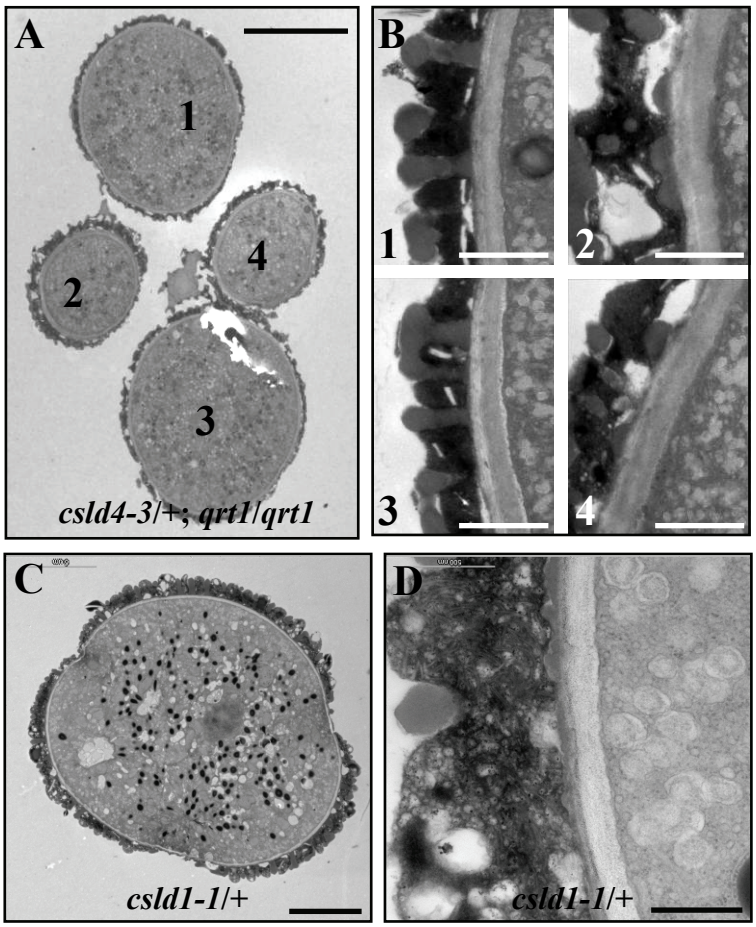
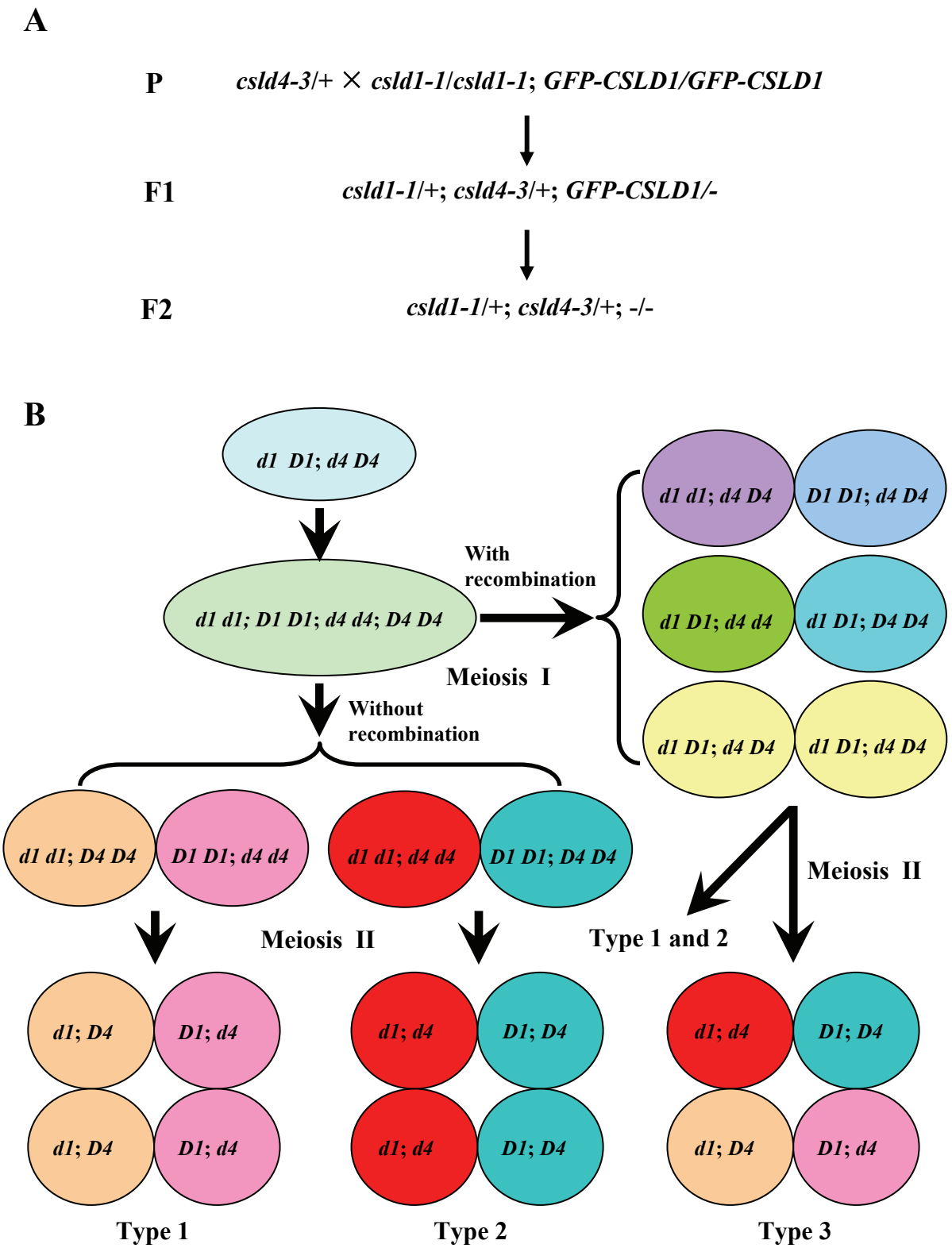
Supplementary Figures

