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Article

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Architecture and dynamics of a desmosome–endoplasmic reticulum complex

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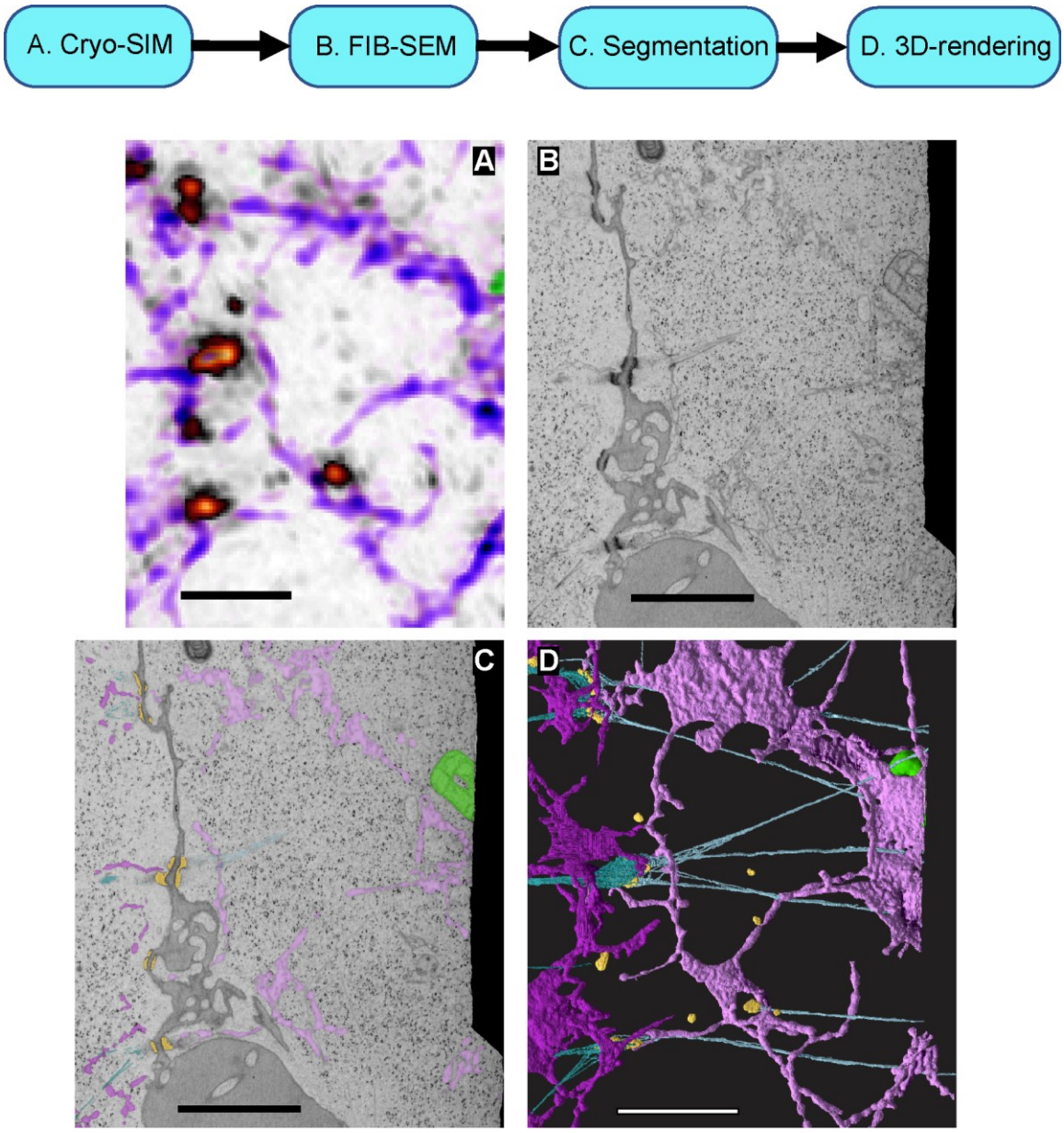
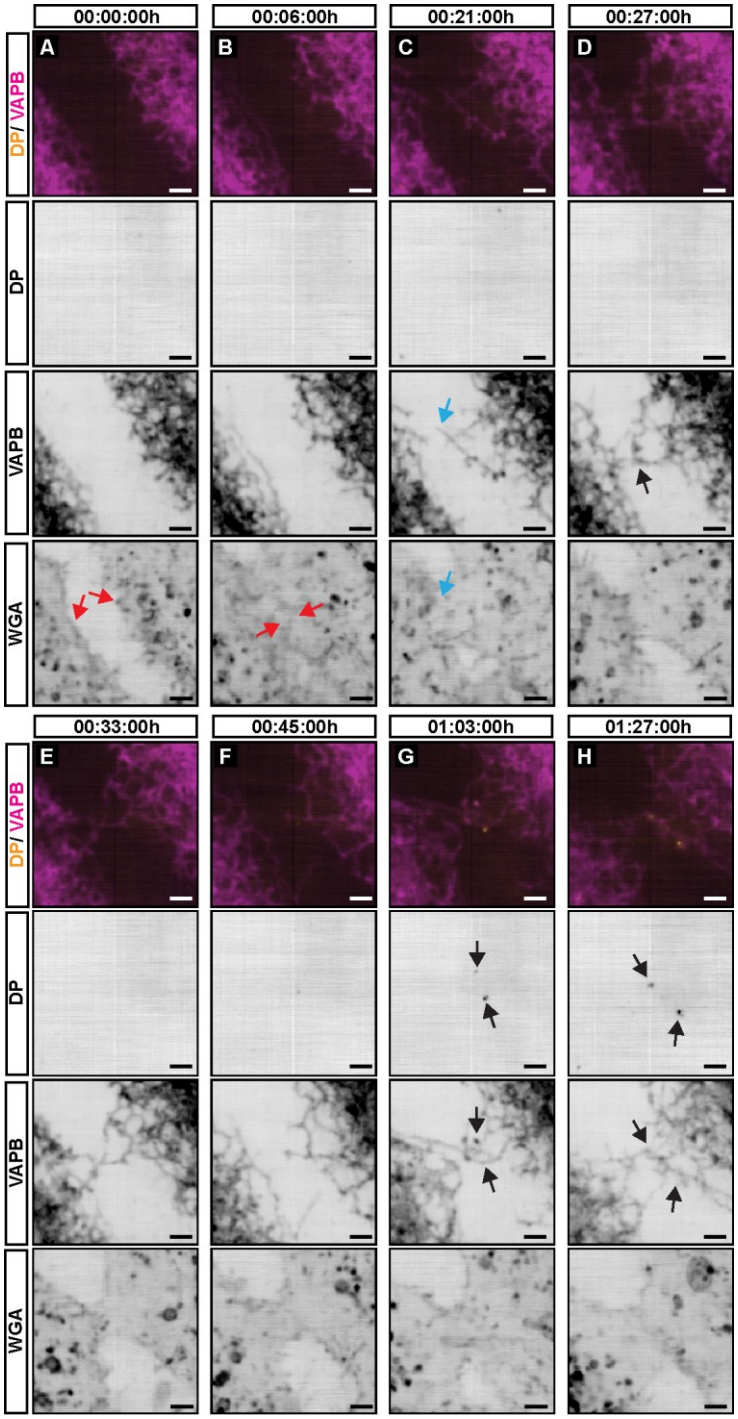
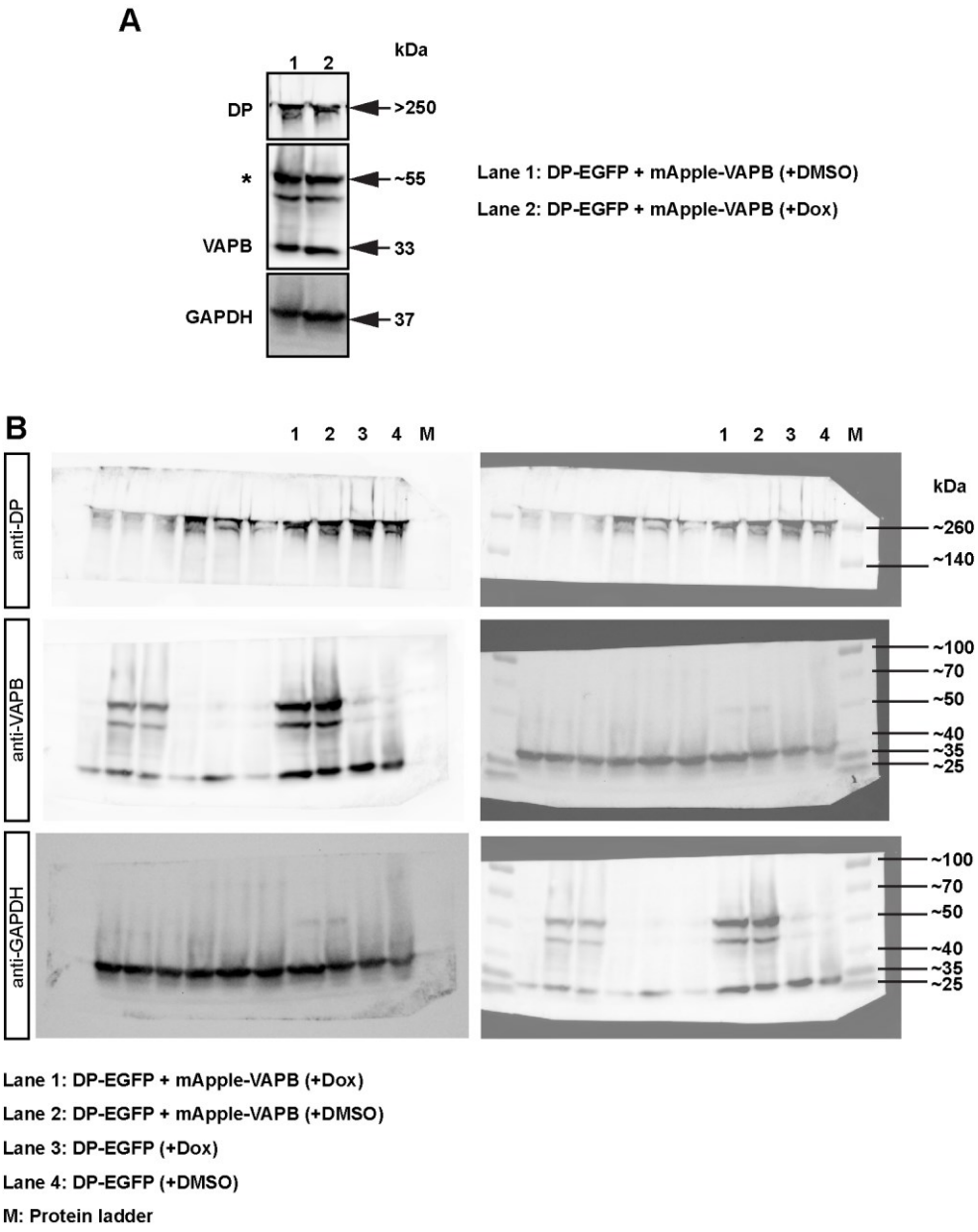


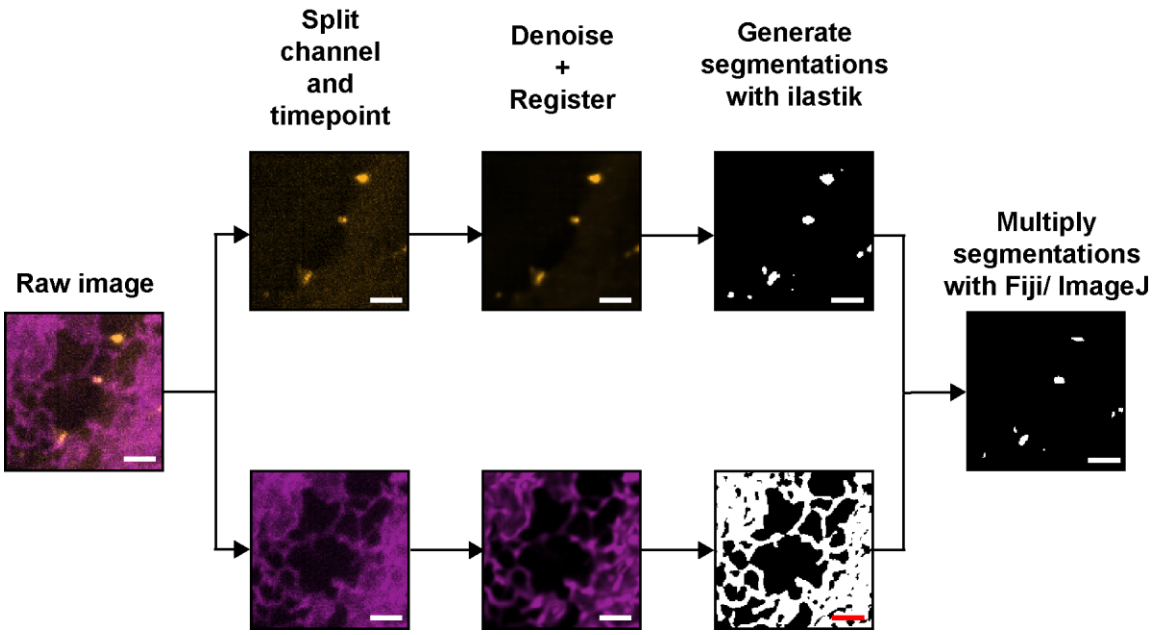
Supplementary Figure 1. **Correlative Light and Electron Microscopy (CLEM) workflow.** (**A**) A fluorescence Cryo-SIM maximum intensity projection of a cell-cell contact with labelled desmosomes (yellow), ER (purple), and mitochondria (green). (**B**) A single FIB-SEM slice of the same region in (**A**). (**C**) Overlay of segmentations