Supplemental Methods:

Reagents: Antibodies include M2 Flag (F-1804, Sigma-Aldrich, St Louis, MO), ApoB (1), Gapdh (SC-25778, Santa Cruz Biotechnology), F4/80 (Abcam) and BrdU (Ab8152, Abcam, Cambridge, MA). AAV8-TBG-Cre (#107787) and AAV8-TBG-LacZ (#105534) were obtained from Addgene (Cambridge, MA). Wild type (E167) and E167K mutant (K167) Tm6sf2 constructs containing a single carboxyl-terminal Flag epitope tag were generated using Tm6sf2 NM_001293795 as a template (GenScript, Piscataway, NJ) and cloned into TBG_PI shuttle plasmid (Penn vector core, Philadelphia). AAV8 was delivered either *IP* (10 to 30 days) or *IV* at 1.3x 10^{11}gc/mouse, with Tm6 mRNA expression verified at sacrifice. Serum ALT and AST levels were measured using kits from Teco (A526-120, A559-150 respectively). Serum lipids (TG, FFA, free and total cholesterol) and glucose were measured using kits from FUJIFILM Wako Diagnostics (Richmond, VA) and serum beta-hydroxybutyrate was measured using a kit from Cayman, #700190 (Cayman Chemical, Ann Arbor, MI).

Dietary studies. Male and female mice were fed high milk fat diet (HMFD, TD.09766 Envigo, Madison, WI) for 3-5 weeks starting at 8-10 weeks of age to induce hepatic steatosis. To induce fibrosis and promote hepatocellular carcinoma, mice were fed a trans-fat, fructose (TFF) supplemented diet containing 22% hydrogenated vegetable oil (TD06303, Envigo) and provided sugar water containing 55% fructose/45% glucose (4.2g/L) as described (2). In other studies, fibrogenesis was induced by feeding a palm oil diet supplemented with fructose and cholesterol (PFC diet, #D09100310 Research diets, New Brunswick, NJ) for 26 weeks, starting at 8 weeks of age. All animal protocols were approved by the Washington University Institutional Animal Care and Use committee and conformed to standards outlined in the Guide for the Care and Use of Laboratory Animals (NIH, 1985).

In vitro studies: Primary hepatocytes were isolated using collagenase perfusion (0.05%, C5138, Sigma Aldrich, St Louis, MO) and plated on collagen-coated dishes (Biocoat™, Corning) as described previously (2). Cells were pulse labeled with 2µCi/ml [^{3}H]-oleate (#ART0198, American Radiolabeled Chemicals, St Louis, MO) and 250µM oleate BSA (O3008, Sigma-Aldrich) for 2 hours, then switched to unlabeled media with 250µM oleate BSA. Cells and media were collected at 0, 4 and 18 hours and extracted with chloroform:methanol (2:1). Lipid classes were resolved using thin layer chromatography (Heptane: Diethyl ether: Acetic acid) and TG identified based on the migration of cold standards. All data were expressed as nmoles TG and normalized to total cellular protein. For ApoB secretion studies, cells were starved for 60 minutes in methionine/cysteine-deficient media (Gibco 21013-024, Thermo Fisher) containing 2mM glutamine and 1mM sodium pyruvate, pulse labeled for 30 minutes in starving media containing 250µCi/ml [^{35}S] -Protein Labeling mix (#NEG772, Perkin Elmer) with 500µM oleate BSA, then chased in media (#11965-084, Thermo Fisher) supplemented with 10mM methionine, 5mM cysteine, and 500µM oleate BSA for 2 and 4 hours (3). Radiolabeled ApoB was isolated by immunoprecipitation from cellular extracts and media, and resolved on 4-15% Criterion gels (#3450027, BioRad). Gels were fixed in methanol/acetic acid, impregnated with EN^{3}HANCE (#6NE9701, PerkinElmer), dried and autoradiographed. To quantitate ApoB, radioactive bands were cut from dried gels, solubilized with 0.5mL Solvable™ (#6NE9100, PerkinElmer) at 50°C for 3h, and counted in Ultima Gold LSC cocktail (#6013329, PerkinElmer). Cellular and secreted ApoB data are presented as % of initial labeled cellular ApoB (15min after chase), corrected to cpm/µg protein.

