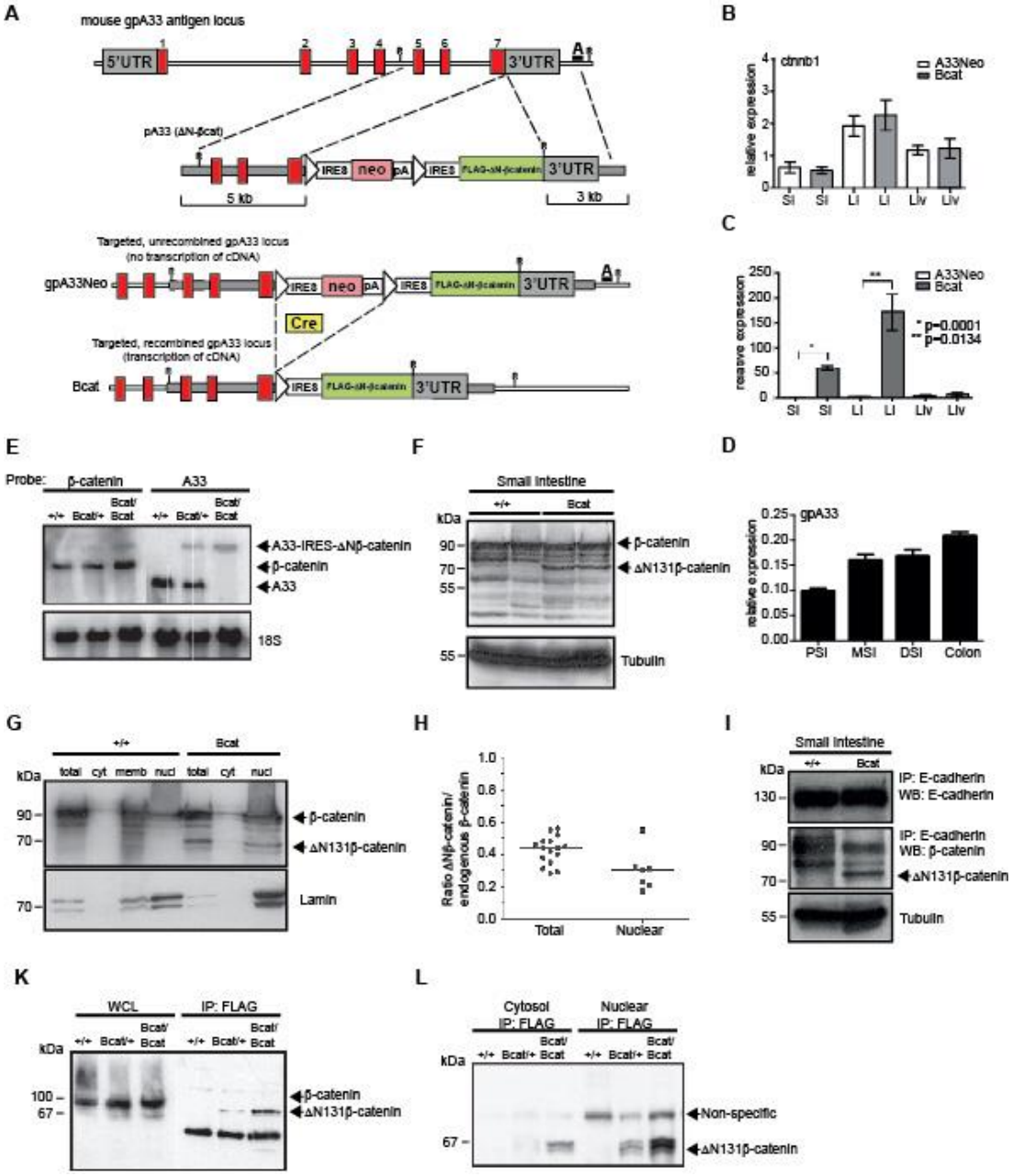

Fig. S1. Organisation of murine *gpA33* locus, targeting strategy and expression analysis. The truncated mutant β-catenin (gpA33ΔN-βcat) is specifically expressed in the intestine of Bcat mice. Transgenic ΔN-Bcat retained the capacity to enter the nucleus, and to bind to E-cadherin at the membrane. **(A)** *Top:* Schematic depiction of murine *gpA33* locus with 5' and 3' UTR (grey box) and position of the seven exons (red boxes) of the *gpA33* gene. *Middle:* The pA33(ΔN-βcat) targeting construct used for homologous recombination in embryonic stem (ES) cells. The first of two internal ribosome entry sites (IRES) immediately downstream of the stop codon of the endogenous *gpA33* gene allows translation of the neomycin (neo) cassette to confer G418 resistance to ES cells after homologous recombination. A transcription termination signal (pA) prevents transcription into the second IRES and sequences further downstream. White triangles represent loxP sites. Bottom: Schematic outline of targeted *gpA33* locus before (referred to as gpA33Neo) and after Cre-mediated excision of the IRES-Neo-pA cassette which creates a bicistronic mRNA leading to expression of mutant β-catenin (ΔN-βcat) under the control of the *gpA33* locus. **(B)** Relative expression of endogenous β-catenin determined by quantitative real-time PCR in small intestine (SI), large (LI) intestine and liver (Liv) from A33Neo control mice (white box) and transgenic Bcat mutant mice (grey box, n=5). Expression is normalized to the median expression of all A33Neo control samples. **(C)** Relative expression of ΔN-Bcat

determined by quantitative real-time PCR in small (SI) and large (LI) intestine, and liver (Liv) from unrecombined A33Neo mice harbouring the neo-cassette, and recombined Bcat following the removal of the neo cassette. Expression is normalized to the median expression of all SI and LI samples from A33neo mice, respectively. All measurements were done in duplicates, n=5 mice per sample and genotype. **(D)** gpA33 is expressed throughout the murine intestine in a rostro-caudal gradient. Relative expression of endogenous gpA33 was determined by quantitative real-time PCR in tissue samples taken from proximal (PSI), middle (MSI) and distal (DSI) small intestine and colon of wild-type mice (n=4). Expression is normalized to the median expression of all