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Supplemental Information

Celastrol inhibits intestinal lipid absorption by reprofiling the gut microbiota to attenuate high-fat diet-induced obesity

Hu Hua, Yue Zhang, Fei Zhao, Ke Chen, Tong Wu, Qianqi Liu, Songming Huang, Aihua Zhang, and Zhanjun Jia

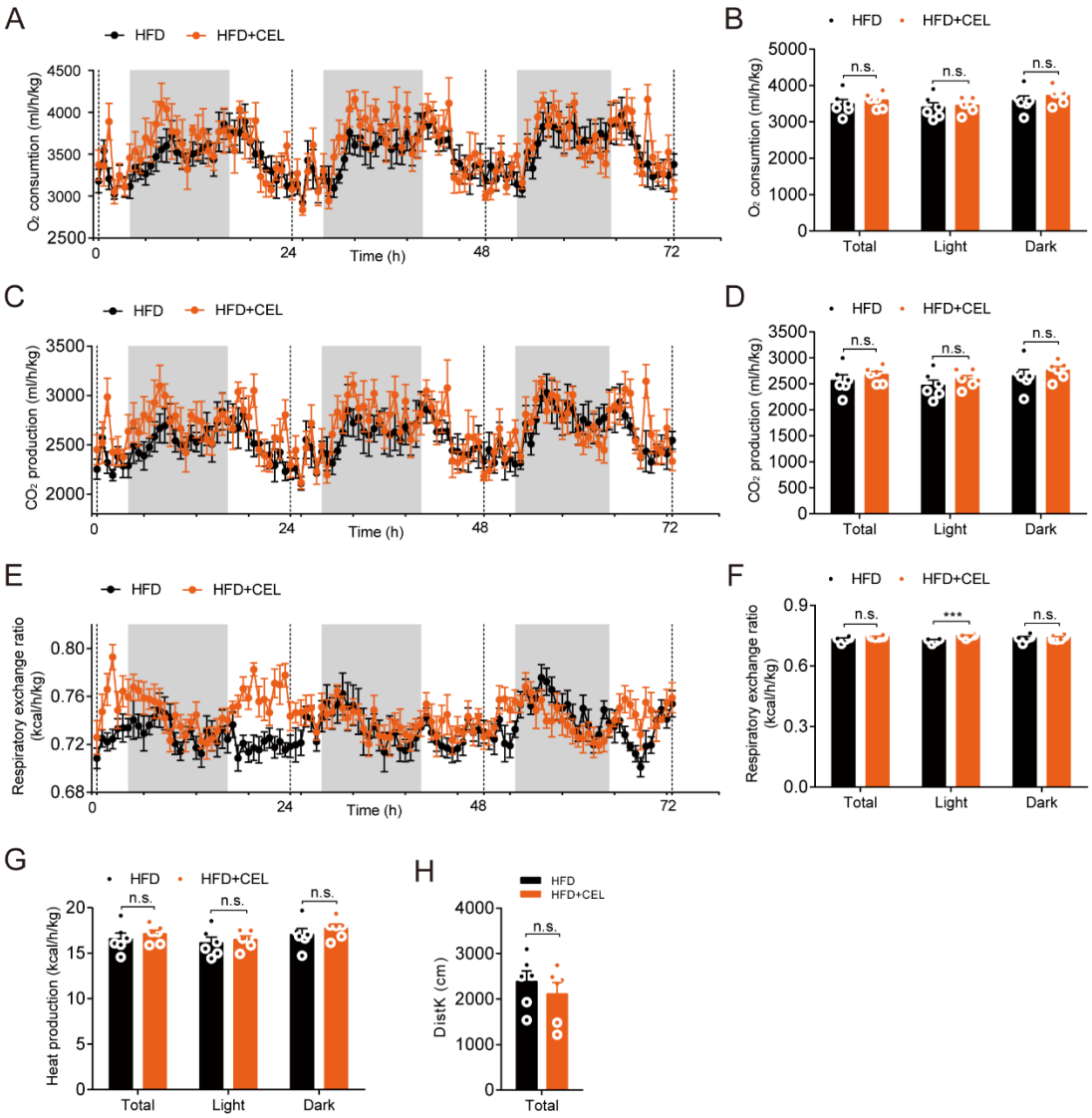

Figure S1. Celastrol does not increase the energy expenditure of DIO C57BL/6 mice, Related to Figure 1.

(A-H) The energy expenditure and home-cage activity of DIO C57BL/6 mice were examined for 3 days using the TSE system (n = 6 for each group). (A and B) Oxygen (O_2) consumption, (C and D) carbon dioxide (CO_2) production, (E and F) respiratory exchange rate, (G) heat production, (H) cumulative moving distance (DistK). The gray parts of figures A, C and E represent the dark phase, and the rest represent the light phase. Error bars represent the mean ± SEM. p values were determined by Student's t test. ***p < 0.001; n.s., not significant (p > 0.05).

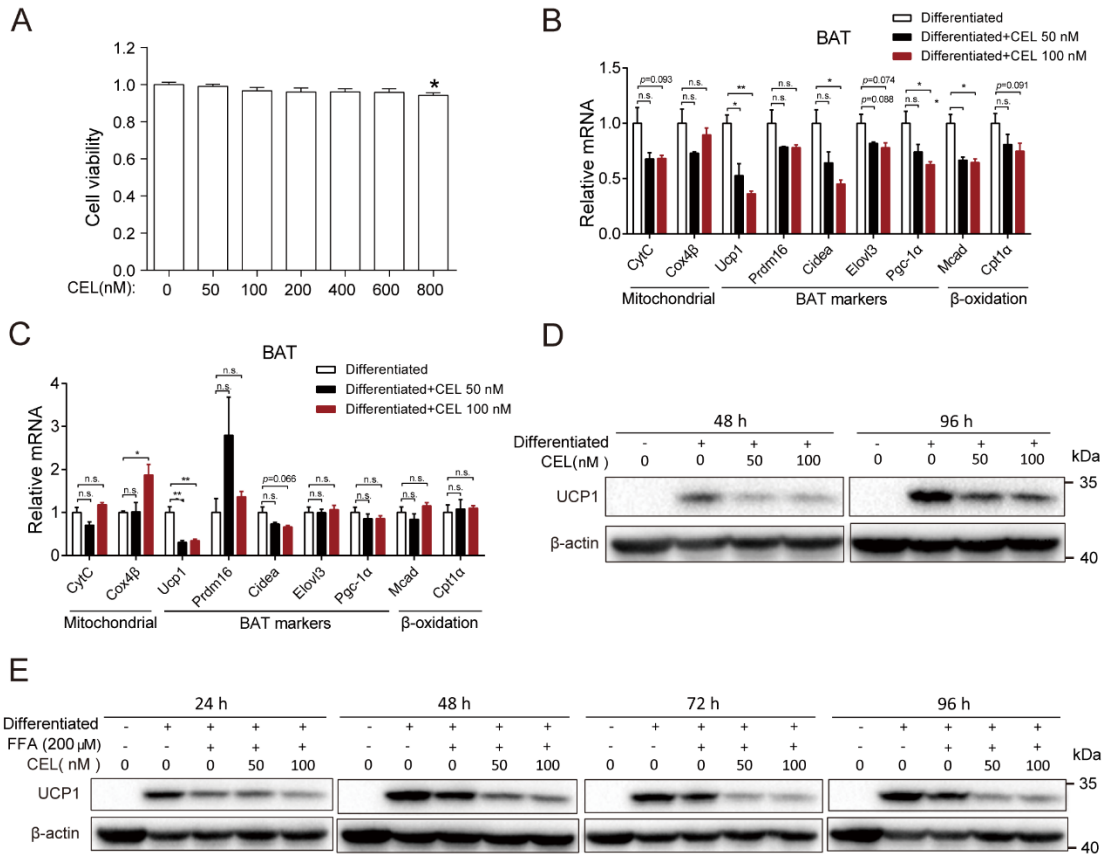

Figure S2. Celastrol does not enhance the function of brown fat in vitro, Related to Figure 1.

