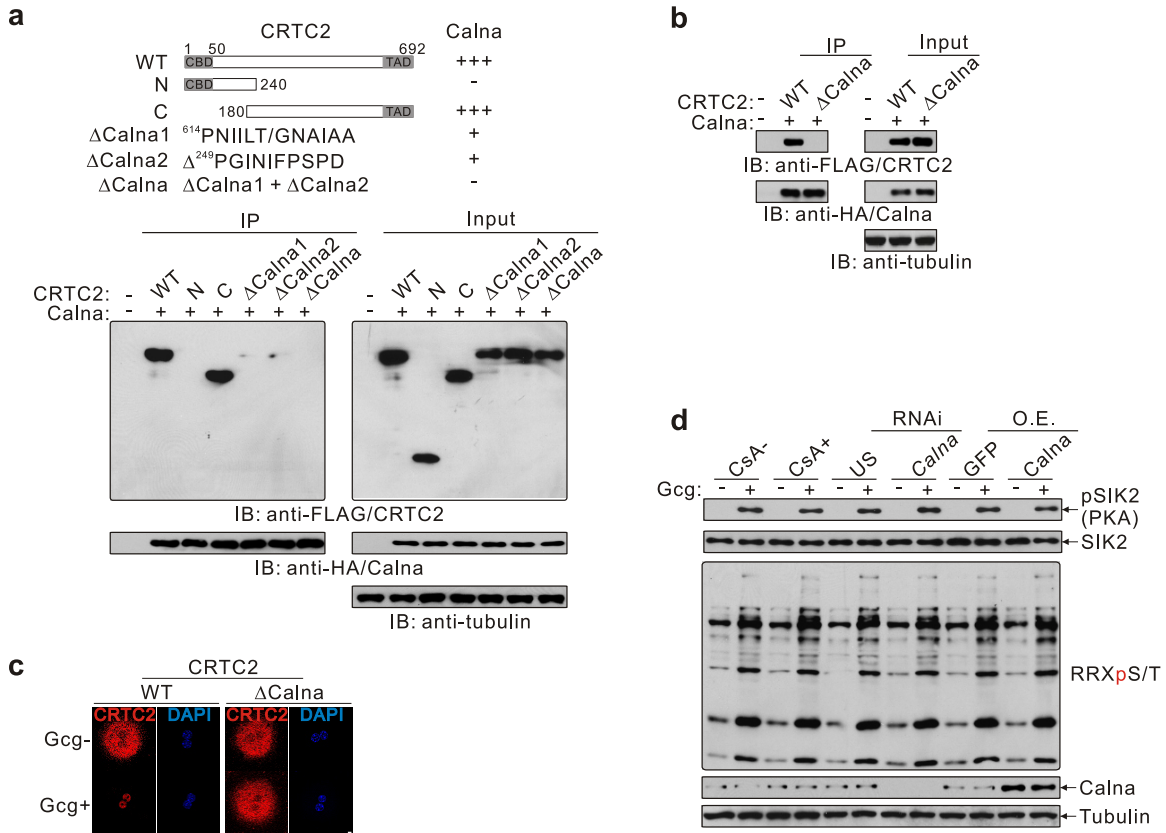
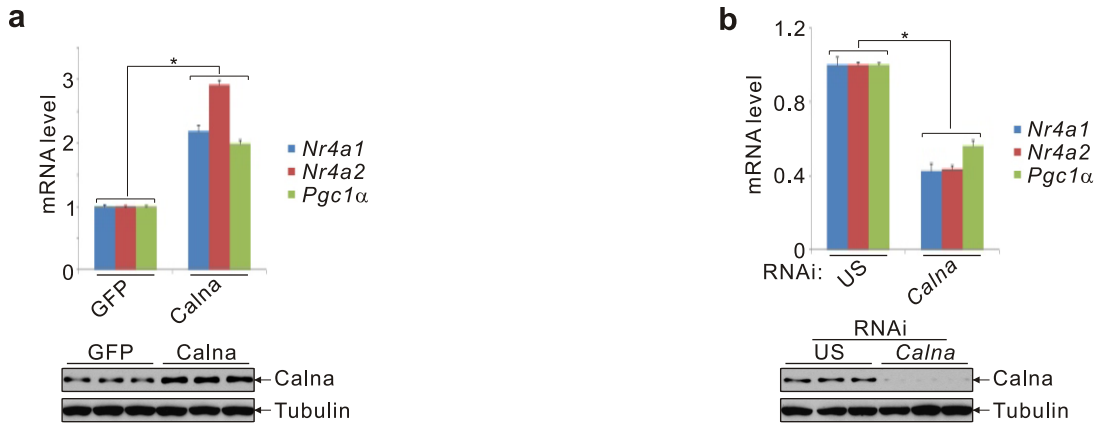


