Supporting Information

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SI Materials and Methods

Generation of *fat-1* Mice Colonies and DNA Genotyping. Hemizygous *fat-1* mice were generated and backcrossed onto a C57BL/6 background as previously described (1). Male *fat-1* mice were mated with WT female mice to obtain offspring, with half being hemizygous *fat-1* mice. Genomic DNA from the ear was isolated using the Omni-Pure Tissue Genomic DNA System (Gene Link) following the manufacturer's protocol and genotyped by PCR. The PCR (20 μL) contained 0.2 μM primers (FAM fluorescent-labeled 5′-CTGCACCACGCCTTCACCAACC-3′ for forward direction and unlabeled 5′-ACACAGCAGCAGATTCCAGA-GATT-3′ for reverse), 0.2 mM dNTPs mix, 1.5 mM $MgCl_2$, and 1 U Platinum Taq DNA Polymerase (Invitrogen). PCR cycle conditions were 15 min at 95 °C followed by 30 cycles of 20 s at 94 °C, 40 s at 62 °C and 1 min at 72 °C, and a final step of 10 min at 72 °C and then cooled to 4 °C. PCR products were analyzed by capillary electrophoresis in a 3130 Genetic Analyzer (Applied Biosystems) (Fig. S7*A*). Genotyping was also performed by conventional PCR in the conditions described above using unlabeled primers, and PCR bands were separated by electrophoresis in 2.5% LM Sieve agarose gels and visualized by GelRed Nucleic Acid Gel Stain (Biotium), using a 100-bp DNA ladder marker (Invitrogen).

Experimental Studies. Male hemizygous *fat-1* (n = 31) and WT (n = 46) mice were housed in wood-chip bedding cages with 50–60% humidity and 12-h light/dark cycles. At 6 wk of age, mice were placed on either a standard rodent chow diet (Chow) (13% kcal from fat) or a HFD (60% kcal from fat; Research Diets) and randomly assigned into two treatment groups receiving either the sEH inhibitor *t*-TUCB or placebo (1% polyethylene glycol 400) for 16 wk. *t*-TUCB was given in the drinking water (10 μg/mL) to yield ∼1.67 mg·kg·d. Drinking water was changed every 2 d and water consumption was monitored throughout the experiment. A schematic diagram of the experimental design is shown in Fig. S7*B*. At the end of the intervention period, mice were euthanized via ketamine/xylazine injection (intraperitoneally, 4:1), and the liver and adipose tissue were excised, rinsed in DPBS, fixed in 10% (vol/vol) formalin, and embedded in paraffin. In addition, portions of liver and adipose tissue were snap-frozen in liquid nitrogen for RNA, protein and fatty acid analyses. An additional group of WT mice (n = 9) received HFD supplemented with EPA/DHA as described in ref. 2. All animal studies were conducted in accordance with the Investigation and Ethics Committee criteria of the Hospital Clínic and European Union legislation.

MRI and Spectroscopy. MR imaging was conducted on a 7.0T BioSpec 70/30 horizontal animal scanner (Bruker BioSpin), equipped with a 12-cm inner diameter actively shielded gradient system (400 mT/m) and a receiver/transceiver coil covering the whole mouse volume. Animals were placed in supine position in a Plexiglas holder with a nose cone for administering anesthetic gases (isofluorane in a mixture of 30% O_2 and 70% CO_2), fixed using a tooth bar, ear bars, and adhesive tape. Tripilot scans were used for accurate positioning of the animal in the isocenter of the magnet. High-resolution T2-weighted images were acquired by RARE (rapid acquisition with rapid enhancement) sequence applying repetition time = 4,534 ms, echo time = 19 ms, RARE factor = 8, eight averages, number of slices = 19 for horizontal view, field-of-view = 100 × 40 mm, matrix size = 640 × 256 pixels, resulting in a spatial resolution of 0.156 × 0.156 mm in 1.5-mm slice thickness. Coronal images included 40 slices, field-of-view = 40 × 40 mm, matrix size = 256 × 256 pixels, with the same slice thickness and same spatial resolution. These two sets of images (coronal and horizontal) were acquired with and without fat suppression to subtract them and quantify the total fat volume. Images were processed in ImageJ software, normalized for signal intensity, subtracted, and binarized. A threshold mask was used over the binary images to select all pixels belonging to fat tissue. Localized ^{1}H MR spectroscopy of the liver was acquired with the same system. The liver slice with the largest gross dimensions was chosen, and MR spectroscopy for water and fat quantification was obtained using a point-resolved spectroscopy sequence. After line broadening and phase and baseline correction, the peak area of the water at 4.77 ppm and fat resonance (FR) at 1.40 ppm were measured. Quantification of the fat content was done by comparing the area of the FR with that of the unsuppressed water. Spectroscopic data were processed using the Paravision 5.1 software (Bruker BioSpin). The hepatic fat percentage was calculated by dividing (FR) by the sum of FR and peak area of water.

Histology and Immunohistochemistry Analysis. Adipose tissue samples fixed in 10% formalin were embedded in paraffin, cut into 5-μm sections, and stained with H&E. Detection of F4/80 by immunohistochemistry was performed in deparaffinized adipose tissue sections which were rehydrated, and pretreated with 0.05% trypsin-0.1% $CaCl_2$ for 20 min at 37 °C to unmask the antigen. Thereafter, sections were incubated in 0.3% H_2O_2 for 25 min at room temperature and dark conditions to block endogenous peroxidase activity and in 2% (wt/vol) BSA for 20 min at room temperature to avoid unspecific binding of the primary antibody. Sections were then incubated overnight at 4 °C with the primary rat anti-mouse F4/80 antibody (dilution 1:400; AbD Serotec), followed by incubation for 30 min at room temperature with a biotinylated rabbit anti-rat IgG secondary antibody (1:200) and incubation with ABC for 30 min at room temperature using the Vectastain ABC Kit (Vector Laboratories). Color was developed using the diaminobenzidine substrate (Dako Diagnostics), and sections were counterstained with Gill's hematoxylin. Fibrosis in adipose tissue was assessed in paraffin sections by Sirius red staining. Briefly, sections were incubated for 10 min in 0.5% thiosemicarbazide and stained in 0.1% Sirius red F3B in saturated picric acid for 1 h, and subsequently washed with an acetic acid solution (0.5%). Fibrosis was also assessed by Masson's trichrome staining at the Pathology Department of the Hospital Clínic. Hepatic lipid content was assessed by Oil Red-O staining in optimal cutting temperature compound-embedded samples. Briefly, cryosections were fixed in 60% (vol/vol) isopropanol for 10 min and stained with 0.3% Oil Red-O in 60% isopropanol for 30 min and subsequently washed with 60% isopropanol. Sections were counterstained with Gill's hematoxylin, washed with acetic acid solution (4%, vol/vol) and mounted with aqueous solution. Sections were visualized at 200× magnification in a Nikon Eclipse E600 microscope and the relative areas of macrophage infiltration, steatosis, and fibrosis were quantitated by histomorphometry using Olympus Cell (Olympus). Adipocyte cross-sectional area was assessed using ImageJ.

