Supplemental Figures and Tables

Hepatic TRAP80 Selectively Regulates Lipogenic Activity of Liver X Receptor

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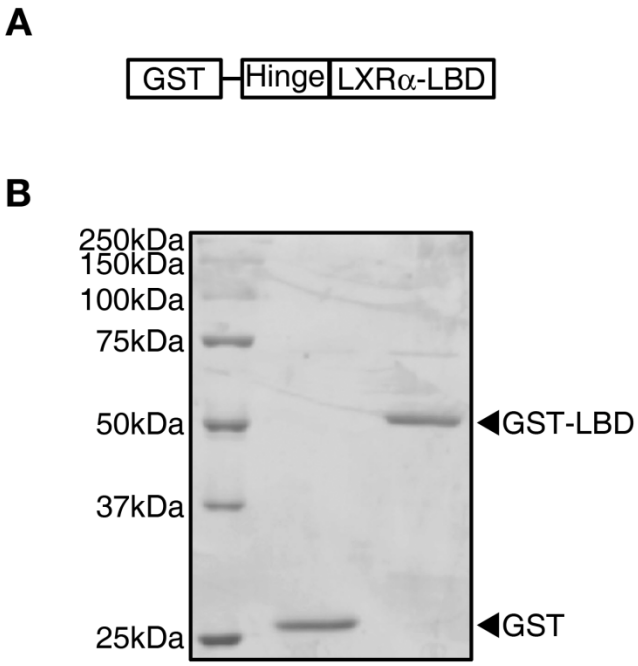
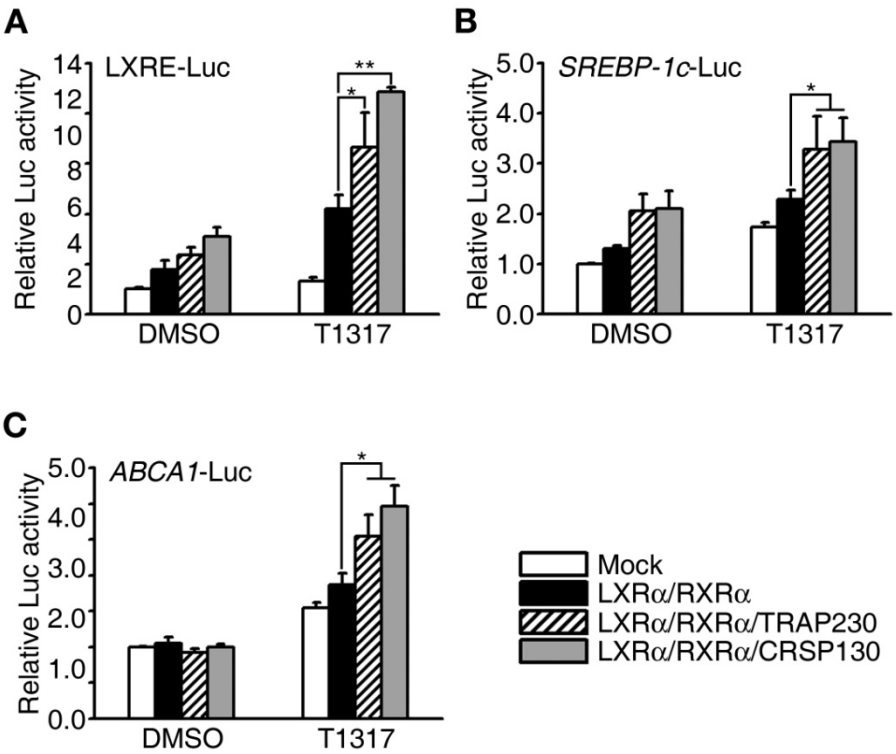
Supplemental Figure 1



