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Supporting information for article:

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Supporting Information

An arginine tetrad as mediator of input-dependent and -independent ATPases in the clock protein KaiC

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Table S1 Interactions of Arg linker motifs in the *S. elongatus* KaiCI and CII halves (interactions are only shown for three of the six independent subunits)

Donor a.a./atom		Acceptor-atom/a.a.		Subunit a[a]	Subunit b[a]	Subunit c[a]
				distances in Å		
R215	NH1	O=C	K232	2.8 (b)[b]	3.2 (c)[b]	3.1 (d)[b]
		OH	Y235	3.3 (b)	3.1 (c)	3.2 (d)
	NH2	O=C	E234	2.7 (b)	2.7 (c)	2.8 (d)
		O=C	E214	2.8 (a)	2.6 (b)	2.8 (c)
R216	NH1	O=C	N209	2.9 (a)	2.8 (b)	2.8 (c)
	NH2	OE1	E221	2.6 (b)	3.1 (c)	2.7 (d)
R217	NH1	OE1	E214	3.3 (f)	3.0 (a)	3.2 (b)
	NH2	OE1	Q394	3.2 (a)	3.2 (b)	3.1 (c)
R218	NH2	O=C	G49	2.5 (a)	2.5 (b)	2.4 (c)
R451	NH1	O=C	G291	3.2 (a)		
		O=C	T292		3.5 (b)	3.2 (c)
	NH2	O2'	ATP	2.7 (a)	2.7 (b)	2.6 (c)

[a] Subunit that Arg linker residue belongs to.

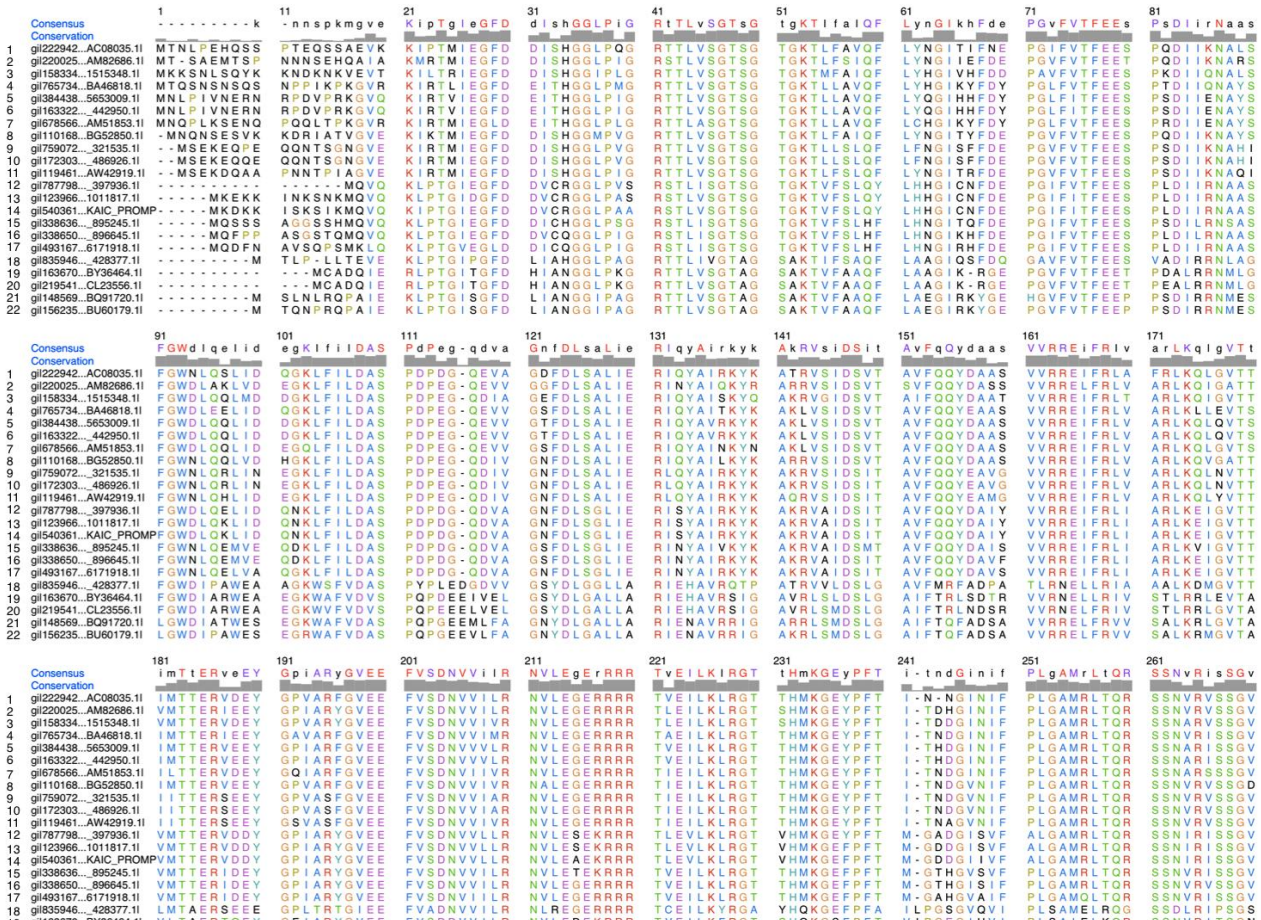
[b] Subunit that acceptor residue belongs to.

