Supplemental Figures and Legends

A

tissue	lncRNAs (FANTOM3 ID)
adipose	2310042I22, 2310079P10, 6030475M24, 8030489O15, 9030024J15, B230114P17
liver	0610005C13, 1700058M13, 1700063J08, 9030024J15, 9130016M20, 9130221J18, 9230101F19, A430069H15, B230114P17, C730010C06, C730016I12, C730036E19
muscle	1110002E14, 1110002E22, 1110020A10, 1110058D09, 2310014F07, 2310040G24, 2310045N14, 2310050B05, 2310058C22, 2310079P10, 6030406G17, A030001D16, B230318C15, D330025O06, D830041H11

B

	Lung	Spleen	Liver	Hippocampus	Heart	Thymus
LncLSTR (lnc3)	0.035688	0.11324	10.6448	0.002875	0.140623	0.028571
lnc1	0	0.0848163	98.1886	0.363708	0	0
lnc2	0.155021	0.471838	85.0819	0.0364536	0.00309765	0.0790134
GAPDH	46.1039	64.1728	72.9469	184.485	343.618	121.635

C

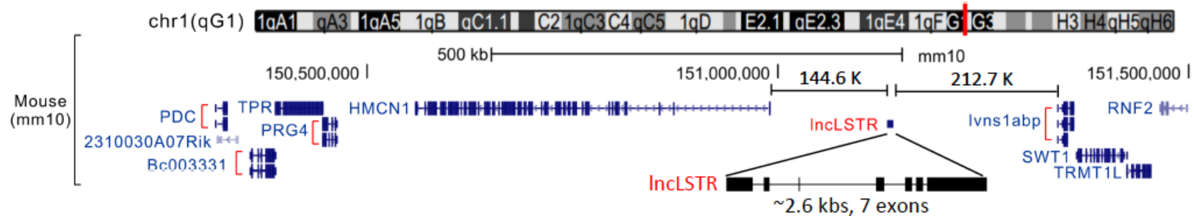


Figure S1, related to Figure 1. *LncRNAs identified in mouse metabolic tissues.* (A) A list of lncRNAs that are enriched in liver, muscle and adipose tissues. (B) A RNA-seq dataset of multiple tissues (Keane et al., 2011) was analyzed to calculate the mean Fragments Per Kilobase of transcript per Million (FPKM) for three liver-enriched lncRNAs as shown in Figure 1A. (C) Genomic region of lncLSTR showing its neighboring genes and genomic structure of lncLSTR.

