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Supporting Information for

The *Drosophila* blood-brain barrier regulates sleep via Moody GPCR signaling

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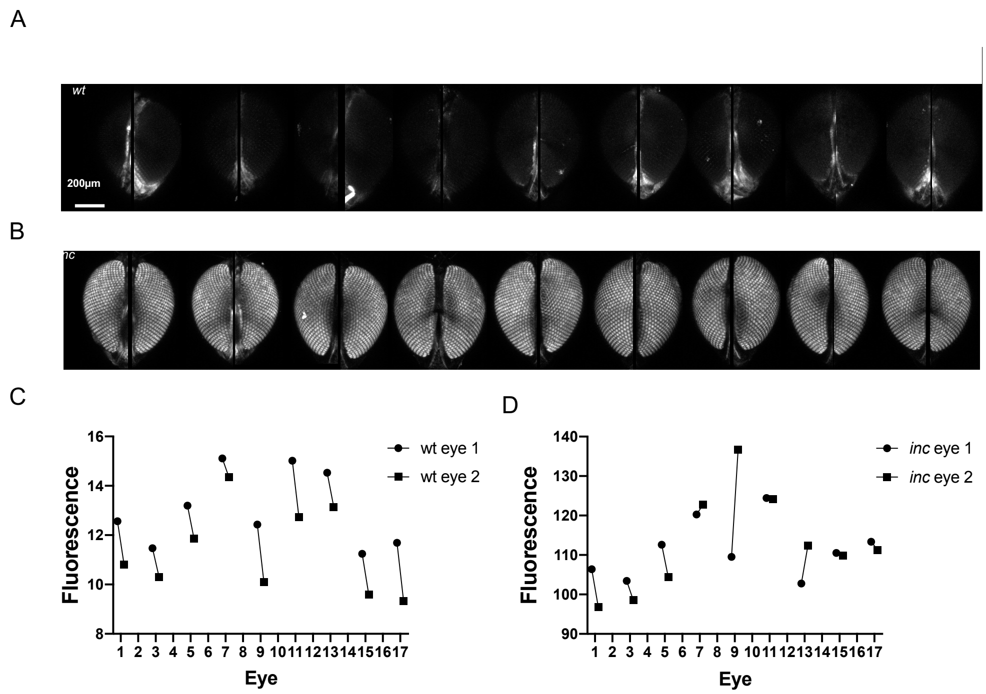

Fig. S1

(A,B) BBB injection assay. Shown is 10 kDa Dextran-TexasRed imaging in 18 eyes from 9 flies for (A) wt and (B) *inc*. Dye permeability is highly consistent between eyes and flies of the same genotype. The variance of the difference between eyes from the same individual is not statistically different from the variance of inter-individual differences (F-test).

(C,D) Quantified fluorescence from eyes #1 and #2 for 9 wt (C) and inc flies (D).

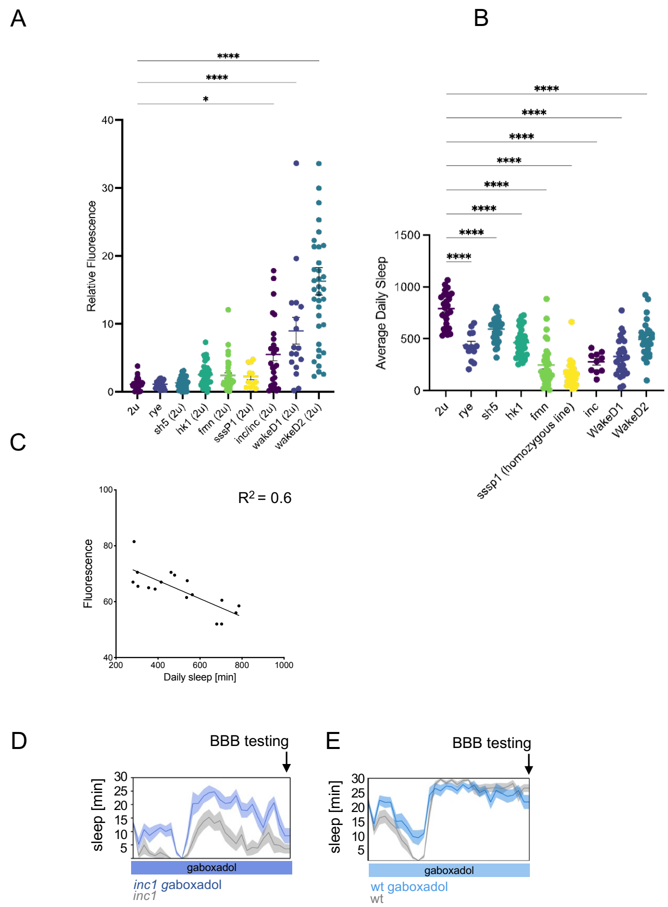

Fig. S2

(A) Scatter plot showing BBB permeability as measured by tracer injection assay for 8 sleep mutants. Shown are data from one representative experiment (2-5 total, see Fig. 3B) with n=30-40 each. Significance was assessed using one-way ANOVA and post hoc Dunn's test.

(B) Scatter plot showing daily sleep for 8 sleep mutants. Shown are data from one representative experiment (4-5 total, see Fig. 3C) with n=32 each. Significance was assessed using one-way ANOVA and post hoc Dunn's test.

(C) BBB permeability correlates with previous sleep. *inc* flies were injected one-by-one after individual sleep recordings. Plotted are total average daily sleep from 4 days prior to injection versus TexasRed 10 kDa fluorescence in eyes as a readout for BBB permeability. R^2=0.6.

