Assembly of D1/D2 complexes of photosystem II: binding of pigments and a network of auxiliary proteins

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SUPPLEMENTAL MATERIAL

Supplemental figures

Supplemental Figure S1. 2D protein analysis of membranes isolated from the CP47-less mutant expressing a native or a His-tagged variant of the D2 protein (ΔCP47 and His-D2/ΔCP47, respectively).

Supplemental Figure S2. 2D protein analysis of the FLAG-Ycf39 preparation isolated from the CP47-null mutant expressing FLAG-Ycf39.

Supplemental Figure S3. 2D protein analysis of the preparation isolated from the CP47-less strain expressing His-tagged D2 protein and lacking Ycf39 (His-D2/ΔCP47/ΔYcf39).

Supplemental Figure S4. 2D protein analysis of the membranes isolated from the CP47-less strain (ΔCP47) after the radioactive pulse-labeling (p) followed by 30 and 60 min of the pulse-chase (pch).

Supplemental Figure S5. 2D protein analysis of the preparation isolated from the CP47-less strain expressing His-tagged D2 protein.

Supplemental Figure S6. 2D protein analysis of the preparation isolated from the CP47-less strain expressing FLAG-tagged PsbI protein using FLAG-specific affinity chromatography.

Supplemental Figure S7. 2D protein analysis of the control preparation isolated by Ni-affinity chromatography from the control CP47-less strain.

Supplemental Figure S8. 2D protein analysis of FLAG-D2 and the control preparation.

Supplemental Figure S9. Separation of RCII complexes by ionex chromatography and their absorption spectra.

Supplemental Figure S10. Room temperature absorption spectra and 77K chlorophyll fluorescence spectra of the FLAG-D2 and control FLAG-free preparations.

Supplemental Figure S11. 2D protein analysis of radioactively labeled membrane proteins of ΔCP47 and ΔCP47/ΔSlr0575 strains.

Supplemental Figure S12. Native gel analysis of His-D2 preparations isolated from membranes of the CP47-less strain expressing His-D2 by using different concentrations of β-dodecyl-maltoside (DM) for solubilization.

Supplemental Figure S13. 2D protein analysis of the preparation isolated from the CP47-less strain lacking FtsH2 and expressing His-tagged D2 protein and the stoichiometry of pigment cofactors in RCII* and RCIIa electrophoretically purified from this preparation.

Supplemental Figure S14. 2D protein analysis of membrane proteins of ΔPSI/ΔCP47/ΔCP43 strain.

Supplemental tables

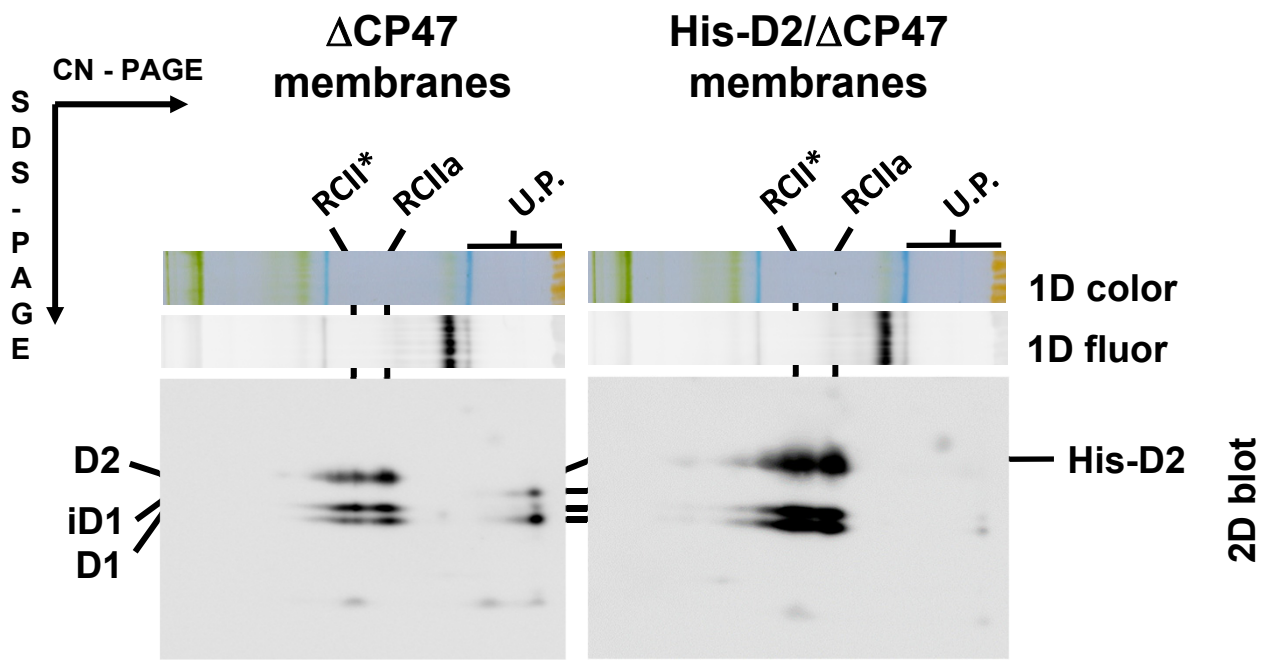
Supplemental Table S1. List of PSII and PSI-related proteins identified by MS and Western blotting in the RCII complexes separated by 2D CN/SDS PAGE from the isolated His-D2/ΔCP47 preparation (see Figure 1, arrows) and identification of Slr1470 in the His-D2/ΔCP47 preparation (see Supplemental Figure S3).

Supplemental Table S2. List of the most abundant 40 proteins identified by MS in the FLAG-PsbI/ΔCP47 preparation.

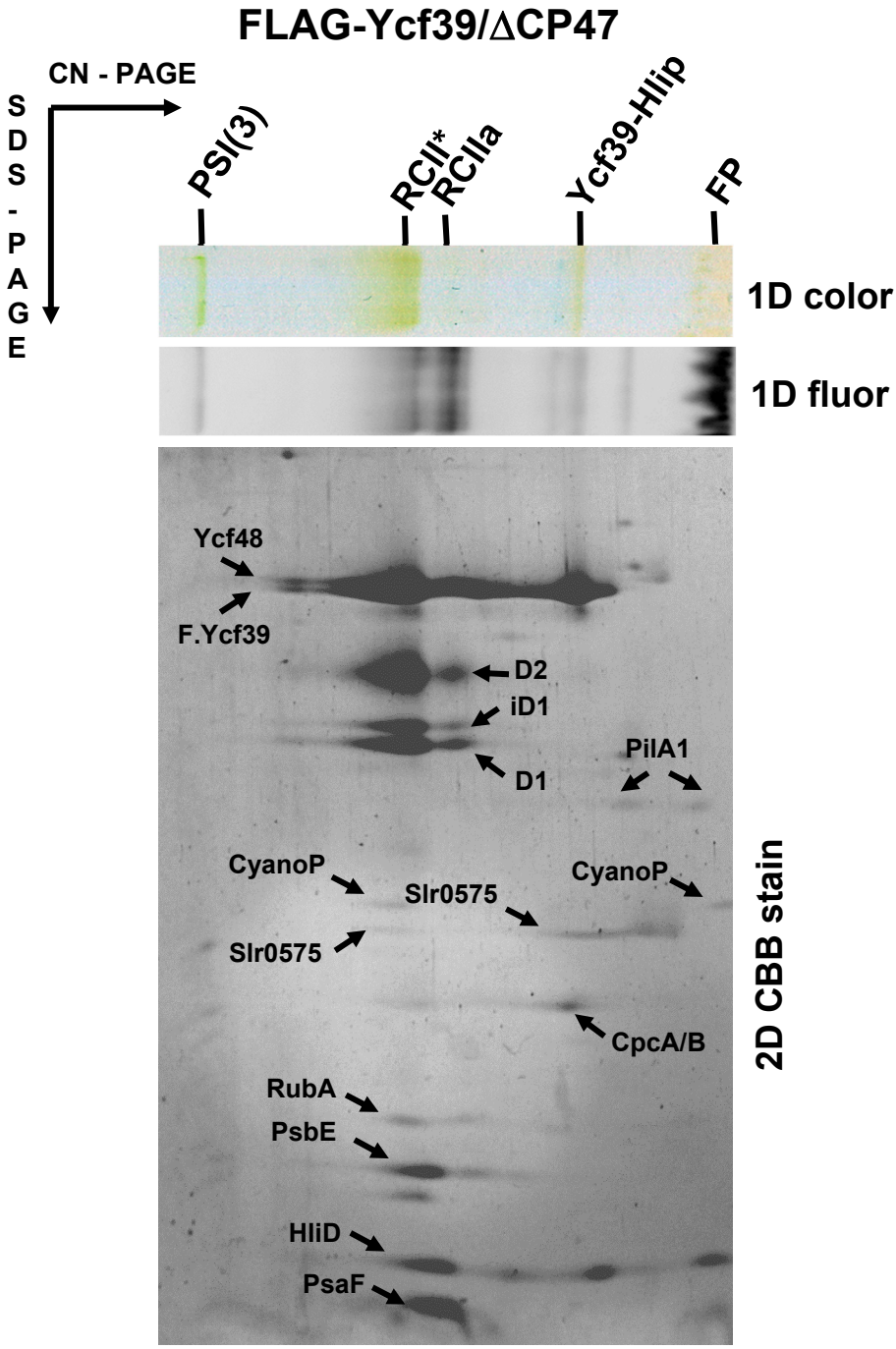
Supplemental Table S3. List of the most abundant 40 proteins identified by MS in the FLAG-D1 preparation.

