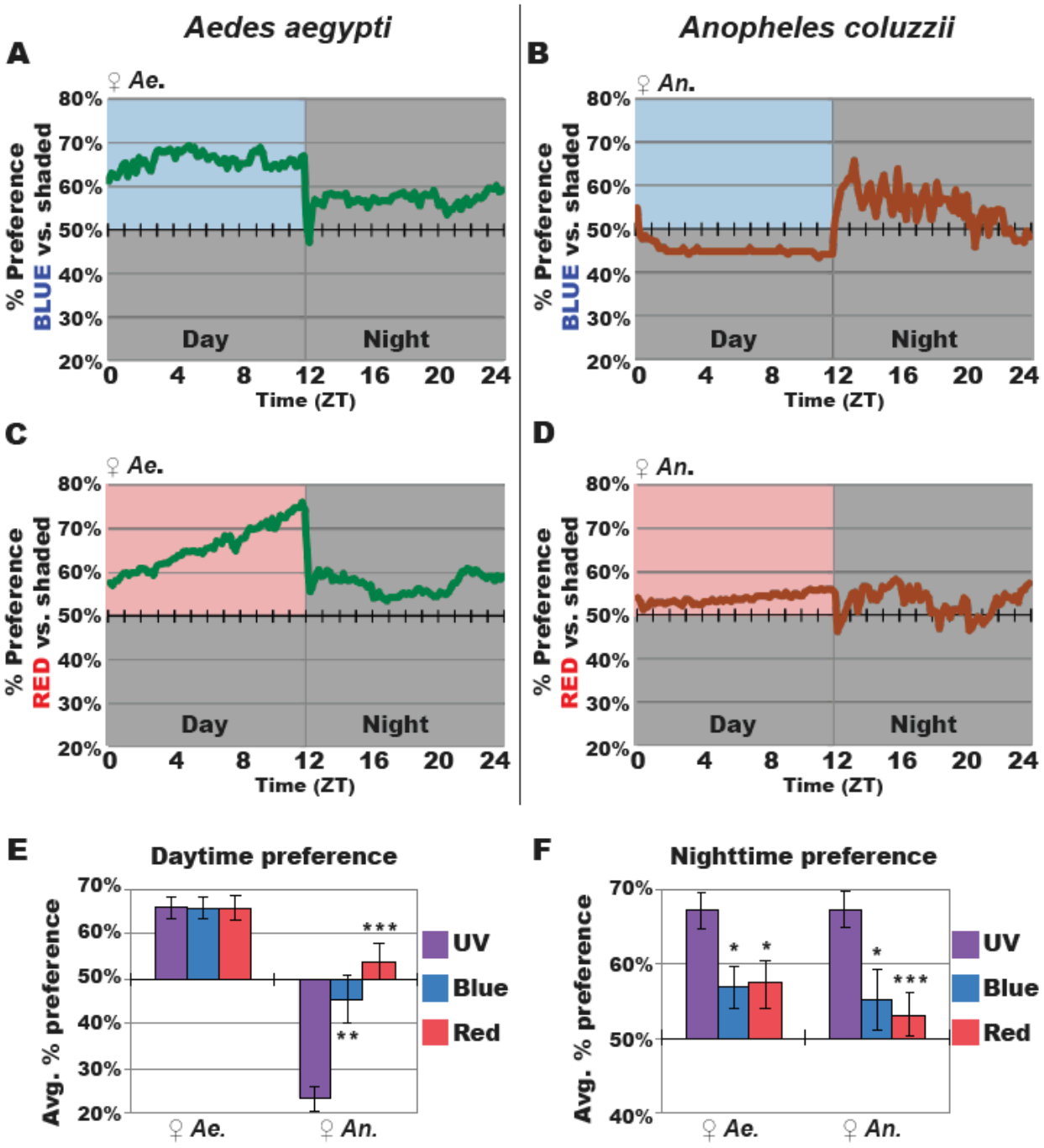

Figure S1. Mosquito light-evoked attraction/avoidance behavior is wavelength-specific, Related to Figure 1.

(A-B) Attraction/avoidance behavior to blue light (450 nm LED, 400 μW/cm^2), measured by % preference in blue light-exposed versus shaded environment throughout 12 hr: 12 hr blue light: dark for female **(A)** *Ae. aegypti* (n=78) and **(B)** *An. coluzzii* (n=34).

