Supplement Method:

Immunohistochemistry and immunofluorescence staining of mouse jejunum

Immunohistochemistry was performed in paraffin-embedded sections of mouse jejunum (56). Before immunostaining antigen retrieval was performed by heating slides in pH 6.0 citrate buffer at 100 °C for 20 min in a microwave oven at 500 W using antigen retrieval solution (10 mM Tris and 1 mM EDTA, pH 9.0). Non-specific antibody binding was blocked for 20 min by incubation with 0.05% w/v BSA in PBS. the slides were incubated with monoclonal anti-Ki67 antibody (M7240 mib1; Dako). Nuclear staining was visualized using streptavidin-peroxidase and diaminobenzidine (DAB) and samples were lightly counterstained with hematoxylin. For γ-H2AX immunofluorescence staining jejunal sections were stained with mouse monoclonal anti-phospho-histone H2A.X (Clone JBW301, Millipore) (1:250) followed by Secondary antibody anti mouse alexa-549 (Thermo Fisher Scientific). A total of 60 crypts were examined per animal.

Immunofluorescence staining of whole mount organoid

40 µl of the matrigel containing human organoids were plated into 1 well each of an 8-well chamber-slide (Thermo Fisher Scientific, Lab-Tek™, catalog number: 154532). Once matrigel was harden in the incubator for 30 mins, 300 µl of organoid media. Media was changed at least every two days. When organoids are ready for fixation, media was removed from each well and 300 µl freshly prepared 4% PFA in 1x PBS buffer (room temperature) was added in each well for 20 min. Fixative was removed and washed once with 1xPBS. 300 µl of 0.5%1x TritonX was added to the well to permeabilize for 20 min. Then 200 ul of 5% animal serum was added each well for blocking the organoid. Rabbit monoclonal anti-Ki67 primary antibody (ab92742, Abcam) and Cleaved Caspase-3 (Asp175) (5A1E) Rabbit

mAb #9664 (Cell signaling) was diluted (1:250) in blocking solution (5% goat serum) and added 150 µl to each well. Chamber-slides were kept overnight in a large, humidified chamber at 4 °C. After overnight incubation, each well was washed 3 times for 5 min each with 300 µl 1x PBS. Prepare secondary antibody anti rabbit Alexa-549 (Thermo fisher scientific) was prepared in blocking Solution (5% goat serum). 150 µl of the solution was added to the well and kept at room temperature in dark for 1 h. To visualize DNA/nuclei, a solution of DAPI (1 µg/ml) in 1xPBS was made and added 300 µl to each well for 5 min. Then proceeded to washing for three times for 5 mins with 1x PBS. Finally, Anti-fade gold mounting medium (Molecular probes) were added on to the slides and coverslip was placed on the slides. Organoids were visualized by confocal microscope (Nikon, A1R) in KUMC imaging core facility.

Mitotic catastrophe assay

Immunofluorescence analysis of multinucleated cells and mitotic catastrophe. Intestinal crypt epithelial cells were seeded into a chambered slides. These cells were cultured overnight in a 37˚C incubator and then exposed to 6 Gy of 60Coγ radiation. Each well were washed twice in PBS, fixed with 4% paraformaldehyde at room temperature for 30 min, washed three times with PBS for 10 min per wash, permeabilized in a 0.3% Triton X-100 solution on ice for 15 min and then blocked in a solution of PBS and 5% goat serum at room temperature for 1 h. Each cell sample was then incubated overnight at 4˚C with a 1:200 dilution of a primary anti-α-tubulin –FITC (cat no. 2168; Sigma). Following this incubation, samples were washed three times in PBS for 10 min per wash. Samples were incubated with Hoechest 33342 (1 mg/ml)(Catalogue no. H1399; Molecular Probes, Eugene, Oregon, USA) for 10 min at room temperature followed by PBS wash for 10 minutes. Then samples were mounted with the coverslip using the Prolong

Gold antifade reagent (catalogue no. P10144, Thermo fisher scientific). Samples were imaged using confocal laser scanning microscopy (Nicon A1R)(KUMC imaging core facility).

