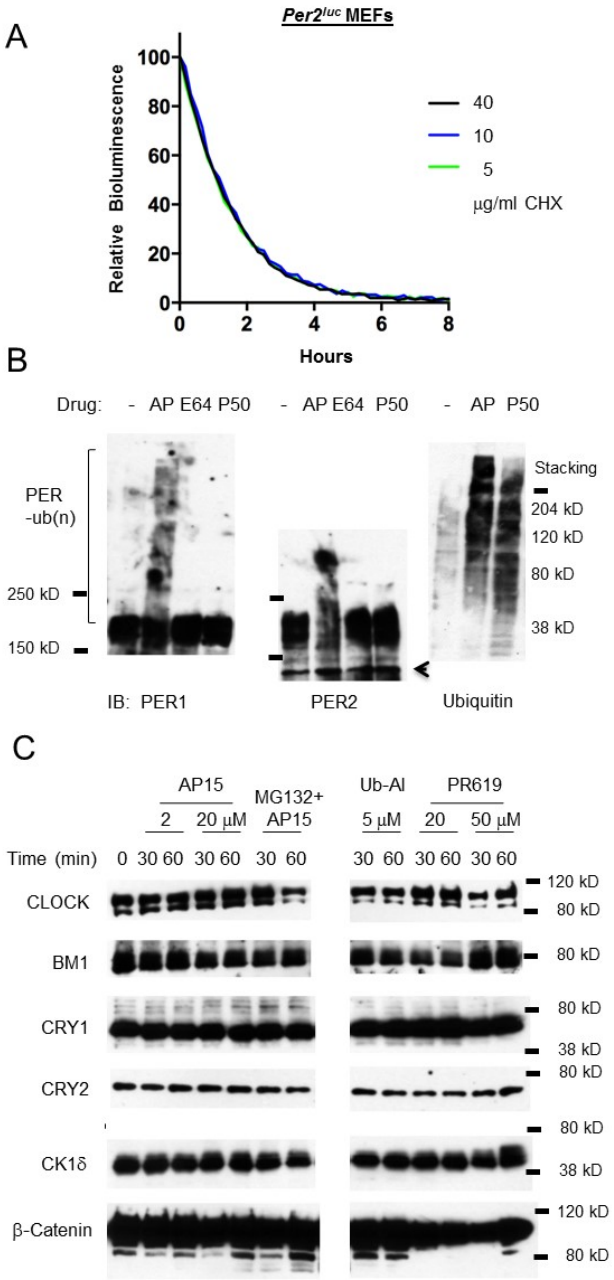


Figure S1. Among essential clock proteins, only PER is subject to rapid ubi/deubiquitination, Related to Figure 1. *Per2*^{Luc} MEFs were used. (A) The level of inhibition for PER2-LUC translation with CHX remained the same across CHX concentrations from 5 to 40 µg/ml based on bioluminescence. Representative results are shown. P>0.05 by one-way ANOVA, n=4 each. (B) Rapid PER ubiquitination and degradation are specifically induced by b-AP15. P5091 (a specific inhibitor of USP7 and USP47) did not induce ubiquitination although it increased general ubiquitination. E-64, an inhibitor of calpain and cysteine proteases did not induce accumulation of PER or ubiquitinated PER. The arrow indicates a nonspecific band in the PER2 blot. (C) None of the other clock proteins were affected by the DUB inhibitors. Ubiquitinated species were not detected even in the presence of MG132 (a proteasome inhibitor, 40 µM) suggesting that ubiquitination of these clock proteins are not regulated by DUBs rather than that ubiquitinated species did not accumulate because they are unstable.

