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Supplementary Tables

Appendix Table S1. Knocking down *Nipped-A* lengthens the period of locomotor rhythm

Genotype	Period(hr)±SEM	Power±SEM	%Rhythmic	N
tim*G4/+;U*dcr2*/U*Nipped-ARNAi-1	25.89±0.07$^{***\#\#\#}$	58.96±4.24***	76	150
tim*G4/+;U*dcr2*/U*Nipped-ARNAi-2	25.27±0.35$^{***\#\#\#}$	7.90±2.11$^{***\#\#\#}$	9	100
tim*G4/+;U*dcr2*/U*Nipped-ARNAi-3	25.25±0.20$^{***\#\#\#}$	11.69±2.11$^{***\#\#\#}$	23	69
tim*G4/+;U*dcr2*/U*Nipped-ARNAi-4	27.25±0.13$^{***\#\#\#}$	40.09±6.42$^{***\#\#\#}$	74	38
tim*G4/+;U*dcr2*/U*Nipped-ARNAi-5	27.25±0.14$^{***\#\#\#}$	52.90±8.12$^{***\#\#\#}$	69	39
tim*G4/U*Nipped-ARNAi*;U*dcr2*/+-*6	25.85±0.08$^{***\#\#\#}$	45.73±4.12$^{***\#\#\#}$	78	81
***tim*G4/+;U*dcr2*/+**	23.61±0.03	138.00±4.68	100	127
U*dcr2*/+;*cry*G4-16/U*Nipped-ARNAi-1*	27.01±0.09$^{***\#\#\#}$	73.62±6.15***	92	86
U*dcr2*/+;*cry*G4-16/U*Nipped-ARNAi-2*	25.77±0.19$^{***\#\#\#}$	27.44±4.24$^{***\#}$	60	60
U*dcr2*/+;*cry*G4-16/U*Nipped-ARNAi-3*	26.58±0.17$^{***\#\#\#}$	47.06±5.33***	80	66
U*dcr2*/+;*cry*G4-16/U*Nipped-ARNAi-4*	26.42±0.14$^{***\#\#\#}$	149.72±9.99**	100	32
U*dcr2*/+;*cry*G4-16/U*Nipped-ARNAi-5*	26.76±0.13$^{***\#\#\#}$	137.31±9.55*	100	48
U*dcr2*/U*Nipped-ARNAi*;*cry*G4-16/+-*6*	26.45±0.07$^{***\#\#\#}$	105.75±5.29	96	99
U*dcr2*/+;*cry*G4-16/+	24.91±0.08	111.18±5.03	98	129
***pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-1*/+**	24.96±0.12$^{*\#\#\#}$	52.49±4.77***	85	82
***pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-2*/+**	24.37±0.17$^{\#\#\#}$	25.34±3.33***	70	63
***pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-3*/+**	24.57±0.18$^{\#\#\#}$	27.78±2.85$^{***\#\#}$	81	42
***pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-4*/+**	25.61±0.15$^{***\#\#\#}$	87.57±8.48$^{*\#\#}$	98	65
***pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-5*/+**	25.56±0.16$^{***\#\#\#}$	86.31±9.81$^{*\#\#\#}$	90	48
pdf*G4-U*dcr2*/U*Nipped-ARNAi-6	25.57±0.10$^{***\#\#\#}$	66.60±5.01$^{***\#\#\#}$	88	113
***pdf*G4-U*dcr2*/+**	24.61±0.02	114.98±5.59	96	147
U*Nipped-ARNAi-1*/+	23.61±0.00	68.70±4.25	99	68
U*Nipped-ARNAi-2*/+	23.60±0.04	50.04±6.18	86	49
U*Nipped-ARNAi-3*/+	23.53±0.04	66.55±7.16	100	43
U*Nipped-ARNAi-4*+	23.94±0.05	126.34±5.63	98	60
U*Nipped-ARNAi-5*/+	23.73±0.03	143.47±4.98	100	92
U*Nipped-ARNAi-6*/+	23.65±0.03	109.76±5.53	99	71
18℃→29℃				
U*dcr2*/+;*cry*G4-16/U*Nipped-ARNAi-4*	26.54±0.13$^{***\#\#\#}$	90.88±10.56	77	56
U*dcr2*/*tub*G80^{ts};*cry*G4-16/U*Nipped-ARNAi-4*	26.29±0.10$^{***\#\#\#}$	99.53±11.99	76	45
U*dcr2*/*tub*G80^{ts};*cry*G4-16/+	25.24±0.08	116.17±13.38	80	40
U*dcr2*/+;*cry*G4-16/+	24.69±0.17	80.98±12.53	79	33
U*Nipped-ARNAi-4*/+	23.51±0.03	106.74±11.58	87	39
***tub*G80^{ts}/+**	23.64±0.03	88.18±9.38	75	63
***tub*G80^{ts}/+;U*Nipped-ARNAi-4*/+**	23.68±0.04	107.89±9.35	93	44
29℃→18℃				
U*dcr2*/+;*cry*G4-16/U*Nipped-ARNAi-4*	26.50±0.28$^{***\#\#\#}$	26.75±4.48	45	51
U*dcr2*/*tub*G80^{ts};*cry*G4-16/U*Nipped-ARNAi-4*	24.73±0.12$^{\#\#\#}$	25.48±3.73$^{\#\#\#}$	61	49

U*dcr2/tub*G80^{ts};*cry*G4-16/+	24.52±0.17	55.27±6.79	83	47
U*dcr2*/+;*cry*G4-16/+	25.64±0.23	31.68±5.81	53	40
U*Nipped-ARNAi-4*/+	24.10±0.10	24.27±3.87	75	32
***tub*G80^{ts}/+**	23.70±0.06	39.91±4.58	93	45
***tub*G80^{ts}/+;U*Nipped-ARNAi-4*/+**	23.72±0.06	34.30±4.13	70	62
Drug treated				
U*dcr2*/+;*pdf*-GS/+	25.08±0.10	94.44±5.09	100	49
U*Nipped-ARNAi*/+-*4*	24.04±0.04	134.11±5.44	100	50
U*dcr2*/+;*pdf*-GS/U*Nipped-ARNAi-4*	25.94±0.12$^{***\#\#\#}$	73.34±7.50$^{***\#\#\#}$	88	41
Ethanol treated				
U*dcr2*/+;*pdf*-GS/+	23.63±0.05	122.34±7.85	97	31
U*Nipped-ARNAi-4*/+	23.84±0.04	115.24±6.16	98	40
U*dcr2*/+;*pdf*-GS/U*Nipped-ARNAi-4*	23.98±0.05$^{\#\#\#}$	119.19±7.97	97	32

One-way ANOVA, $P<0.001$. Tukey's multiple comparison test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared with G4 and U*dcr2*; $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ when compared with U*RNAi.* 18℃→29℃, flies raised at 18℃ and tested at 29℃; 29℃→18℃, flies raised at 29℃ and tested at 18℃. Drug treated, flies were treated with RU486 after eclosion ; ethanol treated, flies were treated with ethanol instead of RU486 after ecloson. $^{***}P<0.001$ when compared with U*dcr2/+;pdf*-GS/+, $^{\#\#\#}P<0.001$ when compared with U*Nipped-ARNAi-4*/+. G4, GAL4; U, UAS.

