Supplementary Materials for

Paneth cells acquire multi-potency upon Notch activation after irradiation

Shiyan Yu [1], Kevin Tong [2], Yanlin Zhao [3], Iyshwarya Balasubramanian [1], George S. Yap [3], Ronaldo P. Ferraris [4], Edward M. Bonder [1], Michael P. Verzi [2, 5], Nan Gao [1, 5, *]

This PDF file includes:

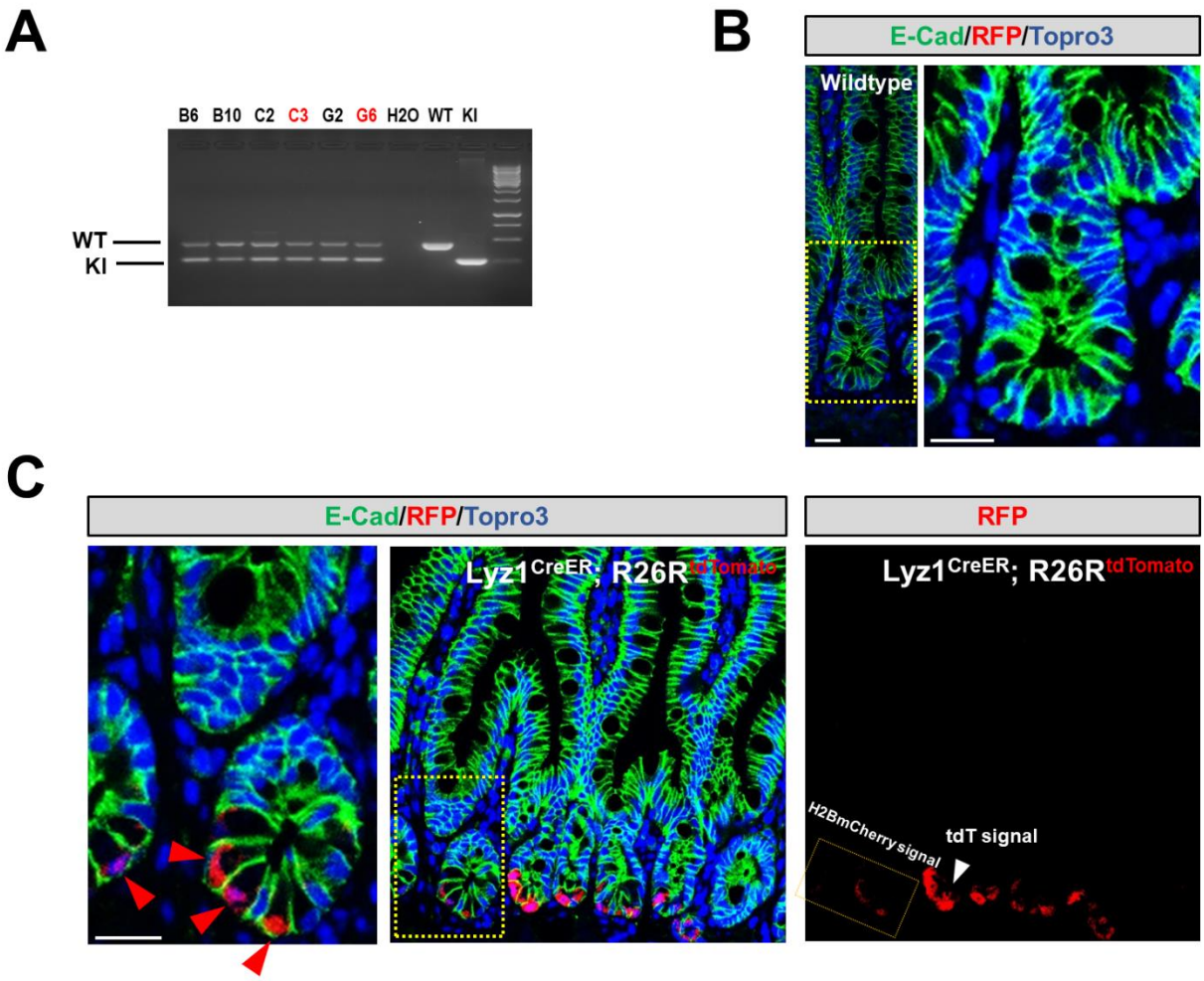

Figure S1. Related to Fig. 1: Nuclear H_2B-mCherry and tdTomato expression
(A) Two positive ES cell clones (C3 and G6) were injected to blastocysts, leading to two independent Lyz1-CreER lines with almost identical labeling patterns.
(B) Anti-RFP antibody did not detect any signals in wild type crypts.
(C) Same antibody detected relatively weak nuclear H_2BmCherry signal (red arrowheads, left panel). Recombined cells showed intense tdTomato signal (white arrowhead, right panel).