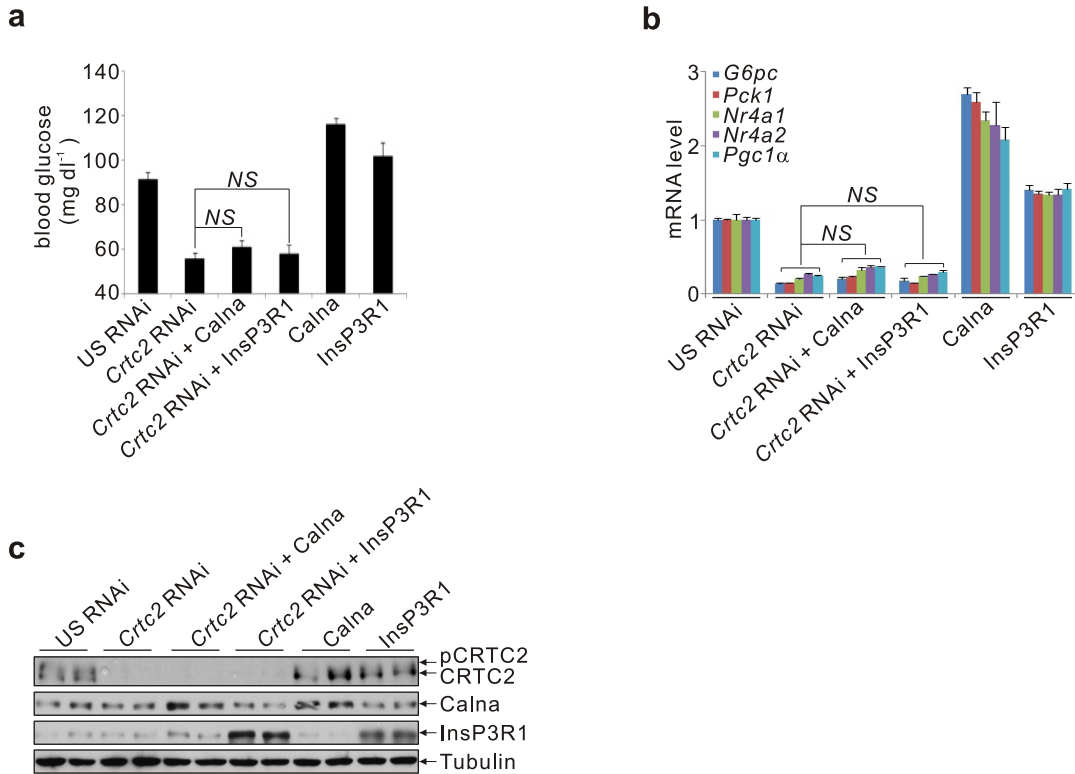
Supplementary Figure 1: Glucagon stimulates CRTC2 activity in hepatocytes via a calcineurin-dependent mechanism. A. Effect of Ser/Thr phosphatase inhibitors (okadaic acid (OA), cyclosporin A (CsA)) on CRTC2 localization in primary mouse hepatocytes exposed to glucagon (Gcg). Scale bar, 5 μ m. B. Effect of calcineurin inhibitors (calcineurin autoinhibitory peptide (CAP), CN585) and PP1/ PP2A inhibitor (calyculin A) on CRE-luciferase reporter activity in primary hepatocytes (* $P < 0.001$; $n=3$). C. and D. Effect of wild-type and phosphorylation-defective (S171A, S275A) active CRTC2 mutants on CRE-luc activity (C) (* $P < 0.001$; $n=3$) and CRTC2 cellular localization (D). Glucagon treatment indicated. Scale bar, 5 μ m. E. Effect of CsA on CRE-luc activity in cells expressing wild-type or constitutively active (S171A/S275A) mutant CRTC2 (* $P < 0.001$; $n=3$). NS, no statistical difference.



