

### Supplemental Figure 1. Characteristics of the *lpa1-2* mutant.

Because the original *lpa1* mutant (Peng et al., 2006) could not be obtained, a new mutant allele, *lpa1-2*, was identified in the Cold Spring Harbor collection and used for the analysis.

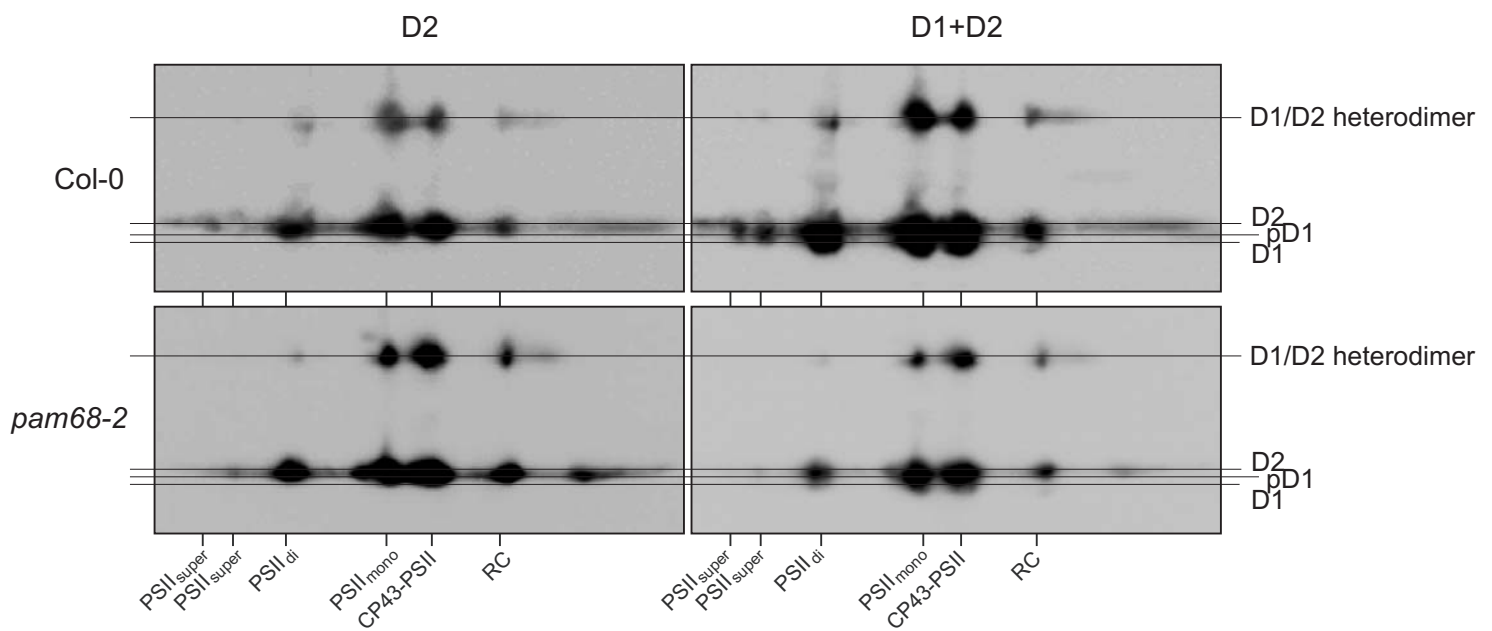
**(A)** T-DNA tagging of *LPA1*. Exons are numbered and shown as white boxes, introns as black lines. Sites and orientations of T-DNA insertions are indicated. The *lpa1-2* line corresponds to CSHL\_ET6851 in the Cold Spring Harbor Gene trap collection.

**(B)** Effect of gene trap insertion on the accumulation of *LPA1* transcript. RT-PCR was performed with primers specific for the *LPA1* coding region located 5' to the gene-trap insertion and with *ACTIN1*-specific primers as control.

