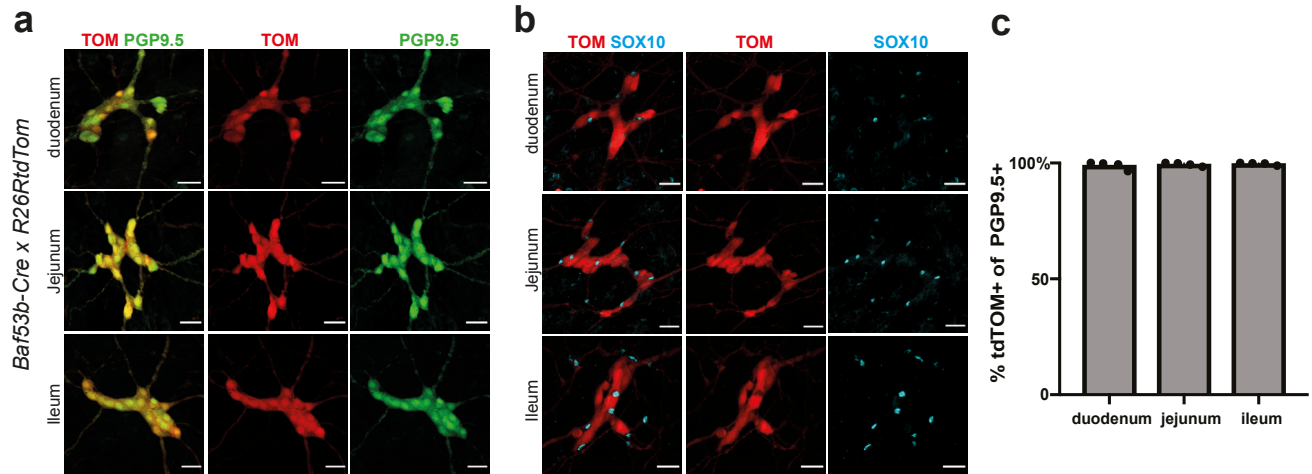


The transcriptomes, connections and development of submucosal neuron classes in the mouse small intestine

In the format provided by the
authors and unedited

Supplementary Figure 1



Supplementary Figure 1: Validation of tdTomato expression in submucosal neurons of *Baf53b-Cre x R26RtdTomato* mice.

Representative pictures from submucosal plexus of duodenum, jejunum and ileum of P24

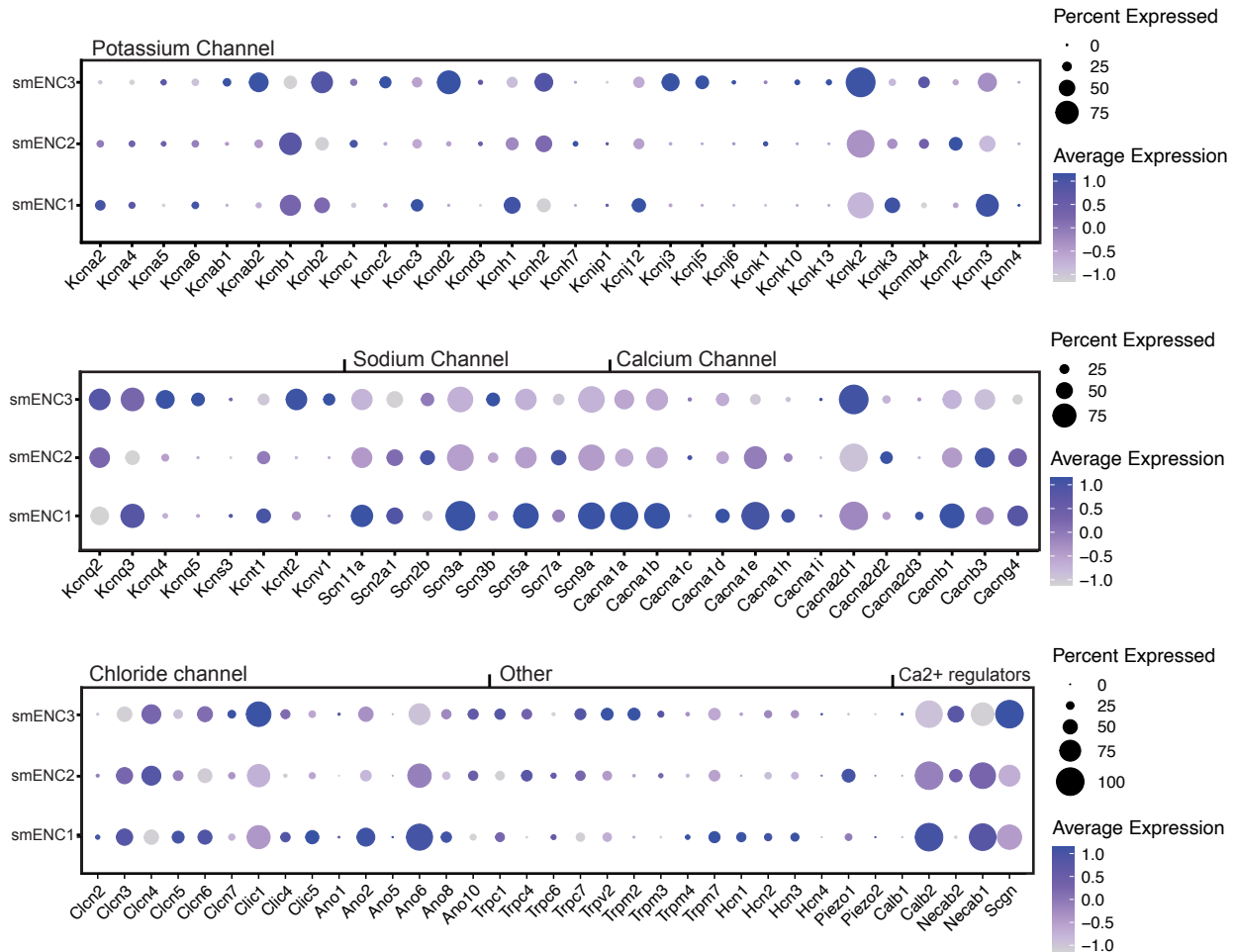
Baf53b-Cre;R26R-Tomato mice, showing tdTomato expression in neurons (**a**) but not in enteric glia (**b**).

c, Graph showing the average percentage of PGP9.5+ cells that express tdTomato within the duodenum (98.9 ± 1.64 ; 2875 cells), jejunum (99.29 ± 0.72 ; 2575 cells) and ileum (99.64 ± 0.50 ; 2585 cells);

