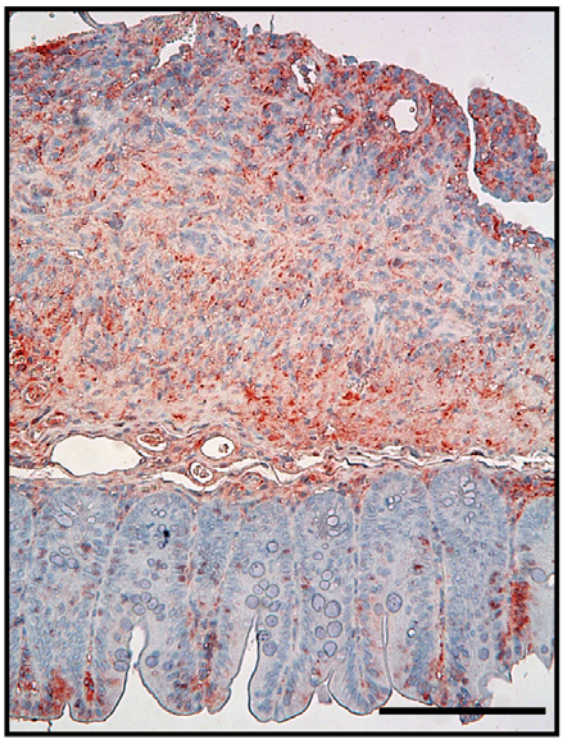
Supplementary Data

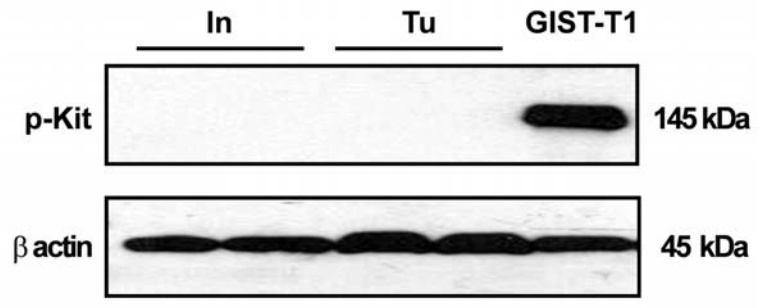
Supplementary Fig.1:

a) Tumors of *Ptch*^{floxflox}*LysMcre*$^{+/-}$ highly expressed Pdgfrα b) Tumors were negative for Kit as revealed by Western Blot analysis of tumors of *Ptch*^{floxflox}*LysMcre*$^{+/-}$ mice compared to normal small intestine. Human GIST-T1 lysates served as positive control for KIT expression. c) Tumors do not express Pdgfrß (see Pdgfrß positive vessels marked by arrow heads).

