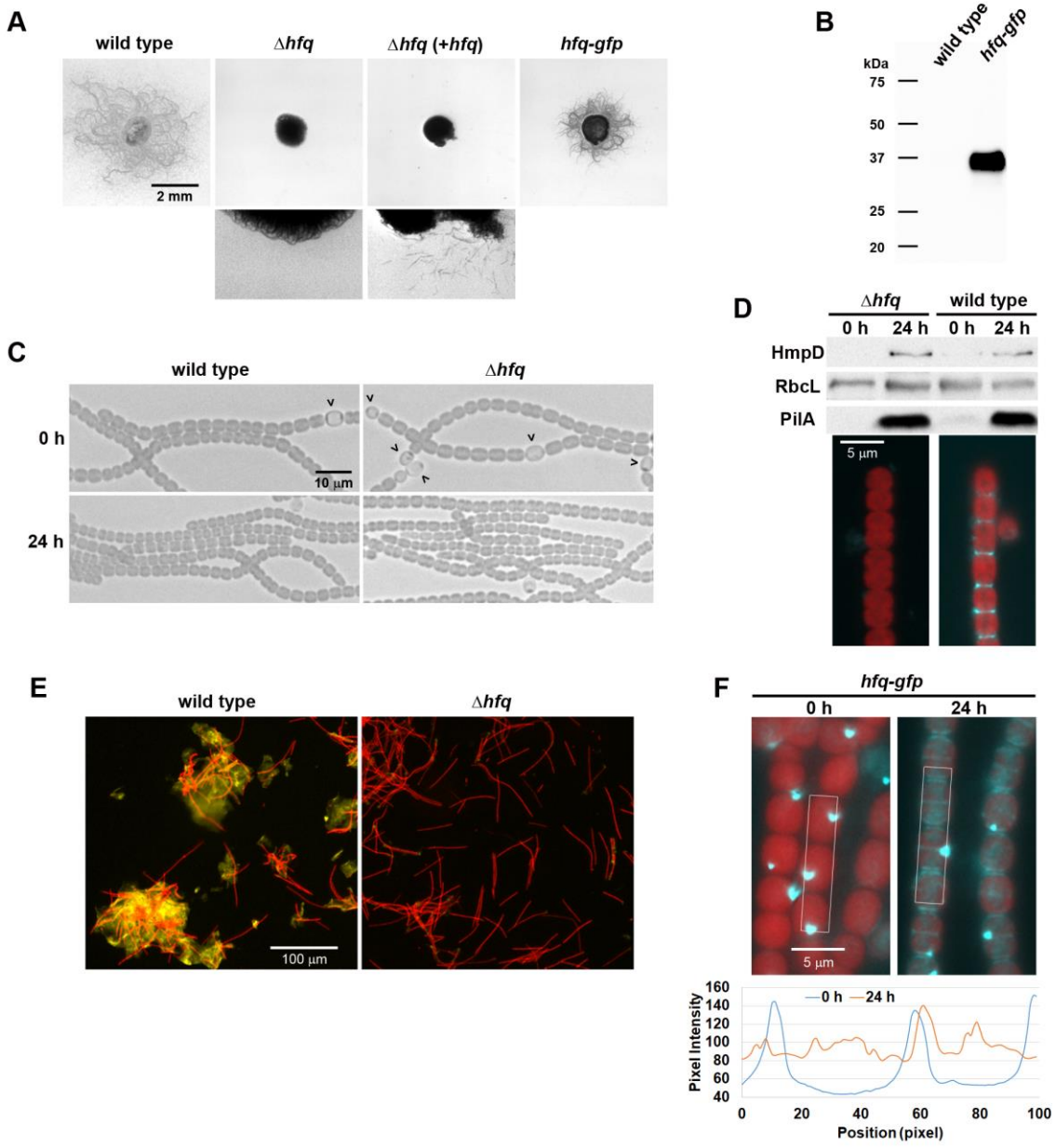


Figure S1. Characterization of *hfq*-deletion and *hfq-gfp* strains. **(A)** Plate motility assays of the wild type, Δ*hfq*, *hfq-gfp*, and complemented Δ*hfq* strains. **(B)** Immunoblot analysis of the wild type and *hfq-gfp* strains with an α-GFP antibody. **(C)** Light micrographs of the filament morphology for the wild type and *hfq*-deletion strains at 0 h and 24 h post hormogonium induction. Carets indicate the presence of heterocysts attached to filaments. Hormogonia can be distinguished from vegetative filaments by the absence of heterocysts, smaller cell size, and presence of tapered cells at the filament termini. **(D)**

