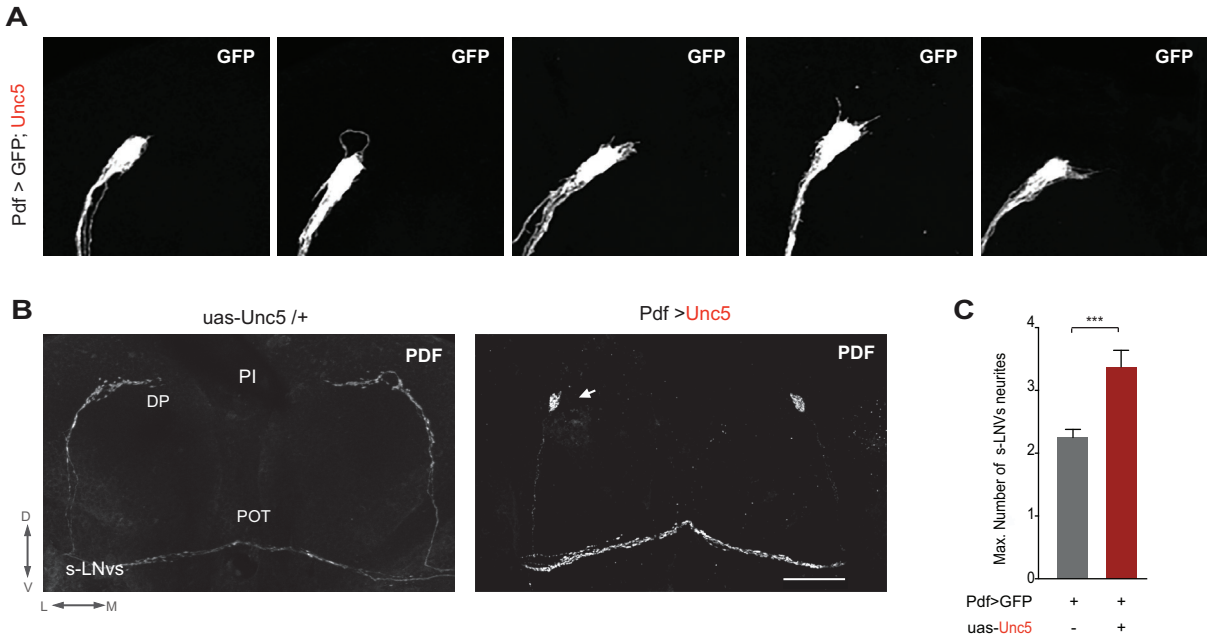

A
Pdf > GFP; Unc5
GFP
GFP
GFP
GFP
GFP
B
uas-Unc5 /+
Pdf >Unc5
PDF
PDF
PI
DP
POT
s-LNvs
D
V
L
M
C
***
Max. Number of s-LNVs neurites
4
3
2
1
0
Pdf>GFP + +
uas-Unc5 - +

Figure S1: Overexpression of the axon guidance receptor Unc5 specifically affects the s-LN_v dorsal termini. Related to Figure 1. (**A**) Confocal Z-series reconstructions of five examples of anti-GFP immunolabeling of brains from *Pdf-Gal4*/*UAS-mCD8::GFP;UAS-Unc5/+* flies revealing the extent to which the development of the dorsal termini of the of s-LN_v dorsal projection was prevented by Unc5 expression. Images represent a scanning area of 75 um x 75 um. All the brains examined (n=40) revealed a complete absence of the dorsal termini. (**B**) The *UAS-Unc5* element alone does not cause arbor phenotypes (left). The posterior optic tract (POT) of the large LN_vs was not affected by the expression of Unc5 (right panel). Scale bar = 50 um. (**C**) Unc5 expressing s-LNvs display a modest de-fasciculation of ascending dorsal projection, consistently displaying more visually distinct, un-fasciculated neurites than controls (see also Figure 1A, lower right panel). *** $P < 0.001$.

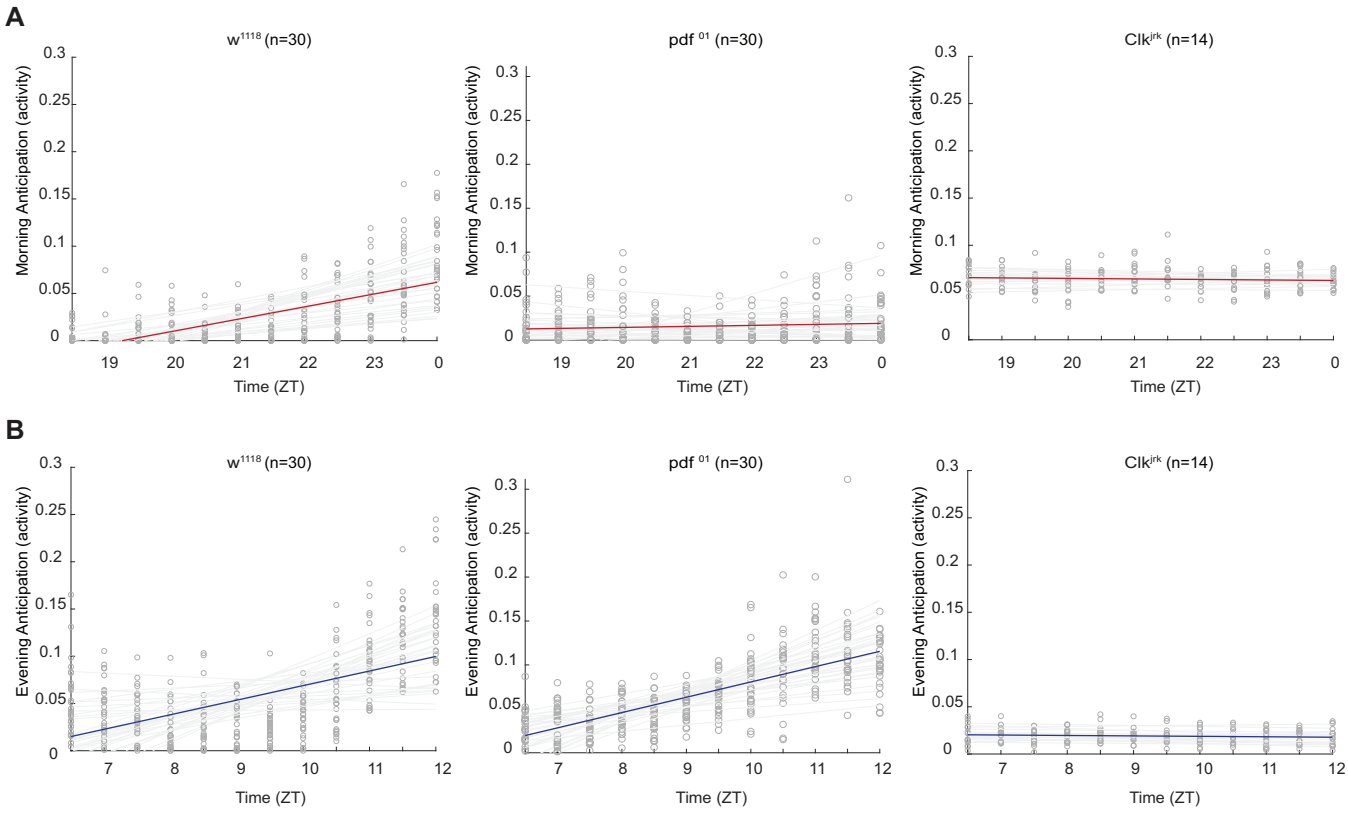

A
w1118 (n=30)
pdf 01 (n=30)
Clkjrk (n=14)
Morning Anticipation (activity)
Time (ZT)
B
Evening Anticipation (activity)

Figure S2: Anticipation indices reflect activity before the lights on and off transitions. Related to Figure 2. (**A**) Least-squares linear regression of normalized 30-min binned activity levels of individual flies (gray points and lines) during the last six hours of the night. Slopes of the individual fly regressions were used to quantify morning anticipation. The averaged regression line is shown in red. As expected, both the Pdf^{01} mutant and the Clk^{jrk} mutant lack the gradual increase in activity seen in wild type flies in the hours before lights on. (**B**) Evening Anticipation Index: an equivalent six-hour analysis of activity during the six hours before the lights-off transition for the same flies shown in A. Least-squares linear regression of normalized 30-min binned activity levels of individual flies are indicated by the gray points and lines. The averaged regression line is shown in blue. While the Clk^{jrk} mutant lacks the gradual increase in evening activity seen in wild type flies, the Pdf^{01} mutant exhibits clear anticipation of lights-off.

