Supplemental Materials and Methods:

Plasma Lipid and Lipoprotein Analyses
Plasma samples were collected by submandibular vein puncture at baseline (6 weeks of age chow-fed animals), and after 4, 8, or 20 weeks of diet and ASO treatment. Plasma lipoprotein separation was achieved by size exclusion chromatography (FPLC), and lipoprotein cholesterol concentrations were measured enzymatically as previously described.1-3 Following FPLC, fractions corresponding to VLDL, LDL, and HDL were collected from individual animals (n=5 per group), and particle size was determined by dynamic light scattering using a zetasizer nano S (Malvern Instruments, United Kingdom). In addition, LDL and HDL were collected and extracted by the method of Bligh and Dyer,4 and fatty acid methyl ester (FAME) analyses were conducted by gas-liquid chromatography (GLC) as described previously.3 Whole plasma immunoblotting was conducted as previously described.1

Glucose homeostasis
Fasting (4 hour fast) plasma insulin levels were measured by ELISA (Crystal Chem, Inc.) in mice that had been treated with diet and ASO for four weeks. Glucose tolerance tests (GTT) were performed after an overnight (10 hour) fast by injecting 1 mg/kg body weight of glucose into the peritoneal cavity. Insulin tolerance tests (ITT) were performed after an overnight (10 hour) fast by injecting 0.75 U/kg body weight into the peritoneal cavity. Plasma glucose levels were measured using a commercial glucometer (Ascensia Countour, Bayer). GTT and ITT were performed on mice that had been treated with diet and ASO for 16 weeks.

Quantification of atherosclerosis
En face morphometric quantification of aortic atherosclerosis was conducted as previously described5,6 with minor modifications. Briefly, lesion area was determined for the whole aorta as described previously,5,6 and lesion area was then examined in specific regions of the aorta including the aortic arch (extending from the aortic valve to a point just distal to the left subclavian artery), the thoracic aorta (extending to the origin of the celiac artery), and the abdominal aorta (extending to the iliac bifurcation). Following en face morphometric analyses, all aortae were subjected to biochemical analysis to determine free and esterified cholesterol mass and fatty acid composition as previously described.5-7 In a subset of animals, hearts and aortas were fixed in 10% neutral buffered formalin and sections were taken from the aorta just distal to the aortic valve for histological evaluation of atherosclerosis. Histological cross sections were stained with Verhoeff-van Gieson's stain. Images were captured using a Sony digital camera (Model DXC-S500) and evaluated for atherosclerosis extent and severity using Scion Image software (Version 1.63).

Hepatic lipid analyses
Liver lipid extracts were made as previously described.1,2 Total cholesterol (TC), cholesterol ester (CE), free cholesterol (FC), triglyceride (TG) mass was measured using enzymatic assays as described previously.1,2 Phospholipid phosphorous mass was determined according to Fiske and Subbarow.8 To determine the fatty acid composition of liver CE, TG, and PL, these lipid classes were separated by thin layer chromatography and the corresponding bands were scraped, methylated, and quantified by GLC as previously described.7

Determination of hepatic lipid secretion rates
Recirculating isolated liver perfusions were performed as previously described1,9 in mice that had undergone four weeks of SFA-enriched diet and ASO treatment.

Peritoneal macrophage isolation and culture
Elicited peritoneal macrophages were collected 4 days after injection of 1ml of 10% thioglycolate into the peritoneal cavities of mice that had been treated with ASOs and fed a saturated diet for 6 weeks as previously described.10 Following 2 hours of culture, non-adherent cells were removed by washing three times with PBS, and remaining adherent macrophages were harvested for membrane fatty acid analysis, immunoblotting, and qPCR. For TLR4 agonist studies, adherent macrophages were maintained in serum free RPMI-1640 for an additional two hours to dampen basal serum-induced signaling. Thereafter, cells were treated with vehicle (PBS) or 10 ng/ml of the defined TLR 4 agonist Kdo2-Lipid A11 for a period of time up to eight hours.