Immunoblot analysis of cellular HmpD, PilA, and RbcL, and immunofluorescence analysis of extracellular PilA in the wild type and Δ*hfq* strains 24 h after hormogonium induction. RbcL is the large subunit of RUBISCO and serves as a protein loading control. Depicted are merged images of fluorescence micrographs acquired using a 63x objective lens from cellular autoflourescence (red) and PilA immunofluorescence (cyan). **(E)** Lectin staining analysis of HPS. Depicted are merged images of fluorescence micrographs acquired using a 10x objective lens from cellular autoflourescence (red) and UEA-fluorescein stained HPS (yellow) 24 h after hormogonium induction. **(F)** Fluorescence micrographs of the *hfq-gfp* strain at 0 and 24 post hormogonium induction. The upper panel depicts merged images of fluorescence micrographs acquired using a 63x objective lens from cellular autoflourescence (red) and GFP fluorescence (cyan). The lower panel depicts quantification of fluorescence intensity derived from Hfq-GFP for the regions indicated with white rectangles in the upper panel.

SMOV1. Time lapse motility assays of individual filaments from the wild type and Δ*hfq* strains.

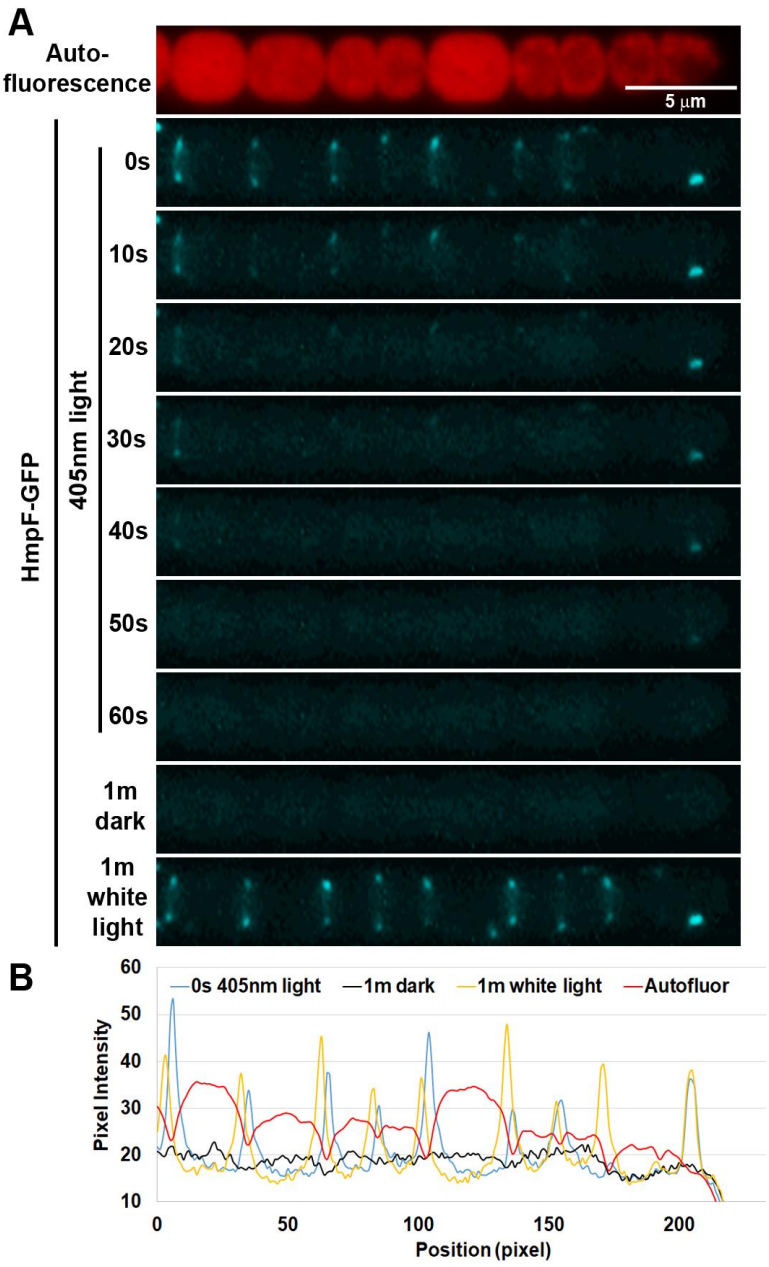


Figure S2. Dynamic localization of HmpF-GFP in response to light in immobilized hormogonium filaments. **(A)** Fluorescence micrographs of cellular autofluorescence (red) and GFP fluorescence (cyan) of the *hmpF-gfp* strain exposed to a light regiment of 405 nm for 1 min, followed by darkness for 1 min, and subsequently incubated in white light for 1 min. **(B)** Quantification of positional fluorescence intensity. Using imageJ, a line was drawn along the length of the filament and the pixel intensity was measured at the indicated time points in the light regimen. Note the bright fluorescent focus at the far