Supplemental Table S4. List of the most abundant 40 proteins identified by MS in the FLAG-D2 preparation.

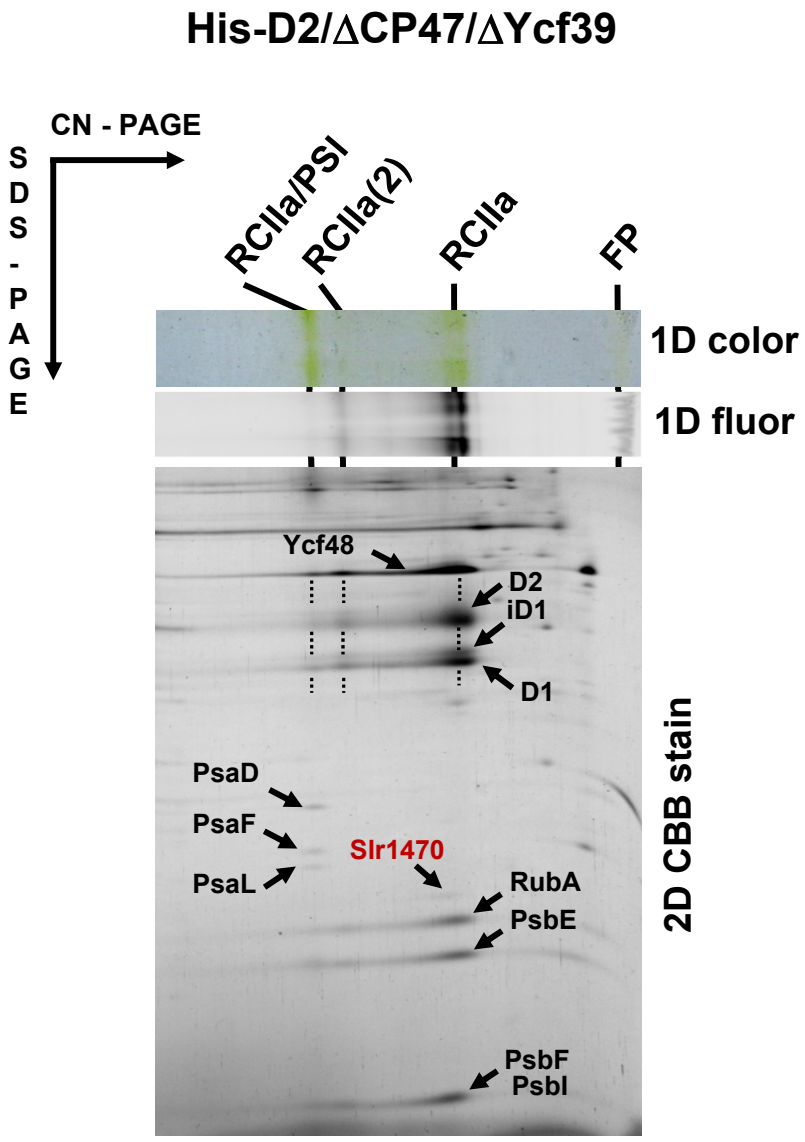
Supplemental Table S5. List of primers.



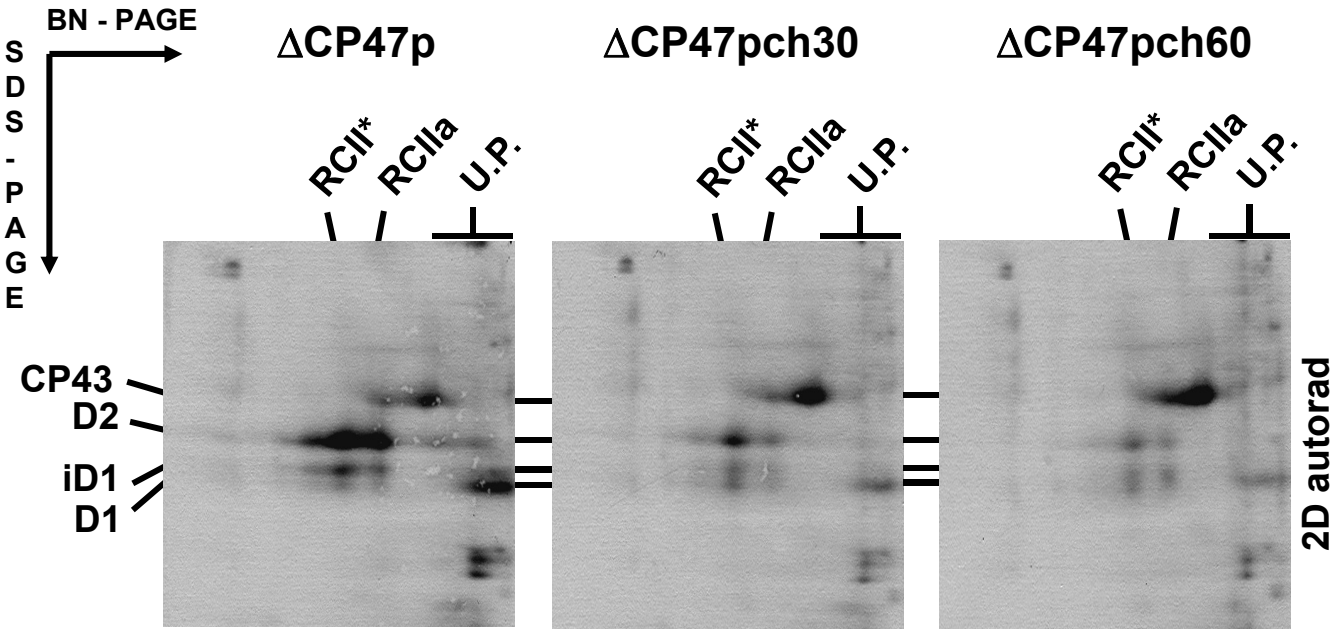
Supplemental Figure S1. 2D protein analysis of membranes isolated from the CP47-less mutant expressing a native or a His-tagged variant of the D2 protein (ΔCP47 and His-D2/ΔCP47, respectively). Membranes were analyzed by CN PAGE in the first dimension, the native gel was photographed (1D color) and scanned by LAS 4000 for fluorescence (1D fluor), after SDS-PAGE in the 2nd dimension the gel was electroblotted and sequentially probed with antibodies against D1 and D2 proteins. 4 µg of Chl were loaded for each sample.



