

Supplementary Information

PATAN-domain regulators interact with the Type IV pilus motor to control phototactic orientation in the cyanobacterium *Synechocystis*

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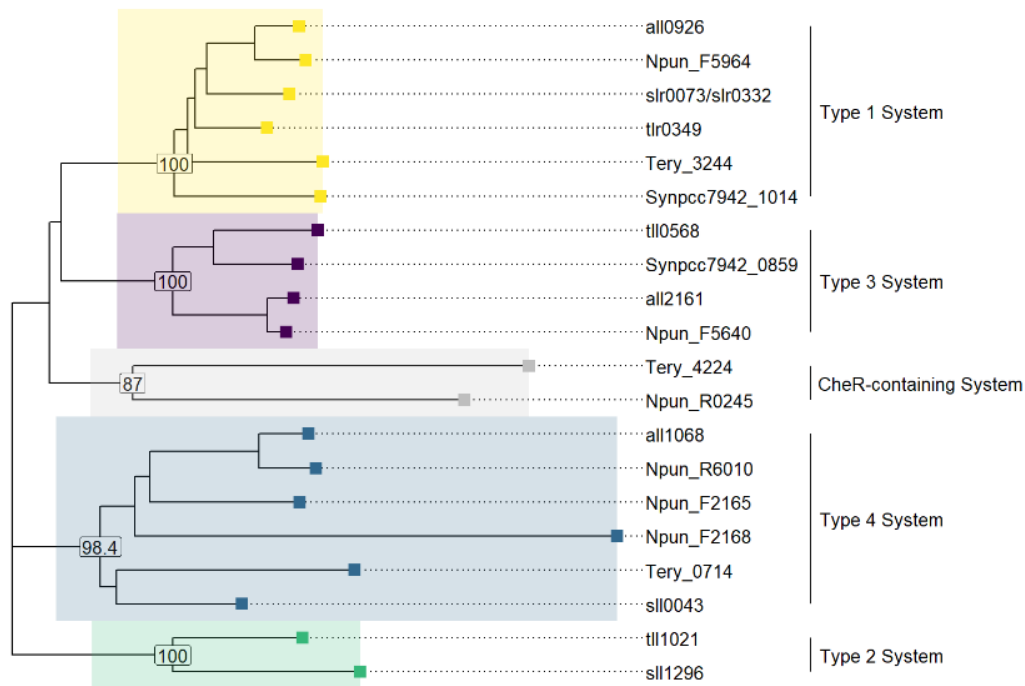


Figure S1: CheA histidine kinases in cyanobacteria are part of distinct chemosensory systems. The maximum likelihood phylogenetic tree was constructed from CheA homologs found in the chemosensory systems of a subset of cyanobacteria listed in the MiST3.0 database (Gumerov et al., 2020). Sequences were aligned with MAFFT and unreliable columns removed by GUIDANCE2 (--bootstraps 100--maxiterate 1000--localpair) (Sela et al., 2015). The evolutionary history was inferred using an LG model (Le and Gascuel, 2008) with a discrete gamma distribution (+G). The percentages at nodes are bootstrap probabilities calculated using 500 replicates. For clarity, the N- and C-terminal PilL sequences from *Synechocystis* (encoded by two annotated open reading frames *slr0073* and *slr0322*) were concatenated before the analysis.

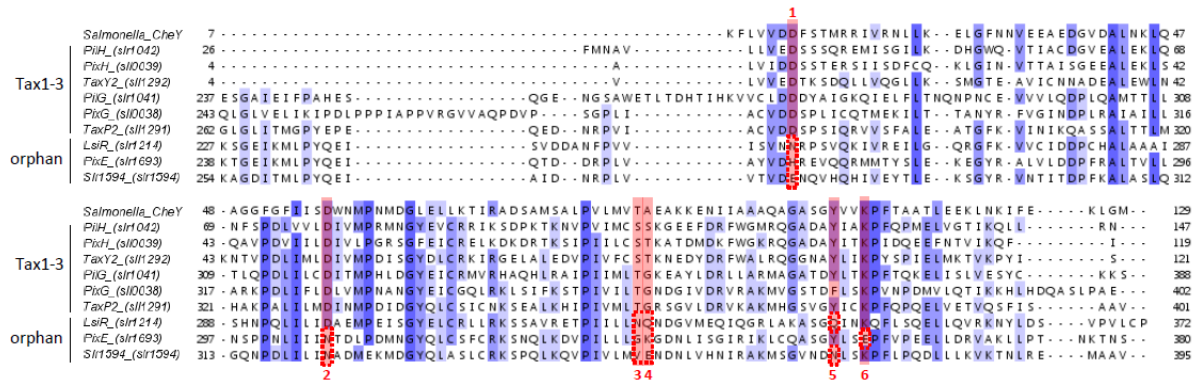


Figure S2: REC domain conservation of CheY- and PatA-type response regulators. Multiple sequence alignment of the REC domains from the *Synechocystis* CheY- and PatA-type proteins compared to *Salmonella* CheY. Sequence identity is shown in blue and columns implicated in protein phosphorylation are shaded red. (1) D critical for metal ion binding; (2) conserved phosphor-accepting D; (3) T/S interact with phosphoryl group; (4) typically A/G but sometimes S/T: allows access to phosphorylation site; (5-6) (F/Y)xxK motif important for phosphorylation-mediated conformational changes.

