Supplemental information (SI)

SI Material and Methods

Fluorescein isothiocyanate (FITC)–dextran-based permeability assay

The mice were fasted for 4 hours, then oral gavaged with 200 mg/kg of 4 kDa FITC–dextran (Sigma-Aldrich, St. Louis, MO). After 90 min, the mice were sacrificed and blood was collected, centrifuged at 3000 *g* for 10 min to prepare the plasma. The fluorescence signal (excitation wavelength 485 nm and emission wavelength 520 nm) in the plasma was measured using microplate spectrofluorometer (Molecular Devices, San Jose, CA), and the concentration of FITC-dextran in the plasma was calculated against a standard curve.

Real-time PCR analysis of gene expression in adipose and colon tissues

Gonadal adipose or colon tissues were grounded after frozen by liquid nitrogen. Total RNA was isolated using TRIzol reagent (Ambion, Austin, TX) according to the manufacturer's instructions. Then the RNA was reverse transcripted into cDNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. The quality of the extracted RNA was measured using a NanoDrop Spectrophotometer (Thermo Scientific, Waltham, MA). qRT-PCR was carried out in a DNA Engine Opticon system (Bio-Rad Laboratories, Hercules, CA) with Maxima SYBR-green Master Mix (Thermo Fisher Scientific). The sequences of mouse-specific primers (Thermo Fisher Scientific) in this research were: *Tnf-α* (forward) 5'- CCCTCACACTCAGATCATCTTCT -3' and (reverse) 5'- GCTACGACGTGGGC-TACAG -3', *Mcp-1*(forward) 5'-TTAAAAACCTGGATCGGAACCAA -3' and (reverse) 5'-GCATT-AGCTTCAGATTTACGGGT -3', *Tlr-4* (forward) 5'- ATGGCATGGCTTACACCACC -3' and (reverse) 5'-GAGGCCAATTTTGTCTCCACA -3', *Tlr-5* (forward) 5'- GCAGGATCATGGCATGTCAAC -3' and (reverse) 5'-ATCTGGGTGAGGTTACAGCCT -3', *Claudin-1* (forward) 5'- GGGGACAACATCGTGACCG -3' and (reverse) 5'-AGGAGTCGAAGACTTTGCACT -3', *Claudin-5* (forward) 5'- GCAAGGTG-TATGAATCTGTGCT -3' and (reverse) 5'-*GTCAAGGTAACAAAGAGTGCCA* -3'. The results of target genes were normalized to glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) and expressed to LFD and vehicle treated mice using the $2^{-\Delta\Delta Ct}$ method. The primer to analyze *Gapdh* is (forward) 5'- AGGTCGGTGTGAAC-GGATTTG-3' and (reverse) 5'- TGTAGACCATGTAGTTGAGGTCA-3'

Real-time PCR analysis of *16S rRNA* in blood and adipose tissues

Blood (~100 µL) and gonadal adipose tissues (~200-300 mg) were collected for anaysis. The total DNA was extracted using QIAamp DNAeasy Blood & Tissue Kit (Qiagen, Germantown, MD) following the manufacturer's instruction with the addition of a bead-beating step. The quality of the extracted DNA was measured using a NanoDrop Spectrophotometer (Thermo Scientific) and 20 µL PCR reactions were made using the Maxima SYBR green Master Mix (Thermo Fisher Scientific), and DNA was normalized to 5 ng/ µL per reaction. The sequences of *16S rRNA* primers were: (forward)5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCC-TACGGGNGGCWGCAG-3' and (reverse) 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAC-TACHVGGGTATCTAATCC-3'.

Flow cytometric analysis of immune cells in gonadal adipose tissues

Adipose tissues were washed with cold PBS, and digested overnight with Hank's-balanced salt solution (HBSS, Lonza, Basel, Switzerland) supplemented with 1 mM dithiothreitol (DTT) and 5 mM ethylenediaminetetraacetic acid (EDTA) at 4 °C. Then the released cells were filtered through 70 µm cell strainers (BD Biosciences, San Jose, CA) to obtain single cell suspensions, which were stained with FITC-conjugated anti-mouse CD45, and isotype control antibody (BioLegend, San Diego, CA). Zombie Violet™ dye from the Zombie Violet™ Fixable Viability Kit (BioLegend) was used to stain dead cells, according to the manufacturer's instructions. Data were acquired using a BD LSRFortessa™ flow cytometer (BD Biosciences) and were analyzed using FlowJo software (FlowJo LLC, Ashland, OR). Gating and cell identification strategies are as follows: cell doublets and

clumps were eliminated using FSC-H vs. FSC-A gating, and debris was eliminated using FSC-A vs. SSC-A. Dead cells were gated out using Zombie Violet™ dye. In our analyses, leukocytes were identified as $CD45^+$ cells.

