SUPPLEMENTAL FIGURES AND TABLE LEGENDS

Figure S1. Neuropeptide imaging of LNv neurons in intact living brains with Dilp2-GFP.

A. i. Dilp2-GFP images of s-LNv terminal projections into the dorsal protocerebrum (Z-projection stack) at ZT 2 and ZT 18. Scale bar = 25 μm. ii. Quantification of neuropeptide content from s-LNv terminals. n = for time points ZT 1, 3, 10, 18, 22, 23 are 10, 22, 5, 7, 7, 7, respectively.

B. i. Dilp2-GFP images of s-LNv somas at ZT2 and ZT23. Scale bar = 10 μm. ii. Quantification of neuropeptide content from s-LNv somata. n = for time points ZT 1, 3, 10, 18, 22, 23 are 18, 11, 10, 13, 17, 14, respectively.

C. i. l-LNv terminal neuropeptide content assessed at 6 time points in a 24-hour period in flies that were entrained 12h light:12h dark. n = for time points ZT 1, 3, 10, 18, 22, 23 are 13, 9, 8, 7, 10, 8, respectively.

ii. l-LNv somatic neuropeptide content assessed at 6 time points over 24 hours in flies that were entrained 12h light:12h dark. n = for time points ZT 1, 3, 10, 18, 22, 23 are 12, 13, 6, 9, 9, 10, respectively.

Fig. S2. Neuropeptide release from l-LNv terminals.

A. Dilp2-FAP (FAP) in l-LNv nerve terminals reveals peptide release. Excised brains started in 0 Ca^{2+}. After 8 min the bath was exchanged with Ca^{2+} containing saline (+Ca) to measure the effect of intrinsic activity. Note that +Ca increases the FAP signal within 4 min. Bar, 5 μm. Dashed yellow outlines show regions of interest (ROIs). B. FAP quantification comparing 0 Ca (n = 4), +Ca (n = 3) and depolarization with high K^+ in the

presence of Ca^{2+} (Hi K) (n = 3).

Fig. S3. Tetanus Toxin (TetTx) attenuates neuropeptide release from LNv somas.

A. Release from UAS-*Dilp2-FAP*; UAS-*TetTx, Pdf*-Gal4 s-LNv somas at ZT23 (n = 12) was 69% less than UAS-*Dilp2-FAP*; UAS-*TetTx^{in}, Pdf*-Gal4, controls (n = 8) expressing inactive mutant toxin. ***p < 0.001, unpaired t test.

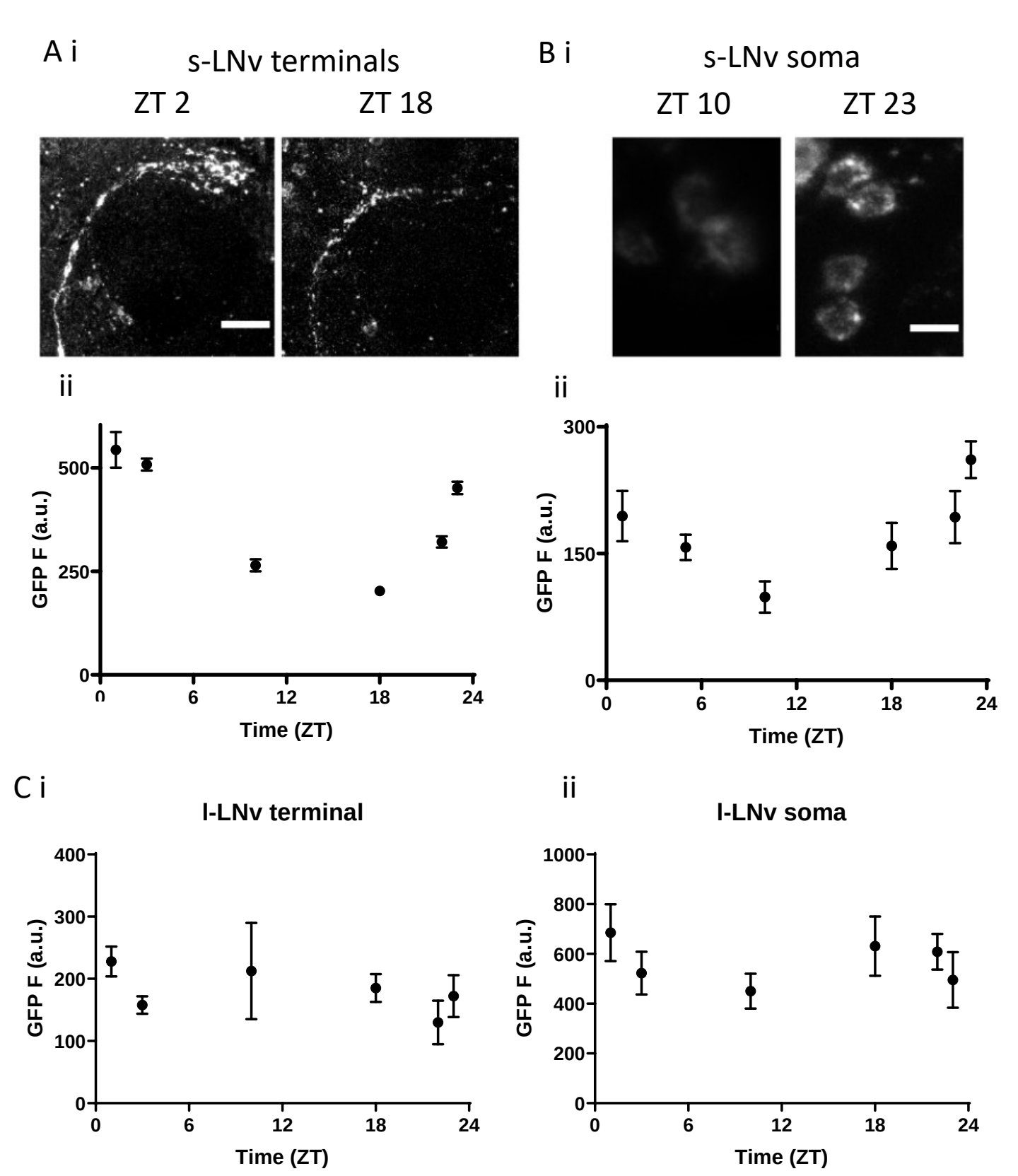
B. Release from l-LNv somas at ZT23 expressing tetanus toxin (n = 22) was 44% less than controls (n = 14) expressing inactive mutant toxin. *p < 0.05, unpaired t test.

