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Supplemental Information

Tumor-Induced Hyperlipidemia Contributes to Tumor Growth

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SUPPLEMENTAL FIGURES

Figure S1, Related to Figure 1

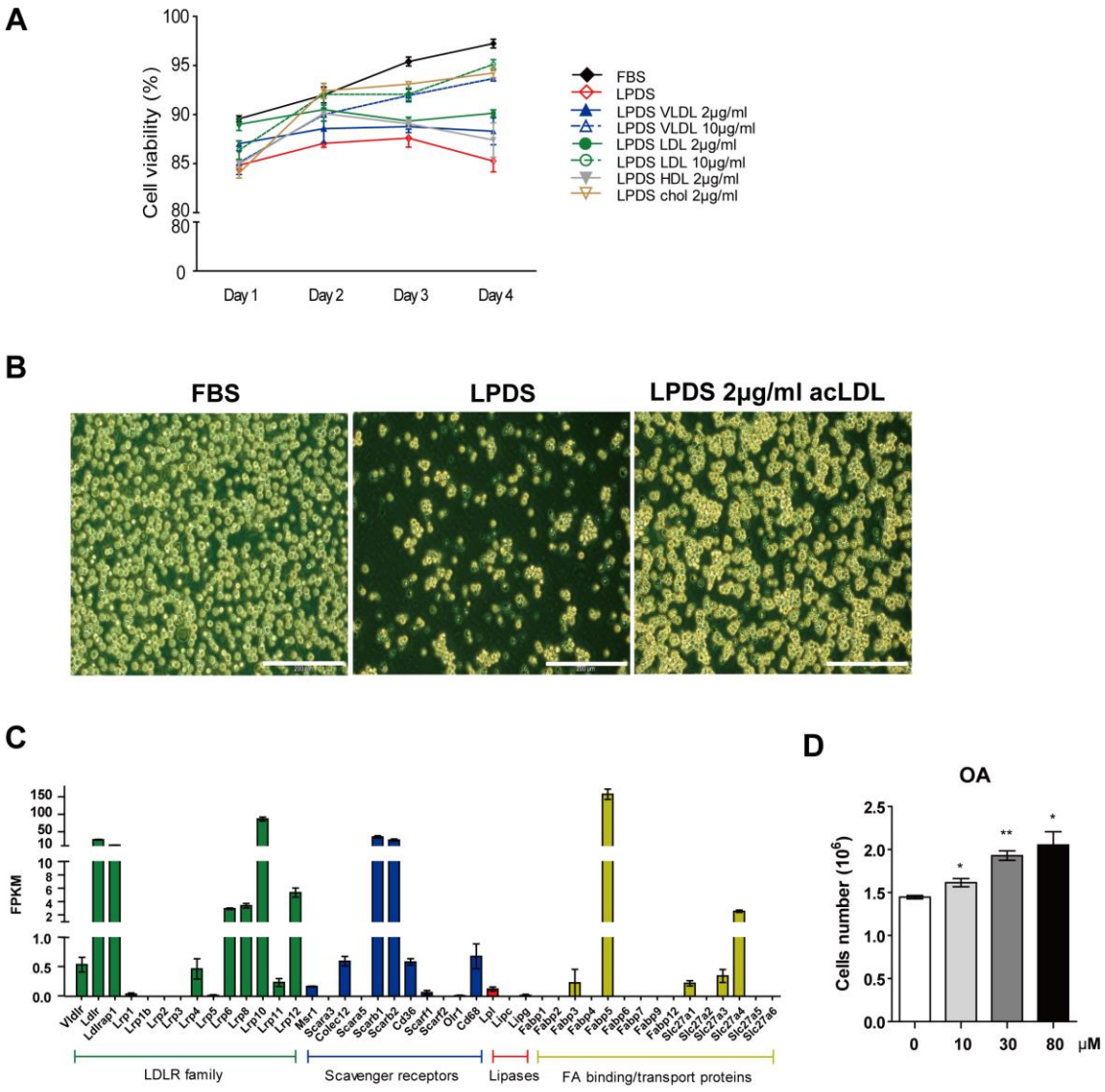

Figure S1. Lipoproteins Are Essential for Growth and Survival of Transformed B-cells, Related to Figure 1

(A) Cell viability of transformed precursor B-cells (TPBCs) treated with 10% FBS or LPDS medium supplemented with 2 or 10 µg/mL cholesterol in the form of VLDL, LDL, HDL and water soluble cholesterol, respectively, was determined by CASY cell counter (n=3).

(B) Morphological appearance of TPBCs treated with 10% FBS or LPDS medium supplemented with/without acetylated LDL (acLDL) containing 2µg/ml cholesterol on day 4 (n=3). Scale bar, 200 µm. Data are representative of two independent experiments with similar results.

(C) RNAseq analysis of expression of genes encoding lipoprotein transporters, lipases and FA uptake-related proteins in TPBC.

(D) Impact of oleic acid (OA, 48 hr) on cell viability of LPDS-treated TPBC.

Error bars represent SEM. Unpaired t-test, ***, $p<0.001$.