Isolation and Incubation of Primary Hepatocytes. Hepatocytes were isolated from WT and *fat-1* mice by a three-step in situ perfusion procedure using 0.03% collagenase IV through the portal vein, as described previously (2). Isolated hepatocytes were seeded on

collagen I-coated plates and cultured in William's E medium supplemented with 10% (vol/vol) FBS, 2 mM L-glutamine, 50 U/mL penicillin, 50 μg/mL streptomycin, 1 μM insulin, 15 mM Hepes, and 50 μM β-mercaptoethanol. Primary hepatocytes were characterized by a combination of phase-contrast microscopy and immunocytochemical analysis, and their viability was determined by Trypan blue exclusion. Hepatocytes were maintained in a humidified 5% CO_2 incubator at 37 °C and cultured in 12-well plates (4×10^5 cells per well) in 1% FBS-William's E medium with 1% fatty acid-free (FAF) BSA and incubated with either vehicle (18.4% FAF-BSA) or 0.5 mM sodium palmitate freshly prepared in DMEM containing 18.4% FAF-BSA for 24 h to induce autophagy. In some experiments, the protease inhibitors E64d (10 μg/mL) and pepstatin A (10 μg/mL) were added. In selected experiments, the cells were also treated with *t*-TUCB (1 μM) alone or in combination with 19,20-EDP (1 μM), 17,18-EEQ (1 μM), 14,15-EET (1 μM), 17(S)-HDHA (1 μM), and DHA (1 and 10 μM) (Cayman Chemical) for 24 h. At the end of the incubation period, hepatocytes were scraped into ice-cold $DPBS^{-/-}$ and resuspended in lysis buffer for protein extraction or stained with Oil Red-O for assessment of intracellular lipid content, as described below.

Differentiation and Incubation of 3T3-L1 Adipocytes. Mouse 3T3-L1 cells were seeded onto six-well plates (250,000 cells per well) in DMEM supplemented with 10% (vol/vol) FBS, 100 U/mL penicillin/streptomycin and 4 mM L-glutamine in a humidified atmosphere of 5% CO_2 at 37 °C and allowed to grow to confluence for 2 d, as described previously (3). Confluent 3T3-L1 cells were cultured in adipocyte induction medium containing insulin (5 μg/mL), isobutylmethylxanthine (0.5 mM), dexamethasone (0.25 μM), penicillin/streptomycin (100 U/mL), and L-glutamine (4 mM) in DMEM supplemented with 10% FBS. After 2 d, the cells were cultured in continuation medium (5 μg/mL insulin) for 72 h and then maintained in DMEM supplemented with 10% FBS until exhibiting an adipocyte phenotype, as characterized by a combination of phase-contrast microscopy and Oil Red-O staining (see below). At day 7–8 of differentiation, 0.5 mM sodium palmitate was added for 24 h to induce autophagy in the absence or presence of protease inhibitors E-64d (10 μg/mL) and pepstatin A (10 μg/mL). In some experiments, the cells received *t*-TUCB (1 μM) alone or in combination with 19,20-EDP (1 μM), 17,18-EEQ (1 μM), 14,15-EET (1 μM), and DHA (1 and 10 μM) for 24 h. At the end of the incubation period, adipocytes were either stained with Oil Red-O or scraped into ice-cold $DPBS^{-/-}$ and resuspended in lysis buffer or TRIzol for protein extraction or RNA isolation, respectively.

Oil Red-O Staining of Hepatocytes and Adipocytes. Cells were washed twice with $DPBS^{-/-}$, exposed to 4% (vol/vol) paraformaldehyde for 1 h, and then washed with 60% isopropanol before incubating with 0.2% Oil Red-O for 30 min at room temperature. To quantify the amount of Oil Red-O retained by the cells, hepatocytes and adipocytes were incubated with isopropanol for 30 min with shaking to elute the stain. The amount of staining was measured by optical density at 500 nm in a FluoStar Optima microplate reader (BMG Labtech). Cells were also grown (1×10^5 cells per well) in Permanox Lab-Teck Chamber Slides (Nalge Nunc), stained with Oil Red-O, counterstained with Gill's hematoxylin, washed with tap water, mounted with aqueous solution, and visualized under a Nikon Eclipse E600 microscope at 200× magnification.

Measurement of 2-Deoxyglucose Uptake. Adipocytes were incubated with either vehicle (18.4% FAF-BSA) or 0.5 mM sodium palmitate in the absence or presence of 19,20-EDP (1 μM) or *t*-TUCB (1 μM) for 24 h. Thereafter, the cells were washed twice with $DPBS^{-/-}$ and incubated with 2-deoxyglucose (2DG) (1 mM) with or without insulin (1 μM) in Krebs Ringer Phosphate Hepes buffer containing 2% BSA for 20 min at 37 °C. Cells were washed three times with cooled PBS containing the glucose uptake inhibitor phloretin (200 μM), scraped and resuspended in Tris·HCl buffer (10 mM, pH8). Cell lysates were heat-treated (80 °C, 15 min) and centrifuged (15,000 × *g*, 20 min, 4 °C), and 2DG uptake was determined in supernatants (diluted 1:4) using a 2DG Uptake Measurement Kit from Cosmo Bio, based on an enzymatic method for the direct measurement of 2DG-6-phosphate.

