Supplemental Information

Autophagy regulates the liver clock and glucose metabolism by degrading CRY1

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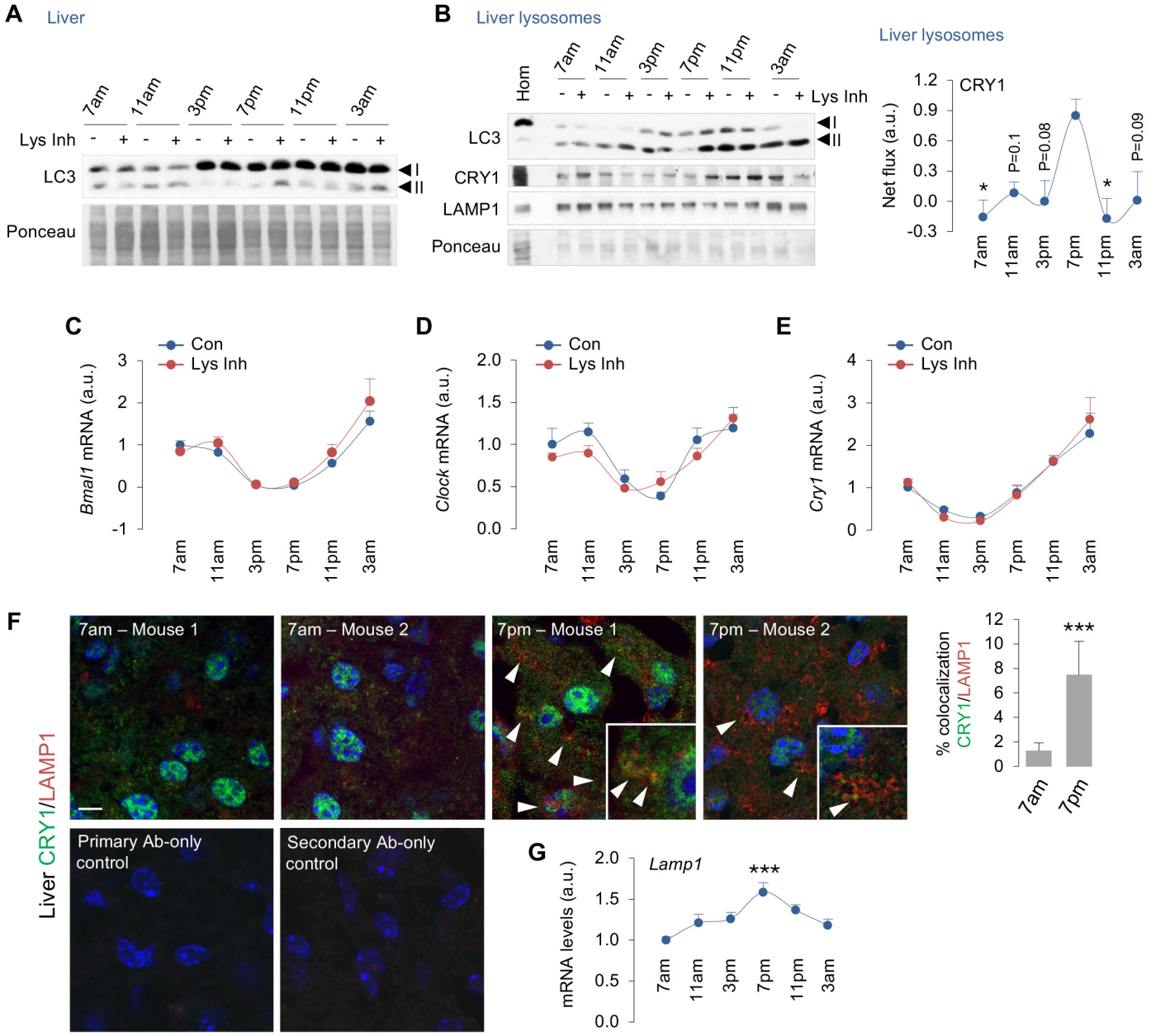

Figure S1, related to Fig. 1. Time-restricted degradation of core circadian proteins in lysosomes

(A, B) Immunoblot (IB) for indicated proteins in liver homogenates and liver lysosome fractions isolated at indicated timepoints in *ad libitum*-fed male mice injected with vehicle or lysosomal inhibitors (Lys Inh) intraperitoneally (i.p.) as shown in Fig. 1A, n=3.

(C-E) RT-PCR for indicated genes from livers harvested at the six indicated timepoints across 24 hr from *ad libitum*-fed male mice injected with vehicle or Lys Inh as depicted in Fig. 1A, n=3.