Figure S1



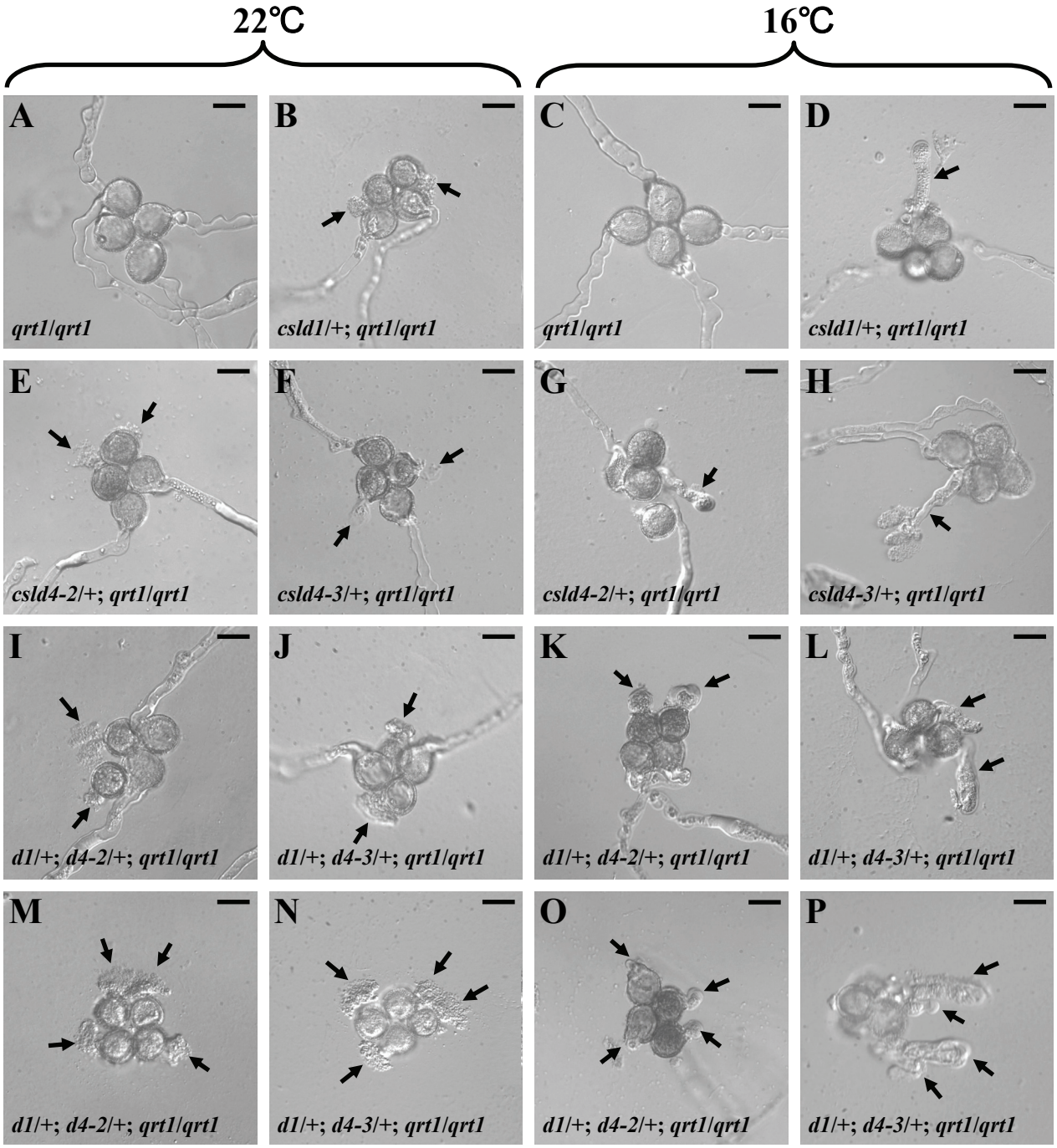
Supplementary Fig. S1. TEM observation of mutant pollen grains before germination.
(A) Overview of a quartet from *csld4-3*/+; *qrt1*/*qrt1* plants before germination.
(B) Close-up of (A). All of the four pollen grains have normal cell wall.
(C) Overview of a pollen grain from *csld1-1*/+ plants before germination.
(D) Highly-magnified images of the pollen grains in (C). The cell wall is normal.
Bars: (A) 10 μm; (B) 1 μm; (C) 5 μm; (D) 500 nm.

Figure S2



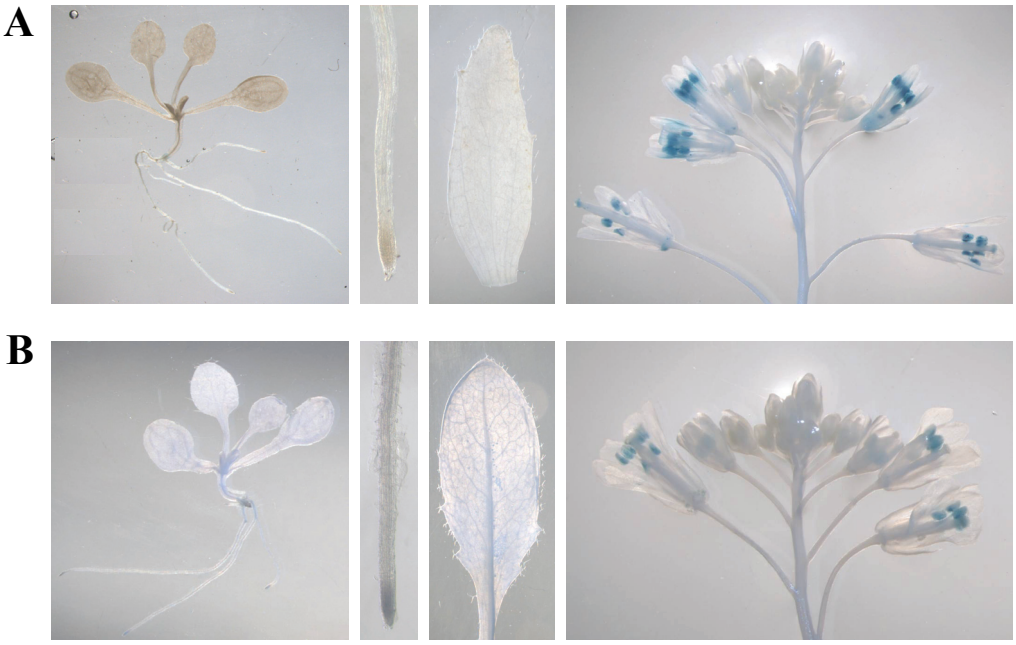
Supplementary Fig. S2. Generation of *csld1 csld4* double mutants.
(A) Diagram of generation of *csld1* and *csld4* double mutants.
(B) Diagram of formation of three types of quartets from *csld1*/+; *csld4*/+; *qrt1*/*qrt1* plants. *D1*, *D4*, *d1* and *d4* represent *CSLD1*, *CSLD1*, *csld1* and *csld4*, respectively.