ATP assay to measure the viability of organoid

The viability of organoid was measured using CellTiter-Glo® 3D reagent (Promega). Sample volumes and microtissue properties (e.g., size, number, days in culture, etc.) was optimized for experimental conditions. Test compound was added to experimental wells, and incubated according to your culture protocol. The experiment was done according to the manufacturer's protocol.

siRNA transfection in human colon organoid

The single cell suspension from human normal and tumor colon derived organoid was prepared by dissociating the organoid with cell dissociation media (Corning Cellgro, Manassas, VA, USA) according to manufacturer provided protocol. siRNA complexes were formed using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA) in Opti-MEM (reduced serum) medium and then added to the crypt cells followed by incubation for 6hrs at 37°C.

For each transfection sample, oligomer-Lipofectamine 2000 complexes were prepared as follows:

20 pmol siRNA oligomer was diluted in 50 µl Opti-MEM I (Gibco™) Reduced Serum Medium without serum which results in final concentration of 400 nM). This was mixed gently.

Lipofectamine 2000 was gently mixed before use, then diluted 1 µl in 50 µl OptiMEM I Reduced Serum Medium. Gently mixed and incubated for 5 minutes at room temperature. After the 5-minute incubation, the diluted oligomer was mixed gently with the diluted Lipofectamine 2000 and incubated for 20 minutes at room temperature (solution may appear cloudy).

Then oligomer-Lipofectamine 2000 complexes were added to each well containing organoid and medium. Plate was incubated at 37°C in a CO2 incubator for 24-96 hours until ready to do the assay for gene knockdown.

In all conditions antibiotics were omitted as recommended by the manufacturer to preserve viability during the transfection process. Then cells were collected by centrifugation. Cells were re-suspended in mixture of regular organoid culture media and matrigel and plated in pre-warmed 24 well plate. After 30 mins regular organoid culture media was overlaid on matrigel containing the organoids and kept at 37oC.

siRNAs were procured from Santa Cruz Biotechnology Dallas, TX.

List of siRNA sequences. all sequences are provided in 5′ → 3′ orientation.

sc-44214 : p21 siRNA (h2) is a pool of 2 different siRNA duplexes:

sc-44214A:

• Sense: CCAGUUCAUUGCACUUUGAtt

• Antisense: UCAAAGUGCAAUGAACUGGtt

sc-44214B:

• Sense: CCACCUAGACUGUAAACCUtt

• Antisense: AGGUUUACAGUCUAGGUGGtt

c-44218 : p53 siRNA (h2) is a pool of 3 different siRNA duplexes:

sc-44218A:

• Sense: GUACCACCAUCCACUACAAtt

• Antisense: UUGUAGUGGAUGGUGGUACtt

sc-44218B:

• Sense: GUUUGGGAGAUGUAAGAAAtt

• Antisense: UUUCUUACAUCUCCCAAACtt

sc-44218C:

• Sense: CAAGGCCCAUAUCUGUGAAtt

• Antisense: UUCACAGAUAUGGGCCUUGtt

sc-36213 : PERK siRNA (h) is a pool of 3 different siRNA duplexes:

sc-36213A:

• Sense: CGAGAGCCGGAUUUAUUGAtt

• Antisense: UCAAUAAAUCCGGCUCUCGtt

sc-36213B:

• Sense: GGAUGAAAUUUGGCUGAAAtt

• Antisense: UUUCAGCCAAAUUUCAUCCtt

sc-36213C:

• Sense: CAGACACACAGGACAAGUAtt

• Antisense: UACUUGUCCUGUGUGUCUGtt

sc-37153 : PUMA siRNA (h) is a pool of 3 different siRNA duplexes:

sc-37153A:

• Sense: GAAGAGCAAAUGAGCCAAAtt

• Antisense: UUUGGCUCAUUUGCUCUUCtt

sc-37153B:

• Sense: CCCAUCAAUCCCAUUGCAUtt

• Antisense: AUGCAAUGGGAUUGAUGGGtt

sc-37153C:

• Sense: CUGUAGAUACCGGAAUGAAtt

• Antisense: UUCAUUCCGGUAUCUACAGtt

sc-37967 : TIP60 siRNA (m) is a pool of 3 different siRNA duplexes:

sc-37967A:

• Sense: CCCUUCCUCUUCUACGUAAtt

• Antisense: UUACGUAGAAGAGGAAGGGtt

sc-37967B:

• Sense: GCAAGCUGCUUAUUGAGUUtt

• Antisense: AACUCAAUAAGCAGCUUGCtt

sc-37967C:

• Sense: CCACACUGCAGUAUCUCAAtt

• Antisense: UUGAGAUACUGCAGUGUGGtt

sc-sc37007

Scramble siRNA