SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: Left, bar graph showing relative Ad-CRE-luc activity in 6 and 24 hour fasted mice normalized to adenovirally encoded RSV β-galactosidase activity. Effect of IP glucagon injection shown. Middle, effect of short (6 hr.) and long term (24 hr) fasting on hepatic G6Pase and PEPCK mRNA amounts. (n=6 mice per group, (**); *P*<.02 for 6 hr. compared to 24 hr. fasting mRNA; data are means ± s.e.m.). Right, circulating blood glucose concentrations in 6 or 24 hour fasted relative to ad libitum fed mice (n=6, *P*<.05; data are means +s.e.m.).

Supplementary Figure 2: Left, relative rate of gluconeogenesis from alanine in ad libitum fed and 6 or 24 hour-fasted mice, evaluated by measuring conversion of ^{14}C-labeled alanine into ^{14}C-glucose. (for 6-hr versus 24 hr. fasted, *P*< .01, n=3 per group; data are means ±s.e.m.). Results are expressed as micromoles of glucose synthesized per hour per gram body weight. Right, ketone body (β-hydroxybutyrate) concentrations in mice fasted for various times (0, 4, 8, 18, 48 hrs). (n=6, *P*<.05; data are means ± s.e.m.) .

Supplementary Figure 3: Left, immunoblot showing relative amounts of CRTC2 and FOXO1 in ad libitum fed and 6 or 24 hour fasted mice. Right, effect of short and long term fasting relative to refeeding on amounts of ubiquitinated and total CRTC2 protein in the liver.

Supplementary Figure 4: Immunoblot of CRTC2 protein amounts in primary hepatocytes exposed to glucagon for times indicated. Effect of proteasome inhibitor MG132 shown.

Supplementary Figure 5: Immunoblot showing effect of COP1 depletion by adenovirally encoded COP1 RNAi on CRTC2 protein amounts in primary mouse hepatocytes exposed to glucagon (100nM) for various times. Bottom, amounts of CRTC2 recovered from immunoprecipitates of P300 prepared from corresponding hepatocyte extracts.

Supplementary Figure 6: Live imaging of mice expressing adenovirally encoded G6Pase luciferase reporter. Relative luciferase activity in ad libitum fed and 6 hour fasted mice shown.

Supplementary Figure 7: Left, immunoblot showing effect of adenovirally encoded CRTC2 RNAi, FOXO RNAi, and unspecific RNAi (USi) on amounts of hepatic CRTC2 and FOXO1. Right, PEPCK mRNA amounts in livers of short and long-term fasted mice. Effect of Ad-CRTC2 RNAi and FOXO1 RNAi (FOXOi) relative to control (Ad-USi) indicated.

Supplementary Figure 8: Left, transient assay of wild-type and mutant G6Pase reporter activity in HepG2 hepatocytes exposed to FSK for 6 or 18 hours. (n=3, (*); *P*<.001; (**); *P*<.02 for 6 hr. compared to 18 hr. treatment; data are means ± s.e.m.). Middle and right, bar graphs showing relative occupancy of CRTC2 and FOXO1 on G6Pase promoter in HepG2 cells following 6hr or 18hr exposure to forskolin (fig. 1d). Occupancy normalized to DNA input.

Supplementary Figure 9: Immunoblot showing effect of glucagon (100nM, 1hr) followed by insulin (100nM, 1hr) exposure on CRTC2 acetylation and total CRTC2 protein amounts in primary hepatocytes.

Supplementary Figure 10: Acetyl-lysine containing CRTC2 peptide sequences recovered from proteomic analysis of HA-CRTC2 immunoprecipitates prepared from HEK293T cells. Number of peptides containing each sequence indicated. Asterisk marks acetylated Lys residue (Lys 628).

Supplementary Figure 11: Left, bar graphs from in vivo imaging study (fig. 2b) showing hepatic CRE-luciferase activity in 8 or 24 hour fasted mice expressing wild-type or acetylation defective Lys628Arg mutant CRTC2 (n=4; p<.05). Right, immunoblot showing relative amounts of adenoviral wild-type and mutant (K628R) CRTC2 protein in 18 hour fasted and 2 hour refed mice.

Supplementary Figure 12: Immunoblot showing amounts of CRTC2 recovered from immunoprecipitates of CBP prepared from cultured primary mouse hepatocytes exposed to FSK and/or insulin for 1 hour.

