Supplementary Material

Notch receptor regulation of intestinal stem cell homeostasis and crypt regeneration

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Supplementary Table 1. Genotyping primer sequences

Allele	Forward primer sequence	Reverse primer sequence
Villin-CreER^{T2}	ACAGGCACTAAGGGAGCCAATG	GTTCTTGCGAACCTCATCACT
Lgr5-GFP	CTGCTCTCTGCTCCCAGTCT	GAACTTCAGGGTCAGCTTGC
Floxed-N1	CCAACTGCACTCTTCTCCCAGTAATCGAAG	TGCCTCAGTTCAAACACAAGATACGAGGGG
Floxed-N2	ACCCTGTCAGAAAGTTGGCTGGTCAGGTTT	TAGAGGACGCACTGACTGCTCATCTGACAA
Floxed-Rbpj	CTTGATAATTCTGTAAAGAGA	ACATTGCATTTTCACATAAAAAAGC

Supplementary Figure legends

Supplementary Fig. 1. N1 deletion in the intestinal epithelium results in increased goblet cells throughout the intestine. Villin$^{+/+}$; N1$^{F/F}$ (Control) and Villin-CreER^{T2}; N1$^{F/F}$ (N1$^{\Delta/\Delta}$) animals were treated for 5 days with 100mg/kg tamoxifen (TAM) and tissue was harvested on day 6 or day 8. (A-H) PAS/AB staining for goblet cells is shown for control (A-D) and N1$^{\Delta/\Delta}$ (E-H) intestine. Representative paraffin sections are shown from duodenum (A, E), jejunum (B, F), ileum (C, G), and proximal colon (D, H). Goblet cell hyperplasia was observed in all segments of N1$^{\Delta/\Delta}$ intestine. (I,J) Staining for the Paneth cell marker MMP7 showed increased Paneth cells in N1$^{\Delta/\Delta}$ ileum compared to control. (K,L) Control and N1$^{\Delta/\Delta}$ ileum were stained for the endocrine cell marker CHGA (white arrows). (M) Quantification of CHGA^{+} cells. There were significantly more CHGA+ cells

per crypt in $N1^{\Delta/\Delta}$ ileum. Quantitative data are compared with Student's *t* test. N = 4/group. Scale bar =100μm.

Supplementary Fig. 2. Complete blockade of Notch signaling by Rbpj deletion results in goblet cell hyperplasia that normalizes over time. (A) Villin-$CreER^{T2}$; $Rbpj^{F/F}$ mice were treated with two doses of 100mg/kg TAM ($Rbpj^{\Delta/\Delta}$) and tissues were harvested on day 7, 9, and 14. (B-G) PAS/AB staining for goblet cells in duodenum (B-D) and ileum (E-G) at the time points indicated showed transient goblet cell hyperplasia. Arrows indicate patches of villi that lack goblet cell hyperplasia. Arrowheads denote crypts that maintain goblet cell hyperplasia. N=1 for day 9, N=4-5 for days 9 and 14. Scale bar =100μm.

Supplementary Fig. 3. N1 deletion results in production of aberrantly differentiated goblet cells. $Villin^{+/+}$; $N1^{F/F}$ (control) or $N1^{\Delta/\Delta}$ animals were treated with 100mg/kg tamoxifen for 5 days and tissues were harvested on day 8 (A,B) and day 60 (C-F). Tissue was co-stained for goblet (MUC2, green) and Paneth cell (MMP7, red) markers. While rare double-stained cells are seen in control ileum (A,C) and colon (E), abundant co-staining is seen throughout $N1^{\Delta/\Delta}$ crypts in ileum (B,D) and colon (F), indicating expansion of aberrant goblet/Paneth-like cells. N = 4 animals/group. Scale bar =100μm.

