

Supplemental Figure 1. Complementation of pam71-1 and pam71-2

- A. Four-week-old complemented lines ($pam71-1_{Pro35S:PAM71}$, $pam71-2_{Pro35S:PAM71}$) were grown on a 12h/12h dark/light cycle at 21°C/18°C and at 90 µmol photons m⁻² s⁻¹, and the minimal chlorophyll *a* fluorescence (F₀) (middle panel) and maximum quantum yield of PSII (F_V/F_M) (right panel) were recorded. The color scale at the bottom indicates the signal intensities.
- B. Chlorophyll *a* fluorescence induction curves (top panel) and P₇₀₀ redox kinetics (bottom panel) were recorded from leaves of the two genotypes. F_V/F_M values are indicated. For chlorophyll *a* fluorescence measurement, the minimal level of fluorescence of dark-adapted leaves is indicated by dashed lines. Saturating pulses (SP) and actinic light (AL, 100 photons m⁻² s⁻¹) were applied as indicated. For P₇₀₀ absorption measurements, leaves were first illuminated with actinic light (AL, 100 µmol photons m⁻² s⁻¹) for 4 min and absorbance changes (at 830 nm) induced by exposure to far-red light (FR, 720 nm) were recorded. Mean values (± SD) are each based on five individual plants.



Supplemental Figure 2. Characterization of *psbo1 psbo2* and *psad1-1* with respect to chlorophyll *a* fluorescence parameters, thylakoid protein complex accumulation and ultrastructure, and oxygen evolution

- A. Chlorophyll *a* fluorescence induction curves (top panel) and P₇₀₀ redox kinetics (bottom panel) were recorded from leaves of the indicated genotypes (WT, *psad1-1*, *psbo1 psbo2*). F_v/F_M values are indicated for each genotype. For chlorophyll *a* fluorescence measurement, the minimal level of fluorescence of dark-adapted leaves is indicated by dashed lines. Application of saturating pulses (SP) and actinic light (AL, 100 µmol photons m⁻² s⁻¹) are indicated. For P700 absorption measurements leaves were first illuminated with actinic light (AL, 100 µmol photons m⁻² s⁻¹) for 4 min and absorbance changes at 830 nm induced by exposure to far-red light (FR, 720 nm) were recorded. Mean values ((± SD) are each based on five individual plants.
- B. Accumulation of thylakoid membrane complexes in wild type (WT), *psad1-1* and *psbo1 psbo2* lines. Thylakoid membranes (25 μg of chlorophyll) were solubilized with 1% [w/v] β-dodecyl maltoside, and protein complexes were fractionated by Blue-native gel electrophoresis. The positions of protein complexes (trimeric LHCII = LHCII_{trimer}, CP43-free PSII complexes = PSII-CP43, PSII monomers = PSII_{mono}, PSII dimers = PSIIdi, PSI monomers = PSII_{mono}, PSII supercomplexes = PSII_{super} and PSI-NDH) are indicated.
- C. Electron micrographs of WT, *psad1-1* and *psbo1 psbo2* leaf sections are shown and starch (St), grana thylakoids (GT) and stroma thylakoids (ST) are indicated.
- D. Oxygen evolution [nmol O₂ mL⁻¹] of WT, *psad1-1 and psbo1 psbo2* was measured over the indicated time in isolated thylakoids adjusted to 30 μg Chl mL⁻¹ and using DCBQ as external electron acceptor (left panel). Reactions were started with the application of actinic light at 500 μmol photons m⁻² s⁻¹. Oxygen evolution rate [μmol O₂ mg Chl⁻¹ h⁻¹] was calculated from two independent thylakoid preparations including three replicates (±SD) (right panel).



Supplemental Figure 3. Analysis of the CP43 subunit in *pam71-1*

- A. Immunodetection of the thylakoid protein CP43 in WT, pam71-1, pam71-2 and pam71-1_{Pro35S:PAM71-GFP}. Samples equivalent to 15 μg protein were loaded in all lanes, except in those marked WT ½, where only half as much was loaded. As loading control, LHCII was visualized by Coomassie blue (C.B.B.).
- B. Thylakoid membrane fractionation of WT and *pam71-1*. Thylakoids (1 mg Chl mL⁻¹) were solubilized with 1% [w/v] β-dodecyl maltoside, and complexes were separated in a linear 0.1 to 1 M sucrose gradient. Nineteen fractions were collected, proteins from each fraction were precipitated, and immunoblot analysis was performed using an anti-CP43 antibody. The positions of molecular mass markers and of the major complexes (LHCII, PSII, PSI) are indicated.



