Expanded View Figures

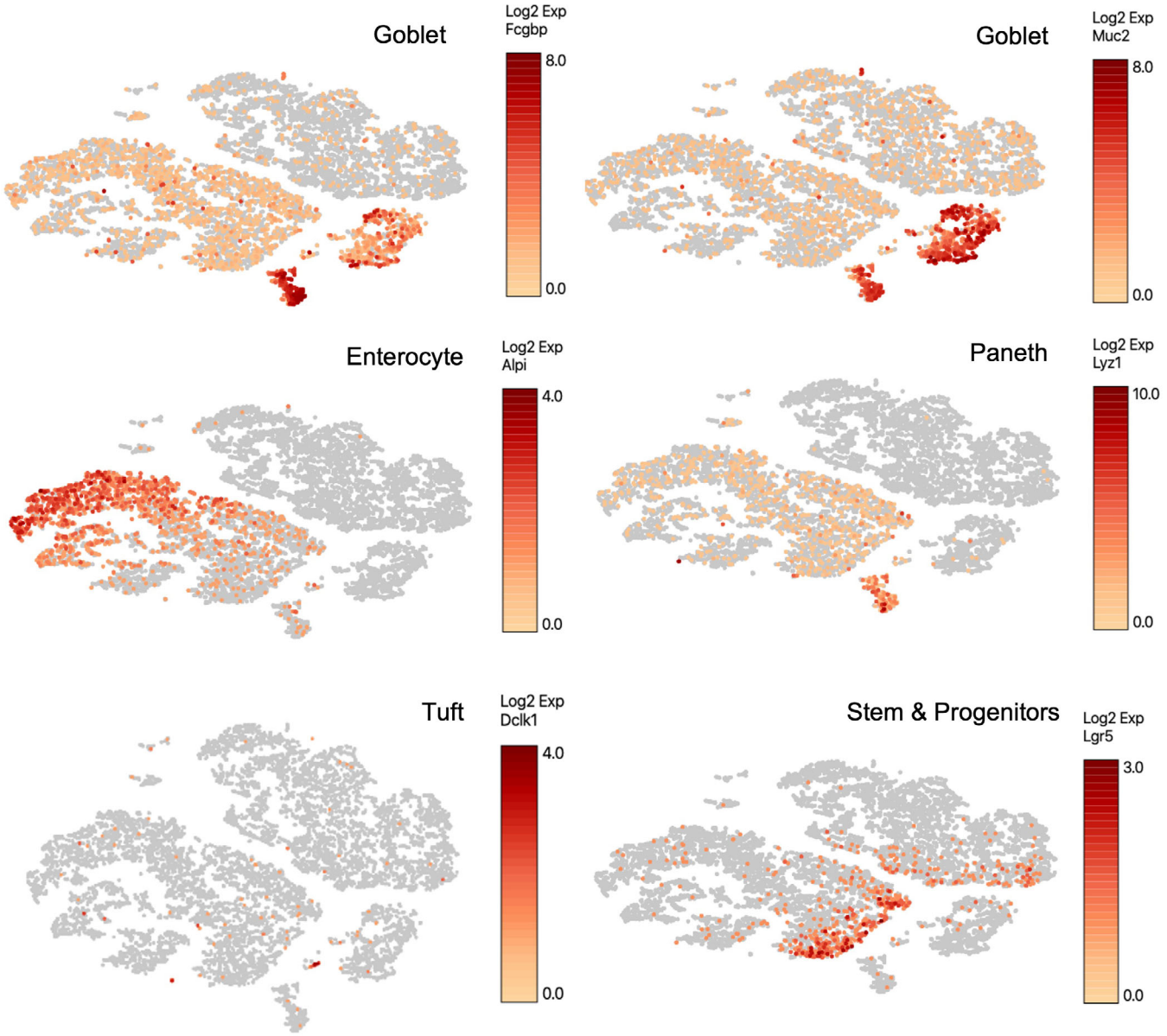