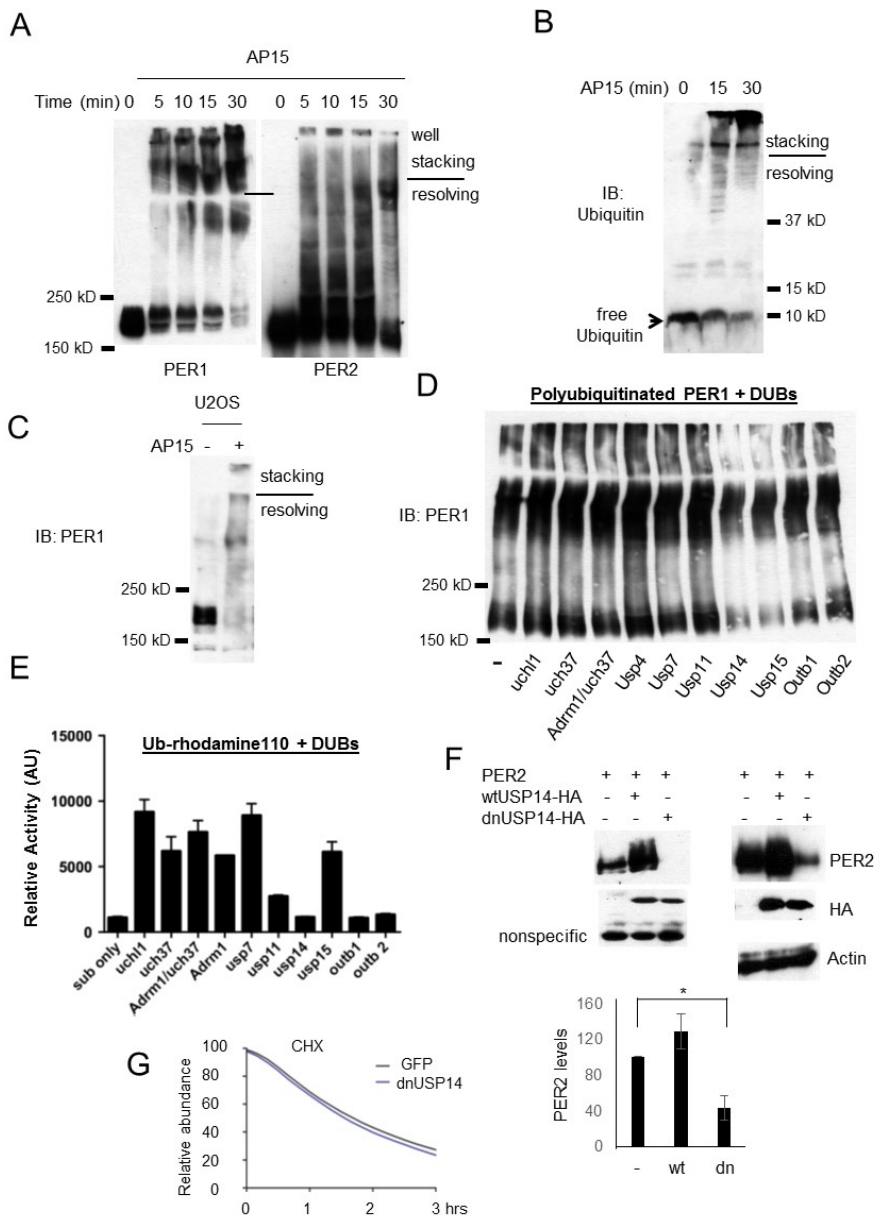


Figure S2. Rapid ubi/deubiquitination of PER is conserved in MEFs, HEK293a and U2OS cells, Related to Figure 1 and 2. (A) Kinetics of PER ubiquitination was measured in MEFs with 10 μM b-AP15. (B) Endogenous Ubiquitin is limiting. Endogenous Ubiquitin becomes almost depleted in 30 min with 10 μM b-AP15 treatment. (C) PER ubi/deubiquitination occurs in U2OS cells. (D) Polyubiquitinated PER1 was mixed with purified DUBs. Note that Usp14 and 15 lanes were underloaded. Adrm1 is an activator of UCH37 [S1]. Representative of two experiments. (E) An artificial DUB substrate, UB-Rhodamine 110 was mixed with DUBs. Data are mean+/-SEM, n=3. All showed significant activity ($p<0.05$) towards the artificial substrate except USP14, OUTB1 and 2. (F) PER2 levels are downregulated when co-expressed with dnUSP14. Quantification data are shown in the graph: n=3, $p<0.05$. (G) PER2-Luc half life is shortened when co-expressed with dnUSP14. GFP and dnUSP14 were expressed for 5 days before CHX is added. Average half life was ~10 min shorter with dnUSP14. Means are shown. n=6; $p<0.05$.