Figure S2, Related to Figure 2

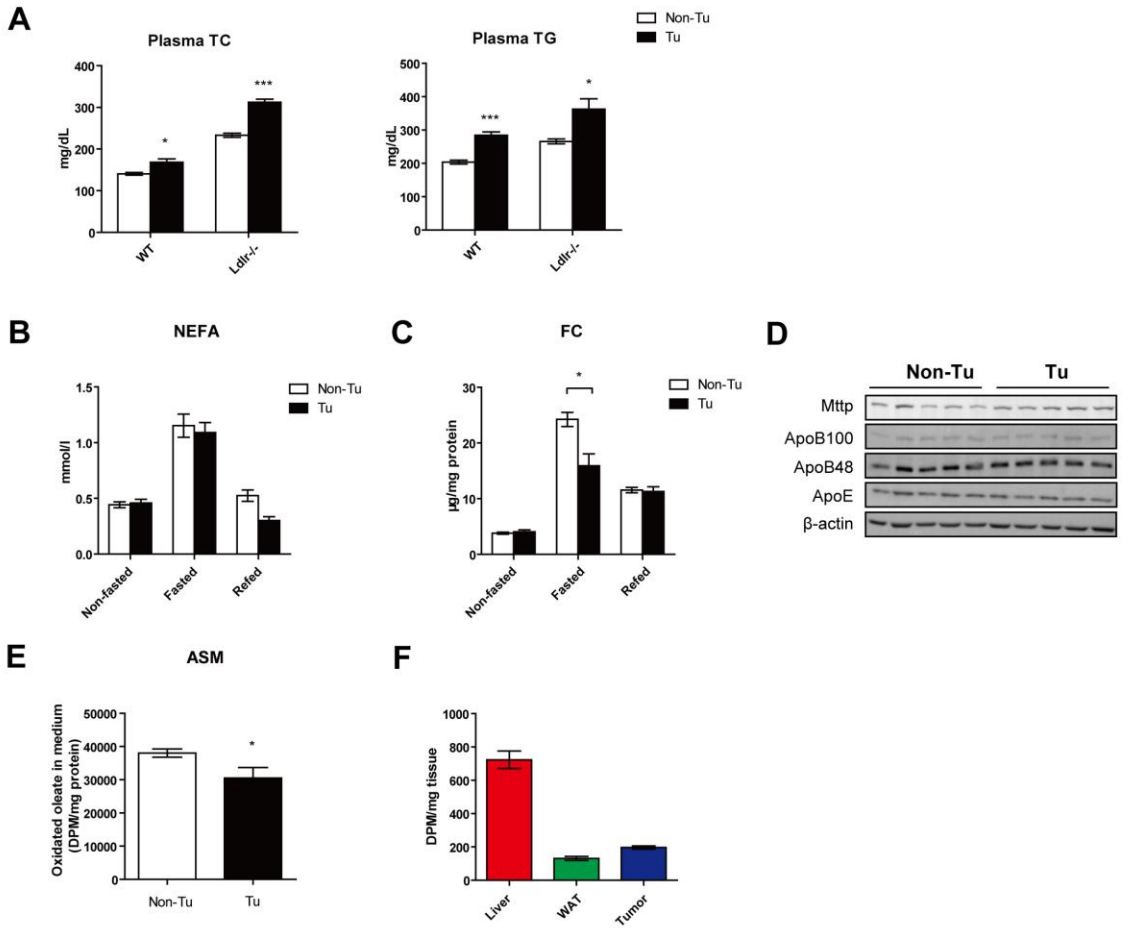

Figure S2. TPBC Tumors Increase Plasma VLDL and CM Lipids, Related to Figure 2

(A) TC and TG concentrations in plasma in *WT* or *Ldlr*$^{-/-}$ mice (14 days post-injection of TPBC or PBS as Non-Tu controls, n=5).

(B and C) Plasma NEFA and liver FC concentrations from non-fasted (NF), overnight (o/n) fasted and refed (10 hr) *WT* mice with/without tumors (day 14, n=4-5).

(D) Immunoblot analysis of VLDL assembly-related proteins (Mttp, ApoB100/48 and ApoE) and loading controls (β-actin) in liver tissues (n=5) from non-tumor (Non-Tu) and tumor (Tu) bearing wild-type (*WT*) mice.

(E) Determination of acid soluble metabolites (ASM)-derived from incomplete oxidation of [^{3}H] oleate. Data (n=3) are representative for two independent experiments with similar results.

(F) Radioactivity distribution after FTT (n=7). Liver, gonadal white adipose tissue (WAT) and tumor were dissected from mice sacrificed 6 hr after oral gavage of [^{3}H] triolein-containing olive oil. Tissues were minced and dissolved in lysis buffer (1% SDS, 0.1N NaOH) at 60°C overnight. Radioactivity was determined by liquid scintillation counting and normalized to protein content. Error bars represent SEM. Unpaired t-test in A, B, D, **, $p<0.05$*.

