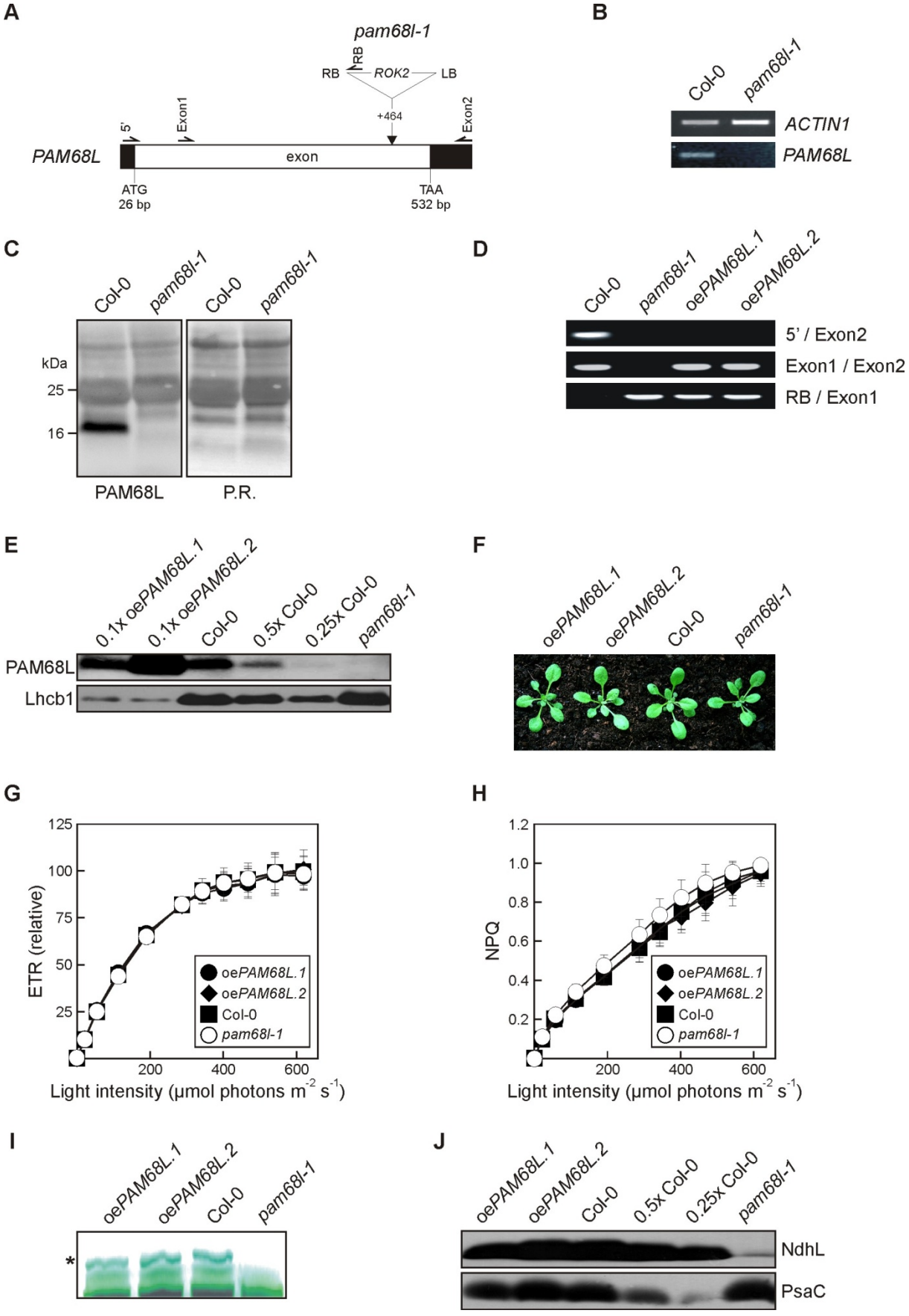