**(C)** Five-week-old WT (*Ler*) and mutant (*lpa1-2*) plants grown in the greenhouse (upper panel) were analyzed for minimum Chl *a* fluorescence ( $F_0$ ) and maximum quantum yield of PSII ( $F_v/F_m$ ) as described in the Methods section. Signal intensities for  $F_0$  and  $F_v/F_m$  are indicated in accordance with the color scale at the bottom of the figure.

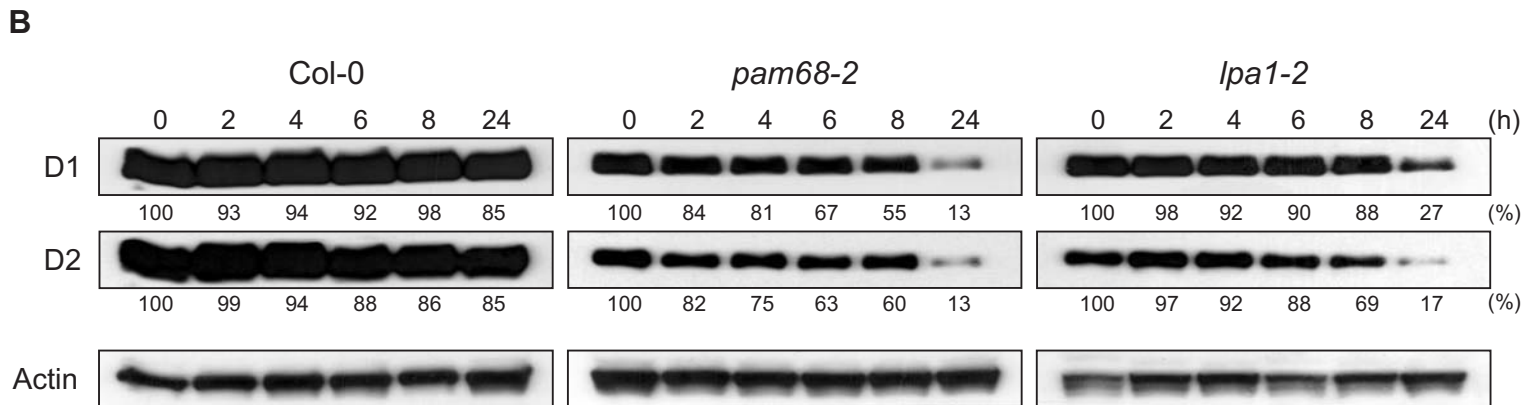
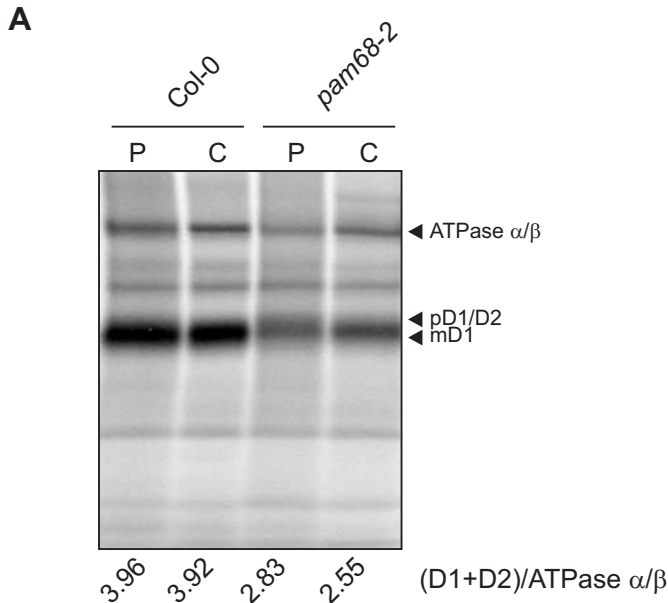
**(D)** Chl *a* fluorescence induction curves for WT (*Ler*) and mutant (*lpa1-2*) leaves. The white bar indicates exposure to actinic light ( $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), the lightning symbols the application of saturation light pulses (0.8 s;  $5000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  white light). Compared to *pam68-2*, *lpa1-1* and *lpa1-2* mutants display a less pronounced reduction in  $F_v/F_m$  (*lpa1-1*, 0.53; *Col-0*, 0.82 [Peng et al., 2006]; *lpa1-2*:  $0.56 \pm 0.01$ ; *Ler*,  $0.83 \pm 0.01$ ).

