Targeting Serine-Arginine Protein Kinase 2 Ameliorates Alcohol-Associated Liver Disease by Alternative Splicing Control of Lipogenesis

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Animal Model for Chronic-Plus-Binge Alcohol Feeding—The Bin-binge mouse model of ALD was developed as described previously (1, 2). This pre-clinical mouse model of chronic-plus-binge alcohol feeding, which mimics drinking patterns and liver pathological changes seen in humans with ALD, such as steatosis and inflammation (1), was used. Male and female mice at 3-5 months of age were acclimated on a control liquid diet (Bio-Serv, Cat#F1259SP, Frenchtown, NJ, USA) for 5 days. The mice were fed a Lieber-DeCarli ethanol diet *ad libitum* (Bio-Serv, Cat#F1258SP, Frenchtown, NJ, USA, ethanol content: 5%) or subjected to a pair-fed control diet. After 10 days of ethanol feeding, mice were gavaged with a single dose of ethanol binge (5 g/kg body weight) in the early morning of the last day. Meanwhile, pair-fed mice with a control liquid diet were gavaged with an isocaloric maltose dextrin solution. All mice were sacrificed 9 hours post-gavage. During this period, both control mice and alcohol-fed mice had free access to the original liquid diet feeding tubes.

All mice were housed in a temperature-controlled environment with a 12-h light/dark cycle. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas Health San Antonio (UTHSA).

Animal Models with FGF21 Gain-of-function and Loss-of-function Strategies—We used an FGF21 transgenic mouse model (FGF21-Tg), in which human FGF21 was predominantly overexpressed in the liver under the control of the human *ApoE* promoter including its hepatic control region(3). As a result, FGF21-Tg mice exhibited elevated plasma concentrations of FGF-21 (70-150 ng/ml) (3). We also used a genetically engineered FGF21 knockout (FGF21 KO) mouse model, as previously described (4). These mice were provided by Eli Lilly and Company, Indianapolis, Indiana. After 5 days of acclimation on a control liquid diet, these mice were subjected to either a Lieber-DeCaril alcohol liquid diet for 10 days, along with one dose of alcohol binge feeding, or a pair-fed control diet plus one dose of an isocaloric maltose dextrin solution at the end of experiments.

***In Vivo* Adenoviral Gene Transfer—**Adenovirus-mediated gene transfer was performed in livers of mice through tail vein injection as described previously(2, 5). To determine the *in vivo* effect of hepatic SRPK2 knockdown, an adenovirus expressing short hairpin RNA targeting SRPK2 (shSRPK2) was purchased from Vector BioLabs (PA, USA). The target sequence for the designed mouse SRPK2 shRNA is GCAGAGAGTGATTACACGTAT. The adenovirus encoding shRNA SRPK2 (1×10^9 ~ 5×10^9 pfu) was administered by tail vein injection into mice using a 0.1-ml syringe with a 29.5-gauge needle on the last day of acclimation. Control mice received the injection with an adenovirus expressing scramble shRNA. At 24 h post-injection, these mice were subjected to a Lieber-DeCaril alcohol liquid diet for 10 days, followed by one dose of alcohol binge feeding at the end of experiments.

Isolation of Primary Mouse Hepatocytes—Primary mouse hepatocytes were isolated using a modified protocol from previous studies(6). After young C57BL/6 mice were anesthetized using isoflurane, the portal vein was cannulated with a 24G IV catheter. Subsequently, the liver was

perfused with 50 mL of PBS (no Calcium) with 0.5 mM EGTA (ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid). After that, the liver was perfused with a collagenase buffer (66.7 mM NaCl, 6.7 mM KCl, 6.3 mM $CaCl_2$, 0.05% collagenase type 2, 0.226 mM BSA, 100 mM HEPES, pH 7.4) containing collagenase type 2 (Worthington Biochemical Corporation, NJ, USA) for 3-5 mins. Once collagenase digestion was completed, the liver was removed and transferred to a 100 mm petri dish, and hepatocytes were released and filtered through 100 and 40 μm nylon mesh cell strainers. The hepatocytes were washed and centrifuged at 50 *g* at 4°C for 3 mins. After centrifugation, the cell pellet was washed three times and resuspended with DMEM containing 10% FBS. Cell number and viability were determined by Trypan Blue staining. The cells were grown and maintained in a humidified chamber with 5% CO_2 at 37°C.