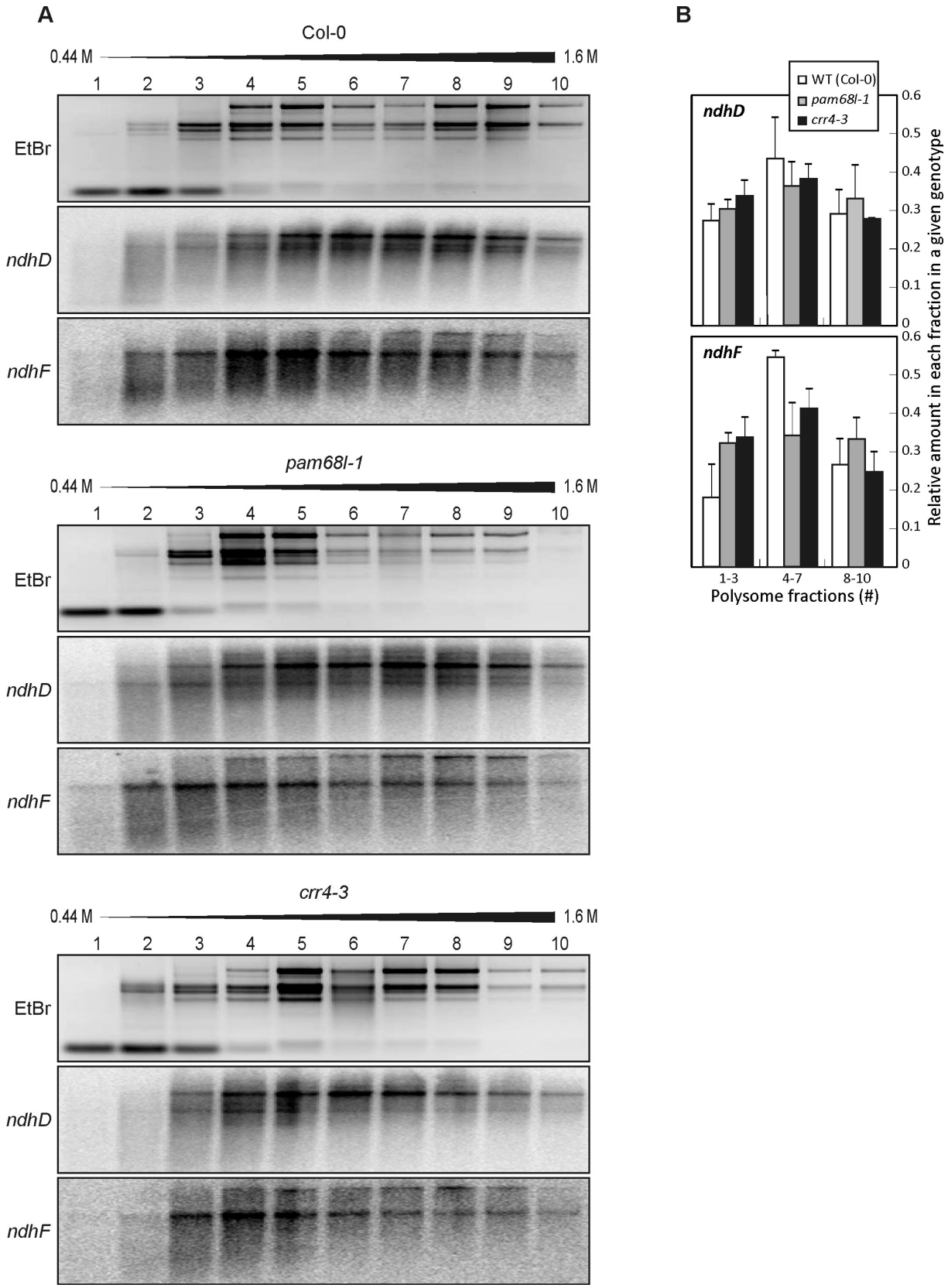


