Supplementary Table, Figures and Videos

Table S1. Oligonucleotides used for different approaches. (A) RT-qPCR study. (B) qPCR study after ChIP assay. (C) Probes used for EMSA.

S1A. Oligonucleotides used for the RT-qPCR study

cDNA detected (gene symbol)	Nucleotide sequence	Fragment length
Alkaline Phosphatase (intestinal-type)	F: CCAGCTTACCAATGAGAAGGA R: CTGGACCATTGCCATAGAGAA	146
Dll1	F: ATCTCCTTTCTCCTCTTTCC R: ACAACTTTCGGTTTCCTCTT	111
Dll4	F: CCCTCACCTGGATTACCTAC R: GAATCTGCTTGTTAGGGATG	147
emGFP	F: AGGACGACGGCAACTACAAG R: CTTGTGCCCCAGGATGTT	119
Glucagon	F: CGCTGATGGCTCCTTCTCTGAC R: CAAGTGACTGGCACGAGATGTTG	156
Hes1	F: CTACCCGTAAAGTCCCTAGCC R: AAAGCAACAAAATAACCACCAAA	103
Jag1	F: ACCAAGCTCAAGATCAAAAA R: TTTATTGCCAGGAACAACAC	141
Jag2	F: TGTCAGGCGGAAAACAAC R: GAGGACACACACACACACAC	122
Lysozyme	F: GCCAAGGTCTACAATCGTTGTGAGTTG R: CAGTCAGCCAGCTTGACACCACG	86
Muc2	F: AGAACGATGCCTACACCAAG R: CATTGAAGTCCCCGCAGAG	132
Notch1	F: TGTGGTGCCTCCTAGAGAAAA R: CTTTGGCAGTCAGGTGTTAGG	131
Notch2	F: GGGGGCAGGGTAGAGCAC R: GACGCAGATCGGGCATCTT	100
Ppib	F: CACCAATGGCTCACAGTTCTT R: ATGACATCCTTCAGTGGCTTG	156

S1B. Oligonucleotides used for qPCR study after ChIP assay

DNA detected (gene symbol)	Nucleotide sequence	Fragment length
Ctnnb1-TRE	F: ATGTGTGCTCAGGAAAACTGG R: CACTAGGTGATGGGCAGAGAC	236
Jag1-TRE1	F: CATGGCTCAGTTTTGATTGCT R: ATGCCTAGAAAACGCCCTACT	98
Jag1-TRE3	F: GGGTTCCCTTTGACCTTGATT R: CACAGAACTTGGCTTCTCCTG	115
Ppia	F: CCACTGTCGCTTTTCGCCGC R: TGCAAACAGCTCGAAGGAGACGC	109
Rplp0 (36B4)	F: TAAAAGATGTCCGCTCTCCTG R: TCCTTCAGCTCTTCTTTGCTC	110
Sfrp2-TRE	F: CTGGCACCTTACAATCCACTT R: TGGTCACCCATCCTGGTC	104
Villin	F: CAGACATACATGCAGGCAAAA R: CCAGATCCCTCTTCAGTGTGT	137

S1C. Probes used for EMSA

Gene	Nucleotide sequence
Ctnnb1-TRE	F: TGCTGAGGTGAGGTGAGGTGAGGCCAGGTCGTGGTC R: GACCACGACCTGGCCTCACCTCACCTCACCTCAGCA
Jag1-TRE1	F: TGCTGTGGGAGCTACTACTTTGACCTGACTTAATA R: TATTAAGTCAGGTCAAAGTAGTAGCTCCCACAGCA
Jag1-TRE2	F: ACATTGGCTTTGAACTTAGAGTGACCCTCCGGCCTCC R: GGAGGCCGGAGGGTCACTCTAAGTTCAAAGCCAATGT
Jag1-TRE3	F: GGGTTCCCTTTGACCTGATTTGACCAGGAGTGTCGC R: GCGACACTCCTGGTCAAATCAGGTCAAAGGGAACCC
Jag1-TRE3-Mut	F: GGGTTCCCTTTGGTCTGATTTGGTCAGGAGTGTCGC R: GCGACACTCCTGACCAAATCAGACCAAAGGGAACCC

