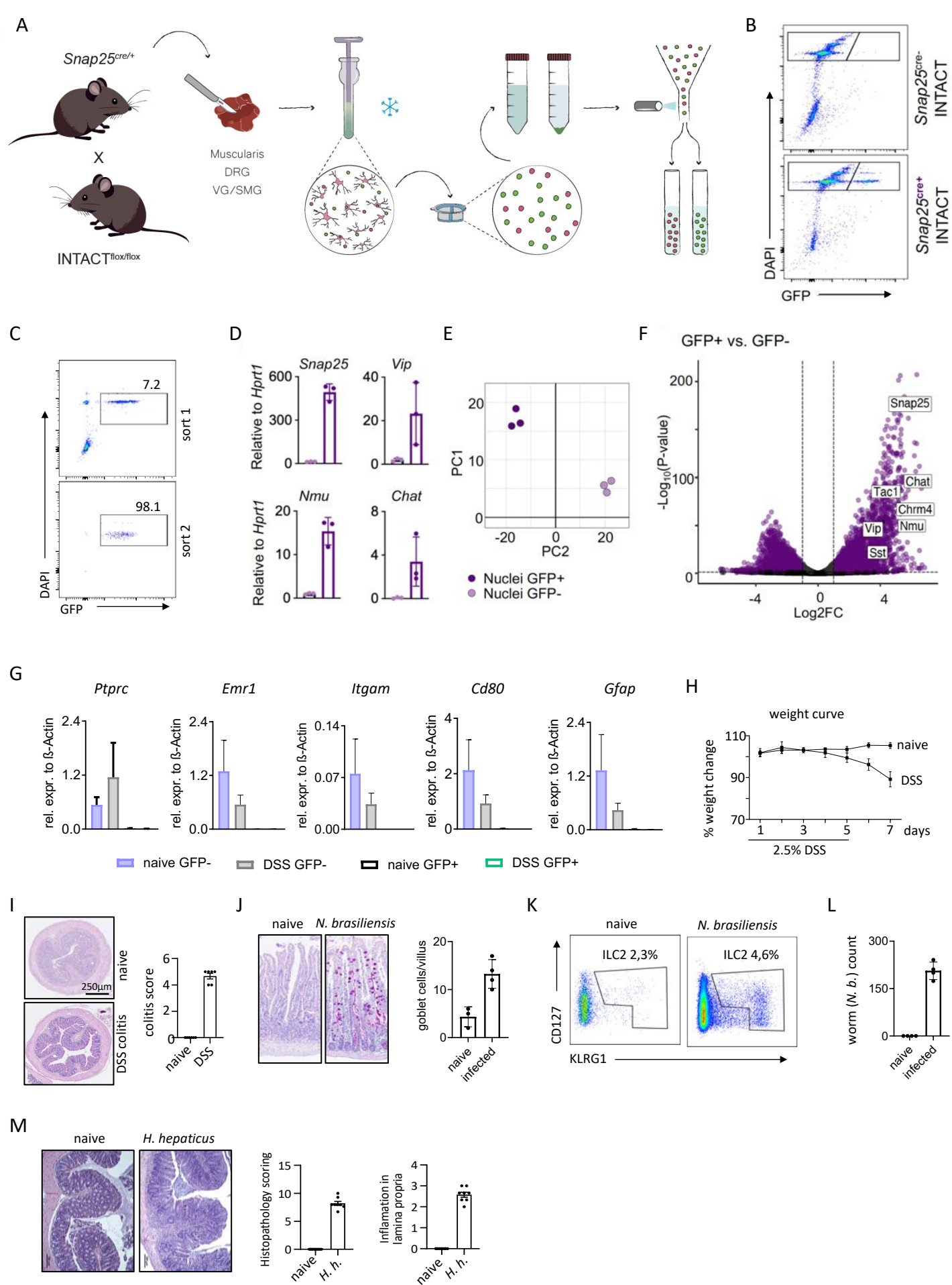


Supplemental information

**A transcriptional atlas of gut-innervating neurons
reveals activation of interferon signaling
and ferroptosis during intestinal inflammation**

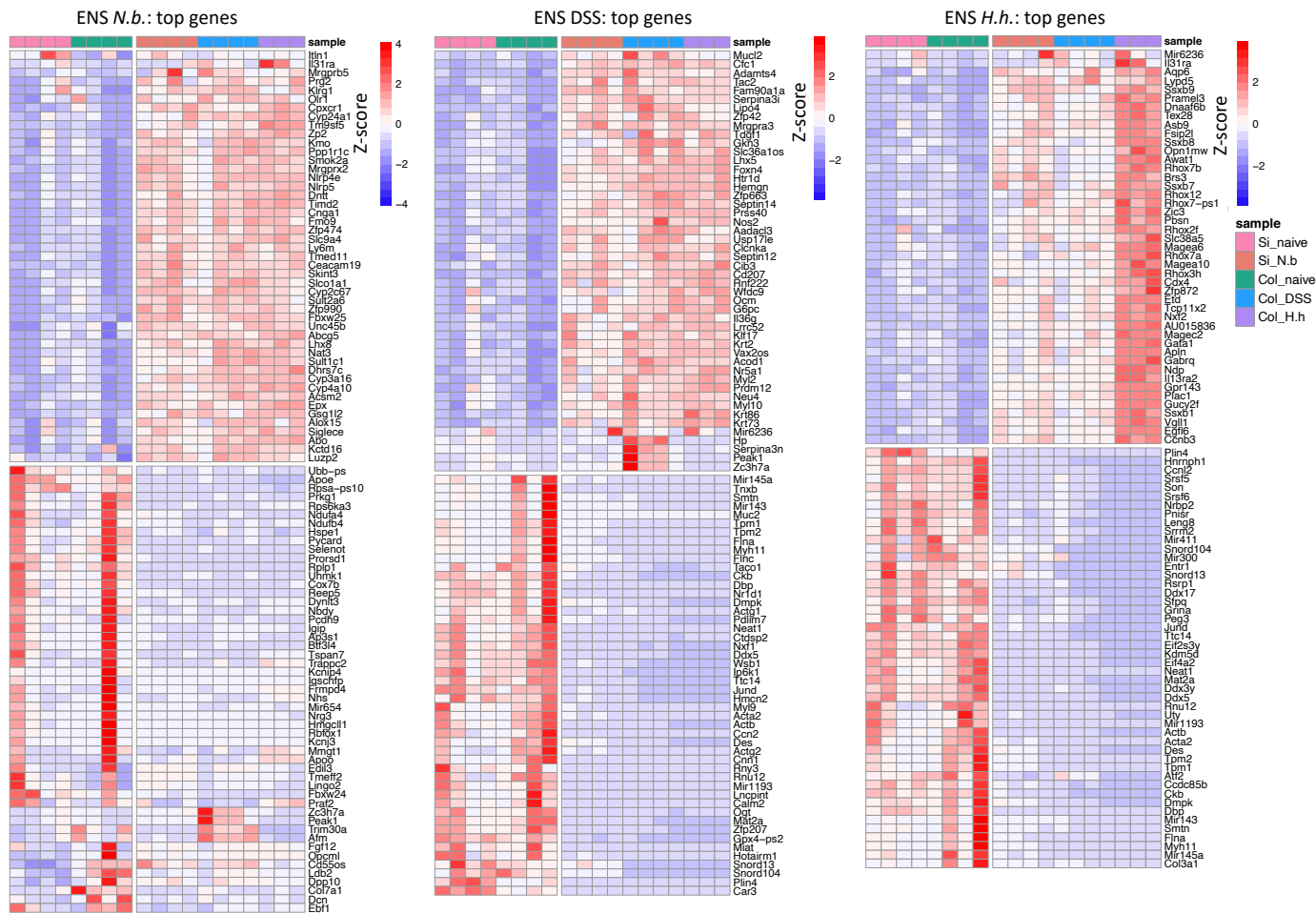
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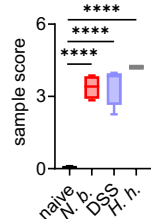
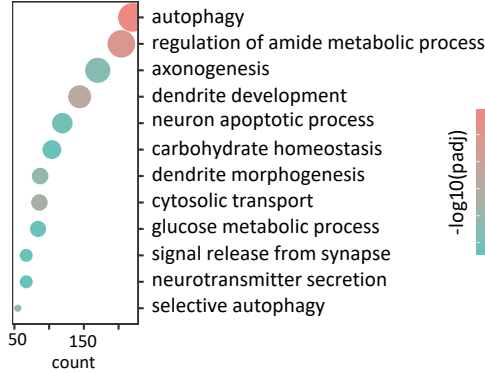
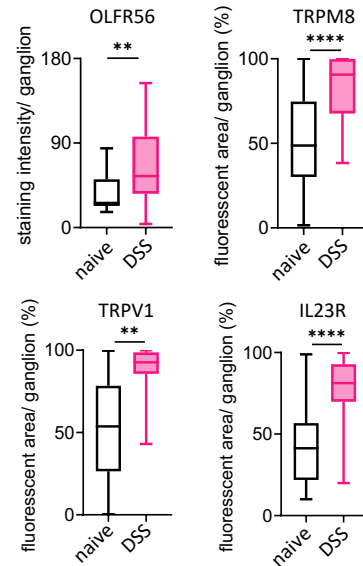
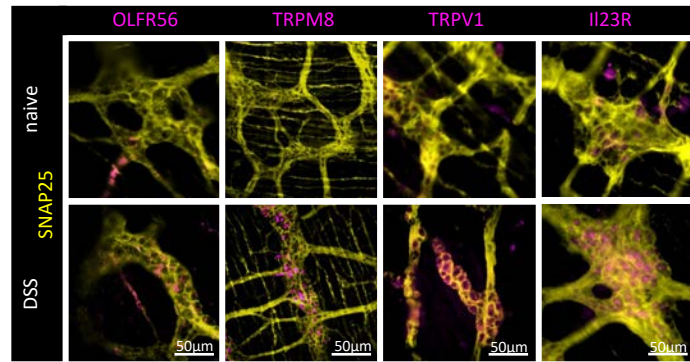
Supp. Figure 1