**(E)** Detection of PSII assembly complexes in *Ler* and *lpa1-2* plants. Immunoblot analyses were performed as in Figure 4C with antibodies against D1, D2, PsbE, PsbI, CP47 and CP43. The positions of PSII assembly complexes and free proteins (f.p.) are indicated as in Figure 4C. Signals were obtained by chemiluminescence. Exposure times were  $\sim 2$  min (WT) and  $\sim 10$  min (mutant).



**Supplemental Figure 2. Discrimination of signals for D1 and D2 proteins.**

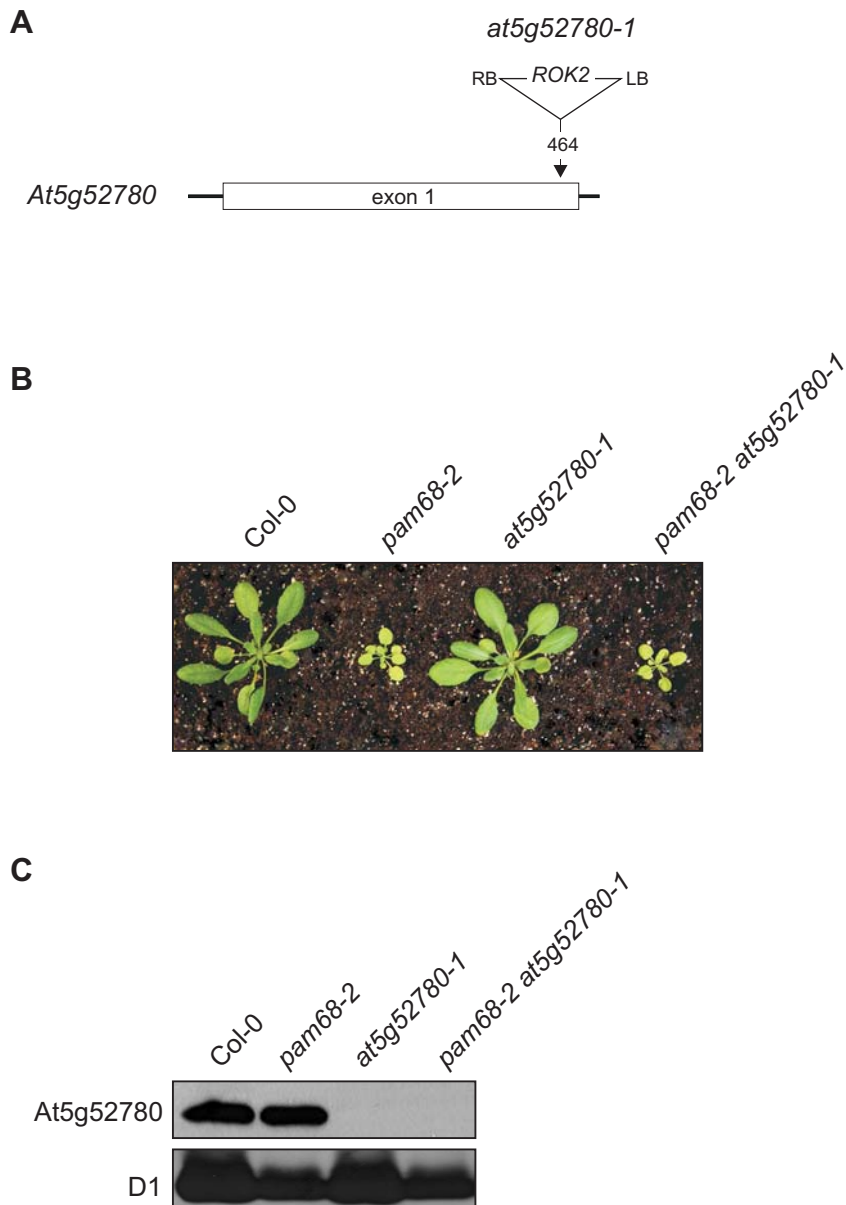
Thylakoid proteins were fractionated by 2D BN/SDS PAGE, and blots were probed with either an antibody raised against D2 or with the same antibody in combination with an antibody raised against D1. Signals were aligned according to the position of the D1/D2 heterodimer.