Immunoblotting
Whole tissue homogenates or microsomes were made from tissues (liver, adipose, skeletal muscles, or skin) as previously described.1,12 Whole cell lysates were prepared from primary macrophage cultures as previously described.13 Microsomal preparations (25 □g loaded per lane) were used to detect SCD1, PDI, SREBP1c, and ACAT2, while whole tissue or cell homogenates (50 □g per lane) were used for detection of all other proteins. Immunoblotting was performed as previously described.1,12,13

Antibodies used for immunoblotting
1) goat anti-human apoB IgG (Academy Biomedical Co.), 2) rabbit anti-rat apoE serum (kindly provided by J. Herz, University of Texas Southwestern Medical Center, Dallas, Texas, USA), 3) rabbit anti-human apoAI IgG (Biodesign), or 4) rabbit anti-mouse LCAT serum (provided by John S. Parks) goat anti-mouse SCD1 (provided by Dr. James Ntambi, University of Wisconsin-Madison), 5) rabbit polyclonal antibody recognizing both SCD1 and SCD2 (provided by Dr. Alan Tall, Columbia University) 6) rabbit polyclonal antibody recognizing sterol regulatory element binding protein 1c (SREBP1c) (provided by Dr. Jay Horton, University of Texas Southwestern Medical Center), 7) polyclonal anti-acetyl-CoA carboxylase 1/2 (ACC1/2, Cell Signaling Technologies # 3662), 8) phospho-specific AMP-activated protein kinase AMPKa1(Ser485)/AMPKa2(Ser491) antibody (Cell Signaling Technologies #4185), 9) rabbit polyclonal antipeptide antibody against scavenger receptor BI (SR-BI) (provided

by Dr. Helen Hobbs, University of Texas Southwestern Medical Center), 10) rabbit polyclonal antibody against mouse ATP-binding cassette G5 (ABCG5) (provided by Dr. Helen Hobbs, University of Texas Southwestern Medical Center), 11) rabbit anti-African green monkey ACAT2 IgG50, 12) polyclonal anti-protein disulfide isomerase (PDI, Cell Signaling Technologies # 2446), 13) phospho-specific p44/42 ERK MAP kinase (Thr202/Tyr204) (Cell Signaling Technologies # 4370), 14) phospho-specific p38 MAP kinase (Thr180/Tyr182) (Cell Signaling Technologies # 9211), 15) phospho-specific JNK MAP kinase (Thr183/Tyr185) (Cell Signaling Technologies # 4668), 16) phospho-specific IKKa/b (Ser176/180) antibody (Cell Signaling Technologies # 2687), 17) rabbit polyclonal anti-IkBa (Cell Signaling Technologies # 9242), 18) phospho-specific Stat1 (Tyr701) antibody (Cell Signaling Technologies # 9171), 19) rabbit polyclonal anti-Toll-like Receptor 4 (TLR4) (Cell Signaling Technologies # 2246), 20) rabbit polyclonal anti-MyD88 (Sigma # M9934), 21) rabbit polyclonal anti-CD14 (Abcam # AB25092) and 22) rabbit polyclonal anti-b-actin (Cell Signaling Technologies # 4967).

Cytokine Array
Thioglycollate-elicited macrophages were seeded at a density of 1.2 x 106 cells/plate in 35-mm tissue culture plates as described under peritoneal macrophage isolation and culture. Cells were subsequently maintained in serum-free RPMI-1640 for an additional two hours to dampen basal serum-induced signaling. Thereafter, cells were treated with vehicle (PBS) or 10 ng/ml Kdo2-Lipid A for an additional eight hours. Conditioned media were collected, and cell debris was pelleted by centrifugation. Cytokine detection was carried out according to the manufacturer's (RayBiotech Inc.) recommendations. For a complete map of the 32 cytokine array see
http://www.raybiotech.com/map/mouse_2_map.pdf

SCD Activity Assay
Liver microsomes were isolated as previously described.12 Reactions were performed at 37 °C for 30 min with 200 µg of microsomal protein and 27 nM of [1-14C]stearoyl-CoA (60,000 dpm), 1mM of NADH, 1 mg fatty-acid free BSA, 100mM of Tris/HCl buffer, pH 7.4. Thereafter, fatty acids were saponified, extracted, and then methylated with 15% boron triflouride in methanol. Saturated fatty acid and monounsaturated fatty acid methyl esters were separated by 10% AgNO3-impregnated TLC using 100% hexane as developing solution. The plates were sprayed with 0.2% 2',7'-dichlorofluorescein in 95% ethanol, and the oleate and stearate bands were identified under UV light. The fractions were scraped, extracted from the silica gel, and measured by scintillation counting. SCD enzyme activity is expressed as nmol / minute / mg protein.

