Expanded View Figures

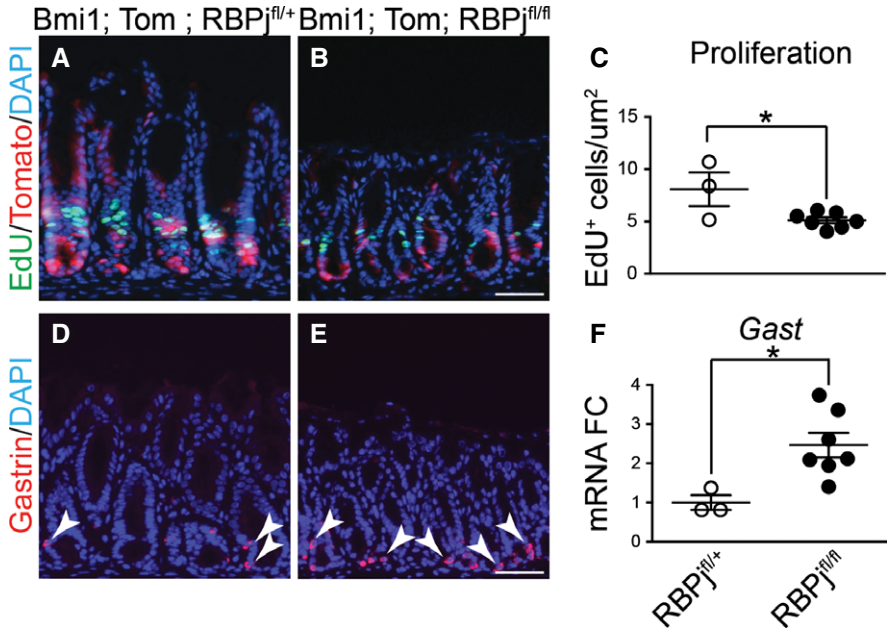

Figure EV1. Genetic Notch inhibition decreases cell proliferation and induces endocrine cell differentiation.

Bmi1; ROSA^{Tom}; RBPj$^{fl/+}$ (control; $n = 3$) and *Bmi1; ROSA^{Tom}; RBPj$^{fl/fl}$* (Notch-inhibited; $n = 7$) mice were treated with 100 mg/kg TX for 5 days and stomachs collected the next day.

A–C Proliferation was measured in antral paraffin sections via EdU detection after incorporation for 2 h (mean ± SEM). *$P = 0.023$ versus *RBPj$^{fl/+}$* using Student's *t*-test.

D, E Cellular differentiation was measured by immunostaining for gastrin-expressing endocrine cells. Scale bars: 50 μm.

F Gastrin gene expression was measured by qRT–PCR analysis of antral RNA (mean ± SEM; $n = 3$–7 mice). *$P = 0.02$ versus *RBPj$^{fl/+}$* using Student's *t*-test.

Data information: Arrowheads indicate gastrin-expressing endocrine cells.

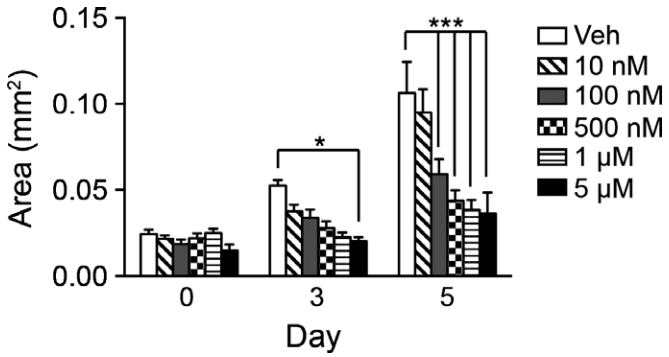

Figure EV2. Notch regulates antral organoid growth.

Antral organoid growth was measured in response to vehicle or increasing concentrations of DAPT (10 nM–5 μM) (mean ± SEM; $n = 16$–30 organoids per group). *$P < 0.05$, ***$P < 0.001$ versus vehicle using two-way ANOVA.