Supplementary Figure 1, related to main Figure 1: Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+} mice represent a nice tool to sort-purify and sequence neuronal nuclei

A Graphical abstract of the workflow used to isolate nuclei from tissues of interest used for bulk and single-nucleus RNA-seq. **B** Flow cytometric plots showing GFP⁻ and GFP⁺ nuclei from *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice co-stained with DAPI (n=3). **C** Nuclei from *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice were sort-purified using the flow-sorter ARIA fusion based on the DAPI and GFP signal. After sort-purification, purity was checked showing purity of >98%. **D** qPCR of neuronal genes after sort-purification of GFP⁻ and GFP⁺ nuclei from *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice (n=3, data are representative of two independent experiments). **E** PCA of bulk RNA-seq of GFP⁻ and GFP⁺ nuclei isolated from *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice (n=3). **F** Volcano plots of bulk RNA-seq of GFP⁻ and GFP⁺ nuclei from *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice where GFP⁺ nuclei showed neuronal marker genes. **G** GFP⁺ and GFP⁻ nuclei from the enteric nervous system in steady state and following DSS-induced colitis were sort-purified and qPCR for indicated immune cell and glial cell genes was performed (n=5). **H,I**, DSS-induced colitis (2.5% DSS) was applied in the drinking water for 5 days. Weight measured daily and compared to the starting weight. Representative images and quantification of the colitis score by Hematoxylin and Eosin staining of the colon (n=6). **J-L** *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice were infected with 500 larvae of *N. b.* s.c. and harvested at day 7 post-infection (n=4). **J** Representative images and quantification of periodic acid–Schiff stain of the small intestine. **K** Flow cytometric plots of mesenteric lymph node innate lymphoid cells type 2 (Pre-gated on live CD45⁺ Lin⁻ cells). **L** Worm count was quantified in the feces of the mice. **M**, *H. h.* was delivered twice by oral gavage and Il-10R-blocking antibody was administered weekly. Tissues were harvested 24 days after disease induction. Histology shows Hematoxylin and Eosin stains and inflammation was quantified at different sections of the colon (n=8). DSS – Dextran Sulfate Sodium-induced colitis, *N. brasiliensis* - *Nippostrongylus brasiliensis*, *H. hepaticus* – *Helicobacter hepaticus*.