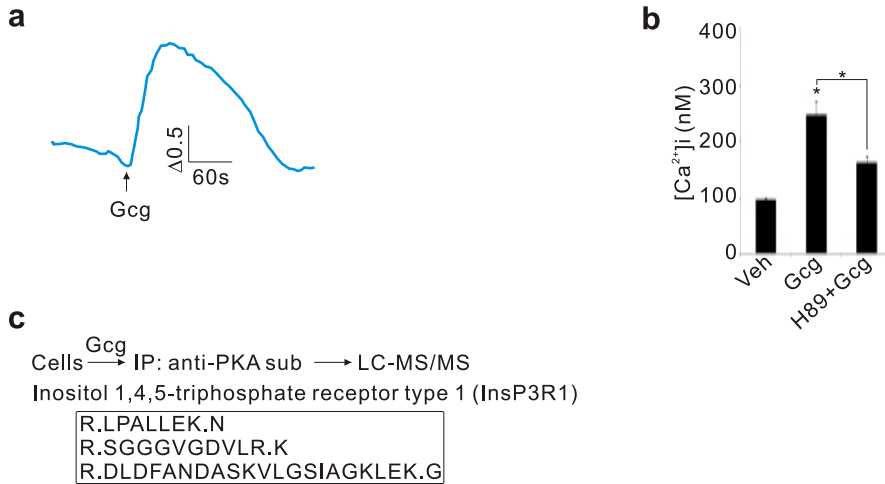
Supplementary Figure 2: Calcineurin modulates CRTC2 activity in hepatocytes. A. Analysis of calcineurin binding sites in CRTC2. Different CRTC2 polypeptides and amino acid substitutions/deletions indicated in schematic. Immunoblot of flag-tagged CRTC2 recovered from IPs of HA-tagged calcineurin (Calna) in HEK293T cells shown. B. Co-immunoprecipitation assay showing relative binding of wild-type and mutant CRTC2 polypeptides to calcineurin in cultures of primary hepatocytes. Cells infected with adenovirus encoding wild-type or calcineurin-defective (Δ Calna) Flag-tagged CRTC2 plus HA-tagged calcineurin A (Calna) indicated. Recovery of CRTC2 polypeptides from HA-Calcineurin IPs shown. C. Nuclear shuttling of wild-type (WT) and calcineurin-defective (Δ Calna) CRTC2 in hepatocytes exposed to glucagon. Scale bar, 5 μ m. D. Effect of Calna over-expression (O.E.) or depletion by RNAi-mediated knockdown on downstream PKA signaling in hepatocytes using phospho-PKA substrate antibody (anti-RRXpS/T).



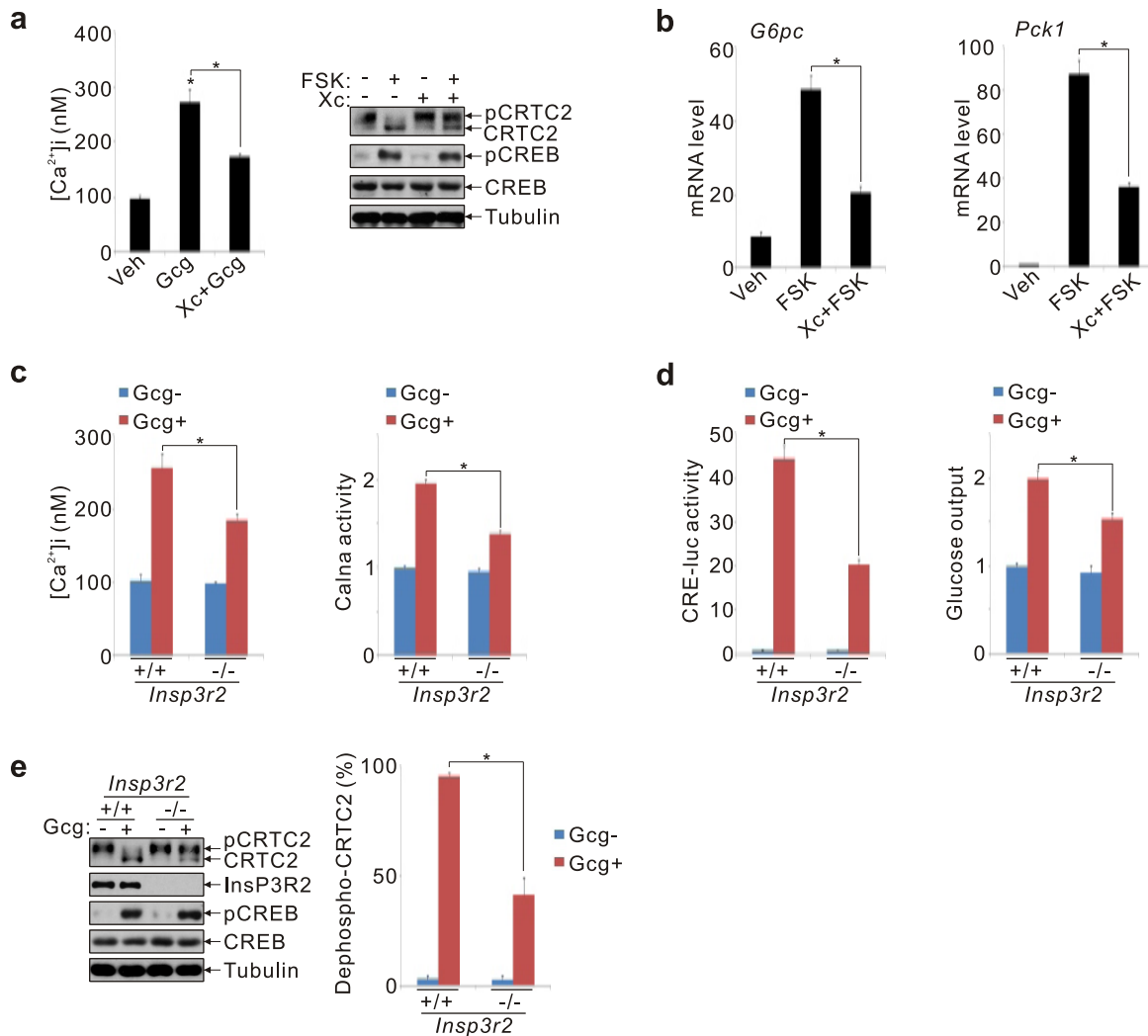
Supplementary Figure 3: Calcineurin regulates CRTC2 activity in liver. Effect of calcineurin (Calna) over-expression (A) or RNAi-mediated depletion (B) on mRNA amounts for fasting-inducible CREB target genes (*Nr4a1*, *Nr4a2*, *Pgc1α*) in livers of fasted mice ($*P < 0.01$; $n=5$). Immunoblot showing relative calcineurin protein amounts in livers from calcineurin over-expressing or knockdown mice compared to controls.