### Supplemental Figure 3. Stability of the D1 protein.

**(A)** Incorporation of [ $^{35}\text{S}$ ]methionine into thylakoid membrane proteins of 4-week-old WT (Col-0) and mutant (*pam68-2*) plants. A 20-min pulse (P) was followed by 30 min chase (C) with cold methionine as in Figure 5. Then, thylakoid membranes were isolated, separated by SDS-PAGE, and visualized autoradiographically. Signal intensities for ATPase  $\alpha/\beta$  and D1 + D2 were quantified and the ratio (D1 + D2)/ATPase  $\alpha/\beta$  calculated.

**(B)** *Arabidopsis* leaves were illuminated in the presence of 100  $\mu\text{g/ml}$  lincomycin with 120  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  for the times indicated. Total proteins were isolated, and the contents of D1, D2 and Actin as loading control were determined by immunoblot analysis. Signals were detected and quantified as described in the Methods section. The percentages of protein levels shown below the lanes were calculated relative to those of samples taken at time 0. Note that *Ler* behaved identical to Col-0.

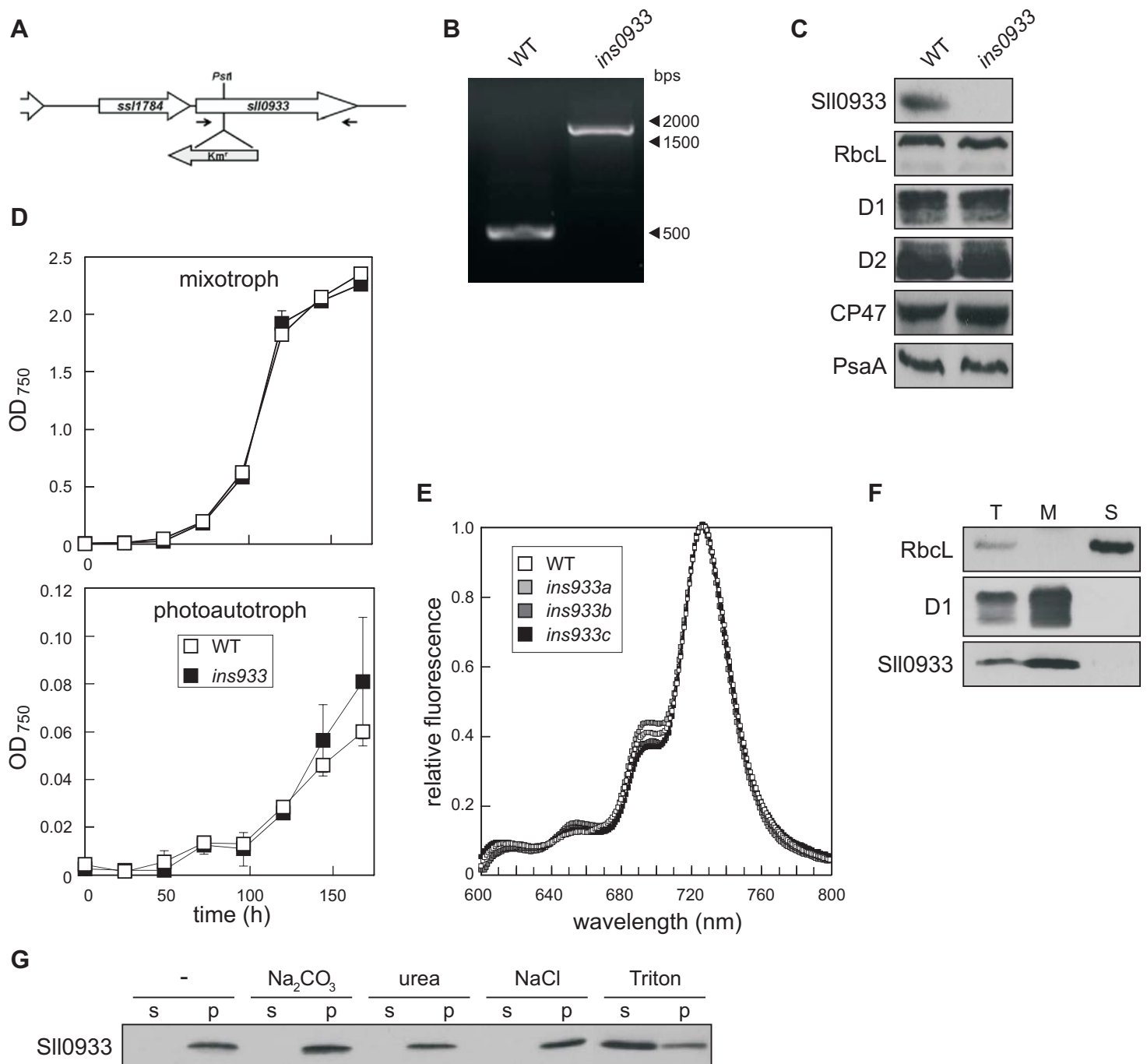


