Supplemental Material

Generation of APPL2^{HepKO} mice and APPL2^{HepKO}/Ad^{KO} mice

To generate APPL2 Loxp mice, we used an APPL2 targeting vector containing the *Appl2* gene spanning from the exon 3 to exon 8. We made the 'floxed' targeting vector by inserting a Loxp site in intron 4 and floxed-neo in intron 5 (Supplementary Fig. 1*A*). Male chimeras were bred with C56BL/6J mice and the offspring containing 'floxed-neo allele' were selected by genotyping and Southern blot analysis (Supplementary Fig. 1*B*). The confirmed progeny were bred with FLP mice (Jackson Lab; Stock number 003946) to remove floxed-neo cassette and then backcrossed with C57BL/6J for 6 generations. Hepatic specific APPL2 knock out (APPL2^{HepKO}) mice were generated by crossing the APPL2 Loxp mice with albumin-cre mice (Jackson Lab; Stock number 003574). Adiponectin-deficient APPL2^{HepKO} (APPL2^{HepKO}/Ad^{KO}) mice were made by breeding APPL2^{HepKO} mice with Adiponectin null mice (Ad^{KO}) to obtain APPL2^{HepKO}/Ad$^{+/-}$ mice first. The later then were bred with APPL2^{HepKO}/Ad$^{+/-}$ mice to generate APPL2^{HepKO}/Ad$^{+/+}$ and APPL2^{HepKO}/Ad^{KO} mice as littermates. Animals were maintained on a 12 hours light/dark cycle and had free access to standard normal chow and water. All experiments were performed using male mice. Animal procedures and housing were carried out in adherence to institutional guidelines at the University of Texas Health San Antonio.

Food intake, body weight, and body composition

Mouse body weight and food intake were measured on a weekly basis. Mouse body composition was measured using dual-energy X-ray absorptiometry (DEXA) (GE Medical Systems, Madison, WI).

Glucose, Insulin and Pyruvate Tolerance Tests

For glucose tolerance test (GTT), mice were fasted for 16 hours and injected intraperitoneally with glucose (2 g/kg). Tail vein blood glucose levels were checked using an automatic glucometer (Biome GM550) at indicated times. For insulin tolerance test (ITT), mice were fasted for 5 hours and injected intraperitoneally with 0.75 U/kg insulin (Humulin R; Eli Lilly, Indianapolis, IN). Tail vein blood glucose levels were monitored at indicated times. For pyruvate tolerance test (PTT), mice were fasted for 16 hours and injected with 2 g/kg sodium pyruvate (Sigma) in saline intraperitoneally. Tail vein blood glucose levels were measured at indicated times.

Analysis of mouse serum profiles

Sera were collected from mice after 16-hr fasting. Circulating metabolic factors were measured by the Insulin (Mouse) Ultrasensitive EIA (ALPCO Diagnostics^{TM}, Salem, NH), Triglyceride Assay Kit (Cayman Chemical Company, Ann Arbor, MI), Cholesterol Assay Kit (Cayman Chemical Company, Ann Arbor, MI), Free fatty acid Quantification Kit (Biovision, Mountain View, CA), HDL and LDL/VLDL Cholesterol Quantification Kit (Biovision, Mountain View, CA), and Leptin (Mouse) ElA Kit (assay designs, Ann Arbor, MI). Relative serum adiponectin levels were detected with Western blot and the band intensities were measured by using Image J.

Quantitative real-time PCR

Total RNAs were isolated from the liver using TriZol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol. cDNAs were synthesized with 1 μg of RNA using Quantitech Reverse Transcription kit (Qiagen, #205313). Real time PCR was performed with specific primers using SYBR green (Applied Biosystem) and quantitated using Bio-Rad CFX qPCR system. PCR was carried out for 40 cycles at 95 °C for 15 s and 60 °C for 1 min each cycle. Data were normalized by using β-actin as an internal control and $^{\Delta\Delta}$Ct (delta-delta-CT) method

was employed for data analysis. The full list of primer sequences is provided in Supplemental data Table 1.

Histological analysis and macrophage (F4/80) staining in liver tissue

Tissue samples were freshly collected from animals and fixed in 10% formalin for 20 hours. Tissue samples were embedded in paraffin, sectioned at 10 μm in thickness, and stained with Hematoxylin and Eosin. For Oil-red-O staining experiments, freshly collected tissues were immediately frozen and sectioned at 8 μm in thickness. Tissue sections were fixed with 10% neutral-buffered formalin and quickly dipped in 60% isopropanol. Tissue sections were stained with Oil-red-O staining solution for 15 min and rinsed once with 60% isopropanol. After staining with hematoxylin for 1 min, the slides were examined under an optical microscope. For the immunofluorescent staining of macrophages in liver tissue, the tissue sections were deparaffinized prior to antigen retrieval. The antigen retrieval was performed by boiling with 10 mM sodium citrate (pH 6.0). Tissue sections were blocked with 10% normal goat serum for one hour and then incubated with 1:100 diluted anti-F4/80 antibodies (Abcam, #ab6640) for overnight at 4^{o}C. The sections were then washed with PBST (phosphate buffered saline containing 0.2% Tween 20) 3 times and incubated with 1:200 diluted FITC-conjugated anti-Rat IgG antibodies (Thermo Fisher, #31629) for 1 hr at room temperature. After washing 3 times with PBST, tissue slides were mounted with anti-fade with DAPI (Thermo Fisher, P36935).