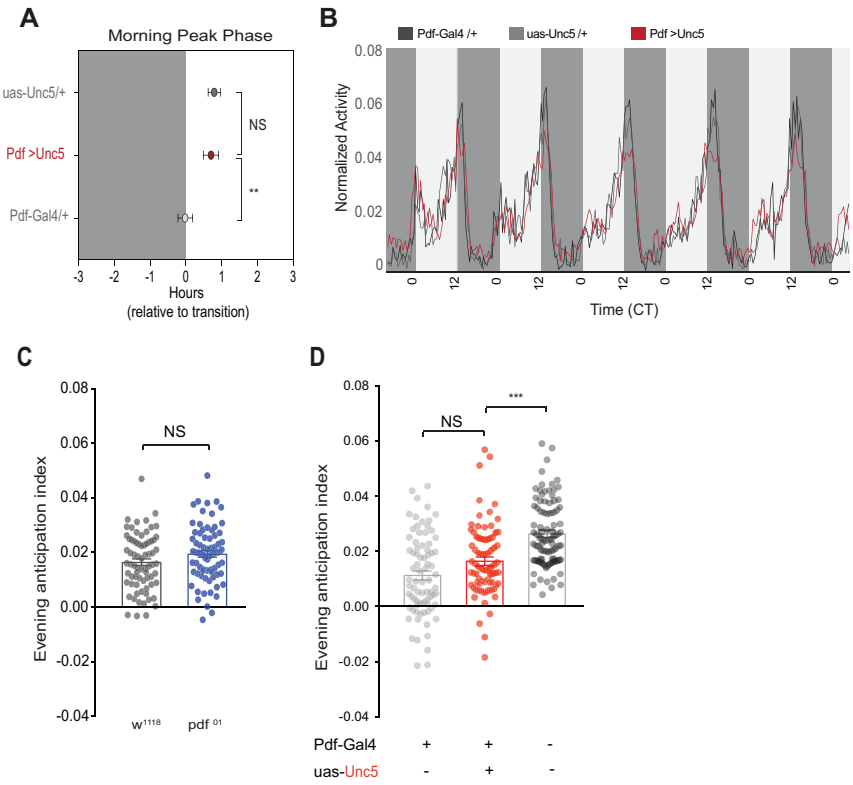

A
Morning Peak Phase
uas-Unc5/+
Pdf >Unc5
Pdf-Gal4/+
NS
**
-3
-2
-1
0
1
2
3
Hours
(relative to transition)
B
Pdf-Gal4 /+
uas-Unc5 /+
Pdf >Unc5
Normalized Activity
0.08
0.06
0.04
0.02
0
0
12
Time (CT)
C
Evening anticipation index
NS
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
w1118
pdf 01
D
Evening anticipation index
NS
***
Pdf-Gal4 + + -
uas-Unc5 - + -

Figure S3: Neither morning nor evening anticipation are affected by Unc5 overexpression in Pdf+ cells. Related to Figure 2. (**A**) The mean morning peak phase of experimental *;Pdf-Gal4/+;UAS-Unc5/+* flies is not significantly different than that of *;;uas-Unc5/+ controls*. **(B**) *Pdf-Gal4/+;UAS-Unc5/+* flies display robust free-running rhythms of locomotor activity, indistinguishable from their parental controls. (**C**) The least-squares regression approach to the quantification of evening peak reveals robust anticipation in both wild-type (w^{1118}) and Pdf^{01} mutant flies. (**D**) Evening anticipation indices were not significantly different between *;Pdf-Gal4/+;UAS-Unc5/+* experimental flies and *Pdf-Gal4/+* controls. *** $P < 0.001$ and NS indicates no significant difference between groups.

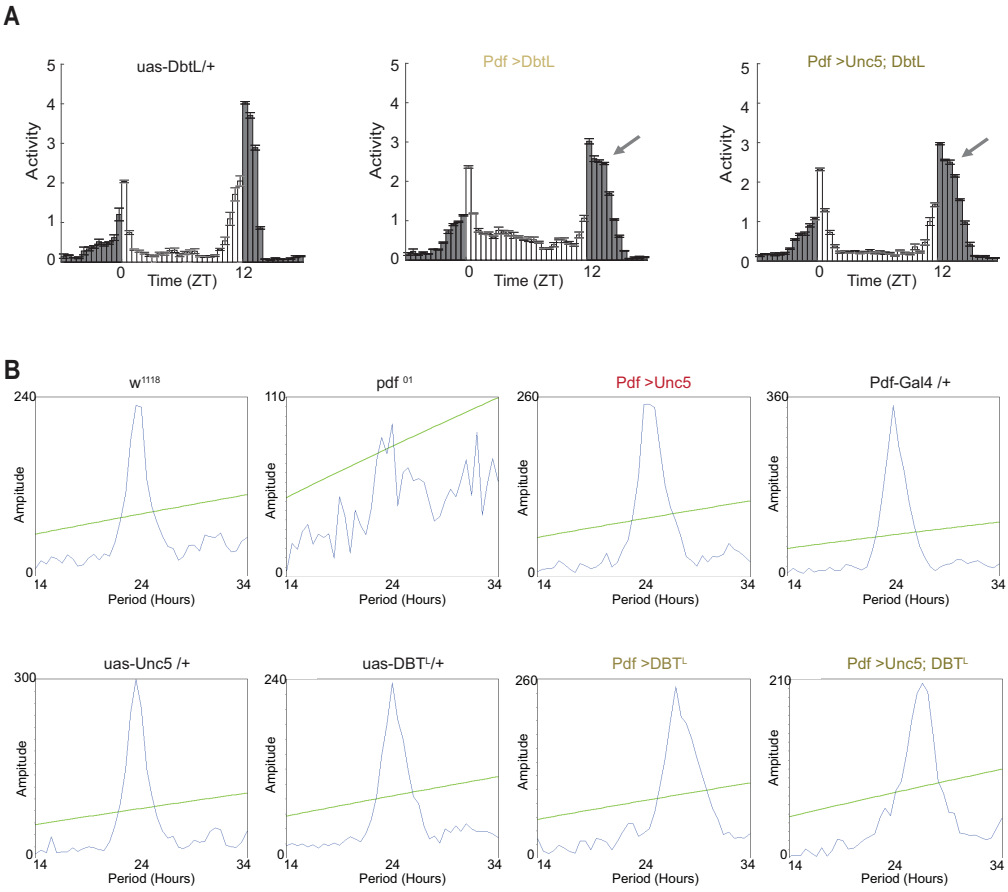

A
uas-DbtL/+
Pdf >DbtL
Pdf >Unc5; DbtL
Activity
Time (ZT)
B
w1118
pdf 01
Pdf >Unc5
Pdf-Gal4 /+
uas-Unc5 /+
uas-DBTL/+
Pdf >DBTL
Pdf >Unc5; DBTL
Amplitude
Period (Hours)

Figure S4: Unc5 expression in the LN_vs does not prevent a slow molecular clock from inducing a long free running period of activity rhythms. Related to Figure 3. (**A**) Population averaged activity profiles of ;;*UAS-Dbt^{LONG}*/+ controls (left), *Pdf-Gal4*/*UAS-Dbt^{LONG}* flies (center), and ;*Pdf-Gal4*/+;*UAS-Dbt^{LONG}/UAS-Unc5* (right). The expression of Unc5 did not prevent the resetting of the evening peak (arrows) by the *Pdf*-expressing LN_vs. (**B**) Representative -square periodograms for flies under seven days of free-running conditions (DD). Genotypes are indicated above the periodograms. Both ;*Pdf-Gal4/+;UAS-Dbt^{LONG}*/+ and ;*Pdf-Gal4/+;UAS-Dbt^{LONG}*/*UAS-Unc5* flies exhibit significantly longer free-running periods compared to all parental controls. See Table S1 for statistical information and sample sizes.