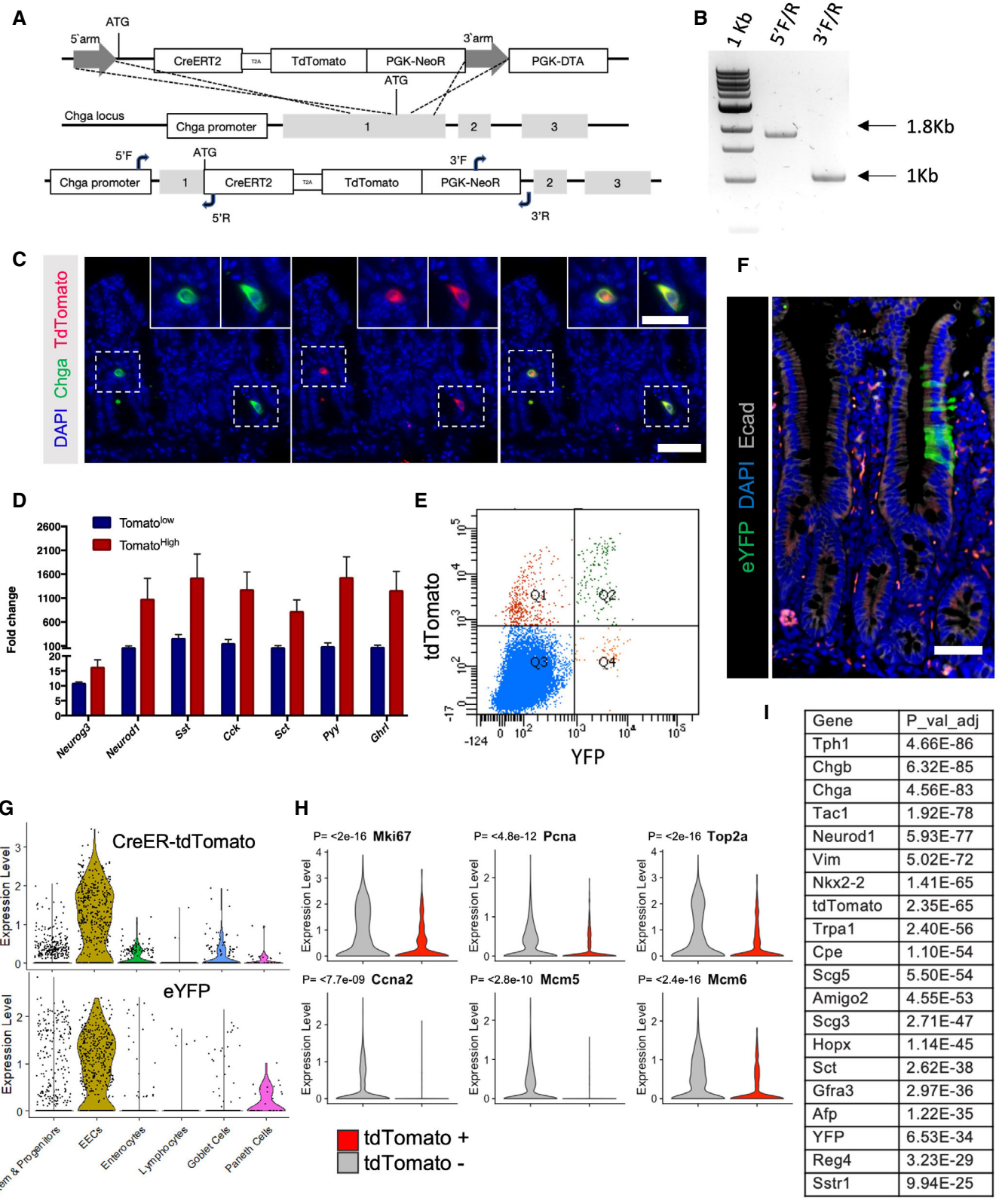


