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Supplemental information

A dietary change to a high-fat diet initiates a rapid adaptation of the intestine

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Supplementary Figure 1- Additional metabolic cage information and morphometric analysis of proximal intestine during acute HFD, related to Figure 1

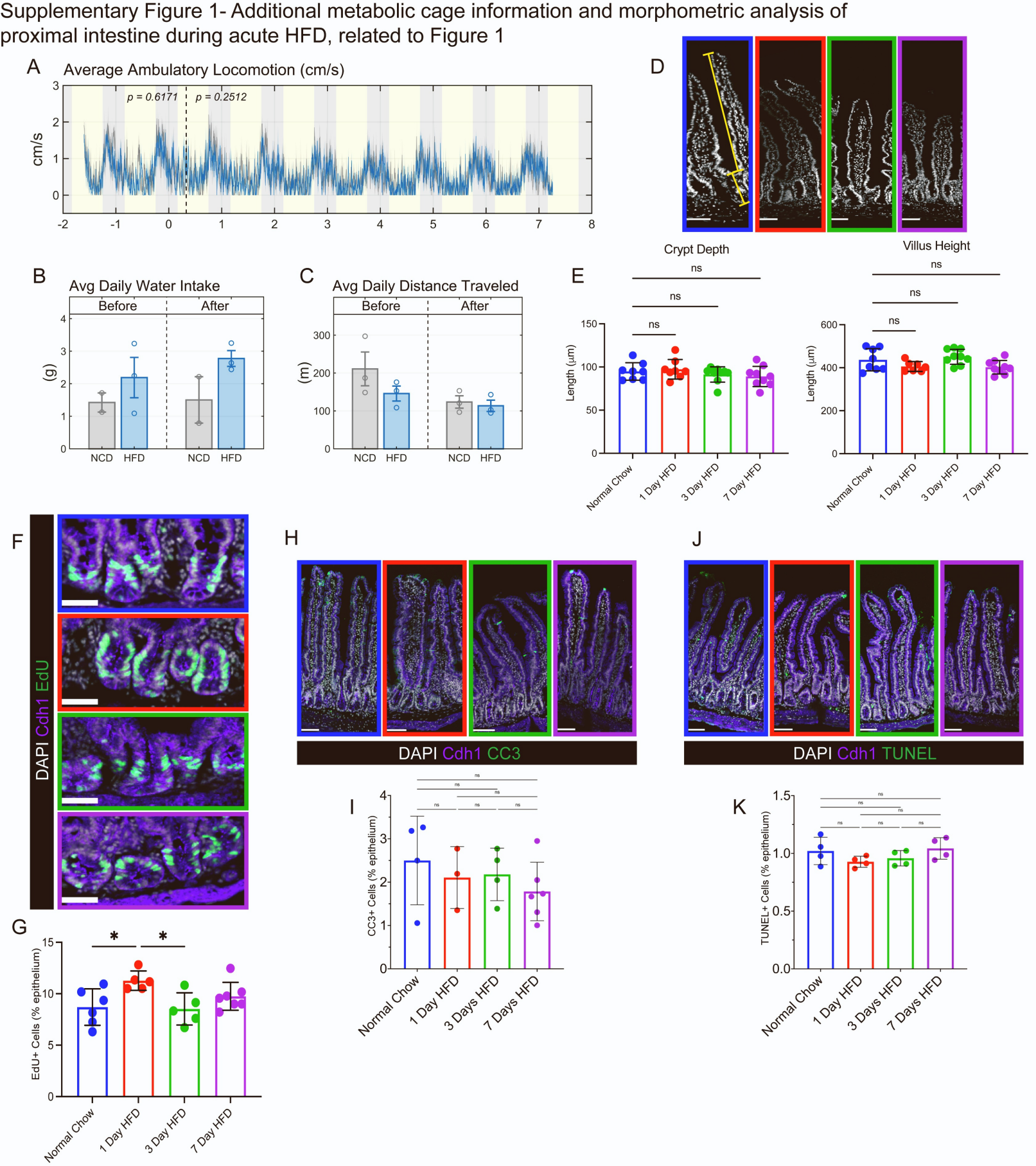

Figure S1- Additional metabolic cage information and morphometric analysis of proximal intestine during acute HFD, related to Figure 1

