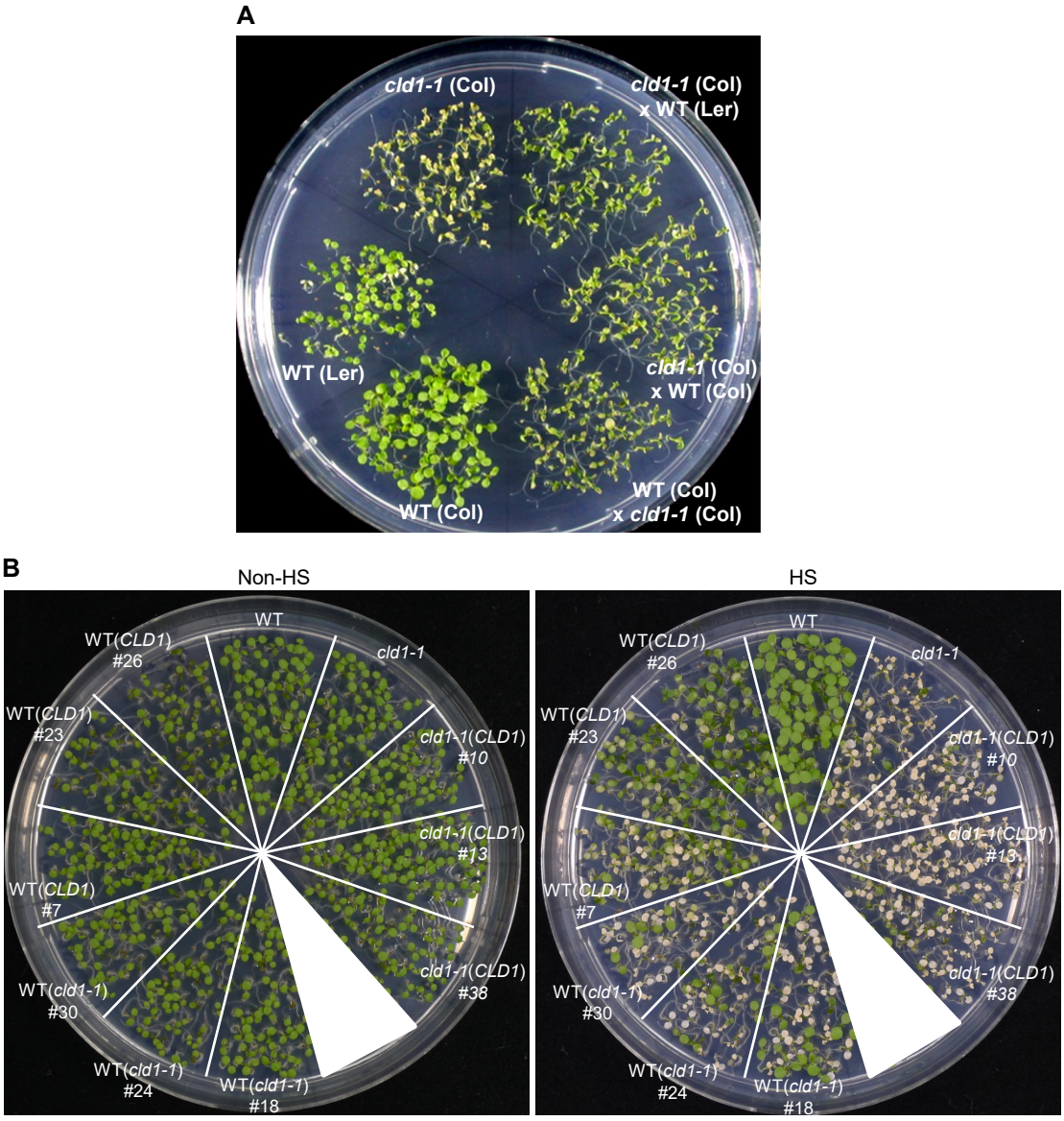
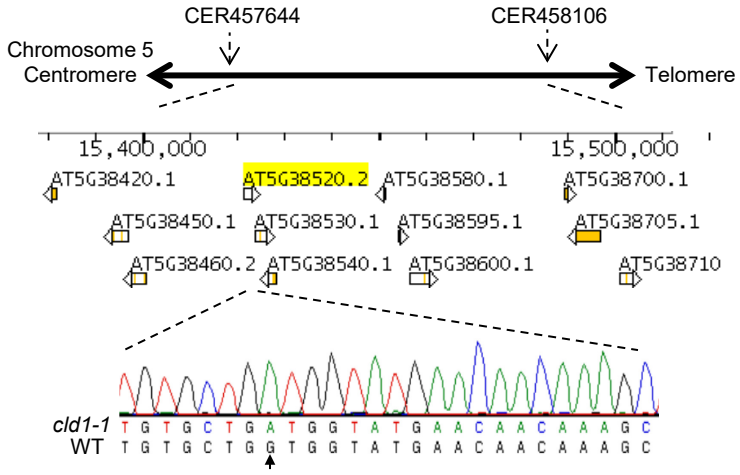
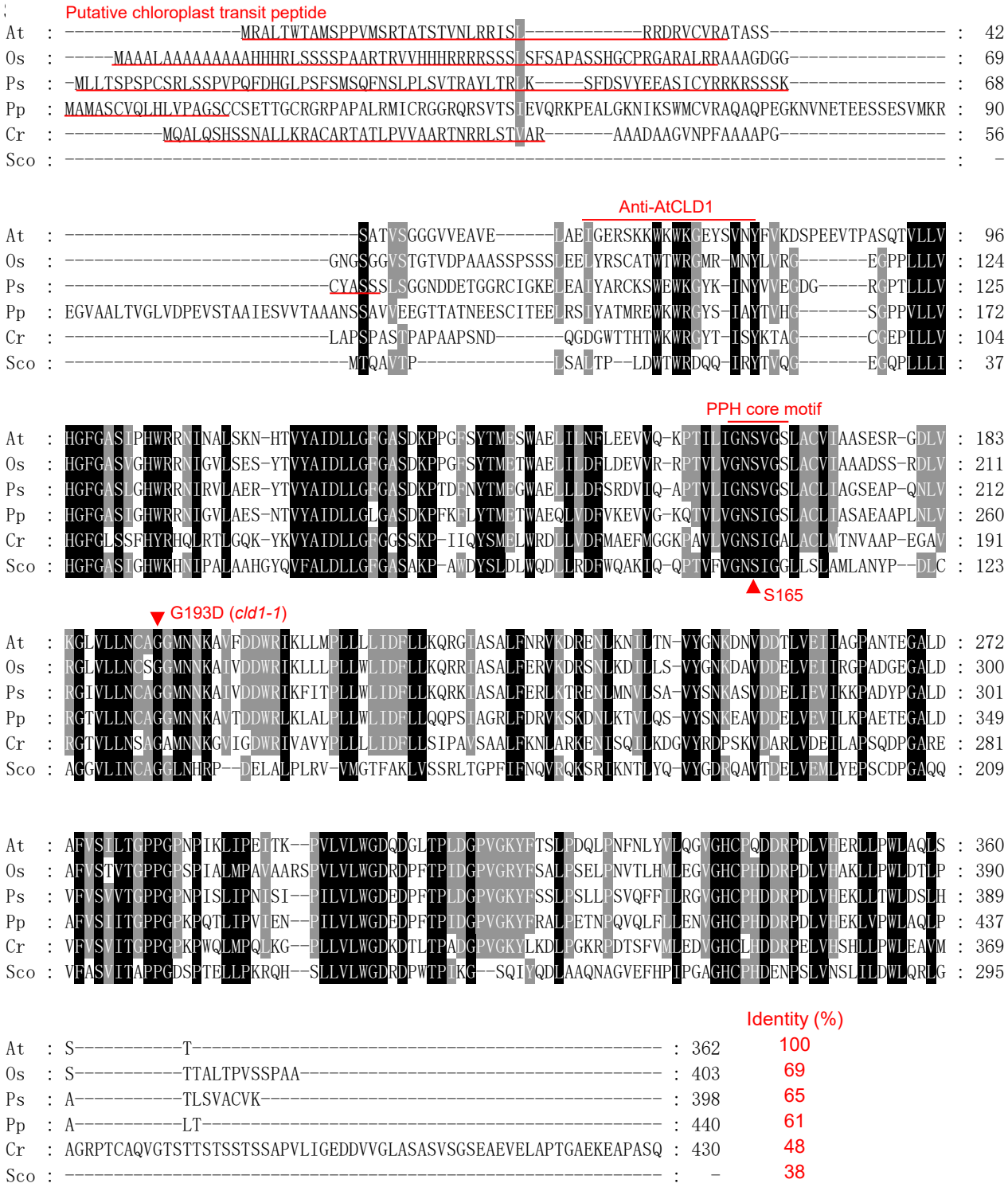