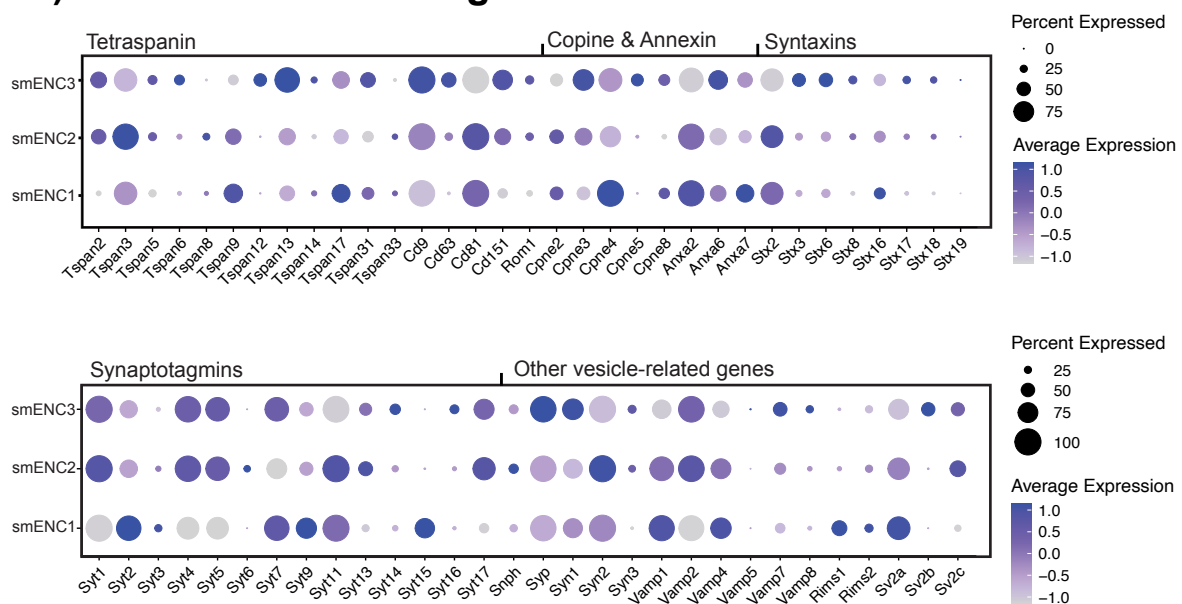
n=4 mice; TOM:tdTomato; Scale bars:30 μ m

Supplementary Figure 2

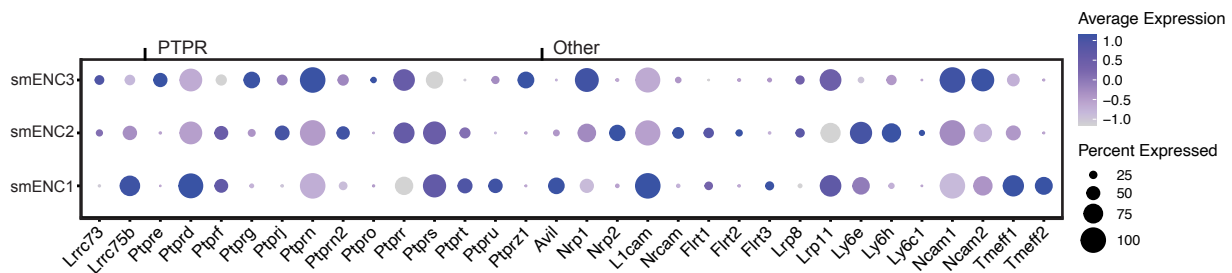
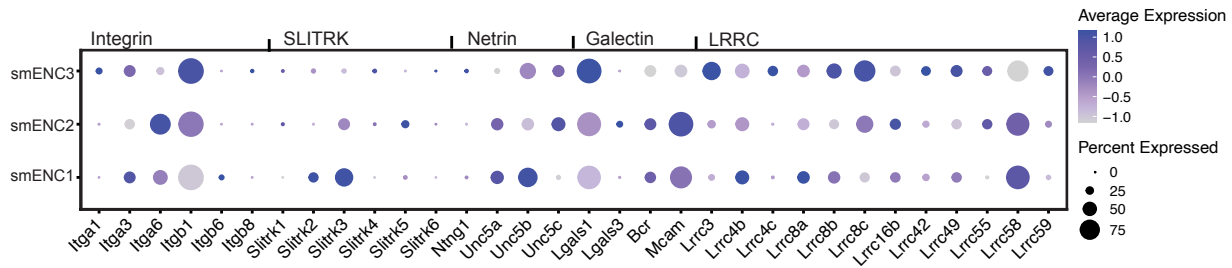
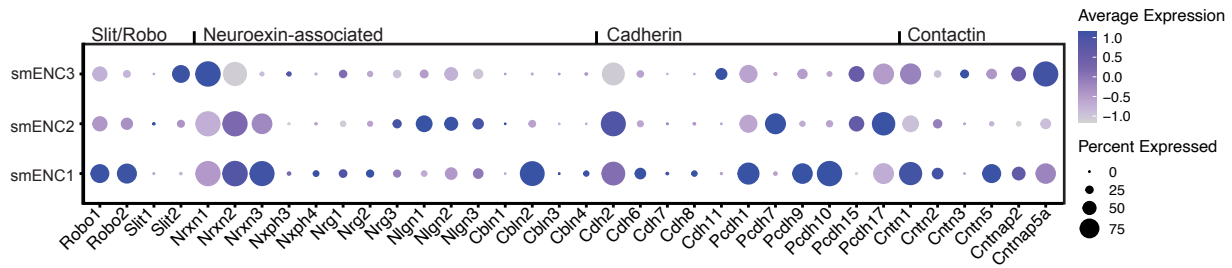
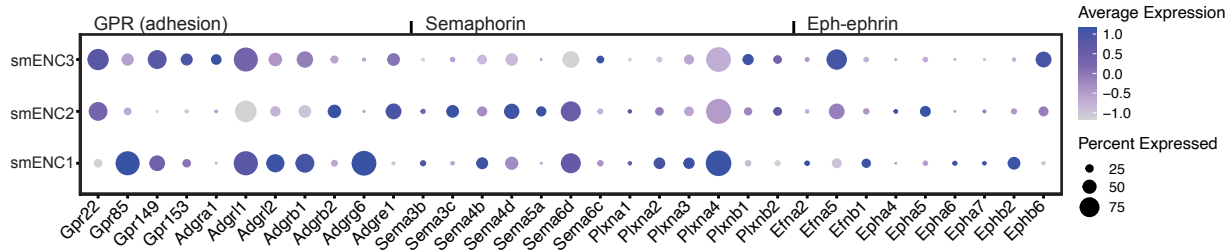
a) Ion Channels