control small or large intestine samples, respectively. All measurements were done in duplicates. Mean ± SEM**. (E)** Northern blot analysis of mRNA extracted from the intestines of wildtype (+/+), Bcat heterozygous (Bcat/+) and Bcat homozygous (Bcat/Bcat) mice and probed with antisense RNA specific for β-catenin or A33 message. A bicistronic RNA encoding gpA33 and mutant β-catenin is detectable in heterozygous and homozygous intestines. **(F)** Immunoblot analysis in whole cell lysate showing total and mutant β-catenin levels in the small intestine of wildtype (+/+) and Bcat homozygous (Bcat) mice using an anti-β-catenin specific antibody. Note that the amounts of endogenous β-catenin (approx. 90 kDa) are essentially unchanged. Expression of mutant β-catenin (approx. 70 kDa) is only detectable in Bcat mice. (**G**) Immunoblot of cytosolic (cyt), membrane (memb) and nuclear (nuc) fractions of total tissue lysates (total) from SI of A33Neo (+/+) and Bcat mice. Mutant ΔN131βcatenin is detectable in the nuclear fraction of Bcat mice. Tubulin and Laminin staining are used as negative and positive controls for nuclear fraction. (**H**) Scatter plot analysis based on western blots similar to (G) showing the relative abundance of total and nuclear mutant ΔN131βcatenin normalised to the expression level of endogenous wild type β-catenin. (n=16 for ratio of total and n=7 for nuclear). **(I)** Immunoprecipitation of E-cadherin and mutant β-catenin with anti-E-cadherin antibody. Mutant β-catenin (ΔN131βcatenin) binds to E-cadherin like endogenous β-catenin. Tubulin staining of tissue lysates is used as loading control. **(K)** Immunoblot analysis in whole cell lysate (WCL) and anti-FLAG immunoprecipitates of total and mutant β-catenin levels in the small intestine of A33Neo (+/+), Bcat heterozygous (Bcat/+) and Bcat homozygous (Bcat/Bcat) mice using an anti-β-catenin specific antibody. Note that the amounts of endogenous β-catenin (approx. 90 kDa) are essentially unchanged. Expression of mutant β-catenin (approx. 70 kDa) is only detectable in Bcat mice in a gene dosage dependent manner. **(L)** Immunoblot of cytosolic (cyt) and nuclear (nuc) anti-FLAG immunocprecipitates from the intestines of A33Neo (+/+), Bcat heterozygous (Bcat/+) and Bcat homozygous (Bcat/Bcat) mice. Mutant β-catenin (ΔN131βcatenin) is detectable in the cytosolic and nuclear fraction of Bcat mice.