Figure EV1. Expression of marker genes in bulk small and large intestinal epithelium.

Marker genes used to identify cell clusters in the single-cell RNAseq dataset included Fcgbp and Muc2 (Goblet cells), Alpi (enterocytes), Lyz1 (Paneth cells), Dclk1 (Tuft cells), and Lgr5 (Crypt base columnar stem cells).

Figure EV2. Generation and validation of a novel Chga-CreER-2A-tdTomato allele.

A Targeting strategy to insert a CreERT2-2A-tdTomato sequence into the translational start site of the endogenous Chga locus.
B PCR to confirm proper targeting of the CreERT2-2A-tdTomato sequence, as demonstrated in (A). Expected 5′ fragment = 1.8 kb, expected 3′ fragment = 1 kb.
C Immunofluorescence staining of small intestine demonstrating overlap of Chga protein and tdTomato reporter in the same cells. Scale bar = 50 μm. Inset scale bar = 25 μm.
D Crypt enriched epithelial cell suspensions were generated from ChgaCreER-2A-tdTomato mice, sorted tdTomato+ cells, and qPCR was performed for transcription factors (Neurog3, Neurod1) and hormones (Sst, Cck, Sct, Pyy, Ghrl). *Y*-axis is fold change relative to tdTomato negative cells. *N* = 3 technical replicates/group. Data are expressed as mean ± SEM.
E Flow cytometry analysis of ChgaCreER-2A-tdTomato::R26RLSL-eYFP intestinal epithelial cells 48 h after five consecutive daily tamoxifen doses. Single-cell RNA sequencing was performed on cells sorted from Q2 + Q4 and a bulk population (all quadrants).
F Immunofluorescence staining of small intestine from a ChgaCreER-2A-tdTomato::R26RLSL-eYFP mouse 48 h after five tamoxifen doses. Scale bar = 50 μm.
G Violin plots demonstrating CreER-tdTomato and eYFP expression by cell type.
H Violin plots of cell cycle related genes in tdTomato+ and tdTomato− populations. *N* = 1 mouse/group, tomato− = 1,907 cells/group, tomato+ = 429 cells/group. *P*-value generated by two-tailed Student's *t*-test.
I Table of EEC related genes enriched in the tdTomato+ versus tdTomato− cell population.



I

Gene	P_val_adj
Tph1	4.66E-86
Chgb	6.32E-85
Chga	4.56E-83
Tac1	1.92E-78
Neurod1	5.93E-77
Vim	5.02E-72
Nkx2-2	1.41E-65
tdTomato	2.35E-65
Trpa1	2.40E-56
Cpe	1.10E-54
Scg5	5.50E-54
Amigo2	4.55E-53
Scg3	2.71E-47
Hopx	1.14E-45
Sct	2.62E-38
Gfra3	2.97E-36
Afp	1.22E-35
YFP	6.53E-34
Reg4	3.23E-29
Sstr1	9.94E-25

Figure EV2.

