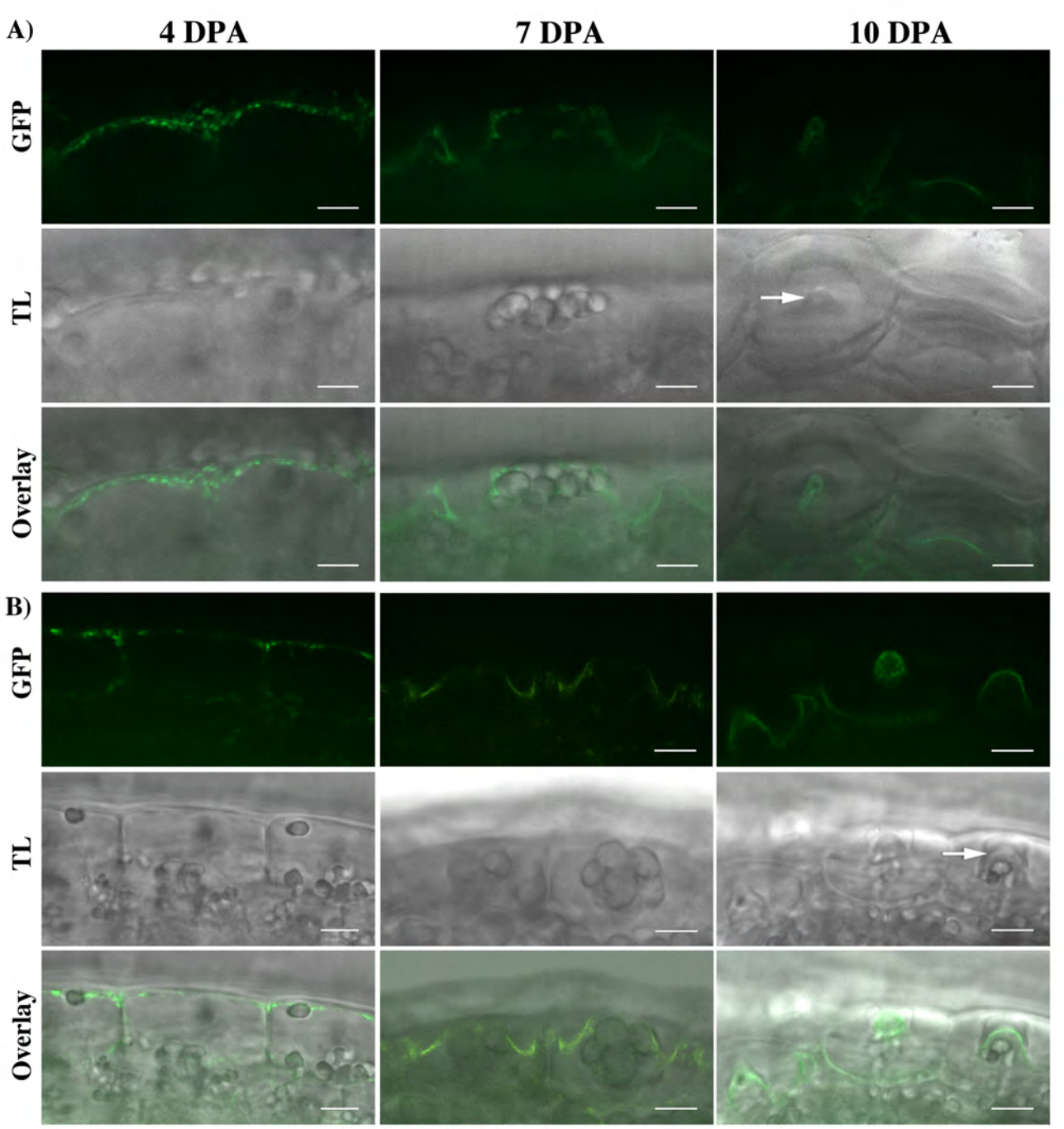
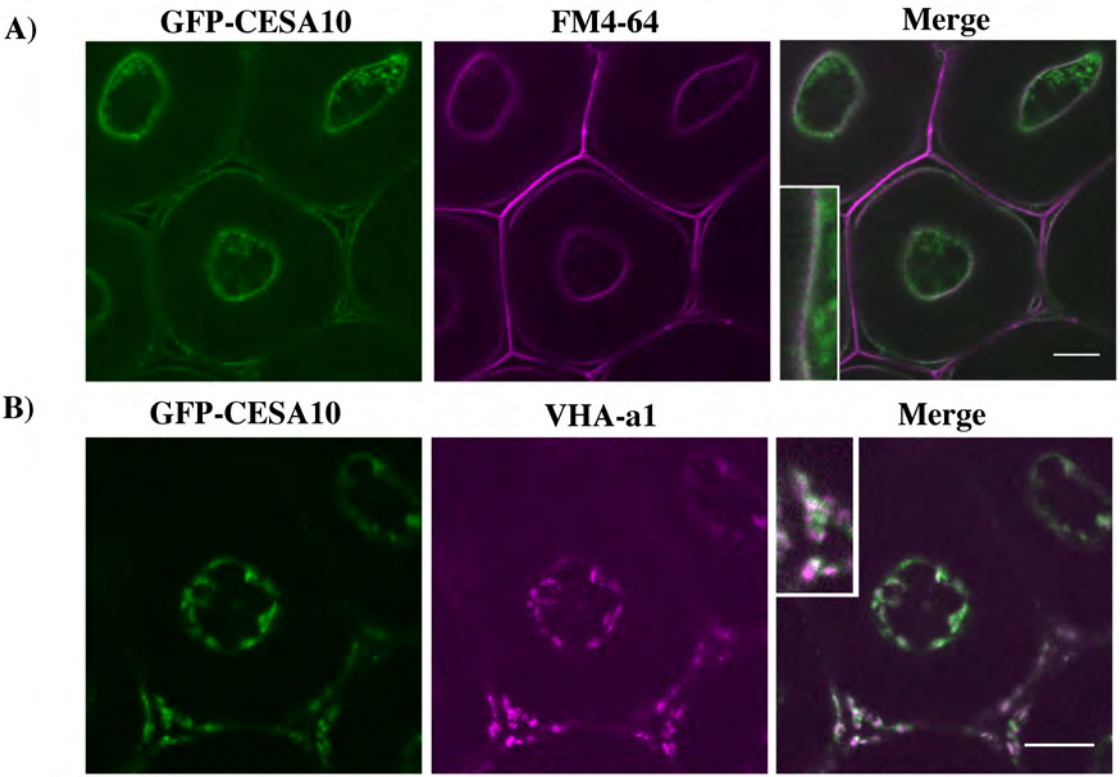


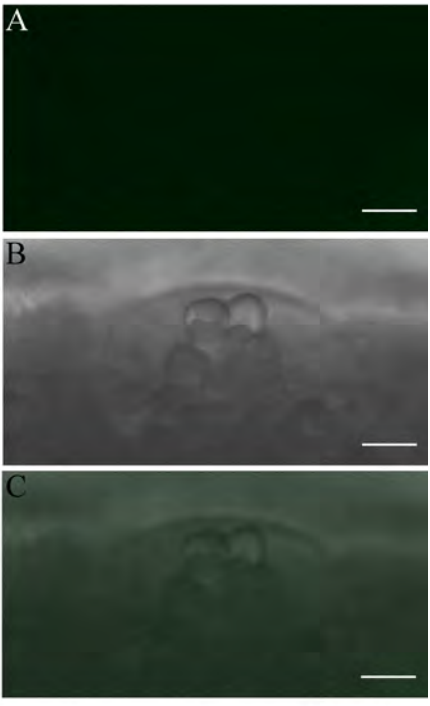
Supplemental Figure 1. GFP-CESA10 and GFP-CESA3 are expressed throughout seed coat epidermal cell development

A) Optical cross sections of the green fluorescence channel (GFP), transmitted light (TL), and overlayed images of A) GFP-CESA10 and B) GFP-CESA3 localization in seed coat epidermal cells at 4 DPA, 7 DPA, and 11 DPA. Arrows indicate regions of columella deposition. Bar = 10 μm.