(A) Average ambulatory locomotion of mice fed Normal Chow (gray line) and HFD (blue line) before and after dietary treatment. Yellow color indicates light cycle whereas gray color indicates night cycle. (B) Average daily water intake before and after dietary treatment. NCD = Normal Chow Diet, HFD = High Fat Diet. (C) Average daily total distance traveled in metabolic cages before and after dietary treatment. (D) Representative images of jejunum used for morphometric analysis. Yellow bars indicate representative depth and height measurement. Normal Chow (Blue Border), 1 Day HFD (Red Border), 3 Days HFD (Green Border), 7 Days HFD (Purple Border) with all images showing DAPI (White). Scale bars = 100μm. (E) Quantification of crypt depth (left panel) and villus height (right panel) between dietary conditions; n = 8 for Normal Chow and 1 Day HFD, n = 9 for 3 Days and 7 Days HFD (F) Representative images of jejunal crypts used for 5-ethynyl-2'-deoxyuridine (EdU) analysis. Normal Chow (Blue Border), 1 Day HFD (Red Border), 3 Days HFD (Green Border), 7 Days HFD (Purple Border) with all images showing DAPI (White), Cdh1 (Epithelium), EdU (Green). Scale bars = 50μm. (G) Quantification of EdU incorporation after a 2h pulse between all conditions; n = 6 for Normal Chow, n = 5 for 1 Day and 3 Days HFD, n = 7 for 7 Days HFD. (H) Representative images of jejunum using CleavedCaspase3 (CC3) staining analysis. Coloration and labels are similar to previous panels, except CC3 (Green). Scale bars = 100μm. (I) Quantification of CC3 between all conditions; n = 4 for Normal Chow, n = 3 for 1 Day HFD, n = 4 for 3 Days HFD, n = 6 for 7 Days HFD. (J) Representative images of jejunum using TUNEL Assay Kit. Coloration of labels are similar to previous panels, except TUNEL positive cells (Green). Scale bars = 100μm. (K) Quantification of TUNEL Assay between all conditions; n = 4 for all conditions. (E, G, I, K) Error bars are SD. One-Way ANOVA: ns- no significance; p-value * < 0.05

Supplementary Figure 2- Intestinal populations of the proximal intestine during acute HFD, related to Figure 2

