Supplementary information.

A basal gradient of Wnt and stem-cell number influences regional tumor distribution in human and mouse intestinal tracts

Supplementary Methods

Ethics and animal husbandry

All mouse work was approved by the UK Home Office and local ethics committee. Mice were housed in the barrier unit of Cancer Research UK, Clare Hall Laboratories. Human archival tissue was obtained from St Marks Hospital, Harrow with multicentre ethics approval (MREC05/Q1606/66). Endoscopic biopsy tissue was obtained from the John Radcliffe Hospital, Oxford with local REC approval (REC 10/H0604/72)

Transgenic stabilized *β-catenin* mouse

Animal breeding and collection of samples

Mice were genotyped for exon 3 excision using the following primers: β-catenin exon 3 forward GCTGCGTGGACAATGGCTAC, reverse GCTTTTCTGTCCGGCTCCAT giving approximately 350bp fragment for mutant alleles and a 550bp fragment for wild-type alleles. Cre recombinase was detected with: forward CGGTCGATGCAACGAGTGATGAGG and reverse CCAGAGACGGAAATCCATCGCTCG primers. Intra peritoneal tamoxifen (5 consecutive days of 100μl of 10mg/ml tamoxifen) initated Cre recombination in 6 week old mice, as mice of this age have undergone post-natal intestinal crypt clonal purification and are maintained on an adult diet. Mice were sacrificed at a specified time after recombination or when symptomatic (anaemic secondary to intestinal lesions or experiencing rectal prolapse). The mean time to sacrifice was 5 weeks. The intestinal tract was divided into four segments - three equal length segments of small bowel (proximal SB1, middle SB2 and distal SB3) and the colon. Each segment was flushed with phosphate buffered saline, opened longitudinally and laid out on filter paper. The samples were fixed in 10% neutral buffered formalin for 24 hours and stored in 70% ethanol. Histology samples were processed using standard methods.

Assessment of colonic Cre-mediated recombination.

Formalin fixed paraffin embedded (FFPE) colonic sections from three heterozygotes (*Ctnnb1*$^{\Delta ex3/+}$) and four wild-type mice were digested overnight with 30μL of Picopure™ proteinase K. TaqMan assays were designed and optimized by Applied Biosystems (Applied Biosystems, Carlsbad, Ca, USA) to the exon 3 region of the *β-catenin* gene, which is deleted in the genetically modified mice and to the exon 6 region of *ribonuclease P protein subunit p30* gene (*Rpp30*), chosen as an endogenous control following the manufacturer's recommendation. Standard curves were set up using six consecutive 2-fold dilutions of DNA extracted from a wild type mouse and were run for both *β-catenin* and *Rpp30* at all times. Colonic epithelial tissue was laser dissected away from the contaminating stroma and extracted DNA from wild-type and heterozygous mutant mice were run on the same plates and the amount of residual *β-catenin* exon 3 DNA was automatically inferred by the program from the standard curve for each gene. Values for *β-catenin* were then normalized to those for the endogenous control *Rpp30.*

Immunohistochemistry

Gut roll sections were de-waxed and rehydrated by standard methods. Endogenous peroxidase was blocked with 3% H_2O_2 in methanol for 10 minutes. Antigen retrieval was achieved by 10 minutes pressure-cooking in sodium citrate buffer at pH 6. Slides were incubated in 3% bovine serum albumin in phosphate buffered saline (PBS) for 15 minutes. Slides underwent primary antibody incubation with varying dilutions of different mouse monoclonal antibodies (Supplementary table 1). This was followed by 1:500 dilution biotinylated rabbit anti-mouse secondary antibodies (DAKO, Glostrup, Denmark) before application of a 1:500 dilution of the tertiary layer of peroxidase-conjugated streptavidin (strep-HRP: DAKO, Denmark). Each layer was applied for 45 minutes and three 5-minute PBS washes were performed between layers. Sections were then developed with 3,3-diaminobenzidine-tetrahydrochloride solution (DAB; Sigma, Gillingham, UK) for 2 minutes, followed by rinsing in tap

water and light haematoxylin counterstaining. Negative controls underwent all steps but were incubated with PBS instead of the primary antibody solution.

In situ hybridization

4μm serial sections of formalin fixed paraffin-embedded human duodenal, ileal and colonic biopsies from 11 different patients and wild-type and transgenic mouse gut rolls were cut. Exon-spanning riboprobes were designed for human and mouse *Lgr5* and a *β-actin* probe was used for hybridization control. Riboprobes were generated by in vitro transcription using SP6 polymerase and labeled with S^{35}-UTP (human and mouse *Lgr5*) or H^3-UTP (mouse *Lgr5* only) (GE Healthcare, Chalfont St Giles, UK). Other methods were as described by Poulsom *et al* [1] In H^3-UTP based assays on normal mouse intestines, the number of *Lgr5* positive cells (≥4 silver granules present) per crypt were counted by three different investigators (PRC, AL, SJL) on 100 crypts from each region in three different wild-type animals.

Normal mouse and human whole mount crypt analysis.

Tissue processing

Tissue was incubated in 5mls of Dulbecco's modified Eagle medium (Invitrogen) with added 30mmol/L ethylenediaminetetraacetic acid (EDTA; Sigma, UK), 0.1 mol/L dithiothreitol, and 100μl RNA later solution (Ambion, Austin, TX, USA) for 15 minutes. Tissue was transferred to phosphate buffered saline (PBS) and then vigorously shaken for 20 seconds. Individual intestinal crypts were then drawn up using glass pipettes and transferred to RNA lysis buffer (Qiagen, Crawley, UK). After several cycles of shaking with fresh PBS washes, complete epithelial denudement occurred and the residual mesenchymal tissue was collected and processed.

