Supporting Information

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SI Methods

Hyperinsulinemic-Euglycemic Clamp Studies. A juglar venous catheter was implanted 6–7 d before the hyperinsulinemic-euglycemic clamps. To assess basal whole-body glucose turnover, [3-^{3}H]-glucose (HPLC-purified; Perkin-Elmer Life Science) was infused at a rate of 0.05 μCi/min for 120 min into the juglar catheter. Following the basal period, hyperinsulinemic-euglycemic clamps were conducted in conscious mice for 140 min with a 4-min prime (29 mU/kg) followed by a continuous [3 mU/(kg-min)] infusion of human insulin (Novolin; Novo Nordisk), a continuous infusion of [3-^{3}H]-glucose (0.1 μCi/min), and a variable infusion of 20% dextrose to maintain euglycemia (100–120 mg/dL). Plasma samples were obtained from the tip of the tail at 0, 30, 50, 65, 80, 90, 100, 110, 120, 130, and 140 min. The tail incision was made at least 2 h before the first blood sample was taken to allow for acclimatization, according to standard operating procedures. In addition, mice received an intravenous albumin-containing solution mimicking artificial plasma during the insulin-stimulated period of the clamp to compensate for volume loss secondary to blood sampling (1). At the end of the clamp, mice were anesthetized with sodium pentobarbital and tissues were snap-frozen in liquid nitrogen.

Biochemical Analysis and Calculations. Plasma glucose (10 μL per sample) was measured using a YSI 2700D glucose analyzer. Plasma fatty acids were determined with the NEFA C kit (Wako Pure chemical Industries). Plasma insulin concentrations were measured by a RIA kit (Millipore). Serum triglycerides and cholesterol panel were analyzed using COBASMira Plus (Roche). Plasma adiponectin was measured using a mouse multiplex assay kit (Meso Scale Discovery). For the determination of ^{3}H-glucose, plasma was deproteinized with $ZnSO_4$ and Ba (OH) 2, dried to remove ^3H_2O, resuspended in water, and counted in scintillation fluid (Ultima Gold; Perkin-Elmer Life Sciences). Rates of basal and insulin-stimulated whole-body glucose turnover were determined as the ratio of the [3-^{3}H]-glucose infusion rate (disintegrations per minute, dpm) to the specific activity of plasma glucose (dpm/mg) at the end of the basal period and during the final 30 min of steady state of the clamp, respectively. Endogenous glucose production was calculated by subtracting the glucose infusion rate from the whole-body insulin-stimulated glucose disposal.

Animals and ASO Treatment. The protocols used here were reviewed and approved by the Institutional Animal Care and Use Committee of Yale University School of Medicine. Male C57BL/6 were purchased from Jackson Labs at 7 wk of age. At 8 wk of age, mice received biweekly intraperitoneal injections of ASO targeting CGI-58 (CGI-58) or a nontargeting control ASO (control) for 8 wk. The mice were maintained on a 12:12-h light/dark cycle (lights on at 7:00 AM). The mice received either regular rodent chow (60% carbohydrate, 10% fat, 30% protein calories), a high-fat diet (20.0% carbohydrate, 60.0% fat, 20.0% protein calories, D. 12492; Research Diets) for 7–10 d. Body composition was assessed by ^{1}H magnetic resonance spectroscopy using a Bruker Minispec analyzer. Metabolic parameters and physical activity were measured using the Columbus Labs Animal Monitoring System. All experiments were done in 6-h fasted animals (7:00 AM to 1:00 PM) at 16 wk of age.

