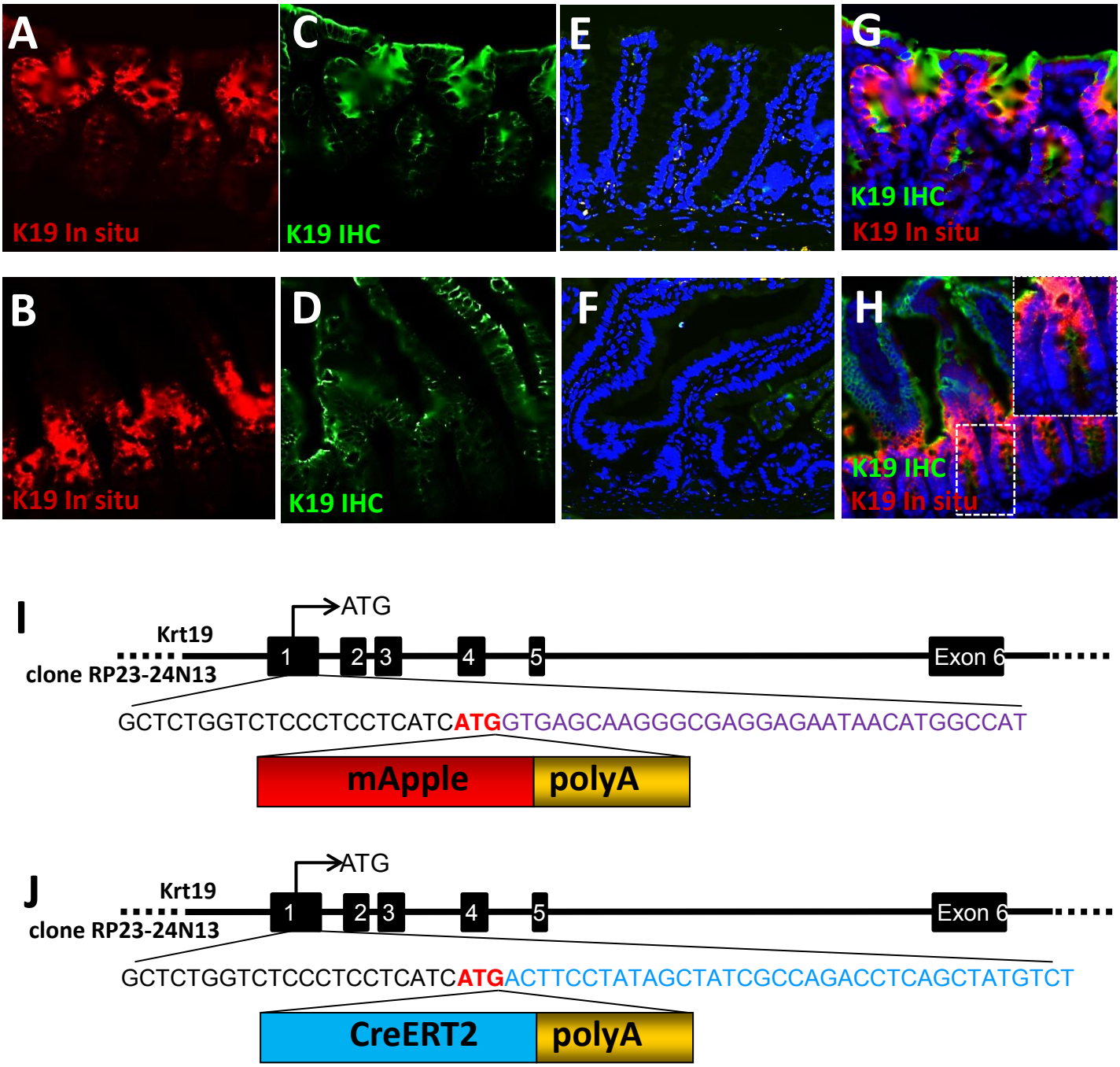
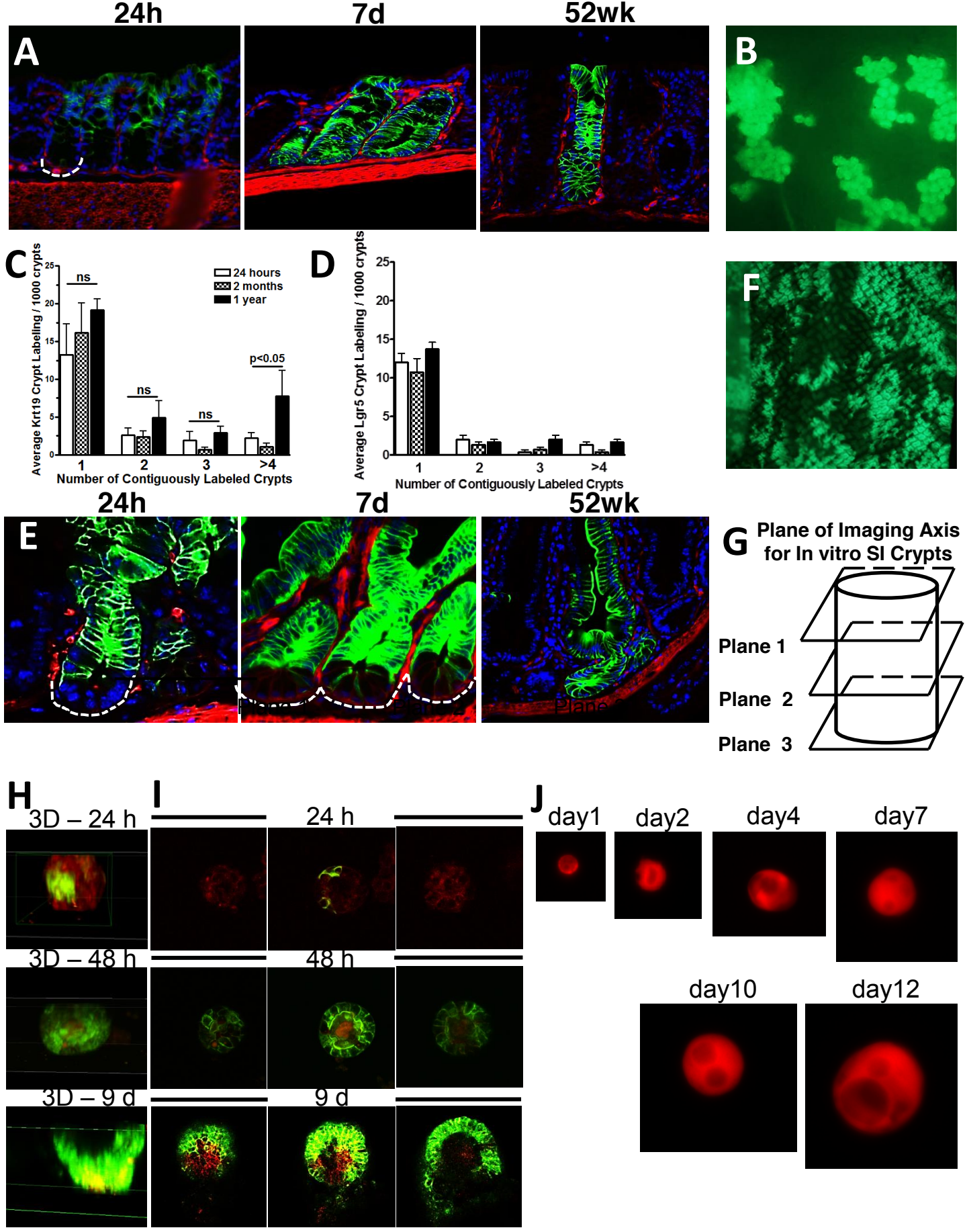