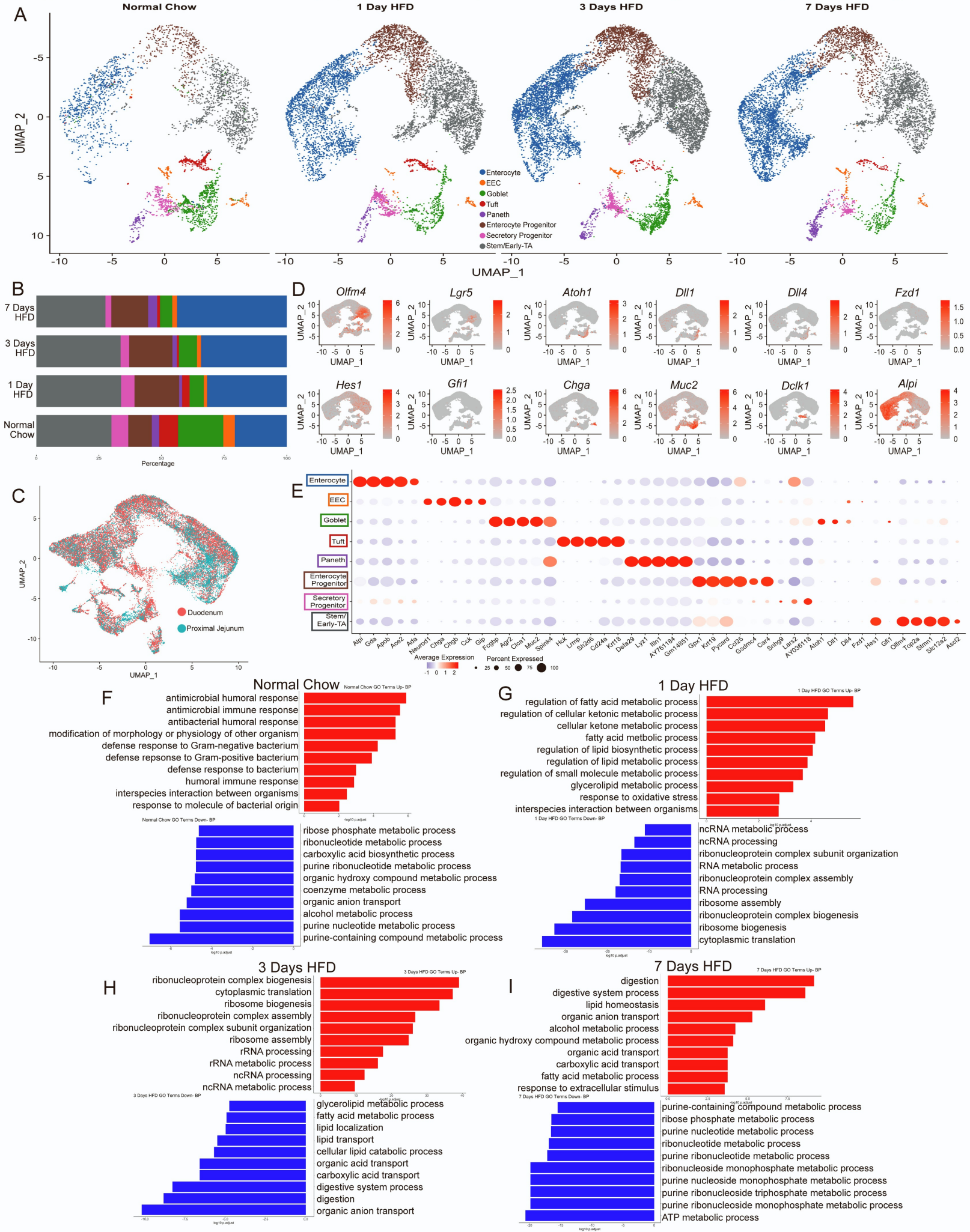

Figure S2- Intestinal populations of the proximal intestine during acute HFD, related to Figure 2

(A) UMAP of integrated analysis of proximal intestine (reference dataset- Normal Chow) split by dietary condition and colorized by cluster identity. (B) Percent proportions of total cells for each cluster over time. (C) UMAP distinguishing between duodenum and jejunum (D) Feature Plots of known genes known to distinguish cell type heterogeneity within the intestinal epithelium. Color gradient depicts gene expression from low (gray) to high (red). (E) Dot plot depicting average gene expression of top genes expressed by each cluster in proximal intestine. (F-I) Top 10 Gene Ontology (GO) Terms- Biological Process for overall clusters comparing between each condition in the jejunum. Red bars indicate significantly upregulated whereas blue bars indicate significantly down regulated. X-axis indicates log10 adjusted-p value (cutoff < 0.05).

Supplemental Figure 3- Enterocyte sub-cluster analysis in response to acute HFD, related to Figure 4

