SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. *Identification of GATA3-expressing cell populations in the gastric mucosa.*

(A) Flow cytometric analysis of isolated fixed CD45-positive cells from wild-type C57BL/6J mice for lineage markers and GATA3. GATA3-positive cells are negative for lineage markers. **(B)** Immunofluorescence staining for GATA3 (red) and T cell marker CD3 (green) **(C)** GATA3 (red) and NK cell marker CD161 (green) **(D)** GATA3 (red) and macrophage marker F4/80 (green) **(E)** GATA3 (red) and mast cell marker MCC (green) in untreated and 3-day L635-treated wild-type C57BL6/J mice (n=6 per group) with nuclear counter stain Hoechst (blue) (scale bars = 100 µm). **(F)** Percent of GATA3-positive cells that are dual positive for each marker. Statistical significance determined by student's t-test (n=6 per group). N.S. for not significant $p \geq 0.05$. Error bars represent mean ± SD.

Supplemental Figure 2. *Enrichment of transcripts in ILC2 populations.*

A heatmap of quantitative PCR relative expression values for eight ILC2 marker genes, five genes involved in ILC2 function, and sixteen genes that were identified as being highly expressed in ILC2 in the single cell RNA-seq data. Three populations were sorted from the gastric mucosa of wild-type C57BL/6J mice by fluorescence activated cell sorting: $CD45^{-}Lin^{-}CD127^{-}ICOS^{-}$ Non-ILC2s (red), $CD45^{+}Lin^{-}CD127^{+}ICOS^{+}$ ILC2 Control (green), and $CD45^{+}Lin^{-}CD127^{+}ICOS^{+}$ ILC2 L635 (blue) from mice treated with 1 day of L635. Expression values normalized to Non-ILC2 population.

Supplemental Figure 3. *Validation of protein expression in populations of gastric ILC2s.*

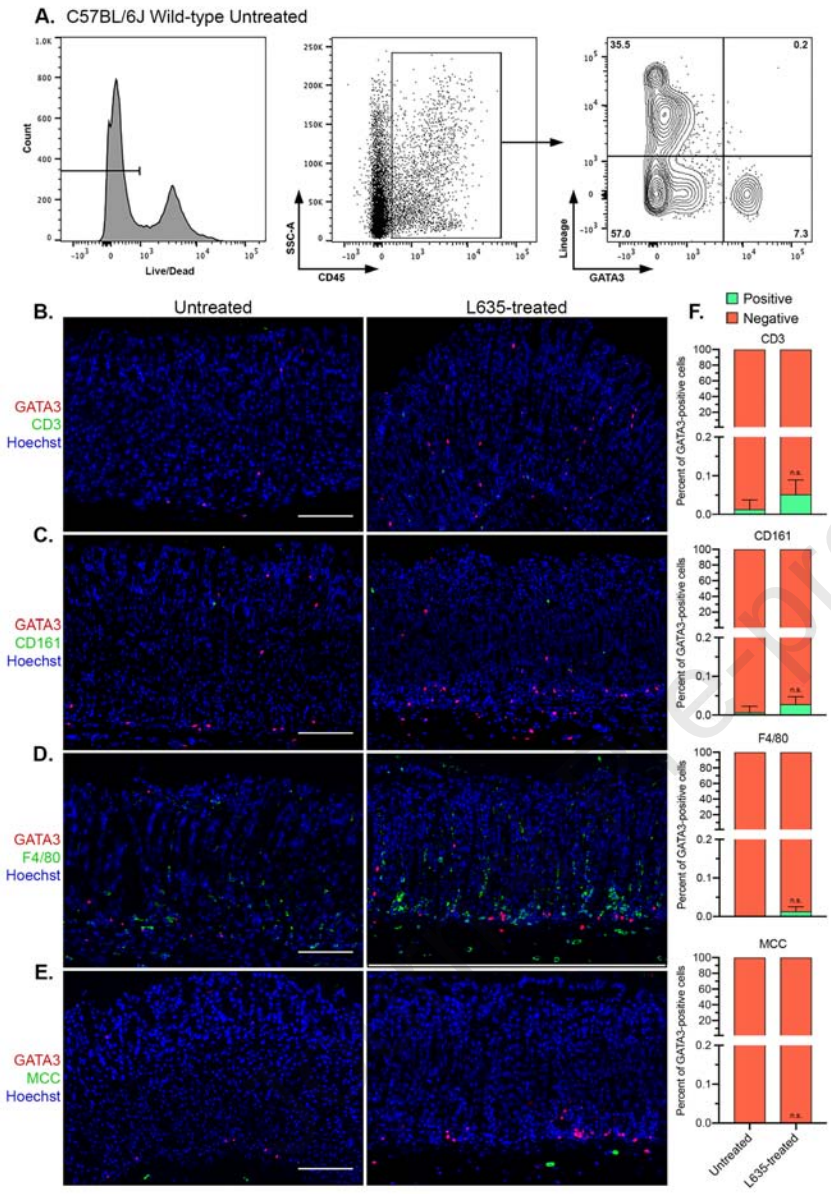
Immunostained sections from untreated mice (n=6) and 3-day L635-treated mice (n=6) **(A)** Representative images for GATA3 (red), ICOS, IL1RL1 (ST2), IL17RB, IL7R (CD127), IL2RB, PD1, DGAT2, and PCNA (green), with nuclear counter stain Hoechst (blue). Magnified *inset* of GATA3-positive ILC2s (*right*). Arrows indicate GATA3-positive ILC2s that are dual positive for each marker**. (B)** Quantification of the percent of GATA3-positive cells that are dual positive for each marker. Statistical significance determined by student's t-test. N.S. for not significant $p \geq 0.05$, ** for $p < 0.01$, and **** for $p < 0.0001$. Error bars represent mean ± SD.