right of the filament (in panel A), which delocalizes, and re-localizes to the same position in the filament, whereas the bands of polar fluorescence show a positional shift indicating the relocalization of HmpF-GFP to the opposite pole of the cell.

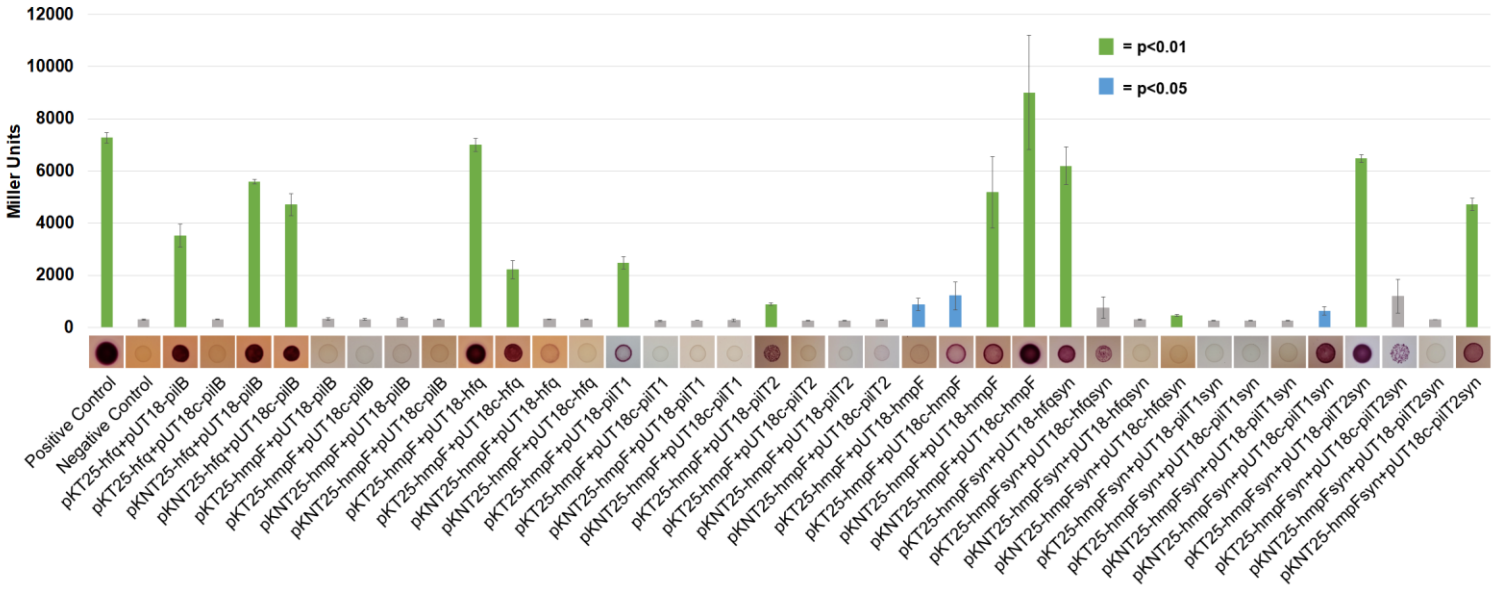
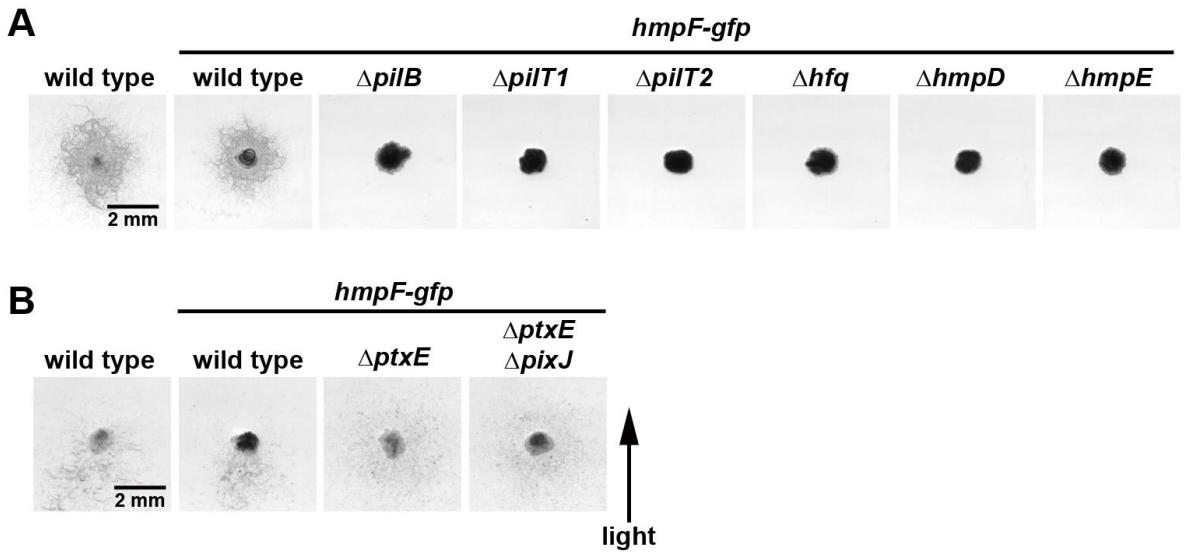