Quantitative Real-Time PCR (qPCR)
RNA extraction and qPCR was conducted as previously described1-3 on pooled samples (n=5 per group).

Cyclophilin or GAPDH were used as internal controls for these studies, and expression levels were calculated based on the □□-CT method. Messenger RNA levels for each gene represent the amount relative to the amount in the control ASO treated group, which

was arbitrarily standardized to 100%. Primer sequences used for qPCR are as provided in Supplemental Table II.

Statistical Analysis

Data are expressed as the mean ± standard error of the mean (SEM), and were analyzed using one-way analysis of variance (ANOVA) followed by Student's t tests for post hoc analysis. Differences were considered significant at $p < 0.05$. All analyses were performed using JMP version 5.0.12 (SAS Institute; Cary, NC) software.

Online Supplement References:

1. Brown JM, Bell TA III, Alger HM, Sawyer JK, Smith TL, Kelley K, Shah R, Wilson MD, Davis MA, Lee RG, Graham MJ, Crooke RM, Rudel LL. Targeted depletion of hepatic ACAT2-driven cholesterol esterification reveals a non-biliary route for fecal sterol loss. J. Biol. Chem. 2008;283:10522-10534.
2. Temel RE, Tang W, Ma Y, Rudel LL, Willingham MC, Ioannou YA, Davies JP, Nilsson LM, Yu L. Hepatic Niemann-Pick C1-like 1 regulates biliary cholesterol concentration and is a target of ezetimibe. J. Clin. Invest. 2007;117:1968-1978.
3. Temel RE, Lee RG, Kelley KL, Davis MA, Shah R, Sawyer JK, Wilson MD, Rudel LL. Intestinal cholesterol absorption is substantially reduced in mice deficient in both ABCA1 and ACAT2. J. Lipid Res. 2005;46:2423-2431.
4. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 1959;37:911-917.
5. Willner E, Tow B, Buhman KK, Wilson M, Sanan DA, Rudel LL, Farese RV Jr. Deficiency of acyl-CoA:cholesterol acyltransferase 2 prevents atherosclerosis in apolipoprotein E-deficient mice. Proc. Natl. Acad. Sci. USA 2003;100:1262-1267.
6. Lee RG, Kelley KK, Sawyer JK, Farese RV Jr, Parks JS, Rudel LL. Plasma cholesterol esters provided by lecithin:cholesterol acyltransferase and acyl-CoA:cholesterol acyltransferase have opposite atherosclerostic potential. Circ. Res. 2004;95:998-1004.
7. Bell TA III, Kelley K, Wilson MD, Sawyer JK, Rudel LL. Dietary fat-induced alterations in atherosclerosis are abolished by ACAT2-deficiency in ApoB100 only, LDLr-/- mice. Arterioscler. Thromb. Vasc. Biol. 2007;27:1396-1402.
8. Fiske CA, Subbarow, Y. The colorimetric determination of phosphorus. J. Biol. Chem. 1925;66:375-400.
9. Lee RG, Shah R, Sawyer JK, Hamilton RL, Parks JS, Rudel LL. ACAT2 contributues cholesteryl esters to newly secreted VLDL, whereas LCAT adds cholesteryl ester to LDL in mice. J. Lipid Res. 2005;46:1205-1212.
10. Timmins JM, Lee JY, Boudyguina E, Kluckman KD, Brunham LR, Mulya A, Gebre AK, Coutinho JM, Colvin PL, Smith TL, et al. Targeted inactivation of hepatic Abca1 causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. J. Clin. Invest. 2005;115:1333-1342.
11. Raetz CR, Garrett TA, Reynolds CM, Shaw WA, Moore JD, Smith DC Jr, Ribeiro AA, Murphy RC, Ulevitch RJ, Fearns C, et al. Kdo2-Lipid A of Escherichia coli,

a defined endotoxin that activates macrophages via TLR-4. J. Lipid Res. 2006;47:1097-1111.
12. Lee RG, Willingham MC, Davis MA, Skinner KA, Rudel LL. Differential expression of ACAT1 and ACAT2 among cells within liver, intestine, kidney, and adrenal on nonhuman primates. J. Lipid Res. 2000;41:1991-2001.
13. Brown JM, Boysen MS, Chung S, Fabiyi O, Morrison RF, Mandrup S, McIntosh MK. Conjugated linoleic acid induces human adipocyte delipidation: autocrine/paracrine regulation of MEK/ERK signaling by adipocytokines. J. Biol. Chem. 2004;279:26735-26747.