(A) The viability of primary brown adipose precursor cells was analyzed by the CCK-8 assay after treatment with celastrol for 24 h at concentrations ranging from 50 nM to 800 nM (n = 6 for each group). (B and C) Gene expression analysis of mitochondrial and brown fat gene programs in differentiated primary brown fat cells treated with celastrol for (B) 48 h and (C) 96 h (n = 3 for each group). (D) Representative western blot of UCP1 in differentiated primary brown fat cells treated with celastrol for 48 h and 96 h. (E) Western blots of UCP1 in differentiated primary brown fat cells exposed to FFA (200 μM) and treated with celastrol for 24, 48, 72 and 96 h. Error bars represent the mean ± SEM. p values were determined by Student's t test. *p < 0.05, **p < 0.01; n.s., not significant (p > 0.05).

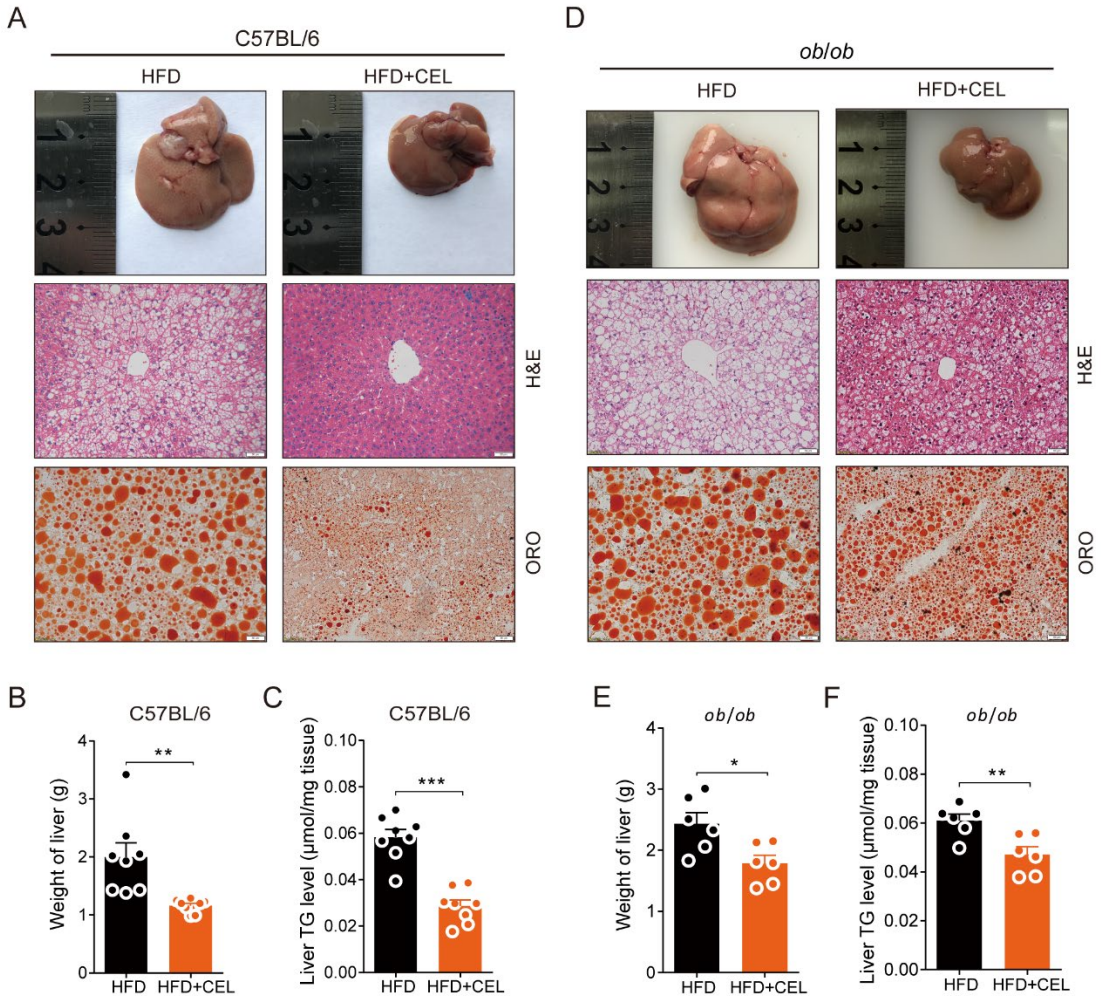

Figure S3. Celastrol alleviates hepatic steatosis in DIO C57BL/6 mice or *ob*/*ob* mice fed a HFD, Related to Figure 1 and 4.

(A-C) DIO C57BL/6 mice were subjected to oral administration of celastrol (3 mg/kg/day) for 3 weeks. (A) The representative gross appearance, representative H&E-stained images (200×) and representative Oil red O-stained images (200×) of liver from DIO C57BL/6 mice treated with celastrol or not. Scale bar, 50 μm. (B) The liver weight and (C) liver TG levels of DIO C57BL/6 mice treated with celastrol or not (n = 8 for each group). (D-F) HFD-fed obese *ob/ob* mice were subjected to oral administration of celastrol for 6 weeks. (D) The representative gross appearance, representative H&E-stained images (200×) and representative Oil red O-stained images (200×) of liver from HFD-fed obese *ob/ob* mice treated with celastrol or not. Scale bar, 50 μm. (E) The liver weight and (F) liver TG levels of HFD-fed obese *ob/ob* mice treated with celastrol or not (n = 6 for each group). Error bars represent the mean ± SEM. p values were determined by Student's t test. *p < 0.05, **p < 0.01, ***p < 0.001.