Primary Hepatocyte Treatment and Adenoviral transduction—Isolated primary mouse hepatocytes were seeded in 6-well plates and cultured in DMEM (Gibco, 4.5 g/L D-glucose, MA, USA) supplemented with 10% FBS and 1% penicillin/streptomycin for 4 h, and then cultured in DMEM medium containing 2% FBS overnight. For ethanol treatment, primary hepatocytes were incubated for 24 h with increasing concentrations of ethanol (50-100 mM) in medium containing 2% FBS. For adenovirus-mediated overexpression, an adenovirus from Vector BioLabs (Malvern, PA, USA) was used. To examine the effect of overexpressing SRPK2, primary mouse hepatocytes were cultured overnight in a 6-well plate, and then transduced for 24 hours with an adenovirus carrying the full-length mouse SRPK2 in medium containing 2% FBS at varying multiplicities of infection (MOI) of 1-5, as previously described (7-10). As a control group, cells were transduced for 24 h with Ad-GFP at an MOI of 5 in the medium containing 2% FBS.

AML12 Hepatocyte Treatment and Adenoviral Transduction—The alpha mouse liver 12 (AML12) hepatocytes purchased from American Type Culture Collection were cultured in 1:1 mixture of DMEM (Gibco, 4.5 g/L D-glucose, MA, USA) and Ham's F12 medium (Hyclone, UT, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen, MA, USA), 40 ng/mL Dexamethasone, 0.005 mg/mL transferrin (Invitrogen, MA, USA), 5 ng/mL selenium, and 1% penicillin/streptomycin (Invitrogen, MA, USA) as described previously (2). The cells were grown and maintained in a humidified chamber with 5% CO_2 at 37°C. For ethanol treatment, cells were seeded in 6-well plates and cultured overnight to reach ~80% confluence, and then incubated for 24 h with increasing concentrations of ethanol (25-100 mM) in medium containing 2% FBS. For overexpression or knockdown of SRPK2 in AML12 hepatocytes, adenoviruses from Vector BioLabs (Malvern, PA, USA) were used. AML12 hepatocytes were seeded and cultured overnight. Adenovirus expressing full-length mouse SRPK2, adenovirus encoding shRNA SRPK2, or control adenoviruses were added into serum-free medium, and AML12 cells were cultured for 6-8 h. Subsequently, AML12 cells were incubated in medium containing 10% FBS, and cells were harvested 48 h post-transduction. Adenovirus-mediated overexpression of FGF21 was also performed in the same method. If ethanol treatment was involved in adenovirus-mediated transduction, AML12 cells were transduced with adenovirus vectors for 24 h, and then incubated with or without ethanol (100 mM) in medium containing 2% FBS for an additional 24 h. For treatments with cycloheximide (CHX) or rapamycin, AML12 cells were seeded in 6-well or 12-well plates and cultured overnight to reach ~80% confluence. The cells were then incubated with CHX (50 μg/ml) in the absence or presence of rapamycin (20 nm) for the duration specified in the figure legends.

Hepatic Lipids Extraction and Measurement—Hepatic lipids were extracted using chloroform and methanol (1:1) solution. First, 20 mg of liver tissue samples were homogenized in 2 mL of PBS buffer. The homogenates were transferred to clear glass tubes (BD Vacutainer, NJ, USA) and centrifuged at 13,000 rpm to remove cell debris. Second, the supernatant was mixed with 4 mL of a chloroform: methanol solution (PBS: chloroform: methanol = 1:1:1), and vigorously