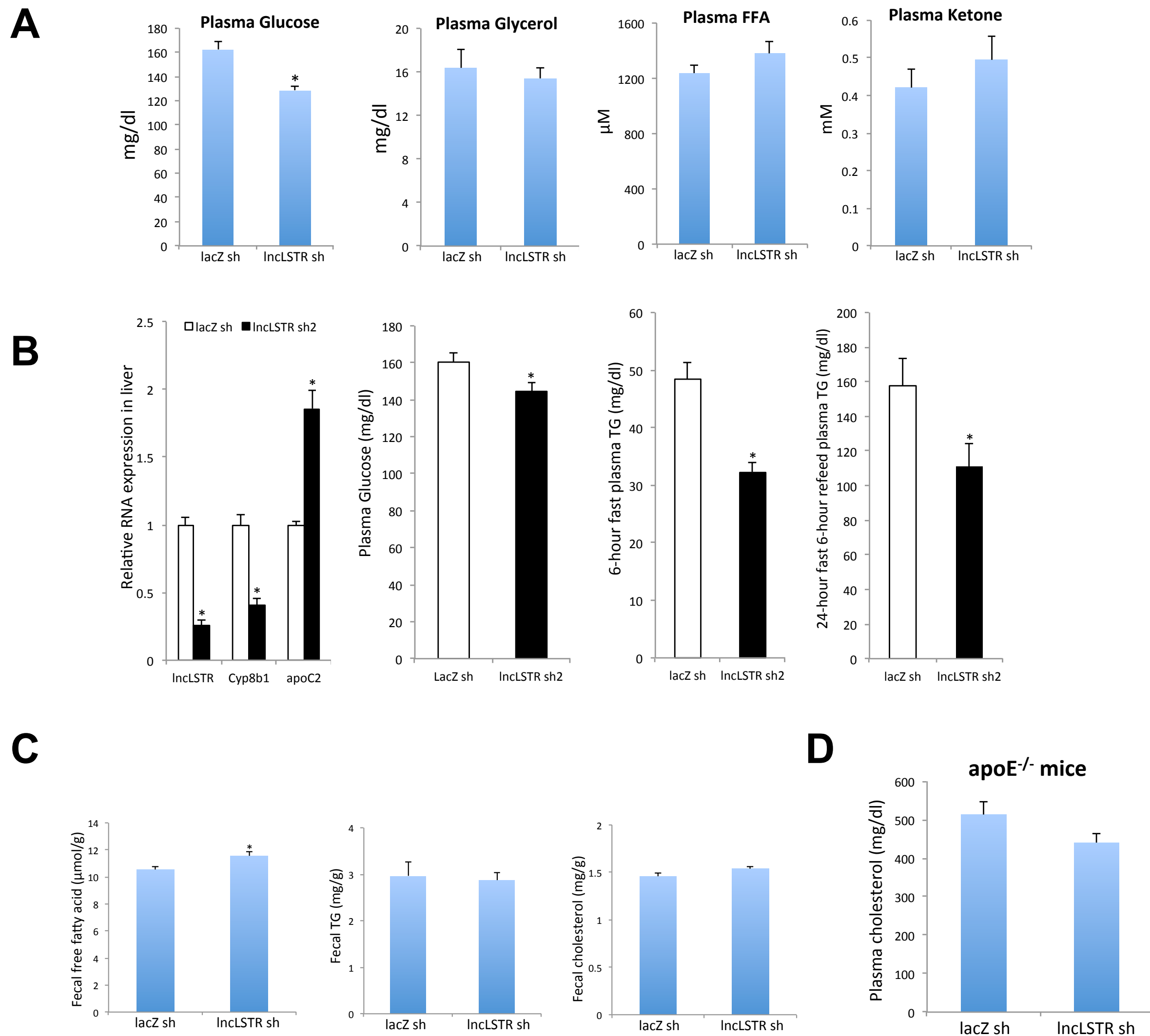


Figure S2, related to Figure 2. *Metabolic profile in control and lncLSTR KD mice.* (A) Plasma glucose, glycerol, free fatty acid (FFA) and ketone levels in control and lncLSTR KD mice after a 6-hour food withdrawal (n=7). (B) Hepatic gene expression (n=6), plasma glucose and TG levels after a 6-hour food withdrawal or plasma TG levels after fasted for 24 hours followed by a 4-hour refeed in control and lncLSTR sh2 KD mice (n=7). (C) Fecal free fatty acid, TG and cholesterol levels in control and lncLSTR KD mice were quantified using a colorimetric assay system (n=10) after 100mg dried feces from individually housed mice were incubated in 1ml 70% ethanol at 50°C for two hours. (D) Total plasma cholesterol levels in control (n=8) and lncLSTR KD (n=6) ApoE$^{-/-}$ mice were quantified using a colorimetric assay system. Error bars represent SEM, *p<0.05.

