

Expanded View Figures

Figure EV1. *Lgr5*-negative/*Krt19*⁺ and *Atoh1*⁺ cells show colonic epithelium renewal capacity in homeostasis and injury.

- A Illustration of experimental protocol outlining DT treatment of *Krt19*^{BAC-CreERT2};*ROSA*^{tdTomato};*Lgr5*^{DTR-GFP} mice.
- B, C In control conditions and after *Lgr5*⁺ stem cell ablation, *Krt19*⁺ cells form crypt ribbons and give rise to new *Lgr5*⁺ stem cells (*N* = 2 per time point).
- D Illustration of experimental protocol outlining DT treatment of *Krt19*^{BAC-CreERT2} or *Atoh1*^{CreERT2} mice.
- E, F Rare proliferating *Krt19*⁺ or *Atoh1*⁺ cells (insets, arrowheads) are found in the transit-amplifying zone of control crypts, and none are present at the bottom of crypts. At d4 of DT treatment, more proliferating *Krt19*⁺ or *Atoh1*⁺ cells (insets, arrowheads) are found in the transit-amplifying zone and the crypts are devoid of proliferating cells. By d8 of DT treatment, proliferating *Atoh1*⁺ cells (insets, arrowheads) are found at the bottom of crypts.

Data information: Scale bars (B, E) = 45 μm; scale bars (F) = 50 μm. Data are represented as mean ± SEM.

