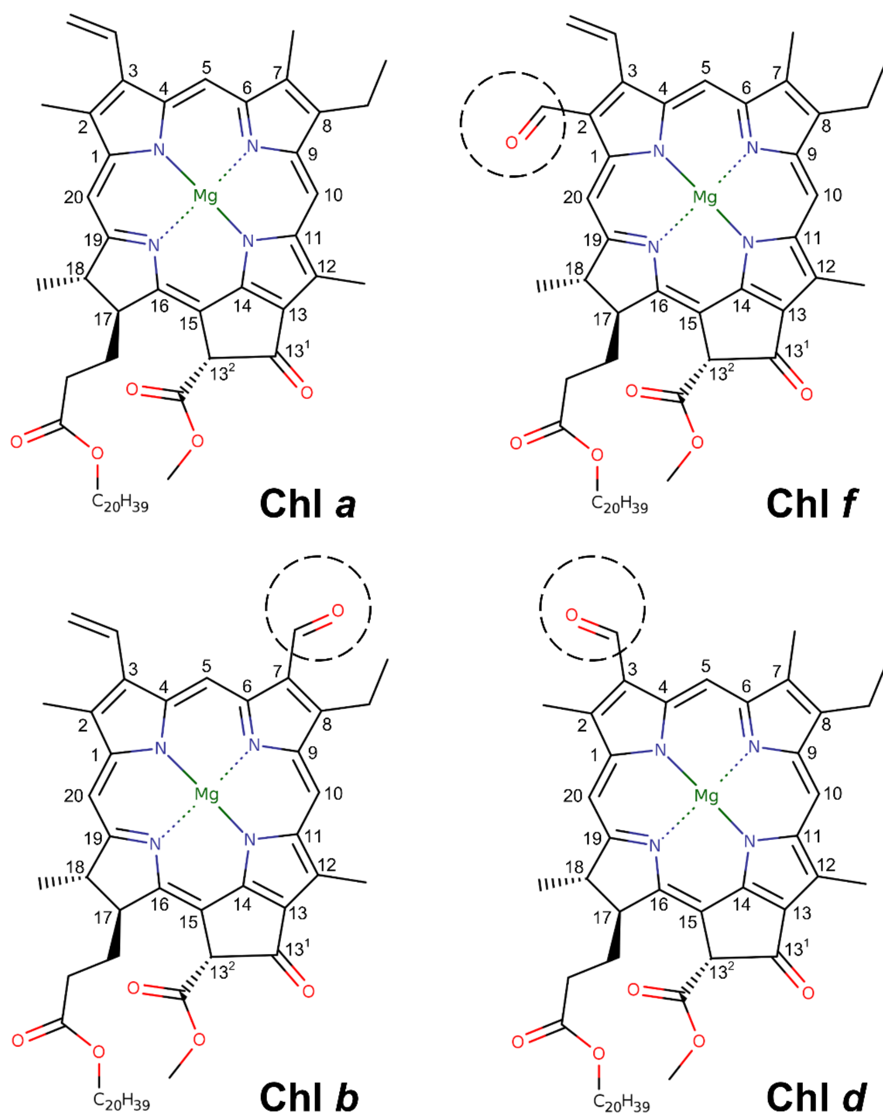
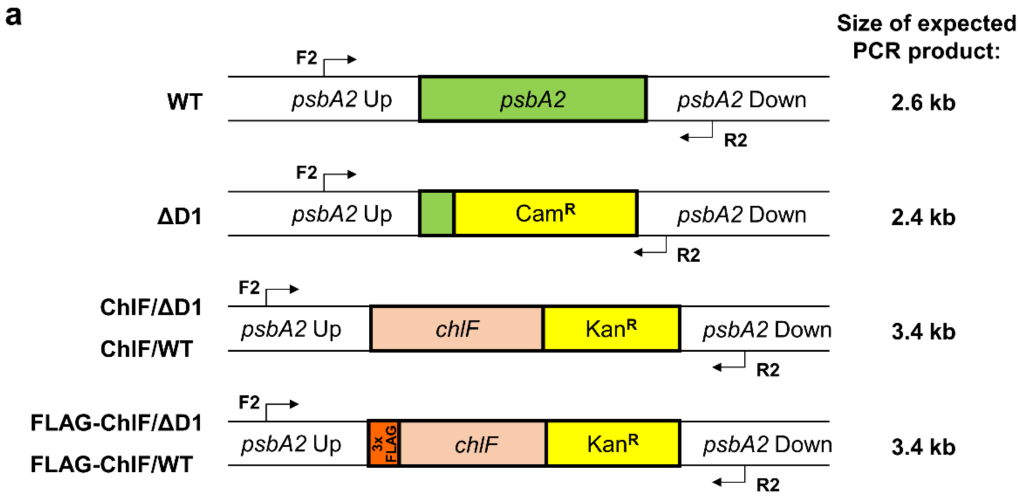
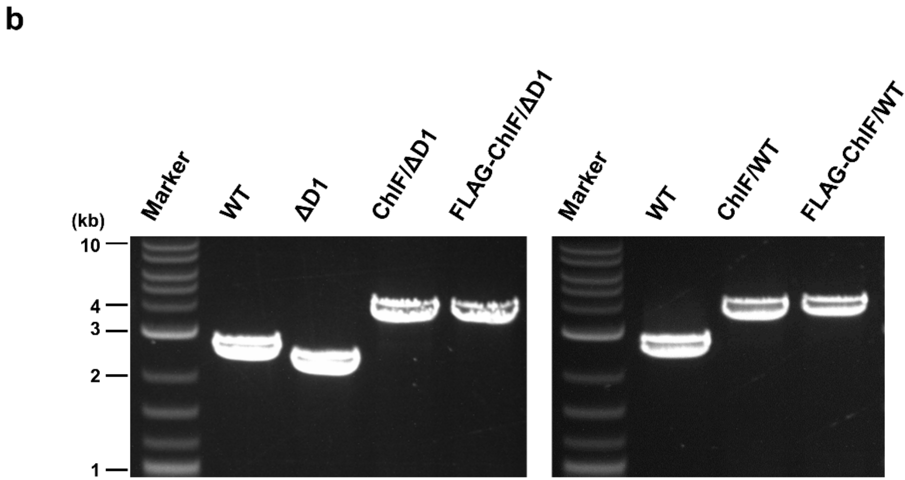
Supplementary Information – Figures and Tables

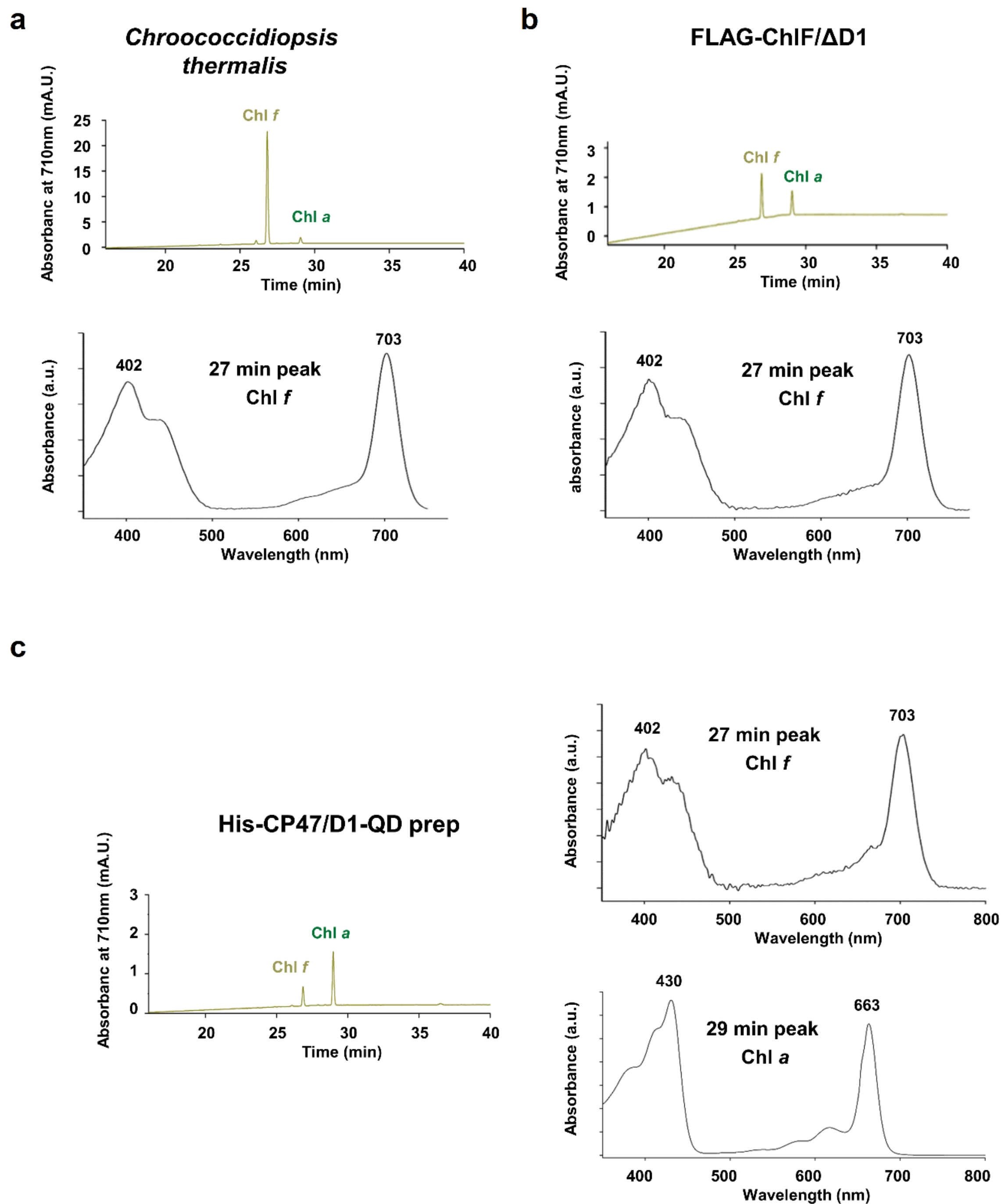


Supplementary Fig. 1. Structures of the chlorophyll molecules involved in oxygenic photosynthesis. In Chl *b* the methyl group of Chl *a* on C7 is oxidised to a formyl group, for Chl *f* the C2 methyl group is