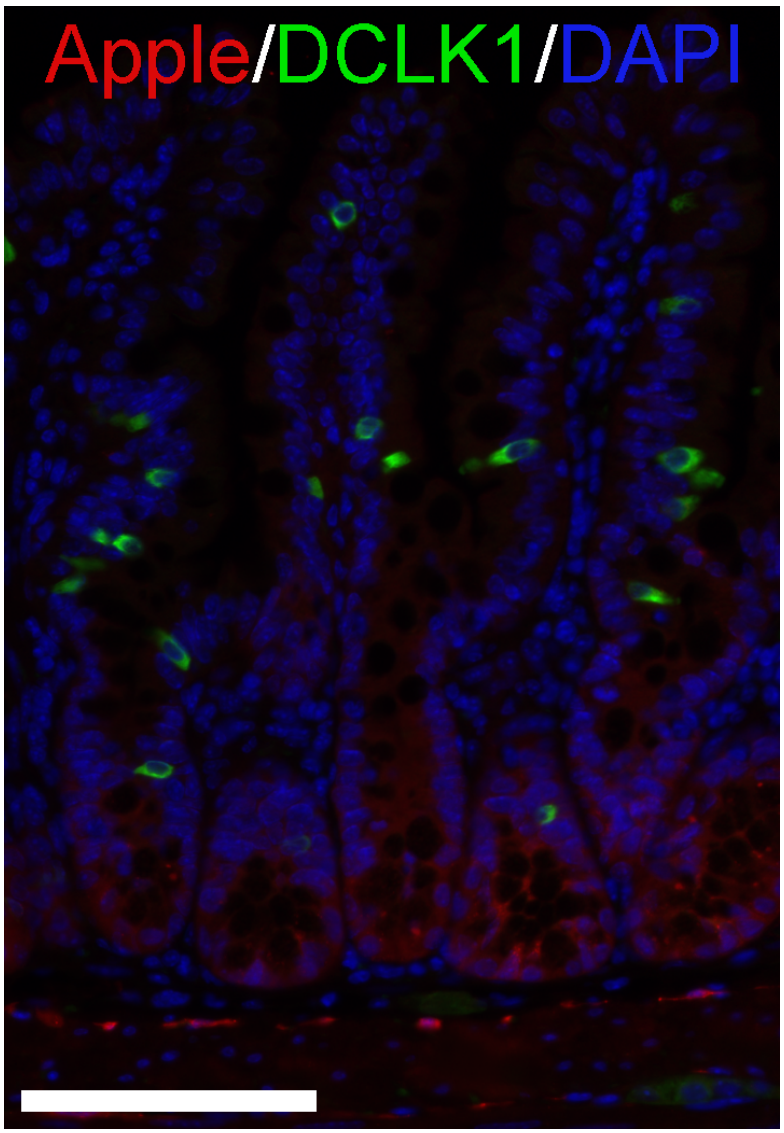
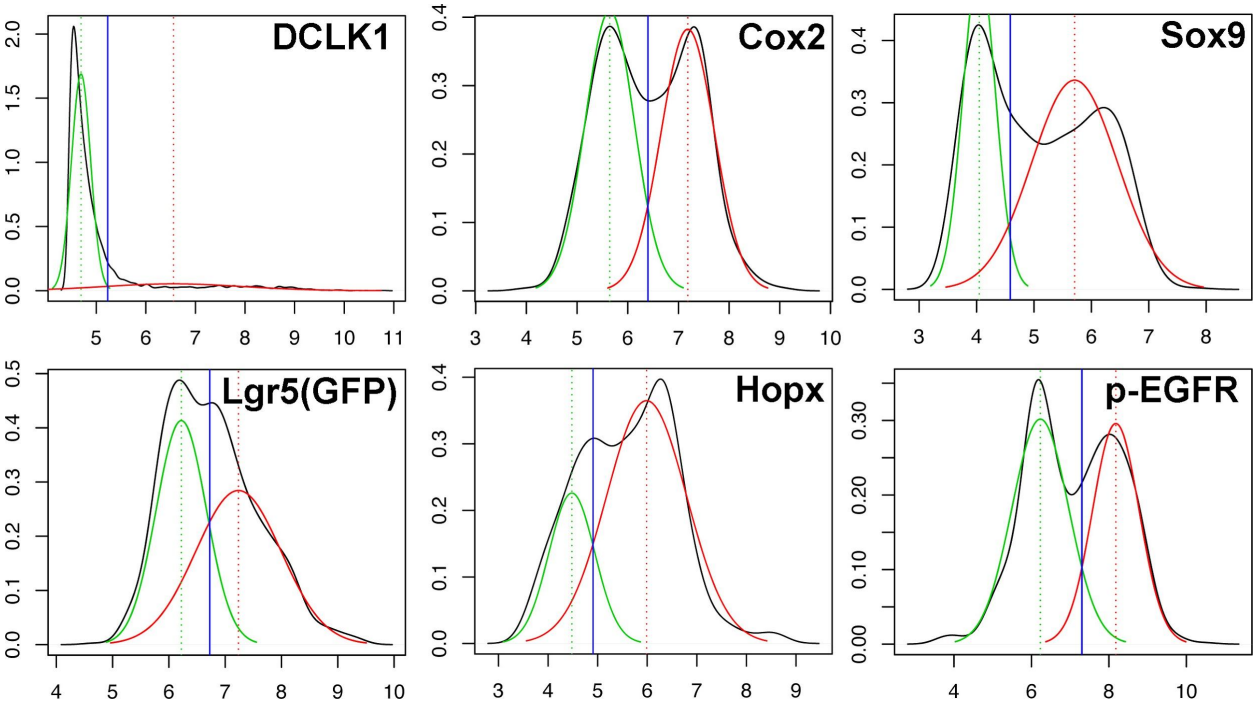
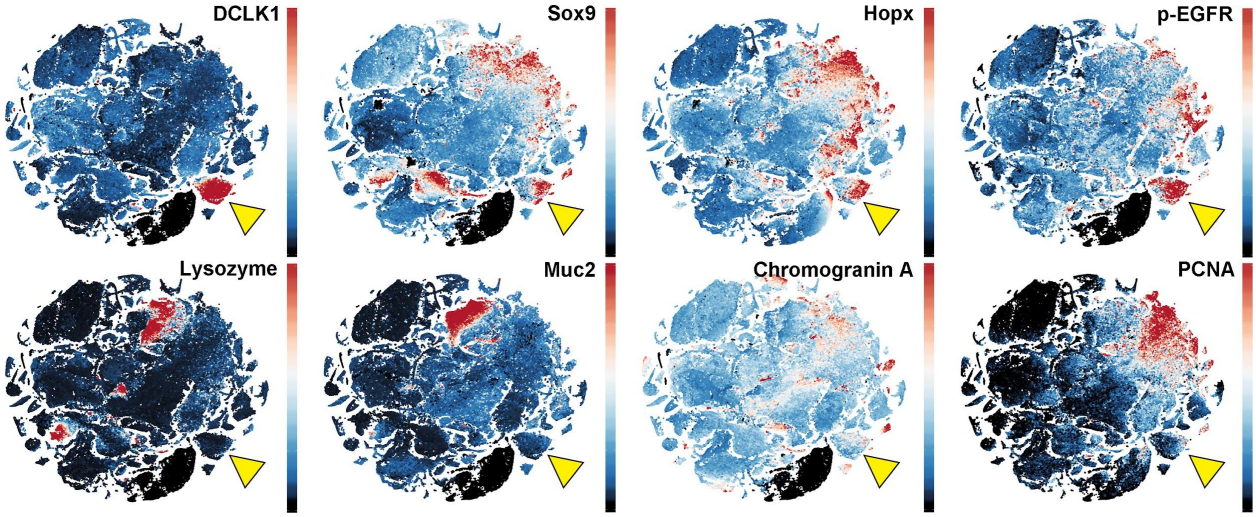


