

Supporting Information for

**Carbon metabolism shapes FtsZ levels and cell division in the
cyanobacterium *Anabaena* PCC 7120**

Author/Affiliation

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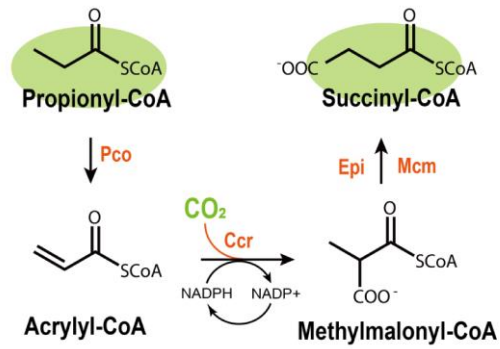
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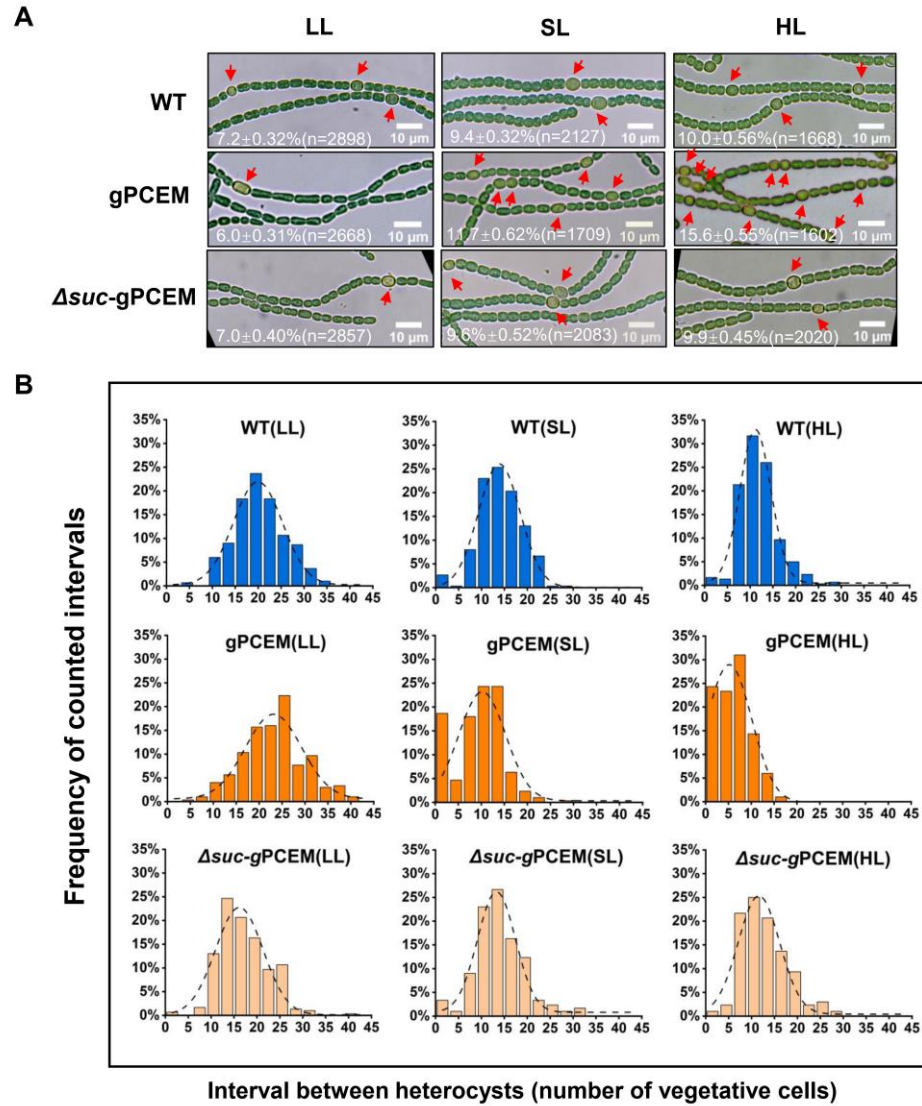
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Supplementary Figure 1 to 16

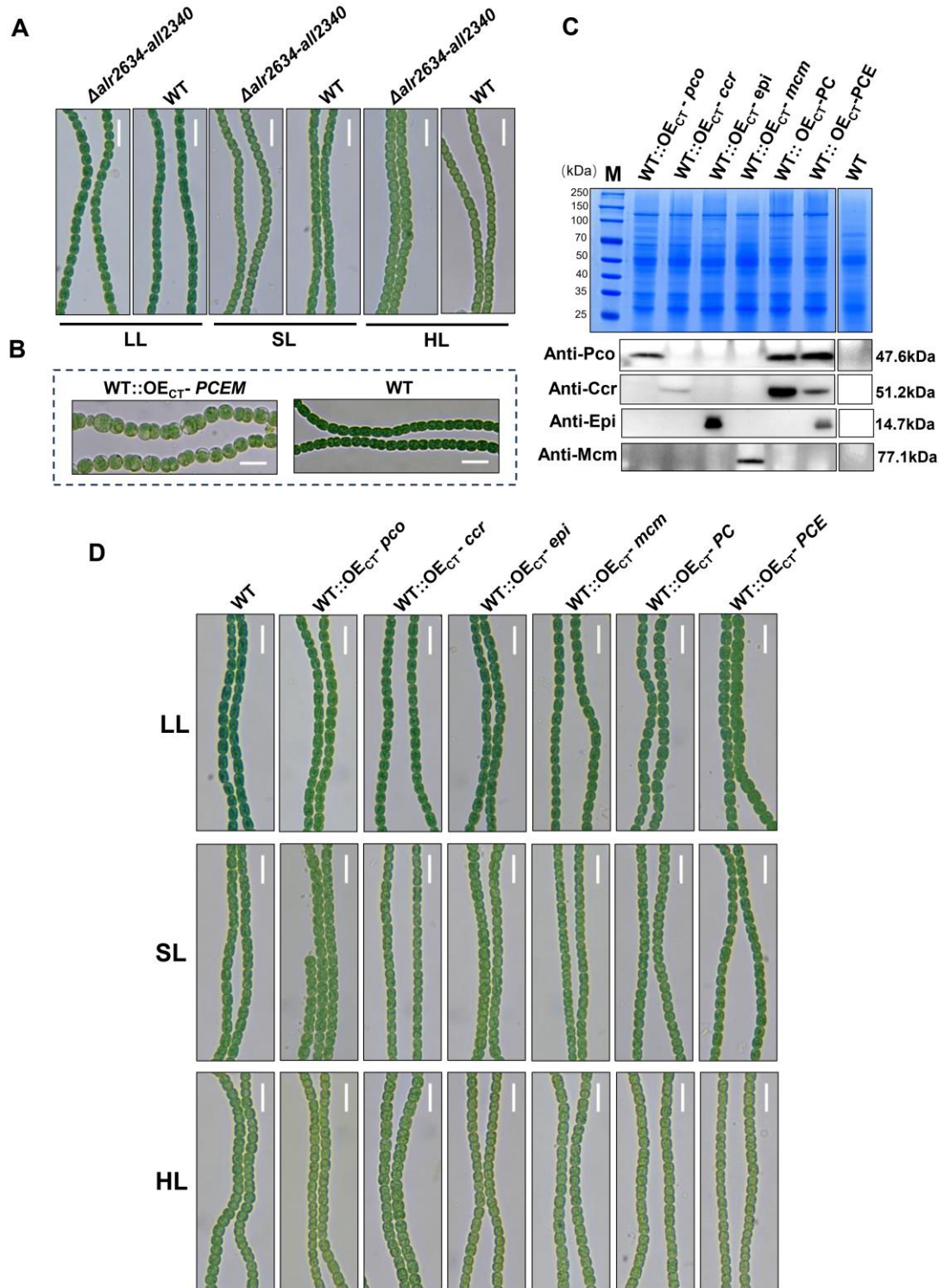
Supplementary Table 1 to 3



Supplementary Figure 1. Catalytic Reactions of the PCEM module. The PCEM module is involved in three individual reaction steps through four enzymes, as indicated. Pco: propionyl-CoA oxidase, Ccr: crotonyl-CoA carboxylase, Epi: emC/mmC epimerase, and Mcm: methyl malonyl-CoA isomerase.



