

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Colonic Treg cells localize close to neurons

Related to Figure 1

A-B Cleared whole-mount of the ENS in colon segments from *Foxp3-gfp* mice, stained for Tuj1 (white), GFP (green) and F4/80 (red). **A:** Z stacks images (0.5 μm) in the lamina propria, max projection to 5 mm total thickness; **B:** 35 mm max projections in the myenteric plexus. Data representative of 2 independent experiments.

C: Cross-section of colon from *Foxp3-gfp* mouse stained for NOS1 (red) shows that the majority of NOS1-immunoreactive fibers are in the muscularis externa but a subset is also detectable in the lamina propria.

Figure S2. Enteric neurons inhibit iTreg induction

Related to Figure 2

A Indicated titrations of ENS neurons from colon MMP were adapted to culture, then co-cultured along with CD4^+ T cells in 72 hr iTreg induction cultures, and the numbers of CD4^+ T cells counted by flow cytometry. Each dot is an independent culture, composite of 3 experiments.

B 10^4 ENS neurons from colon MMP were adapted to culture, then co-cultured along with Foxp3^+ Treg cells sorted from the spleen of *Foxp3-gfp* reporter mice in 72 hr Treg cultures, and the numbers of Foxp3^+ Treg cells counted by flow cytometry. Each dot is an independent culture, composite of 3 experiments.

C Confocal imaging of MMP neuron cultures immunostained for Tuj1 (lower right), EpCAM (lower left), CD45 (upper right) and DAPI (upper right). Representative of 2 or more fields in 3 independent cultures.

Figure S3. Enteric neurons do not inhibit iTregs through classic neurotransmitters

Related to Figure 3

Frequency of Foxp3⁺ cells and viability in 72 hr iTreg induction cultures, supplemented with increased concentrations of different classic neurotransmitters, including CGRP, VIP, Substance P, Neurokinin, GABA, Adrenaline, Acetylcholine and NOS agonist Diethylenetriamine. Each dot is the average value of 3 or more independent preparations.

Figure S4. Enteric neuron supernatants modulate ROR γ ⁺ Treg induction

Related to Figure 5

Representative flow cytometry profiles after 72 hrs of iTreg cultures (soluble α CD3 + splenic APCs) supplemented with MMP neuronal SN and stained for FoxP3 and ROR γ (Each dot is an independent culture, compiled data from 3 experiments plotted at right).

Figure S5. Frequency of colonic ROR γ ⁺ Treg within Treg in Vip-, Calca-, Calcb-deficient mice

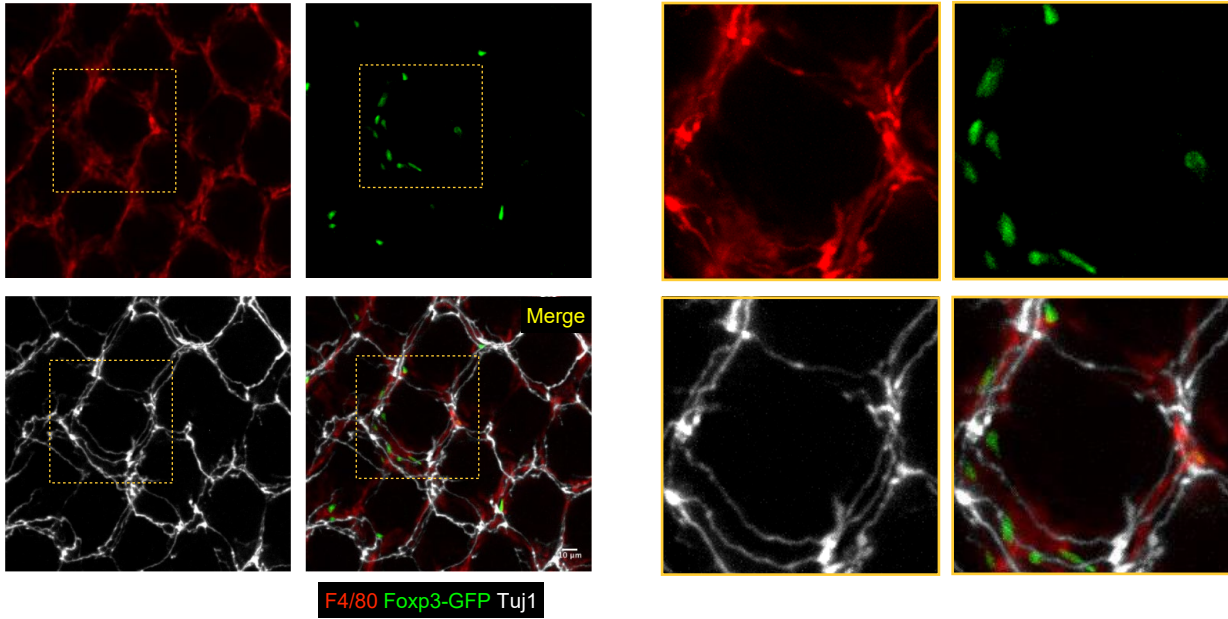
Related to Figure 7

Frequency of colonic ROR γ ⁺ Treg within Treg in Vip-, Calca-, Calcb-deficient mice or wild-type control littermates; p-value from paired Student's t test, each dot represents an individual mouse.