(C-D) Attraction/avoidance behavior to red light (620 nm LED, 400 μW/cm^2), measured by % preference in red light-exposed versus shaded environment throughout 12hr: 12hr red light:dark for female **(C)** *Ae. aegypti* (n=62) and **(D)** *An. coluzzii* (n=52).

(E-F) Average attraction/avoidance behavioral preference to light-exposed (UV, blue and red) versus shaded-environment for **(E)** daytime and **(F)** nighttime in *Ae. aegypti* and *An. coluzzii* female mosquitoes. Data are represented as mean ± S.E.M. *p < 0.05; **p < 0.01; ***p < 0.001 vs. UV.

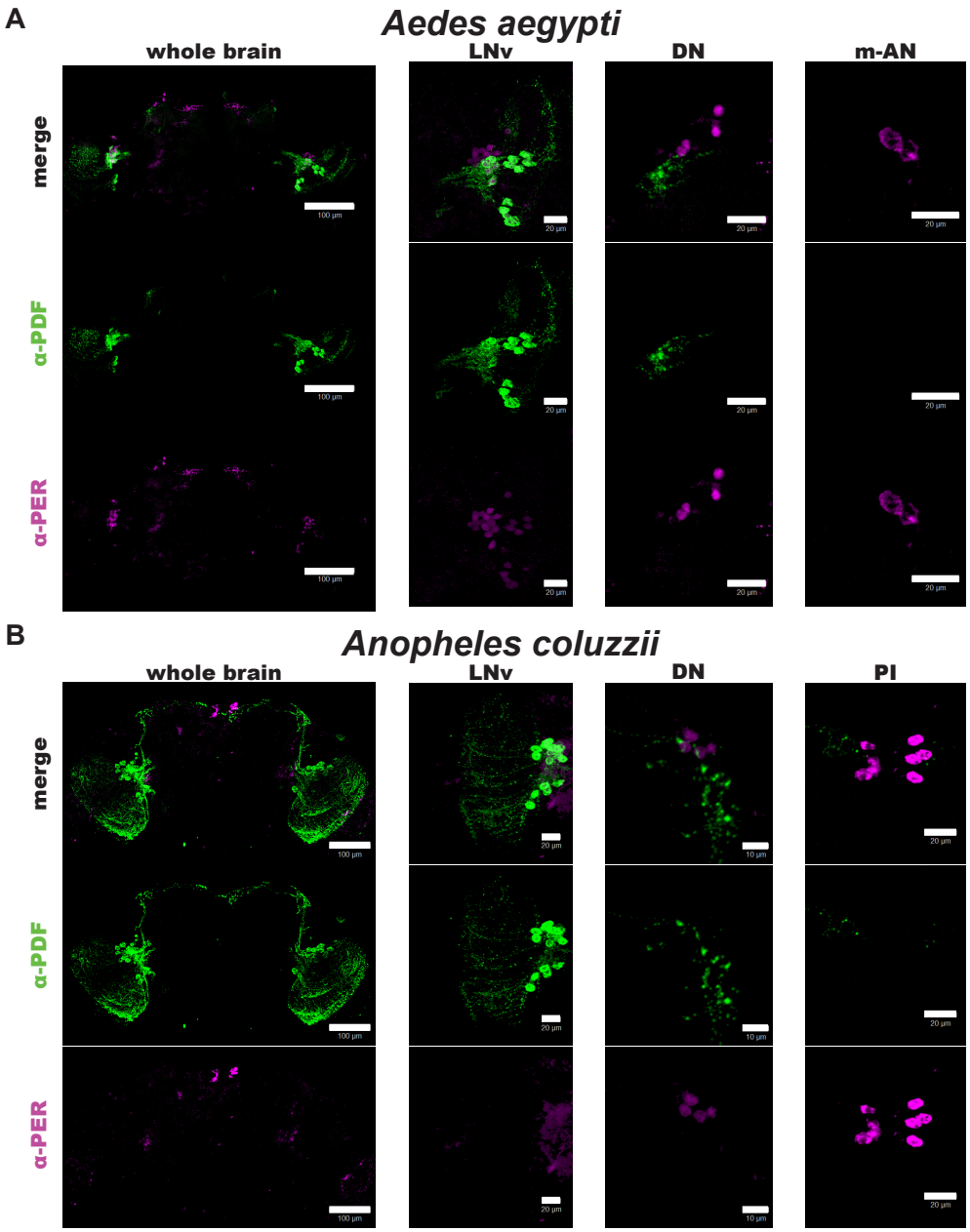

Figure S2. Circadian neuronal circuit of diurnal and nocturnal mosquito brains, Related to Figure 2, Figure 3 and Figure 4.