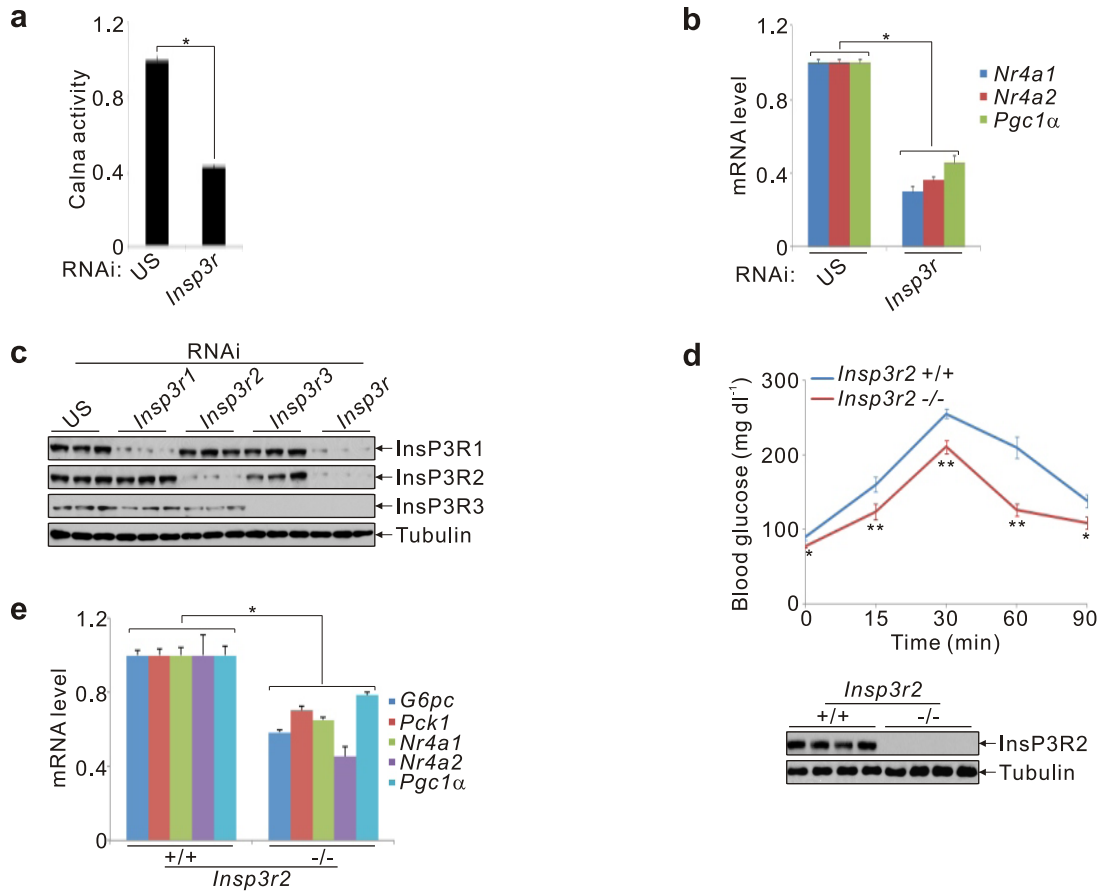
Supplementary Figure 4: Calcineurin and InsP3Rs regulate gluconeogenesis by modulating CRTC2 activity. A-B. Effect of adenovirally encoded *Crtc2* RNAi on blood glucose concentrations (A) and hepatic gene expression (B), in fasted mice expressing adenovirally encoded calcineurin or InsP3R1, as indicated. n=4. NS, no statistical difference. C. Immunoblot showing hepatic protein amounts for CRTC2, Calcineurin (Calna), and InsP3R1 in mice characterized in panels A and B.