Supplemental Figure 1. *cld1-1* is a Semi-dominant Mutation Revealed by Genetic Crossing and Complementation Test.
(A) Different cotyledon bleaching phenotypes of 5-d-old seedlings of the indicated lines subjected to heat-shock (HS) treatment. The image was obtained 3 d after HS treatment. F1 seedlings from the reciprocal crosses between the wild type (WT, of Col or L*er* background) and *cld1-1* showed slow cotyledon bleaching phenotype as compared with the WT (non-bleaching) and *cld1-1* (fast bleaching). **(B)** Phenotyping of transgenic lines with or without HS treatment. The transgenic lines are labeled as *cld1-1*(*CLD1*), *cld1-1* transformed with ectopic *CLD1* genomic DNA; WT(*cld1-1*), WT transformed with ectopic *cld1-1* genomic DNA; WT(*CLD1*), WT transformed with ectopic *CLD1* genomic DNA. Numbers after the symbol # indicate the line designations derived from independent transformation events.



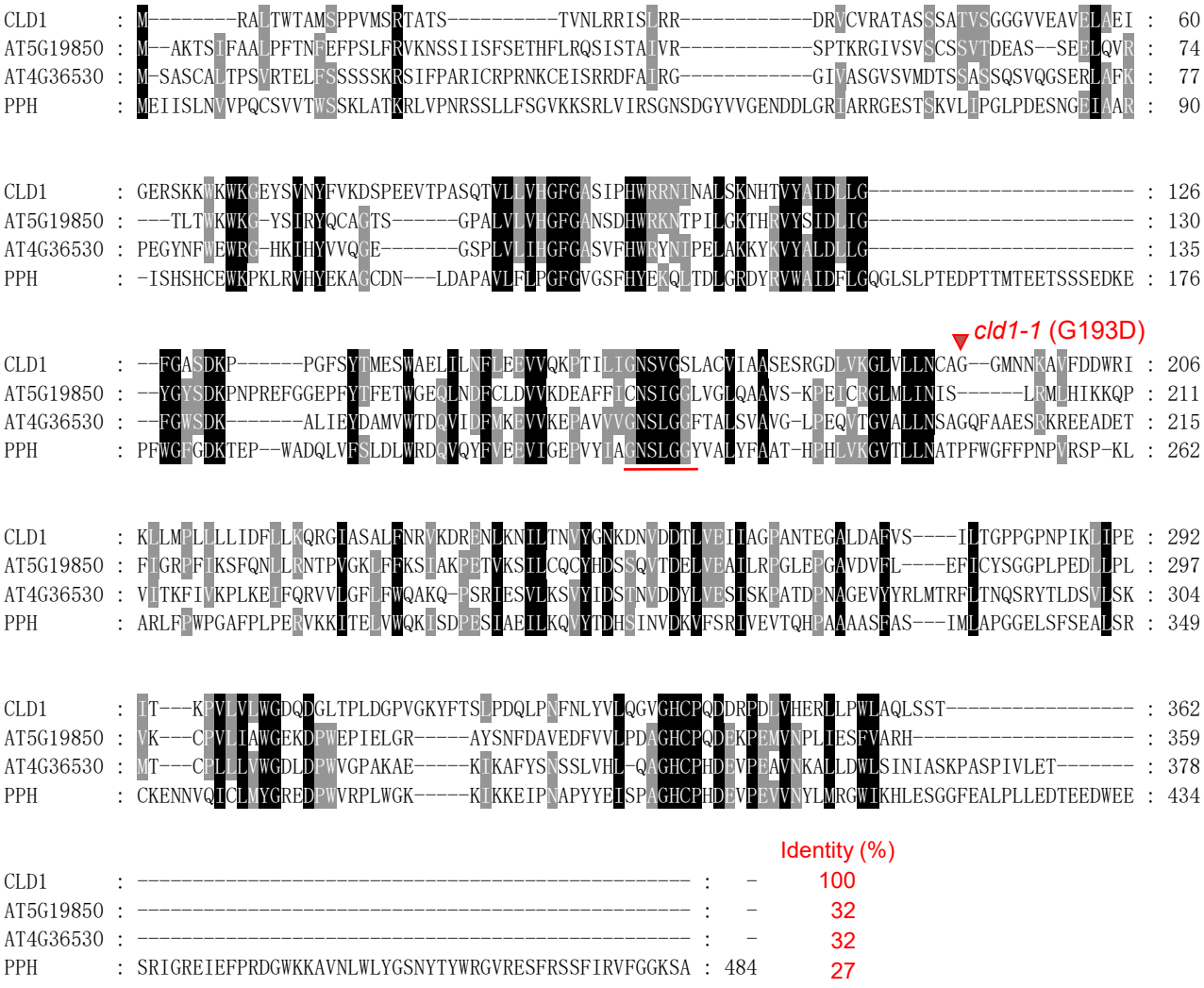
Supplemental Figure 2. Map-based Cloning of *cld1-1*.

The mutated site in *cld1-1* was mapped and narrowed down to a region flanked by CER457644 and CER458106 on chromosome 5. Genes within the two flanking markers were sequenced, and one G-to-A transition (arrowed) was identified in locus AT5G38520. The diagram of gene arrangement was cropped from TAIR.



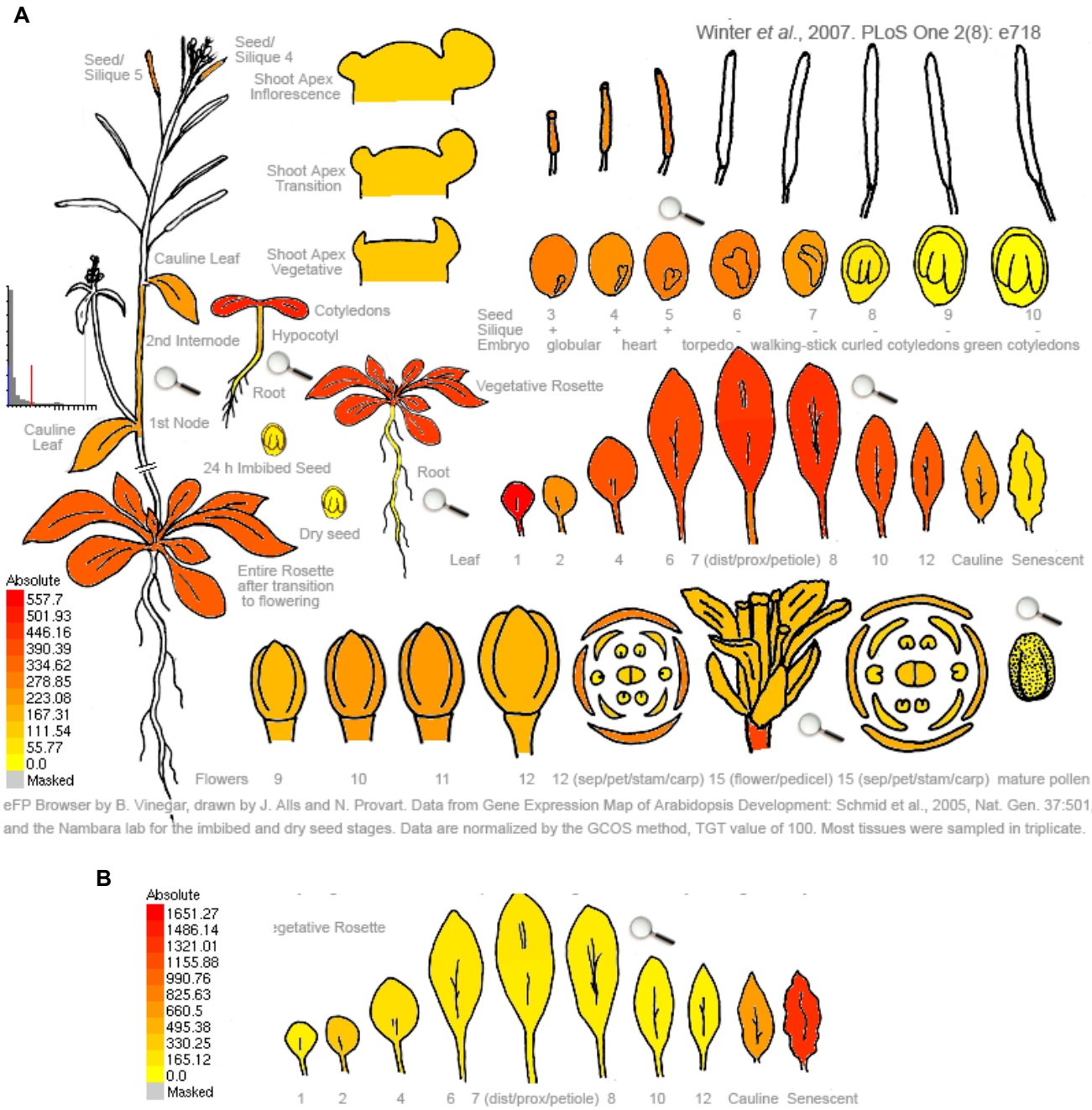
Supplemental Figure 3. Alignment of the Amino Acid Sequences of CLD1 Orthologs from Different Species.