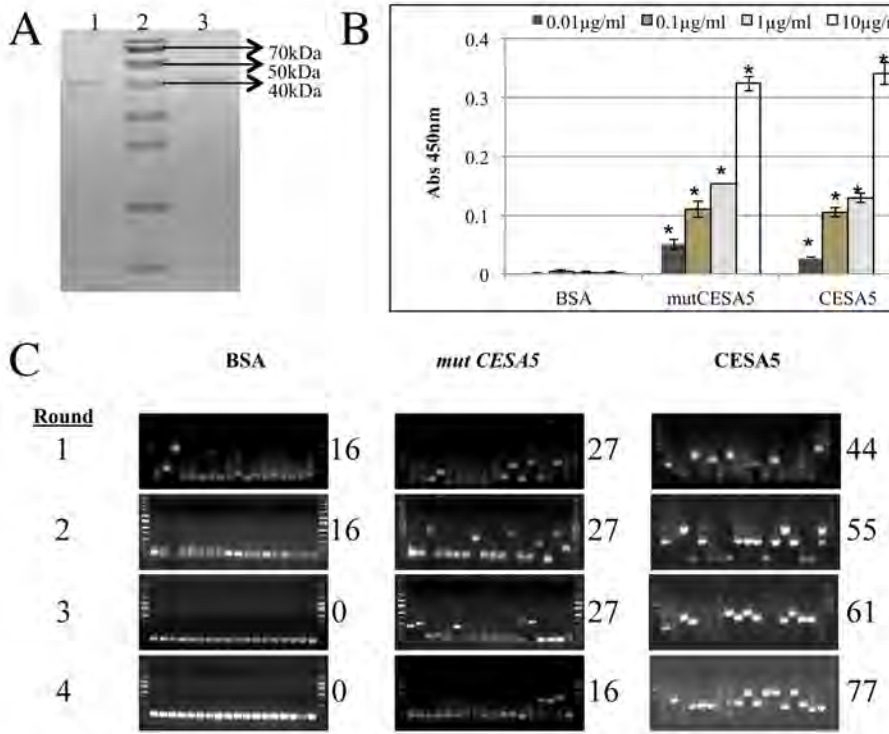


Supplemental Figure 2. GFP-CESA10 is localized to the Golgi and plasma membrane in the cytoplasmic column.

A) GFP-CESA10 seeds stained with the plasma membrane marker FM4-64. Inset shows a magnified region of the plasma membrane of the cytoplasmic column. Inset is 10 μm high. B) Seed coat epidermal cells expressing GFP-CESA10 and RFP-VHA-a1. Inset shows a magnified region showing colocalization. Inset is 10 μm high. Bar = 10 μm.

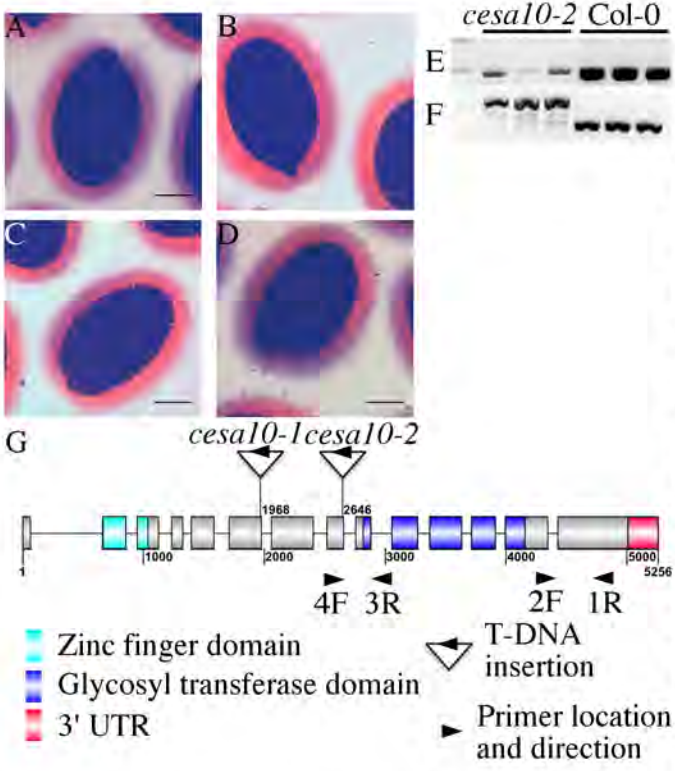


Supplemental Figure 3. GFP-CESA6 is not expressed in seed coat epidermal cells during mucilage production. GFP-CESA6 imaged on the A) GFP channel, B) transmitted light channel, C) Overlay of GFP and transmitted light channels. Bar = 10 μm.

