Supplemental Methods

Systemic tests

For OGTTs, mice were fasted for 3 hours prior to administration of glucose (2.5 g/kg body-weight by gastric gavage). At the indicated time-points, venous blood samples were collected in heparin-coated capillary tubes from the tail-vein. Glucose levels were measured using an oxidase-peroxidase assay (Sigma-Aldrich). Mice did not have access to food throughout the experiment. Glucose levels were determined using an oxidase-peroxidase assay (Sigma-Aldrich). Insulin levels were measured using commercial ELISA kits (Millipore Linco Research). For triglyceride clearance, mice were fasted overnight (~15 h), then gavaged with 15 ul/g body weight of 20% intralipid (Fresenius Kabi Clyton, L.P.). Blood was collected hourly and then assayed for triglyceride (Infinity, Thermo Fisher Scientific). In the hepatic VLDL-TG production assay, mice were fasted for 5 hours, followed by intravenous injection of 10% tyloxapol (Sigma-Aldrich) at 500 mg/kg body weight. Blood was collected hourly and then assayed for plasma triglyceride. The lipolysis suppression assay was initiated by i.p. injection of bovine insulin (0.1 mU/g) and serum was collected at 0, 15, 30, 60, and 120 mins for glycerol assays.

Hyperinsulinemic-euglycemic clamps

Hyperinsulinemic-euglycemic clamps were performed on conscious, unrestrained male C57 black WT and Alb-AC mice, as previously described (Holland et al., 2011; Kusminski et al., 2012).

^{3}H-triolein uptake and β-oxidation

For measurements of endogenous triolein clearance rates, tissue-specific lipid uptake and β-oxidation rates in transgenic tissues, methodologies were adapted from previously detailed studies. Briefly, ^{3}H-triolein was tail-vein injected (2 uCi per mouse in 100 ul of 5% intralipid) into mice after a 5-hour fast. Briefly, blood samples (10 ul) were then collected at 1, 2, 5, 10 and 15 min after injection. At 20 minutes following injection, mice were euthanized, blood samples were taken and tissues were quickly excised, weighed and frozen at −80°C until processing. Lipids were then extracted using a chloroform-to-methanol based extraction method. The radioactivity content of tissues, including blood samples, was quantified as described previously (Kusminski et al., 2012).

Quantitative real-time PCR

Tissues were excised from mice and snap-frozen in liquid nitrogen. Total RNA was isolated following tissue homogenization in Trizol (Invitrogen, Carlsbad, CA) using a TissueLyser (MagNA Lyser, Roche), then isolated using an RNeasy RNA extraction kit (Qiagen). The quality and quantity of the RNA was determined by absorbance at 260/280 nm. cDNA was prepared by reverse transcribing 1 ug of RNA with an iScript cDNA Synthesis Kit (BioRad). **Supplementary Table 1** details the primer sequences that were utilized for quantitative RT-PCR. Results were calculated using the threshold cycle method (Livak and Schmittgen, 2001), with β-actin used for normalization.

Histology, immunohistochemistry (IHC) and immunofluorescence (IF)

The relevant adipose and liver tissues were excised and fixed in 10% PBS-buffered formalin for 24 h. Following paraffin embedding and sectioning (5 μm), tissues were stained with H&E or a Masson's trichrome stain. For IHC, paraffin-embedded

sections were stained using monoclonal antibodies to Mac2 (1:500, CL8942AP, CEDARLANE Laboratories USA Inc.). For IF, cells were grown on glass bottom culture dishes (MatTek Corporation) and were stained with polyclonal antibodies to CD36 (1:200, Novus Biologicals, USA).

Immunoblotting

Frozen tissue was homogenized in TNET buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 5 mM EDTA, phosphatase inhibitors (Sigma-Aldrich) and protease inhibitors (Roche) and then centrifuged to remove any adipose layer present. After the addition of Triton X-100 (final concentration of 1%), protein concentrations were determined using a bicinchoninic acid assay (BCA) kit (Pierce). Proteins were resolved on 4–20% TGX gel (Bio-Rad) then transferred to nitrocellulose membranes (Protran). pAkt (Ser473, 4060) and total Akt (2920) (Cell Signaling Technology, Inc.) were used (1:1,000) for insulin signaling studies. Anti-CD36 (Novus Biologicals, USA) and anti-PKC□ (Santa Cruz, USA) polyclonal antibodies were used (1:1000) for Westerns and immunoprecipitations. Primary antibodies were detected using secondary IgG labeled with infrared dyes emitting at 700 nm (926-32220) or 800 nm (926-32211) (both at 1:5,000 dilutions) (Li-Cor Bioscience) and then visualized on a Li-Cor Odyssey infrared scanner (Li-Cor Bioscience). The scanned data were analyzed and quantitated using Odyssey Version 2.1 software (Li-Cor Bioscience).