RNA Isolation, Reverse Transcription, and Real-Time PCR. Isolation of total RNA from liver, adipose tissue, hepatocytes, and adipocytes was performed using the TRIzol reagent. RNA concentration was assessed in a NanoDrop-1000 spectrophotometer (NanoDrop Technologies), and its integrity tested with a RNA 6000 Nano Assay in a Bioanalyzer 2100 (Agilent Technologies). cDNA synthesis from 1 μg of total RNA was performed using the High-Capacity cDNA Archive Kit (Applied Biosystems). Validated and predesigned TaqMan primers and probes from Assays-on-Demand were used to quantify CYP1A1 (Mm00487218), CYP2E1 (Mm00491127), CYP2U1 (Mm01310397), F4/80 (Emr1; Mm00802529), MCP-1 (*Ccl2*; Mm00441242), Δ5 and Δ6 desaturases (*Fads1*; Mm00507605 and *Fads2*; Mm00517221), TNFα (Mm00443258), IL-1β (Mm01336189), IL-6 (Mm00446190), MGL1 (Mm00546124), CD206 (Mannose Receptor type 1; Mm00485148), IL-10 (Mm00439614), RELMα (Mm00445109), Arg1 (Mm00475988), Ym1 (Chitinase-3 like protein 3; Mm00657889), GLUT-4 (Mm00436615), and IRS-1 (Mm01278327), using β-actin (ID Mm00607939) as the endogenous control. Real-time PCR amplifications were carried out in an ABI Prism 7900HT Sequence Detection System and the results were analyzed with the Sequence Detector Software version 2.1 (Applied Biosystems). Relative quantification of gene expression was performed using the comparative Ct method. The amount of target gene, normalized to β-actin and relative to a calibrator, was determined by the arithmetic equation $2^{-\Delta\Delta Ct}$ described in the comparative Ct Method (docs.appliedbiosystems.com/pebiodocs/04303859.pdf).

Analysis of Protein Expression by Western Blot. Total protein from liver and adipose tissue was extracted using a lysis buffer containing 50 mM Hepes, 20 mM β-glycerophosphate, 2 mM EDTA, 1% Igepal, 10% (vol/vol) glycerol, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 150 mM NaCl, supplemented with protease inhibitor (Complete Mini; Roche Diagnostics) and phosphatase inhibitor (PhosSTOP; Roche Diagnostics) mixtures. For protein isolation from hepatocytes and adipocytes, cells were scraped into ice-cold DPBS and resuspended in 150 μL of lysis buffer. Homogenates were incubated on ice for 10–15 min and centrifuged at either 1,000 × *g* for 2 min (cells) or 9,300 × *g* for 20 min (tissue) at 4 °C. Total protein (50 μg) from supernatants was placed in SDS-containing Laemmli sample buffer, heated for 5 min at 95 °C, and separated by 10% (tissue) or 15% (cells) (vol/vol) SDS/PAGE for 90 min at 120 V. Transfer was performed by the iBlot Dry Blotting System (Invitrogen) onto PVDF membranes at 20 V over 5–7 min, and the efficiency of the transfer was visualized by Ponceau S staining. The membranes were then soaked for 1 h at room temperature in 0.1% T-TBS and 5% (wt/vol) nonfat dry milk. Blots were washed three times for 5 min each with 0.1% T-TBS and subsequently incubated overnight at 4 °C with primary rabbit anti-mouse Δ5 desaturase (ab126706; dilution 1/1000; Abcam), Δ6 desaturase (M-50; dilution 1:200; Santa Cruz Biotechnology), sEH (Ab155280; Abcam), Atg7 (8558; dilution 1/1000; Cell Signaling), Atg12/Atg5 (4180; dilution 1/1000; Cell Signaling), rabbit polyclonal phospho-IRE-1α (NB100-2323; dilution 1/1000; Novus Biologicals), rabbit polyclonal phospho-eIF2α (ab4837; dilution 1/1000; Abcam), mouse monoclonal eIF2α (sc-133132; dilution 1/200; Santa

Cruz Biotechnology), p62 (Ab109012; dil 1/10,000, Abcam), and LC3I/II (12741S; dil 1/1000; Cell Signaling) in 0.1% T-TBS containing 5% BSA. Thereafter, the blots were washed three times for 5 min each with 0.1% T-TBS and incubated for 1 h at room temperature with a horseradish-peroxidase-linked donkey anti-rabbit antibody (1:2,000) in 0.1% T-TBS containing 5% nonfat dry milk, and the bands were visualized using the EZ-ECL chemiluminescence detection kit (Biological Industries). To assess housekeeping protein expression, the membranes were stripped at 50 °C for 20 min in 100 mM β-mercaptoethanol, 2% (vol/vol) SDS, and 62.5 mM Tris·HCl (pH 6.8) and reblotted overnight at 4 °C with primary rabbit anti-mouse GAPDH (ab9485; dilution 1/2500; Abcam) antibody, β-actin HRP conjugate (5125; dilution 1/1000; Cell Signaling), and primary mouse anti-mouse α-tubulin (T6074; dilution 1/1000; Sigma-Aldrich) and the membranes visualized, as described above.

Analysis of CYP Metabolites by LC-ESI-MS/MS. Thirty milligrams of frozen adipose tissue were homogenized in liquid nitrogen and 14,15-EET-d8 (Cayman Chemical) (10 ng) were added as internal standard in 300 μL of 10 M sodium hydroxide solution and heated for 30 min at 60 °C for alkaline hydrolysis. The samples were brought to pH6 with 500 μl of 1 M sodium acetate buffer and acetic acid for hydrolyzed samples. After centrifugation, the supernatant was added to Bond Elute Certify II columns (Agilent Technologies) for solid-phase extraction (SPE) in a SPE Vacuum Manifold. The columns were preconditioned with 3 mL methanol, followed by 3 mL of 0.1 M sodium acetate buffer containing 5% (vol/vol) methanol (pH6). The SPE-columns were then washed with 3 mL methanol/H_2O (50/50, vol/vol) and eluted with 2 mL of n-hexane:ethyl acetate (25:75, vol/vol) with 1% acetic acid. The eluate was evaporated on a heating block at 40 °C under a stream of nitrogen to obtain a solid residue. The residue was dissolved in 70 μL acetonitrile and analyzed using an Agilent 1200 HPLC system with binary pump, degasser, autosampler and column thermostat with a Kinetex C-18, 2.1 × 150 mm, 2.6 μm column (Phenomenex), using a solvent system of aqueous formic acid (0.1%) and acetonitrile. The elution gradient was started with 5% (vol/vol) acetonitrile, which was increased within 0.5 min to 55, 14.5 min to 69%, 14.6 min to 95% and held there for 5.4 min. The flow rate was set at 0.3 mL/min; the injection volume was 7.5 μL. The HPLC was coupled with an Agilent 6460 Triplequad mass spectrometer (Agilent Technologies) with ESI source. Analysis was performed with Multiple Reaction Monitoring in negative mode with the following source parameters (drying gas: 250 °C/10 l/min, sheath gas: 400 °C/10 l/min, capillary voltage: 4500 V, nozzel voltage: 1500 V and nebulizer pressure: 30 psi). The LC-MS/MS conditions used to profile the omega-3 and omega-6 epoxides are described in Table S2. Quantification was performed using standard calibration curves for each corresponding synthetic epoxide.

