Supplementary Information

Supplementary Materials and Methods

Generation of SDF-1/CXCL12 transgenic mice

We first generated an H/K-ATPaseβ/SDF-1 transgene that targeted constitutively secreted murine SDF-1, specifically to parietal cells of the stomach. To generate SDF-1 transgenic mice, we amplified ORF of human SDF-1/CXCL12 (BC039893) using the plasmid isolated from clone# 5729604 (Open Biosystems, AL, USA), and then introduced it in frame to create a fused SDF-1-V5-Hisx6 protein into pEFV5x6HisC (Invitrogen, CA, USA). We used this construct to amplify a shorter version of fused protein SDF-V5 (lacking the his tag but flanked with BamHI sites). The BamHI sites were used to introduce SDF-V5 into the construct with H^+K^+-ATPaseβ promoter. The final construct was sequenced before injection to exclude mutations (data not shown). Potential founders were screened using PCR 3 different primer pairs. Two lines exhibiting high levels of SDF-1-expression (Line 3 and 6) were selected and, initially on a mixed C57BL/6 x CBA background, were later backcrossed to C57BL/6J mice. There were no histological alterations in other organs examined, including intestine, liver, lung, and kidney in SDF-Tg mice (data not shown). Line 6 SDF-Tg mice were crossed to HK-IL-1b mice to generate SDF; IL-1b double transgenic

mice (C57BL/6J background). To generate SDF/TFF2-/- mice, Line 6 SDF-Tg mice were crossed to TFF2 knockout mice (C57BL/6J). The HK-IL-1beta transgenic mice and TFF2 knockout mice were described previously (8, 15). UBC-GFP mice were purchased from Jackson laboratory and used for bone marrow transplantation as described previously (16). All animal studies were performed in Institutional Animal Care and Use Committee (IACUC)-approved facilities at Columbia University (protocol#AAAA-8153).

Recombinant SDF-1 and chemotaxis assay

SDF-1V5x6His fusion gene was cloned into the pET-21D vector and expressed in *E. coli* cells. Met-SDF1-V5x6His containing inclusion bodies were isolated from bacterial cultures, solubilized in guanidinium chloride (6M) and purified using affinity Talon columns (Clontech). Purified protein was refolded by gradual dialysis against 8M, 6M, 4M, 2M, and 0M urea in 100mM Tris-HCl (pH7.5). The final product was tested for purity (>95%) and degree of refolding (>50%) by electrophoresis in reducing and non-reducing conditions (data not shown). For the migration assay, we used splenocytes or MSCs isolated from the bone marrow of wild type mice. Cells were washed, and $3x10^6$ cells/ml were suspended in RPMI 1640 medium with 1% bovine serum albumin.

100μL aliquots of cell suspension were applied to the upper chambers 24-well Transwell plates (Boyden chamber, Costar 3422, 5μm-diameter pore size). 600μL of RPMI 1640 medium supplemented with the indicated concentrations of recombinant SDF-1 or the gastric mixture from either wild-type and SDF-Tg mice were loaded into lower chambers. Mesencult medium was used as an experimental control. Cells were allowed to migrate for 3h at 37°C. After incubation, the porous inserts were removed carefully, and the viable cells were counted using a hemocytometer. The experiments were performed three times in duplicate. The results were expressed as the percentage of cells that migrated to the bottom chamber. Cells were fixed and stained with crystal violet, and positive staining area was calculated by Image J (NCI.).

Bacterial Culture

The *H. felis* strain (ATCC 49179) used in this study has been previously described (17). The organism was grown for 48 hours at 37°C under microaerobic conditions on 5% sheep blood agar supplemented with antibiotics. Bacteria were harvested and aliquoted at a titer of 10^{10} organisms/mL in Trypticase Soy broth with 10% glycerol, and bacterial suspension was stored at -70°C. Before use, aliquots were

thawed, analyzed for motility, and cultured for evidence of aerobic or anaerobic microbial contamination.

Murine gastric cancer model (Chronic H. felis infection and IL-1b transgenic mice)

Mice from each group (SDF-Tg and littermate WT controls) were inoculated with *H. felis* or sterile broth as a control as described previously (18). In brief, three inoculas (0.2mL of *H. felis*, 10^{10} colony-forming units/mL) were delivered every other day by oral gavage using a sterile gavage needle. Animals (n=8 to 10 per each group) were sacrificed 3, 6, 12, and 18 months post-infection by CO_2 asphyxiation and necropsied. In order to inhibit SDF/CXCR4 pathway, AMD3100 was administered 5mg/kg every day and mice were sacrificed at day15. SDF-Tg mice were crossed to HK-IL-1b mice to generate SDF/IL-1b double transgenic mice. Blood was obtained in CO_2-anesthetized mice by cardiac puncture, and sera were collected and frozen at -80°C. At necropsy, stomachs were removed *en bloc* and opened. The animals were examined for gross changes, and the tissue specimens collected consisted of the gastric mucosa beginning at the gastroesophageal junction through the gastroduodenal junction. Stomach tissue specimens were fixed in neutral buffered 10% formalin, processed by standard methods, embedded in paraffin, sectioned at 5μm, and stained with H&E.

