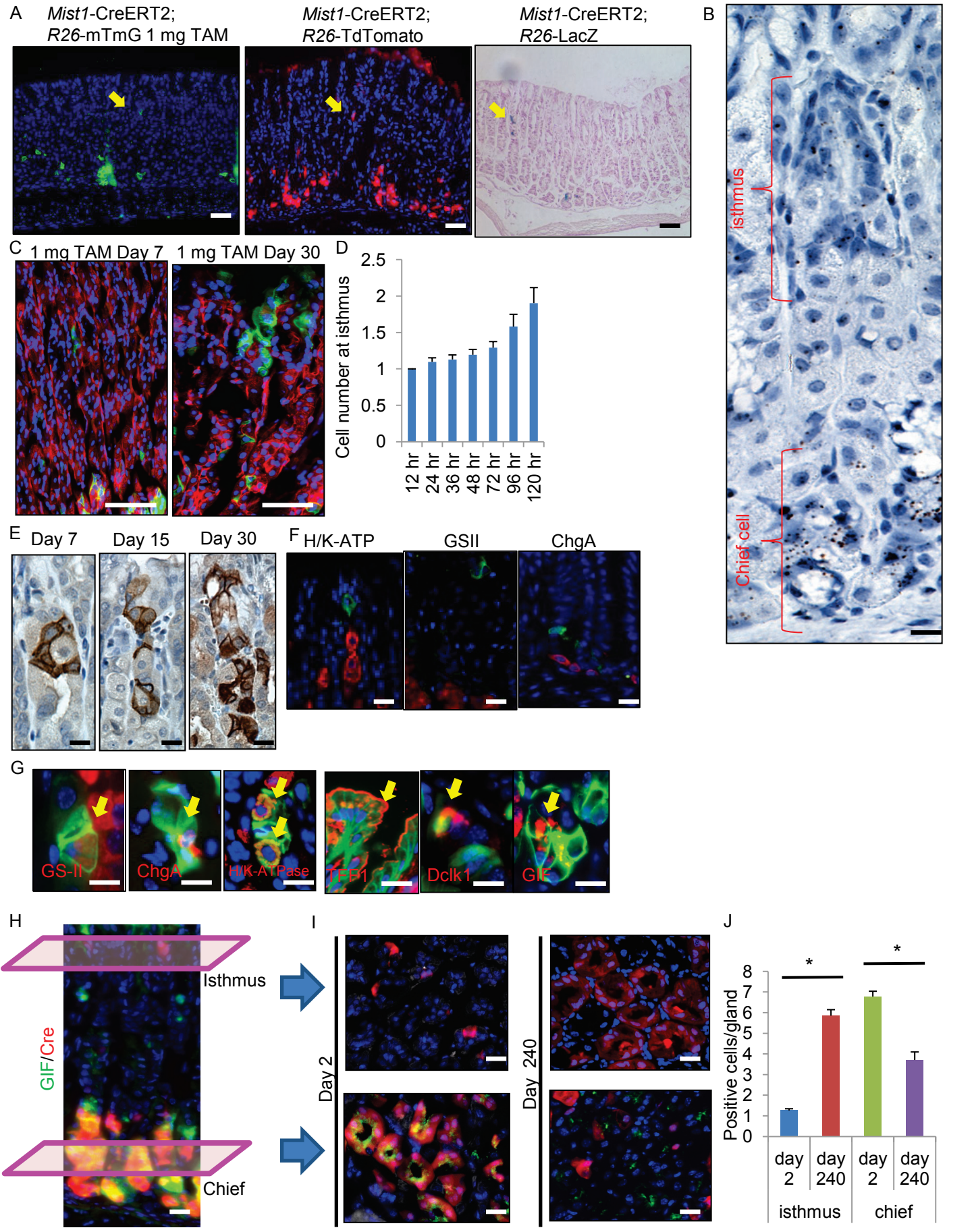
**Supplemetal Data**



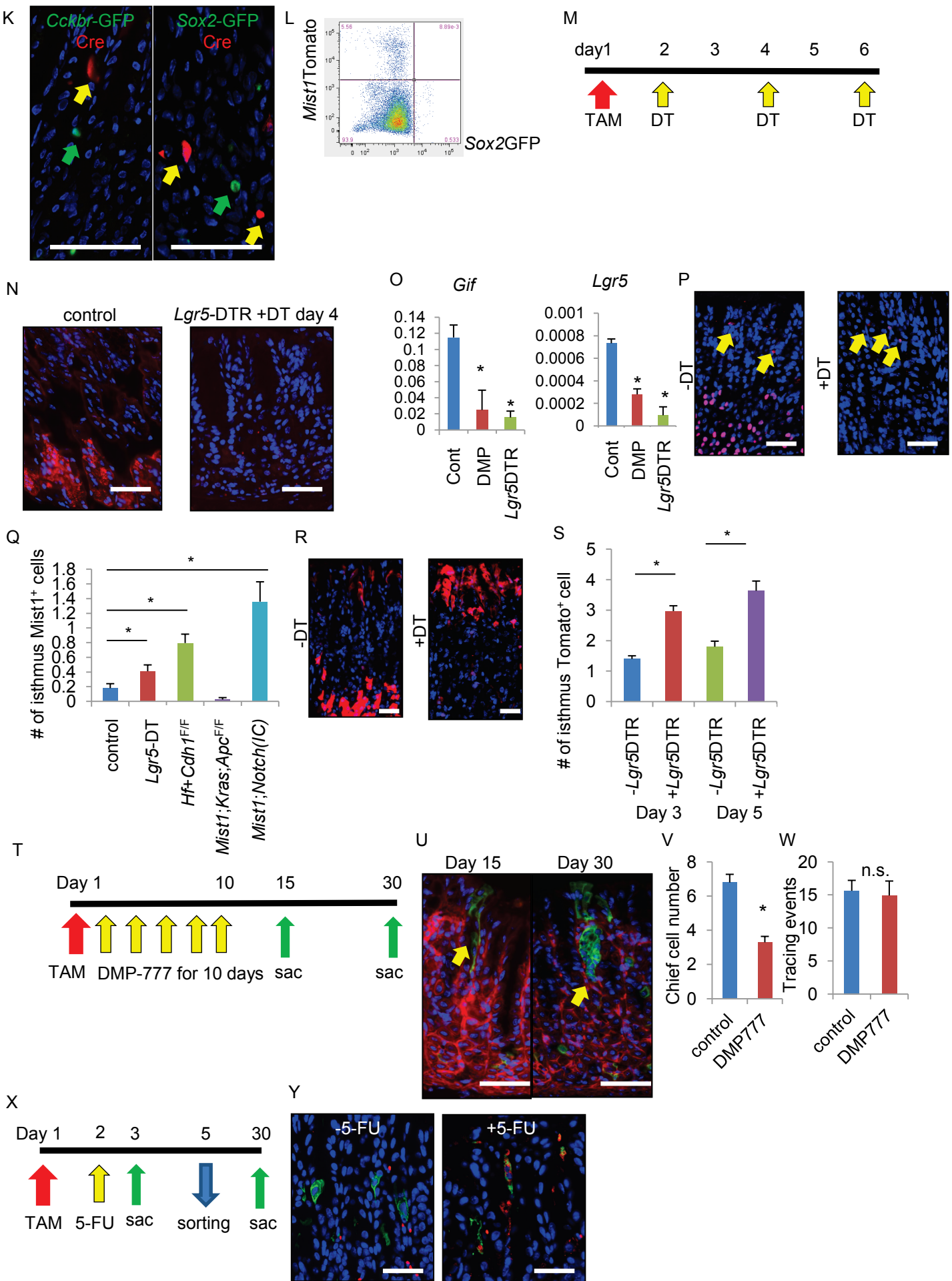

K
Cckbr-GFP Cre
Sox2-GFP Cre
L
Mist1Tomato
Sox2GFP
M
day1 2 3 4 5 6
TAM DT DT DT
N
control
Lgr5-DTR +DT day 4
O
Gif
Lgr5
Cont DMP Lgr5DTR
P
-DT
+DT
Q
# of isthmus Mist1+ cells
control
Lgr5-DT
Hf+Cdh1F/F
Mist1;Kras;ApcF/F
Mist1;Notch(IC)
R
-DT
+DT
S
# of isthmus Tomato+ cell
-Lgr5DTR +Lgr5DTR -Lgr5DTR +Lgr5DTR
Day 3 Day 5
T
Day 1 10 15 30
TAM DMP-777 for 10 days sac sac
U
Day 15 Day 30
V
Chief cell number
control DMP777
W
Tracing events
n.s.
control DMP777
X
Day 1 2 3 5 30
TAM 5-FU sac sorting sac
Y
-5-FU
+5-FU