**Supplemental Figure 4. Characterization of *at5g52780-1* mutants and the double mutant *pam68-2 at5g52780-1*.**

**(A)** T-DNA tagging of *At5g52780*. The exon is shown as a white box, 5' and 3' regions as black lines. Sites and orientations of T-DNA insertions are depicted. The *at5g52780-1* line corresponds to SALK\_143426 from the SALK T-DNA collection.

**(B)** Five-week-old WT (Col-0) and mutant (*pam68-2*, *at5g52780-1*, and *pam68-2 at5g52780-1*) plants grown in the greenhouse.

**(C)** Immunodetection of D1 and *At5g52780* in WT (Col-0), single (*pam68-2* and *at5g52780-1*) and double (*pam68-2 at5g52780-1*) mutant plants. In each case, thylakoid proteins corresponding to 10  $\mu$ g Chl were fractionated by SDS-PAGE and visualized with antibodies against *At5g52780* and D1.



**Supplemental Figure 5. *Synechocystis* SII0933: generation and characterization of knock-out lines (*ins0933*) and subcellular localization and topology.**

(A) Schematic representation of the construct used for inactivation of *sII0933*. *Km<sup>r</sup>*, kanamycin-resistance cassette. Open arrows indicate directions of transcription; solid arrows designate binding sites of primers used for PCR-based segregation analysis (B).