Immunohistochemistry
Dissected colon tissues were cut open longitudinally, washed in ice-cold PBS and fixed in 4% formalin (Thermo Fisher Scientific). The adipose tissues were fixed in 4% formalin (Thermo Fisher Scientific) directly. Formalin-fixed tissue was embedded in paraffin (Thermo Fisher Scientific), sliced to 5-µm sections, and dewaxed in serial xylene (Thermo Fisher Scientific), rehydrated through graded ethanol solutions (Pharmco-Aaper, Shelbyville, KY). For H&E staining, the slides are stained with hematoxylin and eosin (Sigma-Aldrich), and examined with a light microscopy. For Immunohistochemistry, antigen retrieval was performed by heating the sections in 0.01 M citrate buffer (pH 6.0) to 95°C for 10 minutes. Samples were incubated with primary antibodies against Claudin-1 (Cell Signaling, Danvers, MA) and Claudin-5 (Thermo Fisher Scientific) overnight at 4 °C. Horseradish peroxidase (HRP)-conjugated secondary antibodies were then applied to the sections, followed by the chromogen 4-diaminobenzidine staining according to the instruction of HRP/DAB (ABC) Detection IHC kit (Abcam, Cambridge, MA). Sections were then counterstained with hematoxylin for 1 minute. Positive expression of Claudin-1 and Claudin-5 were observed under light microscope.

LPS determination in plasma
To analyze LPS in plasma, blood samples were harvested via cardiac puncture of the mice anesthetized with isoflurane and collected in blood collection tubes. The plasma fractions were prepared by centrifugation of the harvested blood at 2,000 *g* for 10 min at 4 ˚C. The LPS in plasma was detected using the LPS ELISA kit (MBS261904, MyBiosource) according to the manufacturer's instructions.

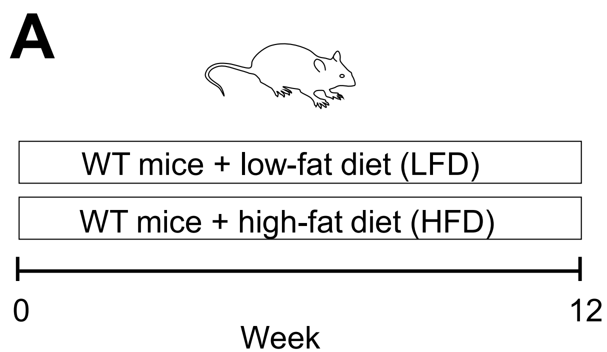
Chemical synthesis of DHETs
The scheme for chemical synthesis of DHETs is shown in Scheme S1. Methyl arachidonate (1.00 g, 3.13 mmol) was diluted in CH_2Cl_2 (80 mL) and *m*-CPBA (~70%, 0.750 g, approx. 2.82 mmol) was added in a single portion. The mixture was stirred at room temperature under argon for 50 min and then quenched by addition of an equivalent volume of saturated aqueous $NaHCO_3$. The mixture was stirred 5 min, and the layers were separated. The aqueous layer was extracted three times with CH_2Cl_2 (50 mL each), and the combined organic layer was then washed sequentially with saturated aqueous $NaHCO_3$, H_2O, and saturated aqueous NaCl (100 mL each), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by flash column chromatography (40 g SiO_2 cartridge), eluting with a gradient of hexanes-5% EtOAc in hexanes (30 min) and then EtOAc (5 min). Two fractions were obtained, both clear and colorless oils: a mixture of 8,9-, 11,12-, and 14,15-methyl-EETs (0.429 g), and 5,6-methyl-EET (0.098 g). The mass of the total EET obtained was 0.527 g (50%). Unreacted methyl arachidonate and over-oxidation products were obtained, although those were not quantified.