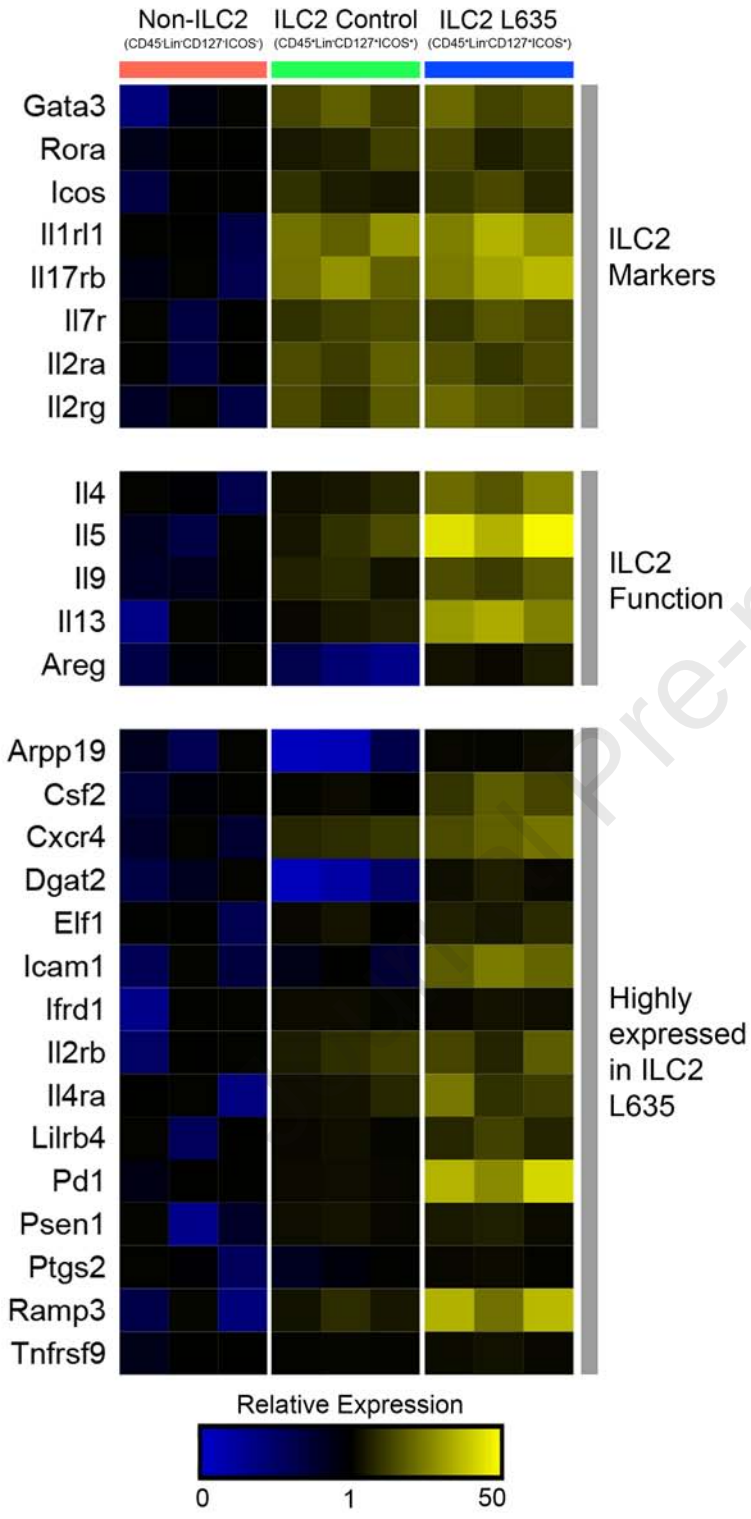
Supplemental Figure 4. *Expression of an immune checkpoint protein in ILC2-depleted tissues.*

