

#### Supplemental Figure 1: Larvae reared on low sugar diet develop normally.

(A) Feeding amount of L2 controls, L3 raised on regular (ctrl) food, and L3 raised on low sugar (L.S.) food at CT1 and CT13. (B, C) Sleep bout number (B) and bout length (C) at CT1 and CT13 in L3 raised on regular (ctrl) and L.S. food. (D) Total body weight of early L3 (in groups of 5) raised on ctrl or L.S. food. (E) Total body length of early L3 raised on ctrl or L.S. food. (F) Developmental analysis of time to pupal formation of animals raised on ctrl or L.S. food. B-C, n=29-34 larvae; D, n=30 larvae per food condition; E, n=33-40 larvae; F, n=100-170 larvae; G-I, n=6 PREFs (180 larvae) per genotype. Two-way ANOVAs followed by Sidak's multiple comparison test [(B-C)]; Unpaired two-tailed Student's *t*-tests [(D-E) and (G-I)].



# Supplemental Figure 2: Baseline odor preferences, feeding, and sleep are not affected by *npf*-Gal4 manipulations.

(A) Feeding rate of L3 expressing *npf*-Gal4>*UAS-TrpA1* and genetic controls at 22°C at CT13. (B-E) Sleep duration (B), bout number (C), bout length (D), and arousal threshold (E) in L3 expressing *npf*-Gal4>*UAS-TrpA1* and genetic controls at CT1 and CT13 at 22°C (temperature controls). (F) Long-term aversive memory performance in L3 expressing *npf*-Gal4>*UAS-TrpA1* and genetic controls at 22°C (temperature controls). (F) Long-term aversive memory performance in L3 expressing *npf*-Gal4>*UAS-TrpA1* and genetic controls at 22°C (temperature controls). (G-I) Naïve OCT, AM, and quinine preference in L3 expressing *npf*-Gal4>*UAS-TrpA1* and genetic controls at 30°C. A, n=18-20 larvae; B-D, n=22-27 larvae; E, n=120-205 sleep episodes, 18 larvae per genotype; F, n=8 PIs (240 larvae) per genotype; G-I, n=6 PREFs (180 larvae) per genotype. One-way ANOVAs followed by Sidak's multiple comparisons tests [(A), (E), and (F-I)]; Two-way ANOVAs followed by Sidak's multiple comparison test [(B-D)].



# Supplemental Figure 3: Baseline sleep and odor preferences are not disrupted by *R76G11*-Gal4 manipulations.

(A, B) Sleep bout number (A) and bout length (B) of L2 control fed vehicle control (L2) or Gaboxadol (L2 Gab). (C, D) Sleep bout number (C) and bout length (D) of L2 expressing *R76G11*-Gal4>*UAS-TrpA1* and genetic controls at 30°C. (E-H) Sleep duration (E), bout number (F), bout length (G), and arousal threshold (H) of L2 expressing *R76G11*-Gal4>*UAS-TrpA1* and genetic controls at 22°C (temperature controls). (I-K) Naïve OCT, AM, and quinine preference in L2 expressing *R76G11*-Gal4>*UAS-TrpA1* and genetic controls at 30°C. (L) Short-term aversive memory performance of L2 expressing *R76G11*-Gal4>*UAS-TrpA1* and genetic controls at 30°C. A, B, n=28 larvae; C-D, n=33-36 larvae; E-G, n=27-29 larvae; H, n=145-316 sleep episodes, 30-40 larvae per genotype; I-K, n=6 PREFs (180 larvae) per genotype; L, n=8

PIs (240 larvae) per genotype. Unpaired two-tailed Student's *t*-tests [(A-B)]; One-way ANOVAs followed by Sidak's multiple comparisons tests [(C-L)].



#### Supplemental Figure 4: Glucose metabolic gene manipulations affect L3 sleep.

(A, B) Sleep bout number (A) and bout length (B) in L3 expressing UAS-Glut1-RNAi with Dh44-Gal4 and genetic controls at CT1 and CT13. (C, D) Sleep bout number (C) and bout length (D) in L3 expressing UAS-Hex-C-RNAi with Dh44-Gal4 and genetic controls at CT1 and CT13. (E, F) Sleep bout number (E) and bout length (F) in L3 expressing UAS-PyK-RNAi with Dh44-Gal4 and genetic controls as CT1 and CT13. (G-I) Sleep duration (G), sleep bout number (H), and bout length (I) in L3 expressing UAS-Hex-C-RNAi with cry-Gal4 and genetic controls at CT1 and CT13. (J, K) Sleep bout number (J) and bout length (K) in L2 expressing UAS-Glut1-RNAi with Dh44-Gal4 and genetic controls at CT1 and CT13. (I, M) Sleep bout number (L) and bout length (M) in L2 expressing UAS-Hex-C-RNAi with Dh44-Gal4 and genetic controls at CT1 and CT13. (N, O) Sleep bout number (N) and bout length (O) in L2 expressing UAS-PyK-RNAi with Dh44-Gal4 and genetic controls at CT1 and CT13. A-O, n=32-40 larvae. Two-way ANOVAs followed by Sidak's multiple comparison test [(A-O)].



Supplemental Figure 5: Amino acid sensing gene manipulations do not affect L3 sleep.

(A, B) Sleep bout number (A) and bout length (B) in L3 expressing UAS-GCN2-RNAi with Dh44-Gal4 and genetic controls at CT1 and CT13. (C, D) Sleep bout number (C) and bout length (D) in L3 expressing UAS-crc-RNAi with Dh44-Gal4 and genetic controls at CT1 and CT13. A-D, n=32-40 larvae. Two-way ANOVAs followed by Sidak's multiple comparison test [(A-D)].



### Supplemental Figure 6: Model Figure

Clock cells in the larval brain (s-LNv, DN2, and DN1a) communicate to coordinate circadian rhythms. In third instar larvae (L3), a new connection is formed between DN1as and Dh44 neurons, generating daily neural activity rhythms in Dh44 cells that drive sleep-wake patterns, deep sleep, and more enduring memories. In the setting of reduced nutrient availability, the functional connection between the clock (DN1a) and arousal output (Dh44) is not present, facilitating a more constant feeding strategy that benefits the animal under such conditions. However, without clock control of sleep at this stage, deep sleep is lost, as is the ability to exhibit long-term memory.