Table S2 Primers used for KaiC mutagenesis

KaiC mutation	Oligonucleotides[a]
R215A	Forward: 5'-AACGTTTTGGAAGGGGAGGCTCGTCGCCGCACCCTCGAAAT-3' Reverse: 5'-ATTTCGAGGGTGCGGCGACGAGCCTCCCCTTCCAAAACGTT-3'
R216A	Forward: 5'-ACGTTTTGGAAGGGGAGCGCGCTCGCCGCACCCTCGAAATC-3' Reverse: 5'-GATTTCGAGGGTGCGGCGAGCGCGCTCCCCTTCCAAAACGT-3'
R217A	Forward: 5'-GTTTTGGAAGGGGAGCGCCGTGCTCGCACCCTCGAAATCCTCAA-3' Reverse: 5'-TTGAGGATTTCGAGGGTGCGAGCACGGCGCTCCCCTTCCAAAAC-3'
R218A	Forward: 5'-TTGGAAGGGGAGCGCCGTCGCGCTACCCTCGAAATCCTCAAGCTA-3' Reverse: 5'- TAGCTTGAGGATTTCGAGGGTAGCGCGACGGCGCTCCCCTTCCAA-3'
R226A	Forward: 5'-CTCGAAATCCTCAAGCTAGCTGGCACCAGCCACATGAAAG-3' Reverse: 5'-CTTTCATGTGGCTGGTGCCAGCTAGCTTGAGGATTTCGAG-3'
R451A	Forward: 5'-AGATTCGTGGCGAAATGTCCGCAGCCATTAACGTCTTCAAGATG-3' Reverse: 5'- CATCTTGAAGACGTTAATGGCTGCGGACATTTCGCCACGAATCT-3'
R459A	Forward: 5'-CCATTAACGTCTTCAAGATGGCTGGATCTTGGCATGACAAAGCA-3' Reverse: 5'-TGCTTTGTCATGCCAAGATCCAGCCATCTTGAAGACGTTAATGG-3'

[a] Changed nucleotides are indicated in red.

