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Supplementary Information for

Molecular bases of an alternative dual-enzyme system for light color acclimation of marine *Synechococcus* cyanobacteria

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Datasets S1 to S4

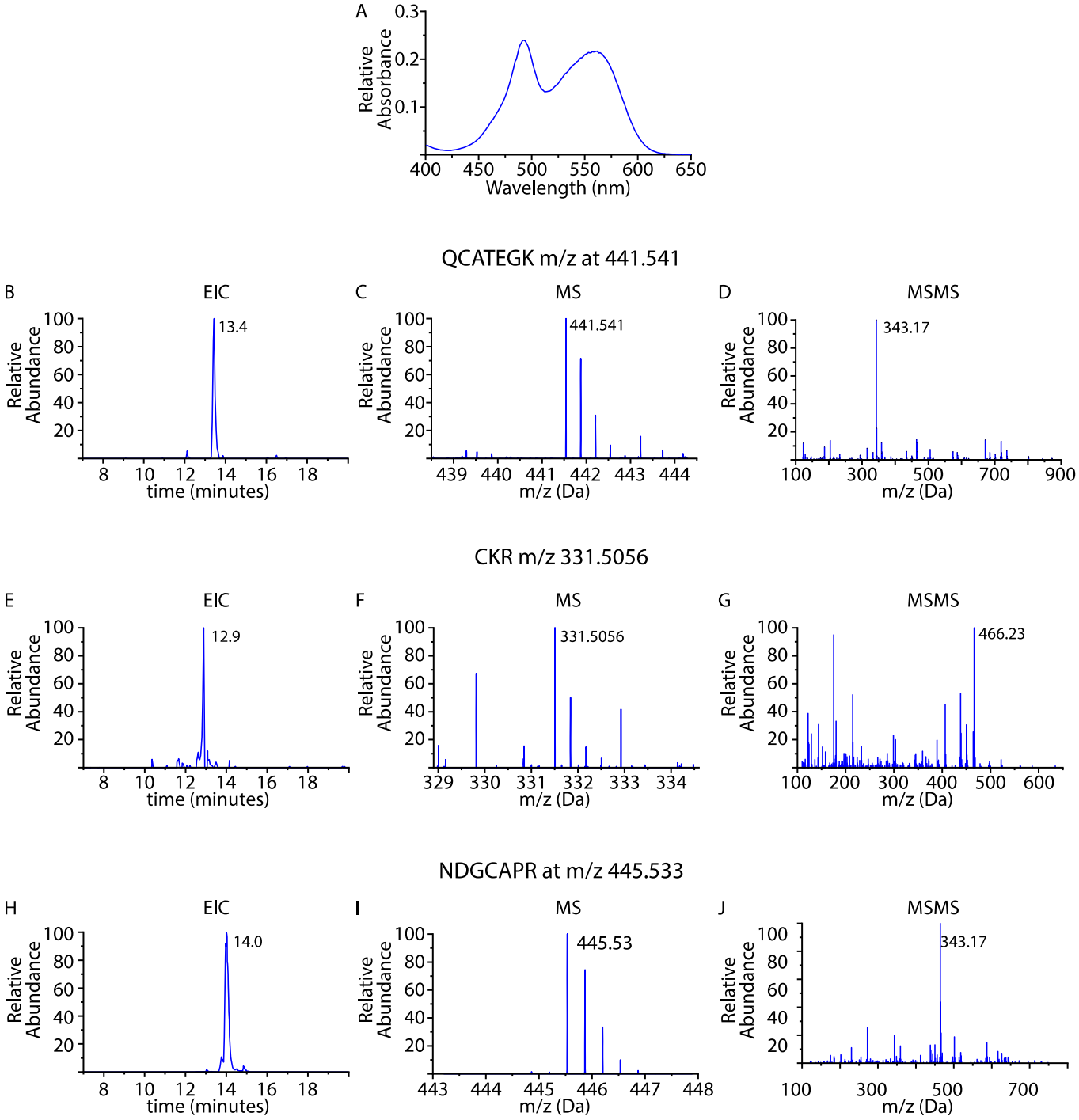


Fig. S1. Absorption spectrum and mass spectrometry analysis of bilin chromophorylation of *Synechococcus* sp. WH7803 phycoerythrin-II α-subunit (MpeA). (*A*) UV-VIS spectrum from the purified *Synechococcus* WH7803 MpeA showing absorbance peaks at 490 and 550 nm, indicating that the protein is binding both PUB and PEB. (*B*) LC-MS analysis showing the EIC at mass 441.541 for the bilin-modified peptide QC*$_{75}$ATEGK showing an elution peak at 13.4 min. (*C*) MS scan of the elution at 13.4 min showing the isotope profile of a peptide with m/z 441.541. (*D*) Tandem mass spectrum of m/z 441.5410 at 13.4 min. The relatively early elution and strong diagnostic ion at m/z 343.17 indicate that it binds with PUB. (*E*) LC-MS analysis showing the EIC at mass 331.506 for the bilin-modified peptide C*$_{83}$KR showing primarily a single species eluting at 12.9 min. (*F*) The MS scan confirms the expected mass at 331.506. (*G*) The strong relative abundance of the diagnostic ion at m/z 466.23, demonstrated the presence of PEB. (*H*) LC-MS analysis showing the EIC at mass 445.533 for the bilin-modified peptide NDGC*$_{140}$APR showing an elution peak at 14.0 min. (*I*) MS scan of the elution at 14.0 min showing the isotope profile of a peptide with m/z 445.533. (*J*) Database interpretation of the MS/MS fragmentation spectra from the 445.533 peptide confirmed the sequence as NDGC*$_{140}$APR. The relatively early elution and strong diagnostic ion at m/z 343.17 indicate modification with PUB. Abbreviations: EIC, extracted ion chromatograph; LC, liquid chromatography; MS, mass spectrometry; PEB, phycoerythrobilin; PUB, phycourobilin.

