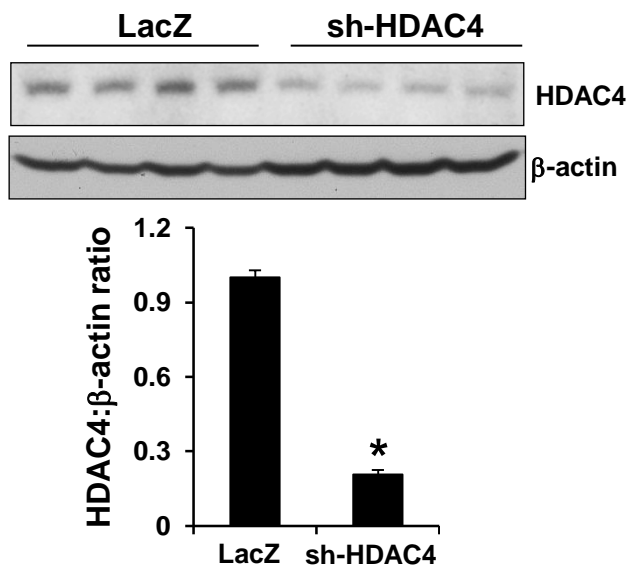
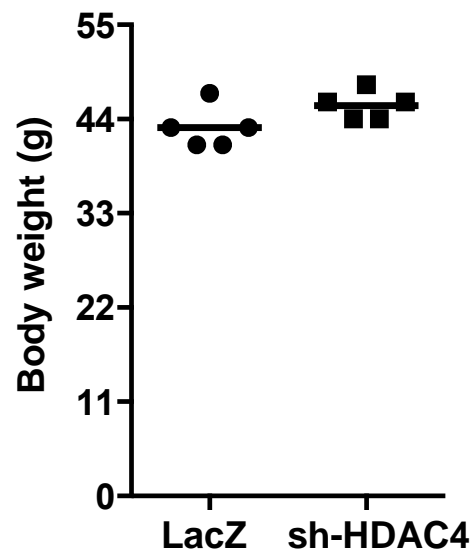
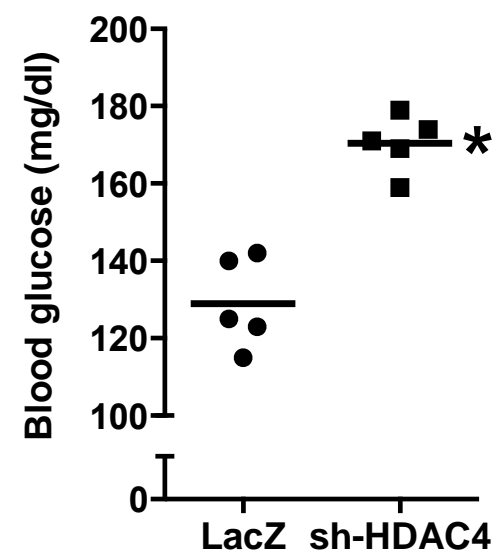
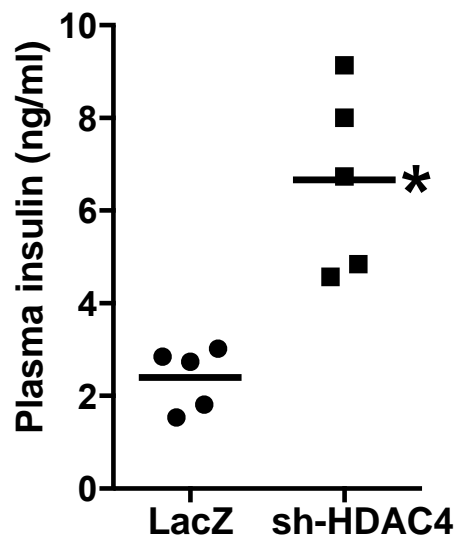
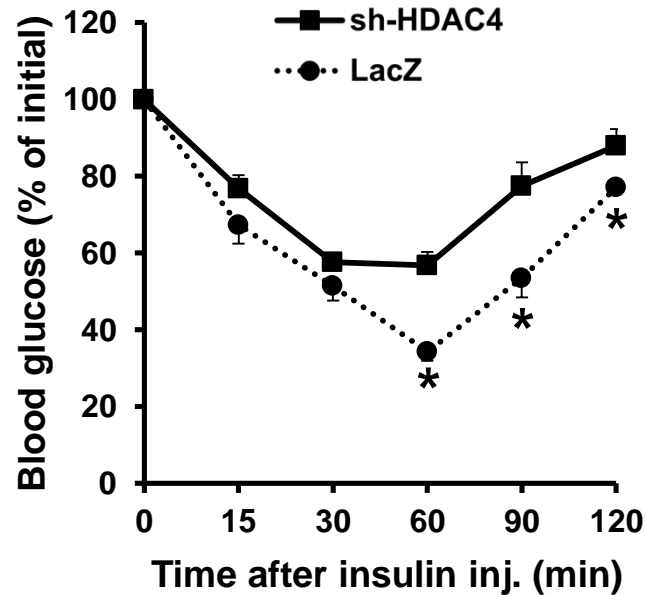
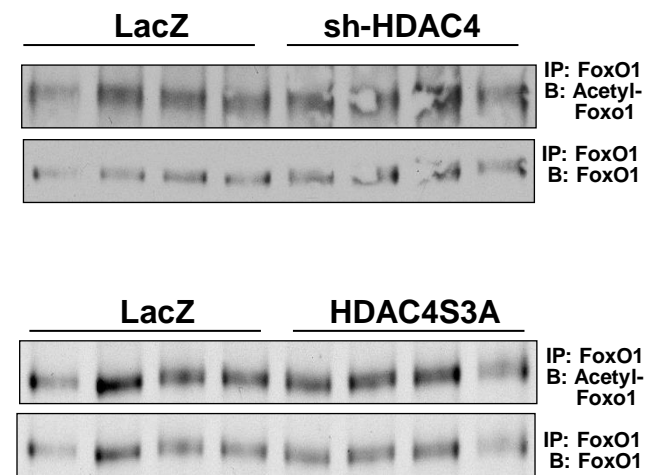
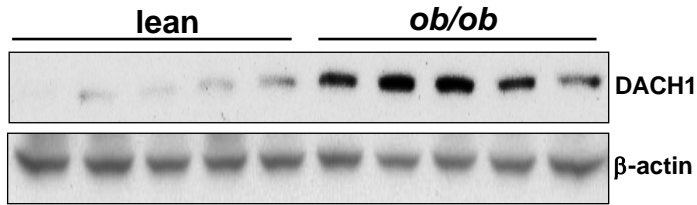
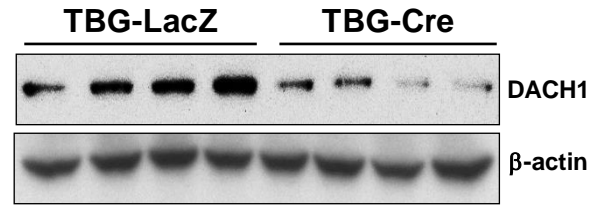