Figure S3, Related to Figure 3

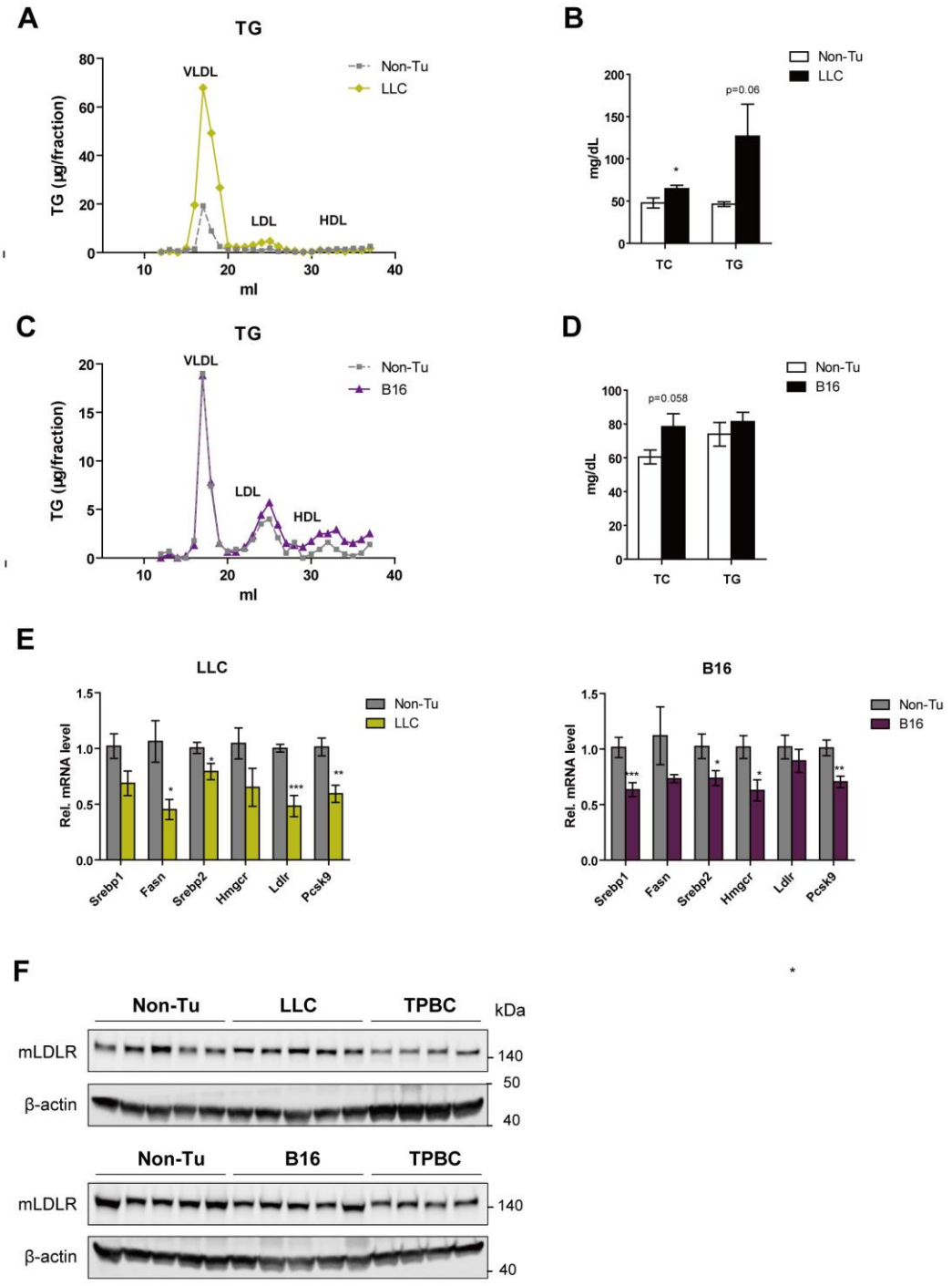

Figure S3. Plasma Lipid Levels, Hepatic Lipogenic Gene Expression and LDLR Abundance in LLC- and B16- bearing Mice, Related to Figure 3

Lipoprotein TG concentrations in plasma pooled from 5 mice after separation by FPLC in (A) LLC and Non-Tu control mice, (C) B16 and Non-Tu control mice. Plasma was prepared 14 days post-injection.

Total plasma TC and TG concentrations in (B) LLC and Non-Tu control mice and (D) B16 and Non-Tu control mice described above (n=5).

(E) Hepatic expression of *Srebp1/2* and their target genes in non-fasted LLC- and B16- bearing mice (n=5).

(F) Hepatic LDLR protein abundance in non-fasted LLC- and B16- bearing and respective Non-Tu control mice (n=5). TPBC tumor served as a positive control (n=4).

Error bars represent SEM. Unpaired t-test in A, B, D and E *, $p<0.05$.

