Supplemental Figure 1: Dclk1-Cre & Dclk1-CreTM label long-lived epithelial tuft cells

A) Cloning strategy of Dclk1-CreGFP and Dclk1-CreERT mice and depiction of Cre-mediated recombination in Dclk1-CreERT mice following induction with Tamoxifen. **B**) LacZ staining of stomach tissue from Dclk1 R26LacZ mice at various time points post induction with Tamoxifen. **C&D** Representative fluorescent photographs of small intestinal (C) and colonic (D) sections from 12 months old Dclk1-CreERT R26mTomatomGFP mice without administration of Tamoxifen. No recombination (as indicated by absence of membranous GFP) can be seen. **E&F)** Representative FACS plot of intestinal (E) and colonic (F) cell preparations from Dclk1 R26tdTom mice. Cells were gated based on red-fluorescence (Dclk1) and CD326 (EpCam) expression.

Supplemental Figure 2: Dclk1-Cre & Dclk1-CreTM labeled cells express tuft cell markers

A-C) Immunofluorescence for epithelial tuft cell markers (Dclk1, alpha-gustducin & acetylated tubulin) in the intestine (A), colon (B) and stomach (C) of Dclk1-CreGFP mice. **D)** Cox2 staining (red) on Dclk1 R26TGFP mice. **E)** Quantification of overlap between Cox2 and Dclk1 recombined cells in the small intestine of Dclk1 R26tdTom mice.

Supplemental Figure 3: Small intestinal Dclk1+ require neural input for survival

A) Small intestinal organoids from Dclk1 R26tdTom mice cultured in the presence of GFP positive neurons 5 days (left panel) and 10 days (right panel) after isolation. **B)** Quantification of Dclk1 cells in intestinal organoids in the presence and absence of neurons 10 days post isolation. **C-F)** Intestinal organoids derived from Dclk1 R26DTA mice cultured in the presence (D&F) and absence of neurons (C&E). 4OH-Tamoxifen was added to induce expression of DTA in Dclk1+ cells (C&E). **G)** Quantification of intestinal organoids in the presence and absence of neurons (left panel) and after Dclk1 cell ablation (right panel).

Supplemental Figure 4. Dckl1+ cells require proper innervation for survival

A) Immunohistochemistry of PGP 9.5 (left) and Dclk1 (right) on biopsy samples from intestinal transplant patients. Upper panels show samples from native, lower panels from graft intestine. **B)** Quantification of Dclk1 positive cells in biopsy samples (n=2) depicted

in A). **C)** Immunofluorescence for peripherin (green) on wildtype (left) and $RET^{-/-}$ mice (right) **D)** Immunofluorescence for Cox2 (red) on wildtype (left) and $RET^{-/-}$ mice (right). **E)** Quantification of intestinal tuft cells in wildtype and $RET^{-/-}$ mice.

Supplemental Figure 5: Gastric Dclk1+ cells require neural input for survival

A-D) Gastric organoids derived from Dclk1 R26tdTom mice cultured in the absence (A&C) and presence of neurons after 3 (A&B) and 14 (C&D) days in culture. Dclk1+ cells express tdTomato (red) neurons express GFP (green). **E)** Immunohistochemistry for Dclk1 on paraffin embedded gastric organoids in the absence (control) and presence of neurons (+nerve). Arrowheads mark Dclk1 positive cells. **F)** Quantification of Dclk1+ cells in gastric organoids in the presence of absence of neurons. **G&H)** Representative photographs of intestinal organoids derived from Dclk1-CreERT x R26tdTomato mice cultured in the presence (H) and absence of 100μM Pilocarpine (G). **I)** Quantification of Dclk1+ cells in (G) and (H).

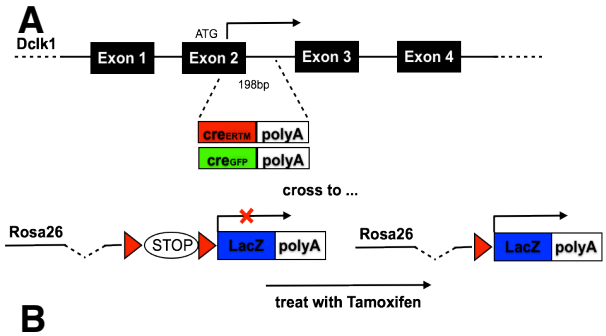
Supplemental Figure 6: Dclk1 cells expand in response to chronic inflammation and in preneoplastic states