**Figure S1, Related to Figure 1. Mist1$^{+}$ lineage tracing in the corpus isthmus.**
(A) Lineage tracing on day 1 in *Mist1*-CreERT2;*R26*-mTmG (1 mg TAM), *R26*-TdTomato, and *R26*-LacZ mice. Arrows indicage Mist1$^{+}$ isthmus cells. (B) In situ hybridization of *Mist1* in the corpus glands. (C) Lineage tracing in *Mist1*-CreERT2;*R26*-mTmG 7 and 30 days after 1 mg TAM induction. (D) Mist1$^{+}$ cell numbers of the isthmus at the indicated time points. Means ± SEM. 30 glands are analyzed at each time point. (E) GFP staining of lineage tracing in *Mist1*-CreERT2;*R26*-mTmG 7, 15, 30 days after TAM induction. (F) H/K-ATPase, GS-II, and Chg-A staining (red) with GFP staining (green) in *Mist1*-CreERT2;*R26*-mTmG mouse corpus on day 1. (G) Immunofluorescence of the indicated markers (red) with GFP staining (green) in *Mist1*-CreERT2;*R26*-mTmG mice 12 months after TAM. Arrows indicate double-positive cells. (H-I) Cross sectional images of *Mist1*-CreERT2;*R26*-Tdtomato mice at day 2 and 240. Sections are stained with GIF (green). (J) The numbers of TdTomato$^{+}$ cells per gland in the cross section at day 2 and 240. 50 glands/group are analyzed at each time point. (K) The corpus images of *Mist1*-CreERT2;*R26*-TdTomato mice crossed to *Cckbr*-GFP and *Sox2*-GFP mice 24 hr after TAM. Yellow arrows indicate Mist1$^{+}$ isthmus cells and green arrows indicate Cckbr$^{+}$ or Sox2$^{+}$ cells. (L) FACS plot of *Mist1*-CreERT;*Sox2*-GFP;*R26*-TdTomato mouse corpus. Mice were sacrificed 24 hr after TAM. (M) Protocol for TAM and DT treatments. (N) GIF staining (red) in control and DT-treated (day 4) *Lgr5*-DTR mouse corpus glands. (O) Relative gene expressions in the corpus glands of control, DMP-777-treated, and DT-treated *Lgr5*-DTR mice. n = 3/group. (P) Mist1 staining (red) in control and DT-treated (day 4) *Lgr5*-DTR mouse corpus glands. Arrows indicate isthmus Mist1$^{+}$ cells. (Q) The numbers of isthmus Mist1$^{+}$ cells in control, DT-treated (day 4) *Lgr5*-DTR mouse, *Hf*-infected *Cdh1*$^{\Delta Mist1}$ (3 months), *Mist1*-CreERT2;LSL-*Kras*$^{G12D}$;*Apc*$^{flox/flox}$, (3 months) and *Mist1*-CreERT2;LSL-*Notch1(IC)* (3 months) mice. Total 50 glands /group were analyzed. (R and S) *Mist1*-CreERT2;*Lgr5*-DTR;*R26*-TdTomato mice were treated with TAM at day1, and given DT at day 2, then sacrificed at day 3 or day 5. Representative tracing (red) at day 3 (R) and the numbers of TdTomato$^{+}$ cells in the isthmus (S) are shown. (T) Protocol for DMP-777 and TAM treatments. (U) Day 15 and 30 lineage tracing of DMP-777-treated *Mist1*-CreERT2;*R26*-mTmG mice. Refer to Fig. 1C for control tracing images. Arrows indicate isthmus cell lineage. (V-W) The number of GFP$^{+}$ chief cells per gland (V) and tracing events per 100 glands (W) in day 30 *Mist1*-CreERT2;*R26*-mTmG mice treated with or without DMP-777. Total 90 glands from 3 mice /group are analyzed for chief cell number, and total 300 glands from 3 mice /group are analyzed for tracing events. (X) Protocol for TAM and 5-FU treatments. (Y) Immunostaining of cleaved caspase 3 (red) and GFP (green) in *Mist1*-CreERT2;*R26*-mTmG mice. Mice were treated with TAM at day 1, and with 5-FU or PBS at day 2, then sacrificed at day 3. Note that Mist1$^{+}$ cells become cleaved caspase 3-positive after 5-FU treatment. Bars=50 μm (A, C, K, N, P, R, U, Y), 10 μm (B, E-I). Means ± SEM. *$p < 0.05$.

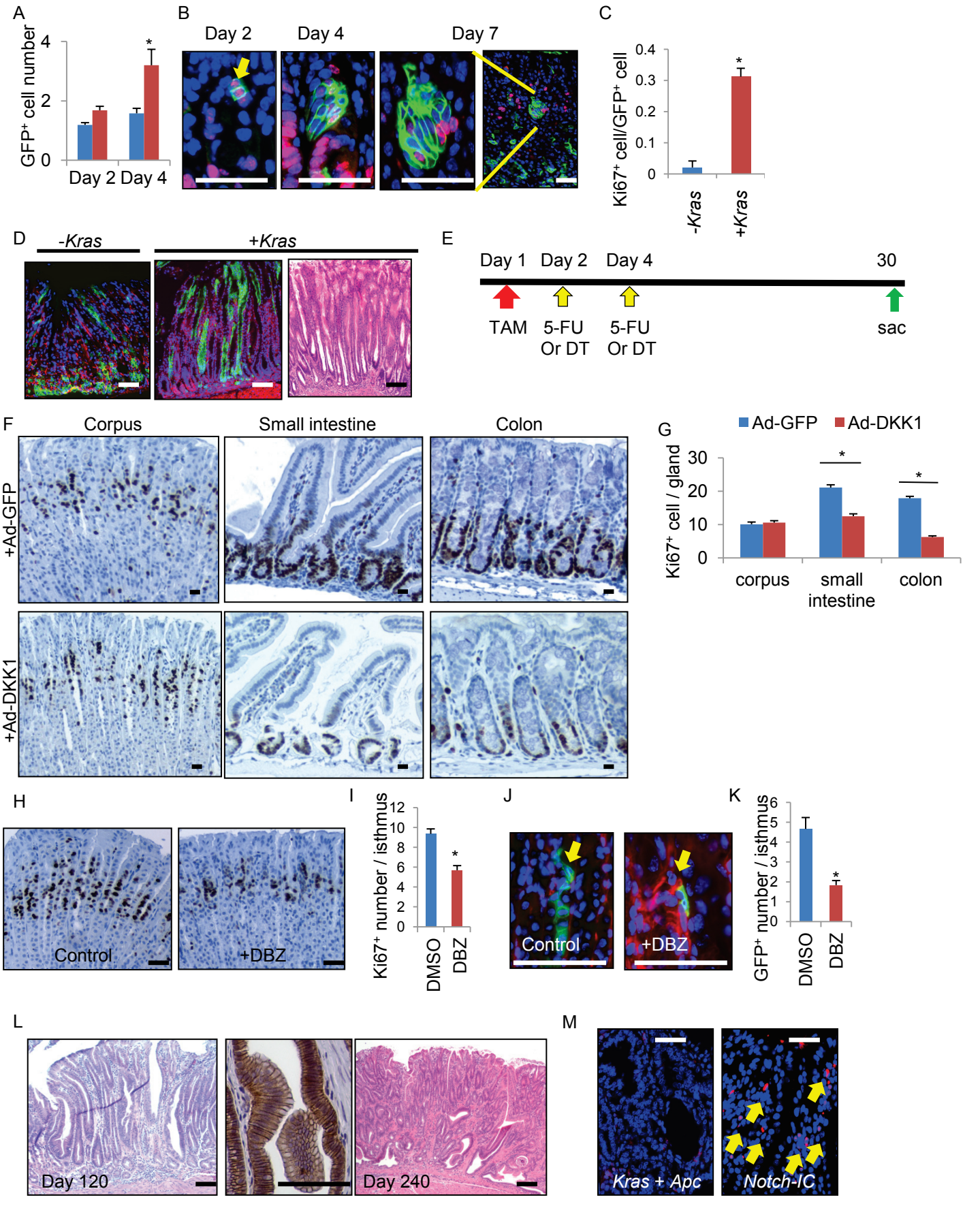

A
GFP+ cell number
4
2
0
*
Day 2 Day 4
B
Day 2
Day 4
Day 7
C
Ki67+ cell/GFP+ cell
0.4
0.3
0.2
0.1
0
*
-Kras
+Kras
D
-Kras
+Kras
E
Day 1 Day 2 Day 4 30
TAM
5-FU Or DT
5-FU Or DT
sac
F
Corpus
Small intestine
Colon
+Ad-GFP
+Ad-DKK1
G
Ad-GFP
Ad-DKK1
Ki67+ cell / gland
30
20
10
0
*
*
corpus
small intestine
colon
H
Control
+DBZ
I
Ki67+ number / isthmus
12
10
8
6
4
2
0
*
DMSO
DBZ
J
Control
+DBZ
K
GFP+ number / isthmus
6
5
4
3
2
1
0
*
DMSO
DBZ
L
Day 120
Day 240
M
Kras + Apc
Notch-IC