Supplementary Figure 13: Immunoblot showing effect of the Ser/Thr kinase SIK2 on Ser89 phosphorylation of HA-tagged P300 in vitro. HA-P300 and HA-SIK2 were expressed and immunoprecipitated from HEK293T cells. P300 phosphorylation detected with phospho (Ser89) specific P300 antiserum.

Supplementary Figure 14: Immunoblot showing recovery of CRTC2 from immunoprecipitates of wild-type and Ser89Ala mutant HA-tagged P300 prepared from primary mouse hepatocytes. Exposure to glucagon (1hr.) followed by insulin (1hr.).

Supplementary Figure 15: Left, immunoblot showing effect of CBP over-expression on amounts of total and acetylated CRTC2 in HEK293T cells exposed for 1 hour to FSK or SIK2 kinase inhibitor staurosporine (STS) relative to control (DMSO). Middle, Immunoblot showing in vitro acetylation of purified recombinant GST-CRTC2 (aa. 601-692) or control GST polypeptides following incubation with purified baculovirus-expressed P300. Acetylated CRTC2 detected with anti-acetyl lysine antibody. Right, densitometric analysis of immunoblot in fig. 2d showing relative P300 protein amounts in control (USi) and Ad-P300 RNAi expressing hepatocytes.

Supplementary Figure 16: Immunoblot showing effect of CBP and P300 RNAi-mediated knockdown on amounts of CRTC2 and FOXO1 in livers of fasted mice shown in figure 3a.

Supplementary Figure 17: Left, graphic analysis of Ad-CRE luc activity from in vivo imaging experiment (fig.3d) of fasted mice injected IP with P300 HAT inhibitor Lys-CoA TAT or control (TAT) peptide. (n=3, *P* <.05; data are means ± s.e.m.) Middle, G6Pase and PEPCK mRNA levels in 8 hr. fasted mice injected with Lys-CoA-TAT or TAT control. For Q-PCR data (n=3; *P* < .02). Right, CRE-luciferase reporter activity in primary hepatocytes expressing wild-type (WT) or K628R mutant CRTC2. Luciferase activity normalized to β-galactosidase activity from adenovirally encoded RSV-βgal vector. Effect of Lys-CoA-TAT (20μM) and SRT1720 (SRT; 1μM) on glucagon-stimulated CRE reporter activity shown. Cells were pre-treated with Lys-CoA-TAT or SRT for 1 hour followed by glucagon treatment for 6 hours.

Supplementary Figure 18: Left, densitometric analysis of acetylated CRTC2 protein amounts in primary hepatocytes exposed to glucagon. Effect of Ad-SIRT1 or control Ad-GFP expression shown. Right, effect of SIRT1 over-expression on Ad-CRE-luc reporter activity in primary hepatocytes exposed to glucagon as indicated. (n=4, *P* <.01; data are means ± s.e.m.)

Supplementary Figure 19: Immunoblot (left) and densitometric analysis (middle) showing effect of Ad-SIRT1 expression on total amounts of CRTC2 protein in primary hepatocytes exposed to glucagon for 2 hours. Right, circulating glucose levels in 8 hr. fasted mice expressing Ad-SIRT1 or Ad-GFP control. (n=4, *; *P*<.05; data are means ± s.e.m.).

Supplementary Figure 20: Left, immunoblot showing effect of 1hr. pre-treatment with SIRT1 activator SRT1720 (SRT; 1μM) on amounts of acetylated and total CRTC2 protein in primary hepatocytes exposed subsequently to glucagon (100nM) for 2 hours. Right, immunoblot showing effect of pre-treatment with SIRT1 agonist resveratrol (1 hr) on CRTC2 acetylation and total CRTC2 protein amounts in primary hepatocytes exposed subsequently to FSK for 1 hr.

Supplementary Figure 21: Effect of exposure to SRT1720 (1 hr.) followed by glucagon (4 hrs.) on amounts of CRTC2 protein (left) and glucose output (right) from primary hepatocytes expressing wild-type or acetylation-defective Lys628Arg CRTC2. (n=3, *P* <.01; data are means ± s.e.m.)