Supplemental Figure 4. Transcription and translation of chloroplast-encoded proteins

- A. Transcript analysis in WT and *pam71-1*. Fifteen μg of total leaf RNA was subjected to RNA gel blot analysis and hybridized with probes specific for *psbA*, *psbB*, *psbC* and *rbcL*. As loading control, blots were stained with methylene blue (M.B).
- B. Association of *psbA*, *psbC* and *rbcL* mRNAs with polysomes. Extracts of WT and *pam71-1* leaves were fractionated in a sucrose gradient and RNA was isolated from 11 fractions. As loading control, blots were stained with methylene blue (M.B).
- C. Incorporation of [³⁵S]Met into thylakoid membrane proteins of 4-week old plants. Pulse-labelling was performed for 20 min in the presence of cycloheximide. WT samples corresponding to 100,000 cpm were loaded in lanes marked 1 and the corresponding dilutions in lanes marked ½ and 1/4.



Supplemental Figure 5. Fast chlorophyll a fluorescence kinetics

Fast chlorophyll *a* fluorescence kinetics (*O-K-J-I-P*) of leaves of WT, *psbo1 psbo2* and *pam71-1* which were dark-adapted for 10 min. Development of the *K* peak after approximately 300 μ s is observed in *psbo1 psbo2* and *pam71-1*. The occurrence of the *K* peak is generally supposed to be an indicator of damage to the OEC (Bukhov et al., 2004). Maximum fluorescence was normalized to one in each sample.



Supplemental Figure 6. Co-immunoprecipitation analysis using *pam71-*1_{Pro35S:PAM71-GFP}

Thylakoid membrane proteins were solubilized and incubated with anti-GFP antibody. The precipitated proteins were separated and analyzed by immunodetection using anti-PsbO and anti-PsbP antibodies. Immunodetection using anti-GFP served as the positive control, and thylakoid membrane proteins isolated from wild-type (WT) served as the negative control. Cp, total chloroplast fraction; Co-IP, co-immunoprecipitated proteins; S.n. supernatant.



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Supplemental Figure 7. Phylogenetic analysis

The phylogenetic tree was constructed using the program MEGA (version 6) (Tamura, 2013). The evolutionary history was inferred by using the maximum-likelihood method based on the General Reverse Transcriptase + Freq. model (Dimmic et al., 2002). The tree with the highest log likelihood (-3694,0511) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The scale bar is an indicator for the evolutionary distance in substitutions per site. The analysis involved 32 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 104 positions in the final dataset. Bootstrap values (based on 1000 iterations) are shown for corresponding nodes, e.g. the percentage of trees in which the associated taxa clustered together. The analysis involved 32 amino Arabidopsis thaliana-1 acid sequences (Accession numbers: NP 564825.1 (At1q64150/PAM71), NP 001241025.1; vinifera-1 Glycine max-1 Vitis XP 002272191.1; Oryza sativa-1 EEC70197.1; Brachypodium distachyon-1 XP 003565685.1; XP_008655787.1; Zea mays-1 Physcomitrella patens-1 XP 001756332.1; Chlamydomonas reinhardtii-1 XP_001701915.1/XM_001701863 (CGLD1); Volvox carteri-1 XP_002953583.1; Galdieria sulphuraria EME31602.1; Arabidopsis thaliana-2 NP_193095.2 (At4g13590); Oryza sativa-2 NP_001068053.1; Glycine max-2 XP_003535962.1; Vitis vinifera-2 XP 002285620.1; Zea mays-2 ACG35639.1: Brachypodium distachyon-2 XP 003570533.1: Physcomitrella patens-2 XP 001770923.1; Chlamydomonas reinhardtii-2 XP_001697928.1; Volvox carteri-2 XP 002949806.1; Synechocystis Thermosynechococcus sp. WP 010873575.1; elongatus NP_681169.1; Anabaena sp. WP_016950136.1; Homo sapiens NP 060945.2 (TMEM165); Mus musculus AAI41082.1; Drosophila melanogaster Caenorhabditis elegans NP_497567.1; Saccharomyces cerevisiae ACR20070.1; AHY74663.1 (GDT1); Neurospora crassa CAE76363.1; Aspergillus nidulans Geobacter sulfurreducens NP 951914.1; Mycobacterium XP 662418.1; vaccae EJZ12059.1; Thermomonospora curvata WP_012855029.1.