Infection status was confirmed by microscopy, and by qRT-PCR assays of gastric corpus tissue at necropsy as described previously (18). Additional sections were cut for immunohistochemistry, and additional samples were processed for qRT-PCR.

Histological Evaluation and Immunohistochemical Studies

Inflammation, atrophy, hyperplasia, and dysplasia in the gastric corpus were each scored on an ordinal scale from 0 to 4 in increments of 0.5 as previously described (19) by a single veterinary pathologist (S.M) blinded to genotyping groups. Three sections through the squamocolumnar junction at the lesser curvature were evaluated for each mouse, and the worst score was recorded for each section evaluated. Cell proliferation was assessed by Ki67 immunohistochemistry. Ki67 positive cells were scored per high power field (20 corpus glands). All immunohistochemistry was performed with Vectastain ABC Kit (Vector Laboratories, Inc, Burlingame, CA, USA) according to the manufacturer's instructions. Antigen retrieval was achieved by boiling sections for 15 min in 0.01M citrate buffer, pH 6.0 (or 0.8M urea for F4/80 staining). We used alpha-smooth muscle actin antibody to stain myofibroblasts (1:500, Abcam, Cambridge, USA), F4/80 (1:100, Abcam, Cambridge, USA) to stain mature macrophages, CD11b (1:100, BD Bioscience, USA) to stain myeloid cells, p-Akt (1:100,

cell signaling technology, USA) to stain p-Akt, p-Erk (1:400, cell signaling technology, USA) to stain p-ERK, CXCR4 (1:200, Acris Antibodies, Germany) to stain the CXCR4 receptor. Binding of the primary Ab was detected with corresponding HRP-conjugated anti-rabbit/mouse IgG Abs (1/500 dilution; GE Healthcare, Piscataway, NJ) followed by visualization with 3, 3'-diaminobenzidine (DAB) and counterstaining with hematoxylin.

Single-Cell Preparation and FACS Analysis

For single-cell suspension preparation from stomach tissues, the mucosa of whole stomach was gently scraped free from the serosa; minced; digested for 1 hr in 1 mM DTT, 1 mM EDTA, 5% FBS in PBS at 37 degree; filtered through a 40 mm nylon mesh strainer; and resuspended in Dulbecco's PBS (D-PBS). For FACS analysis, single-cell suspensions were stained with fluorescence-labeled FITC-CD45, PE-CD3, APC-CD19, PE-Cy7-CD8, Alexa 700-CD4, APC-CD11b, or PerCP-Ly-6G antibodies (BD Pharmingen) and detected using a LSRII flow cytometer (BD Biosciences). Data were analyzed using FlowJo7 software (Tree Star).

Myeloperoxidase (MPO) activity assay

Fresh gastric tissue was isolated and rinsed with chilled PBS, and homogenized in 0.5% hexadecyltrimethylammonium bromide (HTAB) solution (1 ml per 50 mg of tissue). The homogenate was centrifuged at 11,000g for 15 min at 4 °C and supernatant was stored at -80 °C until further processing. The MPO activity was determined using a myeloperoxidase substrate solution as described previously (20).

qRT- PCR, and ELISA of Proinflammatory CC-Chemokines, and Cytokine

Total RNAs were extracted from a sample of whole stomach from each animal with TRIzol reagent (Invitrogen, Carlsbad, CA), and 4 μg of total RNA were used for first-strand complementary DNA synthesis using the Superscript III cDNA Amplification System (Invitrogen) following the manufacturer's instructions. qRT-PCR was performed as previously described using qRT-PCR primers for each gene. All primers were designed using Lasergene® version 5.0 software (DNASTAR Inc, Madison, WI). Sequences of qRT-PCR primers are available upon request. Results were expressed by the copy number of each gene versus 10^4 GAPDH. Cytokine levels were measured by ELISA as manufacturer's instructions.