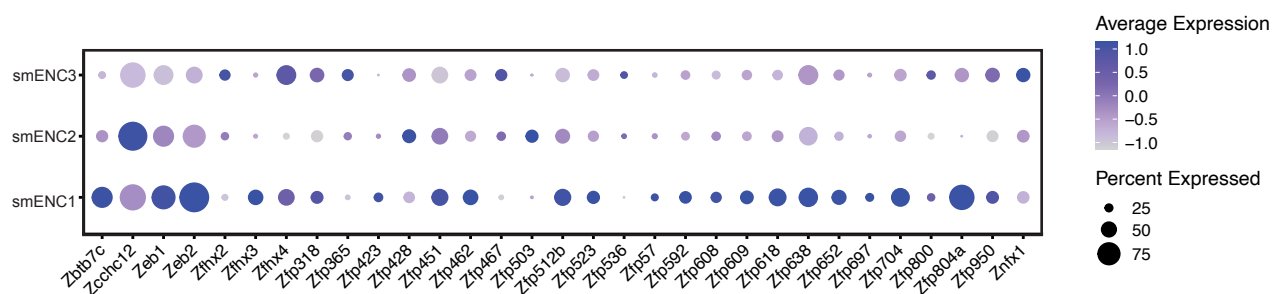
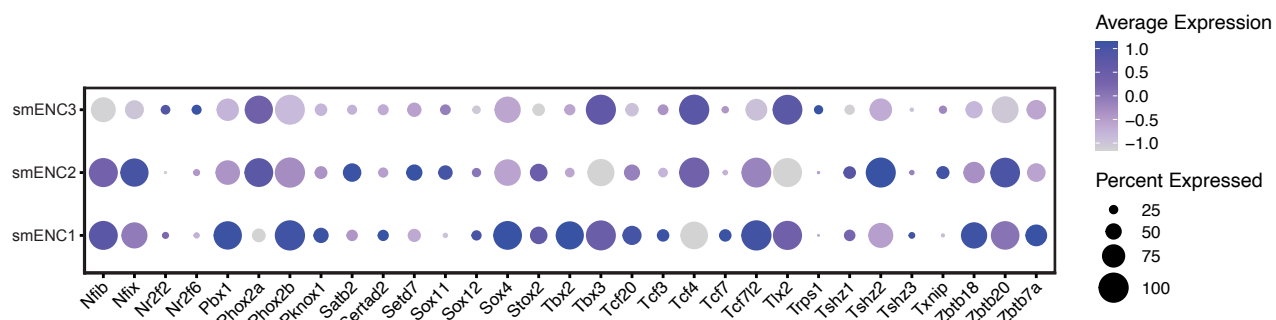
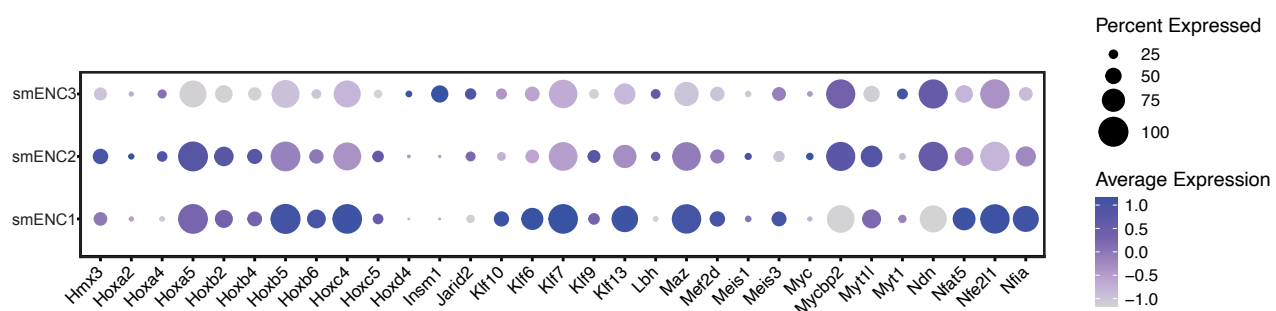
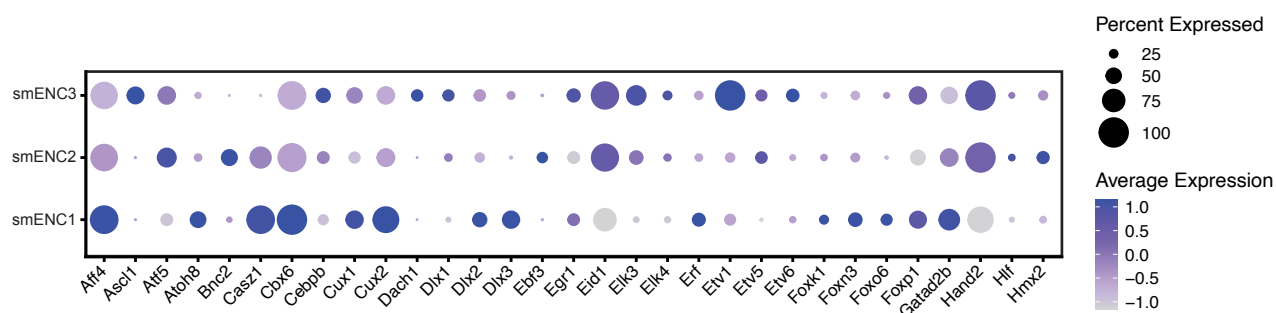
b) Membrane Trafficking



