Supporting Information

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SI Text

Tissue Collection, RNA Isolation, RT-PCR, and mRNA Expression Analyses. Mice of the indicated genotypes were entrained to a 12-h light/12-h dark cycle for at least 7 d before being transferred into DD. Tissues were collected at 4-h intervals on the first day of DD at circadian times (CT) 0, 4, 8, 12, 16, 20, and 24, where CT12 corresponds to the onset of subjective night. Data shown in the various panels were obtained using samples collected from mice of different genotypes and carried out at different times, so the relative values in different experiment/panels are not directly comparable. Each time point for each genotype is from at least three independent mice. The quantitative PCR and RT-PCR results shown are representative of experiments repeated at least three times with similar results.

In Vitro DNA-Mediated Pull Down. DNA-mediated pull-down assays were performed using a DNA-binding Protein Purification kit (Roche; 11835513001). The long concatamers tethered to the magnetic beads were amplified by PCR using the primers: Forward: CTGCCTTCCGTCCCTGACCTACTTTCTGCCTTCCGTCCC-TGACCTACTTT; Reverse: AAAGTAGGTCAGGGACGGA-AGGCAGAAAGTAGGTCAGGGACGGAAGGCAG. Elution of the DNA-binding protein was subject to Western blot.

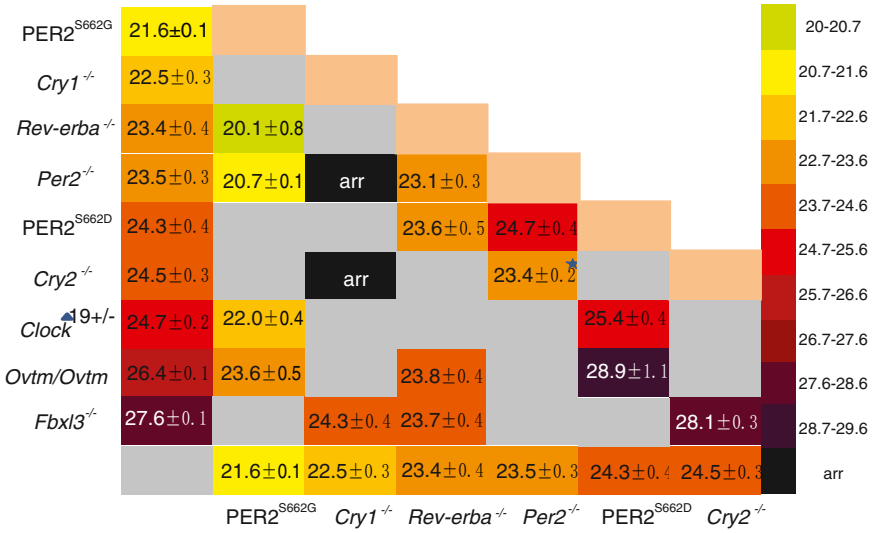

Fig. S1. Period lengths in established mutant mice. The synthetic period lengths are shown in individual boxes for different combinations of genetic mutation. All of the mice were backcrossed for more than six generations. Behavioral analyses revealed high consistency across the different generations. Values represent means ± SD (n = 6–12). All mice were entrained in light/dark cycles (LD) for 7–10 d and then were released into constant darkness (DD) for an additional 3 wk. Wheel-running activity assays were performed as described previously using ClockLab (Actimetrics) software (1–3). *Per2, Period2*; PER2$^{S662G/D}$, human PERIOD2 S662G/D transgenic[2].

1. Xu Y, et al. (2005) Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. *Nature* 434(7033):640–644.
2. Xu Y, et al. (2007) Modeling of a human circadian mutation yields insights into clock regulation by PER2. *Cell* 128(1):59–70.
3. Wang X, et al. (2010) Interaction of MAGED1 with nuclear receptors affects circadian clock function. *EMBO J* 29(8):1389–1400.

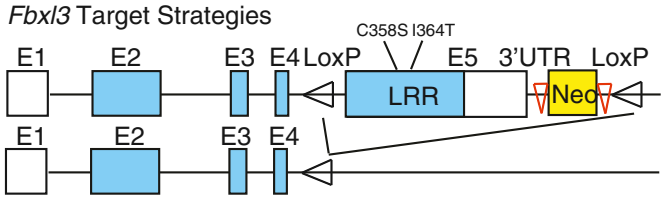

Fig. S2. Targeting strategy for insertion of point mutations in the LRR domain of the *Fbxl3* allele and *LoxP* sites. Exon 5 is flanked by two *loxP* sites, and the neomycin cassette is bound by two flippase recognition target sites as indicated. Target region is exon 5 including whole 3′-UTR.

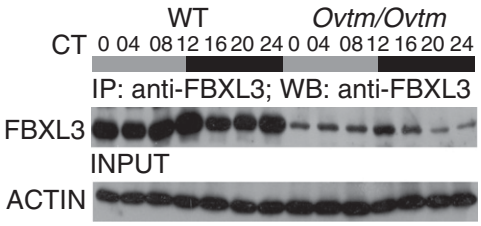

Fig. S3. Altered FBXL3 protein stability in *Overtime* Mice. Representative circadian profile of the FBXL3 protein in the livers of wild-type and *Overtime* mice in DD. Blot against actin was served as the loading control.