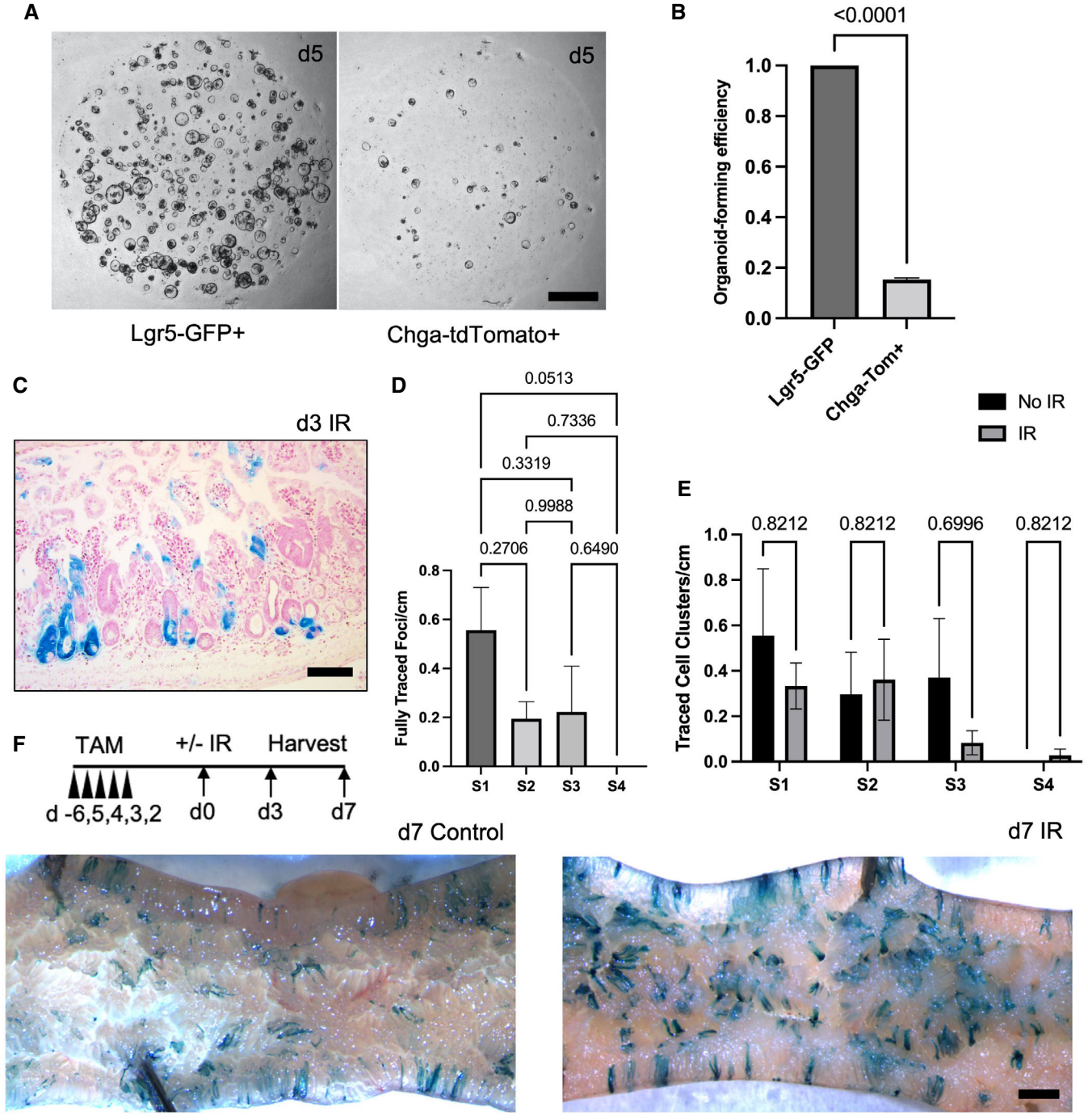


Figure EV3. EECs contribute to tissue mass pre- and post-DNA damaging injury.

A Representative images of enteroids grown from single sorted Lgr5-GFP^{High} or Chga-tdTomato+ cells. Scale bar = 1 mm.