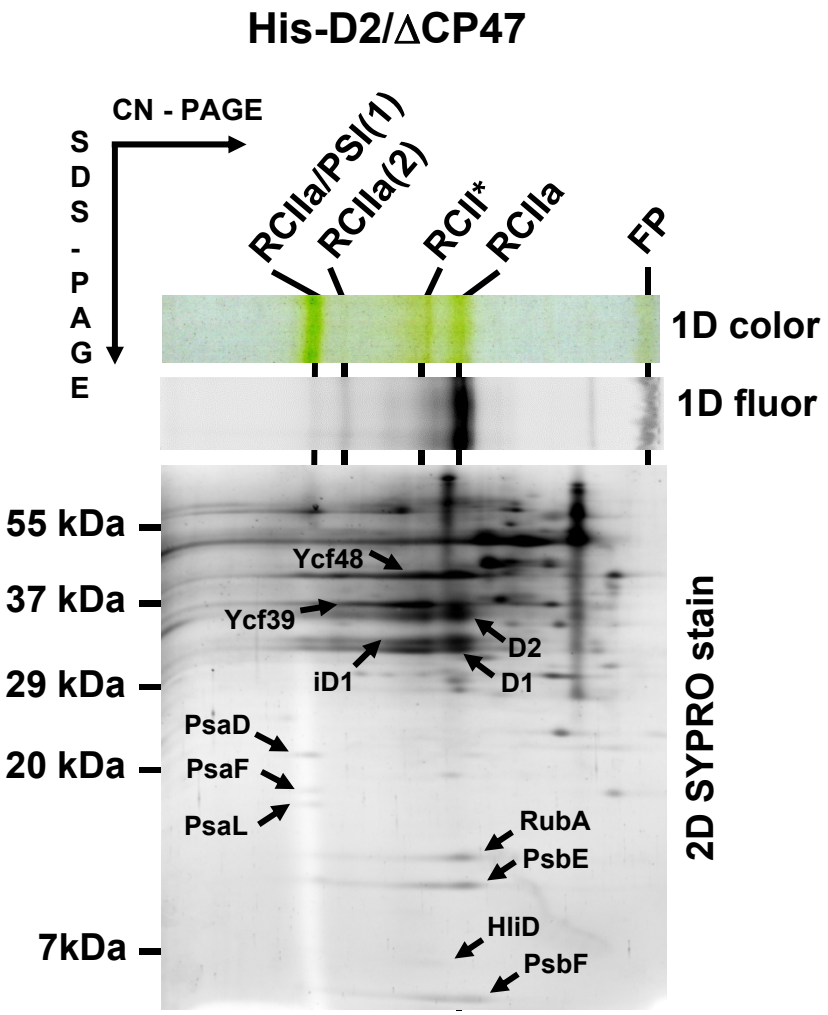
Supplemental Figure S2. 2D protein analysis of the FLAG-Ycf39 preparation isolated from the CP47-null mutant expressing FLAG-Ycf39. The preparation was isolated using anti-FLAG affinity resin and was subsequently analyzed by 2D CN/SDS-PAGE. The one dimension native gel was photographed (1D color) and scanned by LAS 4000 for Chl fluorescence (1D fluor). After the SDS-PAGE in the 2nd dimension the gel was stained by Coomassie blue (2D CBB stain) and the designated spots were identified by MS. FP are free pigments, other designation as in Figure 1. 0.5 µg of Chl were loaded onto the gel.



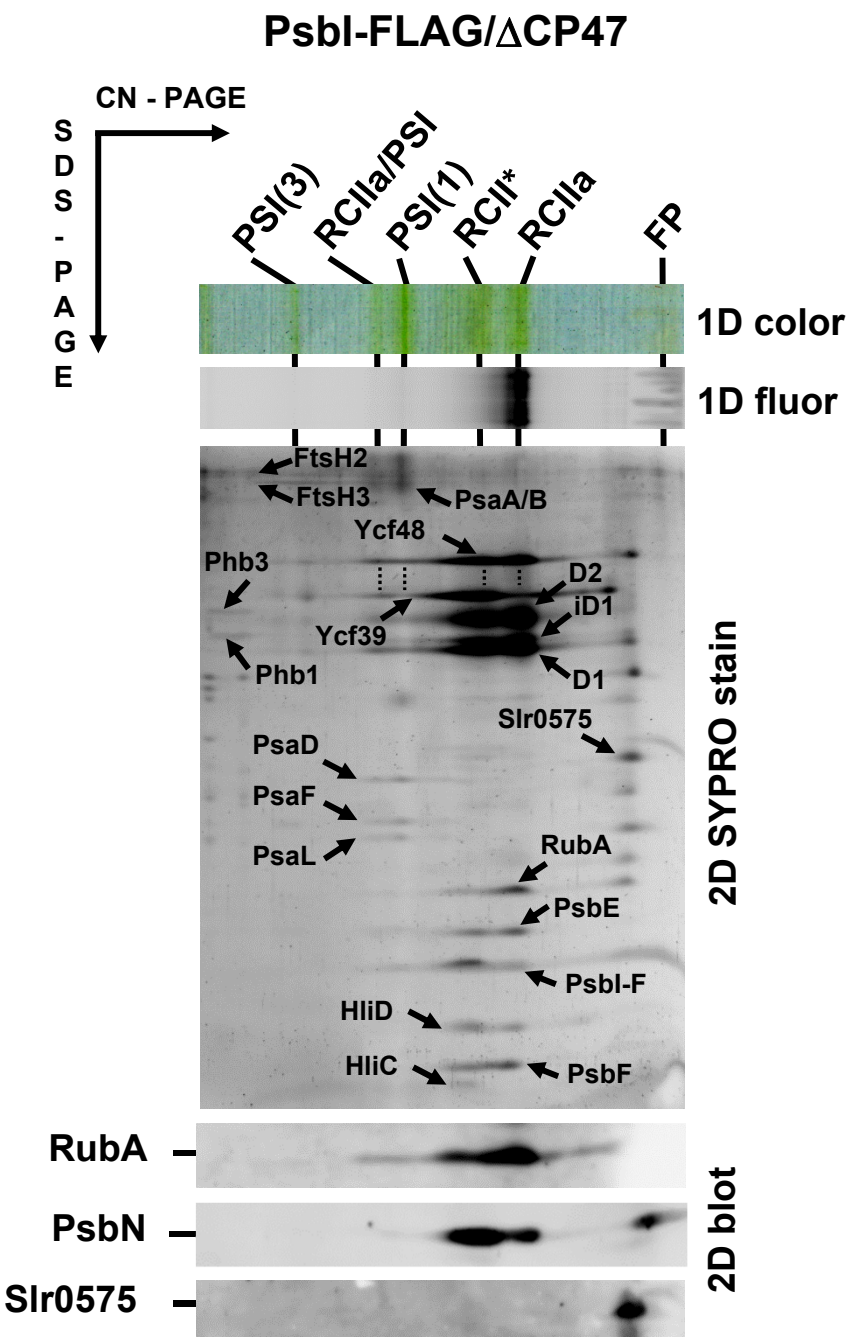
Supplemental Figure S3. 2D protein analysis of the preparation isolated from the CP47-less strains expressing His-tagged D2 protein and lacking Ycf39 (His-D2/ΔCP47/ΔYcf39). The preparation isolated using Ni-affinity chromatography was analyzed by CN PAGE in the first dimension, the native gel was photographed (1D color) and scanned by LAS 4000 for fluorescence (1D fluor), after SDS-PAGE in the 2nd dimension the gel was stained by Coomassie Blue (2D CBB stain). Designation of the complexes as in Figures 1 and 2. Slr1470 was identified by MS (Table S1). 0.5 µg of Chl were loaded onto the gel.