A (livers from DIO mice)**B** (DIO mice)**C** (DIO mice)**D** (DIO mice)**E** (DIO mice)**F** (livers from DIO mice)

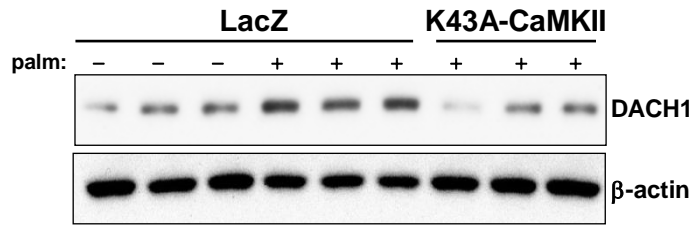
A (liver)

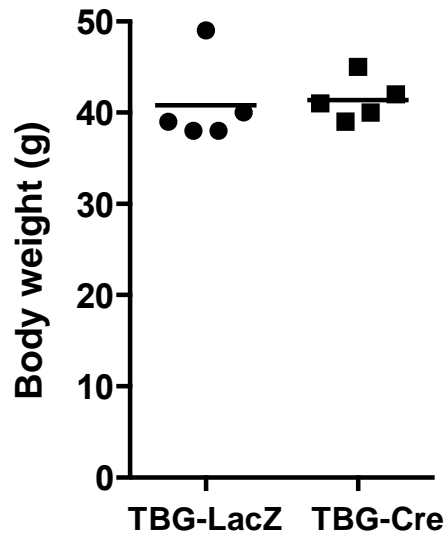
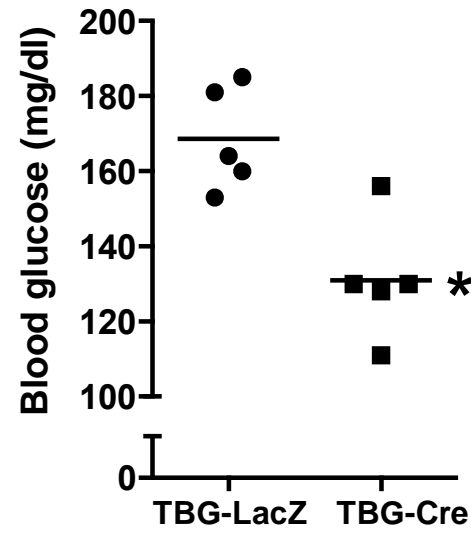
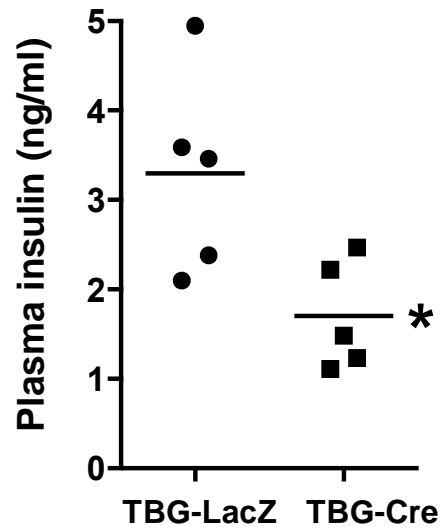
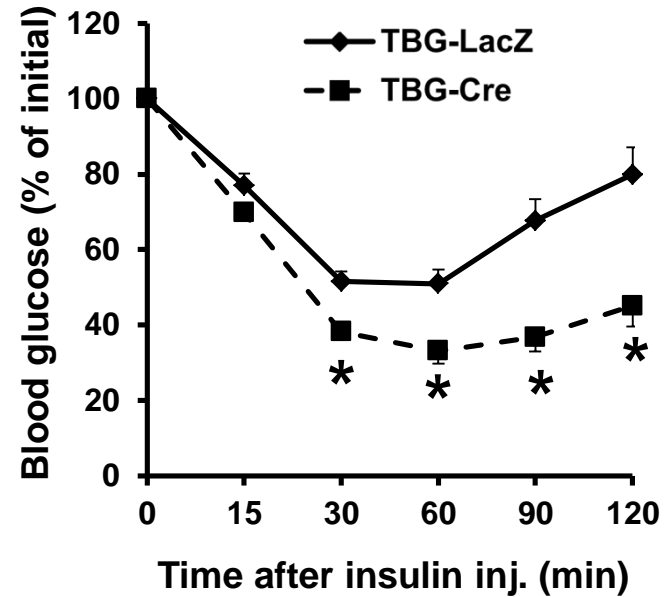