Supplemental Figure 8. Proton motive force size and composition

- A. ECS [515-550 nm Δ I/I 10³] was determined after actinic light (AL) was switched off. Before AL was switched off, leaves were 2 min illuminated at 90 µmol photons m⁻² s⁻¹. Both components of PMF ($\Delta \psi$ and ΔpH) are indicated.
- B. Magnitude of the PMF [ECS_t]. Leaves from plants that were dark-adapted overnight were illuminated for 2 min at increasing light intensities. The size of the proton motive force was determined. The data are means \pm SEM (n=9).



Supplemental Figure 9. Light-dependent accumulation of ⁴⁵Ca²⁺ across thylakoid membranes of pea and Arabidopsis

⁴⁵Ca²⁺ uptake [cpm] in intact chloroplasts. Chlroplasts were isolated, incubated for 10 min on ice in 10 mM HEPES/NaOH [pH 7.6] containing 5 mM MgCl₂, and vortexed to release thylakoids. Reactions were initiated by adding ⁴⁵Ca²⁺ (1.5 μM final concentration) to thylakoid membranes adjusted to 0.3 mg Chl mL⁻¹ and illuminating the samples at 90 μmol photons m⁻² s⁻¹. After the indicated times, the reactions were stopped by adding 50 mM EGTA. A value of 10⁶ cpm corresponds to 0.02 μl ⁴⁵Ca²⁺. Values represent three experimental replicates (±SD). Similar results were obtained in an independent experiment.



Supplemental Figure 10. Cation partitioning in pam71-2 and pam71-1_{Pro35S:PAM71-GFP}

⁴⁵Ca²⁺ and ⁵⁴Mn²⁺ uptake was performed as described in Figure 7 legend and Methods. ⁴⁵Ca²⁺ and ⁵⁴Mn²⁺ partitioning between stroma (Str) and thylakoid (Thy) in *pam*71-2 and *pam*71-1_{Pro35S:PAM71-GFP} chloroplasts [%] is shown. Values are deduced from samples that were incubated for 30 min at 90 µmol photons m⁻² s⁻¹.



Supplemental Figure 11. Calcium deprivation does not restore photosynthesis in *pam71*

- A. Seeds of WT, *pam71-1*, *pam71-2* and *pam71-1*_{Pro35S:PAM71-GFP} were surface sterilized and grown for 2 weeks on MS medium containing either 1x CaCl₂ (0.332 g L⁻¹) and 1x MnSO₄ (0.017 g L⁻¹), or 0.1x CaCl₂ (0.0332 g L⁻¹) and 1 x MnSO₄. At the end of the growth period, the maximum quantum yield of PSII (F_V/F_M) was recorded.
- B. F_V/F_M values are given for WT, pam71-1, psbo1 psbo2 pam71-2 and pam71-1_{Pro35S:PAM71-GFP} grown on the indicated MS medium. Bars represent mean values (±SD) of three experiments, each including five individual plants. Asterisks indicate statistical significance (t-test, p<0.001) of differences between WT and mutant plants.</p>

PAR	WT	pam71-1
0	0	0
37	0.22 ± 0.04	0.53 ± 0.08
53	0.33 ± 0.08	0.97 ± 0.15
84	0.48 ± 0.14	1.10 ± 0.16
126	0.70 ± 0.15	1.18 ± 0.16
157	0.84 ± 0.14	1.20 ± 0.15
247	1.10 ± 0.14	1.25 ± 0.15
370	1.24 ± 0.21	1.28 ± 0.14
562	1.30 ± 0.24	1.32 ± 0.14
856	1.34 ± 0.24	1.35 ± 0.14
Leaves of five	المعدد معتمد متعام المام بالمعاد	

Supplemental Table 1. Non photochemical quenching under transient illumination in WT and *pam71-1* plants.

