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Supplemental Data

PDF Cells Are a GABA-Responsive Wake-Promoting Component of the *Drosophila* Sleep Circuit

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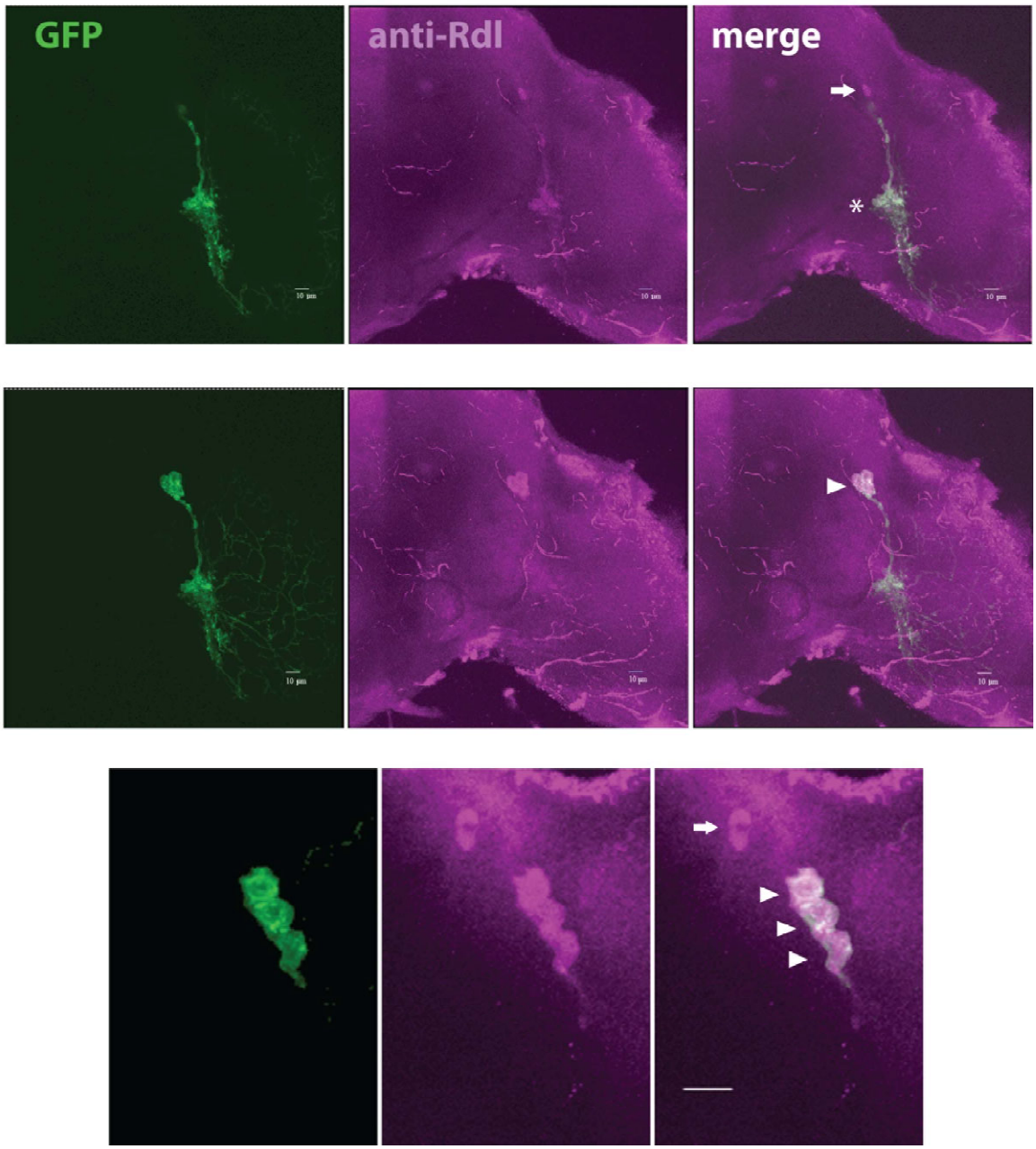
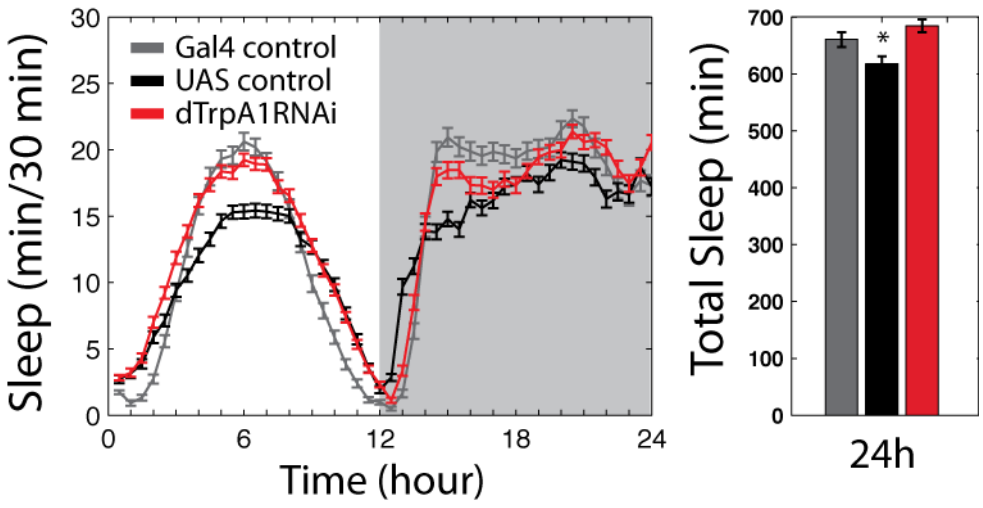
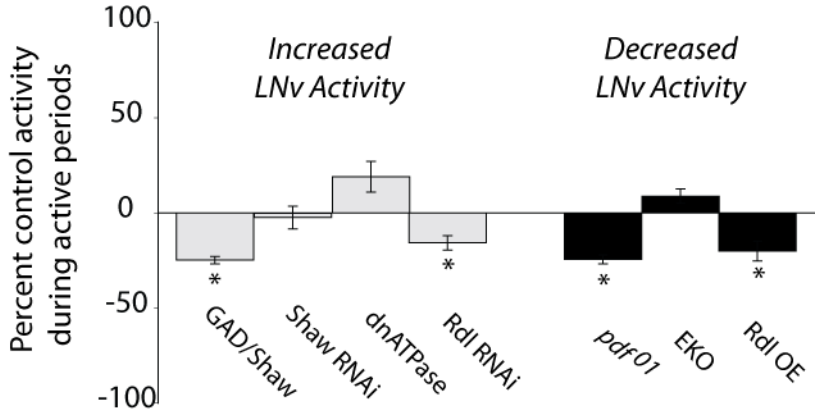