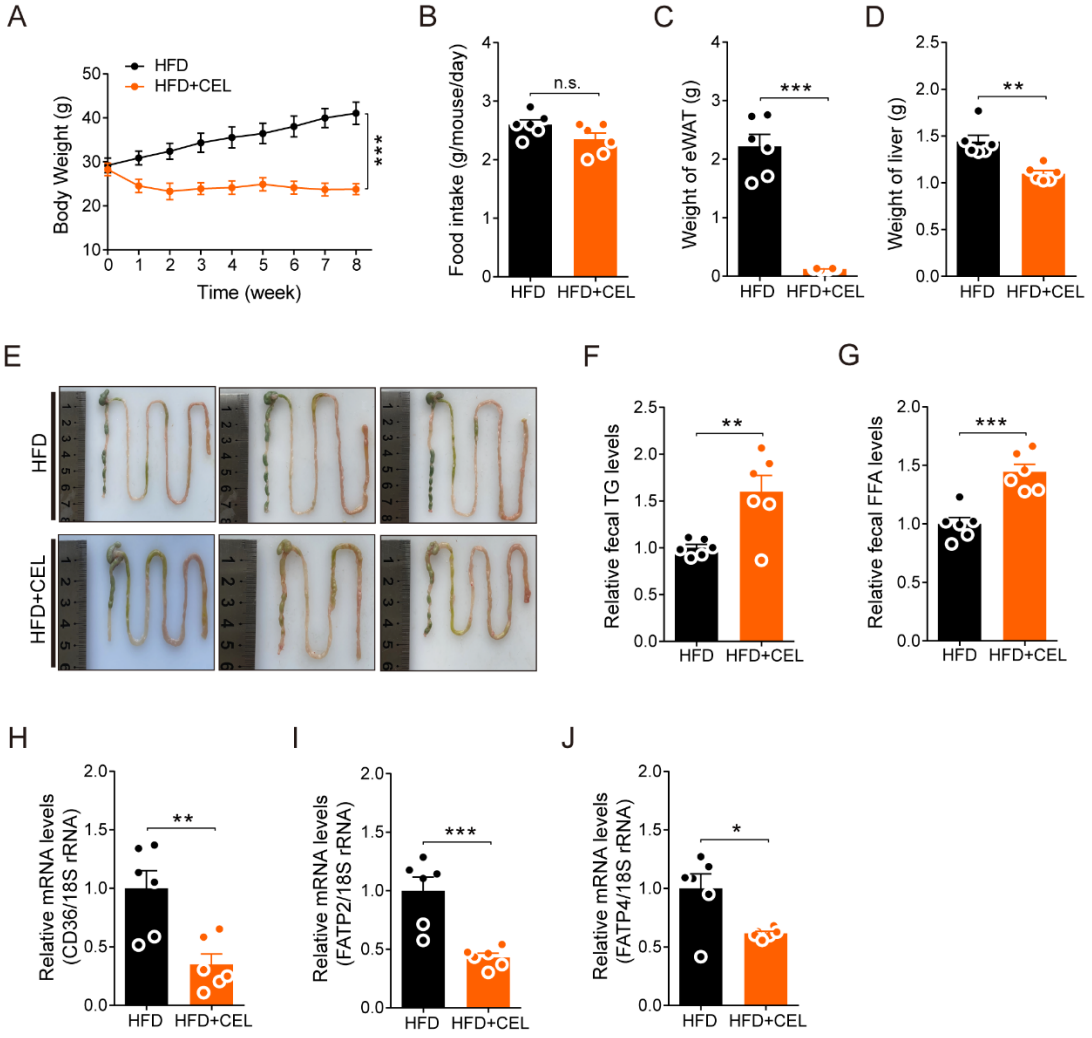

Figure S4. Celastrol treatment by intraperitoneal injection inhibits body weight gain and intestinal lipid transporter expression in mice fed a HFD, Related to Figure 1 and Figure 2.

(A-J) HFD-fed C57BL/6 mice were daily treated with vehicle or celastrol (100 μg/kg) intraperitoneally (i.p.) for 8 weeks. (A) The BW of each group of mice during the treatment. (B) The average 3-day food intake of each group of mice during the first week of treatment. (C-D) Weights of (C) eWAT and (D) liver of each group of mice. (E) Representative pictures of isolated intestines of mice in each group. (F-G) The relative fecal (F) TG and (G) FFA levels in each group of mice. (H-J) mRNA levels of intestinal lipid transport-related genes of mice treated with celastrol or not. n=6 for each group. Error bars represent the mean ± SEM. p values were determined by two-way ANOVA (A) or Student's t test. *p < 0.05, **p < 0.01, ***p < 0.001; n.s., not significant (p > 0.05).

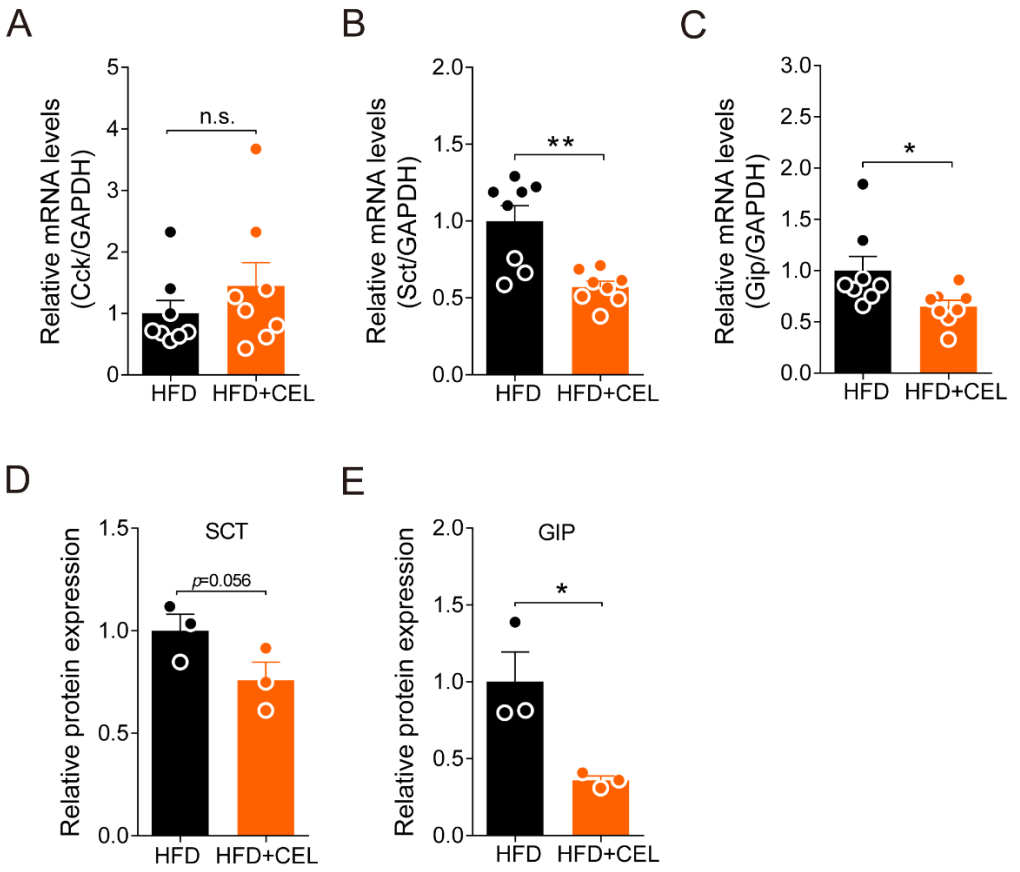

Figure S5. Effect of celastrol treatment on the expression of enteroendocrine related genes in HFD mice, Related to Figure 3.

(A-C) mRNA levels of intestinal enteroendocrine related genes in HFD mice treated with or without celastrol (n = 8 for each group). (D-E) The relative intestinal protein levels of Sct and Gip analyzed by proteomics (n = 3 for each group). Error bars represent the mean ± SEM. p values were determined by Student's t test. *p < 0.05, **p < 0.01; n.s., not significant (p > 0.05).