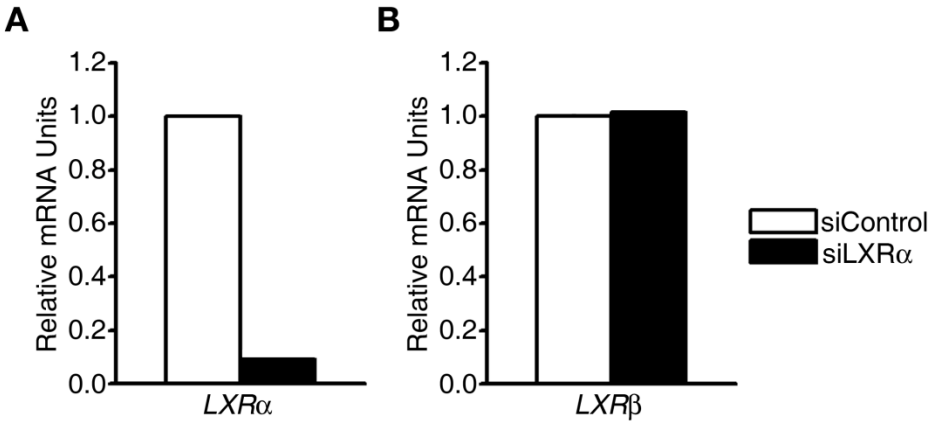
Supplemental Figure 1. Expression and purification of the LBD of LXRα fused to GST. (**A**) Schematic representation of LXRα LBD fused to GST. The carboxyl terminus (residues 159–445) of LXRα is fused to GST. (**B**) Overexpression and purification of GST–LXRα LBD (GST-LBD). Bacterially expressed GST-LXRα LBD was purified on glutathione-Sepharose beads and used as immobilized bait.

Supplemental Figure 2



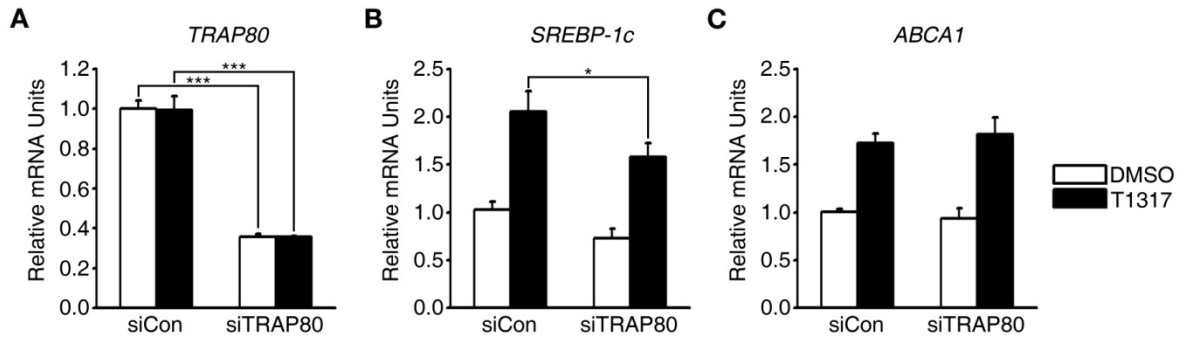
Supplemental Figure 2. TRAP230 and CRSP130 stimulate ligand-dependent activation of the *SREBP-1c* and *ABCA1* promoter. HepG2 cells were transfected with a luciferase reporter plasmid under the control of synthetic LXRE (**A**, $n = 5$), S*REBP-1c* promoter (**B**, $n = 7$), or *ABCA1* promoter (**C**, $n = 6$). Data are presented as the mean ± SEM. T1317, T0901317. *$P < 0.05$, **$P<0.01$, one-way ANOVA.

Supplemental Figure 3



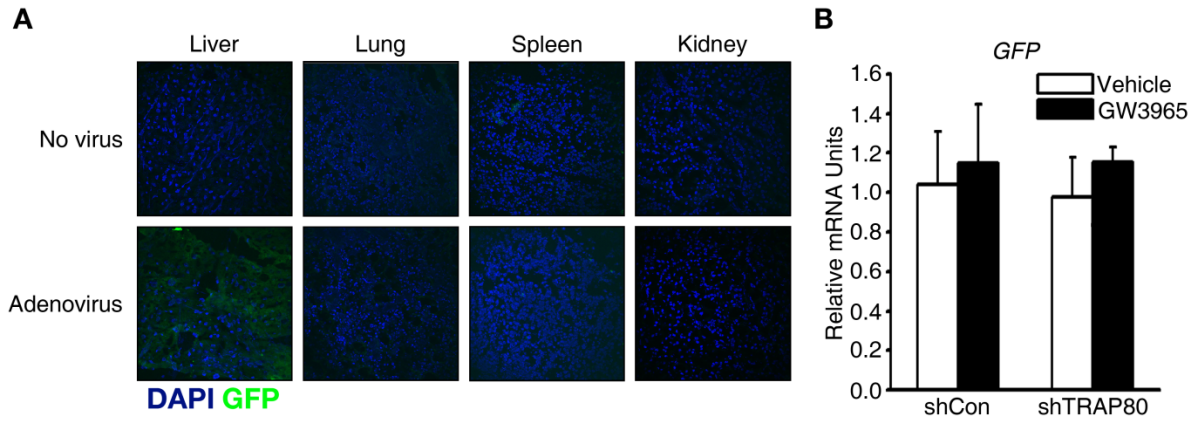
Supplemental Figure 3. LXRα-specific knockdown using siRNA in primary hepatocytes. Mouse primary hepatocytes were transfected with LXRα siRNA (sc-38829, Santa Cruz) or control siRNA (sc-37007, Santa Cruz). The transcript levels of LXRα (**A**) and LXRβ (**B**) were analyzed by qRT-PCR.

