

Supplemental Data

Supplementary Figure legend

Sup Figure 1. Hepatic SIK2 silencing impairs liver energy homeostasis.

Mice were injected with either unspecific shRNA (USi) or SIK2 shRNA adenovirus (SIK2i) and were studied 7 days later in the fed state.

(A) Liver glycogen content and plasma concentration of β hydroxybutyrate (n=8 per group - data represent mean \pm SEM, *p<0.05).

(B) (C) SIK2i treated mice display increased circulating levels of proinflammatory cytokines and transaminases. Plasma from USi and SIK2i mice were analyzed for TNF α , IL6 and resistin by multiplex assay and for ALAT (alanine aminotransferase) and ASAT (aspartate aminotransferase) by ELISA (n=8 per group - data represent mean \pm SEM, *p<0.01).

(D) Relative expression of genes encoding key proteins in hepatic glycolysis, lipogenesis, fatty acid β oxidation pathways and macrophage markers measured by quantitative PCR (n=8 per group - data represent mean \pm SEM, *p<0.01).

Sup Figure 2. SIK2 promotes p300 Ser-89 phosphorylation and inhibits p300 activity.

(A) Inhibition of p300 HAT activity via phosphorylation at Ser-89 by SIK2. HAT assays for either wt or S89A p300 after phosphorylation by SIK2 in the presence of GST-CRTC2 (substrate). Data are the average of three independent experiments.

(B) Regulation of p300, CBP, PCAF and GCN5 HAT activities by SIK2. Either p300, CBP, PCAF or GCN5 were overexpressed in HepG2 cells with or without SIK2 expression vector. These HAT proteins were then immunoprecipitated and used for HAT assays on core histones proteins. Data are the average of three independent experiments (mean \pm SEM, *p<0.05).

Sup Figure. 3 p300 promotes fatty acid synthesis through the regulation of ChREBP activity by acetylation.

Effects of p300 overexpression or p300 silencing on glucose and lipid metabolism were studied in cultured hepatocytes incubated in the presence of insulin (100 nM) and 5 or 25 mM glucose for 18h.

(A, B, and C) H4K8, H3K9 and RNA pol II recruitment to the promoter of LPK was measured by ChIP studies. Immunoprecipitated LPK promoter sequence was analyzed by

quantitative PCR. The amount of immunoprecipitated H3 and H4 at the DNA was unchanged upon p300 overexpression or silencing and was used as control to normalize H3K9 and H4K8 acetylation levels to the LPK promoter. Data are the average of three independent experiments (mean \pm SEM, * $p < 0.05$).

(D) Levels of acetylated ChREBP at the ChoRE-containing region of the LPK promoter determined in a seq-ChIP experiments. ChREBP was immunoprecipitated with ChREBP antibody followed by a second immunoprecipitation with acetylated lysine antibody. Data are the mean \pm SEM of three independent experiments (* $p < 0.05$).

(E, F) ChREBP, p300 and RNA pol II become co-resident on the LPK promoter in a glucose-dependent manner. Seq-ChIP analysis showing co-occupancy of ChREBP/p300 and p300/RNA pol II at the ChoRE-containing region of the LPK promoter. Data are the mean \pm SEM of three independent experiments (* $p < 0.05$).

(G) Co-immunoprecipitation assay in HepG2 cells using epitope-tagged p300 and ChREBP proteins. Amount of p300 recovered from IPs of ChREBP and levels of acetylated ChREBP are shown. Data are representative of three independent experiments.

(H) Quantification of ChREBP acetylation levels presented in Fig. 3D.

Sup Figure. 4 ChREBP promotes p300 recruitment to the LPK promoter in a glucose dependent manner.

Effects of ChREBP silencing on glucose and lipid metabolism were studied in cultured hepatocytes incubated in the presence of 100 nM insulin and 25 mM glucose for 18h.

(A) Levels of ChREBP protein content are shown. Data are representative of three independent experiments.

(B) ChoRE-luciferase (ChoRE-luc) reporter activity and LPK and FAS gene expression measured by quantitative PCR. Data are the average of three independent experiments (mean \pm SEM, * $p < 0.05$).

(C) ChREBP and p300 recruitment to the ChoRE containing region of the LPK promoter was measured by ChIP studies. Immunoprecipitated LPK promoter sequence was analyzed by quantitative PCR. Data are the average of three independent experiments (mean \pm SEM, * $p < 0.05$).

(D) H4K8, H3K9 and RNA pol II recruitment to the LPK promoter was measured by ChIP studies. Immunoprecipitated LPK promoter sequence was analyzed by quantitative PCR. The amount of immunoprecipitated H3 and H4 at the DNA was unchanged upon p300

overexpression or silencing and was used as control to normalize H3K9 and H4K8 acetylation levels at the LPK promoter. Data are the average of three independent experiments (mean \pm SEM, * $p < 0.05$).

(E) Relative effect of either wt-p300 or Δ HAT-p300 on ChoRE-luciferase (ChoRE-luc) reporter activity and ChREBP acetylation levels. Data are the average of three independent experiments (mean \pm SEM, * $p < 0.05$).

Sup Figure. 5 ChREBP is a specific substrate of p300.

(A) (top panel) Relative effect of either p300, CBP, GCN5 and PCAF on ChREBP acetylation levels in HepG2 cells incubated in the presence of 100 nM insulin with 25 mM glucose. Data are representative of three independent experiments. (bottom panel) HepG2 cells overexpressing either epitope-tagged p300, CBP, GCN5 or PCAF with ChREBP were incubated 1h in the presence of ^3H -acetate. FLAG-ChREBP was immunoprecipitated. Autoradiogram of ^3H -acetylated-FLAG-ChREBP is shown. Data are representative of three independent experiments.

(B) p300, CBP, GCN5 and PCAF recruitment to the promoter of LPK was measured by ChIP studies. Immunoprecipitated LPK promoter sequence was analyzed by quantitative PCR. Data are the average of three independent experiments (mean \pm SEM, * $p < 0.05$).

(C) Effects of p300, CBP, GCN5 and PCAF on ChREBP occupancy to the ChoRE containing region of the LPK promoter in HepG2 cells incubated in the presence of 100 nM insulin with 25 mM glucose. Data are the average of three independent experiments (mean \pm SEM, * $p < 0.05$).

(D) (E) Relative effect of either p300, CBP, GCN5 and PCAF on Gal4-ChREBP and on ChoRE-luc reporter activity in HepG2 cells incubated in the presence of 100 nM insulin with 25 mM glucose. Data are the average of three independent experiments (mean \pm SEM, * $p < 0.05$).

Sup Figure. 6 Identification of ChREBP acetylated lysines.

(A) From all the MS/MS analyses performed, 46% total coverage was achieved for ChREBP. Peptides generated using trypsin and recovered during the MS/MS analyses are shaded green.

(B) Quantification of ChREBP acetylation levels presented in Fig. 4B.

(C) Functional activity of ChREBP was assessed using a competition assay, in HepG2 cells overexpressing p300. HepG2 cells were transfected with constant quantity of wt-ChREBP

(25ng) and with various amounts of plasmids (25ng, 50ng and 75ng) expressing either DN or K672R ChREBP mutants. Data are the average of three independent experiments (mean \pm SEM, * p <0.01).

(D) Quantification of ChREBP acetylation levels presented in Fig. 4F.

Sup Figure. 7 p300 regulates lipogenesis in primary cultured hepatocytes

Cultured hepatocytes were stimulated with TAT-lys-CoA or curcumin (10 μ M final concentration) in the presence of 5 or 25 mM glucose with or without 100 nM insulin for 18h. For all experiments presented in sup Fig. 7, data are the average of three independent experiments (mean \pm SEM, * p <0.01).

(A) (E) Effect of TAT-lys-CoA or curcumin on ChREBP acetylation levels.