Appendix Table S2. Over-expressing *Nipped-A* rescues the long period phenotype caused by knocking down *Nipped-A*

Genotype	Period(hr)±SEM	Power±SEM	%Rhythmic	N
U*Nipped-A*/+	23.61±0.03	106.69±5.63	98	61
U*Nipped-ARNAi-4*/+	23.76±0.05	110.54±7.57	97	36
U*Nipped-A*/+;U*Nipped-ARNAi-4*/+	23.85±0.04	102.41±6.99	96	68
***tim*G4/+;U*dcr2*/+**	24.63±0.05	125.82±8.47	100	47
***tim*G4/U*Nipped-A*;U*dcr2*/+**	24.51±0.05$^{\#\#\#}$	100.12±7.89	95	43
tim*G4/+;U*dcr2*/U*Nipped-ARNAi-4	27.12±0.15$^{***\#\#\#}$	51.72±4.14$^{***\#\#\#}$	84	74
tim*G4/U*Nipped-A*;U*dcr2*/U*Nipped-ARNAi-4	24.09±0.12$^{+++\dagger\dagger}$	35.33±8.59†††	61	28
***pdf*G4-U*dcr2*/+**	24.58±0.03	166.35±7.16	100	76
pdf*G4-U*dcr2*/U*Nipped-A	24.58±0.04$^{\#\#\#}$	109.89±7.05***	98	60
***pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-4*/+**	25.88±0.16$^{***\#\#\#}$	84.51±7.67***	96	53
***pdf*G4-U*dcr2*/U*Nipped-A*;U*Nipped-ARNAi-4*/+**	24.87±0.04$^{+++\dagger}$	115.20±7.82^{+}	97	70

One-way ANOVA, *P*<0.001, Tukey's multiple comparison test,****P*<0.001 when compared with G4 control, $^{\#\#\#}$*P*<0.001 when compared with UAS control; $^{+++}$*P*<0.001 when compared with *tim*G4/+;U*dcr2*/U*Nipped-ARNAi-4 or pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-4*/+ , †††*P*<0.001 when compared with *tim*G4/U*Nipped-A;*U*dcr2*/+ or *pdf*G4-U*dcr2*/U*Nipped-A.* G4, GAL4; U, UAS.

Appendix Table S3. Genetic interaction between *Nipped-A* and *GFP*

Genotype	Period(hr)±SEM	Power±SEM	%Rhythmic	N
pdf*G4-U*dcr2/UNipped-ARNAi-6	25.78±0.13$^{***\#\#\#}$	48.62±5.11***	82	61
pdf*G4-U*dcr2/+;UGFP/+	24.36±0.04$^{\#\#\#}$	78.51±6.18	88	56
pdf*G4-U*dcr2/UNipped-ARNAi-6;UGFP/+	25.88±0.12†††	52.72±5.77	89	28
pdf*G4-U*dcr2/+	24.54±0.04	73.79±5.41	89	66
U*Nipped-ARNAi-6/+*	23.68±0.04	108.01±6.81	100	40
U*GFP/+*	23.53±0.02	90.17±10.49	91	33
U*Nipped-ARNAi-6/+;UGFP/+*	23.77±0.04	102.76±8.15	88	40
tim*G4/U*Nipped-ARNAi-6;Udcr2/+	25.95±0.11$^{***\#\#\#}$	49.88±6.71$^{\#\#\#}$	86	37
tim*G4/+;U*dcr2/UGFP	24.15±0.06$^{***\#\#\#}$	82.16±7.06	95	39
tim*G4/U*Nipped-ARNAi-6;Udcr2/UGFP	26.19±0.14†††	38.42±4.42†††	78	60
timG4/+;Udcr2/+	24.65±0.05	69.32±7.49	88	41

One-way ANOVA, *P*<0.001, Tukey's multiple comparison test, ****P*<0.001 when compared with G4 control, $^{\#\#\#}$*P*<0.001 when compared with UAS control, $^{+++}$*P*<0.001 when compared with *pdf*G4-U*dcr2/UNipped-ARNAi-6 or tim*G4/U*Nipped-ARNAi-6;*U*dcr2/+,* †††*P*<0.001 when compared with *pdf*G4-U*dcr2/+;UGFP/+ or tim*G4/+;U*dcr2/UGFP.* G4, GAL4; U, UAS.

Appendix Table S4. Related to Figure 3. Genetic interaction between *Nipped-A* and *tim*

Genotype	**Period(hr)**±SEM	**Power**±SEM	**%Rhythmic**	**N**
***pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-5*/+**	25.52±0.07$^{***\#\#\#}$	74.65±6.77$^{\#\#\#}$	88	43
pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-5/Ptim	24.57±0.17$^{+++\dagger\dagger\dagger}$	45.21±5.65$^{+\dagger\dagger\dagger}$	79	34
***pdf*G4-U*dcr2*/+;*Ptim*/+**	24.11±0.06$^{***\#\#\#}$	108.27±8.83$^{***\#\#\#}$	100	49
***pdf*G4-U*dcr2*/+**	24.54±0.04	73.79±5.41	89	66
***Ptim*/+**	23.42±0.03	83.45±7.76	98	43
U*Nipped-ARNAi-5/Ptim*	23.54±0.02	141.09±7.13	100	24
***tim*G4/+;U*dcr2*/+**	23.80±0.05	111.44±7.49	93	46
tim*G4/+;U*dcr2*/U*Nipped-ARNAi-4	26.76±0.12$^{***\#\#\#}$	62.99±7.11$^{***\#\#\#}$	80	55
tim*G4/*tim*^{01};U*dcr2*/U*Nipped-ARNAI-4	26.74±0.11^{+++}	22.86±4.04$^{+++\dagger\dagger\dagger}$	46	72
timG4/+;Udcr2/UNipped-ARNAi-5	26.77±0.11$^{***\#\#\#}$	62.17±7.40$^{***\#\#\#}$	76	49
tim*G4/*tim*^{01};U*dcr2*/U*Nipped-ARNAi-5	26.77±0.05^{+++}	23.49±4.66$^{+++\dagger\dagger\dagger}$	43	56
***tim*G4/*tim*^{01};U*dcr2*/+**	24.17±0.03**	148.68±6.89**	96	53
***tim*^{01}/+**	24.08±0.03	110.34±6.11	98	53
U*Nipped-ARNAi-4*/+	24.00±0.05	124.97±6.91	98	43
U*Nipped-ARNAi-5*/+	23.82±0.04	123.58±5.71	100	47
***tim*^{01}/+;U*Nipped-ARNAi-4*/+**	24.22±0.05	91.04±6.53	97	39
***tim*^{01}/+;U*Nipped-ARNAi-5*/+**	24.01±0.04	96.27±5.85	98	47