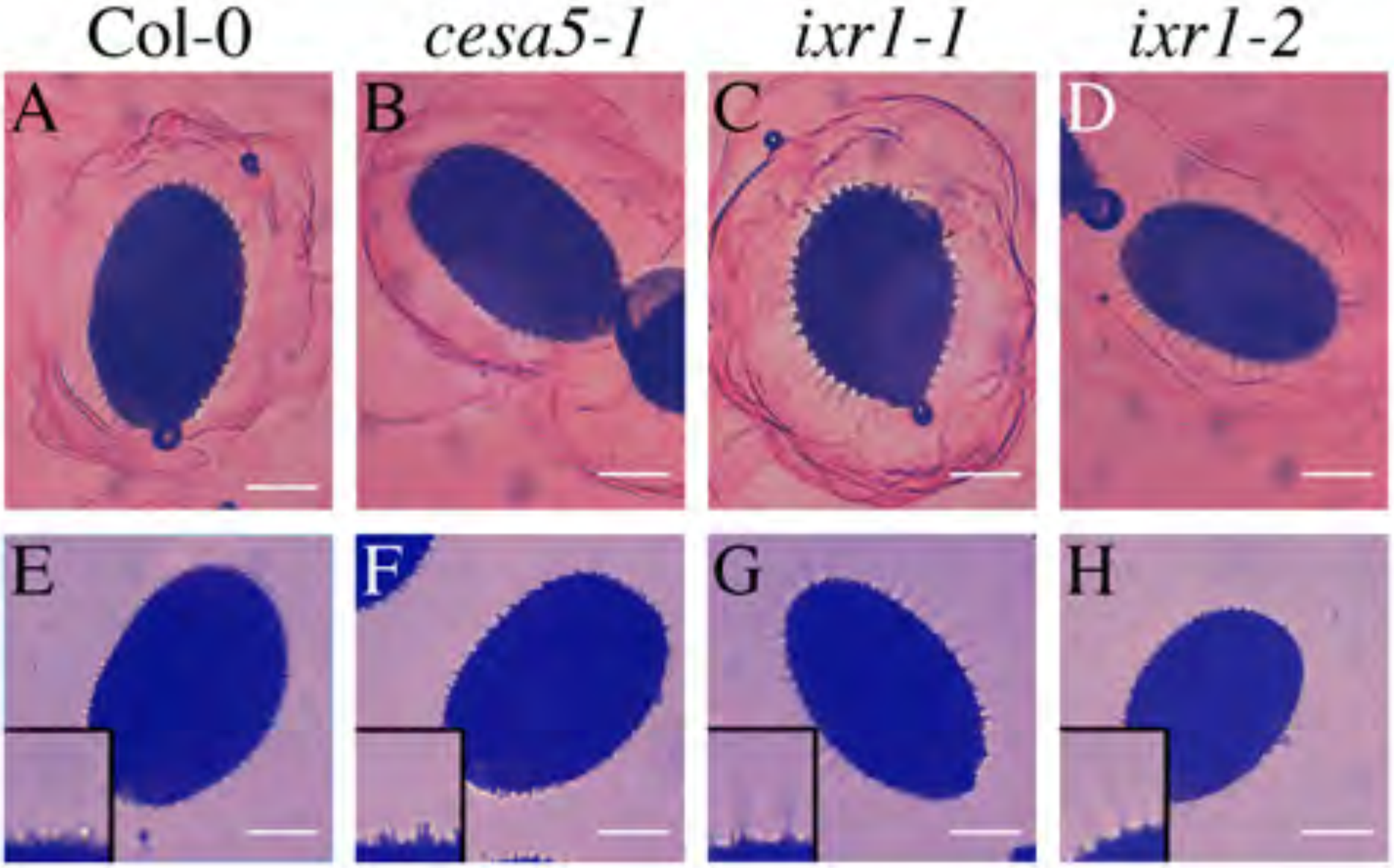


Supplemental Figure 4. Phage display demonstrates that N-CESA5 selectively binds to proteins.

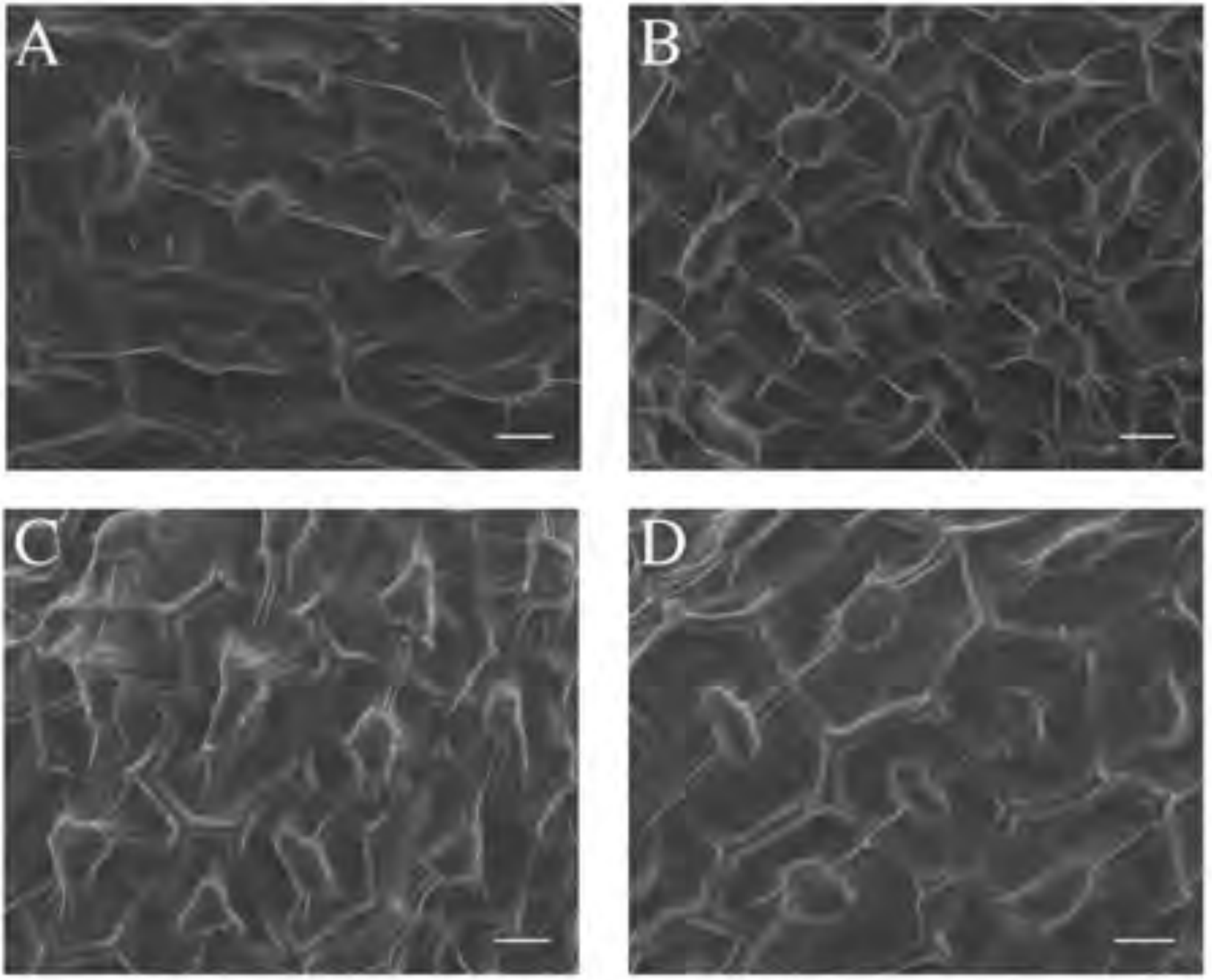
A) 12% SDS-PAGE gel showing recombinant protein purification used for biopanning bait lanes: (1) CESA5, (2) marker, and (3) *mut*CESA5. B) ELISA assay showing the quantity of Bait proteins (BSA, *mut*CESA5, and N-CESA5) attached to microtiter plate wells. Error bars indicate standard error. Asterisks indicate a significant difference from BSA treated samples (t-test $p < 0.05$)C) Agarose gel of inserts isolated from phage bound to BSA, *mut*CESA5 and N-CESA5, from 4 successive rounds of biopanning. At the right of each gel, numbers indicate the percentage of phage containing inserts.



