

## Supplemental Experimental Procedures

### Whole Mount Immunofluorescence

Mice were sacrificed by cervical dislocation and the small intestine or SMG-CG was removed and placed in HBSS  $Mg^{2+}Ca^{2+}$  (Gibco) + 5% FCS. The intestine was cut open longitudinally and the luminal contents washed away in complete media with 1mM DTT (Sigma-Aldrich). The *muscularis externa* was carefully removed from the underlying mucosa. The *muscularis*, mucosal tissue, and SMG-CG were pinned down in a plate coated with Sylgard and then fixed for 15min with 4% PFA. Whole mount samples were then permeabilized in 0.1% Triton X-100 for 1-2 hours. After washing in 1X DPBS, the samples were blocked for 1 h in 5% BSA/10% Normal Goat Serum or 10% BSA/5% Normal Goat Serum/0.1% Triton X-100. Antibodies were added to the blocking buffer at appropriate concentrations and incubated overnight at 4°C. The following day the tissue was washed 3X in 1X DPBS and then incubated in blocking buffer with secondary antibody at 1:200. Samples were again washed 3X in 1X DPBS and mounted on slides with Fluormount-G with or without DAPI, cover slipped, and sealed. Images were taken on a Leica inverted TCS SP8 laser scanning confocal microscope and adjusted post-hoc in Image-J. The following unconjugated (unless otherwise stated) primary antibodies were used to stain the intestine and sympathetic ganglia: Abcam (anti- $\beta$ III Tubulin, mouse 2G10 and rabbit polyclonal; anti-SNAP25, SMI 81), Millipore (anti-TH rabbit polyclonal; anti-MHCII rat M5/114), Thermo Fisher Scientific (anti-HuC/D mouse 16A11; anti-GFP rabbit A-21311), Immunostar (anti-DBH rabbit polyclonal), Aves Labs (anti-GFP chicken polyclonal).

### Intravital Two-Photon Imaging

Mice were anesthetized with a ketamine/xylazine/acepromazine cocktail at 10  $\mu$ l/g of body weight (Farache et al., 2013). 10 min following induction of anesthesia, mice were placed on a custom platform heated to 37°C. Isoflurane was given through a nose cone at a dose of 1.5% with 1% oxygen. Upon loss of recoil to paw compression, a small incision was made in the abdomen. The *ileum* entrance to the *caecum* was located and a loop of *ileum* was exposed and placed onto a raised block of thermal paste covered with a wetted wipe. A coverslip was placed on top of the loop to immobilize the intestine. The platform was then transferred to the FV1000MPE Twin upright multiphoton system (Olympus) heated stage. A 25x heated objective was used and images were taken at 4-8 $\mu$ s/pixel.

### Diphtheria Toxin (DT) Administration.

The first dose of DT (Sigma, D0564) was delivered intraperitoneally (i.p.) as 500 ng in 200  $\mu$ l PBS/ 20 g body weight followed by two doses of 100 ng in 200  $\mu$ l PBS/ 20 g body weight, 48h and 12h before analysis. Depletion efficiency was monitored in each animal by the serum Flt3 ligand surge upon cDC depletion (zDC<sup>DTR</sup>) and the loss of blood monocytes 24h post DT administration in *Iy2<sup>csfr1-DTR</sup>* mice (Schreiber et al., 2013).

### Single Cell Suspension of Intestinal Macrophages

After euthanasia the small intestine was carefully removed, cleaned, cut open longitudinally and washed 2X in HBSS  $Mg^{2+}Ca^{2+}$  (Gibco) and 1X in HBSS  $Mg^{2+}Ca^{2+}$  with 1mM DTT (Sigma-Aldrich). The tissue was cut in two and the *muscularis* region was carefully dissected from the underlying mucosa. Each region was then finely cut and digested in HBSS  $Mg^{2+}Ca^{2+}$  + 5% FBS + 1x NaPyr + 25mM HEPES + 50  $\mu$ g/ml DNaseI (Roche) + 400U/ml Collagenase D (Roche) + 2.5U/ml Dispase (Corning) at 37°C. The *muscularis* was digested for 40 min and mucosa for 20 min. The tissue was then homogenized with an 18-gauge needle and filtered through a 70  $\mu$ m cell strainer and washed with HBSS  $Mg^{2+}Ca^{2+}$ . The cells were incubated with Fc block and antibodies against the indicated cell surface markers in FACS buffer (PBS, 1% BSA, 10 mM EDTA, 0.02% sodium azide).