Isolation of primary macrophages and cell proliferation assay (WST-8)

Isolation of Macrophages were generated as described (21). To isolate macrophages, mice were i.p. injected with 1 ml of 4% thioglycollate (DIFCO) and peritoneal cells were flushed out 4 days later. The anti-apoptotic effect of SDF-1 on cells was assessed by WST-8 cell proliferation assay, using manufacturer's instructions (DOJIN, Kumamoto, Japan). In brief, the WST-8 assay is a colorimetric method in which the intensity of the dye is proportional to the number of the viable cells. 100 µl of cell suspension of macrophages was seeded into a 96-well plate at a density of $2.5x10^3$ cells/well. After 2hr incubation, medium was changed to 100 µl RPMI without FBS, then either gastric mixture extracted from mice stomach or recombinant SDF-1 was added (final concentration at 50nM). After incubation for 48 hrs at 37°C, 10 µl of Cell Counting Kit-8 (CCK-8) solution was added to each well, and the plate was incubated for further 3 hrs at 37°C. Then the optical density was measured at 450nm using MULTISKAN EX spectrophotometer (MTX Lab Systems, Inc., VA, USA). Each experiment was performed using six replicate wells for each stimulant exposure.

Statistical Analysis

Results are expressed as mean ± SE unless otherwise stated. Student *t* test was used to evaluate statistical significance. Values of $p<0.05$ were considered statistically significant.

Supplementary Figure Legends

Supplementary Figure 1 Derivation of Gastric Epithelial Cell SDF-1 transgenic Mice (A) Generation of gastric epithelial (parietal) cell specific SDF-1 transgenic Mice (SDF-Tg). The construct pBS/H/K-ATPaseβ/SDF-1α contains the mouse H+/K+-ATPase β subunit gene promoter and human SDF-1 cDNA. (B) Expression of SDF-1 protein in the gastric tissue of the 4-months–old SDF-Tg and WT mice confirmed by ELISA. (C) Expression of SDF-1 in SDF-Tg mice serum. (D) The correlation of SDF-1 level over the detecting methods between SDF-1 ELISA and v5 ELISA. (E) SDF-1 level in bone marrow and stomach from the SDF-Tg and WT mice determined by the SDF-1 qRT-PCR.

Supplementary Figure 2 (A) No differences in the recruitment of immune cells in young mice. Immunohistochemistry for F4/80 or CD11b in the WT and SDF-Tg mice at the age of 6 months. The number of F4/80 or CD11b positive cells per HPFs was determined by counting 10 HPF per mice. n=5 per each groups. Data represent means + S.E. *$p<0.05$. *Scale bars*, 100μm. (B) Immune cells recruited to the stomach were analyzed by FACS. (C) SDF-1 promoted infiltration of F4/80 positive macrophages and neutrophil activity in older mice. Immunohistochemistry for F4/80 in the WT and

SDF-Tg mice at the age of 15 months. The number of F4/80 positive cells per HPF was determined by counting 10 HPFs per mice. n=5 per each groups. (D) Myeloperoxidase (MPO) activity in the stomach from WT or SDF-Tg at 12 mo was analyzed. N=5 per each group. Data represent means ± S.E. *$p<0.05$. *Scale bars*, 100µm.

Supplementary Figure 3 SDF/IL-1beta-Tg developed severe gastric inflammation and metaplasia (A) Histology from the IL-1beta, or SDF/IL-1beta-Tg mice at the age of 1 month. (Original magnifications, x100) (B) Summary of histological scoring. (C) IL-6 mRNA level in the stomach was upregulated in SDF-Tg mice compared to WT mice, determined by qRT-PCR. *Scale bars*, 100µm.

Supplementary Figure 4 SDF-1 promoted survival/cytokine production in macrophages (A) Primary macrophages were plated in 96 wells and incubated with either rSDF-1 (lt) or gastric extract from WT or SDF-Tg mice (rt) for 2 days; and then WST-8 assay was performed. (B) Cell culture supernatant after LPS stimulation was isolated and was measured IL-6 protein using ELISA kit as manufacturer's instructions. Data represent three experiments.

Supplementary Figure 5 (A) WT and SDF-Tg mice infected with *H. felis* were stained with Ki67. The number of Ki67 positive cells per gland was determined by counting 10 glands per mice. n=5 per each groups, 3 mo of mice. (B) SDF-Tg showed significant increase of K19-positive cells in the pit cell area compared to the WT. WT and SDF-Tg mice were stained with CK19 antibody. The number of CK19-positive cells per gland was determined by counting 10 glands per mice. n=5 per each groups, 4mo of mice. Data represent means ± S.E. *p<0.05. *Scale bars*, 100µm.

Supplementary Figure 6 Immunohistochemistry for phosphor-Erk, and phosphor-Akt WT and SDF-Tg mice treated with or without AMD3100 were stained with either phosphor-Erk, or phosphor-Akt.

