miR-263b Controls Circadian Behavior and the Structural Plasticity of Pacemaker Neurons by Regulating the LIM-Only Protein Beadex

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Supplementary Materials

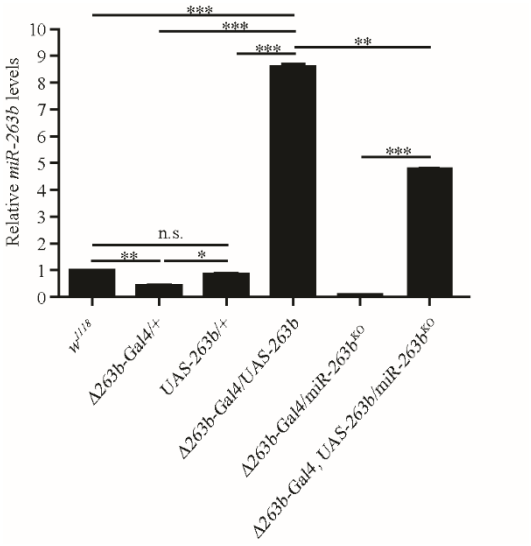

Figure S1. *miR-263b* expression level in relevant flies. Quantitative real-time PCR analysis of total RNA prepared from adult brains at ZT13. The relative expression levels were normalized to *2s* RNA levels and were further normalized to w^{1118} control. Data represent means ± SEM; n.s. not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ determined by Student's *t*-test.

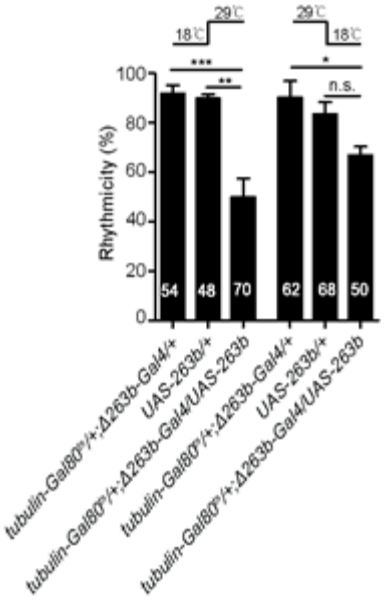

Figure S2. *miR-263b* is required during adulthood, but not development. Left, flies were grown at 18 °C to inhibit *miR-263b* expression during development and transferred at 29 °C after eclosion to allow *miR-263b* overexpression in adult flies. Right, flies were grown at 29 °C to inactivate *Gal80*, thus allowing *miR-263b* overexpression during development, and then transferred to 18 °C to block *miR-263b* overexpression after eclosion. Data represent means ± SEM; n.s. not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ determined by Student's *t*-test. Percentage of rhythmicity is indicated above the bars, and the number of flies tested is shown in the bars.