### **Salmonella Typhimurium Infections**

Mice were intragastrically exposed to  $10^9$  of either mutant strain of *Salmonella* Typhimurium, *Spib* or *invA*. Briefly, a single colony of *Salmonella* was grown in 7ml of LB for 5h at 37°C with agitation, and then the bacteria were sub-cultured (1:1000) into 50ml of LB for 18h at 37°C with no agitation. The bacteria were centrifuged and resuspended in 1.5ml of DPBS. Bacteria were inoculated by gavage into recipient mice in a total volume of 100 $\mu$ l.

### **Total RNA Sequencing Analysis**

RNA libraries from biological replicates were prepared using the SMARTer Ultra Low Input RNA for Illumina Sequencing kit (Clontech Laboratories) and sequenced using 50 base pair paired end reading on a HiSeq 2500 instrument (Illumina). The reads were aligned using the STAR version 2.3.0 software that permits unique alignments to Mouse Ensembl genes. Differential expression was determined by use of the Cufflinks software with default settings. Gene Ontology analysis was performed using DAVID (Database for Annotation, Visualization and Integrated Discovery).

### **Antibodies and Flow Cytometry Analysis**

Fluorescent-dye-conjugated antibodies were purchased from BD-Pharmingen (USA) (anti-CD45.2, 104; anti-CD45R, RA3-6B2); eBioscience (USA) (anti-CD103, 2E7; anti-MHCII, M5; anti-F4/80, BM8; anti-CD11b, M1/70; anti-CD11c, N418; anti-SiglecF, E50-2440; anti-CD3e, 145-2C11; anti-LY6G, RB6-8C5) or BioLegend (USA) (anti-CD64 X54-5/7.1). Rhodamine 123 (Invitrogen) was used to measure oxidative burst. Live/Dead staining was done using Aqua fixable dead cell stain (Invitrogen) and 7-AAD (eBioscience). Flow cytometry data were acquired on a LSR-II flow cytometer (Becton Dickinson) and analyzed using FlowJo software package (Tree Star). Macrophages were sorted as Aqua<sup>-</sup>CD45<sup>+</sup>Lin<sup>-</sup>(CD3<sup>-</sup>B220<sup>-</sup>SiglecF<sup>-</sup>LY6G<sup>-</sup>) MHCII<sup>+</sup>F4/80<sup>+</sup>CD11b<sup>+</sup>CD11c<sup>+</sup>CD103<sup>-</sup>) using a FACS Aria cell sorter flow cytometer (Becton Dickinson). 7AAD was used to further confirm cell viability (Figure S1).

### **RiboTag**

Heterozygous or homozygous *Rpl22*<sup>tm1.1P<sub>sam</sub>/J</sup> (RiboTag) mice were used for polysome analysis, and we found no differences in *pulldown* efficiency between either genotype. Isolation of HA-tagged polysomes was performed as previously described (Sanz et al., 2009) with the following modifications pertaining to the intestinal tissue. A 2 cm piece was isolated from the duodenum, jejunum, and ileum. The *muscularis* was separated from the mucosa and placed into 2.5 mL supplemented homogenization buffer (HBS) containing 50 $\mu$ L protease inhibitor (Sigma P8340) and SUPERase<sup>•</sup> In RNase inhibitor (Ambion AM2696), as well as 3 mg/mL heparin as described in the most recent RiboTag protocol (January 7, 2014). The samples were then homogenized using a motorized bounce pestle. The homogenized and cleared samples were incubated with monoclonal anti-HA antibody (Covance) for 1 h on rotation at 4°C. Protein G or A/G beads (Thermo Scientific) were added to the solution for 30 minutes on rotation at 4°C. Bound polysome-associated mRNA was then washed 4 times with high salt buffer (Sanz et al., 2009) and subsequently eluted with RLT Plus Lysis Buffer (Qiagen RNeasy Plus Micro Kit: 74034) with betamercaptoethanol. Eluted mRNA was purified using the RNeasy Micro Kit Plus (Qiagen) according to the manufacturer's instructions, quantified with a Nanodrop spectrophotometer, and processed for qPCR analysis.