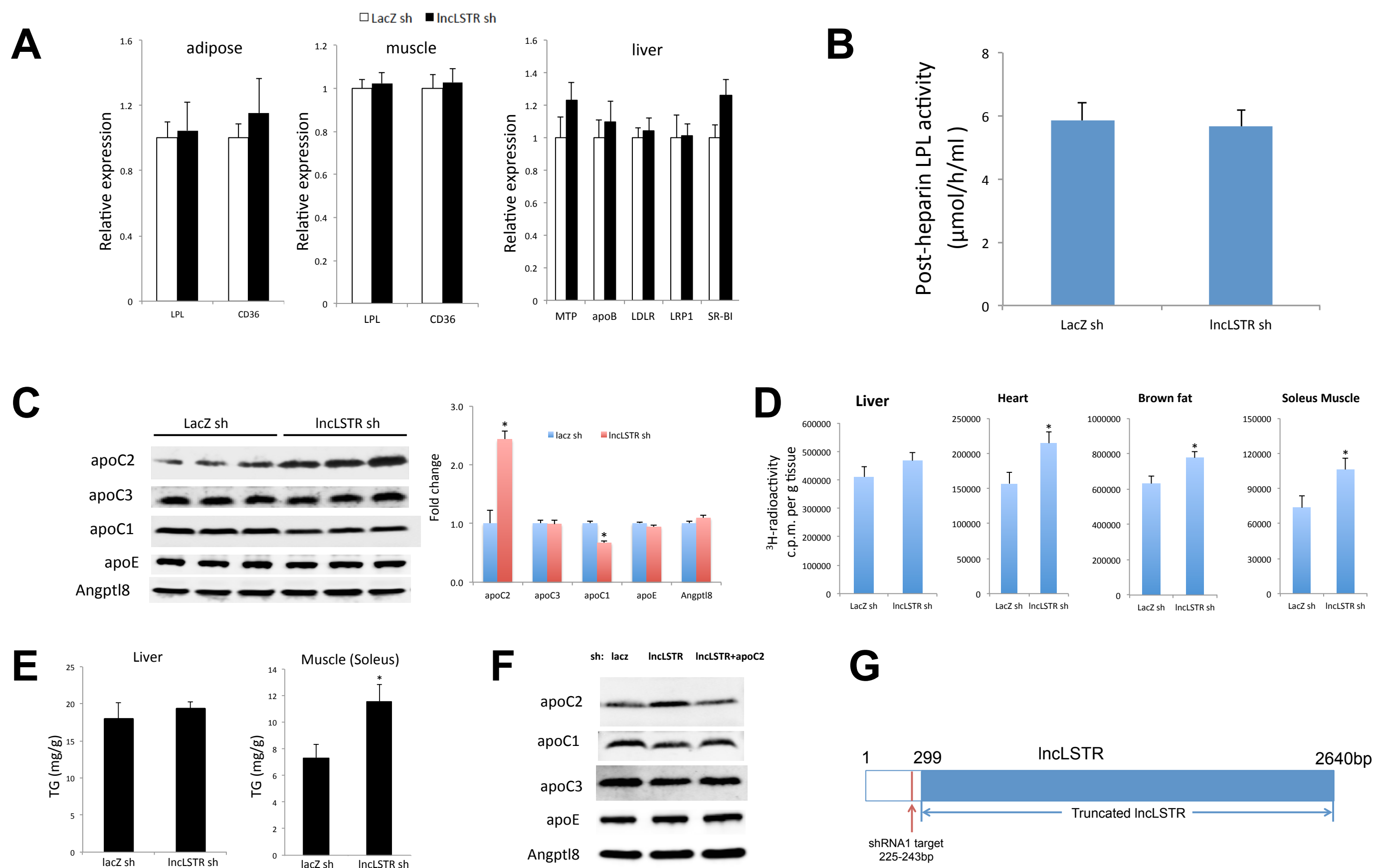


Figure S3, related to Figure 3. *Enhanced apoC2 expression and peripheral TG uptake in lncLSTR KD mice.* (A) Gene expression levels in adipose, muscle or liver tissues of control (lacZ sh) (n=6) and lncLSTR KD (lncLSTR sh) mice (n=5) after a 6-hour food withdrawal. (B) Post-heparin LPL activities in control and lncLSTR KD mice were measured as released free fatty acid from purified human VLDL (n=5). (C) Levels of apoC2, apoC3, apoC1, apoE and Angptl8 in plasma samples from individual mouse in control and lncLSTR KD group were analyzed by immunoblotting, with quantitative analysis on the right. (D) ^{3}H-radioactivities derived from triolein were analyzed in liver, heart, brown fat and soleus muscle from control and lncLSTR KD mice 2 hours after a gavage of 100μl olive oil with 10μCi per mouse of 3H-triolein (n=6). (E) Total TG in the liver (n=6) and soleus muscle (n=8) of lncLSTR KD or control mice was quantified using a colorimetric assay system. (F) Levels of apoC2, apoC3, apoC1, apoE and Angptl8 in plasma pooled from 5-6 mice of each group (control, lncLSTR KD or lncLSTR and apoC2 double KD mice) were analyzed by immunoblotting. (G) A cartoon showing full length and truncated lncLSTR and the site of the shRNA target sequence. Error bars represent SEM, *p<0.05.

lncLSTR Slc10a1
lncLSTR Mbl2
lncLSTR Amdhd1
lncLSTR Slco1b2
lncLSTR Rdh7
lncLSTR F9
lncLSTR Cyp2a12
lncLSTR Cyp2d13
lncLSTR Slc27a5
lncLSTR Fgl1
lncLSTR Cyp2c37
lncLSTR Ugt2b1
lncLSTR Tat
lncLSTR Akr1c6
lncLSTR Cyp8b1
lncLSTR Hao1
lncLSTR Klkb1
lncLSTR Cpn2
lncLSTR Crp
lncLSTR Es1
lncLSTR Cfhr1
lncLSTR Ugt2a3
lncLSTR Cml2
lncLSTR 1300002K09Rik