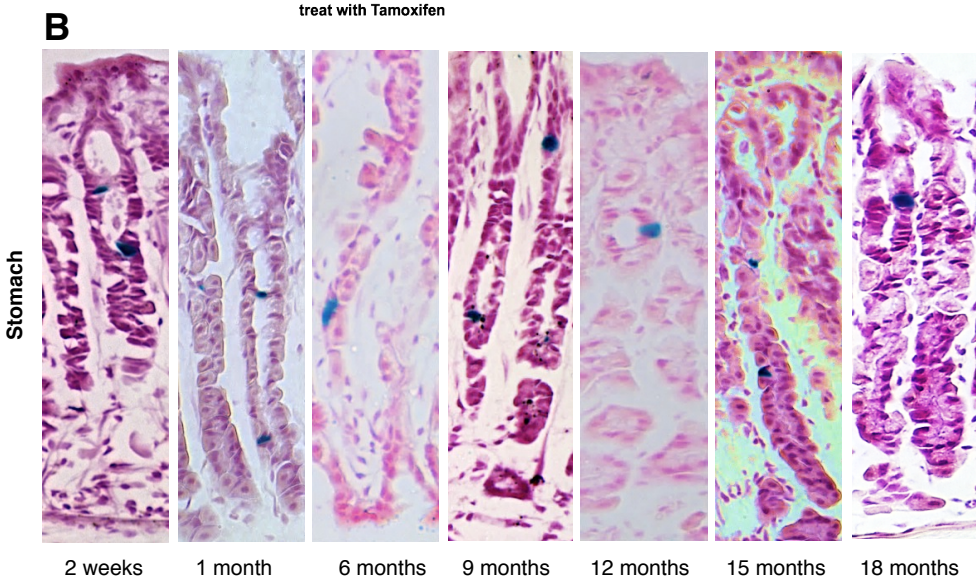
A) Immunohistochemistry for Dclk1 on the corpus of HKATPase-IL1β mice infected with H. felis. **B)** Immunohistochemistry for Dclk1 Barrett's lesions in P2-IL1β mice. **C&D)** Immunofluorescence for Dclk1 on intestinal sections from B6 mice infected with H. hepaticus (D) and controls (C). **E)** Quantification of Dclk1+ cells in mice infected with H. hepaticus and controls. **F&G)** Immunohistochemistry for Dclk1 on colonic sections from mice infected with B. fragilis (G) and controls (F). **H)** Quantification of Dck1+ cells in (F&G) (Scale bars: 50 μm).

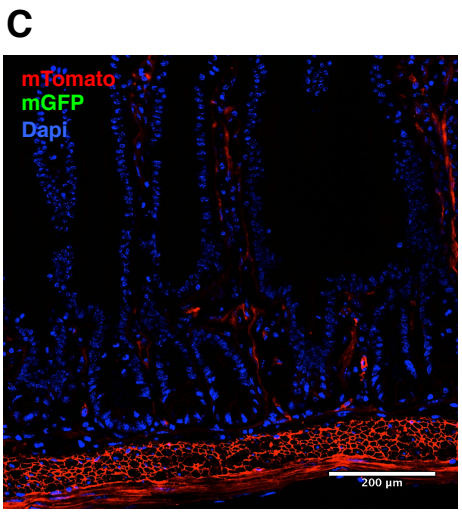
Supplemental Figure 7: Pathology of murine models of colonic neoplasia

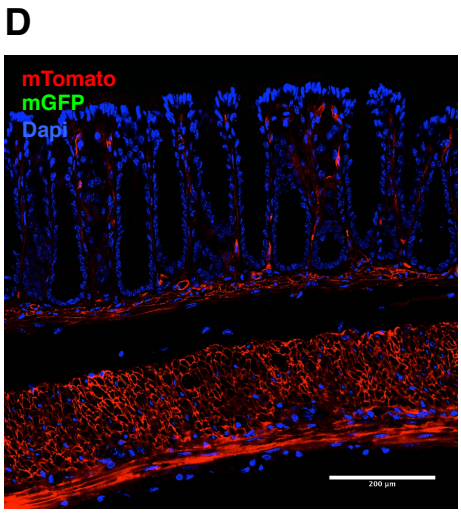
A) Early colonic polyps in APC^{min} mice. Proliferation with evidence of surface maturation is seen. An early sessile proliferative lesion is characterized by increase in proliferative zone of polyp in (A). Right panel: Typical early polypoid proliferative lesion. **B)** H&E of colonic lesions in Lgr5-CreERT x $APC^{flox/flox}$ mice: Numerous colonic adenomas are present. Left panel: Numerous small adenomas with low grade dysplasia arising in the background of colonic mucosa with multifocal low grade dysplasia Right panel: Higher power magnification shows minute adenoma composed of two dysplastic crypts.