**Figure. S2, Related to Figure 2. Mist1$^{+}$ lineage tracing with oncogenic mutations.**
(A) Numbers of GFP$^{+}$ cells in the corpus gland 2 and 4 days after TAM induction in *Mist1*-CreERT2;*R26*-mTmG mice (blue) and *Mist1*-CreERT2;LSL-*Kras*$^{G12D}$;*R26*-mTmG mice (red). n = 30 glands/group. (B) Lineage tracing and Ki67 staining in *Mist1*-CreERT2;*R26*-mTmG mice with or without LSL-*Kras*$^{G12D}$ mutation. Arrow indicates isthmus stem cell. (C) The numbers of Ki67 and GFP double-positive cells per total number of GFP$^{+}$ cells with or without *Kras* mutation on day 2. Total 100 glands from 5 animals/group are analyzed. (D) Lineage tracing and H&E staining in *Mist1*-CreERT2;*R26*-mTmG mice with or without *Kras* mutation after 3 months. (E) Protocol for TAM and 5-FU or DT treatment. (F) Ki67 staining in the corpus gland, small intestine, and colon of mice 2 days after injection of GFP-expressing (Ad-GFP) and DKK1-expressing adenoviruses (Ad-DKK1). (G) Number of Ki67$^{+}$ cells per gland. n = 30 glands/group. (H and I) Ki67 staining (H) and Ki67$^{+}$ cell numbers (I) in mouse corpus glands treated with or without 14 days DBZ. n = 30 glands/group. (J-K) Lineage tracing images (J) and the number of GFP$^{+}$ cells (K) in *Mist1*-CreERT2;*R26*-mTmG mice with or without DBZ treatment for 14 days. Total 30 glands /group are analyzed. (L) H&E and β-catenin staining in *Mist1*-CreERT2;*Eef1a1*-LSL-*Notch1(IC)* mouse corpus on day 120 and 240 post-induction. (M) Mist1 staining (red) of *Mist1*-CreERT2;LSL-*Kras*$^{G12D}$;*Apc*$^{flox/flox}$ and *Mist1*-CreERT2;LSL-*Notch1(IC)* mice 3 months after TAM. Arrows indicate Mist1$^{+}$ cell. Means ± SEM. *$p < 0.05$. Bars=50 μm.

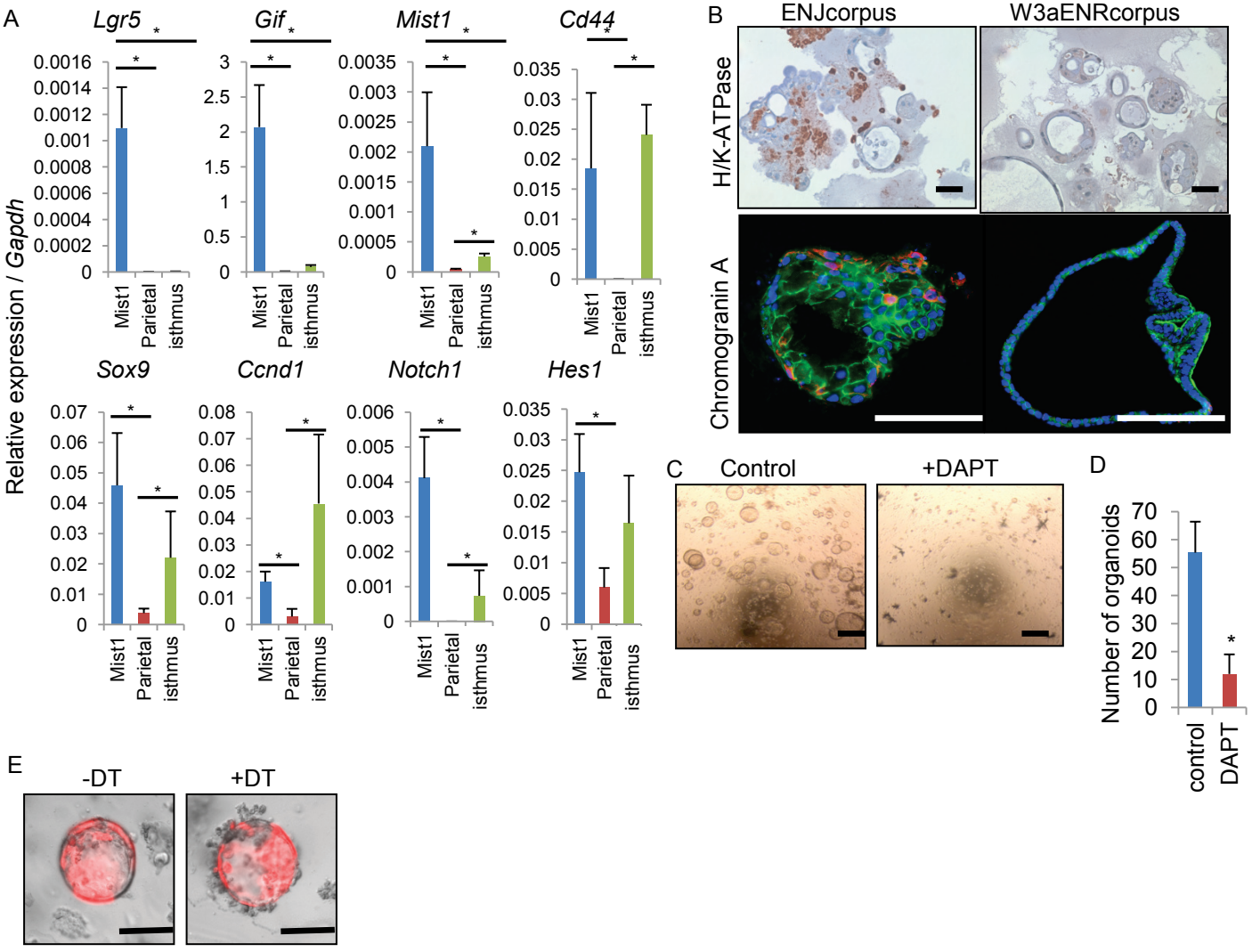
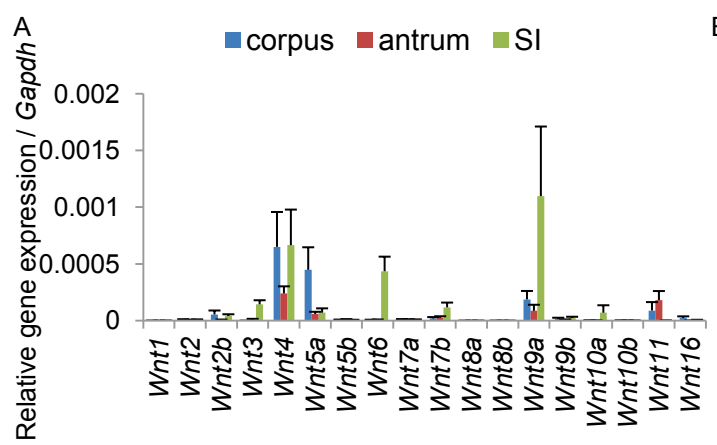
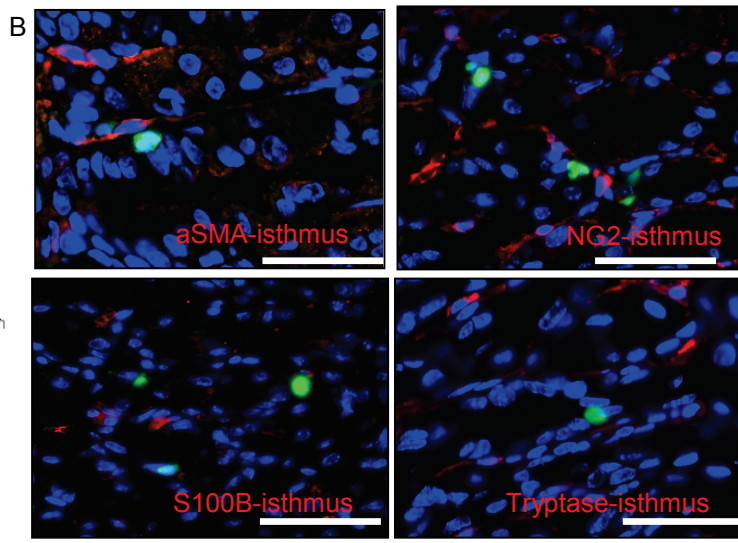
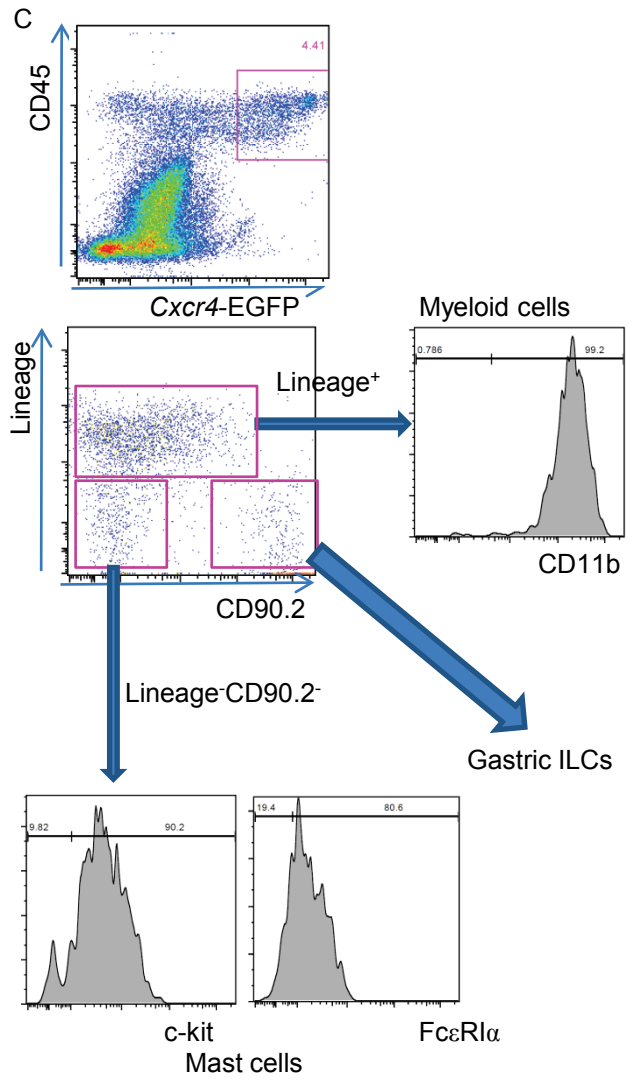