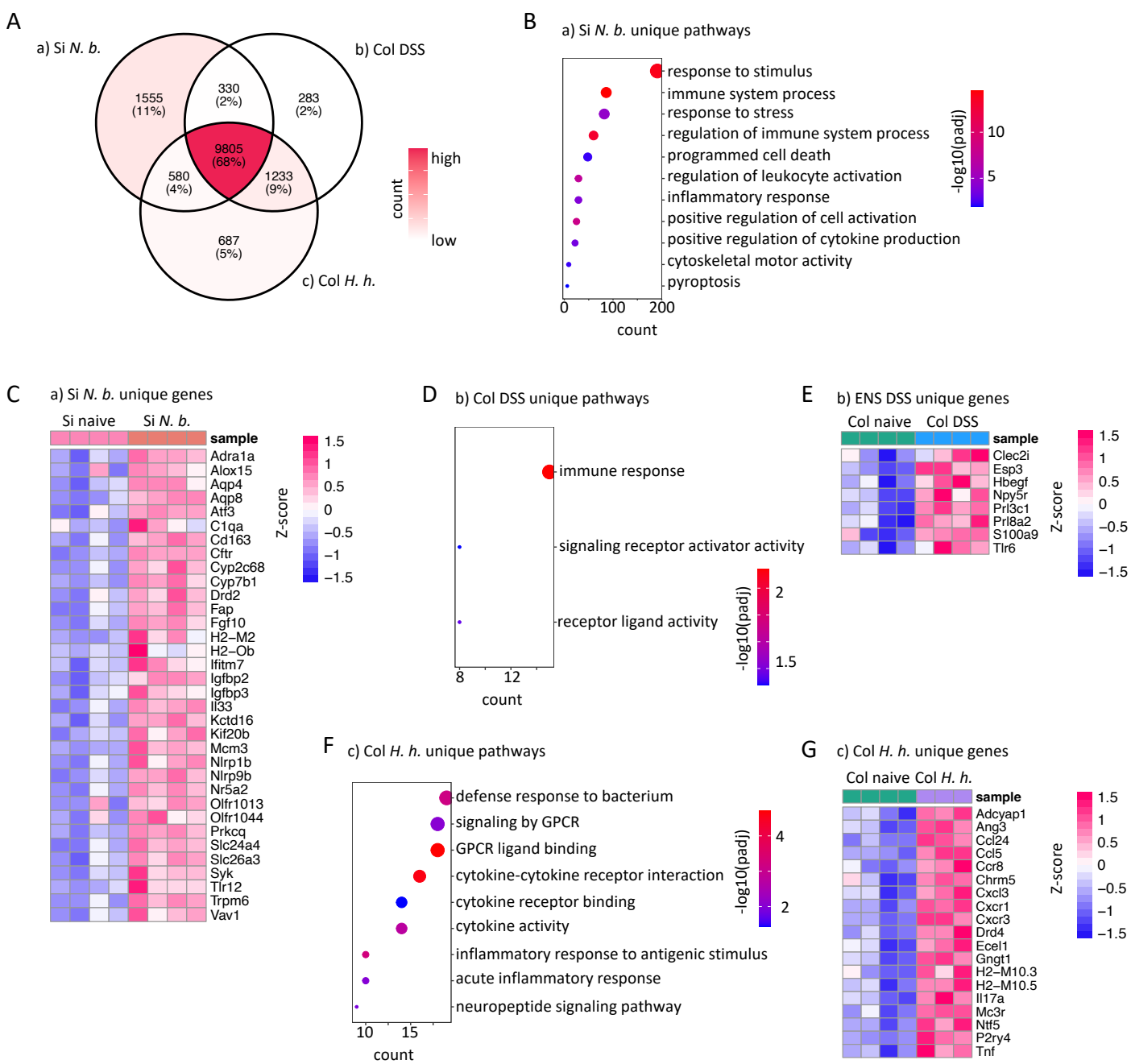
A**B**

Perturbance of ENS

**C****D**

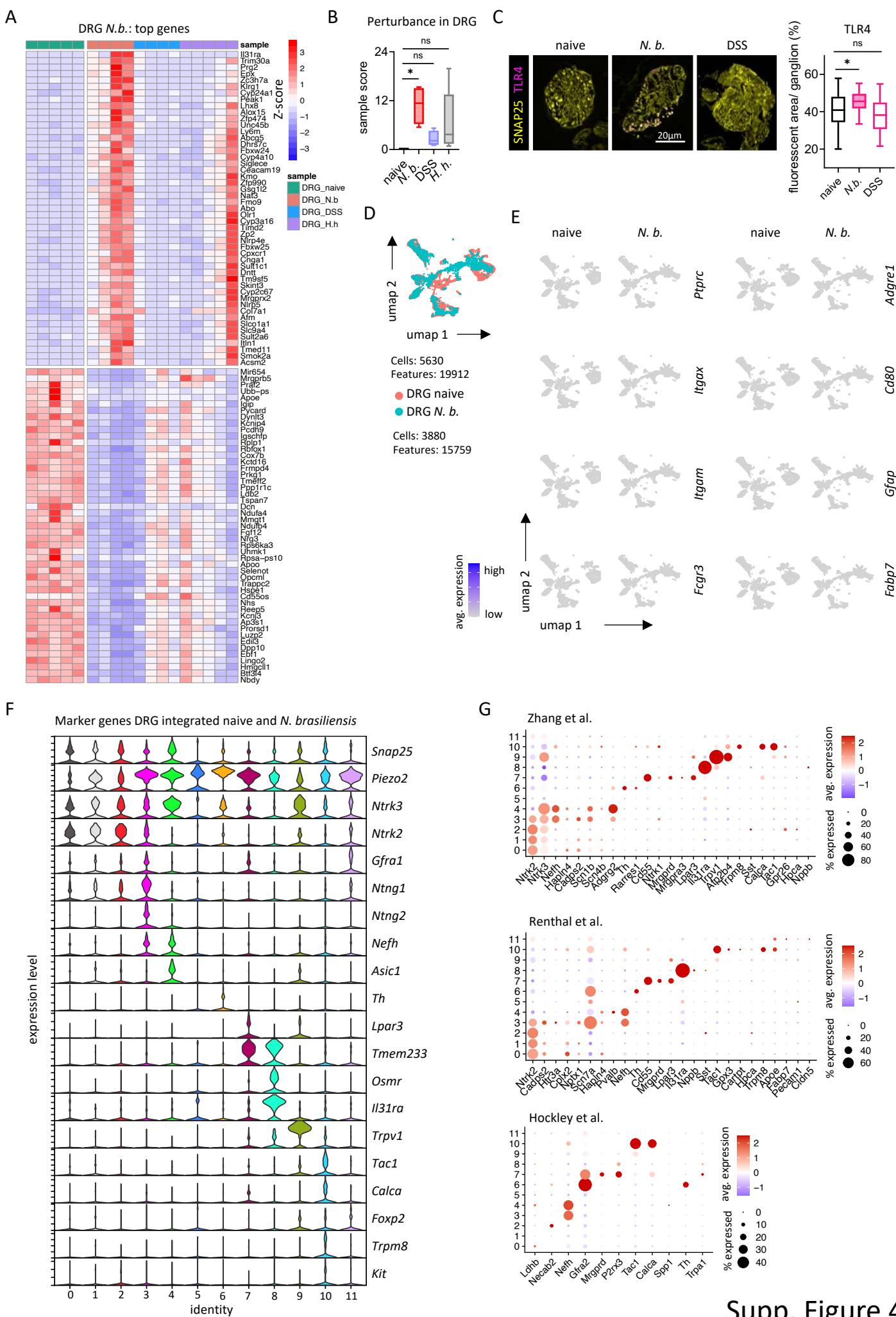
Supplementary Figure 2, related to main Figure 2: Myenteric neurons show a similar altered gene profile across different inflammation models

A Heatmaps of top up- and downregulated genes in the ENS of infected *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice compared to naive mice. Mice were treated with *N. b.* (left), DSS (middle) *H. h.* (right). Each heatmap column represents data from one mouse. **B** Molecular degree of perturbation score for the bulk RNA-seq in the ENS comparing the three different diseases models. **C** Gene Ontology (GO) analysis of bulk-RNA-seq of downregulated genes during inflammation in all disease models in the ENS. **D** Immunofluorescence stainings and quantification of murine whole mount colonic muscularis showing indicated markers (magenta) and SNAP25 (yellow). Quantification was performed measuring the % area of the protein of interest (naive n=5, DSS n=5, data are representative of two independent experiments). ENS – enteric nervous system, DSS – Dextran Sulfate Sodium-induced colitis, *N. b.* - *Nippostrongylus brasiliensis*, *H. h.* – *Helicobacter hepaticus*. Mean +/- SD, one-way ANOVA (B) and Student's t-test (D), ** p<0.01, **** p<0.0001.



Supplementary Figure 3 related to main Figure 2: Unique signaling pathways in the ENS in response to inflammatory diseases.