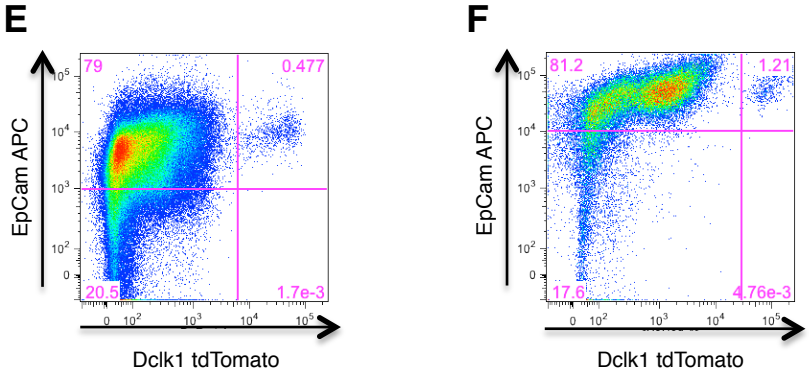
C) H&E of colonic lesions in Dclk1-CreERT x $APC^{flox/flox}$: Few adenomas are present with features of invasive carcinoma and complex architectural patterns of growth and associated desmoplastic stroma. Left panel: A large advanced polyp with marked architectural complexity arising in the background of normal mucosa Right panel: Higher power magnification shows complex cribriform structures associated with focal tumor cell necrosis. The surrounding fibroblastic tissue reaction characterizing desmoplasia surrounding the neoplastic epithelium is a feature of invasive carcinoma (Original magnifications 40x, 200x)

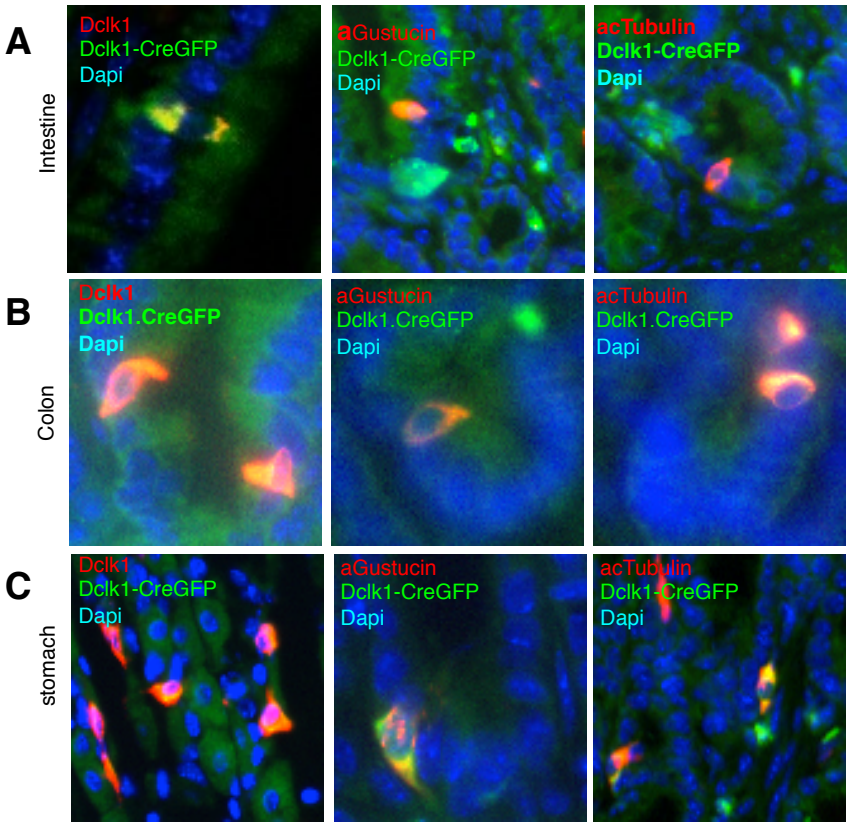

A
Dclk1
Exon 1
Exon 2
Exon 3
Exon 4
ATG
198bp
creERTM
polyA
creGFP
polyA
cross to ...
Rosa26
STOP
LacZ
polyA
Rosa26
LacZ
polyA
treat with Tamoxifen