Figure S3. BACTH analysis between various proteins of *N. punctiforme* and *Synechocystis* sp. strain PCC 6803. Depicted are the results from qualitative assays on MacConkey agar and quantitative analysis of β-galactosidase activity. The positive control strain harbors plasmids pKT25-zip and pUT18c-zip, while the negative control strain harbors the empty vectors pKT25 and pUT18c. Error bars = +/- 1 S.D., p-values determined by two-tailed Student's t-Test between the negative control and each experimental combination of plasmids, n=3.



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69 **Figure S4. (A)** Plate motility and **(B)** phototaxis assays of the wild-type and *hmpF-gfp* strain harboring

70 deletions in genes encoding components of the T4P, Hmp, Ptx, and Pix systems. Images were taken at 48

71 h (A) or 72 h (B) post hormogonium induction.

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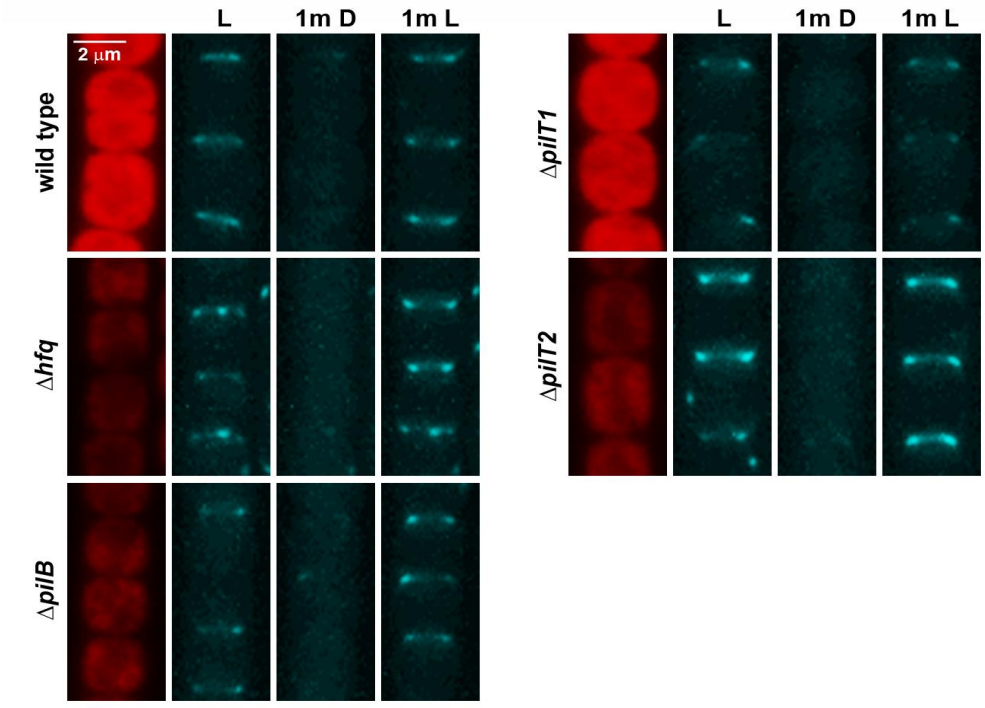


Figure S5. Localization of HmpF-GFP in response to light in strains harboring deletions in genes encoding components of the T4P system. Depicted are fluorescence micrographs of cellular autofluorescence (red) and GFP fluorescence (cyan) at each interval of a light regiment with exposure to white light for 1 min (L), followed by darkness for 1 min (1m D), and subsequently white light for 1 min (1m L).

