

SUPPLEMENTAL INFORMATION

Fluorescence correlation spectroscopy to monitor Kai protein–based circadian oscillations in real time

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Supplemental Figure Legends

Supplemental Fig. S1. No interaction was detected between KaiB and KaiA using

FCS analysis

Diffusion times obtained with FCS analysis over a 5-d period and protein mixtures containing TAMRA-KaiB-His₆ and 3.5 μ M KaiB (open circles; Fig. 3a), or TAMRA-KaiB-His₆, 1.2 μ M KaiA, and 3.5 μ M KaiB (filled circles). A representative example from two independent experiments is shown. Each data point represents the mean \pm SD from 10 measurements at the indicated time point.

Supplemental Fig. S2. Oscillation of the diffusion time despite prolonged

exposure to the laser

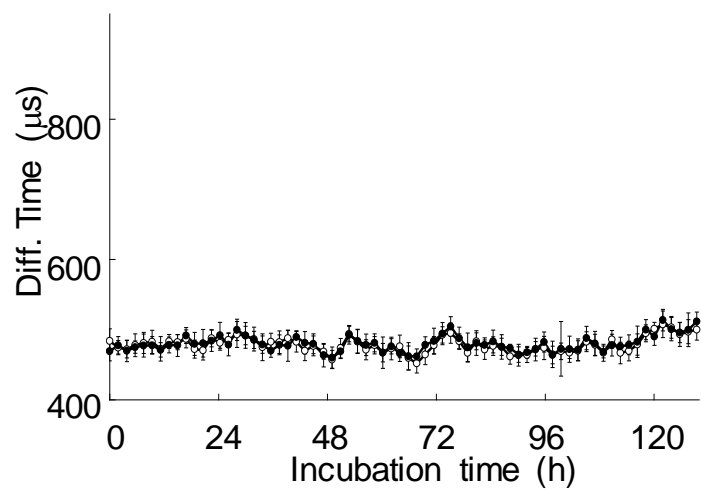
The diffusion time was assessed every \sim 2 h over 4 d using FCS and the standard mixture with TAMRA-KaiB-His₆. Measurement times were 5 \times 15 s (a), 10 \times 15 s (b), 20 \times 15 s (c), 50 \times 15 s (d), and 100 \times 15 s (e) for each data point. Each data point represents the mean \pm SD for

measurements obtained at the indicated time point.