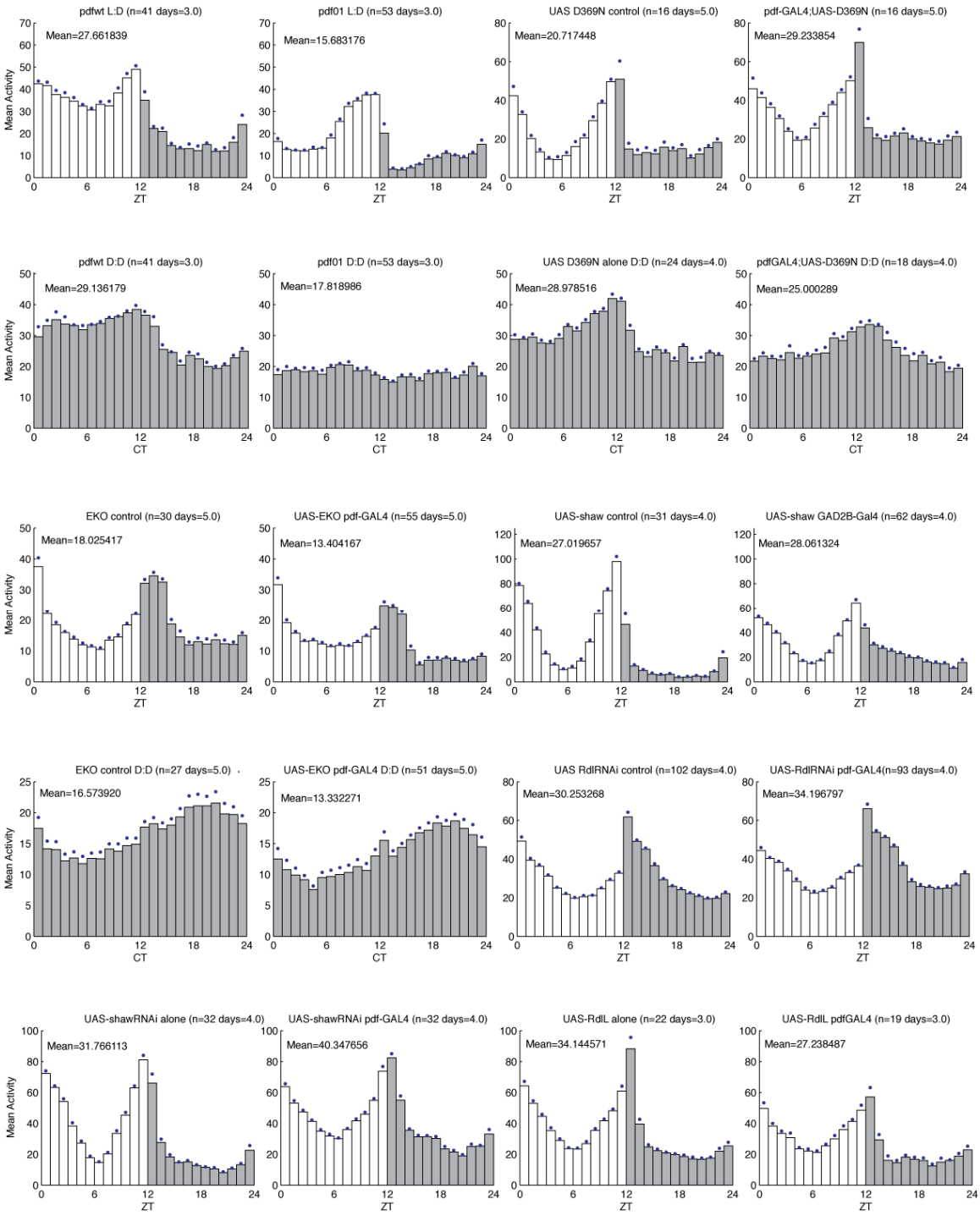
Supplemental Figure 1. Rdl expression in LNvs. Adult brains from *pdf-GAL4;UAS-mCD8GFP* animals were stained with anti-GFP (green) and anti-Rdl (magenta) as described in Materials and Methods. Top and middle panels show two confocal stacks from the same brain. Top panel is taken at the level of small LNvs (16 μm of tissue) and middle at the level of large LNvs (29 μm of tissue). Bottom panel shows a confocal stack of 17 μm from a different brain zoomed 4X. All images were acquired at 63X. Asterisks indicate cell bodies of the small LNvs, arrowheads indicate cell bodies of the large LNvs and arrows indicate the putative 5th small LNv. Scale bars are 10 μm.

