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Supplementary Materials for

Transplantability of a circadian clock to a noncircadian organism

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The PDF file includes:

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Other Supplementary Material for this manuscript includes the following:
(available at www.advances.sciencemag.org/cgi/content/full/1/5/e1500358/DC1)

Movie S1 (.mov format). Circadian oscillations visualized in single *E. coli* cells using a microfluidic device.

Supplementary Materials:

Supplemental Figures

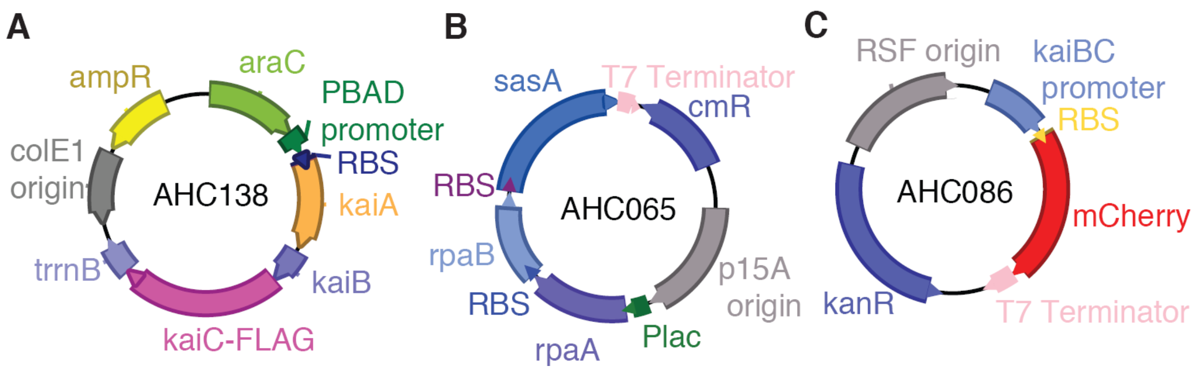

Figure S1. Plasmids built for reconstruction of the circadian oscillator in *E. coli*.

(A) Core oscillator components, *kaiABC*, are expressed in an operon driven by an arabinose inducible promoter. **(B)** Additional native cyanobacterial components, *sasA*, *rpaA*, *rpaB*, are expressed in a synthetic operon driven by an IPTG inducible promoter. **(C)** *mCherry* reporter is driven by a circadian responsive promoter, such as *kaiBC*.