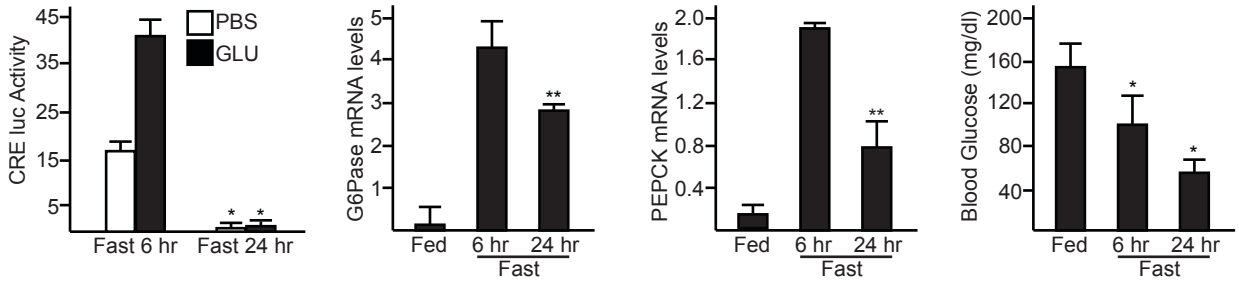
Supplementary Figure 22: Transient assay of primary hepatocytes showing effect of SIRT1 antagonists sirtinol and nicotinamide (NAM) on Ad-CRE-luc reporter activity in cells exposed to glucagon. (n=3, *P* <.01; data are means ± s.e.m.)

Supplementary Figure 23: Left, immunoblot of CRTC2 protein amounts in fasted liver-specific *Sirt1* $^{-/-}$ mice relative to control littermates. Right, Ad-CRE-luc reporter activity in fasted liver-specific *Sirt1* $^{-/-}$ mice relative to control littermates. IP administration of sirtinol indicated. (n=2 mice per group; P <.05; data are means $\pm$ s.e.m.)

Supplementary Figure 24: Q-PCR analysis of G6Pase and PEPCK mRNA levels in wild-type and *Sirt1* $^{-/-}$ hepatocytes exposed to glucagon for 6 hours. (n=4, P <.05; data are means $\pm$ s.e.m.)

Supplementary Figure 25: Sequential activation of CRTC2 and FOXO1 during fasting. Under ad libitum feeding conditions, CRTC2 and FOXO1 are sequestered in the cytoplasm by phosphorylation dependent associations with 14-3-3 proteins. During early fasting, glucagon triggers the dephosphorylation and nuclear entry of CRTC2. Decreases in circulating insulin concentrations also stimulate the dephosphorylation and nuclear entry of FOXO1. P300 modulates fasting gluconeogenesis by acetylating CRTC2 and thereby protecting it from ubiquitin-mediated degradation. Parallel increases in P300-mediated FOXO1 acetylation appear to inhibit its transcriptional activity. During late fasting, the deacetylation of CRTC2 by SIRT1 promotes its ubiquitin-dependent degradation, whereas FOXO1 deacetylation by SIRT1 augments its transcriptional activity.

Supplementary Figure 1



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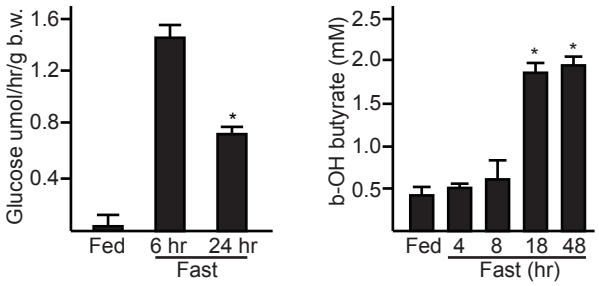

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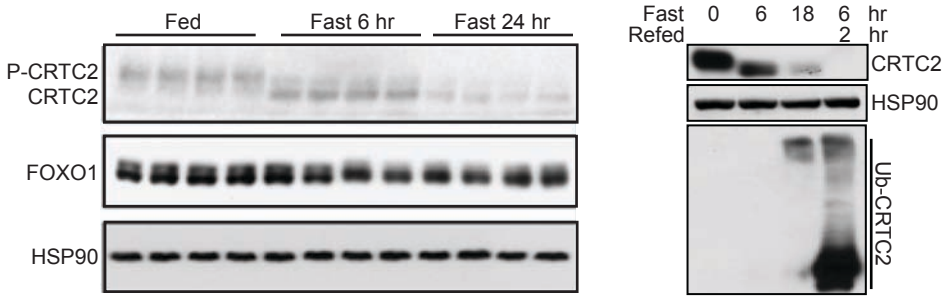

