

Figure S1. Comparison of locomotor activity in isogenic sub-lines A and B. Average (\pm SEM) profile of activity (A1-D1 and A3-D3) and total day and night activity (A2-D2 and A4-D4) under 12:12 LD conditions, for axenic and conventional flies from isogenic sub-line A (A1-D1 and A2-D2) and B (A3-D3 and A4-D4). Results for axenic flies are colored as indicated; corresponding results for conventional flies are indicated using a gray line and fill regardless of genotype. Number of flies tested is indicated in panels 1 and 3 of every row. Data were analyzed by a two-way RM ANOVA and Sidak test where * $p < 0.0332$, ** $p < 0.0021$, *** $p < 0.0002$, and **** $p < 0.0001$. S, M, L: small, medium, and large effect size, respectively, Hedges' g effect size analysis; no letter or symbol means no effect. See Table S1 for values.

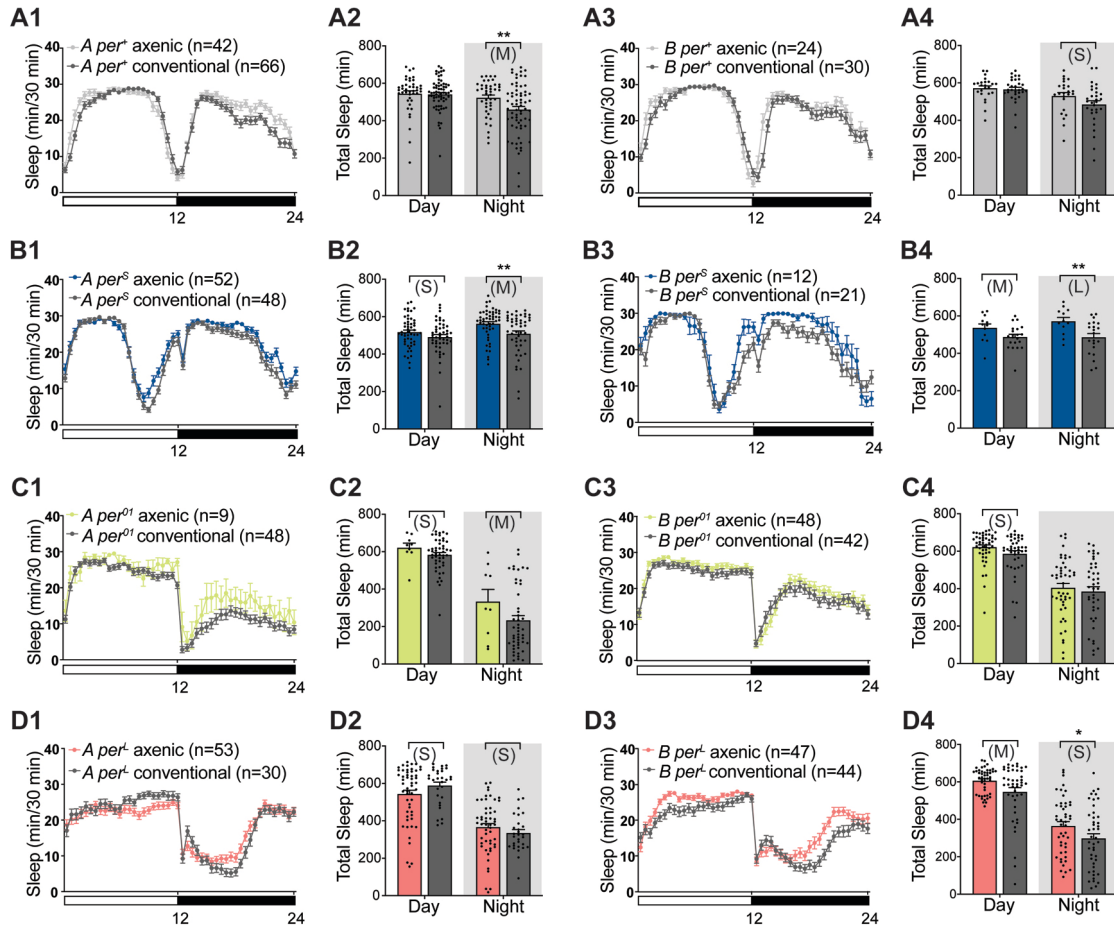


Figure S2. Comparison of sleep behavior in isogenic sub-lines A and B. Average (\pm SEM) sleep per 30 min (A1-D1 and A3-D3), and total day and night sleep (A2-D2 and A4-D4) under 12:12 h L:D conditions, for axenic and conventional flies from isogenic sub-line A (A1-D1 and A2-D2) and B (A3-D3 and A4-D4). Results for axenic flies are colored as indicated; corresponding results for conventional flies are indicated using a gray line and fill regardless of genotype. Number of flies tested is indicated in panels 1 and 3 of every row. Data were analyzed by a two-way RM ANOVA and Sidak test where * $p < 0.0332$, ** $p < 0.0021$, *** $p < 0.0002$, and **** $p < 0.0001$. S, M, L: small, medium, and large effect size, respectively, Hedges' g effect size analysis; no letter or symbol means no effect. See Table S1 for values.