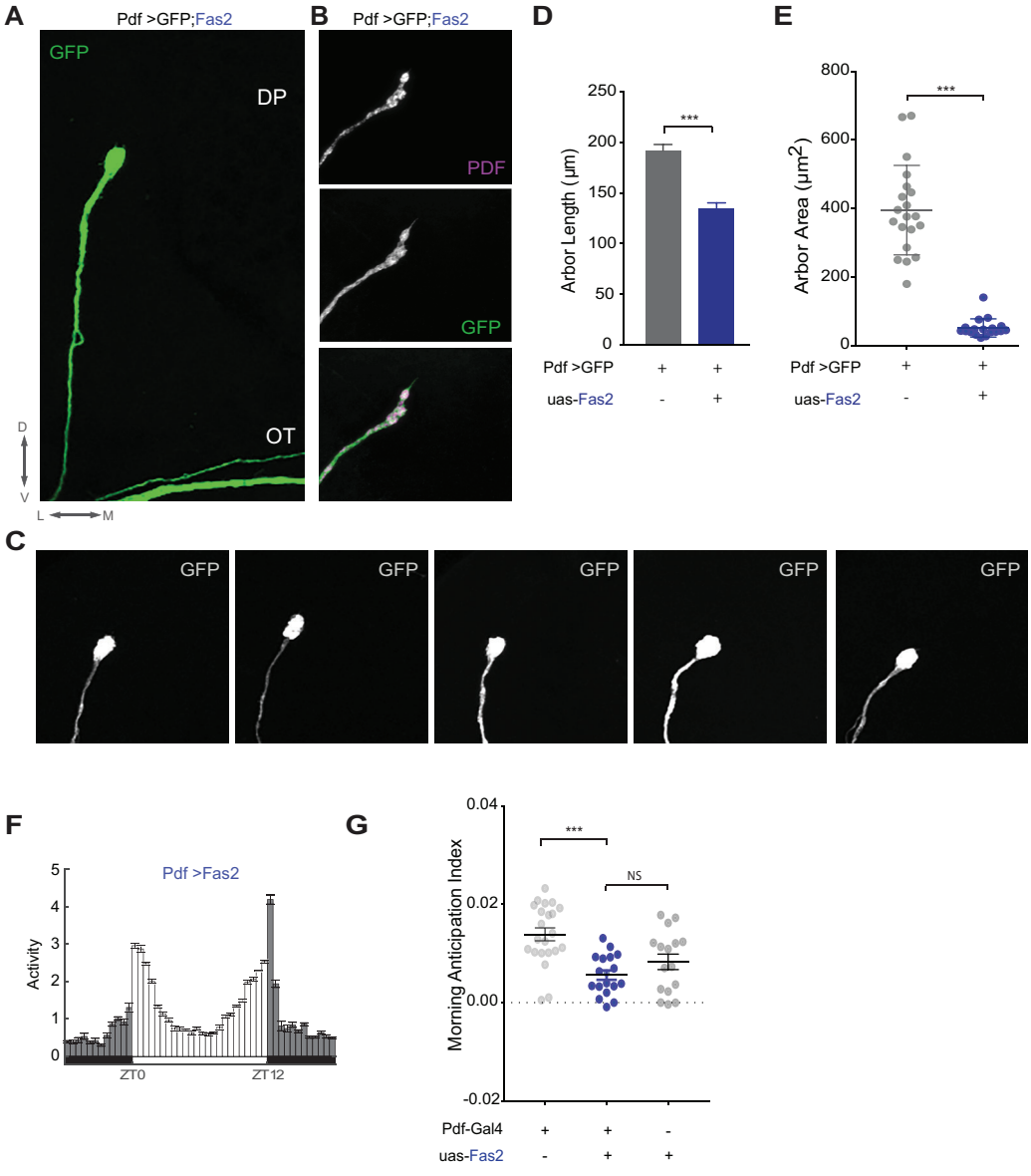

A
Pdf >GFP;Fas2
GFP
DP
OT
D
V
L
M
B
Pdf >GFP;Fas2
PDF
GFP
D
Arbor Length (µm)
250
200
150
100
50
0
***
Pdf >GFP + +
uas-Fas2 - +
E
Arbor Area (µm2)
800
600
400
200
0
***
Pdf >GFP + +
uas-Fas2 - +
C
GFP
GFP
GFP
GFP
GFP
F
Pdf >Fas2
Activity
5
4
3
2
1
0
ZT0
ZT12
G
Morning Anticipation Index
0.04
0.02
0.00
-0.02
***
NS
Pdf-Gal4 + + -
uas-Fas2 - + +

Figure S5: Fas2-mediated elimination the dorsal termini of the s-LN$_v$s does not affect the timing of activity under LD cycles. Related to Figure 5. (**A-C**) Representative confocal images of an anti-GFP immunostaining showing the left hemisphere of a ;*Pdf-Gal4/+*;*UAS-mCD8::GFP,UAS-Fas2/+* adult brain (**A**) and a magnified image of the s-LN$_v$ dorsal projection (**B**) top panel, anti- PDF staining middle panel, anti-GPF staining bottom panel, merged images with PDF shown in magenta and GFP shown in green. (**C**) Examples of the absence of s-LNv dorsal termini ramification in five brains from ;*Pdf-Gal4/+UAS-mCD8::GFP;UAS-Fas2/+* flies. Images represent an area of 75 um x 75 um. (**D**) Quantification of the length of the s-LN$_v$s projection for control ;*Pdf-Gal4/UAS-mCD8::GFP;* and experimental *;Pdf-Gal4/UAS-mCD8::GFP;UAS-Fas2/+* brains. (**E**) Quantification of area of s-LN$_v$s dorsal terminal innervation for the genotypes shown in D. (**F**) Population averaged activity plot for ;*Pdf-Gal4;/+;UAS-Fas2/+* flies during days 3-5 of a 12h:12h LD cycle at a constant 25 ºC. Neither the morning nor the evening peak are affected by the expression of Fas2. (**G**) Morning anticipation indices for ;*Pdf-Gal4;/+;UAS-Fas2/+* (blue) and for ;*Pdf-Gal4;/+;* and ;*;UAS-Fas2/+* controls (gray). See Table S1 for sample sizes and statistical information. *** $P < 0.001$ and NS = Not Significant. Error bars represent SEM.