(B) (F) Relative effect of TAT-lys-CoA or curcumin on glucose-induced LPK and FAS expression measured by quantitative PCR.

(C) (G) Relative effect of TAT-lys-CoA or curcumin on glucose induced ChoRE-luc reporter activity.

(D) (H) Triglyceride content in hepatocytes incubated with TAT-lys-CoA or curcumin.

Sup Figure. 8 SIK2 regulates ChREBP transactivation activity through p300 S89 phosphorylation

Effects of SIK2 and p300 silencing on glucose and lipid metabolism were studied in hepatocytes incubated in the presence of insulin (100 nM) and 5 or 25 mM glucose for 18h.

(A) p300 recruitment to the ChoRE containing region of the LPK promoter. Data are the average of three independent experiments (mean \pm SEM, * p <0.05).

(B) Quantification of ChREBP acetylation levels presented in Fig. 5A.

(C) H3K9 and H4K8 acetylation levels on the LPK promoter measured by ChIP studies. Immunoprecipitated LPK promoter sequence was analyzed by quantitative PCR. Data are the average of three independent experiments (mean \pm SEM, * p <0.05).

(D) ACC gene expression measured by quantitative PCR. Data are the average of three independent experiments (mean \pm SEM, * p <0.05).

(E) Effect of SIK2 and p300 silencing on lipid metabolism. Oil red O staining of neutral lipids was performed in hepatocytes. Data are representative of three independent experiments.

Sup Figure. 9 Down regulation of SIK2 activity in liver of two mouse models of obesity and type 2 diabetes

(A) Relative SIK2 kinase activity from liver of ob/ob and HFD mice under fed state. GST-CRTC2 was used as a SIK2 substrate for an in vitro kinase assay after immunoprecipitation of SIK2 from livers of both fed ob/ob and HFD mice. SIK2 kinase activity was normalized for total SIK2 protein content measured by western blot as described below (sup fig. 9C and D). (n=3 per group - data represent mean \pm SEM, *p<0.01).

(B) Plasma insulin and glucagon levels of fed ob/ob and HFD mice. (n=5 per group - data represent mean \pm SEM, *p<0.01).

(C) (left panel) Western blot showing levels of SIK2 protein content, amounts of Ser-89 phosphorylated p300, acetylated ChREBP and levels of phosphorylated Akt on threonine 308 in liver of fed ob/ob mice (n=4 per group). (right panel) Relative expression of genes encoding key proteins in hepatic glycolysis and lipogenesis measured by quantitative PCR in liver of fed ob/ob mice (n=6 per group - data represent mean \pm SEM, *p<0.01).

(D) (left panel) Western blot showing levels of SIK2 protein content, amounts of Ser-89 phosphorylated p300, acetylated ChREBP and levels of phosphorylated Akt on threonine 308 in liver of fed HFD mice (n=4 per group). (right panel) Relative expression of genes encoding key proteins in hepatic glycolysis and lipogenesis measured by quantitative PCR in liver of fed HFD mice (n=6 per group - data represent mean \pm SEM, *p<0.01).

(E) Relative PKA activity in liver of both fed ob/ob and HFD mice (n=4 per group - data represent mean \pm SEM, *p<0.01).

(F) Relative p300 HAT activity in liver of both fed ob/ob and HFD mice (n=4 per group - data represent mean \pm SEM, *p<0.01).

Sup Figure. 10 Liver p300 overexpression impairs lipid homeostasis and leads to hepatic steatosis

Mice were injected with either p300 and/or SIK2 overexpressing adenovirus and studied 7 days later in fed state.

(A) Plasma and liver concentrations of free fatty acids (NEFA) (n=6 per group - data represent mean \pm SEM, *p<0.01).

(B) (left panel) p300 and RNA pol II recruitment to the ChoRE containing region of the promoter of LPK was measured by ChIP studies. Immunoprecipitated LPK promoter sequence was analyzed by quantitative PCR. Data are the average of three independent

experiments (mean \pm SEM, * $p < 0.05$). (right panel) Tubulin acetylation levels in liver of p300 and p300/SIK2 overexpressing mice (n=3 per group).

(C) Relative expression of genes encoding for ChREBP, SREBP-1c, ACC and ACL measured by quantitative PCR (n=6 per group – data represent mean \pm , * $p < 0.01$).

(D) (E) p300 overexpressing mice display increased circulating levels of proinflammatory cytokines and transaminases. Plasma from fed p300 and p300/SIK2 overexpressing mice were analyzed for TNF α , IL6 and resistin by multiplex assay and for ALAT (alanine aminotransferase) and ASAT (aspartate aminotransferase) by ELISA (n=6 per group - data represent mean \pm SEM, * $p < 0.01$).

(F) Liver glycogen content from fed p300 and p300/SIK2 overexpressing mice (n=6 per group - data represent mean \pm SEM, * $p < 0.01$).

(G) Effect of overnight fasting and 18h of refeeding on ChREBP acetylation levels in liver of ob/+ and ob/ob mice. Amount of total and acetylated ChREBP are shown. (n=3 per group).

Supplemental Experimental Procedures

Mouse Strains and Adenoviruses.

5 X 10⁸ plaque forming units (pfu) of SIK2 shRNA (SIK2i), unspecific shRNA (USi), GFP, p300 or SIK2 adenovirus were delivered to 8 weeks old male C57BL/6J, or BKS.Cg-m +/+ ob/ob mice by tail vein injection as previously described (1). These mice were purchased from Elevage Janvier (France). All mice were adapted to their environment for 1 week before study and were housed in colony cages with 12h light/dark cycle in a temperature-controlled environment. All procedures were carried out according to the French guidelines for the care and use of experimental animals. All animal studies were approved by the “Direction départementale des services vétérinaires de Paris”. Mice had free access to water and regular diet (65% carbohydrate, 11% fat, 24% protein) unless otherwise specified. For high fat diet studies, mice were maintained on 60% fat/20% carbohydrate/20% protein (Research Diets D12492) for 4 months. Wild-type p300, mutant S89A p300, Ad-SIK2, Ad-SIK2i and Ad-p300i adenoviruses have been described previously (2). Mice injected (i.p.) with 50 mg kg⁻¹ Nembutal (Abbott Laboratories), were studied on day 7–10 after adenovirus delivery in fed state.

Analytical procedures.

Blood glucose values were determined using an ACCU-Check glucose monitor (Roche Diagnostic Inc.). Serum triglycerides (TG), free fatty acids (NEFA), β -hydroxybutyrate, alanine aminotransferase (Alat) and aspartate aminotransferase (Asat) concentrations were determined using an automated Monarch device (Laboratoire de Biochimie, Faculté de Médecine Bichat, France). Glycogen concentrations were determined in liver extracts as previously described (3). Hepatic TG and NEFA were extracted with acetone, and were quantified with a colorimetric diagnostic kit according to the manufacturer's instructions (Triglycerides FS; Diasys). Plasma insulin, glucagon, TNF α , IL6 and resistin concentrations were determined using a bead-based multiplex immunoassays using adipokines and cytokine assay kit (millipore Inc.) in conjunction with flow-based protein detection and the Luminex LabMAP multiplex system according to the manufacturer's instructions (Plate-forme phénotypage du petit animal et microdosages. Faculté de médecine Saint-Antoine, France).

Glucose and insulin tolerance tests.

Glucose tolerance tests were performed by oral administration of glucose (1g D-glucose/kg body weight) after overnight fasting. Insulin tolerance tests were performed by intraperitoneal injection of human regular insulin (0.5 unit insulin/kg body wt, Actrapid Penfill, NovoNordisk) 6h after food removal.

Liver lysates.