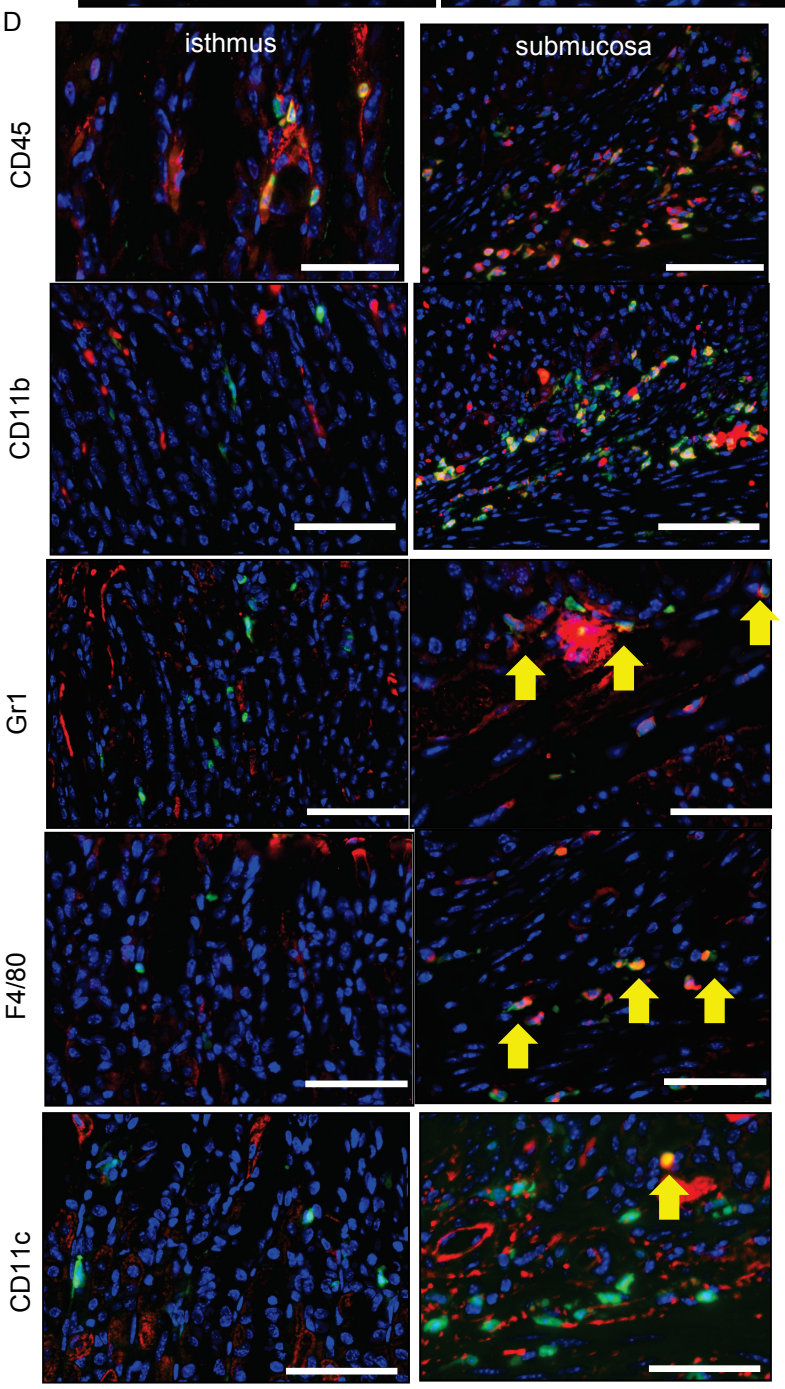

**Figure S3, Related to Figure 3. Notch signaling is important for the Mist1⁺ stem cell maintenance.**

(A) Relative gene expression per *Gapdh* in sorted total Mist1⁺ population, parietal cell population, and Mist1⁺ isthmus cell population after DT ablation. n = 3/group. (B) H/K-ATPase (brown; counter-stained by hematoxylene) and Chromogranin A (red; counter-stained by DAPI (blue) and phalloidin (green)) staining in the corpus organoids cultured with W3aENR and ENJ media for 20 days. (C and D) Corpus gland cultures in W3aENR media treated with vehicle or DAPT on day 10. Representative images (C) and numbers of organoids per well (D) are shown. n = 3/group. (E) In vitro lineage tracing of *Mist1*-CreERT2;*R26*-TdTomato corpus. Glands were isolated 1 day after tamoxifen and cultured with ENJ media with or without DT. Bars=50 μm (B, C), 100 μm (E). Means ± SEM. *p < 0.05.


A
corpus
antrum
SI
Relative gene expression / Gapdh
0.002
0.0015
0.001
0.0005
0
Wnt1
Wnt2
Wnt2b
Wnt3
Wnt4
Wnt5a
Wnt5b
Wnt6
Wnt7a
Wnt7b
Wnt8a
Wnt8b
Wnt9a
Wnt9b
Wnt10a
Wnt10b
Wnt11
Wnt16


B
aSMA-isthmus
NG2-isthmus
S100B-isthmus
Tryptase-isthmus


C
CD45
4.41
Cxcr4-EGFP
Lineage
Lineage+
Myeloid cells
0.786
99.2
CD11b
CD90.2
Lineage-CD90.2-
Gastric ILCs
9.82
90.2
19.4
80.6
c-kit
FcεRIα
Mast cells