Yu_Fig. S2

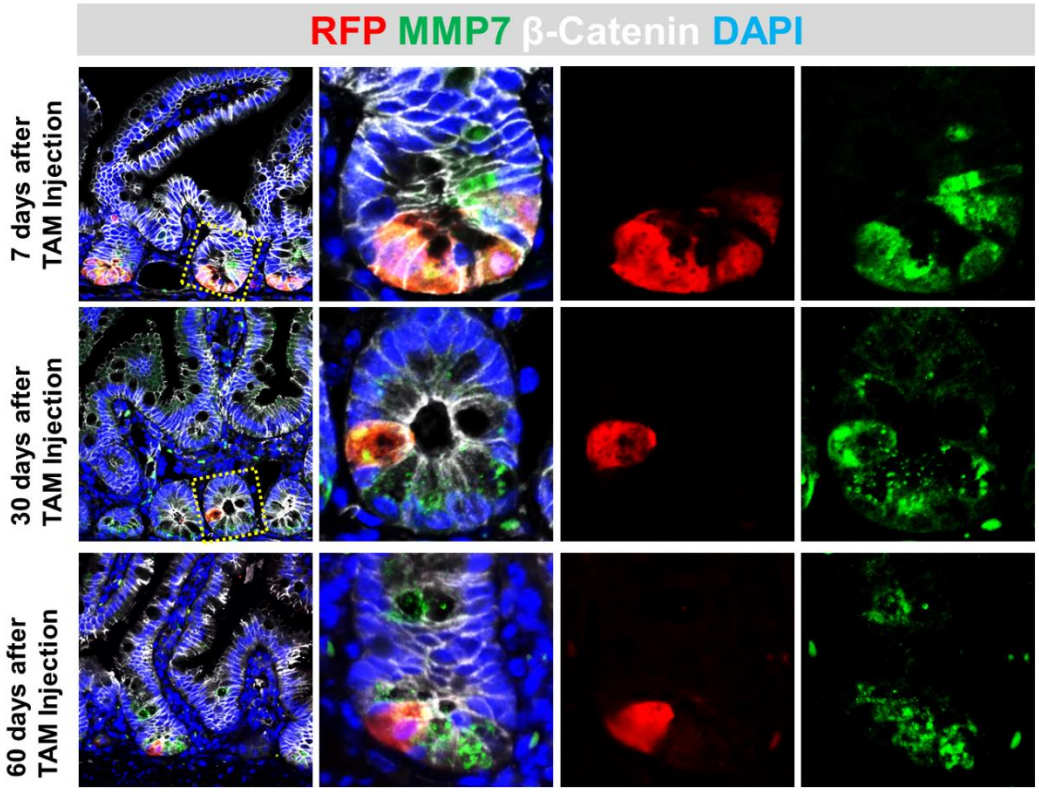

Figure S2. Related to Fig. 1: Determine tdT$^+$ cells after different tracing periods.

Mice were sacrificed 7, 30, and 60 days after tamoxifen injection to examine recombined tdT$^+$ cells in the intestines. Sections were stained for tdT, MMP7, and beta-catenin.