oxidised to a formyl group and in Chl *d* the vinyl group on C3 is oxidised to a formyl group. Modifications are circled.

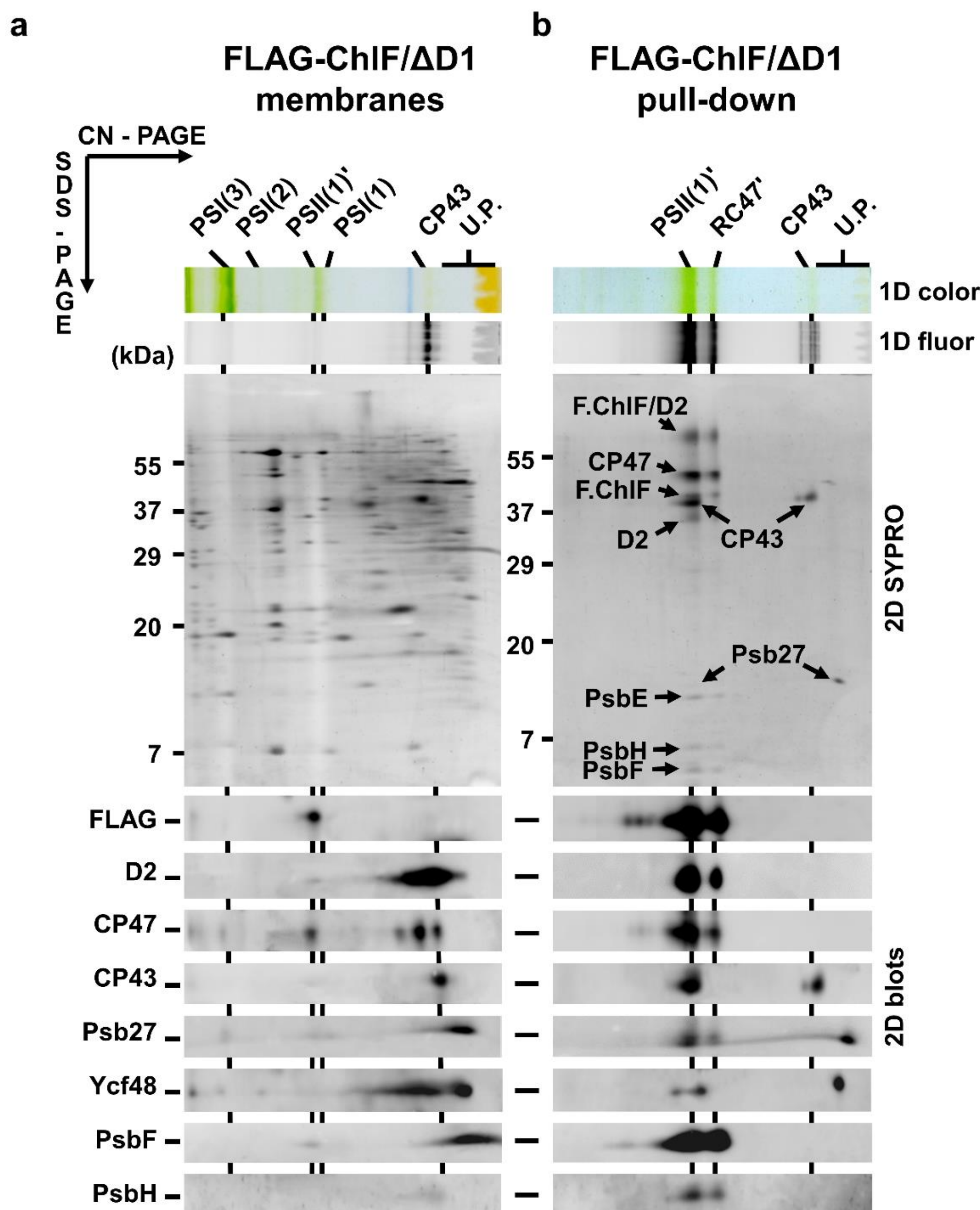




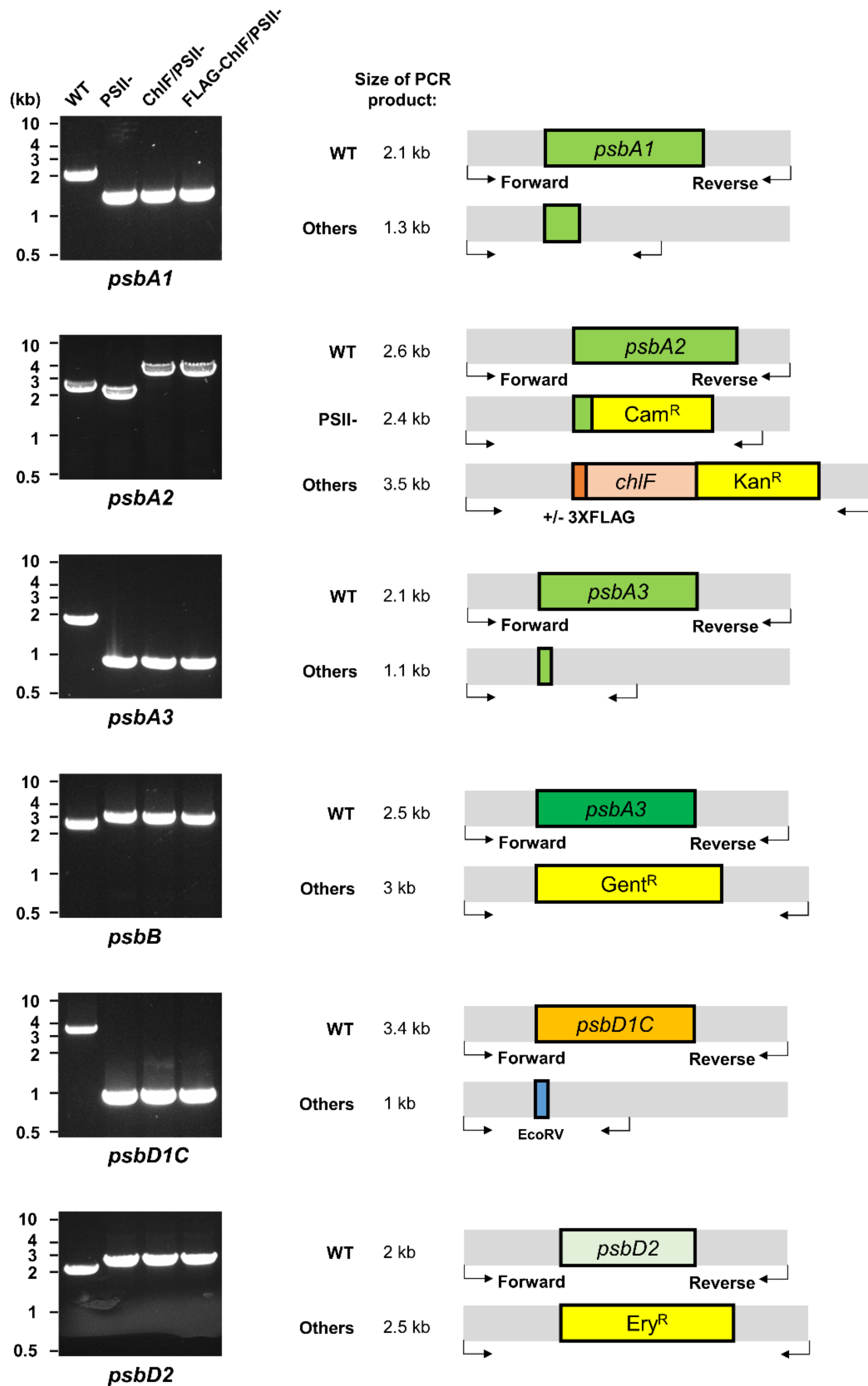
Supplementary Fig. 2. Construction and validation of the FLAG-ChlF/ΔD1 and FLAG-ChlF/WT strains. (a) Cartoon showing the *psbA2* locus found in the parental WT Syn6803 and ΔD1 strains and after insertion of *chlF* in the