D
isthmus
submucosa
CD45
CD11b
Gr1
F4/80
CD11c

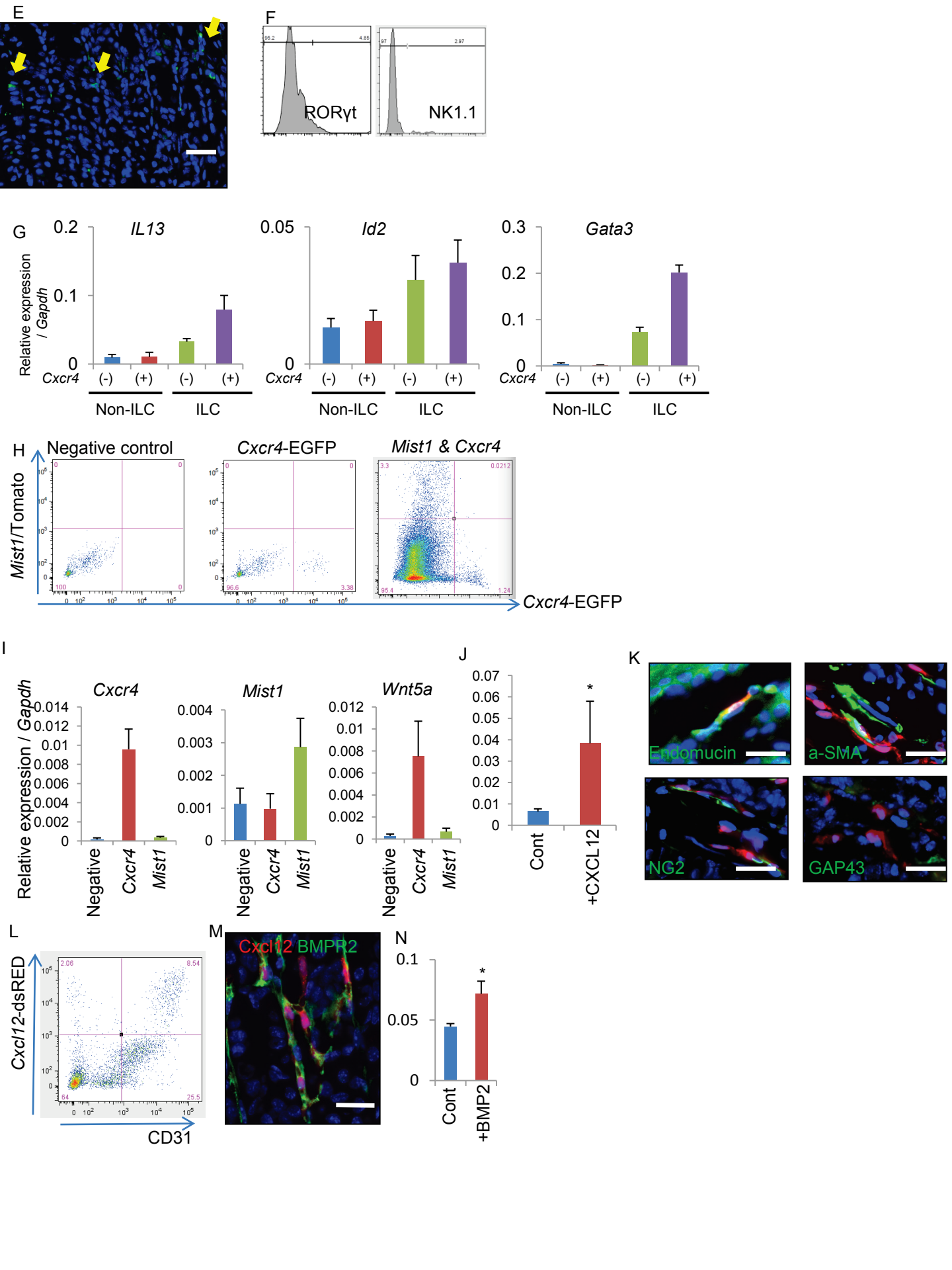

E
F
RORγt
NK1.1
G
IL13
Id2
Gata3
Relative expression / Gapdh
Cxcr4
(-)
(+)
Non-ILC
ILC
H
Negative control
Cxcr4-EGFP
Mist1 & Cxcr4
Mist1/Tomato
Cxcr4-EGFP
I
Cxcr4
Mist1
Wnt5a
Relative expression / Gapdh
Negative
J
Cont
+CXCL12
K
Endomucin
a-SMA
NG2
GAP43
L
Cxcl12-dsRED
CD31
M
Cxcl12 BMPR2
N
Cont
+BMP2

**Figure S4, Related to Figure 4. Cxcr4$^{+}$ cell population in the corpus.**
(A) Relative Wnt ligand expression per *Gapdh* in mouse corpus, antrum, and small intestine. n = 3/group. Means ± SEM. (B) Negative control image of *Wnt5a* in situ hybridyzation. (C) Immunostaining with the indicated markers (red) of *Cxcr4*-EGFP corpus isthmus. (C) FACS plot with CD11b, c-kit, and FcεRIα. Lineage$^{+}$Cxcr4$^{+}$ population is CD11b$^{+}$ myeloid cells. Lineage$^{-}$ CD90.2$^{-}$Cxcr4$^{+}$ population are mast cells. (D) CD45, CD11b, Gr1, F4/80, and CD11c staining (red) in the corpus (isthmus and submucosal area) of *Cxcr4*-EGFP mice. Arrows indicate double positive cells. (E) *Id2*-GFP mouse corpus. Arrows indicate GFP$^{+}$ cells. (F) FACS histogram with RORγt and NK1.1 of CD45$^{+}$Cxcr4$^{+}$Lin$^{-}$CD127$^{+}$ population. (G) Gene expression of type2 ILC markers in Cxcr4$^{+/-}$ ILCs and non-ILCs. n = 3/group. (H-I) FACS plot (H) of WT (negative control), *Cxcr4*-EGFP mice, and *Mist1*-CreERT;*Cxcr4*-EGFP;*R26*-TdTomato mice corpus. Refer to Fig. 3D as TdTomato alone control. Relative gene expression (I) of TdTomato$^{+}$, GFP$^{+}$, and double-negative cells sorted from *Mist1*-CreERT2;*Cxcr4*-EGFP;*R26*-TdTomato mice 1 day after TAM induction. n = 3/group. (J) *Wnt5a* gene expression in sorted Cxcr4$^{+}$ cells from the stomach. Cells were treated with Cxcl12 and harvested 24 hr later. n = 3/group. (K) Immunostaining with the indicated markers (green) of *Cxcl12*-dsRED cells. (L) FACS plot of dsRED and CD31 from *Cxcl12*-dsRED mouse stomach. (M) BMPR2 immunostaining (green) of *Cxcl12*-dsRED mouse stomach. (N) *Cxcl12* gene expression in cultured *Cxcl12*-dsRED cells treated with or without BMP2 for 24 hr. n = 3/group. Means ± SEM. *$p < 0.05$ compared to control. Bars=20 μm (K, M), 50 μm (B, D-E).

**Table S1, Related to Figure 4. qRT-PCR array comparing gene expression between Cxcl12$^{+}$CD31$^{+}$ cells and Cxcl12$^{-}$CD31$^{+}$ cells.**

| Symbol | Fold Change (Cxcl12$^{+}$CD31$^{+}$ cells vs Cxcl12$^{-}$CD31$^{+}$ cells) |
|---|---|
| *Ar* | 13.6895 |
| *Arnt* | 1.3803 |
| *Atf1* | 0.933 |
| *Atf2* | 1.3947 |
| *Atf3* | 0.4047 |
| *Atf4* | 1.9588 |
| *Cebpa* | 0.0899 |
| *Cebpb* | 0.2269 |
| *Cebpg* | 1.9119 |
| *Clasrp* | 0.8888 |
| *Creb1* | 1.3519 |
| *Crebbp* | 1.2483 |
| *Ctnnb1* | 4.2281 |
| *Dr1* | 0.8827 |
| *E2f1* | 0.3231 |
| *E2f6* | 1.0534 |
| *Egr1* | 1.1329 |
| *Esr1* | 0.2932 |
| *Ets1* | 11.5915 |
| *Ets2* | 1.6586 |
| *Fos* | 0.8066 |
| *Foxa2* | 1.2058 |
| *Foxg1* | 0.9138 |
| *Gata1* | 0.0988 |
| *Gata2* | 1.1933 |
| *Gata3* | 1.0105 |
| *Gli1* | 0.6666 |
| *Gtf2b* | 1.5476 |
| *Gtf2f1* | 0.8011 |
| *Hand1* | 0.3242 |
| *Hand2* | 1.454 |
| *Hdac1* | 1.2269 |
| *Hif1a* | 1.3379 |

| | |
|---|---|
| *Hnf1a* | 0.75 |
| *Hnf4a* | 1.3755 |
| *Hoxa5* | 11.0425 |
| *Hsf1* | 1.3149 |
| *Id1* | 1.8856 |
| *Irf1* | 2.8779 |
| *Jun* | 1.5052 |
| *Junb* | 0.7346 |
| *Jund* | 1.1019 |
| *Kcnh8* | 0.3287 |
| *Max* | 1.2924 |
| *Mef2a* | 1.3241 |
| *Mef2b* | 2.0069 |
| *Mef2c* | 7.4643 |
| *Myc* | 0.6902 |
| *Myf5* | 0.8467 |
| *Myod1* | 0.9138 |
| *Nanos2* | 1.8596 |
| *Nfat5* | 2.2815 |
| *Nfatc2* | 0.1811 |
| *Nfatc3* | 1.0389 |
| *Nfatc4* | 0.4665 |
| *Nfkb1* | 0.7765 |
| *Nfyb* | 1.7532 |
| *Nr3c1* | 1.9386 |
| *Pax6* | 0.473 |
| *Pou2af1* | 0.0527 |
| *Ppara* | 0.8827 |
| *Pparg* | 1.676 |
| *Rb1* | 2.166 |
| *Rel* | 0.2154 |
| *Rela* | 1.007 |
| *Smad1* | 1.8088 |
| *Smad4* | 2.395 |
| *Smad5* | 2.0491 |
| *Smad9* | 1.0461 |
| *Sp1* | 1.1487 |
| *Sp3* | 1.834 |

| | |
|---|---|
| *Stat1* | 1.3613 |
| *Stat2* | 1.5801 |
| *Stat3* | 1.5263 |
| *Stat4* | 0.2793 |
| *Stat5a* | 0.8409 |
| *Stat5b* | 0.9862 |
| *Stat6* | 1.0718 |
| *Tbp* | 1.1728 |
| *Tcf7l2* | 1.083 |
| *Tfap2a* | 2.114 |
| *Tgif1* | 0.6878 |
| *Trp53* | 0.6329 |
| *Yy1* | 1.2311 |

* Upregulated genes (> 2 fold) are shown in red, and downregulated genes (< 0.5 fold) are shown in blue.