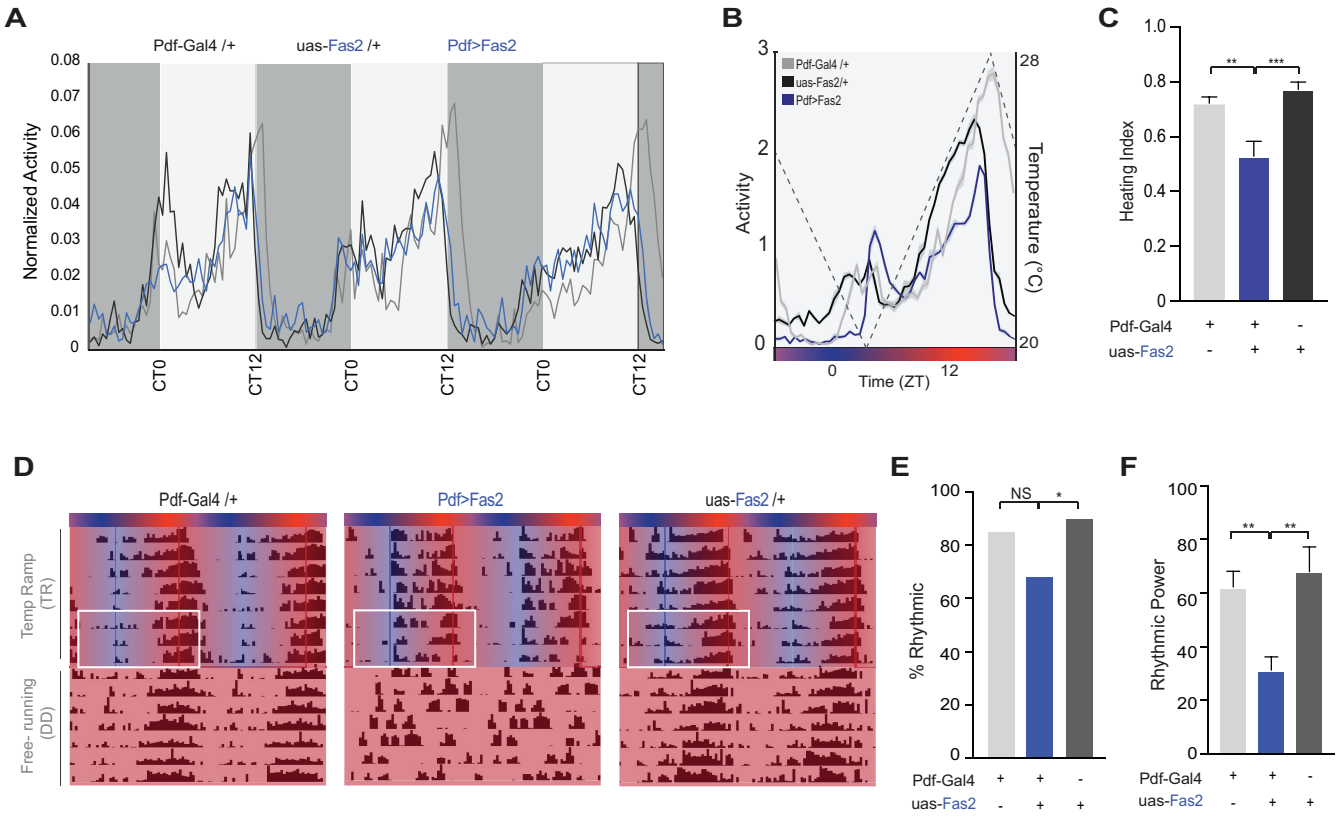
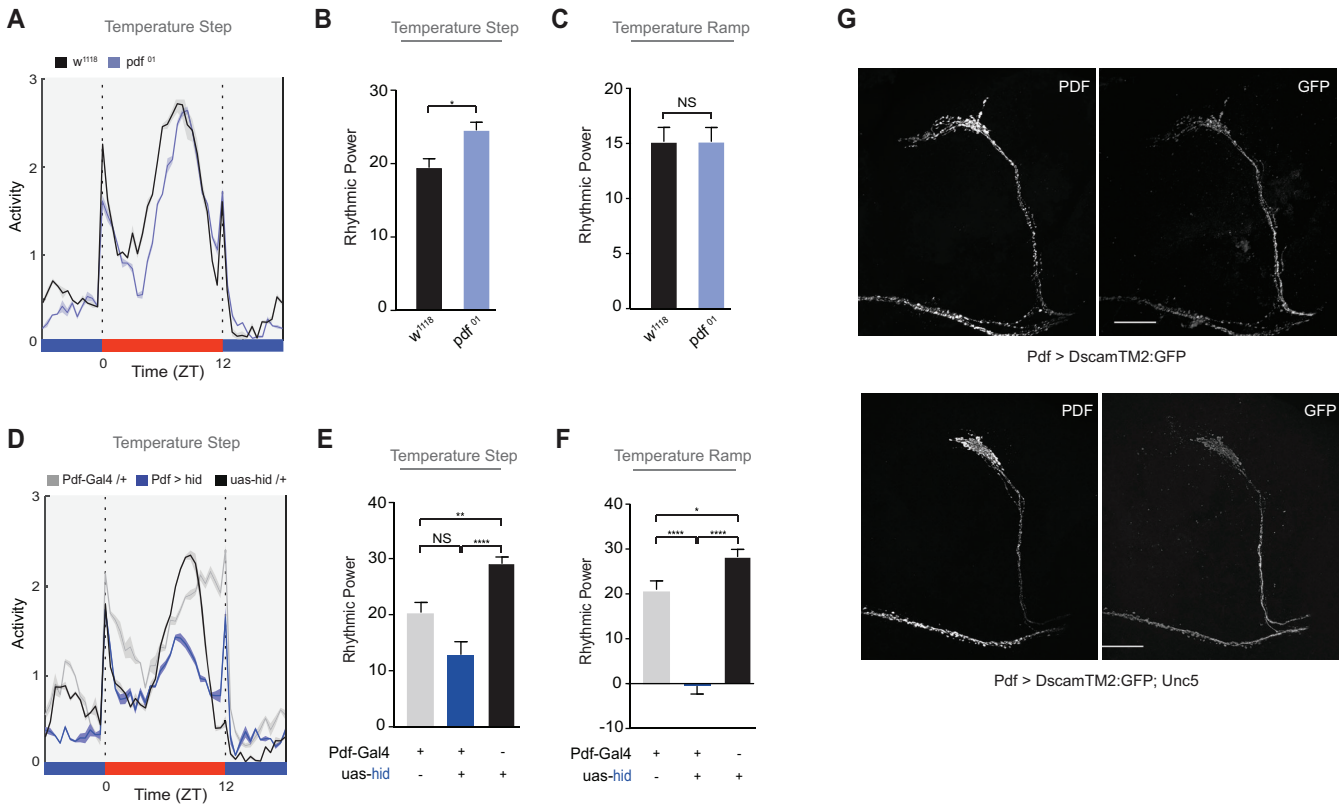

A
Pdf-Gal4 /+
uas-Fas2 /+
Pdf>Fas2
Normalized Activity
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
CT0
CT12
CT0
CT12
CT0
CT12
B
Pdf-Gal4 /+
uas-Fas2/+
Pdf>Fas2
Activity
Temperature (°C)
3
2
1
0
28
20
0
12
Time (ZT)
C
Heating Index
1.0
0.8
0.6
0.4
0.2
0
**
***
Pdf-Gal4 + + -
uas-Fas2 - + +
D
Pdf-Gal4 /+
Pdf>Fas2
uas-Fas2 /+
Temp Ramp (TR)
Free- running (DD)
E
% Rhythmic
100
80
60
40
20
0
NS
*
Pdf-Gal4 + + -
uas-Fas2 - + +
F
Rhythmic Power
100
80
60
40
20
0
**
**
Pdf-Gal4 + + -
uas-Fas2 - + +

Figure S6: Fas2-mediated elimination the dorsal termini of the s-LN$_v$s does impairs entrainment to temperature ramps and affects endogenous circadian timekeeping. Related to Figure 5. (**A**) Normalized activity during the first three days of free-running conditions under DD. Dark gray indicates subjective night and light gray indicates subjective day. **(B)** Averaged population activity plots under ramping temperature cycles for experimental *;Pdf-Gal4/+;UAS-Fas2/+* (blue, N=32) and their parental heterozygous controls ;*Pdf-Gal4/+* (light gray, N=32) and *;;UAS-Fas2/+;* (dark gray, N=31). Plots represent the last three days of entrainment to a ramping temperature cycle (days 6-8), wherein temperature progressively increased from 20 ºC to 28 ºC between ZT 0-12 and gradually decreased from 28 ºC to 20 ºC between ZT 12-0. Blue to red gradients indicate heating phase, red to blue gradients indicate cooling phase. ZT0 is the beginning of the heating phase (T= 20 ºC), ZT12 is the end of the heating phase (T= 28 ºC). (**C**) Heating indices for the genotypes shown in B, which reflect the correlation between environmental heating and increases in locomotor activity. (**D**) Representative actograms of single flies entrained for 8-days to constantly changing temperature ramps under DD followed by one week of free running at 25 ºC under DD. During entrainment, temperature progressively increased from 20 ºC to 28 ºC between ZT 0-12 and gradually decreased from 28 ºC to 20 ºC between ZT 12-0. Blue to red gradients indicate heating phase, red to blue gradients indicate cooling phase. Genotypes are indicated above actograms. White boxes indicate the days used for the analysis shown in B and C. **(E)** Summary of the percentage of flies displaying significant circadian periodicity under DD following entrainment to temperature ramp cycles. **(F)** Summary of rhythmic power under DD following entrainment to temperature ramp cycles. For all histograms, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and NS indicates not significantly different. For all activity plots, lines represent mean ± SEM. See Table S1 for statistical information.