RNA extraction, reverse transcription and quantative PCR (qRT-PCR)

RNA was extracted from individual whole mount crypts using the RNAeasy Micro kit (Qiagen, UK) which includes an on-column DNAse step. Obtained RNA quality was assessed on a Bioanalyser (Agilent, Santa Clara, Ca, USA). Reverse transcription was completed straight after RNA extraction using ABI high

capacity RNA to cDNA kit (Applied Biosystems, Carlsbad, Ca, USA). Taqman Pre-Amp Mastermix (Applied Biosystems, USA) was used to pre-amplify individual crypt cDNA. The intron-spanning pre-optimized Taqman probes used, were assessed using control cDNA to ensure that no amplification bias was introduced. Quantative RT-PCR was carried out on a 7900 Fast real-time PCR machine (Applied Biosystems, USA) using the same Taqman probes used for pre-amplification. Results were normalized to *GAPDH* and analysis was completed using the standard ΔΔCt method.

Tissue culture

The rat RIE-1 small intestinal cell line was grown in RPMI medium with 10% foetal calf serum (FCS). Cells were plated out and left to grow to about 80% confluence. Following serum starvation for 24 hours, fresh serum-free medium supplemented with increasing doses of recombinant mouse Sfrp2 was added (Sfrp2 at 15nM. 150nM and 300nM concentrations). Cells were harvested after 4 hours, RNA was extracted and underwent DNAse treatment and reverse transcription. qRT-PCR was then carried out for *Axin2*, an established Wnt target gene in these cells (Leedham 2010, unpublished) to assess the effect of Sfrp2 on endogenous Wnt target gene expression.

Archival human sporadic and FAP associated polyps and tumors

Tissue processing

Serial sections were cut from each block, dewaxed and carefully needle dissected using an H&E stained slide as a guide. Muscularis mucosa tissue was taken from colectomy specimens for constitutional DNA. Dissected tissue was digested overnight in 40μL of Picopure™ proteinase K (Arcturus Bioscience, Mt View, CA). DNA was extracted and used for *APC* sequencing and 5qLOH analyisis

APC sequencing and 5qLOH analysis

DNA underwent sequencing of the mutation cluster region of *APC* using previously described primers and PCR conditions [2]. All samples were sequenced directly in forward and reverse orientation from a new PCR product. *APC* codons 1-1220 were also sequenced in one sample in an attempt to find mutations 5' to

the MCR. All samples underwent 5q LOH assessment using microsatellite markers D5S346, D5S421 and D5S646. Forward primers were labeled with a FAM or HEX fluorescent tag allowing identification of the two separated alleles using GENOTYPER software (Perkin-Elmer, Waltham, MASS, USA). Constitutionally homozygous markers were scored as non-informative. LOH at each marker was considered present if the area under one allelic peak in the affected crypt was less than 0.5 times or greater than 2 times that of the other allele, after normalizing the peak areas relative to constitutional DNA.

Data collection from public mutation databases (COSMIC v53)

Data were selected if both the mutation status of each *APC* allele was known (truncating mutation or LOH), and if adequate positional information to determine tumour location in the colon (right colon or left colon) was available. The number of retained 20AAR's on each mutated allele was calculated as described in the supplementary methods.

Calculation of retained 20AAR's

The total number of remaining 20AARs on the mutated allele(s) was calculated for each tumor; in cases where the tumor had two truncating APC mutations this was the sum of the number of 20AARs remaining on each allele; in cases where the tumor had LOH the total number of retained 20AARs was assumed to be twice the number retained on the truncated allele due to mitotic recombination.

Supplementary results

Recombinant Sfrp2 antagonises endogenous Wnt activity in RIE-1 cells.

The secreted frizzled related proteins share sequence homology with the frizzled Wnt receptor and were initially identified as secreted extracellular attenuators of Wnt, exerting their antagonistic effect by binding and sequestering Wnt proteins. In a comprehensive assessment of Sfrp action in chick neural tube cells, Sfrp2 was shown to be the most effective wnt antagonist inhibiting both endogenous and ectopic Wnt-3a induced β-catenin accumulation [3]. However subsequent studies in neonatal mouse intestinal cells [4] kidney and salivary gland

cells [5] demonstrated that Sfrp2 enhanced the effect of recombinant Wnt-3a promoting nuclear β-catenin accumulation and resulting in Wnt target gene expression.

We have shown that physiological *Sfrp2* expression varies considerably in the mouse intestine with colonic mesenchymal expression more than 130 fold greater than that seen in the small intestine. Furthermore this gradient is the reverse of that seen in the human (supplementary figure 1). In order to assess the effects of Sfrp2 on endogenous Wnt activity in a normal rodent intestine cell system we measured Wnt target gene expression in response to physiological and supra-physiological doses of recombinant Sfrp2. In this cell system we saw a dose dependent decrease in wnt target gene activity following incubation with recombinant Sfrp2, demonstrating that in rodent intestinal cells, Sfrp2 antagonises endogenous Wnt activity.

Supplementary table 1

Antibody	Specificity	Species	Dilution	Antigen retrieval	Source
Primary antibody β-catenin (610154)	Activation of Wnt pathway	Mouse	1:100	Pressure cook in sodium citrate buffer	BD Biosciences
Ki-67 (MIB1) (M7249)	S-phase marker	Rat	1:125	Pressure cook in sodium citrate buffer	DAKO
Cleaved caspase 3 (AF835)	Apoptotic cells	Rabbit	1:800	Pressure cook in sodium citrate buffer	R&D systems
Lysozyme (A0099)	Paneth cells	Rabbit	1:500	Pressure cook in sodium citrate buffer	DAKO
Chromogranin A (15160)	Entero-endocrine cells	Rabbit	1:1000	Pressure cook in sodium citrate buffer	Abcam
Secondary antibody IgG Biotin conjugate	Anti-mouse	Rabbit	1:300	Applied as secondary layer	DAKO
IgG Biotin Conjugate	Anti-rabbit	Goat	1:300	Applied as secondary layer	Molecular Probes

Supplementary table 1. Antibodies, dilutions and conditions.