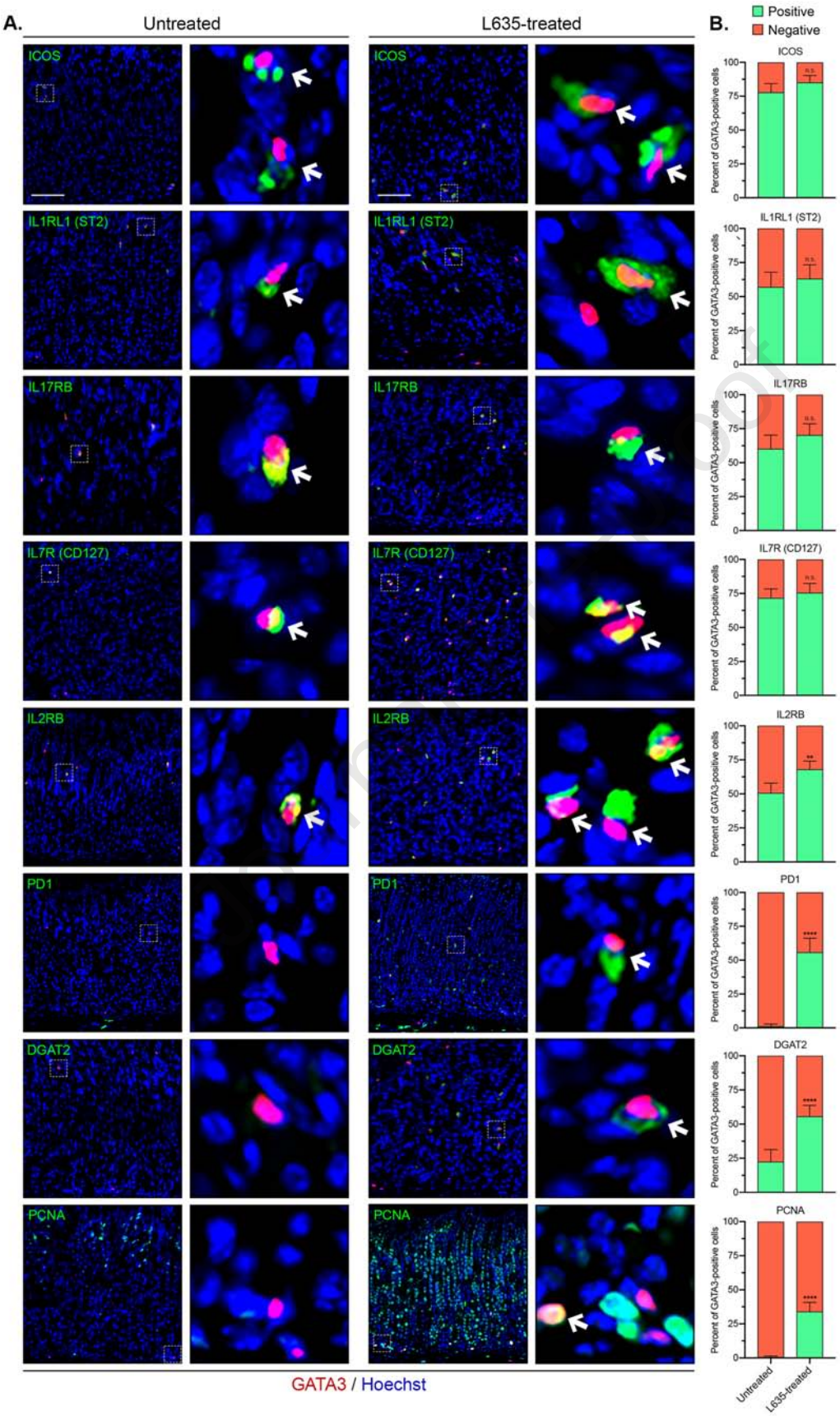
Immunostained sections from Rat IgG2b (n=4), Anti-CD90.2 (n=4), Rat IgG2b + L635 (n=3), and Anti-CD90.2 + L635 (n=4) treated C57BL/6J mice. Representative images of ILC2 marker GATA3 (red), immune checkpoint protein PD1 (green), with nuclear counter stain Hoechst (blue) (scale bars = 100 µm). Magnified *inset* of gland base (*bottom*). Arrow indicates a GATA3-positive iLC2 dual positive for PD1 in the Rat IgG2b + L635 sample.