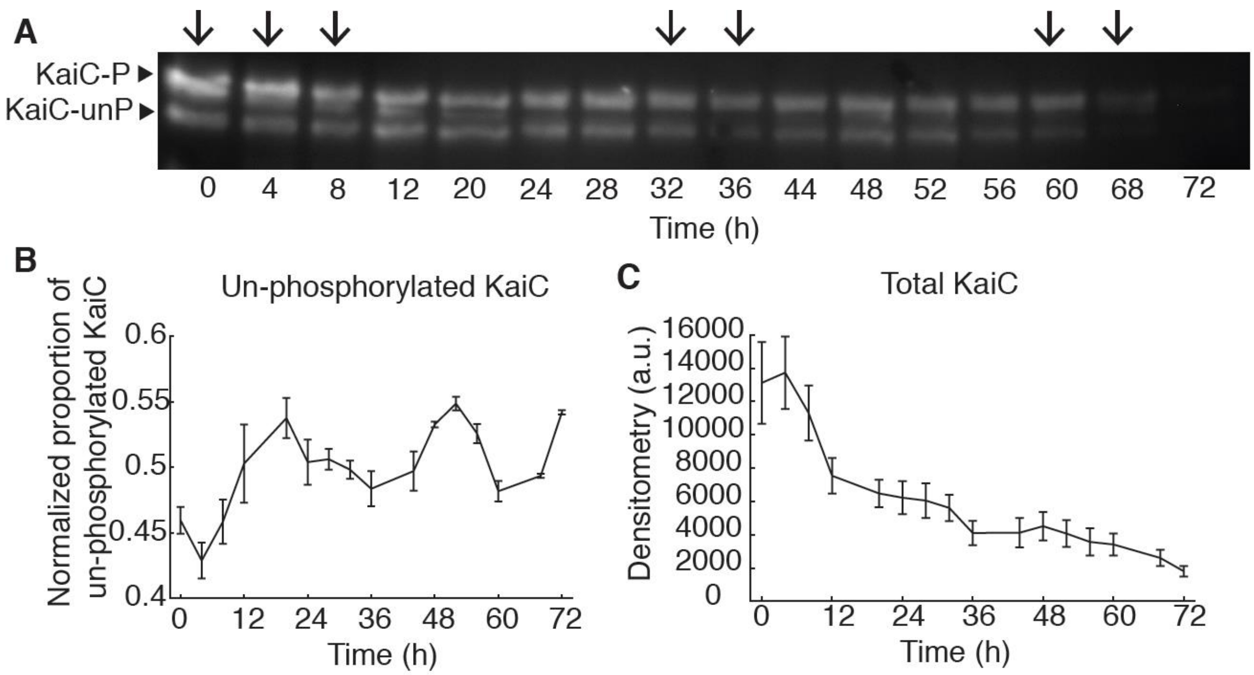

Figure S2. Additional data and quantifications of KaiC phosphorylation in *E. coli* expressing KaiABC.

(A) Western blots, which were quantified in Fig. 1D, show phosphorylated and unphosphorylated KaiC over time in *E. coli* coexpressing KaiA and KaiB. Time t=0h corresponds to synchronization. Arrows indicate timepoints with high proportion of phosphorylated KaiC. (B) Proportion of unphosphorylated KaiC over time in *E. coli* co-expressing KaiA and KaiB, after synchronization (t=0h). The mean ratio of unphosphorylated KaiC to total KaiC across biological replicates, mean normalized for each time-trace, is plotted. (C) Total KaiC quantified over time in *E. coli* co-expressing KaiA and KaiB, after synchronization (t=0h). No statistically significant oscillations were found when analyzed using RAIN (P=0.35). Error bars, s.e.m. (n=3).

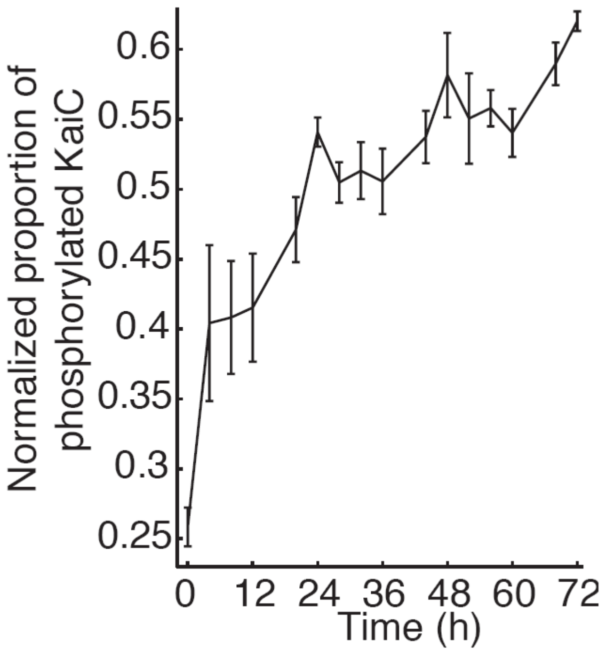

Figure S3. Circadian phosphorylation of KaiC over time requires KaiA and KaiB.

KaiC phosphorylation over time, after synchronization, in *E. coli* expressing only KaiC without other Kai clock components. The mean ratio of phosphorylated KaiC to total KaiC across three biological replicates, mean normalized for each time-trace, is plotted. Error bars, s.e.m. (n=3). Circadian oscillations were not statistically significant as analyzed by RAIN (P>0.99).

