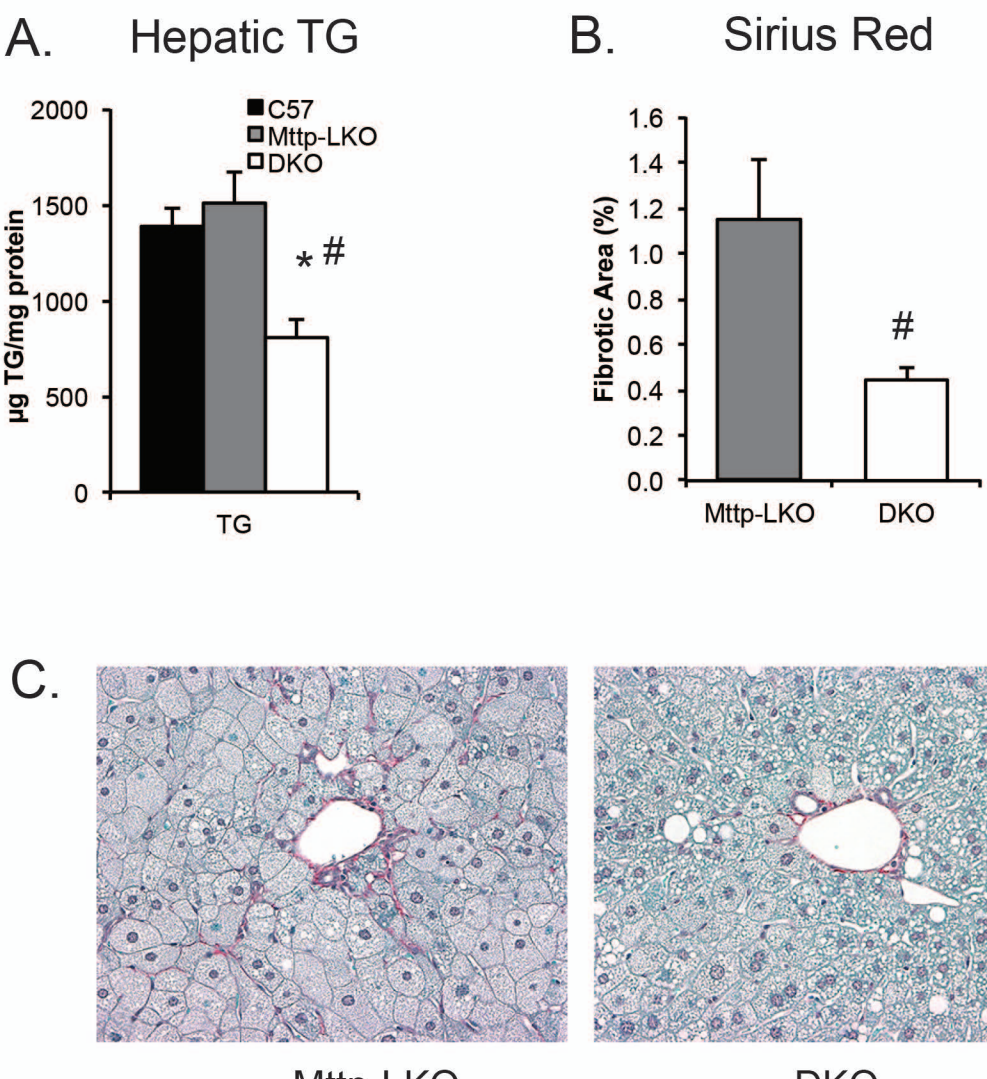
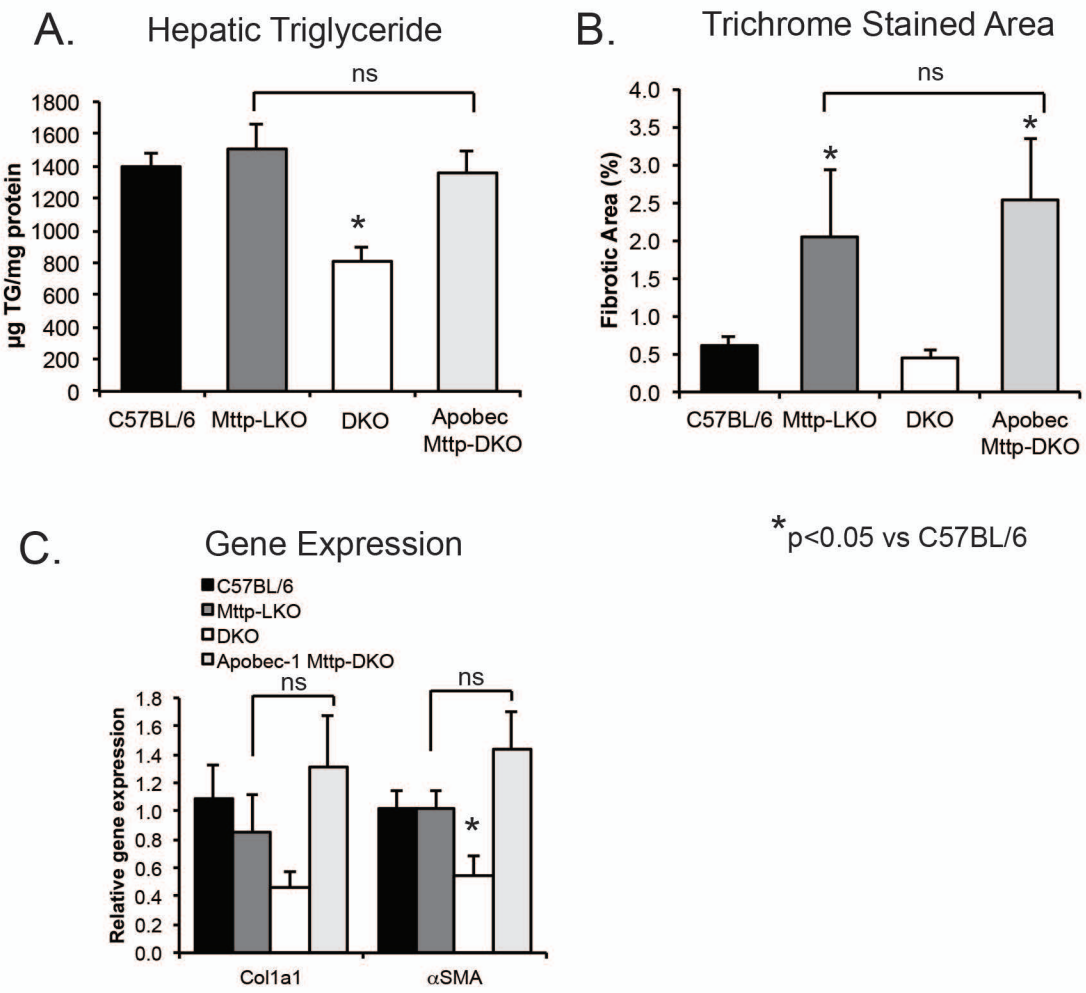
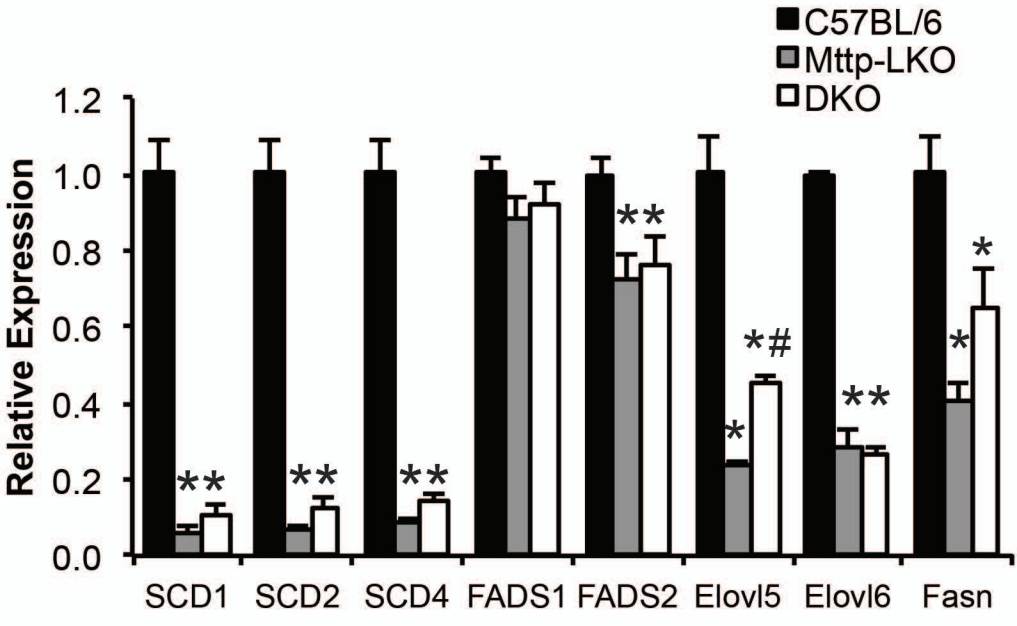
Supplemental Figure 1