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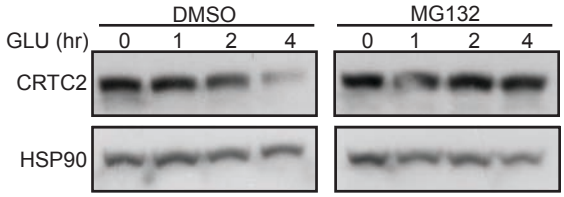

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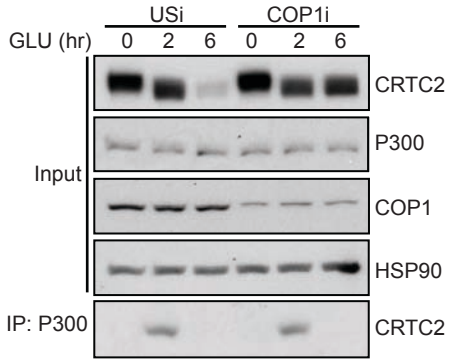

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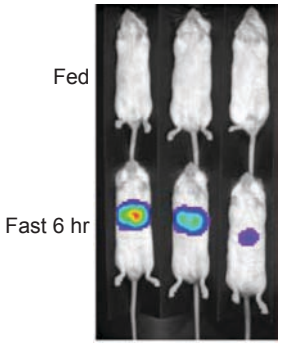

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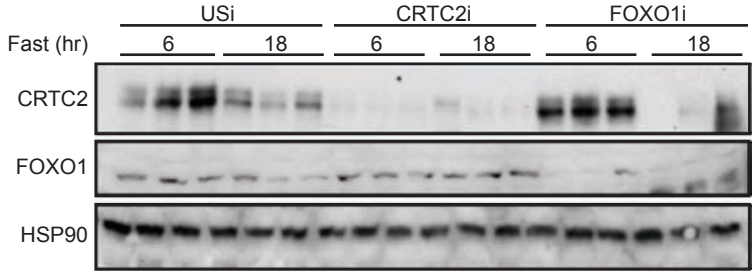

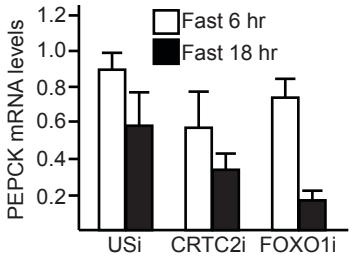

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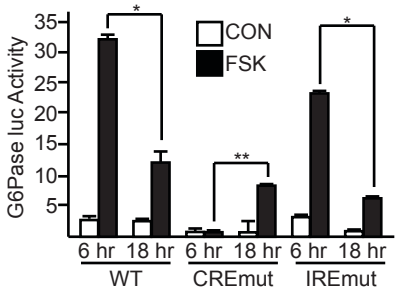

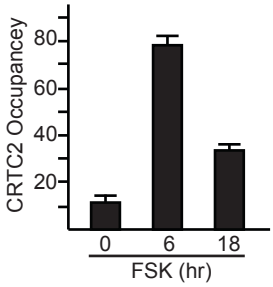

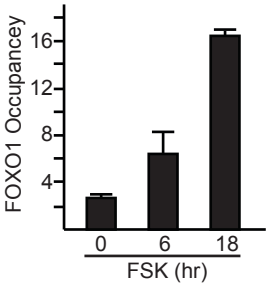

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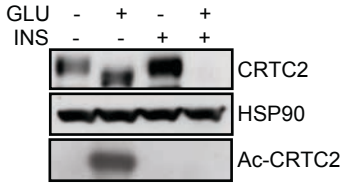

Supplementary Figure 10

	# OF PEPTIDE
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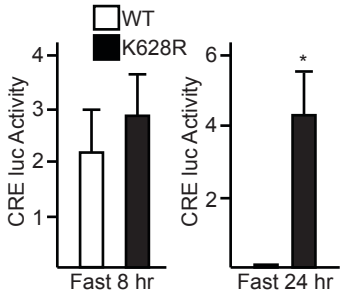
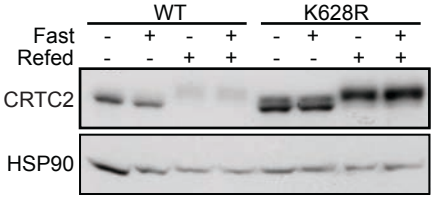
623-SPGFSK*EIAAALAGVPGFEVSASGLELGLGLEDELRM	3

