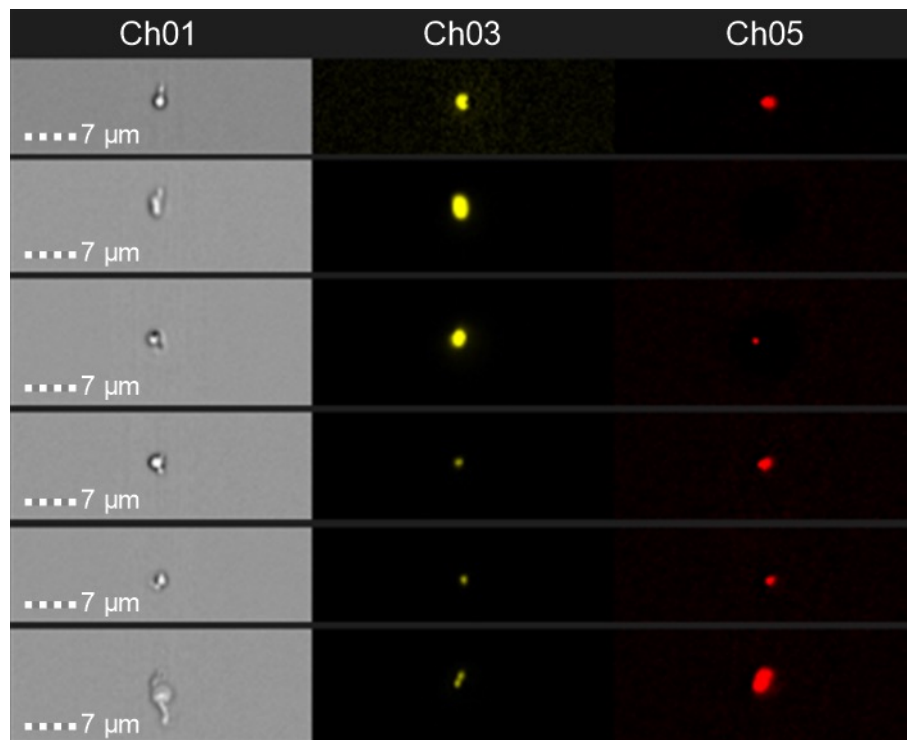
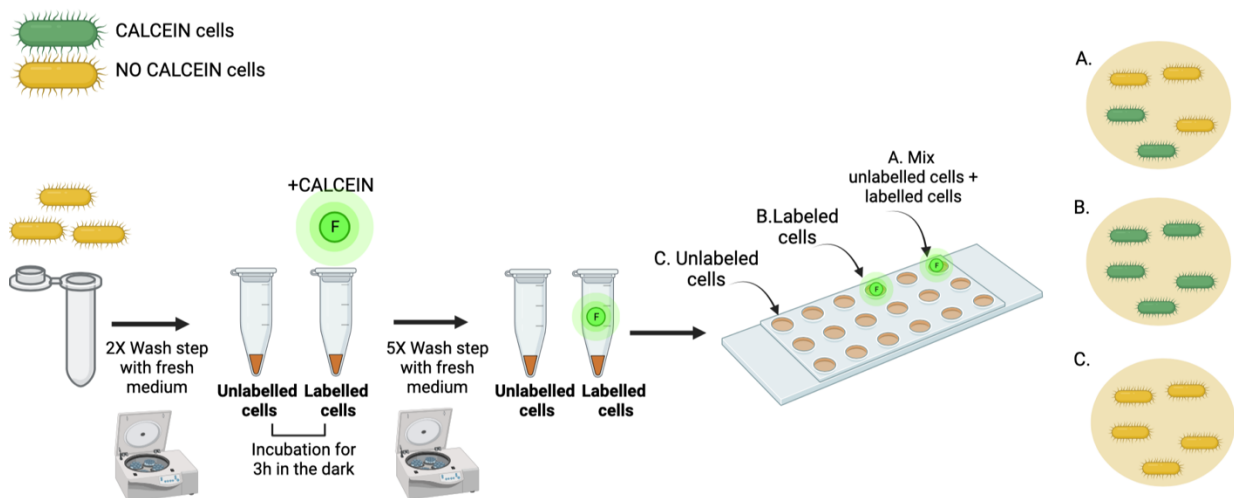