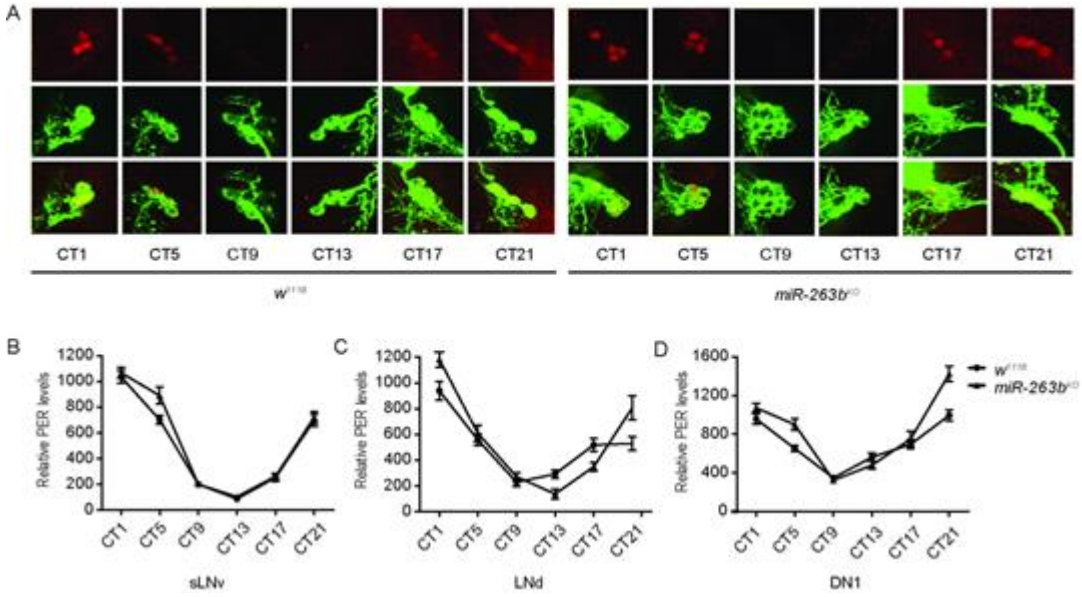

Figure S3. The molecular pacemaker is not affected in *miR-263b*^{KO} flies. The molecular pacemaker is not affected in *miR-263b*^{KO} flies. (A) Representative confocal images of sLNvs from *w*^{1118} and *miR-263b*^{KO} flies dissected at six time points (circadian time, CT) during the second day of DD and stained with anti-PDF (green) and anti-PER (red) antibodies; scale bar, 10 μm. (B–D) Quantification of PER staining in sLNvs, LNds, and DN1s at different circadian time points. Data represent means ± SEM (n = 16–19).

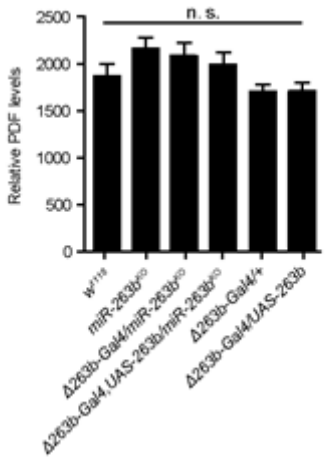

Figure S4. PDF levels in the sLNvs soma are not significantly changed under LD conditions. Quantification of PDF staining in sLNvs at ZT2. Data represent means ± SEM (n = 18–21); n.s. not significant determined by Student's t-test.

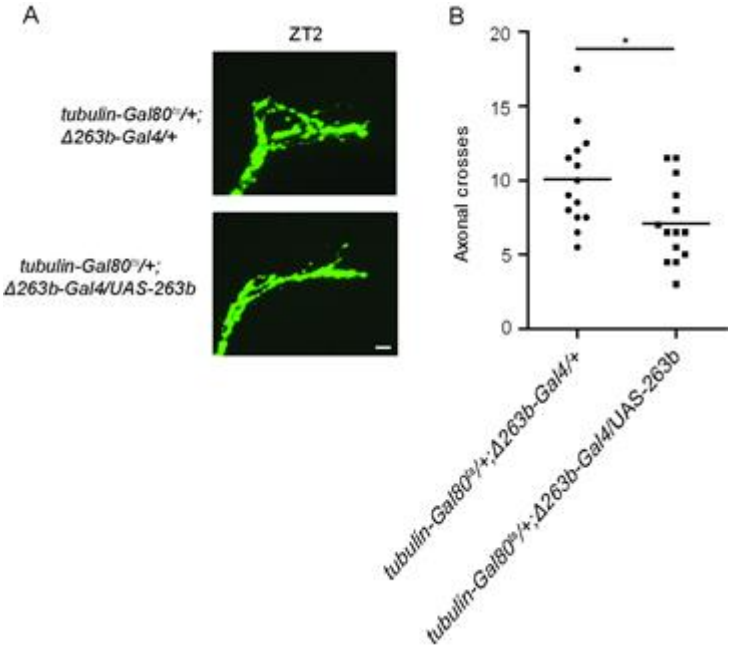

Figure S5. *miR-263b* can drive changes of sLNv axonal projections in short time. (A) Representative images of sLNv dorsal projections from the indicated genotypes stained with anti-PDF at ZT2; scale bar, 10 μm. (B) Quantification of axonal morphology (fasciculation) of sLNv dorsal termini in LD conditions by Sholl's analysis. Data represent means ± SEM (n = 14); * $p < 0.05$ determined by Student's t-test.

