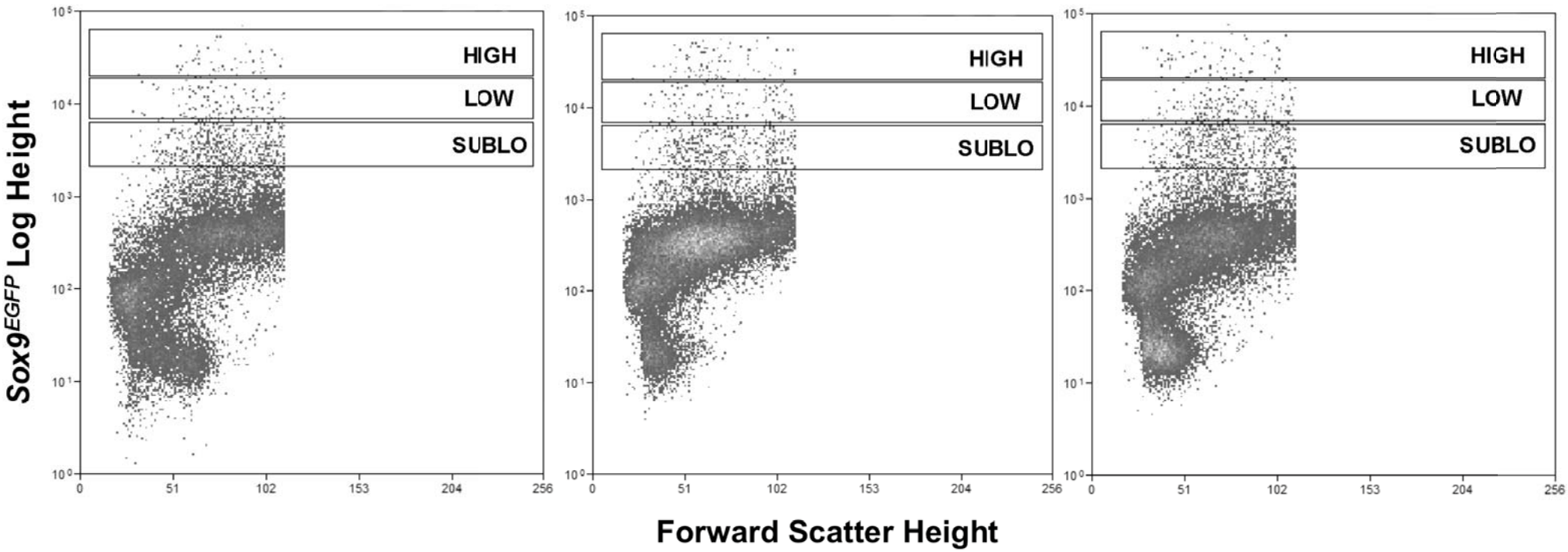
A



B

Population	Experiment 1	Experiment 2	Experiment 3	Average
Sox9^{EGFPhi}	0.25%	0.35%	0.32%	0.31% ± 0.05%
Sox9^{EGFPlo}	0.62%	0.59%	0.85%	0.69% ± 0.14%
Sox9^{EGFPsubLo}	2.96%	1.72%	2.58%	2.42% ± 0.64%