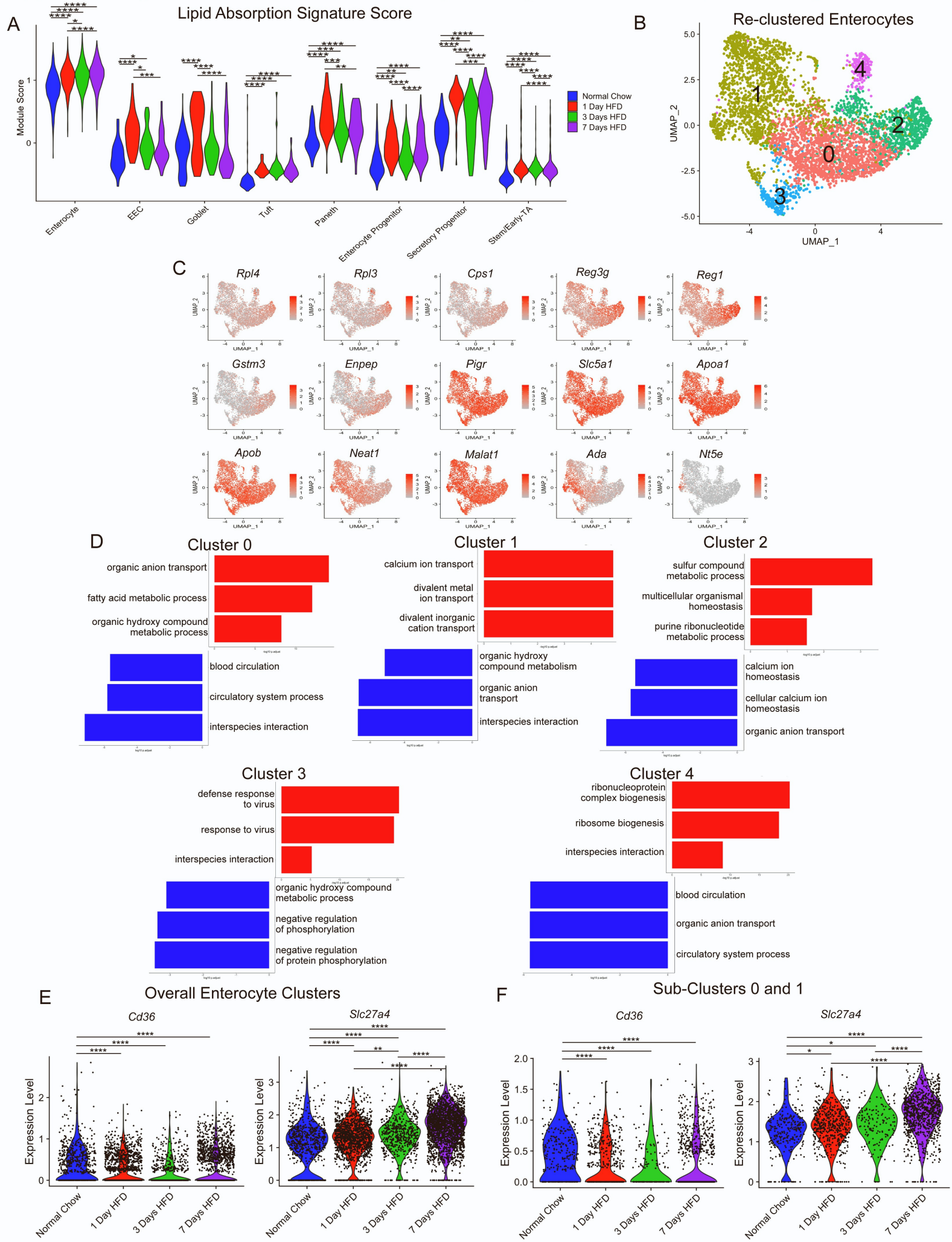

Figure S3- Enterocyte sub-cluster analysis in response to acute HFD, related to Figure 4

(A) Lipid absorption signature scores for each cluster of the intestinal epithelial dataset compared between conditions. (B) UMAP of re-clustered Enterocyte subset extracted from jejunal epithelial dataset (C) UMAP of hallmark genes noted by *Moor et.* al (2018) to distinguish Enterocyte zonation along the intestinal villus axis. Color gradient depicts gene expression from low (grey) to high (red). (D) Top 3 Gene Ontology (GO) Terms-Biological Process for each cluster for the Enterocyte subset comparing between each cluster. Red bars indicate significantly upregulated whereas blue bars indicate significantly downregulated. X-axis indicates log10 adjusted-p value (cutoff < 0.05). Extracted from jejunal epithelial dataset. (E) Violin Plots extracted from Enterocyte subset showing gene expression of fatty acid receptors *Cd36* (Cluster of differentiation-36) and *Slc27a4* (Solute carrier Family 27-Member 4) split between conditions Normal Chow, 1 Day HFD, 3 Days HFD, 7 Days HFD. (F) Violin Plots depicting gene expression of each cell (black dot) from Enterocyte subset showing gene expression of fatty acid receptors *Cd36* and *Slc27a4* split between conditions Normal Chow, 1 Day HFD, 3 Days HFD, 7 Days HFD. (A-B, C-D) One-Way ANOVA: ns- no significance; p-value * < 0.05, ** < 0.007, *** < 0.0006, **** < 0.0001

Supplementary Figure 4- *Neurog3* lineage tracing and cell sorting enriches for secretory lineages, related to Figure 5

