

**Supplemental Figure 1. Genotyping of the *clb5-1* complemented plants. (A)**

The structure of *ZDS* gene is shown with exons by the black boxes, introns with the dark line and the 3'UTR by the dashed line. The G to A change found in *clb5-1* results in the lost of a *Bam*HI restriction site (indicated by the box in the wild-type sequence). The gray line corresponds to the 1.9 Kb fragment used for *Bam*HI digestion amplified using the primers ZDST5 and ZDST3 of *CLB5* sequence. (B) PCR fragments digested with *Bam*HI from wild-type (Wt), *clb5-1* (5-1) and six independent green kanamycin-resistant 35S:*ZDS* complemented lines. L6 and L1 35S::*ZDS* lines correspond to complemented plants homozygous for *clb5* mutation, L3 corresponds to a complemented line homozygous for a wild-type *CLB5* allele and L8 is a complemented plant heterozygous for the mutation.

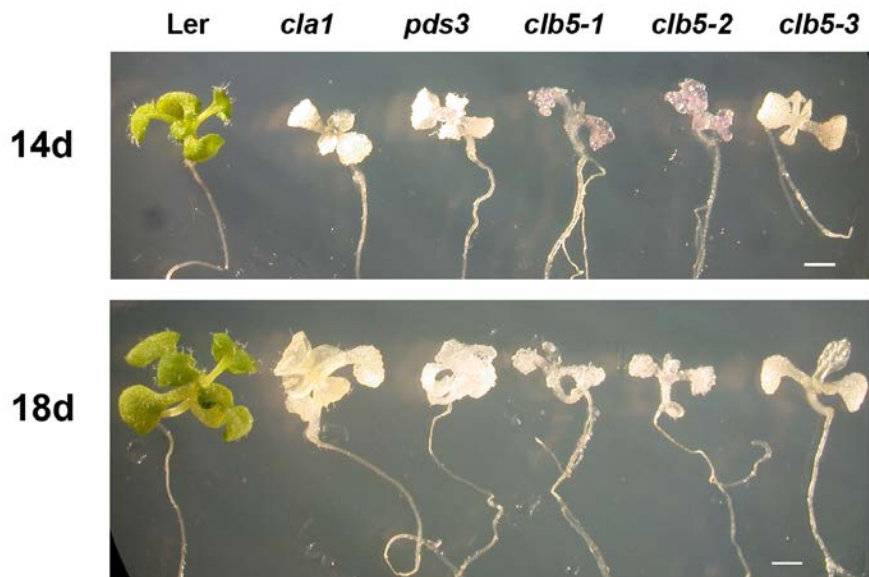
## A

Cs ZDS	YESRSFIGGKVGVSFVDKRGNHIEMGLHVFFGTCYNNLFRLLMCKKVGADKNLLVKDHTHTTFVN
Gl ZDS	YESRPFIGGKVGVSFVDKGGNHIEMLGHVFFGTCYNNLFRLLMCKKVGAEKNLLVKDHTHTTFVN
CapZDS	YESRTFIGGKVGVSFVDKRGNHIEMGLHVFFGTCYNNLFRLLMCKKVGAEKNLLVKEHTHTTFVN
Sl ZDS	YESRTFIGGKVGVSFVDRRGNHIEMGLHVFFGTCYNNLFRLLKCKVGAENLLVKEHTHTTFVN
Dc ZDS1	YESRPFIGGKVGVSFTDKRGNHIEMGLHVFFGTCYNNLFRLLKCKVGADKNLLVKEHTHTTFVN
Dc ZDS2	YESRPFIGGKVGVSFVDKRGNHIEMGLHVFFGTCYNNLFRLLKCKVGAENLLVKDHTHTTFVN
Ha ZDS	YESRTFIGGKVGVSFVDKQGNHIEMGLHVFFGTCYNNLFRLLKCKVGAENLLVKDHTHTTFVN
Te ZDS	YESRTFIGGKVGVSFVDKQGNHIEMGLHVFFGTCYNNLFRLLKCKVGAENLLVKDHTHTTFVN
Cm ZDS	YESRTFIGGKVGVSFVDKGGNHIEMLGHVFFGTCYNNLFRLLKCKVGAENLLVKDHTHTTFVN
At ZDS	YDSRTFIGGKVGVSFVDRRGNHIEMGLHVFFGTCYNNLFRLLMCKKVGAEKNLLVKDHTHTTFIN
Os ZDS	YESRPFIGGKVGVSFVDRKGNHIEMGLHVFFGTCYNNLFRLLMCKKVGADNNLLVKEHTHTTFVN
Mz ZDS	YESRPFIGGKVGVSFVDRQGNHIEMGLHVFFGTCYNNLFRLLMCKKVGADNNLLVKEHTHTTFVN
Ta ZDS	YDSRTFIGGKVGVSFVDKHGNHIEMGLHVFFGTCYNNLFRLLMCKKVGADNNLLVKEHTHTTFVN

