Article:
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Supplementary Tables (S1-S2) and Figures (S1-S13)

Table S1. Primers and gBLOCKS used in this study.

Name	Sequence (5' to 3')	Details
*psbAII*_F	AAACGCCCTCTGTTTACCCA	*psbAII* locus screening
*psbAII*_ R	TCAACCCGGTACAGAGCTTC	primers
*chlG*_ F	TGCAAGCAACCCGTTACCA	*chlG* locus screening
*chlG*_ R	CCCTTTAGATTTTAGGACGGCGA	primers
*hliD*_ F	ATCGCCAATTTTCGGGTGATTC	*hliD* locus screening
*hliD*_ R	TGCAATCCATCGGCAAATTACG	primers
*crtR*_us_F	TGTCAAGTCTGTTGACCAAAAGAGC	*crtR* upstream homology
*crtR*_us_R	*CTGCAATCGGATGCGATTATTGAATA*GCACCGAACAACT AAAACAAAGC	region amplification primers with overlap with Em^R (italics)
*crtR*_ds_F	*GCATCCCTTAACTTGTTTTTCGTGTGCTT*GGTATCAGTAC CAAAACACC	*crtR* downstream homology region
*crtR*_ds_R	TCCGTCAATACACCATCTGGC	amplification primers with overlap with Em^R (italics)
*cruF*_us_F	AACAAACTCCCACAACACCTC	*cruF* upstream homology
*cruF*_us_R	*TAAACAAATAGCTAGCTCACTCGGT*ACCACCAGCCATAG ACC	region amplification primers with overlap with Sm^R (italics)
*cruF*_ds_F	*GATCACCAAGGTAGTCGGCAAATAA*TTGCCATGGTGAT GAGC	*cruF* downstream homology region
*cruF*_ds_R	AGTTGAGTCTTTTACACTCGATCG	amplification primers with overlap with Sm^R (italics)
*cruG*_us_F	TTCAAGATTATGGCATAGGTATTCC	*cruG* upstream homology
*cruG*_us_R	*TAAACAAATAGCTAGCTCACTCGGT*CAAGCAAACAAGG AGTTACC	region amplification primers with overlap with Sm^R (italics)
*cruG*_ds_F	*GATCACCAAGGTAGTCGGCAAATAA*TCCCTGTTGTTGAT GCTTTG	*cruG* downstream homology region
*cruG*_ds_R	TTCGTAGGCTTCTTCCACC	amplification primers with overlap with Sm^R (italics)

*crtO*_us_F *crtO*_us_R	ACGGTCAGGATAAAATGTTCTACC *AAAGTTGGCCCAGGGCTTCCCGGTA*AGCATTAAAAGCAGGCTGG	*crtO* upstream homology region amplification primers with overlap with Cm^R (italics)
*crtO*_ds_F *crtO*_ds_R	*CGATGAGTGGCAGGGCGGGGCGTAA*TGCTAATCCGTCTTTATATTTGG AGTATGTTGATTTAGGCTTTCTGC	*crtO* downstream homology region amplification primers with overlap with Cm^R (italics)
His_{10}-*chlG*	TACTGACATATGCATCACCATCACCATCACCATCACCATCAC*G CGGCCGCA*TCTGACACACAAAATACCGGCCAAAACCAAGCCAAGGCTCGGCAGTTACTGGGCATGAAGGGGGCCGCCCCGGGGGAAAGTTCCATTTGGAAAATTCGTCTTCAGTTGATGAAGCCCATCACTTGGATTCCCCTGATCTGGGGGGTGGTCTGTGGGGCCGCTTCTTCCGGGGGCTACATCTGGTCAGTGGAGGATTTCCTTAAAGCCCTCACCTGTATGTTGTTGTCCGGCCCGTTAATGACCGGTTATACCCAAACCCTCAATGATTTTTACGACCGGGACATCGACGCCATCAATGAACCCTACCGACCTATTCCTTCCGGGGCCATTTCCGTGCCCCAGGTGGTGACCCAAATTTTAATTCTGTTGGTGGCTGGTATTGGCGTTGCCTACGGCTTGGACGTGTGGGCCCAGCACGATTTTCCCATTATGATGGTGCTCACCTTGGGGGGAGCCTTTGTTGCTTATATTTACTCTGCACCACCGTTGAAGCTAAAACAAAATGGCTGGTTGGGTAACTATGCTCTGGGGGCTAGCTACATTGCCCTACCGTGGTGGGCGGGCCATGCTCTGTTTGGCACCCTCAATCCCACCATTATGGTCTTGACCCTAATTTATAGCTTGGCTGGTTTGGGCATTGCGGTGGTTAACGATTTCAAAAGTGTGGAAGGCGATCGCCAATTGGGGCTCAAGTCCTTGCCGGTGATGTTTGGTATCGGGACAGCAGCTTGGATCTGCGTGATCATGATCGACGTTTTCCAAGCGGGTATTGCGGGATACCTGATTTATGTTCACCAACAGCTTTATGCCACCATTGTGTTGCTATTGCTCATTCCCCAGATAACTTTTCAGGATATGTATTTTCTCCGTAATCCCCTGGAAAATGATGTCAAATACCAAGCCAGTGCCCAGCCATTTCTAGTTTTTGGTATGTTAGCCACCGGTCTGGCCCTAGGCCATGCGGGGATTTGAAGATCTGATCA	gBLOCK (Integrated DNA Technologies) used to replace sequence encoding 3×FLAG-*chlG* with sequence encoding 10×His-*chlG* by replacement of the *Nde*I-*Bgl*II fragment in pPD-NFLAG::*chlG* (Chidgey et al., 2014). The flanking *Nde*I and *Bgl*II sites are underlined. The 10×His tag (red) is separated from *chlG* (green) by sequence encoding a 3x alanine linker (black italics).

Table S2. Ion intensities for F.ChlG, HliD and Ycf39 in anti-FLAG co-immunoprecipitation eluates.

Protein	F.ChlG/Δ*chlG* (control)			F.ChlG/Δ*chlG*/Δ*crtR*			F.ChlG/Δ*chlG*/Δ*cruF*			F.ChlG/ΔchlG/ΔcrtR/ΔcruF		
	1	2	3	1	2	3	1	2	3	1	2	3
F.ChlG	2.48E+07	2.80E+07	2.40E+07	8.07E+07	8.18E+07	8.30E+07	2.61E+07	2.71E+07	3.19E+07	4.20E+07	4.27E+07	4.28E+07
HliD	2.43E+09	2.73E+09	2.62E+09	2.16E+08	2.38E+08	2.06E+08	2.54E+09	2.49E+09	2.13E+09	9.72E+07	1.09E+08	1.14E+08
Ycf39	1.36E+07	2.88E+07	2.63E+07	1.42E+07	1.42E+07	1.46E+07	2.74E+07	2.97E+07	2.69E+07	2.89E+06	4.54E+06	4.38E+06
HliD:ChlG	97.87	97.59	109.23	2.68	2.91	2.49	97.24	92.15	66.78	2.32	2.56	2.68
Ycf39:ChlG	0.55	1.03	1.10	0.18	0.17	0.18	1.05	1.10	0.84	0.07	0.11	0.10

Proteins were extracted from the specified eluates, digested with endoproteinase Lys-C/trypsin and the resultant peptides analysed by nano-flow reverse phase chromatography coupled to mass spectrometry. Proteins were identified and quantified using MaxQuant (v. 1.5.3.30). The ion intensities shown are calculated by MaxQuant from the summed intensities of the peptide ions that map to the proteins. The HliD:ChlG and Ycf39:ChlG ratios are plotted in Figure 2F (main article). The mass spectrometer data and MaxQuant output files have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (http://proteomecentral.proteomexchange.org) with the data set identifier PXD020471.