Fatty Acid Profiling by Gas Chromatography. Total lipids were extracted from frozen liver and adipose tissues by the Folch method, with modifications (4). Briefly, chloroform/methanol (2:1 vol/vol) containing 0.005% butylated hydroxytoluene (as antioxidant) was added and mixed vigorously for 30 s before adding 100 μL of 0.25% $MgCl_2$ and 1 mL of 0.01N HCl and mixed again. The chloroform phase containing lipids was collected. The remains were extracted with 3 mL of chloroform. The chloroform phases were pooled and dried under nitrogen and subjected to methylation. Fatty acid methyl esters were prepared by methods similar to those described previously (2, 3) using methanol containing 14% boron trifluoride (BF_3/MeOH). The extracted lipid samples were mixed with BF_3/MeOH reagent (1 mL), and the mixtures were heated at 100 °C in a metal block for 1 h, cooled to room temperature, and methyl esters were extracted twice in the upper (hexane) layer after addition of 1 mL H_2O. The samples were centrifuged at 1,200 × *g* for 10 min and then the upper hexane layer was removed and evaporated under nitrogen. Recovery yields were similar for tissue samples from both WT and *fat-1* mice (85.9 ± 0.9 for WT and 93.3 ± 0.8 for *fat-1*). Fatty acid methyl esters were analyzed by flame ionization gas chromatography (GC). GC analysis was carried out with an Agilent 7890 Autosampler apparatus (Agilent Technologies) equipped with a capillary column (SupraWAX-280, Teknokroma), length 30 m, 0.25 mm i.d., and film thickness 0.25 μm. Column conditions were: initial temperature, 120 °C for 1.0 min; ramp 15 °C/min to 210 °C hold to 35 min; carrier gas, helium. A mixture of standard fatty acids methyl esters (Restek Corporation) was included as an external standard with each run for peak identification by comparison of their retention times. Data acquisition and processing were performed with AgilentChemstation software for GC systems. Each fatty acid was expressed as percentage of total fatty acids.

Statistical analysis of the results was performed by analysis of variance (one-way or two-way ANOVA) or unpaired Student's *t* test. Results are expressed as mean ± SEM and differences considered significant at $P < 0.05$.

1. Kang JX, Wang J, Wu L, Kang ZB (2004) Transgenic mice: *fat-1* mice convert n-6 to n-3 fatty acids. *Nature* 427(6974):504.
2. López-Vicario C, et al. (2014) Molecular interplay between Δ5/Δ6 desaturases and long-chain fatty acids in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 63(2):344–355.
3. García-Alonso V, et al. (2013) Coordinate functional regulation between microsomal prostaglandin E synthase-1 (mPGES-1) and peroxisome proliferator-activated receptor γ (PPARγ) in the conversion of white-to-brown adipocytes. *J Biol Chem* 288(39): 28230–28242.
4. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226(1):497–509.

