Supplementary Methods

Animal model. The animal protocols used in this study were approved by the Stowers Institute for Medical Research Institute Animal Use and Care Committee (IACUC). Induction of PTEN conditional inactivation was performed following the procedure described previously[1]. Briefly, *Mx1-Cre*:*Pten*$^{fl/fl}$ mice[2-4] and control animals were injected intraperitoneally with poly inosine-cytidine (pIpC) 250μg/mouse for 5 times at an interval of every other day, beginning at or prior to weaning. Z/EG mice have been described[5]. Verification of targeting efficiency was performed using the following primers: P1: actca aggca gggat gagc; P2: aatct agggc ctctt gtgcc; P3: gcttg atatc gaatt cctgc agc. Product sizes are: wildtype, 900bp; Loxp, 1000bp; No PTEN Loxp, 300bp.

Intestinal specimen preparation and immunohistochemical/immunofluorescent staining. This work was performed mainly following the procedures described in our previous report[1]. Here we describe new antibodies used and the related staining conditions, and provide details the for immunohistochemical staining of nuclear β-catenin.

Immunohistochemical staining. The condition for unmasking the epitopes varied depending on the primary antibody used.

Immunohistochemical staining of nuclear β-catenin.

Raising and purifying the anti-p-β-Catenin-S^{552} antibody. A phospho-peptide (pSer^{552}-Catnb: [C]HQDTQRRTpSMGG]) was synthesized by Zymed and anti-serum was raised against the phospho-peptide conjugated with Cys-KLH. The anti-serum was first purified using affinity column and passed a column filled with phospho-peptide-matrix gel to enrich the anti-phospho-peptide antibody. Finally, the non-specific antibody was removed using a column filled with non-phospho-peptide-matrix gel (Zymed).

A Staining procedure for p-β-cat-S^{552}.

1. Paraffin-embedded intestinal tissues were deparaffinized, rehydrated, and blocked using a 3-serum blocker (2% mouse serum + 10% goat serum + 10% donkey serum in PBS) for 30 minutes to 1 hour.
2. Antigen retrieval was performed in a microwave oven (BioGnex, EZ Retriever^{TM}) for 10 minutes at 95°C in Citrate buffer (pH 6.0; 82 ml of 0.1M sodium citrate /18 ml of 0.1M citric acid in ml 1000 ml distilled water)
    - Wash with distilled water 3 times.
    - Circle tissue with a PAP pen (Biocare Medical Cat. No. PEN1111).
    - Block with 3-serum blocker (see #1 above).
3. Add 150μl of anti-p-β-cat-S^{552} Ab (1:1000 dilution) onto the tissue sections until they are covered. Leave at room temperature for 1 hour then at 4°C for overnight.
4. Wash with PBS containing Tween20 for 15 minutes x 3 times (total 45 minutes).
5. Add Rabbit-HRP (DAKO, En Vision, Rabbit Peroxidase, K4003) and leave at room temperature for 1 hour.
6. Wash with PBS containing Tween 20 for 15 minutes x 3 times (total 45 minutes).
7. Develop with DAB (3,3'-Diaminobenzidine) until color develops on the tissue (3-5 minutes).
8. Counterstain as desired (e.g. Mayer's hemotoxylin; Sigma Cat. No. MHS32-1L).
9. Mount with Crystal Mount (Biomeda Cat. No. M02). Air Dry. – *Or* -- Mount and cover with cover slip using Cytoseal-60 (Richard Allen Cat. No. 8310-4). Air dry.

B. Staining procedure for Np-NT β-catenin (Upstate Tachnology).

The variation from the procedure for anti-p-β-cat-S^{552} is as follows:

1. Antigen retrieval was performed in a Decloaking Chamber (Biocare Cat. No. DC2002) by decloaking in citrate buffer (pH 6.0; 82 ml of 0.1M sodium citrate /18 ml of 0.1M citric acid in ml 1000 ml distilled water) using the following program:
   - SP1 125°C for 4 minutes and SP2 125°C for another 1 minute.
   - After the program is run, the pressure will start to go down. When the pressure reaches zero (0), wait for another 60 minutes before opening the Decloaking Chamber. Let the slides cool to room temperature.
2. Biotinylate the primary antibody (β-catenin, Upstate Cat. No. 05-665, mouse monoclonal antibody) as follows, using the Biotinylation Kit from DAKO (Cat. No. K3954). Antibody concentration is 1000 μg/ml: β-catenin 3 μl; Biotin 30 μl; Diluents (DAKO Cat. No. S0809) 118 μl.
   - Leave at room temperature for 30 minutes.
   - Add 6 μl of blocking serum at room temperature for 10 minutes.
   - Tip off the blocking serum and blot excess using a tissue (such as Kimwipes or Kleenex).
3. Primary antibody. Add 150μl of the Biotinylated β-catenin antibody onto the tissue sections until they are covered. Leave at room temperature for 1 to 3 hours, then at 4°C for 36 hours.

Secondary antibody. Add the streptavidin-peroxidase from the kit (DAKO Biotinylation Kit) and leave at room temperature for 1-2 hours, then at 4°C overnight.

Immunofluorescent staining. Co-staining of 14-3-3ζ with Musashi and co-staining of Musashi with BrdU and β-catenin, $p27^{kip1}$ was carried out following the method described in our previous report [1].

