

## Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: Representative LLSM movie of 2-celled MDCK spheroid expressing Lifeact-RFP showing non-adherent apical surfaces. Left pane is a XY slice through the lumen and the right plane is a YZ slice of the same spheroid. Scale bar is 5  $\mu\text{m}$ , movie lasts 10 s in real time.

File Name: Supplementary Movie 2

Description: Representative movies of MDCK spheroids expressing Lifeact-RFP treated with DMSO vehicle control (**left**) and cytoskeletal inhibitor cocktail (latrunculin A, Y-27632, ML-7, and nocodazole, **right**). Scale bar is 10  $\mu\text{m}$ .