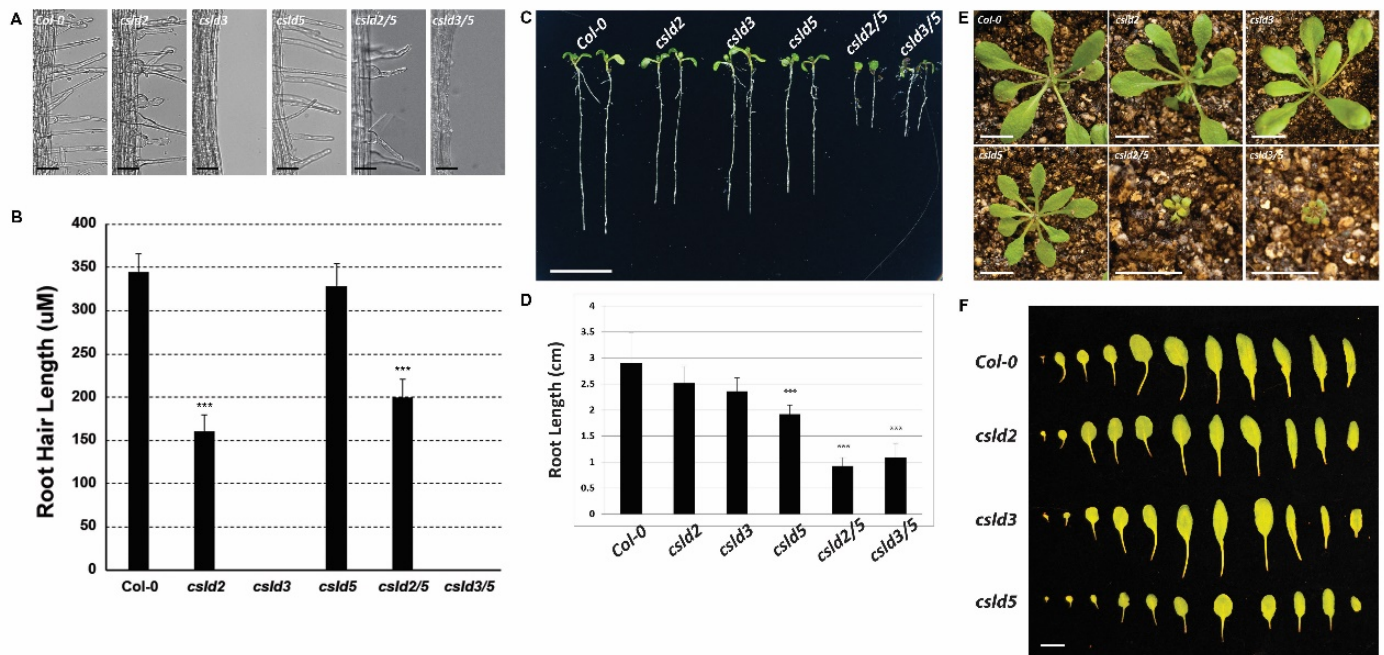


## SUPPLEMENTAL FIGURES AND LEGENDS

Supplemental Figure-1(Nielsen)