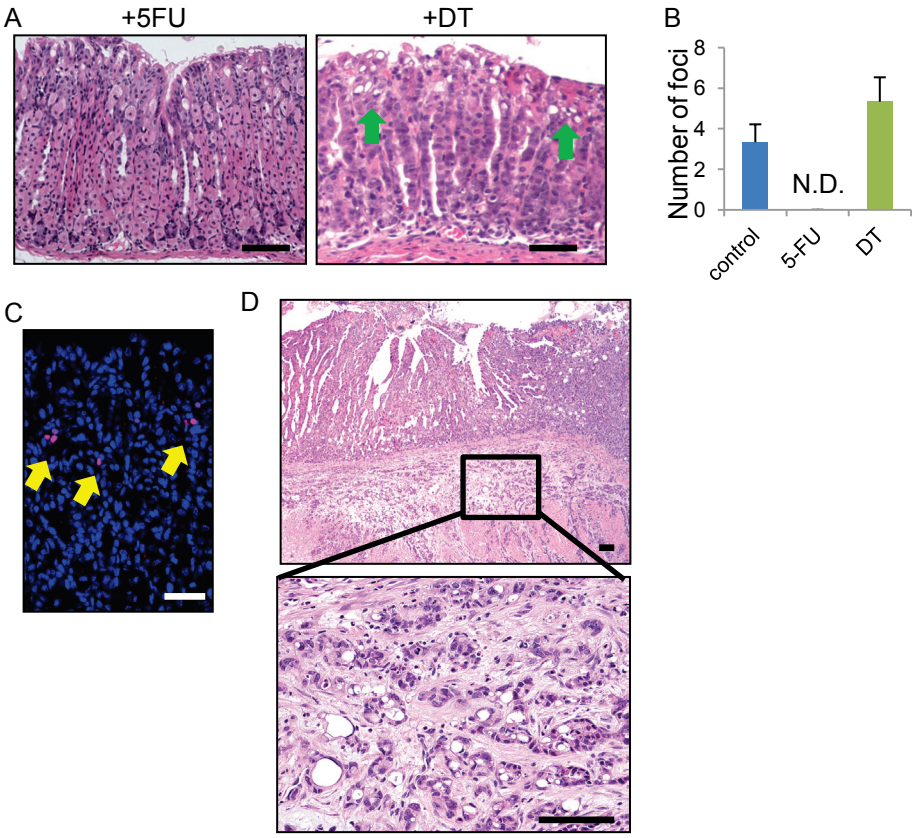
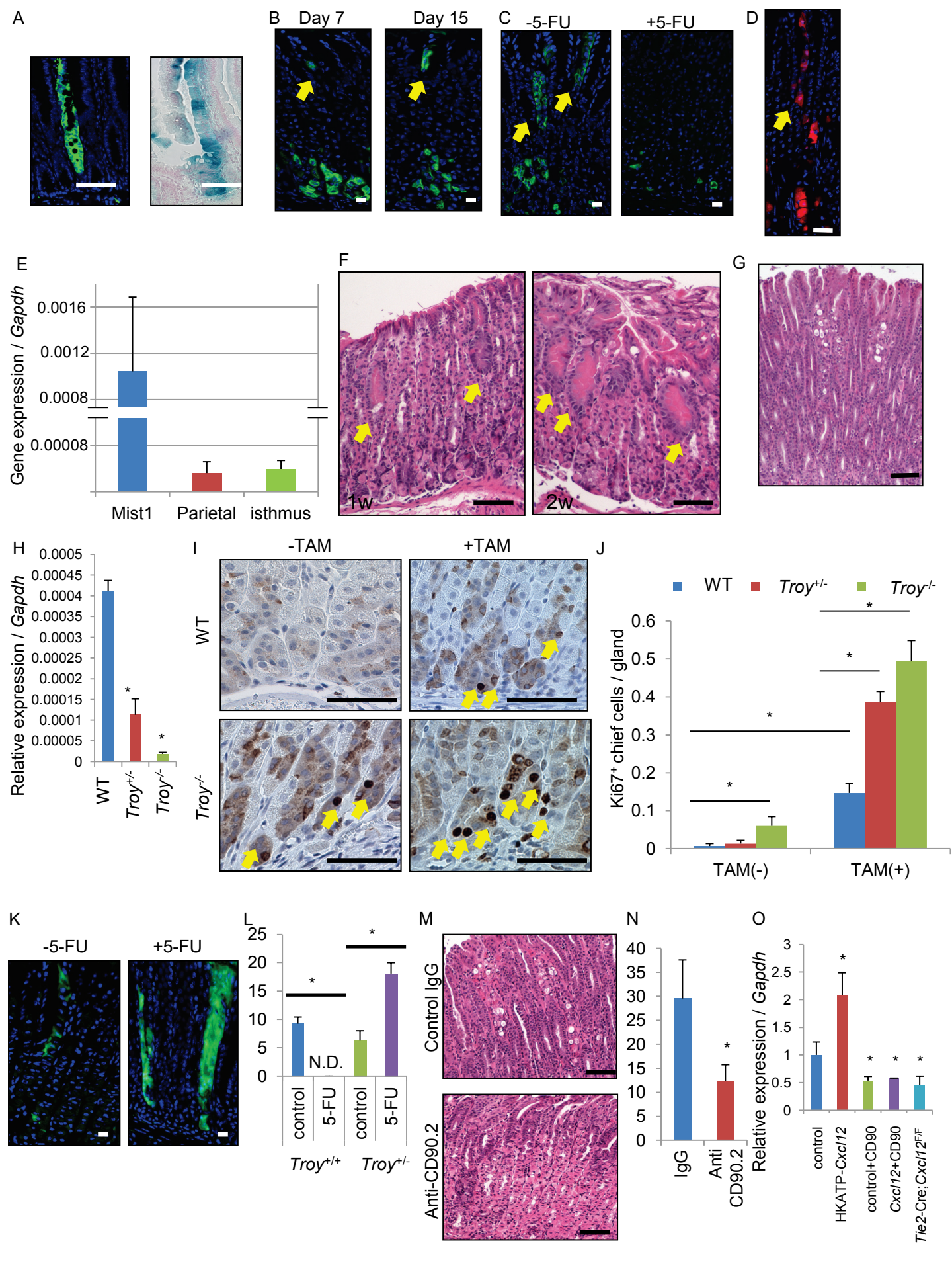



**Figure S5, Related to Figure 5. Cxcl12/Cxcr4 perivascular niche in the stomach and DGC development.**

(A-B) *Cdh1*$^{\Delta Mist1}$ crossed to *Lgr5*-DTR mice were treated with 5-FU or DT after TAM plus *Hf* treatment. Day 30 H&E images (A) and numbers of atypical foci (B). n = 3 mice/group and 4 sections/mouse are analyzed. Arrows indicate atypical foci in the isthmus. (C) Mist1 staining (red) of *Hf*-infected *Cdh1*$^{\Delta Mist1}$ mice 3 months after TAM. Arrows indicate isthmus Mist1$^{+}$ cells. (D) H&E staining of *Hf*-infected *Cdh1*$^{\Delta Mist1}$ crossed to LSL-*Trp53*$^{R172H}$ mice 9 months after TAM. The lower panel shows higher magnification of box area in the upper panel, showing that signet-ring cancer cells invade into submucosa. Means ± SEM. *p < 0.05 compared to control. Bar=50 μm (C-D), 100 μm (A).


