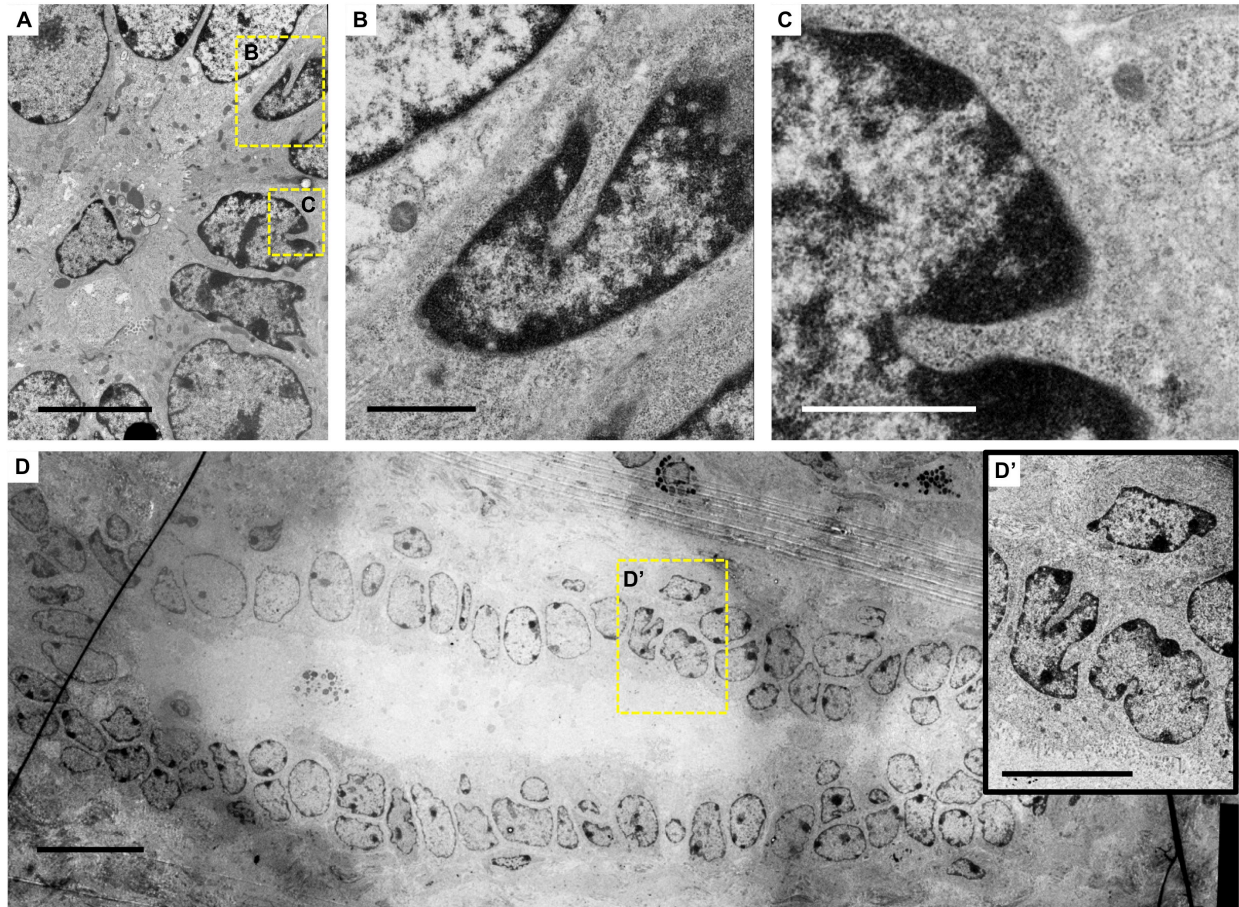
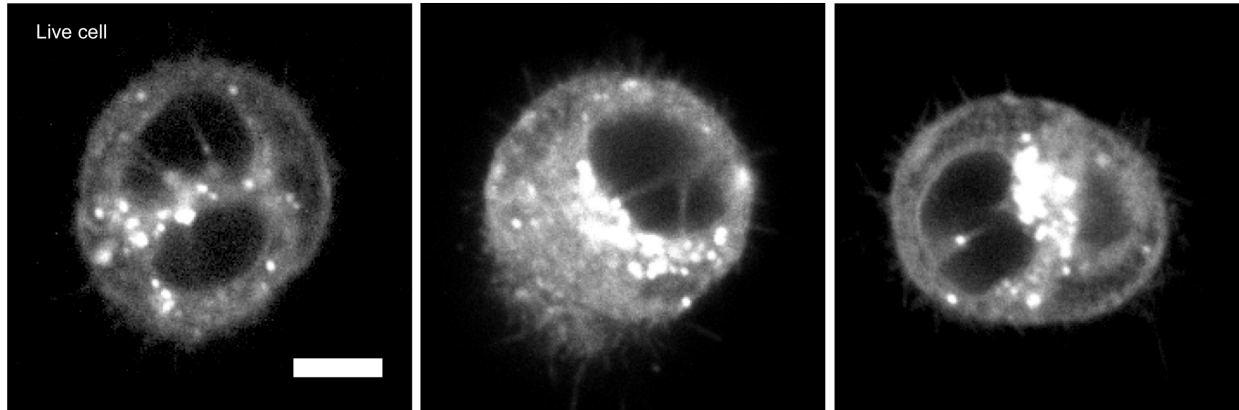