One-way ANOVA, P<0.001. Tukey's multiple comparison test, $^{***}P$<0.001 when compared with G4 control; $^{\#\#\#}P$<0.001 when compared with *Ptim* or *tim*^{01}, ^{+++}P<0.001 when compared with *pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-5*/+ or *tim*G4/+;U*dcr2*/U*Nipped-ARNAi*, $^{\dagger\dagger\dagger}P$<0.001 when compared with *pdf*G4-U*dcr2*/+;*Ptim*/+ or *tim*G4/*tim*^{01};U*dcr2*/+. G4, GAL4; U, UAS.

Appendix Table S5. Genetic interaction between *Nipped-A* and *Pdp1ε*

Genotype	Period(hr)±SEM	Power±SEM	%Rhythmic	N
pdf*G4-U*dcr2/* U*Nipped-ARNAi-4/+	25.65±0.10$^{***\#\#\#}$	59.95±6.55$^{\#\#\#}$	85	54
pdf*G4-U*dcr2/UPdp1ε;* U*Nipped-ARNAi-4/+	23.50±0.11$^{+++\dagger\dagger\dagger}$	26.69±2.88$^{+++\dagger}$	58	106
pdf*G4-U*dcr2/UPdp1ε	24.16±0.05$^{***\#\#\#}$	46.95±4.43$^{***\#\#\#}$	73	93
pdf*G4-U*dcr2/+	24.54±0.04	73.79±5.41	89	66
U*Pdp1ε/+*	23.57±0.02	103.79±5.30	97	75
U*Nipped-ARNAi-4/+*	23.92±0.03	115.44±4.48	97	76
U*Pdp1ε/+; UNipped-ARNAi-4/+*	23.75±0.05	96.05±5.88	100	44
U*dcr2/UNipped-ARNAi-6;cry*G4-16/+	26.53±0.08$^{***\#\#\#}$	102.5±6.67	95	68
U*dcr2/+;cry*G4-16/*Pdp1* ^{3135}	25.76±0.09$^{\#\#\#}$	79.3±5.47	96	59
U*dcr2/UNipped-ARNAi-6;cry*G4-16/*Pdp1* ^{3135}	28.42±0.19$^{+++\dagger\dagger\dagger}$	39.84±3.84$^{+++\dagger\dagger\dagger}$	82	79
U*dcr2/+;cry*G4-16/+	25.59±0.15	94.36±6.21	100	56
U*Nipped-ARNAi-6/+*	23.68±0.04	108.01±6.81	100	40
Pdp1 ^{3135}**/+**	23.79±0.05	66.38±5.21	90	61
U*Nipped-ARNAi-6/+;Pdp1* ^{3135}**/+**	23.75±0.05	79.95±4.85	97	46

One-way ANOVA, *P*<0.001, Tukey's multiple comparison test, ****P*<0.001 when compared with G4 control, $^{\#\#\#}$*P*<0.001 when compared with UAS control, $^{+++}$*P*<0.001 when compared with *pdf*G4-U*dcr2/UNipped-ARNAi-4/+* or U*dcr2/UNipped-ARNAi-6*;*cry*G4-16/+, †††*P*<0.001 when compared with *pdf*G4-U*dcr2/UPdp1ε* or U*dcr2/+;cry*G4-16/*Pdp1*^{3135}. G4, GAL4; U, UAS.

Appendix Table S6. Genetic interaction between *Nipped-A* and *per*

Genotype	Period(hr)±SEM	Power±SEM	%Rhythmic	N
pdf*G4-U*dcr2/UNipped-ARNAi-6	25.78±0.13$^{***\#\#\#}$	48.62±5.11$^{***\#\#\#}$	82	61
pdf*G4-U*dcr2/+;Uper/+	23.83±0.21***	4.92±1.68$^{***\#\#\#}$	13	46
pdf*G4-U*dcr2/UNipped-ARNAi-6;Uper/+	23.73±0.11^{+++}	11.65±2.42^{+++}	31	60
pdf*G4-U*dcr2/+	24.54±0.04	73.79±5.41	89	66
U*Nipped-ARNAi-6/+*	23.68±0.04	108.01±6.81	100	40
U*per/+*	23.74±0.05	61.82±5.74	95	46
U*Nipped-ARNAi-6/+;Uper/+*	23.71±0.04	79.63±4.92	97	46
U*dcr2/UNipped-ARNAi-6;cry*G4-16/+	26.53±0.08$^{***\#\#\#}$	102.50±6.67	95	68
***per^{L};Udcr2/+;cry*G4-16/+**	30.59±0.19$^{***\#\#\#}$	83.51±7.64	93	44
***per^{L};Udcr2/UNipped-ARNAi-6;cry*G4-16/+**	32.93±0.16$^{+++\dagger\dagger\dagger}$	68.49±5.85^{+++}	85	71
per^{L}	29.16±0.06	72.48±5.72	91	45
per^{L};UNipped-ARNAi-6/+	29.38±0.07	94.60±7.69	97	40
U*dcr2/+;cry*G4-16/+	25.58±0.14	94.36±6.21	100	56
U*Nipped-ARNAi-6/+*	23.68±0.04	108.00±6.80	100	40

One-way ANOVA, $P<0.001$. Tukey's multiple comparison test, $^{***}P<0.001$ when compared with G4 control, $^{\#\#\#}P<0.001$ when compared with UAS control, $^{+++}P<0.001$ when compared with *pdf*G4-U*dcr2/UNipped-ARNAi-6* or U*dcr2/UNipped-ARNAi-6;cry*G4-16/+, $^{\dagger\dagger\dagger}P<0.001$ when compared with *per^{L};Udcr2/+;cry*G4-16/+. G4, GAL4; U, UAS.