Supplemental Figure 5. *cesa10* and *cesa1* seeds show no major difference in mucilage properties from wild type. A-D) RR stained seeds. A) Col-0; B) *any1-1*; C) *cesa10-1*; D) *cesa10-2*. Bar = 100 µm. E) RT-PCR of wild type and *cesa10-2* seed coat cDNA using primers 2F and 1R, demonstrating that *CESA10* transcript is still produced in *cesa10-2* seeds. F) RT-PCR of wild type and *cesa10-2* cDNA using primers 4F and 3R. The PCR product on *cesa10-2* seeds is larger than in wild type, demonstrating that the *cesa10-2* insertion results in an altered *CESA10* transcript. G) Diagram of the gene structure of *CESA10* and predicted protein domains. Grey domains indicate exons, lines indicate introns, red domains indicate 3` UTR. Numbers indicate position in nucleotides. Arrowheads denote the approximate position of primers used for RT-PCR. H) *cesa10-2* T-DNA insertion sequence, showing the altered mRNA splicing of *cesa10-2* transcripts and the premature stop codon. Uppercase letters were detected when sequencing *cesa10-2* genomic DNA and cDNA using the 4F and 3R primers. Lowercase letters were only detected from sequencing genomic DNA and are not present in the mature mRNA. Underligned sequence represents the T-DNA insertion. Letters below nucleotides represent translated amino acid sequence predicted from the cDNA sequence. Periods in amino acid sequence represent stop codons. Numbers represent the nucleotide position based on the *CESA10* genomic DNA transcription start site.

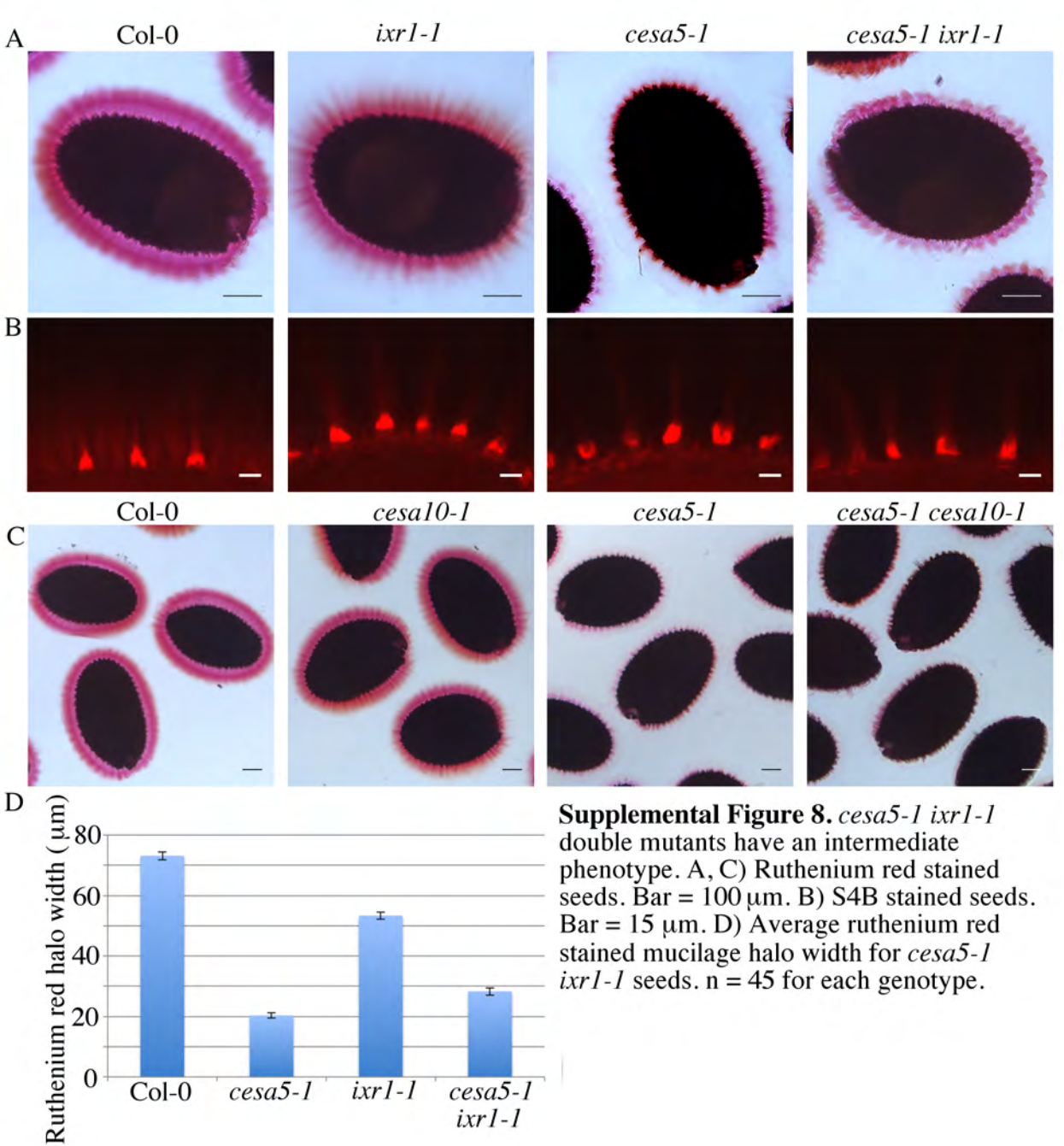


Supplemental Figure 6. Initial hydration and unstained images of *ixr1-1* and *ixr1-2* seeds. A-D) Initial hydration in RR. E-H) Unstained. Bar = 100 µm. Inset shows magnified image of rays. Inset is 65 µm wide.