Supplemental Figure 1. Analysis of mutants and overexpressors of *Arabidopsis PAM68L*.

(A) T-DNA tagging of the *PAM68L*/*At5g52780* locus. The exon is shown as a white box and UTRs as black boxes. Location and orientation of the T-DNA insertion and of primers used for genotyping are indicated. The *pam68l-1* line corresponds to SALK_143426 from the Salk T-DNA collection. Note that the T-DNA is not drawn to scale.
(B) RT-PCR analysis was performed on transcripts from WT (Col-0) and *pam68l-1* plants using the gene-specific primers (Exon1 and Exon2) and *ACTIN1* primers as a positive control.
(C) Thylakoid proteins corresponding to 10 µg of total Chl from WT (Col-0) and *pam68l-1* plants were fractionated by SDS-PAGE, blotted onto membrane and visualized with antibodies raised against PAM68L. The Ponceau Red stain (P.R.) of the membrane prior to immunodetection is shown as loading control.
(D) PCR on genomic DNA from WT, *pam68l-1* and two independent *35S:PAM68L pam68l-1* (oe*PAM68L*) plants using primer combinations specific for either the original *PAM68L* locus (5'/ Exon2), the protein-encoding sequence (Exon1 / Exon2) or the *pam68l-1* mutant allele (RB / Exon1).
(E) Thylakoid proteins corresponding to 10 µg of total Chl from WT (Col-0) and *pam68l-1*, and to 1 µg of total Chl (0.1x) from the two oe*PAM68L* plants were fractionated by SDS-PAGE, blotted and visualized with antibodies raised against PAM68L and, as control, Lhcb1. Decreasing amounts of WT proteins were added to lanes marked 0.5x Col-0 and 0.25x Col-0.
(F) Four-week-old WT (Col-0), *pam68l-1* and the two oe*PAM68L* plants grown in the greenhouse.
(G) Dependence of the relative electron transport rate (ETR) on light intensity. ETR is expressed relative to the maximum ETR in the WT (Col-0) (100%). Values represent averages of twelve measured biological replicates and error bars indicate standard deviations.
(H) Dependence of non-photochemical quenching (NPQ) on light intensity. Values represent averages of twelve measured biological replicates and error bars indicate standard deviations.
(I) Magnification of the top section of the BN-PAGE gel shown in Figure 2B. The star symbol indicates the PSI-NDH complex.
(J) Thylakoid proteins corresponding to 10 µg of total Chl from WT (Col-0), *pam68l-1* plants and two oe*PAM68L* lines were fractionated by SDS-PAGE, blotted and immunodetected with antibodies recognizing NdhL or PsaC (as control). Decreasing amounts of WT proteins were added to the lanes marked 0.5x Col-0 and 0.25x Col-0.