Supplementary Figure 1



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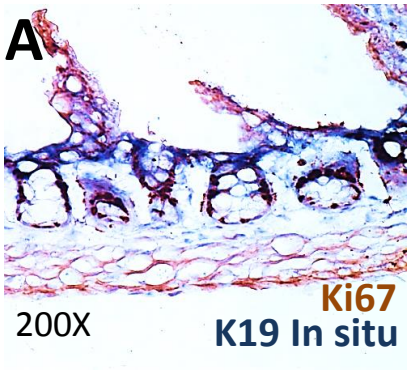
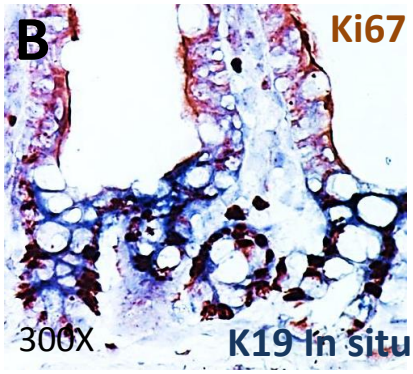
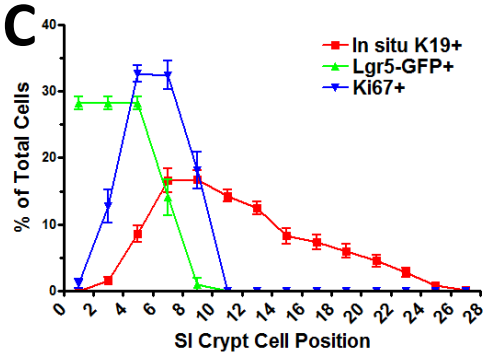
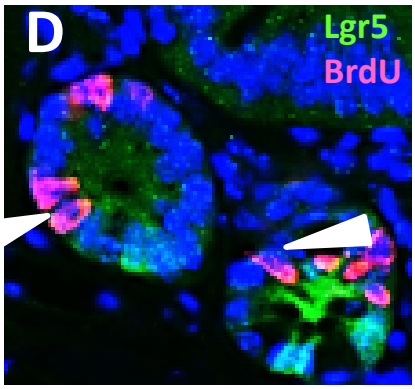
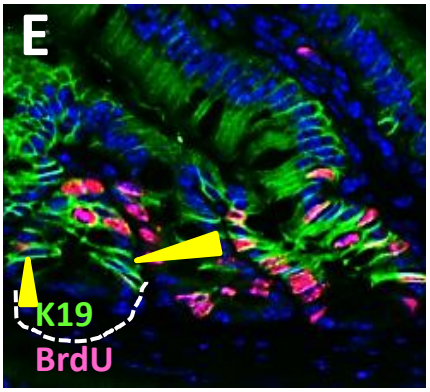
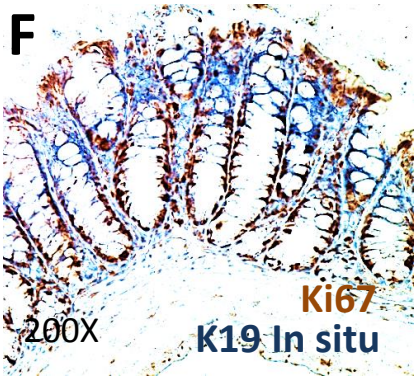


