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Supplemental information

The *Drosophila* microRNA bantam regulates excitability in adult mushroom body output neurons to promote early night sleep

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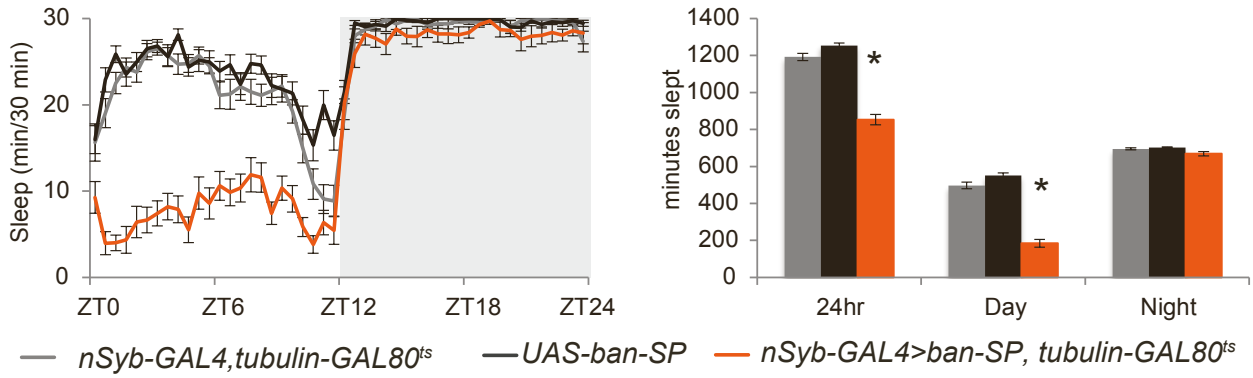
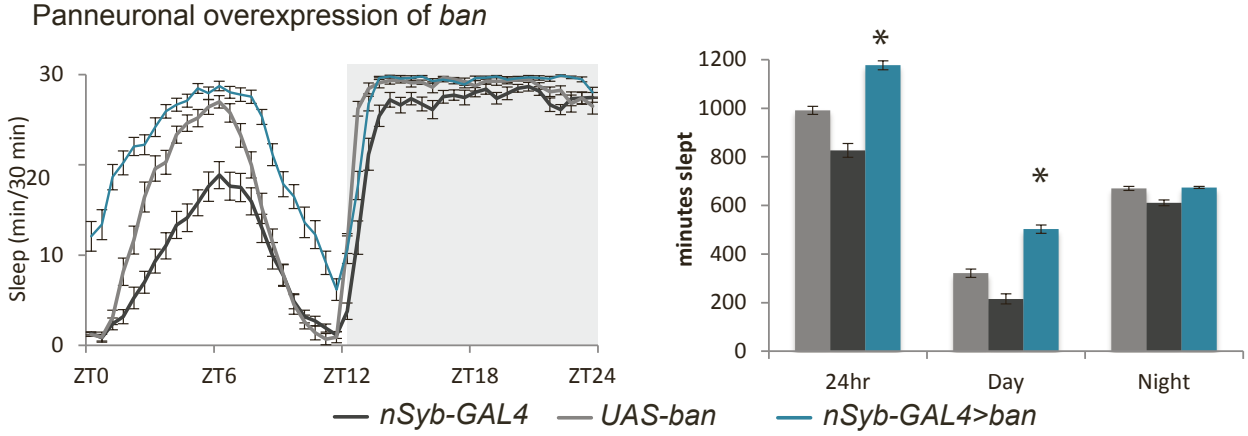
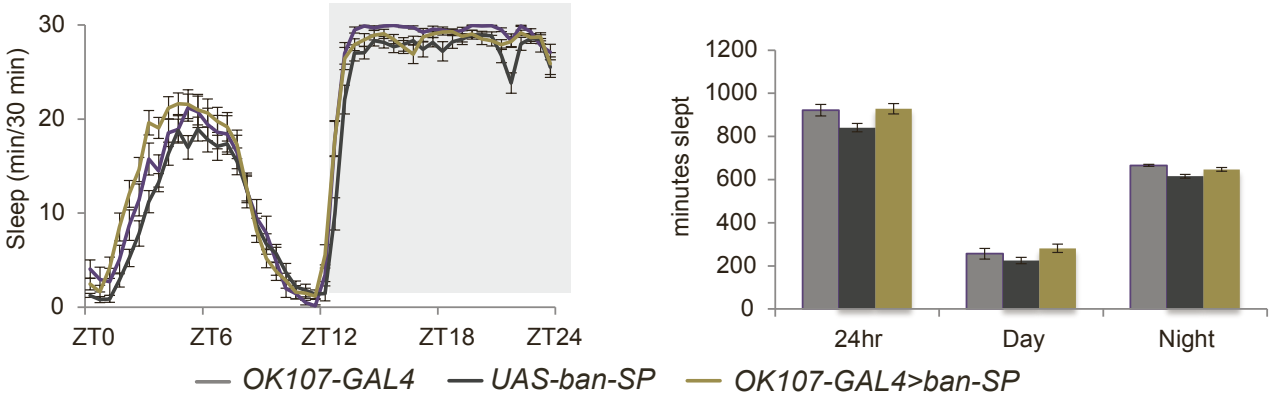
A Panneuronal Developmental KD