**Figure S1. Apically oriented centrosomes and primary cilia in growth arrested acinus.** (A – D) FIB-SEM dataset of growth arrested acinus in which upon the apical surface of four cells either primary cilia or centrioles are observed. (A) A single 4 nm slice of a FIB-SEM dataset, with (A') primary cilium in apical location of central cell (blue arrows, basal body components). (A'') The primary cilium 3D structure displayed as a segmented volume (blue, cilium; collar, yellow; basal body, orange). (B – B') Centrosome (blue arrows) of the left-most cell is visible. (C – C') One basal body (blue arrow) in the right-most cell is observed. (D – D') The primary cilium (blue arrows, defined upper length of the cilium) of the uppermost cell is visible.



**Figure S2. Wild type murine mammary gland TEM displays nucleoplasmic reticulum type II.** (A) Cross-sectional view of the luminal space of a mammary duct in which two cells (B, C) have prominent type II nucleoplasmic reticula (NR). The cytoplasm is dense and filled with ribosomes in both cells. (D) Longitudinal view of a mammary duct with few cells (D') displaying NR type II. Both (A) and (D) are TEM of 90 nm sections and indicate that NR can be difficult to properly visualize by 2D imaging alone. Scale bars for (A) 5  $\mu\text{m}$ , (B) 1  $\mu\text{m}$ , (C) 1  $\mu\text{m}$ , (D) 10  $\mu\text{m}$ , (D') 5  $\mu\text{m}$ .