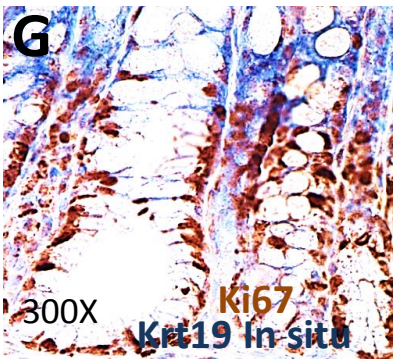


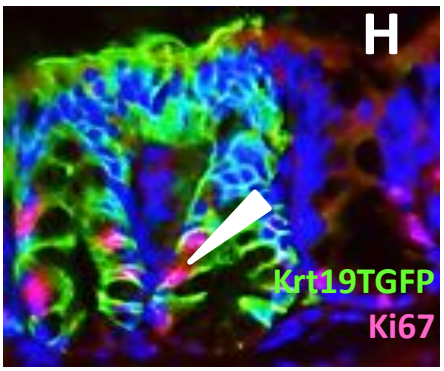


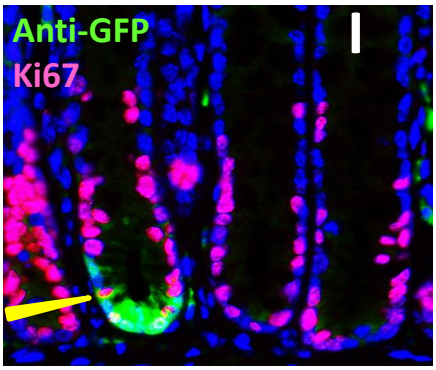


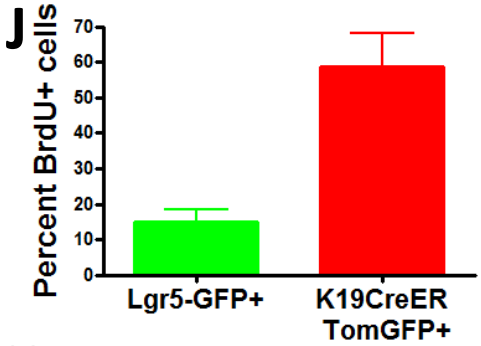


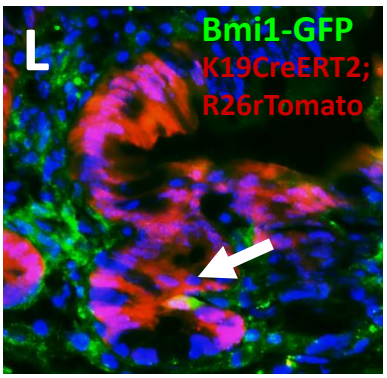


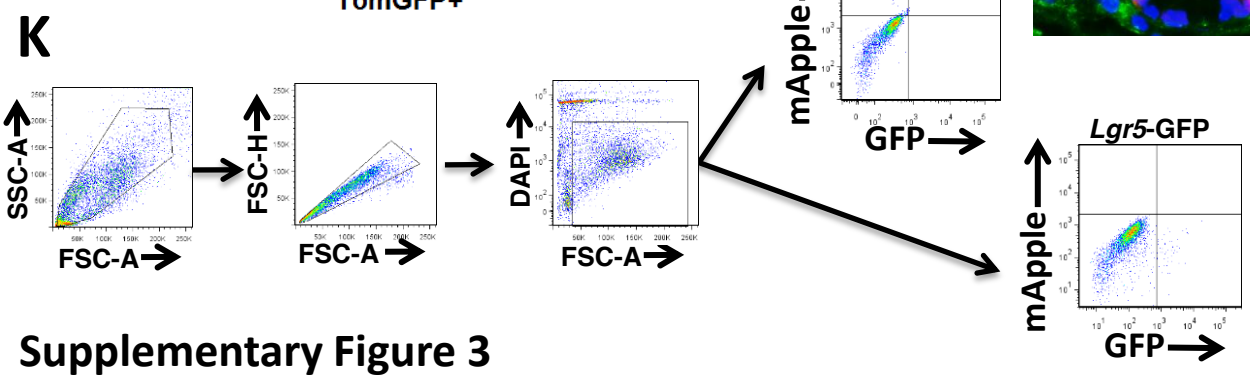




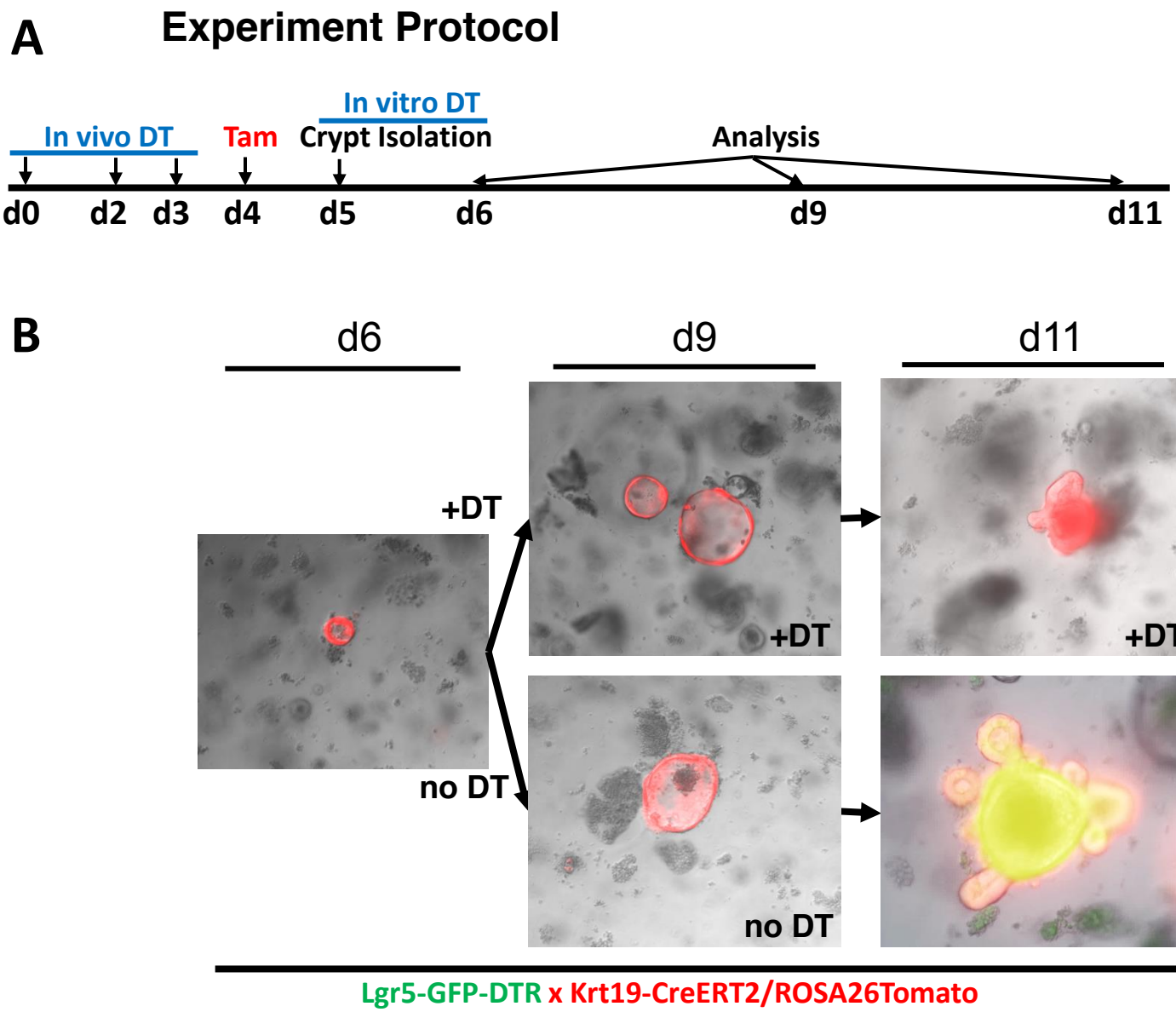




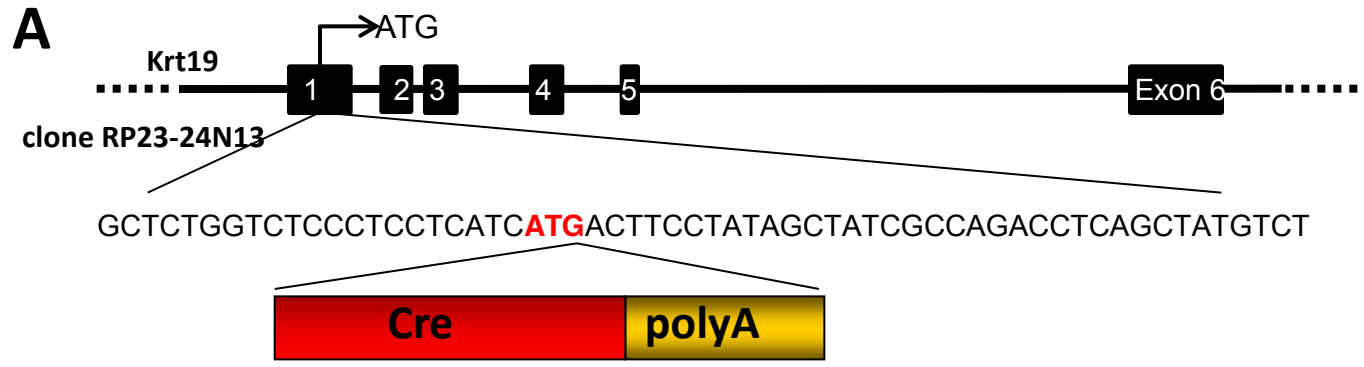


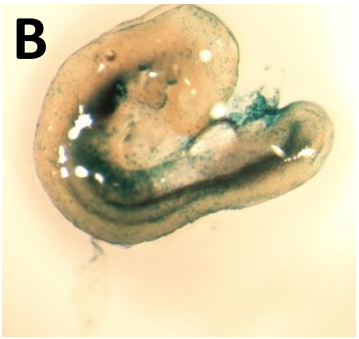


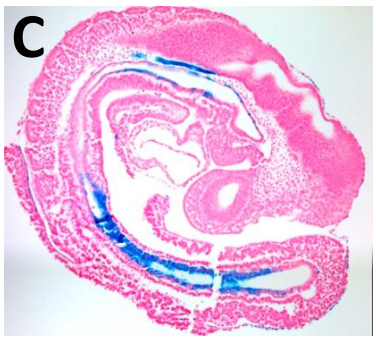
Supplementary Figure 3

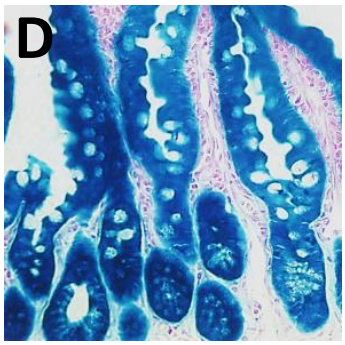


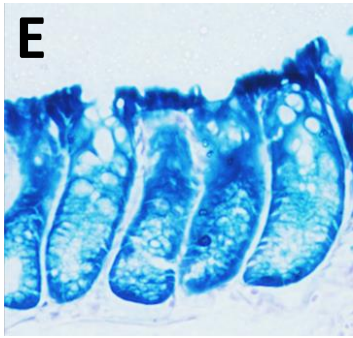
Supplementary Figure 4

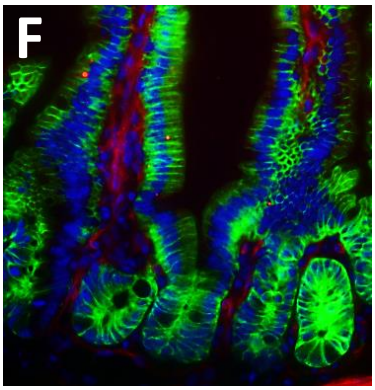


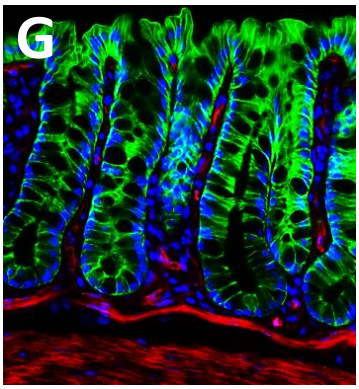


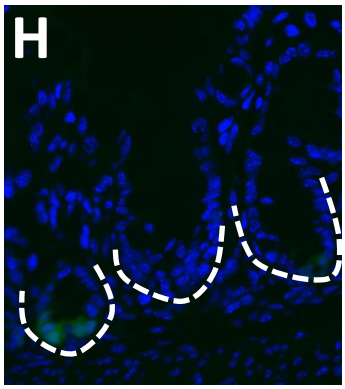


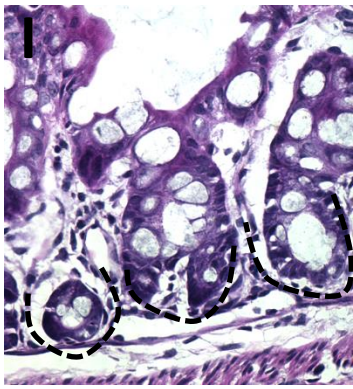




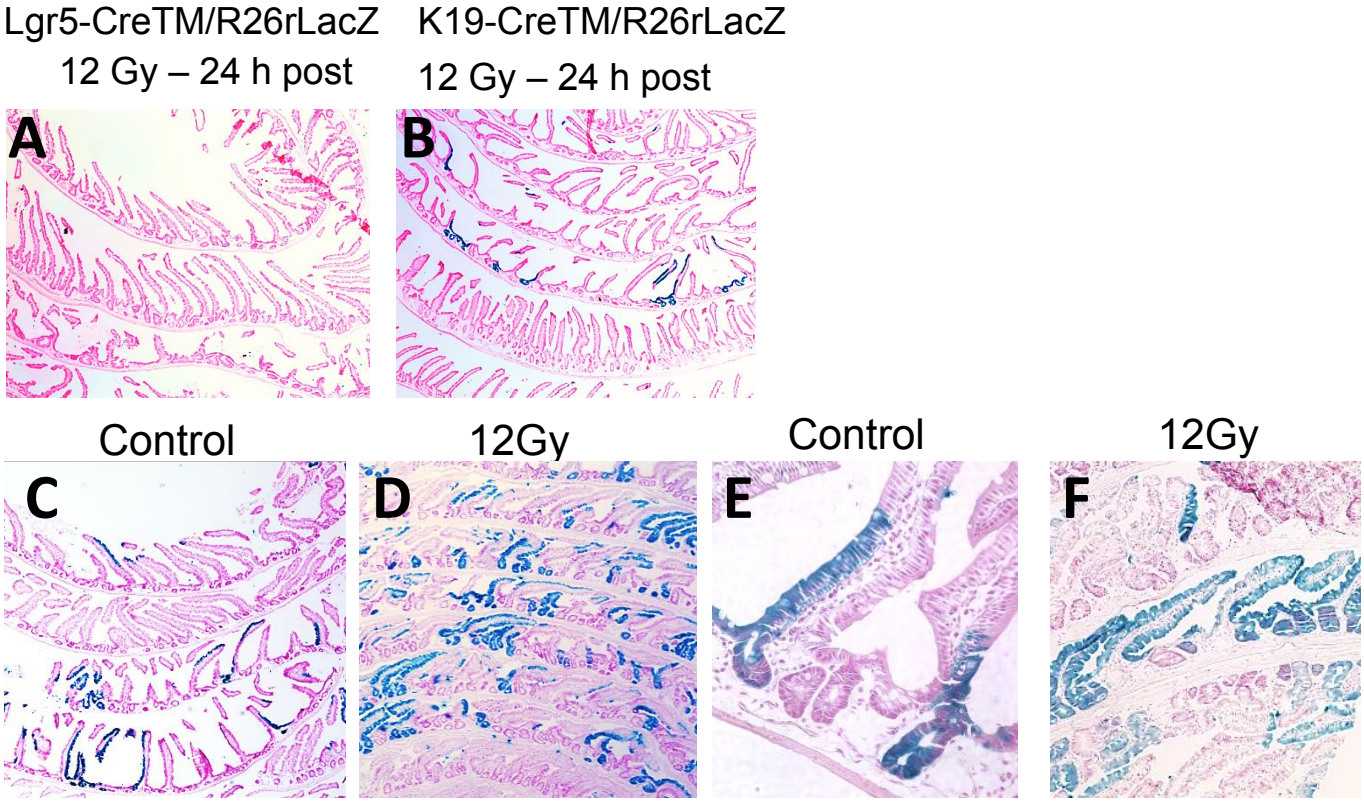








Supplementary Figure 5



Supplementary Figure 6

Supplementary Materials

Supplementary Figure 1

Krt19 mRNA localizes to the colonic and intestinal stem cell zone

Related to Figure 1. *Krt19* mRNA (red) expression pattern in the colonic (A) and intestinal (B) crypt. *Krt19* protein (green) expression pattern in the colonic (C) and intestinal (D) crypt. Respective DAPI (E and F) and overlay images of the colonic (G) and intestinal (H) crypts are shown; ($n \geq 3$ per group). Note that *Krt19* mRNA positive cells are restricted to the intestinal and colonic isthmus. Bacterial artificial chromosome (BAC) transgenic approach used to generate *Krt19*-mApple mice (I) and *Krt19*-CreERT mice (J). A tamoxifen-inducible Cre (CreERT2) from the pl451 vector plasmid was targeted into the translational initiation site of a *Krt19* containing BAC (RP23-24N13) using a Red bacteriophage system (J), whereas an mApple reporter from the pl451 vector plasmid was targeted into the translational initiation site of a *Krt19* containing BAC (RP23-24N13) by BAC recombineering (I). Differentiating the expression pattern of *Krt19* protein (green) expressing cells and *Krt19*$^{+}$ cell lineage tracing in a *Krt19*-CreERT;R26-Tomato mice 24h post tamoxifen (K).