(F) Indirect immunofluorescence for CRY1 (green) and LAMP1 (red) in liver sections from mice sacrificed at 7am and 7pm. Data from livers from two distinct animals for each time point are shown. Primary and secondary antibody-only controls are shown. Quantification for colocalization of CRY1 with LAMP1 at the indicated timepoints is depicted, n=4. Scale bar: 5 μm

(G) RT-PCR for *Lamp1* from livers at the six indicated timepoints across 24 hr from *ad libitum*-fed male mice, n=5.

Values are mean ± s.e.m., *P<0.05, ***P<0.001. In panels S1B, comparisons are made to the 7pm value. One-way and Two-way ANOVA and Bonferroni correction.

Figure S1

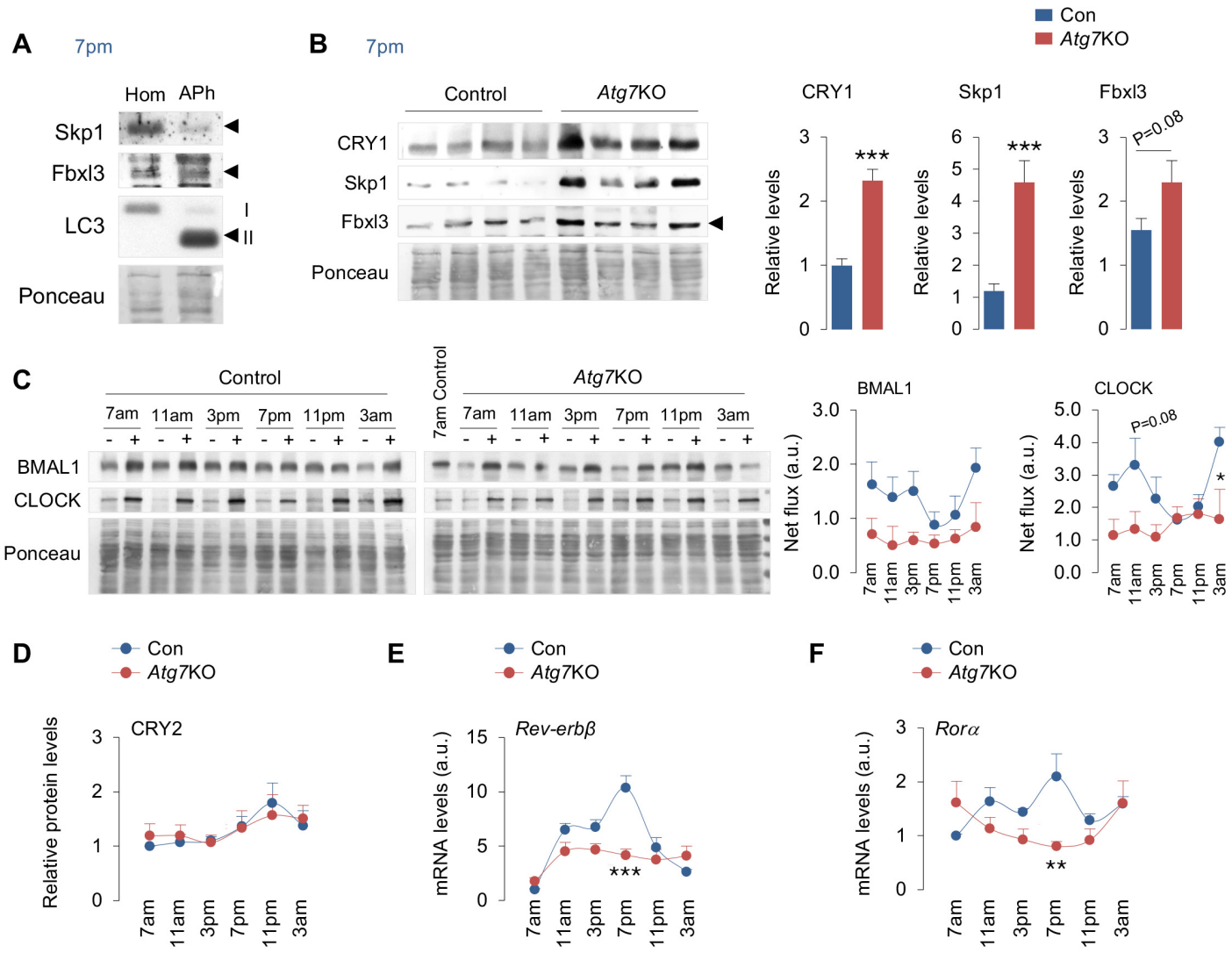

Figure S2, related to Fig. 2. Degradation of CRY1 by autophagy, and effects of loss of autophagy on the liver clock.