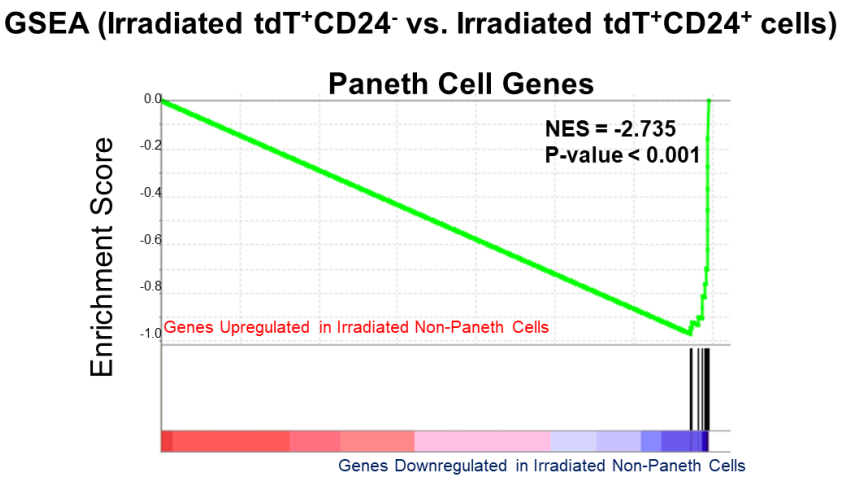

Figure S3. Related to Fig. 4: GSEA showed that tdT$^+$CD24$^-$ cells lost their Paneth cell gene expression when compared to tdT$^+$CD24$^+$ cells in irradiated mice.

GSEA confirms that tdT$^+$CD24$^-$ cells lack Paneth cell gene expression compared to tdT$^+$CD24$^+$ cells.

