Supporting Information

Shaked et al. 10.1073/pnas.1211509109

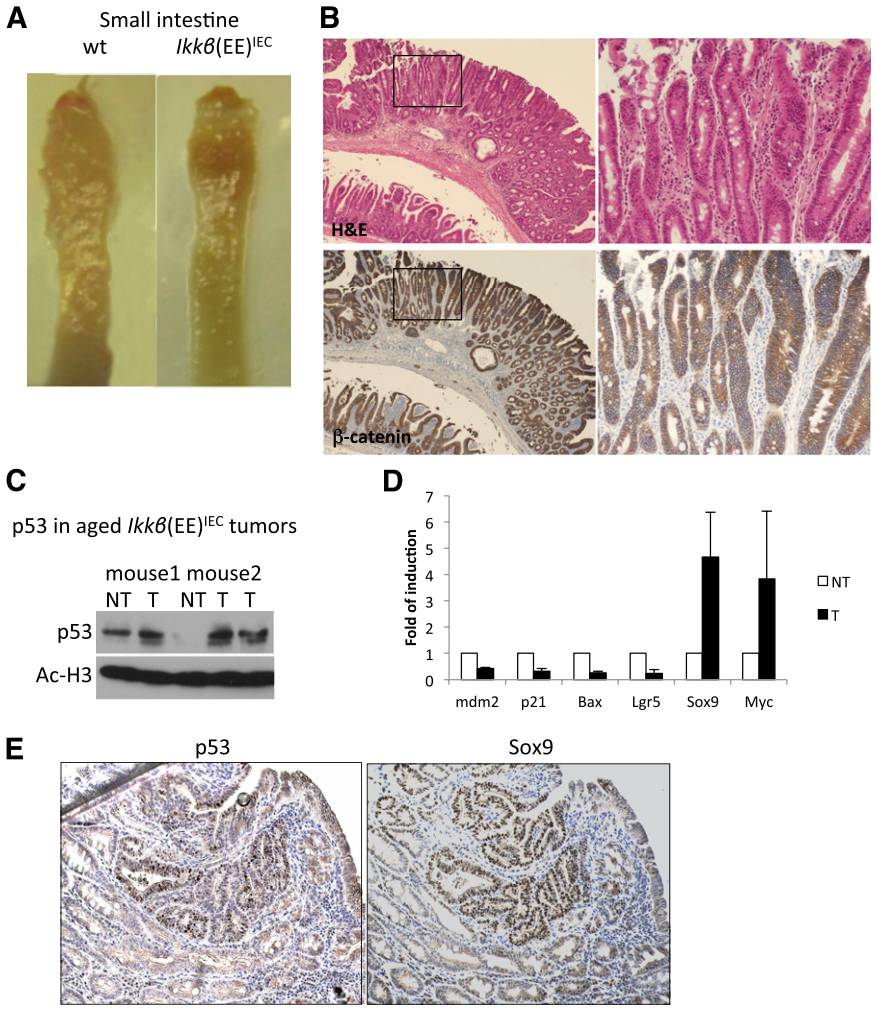

Fig. S1. Spontaneous tumors in mice expressing constitutively active IKKβ in their intestinal intestinal epithelial cells [*Ikkβ*(EE)^{IEC} mice]. (*A*) Tumors in aged *Ikkβ(EE)*^{IEC} mice; a representative pair of small intestines from 1-y-old WT and *Ikkβ(EE)*^{IEC} mice are shown. (*B*) H&E staining (*Upper*) and β-catenin staining (*Lower*) of serial sections with tumors from *Ikkβ(EE)*^{IEC} mice. (*C*) p53 expression in tumor and adjacent nontumor tissue of *Ikkβ(EE)*^{IEC} mice. (*D*) Gene expression in tumors from four 1-y-old *Ikkβ(EE)*^{IEC} mice relative to adjacent nontumor tissue. (*E*) IHC for p53 and Sox9 of sequential sections with tumors from 12-mo-old *Ikkβ(EE)*^{IEC} mice.