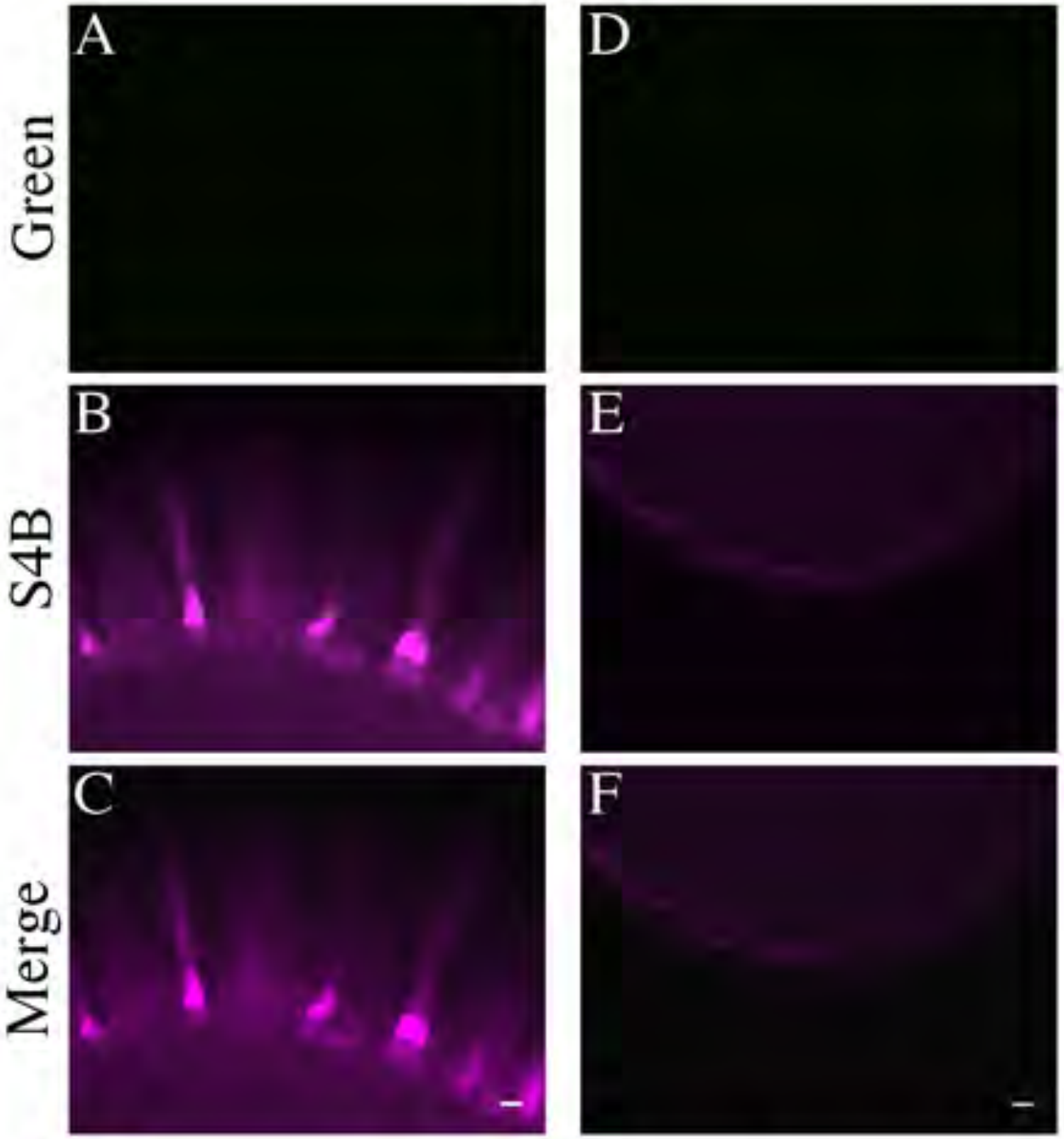


Supplemental Figure 7. SEM images of mature *ixr1-1* and *ixr1-2* seed coat epidermal cells.

A) Col-0, B) *cesa5-1*, C) *ixr1-1*, D) *ixr1-2*. Bar = 10 μm.

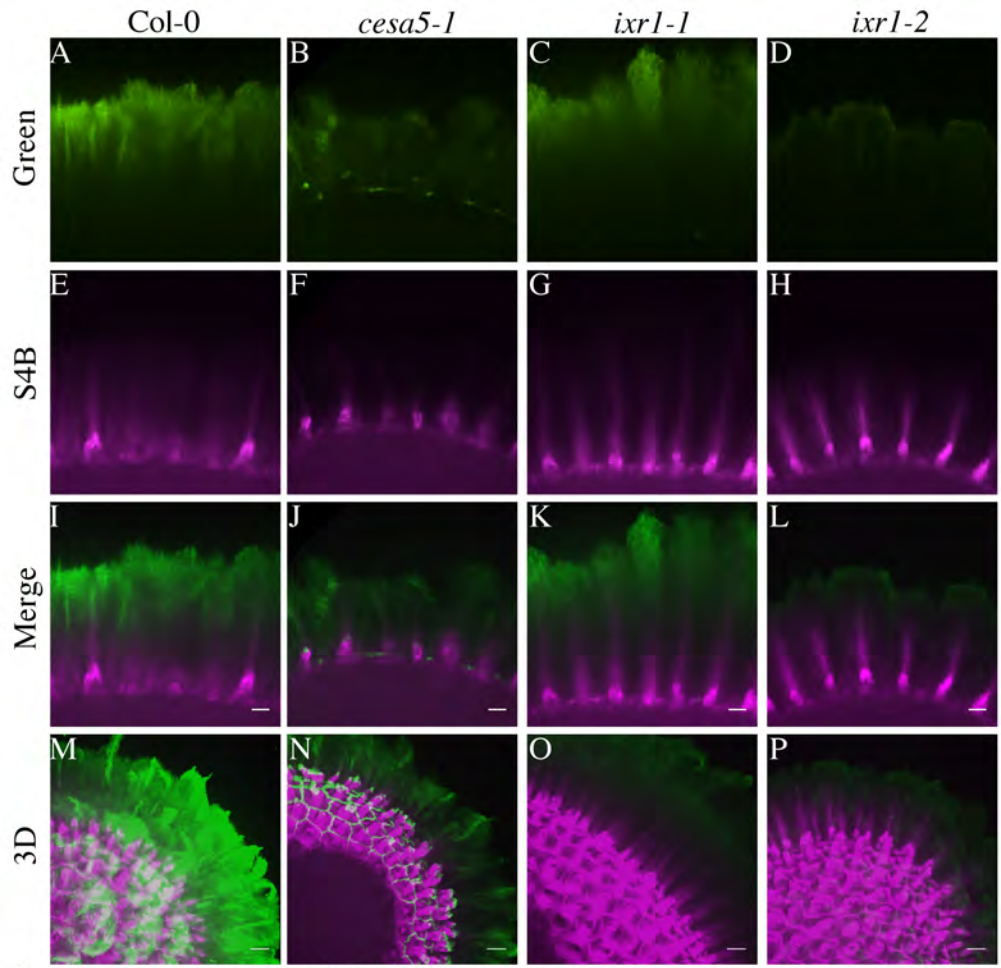


Supplemental Figure 8. *cesa5-1 ixr1-1* double mutants have an intermediate phenotype. A, C) Ruthenium red stained seeds. Bar = 100 μm. B) S4B stained seeds. Bar = 15 μm. D) Average ruthenium red stained mucilage halo width for *cesa5-1 ixr1-1* seeds. n = 45 for each genotype.