Supplemental Figure 4



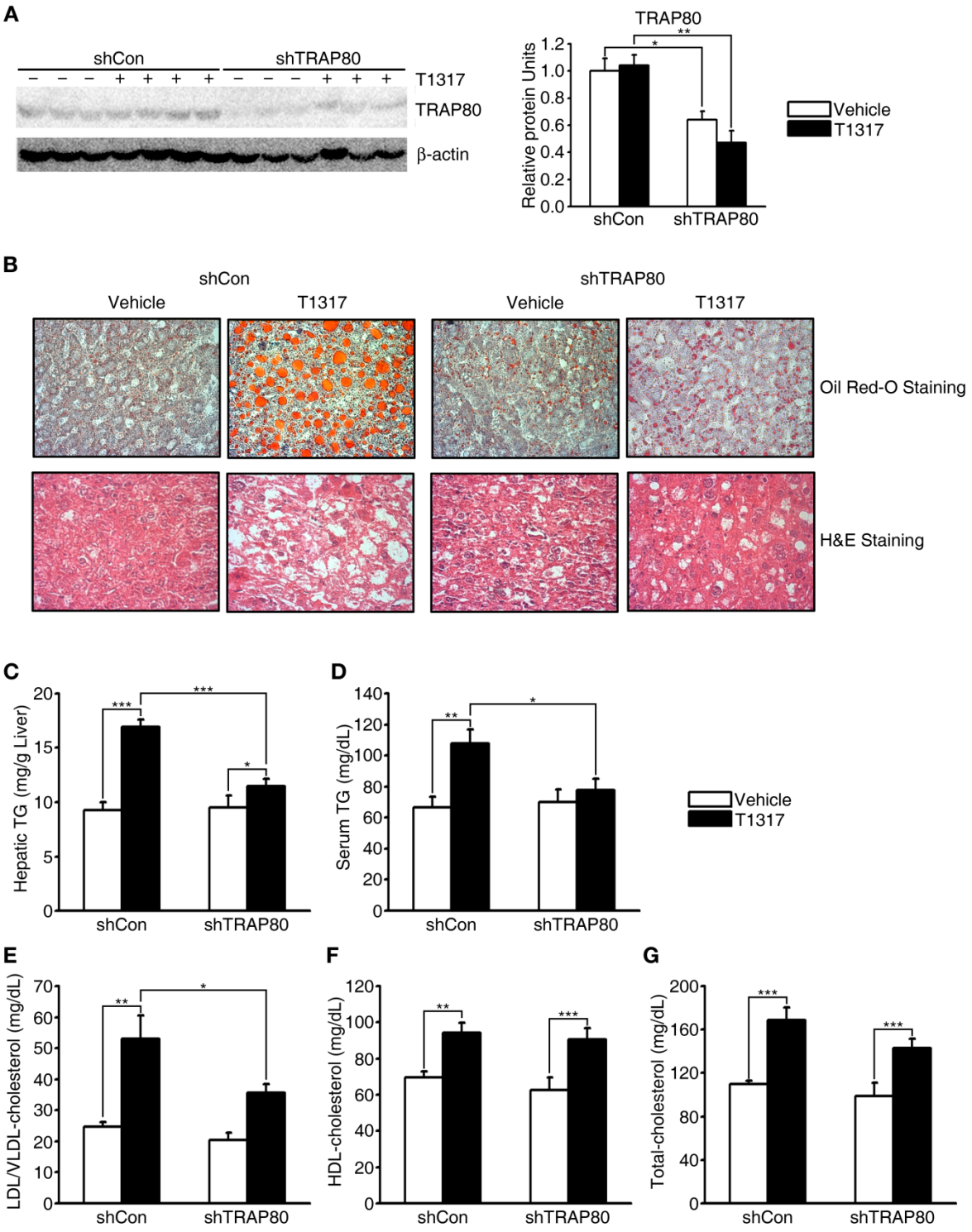
Supplemental Figure 4. Differential effects of TRAP80 siRNA on SREBP-1c and ABCA1 transcript levels. HepG2 cells were transfected with TRAP80 siRNA (sc-38575, Santa Cruz) or control siRNA (sc-37007, Santa Cruz) (n = 3) and the transcript levels of TRAP80 (**A**), SREBP-1c (**B**), and ABCA1 (**C**) in the presence or absence of T0901317 were analyzed by qRT-PCR. Data are presented as the mean ± SEM. *P < 0.05, ***P < 0.001, one-way ANOVA.

