

## SUPPLEMENTARY INFORMATION

**Title:** Complexome profiling on the *Chlamydomonas lpa2* mutant reveals insights into PSII biogenesis and new PSII associated proteins

**Authors:** Benjamin Spaniol<sup>1,§</sup>, Julia Lang<sup>1,§</sup>, Benedikt Venn<sup>2,§</sup>, Lara Schake<sup>1</sup>, Frederik Sommer<sup>1</sup>, Matthieu Mustas<sup>3</sup>, Stefan Geimer<sup>4</sup>, Francis-André Wollman<sup>3</sup>, Yves Choquet<sup>3</sup>, Timo Mühlhaus<sup>2</sup>, and Michael Schroda<sup>1, ¶</sup>

### **Affiliations:**

<sup>1</sup> Molekulare Biotechnologie & Systembiologie, TU Kaiserslautern, Paul-Ehrlich Straße 23, D-67663 Kaiserslautern, Germany

<sup>2</sup> Computational Systems Biology, TU Kaiserslautern, Paul-Ehrlich Straße 23, D-67663 Kaiserslautern, Germany

<sup>3</sup> Biologie du Chloroplaste et Perception de la Lumière chez les Microalgues, Institut de Biologie Physico-Chimique, UMR CNRS/UPMC 7141, Paris, France

<sup>4</sup> Zellbiologie/Elektronenmikroskopie, Universität Bayreuth, 95440 Bayreuth, Germany

§ These authors contributed equally to this work

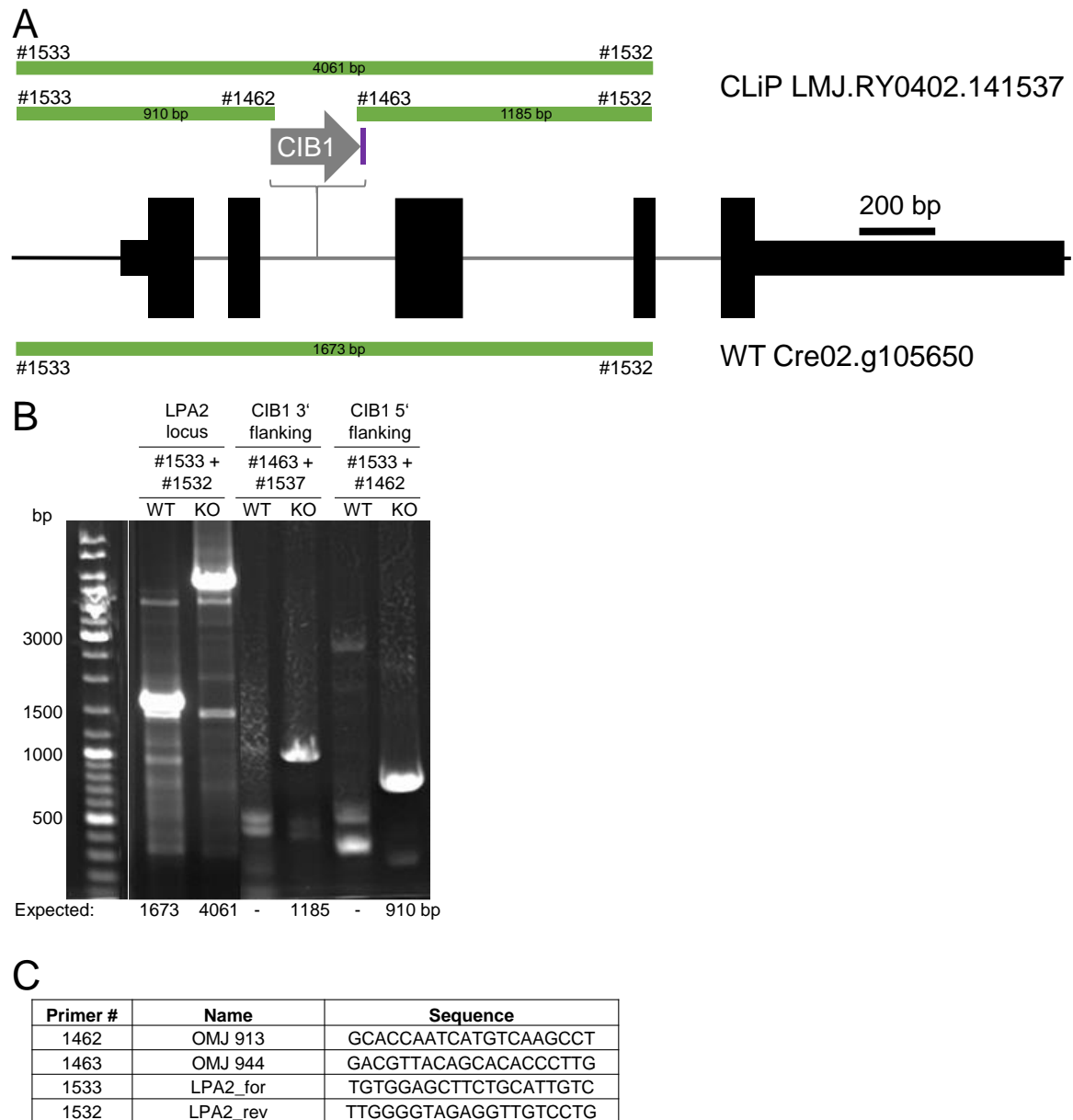
§ These authors contributed equally to this work