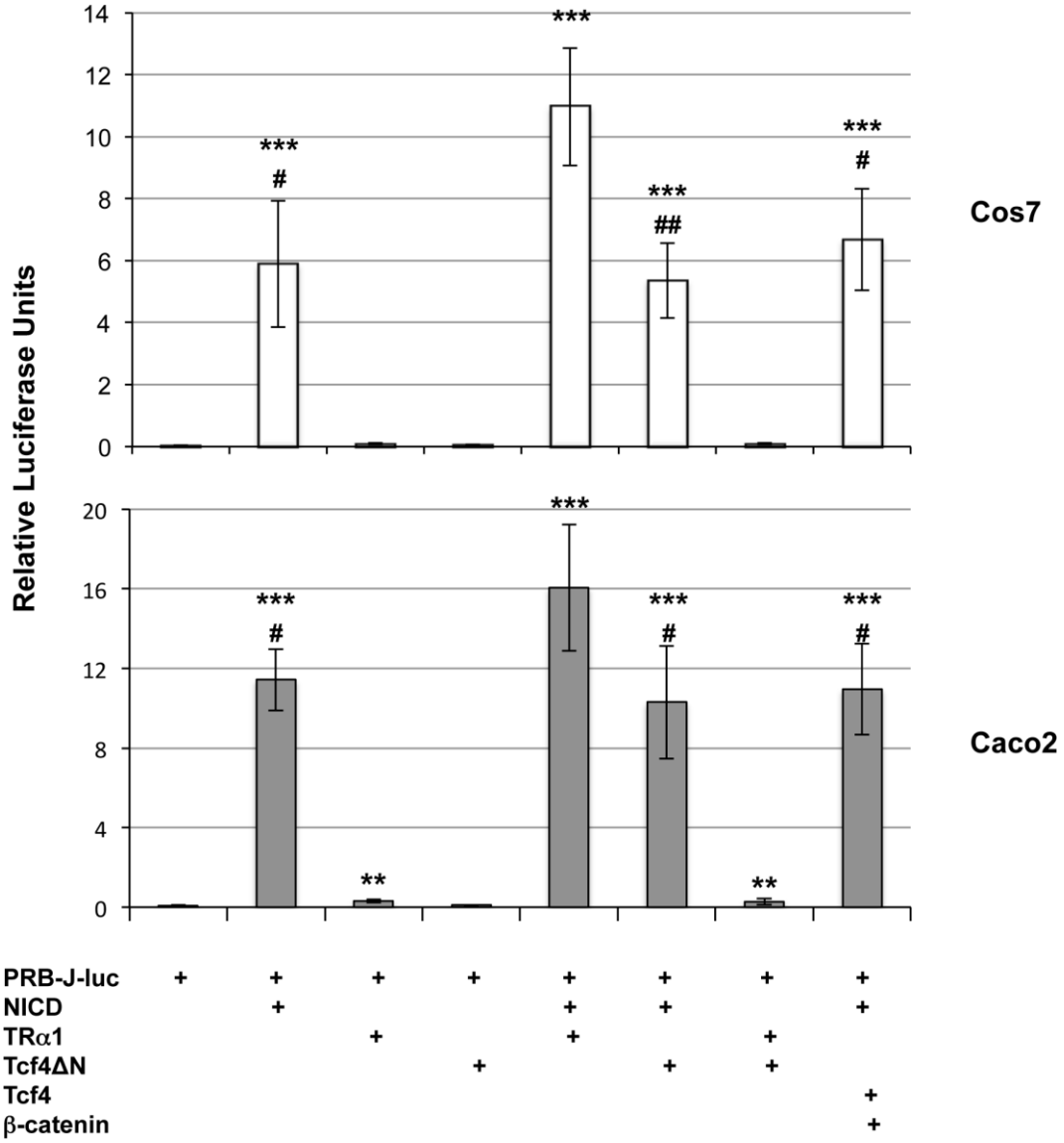


Figure S1. Notch activation by T3 and TRα1 *in vitro* is independent of the Wnt pathway.

The RBP-J luciferase reporter was transfected into Cos7 (upper panel) or Caco2 (lower panel) cells together with TRα1, NICD, a dominant-negative form of Tcf7l2 (Tcf4ΔN) or wild type Tcf7l2 (Tcf4) and β-catenin (the Wnt effectors) expression vectors as indicated. The cells were maintained in non-treated foetal serum containing physiological concentrations of T3. Pictures are representative of two independent experiments each conducted on six replicates; histograms depict the mean ± SD (n=6). *, P<0.05 and **, P<0.01 compared to the control condition (PRB-J-luc alone); #, P<0.05 and ##, P<0.01 compared to the NICD+TRα1 condition.

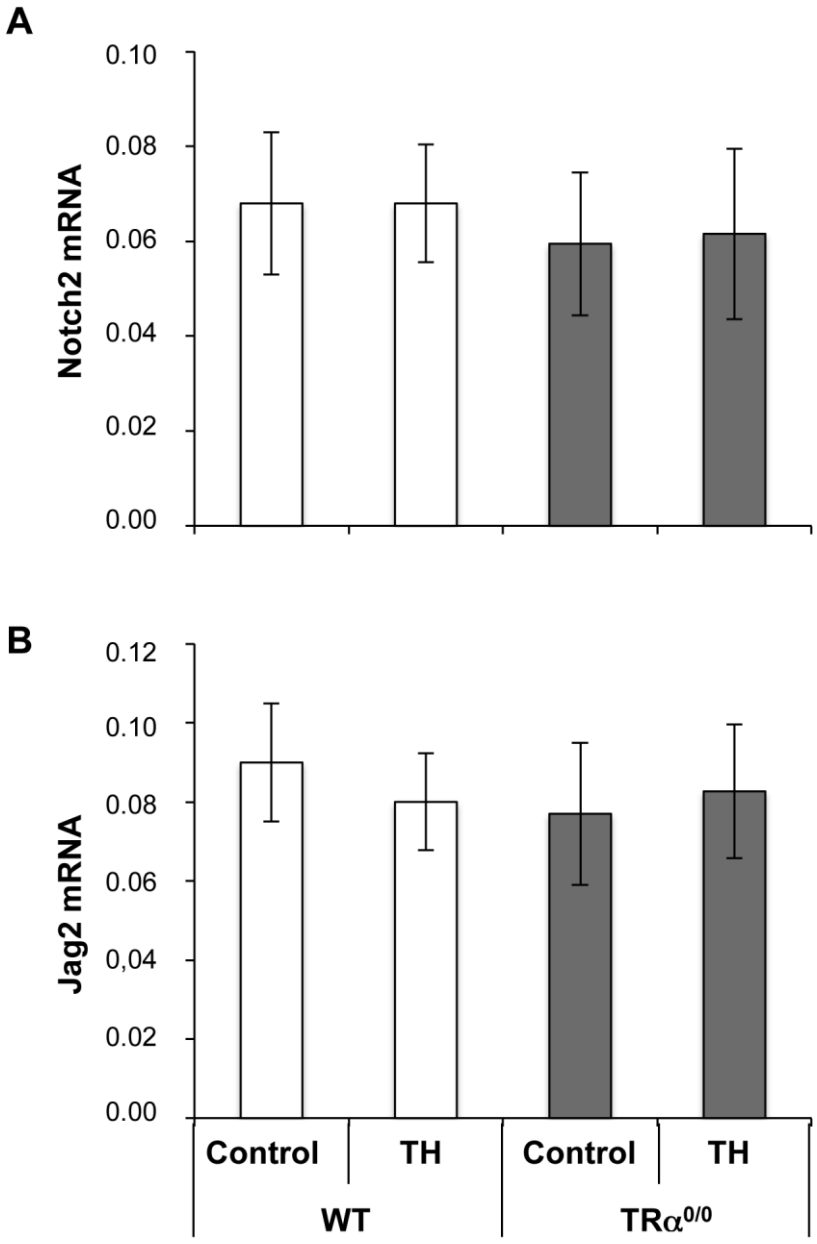


Figure S2. Analysis of Notch2 and Jag2 mRNA expression in WT and TRα$^{0/0}$ intestine. RT-qPCR experiments were performed to analyze the expression of Notch2 (A) and Jag2 (B), in WT (white bars) and TRα$^{0/0}$ (grey bars) animals treated or not with TH, as indicated. Histograms represent mean ± SD, N=3, after normalization with *Ppib*.

