Figure S1

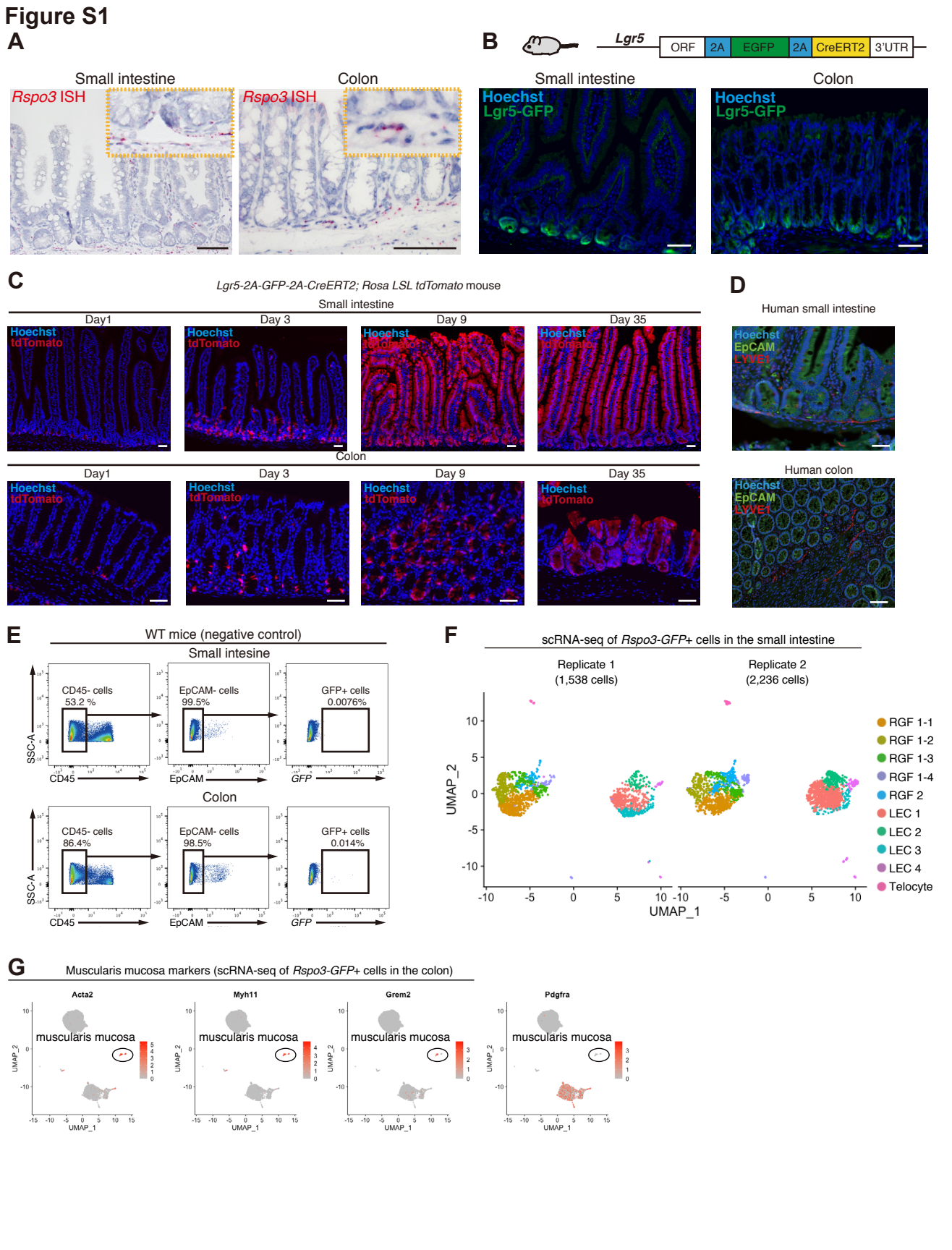

A
Small intestine
Rspo3 ISH
Colon
Rspo3 ISH
B
Lgr5
ORF
2A
EGFP
2A
CreERT2
3'UTR
Small intestine
Hoechst
Lgr5-GFP
Colon
Hoechst
Lgr5-GFP
C
Lgr5-2A-GFP-2A-CreERT2; Rosa LSL tdTomato mouse
Small intestine
Day1
Day 3
Day 9
Day 35
Hoechst
tdTomato
Colon
Day1
Day 3
Day 9
Day 35
Hoechst
tdTomato
D
Human small intestine
Hoechst
EpCAM
LYVE1
Human colon
Hoechst
EpCAM
LYVE1
E
WT mice (negative control)
Small intesine
CD45- cells
53.2 %
EpCAM- cells
99.5%
GFP+ cells
0.0076%
SSC-A
CD45
EpCAM
GFP
Colon
CD45- cells
86.4%
EpCAM- cells
98.5%
GFP+ cells
0.014%
SSC-A
CD45
EpCAM
GFP
F
scRNA-seq of Rspo3-GFP+ cells in the small intestine
Replicate 1
(1,538 cells)
Replicate 2
(2,236 cells)
UMAP_2
UMAP_1
RGF 1-1
RGF 1-2
RGF 1-3
RGF 1-4
RGF 2
LEC 1
LEC 2
LEC 3
LEC 4
Telocyte
G
Muscularis mucosa markers (scRNA-seq of Rspo3-GFP+ cells in the colon)
Acta2
Myh11
Grem2
Pdgfra
muscularis mucosa
UMAP_2
UMAP_1

Figure S1. RSPO3+ LECs reside near Lgr5+ ISCs, Related to Figure 1 and Figure 2

(A) *Rspo3* mRNA expression in the small intestine and colon by ISH.

(B) Schematic (top) of new *Lgr5-2A-GFP-2A-CreERT2* mice. Immunofluorescence (IF) shows that *Lgr5-GFP* is expressed in nearly all the crypts of both the small intestine and colon. The image represents one of 6 biological replicates.

(C) Lineage tracing using *Lgr5-2A-GFP-2A-CreERT2; Rosa-LSL-tdTomato* mice reveals that *Lgr5-GFP*+ cells self-renew for the long-term and give rise to differentiated progeny cells of the small intestine and colon. The image represents one of 3 biological replicates.

(D) IF for LYVE1 and EpCAM in the human small intestine and colon. Related to Figure 1D.

(E) Control (mouse without *Rspo3-GFP*) FACS plot to show that GFP+ cells of the *Rspo3-GFP* mice in Figure 1E are not detected in control mice. Related to Figure 1E.

(F) Individual UMAP plots for each replicate of scRNA-seq of the small intestinal *Rspo3-GFP*+ stromal cells demonstrate the consistent clustering patterns among the replicates. Related to Figure 2A.

(G) Relative expression of *Acta2*, *Myh11*, and *Grem2* (muscularis mucosa cell marker genes), and *Pdgfra* (negative marker for muscularis mucosa cells) onto the UMAP plot of scRNA-seq of colonic *Rspo3-GFP*+ cells. Related to Figure 2E.

Scale bar, 50 μm (A, B, C, D).

