# Supporting Information

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## SI Text

**Prediction of the Range of Entrainment by Various Temperature Cycles.** Based on the non-parametric entrainment model, we analyzed whether the oscillator of *in vitro* KaiC phosphorylation rhythm can be entrained by temperature cycles with arbitrary length of high/low temperature period. We assumed an oscillator with free-running period $\tau$ [hour] is subjected to temperature cycles of A [hour] at high temperature and B [hour] at low temperature. Based on the data shown in Fig. 2*A*, we assumed that the period of the oscillator are identical under various ambient temperatures and that phase shifts caused by temperature shift complete rapidly. In Fig. 2, we obtained phase shifts $f(\phi)$ and $g(\phi)$ for step-up and step-down, respectively. If the first step-up is given at phase $\phi$, the oscillator promptly jumps to new phase $\phi+f(\phi)$ and, after A hours, the second phase shift by step-down $g(\phi+f(\phi)+24A/\tau)$ is induced. Thus, after B hours, when the first temperature cycle is complete, the oscillator phase $\phi'$ should be described as

$$\phi' = \phi + f(\phi) + 24A/\tau + g(\phi + f(\phi) + 24A/\tau) + 24B/\tau. \quad \textbf{[S1]}$$

When the oscillator is entrained by the temperature cycle, the oscillator should run one complete cycle (24 in CT) during one temperature cycle.

$$\phi' - \phi = 24 \quad \textbf{[S2]}$$

By substituting Eq. **S1** into **S2**, we obtain Eq. **2** shown in the Results section,

$$\tau - (A + B) = (f(\phi) + g(\phi + f(\phi) + 24A/\tau))(\tau/24).$$

This equation can be rewritten as

$$\Delta(\phi) = f(\phi) + g(\phi + f(\phi_s) + 24A/\tau) - 24(\tau - A - B)/\tau = 0. \quad \textbf{[S3]}$$

To analyze entrainment for any given temperature cycle (A hour at high-temperature and B hour at low-temperature), we determined whether any $\phi$ (0–24 in CT) would satisfy Eq. **S3**. Based on $f(\phi)$ and $g(\phi)$, we computed $\Delta(\phi)$ for every value (0–24) of $\phi$ and plotted $\Delta(\phi)$ versus $\phi$. It should be noted that $\Delta$ (0) and $\Delta$ (24) should be the same value, because $\Delta$ should also cycle in 24 hours of CT. Thus, as shown in Fig. S5, the profile of $\Delta(\phi)$ can take on several patterns. For some temperature cycles, $\Delta(\phi)$ remains constitutively positive or negative for all values of $\phi$ (upper profile). For these temperature cycles, no stable entrainment is expected. For another group of temperature cycles, $\Delta(\phi)$ could be both negative and positive values (middle profile). In this case, as shown in the profile, Eq. **S3** can be satisfied by two $\phi$. Stable entrainment is attained by $\phi_s$ which satisfies $d\Delta(\phi_s)/d\phi < 0$ (closed points) but is not attained by $\phi_u$ which satisfies $d\Delta(\phi_u)/d\phi \geq 0$ (open points). Because, in the former case, even if the $\phi$ is displaced a small distance from $\phi_s$, the response to repeated cycles force $\phi$ to $\phi_s$. In contrast, in the latter case, the phase shifts by temperature shift move $\phi$ to further away from $\phi_u$. As repeating this process would result in an unstable equilibrium, stable entrainment is not permitted for this type of $\phi$. As shown in Fig. S5*C* (bottom profile), a third case of bi-stable entrainment was numerically observed. However, we excluded these cases, because the entrainments we observed in this study were those having unique phase angle between the oscillator and temperature cycles.