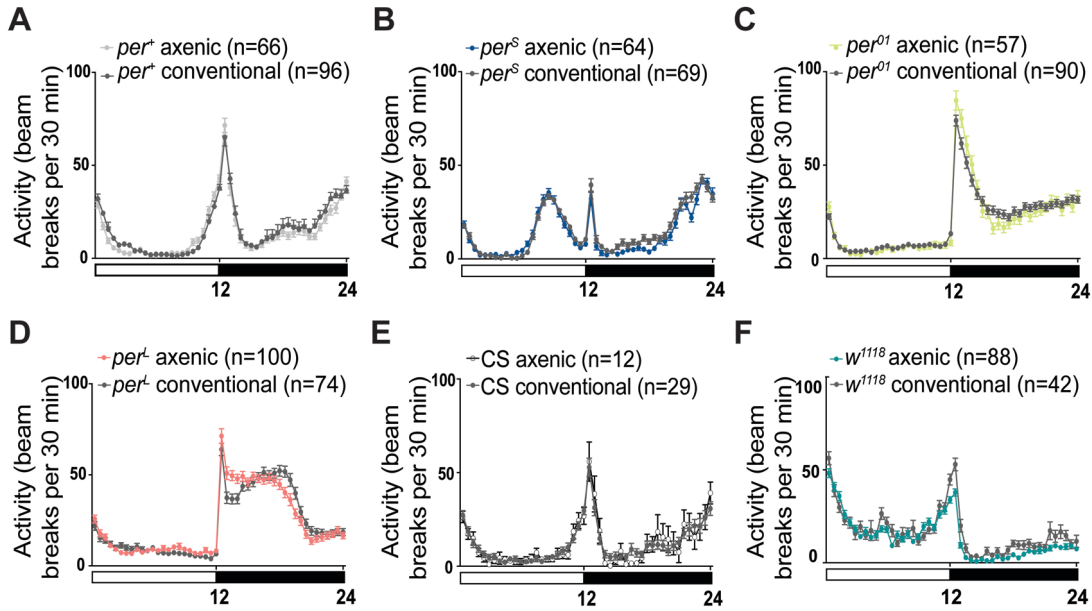


Figure S3. Profiles of locomotor activity of axenic and conventional flies. (A-F) Profiles of locomotor activity under 12:12 LD conditions, averaged over 2 days (\pm SEM), for axenic and conventional flies. Results for axenic flies are colored as indicated; corresponding results for conventional flies are indicated using a gray line regardless of genotype. Flies used for results shown in panels A-D (per^+ , per^S , per^{O1} , and per^L) are from isogenic strains, and E-F are non-isogenic controls (CS and w^{1118}). n indicates the number of flies tested. (Results for isogenic sub-lines A and B are shown separately in Figure S1).

