APPENDIX MATERIAL FOR:

Notch Signaling Regulates Gastric Antral LGR5 Stem Cell Function

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Appendix Contents

Appendix Materials and Methods

Immunostaining

To analyze proliferating LGR5^{+} stem cells, 5 μm frozen tissue sections were immunostained for GFP for 2 hr at room temperature, washed in 0.01% Triton X-100 in PBS (TPBS), and immunostained for Ki67 overnight at 4 °C. Appropriate secondary antibodies (1:400, Invitrogen) were applied for 30 min. at room temperature the following day, and sections were mounted with ProLong Gold containing 4,6-diamidino-2-phenylindole dihydrochloride (DAPI, Invitrogen). For Ki67 immunostaining, antigen retrieval was performed with Trilogy solution (Cell Marque, Rocklin, CA). For all other antibodies, antigen retrieval was performed with Antigen Unmasking Solution (Vector Laboratories, Burlingame, CA). Imaging by digital microscopy was done as previously described (Lopez-Diaz et al. 2006). To analyze goblet cell differentiation in *Lgr5; ROSA^{NICD}* mice, paraffin sections were immunostained for GFP as listed above. Visualization of GFP and Alcian Blue staining of goblet cells was performed as previously described (Carulli et al. 2015).

In situ *hybridization*

Lgr5 probes were made (DIG labeling kit, Roche) and purified (Mini-Elute Gel Extraction kit, Qiagen) according to manufacturer's instructions. Frozen sections were air-dried for 2 hours at room temperature and fixed in 4% PFA for 10 min. *Lgr5* probe was diluted in hybridization buffer (1:500) and incubated overnight at 68 °C. Tissue sections were washed, incubated in blocking solution in buffer (0.1M Maleic acid pH7.5, 0.15M NaCl, 0.1% Tween 20 in sterile H_2O) for 1 hr, and treated with anti-DIG antibody (1:2000, Roche) overnight at 4 °C. Slides were washed, developed and mounted with ProLong Gold.

I. **Appendix Table S1**. List of mouse strains and strain abbreviations used.

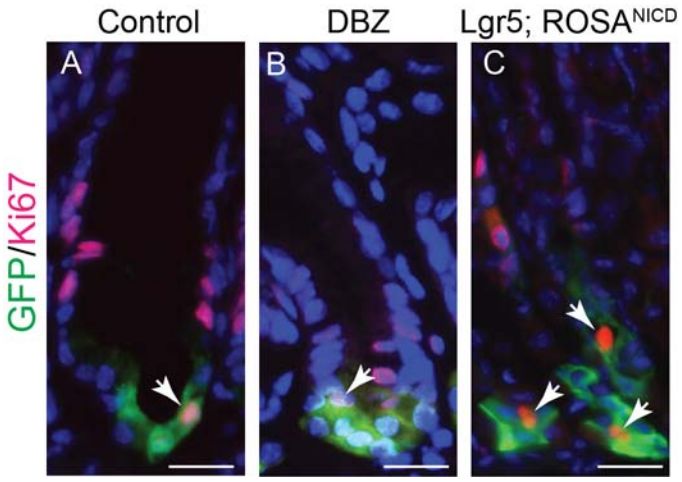
Mouse strains	**Abbreviation**
NIP1::CreERT2; ROSA-LSL-EYFP	*NIP1::CreERT2; ROSA^{EYFP}*
Lgr5-EGFP-IRES-CreERT2; ROSA^{NotchIC}	*Lgr5; ROSA^{NICD}*
Bmi1-CreER; ROSA-CAG-LSL-tdTomato-WPRE; floxed-RBPJκ	*Bmi1; ROSA^{Tom}; RBPj$^{fl/+}$ or Bmi1; ROSA^{Tom}; RBPj$^{fl/fl}$*
Lgr5-EGFP-IRES-CreERT2; ROSA-LSL-Confetti	*Lgr5; ROSA^{Con}*
Lgr5-EGFP-IRES-CreERT2; ROSA-LSL-Confetti; ROSA^{NotchIC}	*Lgr5; ROSA^{Con}; ROSA^{NICD}*