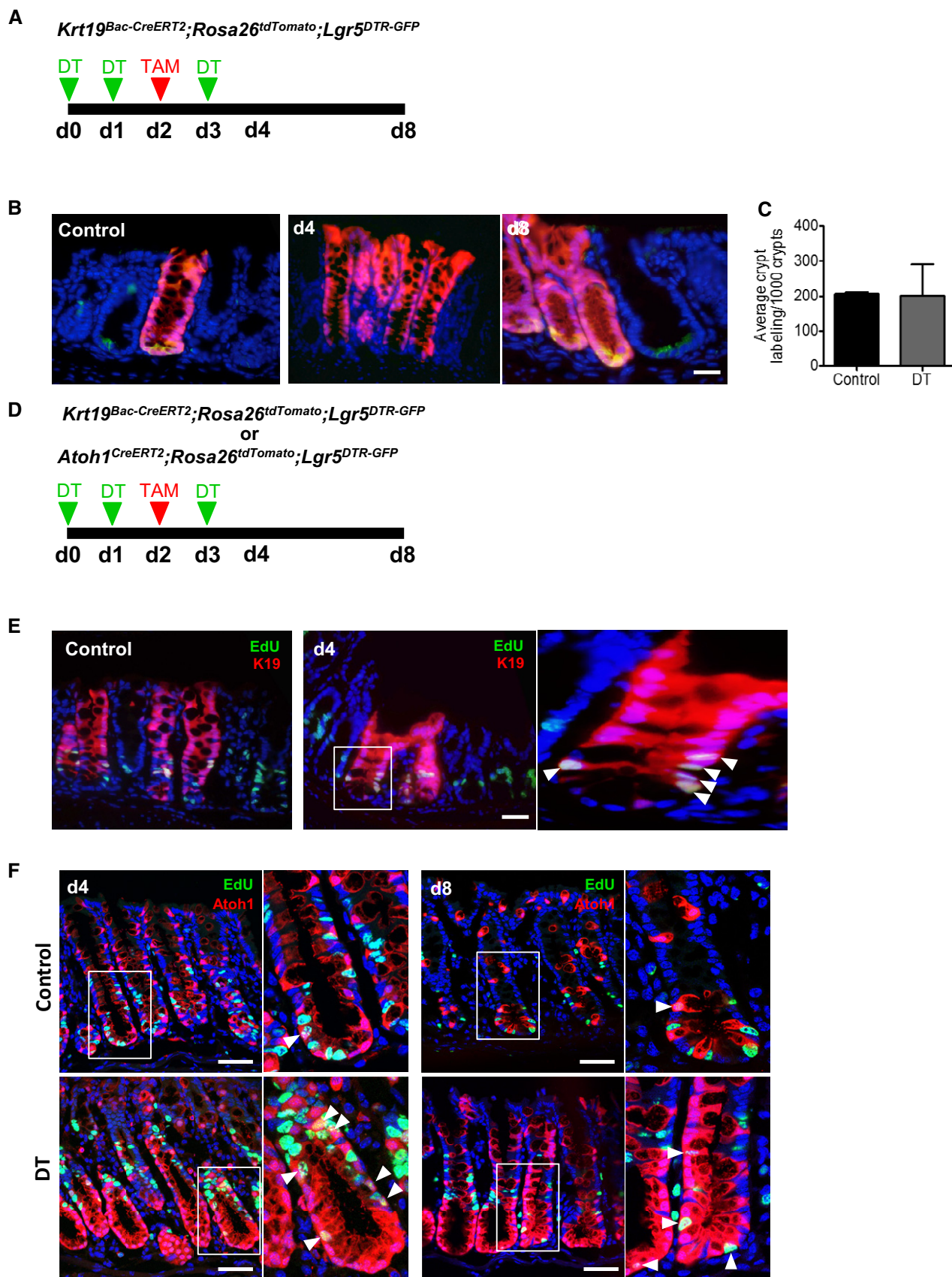


Figure EV1.

Figure EV2. Krt19-expressing cells include multiple secretory cell types in the colon and small intestine.

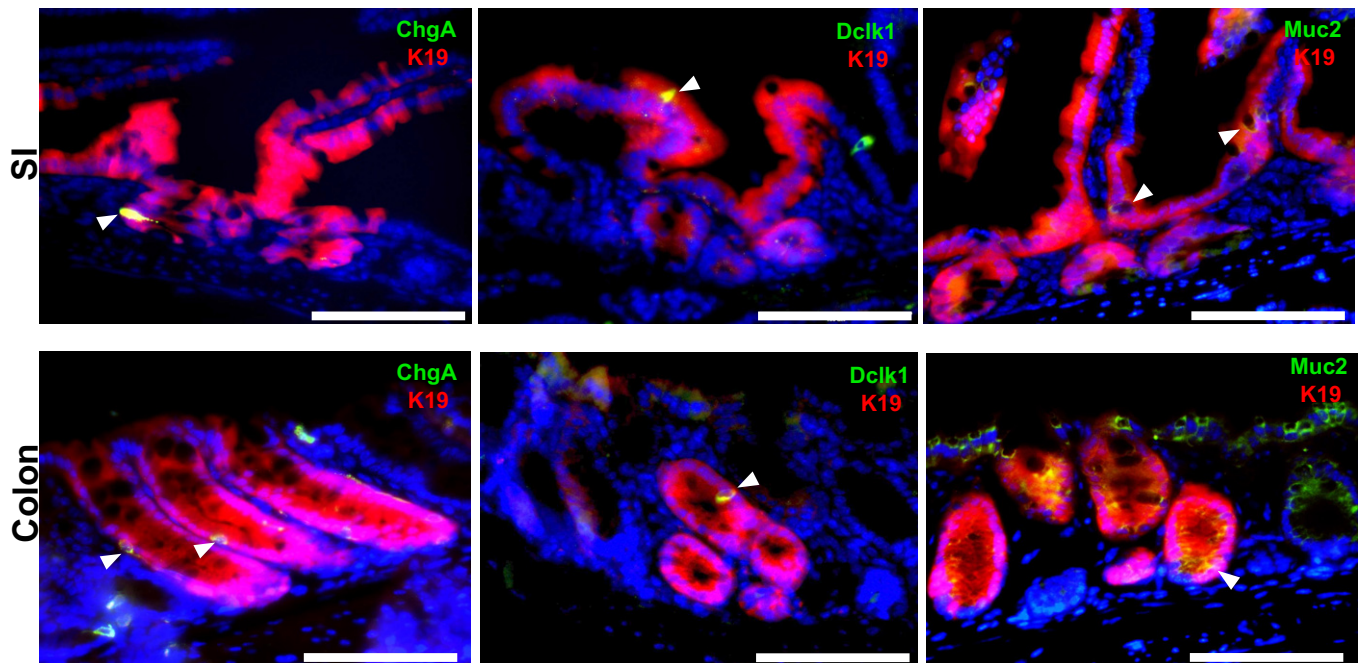
- A Illustration of experimental protocol outlining tamoxifen induction of *Krt19*^{BAC-CreERT2};*ROSA26*^{tdTomato} mice.
- B Immunofluorescence staining showing co-localization of *Krt19*⁺ cells with *ChgA*⁺, *Dclk1*⁺, and *Muc2*⁺ secretory cells in the small intestine and colon (arrowheads).
- C Illustration of experimental protocol outlining DSS-induced colitis in wild-type mice.
- D Effects of DSS colitis on RNA expression levels of various secretory (*Prox1*, *Neurog3*, *Bmi1*) cell markers. *Neurog3* expression is significantly increased acutely post-DSS (d5), whereas *Prox1* expression is significantly increased during late recovery (d19). *Bmi1* expression is not affected by DSS colitis.