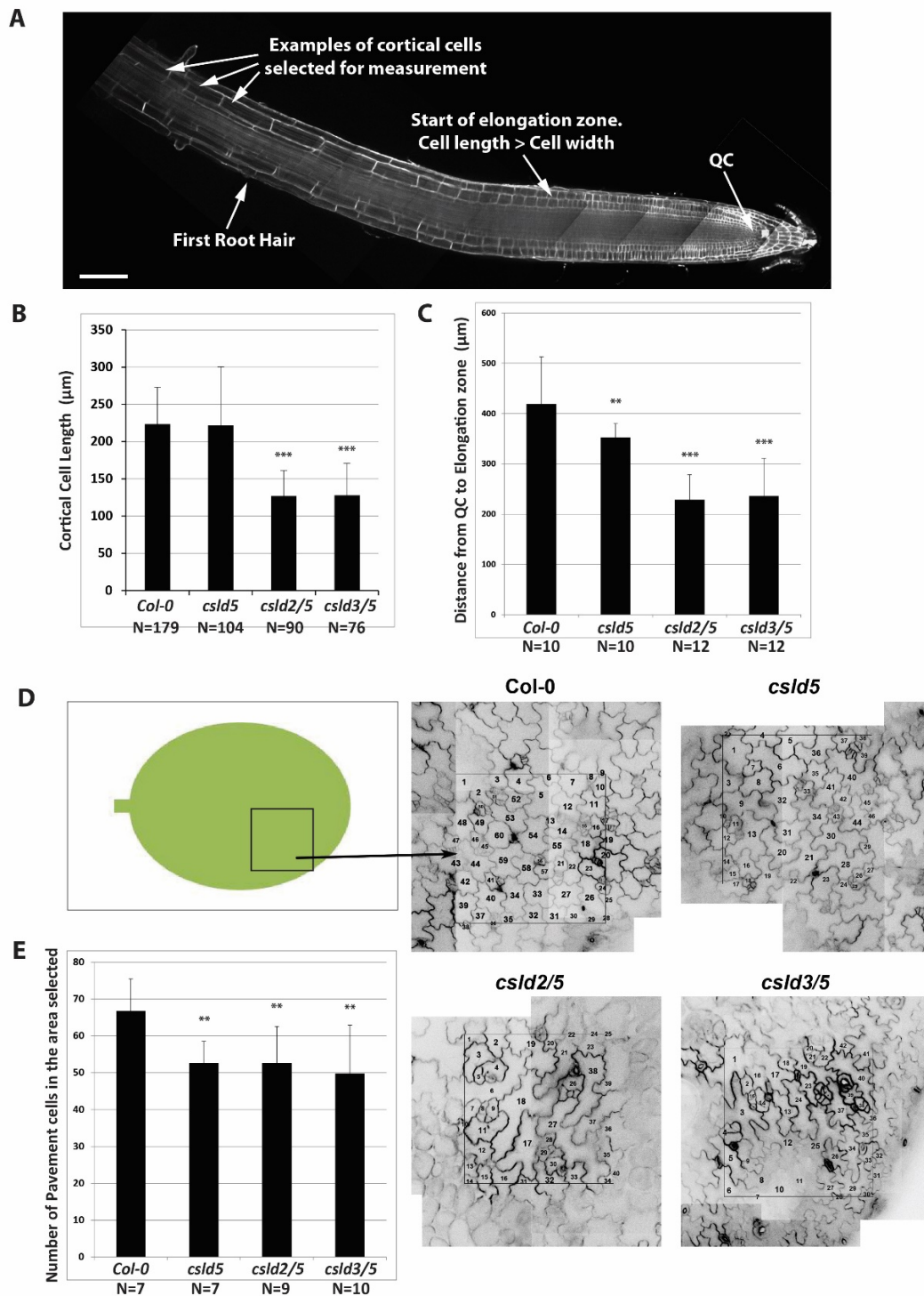


**Figure S1. Phenotypic analysis of *csld* mutants.** (A) Root hair morphology was examined in 5-day-old *Col-0*, *csld2*, *csld3*, *csld5*, *csld2/5*, and *csld3/5* seedlings. Root hairs in *csld2* were shorter and often displayed bulges at the base of the hair, while in *csld3* mutants no root hairs were apparent. Root hairs in *csld5* were indistinguishable from wild-type *Col-0*. (B) Quantitative analysis of root hair defects indicated that there was no significant changes in root hair length in either *csld2/5* or *csld3/5* double mutants ( $n = 8$  seedlings;  $>800$  individual root hairs measured). (C, D) Root lengths of seven-day-old *Col-0*, *csld2*, *csld3*, *csld5*, *csld2/5*, and *csld3/5* seedlings were measured. The roots of *csld5* single and both *csld2/5* and *csld3/5* double mutants were significantly shorter compared to *Col-0* (C). (D) Rosette size of 3-week-old *Col-0*, *csld2*, *csld3*, *csld5*, *csld2/5*, and *csld3/5* plants were compared. While *csld5* rosettes were slightly smaller than wild-type *Col-0*, the reduced rosette size was dramatically enhanced in *csld2/5* and *csld3/5* double mutants. (E) Leaves from *csld5* plants were smaller compared to their counterparts from *Col-0*, *csld2*, and *csld3*. Scale bar: (A) 50  $\mu\text{m}$ ; (B), (D), and (E) 1 cm.