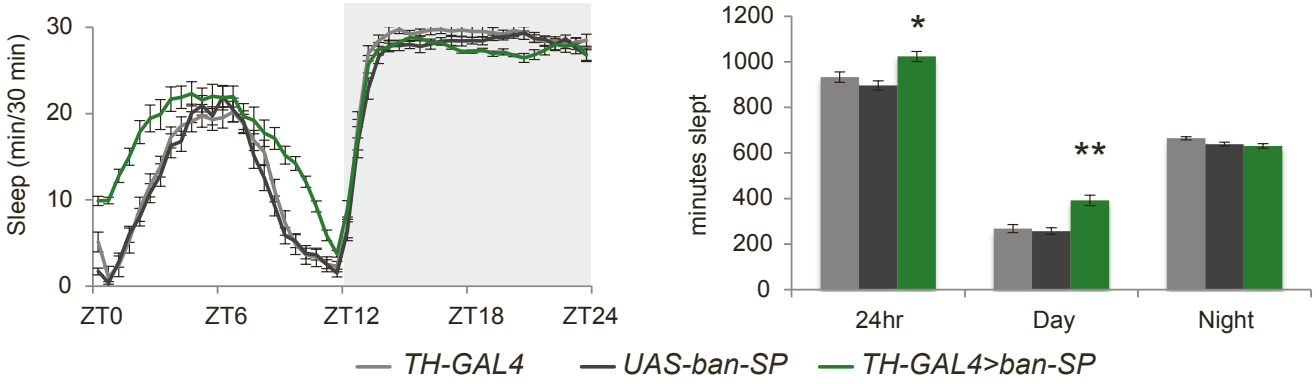
B Panneuronal overexpression of *ban*



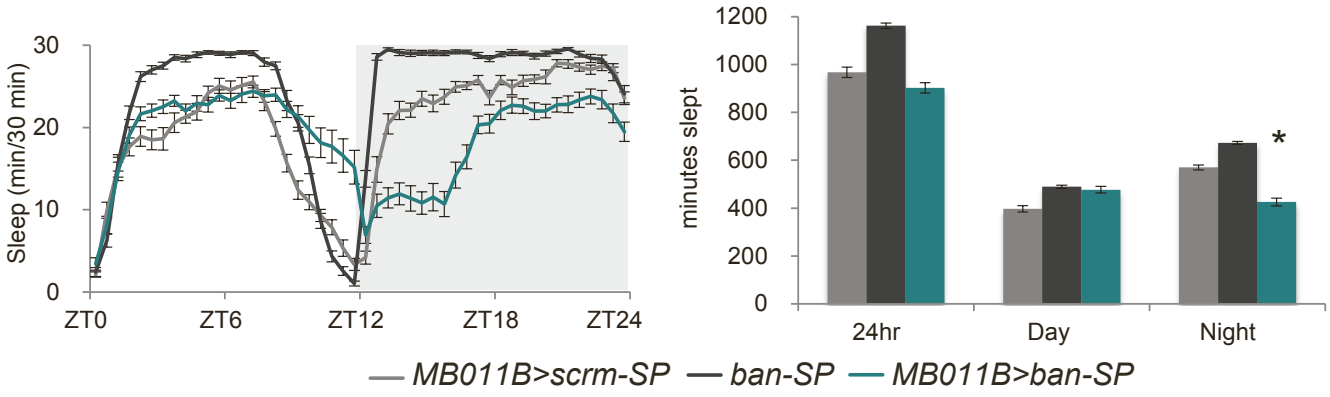
C MB Kenyon Cell KD



D Dopaminergic Neuron KD



E Male MBON KD



F P(wake) with MBON KD

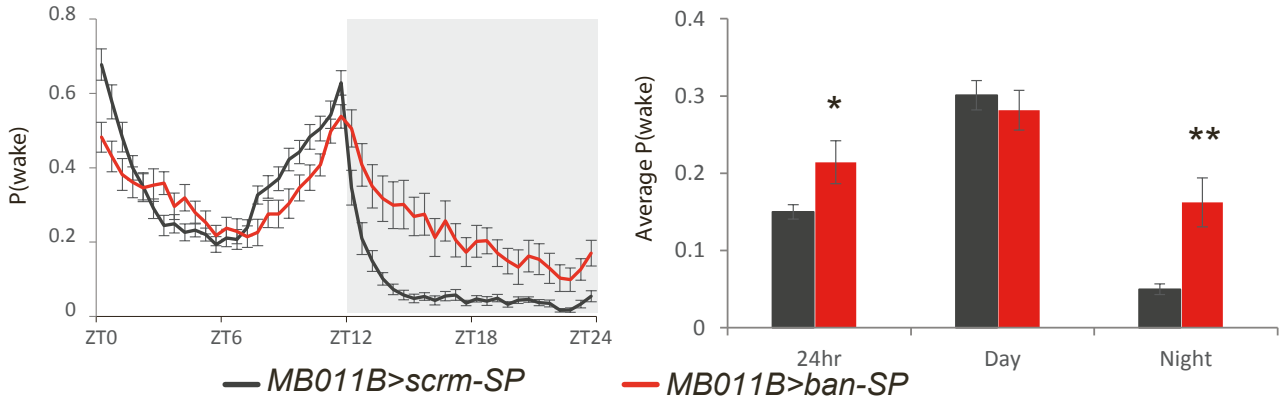


Figure S1. Related to Figure 1.

***Ban* has multiple roles in regulation of sleep.** Knockdown of ban during development and in diverse adult cell groups demonstrates that *ban* has roles at multiple stages of development and in several distinct neuronal populations. This demonstrates the utility of the cell-specific sponge approach. Note that the role of ban in dopaminergic neurons appears to be opposite to its role in MBONs i.e. it is wake-promoting rather than sleep promoting.

(A) Developmental knockdown of *ban* primarily affects daytime sleep. Sleep data for *nSyb>ban-SP+tubulin-GAL80^{ts}* (n=26), *nSyb>scr-SP+tubulin-GAL80^{ts}* (n=27) and *UAS-ban-SP* control (n=31) raised at 29°C and tested at 18°C (mean± SEM). * represents P≤.0001 using a one-way ANOVA with Tukey multiple comparisons test.