*** Percentages represent the numbers of cells within each gate**

FIGURE S1

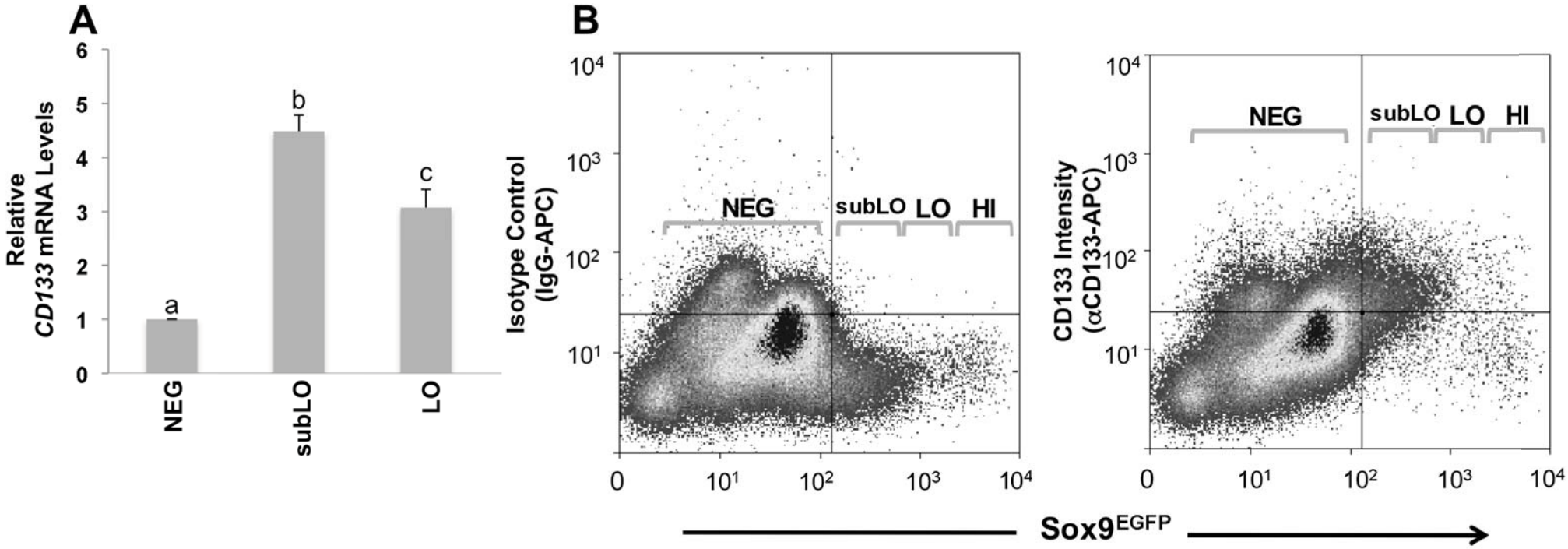

A
Relative
CD133 mRNA Levels
6
5
4
3
2
1
0
a
b
c
NEG
subLO
LO
B
Isotype Control
(IgG-APC)
CD133 Intensity
(αCD133-APC)
NEG
subLO
LO
HI
10⁴
10³
10²
10¹
0
Sox9EGFP

