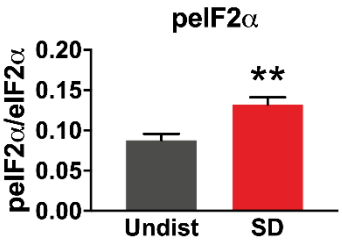

Figure S1. Sleep deprivation leads to enhanced eIF2α phosphorylation in Drosophila heads. Related to Figure 1. Quantification from western blot analysis of peIF2α expression in Drosophila heads collected at ZT0 that are either undisturbed (Undist) or sleep-deprived (SD) from ZT18-ZT24. peIF2α protein level is 50% higher following sleep deprivation (Undist, N=6 biological replicates; SD, N=5 biological replicates; each replicate consisted of 10 pooled heads and 20ug protein was loaded from each pool; **P<0.01, student's t-test). Bar graph shows mean ± SEM.

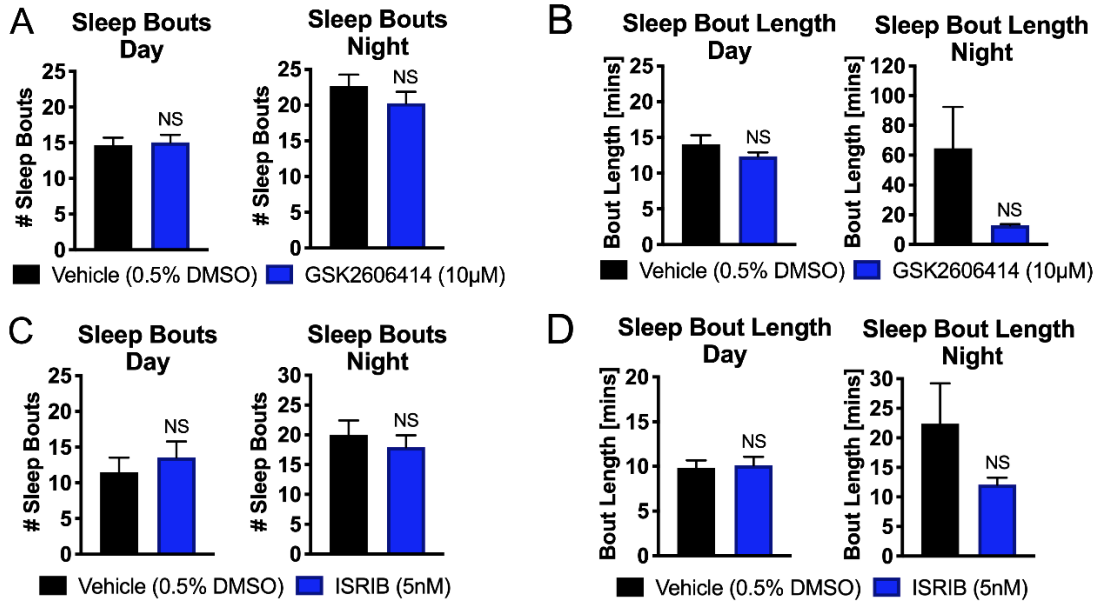

Figure S2. Pharmacological inhibition of the PERK pathway by treatment with GSK2606414 or ISRIB does not significantly affect sleep architecture in *Drosophila.* Related to Figures 2 and 3.
(A) Administration of GSK2606414 does not significantly affect (A) daytime and nighttime sleep bout number or (B) daytime and nighttime sleep bout length relative to vehicle controls *(N=42 animals, NS= not significant; student's t-test).* (C) Administration of ISRIB does not significantly affect (C) daytime and nighttime sleep bout number or (D) daytime and nighttime sleep bout length relative to vehicle controls *(N=28 animals, NS= not significant; student's t-test).* Bar graphs show mean ± SEM.