II. **Appendix Table S2**. List of primary antibodies used for immunostaining.

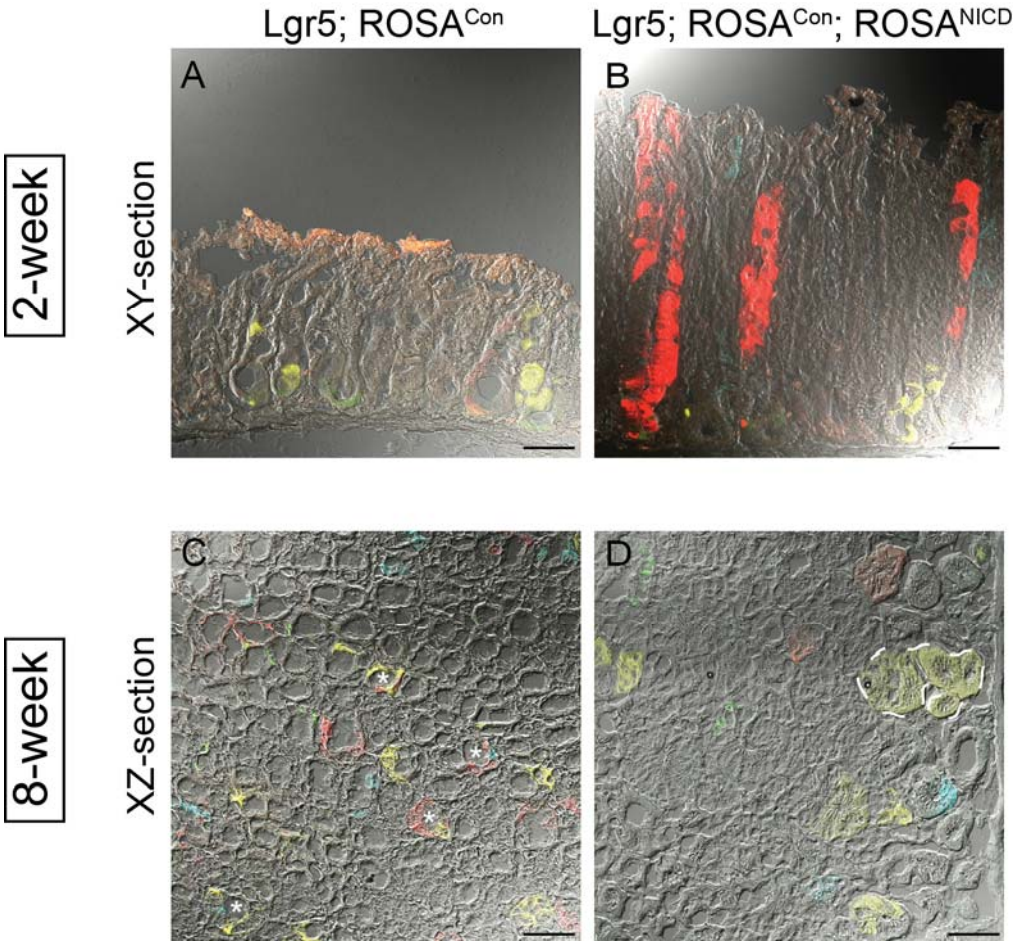
Antibody	Species	Source	Dilution	Reference
Anti-CD44	Rat	BD Pharmingen	1:50	(Qiao and Gumucio 2011)
Anti-E-cadherin	Rat	Invitrogen	1:1000	Figs 3 and 5
Anti-Gastrin	Rabbit	Dako	1:400	(Muller et al. 1987)
Anti-GFP	Chicken	Abcam	1:750	(Scopelliti et al. 2014)
Anti-GFP, 488 conjugate	Rabbit	Invitrogen	1:200	Figs EV4 and EV5
Anti-HES1	Rabbit	Abcam	1:50	(Dailey et al. 2013)
Anti-Ki67	Rabbit	Thermo Scientific	1:200	(VanDussen et al. 2012; Carulli et al. 2015)
Anti-Muc5AC	Mouse	Novacastra	1:75	(Keeley and Samuelson 2010)
Anti-pS6	Rabbit	Cell Signaling	1:300	Figs 9 and EV5
Anti-Sox9	Rabbit	Millipore	1:150	(VanDussen et al. 2012)
Anti-TFF2	Mouse	Abcam	1:100	(Keeley and Samuelson 2010)