Supplementary Fig. 4. Goblet cells in N1 deleted intestine do not co-express enteroendocrine cell markers. $Villin^{+/+}$; $N1^{F/F}$ (control) and $N1^{\Delta/\Delta}$ were treated with tamoxifen for 5 days and harvested on day 8. (A,B) Ileal tissue was co-stained for the goblet cell marker AB (blue) and the endocrine cell marker CHGA (brown). Although both markers are increased in $N1^{\Delta/\Delta}$ intestine, no $AB^{+}/CHGA^{+}$ co-stained cells were observed. (C,D) Analysis of MUC2 (red) with the proliferation marker EdU (green) suggests that the goblet-like cells are not uniformly proliferating. N = 4 animals/group. Scale bar =50μm.

Supplementary Fig. 5. N1 deletion results in patchy recovery of stem cell marker expression. (A-D) Quantitative RT-PCR analysis of Notch target genes and stem cell markers in tamoxifen-treated *Villin*$^{+/+}$; $N1^{F/F}$ (Control) and $N1^{\Delta/\Delta}$ animals at the time points indicated. RNA was isolated from jejunal crypts. Comparisons were made by Student's *t* test compared to control at each time point. N= 4 animals/group. (E,F) *In situ* hybridization for CBC stem cell marker *Olfm4* in control and $N1^{\Delta/\Delta}$ ileum 60 days after tamoxifen treatment. Although *Olfm4* expression was completely abolished immediately after N1 deletion (Figure 6), some crypts had recovered *Olfm4* by day 60, consistent with the patchy recovery of the differentiation program. N = 4 animals/group. Scale bar =100μm.

Supplementary Fig. 6. Nontransgenic mice survive 8 days post-irradiation. Nontransgenic C57BL/6 mice were administered one dose of 12Gy irradiation and harvested at various timepoints or monitored until moribund. (A) Survival curve of irradiated animals. (B) Weight curve of irradiated animals harvested at

0.5, 1, 2, 5, and 8 days post-irradiation. Data is displayed as fasted overnight weight as a percent of initial weight. Although animals do not survive past day 9, weights begin to rebound on day 8. (C,D) Quantitative RT-PCR analysis of the CBC markers *Lgr5* and *Olfm4* in full thickness ileum. Similar to the weight recovery, stem cell markers recover on day 8. Quantitative data are compared with ordinary one-way ANOVA and Dunnett's post-test. N=3-15 animals per time point.

Supplementary Fig. 7. N1 deletion in juvenile mice has a mild but apparent secretory cell phenotype. 10-day old Villin$^{+/+}$; N1$^{F/F}$ (control) or N1$^{\Delta/\Delta}$ animals were treated with 100mg/kg tamoxifen for 5 days and tissues were harvested on day 6. (A-D) PAS/AB staining for goblet cells in control (A, C) and N1$^{\Delta/\Delta}$ (B,D) intestine. Increased goblet cell abundance is observed in both duodenum (B) and ileum (D) of juvenile N1$^{\Delta/\Delta}$ intestine. Interestingly N1$^{\Delta/\Delta}$ mice also appear to have more developed crypts than controls, which may be due to the altered differentiation program. N=1 control, 2 N1$^{\Delta/\Delta}$. Scale bar =100μm.