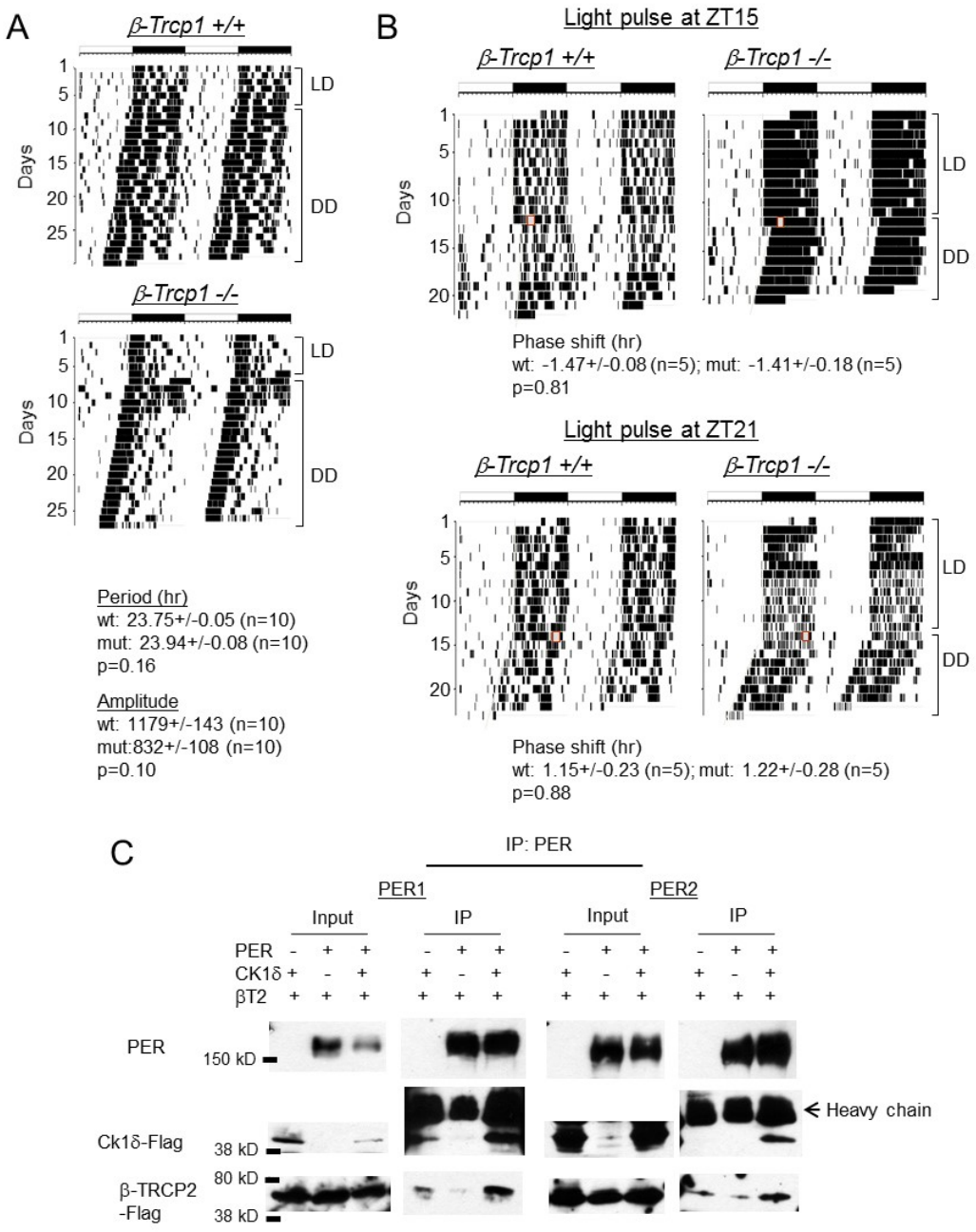


Figure S3. *β-Trcp1* mutant mice do not exhibit circadian phenotypes, Related to Figure 3. Free running rhythms (A) and phase shifts by a 30 min light pulse (red box) (B) were measured in *β-Trcp1* mutant and matching wt control mice. Note that the actogram formats are different in this figure because they were generated using the Stanford software, different from the ClockLab software used in other