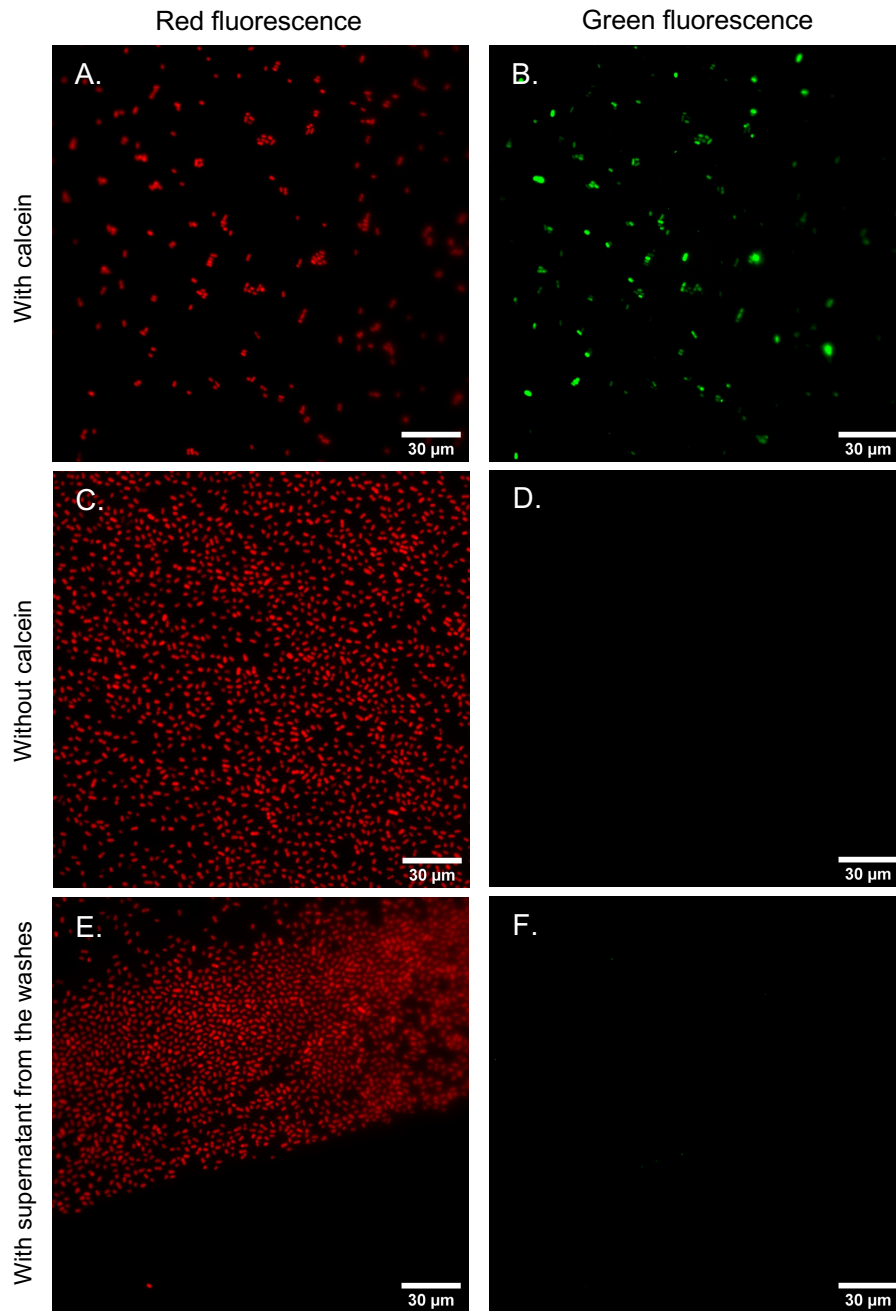


Extended Data Figure 1. Nanotubes identified in *Synechococcus* sp. WH7803 cultures by Imaging Flow Cytometry. Channel 1 (Ch01) shows brightfield images at 60X magnification of *Synechococcus* sp. WH7803 cells with NTs, channel 3 (Ch03) shows phycoerythrin autofluorescence and channel 5 (Ch05) shows chlorophyll autofluorescence.

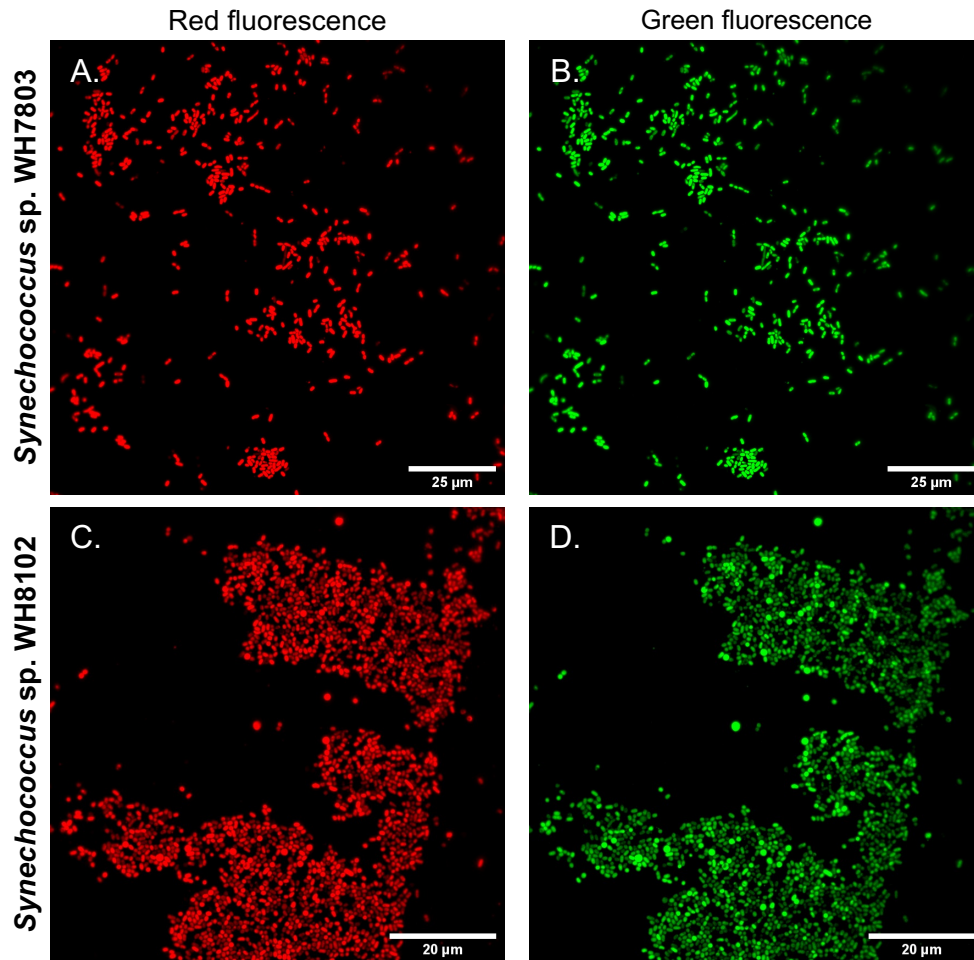


Extended Data Figure 2. Schematic illustration of the experimental design for the calcein experiments