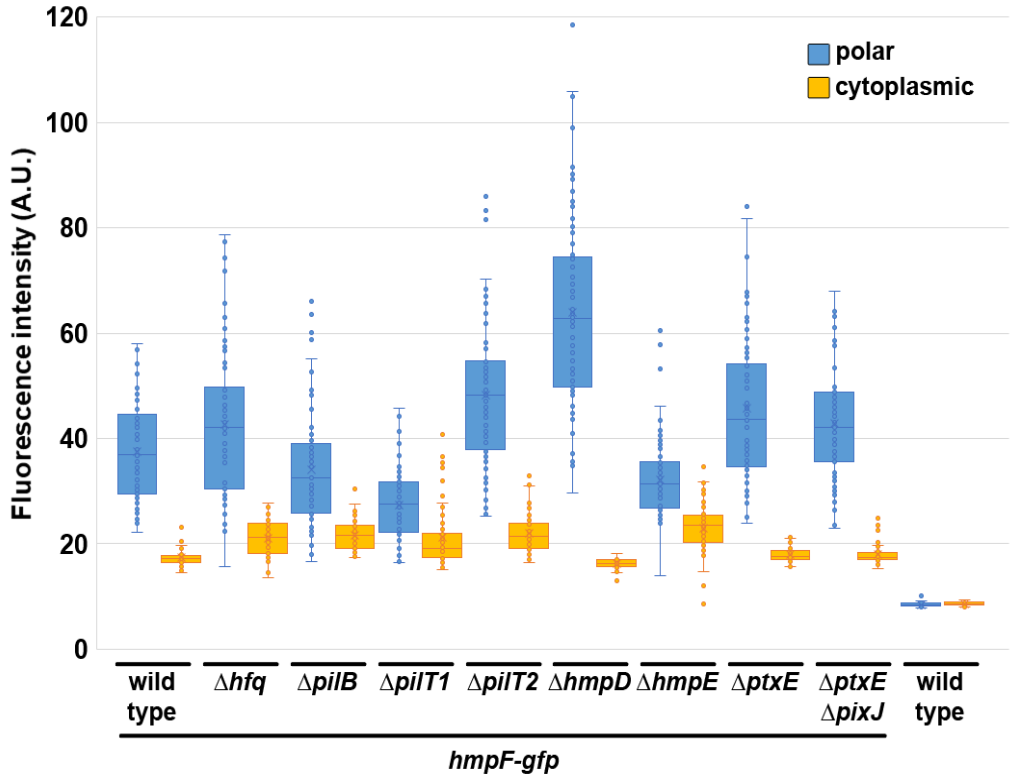
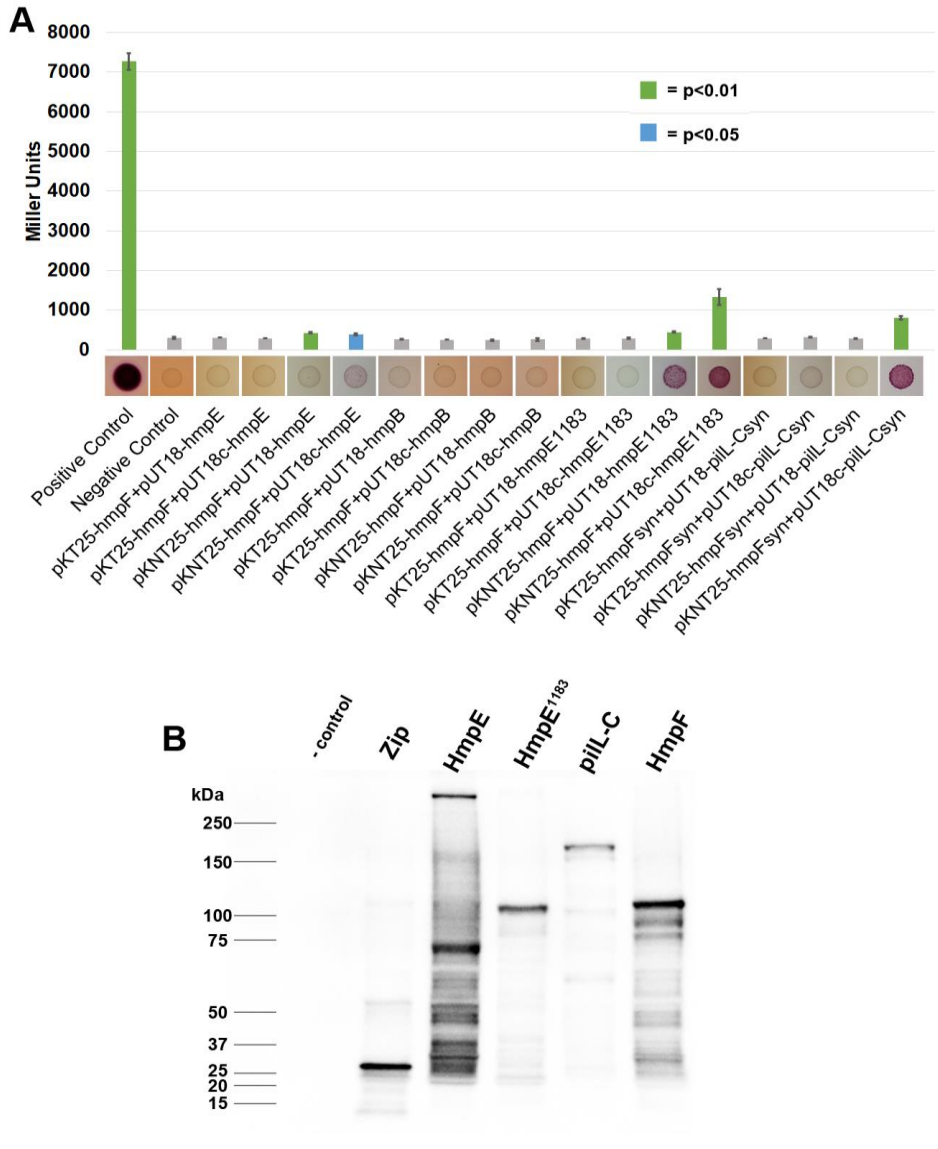


