Supporting Information

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SI Materials and Methods

Mice. Genotypes were assayed by PCR using Takara Ex Taq DNA Polymerase (Takara Bio). The wild-type *Angptl8* allele was detected using the following oligonucleotides: 5′-AAACCCAGAACAGAGGAAAC-3′ (sense) and 5′-CCTTTATTCCCAGTGCTAGG-3′ (antisense) to amplify a 518-bp fragment; for the mutated allele, 5′-CCTATCCCATTACGGTCAATC-3′ (sense) and 5′-TGATTTGTGTAGTCGGTTTATG-3′ (antisense) in the LacZ gene were used to amplify a 357-bp fragment. The amplification products were size-fractionated on 2% (wt/vol) agarose gels and visualized by UV illumination after staining with ethidium bromide.

Indirect Calorimetry. For measurements of energy homeostasis, mice were acclimated in metabolic cages (TSE Systems; LabMaster System) for 3 d before data collection. Indirect calorimetry O_2 consumption and CO_2 production, food intake, water intake, and physical activity were monitored for 1 min at 30-min intervals for 5 consecutive days. Body composition was determined by nuclear magnetic resonance using a minispec analyzer (Brucker).

Core Body Temperature. Core body temperature was measured using an implantable temperature sensor (temperature transponder; IPTT-300; Bio Medic Data Systems) according to the manufacturer's instructions. Mice were anesthetized with isoflurane and the transponder was implanted under the skin on the back. Temperature was recorded 1 d later.

Blood and Tissue Chemistries. Plasma and tissue lipids were measured using commercial reagents as described in *Materials and Methods*. Venous blood was collected in EDTA tubes from the retroorbital plexus or the tail vein, and plasma lipids were measured using enzymatic assays. Plasma was isolated by centrifugation (4,000 rpm) for 10 min. Plasma triglyceride (TG), cholesterol, and glucose levels were measured using the Vitros 250 System (GMI). Nonesterified fatty acids (NEFAs) were measured by use of an HR Series NEFA-HR Kit (Wako Diagnostics).

Tissues were collected and immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Lipids were extracted (1) and then measured using enzymatic assays [TG and cholesterol by Infinity (Thermo Scientific) and phosphatidylcholine by Phospholipid C Kit (Wako)] and normalized to wet tissue weight. In some experiments, plasma was separated by FPLC using a Superose 6 column (GE Healthcare), and the cholesterol and TG content of each fraction was measured enzymatically (Infinity; Thermo Scientific).

Whole-Transcriptome Sequencing. Total RNA was extracted from livers of 10-wk-old male C57BL/6 mice ($n = 3$ per group) using the Qiagen RNeasy Midi Kit. The samples were pooled and the RNA integrity and quantity were determined using an Agilent 2100 Bioanalyzer and Qubit 2.0 fluorometer from Invitrogen. Poly(A)-based library preparation was carried out using TruSeq RNA Sample Preparation Kit version 2 (RS-122-2001) according to the manufacturer's instructions. Four micrograms of total RNA for each sample was used for oligo(dT)-based poly(A) mRNA selection followed by divalent cation thermal mRNA fragmentation. Single-stranded cDNA was synthesized using reverse transcriptase (SuperScript II) and random primers. cDNA was converted into double-stranded DNA using the reagents supplied in the kit, and the resulting dsDNA was end-repaired and the 3′ ends were adenylated. Indexed adapters were ligated to the samples to enable multiplexed sequencing. After quantitative PCR, cluster generation was performed on a cBot (Illumina) and then the samples were sequenced on the Illumina HiSeq 2000 using standard 100 × 100 paired-end sequencing. Raw sequence files were produced (.fasq files) and demultiplexed using CASAVA (http://support.illumina.com/sequencing/sequencing_software/casava.ilmn). Quality control metrics were generated for each sample using FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were then mapped and aligned to the reference genome using TOPHAT (http://tophat.cbcb.umd.edu/). The mapped reads were quality-filtered using SAMTOOLS (http://samtools.sourceforge.net/), and the duplicates were marked but not removed using PICARD (http://picard.sourceforge.net/). Transcript assembly was performed using CUFFLINKS (http://cufflinks.cbcb.umd.edu/) and expression values were calculated. Differential expression between samples was calculated using CUFFDIFF (http://cufflinks.cbcb.umd.edu/). Transcript levels are expressed as fragments per kilobase of exon per million fragments mapped (2).