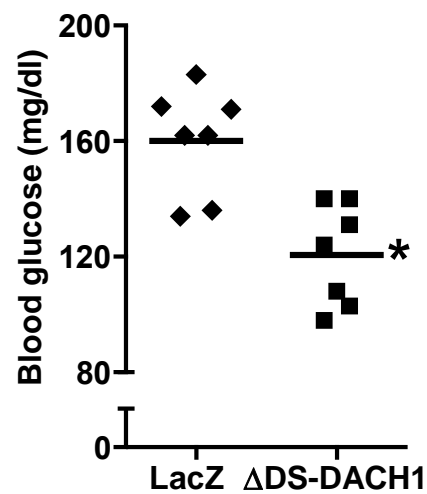
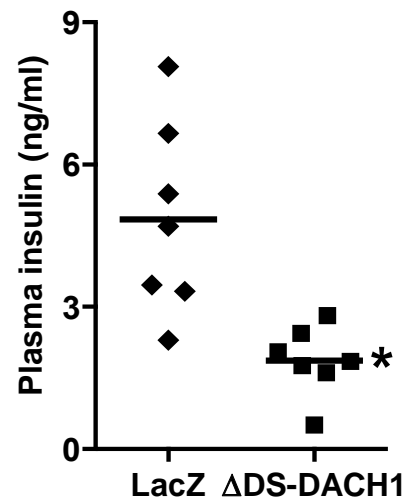
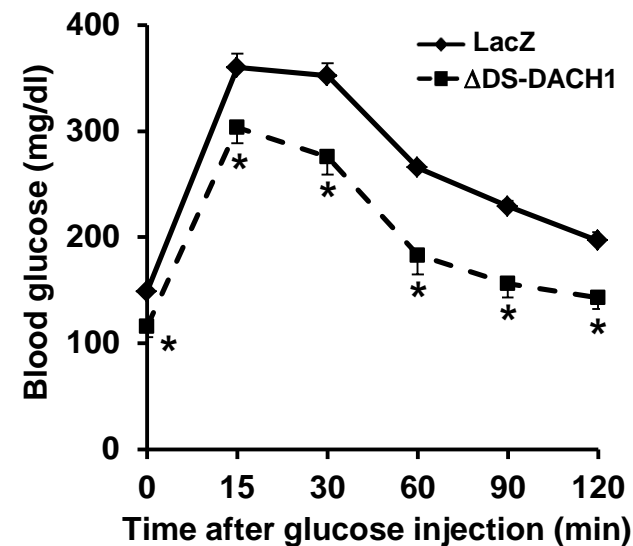
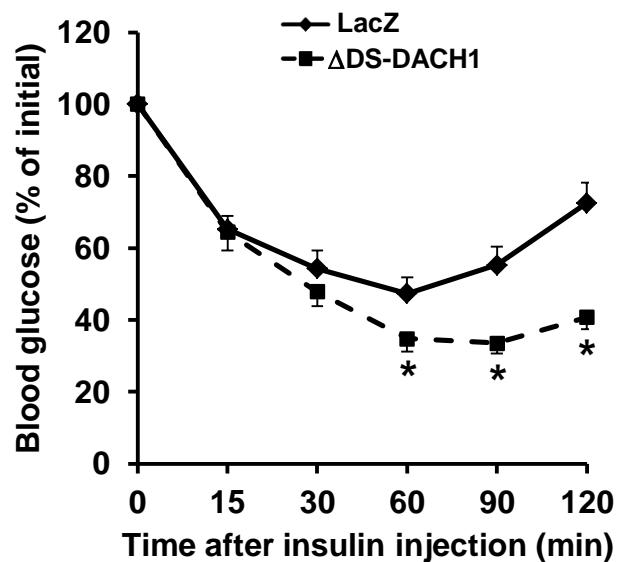
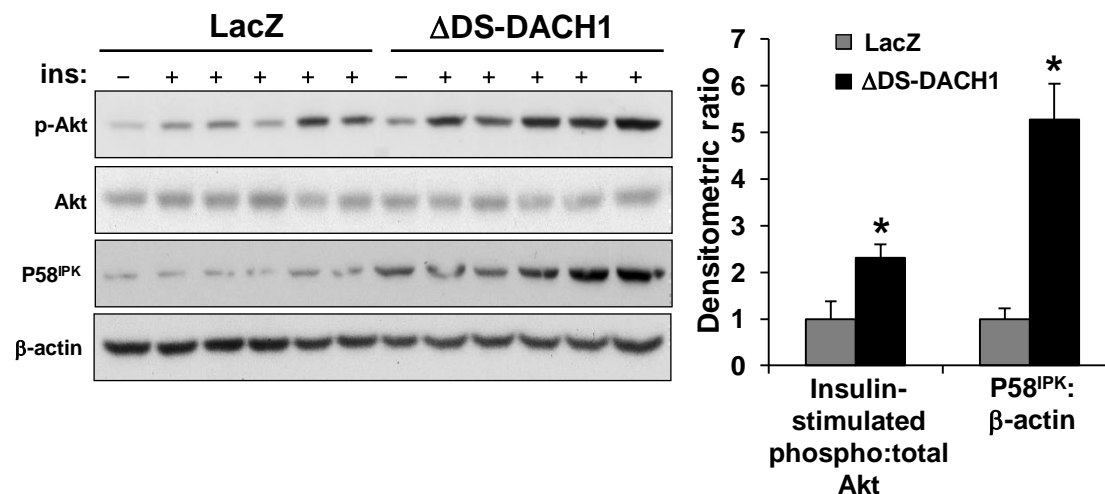
B (livers from DIO *Camk2g^{fl/fl}* mice)