Tissue Lipid Measurement. Tissue triglyceride was extracted by the method of Bligh and Dyer (2) and measured using the Triglyceride-sl assay (Genzyme Diagnostics). The DAG and ceramide extraction and analysis were performed as previously described (3). Sn-1,2 and 1,3 DAG were analyzed as previously described (4) with slight modification. Three to 6 mg of tissue was weighed out, internal standard added (~1 nmol d5 1,3 19:0/19:0 DAG), samples were homogenized in HBSS by sonication, and extracted three times in 1:1 Hexane:MTBE. The extracts were then combined, dried, and derivatized in 1 mL dichloromethane with 100 μg 2,4 difluorophenyl isocyanate and 100 μg dimethyl 4-aminopyridine, overnight at 25 °C. Samples were then dried under nitrogen and resuspended in isooctane for analysis by LC/MS/MS. Samples were run on a Kinetix 2.6 μg 100 Å 50 × 2.1 mm HILIC column with a gradient of mobile phase A, isooctane, and mobile phase B, MTBE:isooctane (1:1). A 10-min linear gradient of 5% B to 30% B was followed by a 3-min linear gradient to 80% B. Ammonium acetate (10 mM in 95% acetonitrile, 5% water) at a flow rate of 30 μL/min was provided postcolumn to enhance ionization. Quantitative analysis of DAG species was performed with the mass spectrometer (AB Sciex 3200) monitoring of ions with neutral loss of 190.1 Da. Peak areas for individual 1,2 DAG and 1,3 DAG molecules present in the samples were compared with the peak area for internal standard (d5 1,3 19:0/19:0 DAG), and quantities present were calculated based on a standard curve made with 1,3 16:0/16:0 DAG.

Total RNA Preparation and RT-PCR Analysis. Total RNA was extracted using RNeasy 96-kit (Qiagen), then 1 μg of RNA was reverse-transcribed into cDNA with the use of the Quantitect RT kit (Qiagen) as per the manufacturer's protocol. The abundance of transcripts was assessed by real-time PCR on a 7500 Real-Time PCR system (Applied Biosystems) with a SYBR Green detection system. Samples were run in duplicate for both the gene of interest and loading control (Tata box binding protein) and data were normalized for the efficiency of amplification. Primers were chosen from the primerbank online database (5) and can be supplied upon request.

Immunoblotting. Liver and adipose tissue were homogenized in 1 mL of lysis buffer [20 mM Tris•HCl buffer (pH 7.5 at 4 °C), 150 mM NaCl, 50 mM NaF, 5 mM NaPPi, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM phenylmethanesulfonyl fluoride (PMSF), phosphatase and protease inhibitor mixture] and homogenized for 30 s. Homogenates were spun at 20,800 × g for 15 min at 4 °C, and protein concentration was determined using the BCA protein assay kit (Pierce). SDS/PAGE was performed by using precast 4–12% Tris•Glycine gradient polyacrylamide gels (Invitrogen). After electrotransfer to nitrocellulose membranes, membranes were incubated in blocking buffer (5% milk) for 1 h and immunoblotted with antibody overnight at 4 °C against CGI-58 (NOVUS Biologicals), GAPDH (Cell Signaling Technology), Phospho S474 AKT2 (SAB Signalway), AKT2 (Cell Signaling), PKCε (BD Transduction), NaK ATPase (Abcam), ADRP (Abnova), GS28 (BD Transduction), or calnexin (Abcam), as indicated. After the incubation with the primary antibody, the membranes were washed three times for 30 min with TBS (10 mM Tris•HCl, pH 7.4, 0.5 M NaCl) plus Tween 20 [0.2% (vol/vol)] (TBST) and incubated with a corresponding IgG-conjugated secondary antibody. The membranes were then washed three times with TBST. Proteins were then detected with enhanced chemiluminescence (Thermo Fisher

Scientific), and autoradiographs were quantified by using densitometry (ImageJ).