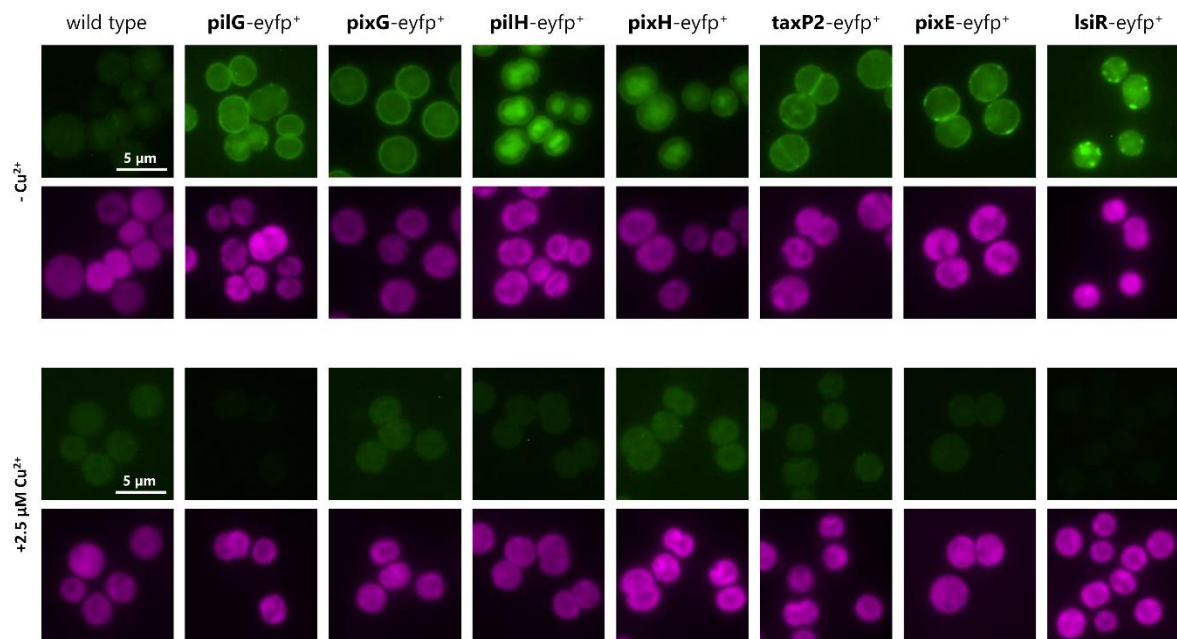


Figure S3: Inducible expression of tagged response regulator proteins. The proteins harboring a C-terminal eYFP fusion are expressed under the control of the copper-repressed *petJ* promoter. The expression cassette was inserted into a neutral genomic locus in wild-type *Synechocystis* cells via homologous recombination. Cells were grown on 0.5% BG11 agar plates without copper to induce gene expression or with the addition of 2.5 μM CuSO₄ to repress the *petJ* promoter. eYFP fluorescence is shown in green and chlorophyll fluorescence in magenta. Scale bars = 5 μm.