Supplementary Figure 7 Loss of TFF2 accelerates the gastric atrophy and dysplasia mediating the SDF-CXCR4 signaling (A) Histology from the SDF-Tg and SDF-Tg/TFF2KO mice infected with *H. felis* for 12 months. (Original magnifications, x100) (B) Summary of the histological scoring from SDF-Tg and SDF-Tg/TFF2KO mice. (C, D) SDF-Tg and SDF-Tg/TFF2KO mice infected with *H. felis* were stained with Ki67. The number of Ki67 positive cells per gland was determined by counting 10

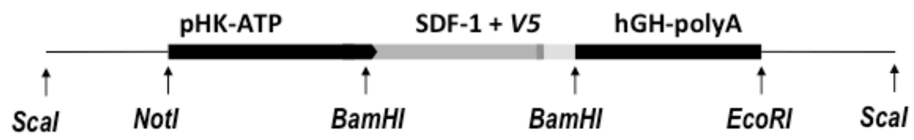
glands per mice. n=5 per each groups, 12 mo after *H. felis* infection. Data represent means ± S.E. *$p<0.05$ in each comparison indicated. *Scale bars*, 100µm.

Supplementary Figure 8 Localization of CXCR4-EGFP positive cells in stomach. CXCR4-EGFP was detected in stromal lesion in corpus, and in epithelial lesion in antrum (A, top) CXCR4-EGFP BAC transgenic mice were analyzed by fluorescent microscopy. CXCR4-EGFP expression was detected at the bottom of the gland in antrum, whereas only few cells were positive in corpus. Green; GFP (CXCR4), and blue; DAPI. (A, middle) E-cadherin immunofluorescent staining on CXCR4-EGFP transgenic mice. Red; E-cadherin, Green; GFP (CXCR4), and blue; DAPI. (A, bottom) alpha-SMA immunofluorescent staining on CXCR4-EGFP transgenic mice. Red; alpha-SMA, Green; GFP (CXCR4), and blue; DAPI. (B, C) The number of CXCR4-EGFP (+) cells in the stomach within CXCR4-EGFP mice or CXCR4/SDF-Tg double transgenic mice; (B) corpus, and (C) antrum. Original magnification x100. Data represent means ± S.E. *$p<0.05$. *Scale bars*, 100µm.

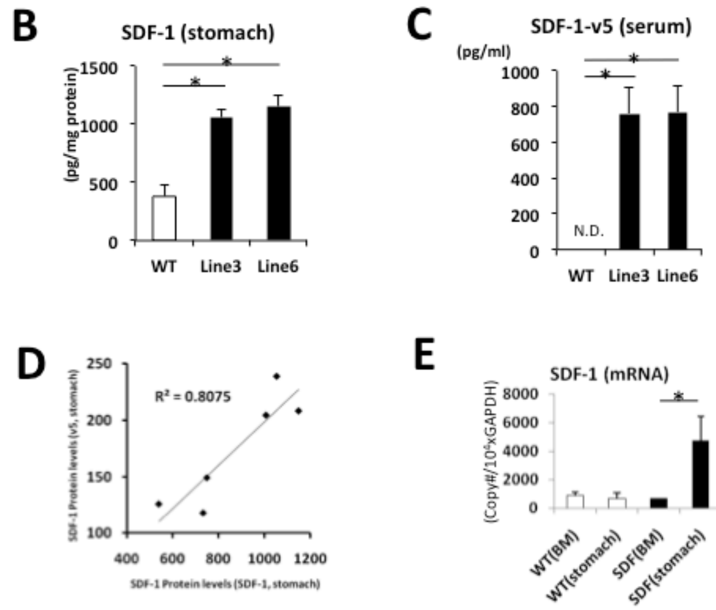
Supplementary Figure 9 (A) Bone marrow mesenchymal stem cells isolated from CXCR4-EGFP mice were observed under fluorescent microscopy. Green fluorescence

confirmed the expression of CXCR4. (B) A section of the gastric corpus taken from a CXCR4-EGFP mouse infected with *H. felis* for 15 months, confirming co-localization (white arrows) of Gremlin 1 (immunostaining, red) with CXCR4 (expression, green) in stromal cells within the gastric corpus. Green = CXCR4, Red = Gremlin 1 immunostaining, Blue = Dapi. Original magnification 300x.