Supplementary Figure 11





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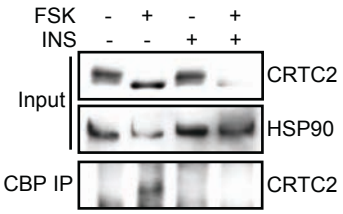

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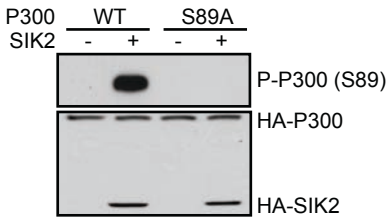

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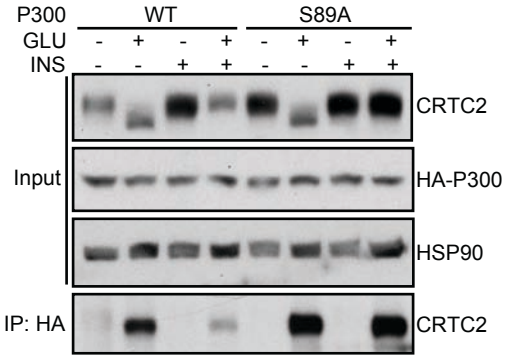

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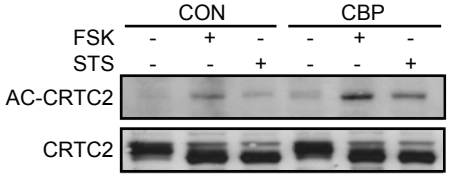

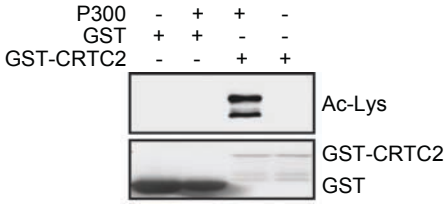

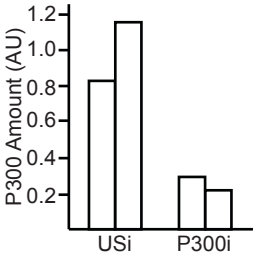

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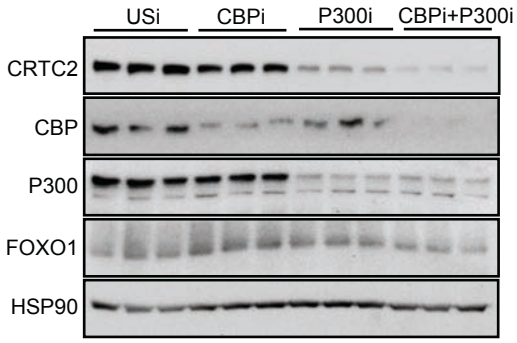

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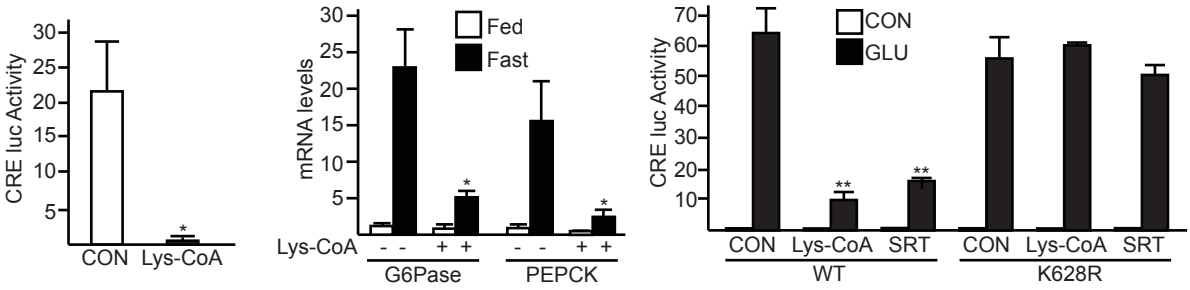