C. Release from UAS-*Dilp2-FAP*; UAS-*TetTx, Pdf*-Gal4, s-LNv terminals at ZT3 expressing tetanus toxin (n = 9) was 82% less than UAS-*Dilp2-FAP*; UAS-*TetTx^{in}, Pdf*-Gal4, controls (n = 4) expressing inactive mutant toxin. ***p < 0.001, unpaired t test.

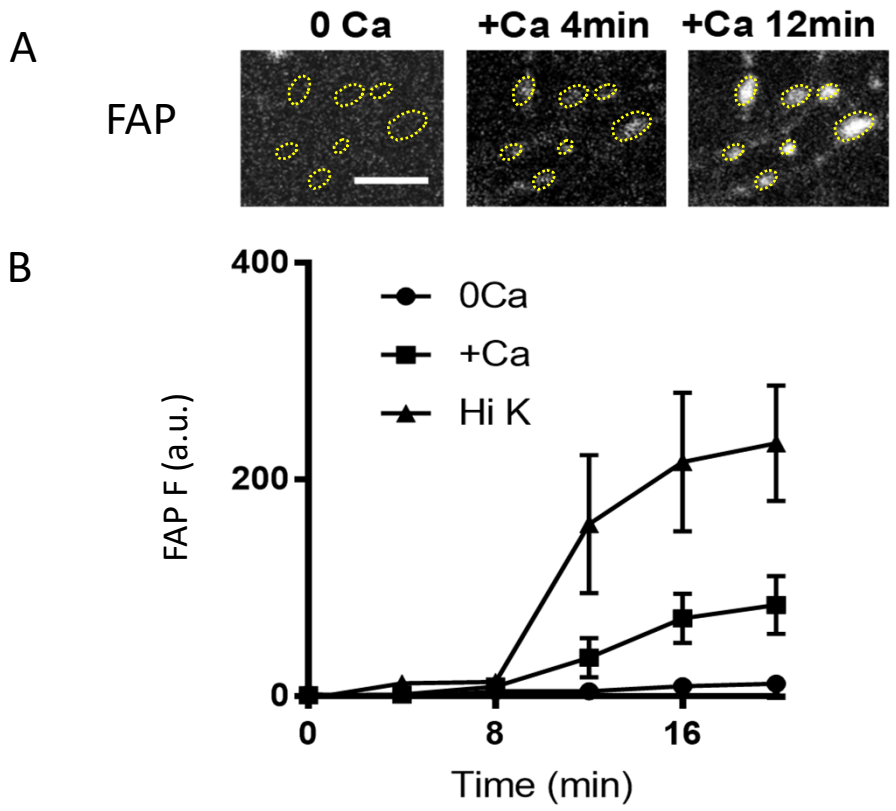
D. Release from l-LNv terminals at ZT23 expressing tetanus toxin (n = 22) was not significantly different from controls (n = 14) expressing inactive mutant toxin.