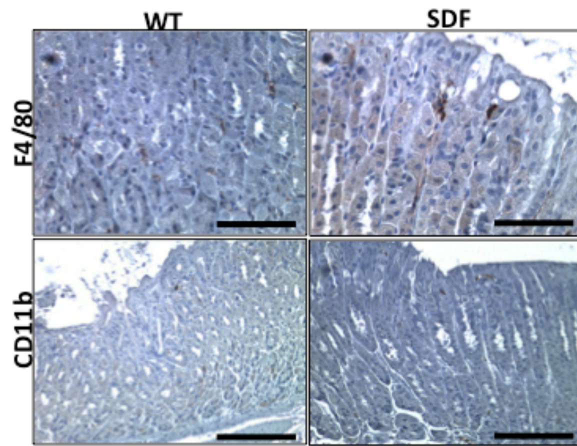
Supplemental Figure 1A

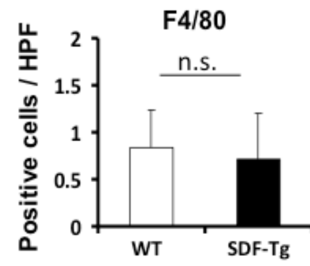


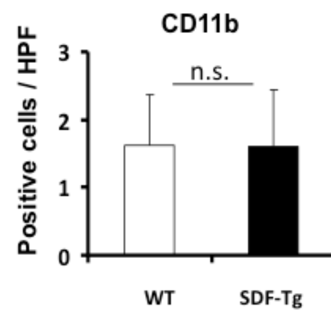
Supplemental Figure 1B-E



Supplemental Figure 2A

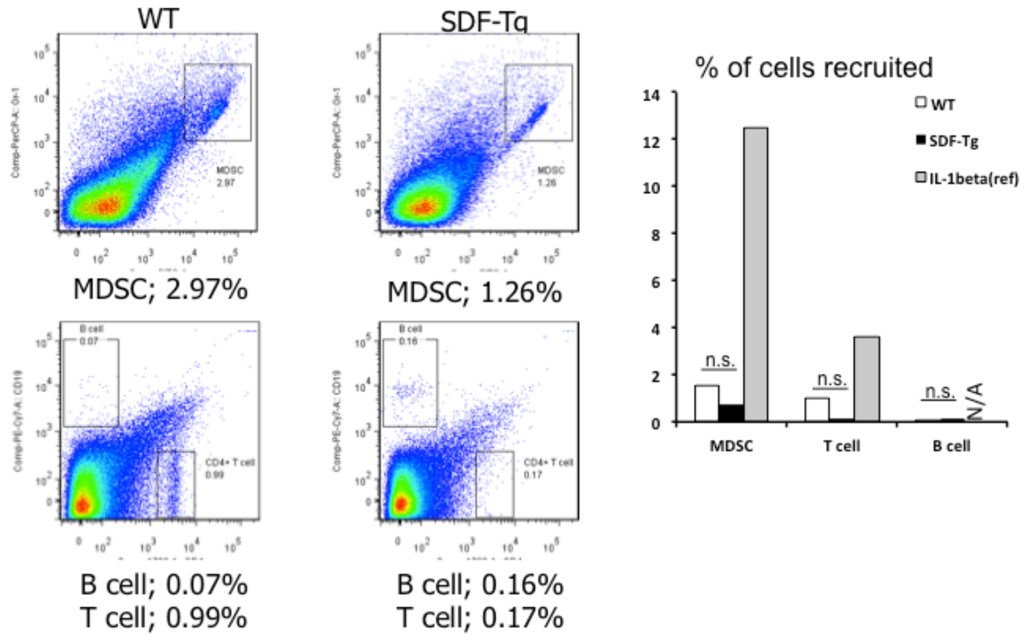






Supplemental Figure 2B

Stomach



Supplemental Figure 2 (cont.)

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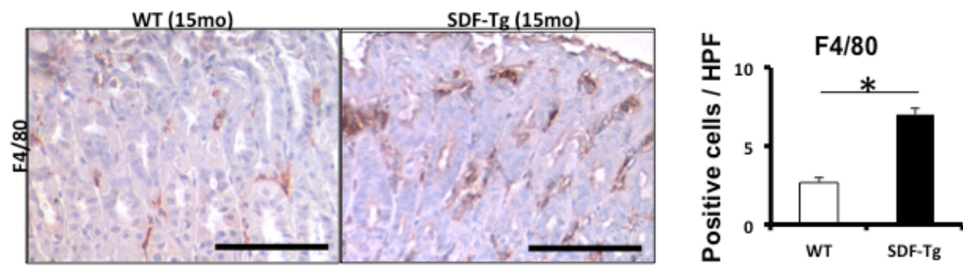

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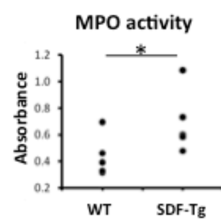