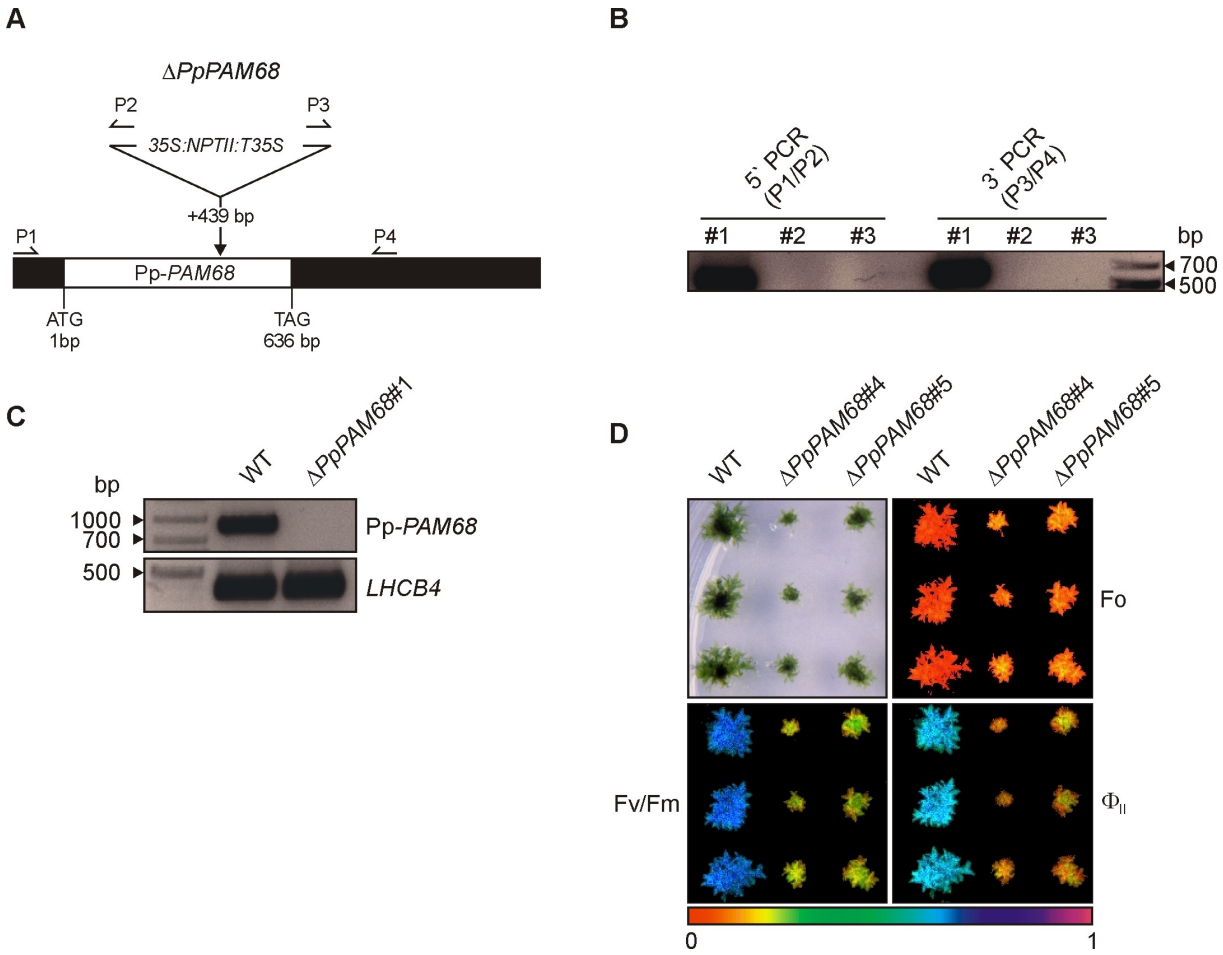


Supplemental Figure 2. Analysis of plastid *ndh* transcript association with polysomes.

(A) Association of *ndhD* (*ndhH/A/I/G/E/psaC/ndhD*) and *ndhF* mRNAs with polysomes. Whole-cell extracts from WT (Col-0) and mutant plants (*pam68l-1* and *crr4-3*) were fractionated in linear 0.44 M to 1.6 M (15% to 55%) sucrose gradients by ultracentrifugation. Gradients were divided into 10 fractions, and RNA was isolated from equal volumes of each. RNA gels were stained with ethidium

bromide (EtBr) to visualize the distribution of rRNAs, blotted and then hybridized with probes specific for *ndhD* and *ndhF*.

(B) Signal intensities for *ndhD* and *ndhF* transcripts were normalized relative to rRNA signals (see Methods), and values for fractions 1-3, 4-7, and 8-10 (relative to the total signal) were calculated for each genotype. Averages of three biological replicates are shown; error bars indicate standard deviations.



Supplemental Figure 3. Generation and characterization of the *Physcomitrella* knockout mutant Δ*PpPAM68*.

(A) Site of integration of the *35S:NPTII:T35S* cassette in the Δ*PpPAM68* line. The intron-free Pp-*PAM68* coding sequence is shown as a white box, UTR regions are indicated as black boxes. The location and orientation of primers used for PCR assays are indicated.

(B) Screening of *PpPAM68* knockout mutant lines. Genomic DNA was isolated from three kanamycin-resistant moss plants and homologous recombination of the selection cassette was verified by PCR using the primer pairs P1/P2 and P3/P4 shown in panel **A**. Primer sequences are provided in Supplemental Table 3 online. Note that Δ*PpPAM68*#4 and Δ*PpPAM68*#5 (shown in panel D), as well as Δ*PpPAM68*#6 and Δ*PpPAM68*#7, behave like Δ*PpPAM68*#1.