**Prediction for Peak Time of Entrained Oscillation Under Various Temperature Cycles.** We analyzed the peak phase of the oscillation in temperature cycle that permitted stable entrainment, using $\phi_s$ as defined above. For calculating the phase progress of the oscillation, transition time of phase shifting is apparently important because the phosphorylation state of KaiC did not change discontinuously, as mentioned in the Discussion. To discuss phase progress of the oscillator, we hypothesized that it took 4 hours to complete phase shifting after the stimuli (the slowest case). As shown by solid line in Fig. S6, from the onset of high-temperature period (hour 0 on abscissa), the phase proceeds from $\phi_s$ with angular velocity $\omega$ plus phase shift accelerant (assuming the phase shift is completed in 4 hours), followed by progress with $\omega$ until the step-down at A hour. At the low temperature (A to A+B hour), the phase proceeds in a similar fashion to resume $\phi_s$ at hour A+B. Based on this process, we could predict the time of the peak in the temperature cycle ($T_p$) as the time when $\phi$ [CT] is 16. Note that we could hypothesize acute phase shifting (red dashed line) to predict the PRC of temperature pulse and this assumption successively predict the actual entrainment. Even if the transition is assumed to take for 4 hours, the results of the prediction remains unchanged because we only discuss experiments of pulse duration longer than 4 hours. In the other words, in either case, the phase of the oscillator should be identical at times 4 hours after the step-up/step-down stimuli.

## Supplemental Methods

**Estimation of the Period and Peak Phase of the *In Vitro* Oscillation.** To estimate period length ($T_p$) and phase of the peak ($\phi_p$) of the KaiC phosphorylation rhythm, time courses of the phosphorylation ratio (Y) of each experiment were fitted to the cosine function of incubation time (t) by nonlinear least-squares method (G-Newton algorithm using open-source statistical software R, version 2.2.0).

$$Y(t) = a^* \cos(2\pi(t - \phi_p)/T_p) + b$$

where a and b represent the amplitude of the rhythm and non-rhythmic component, respectively. Note that the peak of the mixture repeated to occur with regular interval after the second cycle (Fig. 2). Thus, we did not include data for the first 12 hours of incubation for curve-fitting analyses.

**Phase Shift by Temperature Steps and Temperature Pulses.** To examine the effects of temperature step on phase of the KaiC phosphorylation rhythm, the peak positions of temperature-treated rhythms and control were compared after fitting the rhythm data to cosine curves (data points within 2 hours after the step were excluded from the fitting) and normalizing the period difference of each sample. Phase differences of the treated sample were considered as phase shifts. Normalized phase and phase shifts were represented on a circadian time scale (CT) with the peak of the KaiC phosphorylation rhythm set to CT 16. The shifts were then plotted against the phase of the temperature steps. We constructed PRC for temperature step-up by smoothing-spline interpolation for periodic function (smoothing parameter = 0.6). Phase shifting by temperature pulses was examined by administering 4-hour pulses at 45°C and phase shifts were estimated using the same procedure as for temperature step phase shifts.