Figure S3



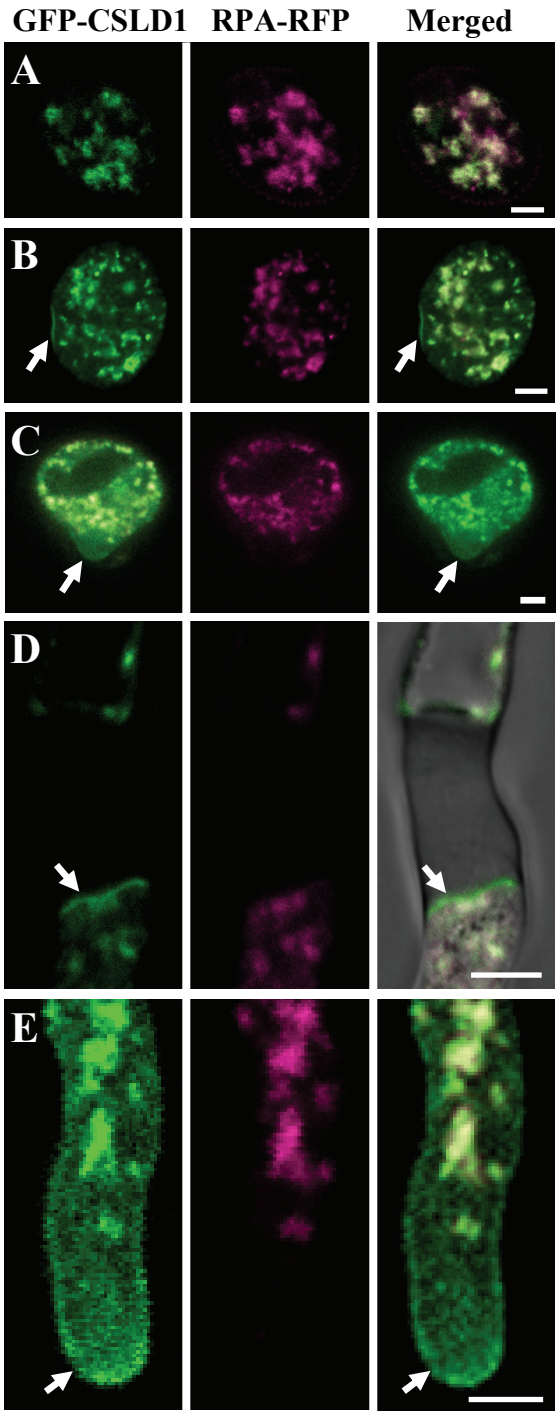
Supplementary Fig. S3. *In vitro* germination of quartets from mutants and wild-type plants at different temperatures.
(A-B, E-F, I-J and M-N) *in vitro* germination of quartets at 22° C. These quartets were obtained from *qrt1*/*qrt1* (A), *csld1-1*/+; *qrt1*/*qrt1* (B), *csld4-2*/+; *qrt1*/*qrt1* (E), *csld4-3*/+; *qrt1*/*qrt1* (F), *csld4-2*/+; *csld1-1*/+; *qrt1*/*qrt1* (I, M) and *csld4-3*/+; *csld1-1*/+; *qrt1*/*qrt1* (J, N) plants. Arrows indicated ruptured pollen tubes.
(C-D, G-H, K-L and O-P) *in vitro* germination of quartets at 16° C. These quartets were obtained from *qrt1*/*qrt1* (C), *csld1-1*/+; *qrt1*/*qrt1* (D), *csld4-2*/+; *qrt1*/*qrt1* (G), *csld4-3*/+; *qrt1*/*qrt1* (H), *csld4-2*/+; *csld1-1*/+; *qrt1*/*qrt1* (K, O) and *csld4-3*/+; *csld1-1*/+; *qrt1*/*qrt1* (L, P) plants. Arrows indicate short aberrant pollen tubes.
Bars: 20 μm.

Figure S4



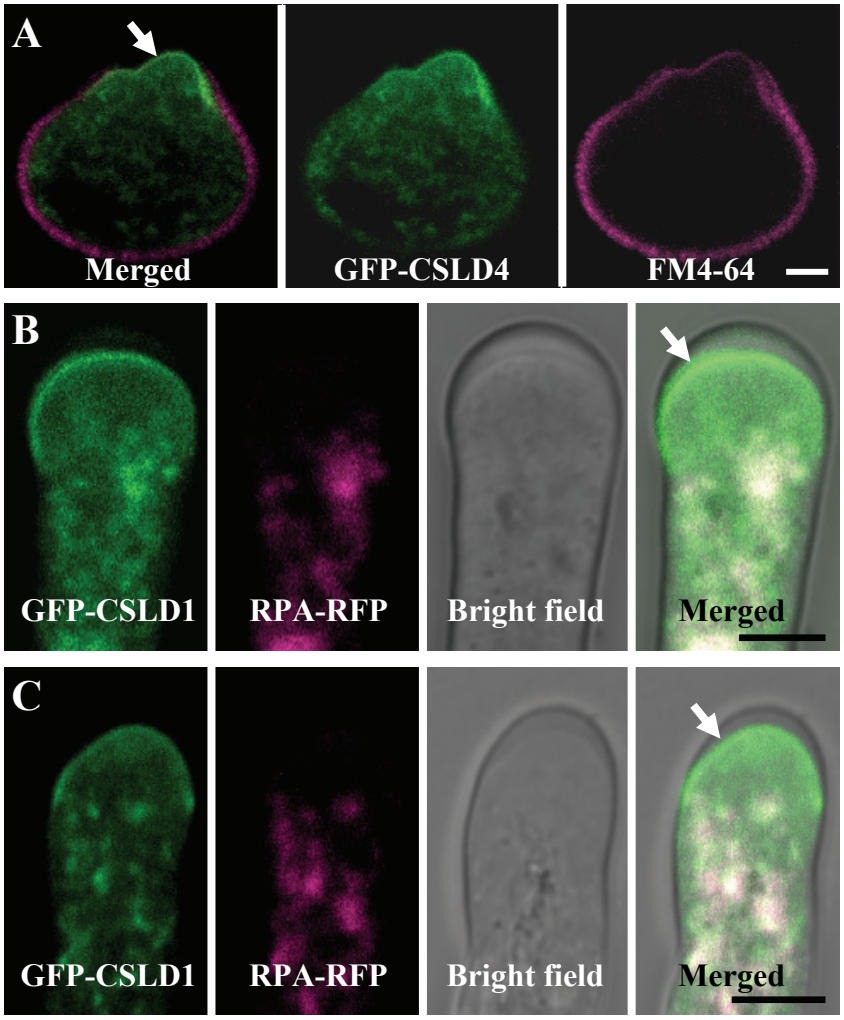
Supplementary Fig. S4. Expression patterns of *CSLD1* and *CSLD4*.
(A) p*CSLD1:GUS* transgenic plants, showing GUS stains in the anthers.
(B) p*CSLD4:GUS* transgenic plants, showing GUS stains in the anthers.