Livers were frozen in liquid nitrogen and kept at -80°C until use. Mouse tissues were sonicated 3 times for 10 sec each at 4°C in lysis buffer [150mM NaCl, 50mM Tris-HCl pH 7.5, 5mM EDTA, 30mM Sodium pyrophosphate, 30mM Sodium Fluoride, 1% Triton X 100, and protease inhibitor cocktail #P8340 (Sigma, St. Louis, MO)]. Lysates were centrifuged 16,000 X G at 4°C for 20 minutes and supernatants were reserved for protein determination, and SDS PAGE analysis.

Reporter assay.

The ChoRE-luc (Carbohydrate response element of the L-pyruvate kinase promoter (ChoRE) luciferase) reporter gene, pNFkBLuc (clontech), the Gal4-p300 (wt or S89A mutant), Gal4-ChREBP and the UAS5E1B-TATALUC were described previously (4, 5). The reporter constructs used were the PG5 luciferase reporter vector that contains five Gal4 DNA binding sites (PG5LUC, Promega) and RSV β galactosidase (RSV β -gal) which serve as constitutive control reporters. All of the Gal4-p300 constructs are derivatives of the pVR-1012gal4-p300 expression vector that expressed full-length p300 fused to the gal4-DBD as previously described (4, 5). DN ChREBP (dominant negative ChREBP- R673A/R674G) and acetylated defective ChREBP mutants (K658R, K672R and K678R) were constructed using the QuickChange site directed mutagenesis kit from Stratagene.

β -galactosidase determination.

β -gal assays for normalization of ChoRE-luciferase activity were performed using 10 μl of hepatocyte lysates, 50 μl 2X buffer (1.33mg/ml 2-Nitrophenyl β -D-galactopyranoside, 100mM 2-mercaptoethanol, 2mM Magnesium Chloride, 200mM sodium phosphate pH 7.5, all purchased from Sigma, St. Louis MO) and 40 μl water in each well of a clear 96-well plate. The plate was covered and incubated 30 minutes at 37°C , and absorbance at 405nm was determined with a Biorad Lumimark Plus plate reader. Lysate samples were assayed in triplicate. Lysates from untransfected cells were used as controls for background activity. β -Gal activity was expressed as A405 units/mg protein.

Cell Culture and Transfection.

Mouse hepatocytes were harvested, cultured, and infected with adenoviruses as previously described (6). HEK293T cells were maintained and transfections were carried out as described (1). HepG2 hepatoma cells were transfected as previously described (1). Primary cultured hepatocytes, HEK293T or HepG2 cells were transiently transfected with the appropriate combination of reporters, expression vectors, and control vectors with lipofectamin 2000 or Fugene HD according to the manufacturer's instructions. 24h post-transfection, luciferase assays were performed at room temperature. Luciferase activity was normalized for transfection efficiency using a β -galactosidase reporter (RSV β -gal) as an internal control. Experimental data are mean of at least 3 independent experiments with luciferase activity normalized to β -galactosidase activity, conducted in triplicate. Hepatocytes were also infected with Ad-GFP, Ad-p300, Ad-SIK2, Ad-p300i or Ad-SIK2i adenovirus (1pfu/cell). 24h after infection hepatocytes were cultured with 100 nM insulin and either 5 or 25 mM glucose for 18h. For drug studies, hepatocytes were pre-treated with Lys-CoA-TAT (10 μ M) or curcumin (10 μ M) for 18h, and then incubated with 5 mM or 25 mM glucose with either no or 100 nM insulin for 18h.

Chromatin Immunoprecipitation.

Primary cultured hepatocytes were cultured for 18h in the presence of 100 nM insulin and either 5 or 25 mM glucose before cross-linking with 1% formaldehyde. Chromatin immunoprecipitation (ChIP) assays were performed as previously described (7). The ChoRE containing portion of the LPK promoter and a fragment of the coding region 5kb downstream of the LPK transcriptional start site were targeted for amplification. Forward and reverse primers used to amplify the ChoRE-containing region of the LPK gene promoter were as follows: 5'-CCCACTGACAAAGGCAGAGT-3' and 5'-CCTCCAAGTTCCTCCATCT-3' (upstream and downstream respectively). Antibodies used for immunoprecipitation of p300 were from Santa Cruz biotechnology, and ChREBP was from Novus Biologicals. After removing crosslinks, DNA was extracted using phenol-chloroform and ethanol precipitated. ChREBP occupancy over the LPK promoter was quantified using SYBR green real-time PCR with the primers flanking the ChoRE site of mouse LPK promoter. All signals were normalized to input chromatin signals.

In seq-ChIP assays, chromatin was first immunoprecipitated with antisera to ChREBP, eluted

with 100µl of elution buffer with 10 mM DDT at 37°C for 30 min, diluted (25 fold) with dilution buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% triton X-100), and re-immunoprecipitated with IgG or either p300 or acetylated-lysine antibody. ChIP experiments were repeated at least three times with reproducible results.

Isolation of total RNA and analysis of mRNA expression by quantitative PCR.

Total cellular RNAs from whole liver or from primary cultured hepatocytes were extracted using the RNeasy kit (Qiagen). Total RNA (1 µg) was reverse-transcribed for 1h at 42°C in a 20 µl final volume reaction containing 50 mM Tris- HCl, 75 mM KCl, 3 mM MgCl₂, 10 mM dithiotreitol, 250 mM random hexamers (Promega), 250 ng of oligo(dT) (Promega), 2 mM of each dNTPs, and 100 units of superscript II reverse transcriptase (Invitrogen). mRNA levels were measured as previously described using a Roche Light Cycler (6). Primer sequences are available on request.

Cell fractionation:

Hepatocytes were washed 2 times with ice cold PBS. Cells were collected in PBS and pelleted at 3000 rpm at 4°C. Cell pellets were resuspended in hypotonic lysis buffer (50 mM Hepes pH 7.4, 10 mM NaF, 1 mM EDTA, 0.5 mM DTT with protease inhibitors). Cells were lysed using a dounce homogenizer and centrifuged at 4200 rpm for 10 min at 4°C. Cytosolic supernatants were collected. Nuclear pellets were washed 3 times in 1 ml of hypotonic lysis buffer and resuspended in 200 µl of nuclear extraction buffer (50mM Hepes pH 7.4, 420 mM NaCl, 10 mM NaF, 1 mM EDTA, 0.5 mM DTT). Samples were sonicated and centrifuged at 13,000 rpm for 30 min. Nuclear supernatants were collected.

Western blot, Immunoprecipitation, in Vitro Kinase Assays:

Western blot and immunoprecipitations as well as in vitro kinase assays with IPs of p300 or ChREBP plus recombinant SIK2 were performed as previously described (1). For immunoprecipitation of acetylated protein, hepatocytes, previously treated as indicated, were washed with PBS and then lysed with lysis buffer (150mM NaCl, 1% Triton X-100, 1mM EDTA, 50mM Tris pH7.5, and protease inhibitor cocktail). After pre-clearing with protein G agarose (Invitrogen) for 30 min at 4°C, lysates (1mg) were incubated for 2h on a rocker with 5 µl of mouse monoclonal acetylated lysine antibodies (Cell Signalling), which selectively bind acetylated proteins. After 2h, 40 µl of protein G agarose (Invitrogen) were added to the tubes, and incubations were continued overnight. Immunoprecipitates were washed

extensively in lysis buffer. After the final washing step, 15 μ l of 2X sample buffer (100 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 20mg 1-bromophenol blue) was added to each tube. Samples were vortexed for 30s, boiled for 5 min, vortexed for 30s and store at -20°C prior to Western blot analysis. For immunoprecipitations using liver tissue, liver lysates were centrifuged at 13,000rpm for 30min at 4°C . Lysates (1mg) were precleared with control serum for 3h and protein G-agarose for 2h. Pre-cleared lysates were incubated with specific antibodies (ChREBP, p300) at 4°C overnight followed by addition of protein G agarose for 4h. Immunoprecipitates were washed extensively in lysis buffer, eluted with 2X SDS-sample buffer, and analyzed by Western blot assay. For Western blot assays, whole cell and tissue lysates (40 μ g) were analyzed using antibodies against: ACC (1:1000, Cell Signaling), Akt (1:5000, Cell Signaling), P-T308-Akt (1:1000, Cell Signaling), HSP90 (1:5000, Santa Cruz), p300 (1:1000, Santa Cruz), P-S89-p300 (1:300, Santa Cruz) followed by the secondary antibodies. The following antibodies were obtained from Upstate Cell Signalling: histone H3, histone H4, acetyl-histone H4 (lys8), acetyl-histone H3 (lys9). Phospho (Ser171) specific and non-discriminating CRTC2 antisera have been previously described (1).