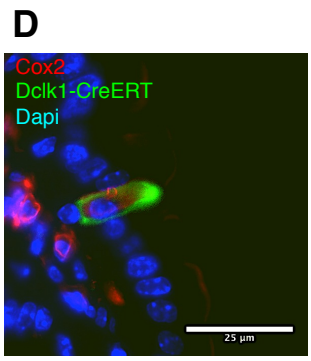

B
Stomach
2 weeks
1 month
6 months
9 months
12 months
15 months
18 months

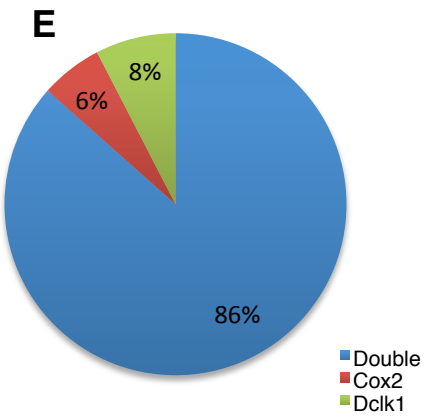

C
mTomato
mGFP
Dapi
200 µm
Small Intestine

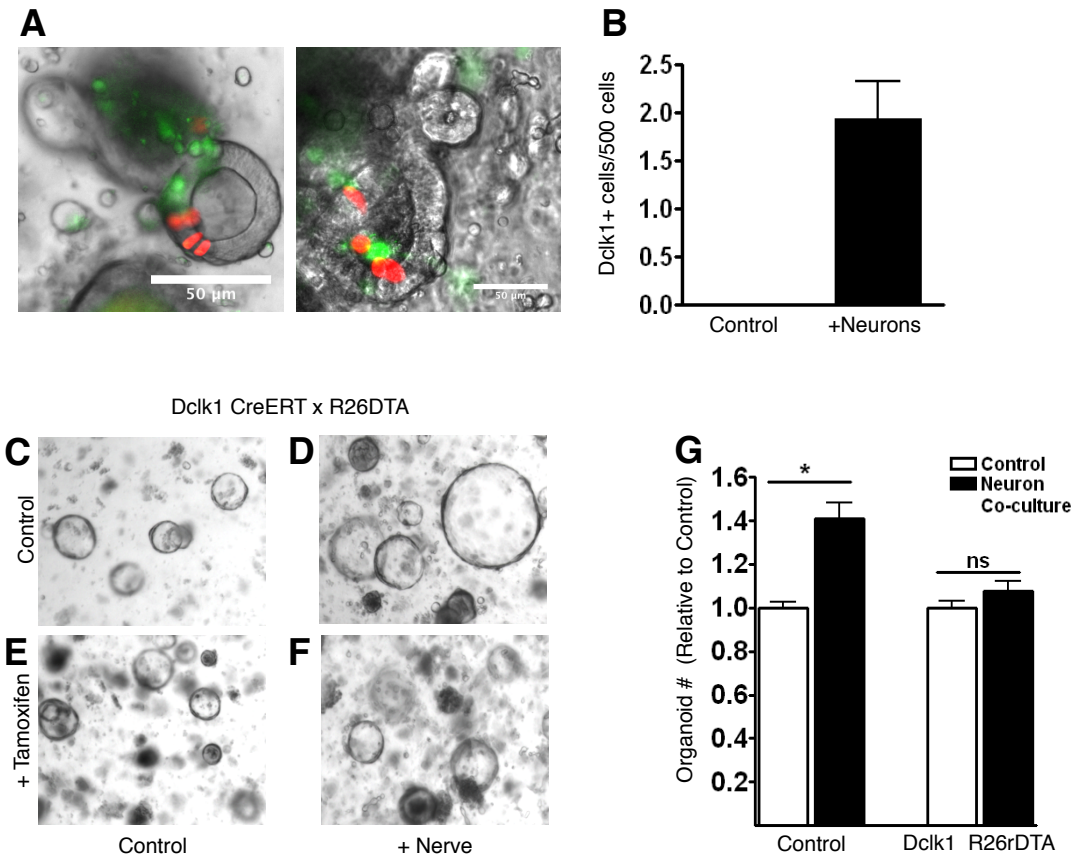

D
mTomato
mGFP
Dapi
200 µm
Colon

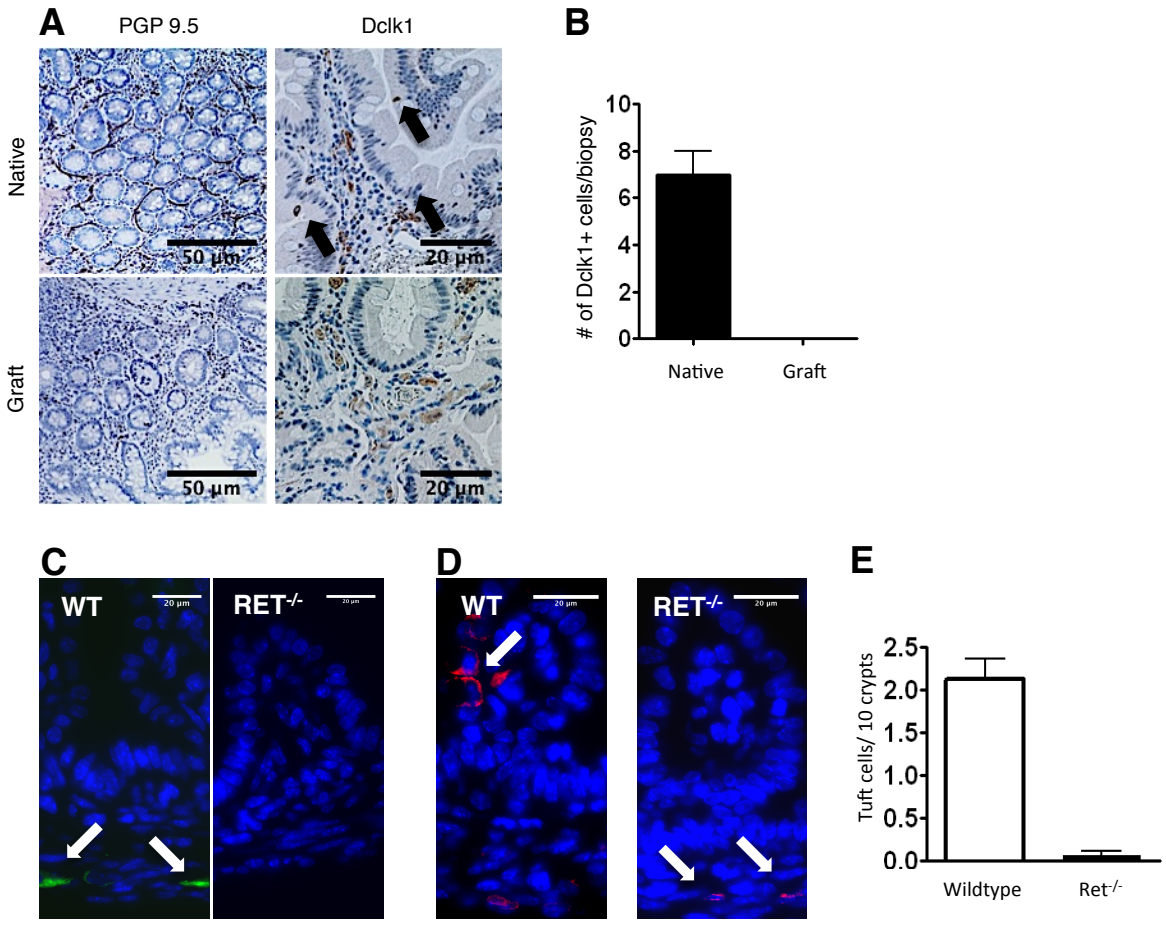

E
79
0.477
20.5
1.7e-3
EpCam APC
Dclk1 tdTomato