CLD1 orthologs from representative species mentioned in Figure 2A were aligned. At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Ps, *Picea sitchensis*; Pp, *Physcomitrella patens*; Cr, *Chlamydomonas reinhardtii*; Sco, *Synechococcus* sp. PCC 7002. Sequence identities shared between AtCLD1 and its orthologs were calculated from sequences without the putative chloroplast transit peptide (underlined). The peptide sequence for induction of anti-AtCLD1 antibody is shown on top of the alignment .



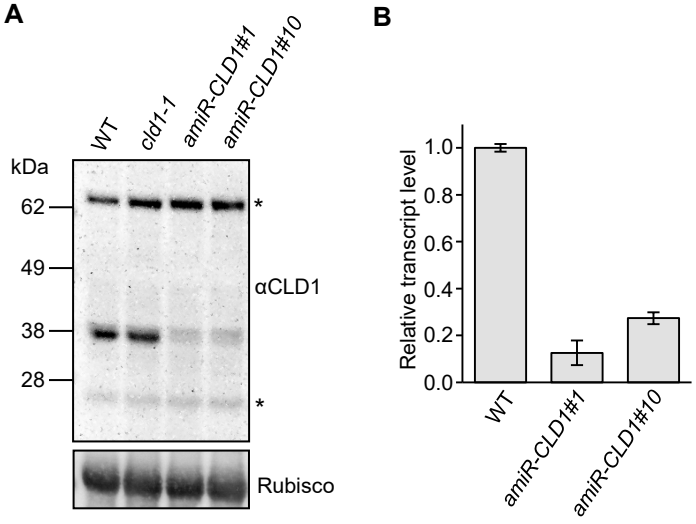
Supplemental Figure 4. Alignment of the Amino Acid Sequences of Arabiodopsis CLD1 Homologs.

CLD1 homologous sequences were obtained from the TAIR database and aligned by using MEGA6. The PPH core motif is underlined. Sequence identities shared between CLD1 and its homologs were estimated from sequences without the putative chloroplast transit peptide.



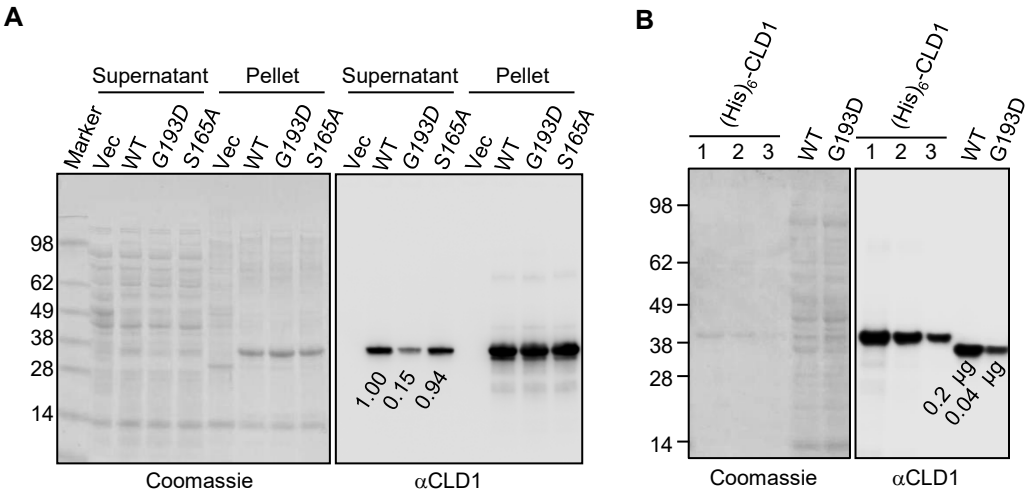
Supplemental Figure 5. Abundance of *CLD1* mRNA in Different Tissues and Developmental Stages in Arabidopsis.

(A) The expression profile of *CLD1* is from the developmental map of Arabidopsis eFP Browser (http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi). Transcript levels are shown in red. **(B)** The expression profile of *PPH* in leaves at different developmental stages.



