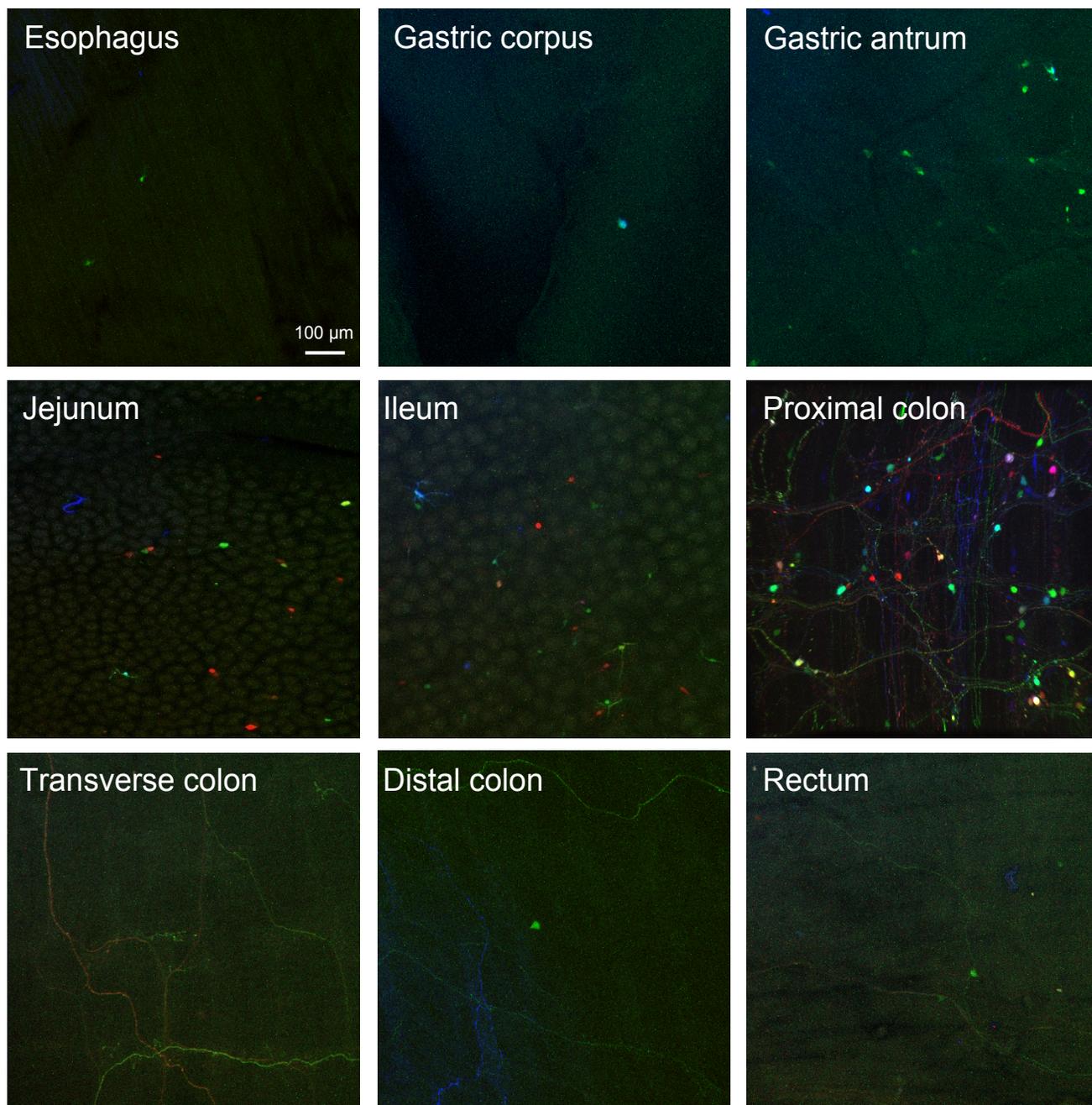
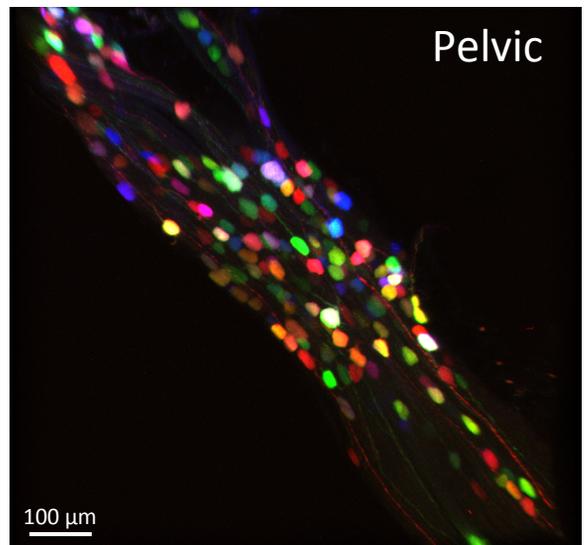
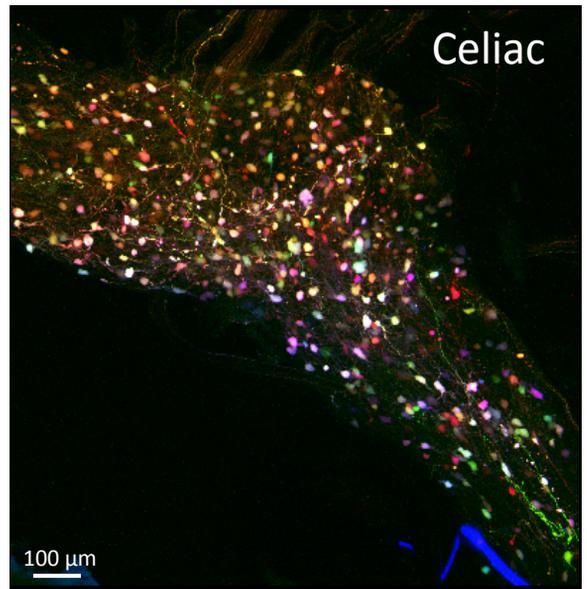
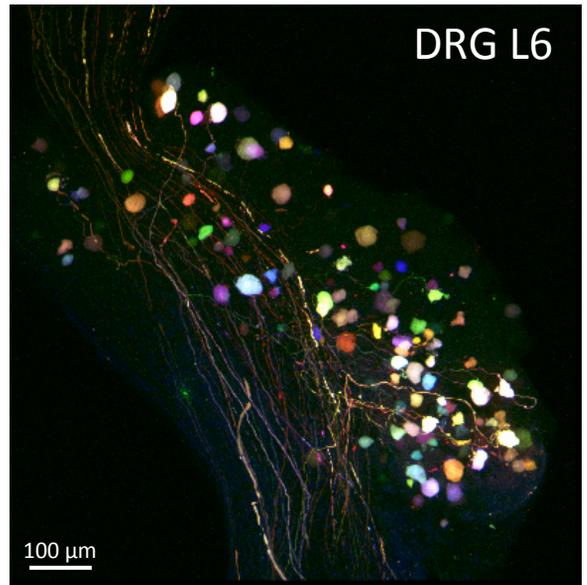


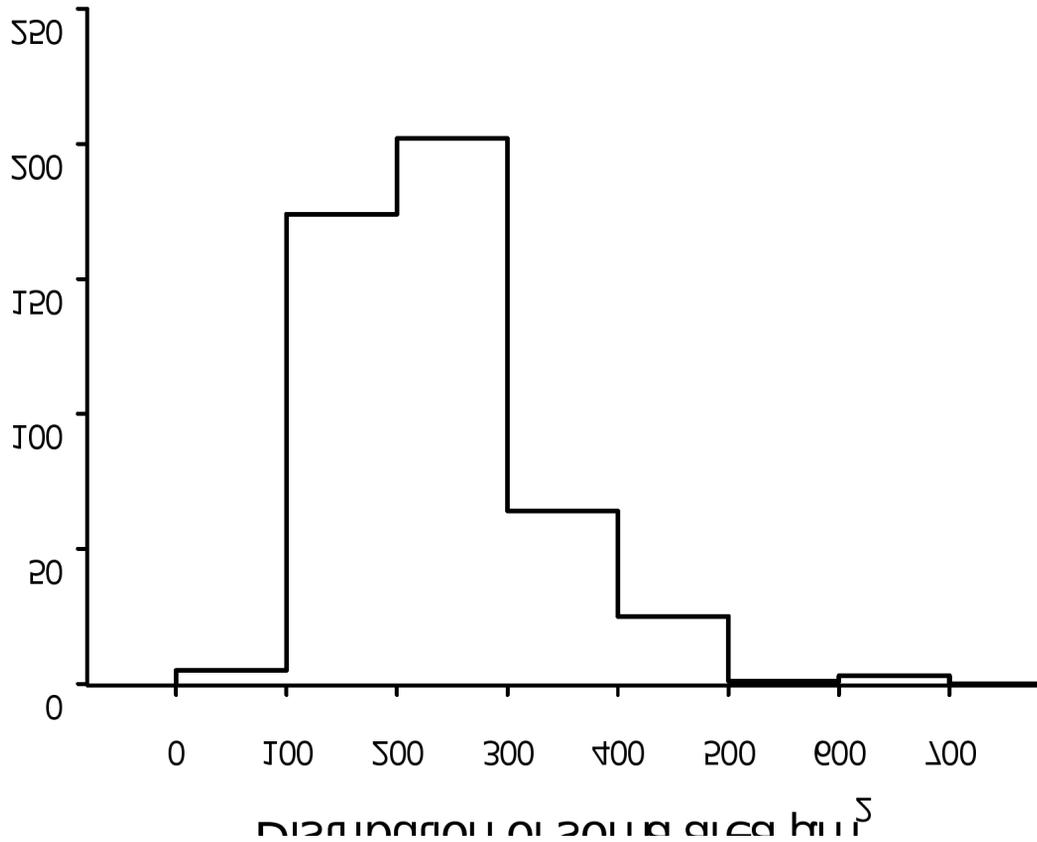
## Supporting Information: Figures



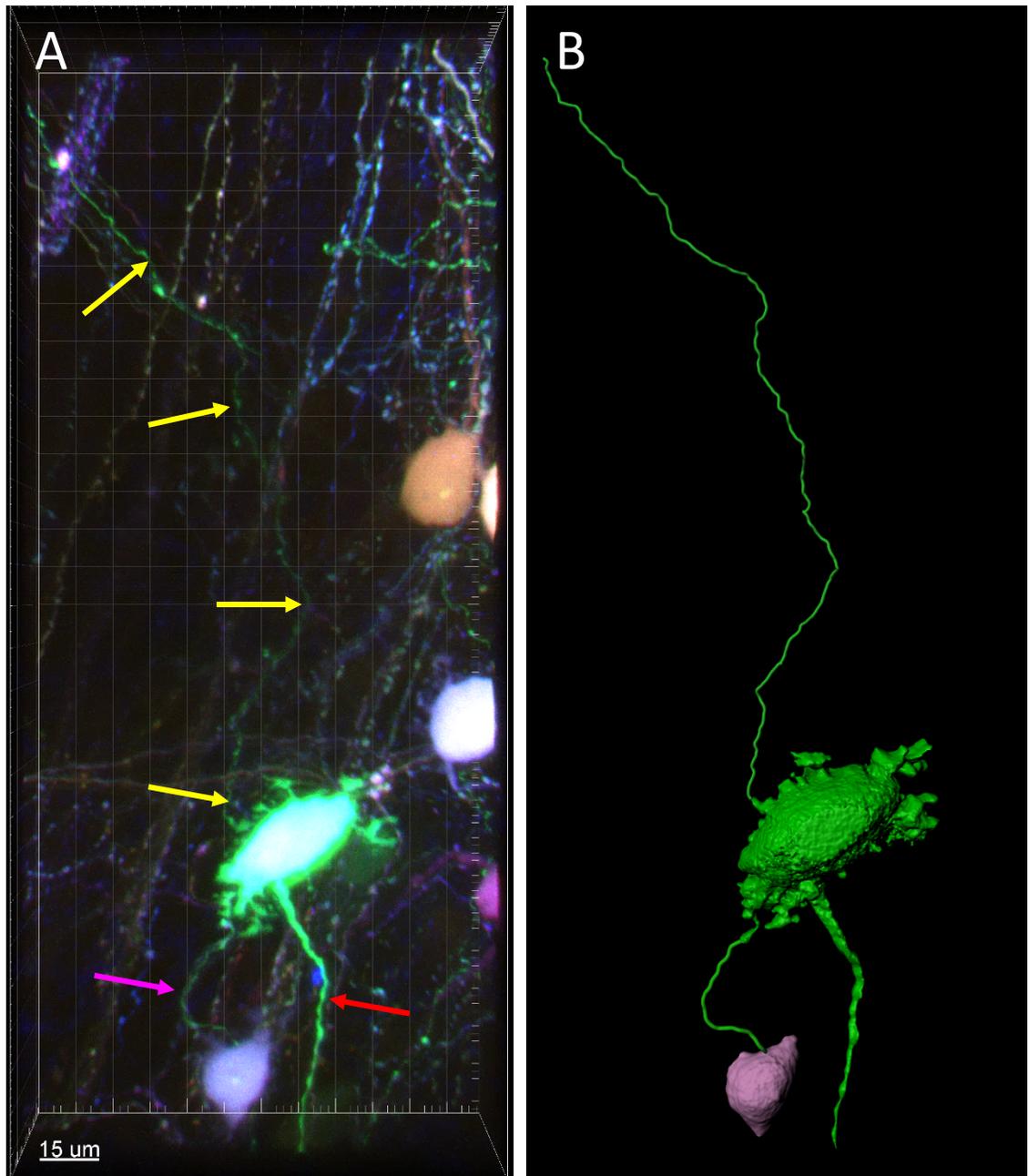
**Fig. S1.** Representative mapping of random sparse labeling of multicolor AAV transduction in the mouse gastrointestinal (GI) tract from a male mouse. A multicolor 4-AAV vectors system (AAV-PHP.S:hSyn-tTA:TRE-XFP; inducer dose was  $1 \times 10^{11}$  GC/mouse) was retro-orbitally injected 3 weeks before. The proximal colon had more labeled neurons than other segments (antrum, jejunum, ileum), while the esophagus, gastric corpus, transverse and distal colon and rectum contained few labeling. The mucosa of the jejunum and ileum was remained because it could support the sample to be comparatively flat when embedded in the viscous mounting medium.



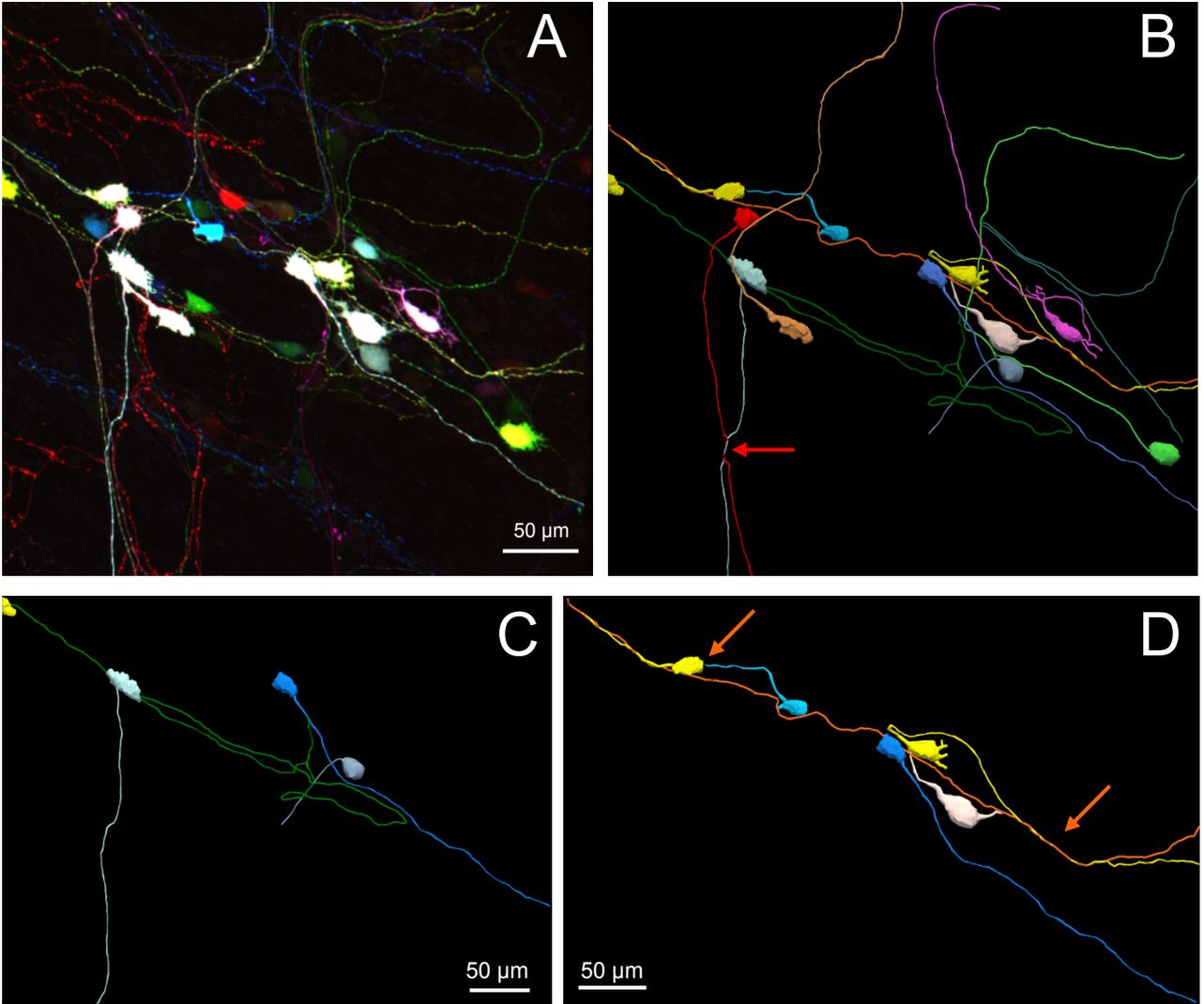