(B) Overexpression of *ban* in all neurons increases sleep. (B) Sleep data for *nSyb>ban* (n=29), *nSyb-GAL4* (n=30) and *UAS-ban* (n=29) (mean± SEM). * represents P≤.0001 using a one-way ANOVA with Tukey multiple comparison test.

(C) Knockdown of *ban* in the intrinsic cells of the mushroom body. Sleep data for *OK107>ban-SP* (n=30), *OK107-GAL4* control (n=28) and *UAS-ban-SP* control (n=30) (mean ± SEM).

(D) Knockdown of *ban* in dopaminergic neurons. Sleep data for *TH>ban-SP* (n=32), *TH-GAL4* (n=31) and *UAS-ban-SP* (n=30) (mean± SEM). * represents P≤.05 using the Kruskal-Wallis Test with Dunn's multiple comparison test. ** represents P≤.0001 using a one-way ANOVA with Tukey's multiple comparison test.

(E) Knockdown of *ban* in MBONs is not sexually dimorphic. Sleep data for male *MB011B>UAS-ban-SP* (n=31), *MB011B>UAS-scr-SP* (n=32) and *UAS-ban-SP* (n=32) (mean± SEM). * represents P≤.0001 using the Kruskal-Wallis Test with Dunn's multiple comparison test.

