Expanded View Figures

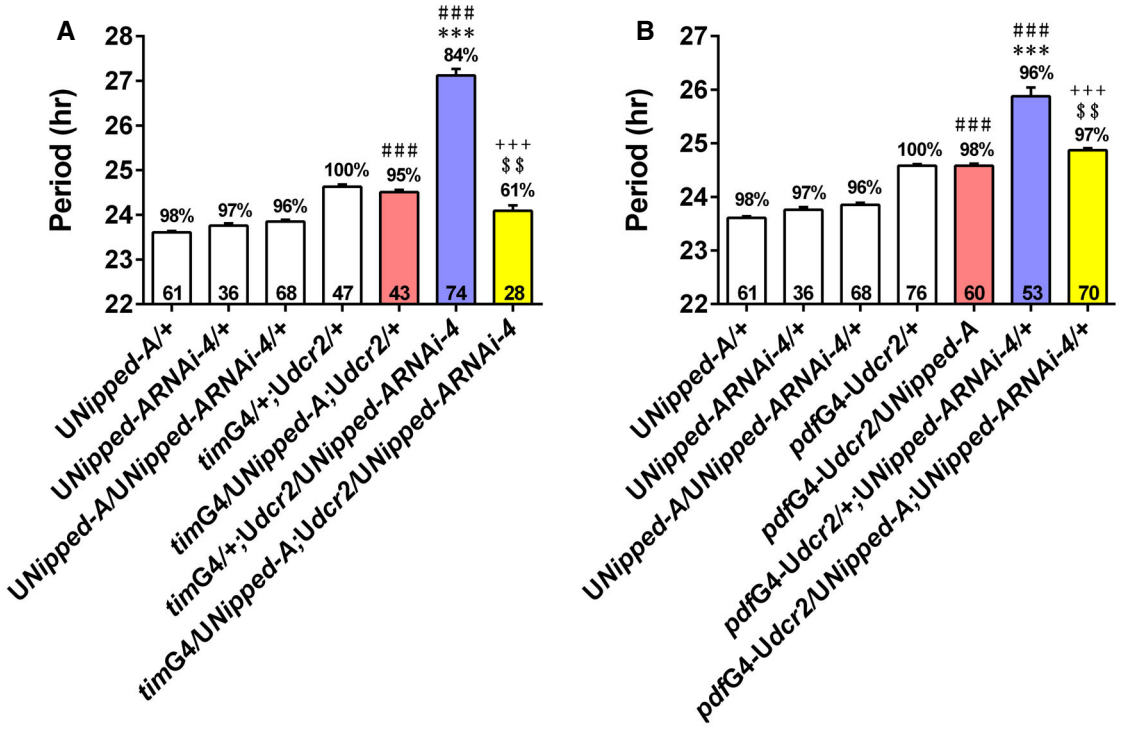

Figure EV1. Over-expressing *Nipped-A* rescues the long period induced by knocking down *Nipped-A*.

A, B The period of DD locomotor rhythm of *Nipped-A* RNAi flies when over-expressing *Nipped-A* using *tim*G4 (A) and *pdf*G4(B).Error bars represent SEM. Digits on the bar 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, $^{\$\$}P < 0.01$, $^{***/\#\#\#/+++}P < 0.001$, * compared with the G4 control; # compared with the UAS control, + compared with the *Nipped-A* RNAi flies, $ compared with the over-expression flies. White bar indicates UAS or GAL4 controls. Red bar indicates *Nipped-A* over-expressing flies. Blue bar indicates flies with *Nipped-A* knocked down. Yellow bar indicates flies with *Nipped-A* over-expressing and knocked down at the same time. G4, GAL4; U, UAS.

