

**Hepatic SIRT1 Deficiency Results in Hyperglycemia, Oxidative Damage, and  
Insulin Resistance Mediated by mTORC2/AKT Signaling**

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Key words: SIRT1, Rictor, AKT, Glucose, Insulin, FOXO1

Supplementary table

<b>Primers for Rictor ChIP</b>	Forward	Reverse
mRictor 1897-1777	TAAACAGGCGAGGATGGAC	TTCATCCCTCCACACATCAC
mRictor 1524-1379	AGTACCCCAAGAGAGCAGCAA	TGCTTGGTCACCTACAACCA
mRictor 803-662	GGGTTAAGGGCCAAATGAGT	CCTCGTCCTGGCTTTCTGTT
mRictor 716-543	GATCCTCGACATCGGCATCAG	GCAAGGAGTGCAGAAAGCGGG
mRictor 550-374	CTTTCTGCACTCCTTGACAG	GCCCCGAGTGAACACGTAAC
<b>Primers for qRT-PCR</b>		
mG6Pase	TCCTGGGACAGACACACAAG	CAACTTTAATATACGCTATTGG
mPEPCK	CTGGCACCTCAGTGAAGACA	TCGATGCCTTCCCAGTAAAC
mPGC1a	GGGGTCAGAGGAAGAGATAAAGTTG	GAAAACAGGAACAGCAGCAGAGAC
p21	CACAGCTCAGTGGACTGGAA	ACCCTAGACCCACAATGCAG
CyclinD	CAACAGGTTGTAGGGCTGGT	GGTAATGCCATCATGGTTCC
Rictor	TCTGTGCTTCAGGTTGTTG	GCTTCCTTTGCTTTGTCCAG
IGFBP1	GGCGTTCCACAGGATGGGCTG	CCAGGGATCCAGCTGCCGTGCG
mSIRT1	GACGCTGTGGCAGATTGTTA	GGAATCCCACAGGAGACAGA
18S for human, rat, mouse	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA

## Supplementary figure legends

**Figure S1. More parameters for clamp experiment.** (This supplementary figure is related to Figure 2A)

(A) Blood glucose level before and during clamping experiment in both wild-type and *Sirt1*<sup>LKO</sup> mice.

(B) Glucose infusion rate during clamping experiment in both wild-type and *Sirt1*<sup>LKO</sup> mice.

(C) Measurement of plasma insulin level under both basal and clamping condition.

(D) Glucose uptake in muscle and white adipose tissue (WAT).

(E) Glucose uptake in brown adipose tissue (BAT).

**Figure S2. Downstream genes of AKT-T308 are not changed in *Sirt1*<sup>LKO</sup> livers.** (This supplementary figure is related to Figure 3)

Western blot analysis showing protein levels in SIRT1 mutant and wild-type mice. The phosphorylation level of S6kinase on T389 was quantified based on 10 pairs of mice and presented as a bar graph on the right.

**Figure S3. Rictor level is regulated by nutrient availability in vitro.** (This supplementary figure is related to Figure 5)

(A) Hepa G2, Hepa1-6 and 293 cells were starved in glucose free medium for overnight, then fed with 5mM or 20mM glucose for an hour. Samples were taken from different treatment, and total cellular protein was analyzed with western blotting to assess the

relationship between SIRT1 and Rictor.

(B) Over expression of SIRT1 up-regulates intracellular Rictor level. 239 Cells were transfected with either GFP or SIRT1 plasmids. Twenty-four or forty-eight hours post transfection, the cells were collected and proteins were analyzed with western blotting with antibodies against Rictor and SIRT1. The quantification of 4 independent experiments is presented as a bar graph on the right.

(C) Knocking down SIRT1 dramatically reduced Rictor mRNA level.

**Figure S4. SIRT1 level does not affect total amount of NRF1.** (This supplementary figure is related to Figure 5).

(A) In the liver of *Sirt1*<sup>LKO</sup> mice, NRF1 total level does not change revealed by Western blot analysis.

(B) Knocking down SIRT1 (T1KD) in Hepa1-6 cells does affect the mRNA level of NRF1.

(C) In Hepa1-6 cells, over-expression of NRF1 does not affect Rictor promoter luciferase activity; when SIRT1 was knocked down, over-expressing NRF1 also cannot over-ride the reduction of luciferase activity due to SIRT1 reduction. Levels of SIRT1 and NRF1 are indicated in the inserts.