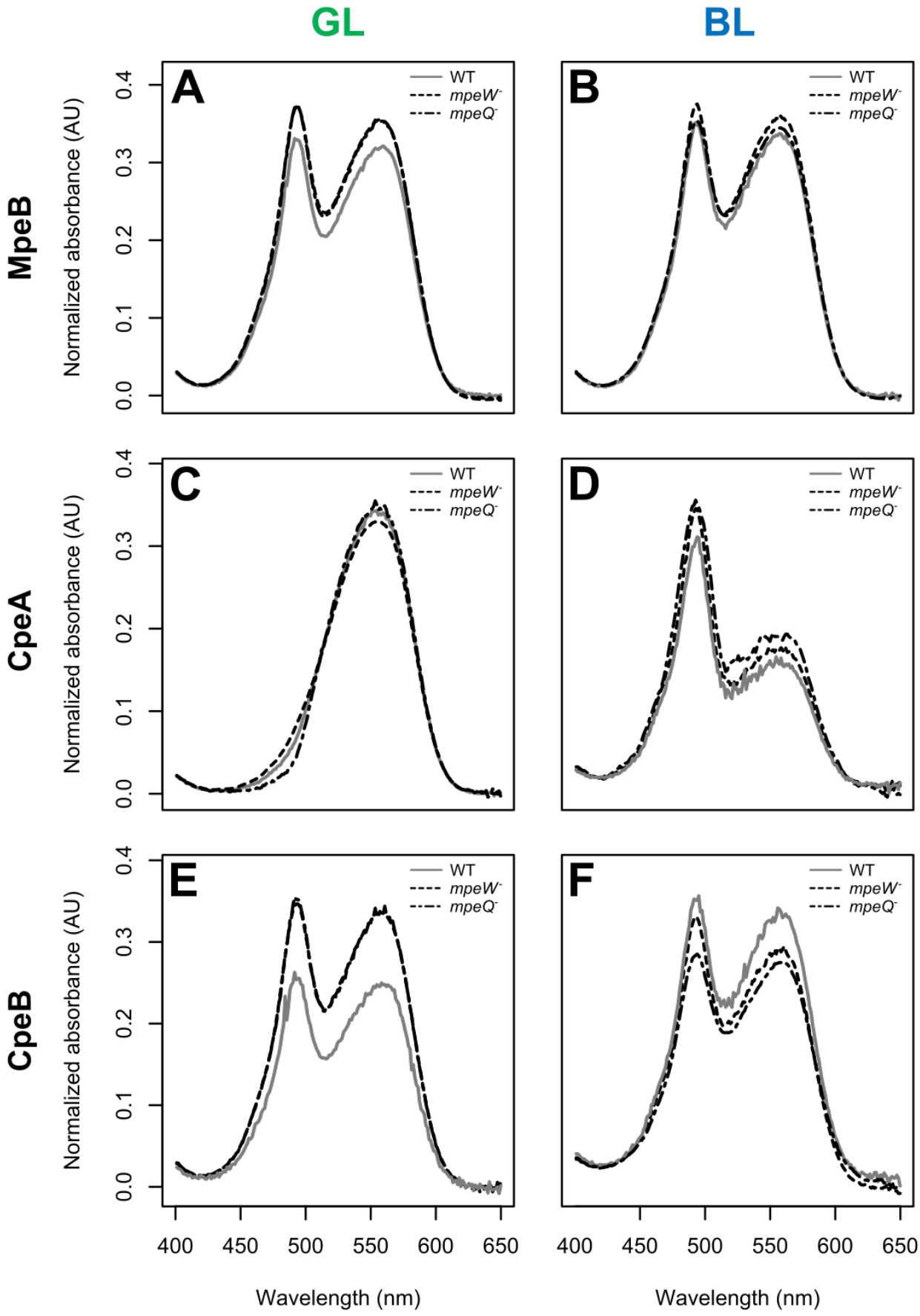


Fig. S2. Absorbance spectra of HPLC-purified phycoerythrin-II β-subunit (MpeB), phycoerythrin-I α- (CpeA) and β- (CpeB) subunits of *Synechococcus* sp. A15-62 WT, *mpeW* inactivation mutant (*mpeW*$^-$) and *mpeQ* inactivation mutant (*mpeQ*$^-$) cells grown under GL or BL. (*A*, *C* and *E*) WT or mutant grown in GL. (*B*, *D* and *F*) WT or mutant grown in BL. (*A* and *B*) MpeB. (*C* and *D*) CpeA. (*E* and *F*) CpeB. See Fig. 2 for absorbance spectra of MpeA. Abbreviations: BL, blue light; GL, green light.

