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## Section D

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Supporting information for article:

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

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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)

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

<sup>a</sup> Subunit that Arg linker residue belongs to.

<sup>b</sup> Subunit that acceptor residue belongs to.

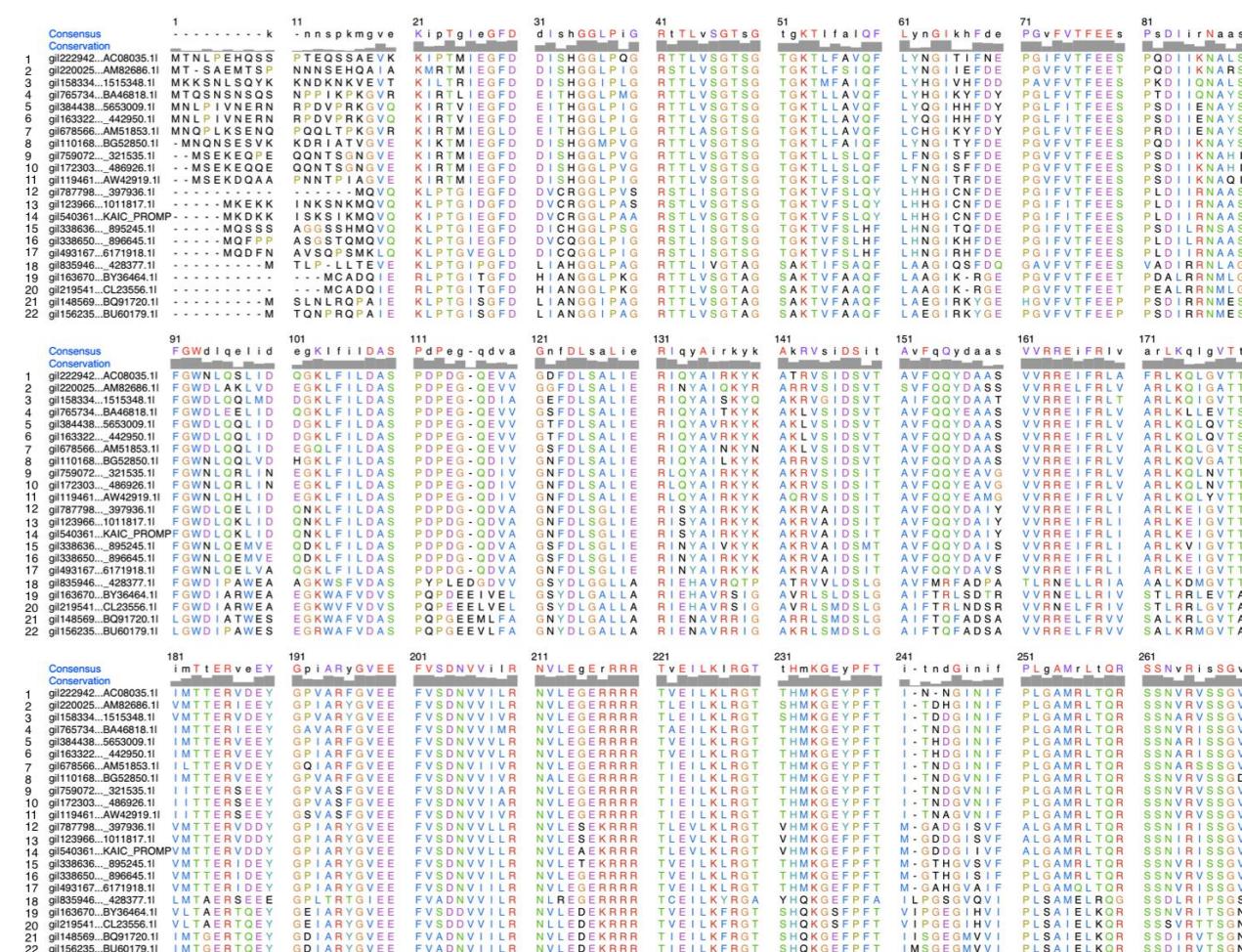
**Table S2** Primers used for KaiC mutagenesis