Figure S6. Box plots depicting the quantitative analysis of polar and cytoplasmic fluorescence from HmpF-GFP in strain harboring deletions in genes encoding components of the T4P, Hmp, Ptx, and Pix systems.



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105 **Figure S7.** BACTH analysis between various proteins of *N. punctiforme* and *Synechocystis* sp. strain
106 PCC6803. **(A)** Depicted are the results from qualitative assays on MacConkey agar and quantitative
107 analysis of β-galactosidase activity. The positive control strain harbors plasmids pKT25-zip and pUT18c-
108 zip, while the negative control strain harbors the empty vectors pKT25 and pUT18c. Error bars = +/- 1
109 S.D. p-values determined by two-tailed Student's t-Test between the negative control and each
110 experimental combination of plasmids, n=3. **(B)** Immunoblot analysis of protein expression in *E. coli*
111 BL21(DE3) harboring pUT18c plasmids expressing the indicated protein. – control is BL21(DE3) not
112 harboring any pUT18c derivatives.

SMOV2. Time lapse motility assays of individual filaments from the *hmpF-gfp* strain treated with either CCCP or DMSO alone (- control).

Table S1. Strains and plasmids used in this study

Strains	Relevant Characteristic(s)	source
Nostoc punctiforme strains		
ATCC 29133	wild type	ATCC
UCD153	Laboratory derivative of *N. punctiforme* ATCC 29133 with reduced motility	(1)
UCD598	Δ*ptxE* (Npun_F2165) *	(2)
TNM703	UCD153 with a Tn5-1063 insertion after nucleotide 242 of *hfq* (Npun_F5230)	This study
UOP157	*hmpF-gfp* (Npun_R5959)	(3)
UOP160	Δ*ptxE*, *hmpF-gfp*	This study
UOP161	Δ*pilB*, *hmpF-gfp*	This study
UOP162	Δ*hmpD* (Npun_F5963), *hmpF-gfp*	This study
UOP163	Δ*pilT1* (Npun_R0117), *hmpF-gfp*	This study
UOP164	Δ*pilT2* (Npun_F2507), *hmpF-gfp*	This study
UOP173	Δ*ptxE*, Δ*pixJ* (Npun_R6012), *hmpF-gfp*	This study
UOP185	Δ*hfq*	This study
UOP191	Δ*hmpE* (Npun_F5964), *hmpF-gfp*	This study
UOP 193	*hfq-gfp*	This study
UOP200	Δ*hfq*, *hmpF-gfp*	This study
Plasmids		
pAM504	Mobilizable shuttle vector	(4)
pRL278	Mobilizable suicide vector	(5)
pRL1063a	Suicide vector carrying Tn5-1063, a Tn5 derivative transposon	(6)
pSCR569	Mobilizable suicide vector for C-terminal *gfpuv* translational fusions	(7)
pSCR583	Suicide vector for in-frame deletion of *hmpD*	(8)
pDDR136	Suicide vector for in-frame deletion of *hmpE*	(9)
pDDR293	Suicide vector for in-frame deletion of *pilT2*	(10)
pDDR354	Suicide vector for in-frame deletion of *pilT1*	(10)