Tissue sections were incubated with primary antibodies overnight at 4°C. After several washes with 1× PBS, the slides were incubated in Cy™ -2 conjugated AffiniPure donkey anti-rabbit IgG (1:300) and Cy™- 3 conjugated AffiniPure donkey anti-sheep IgG (1:600) for 30 minutes at 37°C (Jackson ImmunoResearch Laboratories, West Grove, PA; cat. #711-225-152 and 713-165-003 respectively). Slides were washed with 1× PBS, mounted, and viewed.

Top-Gal staining.

1. Collect intestinal tissue and wash thoroughly with PBS*.
2. Fix tissue in LacZ fixative buffer (0.4 ml 25% glutaraldehyde in 44.1 ml PBS with 0.5 ml 0.5M EGTA and 5 ml 1M MgCl2) at 4°C for 2 hours. (All fixation was performed at 4°C on a shaker/rotator.)
3. Rinse with PBS three times then rinse with LacZ wash buffer (in 500 ml buffer, add 1 ml 1M MgCl2, 5 ml 1% NaDOC, 5 ml 2% NP40, and 489 ml PBS).
4. Stain in LacZ staining solution at 33°C for 6 hours in a dark room. Staining solution: 96 ml LacZ wash buffer, 4.0 ml 25 mg/ml X-gal [dissolved in DMSO], 0.21 g Potassium Ferro cyanide, 0.16 g Potassium Ferro cyanide.
5. Wash with PBS three times, and fix in Zinc Formalin.
6. The remaining procedure was the same as paraffin section preparation.

   * We found that intestine sections collected from mice exposed to low dose irradiation allowed for more robust detection of Top-Gal activity using X-gal staining.

Whole mount immunofluorescent staining.
Crypts were isolated following the procedure described previously[6]. Isolated intestinal crypts were fixed in 4% paraformaldehyde for 1 hr at 4°C, and permeabilized using 0.5% Triton-X100 in PBS. The crypts were blocked by incubation in blocking buffer (Universal blocking reagent, BioGenex) for 1 hr. Primary antibodies were diluted in 0.5% Triton-X100 PBS and the crypts were incubated with gentle agitation overnight at 4°C. Whole mount crypts were then washed for at least 2 hours in PBS containing Tween 20, changing the buffer several times. Fluorescent conjugated secondary antibodies were incubated in the same way. For counter-staining, the crypts were incubated with DAPI (4',6-Diamidino-2-phenyindole) in PBS for 10 minutes, followed by mounting with aqueous mounting medium (Biomedia). Images were acquired using a Zeiss 510 confocal microscope and reconstructed using 30 optical sections of each crypt.

Microarray and RT-PCR assays. The procedures for both microarray and RT-PCR assays have been previously described [7]. Microarray analysis compared the gene expression profiles of polyp regions from three PTEN mutant animals with intestines from two control animals. Genes were considered up-regulated or down-regulated if all of the following conditions were met: 1) There was at least a two-fold change in the average probe signal measured between controls and mutants; 2) There was no overlap between the range of mutant and control data; and 3) The control mean was outside the 95% confidence interval of the PTEN-mutant mean. By these criteria, 1243 known genes were up-regulated and 1,053 were down-regulated. Gene ontology terms were analyzed using Onto-Express[8]. WebGestalt[9] was also used to identify significantly enriched classes of ontology terms by BioCarta mapping. Additional up-regulated genes shown shared the enriched BioCarta term "Cell cycle: G1/S check point" (hypergeometric test; p=0.0496). The microarray signals were plotted with Heatmap Builder. RT-PCR primers were as shown in the table below.

Gene	Forward primer	Reverse Primer
c-Myc	5'-GGACTGTATGTGGAGCGGTTTC-3'	5'-CTGGTAGGAGGCCAGCTTCTC-3'
Cyclin D1	5'-AGTTCATTTCCAACCCACCCTCA-3'	5'-TCTGGAAAGAAAGTGCGTTGTGCG-3'
GAPDH	5'-GAAGGTGAAGGTCGGAGTC-3'	5'-GAAGATGGTGATGGGATTTC-3'

Western blot analysis. Intestinal tissue was homogenized in 1ml lysis cocktail (100 mM Tris-Hcl [pH 6.8], 2% SDS, and a proteinase inhibitor cocktail supplied by Roche). The supernatant was collected after centrifugation. Protein extracts (20μg/well) were fractionated on SDS-PAGE gel and transferred onto nitrocellulose membrane. The membrane was blocked using casein blocker (Pierce) and was incubated with appropriate primary (p-Akt,1:1000, p-GSK,1:2000, Cyclin D1,1:3000 [Cell Signaling], Active-β-catein,1:3000 [Upstate]; p27kip1, 1:4000 [Invitrogen]; p-β-catenin S^{552}, 1;2000 [custom-made by Zymed]) and secondary antibodies (1:4,000 dilutions) in casein or 5% BSA blocker. The membrane was developed using chemoluminescent reagents (Pierce) after washing with TBS-T solution (TBS plus 0.05% Tween-20).

Bioinformatics and mass-spectrometry. To identify potential Akt phosphorylation sites iṇ β-catenin we used Scansite 2.0[8] and performed a medium stringency motif search of the human β-

catenin protein sequence, for identification of potential Akt phosphorylation sites. The procedure for analysis of phospho-peptide using mass-spectrometry has been described previously[10].

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