figures. (C) PER2-β-TRCP2 interaction is enhanced by CK1δ. *Per*, *CK1δ* and *β-Trcp2* were co-transfected into HEK293a cells and coimmunoprecipitation was used to evaluate protein interactions. Endogenous antibodies were used for PER and CK1δ, but anti-Flag antibody was used for β-TRCP2. Note that more β-TRCP2 was copurified with PER in the presence of CK1δ.

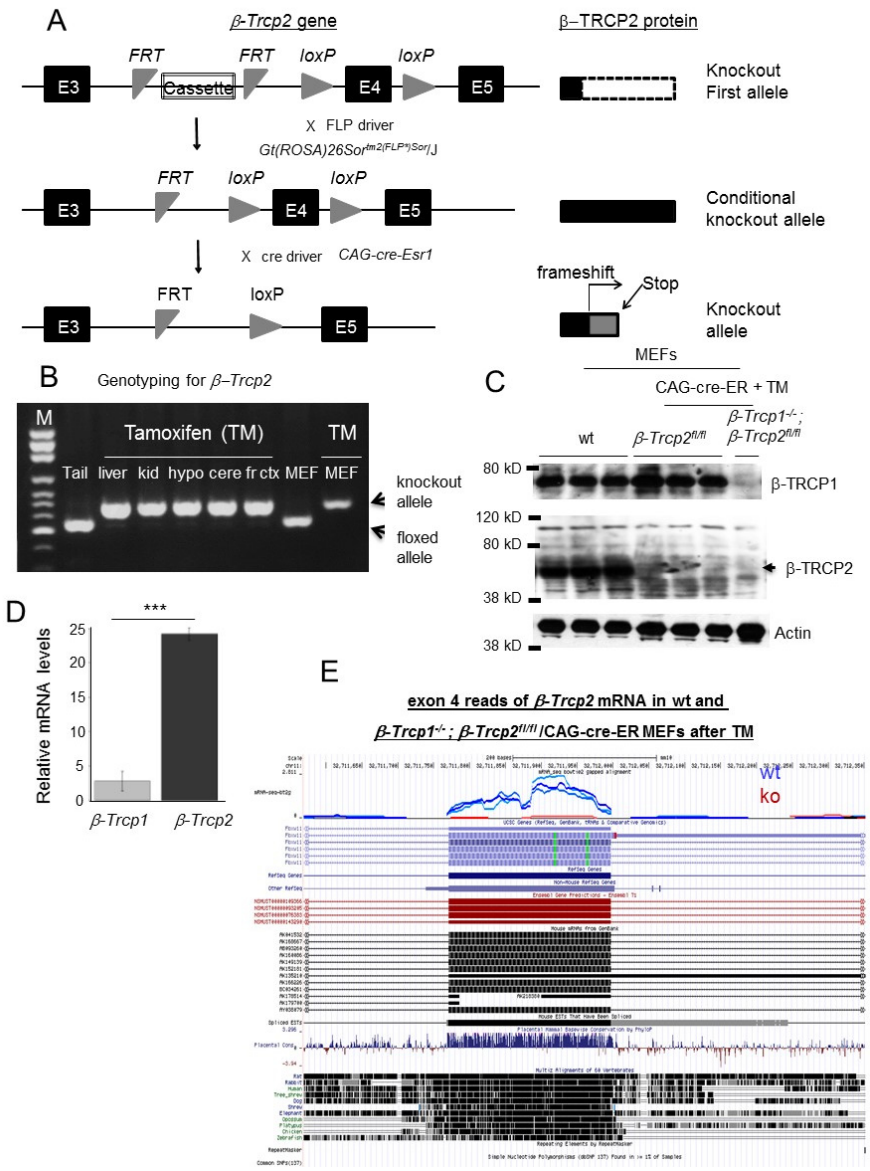
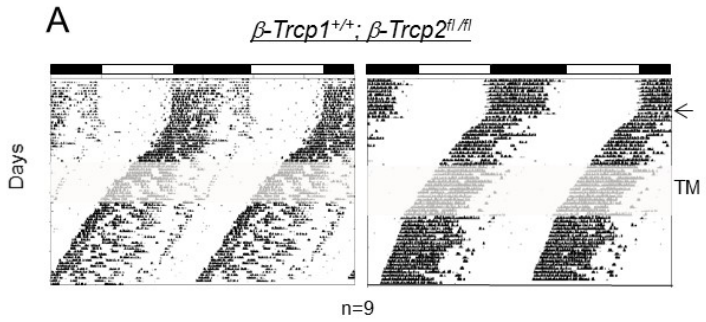