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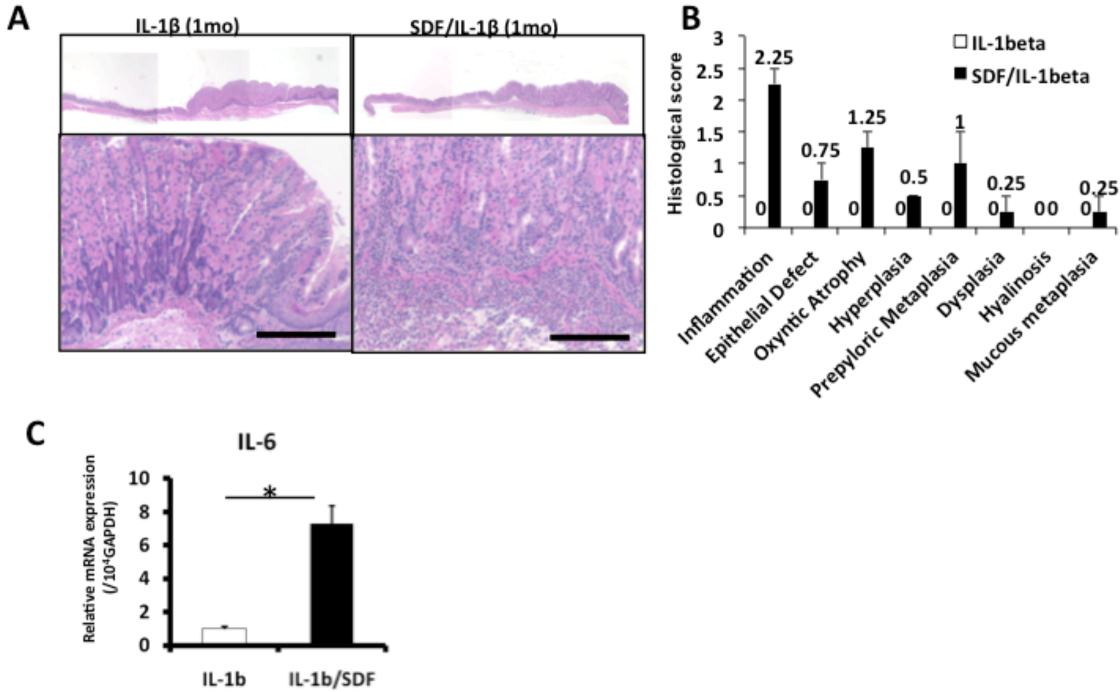
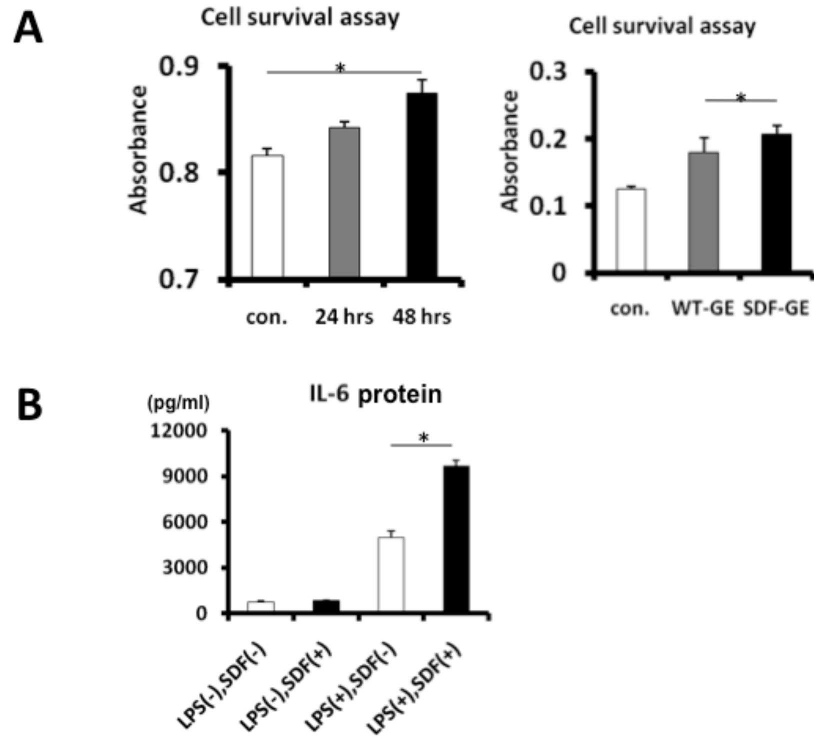