A mixture of methyl-EETs (0.150 g mixture of 8,9-, 11,12-, and 14,15-methyl-EET and 0.050 g 5,6-methyl-EET, total of 0.200 g, 0.598 mmol) was diluted in anhydrous DMF (2 mL) and added to dry cesium propionate (0.621 g, 2.98 mmol) in DMF (5 mL) in a sealable tube. The tube was flushed with argon, sealed, and heated to 120 ˚C for 68 h. The mixture was cooled, poured into H_2O (20 mL) and extracted with EtOAc (3 x 30 mL). The organic phase was washed with 5% NaCl (2 x 50 mL) and saturated aqueous NaCl (50 mL), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by flash column chromatography (25 g SiO_2 cartridge), eluting with a gradient of hexanes-40% EtOAc in hexanes (25 min) to yield the EET propionate mixture as a pale-yellow syrup (0.220 g, 90%) after drying in vacuo. This was immediately used in the next step.

The mixture of EET propionates was then diluted in THF/H_2O (5.5/1.4 mL) and cooled to 0 ˚C under argon. LiOH (1.21 mL of a 2 M solution in H_2O, 2.42 mmol) was added, and the mixture was warmed to room temperature and stirred overnight. The mixture was then quenched by the dropwise addition of formic acid, until the pH of the mixture was approximately 4. H_2O and EtOAc (10 mL each) were then added, and the layers were separated. The aqueous layer was extracted with EtOAc (4 x 10 mL) and the organic phase was washed with

saturated aqueous NaCl (40 mL), dried over anhydrous sodium sulfate, concentrated, and azeotroped with hexanes (3 x 20 mL) to remove residual formic acid. The residue was then purified by flash column chromatography (25 g SiO_2 cartridge), eluting with a gradient of 40-75% EtOAc in hexanes (25 min) to yield the DHET mixture (0.119 g, 65%) after azeotroping with toluene and drying in vacuo. ^{1}H-NMR indicated that only 8,9-, 11,12-, and 14,15-DHET are present (presumably due to lactonization of 5,6-DHET, which was then removed by chromatography). Based on LC-MS/MS analysis, the ratio of 8,9-:11,12-:14,15-DHET is 1:1.6:1.8 (1).

1.Taha AY*, et al.* (2018) Regulation of rat plasma and cerebral cortex oxylipin concentrations with increasing levels of dietary linoleic acid. *Prostaglandins, leukotrienes, and essential fatty acids* 138:71-80.