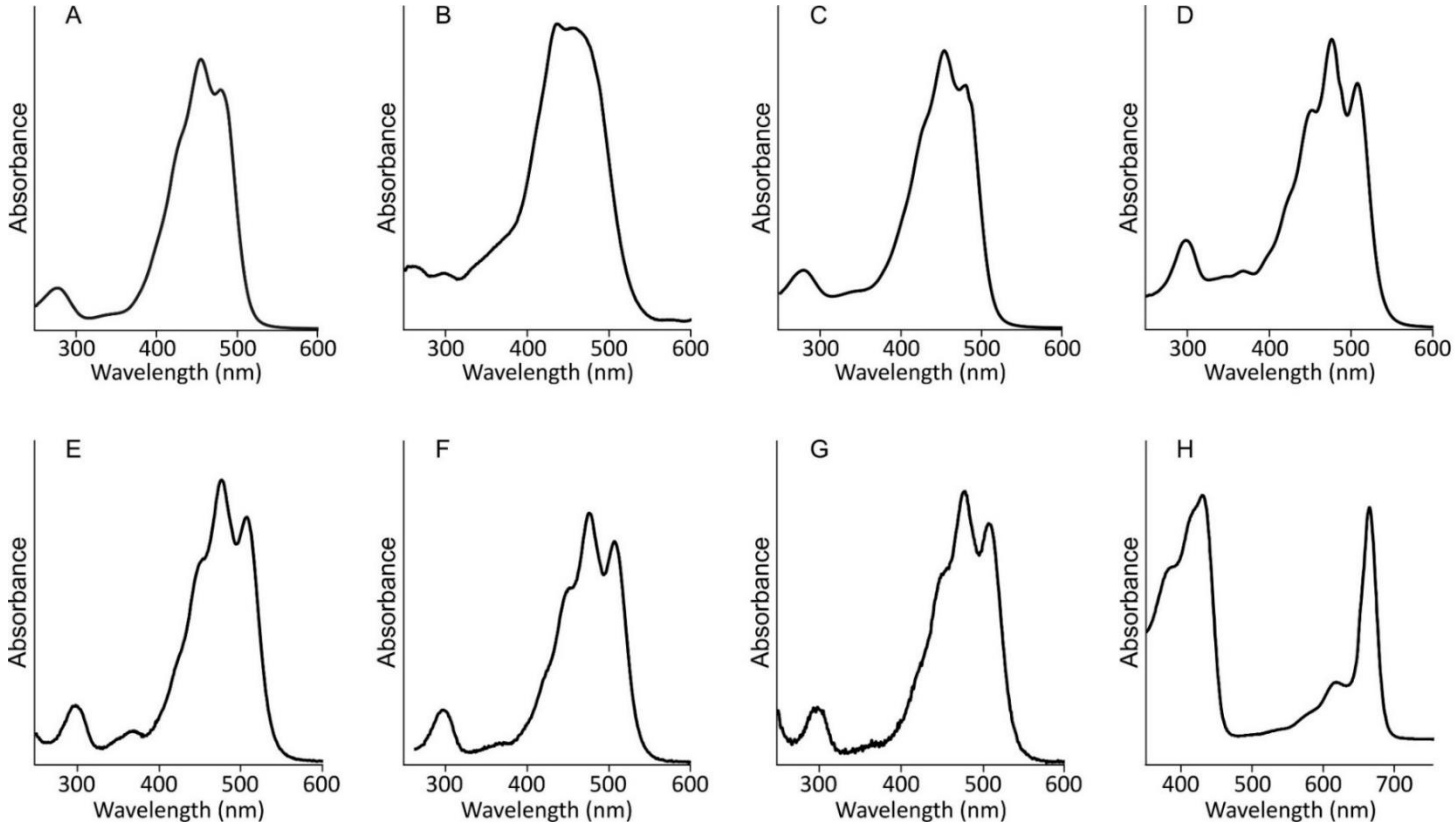

Figure S1. Absorbance spectra of pigments identified by RP-HPLC in this study. (A) β-carotene. (B) Echinenone. (C) Zeaxanthin. (D) Myxoxanthophyll. (E) Dehydroxy-myxoxanthophyll. (F) Myxol. (G) Dehydroxy-myxol. (H) Chl *a*.