F
81.2
1.21
17.6
4.76e-3
EpCam APC
Dclk1 tdTomato

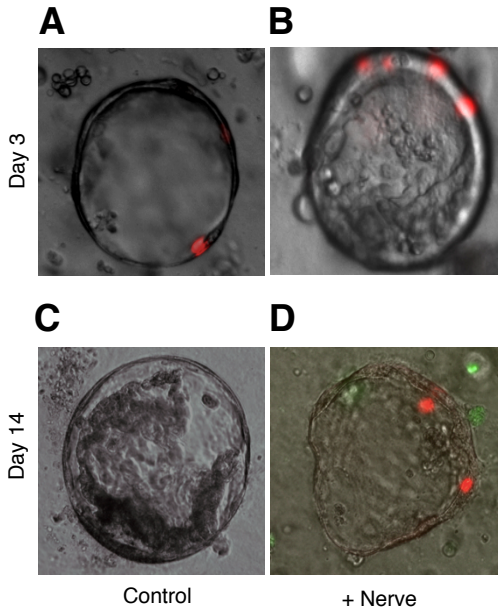

A
Intestine
Dclk1
Dclk1-CreGFP
Dapi
aGustucin
Dclk1-CreGFP
Dapi
acTubulin
Dclk1-CreGFP
Dapi
B
Colon
Dclk1
Dclk1.CreGFP
Dapi
aGustucin
Dclk1.CreGFP
Dapi
acTubulin
Dclk1.CreGFP
Dapi
C
stomach
Dclk1
Dclk1-CreGFP
Dapi
aGustucin
Dclk1-CreGFP
Dapi
acTubulin
Dclk1-CreGFP
Dapi