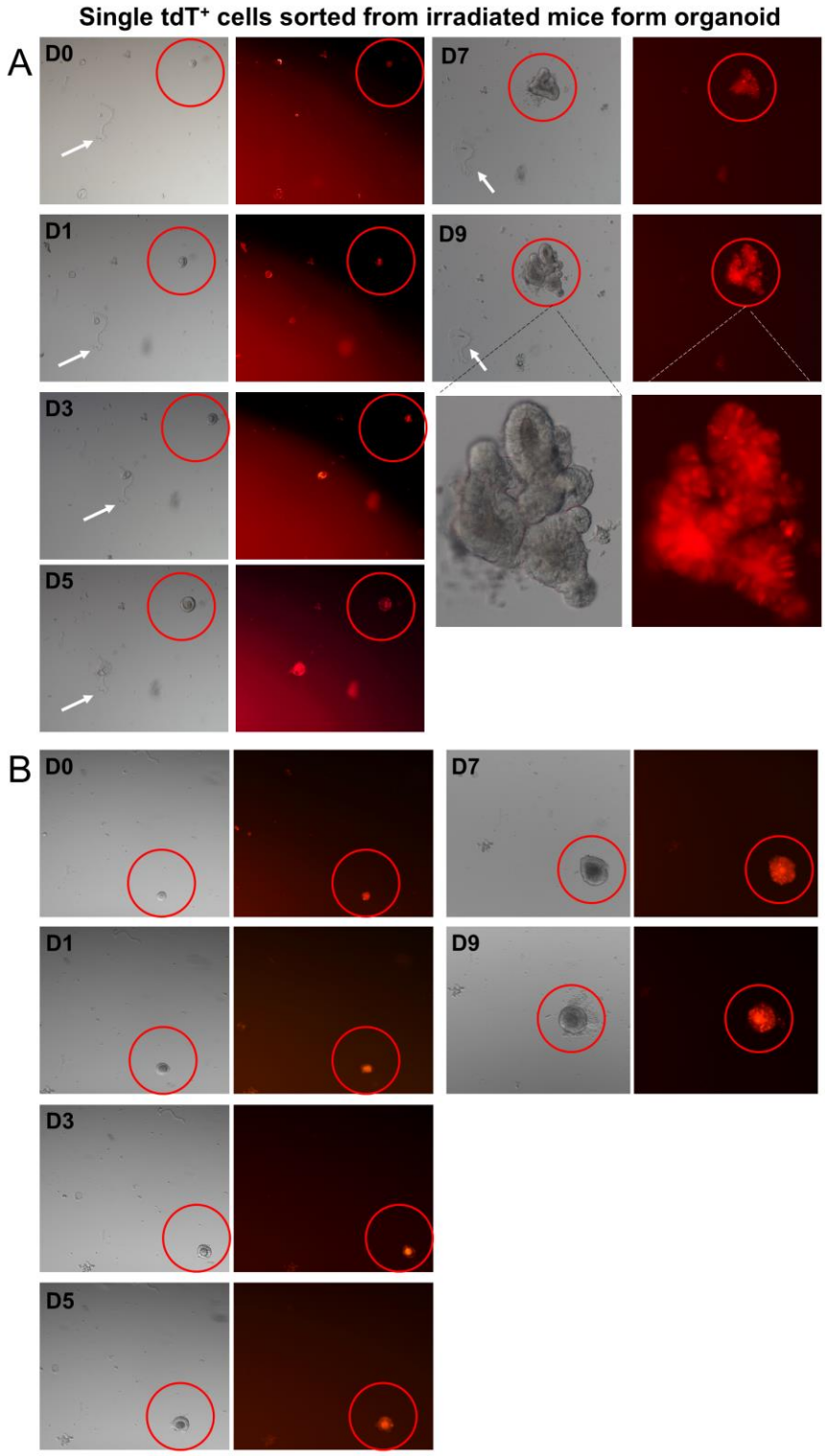

Figure S4. Related to Fig. 4: Single tdT$^+$ cells from irradiated mice formed organoids in vitro.
Two different examples were shown: one formed branches (A) and the other did not (B). White arrows pointed to some marks that were used to track the field. D: day

