

Figure S1.

(A) *PIK3ca*^{*} encodes a dominant active form of PI3K (p110*). p110α is the native subunit of PI3K that was modified to create p110*, which consists of p110α (green and blue rectangles) bound by a glycine kinker (red rectangle) to the iSH2 domain of the p85 subunit of PI3K (purple rectangle).

(B) *FC*⁺ *PIK3ca*^{+/+} mice die very young. The Kaplan-Meier survival curve demonstrates that most *FC*⁺ *PIK3ca*^{+/+} mice become moribund by 60 days of age. Only a few *FC*⁺ *PIK3ca*^{+/+} mice (squares, 3/17) survived up to 100 days of age, whereas all *FC*⁰ *PIK3ca*^{+/+} littermates (diamonds, 5/5) were healthy at that time. The three *FC*⁺ *PIK3ca*^{+/+} mice that survived to 100 days were sacrificed; one was noted to have severe intestinal obstruction but had not yet become moribund, whereas the other two did not possess grossly visible colon tumors. Note that *FC*⁺ *PIK3ca*^{+/+} mice were indistinguishable from *FC*⁰ *PIK3ca*^{+/+} littermates with respect to size, weight, and activity level until they became moribund.

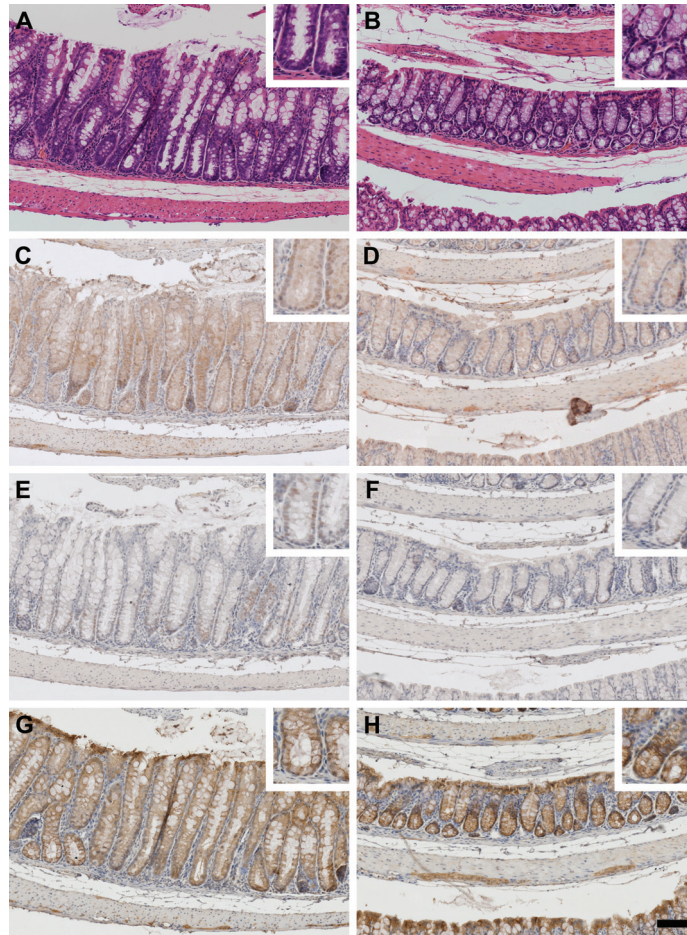


Figure S2. The colon in $FC^+ PIK3ca^{*+}$ mice shows marked hyperproliferation. A mutant (A, C, E, G) and its wild-type littermate (B, D, F, H) were sacrificed at 58 days of age. The colon was removed, splayed open, rolled, embedded in paraffin, and cut. Sections were stained for H&E (A and B). The crypts in mutants were approximately three times longer than those in controls. Immunohistochemistry (IHC) reveals that this change correlates with AKT being more highly phosphorylated (C and D) and having increased cellular proliferation (E and F) by antibodies against pAKT and Ki67, respectively. This hyperproliferation does not appear to rely on aberrant WNT signaling, as β -catenin is not elevated or nuclear as shown by IHC (G and H). Scale bar: 100 μ m.