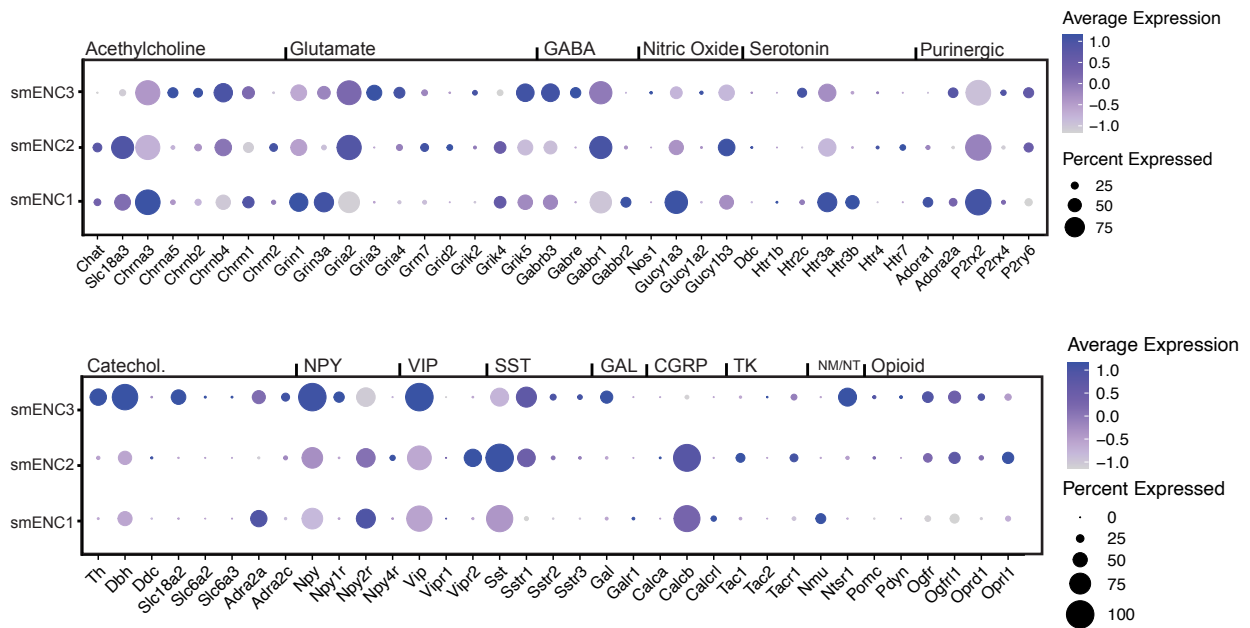
c) Adhesion molecules



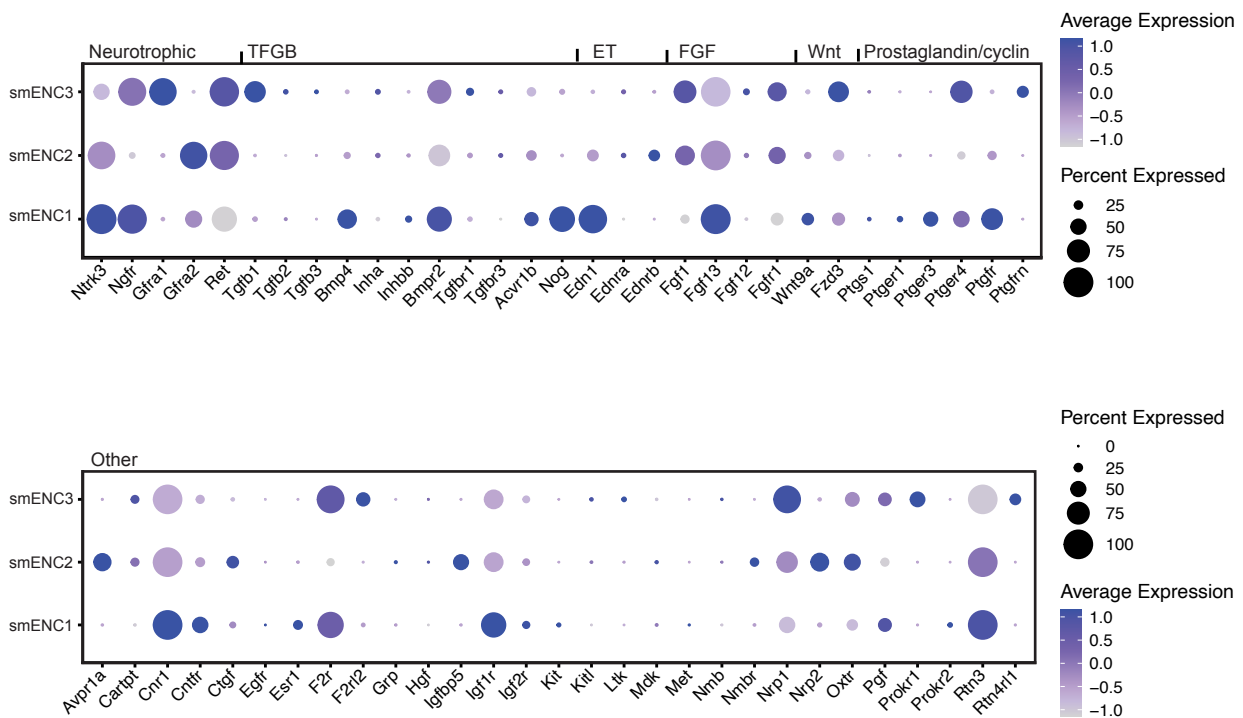
d) Transcription Factors



e) Neurotransmission



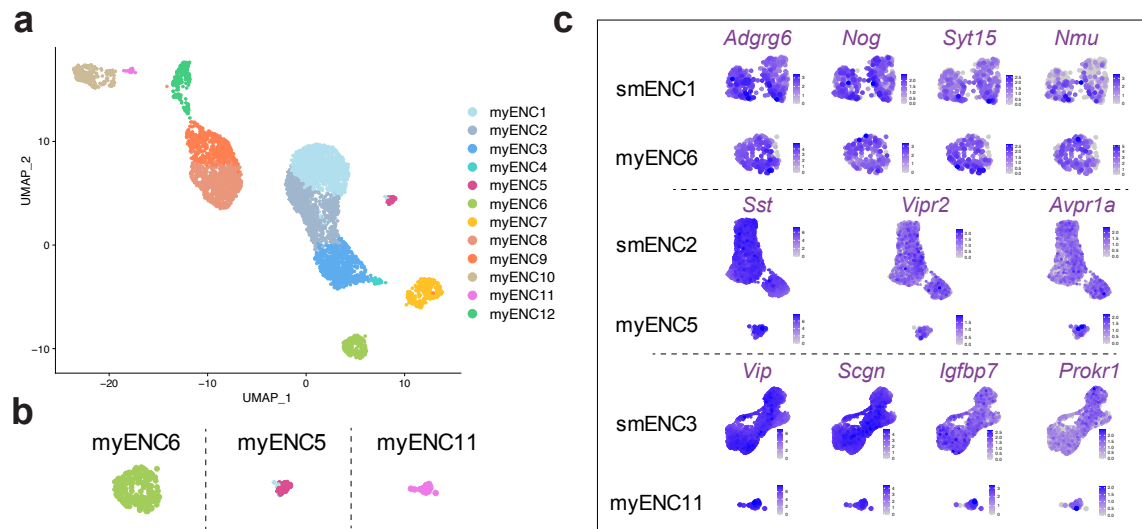
f) Cell-cell signaling



Supplementary Figure 2. Dot plots displaying expression of genes conferring neuronal phenotypes in smENC1-3.