Figure S1 Sequence alignment for *Th*KaiC proteins from various cyanobacterial strains. 1. *Thermosynechococcus elongatus* BP-1; 2. *Synechococcus elongatus* PCC 7942; 3. *Anabaena variabilis* ATCC 29413; 4. *Nostoc* sp. PCC 7120; 5. *Nodularia spumigena* CCY9414; 6. *Trichodesmium erythraeum* IMS101; 7. *Microcystis aeruginosa* PCC 7820; 8. *Acaryochloris marina* MBIC11017; 9. *Prochlorococcus marinus* str. MIT 9312; 10. *Prochlorococcus marinus* str. MIT 9515; 11. *Prochlorococcus marinus* MED4; 12. *Synechococcus sp.* WH 8102; 13. *Synechococcus* sp. WH 5701; 14. *Prochlorococcus marinus* str. MIT 9313; 15. *Rhodospirillum rubrum* ATCC 1117017. 16. *Synechocystis* sp. PCC 6803; 17. *Crocosphaera watsonii* WH 8501; 18. *Chloroflexus aurantiacus* J-10-fl; 19. *Chloroflexus aggregans* DSM 9485; 20. *Roseiflexus* sp. RS-1; 21. *Roseiflexus castenholzii* DSM 13941; 22. *Bradyrhizobium* sp. BTAi1. The sequence alignment was generated with CLUSTAL Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers et al, 2011).



