

Supplemental information

Figure S1: MDCK cells do not grow under parafilm at day 1. MDCK cells patterned using parafilm method at day 1. Cells were fixed and stained with dapi, followed by removal of parafilm insert. Removal of parafilm caused some damage to the circular pattern, but revealed absence of cells in the areas previously bonded to parafilm. Scale bar is 50 μm .

Figure S2. Overtime cells form a double layer at the edges of the patterns. This picture shows MDCK cells patterned with the agarose method at day 7. *Green-ZO-1, Blue-Dapi*. Scale bar 5 μm .

Figure S3: ZO-1 staining in patterned and un-patterned MDCK cells at different days. A-F show a single confocal plane of ZO-1 over time: (A, B and C) ZO-1 staining on patterned MDCK cells at Day 1, 7 and 15, respectively. (D,E and F) ZO-1 staining on un-patterned MDCK cells at Day 1, 7 and 15, respectively. Scale bar is 50 μm .

Figure S4. Cells grow underneath the parafilm insert prepared without heating. Cells were patterned using a parafilm insert without heating step when adhering the parafilm insert to the filter insert's membrane. MDCK cells were cultured overnight and stained for β -catenin, followed by removal of parafilm insert. Scale bar is 25 μm .