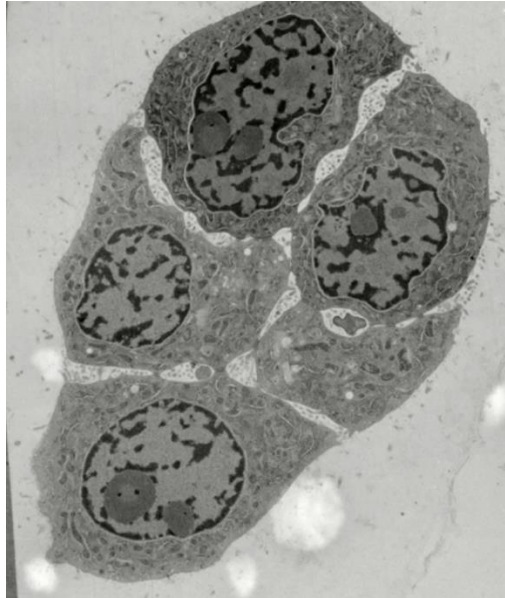




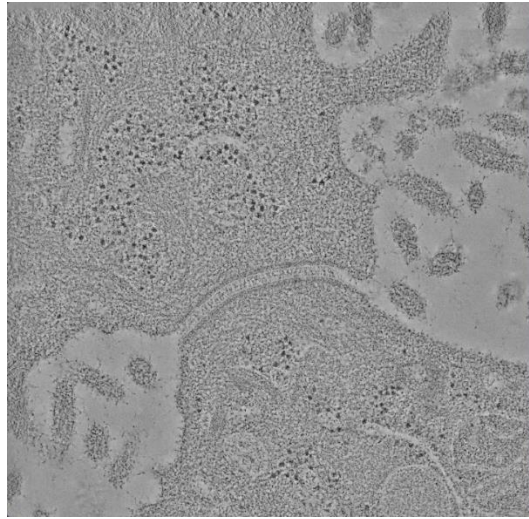
**Figure S3. Live cell imaging of nuclear membrane in S1 cells.** S1 cells were labeled with lipophilic membrane dye Vybrant Dil 24 hours prior to plating in 3D IrECM. Once plated in 3D IrECM cells were allowed to grow for 24 hours and then imaged. We find after 48 hours of labeling the Vybrant Dil membrane dye is internalized and labels internal membranes. Imaging of the dye shows multiple nuclear membrane tunnels traversing S1 nuclei. In some cases Golgi (brightly labeled organelles) appear to reside inside the tunnels. Scale bar 5  $\mu\text{m}$ .

The full movie collection can be viewed on FigShare:

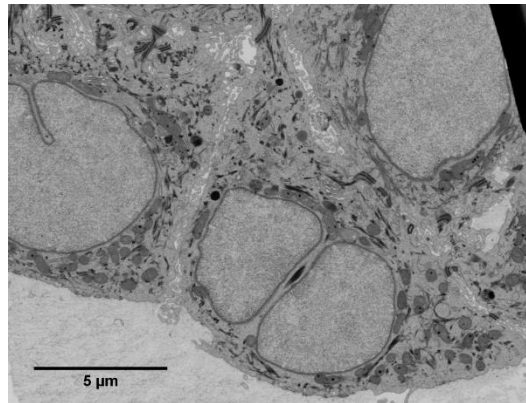
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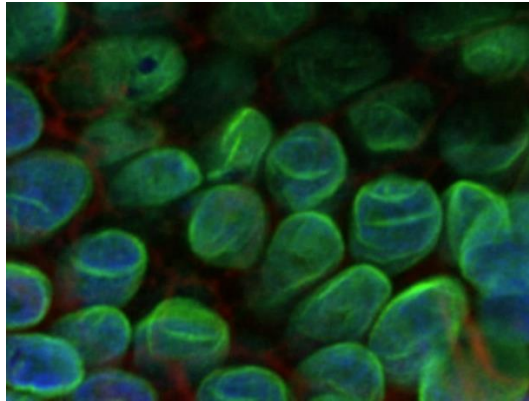
**Movie 1: Serial section TEM of S1 acinus.** Total depth of 2.2 micron of S1 acinus surveyed by serial section TEM. Twenty-two 100 nm thin sections sequentially cut and imaged by TEM. Images were overlaid with ImageJ (<https://imagej.nih.gov/ij/>). Serial sectioning provided 3-dimensional view across middle of an acinus, demonstrating lack of apical polarity and highlighting the spot-like adhesion points on lateral cell surfaces. Movie 1 (doi: 10.6084/m9.figshare.3141928) can be viewed at FigShare: [https://figshare.com/articles/Jorgens et al JCS 2016 Movie 1/3141928](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_1/3141928)



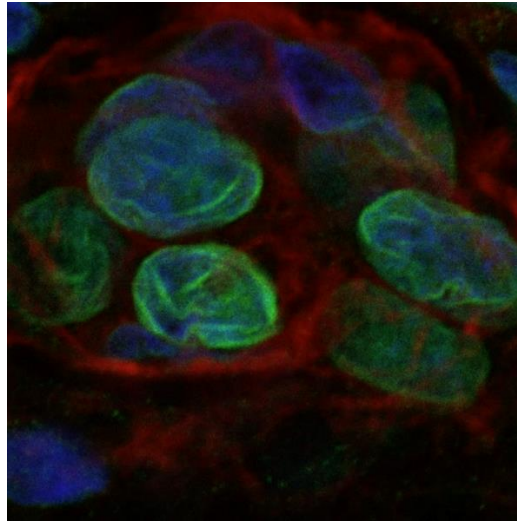
**Movie 2: High resolution electron tomography of desmosome cell junction.** Electron tomogram of a 100 nm thin section imaged every 1° from 70° to 70°. The nominal setting of defocus was 1 micrometer, and the pixel size of the data corresponded to 1 nm. The ultrastructural composition of the desmosome junction is visualized. Filaments between electron dense plaques on adjacent cell membranes are observed to span a gap of ~ 30 nm. Cytoskeletal filaments are observed linking into the electron dense plaques. Movie 2 (doi: 10.6084/m9.figshare.3167950) can be viewed at FigShare: [https://figshare.com/articles/Jorgens\\_et\\_al\\_JCS\\_2016\\_Movie\\_2/3167950](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_2/3167950)