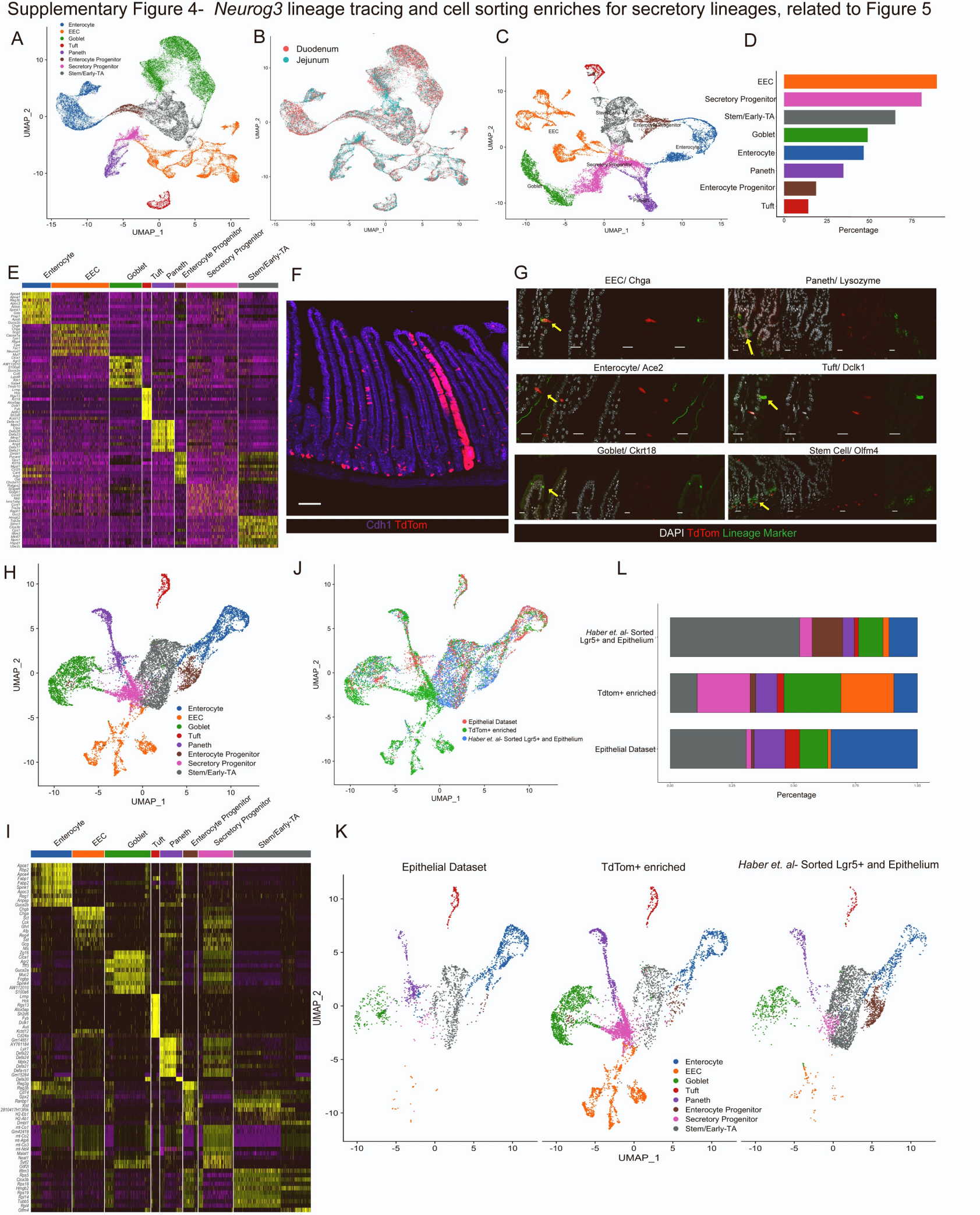

Figure S4- *Neurog3* lineage tracing and cell sorting enriches for secretory lineages, related to Figure 5

(A) UMAP of sorted *Neurog3Cre*-TdTom dataset of proximal intestine colored by various cell types. (B) UMAP distinguishing between duodenum and jejunum (C) UMAP of jejunum subset visualizing 8 clusters: EEC (4915 cells), Enterocyte (2437 cells), Enterocyte Progenitor (983 cells), Goblet (2723 cells), Paneth (1890 cells), Secretory Progenitor (4971 cells), Stem/Early-TA Zone (2741 cells), Tuft (766 cells). (D) Percent proportion of each cluster ordered by abundance from top to bottom. (E) Heatmap related to Panel C showing top 10 genes expressed by each cluster. Gradient coloration low (purple) to high (yellow). (F) Immunofluorescence images of proximal intestine from *Neurog3Cre*-TdTom animals. Cdh1 labels epithelium in purple. Scale bars = 100 μm. (G) Panels of various intestinal epithelial cell types stained in green- Enteroendocrine cell (EEC) stained by Chromogranin-A (*Chga*), Paneth stained by Lysozyme (Lys), Enterocyte stained by Angiotensin-converting enzyme 2 (Ace-2), Tuft stained by Doublecortin Like Kinase 1 (Dclk1), Goblet stained by Cytokeratin-18 (Ckrt18), Stem Cells stained by Olfactomedin 4 (Olfm4). DAPI counterstains nuclei in white. Scale bars = 20 μm. (H) UMAP colored by cell type after integrated analysis between non-labeled intestinal epithelial cells, sorted TdTomato cells, and *Haber et. al, 2017* dataset. (I) Heatmap related to Panel G of top 10 highly expressed genes for each cell type. (J) UMAP colored by various datasets. (K) UMAP split between each dataset and colored by each cell type. (L) Barplot showing percent abundance of each cell type split between datasets.

