Supporting Information

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β-Galactosidase Staining. The intestinal tract was removed, opened longitudinally, and washed with PBS. Samples were fixed in 4% paraformaldehyde for 1 h at 4° C and washed three times with PBS. Intestines were stained in X-gal solution overnight at 37° C and fixed in 10% formalin overnight at 4° C. Fixed samples were embedded in paraffin, sectioned, and stained with eosin. Slides were examined with a BX51 System Microscope (Olympus).

Kras Sequencing. Total RNA was extracted from tumors using TRIZOL (Invitrogen) and the PureLink total RNA purification system (Invitrogen). cDNA was reverse transcribed and amplified using the SuperScript one-step RT-PCR with platinum Taq high fidelity (Invitrogen) with the following primers F-gcaattaaccct-cactaaagggCCTGCTGAAAATGACTGAGTAT and R-aagctaatacgactcactatagggTTAATTTGTTCTCTATAATGGTGAA. Samples were sequenced at the Harvard Biopolymers Facility using the following primers T3-AAT TAA CCC TCA CTA AAG GG and T7-TAA TAC GAC TCA CTA TAG GG.

Western Blot. Whole tumors were processed in lysis buffer containing complete protease inhibitor mixture (Roche) using a TH tissue homogenizer (Omni International). Twenty-five micrograms of protein was separated on 10% SDS/PAGE, transferred

to a nitrocellulose membrane, and incubated with primary antibody overnight at 4°C, and then secondary antibody for 1 h at room temperature. Detection was performed using the ECL Western blot detection kit (GE Healthcare). Antibodies for p-ERK, total ERK, p-S6, and total S6 were purchased from Cell Signaling Technologies.

Immunohistochemistry. Five-micrometer paraffin-embedded tissue sections were deparaffinized in xylene, followed by alcohol rehydration. After quenching endogenous peroxidases in 3% H_2O_2 in methanol, the slides were rinsed in distilled water, and an antigen retrieval step was carried out in a microwave oven for a total of 10 min in preheated citrate buffer (pH 6.0). The slides were then incubated with primary antibody at room temperature overnight. After PBS wash, slides were incubated with secondary antibody for 1 h at room temperature. The Vectastain Elite ABC kit (Vector Laboratories) was used for detection per manufacturer's instructions. The slides were stained with DAB and counterstained with Mayer's hematoxylin. Antibodies for p-ERK, total ERK, p-S6, and total S6 were purchased from Cell Signaling Technologies. β -catenin antibody was purchased from BD Biosciences.



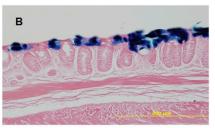




Fig. S1. Adenovirus infects basal colonic crypt. (A) Surgical procedure using clips to limit adenoviral infection to distal colon. Red arrow denotes infection site for adenovirus during incubation period. (B) X-gal staining after adeno-lacZ demonstrates infection predominates in superficial epithelium. (C) X-gal staining after adeno-lacZ demonstrates infection in basal colonic crypt marked by red arrow.