FLAG-ChlF/ΔD1 and FLAG-ChlF/WT strains. Binding sites of the forward (F2) and reverse (R2) primers (Supplementary Table 3) used for PCR genotyping and predicted sizes of PCR fragments are indicated. (b) Agarose gel of PCR fragments confirming the genotype of the FLAG-ChlF/ΔD1 and FLAG-ChlF/WT strains



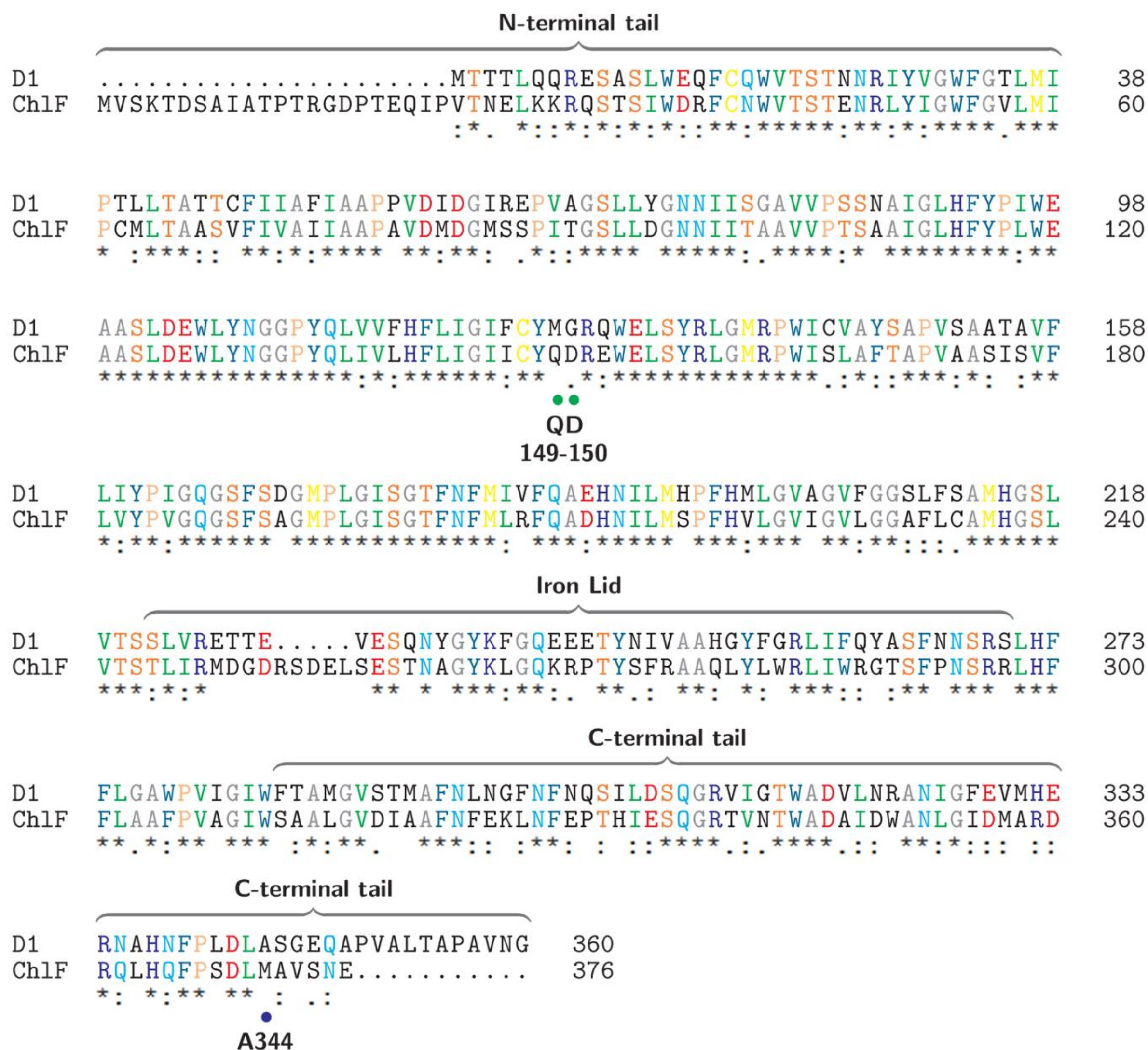
Supplementary Fig. 3. Spectral properties of Chl *f* and Chl *a*. Absorption spectra