(**A**) Immunoblot (IB) for indicated proteins in homogenate (Hom) and autophagosome (APh) fractions from liver at 7pm, n=3 livers per APh sample.

(**B**) IB for indicated proteins at indicated timepoints in homogenate (Hom) from *ad libitum*-fed male mice injected with control or *Cre*-expressing AAVs (*Atg7*KO) for 13 days, n=8-12. Quantification for levels of indicated proteins are shown.

(C) IB and quantification for indicated proteins at indicated timepoints in homogenate (Hom) from *ad libitum*-fed male mice injected with control or *Cre*-expressing AAVs (*Atg7*KO) for 13 days, n=4-7. Densitometry values of signal in blots from *Atg7*KO mice were normalized to the 7am control sample included in each gel.

(D) Quantification for CRY2 immunoblots in Figure 2C, n=5-7.

(E) RT-PCR for *Rev-erbβ* in livers at six indicated timepoints across 24 hr from *ad libitum*-fed male mice injected with control or *Cre*-expressing AAVs (*Atg7*KO) for 13 days, n=5-7.

(F) RT-PCR for *Rorα* in livers at six indicated timepoints across 24 hr from *ad libitum*-fed male mice injected with control or *Cre*-expressing AAVs (*Atg7*KO) for 13 days, n=5-9.

Values are mean ± s.e.m. *P<0.05, **P<0.01, ***P<0.001 compare values in *Atg7*KO mice to corresponding values in Con mice. Student's T-test or Two-way ANOVA and Bonferroni correction.