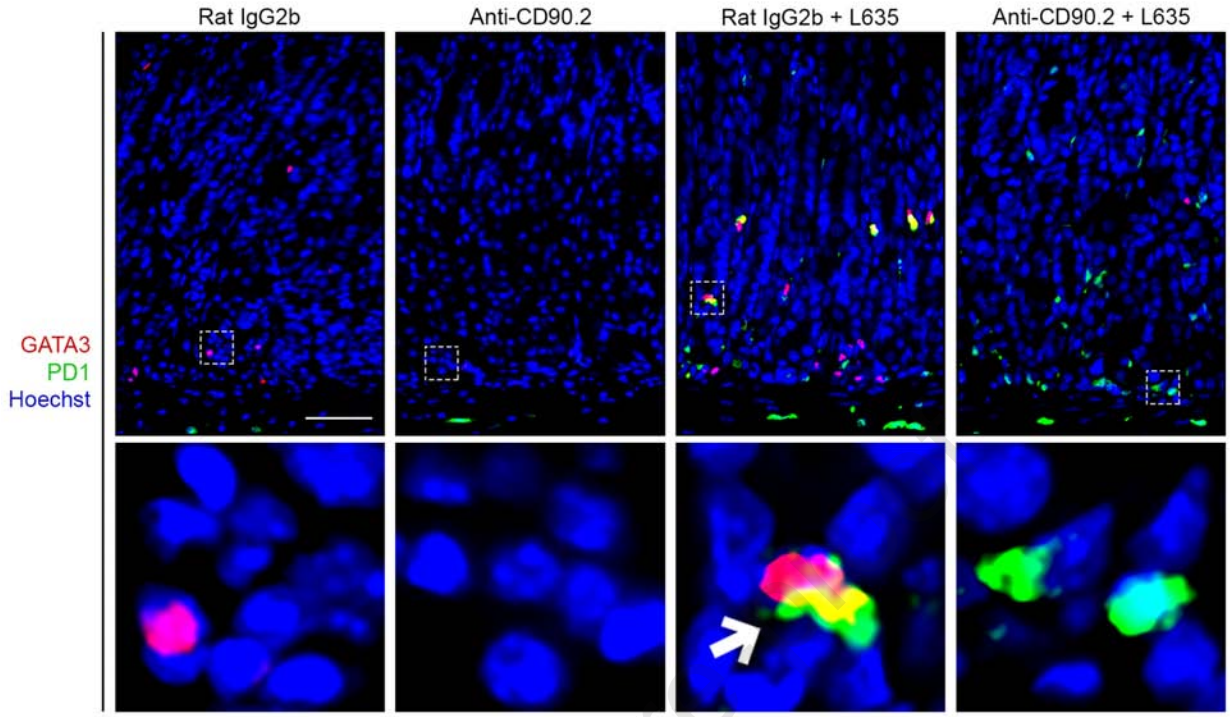
Supplemental Figure 5. *ILC2 depletion blocks development of L635-induced metaplasia in Rag1 knockout mice.*