(C) RT-PCR analysis of the mutant line Δ*PpPAM68*#1. RNA isolation and cDNA synthesis was carried out as described in the Methods section. Pp-*PAM68* transcript abundance was examined by PCR using WT or Δ*PpPAM68*#1 cDNA as template with the primer pair P1/P4. As control, *LHCB4* transcripts were detected with appropriate primers (see Supplemental Table 3 online). Note that Δ*PpPAM68*#4, #5 #6 and #7 behave like Δ*PpPAM68*#1.

(D) Characterization of the photosynthetic phenotype in two additional, independent knockout moss plants (Δ*PpPAM68*#4 and Δ*PpPAM68*#5). The photosynthetic parameters Fo, Fv/Fm and Φ_{II} were determined using an Imaging PAM system as in Figure 7C. Three replicates of each genotype were analyzed. Note that Δ*PpPAM68*#6 and Δ*PpPAM68*#7 behave like Δ*PpPAM68*#1.

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PAM68L      RTSEKPGRPDPDPEDDPPIPQEVFERMMGRIVVSVGTPLGLGVAILKVLE
Al68L       -SSENPGRPDPDQEDDPPIPQEVFERMMGRIVVSVGTPLGLGVAILKILE
Zm68L       -AKQDGGGSNGEVDDDDELPQPVFDRILRRIAFTVGLPMASGVALLNVYD
Os68L       -EALDVKSGGEVDEDDDELPQPVFDRILRRIMFMVGVPMASGVGLLNLYG
Vv68L       -TVPRKNSGNGDDDDDEKIPQVVFDRMIVRILFFVGAPMGIGVALLNLFG
Pt68L       -SSIKKTSKNTNNNDDEEIPEEVLYRIIKRVLVSVGAPMALAFASMNVIG
PAM68       -DEDEDDDDEEDERERGVIPEIVTNRMISRMGFTVGLPLFIGLLFFPFFY
Al68        -NQSDKEDDDEDERERGVIPEIVTNRMISRMGFTVGLPLFIGLLFFPFFY
Vv68        -FYEEEEEEEGAEAEEGVIPEIVTNRMISRMGFSVGIPLMIGLLFFPFFY
Pt68        -DDDEDEEEEQEEPDAGVIPEVVTNRMMTRMGISVGAPLFVGVLFFPFFY
Zm68        -EEEEEEDEDEEEERDATIPEVVTNRMMRRVGASVGLPLALGVGLFPVFY
Os68        -EGSEEEDEEEEEERDVAIPEVVTNRMMRRVGVSVGAPLAVGVAFLPAFY
Pp          -GTQADPQDERQEPEDDVVPEVVTNRMLKRIAFTVGIPFAIGVAFFVLYY
Mp          -ERMEEKRAQYQAASAKGIPQVVTDRMLKRITIFSGVPLLLGFSTGPLFY
Ot          AAKRDAFDAKYAQRSAQGIPQIVTDRMLKRVGIFCGTPLMLGFTTGPLFY
Cr          -PEPASAPAPARRAVVRETPQIVVDRMFRRILVFTGVPVFTGMALFPLFY
Vc          -EAAQSGGPVPRTRVVRETPQVVVDRMFRRILVCTGVPVFTGMALFPLFY
Pt          -VRELLDADQQVQEAAAAIPERVAQRMGARMLPFVGLPFFLGMGVFVGFW
Tp          RDLRATDSLLKEDPNAAAIPEKVAQRMGKRMLPFVGVPLFGTFATFIGFW
Es          -SVMQAKAATEGDPDAGSIPEIVSNRMLSRMVPFFVLPALGGVGVFVTVY
Ma          SPVPPPLKNRSEETSLKAIPQVVSQRMAKRMAVFCGLPTALGIASFFGFY
Cy          VKNPEKQKKRSNDASLSAIPDSVSKRMIKRMAIFSGIPTGLGMSSFFVFY
Sy          -KTSGAKDKKGRRSADSGIPAVVSQRMVKRMALFSGIPTGLGMLSFVLFY
Np          -SGKQADKKLTYTKQEMAIPQVVSQRMIRRVAGFCGVPTALGISVLVVSY
Av          -ADKQDKKQLPYTKEEMAIPQVVSQRMIRRVAAFCGIPTALGITTLVSSY