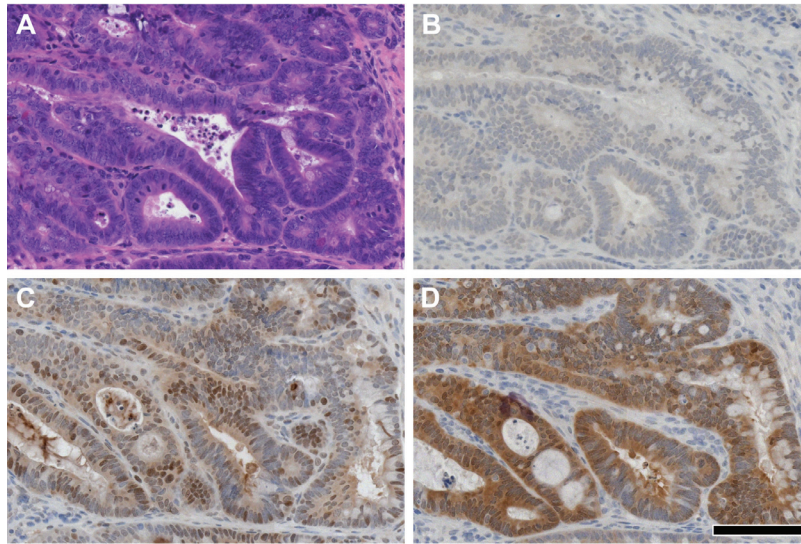


Figure S3. PI3K/AKT signaling appears unperturbed in colon adenocarcinomas from F1 *Apc*^{Min/+} mice. Sections of an adenocarcinoma from an F1 *Apc*^{Min/+} mouse were stained with H&E (A). Immunohistochemical (IHC) staining using antibodies against pAKT and Ki67 revealed that the level of pAKT was relatively low (B), whereas the level of cellular proliferation was moderately high (C), respectively. The neoplastic transformation was coupled to the translocation of the β -catenin to the nucleus which indicative of aberrant WNT signaling as shown by IHC (D). Scale bar: 100 μ m