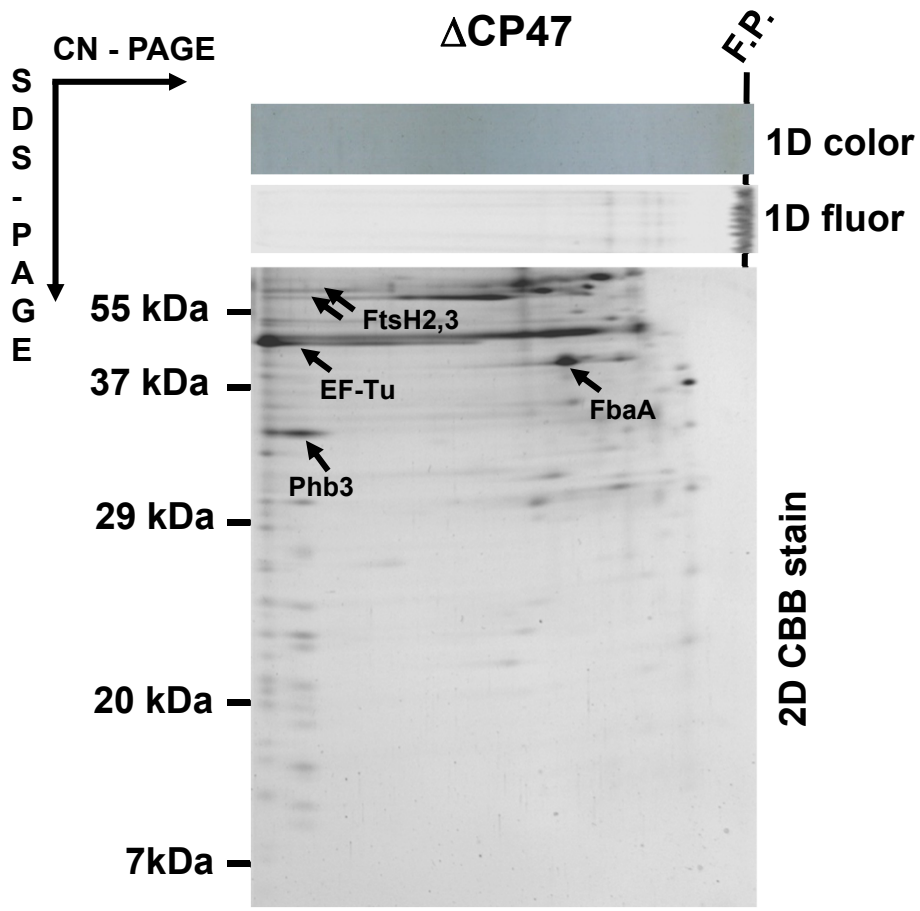
Supplemental Figure S4. 2D protein analysis of the membranes isolated from the CP47-less strain (ΔCP47) after the radioactive pulse-labeling (p) followed by 30 and 60 min of the pulse-chase (pch). Radioactively labeled membranes were analyzed by CN PAGE in the first dimension. After SDS-PAGE in the 2nd dimension the gel was stained by Coomassie Blue and exposed to a Phosphorimager plate (2D autorad). Designation of complexes as in Figures 1 and S1. 4 µg of Chl were loaded onto the gel for each sample.



Supplemental Figure S5. 2D protein analysis of the preparation isolated from the CP47-less strains expressing His-tagged D2 protein. The preparation was isolated using Ni affinity chromatography and analyzed by CN PAGE in the first dimension, the native gel was photographed (1D color) and scanned by LAS 4000 for fluorescence (1D fluor), after SDS-PAGE in the 2nd dimension the gel was stained by SYPRO Orange (2D SYPRO stain), Designation of complexes as in Figure 1. Note the high amount of the RCIIa/PSI(1) in the preparation. 0.5 µg of Chl were loaded onto the gel.

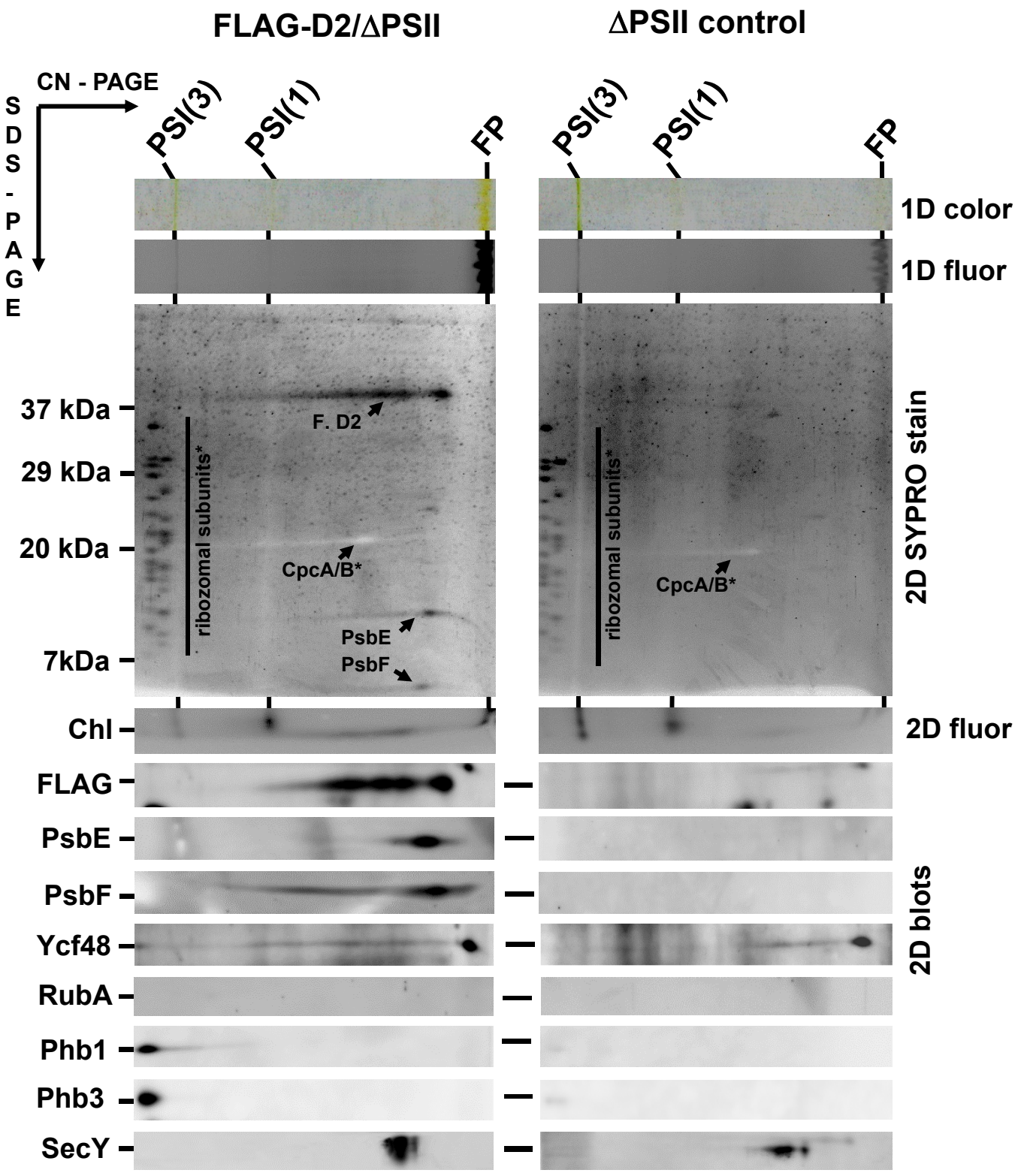


Supplemental Figure S6. 2D protein analysis of the preparation isolated from the CP47-less strains expressing FLAG-tagged PsbI protein using FLAG-specific affinity chromatography. The preparation was analyzed by CN PAGE in the first dimension, the native gel was photographed (1D color) and scanned by LAS 4000 for fluorescence (1D fluor), after SDS-PAGE in the 2nd dimension the gel was stained with SYPRO Orange (2D SYPRO stain) electroblotted and the blot was probed with designated antibodies. Designation of the complexes as in Figure 1. 0.8 µg of Chl were loaded onto the gel.