Supplemental Figure 4



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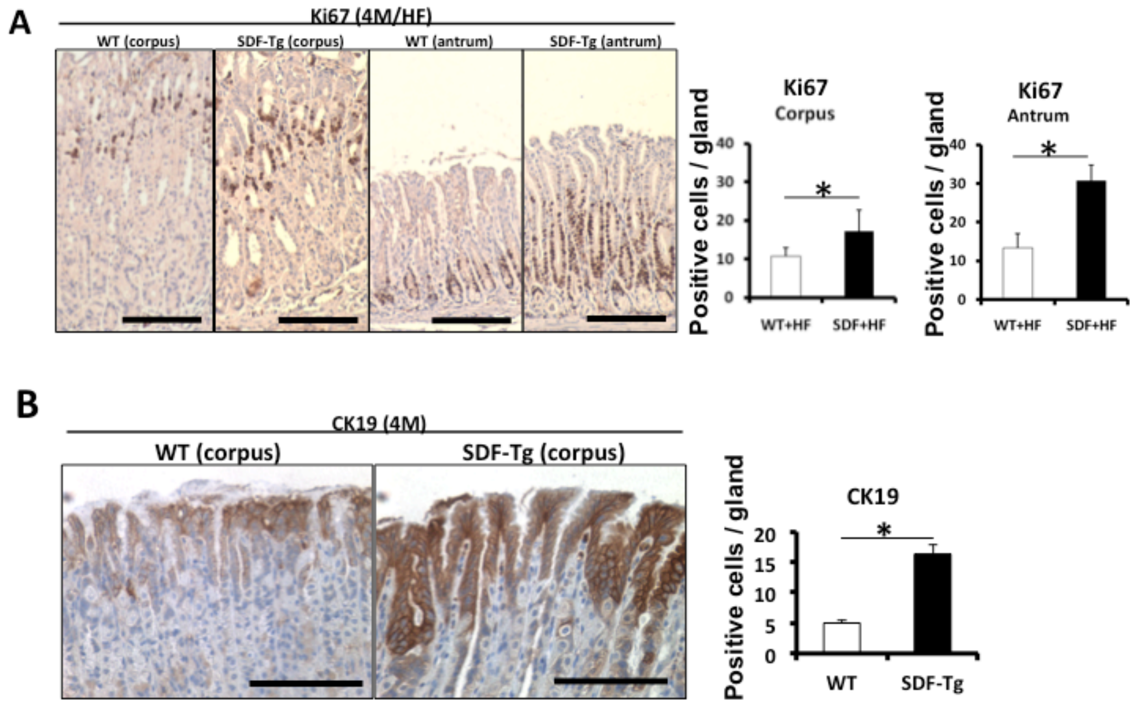

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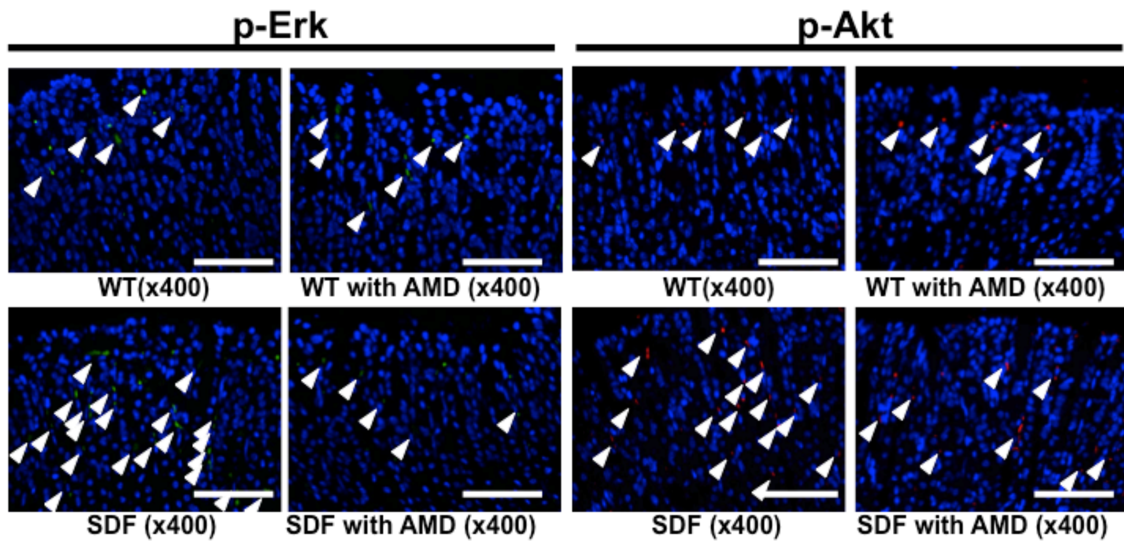