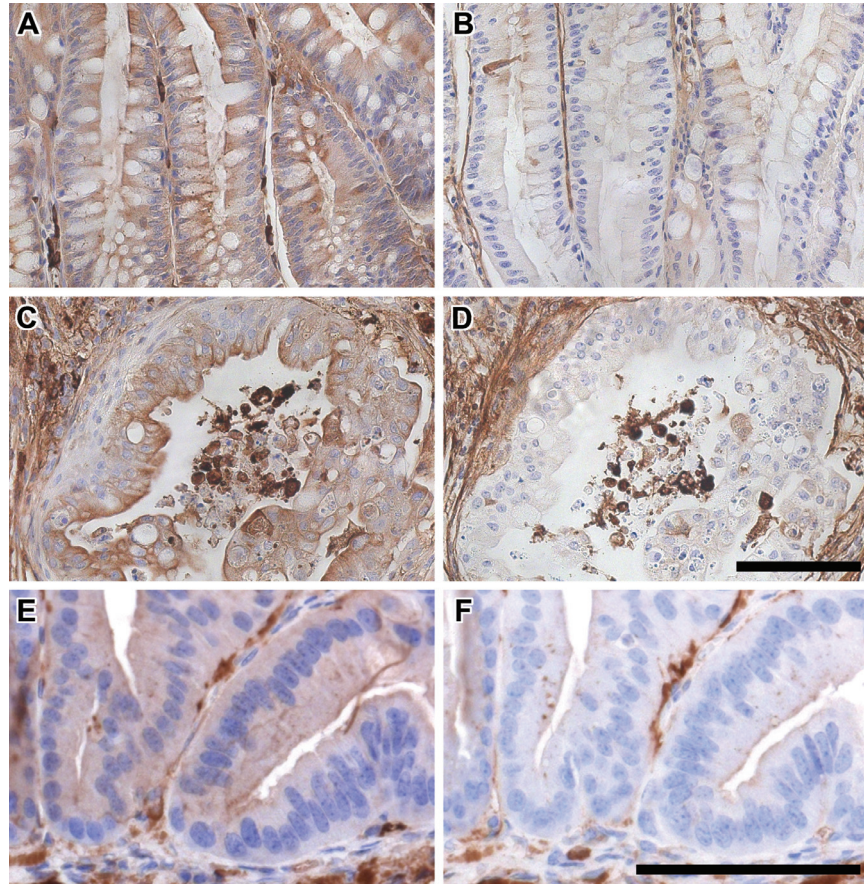


Figure S4. Cytokeratin staining of the colon epithelium and tumor of $FC^+ PIK3ca^{*+}$ mice. The colonic epithelium (A and B), a proximal colon tumor (C and D), a tumor extending into the ovary (E and F) of $FC^+ PIK3ca^{*+}$ mice were stained for cytokeratin 20 (A, C, E) and cytokeratin 7 (B, D, F). The tumor tissue demonstrates 1+ cytokeratin 20 staining and negative cytokeratin 7 signaling indicating that these tumors are of intestinal origin. Scale bar (A-D): 100 μ m. Scale bar (E-F): 100 μ m.

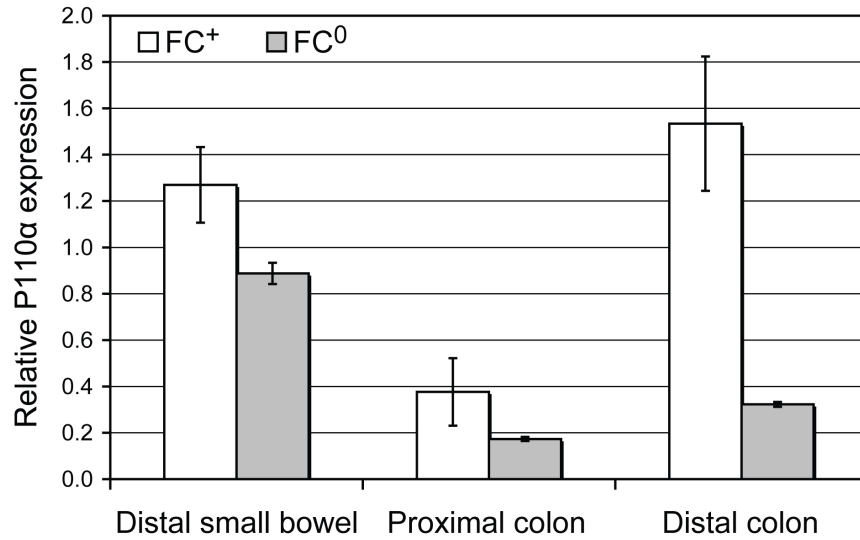


Figure S5. Expression of p110α is greater in the colon of FC^+ $PIK3ca^{*+}$ mice than FC^0 $PIK3ca^{*+}$ mice. Quantitative PCR revealed that transcription of p110α was higher in experimental animals (FC^+) than controls (FC^0). P-values (two-tailed Student's t-test) = 0.02 for distal small bowel, 0.07 for proximal colon and 0.002 for distal colon.

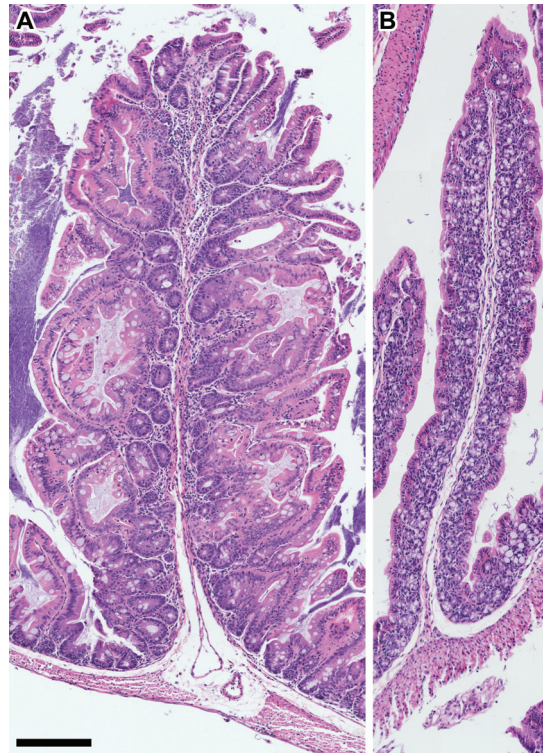


Figure S6. $PIK3ca^*$ induces mucosal hyperplasia throughout the colon. A $FC^+ PIK3ca^{*+}$ mouse and its wild-type littermate were sacrificed at 20 days of age. A H&E-stained cross-section of a rugal fold from the proximal colon of a $FC^+ PIK3ca^{*+}$ mouse exhibited a villiform hyperplasia with numerous dilated crypts filled with mucin (A). These lesions are reminiscent of sessile serrated adenomas in humans, which are also preferentially located in the right colon and are associated with mucinous adenocarcinoma. Such lesions were never observed in the proximal colon of controls (B). Scale bar: 200 μ m.