Supplemental Figure 5



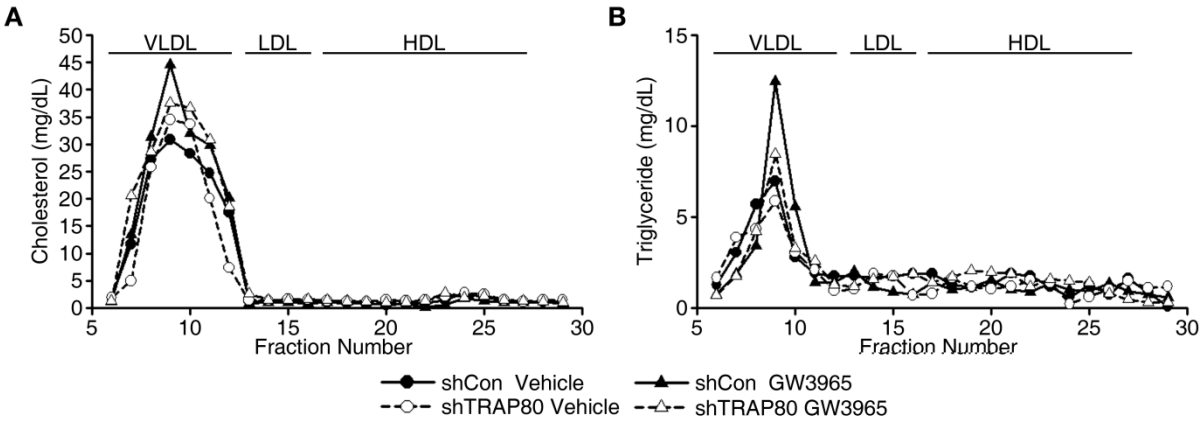
Supplemental Figure 5. Delivery of Ad-shTRAP80 to the liver via tail vein injection. (**A**) Mice were injected with Ad-shTRAP80 (1×10^{11} particles/20 g) via the tail vein. Five days after injection, liver, lung, spleen, and kidney tissues were harvested and cryo-sections of each tissue were analyzed for GFP and DAPI staining by confocal microscopy. Original magnification, ×400. (**B**) Equivalent delivery of adenovirus to the livers of each experimental group (n = 5). Following administration of 50 mg/kg GW3965 to Ad-shCon or Ad-shTRAP80 injected mice, the livers were harvested and the mRNA levels of GFP were determined by qRT-PCR. Data are presented as the mean ± SEM.