Supplemental Table. 1. Rhythmic Locomotor Activity in days DD2-DD8 for flies expressing IP_3 sponge to alter neuropeptide release.

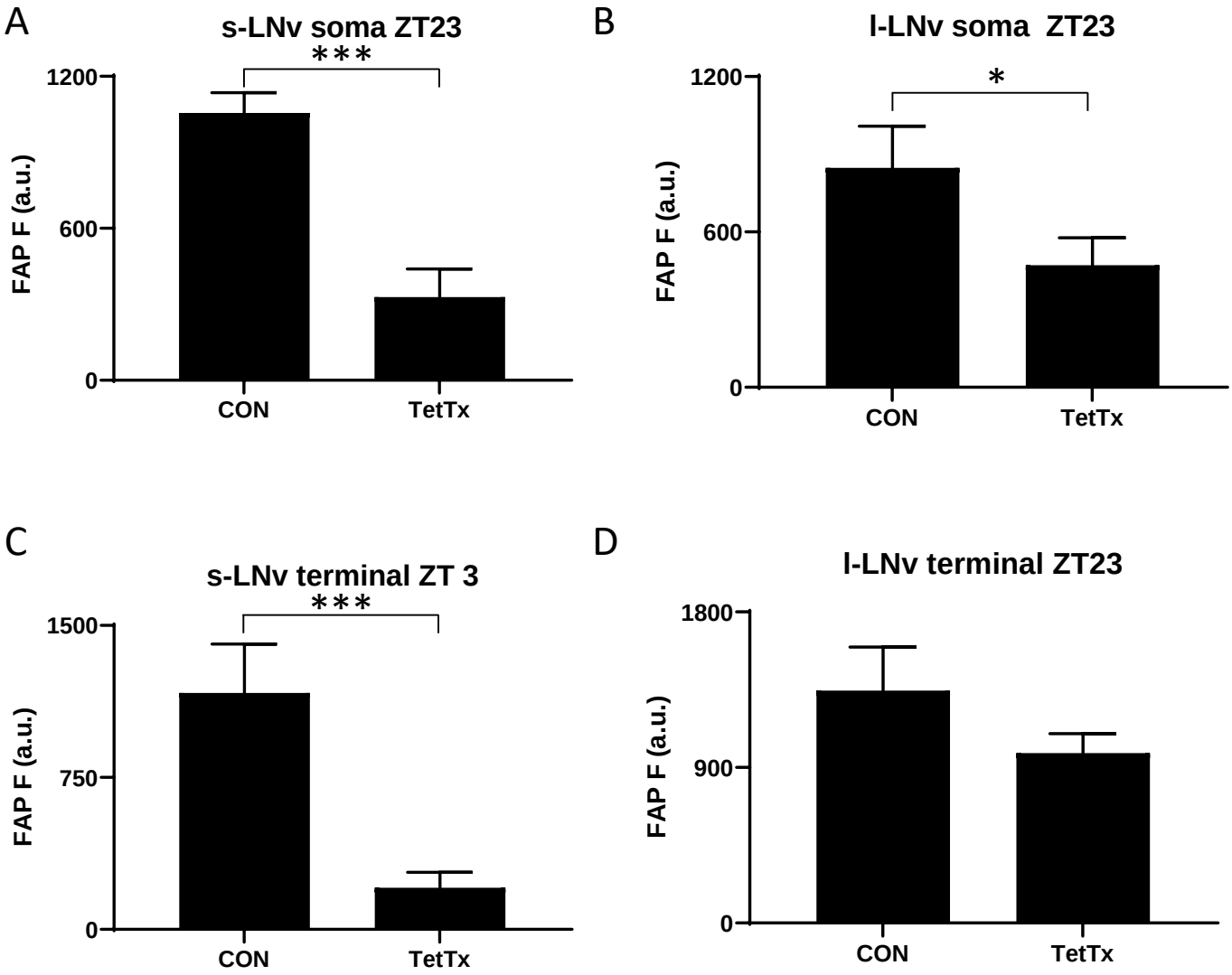
Chi-squared period testing range was 18-30h, resolution was 0.2h, and threshold for filtering arrhythmic flies was set at a rhythm strength of 1, thus any flies below this threshold were not considered rhythmic and did not contribute to the calculation of period. n = # of flies; N = # of experiments; AR% = % of arrhythmic flies; Tau = period of rhythmic flies.



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

	n	N	TAU	SEM	%AR	Rhythm strength	SEM
UAS-IP3 sponge	120	5	23.71	0.23	7	1.35	0.04
UAS-FAP; Pdf>	107	5	24.08	0.16	19	1.34	0.03
UAS-FAP; Pdf> /UAS-IP3 sponge	120	5	24.06	0.14	50	1.11	0.01

Table S1