Appendix Table S7. Genetic interaction between *Nipped-A* and *clk*

Genotype	Period(hr)±SEM	Power±SEM	%Rhythmic	N
***pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-4*/+**	25.78±0.07$^{***\#\#\#}$	73.08±6.77$^{\#\#\#}$	85	49
***pdf*G4-U*dcr2*/+;U*clk*/+**	22.83±0.06$^{***\#\#\#}$	61.21±6.06	92	57
pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-4*/U*clk	23.86±0.06$^{+++\dagger\dagger\dagger}$	74.12±5.88	91	61
***pdf*G4-U*dcr2*/+**	24.31±0.04	84.29±7.29	87	47
U*Nipped-ARNAi-4*/+	23.88±0.05	114.65±5.63	98	47
U*clk*/+	23.80±0.05	73.08±5.46	96	32
U*Nipped-ARNAi-4*/U*clk*	23.97±0.03	130.51±4.47	100	62

One-way ANOVA, $P<0.001$, Tukey's multiple comparison test, $^{***}P<0.001$ when compared with G4 control, $^{\#\#\#}P<0.001$ when compared with UAS control, $^{+++}P<0.001$ when compared with *pdfG4-Udcr2/+;UNipped-ARNAi-4/+*, $^{\dagger\dagger\dagger}P<0.001$ when compared with *pdf*G4-U*dcr2*/+;U*clk*/+. G4, GAL4; U, UAS.

Appendix Table S8. Knocking down *not* reduces the power of locomotor rhythm

Genotype	Period(hr)±SEM	Power±SEM	%Rhythmic	N
U*notRNAi-1*/+	23.37±0.03	54.44±5.31	78	89
U*notRNAi-2*/+	23.54±0.05	115.44±6.31	100	47
***tim*G4/+;U*dcr2*/+**	24.02±0.04	77.33±3.45	97	134
U*dcr2*/+;*cry*G4-16/+	25.37±0.04	126.94±3.38	100	183
***pdf*G4-U*dcr2*/+**	24.36±0.02	104.17±3.81	96	123
tim*G4/+;U*dcr2/UnotRNAi-1	N/A	1.77±0.50$^{***###}$	0	94
***tim*G4/*UnotRNAi-2*;U*dcr2*/+**	Lethal	Lethal	Lethal	0
U*dcr2*/+;*cry*G4-16//*UnotRNAi-1*	26.16±0.09$^{***###}$	16.51±1.82$^{***###}$	42	150
U*dcr2/UnotRNAi-2;cry*G4-16/+	23.72±0.2***	20.82±4.41$^{***###}$	53	30
***pdf*G4-U*dcr2*/+;*UnotRNAi-1*/+**	24.74±0.08$^{***###}$	33.04±3.05$^{***##}$	66	112
pdf*G4-U*dcr2/UnotRNAi-2	24.44±0.07$^{###}$	102.35±9.01	94	35

One-way ANOVA, $P<0.001$, Tukey's multiple comparison test, $^{***}P<0.001$ when compared with G4 and U*dcr2*, $^{##}P<0.01$, $^{###}P<0.001$ when compared with U*RNAi.* G4, GAL4; U, UAS.

Appendix Table S9. Genetic interaction between *Nipped-A* and *not*

Genotype	Period(hr)±SEM	Power±SEM	%Rhythmic	N
U*not*/+	23.58±0.04	95.70±5.88	100	46
U*Nipped-ARNAi-4*/+	23.88±0.04	119.37±5.58	98	45
*pdf*G4-U*dcr2*/+;U*not*/+	24.06±0.05$^{***\#\#\#}$	87.93±5.36	97	59
*pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-4*/+	25.66±0.07$^{***\#\#\#}$	103.14±7.01	97	35
*pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-4*/U*not*	24.82±0.03$^{+++\dagger\dagger\dagger}$	133.26±4.68$^{+\dagger\dagger\dagger}$	99	73
U*Nipped-ARNAi-6*/+;U*not*/+	23.55±0.03	118.16±9.82	97	32
*tim*G4/+;U*dcr2*/+	24.65±0.05	69.32±7.49	88	41
*tim*G4/+;U*dcr2*/U*not*	23.72±0.06***	92.48±7.63	100	39
*tim*G4/U*Nipped-ARNAi-6*;U*dcr2*/+	25.95±0.11$^{***\#\#\#}$	49.88±6.71$^{\#\#\#}$	86	37
*tim*G4/U*Nipped-ARNAi-6*;U*dcr2*/U*not*	24.91±0.10$^{+++\dagger\dagger\dagger}$	46.36±6.28†††	85	40
U*notRNAi-1*/+	23.37±0.03	54.44±5.31	78	89
U*Nipped-ARNAi-6*/+	23.64±0.05	111.55±7.61	97	39
U*Nipped-ARNAi-6*/U*notRNAi-1*	23.50±0.02	77.34±7.19	89	46
*pdf*G4-U*dcr2*/+	24.44±0.05	100.70±6.07	98	55
*pdf*G4-U*dcr2*/+;U*notRNAi-1*/+	24.74±0.08$^{\#\#\#}$	33.04±3.05$^{***\#\#}$	66	112
*pdf*G4-U*dcr2*/U*Nipped-ARNAi-6*	25.45±0.10$^{***\#\#\#}$	79.68±8.66$^{\#\#}$	91	36
*pdf*G4-U*dcr2*/U*Nipped-ARNAi-6*;U*notRNAi-1*/+	26.60±0.17$^{+++\dagger\dagger\dagger}$	22.57±3.93^{+++}	69	32

One-way ANOVA, P<0.001, Tukey's multiple comparison test, $^{***}P$<0.001 when compared with G4 control, $^{\#\#\#}P$<0.001 when compared with UAS control, ^{+++}P<0.001 when compared with *pdfG4-Udcr2/+;UNipped-ARNAi-4/+*, *tim*G4/U*Nipped-ARNAi-6*;U*dcr2*/+ or *pdf*G4-U*dcr2*/U*Nipped-ARNAi-6,* †††p<0.001 when compared with *pdf*G4-U*dcr2*/+;U*not*/+, *tim*G4/+;U*dcr2*/U*not or pdf*G4-U*dcr2*/+;U*notRNAi-1*/+. G4, GAL4; U, UAS.