Figure S4, Related to Figure 4

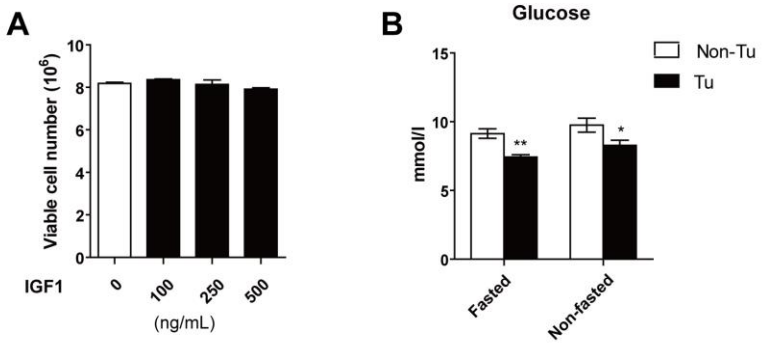

Figure S4. Glucose Concentration in TPBC Tumor-induced Hyperlipidemia, Related to Figure 4

(A) Impact of 48 hr incubation with recombinant IGF-1 (1% FBS medium) on cell growth.

(B) Blood glucose concentration from o/n fasted and non-fasted mice (n=6-7).

Error bars represent SEM. Unpaired t-test. **, $p<0.05$*; ***, $p<0.01$*.

Figure S5, Related to Figure 5

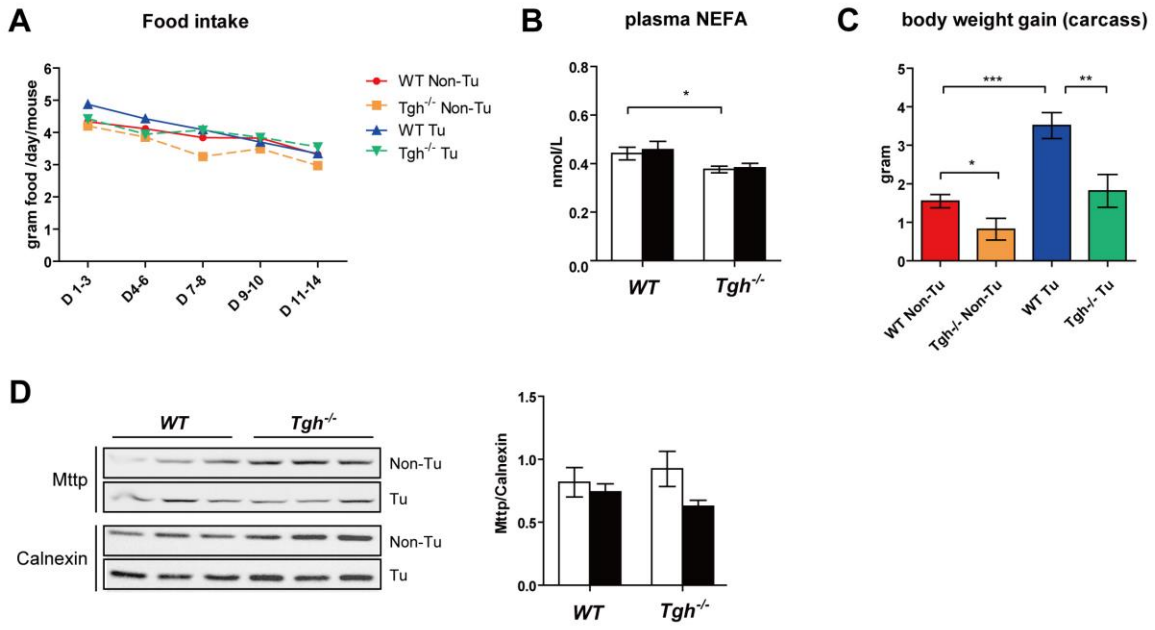

Figure S5. Food Intake, Body Weight Gain, Plasma NEFA Concentration and MTTP Abundance in WT and Ces3/TGH Deficient Non-Tu and Tu-bearing Mice, Related to Figure 5

(A) Average food intake per each mouse per day during the experimental period was calculated (n=5-8).

(B) Plasma NEFA concentrations from daytime (8 a.m. – 1 p.m.) fasted mice (n=8-12).

(C) Carcass body weight gain (total body weight gain subtracted by tumor weight) was measured on day 14 post- tumor cell injection (n=5-8).

(D) Immunoblot analysis of Mttp (99 kDa) in liver lysates from Tu/Non-Tu-bearing *WT* and *Ces3/Tgh*$^{-/-}$ mice (n=5). Calnexin served as a protein loading control.

