**Histology and Immunohistochemistry Staining**

Tissues and organoids were processed for frozen sections or paraffin sectioning using following protocols. For frozen sections, organoids were fixed in 4% paraformaldehyde in PBS at 4°C for 6 h, then placed in 15% sucrose in PBS overnight, centrifuged and resuspended into bacto-agar and embedded in OCT (Tissue-Tek). The frozen sections were stained for F-actin using phalloidin. For paraffin sectioning, tissues were fixed in 10% formalin overnight and embedded in paraffin, whereas organoids were fixed in 10% formalin overnight, resuspended in bacto-agar, then embedded in paraffin. Standard protocols for sectioning paraffin-embedded tissues and staining with H&E, periodic acid-Schiff (PAS) or Alcian Blue staining were used. For Ki67 immunohistochemistry staining, slide deparaffinization was followed by standard protocol. For the antigen retrieval: 250 mL of citric acid buffer was placed into a slide container, which was filled with up to 12 slides. The buffer solution was warmed in a microwave to 100°C for 10 min, then cooled for 5 min, warmed again for 2 min, cooled for 5 min, warmed again for 2 min, then cooled down to RT. Citric acid was replaced with PBS, the slide container was placed onto a rocker, and the slides were washed with PBS. The slides were then incubated in 10% H2O2 RT for 15 min followed by 3 washes in PBS. The slides were placed in a slide box and the tissue was circled using an oil pen. The tissue was incubated in BSA for 20 min, followed by incubation in the primary antibody Ki67 (GB13030-2, 1:1000) overnight at 4°C. The slides were washed 3 times with PBS, incubated in secondary antibody (anti-rabbit serum, 1:200 in PBS) for 50 min, stained with DAB (1:100), washed with H2O, stained with hematoxylin, and then mounted using standard protocol.

**Immunofluorescence Staining**

***For cell lines:*** NIH/3T3 cells stably infected with pCDH-RHOA vectors encoding wild-type and mutant RHOA were plated on 10 μg/ml fibronectin-coated glass coverslips. Cells were washed twice with PBS, fixed in 3.7% formaldehyde (Fisher Scientific) for 10 min at RT, washed with PBS and permeabilized with 0.1% Triton-X100 (Sigma) for 5 min at RT. Non-specific signals were blocked using 2% BSA (Sigma) in PBS for 30 min at RT. Cells were incubated with primary antibody (diluted in 2% BSA-PBS) for 60 min at RT, followed by three washes with PBS and incubation with the secondary antibody (diluted in 2% BSA-PBS) for 45 min at RT. After washing three times with PBS, cells were mounted with Mowiol (Sigma). Focal adhesions were visualized by anti-vinculin antibody (mouse, Sigma) followed by an Alexa-Fluor-568 secondary antibody (Invitrogen). Phalloidin conjugated with an Alexa-488 fluorophore (Invitrogen) was used to label F-actin. HA-tagged RHOA localization was visualized with a mouse anti-HA antibody (HA-7, Sigma), followed by an Alexa-Fluor-488 secondary antibody (Invitrogen). ***For organoids:*** Organoids were fixed in 10% formalin overnight, then resuspended in bacto-agar and embedded in paraffin. The slides were cut, deparaffinized and hydrated using standard protocol. Antigen retrieval was performed by boiling the slides for 10 min in Tris-EDTA pH 9 in a pressure cooker. The sections were blocked with 5% normal donkey serum (Jackson ImmunoResearch Lab Inc) for 1 at RT. Slides were then incubated with rabbit anti-beta-catenin (1:400, Cell Signaling Technology) or rabbit anti-active YAP1 (1:1000, Abcam) and goat anti-E-cadherin (1:200, R&D systems) overnight at 4oC. The slides were washed three times in TBS and incubated with Alexa 647-conjugated donkey anti-rabbit and CY3-conjugated donkey anti-goat secondary antibodies (1:300, Jackson ImmunoResearch). Samples were counter-stained with Hoechst 33342 (1:10,000, Invitrogen) and washed three times with TBS before mounting in Prolong Gold anti-fade mounting media (Invitrogen). Immunofluorescence images were acquired on an LSM 880 confocal microscope.