Appendix Table S10. Knocking down *Sgf11* reduces the power of locomotor rhythm

Genotype	Period(hr)±SEM	Power±SEM	%Rhythmic	N
U*Sgf11RNAi-1*/+	23.47±0.03	68.56±4.00	97	69
U*Sgf11RNAi-2*/+	23.65±0.04	104.73±7.74	95	42
***tim*G4/+;U*dcr2*/+**	24.47±0.05	78.66±7.36	100	38
U*dcr2*/+;*cry*G4-16/+	25.36±0.09	121.32±6.21	98	43
***pdf*G4-U*dcr2*/+**	24.50±0.04	116.80±7.84	97	32
***tim*G4/U*Sgf11RNAi-1*;U*dcr2*/+**	24.86±0.18$^{\#\#\#}$	6.33±1.33$^{***\#\#\#}$	18	79
***tim*G4/U*Sgf11RNAi-2*;U*dcr2*/+**	24.81±0.06$^{*\#\#\#}$	44.44±5.60$^{**\#\#\#}$	70	61
U*dcr2*/U*Sgf11RNAi-1*;*cry*G4-16/+	25.21±0.07$^{\#\#\#}$	78.48±6.33***	91	46
U*dcr2*/U*Sgf11RNAi-2*;*cry*G4-16/+	25.29±0.14$^{\#\#\#}$	110.66±9.90	97	30
pdf*G4-U*dcr2*/U*Sgf11RNAi-1	24.61±0.17$^{\#\#\#}$	35.16±4.44$^{***\#\#}$	77	30
pdf*G4-U*dcr2*/U*Sgf11RNAi-2	24.55±0.08$^{\#\#\#}$	94.83±9.26	97	30

One-way ANOVA, P<0.001, Tukey's multiple comparison test, $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 when compared with G4 and U*dcr*2; $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 when compared with U*RNAi.* G4, GAL4; U, UAS.

Appendix Table S11. Genetic interaction between *Nipped-A* and *Sgf11*

Genotype	Period(hr)±SEM	Power±SEM	%Rhythmic	N
U*Sgf11RNAi-1*/+	23.47±0.03	68.56±4.00	97	69
U*Nipped-ARNAi-4*/+	23.80±0.05	103.70±7.75	97	34
U*Sgf11RNAi-1*/+;U*Nipped-ARNAi-4*/+	23.68±0.06	96.65±6.71	98	42
*pdf*G4-U*dcr2*/+	24.38±0.05	100.63±6.15	98	45
*pdf*G4-U*dcr2*/U*Sgf11RNAi-1*	24.61±0.17$^{\#\#\#}$	35.16±4.44$^{***\#\#}$	77	30
*pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-4*/+	25.62±0.0.08$^{***\#\#\#}$	93.19±6.15	96	69
*pdf*G4-U*dcr2*/U*Sgf11RNAi-1*;U*Nipped-ARNAi-4*/+	26.59±0.10$^{+++\dagger\dagger\dagger}$	36.54±4.23^{+++}	76	42
Sgf11^{e01308}/+	23.73±0.05	93.68±8.43	97	36
U*Nipped-ARNAi-4*/*Sgf11*^{e01308}	24.10±0.08	127.98±9.89	93	27
*pdf*G4-U*dcr2*/+;*Sgf11*^{e01308}/+	24.69±0.05$^{*\#\#\#}$	130.40±8.02$^{*\#\#}$	100	35
*pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-4*/*Sgf11*^{e01308}	26.58±0.07$^{+++\dagger\dagger\dagger}$	80.19±4.43†††	96	68

One-way ANOVA, P<0.001, Tukey's multiple comparison test, $^{***}P$<0.001 when compared with G4 and U*dcr*2, $^{\#\#\#}P$<0.001 when compared with U*Sgf11RNAi-1*/+ or *Sgf11*^{e01308}/+, ^{+++}P<0.001 when compared with *pdf*G4-U*dcr2*/+;U*Nipped-ARNAi-4*/+, $^{\dagger\dagger\dagger}P$<0.001 when compared with *pdf*G4-U*dcr2*/U*Sgf11RNAi-1 or pdf*G4-U*dcr2*/+;*Sgf11*^{e01308}/+. G4, GAL4; U, UAS.

Appendix Table S12. Oligonucleotides used in this study

Genes	Sense (5'-3')	Antisense (5'-3')	Source
si-Nipped-A	GCGUGUAAAUUGCCCAUAATT	UUAUGGGCAAUUUACACGCTT	This paper
Nipped-A	CGCGAAAAACATAAAACTGC	GGAATGTTTCCGTGACTTGC	This paper
tim	CTGGGGAGTGACCATGG	GCTGGAATCGCCACTG	This paper
per	CAGCAGCAGCCTAATCG	GAGTCGGACACCTTGG	This paper
clk	TACTGCGTGAGGATATCG	GTTGTTGTTCTGGTTGC	This paper
cyc	GAGGTCTTCGTCGGAAAGG	AAAGCACATGGGAATCATGG	This paper
Pdp1ε	GAACCCAAGTGTAAAGACAATGCG	CTGGAAATACTGCGACAATGTGG	This paper
vri	TGTTTTTTGCCGCTTCGGTCA	TTACGACACCAAACGATCGA	This paper
cry	TGCAGGTACCAAGAATGTGG	GGAAGATATAAGCCGGTTCG	This paper
not	CCACTTGCTTCATGAACTGC	ACATTTGTGCGATGACTTGC	This paper
sgf11	TGCACTCTTCGGGAAATACC	TTTGCCATTGTTCTTCTTGG	This paper
actin	CTAACCTCGCCCTCTCCTCT	GCAGCCAAGTGTGAGTGTGT	This paper
rp49	TACAGGCCCAAGATCGTGAA	GCACTCTGTTGTCGATACCC	This paper
pre-tim	ACTGCTTTCCAATGCGGTATG	AAGCCTCCGAAAAACATATGAAAA	(Andreazza, Bouleau et al., 2015)
pre-pdp1	GGCCGAGTCAAGTGAGTATAGC	CATGTGATCATGGTTTGTTTTACG	This paper
cbp20	AGATCCACGAGCTCTTCTCC	CCGATCGGACATAGTACTCC	This paper
tim-E box	ACACTGACCGAAACACCCACTC	GCGGCACGTTGTGATTACACG	(Zhou, Yu et al., 2016)
tim-Tss	TGCTATTGAAGTTGCATTTTACATAAGCCAACAACG	TCGTGTTATAGTTTCAGTGCTGTTATATACACTGATG	This paper
timbody	GAGGAGCTGGCCCTTATACC	ATCGAGTTGCAGTGCTTCG	This paper
Ppd1ε E-box	GCACTCTCATTCTCTCTGTCG	ACGCGGATTTCAATATGACC	(Zhou et al., 2016)
Ppd1ε TSS	TTTCAGTTACGCGTTGTTGC	ACCTCGCACGGATAAGTCC	This paper
Ppd1ε body	GCAACTGGTAATGGAAATGGTG	CTGTTCAAATGGTTGTGATGCTC	This paper

