Supplementary Information

Supplementary Figure Legends:

Supplementary Figure 1. (A) 293T stable cells expressing various FLAG-tagged clock proteins were exposed to serum shock before immunoblotting of FLAG-tagged proteins, PER1-FLAG, PER2-FLAG, and BMAL1-FLAG.

Supplementary Figure 2. (A) CRY1 protein induction by serum shock was observed in three additional cell types: NIH3T3, PMH (primary mouse hepatocytes) and MEF (mouse embryonic fibroblasts). **(B)** Testing the specificity of CRY1 antibody. Hepa-1 cells were first transfected with either *shCon* or *shCry1* vector for 48 hr and then treated with 2-hr serum shock. The endogenous CRY1 protein levels were determined by immunoblotting with anti-CRY1.

Supplementary Figure 3. (A) The mRNA levels of major human core clock genes in 293T cells after serum shock treatment were determined by QPCR: *Bmal1, Clock, Per1* and *Dbp*. The data were plotted as Mean ± S.D. (n=3). * *p*-value < 0.05 by Students' *t*-test. **(B)** The mRNA level of *Fbxl3* in 293T cells after serum shock treatment was determined by QPCR. The data were plotted as Mean ± S.D. of triplicates. * *p*-value < 0.05 by Student's *t*-test. **(C)** The effects of *Fbxl3* depletion on the CRY1 protein following serum shock treatment. Hepa-1 cells were transfected with *shLacz* or *shFbxl3* before treatment with serum shock. The protein levels for both CRY1 and FBXL3 were detected by immunoblotting.

Supplementary Figure 4. (A) The mRNA levels of *Usp2a* were significantly induced in Hepa-1 cells upon serum shock. The data were plotted as Mean ± S.D. of triplicates. * *p*-value < 0.05.

(B) Serum shock induced the expression of USP2a, the 69-kD isoform of USP2 protein, in Hepa-1 cells.

Supplementary Figure 5. USP2a does not regulate the de-ubiquitination process of clock proteins, CRY2, BMAL1 and PER1. The expression vectors encoding the clock genes were co-transfected with Myc-tagged *Usp2a* expression vector in the *shUsp2a*-expressing *293T* stable cell line (the same cell line used in Figure 2C). Following the transfection, cells were then treated with MG132 and subjected to denaturing IP for detecting clock protein-ubiquitin conjugates. The input level of CRY2-FLAG, BMAL1-FLAG and PER1-FLAG were detected by immunoblotting with anti-FLAG antibody.

Supplementary Figure 6. The mRNA expression of *Usp2a* and *Cry1* in Hepa-1 cells after synchronization by serum shock. The cells were harvested at the indicated times following 2-h serum shock. The mRNA levels of *Usp2a* and *Cry1* were determined by the QPCR. The results were plotted as Mean ± Range (n=2).

Supplementary Figure 7. (A) Adenoviral-CRY1 mediated dose-dependent over-expression of FLAG-CRY1 protein in Hepa-1 cells. **(B)** Over-expression of CRY1 in Hepa-1cells down-regulates its target genes *Per2* and *Dbp*. The data were plotted as Mean ± S.D. of triplicate samples. * p-value < 0.05 by Students' t-test.

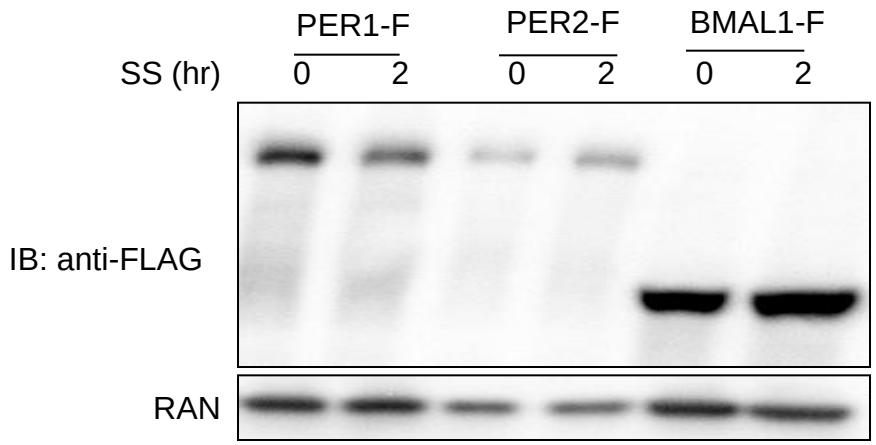
Supplementary Figure 8. **(A)** The mRNA expression pattern of *Dbp*, the other CRY1 target, in the synchronized Hepa-1 cells that were infected with adenovirus expressing either *shLacZ* or *shUsp2a*. AUC analysis for *Dbp* expression during the entire time course was calculated and shown on the right. * *p-value*< 0.05. **(B)** The mRNA expression pattern of *Rev-erbα* in synchronized Hepa-1cells that were infected with adenovirus expressing either *shLacZ* or

