Expanded View Figures

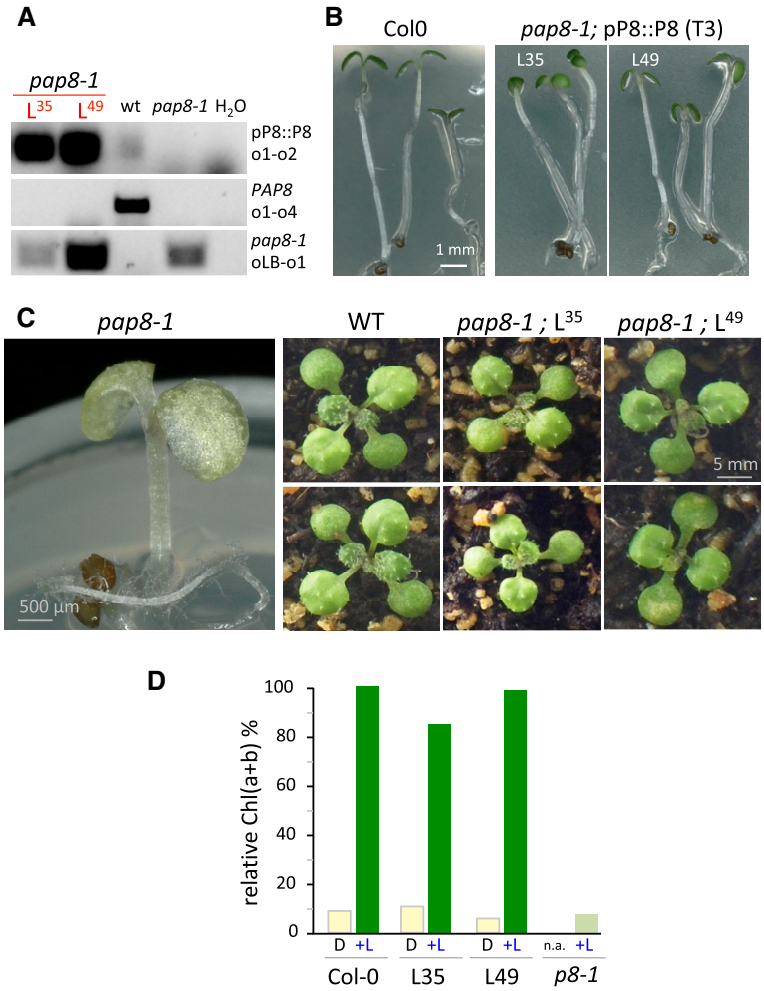


Figure EV1. (Related to Fig 1) Functional complementation of *pap8-1*.

The construction used for complementation is pPAP8::PAP8^{cds} (pP8::P8 in short): PAP8 coding sequence under control of a 1.1-kb upstream region used as promoter (see pBB389 in Appendix Table S1 and Fig 2A for the description of the regulatory region used as promoter).

A PCR on genomic DNA; L35, L49: Two independent "pBB389" transgenic lines; primers are the same as in Fig 1B and o4: op8i2_R.

B Greening assay on wild type and rescued *pap8-1* homozygous plants from third generation transgenic lines (T3) grown *in vitro* 3 days in the dark followed with a 30-h light treatment. L35 and L49 are two independent rescued lines.

C Phenotypes of *pap8-1* homozygous plant grown *in vitro*, and two representative plants of wild type or *pap8-1*/pP8::PAP8 (line L35 or line L49) grown on soil.

D Content of total chlorophylls (Chl(a+b)) normalized to fresh weight and relative to wild type in the given genotypes grown in the dark (D) or grown in the dark followed with 30 h of white light treatment (+L); n.a. not applicable.

E Spectrophotometric analysis of pigments: absorption spectra of acetone-soluble extracts from seedling grown *in vitro* 3 days in the dark (D) or 3 days in the dark plus 30 h of white light (L) Col-0, wild type; p8-1/p8-1, homozygous mutant *pap8-1*; L35 and L49, two lines of *pap8-1*/pPAP8::PAP8; n.a., not applicable. Absorbance was normalized to fresh weight (FW); Chl*a*, chlorophyll *a*; Chl*b*, chlorophyll *b*; Car, carotenoids.

Source data are available online for this figure.

Figure EV2. (Related to Fig 3) Sub-cellular localization of PAP8 variants.