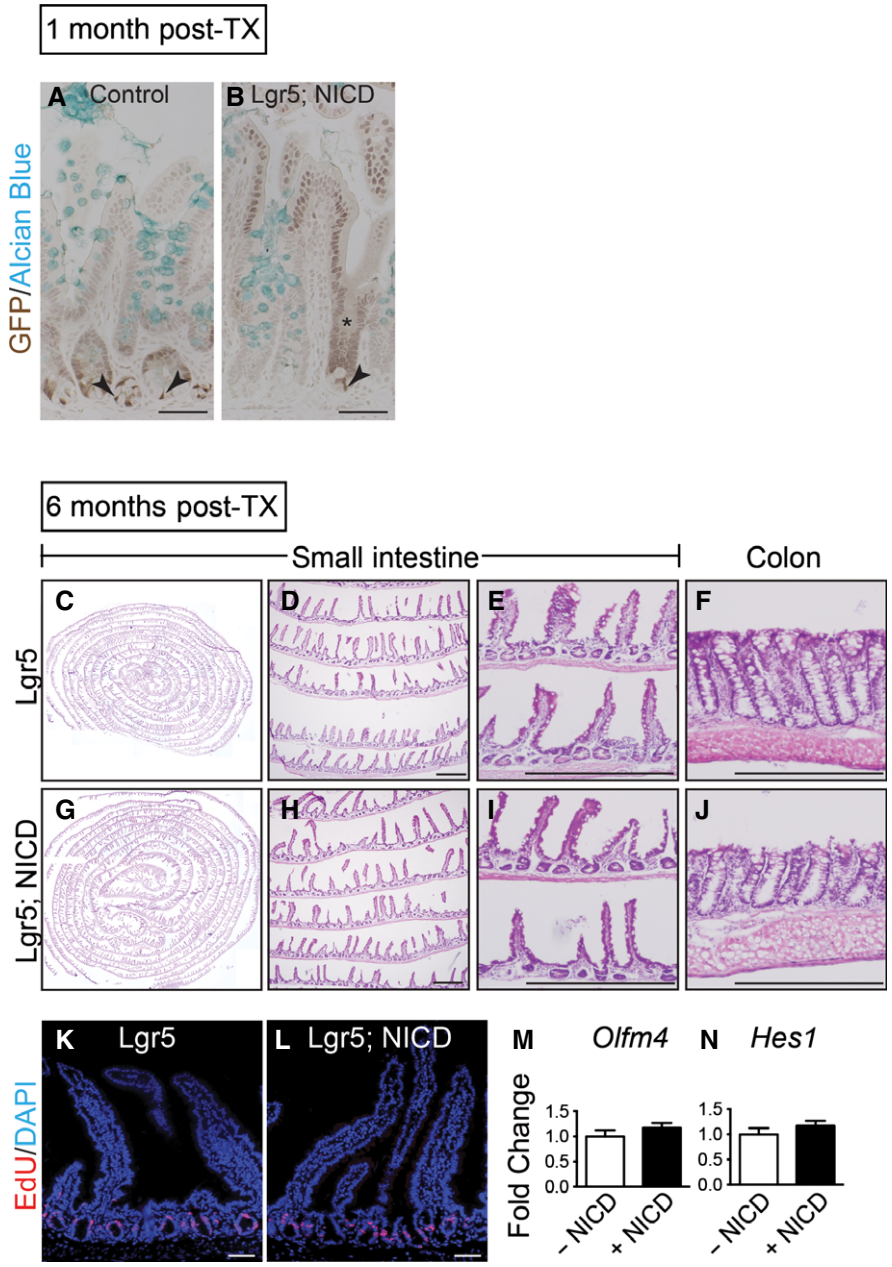

Figure EV3. Chronic Notch activation in LGR5$^+$ stem cells does not induce intestinal polyps.

A, B Goblet cells in NICD$^+$ ileal crypts were analyzed by co-staining for GFP (cytoplasmic, Lgr5; nuclear, NICD) and Alcian Blue in control (n = 3) or *Lgr5*; *ROSA*^{NICD} (n = 3) mice 1 month post-TX. Nuclear GFP was observed in a patchy pattern consistent with the known mosaic expression pattern of *Lgr5-EGFP-IRES-CreERT2*. Recombined crypts with NICD-GFP expression (asterisk) in *Lgr5*; *ROSA*^{NICD} mice exhibited a reduction in goblet cells, in accordance with the known effect of Notch signaling to block secretory cell differentiation. Arrowheads indicate LGR5-GFP$^+$ intestinal stem cells at the base of the crypts. Scale bars: 50 μm.

C–J No polyps were observed in intestine 6 months after TX induction of NICD. H&E analysis of *Lgr5* control (n = 7) (C–F) and *Lgr5*; *ROSA*^{NICD} (n = 7). (G–J) small intestine (C–E; G–I) and colon (F, J). Scale bars: 100 μm.