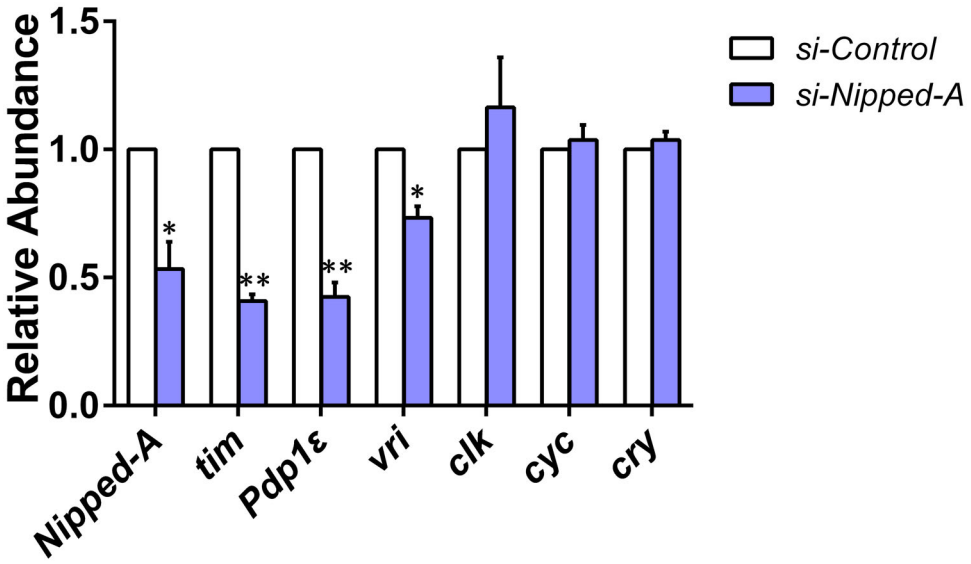

Figure EV2. Knocking down *Nipped-A* decreases *tim*, *Pdp1ε*, and *vri* mRNA levels in cultured cells.

Relative mRNA abundance of clock genes determined by qRT–PCR in S2 cells transfected with small interfering RNA targeting *Nipped-A* (si-*Nipped-A*; $n = 3$). Error bars represent SEM. Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$.