(A-B) Representative confocal images of adult female **(A)** *Ae. aegypti* and **(B)** *An. coluzzii* mosquito brains immunocytochemistry stained with α–PER (magenta) and α–PDF (green) antibodies. Similar to *Drosophila*, neurites from PDF^{+} LNv neurons project dorsally towards the DNs in *Ae. aegypti*. In *An. coluzzii* brains, PDF^{+} LNv neurites project

dorsally towards to the DNs and then extend medially towards the PER^{+} PI neurons. *Scale bars indicate 100μm for whole brains, 10μm for *An. coluzzii* DN, and 20μm all others.

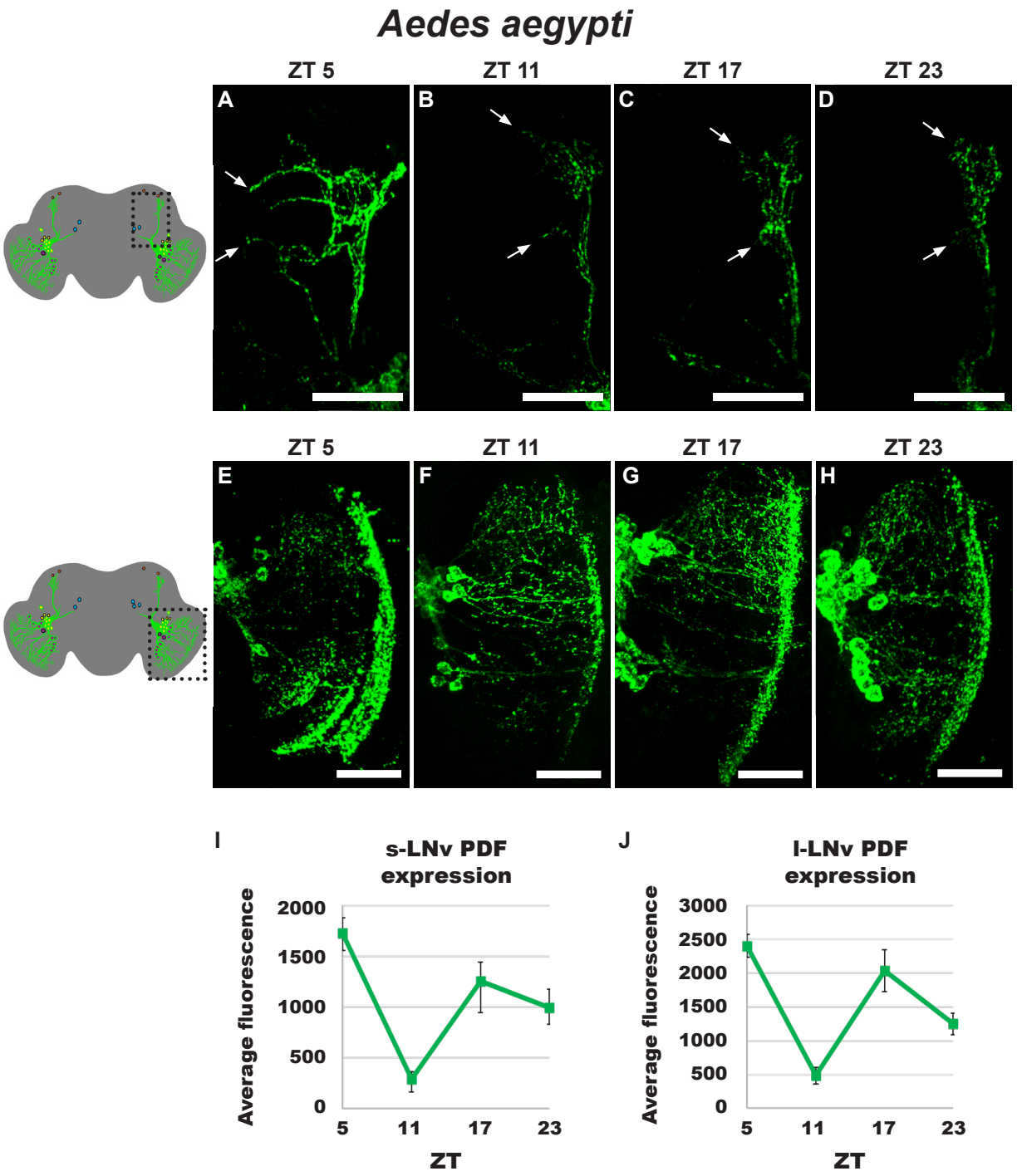

Figure S3. PDF^{+} neurons and in *Aedes aegypti* female brains, Related to Figure 2 and Figure 3.