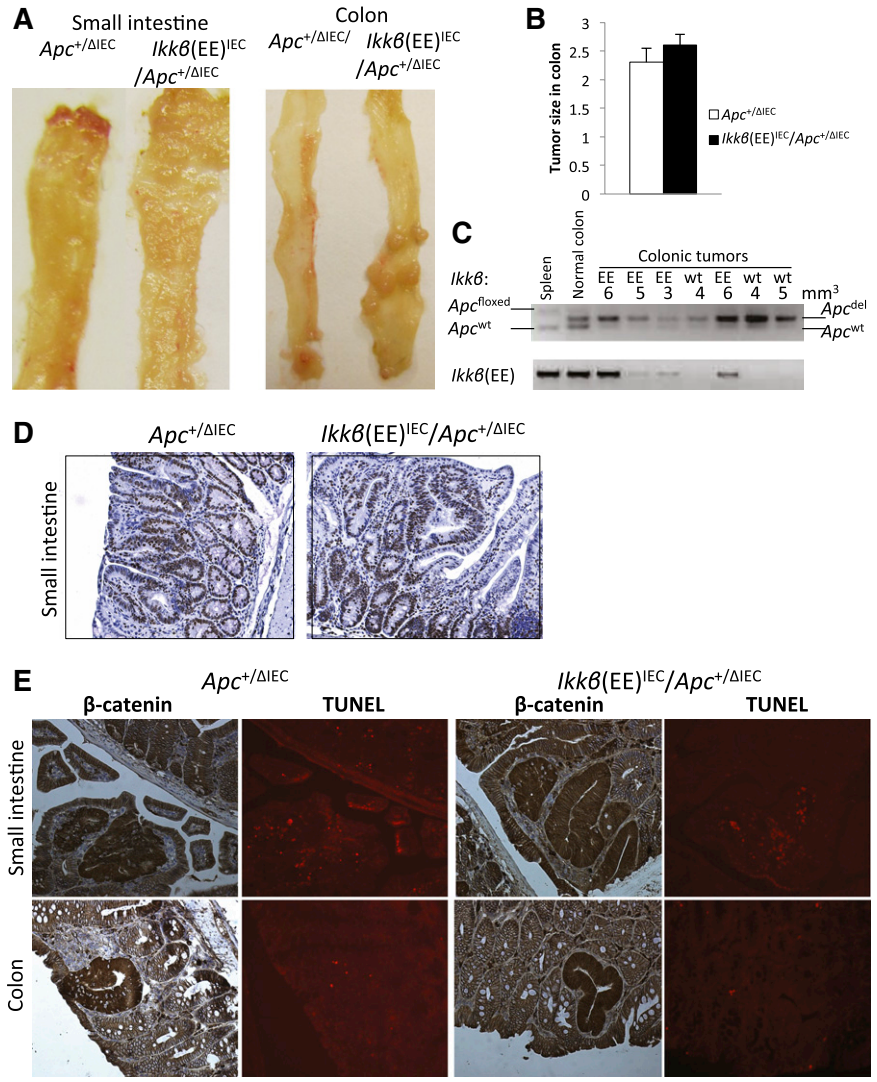

Fig. S2. Tumors and premalignant lesions in *Ikkβ(EE)*^{IEC}/*Apc*$^{+/\Delta IEC}$ and *Apc*$^{+/\Delta IEC}$ mice. (*A*) Tumors in small intestine (SI) and colons of *Ikkβ(EE)*^{IEC}/*Apc*$^{+/\Delta IEC}$ and *Apc*$^{+/\Delta IEC}$ mice. (*B*) Diameter (millimeters) of colonic tumors in *Ikkβ(EE)*^{IEC}/*Apc*$^{+/\Delta IEC}$ and *Apc*$^{+/\Delta IEC}$ mice. (*C*) Loss of the WT *Apc* allele in colonic tumors from *Ikkβ(EE)*^{IEC}/*Apc*$^{+/\Delta IEC}$ and *Apc*$^{+/\Delta IEC}$ 3-mo-old mice. (*D*) Immunohistochemistry (IHC) analysis with Ki67 antibody of premalignant lesions in 6-wk-old mice of the indicated genotypes. (*E*) β-Catenin IHC and in situ TUNEL assay were performed on sequential sections containing premalignant lesions of 6-wk-old mice of the indicated genotypes.