(D) In Hepa1-6 cells, NRF1 mRNA level does not change with SIRT1 over-expression compared with transfection of a GFP construct control.

**Figure S5. Anti-oxidant treatments do not affect intrinsic hepatic glucose over-production in *Sirt1*<sup>LKO</sup> mice.** (This supplementary figure is related to Figure 7).

- (A) In 2 months old *Sirt1*<sup>LKO</sup> mice, increased ROS was only observed in BAT.
- (B) Total protein level of each mTORC2 component is not altered in old *Sirt1*<sup>LKO</sup> mice. Proteins from WAT, BAT and muscle were analyzed.
- (C) NAC or Resveratrol treatment did not overcome hepatic glucose overproduction in SIRT1 mutant mice compared with wild type mice.
- (D) NAC or Resveratrol treatment did not improve the phosphorylation of S473 on AKT.

**Figure S6. In *Sirt1*<sup>LKO</sup> mice, FOXO1 acetylation level only increased after 24 h fasting.**

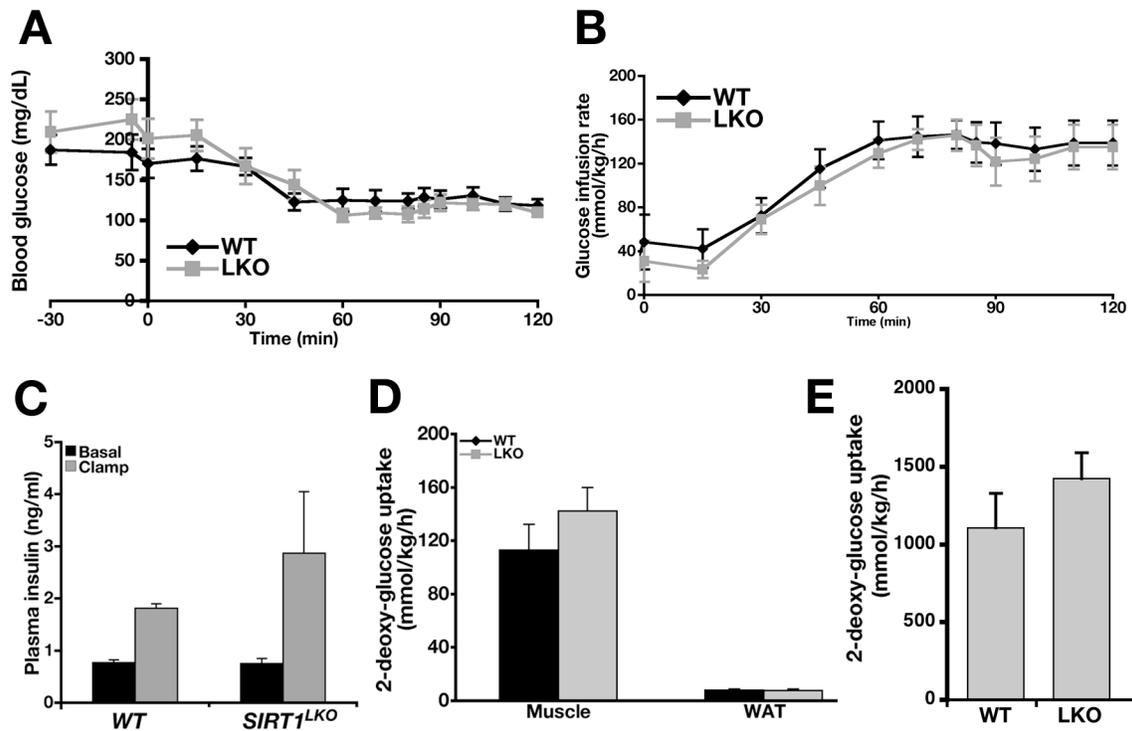
- (A) Acetylation of FOXO1 in 2-month-old mice under different length of fasting by Western blot analysis.
- (B) FOXO1 acetylation in 2-month, 6-month and 12-month old mice under different length of fasting. Bar graph represents quantification of western blots. \* $p < 0.05$ .