## B

At ZDS	DSRTFIGGKVGVSFVDRRGNHIEMGLHVFFGTCYNNLFRLLMCKKVGAEKNLLVKDHTHTTFIN <b>K</b>
Pp ZDS	ESRKFIGGKVGVSFKDKNGNHIEMLGHVFFGTCYNNLFRLLTKVGADNNLLVKDHIHTTFIN <b>K</b>
Sy CRTQ	EARSFIGGKVGSWVDGDGNHIEMGLHVFFGTCYNNLFRLLMEKVGAKQNLRLKEHTHTTFVN <b>Q</b>
An CRTQ	ESRPFVGGKVGSWIDGDGNHIEMLGHVFFGTCYNNLFRLLMCKKVGAFSHLRLKEHTHTTFVN <b>K</b>
Cr ZDS	EGRQWIGGKVASFVVDKGNHIEMGLHVFFGTCYNNLFRLLMAKCGVLENLLVKEHTHTTF <b>CNN</b>
Pc CRTQ	EGRPFIGGKVGSWEDTDGNHIEMGLHVFFGTCYNNLFRLLMCKKVGADNNLLVKDHTHT <b>IFINR</b>

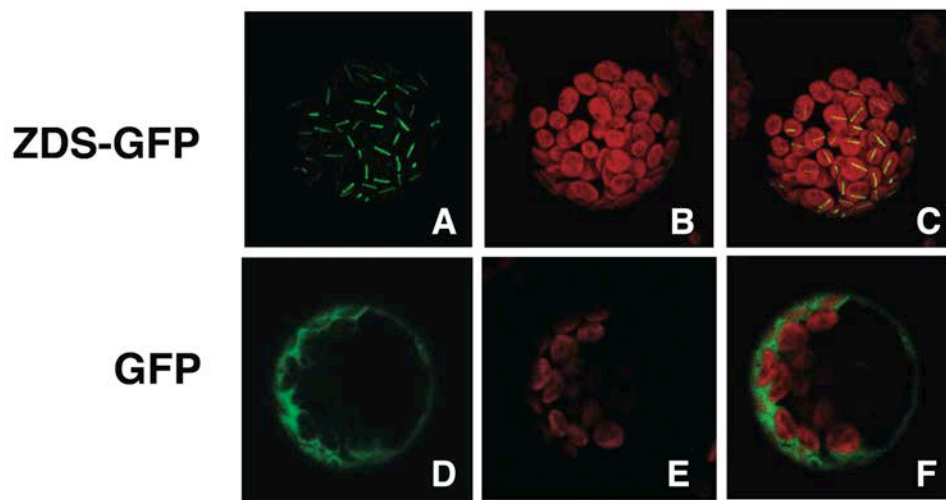
**Supplemental Figure 2. Amino acid alignment of the conserved region of the ZDS.** (A) Alignment of ZDS from Arabidopsis (At ZDS) and different plants. (B) Alignment of At ZDS with the ZDS from photosynthetic organisms. The amino acid substitution of Lys (K) to Glu that results from the A to G mutation in the *clb5-3* mutant allele is marked in bold. Species designation and GenBank accession number are as follows: *Anabaena* sp. (An) CRTQ, Q9R6X4; *Arabidopsis thaliana* (At), Q38893; *Capsicum annuum* (Ca), CAA61985; *Chlamydomonas reinhardtii* (Cr) ZDS, A81647; *Chrysanthemum x morifolium* (Cm), BAE79555; *Citrus sinensis* (Cs), CAC85667; *Daucus carota* (Dc1) ZDS1, ABB52083 and ZDS2, ABB52070; *Gentiana lutea* (Gl), ACF21785; *Helianthus annuus* (Ha), CAD55814; *Oryza sativa* (Os), BAF21059; *Paulinella chromatophora* (Pc) CRTQ, B1X467; *Physcomitrella patens* (Pp) ZDS, A9TE14; *Solanum lycopersicum* (Sl), ABD67160; *Synechocystis* sp. (Sy) CRTQ, P74306; *Tagetes erecta* (Te), Q9FV46; *Triticum aestivum* (Ta), ACI04664 and *Zea mays* (Zm), AAD02462.