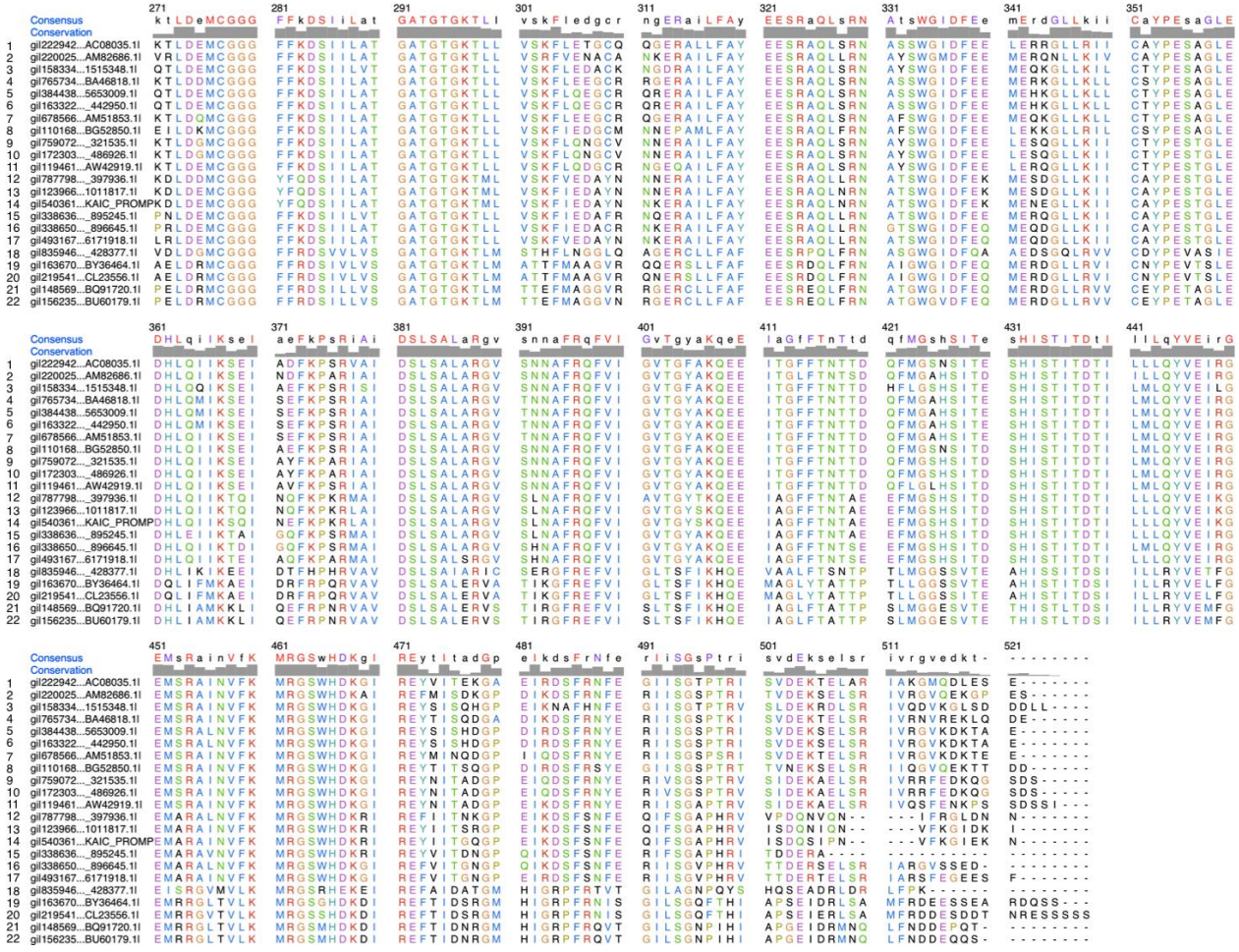

Consensus
Conservation
gil222942...AC08035.1|
gil220025...AM82686.1|
gil158334...1515348.1|
gil765734...BA46818.1|
gil384438...5653009.1|
gil163322..._442950.1|
gil678566...AM51853.1|
gil110168...BG52850.1|
gil759072..._321535.1|
gil172303..._486926.1|
gil119461...AW42919.1|
gil787798..._397936.1|
gil123966...1011817.1|
gil540361...KAIC_PROMP
gil338636..._895245.1|
gil338650..._896645.1|
gil493167...6171918.1|
gil835946..._428377.1|
gil163670...BY36464.1|
gil219541...CL23556.1|
gil148569...BQ91720.1|
gil156235...BU60179.1|

Figure S2 Conformational differences in the crystal structures of *T. and S. elongatus* KaiC. Trimers composed of subunits a-c from the structures of *T. elongatus* (CI and CII ribbons colored in gray ad pink, respectively) and *S. elongatus* KaiC (cyan ribbon) were superimposed by aligning residues 252-498 from all three CII domains. Carbon atoms of ATP molecules bound between CI and CII domains of *T. elongatus* KaiC subunits are colored in black and purple, respectively, and carbon atoms of ATP molecules bound between *S. elongatus* KaiC subunits are colored in cyan. Deviations in the orientations of CI portions and the positions of ATP molecules bound there in the two structures are clearly apparent (arrow).

