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## Supplemental Information

# MicroRNAs Regulate Sleep and Sleep Homeostasis in *Drosophila*

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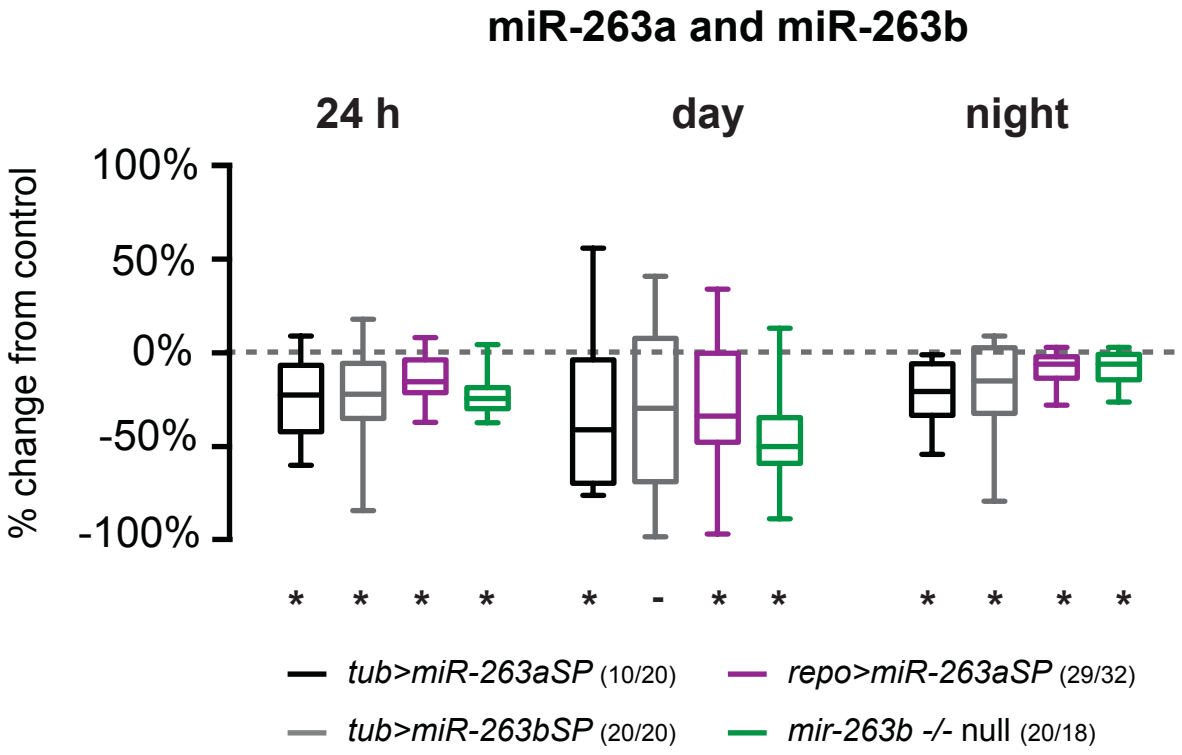
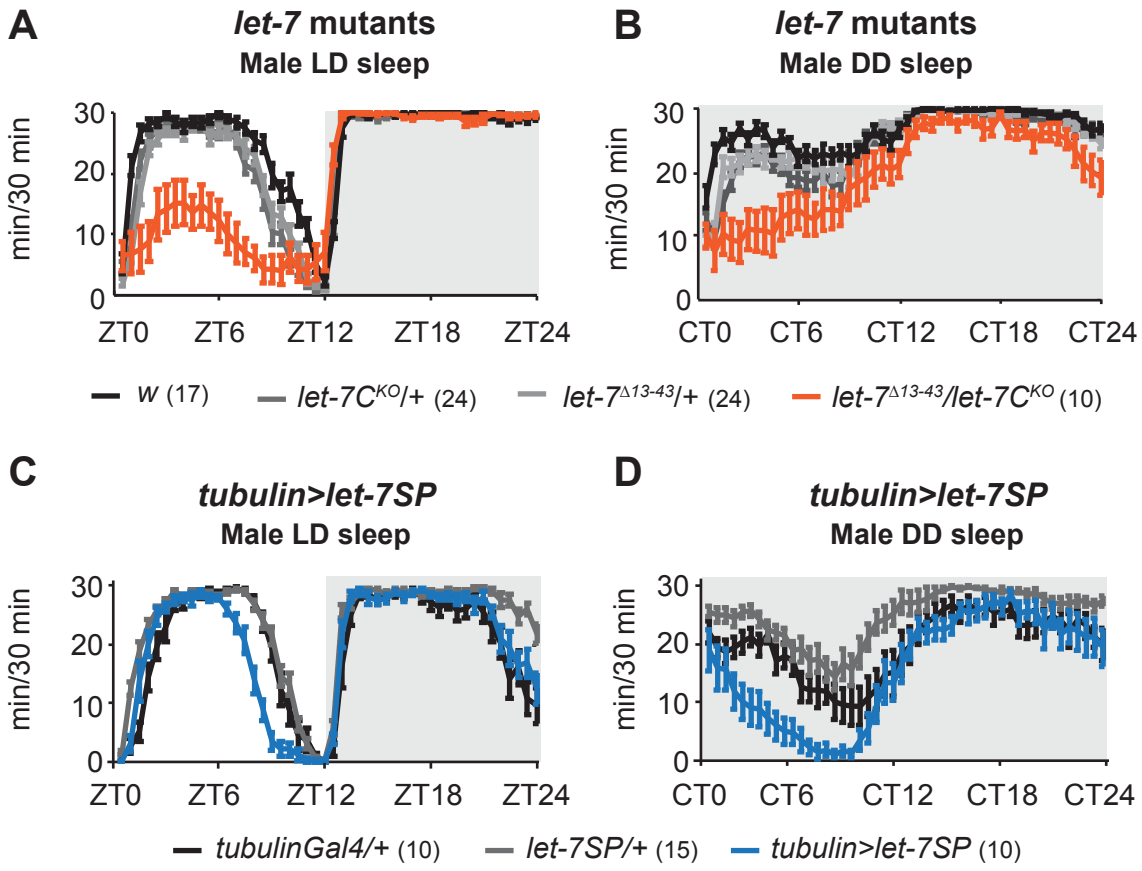
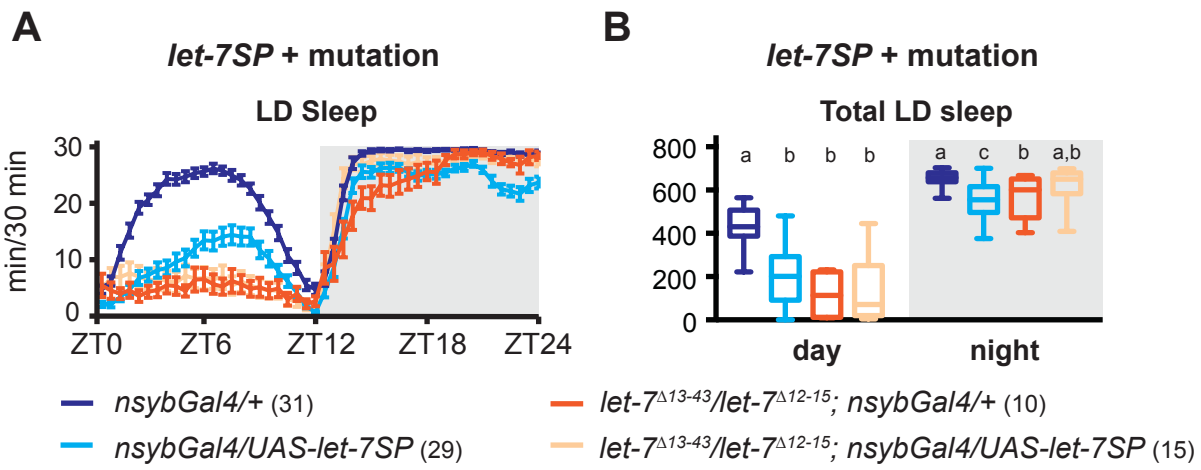