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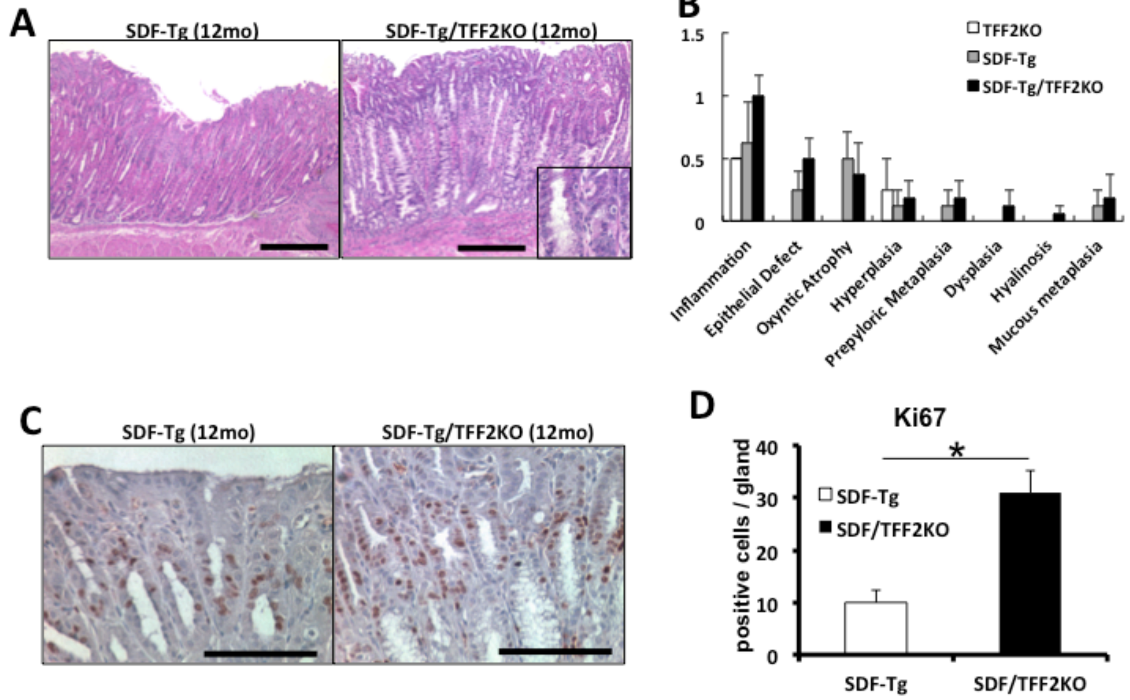
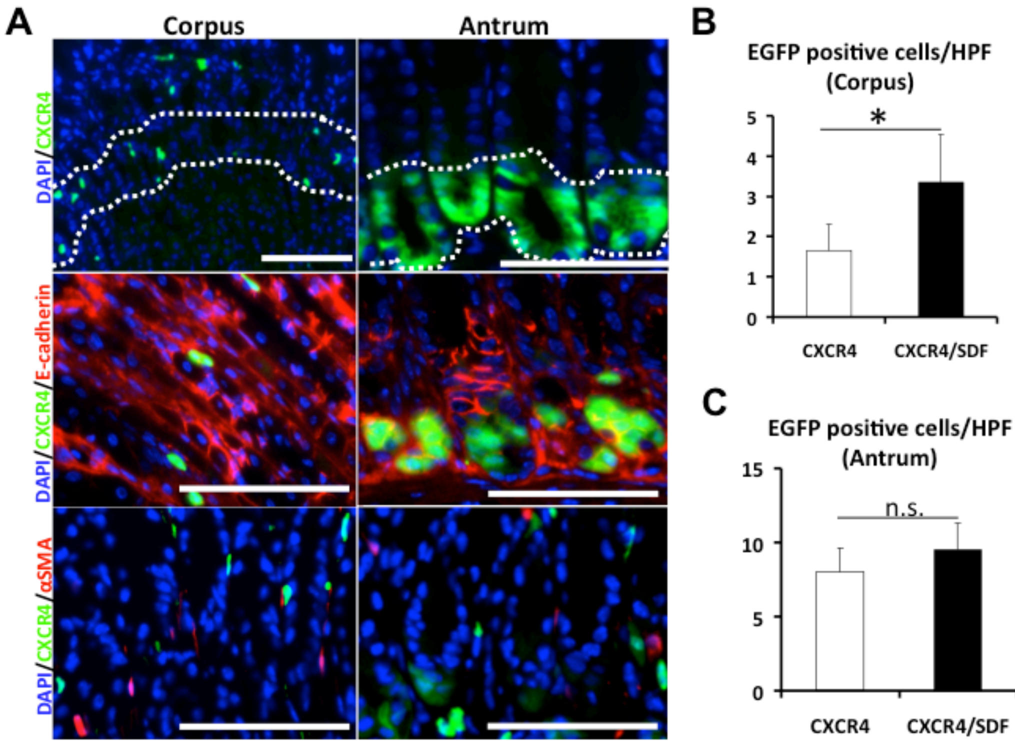
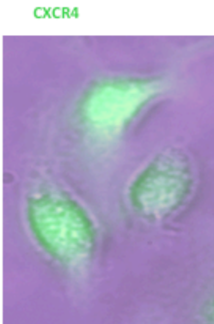


Supplemental Figure 8



Supplemental Figure 9

A



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