Supplementary Materials

Calorie Restriction on Normal Body Weight Mice Prevents Body Weight Regain on a Follow-up High-fat Diet by Shaping an Obesity-resistant-like Gut Microbiota Profile

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The following are available online at www.mdpi.com/xxx/s1, Figure S1：^{1}H-NMR chemical shift attribution spectra of cecal contents in the (A) 5–10 ppm, (B) 3–5 ppm, and (C) 0–3 ppm. 1:1,3-Dihydroxyacetone, 2: Acetate, 3: Acetone, 4: Adenine, 5: Alanine, 6: Aspartate, 7: Betaine, 8: Butyrate, 9: Cholate, 10: Choline, 11: Creatine, 12: Cytidine, 13: Ethanol, 14: Formate, 15: Fumarate, 16: Glucose, 17: Glutamate, 18: Glutamine, 19: Glycine, 20: Hypoxanthine, 21: Isobutyrate, 22: Isoleucine, 23: Isovalerate, 24: Lactate, 25: Leucine, 26: Lysine, 27: Methanol, 28: Methionine, 29: Methylamine, 30: Nicotinate, 31: Phenylacetate, 32: Phenylalanine, 33: Proline, 34: Propionate, 35: Ribose, 36: Sarcosine, 37: Succinate, 38: Taurine, 39: Threonine, 40: Tryptophan, 41: Tyrosine, 42: Uracil, 43: Uridine, 44: Valerate, 45: Valine, 46: Xylose, 47: myo-Inositol, and 48: β-Alanine. Figure S2: Linear correlation between (A) body weight and succinate, (B) visceral fat mass and succinate, (C) body weight and fumarate, as well as (D) visceral fat mass and fumarate. Figure S3: Change in AUC with varying number of variables in the random forest model. The model with the highest AUC contained seven families. Figure S4: Order-level network diagram of the correlation. Positive correlations are in red, negative correlations are in green.

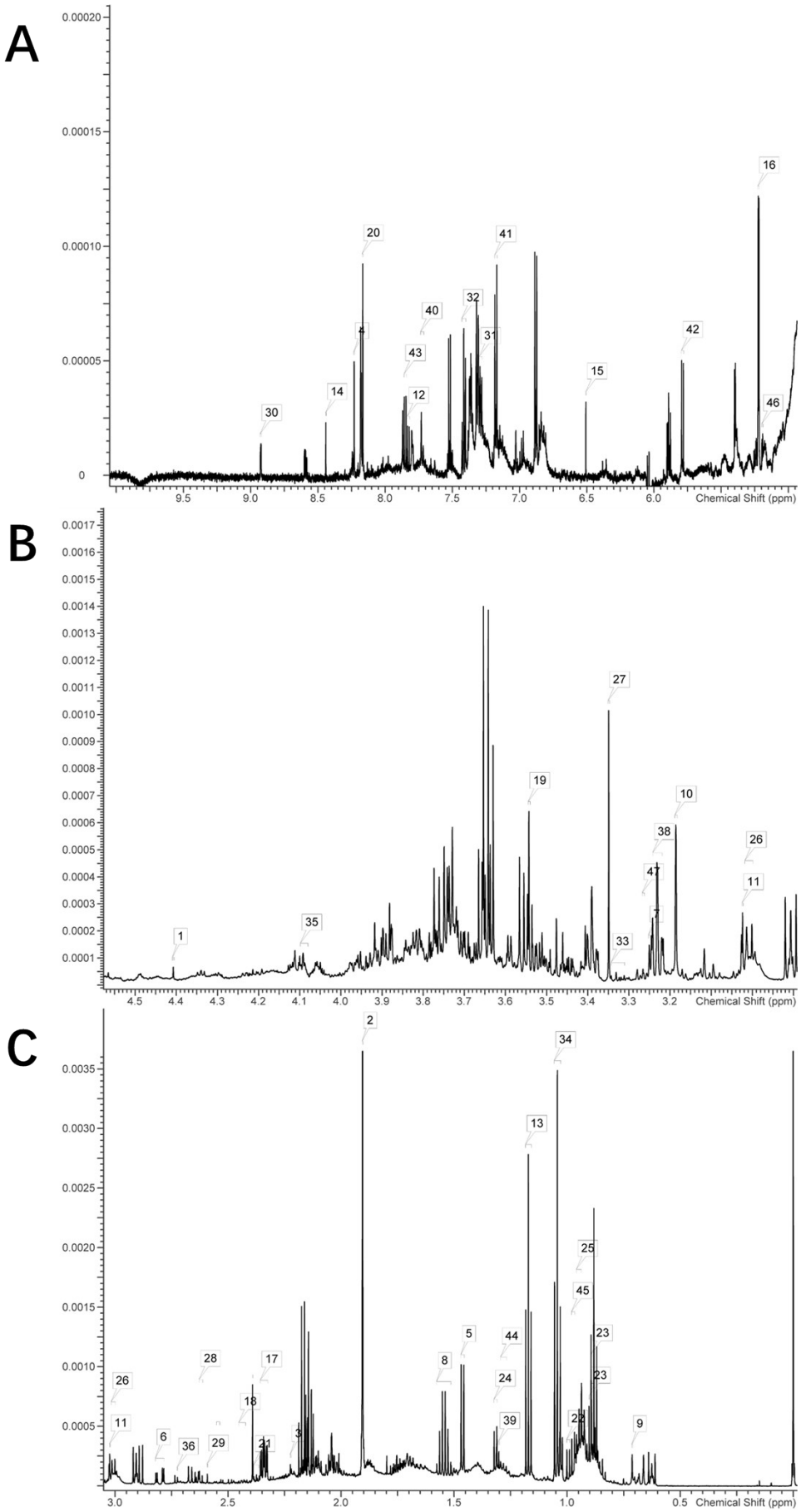
A
B
C
Chemical Shift (ppm)
Chemical Shift (ppm)
Chemical Shift (ppm)

