Genotype
Period length (h) Average power \# of rhythmic flies/ $\pm$ S.D. of rhythmicity total number tested

A. | $w^{1118}$ | $23.40 \pm 0.33$ | 107.97 | $31 / 31$ |
| :--- | :---: | :---: | :---: |
| per ${ }^{01}$ | $23.41 \pm 2.33$ | 37.50 | $23 / 100$ |
| tim-Gal4 | $24.21 \pm 0.31$ | 77.43 | $109 / 129$ |
| UAS-Nab2 ${ }^{1 R}$ | $23.55 \pm 0.30$ | 158.13 | $30 / 31$ |
| UAS-Atx2 | $23.63 \pm 0.30$ | 96.77 | $30 / 31$ |
| tim-Gal4 > UAS-Nab2 |  |  |  |
| tim-Gal4 > UAS-Atx2 | $24.17 \pm 0.36$ | 99.16 | $118 / 119$ |
| tim-Gal4 > UAS-Nab2 ${ }^{1 R}+$ UAS-Atx2 ${ }^{1 R}$ | $25.25 \pm 0.97$ | 30.50 | $3 / 73$ |



Figure S1: (Associated with Figure 3) Period length and power of rhythmicity of flies with RNAi depletion of Nab2, Atx2, or both RBPs in circadian neurons. (A) Free-running circadian period length, average power of rhythmicity, and rhythmic number were calculated using chi-squared periodograms and the Rethomics $R$ package (GEISMANN, 2019). Only data from seven 24 -hour dark periods was used to calculate circadian parameters. (B) Box and whisker plots showing median period length (hours), interquartile range, and min/max for each genotype. Data points for each
of the selected genotypes are also plotted. Decreased expression of both Nab2 and Atx2 in circadian neurons significantly extended period length compared to tim-Gal4,UAS-Nab2IR, or UAS-Atx2IR flies alone. (C) Box and whisker plots showing median power measurements, interquartile range, and min/maxfor each genotype. Data points for each of the selected genotypes are also plotted. The power of rhythmicity was significantly decreased for flies lacking both Nab2 and Atx2 in circadian neurons compared to tim-Gal4 controls. The period length and power data was analyzed using non-parametric Kruskal Wallis tests and pairwise Wilcoxon Rank Sum tests with