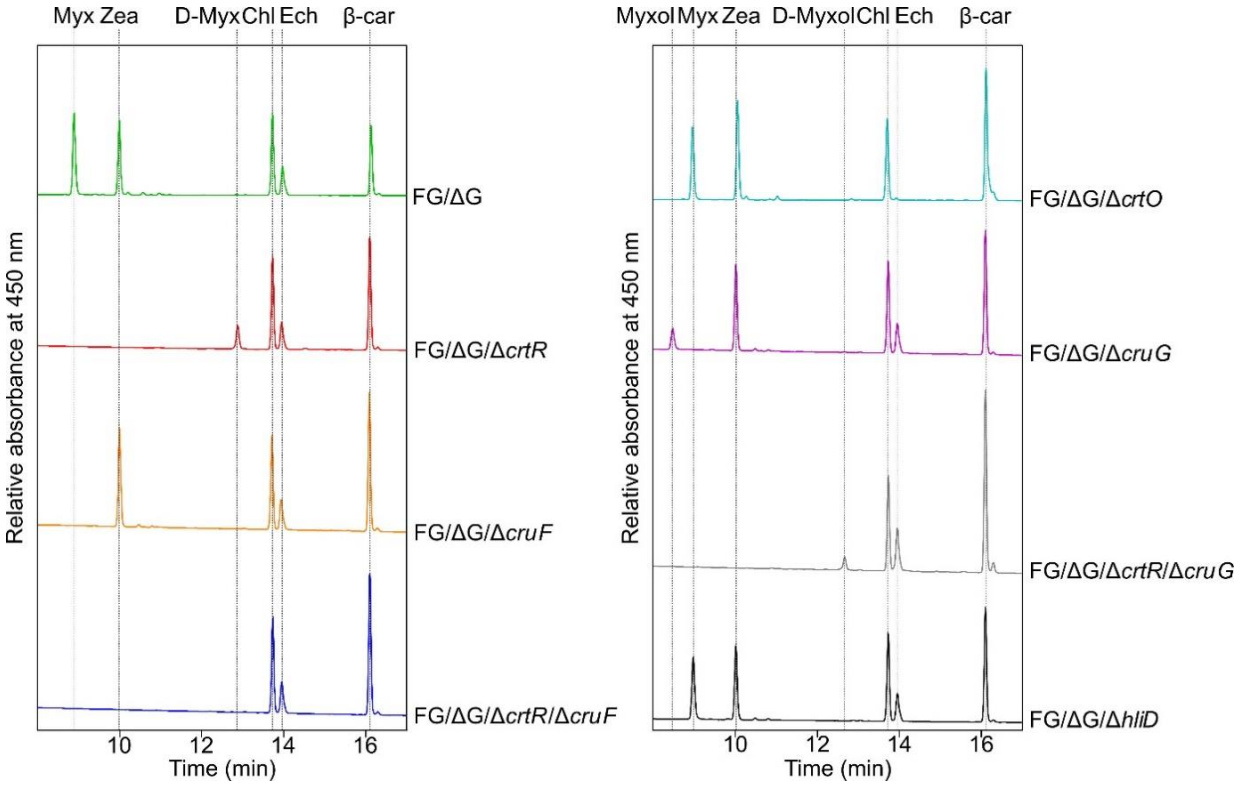

Figure S2. **Pigment profiles of strains of *Synechocystis* analysed in this study**. Pigments were extracted from cell pellets in 100% methanol and analysed by HP-RPLC according to the protocol described in the Materials and Methods section. Strains analysed are: FLAG-*chlG* Δ*chlG* (FG/ΔG), FLAG-*chlG* Δ*chlG* Δ*crtR* (FG/ΔG/Δ*crtR*), FLAG-*chlG* Δ*chlG* Δ*cruF* (FG/ΔG/Δ*cruF*), FLAG-*chlG* Δ*chlG* Δ*crtR* Δ*cruF* (FG/ΔG/Δ*crtR*/Δ*cruF*), FLAG-*chlG* Δ*chlG* Δ*crtO* (FG/ΔG/Δ*crtO*), FLAG-*chlG* Δ*chlG* Δ*cruG* (FG/ΔG/Δ*cruG*), FLAG-*chlG* Δ*chlG* Δ*crtR* Δ*cruG* (FG/ΔG/Δ*crtR*/Δ*cruG*) and FLAG-*chlG* Δ*chlG* Δ*hliD* (FG/ΔG/Δ*hliD*). Pigments were identified by their known absorbance spectra (see Figure S12) and comparison to literature results as myxoxanthophyll (Myx), zeaxanthin (Zea), deoxymyxoxanthophyll (D-Myx), myxol, deoxymyxol (D-Myxol), chlorophyll *a* (Chl), echinenone (Ech) and β-carotene (β-car).

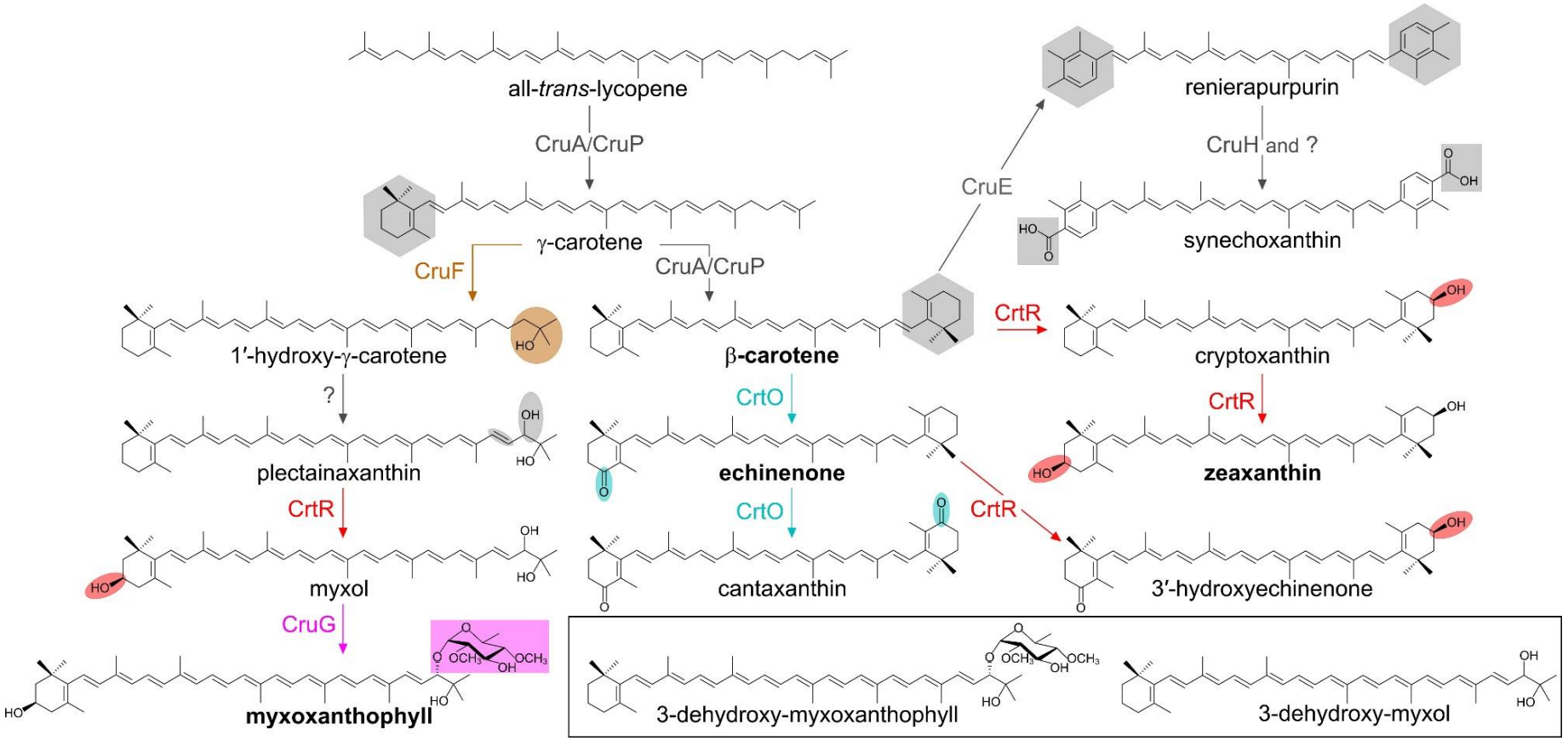

Figure S3. Carotenoid biosynthesis in *Synechocystis*. Pathway for the biosynthesis of the four major carotenoids in *Synechocystis*, β-carotene, myxoxanthophyll, zeaxanthin and echinenone (bold). Protein products of genes targeted for deletion in this study are coloured as follows: CrtR (red), CruF (orange), CruG (pink) and CrtO (cyan). Equivalently coloured boxes indicate sites of chemical modification by these proteins. The inset panel shows carotenoid species that accumulate in the *crtR* and *crtR*/*cruG* strain.