PAM68L      VLKDRNVWDVPLWVPYLTTLVTFGSSALGIAYGSLSTNLDPAKTNSLFGL
Al68L       VLKDRKVWDVPLWVPFLTTLVTFGSSALGIAYGSLSTNLDPTKTNSLFGL
Zm68L       ALKRGQGVVVPSWVPLLTILVAFGTSALGIAYGTLSASWDPDKEGSLLGI
Os68L       ALERGRGVAVPSWLPLLTILVAFGTSALGIAFGTLSASWDPEKEGSLLGL
Vv68L       AVKDQHLWDVPVWLPFLTTLIAFGASALGIAYGTLSTSWDAEKKGSLLGL
Pt68L       LVMEQHIWNVPKWFMFLTLFLTLGASVCGIAYGALSTSMDPNEKGSFLGF
PAM68       YLKVGLKVDVPTWVPFIVSFVFFGTALAGVSYGIVSSSWDPLREGSLLGW
Al68        YLKVGLKVDVPTWVPFIVSFVFFGTALAGVSYGIVSSSWDPLRKGSLLGW
Vv68        YLKVGLKIDVPTWVPFIVSFFFFGSALLGVSYGIVSSSWDPLREGSFLGW
Pt68        YLKVGLKIDVPTWVPFIVSFIFFGSALLGVSYGIVSSSWDPKREGSFWGW
Zm68        YLKAVAKVDVPTWIPFGVSFVFFGAALAGVSYGIVSASWDPAREGSLLGW
Os68        YLKKAAKVDVPTWIPFGVSFLFFGAALLGVSYGIVSASWDPAREGSLLGW
Pp          YLKAVKKVDIPEWLPLFTSFLTFGSAGAGITYGVLSASWDPKREGSLLGW
Mp          LGKTVAHFDLAPWQFFFASTATFGAALVGITYGVLSASWEPGREGSFWGV
Ot          YFKAVRHVDVPPWVFFTASTATFGAAFIGISYGVLSASWDPRREGTFWGG
Cr          YLRVVMDIEYPLWIVYVSQVLTFGGGLFGITYGALSASWDPTREGSAMGW
Vc          WLRVVQDIDYPLWIVYIAQVLTFGGGLLGITYGALSASWDPSREGSALGW
Pt          YMATYRNLEYQPALVAASTIVVLLLGLVGITYSILSASWDPDREGSLLGT
Tp          YAAVYKDMEFQPAIVASTSFVFLAIGLLGITYSVMSSSWDEDREGSGLGF
Es          VLSHKYDYTIPAYIVAYATQAPFFVALAGITYAIMSASWDEDREGTFFGF
Ma          WIISHDLLEIPSYVAMLVSLSLFGLGFIGLSYGIFSASWDEDRVGDWLGW
Cy          WIVSHDWLDIPTAAVGAVSLGLFGLGVLGLSYGIFSSSWDEHRAGGWWGW
Sy          LVVSRDWFEIPTYVVFSVSLLFFGLGVVGLSYGIFSTSWEDE-PGSVWGW
Np          LLAIYSDIQLAPIAVLLVNMGLFGLGVLGITYGVLSASWDEERVGSLLGL
Av          LLTIYSDIQLAPIAVLLVNMGFFGLGVLGITYGVLSASWDEERTGSLLGL
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PAM68L      KEAKENWVEMWKEDQ--
Al68L       KEAKENWVEMWKE----
Zm68L       DEARANWPVLWQEEIE-
Os68L       EEARANWPVLWEEEIE-
Vv68L       EEAQQNWVDVWKEE---
Pt68L       EQVQKNWVEMWKEEDE-
PAM68       NEAKKNWPVFWQSFWN-
Al68        NEAKKNWPVFWQSFRN-
Vv68        TEAQNNWPVFWQSLWG-
Pt68        NEAQKNWPVFWQSLRG-
Zm68        NEARRNWPVFWDSLRG-
Os68        NEARRNWPVFWDSLRG-
Pp          KEAQLNWPVFWDTFRG-
Mp          TEVKANIPILWQTILGK
Ot          SEFKENVPVVISTIMNK
Cr          SEMQANLSILLNRNKQ-
Vc          TELQANLAILLNRNKN-
Pt          DEFSKNIENIRDGLKR-
Tp          DEFSKNVDSLKEGLSR-
Es          DEAKRNFGNIVEGLRR-
Ma          QEFQANFGRTIAAWRSG
Cy          QEFTQNLGRTIQAWRSA
Sy          PEFRLNLSRTIAVWRNA
Np          GEFNTNWGRMVTVWRE-
Av          GEFGTNWGRMVEGWRE-
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Supplemental Figure 4. Alignment of orthologous PAM68 and PAM68L protein sequences for phylogenetic analyses (see Figure 1A).