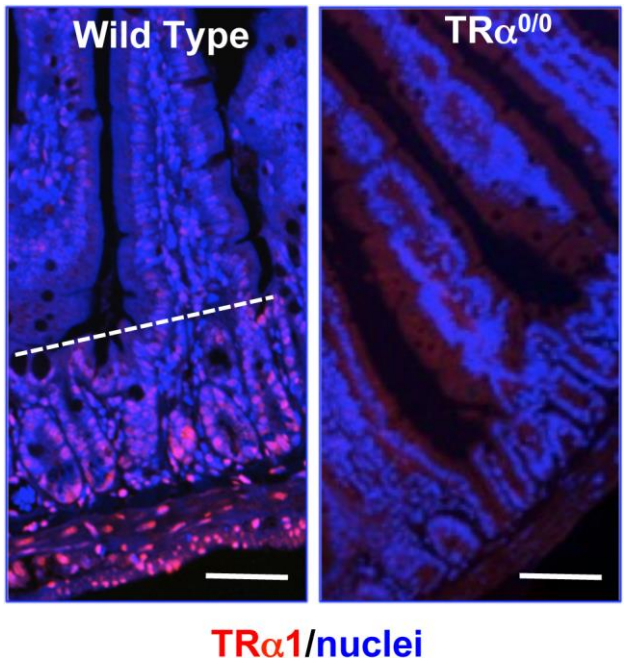


Figure S3. TRα1 is expressed in crypt epithelial cells. In the intestinal epithelium of wild-type animals, TRα1 is highly expressed in nuclei of crypt cells. However, it is worth highlighting that TRα1 is also expressed in nuclei of myofibroblasts and smooth muscle cells. As expected, TRα1 labeling is absent from intestinal sections of TRα$^{0/0}$ animals, confirming the specificity of the antibody used. The white dotted bar in the WT section shows the border between the crypts and the villi. Bars=30 μm.

Figure S4. Localization and *in vitro* analysis of the putative TRα1-binding sites on the *Jag1* promoter. A) A 5 kb region upstream of the *Jag1* start site was analyzed using NUBISCAN software, revealing three putative binding sites. The arrows indicate the location of the binding sites (TRE1, TRE2, TRE3), and the sequences underlined in bold show the arrangement of the half-sites as a typical DR4. The first exon is in red, and the first coding ATG is underlined. B) EMSA analysis using labeled TRE1, TRE2 and TRE3 probes and *in vitro*-transcribed/translated TRα1. Arrows indicate TRα1 binding at TRE1 and TRE3. The *Ctnnb1*-TRE probe was used as a positive control.

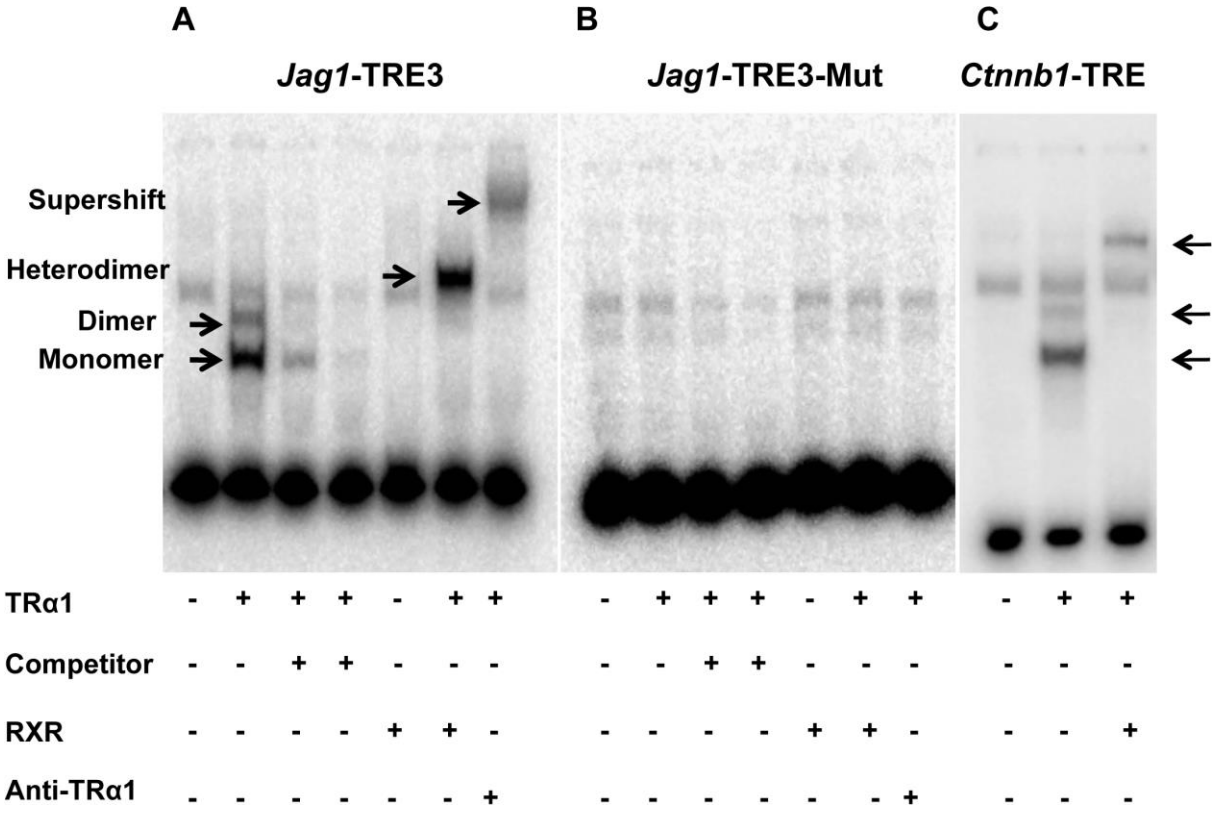


Figure S5. Electrophoretic mobility shift assay on the TRE3 present in the *Jag1* promoter. EMSA analysis using labeled Jag1-TRE3 (A) or Jag1-TRE-Mut (B) probes and in vitro-transcribed/translated proteins as indicated. Addition of specific cold sequences at increasing molar excess was used to assess the specificity of the binding. +, present; -, absent. Arrows indicate the TRα1 monomer and homodimer or the TRα1/RXR heterodimer binding the *Jag1*-TRE3. The addition of an anti-TRα1 antibody is able to induce a supershift of the TRα1-bound probe. The *Ctnnb1*-TRE (C) probe was used as a positive control.