**Figure S7. Phenotype analysis of *Sirt1*<sup>LKO</sup> mice in C57BL/6 background.**

- (A) Blood glucose content in fed condition (n=8 pairs).
- (B) qRT-PCR analysis of gene expression between SIRT1 mutant and wild type control mice.
- (C) Western blot shows the livers of *Sirt1*<sup>LKO</sup> mice still display reduced Rictor protein level, decreased phosphorylation on S473 of AKT, increased level of G6pase, Pepck and p21.
- (D) GTT analysis demonstrates *Sirt1*<sup>LKO</sup> mice are still glucose intolerant. (n=8 pairs). \* $p < 0.05$ .

Supplementary Figures

Supplementary Figure 1



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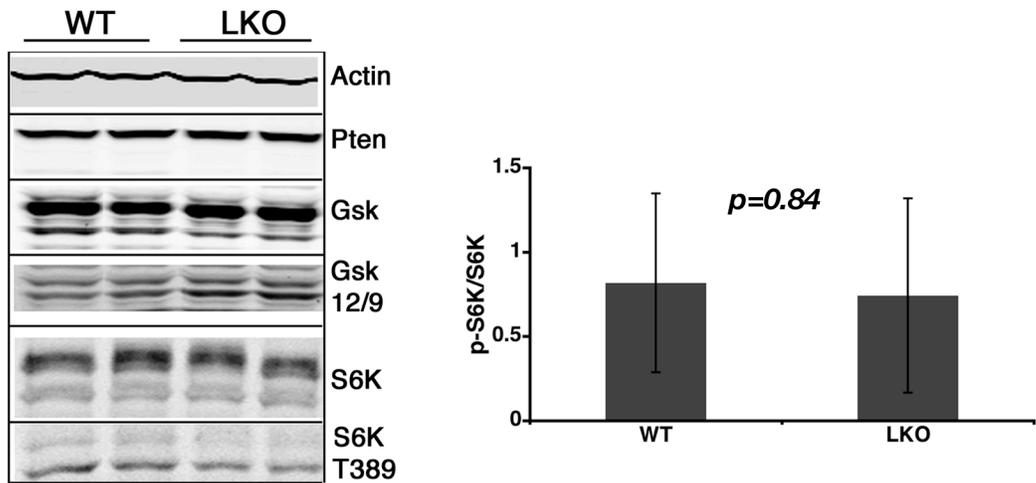
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Supplementary Figure 2

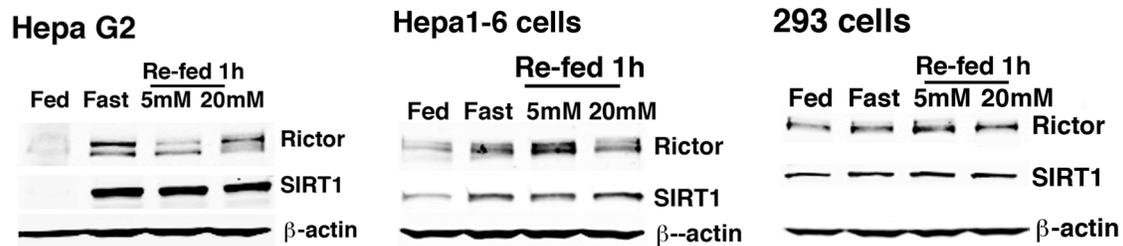


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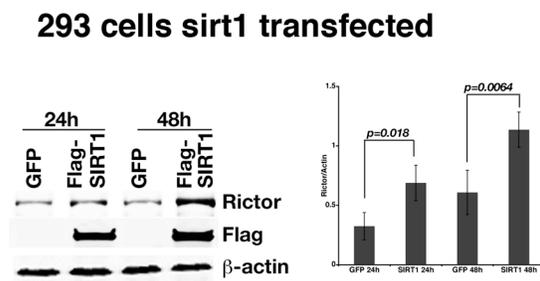
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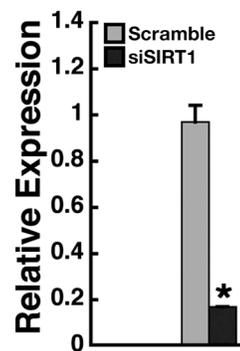
**A**