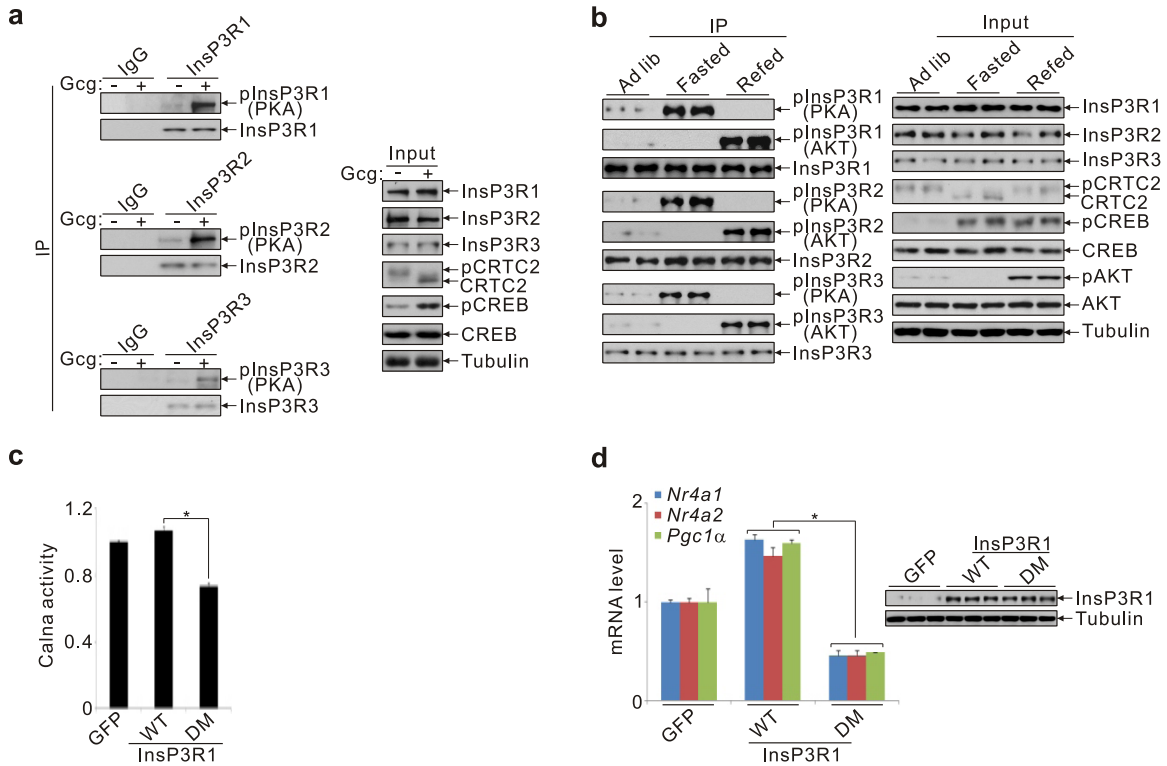
Supplementary Figure 5: Glucagon stimulates calcium mobilization in hepatocytes. A. and B. Effect of glucagon (Gcg) and PKA inhibitor H89 on calcium mobilization in primary hepatocytes by fluorescence imaging ($*P < 0.001$; $n=3$). C. InsP3R1 peptides identified by MS analysis of immunoprecipitates prepared with phospho-PKA substrate antiserum on lysates from primary hepatocytes exposed to glucagon (Gcg).