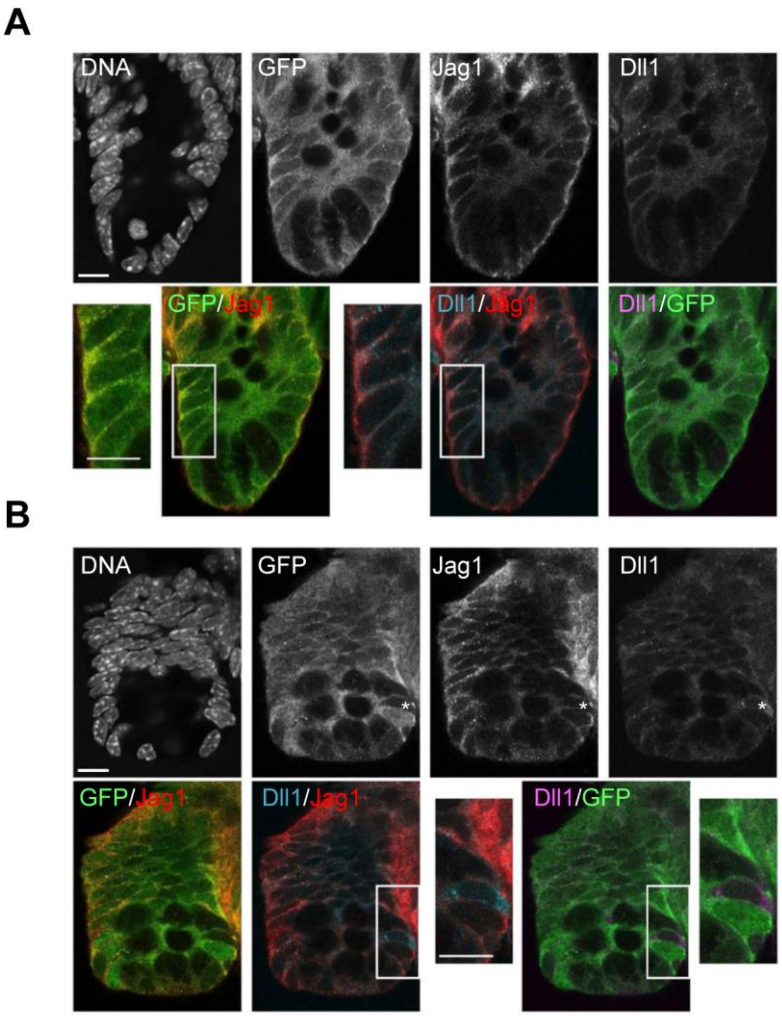


Figure S6. Pattern of Jag1 and Dll1 expression in the intestinal crypts. (A, B) *In toto* crypt immunostaining for Jag1 (red), GFP/Hes1 (green), Dll1 (cyan) and DNA, as indicated. All images represent maximum intensity projections of 3 consecutive focal planes (z= 0.48 μ m). White stars in the upper B panels indicate an exclusive Dll1-expressing cell. Bars = 10 μ m.

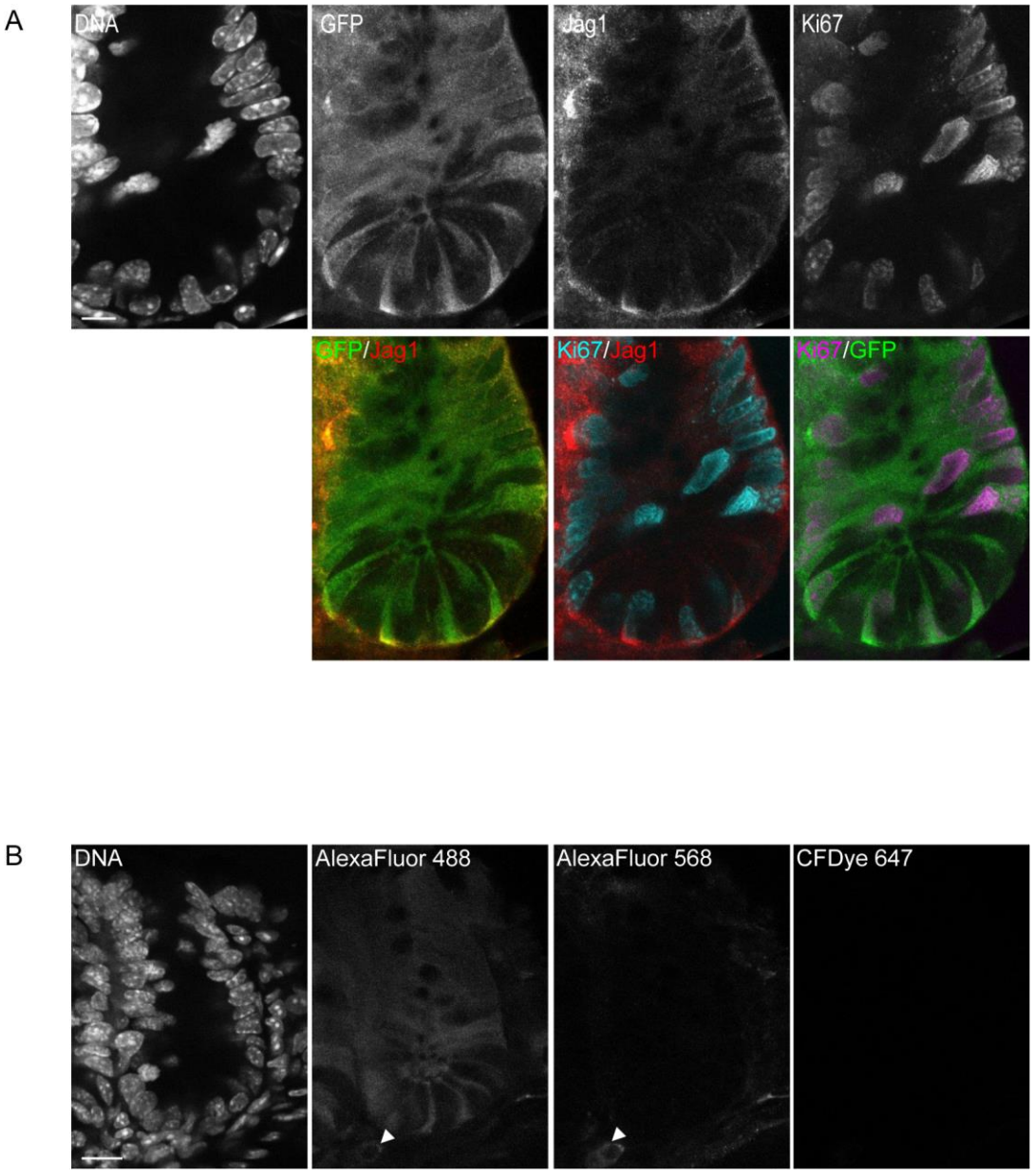


Figure S7. Complementary immunolabeling analysis in intestinal crypts. (A) Immunostaining for Jag1 (red), GFP/Hes1 (green), Ki67 (cyan) and DNA as indicated. (B) Images show negative control experiments to assess the specificity of the secondary fluorescent antibodies; these experiments were performed in the absence of primary antibodies. The native GFP/Hes1-positive cells are visualized with 488-nm laser illumination. White arrowheads indicate cells displaying non-specific labeling under 488-nm and 568-nm laser illumination. All images represent maximum intensity projections of 3 consecutive focal planes (z= 0.48 μm). Bars = 10 μm.