Leaves of five week old plants were used and NPQ was determined in the range 37-856 µmol photons m⁻² s⁻¹ photosynthetically active radiation (PAR). The plants were dark-adapted for 30 min prior to measurement. NPQ was recorded following illumination for 30 s at the appropriate PAR. The NPQ parameter was calculated according to the equation: NPQ = $(F_M - F_M)/F_M$ (where F_M and F_M represents the maximal fluorescence of dark adapted or illuminated samples). Values are means (±SD) from 8 individual plants.

Subcellular locali- sation of proteins:	Arabidopsis thaliana	Vitis vinifera	Zea mays	Oryza sativa	Brachypodium distachyon
Chloroplast	At1g64150 (PAM71) [1]	Vv_02s0025g00720* [1]	Zm2g464891 [1]	Os01g12220 [1]	Bradi2g07266 [1]
	At4g13590 (PAM71-HL) [2]	Vv_05s0051g00440* [2]	Zm2g097674* [2]	Os11g34180 [2]	Bradi3g57430* [2]
Secretory pathway	At1g25520	Vv_01s0011g03200	Zm2g095552	Os08g33630	Bradi3g41030
	At1g68650	Vv_04s0023g00380*	Zm2g151863	Os08g41670	Bradi3g36630
	At5g36290		Zm2g160560	Os11g28300	Bradi4g19090
			Zm5g872373		Bradi5g03610

Supplemental Table 2. Homologous PAM71 genes/proteins found in plants

Data were extracted from the Aramemnon database (<u>http://aramemnon.botanik.uni-koeln.de/</u>) and gene numbers given accordingly, except for *Vitis vinifera*, where gene numbering is according to UniProtKB (<u>http://www.uniprot.org/uniprot/</u>). Numbers in squared brackets correspond to numbers given in the phylogenetic tree (Supplemental Figure 7).*A subcellular localisation to mitochondria is additionally predicted.

Supplemental Table 3. Primers used in this study

Primer name	Sequence (5'-3')	Application	
1g64150-F1	AAT TCT CCT GAA ACT TGG AAG ACA		
1g64150-R1	TTC CGT CTT TAG AAC ACA CTC TCT	Arabidopsis	
1g64150-F2	TCT TGA TGT TCT GGT TGA GTC ATC G	Genotyping	
1g64150-R2	ACT GAA CCA AAA CCA AAA CAA AAC		
GK-LB	ATA TTG ACC ATC ATA CTC ATT GC		
35S-F	GCA AGA CCC TC CTC TAT ATA AG		
RT-Ubi-F	GGA AAA AGG TCT GAC CGA CA	RT-PCR	
RT-Ubi-R	CTG TTC ACG GAA CCC AAT TC		
RT-1g64150-F	CAA GAA GAG AGA TTC AGA GAG TTC		
RT-1g64150-R	GAA ACA GAA CTC CTC CGA CAT AAG		
1g64150-GW-F	CAC CAT GCT AAG TTT GAA TCT CTC GGA G	Cloning	
1g64150-GW-R1	CGT AAC GAT CTC AGC CAC CGT CAC		
1g64150-GW-R2	TTA CGT AAC GAT CTC AGC CAC CGT CAC		
P1:CrCGDL1-ins-for	AGG TGC AAC GAT GAG CTG TG	Chlamydomonas	
P2: CrCGDL1-ins-rev	ATG GGG GAG TTC GGA TGA CA	Genotyping	
P3: RMD326-pBC1	CAA GCC TCA GCG AGC TCC CCG CTC GAG		
PAM71-F	AGG AGT CGA CAT GCT AAG TTT GAA TCT CTC GG	Yeast	
PAM71-R	AGG AAC TAG TTT ACG TAA CGA TCT CAG CCA C	Complementation CA C	
psbA-F	CGG CCA AAA TAA CCG TGG C	Probe generation	
psbA-R	TAT ACA ACG GCG GTC CTT ATG		
psbB-F	GTT CTT GAT CCA ATG TGG AG		
psbB-R	TTC CGG TTA GTC CAT AAG GG		
psbC-F	TAG CTT TAG CTG GTC GTG AC		
psbC-R	GAA AGG CAC CTA CAC CTA AC		
rbcL-F	CGT TGG AGA GAC CGT TTC TT		
rbcL-R	CAA AGC CCA AAG TTG ACT CC		