Gene categories involved in a) ion transport, b) membrane trafficking, c) adhesion d) transcription e) neurotransmission f) other cell-cell signaling. Color scale represents z-score and dot size represents percent of cells with non-zero expression within a given class. smENC: submucosal Enteric Neuron Class; GPR: G-protein coupled receptor; GABA: Gamma-aminobutyric acid; NPY: Neuropeptide Y; TK: Tachykinin; CGRP: Calcitonin gene-related peptide; GAL: Galanin; VIP: Vasoactive intestinal peptide; SST: Somatostatin; NM: Neuromedin U; NT: Neurotensin; CCK: Cholecystokinin; TGFB: Transforming growth factor beta; ET: Endothelin; FGF: Fibroblast growth factor; LRRC: Leucine Rich Repeat Containing; PTPR: Protein tyrosine phosphatase receptor.

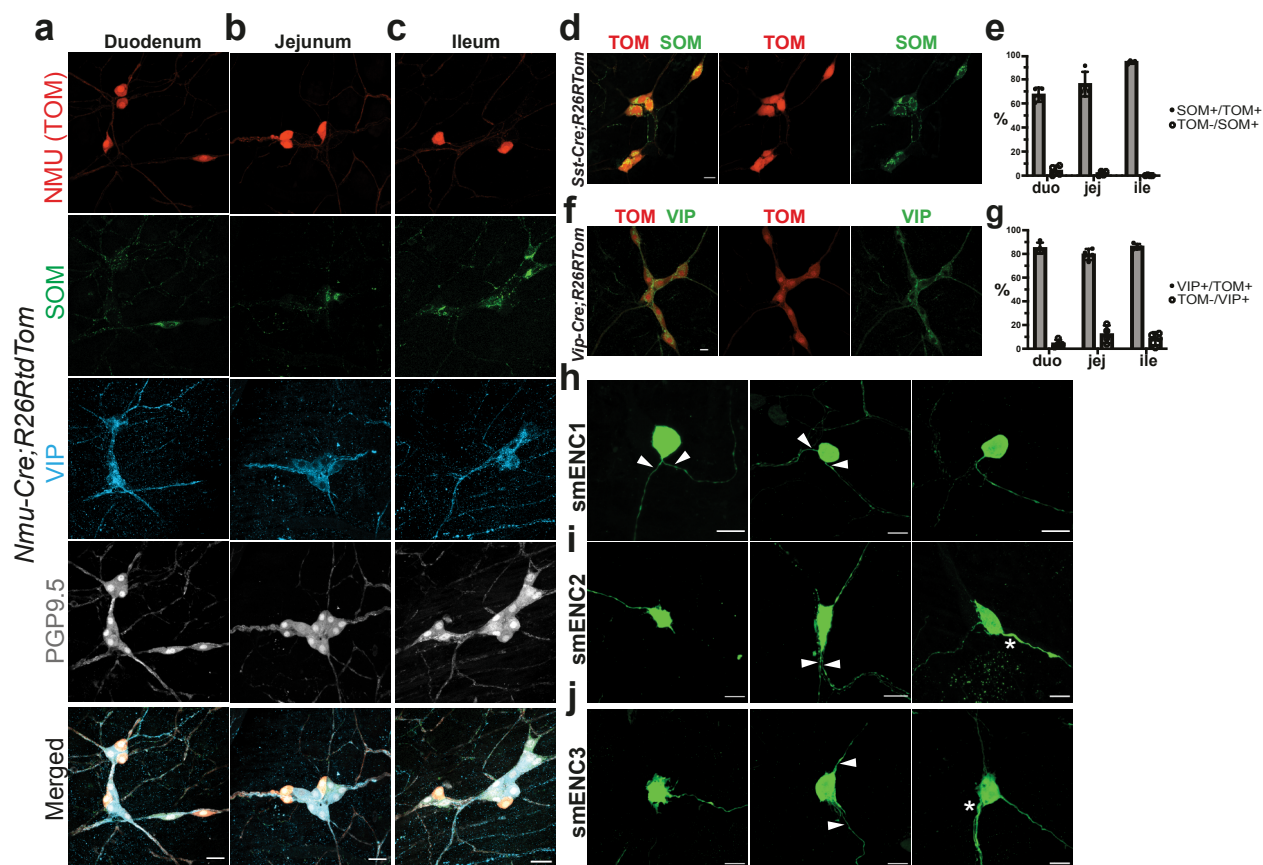
Supplementary Figure 3



Supplementary Figure 3. Supportive Data related to Figure 2.

a, UMAP representing scRNA-seq analysis of neurons in the juvenile myenteric plexus (Morarach et al., 2021). **b**, Closeup of the three myenteric clusters that are transcriptionally similar to submucosal clusters. **c**, Feature plot assembly showing examples of gene expression that is shared between transcriptionally similar smENCs and myENCs.