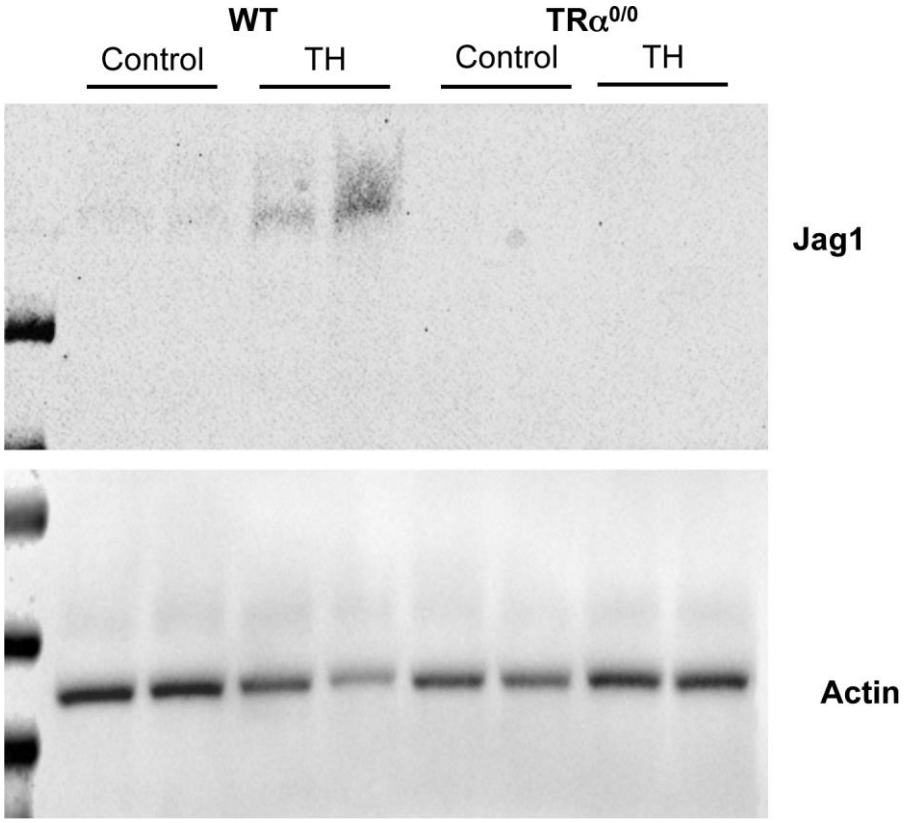
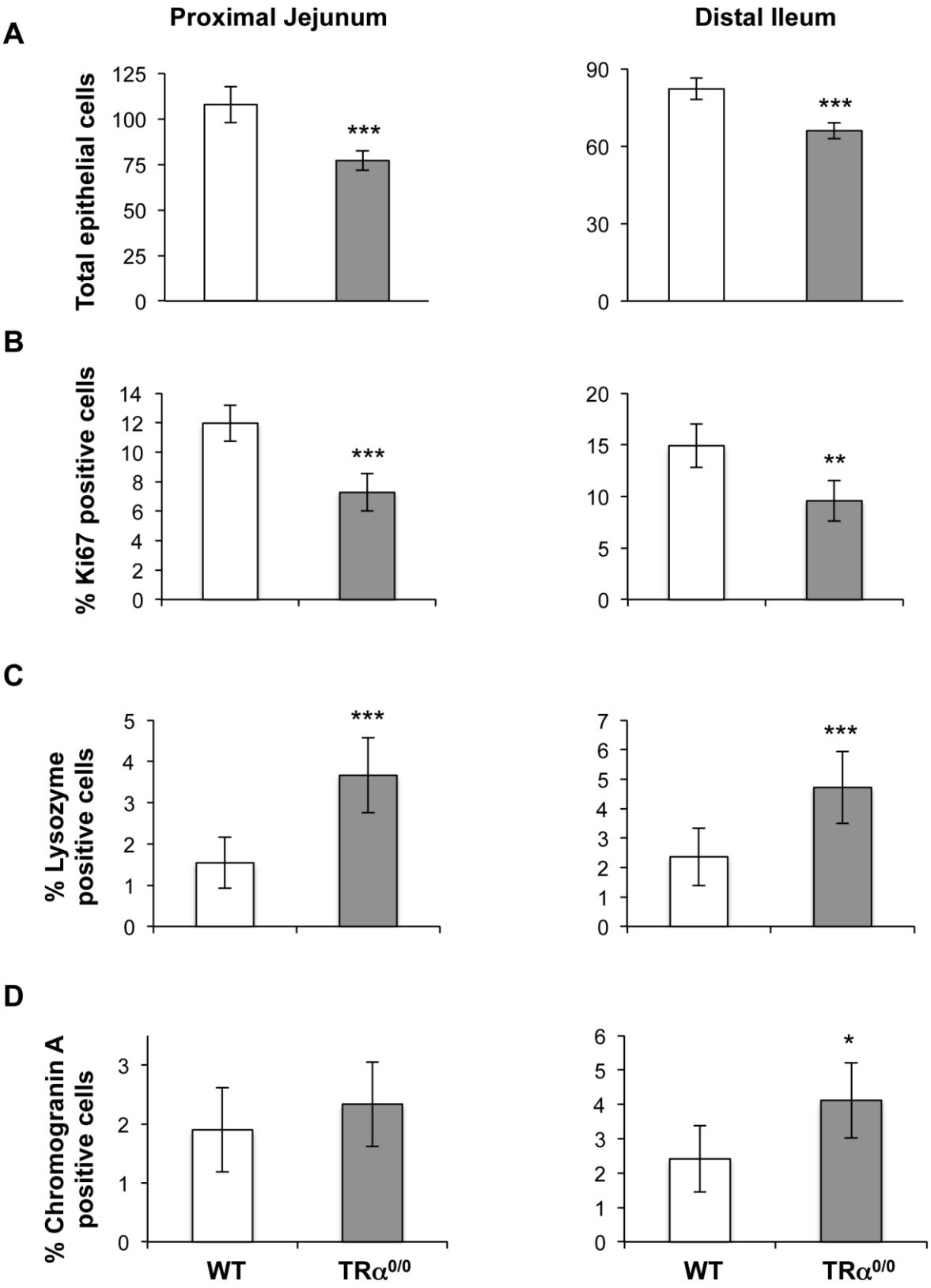


Figure S8. Study of Jag1 protein expression by western blot. WT and TRα$^{0/0}$ mice were treated or not with TH as indicated, and Jag1 expression was analysed in whole protein extracts from the intestinal mucosa. Actin was used as the loading control.


