

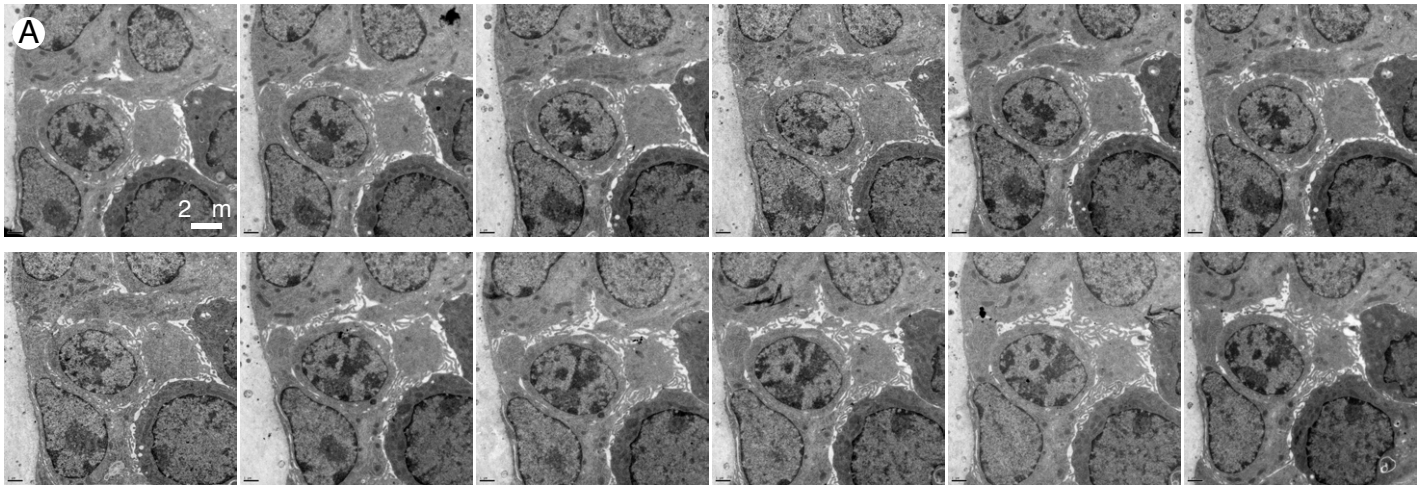
Fig. S1. Lateral membrane protrusions represent extensive three dimensional membrane extensions. (A-B) Using both serial section TEM and (A) and serial block face SEM (B) we observed lateral membrane protrusions to extend through 5-30 thin sections, corresponding to a thickness of 500-2500 nm. Individual protrusions continuously vary from linear to branched morphologies. The image series used to generate Panel B has been uploaded to the ASCB's The Cell: An Image Library.

Fig. S2. Thin protrusions on single sections extended into thick membrane extensions. (A-B) Both in 3D reconstructions (A) and in individual serial block face SEM images (B), we observed thin membrane protrusions continuing through multiple sections. Once reconstructed, apparently thin protrusions (red arrowheads) were revealed to be 3D membrane extensions.

Fig. S3. Inhibition of Rac or myosin light chain kinase (MLCK) signaling resulted in distinct epithelial architectures. (A-C) Inhibition of MLCK with 1 μ M ML-7 resulted in luminal filling, a failure of ductal initiation, and subsequent luminal clearing back to a well-polarized cyst with tight junctions. **(D-F)** Inhibition of Rac signaling with 100 μ M Rac inhibitor resulted in luminal filling, a failure of ductal initiation, and a persistently filled lumen. Cells made extensive contact via complex interdigitating membrane protrusions.

Fig. S4. Inhibition of ROCK abolishes simple epithelial organization. (A-C) Treatment with the ROCK inhibitors H1152 (B) or Y27632 (C) both block lumen formation and simple epithelial organization relative to control (A). In both inhibitor-treated samples there are small microlumens. **(D)** ROCK inhibitor treated organoids have multiple poorly organized buds. The main difference from control is the organization of the epithelium, not the number of buds.

Serial Sections, 90 nm apart, Consecutively Imaged by TEM



Serial Block Face SEM, Images Every 50 nm