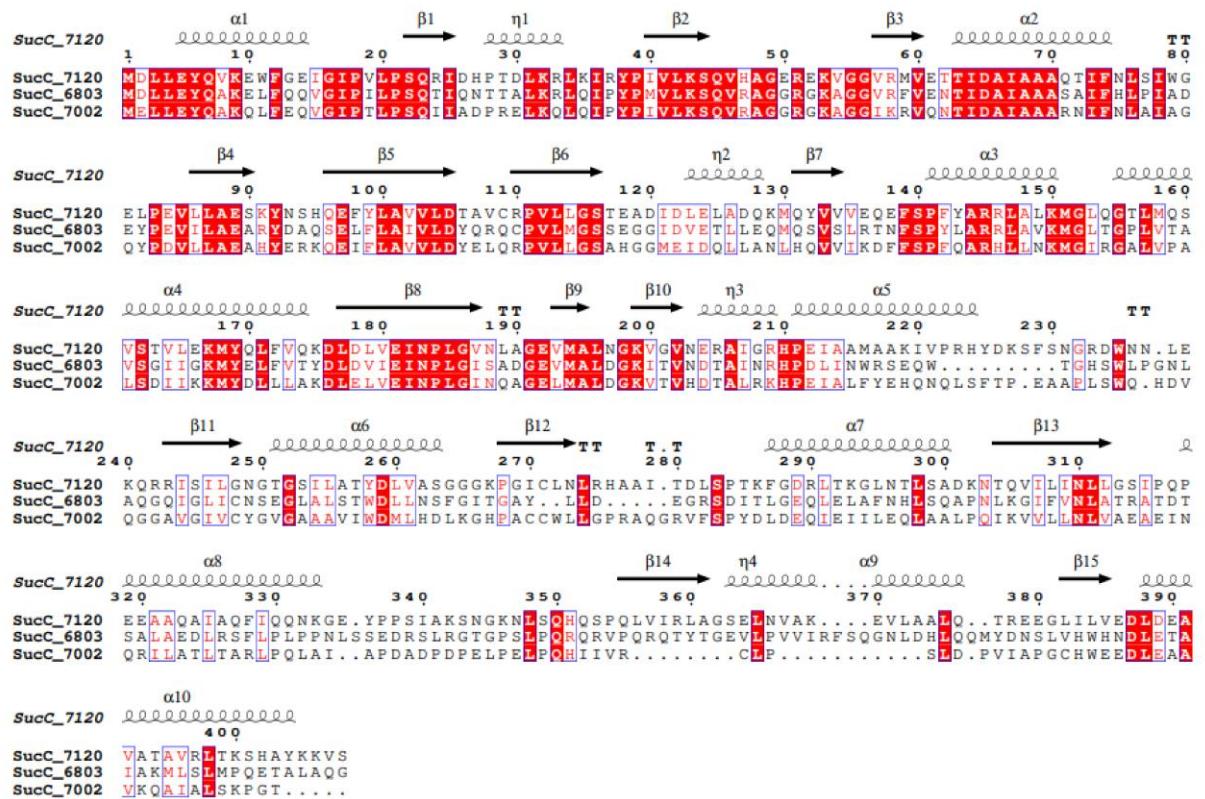
Supplementary Figure 2. Heterocyst differentiation in the gPCEM, WT, and Δ suc-gPCEM strains at different light conditions. (A) Light microscopic images of filaments from the indicated strains at 48 h after initiation of heterocyst differentiation induced by the deprivation of combined nitrogen under different light conditions. Heterocysts are indicated by red arrows. The heterocyst frequency of the strain is indicated with white characters. LL: low light, SL: standard light, HL: high light. n: the number of the counted cells (B) Analysis of heterocyst pattern in different strains, as in (A), at 48 h after initiation of heterocyst differentiation. The number of vegetative cells between two heterocysts (interval between heterocysts) was counted, and the distribution of heterocyst intervals was analyzed according to the frequency of counted intervals.



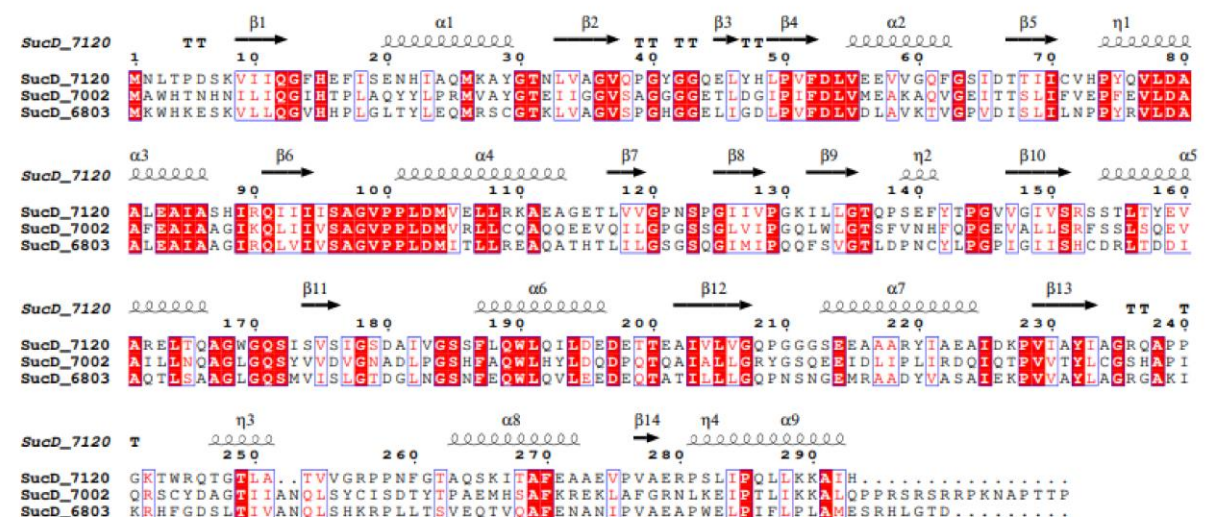
Supplementary Figure 3. Cell morphology defects of the gPCEM strain are not caused by the ectopic protein expression. (A) Microscopic images of *Anabaena* filaments of the WT strain and the $\Delta all2634-all2640$ strain culturing under LL (low light), SL (standard light), and HL (high light). Scale bars represent 10 μ m. (B) Microscopic image of *Anabaena* filaments of the WT::OE_{CT}-PCEM strain from the transformation

colonies on the BG11 plate. (C) Western blotting analysis of the levels of the indicated proteins in the indicated strains. Similar amounts of total proteins extracted from different strains were loaded on the gel, stained with Coomassie Brilliant Blue (up, CBB), or probed with polyclonal antibodies against Pco, Ccr, Epi, or Mcm (down). (D) Microscopic image of *Anabaena* filaments of the indicated overexpression strains culturing under LL, SL and HL. All the overexpression strains under the control of the CT promoter were induced with 1 mM TP (theophylline), 0.3 μ M Cu²⁺. All images were observed at 96 h after the addition of the inducers. PC: *pco-ccr*; PCE: *pco-ccr-epi*. Scale bars represent 10 μ m.