The purified wild type or S89A mutant of p300 from HEK293T cells were phosphorylated with purified recombinant SIK2 protein in an in vitro kinase assay using labeled ^{32}P -ATP. The serine/threonine kinase activity of SIK2 was confirmed by phosphorylation of GST-CRCT2 protein as a positif control (data not shown). The phosphorylation reaction was carried out at 30°C for 20 min in kinase buffer (20 mM Hepes, pH 7.4, 10 mM magnesium acetate, 1 mM DTT, and 20 μ M of ^{32}P -ATP supplemented with 0.5 mM CaCl_2 , 100 ng/ml PMA, 100 μ g/ml phosphatidylserine. After phosphorylation, p300 were analyzed by 6% SDS-PAGE by autoradiography or by Western blot analysis using phospho-specific Ser-89 p300 antibody.

P300 histone acetyltransferase activity.

Histone acetyltransferase assays were performed to determine p300 HAT activity both in vitro and in vivo. Briefly either HEK293T, HepG2 cells, primary cultured hepatocytes were co-transfected with expression vector for p300. 24h after transfection cells were collected by scraping in 1ml of ice-cold phosphate buffered saline, and pelleted by centrifugation. The cells were resuspended in 300 μ l of cell lysis buffer (50 mM tris-HCl, pH8.0, 120 mM NaCl, 0.5% nonidet P-40, 2 mg/ml leupetin, 2 mg/ml aprotinin, 100 mM PMSF, 2 mM DTT) and incubated on ice for 30 min to extract the whole cells proteins. For immunoprecipitation the

proteins concentrations was adjusted to 1 mg/ml in 500 µl cell lysis buffer and was incubated with 5 µg of p300 antibodies (N15, Santa Cruz Biotechnology) at 4°C overnight with rotation. Protein A-agarose was then added, and the mixture was rotated slowly for 4h. The immune complexes were pelleted by gentle centrifugation and washed three times with cell lysis buffer. The beads were then washed with HAT buffer (1 mM phenylmethylsulfonyl fluoride, 50 mM Tris-HCl, pH 8.0, 10% glycerol, 10 mM butyric acid, 0.2 mM EDTA, 1 mM DTT). For HAT assay, the immune complexes were incubated with 1.25 ml of 20 mg/ml core histones (mixture of H2A/B, H3 and H4) and ¹⁴C acetyl-CoA (Amersham Biosciences) at 30°C for 1h. Reactions were then subjected to 12% SDS-PAGE followed by autoradiography. p300 HAT activity was also directly quantified with a colorimetric diagnostic kit according to the manufacturer's instructions (HAT activity colorimetric assay; bioVision).

In vitro acetylation assays of ChREBP

Immunoprecipitated ChREBP from HEK293T cells was incubated with purified recombinant HAT fragment of p300 (Upstate biotechnology, 5U) in the presence of labeled ³H-acetate (20 µM) (27.5 Ci/mmol, 1.0 mCi/ml, Sigma) for 1h at 30°C. The reactions were performed in a buffer containing 50 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 1 mM DTT, 10% glycerol. Reactions were stopped by adding SDS-sample buffer, and proteins were resolved by SDS/PAGE and stained with Coomassie brilliant blue. For autoradiography, stained SDS/polyacrylamide gels were further soaked in « amplifly » solution (Amersham Biosciences) for 30 min, quickly rinsed with water, dried under vacuum, and exposed to X-ray films with an intensifying screen for at least 24h at -80°C. Unlabelled acetylation reactions were performed similarly (but with unlabelled acetate) and acetylated proteins were detected by western blot using a specific acetyl-lysine antibody, as described above.

Acetylation of endogenous ChREBP

Livers or primary cultured hepatocytes were lysed in the lysis buffer as described above. Cell extracts were subjected to immunoprecipitation with ChREBP antibody. The immune complexes were analyzed by western-blotting with anti-acetyl lysine antibodies. Alternatively, cell extracts were subjected to immunoprecipitation with the anti-acetyl lysine antibodies and the immune complexes were analyzed by western blotting with rabbit polyclonal antibody against ChREBP.

Measurement of cAMP-Dependent Protein Kinase

PKA activity was measured from liver samples of ob/ob or HFD fed mice under post-prandial state using PepTag assay for non-radioactive detection of PKA according to the manufacturer's instructions (Promega).

Measurement of Acetyl-CoA content

Acetyl-CoA content was measured both in vivo and in vitro using the fluorometric acetyl-CoA assay kit according to the manufacturer's instructions (BioVision).

Fatty acid synthesis in vivo.

Rate of fatty acid synthesis was measured by intraperitoneally injecting 150 μ Ci of 3 H-labeled water to mice during the early light cycle. Two hours later, blood was drawn from the inferior cava vein to determine the plasma 3 H-labeled water-specific activity. Rates of fatty acid synthesis were calculated as micromoles of 3 H-radioactivity incorporated into fatty acids per hour per gram of tissue.

Staining techniques.

For histology studies, tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Then, 7 μ m sections were cut and stained with hematoxylin eosin. For the detection of neutral lipids, liver cryosections were stained with the Oil Red O technique, using 0.23% dye dissolved in 65% isopropyl alcohol for 10 min.