A
Temperature Step
w1118
pdf 01
Activity
Time (ZT)
B
Temperature Step
Rhythmic Power
C
Temperature Ramp
NS
Rhythmic Power
G
PDF
GFP
Pdf > DscamTM2:GFP
PDF
GFP
Pdf > DscamTM2:GFP; Unc5
D
Temperature Step
Pdf-Gal4 /+
Pdf > hid
uas-hid /+
Activity
Time (ZT)
E
Temperature Step
NS
Rhythmic Power
Pdf-Gal4
uas-hid
F
Temperature Ramp
Rhythmic Power
Pdf-Gal4
uas-hid

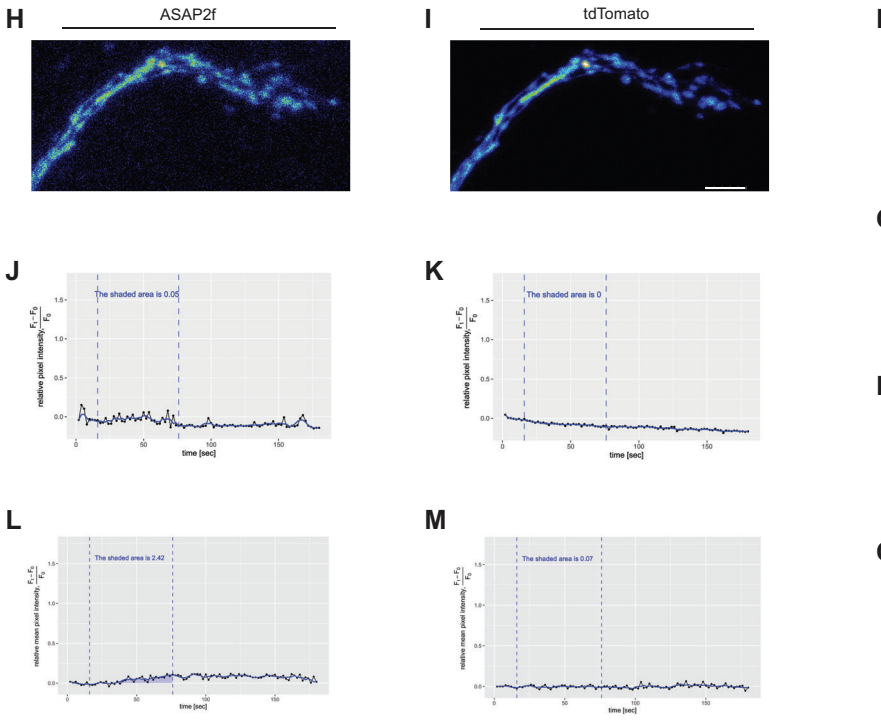

H
ASAP2f
I
tdTomato
J
The shaded area is 0.05
K
The shaded area is 0
L
The shaded area is 2.42
M
The shaded area is 0.07
N
O
P
Q

Figure S7: Effect of *pdf* mutation vs ablation of PDF-expressing LN_v neurons in entrainment under temperature step and temperature ramp conditions glutamate directly inhibits cholinergic excitation of the s-LN_v dorsal termini. Related to Figures 5 and 6. (**A**) Average population activity plots for days 6 to 8 of entrainment to a temperature step cycle for pdf^{01} mutants and their genetic background control, w^{1118}. Temperature was held at a constant 28ºC between ZT 0-12 and 20ºC between ZT 12-0. Red bar indicates 28ºC phase, blue bar indicates 20ºC phase. Dashed vertical lines indicate transition points between temperature steps. **(B)** Summary of rhythmic power during days 6-8 of temperature step cycle. Rhythmic power was used to provide a direct comparison between step and ramping conditions. Remarkably, pdf^{01} mutants displayed slightly higher rhythmic power under entrainment to temperature steps. **(C)** Summary of rhythmic power during days 6-8 of 20ºC/28ºC temperature ramp cycle. **(D)** Average population activity plots for days 6 to 8 of 20ºC/28ºC temperature step cycle for flies in which the proapoptotic gene *hid* was expressed in the PDF expressing LN_vs compared to heterozygote parental controls. **(E)** Summary of rhythmic power during days 6-8 of temperature step cycle. Though there was a trend toward lower rhythmic power in the ablated experimental flies, they were not significantly different from *Pdf-GAL4*/+ controls. **(F)** Summary of rhythmic power during days 6-8 of 20ºC/28ºC temperature ramp cycle. Experimental flies are significantly different than both parental controls under ramping temperature cycles. Data from **A-C** and **D-F** were obtained from parallel temperature step and ramp experiments in which flies of the same genotype and were progeny of the same crosses. For activity plots, lines represent mean ± SEM. For all bar graphs, * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, and NS indicates not significantly different. Error bars represent SEM. See Table S1 for statistical information and sample sizes. **(G)** Confocal reconstruction of the expression of the dendritic reporter DSCAM-TM1-GFP driven by *Pdf-GAL4* in the dorsal projections of normal (top panels) or projections truncated by the expression of Unc5 (bottom panels). Brains were immunolabeled for PDF (left panels) and GFP (right panels). (**H**) Rapid Z-series reconstruction of ASAP2f expression in a living *Pdf(M)-Gal5/UAS-tdTomato/+;UAS-ASAP2f/+* brain from the first timepoint of a representative volumetric timeseries of the s-LN_v dorsal termini. (**I**) The same brain volume as H scanned simultaneously for td-Tomato expression. tdTomato fluorescence was used to define the region of interest for GFP sensor fluorescence intensities at each timepoint for all imaging experiments in this study. (**J**) A representative trace of ASAP2f fluorescence for s-LN_v dorsal termini treated with 1mM GABA, which was applied from 15-45s during the time-course. "Shaded area" refers to the area under the trace (from 0 to positive trace values) within the time-points indicated by the dashed blue lines. This value was used as measure of excitatory response magnitude in other experiments below. GABAergic inhibition would increase ASAP2f fluorescence. No robust response was apparent. (**K**) as for J but treated with 1mM glutamate from 15-45s, again with no clear response. (**L**) A GCaMP6f fluorescence trace from the dorsal projection of a *Pdf(M)-Gal5/UAS-tdTomato/+;UAS-GCaMP6f/+* brain treated with a control vehicle

perfusion from 15-45s. (**M**) GCaMP6f fluorescence trace from the same dorsal projection shown in L treated with a 1mM GABA from 15-45s. GABAergic inhibition would decrease GCaMP6f fluorescence. No clear response was apparent. (**N**) A GCaMP6f fluorescence trace from the dorsal projection of a *Pdf(M)-Gal5/UAS-tdTomato/+;UAS-GCaMP6f/+* brain treated with 0.025mM Carbachol (CCh) in the presence of 1mM Glutamate. (**O**) GCaMP6f fluorescence trace from the same dorsal projection treated with 0.025mM CCh alone, immediately after the trace in N, revealing that glutamate had completely abrogated the CCh response in the previous time-course. (**P**) A GCaMP6f fluorescence trace from the dorsal projection of a *Pdf(M)-Gal5/UAS-tdTomato/+;UAS-GCaMP6f/+* brain treated with 0.025mM Carbachol (CCh) in the presence of 1mM Glutamate. (**Q**) GCaMP6f fluorescence trace from the same dorsal projection treated with 0.025mM CCh alone, immediately after the trace in P, revealing that glutamate had reduced but not completely abrogated the CCh response in the previous time-course.

Figure 1	Treatment	Genotype	n	Mean	Std. Dev	S.E.M.	Gaussian distr.	Test			
1B	Full projection length							unpaired t- test	Significant?	Summary	P value
		Pdf>mCD8:GFP (ZT14)	9	206.2	14.31	4.769	yes	Pdf>mCD8:GFP vs.	yes	***	<0.0001
		Pdf>mCD8:GFP;Unc5 (ZT14)	12	164.6	15.35	4.43	yes	Pdf>Unc5;mCD8:GFP			
1D	Arbor Area							ANOVA with Tukey's Test	Significant?	Summary	P value
		Pdf>mCD8:GFP (ZT2)	10	612.7	189.7	59.99	yes	Pdf>GFP (ZT2) vs. Pdf>GFP (ZT14)	Yes	*	0.0173
		Pdf>mCD8:GFP (ZT14)	9	461.9	70.26	23.42	yes	Pdf>GFP (ZT2) vs. Pdf>GFP;Unc5 (ZT2)	Yes	***	<0.0001
		Pdf>mCD8:GFP;Unc5 (ZT2)	8	169.1	31.75	11.23	yes	Pdf>GFP (ZT2) vs. Pdf>GFP;Unc5 (ZT14)	Yes	***	<0.0001
		Pdf>mCD8:GFP;Unc5 (ZT14)	12	166	33.49	9.666	yes	Pdf>GFP (ZT14) vs. Pdf>GFP;Unc5 (ZT2)	Yes	***	<0.0001
								Pdf>GFP (ZT14) vs. Pdf>GFP;Unc5 (ZT14)	Yes	***	<0.0001
								Pdf>GFP;Unc5 (ZT2) vs. Pdf>GFP;Unc5 (ZT14)	No	ns	0.9999
1E	3D spread							Mann-Whitney	Significant?	Summary	P value
		Pdf>mCD8:GFP (ZT2)	10	36671	12734	4245	yes	Pdf>GFP (ZT2) vs. Pdf>GFP (ZT14)	Yes	***	0.0002
		Pdf>mCD8:GFP (ZT14)	9	18427	9506	3169	yes	Pdf>GFP (ZT2) vs. Pdf>GFP;Unc5 (ZT2)	Yes	***	<0.0001
		Pdf>mCD8:GFP;Unc5 (ZT2)	8	4281	3041	1075	yes	Pdf>GFP (ZT2) vs. Pdf>GFP;Unc5 (ZT14)	Yes	***	<0.0001
		Pdf>mCD8:GFP;Unc5 (ZT14)	12	5003	3357	969.2	yes	Pdf>GFP (ZT14) vs. Pdf>GFP;Unc5 (ZT2)	Yes	**	0.0051
								Pdf>GFP (ZT14) vs. Pdf>GFP;Unc5 (ZT14)	Yes	**	0.0033
								Pdf>GFP;Unc5 (ZT2) vs. Pdf>GFP;Unc5 (ZT14)	No	ns	0.9973