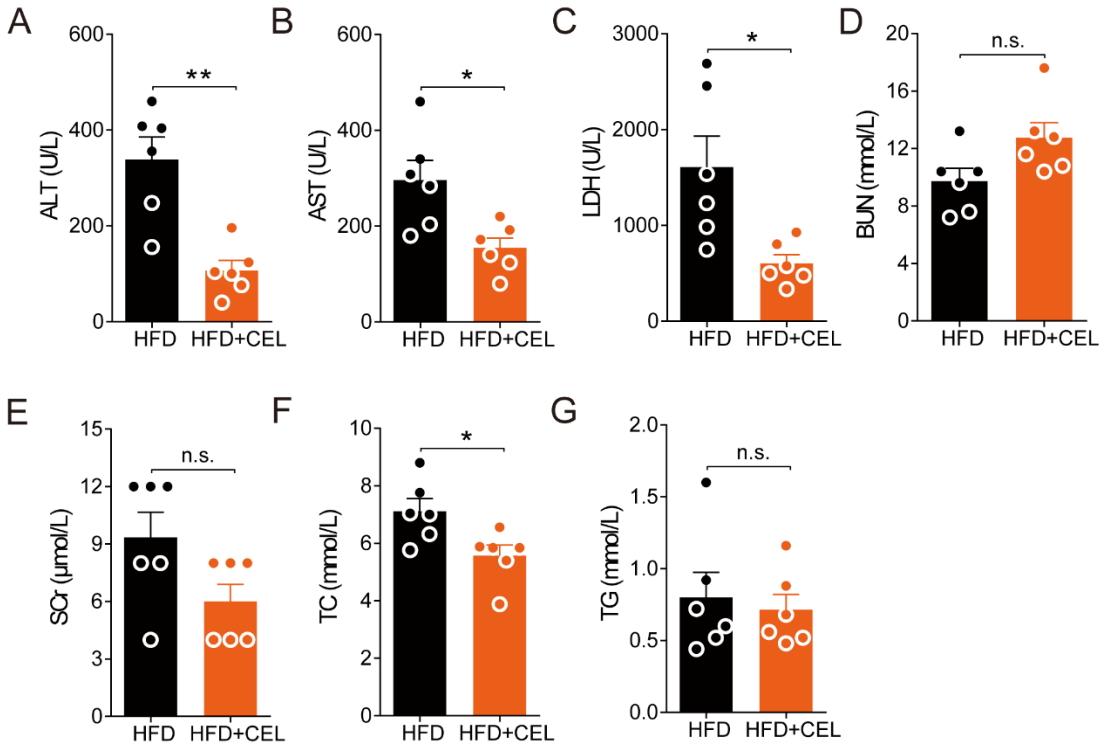

Figure S6. Safety evaluation of celastrol treatment in HFD-fed *ob/ob* mice, Related to Figure 4.

(A-G) Serum levels of (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST), (C) lactate dehydrogenase (LDH), (D) blood urea nitrogen (BUN), (E) serum creatinine (SCr), (F) total cholesterol (TC) and (G) triglyceride (TG) in HFD-fed *ob/ob* mice treated with celastrol or not (n = 6 for each group). Error bars represent the mean ± SEM. p values were determined by Student's t test. *$p < 0.05$, **$p < 0.01$; n.s., not significant ($p > 0.05$).

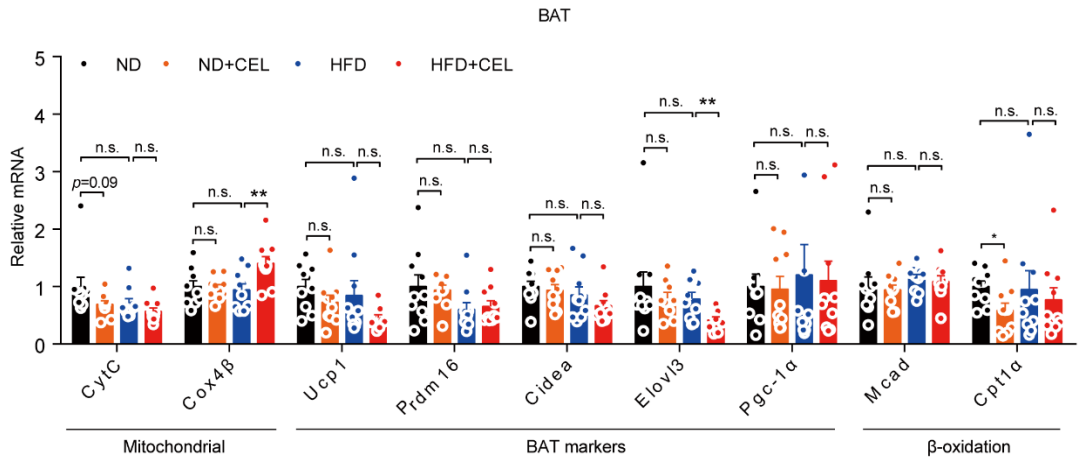

Figure S7. Early celastrol intervention did not upregulate the expression of the mitochondrial and brown fat genes, Related to Figure 6.

ND- or HFD-fed C57BL/6 mice were subjected to oral administration of celastrol (3 mg/kg/day) for 24 days. Gene expression analysis of mitochondrial and brown fat gene programs in BAT of each group of mice by qRT-PCR (n = 10 for each group). Error bars represent the mean ± SEM. p values were determined by Student's t test. **p < 0.01; n.s., not significant (p > 0.05).

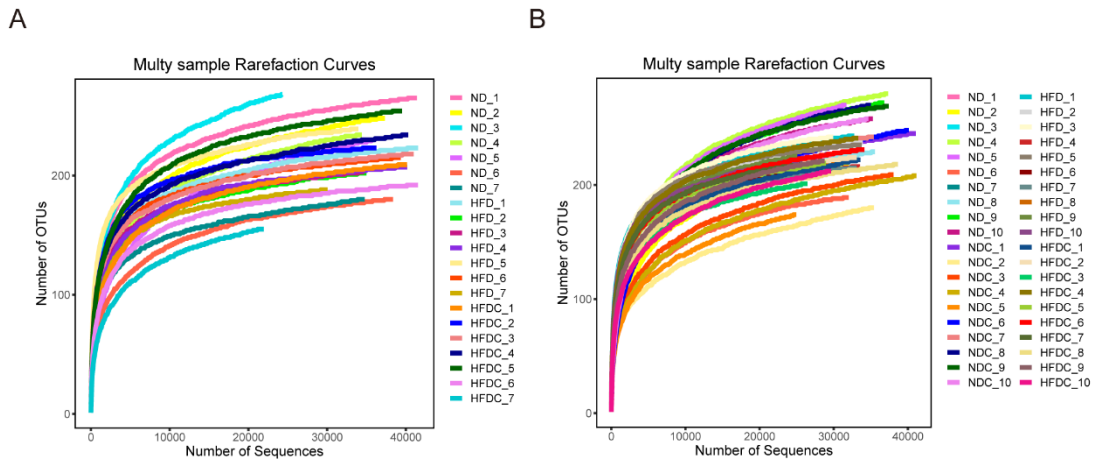

Figure S8. Rarefaction curves for feces samples, Related to Figure 8.

(A-B) Rarefaction curves for samples from (A) DIO mice treated with celastrol (n = 7 for each group) or (B) early celastrol-treated HFD-fed mice (n = 10 for each group). ND, normal diet; NDC, normal diet plus celastrol; HFD, high-fat diet; HFDC, high-fat diet plus celastrol.