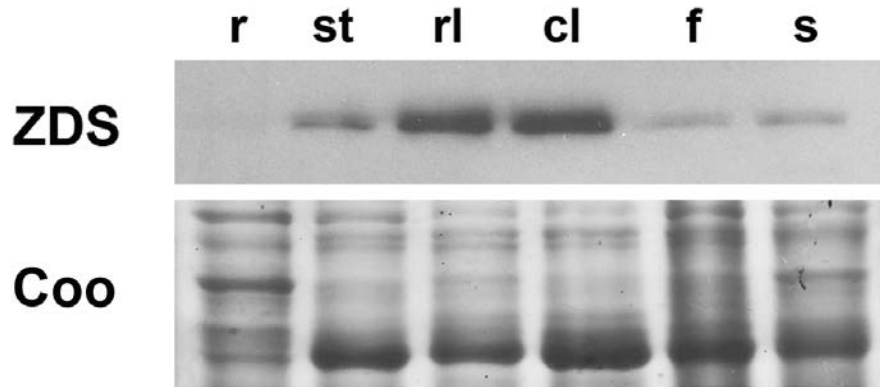
**Supplemental Figure 3. Phenotype of the different *clb5* alleles.** Homozygous mutant seedlings of DXS1 (*cla1*), PDS (*pds3*) and ZDS (*clb5-1*, *clb5-2* and *clb5-3*) were grown under standard light conditions ( $120 \mu\text{mol m}^{-2} \text{sec}^{-1}$ ). Pictures were taken at 14 and 18 d after germination. Scale bar corresponds to 1 mm.



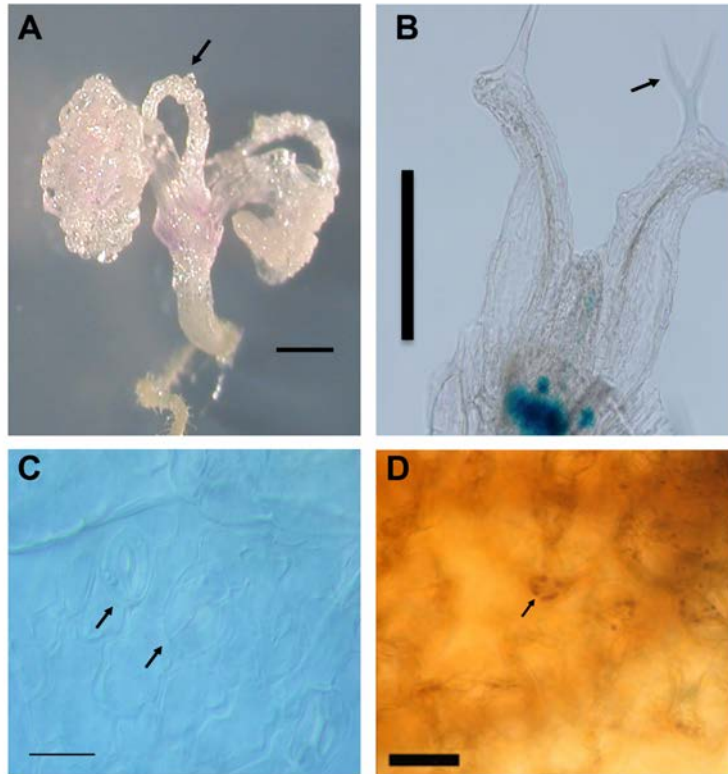
**Supplemental Figure 4. Phenotype of 8-week-old *clb5-1* mutant plant.** *clb5-1* mutant seedling was grown under standard light conditions ( $120 \mu\text{mol m}^{-2} \text{sec}^{-1}$ ). Picture was taken at 8 weeks after plant germination. Photograph of representative seedling was taken with a Nikon SMZ1500 stereoscopic microscope equipped with a digital SIGHT DS-Fi1c camera. Scale bar corresponds to 1 mm.