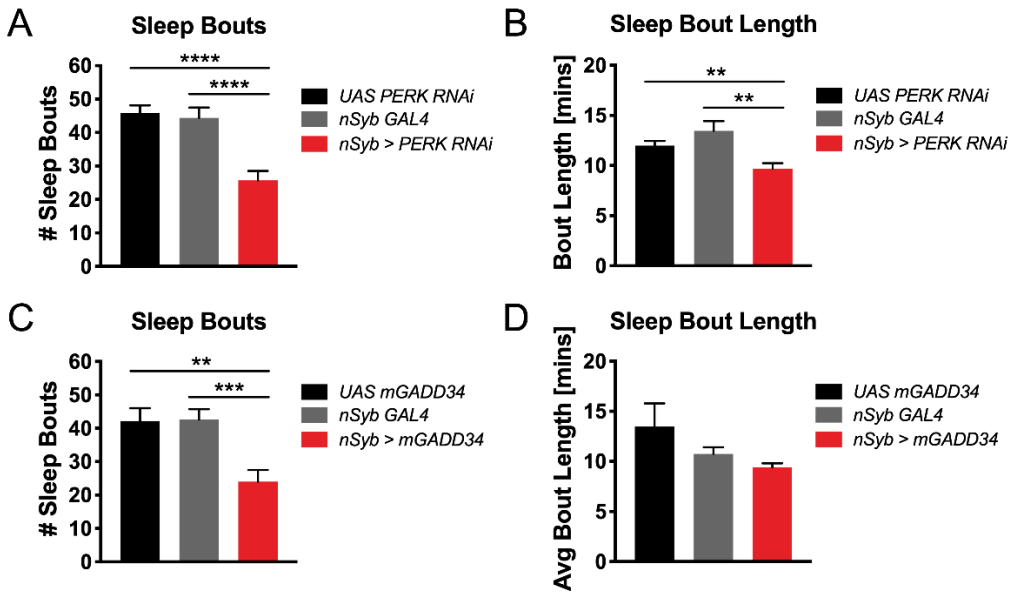

Figure S3. Genetic inhibition of peIF2α reduces the length and number of sleep bouts. Related to Figure 4.

(A) The average number of daily sleep bouts is reduced in flies expressing PERK RNAi in neurons relative to parental controls *(N=27 animals, P<0.0001, one-way ANOVA; **P<0.01, ****P<0.0001).* (B) PERK RNAi expression in neurons decreases average daily sleep bout length relative to parental controls *(N=27 animals, P<0.01, one-way ANOVA; **P<.01).* (C) The average number of sleep bouts is reduced in flies expressing mGADD34 in neurons relative to parental controls *(N=18 animals, P<0.001, one-way ANOVA; **P<0.01, ***P<.001).* (D) mGADD34 overexpression in neurons does not significantly alter average daily sleep bout length relative to parental controls *(N=18 animals, P=.0914, one-way ANOVA).* Bar graphs show mean ± SEM.

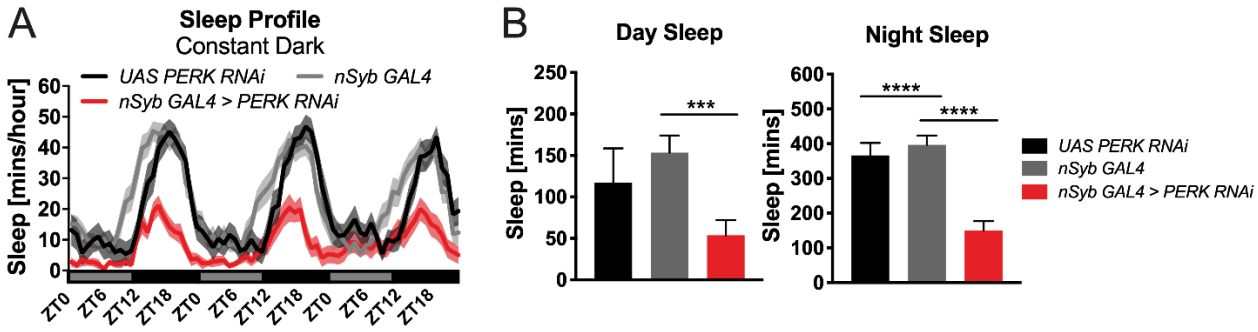

Figure S4. PERK knockdown reduces sleep amount under constant conditions. Related to Figure 4.