Figure 2	Treatment	Genotype	n	Mean	Std. Deviation	S.E.M.	Gaussian distr.	Test			
2B	Morning Anticipation							Mann Whitney	Significant?	Summary	P value
		w1118	72	0.0106	0.0058	0.0007	yes	w1118 vs. Pdf 01	yes	***	<0.0001
		pdf01	75	-0.00001765	0.0056	0.0006	no				
2D	Morning Anticipation							Kruskal Wallis with Dunn's post test	Significant?	Summary	P Value
		Pdf-Gal4/+	79	0.0152	0.0080	0.0009	no	Pdf-Gal4/+ vs. Pdf>Unc5	yes	****	<0.0001
		Pdf>Unc5	75	0.0062	0.0079	0.0009	yes	Pdf-Gal4/+ vs. uas-Unc5/+	yes	****	<0.0001
		uas-Unc5/+	92	0.0058	0.0059	0.0006	no	Pdf>Unc5 vs. uas-Unc5/+	no	ns	>0.9999
2E	Evening Phase							Kruskal Wallis with Dunn's post test	Significant?	Summary	P Value
		Pdf-Gal4/+	49	-0.4286	0.7706	0.1101	no	Pdf-Gal4/+ vs. Pdf>Unc5	no	ns	>0.9999
		Pdf>Unc5	42	-0.4286	0.7034	0.1085	yes	Pdf-Gal4/+ vs. uas-Unc5/+	yes	****	<0.0001
		uas-Unc5/+	48	-1.365	0.8736	0.1261	no	Pdf>Unc5 vs. uas-Unc5/+	yes	****	<0.0001

Figure 3	Treatment	Genotype	n	Mean	Std. Deviation	S.E.M.	Gaussian distr.	Test		Watson-Wheeler	Watson's Two-Sample
3B	Phase Analysis							Both circular statistics tests	Significant?	P-value	P-value
		Pdf-Gal4 /+;	53	0.0377	0.7522	0.1033	N/A	Pdf>Unc5 vs. Pdf-Gal4/+	no	0.167	<0.10
		uas-Unc5/+	60	-1.4250	0.6299	0.0813	N/A	Pdf>Unc5 vs. uasUnc5	yes	<0.0001	<0.001
		Pdf-Gal4 /+;Unc5/+	41	0.4268	0.9392	0.1467	N/A	Pdf>DbtL vs. Pdf-Gal4	yes	<0.0001	<0.001
		uas-DbtL/+	59	-0.9068	0.8172	0.1064	N/A	Pdf>DbtL vs. uas-DbtL	yes	<0.0001	<0.001
		uas-DbtL/+;uas-Unc5/+	49	-1.3270	0.9385	0.1341	N/A	Pdf>Unc5; DbtL vs. Pdf>Unc5	yes	0.0002905	<0.001
		Pdf-Gal4 /uas-DbtL	35	2.0140	0.8444	0.1427	N/A	Pdf>Unc5; DbtL vs. Pdf>DbtL	no	0.4324	0.113
		Pdf-Gal4 /DbtL;Unc5/+	60	1.3000	1.1760	0.1518	N/A	Pdf>Unc5; DbtL vs. Pdf-Gal4	yes	<0.0001	<0.001
								Pdf>Unc5; DbtL vs. uas-DbtL	yes	<0.0001	<0.001
								Pdf>Unc5; DbtL vs. uasUnc5	yes	<0.0001	<0.001
3C	Free- Running Period							Kruskall-Wallis + Dunn's post-test	Significant?	Summary	P Value
		Pdf-Gal4/+	55	24.0300	0.1146	0.0155	no	Pdf-Gal4/+ vs. uas-Unc5/+	yes	****	<0.0001
		uas-Unc5/+	61	23.5900	0.2142	0.0274	no	Pdf-Gal4/+ vs. uas-DbtL/+	no	ns	>0.9999
		uas-DbtL/+	58	23.9100	0.1977	0.0260	no	Pdf-Gal4/+ vs. Unc5/+;DbtL/+	yes	****	<0.0001
		Unc5/+;DbtL/+	54	23.5900	0.2394	0.0326	yes	Pdf-Gal4/+ vs. Pdf>Unc5	no	ns	>0.9999
		Pdf>Unc5	40	24.0400	0.2628	0.0416	no	Pdf-Gal4/+ vs. Pdf>DbtL	yes	****	<0.0001
		Pdf>DbtL	28	27.2100	0.5998	0.1134	no	Pdf-Gal4/+ vs. Pdf>DbtL;Unc5	yes	***	0.0009
		Pdf>DbtL;Unc5	58	26.2400	1.5220	0.1999	no	uas-Unc5/+ vs. uas-DbtL/+	yes	****	<0.0001
								uas-Unc5/+ vs. Unc5/+;DbtL/+	no	ns	>0.9999
								uas-DbtL/+ vs. Pdf>DbtL;Unc5	yes	****	<0.0001
								Unc5/+;DbtL/+ vs. Pdf>Unc5	yes	****	<0.0001
								Unc5/+;DbtL/+ vs. Pdf>DbtL	yes	****	<0.0001
								Unc5/+;DbtL/+ vs. Pdf>DbtL;Unc5	yes	****	<0.0001
								Pdf>Unc5 vs. Pdf>DbtL	yes	****	<0.0001
								Pdf>Unc5 vs. Pdf>DbtL;Unc5	yes	**	0.0031
								Pdf>DbtL vs. Pdf>DbtL;Unc5	no	ns	0.4648
								uas-Unc5/+ vs. Pdf>Unc5	yes	****	<0.0001
								uas-Unc5/+ vs. Pdf>DbtL	yes	****	<0.0001
								uas-Unc5/+ vs. Pdf>DbtL;Unc5	yes	****	<0.0001
								uas-DbtL/+ vs. Unc5/+;DbtL/+	yes	***	0.0001
								uas-DbtL/+ vs. Pdf>Unc5	no	ns	>0.9999
								uas-DbtL/+ vs. Pdf>DbtL	yes	****	<0.0001

Figure 5	Treatment	Genotype	n	Mean	Std. Deviation	S.E.M.	Gaussian distr.	Test			
5C	Heating Index							Kruskal Wallis with Dunn's post test	Significant?	Summary	P Value