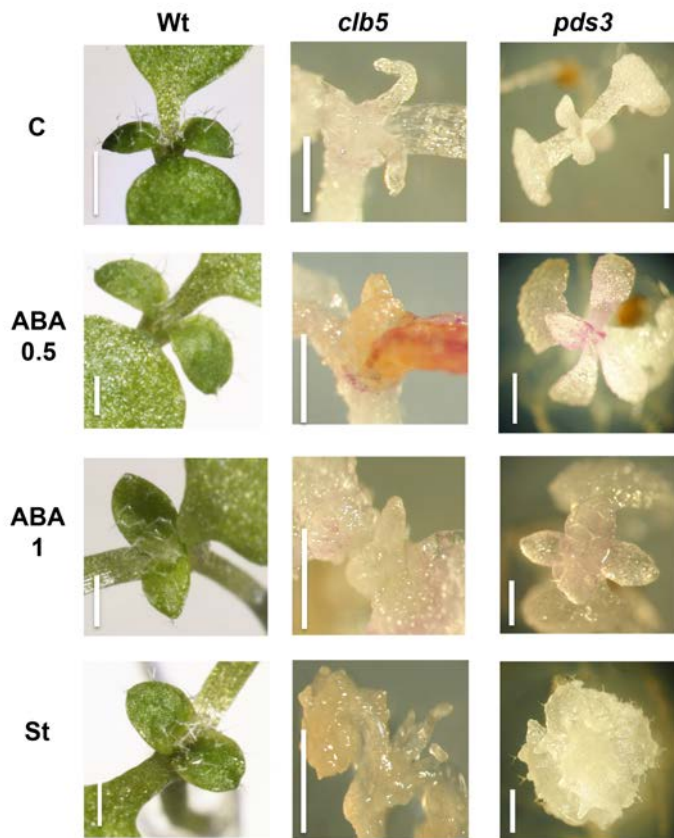
**Supplemental Figure 5. Subcellular localization of ZDS.** (A-C) Transient expression of the ZDS-GFP fusion protein in Arabidopsis mesophyll protoplasts. The empty pEG103 vector (D-F) was used as cytoplasmic control. Confocal microscope images of the GFP fluorescence (A and D), chlorophyll autofluorescence (B and E) and the merged images for GFP and chlorophyll for each construct (C and F) are shown.



**Supplemental Figure 6. ZDS protein abundance in Arabidopsis tissues.** Total protein extracts were isolated from wild-type (*Ler*) plants from roots (r), stem (st); rosette leaves (rl), cauline leaves (cl), flowers (f) and siliques (s). Immunoblots were performed with antibodies against the ZDS protein. Each lane contains 10  $\mu$ g of total protein extract. A Coomassie blue stained gel run in parallel with the same samples is shown as a loading control (Coo). This gel is representative of two biological independent experiments.