Supplementary Figure 1

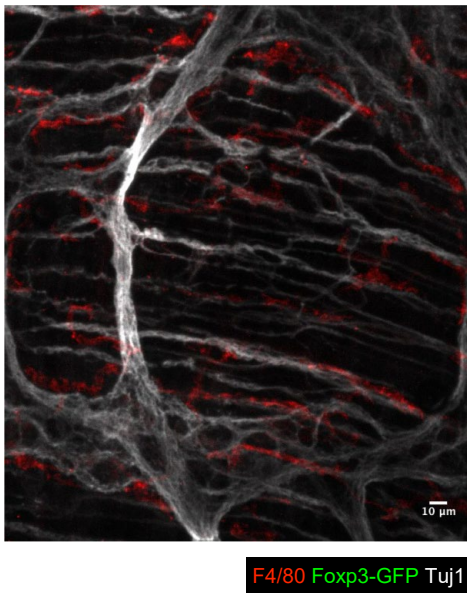
A

Lamina propria



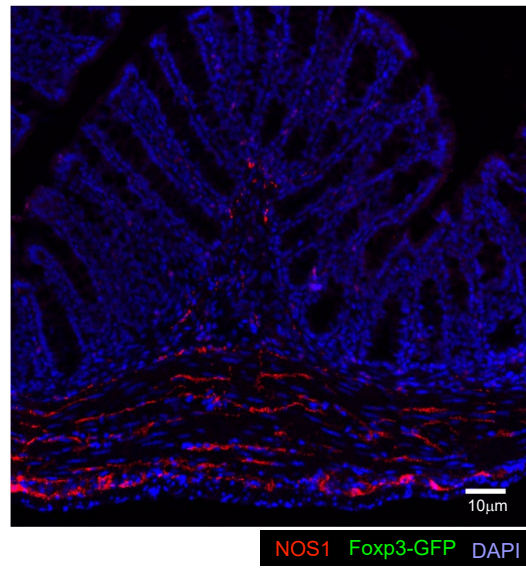
B

Myenteric plexus

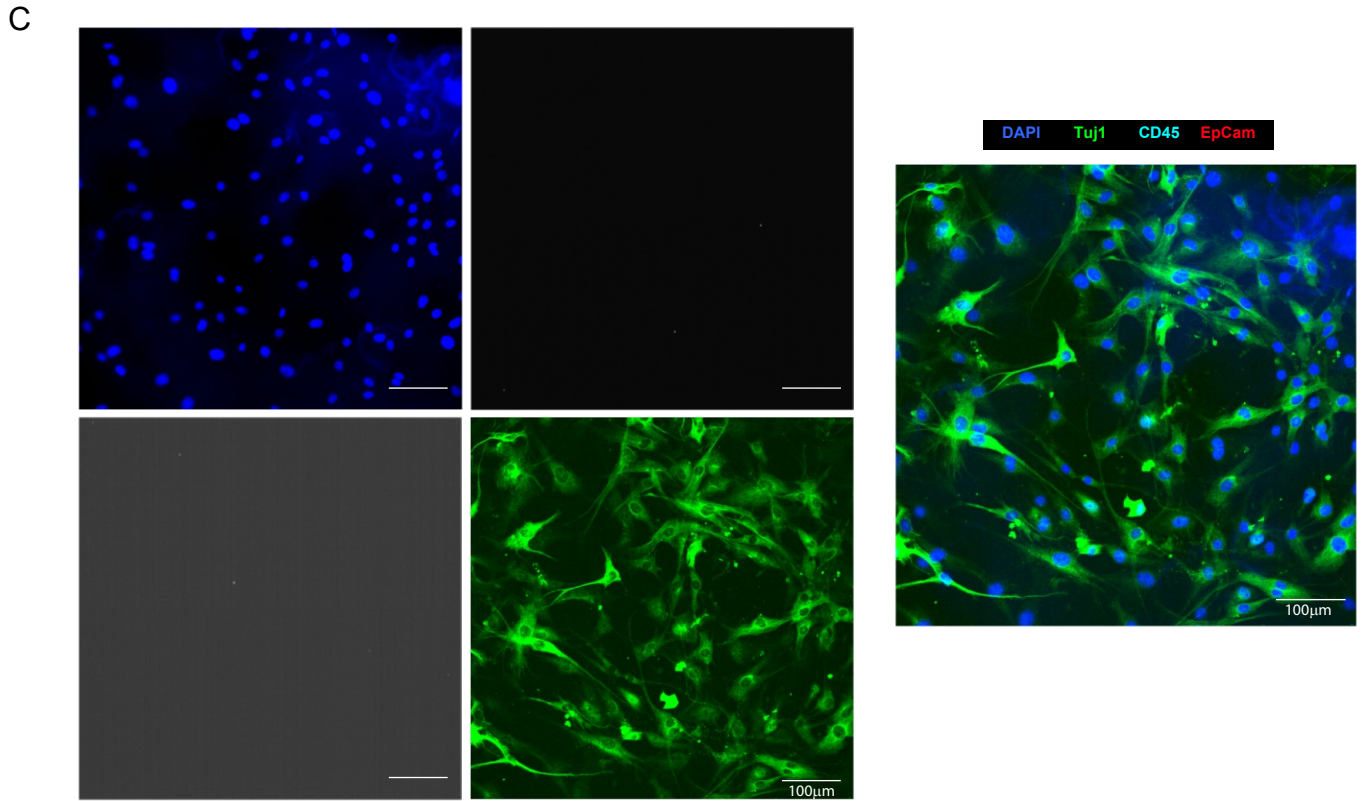
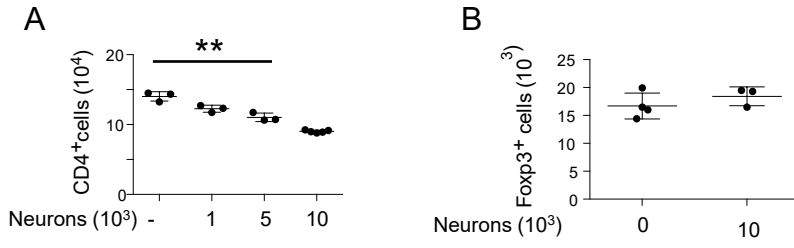