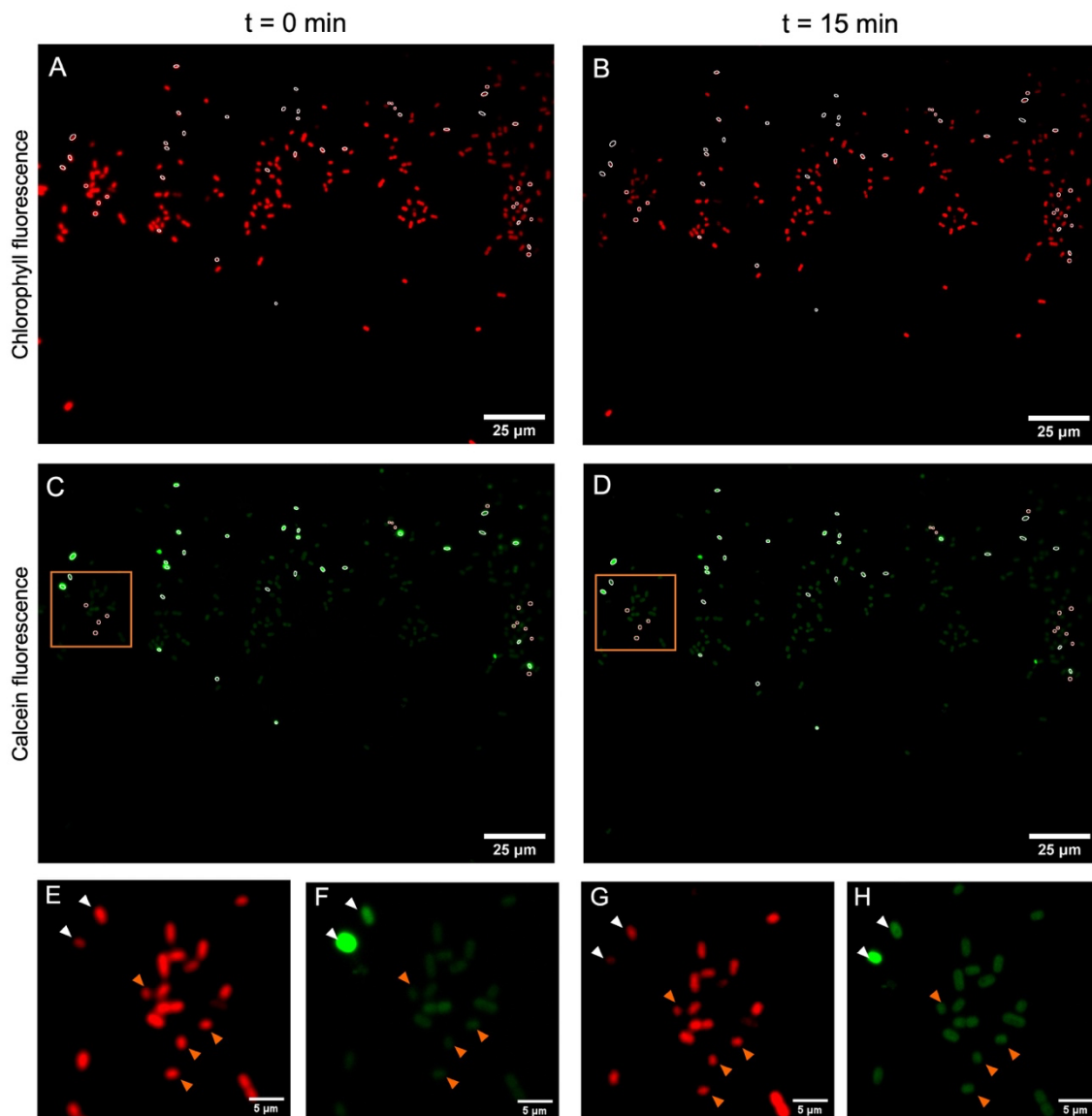
***Synechococcus* sp. PCC7002**



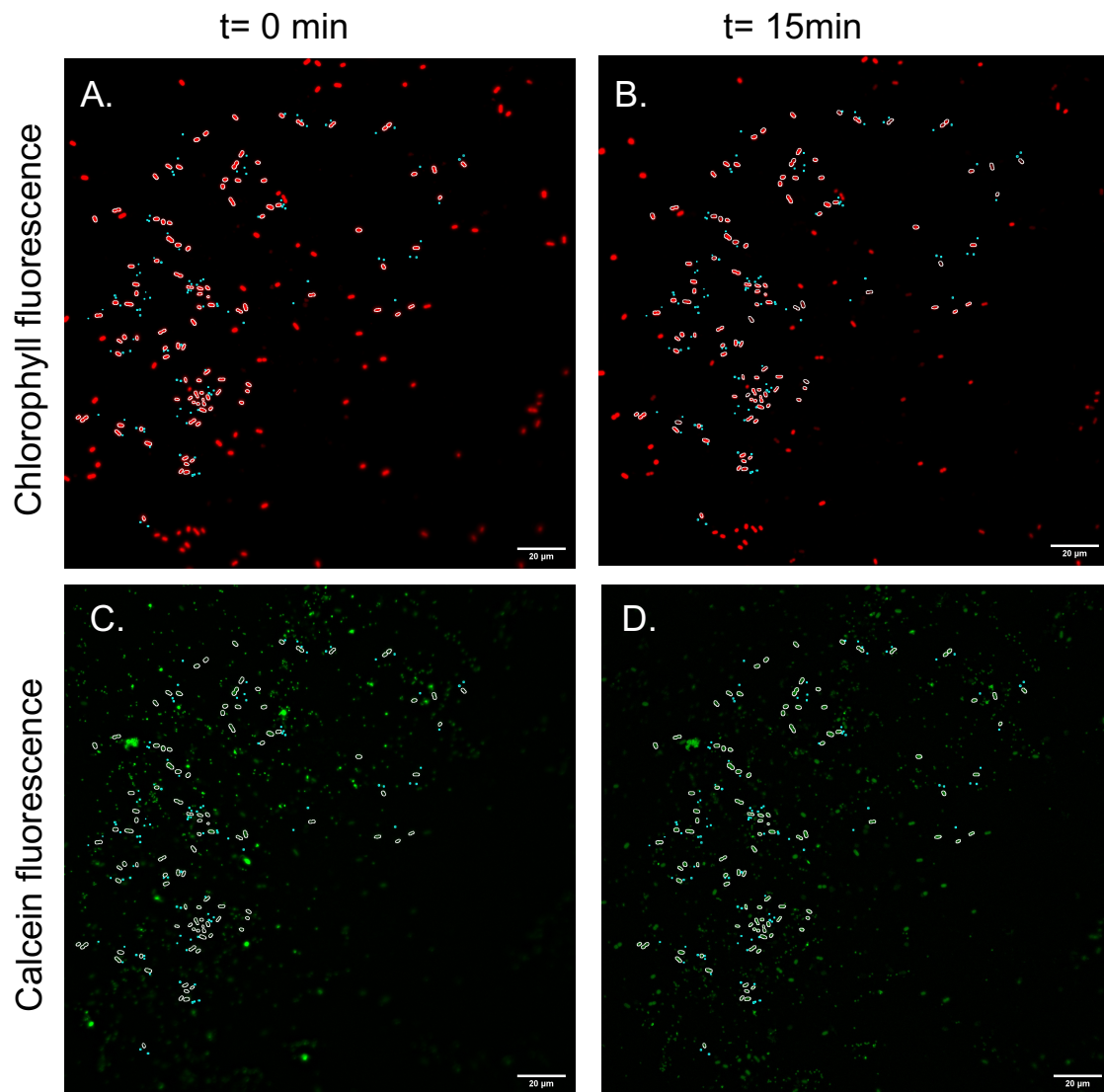
Extended Data Figure 3. *Synechococcus* sp. PCC 7002 cells are efficiently stained with Calcein-AM. Fluorescence microscopy images of *Synechococcus* sp. PCC 7002 cells. Left column shows red chlorophyll fluorescence (autofluorescence) and right column shows green fluorescence (Calcein-AM). (A-B) Cells labeled with calcein. (C-D) Cells not labeled with calcein. (E-F) Cells incubated with the supernatant from the calcein-labeled cells.