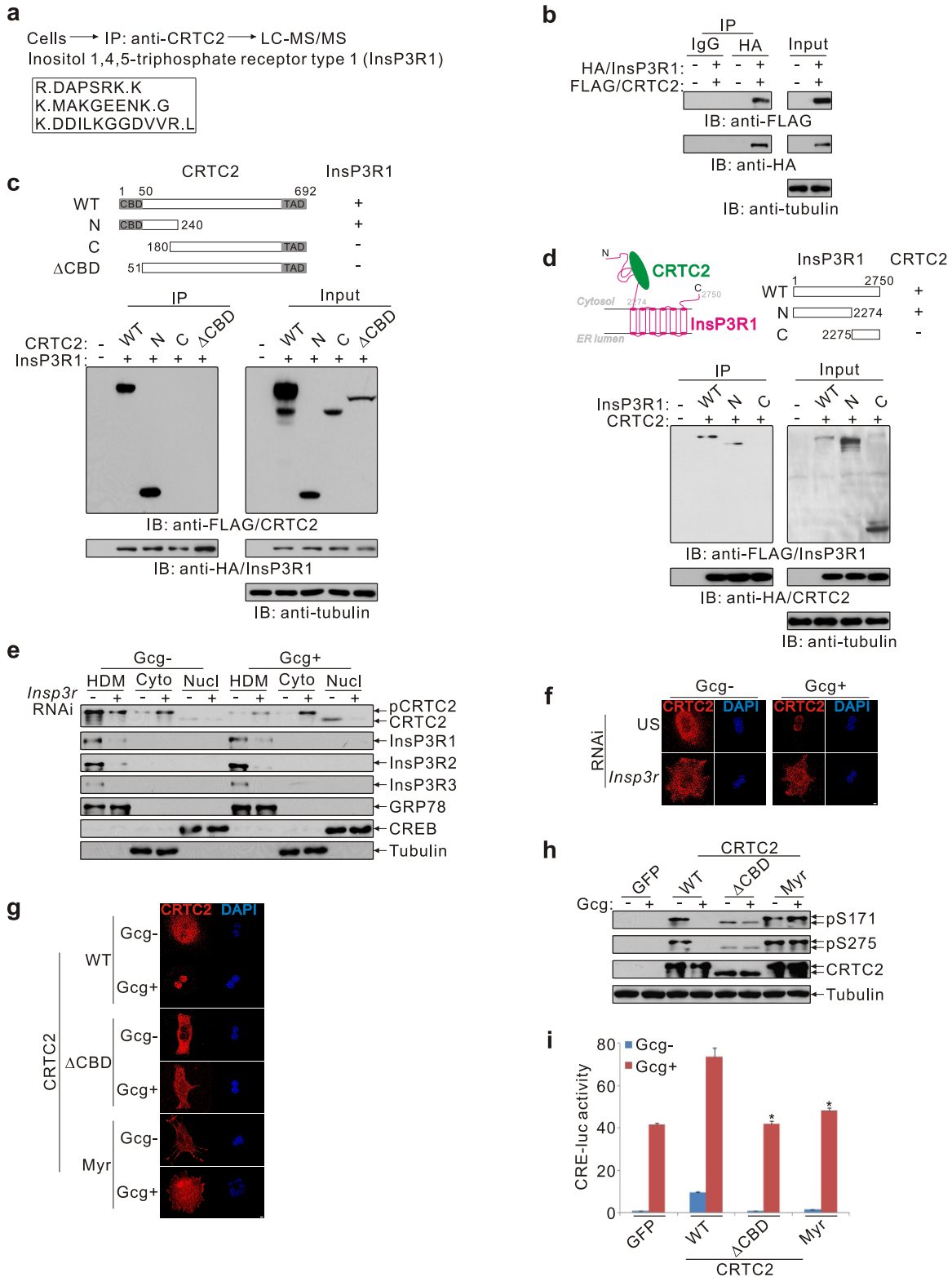
Supplementary Figure 6: InsP3 Receptors are required for CRTC2 activation in response to cAMP agonists. A, B. Effect of Xestospongin C (Xc) on calcium mobilization and CRTC2 dephosphorylation (A) and on mRNA amounts for glucose-6-phosphatase (*G6pc*) and PEPCK (*Pck1*) (B) in primary hepatocytes exposed to Forskolin (FSK) (**P* < 0.001; n=3). C-E. Effects of glucagon on calcium mobilization and calcineurin activation (C) (**P* < 0.001; n=4) as well as CRE-luc reporter activation and glucose secretion (D) (**P* < 0.001; n=4) and CRTC2 dephosphorylation (E) in primary hepatocytes from *Insp3r2* knockout mice and control littermates. E. Bar graph on right shows relative dephosphorylation of CRTC2 in wild-type and *Insp3r2* knockout hepatocytes exposed to glucagon, determined by densitometry (**P* < 0.01; n=3).



Supplementary Figure 7: InsP3Rs modulate hepatic gluconeogenesis. A. Effect of RNAi-mediated depletion of all three InsP3R family members on calcineurin activation in fasted livers ($*P < 0.001$; $n=5$). B and C. Effect of hepatic InsP3R knockdown on mRNA amounts for fasting inducible CREB target genes (B) and on InsP3R protein amounts (C) in livers of mice ($*P < 0.01$; $n=5$). D. and E. Hepatic gluconeogenesis, measured by pyruvate tolerance testing (D) ($*P < 0.02$; $**P < 0.01$; $n=4$) and gluconeogenic gene expression (E) ($*P < 0.01$; $n=4$) in wild-type and InsP3R2 knockout mice. Immunoblot shows InsP3R2 protein amounts in hepatic extracts from control and InsP3R2 knockout mice.