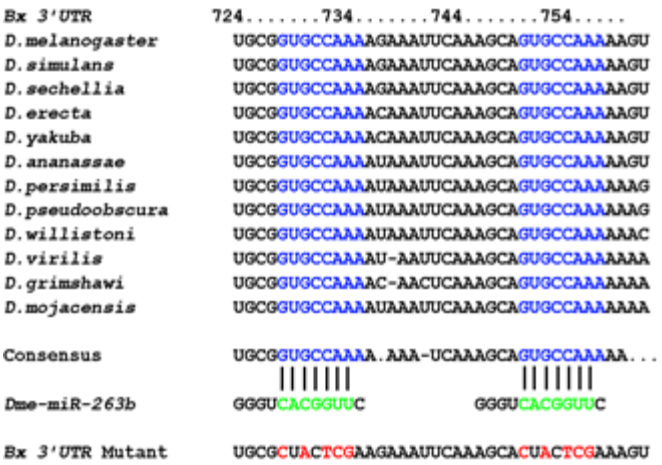

Figure S6. Predicted *miR-263b* binding site conservation in the *Bx* 3′ UTR among *Drosophila* species. Blue letters indicate conserved sequences, green letters indicate *miR-263b* seed region, and red letters indicate positions of mutations in *Bx* 3′ UTR made for S2 cell reporter gene assay.

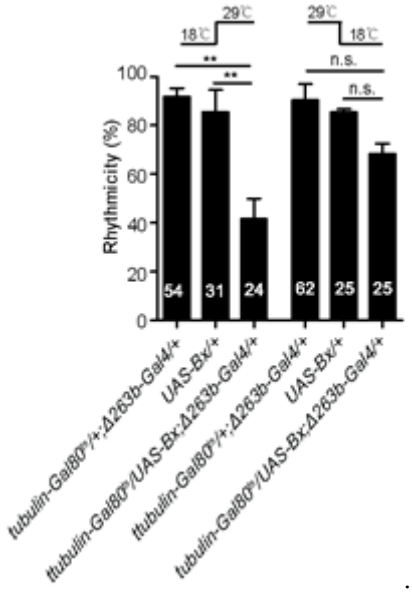

Figure S7. *Bx* is required during adulthood, but not during development. Left, flies were grown at 18 °C to inhibit *Bx* expression during development and transferred at 29 °C after eclosion to allow *Bx* overexpression in adult flies. Right, flies were grown at 29 °C to inactivate Gal80^{ts}, thus allowing *Bx* overexpression during development, and then transferred to 18 °C to block *Bx* overexpression after eclosion. Data represent means ± SEM; n.s. not significant, ** $p < 0.01$, determined by Student's *t*-test. Percentage of rhythmicity is indicated above the bars, and the number of flies tested is shown in the bars.

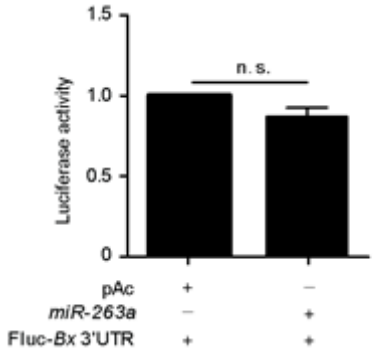

Figure S8. *miR-263a* cannot inhibit the expression of *Bx* in S2 cells. pAC or pAC-*miR-263a* was co-transfected with pAc-fluc-*Bx* 3′ UTR into S2 cells. After two days, luciferase activity was quantified. For each condition, a normalized firefly/*Renilla* luciferase value is plotted with SEM.