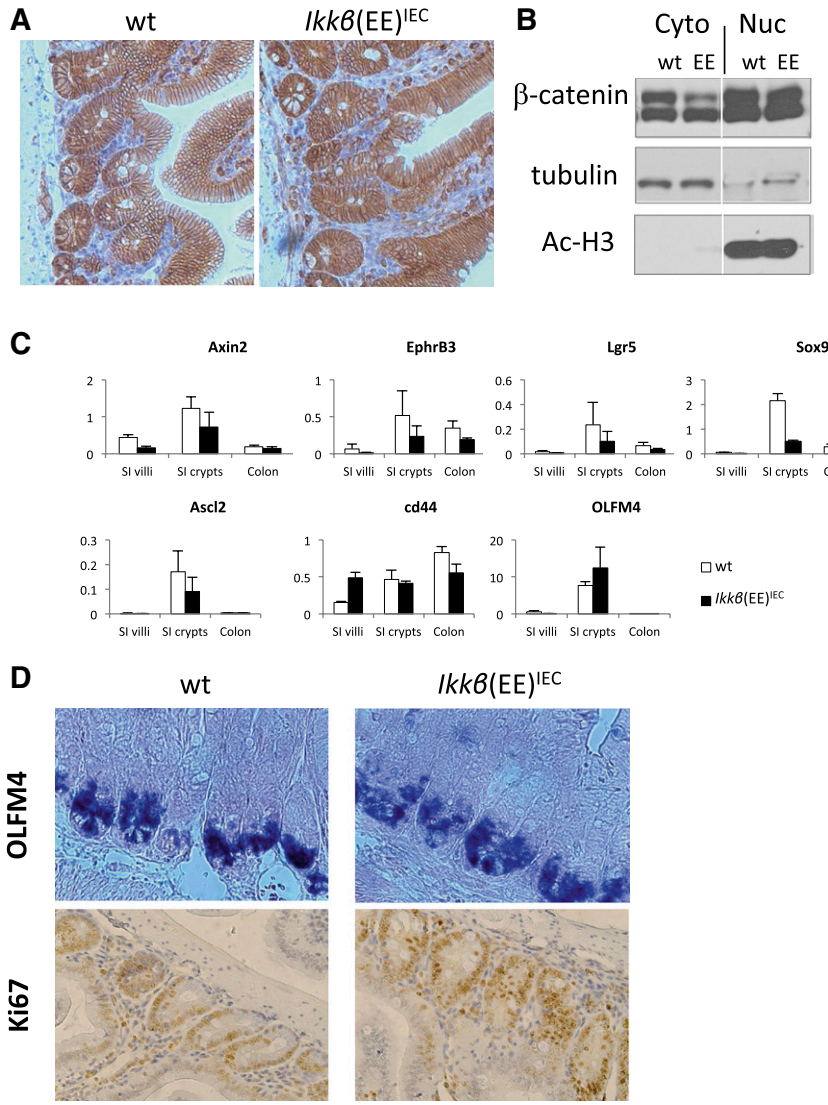

Fig. S3. *Ikkβ(EE)*^{IEC} mice do not exhibit β-catenin activation or elevated stem cell numbers. (*A*) IHC for β-catenin in SI of 2-mo-old WT and *Ikkβ(EE)*^{IEC} mice. (*B*) Immunoblot analysis of nuclear and cytoplasmic fractions from SI crypt cells of WT and *Ikkβ(EE)*^{IEC} mice. (*C*) qRT-PCR analysis of β-catenin target genes in RNA isolated from intestinal epithelial cells (IECs) of SI villi, SI crypts, or colonic crypts of 2-mo-old WT and *Ikkβ(EE)*^{IEC} mice; normalized to the Hprt gene. (*D*) In situ hybridization for the stem cell marker OLFM4 and IHC analysis of the proliferation marker Ki67 in SI of 2-mo-old WT and *Ikkβ(EE)*^{IEC} mice.