**Fig. S2.** Numerous spectral colored neurons in peripheral ganglia, i.e dorsal root ganglia (DRG) at L6, celiac and pelvic ganglia transduced by retro-orbitally injected AAV-PHP.S:hSyn-tTA:TRE-XFP. DRG and celiac ganglion were from a female mouse injected with the inducer vector at  $1 \times 10^{10}$  GC, and pelvic ganglion from a male mouse with the inducer at  $1 \times 10^{11}$  GC.



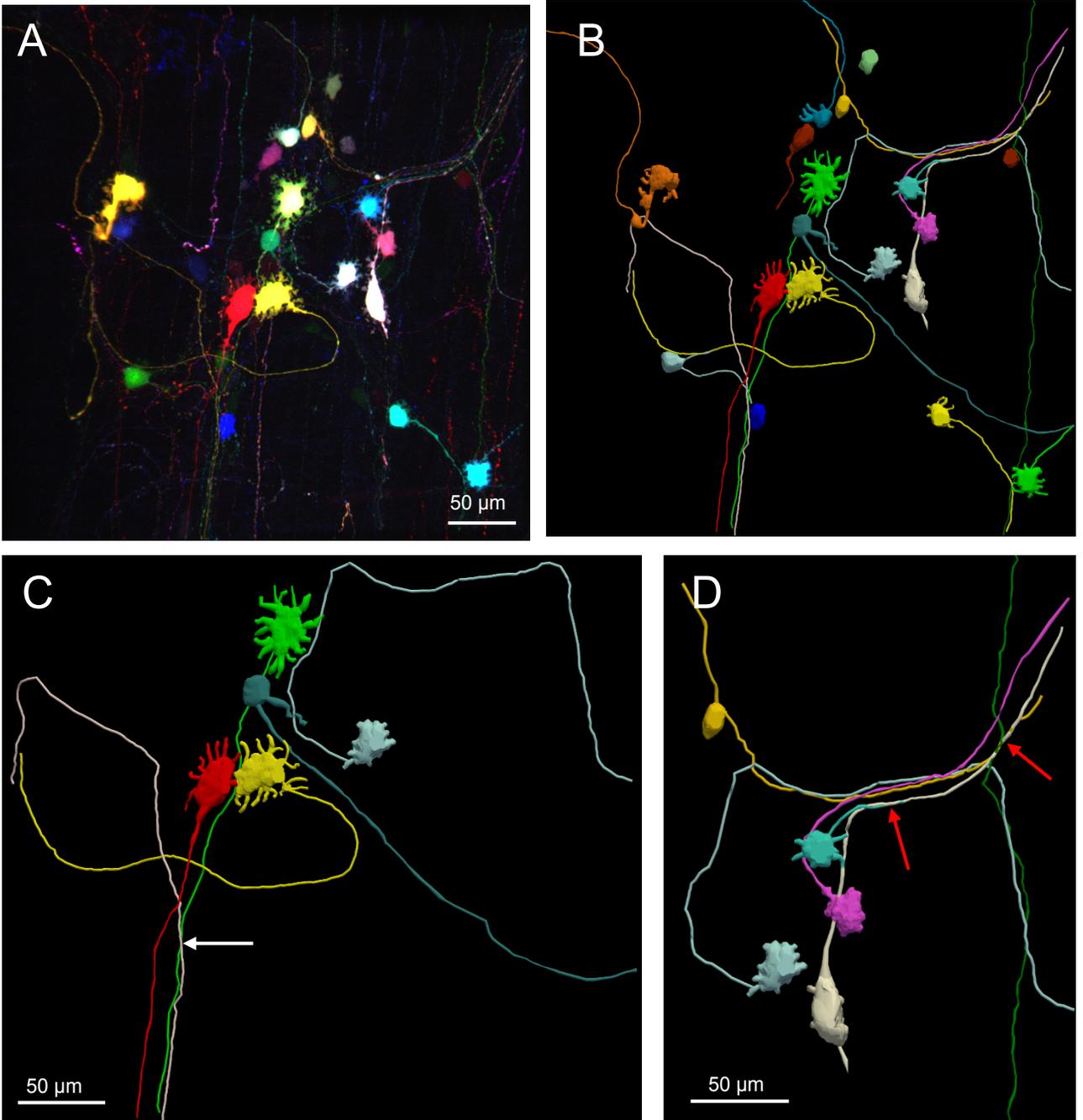
**Fig. S3.** Histograms showing the range and distribution of neuronal soma area sizes in myenteric plexus of the mouse proximal colon. Mice were injected retro-orbitally with the inducer vector, AAV-PHP.S-hSyn-tTA ( $1 \times 10^{10}$ ,  $5 \times 10^{10}$  and  $1 \times 10^{11}$  GC/mouse) mixed with the fluorophores-carrying vectors, PHP.S-TRE-XFP (total dose of  $1 \times 10^{10}$  GC/mouse). The somas of 675 neurons were digitally traced from 8 mice (male 4 and female 4) in NeuroLucida 360 and measured in NeuroLucida Explorer.