Error bars represent standard deviation.

Asterisks represent p-value (\*\*\*) = less than 0.005 as determined by Student's t-test

Supplemental Figure-2(Nielsen)



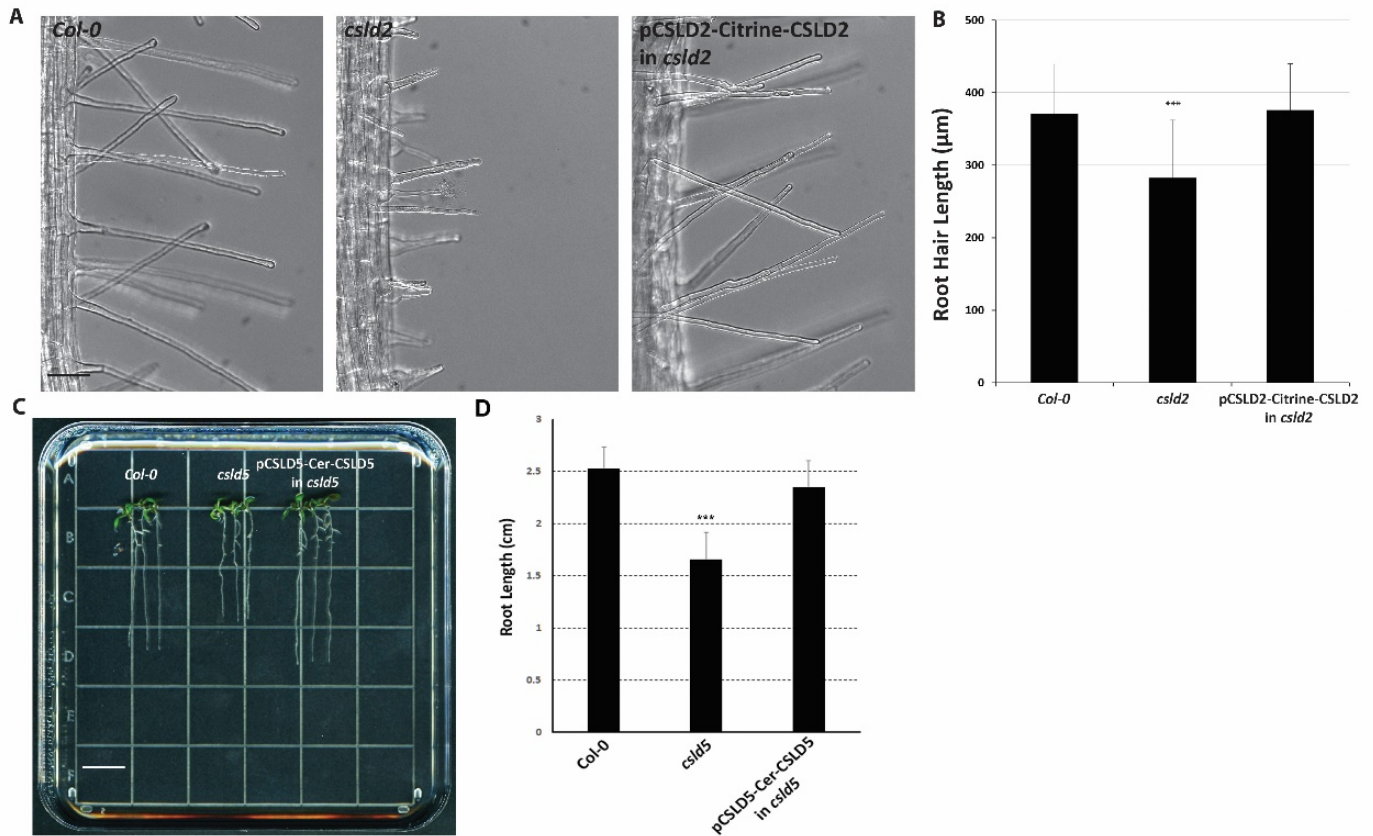
**Figure S2. Cell division is suppressed in *csld5* mutants.** (A) A representative mosaic image of a root indicating where fully expanded root cortical cell measurements were collected and how distance from QC to zone of elongation was measured. (B) Lengths of fully expanded cortical cells were not significantly different between *Col-0* and *csld5*. The length of 5-10 root cortical cells above the first root hair was measured in (n = 10) individual seedlings. T-test analysis indicated that length was not significantly different between *Col-0* and *csld5*, but mature cortical cell lengths in *csld2/5* and *csld3/5* double mutants were significantly shorter than *Col-0*, indicating loss of either *CSLD2* or *CSLD3* activity affected root cortical cell expansion. (C) The distance from QC to the elongation zone was measured in *Col-0*, *csld5*, *csld2/5*, and *csld3/5* seedlings. The initiation site of the elongation zone was defined as the place where the cortical cell length is larger than its width. T-test analysis indicated that the distance from QC to the elongation zone in *csld5* mutants was significantly shorter compared to that in *Col-0*, and that this reduced distance was enhanced in *csld2/5* and *csld3/5* double