(F) P(Wake) is significantly reduced at night by *ban* knockdown in MBONs. Data for *MB011B>ban-SP* (N=17) and scramble controls (N=24) shown as mean± SEM. * represents P≤.05 and ** represents P≤.0001 using the Mann-Whitney Test.

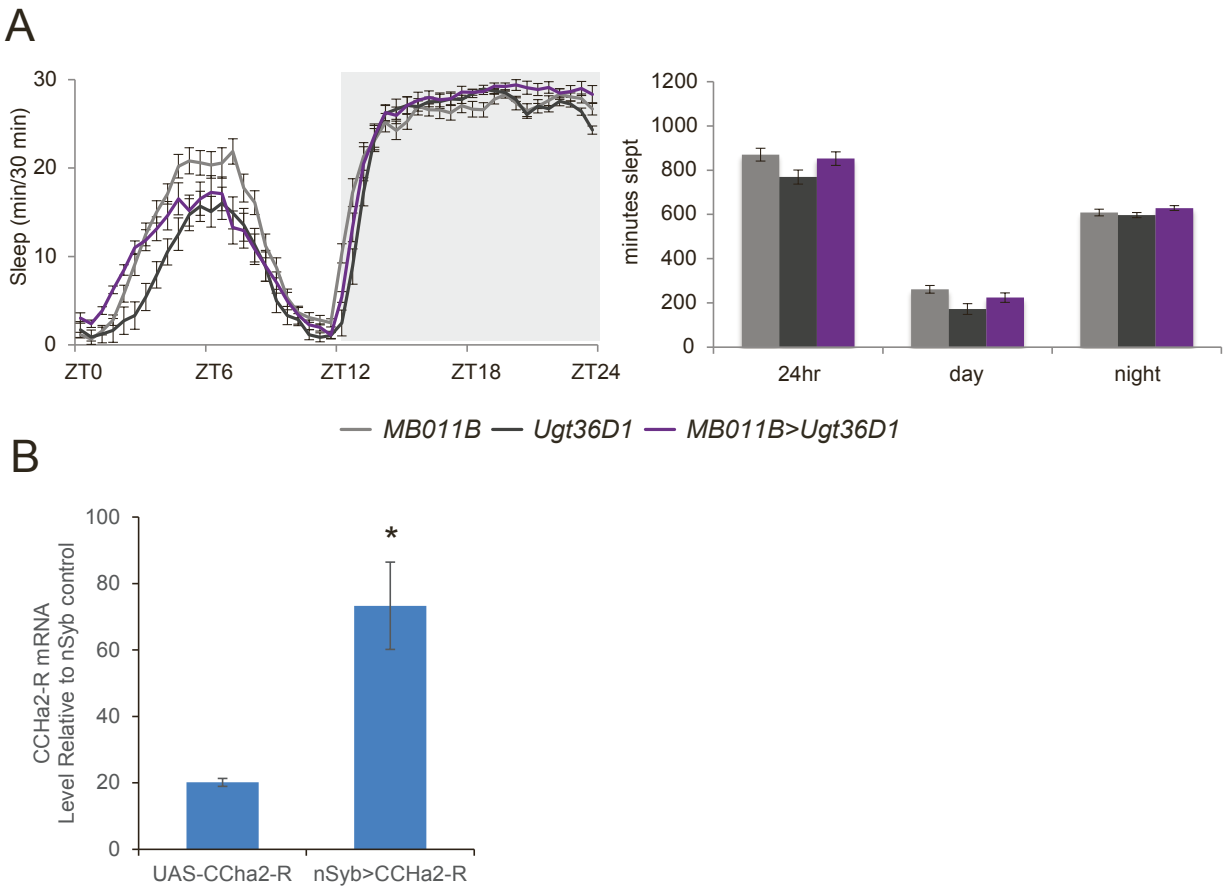


Figure S2. Related to Figure 2.

Effects of overexpression of putative ban targets on sleep and effectiveness of overexpression transgene. (A) Ugt36D1 does not affect sleep. Sleep data for *MB011B> Ugt36D1* (n=24), *MB011B/+* control (n=27) and *UAS-Ug36D1/+* control (n=28) (mean± SEM). Overexpression of this gene product in MBONs has no effect on sleep. Note that the GAL4 control data set is the same as shown in Figure 2D as these lines were all run concurrently. (B) qPCR for mRNA from *nSyb*, *nSyb>CCHa2-R* and *UAS-CCHa2R* adult flies using CCHa2-R primers. Mean value (± SEM) for three replications.
* represents P≤.05 using One-Way ANOVA with Tukey-Kramer test.