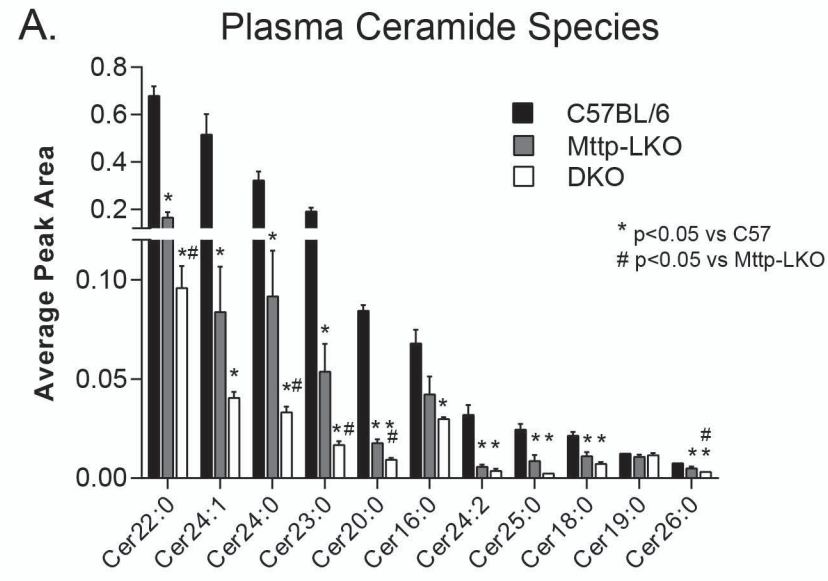
Supplemental Figure 2

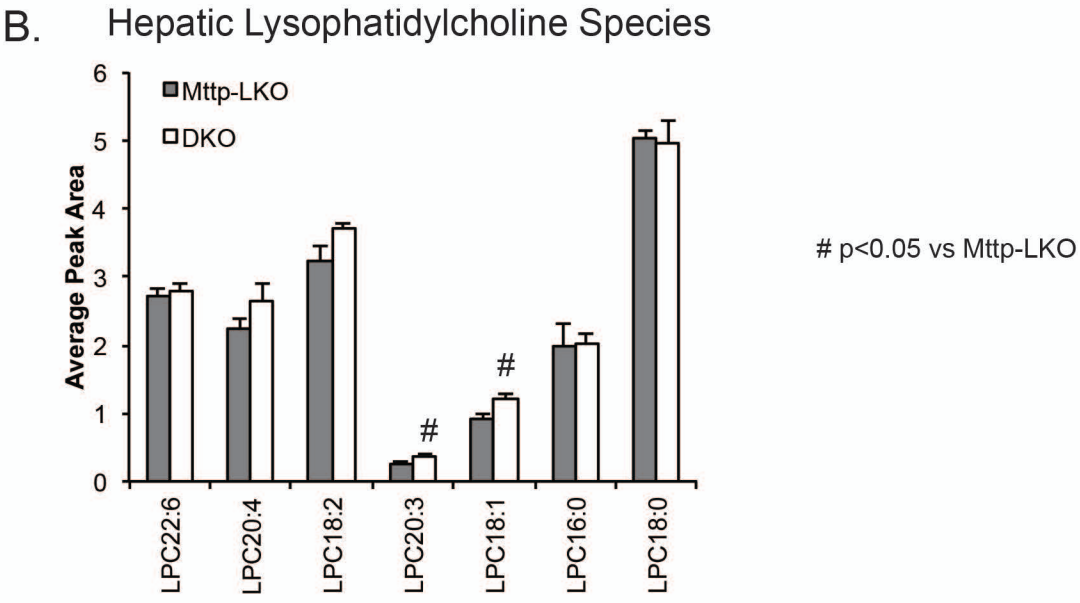


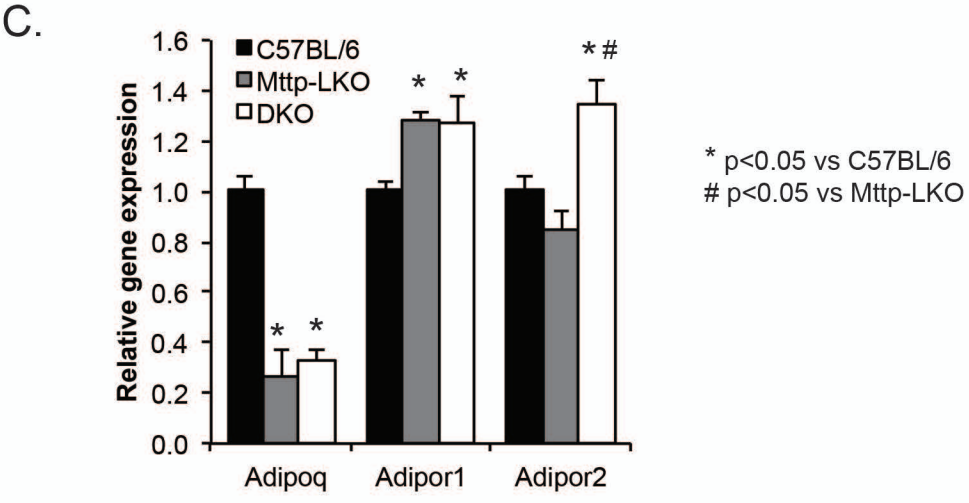
Supplemental Figure 3



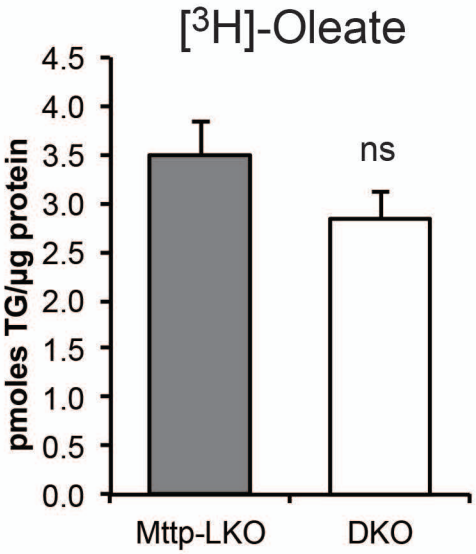
Supplemental Figure 4

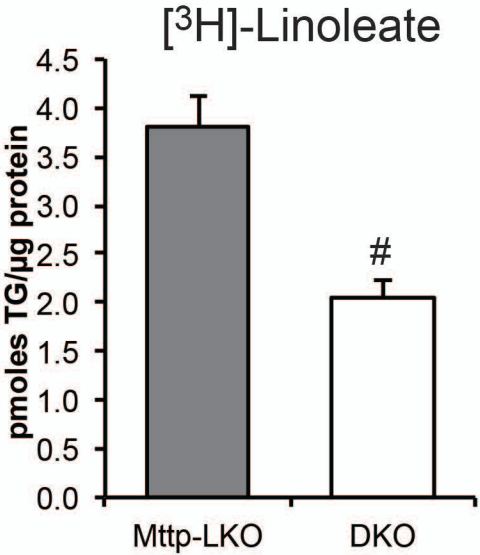




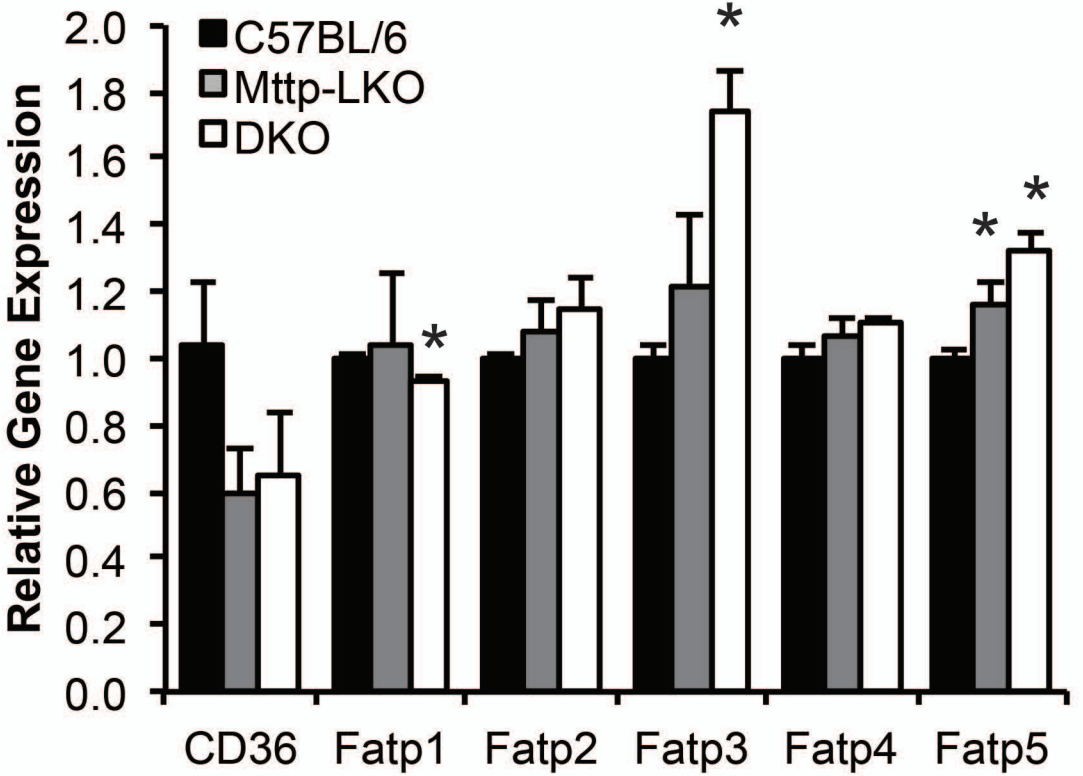


Supplemental Figure 5



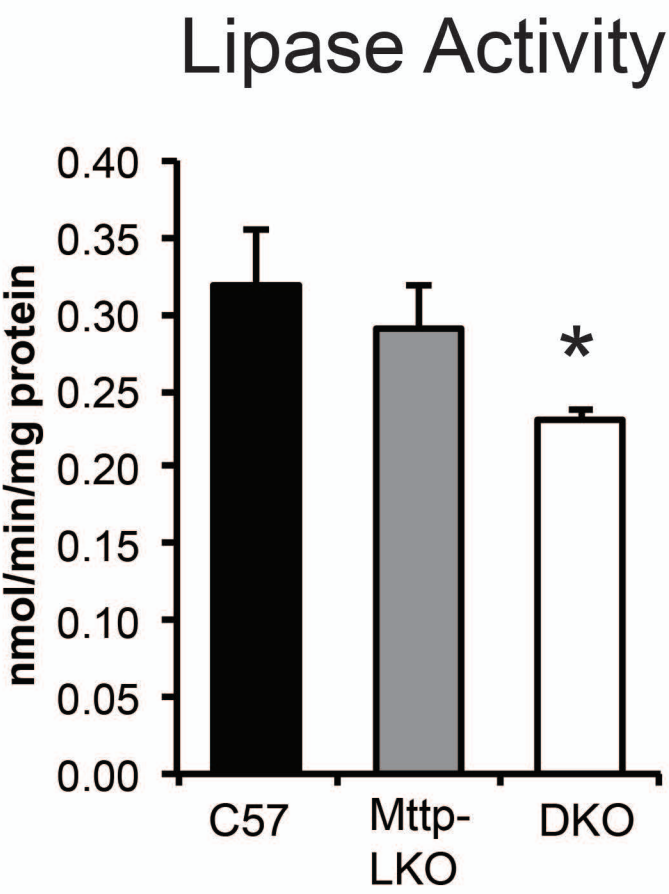


Supplemental Figure 6

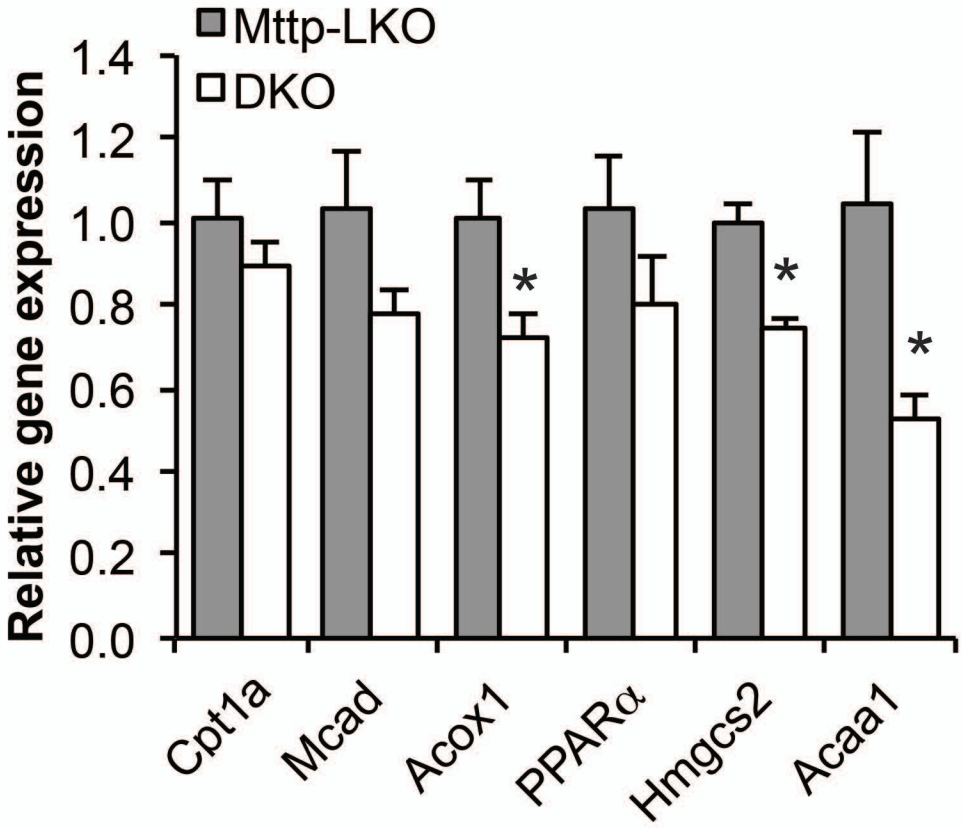


* indicates p<0.05 vs C57BL/6

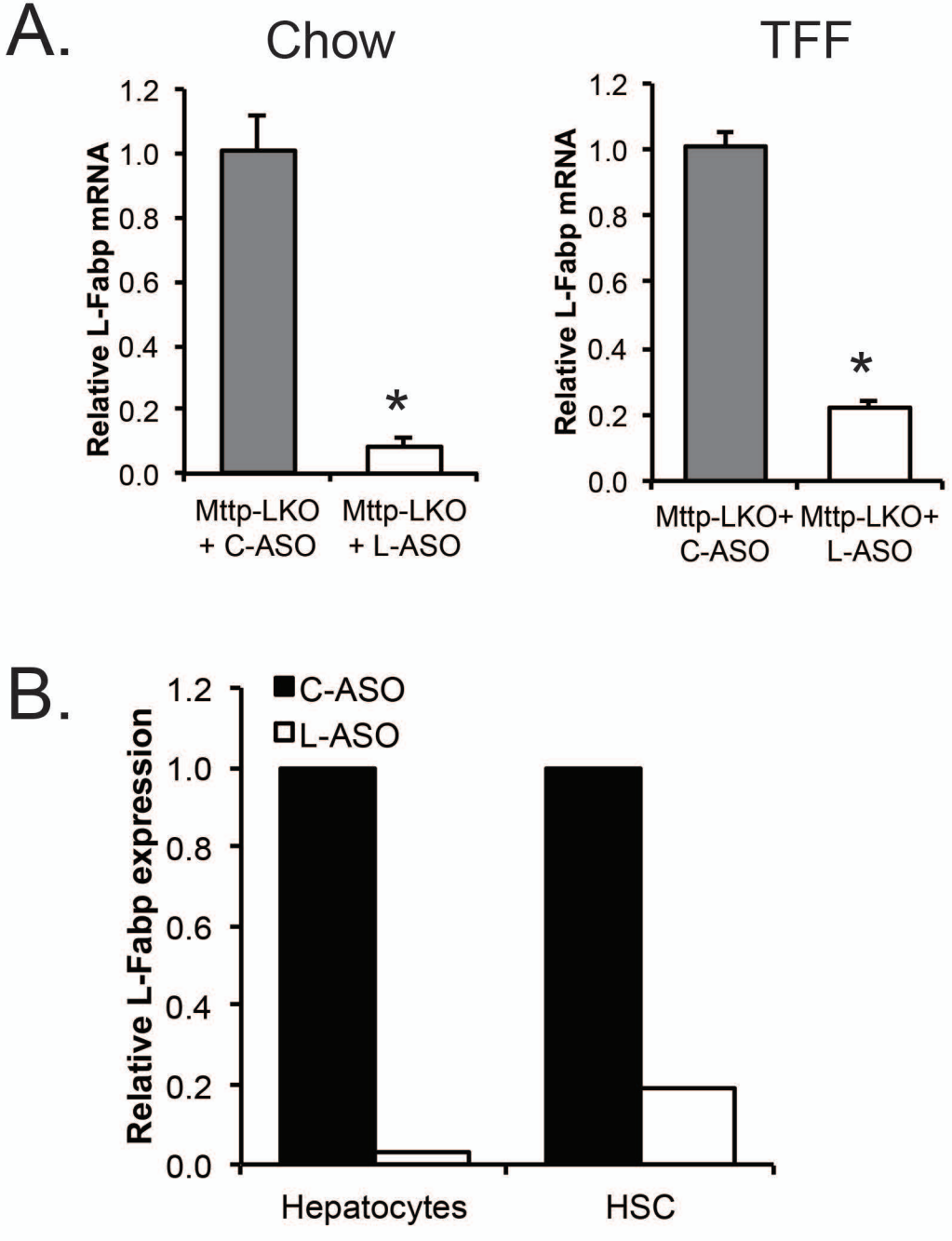
Supplemental Figure 7



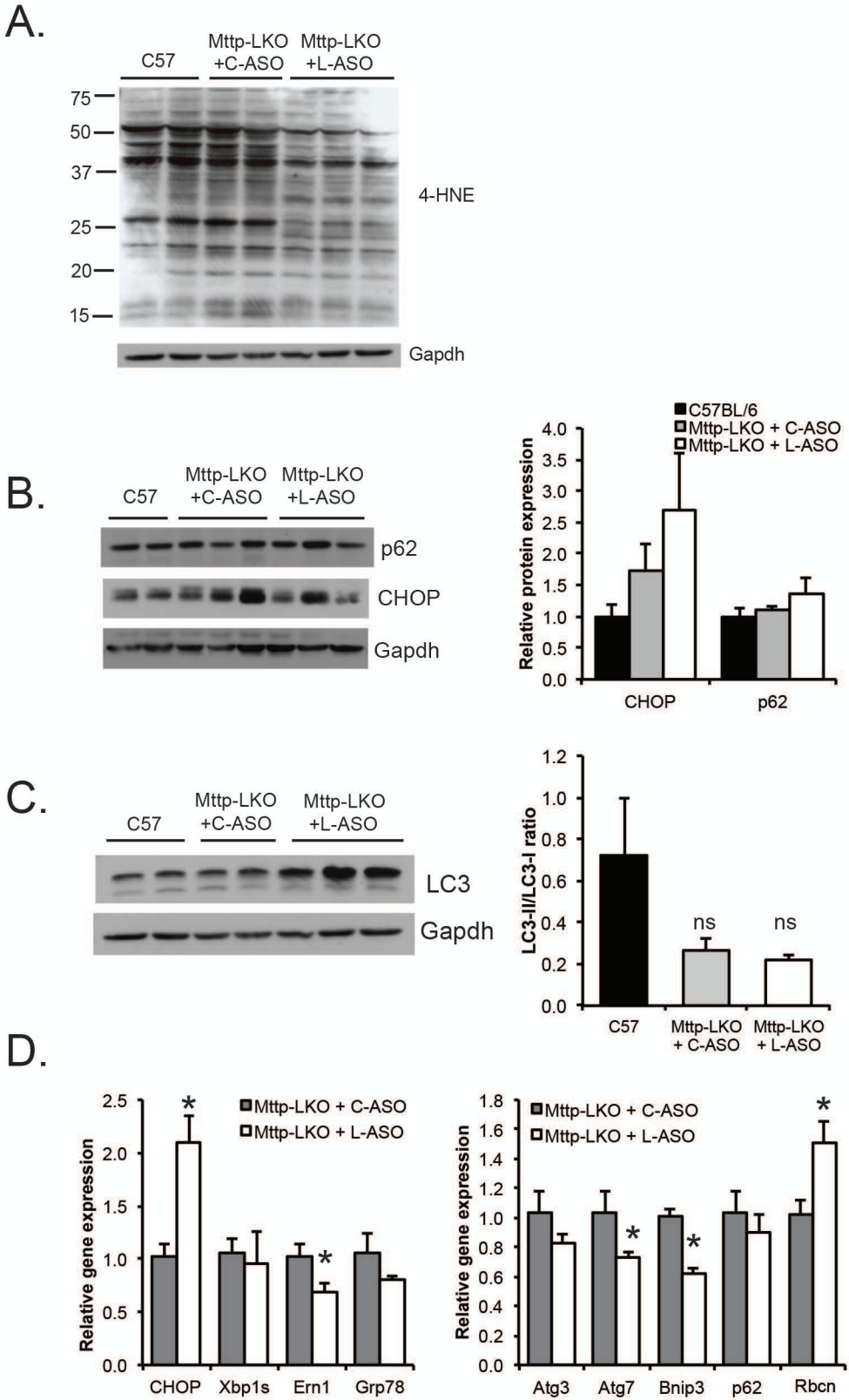
Supplemental Figure 8



Supplemental Figure 9



Supplemental Figure 10



Supplemental Table 1: Serum ALT