Lipase Assays. Postheparin plasma was collected 15 min after heparin (Sigma) injection (1 U/g). Total TG lipase activity was determined by incubating 5 μL of pre- and postheparin plasma from each mouse with a glycerol-stabilized emulsion composed of 9,10-[^{3}H]triolein (American Radiolabeled Chemicals), triolein, and phosphatidylcholine (3) at 37 °C for 1 h. The reaction was stopped by adding 3.25 mL of heptane:chloroform:methanol (1:1.25:1.41) followed by 0.1 M K_2CO_3 (pH 10.5) (1 mL). Samples were vortexed for 30 s and then centrifuged at 1,800 × *g* for 15 min. A 1-mL aliquot of the upper phase was placed in a scintillation counter and the amount of free fatty acids liberated was calculated as described (4).

To separate hepatic lipase (HL) and lipoprotein lipase (LPL) activity, 500 μL of pooled postheparin plasma was loaded onto a 1.0-mL heparin-Sepharose HiTrap column (GE Healthcare) that had been equilibrated with 0.25 M NaCl, 20% glycerol, 1% BSA, 10 mM sodium phosphate (pH 6.5). The column was washed with 10 mL of the equilibration buffer and eluted with a 30-mL NaCl gradient [0.25–1.5 M in 20% (vol/vol) glycerol, 1% (wt/vol) BSA, 10 mM sodium phosphate, pH 6.5] (5). Forty microliters of each fraction was assayed for lipolytic activity in a total of 200 μL of reaction system.

Preparation of Radiolabeled Very Low Density Lipoprotein. Wild-type male mice ($n = 12$) were injected with 0.1 mCi of [9,10-^{3}H]palmitic acid (American Radiolabeled Chemicals) complexed with 2 mg/mL BSA in 200 μL of saline via the tail vein. Blood was collected 25 min after injection, plasma was pooled, and very low density lipoprotein (VLDL) was isolated by ultracentrifugation (200,000 × *g*, 24 h, 4 °C) at a density of 1.006. Analysis of VLDL lipids by TLC on silica gel G plates (Macherry-Nagel) confirmed that more than 90% of total counts in labeled VLDL were in the TG fraction.

Immunoblot Analysis. Serum from the mice was diluted 10-fold in saline and then incubated at 95 °C for 5 min in sample buffer [31 mM Tris·Cl, pH 6.8, 1% (wt/vol) SDS, 12.5% (vol/vol) glycerol]. A total of 2 μL of plasma was size-fractionated on a 10% SDS-polyacrylmide gel and transferred to nitrocellulose (GE Healthcare) before incubation overnight at 4 °C with a goat anti-mouse ANGPTL3 polyclonal antibody (1:1,000) (R&D Systems; AF136) in PBST (Sigma) plus 5% (wt/vol) fat-free milk. The filter was then washed in PBST for 10 min thrice before adding HRP-conjugated bovine anti-goat IgG (1:5,000) (Jackson ImmunoResearch) in phosphate-buffered saline with TWEEN-20 plus 5% fat-free milk. After a 1-h incubation at room temperature, the

filter was washed again with PBST and the membrane was exposed to F-BX810TM Blue X-Ray films (Phoenix Research Products). To quantify the immunoblot signals, films were scanned using an HP Scanjet 5590 and quantified using ImageJ (http://rsbweb.nih.gov/ij). The intensity of each band was corrected for background using a blank from the same film.

Real-Time PCR. Total RNA from liver was isolated using a commercial reagent (RNeasy Plus Universal; Qiagen). cDNA was synthesized from 2 μg of RNA using TaqMan (Applied Biosystems) with random hexamer primers and then amplified by PCR in 2× SYBR Master Mix (Applied Biosystems) according to the manufacturer's instructions. The specific oligonucleotides for each transcript are available on request. The levels of each target mRNA were normalized to the levels of 36B4 (liver), GAPDH (heart), or β-actin (white adipose tissue, skeletal muscle). For each transcript, the mean level of the wild-type animals was set to 1.

1. 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.
2. Trapnell C, et al. (2010) Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat Biotechnol* 28(5):511–515.
3. Nilsson-Ehle P, Schotz MC (1976) A stable, radioactive substrate emulsion for assay of lipoprotein lipase. *J Lipid Res* 17(5):536–541.
4. Quagliarini F, et al. (2012) Atypical angiopoietin-like protein that regulates ANGPTL3. *Proc Natl Acad Sci USA* 109(48):19751–19756.
5. Beigneux AP, et al. (2007) Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. *Cell Metab* 5(4):279–291.