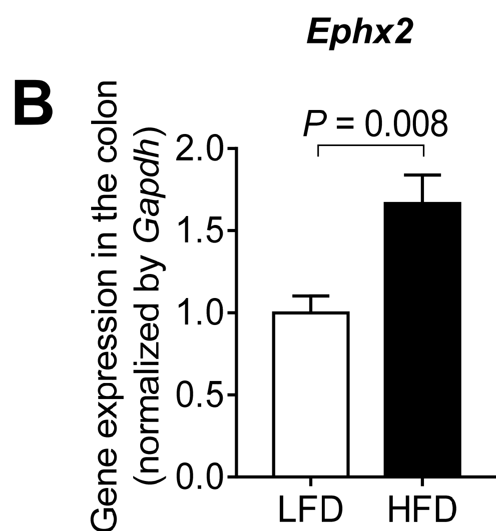
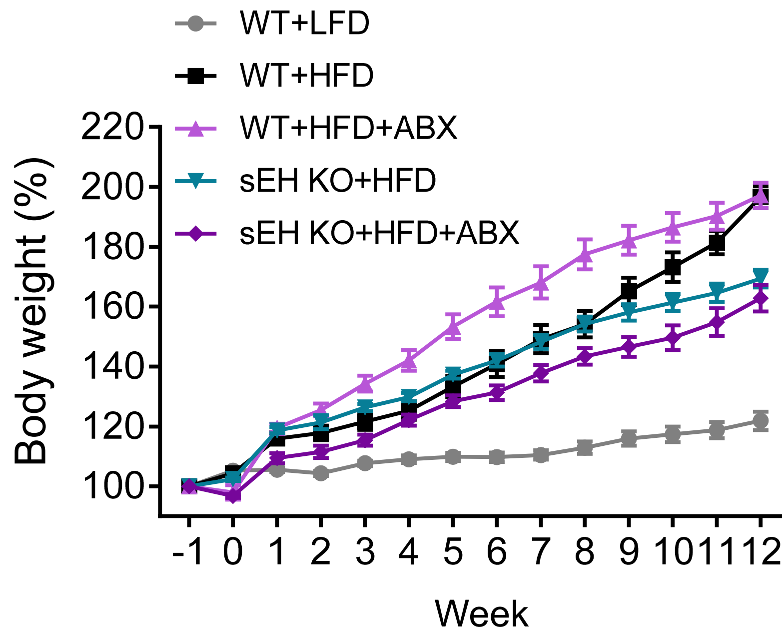
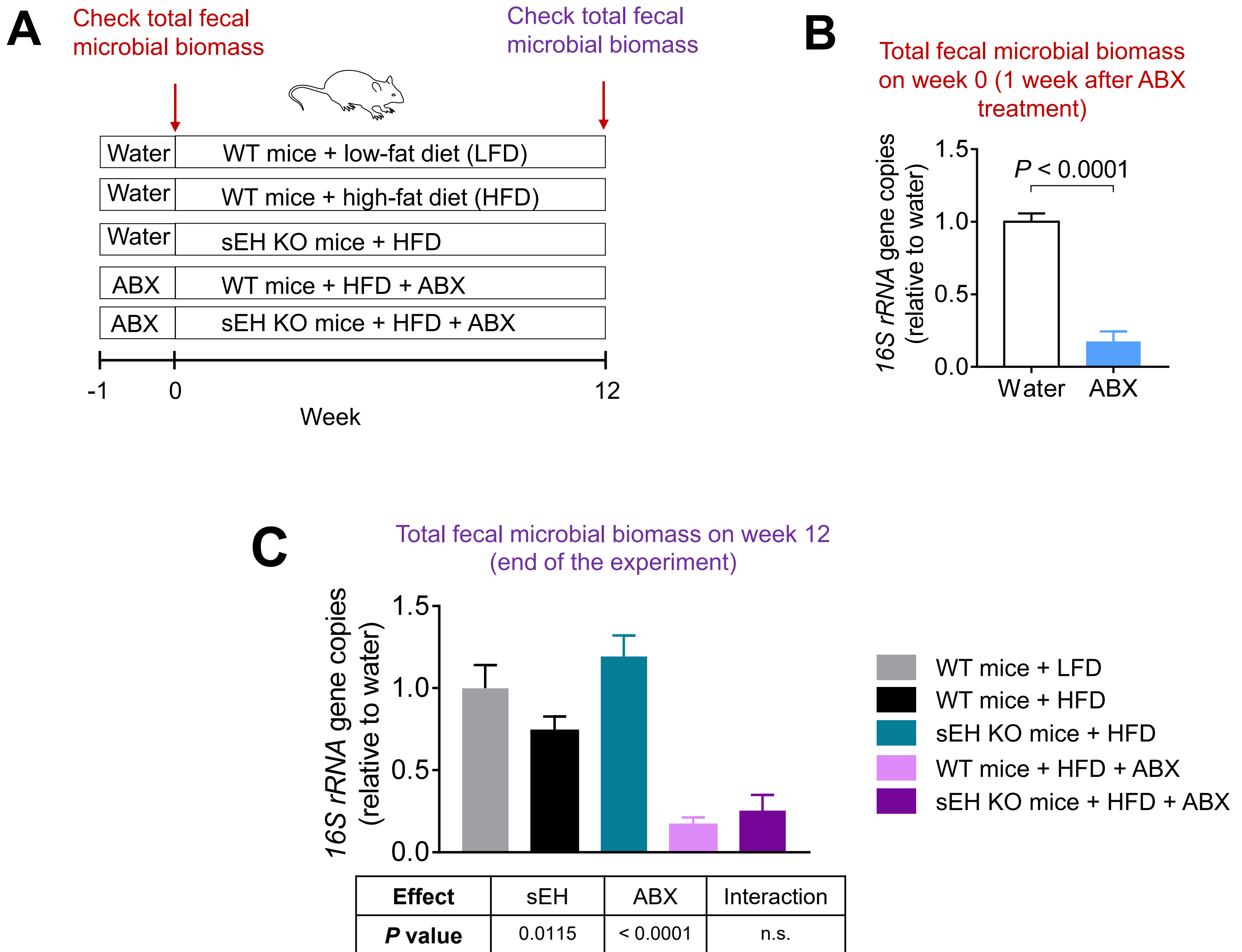


Figure S1. HFD increased expression of *Ephx2* in colon tissues of mice. (A) Scheme of animal experiment (adapted from Fig. 1A). (B) Gene expression of *Ephx2* in colon. The data are mean ± SEM, n = 6 mice per group.