(D) *inc* or (E) wild-type flies received 1 mg/ml gaboxadol by feeding for 15 h. Shown is average sleep in 16 flies binned as sleep in 30 min.

For quantification of BBB permeability, fluorescence was measured for both eyes of each fly. Each dot represents normalized fluorescence in one eye. Significance levels are P<0.05: *, <0.01: **, <0.001: ***, <0.0001: ****.

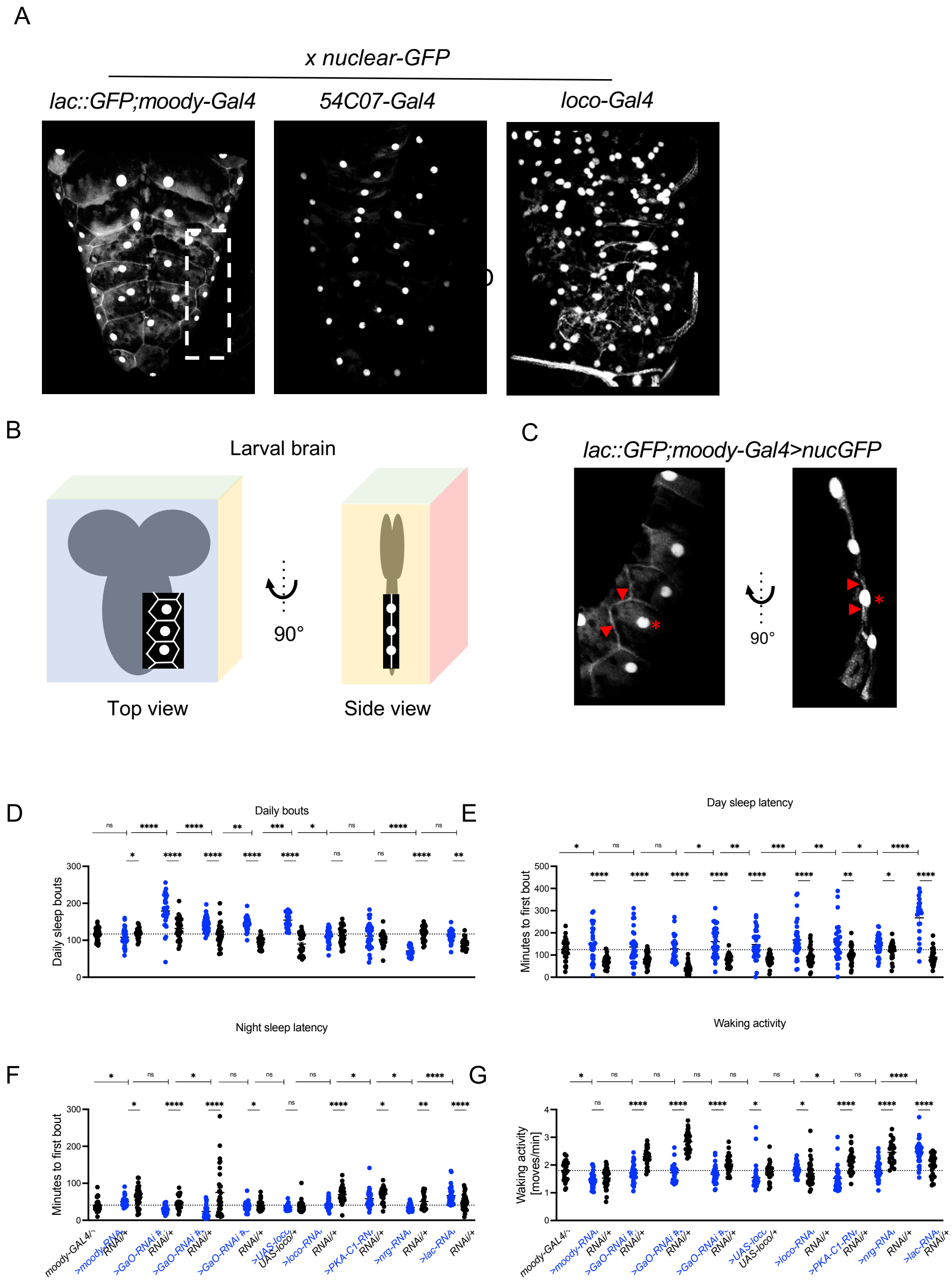

Fig. S3

(A-C) Specificity of the *moody-Gal4* driver.

(A) *lachesin-GFP;moody-Gal4*, the SPG driver *57C07-Gal4* and the pan-glial driver *loco-Gal4* were crossed to *UAS-nuclear-GFP*. 3rd instar larval brains of the F1 progeny were dissected and the ventral nerve cord was imaged live using confocal microscopy. *moody-Gal4* specifically localizes to lachesin-GFP marked, *54C07-Gal4* positive cells. *loco-Gal4* labels additional glia.

(B-C) 90º rotation of the confocal stack as displayed in **(B)** reveals that *moody-Gal4* labeled nuclei are inside the lachesin-GFP marker delineating SPG boundaries. **(D)** Daily sleep bouts, **(E)** Day and **(F)** Night sleep latency as minutes to first bout, and **(G)** Waking activity as moves per waking minute in flies with transgenic RNAi knockdown of the indicated *moody* pathway components in the BBB using *moody-Gal4,dicer.*

Significance was assessed using one-way ANOVA and post hoc Dunn's test. Significance levels are P<0.05: *, <0.01: **, <0.001: ***, <0.0001: ****. For D-G, ⊣ indicates comparison to *moody-GAL4/+*. Horizontal dashed lines in D-G indicate mean of *moody-Gal4/+* control.