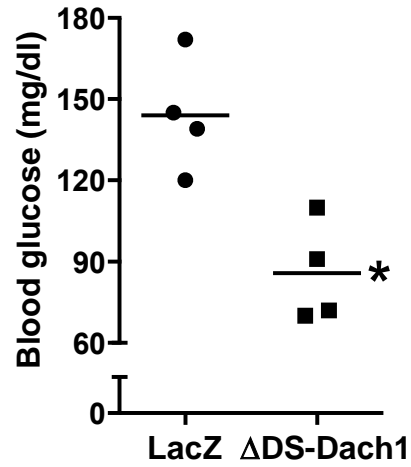
C (Human HCs)



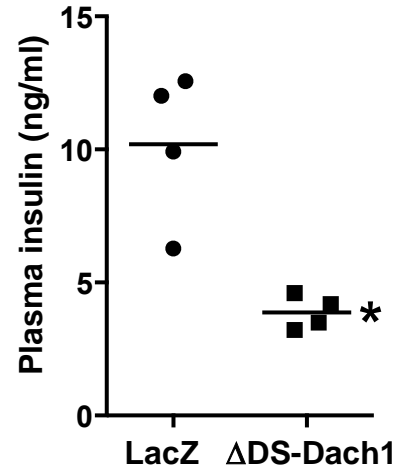
A (DIO *Dach1^{fl/fl}* mice)**B** (DIO *Dach1^{fl/fl}* mice)**C** (DIO *Dach1^{fl/fl}* mice)**D** (DIO *Dach1^{fl/fl}* mice)

A (DIO mice)**B** (DIO mice)**C** (DIO mice)**D** (DIO mice)**E** (livers from DIO mice)

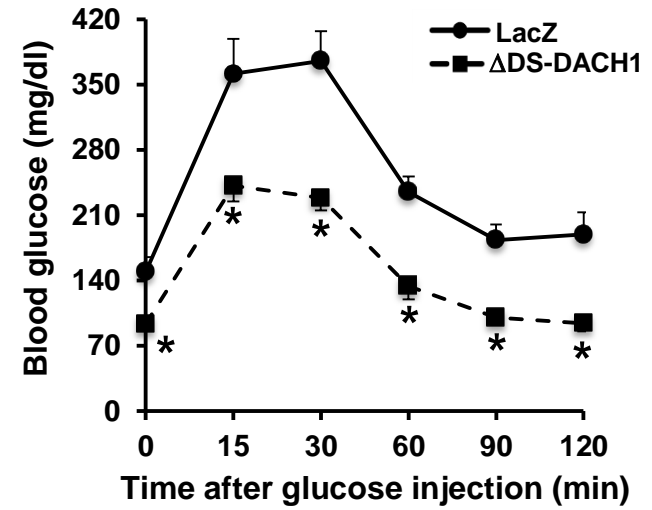
A (*ob/ob* mice)



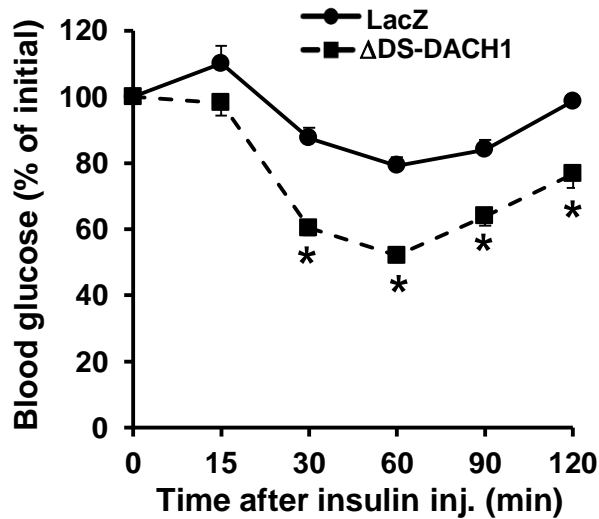
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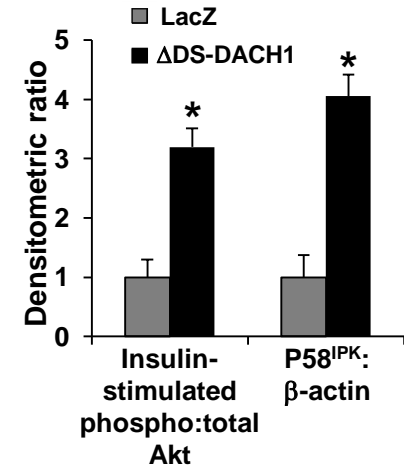
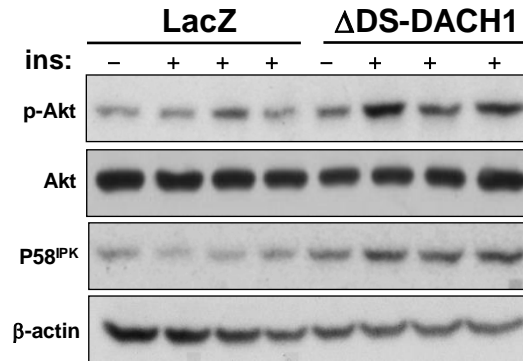
C (*ob/ob* mice)