¶ Corresponding author : Michael Schroda ([schroda@bio.uni-kl.de](mailto:schroda@bio.uni-kl.de))

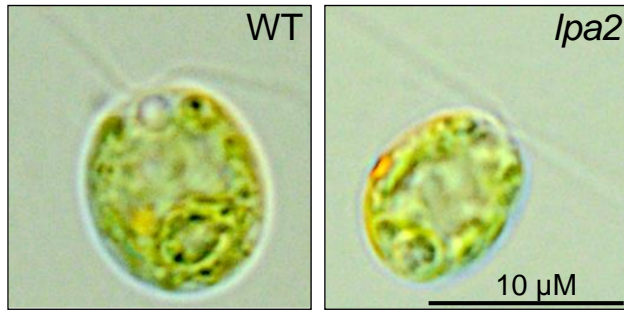
**Supplementary Table S1.** Proteins involved in PSII assembly, repair, or complex dynamics that have clear homologs in *Chlamydomonas* and are present in the complexome profiling dataset. The data are from Lu (2016). Ath – *Arabidopsis thaliana*; Syn – *Synechocystis*; Cre – *Chlamydomonas reinhardtii*.

Locus Ath	Name Ath/ Syn	Name Cre	Locus Cre	Description from Lu (2016) for Ath homolog
At2g47450	cpSRP43	SRP43	Cre04.g231026	Insertion and assembly of PSII proteins such as D1, D2, and CP47, and LHCII subunits
At2g28800	ALB3	ALB3.1	Cre06.g251900	Insertion and assembly of PSII proteins such as D1, D2, and CP47, and LHCII subunits
		ALB3.2	Cre17.g729800	
At2g18710	cpSecY1	SECY1	Cre16.g681900	Insertion and assembly of PSII proteins such as PsbO
At5g28750	Tha4	TATA	Cre10.g438550	Insertion and assembly of PSII proteins such as PsbP and PsbQ
At5g52440	HCF106	TATB	Cre08.g371650	Insertion and assembly of PSII proteins such as PsbP and PsbQ
At2g01110	cpTatC	TATC	Cre03.g145887	Insertion and assembly of PSII proteins such as PsbP and PsbQ
At1g05810	cpRabA5e	RAB11	Cre03.g189250	Transport of PSII proteins such as LHCB1, LHCB3 and CP47 to and from thylakoids
At2g20890	THF1/PSB29	THF1, PSB29	Cre13.g562850	Dynamics of PSII-LHCII supercomplexes
At5g12130	TERC	TERC	Cre17.g712400	Co-translational insertion of PSII proteins such as D1, D2, and CP43
At1g65260	VIPP1	VIPP1	Cre13.g583550	Lipid microdomain
At3g22840	ELIP1	ELIP1	Cre16.g679250	Binding of chlorophyll and/or stability of pigment-binding proteins and complexes during photoinhibition?
At4g14690	ELIP2	ELIP2	Cre09.g393173	Binding of chlorophyll and/or stability of pigment-binding proteins and complexes during photoinhibition?
At4g17600	SEP3.1/LIL3.1	LHL3, LIL3	Cre03.g199535	Anchoring geranylgeranyl reductase to thylakoid membranes; stabilizing LHCII
At1g02910	LPA1/PratA	REP27, LPA1	Cre10.g430150	Biogenesis and assembly of the D1 protein
At1g55480	MET1	TEF30, MET1	Cre01.g031100	Supercomplex formation in PSII repair