Figure S2

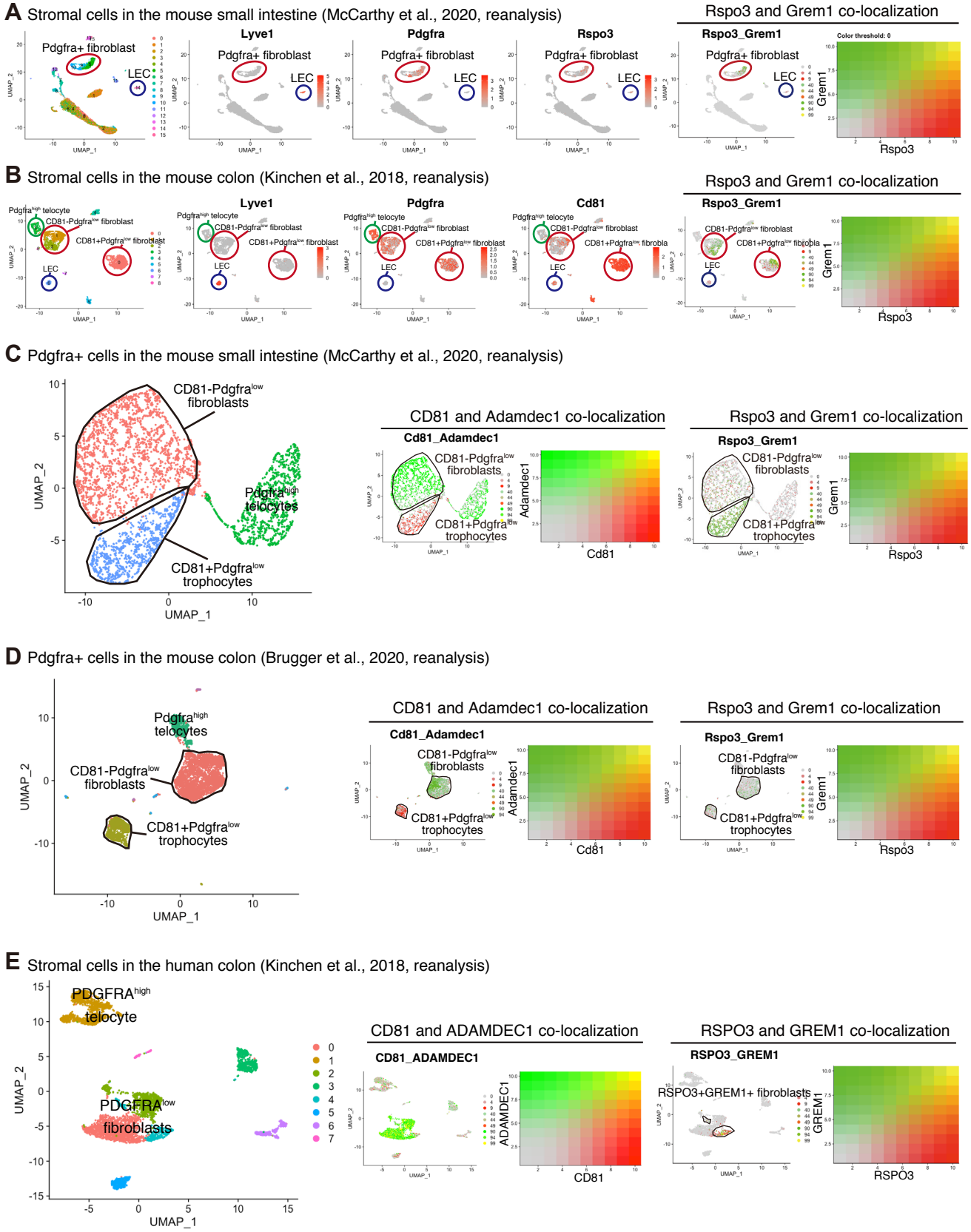

A Stromal cells in the mouse small intestine (McCarthy et al., 2020, reanalysis)
Pdgfra+ fibroblast
LEC
Lyve1
Pdgfra
Rspo3
Rspo3 and Grem1 co-localization
Rspo3_Grem1
Color threshold: 0
Grem1
Rspo3
B Stromal cells in the mouse colon (Kinchen et al., 2018, reanalysis)
Pdgfra^high telocyte
CD81-Pdgfra^low fibroblast
CD81+Pdgfra^low fibroblast
Cd81
C Pdgfra+ cells in the mouse small intestine (McCarthy et al., 2020, reanalysis)
CD81-Pdgfra^low fibroblasts
Pdgfra^high telocytes
CD81+Pdgfra^low trophocytes
CD81 and Adamdec1 co-localization
Cd81_Adamdec1
Adamdec1
Cd81
D Pdgfra+ cells in the mouse colon (Brugger et al., 2020, reanalysis)
E Stromal cells in the human colon (Kinchen et al., 2018, reanalysis)
PDGFRA^high telocyte
PDGFRA^low fibroblasts
CD81 and ADAMDEC1 co-localization
CD81_ADAMDEC1
ADAMDEC1
CD81
RSPO3 and GREM1 co-localization
RSPO3_GREM1
RSPO3+GREM1+ fibroblasts
GREM1
RSPO3
UMAP_1
UMAP_2

Figure S2. RSPO3 expression in the small intestinal and colonic stromal cells (reanalysis of public datasets). Related to Figure 1 and Figure 2

(A) Uniform manifold approximation and projection (UMAP) of scRNA-seq of small intestinal stromal cells (McCarthy et al., 2020, reanalysis). *Rspo3* is expressed by *Lyve1*+ LEC cluster and by a subset of *Pdgfra*+ fibroblast cluster. *Rspo3*+ cells in the *Pdgfra*+ fibroblasts cluster co-express *Grem1*.

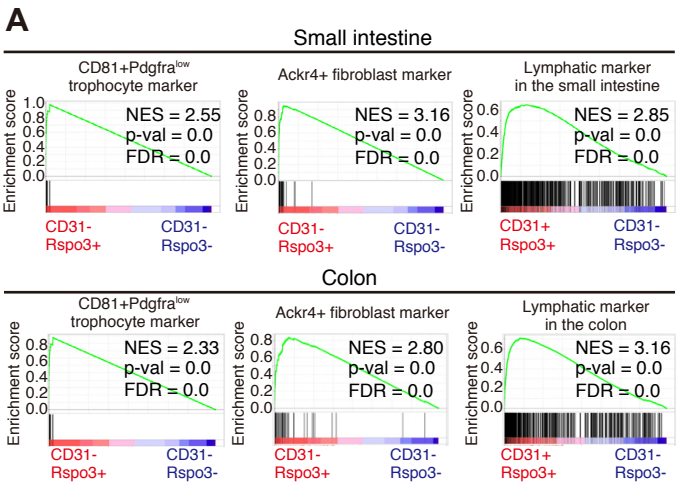
(B) UMAP of scRNA-seq of colonic stromal cells (Kinchen et al., 2018, reanalysis). *Rspo3* is expressed by *Lyve1*+ LEC cluster and by a subset of *Pdgfra*^{low} fibroblasts (both *Cd81-Pdgfra*^{low} fibroblasts and *Cd81+Pdgfra*^{low} fibroblasts). A subset of *Rspo3*+ cells in the *Pdgfra*^{low} fibroblast clusters co-express *Grem1*.