	C57BL/6J	Mttp-LKO	DKO	Apobec1 Mttp DKO
Chow diet, females	26.8 ± 7.5 [a]	5.4 ± 2.1[b]	14.9 ± 3.6[a]	12.8 ± 0.7[a]
Chow diet, males	7.1 ± 3.1[a]	12.5 ± 2.9[a]	33.9 ± 5.8[b]	23.8 ± 5.0[b]
TFF diet, females	15.6 ± 4.1 [a]	28.6 ± 8.4 [a]	21.0 ± 5.0 [a]	129.2 ± 27.8 [b]
TFF diet, males	114.5 ± 18.6 [a]	106.0 ± 38.7 [a]	67.0 ± 19.1 [a]	137.0 ± 20.1 [a]

Units are IU/L. Distinct letters indicate significant differences between groups.

Supplemental Table 2: Q PCR Primers

Gene	Ref Seq	Forward (5' to 3')	Reverse (5' to 3')
Acaa1	NM_130864	TTCTGTCGGCCGTGTTGAC	CATTGCCCACGGAGATGTC
Acox1	NM_015729	GGATGGTAGTCCGGAGAACA	AGTCTGGATCGTTCAGAATCAAG
Adpn		TTACCTATGGCTGTAGGATTGGAT ACT	CACCCTTGCTTCCCTTCTTG