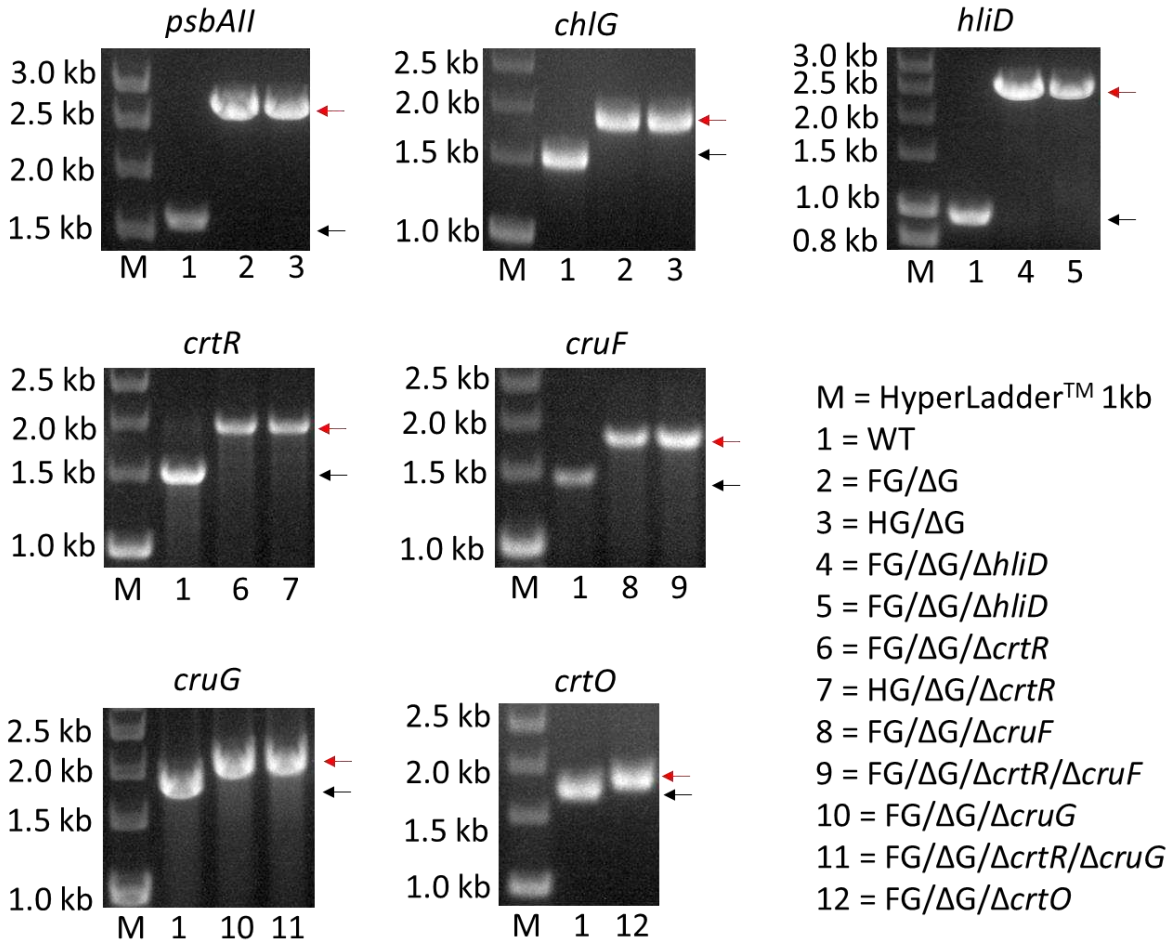

Figure S4. Confirmation of mutant strains used in this study. PCR analysis of the *psbAII*, *chlG*, *hliD*, *crtR*, *cruF*, *cruG* and *crtO* loci (locus indicated above the agarose gel images). Genomic DNA of the strain in the numbered key was used as template with gene-specific screening primers (see Table S1 for primer details). In all cases the WT PCR product is shown with a black arrow and the larger mutant PCR product with a red arrow; absence of a WT PCR product indicates full segregation of the mutant allele at each locus.

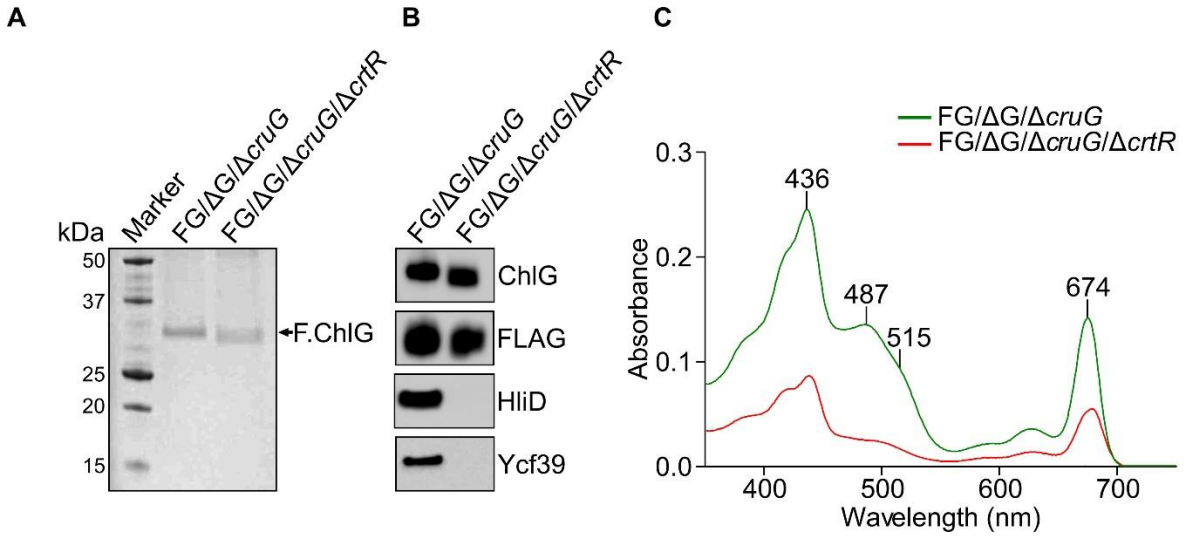

Figure S5. Analysis of FLAG immunoprecipitation eluates isolated from *Synechocystis* Δ*cruG* mutants. Strains without CruG lack the fucose group on (dehydroxy)-myxoxanthophyll and accumulate (dehydroxy)-myxol (see inset panel in Figure S1). (A) Coomassie stained SDS-PAGE analysis of FLAG immunoprecipitation eluates from the FLAG-*chlG* Δ*chlG* Δ*cruG* (FG/ΔG/Δ*crtG*) and FLAG-*chlG* Δ*chlG* Δ*cruG* Δ*crtR* (FG/ΔG/Δ*cruG*/Δ*crtR*) strains. FLAG-ChlG (F.ChlG) is indicated with an arrow. (B) Immunoblotting with anti-ChlG and anti-FLAG specific primary antibodies confirmed the presence of the bait protein; HliD and Ycf39 were detected with protein specific primary antibodies. (C) Absorbance spectra of FG/ΔG/Δ*cruG* (green) and FG/ΔG/Δ*cruG*/Δ*crtR* (red) immunoprecipitation eluates.