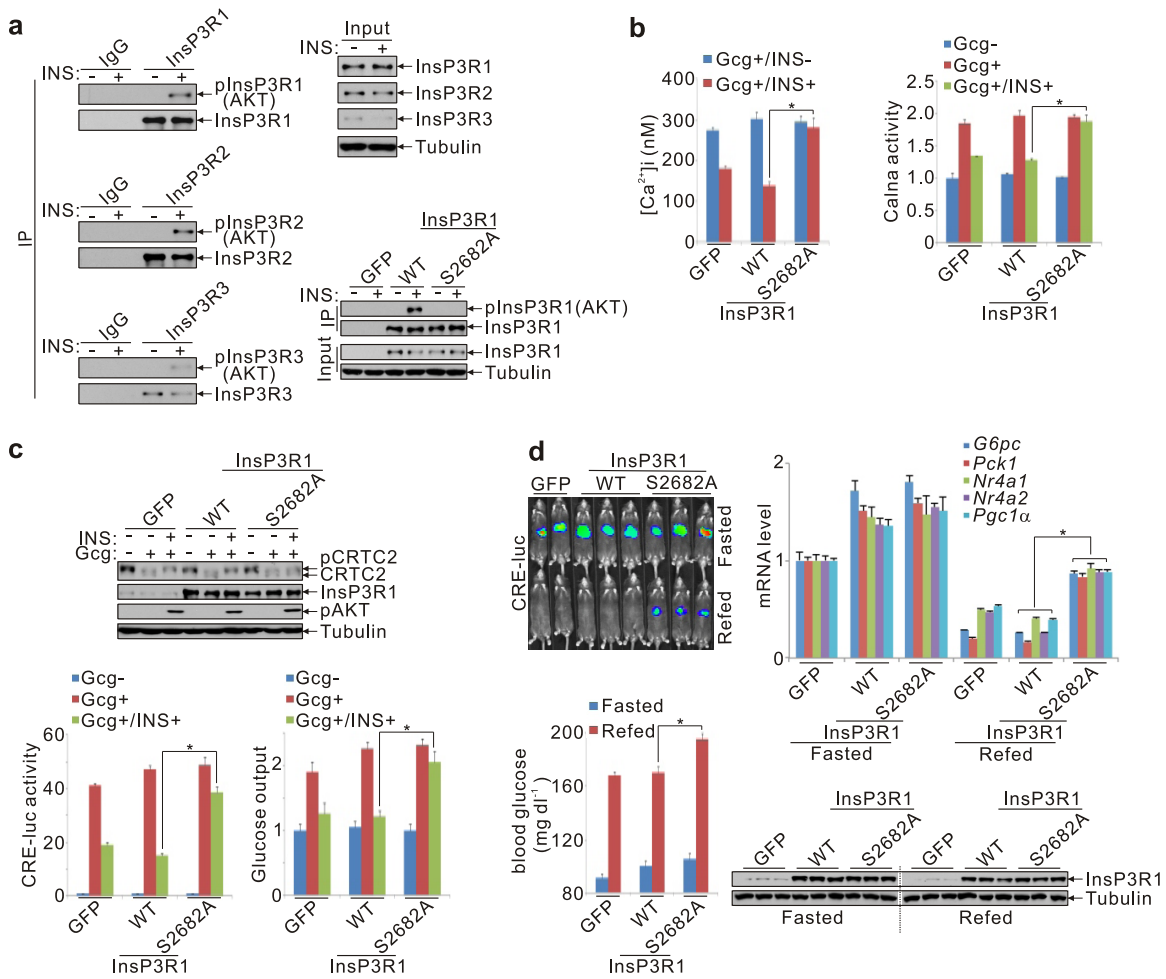


Supplementary Figure 8: Glucagon modulates InsP3R activity via PKA-mediated phosphorylation. A. Immunoblots showing effect of glucagon (Gcg) on InsP3R phosphorylation, using phospho-PKA substrate antiserum on immunoprecipitates of InsP3Rs prepared from primary hepatocytes. B. Immunoblots showing phosphorylation of InsP3R at PKA or AKT sites in livers of 6-8 hour fasted or 2 hour-refed mouse livers. C. and D. Effect of wild-type and PKA-defective (DM) InsP3R1 on calcineurin (Calna) activation (C, $*P < 0.001$, $n=5$) and CREB target genes (D, $*P < 0.01$, $n=5$) in fasted livers. Right, immunoblot showing relative InsP3R protein amounts in livers of mice injected with adenovirus encoding wild-type or PKA-defective (DM) InsP3R1.

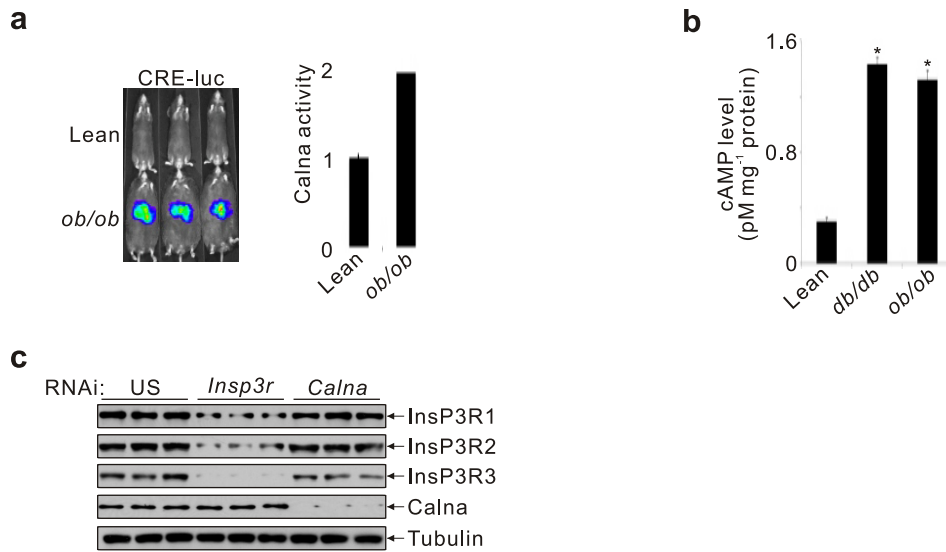


Supplementary Figure 9: Association of InsP3Rs with CRTC2 in hepatocytes. A. InsP3R1 peptides recovered from anti-CRTC2 immunoprecipitates by MS analysis. B. Co-immunoprecipitation assay showing amounts of Flag-tagged CRTC2 recovered from