**B**



**C**



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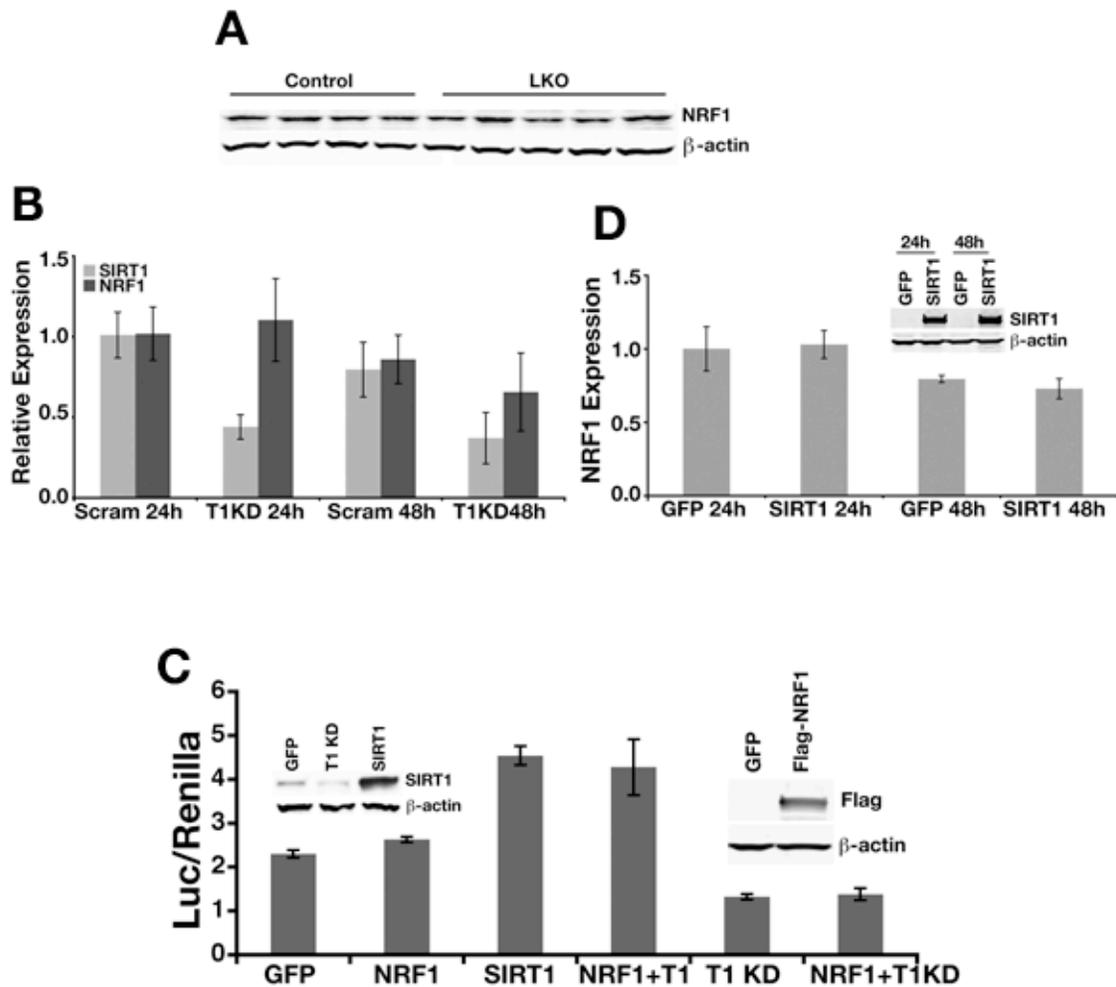
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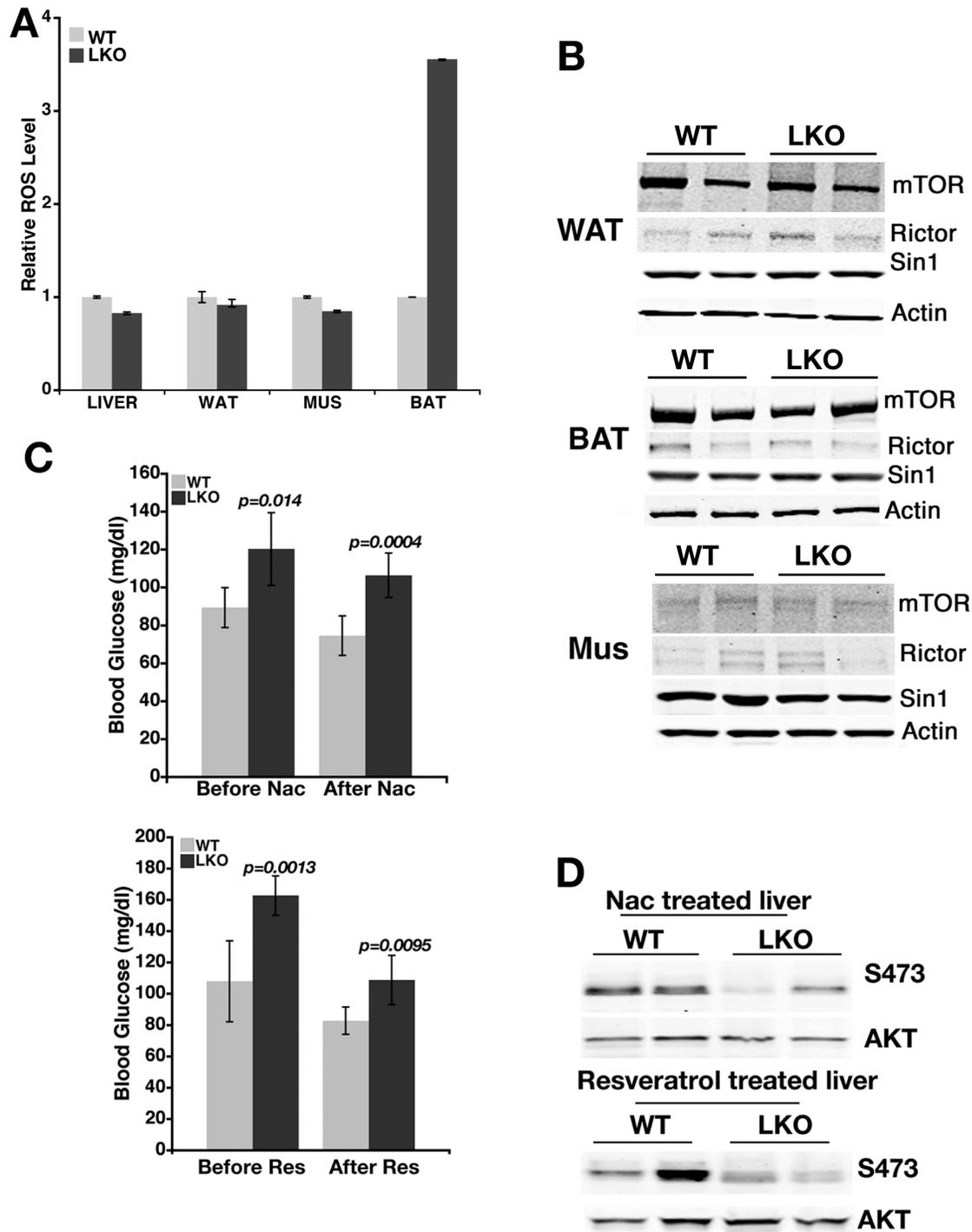