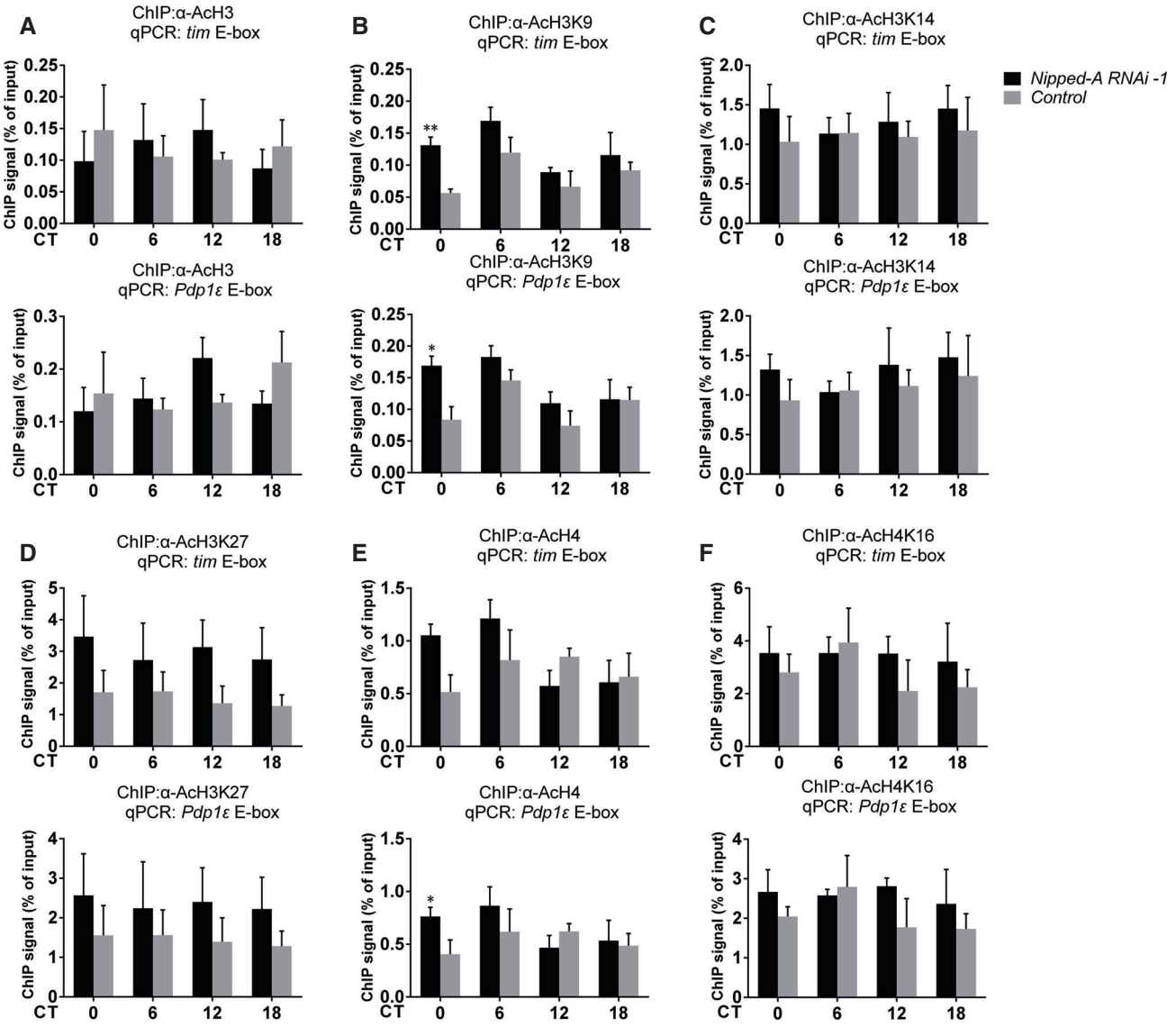

Figure EV3. Knocking down *Nipped-A* increases acetyl-H3K9 and acetyl-H3K27 at the promoters of *tim* and *Pdp1ε*.

A ChIP assays to detect acetyl-H3 binding at E-box elements in *tim* and *Pdp1ε* promoters of *Nipped-A* RNAi (*tim*G4/+;U*dcr2*/U*Nipped-ARNAi-1*) and control (*tim*G4/+; U*dcr2*/+) flies (n = 3).

B ChIP assays to detect acetyl-H3K9 binding at E-box elements in *tim* and *Pdp1ε* promoters of *Nipped-A* RNAi (*tim*G4/+;U*dcr2*/U*Nipped-ARNAi-1*) and control (*tim*G4/+; U*dcr2*/+) flies (n = 3).

C ChIP assays to detect acetyl-H3K14 binding at E-box elements in *tim* and *Pdp1ε* promoters of *Nipped-A* RNAi and control flies (n = 4).

D ChIP assays to detect acetyl-H3K27 binding at E-box elements in *tim* and *Pdp1ε* promoters of *Nipped-A* RNAi and control flies (n = 4).

E ChIP assays to detect acetyl-H4 binding at E-box elements in *tim* and *Pdp1ε* promoters of *Nipped-A* RNAi and control flies (n = 4).

F ChIP assays to detect acetyl-H4K16 binding at E-box elements in *tim* and *Pdp1ε* promoters of *Nipped-A* RNAi and control flies (n = 3).

Data information: Error bars represent SEM. Two-way ANOVA and significant effect of genotypes were found for *tim* E-box ($P < 0.01$) and *Pdp1ε* E-box ($P < 0.05$) (B), as well as for *tim* E-box ($P < 0.05$) (D). Student's t-test, $^*P < 0.05$, $^{**}P < 0.01$.