(A-D) Representative confocal images of adult female *Ae. aegypti* brains immunocytochemistry stained with α–PDF (green) antibody, zoomed in on LNv dorsal

projections at **(A)** ZT5, **(B)** ZT11, **(C)** ZT17, and **(D)** ZT23. White arrows indicate the ends of LNv neuronal arbors. These images were enhanced for intensity and contrast to better show the ends of the arbors. *Scale bars indicate 50 μm.

(E-H) Representative confocal images of adult female *Ae. aegypti* brains immunocytochemistry stained with α–PDF (green) antibody, zoomed in on an optic lobe at **(E)** ZT5, **(F)** ZT11, **(G)** ZT17, and **(H)** ZT23. These images were enhanced for intensity and contrast to better show the ends of the arbors. *Scale bars indicate 50 μm.

(I-J) PDF expression levels over 24 hrs time for *Ae. aegypti* (ZT5, n=27; ZT11, n=17; ZT17, n=6, ZT23, n=7) **(I)** s-LNv and **(J)** l-LNv. Data are represented as mean ± S.E.M.

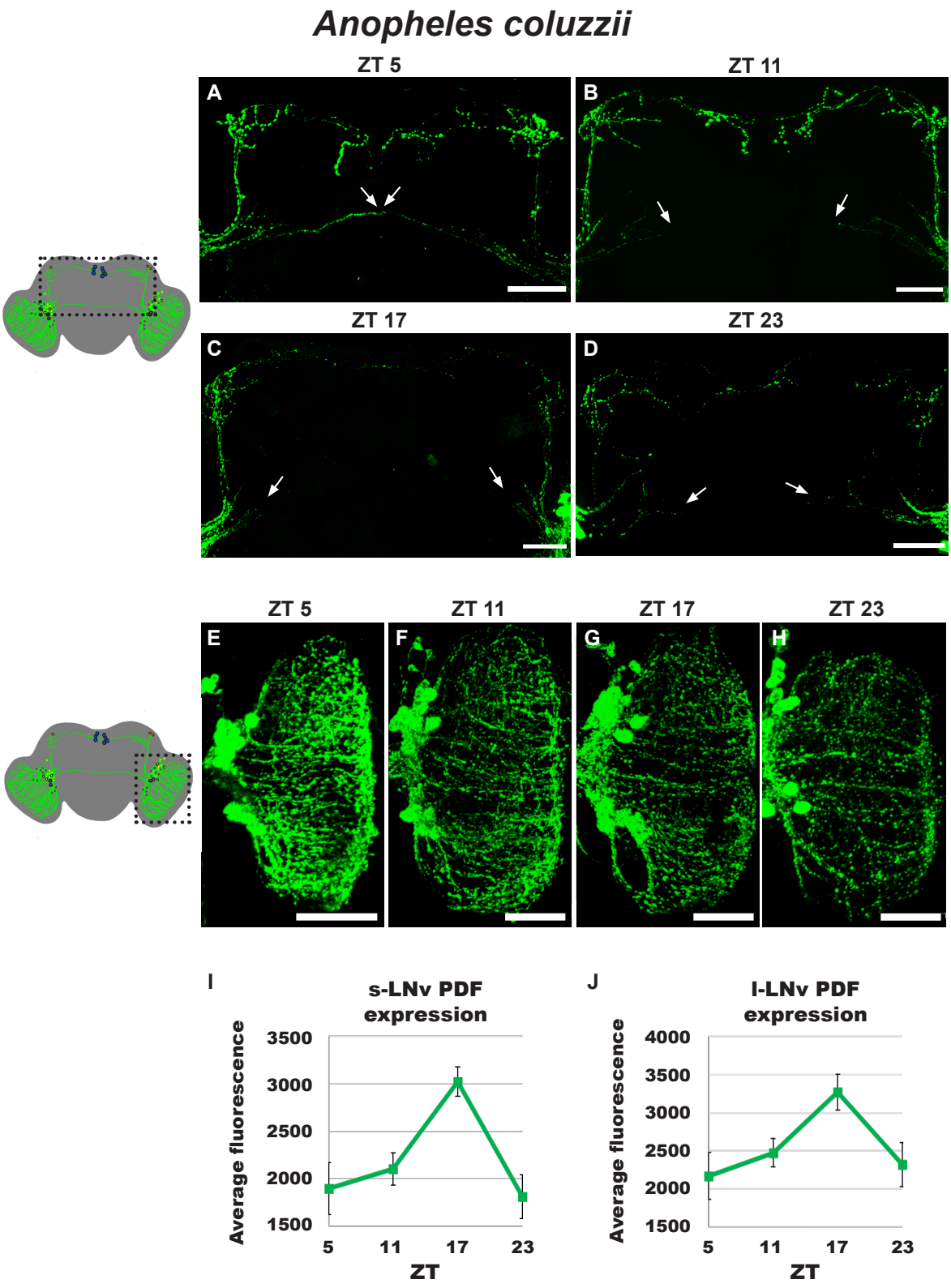

Figure S4. PDF^{+} neurons and in *Anopheles coluzzii* female brains, Related to Figure 2 and Figure 3.

(A-D) Representative confocal images of adult female *An. coluzzii* brains immunocytochemistry stained with α–PDF (green) antibody, zoomed in on LNv dorsal projections at **(A)** ZT5, **(B)** ZT11, **(C)** ZT17, and **(D)** ZT23. White arrows indicate the ends of LNv neuronal arbors. These images were enhanced for intensity and contrast to better show the ends of the arbors. *Scale bars indicate 50 μm.

(E-H) Representative confocal images of adult female *An. coluzzii* brains immunocytochemistry stained with α–PDF (green) antibody, zoomed in on an optic lobe at **(E)** ZT5, **(F)** ZT11, **(G)** ZT17, and **(H)** ZT23. These images were enhanced for intensity and contrast to better show the ends of the arbors. *Scale bars indicate 50 μm.