Extended Data Figure 4. *Synechococcus* sp. WH7803 and WH8102 cells without calcein addition showing autofluorescence in red and green channels. The red channel corresponds with chlorophyll and green with phycoerythrin and phycocyanin pigments, the later overlapping with the calcein channel.

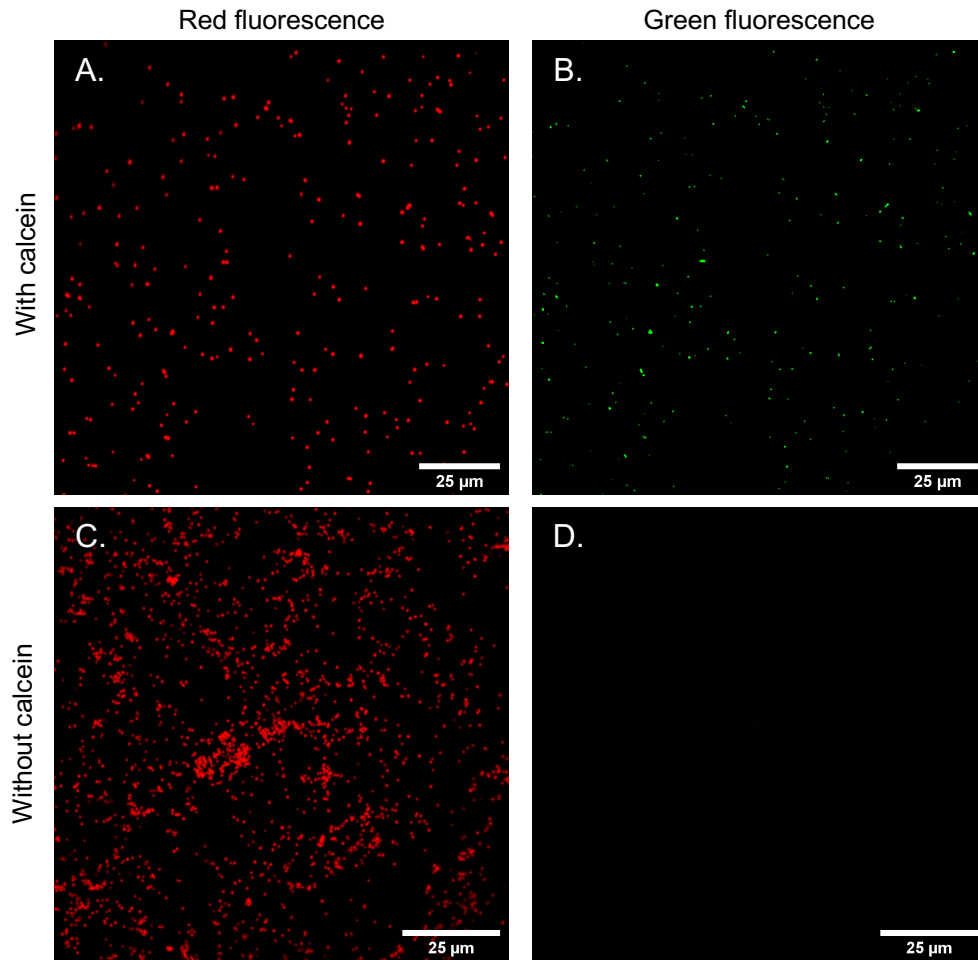


Extended Data Figure 5. Transfer of calcein between *Synechococcus* sp. PCC 7002 and *Prochlorococcus* sp. MIT9313 cells. (A, B, C, D) *Synechococcus* sp. PCC 7002 calcein-labeled cells were mixed with unlabeled *Prochlorococcus* sp. MIT9313. 100 cells were selected: 50 calcein-labeled *Synechococcus* cells (white circles) and 50 unlabeled *Prochlorococcus* cells (pink circles). The average fluorescence intensity at $t = 0$ min vs $t = 15$ min (after the mix) was compared (Extended Data). (E-H) A magnification of the orange squares in C and D. White arrows highlight *Synechococcus* cells whose average fluorescence intensity decreases after 15 minutes. Orange arrows highlight *Prochlorococcus* cells whose average fluorescence intensity increases after 15 minutes. In panels E-G, *Synechococcus* cells appear with a size clearly bigger than *Prochlorococcus*.

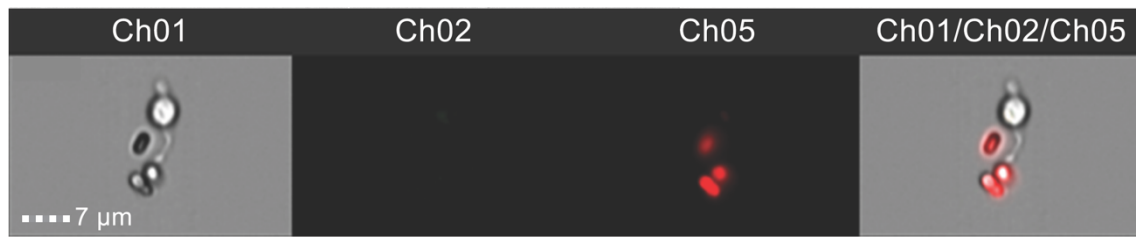


Extended Data Figure 6. Transfer of calcein between *Prochlorococcus* sp. SS120 cells and *Synechococcus* sp. PCC 7002. *Prochlorococcus* sp. SS120 calcein-labeled cells were mixed with unlabeled *Synechococcus* sp. PCC 7002. 100 cells were selected: 50 calcein-labeled *Prochlorococcus* cells (cyan circle) and 50 unlabeled *Prochlorococcus* cells (white circle); and compared the average fluorescence intensity at t= 0 min (A, C) vs t = 15 min (B, D) (after the mix). Top images correspond with the autofluorescence (chlorophyll) and below images with the immunofluorescence (calcein-AM).