C

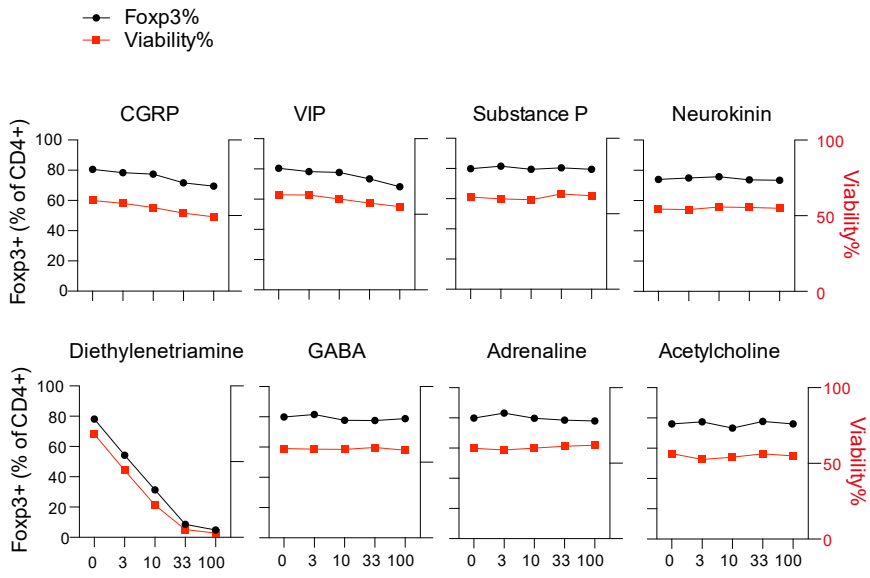
Colon



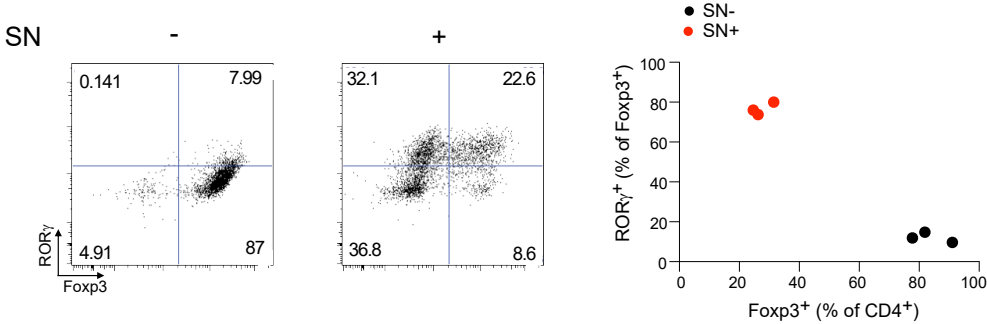
Supplementary figure 2



Supplementary figure 3



Supplementary figure 4



Supplementary figure 5