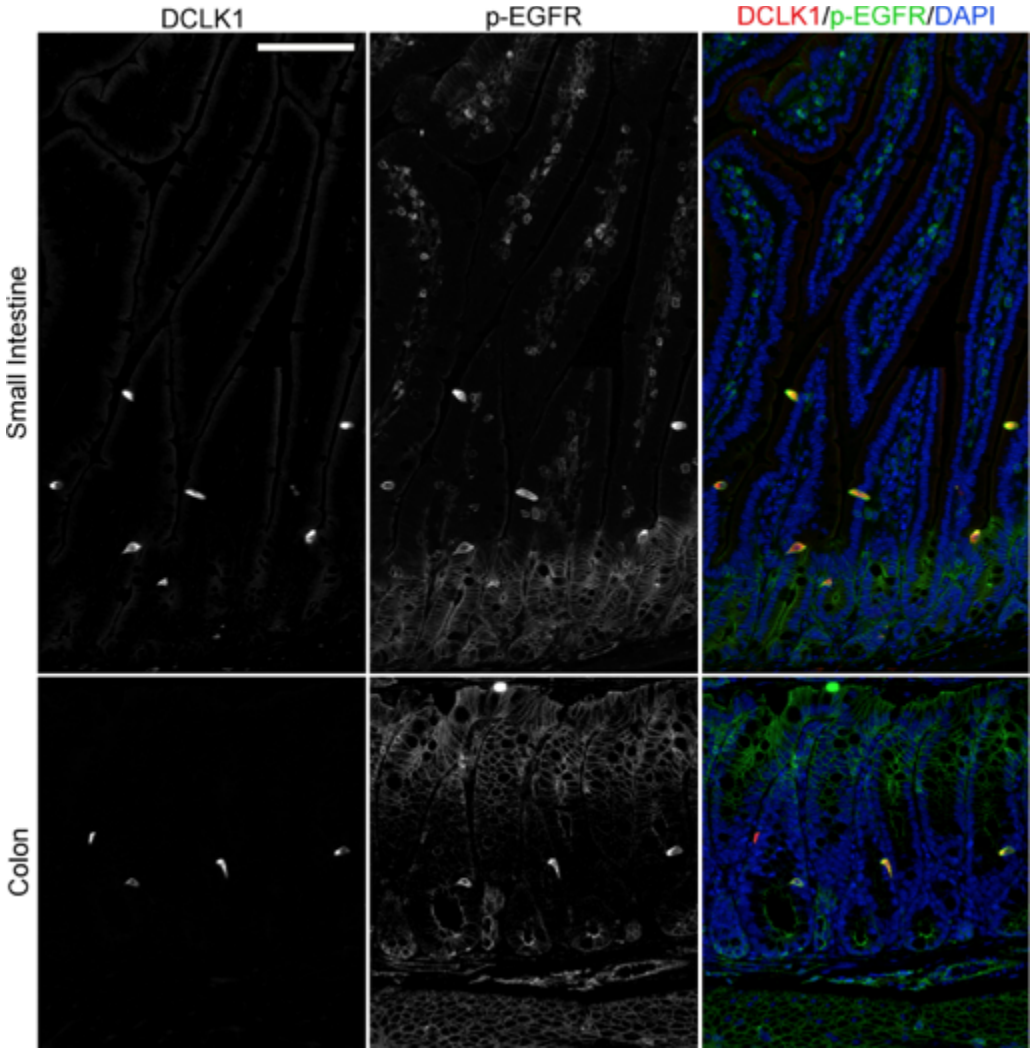
Supplemental Figure 1: Lrig1-Apple expression in small intestine. Lrig1-Apple is observed at the crypt base and in insterstial cells of Cajal, but is not co-expressed in DCLK1-positive tuft cells. Scale bar: 100 μm



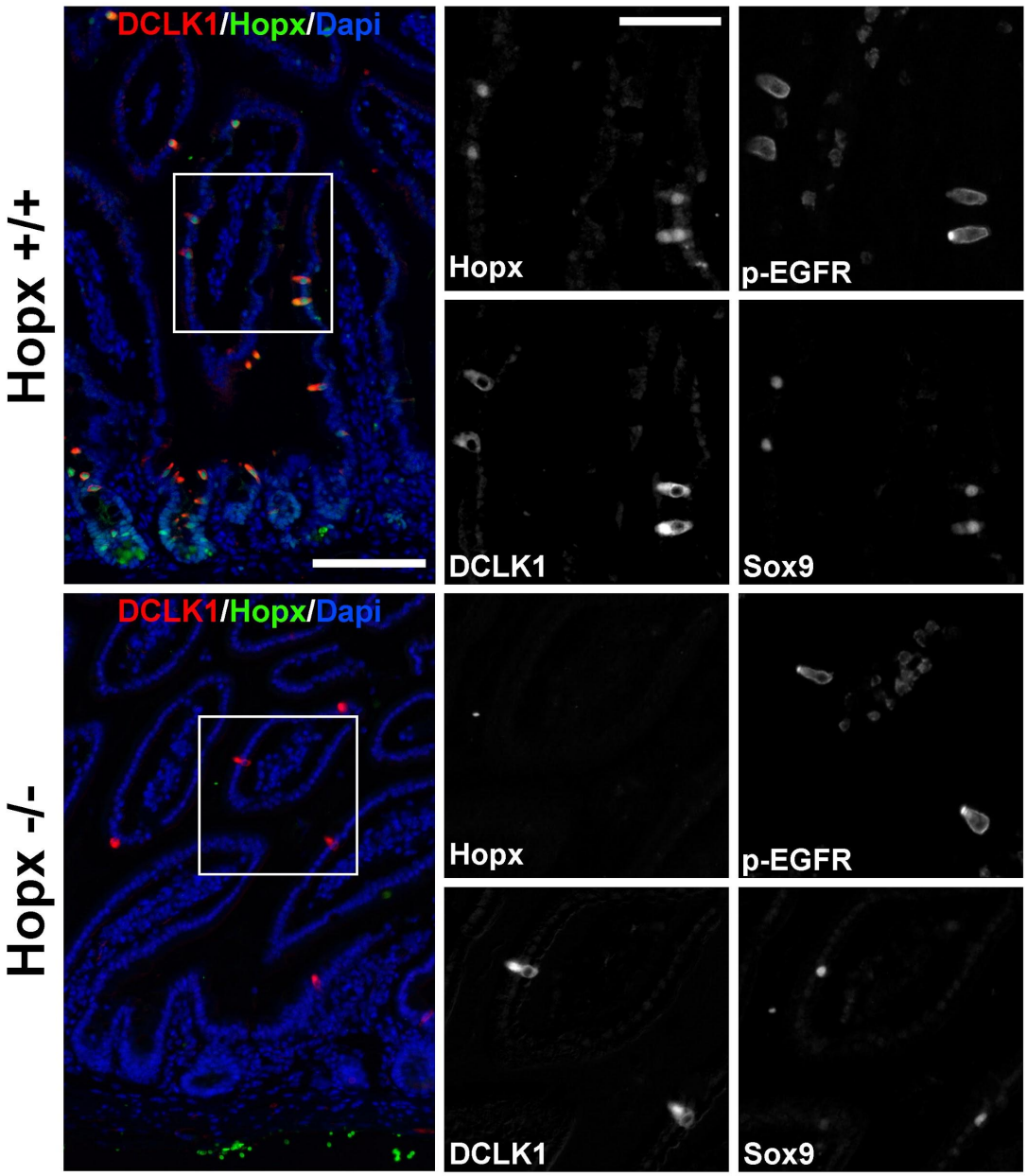
Supplemental Figure 2: Gaussian model based deconvolution. Representative results of automated model based deconvolution to determine positive thresholds. The top 3% DCLK1-expressing cells were isolated from the entire epithelial cell population to obtain a DCLK1-positive threshold (blue line). Subsequent deconvolution steps for the other tuft cell markers, Cox2, So9, Lgr5(GFP), Hopx, and pEGFR were conducted only on the DCLK1-positive cells.