Streptozotocin (STZ) administration

Mice were fasted for 6 hrs and subjected to a single i.p. injection of streptozotocin (STZ, Sigma #S1030, St. Louis, MO) at the dose of 135 ug/g BDW. STZ

was stored at -20 °C as powder, and freshly diluted in ice-cold sodium citrate buffer (0.1M, pH 4.5) before injection as previously described (Ye et al., 2014).

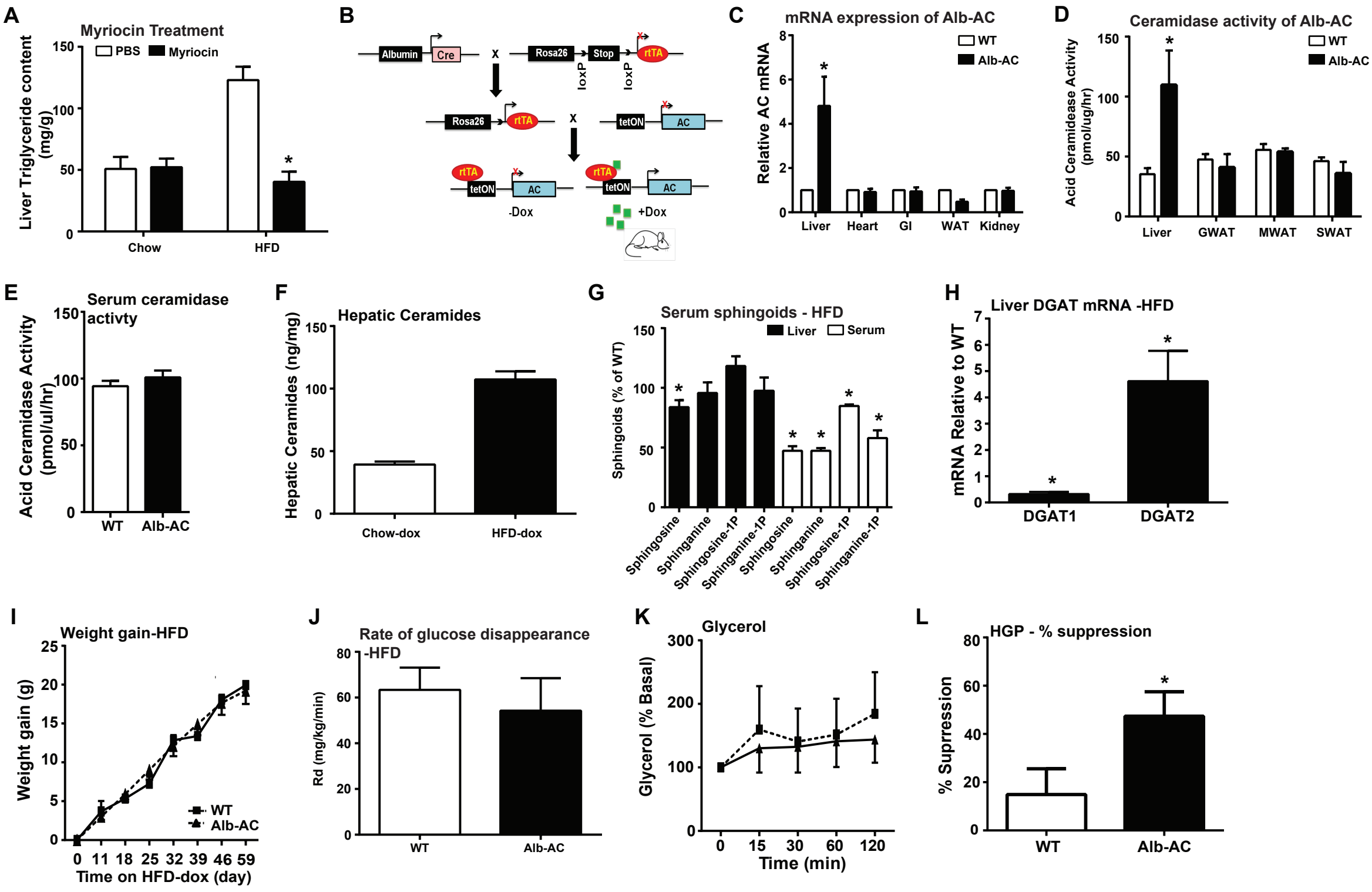
Cell Culture

For experiments, cells were grown to 90-100% confluence in 6 well culture dishes. Cells were removed into serum-free media supplemented with 0.2% fatty acid-free BSA for 90 minutes in the presence or absence of PKCζ pseudosubstrate inhibitor. Following 90-minute stepdown from serum cells were stimulated with C2-ceramide (100 uM) or sphingosine (50 uM) for 60 minutes. For lipid uptake, palmitate 50 uM 3 uCi was included for 5 minutes. Cells were washed 3x in ice cold PBS and harvested in triton-free TNET. Then, 10 uL of homogeneous lysate was counted. To evaluate background, a set of cells was harvested immediately after addition of palmitate for each experiment. Remaining TNET was processed for western blot confirmation of PKCζ overexpression, and for PKCζ activity assays.