III. Appendix Table S3. List of oligonucleotide sequences used for qRT-PCR.

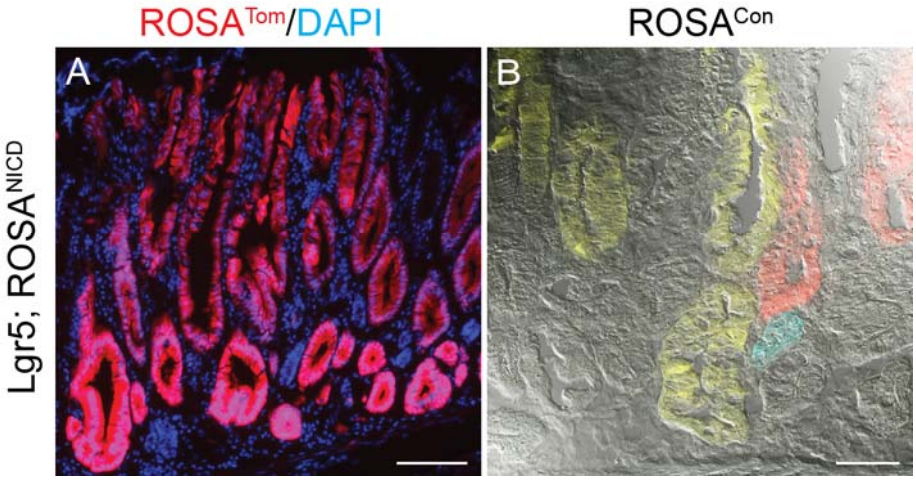
	Forward (5' to 3')	Reverse (5' to 3')	Amplicon Size (bp)
Axin2	AAGAGAAGCGACCCAGTCAATCC	GGTTCCACAGGCGTCATCTCC	278
FoxQ1	TGGCGGAGATCAACGAGTACC	CGCAGCACCTTGACGAAGC	121
Gastrin	GGACCAGGGACCAATGAGG	CCAAAGTCCATCCATCCGTAGG	173
Hes1	GCTCACTTCGGACTCCATGTG	GCTAGGGACTTTACGGGTAGCA	75
Hey1	GCCGACGAGACCGAATCAATAAC	CCCAAACTCCGATAGTCCATAGCC	199
Hey2	ATTACCCTGGGCACGCTACAAG	GGCAAGAGCATGGGCATCAAAG	281
HeyL	AGAGACCGCATCAACAGTAGCC	TCAGTGAGGCATTCCCGAAACC	215
Lgr5	CGAGCCTTACAGAGCCTGATACC	TTGCCGTCGTCTTTATTCCATTGG	143
Muc5ac	GCCGTGTCCAGGAGTCTAATACC	CAGCCTAGCCACCACCTTCAG	133
Muc6	CCGGCGATGCAGCATGACTGG	CGCACTCCTGGTACACTTGGTTGG	101
Neurog3	ACCCTATCCACTGCTGCTTGTC	CGGGAAAAGGTTGTTGTGTCTCTG	136
Olfm4	GCCACTTTCCAATTTCAC	GAGCCTCTTCTCATACAC	199
Spdef	GGACGGACGACTCTTCTGACAG	GCTCCTGATGCTGCCTTCTCC	166
Tff2	TGCTTTGATCTTGGATGCTG	GGAAAAGCAGCAGTTTCGAC	174