### **Quantitative PCR**

Total RNA was isolated with TRIzol (Invitrogen), from which cDNA libraries were reverse transcribed using Superscript II (Invitrogen) and random primers following the instructions provided by the manufacturer. Quantitative PCR was performed using SYBR green (Bio-Rad

Laboratories). Data were collected and analyzed on a QuantStudio 3 (Thermo Scientific). *Rpl32* housekeeping gene was used to normalize samples. Primers used included:

*Rpl32*-forward 5'-ACAATGTCAAGGAGCTGGAG-3', *Rpl32*-reverse 5'-TTGGGATTGGTACTCTGATG-3'; (Set1) *Arg1*-forward 5'-CTCCAAGCCAAAGTCCTTAGAG-3', (Set 1) *Arg1*-reverse 5'-AGGAGCTGTCATTAGGGACATC-3'; (Set 2) *Arg1*-forward 5'-GGAGGCCTATCTTACAGAGAAGGTC-3', (Set2) *Arg1*-reverse 5'-CGAAGCAAGCCAAGGTTAAAGCCAC-3'; *Ym1*-forward 5'-AGACTTGCGTGACTATGAAGCATT-3', *Ym1*-reverse 5'-GCAGGTCCAAACTTCCATCCTC-3'; *Fizz1*-forward 5'-CTGGATTGGCAAGAAGTTCC-3', *Fizz1*-reverse 5'-CCAGGATGCCAACTTTGA-3'; *Tnf*-forward 5'-AGGGTCTGGGCCATAGAACT-3', *Tnf*-reverse 5'-CCACCACGCTCTTCTGTCTAC-3'; *Nos2*-forward 5'-GTTCTCAGCCCAACAATACAAGA-3', *Nos2*-reverse 5'-GTGGACGGGTCGATGTCAC-3'; *Il12b*-forward 5'-TGGTTTGCCATCGTTTTGCTG-3', *Il12b*-reverse 5'-ACAGGTGAGGTTCACTGTTTCT-3'; *Adrb2*-forward 5'-TAGCGATCCACTGCAATCAC-3', *Adrb2*-reverse 5'-ATTTTGGCAACTTCT-3'; *Il10*-forward 5'-CCCATTCTCGTCACGATCTC-3', *Il10*-reverse 5'-TCAGACTGGTTTGGGATAGGTTT-3'; *Il1b*-forward 5'-TCGTGCTGTCGGACCCATAT-3', *Il1b*-reverse 5'-GTCGTTGCTTGTTCTCCTTGT-3'; *Il6*-forward 5'-ACCAGAGGAAATTTTCAATAGGC-3', *Il6*-reverse 5'-TGATGCACTTGCAGAAAA-3'; *Th*-forward 5'-CAGGAECTATGCCTCTCGTATC-3', *Th*-reverse 5'-GAGGACTGTCCAGTACATCAAT-3'; *Dbh*-forward 5'-GATCTCATCATGCTCTGGACTG-3', *Dbh*-reverse 5'-CTG GTA GTC TTG CTG GGA ATC-3'.

### **cFos Quantification**

*Fos*<sup>GFP</sup> mice were given *Spib* or PBS by intragastric gavage and SMG-CG along with 6 cm of terminal ileum were dissected after 2 hours. The samples were then stained with anti-GFP-AF488 or anti-ELAVL4/TH followed by streptavidin-AF568 or goat-anti rabbit AF568 secondary antibodies, respectively. We attempted to capture all sympathetic neurons associated with the SMG-CG in separate images and took 10 random images from across each ileal sample. Fluorescent images of the SMG-CG and ileum were analyzed in Image-J. Each image was separated into two channels; GFP-AF488 and ELAVL4/TH-AF568. Using the Cell Counter plugin, the number of neurons and GFP+ nuclei were counted. The number of GFP+ nuclei were divided by the total number of neurons to give a percentage of activated neurons. Data from SMG-CG were represented with each GFP+ neuronal nucleus quantified as an independent data point to properly capture all sympathetic neurons within this anatomical location.