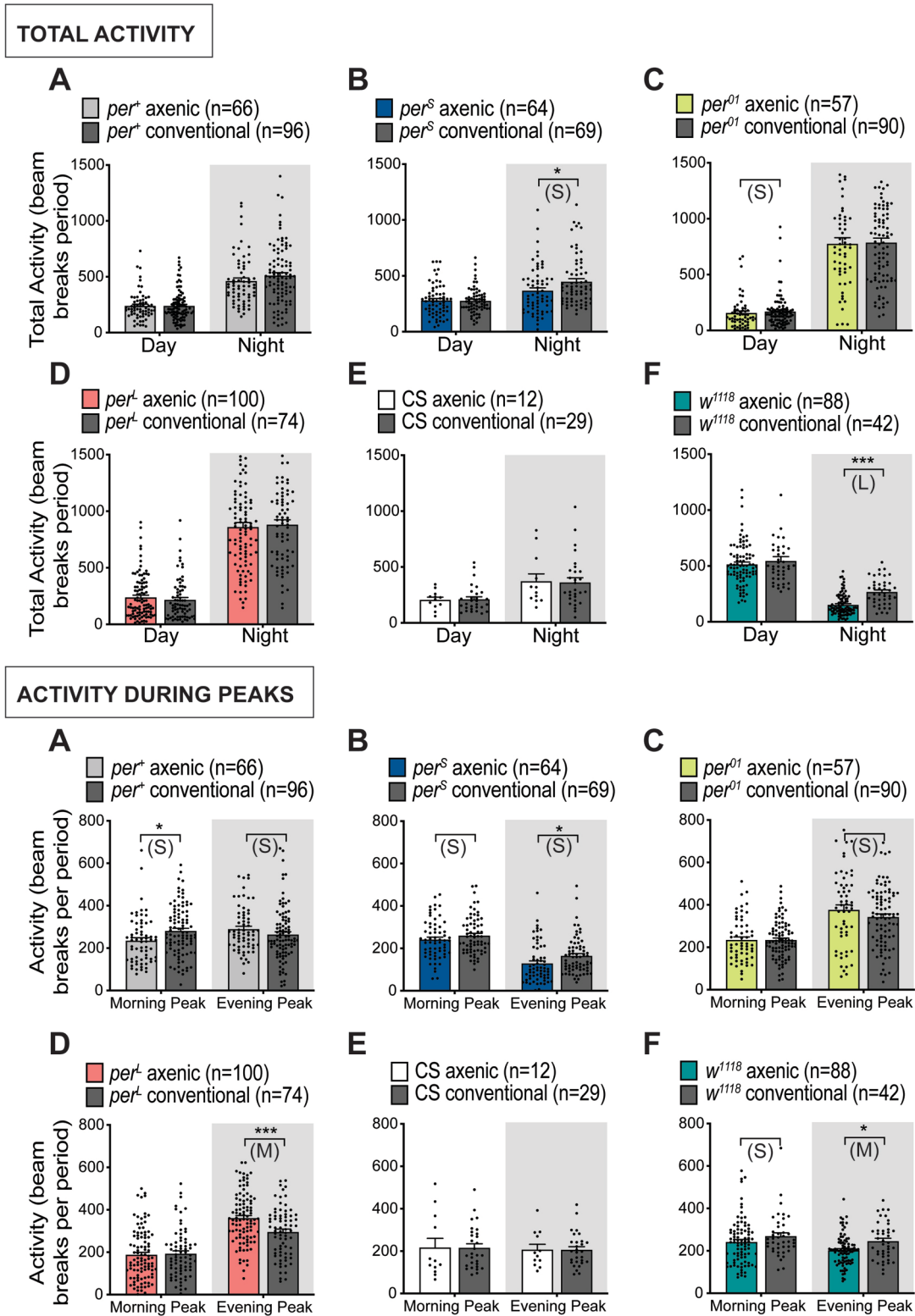


Figure S4. Effect of the microbiome on the daily distribution of locomotor activity. Average activity (\pm SEM) during the day and night (A-F) and in the morning and evening (G-L) under 12:12 LD conditions averaged over 2 days, for axenic and conventional flies. Results for axenic flies are colored as indicated; corresponding results for conventional flies are indicated using a gray fill regardless of genotype. Flies used for results shown in panels A-D and G-J (per^+ , per^S , per^{01} , and per^L) are from isogenic strains, E-F and K-L are non-isogenic controls (CS and w^{1118}). n indicates the number of flies tested. All the data were analyzed by a two-way RM ANOVA and Sidak test where * $p < 0.0332$, ** $p < 0.0021$, *** $p < 0.0002$, and **** $p < 0.0001$. S, M, L: small, medium, and large effect size, respectively, Hedges' g effect size analysis; no letter or symbol means no effect. See Table S1 for values.

