

Supplementary Figures

Figure S1. Expression of active β -catenin in response to tamoxifen treatment.

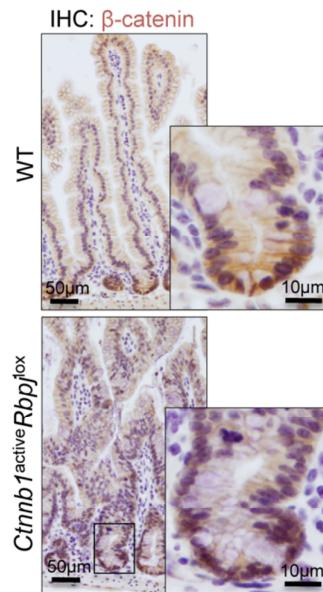


Figure S1: Active β -catenin expression in the intestinal cells detected by IHC, 4 days after tamoxifen treatment. $Ctnnb1^{\text{active}}$ corresponds to the β -catenin GOF mutant $Ctnnb1^{\text{lox(ex3)}}$ and $Rbpj^{\text{lox}}$ to the Notch LOF mutant.

Figure S2: Notch and Wnt/β-catenin co-regulate gene expression.

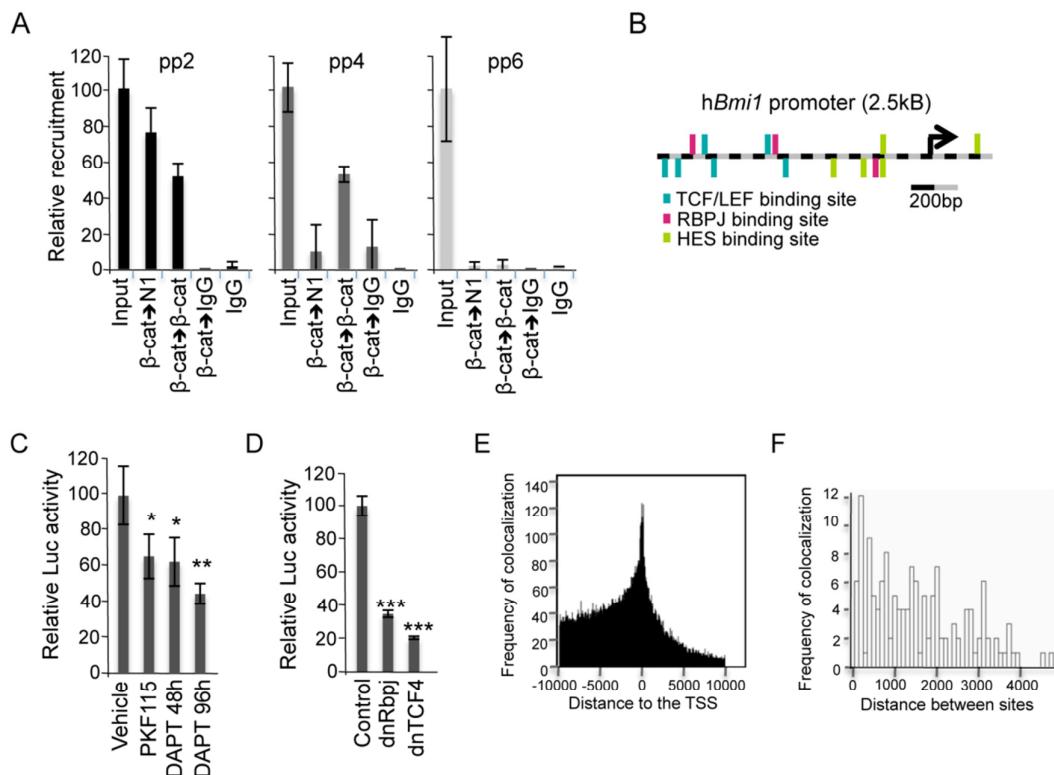


Figure S2 (A) Quantitative analysis of β-catenin and β-catenin/Notch1 co-recruitment to the *Bmi1* promoter region as determined by q-PCR analysis with the indicated primer pairs (see details in Figure 2B). Average and standard deviation values obtained from two independent ChIP experiments are shown. (B) Scheme of the 2-kb proximal promoter of the indicated genes showing the position of RBPJ- and TCF4-binding sites and TSS. Red boxes represent RBPJ-binding sites and green boxes, TCF4-binding sites predicted with Genomatix Software. (C-D) *Bmi1* reporter assay to test the effect of pharmacological inhibitors (C) or the indicated constructs (D). (E-F) Bioinformatic analysis of the whole human genome sequence demonstrating that TCF and RBPJ binding consensus colocalized close to the TSS of the gene promoters (E) and determine the presence of adjacent consensus sequences (F).

Figure S3. Knockout phenotype is detected from the stage of villogenesis and results in intestinal defects at birth.