Supplementary Figure 5- Secretory cell types maintain cell number during acute HFD, related to Figure 5

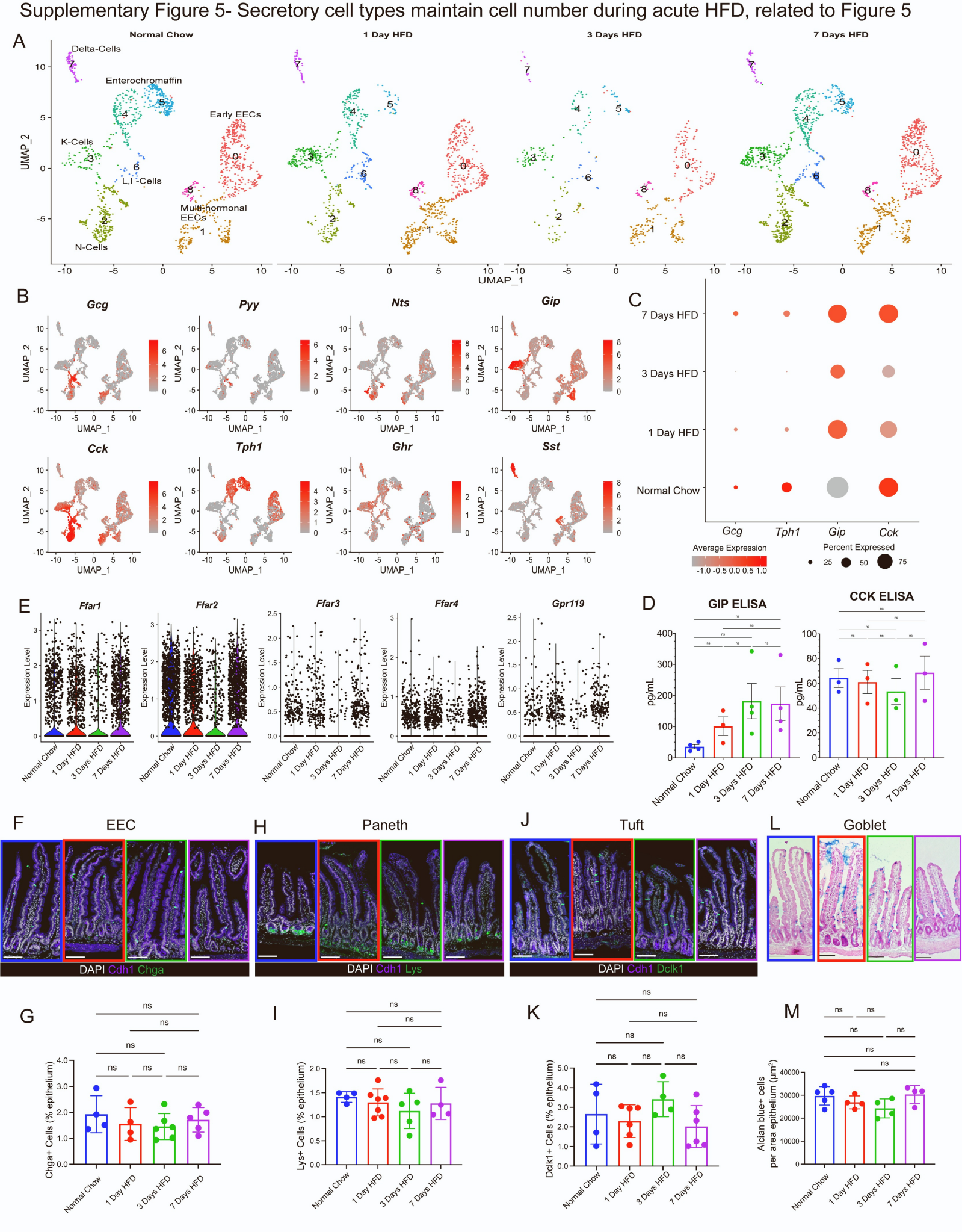

Figure S5- Secretory cell types maintain cell number during acute HFD, related to Figure 5