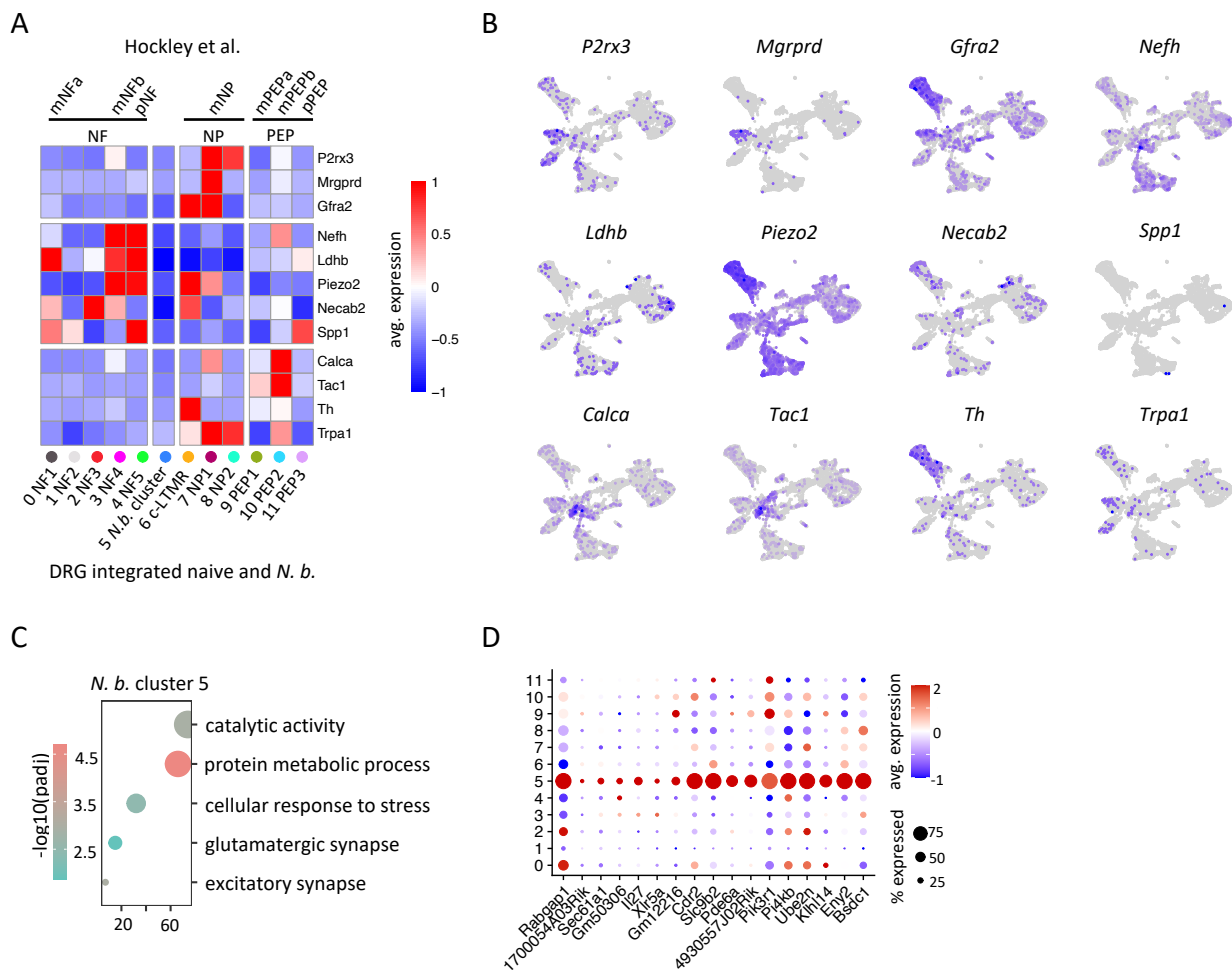
A, Venn Diagram of dysregulated genes in sort-purified nuclei of *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice following *N. b.*-infection, DSS-induced colitis or *H. h.* infection compared to naive mice. **B,D,F** Exclusive pathways regulated during **B**, *N. b.*, **D**, DSS-induced colitis and **F**, *H. h.* **C,E,G**, Heatmaps of important unique dysregulated genes during the indicated diseases from B,D,F. Each heatmap column represents data from one mouse. ENS – enteric nervous system, DSS – Dextran Sulfate Sodium-induced colitis, *N. b.* - *Nippostrongylus brasiliensis*, *H. h.* – *Helicobacter hepaticus*.



Supp. Figure 4

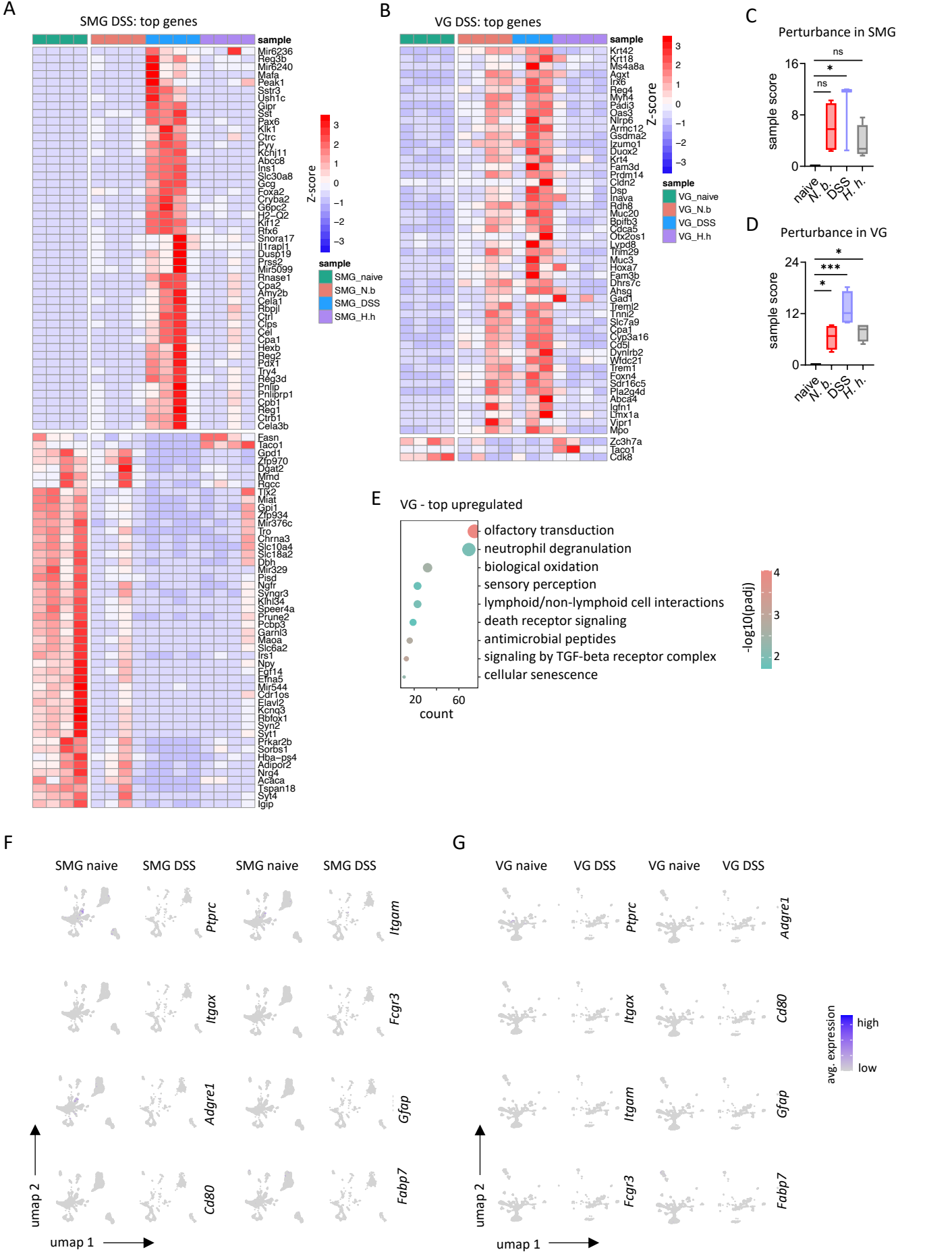
Supplementary Figure 4 related to main Figure 3: Dorsal root ganglia primarily react during *Nippostrongylus brasiliensis* infection