Supplementary table 2A: Mouse whole mount crypt gene expression by qRT-PCR

Epithelial Gene/patient	SB1		SB2		SB3		Caecum		Rectum		ANOVA
	Mean (SEM) ΔCt	ΔΔCt	Mean (SEM) ΔCt	ΔΔCt	Mean (SEM) ΔCt	ΔΔCt	Mean (SEM) ΔCt	ΔΔCt	Mean (SEM) ΔCt	ΔΔCt	
Ascl2											
Mouse 1	5.6 (0.09)	-1.59	5.8 (0.16)	-1.39	5.7 (0.49)	-1.5	7.2 (0.17)	0	7.2 (0.36)	-0.03	
Mouse 2	5.2 (0.26)	-3.67	6.7 (0.07)	-2.13	7.9 (0.17)	-1	8.9 (0.27)	0	7.6 (0.32)	-1.26	p<0.001
Mouse 3	4.5 (0.32)	-3.18	5.9 (0.15)	-1.72	3.1 (0.54)	-2.5	7.6 (0.56)	0	6.2 (0.1)	-1.42	
Mean (SEM)	5.1 (0.35)	-2.8 (0.62)	6.16 (0.29)	-1.75 (0.21)	6.2 (0.84)	-1.7 (0.44)	7.9 (0.49)	0	7 (0.42)	-0.9 (0.44)	
Lgr5											
Mouse 1	6.7 (0.17)	-1.8	7.3 (0.39)	-1.2	6.5 (0.4)	-2.02	8 (0.23)	-0.54	8.5 (0.31)	0	
Mouse 2	7.6 (0.05)	-2.2	7.3 (0.28)	-2.6	7 (0.18)	-2.86	9.8 (0.31)	0	9 (0.41)	-0.84	p<0.001
Mouse 3	7.4 (0.17)	-2	8.6 (0.19)	-0.7	6.9 (0.4)	-2.47	9.4(0.64)	0	8.3 (0.23)	-1.04	
Mean (SEM)	7.23 (0.26)	-2 (0.13)	7.73 (0.45)	-1.51 (0.55)	6.79 (0.15)	-2.45 (0.24)	9.05 (0.56)	-0.18 (0.18)	8.61 (0.2)	-0.63 (0.32)	
Olfm4											
Mouse 1	-0.6 (0.1)	-7.39	-1 (0.25)	-7.8	-0.6(0.59)	-7.4	6.5 (0.36)	-0.29	6.8 (0.38)	0	
Mouse 2	-0.2 (0.32)	-11.79	-0.6 (0.08)	-12.15	1 (0.27)	-10.6	11.6 (0.2)	0	9.3 (0.27)	-2.21	p<0.001
Mouse 3	-0.3 (0.27)	-9.28	0.3 (0.06)	-8.7	-0.6(0.45)	-9.5	8.95 (1.12)	0	7.5 (1.45)	-1.49	
Mean (SEM)	0.08 (0.42)	-9.49 (1.27)	-0.44 (0.37)	-9.54 (1.33)	-0.66 (0.58)	-9.15 (0.9)	11.46 (1.54)	-0.1 (0.1)	7.87 (0.76)	-1.23 (0.65)	
Sox4											
Mouse 1	9.1 (0.01)	-2.9	10.1 (0.27)	-1.9	10.0 (0.39)	-2	11.6 (0.2)	-0.4	12.0 (0.2)	0	
Mouse 2	9.9 (0.02)	-2.8	10.6 (0.22)	-2.1	10.6 (0.21)	-2	12.6 (0.4)	0	11.6 (0.3)	-1	p<0.001
Mouse 3	9.2 (0.32)	-3.3	11 (0.21)	-1.6	10.3 (0.28)	-2.2	12.5 (0.3)	0	10.7 (0.2)	-1.9	
Mean (SEM)	9.4 (0.24)	-2.99 (0.18)	10.55 (0.25)	-1.84 (0.16)	10.3 (0.17)	-2.09 (0.07)	12.26 (0.33)	-0.13 (0.13)	11.43 (0.39)	-0.97 (0.53)	
Bmp2											
Mouse 1	9.2 (0.32)	0	9.2 (0.09)	-0.1	7.3 (0.1)	-1.9	7.5 (0.2)	-1.7	6.3 (0.5)	-2.9	
Mouse 2	8.8 (0.11)	0	8.6 (0.22)	-0.3	7.2 (0.22)	-1.6	8.5 (0.6)	-0.3	5.7 (0.2)	-3.1	p<0.001
Mouse 3	10.3 (0.27)	0	9.7 (0.41)	-0.6	8 (0.62)	-2.2	7.2 (0.4)	-3.1	5.9 (0.3)	-4.4	
Mean (SEM)	9.44 (0.43)	0	9.14 (0.32)	-0.3 (0.15)	9.01 (0.34)	-1.9 (0.17)	7.71 (0.4)	-1.73 (0.8)	5.96 (0.16)	3.48 (0.45)	
Axin2											
Mouse 1	4.05 (0.04)	-2.4	4.03 (0.34)	-2.4	4 (0.28)	-2.5	6.2 (0.28)	-0.31	6.47 (0.1)	0	
Mouse 2	4.32 (0.09)	-3.1	4.5 (0.24)	-2.9	4.3 (0.14)	-3.1	7.4 (0.19)	0	5.79 (0.2)	-1.6	p<0.001
Mouse 3	3.73 (0.42)	-4.3	4 (0.3)	-4	4.3 (0.34)	-3.8	8.1 (0.1)	0	6.37 (0.55)	-1.7	
Mean (SEM)	4.03 (0.17)	-3.28 (0.56)	4.19 (0.15)	-3.13 (0.47)	4.18 (0.09)	-3.14 (0.37)	7.21 (0.34)	-0.1 (0.1)	6.21 (0.21)	-1.1 (0.55)	
Ccnd1											
Mouse 1	4.8 (0.13)	-2.1	4.8 (0.25)	-2.1	4.2 (0.27)	-2.7	6.3 (0.08)	-0.6	6.9 (0.22)	0	
Mouse 2	5.1 (0.19)	-1.6	4.15 (0.19)	-2.5	3.7 (0.18)	-3	6.7 (0.21)	0	5.7 (0.07)	-1	p<0.001
Mouse 3	4.95 (0.33)	-2.4	5.8 (0.14)	-1.5	3.9 (0.22)	-3.9	7.3 (0.44)	0	6.4 (0.19)	-0.9	
Mean (SEM)	4.96 (0.08)	-2.02 (0.25)	4.93 (0.48)	-2.05 (0.29)	3.78 (0.24)	-3.2 (0.37)	6.78 (0.29)	-0.2 (0.2)	6.33 (0.37)	-0.65 (0.32)	