### **Norepinephrine ELISA**

*Spib* or DPBS was given by intragastric gavage and the cecum was removed after 2 hours. One third of the cecum was dissected including the ileum-cecal and colon-cecal junctions. Contents were removed and placed into 600  $\mu$ L of norepinephrine stabilization buffer (10 mM HCl, 1 mM EDTA, 4 mM Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>) and then homogenized. Homogenized cecal samples were spun at 4°C for 5 minutes 2,300 RCF. The supernatant was then collected and 100  $\mu$ L was used to run a NE ELISA according to the manufacturer protocol (Labor Diagnostika Nord: BA E-5200 Noradrenaline Research ELISA).

### **Gastrointestinal Total Transit Time**

Gastrointestinal transit time was determined as previously described (Li et al., 2011). *Spib* was given by intragastric gavage 30 minutes before gavage of the carmine red test meal. Infected and control groups were age and sex matched.

### Neuron Calcium Signaling Quantification

IVM movies from *Hand2<sup>GCaMP3</sup>xCD11c<sup>eYFP</sup>* were analyzed using ImageJ. Circular regions of interest (ROI) were drawn over neuronal cell bodies. A separate ROI of identical size was placed over an area of the image corresponding to background. Gray scale values were determined for both neuronal ROIs and background ROIs at the initial frame as well as each subsequent frame. The following calculation was done to determine the fluorescence at each time point:  $(F_n)_{10, n=1} = (EN_{ROI} - Background_{ROI})_n$

### Enteric-associated neurons - Neurosphere Culture and Differentiation

Pregnant females approximately 18 days post-coitum were euthanized, their uteri were removed, placed on cold HBSS and the embryos subsequently dissected. The intestines were removed by manual dissection and placed in a 1.5 ml tube containing 1 ml of HBSS + 2% FBS. After chopping into small pieces, the tissue was centrifuged for 4 min at 400g and the supernatant was discarded. This process was repeated at least 3 times. The tissue was placed in a 48 well plate with 1ml HBSS + 0,05% trypsin-EDTA (Gibco) + 50µg/ml DNaseI (Roche) and digested for 15 min at 37°C. The cells were then mechanically dissociated, washed and resuspended in DMEM/F12 + 1X N2 (Gibco) + EGF-eFGF (20ng/ml) + 1x Antibiotic/Antimycotic (Gibco) and plated on a non-adherent plate. New media was added on the next day and every 4-5 days. EGF-eFGF (20ng/ml) was added every other day. After neurosphere expansion, cells were dissociated and plated on a 24-well adherent plate previously coated with fibronectin in NEUROBASAL media (Gibco) + 1X B27 (Gibco) for differentiation. After 15 days, cells were ready to be used for the co-culture experiments.

### Cell culture and co-culture conditions

RAW 264 macrophages were cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco) supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (all Sigma). Peritoneal macrophages were cultured on RPMI (Gibco) supplemented in the same way as DMEM. For the co-culture experiments, either peritoneal or RAW 264 macrophages were seeded into the plate that contained the differentiated enteric neurons. The co-culture was maintained for 24 h in NEUROBASAL media (Gibco), and after this period, macrophages were sorted using a FACS Aria cell sorter flow cytometer (Becton Dickinson), and the conditioned media was collected and applied to previously cultured macrophages for 24 h, followed by RNA extraction using TRIZol (Invitrogen).

### Drug Administrations

Butaxamine was used *in vivo* at a dose of 5mg/kg i.p., 5-10 min before gavage with the bacteria. For *in vitro* studies 100µM was added 30min prior to the co-culture experiments. Plated peritoneal macrophages were exposed to 100µM of Norepinephrine (Sigma) or 10µM of Salbutamol (Sigma) for 1 hour prior to analysis.

## Supplemental References

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