Error bars represent SEM. Unpaired t-test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Figure S6, Related to Figure 6

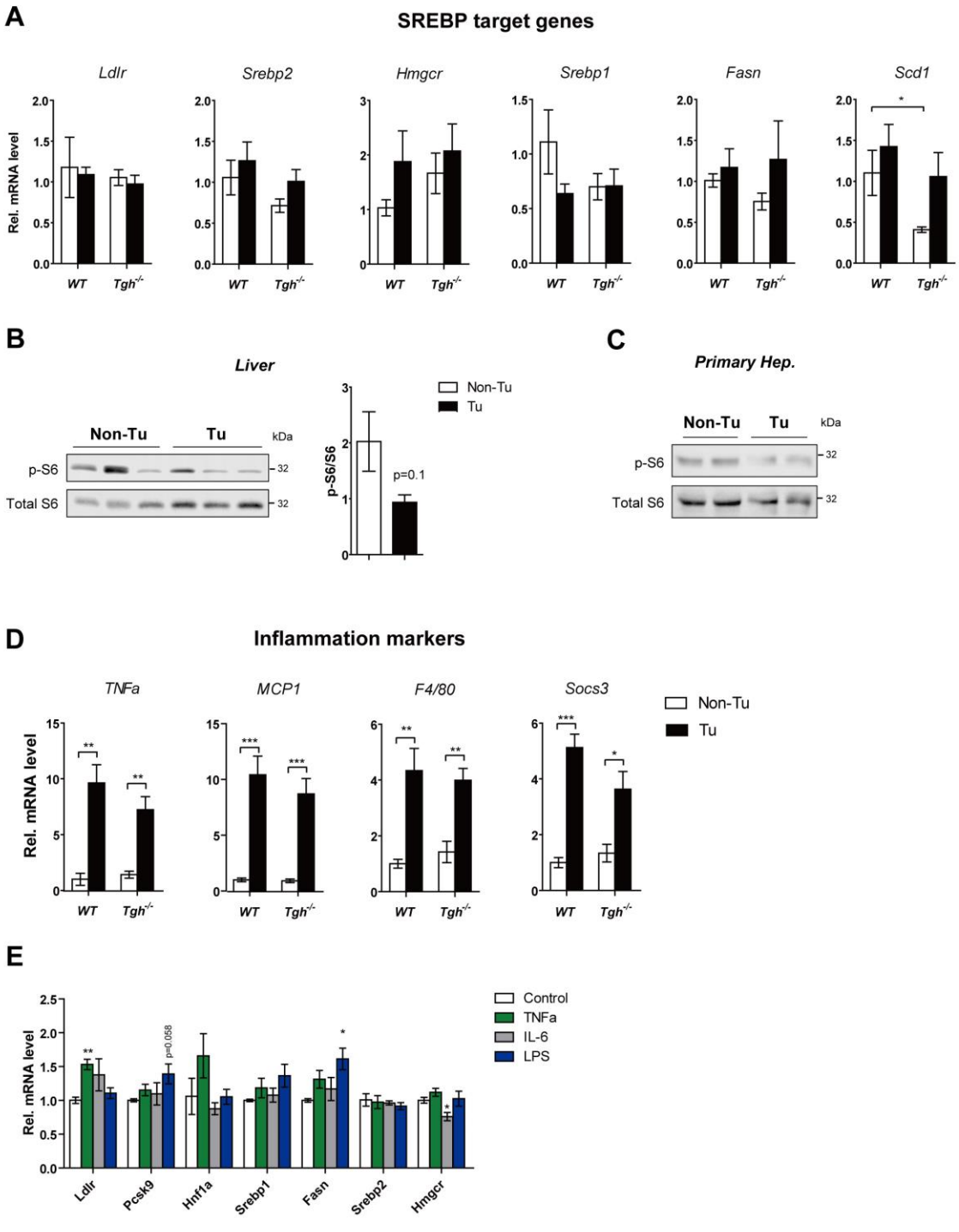

Figure S6. Transcription of Lipogenic and Inflammation Genes in Livers from Non-Tu and Tu WT and Ces3/TGH Deficient Mice and S6 Phosphorylation, Related to Figure 6

(A) Hepatic expression of Srebp1/2 and their target genes (n=5), relative to cyclophilin A mRNA expression.

(B and C) S6 immunoblot analysis of Ser235/236 phosphorylated(p-)/total ribosomal protein S6 (32 kDa; n=3) from livers of o/n fasted WT mice (B) and primary hepatocytes isolated from non-fasted WT mice (C). Primary hepatocytes were pooled from two mice. After 4 hr of attachment, cells were subjected to 6 hr serum starvation before cell harvesting.

(D) Expression of inflammatory marker genes in livers from *WT* and *Ces3/Tgh*$^{-/-}$ mice (n=5-7).

(E) Impacts of inflammatory factors on mRNA expression of *Pcsk9*, *Ldlr* and their upstream regulators (*Srebp1/2* and *Hnf1a*) in immortalized AML-12 hepatocytes. Cells were treated with recombinant TNFα (10 ng/ml), IL-6 (20 ng/ml) and LPS (10 ng/ml) for 48 hr before cell harvesting (n=3).