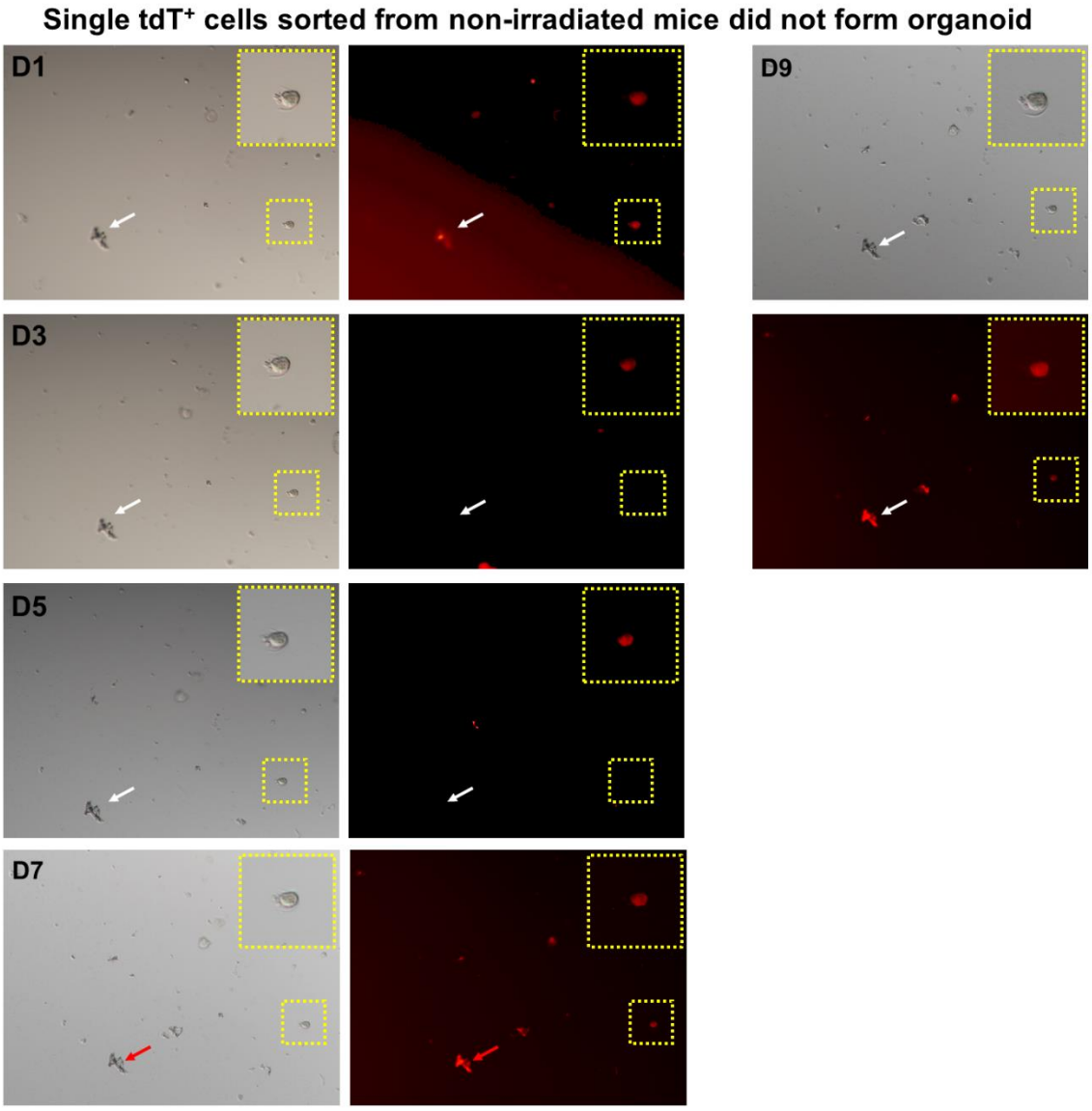

Figure S5. Related to Fig. 4 Single tdT$^+$ cells from non-irradiated mice did not form organoids.
A single tdT$^+$ cell was followed from Day 1 to Day 9.
White arrows pointed to the landmark that was used to track the field. D: day