A

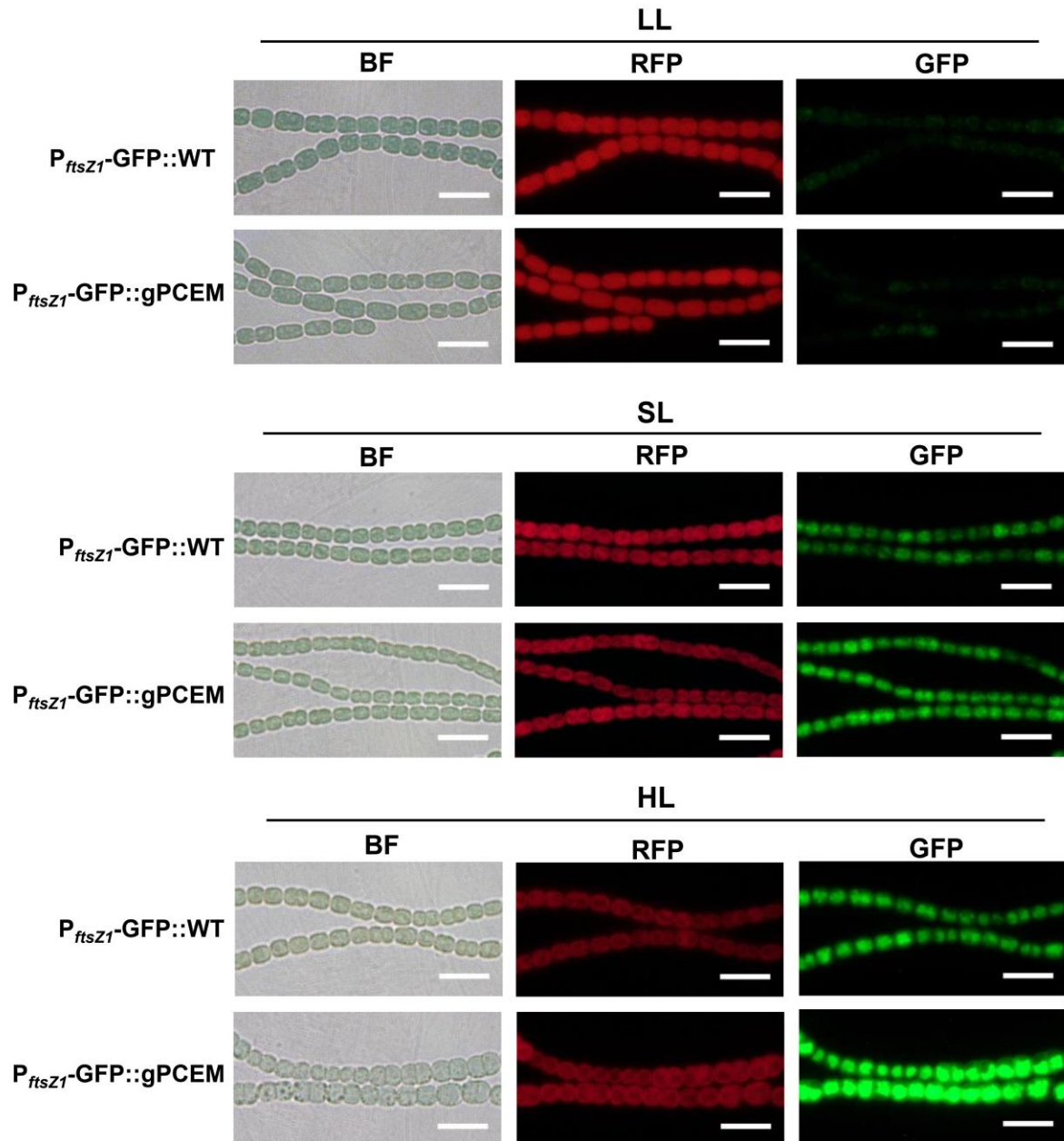


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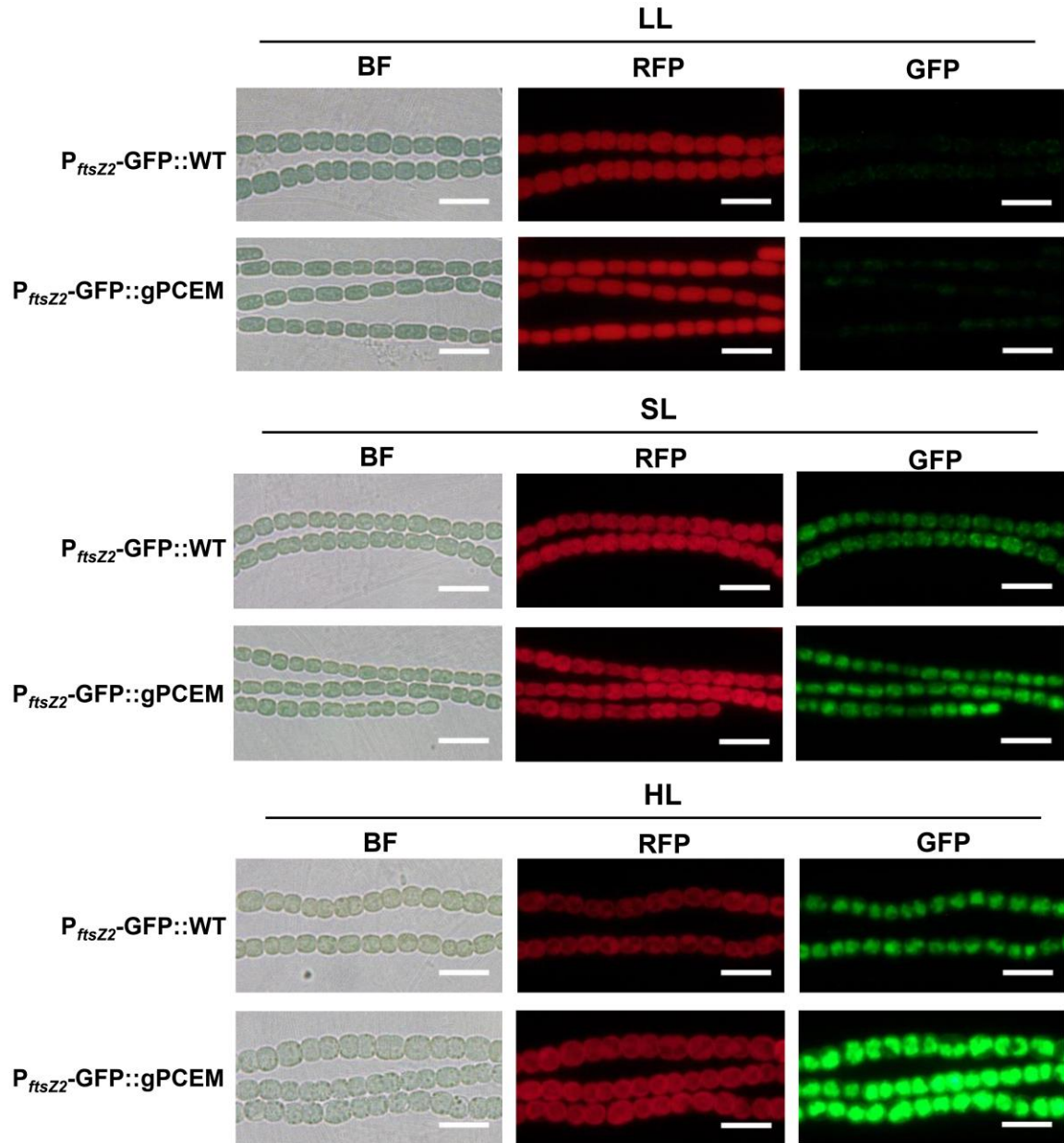
Supplementary Figure 4. Sequence alignment of SucC and SucD. (A) Sequence alignment of SucC from *Anabaena* sp. PCC 7120, *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002. (B) Sequence alignment of SucD from *Anabaena* sp. PCC 7120, *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002. These sequences were aligned using CLUSTAL OMEGA and visualized with ESPrpt 3. X

(<https://esprict.ibcp.fr/ESPrict/cgi-bin/ESPrict.cgi>). Identical residues are shown as white characters on a red background, and similar residues are shown as red characters. The secondary structural elements (top) correspond to those deduced from the structure predicted by AlphaFold 2.



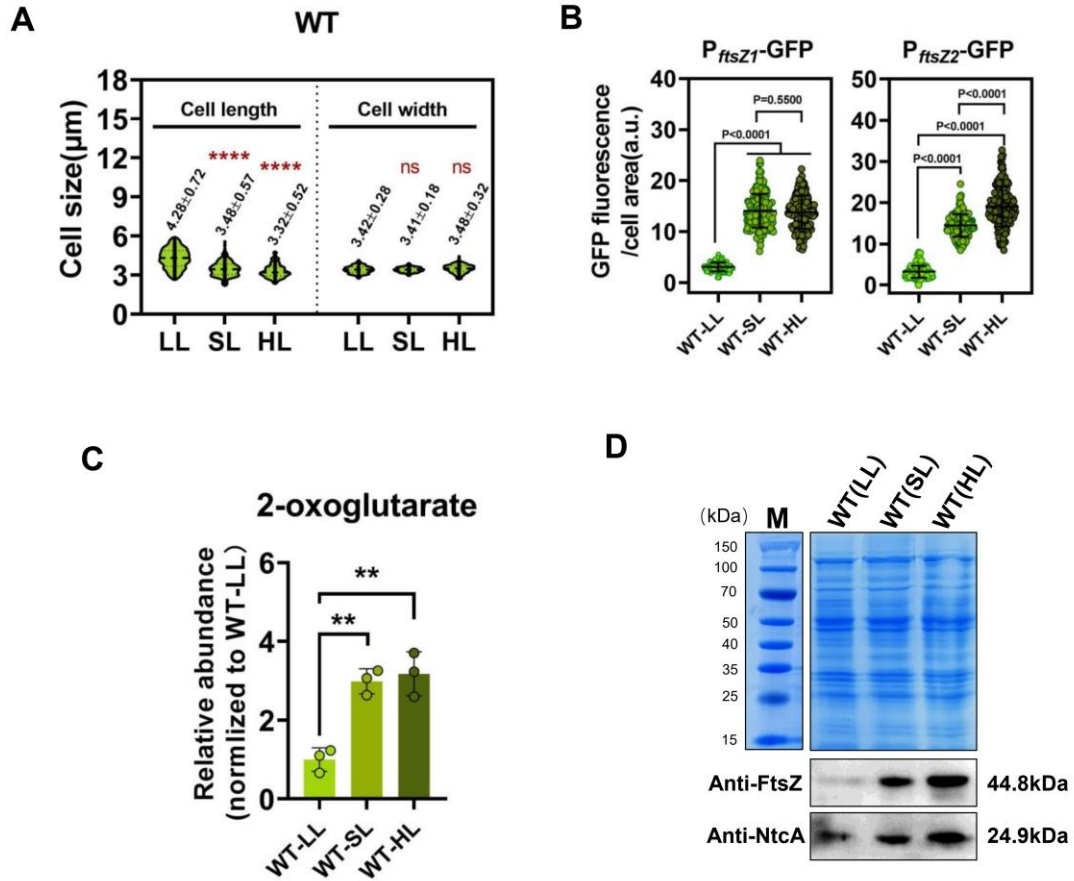
Supplementary Figure 5. Light microscopic images of *PftsZ1-GFP::WT* and *PftsZ1-GFP::gPCEM* strains. Bright-field (BF, left panel), pigment fluorescence (PF, middle panel), and GFP fluorescence (GFP, right panel) microscopic images of *PftsZ1-GFP::WT* and *PftsZ1-GFP::gPCEM* strains under LL (upper), SL (middle), and HL

74 (lower), respectively. Images were taken with the same settings from cultures that had
 75 been induced with 1 mM TP (theophylline), 0.3 μM Cu^{2+} , of combined nitrogen for 12
 76 h (B) or 24 h (C). Scale bars represent 10 μm .