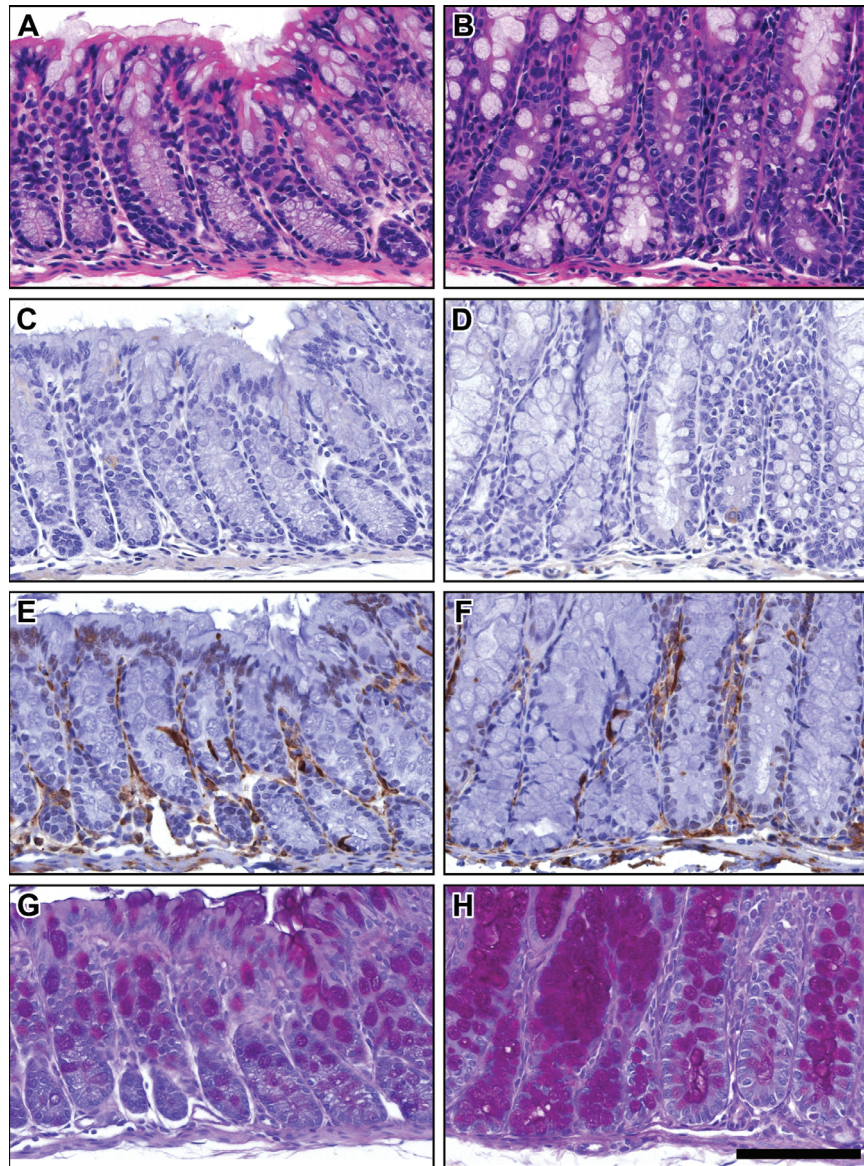


Figure S7. Intestinal cell fate is maintained in the intestines of $FC^+ PIK3ca^{++}$ mice. Experimental mice (right panel) and controls (left panel) were sacrificed at 30 days of age. The intestinal tract was isolated, rolled, fixed, processed, embedded in paraffin, and sectioned. Sections were stained with H&E (A and B), for lysozyme (C and D), for synaptophysin (E and F), and with PAS (G and H) to assess absorptive, Paneth, enteroendocrine, and goblet cells. Scale bar: 100 μ m.