A Transient assay in onion epidermal cells, pictures are given at the same scale. FL, full-length ORF; −G, translational fusion with GFP; ΔNLS, deletion of the NLS; ΔcTP, deletion of the cTP, ΔΔ, deletion of both the NLS and the cTP. Onion cell co-transfected with the corresponding variant fused to GFP and a plastid control fused to RFP (PAP10, PAP10-RFP or RecA, RecA-RFP) Merge, merged channels; DIC, differential interference contrast microscopy pictures to reveal the position of the nucleus within the cell when fluorescent nuclei were observed (marked with white arrowheads).

B, C Confocal imaging of stably expressed CaMV35S::PAP8$^{\Delta cTP}$-GFP (B) or pPAP8::PAP8$^{\Delta nls}$-GFP (C) in cotyledons of *Arabidopsis thaliana*; white arrowheads indicate nuclei; green arrowheads indicate sub-plastidial localization. Observations similar to (C) were recorded for pPAP8::PAP8^{FL}-GFP.

D Confocal imaging of stably expressed CaMV35S::PAP10-GFP in cotyledons of *Arabidopsis thaliana* showing the PEP-PAP complex; the green arrowhead indicates the putative location of PEP-PAP complexes within one chloroplast.

E–H PAP8-GFP in stromules of onion epidermal cells expressed alone (E) or in co-localization with PAP10-GFP (F–H); stromules (str, white arrowheads) are only marked by PAP8-GFP.

Source data are available online for this figure.