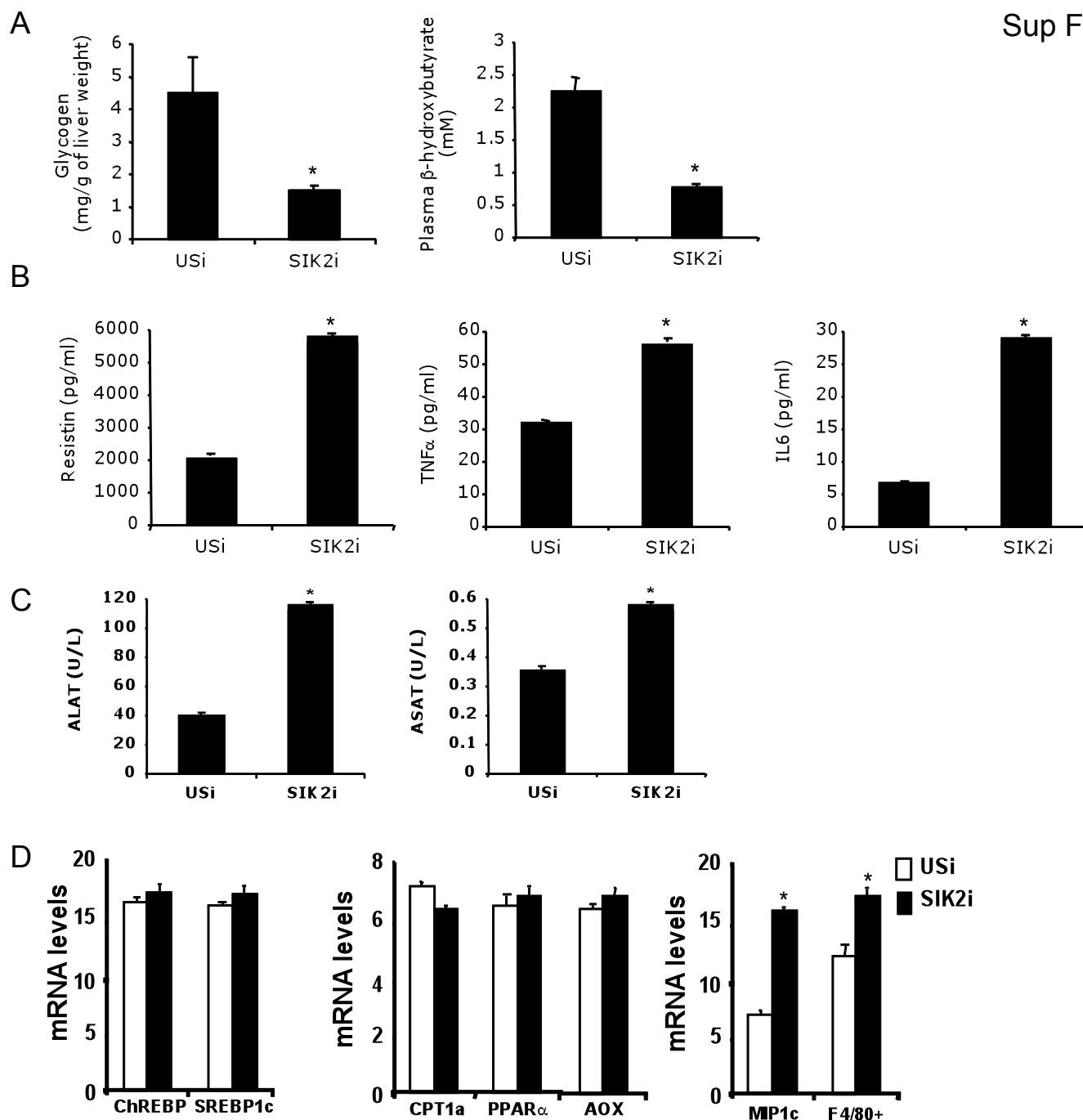
Statistical analyses

Results are expressed as mean \pm SEM. Statistical significance was assessed using the ANOVA test (StatView). Differences were considered statistically significant at $P < 0.05$. All experiments were performed on at least three independent occasions.

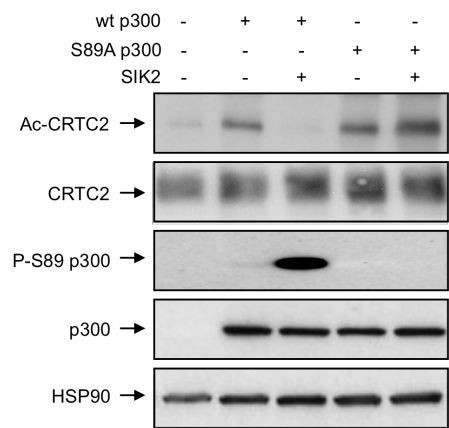
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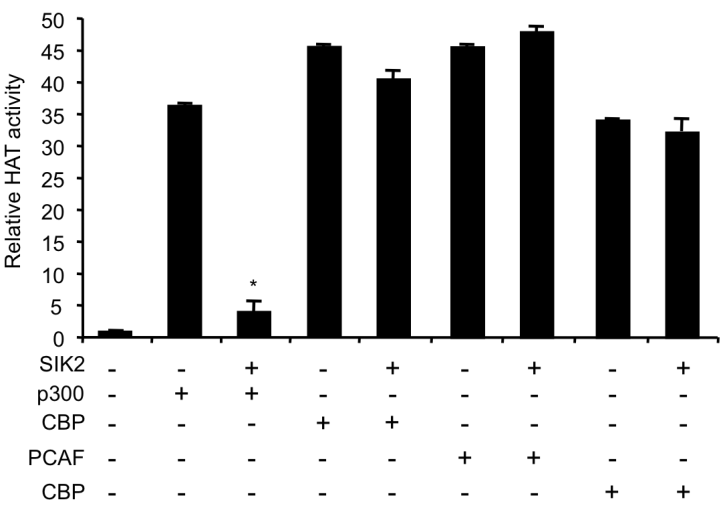
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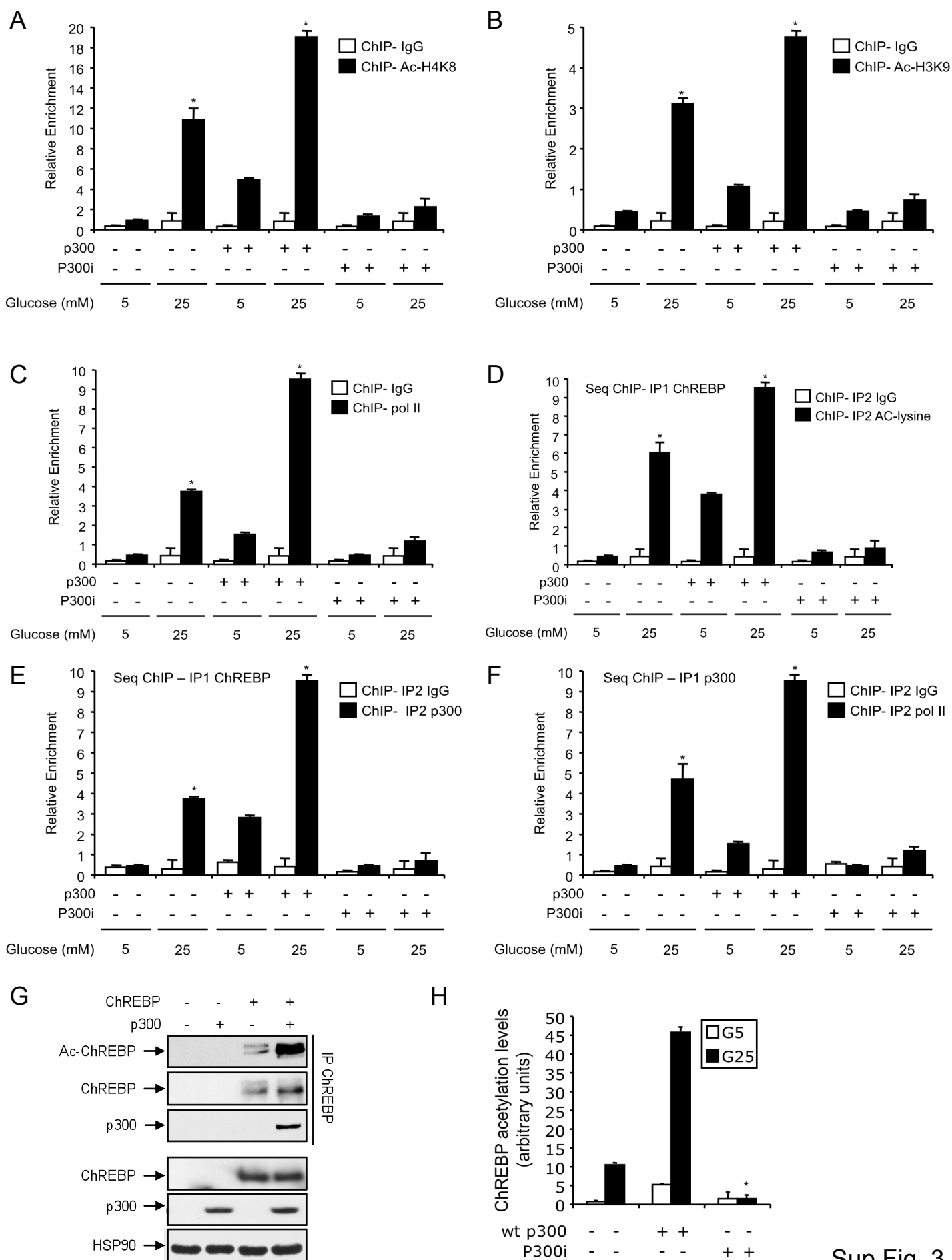