Figure S5



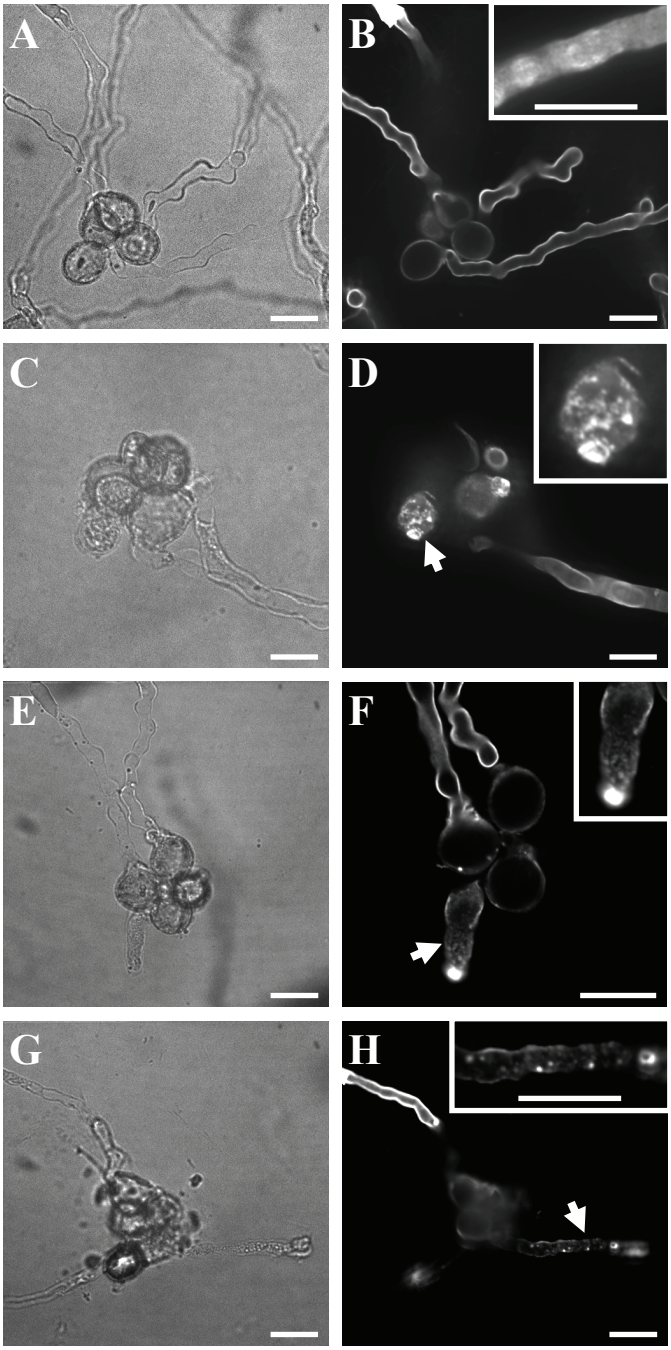
Supplementary Fig. S5. Subcellular localization of CSLD1 in Arabidopsis pollen grains and pollen tubes.
(A) An transgenic pollen grain before germination, showing the GFP-CSLD1 signals colocalized with RPA-DsRed2 in Golgi bodies of the pollen grain.
(B and C) A germinating transgenic pollen grain, showing the polar localization of the GFP-CSLD1 signals in the cell periphery (B, arrow) and the plasma membrane (C, arrow) at the germinating point.
(D) A pollen tube, showing that the GFP-CSLD1 signals were found at the plasma membrane adjacent to the periphery of the pollen tube plugs (arrow).
(E) The tip region of a pollen tube, showing that the GFP-CSLD1 signals were found colocalized with RPA-DsRed2 signal only in the shank region where RPA-DsRed2 signal also appeared, the clear zone and the plasma membrane (arrow) where RPA-DsRed2 signal was absent.
Bars: 5 μm.