At1g75690	LQY1	ZNJ1, LQY1	Cre11.g475850	Disassembly, folding, and/or reassembly of cysteine-containing PSII subunits and complexes and/or D1 synthesis and turnover during PSII repair
At1g77510	PDI6/PDIL1-2	RB60	Cre02.g088200	Regulation of D1 synthesis
At4g35760	LTO1	CPLD41, VKE1, LTO1	Cre12.g493150	Disulfide bond formation in PsbO
At1g54500	RBD1	RBD1	Cre07.g315150	PSII assembly and stability
At3g01480	CYP38/TLP40	CYN38, TLP40	Cre03.g189800	PPIase; Inhibiting dephosphorylation of PSII subunits during PSII repair; conversion of PSII core monomers to PSII supercomplexes
At3g60370	FKBP20-2	FKB20	Cre13.g577850	PPIase; Formation of PSII-LHCII supercomplexes under normal and high light
At5g01920	STN8	STN7	Cre12.g483650	Phosphorylation of D1, D2, CP43, and PsbH
At1g54780	TLP18.3	TEF8, TLP18.3	Cre03.g182150	D1 degradation and PSII dimerization; dephosphorylation of PSII core proteins (e.g., D1 and D2)
At1g50250	FtsH1	FTSH1	Cre12.g485800	Degradation of photodamaged D1
At2g30950	FtsH2/VAR2	FTSH2	Cre17.g720050	Chloroplast biogenesis; thylakoid formation; degradation of photodamaged D1
At3g27925	Deg1	DEG1B	Cre14.g630550	Degradation of plastocyanin and PsbO, and photodamaged D1; integration of newly synthesized PSII subunits such as D1, D2, CP43, and CP47, into PSII complexes
		DEG1C	Cre12.g498500	Stress-induced degradation of LHCB6; a minor protease in in vivo degradation of photodamaged D1
At1g03600	PSB27	CPLD45, PSB27	Cre05.g243800	C-terminal processing of D1 during PSII repair?
At1g05385	PSB27-H2/LPA19	CGL54, LPA19	Cre02.g073850	C-terminal processing during de novo PSII assembly
At5g23120	HCF136 (YCF48)	HCF136, YCF48	Cre06.g273700	Assembly of PSII reaction-center complexes such as RC, RC47a, and RC47b
AtCg00700	PsbN/PBF1	PsbN	cp-encoded	Assembly of PSII minimal reaction-center complexes; regulation of PSII core and antenna protein phosphorylation
At4g28660	PSB28	PSB28	Cre10.g440450	Biogenesis of chlorophyll-binding proteins such as CP47, PsbA, and PsbB
At5g51545	LPA2	LPA2	Cre02.g105650	Synthesis and assembly of CP43
At1g71500	PSB33	TEF5, PSB33	Cre09.g411200	Association of LHCII with PSII