Adipor1	NM_028320	CTACATGGCCACAGACCACCTA	CTCTGTGTGGATGCGGAAGA
Adipor2	NM_197985	TCCCAGGAAGATGAAGGGTTTAT	CTCTTCCATTCGTTCCATAGCAT
Atf4	NM_009716	CGAGTTAAGCACATTCCTCGAATC	TTCGCTGTTCAGGAAGCTCAT
Atg3	NM_026402	CCAGCCCCAGGATGCA	CAGGTACTCGGCCACTTCCA
Atg7	NM_023566	CGCCAAGATCTCCTACTCCAA	CCACCCCCTAGACAATCTTCAA
Bmp2			
Bnip3	NM_009760	GATTGGATATGGGATTGGTCAAG	GCTTCGGGTGTTTAAAAAGGAA
CD36			
Chop/Ddit	NM_007837	CCACCACACCTGAAAGCAGAA	TGAAAGGCAGGGACTCA
Col1a1	NM_007742	CACGGCTGTGTGCGATGA	TCGCCCTCCCGTCTTTG
Col4a1	NM_009931	CCAGGATGCAACGGTACAAA	AACGTGGCCGAGAATTTCAC
Cpt1a	NM_013495	TGAGTGGCGTCCTCTTTGG	CAGCGAGTAGCGCATAGTCATG
Ctgf	NM_010217	CCCACACAAGGGCCTCTTC	CCATCTTTGGCAGTGCACACT
Elovl5	NM_134255	CTTCTCCAAACTCATCGAATTCATG	CGGTGATCTGGTGGTTGTTCT