K, L Proliferation was measured by EdU incorporation and found to be unchanged in *Lgr5*; *ROSA*^{NICD} mice. Scale bars: 50 μm.

M, N qRT–PCR analysis of *Olfm4* and *Hes1* in *Lgr5* and *Lgr5*; *ROSA*^{NICD} intestine revealed no change to stem cell marker or Notch target gene expression. Data are expressed as mean ± SEM (n = 7 mice per group).

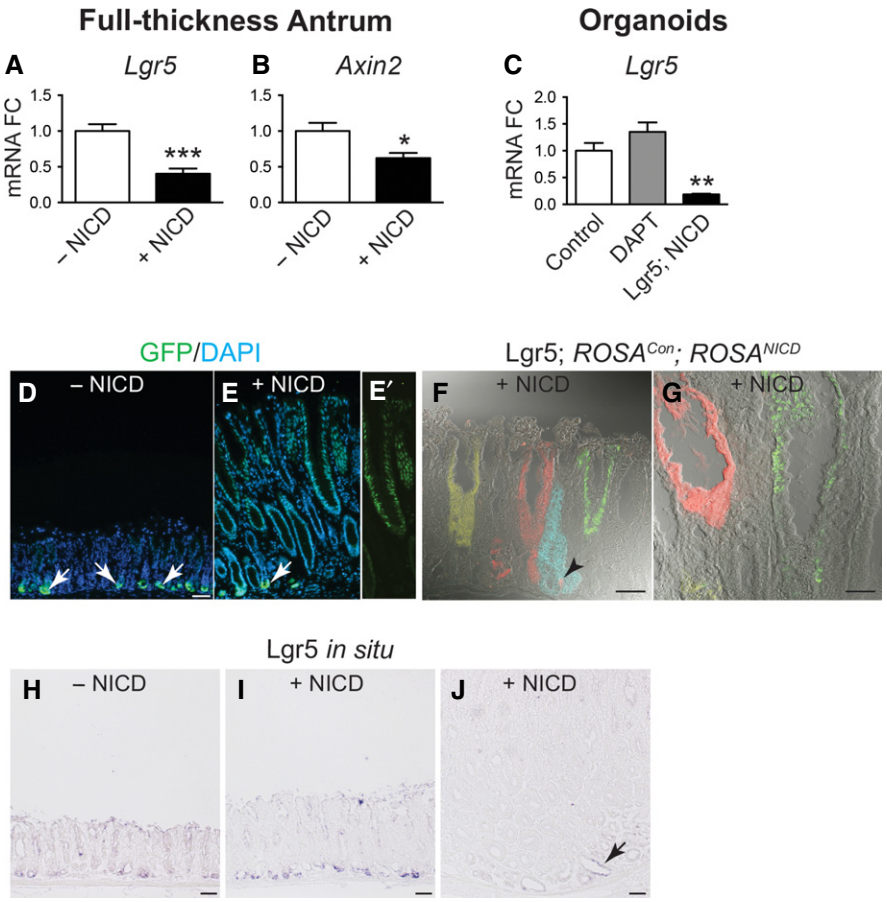

Figure EV4. Chronic Notch activation in LGR5$^+$ stem cells decreases Wnt signaling.

A–C qRT–PCR analysis for Wnt target genes Lgr5 (A) and Axin2 (B) in control (n = 7) and *Lgr5; ROSA^{NICD}* (n = 7) mice 1 year post-TX, or Lgr5 (C) in control, DAPT-treated and *Lgr5; ROSA^{NICD}* antral organoids (n = 8 per group) (mean ± SE). *P < 0.05 and ***P < 0.001 vs. −NICD using Student's t-test. **P < 0.01 vs. Control using one-way ANOVA.

D, E GFP immunostaining in control and *Lgr5; ROSA^{NICD}* mice 1 year post-TX. NICD activation showed lack of cytoplasmic Lgr5-GFP expression in hyperplastic polyps. Arrows indicate cytoplasmic Lgr5-GFP$^+$ stem cells at the gland base. (E′) Prominent nuclear GFP resulting from NICD activation in *Lgr5; ROSA^{NICD}* antral polyp. Scale bars: 50 μm.

F, G Lgr5 re-tracing with the ROSA^{Con} reporter in *Lgr5; ROSA^{NICD}* mice. Re-tracing was observed in non-polyp tissue (F; arrowhead), but not in a polyp region (G). Scale bars: 50 μm.