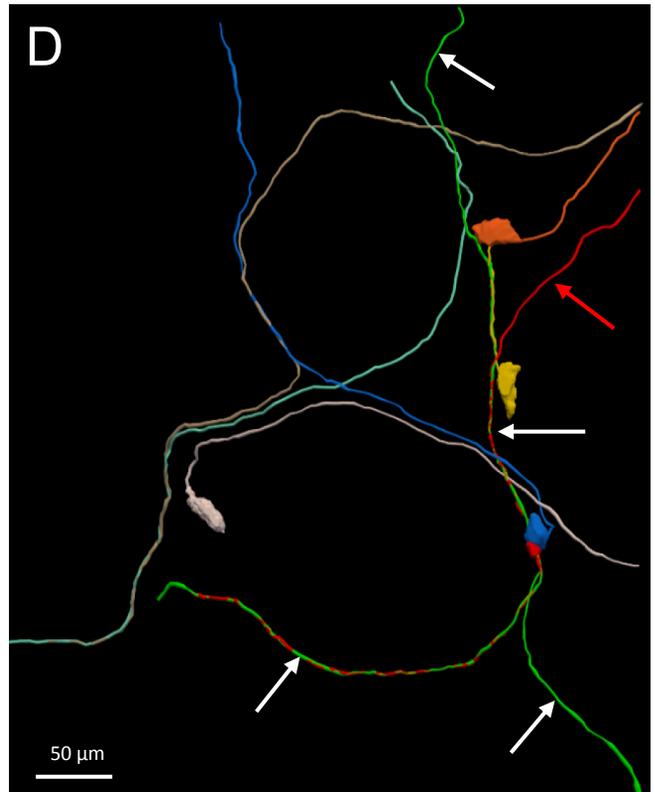
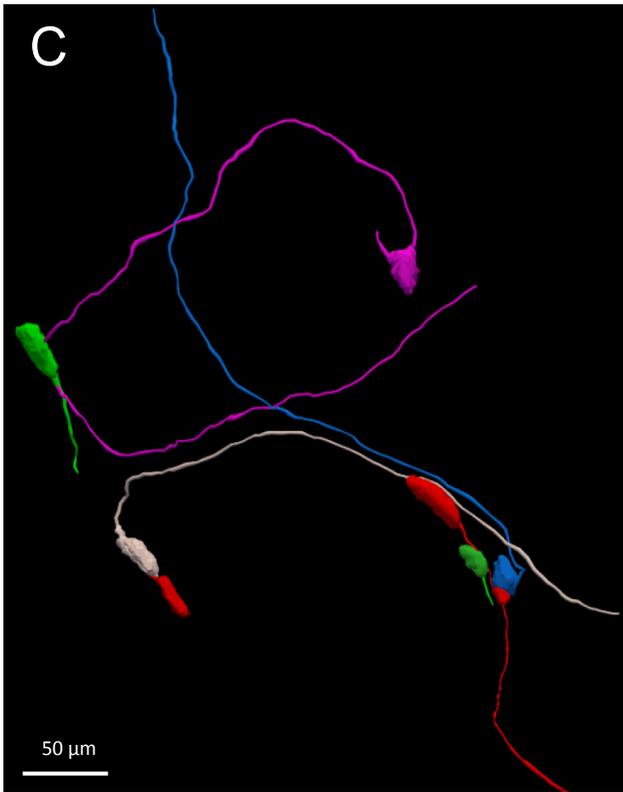
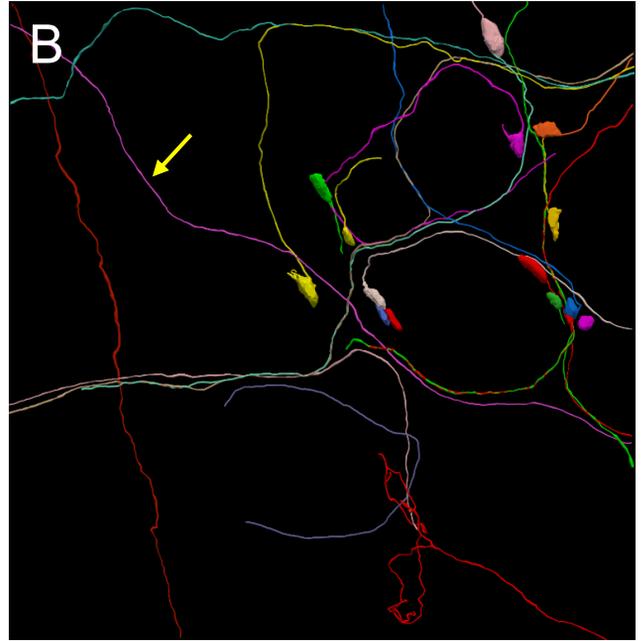
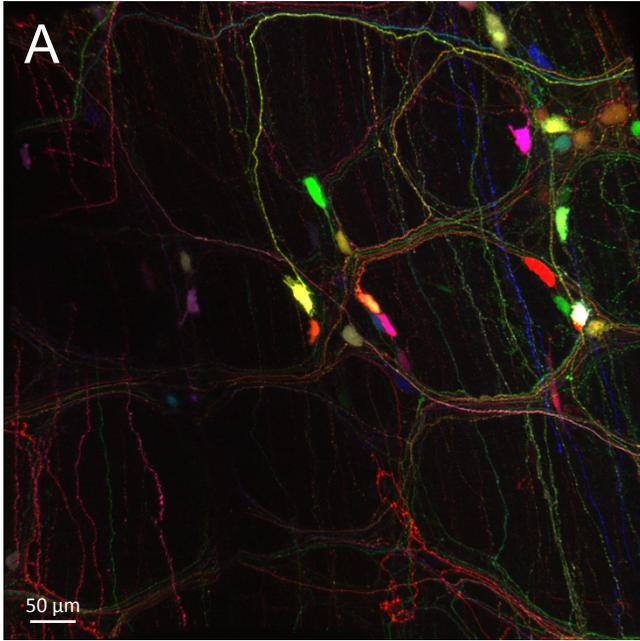
**Fig. S4.** Example of 3D digital tracing in Imaris. **A.** Confocal microscopic image cropped in 3D from Fig. 2A. **B.** digital traces in image A, using Imaris 9.2. A large neuron (#11 in Fig. 2A) has short thick dendrites and a few long processes.

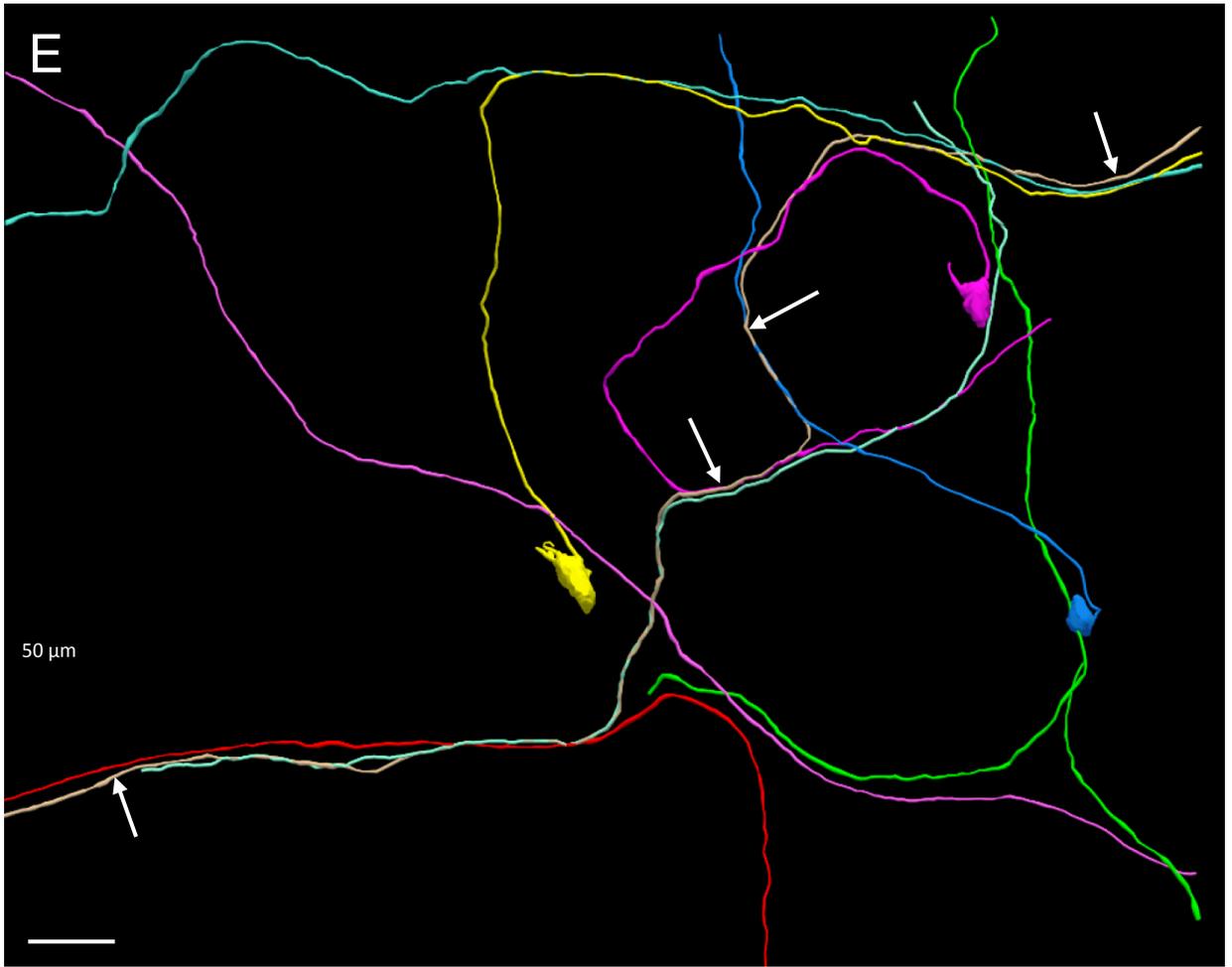


**Fig S5. A.** Confocal microphotograph of random and sparse AAV-transduced multicolored neurons and fibers in a mouse proximal colon. **B.** Digital traces in A (same scale). The sample was obtained from a female mouse injected with the inducer vector at  $1 \times 10^{10}$  GC. The 3D digital tracing was performed in Neurolucida 360. The red arrow indicates an axon-axon contact. **C** and **D**: Digital traces demonstrate one nerve fiber (dark green in C and orange in D) contacted several neurons and/or run together with other fibers (orange arrows in D).