Figure S4. Generation of a *β-Trcp2* mutant mouse, Related to Figure 3. (A) Schematic of knockout strategy. Targeted ES cells harboring a mutant *Fbxw11* (*β-Trcp2*) allele (*Fbxw11*$^{tm1a(KOMP)Wtsi}$) were used to generate the first *β-Trcp2* mutant mouse. The knockout-first allele was then converted into a conditional allele by a Flp recombinase transgene (The Jackson Laboratory # 005703). This conditional *β-Trcp2* mutant mouse was crossed with cre drivers to generate final inducible *β-Trcp2* mutant mice in wt or *β-Trcp1*$^{-/-}$ background. Deletion of exon4 of *Fbxw11* causes a frameshift mutation in the downstream cDNA. (B) Tamoxifen in the mouse chow and 4-OH tamoxifen in culture medium induced ~100 % recombination in various tissues and cultured MEFs, respectively. DNA was extracted from liver, kidney, hypothalamus, cerebellum, frontal cortex, and MEFs. (C) Confirmation of β-TRCP2 deletion in MEFs. The inducible *β-Trcp2* mutant cells were harvested 5 days after TM treatment. (D) mRNA levels of *β-Trcp1* and *2* in wt MEFs. Mean+/-SEM; n=3. P<0.001 (Student's t test). (E) Exon 4 of the *β-Trcp2* gene is missing in TM-treated *β-Trcp2*$^{fl/fl}$ MEFs**.** Sequencing reads for exon 4 are absent in the mutant cells after TM treatment. The top graph represents the number of RNA seq reads (Y-axis) along the exon 4 (X-axis).



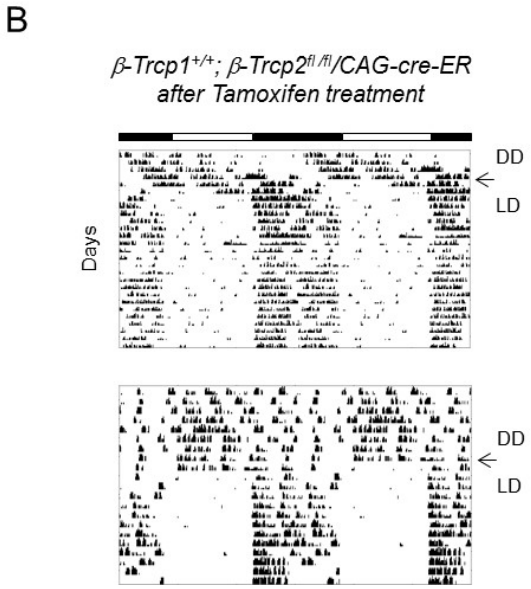


Figure S5. Actograms of *β-Trcp2*$^{fl/fl}$ control mice before and after TM treatment (A), and intact photic transduction pathway in the *β-Trcp2* mutant mice (B) , Related to Figure 3.