Data information: Scale bars (B) = 100 μ m. Data are represented as mean \pm SEM analyzed by one-way ANOVA. * $P \leq 0.05$, *** $P \leq 0.0001$.

A *Krt19*^{Bac-CreERT2};*Rosa26*^{tdTomato}



B



C *wt*

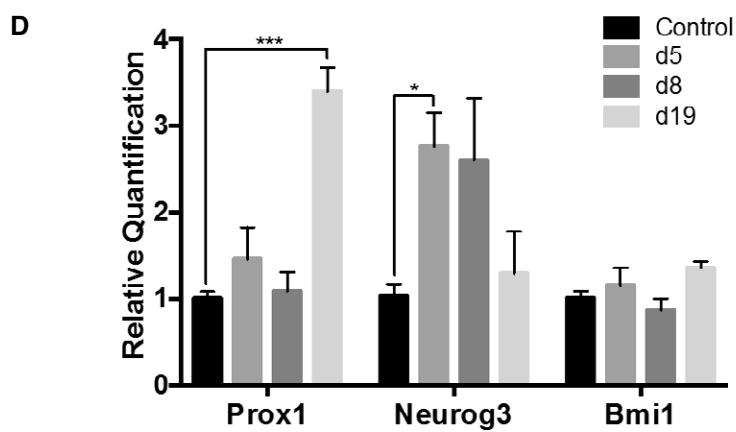
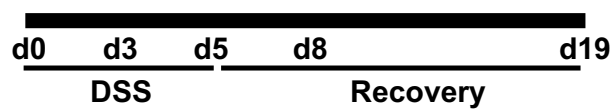


Figure EV2.