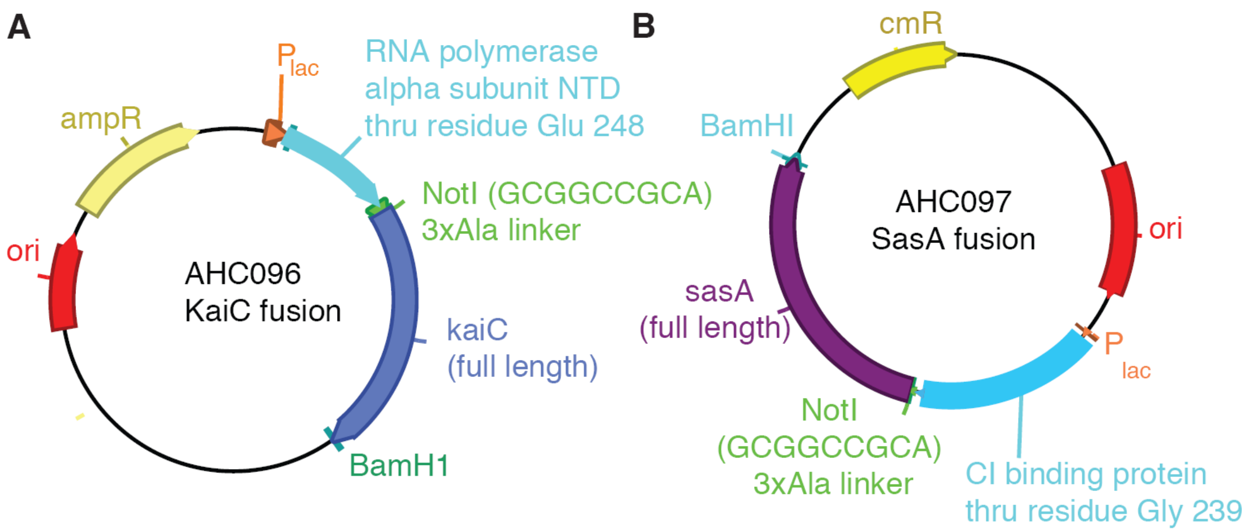

Figure S4: Plasmids built for the synthetic oscillator utilizing a modified bacterial two-hybrid system

(A) Plasmid expressing full length KaiC C-terminally fused to the α subunit N-terminal domain of RNA polymerase (KaiC-αNTD). (B) Plasmid expressing full length SasA C-terminally fused to λCI protein (SasA-CI). The two parts of the fusions are connected via 3X alanine linkers and their corresponding genes are driven by lac promoters.

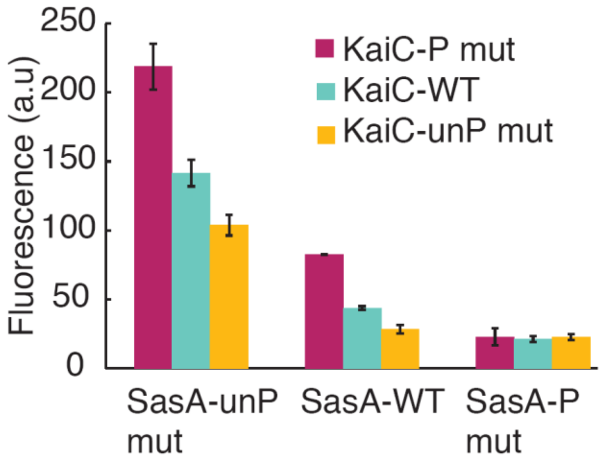

Figure S5: KaiC and SasA phosphorylation states affect reporter output.

OD normalized fluorescent reporter output of interactions between combinations of phosphomimic (-P mut, KaiC: S431A, T432E; SasA: H161D), non-phosphorylatable mutant (-unP mut, KaiC: S431A, T432A; SasA: H161A) and wild-type (WT) KaiC- αNTD and SasA-CI. Error bars, s.e.m. (n=3)

Table S1 Raw data for Fig. 2D.