EphB2											
Mouse 1	4.8 (0.16)	-0.5	4.8 (0.25)	-0.5	4.3 (0.3)	-1	4.5 (0.2)	-0.82	5.3 (0.14)	0	
Mouse 2	2 (0.89)	-3.7	4.1 (0.25)	-1.6	4.1 (0.15)	-1.56	5.7 (0.4)	0	5.1 (0.31)	-0.56	p=0.1
Mouse 3	4.5 (0.24)	-1.85	5.3 (0.13)	-1	4.6 (0.42)	-1.8	6.3 (0.02)	0	5.15 (0.20	-1.2	
Mean (SEM)	3.75 (0.88)	-2.02 (0.91)	4.74 (0.37)	-1.03 (0.34)	4.33 (0.12)	-1.44 (0.23)	5.49 (0.54)	-0.27 (0.27)	5.19 (0.06)	-0.58 (0.33)	
Lef1											
Mouse 1	11.8 (0.23)	-1.2	12.1(2)	-0.9	12.6 (0.28)	-0.4	13 (0.34)	0	12.8 (1.2)	-0.2	
Mouse 2	14.4 (1.12)	-0.4	13.2 (0.84)	-1.6	13.5 (1.09)	-1.4	14.8 (0.54)	0	14.3 (0.3)	-0.55	p=0.7
Mouse 3	12.6 (0.07)	-2.1	14.6 (0.68)	-0.1	14 (0.95)	-0.7	13.2 (1.02)	-1.5	14.7 (1.1)	0	
Mean (SEM)	12.92 (0.78)	-1.27 (0.49)	13.29 (0.72)	-0.9 (0.43)	13.36 (0.41)	-0.82 (0.28)	13.68 (0.59)	-0.51 (0.51)	13.94 (0.58)	-0.25 (0.16)	
EphB3											
Mouse 1	8.7 (0.17)	-1	8.1 (0.4)	-1.6	7.8 (0.3)	-1.9	9.7 (0.27)	0	8.4 (0.1)	-1.3	
Mouse 2	7.4(0.13)	-1.9	7.9 (0.2)	-1.3	7.8 (0.31)	-1.4	9.2 (0.35)	0	7.6 (0.2)	-1.6	p=0.1
Mouse 3	5.7 (0.14)	-4.3	9.8 (0.23)	-0.1	8.4 (0.43)	-1.6	10 (0.35)	0	8.1 (0.37)	-1.8	
Mean (SEM)	7.25 (0.88)	-2.38 (0.98)	8.6 (0.62)	-1.03 (0.46)	8 (0.17)	-1.63 (0.14)	9.63 (0.22)	0	8.06 (0.23)	-1.57 (0.16)	
Mmp7											
Mouse 1	5.6 (0.44)	-5.9	4.4 (0.85)	-7.1	4.9 (0.31)	-6.6	11.3 (0.28)	-0.2	11.5 (0.55)	0	
Mouse 2	5.3 (0.04)	-8.9	4 (0.28)	-10.2	4.4 (0.37)	-9.8	14.14(0.79)	0	14.1 (1.13)	-0.04	p<0.001
Mouse 3	6.05 (0.52)	-8	8.3 (1.04)	-5.8	7.35 (0.15)	-6.7	14.1 (1.01)	0	12.6 (1.28)	-1.45	
Mean (SEM)	5.63 (0.23)	-7.6 (0.88)	5.5 (1.37)	-7.7 (1.3)	5.55 (0.91)	-7.68 (1.04)	13.17 (0.94)	-0.06 (0.06)	12.74 (0.76)	-0.5 (0.5)	
Grem 1 (m)											
Mouse 1	4.8	-1.1	5.7	0	2.5	-3.2	4.3	-1.4	5.0	0.7	
Mouse 2	7.3	0	4.5	-2.8	3.3	-4	4.7	-2.6	3.2	-4.1	p=0.36
Mouse 3	4.4	-1	5.4	0	5	-0.4	3.8	-1.6	4.9	-0.5	
Mean (SEM)	5.5 (0.9)	0.7 (0.35)	5.2 (0.4)	0.93 (0.9)	3.6 (0.7)	-2.53 (1.1)	4.3 (0.3)	-1.86 (0.4)	4.4 (0.6)	-1.3 (1.44)	
Grem 2 (m)											
Mouse 1	7.0	0	6.0	-1	5.6	-1.4	3.1	-3.9	5.6	-1.4	
Mouse 2	11.0	0	7.1	-3.9	7.1	-3.9	3.6	-7.4	5.4	-5.6	p=0.05
Mouse 3	7.8	0	7.6	-0.2	6.5	-1.3	3.6	-4.2	7.4	-0.4	
Mean (SEM)	8.6 (1.2)	0	6.9 (0.5)	-1.7 (1.1)	6.4 (0.4)	-2.2 (0.85)	3.4 (0.2)	-5.2 (1.12)	6.1 (0.6)	-2.5 (1.6)	
Hgf (m)											
Mouse 1	8.0	-1.9	7.4	-2.5	8.4	-1.5	8.4	-1.5	9.9	0	
Mouse 2	7.8	-2	8.4	-1.5	9.8	0	8.9	-0.9	9.7	-0.1	p=0.04
Mouse 3	8.2	-3.4	8.7	-2.9	9	-2.6	8.6	-3	11.6	0	
Mean (SEM)	8 (0.1)	-2.4 (0.5)	8.2 (0.4)	-2.3 (1.7)	9.1 (0.4)	-1.36 (0.75)	8.6 (0.1)	-1.8 (0.6)	10.4 (0.6)	-0.03 (0.03)	
Sfrp2 (m)											
Mouse 1	13.7	0	13.7	0	11.8	-1.9	7.4	-6.3	6.9	-6.8	
Mouse 2	15.6	0	12.3	-3.3	14.3	-1.3	8.6	-7	5.9	-9.7	p=0.035
Mouse 3	10.8	4.9	15.7	-4.9	14.3	-1.4	2.5	-13.2	7.1	-8.	
Mean (SEM)	13.4 (1.4)	- 1.63 (1.6)	15.6 (2.6)	-2.7 (1.4)	13.5 (0.8)	-1.5 (0.18)	6.2 (1.9)	-8.8 (2.2)	6.6 (0.4)	-8.4 (0.8)	