pDDR368	Suicide vector for in-frame deletion of *pilB*	(10)
pDDR465	Suicide vector for allelic substitution of *hmpF* with *hmpF-gfpuv*	(3)
pDDR476	Suicide vector for in-frame deletion of *hfq* [1-4] †	This study

pTVH102	Suicide vector for in-frame deletion of *pixJ* [5-8]	This study
pEZ101	Suicide vector for allelic substitution of *hfq* with *hfq-gfpuv* [5,8,9,10]	This study
pEZ102	Shuttle vector containing *hfq* and the 241 bp 5' to the start codon [11-12]	This study
pEZ103	pKT25-*hfq* [13-14]	This study
pEZ104	pKNT25-*hfq* [13-14]	This study
pEZ105	pUT18-*hfq* [13-14]	This study
pEZ106	pUT18c-*hfq* [13-14]	This study
pEZ109	pUT18-*pilB* [15-16]	This study
pEZ110	pUT18c-*pilB* [15-16]	This study
pTVH105	pUT18-*hmpF* [17-18]	This study
pTVH106	pUT18c-*hmpF* [17-18]	This study
pTVH107	pKT25-*hmpF* [17-18]	This study
pTVH108	pKNT25-*hmpF* [17-18]	This study
pTVH109	pUT18-*hmpE* [19-20]	This study
pTVH110	pUT18c-*hmpE* [19-20]	This study
pTVH113	pUT18-*hmpE*1183-1865 [20-21]	This study
pTVH114	pUT18c-*hmpE*1183-1865 [20-21]	This study
pDDR480	pUT18-*hfq* (ssr3341, *Synechocystis sp.* strain PCC6803) [22-23]	This study
pDDR481	pUT18c-*hfq* (ssr3341, *Synechocystis sp.* strain PCC6803) [22-23]	This study
pDDR482	pUT18-*pilL-C* (slr0322, *Synechocystis sp.* strain PCC6803) [24-25]	This study
pDDR483	pUT18c-*pilL-C* (slr0322, *Synechocystis sp.* strain PCC6803) [24-25]	This study
pDDR484	pKT25-*hmpF* (slr1301, *Synechocystis sp.* strain PCC6803) [26-27]	This study
pDDR485	pKNT25-*hmpF* (slr1301, *Synechocystis sp.* strain PCC6803) [26-27]	This study
pDDR486	pUT18-*pilT1* [28-29]	This study
pDDR487	pUT18c-*pilT1* [28-29]	This study
pDDR488	pUT18-*pilT1* (slr0161, *Synechocystis sp.* strain PCC6803) [30-31]	This study
pDDR489	pUT18c-*pilT1* (slr0161, *Synechocystis sp.* strain PCC6803) [30-31]	This study
pDDR490	pUT18-*pilT2* [32-33]	This study
pDDR491	pUT18c-*pilT2* [32-33]	This study
pDDR492	pUT18-*pilT2* (sll1533, *Synechocystis sp.* strain PCC6803) [34-35]	This study
pDDR493	pUT18c-*pilT2* (sll1533, *Synechocystis sp.* strain PCC6803) [34-35]	This study

pDDR494	pUT18-*hmpB* (Npun_F5961) [36-37]	This study
pDDR495	pUT18c-*hmpB* [36-37]	This study

* locus tag and genes derived from *Synechocystis* sp. strain PCC6803 rather than *N. punctiforme* denoted in parentheses

† numbers in brackets correspond to primers used to construct plasmid. Detailed information on primers can be found in Table S2