Figure S6



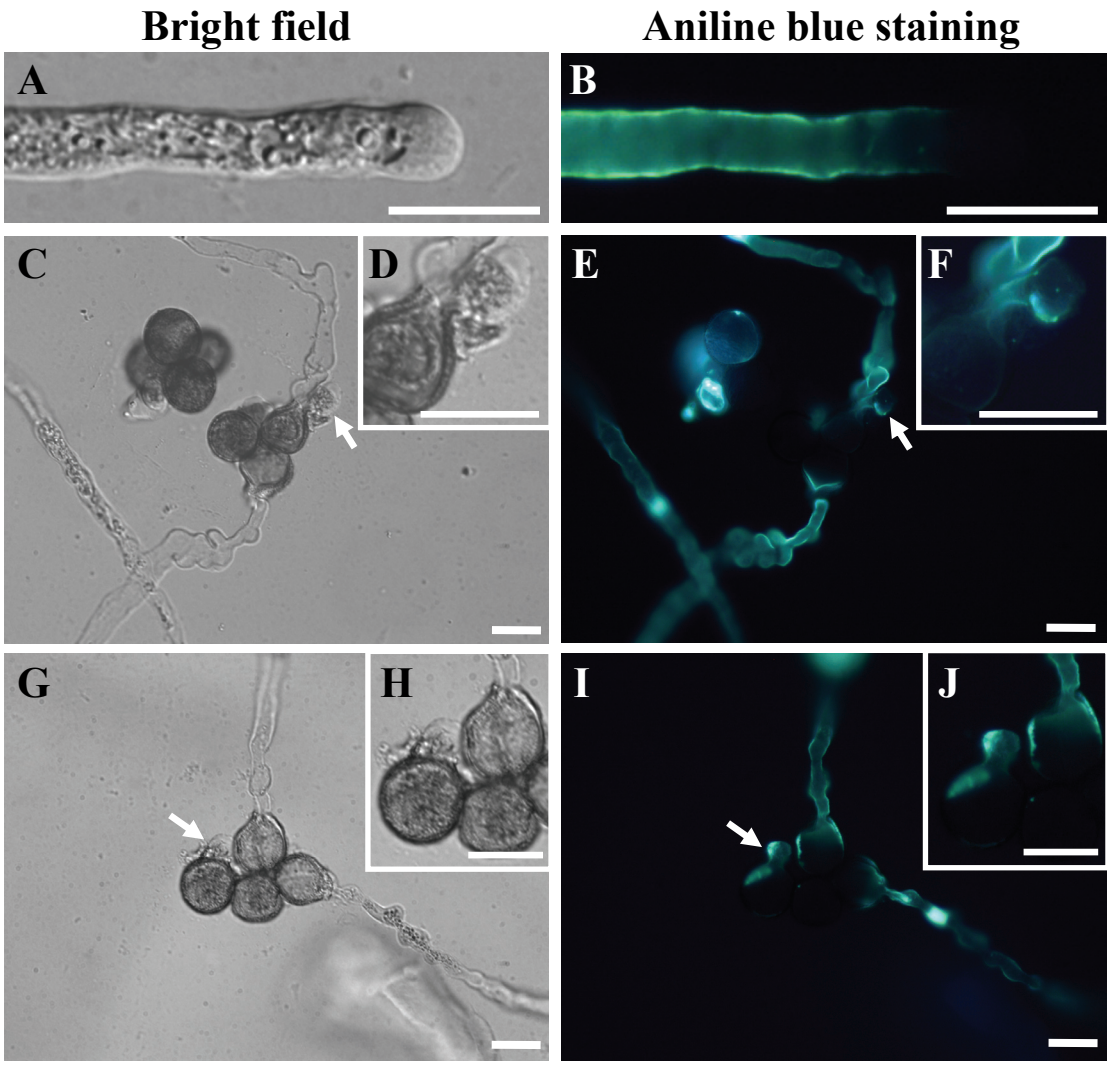
Supplementary Fig. S6. GFP-CSLD1 and GFP-CSLD4 are located at the plasma membrane of the pollen tube tip.
(A) Showing the colocalization of GFP-CSLD4 (green) with FM4-64 staining (magenta) in the PM of an emerging pollen tube (arrow). Image was taken after incubation with FM4-64 for about 1 min.
(B and C) Plasmolysis of the pollen tubes co-expressing GFP-CSLD1 (B, green) or GFP-CSLD4 (C, green) and Golgi-specific RPA-DsRed2 (magenta). Arrows indicate shrinkage of pollen tube cytoplasm.
Bars: 5 μm.

Figure S7



Supplementary Fig. S7. Calcofluor staining of wild-type and mutant pollen tubes.
(A-H) Showing the bright field and spinning disk confocal images of wild-type *qrt1*/*qrt1* (A, B), *csld4-3*/+; *qrt1*/*qrt1* (C, D), *csld1-1*/+; *qrt1*/*qrt1* (E, F) and *csld1-1*/+; *csld4-3*/+; *qrt1*/*qrt1* (G, H) pollen tubes stained with Calcofluor. Arrows indicate abnormal mutant pollen tubes with punctate fluorescence signals. White box in (B) shows an image taken at another focal plane of a pollen tube in (B). White boxes in (D, F, H) show the close-up of the pollen tubes indicated by arrows in the same images respectively.
Bars: 20 μm.