Supplementary table 2B: Human whole mount crypt gene expression by qRT-PCR

Epithelial Gene/patient	Duodenum		Ileum		Caecum		Rectum		ANOVA
	Mean (SEM) ΔCt	ΔΔCt	Mean (SEM) ΔCt	ΔΔCt	Mean (SEM) ΔCt	ΔΔCt	Mean (SEM) ΔCt	ΔΔCt	
LGR5									
Patient 1	6.7 (0.64)	-2.4	5.5 (0.27)	-3.6	6.2 (0.61)	-3	9.1 (1.1)	0	p<0.001
Patient 2	7.5 (0.74)	-0.8	5.3 (0.95)	-3	7.7 (0.46)	-0.56	8.3 (0.2)	0	
Patient 3	9 (0.55)	-1.4	5.5 (0.38)	-3.9	9.4 (0.21)	0	9.4 (0.2)	0	
Patient 4	6.6 (0.23)	-2.3	6.2 (0.22)	-2.7	7.8 (0.25)	-1.1	8.9 (0.4)	0	
Mean (SEM)	7.2 (0.32)	-1.74 (0.38)	5.6 (0.2)	-3.32 (0.29)	7.8 (0.66)	-1.17 (0.65)	8.94 (0.24)	0	
OLFM4									
Patient 1	-2 (0.5)	-4.5	-4.1 (0.23)	-6.6	1.6 (0.6)	-0.9	2.5 (1.5)	0	p<0.001
Patient 2	-2.1(0.2)	-3.9	-2.1 (0.37)	-3.9	-1.9 (0.4)	-3.6	1.8 (1.16)	0	
Patient 3	0.01 (0.2)	-4	-1.8 (0.33)	-5.8	-0.6 (0.8)	-4.6	4 (0.3)	0	
Patient 4	-1 (0.6)	-6.6	-1.9 (0.1)	-7.5	-0.6 (0.4)	-6.2	5.5 (0.25)	0	
Mean (SEM)	-1.29 (0.5)	-4.8 (0.62)	-2.5 (0.55)	-5.94 (0.77)	-0.37 (0.73)	-3.83 (1.11)	3.46 (0.84)	0	
BMP2									
Patient 1	7.8 (0.2)	0	7.6 (0.2)	-0.14	5 (0.6)	-2.8	4.8 (0.1)	-3	p<0.001
Patient 2	6.5 (0.7)	-0.9	7.3 (0.4)	0	5.3 (0.3)	-2	4.9 (0.2)	-2.4	
Patient 3	7.9 (0.5)	-2.9	10.7(0.7)	0	6.7 (0.4)	-4	5.1 (0.1)	-5.6	
Patient 4	8.7 (0.1)	-0.3	9 (0.2)	0	8.5 (0.3)	-0.5	5.4 (0.5)	-3.6	
Mean (SEM)	7.7 (0.46)	-0.99 (0.61)	8.65 (0.76)	-0.04 (0.04)	6.39 (0.8)	-2.3 (0.74)	5.04 (0.13)	-3.65 (0.69)	
AXIN2									
Patient 1	5.2 (0.4)	0	3.8 (0.16)	-1.4	3.9 (0.09)	-1.3	4.2 (0.2)	-0.9	p=0.3
Patient 2	5.3 (0.6)	0	4.2 (0.1)	-1.1	4.6 (0.2)	-0.7	3.5 (0.34)	-1.8	
Patient 3	6.5 (0.1)	-1.74	4.5 (0.2)	-3.8	6.3 (0.4)	-2	8.3 (0.2)	0	
Patient 4	5.2 (0.2)	0	4.2 (0.3)	-1	5.2 (0.5)	-0.1	3.8 (0.5)	-1.4	
Mean (SEM)	5.56 (0.33)	-0.44 (0.44)	4.17 (0.14)	-1.8 (0.67)	4.98 (0.5)	-1.02 (0.42)	4.95 (1.12)	-1.04 (0.39)	
CCND1									
Patient 1	4.6 (0.21)	-1.2	5.8 (0.18)	0	5.4 (0.18)	-0.37	5.6 (0.18)	-0.2	p=0.8
Patient 2	4.3 (0.19)	-0.6	4.9 (0.39)	0	4.6 (0.5)	-0.34	4.6 (0.36)	-0.34	
Patient 3	5.6 (0.15)	-0.8	6.1 (0.35)	-0.35	6.4 (0.1)	0	6 (0.07)	-0.45	
Patient 4	5.3 (0.17)	-0.6	5.4 (0.19)	-0.6	6 (0.08)	0	5.9 (0.07)	-0.01	
Mean (SEM)	4.95 (0.3)	-0.81 (0.13)	5.52 (0.25)	-0.24 (0.15)	5.58 (0.39)	0.18 (0.1)	5.51 (0.32)	-0.25 (0.09)	
EPHB2									
Patient 1	1.7 (0.4)	-1.1	1.2 (0.2)	-1.6	2.1 (0.3)	-0.7	2.8 (0.5)	0	p=0.3
Patient 2	1.4 (0.3)	-1.2	1.3 (0.01)	-1.2	1.8 (0.2)	-0.7	2.5 (0.2)	0	
Patient 3	3.5 (0.2)	-1	2 (0.1)	-2.5	3.5 (0.2)	-1	4.5 (0.04)	0	
Patient 4	3.2 (0.44)	-0.7	2.4 (0.1)	-1.5	2.7 (0.3)	-1.1	3.8(0.2)	0	
Mean (SEM)	2.43 (0.52)	-0.97 (0.11)	1.74 (0.28)	-1.67 (0.28)	2.53 (0.36)	-0.88 (0.11)	3.4 (0.46)	0	