A
B Day 7
Day 15
C -5-FU
+5-FU
D
E
Gene expression / Gapdh
0.0016
0.0012
0.0008
0.00008
Mist1
Parietal
isthmus
F
1w
2w
G
H
Relative expression / Gapdh
0.0005
0.00045
0.0004
0.00035
0.0003
0.00025
0.0002
0.00015
0.0001
0.00005
0
WT
Troy+/-
Troy-/-
I
-TAM
+TAM
WT
Troy-/-
J
WT
Troy+/-
Troy-/-
Ki67+ chief cells / gland
0.6
0.5
0.4
0.3
0.2
0.1
0
TAM(-)
TAM(+)
K
-5-FU
+5-FU
L
25
20
15
10
5
0
N.D.
control
5-FU
control
5-FU
Troy+/+
Troy+/-
M
Control IgG
Anti-CD90.2
N
40
35
30
25
20
15
10
5
0
IgG
Anti CD90.2
O
Relative expression / Gapdh
3
2.5
2
1.5
1
0.5
0
control
HKATP-Cxcl12
control+CD90
Cxcl12+CD90
Tie2-Cre;Cxcl12F/F

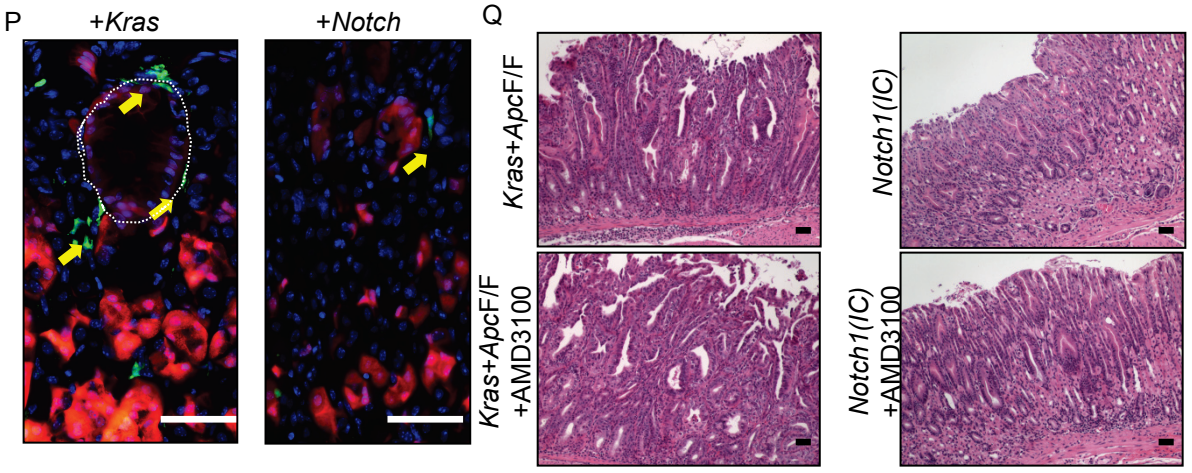



**Figure S6, Related to Figure 6. Troy$^{+}$ isthmus stem cells give rise to gastric cancers and loss of *Troy* expression leads to increased proliferation and lineage tracing of chief cells.** (A) Lineage tracing in the small intestine of *Troy*-BAC-CreERT2 mice 30 days after TAM induction. (B) Lineage tracing (day 7, 15) in the corpus glands of *Troy*-BAC-CreERT2;*R26*-mTmG mice. Arrows indicate isthmus Troy$^{+}$ cells. (C) Lineage tracing (day 30) in the *Troy*-BAC-CreERT2;*Troy*$^{+/+}$;*R26*-mTmG mice with or without 5-FU. Arrows indicate isthmus Troy$^{+}$ cells. (D) Day 30 lineage tracing of *Troy*-CreERT2;*Lgr5*-DTR;*R26*-TdTomato mice after DT ablation. TAM and DT were given as shown in Fig. S2S. Arrows indicate isthmus Troy$^{+}$ cells. (E) *Troy* gene expression per *Gapdh* in sorted total Mist1$^{+}$ population, parietal cell population, and Mist1$^{+}$ isthmus cell population after DT ablation. n = 3/group. (F) H&E staining of *Troy*-CreERT;LSL-*Kras*$^{G12D}$ mice 1 week and 2 weeks after TAM. Arrows indicate isthmus Troy$^{+}$ cell-derived metaplasia. (G) H&E staining of *Troy*-CreERT;*Cdh1*$^{flox/flox}$ mice treated with *Hf* and TAM (day 30). (H) Relative *Troy* expression in WT, *Troy*$^{+/-}$, and *Troy*$^{-/-}$ mouse corpus glands. n = 3/group. (I-J) WT, *Troy*$^{+/-}$, and *Troy*$^{-/-}$ mice were treated with 5 mg TAM and sacrificed after 4 days. Ki67 staining (I) and the number of Ki67$^{+}$ chief cells per gland (J) are shown. Total 90 glands from 3 mice /group are analyzed. Arrows indicate Ki67$^{+}$ chief cells. (K) Lineage tracing in the*Troy*-BAC-CreERT2;*Troy*$^{+/-}$ mice with or without 5-FU. (L) The numbers of lineage tracing events per 100 glands in *Troy*-BAC-CreERT2;*Troy*$^{+/+}$ or *Troy*-BAC-CreERT2;*Troy*$^{+/-}$ mice with or without 5-FU at day 30. Total 500 glands from 5 mice/group are analyzed. (M and N) H&E staining (M) and the numbers of atypical foci per section (N) of *Hf*-infected *Cdh1*$^{\Delta Mist1}$ crossed to H/K-ATPase-*Cxcl12* mice treated with control or anti-CD90.2 antibodies. n = 4 mice/group and 4 sections/mouse are analyzed. (O) Relative gene expression of *Wnt5a* in WT, H/K-ATPase-*Cxcl12*, or *Tie2*-Cre;*Cxcl12*$^{flox/flox}$ mouse stomach treated with control or anti-CD90.2 antibodies. The expression in control group is set as 1.0. n =3 /group. (P) *Mist1*-CreERT2;LSL-*Kras*$^{G12D}$;*Cxcr4*-EGFP;*R26*-TdTomato and *Mist1*-CreERT2;LSL-*Notch1(IC)*;*Cxcr4*-EGFP;*R26*-TdTomato mouse corpus 30 days after TAM. Note that the number of Cxcr4$^{+}$ cells (arrows) in the isthmus is increased in mutant *Kras* mice (metaplastic area is indicated by white line) but not in *Notch1(IC)* mice. (Q) H&E staining of *Mist1*-CreERT2;LSL-*Kras*$^{G12D}$;*Apc*$^{flox/flox}$ and *Mist1*-CreERT2;LSL-*Notch1(IC)* mice 30 days after TAM. Mice were treated with vehicle or AMD3100 for 2 weeks (day 7 to 21) as shown in Experimental procedures. Histological changes were not improved by AMD3100 in these models. Means ± SEM. *p < 0.05. Bars=10 μm (B-D, K), 50 μm (A, F, M, P-Q), and 100 μm (G, I).

## Supplemental Experimental Procedures

### Gland isolation and in vitro culture system

The harvested mouse stomachs were opened longitudinally and washed with cold PBS. The tissue was chopped into approximately 5 mm pieces, washed with cold PBS, and incubated in 8 mM EDTA in PBS for 60 min on ice. The tissue fragments were suspended vigorously in cold 10 % FBS using a 10-mL pipette, yielding supernatants enriched in crypts. Gland fractions were centrifuged at 900 rpm for 6 min at 4 °C and diluted with advanced DMEM/F12 (Invitrogen) containing B27, N2, 1 μM n-Acetylcysteine, 10 mM HEPES, penicillin/streptomycin, and Glutamax (all from Invitrogen). Samples were passed through 100-μm filters (BD Biosciences) and centrifuged at 720 rpm for 5 min at 4°C, and single cells were discarded. Glands were embedded in Matrigel (provided from NCI) and 500 glands/well were seeded in a pre-warmed 24-well plate. After the Matrigel solidified, it was overlaid with advanced DMEM/F12 medium containing 50 ng/mL EGF (Invitrogen), 100 ng/mL Noggin (Peprotech), 1 μg/mL R-spondin 1, and 10 μg/mL Wnt3a. Growth factors were added every other day, and all medium contents were changed twice a week. Murine Cxcl12 (Peprotech), Wnt5a (Millipore), and Jagged-1 were added to the culture media in the indicated experiments. Organoids were treated with vehicle (DMSO) or 25 μM DAPT (Stengent) for 10 days in the indicated experiments. For $Lgr5^{+}$ cell ablation, DT was

added to the culture medium at 100 μg/mL. Gastric single cells were isolated and cultured as described previously (van Es et al., 2012). Glands were dissociated using TrypLE express (Invitrogen) including 1 mg/mL DNase I (Roche) for 10 min at 37 °C. Dissociated cells were passed through a 20-μm cell strainer and washed with 2 % FBS/PBS. Parietal cell isolation was performed as described previously (Hinkle et al., 2003). For the isolation of $Cxcr4^+$ cells and $Cxcl12^+$ cells, stomach was chopped into approximately 5 mm pieces, washed with cold HBSS, and incubated in 0.1 mg/ml dispase and 3.5 mg/ml collagenase 4 in HBSS for 30 min at 37 °C. The tissue fragments were suspended vigorously in 10 % FBS using a 10-mL pipette, and the supernatant was centrifuged at 1300 rpm for 5 min, resuspended in 5 ml of 40 % Percoll, and then centrifuged at 2000 g for 10 min, yielding the lymphocytes or endothelial cell fraction. Viable epithelial single cells were gated by forward scatter, side scatter and a pulse-width parameter, and propidium iodide-negative staining. For staining, cell suspensions were incubated with conjugated monoclonal antibodies against CD4 (RM4-5 or GK1.5), CD11b (M1/70), CD11c (N418), CD19 (6D5), CD31 (390), CD45 (30-F11), F4/80 (BM8), FcεRIα (MAR-1), c-kit (2B8), Gr-1 (RB6-8C5), ICOS (C398.4A), IL-7Ra (A7R34), KLRG1 (2F1), NK1.1 (PK136), NKp46 (29A1.4), Sca-1 (D7), CD90.2 (53-2.1), and lineage-cocktail antibodies (purchased from BD Biosciences, BioLegend, eBioscience or R&D Systems). For intracellular staining,

cells were fixed and permeabilized via a Foxp3 staining kit (eBioscience) and stained with an antibody to RORgt (eBioscience). Sorted cells were collected, pelleted and embedded in extracellular matrix, followed by seeding in a 48-well plate (100-3000 singlets per well). The images of gastric organoids were acquired using fluorescent microscopy (Nikon, TE2000-U) and two-photon microscopy (Nikon, A1RMP). $Cxcl12^{+}CD31^{+}$ cells were cultured on collagen-coated plates with mouse endothelial cell medium (Cell biologics).