**Supplemental Figure 1. Sponges targeting miRs in the same family have similar effects on sleep. Related to Figure 3.** Ubiquitous expression of miR-263aSP decreased 24 h, day, and night sleep. Although miR-263bSP did not satisfy all the criteria to be considered a hit, it was very close to the cut-off (24 h sleep for control group: -22%, $p<0.05$; 24 h sleep for SD group: -19%, $p=0.057$). We found that miR-263a is not required in neurons for its effect on sleep (Table 1). However, inhibition of miR-263a in glia via expression of miR-263aSP with repo Gal4 significantly reduced 24 h, day, and night sleep. We were not able to investigate the effect of mir-263a mutations on sleep, because homozygous mutants are not viable as adults; however mir-263b mutants displayed reduced 24 h, day, and night sleep relative to controls. These results support a glial role for the miR-263 family in sleep.

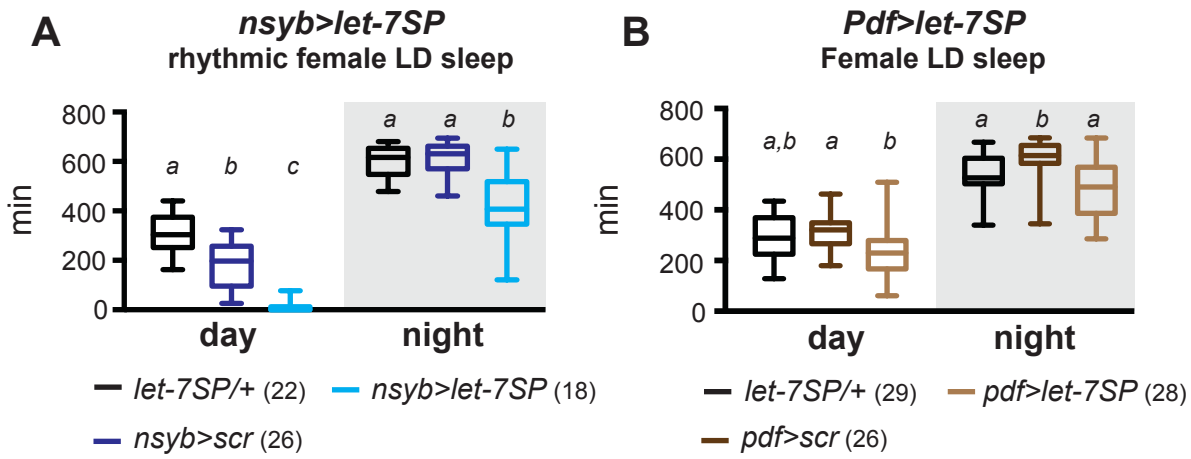
Figure shows percent change in 24 h, day, and night sleep relative to scramble or genetic background control after inhibition of miR-236a and miR-263b by expression of sponges with *tubulinGal4* (grey plots), glia-specific *repoGal4* (purple plots), or through knockout of the *mir-263b* gene (green). '*' $p<0.05$, '-' $p>0.05$ using Mann-Whitney test. N values are reported next to genotype.




**Supplemental Figure 2. Let-7 regulates sleep in males. Related to Figure 4.** Sleep per 30 min in *let-7CKO/let-7Δ13-43*, *let-7CKO/+*, *let-7Δ13-43/+*, and *w* male flies in L:D (**A**) and D:D (**B**). Sleep per 30 min in *tubulin>let-7SP*, *tubulinGal4/+*, and *UAS-let-7SP* male flies in L:D (**C**) and D:D (**D**). N values are reported next to genotype.




**Supplemental Figure 3. Let-7SP and let-7 mutations affect sleep through the same pathway. Related to Figure 4.** Sleep per 30 min (A) and total sleep (B) for female let-7 mutant flies expressing let-7SP in neurons (w; let-7Δ13-43/ let-7Δ12-15; nsybGal4/UAS-let-7SP), let-7 mutant flies (w; let-7Δ13-43/ let-7Δ12-15; nsybGal4/+), flies expressing let-7SP in neurons (w;; nsybGal4/ UAS-let-7SP) and control (nsybGal4/+) flies. Flies with both the sponge and mutations were not significantly different from flies with the sponge alone or mutations alone (Kruskal-Wallis, $p<0.05$). N values are reported next to genotype.




**Supplemental Figure 4. Related to Table 2** (**A**) *Nsyb>let-7SP* flies with normal circadian rhythms have reduced sleep. Total sleep in *nsyb>let-7SP, nsyb>scramble*, and *UAS-let-7SP/+* females in L:D after excluding any flies that were later arrhythmic in D:D. (**B**) Let-7 is not required in PDF+ neurons to promote sleep. Total sleep in *PDF>let-7SP, PDF>scramble,* and *UAS-let-7SP/+* female flies in L:D.