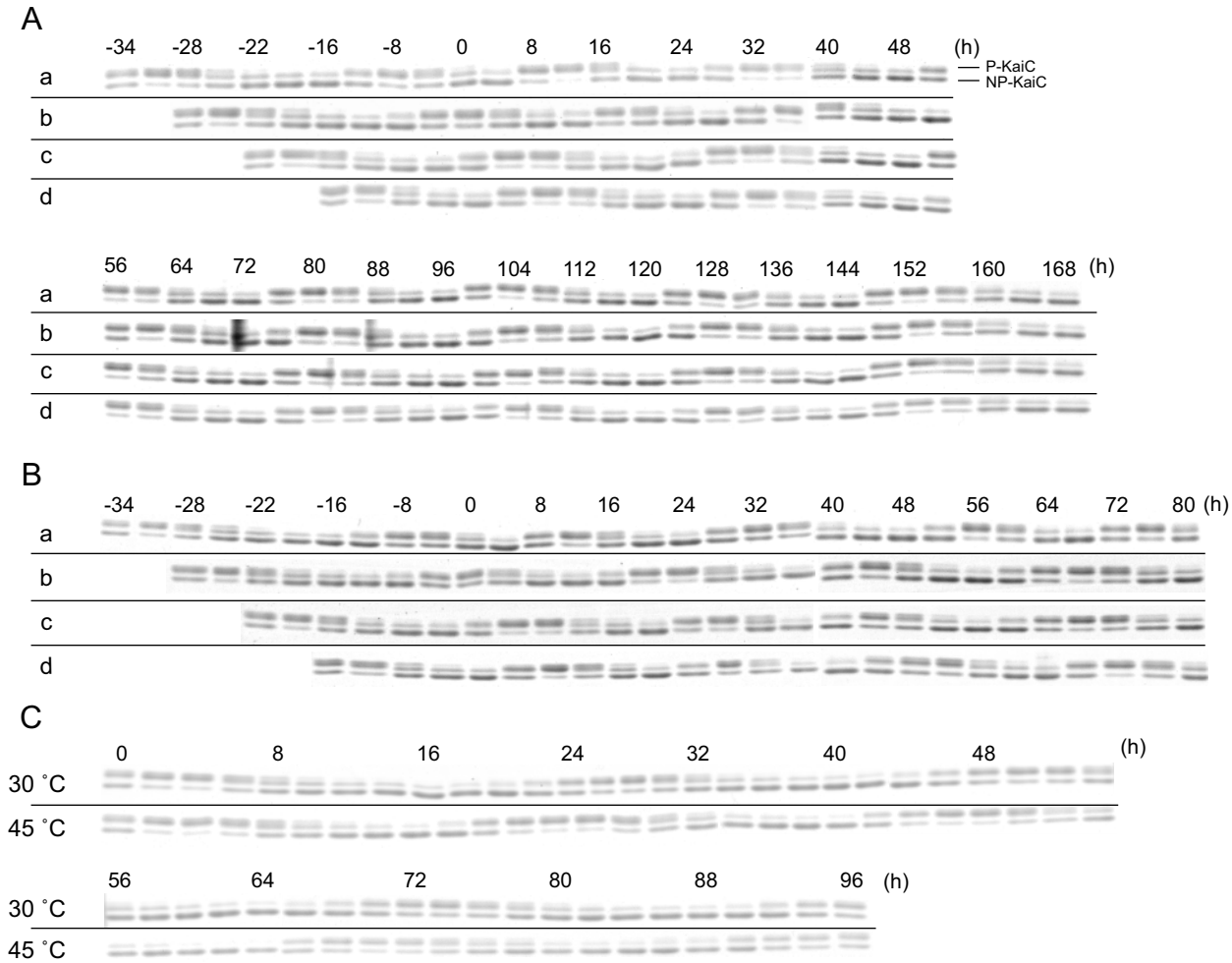


**Fig. S1.** KaiC phosphorylation under various temperature cycles. Four mixtures of Kai proteins (a–d) were prepared at 6-hour intervals and incubated at 30°C for 34 hours (a), 28 hours (b), 22 hours (c), and 16 hours (d), then subjected to 45°C/30°C cycles of 12H12L (12 hours 45°C and 12 hours 30°C) (*A* and Fig. 1*B*), and 8H8L (8 hours 45°C and 8 hours 30°C) (*B* and Fig. 1*D*). In each experiment, aliquots of reaction mixtures were collected every 3 hours or 4 hours and subjected to SDS/PAGE and CBB staining. Zero represents time in temperature cycle. (*C*) A mixture of Kai proteins was prepared and divided into two fractions, which were incubated at 30°C and 45°C (Fig. 2*A*). Aliquots of the mixtures were collected every 2 hours and subjected to SDS/PAGE and CBB staining. Time represents hours in incubation. Upper and lower bands correspond to phosphorylated (P-KaiC) and unphosphorylated KaiC (NP-KaiC), respectively.