of Chl *f* and Chl *a* extracted from (a) far-red-light-treated cells of *Chroococcidiopsis thermalis* (b) cells of FLAG-ChlF/ΔD1 and (c) His-tagged PSII complexes isolated from the His-CP47/D1-QD mutant, separated by HPLC, and eluted from the column at a time of 27 min for Chl *f* and 29 min for Chl *a*.



Supplementary Fig. 4. Immunoblot analysis of PSII subunits in the FLAG-ChlF/ΔD1 strain and immunopurified FLAG-ChlF complexes. (a) Detergent-solubilized membrane proteins from FLAG-ChlF/ΔD1 or (b) FLAG-ChlF complexes isolated from the FLAG-ChlF/ΔD1 strain were separated by clear-native polyacrylamide gel electrophoresis (CN-PAGE) in the first dimension (1D color) and visualized by fluorescence (1D fluor) then denatured, separated by SDS-PAGE in the second dimension and protein subunits stained by Sypro Orange (2D SYPRO), then transferred to PVDF membranes for immunochemical detection using specific antibodies. Proteins were identified on the stained gel by immunoblotting and mass spectrometry. Abbreviations: F.ChlF, FLAG-tagged ChlF; PSI(3), trimeric PSI; PSII(2), dimeric PSII complex; PSII(1)', monomeric PSII-like complex containing ChlF; PSI(1), monomeric PSI complex; RC47', PSII(1)' complex lacking CP43; CP43, CP43 module; U.P., unassembled proteins.