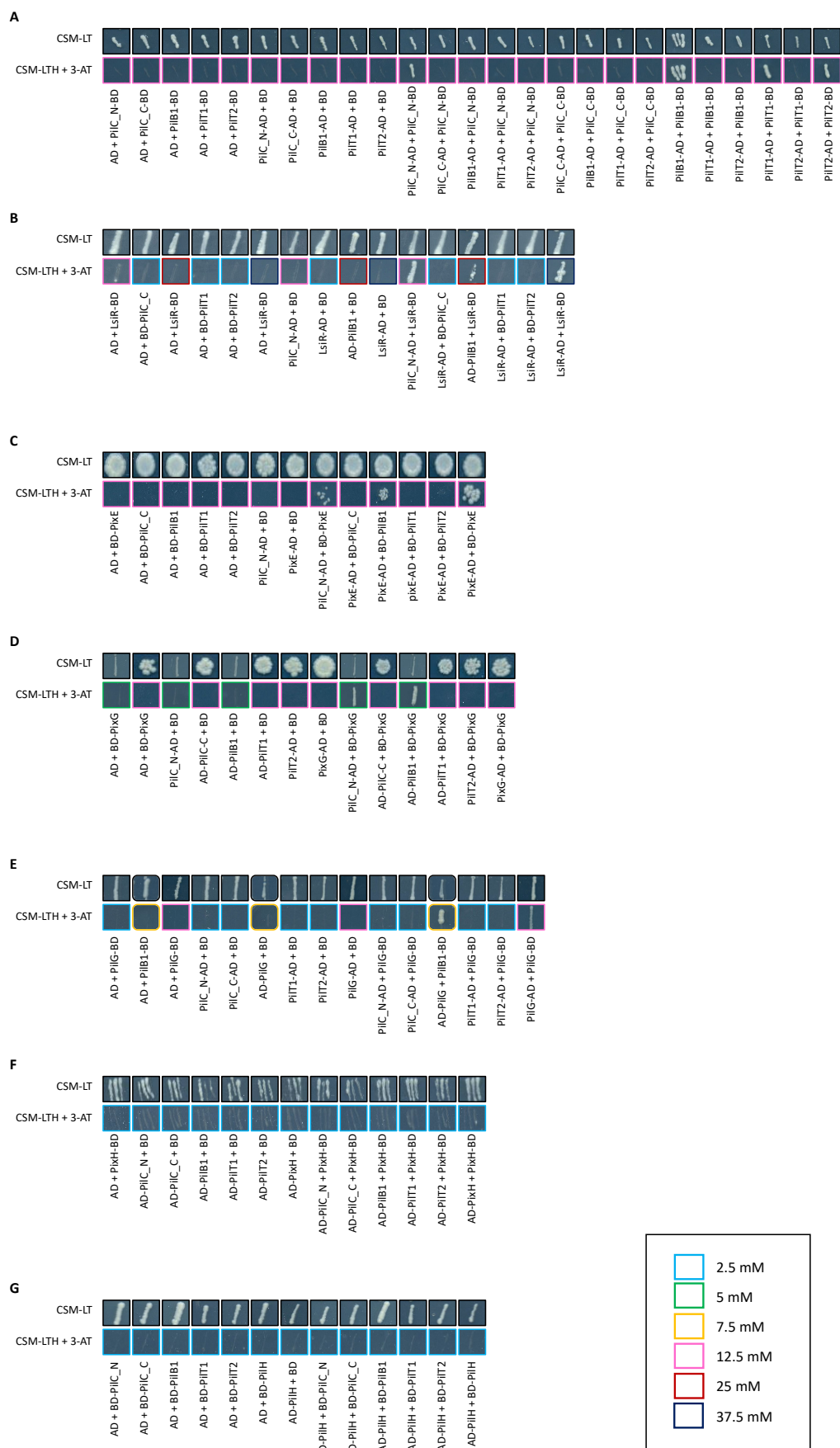


Figure S4: Y2H analysis showing interactions between response regulators and TFP components. Interactions between (A) pili proteins, and (B) LsiR, (C) PixE, (D) PixG, (E) PilG, (F) PixH and (G) PilH CheY-like response regulators. Respective proteins were fused with AD (GAL4 activation domain) or BD (GAL4 DNA binding domain) at either N- or C-terminus and expressed in yeast strain AH109. For positive interactions, representative results using the highest used 3-AT concentration are shown. For negative interactions, the lowest used 3-AT concentration and only one representative combination is shown. The positions of the fused AD and BD domains are displayed below each rectangle and the used concentrations of 3-AT are represented by different colors, respectively (blue, 2.5 mM; green, 5 mM; orange, 7.5 mM; pink, 12.5 mM; dark red, 25 mM; dark blue, 37.5 mM). Yeast cells grew on CSM-LT (complete supplement medium lacking leucine and tryptophan) and CSM-LTH (complete supplement medium lacking leucine, tryptophan, and histidine) supplemented with 3-AT for 6-7 days at 30°C. Rounded rectangle (PilG-PilB1 interaction): the result is ambiguous because only 4 out of 6 colonies grew on CSM-LTH plates supplemented with 3-AT.