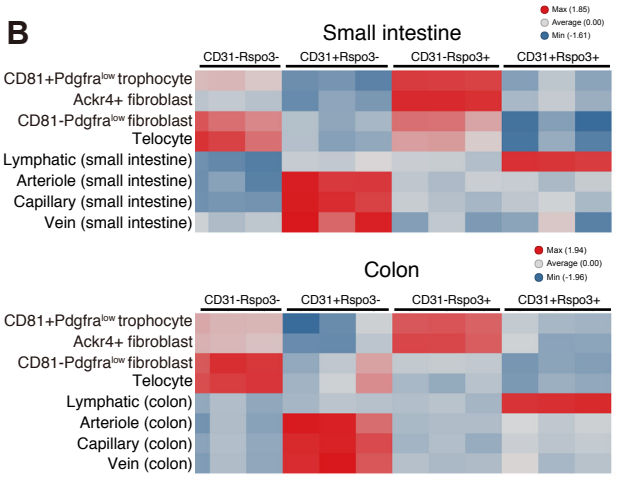
(C) UMAP of scRNA-seq of small intestinal *Pdgfra*+ cells (McCarthy et al., 2020, reanalysis). The expressions of *Cd81* and *Adamdec1* are mutually exclusive. *Rspo3*+ cells are detected both in the *Cd81*$^{+}$*Adamdec1*$^{-}$*Pdgfra*^{low} trophocyte and *Cd81*$^{-}$*Adamdec1*$^{+}$*Pdgfra*^{low} fibroblast clusters, and these *Rspo3*+ cells frequently co-express *Grem1*.

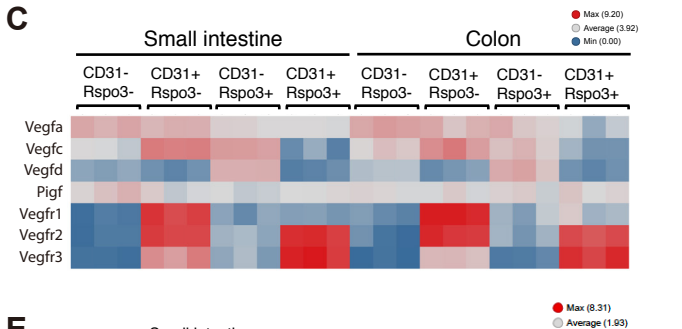
(D) UMAP of scRNA-seq of colonic *Pdgfra*+ cells (Brugger et al., 2020, reanalysis). The expressions of *Cd81* and *Adamdec1* are mutually exclusive. *Rspo3*+ cells are detected both in the *Cd81*$^{+}$*Adamdec1*$^{-}$*Pdgfra*^{low} trophocyte and *Cd81*$^{-}$*Adamdec1*$^{+}$*Pdgfra*^{low} fibroblast clusters, and these *Rspo3*+ cells frequently co-express *Grem1*.

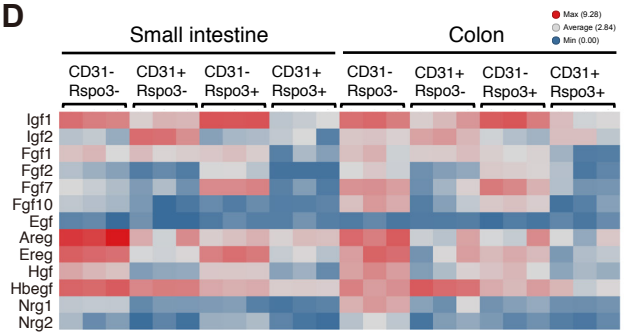
(E) UMAP of scRNA-seq of human colonic stromal cells (Kinchen et al., 2018, reanalysis). The expressions of *CD81* and *ADAMDEC1* are ubiquitous in *PDGFRA*^{low} fibroblasts and not mutually exclusive. *RSPO3*+*GREM1*+ cells are detected in a subset of *PDGFRA*^{low} fibroblasts.

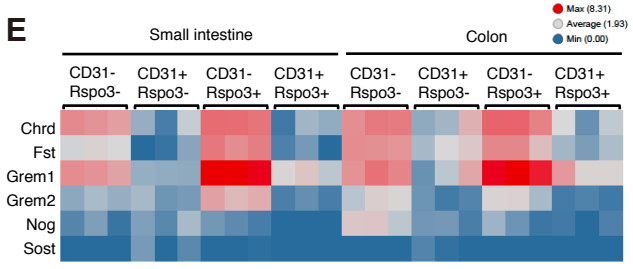
Figure S3

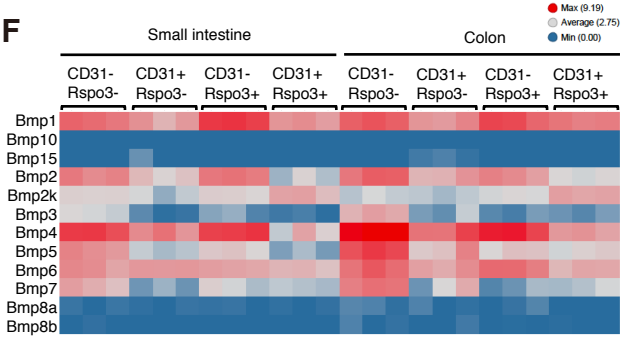

A
Small intestine
CD81+Pdgfra^low trophocyte marker
NES = 2.55
p-val = 0.0
FDR = 0.0
Ackr4+ fibroblast marker
NES = 3.16
p-val = 0.0
FDR = 0.0
Lymphatic marker in the small intestine
NES = 2.85
p-val = 0.0
FDR = 0.0
Enrichment score
CD31- Rspo3+
CD31- Rspo3-
CD31+ Rspo3+
Colon
CD81+Pdgfra^low trophocyte marker
NES = 2.33
p-val = 0.0
FDR = 0.0
Ackr4+ fibroblast marker
NES = 2.80
p-val = 0.0
FDR = 0.0
Lymphatic marker in the colon
NES = 3.16
p-val = 0.0
FDR = 0.0

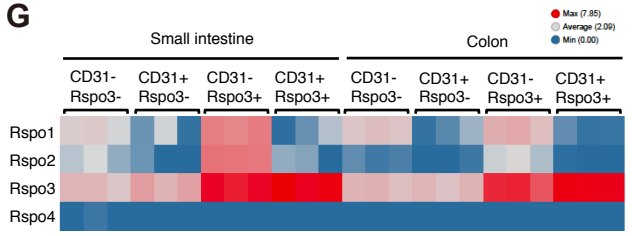

B
Small intestine
Colon
CD31-Rspo3-
CD31+Rspo3-
CD31-Rspo3+
CD31+Rspo3+
CD81+Pdgfra^low trophocyte
Ackr4+ fibroblast
CD81-Pdgfra^low fibroblast
Telocyte
Lymphatic (small intestine)
Arteriole (small intestine)
Capillary (small intestine)
Vein (small intestine)
Lymphatic (colon)
Arteriole (colon)
Capillary (colon)
Vein (colon)