Supplementary Figure 2

Krt19 marks long-lived stem cells *in vivo* and *in vitro*

Related to Figure 1. Lineage tracing in the colon was performed using *Krt19*-CreERT mice crossed to R26-mT/mG reporter mice. Representative sections of high-power view of lineage-labeled colon at different time points (24 h, 7 days, 52 weeks) following administration of tamoxifen. Macroscopic views of EGFP+ whole mount colon (B) of *Krt19*-CreERT;R26-mT/mG mice 6 months following tamoxifen; ($n \geq 3$ per group). Quantitative analysis of small intestinal contiguously labeled β-gal^{+} *Krt19* (C) or *Lgr5* (D) labeled crypts over time. Related to Figure S1E, Lineage tracing in the colon was performed using *Krt19*-CreERT mice crossed to R26-mT/mG reporter mice. Representative sections of high-power view of lineage-labeled colon at different time points (24 h, 7 days, 52 weeks) following administration of tamoxifen. Macroscopic views of EGFP+ whole mount colon (F) of *Krt19*-CreERT;R26-mT/mG mice 6

months following tamoxifen; (n ≥ 3 per group). Schematic illustration of an intestinal crypt imaged in 3 planes as shown using two photon microscopy (G). Related to Figures (H-I), Representative two photon microscopic images of *in vitro* cultured intestinal crypts isolated from *Krt19*-CreERT;R26-mT/mG mice administered tamoxifen 24 h prior to sacrifice. 3-dimensional reconstructed image of small intestinal crypts 24 and 48 hours and 9 days following tamoxifen induction and EGFP labeling of Krt19$^+$ cells (B). Representative two photon microscopic images of intestinal crypts at different time points (24 hours, 48 hours, and 9 days) following administration of 1 mg tamoxifen (p.o.) induction is shown (I). Fluorescent microscopic images of intestinal enteroids derived from *Krt19*-mApple$^+$ single-cells isolated from *Krt19*-mApple reporter mice (J); (n ≥ 3 per group).

Supplementary Figure 3

***Krt19*+ and *Lgr5*+ mark distinct cellular populations within the intestinal crypt and the majority of *Krt19*+ but not *Lgr5*+ crypt based columnar cells are located within the intestinal crypt active proliferation zone**

Related to Figure 2. Representative low (A) and high (B) power *Krt19 in situ* hybridization images colocalized with Ki67$^+$ (brown) cells in the intestinal crypt. *Krt19* mRNA expressing cells (dark blue) and Ki67$^+$ cells (brown) are shown in the crypts (A-B). Quantification of average cell position of *Lgr5*-EGFP$^+$ (green) cells, in situ *Krt19* mRNA$^+$ cells (red) and Ki67$^+$ cells (blue) within the intestinal crypt (C). Representative high power immunofluorescent images colocalizing BrdU$^+$ (red), *Lgr5*-EGFP$^+$ (green) cells in the small intestine of *Lgr5*-EGFP-IRES-CreERT2 mice (D) versus BrdU+ (red), *Krt19*$^+$ (green) cells expressing EGFP 20 h following tamoxifen induction in the small intestine of *Krt19*-CreERT;R26-mT/mG mice (E). Note white arrows point to rare double positive (Lgr5-GFP$^+$, Ki67$^+$) cells while yellow arrows point to rare (Ki67$^-$, *Krt19*-GFP$^+$) cells. Representative low (F) and high (G) power *in situ* hybridization images colocalizing Ki67$^+$ (brown), *Krt19* mRNA expressing cells (dark blue) cells in the colonic crypt of wild-type mice. Representative high power immunofluorescent images colocalizing Ki67$^+$ (red), *Krt19*$^+$ (green) cells expressing EGFP 20 h following tamoxifen induction in the colon of

Krt19-CreERT;R26-mT/mG mice (H) versus Ki67$^+$ (red), *Lgr5*-EGFP$^+$ (green) cells in the colon of *Lgr5*-EGFP-IRES-CreERT2 mice (I). Note white arrow point to a double positive (Ki67$^+$, Krt19-GFP$^+$) cell and yellow arrow points to a rare double positive (Lgr5-GFP$^+$, Ki67$^+$) cell. Quantification of BrdU$^+$, *Lgr5*-EGFP$^+$ cells versus Ki67$^+$, *Krt19* EGFP$^+$ cells in the small intestine is shown (J); ($n \geq 3$ per group). FACS plots of gating strategy used for sorting intestinal epithelial cells including from control *Krt19*-mApple or *Lgr5*-EGFP-IRES-CreERT2 mice as shown (K). Colocalization of *Bmi1*-GFP$^+$ and *Krt19*$^+$ cells in a *Krt19*-CreERT;R26-tdTomato;*Bmi1*-GFP mice 24h post tamoxifen (L).

Supplementary Figure 4

***Krt19*$^+$ stem cells render *Lgr5*$^+$ stem cells dispensable *in vitro* independent of *Dll1*$^+$ progenitors**

Related to Figure 3. (A)Diptheria toxin (DT) and tamoxifen (TAM) treatment protocol used for in vitro enteroid culture experiments using intestinal crypts isolated from *Lgr5*-DTR-EGFP; *Krt19*-CreERT;R26-Tomato mice. Representative fluorescent microscopy images of enteroids from *Lgr5*-DTR-EGFP;*Krt19*-CreERT;R26-Tomato mice treated with DT (500 ng i.p. x 3) in vivo followed by in vitro DT (20 ng/ml x 2 days). (B)Tamoxifen (2 mg) labeling of *Krt19*$^+$ stem cells was done in vivo 24 h prior to sacrifice and isolation of crypts. Images of enteroids were taken 6 days (d6), 9 days (d9) or 11 days (d11) after the first *in vivo* DT dose. Note the dead cells extruded into the internal lumen of large enteroids appear as autofluorescent yellow cells. * indicates $p<0.05$; ($n \geq 5$ per group). (C) Efficacy of in vitro DT ablation of *Lgr5*$^+$ cells as assessed by Lgr5 mRNA expression. Low power views of β-gal$^+$ small intestinal crypts from control (D) versus irradiated (12 Gy) (E) *Krt19*-CreERT;R26LacZ mice. (D) Representative high power view of Ki67$^+$ cells in the small intestine of control (left) versus 5-FU treated (right) mice for transit amplifying (TA) cell ablation.

Supplementary Figure 5

***Krt19* marks an early gastrointestinal stem cell during embryonic development of the gut**

Related to Figure 3. (A)Bacterial artificial chromosome (BAC) transgenic approach used to generate constitutive Cre expressing *Krt19*-BAC-Cre mice. A constitutively expressed Cre recombinase enzyme

was targeted into the translational initiation site of a *Krt19* containing BAC (RP23-24N13) using a Red bacteriophage system. (B)Whole mount macroscopic picture of a *Krt19*-Cre; R26LacZ embryo at E9.5 stained with X-gal shows the entire gut tube is β-gal$^+$. (C)Representative section of an E9.5 day old embryo demonstrating β-gal$^+$ gut epithelium. β-gal$^+$ small intestinal and colonic epithelium in 8 week old *Krt19*-Cre;R26LacZ (D-E) adult mice or *Krt19*-Cre;R26-mT/mG mice (F-G). (H)Representative high-power view section of neonatal *Lgr5*-EGFP-IRES-CreERT2 mice at P5 demonstrating the first detection of Lgr5-GFP+ cells within the early intestinal gut. (I) Serial section of P5 intestine stained for H & E is shown; ($n \geq 4$ per group).

Supplementary Figure 6

***Krt19*$^+$ and *Lgr5*$^+$ intestinal stem cells expand with radiation-induced injury**

Related to Figure 4. Representative low power view of β-gal$^+$ small intestinal crypts of *Lgr5*-EGFP-IRES-CreERT2;R26RLacZ mice following high dose radiation exposure 24 h post tamoxifen demonstrating the lack of β-gal$^+$ crypts following irradiation (A), in contrast to β-gal$^+$ crypt lineage tracing in *Krt19*-BAC-CreERT2/R26RLacZ mice (B). Low power views of β-gal$^+$ small intestinal crypts from mice irradiated 2 weeks after tamoxifen (control (C) versus irradiated (12 Gy) (D) *Lgr5*-EGFP-IRES-CreERT2;R26RLacZ mice are shown). Bone marrow transplantation from wild-type C57BL/6 mice was performed to sustain mice following lethal irradiation. Radiation exposure for above experiments was carried out 2 weeks following tamoxifen induction (6 mg p.o x 3) and epithelial lineage tracing examined 2 months following tamoxifen induction; ($n \geq 6$ per group). High power views of β-gal$^+$ small intestinal crypts from control (E) versus irradiated (12 Gy) (F) *Krt19*-CreERT;R26RLacZ mice irradiated 2 weeks after tamoxifen. *Krt19*-CreERT;R26RLacZ mice were irradiated 2 weeks after tamoxifen and lineage tracing examined 2 months following tamoxifen.