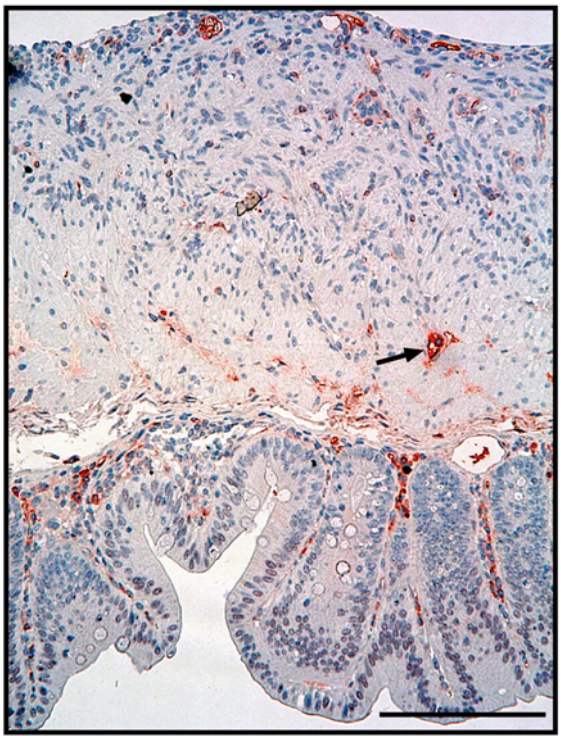
a



b



c



Supplementary Table 1: Oligonucleotides used for genotyping and RT-PCR

Genotyping		
Primer Name	***Primer Sequence (5'-3' orientation)***	***Application***
mLys1	CTTGGGCTGCCAGAATTTCTC	Genotyping of *LysMcre* mice
mLys2	TTACAGTCGGCCAGGCTGAC	
Cre8	CCCAGAAATGCCAGATTACG	
Rosa1	AAAGTCGCTCTGAGTTGTTAT	Genotyping of *LacZ* and *YFP* knock-in mice
Rosa2	GCGAAGAGTTTGTCCTCAACC	
Rosa3	GGAGCGGGAGAAATGGATATG	
p910F.4	GCGAAGAGTTTGTCCTCAACC	Genotyping of $Ptch^{flox}$ mice
p1011R.2	GGAGCGGGAGAAATGGATATG	
Neo-F	CGTGATATTGCTGAAGAGCTTGG	
Neo-R	GCATCAGAGCAGCCGATTGTCTG	
Exon 7-F	AGGAAGTATATGCATTGGCAGGAG	
mPTCwt_r.2	ACACAACAGGGTGGAGACCAC T	
mPTCNx_f	TGGTAATTCTGGGCTCCCGT	
mPTCNx_r	CCGGTAGAATTAGCTTGAAGTTCCT	

Semi-Quantitative RT-PCR		
Primer Name	***Primer Sequence (5'-3' orientation)***	***Application***
mCD21F.1	CTGTGAGAGTGATTTCCCTCTGGA	murine *CD21* expression analysis
mCD21R.2	GCAAATAGCCAGGTTCACAACTGTA	
mCD23F.1	ATTTCAAAGGGAACTGCATGCA	murine *CD23* expression analysis
mCD23R.1	ACTAGTCGCCCTTGCAGGTCA	
mCD35F.1	TTGTAATCAAGGATACCGCCTCATT	murine *CD35* expression analysis
mCD35R.1	AGAAATCTCCATTGGGAATGCCT	
Krt 7 F1	GCCTGGAGGTGGAACTGCGGAAC	murine *Krt7* expression analysis
Krt 7 F2	CAGCTCGAGACACTGCAGCTGGAT	

Semi-Quantitative RT-PCR

Primer Name	***Primer Sequence (5'-3' orientation)***	***Application***
Gapdh-F	ATCTTCTTGTGCAGTGCCAG	murine *Gapdh* expression analysis
Gapdh-R	ATGGCATGGACTGTGGTCAT	

Quantitative RT-PCR

Primer Name	***Primer Sequence (5'-3' orientation)***	***Application***
mHand2F.1	TGGCCAAGGACGACCAGAA	murine *Hand2* expression analysis
mHand2R.1	TTCAAGATCTCATTCAGCTCTTTCTTC	
mFoxf1F.1	AACAGCCTCTGTCCCCTTGC	murine *FoxF1* expression analysis
mFoxf1R.1	CGAGGGATGCCTTGCAGTTCT	
mGli1-tq-F	TACATGCTGGTGGTGCACATG	murine *Gli1* expression analysis
mGli1-tq-r	ACCGAAGGTGCGTCTTGAGG	
mPTC10	TACAGTCCGGGACAGCATACC	murine *Ptch* expression analysis
mPTC11R	GTACCCATGGCCAACTTCGGCTTT	
mPdgfa F	GCAAGACCAGGACGGTCATTTAC	murine *Pdgfa* expression analysis
mPdgfa R	GGCTTCTTCCTGACATACTC	
mPdgfb F	GGCTGCTGCAATAACCGCAATG	murine *Pdgfb* expression analysis
mPdgfb R	CCTGGATGTCCCAGGACTTCTAG	
mPdgfc F	GTACCTAGAGCCAGATCGATGG	murine *Pdgfc* expression analysis
mPdgfc R	CTCTTCCCGTATGGACACTGAG	
18S-fwd	CGCAAATTACCCACTCCCG	murine *18s* expression analysis
18S-rev2	TTCCAATTACAGGGCCTCGAA	
GLI1 F	CCAGCGCCCAGACAGAG	human *GLI1* expression analysis
GLI1 R	GGCTCGCCATAGCTACTGAT	
PTCH F	TGGGATTAAAAGCAGCGAAC	human *PTCH* expression analysis
PTCH R	TCTCCAATCTTCTGGCGAGT	
HAND2 F	CCTTTGAGGCATCTGCTCC	human *HAND2* expression

Quantitative RT-PCR		
Primer Name	***Primer Sequence (5'-3' orientation)***	***Application***
HAND2 R	GCACACGGGAGTGTCCTC	
FOXF1 F	CGTATCTGCACCAGAACAGC	human *FOXF1* expression analysis
FOXF1 R	GACAAACTCCTTTCGGTCACA	