Supplemental Figure 6



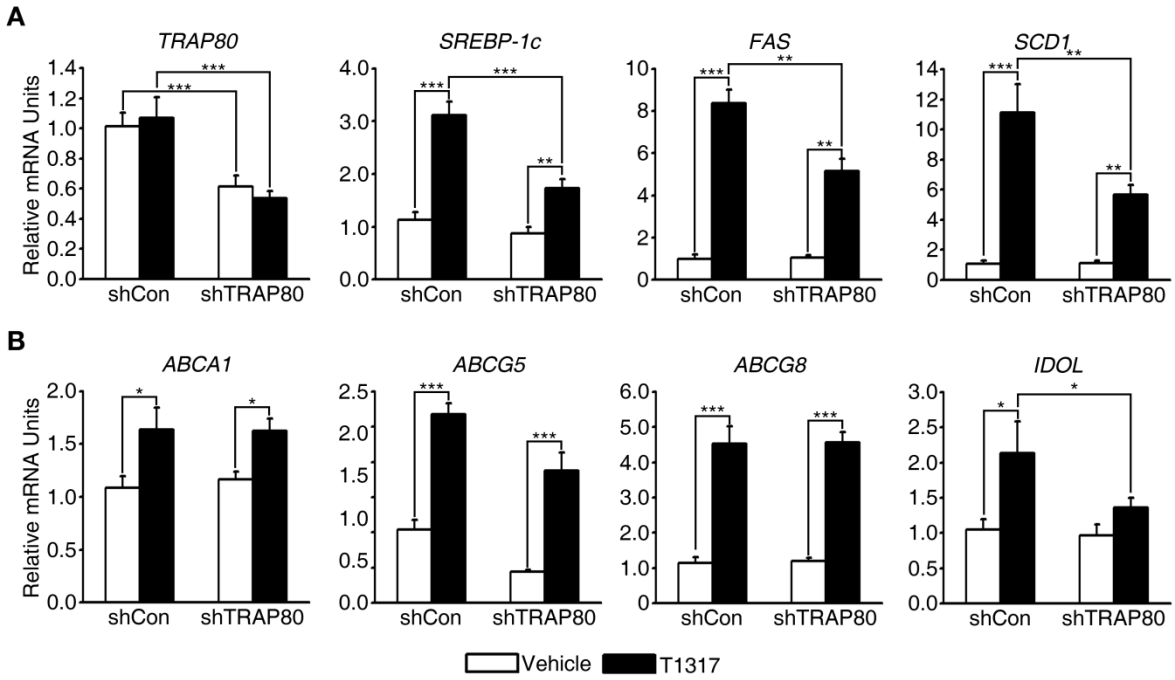
Supplemental Figure 6. Knockdown of hepatic TRAP80 ameliorates T0901317-induced fatty liver and the aberrant serum lipid profile. (**A**) Knockdown of hepatic TRAP80 by tail vein injection of Ad-shTRAP80. After Ad-shTRAP80 injection, the livers were harvested and the protein levels of TRAP80 were determined by immunoblot analysis. Relative band intensities were calculated by Image J. (**B**) Representative images of hematoxylin and eosin (H&E) and oil Red O-stained liver of Ad-shTRAP80 injected mice following the administration of 50 mg/kg T0901317 for 3 days. Original magnification, ×200. (**C**−**G**) Changes in the hepatic triglyceride and serum lipid profile in mice injected with Ad-shTRAP80 via the tail vein (shCon+vehicle, $n = 5$; shCon+T1317, $n = 8$; shTRAP80+vehicle, $n = 5$; shTRAP80+T1317, $n = 9$). Data are presented as the mean ± SEM. TG, triglyceride. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, one-way ANOVA.

Supplemental Figure 7



Supplemental Figure 7. Effects of GW3965 on serum lipoprotein profiles in Ad-shTRAP80 infected ApoE$^{-/-}$ mice. Ad-shCon or Ad-shTRAP80 infected ApoE$^{-/-}$ mice were administered with 50 mg/kg GW3965 for 3 days. Lipoproteins were separated from pooled mouse serum by FPLC (n = 5). The concentration of cholesterol (A) or triglyceride (B) in each eluted fraction was determined enzymatically.

Supplemental Figure 8



Supplemental Figure 8. Effects of TRAP80 on T0901317-dependent expression of metabolic genes in the liver. The effects of TRAP80 knockdown on the transcript levels of lipogenesis- (A) or RCT-related genes (B) in the livers of mice treated with T0901317 (shCon+vehicle, $n = 5$; shCon+T1317, $n = 8$; shTRAP80+vehicle, $n = 5$; shTRAP80+T1317, $n = 9$). The mRNA levels were measured by qRT-PCR. Data are presented as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, one-way ANOVA.

Supplemental Table 1. Results of MALDI-TOF analysis of LXRα−interacting proteins.

Protein	Score[A]	Expect[B]	No. of peaks identified[C]	Masses matched[D]	Protein mass	Accession No.
TRAP230	134	6.2e-10	30	36.6%	247	Q93074
TRAP220	34	6.3	9	23.7%	168	Q15648
CRSP130	100	1.5e-6	18	33.3%	156.4	Q9ULK4
TRAP80	81	1.2e-4	16	19.3%	73	Q9NVC6

[A] Protein score is a measure of the statistical significance of a match. A score greater than 65 is significant. ($p<0.05$, default significance threshold)

[B] Number of matches with equal or better scores expected to occur by chance alone. This is directly equivalent to the E-value in a BLAST search result. The lower the expectation value, the more significant the score.

[C] Number of mass values matched.

[D] Percentage of number of mass values matched in number of mass values searched

Supplemental Table 2. List of primers used for cloning.