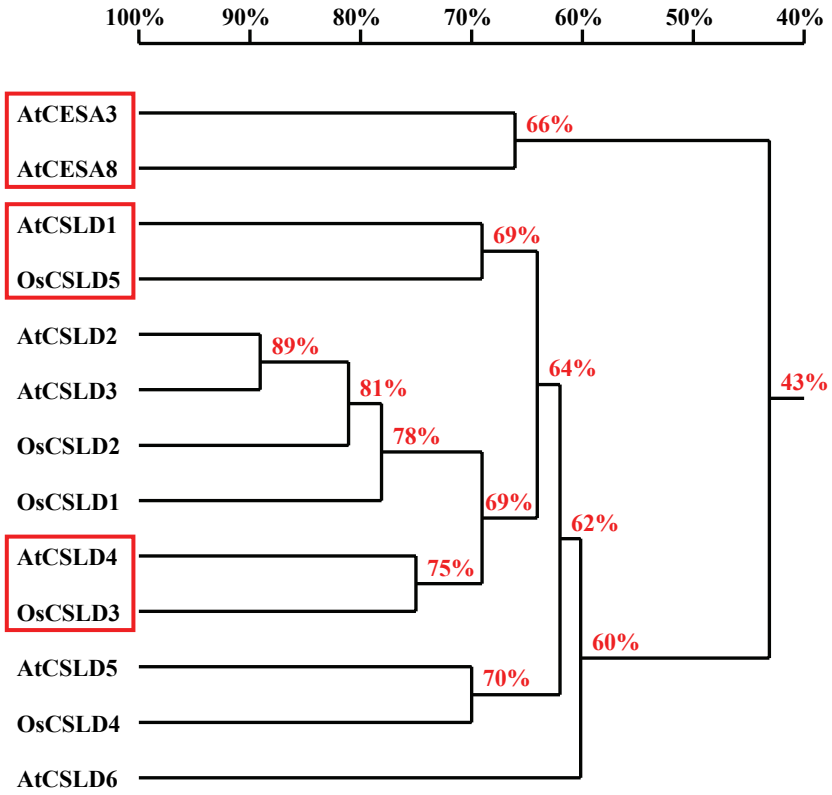
Figure S8



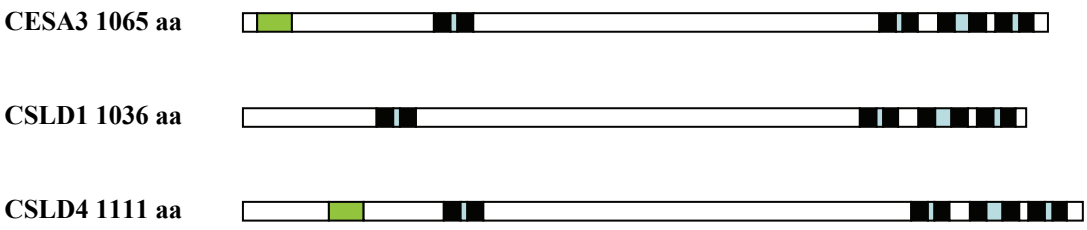
Supplementary Fig. S8. Callose distribution in wild-type and mutant pollen tubes. Wild-type (A, B) and *csld4*/+; *qrt1*/*qrt1* (C-J) pollen tubes were stained with aniline blue. In wild-type pollen tubes, intense callose staining was mainly distributed in the tube shank region and absent from the apical region of the pollen tube (B). By contrast, the distribution of callose staining along *csld4* (C-J) mutant pollen tubes was highly irregular. (C-F) Callose staining of *csld4* pollen tubes was weaker then that of the normal pollen tubes. (G-J) Callose was unevenly accumulated in *csld4* mutant pollen tubes. White boxes in (C, E, G and I) show the close-up of the pollen tubes indicated by arrows in the same images respectively. Bars: 20 μm.