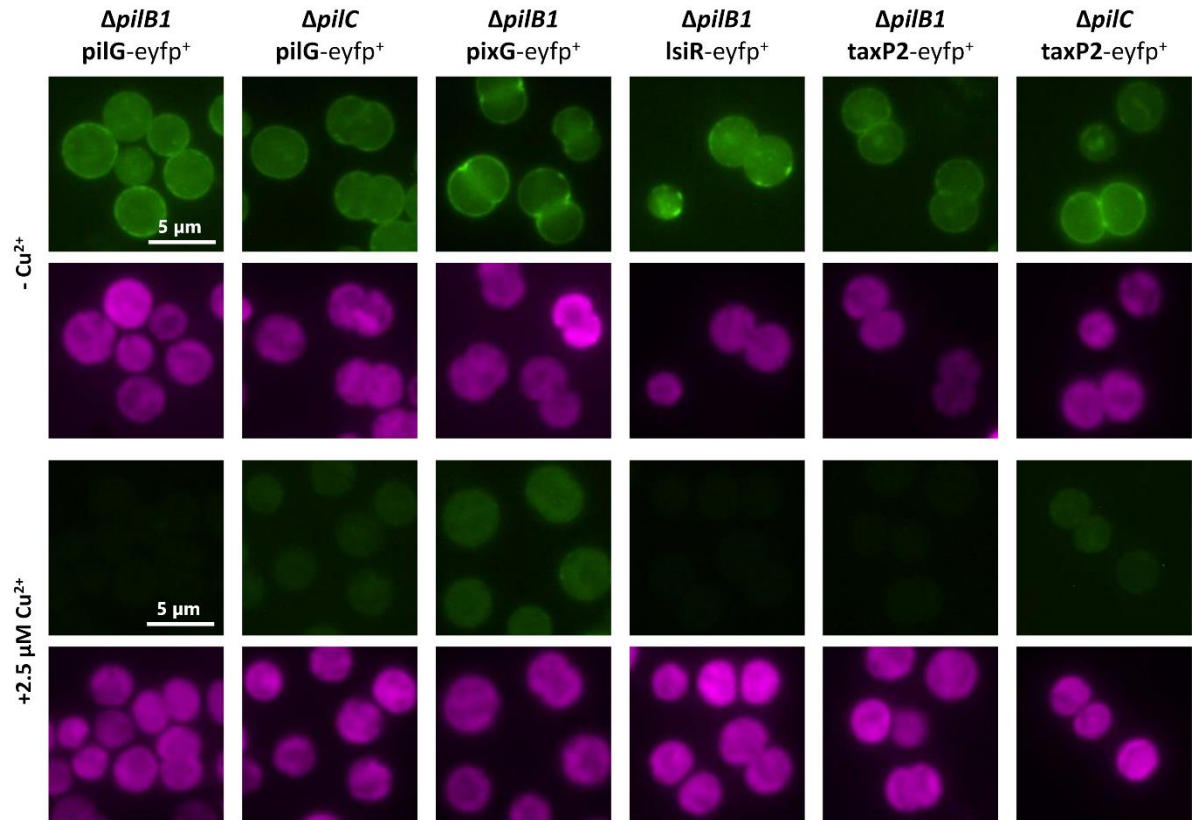


Figure S5: Localization of PatA-type response regulators in $\Delta pilB1$ or $\Delta pilC$ mutant strains. The proteins harboring a C-terminal eYFP fusion are expressed under the control of the copper-repressed *petJ* promoter. The expression cassette was inserted into a neutral genomic locus in wild-type *Synechocystis* cells via homologous recombination. Cells were grown on 0.5% BG11 agar plates without copper to induce gene expression (upper panel) or with the addition of 2.5 μM CuSO_4 to repress the *petJ* promoter (lower panel). eYFP fluorescence is shown in green and chlorophyll fluorescence in magenta. Scale bars = 5 μm .

Table S1. Strains and plasmids used in this study.