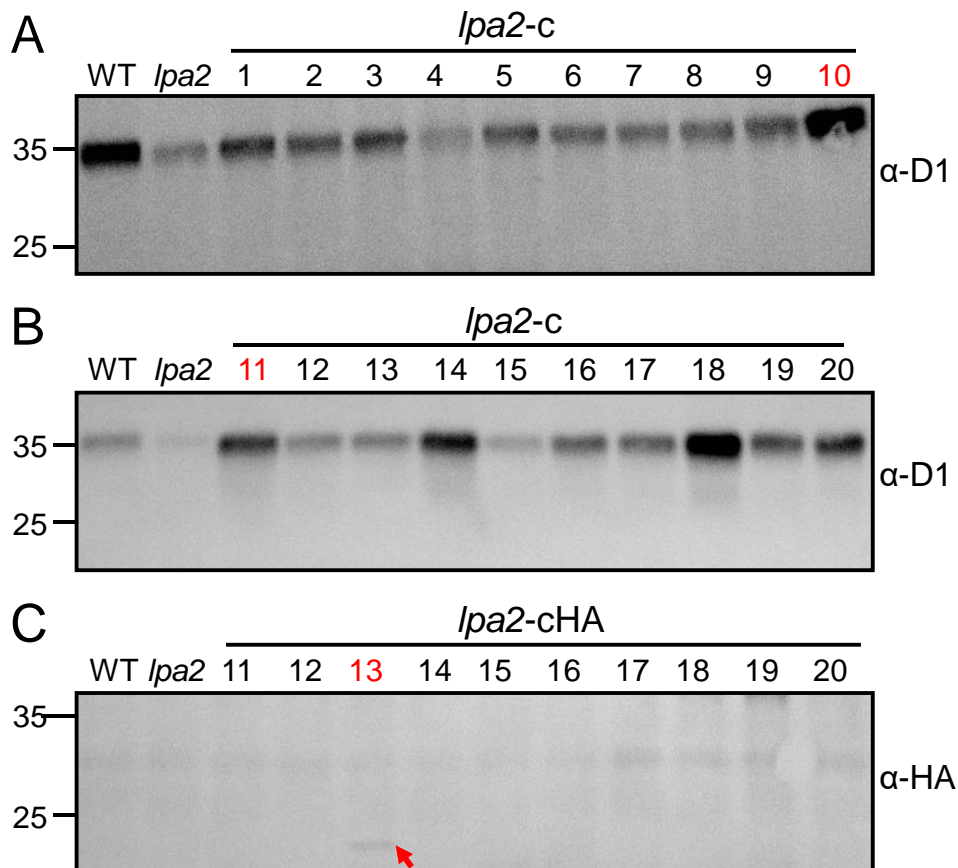
## SUPPLEMENTARY FIGURES AND FIGURE LEGENDS