A Heatmaps of the top up- and downregulated genes in DRGs of infected compared to naive *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice. Mice were treated with *N. b.* (left), DSS (middle) and *H. h.* (right). Each heatmap column represents data from one mouse. **B** Molecular degree of perturbation score for the bulk RNA-seq in DRG comparing the three different disease models. **C** Immunofluorescence stainings of murine DRG sections showing TLR4 (magenta) and SNAP25 (yellow). Quantification was measuring the % area of the protein of interest (naive n= 5, *N. b.* n=4, DSS n=5, data are representative of two independent experiments). **D** Color-coded UMAP of single-nucleus RNA-seq from DRGs of naive and *N. b.*-infected *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice. **E** UMAPs showing immune cell and glial cell markers, conditions as in D. **F** Violin plots showing normalized count distributions of selected genes in single-nucleus RNA-seq of DRGs from *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}*. **G** Dotplots showing normalized count distributions of DRG marker genes in nuclei of DRGs from *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice and compared to the papers of Zhang ³⁸, Renthal ²⁶, Hockley ³⁹. DRG – dorsal root ganglia, DSS – Dextran Sulfate Sodium-induced colitis, *N. b.* - *Nippostrongylus brasiliensis*, *H. h.* – *Helicobacter hepaticus*. Mean +/- SD, one-way ANOVA, * p<0.05.



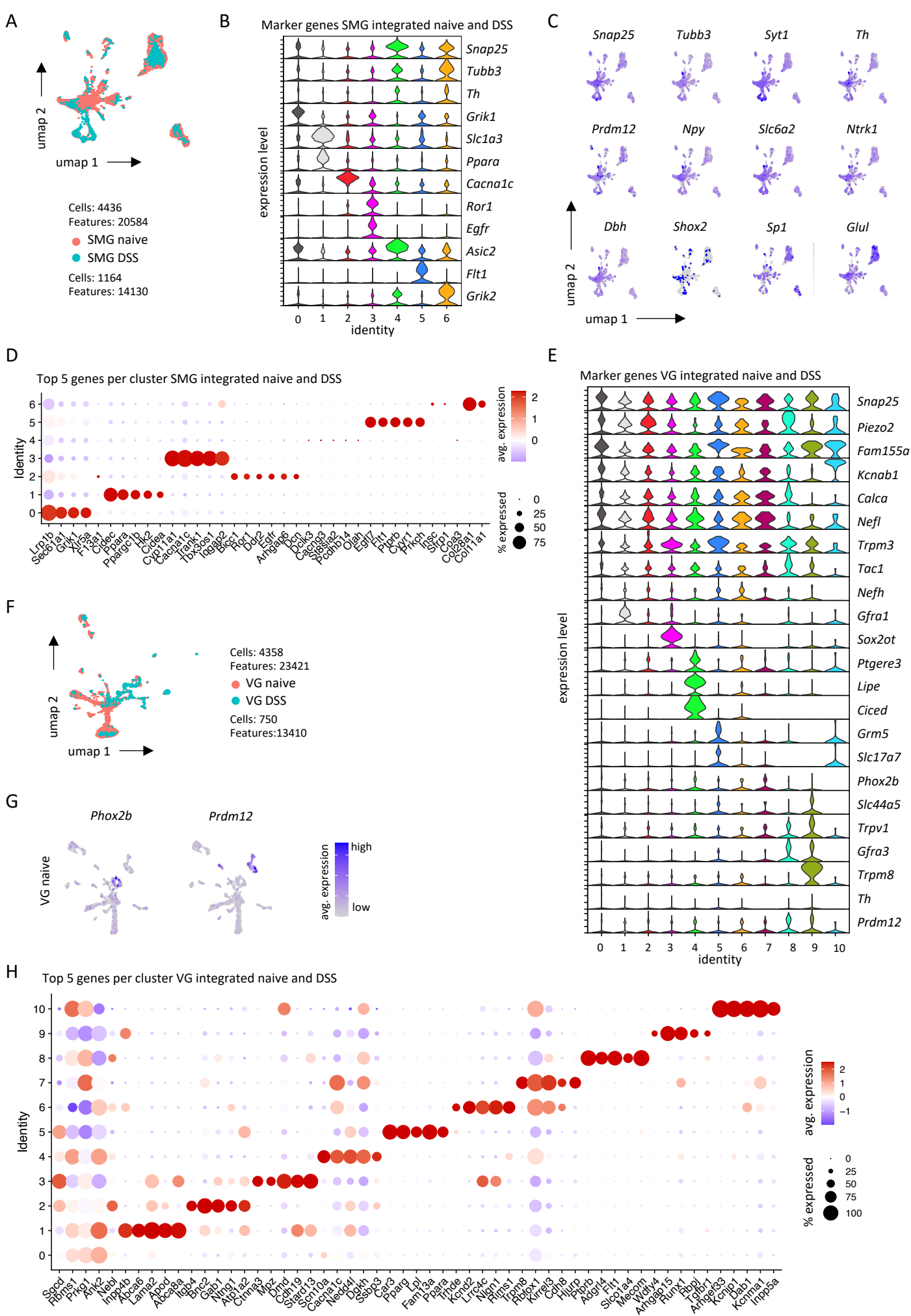
Supplementary Figure 5 related to main Figure 3: Our single-nucleus sequencing data from DRGs contain neurons known to innervate the colon

A Heatmap showing normalized counts of DRG marker genes in nuclei of naive DRGs from *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice and compared to Hockley et al.³⁹ **B**, UMAPs of marker genes in DRGs related to Hockley et al.³⁹ **C,D** The transcriptional profile of the *de novo* cluster 5 was compared to all other clusters. **C**, Selected pathways of altered genes in Cluster 5. **D**, Identification of fingerprint genes of the *N. b.* cluster. DRG – dorsal root ganglia, *N. b.* – *Nippostrongylus brasiliensis*.



Supplementary Figure 6 related to main Figure 4: Sequencing of the superior mesenteric and vagal ganglia are not contaminated by non-neuronal cells

A,B Heatmaps of top up- and downregulated genes in the SMG (A) and VG (B) of *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice upon indicated infection compared to naive mice. Mice were treated with *N. b.* (left), DSS (middle) and *H. h.* (right). For A,B, each heatmap column represents data from one mouse. **C,D** Molecular degree of perturbation score for the bulk RNA-seq data in the SMG (C) and VG (D) comparing the three different disease models. **E** Gene Ontology (GO) analysis of upregulated genes following DSS in the VG. **F,G** UMAPs of single-nucleus RNA-seq from the SMG (F) and VG (G) of naive and after DSS induced-colitis in *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice. UMAPs show classical immune cell and glial cell markers. SMG – superior mesenteric ganglion, VG – vagal ganglia. DSS – Dextran Sulfate Sodium-induced colitis, *N. b.* - *Nippostrongylus brasiliensis*, *H. h.* – *Helicobacter hepaticus*. Mean +/- SD, one-way ANOVA, * p<0.05, *** p<0.001.