of the desmosome outer dense plaque (yellow), keratin filaments (teal), ER (magenta), and mitochondria (green) with the FIB-SEM slice in (**B**). (**D**) Three-dimensional reconstruction of the same structures in (**C**) in a stack of serial FIB-SEM slices. Images are representative of n=1 cell-cell contact. Scale bar = 2μm.



Supplementary Figure 2. **ER tubules extend toward cell periphery after cell-cell contact and associate with desmosomes during assembly.** (**A-H**) Snapshots of a live-cell time-course of desmoplakin (orange) and VAPB (magenta) in A431 cells as they form cell-cell contacts after a switch to high calcium media. Wheat Germ Agglutinin (WGA) labels the plasma membrane (PM). Cell membranes of adjacent cells first extend toward each other (**A**, **B**, red arrows), followed by extension of ER tubules toward the cell-cell contact (**C**, blue arrows). ER tubules then arrange into mirror image-like structures at the cell-cell contact (**D**, black arrow). Images are representative of n=2 independent experiments. Scale bar = 2μm.



Supplementary Figure 3. **Western blots of lentivirus-generated A431 cell lines.** (**A**) Western blots of cell lysates collected from DP-EGFP/ mApple-VAPB A431 cells treated with DMSO (Lane 1) or 4μg/mL Doxycycline (Lane 2) to activate DP-EGFP expression. Asterisk denotes mApple-VAPB band. Levels of mApple-VAPB were found to be ~1.2X to 1.5X that of endogenous VAPB (n=2 independent experiments). GAPDH was used as a loading control. Blots labelled with anti-VAPB were stripped and re-probed for anti-GAPDH. (**B**) Original uncropped images of the blots in (**A**) (left), and composite images with a protein ladder (right).



Supplementary Figure 4. **Schematic flow of image processing and analysis to determine DP and VAPB overlap.** Raw images are first acquired and are then split by channel and timepoint in Fiji/ImageJ. These images are then denoised using 3DRCAN or Noise2Void, followed by registration. Segmentation of channels is performed with ilastik Pixel Classification to obtain binary masks. These image masks are then "Multiplied" in Fiji/ImageJ to detect overlapping pixels, indicating a close association. Scale bar = 2μm.