vortexed for 30s. Then the mixture solution was centrifuged at 3,000 rpm at 4℃ for 10 min to allow for separation into two phases. The lipid extracts were condensed in the chloroform layer at the bottom of the tube and were carefully transferred to a new glass tube. An aliquot of the organic solvent phase was evaporated under nitrogen gas to dry chloroform and prevent oxidation of lipids. Finally, after chloroform was totally dried, lipids were re-dissolved in isopropanol with 1% Triton X-100. To assay lipid concentrations, 3 µl of triglyceride standard or 3 µl of liver lipid extract were added to a 96-well flat-bottom polystyrene plate, and 300 µl of Infinity triglyceride or cholesterol reagents were added to the microplate. Measurement of lipid content was performed using Infinity™ Triglycerides and Cholesterol (Thermo SCIENTIFIC, Cat# TR22421 and Cat# TR13421, MA, USA) according to manufacturer's instructions. The optical density was measured at 515 nm using the Spectramax M5 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). Hepatic lipid concentrations were normalized to liver tissue weight and expressed as mg of lipid/g of liver tissue.

Measurement of Plasma Triglyceride and Cholesterol levels—Measurement of plasma triglyceride and cholesterol content was performed using Infinity™ Triglycerides and Cholesterol (Thermo SCIENTIFIC, Cat# TR22421 and Cat# TR13421, MA, USA) according to manufacturer's instructions.

Measurement of Plasma Alanine Aminotransferase (ALT) Levels—Measurement of plasma ALT levels was performed with ALT assays using Liquid ALT Reagent Set (POINTE SCIENTIFIC, Cat#A7526-150, MI, USA) according to manufacturer's instructions. A ratio of plasma sample: reagent (1:10) was used, and reagents were pre-warmed at 37°C before measurement. Plasma samples and reagents were mixed in a 96-well plate. The optical density (OD) at 340 nm was measured using Spectramax M5 Microplate Reader (Molecular Devices, CA, USA). The plasma ALT levels were calculated as following: ALT (U/L) = ΔOD_{340}/min x 1768.

Immunoblotting Analysis—Immunoblotting analysis was conducted as described previously (2, 5, 11, 12). Briefly, liver samples (100 mg) were homogenized in a lysis buffer (20 mM Tris-HCl at pH8.0, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 1 mM sodium orthovanadate, 25 mM β-glycerolphosphate, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2 βg/mL aprotinin, 2 βg/mL leupeptin, and 1 µg/mL pepstatin), and then lysates were sonicated to break the nuclear membrane. After incubation on ice for 1 h, the lysates were centrifuged at 13,000 rpm for 10 min at 4°C. Following centrifugation, the supernatant was transferred to a new tube, and protein concentration was measured using Bradford assays (Bio-Rad, Cat#5000201, CA, USA) according to manufacturer's instructions. Subsequently, protein samples were separated on 10% SDS-PAGE in a protein electrophoresis running buffer (Bio-Rad, Cat#1610772, CA, USA). Proteins were transferred onto PVDF membranes (Bio-Rad, Cat#1610771, CA, USA) in a Western blot transfer buffer (Bio-Rad, Cat#1610771, CA, USA) at 90V at 4°C for 2 h. After transfer, the PVDF membranes were washed with Tris-buffered saline with 0.1% Tween-20 (TBST) three times, each wash lasting 5 min. The membranes were incubated in Tris-buffered saline (TBST) containing 5% non-fat milk at room temperature for 1 h. After blocking, the PVDF membranes were briefly washed to remove any residual milk and stripped according to target protein's molecular weight. The protein membranes were incubated with a primary antibody in TBST with 3% BSA at 4°C with gentle agitation overnight. After PVDF membranes were washed in TBST 3 times, 5 min each time, the membranes were incubated in horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h. Immunoblots were detected by using Clarity Western ECL Substrate or Clarity Max Western ECL Substrate (Bio-Rad, Cat#170-5061 or Cat#170-5062, CA, USA). Immunoblotting bands were visualized using ChemiDoc MP Imaging System (Bio-Rad, CA, USA). Band intensity was quantified by NIH Image J software. For phosphorylation, phosphorylation levels of SR proteins were quantified by densitometry,

normalized to those of GAPDH, and presented as the fold change relative to the control. For protein levels, the specific band intensity was quantified, normalized to those of GAPDH, and presented as the fold change relative to the control.

