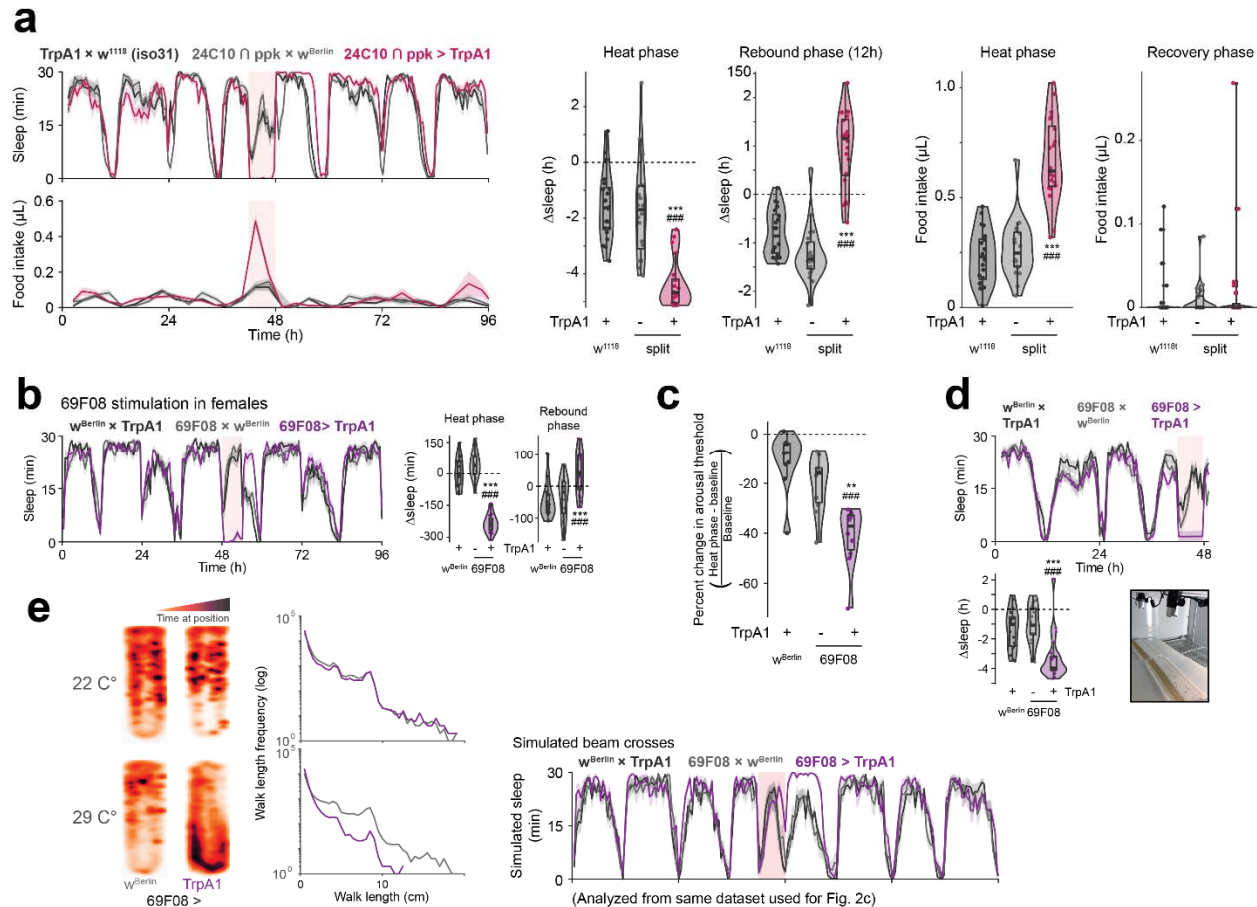


Supplemental Figure S1. Food intake and sleep are altered during and following broad sleep-suppressing manipulations.

(a) Cholinergic stimulation elicits sleep loss, sleep rebound, and post-SD overfeeding in females ($N = 20$ per genotype). (b) Prolonged (12 hr) stimulation of octopaminergic neurons suppresses sleep but does not affect post-SD sleep or food intake, whereas prolonged stimulation of dopaminergic neurons suppresses sleep, increases food intake during the heat phase, and induces rebound sleep. The light pink rectangular background indicates heat phase. Box plots represent median and interquartile range. All lines represent mean and the shaded regions SEM. $N = 15$ males per genotype. Following one-way ANOVA, significant differences were compared post-hoc using Games-Howell (if variance not homogeneous by Levene's test) or Tukey pairwise comparisons (if variance homogeneous) between the experimental and each of the control lines. Significant differences from the post-hoc comparisons are noted as: #, significantly different from TrpA1 control; *, significantly different from GAL4 control. The number of symbols denote the p value of the difference between the control and the sleep-deprived group (***/###, $p < 0.001$).



Supplemental Figure S2. Thermogenetic stimulation of restricted subsets of cholinergic neurons causes hard sleep loss

(a) Stimulation of a more restricted subset of R2 neurons suppresses sleep and increases feeding during the stimulation, followed by sleep rebound. $N = 20$ males per genotype. (b) 69F08 stimulation also elicits sleep loss and rebound sleep in females. $N = 20$ females per genotype. (c) 69F08 stimulation decreases arousal threshold, consistent with sleep loss. $N = 10$ males per genotype. (d) Orienting the ARC chamber horizontally has no effect on the sleep loss observed during 69F08 stimulation, suggesting that sleep loss is not due to restrictions from the vertical orientation of the chamber. $N = 20$ males per genotype. (e) Spatial map (left) and walk length frequency histogram (middle) of animals within ARC chamber during 69F08 stimulation from Fig. 3c. Simulated bisecting beam crosses from the animal movements show unchanged sleep, contrary to the observation from animal tracking. Box plots represent median and interquartile range. Lines represent mean and the shaded regions SEM. Following one-way ANOVA, significant differences were checked for homogeneity of variance using Levene's test and post-hoc comparisons between the experimental and each of the control lines were performed using Tukey's multiple comparisons test. Significant differences from the post-hoc comparisons are noted as: #, significantly different from

TrpA1 control; *, significantly different from GAL4 control. The number of symbols denote the p value of the difference between the control and the sleep-deprived group (*/#, $p < 0.05$; **/##, $p < 0.01$; ***/###, $p < 0.001$).