mutants. (D) Schematic diagram shows the region where cotyledon leaf pavement cell numbers was counted. Area of 300  $\mu\text{m}$  x 300  $\mu\text{m}$  in the distal end from the base was selected, and the number of pavement cells was counted for each genotype, as indicated in the brightfield panels on the right. (E) Quantitative analysis of the leaf epidermal cell number.

\*\* : P-value less than 0.05.

\*\*\* : P-value less than 0.005.

P-values determined by Student's t-test. Error bars represent standard deviation.



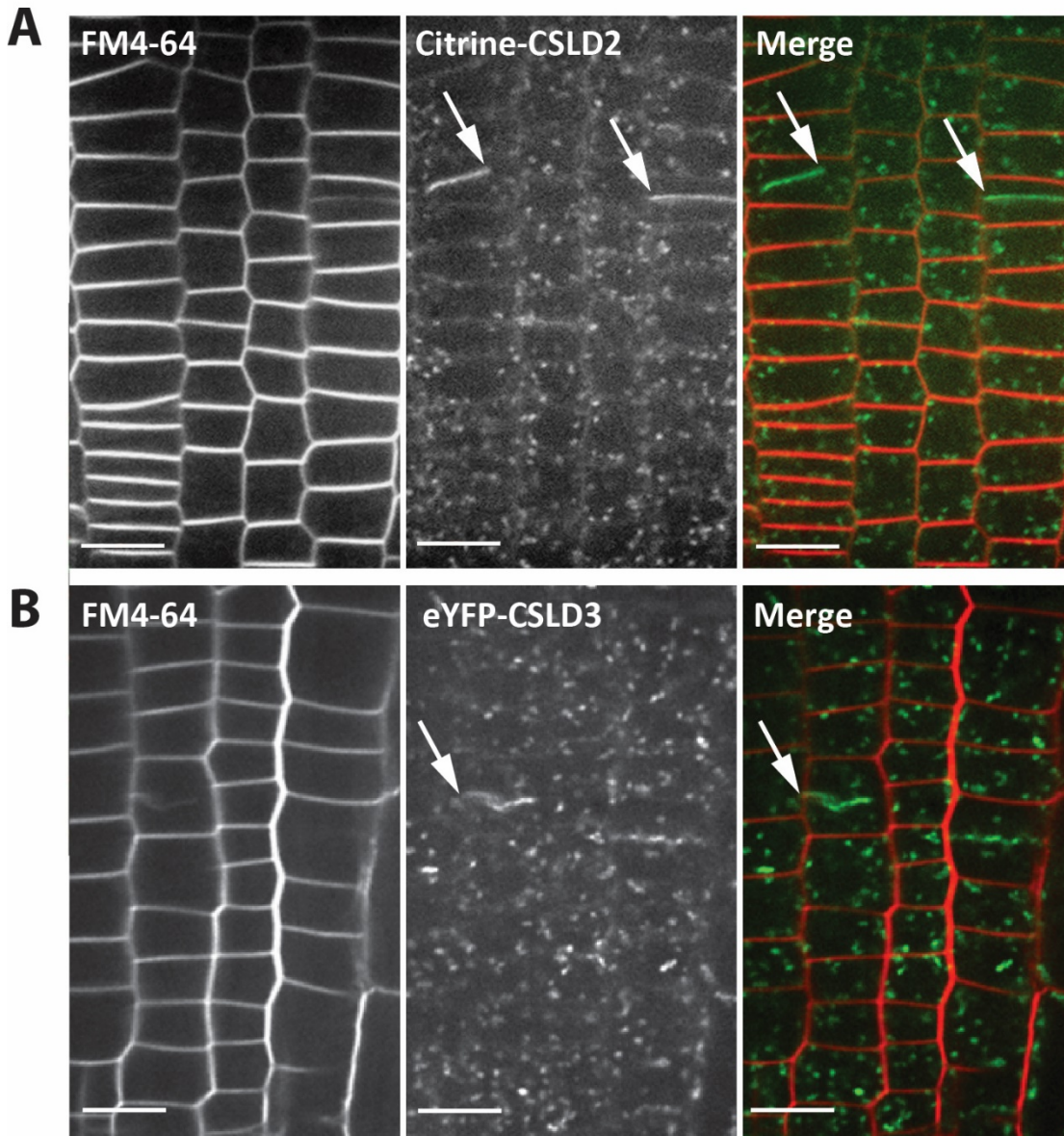
**Figure S3. Citrine-CSLD2 and Cerulean-CSLD5 are functional fluorescent CSLD fusion proteins.** (A, B) Citrine-CSLD2 expressed under control of the *CSLD2* promoter sequences rescues the root hair defects of *csld2*. Representative images were selected to show the rescue of *csld2* phenotype (A). Root hair lengths from 5-day-old seedlings ( $n = 15$  independent seedlings) were measured and displayed in (B). (C) Cerulean-CSLD5 under control of the *CSLD5* promoter sequences rescues the short root defect of *csld5* mutants. Representative 7-day-old seedlings were shown, and root lengths ( $n = 10$  independent seedlings) were measured and displayed in (D).