Supplementary Materials & Methods

Generation of *Krt19*-CreERT2 transgenic mice

For BAC recombineering, a K19 containing BAC clone (BAC RP-23-24N13) was purchased from Children's Hospital Oakland Research Institute (CHORI). The BAC clone RP24-24N13, which was a total size of 303,548 bp and contained DNA sequences ~175 kb upstream and ~125 kb downstream of the K19 gene coding region. The BAC was isolated and transformed into SW105 competent cells as previously described (Sharan et al., 2009). A *Krt19*-CreERT construct was then generated as previously described (Sharan et al., 2009; Thomason et al., 2007). *Briefly*, a CreERT2 cassette was cloned upstream of the kanamycin resistant gene (KANA) in the plasmid vector pKD4. Next, we generated a DNA probe containing a 40 bp sequence homologous to the BAC sequence directly upstream of the ATG and 40 bp downstream of the ATG, with deletion of the 3 bp endogenous ATG sequence of the K19 exon 1 as previously described (Sharan et al., 2009). The CreERT2-KANA fragment was then amplified using the DNA probes containing 40 bp overhang sequences homologous to BAC upstream and downstream of the ATG site. Purified PCR products were transformed into SW105 bacteria containing the BAC RP-23-24N13 with the RED recombineering system as previously described (Thomason et al., 2007). Recombinant clones were then selected on plates containing Kanamycin (30 ug/ml), and bacterial clones screened for correct homologous insertion of PCR generated constructs using PCR to confirm insertion at both ends of the construct. Correct clones were transformed with FLP coding plasmid to delete the KANA gene upon growth in LB medium containing 0.1% arabinose. Clones with correct kanamycin resistance gene deletion were screened by PCR, and sequences of the BAC containing the inserted CreERT2 construct were sequence-verified as previously described (Sharan et al., 2009). The *Krt19*-CreERT DNA was then isolated, linearized and microinjected into the pronucleus of fertilized CBA x C57BL/6J oocytes at the University of Pennsylvania Transgenic Mouse Core Facility. After back-crossing (F6) to C57BL/6 mice, genotype positive founders were mated with B6.129S4-Gt(ROSA)26Sortm1Sor5 /J, referred to as R26rLacZ, or B6.129(Cg)-Gt(ROSA)26Sortm4(ACTB-

tdTomato-EGFP) referred to as R26-mT/mG reporter mice, (Jackson Laboratories) and histological analysis performed following administration of tamoxifen. All animal studies were performed in Institutional Animal Care and Use Committee (IACUC)-approved facilities and completed in accordance to IACUC protocols at Columbia University.

A *Krt19*-mApple reporter construct was then generated in a manner similar to that described above. Briefly, an monomeric Apple (mApple) cassette was cloned upstream of the neomycin resistance gene flanked by *Frt* sites (FNF) in the plasmid vector pl451. Next, we generated a DNA probe containing a 40 bp sequence homologous to the BAC sequence directly upstream of the ATG and 40 bp downstream of the ATG as previously described (Sharan et al., 2009).The mApple-FNF fragment was amplified using the DNA probes containing 40 bp overhang sequences homologous to BAC upstream and downstream of the ATG site. Purified PCR products were transformed into SW105 bacteria containing the *Krt19* BAC RP-23-24N13 and recombineering performed as previously described (Thomason et al., 2007). Clones with correct NEO resistance gene deletion were screened by PCR, and sequences of the BAC containing the inserted mApple construct sequence-verified as previously described (Sharan et al., 2009). The *Krt19*-BAC-mApple DNA was then isolated, linearized and microinjected into the pronucleus of fertilized CBA x C57BL/6J oocytes at the Columbia University Transgenic Mouse Core Facility.

Lineage tracing analysis and assessment of β-galactosidase activity or EGFP immunofluorescence

To assess the stem cell properties of *Krt19* and *Lgr5* expressing cells, we performed lineage tracing analysis of *Krt19* or *Lgr5* positive cells in *Krt19*-CreERT;R26rLacZ or *Lgr5*-EGFP-IRES-CreERT2;R26rLacZ mice as previously described (Barker et al., 2007). Mice were administered 6 mg tamoxifen in 200 μl peanut oil containing 20 mg/ml tamoxifen administered by oral gavage. For short term lineage tracing experiments (7 days to 1 month) mice were administered a single dose of 6 mg tamoxifen (p.o.). For long term time points (4 months – 1 year) mice were administered 6 mg tamoxifen (p.o.) every other day for a total of 3 doses. To ensure, specificity of *Krt19*$^{+}$ cell labeling, lineage tracing

experiments were additionally repeated using low dose tamoxifen (ranging from 0.5-6 mg p.o.). No difference in the pattern of labeling was detected at short or long-term time points (data not shown). Following tamoxifen induction of Cre recombinase in *Krt19*$^{+}$ or *Lgr5*$^{+}$ cells, β-galactosidase labelling was assessed by staining of frozen sections taken from R26rLacZ mice. Briefly, mice were sacrificed at various time points post tamoxifen and for tumor studies mice analyzed at the time points specified. Following intracardiac perfusion of a solution containing 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M Sorensen's phosphate buffer (pH 7.4) (containing 2 mM MgCl2 and 5 mM EGTA), tissues were fixed in 4% PFA at 4°C for two to three hours and cryopreserved in 30% sucrose before embedding in OCT compound. Tissues were then sectioned on a cryostat for enzyme histochemical analysis. Frozen sections (8 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)6.6H2O). Sections were then counterstained with nuclear fast red and visualized using standard light microscopy.

To verify our findings using *Krt19*-CreERT;R26rLacZ mice, *Krt19*-CreERT mice were also crossed to ROSA26-mT/mG reporter mice. Once again, following tamoxifen induction in a manner identical to that used in *Krt19*-CreERT;R26rLacZ mice, we analyzed tissues for EGFP positive Krt19 expressing cells and their progeny at various time points post tamoxifen. In experiments examining the effects of radiation, AOM, DSS or a combination of AOM and DSS on Krt19+ or Lgr5+ stem cell lineage tracing, mice were analyzed at various time points as specified. Briefly, *Krt19*-CreERT;R26-mT/mG mice were assessed following intracardiac perfusion of 20 cc of cold PBS and 40 cc of a solution containing ice cold 4% paraformaldehyde solution. The SI and colon were then removed and immersed in 4% paraformaldehyde at 4^{o}C for an additional 2-4 hours prior to cryopreserving in 30% sucrose and embedding in optimum cutting temperature (OCT) compound. For visualization of tissues, 5 μm frozen sections covered using Vectashield mounting media containing DAPI (Vector Labs) were cut onto slides

and assessed using a standard florescence microscope. For immunofluorescence staining, secondary antibodies conjugated to a far-red A647 fluorescent protein was used in order to avoid overlap with the endogenous Tomato red and EGFP signals.