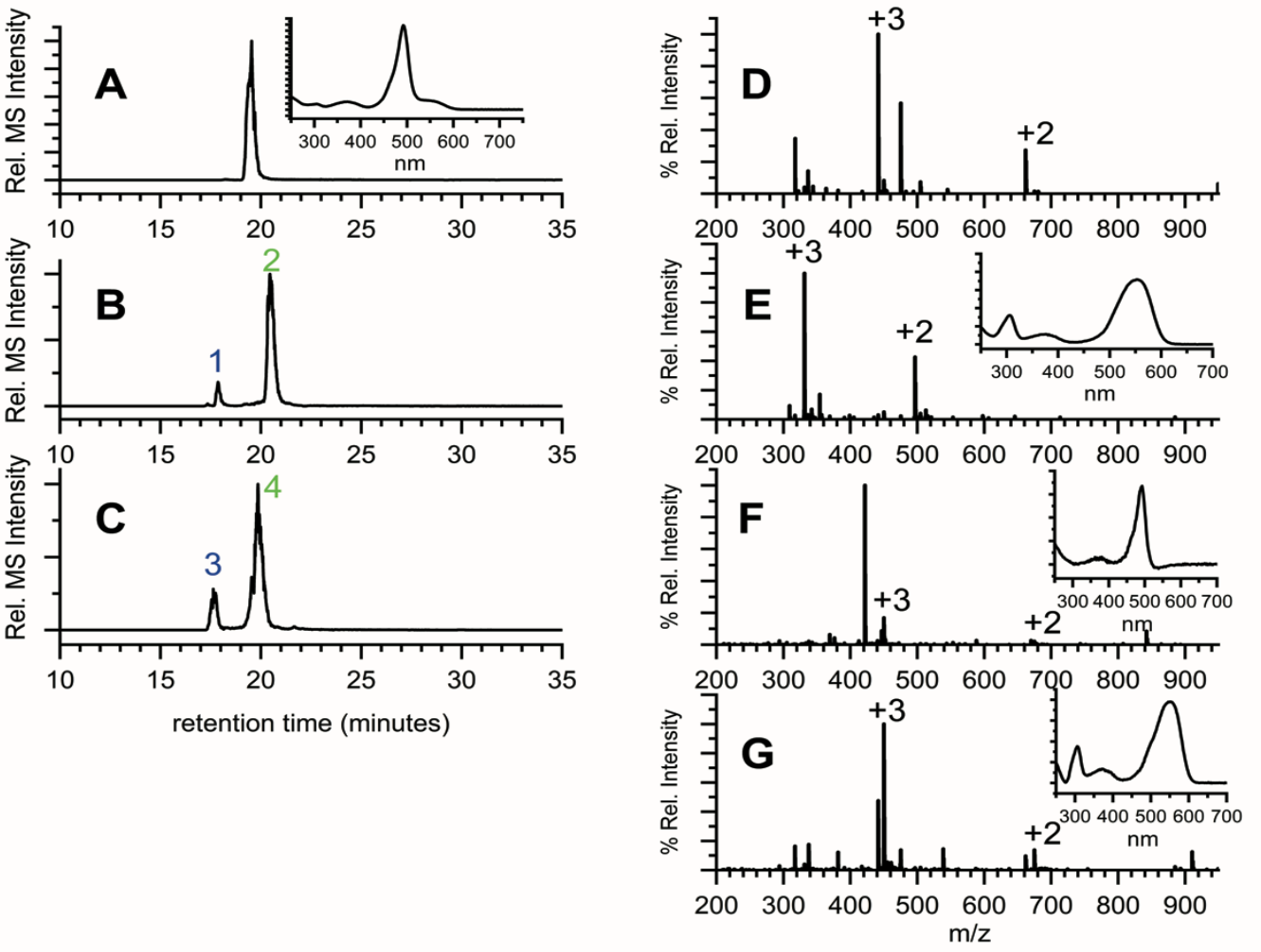


Fig. S3. Mass spectrometry analysis of phycoerythrin-II α-subunit (MpeA) isolated from *Synechococcus* sp. A15-62 wild-type cells grown in GL. (*A*) EIC for m/z 441.5532, KC*$_{75}$ATEGK^{3+} where the * corresponds to a Cys modified by PUB. (*B*) EIC for m/z 336.8372, C*$_{83}$KR^{3+}, where the * corresponds to Cys modified by PUB (peak 1) or PEB and oxidation (peak 2). (*C*) EIC for m/z 450.2204, SRGC*$_{140}$APR, where the * corresponds to Cys modified by PUB (peak 3) or PEB and oxidation (peak 4). (*D*) MS data for the 19.5 min peak in panel *A*. (*E*) MS data for peak 2 in panel *B;* inset UV-VIS spectrum at 19.5 min indicates PEB on Cys$_{140}$. (*F*) MS from 17.60 min peak 3 in C; UV-VIS spectrum indicates PUB modified Cys. (*G*) MS data for the 19.9 min peak 4 in panel *C*; UV-VIS indicates PEB modified Cys. The charge states refer to (peptide+nH)$^{n+}$ ions. Abbreviations: EIC, extracted ion chromatograph; GL, green light; MS, mass spectrometry.