<u>Supplementary Table 2:</u> Primary and secondary antibodies for immunohistochemistry and Western Blot

primary antibodies for IHC	**dilution**	**antigen retrieval**
pAb rabbit anti-Pdgfrα (C-20) Santa Cruz Biotechnologies, SC 338	1:1000	boric acid pH 5.2; 30 min, 60°C
mAb rabbit anti- Pdgfrβ (C82A3) Cell Signaling 4564	1:1000	citric acid pH 6; heat mediated
secondary antibodies for IHC	**dilution**	
En vision+ anti- rabbit/mouse/HRP* Dako K5007	undiluted	
primary antibodies for immunoflourescence	**dilution**	
mAb rat anti- cKit Cedarlane CL8936AP	1:200	
mAb rat anti- PDGFRa Cell signaling 3174	1:100	
pAb goat anti- GFP Rockland 600-101-215	1:400	
secondary antibodies for immunoflourescence	**dilution**	
Rhodamin red conj. anti- rat Jackson Laboratories 712-295-153	1:200	
488 (green) anti- rat Invitrogen A-21208	1:1000	
488 (green) anti- rabbit Invitrogen A-21206	1:1000	
568 (red) anti- goat Invitrogen A-11057	1:1000	
primary antibodies for Western Blot	**dilution**	
pAb rabbit anti- p-cKit (Tyr 719) Cell Signaling, 3319	1:1000	
pAb rabbit anti- p-Pdgfrα (Tyr 754) Santa Cruz Biotechnologies, SC12911	1:500	
pAb rabbit anti- Pdgfrα (C-20) Santa Cruz Biotechnologies, SC 338	1:500	
mAb mouse anti-β actin (C4) Santa Cruz Biotechnologies, SC 47778	1:5000	
mAb rabbit anti- Actin Cell signaling 4970S	1:5000	
secondary antibodies for Western Blot	**dilution**	
pAb mouse anti- rabbit/HRP** Sigma AO545	1:5000	
pAb sheep anti- mouse/HRP** Amersham NA931	1:10000	

* antibody binding was visualized using DAB+ (En vision+ system-HRP, Dako) or aminoethylcarbazol as chromogen.
** signals were visualized using the ECL plus detection system (GE Healthcare).

abbreviations: HRP, horseradish peroxidase; IHC, immunohistochemistry; mAb, monoclonal antibody; pAb, polyclonal antibody

<u>Supplementary Material and Methods:</u> ***In situ*** **hybridization, Western blot, immunoflourescence, immunoperoxidase and LacZ staining**

In situ hybridization was performed as described [1]. The probe sequences for mouse *Gli1* are reported in [2]. The *Ptch* specific probes were a 477 bp fragment spanning exons 2 to 6, and a 250 bp fragment spanning exons 8 and 9 of the *Ptch* gene [3].

LacZ activity was analyzed according to the standard procedures. In short, intestines were fixed for 2h in 4% paraformaldehyde in phosphate buffered saline. Fixed tissues were washed in PBS and soaked in 25% sucrose in PBS overnight. Tissues were frozen in OCT and chilled on dry ice. Sections were cut at 7 μm thickness. Dried tissue sections were post fixed with 0.2% glutaraldehyde in PBS and washed three times in rinse solution (0.005% Nonidet P-40 and 0.01% sodium deoxycholate in PBS). Slides were stained overnight at 30ºC in standard staining solution (5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM MgCl2, 0.4% X-gal in DMSO). Slides were then washed with PBS and mounted.

For Western blot analysis the organs were collected, washed thoroughly with PBS and lysed in RIPA buffer. Fifty μg of total cell lysates were loaded on a denaturing SDS gel. The membranes were blocked with 0.2% casein for 1 hour and probed with the primary antibodies at 4°C overnight. HRP-conjugated secondary antibodies and ECL plus Reagent (Amersham Pharmacia Biotech, GE Healthcare Life Sciences, UK) were used to visualize the signals.

For immunofluorescence analysis tissues were fixed in cold 4% paraformaldehyde in PBS overnight at 4 °C and then incubated in 30% sucrose for 48h at 4 °C. Tissues were frozen in Tissue Tec. After sectioning, slides were stained with antibodies and mounted with *Slowfade* Gold anti-fade reagent containing DAPI (Invitrogen, S36938).

For immunoperoxidase staining and *in situ* hybridization, formalin-fixed tissue samples were embedded in paraffin and sectioned.

All primary and secondary antibodies, the respective dilutions, and if necessary the respective antigen retrieval methods are described in Supplementary Table 2.

Supplementary Literature

1. van Dop, W.A. et al. Depletion of the colonic epithelial precursor cell compartment upon conditional activation of the hedgehog pathway. *Gastroenterology* **136**, 2195-2203 e1-7 (2009).
2. Wijgerde, M., Ooms, M., Hoogerbrugge, J.W. & Grootegoed, J.A. Hedgehog signaling in mouse ovary: Indian hedgehog and desert hedgehog from granulosa cells induce target gene expression in developing theca cells. *Endocrinology* **146**, 3558-66 (2005).
3. Zibat, A. et al. Time-point and dosage of gene inactivation determine the tumor spectrum in conditional Ptch knockouts. *Carcinogenesis* **30**, 918-26 (2009).