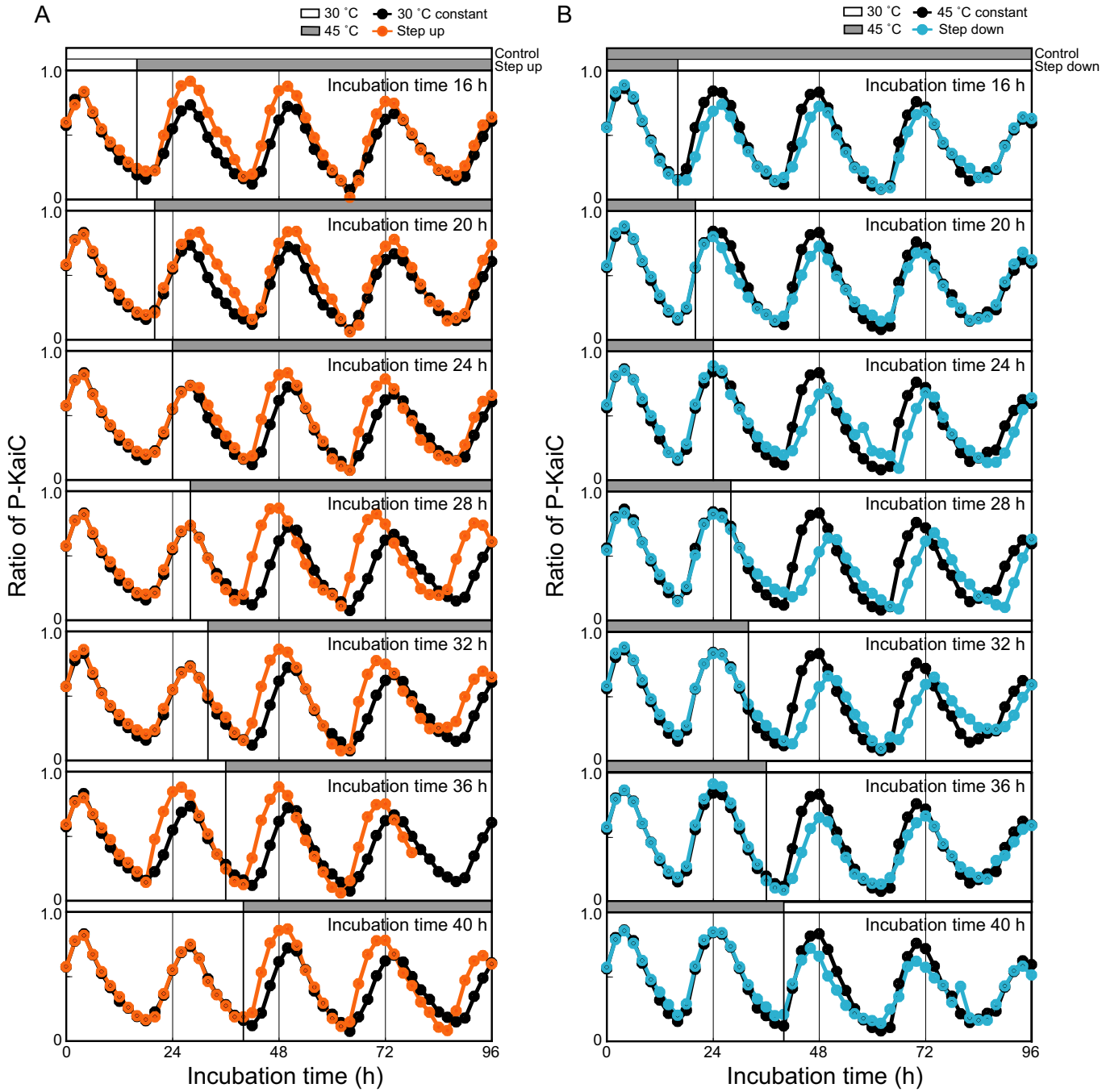


**Fig. S2.** Phase shift of the *in vitro* KaiC phosphorylation rhythm by temperature steps. KaiC phosphorylation rhythms after exposure to temperature step-ups from 30° to 45°C (*A*) and step-downs from 45° to 30°C (*B*). The Kai protein mixtures were incubated under standard conditions at a control temperature of either 30°C (*A*) or 45°C (*B*). The mixtures were either stepped up to 45°C (*A*) or stepped down to 30°C (*B*) at hours 16, 20, 24, 28, 32, 36, and 40 of incubation. Aliquots were collected every 2 hours and subjected to SDS/PAGE as described in Fig. 1. The phosphorylation level of KaiC was plotted against incubation time. Black symbols and lines represent control (untreated) mixtures; red and blue symbols and lines represent stepped-up and stepped-down samples, respectively. Black vertical lines show the temperature change.

