figure S1. HPLC chromatogram of five flavonoids in HTE. A) Chromatogram of standards, B) Chromatogram of HTE. 1. Hyperoside, 2. Isoquercitrin, 3. Astragalin, 4. Quercetin, 5. Kaempferol

Table S2. Calibration curve, LOD and LOQ, and linear range for five standards.

Flavonoids	Linear	R^2	LODs (μg/mL)	LOQs (μg/mL)	Linear Range (μg/mL)
Hyperoside	Y = 19143X - 2764	1	0.22	0.67	0.78-100.20
Isoquercitrin	Y = 24618X - 4438	1	0.29	0.89	1.35-174.00
Astragalin	y = 22263x - 3659.7	1	0.46	1.41	2.04-260.80
Quercetin	y = 30776x - 5591.6	0.9997	0.23	0.71	1.00-32.00
Kaempferol	y = 36009x - 4063.7	0.9998	0.10	0.29	0.83-106.24

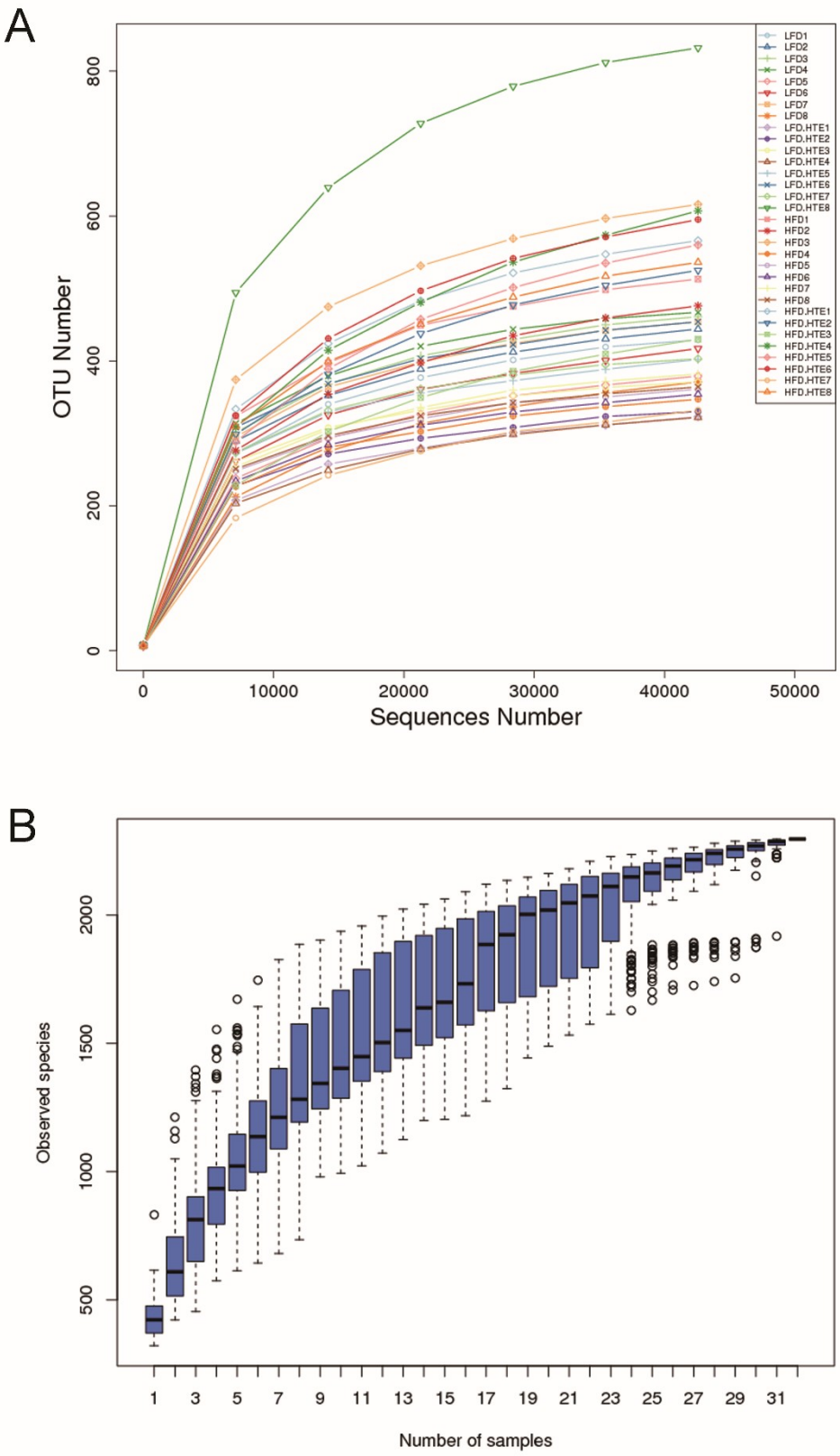


Figure S2. Effect of HTE on gut microbiota of mice with high-fat diet. A) Rarefaction Curve. B) Species accumulation boxplot.

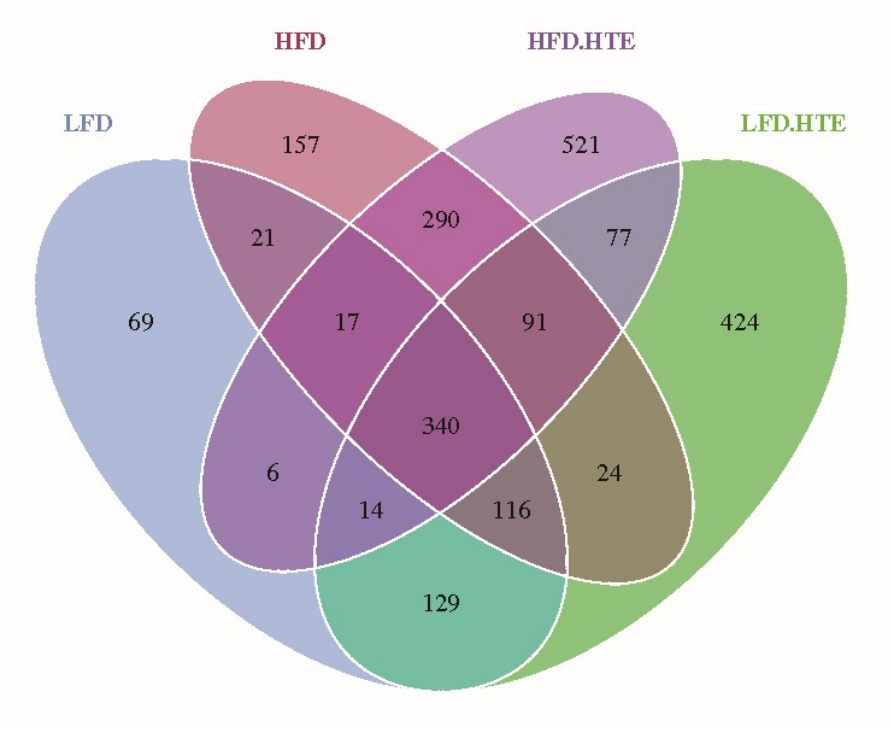


Figure S3. Venn diagram analysis was carried out to better understand the bacterial diversity across all treatment groups