	Fluorescence		
Time	replicate 1	replicate 2	replicate 3
1	16.5298743	-37.989606	-18.859029
2	9.17214036	-32.030356	-23.132065
3	2.88513554	-34.610537	-27.914756
4	3.17460317	-26.682346	-25.12607
5	2.39940938	-33.989081	-27.952593
6	10.4375	-30.440816	-29.121372
7	19.1532258	-26.778584	-22.288246
8	11.4589335	-25.411454	-20.648536
9	11.5794952	-23.843485	-27.503581
10	19.9669421	-23.81736	-20.476034
11	11.0637163	-27.436236	-20.260251
12	12.0967742	-17.495511	-25.319834
13	13.1471944	-20.691749	-24.681413
14	15.1186441	-20.88608	-18.357907
15	17.0940171	-20.380536	-22.887026
16	12.8205128	-11.787177	-20.321454
17	18.5280494	-15.845521	-11.670214
18	21.9890055	-16.734217	-23.688132
19	12.0569086	-13.683353	-13.677615
20	17.1256654	-8.6546374	-11.183774
21	29.6686073	-11.265066	-15.745511
22	23.8523376	-12.068371	2.67181753
23	17.1399594	-3.4443579	-3.1438061
24	16.4835165	3.96783718	11.767629
25	-16.288796	4.87345564	6.54083332
26	-13.413058	6.62623064	6.61988719
27	-18.759824	6.0190977	10.0851131
28	-16.273904	10.7170211	18.8553952
29	2.53569999	20.6133486	20.3001099
30	-5.0505051	19.2628244	26.9689778
31	3.97381954	22.8753833	30.8825358
32	8.33333333	39.3017746	44.3178091
33	23.6963696	32.8105316	40.5225029
34	31.1625076	40.3775453	45.7233114
35	30.9193122	50.8189174	53.9602684
36	38.1355932	46.7314732	54.4222425
37	43.2481371	48.9319714	54.4018628
38	54.5685817	47.5982642	51.3467069
39	59.7117364	51.324421	58.6961962
40	73.8448845	47.7431077	53.3777483
41	72.0897833	54.1100268	47.7631232
42	82.2587817	53.9151737	45.5951135
43	80.9243552	46.5668307	42.3552629
44	86.0787465	32.1872365	37.9470991
45	82.6875969	31.27565	29.105205
46	84.0099609	23.8417993	12.5267343
47	83.1581734	12.7749376	5.52701572
48	80.6428893	15.0923995	2.5424188
49	70.575056	-4.1829313	-4.0683885
50	56.9307482	-5.5846933	-9.0240603
51	48.5938425	-4.7895156	-6.7092226
52	39.9149844	-8.918424	-4.0437291
53	31.1594203	-13.033662	-7.7499961
54	26.0746848	-7.8888933	-3.0120124
55	28.838436	-9.1611522	8.04145466
56	17.571764	-2.0785048	13.3354077
57	20.791124	-3.5061892	1.56066127
58	0.1546073	-2.7176267	9.13702337
59	21.432212	4.98697154	6.25124341
60	23.7064928	6.57052831	-1.3146147
61	19.8291731	6.36647407	-2.0427165
62	18.979804	7.95140669	3.81916039
63	35.499436	19.5033197	-9.1809275
64	32.86221	12.0007051	-16.814713
65	43.44294	16.0723173	-14.764842
66	26.7636479	12.4338231	-15.337846
67	30.7363026	10.4385363	-27.24003
68	59.1458779	11.978234	-28.617678
69	53.6357544	5.82639909	-18.566773
70	62.7539549	10.8418204	-20.514412
71	58.3486671	15.2190976	-18.115157
72	54.9997925	4.34796799	-22.07229
73	46.8780327	1.84394444	-21.675737
74	31.95251	0.31927359	-30.624794
75	20.0404566	2.24881039	-26.964285
76	8.81496195	-0.6814817	-28.162967
77	0.52764813	-9.8476557	-23.432994
78	-9.9605632	-14.586675	-22.697126
79	-11.977503	-17.07131	-11.175086
80	-3.9155606	-21.194207	-8.6847944
81	-10.44642	-24.3239	-10.762348
82	9.16548921	-16.748502	-9.1209008
83	11.0718623	-18.442545	0.49892504
84	8.80489356	-18.999236	-1.4047515
85	18.6376381	-29.084561	-9.2788561
86	66.984227	-28.395516	-2.3372004
87	29.7186655	-18.990072	1.33849634
88	28.4960883	-27.829526	-5.3052775
89	44.7383777	-19.914695	0.88078363
90	56.6814267	-20.462357	7.45559977
91	48.952395	-18.993186	-1.8872343
92	65.3518497	-16.319075	3.45122792
93	62.8797898	-11.057743	2.72668989
94	104.478081	-15.817009	4.67080909
95	201.2486	-6.097194	12.6376633
96	140.17761	-6.6224112	6.03491311