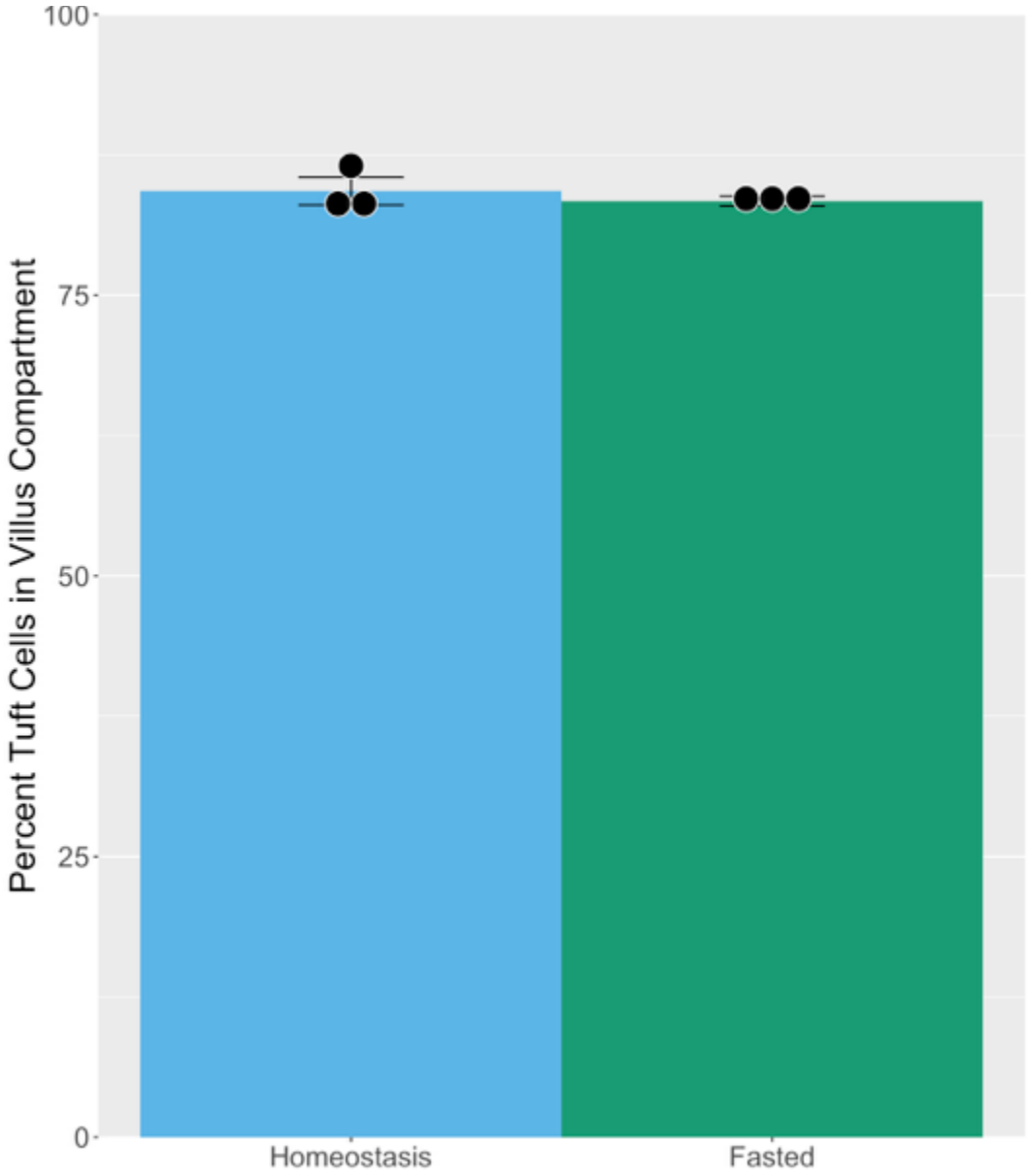
Supplemental Figure 3: t-SNE maps derived from stem cell-marker focused MxIF of mouse small intestine. t-SNE maps derived from MxIF of epithelial cells (N= 199,419) in two murine ilea demonstrated separation of DCLK1-positive tuft cells from the rest of the intestinal epithelial cells. Cells in the tuft cell “island” (yellow arrowhead) also demonstrated expression of the tuft cell marker Sox9 as well as novel markers Hopx and p-EGFR. Other differentiated cell markers for Paneth (Lysozyme), goblet (Muc2), and enteroendocrine (Chromogranin A) cells were not observed in tuft cells. The tuft cells were not proliferative as measured by PCNA staining.



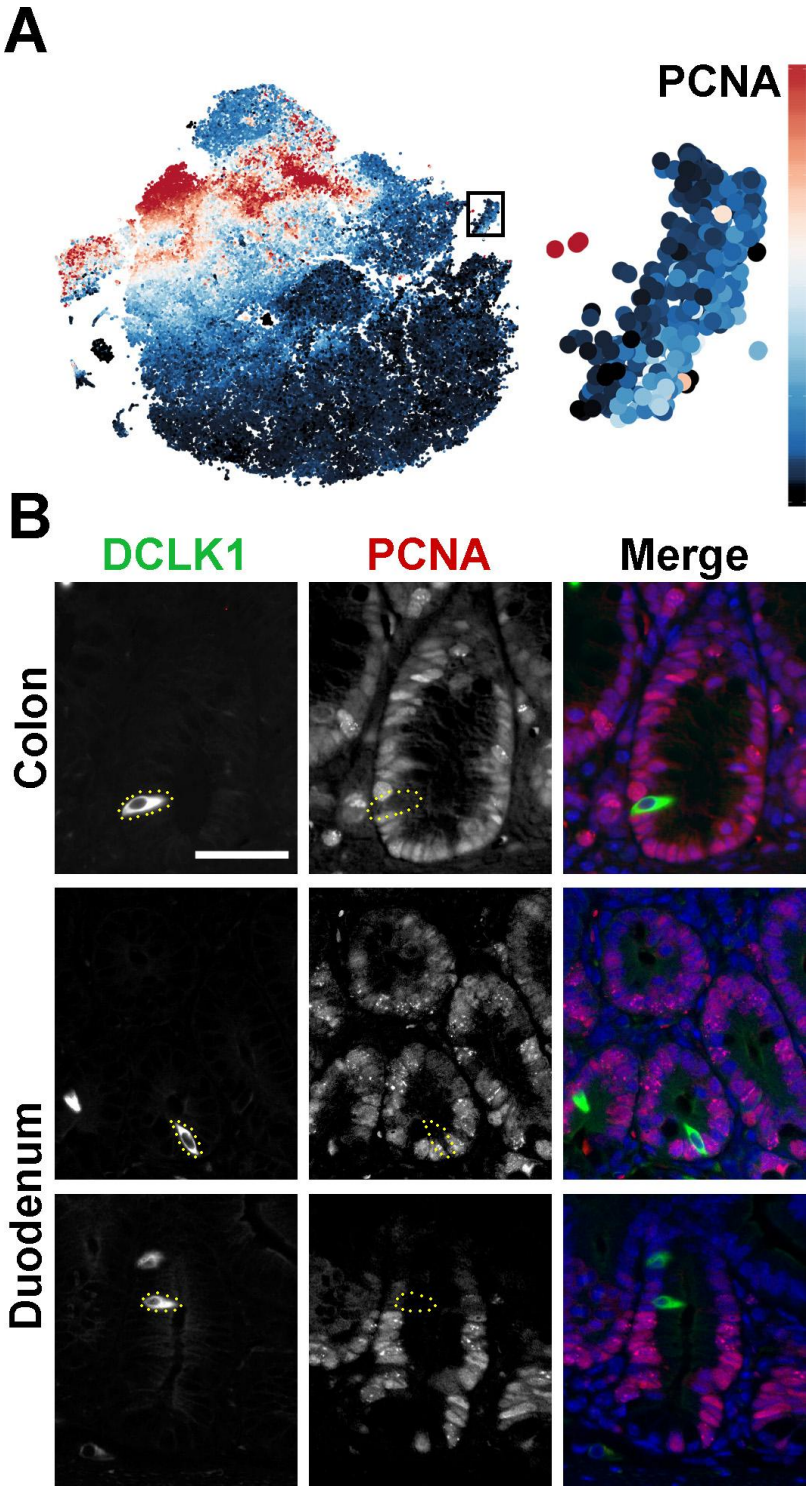
Supplemental Figure 4: p-EGFR staining in mouse small intestine and colon. Representative images of mouse small intestine and colon. DCLK1 staining colocalizes with p-EGFR in tuft cells. In the small intestine, besides tuft cells, p-EGFR is expressed at the crypt base, villus tip, and in stromal cells located in the lamina propria. In the colon, p-EGFR is expressed at the bottom of the base and at the luminal surface of the crypt. Scale bar: 100 μm