Measurements of liver triglyceride content

50 mg of liver tissues were homogenized with 1ml of 2:1 chloroform-methanol and kept at 4^{o}C overnight with gentle shaking. The next day, 500 μl of 0.6% NaCl was added and the samples were vortexed for 1 min. The samples were spun at 800 x g for 20 min at 4^{o}C. After collecting the

organic phase, samples were evaporated under nitrogen and reconstituted in 50 μl of PBS containing 1% Triton X-100. Triglyceride concentrations were examined using Triglyceride Assay Kit (Cayman Chemical Company, Ann Arbor, MI).

Isolation of primary hepatocytes and cell culture

Primary mouse hepatocytes were isolated as previously described (1). Isolated hepatocytes were suspended in Williams' E Medium (Thermo Fisher Gibco. Cat. No. 12551-032) containing 5% fetal bovine serum (FBS) at a density of 7 x 10^5 cells per 35 mm dish. Next day, cells were serum starved for 4 hours and incubated with or without TNF-α for overnight at 37ºC. $TSC2^{KO}$ cell lines were obtained from Dr. David Kwiatkowski (2) and cultured in 10% FBS containing DMEM media.

Migration assay with bone-marrow derived macrophages

Bone marrow cells were isolated from 4 ~ 6 weeks old wild type male C57BL/6J mice. Bone-marrow derived macrophages (BMMs) were prepared by incubating bone marrow cells with 10% FBS DMEM supplemented with 40% L929-conditioned media for 5 days. Isolated primary hepatocytes from control and $APPL2^{HepKO}$ mice were plated to a collagen-coated 6 well plate followed by stimulation with 10 ng/ml TNF-α for 24 hrs, and washed twice with PBS before incubating with DMEM media containing 1% FBS. BMMs were resuspended (1 x 10^6/ml) in DMEM containing 1% FBS. One ml of cells (1 x 10^6/ml) was loaded to the upper compartment of Transwell (Costar, cat#3412) and incubated for 24 hrs. Transwell was fixed with 3.7% Formaldehyde for 10 min and incubated with 70% ethanol for 5 min, followed by staining with Hematoxylin solution for 20 min. After removal of un-migrated cells, the migrated cells were detected under light microscopy.

RNA sequencing analysis

Total RNA was isolated from the liver tissues of Loxp control and APPL2^{HepKO} mice fed with HFD for 16 weeks (n=4/each group). RNA sequencing was performed by Genome sequencing facility in the Greehey Children's Cancer Research Institute at the UT Health San Antonio.

Adenovirus generation and infection

Myc-tagged mouse *APPL2* gene was cloned into pAd-Track plasmid. Purified pAd-Track-APPL2 shuttle vector was introduced to BJ5183 competent cells carrying adenovirus backbone and pAdEasy-1 plasmid. Adenovirus encoding APPL2 was generated by transfection of the recombinant pAd-Easy-APPL2 plasmid into HEK293 cells. Isolated primary hepatocytes were infected with adenovirus encoding GFP or GFP plus APPL2 at a multiplicity of infection of 10. The efficiency of adenovirus infection was assessed by fluorescent GFP expression 24 hours post-infection.

Antibodies and chemicals

Recombinant full-length adiponectin was produced and purified as previous described (1). AdipoRon was purchased from MedChem Express (HY-15848). Human TNF-α was obtained from Peprotech (Cat#300-01A-10UG). Rapamycin was purchased from Sigma (Cat# R0395). Homemade APPL2, Tubulin, GAPDH and anti-myc antibodies were used as previously described (1, 3). Antibodies for ACC (#3662), FASN (#3189), SCD-1(#2794), AKT (#9272), pAKT (T308) (#9275), pAKT (S473) (#9271), p-GSK3β (S9) (#9336), pAMPK (T172) (#2351), AMPK (#2352), β–Actin (#4970), and MCP-1 (#2029) were purchased from Cell Signalling. GSK3 antibodies were obtained from Santa Cruz (Cat# SC-7291). F4/80 antibodies were purchased from Abcam

(#ab6640). The Western blot images were obtained by using either X-ray film or Syngene G:Box Image System.

1. Galan-Davila AK, Ryu J, Dong K, Xiao Y, Dai Z, Zhang D, Li Z, et al. Alternative splicing variant of the scaffold protein APPL1 suppresses hepatic adiponectin signaling and function. J Biol Chem 2018;293:6064-6074.
2. Zhang H, Cicchetti G, Onda H, Koon HB, Asrican K, Bajraszewski N, Vazquez F, et al. Loss of Tsc1/Tsc2 activates mTOR and disrupts PI3K-Akt signaling through downregulation of PDGFR. J Clin Invest 2003;112:1223-1233.
3. Wang C, Xin X, Xiang R, Ramos FJ, Liu M, Lee HJ, Chen H, et al. Yin-Yang regulation of adiponectin signaling by APPL isoforms in muscle cells. J Biol Chem 2009;284:31608-31615.