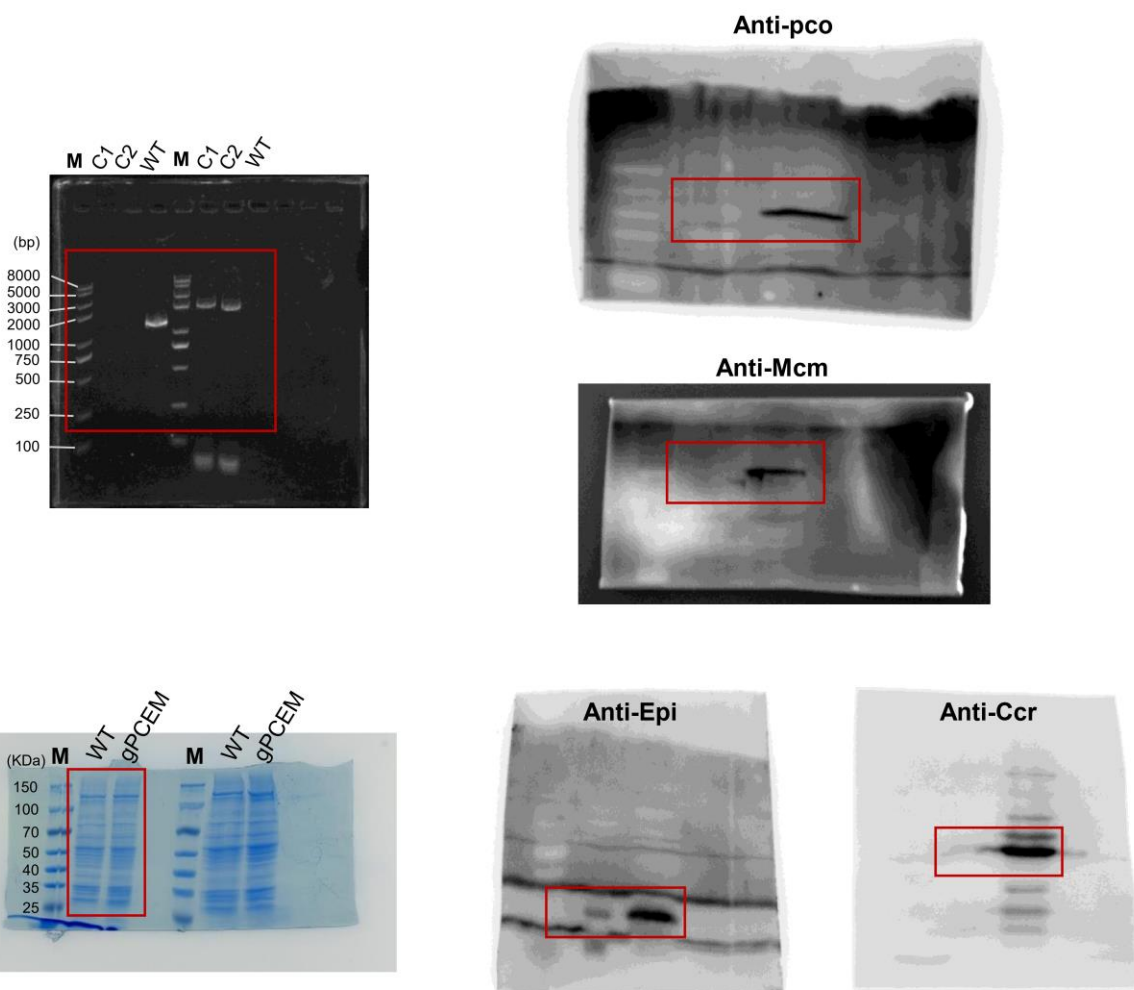


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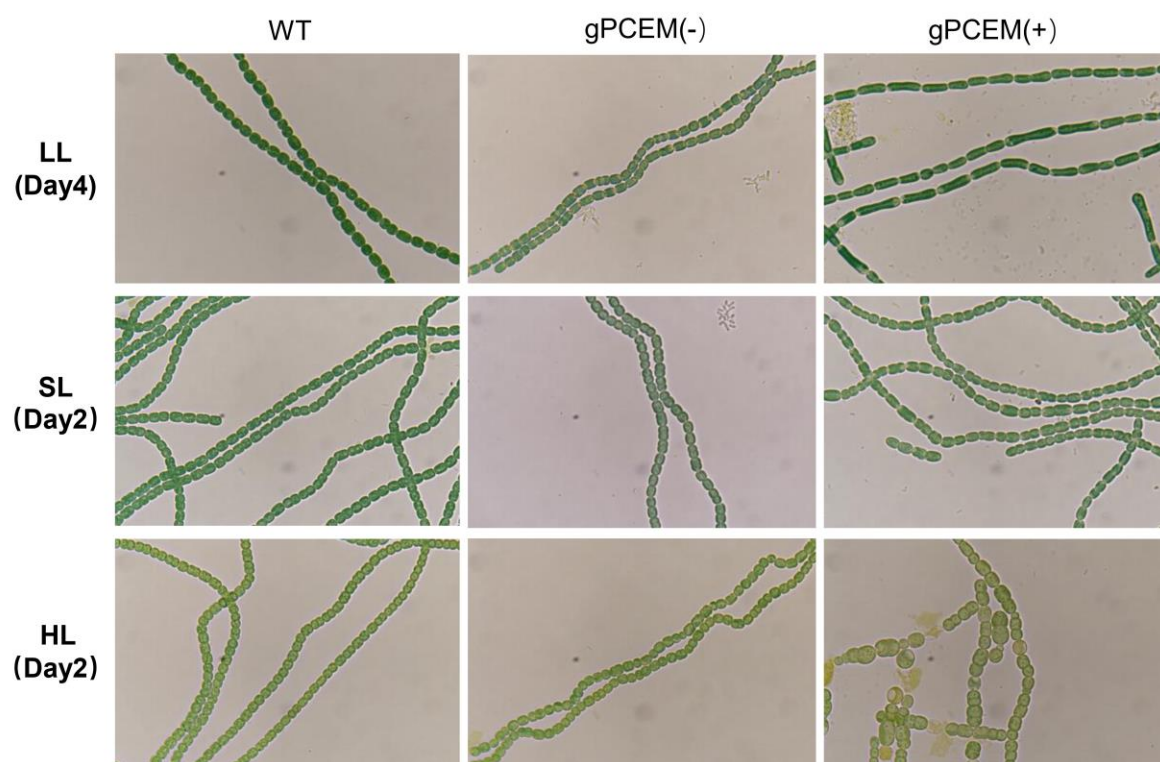
78 **Supplementary Figure 6. Light microscopic images of *PftsZ2*-GFP::WT and *PftsZ2*-**
 79 **GFP::gPCEM.** Bright-field (BF, left panel), pigment fluorescence (PF, middle panel),
 80 and GFP fluorescence (GFP, right panel) microscopic images of *PftsZ2*-GFP::WT and
 81 *PftsZ2*-GFP::gPCEM strains under LL (upper), SL (middle) and HL (lower), respectively,
 82 were acquired and analyzed similarly as in Fig. S5. Scale bars represent 10 μm .