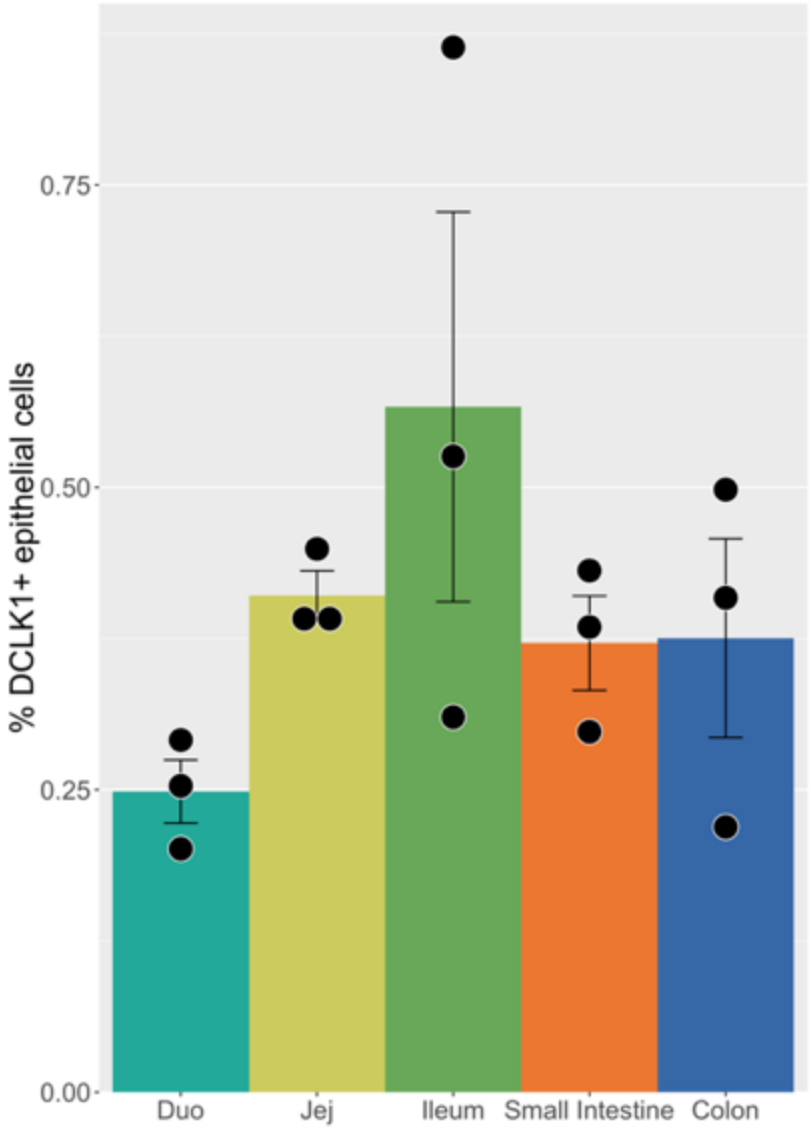
Supplemental Figure 5: Validation of Hopx antibody in mouse tissue. Hopx antibody specificity was confirmed using Hopx-null mouse intestinal staining. In wild type tissue (top), Hopx was clearly visualized in tuft cells as well as at the bottom of the crypt (scale bar: 100 μm). Hopx-positive cells in the villus co-localized with DCLK1, p-EGFR, and Sox9 (scale bar: 50 μm). Hopx null tissue (bottom) demonstrated no antibody staining in tuft cells or at the bottom of the crypt. Other markers of tuft cells remained intact with Hopx deletion.



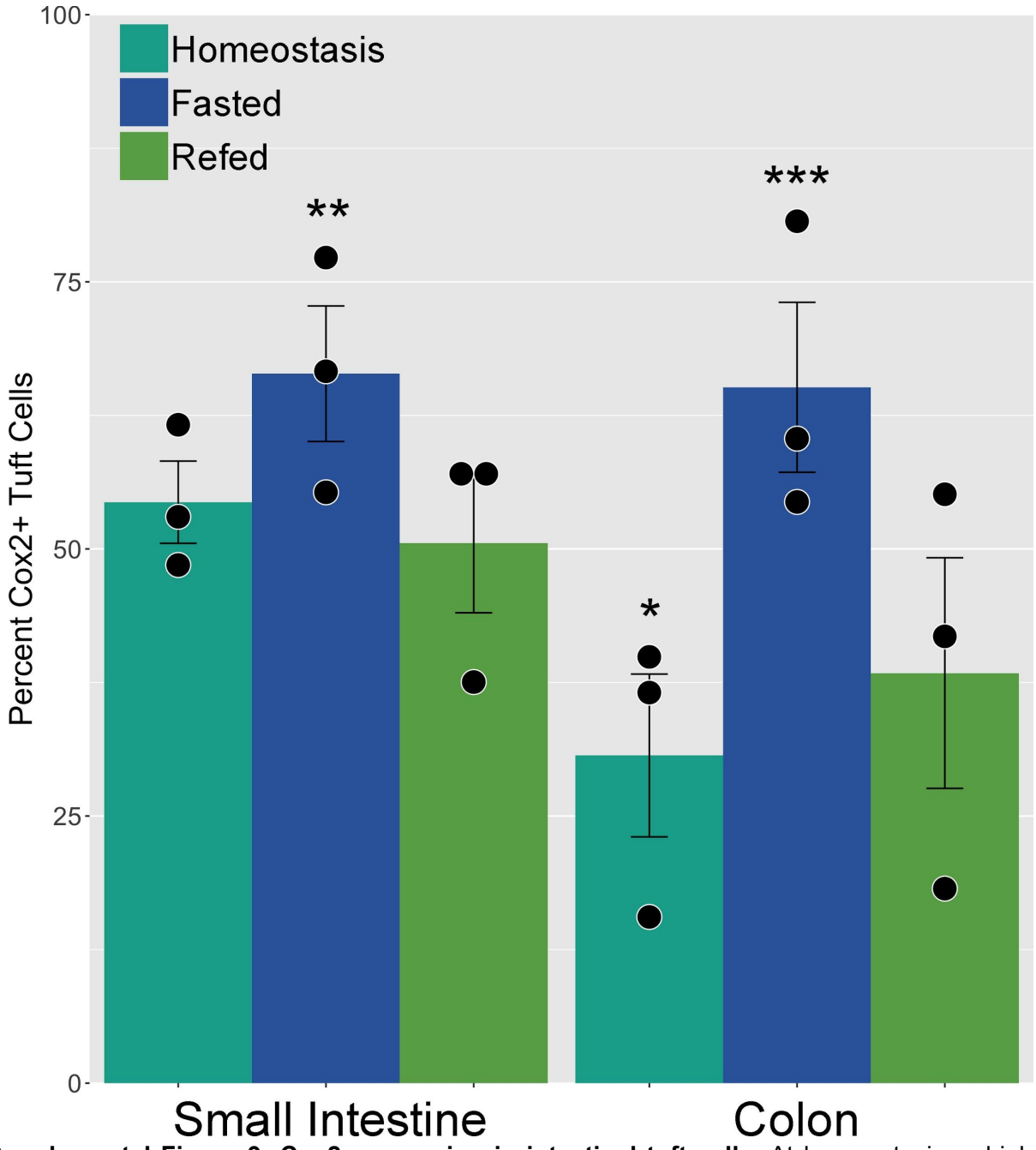
Supplemental Figure 6: Distribution of tuft cells in the villus compartment. At homeostasis, >80% of tuft cells were located in the villus compartment of the crypt. After fasting, there was no change in the distribution of tuft cell localization in the villus or crypt compartment. N=3 for each condition.



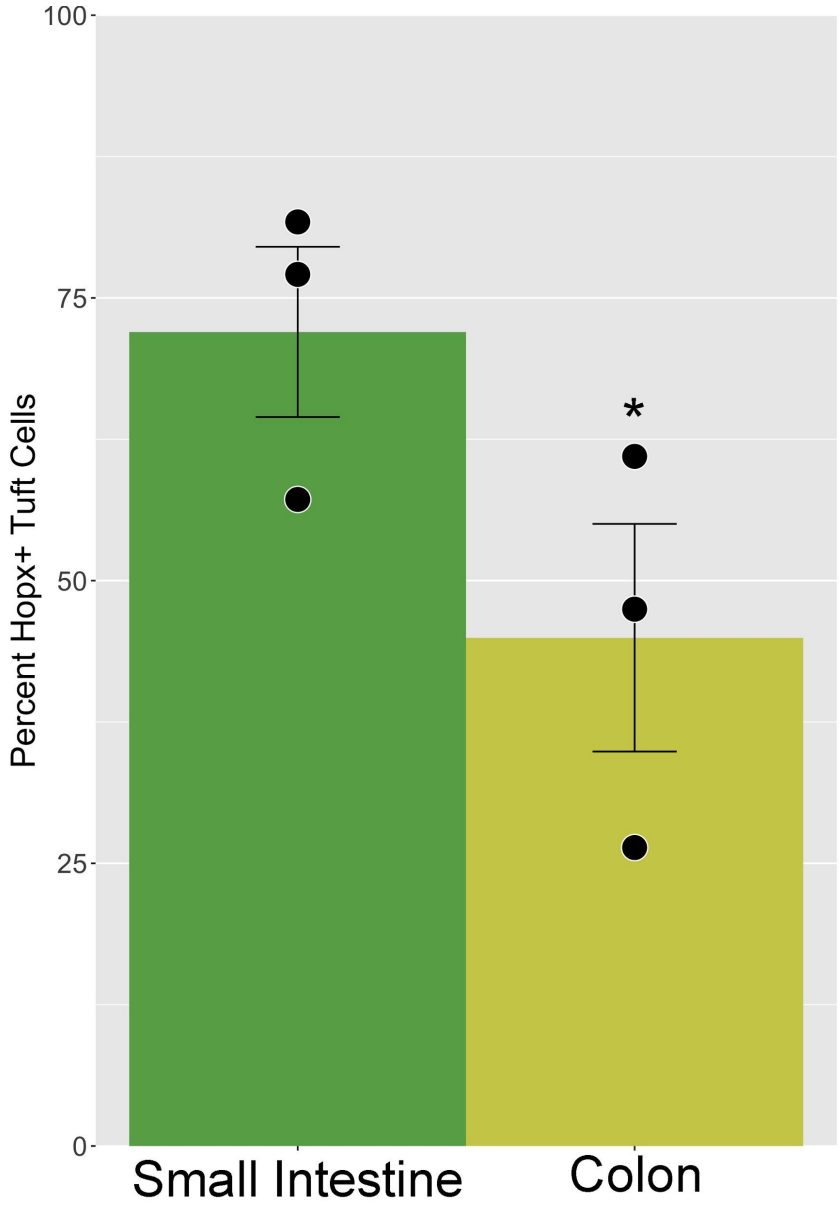
Supplemental Figure 7: Intestinal tuft cells are not proliferative. (**A**) PCNA-positive proliferative cells were largely constrained to the stem/progenitor cell zone as depicted in t-SNE maps of epithelial cells in the ileum (see Figure 1). (**B**) Tuft cells were never observed to express PCNA even when localized near the bottom of the crypt where proliferative stem cells reside or in the transit-amplifying region (scale bar: 50 μm).