Supplementary Fig. 5. Construction and validation of FLAG-ChlF/PSII$^-$ strain lacking D1, D2, CP47 and CP43. Cartoons showing the *psbA1, psbA2, psbA3, psbB, psbD1C* and *psbD2* loci found in the WT, PSII$^-$, FLAG-ChlF/PSII$^-$ and ChlF/PSII$^-$ strains. Binding sites of the forward (Primer F) and reverse (Primer R) primers (Supplementary Table 3) used for PCR genotyping and predicted sizes of PCR products are indicated. For each locus, an agarose gel of PCR fragments is shown confirming the genotype of each of the strains.



Supplementary Fig. 6. Sequence comparison of ChlF from *C. thermalis* and D1 (encoded by *psbA2*) from Syn6803. Sequence alignment was performed using the Clustal Omega tool (https://www.ebi.ac.uk/Tools/msa/clustalo/). Identical residues are marked by an asterisk and similar residues by colon or period according to the degree of similarity. Bracketed regions indicate the regions that were swapped to create the various D1/ChlF chimeric mutants: N-terminal tail mutant; C-terminal tail mutant; Iron Lid mutant, and QD mutant in which residues M127G128 of D1 were replaced by the QD pair found in ChlF at positions 149 and 150.

Supplementary Table 1. Quantification of Chl *f* in various strains. The procedure using standard methanol pigment extraction and HPLC analysis was utilized; Chl *f*/Chl *a* ratio was calculated from the absorbance of Q_y peaks of Chl *a* and Chl *f* recorded at 665 and 710 nm, respectively. (nd, not detected)

Strains/preparations	Presence of PSII core complex containing FLAG-ChlF or D1-QD	Chl *f*/Chl *a* (%)
WT	−	nd
ΔD1	−	nd
FLAG-ChlF/ΔD1	+	0.24±0.07
FLAG-ChlF/ΔD1/ΔCP43	−	nd
FLAG-ChlF/ΔD1/ΔCP47	−	nd
ΔD1/ΔD2/ΔCP47/ΔCP43	−	nd
FLAG-ChlF/ ΔD1/ΔD2/ΔCP47/ΔCP43	−	nd
His-CP47/D1-QD	+	0.07±0.02
FLAG-ChlF/ΔD1 prep	+	1.05±0.07
His-CP47/D1-QD prep	+	0.26±0.06

Supplementary Table 2. Analysis of FLAG-ChlF preparation by mass spectrometry. Proteins identified in the FLAG-ChlF preparation isolated from the D1-less *Synechocystis* strain expressing FLAG-ChlF from *Chroococcidiopsis thermalis.* PLGS score is a statistical measure of peptide assignment accuracy, and is calculated using the Protein Lynx Global Server (PLGS 2.2.3) software (Waters).

Protein UniProtKB No.	Size (Da) Length (AA)	Number of detected peptides	PLGS score
PsbF P09191	4929 44	16	27749.54
CP47 P05429	55903 507	147	18672.52
PsbH P14835	7116 64	13	9585.346
HliA P73183	7868 70	1	7952.975
D2 P09192	39466 352	79	7174.181
ChlF Chro_1030	41507 47	44	6175.665
PsbE P09190	9442 81	15	5626.923
CP43 P16033	39695 360	93	5540.293
Psb27 P74367	14786 134	38	5444.494
Sll1106 P74720	18031 171	15	5111.672
HliC A0A068MZZ7	5150 47	3	4693.248

Supplementary Table 3. Sequence of primers

Primer name	Sequence (5' – 3')	Notes
Primers for constructing the plasmids		
A2_Ct_F	CTTTTCCAGTAGCAGGAATCTGGTTCACTGCTATGGGTGTAAG	To amplify from pPD FLAG-psbA2 the vector backbone to clone the "N-terminal tail" construct.