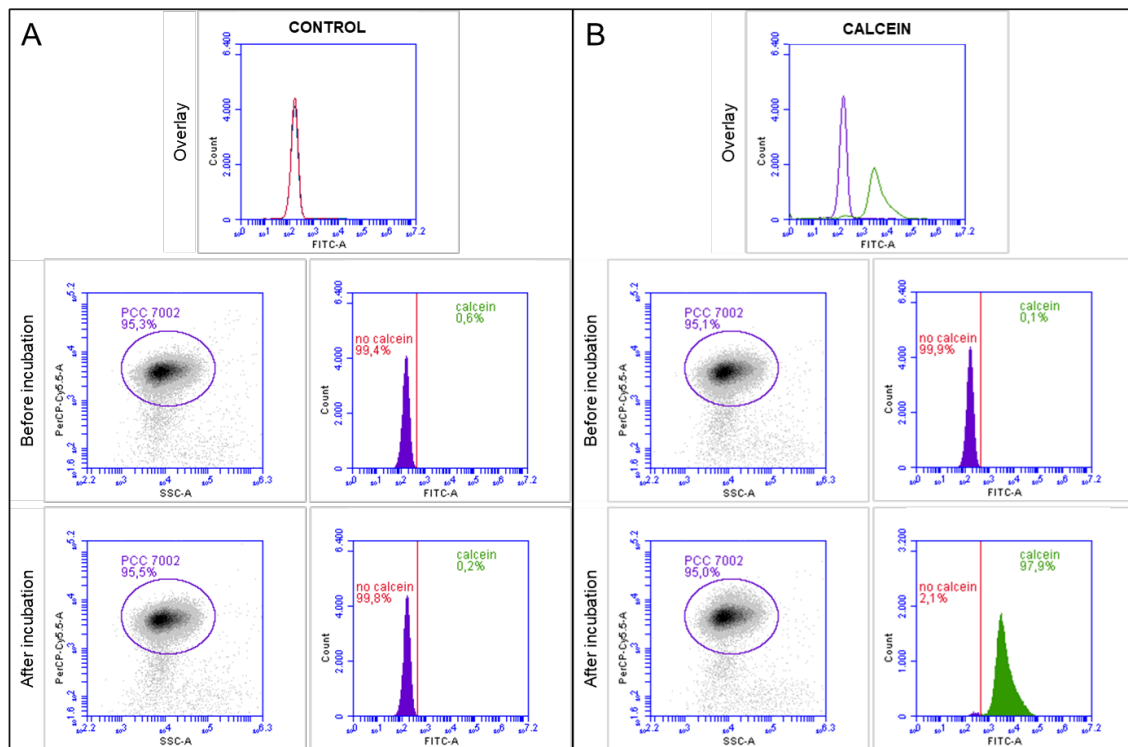
***Prochlorococcus* sp. SS120**



Extended Data Figure 7. *Prochlorococcus* cells are efficiently stained with Calcein-AM. Fluorescence microscopy images of *Prochlorococcus* sp. SS120 cells. Left column shows red chlorophyll fluorescence (autofluorescence) and right column shows green fluorescence (Calcein-AM). (A-B) Cells labeled with calcein. (C-D) Cells not labeled with calcein.

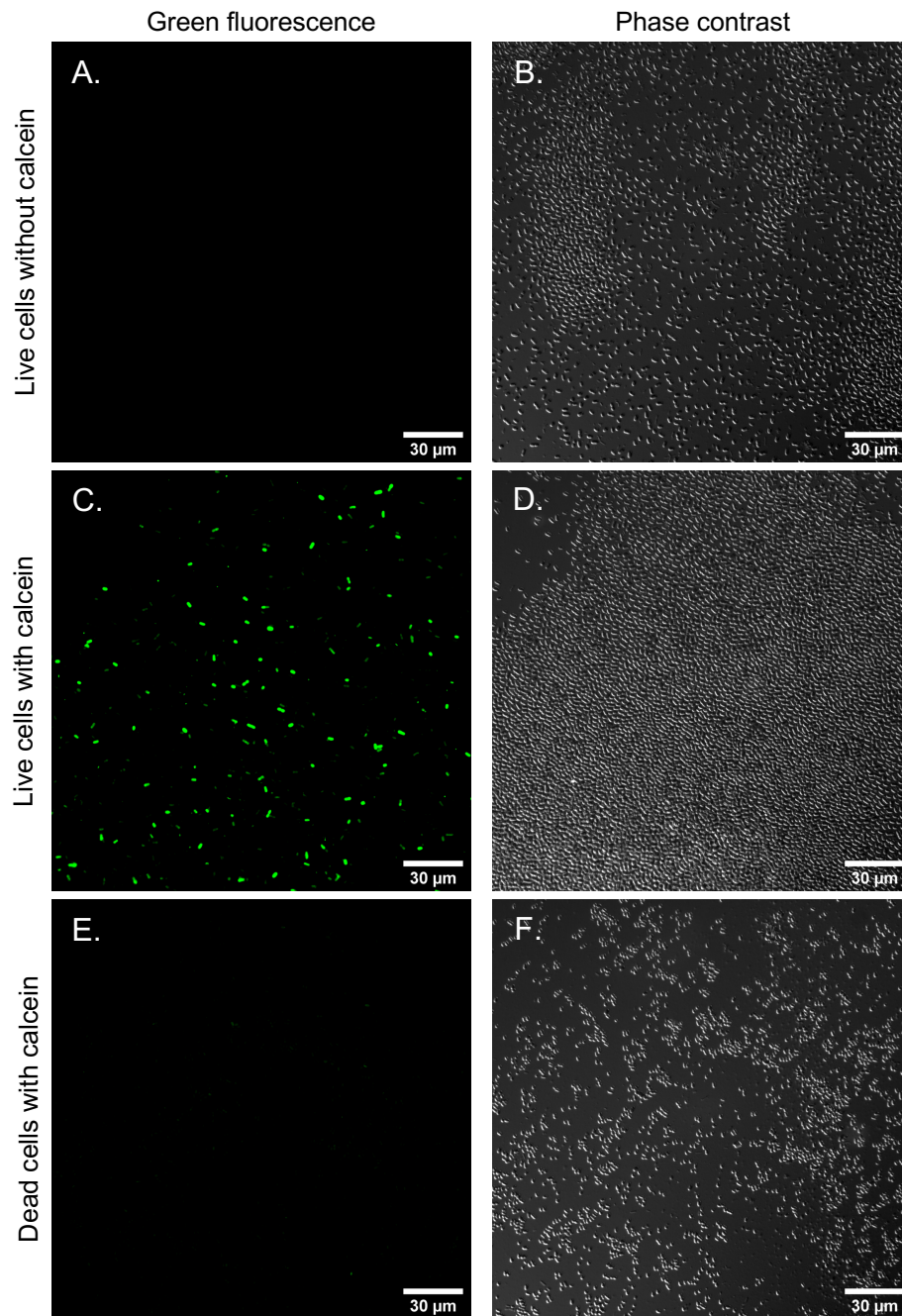


Extended Data Figure 8. Interaction between *Synechococcus* sp. WH7803 and heterotrophic bacteria mediated by nanotubes in non-axenic culture of *Synechococcus* sp. WH7803. Bright field images at 60x magnification of aggregates between *Synechococcus* sp. WH7803 and heterotrophic bacteria observed by Imaging Flow Cytometry.