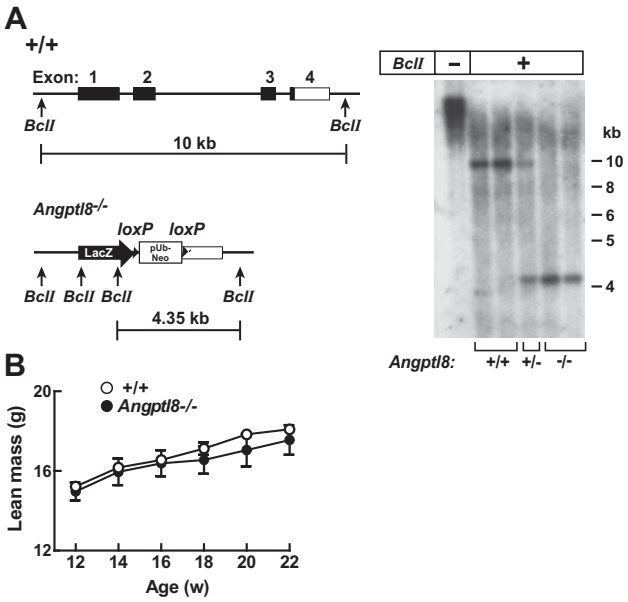

Fig. S1. Southern blotting (*A*) and lean body mass (*B*) in *Angptl8*$^{-/-}$ and wild-type mice. (*A*) Genomic DNA from *Angptl8*$^{-/-}$, *Angptl8*$^{+/-}$, and wild-type mice was digested with BclI, size-fractionated on 0.8% agarose gels, and transferred to Hybond-N nylon membranes. The filter was probed with a 400-bp PCR-amplified fragment corresponding to the central portion of the 3′ UTR of *Angptl8* and exposed to X-ray film. (*B*) Lean body mass was measured using NMR at the indicated ages for the mice described in the figure legend for Fig. 1*C*. Values are means +/− SEM.