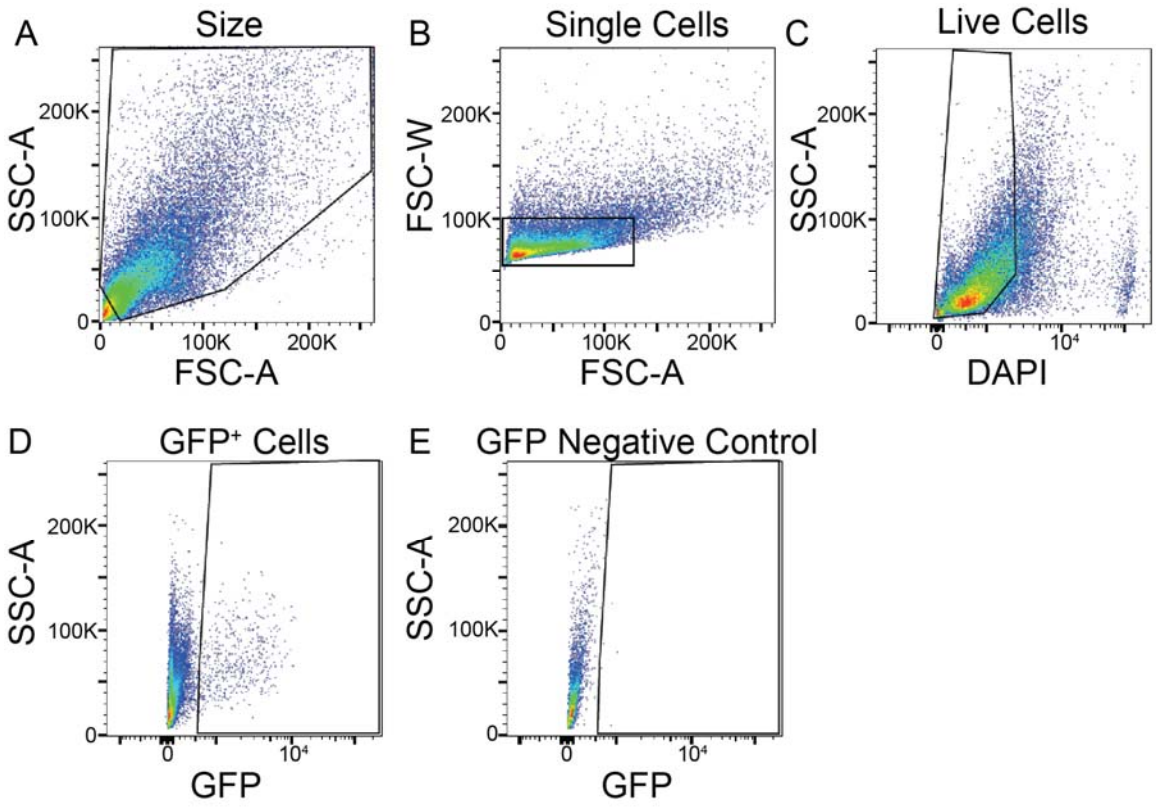
IV. Appendix Figure S1. Co-immunostaining for GFP/Ki67 in antral cryosections from (A) control, (B) DBZ or (C) *Lgr5; ROSA*^{NICD} 1 month post-TX. Arrowheads indicate GFP^{+}/Ki67^{+} cells. Scale bars: 20 μm.



V. Appendix Figure S2. (A&B) Analysis of *ROSA*^{Con} lineage tracing in *Lgr5; ROSA*^{Con} or *Lgr5; ROSA*^{Con}*; ROSA*^{NICD} antra 2-weeks post-TX. (C&D) Low-magnification view of XZ sections from *Lgr5; ROSA*^{Con} or *Lgr5; ROSA*^{Con}*; ROSA*^{NICD} antra 8-weeks post-TX. Asterisks in (C) indicate examples of polyclonal glands in control mice, whereas outlined glands in Notch-activated mice indicate a gland cluster (D). Scale bars: 50 μm.



VI. Appendix Figure S3. Lineage tracing in (A) *Lgr5; ROSA*^{Tom}; *ROSA*^{NICD} or (B) *Lgr5*; *ROSA*^{Con}*; ROSA*^{NICD} stomach 6 months post-TX. Scale bars: 50 μm.



VII. Appendix Figure S4. (A-D) Gating strategy for FACS sorting of single Lgr5-GFP$^+$ antral stem cells for organoid initiation. The GFP$^+$ gate was determined based on analysis of a non-transgenic negative control sample (E).

VIII. Appendix References

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