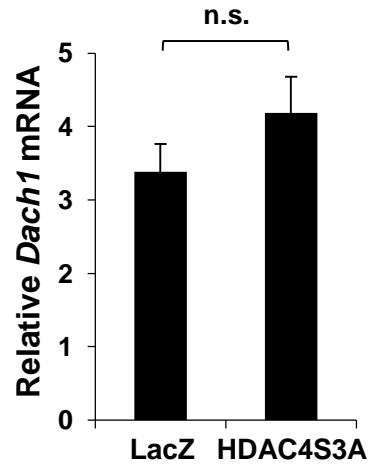
D (*ob/ob* mice)



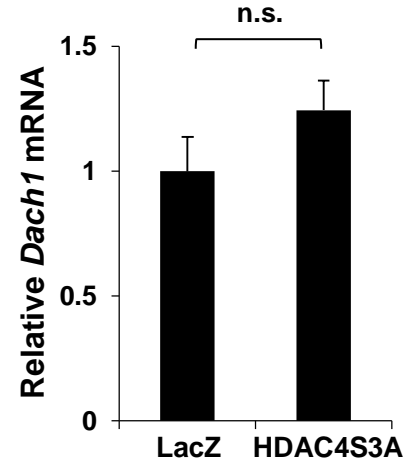
E (livers from *ob/ob* mice)



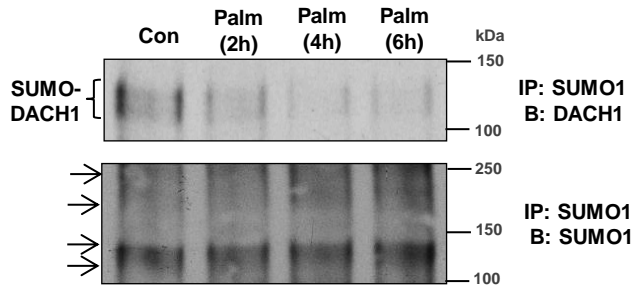
A (livers from DIO mice)



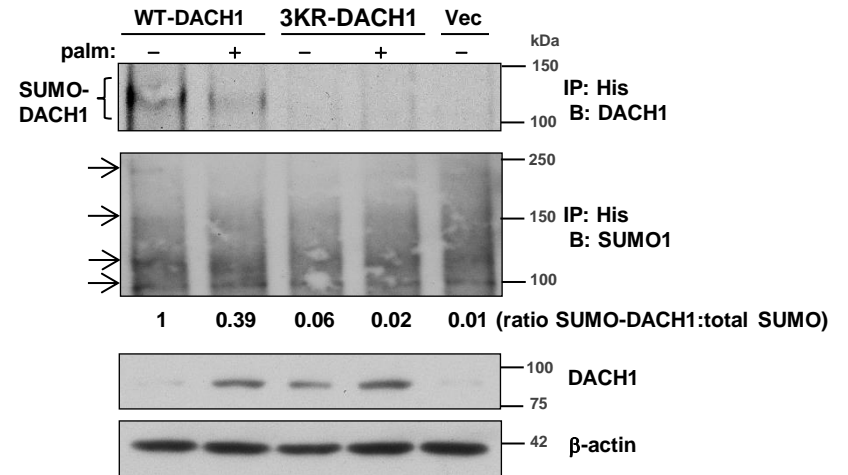
B (palmitate-treated HCs from WT mice)



C (HCs from AAV8-TBG-DACH1-treated mice)



D (HCs from AAV8-TBG-Cre-treated *Dach1*^{fl/fl} mice)



SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Related to Figure 1. ATF6N Overexpression Lowers Blood Glucose and Plasma Insulin Levels in Obese Mice, and *Atf6* mRNA is Induced by CaMKII Inhibition

(A-C) Twenty-week-old WT DIO mice were treated with adeno-LacZ or adeno-ATF6N. Fasting blood glucose and plasma insulin were assayed (A); an insulin tolerance test was conducted (B); and p-Akt and nuclear ATF6 levels were assayed in liver extracts 3 min after intraportal vein injection of insulin (ins) (C). Densitometric quantification of the phospho-Akt:total Akt ratio is shown in the graph (n = 5 mice/group, mean ± SEM, *p < 0.05).