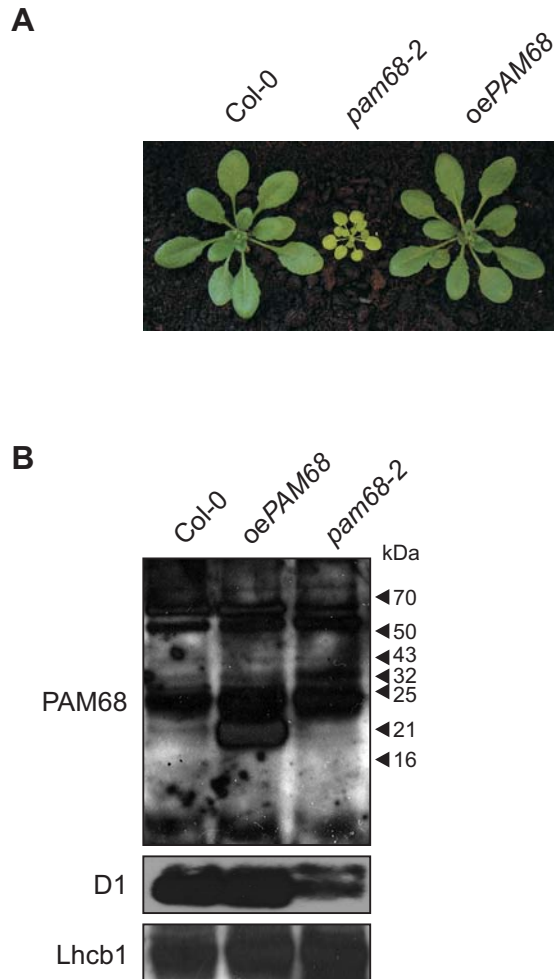
(C) Accumulation of photosynthetic proteins in *ins0933*. Total cellular proteins from *Synechocystis* WT and *ins0933* were isolated as previously reported (Schottkowski et al., 2009b), separated by SDS-PAGE and blots were probed with the indicated antibodies.

(D) Growth behavior of *ins0933*. WT (white rectangles) and *ins0933* (black rectangles) cells were grown under mixotrophic or photoautotrophic conditions in BG11 medium with or without glucose, respectively. Error bars represent the standard deviation of the mean ( $n = 3$ ).

(E) Low temperature fluorescence emission spectra of *Synechocystis* cells. 77-K fluorescence emission spectra of whole cells of WT and three independent *ins0933* transformants (a, b, c) were measured after chlorophyll excitation at 435 nm as described previously (Klinkert et al., 2004). Spectra were normalized to the maxima at 725 nm.

(F) SII0933 is a membrane protein. Total (T), membrane (M) and soluble (S) protein fractions of *Synechocystis* WT cells were prepared according to Schottkowski et al. (2009b) and immunodecorated with the indicated antibodies.

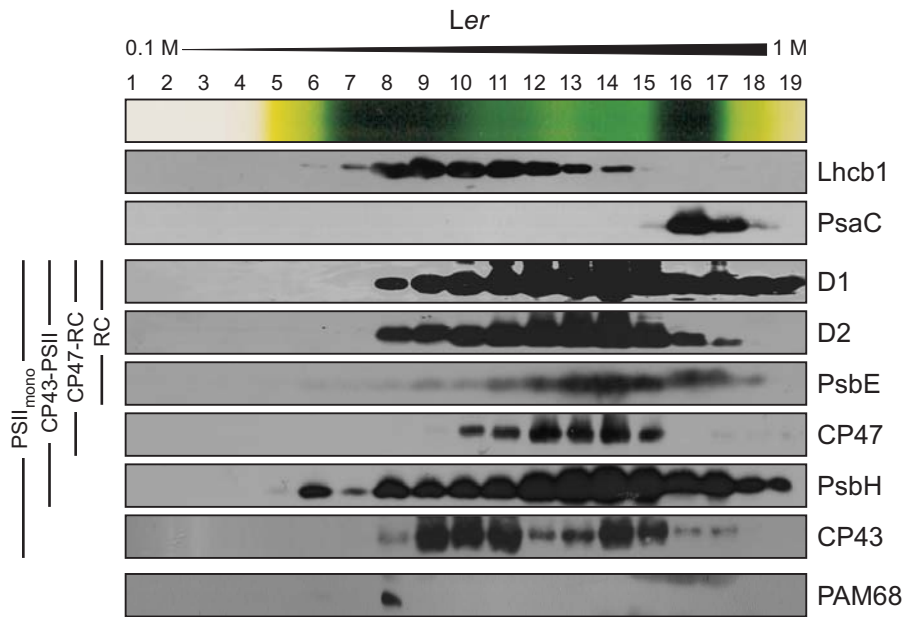
(G) Extraction of membrane proteins. Membrane fractions were treated with the indicated chemicals as described (Schottkowski et al., 2009b) and separated into membrane (p) and soluble (s) fractions by centrifugation.