FIGURE S2

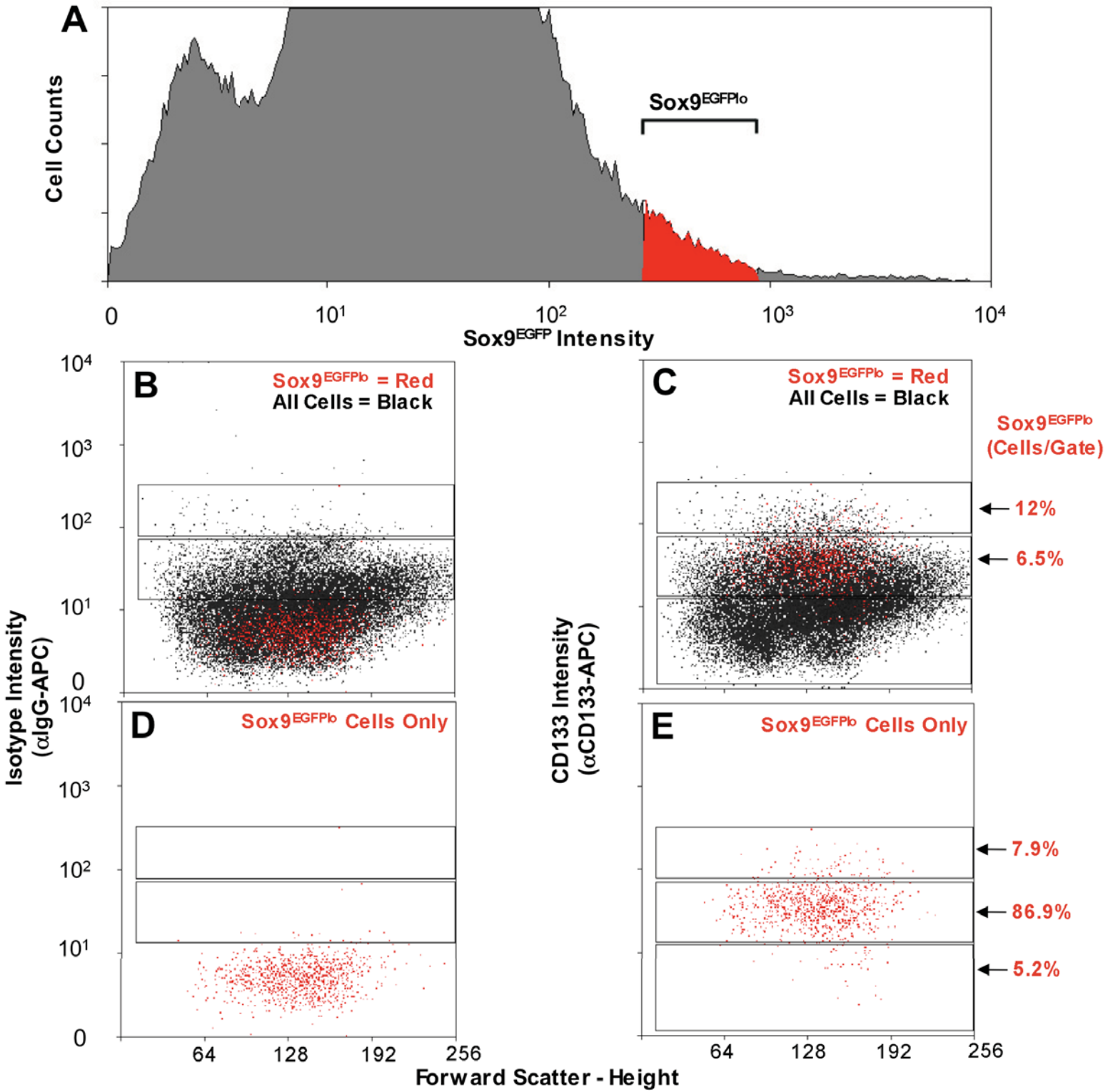

A
Cell Counts
Sox9EGFPlo
0
10¹
10²
10³
10⁴
Sox9EGFP Intensity
B
Sox9EGFPlo = Red
All Cells = Black
C
Sox9EGFPlo = Red
All Cells = Black
Sox9EGFPlo
(Cells/Gate)
12%
6.5%
Isotype Intensity
(αIgG-APC)
CD133 Intensity
(αCD133-APC)
D
Sox9EGFPlo Cells Only
E
Sox9EGFPlo Cells Only
7.9%
86.9%
5.2%
64
128
192
256
Forward Scatter - Height

FIGURE S3

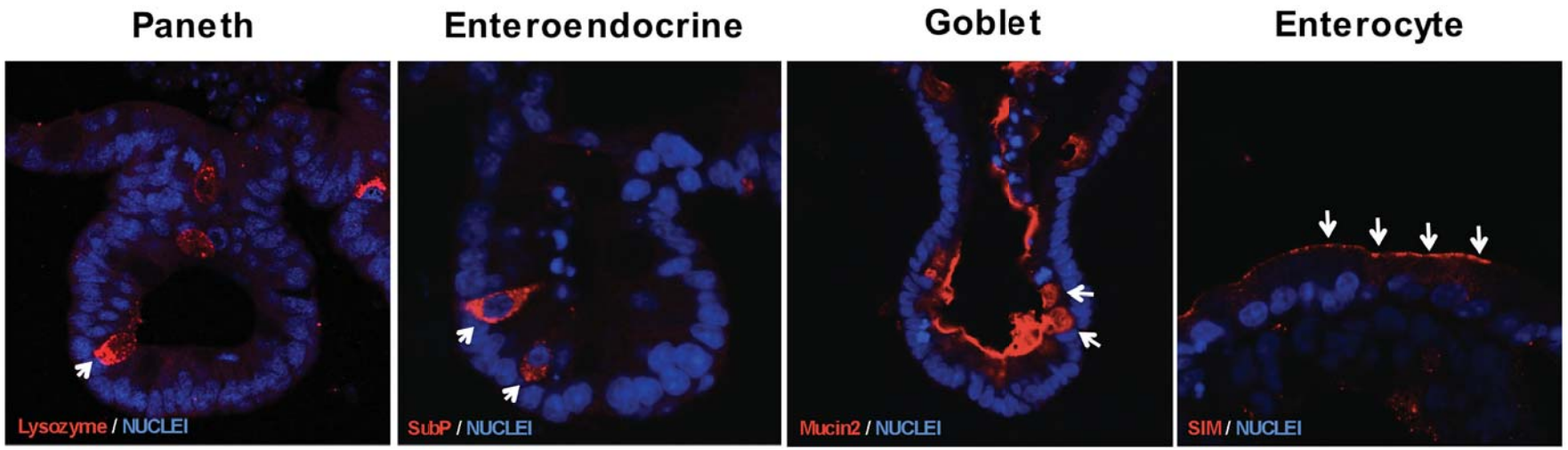

Paneth
Enteroendocrine
Goblet
Enterocyte
Lysozyme / NUCLEI
SubP / NUCLEI
Mucin2 / NUCLEI
SIM / NUCLEI