VLDL characterization. Following a 4h fast, mice were injected with Pluronic F127 (Invitrogen, P6866, 10µl/g body weight, IP). Serum TG was measured in blood collected

at 0h, 2h and 4h after injection. For VLDL imaging, 4h serum was pooled (3-4/genotype), adjusted to a density of 1.019g/ml with sodium bromide and centrifuged at 100,000g (Beckman MLA-130 rotor) to isolate VLDL as described (4). Particles were collected and adsorbed to Copper200 mesh carbon coated grids (PN 01840-F, Ted Pella, Redding, CA) which had been glow discharged for 30 seconds in a GloQube glow discharge unit (EMS, Hatfield, PA), washed 5 times on 50µl ddH_2O water drops by swirling while holding the grids with forceps, rinsed briefly by swirling on a 40µl drop of 0.75% uranyl formate, then negatively stained for 1 minute on a second drop of uranyl formate. Stained grids were dried for 3-5 minutes before imaging in a JEOL JEM-1400 TEM operating at 120kV. Particle area was measured in 8-10 images (magnification 50,000x, 50-150 particles total) per pool using Image J software. For FPLC sizing of lipoprotein particles, serum from 4h post-Pluronic bleed was pooled and separated using Superose 6 Gel filtration column (Amersham Pharmacia). Triglyceride and cholesterol were measured in column fractions. For ApoB western blotting, FPLC fractions or serum (2.5µl of a 1:100 dilution) were separated on Criterion 4-15% gels. Densitometry of autoradiographs was performed using Image J software.

Lipid and protein analyses: Hepatic lipid levels were determined after extraction with chloroform:methanol using commercially available kits, and normalized to cellular protein (DC™ Protein, Biorad, Hercules, CA). For lipidomic profiling (chow fed mice), liver or serum samples were extracted with chloroform methanol (2:1) and analyzed by liquid chromatography-mass spectrometry using a LTQ Orbitrap Elite (Thermo Fisher Scientific) as described (5). Briefly, aliquots were injected onto an Acuity C18 BEH column (Waters Ltd, Warrington, UK) maintained at 55°C with a flow rate of 0.5ml/min. A heated electrospray ionization source was maintained at 375 °C, with the desolvation temperature at 380 °C. Spectra were acquired in positive and negative ion mode in the range of 100-

2000 *m/z*, and species abundance was normalized to protein or volume of serum extracted. Lipidomic profiling of TFF fed mice (Supplemental figure 2) was performed as described previously (2). For Western blot analysis, tissue was homogenized in buffer containing 1% Triton X-100 and 0.1% SDS with protease inhibitors using Bullet Blender® (Next Advance, Troy, NY) and proteins separated on 12% SDS-PAGE gels (60µg/lane) unless otherwise noted.

Gene expression: Total RNA was extracted (TRIzol™, Thermo Fisher) and treated with DNAseI. Real time PCR was performed with Fast SYBR Green (Applied Biosystems, Carlsbad, CA) on an ABI StepOnePlus instrument (Applied Biosystems). mRNA levels were normalized to Gapdh and expressed as percent of control as outlined in User Bulletin 2 (Applied Biosystems). Primers used to detect Tm6sf2 mRNA: Forward, 5-TGCCAGTGTCCTACCTACTCAATC-3; reverse, 5-GCACTCGTCAGCACCACAAA. Other primer pairs have been published previously (2) or will be provided on request.

Histology: Sirius Red staining was performed as described (2), with fibrotic area measured using either Nuance 2.10 multispectral imaging software (Perkin Elmer) or Image J software (NIH). Tumor area was assessed in images obtained from H&E stained sections (6-8/per animal, photographed from 3 or 4 distinct pieces of tissue) at low (40x) magnification, using Image J software.

Electron microscopy: Mice were perfused with Ringer's solution, followed by 0.15M cacodylate buffer containing 2.5% glutaraldehyde, 2% paraformaldehyde, and 2mM $CaCl_2$ at 37°C for 5 minutes. Post-perfusion, liver tissue was carefully excised and fixed again in the same fixative overnight at 4°C. After washing in 0.15M cacodylate buffer (3x 5 minutes), the tissue was post-fixed in 1% osmium tetroxide and 0.3% potassium

ferrocyanide in 0.15M cacodylate buffer with 2mM $CaCl_2$ for 1 hour in the dark followed by 3x 10 minute washes in ddH_2O. The liver tissue was en bloc stained with 2% aqueous uranyl acetate overnight at 4°C and dehydrated in a 30%, 50%, 70% and 100% (3x) Spurr's resin mixed with 100% ethanol, embedded in fresh 100% Spurr's resin in silicon molds, and polymerized at 60°C for 48h. Once the resin blocks were polymerized, they were faced and 70nm ultrathin sections were cut on a Leica EM UC7 ultramicrotome (Leica-Microsystems, Vienna, Austria), picked up on copper formvar/carbon support film grids (PN FCF100H-Cu, Electron Microscopy Sciences, Hatfield, PA) and post-stained with 2% uranyl acetate for 15 minutes, followed by Reynolds lead citrate for 2 minutes (6) and imaged on a JEOL JEM-1400 TEM with an AMT XR111 8 Megapixel scintillated CCD camera. For measurement of intracellular lipid droplet size, 5-6 images/sample (2000x magnification) were obtained and measured using Image J.