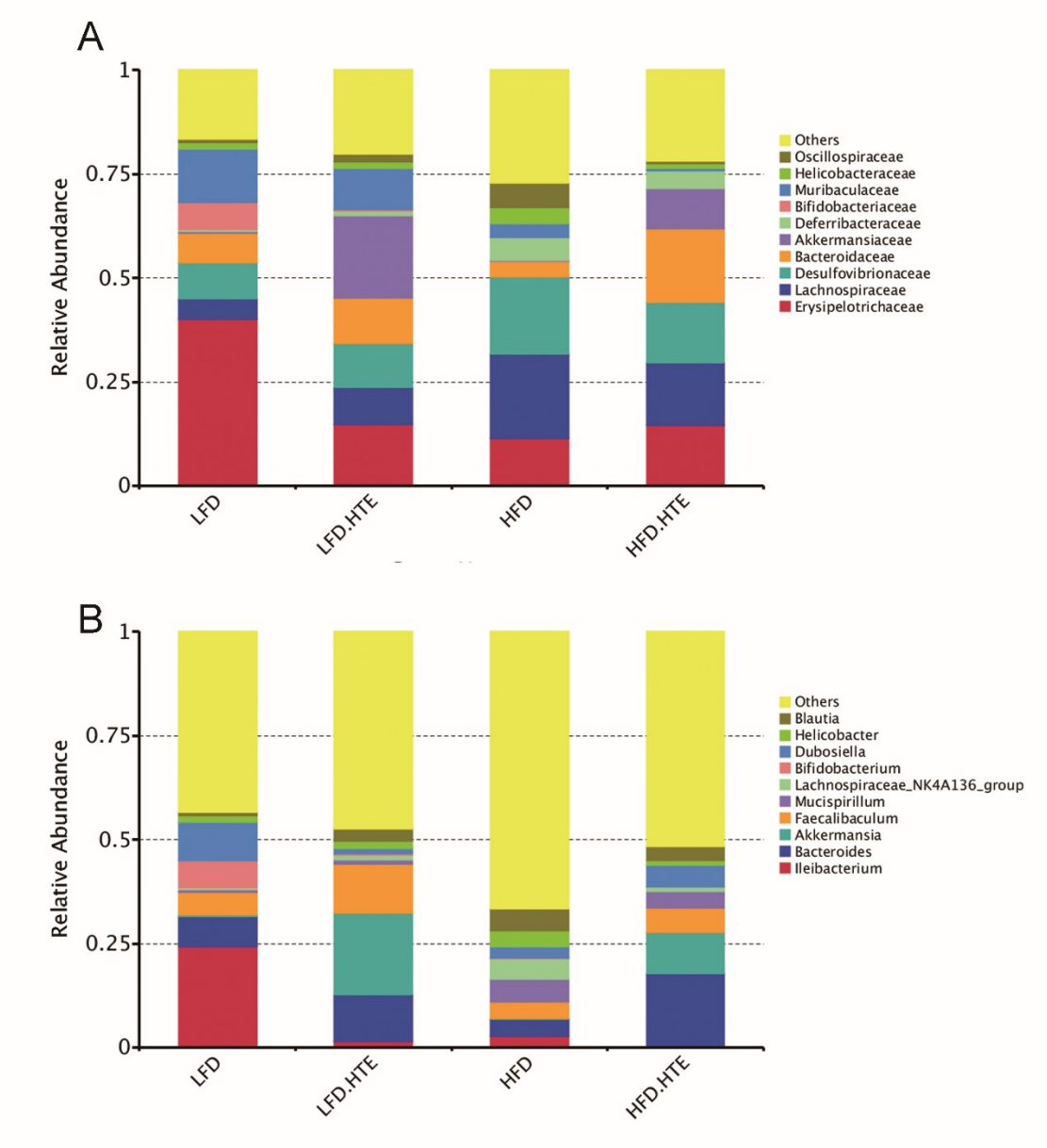


Figure S4. The effect of HTE on the gut microbiota of mice receiving a high-fat diet. A) Relative abundance at the family level. B) Relative abundance at the genus level.