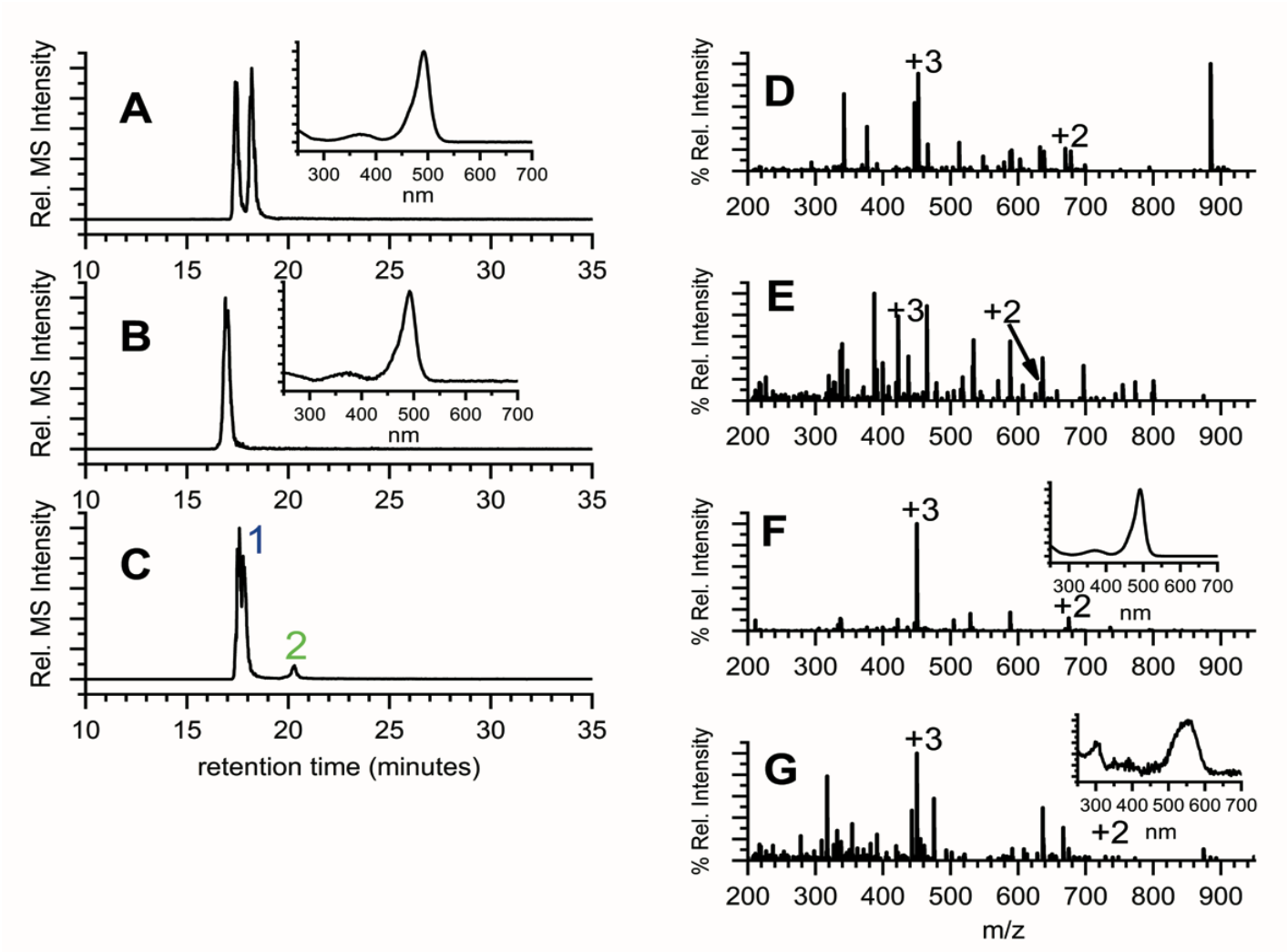


Fig. S4. Mass spectrometry data for phycoerythrin-II α-subunit (MpeA) isolated from *Synechococcus* sp. A15-62 wild-type cells grown in BL. (*A*) EIC for m/z 446.8848, KC*$_{75}$ATEGK^{3+}, where the * corresponds to Cys modified by PUB and oxidation. (*B*) EIC for m/z 422.5498, EKC*$_{83}$KR^{3+}. (*C*) EIC for m/z 450.2204, SRGC*$_{140}$APR. (*D*) MS data for the 18.20 min peak in panel *A* (17.39 min peak in panel *A* gave similar MS, MS-MS, and UV-VIS spectra). (*E*) MS data for the 16.90 min peak in panel *B*. (*F*) MS data for the 17.60 min peak in panel *C* (peak 1); UV-VIS spectrum indicates PUB modified Cys. (*G*) MS data for the 20.31 min peak in panel *C* (peak 2); UV-VIS indicates PEB modified Cys. The charge states indicated in the MS refer to (peptide+nH)$^{n+}$ ions. Abbreviations: BL, blue light; MS, mass spectrometry; EIC, extracted ion chromatograph.

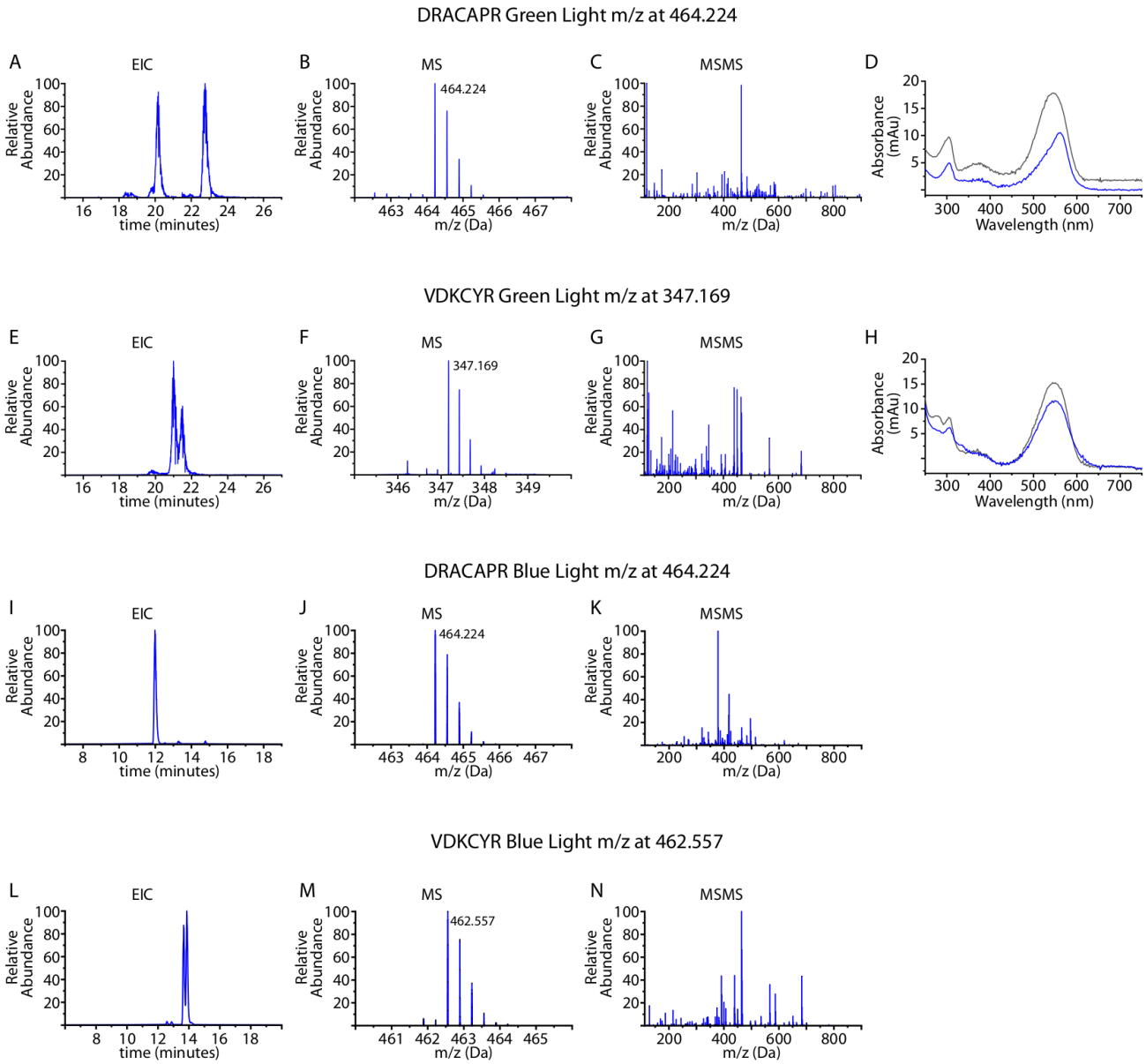


Fig. S5. Tandem mass spectrometry data for phycoerythrin-I α-subunit CpeA-C82 and C139 isolated from for A15-62 WT cells grown in GL and BL. (*A*) LC-MS profile showing the EIC at mass 464.224 for the oxidized version of the bilin-modified peptide DRAC*$_{139}$APR grown in GL with a doublet eluting at 20.1 and 22.8 min. (*B*) MS scan of the elution at 20.1 min showing the isotope profile of a peptide with the expected m/z of 464.224. (*C*) Database interpretation of the MS-MS fragmentation spectra from this 464.224 peptide confirmed the sequence as DRAC*$_{139}$APR. (*D*) UV absorbance spectra at 20.1 min (black) and 22.8 min (blue), with absorbance maxima at 550 nm, indicating both species are modified with PEB. (*E*) LC-MS profile showing the EIC at mass 347.169 for the oxidized version of the bilin-modified peptide VDKC*$_{82}$YR grown in GL with a doublet eluting at 20.1 and 21.1 min. (*F*) MS scan of the elution averaged from 20.9 to 21.2 min showing the isotope profile of a peptide with the expected m/z of 347.169. (*G*) Database interpretation of the MS/MS fragmentation spectra from this 347.2 peptide confirmed the sequence as VDKC*$_{82}$YR. (*H*) UV absorbance spectra at 20.1 min (black) and 21.1 min (blue), with absorbance maxima at 550 nm, indicating that both species are modified with PEB. (*I*) LC-MS profile showing the EIC at mass 464.224 for the oxidized version of the bilin-modified peptide DRAC*$_{139}$APR grown in blue light eluting at 11.88 min. (*J*) MS scan of the elution at 11.88 min showing the isotope profile of a peptide with the expected m/z of 464.224. (*K*) Database interpretation of the MS/MS fragmentation spectra from this 464.224 peptide confirmed the sequence as DRAC*$_{139}$APR. (*L*) LC MS showing the EIC at mass 462.557 for the bilin-modified peptide VDKC*$_{82}$YR grown in BL with a peak eluting in a doublet at 13.65 and 14.17 min. (*M*) MS scan of the elution at 13.65 min showing the isotope profile of a peptide with the expected m/z of 462.557. (*N*) Database interpretation of the MS/MS fragmentation spectra from this 462.557 peptide confirmed the sequence as VDKC*$_{82}$YR. This sample was run without UV/VIS collection, but retention time and tandem MS-MS data from other samples with UV/VIS data were used to differentiate between the PUB and PEB isomers of these peptides. Abbreviations: BL, blue light; EIC, extracted ion chromatograph; GL, green light; LC, liquid chromatography; MS, mass spectrometry; PEB, phycoerythrobilin; PUB, phycourobilin.