shUsp2a. AUC analysis for *Rev-erbα* expression during the entire time course was calculated and shown on the right. There was no statistically significant difference between the two groups.

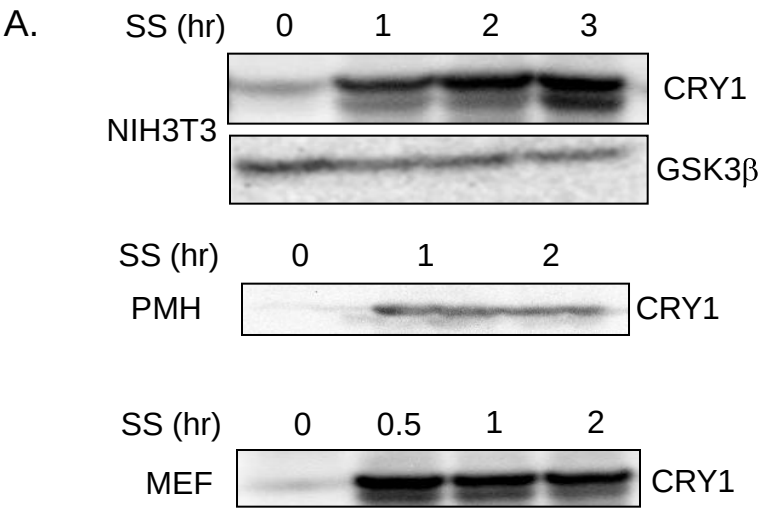
Supplementary Figure 9. **(A)** The *Bmal1*-luc activity was inhibited by over-expression of *Usp2a*-WT but not *290CA* mutant. The luciferase activities were measured and calculated 72 hr after transfection. The data were plotted as Mean ± S.D. (n= 4). * *p* value < 0.05 by Student's *t*-test. **(B)** Effect of *Usp2a* overexpression on the period and amplitude of the *Bmal1*-luciferase in U2OS cells. Manipulation of USP2a protein level was achieved by transfection with *Usp2a* expression vector before synchronization by dexamethasone and forskolin. A representative of three replicates was shown in bioluminescence graph. Amplitude and period length of three replicates were shown as Mean ± S.D. for each treatment. * *p* value < 0.05 was determined by Student's *t*-test.

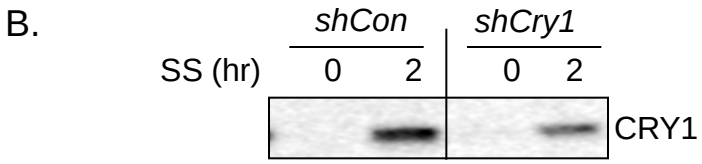
Supplementary Figure 10. (A) TNF-α treatment (10 ng/mL) inhibited BMAL1/CLOCK-mediated activation of the *Per2*-luc reporter activity in Huh7 cells**. (B)** TNF-α treatment lowered *Dbp* but not *Cry1* mRNA levels in Huh7 cells. **(C)** TNF-α dose-dependently induced the CRY1 protein in HepG2 cells. The cells were treated with TNF-α for 16 hr and then harvested for immunoblotting assay. The CRY1 proteins were detected in both cytoplasmic and nuclear fractions. **(D)** TNF-α treatment did not affect the endogenous REV-ERBα protein in Huh7 cells. **(E)** The endogenous *Usp2a* mRNA levels in Huh7 cells following transfection with either *shUSP2a* or *shRNA* control vector. The samples were collected 72 hr post transfection. The data were plotted as Mean ± S.D. of triplicate samples. * *p*-value < 0.05 by Student's *t*-test.