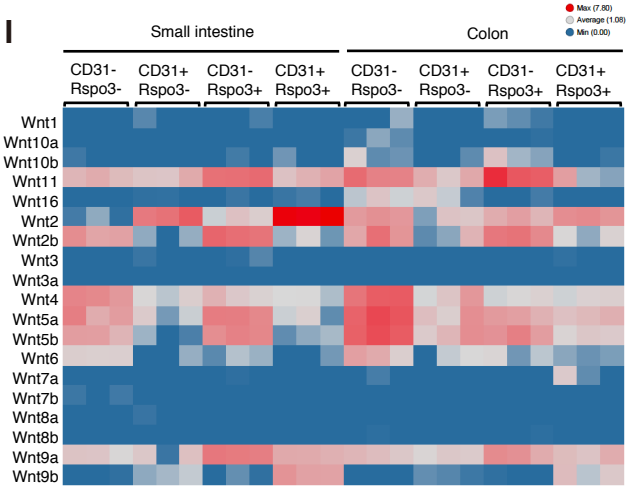

C
Small intestine
Colon
CD31- Rspo3-
CD31+ Rspo3-
CD31- Rspo3+
CD31+ Rspo3+
Vegfa
Vegfc
Vegfd
Pigf
Vegfr1
Vegfr2
Vegfr3


D
Small intestine
Colon
Igf1
Igf2
Fgf1
Fgf2
Fgf7
Fgf10
Egf
Areg
Ereg
Hgf
Hbegf
Nrg1
Nrg2


E
Small intestine
Colon
Chrd
Fst
Grem1
Grem2
Nog
Sost


F
Small intestine
Colon
Bmp1
Bmp10
Bmp15
Bmp2
Bmp2k
Bmp3
Bmp4
Bmp5
Bmp6
Bmp7
Bmp8a
Bmp8b


G
Small intestine
Colon
Rspo1
Rspo2
Rspo3
Rspo4


I
Small intestine
Colon
Wnt1
Wnt10a
Wnt10b
Wnt11
Wnt16
Wnt2
Wnt2b
Wnt3
Wnt3a
Wnt4
Wnt5a
Wnt5b
Wnt6
Wnt7a
Wnt7b
Wnt8a
Wnt8b
Wnt9a
Wnt9b

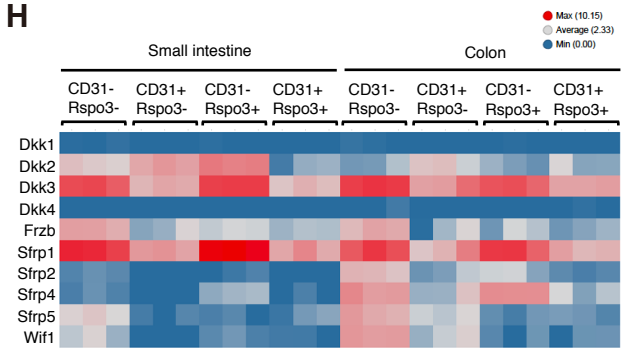

H
Small intestine
Colon
Dkk1
Dkk2
Dkk3
Dkk4
Frzb
Sfrp1
Sfrp2
Sfrp4
Sfrp5
Wif1

Figure S3. RNA-seq on CD31-Rspo3-GFP-, CD31+Rspo3-GFP-, CD31-Rspo3-GFP+, and CD31+Rspo3-GFP+ cells, Related to Figure 3

(A) GSEA of $CD81^{+}Pdgfra^{low}$ trophocyte markers, Ackr4+ fibroblast markers, and lymphatic markers. FDR, false-discovery rate; NES, normalized enrichment score.

(B) The heatmap of single sample GSEA (ssGSEA).

(C-I) The heatmap of gene expressions for angiogenic factors (C), growth factors (D), BMPi (E), BMP (F), RSPO (G), WNT antagonist (H), and WNT (I) using RNA-seq on CD31-*Rspo3-GFP*-, CD31+*Rspo3-GFP*-, CD31-*Rspo3-GFP*+, and CD31+*Rspo3-GFP*+ cells from the small intestine and colon.

n = 3 mice per group.