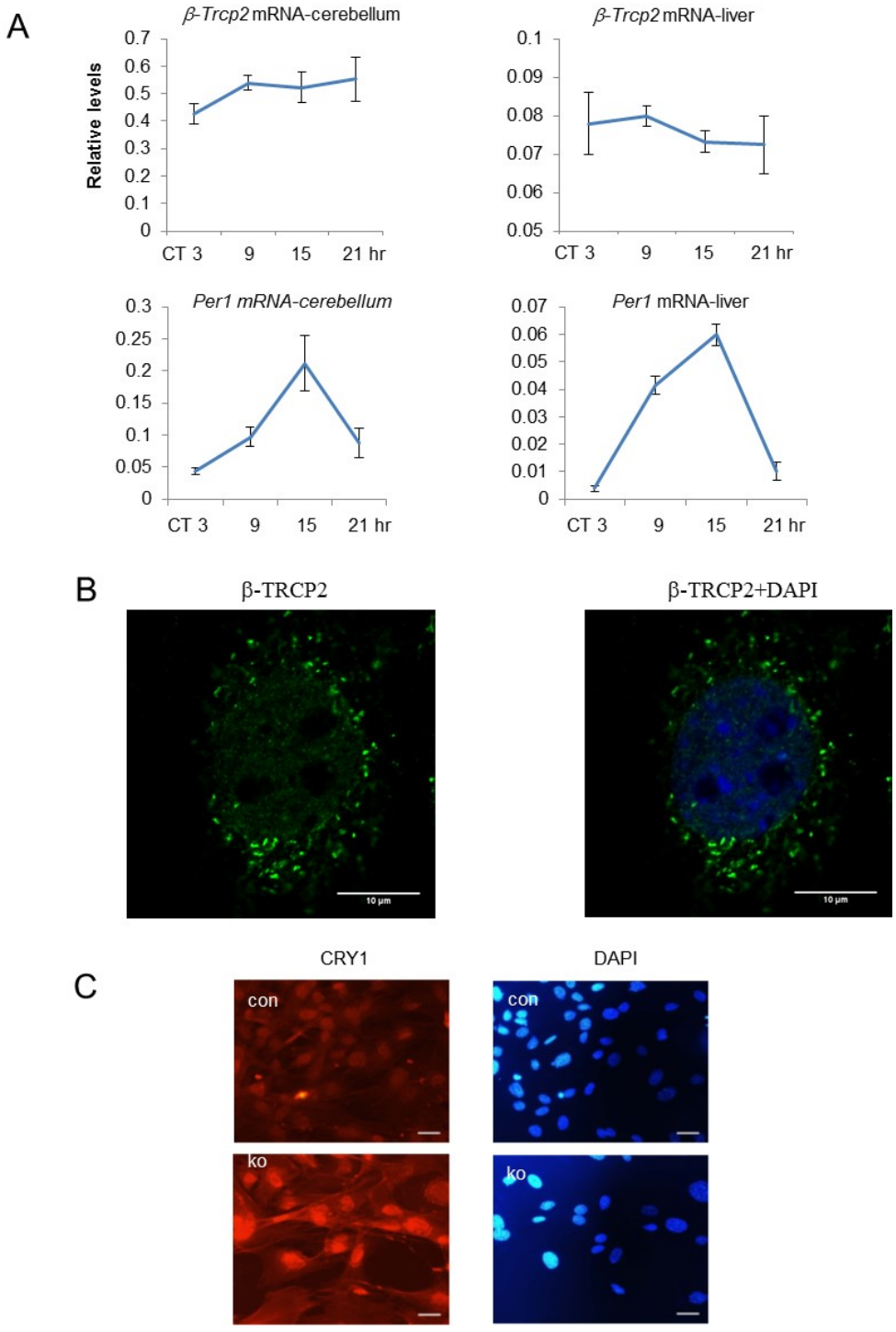


Figure S6. The transcriptional negative feedback loop is constitutively inhibited in *β-Trcp1/2* mutant cells, Related to Figure 5. (A) *β-Trcp2* mRNA does not oscillate in cerebellum and liver. Data are mean+/-SEM, n=3. (B) Representative β-TRCP2 image in control and *β-Trcp1/2* mutant cells. The scale bars represent 10 μm. (C) Immunocytochemistry for CRY1 in control and *β-Trcp1/2* mutant cells. The scale bars represent 20 μm.