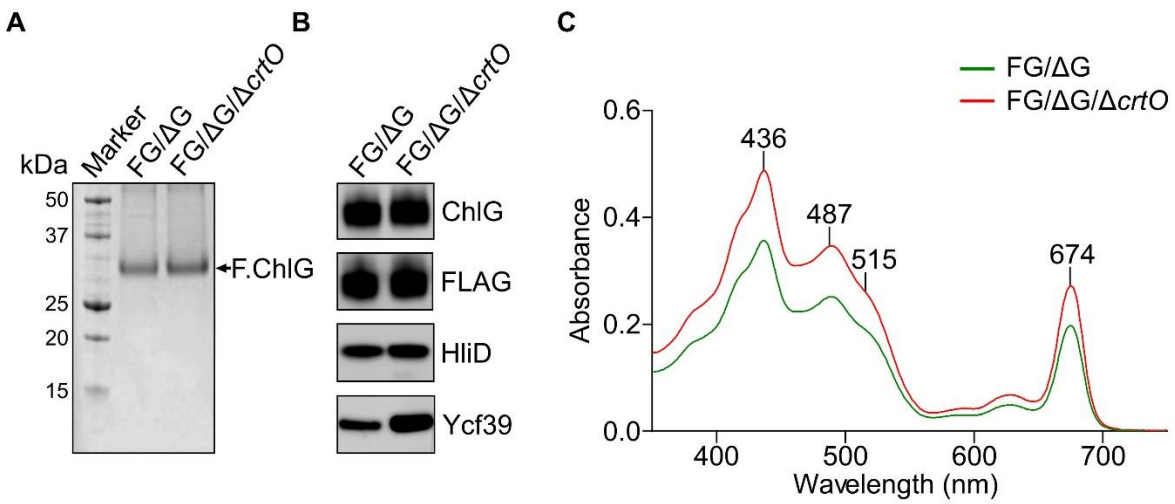

Figure S6. Analysis of FLAG immunoprecipitation eluates isolated from *Synechocystis* Δ*crutO* mutants. Strains without CrtO lack keto-carotenoids (see panel in Figure S1). (A) Coomassie stained SDS-PAGE analysis of FLAG immunoprecipitation eluates from the FLAG-*chlG* Δ*chlG* (FG/ΔG) and FLAG-*chlG* Δ*chlG* Δ*crtO* (FG/ΔG/Δ*crtO*) strains. FLAG-ChlG (F.ChlG) was identified by Coomassie staining and (B) Immunoblotting with anti-ChlG and anti-FLAG specific primary antibodies confirmed the presence of the bait protein; HliD and Ycf39 were detected with protein specific primary antibodies. (C) Absorbance spectra of FG/ΔG (green) and FG/ΔG/Δ*crtO* (red) immunoprecipitation eluates.

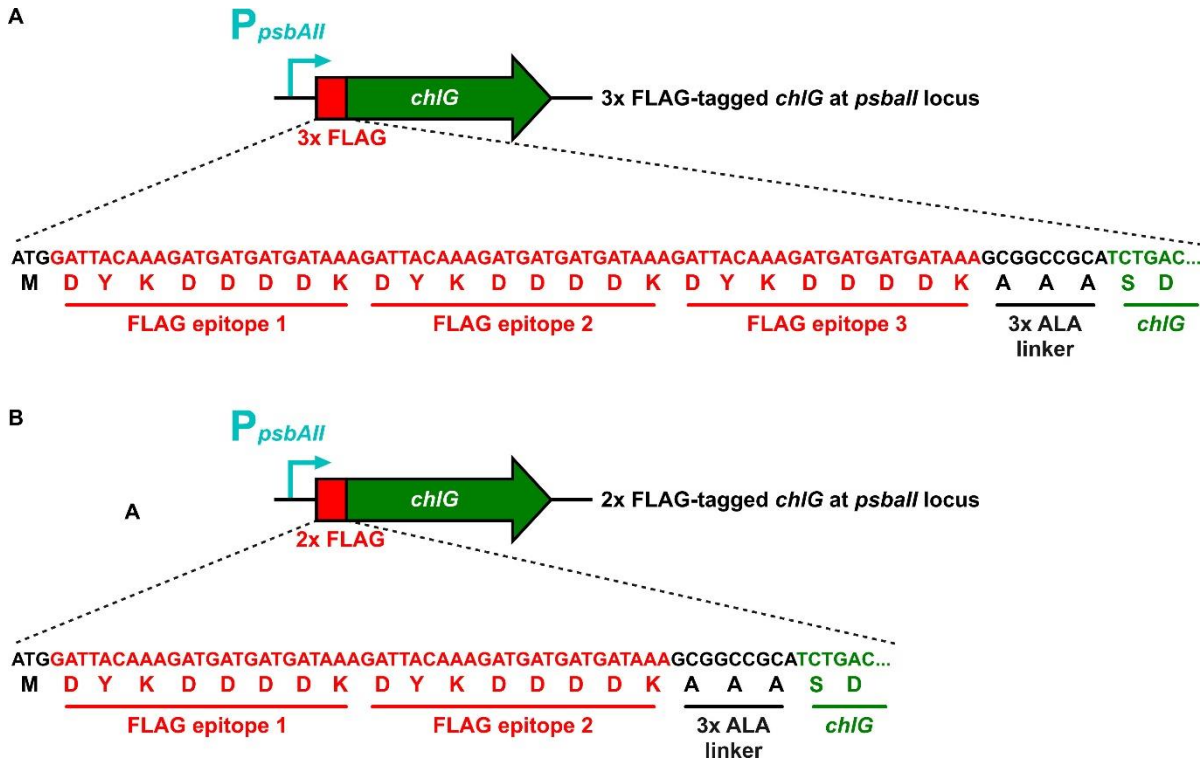

Figure S7. Sequence analysis of the *psbAII* locus in FG/ΔG/Δ*crtR* strains producing 3×FLAG-tagged or 2×FLAG-tagged ChlG. (A) Sequencing of the *psbAII* locus in a strain of FG/ΔG/Δ*crtR* producing 'normal' F.ChlG shows the expected 3×FLAG-tag sequence is present at the 5' end of the *chlG* gene. The translated sequence is shown below the nucleotide sequence. A three-alanine linker (3×ALA) separate the FLAG-tag from ChlG. (B) Sequencing of the *psbAII* locus in a strain of FG/ΔG/Δ*crtR* producing 'smaller' F.ChlG shows that one of the FLAG epitopes is lost, leaving the remaining 2×FLAG-tag sequence in frame with the downstream 3×ALA linker and *chlG* gene.