		uas-Unc5/+	84	0.5839	0.2817	0.0307	no	Unc5/+ vs. Pdf-Gal4/+	yes	**	0.0016	
		Pdf>Unc5	72	0.2477	0.3681	0.0434	yes	Unc5/+ vs. Unc5/Pdf-Gal4	yes	****	<0.0001	
		Pdf-Gal4/+	92	0.7219	0.2173	0.0227	no	Pdf-Gal4/+ vs. Unc5/Pdf-Gal4	yes	****	<0.0001	
5D	% Rythmic							Chi-Square test	Significant?	Summary	P Value	
		uas-Unc5/+	54					Pdf-Gal4/+ vs. Pdf>Unc5	yes	*	0.020258	
		Pdf>Unc5	66					Pdf-Gal4/+ vs. uas-Unc5/+	no	NS	0.814453	
		Pdf-Gal4/+	63					Pdf>Unc5 vs. uas-Unc5/+	yes	**	0.007586	
5E	Rhythmic Power							Kruskal Wallis with Dunn's post test	Significant?	Summary	P Value	
		uas-Unc5/+	54	54.8000	41.4600	5.6410	no	Pdf-Gal4/+ vs. Pdf>Unc5	yes	****	<0.0001	
		Pdf>Unc5	66	24.1600	27.0400	3.3280	yes	Pdf-Gal4/+ vs. uas-Unc5/+	no	NS	>0.9999	
		Pdf-Gal4/+	63	62.2800	46.5100	5.8600	yes	Pdf>Unc5 vs. uas-Unc5/+	yes	****	<0.0001	
5F	Free-running Period							ANOVA with Tukey's Test	Significant?	Summary	P Value	
		uas-Unc5/+	48	23.3800	0.3790	0.0547	yes	Pdf-Gal4/+ vs. Pdf>Unc5	no	NS	0.9533	
		Pdf>Unc5	38	23.6300	0.4455	0.0723	yes	Pdf-Gal4/+ vs. uas-Unc5/+	yes	**	0.0022	
		Pdf-Gal4/+	54	23.6600	0.4214	0.0574	yes	Pdf>Unc5 vs. uas-Unc5/+	yes	*	0.0138	
5I	Arbor Area (T Ramp)							unpaired t- test		Summary	P Value	
		ZT00	18	344.7000	74.8100	17.6300	yes	ZT12 vs.	no	ns	0.2908	
		ZT12	18	377.5000	106.1000	25.0000	yes	ZT00				
5J	Volume							unpaired t- test		Summary	P Value	
		ZT00	18	25058	12303	2900	yes	ZT12 vs.	yes	**	0.0047	
		ZT12	18	14859	7300	1721	yes	ZT00				
5H	Mean Pixel Intensity							Kruskal Wallis with Dunn's post test	Significant?	Summary	P Value	
		pdf>GFP ZT00 (Nuc)	49	1384.0000	699.3000	99.9000	Yes	pdf>GFP ZT00 (Nuc) vs Pdf>Unc5 ZT00 (Nuc)	yes	****	<0.0001	
		pdf>GFP ZT06 (Nuc)	52	1736.0000	618.5000	85.7700	No	pdf>GFP ZT06 (Nuc) vs Pdf>Unc5 ZT06 (Nuc)	yes	*	0.0192	
		pdf>GFP ZT12 (Nuc)	54	242.9000	151.2000	20.5700	No	pdf>GFP ZT12 (Nuc) vs Pdf>Unc5 ZT12 (Nuc)	no	NS	0.446	
		pdf>GFP ZT18 (Nuc)	50	283.4000	119.4000	16.8800	No	pdf>GFP ZT18 (Nuc) vs Pdf>Unc5 ZT18 (Nuc)	no	NS	0.1942	
		Pdf>Unc5 ZT00 (Nuc)	50	308.6000	144.8000	20.4800	No	pdf>GFP ZT00 (Cyt) vs Pdf>Unc5 ZT00 (Cyt)	yes	*	0.0246	
		Pdf>Unc5 ZT06 (Nuc)	62	708.3000	375.4000	47.6800	No	pdf>GFP ZT06 (Cyt) vs Pdf>Unc5 ZT06 (Cyt)	yes	**	0.0017	
		Pdf>Unc5 ZT12 (Nuc)	50	369.2000	268.2000	37.9200	No	pdf>GFP ZT12 (Cyt) vs Pdf>Unc5 ZT12 (Cyt)	no	NS	0.4006	
		Pdf>Unc5 ZT18 (Nuc)	56	417.9000	172.8000	23.0900	No	pdf>GFP ZT18 (Cyt) vs Pdf>Unc5 ZT18 (Cyt)	no	NS	0.2669	
		pdf>GFP ZT00 (Cyt)	49	424.8000	161.8000	23.1200	Yes	pdf>GFP ZT00 (Nuc) vs w1118 ZT06 (Nuc)	no	NS	>0.9999	
		pdf>GFP ZT06 (Cyt)	52	289.2000	154.0000	21.3600	No	pdf>GFP ZT06 (Nuc) vs w1118 ZT12 (Nuc)	yes	****	<0.0001	
		pdf>GFP ZT12 (Cyt)	54	121.0000	51.1200	6.9560	Yes	pdf>GFP ZT12 (Nuc) vs w1118 ZT18 (Nuc)	no	NS	>0.9999	
		pdf>GFP ZT18 (Cyt)	54	308.6000	144.8000	20.4800	No	pdf>GFP ZT00 (Cyt) vs w1118 ZT06 (Cyt)	no	NS	0.3432	
		Pdf>Unc5 ZT00 (Cyt)	56	269.4000	125.4000	16.7500	Yes	pdf>GFP ZT06 (Cyt) vs w1118 ZT12 (Cyt)	yes	****	<0.0001	
		Pdf>Unc5 ZT06 (Cyt)	62	165.8000	80.1700	10.1800	Yes	pdf>GFP ZT12 (Cyt) vs w1118 ZT18 (Cyt)	yes	****	<0.0001	
		Pdf>Unc5 ZT12 (Cyt)	50	210.5000	121.2000	17.1400	No	Pdf>Unc5 ZT00 (Nuc) vs Pdf>Unc5 ZT06 (Nuc)	yes	****	<0.0001	
		Pdf>Unc5 ZT18 (Cyt)	56	441.8000	173.5000	23.1900	No	Pdf>Unc5 ZT06 (Nuc) vs Pdf>Unc5 ZT12 (Nuc)	yes	**	0.0033	
								Pdf>Unc5 ZT12 (Nuc) vs Pdf>Unc5 ZT18 (Nuc)	no	NS	>0.9999	
								Pdf>Unc5 ZT00 (Cyt) vs Pdf>Unc5 ZT06 (Cyt)	yes	*	0.0247	
								Pdf>Unc5 ZT06 (Cyt) vs Pdf>Unc5 ZT12 (Cyt)	no	NS	>0.9999	
								Pdf>Unc5 ZT12 (Cyt) vs Pdf>Unc5 ZT18 (Cyt)	yes	****	<0.0001	
5L	Heating Index							Kruskal Wallis with Dunn's post test	Significant?	Summary	P Value	
		Pdf-Gal4/+	92	0.7219	0.2173	0.0023	no	Pdf-Gal4/+ vs. Pdf>uas-hid	yes	****	<0.0001	
		Pdf>uas-hid	12	-0.0284	0.3955	0.1142	yes	Pdf-Gal4/+ vs. uas-hid/+	no	NS	>0.9999	
		uas-hid/+	16	0.6934	0.1895	0.0474	yes	Pdf>uas-hid vs. uas-hid/+	yes	**	0.0014	
5N	Heating Index							Mann Whitney	Significant?	Summary	P Value	
		w1118	62	0.7023	0.1925	0.0244	no	w1118 vs. pdf01		*	0.0152	
		pdf01	60	0.6119	0.2301	0.0297	no					

Figure 7	**Treatment**	**Genotype**	**n**	**Mean**	**Std. Deviation**	**S.E.M.**	**Gaussian distr.**	**Test**				
7B	Heating Index							Kruskal Wallis with Dunn's post test	Significant?	Summary	P Value	
		uas-GluClRNAi/+	31	0.6461	0.3346	0.0486	no	Uas-GluClRNAi/+ vs. Pdf-Gal4/+	no	NS	>0.9999	
		Pdf>uas-GluClRNAi	13	0.0359	0.4691	0.0601	yes	Uas-GluClRNAi/+ vs. Pdf>Uas-GluClRNAi	yes	****	<0.0001	
		Pdf-Gal4/+	22	0.6454	0.2644	0.0564	no	Pdf-Gal4/+ vs. Pdf>Uas-GluClRNAi	yes	***	0.0007	
7D	Heating Index							Kruskal Wallis with Dunn's post test	Significant?	Summary	P Value	
		uas-GluClRNAi/+	32	0.8399	0.1116	0.0197	no	Uas-GluClRNAi/+ vs. Pdf-Gal4/+	yes	**	0.0066	
		Pdf>uas-GluClRNAi	25	0.3011	0.2897	0.0579	yes	Uas-GluClRNAi/+ vs. Pdf>Uas-GluClRNAi	yes	****	<0.0001	
		Pdf-Gal4/+	22	0.6454	0.2644	0.0564	no	Pdf-Gal4/+ vs. Pdf>Uas-GluClRNAi	yes	**	0.0055	
7F	Heating Index							Kruskal Wallis with Dunn's post test	Significant?	Summary	P Value	
		uas-mGluRRNAi/+	32	0.7691	0.1950	0.0350	no	Uas-mGluRRNAi/+ vs. Pdf-Gal4/+	no	NS	0.2543	
		Pdf>uas-mGluRRNAi	20	0.1749	0.2485	0.0556	yes	Uas-mGluRRNAi/+ vs. Pdf>Uas-mGluRRNAi	yes	****	<0.0001	
		Pdf-Gal4/+	22	0.6454	0.2644	0.0564	no	Pdf-Gal4/+ vs. Pdf>Uas-mGluRRNAi	yes	***	0.0002	
7H	Heating Index							Kruskal Wallis with Dunn's post test	Significant?	Summary	P Value	
		uas-mGluRRNAi/+	31	0.7691	0.1950	0.0350	no	Uas-mGluRRNAi/+ vs. Pdf-Gal4/+	no	NS	0.1715	
		Pdf>uas-mGluRRNAi	30	0.4862	0.3412	0.0623	yes	Uas-mGluRRNAi/+ vs. Pdf>Uas-mGluRRNAi	yes	***	0.0004	
		Pdf-Gal4/+	22	0.6454	0.2644	0.0564	no	Pdf-Gal4/+ vs. Pdf>Uas-mGluRRNAi	no	NS	0.3422	