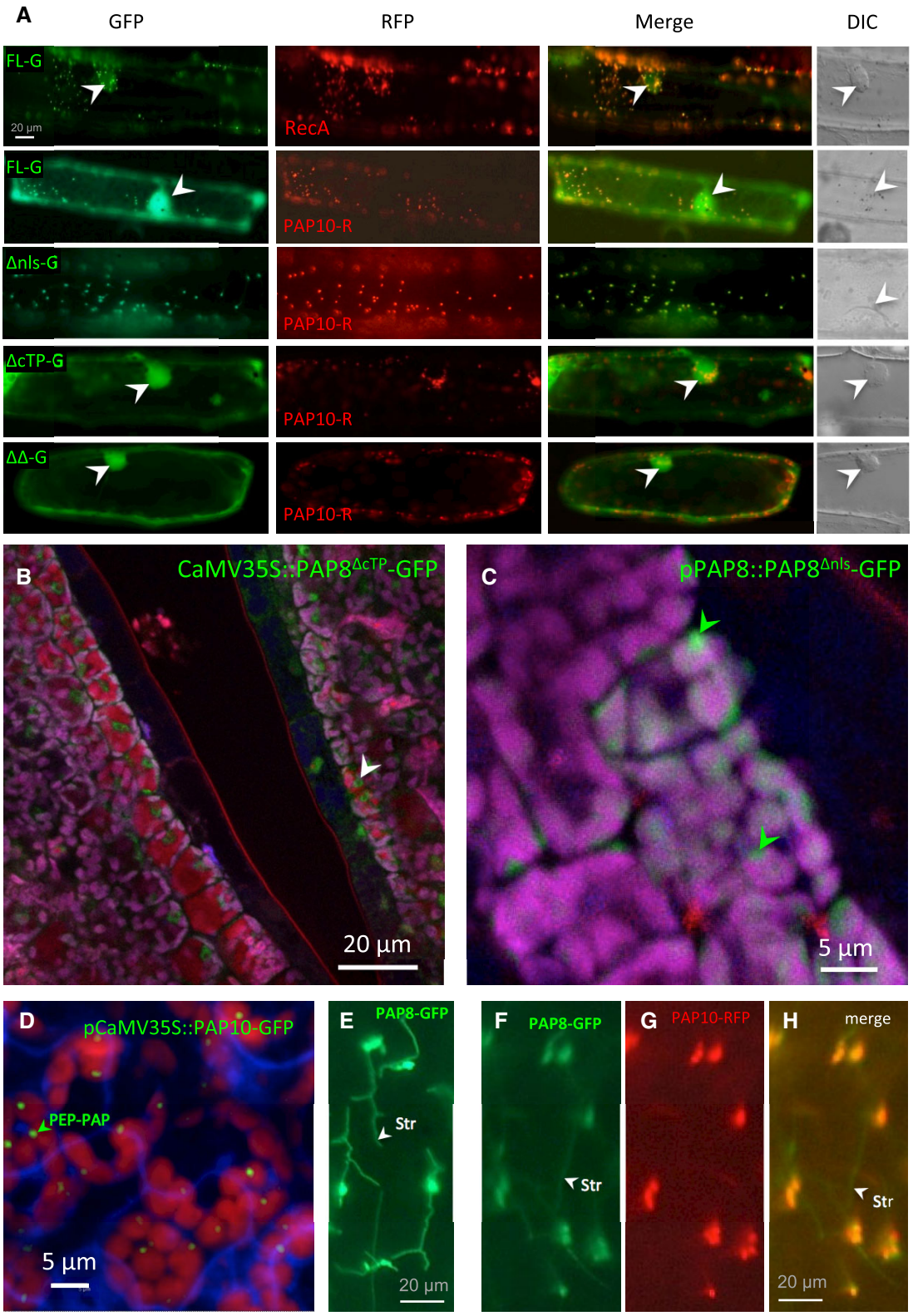


Figure EV2.

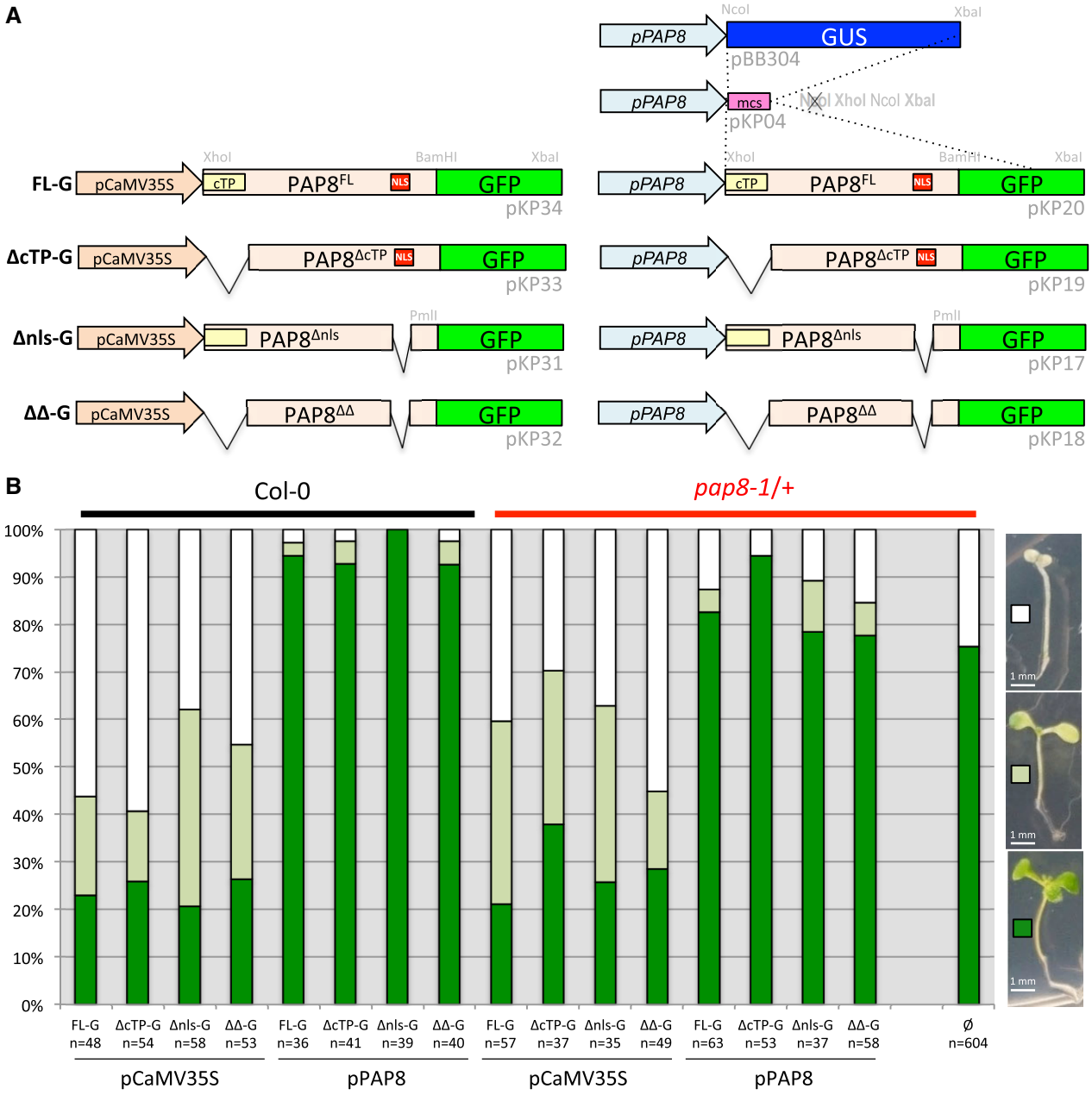
Figure EV3. (Related to Fig 3) PAP8 and its variants fused to GFP.