Supplementary Figure 1



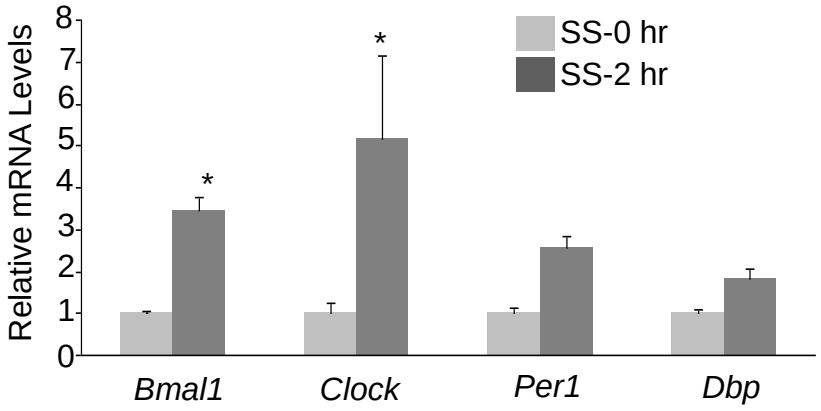
Supplementary Figure 2





Supplementary Figure 3

A.



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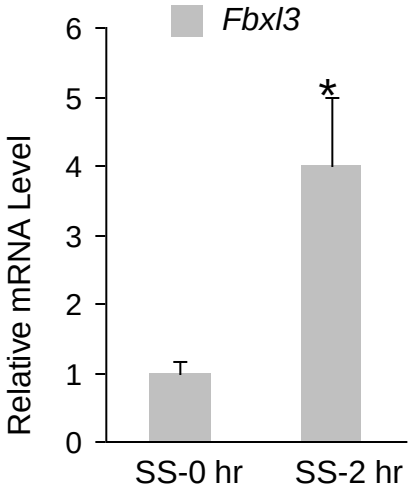

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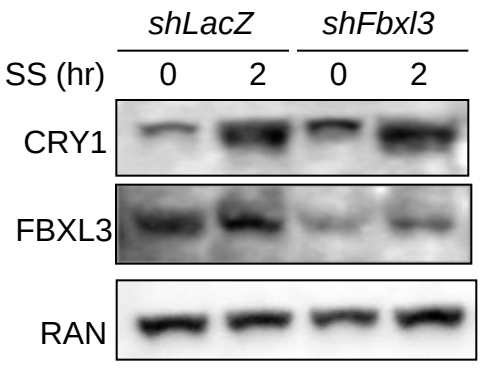