Supplemental Table 1. Quantification of NDH subunit accumulation in different genotypes relative to WT (Col-0) according to Figures 4A and 4B (WT=100%). Immunodetections were repeated two to three times.

	pam68l-1	*crr2-2*	*crr4-3*	*ndhl*	*pnsb2*	*pnsb3*	*ndf5*	*pnsb4*	*pnsl1*	*ndhs*	*ndht*	*ndhu*
NdhA	20	10	20	30	20	20	20	20	0	60	40	50
NdhH	20	10	20	10	20	20	20	20	0	100	60	60
NdhL	20	10	20	0	20	20	40	30	0	40	30	20
PnsB2	20	60	20	80	0	0	40	40	50	80	80	80
PnsL1	40	30	50	100	50	50	50	50	0	100	100	100
PnsL4	30	20	30	60	30	30	30	30	20	100	80	100
Lhca5	50	80	50	90	50	50	50	50	50	100	80	100
NdhS	90	70	70	70	90	100	80	100	100	0	70	80
NdhT	70	80	80	50	50	40	60	60	30	60	0	100

Supplemental Table 2. NDH subunits in *P. patens*.

subcomplex	Protein	AGI code	Present in *P. patens*	Physco identifier	Similarity (%)	Identity (%)	Length Ara	Length Physco	comment or reference
subM	NdhA	ATCG01100	yes	NP_904243	79	71	360	368	
	NdhB1 NdhB2	ATCG00890 ATCG01250	yes	NP_904166	74	65	512 512	501	Only one *ndhB* gene in *P. patens*
	NdhC	ATCG00440	yes	NP_904197	85	75	120	121	
	NdhD	ATCG01050	yes	NP_904238	81	68	506	501	
	NdhE	ATCG01070	yes	NP_904240	80	71	101	100	
	NdhF	ATCG01010	yes	NP_904235	67	58	746	720	
	NdhG	ATCG01080	yes	NP_904241	58	47	176	197	
subA	NdhH	ATCG01110	yes	NP_904244	88	83	393	391	
	NdhI	ATCG01090	yes	NP_904242	76	70	172	181	
	NdhJ	ATCG00420	yes	NP_904199	85	77	158	158	
	NdhK	ATCG00430	yes	NP_904198	67	62	225	261	
	NdhL	AT1G70760	yes	Pp1s101_245V6.1	33	24	191	196	
	NdhM	AT4G37925	yes	Pp1s230_42V6.1	57	44	217	204	
	NdhN	AT5G58260	yes	Pp1s25_204V6.1	58	47	209	255	
	NdhO	AT1G74880	yes	Pp1s230_32V6.1	52	41	158	176	
subB	PnsB1	AT1G15980	yes	Pp1s89_87V6.1	59	46	461	473	
	PnsB2	AT1G64770	yes	Pp1s88_7V6.1	33	20	348	425	
	PnsB3	AT3G16250	yes	Pp1s36_1V6.1	44	30	204	179	
	PnsB4	AT1G18730	yes	Pp1s127_94V6.1	40	31	175	210	
	PnsB5	AT5G43750	yes	Pp1s235_42V6.2	38	26	212	179	
subL	PnsL1	AT2G39470	no						Pp1s30_39V6.1 closer related to PPL1
	PnsL2	AT1G14150	no						Yabuta et al., 2010
	PnsL3	AT3G01440	no						Yabuta et al., 2010
	PnsL4	AT4G39710	no						Pp1s347_30V6.2 closer related to FKB13 (AT5G45680)
	PnsL5	AT5G13120	yes	Pp1s196_68V6.1	66	57	259	247	
subE	NdhS	AT4G23890	yes	Pp1s123_43V6.1	45	35	250	257	
	NdhT	AT4G09350	yes	Pp1s106_74V6.2	47	34	249	251	
	NdhU	AT5G21430	yes	Pp1s91_80V6.1	39	25	218	229	
	Lhca5	AT1G45474	yes	Pp1s284_6G3.1	61	55	256	278	Alboresi et al., 2008
	Lhca6	AT1G19150	no						Alboresi et al., 2008