KaiC mutation	Oligonucleotides <sup>a</sup>
R215A	Forward: 5'-AACGTTTGGAAAGGGGAG <b>GCT</b> CGTCGCCGCACCCCTCGAAAT-3' Reverse: 5'-ATTCGAGGGTGC GGCGACG <b>AGC</b> CTCCCCTCCAAAACGTT-3'
R216A	Forward: 5'-ACGTTTGGAAAGGGAGCGC <b>GCT</b> CGCCGCACCCCTCGAAATC-3' Reverse: 5'-GATTCGAGGGTGC GGCGA <b>GC</b> CGCTCCCCTCCAAAACGT-3'
R217A	Forward: 5'-GTTTTGGAAAGGGAGCGCCGT <b>GCT</b> CGCACCCCTCGAAATCCTCAA-3' Reverse: 5'-TTGAGGATTTCGAGGGTGC <b>G</b> ACGGCGCTCCCCTCCAAAAC-3'
R218A	Forward: 5'-TTGGAAGGGAGCGCCGTCGC <b>GCT</b> ACCCTCGAAATCCTCAAGCTA-3' Reverse: 5'- TAGCTTGAGGATTTCGAGGGT <b>AGC</b> GCGACGGCGCTCCCCTCCAA-3'
R226A	Forward: 5'-CTCGAAATCCTCAAGCTA <b>GCT</b> GGCACCGCCACATGAAAG-3' Reverse: 5'-CTTCATGTGGCTGGTGC <b>C</b> A <b>G</b> CTAGCTTGAGGATTTCGAG-3'
R451A	Forward: 5'-AGATTCGTGGCGAAATGTCC <b>GCA</b> GGCATTAAACGTCTCAAGATG-3' Reverse: 5'- CATCTTGAAGACGTTAATGGCT <b>TG</b> CGGACATTCGCCACGAATCT-3'
R459A	Forward: 5'-CCATTAAACGTCTCAAGATG <b>GCT</b> GGATCTTGGCATGACAAAGCA-3' Reverse: 5'-TGCTTGTCA TGCCAAAGATCC <b>AGC</b> CATCTTGAAGACGTTAATGG-3'

<sup>a</sup> Changed nucleotides are indicated in red.

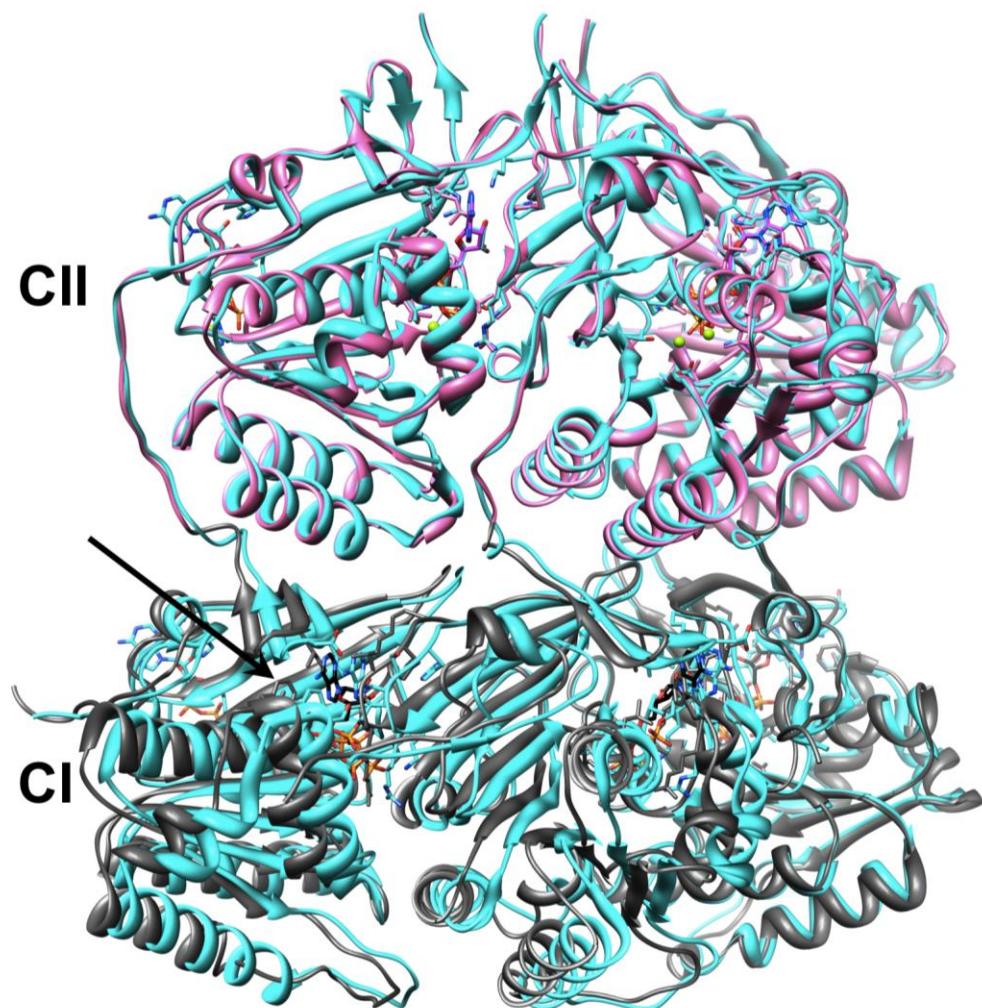
**Figure S1** Sequence alignment for *ThKaiC* proteins from various cyanobacterial strains. 1.*Thermosynechococcus elongatus* BP-1; 2. *Synechococcus elongatus* PCC 7942; 3. *Anabaena variabilis*ATCC 29413; 4. *Nostoc* sp. PCC 7120; 5. *Nodularia spumigena* CCY9414; 6. *Trichodesmium**erythraeum* IMS101; 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. *Crocospaera 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).