Appendix Table S13. Antibodies used in this study

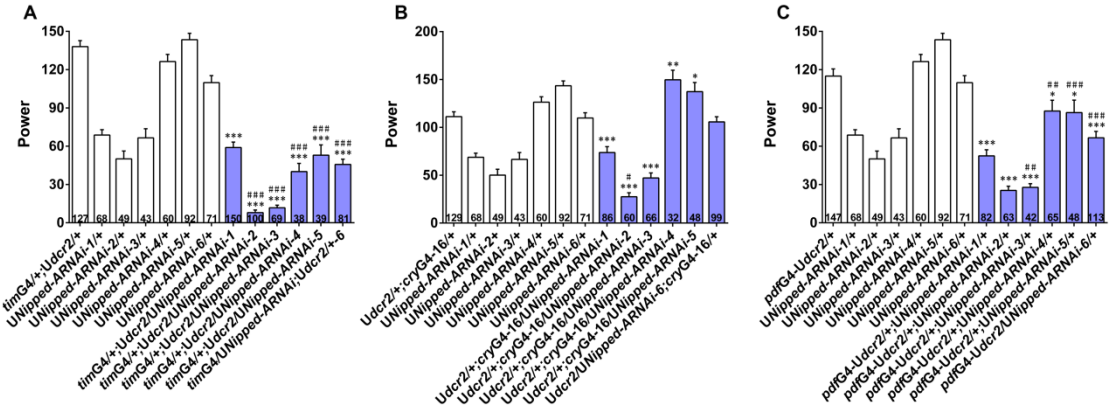
Antibodies	Source	Identifier
Rat anti-TIM	Joanna C. Chiu, University of California, Davis	N/A
Guinea pig anti-PER	Joanna C. Chiu, University of California, Davis	N/A
Guinea pig anti-PDP1ε	Paul E. Hardin, Texas A&M University	N/A
Rabbit anti-Nipped-A	Sigma	Cat#PLA0167, RRID:AB_2209668
Mouse anti-HSP70	Sigma	Cat# SAB4200714
Rabbit anti-acetyl-Histone H3	Millipore	Cat#06-599,RRID: AB_2115283
Rabbit anti-Histone H3 (acetyl K9)	Abcam	Cat#ab4441, RRID:AB_2118292
Rabbit anti-acetyl-Histone H3 (Lys14)	Millipore	Cat#07-353, RRID:AB_310545
Rabbit anti-HistoneH3 (acetyl K27)	Abcam	Cat#ab4729, RRID:AB_2118291
Mouse anti-Ubquityl-Histone H2B, clone56	Millipore	Cat#05-1312-I,RRID:AB_1587119
Rabbit anti-acetyl-Histone H4	Millipore	Cat#06-866,RRID:AB_310270
Rabbit anti-acetyl-Histone H4 (Lys16)	Millipore	Cat#07-329, RRID:AB_310525
Normal mouse IgG	Santa Cruz Biotechnology	Cat#sc-2025, RRID:AB_737182
Normal rabbit IgG	Santa Cruz Biotechnology	Cat#sc-2027, RRID:AB_737197
Donkey anti-mouse Alexa Fluor 488 nm	Molecular Probes	Cat#R37114, RRID:AB_2556542
Donkey anti-mouse Alexa Fluor 594 nm	Molecular Probes	Cat#R37115,RRID:AB_2556543
Donkey anti-rabbit Alexa Fluor 488 nm	Abcam	Cat#ab150073,RRID:AB_2636877
Donkey anti-rat Alexa Fluor 488 nm	Abcam	Cat#ab150153,RRID:AB_2737355
Donkey anti-Guinea pig Alexa Fluor 594 nm	Jackson ImmunoResearch Labs	Cat#706-585-148,RRID:AB_2340474

Supplemental References

Andreazza S, Bouleau S, Martin B, Lamouroux A, Ponien P, Papin C, Chelot E, Jacquet E, Rouyer F (2015) Daytime CLOCK Dephosphorylation Is Controlled by STRIPAK Complexes in Drosophila. Cell Rep 11: 1266-79

Zhou J, Yu W, Hardin PE (2016) CLOCKWORK ORANGE Enhances PERIOD Mediated Rhythms in Transcriptional Repression by Antagonizing E-box Binding by CLOCK-CYCLE. PLoS Genet 12: e1006430

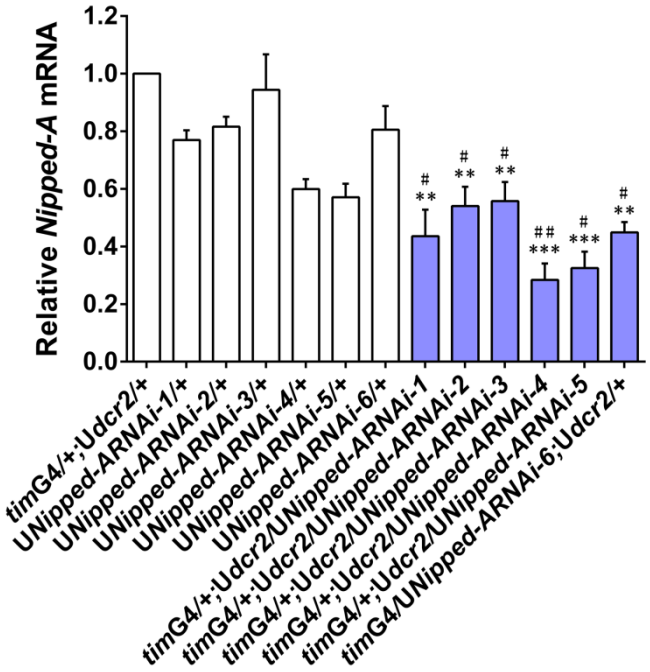
Supplemental Figures



Appendix Figure S1. Knocking down *Nipped-A* reduces the power of locomotor rhythms.