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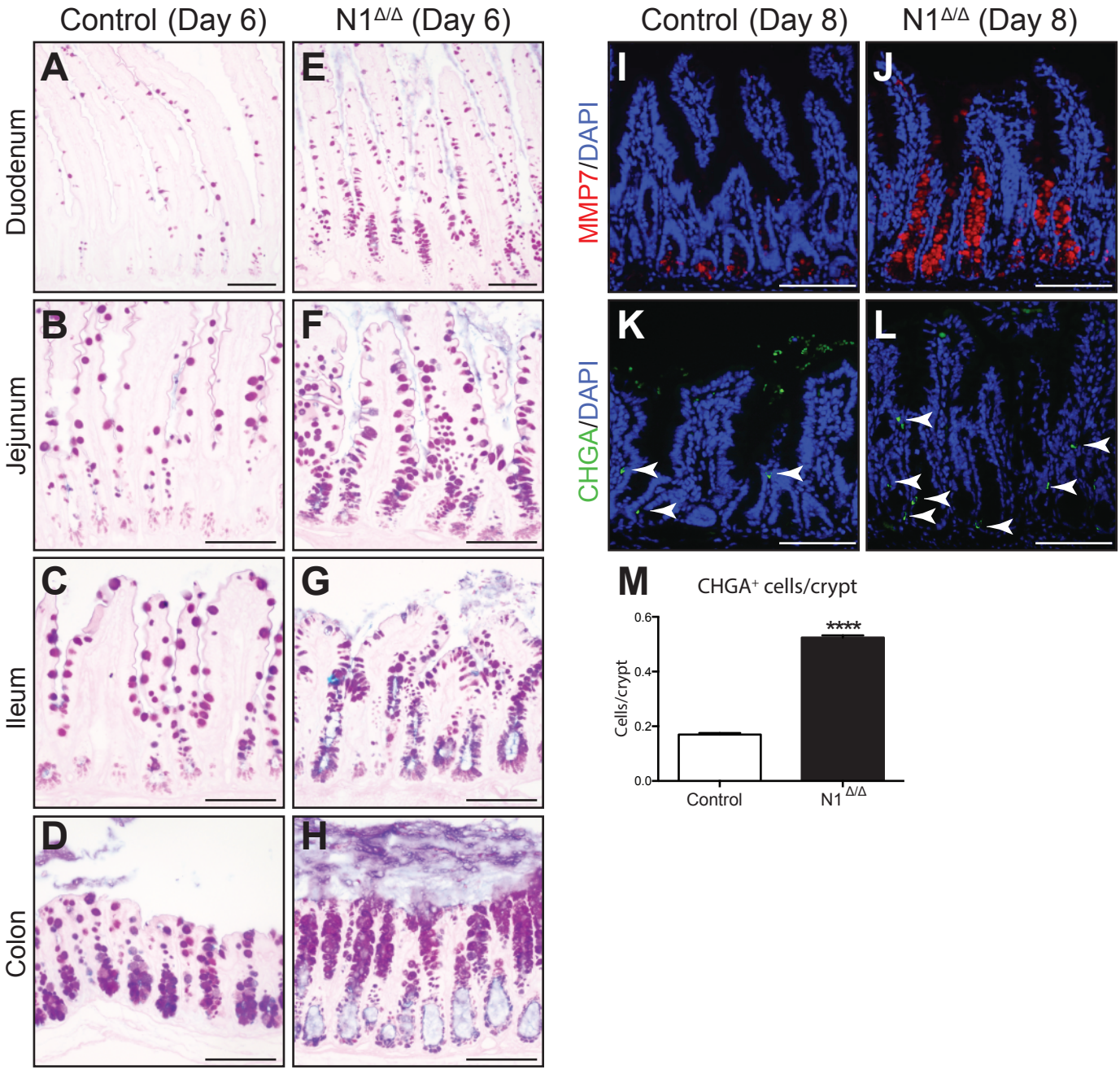
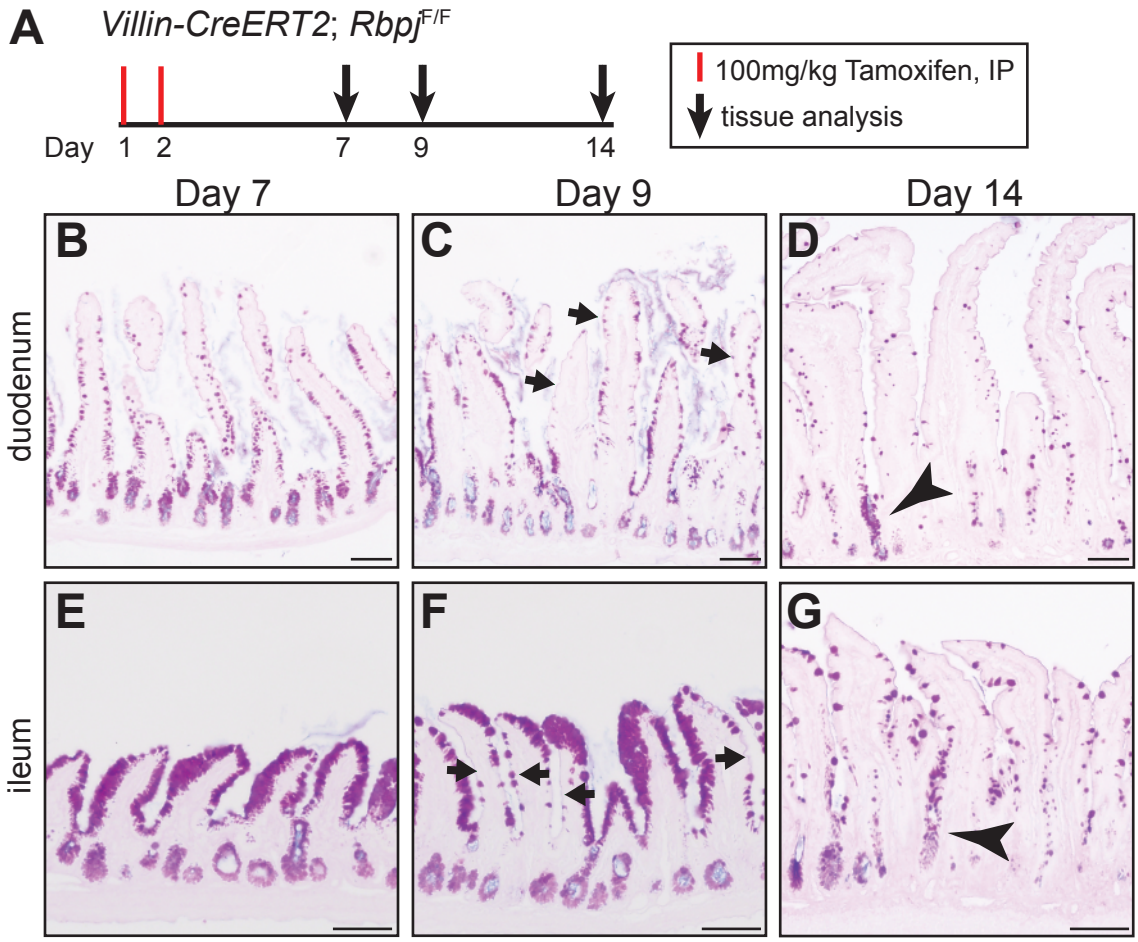