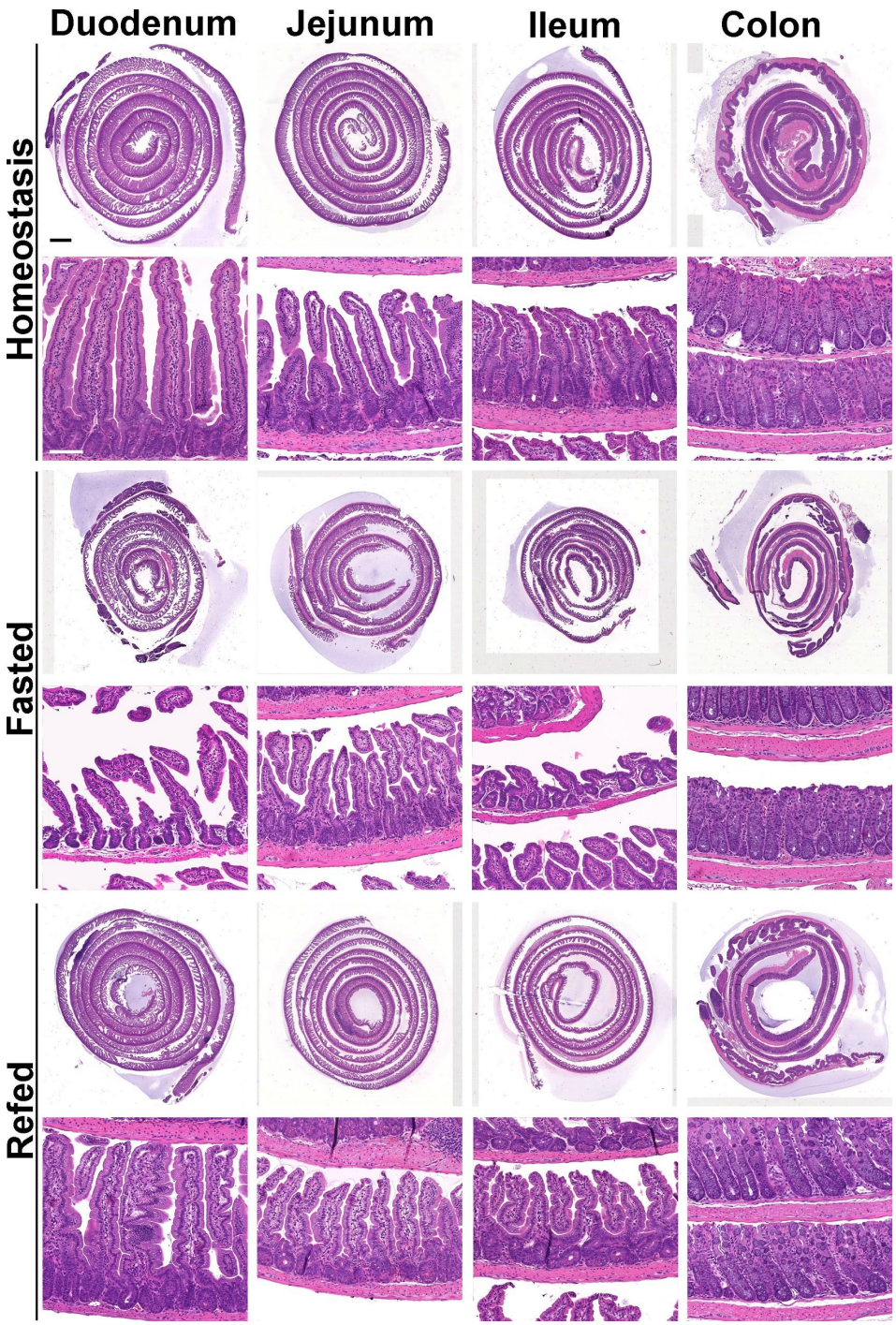
Supplemental Figure 8: Quantification of tuft cell proportion of epithelial cells in the mouse intestine. The proportion of tuft cells in the mouse small intestine increased from the proximal to distal regions. Overall, the proportion of tuft cells in the small intestine and colon were similar at approximately 0.4% of epithelial cells, consistent with prior reports. N=3 for each condition.



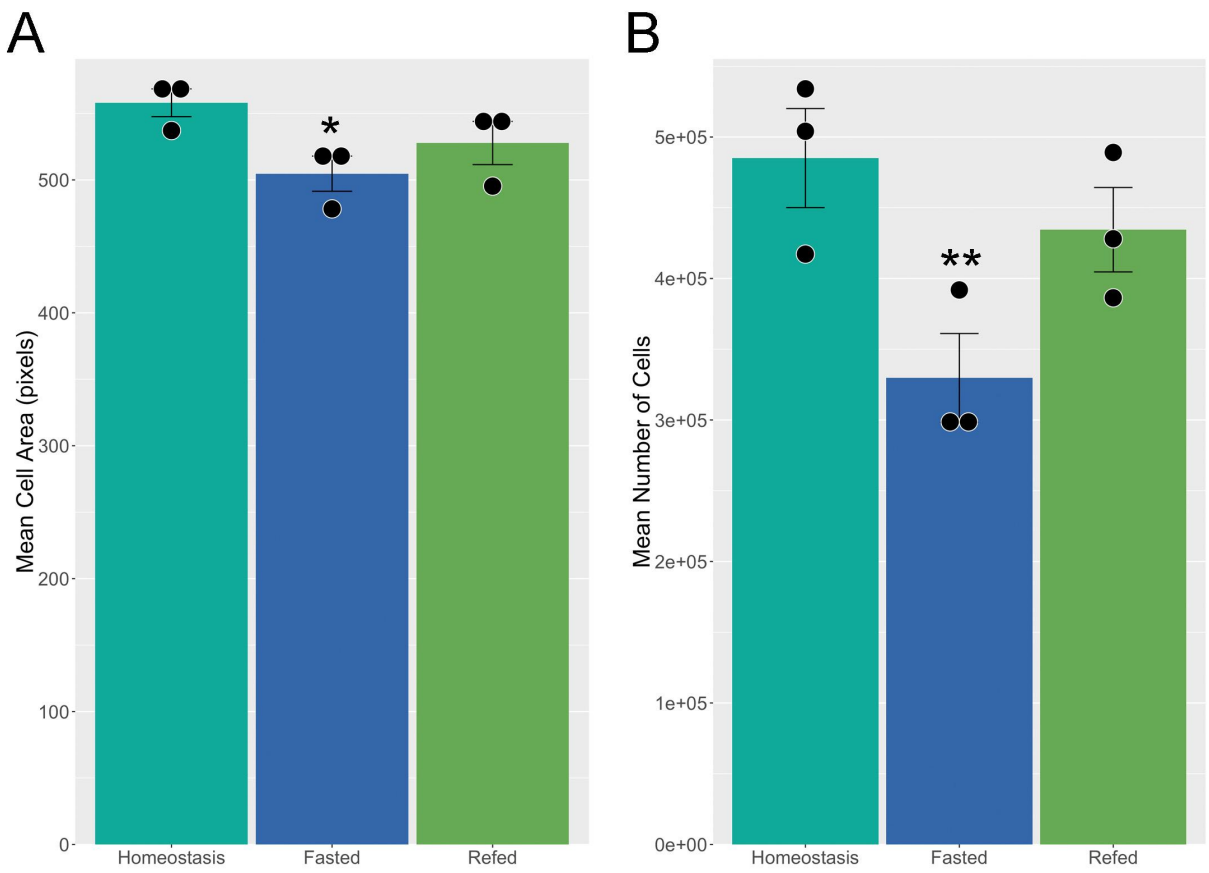
Supplemental Figure 9: Cox2 expression in intestinal tuft cells. At homeostasis, a higher proportion of tuft cells were Cox2-positive in the small intestine than the colon (*P=0.0115). After fasting, the Cox2-positive population increased compared to homeostasis in the small intestine (**P=.0322) and the colon (***P=0.0023). After being refed, Cox2 positivity in tuft cells returned to baseline conditions. N=3 for each condition.