Error bars represent SEM. Unpaired t-test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

SUPPLEMENTAL TABLES:

Table S1. mRNA Expression Correlation between *Ldlr* and SREBPs Target Genes in Human Cancers, Related to Figure 1

Cancer	**SREBP1 target gene**	**SREBP2 target gene**
Diffuse Large B-cell Lymphoma (n = 25)	Scd (r = .425), Fasn (r = .396)	
Acute Myeloid Leukemia (n = 173)	Scd (r = .398), Srebp1 (r = .33)	Hmgcr (r = .315), Hmgcs1 (r = .364), Srebp2 (r = .7)
Prostate Adenocarcinoma (n = 216)	Scd (r = .541), Fasn (r = .371), Acly (r = .46), Srebp1 (r = .351)	Hmgcr (r = .621), Hmgcs1 (r = .604), Srebp2 (r = .658)
Glioblastoma (n = 151)	Scd (r = .39), Fasn (r = .495), Acly (r = .436), Acc (r = .391), Srebp1 (r = .41)	Hmgcr (r = .44), Hmgcs1 (r = .488), Srebp2 (r = .558)
Brain Low Degrade Glioma (n = 376)	Scd (r = .332), Fasn (r = .384), Acc (r = .344)	Hmgcr (r = .621), Hmgcs1 (r = .643), Srebp2 (r = .513)
Pheochromocytoma&Paraganglioma (n = 97)	Scd (r = .613), Fasn (r = .438), Srebp1 (r = .468)	Hmgcr (r = .633), Hmgcs1 (r = .718), Srebp2 (r = .533)
Head & Neck Squamous Cell Carcinoma (n = 279)	Scd (r = .383), Fasn (r = .407), Acly (r = .308)	Hmgcr (r = .441), Hmgcs1 (r = .486)
Adrenocortical Carcinoma (n = 54)	Scd (r = .652), Fasn (r = .666), Acly (r = .633), Acc (r = .543)	Hmgcr (r = .87), Hmgcs1 (r = .87), Srebp2 (r = .63)
Kidney Chromophobe (n = 66)	Scd (r = .673), Fasn (r = .465), Srebp1 (r = .396)	Hmgcr (r = .467), Hmgcs1 (r = .354), Srebp2 (r = .676)
Breast Invasive Carcinoma (n = 992)	Scd (r = .539), Fasn (r = .329), Acly (r = .342)	Hmgcr (r = .412), Hmgcs1 (r = .435), Srebp2 (r = .547)
Ovarian Serous Cystadenocarcinoma (n = 265)	Scd (r = .421), Srebp1 (r = .408)	Hmgcs1 (r = .311), Srebp2 (r = .588)
Uterine Corpus Endometrial Carcinoma (n = 124)	Scd (r = .537), Fasn (r = .338), Acc (r = .376)	Hmgcr (r = .302), Hmgcs1 (r = .454), Srebp2 (r = .53)
Thyroid Carcinoma (n = 496)	Scd (r = .631), Srebp1 (r = .465)	Srebp2 (r = .419)

Correlation was studied by Spearman's correlation. The coefficient value r is shown in brackets. Genes were shown only when $p < 0.05$ and $r > 0.3$.

Scd: stearoyl-CoA desaturase; Acly: ATP-citrate lyase; Srebp: sterol-regulatory element binding protein; Acc: acetyl-CoA carboxylase; Hmgcr: 3-hydroxy-3-methylglutary-CoA reductase; Hmgcs: 3-hydroxy-3-methylglutary-CoA synthase; Ldlr: low-density lipoprotein receptor.

Table S2. qPCR Primer Sets Used in This Study, Related to Figures 3, 6, S3 and S6

Gene	Genbank	Primers
MCP-1	NM_011333.3	F: GGCTGGAGAGCTACAAGAGG R: ATGTCTGGACCCATTCCTTC
F4/80	NM_010130	F: TTGGCCAAGATTCTCTTCCT R: TCACTGCCTCCACTAGCATC
TNF-a	NM_013693	F: GTCTACTGAACTTCGGGGTGA R: CACCACTTGGTGGTTTGCTACGAC
Socs3	NM_007707.3	F: ATTTCGCTTCGGGACTAGC R: AACTTGCTGTGGGTGACCAT
Insig2	NM_133748.2	F: CCCTCAATGAATGTACTGAAGGATT R: TGTGAAGTGAAGCAGACCAATGT
Fbxw7	NM_080428.3	F: AGCGGCGGAGGATTACATCT R: TGAAAGCACATAGAGTGCCAAC
Scap	NM_001001144.3	F: TGGCTACTTCACCCTCGTG R: ACACCAGGCCCACAACAG
Mbtps1	NM_019709.4	F: TCC AGGGGA ATGACTACCTG R: CAATGTCAGGCTTCACACGA
Srebp1	NM_011480.3	F: GAGGCGGCTCTGGAACAG R: CGGGAAGTCACTGTCTTGGT
Srebp2	NM_033218.1	F: AGACCATGGAGACCCTCACG R: GGAACTCTCCCACTTGATTGCT
Ldlr	NM_010700	F: AGGCTGTGGGCTCCATAGG R: TGCGGTCCAGGGTCATCT
Pcsk9	NM_153565.2	F: TTTTATGACCTCTTCCCTGGC R: ATTCGCTCCAGGTTCCATG
Hnf1a	NM_009327.3	F: CGCCTCCACCCTGGTTAT R: ACTCCCCATGCTGTTGATG
Vldlr	NM_001161420	F: CCACAGCAGTATCAGAAGTCAGTGT R: CACCTACTGCTGCCATCACTAAGA
Ldlr	NM_010700	F: AGGCTGTGGGCTCCATAGG R: TGCGGTCCAGGGTCATCT
cyclophilin	NM_008907	F: TCCAAAGACAGCAGAAAACTTTCG R: TCTTCTTGCTGGTCTTGCCATTCC