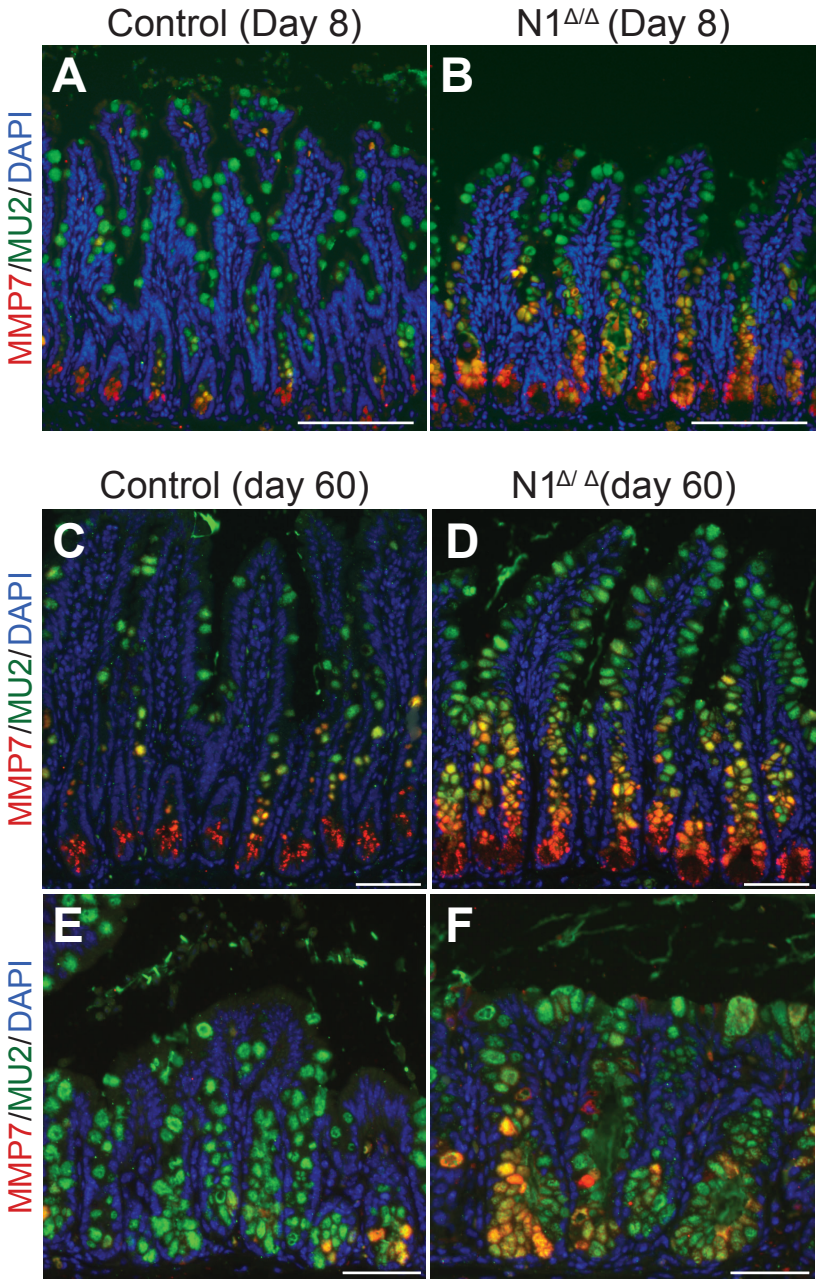
Supplementary Figure 1