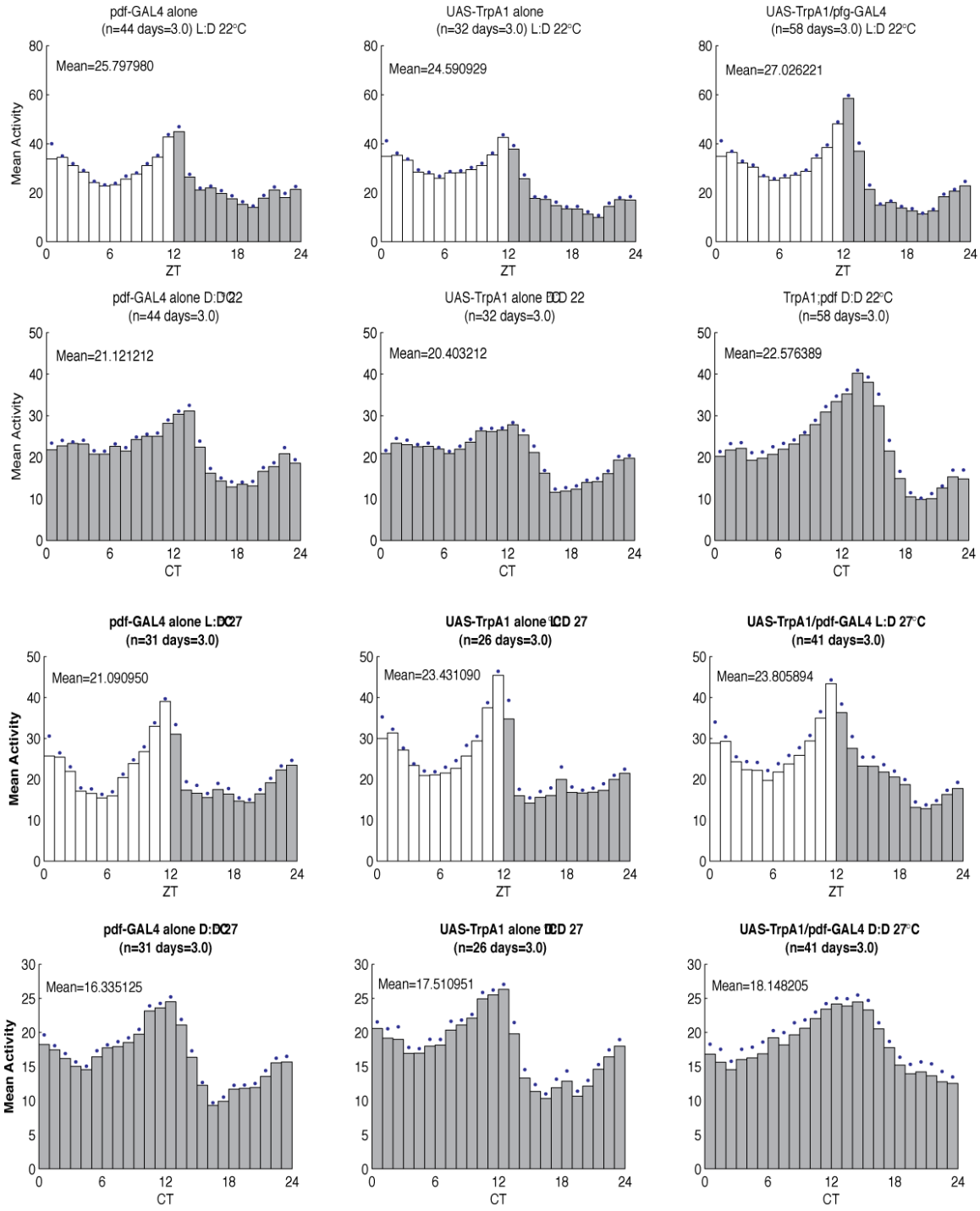


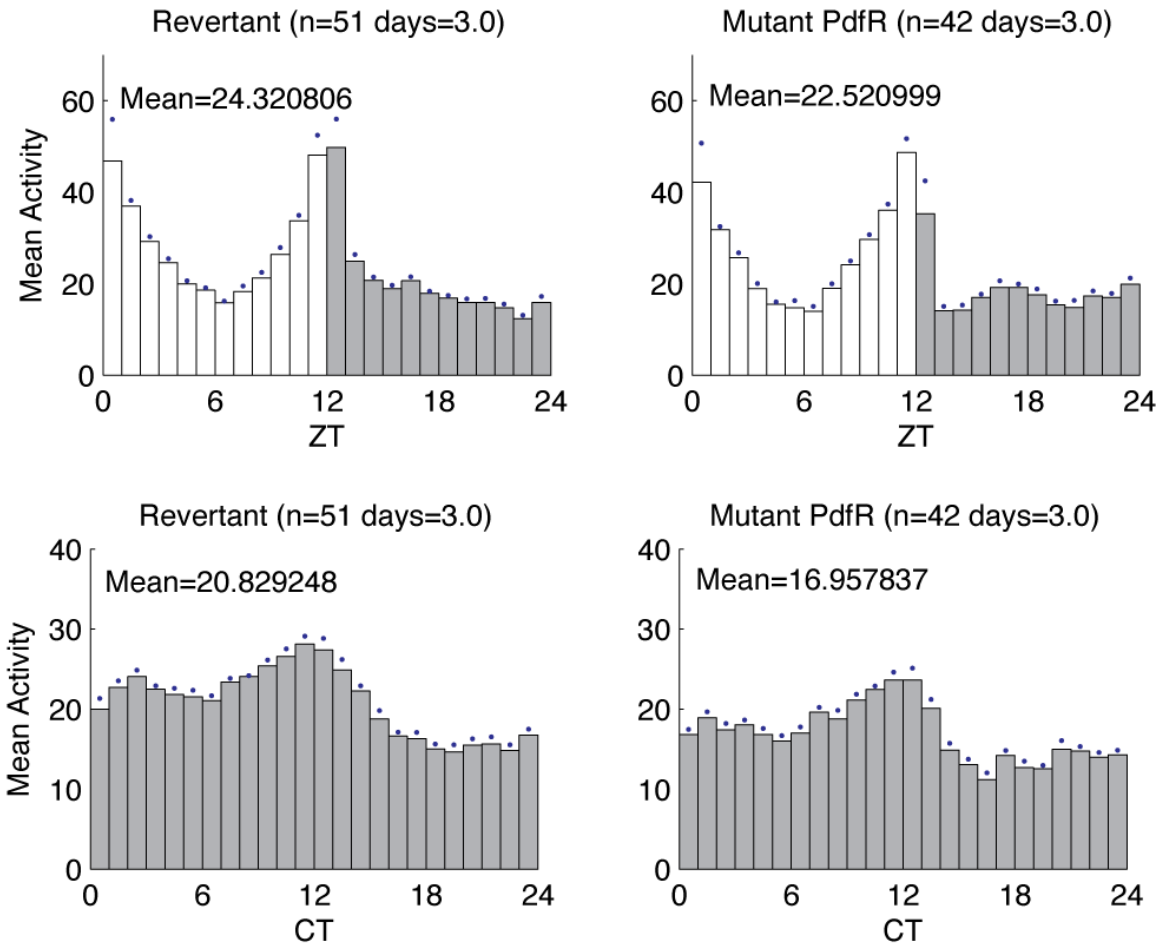
Supplemental Figure 2. Expression of control RNAi does not affect sleep. *UAS-dTrpA1RNAi* was expressed in LNvs driven by *pdf-GAL4*. * indicates $P < 0.5$ for the comparison of the UAS to GAL4 control and experimental by ANOVA with Tukey posthoc test, indicating a mild insertion effect of the UAS transgene. Experimental was not significantly different from GAL4 alone ($P > 0.05$), indicating that expression of control RNAi in LNvs does not affect sleep. n = 69, 77 and 84 for UAS alone, GAL4 alone and experimental respectively.