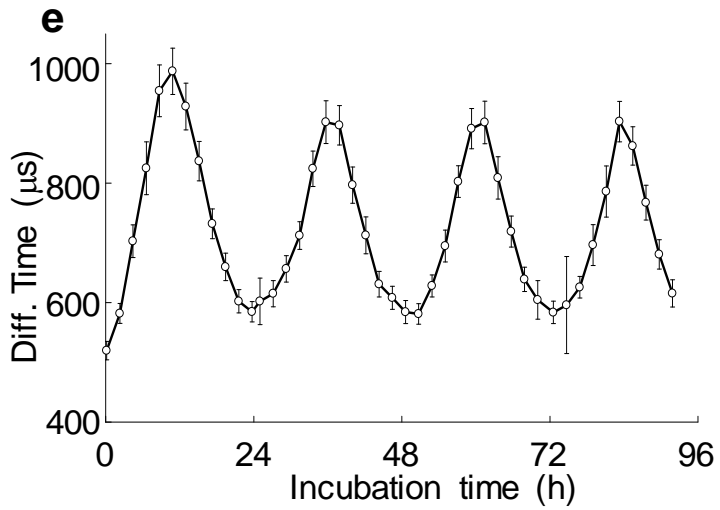
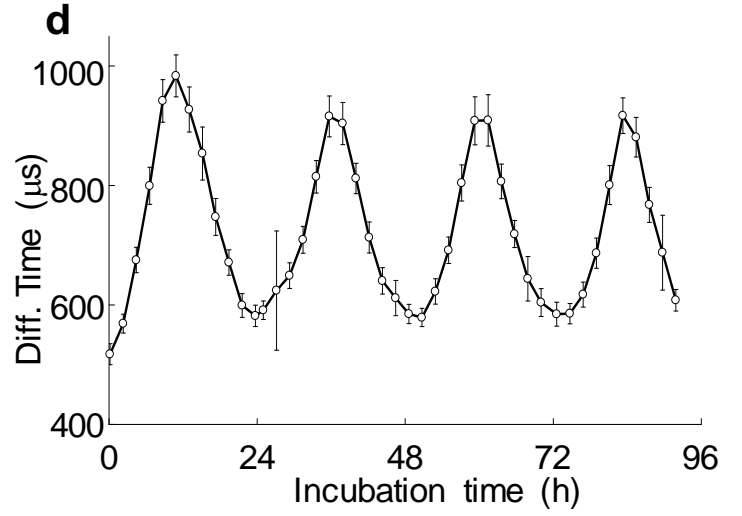
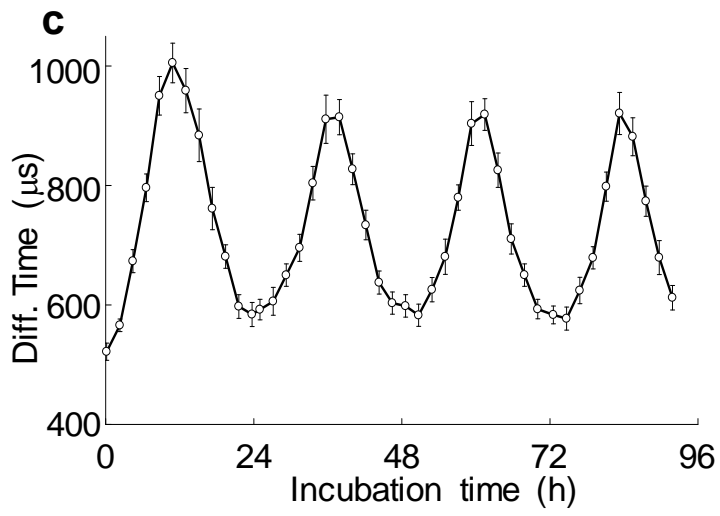
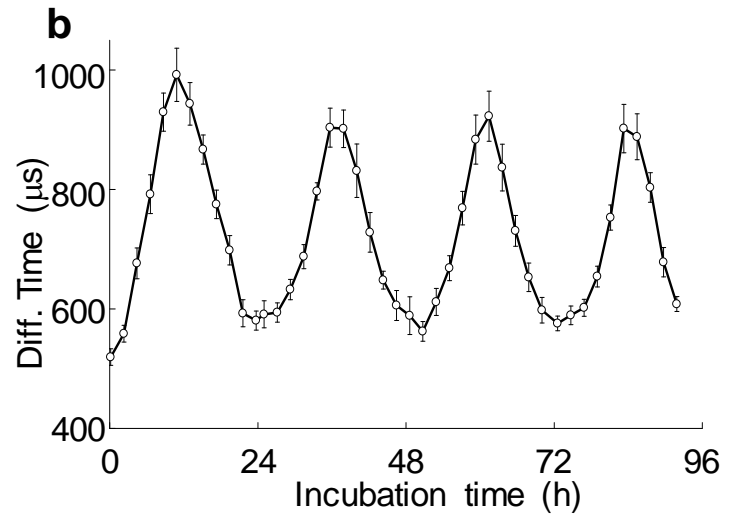
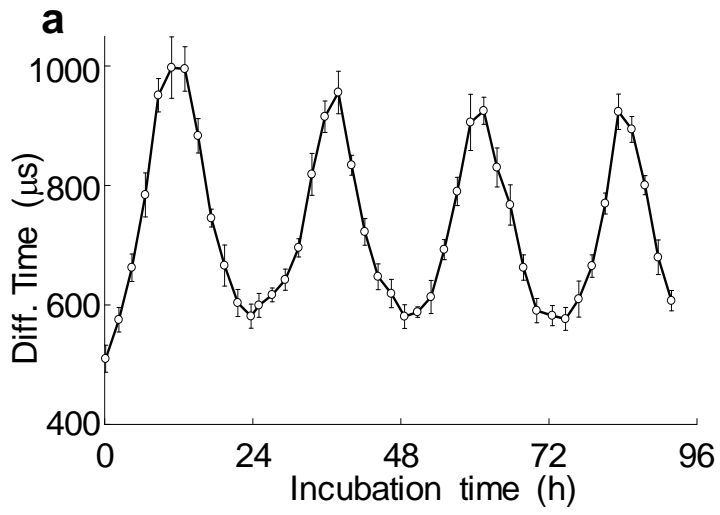
Supplemental Fig. S3. Diffusion time oscillations that showed little phase changes
in response to a high temperature pulse