A2_Ct_R	ATTTGCAGATTATTCCTAGGTTAACCGTTGACAGCAGGAGC	
srD1_no_Ct_F2	CCTAGGAATAATCTGCAAA	To amplify the *C. thermalis chlF* "N-terminal tail" fragment.
srD1 NF N-del R	TGCGGCCGCTTTATCATC	
psbA2_minusCt_F	GGAATTATAACCATATGACAACGACTCTCCAACAG	To amplify from pRD1031Km^R the vector backbone to clone "C-terminal tail" construct.
psbA2_minusCt_R	CCCCAAAGCCGCAGACCAGATGCCGATTACAGGCCAA	
psbA4_Ct_F	TGGTCTGCGGCTTTGG	To amplify the *C. thermalis chlF* "C-terminal tail" fragment.
psbA4_Ct_R	CATATGGTTATAATTCCTTATG	
psbA2_minusFe_F	GGTGCTTGGCCTGTAATCG	To amplify from pRD1031Km^R the vector backbone to clone the "iron lid" construct.
psbA2_minusFe_R	GGAGAACAAGCTACCACCGAAT	
psbA4_Fe_F	TGGTAGCTTGTTCTCCGCTATGCACGGTTCCCT	To amplify the *C. thermalis chlF* "iron lid" fragment.
psbA4_Fe_R	GATTACAGGCCAAGCACCTAGGAAAAAATGCAATCGGC	
QD_F	CAAGATCGTCAGTGGGAACTTTCCT	Make MG to QD mutation in PsbA2 protein of *Synechocystis*
QD_R	GTAGCAGAAAATGCCGATGAG	
psbA4_QD_F	TCATCGGCATTTTCTGCTACAAGATCGCGAATGGGAATTGAG	To amplify (with primer psbA4_Ct_R) the "QD_C-terminal tail" from *C. thermalis chlF*
SDM QD to MG 149150 F	ATGGGTCGCGAATGGGAATTGAGC	To make QD to MG mutation in ChlF
SDM QD to MG 149150 R	ATAGCAAATAATGCCGATCAAG	
pPD psbA4 F	ACATAAGGAATTATAACCATATGGTGTCAAAGACAGACAGT	To make pPD-ChlF and pPD FLAG-Chlf constructs
pPD NFLAG psbA4 F	GATGATAAAGCGGCCGCAATGGTGTCAAAGACAGACAGTGC	
pPD psbA4 R	AGTTGAAGGAAGATCTCTACTCATTACTCACTGCCATCAAG	
Genotyping primers, see also Extended Data Fig. 2a, Extended Data Fig. 3a, Supplementary Figs. 2a and 5.		
F1	GAATCGAGATTGTGCCTGCC	Genotyping of *psbA1* knockout
R1	GAGATTAAAAAATATAAATAACACCACTG	Genotyping of *psbA1* knockout
F2	ACCTTACTTGGATGCCGGTG	Genotyping of *psbA2* knockout
R2	TTGCTGGTTACATTTGACCGG	Genotyping of *psbA2* knockout
F3	CAGTGCTAATTTGTCGGGACAG	Genotyping of *psbA3* knockout
R3	AAAGAGTATGGTGATAAAAACCGGC	Genotyping of *psbA3* knockout
F4	AGTTGCGACAAAATAACCCAGCTCCAGCAA	Genotyping of *psbD1C* knockout
R4	TTGCCAAAGTATTCTCCTGATTTAAATGATATTGAGCA	Genotyping of *psbD1C* knockout
F5	CCATGGCGGATCGAGAAGAT	Genotyping of *psbD2* knockout
R5	CGGGCATCTACCTTGGTTAAC	Genotyping of *psbD2* knockout
F6	TTTTCATTTGTTGTCCCTGGACCGGTAGACAGTA	Genotyping of *psbB* knockout
R6	TGTCAATAGCTCATCTGAGTTGGGAAAAAGCCT	Genotyping of *psbB* knockout