Protein sequences of *A. thaliana* were blasted against *P. patens* protein databases. Pairwise alignments (AlignX, Vector NTI Advance 9.1.0, Invitrogen Corporation) were performed to calculate sequence similarity and identity values (in %). Putative transit peptide sequences of nucleus-encoded proteins were not excluded in the analysis. The full citation of the references listed in this table can be found in the reference list of the main text.

Supplemental Table 3. Overview of oligonucleotides (5' to 3') used in PCR assays.

Gene	Forward primer	Reverse primer	Experiment
ndhA	ndhA_for:	ndhA_rev:	RNA blot analysis
	ATGATAATTTATGCAACAGCAGTCCAAACT	CAGCTCGCAGACCACCTAAAAAAGAATAT	(RBA)
ndhB	ndhB_for:	ndhB_ rev:	RBA
	AAAGCCTTTCATTTGCTTCTCTTCGATG	GCCCCACCCATGAGTAAATATTTCATAGTA	
ndhC	ndhC_for:	ndhC_ rev:	RBA
	CAAGTGCTATTCCTGTTTTGGCATTTC	CCTTTTCGCCATGCATAAACTAAACCA	
ndhD	ndhD_for:	ndhD_ rev:	RBA
	GTGTATCTTGTCTTTACCACG	AGACGTTTCTTTCCACCCCA	
ndhE	ndhE_for:	ndhE_ rev:	RBA
	ATACTCGAACATGTACTTGTTTTGAGTGCC	GGTCGATTGGTTTATGCGAATTGAT	
ndhF	ndhF_for:	ndhF_ rev:	RBA
	AGGTCCTATTGCTAAATCCGC	CGGTTAATCCCGCTGTTGAA	
PAM68L	PAM68HLattB1:	PAM68HLattB2:	overexpression *in*
	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGA	GGGGACCACTTTGTACAAGAAAGCTGGGTC	*planta*
	TGAGAGCTCTCTTGTGC	ACCTGCTCCTTGGTCCTCTTTCCACATC	
PAM68L	pET151_PamHL_for	pET151_PamHL_rev	Expression in *E. coli*
	CACCACCTCTGAAAAACCCGGC	TTATTGGTCCTCTTTCCAC	
PAM68L	Exon1:	Exon2:	Genotyping
	CACAATCCAAAAACCCTATATCC	AGCCAGCTTAAAAGTTTTTATGAG	
PAM68L	5':	Exon2:	Genotyping
	CATTAGAAGAAGAAATAGAAATGAG	AGCCAGCTTAAAAGTTTTTATGAG	
PpPAM68	Pam_PP_KO_s2	Pam_PP_KO_as	Knockout construct
	ATCGACCATGTTGCCCTCCACTGCT	AGCGATCAGATAGAACAGCTGCACC	in *P. patens*
PpPAM68/nptII	Pam68_ver_5'_s (P1):	Pam68_ver_5'_as (P2):	knockout screening
	AAAGCTTAAGCAAGCAAGCGAGGCTGCCT	GGAGGTTTCCCGAAATTACCCTTTGTTGAA	for *PpPAM68* 5' end
PpPAM68/nptII	Pam68_ver_3'_s (P3):	Pam68_ver_3'_as (P4):	knockout screening
	TAGTTTCCCGATAAGGGAAATTAGGGTTCT	CCTGCTGTTAACCATGCAACCCTAAATGC	for *PpPAM68* 3' end
PpLHCB4	PpLhcb4.1-4.4for	PpLhcb4.1-4.4rev	RT-PCR in
	CATCTCGAAGAAGGAGGAGATCG	CGAAGTTCAGTGGGTCGAAGAAC	*P. patens*