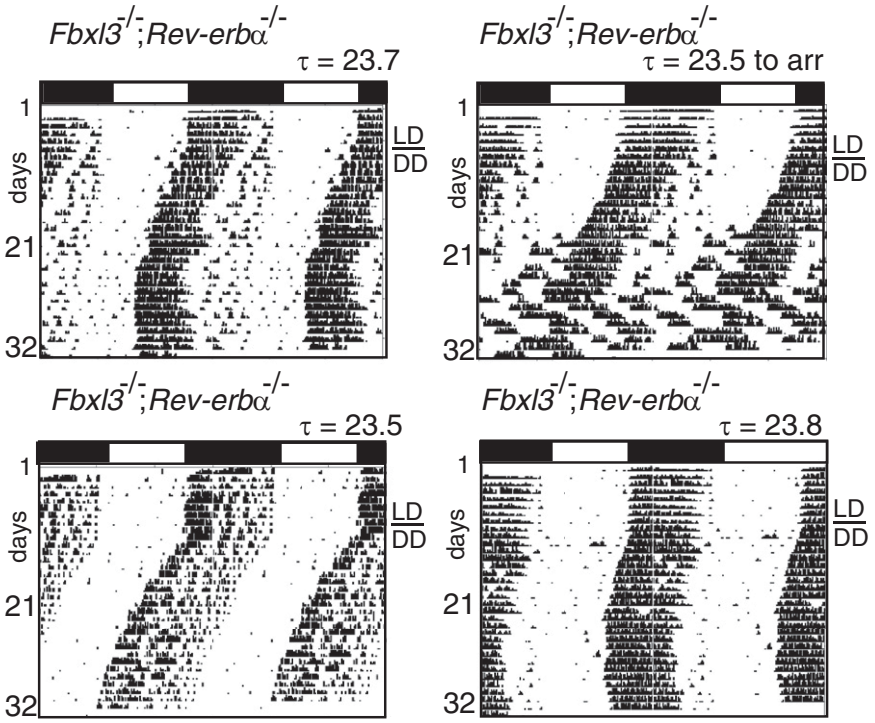

Fig. S4. Representative actograms from established *Fbxl3*$^{-/-}$;*Rev-erbα*$^{-/-}$ mice in locomotor activity across the circadian day (n = 20).

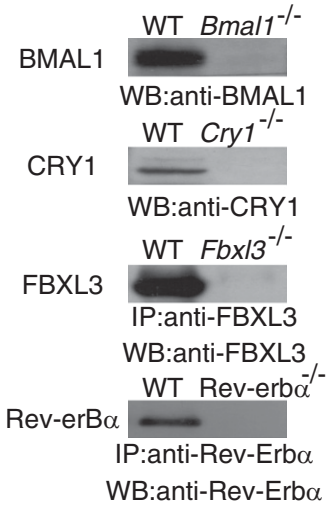

Fig. S5. Validation and confirmation of anti-BMAL1, CRY1, FBXL3, and Rev-Erbα antibodies in the liver tissues in the indicated knockout mice.

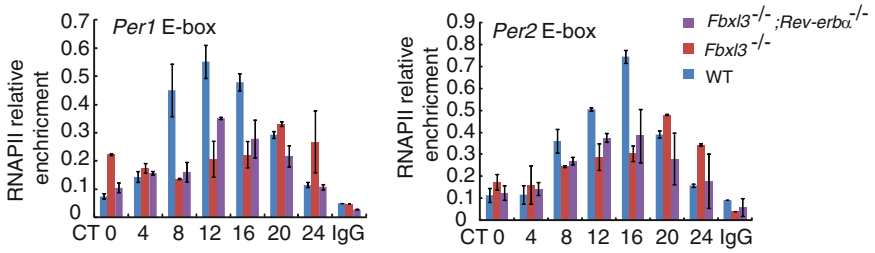

Fig. S6. Altered RNAPII enrichment at the E-box in the *Per1* and *Per2* promoters (mean ± SD, $n = 3$). Each figure shows a representative example from three independent experiments. IgG serves as an internal control.

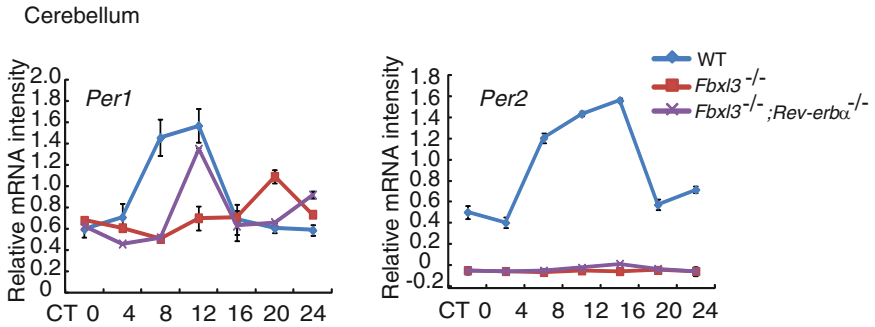

Fig. S7. Quantitative analysis of *Per1* and *Per2* RNA levels in the cerebellum of wild-type, *Fbxl3*$^{-/-}$ and *Fbxl3*$^{-/-}$*;Rev-erbα*$^{-/-}$ mice. Relative levels of RNA were estimated by quantitative RT-PCR and normalized by *Gapdh*. Data represent mean ± SD ($n = 3$).

Table S1. Primers for quantitative PCR

Gene name	Forward primer	Reverse primer
Bmal1 RRE	GGATTGGTCGGAAAGTAGGTT	CGGGTAAACAGGCACCTC
Cry1 RRE in the first intron	TCATTGTGATGGGAGTATGC	TCCAAAAGATGATTTCAACA
Cry1 E-box at the promoter	GCACGCGGGGGTCTGAGCCA	CCGGTCCCGAGGCTGCCCG
Per2 E-box at the promoter	gagcgtagctctcaggttcc	ccgctgtcacatagtggaaa
Per1 E-box at the promoter	tgagacatcctgatcgcatt	tgtcacacacatccctgcac