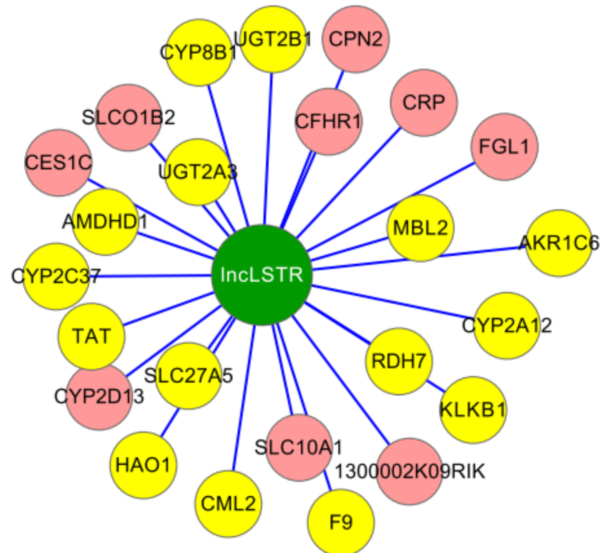
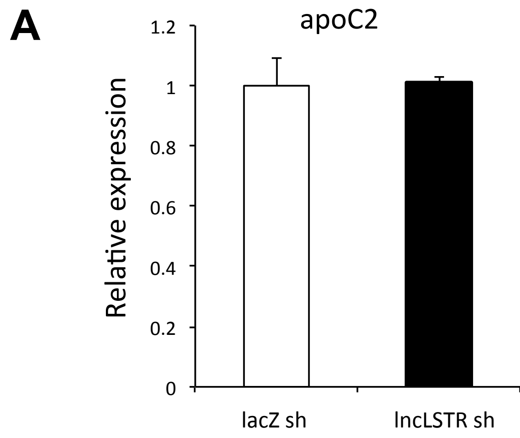
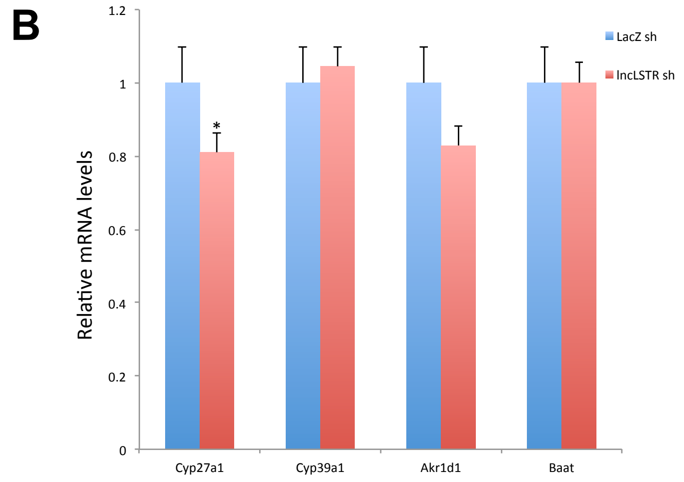
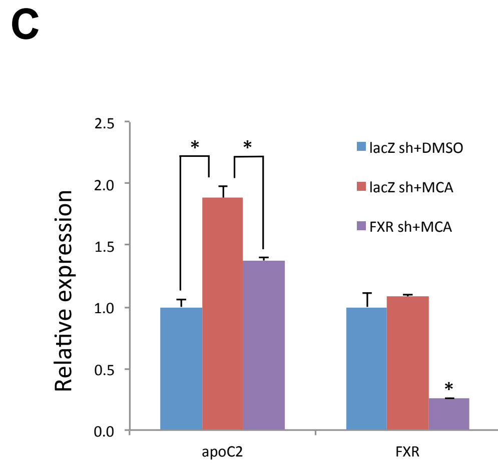
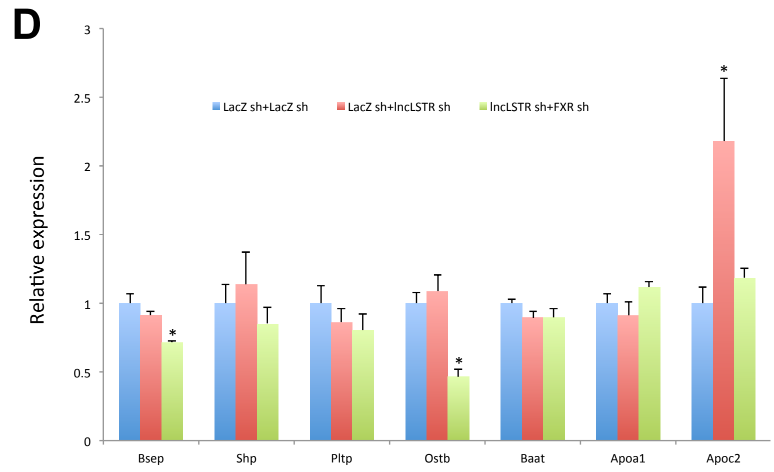


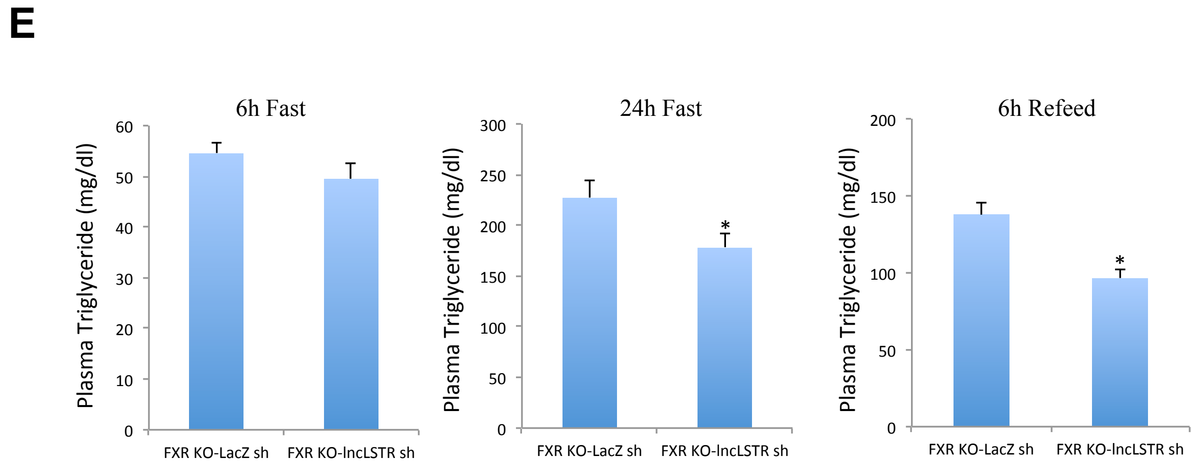
Figure S4, related to Figure 4. *A list of genes whose expressions are correlated with lncLSTR*. Genes with expression correlating with lncLSTR were identified by analyzing gene expression of 2425 samples profiled with Affymetrix Mouse Genome 430 2.0 Array that were deposited in NCBI GEO database. LncLSTR subnetwork consists of LncLSTR (green, center) and its 24 direct neighbors. Genes colored in yellow are known to play a role in metabolic pathways, pink denotes other functions.