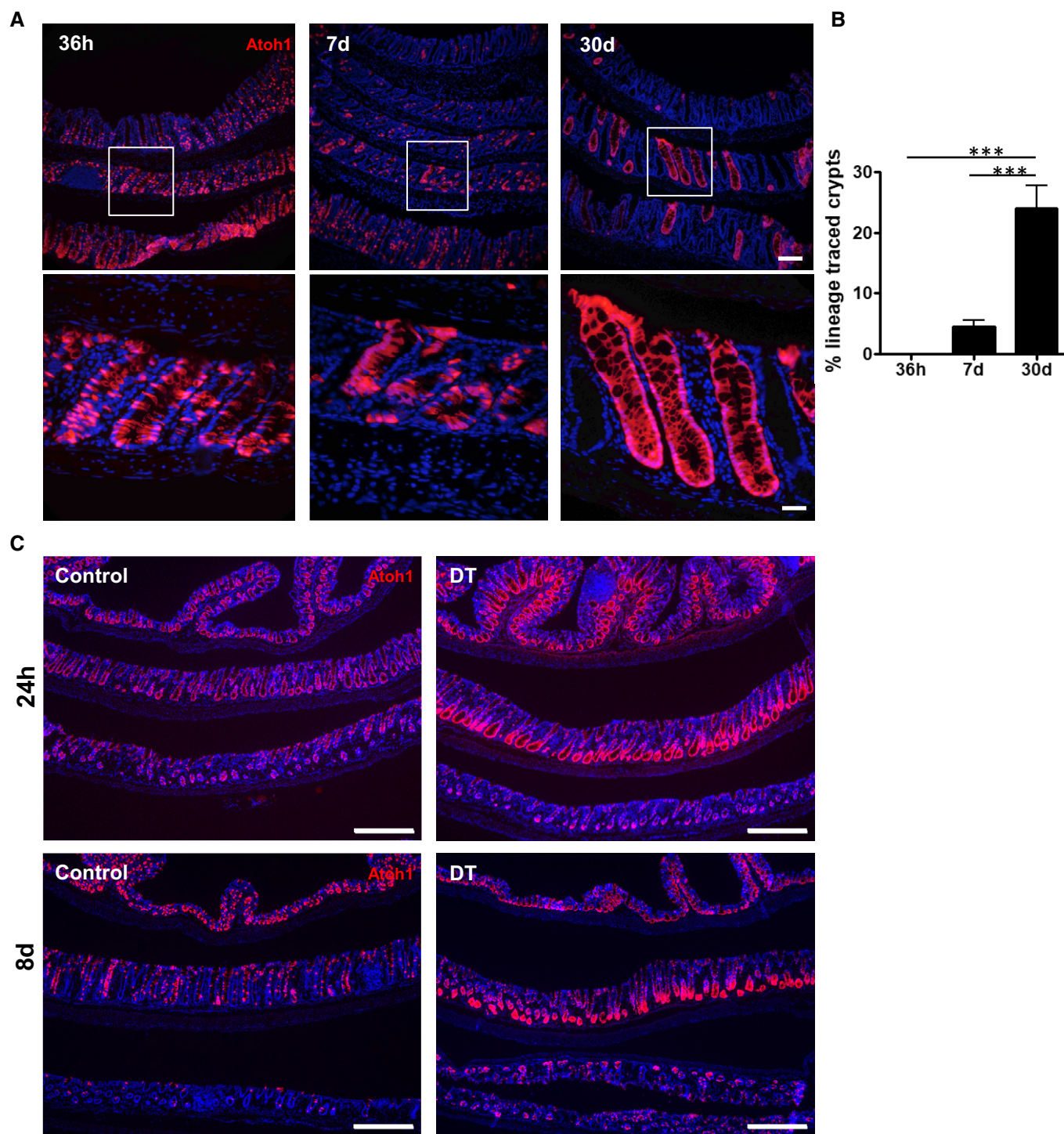


Figure EV3. Atoh1⁺ cells show rare renewal capacity in homeostasis, yet post-injury Atoh1⁺ cells acquire stemness.

A, B Occasionally, Atoh1⁺ cells are able to form crypt ribbons that persist up to 30 days after lineage labeling in homeostasis ($N = 4$ per condition).

C Following DT ablation of *Lgr5*⁺ cells, the majority of crypts are renewed and maintained by Atoh1⁺ lineage.

Data information: Scale bars (A) = 100 μm ; scale bars (C) = 500 μm . Data are represented as mean \pm SEM using one-way ANOVA. *** $P \leq 0.0001$.