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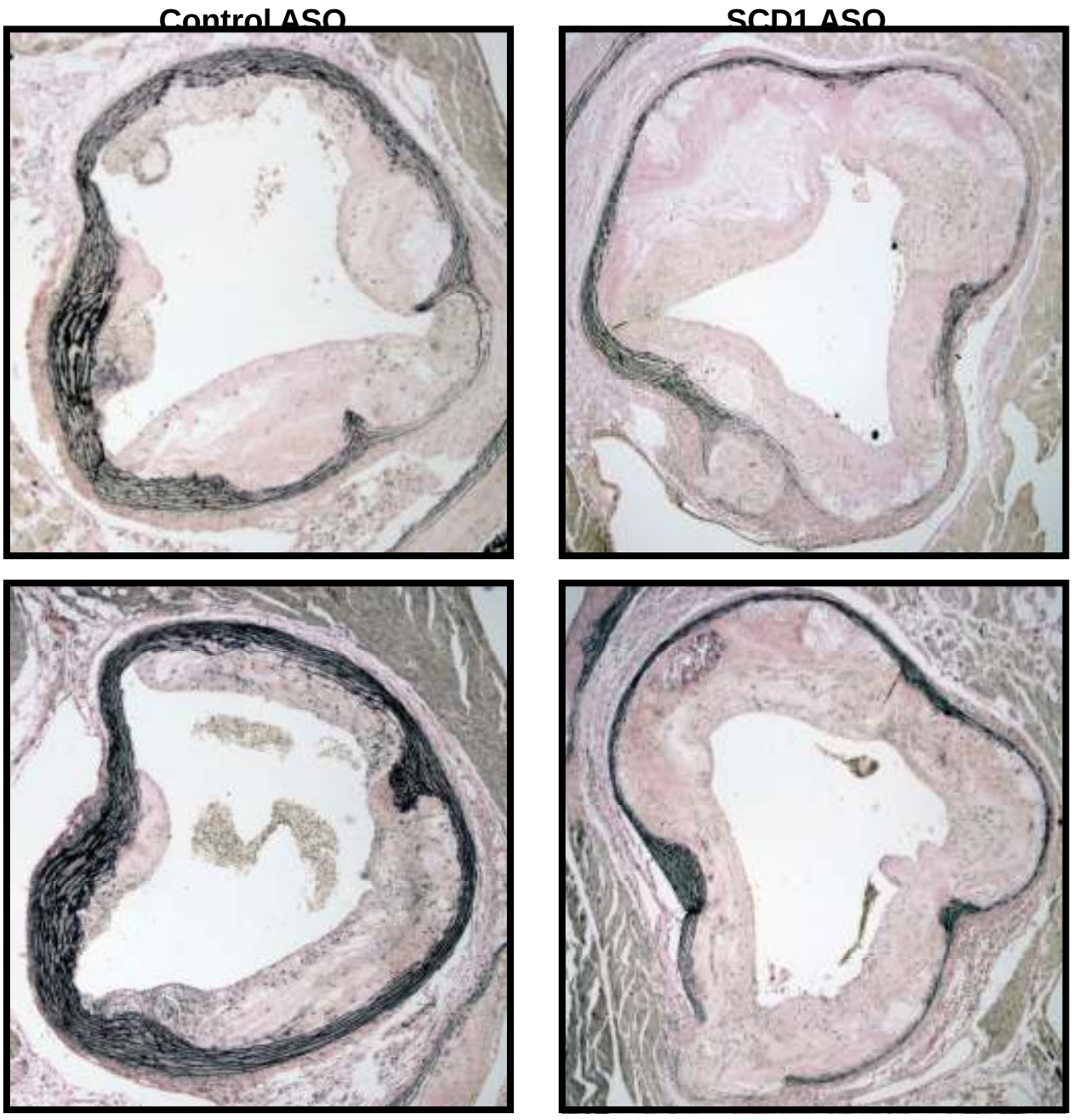
Supplementary Table 1. Fatty acid composition of diets (expressed as % of total FA)