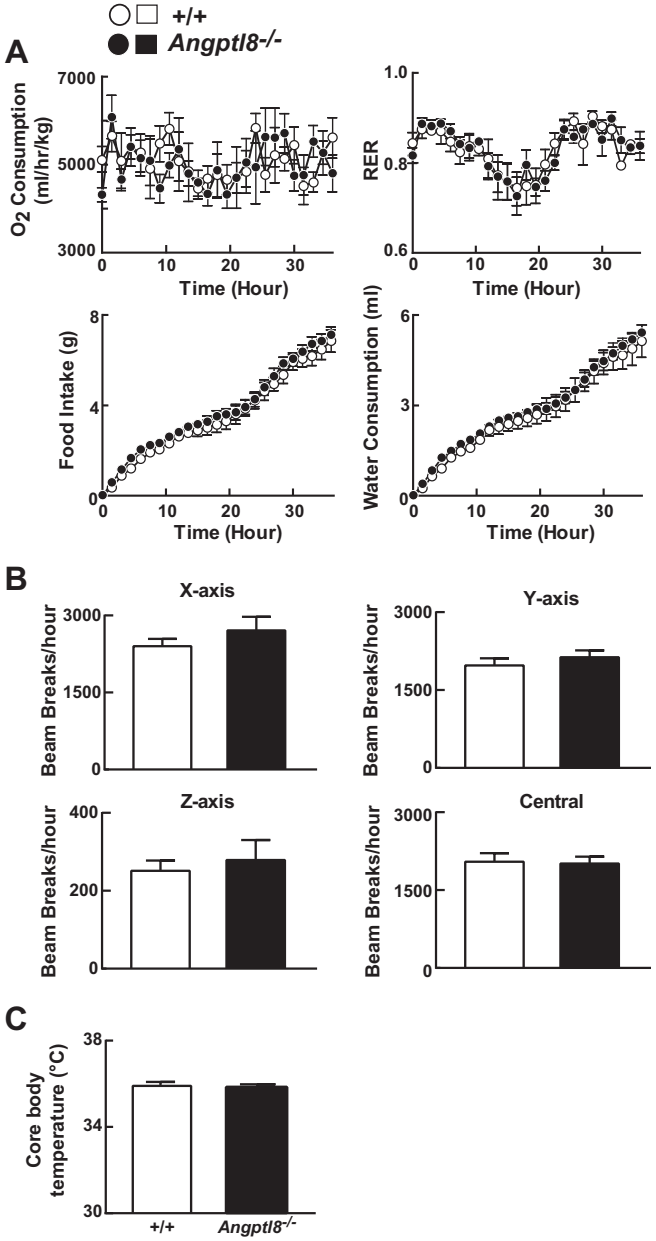

Fig. S2. Energy balance in *Angptl8*$^{-/-}$ and wild-type mice. (*A*) A total of five female *Angptl8*$^{-/-}$ mice and their wild-type littermates (age 12–14 wk) were housed individually in metabolic cages for 7 d. Respiratory exchange ratios were calculated from measurements of oxygen uptake and CO_2 excretion. Food and water were dispensed from continuously monitored hoppers. O_2 and CO_2 were sampled for 1 min at 30-min intervals for 5 consecutive days. (*B*) Ambulatory activity and rearing were measured by continuous monitoring using infrared beams. (*C*) Core body temperatures of female *Angptl8*$^{-/-}$ and wild-type mice were measured using an implantable temperature sensor as described in *SI Materials and Methods* (n = 5 per group, age 17–21 wk). Temperature was recorded at 4:00 PM under ad libitum feeding conditions. Values are means +/− SEM.

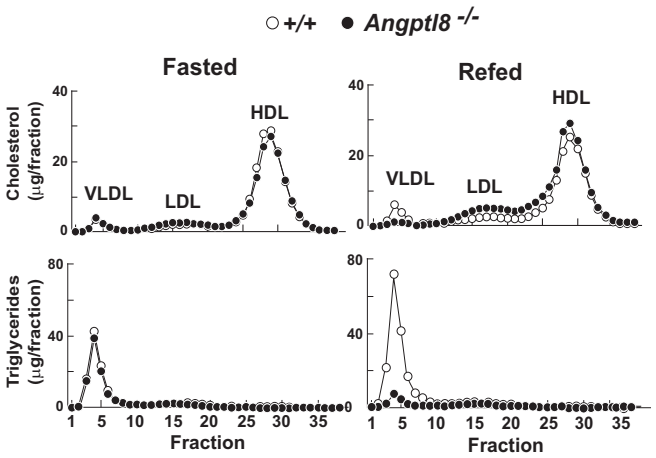

Fig. S3. Lipid levels in fasted and refed *Angptl8*$^{-/-}$ and wild-type mice. Plasma samples obtained from wild-type and *Angptl8*$^{-/-}$ mice after fasting and in the fed state (obtained from the mice shown in Fig. 2*B*) were subjected to FPLC on a Superose 6 column. The cholesterol and TG contents of each fraction were determined enzymatically (Infinity; Thermo Scientific).

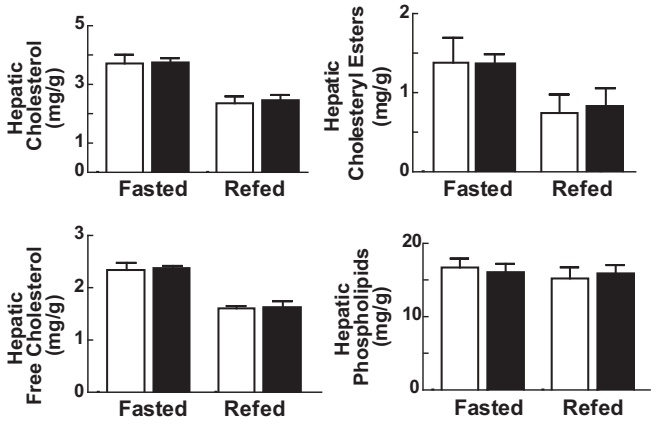

Fig. S4. Hepatic lipid levels in fasted and refed *Angptl8*$^{-/-}$ and wild-type mice. Liver samples from the mice described in the figure legend for Fig. 2*B*. The mice were entrained for 3 d using a regimen in which food was withdrawn during the day (7:00 AM to 7:00 PM) and replaced at night (7:00 PM to 7:00 AM). Livers were removed and lipids were extracted as described in *Materials and Methods*. Levels of free and esterified cholesterol and phospholipids were measured enzymatically as described in *Materials and Methods*. The experiments shown here were repeated with similar results. Values are means +/− SEM.