In vitro PKCζ activity assay

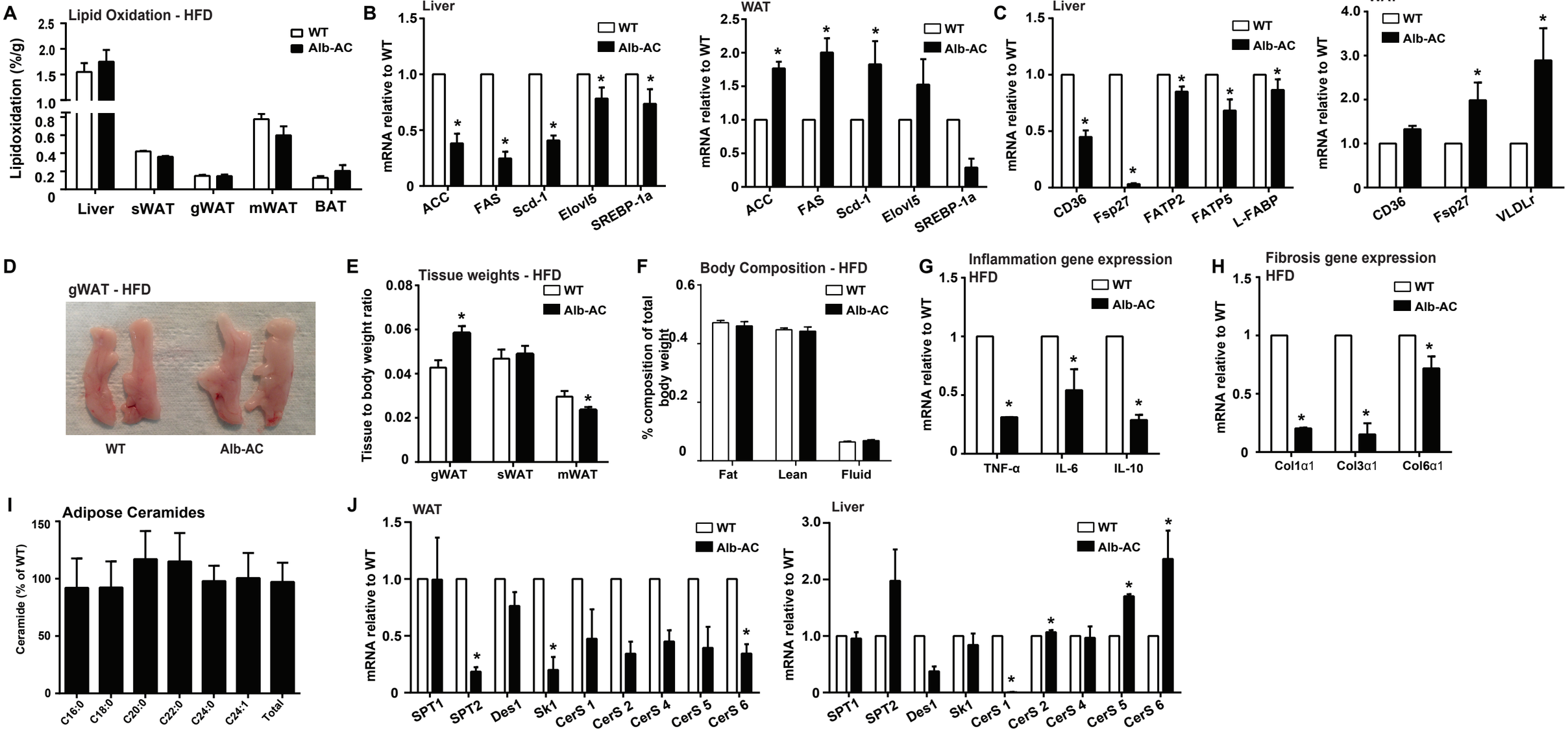
Reactions for atypical PKC activity were performed as reported previously (Luiken et al., 2009; Muller et al., 1995; Sajan et al., 2004). PKCζ peptide substrate was purchased from ENZO life sciences (Farmingdale, NY).