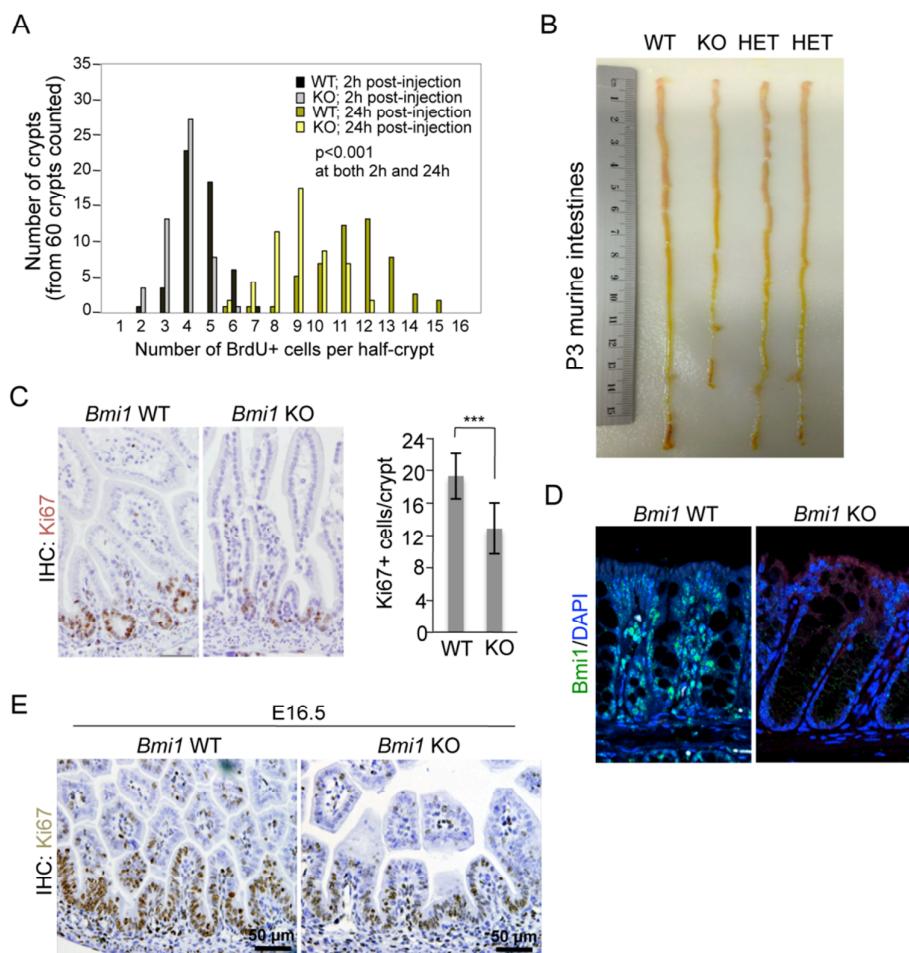


Figure S3: (A) Quantification of the number of crypts with the indicated number of BrdU positive cells in the WT and KO *Bmi1* animals at 2h or 24h after injection. (B-C) Stereoscopical image (B) and (C) IHC of Ki67 in the intestine of the indicated *Bmi1* genotypes (WT: wildtype; KO: knockout; HET: heterozygous) collected at day 3 of age (P3). Quantification of the number of Ki67+ cells per crypt is shown in the right panel. (D) *Bmi1* protein expression in the mouse colonic mucosa. One section of a *Bmi1* deficient colonic mucosa was processed in parallel and is shown as a negative control. (E) Ki67 staining of *Bmi1* WT and KO intestines at day E16.5 of development.

Figure S4. Notch and Bmi1 are active in the ISC and their suppression results in ISC loss in vitro.

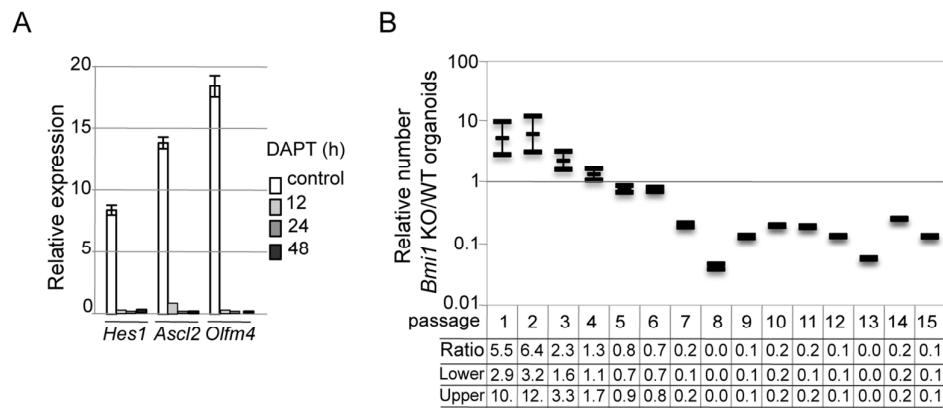


Figure S4: (A) q-RT-PCR to measure the expression levels of the indicated genes in organoid cultures treated with the Notch inhibitor DAPT. (B) Quantification of the average number of organoids obtained from *Bmi1* KO and *Bmi1* WT crypt cells. The ratio between the average number of KO and WT organoids obtained at the different passages (from a minimum of 3 wells counted) is represented. Note the inverted ratio after passage 7.