A-C The power of DD locomotor rhythms of flies with *Nipped-A* knocked down and controls.

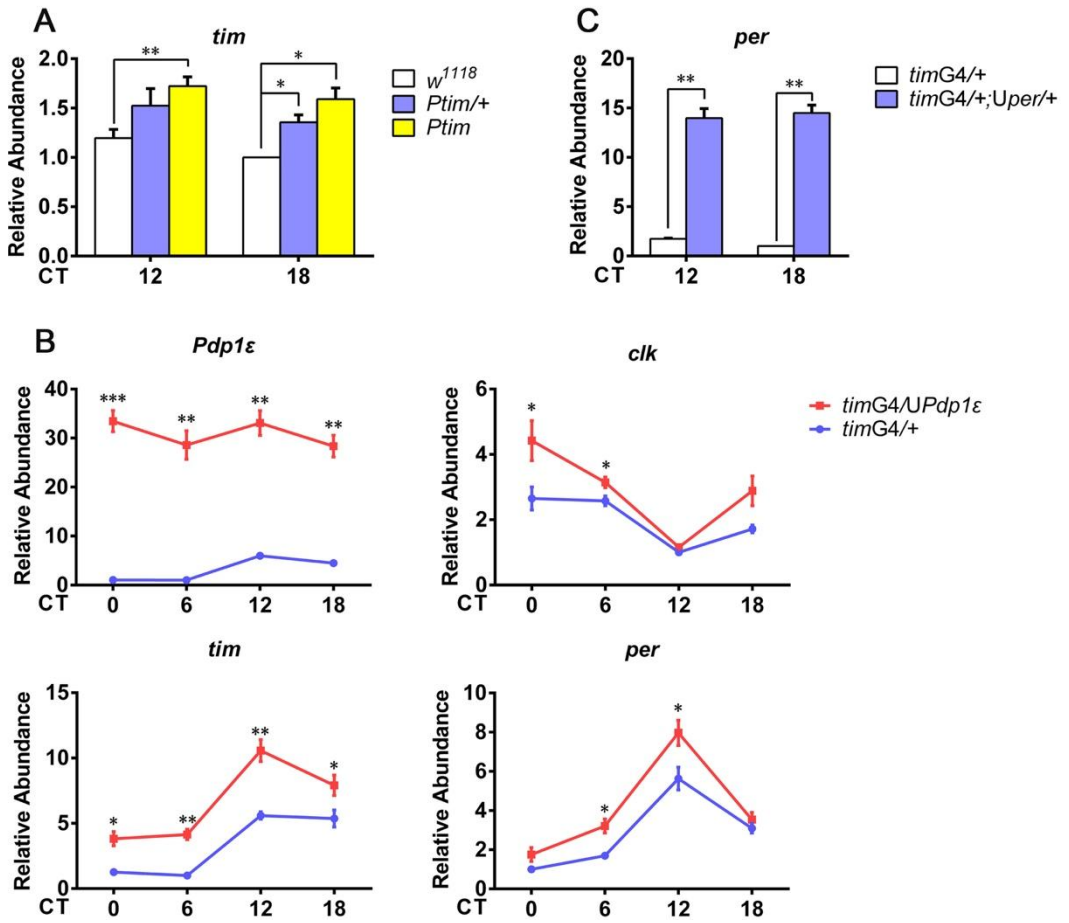
Data information: Digits on the bars are the number of flies tested. Statistical difference is measured using one-way ANOVA, $P<0.001$, Tukey's multiple comparison test, $^{*/\#}P<0.05$, $^{**/\#\#}P<0.01$, $^{***/\#\#\#}P<0.001$. Error bars represent SEM. ∗ compared with the GAL4 controls; # compared with the UAS controls. G4, GAL4; U, UAS.



Appendix Figure S2. Validation of the knock down effects of *Nipped-A* RNAi flies.

Plots of relative mRNA abundance of *Nipped-A* in whole head extracts of flies with *Nipped-A* knocked down and controls determined by qRT-PCR ($n \geq 3$).

Data information: Student's *t* test, $^{*/\#}P<0.05$, $^{**/\#\#}P<0.01$, $^{***}P<0.001$. Error bars represent SEM. ∗ compared with the GAL4 controls; # compared with the UAS controls. G4, GAL4; U, UAS.



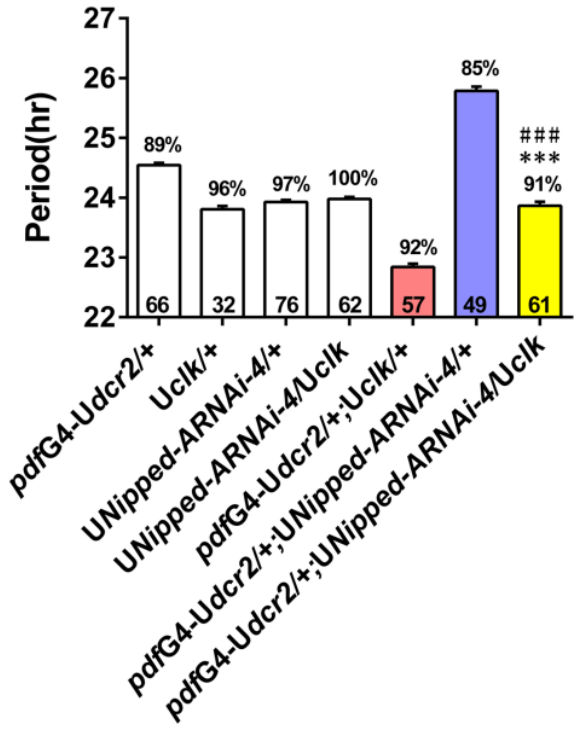
Appendix Figure S3. Validation of the over-expression effects of *tim*, *Pdp1ε* and *per*.

A Plots of relative mRNA abundance of *tim* in whole head extracts of w^{1118}, *Ptim/+ and Ptim* flies determined by qRT-PCR (*n*=4).

B Plots of relative mRNA abundance of *Pdp1ε, clk, tim and per* in whole head extracts of flies with *Pdp1ε* over expression and controls determined by qRT-PCR (*n*=4).

C Plots of relative mRNA abundance of *per* in whole head extracts of flies with *per* over expression and controls determined by qRT-PCR (*n*=3).