FIGURE S4

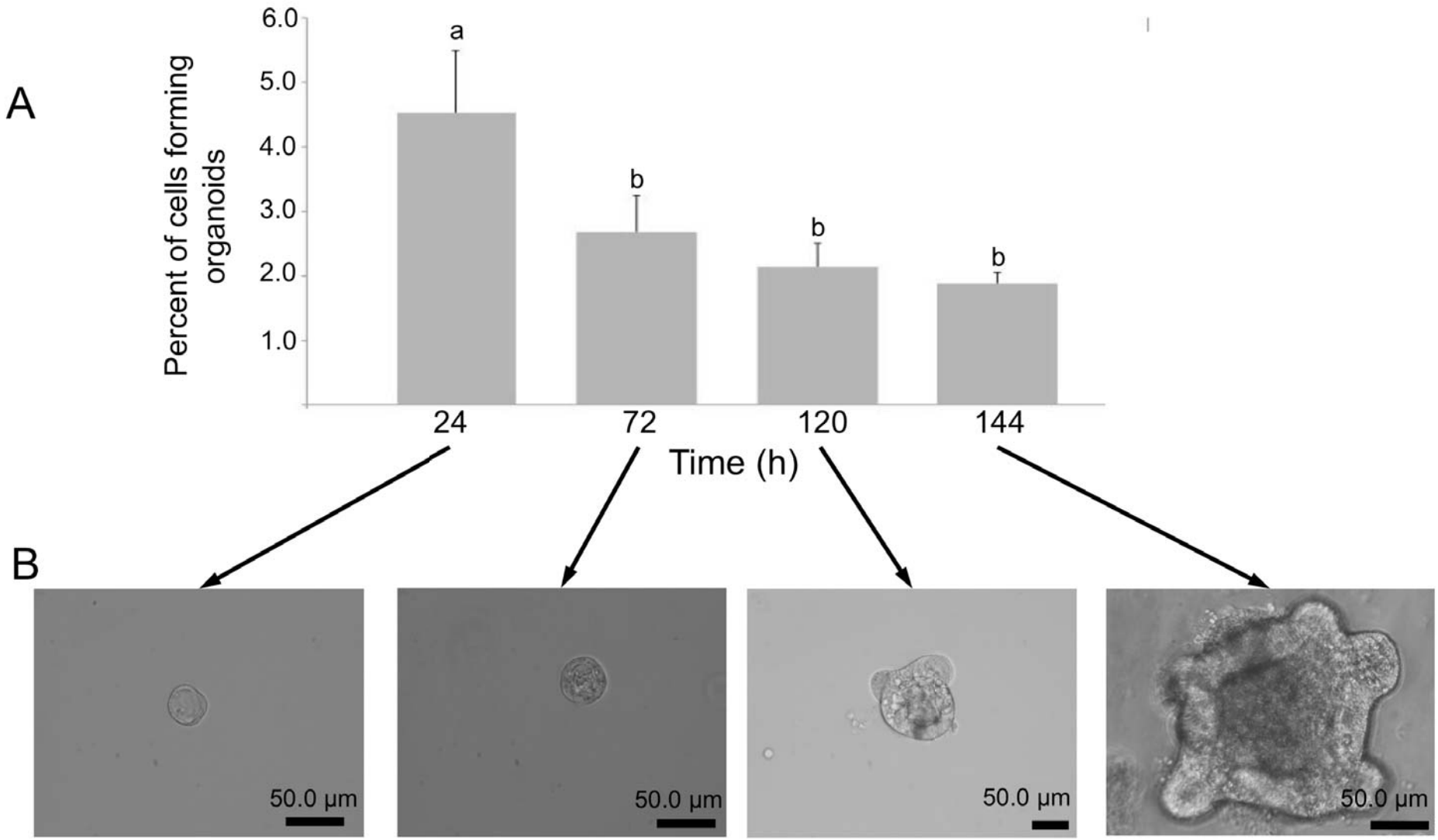

A
Percent of cells forming organoids
6.0
5.0
4.0
3.0
2.0
1.0
a
b
b
b
24
72
120
144
Time (h)
B
50.0 µm
50.0 µm
50.0 µm
50.0 µm