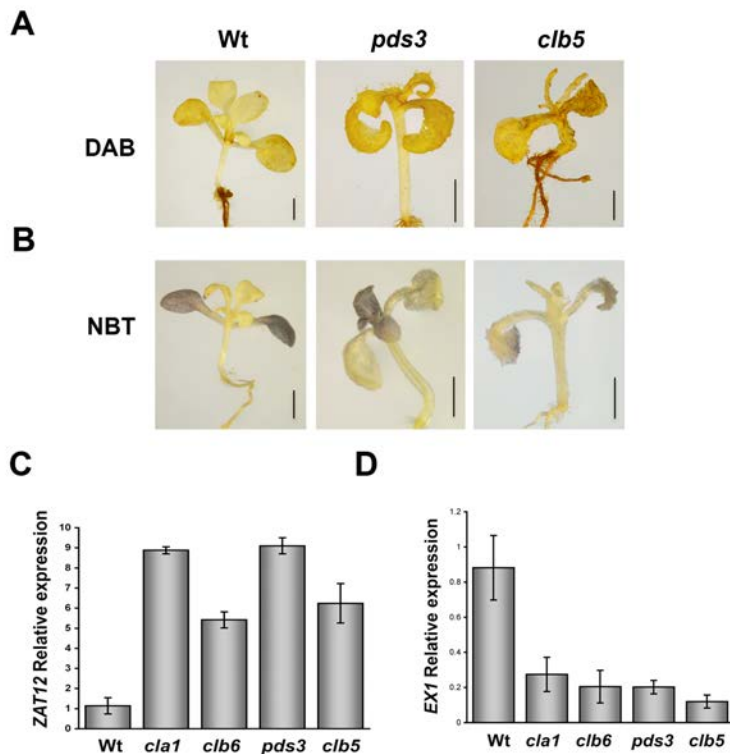


**Supplemental Figure 7. Morphological analysis of the *clb5-1* leaves.** (A) The morphology of the *clb5* leaves display a vitrified appearance with swollen cells that result in an uneven epidermal surface (arrow). (B) Morphology of the first true leaves with the occasional presence of trichomes (arrow), mostly observed at the tip of the leaves. (C) Analysis of the leaf surface demonstrated the presence of stomata in the epidermis (shown by the arrows) of the *clb5-1* leaves. (D) I<sub>2</sub>-KI staining corroborated the accumulation of starch in the epidermal stomata of the *clb5-1* leaves. Scale bar corresponds to 1 mm (A), 0.5 mm (B) and 0.1 mm (C and D).

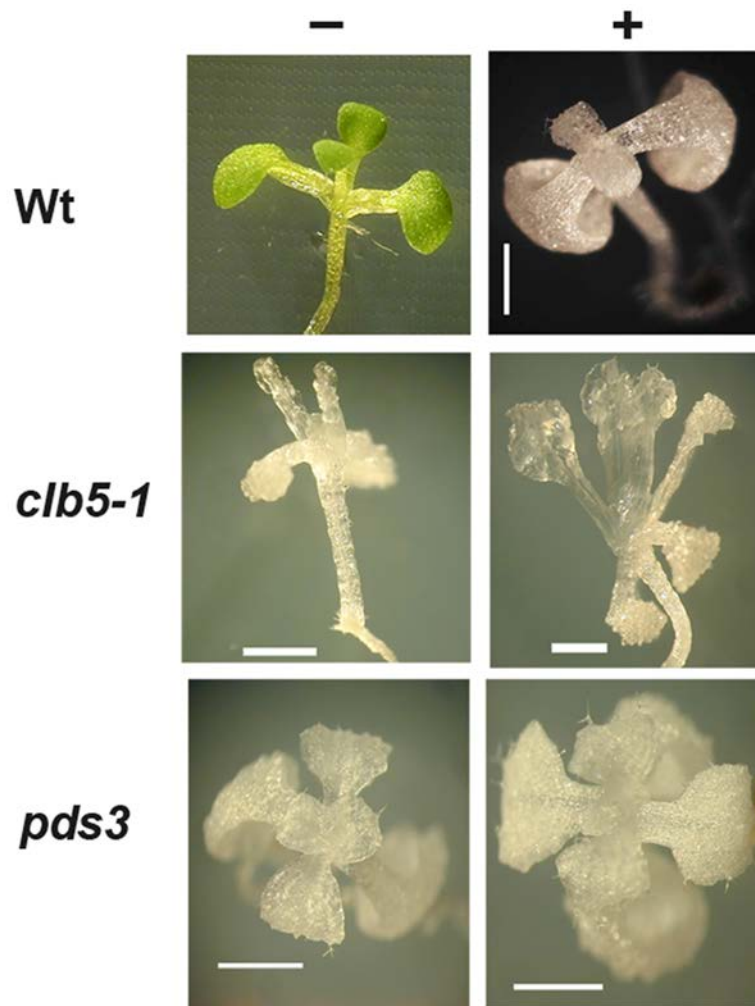


**Supplemental Figure 8. Effect of the ABA and strigolactone hormones over the *clb5* leaf morphology.** Morphology of 8 d wild-type, 14 d *clb5-1* and 10 d *pds3* seedlings grown in MS normal media (C) was compared to 8 d wild-type and 37 d *clb5-1* or *pds3* mutant seedlings grown in 0.5  $\mu$ M (ABA 0.5), 1 $\mu$ M (ABA 1) ABA or 0.5 $\mu$ M GR24 strigolactone (St). Plants were germinated in MS media and transferred to the ABA or strigolactone media 2 d after germination where they were grown in 16:8 light:dark period until morphology was analyzed. Photographs of representative seedlings were taken with a Nikon SMZ1500 stereoscopic microscope equipped with a digital SIGHT DS-Fi1c camera. Scale bar corresponds to 0.5 mm