LEF1									
Patient 1	6.9 (0.42)	-7.4	8.8 (0.4)	-5.6	10.3 (0.5)	-4	14.3 (1.3)	0	p<0.01
Patient 2	9.2 (0.37)	-3	9 (0.6)	-3.2	12.2 (0.6)	0	12.1 (0.3)	-0.1	
Patient 3	8.9 (0.36)	-3.5	7.6 (0.3)	-4.8	11.8 (0.6)	-0.5	12.4 (0.3)	0	
Patient 4	7.4 (0.76)	-3.1	7.5 (0.2)	-3	9.7 (0.4)	-0.8	10.5 (0.4)	0	
Mean (SEM)	8.1 (0.55)	-4.24 (1.04)	8.2 (0.39)	-4.13 (0.62)	11.01 (0.6)	-1.32 (0.92)	12.31 (0.78)	-0.02 (0.02)	
EPHB3									
Patient 1	12.9 (0.59)	-0.54	11.7 (0.19)	-1.7	10 (0.26)	-3.3	13.4 (0.92)	0	p=0.05
Patient 2	14.8 (0.27)	0	10 (0.65)	-4.8	14.5 (0.62)	-0.3	14 (0.16)	-0.8	
Patient 3	16 (0.22)	0	13.4 (0.55)	-2.5	14.8 (0.22)	-1.1	14.4 (0.15)	-1.6	
Patient 4	12.6 (0.55)	-2.22	12 (0.74)	-2.8	14.4 (0.49)	-0.4	14.8 (0.18)	0	
Mean (SEM)	14.05 (0.8)	-0.69 (0.52)	11.78 (0.71)	-2.96 (0.66)	13.47 (1.13)	-1.27 (0.7)	14.15 (0.3)	-0.59 (0.38)	
GREM1									
Patient 1	8	-0.3	7.5	-0.8	7.3	-1	8.3	0	p=0.7
Patient 2	7.4	-0.3	7.1	-0.6	6.5	-1.2	7.7	0	
Patient 3	8.6	-1.3	8.9	-1	9.9	0	9.8	-0.1	
Patient 4	8.2	-2.2	8.3	-2.1	10.4	0	9.9	-0.5	
Mean (SEM)	8.05 (0.25)	-1.03 (0.46)	7.95 (0.4)	1.13 (0.34)	8.53 (0.96)	-0.55 (0.3)	8.9 (0.54)	-0.15 (0.1)	
GREM2									
Patient 1	6.8	-1.1	7.9	0	6	-1.9	6.9	-1	p=0.2
Patient 2	6.4	-2.2	5.3	-3.3	7.3	-1.3	8.6	0	
Patient 3	6	-3.1	6.8	-2.3	7.7	-1.4	9.1	0	
Patient 4	fail	fail	fail	fail	fail	fail	fail	fail	
Mean (SEM)	6.4 (0.2)	-2.1 (0.5)	6.7 (0.65)	-1.9 (0.8)	7 (0.4)	-1.5 (0.16)	8.2 (0.6)	-0.3 (0.3)	
HGF									
Patient 1	5.9	-4.5	6.4	-4	7.1	-3.3	10.4	0	p=0.05
Patient 2	6.4	-2.2	8.6	0	6.2	-2.4	8.5	-0.1	
Patient 3	5.8	-3.4	6	-3.2	5	-4.2	9.2	0	
Patient 4	6	-2.6	5.8	-2.8	5.5	-3.1	8.6	0	
Mean (SEM)	6 (0.16)	-3.4 (0.6)	7 (0.7)	-2.4 (1.06)	6.1 (0.5)	-3.3 (0.45)	9.4 (0.5)	-0.03 (0.03)	
SFRP2									
Patient 1	4.8	-1.7	5.85	-0.65	5.4	-1.1	6.5	0	p=0.25
Patient 2	6.8	-2.4	9.6	0	9.4	-0.2	9.2	-0.4	
Patient 3	7.6	-4.2	8.4	-3.4	10.6	-1.2	11.8	0	
Patient 4	7.8	-4.9	8.7	-4	10.6	-2.1	12.7	0	
Mean (SEM)	6.4 (0.7)	-2.7 (0.6)	7.95 (0.95)	-1.35 (0.9)	8.5 (1.4)	-0.8 (0.3)	9.2 (1.3)	0.13 (0.1)	

Supplementary tables 2A and B.

qRT-PCR gene expression data for mouse (supplementary table 2A) and human (supplementary table 2B) whole mount crypts and mesenchymal sections. Each patient or mouse ΔCt value was calculated from the ΔCt values of 4-5 individual crypts or from a single denuded mesenchymal section, for each gene from each region. Mesenchymal genes are indicated by (m). For calculation of ΔΔCt values the region with the lowest gene expression was considered baseline expression for each individual mouse/patient. ΔΔCt values for other regions were calculated against this baseline expression value. The mean (SEM) ΔΔCt (red text) is the average of the individual mouse/patient values and was used to calculate the fold change values plotted in figure 3. ANOVA was used to calculate p values for differences in absolute gene expression between different regions.