Elovl6	NM_130450	GGAGGAGAGCCCCTGAGCTA	TGATCTTCGGAGTCGCTACGT
Ern1	NM_023913	CGGCAGGCCAACATCCT	CAATGACGTCCTCATGCTTGTC
Fads1	NM_146094	CCCACCAAGAATAAAGCGCTAA	ATGAGGCCCATTCGCTCTACT
Fads2	NM_019699	GCCTGGTTCATCCTCTCGTACT	GCGAGGACAAAGGCTGTGA
Fasn	NM_007988	TCCTGGAACGAGAACACGATCT	GAGACGTGTCACTCCTGGACTTG
Fatp1			
Fatp2			
Fatp3			
Fatp4			
Fatp5			
Gapdh	NM_008084	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGA
Grp78	NM_022310	ACCCCGAGAACACGGTCTT	GCTGCACCGAAGGGTCATT
Habp1			
Hmgcs2	NM_008256	TGGTGGATGGGAAGCTGTCTA	TTCTTGCGGTAGGCTGCATAG
Krt19	NM_008471	TCTCAGACCTGCGTCCCTTT	TGGCGATAGCTATAGGAAGTCATG
L-Fabp	NM_017399	CCAGGAGAACTTTGAGCCATTC	TGTCCTTCCCTTTCTGGATGA