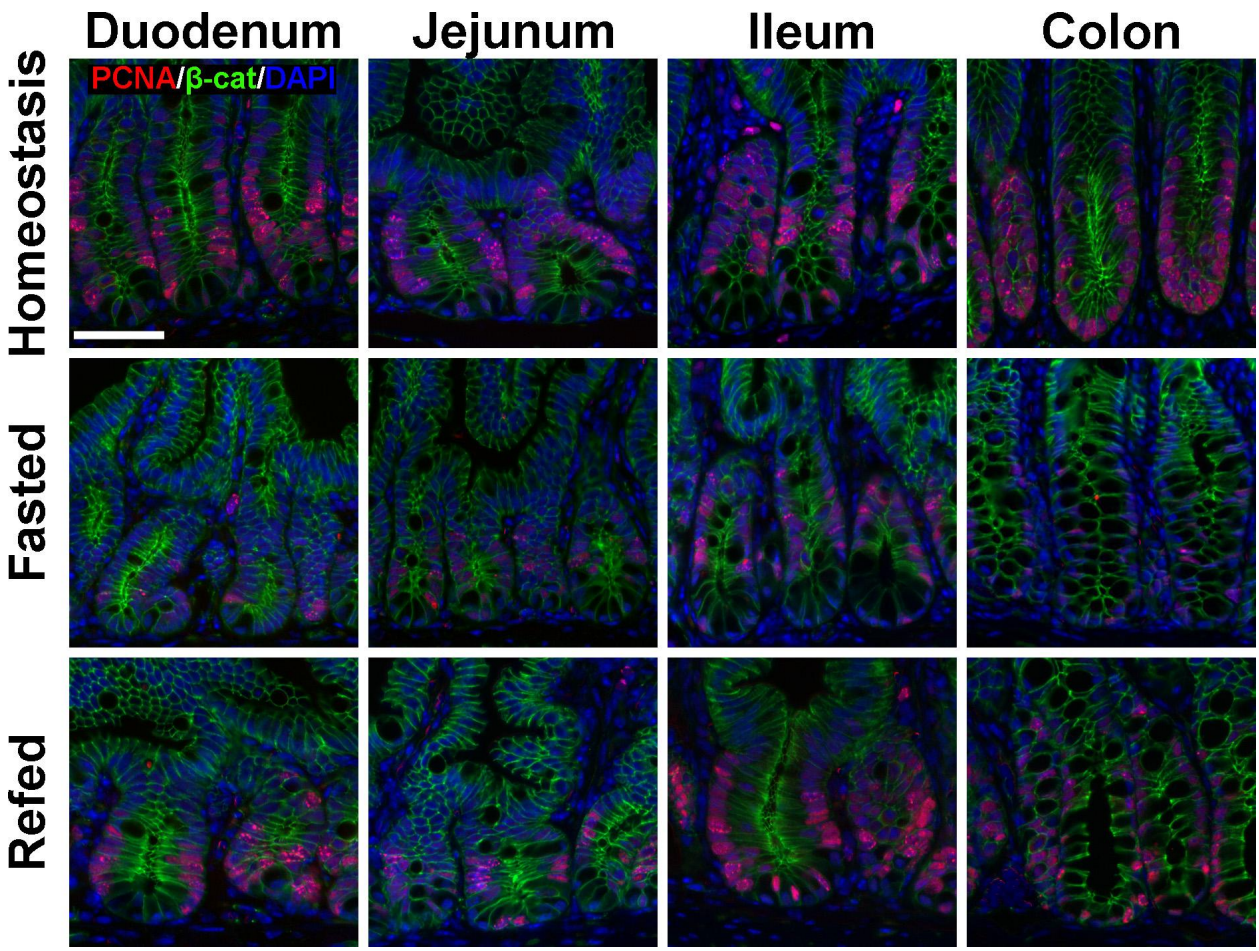
Supplemental Figure 10: Hopx expression is increased in intestinal tuft cells. At homeostasis, a higher proportion of tuft cells were Hopx-positive in the small intestine than the colon (*P=0.0217). N=3 for each condition.



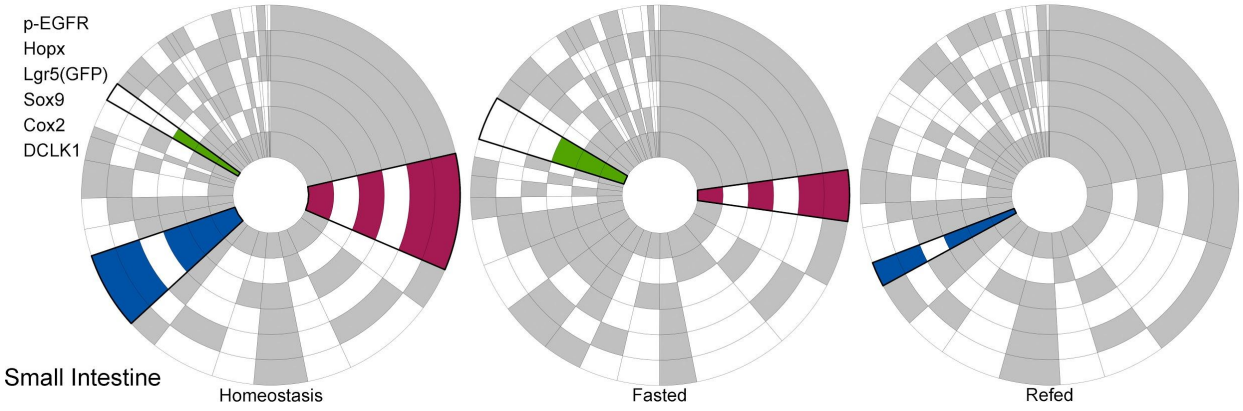
Supplemental Figure 11: Histological evaluation of mouse intestines at homeostasis, fasted, and refed conditions. Hematoxylin and eosin staining demonstrated overall intestinal atrophy in mice fasted for 48 hours compared to homeostasis that was partially recovered following 24 hours of refeeding (black scale bar:1 mm). Severe villus blunting was also observed in the small intestine following fasting that was similarly recovered after refeeding (white scale bar:100 μm).



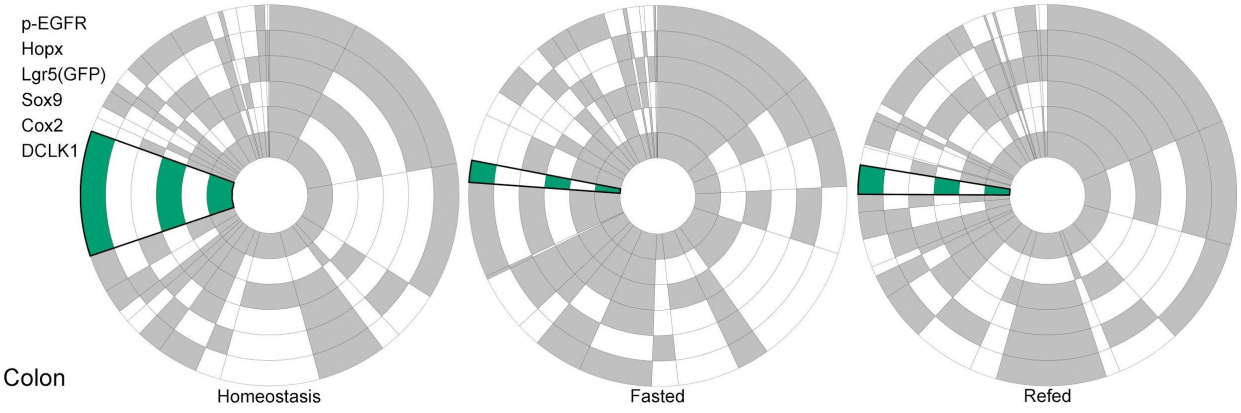
Supplemental Figure 12: Quantification of changes in epithelial cell size and number in mouse intestine. Mean cell area of all epithelial cells over the entire length of the mouse intestine demonstrated reduced cell size in the fasted condition compared to homeostasis (*P=0.0320), that recovered in the refed condition. Similarly the total number of epithelial cells was significantly reduced in fasted mice (**P=0.0140) but was indistinguishable after being refed compared to mice at homeostasis. N=3 for each condition.