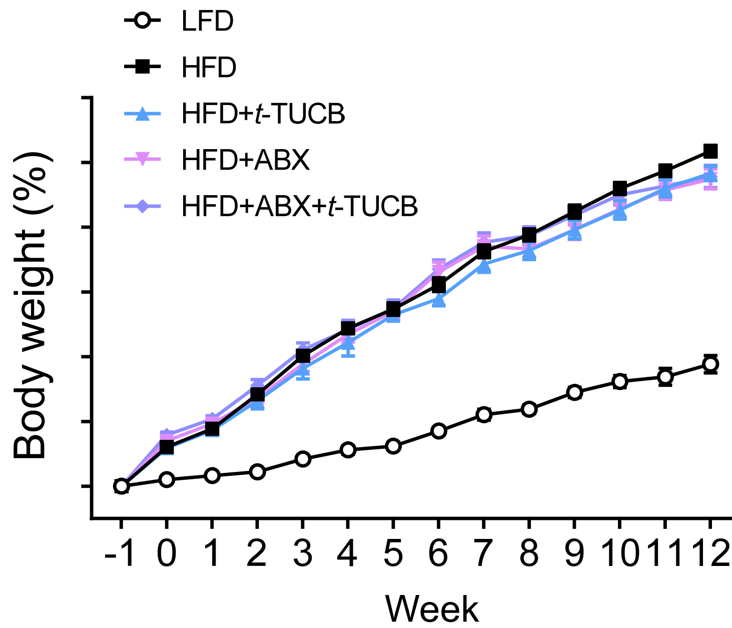
Effect	sEH	ABX	Interaction
***P* value**	< 0.0001	0.4280	n.s.

Figure S2. Body weight of treated mice (see scheme of animal experiment in Fig. 1A). Top panel: body weight of the treated mice over time. Bottom panel: data analysis based on the body weight on week 12. The data are mean ± SEM, n = 10 mice per group. ***Data analysis method for the 5-group comparison:*** The data analysis of sEH (WT mice vs. sEH KO mice) and antibiotics (water vs. ABX) under the high-fat diet (HFD) along with a control group (WT mice + LFD) is performed using the nested two-way ANOVA (with homogeneous/heterogeneous variance) designed for the factorial plus control experiment, followed by (i) the test between the (WT mice + LFD) group and the (WT mice + HFD) group and (ii) the Tukey's multiple comparison test for the comparisons among the four groups (WT mice + HFD, sEH KO mice + HFD, WT mice + HFD + ABX, sEH KO mice + HFD + ABX). Note that the validity of the nested two-way ANOVA model assumptions was assessed by the analysis of residuals and Q-Q plot.



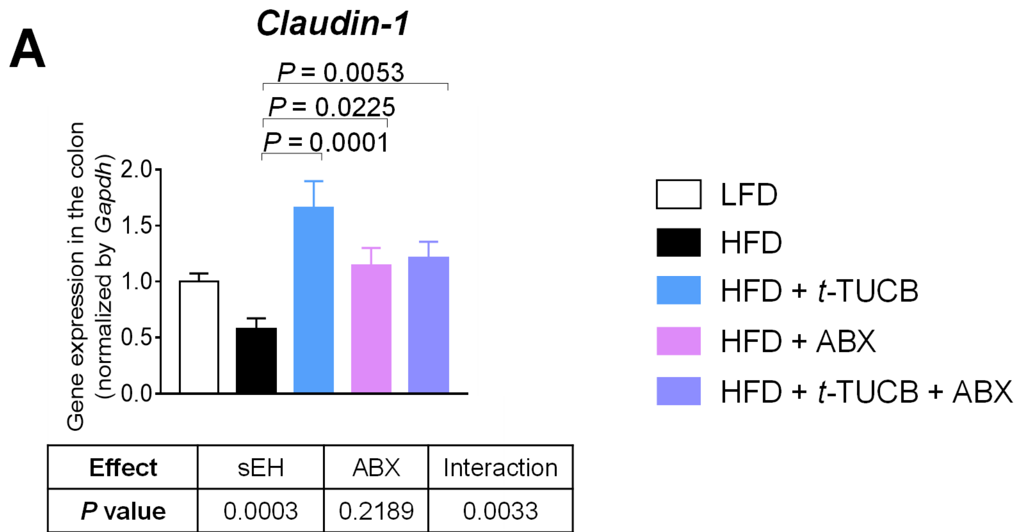
Effect	sEH	ABX	Interaction
***P* value**	0.0115	< 0.0001	n.s.