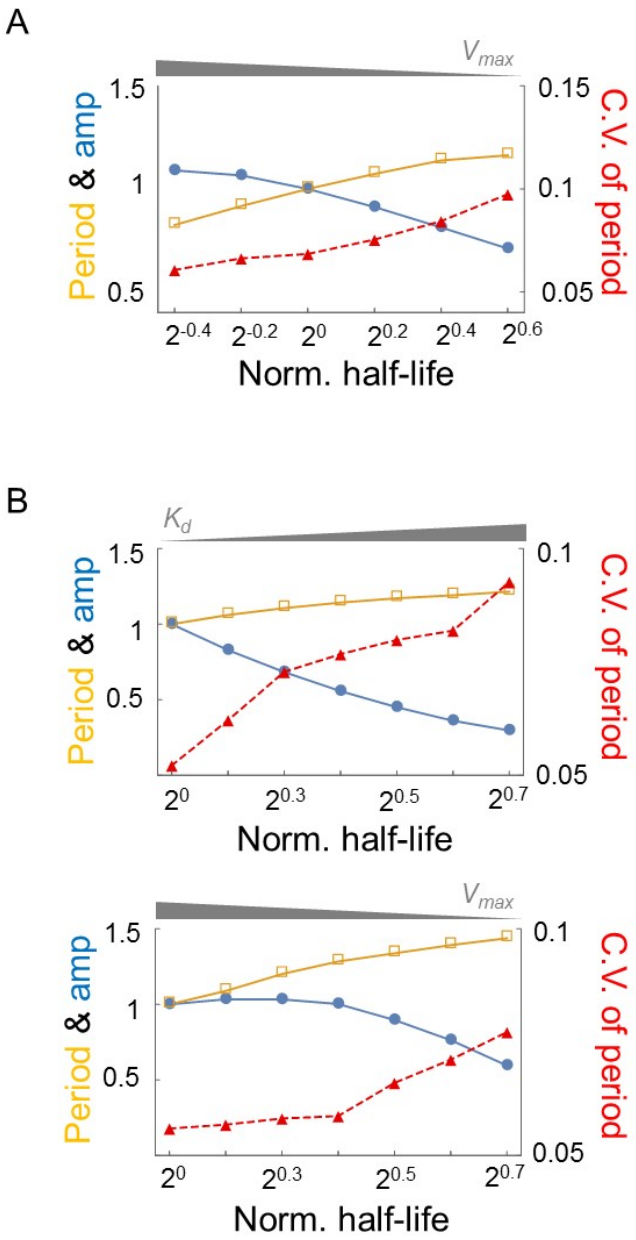


Figure S7. K_d plays a dominant role in the stability of the circadian clock according to mathematical models, Related to Figure 7. (A) The effect of V_{max} on the dynamics of the model (Figure 7A). 10^5 oscillations of stochastic simulation are analyzed. Here, the half-life is measured as the time to reach 50% of the initial concentration of PER protein. The half-life is normalized so that it becomes 1 for the WT model. V_{max} =0.145, 0.108, 0.08, 0.06, 0.047, 0.037. (B) The effect of K_d and V_{max} on the dynamics of the Goodwin model with non-linear degradation (See Methods for details). Varying K_d values leads to a larger change in the C.V. of period than varying V_{max}. On the other hand, varying V_{max} produces a larger change in the mean period than varying K_d. 10^5 oscillations of stochastic simulation were analyzed. Here, the half-life is measured as the time to reach the 50% of the initial concentration of PER protein. Furthermore, the half-life is normalized so that it becomes 1 for WT model. K_d =0.1, 0.16, 0.22, 0.29, 0.36, 0.44, 0.52 and V_{max} =0.27, 0.23, 0.19, 0.16, 0.14, 0.11, 0.09.

Supplemental References

[S1]. Yao, T., Song, L., Xu, W., DeMartino, G.N., Florens, L., Swanson, S.K., Washburn, M.P., Conaway, R.C., Conaway, J.W., and Cohen, R.E. (2006). Proteasome recruitment and activation of the Uch37 deubiquitinating enzyme by Adrm1. Nat Cell Biol *8*, 994-1002.