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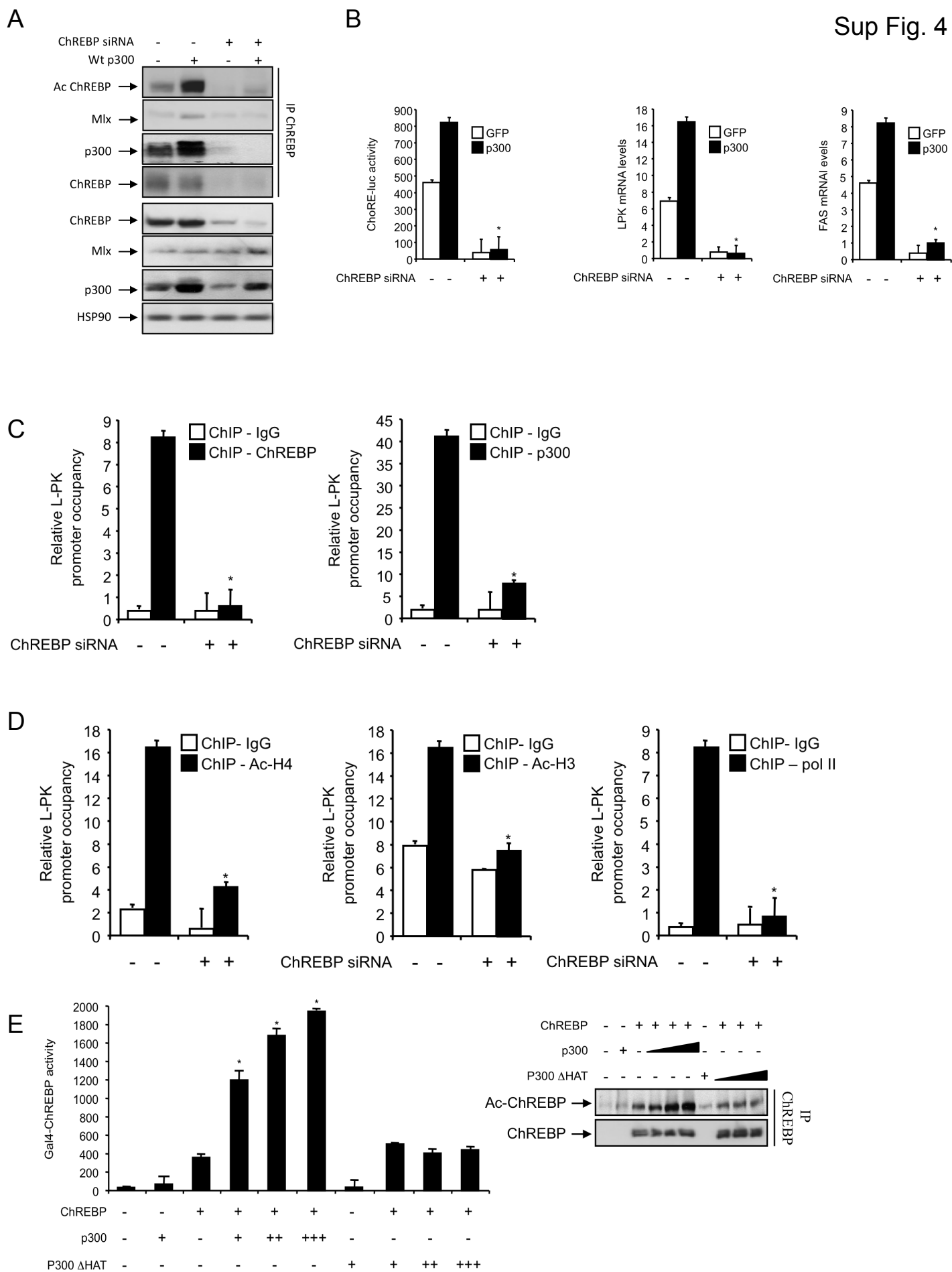


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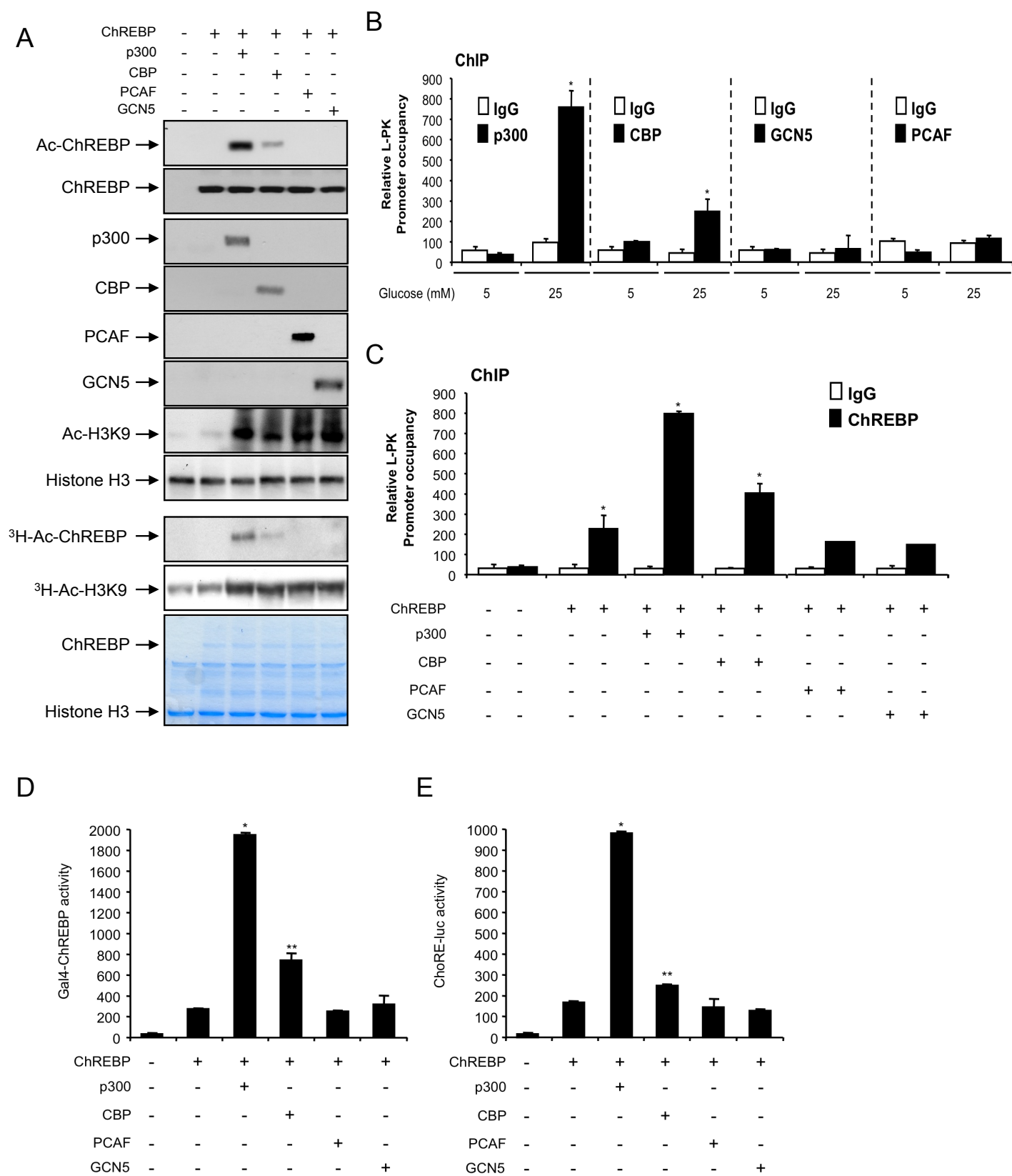