(D) Hepatic *Atf6* mRNA levels were assayed in livers from DIO *Camk2g^{fl/fl}* mice treated with AAV-TBG-Cre or AAV-TBG-LacZ (n = 5 mice/group, mean ± SEM, *p < 0.05).

(E) Hepatocytes (HCs) from *Camk2g^{fl/fl}* mice were transduced with adeno-LacZ or adeno-Cre. Twenty-four hours later, the cells were incubated with BSA control (Con) or 0.3 mM palmitate, and then *Atf6* mRNA was assayed by RT-qPCR (n = 3, mean ± SEM; *p < 0.05; n.s., non-significant).

(F) Palmitate-treated HCs in (E) were subjected to ChIP analysis using anti-RNA polymerase II (Pol II) and anti-H3K27ac. The 5' promoter region of the *Atf6* gene was amplified by quantitative PCR and normalized to the values obtained from the input DNA (n = 3; mean ± SEM, *p < 0.05).

Figure S2: Related to Figure 2. Hepatic HDAC4 Silencing Impairs Glucose Homeostasis in Obese Mice

(A-E) Sixteen-week-old DIO mice were treated with adeno-LacZ or adeno-sh-HDAC4. The mice were then assayed for hepatic HDAC4 (A), body weight (B), 5-h fasting blood glucose (C), 5-h fasting plasma insulin (D), and response of blood glucose to insulin stimulation (E) (n = 5 mice/group, mean ± SEM, *p < 0.05). (F) Liver extracts from DIO mice treated with adeno-LacZ or adeno-sh-HDAC4 (upper blot) or DIO mice treated with adeno-LacZ or adeno-HDAC4S3A (lower blot) were immunoprecipitated for FoxO1 and then assayed by immunoblot for acetyl-FoxO1 or total FoxO1.

Figure S3: Related to Figure 3. Obesity Induces Hepatic DACH1 in a CaMKII-Dependent Manner

(A) Liver extracts from 10-wk-old WT or *ob/ob* mice were assayed by immunoblot for DACH1 and β-actin.

(B) DACH1 and β-actin were assayed in liver extracts from DIO *Camk2g^{fl/fl}* mice treated with AAV-TBG-LacZ or AAV-TBG-Cre.

(C) Primary human HCs were transduced with adeno-LacZ or adeno-K43A-CaMKII and then 36 h later incubated with either BSA control or 0.2 mM palmitate (palm) as indicated by the minus and plus symbols. After 10 h, cell lysates were assayed for DACH1 and β-actin.

Figure S4: Related to Figure 4. Hepatic DACH1 Silencing Lowers Blood Glucose and Insulin and Improves Response to Insulin Challenge in Obese Mice

(A-D) Body weight, fasting blood glucose, fasting plasma insulin, and insulin tolerance test in DIO *Dach1^{fl/fl}* mice treated with AAV-TBG-LacZ or AAV-TBG-Cre (n = 5 mice/group; mean ± SEM, *p < 0.05).

Figure S5: Related to Figure 4. Treatment of DIO Mice with Adeno-ΔDS-DACH1 Improves Glucose Homeostasis

(A-E) Eighteen-week-old WT DIO mice were treated with adeno-LacZ or adeno-ΔDS-DACH1. 5-h fasting blood glucose and 5-h fasting plasma insulin levels were assayed (A-B), glucose tolerance and insulin tolerance tests were conducted (C-D). The mice were fasted for 5 h and injected with insulin through the portal vein. After 3 mins, liver was harvested and liver extracts were assayed for p-Akt, total Akt, P58^{IPK}, and β-actin by immunoblot (E). Densitometric quantification of the immunoblot data is shown in the graph (n = 7 mice/group, mean ± SEM, *p < 0.05).

Figure S6: Related to Figure 4. Treatment of *ob/ob* Mice with Adeno-ΔDS-DACH1 Improves Glucose Homeostasis

(A-E) Ten-week-old *ob/ob* mice were treated with adeno-LacZ or adeno-ΔDS-DACH1 and assayed for the same endpoints as in Figure S5 (n = 4 mice/group; *p < 0.05; mean ± SEM).

Figure S7: Related to Figure 7. HDAC4 Regulates DACH1 Through SUMOylation

(A) *Dach1* mRNA was assayed in the liver extracts from DIO mice treated with adeno-LacZ or adeno-HDAC4S3A (n = 6 mice/group; n.s., non-significant).

(B) Primary HCs from WT mice were transduced with adeno-LacZ or adeno-HDAC4S3A. After 24 h, the cells were incubated with 0.3 mM palmitate for 5 h, and then *Dach1* mRNA was assayed by RT-qPCR (n = 3, n.s., non-significant).

(C) Primary hepatocytes (HCs) from WT mice treated with AAV8-TBG-DACH1 were transfected with HA-tagged SUMO1 and treated with BSA (Con) or palmitate (palm) as indicated. Lysates were immunoprecipitated using anti-SUMO1 and blotted for DACH1 or SUMO1. Arrows indicate SUMOylated proteins.

(D) HCs from *Dach1*^{fl/fl} mice treated with AAV-TBG-Cre were co-transduced with His-tagged SUMO1 and either WT-DACH1, 3KR-mutant DACH1 (3KR-DACH1), or vector (Vec) control. After 48 h, the cells were incubated with BSA control or 0.3 mM palmitate for 5 h as indicated by the minus and plus symbols. Cell lysates were then immunoprecipitated for His and blotted for DACH1 or SUMO1.