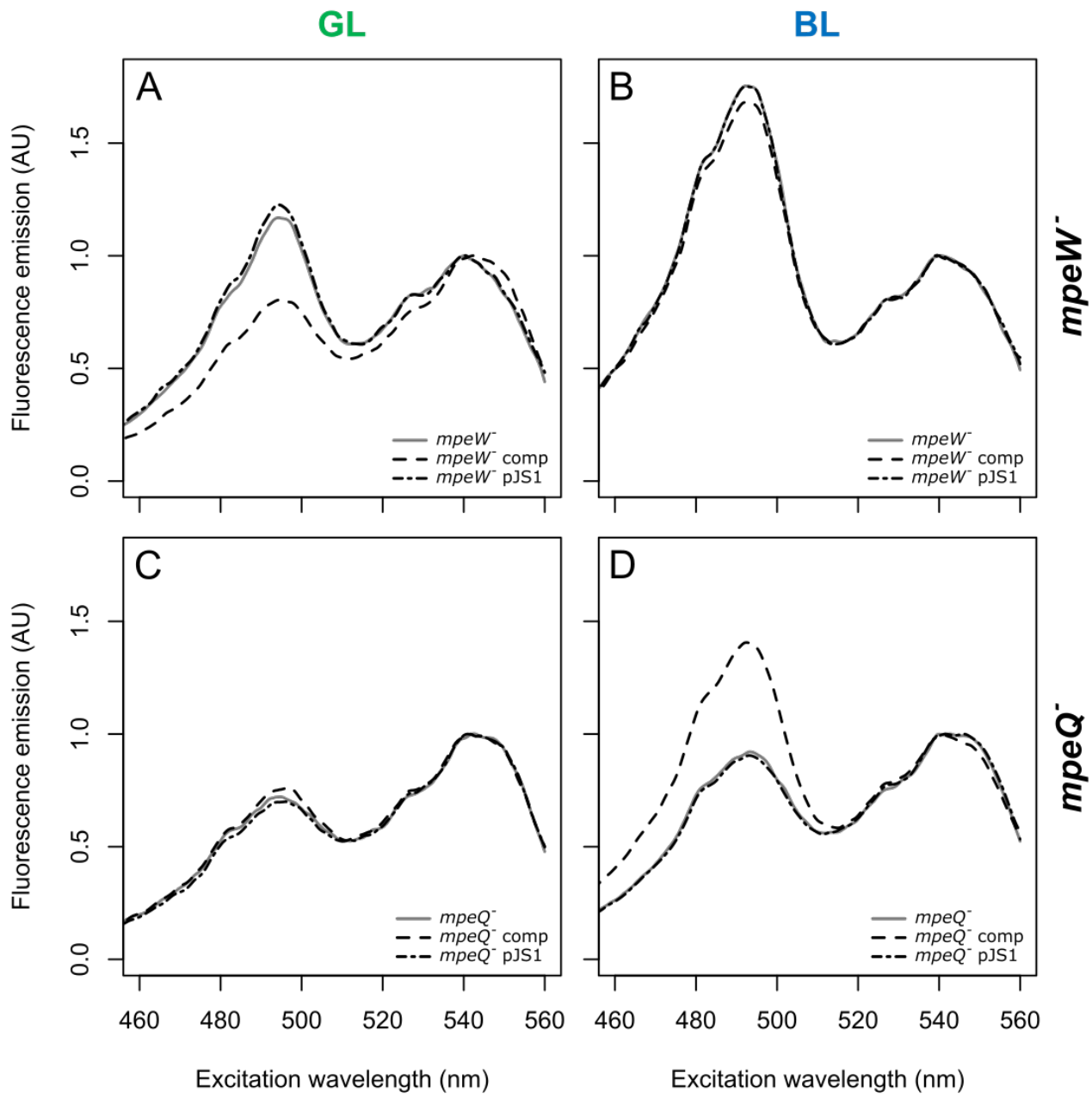


Fig. S6. Whole-cell fluorescence excitation spectra for *Synechococcus* sp. A15-62 mutant and complemented strains. The *mpeW* inactivation mutant (*mpeW*$^-$), *mpeQ* inactivation mutant (*mpeQ*$^-$), complementation strains *mpeW*$^-$ comp (*mpeW*$^-$ mutant with plasmid pTG_A15-62_mpeW_comp) and *mpeQ*$^-$ comp (*mpeQ*$^-$ mutant with plasmid pTG_A15-62_mpeQ_comp) as well as complementation controls *mpeW*$^-$ pJS1 and *mpeQ*$^-$ pJS1 (*mpeW*$^-$ and *mpeQ*$^-$ mutants with empty plasmid pJS1) were acclimated for at least two weeks in either green light (GL) or blue light (BL) before the measurement. (*A, B*) *mpeW*$^-$ mutant, complemented and complementation control strains. (*C, D*) *mpeQ*- mutant, complemented and complementation control strains. Fluorescence emission spectra are normalized at 545 nm for panels *A* and *B* and at 495 nm for panels *C* and *D*. Emission was set at 580 nm. Measurements were repeated twice (biological replicates).