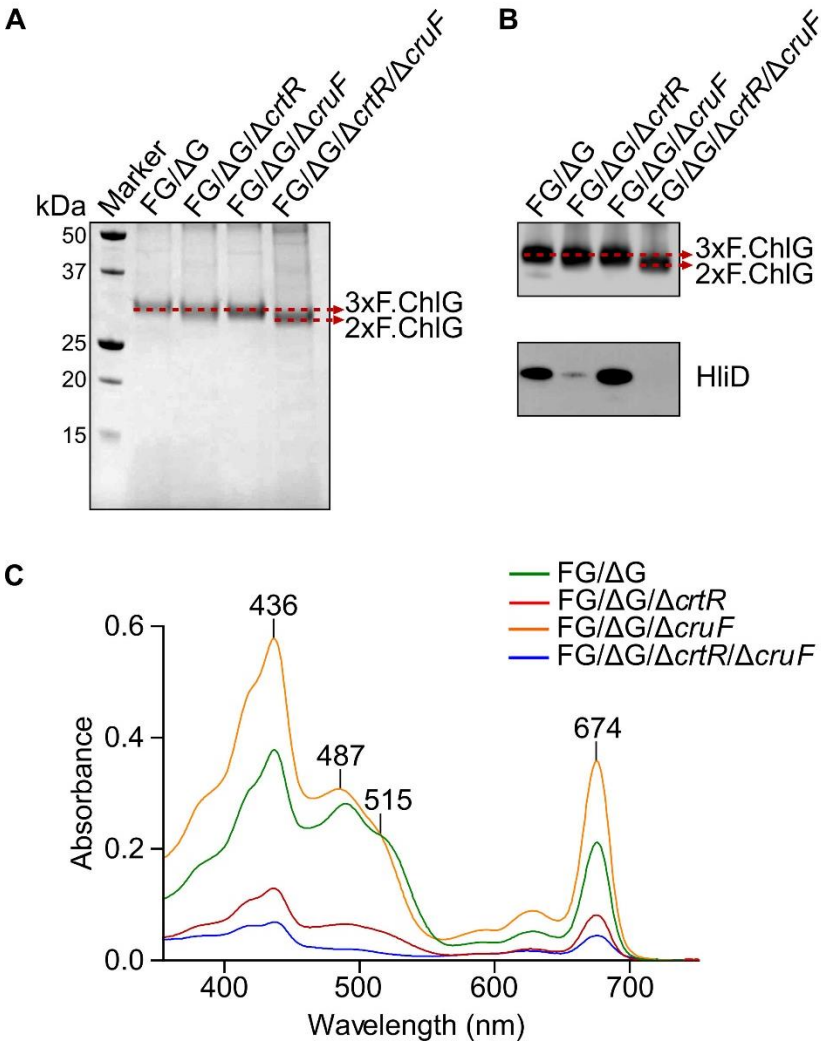

Figure S8. Comparison of immunoprecipitation eluates from strains with 3xFLAG-ChlG (3xF.ChlG) versus one with a 2xFLAG-ChlG (2xF.ChlG). (A) Coomassie stained SDS-PAGE analysis of FLAG immunoprecipitation eluates obtained from 3xFLAG-*chlG* Δ*chlG* (FG/ΔG), 3xFLAG-*chlG* Δ*chlG* Δ*crtR* (FG/ΔG/Δ*crtR*), 3xFLAG-*chlG* Δ*chlG* Δ*cruF* (FG/ΔG/Δ*cruF*), 2xFLAG-*chlG* Δ*chlG* Δ*crtR* Δ*cruF* (FG/ΔG /Δ*crtR*/Δ*cruF*). Red arrows indicate the difference in migration distance between 2xFLAG and 3xFLAG tagged ChlG (see main text for more details). (B) Retrieval of F.ChlG variants (red arrows) was confirmed by immunoblotting with anti-ChlG and anti-FLAG specific primary antibodies. The presence of HliD and Ycf39 was investigated by immunoblotting using protein specific primary antibodies. (C) The pigment content of FG/ΔG (green), FG/ΔG/Δ*crtR* (red), FG/ΔG/Δ*cruF* (orange) and FG/ΔG/Δ*crtR*/Δ*cruF* (blue) were analysed by absorbance spectroscopy. The presence of 2xFLAG tag on the *chlG* gene in the FG/ΔG/Δ*crtR*/Δ*cruF* background was confirmed by gene sequencing (Figure S7).

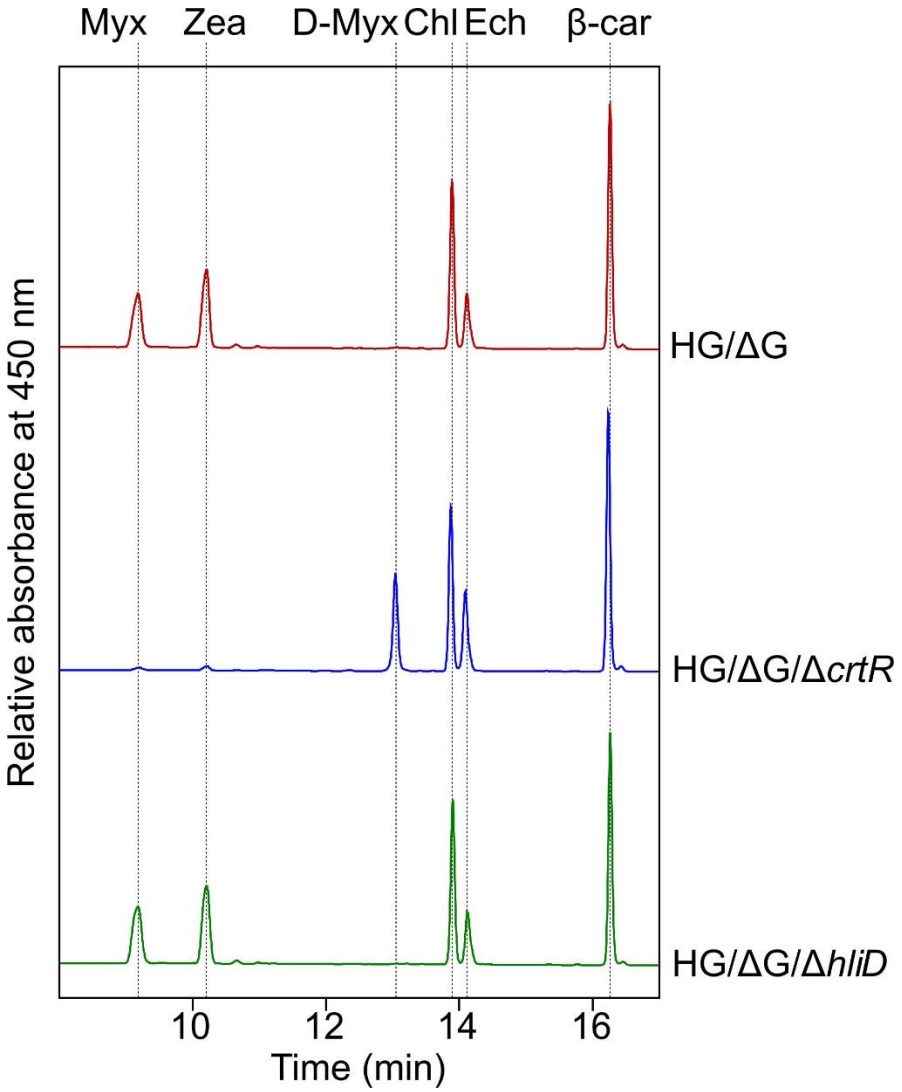

Figure S9. Carotenoid content of strains producing His-tagged ChlG. The HG/ΔG strain (red trace) has the same carotenoid profile as the wild-type organism. Deletion of *crtR* (blue trace) prevents synthesis of myxoxanthophyll (Myx) and zeaxanthin (Zea) and results in accumulation of deoxymyxoxanthophyll (D-Myx). Deletion of *hliD* (green trace) has no effect on the carotenoid profile.