Supplemental Figure 1



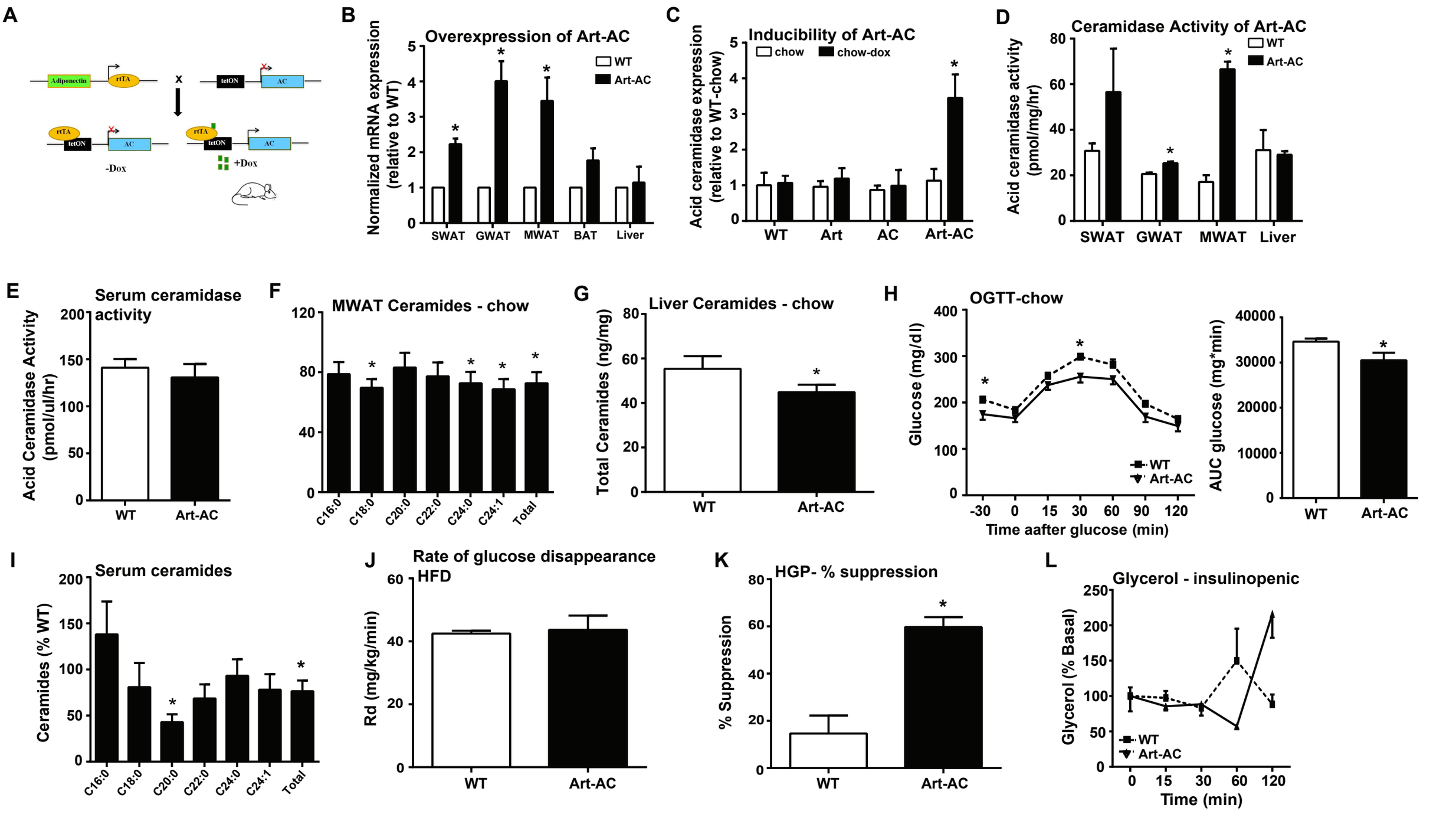
Supplemental Fig. 1 related to Fig. 1: Inducible liver-specific overexpression of acid ceramidase results in significantly reduced $C_{16:0}$ ceramide species in the liver and improves total body glucose homeostasis and insulin sensitivity under HFD feeding. **A)** WT mice fed either a chow or HFD for 8 weeks were sacrificed and liver triglycerides were quantified (n=3, * denotes p<0.05). **B)** Strategy of generating mice with liver-specific inducible expression of AC (Acid Ceramidase). **C)** Mice were fed with doxycycline chow diet for 7 days before sacrifice. mRNA levels for AC in liver, heart, small intestine (SI), white adipose tissue (WAT), and kidneys by qPCR were measured with relative to β-actin. Primers recognize both endogenous and transgenic AC (n = 3, * denotes p<0.05). **D)** Acid ceramidase activity was determined using Rbm 14-12 at 20 uM after incubation for 3 hours with tissue lysates (20 ug protein) of both WT and Alb-AC mice after 8 weeks of HFD-dox. (n = 4, * denotes P<0.05). **E)** Serum acid ceramidase activity was analyzed from Art-AC mice and WT littermates after 8 weeks of HFD-dox diet. **F)** Analysis of hepatic ceramide accumulation after 8 weeks of either chow-dox or HFD-dox diet in mice (n = 6). **G)** Analysis of liver and serum sphingoid species of Alb-AC mice and control littermates after 8 weeks of HFD-dox (n=6, * denotes P<0.05). **H)** mRNA levels of diacyglycerol synthesis genes quantified by qPCR in whole liver tissue, normalized to actin (n=4, *denotes p<0.05). **I)** Weight gain in Alb-AC transgenic mice and WT littermates during 8 weeks of HFD-dox. **J)** Whole body glucose turnover was calculated from ^{3}H glucose infusion administered prior to the clamp and throughout its duration. **K)** Low dose insulin was administered to STZ-treated mice (0s.1 mU/g body weight) and circulating glycerol levels were measured under these conditions. **L)** Hepatic glucose output, represented as % suppression of basal levels, during hyperinsulinemic-euglycemic clamp experiments performed on conscious unrestrained 10-week-old WT and Alb-AC males.