Figure S4

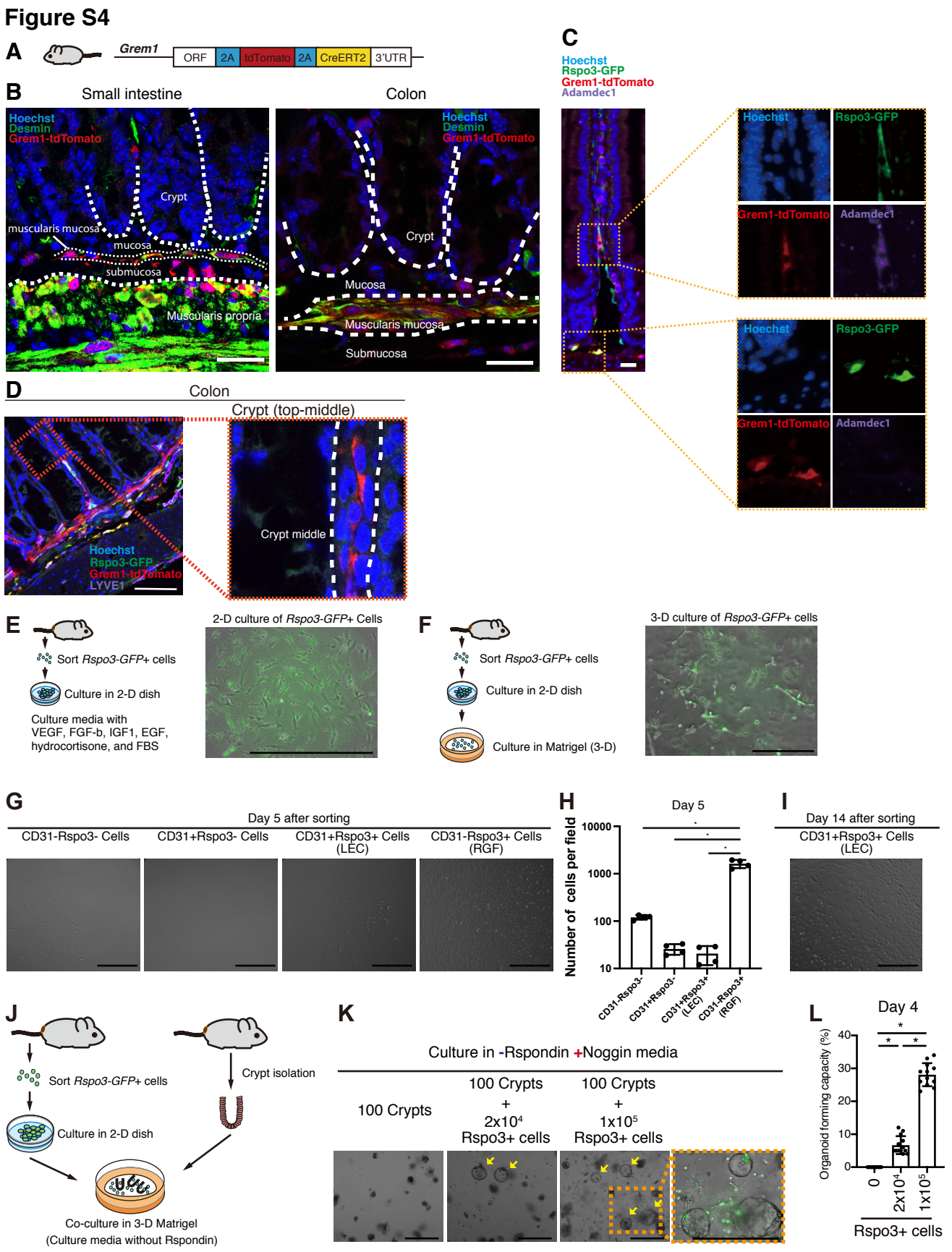

A
Grem1
ORF
2A
tdTomato
2A
CreERT2
3'UTR
B
Small intestine
Colon
Hoechst
Desmin
Grem1-tdTomato
Crypt
muscularis mucosa
mucosa
submucosa
Muscularis propria
Mucosa
Submucosa
C
Hoechst
Rspo3-GFP
Grem1-tdTomato
Adamdec1
D
Colon
Crypt (top-middle)
Crypt middle
LYVE1
E
Sort Rspo3-GFP+ cells
Culture in 2-D dish
Culture media with VEGF, FGF-b, IGF1, EGF, hydrocortisone, and FBS
2-D culture of Rspo3-GFP+ Cells
F
Sort Rspo3-GFP+ cells
Culture in 2-D dish
Culture in Matrigel (3-D)
3-D culture of Rspo3-GFP+ cells
G
Day 5 after sorting
CD31-Rspo3- Cells
CD31+Rspo3- Cells
CD31+Rspo3+ Cells (LEC)
CD31-Rspo3+ Cells (RGF)
H
Day 5
Number of cells per field
10000
1000
100
10
CD31-Rspo3-
CD31+Rspo3-
CD31+Rspo3+ (LEC)
CD31-Rspo3+ (RGF)
I
Day 14 after sorting
CD31+Rspo3+ Cells (LEC)
J
Sort Rspo3-GFP+ cells
Culture in 2-D dish
Crypt isolation
Co-culture in 3-D Matrigel
(Culture media without Rspondin)
K
Culture in -Rspondin +Noggin media
100 Crypts
100 Crypts + 2x10^4 Rspo3+ cells
100 Crypts + 1x10^5 Rspo3+ cells
L
Day 4
Organoid forming capacity (%)
40
30
20
10
0
0
2x10^4
1x10^5
Rspo3+ cells

Figure S4. Expression pattern of GREM1 and RSPO3 and establishment of RSPO3+ stromal cell culture, Related to Figure 3 and Figure 4

(A) Schematic of *Grem1-tdTomato-CreERT2* mouse.

(B) Confocal microscopy of immunofluorescence (IF) for *Grem1-tdTomato* and Desmin, showing that *Grem1-tdTomato* is expressed by a subset of lamina propria and submucosal stromal cells, but is also strongly expressed by Desmin+ muscularis propria and muscularis mucosa cells.

(C) IF for *Rspo3-GFP*, *Grem1-tdTomato*, and Adamdec1 reveals that RGFs that are close to the crypt bottoms do not express Adamdec1 whereas RGFs infrequently detected in the villus core next to lacteals co-express Adamdec1.

(D) GREM1+RSPO3- cells at the middle-top zone of colonic crypts. lower magnification image is adapted from Figure 3E.

(E-F) 2-D (E) and 3-D (F) culture of sorted *Rspo3-GFP*+ stromal cells from the small intestine.

(G-H) Images (G) and quantification (H) of 2-D culture of sorted CD31$^-$*Rspo3-GFP*$^-$, CD31$^+$*Rspo3-GFP*$^-$, CD31$^+$*Rspo3-GFP*$^+$, and CD31$^-$*Rspo3-GFP*$^+$ cells from the small intestine at day 5.

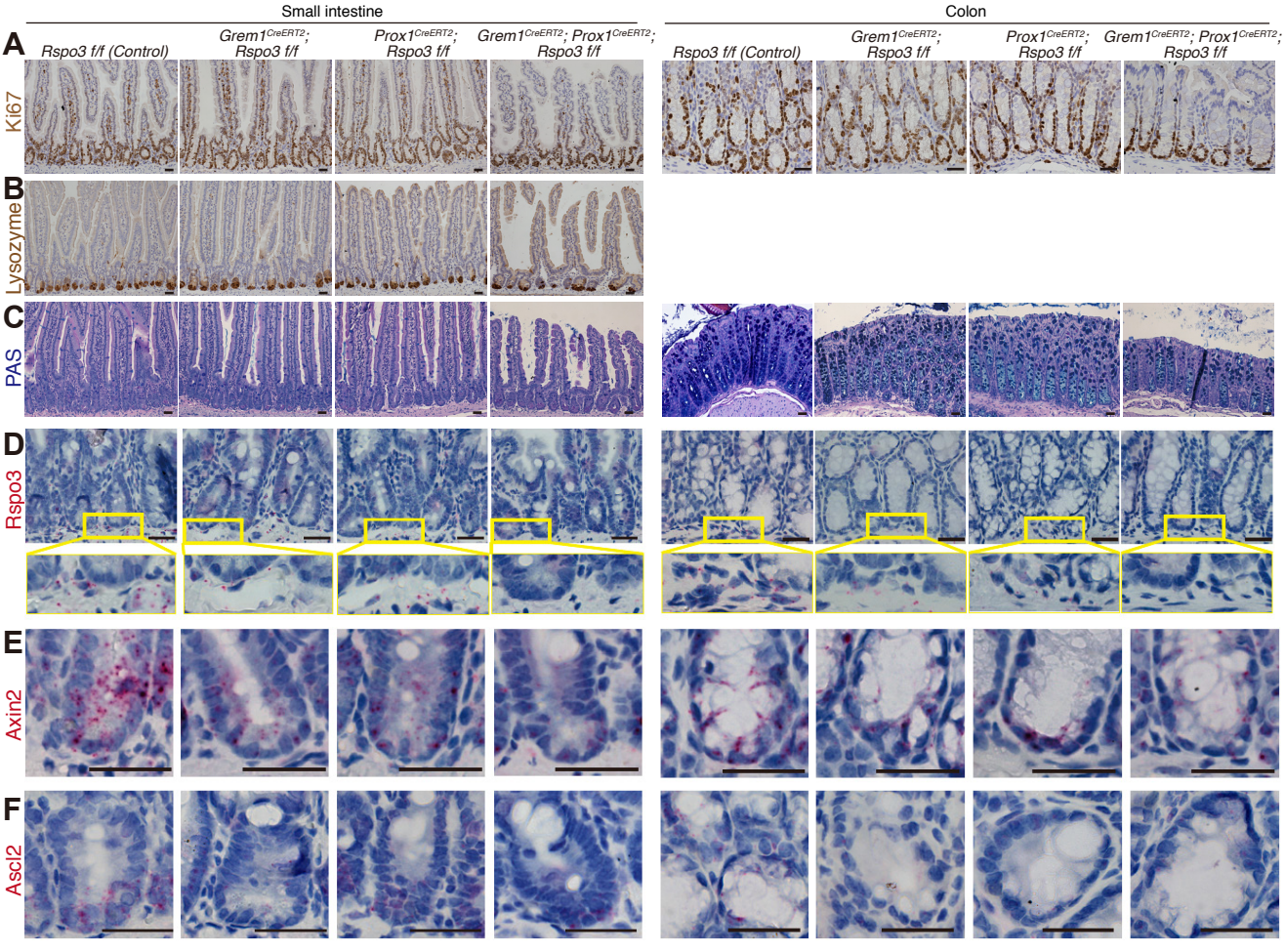
(I) Image of 2-D culture of CD31$^+$*Rspo3-GFP*$^+$ cells (LECs) from the small intestine at day 14 shows the stable expansion of LECs.