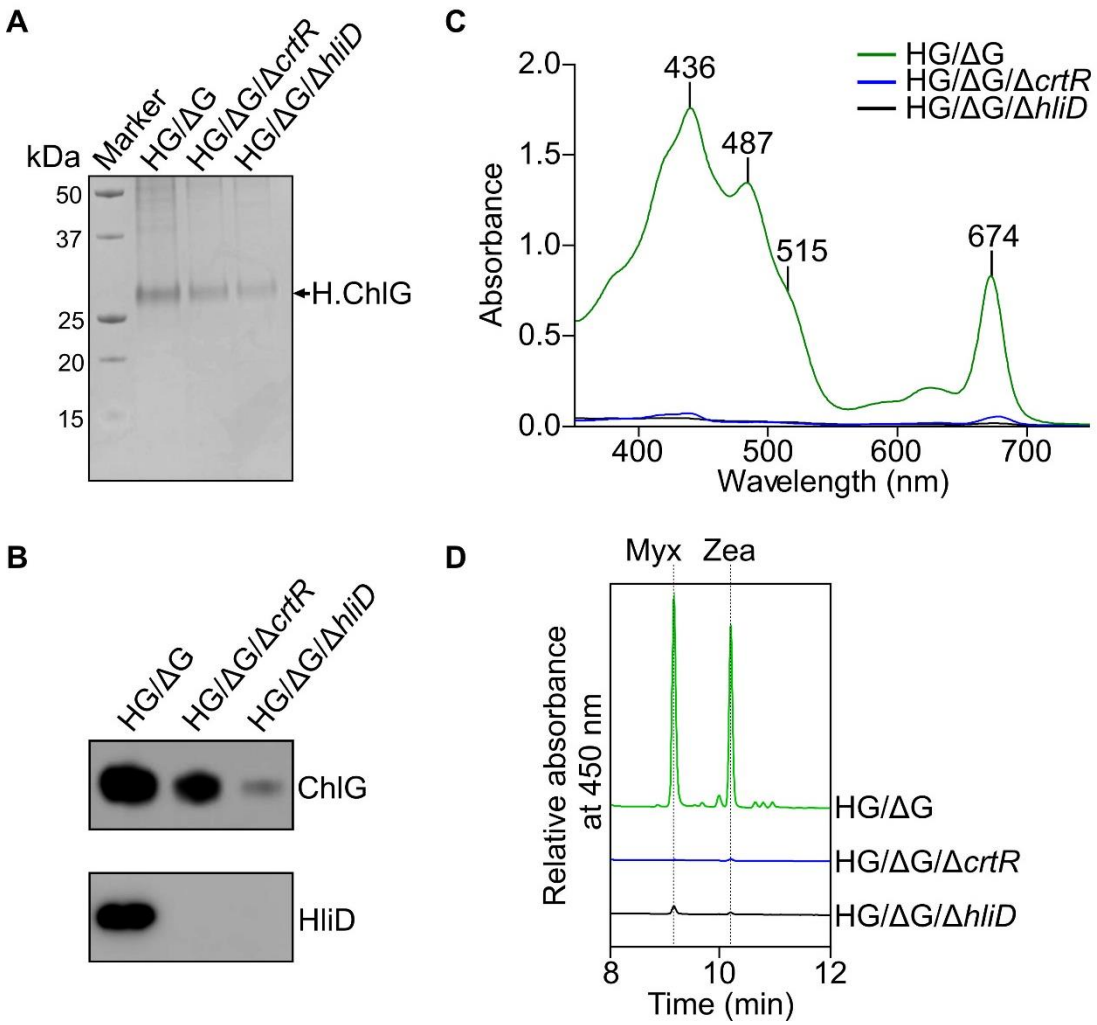

Figure S10. **Substitution of the N-terminal 3xFLAG tag for a 10xHis-tag does not alter the pigment and protein content of the ChlG-HliD complex.** (A) His-ChlG (H.ChlG) was isolated from *Synechocystis* strains His-*chlG* Δ*chlG* (HG/ΔG), His-*chlG* Δ*chlG* Δ*crtR* (HG/ΔG/Δ*crtR*) and His-*chlG* Δ*chlG* Δ*hliD* (HG/ΔG/Δ*hliD*) by IMAC. The eluates were separated by SDS-PAGE and stained with Coomassie blue; H.ChlG is indicated with an arrow. (B) The presence of H.ChlG and HliD in each sample was analysed by immunoblotting with ChlG and HliD specific antibodies. (C) Absorbance spectra of the HG/ΔG (green), HG/ΔG/Δ*crtR* (blue) and HG/ΔG/Δ*hliD* (black). (D) Pigments in the eluates were extracted in methanol and separated by HP-RPLC monitoring absorbance at 450 nm; myxoxanthophyll (Myx) and zeaxanthin (Zea) were only present in the HG/ΔG eluate (green).