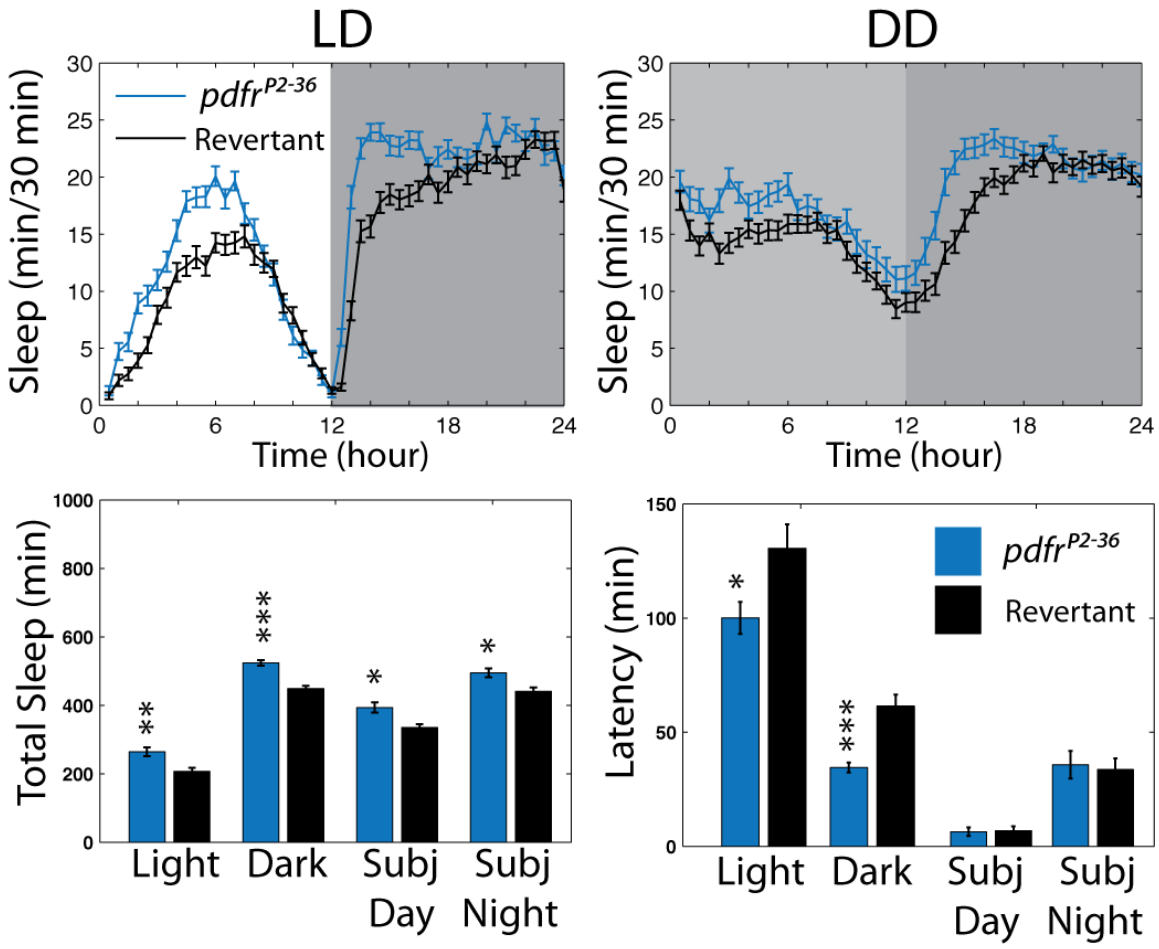
Supplemental Figure 3. Locomotor activity during active (non-sleep) periods. To determine if LNv manipulation was affecting basal motor activity, locomotor activity during wake periods was assessed by counting line crossings during wake periods for each genotype and is expressed as a percentage of the activity of the appropriate genetic control. While some experimental genotypes were significantly less active, no consistent relationship was seen between locomotor activity levels and the neuronal activity of LNvs or sleep. Data were analyzed by ANOVA with Tukey posthoc test; * indicates $P > 0.05$. $n \geq 32$ for each genotype.