The standard oscillatory mixture was subjected to a high temperature pulse (50°C for 30 min) after various incubation times: 12 h (a), 16 h (b), or 32 h (c). Open and filled circles in each plot represent diffusion times observed in mixtures with and without the high temperature stimulus, respectively. Red bars indicate the duration of the pulse. Each data point represents the mean \pm SD from 10 measurements at the indicated time point. Data in a, b, and Fig. 3a were obtained from aliquots of a standard mixture, whereas data in c, Fig. 3b, and Fig. 3c were obtained from aliquots of another.

Supplemental Fig. S1

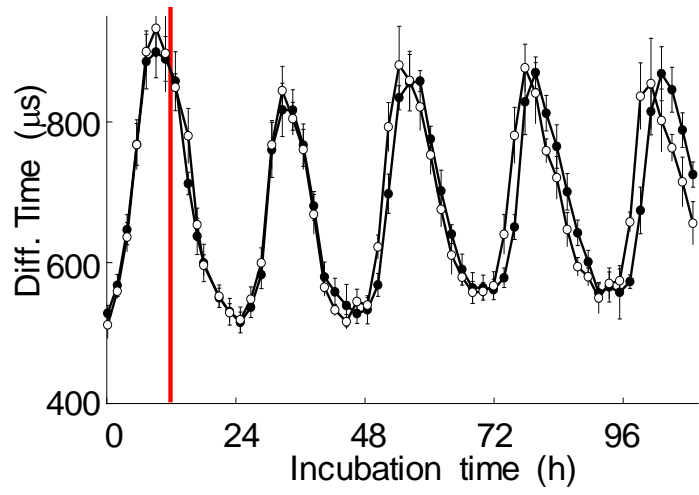


Supplemental Fig. S2

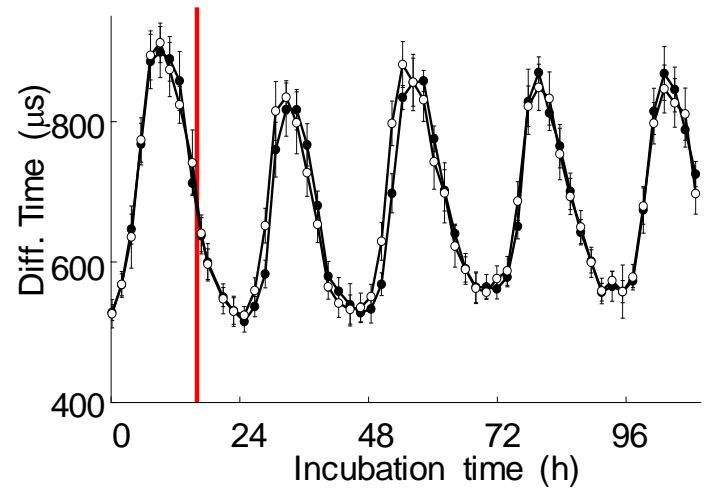


Supplemental Fig. S3

a



b



c