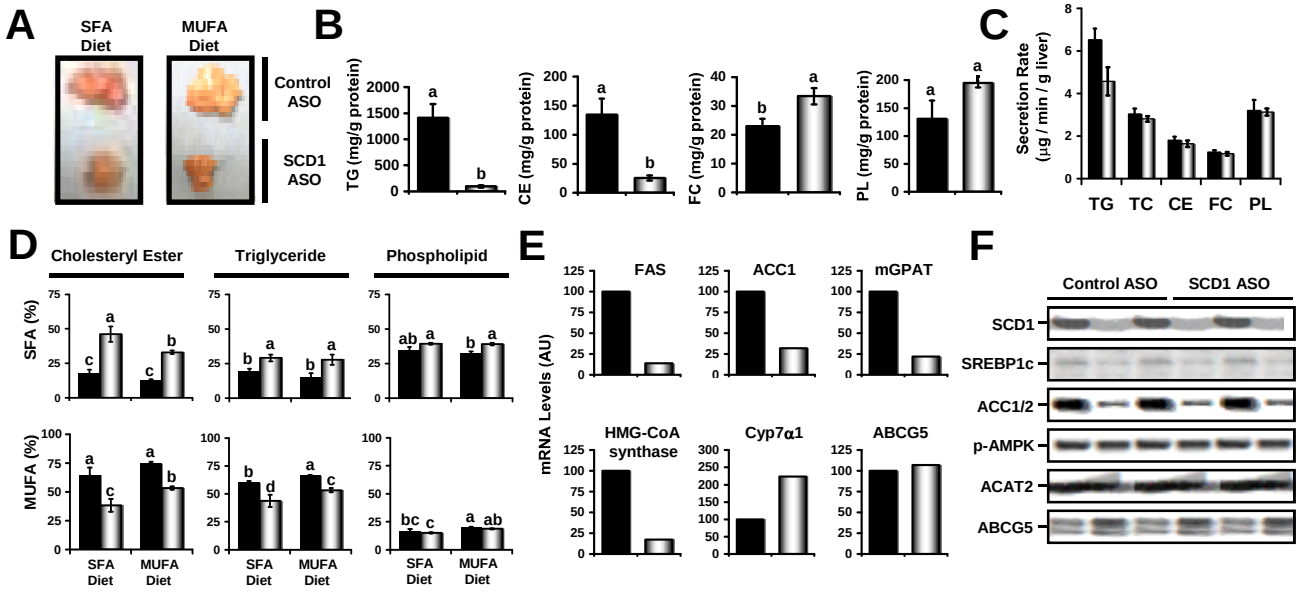
	12:0	14:0	16:0	16:1	18:0	18:1 (n-9)	18:2 (n-6)	20:0	20:5 (n-3)	22:6 (n-3)	Total SFA	Total MUFA
Saturated Diet	8.8	6.4	31.7	0.7	6.1	24.4	17.9	0.2	0.5	0.5	53.4	25.0
MUFA-Rich Diet	0.1	0.7	8.7	0.8	3.1	66.4	15.9	0.4	0.5	0.5	13.0	67.4

Supplementary Table 2. Primers used for quantitative Real Time PCR (qPCR)