**Histopathologic Analysis**

Stomach and other tissues from transgenic and control mice were fixed in either 10 % formalin or 4 % paraformaldehyde, embedded in paraffin or OCT, respectively, and cut into 5 μm sections. Immunohistochemistry and immunofluorescence were performed as described previously (Hayakawa et al., 2015). The primary antibodies used included the following: E-cadherin (1:100, Cell Signaling Technology), Ki67 (1:50, DAKO), TFF1 (1:100, Santa Cruz), lectin GS-II (1:200, Invitrogen), Intrinsic Factor (1:2000, a gift from Dr. D. Alpers, Washington University, USA), H/K-ATPase (1:500, Abcam), Cxcl12 (1:200, R&D), vWF (1:200, Dako), CD31 (1:200, Dako), beta-catenin (1:500, BD laboratories), BrdU (1:500, Dako), GFP (1:400, Invitrogen), GFP (1:400, Abcam), cleaved caspase-3 (1:100, Cell Signaling Technology), F4/80 (1:100, Abcam), αSMA (1:500, Abcam), and Mist1 (1:500, provided by Dr. Konieczny).

Subsequently, the sections were incubated with biotinylated secondary antibodies (Vectastain ABC kit; Vector Laboratories) for 30 min, followed by incubation with avidin-coupled peroxidase (Vector Laboratories) for 30 min. Diaminobenzidine (DAB; Dako) was used as the chromogen, and the slides were counterstained with Mayer's hematoxylin. For immunofluorescence, the slides were incubated with Alexa fluor 488 or 594 secondary antibodies (Invitrogen) and counterstained with 4', 6-diamidino-2-phenylindole (Vector Laboratories). For X-gal staining, a solution containing 4 % paraformaldehyde and 2 % glutaraldehyde in 0.1 M Sorensen's phosphate buffer (pH 7.4) (comprising 2 mM $MgCl_2$ and 5 mM EGTA) was perfused intracardially, the tissues were fixed in 4 % PFA at 4 °C for 2-3 hours and cryopreserved in 30 % sucrose before embedding in OCT compound. Tissues were sectioned on a cryostat for enzyme histochemical analysis. Frozen sections (5 µm) were washed (0.01 % sodium deoxycholate and 0.02 % Nonidet P-40) and incubated overnight at room temperature in a 0.1 % X-gal solution (4 % 4-chloro-5-bromo-3-indolyl-D-galactopyranoside (X-gal) dissolved in dimethylformamide, 5 mM K3Fe(CN)6, 5 mM K4Fe(CN)6.6$H_2O$). Sections were then counterstained with nuclear fast red and visualized using standard light microscopy. In situ hybridization was performed on paraffin-embedded sections using the

RNAScope 2.0 kit (Advanced Cell Diagnostics). Mouse and human DGC were diagnosed by veteran pathologists (A.R.S., H.T., and A.H.).

**Bone marrow transplantation**

Bone marrow cell suspensions were prepared from femurs and tibias, filtered, and counted. Recipient mice received a single intravenous injection of $1 \times 10^7$ bone marrow cells, after being irradiated with 10.5 Gy x-rays. For 4 weeks following the transplant, drinking water was replaced with water containing neomycin sulfate/polymyxin B sulfate mixture. Genomic DNA was extracted from spleen, and bone marrow chimerism was determined by PCR.

***Hf* infection**

Preparation of the *Helicobacter felis* (*Hf*) strain (ATCC 49179) used in this study has been described previously (Hayakawa et al., 2015). Briefly, the organism was grown for 48 hr at 37 °C under microaerobic conditions on 5 % sheep blood agar supplemented with antibiotics. The bacteria were harvested and aliquoted at a titer of $10^{10}$ organisms/mL in trypticase soy broth with 10 % glycerol, and the bacterial suspension was stored at -80 °C. Before use, aliquots were thawed, analyzed for motility, and cultured for evidence of aerobic or anaerobic microbial contamination. Mice were infected by oral gavage with *Hf* in 0.2 mL trypticase broth three times per week, achieving a total dose of 100 million colony-forming units (CFU)/mouse.

**Adenovirus treatment**

Adult (8 weeks old) male WT mice received single i.v. tail vein injection of 2 x $10^9$ pfu of the GFP- or DKK1-expressing vectors (Vector Biolabs).

**Quantitative RT-PCR (qRT-PCR) of proinflammatory cytokines**

Total RNA was extracted from whole stomach samples from each animal using TRIzol reagent (Invitrogen) or the RNAqueous-micro kit (Ambion) and subjected to first-strand complementary DNA synthesis using the Superscript III cDNA Amplification System (Invitrogen) following the manufacturer's instructions. qRT-PCR was performed using a three-step method, an ABI 7300 system and SYBR green (Roche). The qRT-PCR primer sequences are available upon request. The results were expressed as the copy number of each gene relative to that of *Gapdh*. For RT-PCR array, gene expression in gastric *Cxcl12*-dsRED$^+$CD31$^+$ cells and *Cxcl12*-dsRED$^-$CD31$^+$ cells was analyzed by RT$^2$ Profiler$^{TM}$ PCR Array Mouse Transcriptional Factors. n = 2/group. RT² Profiler™ PCR Array Mouse Transcription Factors (SABiosciences) was performed as manufacturer's instructions.

**Electron Microscopy**

Murine corpus tissue was fixed by immersion for 3 hr at room temperature (RT) in a solution of 4 % formaldehyde (from paraformaldehyde) and 0.1 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) containing 6 % sucrose. Fixed specimens were

embedded in 20 % gelatin and 70 µm sections were cut using a vibratome. The sections were incubated for 30 min at RT in a blocking solution containing 10 % normal goat serum in phosphate buffered saline (PBS) followed by 48 hr at 4 °C with rabbit antibodies targeting dsRED (diluted 1:50) in PBS containing 10 % goat serum. Bound primary antibodies were detected using biotinylated secondary antibodies and avidin coupled to horseradish peroxidase (HRP; Vector ABC elite kit). Peroxidase activity was visualized using 3-3'-diaminobenzine (DAB) and glucose/glucose oxidase to generate the peroxide substrate. Sections were washed with PBS, treated with 1 % $OsO_4$ for 1 hr at RT, washed again with PBS and maleate buffer (3 x 5 min) and stained en bloc for 1 hr with iced 2 % aqueous uranyl acetate. The treated sections were finally dehydrated through a graded ethanol series, cleared with propylene oxide, and embedded in an epoxy resin (Epon 812; Electron Microscopy Sciences). Thin (silver) sections were cut, placed on copper grids and examined using a JEOL 1200EX electron microscope.

**Western blotting and immunoprecipitation**

Protein lysates were prepared from cells, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene difluoride membranes (Millipore). The membrane was probed with primary antibodies, and then incubated with the secondary antibody.

Immunocomplexes were detected using the enhanced chemiluminescence system (Amersham Biosciences). For immunoprecipitation, samples were lysed in radioimmunoprecipitation assay buffer and immunoprecipitated with RhoA activation detection kit (Abcam). The beads were washed for 3 times with radioimmunoprecipitation buffer and then analyzed by SDS-PAGE.

**Cell line culture and soft agar assay**

The human cancer cell lines were cultured in Ham's F-12 or IMEM medium supplemented with 10 % fetal bovine serum. Soft agar assay was performed as described previously (Kakiuchi et al., 2014).

**Statistical Analysis**

The differences between the means were compared using either the Student's *t*-test or the Wilcoxon test. p values $< 0.05$ were considered to indicate statistical significance.

**Supplemental References**

Hinkle, K.L., Bane, G.C., Jazayeri, A., and Samuelson, L.C. (2003). Enhanced calcium signaling and acid secretion in parietal cells isolated from gastrin-deficient mice. Am J Physiol Gastrointest Liver Physiol *284*, G145-153.

van Es, J.H., Sato, T., van de Wetering, M., Lyubimova, A., Nee, A.N., Gregorieff, A., Sasaki, N., Zeinstra, L., van den Born, M., Korving, J.*, et al.* (2012). Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. Nat Cell Biol *14*, 1099-1104.