A
apoC2
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
lacZ sh
lncLSTR sh

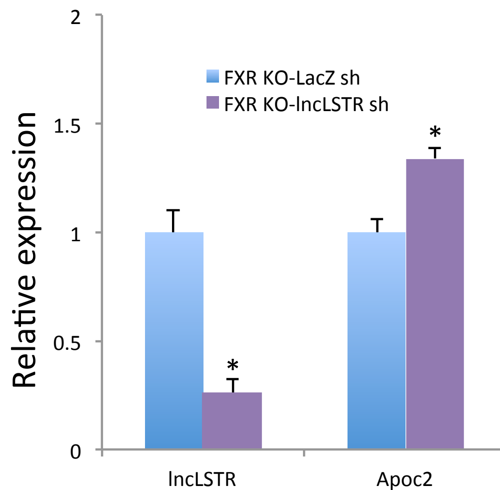

B
Relative mRNA levels
LacZ sh
lncLSTR sh
Cyp27a1
Cyp39a1
Akr1d1
Baat


C
Relative expression
lacZ sh+DMSO
lacZ sh+MCA
FXR sh+MCA
apoC2
FXR


D
Relative expression
LacZ sh+LacZ sh
LacZ sh+lncLSTR sh
lncLSTR sh+FXR sh
Bsep
Shp
Pltp
Ostb
Baat
Apoa1
Apoc2


E
6h Fast
24h Fast
6h Refeed
Plasma Triglyceride (mg/dl)
FXR KO-LacZ sh
FXR KO-lncLSTR sh

F



G

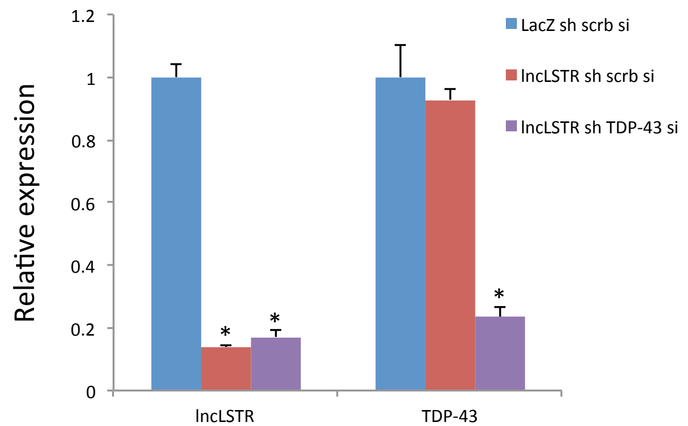


Figure S5, related to Figure 4. *Regulation of lipid metabolism by lncLSTR in mice is bile acid and FXR dependent.* (A) ApoC2 expression levels in primary hepatocytes infected with control or lncLSTR KD adenoviruses. (B) Expression levels of genes involved in bile synthesis in liver tissues from control and lncLSTR KD mice (n=6) were analyzed by real-time PCR. (C) ApoC2 expression in cultured AML12 hepatocytes infected with lacZ or FXR shRNA adenoviruses and treated with DMSO control or MCA. (D) Hepatic expressions of FXR target genes in mice receiving control, lncLSTR KD, or both FXR and lncLSTR KD adenoviruses (n=5). (E) LncLSTR was knocked down in FXR null mice and plasma TG levels were measured in lncLSTR KD (FXR KO-lncLSTR sh) and control (FXR KO-LacZ sh) mice (n=8) after a 6-hour fast (6h Fast), a 24-hour fast (24h Fast), or 6-hour refeeding after a 24-hour fast (6h Refeed). (F) Expression levels of lncLSTR and apoC2 in the livers of FXR null mice received shRNA adenoviruses for LacZ (FXR KO-LacZ sh) and lncLSTR (FXR KO-lncLSTR sh) respectively (n=8). (G) Gene expression in primary hepatocytes receiving lacZ shRNA, lncLSTR shRNA, scramble siRNA (scrb si) or TDP-43 siRNA in combination as indicated. Error bars represent SEM, *$p<0.05$.