Bacterium/plasmids	Relevant characteristic(s)	Reference/Source
<i>Synechocystis</i> strains		
<i>Synechocystis</i> WT	PCC-M wild type	(Trautmann et al., 2012)
<i>pixE</i> _(PATAN) ⁺	WT containing plasmid pUR- <i>pixE</i> _{PATAN} _flag	This study
<i>pixE</i> _(PATAN) - <i>eyfp</i> ⁺	WT transformed with pSK- <i>pixE</i> _{PATAN} _eyfp	This study
<i>pilG</i> - <i>eyfp</i> ⁺	WT transformed with pSK- <i>pilG</i> -eyfp	This study
<i>pixG</i> - <i>eyfp</i> ⁺	WT transformed with pSK- <i>pixG</i> -eyfp	This study
<i>pilH</i> - <i>eyfp</i> ⁺	WT transformed with pSK- <i>pilH</i> -eyfp	This study
<i>pixH</i> - <i>eyfp</i> ⁺	WT transformed with pSK- <i>pixH</i> -eyfp	This study
<i>taxP2</i> - <i>eyfp</i> ⁺	WT transformed with pSK- <i>taxP2</i> -eyfp	This study
<i>pixE</i> - <i>eyfp</i> ⁺	WT transformed with pSK- <i>pixE</i> -eyfp	This study
<i>IsiR</i> - <i>eyfp</i> ⁺	WT transformed with pSK- <i>IsiR</i> -eyfp	This study
$\Delta pilB1$	<i>pilB1</i> knockout mutant (Zeo ^R)	(Linhartová et al., 2014)
$\Delta pilC$	<i>pilC</i> knockout mutant (Str ^R)	(Bhaya et al., 2000)
$\Delta pilB1$ / <i>IsiR</i> - <i>eyfp</i> ⁺	<i>IsiR</i> - <i>eyfp</i> ⁺ transformed with genomic DNA of $\Delta pilB1$	This study
$\Delta pilB1$ / <i>pixG</i> - <i>eyfp</i> ⁺	<i>pixG</i> - <i>eyfp</i> ⁺ transformed with genomic DNA of $\Delta pilB1$	This study
$\Delta pilB1$ / <i>pilG</i> - <i>eyfp</i> ⁺	<i>pilG</i> - <i>eyfp</i> ⁺ transformed with genomic DNA of $\Delta pilB1$	This study
$\Delta pilB1$ / <i>taxP2</i> - <i>eyfp</i> ⁺	<i>taxP2</i> - <i>eyfp</i> ⁺ transformed with genomic DNA of $\Delta pilB1$	This study
$\Delta pilC$ / <i>pilG</i> - <i>eyfp</i> ⁺	<i>pilG</i> - <i>eyfp</i> ⁺ transformed with genomic DNA of $\Delta pilC$	This study
$\Delta pilC$ / <i>taxP2</i> - <i>eyfp</i> ⁺	<i>taxP2</i> - <i>eyfp</i> ⁺ transformed with genomic DNA of $\Delta pilC$	This study
$\Delta pixGH$	<i>Synechocystis</i> transformed with pUC- $\Delta pixGH$	This study
<i>PixG</i> _(WT)	$\Delta pixGH$ transformed with pUC- <i>pixG</i> (WT)H	This study
<i>PixG</i> _(PATAN)	$\Delta pixGH$ transformed with pUC- <i>pixG</i> (PATAN)H	This study
<i>PixG</i> _(REC)	$\Delta pixGH$ transformed with pUC- <i>pixG</i> (REC)H	This study
<i>PixG</i> _(D326A)	$\Delta pixGH$ transformed with pUC- <i>pixG</i> (D326A)H	This study
Y2H plasmids		
pGADT7ah	Expression vector in yeast cells; N_GAL4 AD, <i>LEU2</i> ; Amp ^R	(Hiltbrunner et al., 2005)
pCGADT7ah	Expression vector in yeast cells; C_GAL4 AD, <i>LEU2</i> ; Amp ^R	(Rausenberger et al., 2011)
pGBKT7	Expression vector in yeast cells; N_GAL4 BD, <i>TRP1</i> ; Km ^R	Clontech
pD153	Expression vector in yeast cells; C_GAL4 BD, <i>TRP1</i> ; Amp ^R	(Shimizu-Sato et al., 2002)
pGAD- <i>pilC</i> _N	AD- <i>pilC</i> _N cloned into pGADT7ah	This study
pGAD- <i>pilC</i> _C	AD- <i>pilC</i> _C cloned into pGADT7ah	This study
pGAD- <i>pilT2</i>	AD- <i>pilT2</i> cloned into pGADT7ah	This study
pGAD- <i>IsiR</i>	AD- <i>IsiR</i> cloned into pGADT7ah	This study
pGAD- <i>pilG</i>	AD- <i>pilG</i> cloned into pGADT7ah	This study
pGAD- <i>pilH</i>	AD- <i>pilH</i> cloned into pGADT7ah	This study
pGAD- <i>pilB1</i> _N	AD- <i>pilB1</i> _N cloned into pGADT7ah	This study
pGAD- <i>pilB1</i> _C	AD- <i>pilB1</i> _C cloned into pGADT7ah	This study
pCGAD- <i>pilC</i> _N	<i>pilC</i> _N-AD cloned into pCGADT7ah	This study
pCGAD- <i>pilC</i> _C	<i>pilC</i> _C-AD cloned into pCGADT7ah	This study
pCGAD- <i>pilT2</i>	<i>pilT2</i> -AD cloned into pCGADT7ah	This study
pCGAD- <i>IsiR</i>	<i>IsiR</i> -AD cloned into pCGADT7ah	This study
pCGAD- <i>pilG</i>	<i>pilG</i> -AD cloned into pCGADT7ah	This study
pCGAD- <i>pilH</i>	<i>pilH</i> -AD cloned into pCGADT7ah	This study
pCGAD- <i>pilB1</i> _N	<i>pilB1</i> _N-AD cloned into pCGADT7ah	This study
pCGAD- <i>pilB1</i> _C	<i>pilB1</i> _C-AD cloned into pCGADT7ah	This study
pGBK- <i>pilC</i> _N	BD- <i>pilC</i> _N cloned into pGBKT7	This study
pGBK- <i>pilC</i> _C	BD- <i>pilC</i> _C cloned into pGBKT7	This study
pGBK- <i>pilT2</i>	BD- <i>pilT2</i> cloned into pGBKT7	This study
pGBK- <i>IsiR</i>	BD- <i>IsiR</i> cloned into pGBKT7	This study
pGBK- <i>pilG</i>	BD- <i>pilG</i> cloned into pGBKT7	This study
pGBK- <i>pilH</i>	BD- <i>pilH</i> cloned into pGBKT7	This study
pGBK- <i>pixE</i> -PATAN	BD- <i>pixE</i> -PATAN cloned into pGBKT7	This study
pGBK- <i>pixE</i> -REC	BD- <i>pixE</i> -REC cloned into pGBKT7	This study
pD153- <i>pilC</i> _N	<i>pilC</i> _N-BD cloned into pD153	This study
pD153- <i>pilC</i> _C	<i>pilC</i> _C-BD cloned into pD153	This study