A Schematic illustration of the GFP-fused PAP8 variants used for sub-cellular localization and functional complementation tests FL-G, *PAP8* full-length ORF translationally fused to GFP; Δnls-G, deletion of the NLS (nuclear localization signal), ORF fused to GFP; ΔcTP-G, deletion of the cTP (chloroplastic transit peptide), ORF fused to GFP; ΔΔ-G combined deletion of NLS and cTP in the ORF fused to GFP. The variants are expressed under the control of the CaMV35S promoter or under the native PAP8-1.1-kb promoter. Plasmid identification and relevant restriction sites for cloning are given in light grey (see Appendix Table S1); mcs, multiple cloning site.

B Transgenic lines obtained with the constructions described above. Three phenotypic classes have been recorded corresponding to albino (white squares), pale green (light green squares) and green plants (green squares). Col-0, wild type; *pap8-1/+*, heterozygous mixture; *n*, total number of recorded plants; ø, no transgene.

C Functional complementation output. Hygromycin-resistant plants were transferred on soil and grown under long-day conditions (16-h light/8-h dark; ~ 70 µmol m^{-2} s^{-1}) at 21°C and 60% humidity. Genomic DNA was isolated from true leaves and used for genotyping. The presence of the *pap8-1* allele was confirmed using the primer ortpF/oLBb1.3. PAP8 wild-type allele tested with ortpF/op8i2_R. The insertion of the transgene of interest was tested with oPAP8_rtp_F/oE3_R. The number of the tested plants (Hyg^R#T1), the number of double heterozygous plants (p8-1/+; TG/—) and the number of sesqui-mutant plants (*p8-1/p8-1*; TG/—) are depicted for each construction; p8P8 presented as positive control. None of the 39 T1 plants with pP8::PAP8-GFP were photosynthetic and homozygous *pap8-1*. Therefore, two doubly heterozygous (*pap8-1*/+; TG/—) expressing GFP were tested for their segregation pattern (Line 1: 34% albino, n = 169, and Line 2: 24% albino, n = 199) and compared to that of *pap8-1*/+ (28% albinos, n = 99). In the absence of statistical difference between the samples ($\varepsilon = 0.102 \ll 1.96$ for $\alpha = 0.05$; ε-test, Fisher Yates), PAP8-GFP was declared not functional as opposed to PAP8.

Source data are available online for this figure.



C

Transgene (TG) tested in FC	plasmid ID	Hyg^R# T1	*p8-1/+*; TG/-	*p8-1/p8-1*; TG/-
pPAP8::PAP8Δnls:GFP	pKP17	17	8	0
pPAP8::PAP8ΔΔ:GFP	pKP18	21	9	0
pPAP8::PAP8ΔcTP:GFP	pKP19	32	17	0
pPAP8::PAP8^{FL}:GFP	pKP20	39	9	0
pCaMV35S::PAP8Δnls:GFP	pKP31	10	2	0
pCaMV35S::PAP8ΔΔ:GFP	pKP32	11	7	0
pCaMV35S::PAP8ΔcTP:GFP	pKP33	15	6	0
pCaMV35S::PAP8^{FL}:GFP	pKP34	16	5	0
pPAP8::PAP8 (p8P8)	pBB389	151	36	19

Figure EV3.