Figure S1	Treatment	Genotype	n	Mean	Std. Deviation	S.E.M.	Gaussian distr.	Test			
S1C	Max. # Neurites							unpaired t-test	Significant?	Summary	P value
		Pdf>mCD8:GFP	12	2.2500	0.4523	0.1306	yes	Pdf>mCD8:GFP vs.	yes	***	0.0005
		Pdf>Unc5;mCD8:GFP	8	3.3750	0.7440	0.2631	yes	Pdf>Unc5;mCD8:GFP			

Figure S3	Treatment	Genotype	n	Mean	Std. Deviation	S.E.M.	Gaussian distr.	Test			
S3A	Morning Phase							AnoVA + Tukey's post test			P Value
		Pdf-Gal4/+	49	-0.02041	1.407	0.201	yes	PdfGal4/+ vs. Pdf>Unc5	yes	*	0.0265
		Pdf>Unc5	42	0.7024	1.339	0.2066	yes	PdfGal4/+ vs. uas-Unc5/+	yes	**	0.0078
		uas-Unc5/+	48	0.7917	1.184	0.1709	yes	Pdf>Unc5 vs. uas-Unc5/+	no	ns	0.9445
S3C	Evening Anticipation							Unpaired t-test		Summary	P Value
		w1118	72	0.0165	0.0102	0.0012	yes	w1118 vs. Pdf 01		ns	0.0767
		pdf01	75	0.0195	0.0103	0.0012	yes				
S3D	Evening Anticipation							Kruskal Wallis with Dunn's	Significant?	Summary	P Value
		Pdf-Gal4/+	79	0.0112	0.0147	0.0016	yes	Pdf-Gal4/+ vs. Pdf>Unc5	no	ns	0.1635
		Pdf>Unc5	75	0.0164	0.0132	0.0015	no	Pdf-Gal4/+ vs. uas-Unc5/+	yes	****	<0.0001
		uas-Unc5/+	92	0.0263	0.0121	0.0013	yes	Pdf>Unc5 vs. uas-Unc5/+	yes	****	<0.0001

Figure S5	Treatment	Genotype	n	Mean	Std. Deviation	S.E.M.	Gaussian distr.	Test			
S5D	Arbor Length							Unpaired t-test	Significant?	Summary	P Value
		Pdf>mCD8:GFP	22	192.1	27.5	5.9	yes	Pdf>mCD8:GFP vs	yes	***	<0.0001
		Pdf>Fas2;mCD8:GFP	16	135.2	20.5	5.1	yes	Pdf>Fas2; mCD8:GFP			
S5E	Arbor Area							Mann-Whitney test	Significant?	Summary	P Value
		Pdf>mCD8:GFP	20	395.5	130.7	29.2	yes	Pdf>mCD8:GFP vs	yes	***	<0.0001
		Pdf>Fas2;mCD8:GFP	18	51.4	26.6	6.3	no	Pdf>Fas2; mCD8:GFP			
S5G	Morning Anticipation							AOVAVA with Tukey's post-hoc test	Significant?	Summary	P Value
		Pdf-Gal4/+	22	0.0139	0.0061	0.0013	yes	uas-Fas2/+ vs. Pdf>Fas2	no	ns	0.348
		uas-Fas2/+	18	0.0056	0.0040	0.0010	yes	uas-Fas2/+ vs. Pdf-Gal4/+	yes	*	0.0103
		Pdf>Fas2	16	0.0083	0.0063	0.0016	yes	Pdf>Fas2 vs. Pdf-Gal4/+	yes	****	<0.0001

Figure S6	Treatment	Genotype	n	Mean	Std. Deviation	S.E.M.	Gaussian distr.	Test			
S6C	Heating Index							Kruskal Wallis with Dunn's	Significant?	Summary	P Value
		Pdf-Gal4/+	92	0.7219	0.2173	0.0227	no	uas-Fas2/+ vs. Pdf-Gal4/+	no	NS	0.1692
		Pdf>Fas2	32	0.4454	0.4517	0.0798	no	uas-Fas2/+ vs. Pdf>Fas2	yes	****	<.0001
		uas-Fas2/+	63	0.7724	0.2113	0.0266	no	Pdf-Gal4/+ vs. Pdf>Fas2	yes	**	0.0083
S6E	% Rhthmic							Chi-Square test	Significant?	Summary	P Value
		Pdf-Gal4/+	63					uas-Fas2/+ vs. Pdf-Gal4/+	no	NS	0.495896
		Pdf>Fas2	32					uas-Fas2/+ vs. Pdf>Fas2	yes	*	0.029641
		uas-Fas2/+	31					Pdf-Gal4/+ vs. Pdf>Fas2	no	NS	0.050736
S6F	Rhythmic Power							Kruskal Wallis with Dunn's	Significant?	Summary	P Value
		Pdf-Gal4/+	63	62.2800	46.5100	5.8600	yes	uas-Fas2/+ vs. Pdf-Gal4/+	no	NS	>0.9999
		Pdf>Fas2	32	31.2900	28.0200	4.9520	no	uas-Fas2/+ vs. Pdf>Fas2	yes	**	0.0063
		uas-Fas2/+	31	51.5300	51.5300	9.2550	yes	Pdf-Gal4/+ vs. Pdf>Fas2	yes	**	0.0051

Figure S7	Treatment	Genotype	n	Mean	Std. Deviation	S.E.M.	Gaussian distr.	Test			
S7B	Rhythmic Power							Unpaired t-test	Significant?	Summary	P Value
		w1118	94	19.3400	14.1800	1.4630	yes	w1118 vs. pdf01	yes	*	0.0108
		pdf01	91	24.5100	13.1100	1.3740	yes				
S7C	Rhythmic Power							Unpaired t-test	Significant?	Summary	P Value
		w1118	92	14.9700	14.0000	1.4600	yes	w1118 vs. pdf01	no	NS	0.6914
		pdf01	94	15.0500	13.4400	1.3860	yes				
S7E	Rhythmic Power							Kruskal Wallis with Dunn's	Significant?	Summary	P Value
		Pdf-Gal4/+	60	20.0600	15.9300	2.0570	no	uas-hid/+ vs. Pdf-Gal4/+	yes	**	0.0025
		Pdf>hid	42	12.9400	16.8400	2.5980	yes	uas-hid/+ vs. Pdf>hid	yes	****	<.0001
		uas-hid/+	92	29.1900	13.9300	1.4520	yes	Pdf-Gal4/+ vs. Pdf>hid	no	NS	0.1526
S7F	Rhythmic Power							Kruskal Wallis with Dunn's	Significant?	Summary	P Value
		Pdf-Gal4/+	61	21.1100	14.3400	1.8360	yes	uas-hid/+ vs. Pdf-Gal4/+	yes	*	0.0268
		Pdf>hid	40	-0.6723	10.3100	1.6300	no	uas-hid/+ vs. Pdf>hid	yes	****	<.0001
		uas-hid/+	91	28.4500	14.4200	1.5110	yes	Pdf-Gal4/+ vs. Pdf>hid	yes	****	<.0001

Table S1. Descriptive statistics and statistical tests. Related to Figures 1,2,3 ,5 and 7, and Figures S1, S3, and S5-S7.