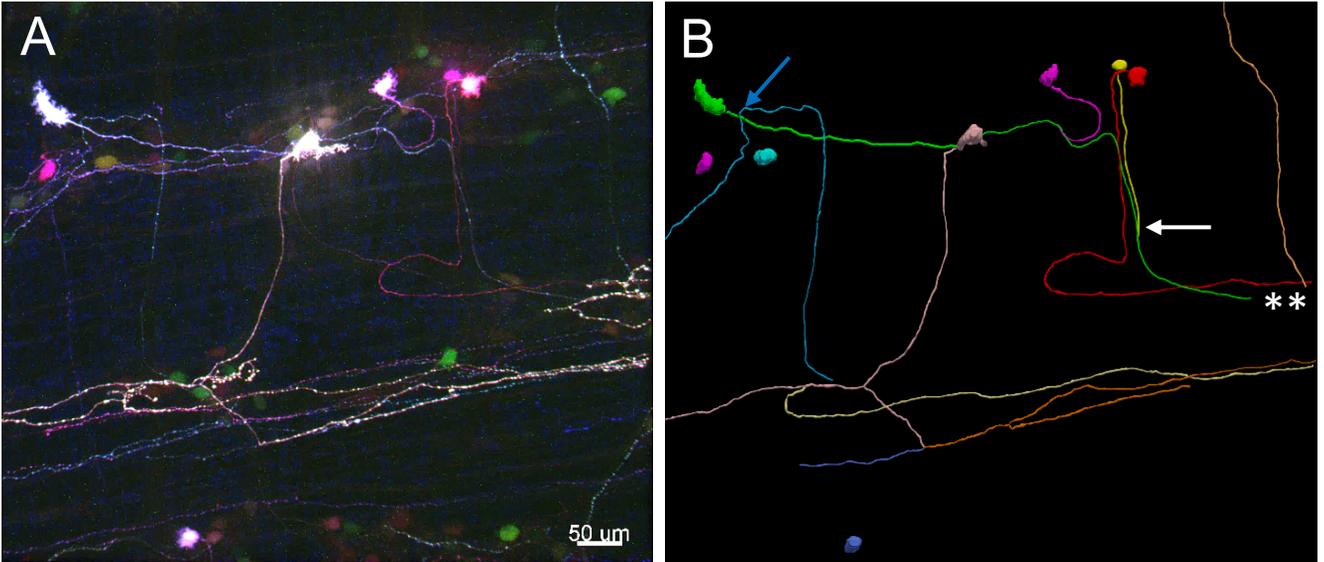


**Fig. S6.** **A.** Confocal microphotograph of random and sparse AAV-transduced multicolored neurons and fibers in a mouse proximal colon. **B.** Digital traces in A (same scale). The sample was obtained from a male mouse injected with the inducer vector at  $5 \times 10^{10}$  GC. The 3D digital tracing was performed in Neurolucida 360. **C** and **D**: Selected digital traces demonstrating one set of direct connections. **C.** One neuron (green) contacted with four others via axon-soma, axon-dendrites and axon-axon connections, as well as one passing fiber (white arrow). **D.** Axons (with contacts) and a long crossing fiber (dark green) joined an interganglionic strand (red arrows).

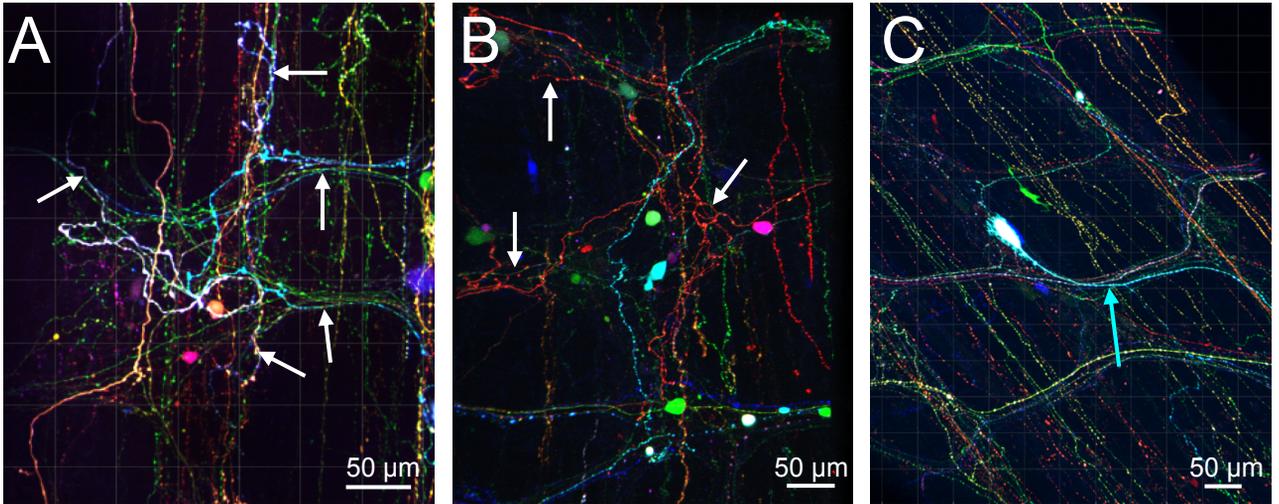




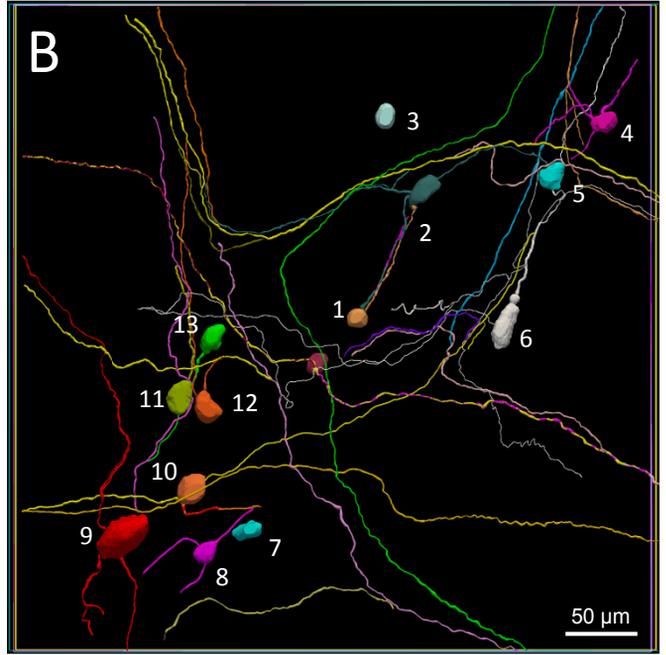
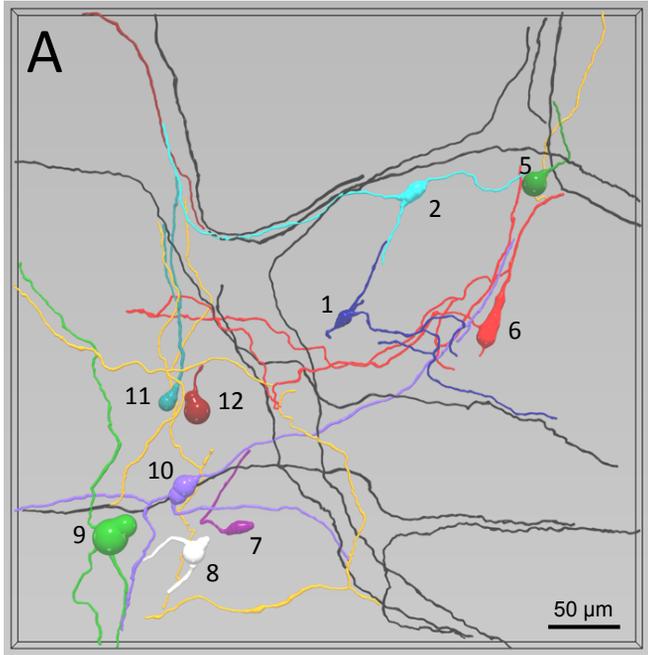
**Fig S7. A.** Confocal microphotograph of random and sparse AAV-transduced multicolored neurons and fibers in a mouse proximal colon. **B.** Digital traces in A (same scale). The sample was obtained from a male mouse injected with the inducer vector at  $1 \times 10^{11}$  GC. The digitally traced 3D in the myenteric plexus of mouse proximal colon, using Neurolucida 360. **C-E:** Selected digital traces demonstrating various types of connections. **C.** Interganglionic connections. **D.** Connections of one ganglion-crossing fiber (green, indicated by white arrows) with neurons in the two ganglia and ganglion-crossing nerve fibers. A nerve fiber (red arrow) run a long distance together with the green one (white arrows). **E.