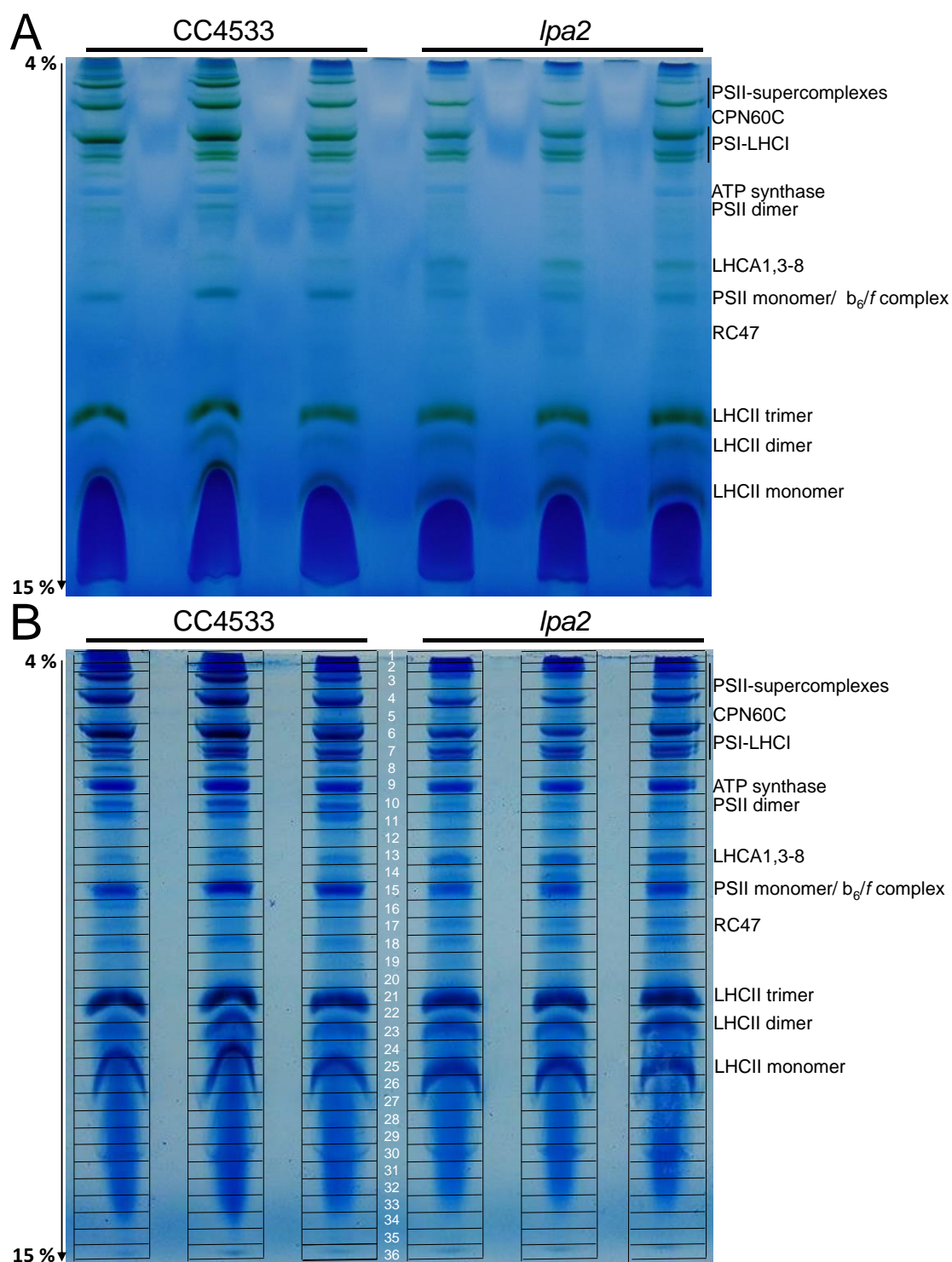
**Supplementary Fig. S1.** Analysis of the CIB1 integration site by PCR. (A) Gene model of the *LPA2* gene with exons shown as black boxes and introns as thin grey lines. The integration site of the CIB1 cassette in the second intron is shown. The purple box indicates a 165-bp fragment derived from the 19<sup>th</sup> intron of gene Cre13.g573450 in reverse orientation that has integrated together with the CIB1 cassette. Green bars indicate the expected PCR products on mutant DNA (CLiP, top) and wild-type DNA (WT, bottom). Numbers in the bars give their sizes and flanking numbers the primer number as provided in the table. (B) PCR products on genomic DNA from wild type (WT) and the *lpa2* mutant from the CLiP collection (KO) were separated on an agarose gel and stained with Gel Red. Expected amplicon sizes are indicated below the gel. (C) Table with primers used.



