Supporting Information

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SI Materials and Methods

For staining intestinal paraffin sections, the following primary antibodies were used: rat anti-Hes1 (1:100; MBL) or rabbit anti-Hes1 (1:500; a generous gift from T. Sudo, Toray Industries, Tokyo), rabbit anti-Ki67 (1:200; Abcys) and rabbit anti-phosphorylated histone H3 (1:500; Upstate Biotechnology). Antigen retrieval was achieved by boiling in 10 mM citrate buffer (20 min) for all antibodies. Horseradish peroxidase-conjugated secondary antibodies were detected by using the DAB peroxidase substrate kit (Vector Laboratories). Identification of goblet cells on paraffin sections was performed by using periodic acid/Schiff and alcian blue stain reagents as recommended by the manufacturer (VWR). Where indicated, nuclei were counterstained with hematoxylin QS (Vector Laboratories). Immunofluorescence was performed on frozen sections by using a polyclonal anti-GFP antibody generated in our laboratory. Cy3- or Alexa Fluor 488-coupled secondary antibodies (Molecular Probes) were detected by epifluorescent microscopy. Identification of goblet cells on frozen sections was performed by using Ulex Europeus agglutinin (UEA) coupled to FITC (1/50) (Sigma). For fluorescence staining, nuclei were stained with DAPI.

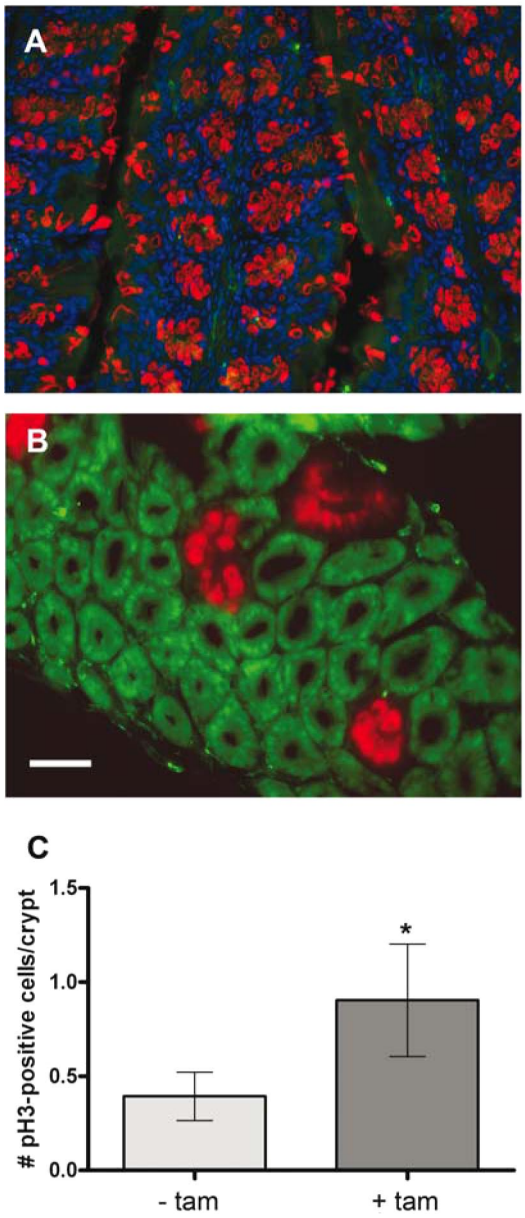

Fig. S1. Conditional Notch activation in adult mice arrests goblet cell differentiation and increases crypt cell proliferation. (*A* and *B*) Frozen tangential sections of adult colon from vilCreERT2/Nic mice either mock-injected (*A*) or induced with tamoxifen (*B*). Immunostaining was performed with a marker of goblet cells (UEA in red) and an antibody anti-GFP to reveal Nic expression (in green in *B*). Nuclei are marked with DAPI in blue in *A*. Nic expression, detected by the nuclear GFP signal in green, is absent in noninduced mice (*A*) and is mosaic in tamoxifen-treated vilCreERT2/Nic mice (green nuclear staining in *B*). The colon presents a very high number of goblet cells (red cells in *A*), and Notch activation completely blocks the differentiation of this cell type (*B*). (Scale bar, 80 μm.) (*C*) Quantification of the number of mitotic cells was performed by counting phosphorylated histone H3-positive nuclei per crypt in vilCreERT2/Nic mice $\pm$ tamoxifen and shows a statistically significant increase in the number of mitotic cells in the crypts of mice induced to express Nic. Ten untreated vilCreERT2/Nic mice and 10 tamoxifen-induced vilCreERT2/Nic mice were processed for immunohistochemistry, and 150 crypts per animal were counted.

Table S1. List of oligonucleotide sequences used for qPCR

Primer name	Target	Species specificity	Primer sequence 5′ to 3′	Product size, bp	Direction
Hes1SF s	Hes1	Mouse	ACACCGGACAAACCAAAGAC	148	Forward
Hes1SF as		Mouse	AATGCCGGGAGCTATCTTTC		Reverse
mBeta2 s	β_2-Microglobulin	Mouse	GCTATCCAGAAAACCCCTCAA	102	Forward
mBeta2 as		Mouse	AGGCGGGTGGAACTGTGTT		Reverse
TFII s	TATA box-binding protein	Mouse	CCACGGACAACTGCGT	236	Forward
TFII as		Mouse	GGCTCATAGCTACTGA		Reverse
hHes1F	Hes1	Human	AGGCGGACATTCTGGAAATG	103	Forward
hHes1R		Human	CGGTACTTCCCCAGCACACTT		Reverse
hHeyLF	HeyL	Human	TCCCCACTGCCTTTGAG	583	Forward
hHeyLR		Human	CTGCTGGGGGCGACA		Reverse
hJ1F	Jagged1	Human	CAACCGTGCCAGTGACTATTTCTGC	250	Forward
hJ1R		Human	TGTTCCCGTGAAGCCTTTGTTACAG		Reverse
hJ2F	Jagged2	Human	TGGGATGCCTGGCACA	550	Forward
hJ2R		Human	CCGGCAGATGCAGGA		Reverse
hN1F	Notch1	Human	CACTGTGGGCGGGTCC	85	Forward
hN1R		Human	GTTGTATTGGTTCGGCACCAT		Reverse
hN2F	Notch2	Human	AATCCCTGACTCCAGAACG	589	Forward
hN2R		Human	TGGTAGACCAAGTCTGTGATGAT		Reverse
hbactF	β-Actin	Human	CGCAAGTACTCCGTGTGGA	337	Forward
hbactR		Human	CGGCCACATTGTGAACTTTG		Reverse
hGAPDHf	GAPDH	Human	GTCATCCCTGAGCTGAACG	347	Forward
hGAPDHr		Human	CTCCTTGGAGGCCATGTG		Reverse
hHPRTf	HPRT*	Human	GCTTTCCTTGGTCAGGCAGTATAAT	142	Forward
hHPRTr		Human	AAGGGCATATCCTACAACAAACTTG		Reverse

*Hypoxyanthine-guanine phosphoribosyltransferase.