Figure S2

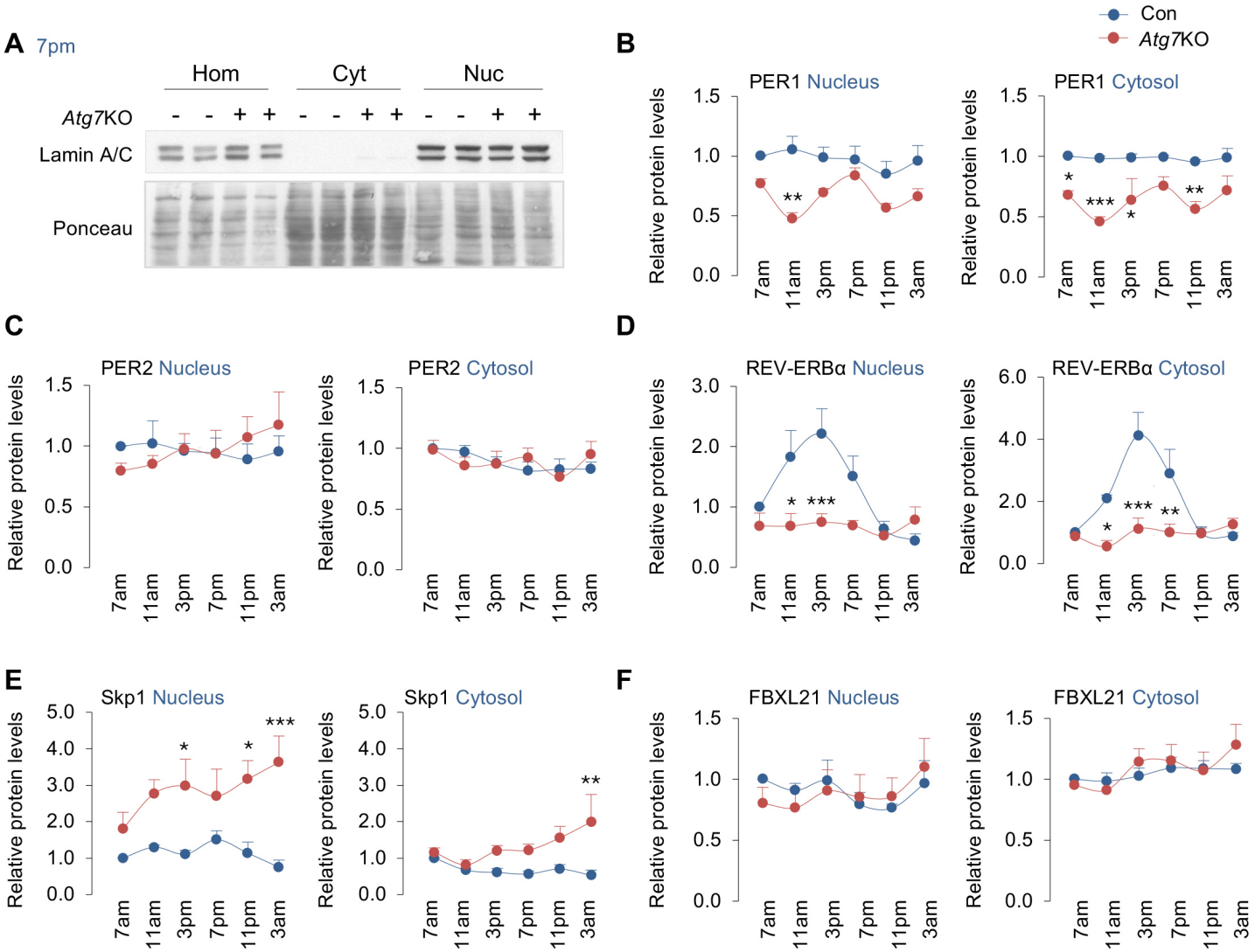

Figure S3, related to Fig. 3. Effects of loss of autophagy on changes in nuclear and cytosolic levels of core circadian proteins, and of FBXL21.

(A) Immunoblots for indicated proteins in homogenates (Hom), cytosol, and nuclear (Nuc) fractions from livers at 7pm from *ad libitum*-fed male mice injected with control or *Cre*-expressing AAVs (*Atg7*KO) for 13 days, n=6.

(B-F) Immunoblots for the indicated proteins in nuclear and cytosolic fractions from livers collected at the indicated timepoints from *ad libitum*-fed male mice injected with control or *Cre*-expressing AAVs (*Atg7*KO) for 13 days, n=4.

Values are mean ± s.e.m. Two-way ANOVA and Bonferroni correction. *P<0.05, **P<0.01, ***P<0.001.