Figure S9

A

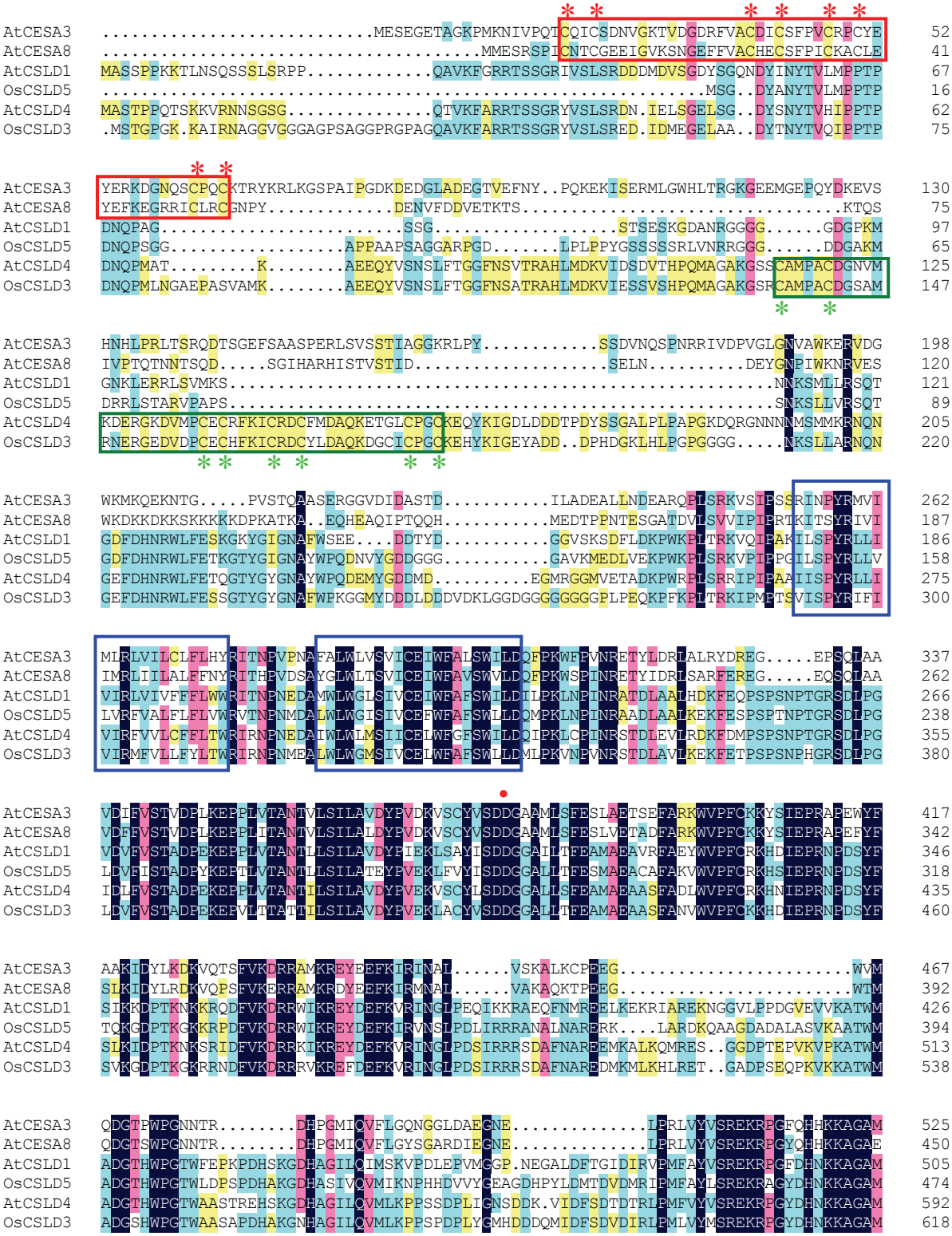


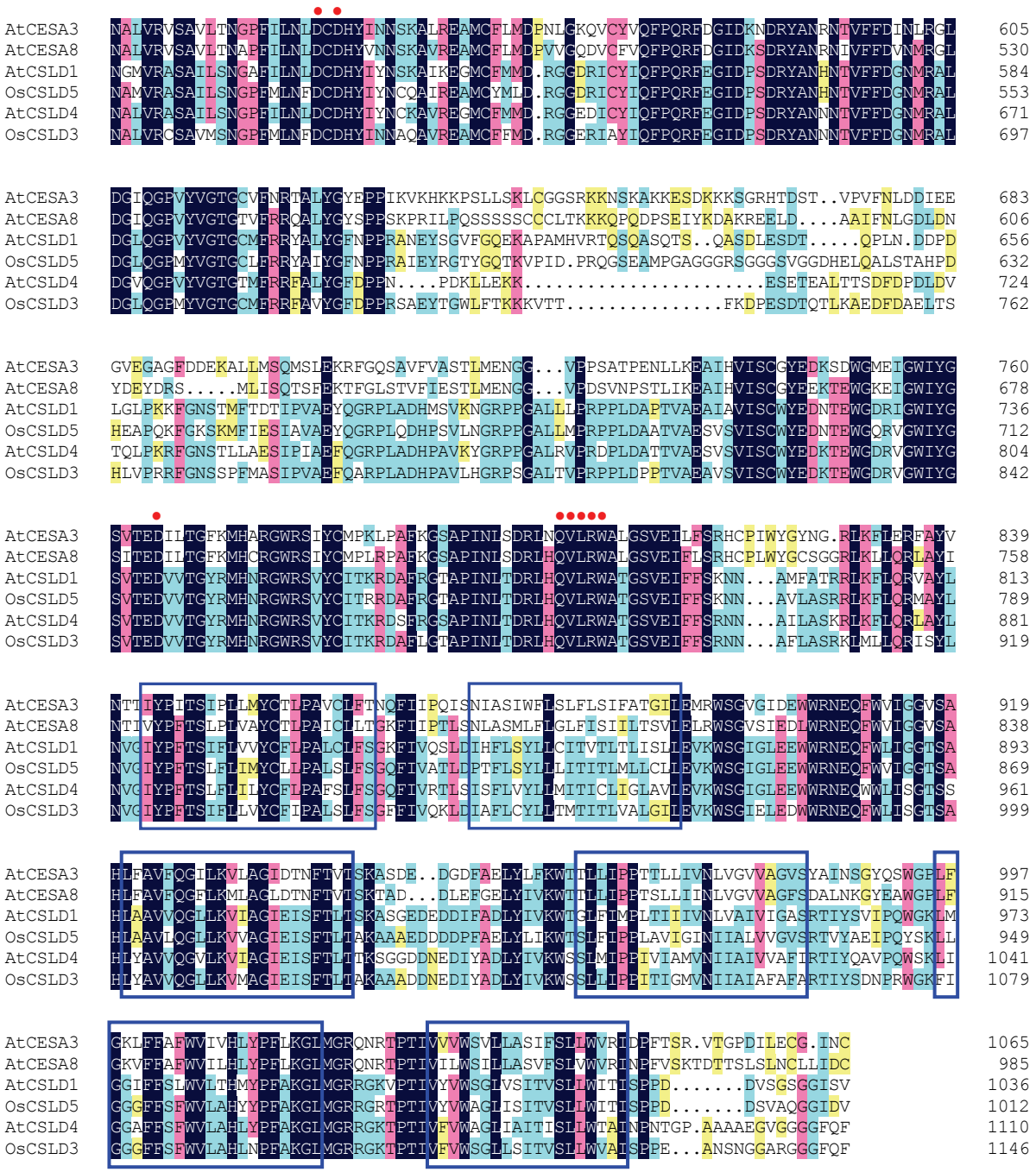
B



Supplementary Fig. S9. Homology of the predicted amino acid sequences of the CSLD and CESA proteins.
(A) A phylogenetic tree of the Arabidopsis CESA, Arabidopsis CSLD and rice CSLD proteins. (B) The structural organization of Arabidopsis CESA3, CSLD1 and CSLD4 proteins is drawn to scale as boxes. Black boxes indicate predicted transmembrane domains (Wang *et al.,* 2001). Green boxes in the N-terminal indicate Cysteine-rich (Cys-rich) Zinc finger domains. White boxes indicate the regions predicted to be located in the cytoplasm. Blue boxes indicate the regions predicted to be located in the cell wall (Doblin *et al.,* 2001).

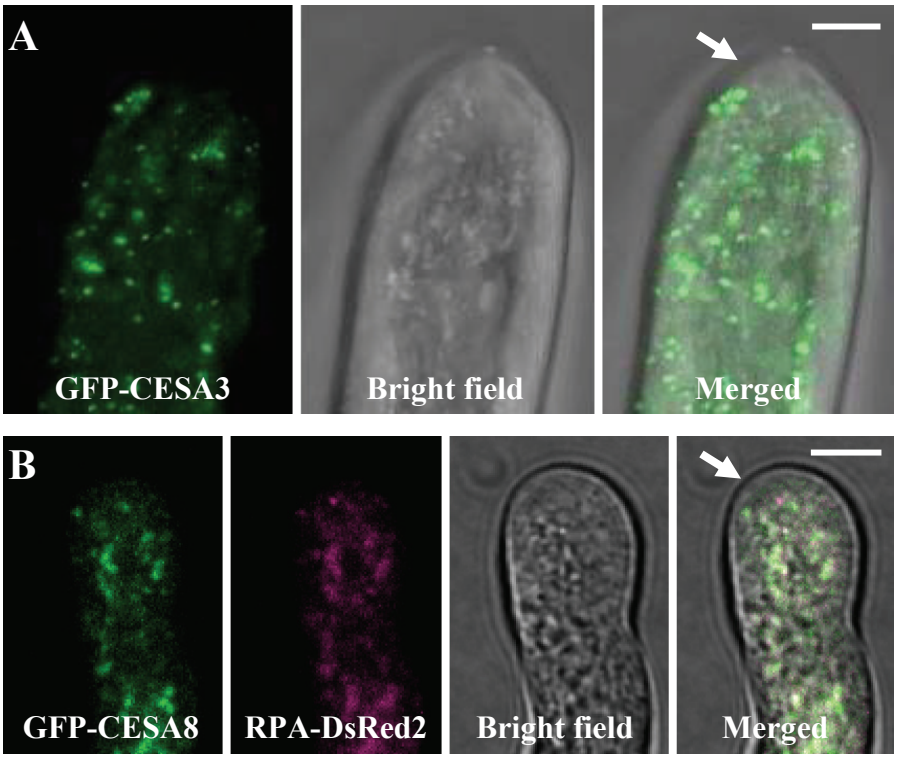
C





(C) A sequence alignment of the Arabidopsis CESA, Arabidopsis CSLD and rice CSLD proteins. The colored characters indicate the identical aa (black), ⩾75% aa similarity (peach), ⩾50% aa similarity (cyan) and ⩾33% aa similarity (yellow), respectively. The typical Cys-rich Zinc finger domains in CESAs are indicated in red boxes and Cys residues are indicated by red asterisks. The similar Cys-rich Zinc finger domains in AtCSLD4 and OsCSLD3 are indicated in green boxes and Cys residues are indicated by green asterisks. It is worth noting that AtCSLD1 and OsCSLD5 do not have the Cysteine-rich (Cys-rich) Zinc finger domain. The eight transmembrane domains are indicated in blue boxes. The positions of the three conserved Asp residues (D) and the QVLRW motifs are indicated by red dots.