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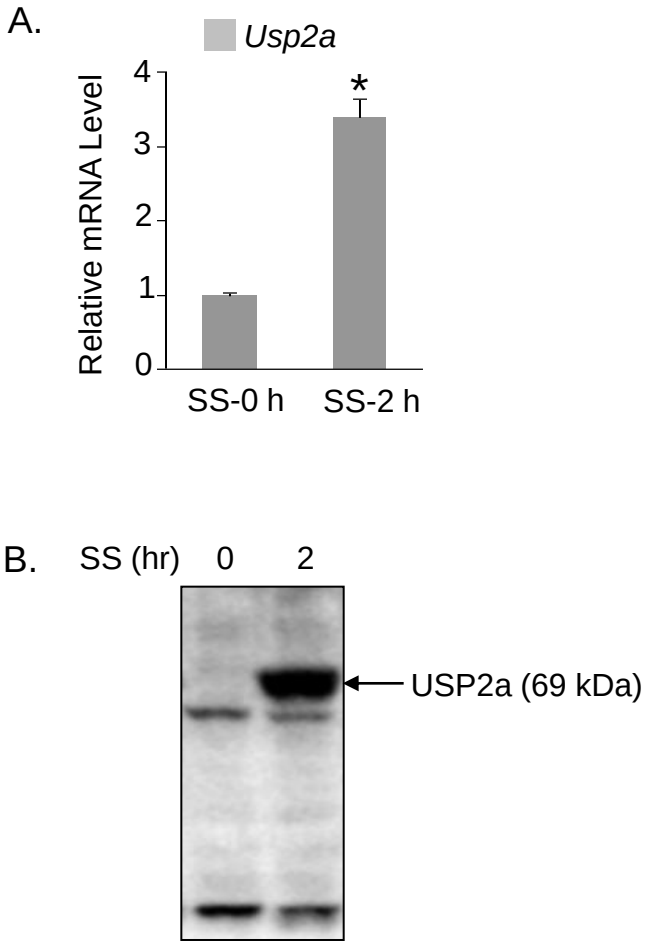
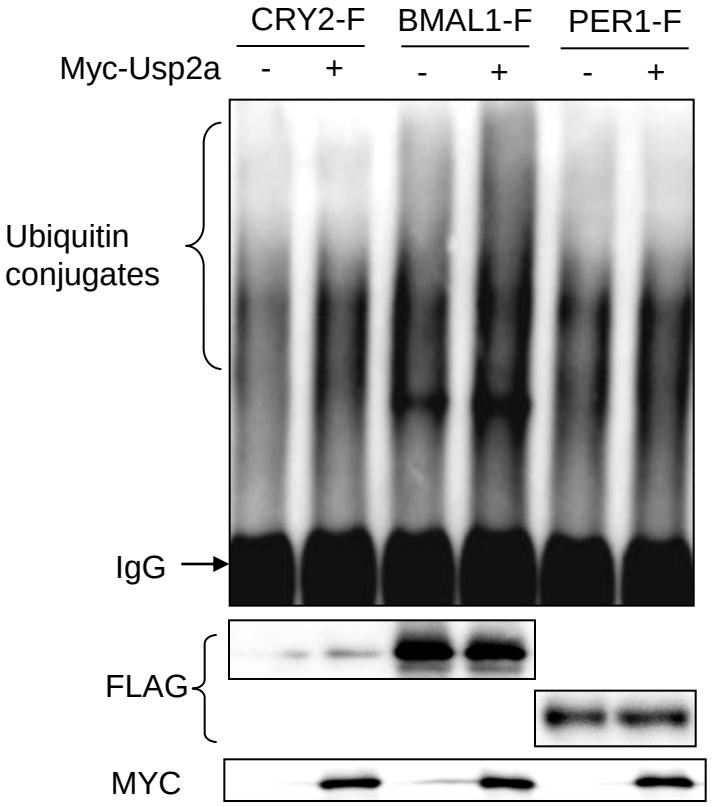
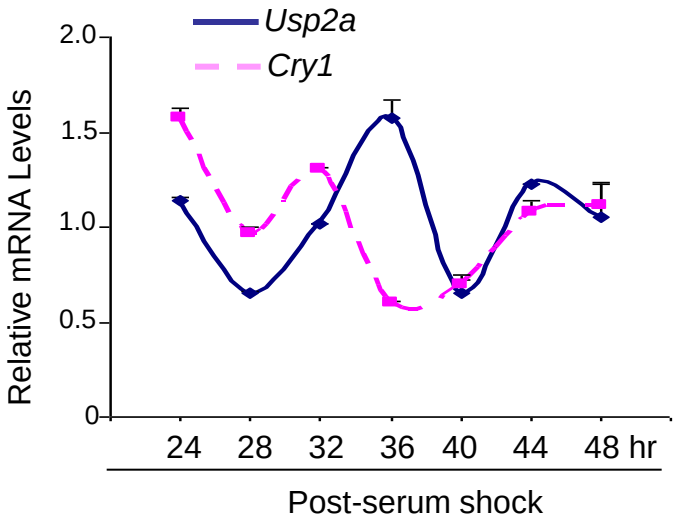