pD153- <i>pilT2</i>	<i>pilT2-BD</i> cloned into pD153	This study
pD153- <i>lsiR</i>	<i>lsiR-BD</i> cloned into pD153	This study
pD153- <i>pilG</i>	<i>pilG-BD</i> cloned into pD153	This study
pD153- <i>pilH</i>	<i>pilH-BD</i> cloned into pD153	This study
Plasmids for the transformation of cyanobacteria		
pSK-hfq-eyfp	pSDC01-derived vector; P _{petJ} , C_eYFP, Ter _{oOp} , Cm ^R	(Schuergers et al., 2014)
pSK- <i>pixE_PATAN</i> -eyfp	<i>pixE-PATAN</i> -eyfp cloned into pSK-hfq-eyfp, Cm ^R	This study
pSK- <i>pilG</i> -eyfp	<i>pilG-eyfp</i> cloned into pSK-hfq-eyfp, Cm ^R	This study
pSK- <i>pixG</i> -eyfp	<i>pixG-eyfp</i> cloned into pSK-hfq-eyfp, Cm ^R	This study
pSK- <i>pilH</i> -eyfp	<i>pilH-eyfp</i> cloned into pSK-hfq-eyfp, Cm ^R	This study
pSK- <i>pixH</i> -eyfp	<i>pixH-eyfp</i> cloned into pSK-hfq-eyfp, Cm ^R	This study
pSK- <i>taxP2</i> -eyfp	<i>taxP2-eyfp</i> cloned into pSK-hfq-eyfp, Cm ^R	This study
pSK- <i>pixE</i> -eyfp	<i>pixE-eyfp</i> cloned into pSK-hfq-eyfp, Cm ^R	This study
pSK- <i>lsiR</i> -eyfp	<i>lsiR-eyfp</i> cloned into pSK-hfq-eyfp, Cm ^R	This study
pUR-C_flag	Expression vector; P _{petJ} , C_FLAG, Ter _{oOp} , Km ^R , Strep ^R	T. Wallner (Uni Freiburg)
pUR- <i>pixE_PATAN</i> _flag	<i>pixE-PATAN</i> _flag cloned into pUR-C_flag, Km ^R , Strep ^R	This study
pUC-ΔpixGH	pUC19-based <i>pixGH</i> knock-out construct containing a kanamycin-resistance cassette flanked by the 650 bp regions upstream of position -285 and downstream of position +1706 from the translation start site of <i>pixG</i> ; Km ^R , Amp ^R	This study
pUC-pixG(WT)H	pUC-ΔpixGH based vector for the complementation of <i>pixGH</i> where the kanamycin resistance cassette is replaced by a gentamycin-resistance cassette flanked by the +36 to -150 region from TSS of <i>slr0031</i> and the -285 to +1706 region from the TSS of <i>pixG</i> ; Gen ^R , Amp ^R	This study
pUC-pixG(PATAN)H	pUC-pixG(WT)H derivative replacing full-length <i>pixG</i> with <i>pixG-PATAN</i> ; Gen ^R , Amp ^R	This study
pUC-pixG(REC)H	pUC-pixG(WT)H derivative replacing full-length <i>pixG</i> with <i>pixG-REC</i> ; Gen ^R , Amp ^R	This study
pUC-pixG(D326A)H	pUC-pixG(WT)H derivative replacing <i>pixG</i> with <i>pixG-D326A</i> ; Gen ^R , Amp ^R	This study