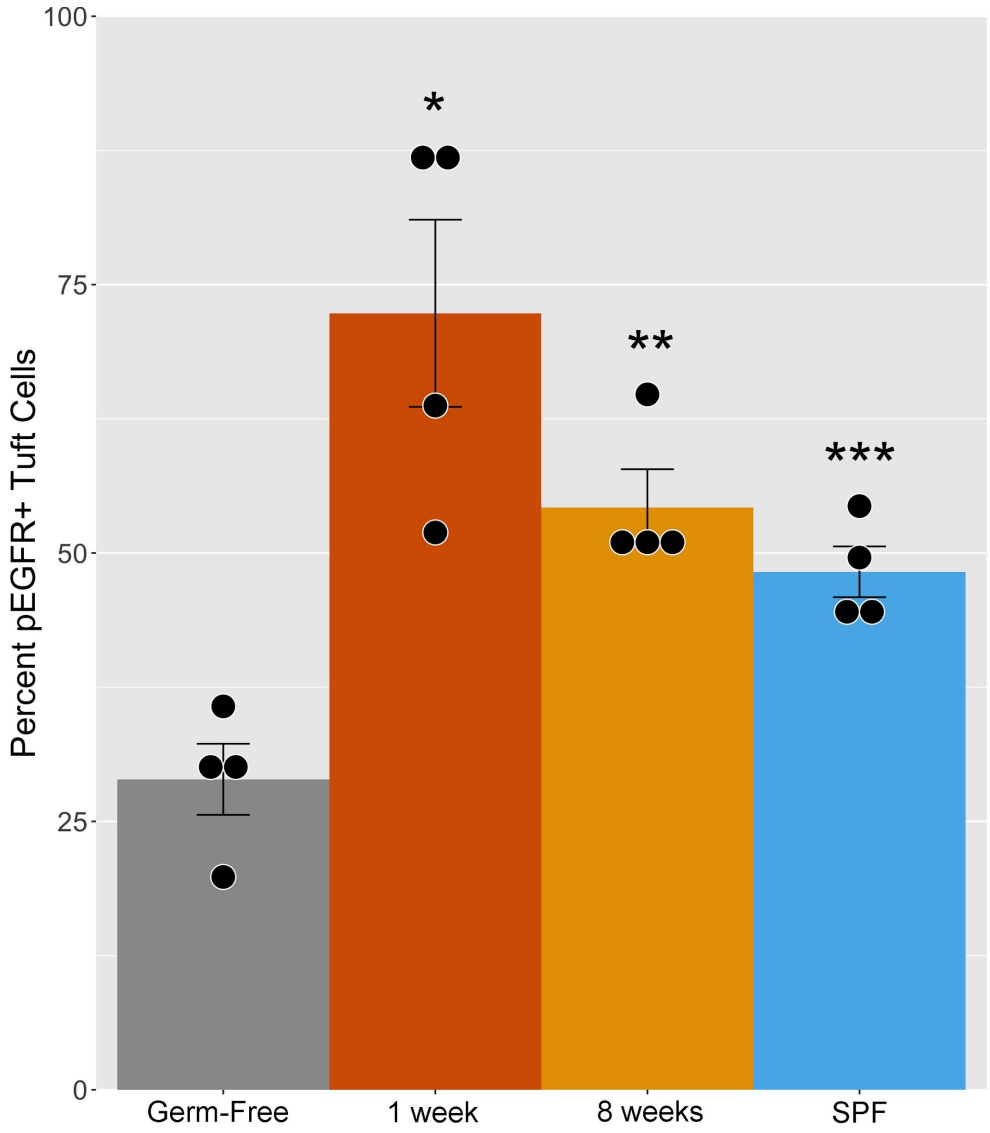
Supplemental Figure 13: Proliferation in crypt base cells at homeostasis and after fasting and refeeding. Robust proliferation was observed in at the crypt base and transit-amplifying cell zones at homeostasis in the small intestine and colon (scale bar: 50μm). After 48 hours of fasting PCNA is greatly decreased across the intestinal crypts and returns to essentially baseline levels 24 hours following refeeding.



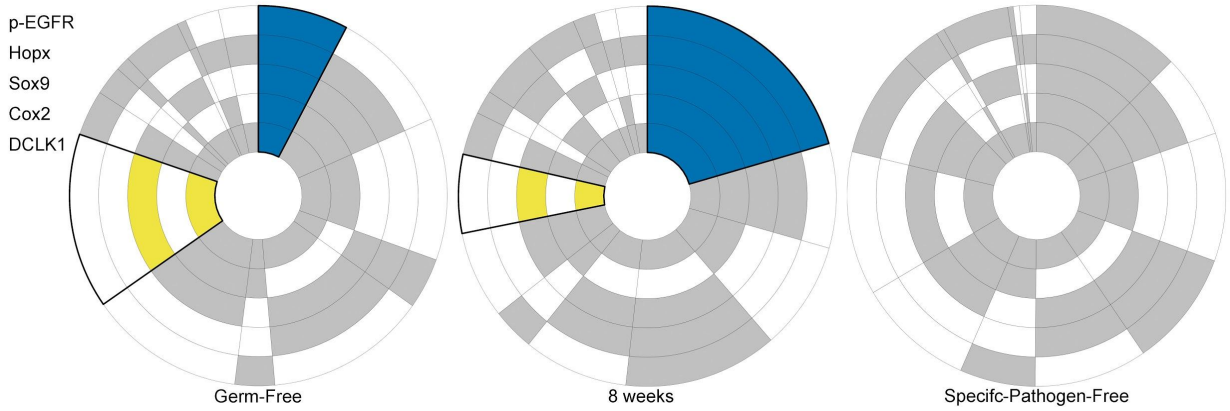
Supplemental Figure 14: Tuft cell expression profiles in the small intestine. Compared to homeostasis, relatively little change was observed in fasted or refed mice. Circular plots demonstrate the proportion of each cell expression profile for each tuft cell marker. Statistically significant changes in expression profiles between sites and conditions are denoted by bold outlines and colors.