** Connections of one ganglion-crossing fiber (beige, indicated by white arrows) with axons of neurons and ganglion-crossing nerve fibers.



**Fig. S8.** Confocal microscopic (**A**) and digital tracing (**B**) images in 3D of multicolor AAV randomly and sparsely transduced neurons and nerve fibers in mouse proximal colon. The AAV-PHP.S inducer dose was  $1 \times 10^{10}$ , and the sample in A was collected from a male mouse. **B**: a long process of the green neuron terminates in varicosity endings (marked by \*\*), passing a nerve fiber (blue arrow), one neuron soma (pink) and processes of 3 neurons (magenta, yellow and red). The axons of 2 neurons contacted (white arrow).



**Fig. S9.** Confocal microscopy images of nerve fibers and endings labeled by a multicolor 4-AAV vectors system (AAV-PHP.S:hSyn-tTA:TRE-XFP). The inducer vector doses were  $1 \times 10^{10}$  (A, female),  $5 \times 10^{10}$  (B, D, two males) and  $1 \times 10^{11}$  (C, female). **A:** A IGNEx consisted of nerve fibers and pericellular varicosity endings of different origins labeled in different colors (arrows). **B:** Varicosity endings branched to form pericellular net in different ganglia (arrows). **C:** Nerve fibers in the circular muscle layer. A labeled neuron in the myenteric plexus has thick varicosity axon (arrow).



**Fig. S10.** Digital 3D neural tracing of myenteric plexus in image of Fig. 2B using NeuTube (A) or Neuroleucida 360 (B). The traces, performed using either NeuTube or Neuroleucida 360 by investigators in different laboratories, were essentially similar.