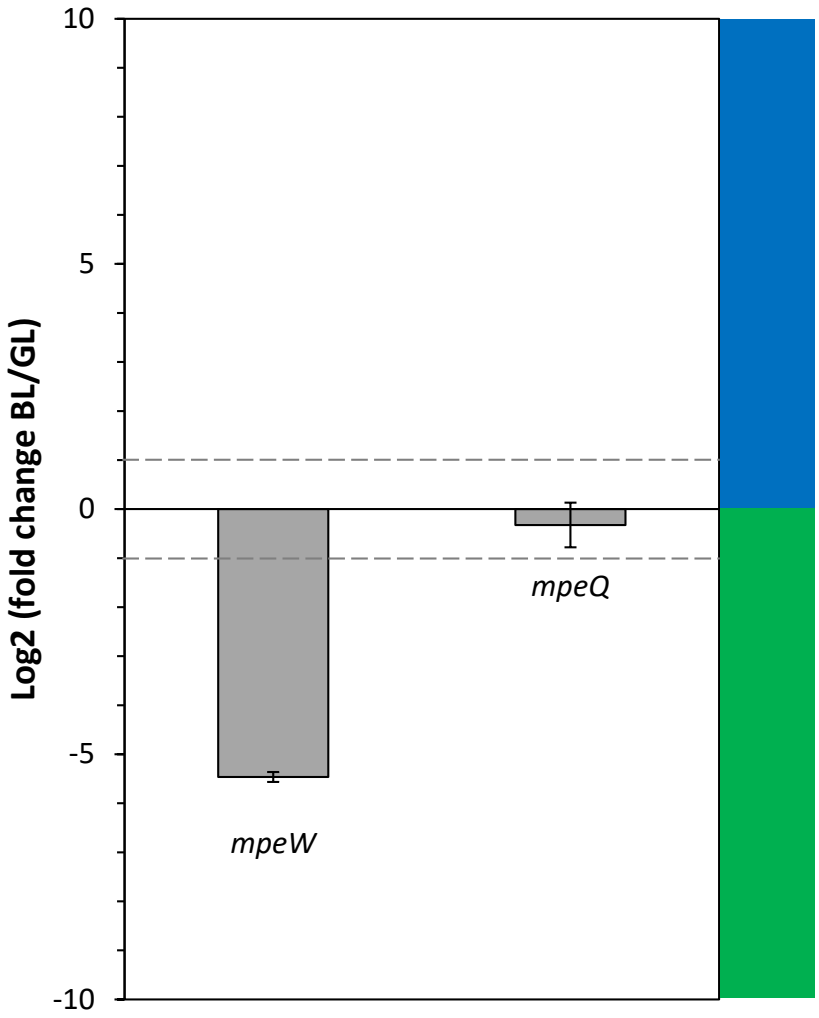


Fig. S7. Differential expression of *mpeW* or *mpeQ* genes in WT *Synechococcus* sp. A15-62 cultures acclimated to 20 µmol photons m^{-2} s^{-1} blue and green light, as measured by real time PCR. Mean and standard deviation were calculated from three biological replicates. Only differential transcript levels above or below the dotted lines (log2(FC) > 1 or < -1) are significant.