A
Proximal Jejunum
Distal Ileum
Total epithelial cells
125
100
75
50
25
0
***
90
60
30
0
***
B
% Ki67 positive cells
14
12
10
8
6
4
2
0
***
20
15
10
5
0
**
C
% Lysozyme positive cells
5
4
3
2
1
0
***
7
6
5
4
3
2
1
0
***
D
% Chromogranin A positive cells
3
2
1
0
WT
TRα0/0
6
5
4
3
2
1
0
*
WT
TRα0/0

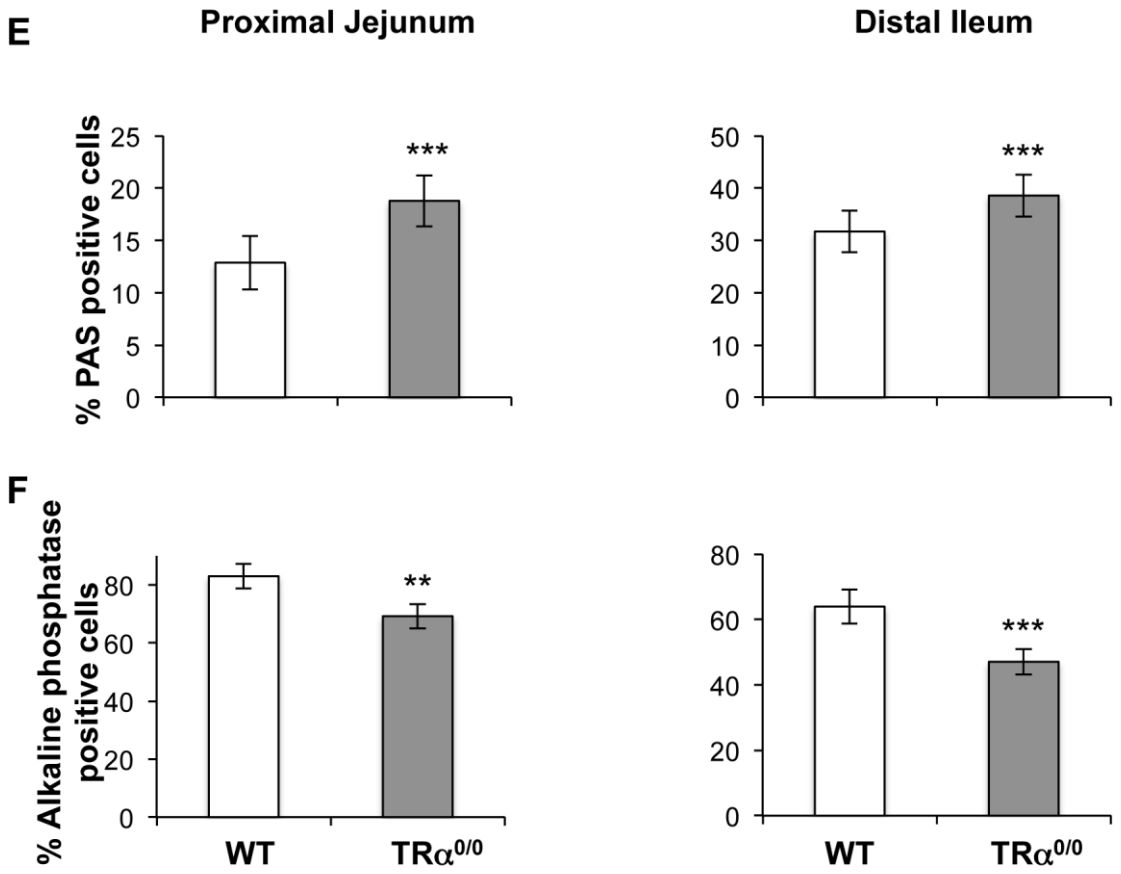


Figure S9. Analysis of intestinal epithelium features of the TRα$^{0/0}$ mice. Intestinal sections from WT (white bars) or TRα$^{0/0}$ (gray bars) mice were analyzed to evaluate alterations in epithelial cell number (A) or in the rates of cell proliferation (B) and cell differentiation (C-F) in both the proximal jejunum and the distal ileum as indicated. For the quantification of total cells and cells positive for each maker in the crypt-villus axes, approximately 30 axes were scored from at least four mice per genotype under the microscope. Histograms represent mean ± SD; N=30. *, P<0.05, **, P<0.01 and ***, P<0.001 compared to the WT.