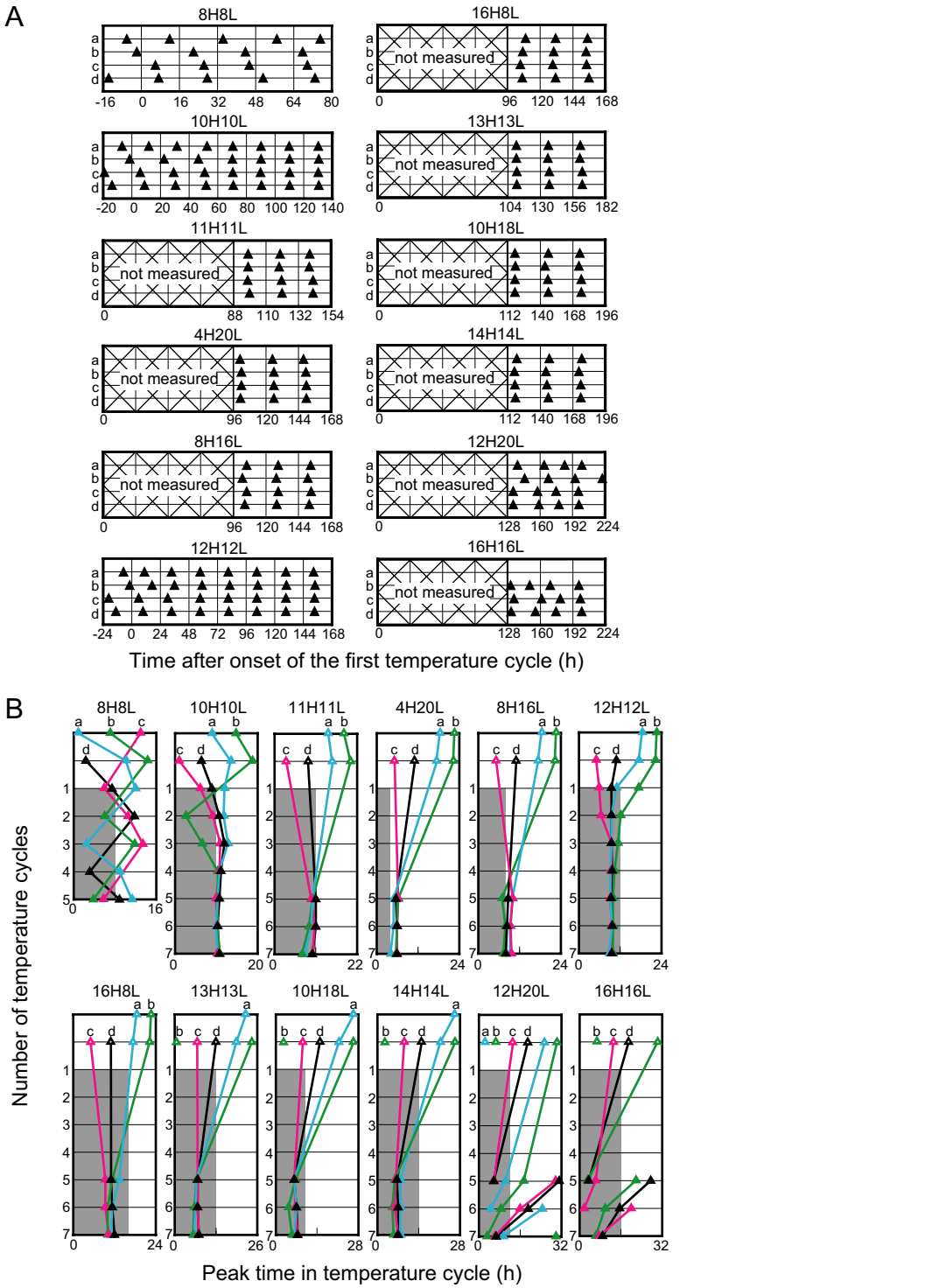


**Fig. S3.** Detailed time course of KaiC phosphorylation rhythms under various temperature cycles. Entrainment of the rhythm was examined for 12 temperature cycles: 8H8L, 10H10L, 11H11L, 4H20L, 8H16L, 12H12L, 16H8L, 13H13L, 10H18L, 14H14L, 12H20L, and 16H16L. For each regimen, four mixtures of the Kai proteins were prepared at 6-hour intervals; after incubation at 30°C, the mixtures were subjected to the temperature cycle at hours 16, 22, 28, and 34 of incubation. Peak positions of each rhythm obtained by cosine fitting were plotted in two ways. (*A*) Peak positions were plotted on extended time scales up to 80–224 hours with closed triangles. (*B*) The elapsed time of experiment was segmented with the length of temperature cycle at every onset of high-temperature period. The high-temperature period of the cycle is shown in gray. The peak positions of each experiment are shown as triangles at the corresponding segments and connected with solid lines. Blue (a), green (b), red (c), and black (d) indicate hours 34, 28, 22, and 16 of incubations at 30°C before administration of the first temperature cycle. Solid symbols indicate experimentally determined peak position, whereas open symbols indicate initial peak position obtained in 12H12L experiments.