H–J Lgr5 *in situ* hybridization in control and *Lgr5; ROSA^{NICD}* mice 1 year post-TX. Lgr5 expression (J; arrow) was markedly reduced in polyp areas. Scale bars: 50 μm.

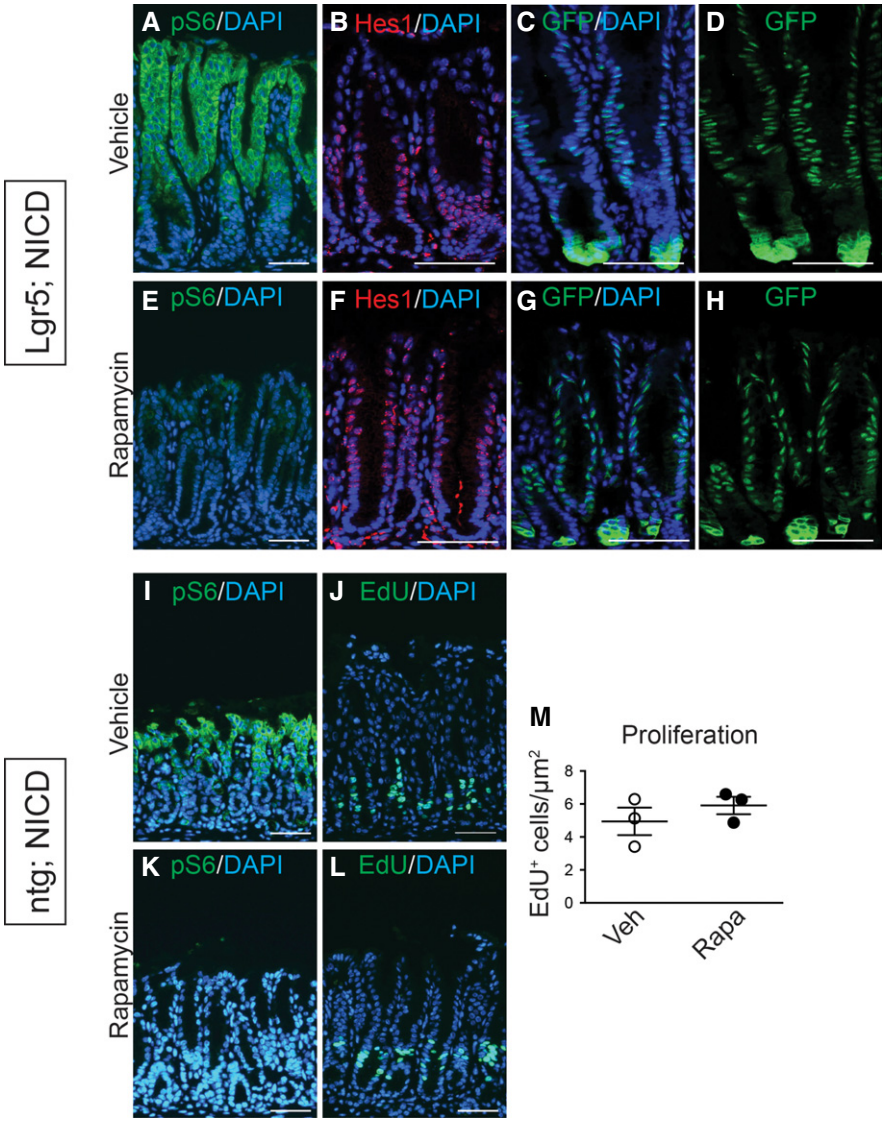

Figure EV5. Rapamycin treatment in *Lgr5; ROSA^{NICD}* and control mice.

A–L pS6 expression in vehicle (A, I) and rapamycin-treated (E, K) *Lgr5; ROSA^{NICD}* or control (*ntg; ROSA^{NICD}*) mice shows effective blockade of mTORC1. Immunostaining for Hes1 (B, F) and NICD-GFP (C, D, G, H) revealed no change to Notch signaling or expression of the *ROSA^{NICD}* transgene (nuclear GFP) during rapamycin treatment of *Lgr5; ROSA^{NICD}* mice. (J, L) Cellular proliferation was measured via EdU incorporation in vehicle or rapamycin-treated control (*ntg; ROSA^{NICD}*) mice, and (M) numbers of EdU$^+$ cells were quantified via morphometric analysis. Data are expressed as mean ± SEM (n = 4 mice per group). Scale bars: 50 µm.