Mcad	NM_007382	TGACGGAGCAGCCAATGA	ATGGCCGCCACATCAGA
Mmp2	NM_008610	TGGGACAAGAACCAGATCACATA	AAAGCATCATCCACGGTTTCA
Opn	NM_009263	TTTCACTCCAATCGTCCCTACA	TCAGTCCATAAGCCAAGCTATCAC
PPARa	NM_011144	TATTCGGCTGAAGCTGGTGTAC	CTGGCATTTGTTCCGGTTCT
p62	NM_011018	TGTGGAACATGGAGGGAAGAG	TGTGCCTGTGCTGGAACTTTC
Rubcn	NM_172615	CCGAGAGATCCAGGAACTGAAG	AGGTTTTTGGTGCGGATCTG
aSMA	NM_007392	CCAGAGCAAGAGAGGGATCCT	TGTCGTCCCAGTTGGTGATG
Smad2			
Smad3			
Smad7			
SCD1	NM_009127	TCGAAGGACCCGAGGTGTT	CACCTCTTAGCAGCTACTTACAGACACT
SCD2	NM_009128	GTACCGCTGGCACATCAACTT	ACACTCTCTTCCGGTCGTAAGC
SCD4	NM_183216	CATCACACGTTCCCCTACGA	TCGATGAAAAACGTGGTGAAGT
Tgfb1			
Tgfbr1			
Tgfbr2			
Xbp1	NM_013842	ATCAGCTTTTACGGGAGAAAACTC	CCATTCCCAAGCGTGTTCTT