Table S2. Oligonucleotides used in this study

Oligonucleotide	Sequence	Number
NpF5230-5'-F	ATATAGGATCCTCGTAGTAAGGACTTTAGTC	1
NpF5230-5'-R-new	CTTGTTTTCGGGGTGTCAAATTCGGTAAGC	2
NpF5230-3'-F-new	GAATTTGACACCCCGAAAACAAGTTAGAAATAG	3
NpF5230-3'-R	ATATAGAGCTCCAGTATTAGGGCAAAACGATTC	4
NpR6012-5'-F	ATATAGGATCCCTGATGGTTATCTATCAAAACC	5
NpR6012-5'-R	CTAAAATCTATTTGACCAAAAATGTCATTTGATTAACCTC	6
NpR6012-3'-F	CAAATGACATTTTTGGTCAAATAGATTTTAGATTTTGG	7
NpR6012-3'-R	ATATAGAGCTCGTTAACAGCTTCAGCAATAC	8
hfq-gfp-SmaI-R	ATATACCCGGGAACTTGTTTTCGGTTTGATG	9
hfq-gfp-3'-F	ATATAACTAGTAAATAGAATTTTGCTTGCGATCG	10
Phfq-BamHI-F	ATATAGGATCCTGCCGTTTTCTCCAGGTAG	11
hfq-SacI-R	ATATAGAGCTCCTAACTTGTTTTCGGTTTGATGTATG	12
hfq-TH-BamHI-F	ATATAGGATCCCATGCTTACCGAATTTGACAC	13
hfq-TH-KpnI-R	ATATAGGTACCCGACTTGTTTTCGGTTTGATGTATG	14
pilB-TH-BamHI-F	ATATAGGATCCCATGACTTACTCGTCACCAC	15
pilB-TH-KpnI-R	ATATAGGTACCCGAAACCGAGATGTCATACAG	16
hmpF-TH-BamHI-F	ATATAGGATCCCGTGCTGTATTTAGCAGAAG	17
hmpF-TH-KpnI-R	ATATAGGTACCCGAGACGCTAATAATTCTGG	18
hmpE-TH-BamHI-F	ATATAGGATCCCATGCTGCCGGAACAACAACAG	19
hmpE-TH-SacI-R	ATATAGAGCTCGGAACATTACTCGTAGTGCTAAC	20
hmpE1183-TH-BamHI-F	ATATAGGATCCCATGGATGAATTTGGTGACTTGGAG	21
hfqsyn-TH-BamHI-F	ATATAGGATCCCATGAGCAGATTTGATAGC	22
hfqsyn-TH-KpnI-R	ATATAGGTACCCGACGGCGGGGAGTAATGTAG	23
pilL-Csyn-TH-BamHI-F	ATATAGGATCCCATGACTAGCGATCCCAATCC	24
pilL-Csyn-TH-SacI-R	ATATAGAGCTCGGCTCGTCTGCACTTAGAGC	25
hmpFsyn-TH-XbaI-F	ATATATCTAGATGTGCTCTATCTGGCTGAAATTAAG	26
hmpFsyn-TH-KpnI-R	ATATAGGTACCCGACCGCCAAACAATAGGGTC	27
pilT1-TH-BamHI-F	ATATAGGATCCCATGGAAATGATGATTGAAGACTTG	28
pilT1-TH-KpnI-R	ATATAGGTACCCGATGTGCTTTGGCAGCCGCAC	29

pilT1syn-TH-BamHI-F	ATATAGGATCCCATGGCTTTGGAATACATGATC	30
pilT1syn-TH-KpnI-R	ATATAGGTACCCGACGACGTTTAGCGGCAACC	31
pilT2-TH-BamHI-F	ATATAGGATCCCATGACAGAATCACAGTCTCC	32
pilT2-TH-KpnI-R	ATATAGGTACCCGAACTCTACCTCGGAGAAAC	33
pilT2syn-TH-BamHI-F	ATATAGGATCCCATGAACCAACCTCCCCG	34
pilT2syn-TH-KpnI-R	ATATAGGTACCCGGGTTCTGCCCCGCAGTC	35
hmpB-TH-BamHI-F	ATATAGGATCCCATGAGTACAGTTCTGATTGTG	36
hmpB-TH-KpnI-R	ATATAGGTACCCGTCCTCGCAGCAGTTGTTTG	37
Tn5-seq-F	CGATGAAGAGCAGAAGTTATC	38
Tn5-seq-R	GGCTCTATTCAGGATAAATC	39
Tn5-seq-F-nest	CGTTACCATGTTAGGAGGTC	40

Supplemental References

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