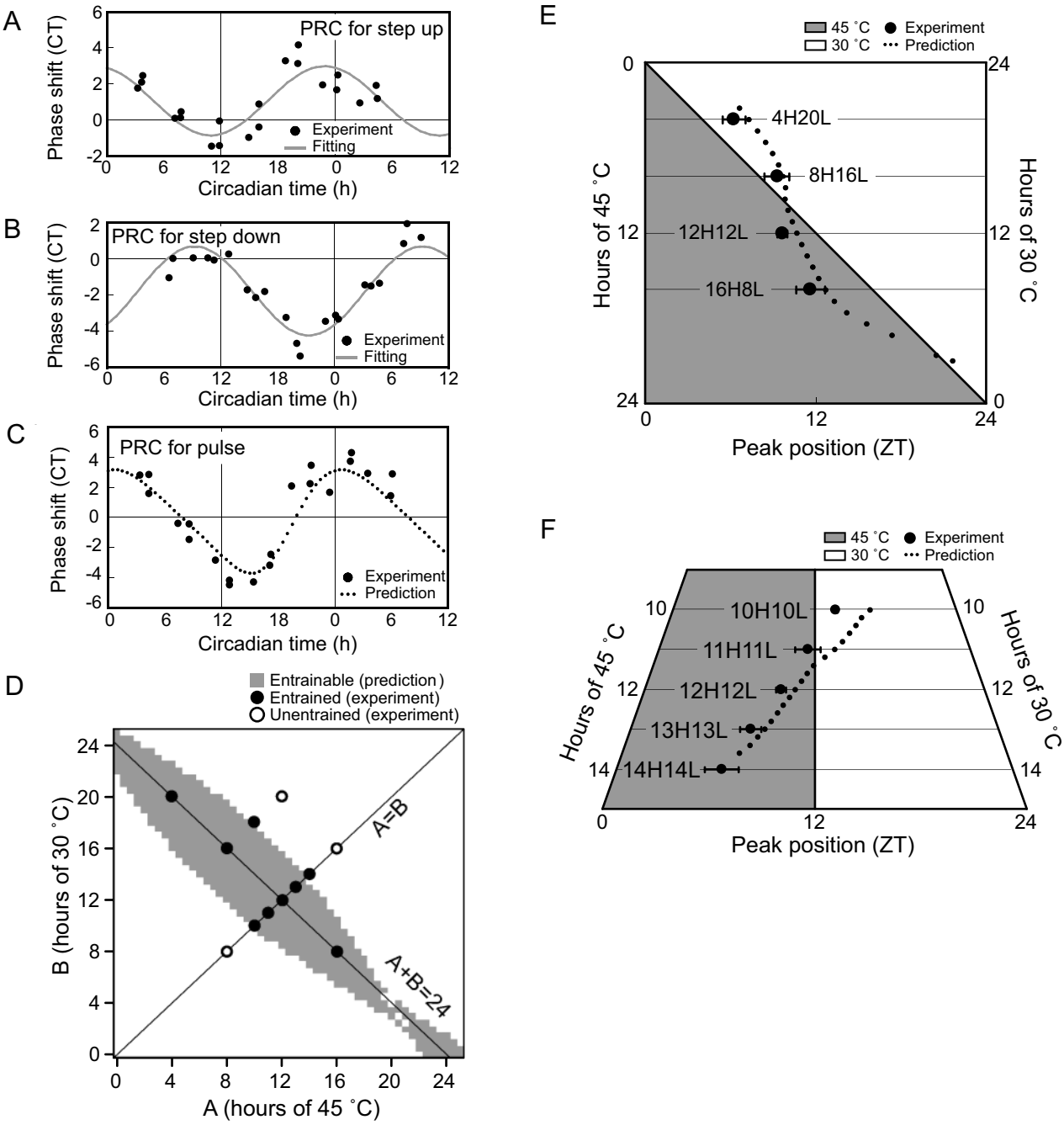


**Fig. S4.** Phase response curves of the KaiC phosphorylation rhythm by high-temperature pulses and entrainment of the rhythm by temperature cycles obtained by fitting to the cosine function. The phase response curves of temperature step-up (*A*) and step-down (*B*) and temperature cycle (*C*) as shown in Figs. 2 and 3, and entrainment patterns as shown in Fig. 4, are again shown. In these plots, PRCs for temperature step-up and step-down were obtained by fitting to the cosine function and PRC for temperature pulse and prediction of the range of entrainment (*D*) and peak positions (*E* and *F*) were computed with PRCs obtained from the fitting. Experimental data are the same as shown in Figs. 2, 3, and 4.