The primary antibodies used were as follows: SRPK2 (BD Transduction Laboratories #611118, 1:1000, CA, USA), phosphoepitope SR proteins (MilliporeSigma #MABE50, 1:2500, MA, USA), SRSF10 (Cell Signaling Technology, #42267, 1:1000, MA, USA), SREBP-1 (Santa Cruz #sc-13551, TX, USA 1:1000), FAS (BD Transduction Laboratories #610963, 1:10000), SCD1 (Cell Signaling Technology, #2794, 1:1000), Cleaved Caspase-3 (Cell Signaling Technology, #9661, 1:1000), Cleaved Caspase-1 p10 (Santa Cruz #514 1:1000), Cleaved Caspase-1 p20 (Cell Signaling Technology, #89332, 1:1000), and GAPDH (MilliporeSigma #MAB374, 1:5000), phospho-Thr389 S6K1 (CST#9234, 1:1000), S6K1 (CST#9202, 1:1000), phospho-Ser235/236S6 (CST#2211, 1:1000), S6 (CST#2212, 1:1000), phospho-Thr37/46 4E-BP1 (CST#9459,1:1000), and 4E-BP1 (CST#9452, 1:1000).

Liver Histology—Hematoxylin& Eosin (H&E) staining was performed on liver tissue sections as described previously(2, 12, 13). Liver tissues were rapidly harvested and fixed in phosphate-buffered 10% formalin (Fisher Chemical, Cat#SF100-20, Cat#SF100-20, MA, USA) for 2 weeks at room temperature. Fixed liver tissues were then embedded in paraffin. Paraffin sections (4 μm) were cut and mounted on glass slides. Paraffin-fixed liver sections were subjected to H&E staining. Staining images were captured and digitalized using a Nikon DS-Fi1 digital camera attached to the Nikon Eclipse 80i microscope.

Immunohistochemistry—Immunohistochemistry of liver sections was performed as described previously (2, 12, 13). Liver sections were deparaffinized and rehydrated. For antigen retrieval, liver sections were heated in 10 mM citric acid buffer (pH 6.0) using a microwave for 3 cycles: 5 mins for the first cycle and 2 mins for the remaining two cycles. After cooling to room temperature, the sections were incubated with 3% hydrogen peroxide solution containing 10% methanol for 10 min at 37°C to eliminate endogenous peroxidase activity. Then, sections were washed with ice-cold phosphate-buffered saline (PBS, pH 7.4) with 0.1% Tween-20 (PBST) 3 times, 5 mins each time. After wash, liver sections were blocked with 2.5% normal horse serum (ImmPRESS Horse Anti-Rabbit IgG Polymer Kit, Vector Laboratories, Cat#MP-7401, CA, USA). For primary antibody incubation, the liver sections were placed in a wet box and incubated with a primary antibody in PBST with 1% BSA at 4°C overnight. The next day, the primary antibodies were removed, and the sections were washed with ice-cold PBST 3 times, 5 mins each time. Subsequently, the sections were incubated at room temperature for 1 h with horse anti-rabbit IgG polymer peroxidase (ImmPRESS Horse Anti-Rabbit IgG Polymer Kit, Vector Laboratories, Cat#MP-7401) according to the manufacturer's instructions. The sections were washed with ice-cold PBST 3 times, 5 mins each time. For color development, the sections were incubated with DAB substrate reaction from the SignalStain® DAB Substrate Kit (Cell Signaling Technology, CST#8059, MA, USA) according to the manufacturer's instructions. Finally, the liver sections were counterstained with hematoxylin and cleared with xylene. After mounting, the positive stained liver sections were observed under a Nikon Eclipse 80i microscope. Staining images were captured and digitalized using a Nikon DS-Fi1 digital camera attached to the Nikon Eclipse 80i microscope.

The primary antibodies used were as follows: SRPK2 (ABclonal # A17536, 1:200, MA, USA), FAS (Cell Signaling Technology, #3180, 1:150), ACLY (Cell Signaling Technology, #13390, 1:600), SCD1 (Cell Signaling Technology, #2794, 1:100), and MPO (Biocare Medical #PP023AA, ready-to-use, CA, USA).