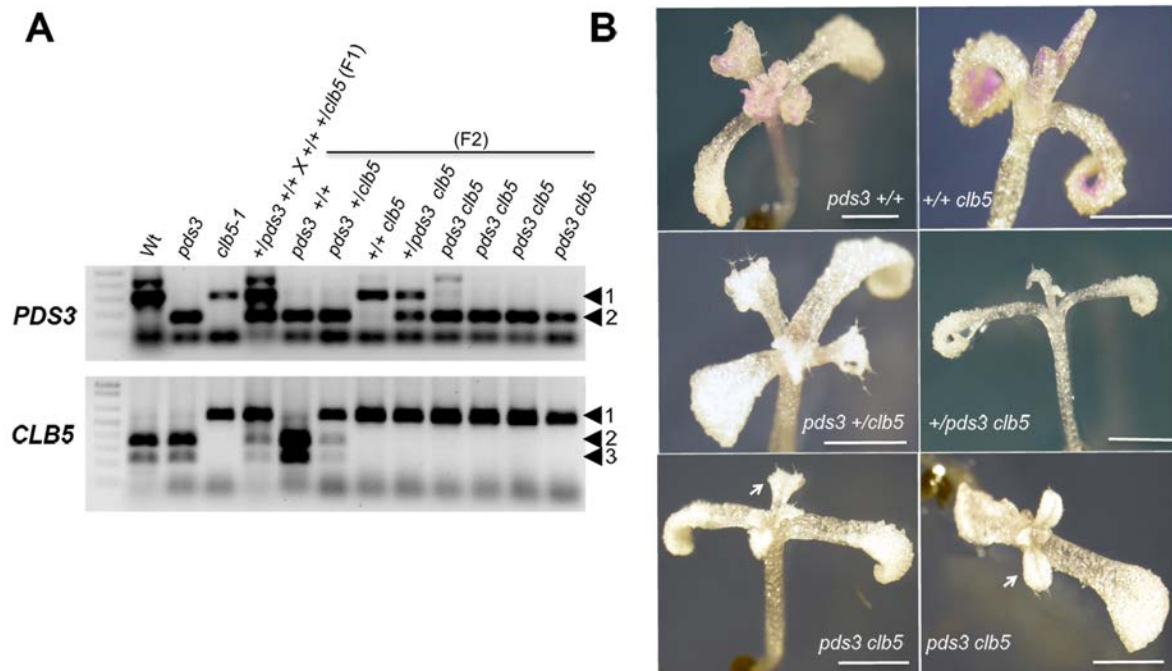




**Supplemental Figure 9. Analysis of the ROS species in *clb5* mutant.** Plants were grown in 16:8 light:dark ( $30 \mu\text{mol m}^{-2} \text{sec}^{-1}$ ) at  $22^\circ\text{C}$  for twelve d wild-type and 21 d *pds3* and *clb5* mutant and exposed to normal light ( $120 \mu\text{mol m}^{-2} \text{sec}^{-1}$ ) for 6 h. After light treatment seedlings were stained with (A) 3, 3'-diaminobenzidine tetrahydrochloride (DAB), for hydrogen peroxide or with (B) nitroblue tetrazolium (NBT) for the presence of superoxide. Scale bar corresponds to 1 mm. Quantitative PCR of the expression of (C) *ZAT12* and (D) *EXECUTER 1 (EX1)* genes in 15 d wild-type (Col) seedlings, *cla1*, *clb6*, *pds3* and *clb5* mutants grown in 16:8 light:dark ( $120 \mu\text{mol m}^{-2} \text{sec}^{-1}$ ) at  $22^\circ\text{C}$ . Error bars represented standard deviation of two biological independent experiments each done in triplicate. Significant differences exist between wild-type (Wt) and the other samples determined by Tukey analysis (confidence 99%).