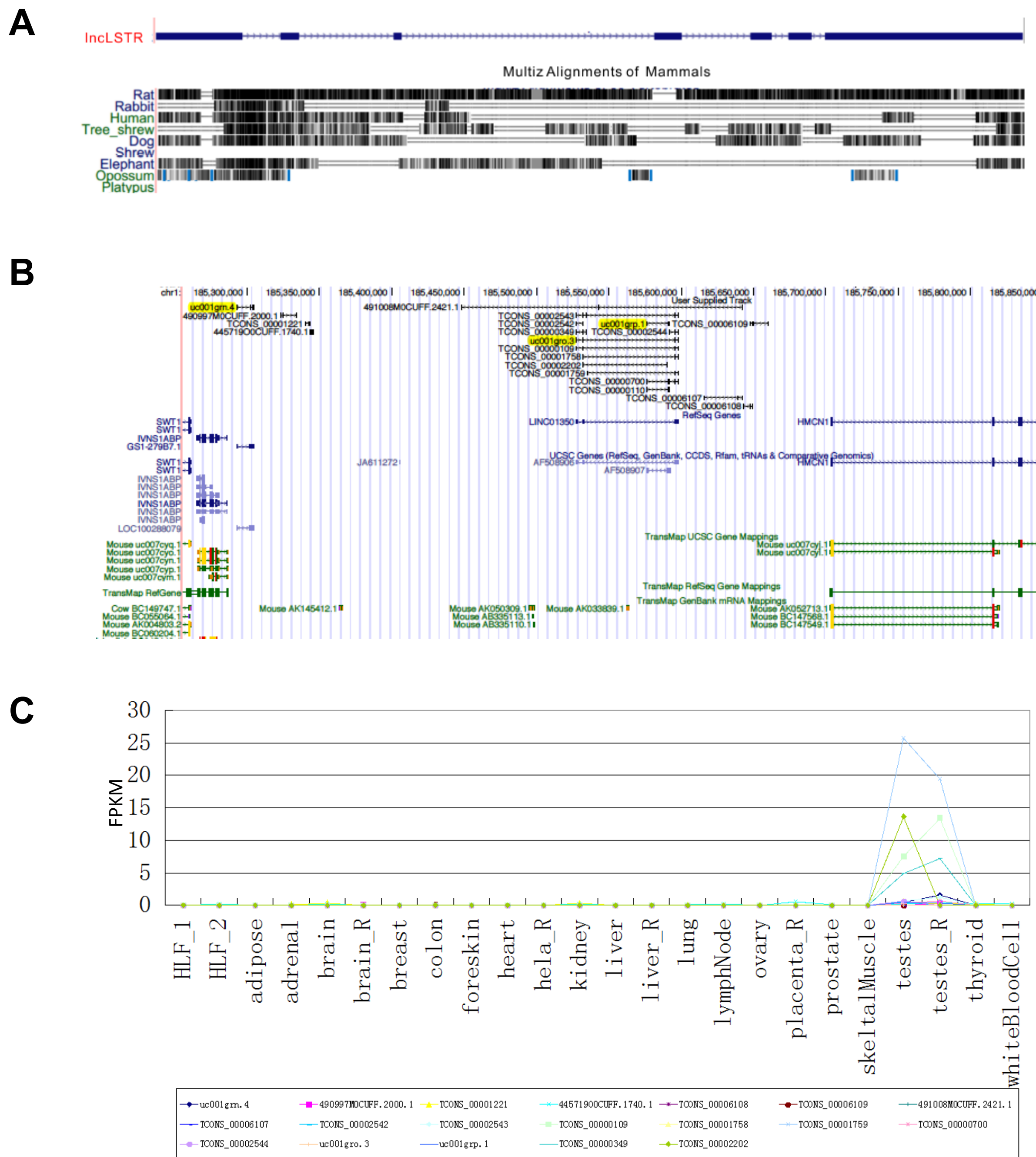


Figure S6, related to Discussion. *Bioinformatic analysis of potential transcripts from the lncLSTR syntenic region in human genome.* (A) Multiple sequence alignment of lncLSTR genomic sequence within major mammalian species. (B) 19 potential transcripts were identified in the human syntenic locus of lncLSTR by transcriptome reconstruction of each sample from two RNA-seq databases (ERP000546 in ArrayExpress and GSE36552 in GEO) for multiple human tissues and cells using Cufflinks. (C) Expression levels (FPKM) of the 19 transcripts were quantified across 16 human tissues and 8 cell lines from Human Body Map 2 project (ERP000546 in ArrayExpress) using Cufflinks.

Supplemental Table

Table S1, related to Experimental Procedures. A list of real-time PCR primers used.