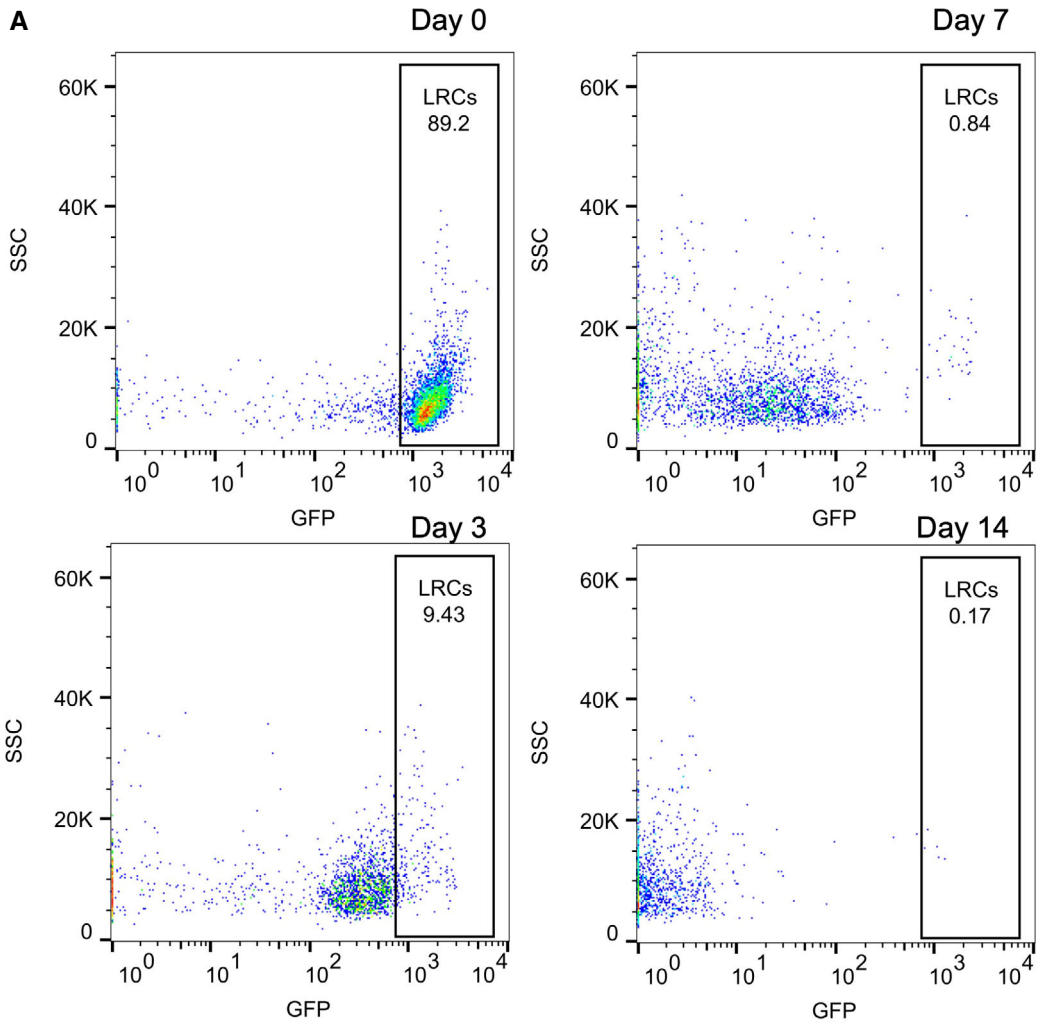
B Organoid formation efficiency of cells plated in (E). N = 3 mice/genotype, N = 3 wells quantified per mouse. Data are expressed as mean ± SEM. P-value generated by two-tailed Student's t-test.

C Example image of d3 small intestine stained with X-Gal, embedded in paraffin, sectioned, and counterstained with neutral red. Scale bar = 100 μm.

D The number of fully traced b-Gal + clonal regenerative foci per cm of small intestine, measured on d3 postirradiation. Small intestines were divided into four equal segments, S1–4, with S1 being the most proximal and S4 the most distal. N = 3 mice. Data are expressed as mean ± SEM. P-value generated by Ordinary one-way ANOVA.

E The number of b-Gal + cell clusters per cm. These cell clusters were considered distinct events from clonally traced crypts. N = 3 mice/group. Data are expressed as mean ± SEM. P-value generated by multiple student's t-tests.

F Whole mount imaging of proximal jejunum from control and irradiated *Chga*$^{CreER\text{-}2A\text{-}tdTomato}$::*R26*$^{LSL\text{-}LacZ}$ mice. Scale bar = 1 mm.



B

Dox Withdrawal Period	%LRCs (Mean +/- SEM)
D0	93.0 +/-1.8
D3	6.5 +/-1.6
D7	1.1 +/- 0.2
D14	0.3 +/- 0.1

Figure EV4. TRE-H2B-GFP label retaining populations decrease over time.

A Representative plots of the distribution of GFP-positive cells from *TRE-H2B-GFP* mice after 0, 3, 7, and 14 day dox withdrawal periods.

B The percentage of label retaining cells in each sample quantified from plots in (A). $N = 3$ mice per time point.