Scale bar: (A) 100  $\mu\text{m}$ ; (C) 1 cm.

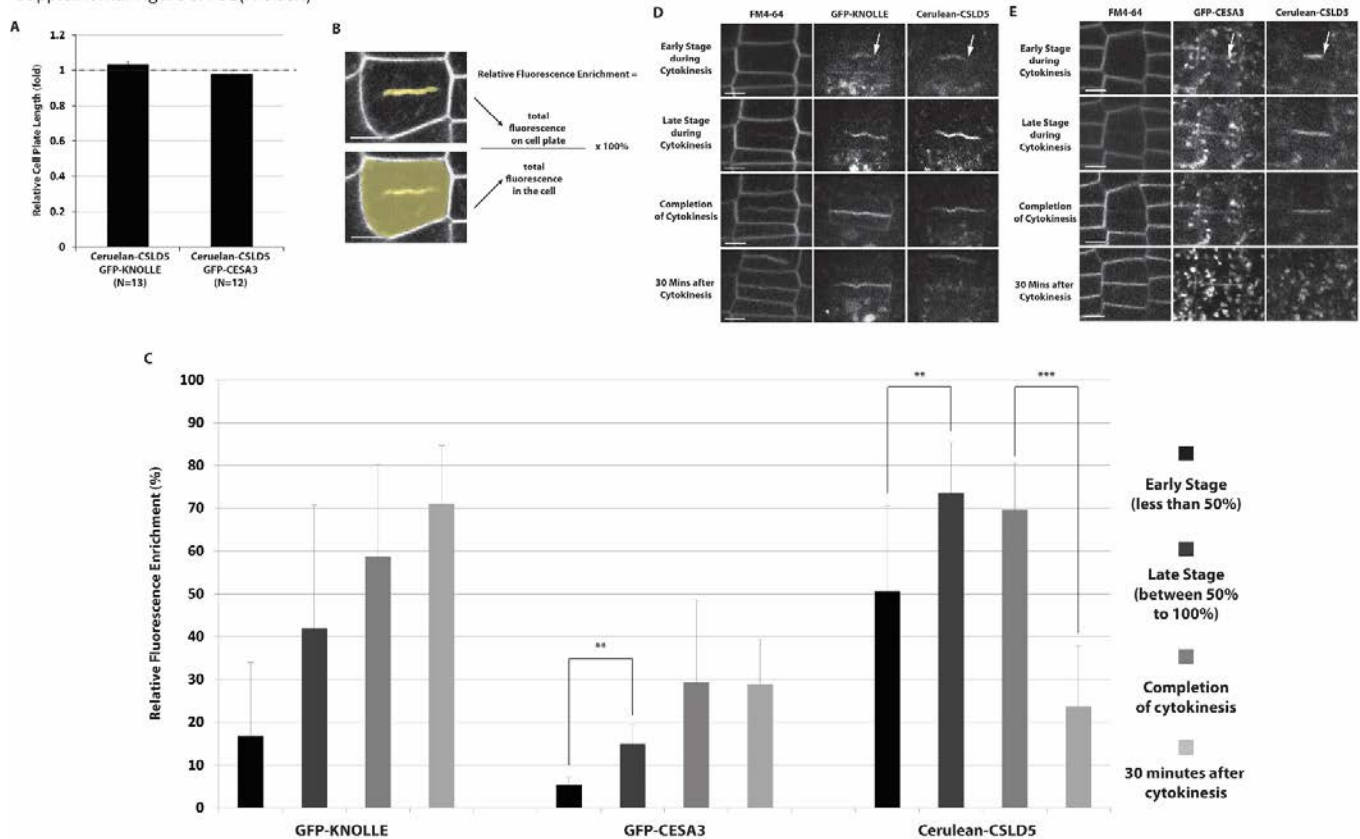
Error bars represent standard deviation.

\*\*\*: P-value less than 0.005 as determined by Student's t-test.

Supplemental Figure-4(Nielsen)



**Figure S4. Citrine-CSLD2 and eYFP-CSLD3 localize to cell plates and punctate sub-cellular compartments in root epidermal cells.** Citrine-CSLD2 (A) and eYFP-CSLD3 (B) were expressed in *Col-0* under the control of their endogenous promoters. Root epidermal cells from 5-day-old seedlings were examined by confocal microscopy. Both Citrine-CSLD2 and eYFP-CSLD3 were localized to cell plates in dividing cells (arrows). Their signal was also visible in punctate sub-cellular compartments in all interphase cells in the field. Scale bar: (A) and (B) 20  $\mu\text{m}$ .



**Figure S5. Quantitative analysis to compare the dynamics of Cerulean-CSLD5, GFP-KNOLLE, and GFP-CESA3.**

(A) Relative lengths of the cell plates labeled by Cerulean-CSLD5 and GFP-KNOLLE/GFP-CESA3 during cell plate elongation in *Col-0* seedlings. The ratio was not significantly different than 1, indicating the co-localization of CSLD5 and KNOLLE/CESA3 during cell plate elongation. (B) Schematic diagram showing the method used for the quantitative analysis of Relative Fluorescence Enrichment (RFE) values. RFE was calculated by dividing the ‘total fluorescence on cell plate’ by the ‘total fluorescence in the cell’. (C) Quantitative analysis comparing the RFE during cell plate elongation and after the completion of