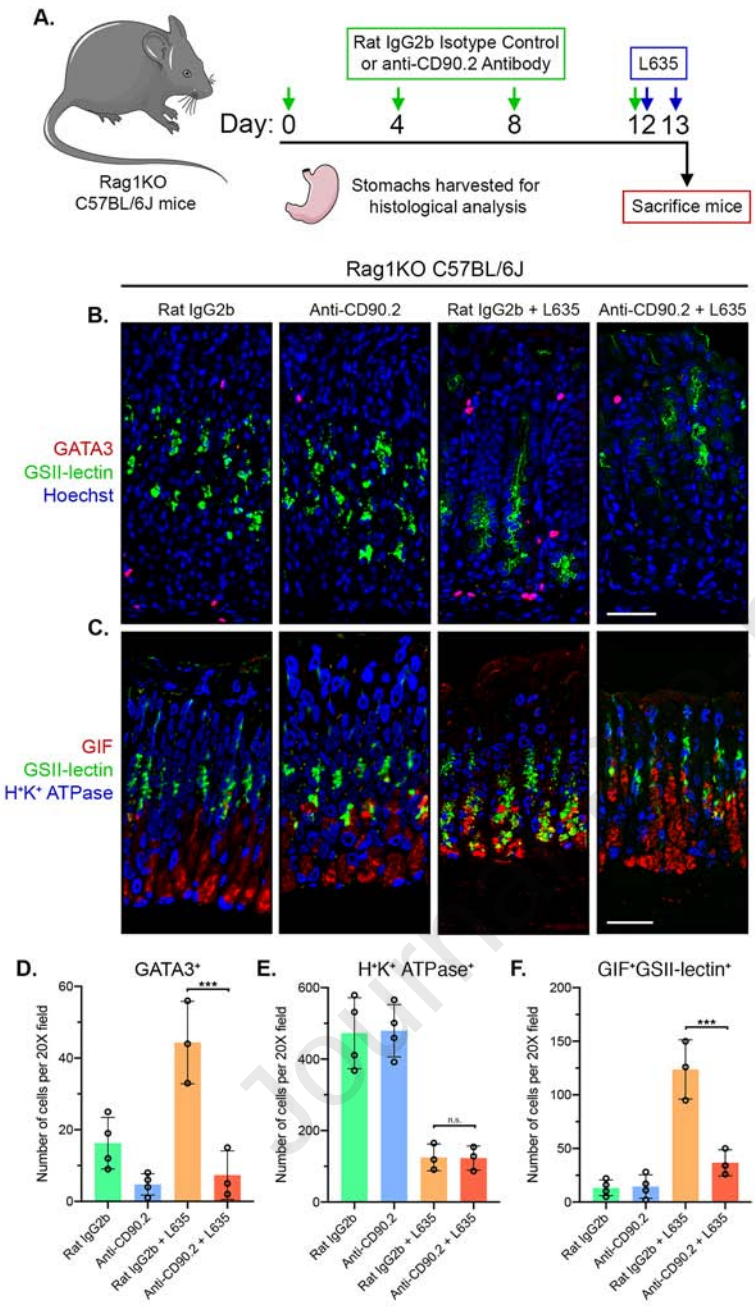
(A) Diagram of ILC2 depletion and drug treatments. Rat IgG2b isotype control antibody or Anti-CD90.2 antibody was administered intraperitoneally to Rag1KO C57BL/6J mice every fourth day for 12 days. Following the final antibody administration, mice were treated with the parietal cell toxic drug L635 by oral gavage daily for two days. Mice were sacrificed 24 hours after final dose of L635, and stomach tissue from Rat IgG2b only mice (n=4), Anti-CD90.2 only mice (n=4), Rat IgG2b + L635 mice (n=3), and Anti-CD90.2 + L635 mice (n=3) was harvested for histological analysis. **(B)** Immunofluorescence staining for GATA3 (red), mucin-6 containing granule marker GSII-lectin (green), with nuclear counter stain Hoechst (blue) (scale bars = 100 µm). **(C)** Immunofluorescence staining for zymogenic granule marker GIF (red), mucin-6 containing granule marker GSII-lectin (green), and parietal cell marker H+/K+-ATPase (blue) (scale bars = 100 µm). Quantification of **(D)** GATA3-positive ILC2, **(E)** H+K+ ATPase-positive parietal cells, and **(F)** GIF and GSII-lectin dual-positive (SPEM) cells per 20X objective field. Statistical significance determined by one-way ANOVA with Bonferroni's post-hoc multiple comparisons test. N.S. for not significant $p \geq 0.05$ and *** for $p < 0.001$. Error bars represent mean ± SD.