**Movie 3: FIB-SEM of part of a S1 acinus with nuclear invaginations and tunnels.** FIB-SEM was collected on the S1 acinus for a final voxel dimension of 4 nm<sup>3</sup>. The left and right most cells each have deep nuclear invaginations (NR type II). A primary cilium is observed at the beginning of the dataset on the apical surface of the central cell. The central displays both nuclear invaginations and cytoskeletal filaments transversing the nucleus within a tunnel. Movie 3 (doi: 10.6084/m9.figshare.3145006) can be viewed at FigShare: [https://figshare.com/articles/Jorgens\\_et\\_al\\_JCS\\_2016\\_Movie\\_3/3145006](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_3/3145006)

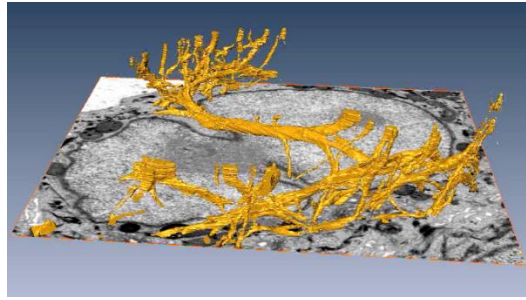


**Movie 4: Lamin B1 stain of human mammary ductal cells.** Confocal stack of a duct in a cryosection of human mammary tissue stained for DNA (DAPI, blue), lamin B1 (green), & F-actin (phalloidin, red). Movie 4 (doi: 10.6084/m9.figshare.3145030) can be viewed at FigShare: [https://figshare.com/articles/Jorgens et al JCS 2016 Movie 4/3145030](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_4/3145030)

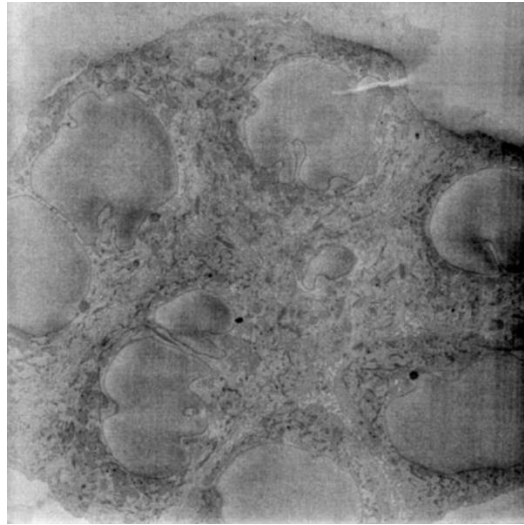


**Movie 5: Lamin B1 stain of human mammary acinar cells.** Confocal stack of an acinus in a cryosection of human mammary tissue stained for DNA (DAPI, blue), lamin B1 (green), & F-actin (phalloidin, red). Movie 5 (doi: 10.6084/m9.figshare.3145027) can be viewed at FigShare: [https://figshare.com/articles/Jorgens\\_et\\_al\\_JCS\\_2016\\_Movie\\_5/3145027](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_5/3145027)