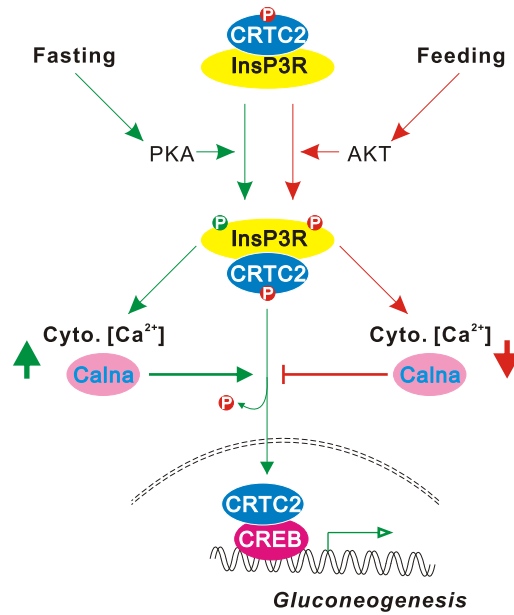
IPs of HA-tagged InsP3R1 in HEK293T cells. C. and D. Deletion analysis of regions in CRTC2 (C) and InsP3R1 (D) required for the CRTC2:InsP3R1 interaction. Interaction-competent CRTC2 and InsP3R1 polypeptides indicated in each schematic (+). E. Effect of InsP3R depletion on CRTC2 protein amounts associated with ER enriched high density microsomes (HDM) as well as cytosolic (Cyto), and nuclear (Nucl) fractions in hepatocytes exposed to glucagon (Gcg). Relative amounts of ER-localized (GRP78), cytosolic (Tubulin), and nuclear (CREB) proteins in each fraction indicated. F. Effect of InsP3R depletion on CRTC2 localization in hepatocytes. Scale bar, 5 μ m. G.- I. Cellular localization (G), phosphorylation state (H) and activity (I) of wild-type, InsP3R-defective (Δ CBD, 51-692 aa), and myristoylated CRTC2 mutant polypeptides ($*P < 0.001$; n=3). Scale bar, 5 μ m.



Supplementary Figure 10: Insulin down-regulates CRTC2 activity via the AKT-mediated phosphorylation of InsP3Rs. A. Left, immunoblots showing effect of insulin (INS) on InsP3R phosphorylation, using phospho-AKT substrate antiserum on immunoprecipitates of InsP3Rs prepared from primary hepatocytes. Bottom right, effect of insulin (INS) on phosphorylation of wild-type and mutant InsP3R1 containing an alanine substitution at the consensus AKT phosphorylation site (Ser2682). B. Effect of insulin on glucagon (Gcg)-induced calcium mobilization and calcineurin (Calna) activation in primary hepatocytes expressing wild-type or AKT-defective (S2682A) InsP3R1 ($*P < 0.001$; $n=3$). C. Effect of wild-type and AKT-defective (S2682A) InsP3R1 on CRTC2 dephosphorylation (top), CRE-luc reporter activation (bottom left), and glucose output (bottom right) from hepatocytes ($*P < 0.001$; $n=3$). D. Effect of wild-type and AKT-defective (S2682A) InsP3R1 on CRE-luc activity, blood glucose, and CREB target gene expression (*G6pc*, *Pck1*, *Nr4a1*, *Nr4a2*, *Pgc1α*) in fasting or refeed mice ($*P < 0.01$; $n=5$). Bottom right, immunoblot showing relative InsP3R protein amounts in livers of mice injected with adenovirus encoding wild-type or AKT defective (S2682A) InsP3R1 ($*P < 0.01$; $n=5$).



Supplementary Figure 11: Hepatic cAMP signaling and calcineurin activity are increased in obesity. A. and B. Hepatic CRE-luc reporter activity and calcineurin activity (A) as well as cAMP content (B) in lean, *db/db*, and *ob/ob* mice (* $P < 0.001$; $n=5$). C. Immunoblot of InsP3R and calcineurin (Calna) proteins amounts in livers of mice injected with *Insp3r* or *Calna* RNAi adenovirus.



Supplementary Figure 12: Fasting and feeding pathways regulate CRTC2-dependent gluconeogenesis through antagonistic effects on InsP3R activity. Fasting signaling activates InsP3Rs via PKA-dependent phosphorylation, leading to increases in calcineurin (Calna) activity and in the subsequent dephosphorylation of CRTC2. By contrast, feeding inhibits InsP3R activity via AKT-dependent phosphorylation, thereby blocking the calcineurin-dependent dephosphorylation of CRTC2.