For *in vivo* experiments examining the effects of *Lgr5*$^+$ cell ablation, we crossed *Lgr5*-DTR-EGFP mice (kindly provided by Genetech) to *Krt19*-CreERT;R26Tomato mice. Baseline characterization of *Lgr5*-EGFP$^+$ versus *Krt19*-Tomato$^+$ cells was assessed in the intestinal and colonic crypts 24h following tamoxifen labeling of *Krt19*$^+$ cells. To assess the effects of *Lgr5*$^+$ cell ablation, diphtheria toxin (DT) (500 ng i.p.) was administered daily on days 0, 2 and 4. On the last day of intraperitoneal DT injection (day 4), a single dose of tamoxifen (4 mg p.o.) was also administered to label *Krt19*$^+$ stem cells. Twenty-four hours following tamoxifen and the last dose of DT, *Lgr5*$^+$ cell ablation and *Krt19*$^+$ stem cell lineage tracing of the intestine and colon were assessed using fluorescence microscopy. For additional experiments examining the efficiency of *Krt19*$^+$ cell lineage tracing in the presence or absence of *Lgr5*$^+$ cell ablation, we administered 5-fluorouracil (5-FU; 150 mg/kg i.p. daily x 2 days) to *Lgr5*-DTR-EGFP; *Krt19*-CreERT;R26-Tomato. Mice were then given tamoxifen 24h following the last dose of 5-FU and sacrificed 24 h after tamoxifen to assess the intestinal and colonic *Krt19*$^+$ cell lineage tracing. Efficiency lineage tracing was determined by quantification of the number of *Krt19*$^+$ lineage traced glands per 1000 crypts counted.

Histology, immunohistochemistry, immunofluorescence and microscopy

For immunohistochemical staining, 5 μm paraffin sections were prepared using a standard protocol. Immunohistochemical staining was performed on paraffin sections using primary antibodies (see list below) and corresponding biotinylated secondary antibodies (Jackson Immunoresearch Laboratories Inc., West Grove, PA) diluted in 2% bovine serum albumin-PBS and incubated one hour each at room temperature. ABC avidin-biotin-DAB detection kit (Vector Labs) was then used for

detection and visualization of staining according to the supplied protocol. Finally, slides were counterstained with hematoxylin and coverslip mounted.

For immunofluorescence staining, 4% PFA-fixed frozen OCT embedded tissue sections were cut in 5 μm sections. Sections were then washed in PBS containing 0.1% Triton X-100, rinsed in PBS and blocked for 30 min with 2% BSA. Primary antibodies (see list below) were diluted in 2% BSA and incubated with tissues overnight at 4^{o}C. Following 5 washes in PBS, florescent protein conjugated secondary antibodies (Invitrogen) were then added to slides for 30 min. Slides were washed with PBS once again and covered with Vectashield mounting media containing DAPI (Vector Labs). Primary antibodies used include Cytokeratin 19 (Invitrogen, 1:100), GFP (Invitrogen, 1:500), Cre (Covance, 1:2000), Chromogranin A (Epitomics, 1:100), Ki-67 (BD Pharmingen, 1:100), Krt19 (University of Iowa, Iowa City, Iowa, 1:100), β-catenin (Transduction Laboratories 1:100), GFP (Clontech 1:300), BrdU (Abcam 1:300). We used secondary antibodies conjugated with AlexaFluor 488 and AlexaFluor 594 and AlexaFluor 647 (Life Technologies). Confocal fluorescence microscopy was performed on a Nikon (Thornwood, NY, USA) LSM 510 NLO multiphoton confocal microscope system based on an Axioskop 2 FS MOT microscope stand. To colocalize *Bmi1*$^{+}$ cells with *Krt19*$^{+}$ *cells* we additionally crossed *Krt19*-CreERT;R26Tomato mice to *Bmi1*-GFP mice generously donated by Irv Weissman's group (Stanford University). The intestine from these triple transgenic mice were then examined for double positive cells 24 h after tamoxifen administration.

***In situ* hybridization**

The cRNA probe for *Krt19* was constructed and labeled as previously described (Joshua and Samuel, 2007). Briefly, linearized template DNA (1 μg) was incubated for 2 h at 37°C in a buffer containing 10 mM DTT, 1 mM digoxigenin-11-NTP (Boehringer-Mannheim; Mannheim, Germany), and 40 U of T7 or Sp6 RNA polymerase. Sense cRNA probe of the same length as the antisense probe was also synthesized to determine specificity. Probe concentrations were estimated by agarose gel

electrophoresis. C57BL/6 male mice were perfusion fixed through left ventricle puncture for 10 min with 4% paraformaldehyde in DEPC-treated PBS, and the SI and colon were excised, postfixed in 4% paraformaldehyde for 2 h, embedded in OCT compound (Sakura Finetek, CA) and frozen on dry ice. Cryostat sections (10 μm) were cut and the sections dried for 20 min. Tissue sections were then prepared for hybridization following a number of washes including (4% PFA fixation for 10 mins, Proteinase K digestion for 5 min, 4% PFA fixation for 5 min, and acetylation solution for 10 min). Sections were treated with an acetylation solution (1.2% triethanolamine and 0.25% acetic anhydride) to block endogenous phosphatases. After being washed with PBS, the sections were pre-hybridized at 60°C for 60 min with hybridization solution containing 50% formamide, 5x sodium citrate saline (SSC; pH 7.0), 25 μg/ml yeast RNA, 0.5 mg/ml sheared salmon sperm DNA, and 5x Denhardt's solution, followed by hybridization at 60°C overnight with the probes (1.5 μg/ml) diluted into the hybridization solution. The sections were washed in 5x SSC at 60°C for 1 min, in 0.2% SSC at 60°C for 1 h, and then at room temperature for 5 min, and then in a solution containing 1.16% maleic acid and 0.9% NaCl, adjusted to pH 7.5 using NaOH, for 5 min. Blocking was performed with blocking solution containing 2% blocking reagent (Roche, Nutley, New Jersey), 0.1% Tween-20, and 10% normal goat serum at room temperature for 1 h. Sections were incubated overnight at 4°C with anti-digoxigenin antibody (Roche) diluted 1:5,000 in the blocking solution. The sections were washed in PBS containing 0.1% Tween-20 for 15 min four times and three times for 5 min each in 100 mM Tris (pH 9.5) containing 50 mM MgCl2, 100 mM NaCl, 0.1% Tween-20, and 1 mM Levamisol. Chromogen was developed at room temperature for 2 h to overnight with BM Purple AP substrate (Roche). The chromogen development was terminated by washing the slides in PBS. The sections were counterstained with nuclear fast red, dehydrated, penetrated, and mounted with Permount (Fischer Scientific).

For florescent probe *in situ* hybridization, a similar protocol to that described above was used with the exception of washes being complete in a Tris buffer (0.1M Tris, 0.15M NaCl and Tween20) followed by blocking 0.5% blocking reagent in 0.1M Tris containing 0.15M NaCl.

***In vitro* intestinal crypt cultures**

To examine the stem cell properties of Krt19 positive cells in the small intestine and colon, intestinal or colonic crypts were isolated from *Krt19*-CreERT;R26-mT/mG mice and cultured as previously described (Jung et al., 2011; Sato et al., 2009). Briefly, tamoxifen 2 mg p.o was administered to *Krt19*-CreERT;R26-mT/mG mice to label *Krt19* expressing cells immediately prior to examination of crypts in vitro. Twelve hours following tamoxifen administration, mice were sacrificed and the small intestine and colon removed. Both the intestine and colon were flushed clean with PBS and cut open longitudinally. In the case of the small intestine, the villi were removed by gently scraping the mucosal surface with a microscope glass coverslip prior to mincing the tissue into ~5 mm sized tissues. The remaining intestinal crypts were then washed in PBS by pipetting tissues up and down in the PBS. Following each wash in PBS, the supernatant was removed and this process repeated 5-7 times until the solution appeared relatively clear following washing. Intestinal tissues were then incubated in a PBS solution containing 2 mM EDTA for 30 min at 4^{o}C. The EDTA containing solution was then removed and the tissues washed in PBS as described above. The tissues were then was then passed through a 70 μm filter (BD Falcon), and centrifuged at 600 g x 5 min to obtain a concentrated intestinal crypt fraction.