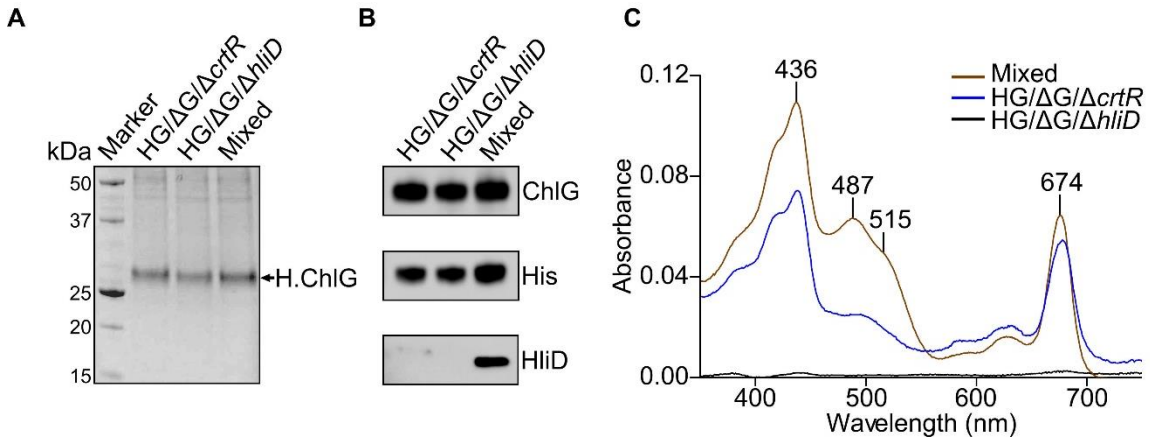

Figure S11. **Reconstitution of H.ChlG with carotenoids and HliD in isolated membranes.** An equivalent reconstitution experiment to that presented in Figure 4 in the main paper but using strains with His-tagged ChlG rather than FLAG-tagged enzyme. Solubilised thylakoid membranes from the His-*chlG* Δ*chlG* strain lacking zeaxanthin and myxoxanthophyll (FG/ΔG/Δ*crtR*) but containing His-ChlG and HliD were mixed with membranes from the HG/ΔG/Δ*hliD* strain, which lacks HliD but contains His-ChlG, myxoxanthophyll and zeaxanthin. (A) Analysis of FLAG immunoprecipitants by SDS-PAGE and Coomassie blue staining; the mixed membranes are labelled as 'Mixed'. (B) Immunodetection of His-ChlG (H.ChlG) and HliD in the immunoprecipitation eluates. HliD is restored in the mixed membrane sample. (C) Absorbance spectra of each immunoprecipitation eluate; an increase in carotenoids is observed for the 'Mixed' membrane elution.

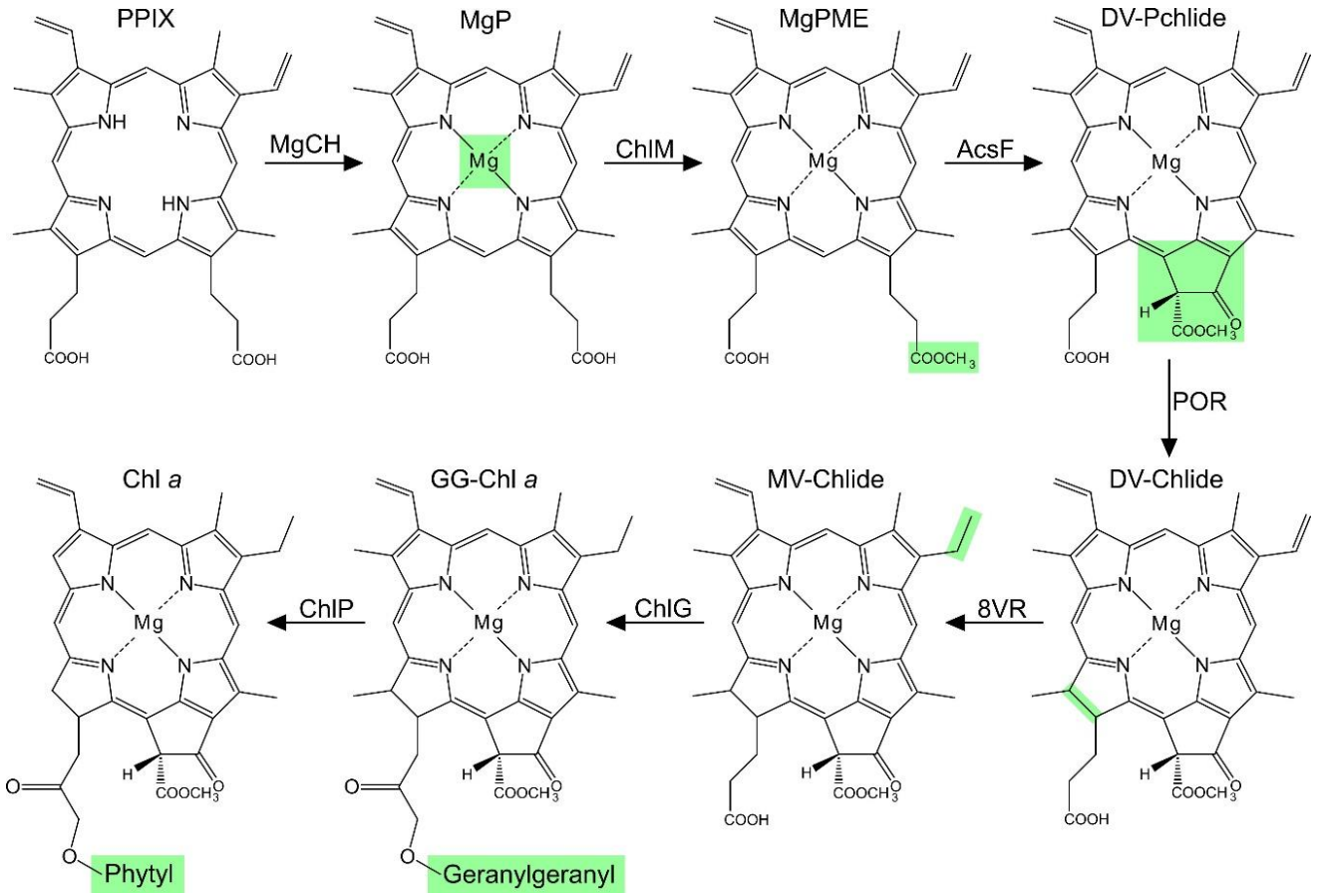

Figure S12. Biosynthesis of chlorophyll *a* from protoporphyrin IX. Green boxes highlight the chemical modification at each step. MgCH, magnesium chelatase; ChlM, Mg-protoporphyrin O-methyltransferase; AscF, MgPME cyclase; POR, protochlorophyllide oxidoreductase; 8VR, 8-vinyl-reductase; ChlG, chlorophyll synthase; ChlP, geranylgeranyl reductase; PPIX, protoporphyrin IX; MgP, Mg-protoporphyrin IX; MgPME, Mg-protoporphyrin monomethyl ester; DV-Pchlide, divinylprotochlorophyllide; DV-Chlide, divinyl chlorophyllide; MV-Chlide, chlorophyllide; GG-Chl *a*, geranylgeranyl chlorophyll *a*; Chl *a*, chlorophyll *a*.

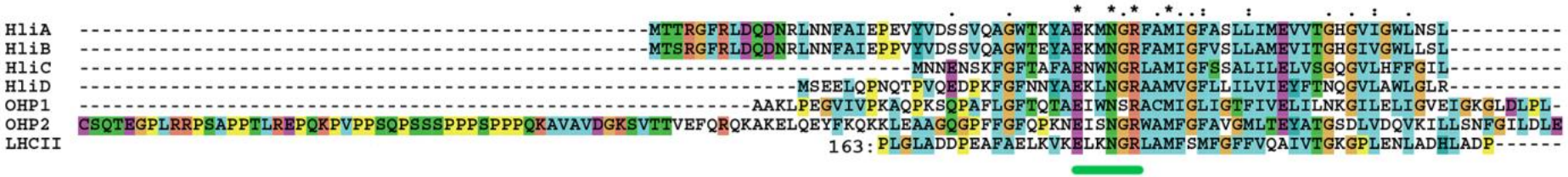

Figure S13. Amino acid sequence alignment of *Synechocystis* Hlips, Arabidopsis OHPs and the third trans-membrane helix of the pea LHCII protein. The conserved Chl-binding motif (ExxNxR) is highlighted by a green bar. The sequences of OHPs are shown without the predicted targeting peptide.