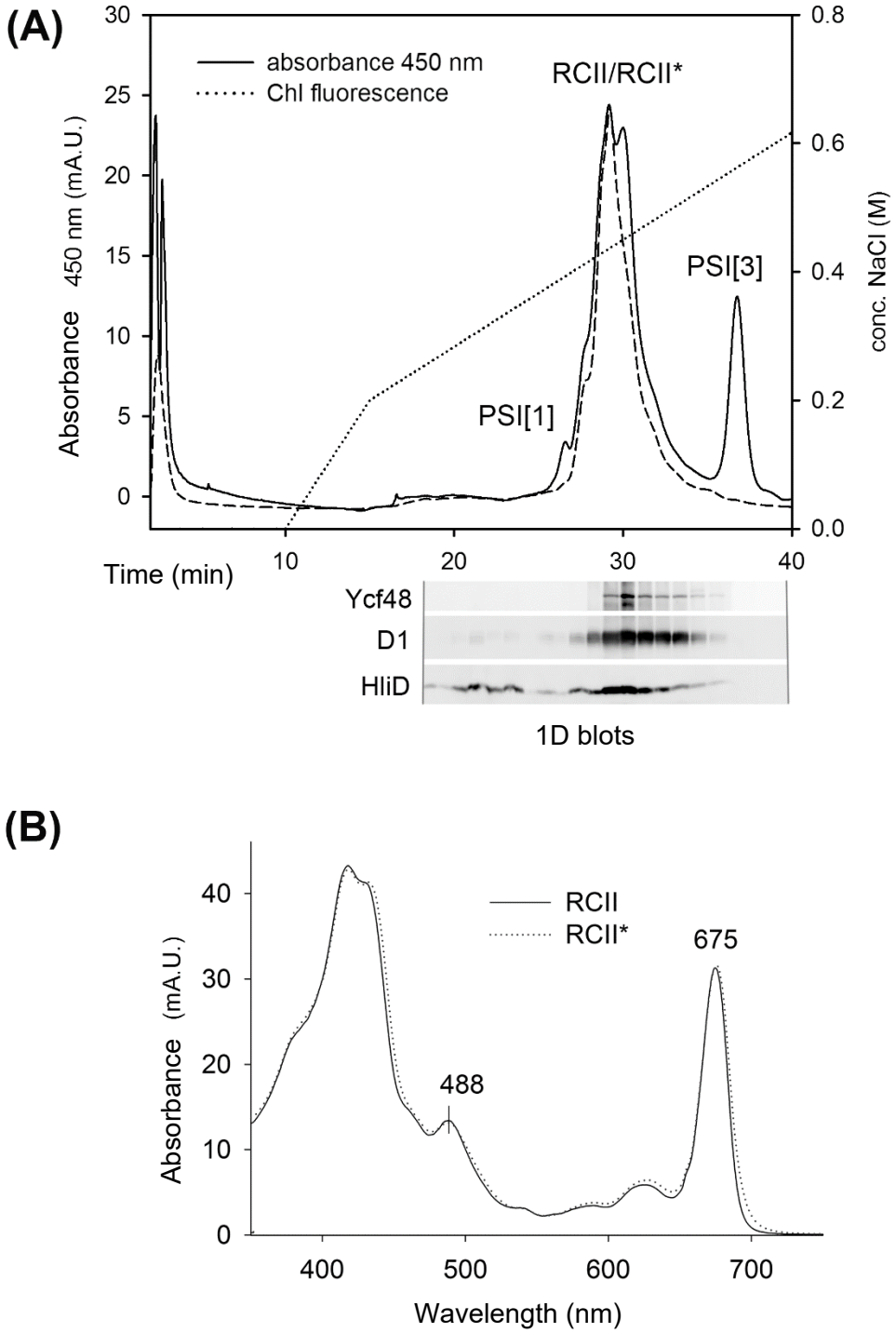


Protein UniProt KB No.	Mass spectrometric analysis				Blot analysis
	Size (Da) Length (AA)	Coverage (%)	Detected/theor.et no. of peptides	PLGS score	
FbaA Q55664	38972 359	29	8/21	691	–
EFTu P74227	43733 399	45	12/37	1266	–
FtsH2 Q55700	68496 627	16	8/48	390	+
FtsH3 P72991	67250 616	21	8/45	384	+
Phb3 P72655	35727 321	31	8/30	311	+

Supplemental Figure S7. 2D protein analysis of the control preparation isolated by Ni-affinity chromatography from the control CP47-less strains. The preparation was analyzed by CN PAGE in the first dimension, the native gel was photographed (1D color) and scanned by LAS 4000 for fluorescence (1D fluor), after SDS-PAGE in the 2nd dimension the gel was stained by Coomassie blue (2D CBB stain). F.P. means free pigments. The main components were identified by MS (see the table), note the absence of PSII-related proteins. The amount of proteins corresponding to that in 0.5 µg of Chl of His-D2 preparation was loaded onto the gel.

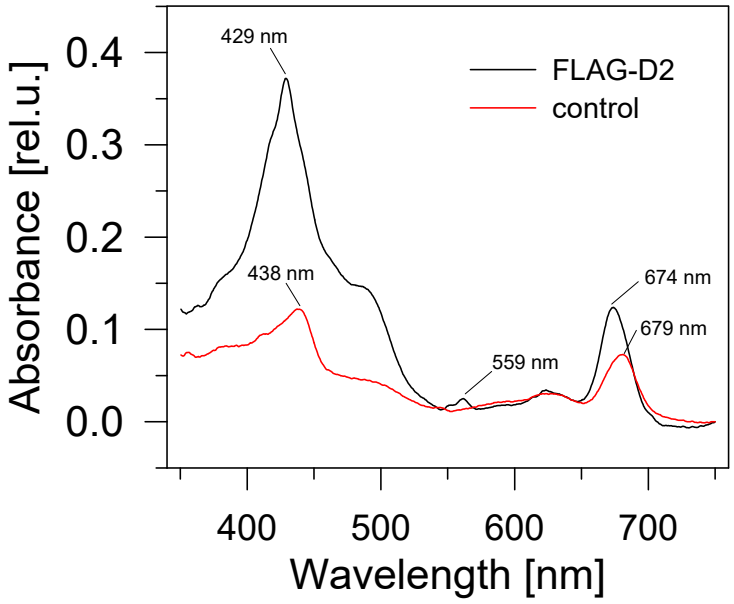


Supplemental Figure S8. 2D protein analysis of FLAG-D2 and the control preparation. The preparations were isolated from the control strain lacking large PSII Chl-binding proteins (ΔPSII control) and from the strain additionally expressing FLAG-D2 (FLAG-D2/ΔPSII). The analysis was performed by CN PAGE in the first dimension, the native gel was photographed (1D color) and scanned by LAS 4000 for fluorescence (1D fluor), after SDS-PAGE in the 2nd dimension the gel was scanned for Chl fluorescence (Chl, 2D fluor), then stained by SYPRO Orange (2D SYPRO stain) and probed with designated antibodies (2D blots). CpcA/B and ribosomes are designated with the asterisk as unspecific contaminants. Both preparations were isolated in parallel from the same amount of cells and the analysis was performed using identical volumes of the obtained preparations. The left panel is identical to the right panel in Figure 6.

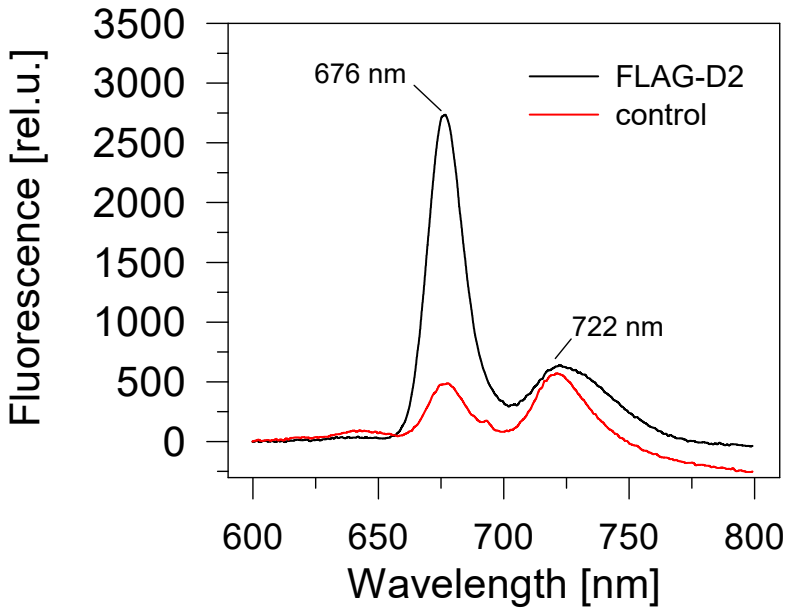


Supplemental Figure S9. Separation of RCII complexes by ionex chromatography and their absorption spectra. A: The His-D2 preparation isolated from the strain lacking CP47 was subjected to HPLC on the ionex MonoQ column. The separation was monitored by measurements of absorption changes at 450 nm (solid line) and fluorescence excited at 440 nm (dashed line). The chromatographic fractions collected between 18 and 40 min were analyzed using SDS-PAGE in combination with immunoblotting using antibodies specific for Ycf48, D1 and HliD. **B:** Absorption spectra measured online by diode array detector in the maxima at 28 (RCII) and 32 (RCII*) min are shown; A.U. - absorbance units. Small blue shift of the RCII* spectrum (dotted line) in comparison with RCII (solid line) one is most probably related to a PSI contamination.