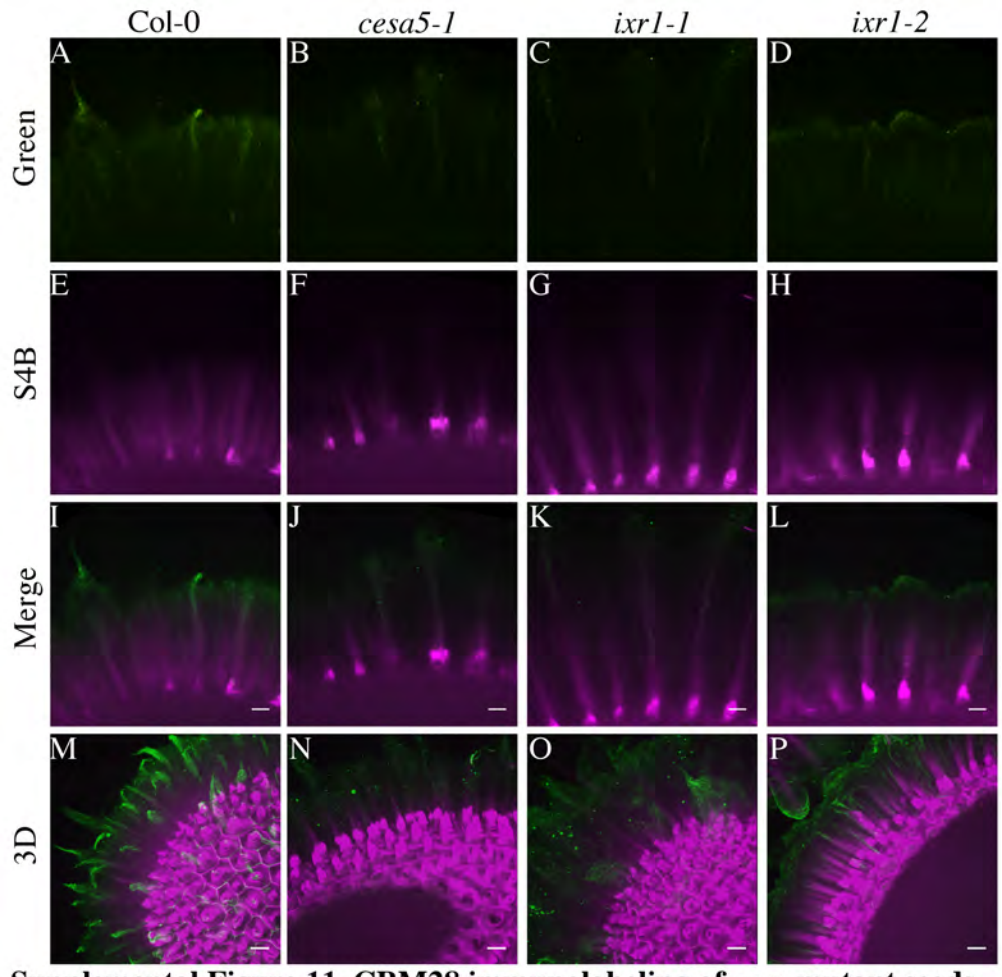


Supplemental Figure 9. Seed mucilage does not significantly autofluoresce.

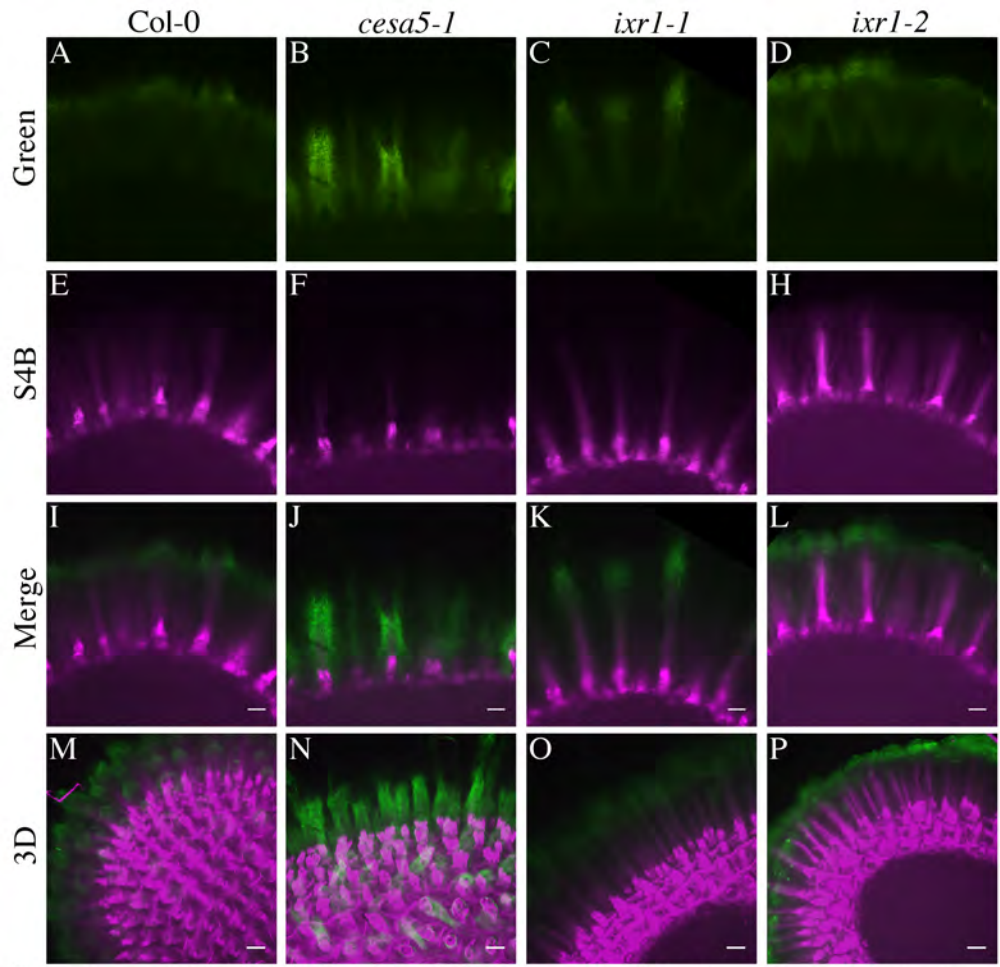
Images of seed mucilage immunolabeled without the primary antibody. A-C) stained with S4B. D-F) No S4B staining. A, D) Green channel; B, E) S4B channel; C, F) Merged image. Bar = 10 μm.



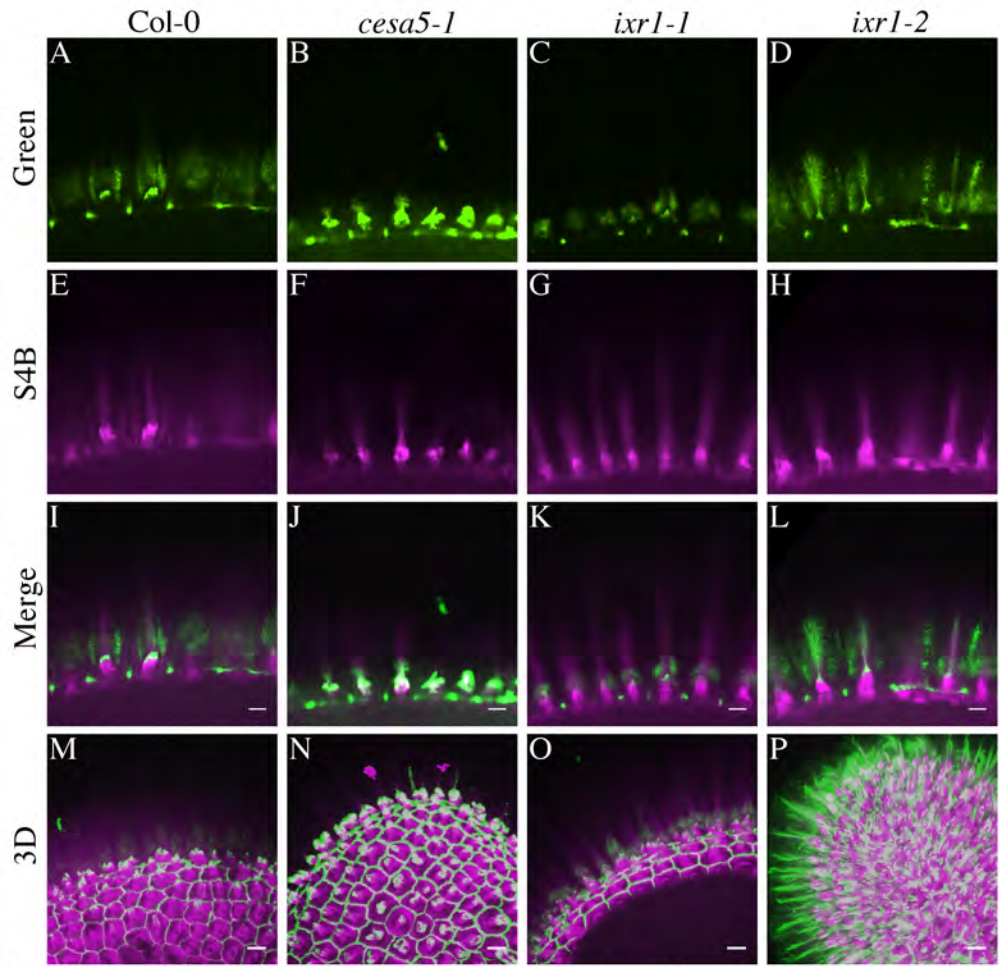
Supplemental Figure 10. CBM3a immunolabeling of *cesa* mutant seeds showing individual light channels and maximum projection of z-stacks. A-D) Green channel; E-H) S4B channel; I-L) Merged image; M-P) maximum projection of z-stacks. A-L) Bar = 15 μm; M-P) Bar = 25 μm.