Fig. S1 ^{1}H-NMR chemical shift attribution spectra of cecal contents in the (A) 5–10 ppm, (B) 3–5 ppm, and (C) 0–3 ppm. 1:1,3-Dihydroxyacetone, 2: Acetate, 3: Acetone, 4: Adenine, 5: Alanine, 6: Aspartate, 7: Betaine, 8: Butyrate, 9: Cholate, 10: Choline, 11: Creatine, 12: Cytidine, 13: Ethanol, 14: Formate, 15: Fumarate, 16: Glucose, 17: Glutamate, 18: Glutamine, 19: Glycine, 20: Hypoxanthine, 21: Isobutyrate, 22: Isoleucine, 23: Isovalerate, 24: Lactate, 25: Leucine, 26: Lysine, 27: Methanol, 28: Methionine, 29: Methylamine, 30: Nicotinate, 31: Phenylacetate, 32: Phenylalanine, 33: Proline, 34: Propionate, 35: Ribose, 36: Sarcosine, 37: Succinate, 38: Taurine, 39: Threonine, 40: Tryptophan, 41: Tyrosine, 42: Uracil, 43: Uridine, 44: Valerate, 45: Valine, 46: Xylose, 47: myo-Inositol, and 48: β-Alanine.

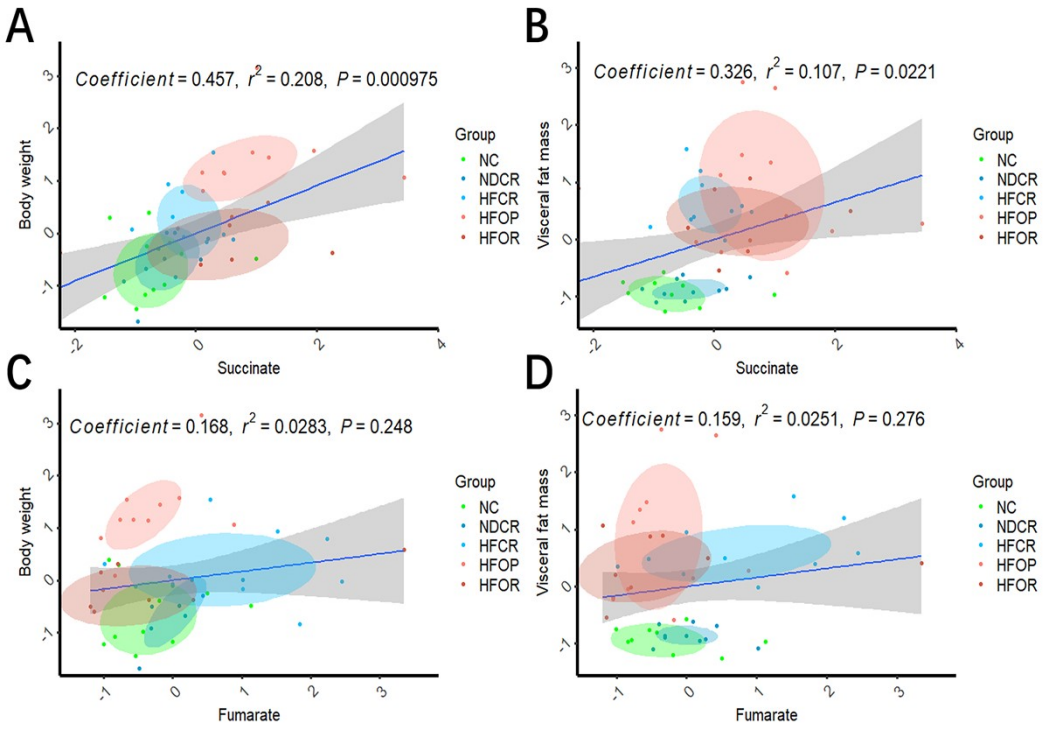

Fig. S2 Linear correlation between (A) body weight and succinate, (B) visceral fat mass and succinate, (C) body weight and fumarate, as well as (D) visceral fat mass and fumarate.

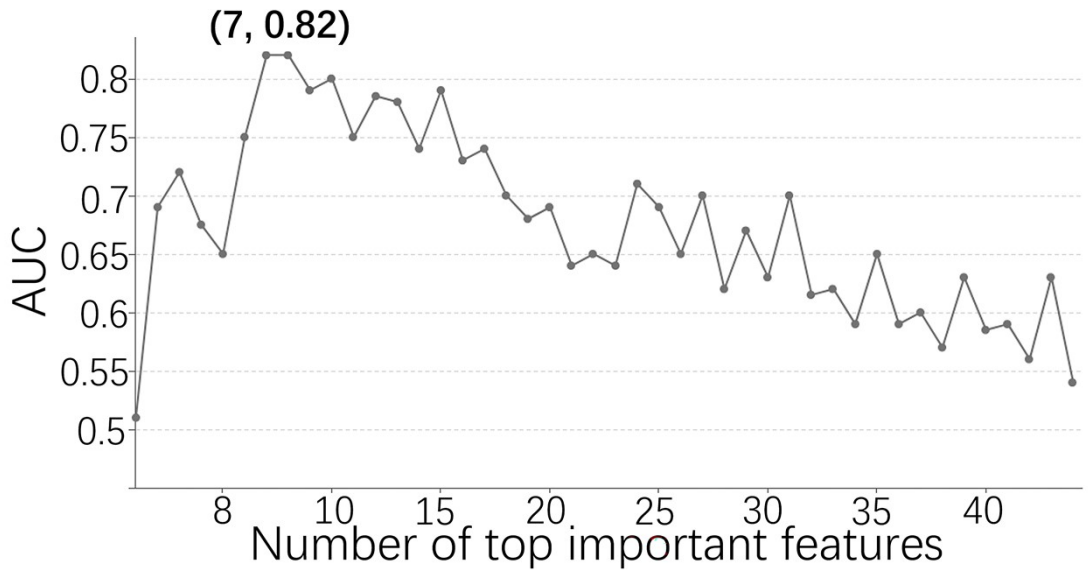

Fig. S3 Change in AUC with varying number of variables in the random forest model. The model with the highest AUC contained seven families.