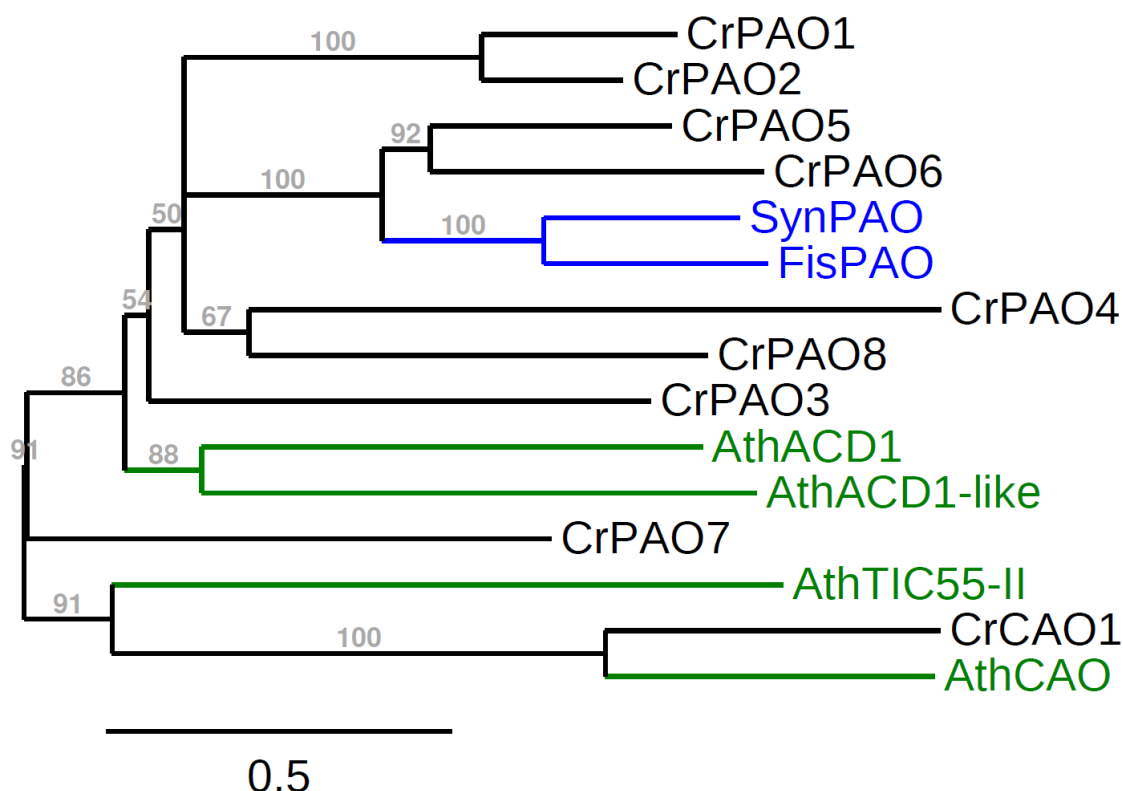
**Supplementary Fig. S2.** Light microscopy images of wild type and *lpa2* mutant cells. Cells were grown in low light ( $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ).



**Supplementary Fig. S3.** Screening of transformants expressing LPA2 with (*lpa2-cHA*) or without (*lpa2-c*) a 3xHA tag. 10  $\mu\text{g}$  whole-cell protein of each transformant were separated by SDS-PAGE and analyzed by immunoblotting using antibodies against the D1 protein (A and B) or the HA epitope (C). The transformant number is given on top of the panels. The number in red indicates the transformant chosen for further analysis. The red arrow points to the weak signal obtained for LPA2-3xHA. Note that the signals for WT and *lpa2* mutant in panel (B) are too weak, probably because of a blotting problem at the gel margin.



**Supplementary Fig. S4.** BN-PAGE for complexome profiling. Thylakoid membranes were isolated from wild type (CC4533) and *lpa2* mutant cells, solubilized with n-Dodecyl  $\alpha$ -D-maltoside, and separated on a 4% to 15% BN gel. 60  $\mu$ g of protein were loaded per lane. (A) Photography of the gel right after the run. (B) Coomassie staining of the gel shown in (A). Each lane was cut into 36 slices according to the grid shown and gel slices were subjected to tryptic in-gel digestion followed by LC-MS/MS. The identity of the indicated complexes derives from the mass spectrometry analysis (Table S1).



**Supplementary Fig. S5.** Phylogenetic tree of pheophorbide a oxygenases. Sequences are from *Chlamydomonas reinhardtii* (Cr), *Arabidopsis thaliana* (Ath) and the closest homologs to CrPAO5 from *Synechococcus* sp. PCC 7335 (Syn) and *Fischerella* sp. PCC 9431 (Fis). Support for the branches is given in bootstrap values based on 1,000 NJ bootstrap replicates. The sequences are documented in Supplemental Table S2. Accession numbers: CrPAO1: Cre17.g724700; CrPAO2: Cre17.g724600; CrPAO3: Cre10.g450550; CrPAO4: Cre03.g173450; CrPAO5: Cre06.g278245; CrPAO6: Cre06.g305650; CrPAO7: Cre11.g476500; CrPAO8: Cre13.g583050; CrCAO1: Cre01.g043350; SynPAO: EDX87484; FisPAO: WP\_155959184; AthACD1: AT3G44880; AthACD1-like: AT4G25650; AthTIC55-II: AT2G24820; AthCAO: AT1G44446.

## SUPPLEMENTARY REFERENCES

**Lu Y.** 2016. Identification and roles of photosystem II assembly, stability, and repair factors in *Arabidopsis*. *Front Plant Sci* 7, 168.