Table S2. Primers used in this study

Name	Sequence (5' → 3')	PCR product
RE cloning of Y2H plasmids		
AD-pilC _N -fw	TACATATGGCTACGTTTGTGCTC	AD-pilC _N
AD-pilC _N -rev	TTCTCGAGTTACACCGGATAAGCCATGG	
AD-pilC _C -fw	TTCATATGAAAAAATATTACGGAACCTATGC	AD-pilC _C
AD-pilC _C -rev	CTCGAGTTACATAGCTGGTTCTATAATAC	
AD-pilT2-fw	TAGGATCCTGAACCAACCTCCCCGC	AD-pilT2
AD-pilT2-rev	TTCTCGAGTTAGGTTCTGCCCCGAG	
AD-LsiR-fw	TAGGATCCTGACTGCTGTGATCACCCG	AD-LsiR
AD-LsiR-rev	TTCTCGAGCTAGGGACAAAGAACAG	
AD-pilG-fw	AGATCTATCAGGGAACCTGAAC	AD-pilG
AD-pilG-rev	CTCGAGTGATTTTTTACAGTAAGATTCAAC	
AD-pilH-fw	AGATCTATATGGAAAATAAACAGG	AD-pilH
AD-pilH-rev	CTCGAGATTGCGCAGGAGTTGTTG	
AD-PilB1-fw	GCAGATCTTGACATCTTCTCTCTC	AD-pilB1 _N
AD-pilB1(1-366)-rev	ATCTCGAGCCGGGCGGCCAATTCCCTTAC	
AD-pilB1(367-672)-fw	GCAGATCTTGCCCTATGGCTTAATGTTGG	AD-pilB1 _C
AD-PilB1-rev	ATCTCGAGGCTAAACCGGGAAG	
pilC _N -BD-fw	TAGGATCCATGGCTACGTTTGTGCTC	pilC _N -AD
pilC _N -AD-rev	CGTCTAGACACCGGATAAGCCATGGCG	
pilC _C -BD-fw	TAGGATCCATGAAAAAATATTACGGAACCTATGC	pilC _C -AD
pilC _C -AD-rev	CGTCTAGACATAGCTGGTTCTATAATAC	
pilT2-AD-fw	TAGGATCCATGAACCAACCTCCCCGC	pilT2-AD
pilT2-AD-rev	CGTCTAGAGGTTCTGCCCCGAGTCG	
LsiR-AD-fw	TAAGATCTATGACTGCTGTGATCAC	LsiR-AD
LsiR-AD-rev	TATCTAGAGGGACAAAGAACAGGGAC	
pilG-AD-fw	AGATCTATGCAGGGAACCTGAAC	pilG-AD
pilG-AD-rev	TCTAGATGATTTTTTACAGTAAGATTCAAC	
pilH-AD-fw	AGATCTATGATGGAAAATAAACAGG	pilH-AD
pilH-AD-rev	TCTAGAATTGCGCAGGAGTTGTTG	
pilB1-AD-fw	GCAGATCTATGACATCTTCTCTCTC	pilB1 _N -AD
pilB1(1-366)-AD-rev	ATGCTAGCCCCGGGCGGCCAATTCCCTTAC	
pilB1(367-672)-AD-fw	GCAGATCTATGCCCTATGGCTTAATGTTGG	pilB1 _C -AD
PilB1-AD-rev	ATGCTAGCGCTAAACCGGGAAG	
BD-pilC _N -fw	TAGGATCCGGCTACGTTTGTGCTCAAG	BD-pilC _N
BD-pilC _N -rev	GCAGTAGTCACCGGATAAGCCATGGCG	
BD-pilC _C -fw	TAGGATCCGAAAAAATATTACGGAACCTATGC	BD-pilC _C
BD-pilC _C -rev	GAGTCGACTTACATAGCTGGTTCTATAATAC	
BD-pilT2-fw	TAGGATCCGAACCAACCTCCCCGC	BD-pilT2
BD-pilT2-rev	GCAGTAGTGGTTCTGCCCCGAGTCG	
BD-LsiR-fw	TAGGATCCGACTGCTGTGATCACCCG	BD-LsiR
BD-LsiR-rev	GCAGTAGTGGGACAAAGAACAGGGAC	
BD-pilG-fw	AGATCTACAGGGAACCTGAACGAAATTG	BD-pilG
BD-pilG-rev	ACTAGTTGATTTTTTACAGTAAGATTCAAC	
BD-pilH-fw	AGATCTAATGGAAAATAAACAG	BD-pilH
BD-pilH-rev	ACTAGTATTGCGCAGGAGTTGTTG	
BD-pixE-fw	TAGGATCCAAGCAATTCAGTTTGTCCAC	BD-pixE-PATAN
BD-pixE-PATAN-rev	GCAGTAGTCACCAAAGGGCGGTCATC	
BD-pixE-REC-fw	TAGGATCCACAAACGGATACCGCCCTTTG	BD-pixE-REC
BD-pixE-rev	GCAGTAGTGGAGTTGGTTTTATTGGTGG	
pilC _N -BD-fw	TAGGATCCATGGCTACGTTTGTGCTC	pilC _N -BD
pilC _N -BD-rev	GAGTCGACAACACCGGATAAGCCATGGCG	
pilC _C -BD-fw	TAGGATCCATGAAAAAATATTACGGAACCTATGC	pilC _C -BD
pilC _C -BD-rev	GAGTCGACAACATAGCTGGTTCTATAATAC	
pilT2-AD-fw	TAGGATCCATGAACCAACCTCCCCGC	pilT2-BD
pilT2-BD-rev	AAGTCGACAAGGTTCTGCCCCGAGTCG	