pdfwt L:D (n=41 days=3.0)
Mean=27.661839
pdf01 L:D (n=53 days=3.0)
Mean=15.683176
UAS D369N control (n=16 days=5.0)
Mean=20.717448
pdf-GAL4;UAS-D369N (n=16 days=5.0)
Mean=29.233854
pdfwt D:D (n=41 days=3.0)
Mean=29.136179
pdf01 D:D (n=53 days=3.0)
Mean=17.818986
UAS D369N alone D:D (n=24 days=4.0)
Mean=28.978516
pdfGAL4;UAS-D369N D:D (n=18 days=4.0)
Mean=25.000289
EKO control (n=30 days=5.0)
Mean=18.025417
UAS-EKO pdf-GAL4 (n=55 days=5.0)
Mean=13.404167
UAS-shaw control (n=31 days=4.0)
Mean=27.019657
UAS-shaw GAD2B-Gal4 (n=62 days=4.0)
Mean=28.061324
EKO control D:D (n=27 days=5.0)
Mean=16.573920
UAS-EKO pdf-GAL4 D:D (n=51 days=5.0)
Mean=13.332271
UAS RdlRNAi control (n=102 days=4.0)
Mean=30.253268
UAS-RdlRNAi pdf-GAL4(n=93 days=4.0)
Mean=34.196797
UAS-shawRNAi alone (n=32 days=4.0)
Mean=31.766113
UAS-shawRNAi pdf-GAL4 (n=32 days=4.0)
Mean=40.347656
UAS-RdlL alone (n=22 days=3.0)
Mean=34.144571
UAS-RdlL pdfGAL4 (n=19 days=3.0)
Mean=27.238487
Mean Activity
ZT
CT


pdf-GAL4 alone
(n=44 days=3.0) L:D 22°C
Mean=25.797980
UAS-TrpA1 alone
(n=32 days=3.0) L:D 22°C
Mean=24.590929
UAS-TrpA1/pfg-GAL4
(n=58 days=3.0) L:D 22°C
Mean=27.026221
pdf-GAL4 alone D:D 22
(n=44 days=3.0)
Mean=21.121212
UAS-TrpA1 alone D:D 22
(n=32 days=3.0)
Mean=20.403212
TrpA1;pdf D:D 22°C
(n=58 days=3.0)
Mean=22.576389
pdf-GAL4 alone L:D 27
(n=31 days=3.0)
Mean=21.090950
UAS-TrpA1 alone L:D 27
(n=26 days=3.0)
Mean=23.431090
UAS-TrpA1/pdf-GAL4 L:D 27°C
(n=41 days=3.0)
Mean=23.805894
pdf-GAL4 alone D:D 27
(n=31 days=3.0)
Mean=16.335125
UAS-TrpA1 alone D:D 27
(n=26 days=3.0)
Mean=17.510951
UAS-TrpA1/pdf-GAL4 D:D 27°C
(n=41 days=3.0)
Mean=18.148205
Mean Activity
ZT
CT



Supplemental Figure 4. Locomotor rhythms of experimental and control genotypes. To determine if activity manipulations and/or mutations altered circadian rhythms we assessed locomotor activity in Trikinetics DAM monitors in LD for all genotypes. DD data are shown for a subset of genotypes.



Supplemental Figure 5. *pdfr* mutants have increased total sleep and decreased sleep latency. Standard sleep plot of mutant (n = 87) and precise excision revertant (n = 102) flies in 12 hour: 12 hour light:dark (LD, left) or in constant darkness (DD, right). Bottom panels show total sleep is increased compared to control in both LD and DD. Sleep latency is decreased in both light and dark conditions in LD, but is not affected in DD. * indicates $P < 0.05$, ** $P < 0.005$ and *** $P < 0.0005$ for comparisons of experimental and control with ANOVA and Tukey posthoc test.