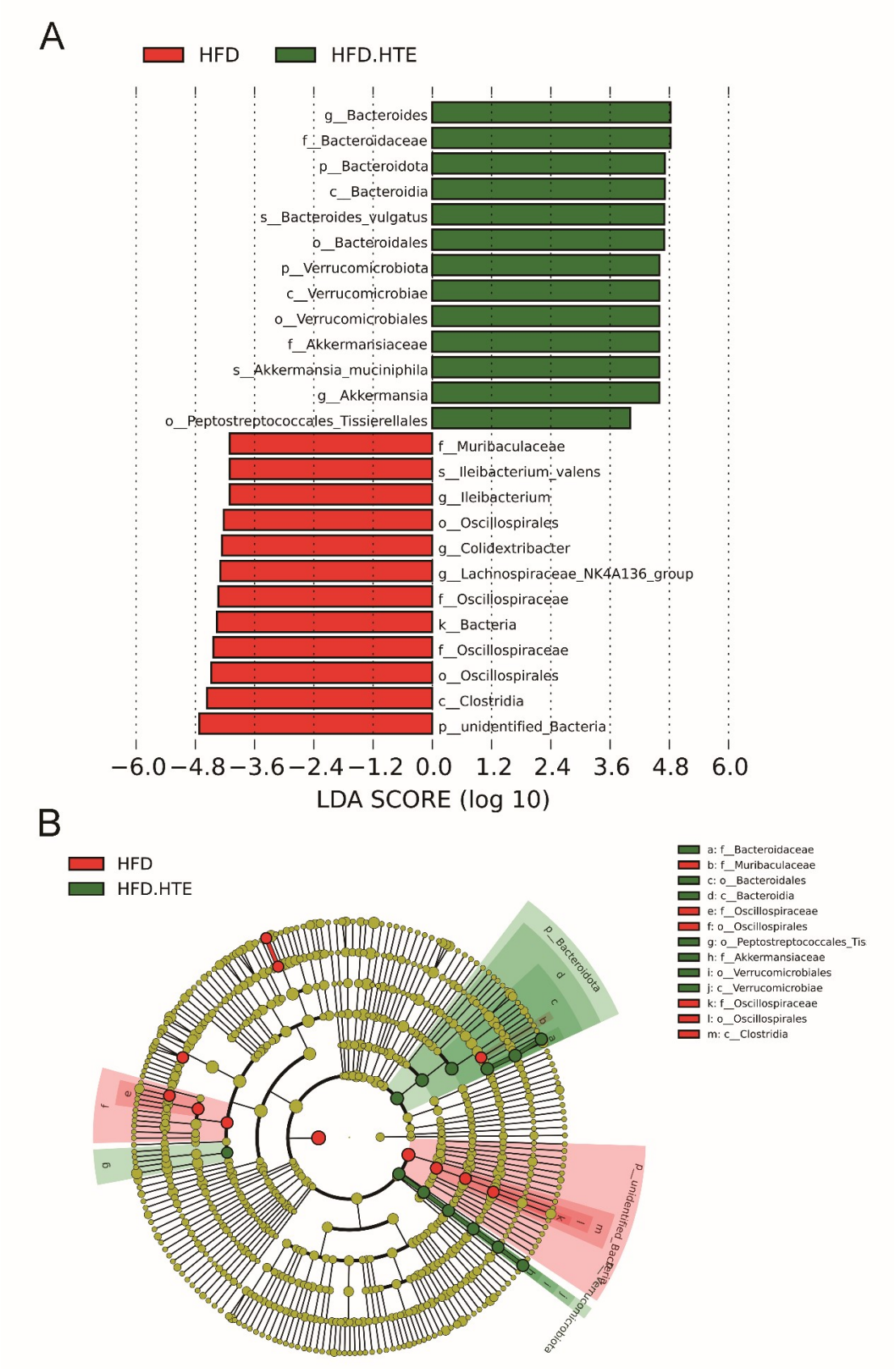


Figure S5. The effect of HTE on the gut microbiota of mice receiving a high-fat diet. A) Value distribution histogram (LDA score > 4). B) Evolutionary branching diagram.