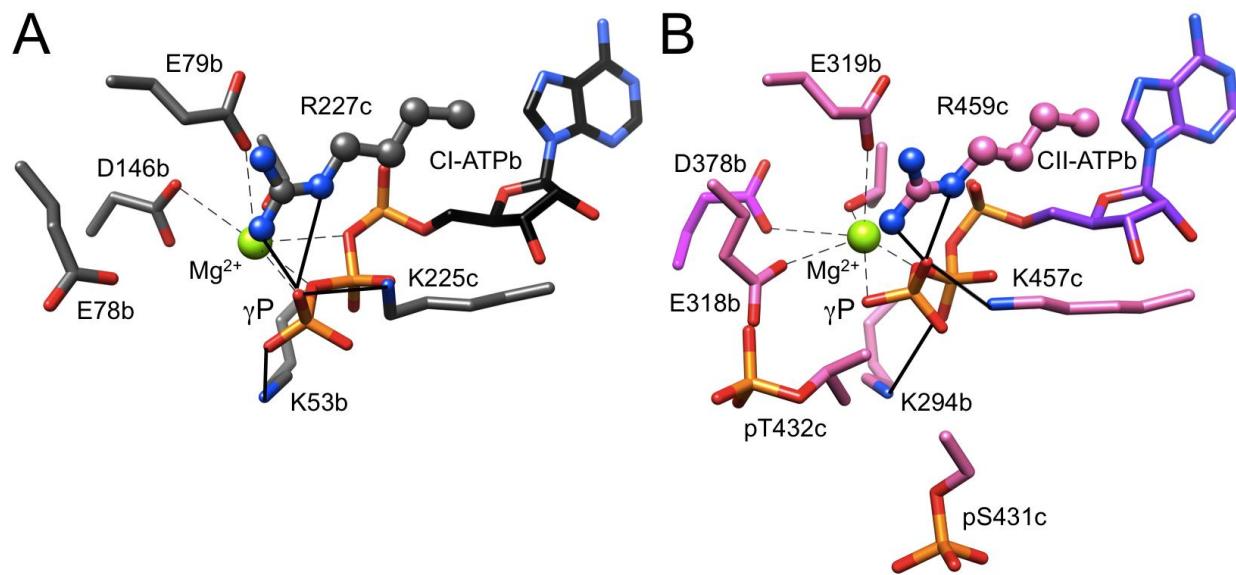


	271	281	291	301	311	321	331	341	351	
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6	g163322..442950.11	Q T L D E M C G G G	F F K D S I I L A T	G A T G T G K T L L	V S K F L O E G C R	O R E R A I L F A Y	E E S R A Q L S R N	A S S W G I D F E E	M E H K G L L K I L	
7	g1678566_AM185.11	K T L D Q M C G G G	F F K D S I I L A T	G A T G T G K T L L	V S K F L E E G C R	O R E R A I L F A Y	E E S R A Q L S R N	A S S W G I D F E E	M E Q K G L L K I L	
8	g1110168_BG52850.11	E I L D K M C G G G	F F K D S I I L A T	G A T G T G K T L L	V S K F L E E G C R	N N E P A M L F A Y	E E S R A Q L F R N	A F S W G I D F E E	C S Y P E S A G L E	
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Consensus	D H L q i I K s e I	a e F k P s R I a i	D S L S A L a R g v	s n n a F R q F V I	G v T g y a K q e e	I a G f f T n T t d	q f M g s h S I T e	s H I S T I T D t I	I I L q V V E I r g	
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Consensus	E M s R a i n V F K	M R G S w H D K g I	R E y t I l a d G p	e l k d s F r N F e	r i l S G s P t r i	s v d E k s e l s r	501	511	521	
Conservation	E M s R a i n V F K	M R G S w H D K g I	R E Y V I T E K G A	E I R D S F R N F E	R I I S C P T R I	S V D E K T E L A R	A K M G D O L E S	E - - -	- - -	- - -
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6	g163322..442950.11	E M s R a i n V F K	M R G S w H D K g I	R E Y M I N D G P	E I I Q D S F R N F E	R I I S C P T R I	S V D E K T E L S R	I V R G V K D K T E	E - - -	- - -
7	g1678566_AM185.11	E M s R a i n V F K	M R G S w H D K g I	R E Y N I T A G P	E I Q D S F R N Y E	R I I S C P T R I	S V D E K T E L S R	I V R G V K D K T E	E - - -	- - -
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9	g175972..32197.11	E M s R a i n V F K	M R G S w H D K g I	R E Y N I T A G P	E I Q D S F R N Y E	R I I S C P T R I	S V D E K T E L S R	I V R G V K D K T A	E - - -	- - -
10	g122842..486929.11	E M s R a i n V F K	M R G S w H D K g I	R E Y N I T A G P	E I R D S F R N Y E	R I I S C P T R I	S V D E K T E L S R	I V R G V K D K T A	E - - -	- - -