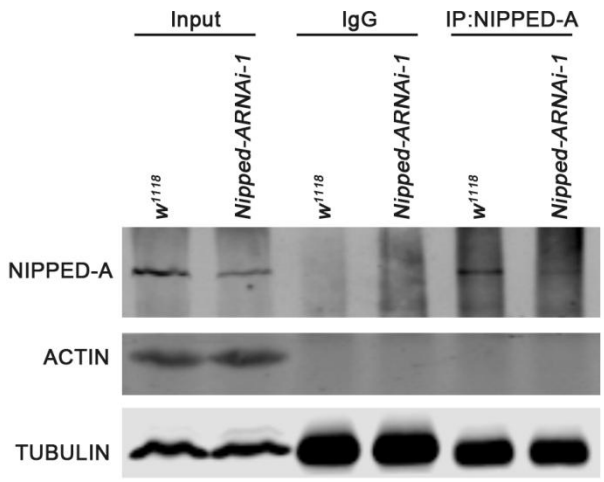
Data information: Student's *t* test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Error bars represent SEM. ∗ compared with *tim*G4/+ or w^{1118} *control.*



Appendix Figure S4. *Nipped-A* does not appear to genetically interact with *clk*.

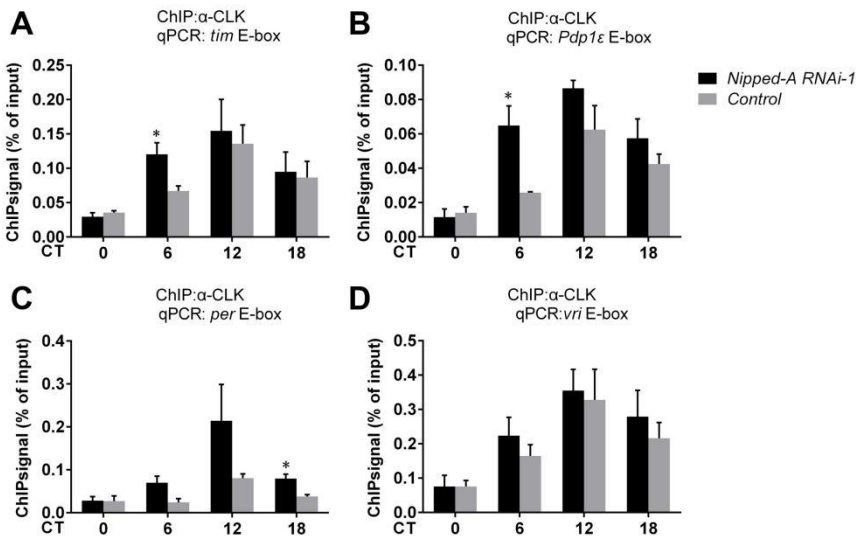
The period of DD locomotor rhythm of *Nipped-A* RNAi flies over-expressing *clk.*

Data information: Digits on the bars are the number of flies tested. Percentage of rhythmicity is indicated above the bars. Statistical difference is measured using one-way ANOVA, $P<0.001$, Tukey's multiple comparison test, $^{***/\#\#\#}P<0.001$, ∗ compared with the *Nipped-A* RNAi flies, # compared with the over-expression flies. Error bars represent SEM. G4, GAL4; U, UAS.



Appendix Figure S5. Validating the specificity of the NIPPED-A antibody.

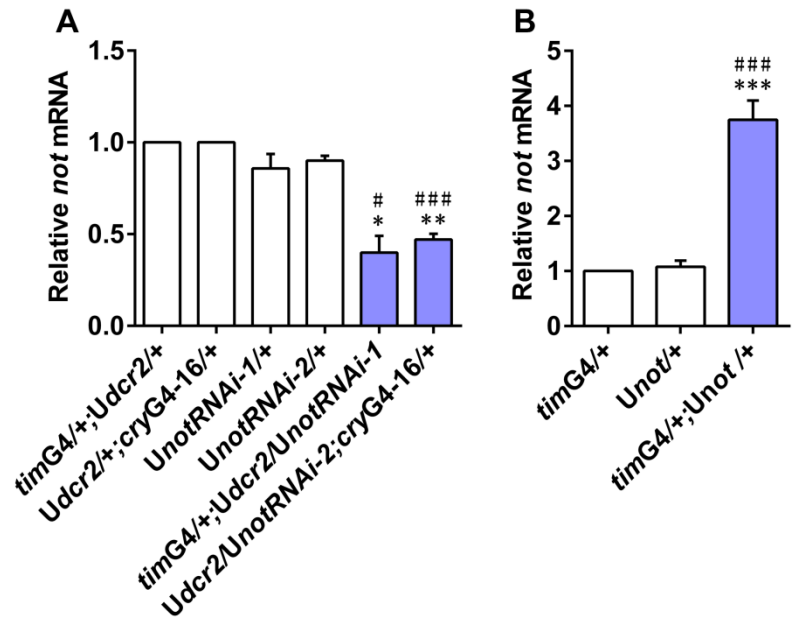
NIPPED-A was immunoprecipitated from whole head extracts of *Nipped-A* RNAi (*tim*G4/+;U*dcr2/UNipped-A RNAi-1*) and control (w^{1118}) flies collected at CT12 and detected in the protein input control and immunoprecipitates by Western blotting.



Appendix Figure S6. Knocking down Nipped-A enhances CLK binding at *Pdp1ε* and *per* promoters.

A-D ChIP assays to detect CLK binding at E-box elements in *tim* (A), *Pdp1ε* (B), *per* (C) and *vri* (D) promoters of *Nipped-A RNAi* (*tim*G4/+;U*dcr2/UNipped-ARNAi-1*) and control (*tim*G4/+;U*dcr2*/+) flies.

Data information: Error bars represent SEM (n=3). Statistical difference for CLK binding at *tim*, *Pdp1ε* and *per* promoters throughout the time course is measured using one-way ANOVA, $P<0.05$. Significant effect of genotype is found for *Pdp1ε* ($P<0.01$) and *per* ($P<0.05$), using two-way ANOVA. Student's *t* test, $^{*}P<0.05$.



Appendix Figure S7. Validation of the knock down or over-expression effects of *not* RNAi or over-expression fly lines.

A, B Plots of relative mRNA abundance of *not* in whole head extracts of flies with *not* knocked down (A) or over-expressed (B) and controls determined by qRT-PCR (n=3). Data information: Student's t-test, $^{*/\#}P<0.05$, $^{**/\#\#}P<0.01$, $^{***/\#\#\#}P<0.001$. Error bars represent SEM. ∗ compared with the GAL4 controls; # compared with the UAS controls. G4, GAL4; U, UAS.