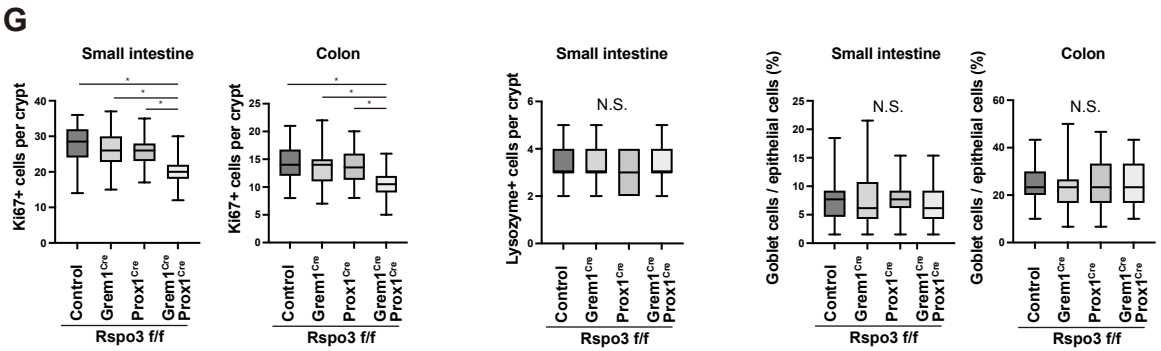
(J) Schematic of heterotypic co-culture of RSPO3+ stromal cells and the intestinal epithelial crypts.

(K-L) Representative images (K) and quantification (L) of co-culture of small intestinal RSPO3+ stromal cells (0, $2x10^4$, $1x10^5$) and the crypts in the culture media supplemented with Noggin, but not with RSPO. n = 12 - 13 from 3 mice per group. Arrows indicate organoid formation.

One-way analysis of variance (ANOVA) with post-hoc Tukey's multiple comparison (H, L). Data are mean ± SD. *$p < 0.05$. Scale bar, 20 μm (B, C, E, F, G, I, K), 50 μm (D).

Figure S5


Small intestine
Colon
A
Rspo3 f/f (Control)
Grem1CreERT2; Rspo3 f/f
Prox1CreERT2; Rspo3 f/f
Grem1CreERT2; Prox1CreERT2; Rspo3 f/f
Ki67
B
Lysozyme
C
PAS
D
Rspo3
E
Axin2
F
Ascl2


G
Small intestine
Colon
Ki67+ cells per crypt
Lysozyme+ cells per crypt
N.S.
Goblet cells / epithelial cells (%)
Control
Grem1Cre
Prox1Cre
Grem1Cre Prox1Cre
Rspo3 f/f

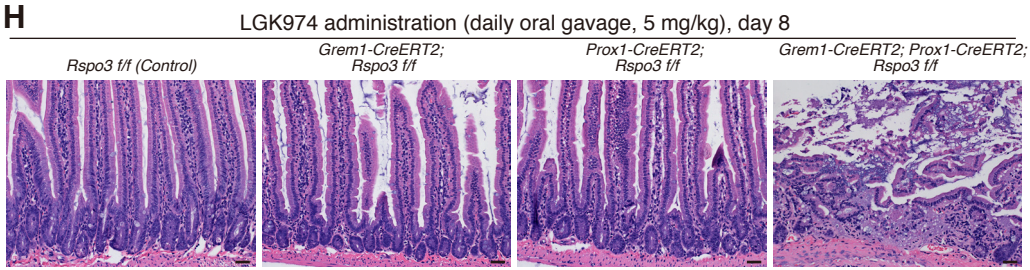

H
LGK974 administration (daily oral gavage, 5 mg/kg), day 8
Rspo3 f/f (Control)
Grem1-CreERT2; Rspo3 f/f
Prox1-CreERT2; Rspo3 f/f
Grem1-CreERT2; Prox1-CreERT2; Rspo3 f/f

Figure S5. Niche RSPO3 loss reduces WNT signaling and proliferation in the crypt, Related to Figure 5.

(A-G) Comprehensive histochemical and immunohistochemical/fluorescence analyses in the small intestine and colon after inducing RSPO3 loss in LECs, RGFs, or both. Immunohistochemistry for Ki67 (A) and Lysozyme (B); PAS staining (C); ISH for Rspo3 (D), Axin2 (E), and Ascl2 (F). Quantification of Ki67+ cells (per crypt), Lysozyme+ cells (per crypt), and goblet cells (percentage in the epithelial cells) (G) (n = 50 from 6 mice per group).

(H) Porcupine inhibitor LGK974 was administered (daily oral gavage, 5 mg/kg) to the mice after RSPO3 deletion. Whereas control mice or the mice with partial RSPO3 stromal loss (i.e., *Grem1-CreERT2; Rspo3 f/f* mice or *Prox1-CreERT2; Rspo3 f/f* mice) did not show any architectural changes, mice with complete RSPO3 stromal loss (i.e., *Grem1-CreERT2; Prox1-CreERT2; Rspo3 f/f* mice) illustrated significant crypt degeneration/drop-out in the intestines on day 8 of treatment. The image represents one of 4 biological replicates per group.

One-way analysis of variance (ANOVA) with post-hoc Tukey's multiple comparison (G). For box-and-whisker plots (G), data were expressed as box-and-whisker from the minimum to the maximum. *$p < 0.05$. N.S. not significant. Scale bar, 20 μm (A, B, C, D, E, F, H).