Supplementary Figure 7, related to main Figure 4: The superior mesenteric ganglion has a sympathetic whereas the vagal ganglia have a sensory neuronal profile

A Color-coded UMAP of single-nucleus RNA-seq from the SMG showing naive nuclei in red and after DSS-induced colitis in green of *Snap25^{Cre/+} Rosa26^{Sun1-Gfp/+}* mice. **B** Violin plots showing normalized count distributions of marker genes in single-nucleus RNA-seq of the SMG. **C** UMAPs of marker genes showing normalized count distributions in single-nucleus RNA-seq of SMG. **D** Dotplot of top five marker genes per cluster in the SMG shown in A. **E** Violin plots showing normalized count distributions of marker genes in single-nucleus RNA-seq of the VG. **F** Color-coded UMAP of single-nucleus RNA-seq from the VG showing naive nuclei in red and after DSS-induced colitis in green. **G** UMAPs of *Phox2b* and *Prdm12* expression in single-nucleus RNA-seq of the VG. **H** Dotplot of top five marker genes per cluster shown in F. SMG – superior mesenteric ganglion, VG – vagal ganglia, DSS – Dextran Sulfate Sodium-induced colitis.

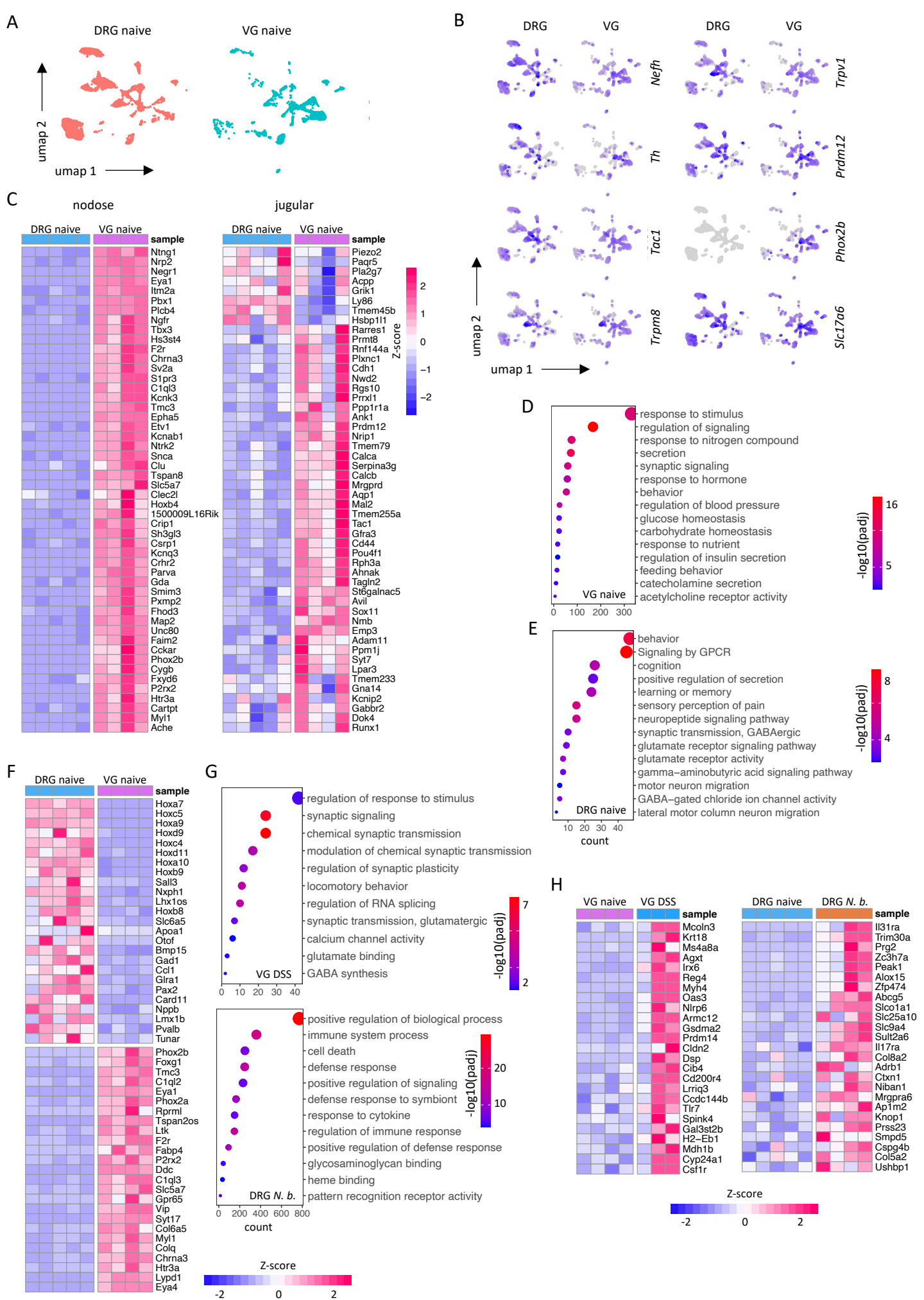
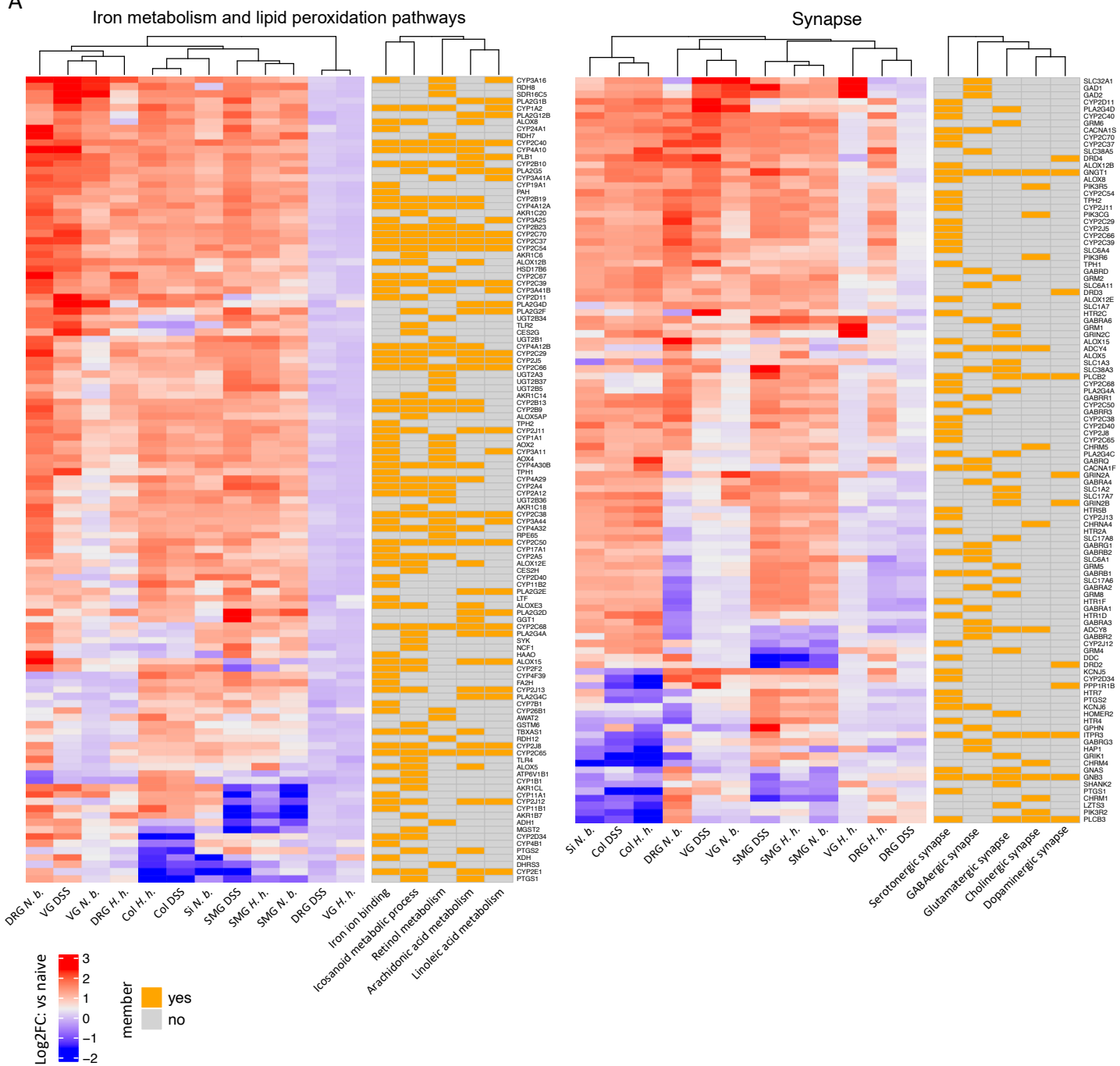