11	g1119461_AW42919.11	E M s R a i n V F K	M R G S w H D K g I	R E Y N I T A G P	E I R D S F R N Y E	R I I S C P T R I	S V D E K T E L S R	I V R G V K D K T A	E - - -	- - -
12	g1787798..397936.11	E M s R a i n V F K	M R G S w H D K g I	R E Y I I T N K G P	E I K D S F N S F E	O I F S C A P H R V	V P D O N V Q N -	N - - -	- - -	- - -
13	g1123966..1011817.11	E M s R a i n V F K	M R G S w H D K g I	R E Y I I T S R G P	E I K D S F N S F E	O I F S C A P H R V	V P D O N V Q N -	N - - -	- - -	- - -
14	g1540361_KAIC_PROMP	E M s R a i n V F K	M R G S w H D K g I	R E Y I I T Q Q P	E I K D S F N S F E	O I F S C A P H R V	I S D O N I Q N -	- - -	- - -	- - -
15	g1338636..996454.11	E M s R a i n V F K	M R G S w H D K g I	R E Y V I T D N G P	E I K D S F N S F E	O I F S C A P H R V	I S D O S I P N -	- - -	- - -	- - -
16	g1338650..996454.11	E M s R a i n V F K	M R G S w H D K g I	R E F V I T G N G P	E I K D S F N S F E	O I F S C A P H R V	T T D E R S E L S R	I A R G V S S E D -	- - -	- - -
17	g1493167..617198.11	E M s R a i n V F K	M R G S w H D K g I	R E F V I T G N G P	E I R D S F N F E	R I I S C P H R V	T T D E R S E L S R	I A R S F E G E E S	F - - -	- - -
18	g162304..397937.11	E M s R a i n V F K	M R G S w H D K g I	R E F A F I T G N G P	E I R D S F N F E	R I I S C P H R V	T T D E R S E L S R	I A R S F E G E E S	F - - -	- - -
19	g163670_BY36464.11	E M R R G L T V L K	M R G S M H D K D I	R E F T I D S R G M	H I G R P F R Q V T	G I L S C N P I H I	A P G E I D R M N Q	L F N D D E P O T -	- - -	- - -
20	g1219541_CL23556.11	E M R R G L T V L K	M R G S M H D K D I	R E F T I D S R G M	H I G R P F R Q V T	G I L S C N P I H I	A P G E I D R M N Q	L F N D D E P O T -	- - -	- - -
21	g148569_BQ91720.11	E M R R G L T V L K	M R G S M H D K D I	R E F T I D S R G M	H I G R P F R Q V T	G I L S C N P I H I	A P G E I D R M N Q	L F N D D E P O T -	- - -	- - -
22	g156235_BU60179.11	E M R R G L T V L K	M R G S M H D K D I	R E F T I D S R G M	H I G R P F R Q V T	G I L S C N P I H I	A P G E I D R M N Q	L F N D D E P Q S -	- - -	- - -