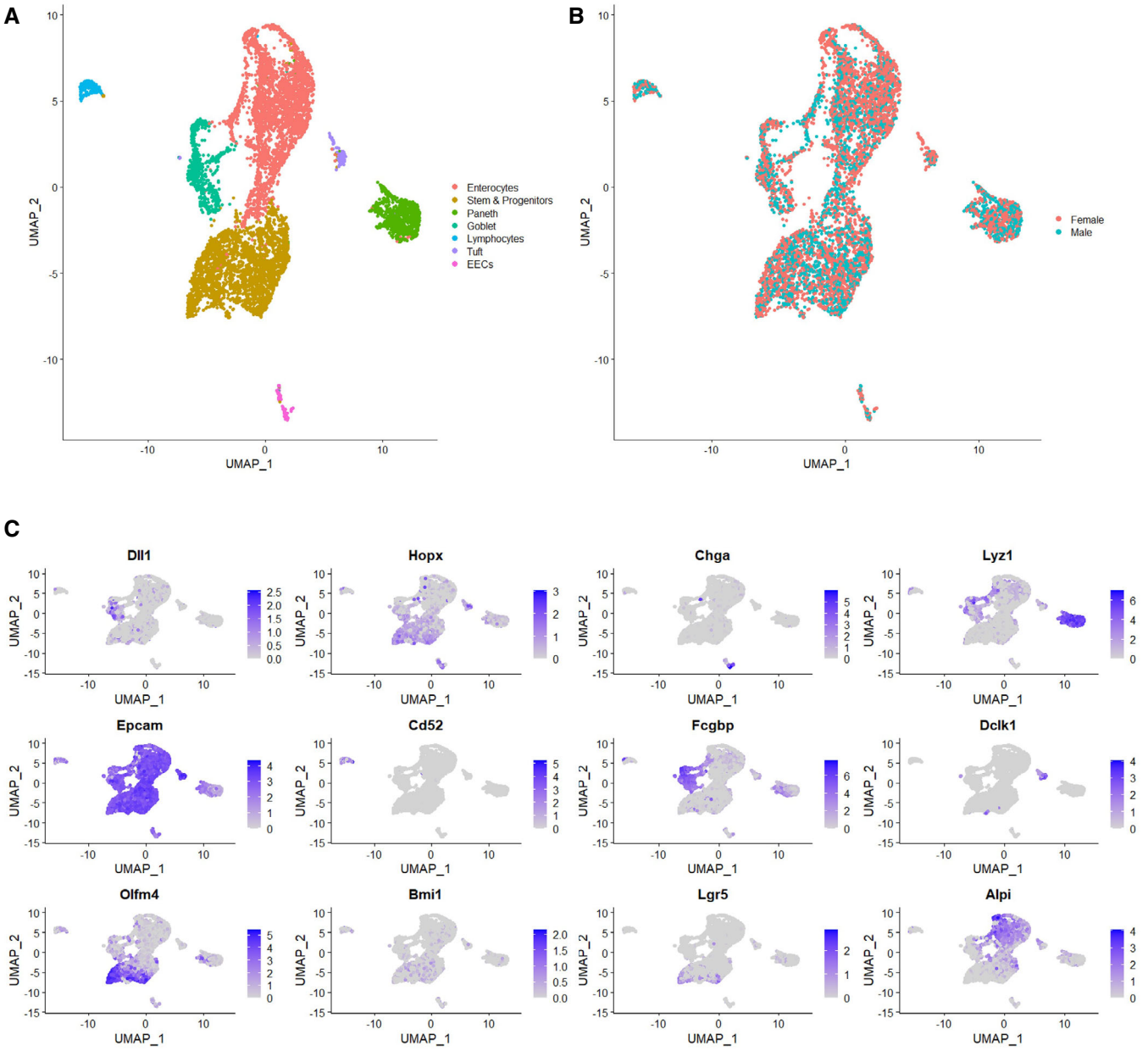


Figure EV5. Marker gene expression used to assign cell type identities to cell clusters.

A UMAP plot of all sequenced cells with cell clusters colored by cell type identity.

B Sequenced cells were a mix of $N = 2$ mice, one male and one female. UMAP of cells colored by sample origin.

C Marker genes used to identify cell type identities.