FIGURE S5

Figure S1 – *Sox9^{EGFP} populations are reproducibly separated by FACS* – **(A)** Three separate FACS experiments performed on three different animals were analyzed to demonstrate that gating protocols for isolating *Sox9^{EGFPsubLo}*, *Sox9^{EGFPlo}*, and *Sox9^{EGFPhi}* are reproducible based on histograms analyzing EGFP fluorescence. **(B)** Numerical analysis of these data shows that the percent of sorted cells falling in each gate is consistent between experiments, demonstrating reproducibility in the separation of *Sox9^{EGFP}* populations. Cut-off of the events on the right side of the histogram is due to gating parameters to exclude potential doublets.

Figure S2 – *Small intestine epithelial stem/progenitor cells express CD133 mRNA and protein* – (A) sqRT-PCR demonstrates *CD133* is expressed 4.4-fold and 3.1-fold higher in the 'subLO' and 'LO' cells respectively when compared to *Sox9^{EGFP}* negative cells. All data points represent means +/- S.E.M. from three independent experiments; statistical analysis was by ANOVA and post-hoc two sample t-tests were then performed. A p-value < 0.05 is considered statistically significant. The different letters above each bar represent data points that are statistically different from each other. **(B)** Flowcytometric analysis indicates that most *Sox9^{EGFP}*-positive cells (94.8%) express *CD133* protein. (*left panel)* APC-conjugated isotype antibody control. αCD133-APC conjugated antibody. (*right panel)* Grey brackets in the histograms represent the FACS gating parameters.

Figure S3 – *Using CD133 as an IESC FACS-enrichment marker is less efficient than CD24* – (A) *Sox9^{EGFP}* expression of dissociated small intestine epithelial cells on a univariate EGFP histogram. Black brackets indicate gate parameters. Color gating *(red)* allows visualization of *Sox9^{EGFPlo}* population in histograms A-F. **(B)** *Sox9^{EGFP}*/CD133 bivariate histogram used to define

the CD133 gate parameters. **(C&E)** IgG control antibody indicates there is no significant non-specific staining. **(D&F)** α*CD133-APC* antibody labels 94.8%% of *Sox9*^{EGFPlo} cells. **(D)** Percentages represent the number of *Sox9*^{EGFPlo}-expressing cells in each gate. **(E&F)** Represent just *Sox9*^{EGFPlo} cells color back-gated onto the *CD133*/FSC histogram to highlight their distribution on the histogram. **(F)** Percentages represent the relative number of all *Sox9*^{EGFPlo} cells in each gate. **(C&D)** Note that although 94.8% of *Sox9*^{EGFPlo} cells express CD133, the shift of the *Sox9*^{EGFPlo} population on the CD133 axis is too small to sufficiently separate the *Sox9*^{EGFPlo} cells away from non-*Sox9*^{EGFPlo} cells. The upper-gates in each histogram represent the region of CD133-expressing cells that fall above the isotype control negative population. The lower-gates in each histogram represents the region of CD133-expressing *Sox9*^{EGFPlo} cells that fall above the isotype control negative population.

Figure S4 – *CD24-expressing IESCs are multipotent* – 14 day organoids derived from CD24-FACSed IESCs were assessed for the presence of the four differentiated cells lineages of the small intestine epithelium by immunostaining. **(A)** Paneth cells are marked by *Lysozyme* expression (red). **(B)** Enteroendocrine cells are marked by *Substance P* (red). **(C)** Goblet cells are marked by *Mucin2* staining (red). **(D)** Absorptive enterocytes are marked by the brush border enzyme *Sucrase Isomaltase* (SIM – red). All images are 1260 X original magnification.

Figure S5 – *Organoid survival is quantified by identification of unique morphology* - (A) Organoids were quantified in culture at 24-hour intervals to determine survival rates. **(A & B)** Following an initial drop, organoid survival at 120-144 hours leveled off at the same time crypt-like budding was observed. **(B)** Organoids were scored as surviving if observed to have a

characteristic morphology, marked by cellular organization in a round structure surrounding an apparently empty (24 hours) or apoptotic (120 hours) pseudo-lumen. All data points represent means +/- S.E.M. from three independent experiments; statistical analysis was by ANOVA and post-hoc two sample t-tests were then performed. A p-value < 0.05 is considered statistically significant. The different letters above each bar represent data points that are statistically different from each other.