Fig. S3. Treatment with the antibiotic mixture (ABX) ablated bacteria in colons of mice. (*A*) Scheme of animal experiment (modified from Fig. 1A): at week 0 (1 week after the ABX treatment) and week 12 (end of the experiment), the total fecal microbial biomass was analyzed by expression of *16S rRNA* gene. (***B***) *16S rRNA* gene copies in mouse feces on week 0 (n = 7-8 mice per group). (***C***) *16S rRNA* gene copies in mouse feces on week 12 (n = 8 mice per group, see data analysis method for the 5-group comparison in Fig. S2). The data are mean ± SEM.

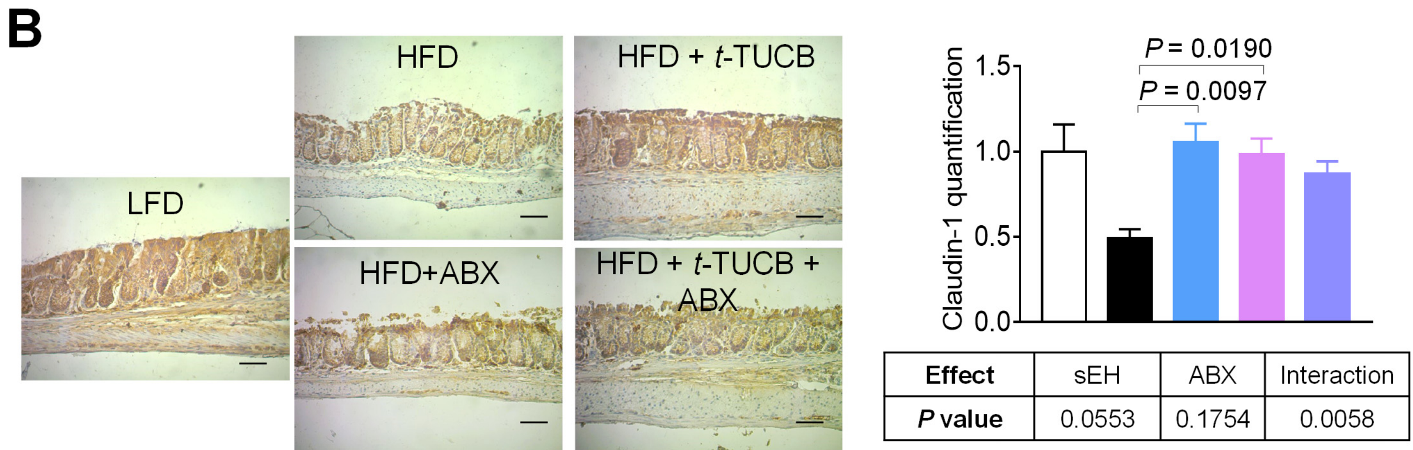


Effect	sEH	ABX	Interaction
***P* value**	0.2678	0.0765	n.s.