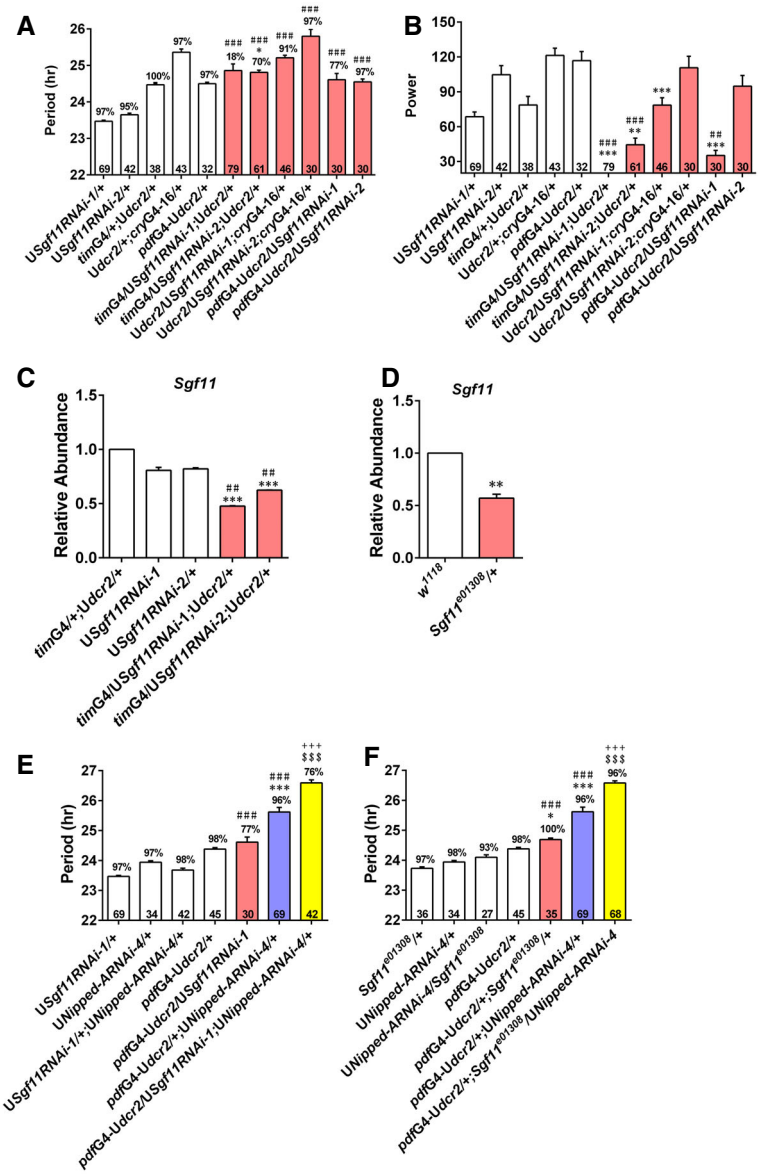

Figure EV4. *Nipped-A* synergistically interacts with *Sgf11* to determine period length.

A, B The period (A) and power (B) of DD locomotor rhythms of flies with *Sgf11* knocked down and controls.

C, D Plots of relative mRNA abundance for *Sgf11* determined by qRT–PCR in whole-head extracts of *Sgf11* RNAi (C) (*sgf11RNAi-1*: $n = 5$; *sgf11RNAi-2*: $n = 3$) and $Sgf11^{e01308}$ (D) flies ($n = 3$).

E The period of DD locomotor rhythm of flies when knocking down *Nipped-A* and *Sgf11*.

F The period of DD locomotor rhythm of *Sgf11* mutant flies with *Nipped-A* knocked down.

Data information: Error bars represent SEM. (A, B, E, F) 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, $^{*}P < 0.05$, $^{**/\#\#}P < 0.01$, $^{***/\#\#\#/+++/\$\$\$}P < 0.001$, * compared with the G4 control, # compared with the UAS control, + compared with the *Nipped-A* RNAi flies, $ compared with the *Sgf11* RNAi or $Sgf11^{e01308/+}$ flies. (C, D) Student's *t*-test, $^{**/\#\#}P < 0.01$, $^{***}P < 0.001$. White bar indicates UAS or GAL4 controls. Red bar indicates flies deficient for *Sgf11*. Blue bar indicates flies with *Nipped-A* knocked down. Yellow bar indicates flies with *Nipped-A* knocked down and deficient for *Sgf11* at the same time. G4, GAL4; U, UAS.