Cloning Oligo Name	Sequence (5'→3')[A]
mSREBP-1c (-550/+42) For	CGGGGTACCCCCCCCTCCTTGAAACAA
mSREBP-1c (-550/+42) Rev	CCGCTCGAGCCTAGGGCGTGCAGACGC
mSREBP-1c (mt LXRE a) For	ACGACAGAGTCCGCCAGAATCCCCAGC
mSREBP-1c (mt LXRE a) Rev	GCTGGGGATTCTGGCGGACTCTGTCGT
mSREBP-1c (mt LXRE b) For	AAGGCGGAAGTCCGCTAGAATCCCCGGC
mSREBP-1c (mt LXRE b) Rev	GCCGGGGATTCTAGCGGACTTCCGCCTT
hABCA1 (-928/+101) For	CGGGGTACCTAAGTTGGAGGTCTGGAG
hABCA1 (-928/+101) Rev	CCGCTCGAGGCTCTGTTGGTGCGCG
hABCA1 (mt LXRE) For	AGGCTTTGTGTGATAGTAACTGCTGCGCT
hABCA1 (mt LXRE) Rev	AGCGCAGCAGTTACTATCACACAAAGCCT
Ad-shTRAP80 (1587) For	CGCGTCAGAGATGGTCGGGTAATCATTGATATCCGTGATTACCCGACCATCT CTTTTTTTCCAAA
Ad-shTRAP80 (1587) Rev	AGCTTTTGGAAAAAAAGAGATGGTCGGGTAATCACGGATATCAATGATTACCC GACCATCTCTGA

[A] Mutated nucleotides are underlined.

Supplemental Table 3. List of primers used for ChIP.

ChIP Oligo Name	Sequence (5'→3')	Notes
mSREBP-1c (LXRE) For	AGGCTCTTTTCGGGGATGG	
mSREBP-1c (LXRE) Rev	TGGGGTTACTGGCGGTCAC	
mABCA1 (LXRE) For	GGGGAAAGAGGGAGAGAACAG	
mABCA1 (LXRE) Rev	GAATTACTGGTTTTTGCCGC	
mSREBP-1c (Exon) For	TTTGTCATTGGCTGTGGTCTTC	Non-LXRE
mSREBP-1c (Exon) Rev	CGGCATGGTCCTGATTGC	Non-LXRE
mABCA1 (Exon) For	GGCAGTGCCTTTGTAGCCTATG	Non-LXRE
mABCA1 (Exon) Rev	GGTTCCACACCAGAGTTTCACA	Non-LXRE

Supplemental Table 4. List of primers used for gene expression analysis by qRT-PCR.

Gene name	Forward primer sequences	Reverse primer sequences
hTRAP80	CCTTCCGAGTAGCTGGGACC	AAATTAGCCAGGCGTGGTTG
hSREBP-1c	CCATCTGTGAGAAGGCCAGTG	GGTGTGGTAGCCAGGCTGTC
hABCA1	GAGAAGCTTTCAACGAGACTAACCAG	TGAAGCGAGATATGGTCCGG
hRPS29	CGCGAAGGATATCGGTTTCA	GCCCCGGATAATCCTCTGA
mTRAP80	CCTTGGGACTCTGTGAGGAA	GTGGACTCTGTTTGGGAGGA
mLXRα	GCAGCTGGGCATGATCG	CTGTTACACTGTTGCTGGGCA
mLXRβ	TACCTCCGCCACGTCACC	GGATCAGTCTCCTGCCCCTC
mSREBP-1c	CAGCCATGGATTGCACATTTG	GTCTTGGTTGTTGATGAGCTGG
mFAS	CTGCAGCTGTCAGTGTGAAGAAG	GCAGCATTTTTACCAGGTTGGT
mSCD1	AGCTCAGTCTCACTCCTTCCCTTA	CAGCCAGCCTCTTGACTATTCC
mABCA1	GACCCGTACTCTCGCAGGG	CCTTGCCGGTATTTTAGCAGG
mABCG1	TTCATCGTCCTGGGCATCTT	GAAATAGGCGATGAGCCGC
mABCG5	TCAATGAGTTTTACGGCCTGAA	GCACATCGGGTGATTTAGCA
mABCG8	CGGAGCACTGTGCCTACGT	CGCAGGTTTGTCAGCCAGTA
mIDOL	TGTGGAGCCTCATCTCATCTT	AGGGACTCTTTAATGTGCAAGAA
mCOX-2	AACCGCATTGCCTCTGAAT	CATGTTCCAGGAGGATGGAG
miNOS	CGAAACGCTTCACTTCCAA	TGAGCCTATATTGCTGTGGCT
mRPS29	CGCAAATACGGGCTGAACA	GCCTATGTCCTTCGCGTACTG