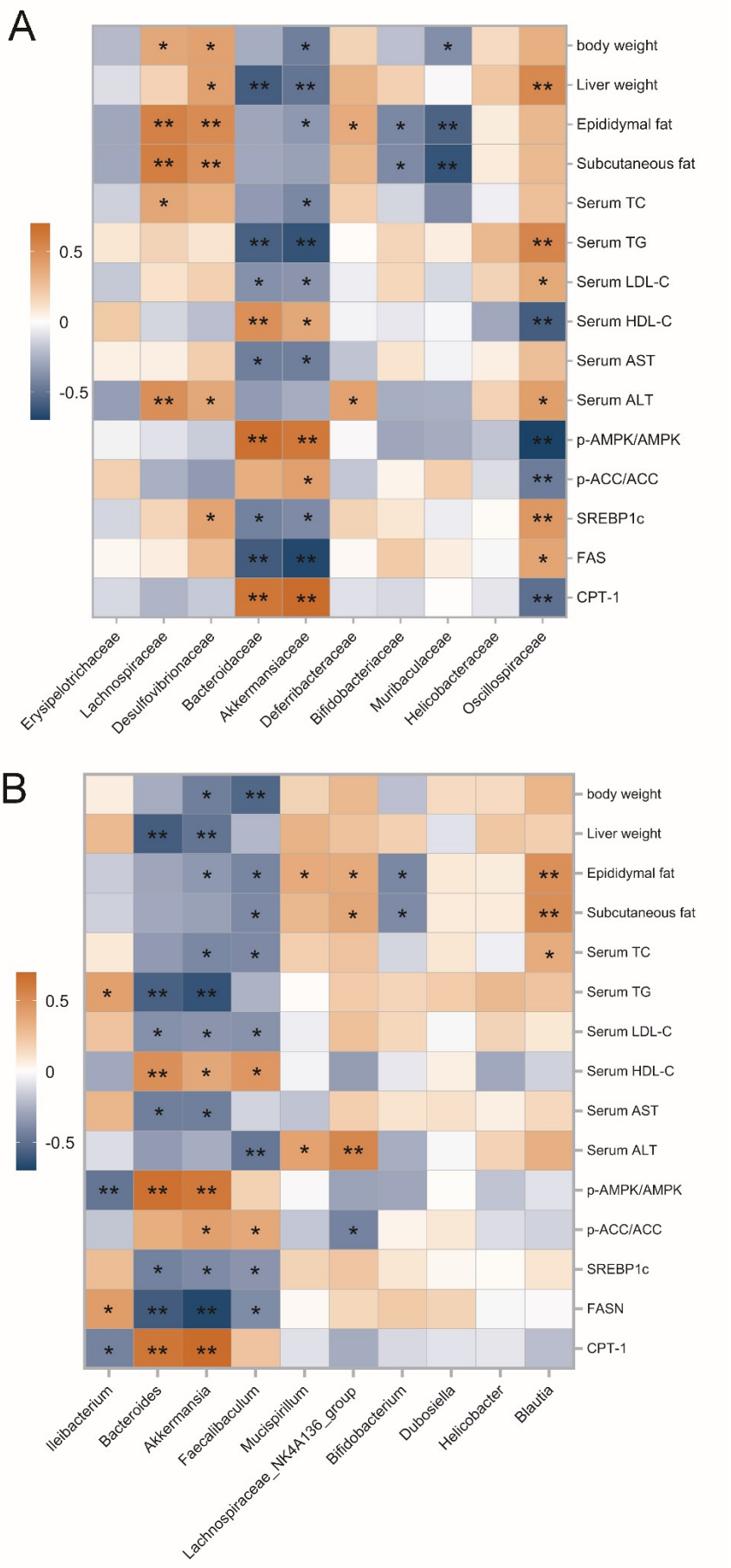
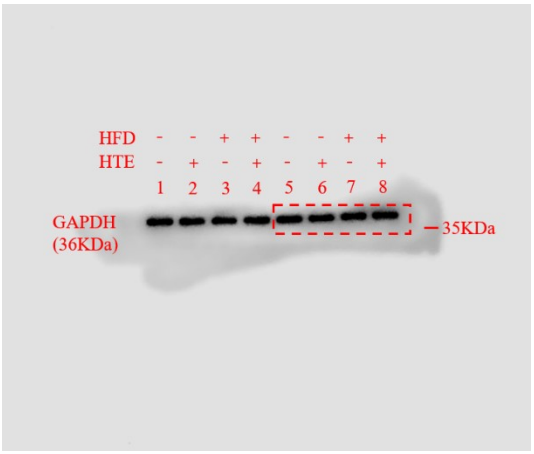
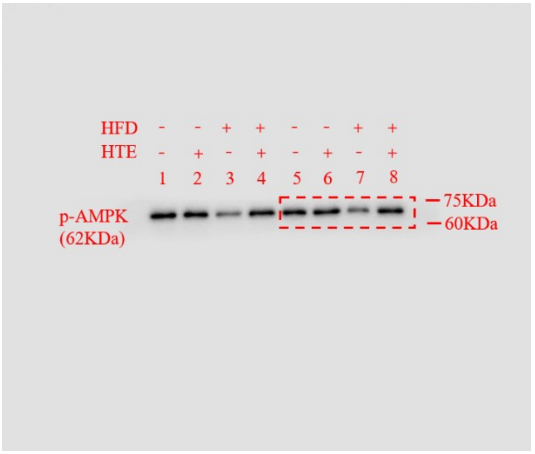
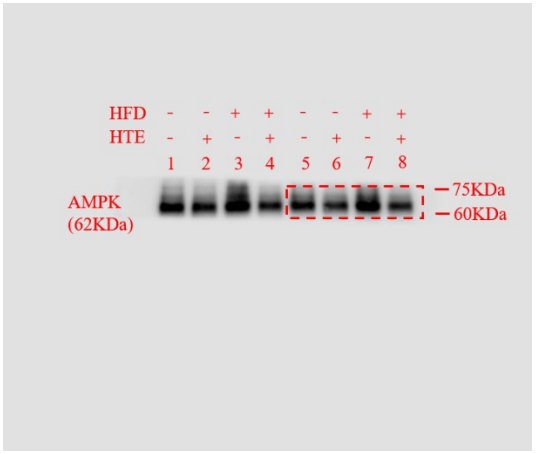