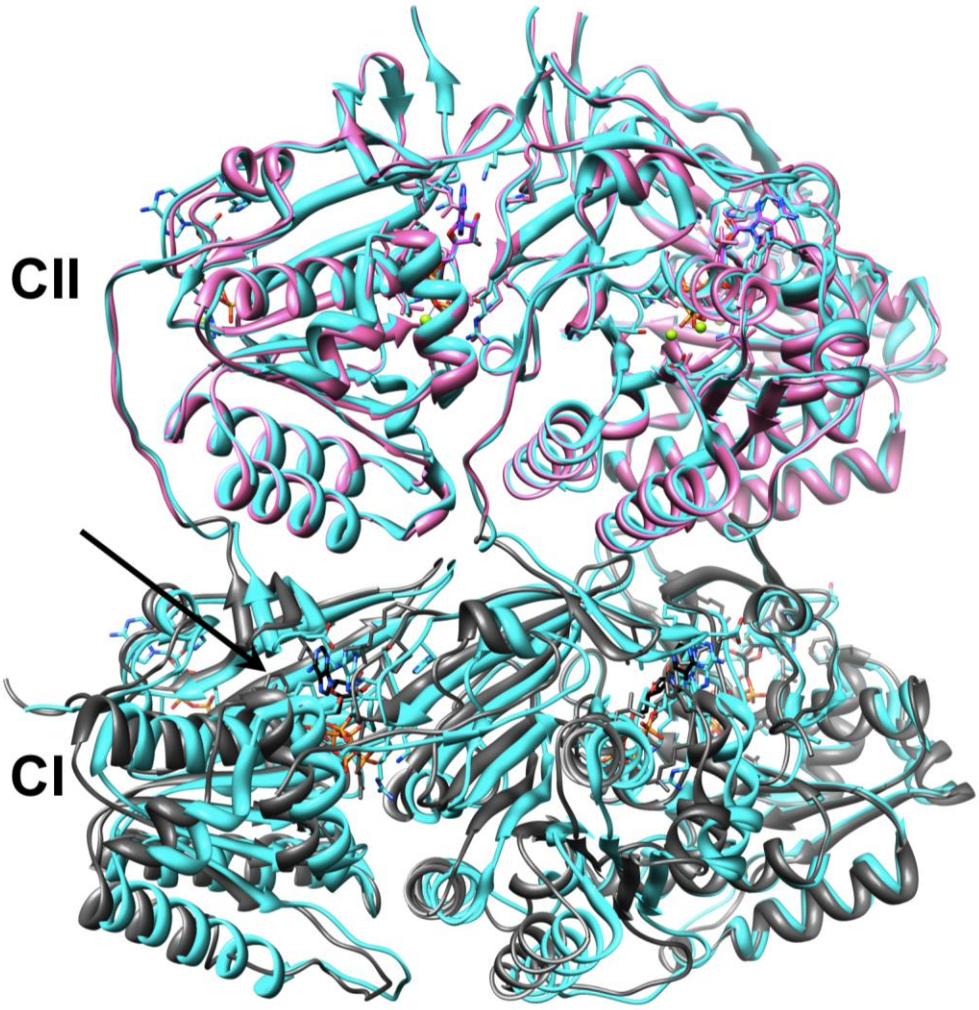


Figure S3 Arginine finger interactions in the CI and CII halves. Arg fingers (A) R227 and (B) R459 at the b/c subunit interface active sites in the CI and CII halves, respectively (only a selection of amino acids is depicted). The color code for residues is identical to that in **Figure 2**; Arg finger side chains are highlighted in ball-and-stick mode, with hydrogen bonds drawn as solid lines. Magnesium ions are green spheres with the coordination geometry indicated by dashed lines.