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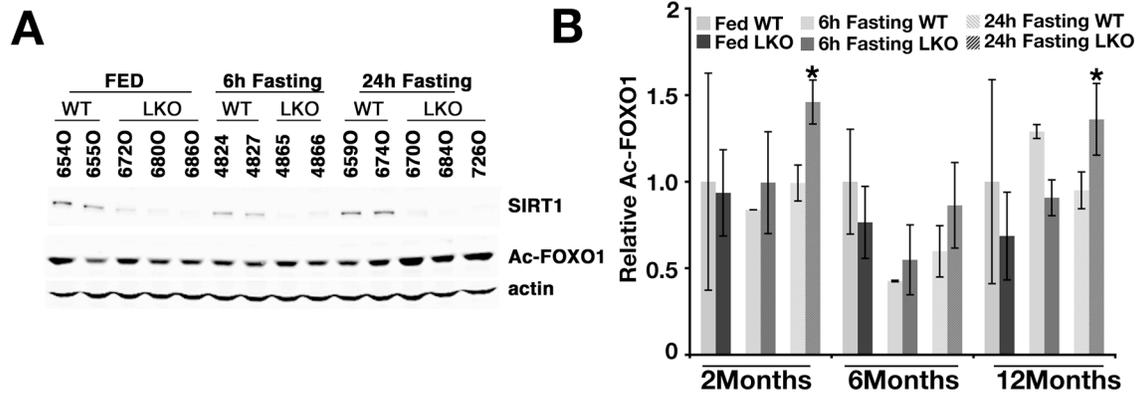
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Supplementary Figure 6