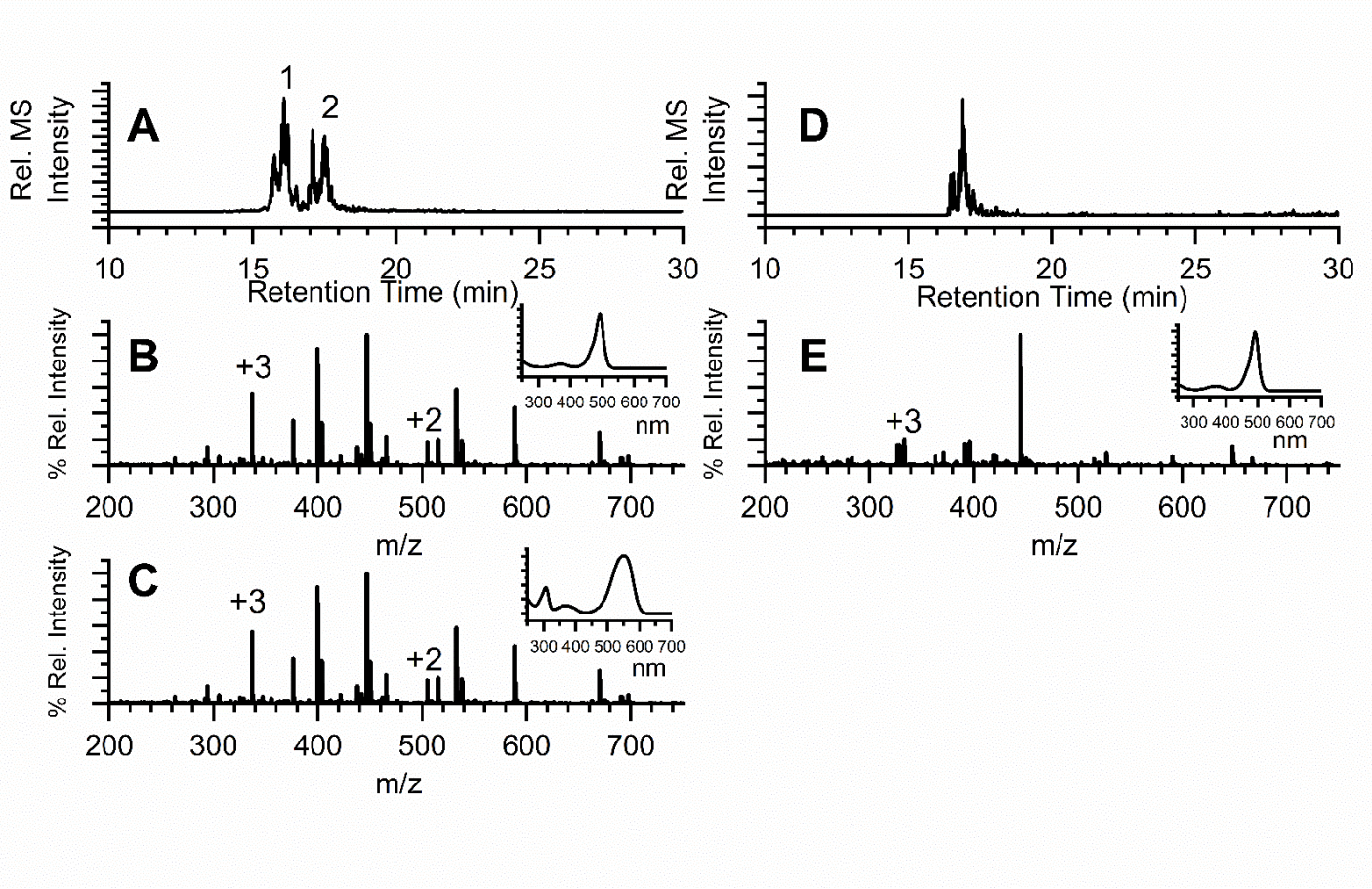


Fig. S8. Mass spectrometry analyses of the phycoerythrin-II α-subunit MpeA-C83 peptides isolated from *Synechococcus* sp. A15-62 *mpeQ*$^{-}$ mutant cells grown in GL and BL. (*A*) EIC for C*$_{83}$KR+bilin+oxygen (m/z 336.8372^{3+}) in GL. (*D*) EIC for C*$_{83}$KR+bilin (m/z 331.5056^{3+}) in BL. (*B*) Mass spectra and UV-VIS spectra (insert) foe the 16.1 min peak (peak 1 in panel *A*). (*C*) Mass spectra and UV-VIS spectra (insert) for the 17.5 min peak (peak 2 in panel *A*). (*E*) Mass spectra and UV-VIS spectra (insert) for the 16.9 min peak in panel (*D*). Charges labeled in mass spectra indicate peaks arising from C*$_{83}$KR peptides. Abbreviations: BL, blue light; EIC, Extracted ion chromatograms; GL, green light.

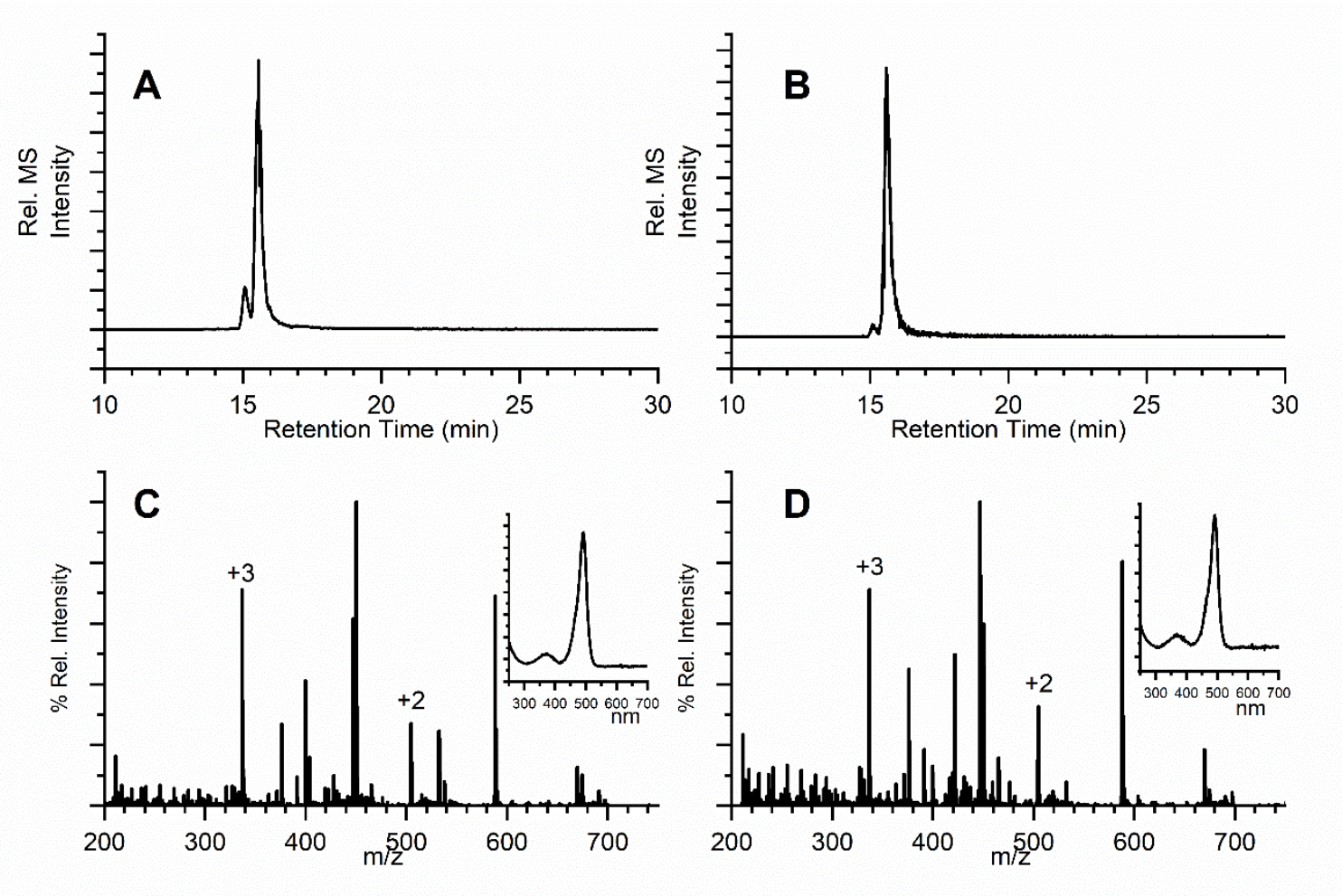


Fig. S9. Mass spectrometry analyses of the phycoerythrin-II α-subunit MpeA-C83 peptides isolated from *Synechococcus* A15-62 *mpeW*$^{-}$ mutant cells grown in GL and BL. EIC for C*$_{83}$KR+bilin+oxygen (m/z 336.8373^{3+}) grown in (*A*) BL and (*B*) GL. (*C-D*) Mass spectra and UV-VIS spectra (insert) from 15.6 min peak in panels *A* and *B*, respectively. Both peptides were shown to contain PUB. BL, blue light; EIC, Extracted ion chromatograms; GL, green light.

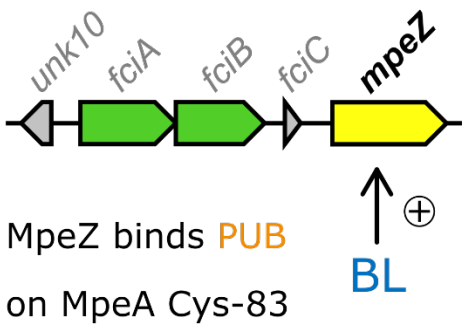
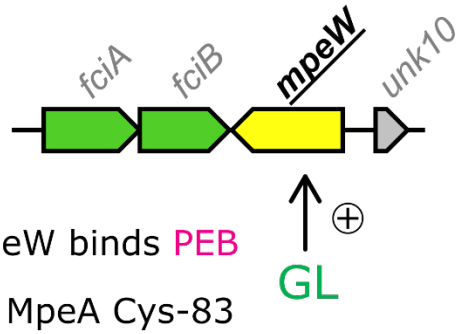
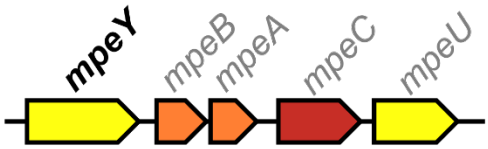
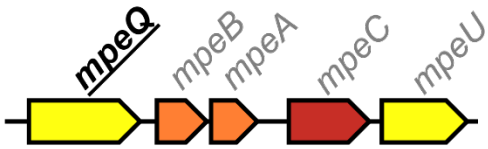
Model strain	RS9916	A15-62
Clade	IX	IIc
Pigment type	3dA	3dB
CA4 island	CA4-A  MpeZ binds PUB on MpeA Cys-83 BL	CA4-B  MpeW binds PEB on MpeA Cys-83 GL
PBS region	 MpeY binds PEB on MpeA Cys-83	 MpeQ binds PUB on MpeA Cys-83
References	(1, 2)	This study