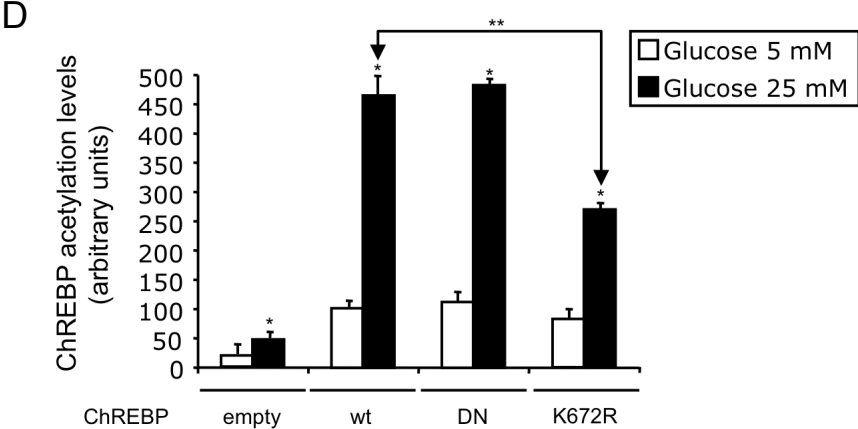
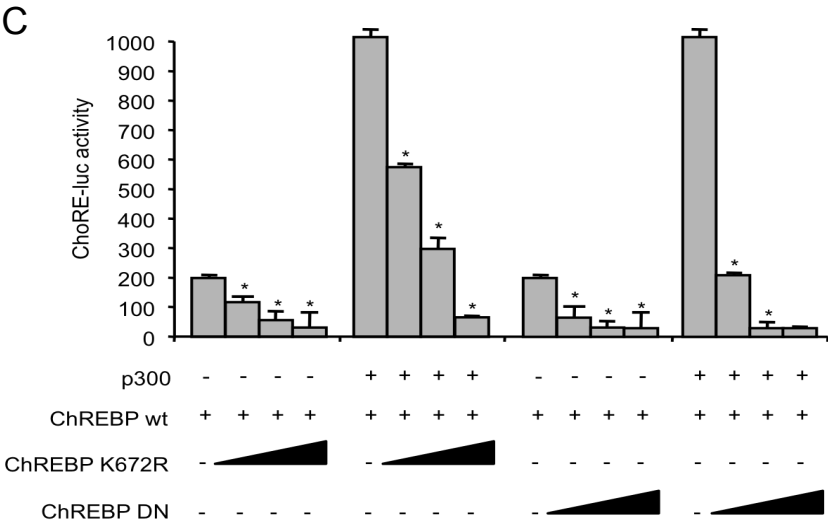
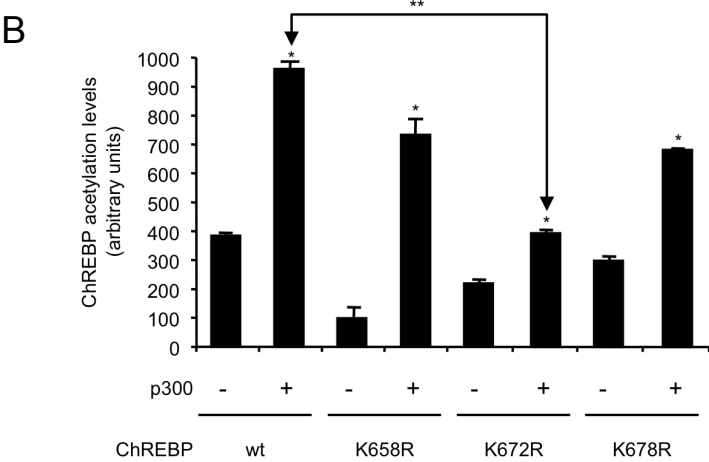