Immunofluorescent staining—AML12 hepatocytes were seeded on coverslips and fixed in 4% paraformaldehyde in PBS (pH 7.4) for 10 min at room temperature, and were washed with ice-cold PBST 3 times, 5 mins each time. After washing, cells were permeabilized with 0.1% Triton X-100 for 10 mins at room temperature. After washing with PBST 3 times, coverslips were blocked using 10% normal goat serum at room temperature for 1 h. The cell samples on coverslips were incubated with a primary antibody at 4°C overnight. The next day, the cell samples were washed with ice-cold PBST 3 times. After being washed, the cell samples on coverslips were incubated with a secondary antibody in darkness for 1 h. After a final wash with PBST, slides were mounted with ProLong™ Gold Antifade Mountant with DAPI (Invitrogen, Cat#P36935, MA, USA). The positive stained liver sections were observed under a Nikon Eclipse 80i microscope. The fluorescence images were taken under a Nikon Eclipse DS-Qi1MC Digital Camera attached to the Nikon Eclipse 80i microscope.

The primary antibodies used were as follows: SRPK2 (BD Transduction Laboratories, #611118, 1:100), and Texas-red dye-conjugated AffiniPure Goat-Anti-Mouse IgG (Jackson ImmunoResearch, Cat#115-076-062, 1:100, discontinued, PA, USA).

Total RNA Extraction and Quantitative Reverse Transcription PCR (qRT-qPCR)— Approximately 20 mg of liver tissue was used for total RNA extraction according to the TRIzol reagent's manufacture instructions (Invitrogen, Cat#15596026, MA, USA). Concentrations of total RNA were measured in a Spectramax M5 Microplate Reader (Molecular Devices, CA, USA). Total RNA (2500 ng) was used for single-strand cDNA synthesis using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Cat#4368813, MA, USA) according to the manufacturer's instructions. The cDNA synthesis was carried out in a Bio-Rad T100 Thermal Cycler (Bio-Rad, Hercules, CA). For quantitative RT-PCR (qRT-PCR), SYBR Green PCR master mix (Thermo Fisher, #A25742, Waltham, MA,) and gene-specific primers were used in a 10 µL reaction mix. The mRNA levels were quantified by real-time PCR analysis in the Bio-Rad CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Quantitative analysis was performed using the ΔΔCt method. The relative mRNA levels of genes were normalized to those of GAPDH and expressed as relative levels to the control. The primers used for qRT-qPCR are presented in **Table S2**.

Human Liver Tissue Samples—Normal human liver samples (n =10) and alcoholic liver disease tissues (n =10) with steatosis, inflammation, and severe fibrosis (F3-4) were obtained from donor livers and recipient's livers, respectively, during liver transplantations from the Liver Tissue Procurement and Distribution System (LTPDS) at the University of Minnesota as described previously(2). Particularly, normal healthy liver samples were obtained from the part of donor livers that were not used for transplantation. Individuals with an alcohol-drinking history (2 to 3 drinks/day) and liver cancer were excluded from the study. Prior to this analysis, all normal controls and patient records were anonymized and de-identified. All snap-freezing liver samples were collected in liquid nitrogen and stored in a -80°C freezer.

Statistical Analysis—Data are presented as means ± standard error. Using GraphPad Prism 6.0 software, results were analyzed by one-way ANOVA between multiple groups; and results were analyzed by a two-tailed Student's *t*-test between two groups. $P < 0.05$ was considered statistically significant.