For Second Harmonic Generation (SHG) studies, images of 50µm liver tissue sections were acquired on a Zeiss LSM 880 NLO microscope system (Carl Zeiss Microscopy, Jena, Germany) using a 20x/0.8NA air objective and a Coherent Discovery Titanium Sapphire mode-locked femto-second laser tuned to 800nm (7). Second Harmonic signal was detected at 400nm on a non-descanned Big.2 detector equipped with a 390/40nm BrightLine band-pass emission filter (Semrock, Rochester, NY).

References

1. Xie Y, Nassir F, Luo J, Buhman K, Davidson NO. Intestinal lipoprotein assembly in apobec-1-/- mice reveals subtle alterations in triglyceride secretion coupled with a shift to larger lipoproteins. Am J Physiol Gastrointest Liver Physiol 2003;285:G735-746.
2. Chen A, Tang Y, Davis V, Hsu FF, Kennedy SM, Song H, Turk J, et al. Liver fatty acid binding protein (L-Fabp) modulates murine stellate cell activation and diet-induced nonalcoholic fatty liver disease. Hepatology 2013;57:2202-2212.
3. Xie Y, Newberry EP, Young SG, Robine S, Hamilton RL, Wong JS, Luo J, et al. Compensatory increase in hepatic lipogenesis in mice with conditional intestine-specific Mttp deficiency. J Biol Chem 2006;281:4075-4086.
4. Blanc V, Xie Y, Luo J, Kennedy S, Davidson NO. Intestine-specific expression of Apobec-1 rescues apolipoprotein B RNA editing and alters chylomicron production in Apobec1 -/- mice. J Lipid Res 2012;53:2643-2655.
5. Hall Z, Chu Y, Griffin JL. Liquid Extraction Surface Analysis Mass Spectrometry Method for Identifying the Presence and Severity of Nonalcoholic Fatty Liver Disease. Anal Chem 2017;89:5161-5170.
6. Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 1963;17:208-212.
7. Gailhouste L, Le Grand Y, Odin C, Guyader D, Turlin B, Ezan F, Desille Y, et al. Fibrillar collagen scoring by second harmonic microscopy: a new tool in the assessment of liver fibrosis. J Hepatol 2010;52:398-406.

Supplemental Table 1:

Tm6sf2 f/f Line 1

Line 1	Flox Female (8)	LKO Female (9)	Flox Male (15)	LKO Male (8)
Body weight (g)	20.8 ± 0.5	20.2 ± 1.0	27.1 ± 0.5	28.1 ± 1.1
Liver (g)	1.0 ± 0.02	0.9 ± 0.08	1.2 ± 0.02	1.4 ± 0.09*
Liver/Body (%)	4.6 ± 0.1	4.6 ± 0.2	4.6 ± 0.1	5.1 ± 0.2*
Hepatic TG (μg/mg protein)	54.0 ± 11.0	209.3 ± 44.9*	47.9 ± 5.0	112.9 ± 17.4*
Hepatic cholesterol (μg/mg protein)	21.8 ± 0.8	23.7 ± 0.9	14.5 ± 0.6	15.1 ± 0.5
Hepatic free cholesterol (μg/mg, n=5)	13.5 ± 0.7	14.0 ± 0.4	11.1 ± 0.9	10.4 ± 0.6
Hepatic FFA (nmol/mg)	67.6 ± 2.9	82.3 ± 10.8	63.8 ± 6.3	55.5 ± 6.1
Serum TG (mg/dL)	54.7 ± 5.1	27.4 ± 2.7*	44.0 ± 4.4	43.5 ± 6.0
Serum cholesterol (mg/dL)	31.2 ± 2.7	23.0 ± 1.5*	40.7 ± 2.4	36.4 ± 5.3
Serum FFA (mmol/L)	0.386 ± 0.04	0.325 ± 0.03	0.279 ± 0.02	0.305 ± 0.04
Serum ALT (IU/L)	9.3 ± 1.2	8.8 ± 1.9	27.8 ± 6.6	29.3 ± 5.1
Serum AST (U/L)	4.7 ± 0.7	5.3 ± 1.0	7.9 ± 1.6	10.8 ± 1.9
Serum β-hydroxybutyrate (mm)	0.219 ± 0.02	0.311 ± 0.04	0.351 ± 0.01	0.365 ± 0.04