cytokinesis. Cell plates were defined as “early stage” when the cell plate length was equal to or less than 50% of the total cell width. “Late stage” cell plates were defined as when the cell plate length was greater than 50% of the total cell width, but not yet fused with the plasma membrane. Cytokinesis was defined as “completed” when elongating cell plates could be observed to establish contact with the limiting plasma membranes in an actively dividing cell. RFE values significantly higher than “random background” were only observed for GFP-KNOLLE and Cerulean-CSLD5 “early stage” cell plates. RFE of GFP-CESA3, GFP-KNOLLE and Cerulean-CSLD5 all significantly increased in “late stage” cell plates. RFE values for Cerulean-CSLD5 were significantly reduced 30 minutes after completion of cytokinesis, while both GFP-CESA3 and GFP-KNOLLE remained high. (n=4 for each condition measured) (D, E) Representative snapshots showing the dynamics of Cerulean-CSLD5 with GFP-KNOLLE (D) and GFP-CESA3 (E) from early stage of cytokinesis to 30 minutes after cytokinesis. These images were used for quantitative analysis. (F – I; see next page) Extra representative images used for quantitative analysis. The images used for comparing Cerulean-CSLD5 and GFP-KNOLLE during cell plate elongation (F), comparing Cerulean-CSLD5 and GFP-KNOLLE after the completion of cytokinesis (G), comparing Cerulean-CSLD5 and GFP-CESA3 during cell plate elongation (H), and comparing Cerulean-CSLD5 and GFP-CESA3 after the completion of cytokinesis (I) were shown.