**Supplemental Figure 6. Overexpression of *PAM68* complements the *pam68-2* phenotype.**

**(A)** Four-week-old WT (Col-0), *pam68-2* and *35S:PAM68 pam68-2* (*oePAM68*) plants grown in the greenhouse.

**(B)** Thylakoid proteins corresponding to 20  $\mu$ g of total Chl from WT (Col-0), *pam68-2* and *oePAM68* plants were fractionated by SDS-PAGE, and visualized with antibodies against PAM68 and D1. Lhcb1 served as loading control. Note that because *pam68-2* plants contain only 65% of WT Chl *a + b* levels, the *pam68-2* sample contained about 50% more protein. The position of molecular weight markers is indicated by arrows; the PAM68 band runs at ~21 kDa.



**Supplemental Figure 7. Analysis of PAM68 complex formation.**

Thylakoids from *Ler* were fractionated and analyzed with the same antibodies as in Figure 9.

**Supplemental Table 1 online.** Relative transcript levels of PSII assembly factors, as well as DEG1 and VAR2, under different light conditions.

Light quality	<i>LPA1</i>	<i>PAM68</i>	<i>HCF136</i>	<i>ALB3</i>	<i>DEG1</i>	<i>VAR2</i>
Far-red	0.1 / 1.07	0.17 / 1.13	0.85 / 1.8	-0.01 / 0.99	1.03 / 2.04	1.49 / 2.81
White	0.12 / 1.09	0.23 / 1.17	0.49 / 1.41	-0.1 / 0.93	0.97 / 1.96	1.15 / 2.21
Blue	0.24 / 1.18	0.17 / 1.13	0.48 / 1.4	-0.14 / 0.91	0.92 / 1.89	1.27 / 2.42
Red	0.22 / 1.16	0.15 / 1.11	0.28 / 1.21	0.02 / 1.02	0.84 / 1.79	0.81 / 1.76
UV-AB	-0.01 / 0.99	0.35 / 1.27	0.28 / 1.21	-0.32 / 0.8	0.79 / 1.73	1.16 / 2.24
UV-A	0.15 / 1.11	0.23 / 1.17	0.15 / 1.11	-0.07 / 0.95	0.71 / 1.63	0.88 / 1.84

Transcript level data were obtained from Genevestigator (TAIR-Accession: ExpressionSet 1007966126) and are provided relative to dark ( $\log_2$ /linear).