Cell Fractionation. Fifty milligrams of fresh liver tissue was homogenized in 700 μL of homogenization buffer A [20 mM Tris•HCl (pH 7.4), 1 mM EDTA, 0.25 mM EGTA, 250 mM sucrose, protease, and phosphatase inhibitor mixture], then 300 μL of 3% sucrose was layered on top of the homogenate, and samples were centrifuged 100,000 × *g* for 1 h at 4 °C. The lipid cake was removed with a 23-G1 needle, and then the cytoplasm was removed. The pellet was resuspended in 700 μL Buffer A for DAG analysis or 700 μL Buffer B [20 mM Tris•HCl buffer (pH 7.5 at 4 °C), 150 mM NaCl, 50 mM NaF, 5 mM NaPPi, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, phosphatase and protease inhibitor mixture] for protein analysis. The samples were then homogenized with a 25-G7/8 needle, and centrifuged 20,800 × *g* for 15 min at 4 °C. The remaining floating lipid was removed with a 23-G1 needle and Triton-X was added to 2% (vol/vol) to the supernatant of the samples for protein analysis, or the supernatant was completely removed and the pellet was resuspended in 700 μL Buffer A for DAG analysis. Membrane samples were then passed through a 28-G1/2 needle and stored at −20 for DAG analysis or, for protein analysis, incubated on ice for 30 min and then centrifuged 20,800 × *g* for 15 min at 4 °C. The supernatant membrane fraction was removed and saved. Proteins in the lipid fraction were precipitated in acetone at −20 °C overnight, centrifuged at 20,800 × *g* for 30 min at 4 °C, the protein pellet was then dried under N_2, and resuspended in Buffer B with 2% Triton-X (vol/vol). Protein concentrations in all three fractions were then determined using the BCA protein assay method and equal concentrations of protein were loaded on a SDS/PAGE gel.

Statistical Analysis. Values are expressed as mean ± SEM. The significance of the differences in mean values among different treatment groups was evaluated by two-way ANOVA using GraphPad Prism, v6.0a. *P* values less than 0.05 were considered significant.

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2. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37(8):911–917.
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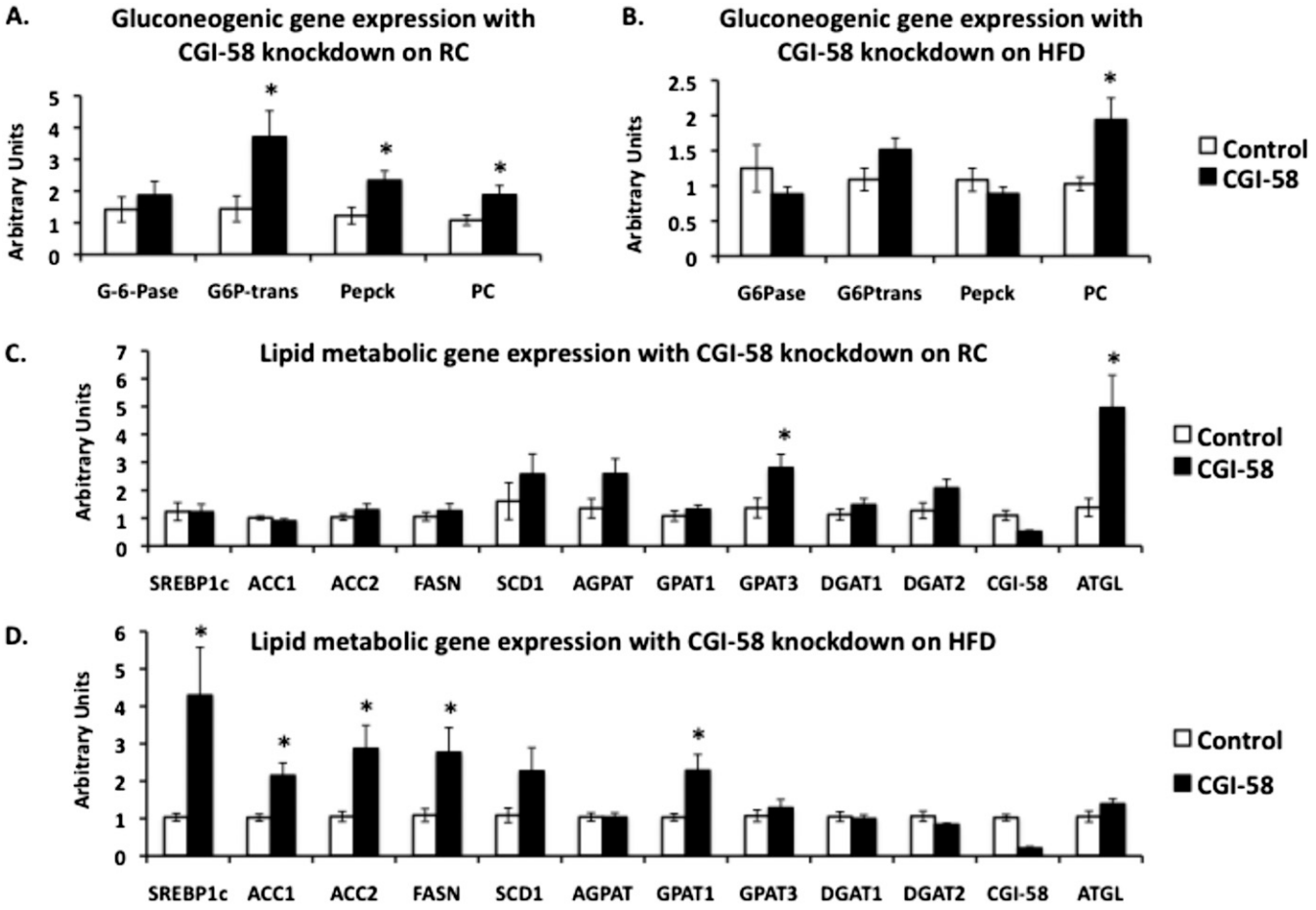