Figure 8, related to main Figure 4: The vagal ganglia are functionally distinct compared to thoracolumbar DRGs

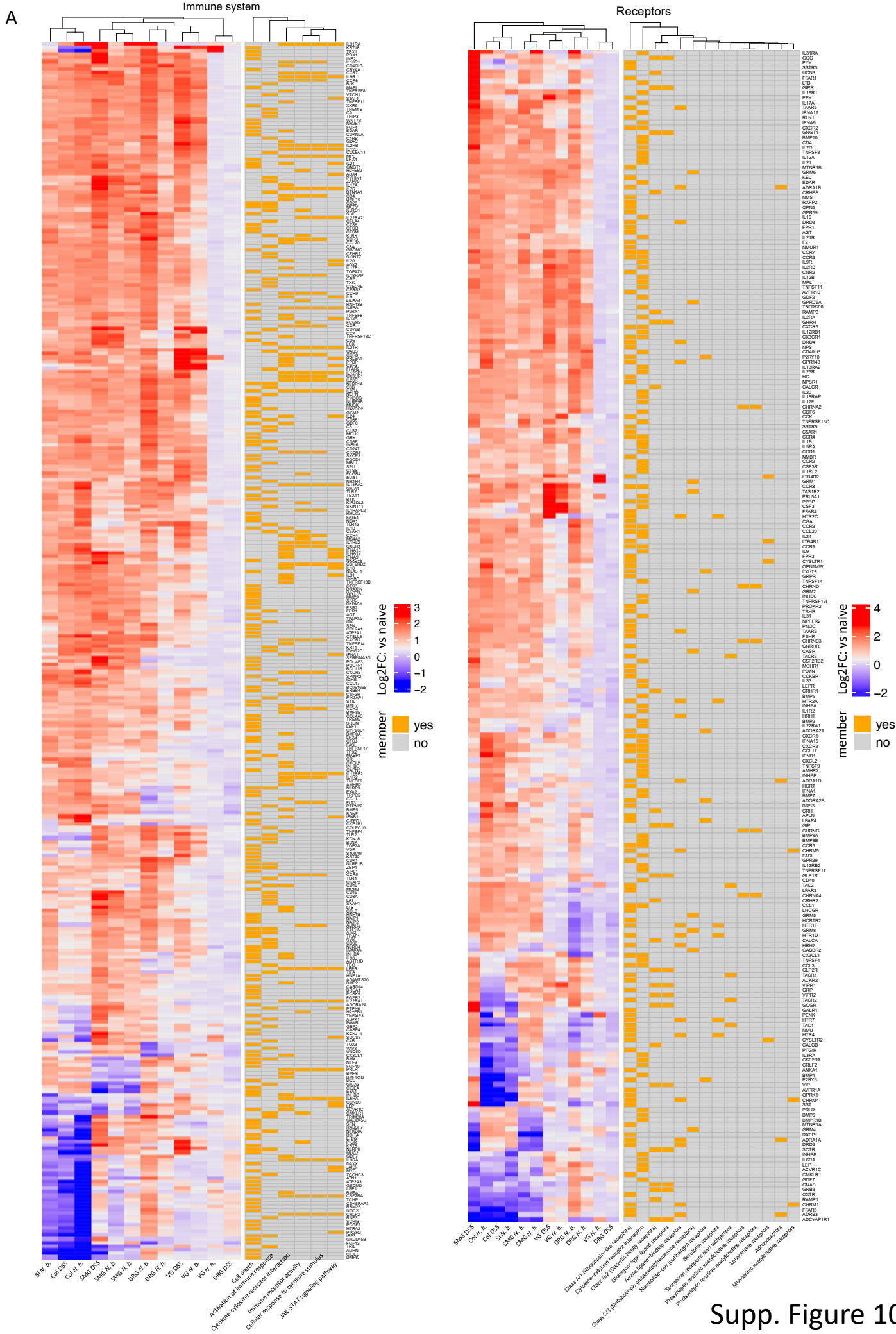
A Color-coded UMAP of integrated single-nucleus RNA-seq from *Snap25*^{Cre/+} *Rosa26*^{Sun1-Gfp/+} mice, showing naive DRG nuclei in red and naive VG in green (split). **B** UMAPs of single-nucleus RNA-seq from A showing typical DRG and VG markers. **C** Heatmaps of nodose and jugular genes from Kupari et al. ²⁹ in the DRG and VG of naive mice. **D,E** Gene Ontology (GO) analysis of bulk RNA-seq upregulated genes comparing naive DRG (D) and naive VG (E). **F** Heatmap of top regulated genes comparing DRG and VG in naive mice. **G** GO analysis of uniquely upregulated genes in DRG compared to the VG during *N. b.* infection (top) or in VG compared to DRG during DSS-induced colitis (bottom). **H** Heatmaps of top uniquely upregulated genes in DRGs comparing to VG during *N. b.* (left) or VG compared to DRG during DSS colitis (left). For C,F,H, each heatmap column represents data from one mouse. DRG – dorsal root ganglia, VG – vagal ganglia, DSS – Dextran Sulfate Sodium-induced colitis, *N. b.* - *Nippostrongylus brasiliensis*.