**Supplemental Table 2 online.** Primers used in this study.

Gene	Forward primer	Reverse primer	Application
<i>ACTIN1</i>	TGCGACAATGGAAGTGGAAATG	GGATAGCATGTGGAAGTGCATACC	RBA
<i>psbA</i>	TCCGGTGCCATTATTCCTAC	CTTCTTCTTGCCCGAATCTG	RBA
<i>psbB</i>	CGGGTCTTTGGAGTTACGAA	TCCAGCAACAACAAAAGCTG	RBA
<i>psbCD</i>	ATGGATGACTGGTTACGGAG	CGAAATATAGCCGCTACACC	RBA
<i>psbC</i>	TAGCTTTAGCTGGTCGTGAC	GGTATCATATACGCCCCCAA	RBA
<i>psbEFJL</i>	ATGTCTGGAAGCACAGGAGAAC	CTAAAAAGATCTACTAAATTCATC	RBA
<i>PAM68</i>	GGCTTCTGTACCATGTTCCCTT	ACACCAGCTAAAGCCGTACC	RBA
<i>PAM68</i>	<u>GGGACAAGTTTGTACAAAAAAGCA</u> <u>GGCTTGATGGCTTCTGTACCATGTT</u> CC	<u>GGGACCACCTTTGTACAAGAAAGC</u> <u>TGGGTCCTATCTCTTGTCTGAGGA</u> ATTC	Over-expression in planta
<i>PAM68</i>	36: CGTCTAGACAAAAATGGATAAAACG AAGATCAAG	214: CGCCATGGATTCTCTTGTCTGAGG AATTCCA	Split Ubiquitin Cub
	122: CGTCTAGACAAAAATGGGATTTACA GTGGGGT	146: CGCCATGGATTTGAGATAGTAAA AGAATGGGA	
<i>LPA1</i>	ATGGATCCGATGCTCTTGTTCAAGTT GA	GCGAATTCGCTCATCTTTCTAACTT GCTGAGA	Split Ubiquitin Nub
<i>LPA2</i>	CGCCCGGGTATCAAAGAATTCAAGC TCTTCC	ATCTCGAGTCACTCTTGACCCTTCA TTTTCC	Split Ubiquitin Nub
<i>HCF136</i>	ATCCCGGGTAGATGAACAGTTATCC GAATG	ATCTCGAGTCAGCCAACATATCGG AGCAA	Split Ubiquitin Nub
<i>PsbB</i>	ATCCCGGGTAGGTTTGCCTTGGTAT CGTGT	ATCTCGAGTCAGACTGCTTGTGCGT TTTGTA	Split Ubiquitin Nub
<i>PsbA</i>	ATGGATCCACTGCAATTTTAGAGAG ACGC	GCGAATTCGCTTATCCATTTGTAGA TGGAGCC	Split Ubiquitin Nub
<i>PsbD</i>	ATGGATCCACTATAGCCCTTGGTAA ATTTAC	GCGAATTCGCTTAAAGAGCGTTTC CACGTGG	Split Ubiquitin Nub
<i>PsbC</i>	ATGGATCCAAAACCTTATATTCCCTG AGGAG	GCGAATTCGCTTAGTTAAGAGGAG TCATGGAA	Split Ubiquitin Nub
<i>PsbE</i>	ATCCCGGGTATCTGGAAGCACAGGA	ATCTCGAGCTAAAAAGATCTACTAA	Split

	GAACG	ATTCATC	Ubiquitin Nub
<i>PsbI</i>	<u>GCGGATCC</u> ATGACTATAGATAGGAC CTA	<u>GCGAATTC</u> TTATCGTTGGATGAACT GCA	Split Ubiquitin Nub
<i>PsbF</i>	<u>GCGGATCC</u> ATGCTTACTCTCAAAC TTT	<u>GCGAATTC</u> TTATTCTTCACGTCCC	Split Ubiquitin Nub
<i>PsbH</i>	<u>AGGGATCC</u> TCCATGAGCACTACTGT AGGGAAGTTATTGA	<u>GACCCGGG</u> CCTAATTCACTGAAAT TCCATC	Split Ubiquitin Nub
<i>PsbO</i>	<u>CACCCGGG</u> CTATGGAGGGAGCTCC AAAGAGAT	<u>GAGAATTC</u> AGATCACTCAAGTTGA CCATACC	Split Ubiquitin Nub
<i>PAM68</i>	GGCTTCTGTACCATGTTCTT	CTATCTCTTGTCTGAGGAATTC	Genotyping
<i>At5g52780</i>	CATTAGAAGAAGAAATAGAAATGAG	AGCCAGCTTAAAAGTTTTTATGAG	Genotyping
<i>LPA1/ At1g02910</i>	GTGAAAGATGCTCTTGTTCACT	GCTGCACAAGTTCAACAACGC	Genotyping

Primer sequences are provided in 5' to 3' orientation. Restriction enzyme or homologous recombination sites used to insert the cloned cDNA into the respective vectors are underlined. RBA, RNA blot analysis.