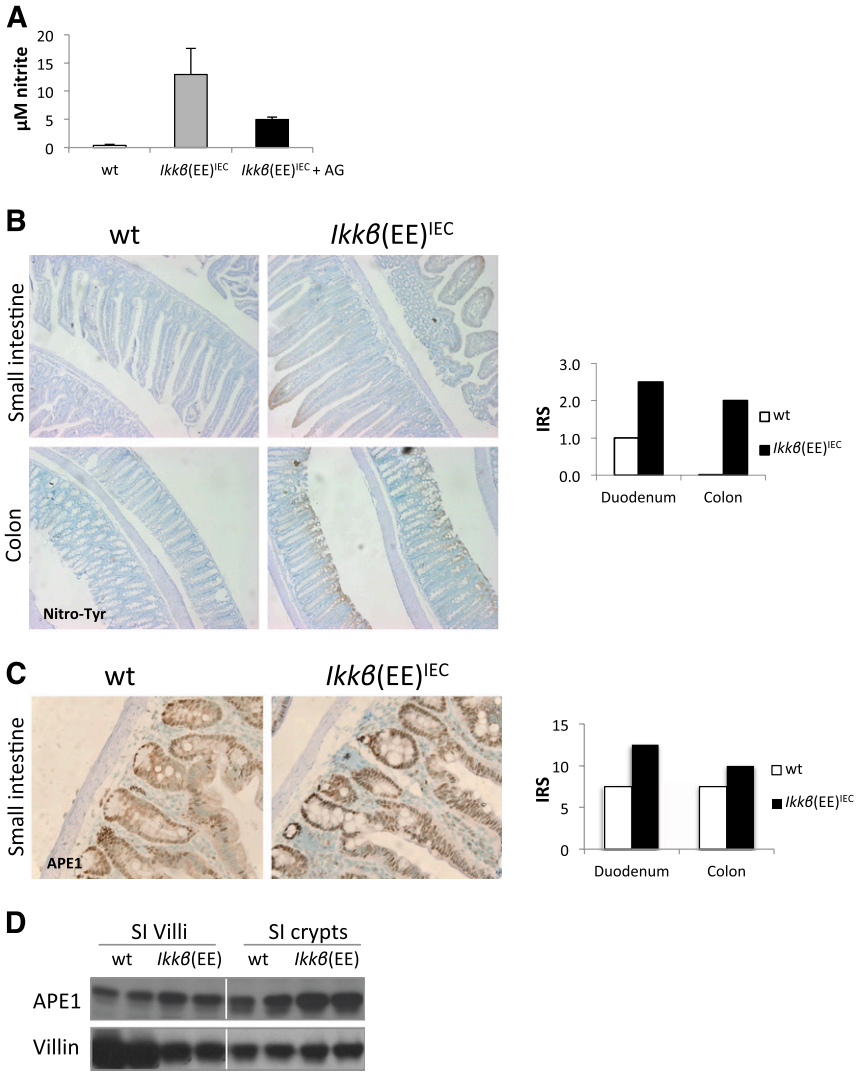

Fig. S4. Nitrosative stress and DNA repair in unchallenged *Ikkβ(EE)*^{IEC} mice. (*A*) Nitrite levels in ultra-filtered plasma as measured by Griess reaction, in WT and *Ikkβ(EE)*^{IEC} mice and *Ikkβ(EE)*^{IEC} mice treated with 2g/L of aminoguanidine (AG) in drinking water for 24 h (n = 3). (*B*) IHC of intestinal sections from 2-mo-old WT and *Ikkβ(EE)*^{IEC} mice with nitrotyrosine antibody. (*C*) IHC of SI sections from 2-mo-old mice with APE1 antibody and blind immunoreactivity score (IRS) of APE1 staining in SI and colon of WT and *Ikkβ(EE)*^{IEC} mice. (*D*) Immunoblot analysis of APE1 in SI villi and crypts of 2-mo-old WT and *Ikkβ(EE)*^{IEC} mice.