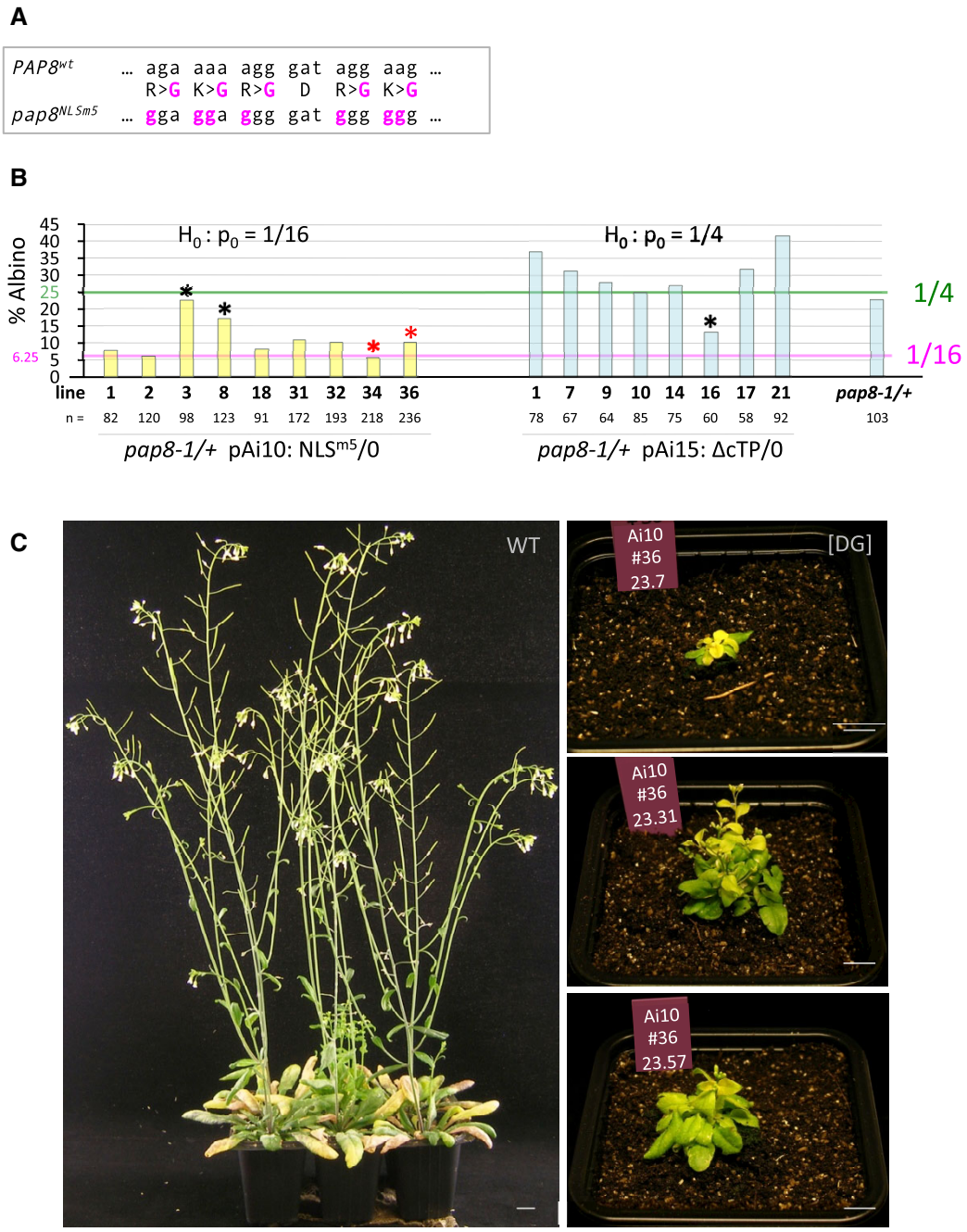


Figure EV4. (Related to Fig 4) Hemi-complementation test in *pap8-1*.

A Genetic alterations of the *pap8*^{NLSm5} allele. Altered codons and corresponding changes in amino acids (central line) are in magenta; the sign ">" indicates aa replacement at the same position.

B Bar graph representing the albino segregation ratios in doubly heterozygous transgenic lines (pap8-1/+; TG/—) obtained with pPAP8::PAP8NLSm5 (NLSm5: pAi10) and pPAP8::PAP8ΔcTP (ΔcTP: pAi15). *pap8-1*/+ used as control; *n*, total number of recorded plants. Segregation pattern were tested using ε-test with null hypothesis set to p0 = 1/16 (greening complementation) for NLSm5 and p0 = 1/4 (no complementation) for ΔcTP (see data source); black *, outliers correspond to the samples that did not pass the statistical test; red * indicate chosen genotypes for follow-up studies.