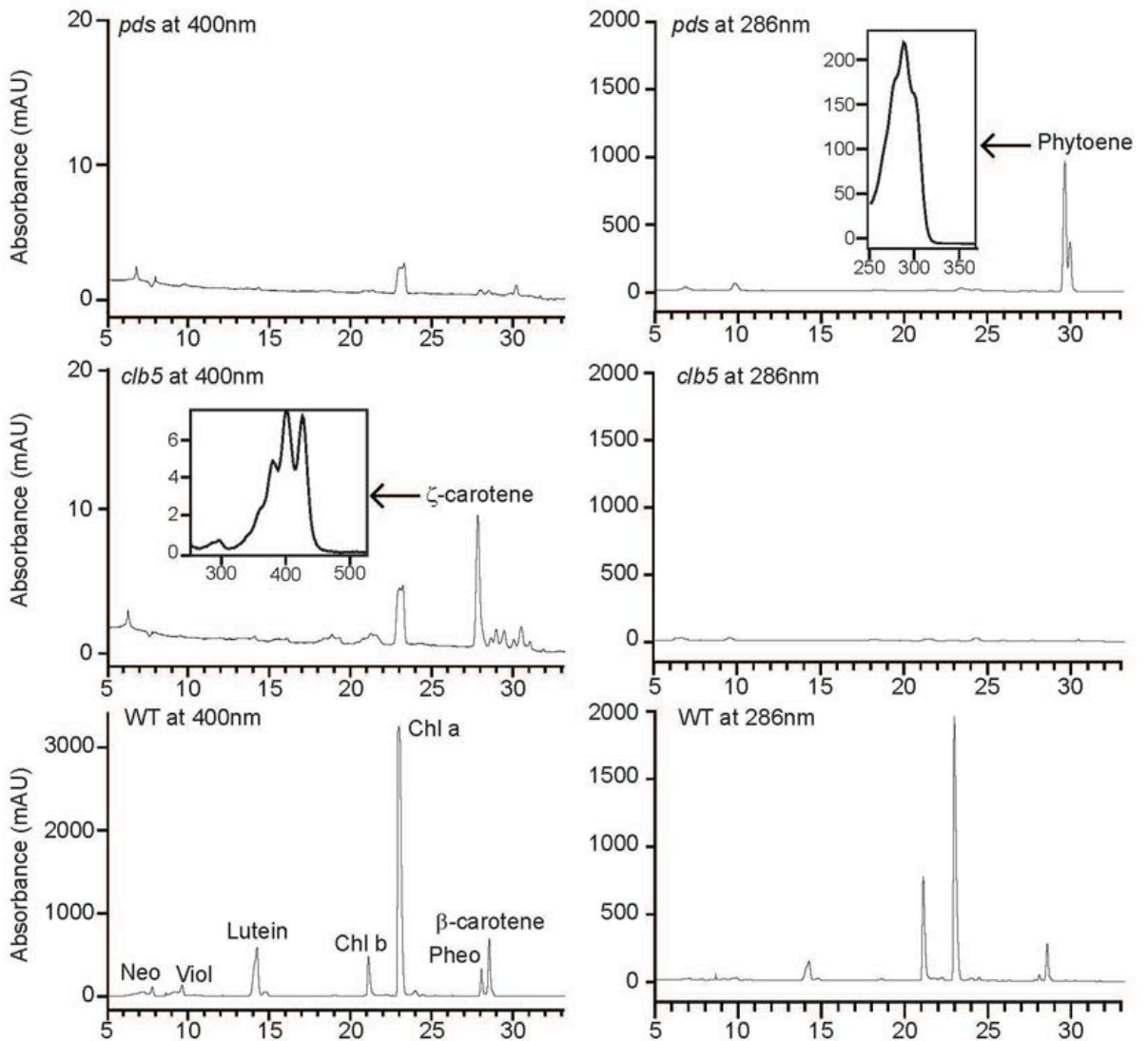


**Supplemental Figure 10. Fluridone treatment.** (a) Morphology of 15 d *clb5-1* and *pds3* seedlings treated with (+) or without (-) fluridone. The picture of the wild-type plant without fluridone corresponds to an 8 d seedling treated under the same conditions. Scale bar corresponds to 1 mm.