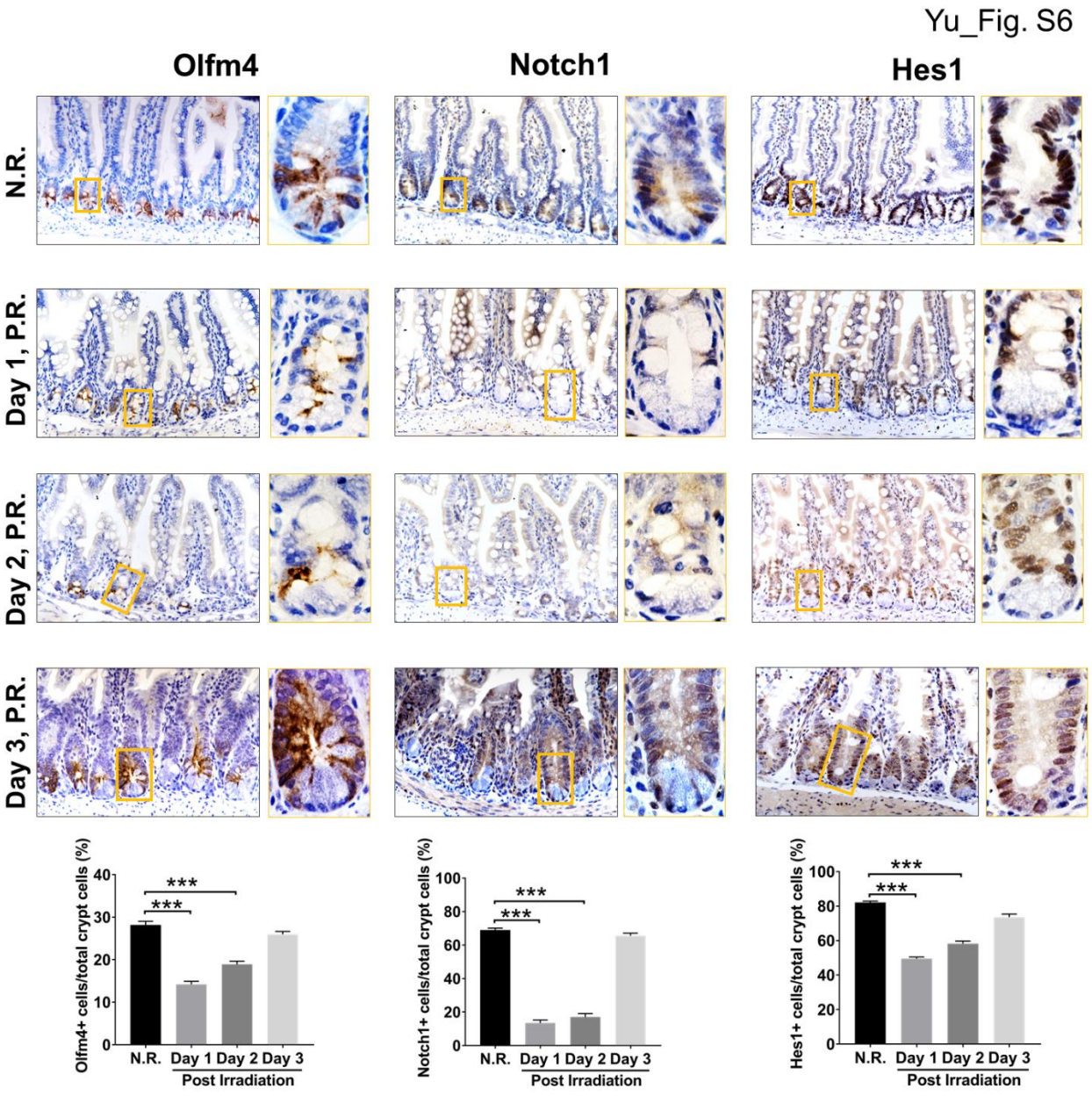

Figure S6. Related to Fig. 6: Irradiation rapidly reduced Olfm4-, Notch1-, and Hes1-expressing crypt cells.

In non-irradiated (N.R.) mice, Olfm4, Notch1, Hes1 expressing cells were detected at the bottom of crypts where CBCs localize. 1-2 days after irradiation, cells expressing these markers were significant decreased. 3 days after irradiation, cells expressing these markers recovered to some extent. ***, $p<0.001$.

Table S1. Detailed information about antibodies for immunofluorescence

Antibody Name	Source	Antigen Retrieval	Dilution
Goat anti-GFP	Abcam (ab6673)	No antigen retrieval on frozen sections	1:100
Rabbit anti-RFP	Rockland (600-401-379)	Citric acid pH6.0 on paraffin sections	1:200
Mouse anti-RFP	Invitrogen (MA5-15257)	Citric acid pH6.0 on paraffin sections	1:200
Rabbit anti-Hes1	Cell signaling (11988)	Citric acid pH6.0 on paraffin sections	1:1,000
Rabbit anti-NICD1	Cell Signaling (4147)	DAKO Target Retrieval Solution (S1699) on paraffin sections	1:100
Rabbit anti-Olfm4	Cell Signaling (39141)	No antigen retrieval on frozen sections; Citric acid pH6.0 on paraffin sections	1:1,000
Rabbit anti-YAP	Cell Signaling (14074)	Citric acid pH6.0 on paraffin sections	1:100
Rabbit anti-Ki67	Abcam (ab16667)	Citric acid pH6.0 on paraffin sections	1:200
Rat anti-Ki67	Invitrogen (14-5698-82)	Citric acid pH6.0 on paraffin sections	1:250
Mouse anti-PCNA	Santa Cruz (sc-56)	Citric acid pH6.0 on paraffin sections	1:200
Rabbit anti-Lysozyme	BioGenex (AR024-10R)	No antigen retrieval on frozen sections; Citric acid pH6.0 on paraffin sections	Read-to-use
Goat anti-Sucrase-isomaltase	Santa Cruz (sc-27603)	No antigen retrieval on frozen sections; Citric acid pH6.0 on paraffin sections	1:200
Rabbit anti-Dclk1	Abcam (ab37994)	No antigen retrieval on frozen sections;	1:200

		Citric acid pH6.0 on paraffin sections	
Rabbit anti-Chromogranin A	ImmunoStar (20085)	No antigen retrieval on frozen sections; Citric acid pH6.0 on paraffin sections	1:2,000
Rabbit anti-Mucin 2	Santa Cruz (sc-15334)	No antigen retrieval on frozen sections; Citric acid pH6.0 on paraffin sections	1:200
Goat anti-MMP7	Santa Cruz (sc-8832)	Citric acid pH6.0 on paraffin sections	1:100
Rat anti-BrdU	Accurate Chemical (OBT0030G)	Citric acid pH6.0 on paraffin sections	1:200
Rabbit anti-phospho-p53 (S18)	R&D systems (AF1043-SP)	Citric acid pH6.0 on paraffin sections	1:200
Rabbit anti-phospho-Stat3 (Y705)	Cell Signaling (9145)	EDTA pH8.0 on paraffin sections	1:100