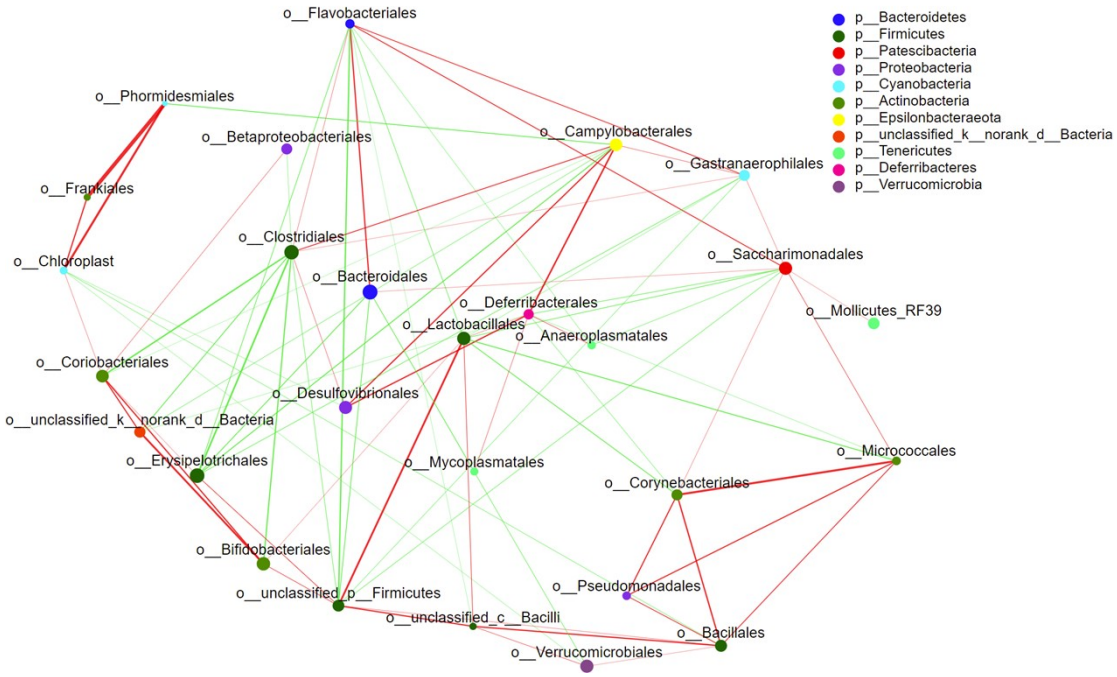

Fig. S4 Order-level network diagram of the correlation. Positive correlations are in red, negative correlations are in green.

Table S1. Composition of the experimental diet (g/kg diet)

Ingredien	Normal Diet	High-Fat Diet
Casein	210	257
L-cystine	3	4
Cornstarch	402	261
Dextrinized cornstarch	132	75
Sucrose	100	54
Soybean oil	55	55
Lard	0	180
Mixed minerals	35	42
Mixed vitamins	10	12
Cellulose	50	57
Choline	3	3
Total	1000	1000
kcal/g diet	3.89	4.72
Calorie from carbohydrate (%)	65.3	33.2
Caloriel from protein (%)	22.0	22.0
Calorie from fat (%)	12.7	44.8

Supplementary Methods

Microbial DNA extraction from stool samples

200 µl stool sample, 300 µl E.Z.N.A. Kit SLX buffer, 100 µL HTR Reagent, and 500 mg glass beads were added to a screw-capped Eppendorf tube and disrupted in a bead beater at room temperature for two minutes. Following subsequent incubation at 70°C for 10 minutes and centrifugation at 3,000 rpm for three minutes at room temperature, 270 µL P2 Buffer was

added to each sample and incubated on ice for five minutes. Samples were then centrifuged at 14,000 × g for five minutes at 4 °C. The supernatant was transferred into microcentrifuge tubes, each containing 0.7x volumes of isopropanol. Following thorough mixing, samples were incubated at room temperature for two minutes and centrifuged at 14,000 × g. The supernatant was aspirated and discarded and the tube was inverted on an absorbent paper for one minute to drain the liquid. Then, 200 µL elution buffer was added and the samples were incubated at 70°C for 10-20 minutes to dissolve the DNA pellet. The remainder of the DNA extraction protocol was carried out in HiBind® DNA Mini Columns.

Cecal content sample preparation

The cecal content samples (50 - 60 mg) were mixed with 600 µL precooled phosphate buffer. After vortex mixing for about 30 s, the mixed slurry was subjected to freeze thaw treatments (three times) and homogenization with a TissueLyser (QIAGEN, Hilden, Germany) at 20 Hz. Following centrifugation (11180g, 4 ℃) for 10 min, the supernatants (550 µL) were transferred into 5 mm NMR tubes directly for NMR analysis.