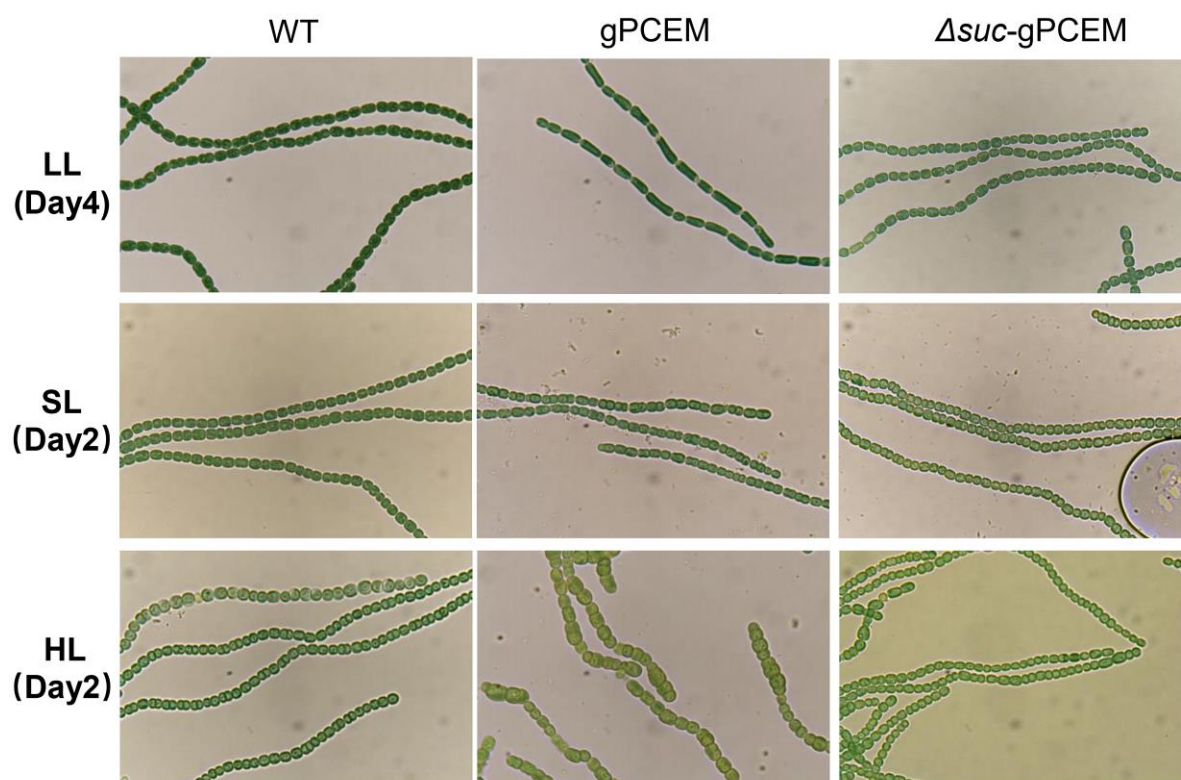
Supplementary Figure 7. NtcA-mediated FtsZ regulation in *Anabaena* under different light conditions. (A) Statistical analysis of cell length and width of the WT *Anabaena* strain at the indicated light conditions. The mean value was indicated with numbers. LL: low light; SL: standard light; HL: high light. Data are derived from Figure 4. The student's t-test examined the differences between LL and SL/HL. Results were considered significant at **, $p<0.05$; **, $p<0.01$; ***, $p<0.001$, **** $p<0.0001$, ns: no significance. (B) Statistical analysis of GFP fluorescence in the WT strain that had a *gfp* gene under the control of the *ftsZ1* (-260bp to -1bp) or the *ftsZ2* (-526bp to -242bp) promoter at the indicated light conditions, data are sourced from Figure 6. (C) Graphical representation of relative changes of the 2-oxoglutarate (2-OG) in WT *Anabaena* under the indicated light conditions. LL: low light; SL: standard light; HL: high light. ** $p<0.01$ indicates statistical significance. (D) Western blotting of FtsZ expression levels in the WT strain under the indicated light conditions.



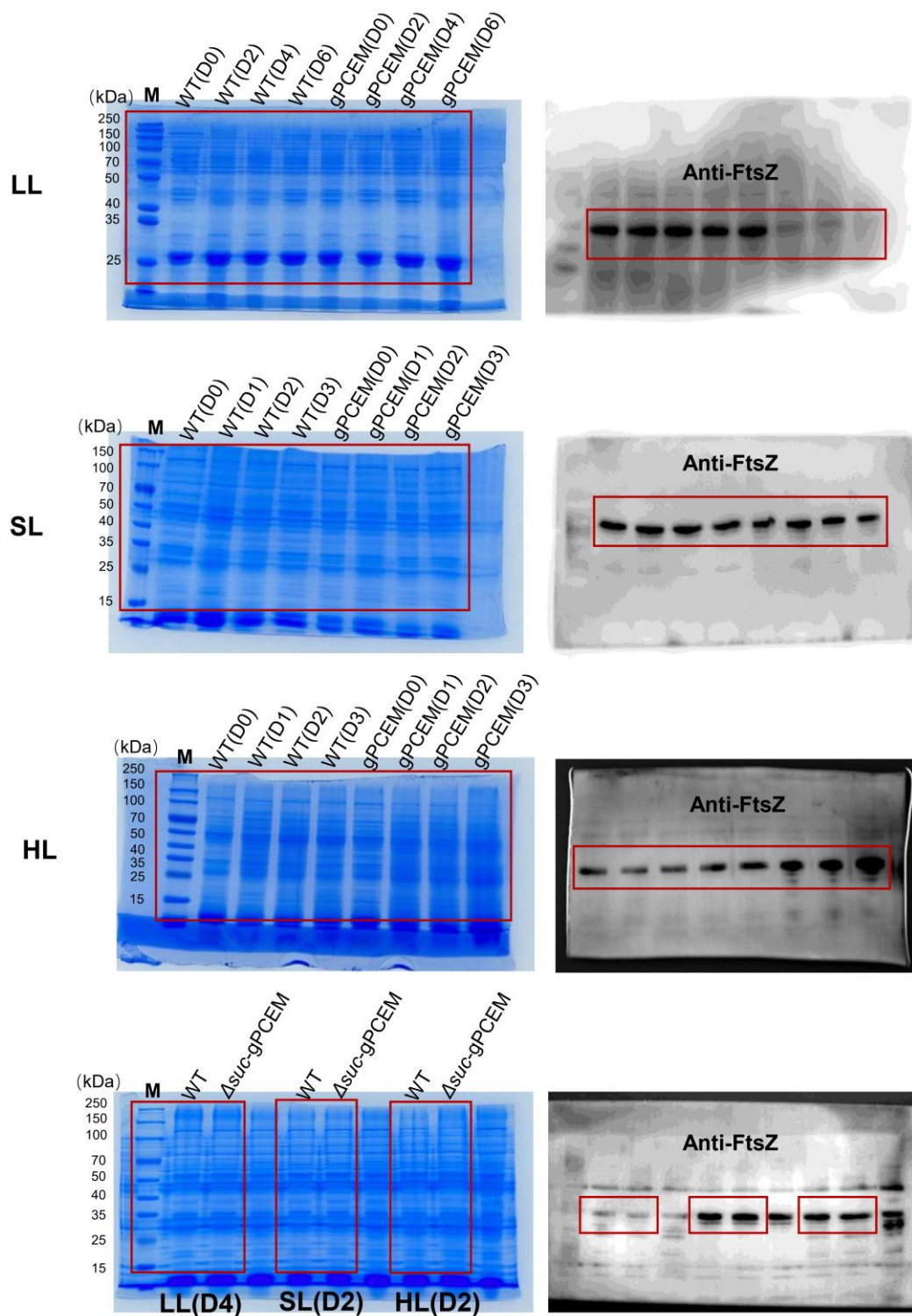
Supplementary Figure 8. Unedited blot/gel images for Fig.1C and 1D.



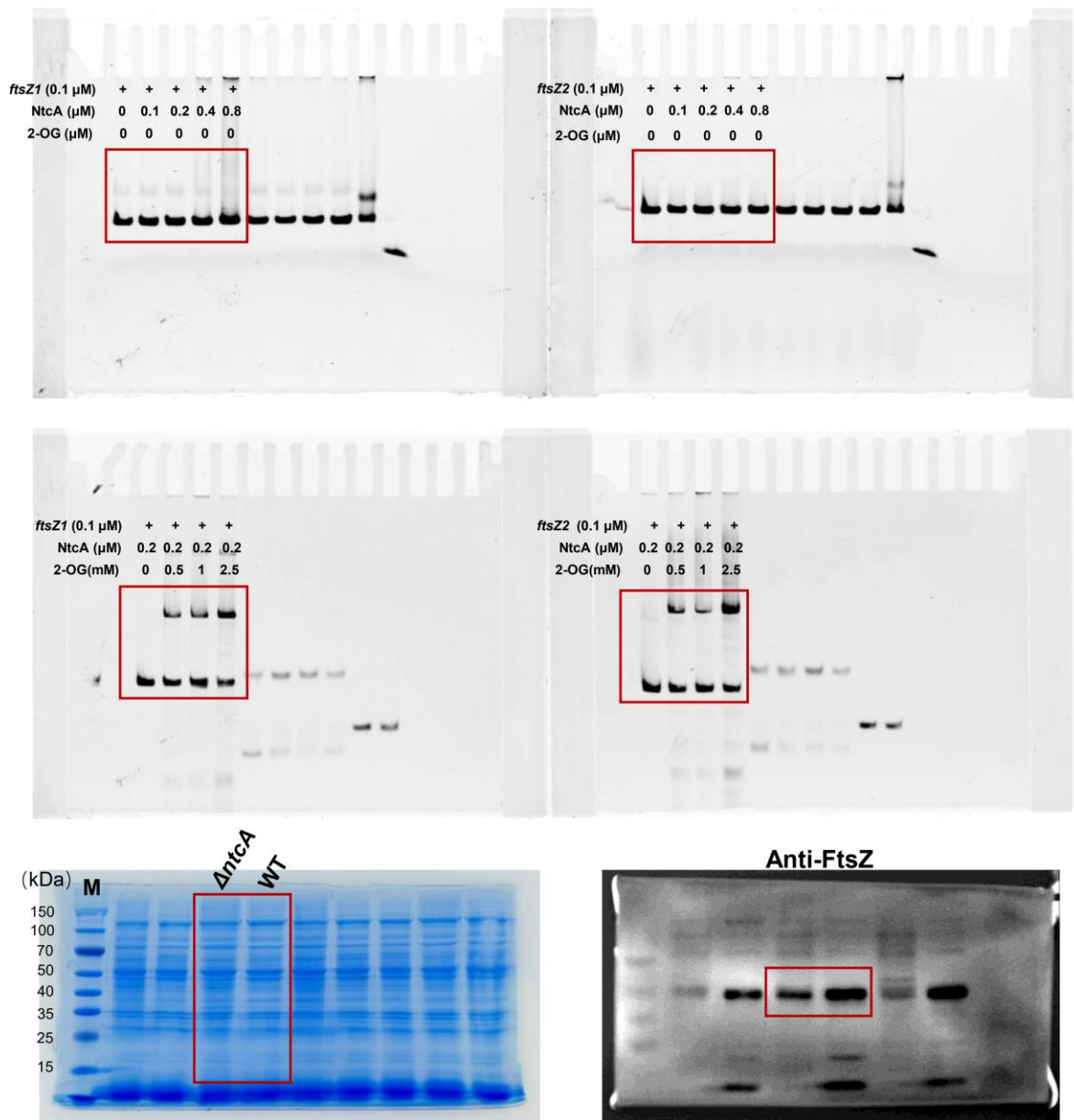
Supplementary Figure 9. Unedited micrographs for Fig.3B.