Error bars represent standard error in (A) and standard deviation in (C).

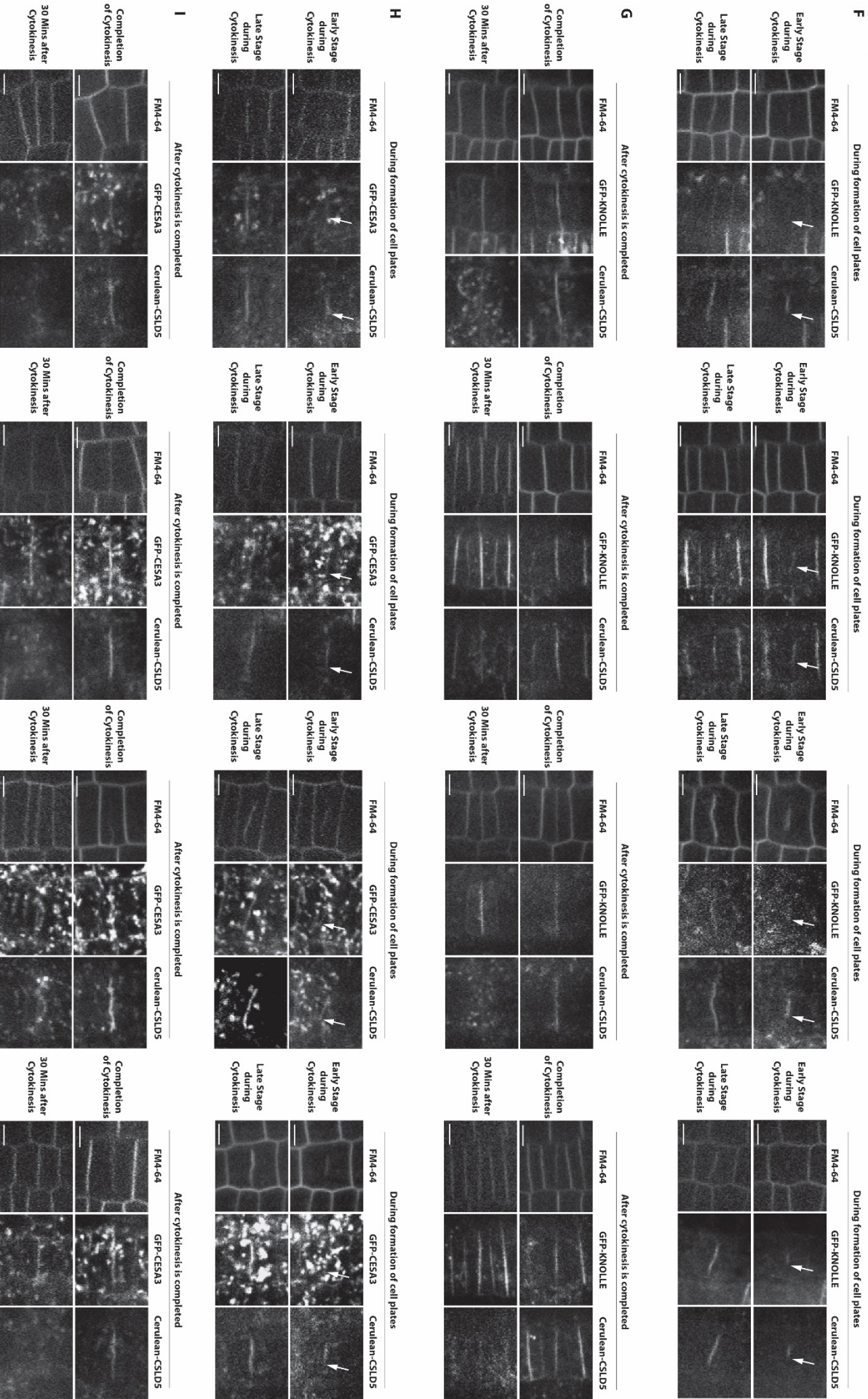
Scale bar: (D– I): 10  $\mu$ m

\*\* : P-value less than 0.05.

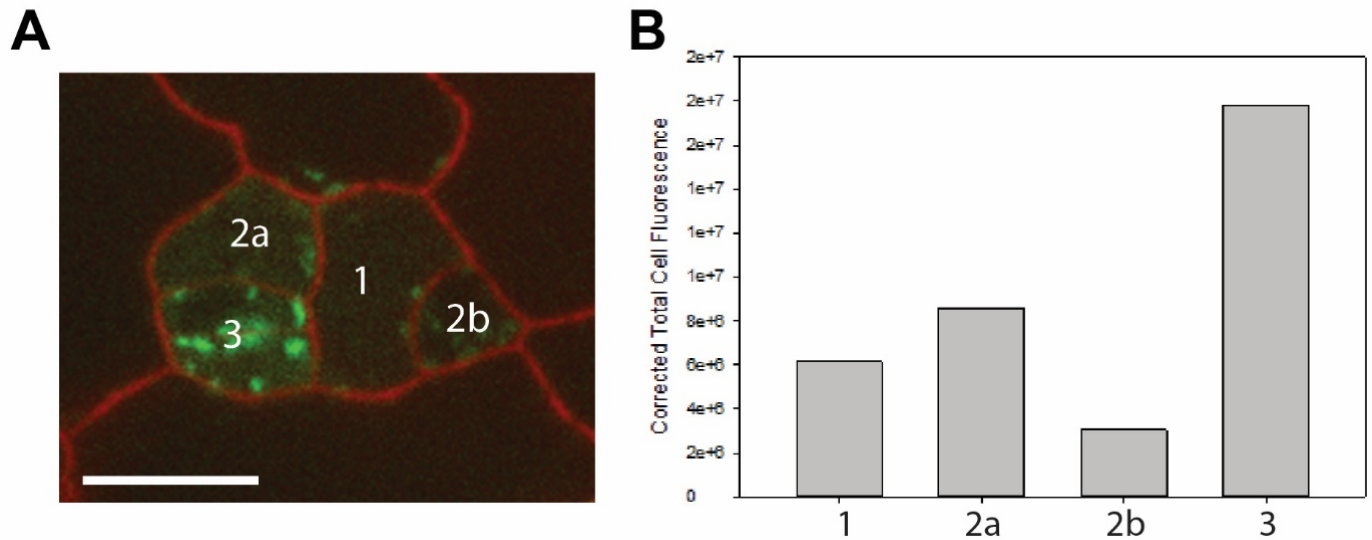
\*\*\* : P-value less than 0.005.

P-values determined by Student’s t-test.

## Supplemental Figure 5F-5I(Nielsen)



## Supplemental Figure-6(Nielsen)



**Figure S6. Quantitative analysis of Cerulean-CSLD5 fluorescence in stomatal lineages.** (A) Representative images of stomatal guard cell lineages from propidium iodide stained 5-day-old seedlings expressing Cerulean-CSLD5 were assigned cell birth order and used for quantification of Cerulean-CSLD5 fluorescence. Scale bar = 10  $\mu\text{m}$  (B) Fluorescence intensity of individual cells was determined using ImageJ. Corrected Total Cell Fluorescence (CTCF) was calculated as  $\text{CTCF} = \text{integrated density of cerulean fluorescence intensity} - (\text{cell area in pixels} * \text{average mean background fluorescence for 3 ROIs outside of the cells})$ .