Legends to Supplemental Figures

Figure S1. A. Hepatic triglyceride content in male mice fed TFF diet for 16 weeks, 5-6 animals/genotype. B. Sirius Red stained fibrotic area in livers of male Mttp-LKO and DKO mice fed TFF diet. N=4/genotype. C. Representative images of Sirius red stained tissue, showing reduced lipid and fibrosis in DKO liver tissue, 200x magnification. The asterisk indicates $p<0.05$ vs C57BL/6; # indicates $p<0.05$ in DKO vs Mttp-LKO.

Figure S2. A. Hepatic triglyceride content in male mice fed TFF diet for 16 weeks, 5-8 animals/genotype. B. Trichrome stained fibrotic area in mice fed TFF diet. N=4-5/genotype. C. Fibrogenic gene expression in livers of TFF fed mice. Asterisks indicate significant differences versus C57BL/6. ns indicates that differences between Apobec-1 DKO and Mttp-LKO are not significant.

Figure S3. Expression of genes involved in FA synthesis, elongation and desaturation in the livers of chow fed mice (n=4/genotype). Asterisks indicate $p<0.05$ vs C57BL/6; # indicates $p<0.05$ in DKO vs Mttp-LKO.

Figure S4. A. Relative abundance of ceramide species in plasma of mice fed TFF diet for 16 weeks (n=5/genotype). B. Relative abundance of lysophosphatidyl choline (LPC) species in livers of Mttp-LKO and DKO mice fed chow diet (n=4-5/genotype). C. Relative expression of adiponectin (Adipoq) and adiponectin receptor (Adipor1/2) mRNAs in liver of TFF-fed mice (n=5/genotype). For all panels, asterisks indicate $p<0.05$ vs C57BL/6; # indicates $p<0.05$ in DKO vs Mttp-LKO.

Figure S5. Incorporation of [^{3}H]-oleate (left) and [^{3}H]-linoleate into cellular triglyceride of primary hepatocytes following a 15 minute labeling period. Data are from 4 independent experiments for oleate and 2 independent experiments for linoleate. # indicates $p<0.05$ in DKO vs Mttp-LKO; ns indicates not significant.

Figure S6. Relative expression of CD36 and Fatp mRNAs in livers of chow fed mice (n=4/genotype). Asterisks indicate $p<0.05$ vs C57BL/6; # indicates $p<0.05$ in DKO vs Mttp-LKO.

Figure S7. Hepatic lipase activity in livers of TFF fed female mice (n=5/genotype). Asterisk indicates $p<0.05$ compared to C57BL/6J.

Figure S8. Expression of genes involved in FA oxidation and ketogenesis in livers of mice fasted 48 hours. Asterisks indicates $p<0.05$ in DKO vs Mttp-LKO; n=4-5/genotype.

Figure S9. A. Expression of L-Fabp mRNA in whole liver of Mttp-LKO mice treated with control (C-ASO) or L-Fabp ASO (L-ASO), fed either chow or TFF diet (10 weeks). n=3-4/group. * indicates $p<0.5$ vs C-ASO B. Expression of L-Fabp mRNA in

primary hepatocyte and hepatic stellate cell pools isolated from (n=5 per group) C57BL/6 mice treated with control or L-Fabp ASO for 3 weeks.

Figure S10. A. Western blot analysis of liver tissue from TFF fed C57BL/6J, Mttp-LKO +C-ASO and Mttp-LKO + L-ASO mice, probed with an antibody to 4-hydroxynonenal. Expression of Gapdh is shown as a loading control. B. Expression of Chop and p62 protein in livers of TFF fed C57BL/6J, Mttp-LKO +C-ASO and Mttp-LKO + L-ASO mice. A representative blot is shown (left), with quantitative data from 4-6 animals per group presented in the bar graph, normalized to levels of Gapdh.

C. Expression of LC3 protein in TFF fed mice. A representative blot is shown (left), with quantitative data (5-6 samples/genotype), showing ratio of LC3-II (lower band) to LC3-I = in the bar graph. D. Expression of genes involved in ER stress (left) and autophagy (right) in livers of Mttp-LKO mice treated ± L-ASO.

SUPPLEMENTAL MATERIALS AND METHODS.

Reagents: Serum and tissue TG, cholesterol, FFA, glucose and b-hydroxybutyrate levels were measured using commercially available kits (Wako Diagnostics) as described previously (1). Serum ALT and AST were measured using colorimetric kits (TECO Diagnostics). Serum insulin levels were determined using an Erenna Immunoassay (Singulex). Total and high molecular weight apdiponectin levels in plasma were measured using an Elisa from Alpco Diagnostics (Salem, NH; #47-ADPMS-E01). Lipase activity and hydroxyproline content in liver tissue was measured using kits from Sigma Aldrich (MAK046, MAK008, respectively). Lipid peroxidation was evaluated by using a kit to measure lipoperoxidase levels (Cayman Chemical, #705002). Triascin C was obtained from Santa Cruz biotechnology (SC-200574). Antibodies for Western blot analysis were obtained from the sources listed below.