Gene	Accession #	Forward Primer	Reverse Primer
stearoyl-CoA Desaturase 1 (SCD1)	NM_009127	5'-ccggagacccttagatcga-3'	5'-tagcctgtaaaagatttctgcaaacc-3'
acetyl-CoA Carboxylase 1 (ACC1)	NM_133360	5'-tggacagactgatcgcagagaaag-3'	5'-tggagagccccacacaca-3'
cholesterol 7-α hydroxylase (Cyp7α1)	NM_007824	5'-agcaactaaacaacctgccagtacta-3'	5'-gtccggatattcaaggatgca-3'
3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGC syn)	NM_145942	5'-gccgtgaactgggtcgaa-3'	5'-gcatatatagcaatgtctcctgcaa-3'
ATP binding cassette transporter G5 (ABCG5)	NM_ 031884	5Õ-tggccctgctcagca-3Õ	5Õ-atttttaaaggaatgggcatctctt-3Õ
mitochondrial glycerol-3-phosphate acyltransferase	NM_008149	5'-agcaagtcctgcgctatcat-3'	5'-ctcgtgtgggtgattgtgac-3'
fatty acid synthase (FAS)	NM_007988.3	5'-gctgcggaaacttcaggaaat-3'	5'-agagacgtgtcactcctggactt-3'
interleukin 1-beta (IL-1β)	NM_008361	5'-gtcacaagaaaccatggcacat-3'	5'-gcccatcagaggcaagga-3'
interleukin 6 (IL-6)	NM_031168.1	5'-ctgcaagagacttccatccagtt-3'	5'-agggaaggccgtggttgt-3'
monocyte chemotactic protein 1 (MCP-1)	NM_011333.3	5'-ttcctccaccaccatgca-3'	5'-ccagccggcaactgtga-3'
inducible nitric oxide synthase (iNOS)	NM_010927.2	5'-gcagctgggctgtaccaaa-3'	5'-agcgtttcgggatctgaat-3'
interferon-induced with tetratricopeptide repeats 1 (Garg-16)	NM_008331.2	5'-aggctggagtgtgctgagat-3'	5'-tctggatttaaccggacagc-3'
(C-X-C motif) ligand 10 (IP-10)	NM_021274.1	5'-ccagccgtggtcacatcag-3'	5'-acctccacatagcttacagtacag-3'
GAPDH	NM_008084	5'-tgtgtccgtcgtggatctga-3'	5'-cctgcttcaccaccttcttgat-3'
cyclolphilin	M60456	5'-tggagagcaccaagacagaca-3'	5'-tgccggagtcgacaatgat-3'



Supplemental Figure 1. Verhoeff-van Giesen stained sections of proximal aortae from LDLr $^{-/-}$, ApoB$^{100/100}$ mice fed diets enriched in 0.1% (w/w) cholesterol and saturated fat for 20 weeks in conjunction with biweekly injections (25 mg/kg) of either a non-targeting control ASO (left panels) or an ASO targeting the knockdown of SCD (right panels).



Supplemental Figure 2. SCD1 inhibition prevents diet-induced hepatic steatosis in LDLr $^{-/-}$, ApoB$^{100/100}$ mice. Starting at six weeks of age, mice were fed diets enriched in 0.1% (w/w) cholesterol and either saturated fatty acids (SFA) or monounsaturated fatty acids (MUFA) for 20 weeks in conjunction with biweekly injections (25 mg/kg) of either a non-targeting control ASO ■ or SCD1 ASO □. **(A)** Representative photographs of livers from mice treated for 20 weeks. **(B)** Hepatic lipid mass measurements were conducted for triglyceride (TG), cholesteryl ester (CE), free cholesterol (FC), and phospholipids (PL) from mice fed the SFA diet, and represent the mean ± SEM from 8 mice per group; values not sharing a common superscript differ significantly ($p<0.05$). **(C)** Hepatic secretion rate of triglyceride (TG), total cholesterol (TC), cholesteryl ester (CE), free cholesterol (FC), and phospholipid (PL) was determined by recirculating isolated liver perfusion in mice (n=5 per group) treated with ASO and fed the SFA diet for 4 weeks; no significant differences were apparent within each lipid class. **(D)** Fatty acid (FA) composition (% of total FA that was SFA or MUFA) of hepatic cholesteryl esters, triglycerides, and phospholipids. Data shown is panel **(D)** represents the mean ± SEM from 8 mice per group, and values not sharing a common superscript differ significantly ($p<0.05$). **(E)** Hepatic gene expression was measured in pooled samples (n=5 per pool) from mice treated with ASO and fed a SFA diet for 20 weeks by qPCR, and normalized to cyclophilin. **(F)** Western blot analysis of hepatic protein expression was conducted in individual animals (n=3 per group) treated with ASOs and fed the SFA diet for 20 weeks.