C Phenotype of *pap8-1* transformed with pPAP8::PAP8^{NLSm5}. WT, 5-week-old Col-0 control; [DG], delayed greening phenotype observed for the partial rescue of *pap8-1* mutant expressing PAP8^{NLSm5} under its endogenous promoter; pictures depict three 15-week-old plants in which the alteration of the greening corresponds to the emergence of white leaves that slowly acquire the photosynthetic apparatus; plants #7, #31 and #57 are siblings of the same genotype (Ai10#34). Scale bars equal 10 mm.

Source data are available online for this figure.

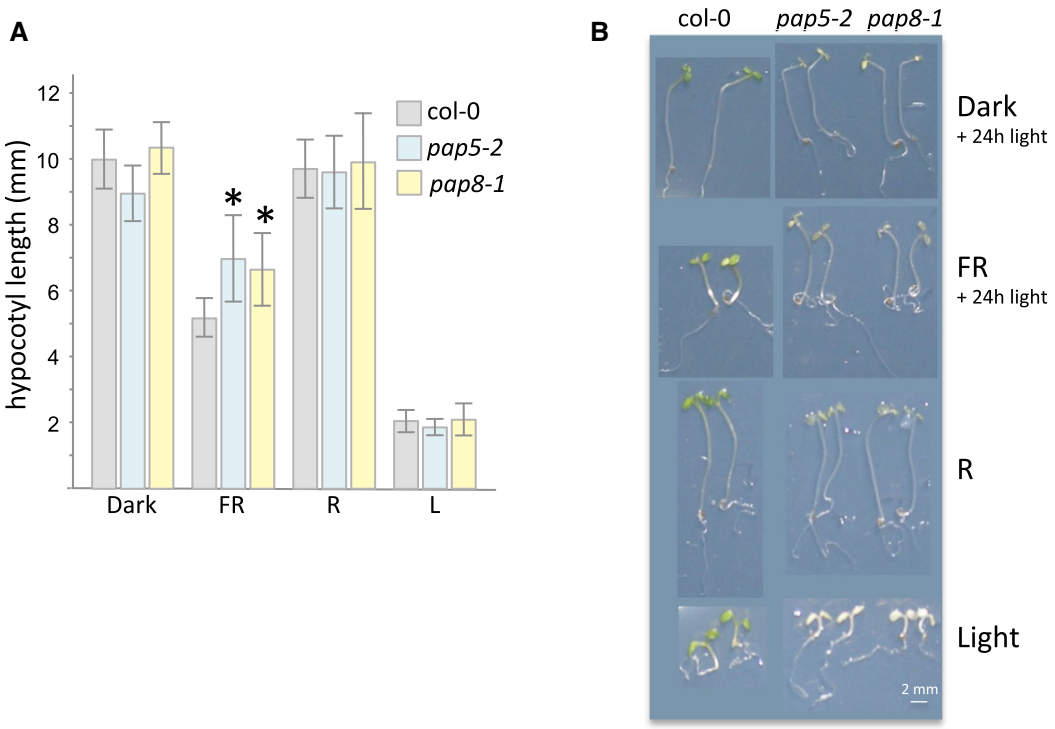


Figure EV5. Hypocotyl growth experiment.

A Hypocotyl length measurements, given as mean ± SD, of genotypes grown under different light sources. Individual measurements and sample sizes are given in source data. Dark, true dark treatment; FR, far-red light (low fluence approx. 10 μmol m^{-2} s^{-1}; peak at 730 nm ± 10 nm); R, red light (8 μmol m^{-2} s^{-1} peak at 660 nm ± 10 nm); L, white light 30 μmol m^{-2} s^{-1}. Whereas *pap5-2* and *pap8-1* are statistically undistinguishable using δ-test for the comparison of the mean (Fisher Yates $\delta = 0.791 \ll U\alpha = 0.05 = 1.96$, not significant at α set to 0.05), both *pap5-2* and *pap8-1* show significant hypocotyl differences compared with wild type ($\delta = 5.374$; $\delta = 5.061$ respectively so that both *P-values $< 10^{-6}$).

B Images of representative seedlings.

Source data are available online for this figure.