Figure S4. Body weight of treated mice (see scheme of animal experiment in Fig. 3A). Top panel: bodyweight of the treated mice over time. Bottom panel: data analysis based on the body weight on week 12. The data are mean ± SEM, n = 11-12 mice per group. ***Data analysis method for the 5-group comparison:*** Analysis of sEH inhibition (*t*-TUCB vs. Vehicle) and antibiotics (water vs. ABX) under the high-fat diet along with a control group (LFD) is performed using the nested two-way ANOVA (with homogeneous/heterogeneous variance) designed for the factorial plus control experiment, followed by (i) the test between the (LFD) group and (HFD) group and (ii) the Tukey's multiple comparison test for the comparisons among the four groups (HFD, HFD + *t*-TUCB, HFD + ABX, HFD + *t*-TUCB + ABX). Note that the validity of the nested two-way ANOVA model assumptions was assessed by the analysis of residuals and Q-Q plot.



Effect	sEH	ABX	Interaction
P value	0.0003	0.2189	0.0033



Effect	sEH	ABX	Interaction
P value	0.0553	0.1754	0.0058

Figure S5. Pharmacologic inhibition of sEH attenuates obesity-induced reduction of tight junction proteins in colon. (***A***) Gene expression of *Claudin-1* in colon (n = 8-10 mice per group). (***B***) Immunohistochemical staining of Claudin-1 in colon (scale bar: 50 µm, n = 5-6 mice per group). The data are mean ± SEM, see data analysis method for the 5-group comparison in Fig. S4.

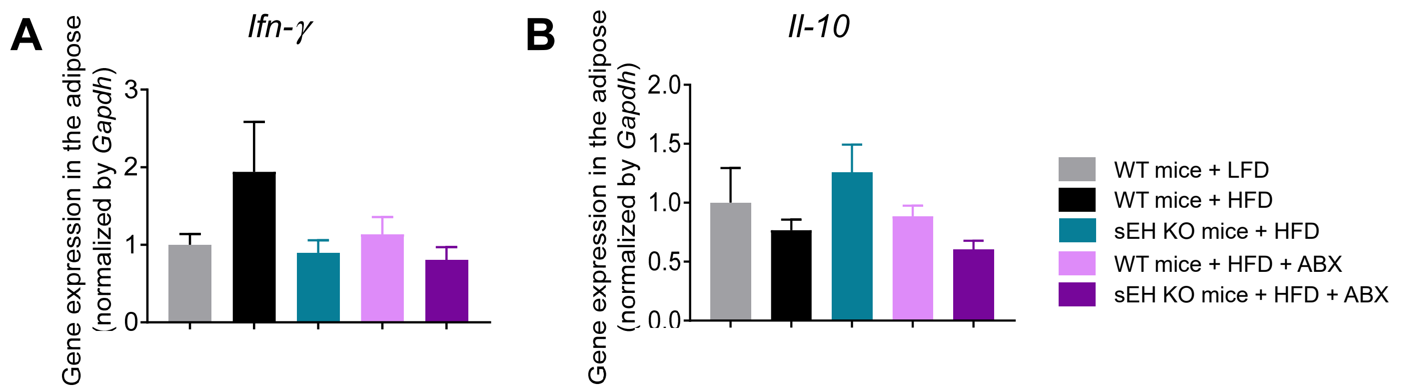


Figure S6. The effect of genetic ablation of sEH on gene expression of cytokines in adipose tissues. (*A*) Gene expression of *Ifn-γ* in adipose (n = 6-7 mice per group). (B) Gene expression of *Il-10* in adipose (n = 5-9 mice per group). The data are mean ± SEM.