Figure S3

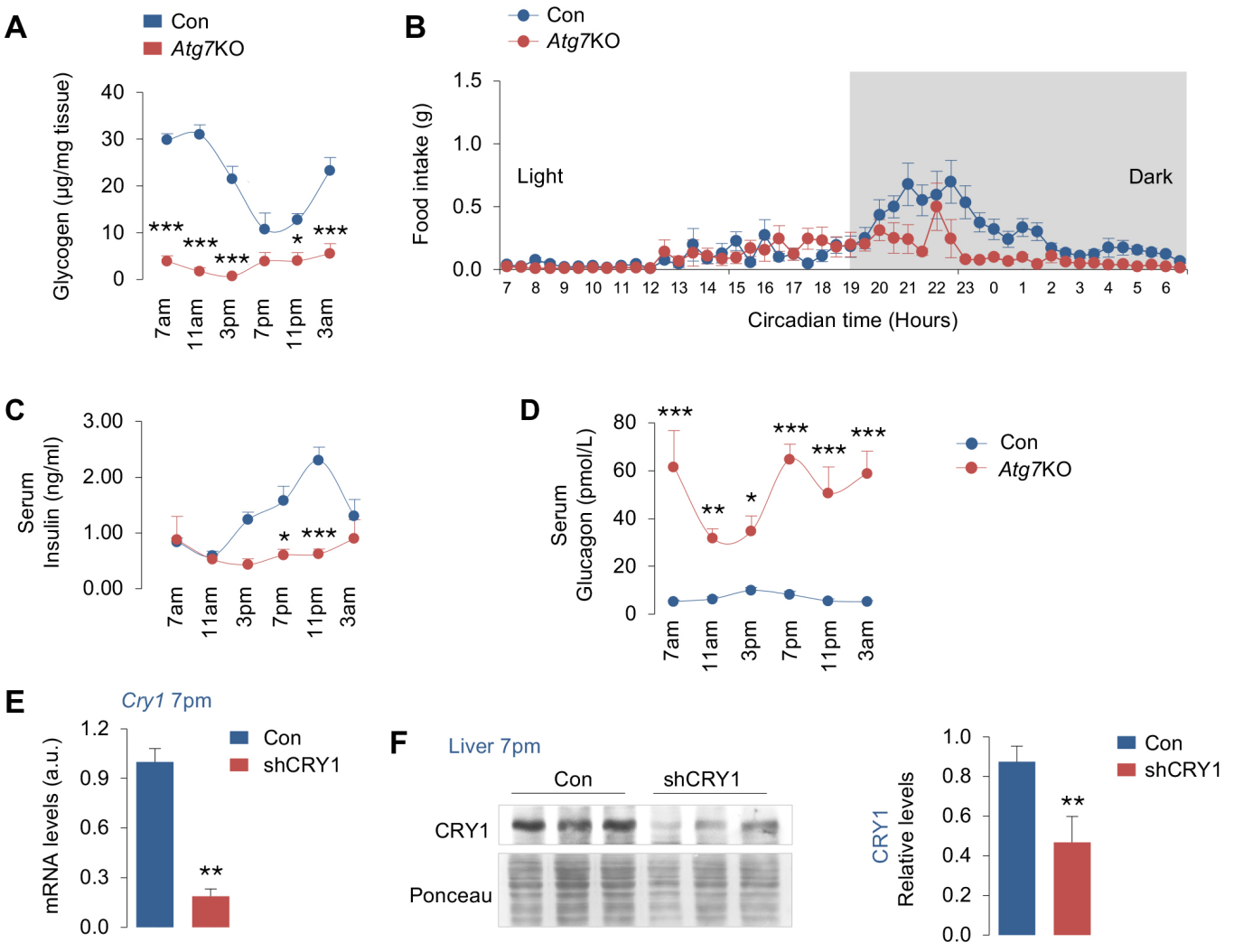

Figure S4, related to Fig. 4. Effects of loss of liver-specific autophagy on liver glycogen, circadian feeding pattern, serum glucagon and insulin levels, and validation of reduction of *Cry1* expression/CRY1 levels after shCRY1 tail vein injections.

(A) Liver glycogen levels at the indicated timepoints from *ad libitum*-fed male mice injected with control or *Cre*-expressing AAVs (*Atg7*KO) for 13 days, n=6-7.

(B) Circadian changes in food intake by *ad libitum*-fed male mice assessed for over 7 days after injections with control or *Cre*-expressing AAVs (*Atg7*KO), n=4.

(C) Serum insulin levels at the indicated timepoints in *ad libitum*-fed male mice injected with control or *Cre*-expressing AAVs (*Atg7*KO) for 13 days, n=3-8.

(D) Serum glucagon at the indicated timepoints in *ad libitum*-fed male mice injected with control or *Cre*-expressing AAVs (*Atg7*KO) for 13 days, n=3-8.

(E, F) RT-PCR for *Cry1* and immunoblot for CRY1 in livers from male mice 1 day after tail vein injections of adenoviruses expressing Con or shCRY1 constructs, n=4-5. Ponceau is the loading control.

Values are mean ± s.e.m. Student's T-test or Two-way ANOVA and Bonferroni correction. *P<0.05, **P<0.01, ***P<0.001.