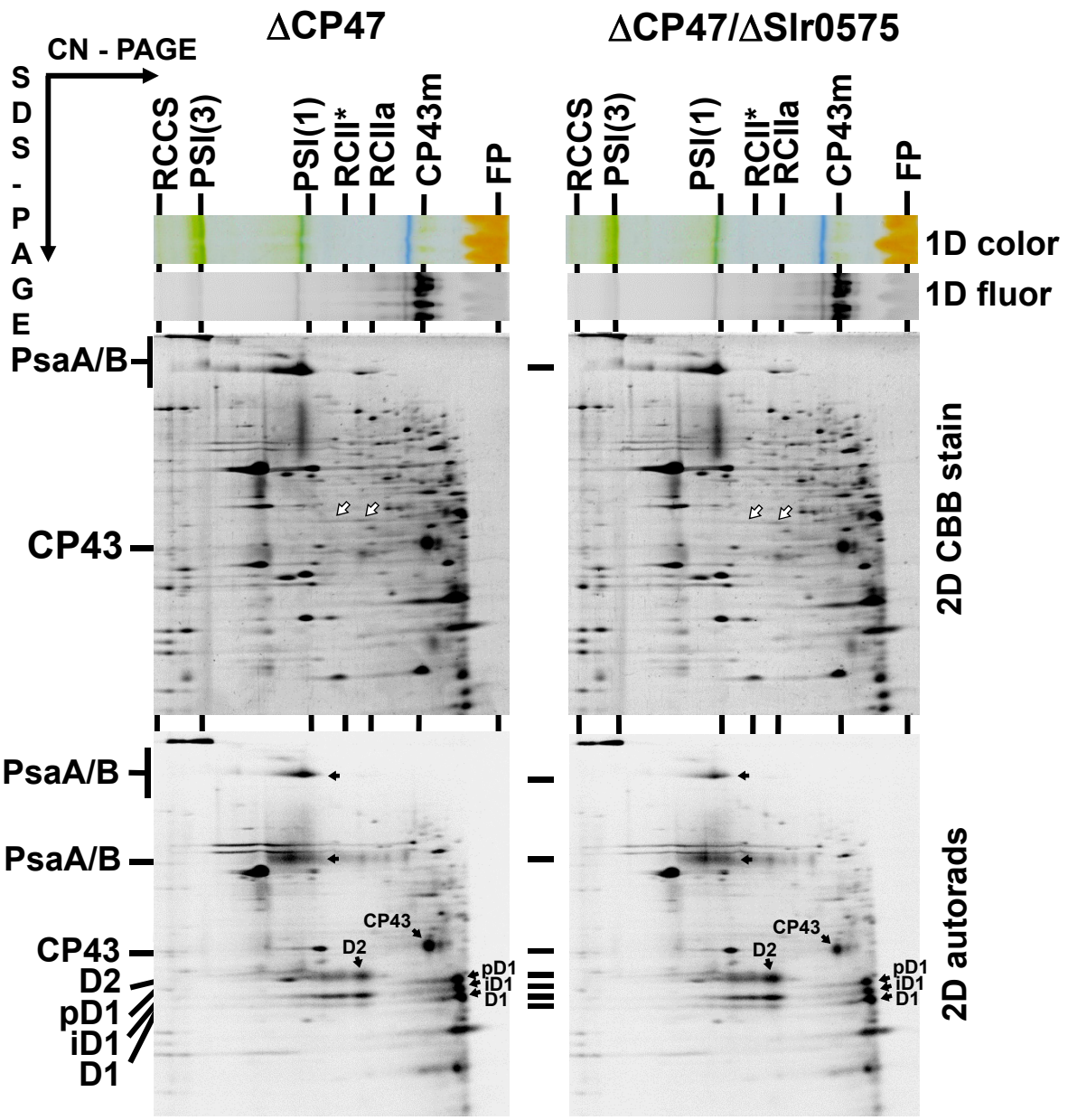
A



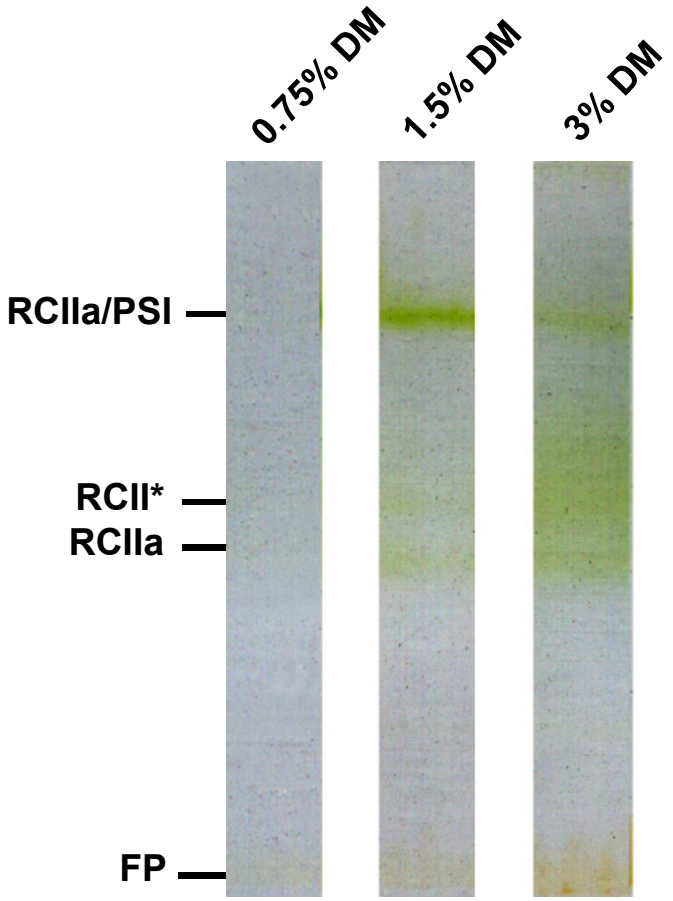
B



Supplemental Fig. S10. Room temperature absorption spectra and 77K chlorophyll fluorescence spectra of the FLAG-D2 and the control FLAG-free preparations. The preparations were isolated using FLAG affinity chromatography from the control strain lacking large PSII Chl-binding proteins (control) and from the strain additionally expressing FLAG-D2 (FLAG-D2). Both preparations were isolated in parallel from the same amount of cells and the measurements were performed using identical volumes of the obtained preparations. **A**: absorption spectra of FLAG-D2 (black line) and control (red line) were measured using Shimadzu UV3000 spectrophotometer. **B**: fluorescence spectra of of FLAG-D2 (black line) and control (red line) were measured using SM 9000 spectrophotometer (Photon Systems Instruments) at an excitation wavelength of 455 nm.