Sup Fig. 3

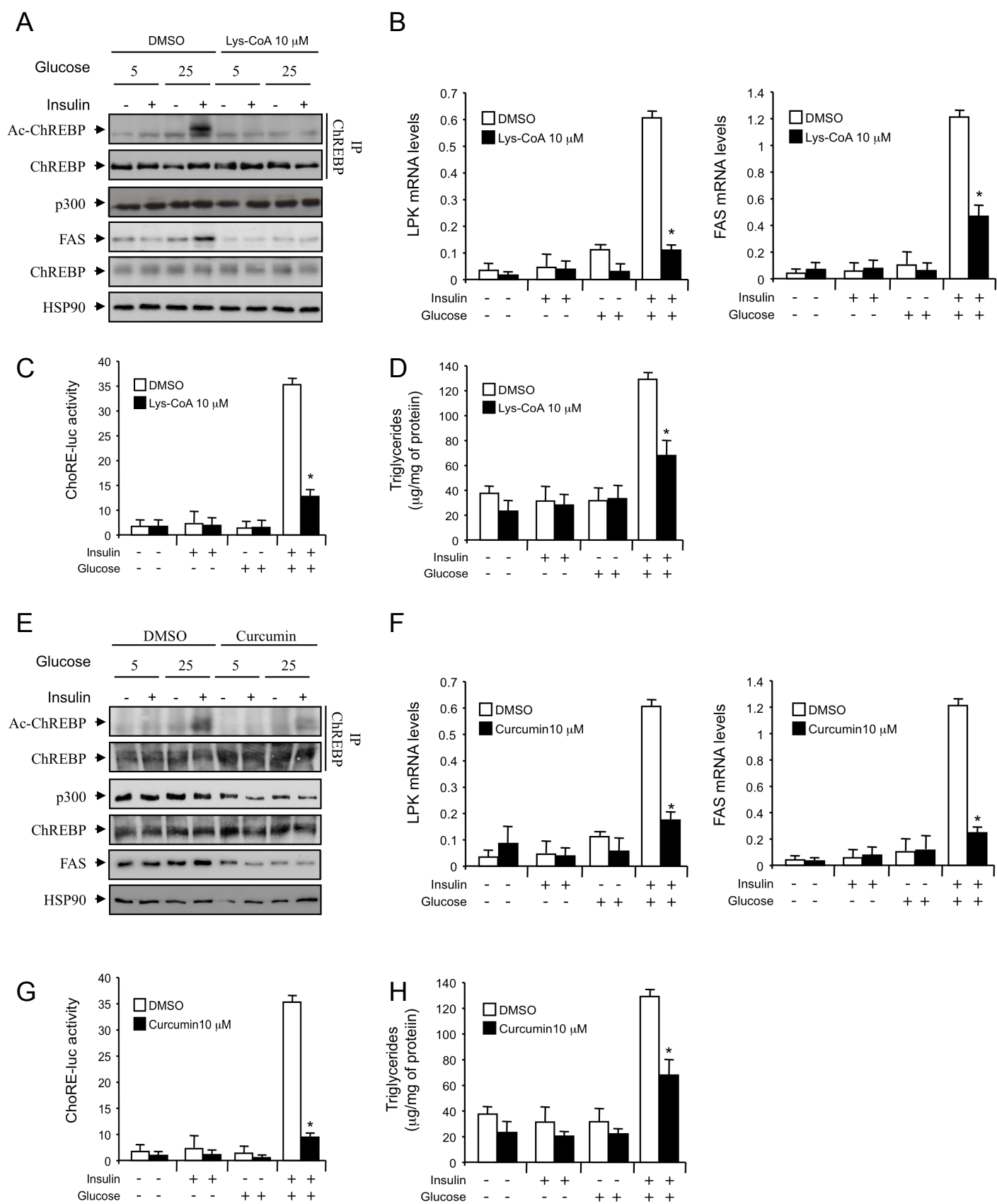


Sup Fig. 5



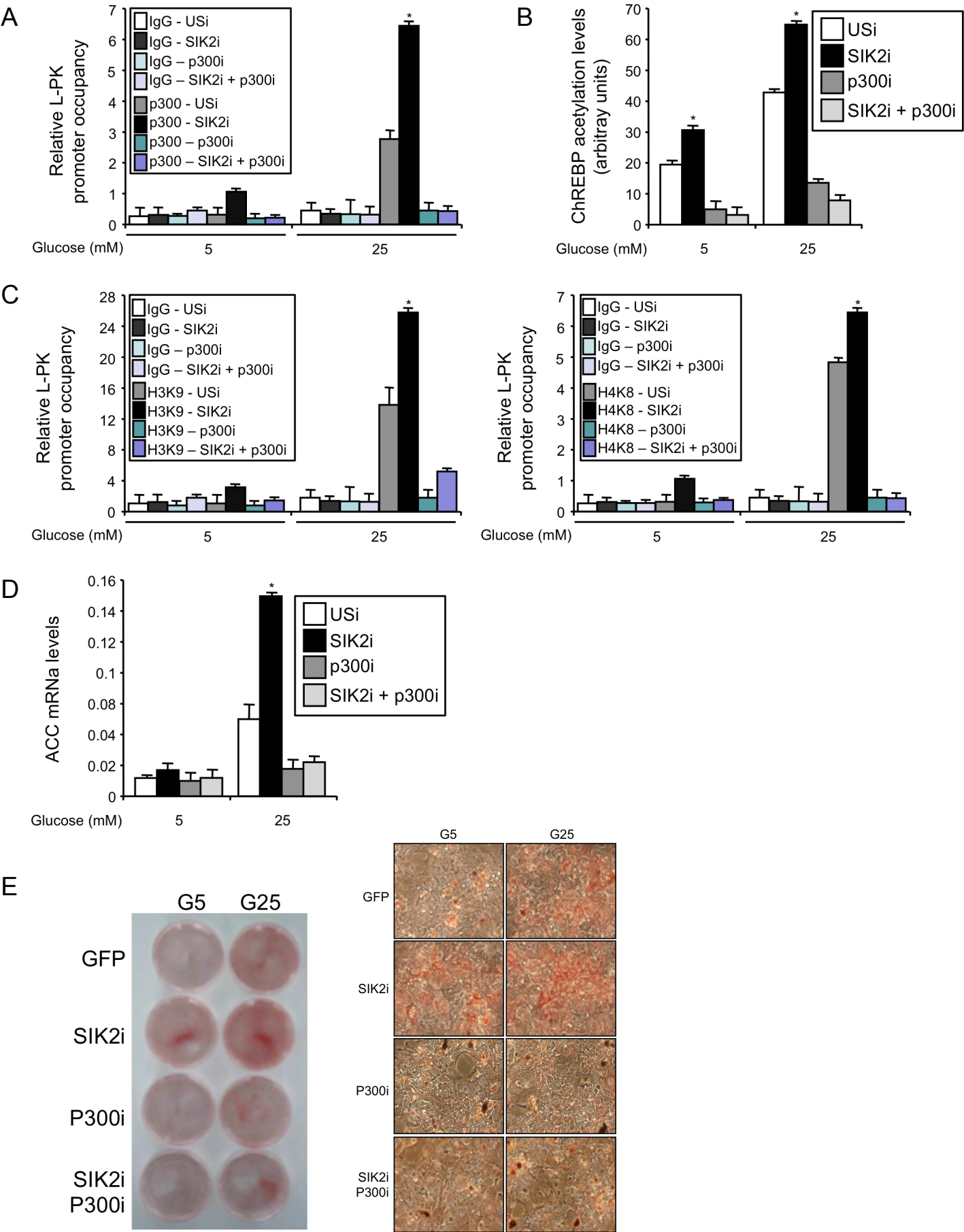


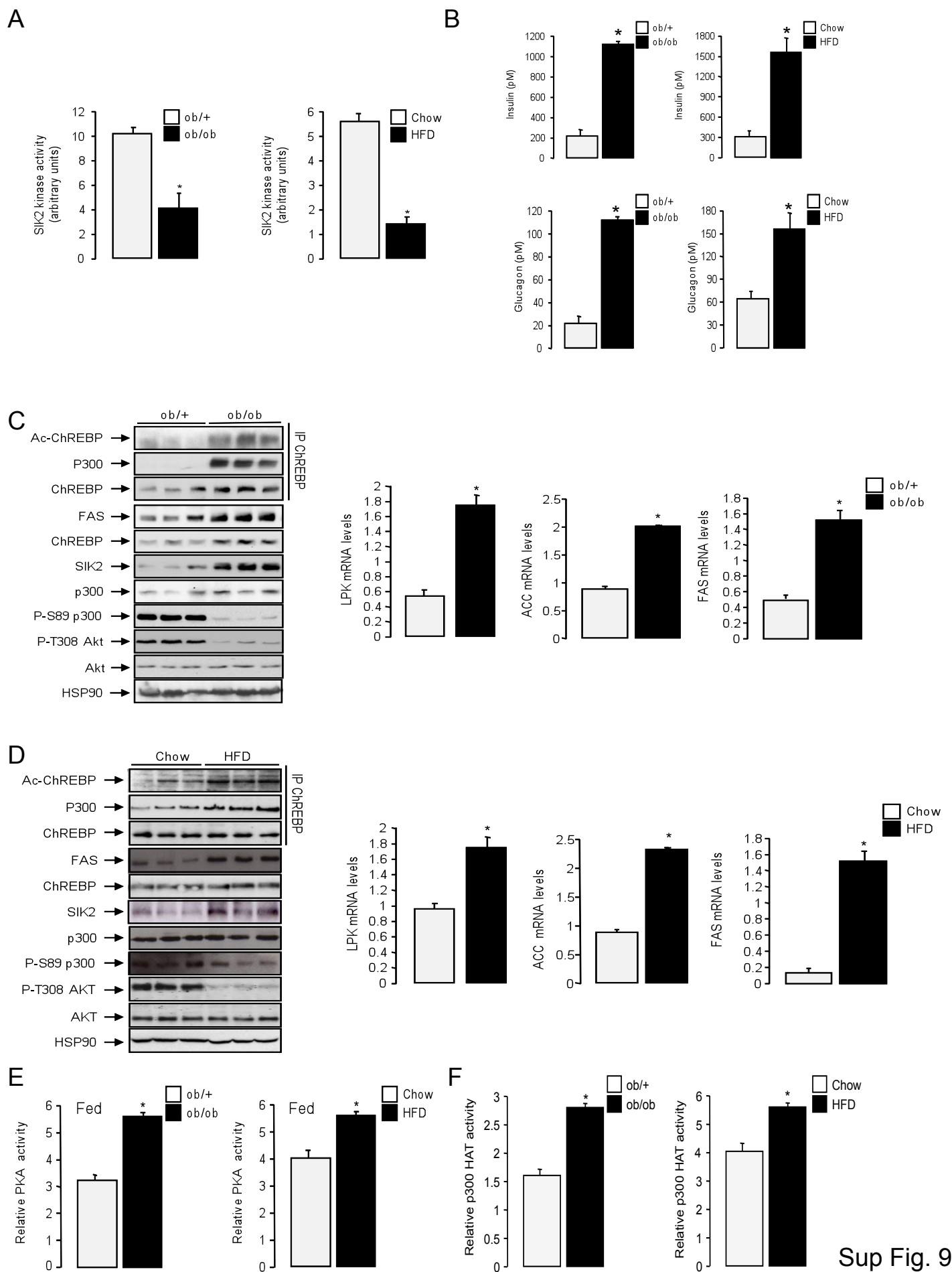
Sup Fig. 6



Sup Fig. 7

Sup Fig. 8





Sup Fig. 9

Sup Fig. 10