Figure S6

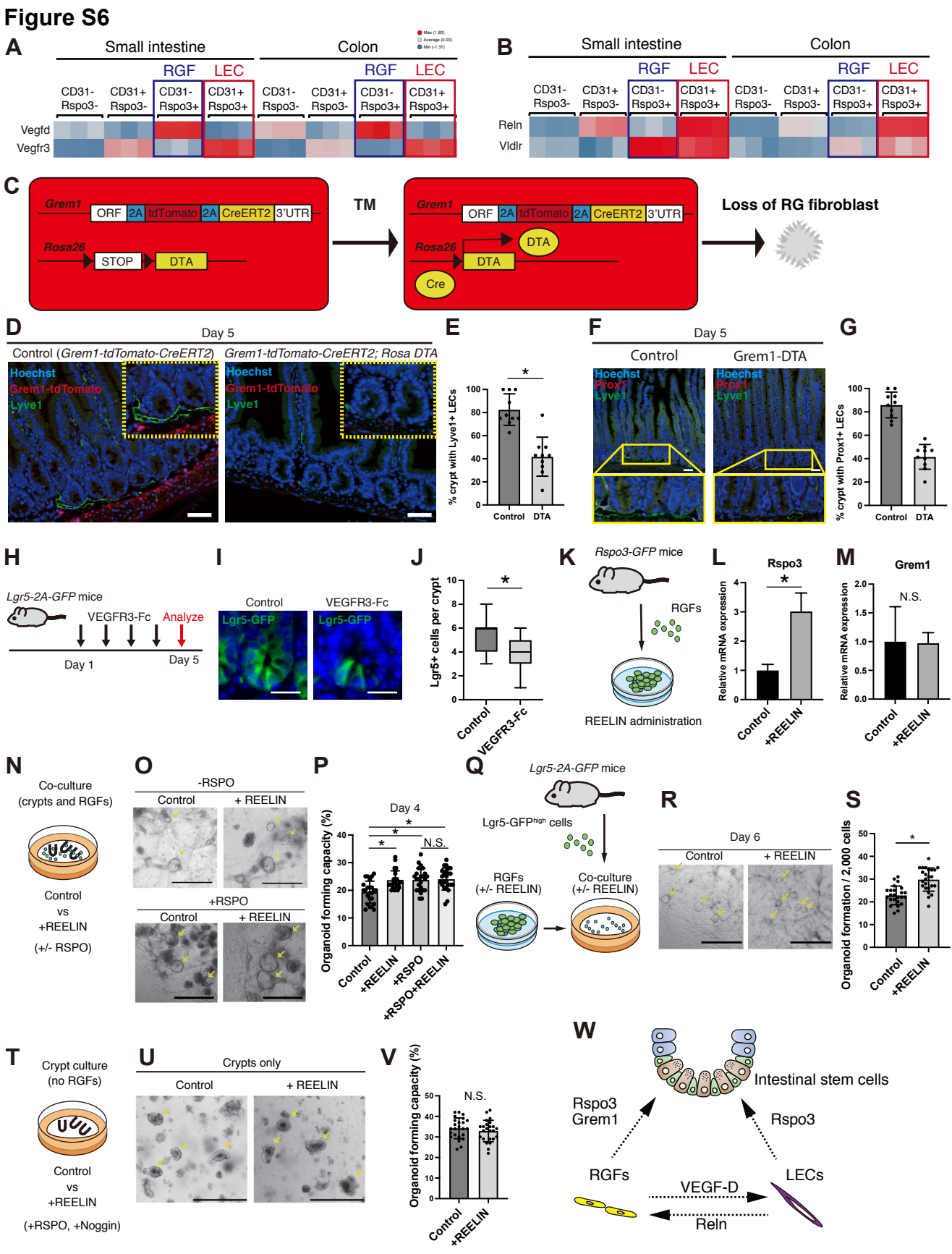

A
Small intestine
Colon
RGF
LEC
CD31- Rspo3-
CD31+ Rspo3-
CD31- Rspo3+
CD31+ Rspo3+
Vegfd
Vegfr3
B
Reln
Vldlr
C
Grem1
ORF
2A
tdTomato
CreERT2
3'UTR
Rosa26
STOP
DTA
TM
Cre
Loss of RG fibroblast
D
Day 5
Control (Grem1-tdTomato-CreERT2)
Grem1-tdTomato-CreERT2; Rosa DTA
Hoechst
Grem1-tdTomato
Lyve1
E
% crypt with Lyve1+ LECs
Control
DTA
F
Day 5
Control
Grem1-DTA
Hoechst
Prox1
Lyve1
G
% crypt with Prox1+ LECs
H
Lgr5-2A-GFP mice
VEGFR3-Fc
Analyze
Day 1
Day 5
I
Control
VEGFR3-Fc
Lgr5-GFP
J
Lgr5+ cells per crypt
Control
VEGFR3-Fc
K
Rspo3-GFP mice
RGFs
REELIN administration
L
Rspo3
Relative mRNA expression
Control
+REELIN
M
Grem1
N.S.
N
Co-culture
(crypts and RGFs)
Control
vs
+REELIN
(+/- RSPO)
O
-RSPO
Control
+ REELIN
+RSPO
P
Day 4
Organoid forming capacity (%)
Control
+REELIN
+RSPO
+RSPO+REELIN
Q
Lgr5-2A-GFP mice
Lgr5-GFPhigh cells
RGFs
(+/- REELIN)
Co-culture
(+/- REELIN)
R
Day 6
S
Organoid formation / 2,000 cells
T
Crypt culture
(no RGFs)
Control
vs
+REELIN
(+RSPO, +Noggin)
U
Crypts only
V
W
Intestinal stem cells
Rspo3
Grem1
RGFs
LECs
VEGF-D
Reln

Figure S6. Cross-regulation between RGFs and LECs, Related to Figure 5

(A-B) Heatmap of RNA-seq on CD31-*Rspo3-GFP-,* CD31+*Rspo3-GFP-,* CD31-*Rspo3-GFP+,* and CD31+*Rspo3-GFP+* cells in the small intestine and colon. RGFs express *Vegfd*; LECs express its receptor (*Vegfr3*) (A). LECs express *Reln*; RGFs express its receptor (*Vldlr*) (B). n = 3 mice per group.

(C) Schematic for RG fibroblast ablation experiment.

(D-G) Immunofluorescence for *Grem1-tdTomato* and LYVE1 (D), Prox1 and LYVE1 (F) and the percentage of crypts with LYVE+ LECs (E) or Prox1+ LECs (G) in *Grem1-tdTomato-CreERT2* (control) and *Grem1-tdTomato-CreERT2; Rosa DTA* mice. n = 10.

(H) Schematic for soluble VEGFR3 receptor administration experiment.

(I-J) Representative images (I) and quantification (J) of *Lgr5-GFP*+ cells after soluble VEGFR3 receptor administration. n = 50 crypts from 4 mice per group.

(K) Schematic for the treatment of RGF culture with REELIN.

(L-M) qRT-PCR of *Rspo3* and *Grem1* in RGFs after REELIN treatment. n = 6 and n = 3, respectively.

(N) Schematic of REELIN treatment to the co-culture of crypts and RGFs in media with/without RSPO.

(O-P) Representative images (O) and quantification of organoid forming capacity (P) in the co-culture of crypts and RGFs in media with/without REELIN and with/without RSPO. n = 24 from 3 mice per group.

(Q) Schematic of co-culture experiments of sorted Lgr5-GFP^{high} ISCs and REELIN-pretreated RGFs.

(R-S) *Lgr5-GFP*^{high} ISC-derived organoid formation is modestly elevated when RGFs were pretreated with REELIN. Representative images (R) and quantification (S). n = 24 from 3 mice per group.

(T) Schematic of REELIN treatment to crypt culture.

(U-V) Representative images (U) and quantification of organoid forming capacity (V) in the crypt culture with Noggin and RSPO supplementation with or without REELIN administration. n = 24 from 3 mice per group.

(W) Schematic of proposed cross-regulation between RGFs and LECs.

Unpaired two-tailed t-tests (E, G, J, L, M, S, V). One-way ANOVA (P). Data are mean ± SD (E, G, P, S, V) and mean ± SEM (L, M). For box-and-whisker plots (J), data were expressed as box-and-whisker from the minimum to the maximum. *$p < 0.05$. N.S. not significant. Scale bar, 50 μm (D, F, I), 20 μm (O, R, U).