Supplemental Experimental Procedures

Reagents and Antibodies

Sodium palmitate, insulin, and MG132 were from Sigma. Anti- β -actin, anti-P58, anti-DACH1, anti-NCOR, anti-Pol II, anti-H3K4me1, antiH3K27Ac, anti-SUMO1 and anti-HA antibodies were from Abcam. Anti-phospho-S473-Akt, anti-Akt, anti-nucleophosmin (Np), anti-phospho-S632-HDAC4, anti-HDAC4 and anti-His antibodies were from Cell Signaling. Anti-phospho CaMKII and anti-ATF6 antibodies were from Novus. Anti-rabbit IgG, anti-FoxO1 and anti-Acetyl-FoxO1 antibodies were from Santa Cruz. Anti-phospho-S467-HDAC4 antibody was a gift from Dr. Tso-Pang Yao (Duke University). The expression plasmids for GFP-DACH1 and GFP-DACH1-DS-domain deleted (Δ -DS) were described previously (Wu et al., 2007). Adenoviruses encoding LacZ and K43A-CaMKII were gifts from Dr. Harold A. Singer (Albany Medical College), adeno-sh-ATF6 and -ATF6-N were gifts from Dr. Marc Montminy (Salk Institute for Biological Studies), and adeno-HDAC4S3A was a gift from Dr. Eric Olson (UT Southwestern). Viruses were amplified by Viraquest, Inc. (North Liberty, IA). Adeno-sh-HDAC4 was a gift from Dr. Reuben Shaw (Salk Institute for Biological Studies) and was amplified by Salk Institute Gene Transfer, Targeting, and Therapeutics Core. Adeno-associated virus subtype 8 (AAV8) containing either hepatocyte-specific TBG-Cre recombinase (AAV-TBG-Cre), the control vector (AAV-TBG-LacZ), or hepatocyte-specific TBG-DACH1 were purchased from the Penn Vector Core. AAV8-shRNA constructs targeting murine DACH1 and NCOR were made by annealing complementary oligonucleotides and then ligating them into the pAAV-RSV-GFP-H1 vector, as described previously (Lisowski et al., 2014). The resultant constructs were amplified by Salk Institute Gene Transfer, Targeting, and Therapeutics Core. siRNA sequences against murine *Hdac4*, *Ubc9*, and *Atf6* were purchased from Qiagen. His-tagged and HA-tagged SUMO-1 plasmid was purchased from Addgene.

Mouse Experiments

Recombinant adenovirus ($1.5\text{-}3 \times 10^9$ plaque-forming unit/mouse) or adeno-associated virus ($1\text{-}2 \times 10^{11}$ genome copy/mouse) was delivered by tail vein injection, and experiments were commenced after 4-12 days. Fasting blood glucose was measured using a glucose meter (One Touch Ultra, Life- scan) in mice that were fasted for 4-5 h, with free access to water. Glucose tolerance tests were performed by intraperitoneal glucose injection (0.5 g/kg for *ob/ob* and 1-1.5 g/kg for DIO) following an overnight fast. Insulin tolerance tests were performed by intraperitoneal insulin injection (0.75-1 IU/kg for DIO and 2 IU/kg for *ob/ob*) following 5-h daytime food withdrawal. Pyruvate tolerance tests were carried out with an i.p. injection of pyruvate (2 g/kg body weight) after 17 h of fasting, followed by measurement of blood glucose levels over the next 2 h. Plasma insulin levels were measured using an ultra-sensitive mouse insulin ELISA Kit (Crystal Chem). Animal studies were conducted in accordance with the Columbia University Animal Research Committee.

Primary HCs

HCs were transfected with siRNA or plasmids or transduced with viral constructs 10-12 h after plating, and experiments were conducted 12 h later. Transfections with scrambled, si-HDAC4, si-Ubc9, and si-ATF6 constructs were carried out using HiPerFect transfection reagent (Qiagen) according to manufacturer's instructions. Transfections with SUMO-1, WT-DACH1 or 3KR-mutant DACH1 were carried out using Lipofectamine LTX Reagent with PLUS Reagent (ThermoFisher Scientific) according to manufacturer's instructions. Metabolism-qualified human hepatocytes were purchased from Life Technologies and cultured according to the manufacturer's instructions.

Human Samples

Human liver samples were obtained from patients undergoing bariatric surgery or clinically indicated laparoscopic

procedures at the New York Presbyterian Hospital, Columbia University Medical Center (New York, NY). Samples were obtained from intra-operative needle biopsies of the liver at a standard anatomic location. The biopsy specimens were frozen immediately in liquid nitrogen and stored at -80°C until subsequent analyses. The Institutional Review Board at the Columbia University Medical Center approved the research protocol, and all participants provided written informed consent.