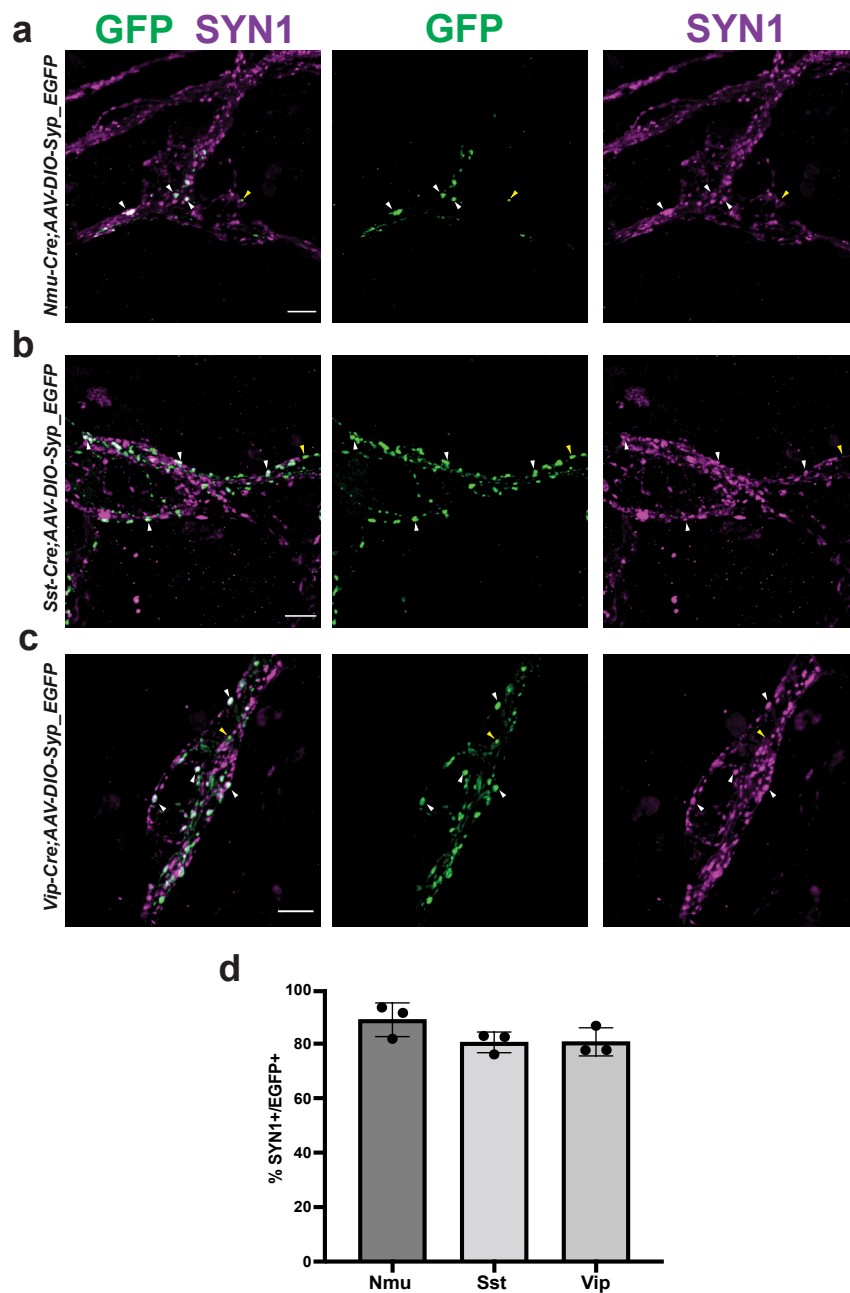
Supplementary Figure 4



Supplementary Figure 4. Supportive Data related to Figure 3.

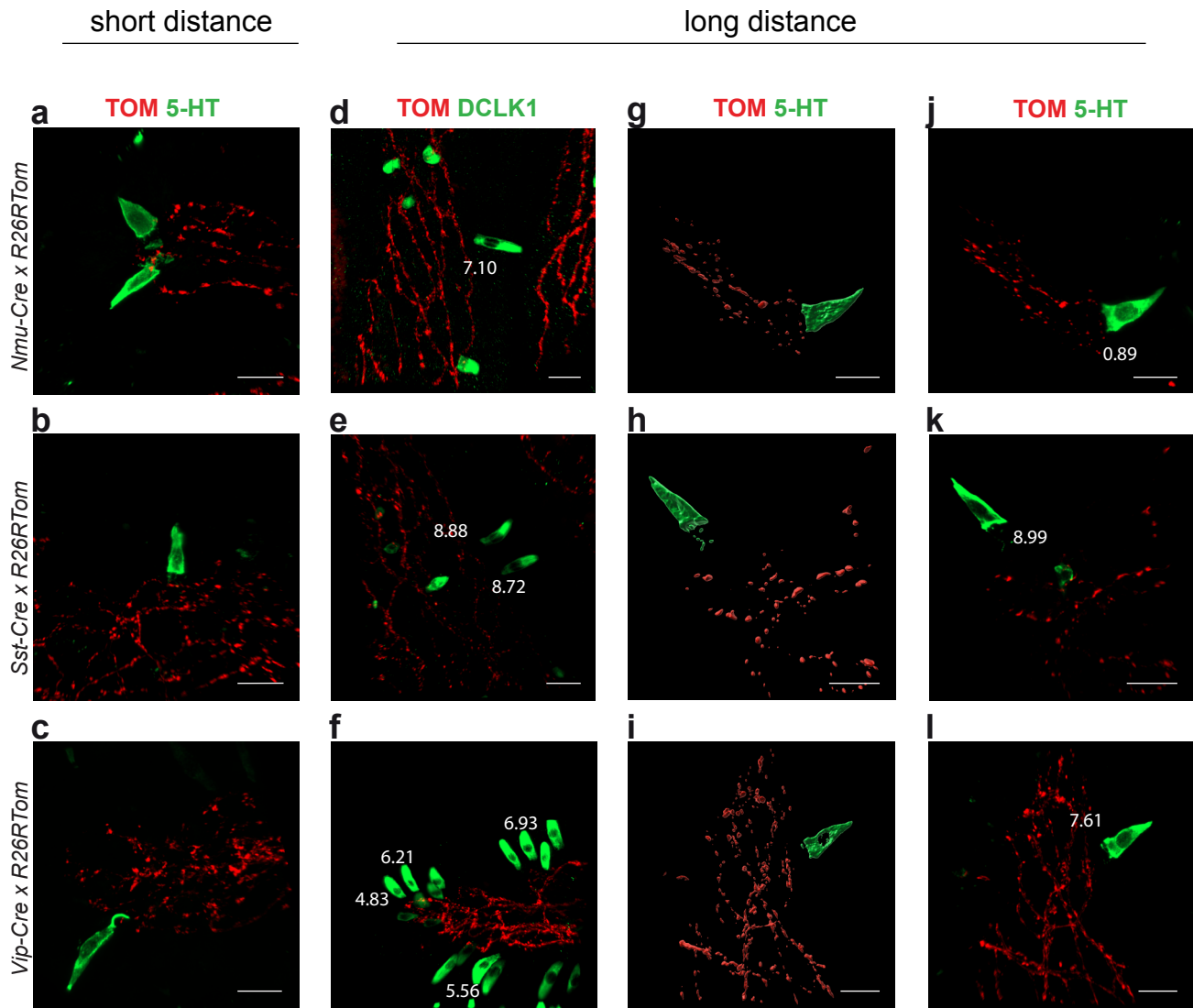
a-c, Representative images showing smENC1(*Nmu-Cre;R26RTomato*), smENC2 (SOM) or smENC3 (VIP) in duodenum (**a**), jejunum (**b**) and ileum (**c**). Scale bars: 30μm. **d**, Representative pictures from ileum submucosal peels showing colocalization of SOM and tdTomato in *Sst-Cre;R26RtdTomato* mice. **e**, Graph showing the percentage of specificity (%SOM+/TOM+) and efficiency (%TOM-/SOM+) of *Sst-Cre;R26RtdTomato* mice (n=4 mice) **f**, Representative pictures from ileum submucosal peels showing colocalization of VIP and tdTomato in *Vip-Cre;R26RtdTomato* mice. **g**, Graph showing the specificity (%VIP+/TOM+) and efficiency(%TOM-/VIP+) of *Vip-Cre;R26RtdTomato* mice (n=4 mice). **h-j**, Images showing examples of neuron morphologies of smENC1-3. Scale bars:20μm. Data are presented as mean values±SD. Arrowheads indicate multiple axons emanating from a single soma. Stars indicate irregular axonal start. duo:duodenum; jej:jejunum; ile: ileum; TOM:tdTomato

Supplementary Figure 5



Supplementary Figure 5. Validation of Syp-EGFP labeling in SYN1+ puncta in the class Cre mouse lines. a,b,c. Representative pictures showing colocalization of SYN1 and Syp-EGFP in *Nmu-Cre*, *Sst-Cre* and *Vip-Cre* mice injected with AAV-PHP.S-DIO-SYP-EGFP. Scale bars:10μm. White arrowhead: SYN1+ Syp-EGFP+ puncta, yellow arrowhead: SYN1- Syp-EGFP+ puncta. **d,** Graph showing the percentage of colocalization (n=3 mice). Data are presented as mean values±SD. SYN1: Synapsin1.