Figure S4

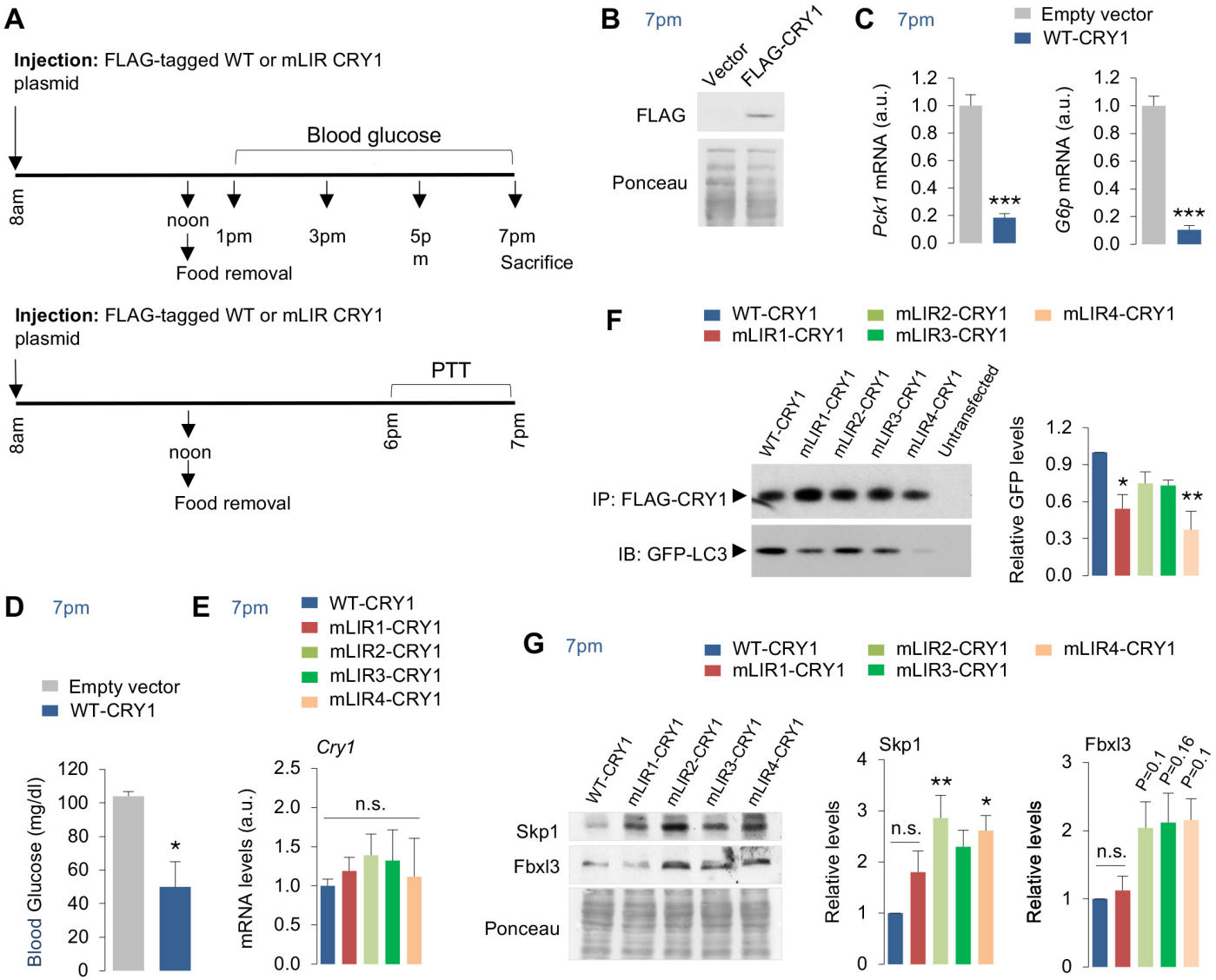

Figure S5, related to Fig. 5. LIR motifs on CRY1 determine their degradation and regulation of gluconeogenesis

(A) Plan depicting injections of plasmids expressing FLAG-tagged WT-CRY1 or mutant LIR (mLIR) CRY1 in male mice followed by blood glucose analyses or PTT.

(B-D) Immunoblots (IB) for FLAG, RT-PCR for indicated genes in liver, and blood glucose levels in mice at 7pm after injections with FLAG-tagged WT-CRY1 plasmid or empty plasmid as per plan in panel S4A, n=4-6.

(E) RT-PCR for *Cry1* at 7pm in livers expressing FLAG-tagged WT-CRY1 or each CRY1 LIR mutant (mLIR1-4) plasmid, n=4-6.

(F) Co-IP of FLAG and GFP in 293T cells untransfected or transfected with FLAG-tagged WT-CRY1 or indicated mLIR CRY1 and GFP-tagged LC3 plasmid. Quantitation for GFP-tagged LC3 is depicted, n=3.

(G) IB for Skp1 and Fbxl3 in livers at 7pm expressing FLAG-tagged WT-CRY1 or each CRY1 LIR mutant (mLIR1-4) plasmid, n=4-6.

Values are mean ± s.e.m. *P<0.05, **P<0.01, ***P<0.001. Student's T-test or One-way ANOVA and Bonferroni correction.