Figure S7

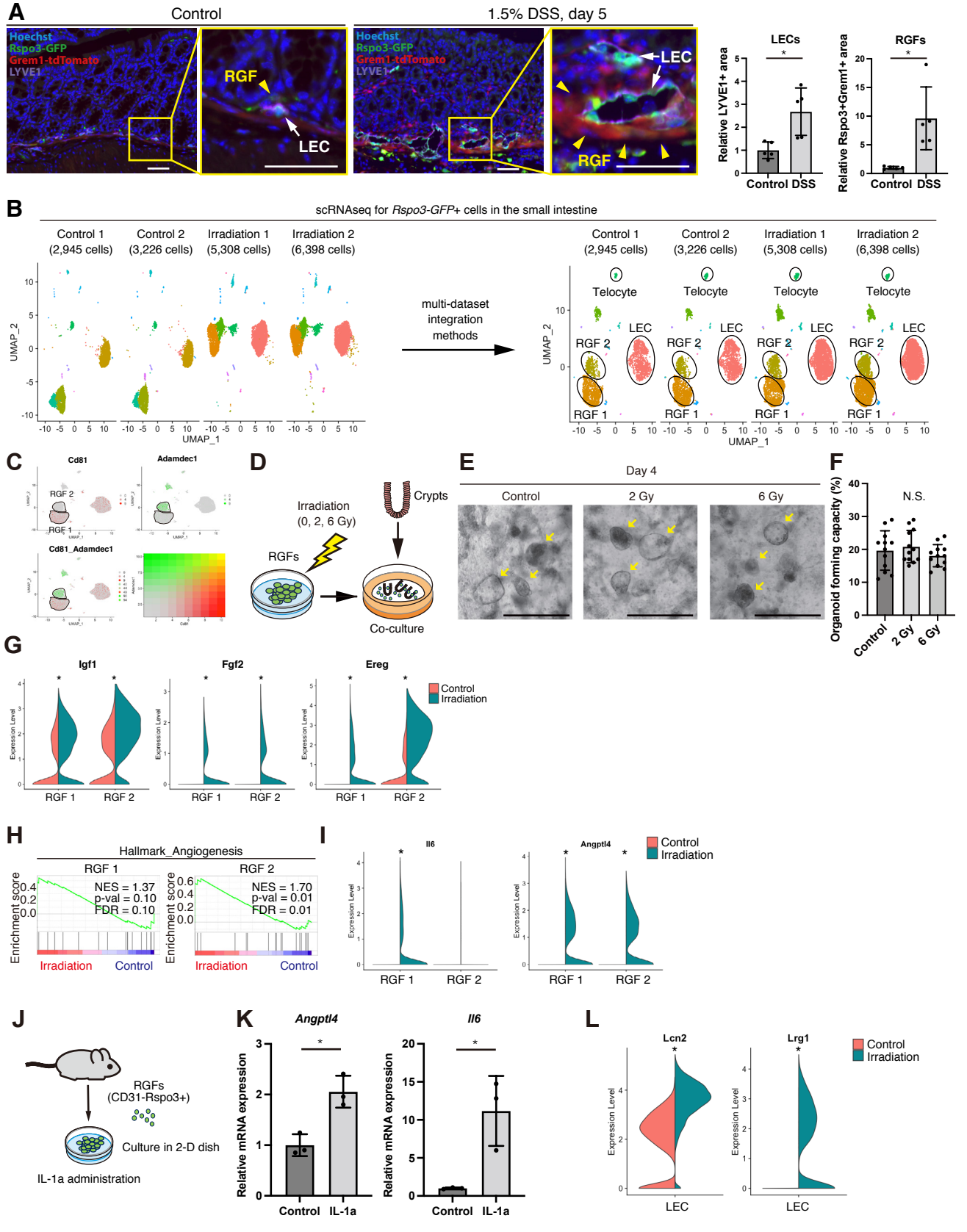

A
Control
1.5% DSS, day 5
Hoechst
Rspo3-GFP
Grem1-tdTomato
LYVE1
RGF
LEC
LECs
RGFs
Relative LYVE1+ area
Relative Rspo3+Grem1+ area
Control DSS
B
scRNAseq for Rspo3-GFP+ cells in the small intestine
Control 1 (2,945 cells)
Control 2 (3,226 cells)
Irradiation 1 (5,308 cells)
Irradiation 2 (6,398 cells)
UMAP_1
UMAP_2
multi-dataset integration methods
Telocyte
LEC
RGF 1
RGF 2
C
Cd81
Adamdec1
Cd81_Adamdec1
D
Irradiation (0, 2, 6 Gy)
RGFs
Crypts
Co-culture
E
Day 4
Control
2 Gy
6 Gy
F
N.S.
Organoid forming capacity (%)
G
Igf1
Fgf2
Ereg
Control
Irradiation
Expression Level
H
Hallmark_Angiogenesis
NES = 1.37
p-val = 0.10
FDR = 0.10
NES = 1.70
p-val = 0.01
FDR = 0.01
Enrichment score
I
Il6
Angptl4
J
RGFs (CD31-Rspo3+)
Culture in 2-D dish
IL-1a administration
K
Angptl4
Il6
Relative mRNA expression
IL-1a
L
Lcn2
Lrg1

Figure S7. Roles of LECs and RGFs in normal homeostasis and regeneration after injury, Related to Figure 6 and Figure 7

(A) Immunofluorescence for *Rspo3-GFP*, *Grem1-tdTomato*, and LYVE1 in the DSS-induced colitis model at day 6 of 1.5% DSS administration. Yellow arrowheads indicate RGFs. White arrows indicate LECs. LYVE1+ areas (LECs) and *Rspo3-GFP*+*Grem1-tdTomato*+ areas (RGFs) were quantified. (n = 5 fields from 3 mice per group)

(B) Individual UMAP plots of scRNA-seq of small intestinal *Rspo3-GFP*+ cells 3 days post-irradiation before (left) and after (right) applying multi-dataset integration methods (Control, n = 2 mice (2,945 cells and 3,226 cells); irradiation, n = 2 mice (5,308 cells and 6,398 cells)).

(C) Relative expression of *Cd81* and *Adamdec1* onto the UMAP plot of *Rspo3-GFP*+ cells from both control and irradiation mice. n = 17,877 cells from 4 mice.

(D) Schematic of co-culture experiments post-irradiation.

(E-F) Representative images (E) and quantification of organoids (F) at day 4 of co-culture of irradiated RGFs and crypts. n = 12 from 3 mice per group.

(G) Violin plots for *Igf1*, *Fgf2*, and *Ereg* expressions.

(H) GSEA of HALLMARK Angiogenesis genes in irradiation vs control mice from RGF 1 cluster (left) and RGF 2 cluster (right) of scRNA-seq.

(I) Violin plots for *Il6* and *Angptl4* expressions.

(J) Schematic for the treatment of RGF culture with IL-1a. Adapted from Figure 7I.

(K) qRT-PCR of *Il6* and *Angptl4* mRNA expressions from RGFs 24 hours after IL-1a treatment. n = 3 mice per group.

(L) Violin plots for *Lcn2* and *Lrg1* expressions comparing control and irradiation mice in LEC cluster of scRNA-seq. One-way analysis of variance (ANOVA) with post-hoc Tukey's multiple comparison (F). Unpaired two-tailed t-tests (A, K). Wilcox test (G, I, L). Data are mean ± SD. *$p < 0.05$. N.S. not significant. Scale bar, 50 μm (A), 20 μm (E).