Supplementary Figure 6



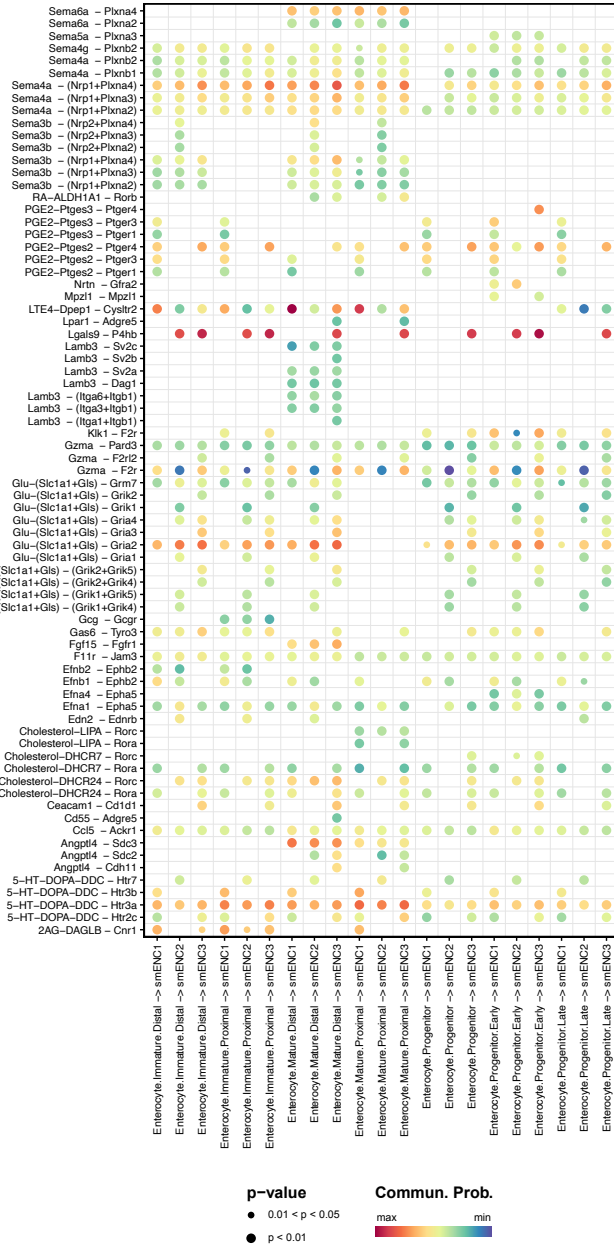
Supplementary Figure 6. Supportive Data to Figure 5.

a-f, Original confocal images of Imaris representations shown in Fig. 5d and e.

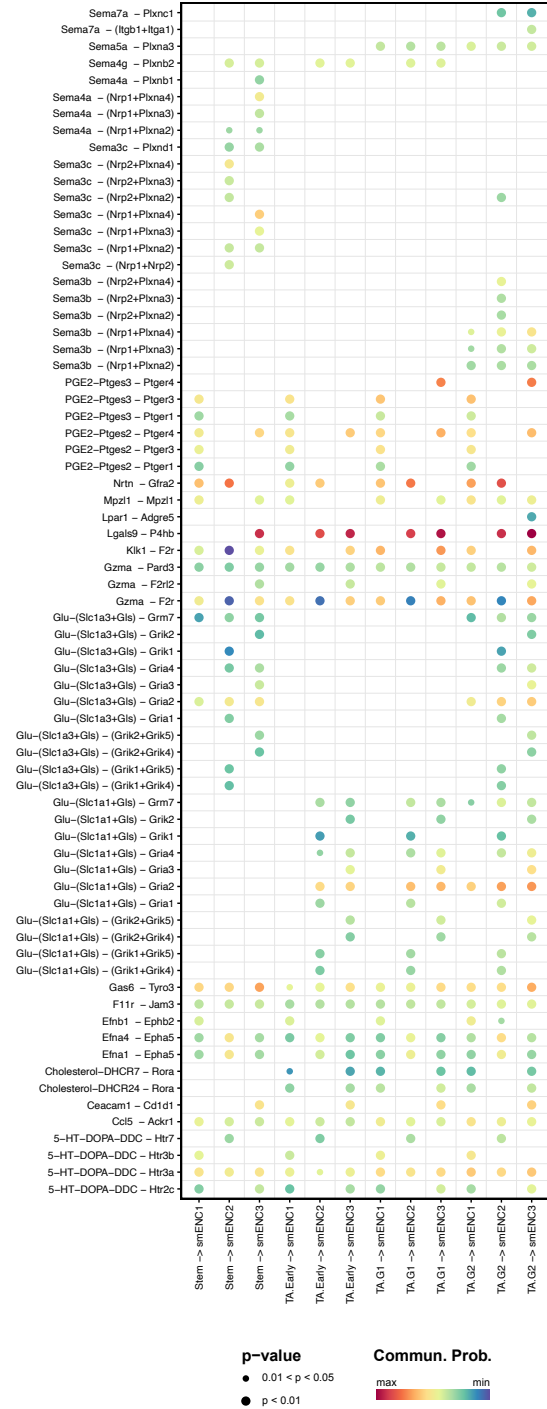
g-l, Examples of enterochromaffin cells with the nerve processes from smENCs at a long distance as Imaris representations (g-i) and original confocal images (j-l). Distances are displayed in μm . Scale bars: $20\mu\text{m}$.