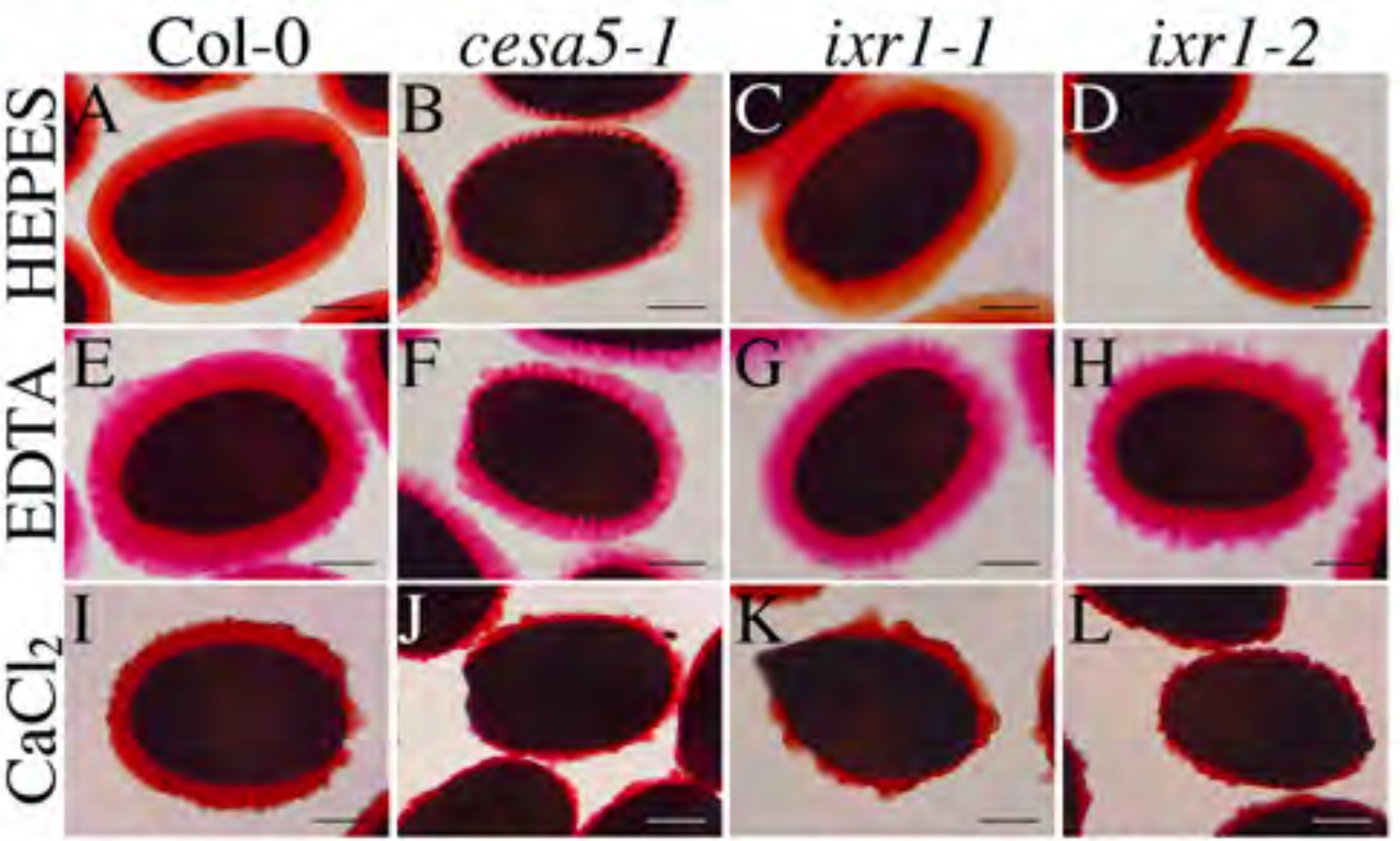
Supplemental Figure 11. CBM28 immunolabeling of *cesa* mutant seeds showing individual light channels and maximum projection of z-stacks. A-D) Green channel; E-H) S4B channel; I-L) Merged image; M-P) Maximum projection of z-stacks. A-L) Bar = 15 μm; M-P) Bar = 25 μm.



Supplemental Figure 12. CCRC-M36 immunolabeling of *cesa* mutant seeds showing individual light channels and maximum projection of z-stacks. A-D) Green channel; E-H) S4B channel; I-L) Merged image; M-P) maximum projection of z-stacks. A-L) Bar = 15 μm; M-P) Bar = 25 μm.