(A) 3-day sleep profile of flies expressing *PERK* RNAi in nSyb-expressing neurons compared to uncrossed parental controls following 5 days in constant darkness *(UAS PERK RNAi, N=15 animals; nSyb GAL4, N=19 animals; nSyb>PERK RNAi, N=21 animals).* (B) Quantification of daytime and nighttime sleep. Daytime sleep is significantly reduced by *PERK RNAi* expression in neurons *(P= 0.0225, one-way ANOVA; *** P<0.001, student's t-test)*. Daytime sleep is significantly reduced by *PERK RNAi* expression in neurons *(P<0.0001, one-way ANOVA; ****P<0.0001, student's t-test).* Line and bar graphs show mean ± SEM. Grey and black boxes under line graph indicate subjective day and night, respectively.

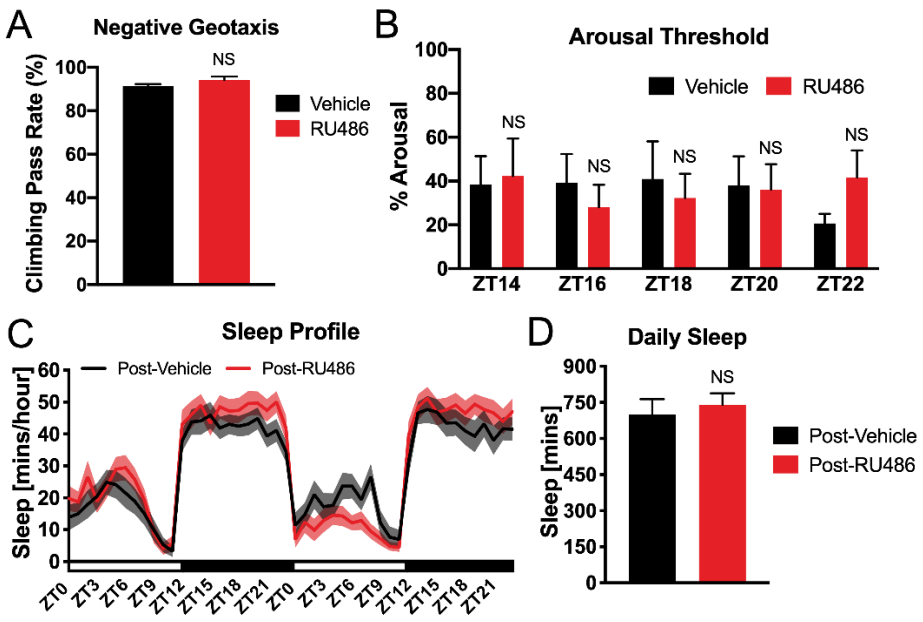

Figure S5. RU486-induction of PERK overexpression does not impair locomotor activity or alter arousal threshold and sleep patterns return to normal levels after RU486 is removed. Related to Figure 6.
(A) RU486 treatment in transgenic flies (*nSyb GeneSwitch>dPERK*) did not impair locomotor activity as measured by climbing pass rate in a negative geotaxis assay *(N=100 animals)* (B) Percentage of *nSyb* GeneSwitch>*dPERK* flies that are aroused by a mechanical kick during sleep is not significantly different between vehicle or RU486 treatment groups (*N= 63 animals, P>0.99, Mann-Whitney U Test; NS= not significant, student's t-test*). (C) 48 hour sleep profiles for transgenic flies after three days on regular dextrose medium. Previously, flies were either administered food containing vehicle (0.8% EtOH) or RU486 (100uM) *(N=19 animals)* (D) Quantification of daily sleep amounts *(N=19 animals, NS= not significant, student's t-test).* Line and bar graphs show mean ± SEM. White and black boxes under line graphs indicate day and night, respectively.