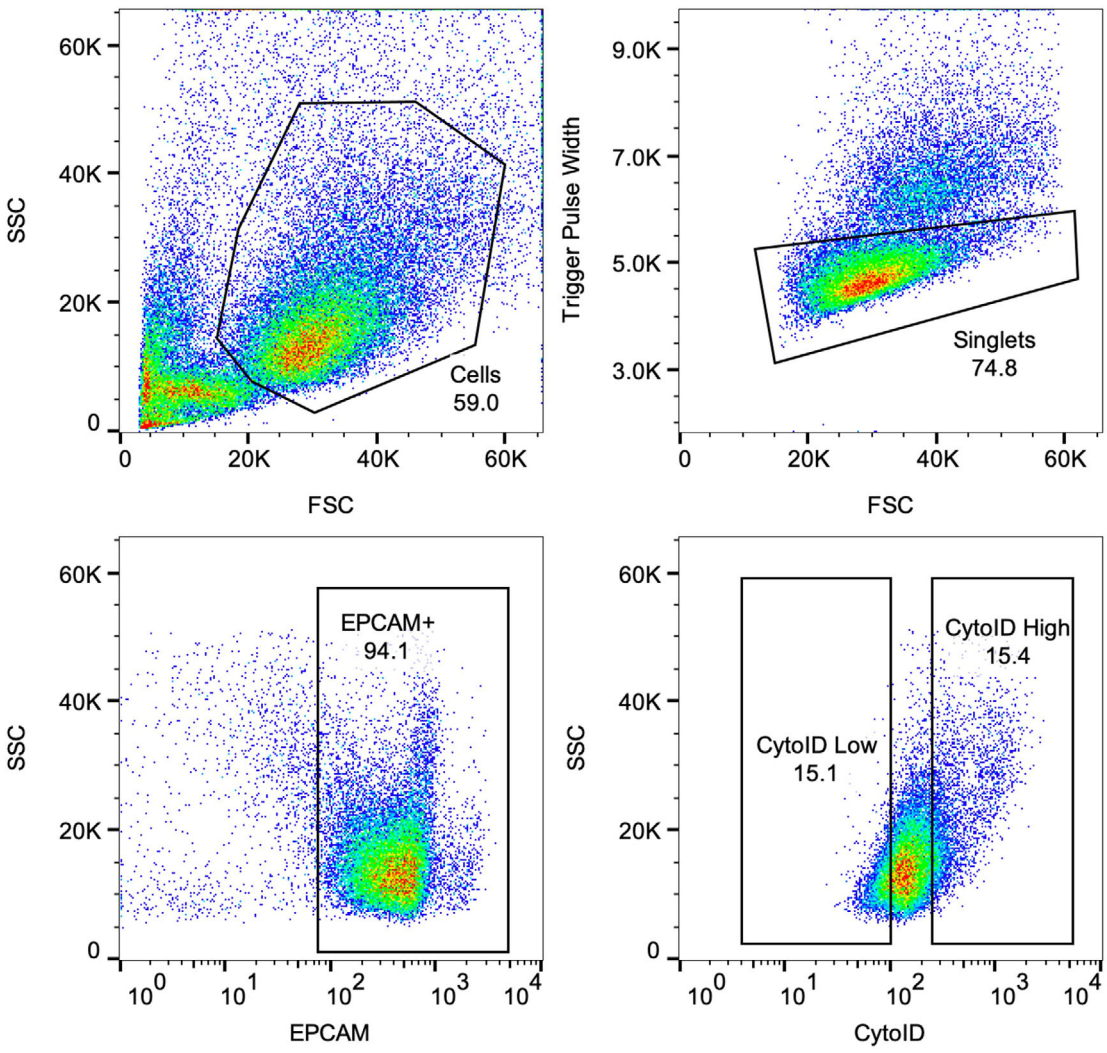


Figure EV6. Gating scheme for isolating CytoID low and high cell populations.

Representative plots of the gating scheme utilized to isolate CytoID low and high experiments for organoid-formation efficiency and single-cell sequencing experiments. Note that panel 4B is repeated in this figure to clearly demonstrate the gating strategy utilized for sorting CytoID low and high cells.