A
Villin-CreERT2; Rbpj F/F
Day 1 2 7 9 14
100mg/kg Tamoxifen, IP
tissue analysis
Day 7
Day 9
Day 14
duodenum
ileum
B
C
D
E
F
G


Control (Day 8)
N1Δ/Δ (Day 8)
A
B
MMP7/MU2/DAPI
Control (day 60)
N1Δ/ Δ(day 60)
C
D
MMP7/MU2/DAPI
E
F
MMP7/MU2/DAPI

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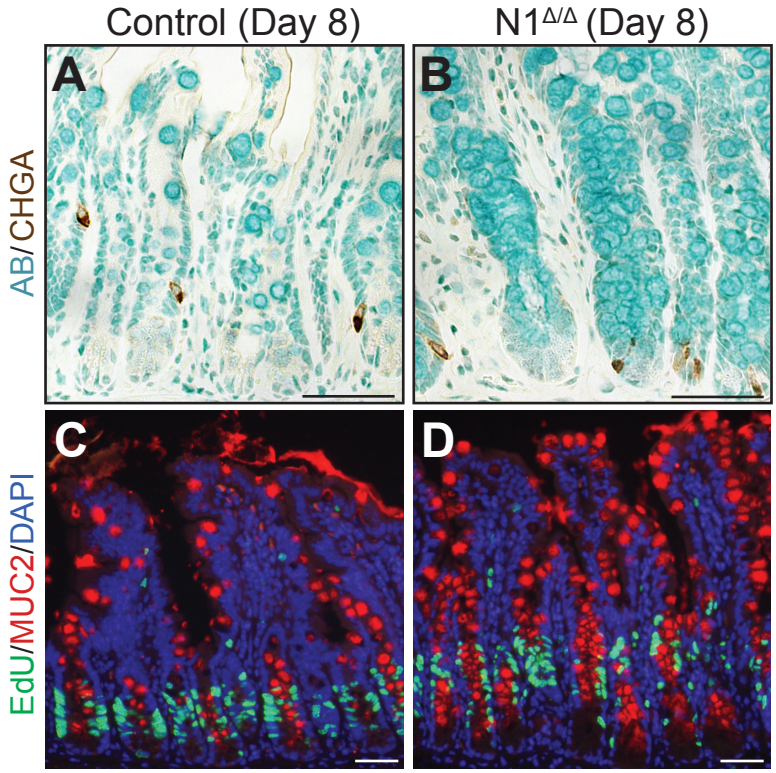
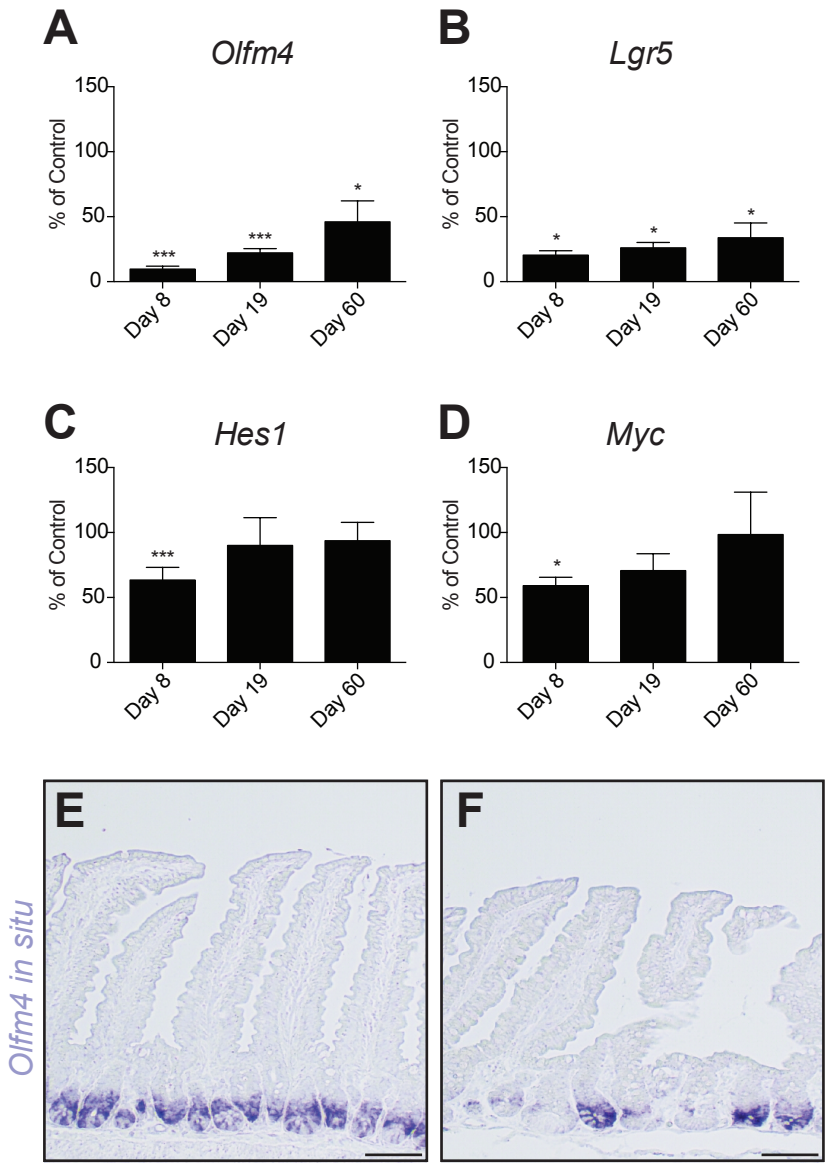
Supplementary Figure 4