Key Resources Table S1

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-SRPK2	BD Transduction Laboratories	Cat#611118; RRID: AB_398429
Anti-SRPK2	Abclonal	Cat#A17536; RRID:AB_2772388
Anti-phosphoepitope SR proteins	MilliporeSigma	Cat#MABE50; RRID:AB_10807429
Anti-FAS	BD Transduction Laboratories	Cat#610963; RRID:AB_398276
Anti-SREBP1	Santa Cruz	Cat#sc-13551; RRID:AB_628282
Anti-SCD1	Cell signaling technology	Cat#2794; RRID:AB_2183099
Anti-Cleaved Caspase-1 p10	Santa Cruz	Cat#514; RRID:AB_2068895
Anti-Cleaved Caspase-1 p20	Cell signaling technology	Cat#89332; RRID:AB_2923067
Anti-Cleaved Caspase-3	Cell signaling technology	Cat#9661; RRID:AB_2341188
Anti-SRSF10	Cell signaling technology	Cat#42267;
Anti-pS6K1 (T389)	Cell signaling technology	Cat#9234; RRID:AB_2269803
Anti-S6K1	Cell signaling technology	Cat#9202; RRID:AB_331676
Anti-pS6 (S235/236)	Cell signaling technology	Cat#2211; RRID:AB_331679
Anti-S6	Cell signaling technology	Cat#2217; RRID:AB_331355
Anti-p4E-BP1 (T37/46)	Cell signaling technology	Cat#2855; RRID:AB_560835
Anti-4E-BP1	Cell signaling technology	Cat#9644; RRID:AB_2097841
HRP conjugated Goat anti Mouse IgG	Bio Rad	Cat#1706516; RRID:AB_11125547
HRP conjugated Goat anti Rabbit IgG	Cell signaling technology	Cat#7074 RRID:AB_2099233
Texas-red dye-conjugated AffiniPure Goat-Anti-Mouse IgG	Jackson ImmunoResearch	Cat#115-076-062
Anti-GAPDH	MilliporeSigma	#MAB374; RRID:AB_2107445
Bacterial and virus strains		
Ad-U6-m-SRPK2-shRNA (Mouse SRPK2 shRNA) NM_009274	VECTOR BIOLABS	Cat# shADV-253411
Ad-m-SRPK2 (Mouse SRPK2)	VECTOR BIOLABS	Cat# ADV-253411
Biological samples		
Human liver samples	Liver Tissue Cell Distribution System	NIH Contract #HHSN276201200017C

Chemicals, peptides, and recombinant proteins		
Dulbecco's Modified Eagle Media (DMEM)	Gibco	Cat# 11965092
Ham's F-12 medium	Hyclone	Cat#SH30026.01
Penicillin-Streptomycin	Gibco	Cat#15140-122
0.25% Trypsin-EDTA	Gibco	Cat#25200-056
Lipofectamine 3000	Invitrogen	Cat#L3000-008
TRIzol	ambion	Cat#15596026
High Capacity cDNA Reverse Transcription Kit	appliedbiosystems	Cat#4368813
PowerUp SYBR Green Master Mix	appliedbiosystems	Cat#A25742
30% Acrylamide/Bis Solution 29:1	Bio Rad	Cat#1610156
Protein Assay Dye Reagent Concentrate	Bio Rad	Cat#5000006
Clarity Western ECL Substrate	Bio Rad	Cat#170-5061
Clarity Max Western ECL Substrate	Bio Rad	Cat#170-5062
ImmPRESS Horse Anti-Rabbit IgG Polymer Kit	VECTOR LABORATORIES	Cat#MP-7401
DAB Substrate Kit	Cell Signaling technology	Cat#8059
Collagenase Type 2	Worthington Biochemical Corporation	Cat#44N15257B
ProLong Gold antifade reagent with DAPI	Invitrogen	Cat#36935
Rapamycin	ALEXIS BIOCHEMICALS	Cat#380-001-M001
Cycloheximide	ThermoFisher SCIENTIFIC	Cat#J66665.03
Critical commercial assays		
LIQUID ALT (SGPT) REAGENT SET	POINTE SCIENTIFIC	Cat#A7526-150
Infinity Triglycerides	Thermo SCIENTIFIC	Cat#TR22421
Infinity Cholesterol	Thermo SCIENTIFIC	Cat#TR13421
Experimental models: Cell lines		
AML12	ATCC	CRL-2254
Experimental models: Organisms/strains		
Mouse: WT C57BL6/J	Jackson Laboratories	JAX: 000664
Mouse: FGF21 transgenic	Kharitonenkov et al. 2005	DOI: 10.1172/JCI23606
Mouse: FGF21 knockout	Fisher et al. 2014	https://doi.org/10.1053/j.gastro.2014.07.044
Oligonucleotides		
qRT-PCR primers are shown in Table S1	This study	N/A
SRPK2 shRNA GCAGAGAGTGATTACACGTAT	Vector BioLabs	Cat# shADV-253411
Recombinant DNA		
pSPORT-mSRPK2	This paper	N/A
Software and algorithms		
ImageJ	NIH	https://ImageJ.nih.gov/ij/
Prism	GraphPad 6.0	