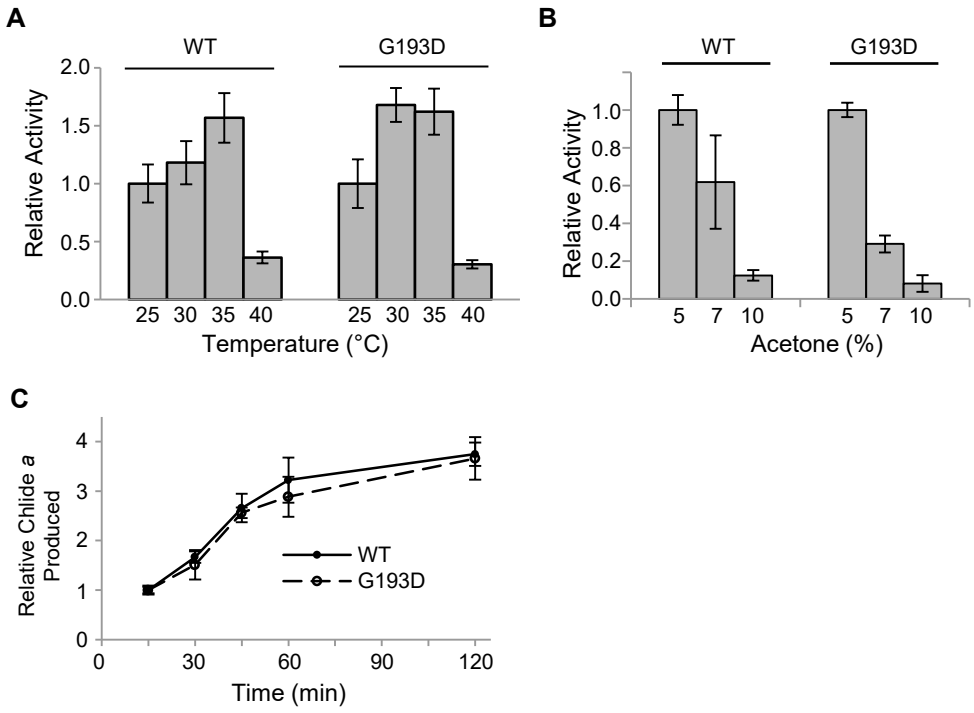
Supplemental Figure 6. Immunoblot and Quantitative RT-PCR Analyses of *CLD1* Expression in *amiR-CLD1* Lines.

(A) Immunoblot analysis of CLD1 in total proteins extracted from 5-d-old seedlings of indicated lines. In each lane, 70 μg of protein was loaded, and Rubisco protein stained by Amido black is shown as loading control. Asterisks indicate non-specific bands. **(B)** Quantitative RT-PCR analysis of *CLD1* transcripts in 5-d-old seedlings of indicated lines. Relative transcript level of *CLD1* was normalized to that of *ACTIN2*, with that of the wild type (WT) set to 1. Data are means ± SD from three replicates.



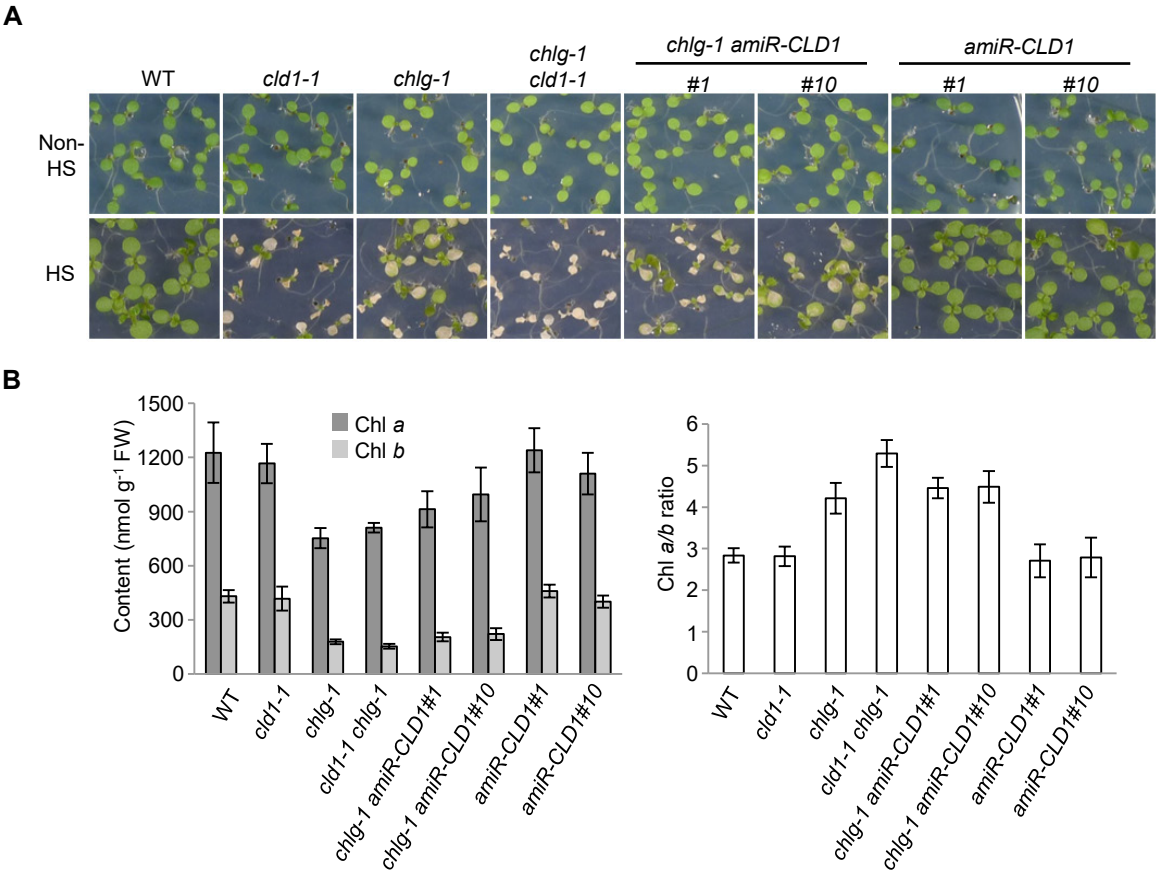
Supplemental Figure 7. Recombinant CLD1 Expressed in *E. coli* Cells and Its Quantification.