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Supplementary Figure 7

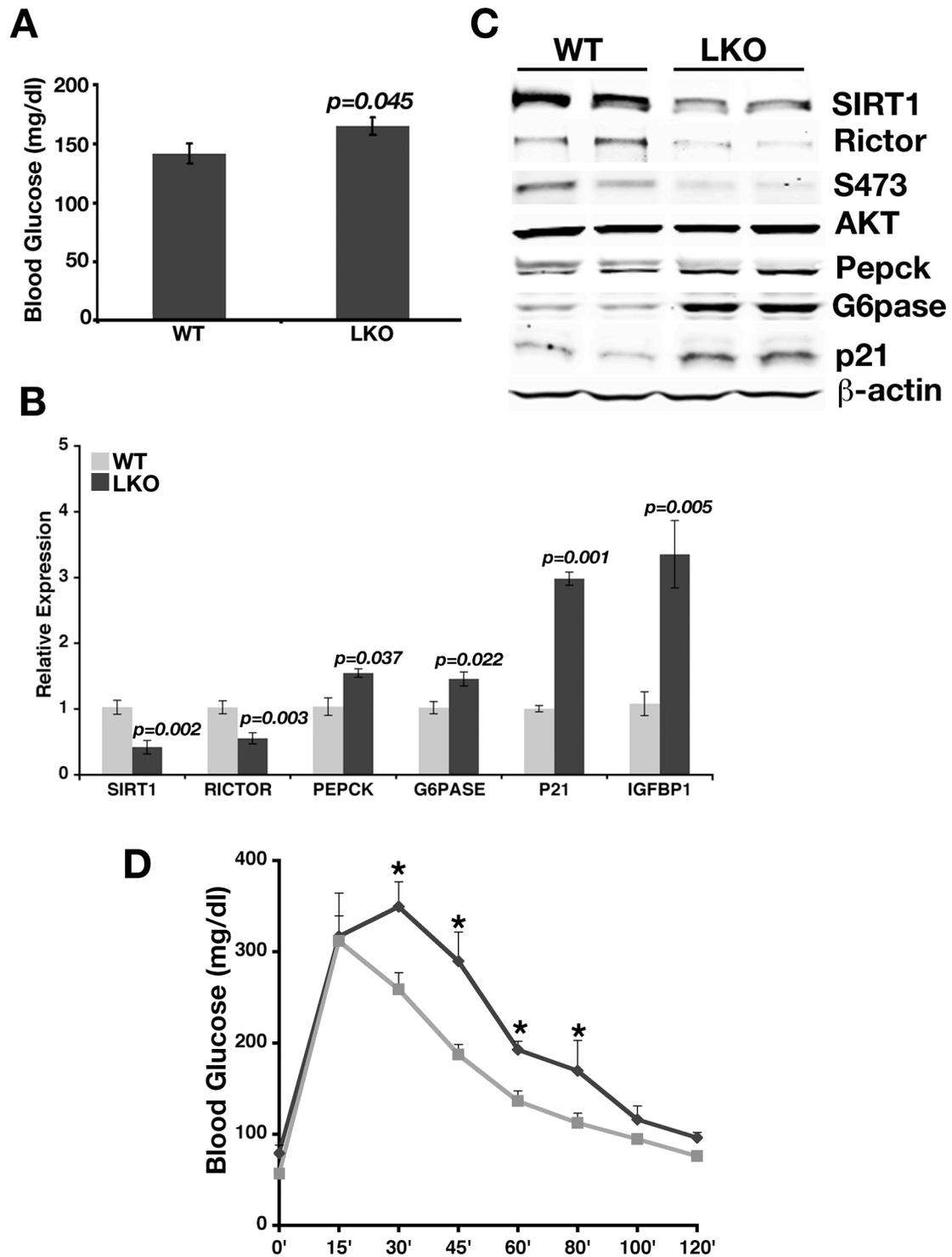


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