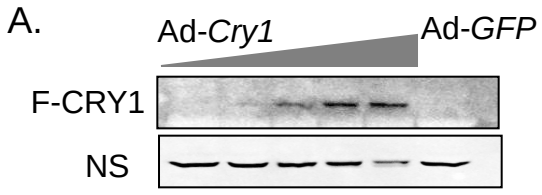
Supplementary Figure 5

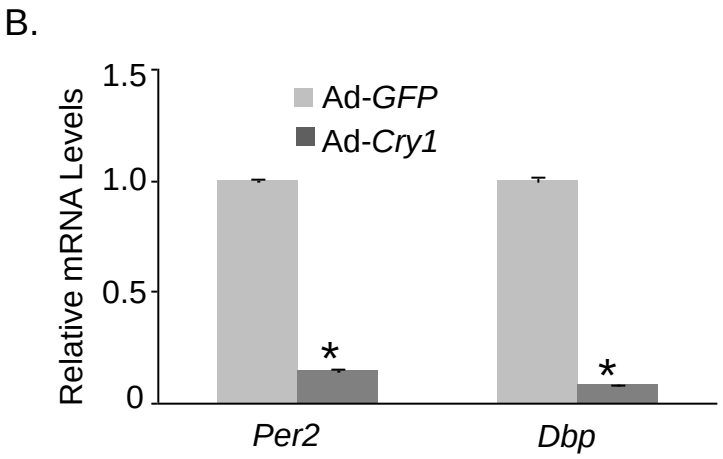


Supplementary Figure 6

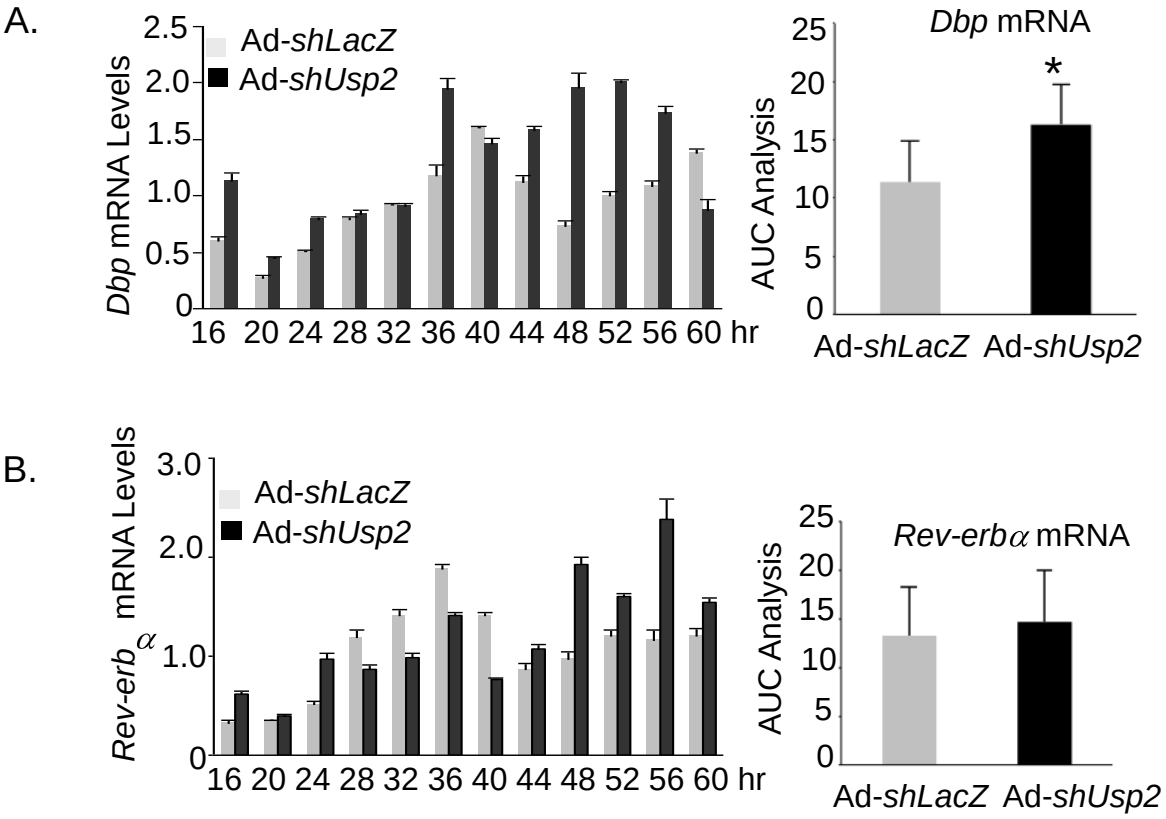