(A) Immunoblot analysis of the lysates of *E. coli* cells harboring constructs encoding recombinant WT or mutated CLD1 proteins (G193D and S165A).The lysates were centrifuged to separate soluble from insoluble proteins present in the supernatant and the pellet, respectively. Proteins were resolved on SDS-PAGE followed by Coomassie blue staining and immunoblotting. Each lane contained 10 μg proteins. The quantification was performed by using ImageJ. The band intensity for each supernatant sample was normalized to that of WT and indicated under the band.
(B) Quantification of CLD1 in *E. coli* expressing WT or G193D protein by immunoblot analysis with the purified $(His)_6$-CLD1 protein as standard. Lane 1, 2, and 3 were loaded with 0.4, 0.2, and 0.08 μg of $(His)_6$-CLD1 protein, respectively. WT and G193D protein in *E. coli* crude extracts (10 μg) were estimated to be 0.2 and 0.04 μg, respectively.



Supplemental Figure 8. Effect of Temperature and Acetone on CLD1 Activity.

(A) The effect of different temperature on the WT and G193D enzyme activity with 5% acetone in the reaction mixtures. The enzyme activities were expressed in relation to that obtained at 25°C. Data are means ± SD of three replicates.
(B) The effect of different acetone concentration on the WT and G193D enzyme activity assayed at 35°C for 1h. The enzyme activities were expressed in relation to that obtained with 5% acetone. Data are means ± SD of three replicates.
(C) Time course analysis of CLD1 enzyme activity with 5% acetone at 35°C. The reaction mixtures contained 55 μM Chl *a* and 0.4 or 0.08 μg of WT or G193D protein, respectively. The amount of Chlide *a* produced by the enzyme was expressed in relation to that produced at 15 min. Data are means ± SD of three replicates.



Supplemental Figure 9. Phenotypes of Arabidopsis Seedlings with Varied CLD1/CHLG Ratios in Response to Heat Shock and Their Chl Content.

(A) Phenotypes of 5-d-old seedlings with and without HS at 40°C for 1 h with 3-d recovery under light/dark cycle. Seedlings in the same row were grown on the same plate and reorganized for presentation. **(B)** Chl *a*/*b* content and ratio of 5-d-old seedlings under normal condition. Data are means ± SD of four independent experiments.

ANOVA for Figure 6A, Chlide *a*

	df	SS	MS	F-values	p-values*
Genotype x treatment	11	23252.10	2113.83	131.22	0
residuals	24	386.61	16.11		0

* p-value lower than 0.0001 is marked with 0.

ANOVA for Figure 6A, Chlide *b*

	df	SS	MS	F-values	p-values*
Genotype x treatment	11	78.94	7.18	14.13	0
residuals	24	12.19	0.51		0

* p-value lower than 0.0001 is marked with 0.

Tukey's HSD groups for Figure 6A

	Chlide *a*		Chlide *b*	
Genotype x treatment	Mean	Group	Mean*	Group
Non-HS WT	1.56	d	nd	
Non-HS WT(*CLD1*)#23	1.89	d	nd	
Non-HS WT(*CLD1*)#26	1.36	d	nd	
Non-HS *cld1-1*	1.73	d	nd	
Non-HS WT(*cld1-1*)#18	1.09	d	nd	
Non-HS WT(*cld1-1*)#24	1.33	d	nd	
HS WT	2.01	d	nd	
HS WT(*CLD1*)#23	12.19	d	0.67	bc
HS WT(*CLD1*)#26	12.49	d	0.79	bc
HS *cld1-1*	30.47	c	2.07	bc
HS WT(*cld1-1*)#18	60.50	b	3.12	ab
HS WT(*cld1-1*)#24	79.98	a	4.68	a

* nd, not detected.

ANOVA for Figure 7B, Chlide *a*

	df	SS	MS	F-values	p-values*
Genotype x treatment	15	8104.00	540.27	93.00	0
residuals	32	185.90	5.81		

* p-value lower than 0.0001 is marked with 0.

Tukey's HSD groups for Figure 7B

	Chlide *a*	
Genotype x treatment	Mean	Group
Non-HS WT	1.05	f
Non-HS *cld1-1*	1.76	f
Non-HS *chlg-1*	4.70	def
Non-HS *cld1-1 chlg-1*	4.63	def
Non-HS *chlg-1 amiR-CLD1#1*	5.01	cdef
Non-HS *chlg-1 amiR-CLD1#10*	4.95	cdef
Non-HS *amiR-CLD1#1*	1.56	f
Non-HS *amiR-CLD1#10*	1.12	f
HS WT	2.22	ef
HS *cld1-1*	30.81	b
HS *chlg-1*	12.50	cd
HS *cld1-1 chlg-1*	50.79	a
HS *chlg-1 amiR-CLD1#1*	13.30	c
HS *chlg-1 amiR-CLD1#10*	10.42	cde
HS *amiR-CLD1#1*	1.78	f
HS *amiR-CLD1#10*	2.14	ef

Supplemental Figure 10. ANOVA tables for Figures 6, 7, and 8.