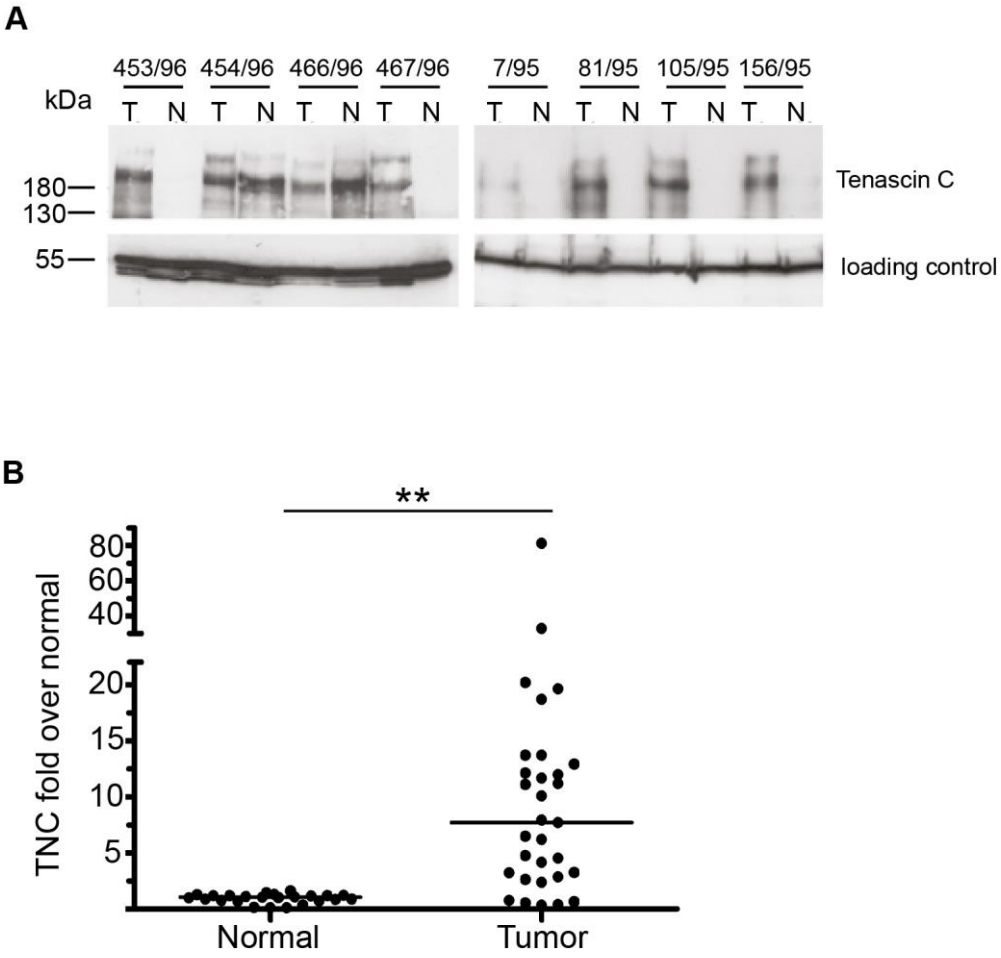

Fig. S2. The Wnt/β-catenin target gene Tenascin-C is upregulated in Bcat mice and in human CRC. **(A)** Immunoblot analysis of TENASCIN-C expression in matched tissues (N, normal colon vs T: tumor tissue) in individual pairs. Upper lane, anti-tenascin-C polyclonal antiserum, lower panel, loading control (anti-tubulin). **(B)** Scatter plot showing quantification of TENASCIN-C (TNC) immunoblots. TNC expression was increased in 24 out of 31 (77%) human CRC samples tested (Mean $\pm$ SEM, ** $p<0.01$).

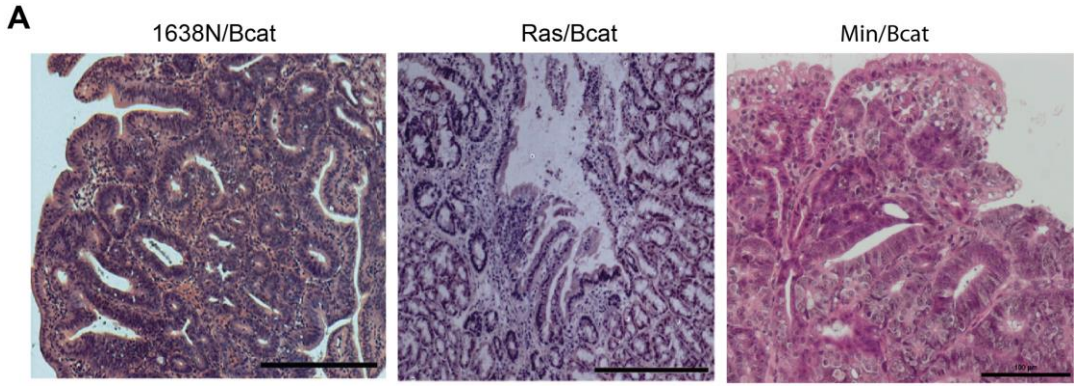

Fig. S3. Morphology of adenomatous polyps is similar between compound mutant mice. **(A)** Hematoxylin and eosin staining of intestinal adenomas from the indicated compound mutant mouse strains (100x magnification, scale bar = 100 µm).