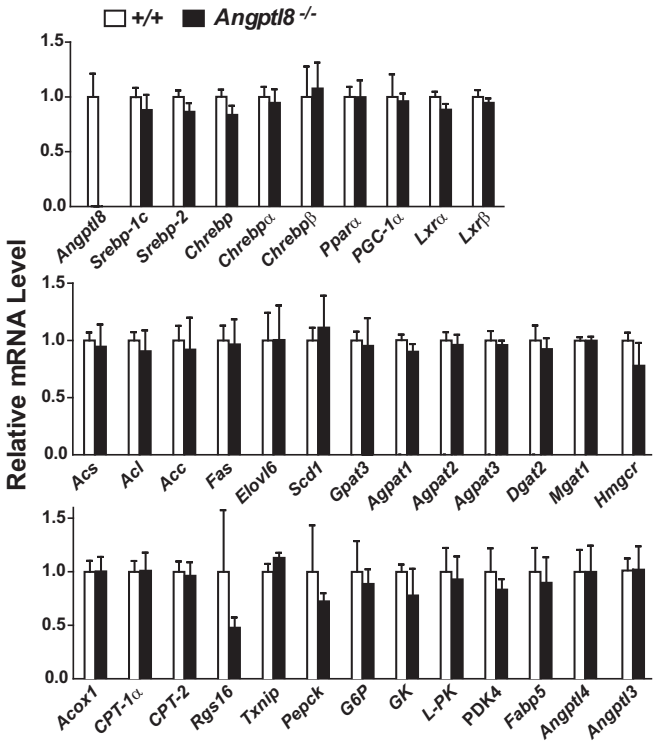

Fig. S5. Expression levels of selected mRNAs in livers of *Angptl8*$^{-/-}$ and wild-type mice. *Angptl8*$^{-/-}$ and wild-type littermates were trained for 3 d to fast during the day and eat at night as described in *Materials and Methods*. Livers were removed and snap-frozen in liquid N_2 and stored at −80 °C until the mRNA was isolated, and the levels of the indicated transcripts were measured by quantitative RT-PCR (1). 36B4 was used as an internal control for this experiment. Expression levels of *Angptl8*$^{-/-}$ were normalized to those of the wild-type mice, which were set to 1. The experiments shown here were repeated twice with similar results. Values are means +/− SEM.

1. Engelking LJ, et al. (2004) Overexpression of Insig-1 in the livers of transgenic mice inhibits SREBP processing and reduces insulin-stimulated lipogenesis. *J Clin Invest* 113(8):1168–1175.

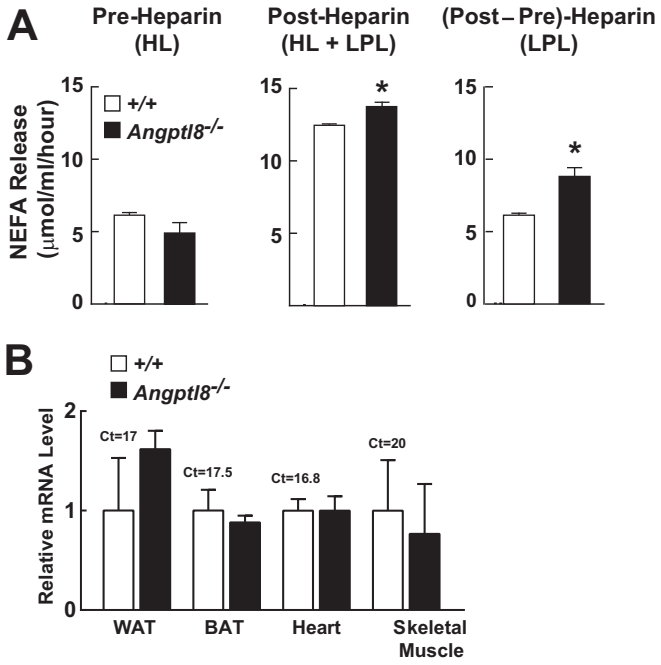

Fig. S6. Lipoprotein lipase activity and mRNA levels. (*A*) Plasma was collected 15 min after heparin injection (1 U/g). Preheparin TG hydrolase activity (representing HL activity) and postheparin lipase activity (total lipase activity: HL and LPL activity) were determined by incubating 5 μL of pre- and postheparin plasma from each mouse ($n = 6$ per group) with a glycerol-stabilized emulsion of triolein. The amount of free fatty acids liberated was calculated as described in *SI Materials and Methods*. LPL activity was estimated by subtracting the preheparin lipase activity from the postheparin lipase activity. *$P < 0.05$. (*B*) LPL mRNA levels in white adipose tissue (WAT), brown adipose tissue (BAT), heart, and skeletal muscle were measured by quantitative RT-PCR as described in *Materials and Methods*. Cyclophilin was used as an internal control for WAT and BAT. GAPDH and β-actin were used for heart and skeletal muscle, respectively. The mean level of the wild-type animals was set to 1. Ct, threshold cycle. Values are means +/− SEM.