Supplemental Figure 2



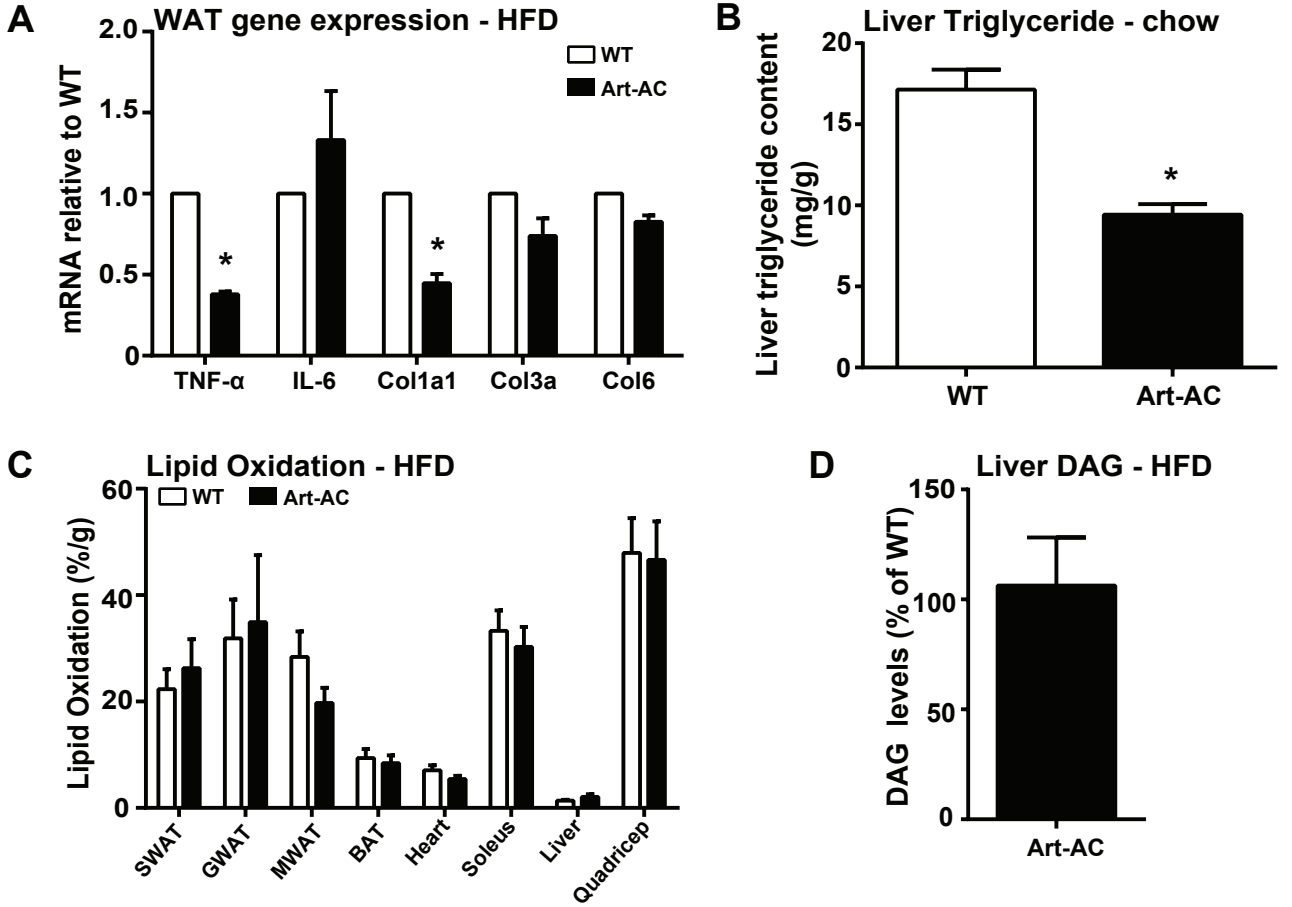
<u>Supplemental Fig. 2 related to Fig. 2:</u> Liver specific overexpression of acid ceramidase significantly reduces hepatic lipid accumulation and improves adipose tissue metabolic health when challenged with HFD. **A)** Total ^{3}H-triolein lipid-oxidation per tissue in WT and Alb-AC males at 15 min after injection (2 µCi per mouse in 100 µl of 5% intralipid, single tail-vein injection) (*n* = 6 mice per group). **B)** mRNA levels of fatty acid synthesis genes quantified using qPCR in whole liver and gWAT tissue, normalized to actin (n=4, *denotes p<0.05). **C)** mRNA levels of fatty acid uptake genes quantified using qPCR in whole liver and gWAT tissue, normalized to actin (n=4, *denotes p<0.05). **D)** Representative gross image of gonadal fat pads. **E)** The masses of the gonadal (gWAT), subcutaneous (sWAT), and mesenteric (mWAT) white adipose tissues of Alb-A mice and WT littermate controls were weighed and normalized to the total body weight of the mice (* denotes p<0.05). **F)** Body composition of fat and lean masses was quantified via NMR. **G)** mRNA levels of inflammatory genes (TNFα, IL-6, IL-10) quantified using qPCR in whole gWAT tissue, normalized to β-actin (n=4, *denotes p<0.05). **H)** mRNA levels of fibrosis genes (Col1α1, Col3α1, Col6α1) were quantified using qPCR in whole gWAT tissue, normalized to β-actin (n=4, *denotes p<0.05). **I)** Analysis of gWAT ceramide species from Alb-AC mice and WT littermates after 8 weeks of HFD-dox. **J)** mRNA levels of sphingolipid synthesis genes quantified using qPCR in whole liver and gWAT tissue, normalized to actin (n=4, *denotes p<0.05). All samples are from Alb-AC mice and WT littermates after 8 weeks of HFD –dox challenge (n = 6), unless specified otherwise.