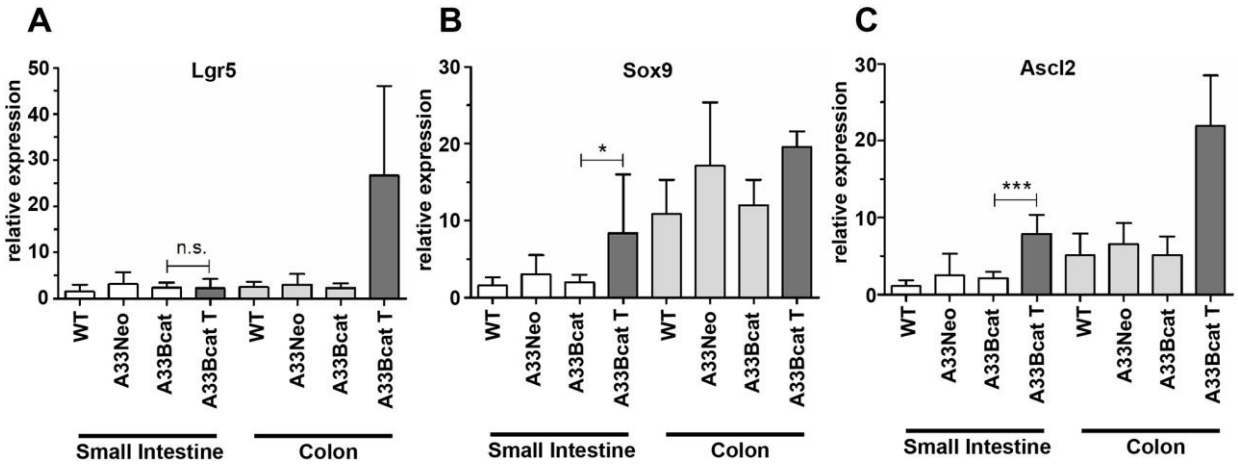

Fig. S4. Intestinal stem cell markers are unchanged in normal tissue of Bcat mice. Relative expression of endogenous Lgr5 **(A)**, Sox9 **(B)** and Ascl2 **(C)** determined by quantitative real-time PCR in unaffected areas and tumor lesions collected from small intestines and colons of wild-type (WT), knock-in controls (A33Neo) as well as transgene expressing (A33Bcat) mice. Expression is normalized to the median expression of all control samples from the small intestine and colon,, respectively. All measurements were performed in duplicates (mean ± s.d, n=5, * $p<0.05$, *** $p<0.001$).