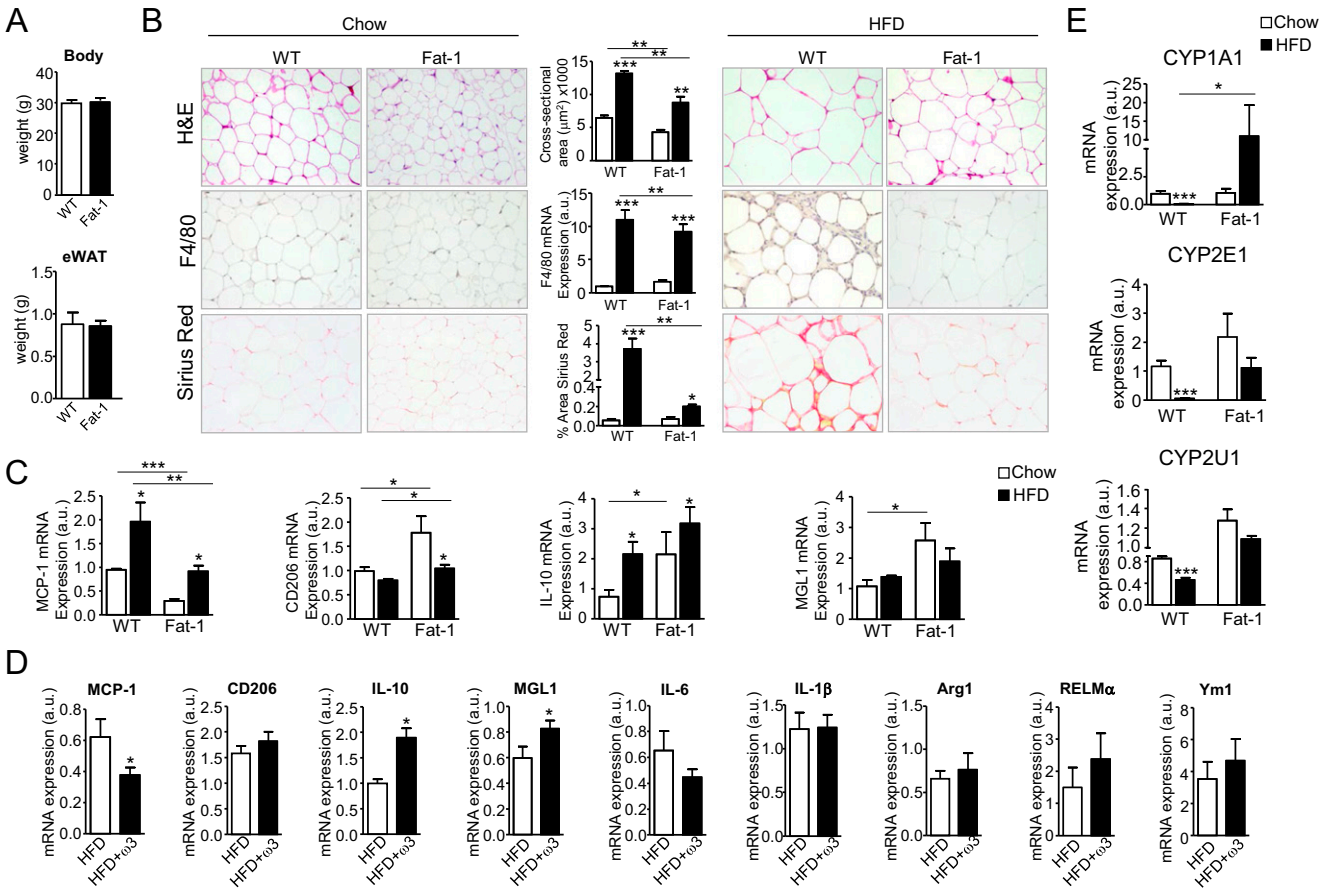

Fig. S1. CYP epoxygenase expression in adipose tissue from *fat-1* mice. (*A*) Body and eWAT weights in WT ($n = 5$) and *fat-1* ($n = 8$) mice under Chow diet for 16 wk. (*B*) Representative photomicrographs of H&E-stained adipose tissue sections (*Top*), F4/80 (*Middle*), and Sirius red (*Bottom*) in WT ($n = 25$) and *fat-1* ($n = 12$) mice after receiving either Chow or HFD for 16 wk. (Magnification: 200×.) Quantification of adipocyte cross-sectional area, Sirius red staining and F4/80 mRNA expression is also shown. (*C*) MCP-1, CD206, IL-10, and MGL1 expression in adipose tissue. (*D*) Gene expression analysis of MCP-1, CD206, IL-10, MGL1, IL-6, IL-1β, Arg1, RELMα, and Ym1 in eWAT from WT mice (HFD) ($n = 10$) and WT mice fed an omega-3–rich diet (HFD+ω3) ($n = 9$) receiving a HFD for 16 wk. (*E*) CYP1A1, CYP2E1 and CYP2U1 expression in adipose tissue. Results are mean ± SEM $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

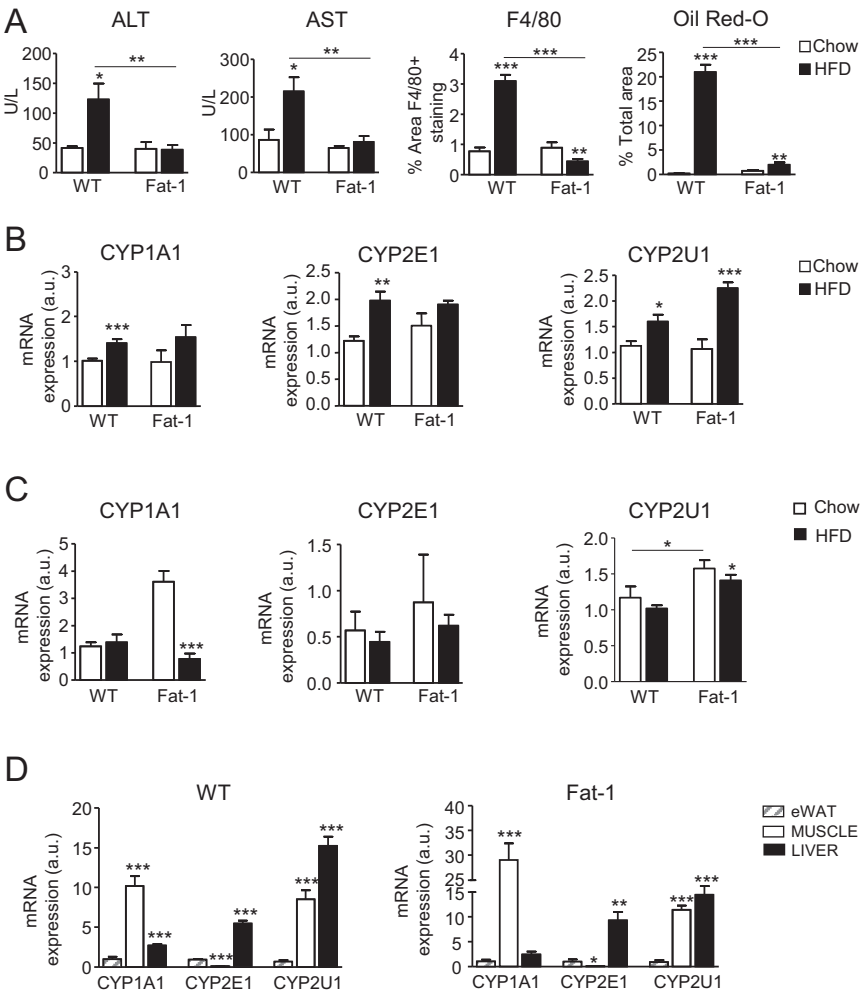

Fig. S2. CYP epoxygenase expression in liver from *fat-1* mice. (*A*) Serum ALT and AST and analysis of hepatic F4/80 and Oil Red-O staining in WT ($n = 25$) and *fat-1* ($n = 12$) mice after receiving either Chow or HFD for 16 wk. (*B*) Hepatic CYP1A1, CYP2E1, and CYP2U1 expression. (*C*) Gene expression of CYP1A1, CYP2E1, and CYP2U1 in skeletal muscle. (*D*) Gene expression of CYP1A1, CYP2E1 and CYP2U1 in eWAT, skeletal muscle and liver from WT and *fat-1* mice under Chow diet. Results are mean ± SEM $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

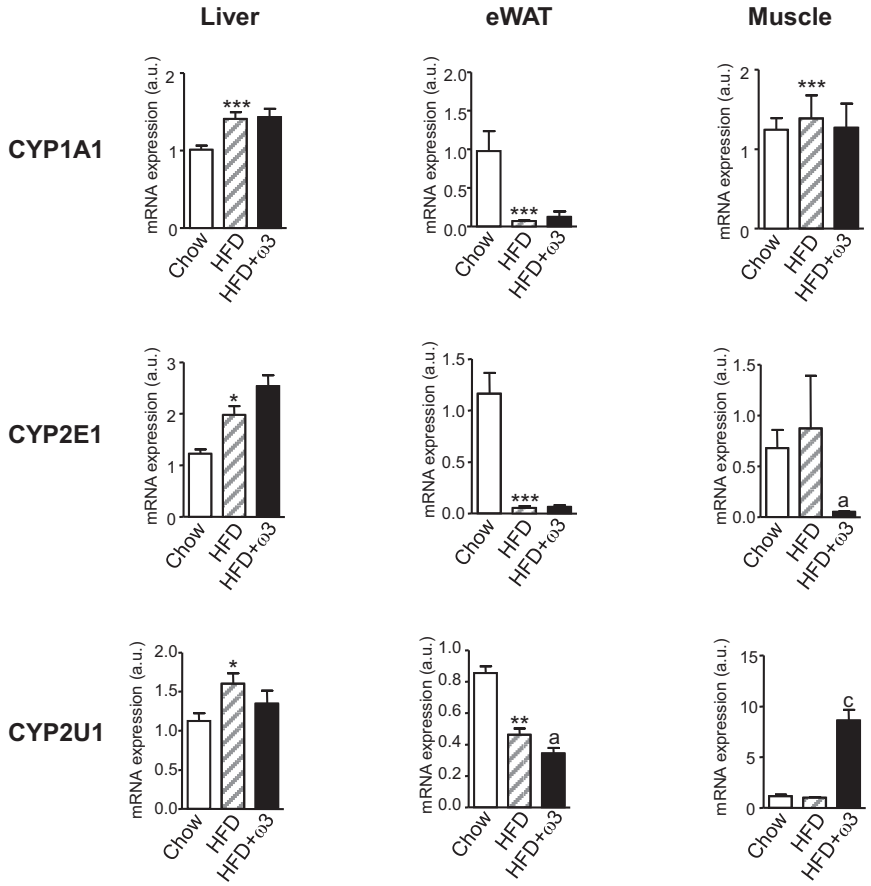

Fig. S3. Gene expression of CYP1A1, CYP2E1, and CYP2U1 in liver, eWAT and skeletal muscle from WT mice receiving either Chow diet ($n = 7$), HFD ($n = 10$), or HFD enriched with omega-3 fatty acids (HFD+ω3) ($n = 9$) for 16 wk. Results are expressed as mean ± SEM *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. Chow and $^{a}P < 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$ vs. HFD.