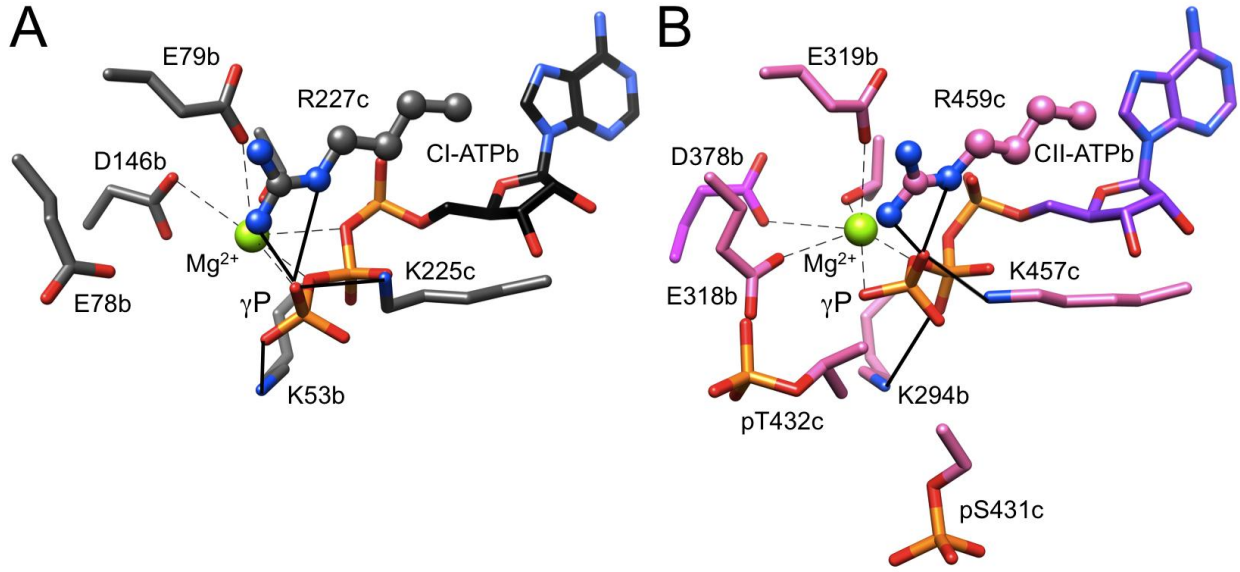


Figure S4 KaiC monomer fold (CI domain). The ribbon diagram is shown in rainbow colors, blue, cyan, green, yellow, orange and red, from N- to C-terminal end and individual α-helices and β-strands are labeled. The CII domain exhibits a similar secondary structure. The ATP molecule is shown in ball and stick mode.

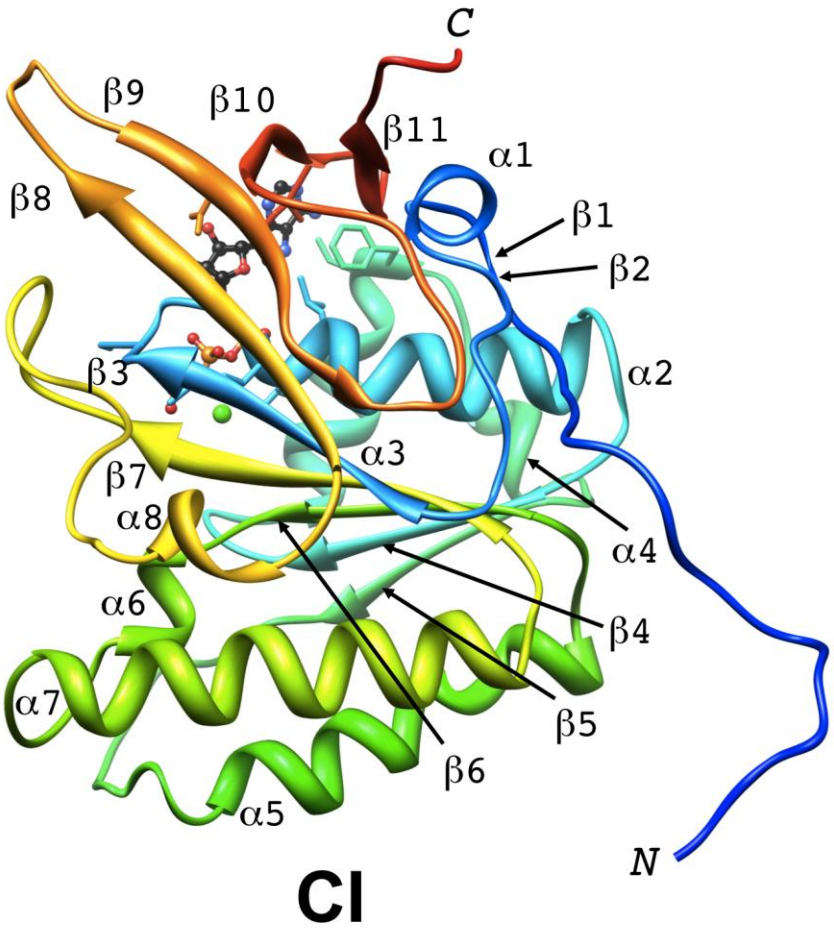


Figure S5 Effects of KaiC Arginine Linker and Finger Alanine Mutants on Circadian Rhythms and Expression Levels of the *kaiBC*p-driving *luxAB* Luminescence in *S. elongatus.* **A.** Original luminescence units. **B**. Normalized luminescence. WT = wild-type KaiC; KaiC mutants include Arg linker mutants (CI: R215A, R216A, R217A, R218 A; CII: R451A) and Arg finger mutants (CI: R226A; CII: 459A). Shown are averages from triple colonies in each case.