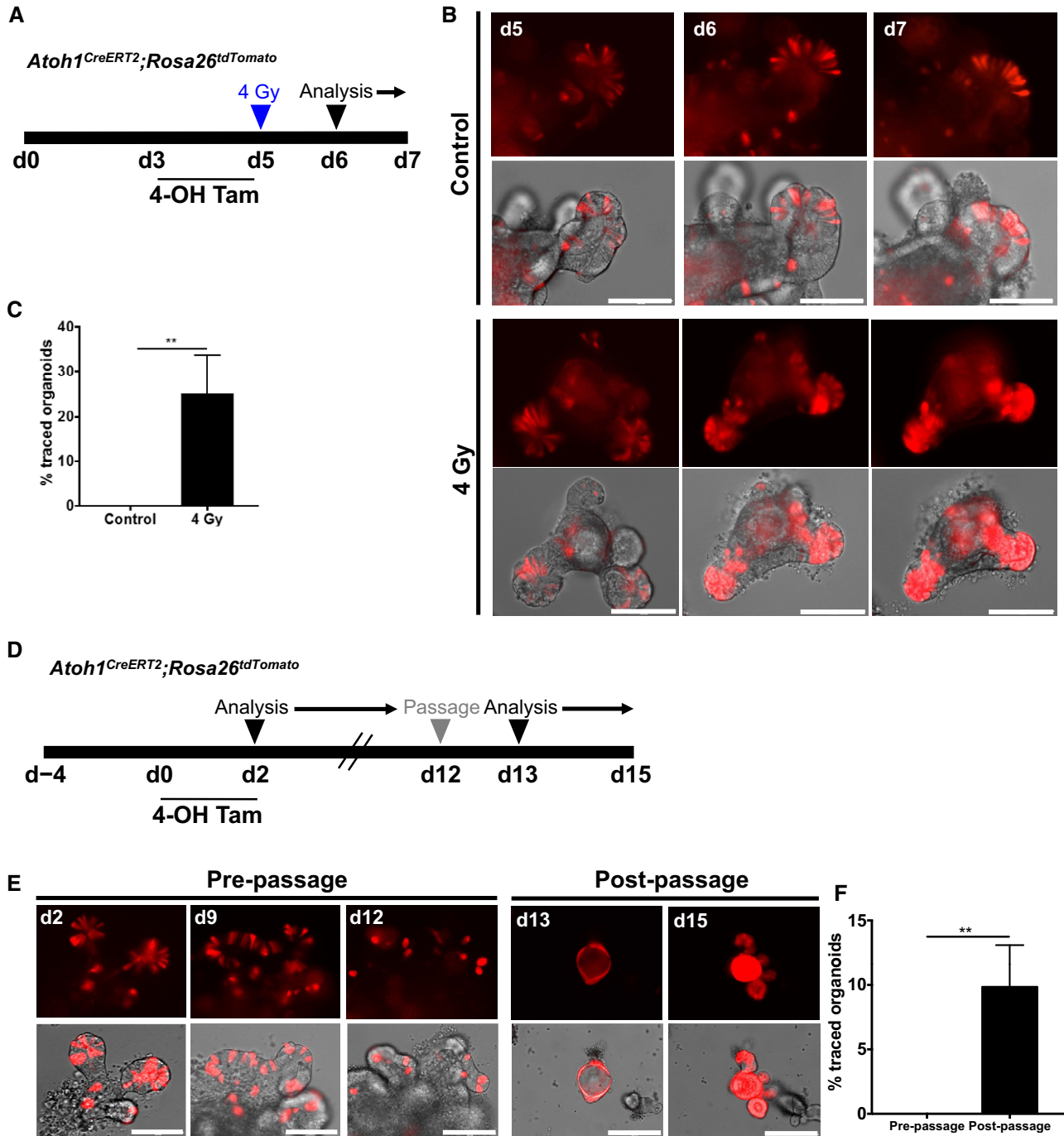


Figure EV4. Small intestinal *Atoh1*⁺ cells show renewal activity following injury *in vitro*.

A Illustration of experimental protocol outlining 4-OHT induction and irradiation of *Atoh1^{CreERT2};Rosa26^{tdTomato}* small intestinal organoids.

B *Atoh1*-tdTomato⁺ cells within crypts and villus regions of unirradiated organoids are scattered among tdTomato-negative cells. After irradiation-induced damage, *Atoh1*-tdTomato⁺ cells are able to acquire stemness and give rise to fully labeled crypts.

C After radiation-induced damage, 25% of organoids show robust lineage labeling (*N* = 3 control; *N* = 4 irradiated; *n* = 3 technical replicates per condition).

D Illustration of experimental protocol outlining 4-OHT induction of *Atoh1^{CreERT2};Rosa26^{tdTomato}* small intestinal organoids.

E, F Pre-passage organoids contain sporadic *Atoh1*-tdTomato⁺ cells interspersed between non-labeled cells. Post-passaging, fully labeled spheroids develop into fully labeled organoids (*N* = 3; *n* = 3 technical replicates per condition).

Data information: Scale bars (B, E) = 100 μ m. Data are represented as mean \pm SEM analyzed by Student's *t*-test. ***P* \leq 0.01.