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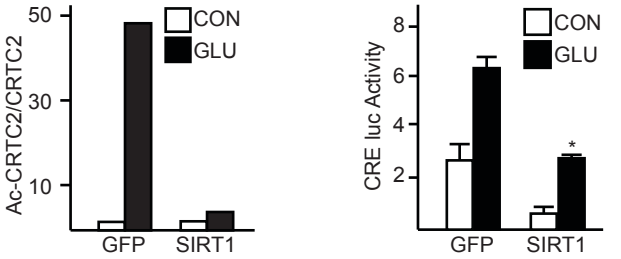

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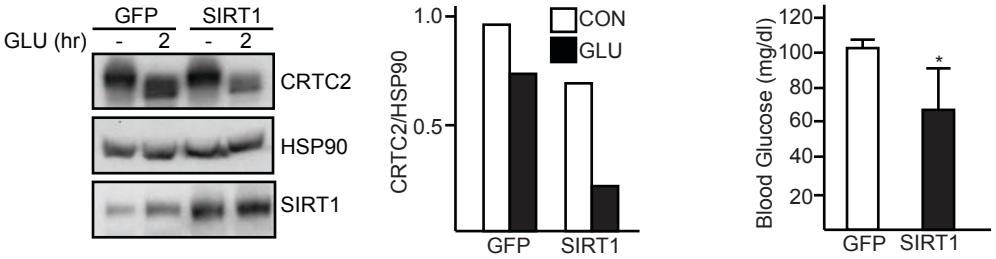

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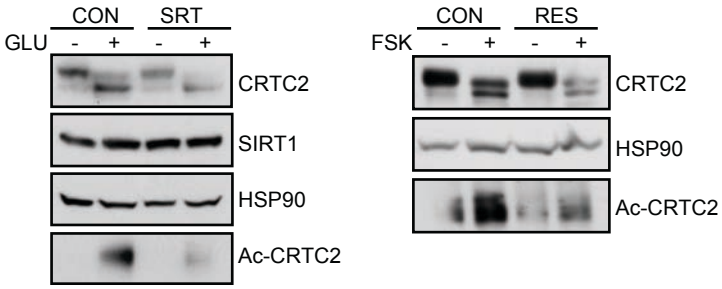

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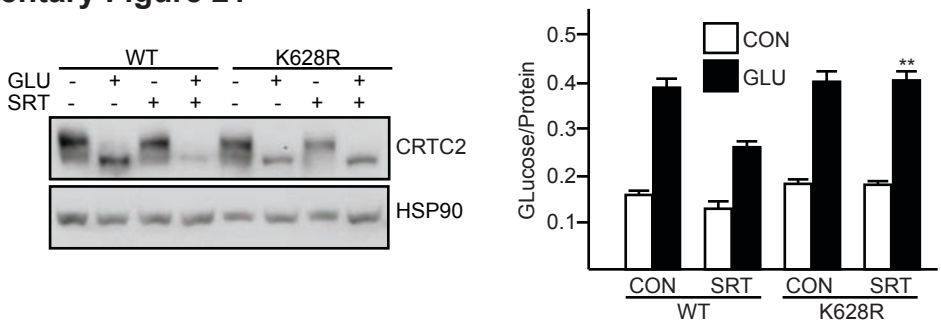

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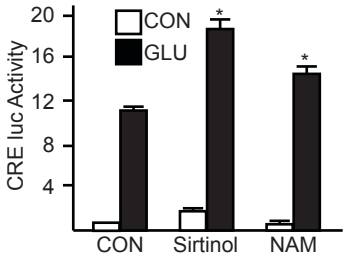

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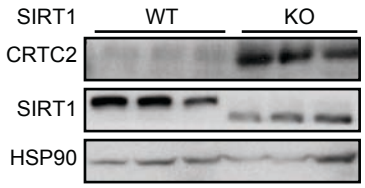

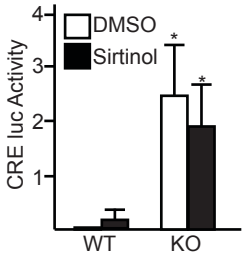

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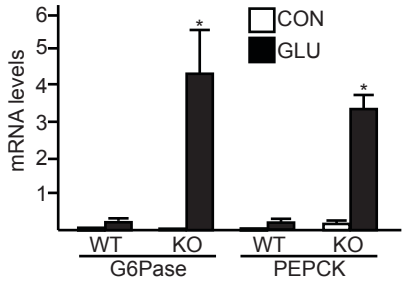

Supplementary Figure 25