Antigen	**Antibody Source/Reference**
Actin	Sigma Aldrich, #A-2066
Albumin	Abcam, #ab83465
CHOP	Cell Signaling, #2895
Gapdh	Santa Cruz Biotechnology, SC-25778
Grp78	Enzo, ALX-210-137
4-HNE	Novus, #NB100-63093
eIF2 alpha	Cell Signaling, #5324
Phospho-eIF2a	Cell Signaling, #3398
LC3b	Cosmo Bio Co, CTB-LC3-1-50
L-Fabp	Dr Jeff Gordon, as described (1)
Mttp	BD Transduction Laboratories, #612022
p62/SQSTM1	Abnova, H00008878-M01
Plin2, 3, 4 and 5	Gift of Dr Nathan Wolins (2)

Animal Studies: Mice were housed in a full barrier facility (12h light:dark cycle) with littermates of same gender (2-5 mice/cage), in standard isolator cages with metal wire racks and corncob bedding. Mice were fed a standard rodent, low fat chow (PicoLab 20, #5053) containing ~4.5% fat (sourced primarily from soybean oil) and given free access to food and water unless otherwise noted. Data is from female mice unless otherwise noted. All animal protocols were approved by the Washington University Animals studies committee, and followed guidelines outlined by the National Institutes of Health. Female mice were fed fructose diet (60% fructose, TD.89247, Envigo, Madison WI) for 3 weeks to stimulate lipogenesis. To induce hepatic steatosis and fibrosis, mice were fed a high transfat, fructose (TFF) diet containing 22% hydrogenated vegetable oil (primarily palm and soybean) (TD.06303, Envigo)] for 16 weeks and given water containing 45% glucose, 55% fructose, 42 g/L, as described (3). Fasting studies were performed as described (1), using male mice 10-14 weeks of age. Anti-sense oligonucleotides were injected as

described (4) for either 2 weeks in chow fed mice or for 12 weeks in mice fed TFF diet, with ASO injections starting 2 weeks prior to TFF feeding. To examine cell type-specific, ASO-mediated knockdown of L-Fabp, male C57BL/6J mice were treated with control or L-Fabp ASO for 3 weeks. Hepatocytes and HSC were isolated as described below and in (3) and expression of L-Fabp mRNA was determined.

Primary Hepatocyte Studies: Livers were perfused via inferior vena cava with HBSS, then digested with 0.05% collagenase (C-5138, Sigma) as described (5). Disrupted cells were washed in Hepatocyte Wash medium (Gibco) and plated on collagen-coated dishes (Biocoat) in 5% fetal calf serum. For FA uptake, cells were labeled with ~1μCi [^{3}H]-oleate or [^{3}H]-linoleate (American Radiolabeled Chemicals, #0198, #0332, respectively) in media containing 250μM unlabeled FA coupled to BSA (Sigma O-3008, L-9530) for 15 minutes or 4 hours. Media and cell extracts were collected. Cells were disrupted using Bullet Blender (NextAdvance) with 1mM glass beads. Lipids were extracted from 50μl of homogenate with chloroform: methanol (2:1), dried under nitrogen, and separated by thin layer chromatography using a mobile phase of hexane: ethyl ether: acetic acid (60:30:1). The TG band was identified by co-migration of authentic TG standard (#1787, Supleco) and normalized to total cellular protein. Levels of FA oxidation were determined by counting the radioactivity in the aqueous phase after extraction with chloroform:methanol, normalized to cellular protein. Oleate incorporation data are from 7 independent hepatocyte isolations per genotype, linoleic acid incorporation data are from 4 isolations, and acetate data are from 5 isolations. In each experiment, assays were performed in duplicate. In some studies, cells were labeled for 4 hours, then chased overnight (18h) in media containing only unlabeled FA, in the presence or absence of Triacsin C to inhibit reutilization of released FA. Data are expressed as percent of radiolabeled TG at 0h (after 4h label) to correct for genotype-dependent differences in incorporation.

In vivo FA trafficking: Mice were fasted for 4h, then injected with 1.7μC [^{3}H]-oleate in saline containing 6% FA-free BSA via the tail vein as described (6). After 10 minutes, liver was perfused with cold PBS, excised and snap frozen in liquid nitrogen. Tissue was homogenized with glass beads and lipid extracted as described above.

Histology: Osmium stained liver tissue was prepared as described previously (3), with droplet size/number measured using Nuance (Version 2.10, Perkin Elmer) and Inform (Version 1.4, Caliper Life Sciences) software. Data were obtained from 3-4 mice/genotype, from 10 photos/slide. Masson's trichrome and Sirius Red staining was performed as described (3), Quantitation of stained area was performed using Nuance multispectral imaging software. 8-10 images were analyzed per sample.

Analysis of Plasma Ceramide Species: 20μl of plasma was extracted using a modified Bligh-Dyer method in the presence of an internal standard (Cer17:0). Measurement of lipids was performed with a Shimadzu 10A HPLC system and a Shimadzu SIL-20AC HT auto-sampler coupled to a Thermo Scientific TSQ Quantum

Ultra triple quadrupole mass spectrometer operated in SRM mode under ESI(+). Data processing was conducted with Xcalibur (Thermo), with data reported as the peak area ratios of the analytes to the internal standard.

Gene expression: Real time PCR reactions were performed using Fast SYBR Green (ThermoFisher) master mix in a Step One Plus (Applied Biosystems) thermocycler. Gene expression levels were expressed as fold change relative to control after normalization to GAPDH as described (3). Primer sequences are listed in Supplemental Table 2.

Statistical analysis: Statistical comparisons were performed using Student t-test (two tailed, unpaired) or one-way ANOVA in Microsoft Excel or GraphPad Prism. Data are presented as mean ± SE unless otherwise noted.

Supplemental References

1. Newberry EP, Xie Y, Kennedy S, et al., J Biol Chem 2003; 278: 51664.
2. Wolins NE, Quaynor BK, Skinner JR et al., J Biol Chem 2005; 280: 19146.
3. Chen A, Tang Y, Davis V, et al., Hepatology 2013; 57: 2202.
4. Newberry EP, Kennedy SM, Xie Y, et al., J Lipid Res 2012; 53: 744.
5. Chen Z, Fitzgerald RL, Averna MR, and Schonfeld G, J Biol Chem 2000; 275: 32807.
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