Supplemental Fig. 3



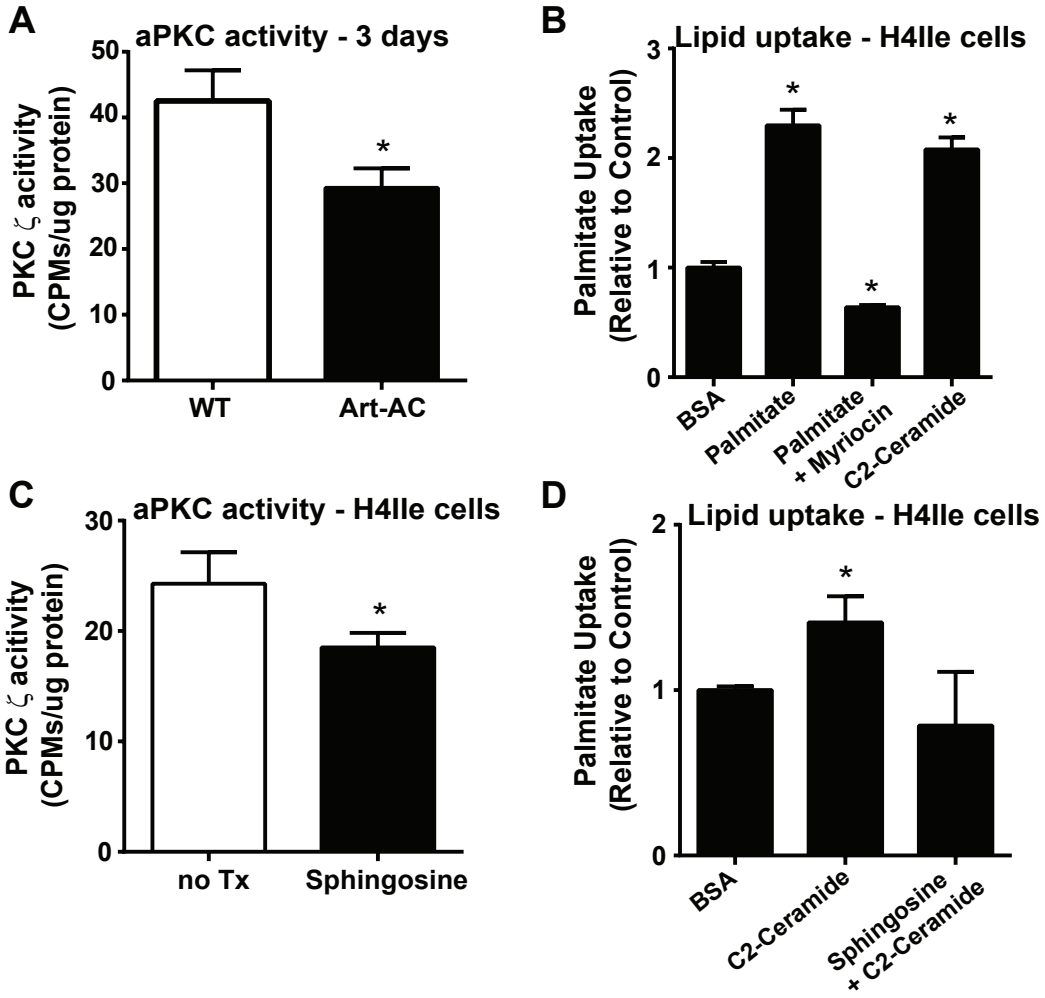
Supplemental Fig. 3 related to Fig. 3: Adipose-specific acid ceramidase overexpression improves glucose homeostasis. **A)** To allow for adipose-restricted expression of acid ceramidase in a doxycycline inducible manner, adiponectin rtTA (Art) mice were crossed with TRE-AC transgenic mice to generate Art-AC double transgenic mice. **B)** Following HFD-Dox treatment, major fat pads and liver were analyzed for expression of acid ceramidase message by qPCR and normalized to GAPDH message (n=6/group, * denotes $p<0.05$). **C)** To verify the doxycycline-restricted expression of the transgene, mesenteric adipose was analyzed by qPCR for acid ceramidase message from Art transgenic, TRE-AC transgenic, Art-AC double transgenic and wildtype mice following 10 days of treatment with dox chow or normal chow (n=4/group, * denotes $p<0.05$). **D)** Following 10 days of treatment with dox chow, acid ceramidase activity was analyzed from lysates of subcutaneous, gonadal, and mesenteric WAT and liver of WT and Art-AC transgenic mice (n=4/group, * denotes $p<0.05$). **E)** Analysis of liver C17 fatty acid levels in Art-AC mice and control littermates after 3 days of HFD-dox. **F)** Serum acid ceramidase activity was analyzed from Art-AC mice and WT littermates after 8 weeks of HFD and 3 days of HFD-dox diet. **G)** Following 3 weeks of dox chow, ceramides were quantified from mesenteric WAT of Art-AC transgenic mice and presented as normalized to wildtype littermates (n=6-8/group, * denotes $p<0.05$). **H)** Following 3 weeks of dox chow, ceramides were quantified from liver of Art-AC transgenic mice and WT mice and presented as sum of all chain