Fig. S1. Comparitive gene identification 58 (CGI-58) antisense oligonucleotide (ASO) treatment alters gluconeogenic and lipid metabolic gene expression. (*A* and *B*) Real-time PCR analysis of gluconeogenic gene expression in mice treated with control or CGI-58 ASO for 8 wk on a regular chow or high-fat diet. (*C* and *D*) Real-time PCR analysis of lipid metabolic gene expression in mice treated with control or CGI-58 ASO for 8 wk on a regular chow or high-fat diet (n = 8–10 per group). *P value < 0.05 as calculated by student t test.

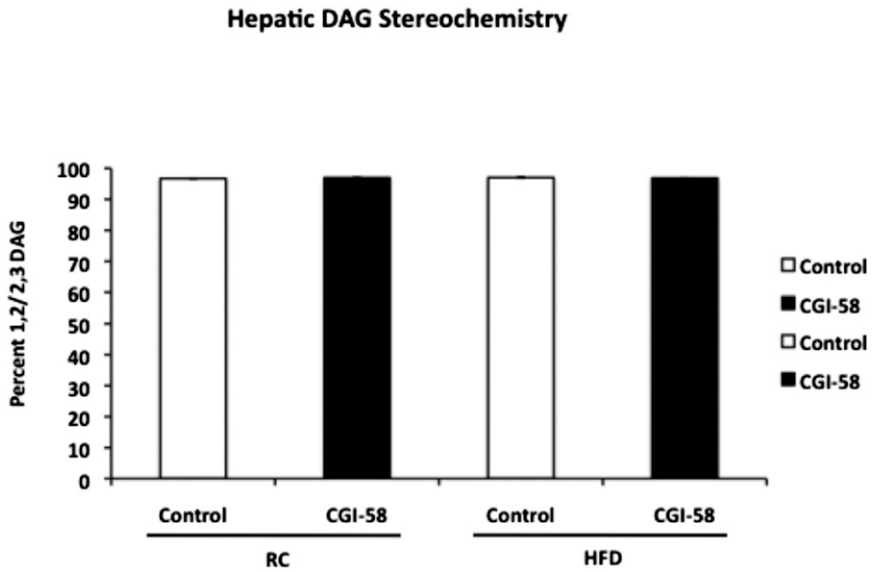

Fig. S2. Differences in fatty-acid position on the glycerol backbone of DAG do not explain the hepatic insulin sensitivity seen with CGI-58 ASO treatment. Hepatic DAGs in the sn-1,2/2,3 or sn-1,3 position were assessed by LC/MS/MS in mice treated with control or CGI-58 ASO for 8 wk (n = 6 per group). Percent sn-1,2/2,3 DAG was calculated based on the total sn-1,2/2,3 and sn-1,3 DAG (nanomole per gram of tissue).