A. C57BL/6J Wild-type Untreated
Count
Live/Dead
SSC-A
CD45
Lineage
GATA3
35.5
0.2
57.0
7.3
B.
Untreated
L635-treated
GATA3
CD3
Hoechst
C.
GATA3
CD161
Hoechst
D.
GATA3
F4/80
Hoechst
E.
GATA3
MCC
Hoechst
F.
Positive
Negative
CD3
CD161
F4/80
MCC
Percent of GATA3-positive cells
n.s.
Untreated
L635-treated




A.
Untreated
L635-treated
ICOS
IL1RL1 (ST2)
IL17RB
IL7R (CD127)
IL2RB
PD1
DGAT2
PCNA
GATA3 / Hoechst
B.
Positive
Negative
Percent of GATA3-positive cells
N.S.
Untreated
L635-treated




A.
Rag1KO
C57BL/6J mice
Rat IgG2b Isotype Control
or anti-CD90.2 Antibody
L635
Day: 0
4
8
12 13
Stomachs harvested for
histological analysis
Sacrifice mice
Rag1KO C57BL/6J
B.
Rat IgG2b
Anti-CD90.2
Rat IgG2b + L635
Anti-CD90.2 + L635
GATA3
GSII-lectin
Hoechst
C.
GIF
GSII-lectin
H+K+ ATPase
D.
GATA3+
E.
H+K+ ATPase+
F.
GIF+GSII-lectin+
Number of cells per 20X field
***
n.s.
Rat IgG2b
Anti-CD90.2
Rat IgG2b + L635
Anti-CD90.2 + L635

Supplemental Methods:

Immunohistochemical Staining

Mouse stomachs were fixed in 4% PFA overnight at 4°C and were transferred into 70% ethanol for subsequent paraffin embedding. Five-micrometer sections were used for all immunohistochemistry studies. Deparaffinization, rehydration, and antigen retrieval were performed as previously described.[24] Five-micrometer tissue sections were blocked in Dako Protein Block Serum-Free at room temperature for 1.5 hours. For mouse primary antibodies, Mouse on Mouse (M.O.M.) blocking reagent was added to slides for 20 minutes at room temperature. Primary antibodies were added overnight at 4°C in Dako Antibody Diluent with Background Reducing Components. Fluorescent donkey secondary antibodies were added in Dako Antibody Diluent at room temperature for one hour or HRP-conjugated secondary antibodies were added for 15 minutes at room temperature. DAB chromogen was added for detection of HRP-conjugated secondary antibodies. The Zeiss Axio Imager M2 microscope with Axiovision digital imaging system or the Leica Aperio Versa 200 Fluorescent Slide Scanner in the Vanderbilt Digital Histology Shared Resource was used to image sections.