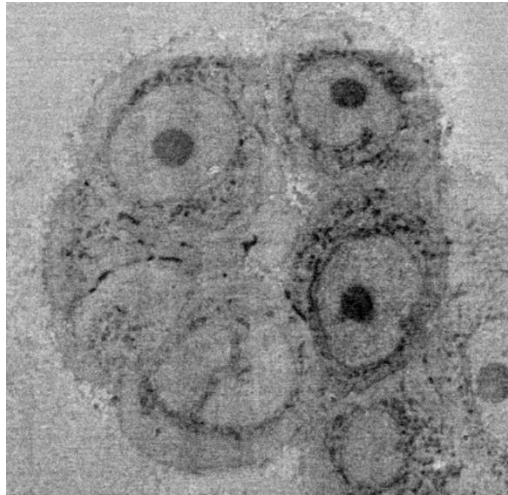


**Movie 6: Segmented nuclear tunnel and cytoskeletal filament from FIB-SEM.** The central cell nuclei from Movie 3 is segmented to reveal the 3D architecture of the cytoskeletal filament transversing the nucleus within a tunnel, rendered in yellow. Visualization of the 3D segmentation was performed with Amira software (FEI, Inc.). Movie 6 (doi: 10.6084/m9.figshare.3145063) can be viewed at FigShare: [https://figshare.com/articles/Jorgens\\_et\\_al\\_JCS\\_2016\\_Movie\\_6/3145063](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_6/3145063)



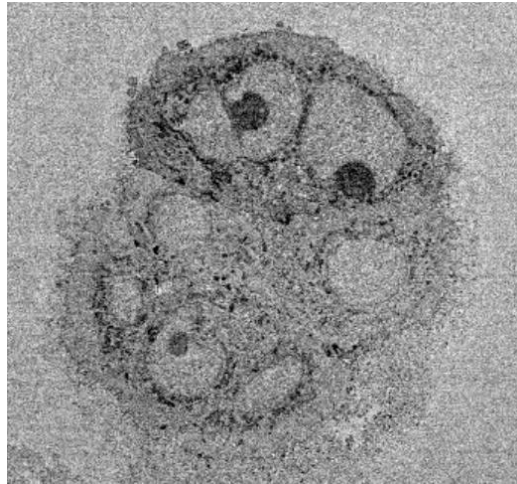
**Movie 7: SBF-SEM of a S1 acinus with nuclear invaginations and tunnels.** An acinus was imaged via SBF-SEM for a final voxel dimension of  $50 \text{ nm}^3$ . Cells are observed at various z-depths without and with deep nuclear invaginations (NR type II) and two cells display nuclear tunnels with cytoskeletal filaments. Movie 7 (doi: 10.6084/m9.figshare.3145012) can be viewed at FigShare:

[https://figshare.com/articles/Jorgens\\_et\\_al\\_JCS\\_2016\\_Movie\\_7/3145012](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_7/3145012)



**Movie 8: S1 acinus imaged by SBF-SEM.** SBF-SEM data set collected of a S1 acinus, displaying eight whole and partially imaged cells. Five of the cells contain nuclear invaginations, three cells (37%) have nuclear tunnels with a total of four tunnels observed (two in one cell and one in each other cell). One cell has neither feature. Pixel size is  $\sim 100 \text{ nm}^3$ . Movie 8 (doi: 10.6084/m9.figshare.3466046) can be viewed at FigShare:

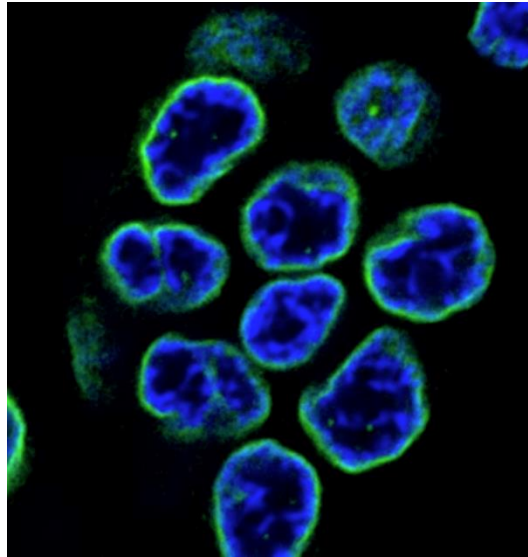
[https://figshare.com/articles/Jorgens et al JCS 2016 Movie S1/3466046](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_S1/3466046)