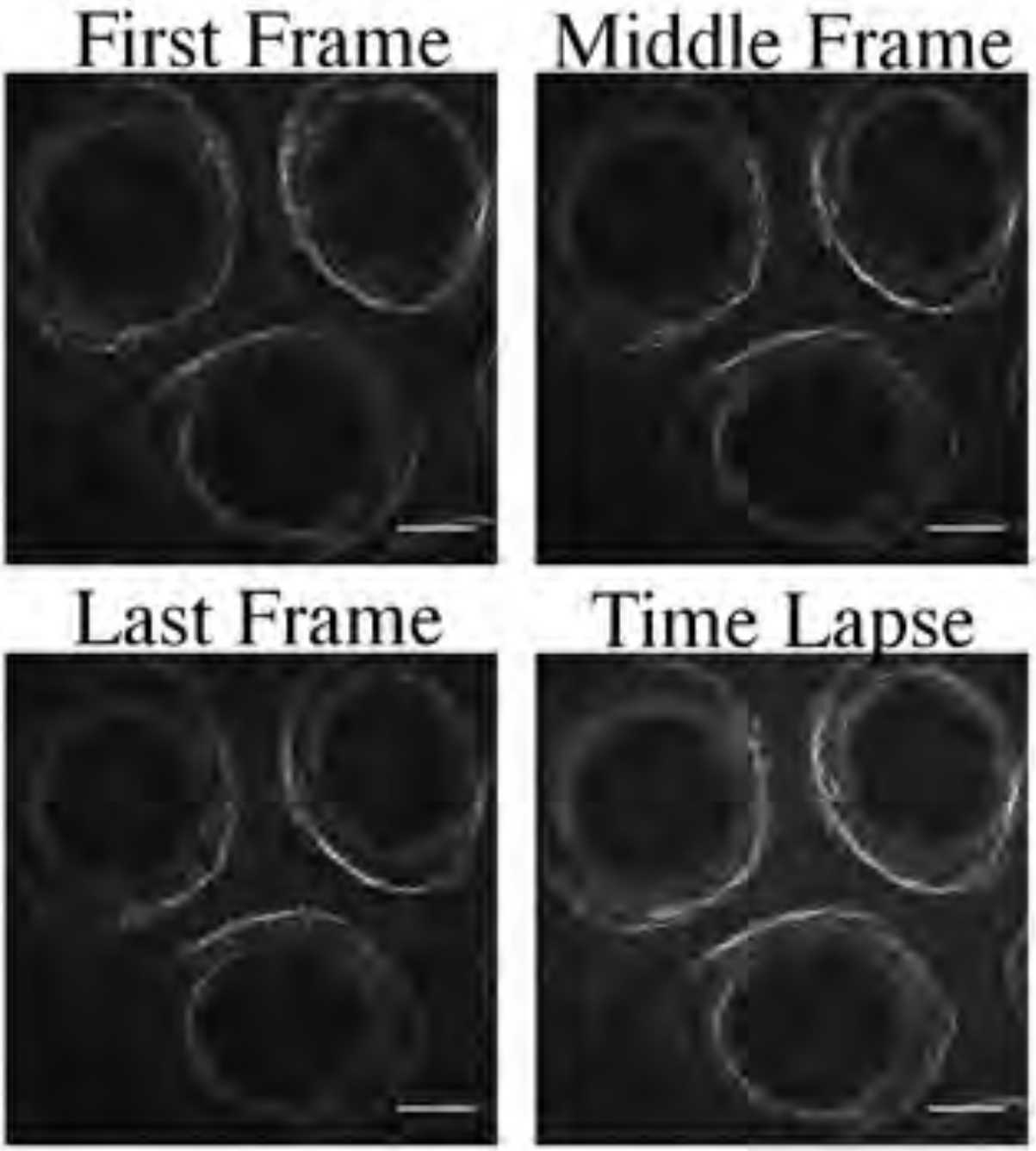


Supplemental Figure 13. JIM5 immunolabeling of *cesa* mutant seeds showing individual light channels and maximum projection of z-stacks. A-D) Green channel; E-H) S4B channel; I-L) Merged image; M-P) maximum projection of z-stacks. A-L) Bar = 10 μm; M-P) Bar = 25 μm.

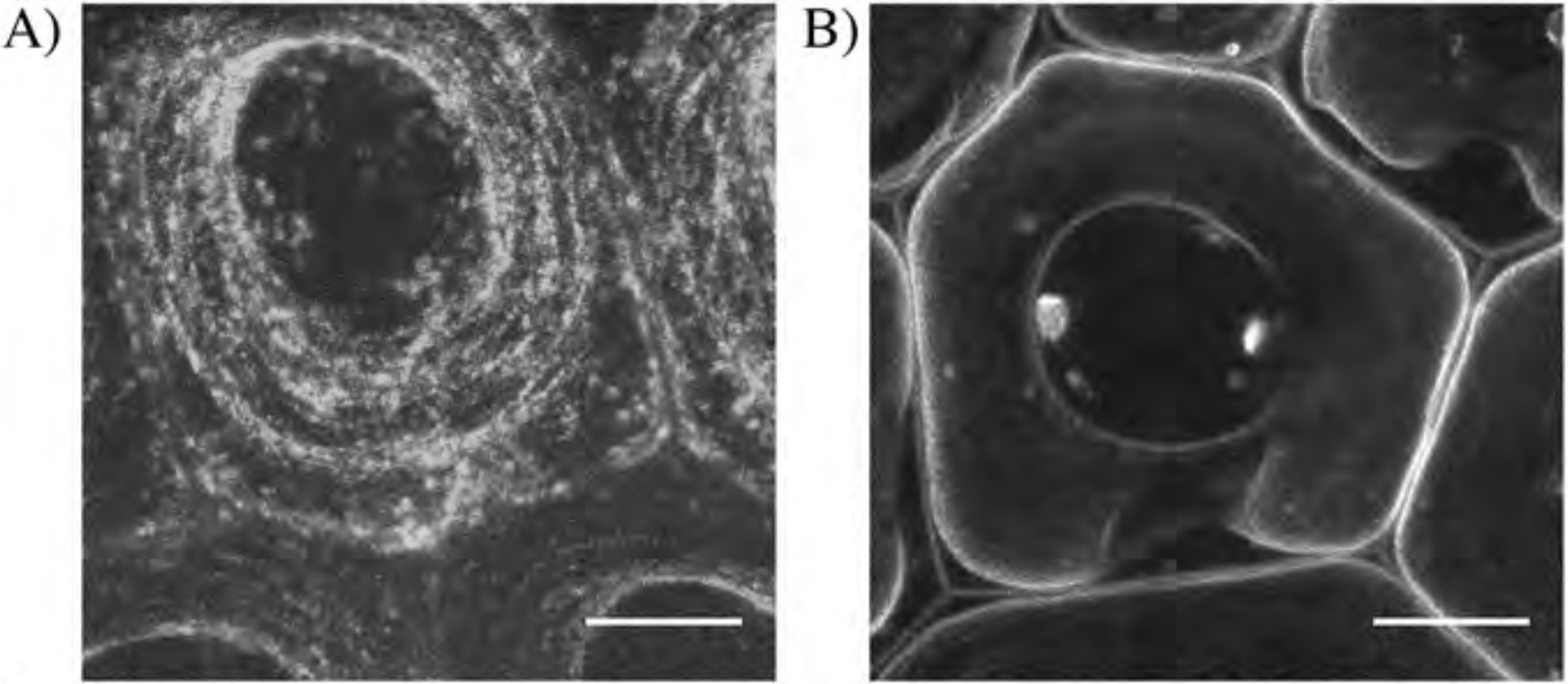


Supplemental Figure 14. *cesa* mutations influence calcium-mediated mucilage expansion.

A) to L) Wild-type and *cesa* mutant seeds hydrated in 0.05M HEPES buffer, 0.05M EDTA, and 0.1M $CaCl_2$ (pH 6.0). Bar = 100 μm.



Supplemental Figure 15: Images of GFP-CESA5 from a 10 min video (Supplemental Video 1) showing the first frame, middle frame, final frame and time lapse.



Supplemental Figure 16: Three dimensional image of the localization of A) GFP-CESA3 and B) GFP-CER5 in seed coat epidermal cells. Bar = 10 μm.