lengths (n=6-8/group, * denotes $p<0.05$). **I)** Glucose tolerance tests were performed after 3 weeks of dox chow on WT and Art-AC transgenic mice (n=8-10 per group, $p<0.05$). The area under the curve during the GTT was calculated ($p<0.05$). **J)** Analysis of serum ceramide species from Art-AC mice and WT littermates after 8 weeks of HFD-dox (n=6/group, * denotes $p<0.05$). **K)** Whole body glucose turnover was calculated from the ^{3}H glucose infusion administered prior to the clamp and throughout its duration. **L)** Hepatic glucose output, represented as % suppression of basal levels, during hyperinsulinemic-euglycemic clamp experiments performed on conscious unrestrained 8-week-old WT and Art-AC males (n=6-8/group, * denotes $p<0.05$). **M)** Low dose of insulin was administered to STZ-treated mice (0.1 mU/g body weight) and circulating glycerol levels were measured under these conditions.

Supplemental Figure 4



Supplemental Fig. 4 related to Fig. 4. Adipose-specific overexpression of acid ceramidase reduces hepatic lipid accumulation and improves adipose tissue metabolic health. A) Following 10 weeks of HFD dox administration, expression of inflammatory genes and fibrosis genes was assessed by qPCR from gonadal WAT, normalized to GAPDH (n=6/group, $p<0.05$). **B)** Liver triglycerides were biochemically measured following 3-week administration of dox chow diets (n=6-9/group, $p<0.05$). **C)** Lipid oxidation was determined for the indicated tissues following bolus injection of ^{3}H-Triolein into WT or Art-AC transgenic mice maintained on HFD-dox. **D)** Analysis of liver and serum diacylglycerol (DAG) levels in Art-AC mice and control littermates after 8 weeks of HFD-dox.