**Movie 9: S1 acinus imaged by SBF-SEM.** SBF-SEM data set collected of a S1 acinus, displaying six whole and partially imaged cells. Four of the cells contain nuclear invaginations, two cells (33%) have nuclear tunnels with a total of four tunnels observed (three in one cell and one in the other). Two cells do not display either feature. Pixel size is  $\sim 100 \text{ nm}^3$ . Movie 9 (doi: 10.6084/m9.figshare.3466049) can be viewed at FigShare:

[https://figshare.com/articles/Jorgens\\_et\\_al\\_JCS\\_2016\\_Movie\\_S2/3466049](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_S2/3466049)

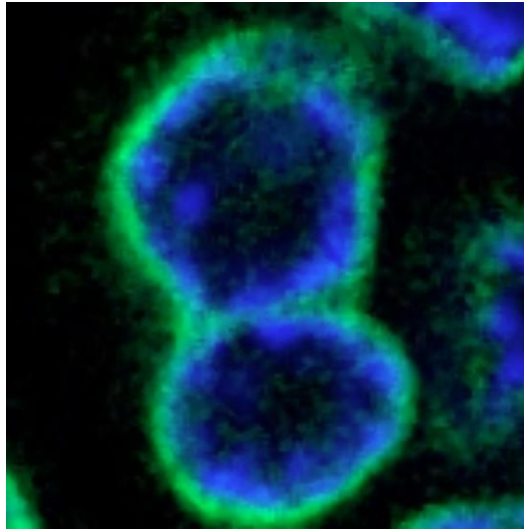




**Movie 10: Lamin B1 stain of S1 acinus illuminates multiple nuclear tunnels and the NR.**

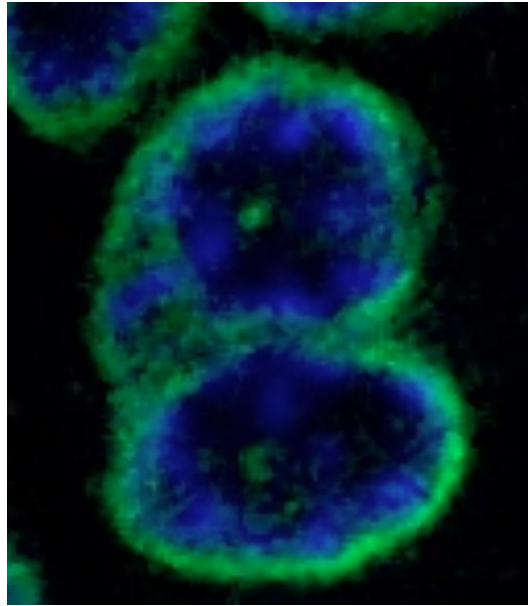
Confocal stack of staining for lamin B1 (green) and DNA (DAPI, blue) in growth arrested S1 acini. NR type II is observed in multiple cells as well as nuclear tunnels transversing the nucleus. Movie 10 (doi: 10.6084/m9.figshare.3145003) can be viewed at FigShare:

[https://figshare.com/articles/Jorgens et al JCS 2015 Movie 8/3145003](https://figshare.com/articles/Jorgens_et_al_JCS_2015_Movie_8/3145003)

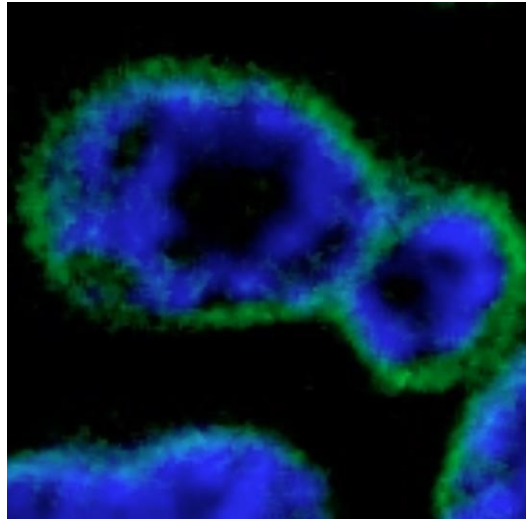


**Movie 11: Lamin B1 stain of single cell illuminates a nuclear tunnel in a S1 acinus.** Close up view of a single cell from the acinus in Movie 8 stained for lamin B1 (green) and DNA (DAPI, blue). A tunnel transverse the nucleus is observed. Movie 11 (doi: 10.6084/m9.figshare.3145021) can be viewed at FigShare:

[https://figshare.com/articles/Jorgens et al JCS 2016 Movie 11/3145015](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_11/3145015)

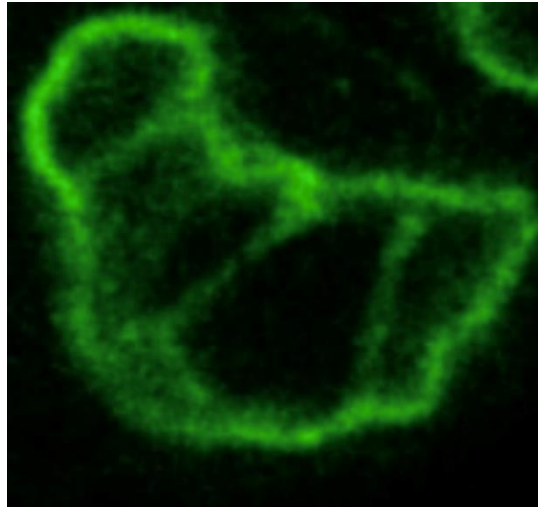


**Movie 12: Lamin B1 stain of single cell illuminates a nuclear tunnel in a S1 acinus.** Close up view of a second cell from the acinus in Movie 8 stained for lamin B1 (green) and DNA (DAPI, blue). A tunnel transversing the nucleus is observed. Movie 12 (doi: 10.6084/m9.figshare.3145018) can be viewed at FigShare: [https://figshare.com/articles/Jorgens\\_et\\_al\\_JCS\\_2016\\_Movie\\_10/3145018](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_10/3145018)



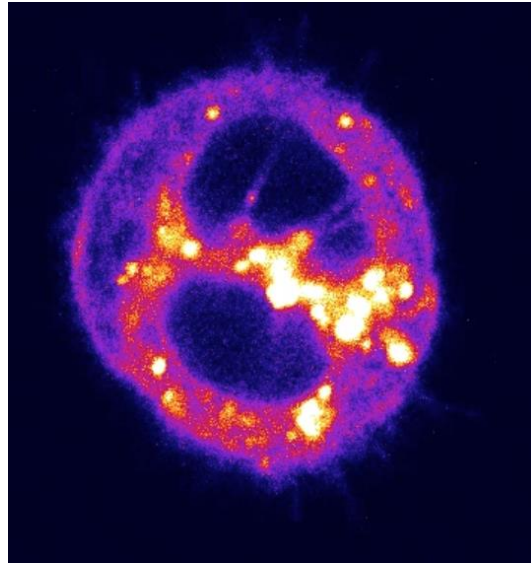
**Movie 13: Lamin B1 stain of single cell illuminates a nuclear tunnel in a S1 acinus.** Close up view of a third cell from the acinus in Movie 8 stained for lamin B1 (green) and DNA (DAPI, blue). A tunnel transversing the nucleus is observed. Movie 13 (doi: [https://figshare.com/articles/Jorgens\\_et\\_al\\_JCS\\_2016\\_Movie\\_9/3145021](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_9/3145021))





**Movie 14: Lamin B1 stain illuminates four nuclear tunnels in a single S1 cell.** Close up view of a single cell in an acinus stained for lamin B1 (green) and DNA (DAPI, blue) and imaged by confocal microscopy. Four tunnels traversing a single nucleus are observed. Movie 14 (doi: 10.6084/m9.figshare.3466052) can be viewed at FigShare:

[https://figshare.com/articles/Jorgens\\_et\\_al\\_JCS\\_2016\\_Movie\\_S3/3466052](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_S3/3466052)



**Movie 15: Live S1 cells stained with a membrane dye.** Confocal stack of live S1 cells stained with the lipophilic membrane dye Vybrant Dil after 24 hours in 3D IrECM culture. The membrane dye stains the nuclear membrane illuminating multiple tunnels transversing the nucleus. Movie 15 (doi: 10.6084/m9.figshare.3145024) can be viewed at FigShare:

[https://figshare.com/articles/Jorgens et al JCS 2016 Movie 12/3145024](https://figshare.com/articles/Jorgens_et_al_JCS_2016_Movie_12/3145024)