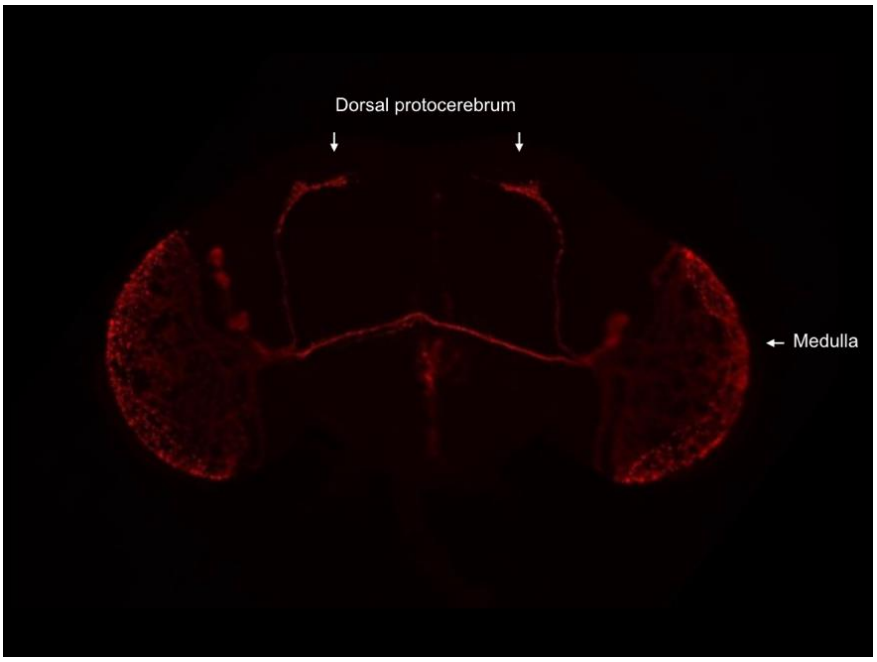

Figure S6. PDF is expressed in a subset of lateral ventral neurons that have projections into the medulla and the dorsal protocerebrum. Related to Figure 7.
Immunohistochemical analysis of PDF demonstrates its known expression pattern in the *Drosophila* brain.

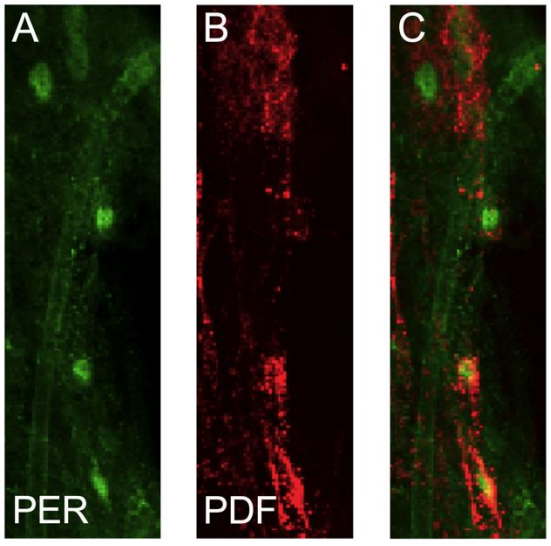

Figure S7. *PERK* overexpression does not induce cell loss of the sLNvs. Related to Figure 7.
Visualization of the sLNvs in the PERK overexpressing transgenic flies. Immunohistochemical analysis of PER and PDF confirms that PERK overexpression does not lead to loss of sLNVs cell bodies. (A) Immunostaining of PER confirms the presence of the sLNvs in the *PDF>dPERK* transgenic line (B) PDF signal is not limited to the cell bodies in the *PDF>dPERK* flies and (C) partially colocalizes with the sLNvs PER signal in cell bodies.