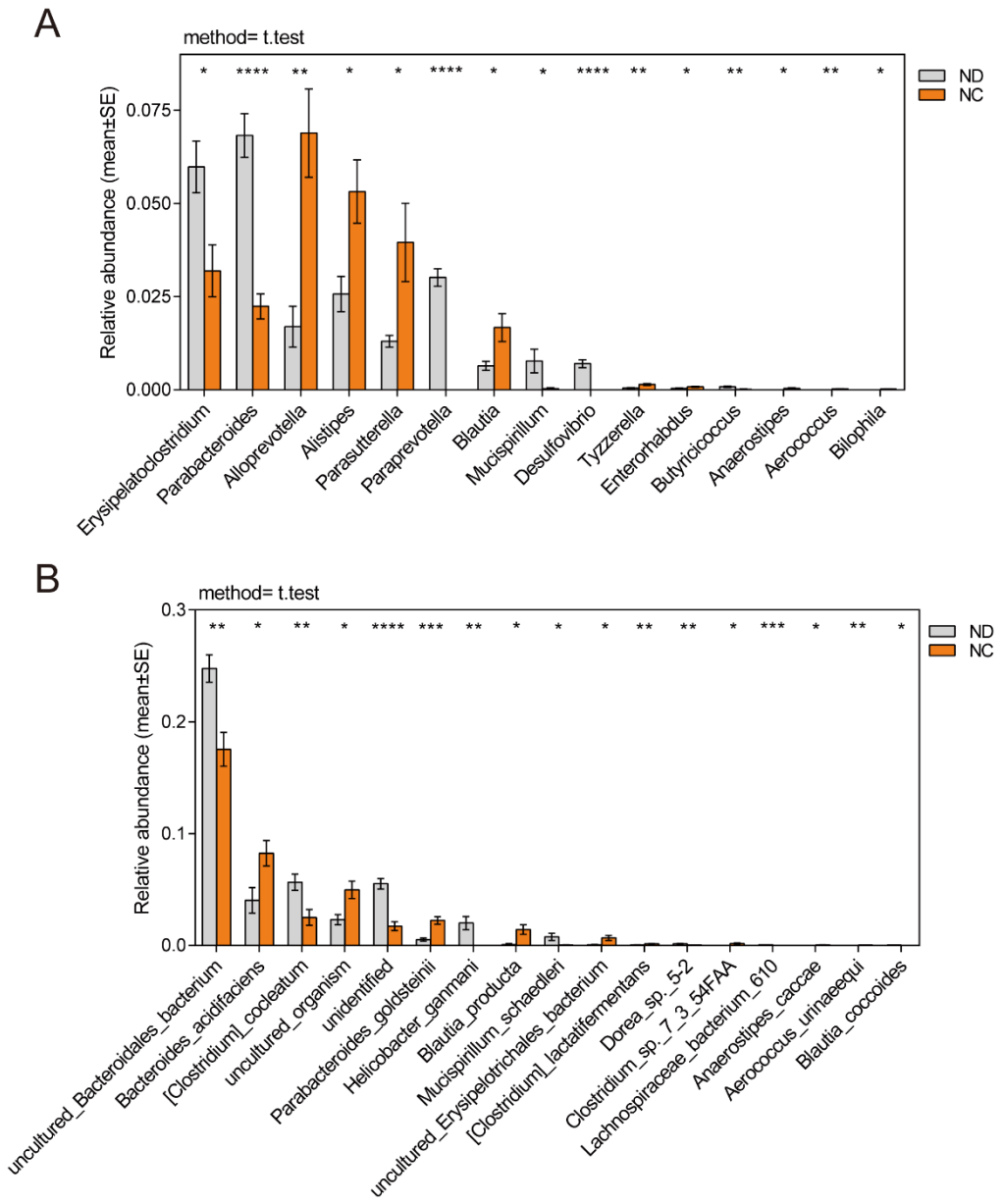

Figure S9. Gut microbiota with significant differences in relative abundance in ND-fed mice treated or not treated with celastrol, Related to Figure 8.

(A-B) Significantly different intestinal microbiota (A) at the genus level or (B) at the species level between ND-fed mice and ND-fed C57BL/6 mice treated with celastrol (3 mg/kg/day). ND, normal diet; NC, normal diet supplemented with celastrol. n = 10 for each group. p values were determined by the t test. 0.01<*p < 0.05, 0.001<**p < 0.01, 0.0001< ***p<0.001, ****p<0.0001.

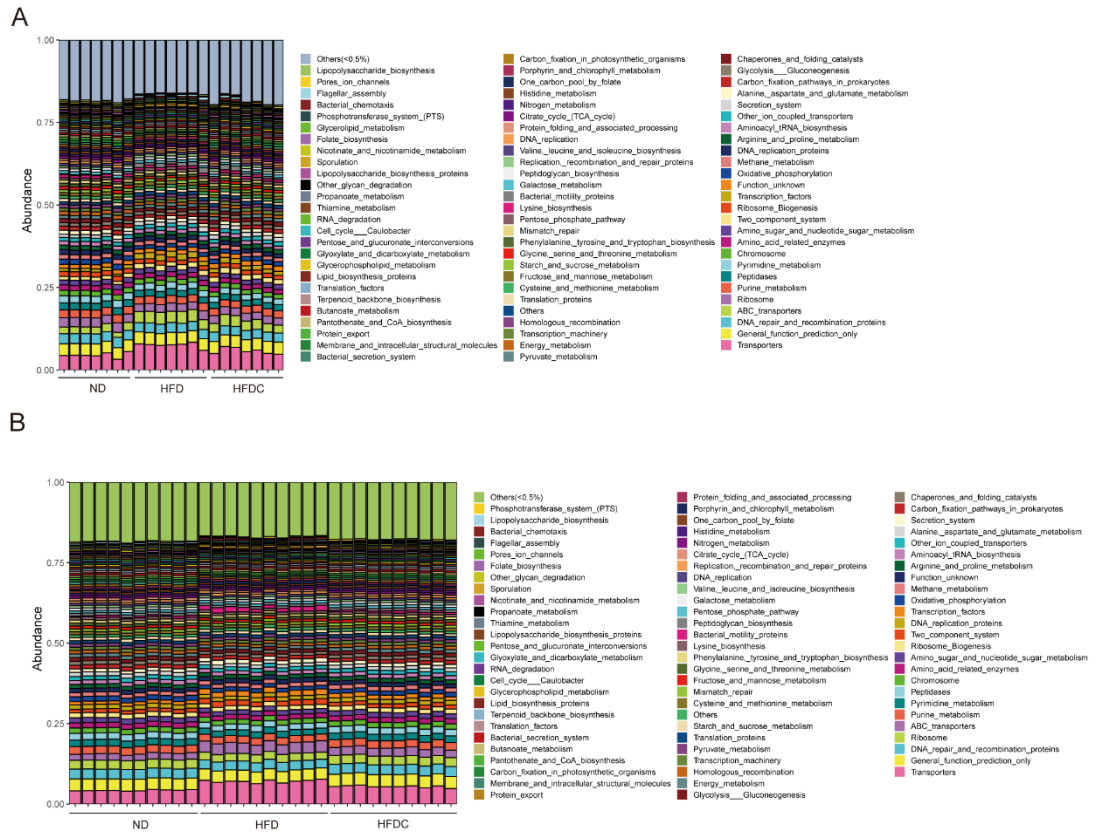

Figure S10. Celastrol treatment promotes functional shifts in the gut microbiota, Related to Figure 8.

(A-B) DIO mice (n=7 for each group) or HFD-fed lean C57BL/6 mice (n=10 for each group) were subjected to oral administration of celastrol (3 mg/kg/day). Mouse fecal samples were taken and subjected to 16S rRNA gene sequencing. (A-B) Pathway-enrichment analysis profiling of samples taken from (A) DIO mice treated with celastrol or (B) early celastrol-treated HFD-fed lean mice. ND, normal diet; HFD, high-fat diet; HFDC, high-fat diet supplemented with celastrol.