Supplementary Figure 10. Unedited micrographs for Fig.4A.

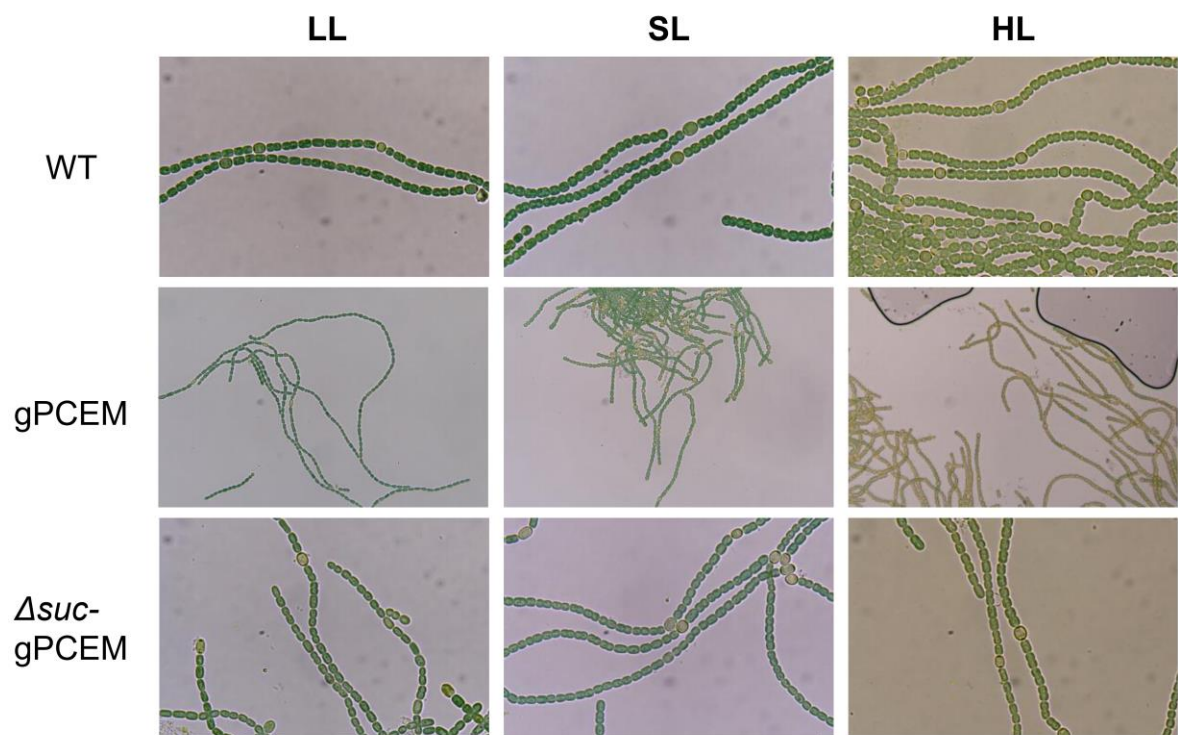


Supplementary Figure 11. Unedited blot/gel images for Fig.5A and 5B.