A
Olfm4
% of Control
150
100
50
0
***
***
*
Day 8
Day 19
Day 60
B
Lgr5
% of Control
150
100
50
0
*
*
*
Day 8
Day 19
Day 60
C
Hes1
% of Control
150
100
50
0
***
Day 8
Day 19
Day 60
D
Myc
% of Control
150
100
50
0
*
Day 8
Day 19
Day 60
E
F
Olfm4 in situ

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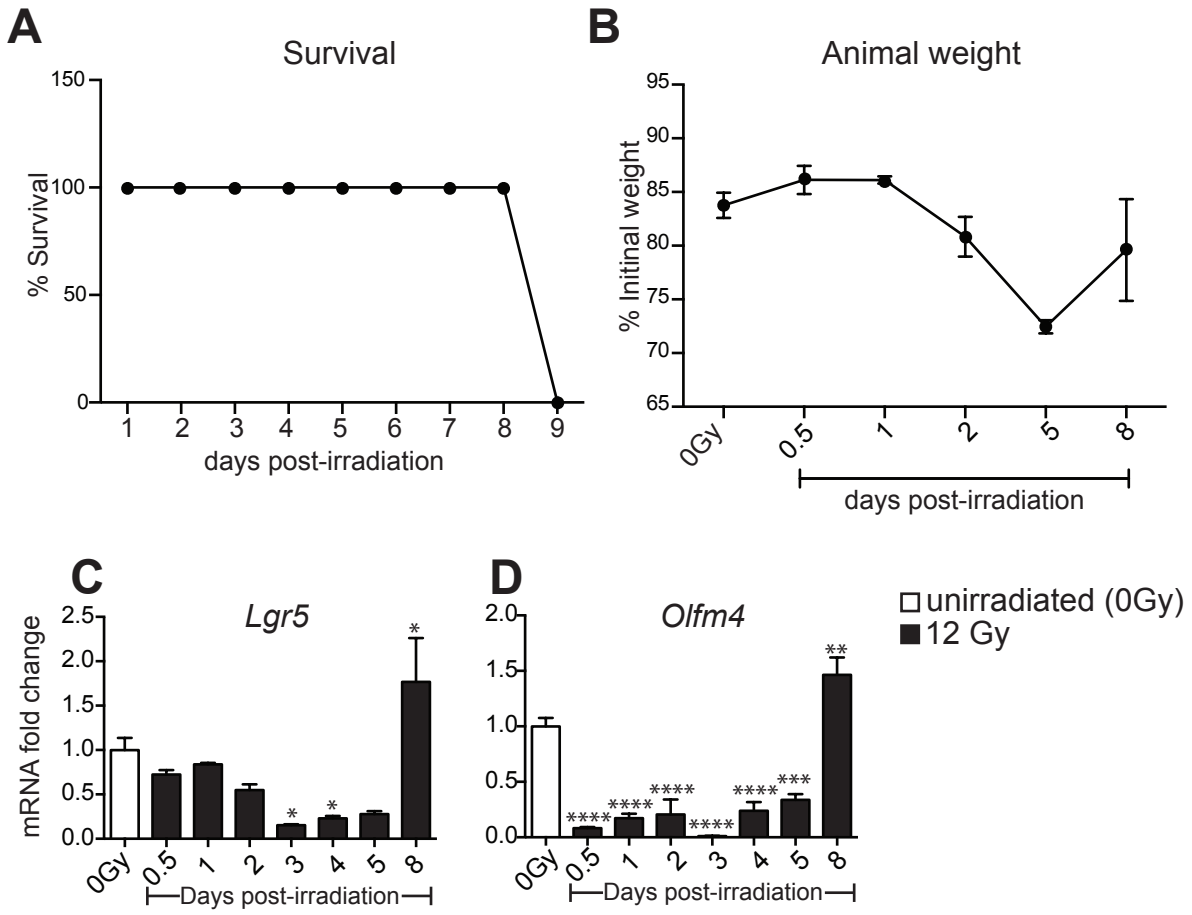
Supplementary Figure 6



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Supplementary Figure 7