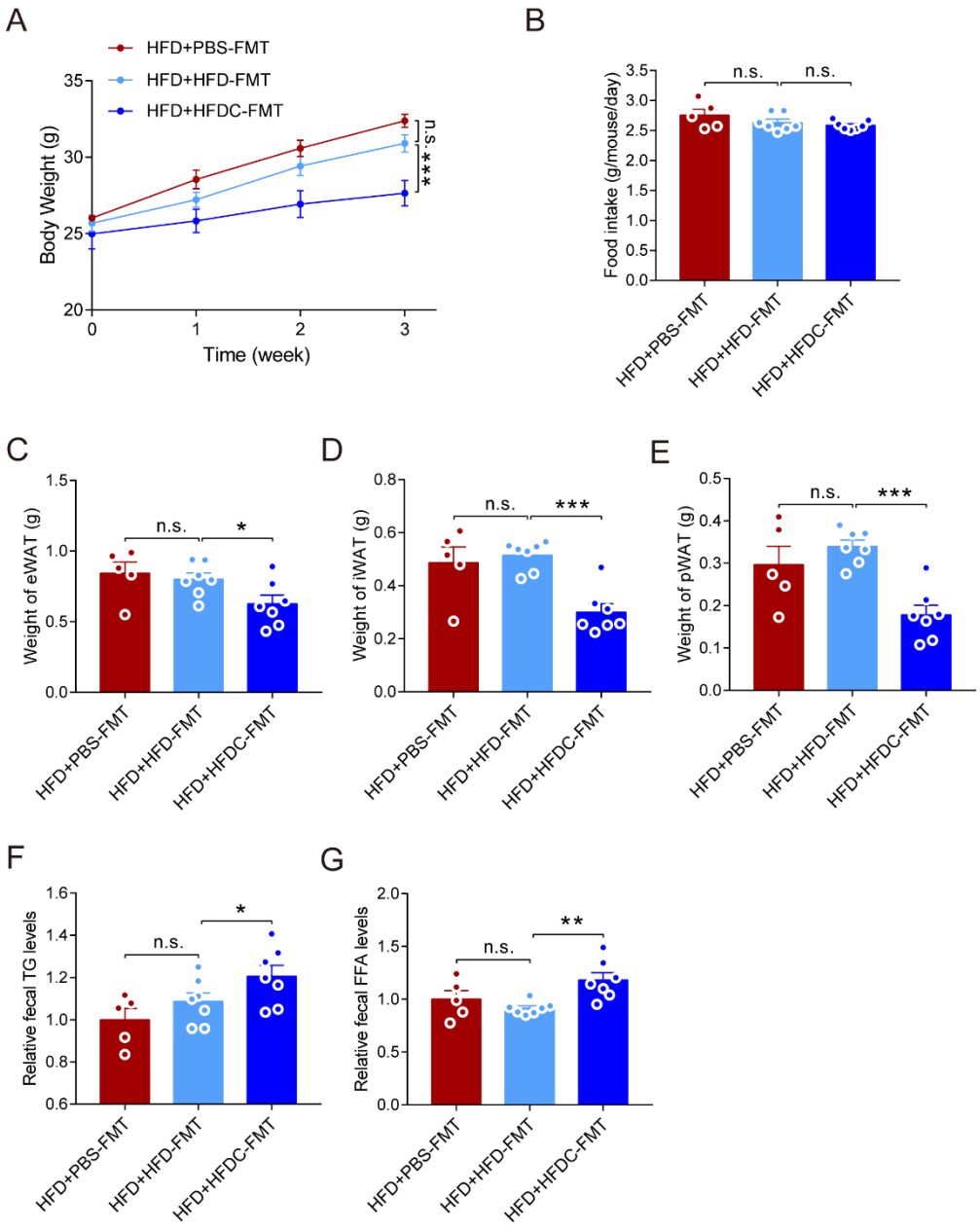

Figure S11. Transplantation of fecal microbiota from celastrol-treated obese mice reduces the body weight gain of HFD mice, Related to Figure 9.

(A-G) HFD-fed C57BL/6 mice were transplanted with fecal bacteria derived from HFD or HFD+CEL mice. (A) The BW of each group of mice during the treatment. (B) The average 3-day food intake of each group of mice during the treatment. (C-E) Weights of (C) eWAT, (D) iWAT and (E) pWAT of each group of mice. (F-G) The fecal (F) TG and (G) FFA levels in each group of mice. n=5 for HFD+PBS-FMT group and n=7 for HFD+HFD-FMT or HFD+HFDC-FMT group. Error bars represent the mean ± SEM. p values were determined by two-way ANOVA (A) or one-way ANOVA (B-G). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; n.s., not significant ($p > 0.05$). HFD+PBS-FMT, PBS gavage plus high-fat diet; HFD+HFD-FMT, transplanted with fecal bacteria derived from HFD mice plus high-fat diet; HFD+HFDC-FMT, transplanted with fecal bacteria derived from HFD+CEL mice plus high-fat diet.

TRANSPARENT METHODS

Animal experiments

(ⅰ) Determination of the effects of celastrol on mouse weight gain

Male C57BL/6J mice (8-week old) and *ob*/*ob* mice (4-week old) were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). The mice were maintained under a 12-h/12-h light/dark cycle at 22 ± 2°C and a relative humidity of 55 ± 10%. For treatments of lean C57BL/6J mice, celastrol was mixed with powdered chow (Beijing Keao Xieli Feed Co., Ltd., China) or HFD chow (60%, ResearchDiets, D12492) at a dose of 3 mg/kg/d. For treatments of *ob*/*ob* mice, celastrol at the same concentration used in DIO C57BL/6J mice (equivalent to 55.2 ppm) was mixed with HFD chow (60%, ResearchDiets, D12492). The food intake of all mice was measured once a day for 3 consecutive days. Parameters such as fat weight and liver weight were measured at the end of the treatment.

(ⅱ) Determination of the causal role of microbiota in the anti-obesity effects of celastrol

(a) Analysis of gut microbiota. (1) DNA extraction. Fecal samples of mice were collected directly into sterile tubes and stored at -80°C until DNA extraction. DNA was extracted using a QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) and then purified as a template for polymerase chain reaction (PCR) amplification. (2) PCR amplification of 16S rRNA genes and MiSeq sequencing. PCR amplification of the V4-V5 region of the 16S rRNA gene was performed using general bacterial primers (515F 5'-GTGCCAGCMGCCGCGGTAA-3' and 926R 5'-CCGTCAATTCMTTTGAGTTT-3'). The recovered product was used as a template for an 8-cycle PCR amplification to add the adapters, sequencing primers, and barcode tag sequences required for sequencing on the Illumina platform to the ends of the target fragment. All PCR products were recovered using an AxyPrep DNA gel recovery kit (Axygen, China), and fluorescence quantification was performed using an FTC-3000TM Real-Time PCR instrument. After homogenization and mixing, the libraries were sequenced by 2×300 bp paired-end sequencing on the MiSeq platform using the MiSeq v3 Reagent Kit (Illumina). (3) Bioinformatic analysis. Sample reads were allocated to the original data based on the barcode to obtain the effective sequence of each sample. Paired-end (PE) reads for all samples were run through Trimmomatic (version 0.35) to remove low-quality base pairs. According to the overlap relationship between PE reads, the paired reads are stitched into a sequence using the FLASH program (version 1.2.11), and the quality of the sequence is filtered using mothur software (version 1.33.3) to obtain optimized sequences. The demultiplexed reads were clustered at 97% sequence identity into operational taxonomic units (OTUs) using UPARSE software. The OTU representative sequences were assigned for taxonomy against the Silva 128 database with a confidence score ≥ 0.6 by the classify.seqs command in mohur. OTU taxonomies (from phylum to species) were determined based on NCBI. Based on the above analysis, a series of statistical and visual analyses of community structure was performed. The alpha-diversity analyses were calculated using mothur and plotted by R, and the beta-diversity metrics were calculated using mothur and visualized with principal coordinate analysis (PCoA).