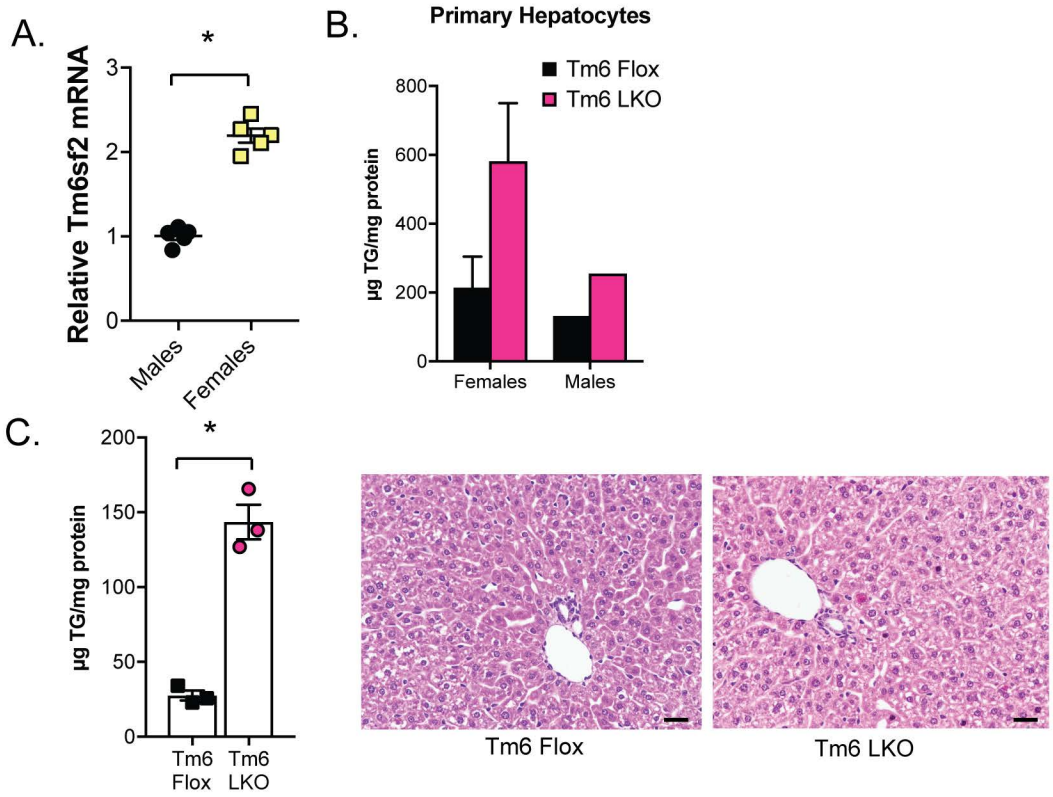
Tm6sf2 f/f Line 2

Line 2	Flox Female (6)	LKO Female (3)	Flox Male (6)	LKO Male (6)
Body weight (g)	21.6 ± 0.7	21.0 ± 0.8	30.2 ± 0.9	26.4 ± 0.5*
Liver (g)	1.0 ± 0.05	1.0 ± 0.03	1.5 ± 0.08	1.3 ± 0.02*
Liver/Body (%)	4.5 ± 0.1	4.6 ± 0.1	4.8 ± 0.2	4.7 ± 0.1
Hepatic TG (μg/mg protein)	90.6 ± 20.6	352.2 ± 33.9*	59.8 ± 15.0	130.2 ± 12.4*
Hepatic cholesterol (μg/mg protein)	21.6 ± 1.1	26.6 ± 3.3	17.4 ± 1.4	15.4 ± 0.2
Hepatic FFA (nmol/mg)	81.7 ± 7.6	131.6 ± 14.5*	49.1 ± 2.4	58.7 ± 3.4*
Serum TG (mg/dL)	41.5 ± 7.6	29.7 ± 4.5	45.4 ± 6.1	28.3 ± 2.4*
Serum cholesterol (mg/dL)	29.7 ± 4.5	13.8 ± 2.3	48.5 ± 2.6	37.2 ± 1.9*
Serum FFA (mmol/L)	0.275 ± 0.013	0.279 ± 0.04	0.380 ± 0.05	0.214 ± 0.04*
Serum ALT (IU/L)	17.8 ± 3.3	13.0 ± 2.5	55.3 ± 18.6	39.7 ± 3.0
Serum AST (U/L)	5.9 ± 1.3	7.1 ± 1.1	31.3 ± 9.4	16.3 ± 6.1