Figure S5

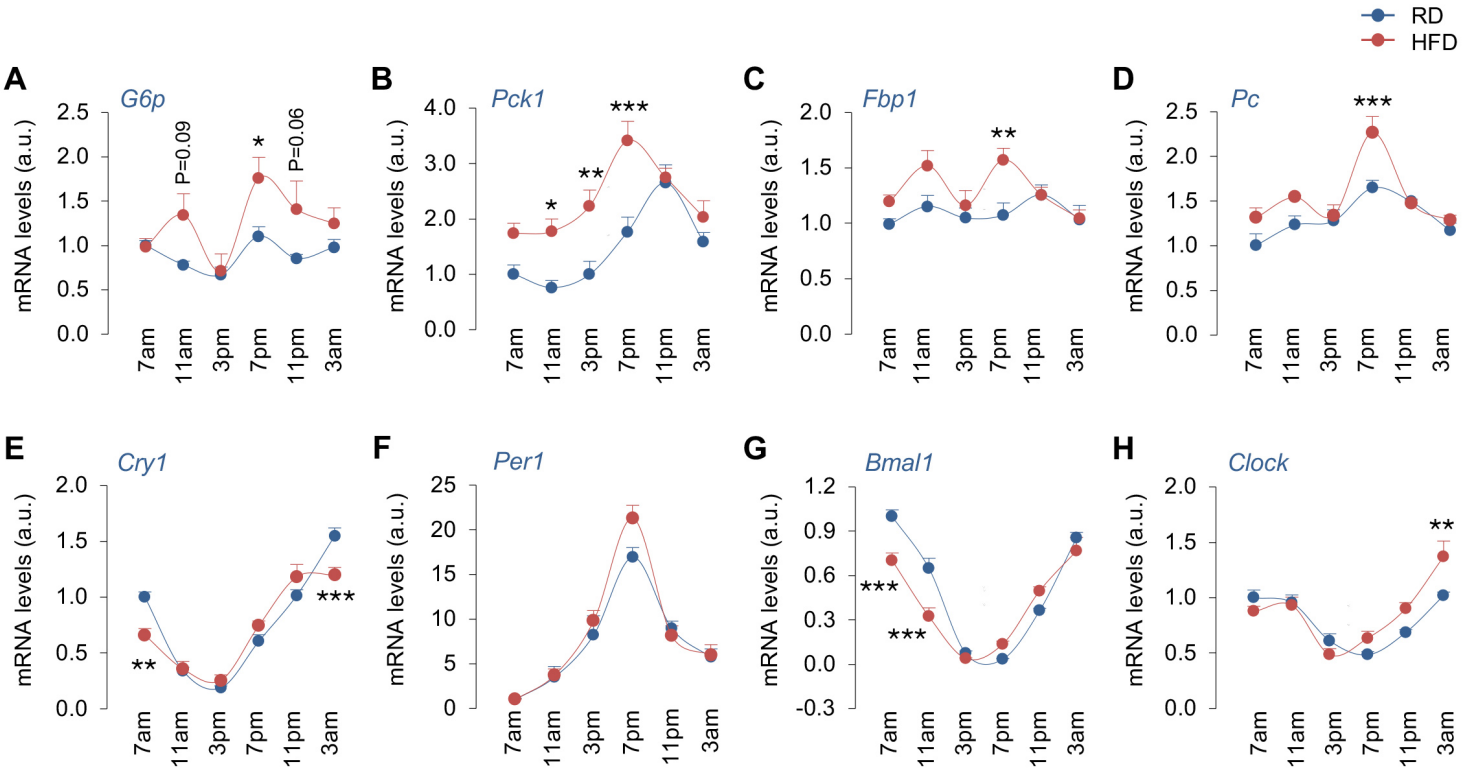

Figure S6, related to Fig. 6. Effect of HFD feeding on expression of gluconeogenic and core circadian genes in liver.

(A-H) RT-PCR for indicated gluconeogenic and circadian genes at indicated timepoints across 24 hr in livers from male mice fed a regular chow diet (RD) or high fat diet (HFD; 60% calories in fat) for 8 weeks, n=5.

Values are mean ± s.e.m. Two-way ANOVA and Bonferroni correction. **P<0.01, ***P<0.001.