Cell Culture

Freshly sorted gastric ILC2s were cultured in RPMI culture media supplemented with 10% FBS, 100 U/ml penicillin/streptomycin, 10 mM HEPES buffer solution, 1xMEM nonessential amino acids, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol and 50

μg/ml gentamycin sulfate in 96-well round bottom plates. Cells were cultured with IL-2 (Peprotech 212-12) or IL-33 (210-33) at 10 ng/ml for 24 hr. IL-13 cytokine levels were measured by ELISA (ThermoFisher BMS6015) and detected on Biotek Synergy 4 instrument.

Quantitative Real-Time PCR

Total RNA from the stomach (oxyntic region) was extracted from paraformaldehyde-fixed paraffin-embedded tissue of three to four mice per experimental group to examine the expression of mRNA transcripts. Five-micrometer sections were taken for H&E stain to identify oxyntic region in tissue block. A 2-mm biopsy punch was then used to extract tissue. The standard Qiagen RNeasy FFPE Kit (73504) protocol was used for purification of total RNA. Alternatively, Qiagen RNeasy Plus Mini Kit was used for purification of total RNA from sorted cells. ThermoFisher High-Capacity cDNA Reverse Transcription Kit (4368814) was used for complementary DNA synthesis. Quantitative real-time polymerase chain reaction was performed with Biorad SsoAdvanced Universal SYBR Green Super Mix (172-5270) and specific primers on the Biorad CFX96 Touch Real-Time PCR Detection System. The expression of mRNA transcripts was normalized to *TBP* expression and displayed as relative expression levels ($2^{\Delta Cq}$).

Antibodies		
CD127 (1:100)	eBioscience	25-1273-82
CD161 (1:500)	Novus Biologicals	NB100-77528SS
CD163 (1:100)	NeoMarkers	163C01
CD3 (1:100)	eBioscience	12-0032-82
CD3 (1:500)	Santa Crus	sc-1127
CD44v9 (1:10,000)	Cosmo Bio USA	CAC-LKG-M001
CD45 (1:200)	Tonbo Biosciences	80-0451
CD90 (1:200)	eBioscience	11-0903-81
DCLK1 (1:2,000)	Abcam	ab109029
DGAT2 (1:500)	Abcam	ab96094
F4/80 (1:100)	Life Technologies	MF48000
FcεRI (1:100)	eBioscience	13-5898-81
GATA3 (1:500)	Abcam	ab199428
GATA3 (1:20)	eBioscience	12-9966-42
GATA3 (1:500)	Proteintech	66400-1-Ig
GIF (1:2,000)	A gift from Dr. David Alpers	N/A
GSII-lectin (1:2,000)	Invitrogen	L32451
H^+K^+ATPase (1:10,000)	A gift from Dr. Adam Smolka	N/A
ICOS (1:50)	eBioscience	25-1273-82
ICOS (1:500)	LSBio	LS-B11916
IL1RL1 (1:500)	Novus Biologicals	NBP1-85252
IL17RB (1:200)	Bioss	bs-2610R

IL2RB (1:500)	Proteintech	13602-1-AP
IL7R (1:1,000)	LSBio	LS-B2831
KI67 (1:1,000)	Cell Signaling Technology	#9129
Lineage Cocktail (1:12.5)	Miltenyi	120-092-613
MBP (1:100)	Mayo Clinic	Clone MR-14.7
MCC (1:500)	Abcam	ab111239
MIST1 (1:1,000)	A gift from Dr. Jason Mills	N/A
PCNA (1:15,000)	Cell Signaling Technology	#2586
PD1 (1:500)	Proteintech	18106-1-AP
tdTomato (1:500)	LSBio	LS-C340696-600
TFF2 (1:500)	A gift from Dr. Nicholas Wright	N/A
UEA1-lectin (1:2,000)	Sigma	L9006