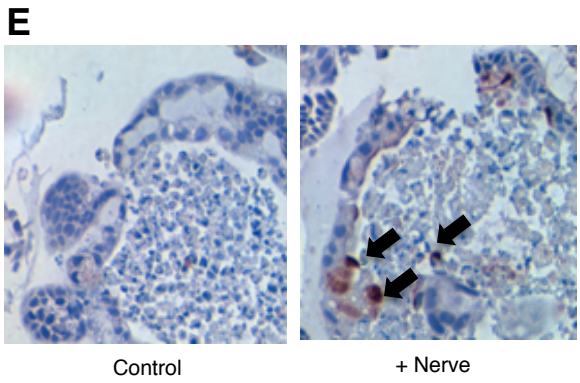

D
Cox2
Dclk1-CreERT
Dapi
25 µm

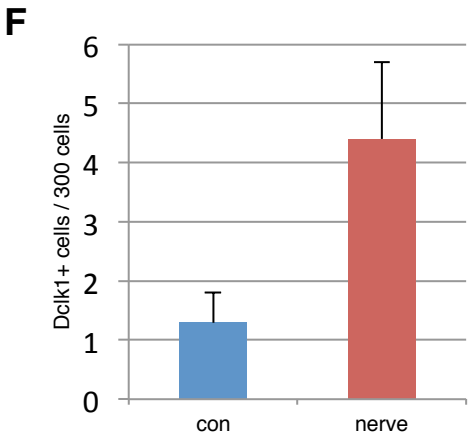

E
8%
6%
86%
Double
Cox2
Dclk1

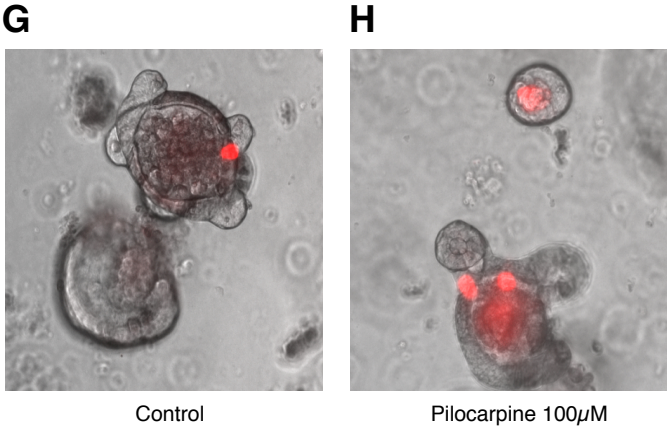

A
50 µm
B
Dclk1+ cells/500 cells
2.5
2.0
1.5
1.0
0.5
0.0
Control
+Neurons
Dclk1 CreERT x R26DTA
C
D
Control
E
F
+ Tamoxifen
Control
+ Nerve
G
Organoid # (Relative to Control)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
*
ns
Control
Neuron
Co-culture
Control
Dclk1 R26rDTA

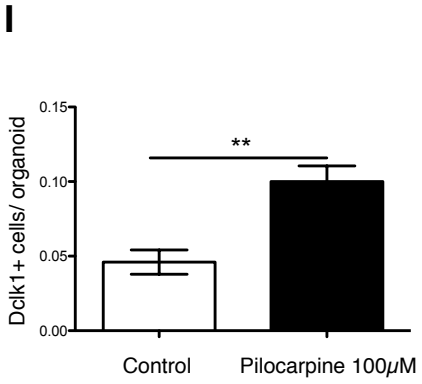

A
PGP 9.5
Dclk1
Native
Graft
50 µm
20 µm
50 µm
20 µm
B
# of Dclk1+ cells/biopsy
10
8
6
4
2
0
Native
Graft
C
WT
RET-/-
D
WT
RET-/-
E
Tuft cells/ 10 crypts
2.5
2.0
1.5
1.0
0.5
0.0
Wildtype
Ret-/-