Animal Diets		
Lieber-DeCarli control diet	Bio-Serv	Cat#F1259SP
Lieber-DeCarli ethanol diet	Bio-Serv	Cat#F1258SP

Table. S2. List of primers used for quantitative real-time PCR and RT-PCR in our study.

Gene Name	Accession No.	Primer Sequence Forward 5'-3'	Primer Sequence Reverse 5'-3'
Srebp1c	NM_001358314.1	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
Acly	NM_001199296.1	GCCAGCGGGAGCACATC	CTTTGCAGGTGCCACTTCATC
Acc1	NM_133360.2	CTCCTGCTGCGATTGCTACC	TCCACAGTCCCAGCACTCAC
Fas	NM_007988.3	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
Elovl6	NM_130450.2	CCCGAACTAGGTGACACGAT	CCAGCGACCATGTCTTTGTA
Scd1	NM_009127.4	GCCTGTACGGGATCATACTG	CAGCCGTGCCTTGTAAGTTC
Dgat2	NM_026384.3	AGTGGCAATGCTATCATCATCGT	TCTTCTGGACCCATCGGCCCCAGGA
Lipin 1	NM_001355598.1	CCCTCGATTTCAACGTACCC	GCAGCCTGTGGCAATTCA
Lipin 1 isoform α	NM_001130412.1	GGTCCCCCAGCCCCAGTCCTT	GCAGCCTGTGGCAATTCA
Lipin 1 isoform β	NM_001355598.1	CAGCCTGGTAGATTGCCAGA	GCAGCCTGTGGCAATTCA
Lipin 1 (RT-PCR)	NM_001355598.1	TCCCAGTTCGGACAGAGAAT	GCCAGAGCATTTCCAGGTTA
Cxcl1	NM_008176.3	TCTCCGTTACTTGGGGAC	CCACACTCAAGAATGGTCGC
Il-1β	NM_008361.4	TTCGTGAATGAGCAGACAGC	GGTTTCTTGTGACCCTGAGC
Tnf-α	NM_001278601.1	CGTCAGCCGATTTGCTATCT	CGGACTCCGCAAAGTCTAAG
Cd11b	NM_001082960.1	CTGCGAAGATCCTAGTTGTC	GGGACTGTGGTTTGTTGAAG
Mpo	NM_010824.2	CCCTAGACCTGCTGAAGAG	GTGATGGTGCGATACTTGTC
Ly6g	NM_001310438.1	TGCGTTGCTCTGGAGATAGA	CAGAGTAGTGGGGCAGATGG
Srpk2	NM_001359172.1	AGAGCCAGAGGAGGAGATTC	CCAGCACAGCCATACAGTAG
Srpk2	NM_001359172.1	GCAGCCGACTTAGAGGACAC	CCTTCTCCGCGTTCTCCTTC
SRSF10	NM_001080387.2	TTCTACACTCGGCGTCCAAG	CAATCTGACGCCCACAAATC
Gapdh	NM_001289726.1	TGCGACTTCAACAGCAACTC	CTTGCTCAGTGTCCTTGCTG
PKM	NM_001253883.2	CACACAGATGCTGGAGAG	GCCAAGTTTACACGAAGG
Atp8a1	NM_001038999.2	AAGGCTGATAATGCTGTGAAC	TCGTAGAGATGTCGGTTTGG
Immt	NM_001253681.1	CTGCCTGTGGCACAGAGCC	CAGTATGTTGGAGTGTG

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