A
body weight
Liver weight
Epididymal fat
Subcutaneous fat
Serum TC
Serum TG
Serum LDL-C
Serum HDL-C
Serum AST
Serum ALT
p-AMPK/AMPK
p-ACC/ACC
SREBP1c
FAS
CPT-1
0.5
0
-0.5
Erysipelotrichaceae
Lachnospiraceae
Desulfovibrionaceae
Bacteroidaceae
Akkermansiaceae
Deferribacteraceae
Bifidobacteriaceae
Muribaculaceae
Helicobacteraceae
Oscillospiraceae
B
body weight
Liver weight
Epididymal fat
Subcutaneous fat
Serum TC
Serum TG
Serum LDL-C
Serum HDL-C
Serum AST
Serum ALT
p-AMPK/AMPK
p-ACC/ACC
SREBP1c
FASN
CPT-1
0.5
0
-0.5
Ileibacterium
Bacteroides
Akkermansia
Faecalibaculum
Mucispirillum
Lachnospiraceae_NK4A136_group
Bifidobacterium
Dubosiella
Helicobacter
Blautia

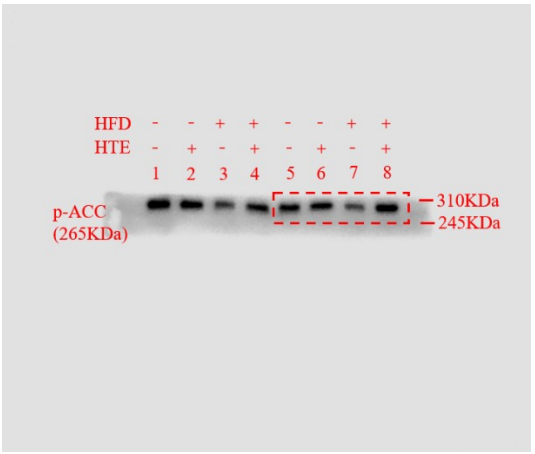
Figure S6. Correlation between the obesity-related indicators and gut microbiota. Correlation between the gut microbiota and biochemical indexes, AMPK/ACC/SREBP1c Signaling pathway protein expression at the family level A), genus level B) (n=8). Significant correlations are indicated by * $p < 0.05$ and ** $p < 0.01$.

