Nuclear bile acid receptor FXR protects against intestinal tumorigenesis.
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Legends to Supplementary Figures

Supplementary Figure 1. Large and small tumors and ACFs in FXR+/-ApcMin/+ mice.
Examples of a large colon tumor (A, 5.5 mm diameter), aberrant crypt focus (B, light microscopy, 250 x after methylene blue staining) and a small tumor (C, light microscopy, 250 x after methylene blue staining) observed in a 4 months old FXR+/-ApcMin/+ mouse, with no tumors observed in a age-matched FXR+/+ApcMin/+ mouse.

Supplementary Figure 2. Susceptibility of FXR-/- to intestinal tumorigenesis is unaffected by cholestyramine-mediated reduction of circulating bile acid levels.
A) Temporal scheme of the protocol used for the AOM-DSS chronic colitis associated colon carcinogenesis mouse model. B) Examples of H&E section of colonic mucosa at light optical microscopy examination (magnification 250x) in FXR+/+ and FXR-/- mice treated with chow diet or 2% cholestyramine containing diet.

Supplementary Figure 3. Intratumoral injection of AdVP16FXR adenovirus reduced tumor growth in a xenograft model.
Ten millions of APC-mutated HT29 human colorectal cancer cells were injected subcutaneously in nude mice. Tumor volume was measured during 16 days after intratumoral injection of adenoviruses VP16FXR and VP16. Tumor growth inhibitory effects were observed for AdVP16FXR versus AdVP16.

Supplementary Figure 4. FXR activation in colon cancer cells via AdVP16FXR and synthetic ligand GW4064 results in apoptosis and reduced cell proliferation.
A) Anti-proliferative and pro-apoptotic effects of AdVP16FXR versus AdVP16 were observed in LS174T by means of BrdU, Nucleosome fold enrichment and Annexin-V assays. B) Significant increased mRNA levels of IBABP, P21 and KLF4 together with a downregulation of cyclin E1 were observed in HT29 treated with GW4064 5mM for 48hrs versus Vehicle. C) Upregulation of P21 protein levels were detected after 48hrs 5mM GW4064 in HT29 cells that expressed FXR after
confluence. D) Anti-proliferative effects of GW4064 at increasing concentrations after 48hrs treatment of postconfluent HT29 cells by [3H]-thymidine incorporation.*P<0.05

**Supplementary Figure 5. FXR activation via synthetic ligand GW4064 induces apoptosis in the intestine.**

3 months old C57B6/J male mice were treated with daily intragastric administration of GW4064 at conc. of 50mg/kg b.w. for 5 days. Tunel assay was performed on intestinal sections. Induction of pro-apototic events was shown by the increased labeling of differentiated enterocytes of the upper mucosa of the ileal villus and epithelial colon.

**Supplementary Figure 6. Gene expression levels in normal and tumor intestinal samples.**

mRNA levels were measured in tumor samples compared to normal adjacent mucosa of the intestine of male ApcMin/+ mice. A significant decrease in FXR and pro-apoptotic genes (P21, P27, FAS, KLF4) was accompanied by the increase of anti-apoptotic Bcl2, pro-inflammatory TNFa and b-catenin target genes, cmyc and cyclin D1.