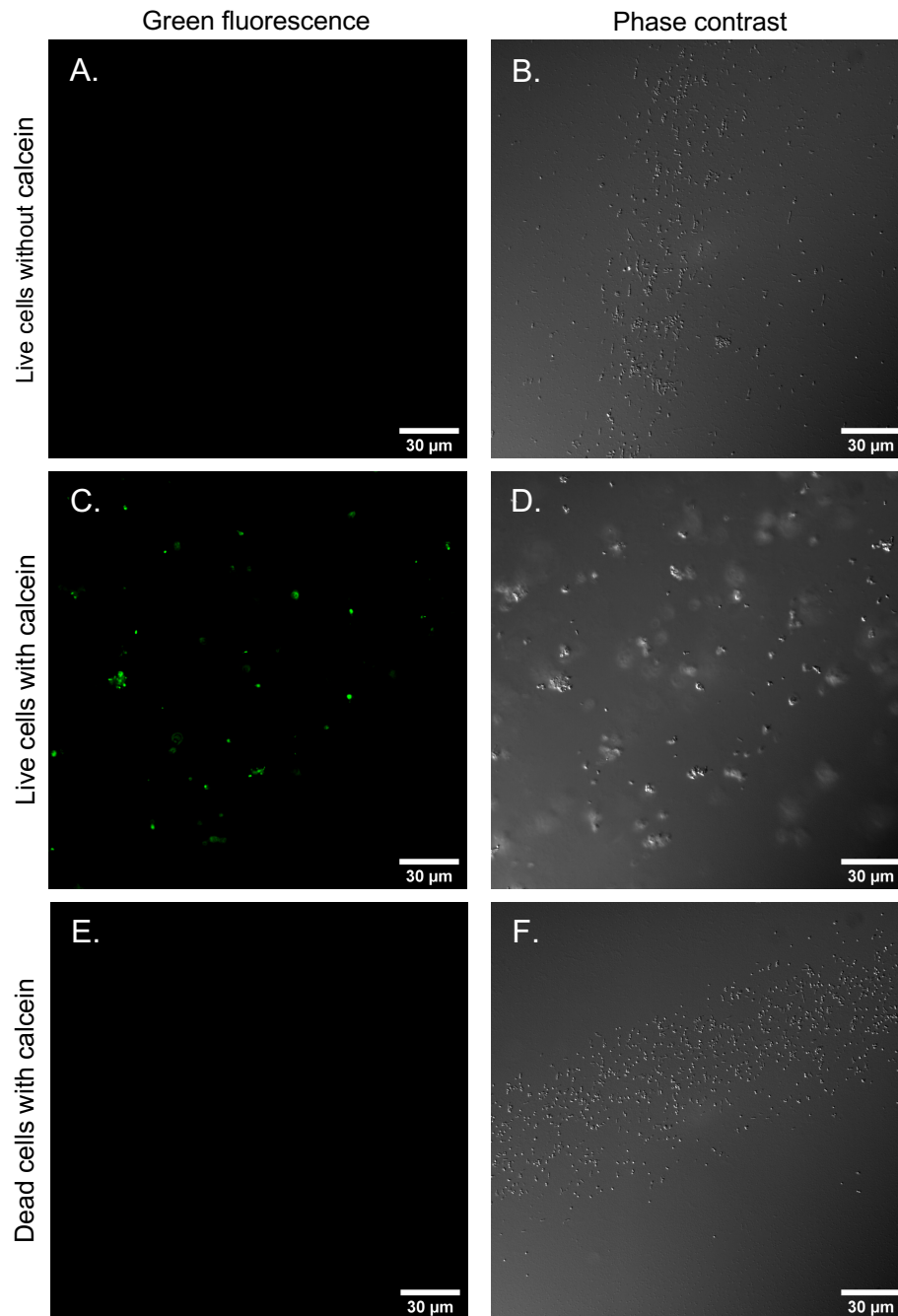
Extended Data Figure 9. Monitoring Calcein-AM staining of *Synechococcus* sp. PCC 7002 by flow cytometry. Measurements were made before and after the 3-hour incubation period for both control (A) and calcein-added (B) sample. For each sample there is an overlay plot of the fluorescence before and after the incubation (first row); a density plot (FSC-A vs PerCP) showing chlorophyll fluorescence of the population and a histogram showing calcein fluorescence (FITC) gated by the population of *Synechococcus* sp. PCC 7002 (second and third rows).

***Synechococcus* sp. PCC7002**



Extended Data Figure 10. Fluorescence microscopy images of *Synechococcus* sp. PCC 7002 cells. Left column shows green fluorescence (calcein) images and right column shows phase contrast images. (A-B) Live cells without calcein. (C-D) Live cells labeled with calcein. (E-F) Dead cells labeled with calcein.

***Prochlorococcus* sp. MIT9313**



Extended Data Figure 11. Fluorescence microscopy images of *Prochlorococcus* sp. MIT9313 cells. Left column shows green (calcein) fluorescence images and right column shows phase contrast images. (A-B) Live cells without calcein. (C-D) Live cells labeled with calcein. (E-F) Dead cells labeled with calcein.

Extended Data Table 1. Average fluorescence intensity (a.u.) of the three combinations of cells used at t=0 min and t= 15 min and difference between timepoints of the two cell populations (labeled and non-labeled with calcein-AM).

Combination 1 <i>Synechococcus</i> sp. PCC 7002 calcein-labeled cells mixed with <i>Synechococcus</i> sp. PCC 7002 non-labeled cells	Population	Average fluorescence intensity (a.u.) of the 50 cells – background fluorescence at t= 0 min	Average fluorescence intensity (a.u.) of the 50 cells – background fluorescence at t= 15 min	Δ over time (a.u)
	Labeled cells	5384.6	1464.7	Decrease of 3191.9
	Non- labeled cells	539.2	734.4	Increase of 195.2
Combination 2 <i>Synechococcus</i> sp. PCC 7002 calcein-labeled cells mixed with <i>Prochlorococcus</i> sp. MIT9313 non-labeled cells	Labeled cells	4697.9	2250.3	Decrease of 2447.6
	Non- labeled cells	256.04	331.55	Increase of 75.51
Combination 3 <i>Prochlorococcus</i> sp. SS120 calcein-labeled cells mixed with <i>Prochlorococcus</i> sp. SS120 non-labeled cells	Labeled cells	769.97	148.85	Decrease of 621.13
	Non- labeled cells	218.3	251.3	Increase of 33.08

Extended Data Table 2. Description of proteins involved in NT formation.