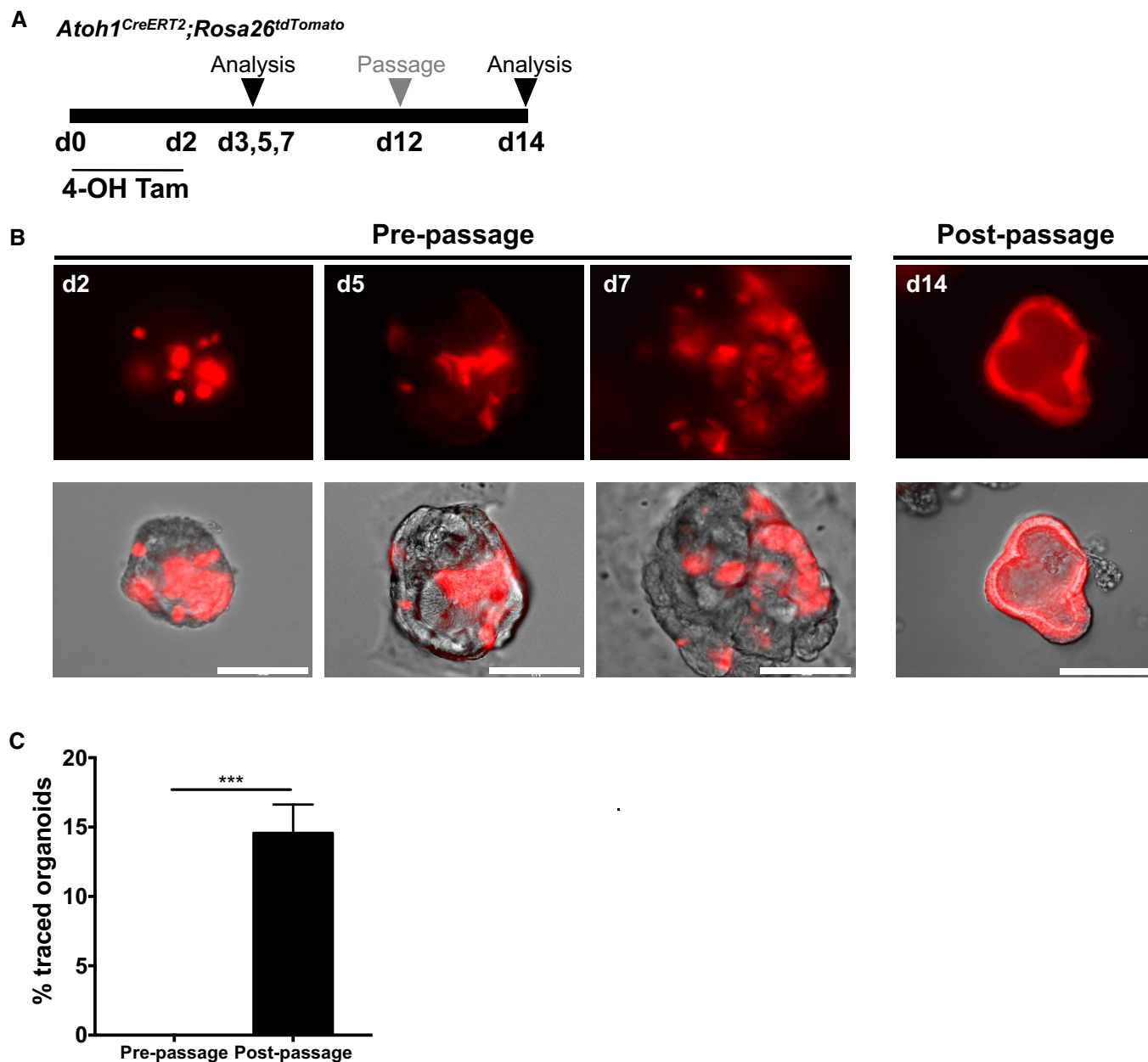


Figure EV5. *Atoh1*⁺ colonic progenitor cells show renewal capacity post-stress induced passaging *in vitro*.

A Illustration of experimental protocol outlining 4-OHT induction of *Atoh1*^{CreERT2};*Rosa*^{tdTomato} colonic organoids.

B, C Pre-passage colonic organoids exhibit lineage labeling of *Atoh1*⁺ cells that is limited to single cells. Following passage, *Atoh1*⁺ progenitors give rise to fully labeled colonic organoids ($N = 3$; $n = 3$ technical replicates per condition).

Data information: Scale bars = 100 μ m. Data are represented as mean \pm SEM analyzed using Student's t-test. *** $P \leq 0.0001$.