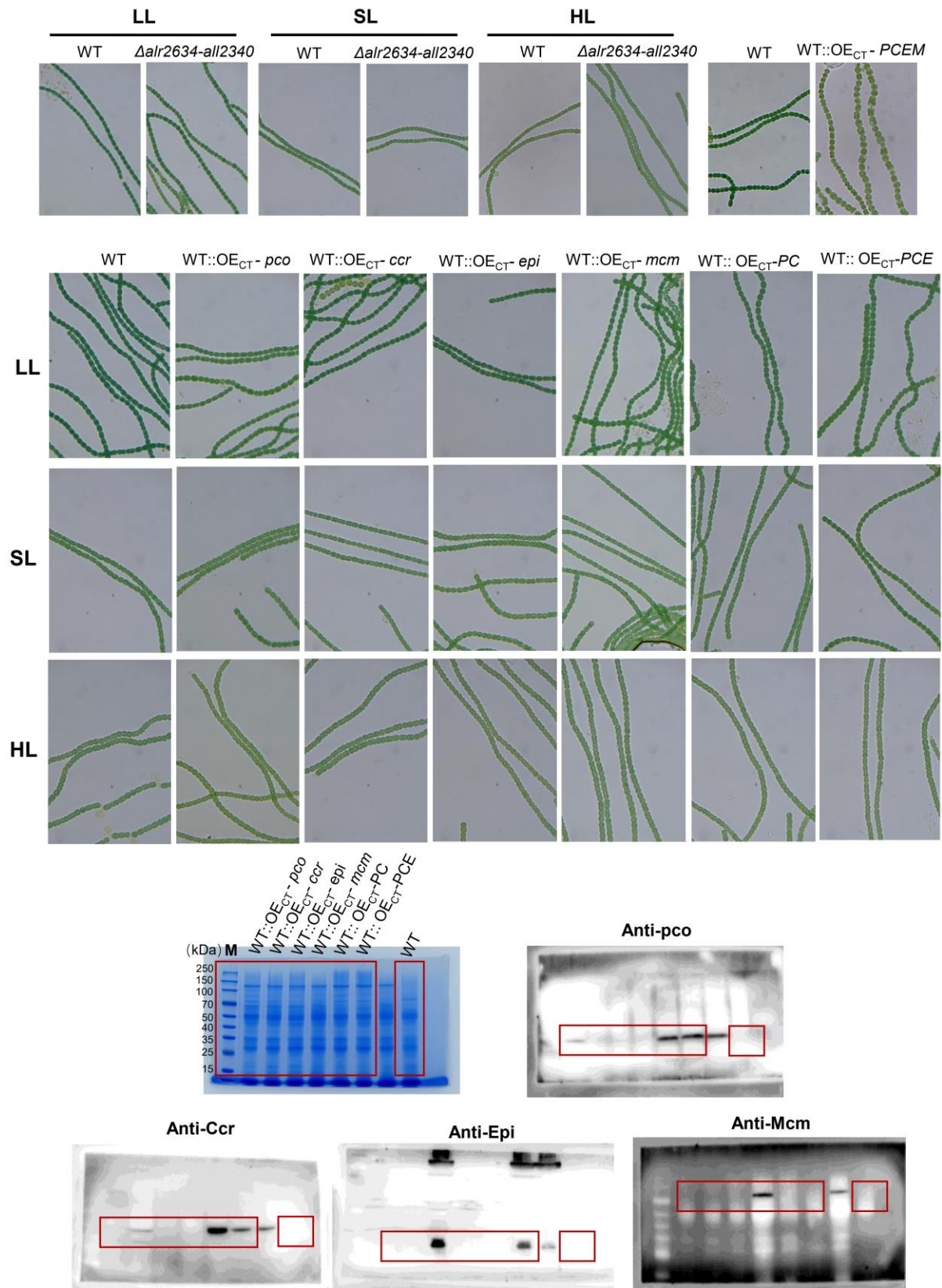


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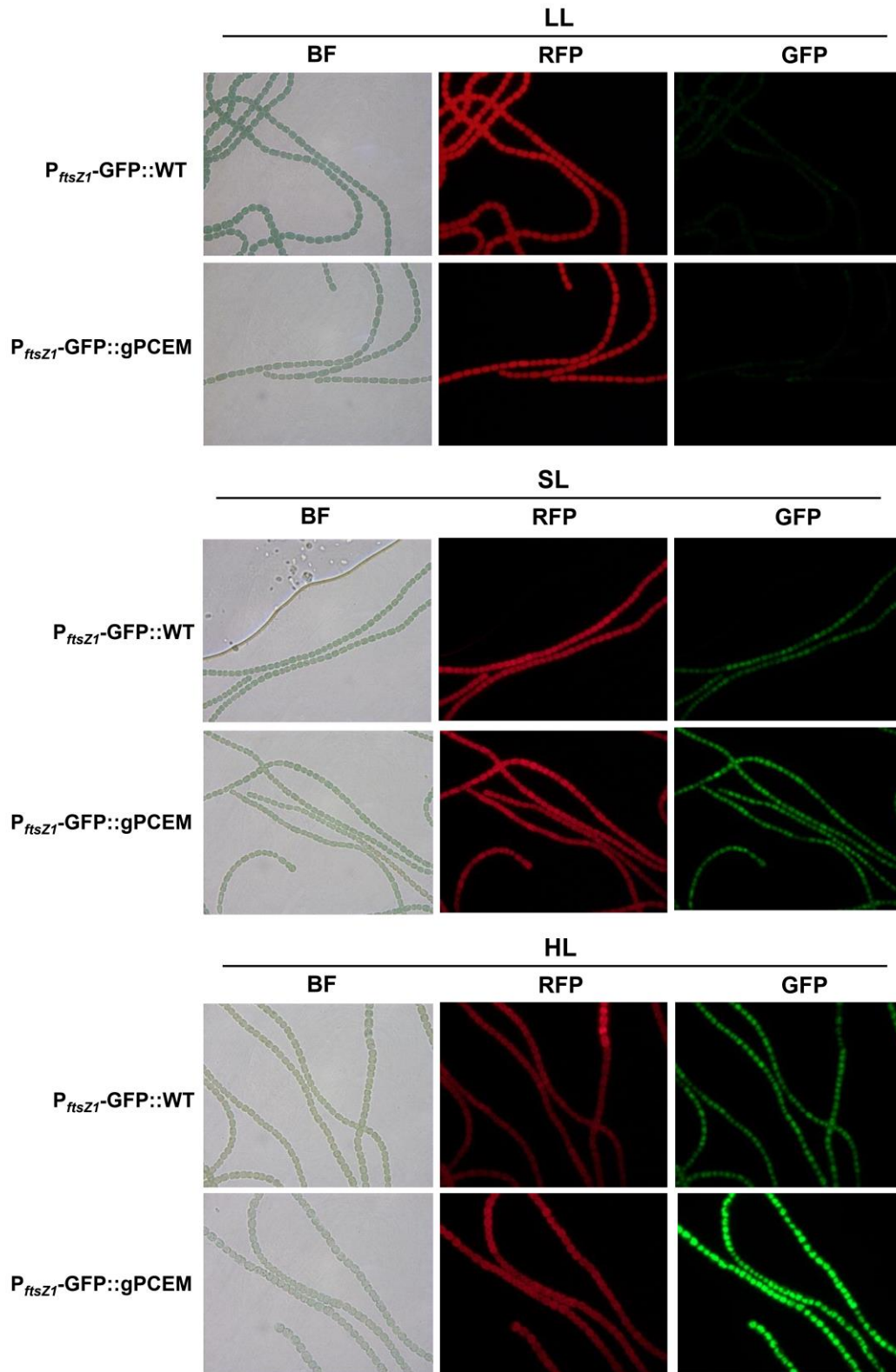
108 **Supplementary Figure 12. Unedited blot/gel images for Fig.6B and 6G.**



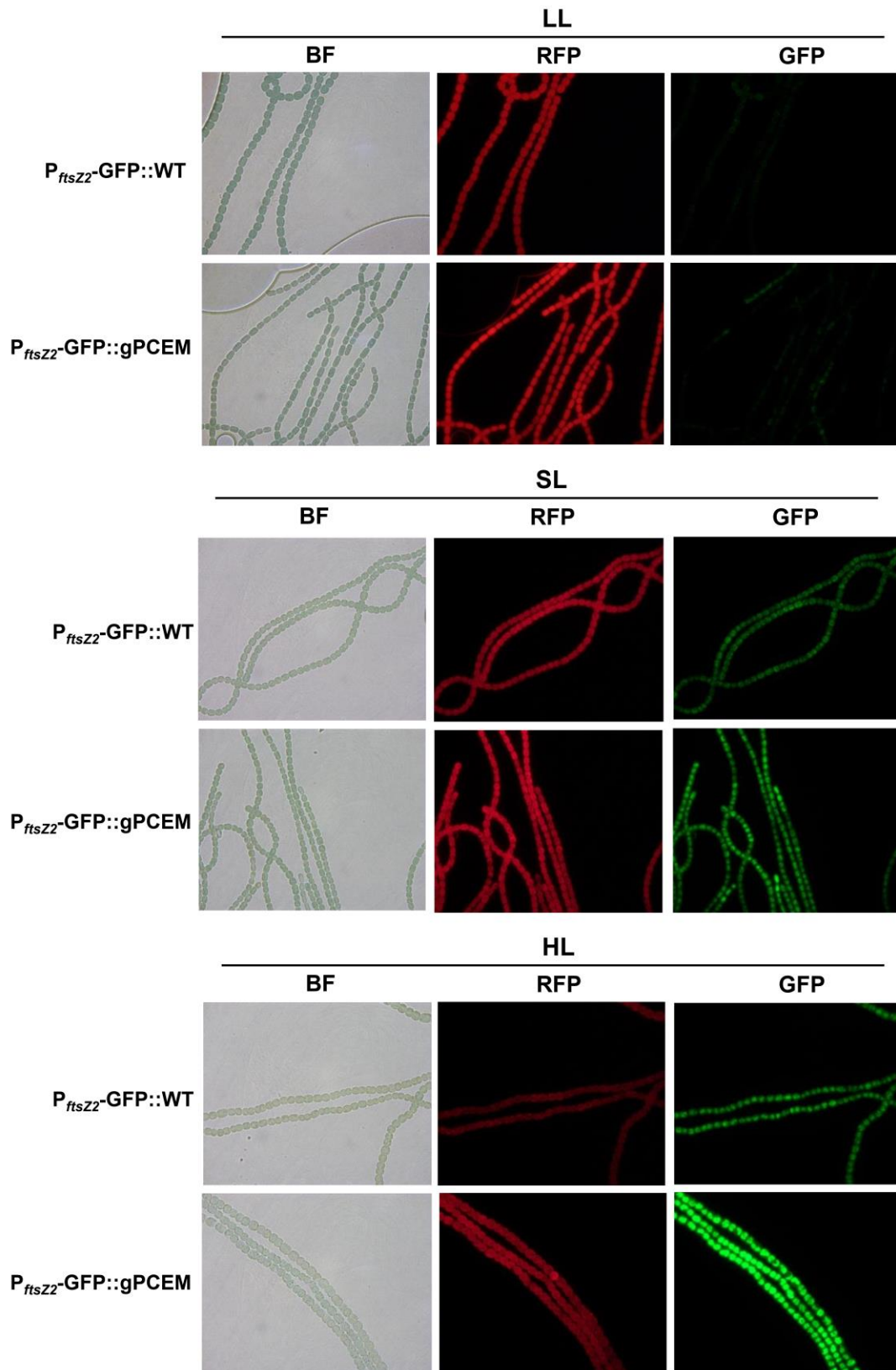
Supplementary Figure 13. Uncropped and unedited micrographs for Supplementary Figure 2.



Supplementary Figure 14. Uncropped and unedited micrographs and blot/gel images for Supplementary Figure 3.



Supplementary Figure 15. Uncropped and unedited micrographs for Supplementary Figure 5.



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120 **Supplementary Figure 16. Uncropped and unedited micrographs for**
 121 **Supplementary Figure 6.**

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Supplementary Table 1. Strains used in this study

Strains	Description	Source
<i>Anabaena</i> PCC 7120	Wild type	Pasteur Culture Collection
gPCEM	A markless insertion mutant by insert PCEM Module	This study
$\Delta alr2634$ - <i>all2640</i>	A markless deletion mutant by removing the ORF <i>Alr2634</i> to <i>All2640</i>	This study
Δsuc -gPCEM	A markless deletion mutant by removing the ORF of <i>sucCD</i> in the gPCEM background	This study
WT::OE _{CT} - <i>pco</i>	Gene <i>pco</i> overexpression strain, under the control of the CT promoter (inducible by copper and theophylline)	This study
WT::OE _{CT} - <i>ccr</i>	Gene <i>ccr</i> overexpression strain, under the control of the CT promoter	This study
WT::OE _{CT} - <i>epi</i>	Gene <i>epi</i> overexpression strain, under the control of the CT promoter	This study
WT::OE _{CT} - <i>mcm</i>	Gene <i>mcm</i> overexpression strain, under the control of the CT promoter	This study
WT::OE _{CT} - <i>PC</i>	Gene <i>pco-ccr</i> overexpression strain, under the control of the CT promoter	This study
WT::OE _{CT} - <i>PCE</i>	Gene <i>pco-epi</i> overexpression strain, under the control of the CT promoter	This study
WT::OE _{CT} - <i>PCEM</i>	Gene <i>pco-mcm</i> overexpression strain, under the control of the CT promoter	This study
P _{<i>ftsZ1</i>} -GFP::WT	WT with <i>ftsZ1</i> -gfp, transcriptional fusion on plasmid pCT;	This study
P _{<i>ftsZ2</i>} -GFP::WT	WT with <i>ftsZ2</i> -gfp, transcriptional fusion on plasmid pCT;	This study
P _{<i>ftsZ1</i>} -GFP::gPCEM	gPCEM with <i>ftsZ1</i> -gfp, transcriptional fusion on plasmid pCT;	This study
P _{<i>ftsZ2</i>} -GFP::gPCEM	gPCEM with <i>ftsZ2</i> -gfp, transcriptional fusion on plasmid pCT;	This study
DH5 α	Used for routine transformation	
BL21 (DE3)	Used for protein expression	
Alice	Used for conjugation	

Supplementary Table 2. Plasmids used in this study.