A



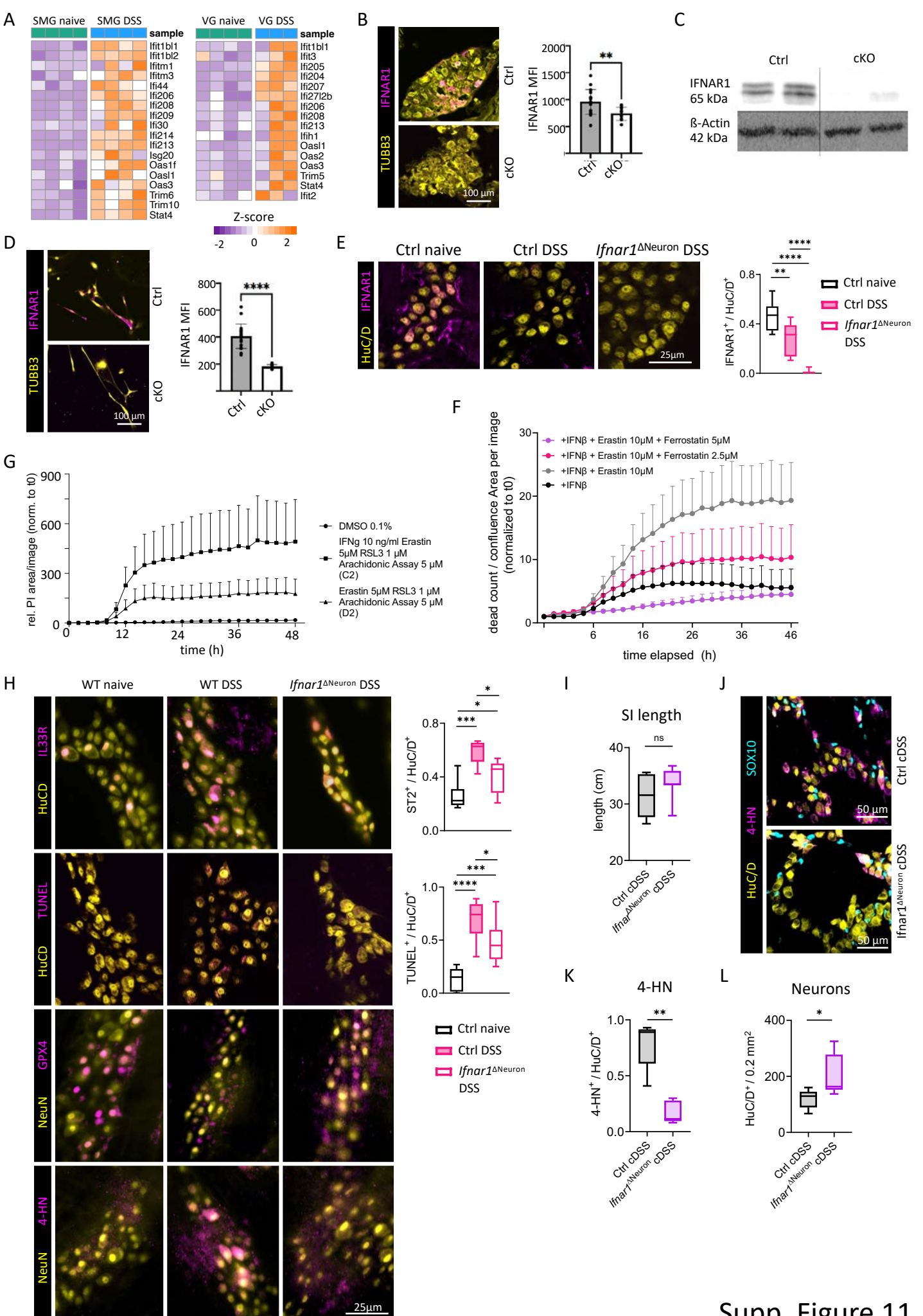
Supplementary Figure 9, related to main Figure 5: Regulation of the iron metabolism, lipid peroxidation and synapse pathways across all inflammation models and gut-innervating neurons

A Heatmaps of the up- and downregulated genes and pathways over all samples shown in Fig 5B. Each heatmap column represents data from 3-5 mice (Si *N. b.* n=4, Col DSS n=4, Col *H. h.* n=4, DRG *N. b.* n=4, DRG DSS n=4, DRG *H. h.* n=5, SMG *N. b.* n=4, SMG DSS n=4, SMG *H. h.* n=4, VG *N. b.* n=4, VG DSS n=3, VG *H. h.* n=4). Si – small intestine, Col – colon, DRG – dorsal root ganglia, SMG – superior mesenteric ganglion. VG – vagal ganglion, DSS – Dextran Sulfate Sodium-induced colitis, *N. b.* - *Nippostrongylus brasiliensis*, *H. h.* – *Helicobacter hepaticus*.



Supplementary Figure 10, related to main Figure 5: Regulation of the immune responses and receptors pathways across all inflammation models and gut-innervating neurons

A Heatmaps of the up- and downregulated genes and pathways over all samples shown in Fig 5B. Each heatmap column represents data from 3-5 mice (Si *N. b.* n=4, Col DSS n=4, Col *H. h.* n=4, DRG *N. b.* n=4, DRG DSS n=4, DRG *H. h.* n=5, SMG *N. b.* n=4, SMG DSS n=4, SMG *H. h.* n=4, VG *N. b.* n=4, VG DSS n=3, VG *H. h.* n=4). Si – small intestine, Col – colon, DRG – dorsal root ganglia, SMG – superior mesenteric ganglion. VG – vagal ganglion, DSS – Dextran Sulfate Sodium-induced colitis, *N. b.* - *Nippostrongylus brasiliensis*, *H. h.* – *Helicobacter hepaticus*.



Supplementary Figure 11, related to main Figure 7: *Ifnar1* on neurons controls ferroptosis

A Heatmaps of upregulated interferon pathway-related genes in the SMG and VG. Each heatmap column represents data from one mouse. **B-D** Validation of the cKO (*Snap25^{Cre/+} Ifnar1^{fl/fl}*) mice **B** Immunofluorescence stainings in murine DRGs from Ctrl (*Snap25^{Cre/+} Ifnar1^{+/+}*) and cKO mice showing IFNAR1 (magenta) and Tuj (TUBB3, yellow). Quantification of A using the MFI (n=3). **C** Western blot of DRGs showing the protein production of IFNAR1 in Ctrl and cKO mice. β -Actin as control (n=2). **D** Immunofluorescence of murine DRG cultures of Ctrl and cKO mice showing IFNAR1 (magenta) and Tuj (TUBB3, yellow). Quantification using MFI (n=3). **E** Validation of the *Ifnar1^{ΔNeuron}* (*Snap25^{Cre/+} Ifnar1^{fl/fl} Rosa26^{Sun1-Gfp/+}*) mouse line in ENS showing IFNAR1 (magenta) and HuC/D (yellow) compared to Ctrl mice (*Snap25^{Cre/+} Ifnar1^{+/+} Rosa26^{Sun1-Gfp/+}*) (Ctrl naive n=4, Ctrl DSS n=5, *Ifnar1^{ΔNeuron}* DSS n=5, data are representative of two independent experiments). **F,G** Myenteric cultures exposed to the indicated compounds and monitored for propidium iodide uptake (n=3). **H**, Representative immunofluorescence stainings of colonic myenteric neurons during steady state and DSS-induced colitis from Ctrl mice and *Ifnar1^{ΔNeuron}* mice showing different markers (magenta) and HuC/D or NeuN (yellow) (Ctrl naive n=4, Ctrl DSS n=5, *Ifnar1^{ΔNeuron}* DSS n=5). **I**, Lengths of the small intestine after chronic DSS-induced colitis (n=8) (belongs to Fig. 7K,L). **J-L** Colon sections of Ctrl mice (*Snap25^{Cre/+} Ifnar1^{+/+} Rosa26^{Sun1-Gfp/+}*) and *Ifnar1^{ΔNeuron}* (*Snap25^{Cre/+} Ifnar1^{fl/fl} Rosa26^{Sun1-Gfp/+}*) after chronic DSS-induced colitis showing 4-HN (magenta), HuC/D (yellow) and SOX10 (glia cells, cyan). **K** 4-HN⁺ was quantified as staining signal per HuC/D⁺ nuclei and **L** HuC/D⁺ nuclei per 0.2 mm² counting the entire slide (n=8). SMG – superior mesenteric ganglion, VG – vagal ganglion, DSS – Dextran Sulfate Sodium-induced colitis. Mean \pm SD, Student's t-test (B,D,I,K,L), one-way ANOVA (E,H) and two-way ANOVA (F) * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.