In the case of colonic crypts, tissues were cut into ~5 mm sized tissues and washed in PBS 5-7 times as described for the small intestine. Following the final wash, the PBS supernatant was removed and colonic tissues incubated in a solution containing 2 mM EDTA for 1 hour at 4^{o}C. The EDTA containing solution was then removed and the tissues washed in PBS as described above. The tissues were then was then passed through a 100 μm filter (BD Falcon), and centrifuged at 600 g x 5 min to remove the PBS and obtain a concentrated colonic crypt fraction.

Colonic or intestinal crypt fractions were then mixed with Matri-gel (BD) at a density appropriate for optimal visualization of intestinal crypts using a Nikon 2-photon microscope. Intestinal crypts were maintained in culture for several weeks as previously described in Advanced DMEM/F12 media containing additional growth factors including B27 supplement (Invitrogen), N2 supplement (Invitrogen),

n-Acetylcysteine (Invitrogen), EGF 50 ng/ml (Invitrogen), mNoggin 100 ng/ml (Peprotech), and R-Spondin 1 ug/ml. Intestinal and colonic crypt cultures were then imaged every 48 h using 2-photon microscope imaging.

For single cell culture experiments, single cells were isolated and cultured as described previously (38). Briefly, crypts were isolated and dissociated by EDTA digestion as described but additionally dissociated with TrypLE Express (Invitrogen) including 1 mg/ml DNase I (Roche Applied Science) for 10 minutes at 37°C. Dissociated cells were then passed through a 20-μm cell strainer, washed with 2% FBS/PBS, and sorted by FACS. Viable *Krt19*-mApple$^+$ or *Lgr5*-GFP$^+$ cells were collected, pelleted, and embedded in Matri-gel, followed by seeding on a 48-well plate (1,000 singlets per well). To perform mRNA expression analysis of *Krt19*-mApple$^+$, *Lgr5*-GFP$^+$ cells or double positive *Krt19*-mApple$^+$/*Lgr5*-GFP$^+$ cells, ~10,000 cells of each cell population were collected into TRizol solution for RNA extraction as described above.

For in vitro experiments examining the effects of *Lgr5*$^+$ cell ablation, we used *Lgr5*-DTR-EGFP; *Krt19*-CreERT;R26Tomato mice. Diphtheria toxin (DT) (500 ng i.p.) was administered daily on days 0, 2 and 4. On the day of the last dose of intraperitoneal DT injection (day 4), a single dose of tamoxifen (4 mg p.o.) was also administered to label *Krt19*+ stem cells. Twenty-four hours following tamoxifen and the last dose of DT, mice were sacrificed and small intestinal and colonic crypts isolated for in vitro culture in the absence or presence of DT (20 ng/ml). Enteroid cultures were then followed by 2-photon microscopy that was then used to detect Tomato-red or EGFP fluorescence. To examine the effects of radiation injury on normal (wildtype) or APC floxed crypts, intestinal crypts from *Krt19*-mApple;*Lgr5*-EGFP-IRES-CreERT2 double transgenic mice or *Krt19*-CreERT;ApcF/F mice were cultured in vitro as described above. Intestinal crypts were irradiated (10 Gy) ~1 week after initial isolation and culture of intestinal crypts. Crypts were then followed using microscopy and/or RNA isolated following crypt dissociation by cooling of Matrigel and mechanical dissociation. To directly test the effects of *Lgr5*$^+$ cell ablation in APC floxed crypts, intestinal crypts from *Lgr5*-DTR-EGFP;*Krt19*-BAC-CreERT2;ApcF/F

mice were isolated and cultured in vitro 24 h after tamoxifen (2 mg p.o.) in the presence or absence of DT (200 ng/ml).

Additional in vitro intestinal crypt culture experiments were carried out in the presence or absence of standard growth factors. Briefly, in experiments examining the growth of crypts in media lacking Noggin and R-spondin, crypts were cultured in Advanced DMEM/F12 media containing all the supplements as described above including EGF 50 ng/ml (Invitrogen), but lacking Noggin and R-spondin. Lastly, to test the effects of *Lgr5*$^{+}$ cell ablation in APC floxed crypts cultured in media lacking R-spondin and Noggin, the intestinal crypts from *Lgr5*-DTR-EGFP; *Krt19*-CreERT;ApcF/F mice were isolated and cultured 24 h after tamoxifen (2 mg p.o.) in the presence or absence of DT (200 ng/ml).

Mouse Models of Intestinal Injury and Analysis of Radiation Effects

To examine the response of *Krt19*$^{+}$ and *Lgr5*$^{+}$ stem cells to intestinal radiation injury, six week old *Krt19*-CreERT;R26rLacZ or *Lgr5*-EGFP-IRES-CreERT2;R26rLacZ mice were first treated with tamoxifen (6 mg p.o. every other day x 3 doses) in order to label these two stem cell populations. Mice were then exposed to lethal dose (12 Gy) irradiation, bone marrow transplanted with C57BL/6 bone marrow in order to sustain these mice alive, and then lineage tracing of the intestine examined ~1 month following irradiation. In a separate group of mice, tamoxifen labeling of *Krt19* or *Lgr5* positive cells was initiated 24 h prior to irradiation, and again lineage tracing from *Krt19* or *Lgr5*, respectively, examined 1, 2 or 4 weeks following irradiation. To assess β-galactosidase activity in *Krt19* and *Lgr5* positive stem cells and their progeny, X-gal staining was carried out as described above. Histologic sections were stained with hematoxylin and eosin (H&E) and additional sections stained for β-catenin prior to histopathologic assessment using light microscopy.

Lastly, to demonstrate the relative radiation-resistance of *Krt19*$^{+}$ cancer initiating cells and radiation-sensitivity of *Lgr5*$^{+}$ cancer initiating cells, the mortality and tumor burden of *Krt19*-CreERT;R26-LacZ;ApcF/F versus *Lgr5*-EGFP-IRES-CreERT2;R26-LacZ;ApcF/F mice exposed to

radiation (12 Gy) 24 h following tamoxifen induction of APC deletion/truncation and labeling of *Krt19* or *Lgr5* positive cells was assessed.

RNA Isolation and Quantitative RT-PCR analysis of gene expression.

Total RNA was extracted with Trizol reagent (Invitrogen, CA) and cDNA was synthesized from 4 μg of total RNA with Superscript III First Strand cDNA synthesis kit (Invitrogen, CA). Expression level of each gene was quantified by real-time RT-PCR assays using PCR conditions: 95 degrees for 15 minutes followed by 40 cycles of 94 degrees for 10 seconds, 55 degrees for 20 seconds and 72 degrees for 30 seconds using QuantiTect SYBR Green PCR kit (Qiagen) and 7300 Real Time PCR System (Applied Biosystems, CA). Primers used included: Krt19: GCAGAATCGCCAGGAATT (F), AGACAGCAGCCCATCAGAC (R); Lgr5: GACGCTGGGTTATTTCAAGTTCAA (F), CAGCCAGCTACCAAATAGGTGCTC (R). Briefly, ΔCt was calculated by the subtraction of GAPDH Ct from the gene-specific Ct, and ΔΔCt was calculated by the subtraction of the gene-specific ΔCt from standard ΔCt (=40 cycles in this study).