(b) Antibiotic-induced gut microbiota depletion. A subset of 8-week-old male C57BL/6J mice were randomly distributed into the following groups: group fed with the ND and treated with antibiotics in drinking water (AN, n=6), group fed with the HFD and treated with antibiotics in

drinking water (AH, n=7) and a group fed with HFD and treated with celastrol (3 mg/kg/d) and a combination of antibiotics in drinking water (AHC, n=7). The ND group (n= 10), ND + celastrol (NC) group (n= 10), HFD group (n= 10), HFD + celastrol (HC) group (n= 10) were also set up as the control groups. The broad-spectrum antibiotic cocktail (Sigma, USA) included ampicillin (500 mg/L), vancomycin (250 mg/L), neomycin sulfate (500 mg/L) and metronidazole (500 mg/L). Body weight was recorded every 3 days, and food intake was recorded once a day for 3 consecutive days.

(c) Fecal microbiota transplantation (FMT). To prepare the recipient mice, a separate cohort of male C57BL/6 mice (8 weeks old) received a cocktail of antibiotics in their drinking water for three consecutive days (ampicillin, 200 mg/kg; neomycin sulfate, 200 mg/kg; metronidazole, 200 mg/kg; and vancomycin, 100 mg/kg). After completion of the antibiotic treatment, mice were fasted overnight and randomly assigned to two groups：one group of mice received FMT from donor HFD-fed mice, the other group received FMT from donor HFD-fed C57BL/6 mice treated with celastrol. Fresh fecal matter was collected from HFD-fed C57BL/6 mice treated with or without celastrol (3 mg/kg/day) for FMT. Each FMT dose was prepared on the same day of FMT. Feces were sliced with a scalpel, homogenized in phosphate-buffered saline (PBS), vortexed twice, and incubated for 15 minutes at 37℃. Then the samples were vortexed again and spun at 800 rpm for 3 minutes. The aqueous fecal extract was given to recipients by gavage (200 μl each). FMT of mice were repeated on consecutive days (without fasting) for a total of seven FMT per mouse. All recipient mice had free access to HFD chow. The HFD mice treated with the same volume of PBS by gavage were used as control. The body weight and food intake were recorded after FMT treatment.

All animal experiments were performed according to the protocols approved by the Institutional Animal Care and Use Committee of Nanjing Medical University (IACUC14030112-1).

In vivo metabolic phenotype analysis

To assess metabolic parameters, 8-week-old male C57BL/6J mice were fed HFD chow with or without celastrol for 2 weeks. Energy expenditure and home-cage activity were assessed using a combined indirect calorimetry system (TSE System, Bad Homburg, Germany). Mice were housed individually and given free access to food and water. O_2 consumption, CO_2 production and heat generation were measured every 40 min for a total of 120 h (including 48 h of adaptation) to determine the respiratory quotient and energy expenditure. Home-cage locomotor activity was determined using a multidimensional infrared light beam system with beams scanning the bottom and top levels of the cage, and activity was expressed as beam breaks.

Western blotting (WB)

We lysed cells or homogenized tissues in protein lysis buffer (50 mmol/L Tris, 150 mmol/L NaCl, 10 mmol/L EDTA, 1% Triton X-100, 200 mmol/L sodium fluoride, supplemented with 1× protease inhibitor cocktail (Roche, 04693132001)). Then, the samples were centrifuged at 4℃ and 14,000 rpm for 15 min, and the supernatant was collected. The protein concentration was determined using a BCA protein assay kit (Beyotime, China), and the samples were diluted in SDS sample buffer. Total protein was separated by SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked by TBS-T (0.1% Tween 20 in TBS) containing 5%

nonfat milk for 1 h at room temperature and then incubated with primary antibodies against CD36 (1/1000; Abcam, UK), ApoB (1/500; Proteintech, China), FATP2 (1/500; Proteintech, China), FATP4 (1/500; Proteintech, China), GLUT2 (1/500; Proteintech, China), GLUT5 (1/500; Proteintech, China), PPARα (1/500; Proteintech, China), and UCP1 (1/1000; Abcam, UK), followed by the addition of HRP-labeled secondary antibodies (1/2500). β-Actin (1/5000; Bioworld, USA) was used as an internal control. The blots were visualized using the Bio-Rad ECL detection system. Densitometric analysis was performed using Image Lab software (Bio-Rad, USA).

Quantitative real-time PCR (qRT-PCR)

Total cell or tissue RNA was extracted with RNAiso Plus reagent (TaKaRa Biotechnology Co., Ltd., Dalian, China) following the manufacturer's protocol. cDNA was synthesized with 1 μg of total RNA using PrimeScript™ Reverse Transcriptase (TaKaRa Biotechnology Co., Ltd., Dalian, China). qRT-PCR was conducted by SYBR Green Master Mix (Vazyme, Nanjing, China) on a QuantStudio 3 real-time PCR System (Applied Biosystems, Foster City, CA, USA). The primers used for PCR amplification are listed in Table S1. The cycling conditions were 95°C for 10 min, followed by 40 repeats of 95°C for 15 s and 60°C for 1 min. The mRNA levels were normalized to a GAPDH or 18S rRNA control and calculated using the comparative cycle threshold (ΔΔCt) method.

Histological examination

Tissues were fixed in 4% paraformaldehyde (PFA) for 24 h at room temperature and then dehydrated by a graded series of ethanol and paraffin embedded. Subsequently, the tissues were cut into 4-μm sections and stained with hematoxylin-eosin (H&E). The images were captured with an Olympus BX51 microscope (Olympus, Center Valley, PA). Adipose tissues were treated with 90% ethanol for 24 h before being fixed with 4% PFA, and the remaining steps were the same as above.

Oil red O staining

Oil red O (ORO) staining was used to observe lipid droplets in liver tissues. First, frozen liver sections (10 μm) were prepared using a Leica CM1900 cryostat (Leica, Germany). Then, the sections were placed in 60% isopropanol for 1 min and stained with the prepared Oil red O dye (Sigma) solution for 20 min. Then, 60% isopropanol was used to wash away the nonspecifically stained Oil red O dye solution, and the tissue sections were photographed using Olympus BX51 microscopy (Olympus, Center Valley, PA) in bright field.

Isolation and differentiation of primary brown fat precursor cells

Brown adipose tissues (BAT) were dissected from 4-week-old C57BL/6J mice, rinsed in phosphate-buffered saline (PBS), minced, and digested for 1 h at 37℃ in 0.2% (w/v) type I collagenase (Sigma) in DMEM/F12 (Gibco) supplemented with 2% fatty-acid-free BSA. Adipocytes were filtered through a 200-mesh screen followed by three washes (centrifuged at 2000 rpm for 10 min) with PBS. The sediment was resuspended in DMEM/F12 with 10% fetal bovine serum (FBS) (Gibco). Two days after reaching confluence (day 0), the cells were induced to differentiate into adipocytes in induction medium Ⅰ (DMEM/F12 containing 10%

FBS, 17 nM insulin (Sigma), 2 μg/ml dexamethasone (Sigma), 0.5 mM isobutylmethylxanthine (Sigma), 0.5 μM rosiglitazone (Sigma), 125 μM indomethacin (Sigma) and 1 nM triiodothyronine (T3) (Sigma)). After 2 days, induction medium Ⅰ was replaced with induction medium Ⅱ (DMEM/F12 supplemented with 10% FBS, 1 nM T3, 17 nM insulin, and 0.5 μM rosiglitazone) and replaced with fresh induction medium Ⅱ every two days. Five days later, the precursor cells were differentiated into mature brown fat cells.

Cell viability

Cell viability was determined by the CCK-8 assay kit (KGA317, KeyGen Biotech, China). Briefly, the primary brown fat precursor cells were treated with celastrol (50 nM to 800 nM) for 24 h, and then 10 μl of CCK-8 reagent was added to the medium and incubated for 2 h. Finally, the absorbance was detected at 450 nm.