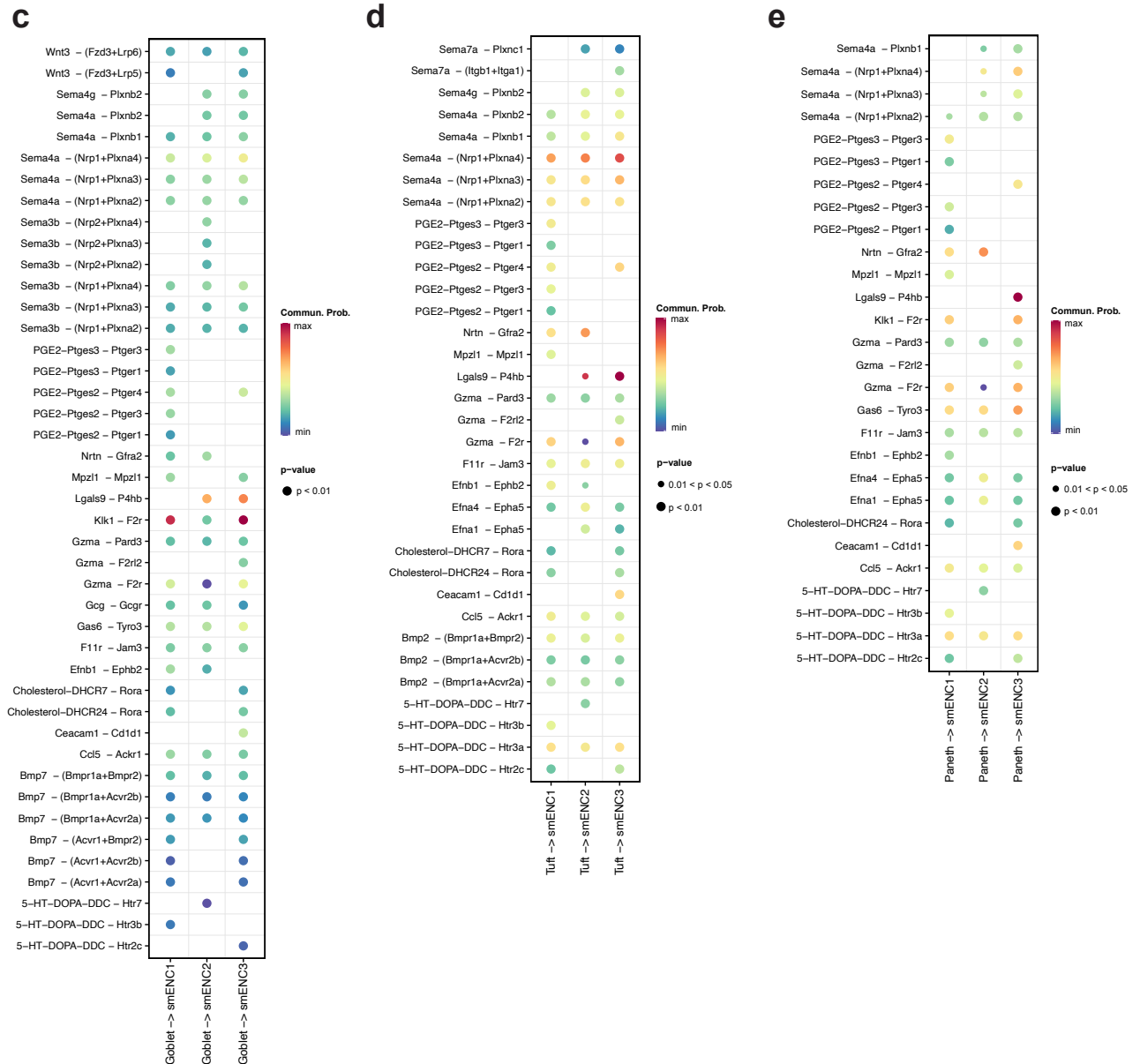
Supplementary Figure 7

a

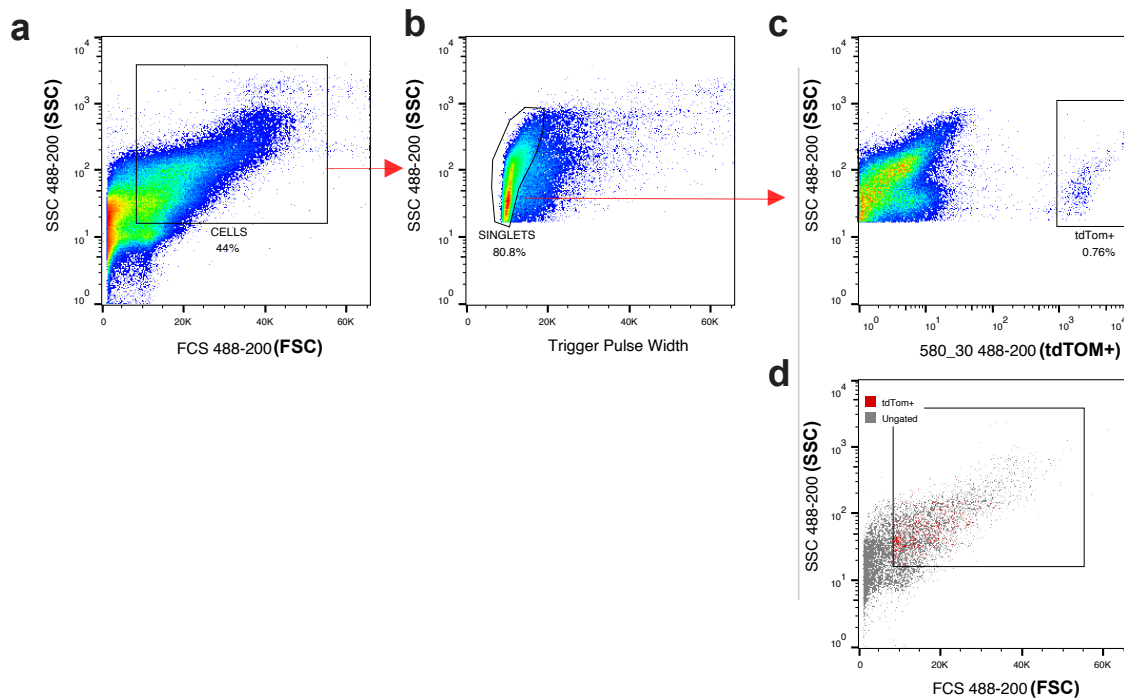


b





Supplementary Figure 8



Supplementary Figure 8: Representative Flow Cytometry Data Plots.

Plots show gating strategy for sorting tdTom⁺ cells from *Baf53b-Cre;R26RtdTom* mice at P24. **a**, Forward vs Side Scatter plot gating on cells **b**, Side Scatter vs Trigger Pulse Width to gate out doublets and multiplets. **c**, Red fluorescence (580/30) to distinguish TOM⁺ cells from autofluorescent cells. **d**, Plot of cells indicating sorted (red, tdTom⁺) versus non-sorted cells (grey, ungated).

SSC: Side-scattered light; FSC: Forward-scattered light