Supplemental Figure 5



Supplemental Fig. 5 related to Fig. 6: Ceramides facilitate lipid uptake by mechanisms involving activation of PKCζ and CD36. A) PKCζ activity was assessed from livers of WT and Art-AC mice following 3 days of HFD-dox. **B)** H4IIe hepatocytes were treated with either palmitate, C2-ceramide (100 μM, in 0.2% BSA) or BSA for 1 hour prior to assessment of ^{3}H-palmitate uptake (n=4, * denotes $p<0.05$). **C)** PKCζ activity was assessed from H4IIe cells with or without 1 hour pre-treatment of sphingosine (* denotes $p<0.05$). **D)** H4IIe hepatocytes were treated with either C2-ceramide (100 μM, in 0.2% BSA), sphingosine and C2-ceramide or BSA for 1 hour prior to assessment of ^{3}H-palmitate uptake (*denotes $p<0.05$).

Supplementary Table 1 related to Main Methods

List of qPCR primers used

β-actin	5’-TGGCATTGTTACCAACTGGG	5’-GGGTCATCTTTTCACGGTTG
SPT1	5’-TACTCAGAGACCTCCAGCTG	5’-CACCAGGGATATGCTGTCATC
SPT2	5’-GGAGATGCTGAAGCGGAAC	5’-GTATGAGCTGCTGACAGGCA
CerS1	5’-CTGTTCTACTTGGCCTGTTG	5’-TCATGCAGGAAGAACACGAG
CerS2	5’-TCTTCTCAAAAAGTTCCGAG	5’-AGTGATGATGAAAACGAATGG
CerS4	5’-TGTCGTTCAGCTTGAGTGAG	5’-AGCAGGCTTCACAGAATTTC
CerS5	5’-CTCCAACGCTCACGAAATTC	5’-ATGCAGACAGAAGATGAGTG
Cers6	5’-GTTCGGAGCATTCAACGCTG	5’-CTGAGTCGTGAAGACAGAGG
Des1	5'-CACCGGTACCTCGGAGCGGA	5'-GTTTGGGATTGATGAACAGGGGT
Sk1	5'-TCCTGGCACTGCTGCACTC	5'-TAACCATCAATTCCCCATCCAC
AC	5’-CAGGACTGTTCAGTCTTTCAC	5’-GAGTGATAAACCCTACCCACT
ACC	5’-GGCAGCTCTGGAGGTGTATG	5’-TCCTTAAGCTGGCGGTGTT
FAS	5’-CCTGGATAGCATTCCGAACCT	5’-AGCACATCTCGAAGGCTACACA
Scd-1	5'-TCGCCCCTACGACAAGAACA	5'-CCGGTCGTAAGCCAGGCCCA
Elovl5	5’-TCAATGCTTTCTTGGACACATG	5’-GGTAAGAGTCCAGCAGGAACCA
SREBP-1a	5’-GGAGCCATGGATTGCACATT	5’-GGCCCGGGAAGTCACTGT
CD36	5’-TGAGACTGGGACCATTGGTGAT	5’-CCCAAGTAAGGCCATCTCTACCAT
Fsp27	5’-CAAGGCCAAGCGCATCGT	5’-TGCCAAGCAGCATGTGACCG
FATP2	5’-CAACACACCGCAGAAACA	5’-ATTTCCCAGGGCTTTTTTCA
FATP5	5’-GACCACTGGACTCCCAAAGC	5’-GACAGCACGTTGCTCACTTGT
L-FABP	5’-GGTGACAACTTTCAAAGGCATAAA	5’-TGTCGCCCAATGTCATGGTA
VLDLr	5’-GAGCCCCTGAAGGAATGCC	5’-CCTATAACTAGGTCTTTGCAGATATGG
TNF-α	5'-TCTGTCTACTGAACTTCGGG	5'-ATCTGAGTGTGAGGGTCTG
IL-6	5'-TCCAGTTGCCTTCTTGGGAC	5'-GTACTCCAGAAGACCAGAGG
IL-10	5'-TGGCCCAGAAATCAAGGAGC	5'-CAGCAGACTCAATACACACT

Col1α1	5’-GATGAGGGTGAAGTGGGAGA	5’-CAGCACGAAGAGGATGTCAA
Col3α1	5’-GGGTTTCCCTGGTCCTAAAG	5’-CCTGGTTTCCCATTTTCTCC
Col6α1	5’-GATGAGGGTGAAGTGGGAGA	5’-CAGCACGAAGAGGATGTCAA