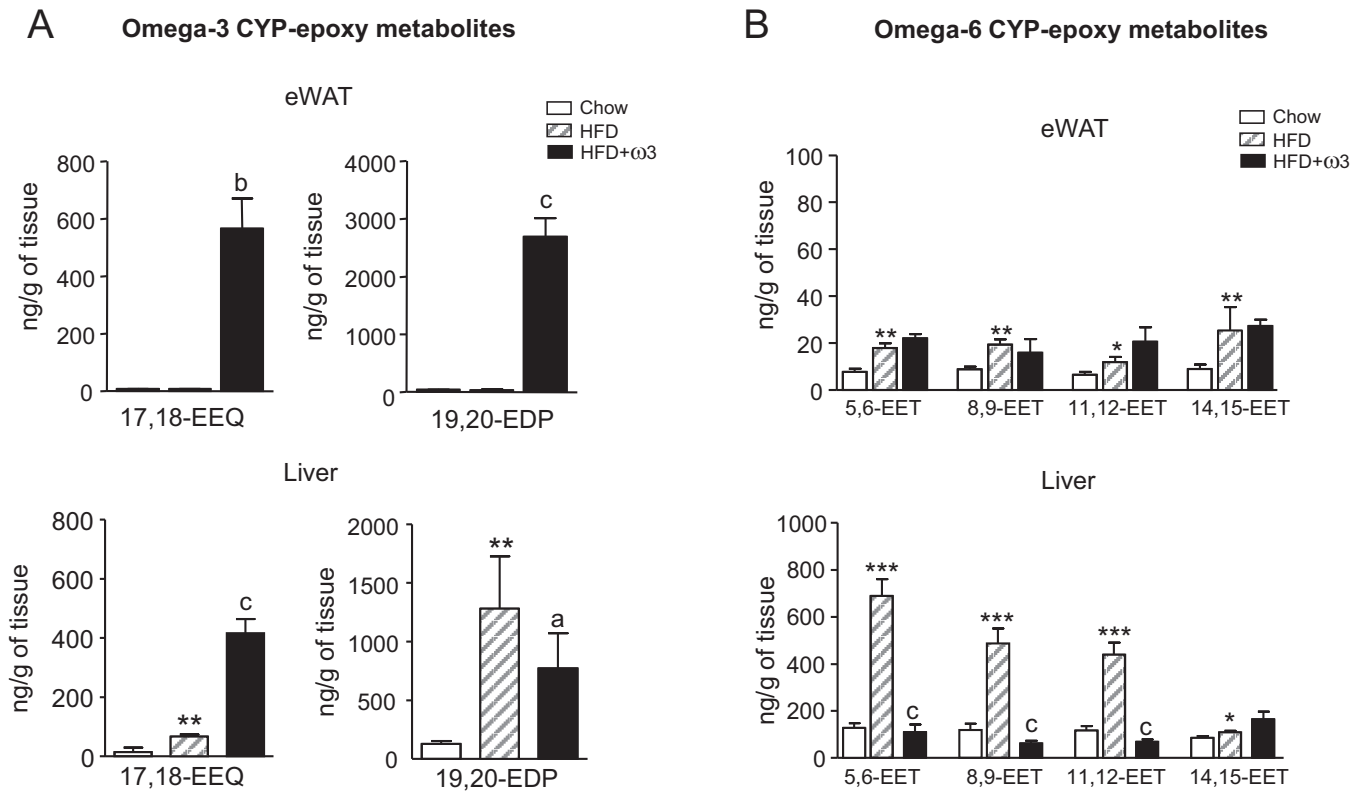

Fig. S4. (*A*) Analysis of the levels of omega-3 CYP-epoxy metabolites (17,18-EEQ and 19,20-EDP) by LC-ESI-MS/MS in eWAT and liver from WT mice receiving either Chow diet ($n = 7$), HFD ($n = 10$), or HFD enriched with omega-3 fatty acids (HFD+ω3) ($n = 9$) for 16 wk. (*B*) Analysis of the levels of omega-6 CYP-epoxy metabolites (5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET) in these animals by LC-ESI-MS/MS. Results are expressed as mean ± SEM $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. Chow and $^{a}P < 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$ vs. HFD.