Fig. S10. Summary of the differences between the two types of chromatic acclimation (CA4-A and –B) occurring in marine *Synechococcus*. The table summarizes the main characteristics of the model strains and recapitulates the results from two previous studies on CA4-A using RS9916 and how they compare to the new results obtained in the present study on CA4-B using A15-62. The 'PBS region' refers to the genomic region that gathers most genes involved in the biosynthesis and regulation of phycobilisome rods (3). Phylogenetically, both strains belong to sub-cluster 5.1 but to different (sub)clades *sensu* (4).

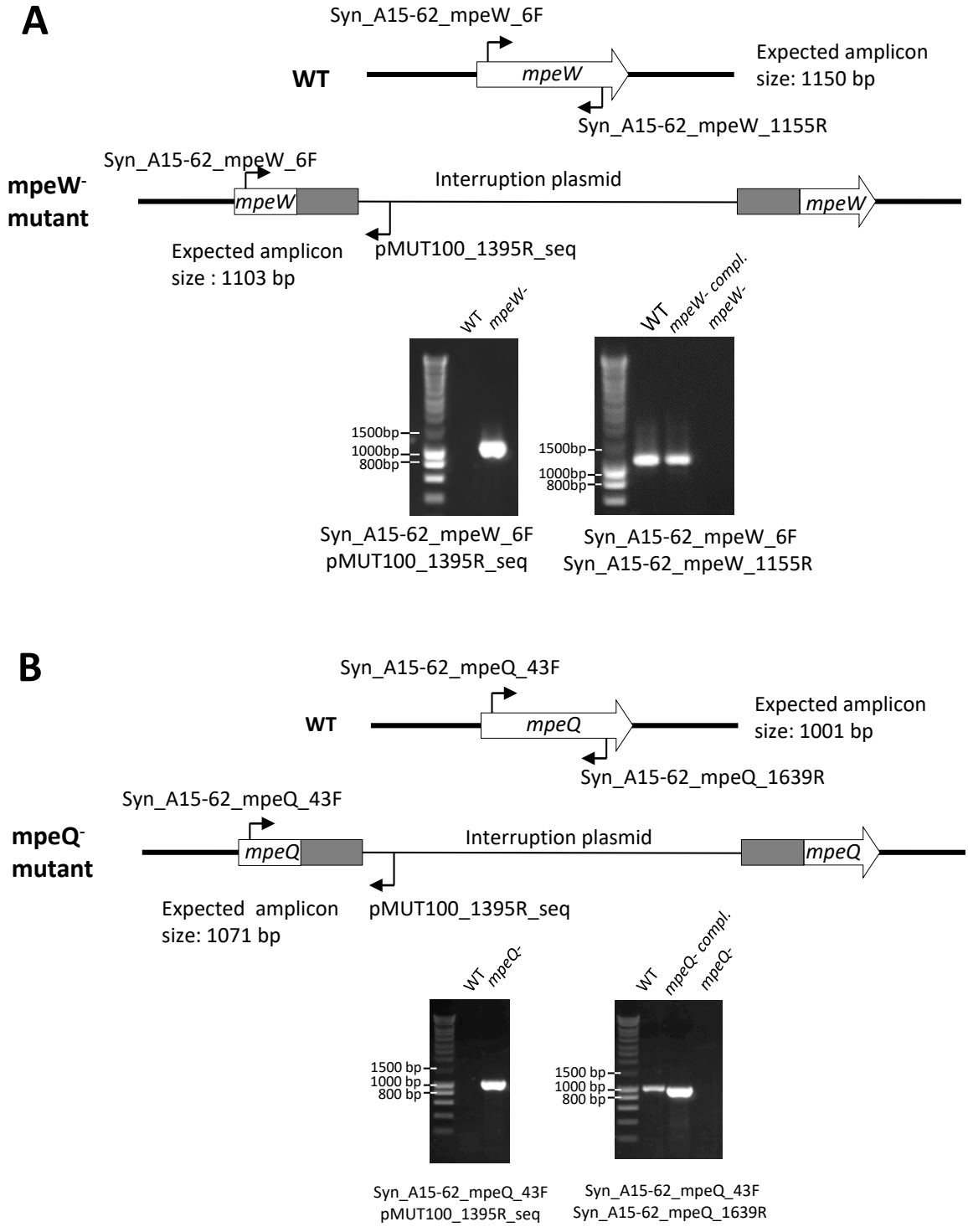


Fig. S11. Constructions of *mpeW*$^{-}$*/Q*$^{-}$ mutants and complementations. (*A*) Scheme of *mpeW* interruption and PCR verification of non-complemented and complemented *mpeW*$^{-}$ mutant. (*B*) Scheme of *mpeQ* interruption and PCR verification of non-complemented and complemented mutant *mpeQ*$^{-}$ mutant.

SI References:

1. G. K. Farrant, *et al.*, Delineating ecologically significant taxonomic units from global patterns of marine picocyanobacteria. *Proc. Natl. Acad. Sci.*, 201524865 (2016).

2. J. E. Sanfilippo, *et al.*, Interplay between differentially expressed enzymes contributes to light color acclimation in marine *Synechococcus*. *Proc. Natl. Acad. Sci.* **116**, 6457–6462 (2019).

3. A. Shukla, *et al.*, Phycoerythrin-specific bilin lyase-isomerase controls blue-green chromatic acclimation in marine *Synechococcus*. *Proc. Natl. Acad. Sci.* **109**, 20136–20141 (2012).

4. C. Six, *et al.*, Diversity and evolution of phycobilisomes in marine *Synechococcus* spp.: a comparative genomics study. *Genome Biol* **8**, R259 (2007)