ANOVA for Figure 8D

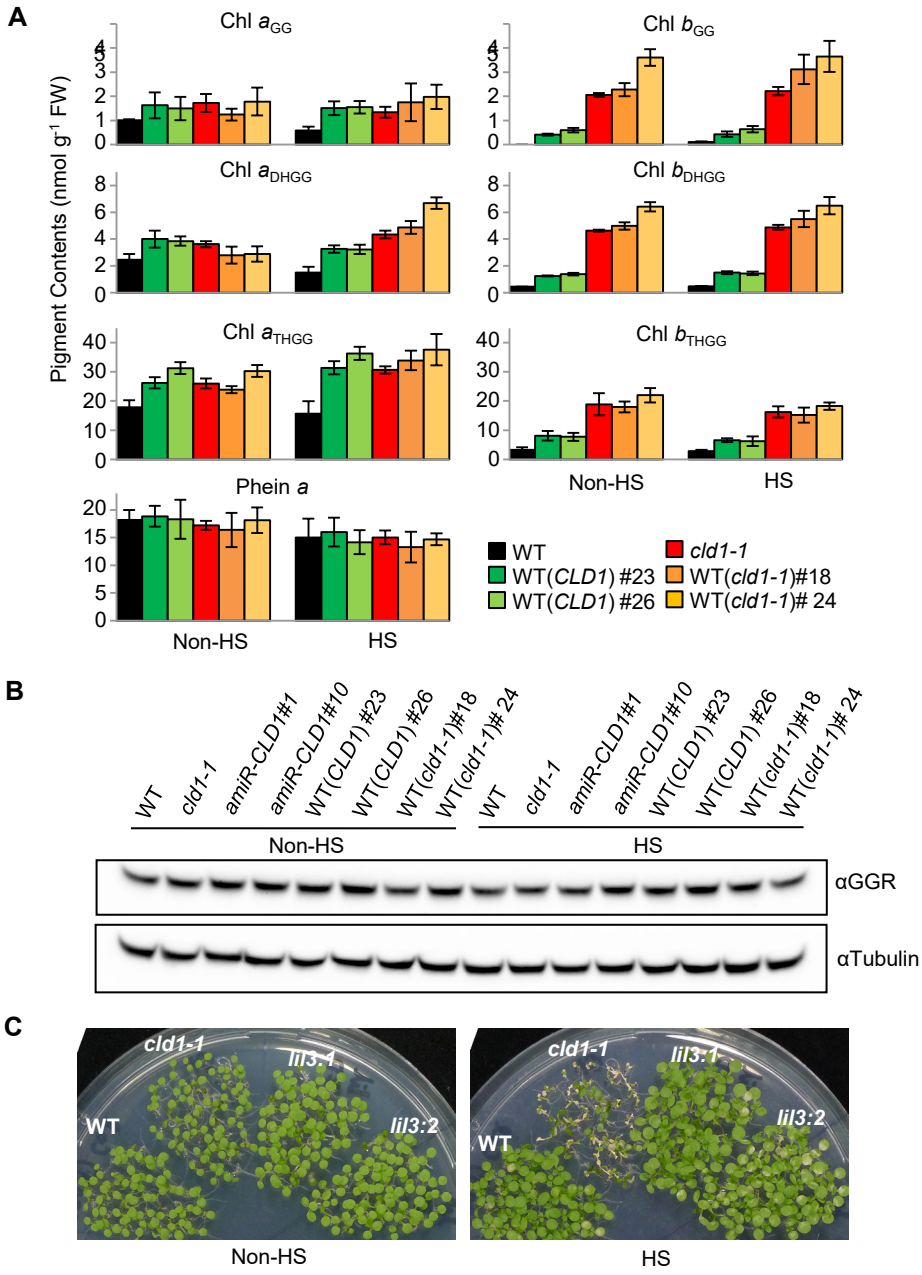
	df	SS	MS	F-values	p-values*
Genotype x treatment	11	3.93	0.36	637.44	0
residuals	48	0.03	5.60		0

* p-value lower than 0.0001 is marked with 0.

Tukey's HSD groups for Figure 8D

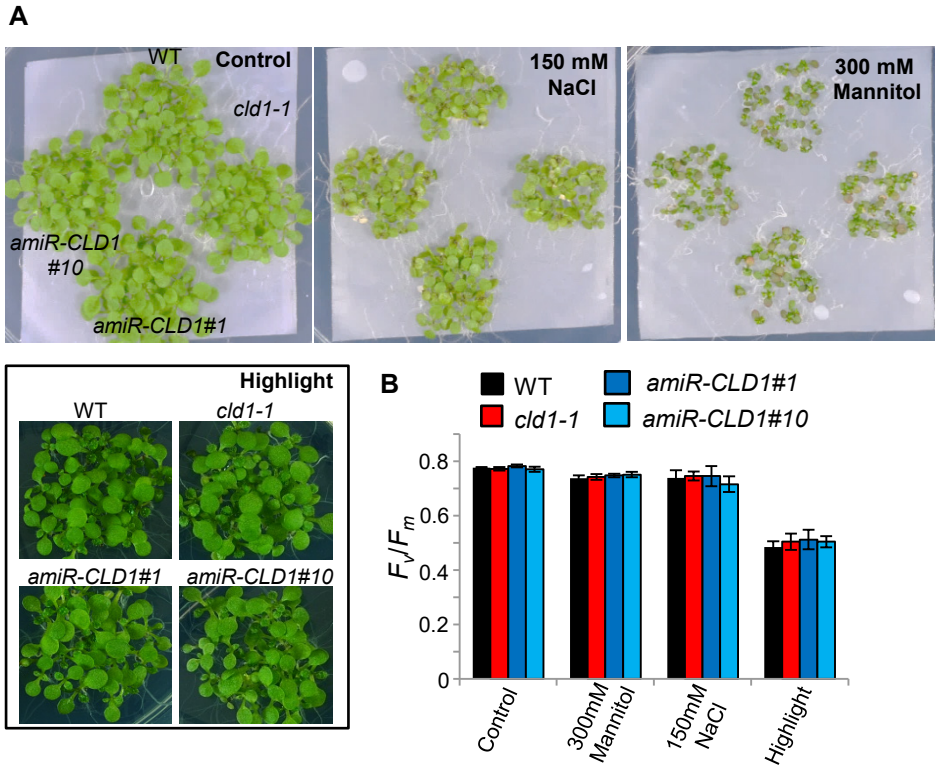
Genotype x treatment	Mean	Group
Non-HS WT	0.789	a
Non-HS *cld1-1*	0.787	a
Non-HS *amiR-CLD1#1*	0.787	a
Non-HS *amiR-CLD1#10*	0.787	a
40˚C 30min WT	0.164	e
40˚C 30min *cld1-1*	0.098	f
40˚C 30min *amiR-CLD1#1*	0.251	d
40˚C 30min *amiR-CLD1#10*	0.222	de
35˚C 5d WT	0.599	b
35˚C 5d *cld1-1*	0.569	b
35˚C 5d *amiR-CLD1#1*	0.387	c
35˚C 5d *amiR-CLD1#10*	0.369	c