Plasmids	Description	Source
pCT	Km ^r Nm ^r ; expression plasmid under the control of the CT promoter	Accession number: MK948095
pCpf1-sp	Sm ^r Sp ^r ; CRISPR-Cpf1-Based Genome Editing vector	#122188 (Addgene)
pCpf1-PCEM-F1143	Sp ^r Sm ^r ; for constructing gPCEM by CRISPR-pCpf1b genome editing system	This study
pCpf1-PCEM-F1574	Sp ^r Sm ^r ; for constructing gPCEM by CRISPR-pCpf1b genome editing system	This study
pCpf1-Malr2631-all2640 -F1143	Km ^r Nm ^r ; for constructing <i>Δalr2631-all2640</i> by CRISPR-pCpf1b genome editing system	This study
pCpf1-Malr2631-all2640 -F1574	Km ^r Nm ^r ; for constructing <i>Δalr2631-all2640</i> by CRISPR-pCpf1b genome editing system	This study
Pcpf1-MsucCD-F94	Km ^r Nm ^r ; for constructing <i>Δsuc</i> by CRISPR-pCpf1b genome editing system	This study
Pcpf1-MsucCD-F340	Km ^r Nm ^r ; for constructing <i>Δsuc</i> by CRISPR-pCpf1b genome editing system	This study
pCT- <i>pco</i>	Km ^r Nm ^r ; for constructing <i>pco</i> overexpression strain	This study
pCT- <i>ccr</i>	Km ^r Nm ^r ; for constructing <i>ccr</i> overexpression strain	This study
pCT- <i>epi</i>	Km ^r Nm ^r ; for constructing <i>epi</i> overexpression strain	This study
pCT- <i>mcm</i>	Km ^r Nm ^r ; for constructing <i>mcm</i> overexpression strain	This study
pCT-PC	Km ^r Nm ^r ; for constructing <i>pco-ccr</i> overexpression strain	This study
pCT-PCE	Km ^r Nm ^r ; for constructing <i>pco-epi</i> overexpression strain	This study
pCT-PCEM	Km ^r Nm ^r ; for constructing <i>pco-mcm</i> overexpression strain	This study
pET28a-Pco	Km ^r ; protein expression plasmid containing the N-terminal His-tagged <i>pco</i> ORF;	This study
pET28a-Ccr	Km ^r ; protein expression plasmid containing the N-terminal His-tagged <i>ccr</i> ORF;	This study
pET28a-Epi	Km ^r ; protein expression plasmid containing the N-terminal His-tagged <i>epi</i> ORF;	This study
pET28a-Mcm	Km ^r ; protein expression plasmid containing the N-terminal His-tagged <i>mcm</i> ORF;	This study

Plasmids	Description	Source
pRL- <i>ftsZ1</i> -gfp	Sp ^r ; transcriptional fusion plasmid that had a gfp gene under control of the <i>ftsZ1</i>	This study
pRL- <i>ftsZ2</i> -gfp	Sp ^r ; transcriptional fusion plasmid that had a gfp gene under control of the <i>ftsZ2</i>	This study

127 ^a Km, kanamycin; Nm, neomycin; Sm, streptomycin; Sp, spectinomycin;

128 **Supplementary Table 3. Primers used in this study**

Primers	Sequence (5'-3')
Palr2634-F	TGGCAGAAATTCGATATCTAGATCCATCCTCAGTTTCACACACTTC
Palr2634-R	GTAAATTTACAGGCTTTAGAGCTACCGTTTGCGATTCA
PCT-Fran	TAAAGCCTGTGAAATTAAC
PCT-PM4R	GCACTTGATAGTACCGCCATCATCTTGTTGTTACCTCCTTAGC
Ppco-ctF	atggcgggtactatcaagtgc
Pmcm-R	TTAGGAACGCGCTTCTCTA
Pall2640-F	TAGAGAAGCGCGTTCCTAA cttcggggatatgtcctctaaca
Pall2640-R	CGCAACGTTGTTGCCATTGCCCCAAACGGACAATTTCTCCTA
cr_all2638F1143F	agatCACAGCACACACCGAAGAAGAT
cr_all2638F1143R	agacATCTTCTTCGGTGTGTGCTGTG
cr_all2635F1574F	agatACCATCGTCGTTGCTTAGTTTG
cr_all2635F1574R	agacCAAATAAGCAACGACGATGGT
Pall2637-F	ttaacatgactctaagttaagttgaaataactctg
Pall2639-R	ATGCGTGAGCTAAAAATCACCTATGA
PccrF-22	tatccgtcgtgaacgtcat
PmcmR-22	CCAGATCGAATGCGACAGAC
Palr2634-R2	TGTTAGAGGACATATCCCCGAAG GAGCTACCGTTTGCGATTCA
Pall2640-F2	cttcggggatatgtcctctaaca
Palr2634-seqF	tttctcttcactttgttgccaacaatgg
Pall3914-upF	TGGCAGAAATTCGATATCTAGATCattaaaaccgggtatcaggaggaggaa
Pall3914-upR	TGCTATGCACCTTAATTTATACGTTTAG
Pall3914-downF	CTAAACGTATAAATTAAGGTGCATAGCAgtaattcaagatgacggatttatgagtt
Pall3914-downR	CGCAACGTTGTTGCCATTGCAGCTATGTGCTTATTTAAGTGACATCAA
cr_F94F	agatAAACGCTTAAAAATTCGCTACC
cr_F94R	agacGGTAGCGAATTTTAAAGCGTTT
cr_F370F	agatGAATTAGCTGATCAAAAAATGC
cr_F370R	agacGCATTTTTTGTATCAGCTAATTC
Pall3913-R1291m	TGAAATCTTACTCACCTATGATAATGTGCG
Pall3913-F1m	atgaacttaacgccagatagtaaag
Pall3913-R778m	AATTAGGTGGACGACCGACAA

Primers	Sequence (5'-3')
Ppco-CTF	TGCTAAGGAGGTAACAACAAGATGATGGCGGTACTATCAAGTGCAGAT
Ppco-CTR	AGTGCTACCACCACCAGAACCCCCTTATAGGCGGCTACGCGTTGC
Pccr-CTF	TGCTAAGGAGGTAACAACAAGATG atggctgcgtcagcagcacc
Pccr-CTR	AGTGCTACCACCACCAGAACCCCCTttggctgaattacatggccttcagagg
Pepi-CTF	TGCTAAGGAGGTAACAACAAGATGatgataggaaggctaaatcacgtag
Pepi-CTR	AGTGCTACCACCACCAGAACCCCCTTAAACCTGTTCCAACCTCCACCA
Pmcm-CTF	TGCTAAGGAGGTAACAACAAGATGatgacagaagatttagacgcttg
Pmcm-CTR	AGTGCTACCACCACCAGAACCCCCTTAGGAACGCGCTTCTCTA
pPM-CTF	TGCTAAGGAGGTAACAACAAGATGatggcggtactatcaagtgc
PV_19	CATCTTGTTGTTACCTCCTTAGCA
PV_20	GGGGGTTCTGGTGGTGGTAGCACT
Palr3858F260m	tgtcaagctacgtgctgaGaattagctgtgcattcacctatg
Palr3858R1m	TGGATTTCGGGAAGGTAGATAAACG
Palr3858F526m	tgtcaagctacgtgctgacagaatagatacctcaaattctcagt
Palr3858R242m	ATAGTGTTTGGCTCACCATAGG
PpCT-R2979	TCCAAAAAAAAAACCCCGCCGAA
PGFP-atgF	atgggatccaaggcctctggtgg
Palr3858F526m-2	ttcgcggggttttttttggga cagaatagatacctcaaattctcagt
Palr3858R242m-2	CCACCAGAGGCCTTGGATCCCAT ATAGTGTTTGGCTCACCATAGG
Palr3858F260m-2	ttcgcggggttttttttggga Gaattagctgtgcattcacctatg
Palr3858R1m-2	CCACCAGAGGCCTTGGATCCCAT TGGATTTCGGGAAGGTAGATAAACG

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