Supplemental Experimental Procedures

Reagents and Antibodies

Fatostatin was obtained from Cayman Chemical (MI, USA), water-soluble cholesterol, hydroxypropyl-β-cyclodextrin (HPCD), lipoprotein-deficient serum (LPDS) and mouse recombinant TNFα were purchased from Sigma-Aldrich (Germany). Mouse recombinant IGF-1 was purchased from eBioscience (Vienna, Austria). All human purified lipoproteins (VLDL, LDL, acLDL, HDL) were from healthy subjects and prepared according to routine protocols. Mouse PCSK9 ELISA Kit was purchased from R&D Systems (MN, USA). The following antibodies were used: Akt (#9272), phospho-Ser473-Akt (#4060), AMPKα (#2532), phospho-Thr172-AMPKα(#2535), ACC (#3662), FASN (#3189), ribosomal protein S6 (#2217), phospho-Ser235/236–ribosomal protein S6 (#2211),β-actin (#4967) all purchased from Cell Signaling (MA, USA); SREBP1(#297) and HMGCR antibodies were kindly provided by Drs. Joseph Goldstein &Michael Brown and Dr. Russell Debose-Boyd (UT Southwestern), respectively; LDLR antibodies were kindly given by Dr. Da-wei Zhang (University of Alberta); all other antibodies were generated in-house.

Generation of Bcr/Abl-transformed Precursor B-cells (TPBC) and Cell Culture

Briefly, bone marrow was isolated from C57BL/6J mice. Cells were transduced using GFP/p185$^{bcr/abl}$ viral supernatant from Φ-NX (Phoenix) cells. GFP is used as a transduction marker for primary positive cell selection. Candidate cell lineages were further sorted by fluorescence-activated cell sorting (FACS) based on expression of the precursor B-cell markers B220, CD43 and CD19.Cells were maintained in RPMI 1640 medium containing 10% FBS. Immortalized non-transformed AML-12 hepatocytes were purchased from ATCC, maintained in DMEM:F12 medium with 0.005 mg/ml insulin, 0.005 mg/ml transferrin, 5 ng/ml selenium, and 40 ng/ml dexamethasone and 10% FBS. Lung carcinoma cell line LLC and melanoma cell line B16 were from the lab stock, cultured in 10% FBS containing DMEM. All cell lines were cultured in a 37°C incubator with 5% CO_2.

mRNA Expression Analysis and RNA Sequencing

Total RNA was extracted by Trizol (Life Technologies, CA). cDNA was synthesized from 2 mg of total RNA by the Superscript Ⅲ Reverse Transcriptase (Invitrogen, CA) using oligo $(dT)_{12\text{-}18}$ primers. Real-time quantitative PCR was performed with the Platinum SYBR Green qPCR SuperMix-UDG kit (Invitrogen) in the Rotor-Gene 3000 instrument (Montreal Biotech, Canada). Primers used in this study are listed in Table S2. Gene expression profile in TPBC was determined by a quantitative RNA-seq analysis on the Ion Torrent platform (Thermo Fisher Scientific, NY).

Apoptosis Assay

Cell apoptosis was assessed by a LSRII flow cytometry (BD Biosciences, USA). Annexin V and 7AAD were purchased from BD Biosciences and used as early and late stage apoptotic markers, respectively.

Subcellular Fractionation and Protein Extraction

Total protein from liver and tumor samples (100 mg each) were homogenized in RIPA lysis buffer containing a protease inhibitor cocktail and phosphatase inhibitor (Roche Diagnostics, Canada). For liver subcellular fractionation, the protocol is modified from previous studies (Darnell and Posner, 2003; Das et al.). 100 mg frozen liver samples were minced in 1 mL sucrose-lysis buffer containing 250 mM sucrose, 50 mM Tris, 1 mM EDTA, pH7.4 supplemented with a protease inhibitor cocktail. Tissue samples were homogenized by three strokes of a teflon pestle in homogenizer at low speed. Tissue lysates were centrifuged at 500×*g* for 15 min at 4°C to separate supernatant (cytoplasmic fraction) and pellet. Pellets were washed gently by the same lysis buffer once to remove the remaining supernatant, and then resuspended in 300 μl hypertonic buffer (10 mM Hepes, pH7.4, 0.42 M NaCl, 1.5 mM $MgCl_2$, 2.5% (vol/vol) glycerol, 1 mM EDTA, 1 mM EGTA and 1 mM dithiothreitol) supplemented with the same protease inhibitor cocktail for nuclear extraction. After gentle shaking on ice for 30 min for nuclei swelling, samples were centrifuged at

$10^5 \times g$ for 30 min at 4°C to yield the supernatant (nuclear fraction). Aliquots were assayed or protein concentrations were determined by a Bradford Protein Assay (Bio-Rad, Canada).