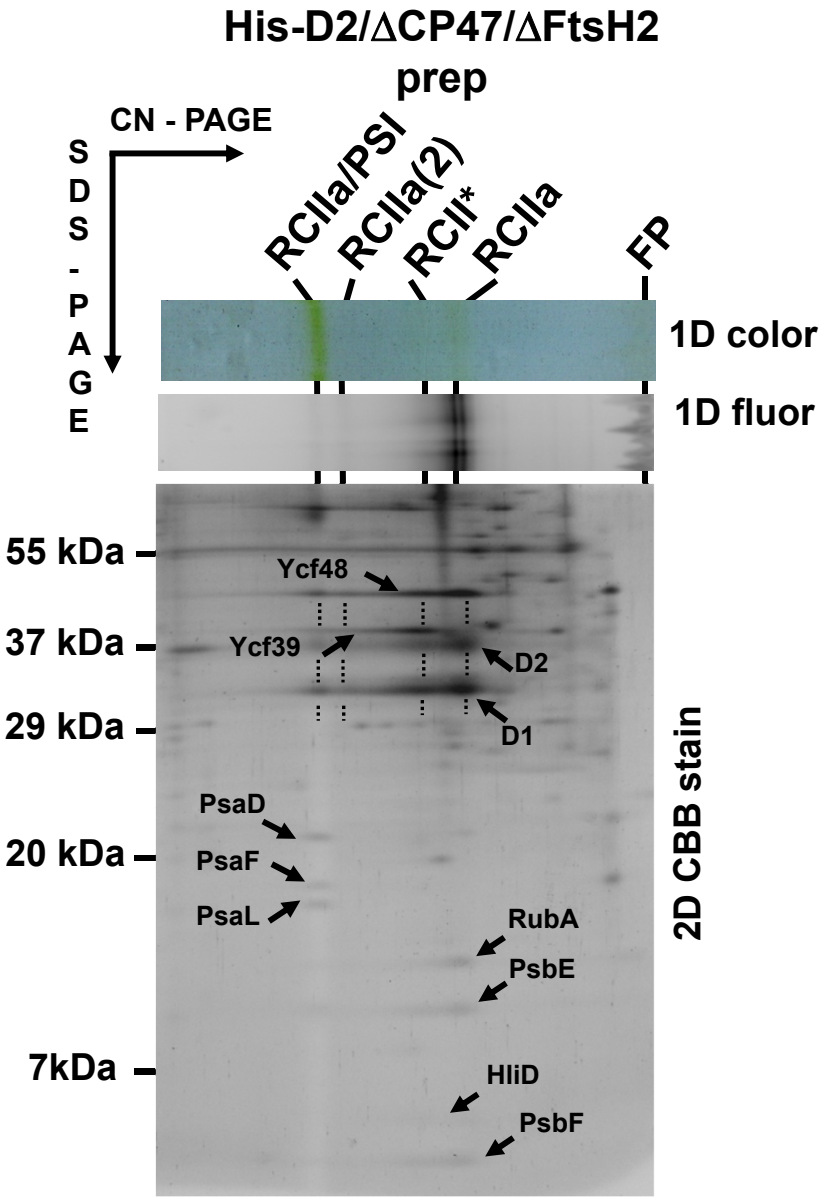


Supplemental Figure S11. 2D protein analysis of radioactively labeled membrane proteins of ΔCP47 and ΔCP47/ΔSlr0575 strains. Membranes isolated from radioactively labeled cells were analysed using 2D-CN/SDS-PAGE. After the first dimension, the gel was photographed (1D colour) and scanned for Chl fluorescence (1D fluor). After separation in the second dimension, the 2D gel was stained with Coomassie Blue (2D CBB stain) and the radiolabeled proteins were subsequently detected by autoradiography (2D autorads). Designation of complexes as described in Figure S1. Empty arrows designate Ycf48 present in RCII complexes in the stained gel, closed arrows designate distinctly synthesized D2, CP43 and PSI proteins. Each loaded sample contained 3.5 µg of Chl.



Supplemental Figure S12. Native gel analysis of His-D2 preparations isolated from membranes of the CP47-less strain expressing His-D2 by using different concentrations of β-dodecyl-maltoside (DM) for solubilization. The preparations were analyzed by CN PAGE. Designation of complexes as in Figure 1. The same volume of the samples prepared using the identical isolation procedure and identical concentration of the preparation. Concentration of Chl in membranes used for solubilization was 870 µg/ml.

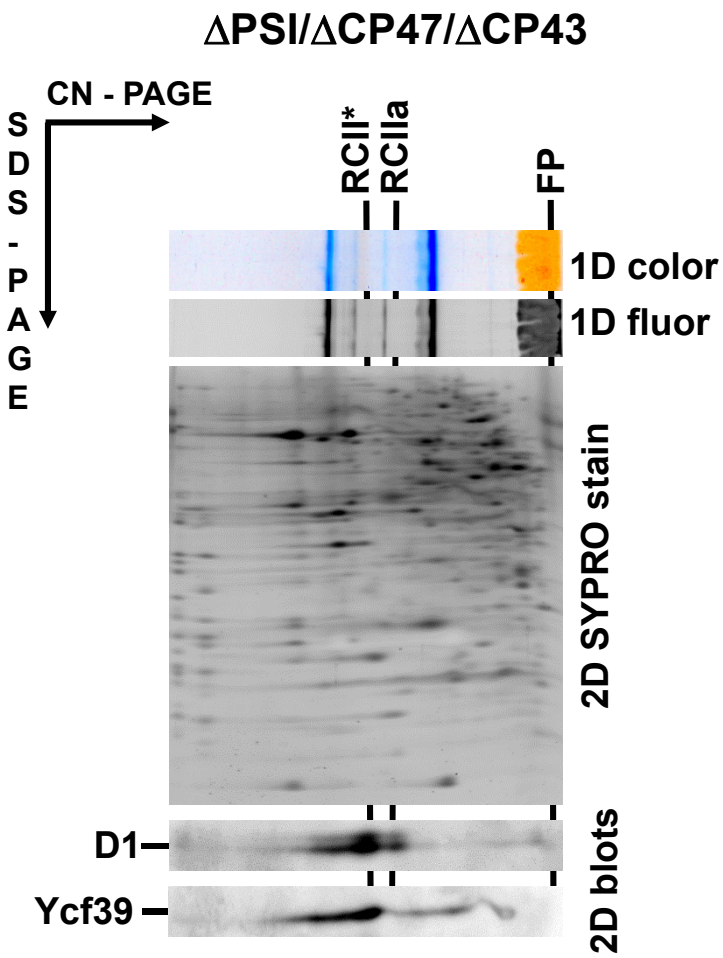
A



B

Preparation	Stoichiometry of pigment cofactors			
	Chl-*a*	**b-carotene**	**Pheophytin**	**Heme-*b***
RCII*(ΔFtsH2)	9.2 ± 0.7	2.0 ± 0.3	1.8 ± 0.1	1.0
RCIIa (ΔFtsH2)	6.7 ± 0.6	1.3 ± 0.3	1.8 ± 0.2	1.0

Supplemental Figure S13. 2D protein analysis of the preparation isolated from the CP47-less strain lacking FtsH2 and expressing His-tagged D2 protein and the stoichiometry of pigment cofactors in RCII* and RCIIa electrophoretically purified from this preparation. A: The preparation was analyzed by CN PAGE in the first dimension, the native gel was photographed (1D color) and scanned by LAS 4000 for fluorescence (1D fluor), after SDS-PAGE in the 2nd dimension the gel was stained by Coomasie Blue (2D CBB stain). Designation of complexes as in Figure 1. 0.5 µg of Chl were loaded onto the gel. **B:** RCII* and RCIIa were electrophoretically purified, eluted, pigments extracted and analyzed by HPLC. The ratio of all pigments is normalized per one heme. Data are shown as mean of three independent replicates.



Supplemental Figure S14. 2D protein analysis of membrane proteins of ΔPSI/ΔCP47/ΔCP43 strain. Membranes isolated from cells were analyzed using 2D-CN/SDS-PAGE. After the first dimension, the gel was photographed (1D color) and scanned for Chl fluorescence (1D fluor). After separation in the second dimension, the 2D gel was stained with SYPRO Orange (2D SYPRO stain) and probed with antibodies specific for D1 and Ycf39. 1 µg of Chl was loaded onto the gel.

Supplemental Table S1. List of PSII and PSI-related proteins identified by MS and Western blotting in the RCII complexes separated by 2D CN/SDS PAGE from the isolated His-D2/ΔCP47 preparation (see Figure 1, arrows) and identification of Slr1470 in the His-D2/ΔCP47 preparation (Supplemental Figure S3).

Uniprot Accession numbers	Mass spectrometric analysis				Blot analysis
	Size (Da) Length (AA)	Coverage (%)	Detected no. of peptides	PLGS score	
Ycf39 P74429	36496 326	33	11	1148	+
Ycf48 P73069	37267 342	36	9	928	+
D2 P09192	39466 352	18	5	560	+
D1 P16033	39695 360	10	3	303	+
CyanoP Slr1418	20747 188	10	2	456	+
PsbE P09190	9442 81	25	2	134	+
PsbF P09191	4929 44	38	1	145	+
PsbI Q54697	4306 38	–	–	–	+
RubA P73068	12570 115	38	2	109	+
HliD P73563	6472 57	44	2	226	+
PsaD P19569	15644 141	25	3	101	+
PsaF P29256	18249 165	30	4	89	+
PsaL P37277	16624 157	25	2	95	+
Slr1470 P74154	14892 188	32	5	400	+

The analysis of protein bands was performed using a NanoAcquity UHPLC (Waters, Milford, MA, USA) on-line coupled to an ESI Q-ToF Premier mass spectrometer (Waters, Milford, MA, USA) PLGS score is calculated by Protein Lynx Global Server (PLGS 2.2.3) software (Waters, Milford, MA, USA) and is a statistical measure of peptide assignment accuracy.

Supplemental Table S2. List of the most abundant 40 proteins identified by MS in the FLAG-PsbI/ΔCP47 preparation.