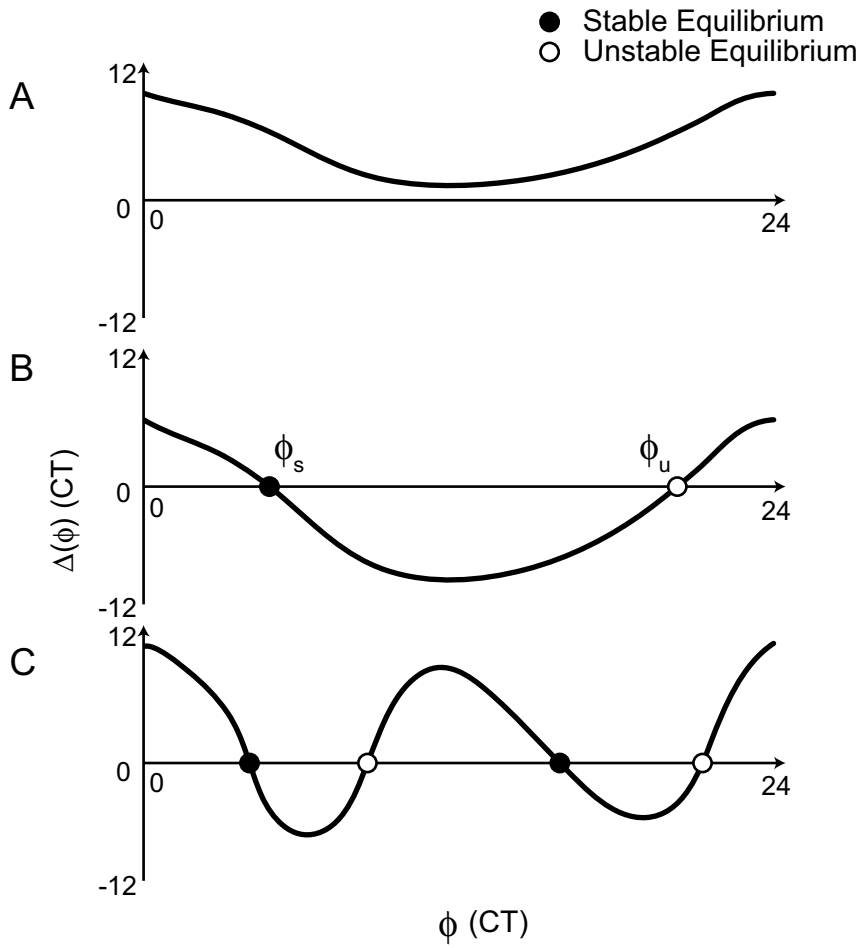


**Fig. S5.** Change in $\Delta(\phi)$ against the initial phase of the KaiC phosphorylation rhythm at the onset of the high-temperature period (see SI Text for explanation).