Supplementary table 3

Patient / Germline mutation	Somatic mutation	Upper GI lesions	Colonic lesions							
Patient 1 Deletion exon 11-13	Truncating	c.4660_61insA p.T1556fs	Rectum	Left colon	TC 1	TC 2	TC 3	Right	AC	Caecum
			c.4393_94del2 p. p.S1465fs	c.3856G>T p.E1286X	c.4265delA p.D1422fs	c.4606G>T p.E1536X	c.4259delC p.P1420fs	c.4483_84insA p.S1495fs	c.3871C>T Q1291X	c.4742_43del2 p.S1581fs
	LOH	No	No	Yes	No	No	No	No	No	No
	Residual 20AARs	3	2	1, 1	2	2	2	3	1	3
Patient 2 c.1660C>T p.R554X,	Truncating	c.4660_61insA p.T1556fs	Rectum 1	Rectum 2	Rectum 3	DC 1	DC 2	AC 1	AC 2	
			p.S1400X	c.4063_64insT p.S1355fs	c.3942delG p.R1314fs	c4467_71del6 p.L1489fs	c.4316delC p.P1439fs	c.4464delAT p.L1488fs	c.4191del2 p.E1397fs	
	LOH	No	No	Yes	No	No	No	No	No	
	Residual 20AARs	3	2	1, 1	1	2	2	2	2	
Patient 3 c.2055del11 p.W685fs	Truncating	c. 4613insG p. E1538fs	Unspecified colonic location							
			c.4192_93del2 p.S1398fs	c.4308delT p.S1436fs	c4221_22del2 p.S1407fs	c.4476delC p. A1492fs	c.3883G>T p.E1295X	Complex del p.L1423fs		
	LOH	No	No	No	No	No	No	No		
	Residual 20AARs	3	2	2	2	2	1	2		
Patient 4 c. 2387 del2 p.796fs	Truncating	c.4291delA p.M1431fs	Unspecified colonic location							
			c.4465_66ins2 p.L1489fs	c.4463delT p.L1488fs						
	LOH	No	No	No						
	Residual 20AARs	2	2	2						
Patient 5 c. 4387del4 p.K1462fs	Truncating	c.4012C>T p.Q1338X	Unspecified							
			c.4348C>T p.R1450X							
	LOH	NI	NI							
	Residual 20AARs	1	2							

Patient 6 c.4630 G>T p.E1544X	Truncating	None	Unspecified colonic location							
			c.2432C>G p.S811X	c.1660C>T p.R554X	c.2432C>G p.S811X	c.694C>T p.R232X	c.994C>T, p.R332X	c.2432C>G p.S811X		
	LOH	Yes	No	No	No	No	No	No		
	Residual 20AARs	3	0	0	0	0	0	0		
Patient 7 c. 8136del2 p.2711-12fs	Truncating	c.4660_61insA p.T1556fs	Rectum 1	Rectum 2	Rectum 3	Rectum 4	Rectum 5	TC 1	TC 2	TC 3
			c.4392del2 p.S1465fs	c.4660_61inA p.T1556fs	c. 4457del2 p.D1486fs	c.4392del2 p.S1465fs	c.4392del2 p.S1465fs	c.4489delC p.P1497fs	c.4489delC p.P1497fs	c.4216C>T p.Q1406X
	LOH	No	No	No	No	No	No	No	No	No
	Residual 20AARs	3	2	3	2	2	2	3	3	2
	Truncating	Colonic lesions continued…	TC 4	TC5	AC 1	AC 2	AC 3	AC 4		
			c.4318delC p.P1440fs	c.3925_29del5 p.E1309fs	c.4473delT p.A1492fs	c.4358delC p.P1453fs	c.4660_61insA p.T1556fs	c4127_28del2 p.1376fs		
	LOH		No	Yes	No	No	No	Yes		
	Residual 20 AARs		2	1, 1	2	2	3	1,1		

Supplementary Table 3. Upper and lower GI polyp mutation profiles in individual FAP patients. Mutations are classified using standard nomenclature where c stands for complementary DNA sequence followed by the nucleotide number and the nucleotide change and p stands for protein sequence followed by the amino acid code letter change flanking the altered amino acid number. The number of retained 20 amino acid repeats (20AARs) is listed under each mutation. Significantly more 20AARs were retained in upper GI lesions than lower GI polyps (Binomial test, p=0.002) Fs - frame shift; DC - descending colon; TC – transverse colon; AC – ascending colon

Supplementary table 4A

Patient age, gender	Dukes stage	Location	*APC* mutation 1	*APC* mutation 2	Retained 20AARs
82 F	NK	Caecum	c.3871C>T p.Q1291X	c.645C>T p.R216X	1, 0
46 F	C	Caecum	c.3340C>T p.R1114X	c.4343delC p.T1448fs	0, 2
NK	NK	Caecum	c.847C>T p.R283X	c.4343delC p.T1448fs	0, 2
77 F	C	Caecum	c.4333insAT p.T1445fs	LOH	2, 2
77 M	NK	Ascending	c.4348C>T p.R1450X	LOH	2, 2
72 M	C	Left	c.4012C>T p.Q1338X	LOH	1, 1
81 M	B	Sigmoid	c.637C>T p.R213X	c.4231delG p.C1410fs	0, 2
71 M	A	Sigmoid	c.3943insA p.R1314fs	c.637C>T p.R213X	1, 0
73 M	B	Sigmoid	c.4659del5 p.E1554fs	c.4147insA p.M1383fs	0, 2
64 M	NK	Sigmoid	c.4334insCT p.1445fs	LOH	2, 2
86 F	B	Sigmoid	c.847C>T p.R283X	c.3949G>T p.E1317	0, 1
75 M	B	Rectum	c.3934G>T p.G1312X	LOH	1, 1
65 F	A	Rectum	c.3927del5 p.E1309fs	LOH	1, 1
56 M	B	Rectum	c.3694insC p.1232fs	LOH	0, 0
57 M	B	Rectum	c.4132C>T p.Q1378X	LOH	1, 1
83 F	C	Rectum	c.2460delT p.T820fs	LOH	0, 0
58 M	B	Rectum	c.1860delT p.L620fs	c.4308delT p.S1436fs	0, 2
75 M	NK	Rectum	c.3340C>T p.R1114X	LOH	0, 0
64 F	NK	Rectum	c.4263delT p.S1421fs	LOH	2, 2