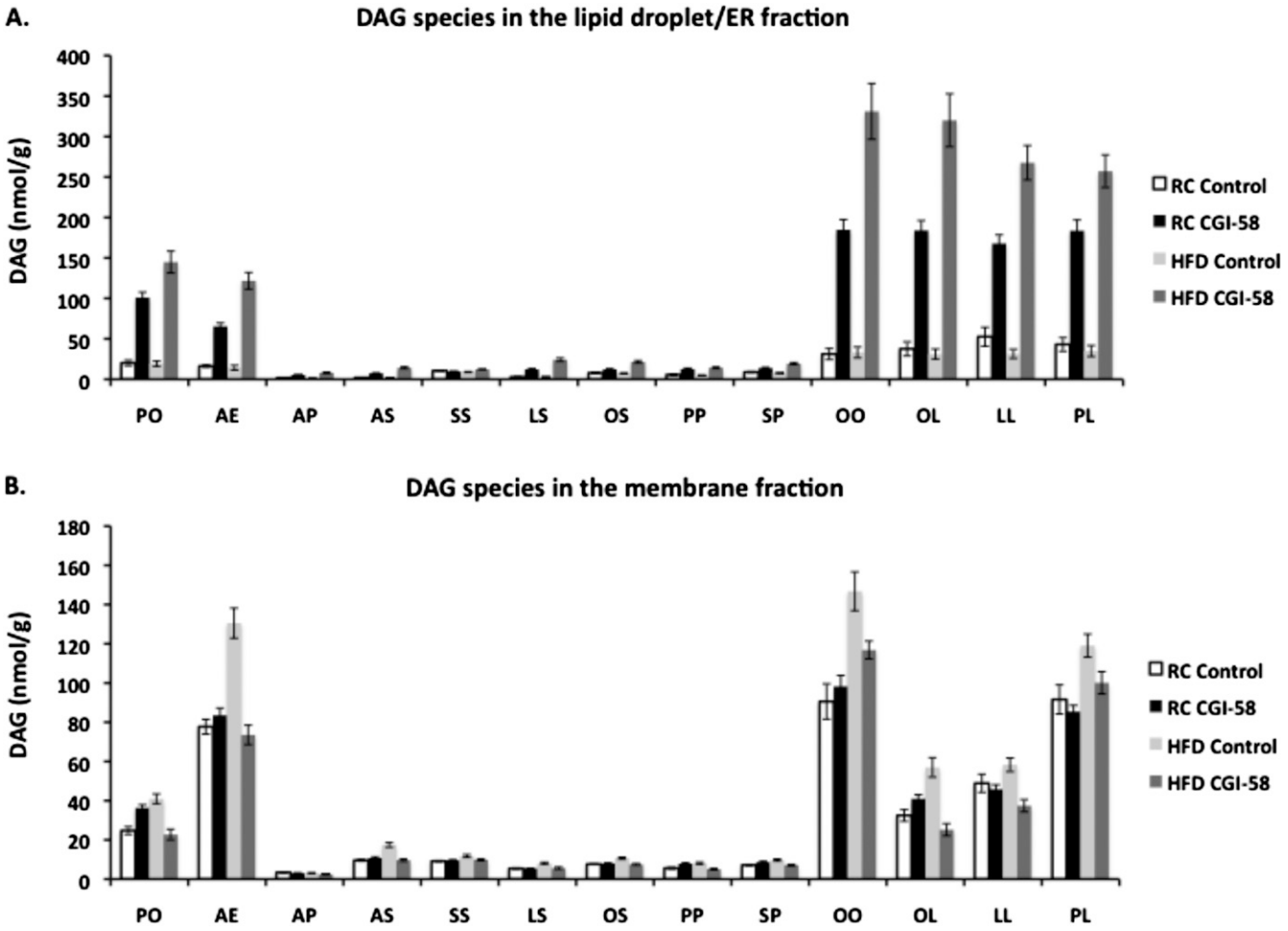

Fig. S3. Differences in DAG species in the membrane and lipid droplet/ER fraction do not explain the repartitioning of PKCε to the lipid droplet fraction with CGI-58 ASO treatment. (*A*) Hepatic diacylglycerol (DAG) concentration in the lipid droplet/endoplasmic reticulum fraction as assessed by LC/MS/MS. (*B*) Hepatic DAG concentration in the membrane fraction as assessed by LC/MS/MS. ($n = 6$ per group). A, arachidonic acid; E, eicosapentaenoic acid; L, linoleate; O, oleate; P, palmitate; S, stearate.