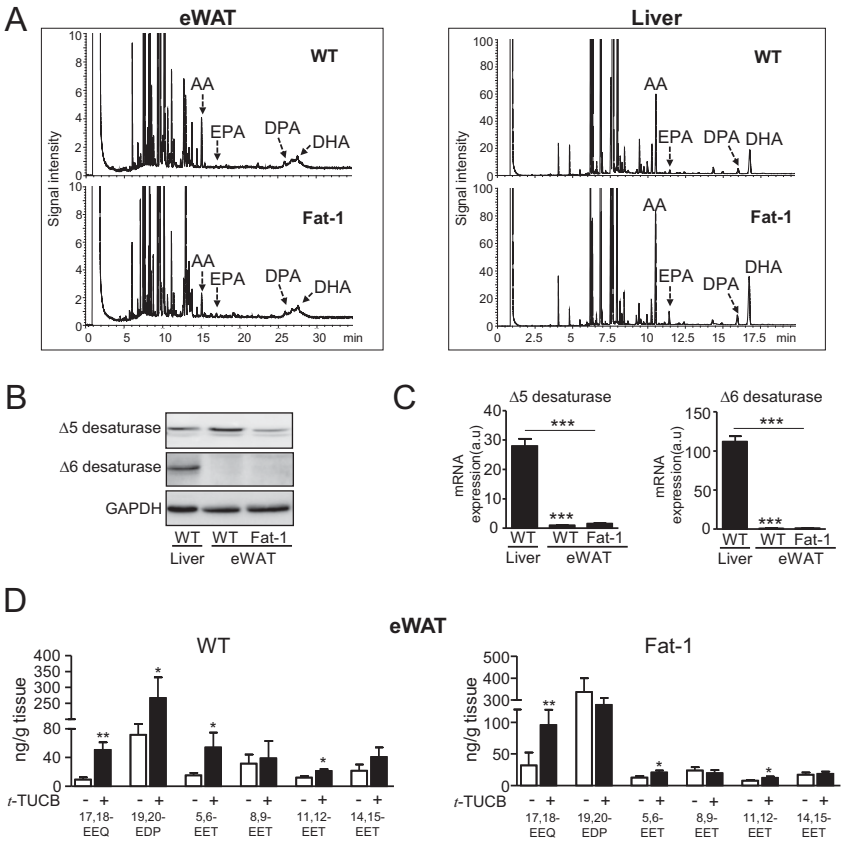

Fig. S5. (*A*) Representative GC chromatograms of eWAT and liver content of omega-6- and omega-3 PUFA in WT and *fat-1* mice. Protein (*B*) and mRNA (*C*) expression of Δ5 and Δ6 desaturases in eWAT and liver from WT and *fat-1* mice. (*D*) LC-ESI-MS/MS analysis of omega-3 and omega-6 epoxides in eWAT from HFD-induced obese WT and *fat-1* mice receiving *t*-TUCB (10 μg/mL) for 16 wk. Results are mean ± SEM $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

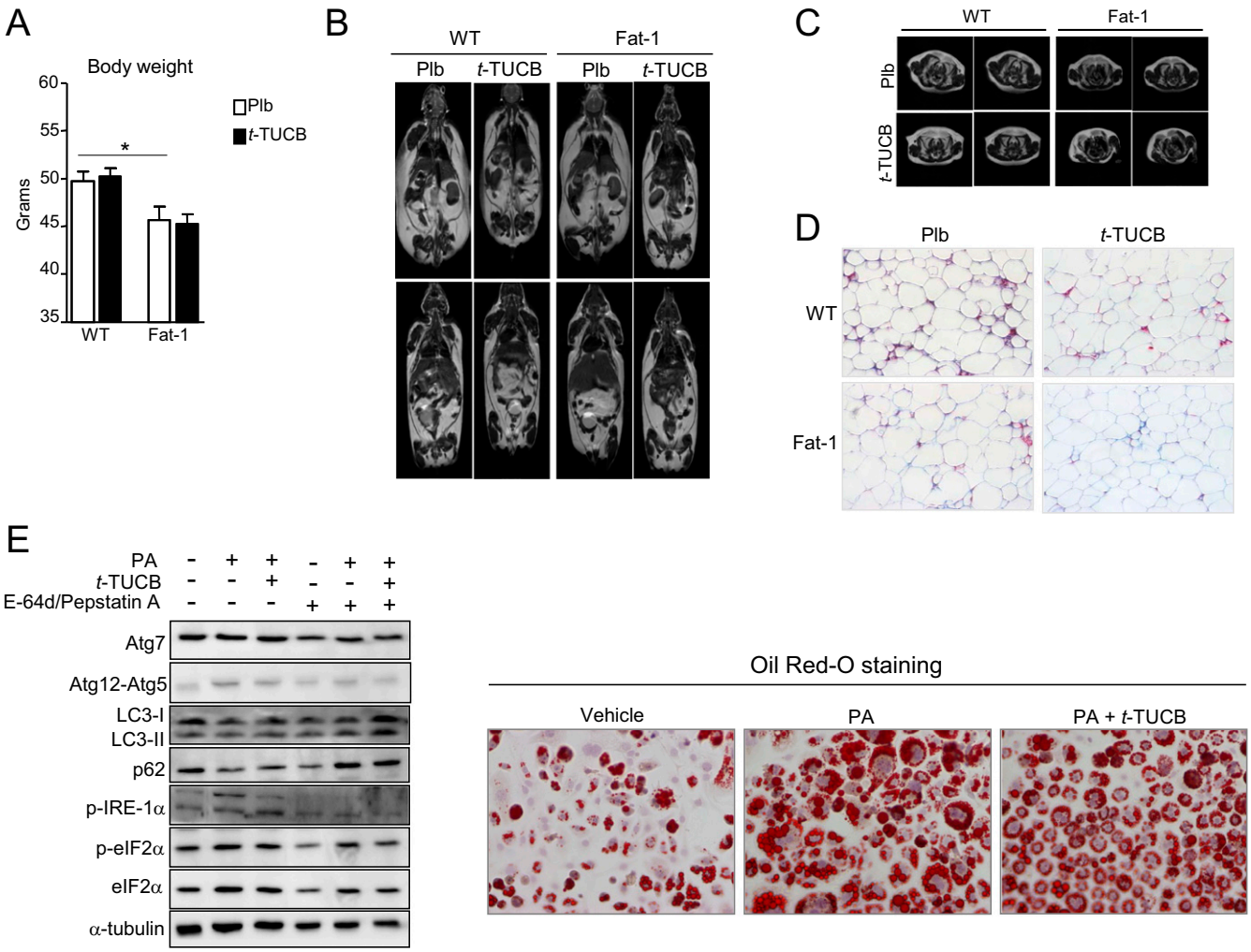

Fig. S6. (*A*) Endpoint body weight for WT ($n = 28$) and *fat-1* ($n = 20$) mice receiving a HFD and treated with either placebo (Plb) or *t*-TUCB for 16 wk. (*B*) Representative MR images of axial sections. (*C*) Representative MR images of coronal sections from the interscapular area. (*D*) Representative photomicrographs of Masson's trichrome-stained adipose tissue sections. (*E*) Protein expression of Atg7, Atg12-Atg5, LC3-I/II, p62, phosphoIRE-1α (p-IRE-1α), phosphoeIF2α (p-eIF2α), total eIF2α (eIF2α), and α-tubulin in differentiated 3T3-L1 adipocytes incubated with either vehicle or sodium palmitate (PA, 0.5 mM) alone or in combination with *t*-TUCB (1 μM) in the absence or presence of the protease inhibitors E-64d/pepstatin A for 24 h. Western blots were performed in 15% SDS/PAGE. Representative photomicrographs of Oil Red-O–stained cells incubated with either vehicle or PA alone or in combination with *t*-TUCB are shown on the right. Results are mean ± SEM $*P < 0.05$. (Magnification: 200×.)