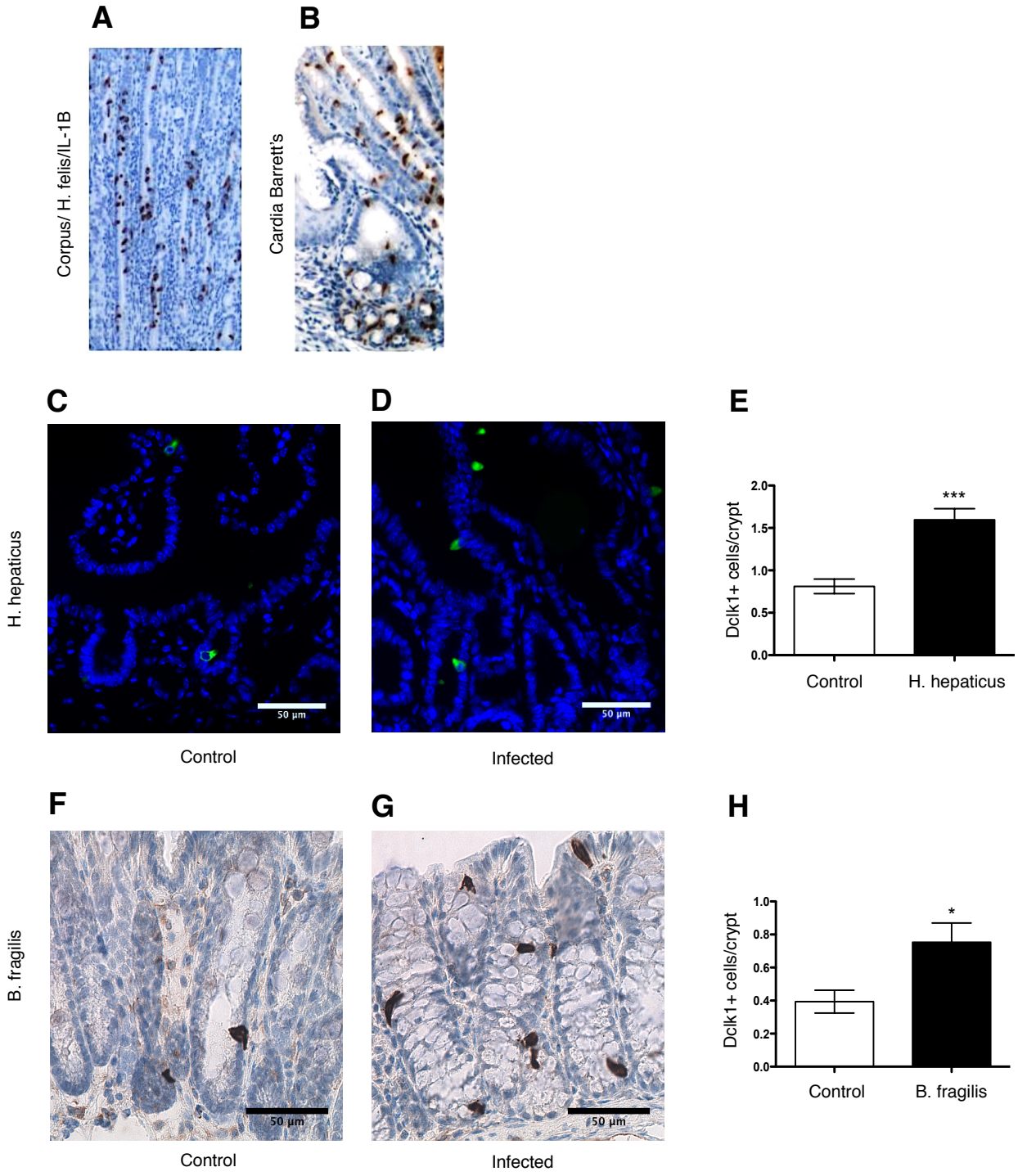

A
B
Day 3
C
D
Day 14
Control
+ Nerve

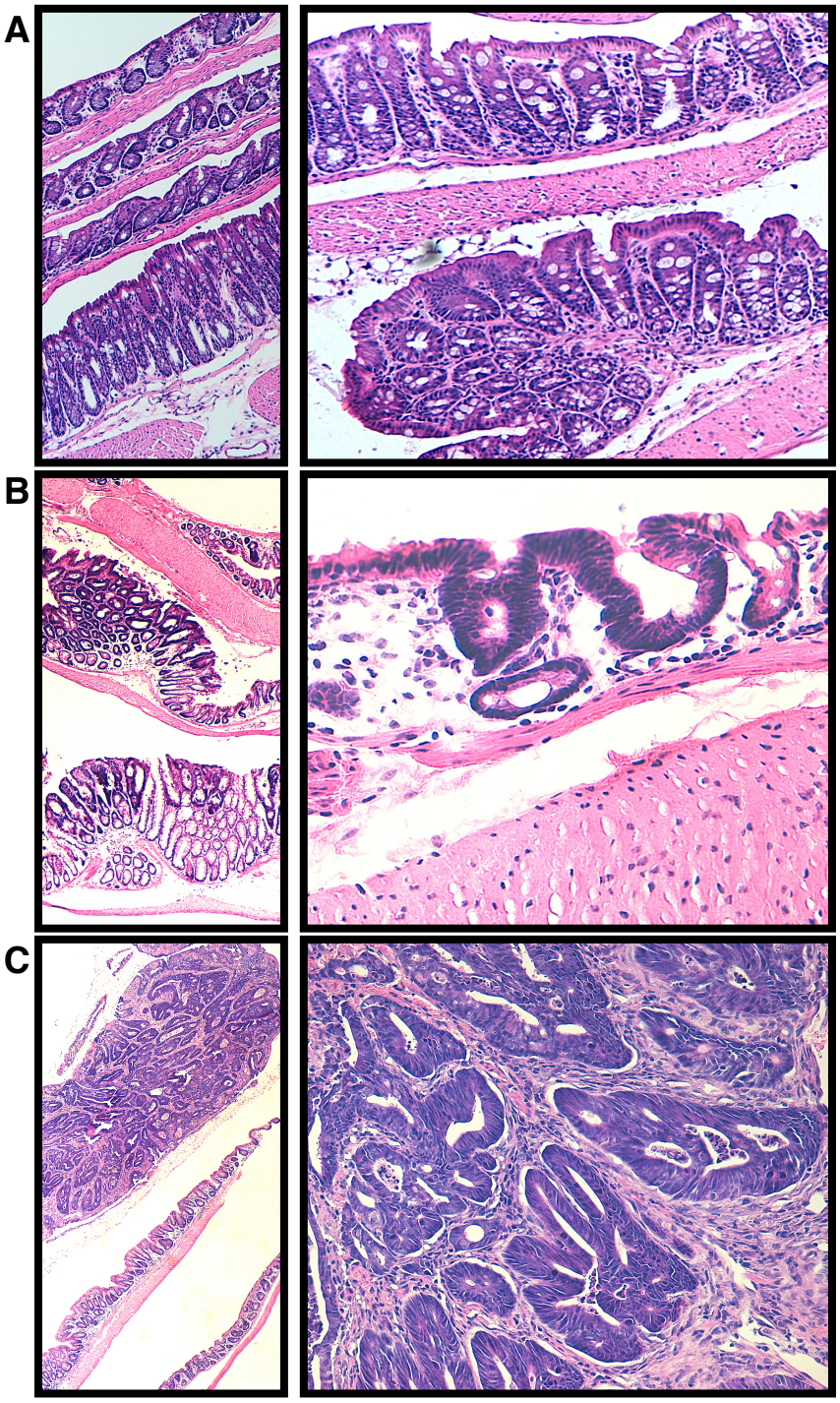

E
Control
+ Nerve


F
Dclk1+ cells / 300 cells
0
1
2
3
4
5
6
con
nerve


G
H
Control
Pilocarpine 100μM


I
Dclk1+ cells/ organoid
0.00
0.05
0.10
0.15
**
Control
Pilocarpine 100μM


A
B
Corpus/ H. felis/IL-1B
Cardia Barrett's
C
D
E
H. hepaticus
50 µm
50 µm
Control
Infected
Dclk1+ cells/crypt
2.0
1.5
1.0
0.5
0.0
***
Control
H. hepaticus
F
G
H
B. fragilis
50 µm
50 µm
Control
Infected
Dclk1+ cells/crypt
1.0
0.8
0.6
0.4
0.2
0.0
*
Control
B. fragilis


A
B
C

Supplemental Table 1: Immunohistochemical analysis of recombined cells in Dclk1-Cre & CreERT mice

Dclk1-CreTM x TGFP	**Recombined cells (100 crypts)**	**Dclk1**	**aGustducin**	**acTubulin**	**ChromA**
Corpus	42	95% ±2%	81% ±4%	98% ±1%	0% ±0%
Antrum	54	98% ±2%	89% ±2%	97% ±2%	5% ±0%
Small Intestine	169	96% ±2%	79% ±5%	93% ±2%	3% ±1%
Colon	81	98% ±2%	94% ±2%	98% ±2%	0% ±0%
Dclk1-CreGFP	**GFP+ cells (100 crypts)**	**Dclk1**	**aGustducin**	**acTubulin**	**ChromA**
Corpus	56	85% ±4%	72% ±4%	82% ±5%	1% ±0%
Antrum	61	87% ±7%	74% ±2%	83% ±5%	2% ±1%
Small Intestine	190	81% ±12%	59% ±4%	81% ±5%	1% ±0%
Colon	108	82% ±6%	61% ±7%	85% ±4%	1% ±0%

Supplemental Table 2: Mouse strains used in the manuscript

Full Name	**Abbrevation**	**Source**	**Consensus Name**
$APC^{flox/flox}$		NCI	B6: Apctm2Rak