**Supplemental Figure 11. Genotyping of the double *pds3 clb5* mutant.** (A) Genotyping of the *pds3 clb5* double mutant. Using PCR we identified in the albino plants segregating from the F2 progeny of the cross, single mutant alleles (*pds3/++* and *clb5/++*), single mutants in heterozygous background for the other allele (*pds3 +/clb5* and *+1/pds3 clb5*) and the double *pds3 clb5* mutant. The PCR amplification product from the wild-type *PDS3* allele (Wt) is indicated by arrowhead 1 (308 bp) and from the *pds3* mutant allele by arrowhead 2 (176 bp). The PCR *Bam*HI digested fragments corresponding to wild-type *CLB5* allele (Wt), are indicated by 2 (343 bp) and 3 (225 bp) arrowheads. The undigested PCR product (586 bp, arrowhead 3) corresponds to mutant *clb5* allele. The single mutants (*pds3*, *clb5*), the F1 and segregating F2 progeny genotypes are marked in the corresponding

lane. (B) Seedling morphology of the single *pds3* (*pds3* +/+ and *pds3* +/*clb5*), *clb5* (+/+ *clb5* and +/*pds3 clb5*) and double *pds3 clb5* mutants is shown. Scale bar corresponds to 1 mm.



**Supplemental Figure 12.** HPLC chromatograms of carotenoids extracted from *pds3* and *clb5-1* mutants as well as wild-type (WT). Pigments were monitored at  $\lambda_{max}$  400 nm (major carotenoids and chlorophyll) and 286 nm (phytoene). Seedlings were grown on MS media under  $120 \mu\text{mol m}^{-2} \text{sec}^{-1}$  light condition. Clearly discernable spectra for  $\zeta$ -carotene (peaks at 380, 402 and 426 nm) in *clb5* and phytoene (peaks at 276, 286 and 297 nm) in *pds* are shown as inserts at

400 nm and 286 nm, respectively. Traces of phytofluene and  $\zeta$ -carotene derivatives and/or isomers were identified in *clb5* at 400nm, however clear spectra could not be generated due to the low resolution of HPLC. Neo, neoxanthin; Viol, violaxanthin; Chl *a*, chlorophyl *a*; Chl *b*, chlorophyl *b*; mAU, milli absorbance units.

**Supplemental Table 1.** Sequence of the oligonucleotides used in this work.

<b>GENE</b>	<b>NAME</b>	<b>SEQUENCE</b>
<i>rpoA</i>	rpoA F	5'-ACTTGGCGAAATAGAAGG-3'
	rpoA R	5'-CTCTTCTTTTTTCATTCCC-3'
<i>rpoC</i>	rpoC1 F	5'-CAATGAAATGTTGGTTGG-3'
	rpoC1 R	5'-CCCCCTTCTATCTGAATG-3'
<i>clpP1</i>	clpP1 F	5'-GTAATGATCCATCAACCCGC-3'
	clpP1 R	5'-TGAACCGCTACAAGATCAAC-3'
<i>CHLH</i>	GUN5-F	5'-CAGGGGTCTTCCGTGATCTCT-3'
	GUN5-R	5'-GTTCTTGCGTCCAGCCTCACT-3'
<i>PSY</i>	PSY F	5'-GAACCGAAGTAGAAGAATTG-3'
	PSY R	5'-GATCATCGAAGTTCTGGTAT-3'
<i>PDS3</i>	PDS3 F	5'-TTGCAGTGAAGGAACACTC-3'
	PDS3 R	5'-ACTCTTAACCGTGCCATCGT-3'
<i>ZDS</i>	ZDS5	5'-CACCATGGCTTCTTCAGTCGTCTTC-3'
	ZDS3	5'-CATCATTAGACCAGACTTAGC-3'
	4870-Exo7F	5'-CGAAGCTAGTGCAAGATCCGC-3'
	ZDST5	5'-CAAAC TAAGGAATTC ACTGG-3'
	ZDST3	5'-CCCATAGTGTCTCAAATCTTC-3'
	4870R	5'-GCTGTCAAGGTTGCGAATGTC-3'
<i>CRTISO</i>	CRTISO-F	5'-GGCATTGAAGGCAGTTGGTTCG-3'
	CRTISO-R	5'-GCCTTGTATTGTATTTCACTG-3'
<i>EX1</i>	EX1-Fq	5'-TCTTCTGACACTTGATGGG-3'
	EX1-R	5'-CCAGTATCTTCCTTCCTTCC-3'
<i>ZAT12</i>	ZAT12-Fq	5'-CCACCATCCCTAGACTCAGA-3'
	ZAT12-R	5'-CCACCATCCCTAGACTCAGA-3'
<i>CCD4</i>	CD4FW	5'-ACAGACTGTGAAATCATCCAC-3'
	SALKLB	5'-GCGTGGACCGCTTGCTGCAACT-3'