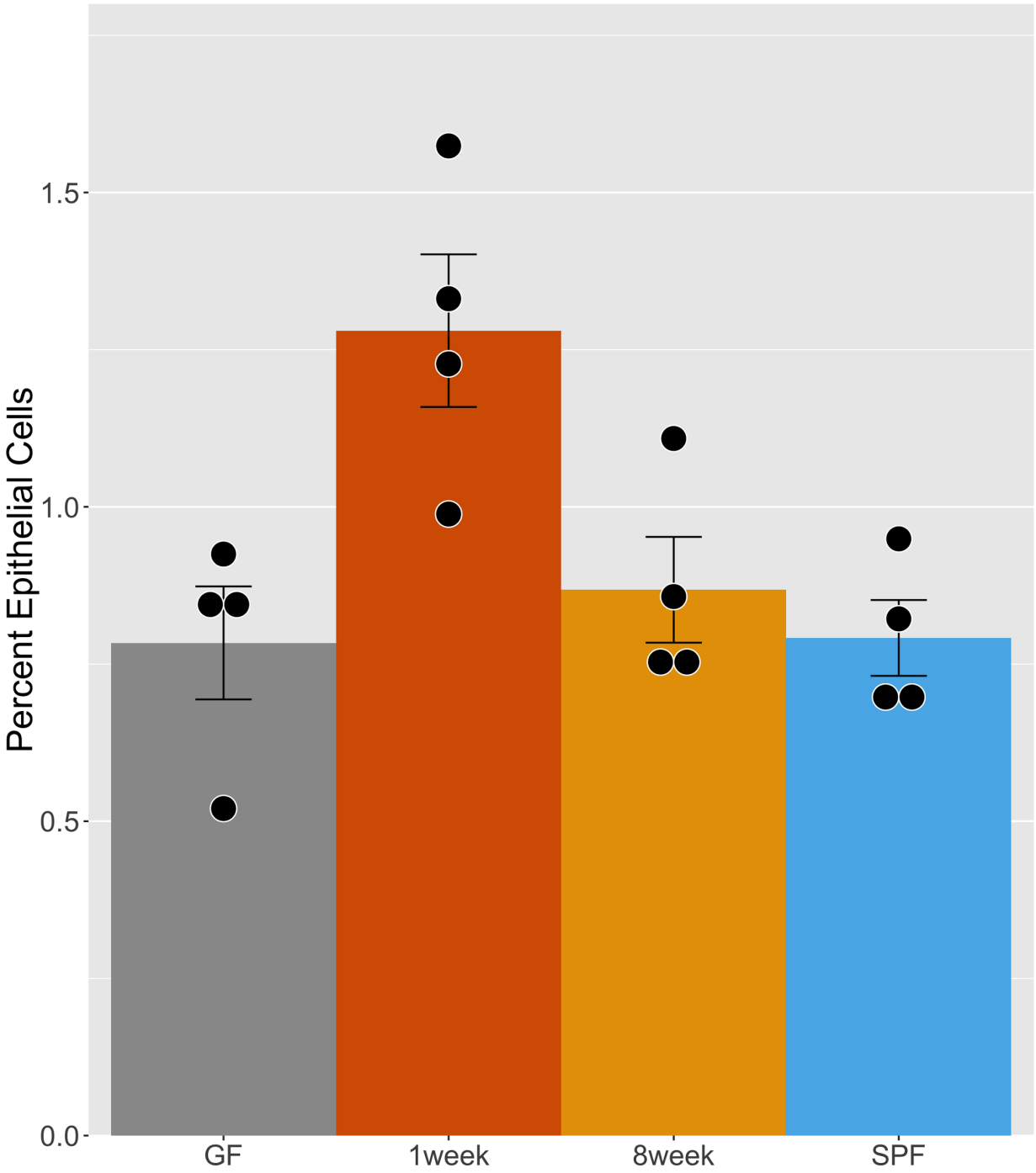
Supplemental Figure 15: Tuft cell expression profiles in the colon. Compared to homeostasis, only one expression profile was reduced in fasted or refed mice. Circular plots demonstrate the proportion of each cell expression profile for each tuft cell marker. Statistically significant changes in expression profiles between sites and conditions are denoted by bold outlines and colors.



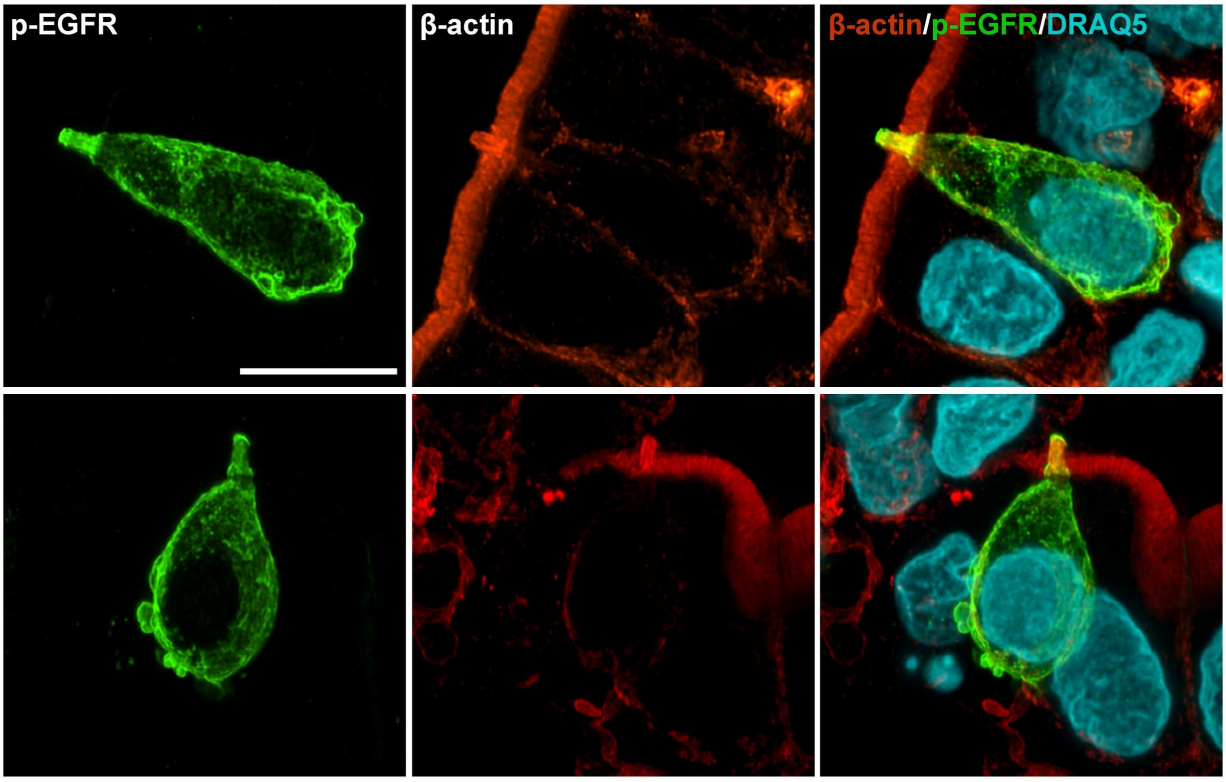
Supplemental Figure 16: pEGFR expression in colonic tuft cells. Compared to germ-free mice, mouse colons collected 1 week (*P=<0.0001), 8 weeks (**P=0.0282), and in specific-pathogen-free (**P=0.0061) conditions demonstrated increased pEGFR positivity in tuft cells. N=4 for each condition.



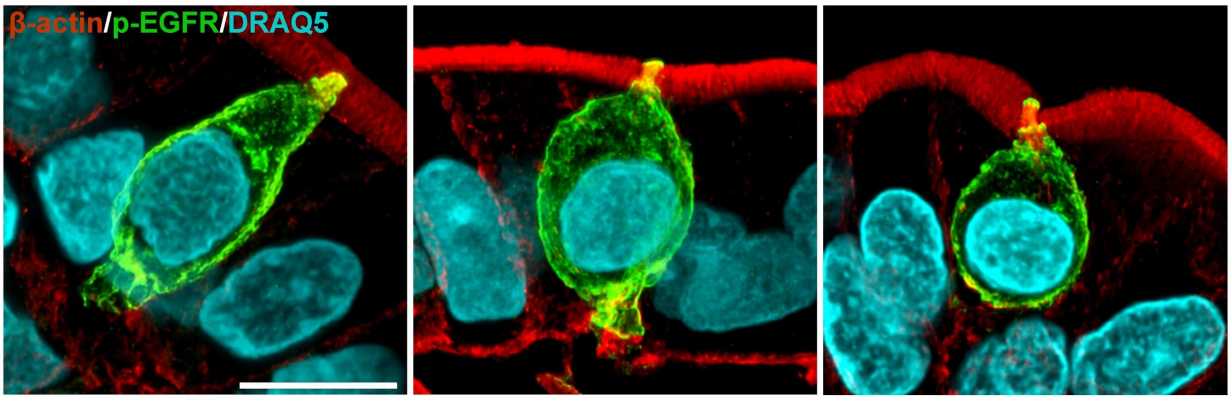
Supplemental Figure 17: Tuft cell expression profiles in the colon. Large tuft cell protein signature changes were observed when comparing germ-free mice to those 1 week following microbiota introduction; mice at 8 weeks following inoculation with microbiota showed relatively little difference in tuft cell profiles. No difference was observed comparing mice housed in germ-free to specific-pathogen-free conditions. Circular plots demonstrate the proportion of each cell expression profile for each tuft cell marker. Statistically significant changes in expression profiles between sites and conditions are denoted by bold outlines and colors.



Supplemental Figure 18: Muc2 positive cells in colon. No change in the percentage of goblet cells in the colon was observed after the introduction of microbiota to previously germ free mice. N=4 for each condition



Supplemental Figure 19: Expression of pEGFR and β-actin in human intestinal tuft cells. Increased pEGFR staining is observed at the apical surface of human tuft cells in the duodenum which colocalizes with increased β-actin expression that extends into the lumen (scale bar:10 μm). Frequently, large membrane blebs are observed on the membranes of tuft cells by pEGFR staining.



Supplemental Figure 20=: Human intestinal tuft cell shapes. Tuft cells in the human duodenum present with many shapes and basal extensions (scale bar: 10 µm).