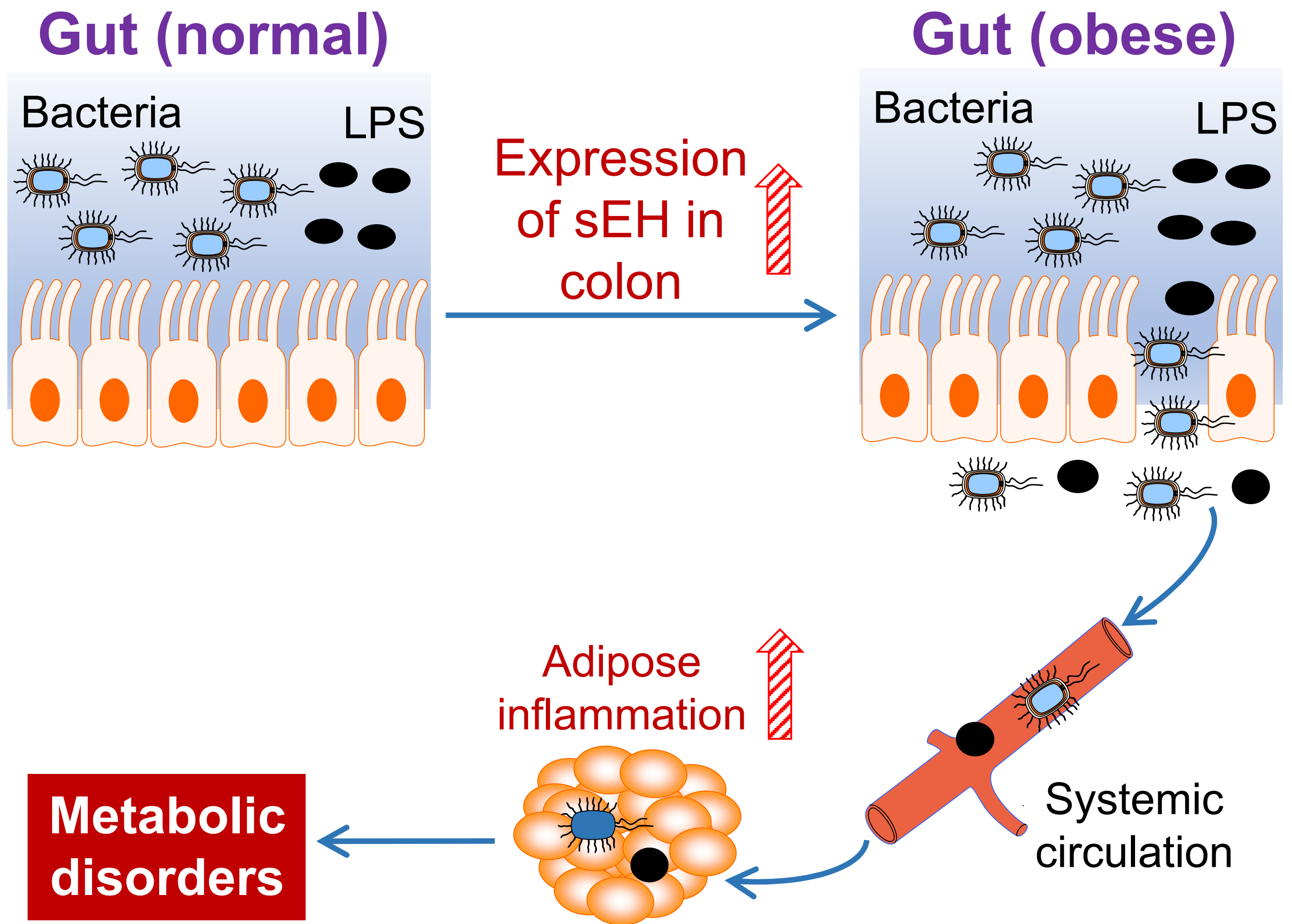
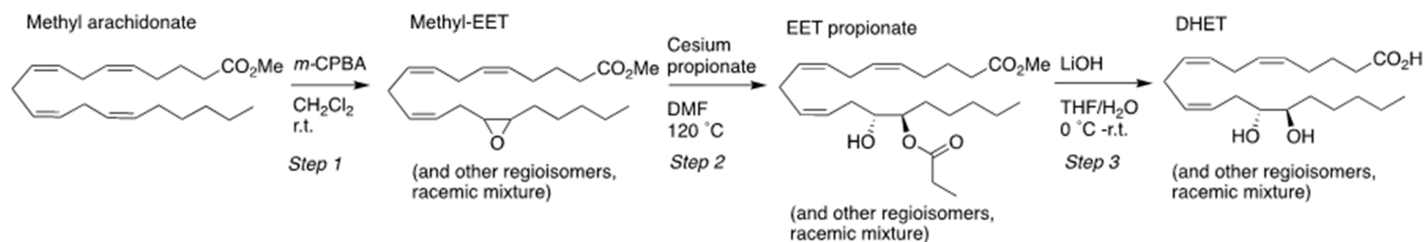


Figure S7. Proposed model: sEH is a novel therapeutic target of obesity-induced intestinal barrier dysfunction.



Scheme S1. Chemical synthesis of mixed DHETs for the animal experiment.