Supplementary table 4B

COSMIC sample ID	Location	Cumulative total retained 20AAR's	COSMIC sample ID	Location	Cumulative total retained 20AAR's
736984	Right	5	1430400	Left	2
874934	Right	2	736987	Right	4
874961	Right	1	908482	Left	2
874963	Right	1	909748	Left	0
874964	Right	1	990137	Right	4
874965	Right	2	995381	Left	2
874967	Right	2	995391	Right	6
874975	Right	1	995397	Left	4
875010	Right	4	995400	Left	0
907794	Right	3	995404	Left	2
908457	Right	2	995408	Left	4
909755	Left	2	995409	Right	2
910783	Left	1	995413	Left	4
995375	Left	2	1206145	Left	4
995385	Right	5	1206183	Left	2
995387	Left	2	1235040	Right	4
995401	Right	3	1235041	Right	4
995412	Left	3	1235045	Right	2
995651	Left	3	1235046	Left	0
1231126	Left	3	1235051	Right	4
1235039	Left	2	1235054	Right	4
1235043	Right	2	1235056	Right	2
1235055	Left	1	1235058	Left	0
1235057	Left	2	1235066	Left	2
1235059	Right	2	1235067	Left	0
1235061	Right	0	1235068	Left	0
1322285	Left	1	1235069	Left	2
1322293	Right	3	1235073	Right	4
1322287	Right	2			

Supplementary table 4A and B: Sporadic colorectal cancers and cell lines

Table 4A. Genotyped sporadic colorectal cancers. 19 sporadic colorectal cancer cases from specified colonic locations were genotyped and two hits at *APC* were identified. **Table 4B. Publically available data from COSMIC v53.** 57 tumors and cell-lines with positional data and two hits at *APC* were identified. The number of retained 20 amino acid repeats (20AARs) resultant from the identified mutations is stated. Mutations are classified using standard nomenclature where "c." stands for cDNA sequence followed by the nucleotide number and the nucleotide change and "p." stands for protein sequence followed by the amino acid code letter change flanking the altered amino acid number.

Abbreviations M, male; F, female; LOH, loss of heterozygosity (determined by 5q microsatelline analysis); NK, not known.

Supplementary figure legends

Supplementary figure 1

Examples of immunohistochemical staining for cell lineage and apoptosis in wild-type and *Ctnnb1*$^{\Delta ex3}$ mice.

In wild type mice lysozyme staining for Paneth cells is restricted to cells at the base of crypts. No such positional restriction is seen in the section of a *Ctnnb1*$^{\Delta ex3}$ mouse where brown stained Paneth cells can be seen the length of the grossly enlarged crypts. Occasional chromogranin A stained cells can be seen in both normal and mutant intestinal crypts but no significant difference in number could be found. Apoptotic cells stained positive for cleaved caspase 3 (black arrows) were uncommon in the normal intestinal crypts but were significantly more prevalent in the dysplastic crypts of *Ctnnb1*$^{\Delta ex3}$ mice.

Supplementary figure 2

A. Physiological mesenchymal *Sfrp2* expression in human and mouse intestinal tracts. Mesenchymal expression of this important Wnt modulator varies considerably along the length of the human and murine intestines, with a reverse gradient seen in the two different species. Mouse colonic mesenchymal *SFRP2* expression is greater than 130-fold that seen in the small intestinal mesenchyme.

B. Recombinant Sfrp2 antagonises endogenous Wnt activity in rodent intestinal epithelial cells. Culturing RIE cells with recombinant SFRP2 at physiological (15nM) and supra-physiolgical (150 and 300nM) concentrations resulted in a dose dependent decrease in Wnt target gene activity as a consequence of endogenous physiological Wnt pathway antagonism.

Supplementary References

1. Poulsom R, Longcroft JM, Jeffery RE et al. A robust method for isotopic riboprobe in situ hybridisation to localise mRNAs in routine pathology specimens. Eur J Histochem. 1998;**42(2)**:121-32.
2. Thirlwell C, Will O, Domingo E et al. Clonality assessment and clonal ordering of individual neoplastic crypts shows polyclonality of colorectal adenomas. Gastroenterology. 2010 Apr;**138(4)**:1441-54, 54.e1-7.
3. Galli LM, Barnes T, Cheng T et al. Differential inhibition of Wnt-3a by Sfrp-1, Sfrp-2, and Sfrp-3. Dev Dyn. 2006 Mar;**235(3)**:681-90.
4. Kress E, Rezza A, Nadjar J et al. The frizzled-related sFRP2 gene is a target of thyroid hormone receptor alpha1 and activates beta-catenin signaling in mouse intestine. J Biol Chem. 2009 Jan 9;**284(2)**:1234-41.
5. von Marschall Z, Fisher LW. Secreted Frizzled-related protein-2 (sFRP2) augments canonical Wnt3a-induced signaling. Biochem Biophys Res Commun. 2010 Sep 24;**400(3)**:299-304.