Supplemental Table 2

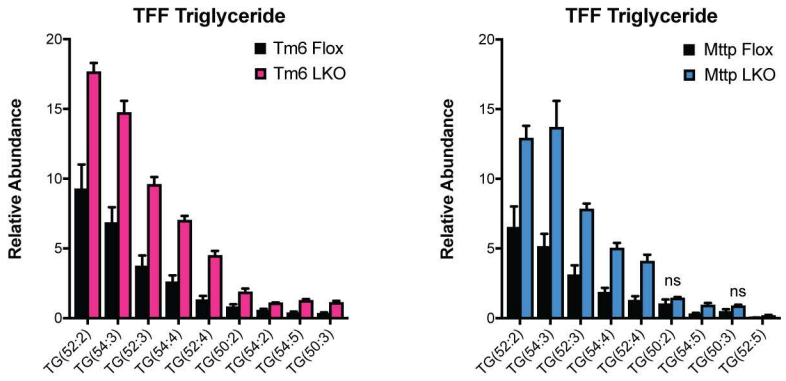
		Female		Male	
Diet/Model	**Parameter**	**Tm6 Flox**	**Tm6 LKO**	**Tm6 Flox**	**Tm6 LKO**
HMFD	Body Weight (g)	24.0 ± 1.3	23.2 ± 0.8	33.1 ± 1.1	34.1 ± 1.2
	Liver Weight (g)	1.0 ± 0.1	1.3 ± 0.1*	1.5 ± 0.1	1.7 ± 0.1
	Liver/Body (%)	4.0 ± 0.2	5.5 ± 0.2*	4.5 ± 0.2	5.0 ± 0.3
	Hepatic TC (µg/mg)	35.9 ± 2.2	68.5 ± 6.7*	29.1 ± 2.8	29.8 ± 3.2
	Hepatic FC (µg/mg)	17.7 ± 1.5	36.0 ± 5.9*	15.2 ± 1.7	16.1 ± 2.4
	Serum Glucose (mg/dL)	174 ± 12	146 ± 7	290 ± 29	234 ± 48
	n	6	6	16	11
TFF	Body Weight (g)	24.4 ± 0.5	23.8 ± 0.9	38.4 ± 0.8	37.3 ± 1.5
	Liver Weight (g)	1.1 ± 0.1	1.3 ± 0.1	1.8 ± 0.1	2.2 ± 0.2
	Liver/Body (%)	4.6 ± 0.2	5.3 ± 0.1*	4.7 ± 0.3	5.8 ± 0.4
	Hepatic TC (µg/mg, n=5)	24.0 ± 1.2	31.8 ± 3.6	27.4 ± 3.5	38.4 ± 4.1
	Hepatic FC (µg/mg, n=5)	12.5 ± 2.5	22.0 ± 3.1*	15.4 ± 1.7	20.1 ± 1.8
	n	8	6	5	7
PFC	Body Weight (g)	34.4 ± 3.1	32.7 ± 2.6	33.8 ± 1.3	39.5 ± 1.9
	Liver Weight (g)	1.8 ± 0.3	2.4 ± 0.3	1.5 ± 0.1	3.5 ± 0.4*
	Liver/Body (%)	5.1 ± 0.5	7.1 ± 0.4*	4.4 ± 0.1	8.8 ± 0.8*
	Hepatic TC (µg/mg)	131 ± 12	156.7 ± 10	108 ± 1.2	174 ± 16*
	Hepatic FC (µg/mg)	73.4 ± 7.7	90 ± 13.3	33.6 ± 3.1	51 ± 3.8*
	Serum Glucose (mg/dL)	178 ± 18	132 ± 24	261 ± 23	271 ± 30
	n	8	7	4	5
STAM,12w	Body Weight (g)			15.4 ± 1.3	22.5 ± 1*
	Liver Weight (g)			1.5 ± 0.1	2.4 ± 0.2*
	Liver/Body (%)			11.1 ± 1.4	10.5 ± 0.9
	Serum Glucose (mg/dL)			445 ± 21	520 ± 24
	n			12	10
STAM,18w	Body Weight (g)			19.9 ± 1.4	24.1 ±0.9*
	Liver Weight (g)			1.4 ± 0.1	2.7 ± 0.3*
	Liver/Body (%)			7.0 ± 0.7	11.2 ± 1*
	Serum Glucose (mg/dL)			452 ± 26	499 ± 8
	n			12	12

Supplemental Figure 1:



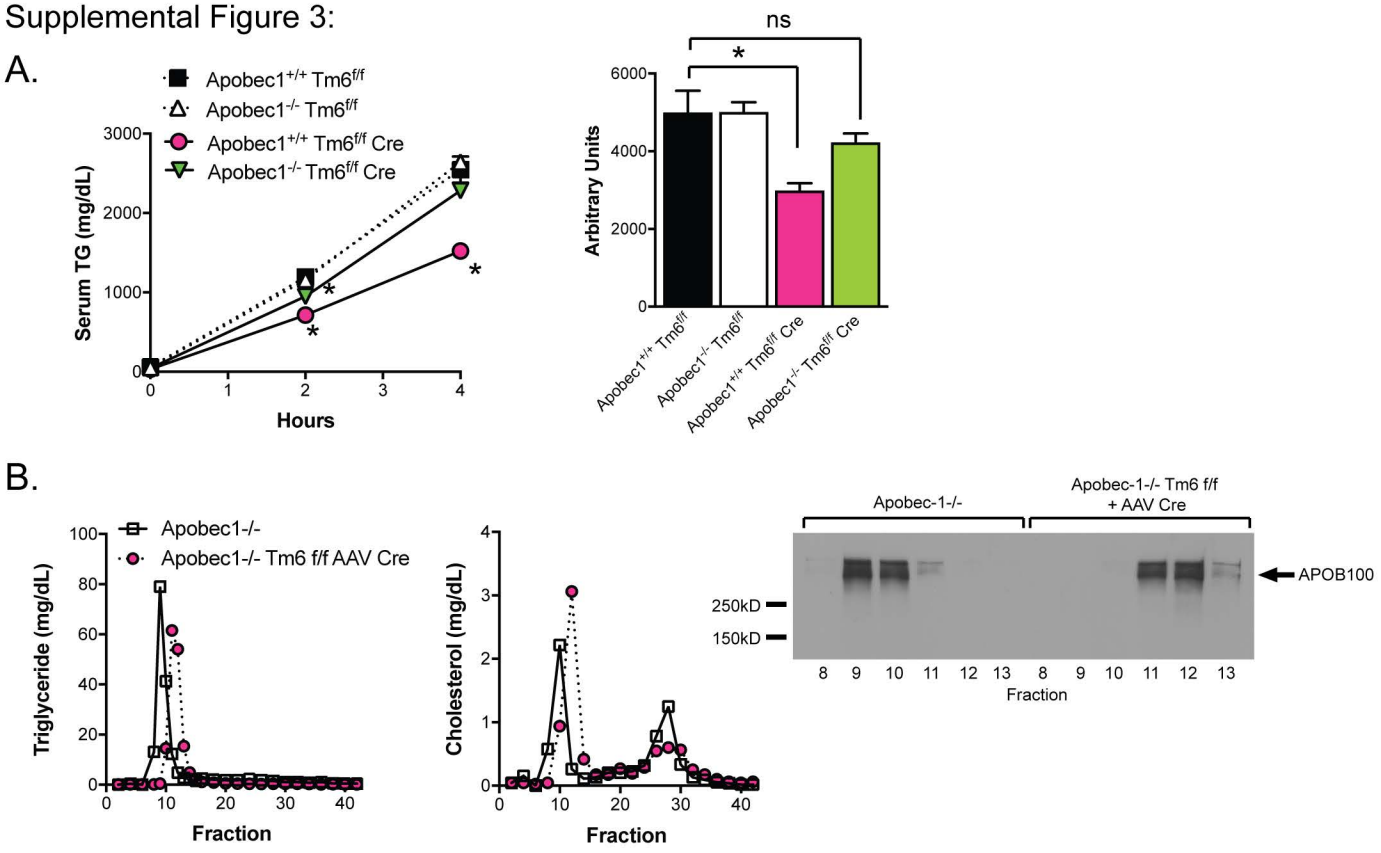
Supplemental Figure 1: A. Tm6sf2 mRNA in male and females Tm6 Flox (control) liver tissue. n=4/group. B. Triglyceride content of primary hepatocytes isolated from male and female Tm6 Flox and Tm6 LKO animals. n= 3 females, 1 male. C. Hepatic triglyceride in 3 week old female mice. n=3/genotype. Representative H&E images are shown (200x magnification, scale bar= 50µm).

Supplemental Figure 2:



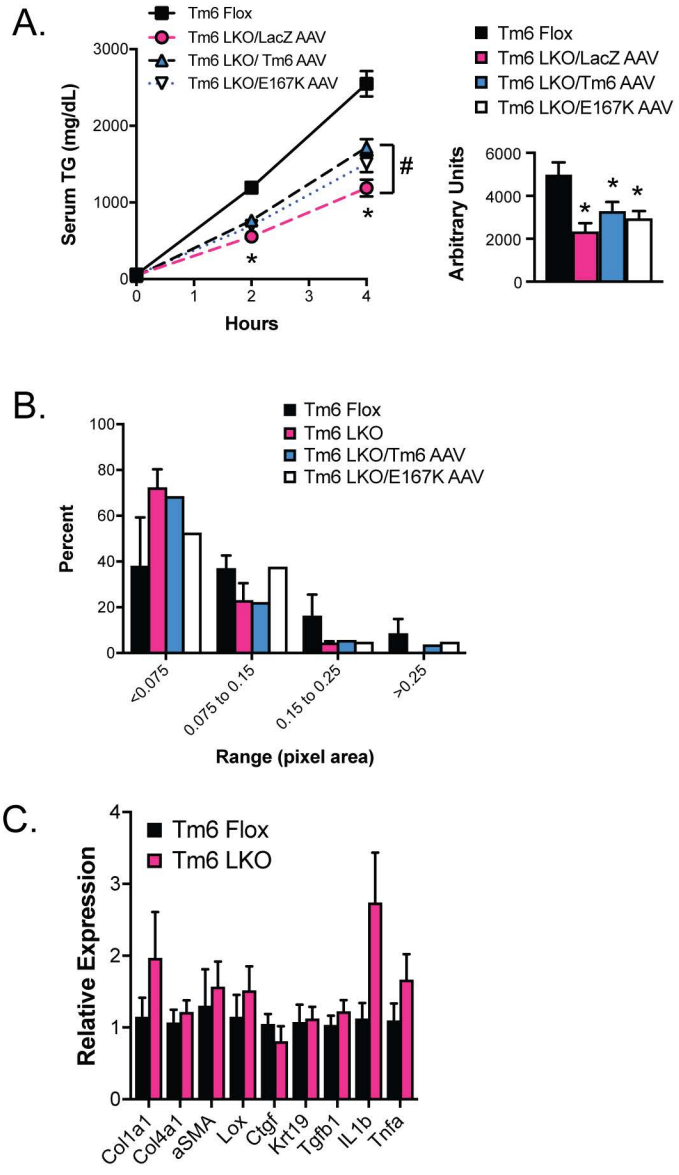
Supplemental Figure 2: Relative abundance of major triglyceride species in liver of control (Flox) mice and mice with liver specific deletion (LKO) of *Tm6sf2* or *Mttp* (right). Data are from 3-5 females per genotype, and are presented as mean ± SEM. Differences betweeen LKO and respective Flox animals are significant unless otherwise indicated (ns).

