**Supplemental Table 1. Small RNA-seq mapping statistics.** Table for each cell population that was sequenced, indicating sample name, cell type identity, 3’ adapter trimming statistics, and mapping statistics.

**Supplemental Table 2. ChRO-seq mapping statistics.** Table containing the statistics on adapter removal, PCR duplicates, genome mapping, and number of identified transcriptional regulatory elements from 6-7 months old male Prox1-GFP mice (n=2).

**Supplemental Table 3.** **Single-cell RNA-seq mapping statistics.** Table containing the number of cells, mapping efficiency, and filtering statistics for 10X single-cell data generated from intestinal crypts from 12 months old male B62J mice (n=2).

**Supplemental Figure 1.** FACS gating strategy for intestinal stem cell populations. **A**, Gating strategy and percentage of total cells gated in isolating Lgr5+ ISCs that have low to medium granularity detected from side scatter (SSC-A) from live, CD31- CD45- Annexin-V- jejunal crypt IECs. **B**, Gating strategy and percentage of total cells gated in isolating Cd24-Low ISCs that are EpCAM+ from live, UEA-I- CD45- jejunal crypt IECs.

**Supplemental Figure 2. FACS gating strategy for intestinal secretory cell populations. A****,** Gating strategy and percentage of total cells gated in isolating SiglecF+ tuft cells that are CD24+ from live, CD45- EpCAM+ CD24- UEA-I+ small intestinal IECs.  **B,** Gating strategy and percentage of total cells gated in isolating Defa6+ Paneth cells that are EpCAM+ from live, CD45- jejunal crypt IECs. **C,** Gating strategy and percentage of total cells gated in isolating UEA-I+ goblet cells and UEA-I- enterocytes (for qPCR analysis) that are both CD24- from live, CD45- EpCAM+ CD166- small intestinal IECs.

**Supplemental Figure 3. Validation of sorted small intestinal cell populations. A**, RT-qPCR data for *Lgr5*, *Chga*, *Lyz1*, and *Muc2* sorted jejunal Lgr5+ cells (ISCs) relative to Lgr5- cells from Lgr5-EGFP mice (n=2). **B,** RT-qPCR data for *Lgr5*, *Chga*, *Dclk1*, *Lyz1*, *Muc2*, and *Sis* in sorted small intestinal live CD45- EpCAM+ SiglecF+ CD24+ cells (tuft cells) relative to live CD45- EpCAM+ UEA-I- CD24- CD166- cells (enterocytes) from C57BL/6 mice (n=4). **C,** RT-qPCR data for *Lgr5*, *Chga*, *Dclk1*, *Lyz1*, *Muc2*, *Sis*,and *Defa20* in sorted jejunal Defa6 expressing tdTomato + cells (Defa6+) (Paneth cells) relative to Defa6 non-expressing tdTomato- cells from Defa6-Cre; CAG-tdTomato mice (Defa6-) (n=8). **D,** RT-qPCR data for *Lgr5*, *Chga*, *Dclk1*, *Lyz1*, *Muc2*, and *Sis* in sorted small intestinal live CD45- EpCAM+ UEA-I+ CD24- CD166- cells (goblet cells) relative to live CD45- EpCAM+ UEA-I- CD24- CD166- cells (enterocytes) from C57BL/6 mice (n=4). **E,** RT-qPCR data for *Lgr5*, *Chga*, *Dclk1*, *Lyz1*, *Muc2*, and *Sis* in sorted jejunal Sox9-neg (enterocytes) cells relative to unsorted Sox9-uns cells from Sox9-EGFP mice (n=3). \*P < 0.05, \*\*P< 0.01, \*\*\*P<0.001 by two-tailed Student's t-test. RQV, relative quantitative value. Sox9-neg = Sox9 negative, Sox9-uns = Sox9 unsorted.

**Supplemental Figure 4. MicroRNA and host gene expression patterns in both mouse and human. A,** UCSC browser images showing the microRNA locus and the host gene coding region (top). The sites of microRNA transcriptional initiation were identified using signal from chromatin run-on sequencing (ChRO-seq) on crypts from 6-7 months old male Prox1-GFP mice (n=2). Normalized ChRO-seq signal on the plus strand is shown in red and on the minus strand is shown in gray. **B,** Expression of host gene (left; log normalized expression) and embedded microRNA (right; DESeq2 normalized expression) for Hnrnpk/miR-7a, *Ank1*/miR-486, *Map2k4*/miR-744, *E130102H24Rik*/miR-101a, *Pank1*/miR-107, and *Pank2*/miR-103. **C,** Expression pattern of four host genes of interest (*VWA5B2*, *COPZ2*, *PANK3*, and *PPARGC1B*) in a single-cell RNA-seq dataset from human ileum epithelium.

**Supplemental Figure 5. FACS gating strategy and validation of sorted Prox1+ secretory progenitor cells. A,** Gating strategy and percentage of total cells gated in isolating Prox1+ cells that have low to medium granularity detected from side scatter (SSC-A) from live, CD31- CD45- Annexin-V- jejunal crypt IECs. **B**, RT-qPCR data for *Lgr5*, *Chga*, *Dclk1*, *Lyz1*, *Muc2*, *Sis*, and *Hopx* in sorted jejunal Prox1+ cells relative to Prox1- cells from Prox1-GFP mice (n=3). \*P< 0.05, \*\*\*P<0.001 by two-tailed Student's t-test. RQV, relative quantitative value.

**Supplemental Figure 6. Diminished miR-486 expression correlates with reduction in small intestinal tuft cells. A**, Schematic of experimental plan for analyzing tuft cells under two different conditions: normal (wild-type, WT) and tuft-diminished (Pou2f3 knockout, Pou2f3KO). Jejunal crypts from wild-type (WT) and Pou2f3 knockout (Pou2f3KO) mice were isolated and subjected to RT-qPCR analysis. **B,** RT-qPCR data for *Lgr5*, *Chga*, *Dclk1*, *Lyz1*, *Muc2*, and *Sis* in jejunal crypts from Pou2f3KO mice (n=2) relative to jejunal crypts from WT mice (n=2). **C,** RT-qPCR data for miR-486 in jejunal crypts from Pou2f3KO mice (n=2) relative to jejunal crypts from WT mice (n=2). **D,** RT-qPCR data for miR-152, miR-194, miR-30b, miR-375, and miR-7 in jejunal crypts from Pou2f3KO mice (n=2) relative to jejunal crypts from WT mice (n=2). \* P < 0.05, \*\* P < 0.01 by two-tailed Student's t-test. RQV, relative quantitative value.

**Supplemental Figure 7. FACS gating strategy and validation of Paneth cell depletion in two mouse genetic models. A,** RT-qPCR data for *Lgr5*, *Chga*, *Dclk1*, *Lyz1*, *Muc2*, and *Sis* in mid-jejunal whole tissue from diphtheria toxin-A (DT) treated Defa6-DTR mice (n=9) relative to sham treated Defa6-DTR mice (n=7). **B,** Gating strategy and percentage of total cells gated in isolating Mist1+ cells that are CD24+ from live, CD45- EpCAM+ jejunal crypt IECs. **C,** RT-qPCR data for *Lgr5*, *Chga*, *Dclk1*, *Lyz1*, *Muc2*, and *Sis* in tdTomato+ cells from Defa6-Cre; CAG-tdTomato mice (n=4) relative to tdTomato+ cells from Mist1-Cre; CAG-tdTomato mice (n=4). \*P < 0.05, \*\*P< 0.01, \*\*\*P<0.001 by two-tailed Student's t-test. RQV, relative quantitative value.