Immunoblotting

Immunoblotting was performed using total or fractionated protein extracts or plasma and probed with antibodies listed above. Immunoreactivity was detected by ECL (Amersham-Pharmacia, Canada) or SuperSignal West Dura (Thermo Scientific, IL) to enhance the signal. Immunoblots were scanned and quantified using G-Box (SYNGENE, UK).

Lipid and lipoprotein Analysis

Plasma TG (Roche Diagnostics, Canada), TC, FC and NEFA concentrations (Wako Diagnostics, USA) were determined spectrophotometrically. To determine plasma lipoprotein profiles, plasma from 5 non-fasted mice was pooled in each group and applied onto size-exclusion fast-protein liquid chromatography (FPLC) (Wei et al., 2007). Determination of isotope-labeled lipids from plasma and hepatocytes were previously described(Ko et al., 2009). For determination of lipids mass, liver and tumor sample were washed with PBS and frozen. Around 100 mg tissue samples were homogenized in 1 mL methanol and total lipids were isolated from homogenates by Folch extraction.

Histology and Immunohistochemistry

Livers were either fixed in 4% neutral-buffered formaldehyde solution followed by paraffin embedding for hematoxylin and eosin (H&E) staining and F4/80 immunohistochemistry IHC or frozen in liquid nitrogen for Ki67 IHC. Ki67 and F4/80 IHC were performed on 3μm thick sections using specific antibodies against Ki67 (Novocastra, UK) and F4/80 (Serotec, USA), respectively, and appropriate secondary antibodies.

mRNA Expression in Human Cancers

mRNA expression (RNA Seq V2RSEM) and patient clinical data from various types of cancer are

publicly available from the Cancer Genome Atlas Project (TCGA). The raw TCGA data sets were accessed and downloaded through the cBioPortal for Cancer Genomics (http://www.cbioportal.org/index.do) (Zak et al.). Tumor clinical records were reviewed and tumor samples ID were sorted for mRNA profiles analysis. Correlation of mRNA expression was tested by non-parametric Spearman's correlation.

Metabolic Labeling Studies in Primary Hepatocytes

Primary hepatocytes were isolated from non-fasted mice by perfusion with 0.8 mg/mL Collagenase Type I (Worthington Biochemical Group, NJ) through the portal vein. Cells were incubated with DMEM containing 15% FBS for 4 hr for cell attachment as described previously (Wei et al., 2010). For incorporation of exogenous substrates into lipids, hepatocytes were washed twice, and then incubated for 4 hr in 2 mL serum-free DMEM containing either 5 μCi [^{3}H] oleate (combined with 400 μM oleate conjugated with 0.5% FA-free BSA) or 5 μCi [^{3}H] acetate (combined with 50 μM sodium acetate). Cells and media (after washing with PBS to remove unabsorbed substrate) were collected for analysis (pulse) or incubated with DMEM for further 12 hr (chase). [^{3}H] labeled lipids were determined following lipid extraction and TLC by liquid scintillation counting as described above.

Post Heparin Plasma TG lipase Activity Assay

Post Heparin Plasma (PHP) was drawn 20 min after subcutaneous injection of sodium heparin (500 U/kg body weight). Enzyme activities of LPL and hepatic lipase in PHP were assayed as previously described (Zechner, 1990).

Hepatic VLDL Secretion

Mice were fasted overnight followed by subcutaneous injection with P-407 (1 mg/g body weight) to inhibit peripheral lipolysis. Plasma samples were drawn right before P-407 injection as initial time point and 1, 2, and 3 hr after injection. Plasma TG concentration at each time point was determined.

In vivo Insulin Signaling

Overnight fasted mice were anesthetized and body temperature was maintained using a lamp. A minimal midline laparotomy was performed, and sterile saline or bovine insulin (0.75 units/kg body weight) was administrated into the portal vein. Exactly 5 min later, liver and muscle were excised and immediately snap frozen in liquid nitrogen. Protein was extracted from tissues by RIPA lysis buffer and insulin signaling was assessed by immunoblotting using anti-Akt and anti-phospho-Akt (ser473) antibodies.

Oral Glucose Tolerance Test and Insulin Tolerance Test

Oral glucose tolerance test (OGTT) was performed in overnight fasted mice. Glucose solution (2g/kg body weight) was administered by oral gavage. Glucose levels were monitored before and 15, 30, 60, and 120 min postgavage using blood glucose strips (Roche Diagnostics, Canada). For insulin tolerance test (ITT), mice were fasted during daytime (8 a.m. - 1 p.m.) and injected intraperitoneally with bovine insulin (1 U/kg body weight). Similarly, glucose levels were determined before and 15, 30, 60, 90, 120, and 180 min after injection.

Fat Tolerance Test

Overnight fasted mice were gavaged with 150 µL olive oil containing 10 µCi [^{3}H]-triolein. Blood was drawn right before gavage as initial time point and 1, 2, 3, 4, and 5 hr post-gavage. Lipids were extracted and separated by TLC. Radioactivity in TG-corresponding bands was counted by liquid scintillation counting.

Supplemental References

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