Supplemental Table 1. *Catalog of antibodies.*

Oligonucleotides
mAreg primers F 5' GCAGATACATCGAGAACCTGGAG 3' R 5' CCTTGTCATCCTCGCTGTGAGT 3'
mArpp19 primers F 5' CTCACTTGGGACAAAAGCCTGG 3' R 5' TGCTTTTGCCATGTTGTAGTCCC 3'
mCsf2 primers F 5' AACCTCCTGGATGACATGCCTG 3' R 5' AAATTGCCCCGTAGACCCTGCT 3'
mCxcr4 primers F 5' GACTGGCATAGTCGGCAATGGA 3' R 5' CAAAGAGGAGGTCAGCCACTGA 3'
mDgat2 primers F 5' CTGTGCTCTACTTCACCTGGCT 3' R 5' CTGGATGGGAAAGTAGTCTCGG 3'
mElf1 primers F 5' ACCCAGCTCTTCCGAACTGTTC 3' R 5' AGGAGACACCACTACTGGAACC 3'
mGata3 primers F 5' TTTACCCTCCGGCTTCATCCTCCT 3' R 5' TGCACCTGATACTTGAGGCACTCT 3'
mIcos primers F 5' GCAGCTTTCGTTGTGGTACTCC 3' R 5' TGTGTTGACTGCCGCCATGAAC 3'
mIcam1 primers F 5' AAACCAGACCCTGGAACTGCAC 3' R 5' GCCTGGCATTTCAGAGTCTGCT 3'
mIfrd1 primers F 5' CTGGCGAATCTTTGGCACTTCTG 3' R 5' ACCGCTGCTTTCTCTTGTCCAC 3'
mIl1rl1 primers F 5' GGATTGAGGTTGCTCTGTTCTGG 3' R 5' TCGGGCAGAGTGTGGTGAACAA 3'
mIl17rb primers F 5' CCATCCCTCCAGATGACAAC 3' R 5' TGCTCCTTCCTTGCCTCCAAGTTA 3'
mIl2ra primers F 5' GCGTTGCTTAGGAAACTCCTGG 3' R 5' GCATAGACTGTGTTGGCTTCTGC 3'
mIl2rb primers F 5' CTCAAGTGCCACATCCCAGATC 3' R 5' AGCACTTCCAGCGGAGAGATCT 3'
mIl2rg primers F 5' GGAGCAACAGAGATCGAAGCTG 3' R 5' CCACAGATTGGGTTATAGCGGC 3'
mIl4 primers F 5' ATCATCGGCATTTTGAACGAGGTC 3' R 5' ACCTTGGAAGCCCTACAGACGA 3'
mIl4ra primers F 5' ACCAGATGGAACTGTGGGCTGA 3' R 5' AGCAGCCATTCGTCGGACACAT 3'
mIl5 primers F 5' GATGAGGCTTCCTGTCCCTACT 3' R 5' TGACAGGTTTTGGAATAGCATTTCC 3'

mIl7r primers F 5' CACAGCCAGTTGGAAGTGGATG 3' R 5' GGCATTTCACTCGTAAAAGAGCC 3'
mIl9 primers F 5' TCCACCGTCAAAATGCAGCTGC 3' R 5' CCGATGGAAAACAGGCAAGAGTC 3'
mIl13 primers F 5' AACGGCAGCATGGTATGGAGTG 3' R 5' TGGGTCCTGTAGATGGCATTGC 3'
mLilrb4 primers F 5' CTGGATGCTGTTACTCCCAACC 3' R 5' TGGGTGTAGAGGACTGGTCCTT 3'
mPd1 primers F 5' CGGTTTCAAGGCATGGTCATTGG 3' R 5' TCAGAGTGTCGTCCTTGCTTCC 3'
mPsen1 primers F 5' GAGACTGGAACACAACCATAGCC 3' R 5' AGAACACGAGCCCGAAGGTGAT 3'
mPtgs2 primers F 5' GCGACATACTCAAGCAGGAGCA 3' R 5' AGTGGTAACCGCTCAGGTGTTG 3'
mRamp3 primers F 5' AGAAGGTGGCTGTCTGGAAGTG 3' R 5' GCCAGTAGCAGCCCATGATGTT 3'
mRora primers F 5' CAGAGCAATGCCACCTACTCCT 3' R 5' CTGCTTCTTGGACATCCGACCA 3'
mTnfrsf9 primers F 5' CCAAGTACCTTCTCCAGCATAGG 3' R 5' GCGTTGTGGGTAGAGGAGCAAA 3'

Supplemental Table 2. *Catalog of qPCR primer sequences.*