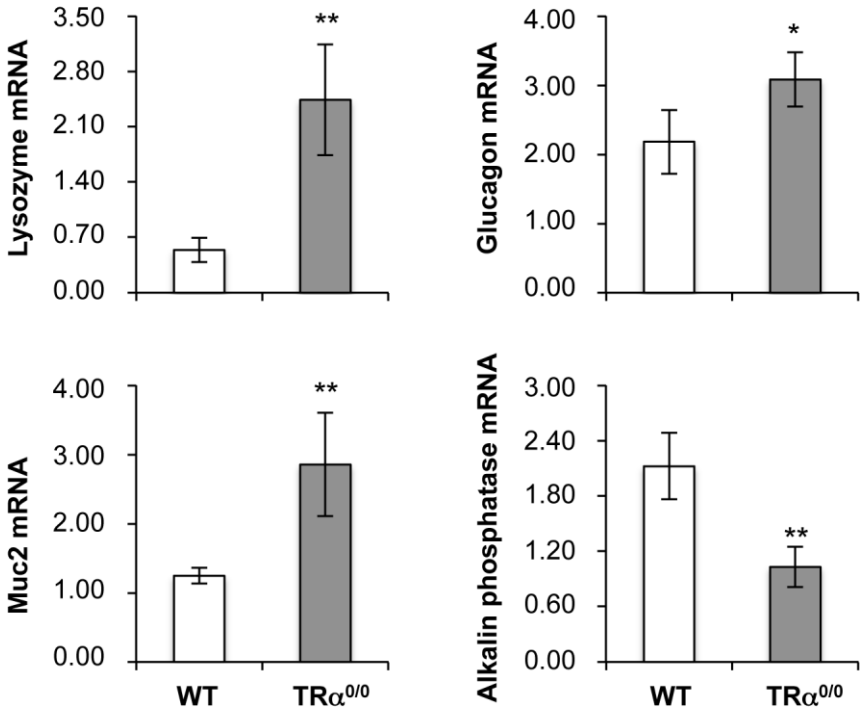
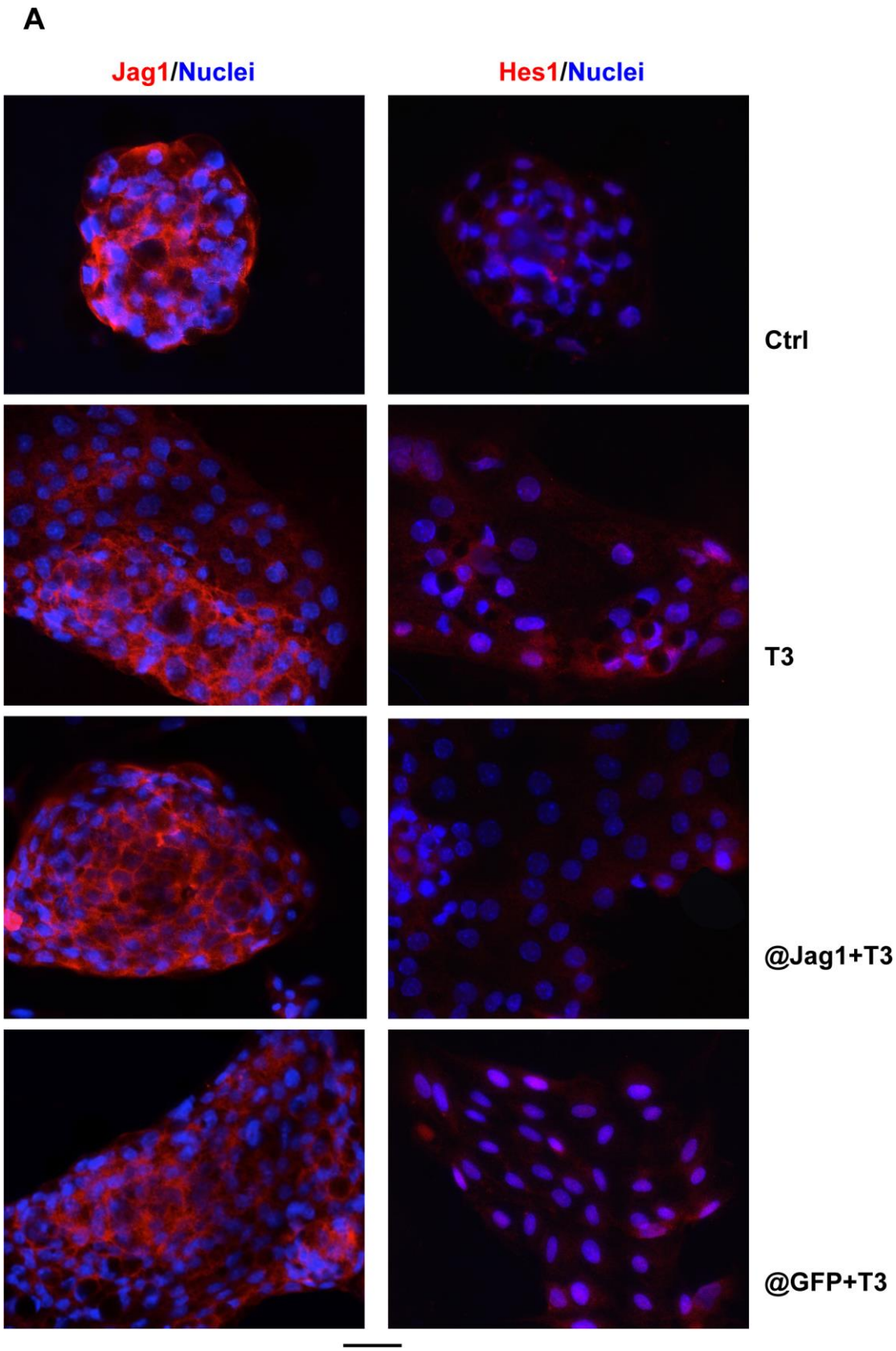


Figure S10. Analysis of differentiation marker's expression in WT and TRα$^{0/0}$ intestine. RT-qPCR experiments were performed to analyse the expression of lysozyme (Paneth cells), glucagon (enteroendocrine cells), Muc2 (goblet cells) and alkaline phosphatase (enterocytes), in the distal ileum of WT (white bars) and TRα$^{0/0}$ (grey bars) animals as indicated. Histograms represent mean ± SD, N=3, after normalization with *Ppib*. *, P<0.05 and **, P<0.01 compared to the WT.