(I-J) PDF expression levels over 24 hrs time for *An. coluzzii* (ZT5, n=13; ZT11, n=31; ZT17, n=9, ZT23, n=8) **(I)** s-LNv**,** and **(J)** l-LNv. Data are represented as mean ± S.E.M.

	Avg. number of neurons per hemisphere				*Avg. number of neurons per brain*		
	PDF^+ l-LNv	PDF^+ s-LNv	PDF^- l-LNv	PDF^- s-LNv	DNs	PI Neurons	m-ANs
Aedes aegypti	8.6 ± 0.4 (n= 18)	9.3 ± 0.5 (n= 18)	3.1 ± 0.4 (n= 19)	5.8 ± 1.1 (n= 19)	4.1 ± 0.3 (n= 30)	-	4.8 ± 0.4 (n= 31)
Anopheles coluzzii	10 ± 0.5 (n= 20)	9.8 ± 0.5 (n= 21)	3.5 ± 0.7 (n= 8)	5.2 ± 0.8 (n= 8)	3.3 ± 0.4 (n= 26)	7.3 ± 0.1 (n= 22)	-

Table S1. Average number of PERIOD-expressing neurons, Related to Figure 2.

Average number of PERIOD-expressing neurons ±SEM (n= #) in *Ae. aegypti* and *An. coluzzii* female brains, per hemisphere for PDF^+ or PDF^- large- and small-LNvs, and per whole brain for DNs, m-ANs, and PI neurons. Female *Ae. aegypti* brains have approximately 8-9 PDF^+ l-LNvs and 9-10 PDF^+s-LNvs, while female *An. coluzzii* brains have approximately 10 PDF^+ l-LNvs and 9-10 PDF^+ s-LNvs per hemisphere. Both species of mosquitoes have larger number of LNvs compared to *Drosophila melanogaster*, which has 5-6 l-LNvs and 4-5 PDF^+ s-LNvs, but otherwise their neuroanatomical features are highly similar to *Drosophila melanogaster* and other insects. In the lateral ventral region amongst the LNv, there are PER^+/PDF^- neurons, again, consistent with a PER^+/PDF^- "5^{th} s-LNv" neuron seen in flies. We find approximately 3 PDF^- putative l-LNvs and 6 PDF^- putative s-LNvs in female *Ae. aegypti*, and approximately 3-4 PDF^- putative l-LNvs and 4-6 PDF^- putative s-LNvs in female *An. coluzzii* in each side of the brain.