Functional group	Protein	NCBI ID	Protein annotation	Organism
CORE	FliP	NP_389517.1	flagellar biosynthesis protein FliP	<i>Bacillus subtilis</i> subsp. subtilis str. 168
CORE	FliQ	NP_389518.1	flagellar type III secretion system protein FliQ	<i>Bacillus subtilis</i> subsp. subtilis str. 168
CORE	FliR	NP_389519.2	flagellar type III secretion system protein FliR	<i>Bacillus subtilis</i> subsp. subtilis str. 168
CORE	FlhA	NP_389521.2	flagellar type III secretion system protein FlhA	<i>Bacillus subtilis</i> subsp. subtilis str. 168
CORE	FlhB	NP_389520.1	flagellar type III secretion system protein FlhB	<i>Bacillus subtilis</i> subsp. subtilis str. 168
CORE	EscR	NP_312610.2	T3SS structure protein EscR	<i>Escherichia coli</i> O157:H7 str. Sakai
CORE	EscS	NP_312609.1	type III secretory pathway, component EscS	<i>Escherichia coli</i> O157:H7 str. Sakai
CORE	EscT	NP_312608.1	type III secretion protein SpaR/YscT/HrcT	<i>Escherichia coli</i> O157:H7 str. Sakai
CORE	EscU	NP_312607.1	EscU/YscU/HrcU family type III secretion system export apparatus switch protein	<i>Escherichia coli</i> O157:H7 str. Sakai
CORE	EscV	NP_312596.1	type III secretory pathway, component EscV	<i>Escherichia coli</i> O157:H7 str. Sakai
Cell wall hydrolysis	LytE	NP_388823.2	cell wall dl-endopeptidase; phosphatase-associated protein (major autolysin)	<i>Bacillus subtilis</i> subsp. subtilis str. 168
Cell wall hydrolysis	LytB	NP_391443.1	putative cell wall-binding domain [Cell wall/membrane/envelope biogenesis]	<i>Bacillus subtilis</i> subsp. subtilis str. 168
Cell wall hydrolysis	LytC	NP_391442.1	N-acetylmuramoyl-L-alanine amidase (major autolysin)	<i>Bacillus subtilis</i> subsp. subtilis str. 168
Regulation	YmdB	WP_003245138	YmdB, a global regulator of late adaptive responses; a phosphodiesterase	<i>Bacillus</i> : Multispecies

Extended Data Table 3. Protein hits in *Synechococcus* PCC7002 (taxid:32049)

Protein	Query ID	Subject ID	Percent identity to query	Query alignment coverage	E value	Notes
FliP	NP_389517.1	NA	NA	NA	NA	delta-blast no hits
FliQ	NP_389518.1	NA	NA	NA	NA	delta-blast no hits
FliR	NP_389519.2	NA	NA	NA	NA	delta-blast no hits
FlhA	NP_389521.2	NA	NA	NA	NA	delta-blast no hits
FlhB	NP_389520.1	NA	NA	NA	NA	delta-blast no hits
EscR	NP_312610.2	NA	NA	NA	NA	delta-blast no hits
EscS	NP_312609.1	NA	NA	NA	NA	delta-blast no hits
EscT	NP_312608.1	NA	NA	NA	NA	delta-blast no hits
EscU	NP_312607.1	NA	NA	NA	NA	delta-blast no hits
EscV	NP_312596.1	NA	NA	NA	NA	delta-blast no hits
LytE	NP_388823.2	WP_012308356.1	25.9	29	1.00E-20	LysM peptidoglycan-binding domain-containing M23 family metallopeptidase; confirmed with Delta-BLAST
LytE	NP_388823.2	WP_012307784.1	30	14	7.00E-03	C40 family peptidase; pfam00877
LytB	NP_391443.1	ACA99878.1	31.3	40	1.00E-29	SpoIID/LytB domain-containing protein; aligns to SpoIID domain only
LytB	NP_391443.1	WP_012307251.1	31.6	40	1.00E-19	SpoIID/LytB domain-containing protein; aligns to SpoIID domain only
LytC	NP_391442.1	WP_012306209.1	46	34	2.00E-38	N-acetylmuramoyl-L-alanine amidase; does not have LyB
LytC	NP_391442.1	WP_012306215.1	31.2	36	1.00E-17	N-acetylmuramoyl-L-alanine amidase; does not have LyB
YmdB	WP_003245138	NA	NA	NA	NA	delta-blast no hits

Extended Data Table 4. Protein hits in *Synechococcus* WH8102 (taxid:84588)

Protein	Query ID	Subject ID	Percent identity to query	Query alignment coverage	E value	Notes
FliP	NP_389517.1	NA	NA	NA	NA	delta-blast no hits
FliQ	NP_389518.1	NA	NA	NA	NA	delta-blast no hits
FliR	NP_389519.2	NA	NA	NA	NA	delta-blast no hits
FlhA	NP_389521.2	NA	NA	NA	NA	delta-blast no hits
FlhB	NP_389520.1	NA	NA	NA	NA	delta-blast no hits
EscR	NP_312610.2	NA	NA	NA	NA	delta-blast no hits
EscS	NP_312609.1	NA	NA	NA	NA	delta-blast no hits
EscT	NP_312608.1	NA	NA	NA	NA	delta-blast no hits
EscU	NP_312607.1	NA	NA	NA	NA	delta-blast no hits
EscV	NP_312596.1	NA	NA	NA	NA	delta-blast no hits
LytE	NP_388823.2	WP_011128815.1	28.9	36	2.00E-12	LysM peptidoglycan-binding domain-containing protein
LytE	NP_388823.2	WP_011128971.1	24.8	27	5.00E-04	C40 family peptidase
LytB	NP_391443.1	WP_083810650.1	30.2	45	2.00E-26	SpoIID/LytB domain-containing protein; aligns to SpoIID domain only
LytB	NP_391443.1	WP_011126937.1	36.4	16	3.00E-15	SpoIID/LytB domain-containing protein; aligns to SpoIID domain only
LytB	NP_391443.1	CAE07054.1	28.7	56	2.00E-26	similar to stage II sporulation protein D
LytC	NP_391442.1	WP_225867742.1	39.4	35	9.00E-31	N-acetylmuramoyl-L-alanine amidase
LytC	NP_391442.1	CAE07524.1	39.4	35	3.94E+01	N-acetylmuramoyl-L-alanine amidase
YmdB	WP_003245138	NA	NA	NA	NA	a weak hit to a hypothetical protein with E value=0.044; SYNW0778

