Figure 1. CESA7-dependent Induction of Secondary Walls in Protoplasts of Arabidopsis thaliana. A) Maximum Z projection of a protoplast synthesizing a banded SCW, imaged 24 hours after induction. Protoplasts were isolated from Arabidopsis plants expressing VND7-GR (inducible secondary wall regeneration) and YFP-CESA7 in a cesa7 knockout background (seeds from Lacey Samuel's lab). Cellulose stained with calcofluor white (blue color), and YFP-CESA7 fluorescence (yellow). B) Individual frame of z-stack at the top of the cell and C) ~3 microns below that show YFP-CESA7 at the plasma membrane under the localized cellulose bands.

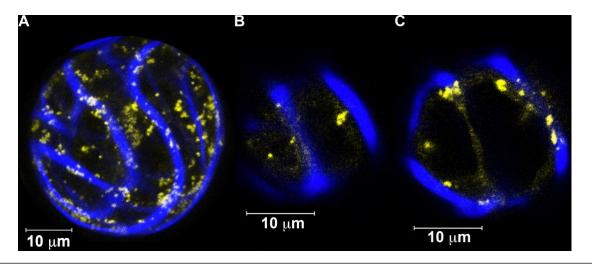
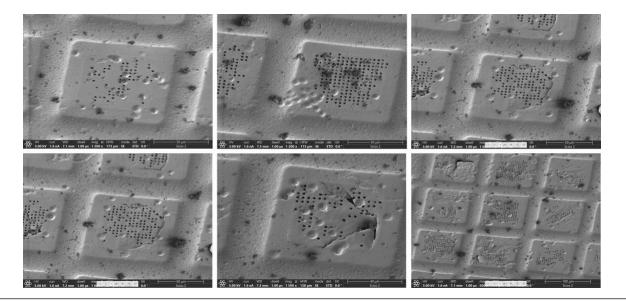


Figure 2. Collage of scanning EM micrographs of vitrified differentiating protoplasts. The panel of images show the quality of thin ice and cell size that one can expect to encounter when using the Aquilos2 integrated cryoFLM/cryoFIB milling device. Cells range from 5 to 15 microns in size.



References

- **1.** Purushotham P, Ho R, Zimmer J. Architecture of a catalytically active homotrimeric plant cellulose synthase complex. Science. 2020 Aug 28;369(6507):1089-1094. doi: 10.1126/science.abb2978.
- **2.** Nixon BT, Mansouri K, Singh A, Du J, Davis JK, Lee JG, Slabaugh E, Vandavasi VG, O'Neill H, Roberts EM, Roberts AW, Yingling YG, Haigler CH. Comparative Structural and Computational Analysis Supports Eighteen Cellulose Synthases in the Plant Cellulose Synthesis Complex. Sci Rep. 2016 Jun 27;6:28696. doi: 10.1038/srep28696.
- **3.** Duncombe SG, Chethan SG, Anderson CT. Super-resolution imaging illuminates new dynamic behaviors of cellulose synthase. Plant Cell. 2021 Sep 15:koab227. doi: 10.1093/plcell/koab227.