A
Jag1/Nuclei
Hes1/Nuclei
Ctrl
T3
@Jag1+T3
@GFP+T3

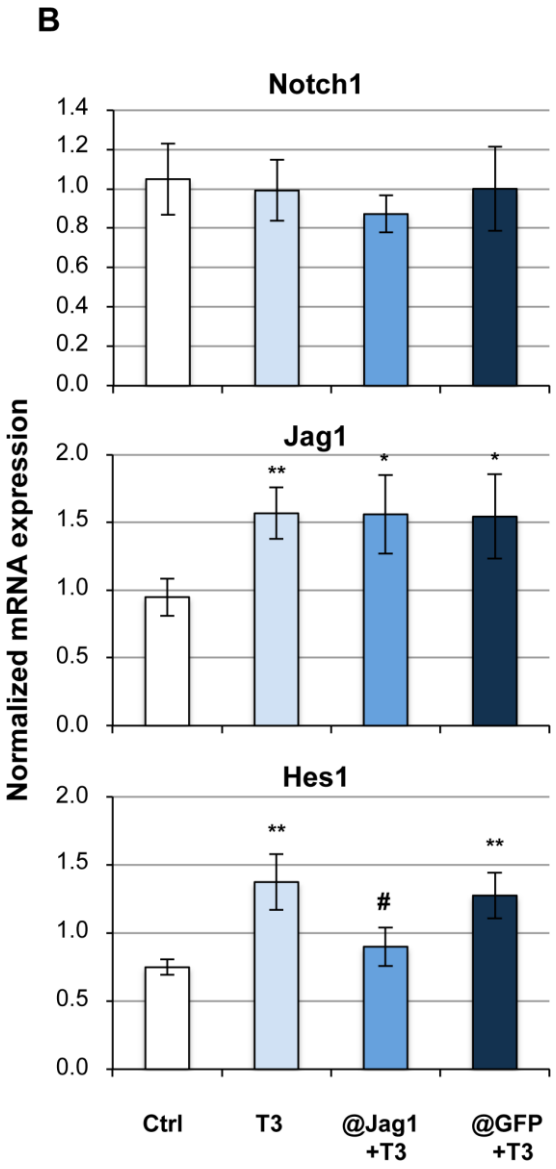
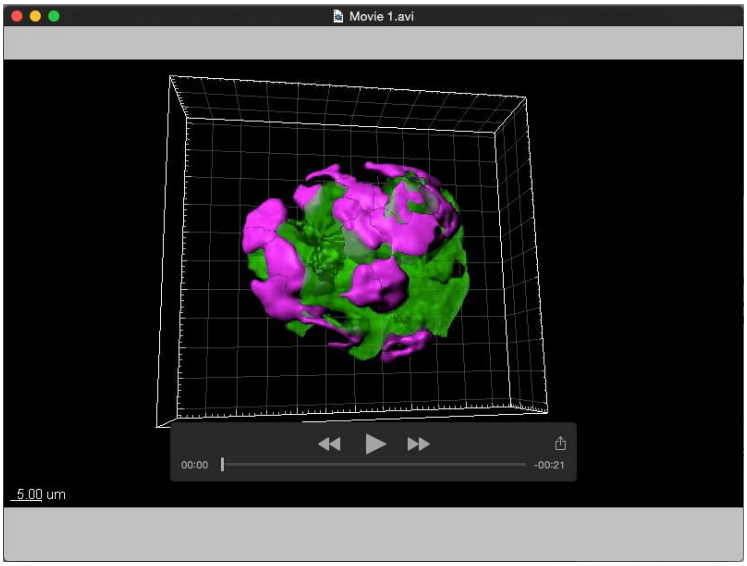
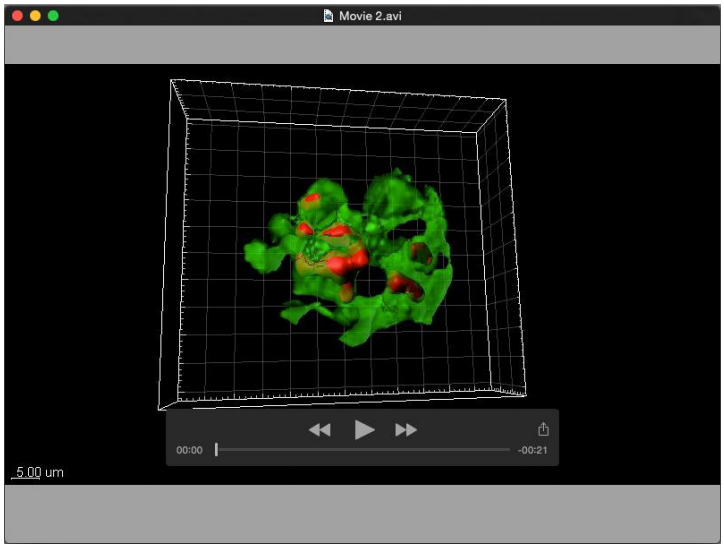


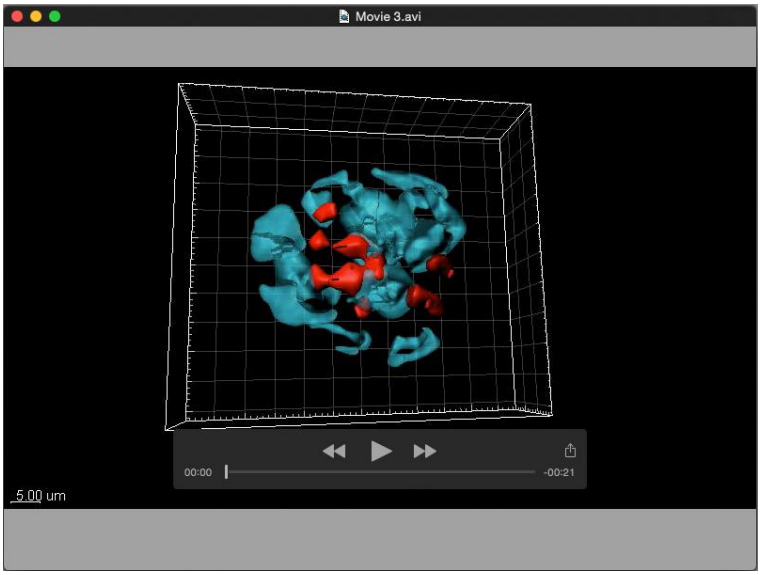
Figure S11. Supplementary data on functional link between T3-dependent Jag1 expression and Notch activity. A) Jag1 and Hes1 immunolabeling of intestinal epithelial primary cultures maintained in different conditions. The images show merged pictures of nuclear staining (blue) and specific staining (red) as indicated. They are representative of two independent experiments each conducted on triplicates. Bars, 20 μm. B) RT-qPCR analysis of the indicated mRNAs in cells maintained in different culture conditions. Graphs are representative of two independent experiments each conducted on duplicates; histograms depict the mean ± SD, N=4, after normalization with *Ppib*. *, $P<0.05$ and **, $P<0.01$ compared to the control condition; #, $P<0.05$ compared with T3 or @GFP+T3 conditions.



Movie 1. GFP/Hes1- and lysozyme-expressing cells in the crypts. 3D isosurface rendering of the fluorescent GFP/Hes1 (green) and lysozyme (magenta) signals observed in Figure 5C.



Movie 2. GFP/Hes1- and Jag1-expressing cells in the crypts. 3D isosurface rendering of the fluorescent GFP/Hes1 (green) and Jag1 (red) signals observed in Figure 5C. The green signal is 50% transparent to better visualize eventual signal overlap.



Movie 3. Jag1- and lysozyme-expressing cells in the crypts. 3D isosurface rendering of the fluorescent Jag1 (red) and lysozyme (cyan) signals observed in Figure 5C. The cyan signal is 50% transparent to better visualize eventual signal overlap.