Uniprot Accession numbers	Protein name	No of peptides	Intensity
P73069	PSII assembly factor Ycf48	18	1242200000
P74429	PSII assembly factor Ycf39	17	605940000
P09192	PSII protein D2	6	521250000
P16033;P07826	PSII protein D1	7	460220000
P73952	PSII assembly factor CyanoP	7	149090000
P09190	Cytochrome b559 alpha subunit	2	129110000
Q55700	ATP-dependent zinc metalloprotease FtsH2	10	78206000
P37277	PSI protein PsaL	3	62678000
P19569	PSI protein PsaD	4	59026000
P29254	PSI protein PsaA	6	50275000
P73068	Rubredoxin	3	44167000
P12975	PSI protein PsaE	1	34431000
P29255	PSI protein PsaB	4	27994000
P74720	Sll1106	5	27463000
P23349	50S ribosomal protein L7/L12	3	25268000
P72991	ATP-dependent zinc metalloprotease FtsH3	10	24798000
P29256	PSI protein PsaF	4	23745000
P32422	PSI protein PsaC	2	20984000
Q54715	C-phycocyanin alpha subunit	4	20784000
P73563	HliC	1	19752000
P73790	Pterin-4-alpha-carbinolamine dehydratase	1	14659000
Q54714	C-phycocyanin beta subunit	4	14427000
Q55584	Cation or drug efflux system protein	2	12120000
P72655	Stomatin slr1128	2	11273000
P72932	HliD	1	10270000
P72805	Sll1665 protein	2	9274200
P73437	ATP-dependent zinc metalloprotease FtsH4	5	8696200
Q6ZEP1	Uncharacterized protein Sll5089	1	8280600
P73704	General secretion pathway protein HofG	2	8268100
P36236	50S ribosomal protein L1	3	7701300
P72771	CemA-like protein Sll1685	1	7358200
Q01951	PSI protein PsaB	2	6715100
Q55176	CurT-like protein Slr0483	2	6641700
P73319	50S ribosomal protein L4	1	6590400
P73655	Tryptophan--tRNA ligase	1	5508400
P09193	PSII protein CP43	3	5168000
P23350	50S ribosomal protein L10	1	5114800
P72680	Tyrosine recombinase Slr0733	1	4748300
P73307	30S ribosomal protein S8	2	4005500

The analysis of proteins precipitated from the preparation was performed using NanoElute UHPLC (Bruker) on-line coupled to a high-resolution mass spectrometer (Bruker Impact HD).

Supplemental Table S3. List of the most abundant 40 proteins identified by MS in the FLAG-D1 preparation.

Uniprot Accession numbers	Protein name	No of peptides	Intensity
P73069	PSII assembly factor Ycf48	8	54533000
P73655	Tryptophan--tRNA ligase	15	42896000
Q54714	Phycocyanin beta subunit	5	37597000
P73713	Diguanylate cyclaseSll1687 protein	22	31041000
P23349	50S ribosomal protein L7/L12	4	27108000
P16033;P07826	PSII protein D1	3	26748000
P73842	Folyl-polyglutamate synthetase	2	23692000
P73304	30S ribosomal protein S5	11	22235000
P73317	50S ribosomal protein L2	10	19645000
P74429	PSII assembly factor Ycf39	9	19328000
P42352	50S ribosomal protein L9	10	19255000
P74754	Slr0607 protein	2	19231000
P74229	30S ribosomal protein S7	8	18638000
P73319	50S ribosomal protein L4	4	18024000
P73299	30S ribosomal protein S13	7	17563000
P72655	Stomatin slr1128	7	17022000
P74071	30S ribosomal protein S2	13	16505000
P26527	ATP synthase beta subunit	11	15186000
Q54715	C-phycocyanin alpha subunit	4	14367000
P73303	50S ribosomal protein L15	7	13402000
P74625	PSI-associated linker protein CpcL	11	13379000
P73312	50S ribosomal protein L29	7	12825000
P74227	Elongation factor Tu	5	11562000
P19569	PSI protein PsaD	8	10917000
P36236	50S ribosomal protein L1	6	10619000
P73145	DNA-binding protein Slr1034	3	10453000
P72851	50S ribosomal protein L28	5	10354000
P37277	PSI protein PsaL	4	10242000
P74386	Urease beta subunit	4	10170000
P72659	Polyribonucleotide nucleotidyltransferase	10	9934600
P48944	30S ribosomal protein S14	6	9892500
Q55499	Single-stranded DNA-binding protein 1	9	9727800
P27179	ATP synthase alpha subunit	7	8980900
P77965	RNA polymerase subunit beta	10	8821900
Q01951;P74551	Allophycocyanin alpha subunit	6	8239100
P73530	30S ribosomal protein S1	5	8025800
P29256	PSI protein PsaF	5	8023300
P29254	PSI protein PsaA	7	7671400
P73314	30S ribosomal protein S3	3	7613900

The analysis of proteins precipitated from the preparation was performed using NanoElute UHPLC (Bruker) on-line coupled to a high-resolution mass spectrometer (Bruker Impact HD).

Supplemental Table S4. List of the most abundant 40 proteins identified by MS in the FLAG-D2 preparation.

Uniprot Accession numbers	Protein name	No of peptides	Intensity
P09192	PSII protein D2	5	333020000
P09190	PSII cytochrome b559 subunit alpha	2	183180000
P36236	50S ribosomal protein L1	11	69920000
P23349	50S ribosomal protein L7/L12	5	68215000
P73308	50S ribosomal protein L5	9	52804000
P73299	30S ribosomal protein S13	6	51161000
P48946	30S ribosomal protein S18	5	48213000
P73307	30S ribosomal protein S8	5	47533000
P73319	50S ribosomal protein L4	4	45939000
P73317	50S ribosomal protein L2	8	45912000
P73298	30S ribosomal protein S11	7	44900000
P73306	50S ribosomal protein L6	8	39285000
Q54715	C-phycocyanin alpha subunit	5	38334000
P42352	50S ribosomal protein L9	7	37343000
P73305	50S ribosomal protein L18	3	36912000
P72866	30S ribosomal protein S15	3	33229000
P74267	50S ribosomal protein L27	3	30195000
P74229	30S ribosomal protein S7	5	30186000
P73676	PSII protein PsbY	1	27901000
P73311	30S ribosomal protein S17	3	24534000
P74410	30S ribosomal protein S16	4	24275000
P73530	30S ribosomal protein S1 h	7	23419000
P73636	30S ribosomal protein S6	4	20741000
P73320	50S ribosomal protein L3	3	20686000
P74230	30S ribosomal protein S12	5	19824000
P72851	50S ribosomal protein L28	4	18770000
P73304	30S ribosomal protein S5	3	18066000
P74227	Elongation factor Tu	7	16956000
Q54714	C-phycocyanin beta subunit	4	16935000
P74518	Ribosome hibernation promotion factor	2	16056000
P73312	50S ribosomal protein L29	2	15798000
P80505	Glyceraldehyde-3-phosphate dehydrogenase	5	13881000
P23350	50S ribosomal protein L10	1	13172000
P37101	Phosphoribulokinase	4	11821000
P48959	50S ribosomal protein L35	5	11341000
P73313	50S ribosomal protein L16	3	9212000
P73314	30S ribosomal protein S3	3	8960200
P48949	30S ribosomal protein S21	2	8592000
P73655	Tryptophan--tRNA ligase	4	8216900

The analysis of proteins precipitated from the preparation was performed using NanoElute UHPLC (Bruker) on-line coupled to a high-resolution mass spectrometer (Bruker Impact HD).

Supplemental Table S5. List of primers. Hexa-*his* tag underlined, EcoRV site in lower case.

Primers	**Sequences (5´-3´)**
psbDC1F	AGTTGCGACAAAATAACCCAGCTCCAGCAA
psbD-His-2R	GCGCGTCCGACTGCAATAGTATGATGATGATGATGATGCATAAAT GCAAATCCTCTTGCGTAGCT
psbD-His-3F	AGCTACGCAAGAGGATTTGCATTTATG<u>CATCATCATCATCATCAT</u> ACTATTGCAGTCGGACGCGC
psbDC4R	TTGCCAAAGTATTCTCCTGATTTAAATGATATTGAGCA
slr0906-1F	TTTTCATTTGTTGTCCCTGGACCGGTAGACAGTA
slr0906-2R	AAGCTGTGGTTAAAAGCTGTGCAAGAAGCACgatatcTGACGCTCC TTCTAGTAACGAATAGTGTT
slr0906-3F	AACACTATTCGTTACTAGAAGGAGCGTCAgatatcGTGCTTCTTGCA CAGCTTTTAACCACAGCTT
slr0906-4R	TGTCAATAGCTCATCTGAGTTGGGAAAAAGCCT
psbDC1F	AGTTGCGACAAAATAACCCAGCTCCAGCAA