Portal Vein Insulin Infusion and Protein Extraction from Tissues

Following 4-5 h of food withdrawal, mice were anesthetized, and then insulin (1 IU/kg/ body weight for DIO and 2 IU/kg body weight for *ob/ob*) or PBS was injected through the portal vein. Three minutes after injection, the liver was removed, frozen in liquid nitrogen, and kept at -80°C until processing. For protein extraction, liver segments were placed in a cold lysis buffer (25 mM Tris-HCl (pH 7.4), 1 mM EGTA, 1 mM EDTA, 10 mM $\text{Na}_4\text{P}_2\text{O}_7$, 10 mM NaF, 2 mM Na_3VO_4 , 1% NP-40, 2 mM PMSF, 5 $\mu\text{g}/\text{ml}$ leupeptin, 10 nM okadaic acid and 5 $\mu\text{g}/\text{ml}$ aprotinin) and then homogenized on ice. The tissue lysates were centrifuged, and the supernatant fractions were used for immunoblot analysis.

Immunoblotting and RT-qPCR

Immunoblot and RT-qPCR assays were performed as previously described (Ozcan et al., 2012). Total RNA was extracted from HCs using the RNeasy kit (Qiagen), and cDNA was synthesized from 1 μg total RNA using oligo (dT) and Superscript II (Invitrogen). Nuclear extraction from liver was performed using the Nuclear Extraction Kit from Panomics according to the manufacturer's instructions.

Immunoprecipitation

Cell or tissue lysates (~1 mg total protein) were brought to a total volume of 1 ml in microfuge tubes with cold lysis buffer. Antibodies (0.5-1 μg) and protein A Sepharose or magnetic beads (50 μl) were added to each tube, and then the tubes were gently rotated at 4°C overnight. Immune complexes were collected by centrifugation at 16,000 \times g and washed 3 times with chilled lysis buffer.

Chromatin Immunoprecipitation Assay in HCs

Chromatin immunoprecipitation analysis from primary HCs was performed following a protocol provided by EMD Millipore. Briefly, HCs were cultured and treated as indicated in the figure legends, and then the cells were cross-linked by directly adding formaldehyde buffer for 10 min at 37°C . The fixative was removed, and the cells were lysed and sonicated to shear DNA to lengths between 200 and 1,000 base pairs. Sonicated DNA was mixed in microfuge tubes with protein A magnetic beads and the indicated antibodies, and the tubes were then gently rotated at 4°C overnight. The beads were collected by centrifugation and washed three times, and then chromatin was eluted using 1% SDS. Crosslinking was reversed by shaking for 2 h at 62°C , and the DNA was purified. The regions of interest in the *Atf6* gene were amplified by real-time PCR using appropriate primer sets.

Mouse Liver Nuclei Preparation and ChIP Assays

Mouse liver tissues were homogenized using a Dounce homogenizer (Wheaton) with loose pestle in 1:10 (w:v) of ice-cold lysis buffer supplemented with protease inhibitor cocktail. The release of nuclei was monitored by DAPI staining and fluorescence microscopy. To purify intact nuclei, lysates were layered over 1M (bottom) and 0.68M (top) sucrose and centrifuged at 4000 rpm for 30 min at 4°C . Following a washing step, nuclear pellets were cross-linked with 1% fresh formaldehyde in PBS for 10 min at room temperature. Cross-linking was terminated by the addition of 200 mM Tris-HCl (pH 9.4) and 1mM DTT; after 10 min, the suspensions were centrifuged at 2500 rpm for 15 min at 4°C . Nuclear pellets were suspended in SDS lysis buffer containing protease inhibitors, incubated for 10 min on ice. DNA was sheared in a cold water bath in a focused-ultrasonicator (Covaris, S2) to obtain DNA fragments with an average size of 500 bps. Fragmented chromatin was pre-cleaned by incubating with normal rabbit IgG for 1 hr at 4°C , followed by 1 hr of incubation with 50 μL protein G magnetic beads (Pierce) at 4°C with rotation. A rabbit anti-DACH1 antibody was used to pull down DACH1-binding complexes, and a control rabbit anti-HA antibody was used to pull down non-specific binding complexes. Immunoprecipitated chromatin fragments were reverse cross-linked, digested by proteinase K, and purified using QIAquick PCR Purification Kit (Qiagen). The presence of DACH1 in *Atf6* intronic and exonic region was quantified by qPCR and expressed relative to the input genomic DNA.

SUMOylation assays

For protein extraction from primary HCs transfected with His- or HA-tagged SUMO-1, cells were washed with ice-

cold PBS containing 20 mM N-ethylmaleimide (freshly added to preserve SUMO) and lysed with lysis buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM NaF, 0.4 mM Na₃VO₄, and 1% Triton X100 with freshly added 2 mM PMSF, 5 µg/ml leupeptin, 10 nM okadaic acid, 5 µg/ml aprotinin and 20 mM N-ethylmaleimide. Cell lysates (~1 mg total protein) were brought to a total volume of 1 ml with lysis buffer. Anti-His (0.5-1 µg), anti-HA or anti-SUMO1 and magnetic beads (50 µl) were added to the tube, which was then rotated at 4°C overnight. Immune complexes were collected by centrifugation at 16,000 x g and washed 3 times with the same lysis buffer as above. The beads were boiled in Laemmli buffer, and the extract was subjected to SDS-PAGE and immunoblot analysis using anti-DACH1 or anti-SUMO1.

Supplemental References

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- Wu, K., Liu, M., Li, A., Donniger, H., Rao, M., Jiao, X., Lisanti, M.P., Cvekl, A., Birrer, M., and Pestell, R.G. (2007). Cell fate determination factor DACH1 inhibits c-Jun-induced contact-independent growth. *Mol. Biol. Cell* 18, 755-767.