Table S1. Related to Experimental Procedures. (A) Primer sequence of different genes used by expression analysis. (B) Primer sequence of different genes used by Chromatin immuno-precipitation assays.

TABLE S1A | qRT-PCR primers

Murine Gene	Sense primer 5'-3'	Antisense primer 5'-3'
<i>Villin</i>	CACCTTGGAAAGCTTCTCG	CTCTCGTTGCCTGAACCTC
<i>Gapdh</i>	TGTTCCCTACCCCCAATGTGT	TGTGAGGGAGATGCTCAGTG
<i>β2microglobulin</i>	CTGACC GG C CTGTATGCTAT	CAGTCTCAGTGGGGTGAAT
<i>Lgr5</i>	CGTCTTGC TG GAAATGCTTGAC	AAGGCGTAGTCTGCTATGTGGTG
<i>Olfm4</i>	GCTGGAAGTGAAGGAGATGC	ACAGAAGGAGCGCTGATGTT
<i>Ascl2</i>	AGCATGGAAGCACACCTTG	AAGTGGACGTTGCACCTC
<i>Bmi1</i>	CCAATGAAGACCGAGGAGAA	TTTCGATCCAATCTGCTCT
<i>EphB2</i>	TTCTCACCTCAGTTGCCTCTG	CAAACCCCCGTCTGTTACATACG
<i>cMyc</i>	TATCACCA GCAACAGCAGAGCGAG	AACATAGGATGGAGAGCAGAGCCC
<i>p16Ink4a</i>	GTCGTACCCCGATT CAGGT	ACCAGCGTGTC CAGGAAG
<i>p19Arf</i>	CATGTTGTTGAGGCTAGAGAGG	ACCAGCGTGTC CAGGAAG
<i>Hopx</i>	GAGGACCAGGTGGAGATCCT	TCCGTAACAGATCTGCATTCC
<i>Lrig1</i>	CCAAAAGCTGCATGAGTTGA	GCACCACTGGTATCCTCGAT
<i>mTert</i>	AGGGTAAGCTGGTGGAGGTT	GATGCTCTGCTCGATGACAA
<i>Hes1</i>	CGGCATTCCAAGCTAGAGAAGG	GGTAGGT CATGGCGTTGATCTG
<i>Lyz1</i>	AGACCGAACCGACTATG	CGGTTTGACATTGTTCG
<i>Muc5</i>	AATCAGATGGCTGTGTTCC	TCAGCACATAGGTGCAGTCC
Human Gene	Sense primer 5'-3'	Antisense primer 5'-3'
<i>β-ACTIN</i>	CGC AAGTACTCCGTGTGGA	CGGCCACATTGTGAACTTTG
<i>BMI1</i>	CACCAGAGAGATGGACTGACAAATG	TGAGGAAACTGTGGATGAGGAGAC
<i>C-MYC</i>	CGTGGTATGTATGGAGATGGCAG	GGACAGTAGGAAAGGAAGTGGGATG
<i>EPHB2</i>	CCAGACAAGCATCCAGGAGAAGTTG	AGATTGGGGAAACCGACAGTGAAGG
<i>EREG</i>	CTGCCTGGGTTCCATCTTCTAC	TGTTATTGACACTTGAGGCCACACG
<i>AMOTL2</i>	AGCAGGTTAAAGGTGCTCCA	TCTGCTTTGTCGCTCACT
<i>HES1</i>	AAATGACAGTGAAGCACCTCCG	GAAGCCTCCAAACACCTAGCC

TABLE S1B | ChIP primers

Human Gene	Sense primer 5'-3'	Antisense primer 5'-3'
<i>BMI1-PRO1</i>	TAGAGCCA ACTCCACGTTCC	CGCTGGAGT GATCATAGCAA
<i>BMI1-PRO2</i>	TGGCTT GAAAATGTCTTGC	TCTGCAGAAGATGCCTTGA
<i>BMI1-PRO3</i>	GGCATCTTCTGCAGAGTCGT	CGGTTATTGCCCCTCACACT
<i>C-MYC-PRO1</i>	TGGATGCATTCTTCCCTGA	GTGTGGGAGCCTCTGCTAAG
<i>C-MYC-PRO2</i>	CCTCCCATATTCTCCC GTCT	TGTGTCTGCCTGTTCCAGAG
<i>C-MYC-PRO3</i>	GCGCCC ATTAATACCCTCT	CAGCCGAGCACTCTAGCTCT
<i>EPHB2-PRO1</i>	CGTTGGTGGGACTGAAA ACT	GTGAGAACATGCGGGT TTTG
<i>EPHB2-PRO2</i>	TGAAT CCTAGGCCAATTG C	AGGGCCAGTGGTTACTTCCT
<i>EPHB2-PRO3</i>	AAGGCCAGTCTCCACT	TACCTGTCA GGGCAGGGAGT