Supplemental Figure 3:



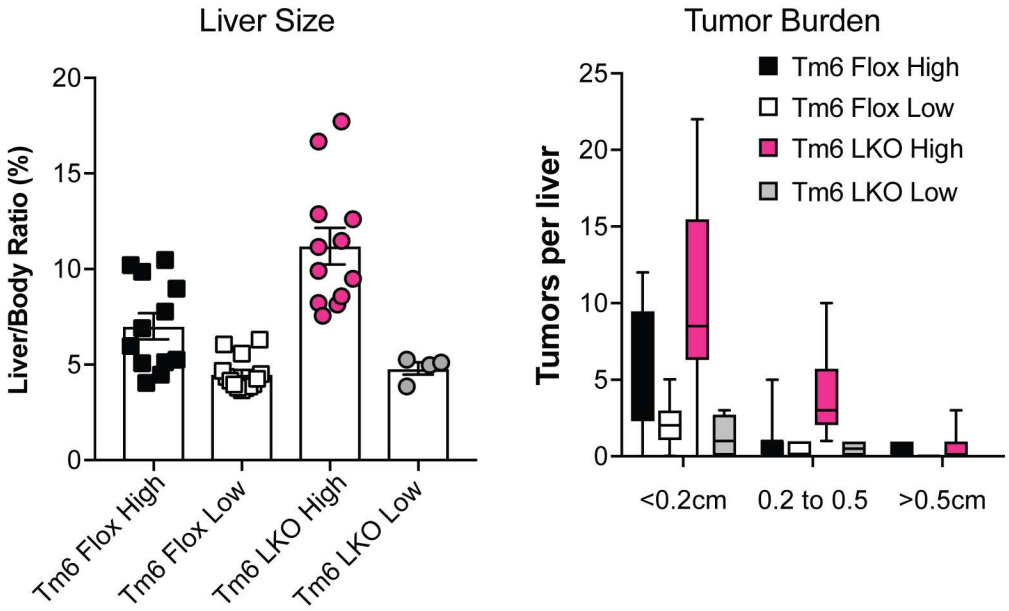
Supplemental Figure 3: A. VLDL secretion in Apobec1$^{+/+}$ Tm6$^{f/f}$, Apobec1$^{-/-}$ Tm6$^{f/f}$, Apobec1$^{+/+}$ Tm6Tm6$^{f/f}$ AAV Cre, and Apobec1$^{-/-}$ Tm6$^{f/f}$ AAV Cre mice fed chow diet (n=4-8/group). Control mice were injected with LacZ AAV or null AAV. Right panel shows average area under the curve for all groups. ns= not significant. Asterisks indicate $p<0.05$ vs Apobec1$^{+/+}$ Tm6$^{f/f}$ control. B. TG and cholesterol concentration in FPLC fractions from chow diet fed Apobec1$^{-/-}$ Tm6$^{f/f}$ and Apobec1$^{-/-}$ Tm6$^{f/f}$ AAV Cre after Pluronic F-127 injection. Western blot (right) shows a corresponding shift in localization of APOB100.

Supplemental Figure 4:



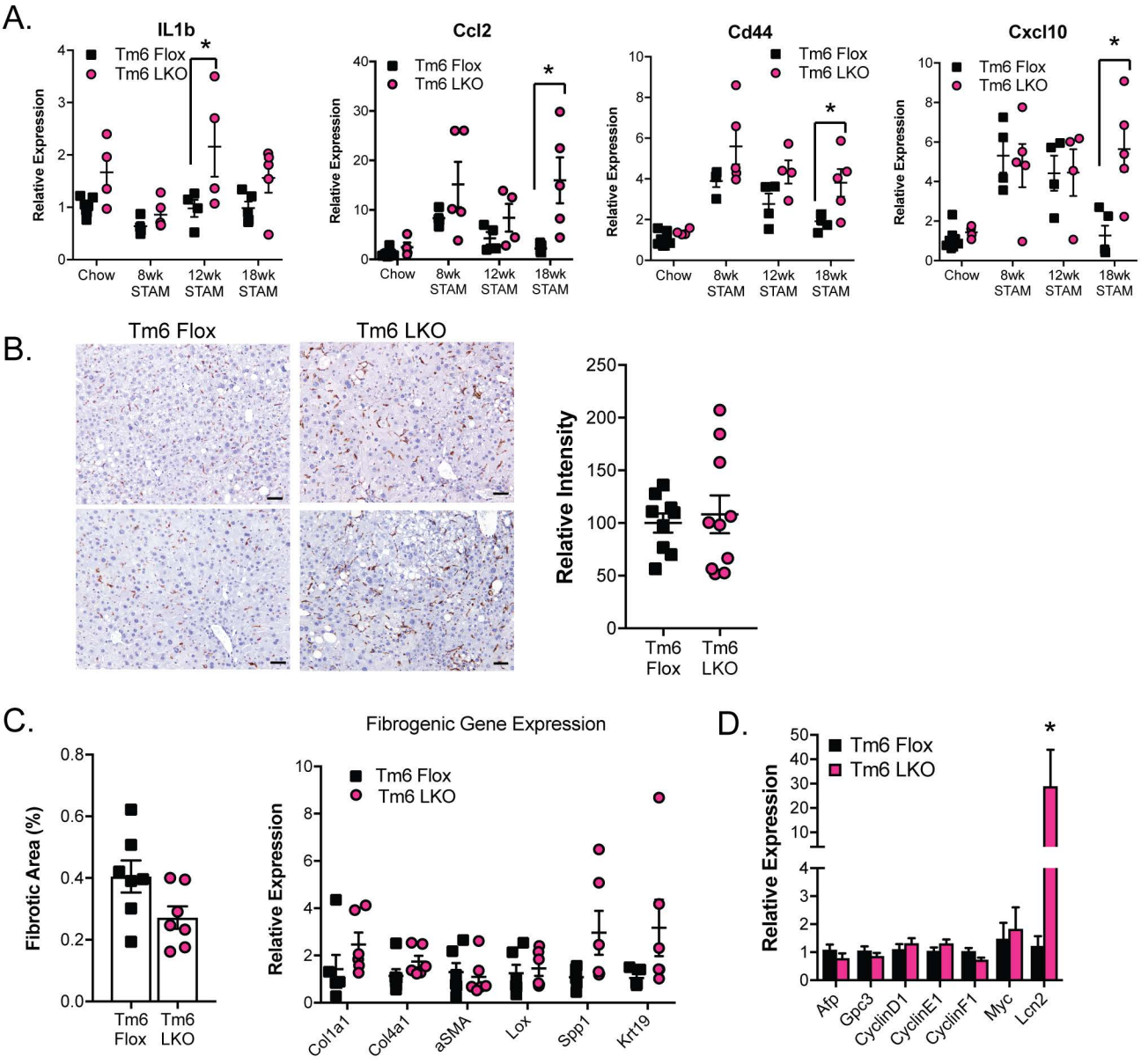
Supplemental Figure 4: A. AAV mediated rescue of impaired VLDL secretion in chow fed Tm6 LKO mice injected with LacZ AAV, WT Tm6 AAV, or E167K Tm6 AAV, compared to Tm6 Flox controls. n = 7-10 mice/group, mixed genders. # indicates $p<0.05$ for LKO + WT Tm6 AAV versus LKO + LacZ AAV. Asterisks indicate $p<0.05$ versus Flox control. Right panel shows area under the curve for each genotype. B. VLDL particle size in Tm6 flox mice and Tm6 LKO mice transduced with Tm6 AAV and fed HMFD for 5 days. Particles were isolated from pooled serum samples collected 4h after Pluronic injection and imaged. C. Expression of fibrogenic and inflammation genes in livers of HMFD fed male mice (n=6-7 mice/genotype).

Supplemental Figure 5:



Supplemental Figure 5: Liver size and visible tumors in STAM model mice segregated by blood glucose levels. Mice with consistently low glucose, particularly at sacrifice, were excluded from further analysis. Serum glucose: Tm6 Flox high (n=10), 452 ± 26 mg/dL; Tm6 Flox low (n=12), 245 ± 16; Tm6 LKO high (n=11), 499 ± 8; Tm6 LKO low (n=4), 371 ± 62.

Supplemental Figure 6:



Supplemental Figure 6: A. Expression of inflammation related genes in control (uninjected mice fed chow diet) and STAM mice at 8, 12, and 18 weeks of age. n=4-5 males/group, normalized to expression in Tm6 Flox controls. B. F4/80 immunostaining in STAM mice at 12 weeks with representative images (200x, scale bar =50μm) and quantification. C. Fibrotic area and fibrogenic gene expression (right) in STAM mice at 18 weeks. Differences in gene expresion are not significant D. Expression of proliferation-related genes in livers of DEN-treated animals at 4 months. n=6-8/genotype. For all panels, asterisks indicate $p<0.05$.