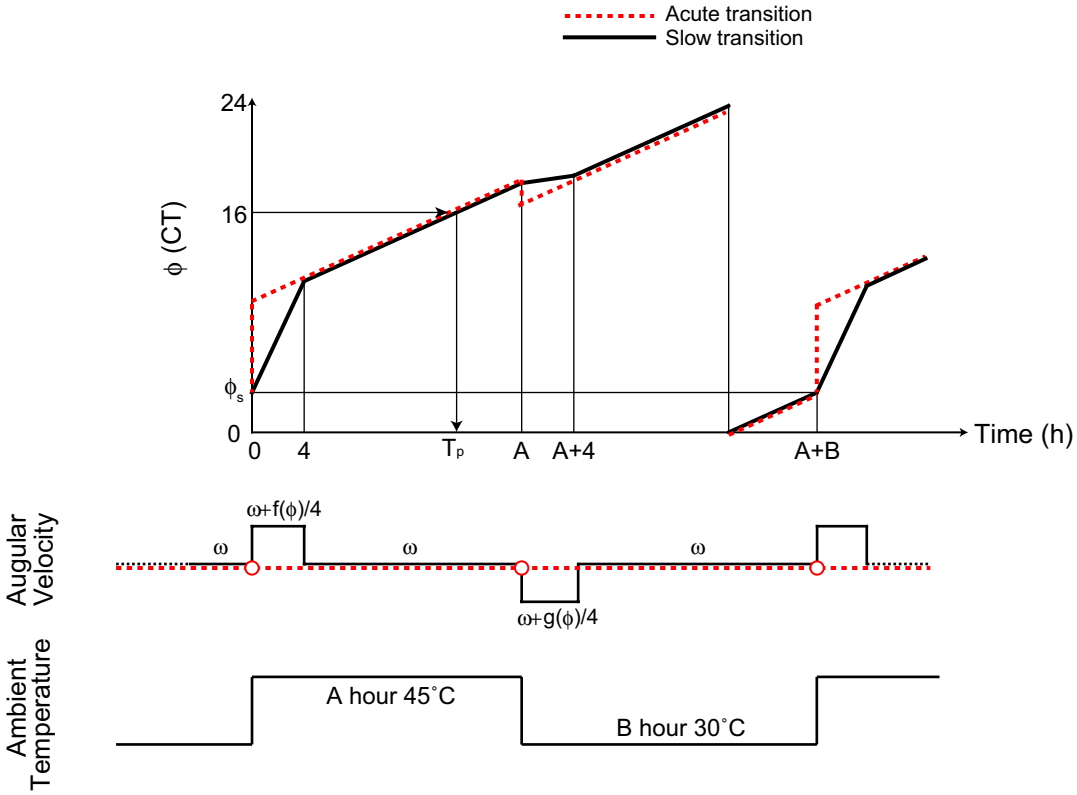


**Fig. S6.** Phase progress of the KaiC phosphorylation rhythm entrained to temperature cycle (see SI Text for explanation).