Figure S6

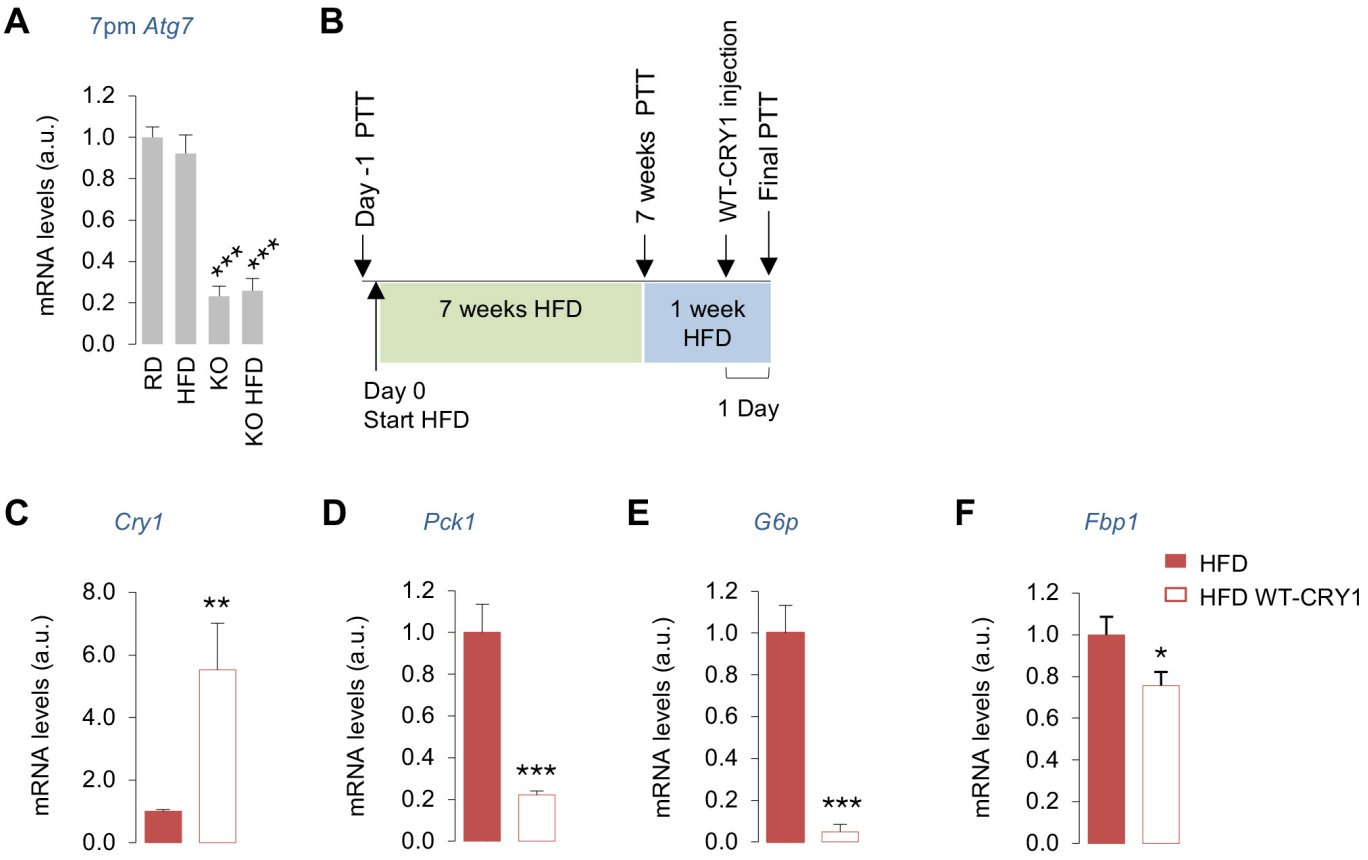

Figure S7, related to Fig. 7. Autophagic degradation of CRY1 and regulation of gluconeogenesis in obese mice.

(A) RT-PCR for *Atg7* in livers from RD or HFD-fed mice, (n=4).

(B) Experimental plan for expression of WT-CRY1 in livers from HFD-fed mice, i.e., first PTT on day -1, HFD initiated for 8 weeks on day 0, second PTT after 7 weeks of HFD feeding, WT-CRY1 injection 1 day before completion of 8 weeks of HFD feeding, and final PTT the following day.

(C-F) RT-PCR for indicated genes in livers collected at 7pm from male mice fed HFD *ad libitum* for 8 weeks and injected with wildtype (WT) CRY1 as shown in plan in panel **B**, n=5-6.

Values are mean ± s.e.m. Student's T-test or Two-way ANOVA and Bonferroni correction. *P<0.05, **P<0.01, ***P<0.001.

Figure S7

TABLE S1. Primers for real-time PCR analysis related to STAR Methods

GENE	PROTEIN	PRIMERS
Atg7	Autophagy related 7	(f) 5'-tccgttgaagtcctctgctt-3' (r) 5'-ccactgaggttcaccatcct-3'
Bmal1	Aryl hydrocarbon receptor nuclear translocator-like protein 1	(f) 5'-aaatccacaggataagaggg-3' (r) 5'-atagtccagtggaaaggaatg-3'
Clock	Circadian locomoter output cycles protein kaput	(f) 5'-aagtgactcattaacccctg-3' (r) 5'-ctatgtgtgcgttgtatagttc-3'
Cry1	Cryptochrome-1	(f) 5'-cgtttggaaaggcatttgg-3' (r) 5'-cttccattttgtcaaagcgtg-3'
G6Pase	Glucose-6-phosphatase	(f) 5'-gtttggtttcgcgcttggat-3' (r) 5'-gccgctcacaccatctctta-3'
Pc	Pyruvate carboxylase	(f) 5'-cagggcggagctaacatctac-3' (r) 5'gacatttggggaggcaacag-3'
Pck1	Phosphoenolpyruvate carboxykinase	(f) 5'-cttgctctaccgtgagcctc-3' (r) 5'-accacaatcaccagatcacc-3'
Per1	Period circadian protein homolog 1	(f) 5'-gttctcatagttcctcttctg-3' (r) 5'-gtgagtttgtactcttgctg-3'
Per2	Period circadian protein homolog 2	(f) 5'-ctttcactgtaagaaggacg-3' (r) 5'-ctgagtgaaagaatctaagcc-3'
Reverbα	REV-ERB alpha	(f) 5'-ctggagggctgcagtatagc-3' (r) 5'-tattggagtccagggtcgtc-3'
Reverbβ	REV-ERB beta	(f) 5'-tcctctagctctgcctccag-3' (r) 5'-tggttttgcctgttttcaca-3'
RORα	Nuclear receptor ROR-alpha	(f) 5'-gcacctgaccgaagacgaaa-3' (r) 5'-gagcgatccgctgacatca-3'