LsiR-AD-fw	TAAGATCTATGACTGCTGTGATCAC	<i>lsiR</i> -BD
LsiR-BD-rev	CCCGGGACGGGACAAAGAACAGGGAC	
pilG-AD-fw	AGATCTATGCAGGGAACCCCTGAAC	<i>pilG</i> -BD
pilG-BD-rev	CCCGGGTTTGATTTTTTACAGTAAGATTCAAC	
pilH-AD-fw	AGATCTATGATGGAAAATAAACCCAGGC	<i>pilH</i> -BD
pilH-BD-rev	CCCGGGTTATTGCGCAGGAGTTGTTTG	
RE cloning of plasmids for expression of fusion proteins		
NdeI-pilG-fw	TGCATATGCAGGGAACCCCTG	<i>pilG_eyfp</i>
XhoI-pilG-rev	GACTCGAGTGATTTTTTACAGTAAGATTC	
NdeI-pixG-fw	TGCATATGACAGCTCCCAACCCCT	<i>pixG_eyfp</i>
XhoI-pixG-rev	GACTCGAGTTCCGCTGGCAGCGATGC	
NdeI-pilH-fw	TGCATATGATGGAAAATAAACCCAGGC	<i>pilH_eyfp</i>
XhoI-pilH-rev	GACTCGAGATTGCGCAGGAGTTGTTTG	
NdeI-pixH-fw	TGCATATGGGCAGCGCACTTGTTA	<i>pixH_eyfp</i>
XhoI-pixH-rev	GACTCGAGGATAAATTGCTTGATTACCGTG	
NdeI-taxP2-fw	TGCATATGCAATCTCCCTGTC	<i>taxP2_eyfp</i>
XhoI-taxP2-rev	GACTCGAGAACAGCTGCGGAGATAAAG	
NdeI-pixE-fw	TGCATATGAGCAATTAGTTTTGTC	<i>pixE_eyfp</i>
XhoI-pixE-rev	GACTCGAGGGAGTTGGTTTTATTGGTG	
NdeI-lsiR-fw	TGCATATGACTGCTGTGATCACCCG	<i>lsiR_eyfp</i>
XhoI-lsiR-rev	GACTCGAGGGGACAAAGAACAGGGAC	
pixE-PATAN-fw	AAAACATATGAGCAATTAGTTTTGTCCAC	<i>pixE_PATAN_flag</i>
pixE-PATAN-rev	AAAAAGATCTCACCAAGGGCGGTCATC	
NdeI-pixE-fw	TGCATATGAGCAATTAGTTTTGTC	<i>pixE_PATAN_eyfp</i>
pixE-PATAN-eyfp-rev	AAAACTCGAGCACCAAGGGCGGTCATC	
Seamless cloning of pUC-ΔpixGH		
pUC19+upward-fw	TTCGAGCTCGGTACCCATACATTACCCCTGGAGG	upstream homology region
Upward+kana-rev	CACGAGGCAGACCTCAACTTTATATCCCCCATGC	
Upward+kana-fw	ATGGGGGATATAAAGTTGAGGTCTGCCTCGTGAAG	Kanamycin cassette
Kana+downward-rev	GGGGCGTTGGCATCGCTGCAAAAGCCACGTTGTG	
Kana+downward-fw	CAACGTGGCTTTTGCAGCGATGCCAACGCCCCAG	downstream homology region
Downward+pUC19-rev	ACTCTAGAGGATCCCCTAGGGTAAATTCCCAAATAGTCC	
Seamless cloning of pUC-pixG(WT)H		
pUC19+upward-fw	TTCGAGCTCGGTACCCATACATTACCCCTGGAGG	upstream homology region
Upward+pixH-rev	CAAGCAATTTATCTGAACTTTATATCCCCCATGC	
Upward+pixH-fw	ATGGGGGATATAAAGTTGAGATAAATTGCTTGATTACCG	<i>pixGH</i> (-285 to +1706 region from <i>pixG</i> TSS)
pixG+gen-rev	TTCGAGCTCGGTACCCCACTTGGTAGTGACAGATG	Gentamycin cassette
pixG+gen-fw	TCTGCACTACCAAGTGGGGTACCGAGCTCGAATTG	
Gen+slr0031-rev	CCCCTCGGTGAGGTCGGGGTACCGAGCTCGAATTG	
Gen+slr0031-fw	TTCGAGCTCGGTACCCGACCTCACCGAGGGGAC	<i>Slr0031</i> (+36 to -150 region from TSS)
Slr0031+downward-rev	GGGGCGTTGGCATCGCCACTTGGTAGTGACAGATG	downstream homology region
Slr0031+downward-fw	TCTGCACTACCAAGTGGCGATGCCAACGCCCCAG	
Downward+pUC19-rev	ACTCTAGAGGATCCCCTAGGGTAAATTCCCAAATAGTCC	
<i>pixG</i> mutagenesis in pUC-pixG(WT)H		
pixG_PATAN-fw	GATCAGGGGACCACTGGGCAC	<i>pixG_PATAN</i>
pixG_PATAN-rev	TAAGTTGACCCAAGGAGGCC	
pixG_REC-fw	CATTTAATCAAAGGAAGAGGCAG	<i>pixG_REC</i>
pixG_REC-rev	GATGTGCCCAGTGGTCCCTG	
pixG_D326A-fw	GATTTTCCTCGCTTTGGTCATGCC	<i>pixG_D326A</i>
pixG_D326A-rev	AAATCCGGCTTCCGGGGC	

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