Glucose tolerance tests (GTT)

For the GTT, mice were fasted for 15 h (from 6 p.m. to 9 a.m.), and fasting blood glucose was detected (0 min). Then, 1.5 g glucose/kg BW was injected i.p., and tail blood glucose was measured using a handheld glucometer (Ascensia Breeze, Bayer Company, Leverkusen, Germany) at 15, 30, 60, 90, 120 and 150 min after glucose injection. The area under the curve (AUC) was determined by GraphPad Prism software (version 7.0, GraphPad Software, La Jolla, CA, USA).

Lipid absorption studies

Lean or DIO C57BL/6 mice were treated with celastrol at a dose of 3 mg/kg/d for 6 days. After fasting for 6 h, blood was collected from the lower jaw of the mice to detect the basal levels of TG. Then, the mice were fed olive oil (17 μl/g BW) by gavage, and blood was collected from the lower jaw 2 h later (2 h). The serum was separated by centrifugation (4°C, 2000 rpm), and the TG content was determined using a liquid sample TG assay kit (E1003, Applygen, Beijing, China) according to the manufacturer's instructions.

TG and free fatty acid (FFA) contents determination

For TG and FFA levels in feces, 200 mg of dried feces was homogenized in 1 ml of the extract reagent provided by the kits. The concentrations of TG and FFA were examined using assay kits (TG assay kit：E1013, Applygen, Beijing, China；FFA assay kit：BC0595, Solarbio, Beijing, China) according to the manufacturers' instructions. All measurements were adjusted to the starting fecal mass. For TG levels in liver tissues, 50 mg of the liver tissue was accurately weighed, and 1 ml of the lysate supplied by the kit was added for homogenization. The homogenized liver tissue was heated and centrifuged in the same manner as the extraction of fecal TG. Ten microliters of supernatant was used to detect TG levels according to the instructions of the kit.

Serum biochemistry

Mouse blood was collected from the inferior vena cava after anesthesia, and serum samples were collected by centrifugation at 2000 rpm for 10 min. The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH),

serum creatinine (SCr), blood urea nitrogen (BUN), triglyceride (TG) and serum total cholesterol (TC) were evaluated using a serum biochemical autoanalyzer (Hitachi 7600 modular chemistry analyzer, Hitachi Ltd., USA).

Enzyme-linked immunosorbent assay (ELISA)

The circulating leptin levels were determined by ELISA kits (E-EL-M0039c, Elabscience, Wuhan, China) according to the manufacturer's instructions.

Proteomics analysis

Three samples (n=3 in DIO or DIO plus Celastrol group) were obtained from DIO mice treated with celastrol or not. For each sample, 20 μg of protein was separated on a 12.5% SDS-PAGE gel, and protein bands were visualized by Coomassie Blue R-250 staining for quality control. The samples were then digested using trypsin, and after protein digestion, peptides were first quantified spectrophotometrically at OD280, and 100 μg of each peptide sample was labeled using a TMT Mass Tagging kit (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's instructions. Peptides of the two groups were labeled with different TMT labels, equivalently mixed and fractionated using a High pH Reversed-Phase Peptide Fractionation kit (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's instructions. Each fraction was loaded onto a reversed-phase trap column connected to the C18-reversed-phase analytical column in buffer A (0.1% formic acid) and separated with a linear gradient of buffer B (84% acetonitrile and 0.1% formic acid) at a flow rate of 300 nl/min for nano LC-MS/MS analysis. Liquid chromatography-mass spectrometry/MS (LC-MS/MS) analysis was performed on a Q Exactive mass spectrometer (Thermo Scientific) that was coupled to Easy nLC. Tandem mass spectrometry (MS/MS) spectra were searched using the MASCOT engine embedded in Proteome Discoverer. The differentially expressed proteins were identified by fold change values greater than ±1.2 and $p < 0.05$. KEGG pathway enrichment analyses were applied based on Fisher's exact test.

Untargeted metabolomics analysis

Eighty milligrams of tissue was weighed, homogenized in 200 μl of water, and vortexed for 60 s. Then, 800 μl of methanol acetonitrile solution was added, followed by vortexing for 60 s, sonicating twice at low temperature for 30 min each time, and incubating at -20℃ for 1 h to precipitate protein. After centrifuging the samples at 14,000 rcf at 4℃ for 20 min, the supernatant was lyophilized and stored at -80℃. The samples were separated using an Agilent 1290 Infinity LC Ultra-High Performance Liquid Chromatograph (UHPLC) HILIC column and analyzed by mass spectrometry using a Triple TOF 5600 mass spectrometer (AB SCIEX). Then, exact mass matching (<25 ppm) and secondary spectrum matching were used to search in the laboratory's self-built database to identify the structure of metabolites. After the data were preprocessed by Pareto-scaling, multidimensional statistical analysis and one-dimensional statistical analysis, including Student's t-test and fold change analysis, were performed. The differential metabolites were identified by $FC > 1.5$ and $p < 0.05$. The volcanic map was drawn using R software.

Statistical analysis

All values are presented as the mean value ± SEM. GraphPad Prism (version 7.0, GraphPad Software, La Jolla, CA, USA) software was used for statistical analyses. Statistical significance was calculated by Student's t test or by one-factor or two-factor ANOVA. Significance was accepted at $P < 0.05$.

Table S1. Primers used for qRT-PCR, Related to Figure 1, 3, 5, S2, S4, S5 and S7

Name	Forward Primer (5'- 3')	Reverse Primer (5'- 3')
CD36	ATGGGCTGTGATCGGAACTG	GTCTTCCCAATAAGCATGTCTCC
ApoB	AAGCACCTCCGAAAGTACGTG	CTCCAGCTCTACCTTACAGTTGA
FATP2	TCCTCCAAGATGTGCGGTACT	TAGGTGAGCGTCTCGTCTCG
FATP4	GCCACTGTCTTGACACCTCA	AACCCTTGTCTGGGTGACTG
SGLT1	ATGCGGCTGACATCTCAGTC	ACCAAGGCGTTCCATTCAAAG
GLUT2	TCAGAAGACAAGATCACCGGA	GCTGGTGTGACTGTAAGTGGG
GLUT5	CCAATATGGGTACAACGTAGCTG	GCGTCAAGGTGAAGGACTCAATA
PPARα	TCAGGGTACCACTACGGAGTTCA	CCGAATAGTTCGCCGAAAGA
CytC	AAATCTCCACGGTCTGTTCGG	GGGTATCCTCTCCCCAGGTG
Cox4β	CTGCCCGGAGTCTGGTAATG	CAGTCAACGTAGGGGGTCATC
Mcad	ATGACGGAGCAGCCAATGAT	TCGTCACCCTTCTTCTCTGCTT
Cpt1α	TTGCCCTACAGCTCTGGCATTTCC	GCACCCAGATGATTGGGATACTGT
UCP1	TACCAAGCTGTGCGATGTCCA	GCACACAAACATGATGACGTTCC
PRDM16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Cidea	TGACATTCATGGGATTGCAGAC	CGAGCTGGATGTATGAGGGG
Elovl3	TTCTCACGCGGGTTAAAAATGG	TCTCGAAGTCATAGGGTTGCAT
PGC-1α	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Cck	AGCGCGATACATCCAGCAG	ACGATGGGTATTCGTAGTCCTC
Sct	AGACACTCAGACGGAATGTTCA	CTGGTCCTCTAAGGGCTTGGA
Gip	TGAGTTCCGATCCCATGCTAA	CCAGTTCACGAAGTCTTGTTGTC
18S rRNA	ACGGACAGGATTGACAGA	CGCTCCACCAACTAAGAA
GAPDH	GTCTTCACTACCATGGAGAAGG	TCATGGATGACCTTGGCCAG