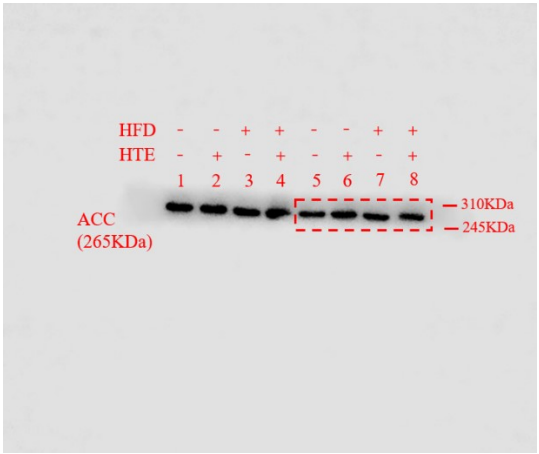
Raw images of western blot in Figure 3A



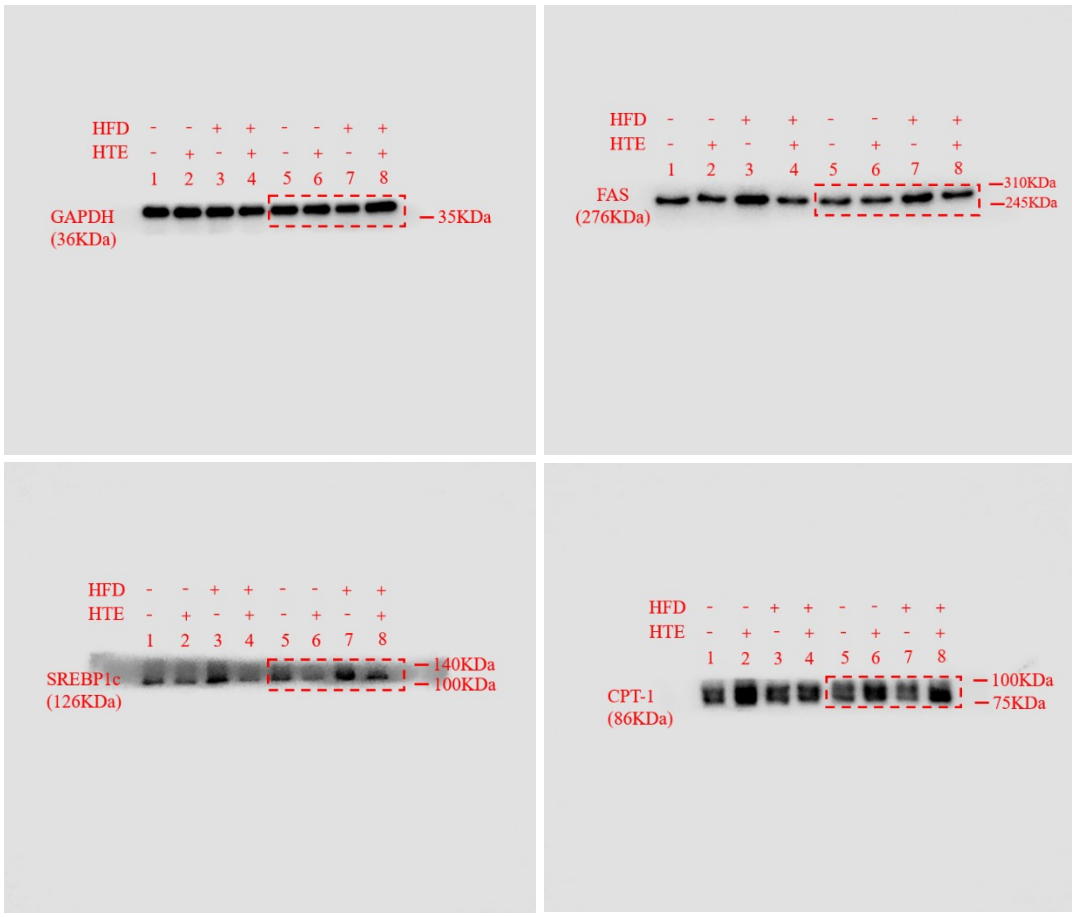








Raw images of western blot in Figure 3B



References

1 P. G. Reeves, F. H. Nielsen and G. C. Fahey, Jr., AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet, *J Nutr*, 1993, **123**, 1939-1951.

2 Z. Chen, D. Zhang, J. J. Guo, W. Tao, R. X. Gong, L. Yao, X. L. Zhang and W. G. Cao, Active Components, Antioxidant, Inhibition on Metabolic Syndrome Related Enzymes, and Monthly Variations in Mature Leaf Hawk Tea, *Molecules*, 2019, **24**.