Extended Data Table 5. Protein hits in *Prochlorococcus* SB (taxid:59926)

Protein	Query ID	Subject ID	Percent identity to query	Query alignment coverage	E value	Notes
FliP	NP_389517.1	NA	NA	NA	NA	delta-blast no hits
FliQ	NP_389518.1	NA	NA	NA	NA	delta-blast no hits
FliR	NP_389519.2	NA	NA	NA	NA	delta-blast no hits
FlhA	NP_389521.2	NA	NA	NA	NA	delta-blast no hits
FlhB	NP_389520.1	NA	NA	NA	NA	delta-blast no hits
EscR	NP_312610.2	NA	NA	NA	NA	delta-blast no hits
EscS	NP_312609.1	NA	NA	NA	NA	delta-blast no hits
EscT	NP_312608.1	NA	NA	NA	NA	delta-blast no hits
EscU	NP_312607.1	NA	NA	NA	NA	delta-blast no hits
EscV	NP_312596.1	NA	NA	NA	NA	delta-blast no hits
LytE	NP_388823.2	WP_032519589.1	30.4	36	2.00E-06	LysM peptidoglycan-binding domain-containing protein
LytE	NP_388823.2	WP_032520089.1	31.7	26	3.00E-06	C40 family peptidase
LytB	NP_391443.1	WP_052043492.1	28.4	44	9.00E-29	SpoIID/LytB domain-containing protein; aligns to SpoIID domain only
LytB	NP_391443.1	WP_032519945.1	25.8	41	4.00E-12	SpoIID/LytB domain-containing protein; aligns to SpoIID domain only
LytC	NP_391442.1	WP_032519270.1	42.3	36	6.00E-34	N-acetylmuramoyl-L-alanine amidase
YmdB	WP_003245138	NA	NA	NA	NA	NA

Extended Data Table 6. Plasmids constructed for the generation of mutant strains of *Synechococcus* sp. PCC 7002.

NAME	DESCRIPTION	COMMENTS
pRLi21a	A derivative of pTrc99A carrying the <i>sf-gfp</i> gene and the C.K1 kanamycin resistance cassette	Used as platform for further constructions
pPC-deliv2	A derivative of pRLi21a carrying the <i>trc</i> promoter upstream of the superfolder-GFP gene, a kanamycin resistance cassette and the <i>rrnB</i> terminator from <i>E. coli</i> flanked by sequences NS1-LEFT and NS1-RIGHT for recombination in a neutral site of the <i>Synechococcus</i> sp. PCC 7002 genome.	Expresses sf-GFP in the cytoplasm of <i>Synechococcus</i>
pPC3	A derivative of pPC-deliv2 where the <i>trc</i> promoter was replaced by the promoter of the RuBisCO large subunit gene and the signal peptide of the GN=SYNPCC7002_A0313 gene upstream of the <i>sf-gfp</i> gene	Expresses sf-GFP in the periplasm of <i>Synechococcus</i>
pPC4	A derivative of pPC-deliv2 where the <i>trc</i> promoter was replaced by promoter of the RuBisCO large subunit gene and the signal peptide of the GN=SYNPCC7002_A0500 gene upstream of the <i>sf-gfp</i> gene	Expresses sf-GFP in the periplasm of <i>Synechococcus</i>
pPC5	A derivative of pPC-deliv2 where the <i>trc</i> promoter was replaced by promoter of the GN=SYNPCC7002_A0740 gene and the signal peptide of the GN=SYNPCC7002_A0313 gene upstream of the <i>sf-gfp</i> gene	Expresses sf-GFP in the periplasm of <i>Synechococcus</i>
pPC6	A derivative of pPC-deliv2 where the <i>trc</i> promoter was replaced by promoter of the GN=SYNPCC7002_A0740 gene and the signal peptide of the	Expresses sf-GFP in the periplasm of <i>Synechococcus</i>

	GN=SYNPCC7002_A0500 gene upstream of the <i>sf-gfp</i> gene	
--	---	--

Extended Data Table 7. Oligonucleotides used in the generation of mutant strains of *Synechococcus* sp. PCC 7002.

NAME	SEQUENCE
NS1-LEFT-F	gcatgcatCAAGCCCAAGGGTTTCGTAGG
NS1-LEFT-R	cgttgccgAGATCGAAAGAAAGAGGATCC
NS1-RIGHT-F	cttttgcAGCTTCATTATATAGAATTTC
NS1-RIGHT-R	gtagaaacTTGCGAATGTGATTGTAGAAG
pPC1-1F	TTCGATCTgcgcaacgcaattaatgtgag
pPC1-1R	TGGGCTTGatgcatgccgcttcgccttcg
pPC1-2F	ATTCGCAAgtttctacaaactcttttgttta
pPC1-2R	ATGAAGCTgcaaaaaggccatccgtcagg
Prom_A0740-A1	TCGGGGGCCCCCGGGGGGATAATGGCGTGATATGATTGCACGTG
Prom-A0740-A2	TCGGGCCCtctCCGGGGATTGGGGTGTTACTTGCCGAATC
Prom_rbc-A1	TCGGGGGCCCCCGGGGGGATTTGTTGCTAAAAGATAAAAAATAAGTCG
Prom_rbc-A2	TCGGGCCCtctCCGGGGATGCGGTTTTCTCCAGCAAAAATGC
PepS_A0313-A2	ATCCCCGgaggaGGGCCCgaATGCCCAATCGCCGCCATTTTTTG
PepS_A0313-A3	AGTTCTTCTCCTTTGCTCATTCGTTGACACCCTCCCAAACACCC
PepS_A0500-A2	ATCCCCGgaggaGGGCCCgaATGTTTAATGGTCTCAAAGGGCTAC
PepS_A0500-A3	AGTTCTTCTCCTTTGCTCATACCAGGTACCTGTTGACAAGCGGC
Vector-A1	ATCCCCCGGGGGCCCCCGAgccagaaccgttatgatgtcggc
Vector-A3	atgagcaaaggagaagaacttttc
Prom_trc_R-A2	TCGGGCCCtctCCGGGGATggtgaattccatggtctgtttc
pPC-F	CGCACTCCCGTTCTGGATAATGTT
pPC-R	GGTCACGCTTTTCGTTGGGATCTT