ONLINE METHODS

Animals. We have described C57BL/6 *Hdac3fl/fl* mice previously[26]. We housed mice under the 12 h light/ 12 h dark cycles (lights on at 7 a.m., lights off at 7 p.m.). We used adult male mice at the age of 3–7 months in all experiments except for where otherwise indicated. The Penn Vector Core generated AAV vectors (AAV2/8-Tbg-GFP and AAV2/8-Tbg-Cre). We injected AAV intravenously at 1.5×10^{11} GC per mouse and characterized mice after 1–3 weeks upon AAV-Cre injection. For Foxo1 overexpression, we injected intravenously adenovirus vectors expressing GFP or Foxo1 (TSS mutant) at 1.3×10^{9} IFU per mouse. Adenovirus for overexpression of Plin2 was described[58]. For Plin2 knockdown, we injected intraperitoneally either control or Plin2 ASO at 25 mg per kg body weight, twice per week, for 4–5 weeks. We harvested tissues at 5 p.m. without restricting mice to food or water, unless otherwise indicated. We performed glucose tolerance tests and insulin tolerance tests after fasting mice for 6 h since 9 a.m. For glucose tolerance test, we injected glucose intraperitoneally at 1.5 g or 2 g per kg body weight. For insulin tolerance test, we injected insulin (Novolin R) intraperitoneally at 0.6 U per kg body weight. We measured blood glucose using glucometer (Onetouch) via sampling from the tail. HFD containing 60 kcal % fat was purchased from Research Diets Inc. (D12492i). All the animal care and use procedures followed the guidelines of the Institutional Animal Care and Use Committee of the University of Pennsylvania in accordance with the guidelines of the US National Institute of Health.

Western Blot, fractionation and gene expression analysis. For western blot of total lysates, we lysed tissues or cells in RIPA buffer supplemented with phosphatase and protease inhibitors, resolved samples by Tris-glycine SDS-PAGE, transferred samples to PVDF membrane, and

blotted with indicated antibodies. Prkce translocation was previously described[59]. For LDs fractionation, we grounded liver tissues in Tris-EDTA buffer supplemented with proteasome inhibitors, and ultra-centrifuged samples againt 250 mM sucrose using Beckman SW 40Ti roter at 35,000 rpm for 1.5 hr at 4 degree. We separated the top lipid droplet fractions, delipidated them, and analyzed them using western blot. We purchased antibodies from commercial resources: Hdac3, Cidec, Fitm1 (Abcam), Plin2 (Progen), Ran (BD), phospho-S473 Akt, total Akt, Hsp90 (Cell Signaling), and G0S2 (Santa Cruz). For RT-qPCR, we extracted total RNA using TRIzol (Invitrogen) and High Pure RNA tissue kit (Roche). We performed reverse transcription and quantitative PCR with High Capacity RT kit, SYBR Green PCR Master Mix, and the PRISM 7500 instrument (ABI) using absolute quantification method with standard curves. We used 36B4 (Arbp) as the housekeeping control. Microarray analysis was previously described[23] and the data is available at Gene Expression Omnibus GSE25937.

Histology. For H&E staining, we fixed tissues in 4% paraformaldehyde for overnight, dehydrated samples, paraffin-embedded them, and prepared 5 μm sections. For ORO staining, we prepared 5 μm frozen sections from snap-frozen liver tissues and fixed them in 10% buffered formalin for 3 min. We then stained the sections in 0.5% ORO in propylene glycerol and then in hematoxylin for nucleus for 5 s. We performed immunohistochemistry and immunofluorescence staining on paraffin sections with antibodies to GFP (Abcam), F4/80 (Invitrogen), Plin2 and Plin3[60] according to standard protocols.

Metabolites measurements. We measured serum triglyceride (Stanbio), ketone bodies (Stanbio), total FFA (Wako), and insulin (Crystal Chem) using commercial kits. For measuring tissue triglyceride, we homogenized tissues in lysis buffer (140 mM NaCl, 50 mM Tris and 1%

Triton X-100, pH 8.0) and quantified triglyceride concentration in the lysates using LiquiColor triglyceride assay kit (Stanbio)[13,23]. For measuring glycogen, we homogenized tissues in 0.5 N KOH, precipitated glycogen by ethanol, and digested glycogen with 0.25 mg ml^{-1} amyloglycosidase (Sigma). We quantified the resultant glucose levels using glucose HK assay (Sigma). DAG was analyzed by MMPC/DRTC Lipid Lab at Vanderbilt. Lipids were extracted using Folch method and separated by thin layer chromatography, followed by analysis on Agilent 7890A gas chromatograph with known standards. Hepatic ceramide was analyzed by MMPC Metabolomics Core at Yale. We extracted acyl-CoAs with chloroform: methanol (2:1, v/v) along with isotope -labeled internal standards and then generated an acyl-CoA profile with flow injection tandem mass spectrometry using the unique neutral loss of m/z507. We determined the acyl-CoA concentrations by the intensity of each acyl-CoA species to the nearest chain-length internal standards, and normalized the values to wet weight of the tissue. We measured plasma acylcarnitines by flow-injection tandem mass spectrometry with analysis of the parent ion of fragment m/z 85, along with isotope -labeled internal standards.

Fatty acid oxidation, uptake and glucose production in hepatocytes. We isolated primary hepatocytes, plated them in triplicates in complete DMEM medium, and washed them with PBS after attachment. For fatty acids oxidation and uptake, we incubated cells in PBS supplemented with 62.5–250 uM ^{3}H-palmitate conjugated on BSA and carnitine for 15–120 min. We delipidated the incubation solution and measured $^{3}H_2O$ using a scintillation counter. We then washed the cells two times with PBS and harvested them. We extracted intracellular lipid by the Folch method and measured ^{3}H in a scintillation counter. For glucose production, we isolated hepatocytes from overnight fasted mice and further starved cells in Krebs buffer for 1 hr after

attachment. We then incubated cells with Krebs buffer supplemented with different substrates for 2 h. We quantified glucose production in the incubation solution using HK assay kit (Sigma).

Hepatic TG secretion. We fasted mice for 4 h and injected intraperitoneal with 400 ul 7.5% Pluonic 407 (Poloxamer P407, Fisher) solution in PBS. We collected blood across a time course, and quantified plasma TG using triglyceride assay kit (StanBio).

***De novo* lipogenesis.** We injected deuterated water intraperitoneally (20 µl per g body weight) and euthanized mice after 4 h. We extracted lipid from liver and analyzed it using GC/MS. We determined the percent contribution of newly-made fatty acid using the equation: % newly-made fatty acid = [total ^{2}H-labeled fatty acid/ (^{2}H-labeled body water ×n)] ×100 where n is the number of exchangeable hydrogens, assumed to equal 22 for palmitate and 23 for oleate. We determined the absolute amount of newly-synthesized fatty acid by multiplying the % newly-made fatty acid by the concentration of the total fatty acid.

Hyperinsulinemic-euglycemic clamp. The clamp study was performed by the Mouse Phenotyping, Physiology and Metabolism Core at the University of Pennsylvania. Human insulin was infused at 2.5 mU per kg body weight per min, and blood glucose levels were maintained between 120–140 mg dl^{-1} by infusing 20% glucose at various rates. ^{3}H-glucose was used to trace hepatic glucose production and glucose turnover.

Statistics. Student's two-tail t-test was performed for all experiments to determine significance of differences between two groups.