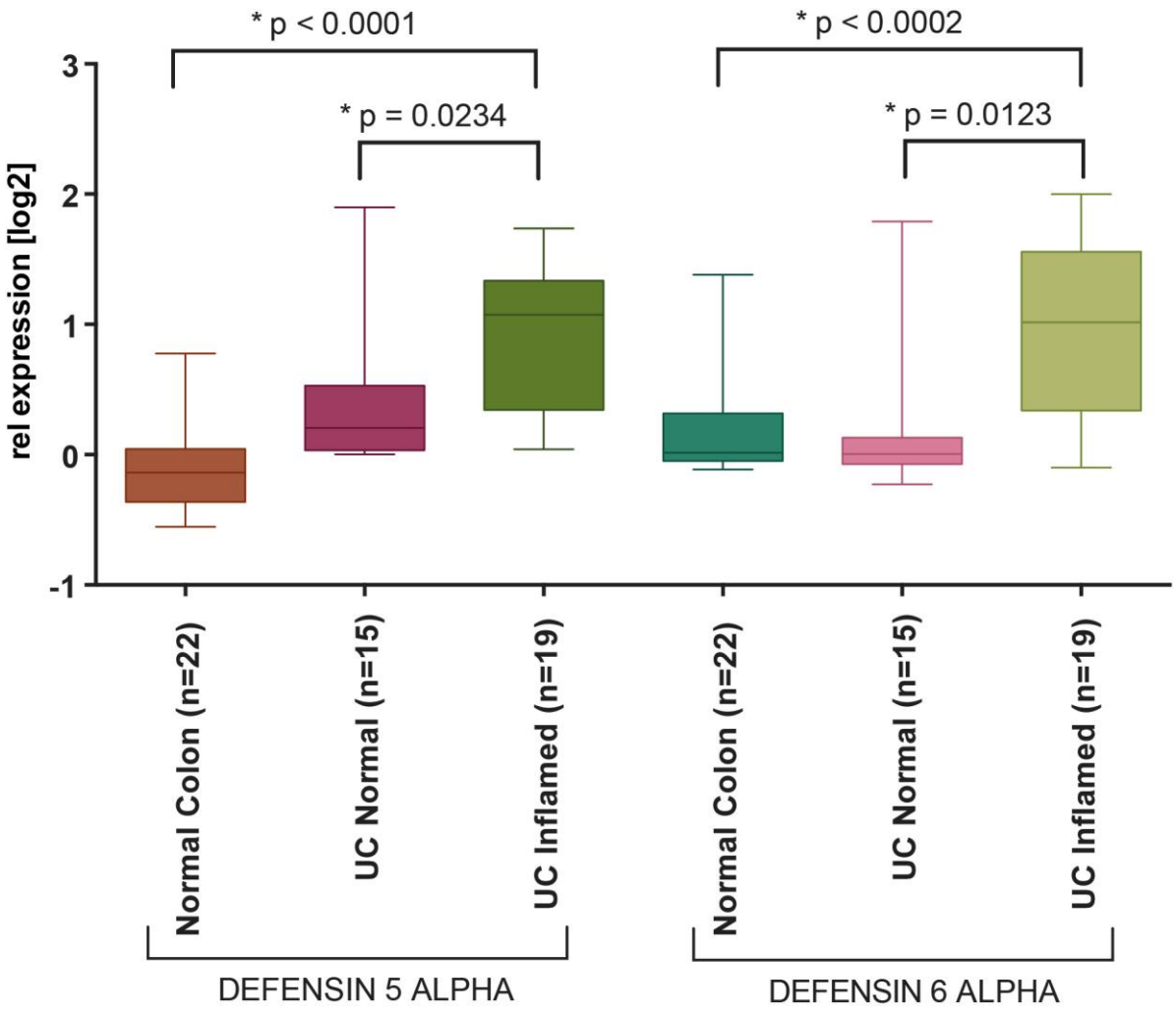

Fig. S5. Expression analysis of antimicrobial genes in normal and inflamed intestines of patients with ulcerative colitis. Whisker and box plots representing the relative expression levels (log2) of DEFENSIN 5 and DEFENSIN 6 in colons of healthy individuals and (normal colon, n=22) and in normal (uninflamed, n=15) and inflamed colons (n=19) of ulcerative colitis patients. Published dataset was used for analysis (Nobel et al 2008; accession number: GDS3268). Student's t-test, unpaired.

Noble, C. L., Abbas, A. R., Cornelius, J., Lees, C. W., Ho, G. T., Toy, K., et al. (2008). Regional variation in gene expression in the healthy colon is dysregulated in ulcerative colitis. *Gut* **57**:1398-405.

Supplementary material Table 1: Incidence and distribution of intestinal neoplasia in wild type and Bcat mice.

Genotype	Number of mice	Mean age (months)	Incidence	Tumors per animal	Avg size of tumors (SD, mm)	Localisation of tumors	
						Small intestine	Colon
WT	16	20	0%	0	0.0±0.0	0%	0%
Bcat/+	31	17	19%	1.5	2.6±1.5	70%	30%
Bcat/Bcat	54	17	37%	1.7	2.3±1.6	40%	60%

Wild type (WT) and mutant mice heterozygous (Bcat het) or homozygous (Bcat hom) for the N-terminally truncated β-catenin knock-in transgene were harvested at the indicated time and the incidence, number, size and localisation of intestinal tumors were determined.

Supplementary material Table 2: Frequency and nature of AOM induced somatic mutations in colonic polyps.

Mutation	Genotype	
	WT	**Bcat**
Kras G12, G13, Q61	3/28	3/26
Ctnnb1 S37, S38	0/16	4/24
Apc Absent/reduced	4/14	15/18

16 weeks after the last of 6 consecutive AOM injections, colonic adenomas of wild type (WT) and mutant (Bcat) mice were harvested and sequenced for the presence of known mutation hotspots in the *Kras* (G12, G13, Q61) and *Ctnnb1* (β-catenin; S37, S38) genes or prepared for immunohistochemical analysis using an antibody raised against the C-terminus of Apc.