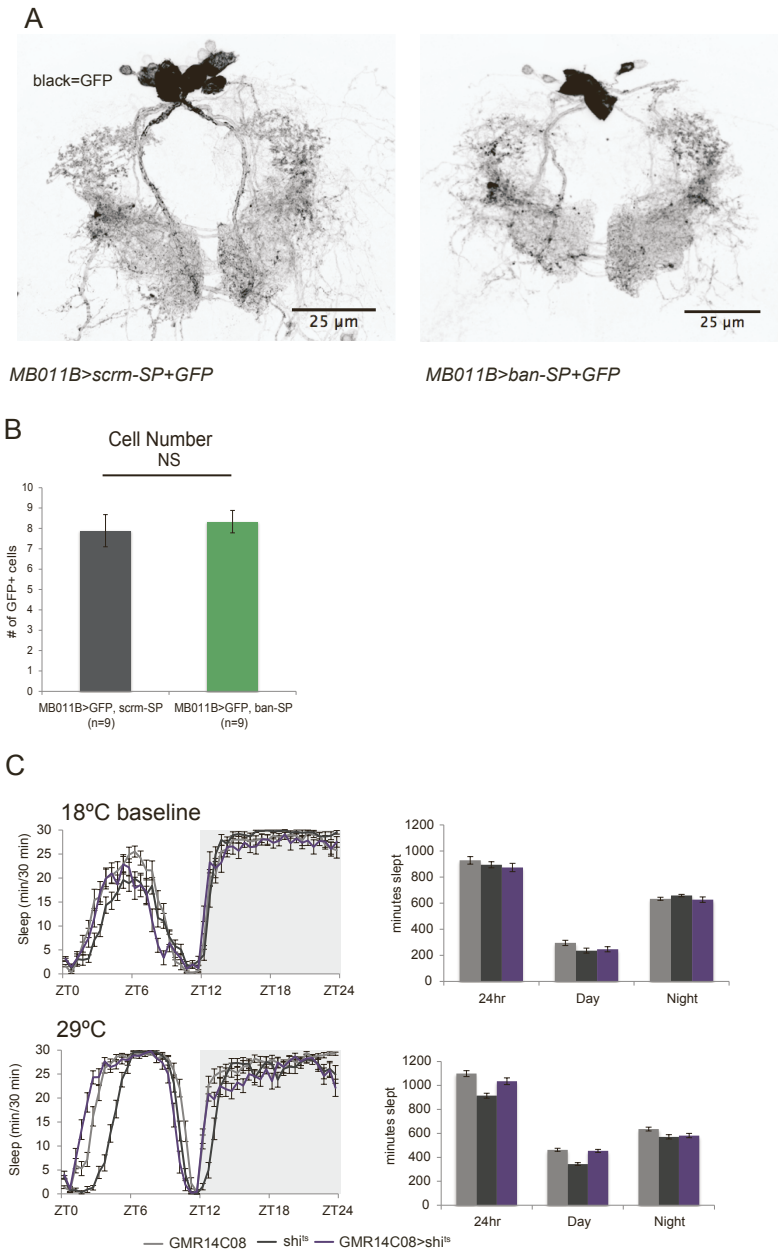


Figure S3. Related to Figure 3.

γ5β′2a/β′2mp/β′2mp_bilateral MBONs develop normally when ban is disrupted. Knockdown of ban in the γ5β′2a/β′2mp/β′2mp_bilateral MBONs does not change cell number or gross morphology. The output of these cells does not contribute significantly to baseline sleep arguing that their activity is recruited in specific contexts to alter early night sleep. (A) IHC anti-GFP staining of representative central brains of *MB011B>CD8::GFP+UAS- scr-SP* and *MB011B>CD8::GFP ban-SP* flies. Scale bar is 25 um. (B) Quantification of the number of GFP-positive cells per brain. Data shown as (mean± SEM). (C) Sleep data for *GMR14C08>shi^{ts}* (n=22) *GMR14C08-GAL4/+* control (n=27) and *UAS-shi^{ts}/+* control (n=31) at 18ºC and 29ºC. Data shown as (mean± SEM). No significant change in sleep was seen at temperatures where neurotransmission is blocked.