Supplemental Figure 10. (continued)

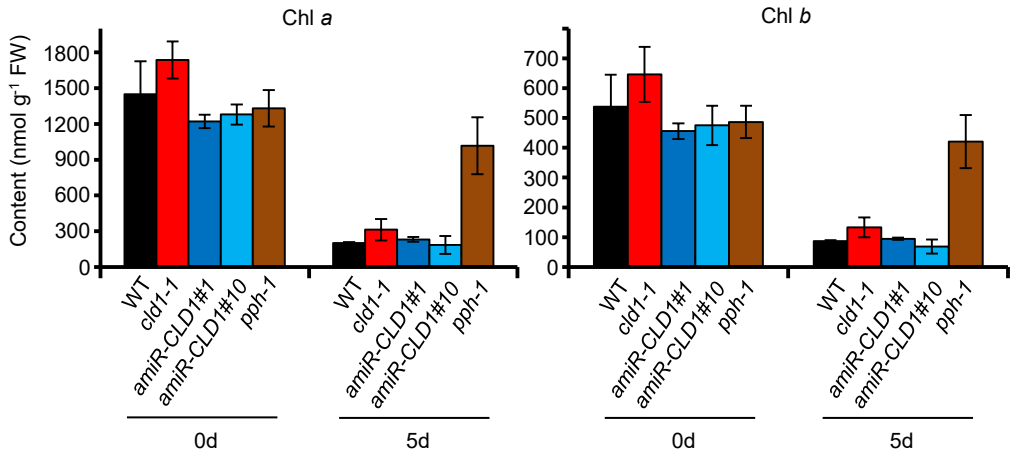


Supplemental Figure 11. Accumulation of Chls with Unsaturated Side Chains and Effect on Cotyledon Bleaching Phenotype.

(A) Total Chls extracted from 5-d-old seedlings with or without HS treatment at 40°C for 1 h were analyzed by HPLC. Data are means ± SD of three independent experiments. **(B)** Immunoblots of total proteins extracted from 5-d-old seedlings of indicated seedling lines before and after HS. In each lane, 50 µg of protein was loaded. **(C)** Phenotyping of 5-d-old seedlings of the indicated lines with or without HS treatment. The images were obtained after 3-d recovery under light/dark cycle after HS.



Supplemental Figure 12. Growth and PSII Maximum Efficiency of *cld1-1* and Two *amiCLD1* Lines under Osmotic, Salt, and High Light Stresses.
(**A**) Seeds were sown on nylon mesh with normal medium and imbibed at 4°C for 3 d in the dark. After growing for 5 d under normal conditions, the seedlings with mesh were transferred to medium with indicated stress factors for another 3 d of growth. For highlight stress assay, 5-d-old seedlings grown on normal plate was incubated at 22°C for 6 h under 1000 μmol m^{-2} s^{-1} light intensity, and the picture was taken after 3-d recovery. (**B**) PSII maximum efficiency of seedlings shown in **(A)**. Data are means ± SD of three independent experiments. For highlight stress assay, the F_v/F_m was analyzed immediately after 6 h treatment.



Supplemental Figure 13. Chl Breakdown in Dark-Induced Senescence Leaves.

The first five rosette leaves were detached from 4-week-old plants of the indicated lines and kept in a humid box for 5 d at 22°C in the dark. Total Chls were extracted and analyzed by spectrometry. Data are means ± SD of three independent experiments.

Supplemental Table 1. Phenotypic Segregation of F2 Seedlings from the Cross Between *cld1-1* (Col) and Wild Type (WT, L*er*) after Heat Shock (HS) treatment. The inheritance model of a single semi-dominant gene was tested.

Cross	Number of F2 seedlings with indicated phenotype			Total	X^2	*P*	Inheritance
	non-bleaching	slow bleaching	fast bleaching				
1	60	96	44	200	2.88	0.24	semi-dominant
2	58	88	49	195	2.69	0.26	semi-dominant
3	52	102	47	201	0.29	0.87	semi-dominant
total	170	286	140	596	1.12	0.57	semi-dominant

Supplemental Table 2. List of oligonucleotide primers used in this study.

Purposes and primer names	Primer sequence (5' to 3')
Complementation test	
YP011	TCAAATTTCGTCGTGTTGGA
YP012	GACCATCAATCATTCATCAACC
amiR-CLD1 construction	
YP021	CGAGGAGATCTGGTCAAAGG
YP022	ATCCGGAAGGGAAGTGAAGT
allele-specific qPCR	(mutated sites are underlined)
YP031 (for *CLD1*)	CTTGTTCTATTGAATTGTGATGG
YP033 (for *cld1-1*)	CTTGTTCTATTGAATTGTGATGA
YP032 (for both *CLD1* and *cld1-1*)	CACCTTCGGTATTTGCTGGTC
YP041 (for *ACT2*)	ACCCAAAGGCCAACAGAGAGA
YP042 (for *ACT2*)	AGAATCCAGCACAATACCTGTT
Subcellular localization of CLD1-GFP	
YP051	ATGAGAGCTCTAACATGGACGGC
YP052	GGTGGAAGAAAGTTGAGCCAGCC
Recombinant proteins production	
WT or G193D	(with *NdeI* site)
YP061	GTCATATGGCCACGGCTTCGTCTAGCGCC
YP062	GACATATGCTAGGTGGAAGAAAGTTGAG
S165A	(mutated site is underlined)
YP071	ATTGGAAACGCTGTTGGAAGCCTTG
YP072	CAAAATAGTCGGTTTCTGAACCACT