Table S2. Bacterial strains and plasmids.

Strain or plasmid	Relevant genotype	Resistance	Reference
E. coli strains			
DH10B/Top10	Expression strain		Invitrogen
MG1655	Parent strain of DH10B for microfluidic device loading and growth		
DP10	Variant of DH10B for arabinose induction		Kizer 2008
Plasmids			
AHC138	pBAD-kaiABC-FLAG	Amp	This work
AHC181	Phosphomutant variant of AHC138 with KaiC S431A, T432A	Amp	This work
AHC82	pTET kaiBC-6XHis	Kan	This work
AHC21	pBAD kaiA	Amp	This work
AHC20	pBAD-kaiABC	Amp	This work
AHC86	*kaiBC* promoter -mcherry	Kan	This work
AHC65	plac-rpaA, rpaB, sasA	Cm	This work
AHC22	AHC20 ΔkaiA	Amp	This work
AHC165	AHC20 ΔkaiB	Amp	This work
AHC166	AHC20 ΔkaiC	Amp	This work
AHC163	AHC65 ΔrpaA	Cm	This work
AHC164	AHC65 ΔsasA	Cm	This work
AHC123	AHC65 ΔrpaB	Cm	This work
AHC170	AHC20 ΔkaiAC	Amp	This work
AHC171	AHC65 ΔSasA ΔRpaB	Cm	This work
AHC172	AHC65 ΔRpaAB	Cm	This work
AHC85	AHC65 ΔSasA ΔRpaA	Cm	This work
AHC205	AHC20 KaiC phosphomimic S431D T432D		
AHC 140	AHC65 RpaA phosphomimic D53E	Cm	This work
AHC141	AHC65 Non-phosphorylatable RpaA D53A	Cm	This work
AHC 142	AHC65 RpaB phosphomimic D56E	Cm	This work
AHC 143	AHC65 Non-phosphorylatable RpaB D56A	Cm	This work
AHC 144	AHC65 SasA phosphomimic H161D	Cm	This work
AHC 145	AHC65 Non-phosphorylatable SasA H161A	Cm	This work
pBR α	P_{lacUV5}/P_{lpp} -directed synthesis of the full length a subunit of *E. coli* RNAP	Amp	Dove et al. 1997
pACλCI	P_{lacUV5}-directed synthesis of the lCI protein	Cm	Dove et al. 1997

AHC096	p*lacUV5*- and p*lpp*-directed synthesis of the αNTD (residues 1-248 of the α subunit of *E. coli* RNAP) fused via three alanines to *S. elongatus* KaiC protein	Amp	This work
AHC097	P_{lacUV5}-directed synthesis of the lCI protein fused via three alanines to *S. elongatus* SasA protein	Cm	This work
AHC157	p*lac*O_L2 driving 3x tandem sfGFP	Kan	This work
AHC177	AHC096 Non-phosphorylatable KaiC S431A, T432A	Amp	This work
AHC178	AHC096 KaiC phosphomimic S431D T432D	Amp	This work
AHC179	AHC097 Non-phosphorylatable SasA H161A	Cm	This work
AHC180	AHC097 Phosphomimic SasA H161D	Cm	This work