Supplementary Figure 7

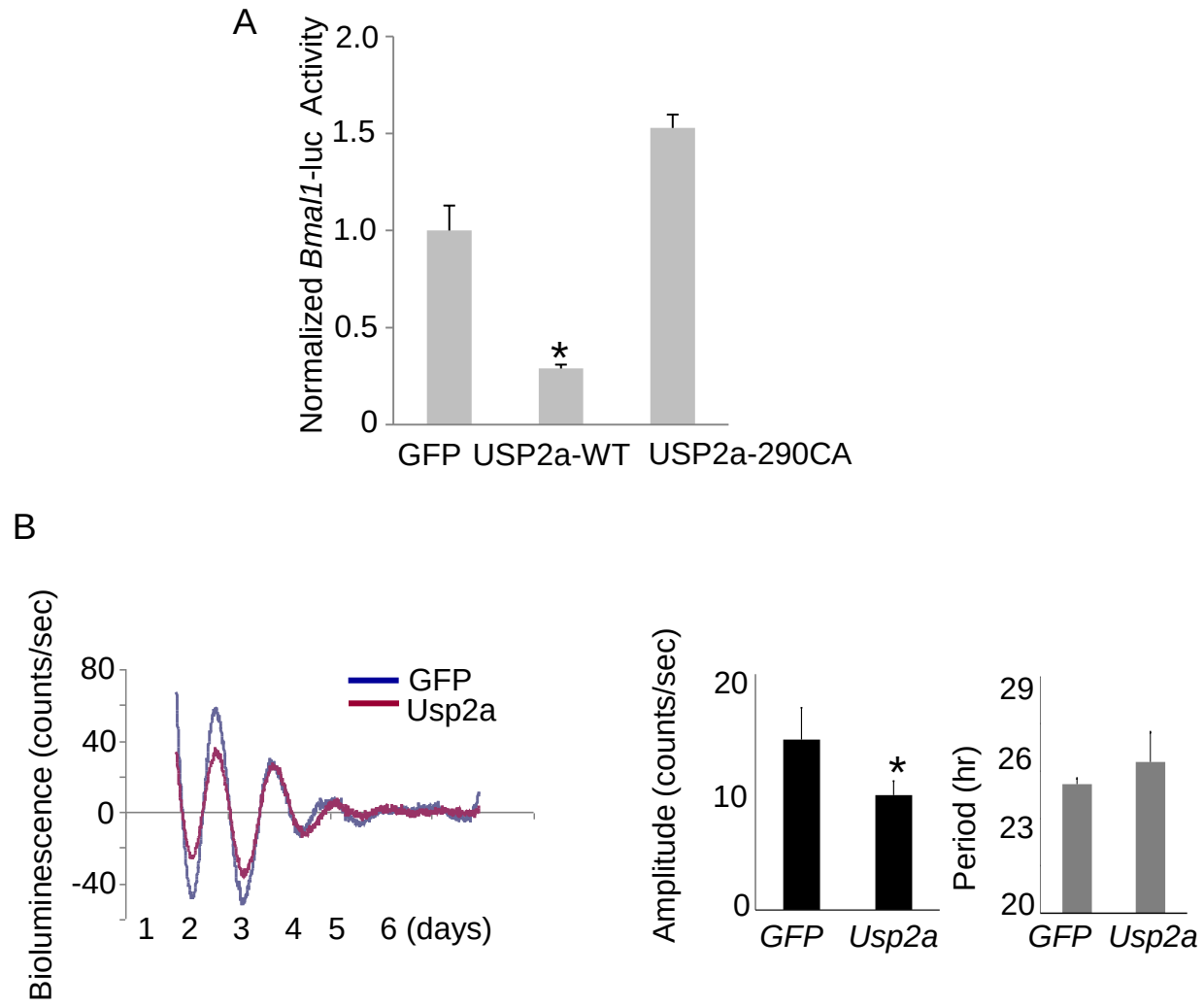




Supplementary Figure 8



Supplementary Figure 9



Supplementary Figure 10