Gene	Forward Primer	Reverse Primer
lncLSTR	TGTAGGAGCCCGCAATGAA	CAACTTAAAGCTGCCCCATCA
apoC2	ATGGGGTCTCGGTTCTTCCT	GTCTTCTGGTACAGGTCTTTGG
apoC3	GCATCTGCCCGAGCTGAAGAG	CTGAAGTGATTGTCCATCCAGC
Angptl3	TCTACTGTGATACCCAATCAGGC	CATGTTTCGTTGAAGTCCTGTGA
Cyp8b1	CCTCTGGACAAGGGTTTTGTG	GCACCGTGAAGACATCCCC
Cyp7a1	GGGATTGCTGTGGTAGTGAGC	GGTATGGAATCAACCCGTTGTC
FXR	TCCGGACATTCAACCATCAC	TCACTGCACATCCCAGATCTC
LPL	GGGAGTTTGGCTCCAGAGTTT	TGTGTCTTCAGGGGTCCTTAG
CD36	ATGGGCTGTGATCGGAACTG	GTCTTCCCAATAAGCATGTCTCC
MTP	AGGTTCCTCTATGCCTGTGGCTTT	TCTTAGCTTCCACCACTGCCTTGA
apoB	CGTGGGCTCCAGCATTCTA	TCACCAGTCATTTCTGCCTTTG
LDLR	TGACTCAGACGAACAAGGCTG	ATCTAGGCAATCTCGGTCTCC
LRP1	ACTATGGATGCCCCTAAAACTTG	GCAATCTCTTTCACCGTCACA
SR-BI	TTTGGAGTGGTAGTAAAAAGGGC	TGACATCAGGGACTCAGAGTAG
Shp	TGGGTCCCAAGGAGTATGC	GCTCCAAGACTTCACACAGTG
Bsep	AAGCTACATCTGCCTTAGACACAGAA	CAATACAGGTCCGACCCTCTCT
Cyp27a1	GCCTCACCTATGGGATCTTCA	TCAAAGCCTGACGCAGATG
Cyp39a1	TGCACTGCATGAGCGACTG	GGTATTGAGTGTGGCTGGATAAA
Akr1d1	TGCACACCACCAAATATCCCT	CTTCACTGCCACATAGGTCTTC
Baat	TGTGATGAATAGCCCCTACCA	AGGACTGACGACTATGTCTTGTA
Pltp	CGCAAAGGGCCACTTTTACTA	GCCCCCATCATATAAGAACCAG
apoA1	CAGGAGATGAAAAGGACCTAG	GCAGCTCTGCAGCTTCTGGCG
Ostb	AGATGCGGCTCCTTGGAATTA	TGGCTGCTTCTTTCGATTTCTG
TDP-43	GCCCTAGCGCCATTTTGTG	GAAGCACAAGGAGGAAGCAC
Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Gapdh	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA
18S	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA

Supplemental Experimental Procedures

Animal experiments

All animal experiments were performed in accordance and with approval from the NHLBI Animal Care and Use Committee. Male C57BL/6 (B6) mice were purchased from Jackson Laboratory at 8 weeks of age, and housed 3-5 mice per cage with free access to water and normal chow diet (NIH-31), and animals were acclimatized to the housing for 10-14 days before experiments. Groups of co-housed mice were randomly assigned to experimental groups with age and weight in accordance between groups. Animal data were excluded from experiments based on pre-established criteria of visible abnormal liver structure during sample harvest or other health issues including fighting wounds. According to the variability of metabolic parameters, group size was determined based on previous studies using similar assays within the laboratory and pilot experiments. Experimenters were not blinded to treatment group. For the fasting and refeeding study, mice were either allowed free access to food or were subjected to a twenty-four hour fast before euthanized for tissue harvest. A third group was fasted for twenty-four hours and then allowed to feed *ad libitum* for another four hours before tissue harvest.

Tissue Triglyceride uptake analysis

TG uptakes in different organs were analyzed as described in (Bartelt et al., 2011). Briefly, the mice were fasted for 24h followed by oral gavage of 100μl olive oil with 10μCi per mouse of ^{3}H-triolein. After 2h, blood was removed by perfusion of the carcass with PBS containing 50U/ml heparin. Next, organs were quickly collected and solubilized in Solvable (PerkinElmer, 0.1ml per 10mg tissue). 200μl tissue lysate were then counted in scintillation fluid for radioactivity, and TG uptake was calculated as c.p.m. per gram of tissue.

HPLC-UV analysis of conjugated bile acids in mouse gallbladder bile

Bile acid (BAs) standards including taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), taurolithocholic acid (TLCA) and glycochenodeoxycholic acid (GCDCA) were purchased from Sigma. Tauroursodeoxycholic acid (TUDCA) was purchased from Calbiochem. Tauro-alpha/beta-muricholic acids (TMCA) were obtained from Steraloids Inc. In mouse bile, 99.6% of BAs are taurine conjugates and the taurine conjugates of CA and MCA constitute 95% of total BAs in the bile. Thus, we used a simplified HPLC-UV method to determine the relative amount of TCA and TMCA (Alnouti et al., 2008). Mouse gallbladder bile samples were extracted using Oasis-HLB SPE cartridges (Waters). As mouse bile contains very little glycine-conjugated acid (less than 0.1%), we used GCDCA as the internal standard for HPLC-UV analysis. Briefly, 100ul of diluted bile (100-fold in H2O) spiked with 20ul of 2.5mM GCDCA was loaded onto 3cc Oasis SPE cartridges (60mg) pre-conditioned with 1ml MeOH and 1ml H2O. Bile salt was eluted with 1.8ml MeOH then washed with 2ml H_2O; the eluate was evaporated under vacuum and resuspended in 100ul 50% MeOH. For analysis of bile acids with HPLC, reverse phase chromatography was performed on an Eclipse Plus C18 (3.0 x 150mm, 3.5mm,

Agilent technologies) at 30°C. The mobile phase consists of two solvents: solvent A (10mM K_2HPO_4 pH8.14, 10mM Sodium Borate) and solvent B (acetonitrile: methanol: water 20:70:10 v/v). The column was connected to the Agilent 1100 series HPLC (Agilent Technologies) and the flow rate was 0.5ml/min. Absorbance was read at 210nm. Chromatography started with a solvent mixture of 50% solvent A and B. The gradient increased up to 95% solvent B within twenty minutes and reached 100% B after one more minute. 100% B was used for five minutes and then was returned to the initial setting within one minute, followed by equilibration of the column for seven minutes. The method was verified with taurine conjugated bile acid standards listed above and all data were analyzed using the Agilent software, Chem Station Rev A 10.02 (Agilent technologies).