(A) Integrated reference analysis for sorted TdTom+ population (secretory enriched) focusing on enteroendocrine cells (EECs) colored by each cluster and then split into dietary conditions. Total of 8 clusters: 0 (Early EECs, 1234 Cells), 1 (Multi-hormonal EECs, 924 cells), 2 (N-Cells, Neurotensin cells, 722 cells), 3 (K-Cells, Gastric-Inhibitory Peptide cells , 538 cells), 4 (Enterochromaffin-1, Serotonin cells, 515 cells), 5 (Enterochromaffin-2, Serotonin cells, 359 cells), 6 (L, l- Cells, Glucagon or Gastric-Inhibitory Peptide cells ,255 cells), 7 (Delta- Cells, Somatostatin cells, 212 cells), 8 (Multi-hormonal EECs, 156 cells); Normal Chow (1448 Cells), 1 Day HFD (1304 cells), 3 Days HFD (502 cells), 7 Days HFD (1661 cells). (B) UMAP feature plots showing gene expression of Glucagon (*Gcg*), Peptide-yy (*Pyy*), Neurotensin (*Nts*), Gastric-Inhibitory Peptide (*Gip*), Cholecystokinin (*Cck*), Tryptophan Hydroxylase 1 (*Tph1*), Ghrelin (*Ghr*), Somatostatin (*Sst*). Colorization is based on normalized expression gradient scale low (gray) to high (red). (C) Average gene expression dot plot for *Gcg*, *Tph1*, *Gip*, and *Cck* split between dietary conditions. Colorization is based on normalized expression gradient scale low (gray) to high (red). Dot size is based on percentage of cells expressing gene of interest. (D) Enzyme linked immunosorbent assay (ELISA) quantification of GIP and CCK from mouse serum measured in pg/mL. n = 3 or 4 per condition. (E) Violin Plots showing expression of EEC fatty acid receptors *Ffar1, Ffar2, Ffar3, Ffar4* (Free-fatty acid receptor 1-4) and *Gpr119* (G-protein Receptor-119) across conditions Normal Chow, 1 Day HFD, 3 Days HFD, and 7 Days HFD. (F) Representative images of jejunum staining for DAPI (white, nuclei), Cdh1 (purple, epithelium), and Chromogranin-A (green, Chg*a*). Normal Chow (Blue Border), 1 Day HFD (Red Border), 3 Days HFD (Green Border), 7 Days HFD (Purple Border). Scale bars = 100 μm. (G) Quantification of Chga between all conditions; n = 4 for Normal Chow and 1 Day HFD, n = 6 for 3 Days HFD, n = 5 for 7 Days HFD. (H) Representative images of jejunum staining for Lysozyme (Lyz1). Border coloration of conditions and scale bars are like previous panels, except Lysozyme positive cells (Green). (I) Quantification of Lyz1 positive cells between all conditions; n = 4 Normal Chow, n = 7 for 1 Day HFD, n = 5 for 3 Days HFD, n = 4 for 7 Days HFD. (J) Representative images of jejunum staining for Doublecortin-like kinase 1 (Dclk1). Border coloration of conditions and scale bars are like previous panels, except Dclk1 positive cells (Green). (K) Quantification of Dclk1 between all conditions; n = 4 for Normal Chow, n = 6 for 1 Day HFD, n = 4 for 3 Days HFD, and n = 6 for 7 Days HFD. (L) Representative images of Alcian Blue staining of jejunum counter stained with Nuclear Fast Red for each condition. Border coloration and scale bars are like previous panels. (M) Quantification of Alcian blue between all conditions; n = 5 for Normal Chow, n = 4 for 1 Day, 3 Days, and 7 Days HFD. (D, G, I, K, M) Error bars are SD. One-Way ANOVA: ns- no significance