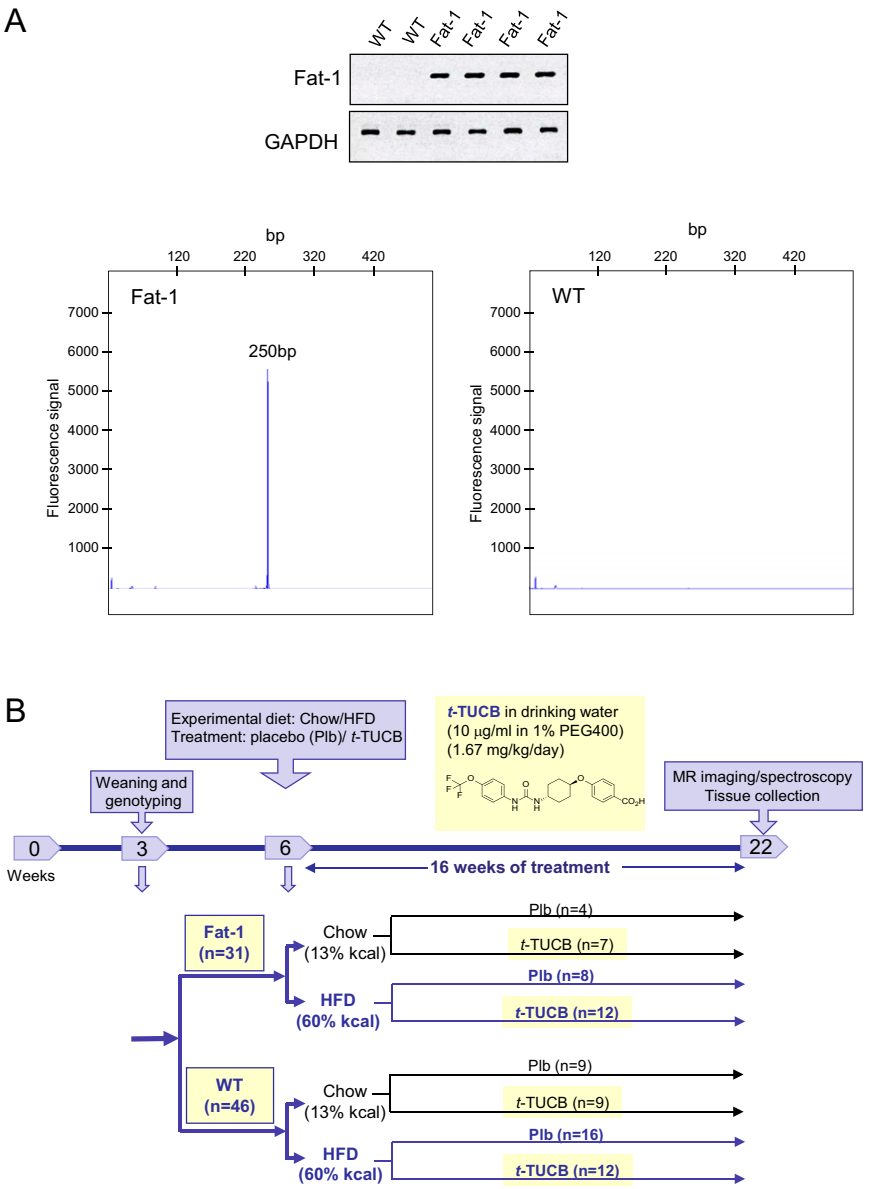

Fig. S7. (*A*) DNA genotyping of transgenic *fat-1* mice by conventional PCR (*Upper*) and by fluorescent-labeled fragment analysis in a ABI 3130 capillary electrophoresis analyzer (*Lower*). (*B*) Schematic diagram of the experimental design of the study. At 3 wk of age, after weaning, male hemizygous *fat-1* and WT mice were genotyped. At 6 wk of age, these animals were placed on either a standard rodent chow diet (Chow) (13% kcal from fat) or HFD (60% kcal from fat) and randomly assigned into two treatment groups receiving either the sEH inhibitor *t*-TUCB or placebo (Plb; 1% polyethylene glycol 400) for 16 wk. *t*-TUCB was given in the drinking water (10 μg/mL) to yield ∼1.67 mg·kg·d. Tissue collection and MR imaging and spectroscopy analysis were carried out at the end of the experimental period.

Table S1. Polyunsaturated fatty acid composition in HFD experimental mice groups

	WT				*fat-1*			
	Liver		eWAT		Liver		eWAT	
PUFA	Placebo	t-TUCB	Placebo	t-TUCB	Placebo	t-TUCB	Placebo	t-TUCB
AA (C20:4n6)	3.47 ± 0.03	3.78 ± 0.53	0.45 ± 0.12	0.43 ± 0.077	3.57 ± 0.08	3.14 ± 0.20	0.42 ± 0.03	0.32 ± 0.05
EPA (C20:5n3)	0.25 ± 0.03	0.28 ± 0.042	1.50 ± 0.54	1.89 ± 0.44	0.33 ± 0.03*	0.39 ± 0.08	1.87 ± 0.01	1.54 ± 0.03
DPA (C22:5n3)	0.51 ± 0.07	0.58 ± 0.08	0.22 ± 0.016	0.26 ± 0.03	0.74 ± 0.05*	0.73 ± 0.04	0.11 ± 0.06	0.18 ± 0.05
DHA (C22:6n3)	1.97 ± 0.02	2.36 ± 0.25	0.58 ± 0.72	0.42 ± 0.11	3.49 ± 0.64*	2.9 ± 0.07	0.33 ± 0.21	0.37 ± 0.11

All values are expressed as percent area/gram tissue.*$P < 0.05$ vs.WT-placebo. AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

Table S2. LC-MS/MS conditions used to determine the epoxides of AA, EPA, and DHA

Compound name	Precursor ion	Product ion	Fragmentor (V)	Collision energy	Cell accelerator (V)	Retention time* (min)
17,18-EEQ	317.2	259.1	120	3	1	10.63
19,20-EDP	343.3	241.0	160	8	2	12.44
14,15-EET	319.2	219.1	120	3	1	12.89
11,12-EET	319.2	167.1	120	2	1	13.67
8,9-EET	319.2	155.1	120	3	1	13.87
5,6-EET	319.2	191.1	120	4	1	14.05
14,15-EET-D8†	327.2	226.1	120	3	8	12.65

*Retention times refer to the Kinetex C-18, 2.1 x 150 mm, 2.6-μm column using a solvent system of aqueous formic acid (0.1%) and acetonitrile. Gradient elution was started with 5% acetonitrile, which was increased within 0.5 min to 55, 14.5 min to 69%, 14.6 min to 95% and held there for 5.4 min. The flow rate was set at 0.3 mL/min.

†Used as internal standard for all epoxides.