Lipoprotein lipase assay

For analysis of post-heparin lipoprotein lipase (LPL) activity, post-heparin plasma was prepared from mice 15min after i.p. injection of 500U/kg sodium heparin. LPL activities were measured as free fatty acids released from purified human VLDL as previously described (Carballo-Jane et al., 2010; Di Filippo et al., 2014).

In order to evaluate the regulatory activity of mouse plasma on LPL, a LPL assay using purified bovine LPL protein was performed as described (Basu et al., 2011) with modifications. Briefly, a 100ul reaction was set up by adding 0.15M NaCl, 20mM Tris-HCl pH8.0, 0.0125% Zwittergent, 1.5% FA-free BSA, 1:500 dilution of a fluorogenic triglyceride analog (Cell Biolabs) and 0.5ul mouse plasma. The reaction was carried out for 10min at 37°C using 175ng LPL (from Bovine milk, Sigma), and the signal of the fluorescent product was measured in a fluorescence microplate reader (Ex.480 nm/Em.525 nm).

Isolation and culture of mouse primary hepatocytes

Primary hepatocytes were isolated from C57BL/6 mice fed with a normal chow diet. Briefly, mice were anesthetized with Ketamine (100mg/kg) and Xylazine (10mg/kg), and the liver was perfused with Krebs Ringer buffer with glucose at a rate of 5ml/min for eight minutes, followed by continuous perfusion with the same buffer containing collagenase (Liberase TM Research Grade, Roche) for 10 minutes. Hepatocytes were harvested and purified with Percoll. The viability of hepatocytes was examined by trypan blue exclusion. Only cell isolates with viability over 90% were used. Hepatocytes were plated onto collagen-coated plates ($1x10^6$ cells/well in 6-well plates and $5x10^5$ cell per well in 12-well plates) in DMEM (no glucose) supplemented with 5.5mM glucose, 1xGlutaMAX™ and 10% Cosmic Calf Serum (CCS). To knock down TDP-43 in hepatocytes, ON-TARGETplus Mouse TDP-43 siRNA (Thermo Fisher Scientific Inc.) was transfected using RNAiMAX, and a scramble siRNA was used as control.

AML12 cell culture and bile treatment

The AML12 cells (ATCC) were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium (Invitrogen) with 10% CCS, ITS (Invitrogen) and dexamethasone (40ng/ml). For treatment of bile acids, 100% confluent AML12 cells were cultured in serum free medium for 48 hours, treated with 100uM CA, MCA or

DMSO as control for 24 hours. RNA were harvested and quantified by real-time PCR analysis.

RNA extraction and quantitative real-time PCR analysis
Total RNA was isolated from liver tissues or cells using Trizol reagent (Invitrogen). After Turbo DNA-free DNase treatment (Ambion), reverse transcription was carried out with SuperScript® III First-Strand Synthesis system (Invitrogen) using 1 μg of RNA. Quantitative real-time RT-PCR was performed on a ViiA™ 7 Real-Time PCR System (Applied Biosystems Inc.) The PCR program was: 2 min 30 s at 95°C for enzyme activation, 40 cycles of 15 s at 95°C, and 1 min at 60°C. Melting curve analysis was performed to confirm the real-time PCR products. All quantitations were normalized to 18S rRNA levels. 18S rRNA levels are in general stable for samples collected under similar conditions, though they may vary across different tissues, so slight variations might be introduced when it is used to normalize tissue-specific expression. Primer sequences used are provided in the Table S1.

Immunoblotting
For Immunoblotting analyses, the cells and tissues were lysed in RIPA buffer (Cell Signaling Technology) containing phosphatase inhibitors (Sigma) and a protease inhibitor cocktail (Roche). The lysate was subjected to SDS–PAGE, transferred to polyvinylidene fluoride (PVDF) membranes, and incubated with the primary antibody followed by the fluorescence conjugated secondary antibody (LI-COR). The bound antibody was visualized using a quantitative fluorescence imaging system (LI-COR). The primary antibodies used are anti-TDP43 (ab41881, Abcam)

For detection of apoproteins and Angptl8 protein levels in mouse plasma, equal amount of plasma from individual mice or pooled plasma of 5-6 mice from the same experimental group was mixed with NuPAGE LDS Sample Buffer and Reducing Agent, heated at 95°C for 10min before loaded to Novex NuPAGE 4–12 % Bis-Tris gels (200V for 30 min in MES buffer), then transferred to PVDF membranes (450mA, 3hr in 0.25X Laemmli buffer, 20% Methanol) for Western Blot analysis. The primary antibodies used are apoC2 (T-12) antibody (SC-19015, Santa Cruz), apoC3 antibody (SC-50378, Santa Cruz), apoC1 antibody (ab20051, Abcam), apoE antibody (SC-6384, Santa Cruz), and Angptl8 antibody (7619, ProSci).

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