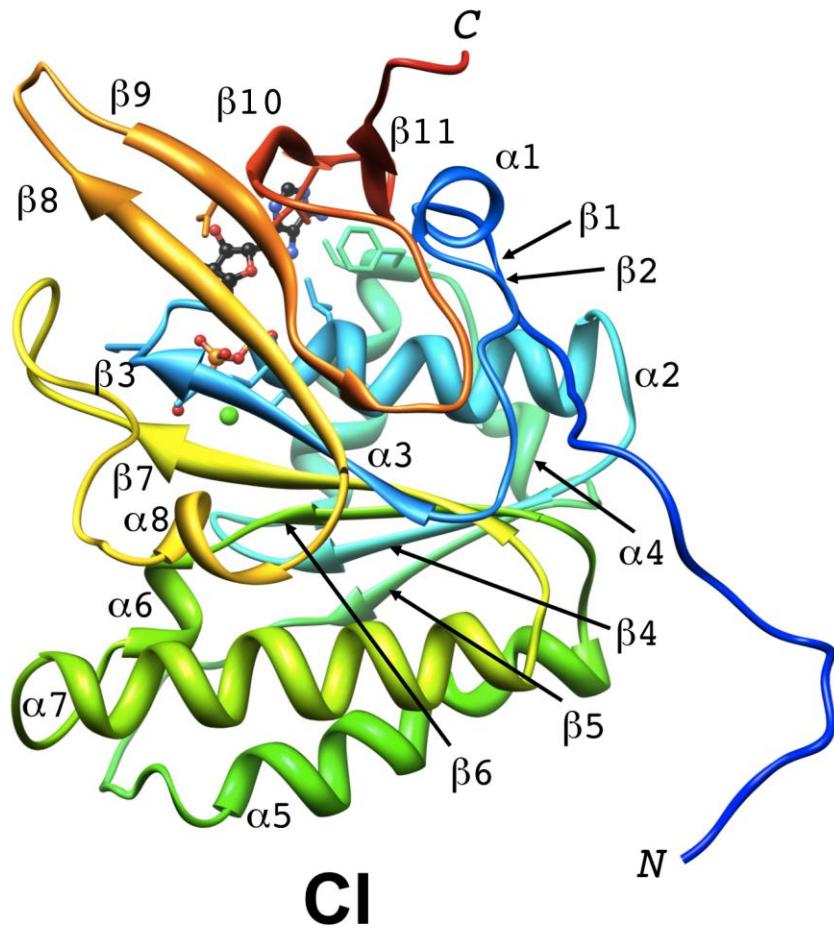
**Figure S2** Conformational differences in the crystal structures of *T. elongatus* KaiC. Trimers composed of subunits a-c from the structures of *T. elongatus* (CI and CII ribbons colored in gray ad pink, respectively) and *S. elongatus* KaiC (cyan ribbon) were superimposed by aligning residues 252-498 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 Cl and CII halves. Arg fingers (A) R227 and (B) R459 at the b/c subunit interface active sites in the Cl 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  $\alpha$ -helices and  $\beta$ -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 *kaiBCp*-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, R218A; CII: R451A) and Arg finger mutants (CI: R226A; CII: 459A). Shown are averages from triple colonies in each case.