Figure S10



Supplementary Fig. S10. Subcellular localization of CESA3 and CESA8 in Arabidopsis pollen tubes.

To determine whether CESAs participate in cellulose biosynthesis in the growing tip region of pollen tubes, we further investigated the localization of CESA proteins in pollen tubes using stably transformed Arabidopsis lines expressing N-terminal GFP fusions of Arabidopsis CESA3 and CESA8 under the control of the *CSLD4* promoter. *CESA3* and *CESA8* cDNAs were cloned using RT-PCR with the gene-specific primer pairs CA3-AF/CA3-AR, CA3-BF/CA3-BR, CA8-AF/CA8-AR and CA8-BF/CA8-BR. These cDNA fragments were then subcloned to create an N-terminal fusion gene with the *GFP* coding sequence downstream of the *CSLD4* promoter in pCAMBIA1300. These constructs could not complement the *csld4* mutant phenotype.

(A) Showing that GFP-CESA3 was not found in the clear zone and PM of pollen tube tip (arrow).

(B) Showing that GFP-CESA8 was colocalized with RPA-DsRed2 in the shank of pollen tubes, but did not present in the clear zone and PM of the pollen tube tip (arrow).

Bars: 5 μm.

Supplementary Tables

Supplementary Table S1. *In vitr*o germination of quartets from mutant and wild-type plants.

Genotype	PG with normal tubes	PG with ruptured PT	Ungerminated PG	Total PG[a]
qrt1/*qrt1*	33.4%	11.2%	55.4%	762
csld1-1/+; *qrt1*/*qrt1*	0.0%	54.8%	45.1%	598
csld4-3/+; *qrt1*/*qrt1*	0.0%	45.0%	55.0%	626
csld1-1/+; *csld4-3*/+; *qrt1*/*qrt1*	0.0%	63.0%	37.0%	216

[a]Total number of the pollen grains from all qurtets examined, each of which had at least two pollen grains with normal tubes.

PG, pollen grains; PT, pollen tubes.

Supplementary Table S2. Primers used for cloning and PCR analysis.

Primer name	Primer sequence (from 5' end to 3' end)
LBa1	TGGTTCACGTAGTGGGCCATCG
D1-S1	CTGCAGCCCATCTAA TGCTCTCATG
D1-S2	TTTGATCTATCTAGTTTTCTCAC
D1-P1	GAATTCCTGTAGGGACTAAGAATTTTG
Ds5-1	CCGTTTACCGTTTTGTATATCCCG
D4-Ds	ATGTTTAGGCGCTTTGCTCTC
D4-P1	TGGATTGAGATGTCATGACTG
D4-P2	GTCGACTTAGGCGGCTGGAATAGGGAT
D4-FAF	CCCAAGCTTAACGCATATTCGACTTCT
D4-FAR	TACGAGTCTGGATTTCGAGG
D4-FBF	TGCTAGCTTTGCGGATCTCTG
D4-FBR	CGGGATCCCGTCGTCATCAACATCTC
D1-FAF	CTGCAGGGTTTGTGGGGGATTCAACTG
D1-FAR	CTCTCGGGCAATTCGCTTCTC
D1-FBF	ACTGAACAGTTCAACATGAGAG
D1-FBR	GGATCCGGAGTAGAACATACCGTGATC
1300HindIII	TGGCGAAAGGGGGATGTGCTG
1300EcoRI	CATGATTACGAATTCGAGCTC
TUB8-F	CTTCGTATTTGGTCAATCCGGTGC
TUB8-R	GAACATGGCTGAGGCTGTCAAGTA
GFP-F	TCTAGAGGATCCAAGGAGATATAACAATGAGT
D4-PF	GCTGCAGCAGATCGCATGGATGAGCTTGTAG
D4-PR	GGATCCTGTGAAGCCAAACAAAG
D4-CAF	ACTAGTATGGCGTCCACGCCTCCTC
D4-CAR	CAAGGTCCGGGTCAAAGTCAC
D4-CBF	ATGTTTAGGCGCTTTGCTCTC
D4-CBR	CGAGCTCGATACAAAGGCTGATTTATACAG
D1-CAF	TCTAGAATGGCTTCAAGTCCACCCAAG
D1-CAR	CTCTCGGGCAATTCGCTTCTC
D1-CBF	ACTGAACAGTTCAACATGAGAG
D1-CBR	GTCGACTTACACTGAGATTCCTCCACTG
CA3-AF	TCTAGAATGGAATCCGAAGGAGAAACC
CA3-AR	TGTGGTGCTGGAATCCTGGTC
CA3-BF	GATGCAGAGGGCAATGAGCTC
CA3-BR	GGTACCTCAACAGTTGATTCCACATTC
CA8-AF	ACTAGTATGATGGAGTCTAGGTCTC
CA8-AR	CAAACGTCTTGACCAACAACAG
CA8-BF	AGCCGTGCGTGAAGCAATGTG
CA8-BR	GTCGACTTAGCAATCGATCAAAAGAC

F, forward primer; R, reverse primer.

Supplementary Video Legends

Supplementary Video S1. GFP-CSLD1-labeled Golgi apparatus and small vesicles/particles move rapidly in a pollen tube.

Supplementary Video S2. GFP-CSLD4-labeled Golgi apparatus and small vesicles/particles move rapidly in a pollen tube.

Supplementary Video S3. FRAP analysis of GFP-CSLD4 in the pollen tube tip of growing pollen tubes. Photobleaching was performed in the apical area of the pollen tube. The fluorescence recovery was obvious in the apical region of the plasma menbrane.

Supplementary Data File Legends

Supplementary Data File S1. Genetic analysis of *CSLD4* transgenic *csld4* mutant lines.

CSLD4 genomic DNA and p*CSLD4:GFP-CSLD4* constructs were introduced into *csld4*/+ and *csld4*/+; *qrt1*/*qrt1* heterozygous plants. Segregation ratio of the progeny from self-pollinated T1 transgenic plants was shown.

kanR: kanamycin-resistant; kanS: kanamycin-sensitive.

Supplementary Data File S2. Expression levels of *CESAs* and *CSLDs* during pollen development.

Expression levels of *CESAs* and *CSLDs* were shown as an average of raw gene expression values from publicly available microarray data (Honys and Twell, 2003; 2004; Qin *et al.,* 2009). Because there is one replicate of microarray data for mature pollen, no average and STDEV values are shown.

Average: average of gene expression values; STDEV: Standard deviation.