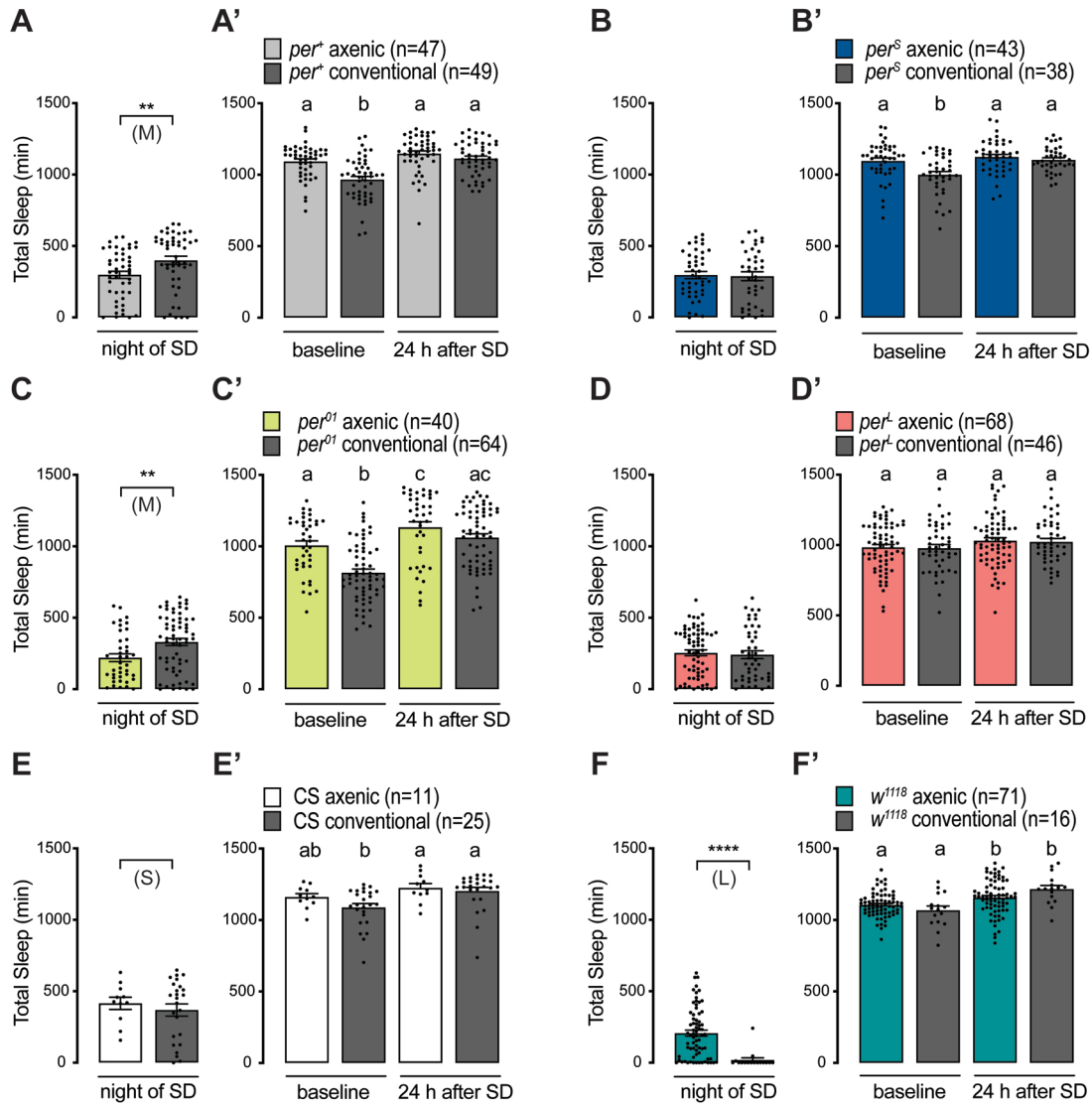


Figure S5. “Sleep” during sleep deprivation, and levels of baseline and rebound sleep. (A-F) Average “sleep” (\pm SEM) during sleep deprivation (SD); (A'-F') Average (\pm SEM) baseline sleep during the 2 days prior to SD (left) and during the 24 h recovery period following SD (right) (A'-F'), for axenic and conventional flies. See text for a discussion regarding the interpretation of “sleep” measured during SD. Results for axenic flies are colored as indicated; corresponding results for conventional flies are indicated using a gray fill regardless of genotype. Flies used for results shown in panels A-D and A'-D' (*per⁺*, *per^S*, *per^{D1}*, and *per^L*) are from isogenic strains, E-F and E'-F' are non-isogenic CS and *w¹¹¹⁸* flies. n indicates the number of flies tested. A-F data were

analyzed by unpaired *t*-test (* $p < 0.0332$, ** $p < 0.0021$, *** $p < 0.0002$, and **** $p < 0.0001$) while A'-F' were analyzed with an ordinary one-way ANOVA followed by Tukey test; different letters indicate statistically different results. S, M, L: small, medium, and large effect size, respectively; Hedges' *g* effect size analysis; no letter or symbol means no effect. See Table S1 for values.

SUPPLEMENTARY TABLES

Table S1. Results of all statistical analyses performed (p values and Hedges' *g* effect size analysis).

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