C57BL/6J	B6	Jackson labs	C57BL/6J
DCLK1-CreERT	Dclk1	Generated	DCLK1-BAC-CreERT
DCLK1-CreGFP		Generated	DCLK1-BAC-CreGFP
$Dclk1^{floxed/floxed}$		Jackson labs	Dclk1tm1.2Jgg/J
Rosa26 DTA	R26DTA	Jackson labs	B6.129P2-Gt(ROSA)26Sortm1(DTA)Lky/J
Rosa26 LacZ	R26LacZ	Jackson labs	129S-Gt(ROSA)26Sortm1Sor/J
Rosa26 mTomato/mGFP	R26-TGFP	Jackson labs	B6.129(Cg)-Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J
Rosa26 tdTomato	R26tdTom	Jackson labs	B6;129S6-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J
Rosa26DTR	R26iDTR	Jackson labs	CBy.B6-Gt(ROSA)26Sortm1(HBEGF)Awai/J
UBC-GFP		Jackson labs	C57BL/6-Tg(UBC-GFP)30Scha/J

Supplemental Table 3: Antibodies used in the manuscript

Name	Company	Order Number	Dilution
Alexa Fluor 488 (F(ab')2 Goat-anti-Rabbit	Invitrogen	A11070	1:500
Alexa Fluor 488 Conjugate anti-GFP	invitrogen	A21311	1:50
Alexa Fluor 546 Donkey-anti-mouse	Invitrogen	A10036	1:500
Alexa Fluor 546 Donkey-anti-Rabbit	Invitrogen	A10040	1:500
anti-acetylated-Tubulin	Sigma-Aldrich	T7451	1:100
anti-alpha-Gustducin	Santa Cruz	sc-395	1:200
anti-BrdU	AbCam	ab6326	1:100
anti-Cox2	Pierce	PA1-20955	1:250
anti-Dcamlk1	Abgent	AP7219b	1:200
anti-Ki67	abcam	ab15580	1:500
anti-Muc2	Santa Cruz	sc-15334	1:500
anti-NGF	Santa Cruz	sc-549	1:100
anti-Peripherin	Millipore	AB1530	1:200
anti-ß-catenin	BD Bioscience	610157	1:100
anti-ß-Gal	AbCam	ab9361	1:1000
anti-TFF3	Santa Cruz	sc-28927	1:500