Briefly, following humane sacrifice, the colon was harvested and opened longitudinally with intestinal contents removed. The tissue was rinsed with ice cold PBS in a 90 mm petri dish, washed with ~20 ml ice cold PBS in a 50 ml tube by vigorous shaking, and then rinsed again with ice cold PBS in a 90 mm petri dish. After washing, tissue was transferred to a fresh petri dish in a biological tissue culture hood and then minced/scraped with glass slides to separate the epithelial cells from underlying muscle. One ml collagenase (Invitrogen) solution was added to suspend tissue fragments, and the petri dish was incubated in a cell culture incubator (37°C) with vigorous mixing every 5–10 min, using a 1,000 μl pipette. Once visible, single epithelial units (crypts/pits) were separated from the larger tissue fragments as seen on a phase or dissection microscope. The epithelial units were passed through a 70 μm cell strainer (BD) using a 1,000 μl pipette and the strainer was washed with 9 ml washing media (penicillin (100 units/ml), streptomycin (0.1 mg/ml), L-glutamine (2 mM), and FBS (10%) in DMEM/F12 (Invitrogen) with HEPES). This filtrate was transferred to a 15 ml centrifuge tube and centrifuged at 20 g for 5 min. The pellet was suspended in 500 μl–1 ml washing media, transferred to a 1.5 ml tube, centrifuged at 200 g for 5 min, placed on ice and the epithelial units resuspended in Matrigel (Corning, 200 μl per well). Fifteen μl of cell-Matrigel suspension was then placed as domes making 8-10 domes for each well of a 6-well plate using a 20 μl pipette. To polymerize the Matrigel, plates were incubated upside down to avoid attachment of epithelial units to the plate surface. After 10 min, plates were returned to the upright orientation and 2000 μl of 50% L-WRN conditioned medium (1) (a 1:1 mix of L-WRN conditioned medium and Advanced DMEM/F-12 with 20% FBS) were added to each well, and the medium subsequently changed at least every 48 h.