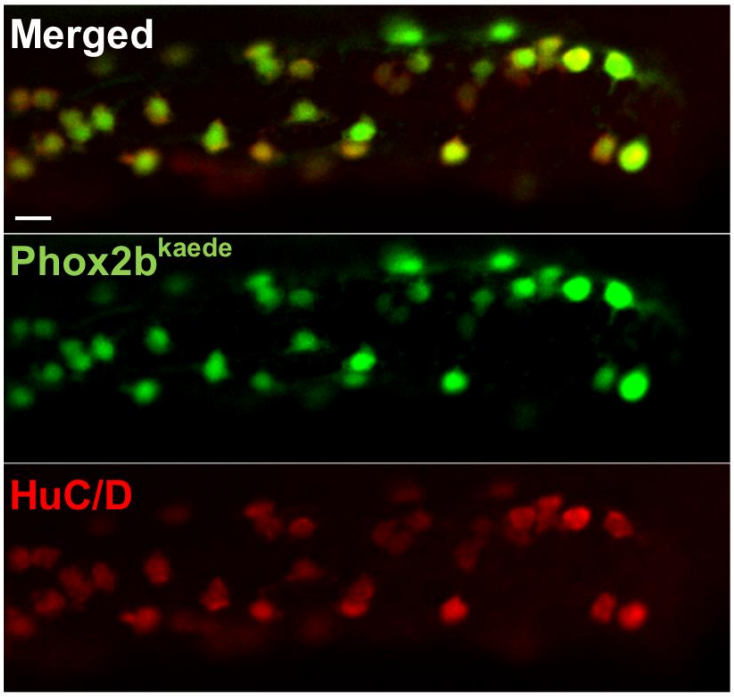


Fig S1. HuC/D co-localizes with all *Tg(-8.3bphox2b:Kaede)* (Phox2b-kaede) enteric neurons at 5 dpf.

Phox2b-kaede fish were fixed at 5 dpf and underwent IHC for HuC/D, and imaged for the endogenous kaede fluorescence and HuC/D. All Phox2b-kaede cells co-localized with HuC/D, indicating that at this stage, Phox2b represents differentiated enteric neurons.

Scale bar: 10 um

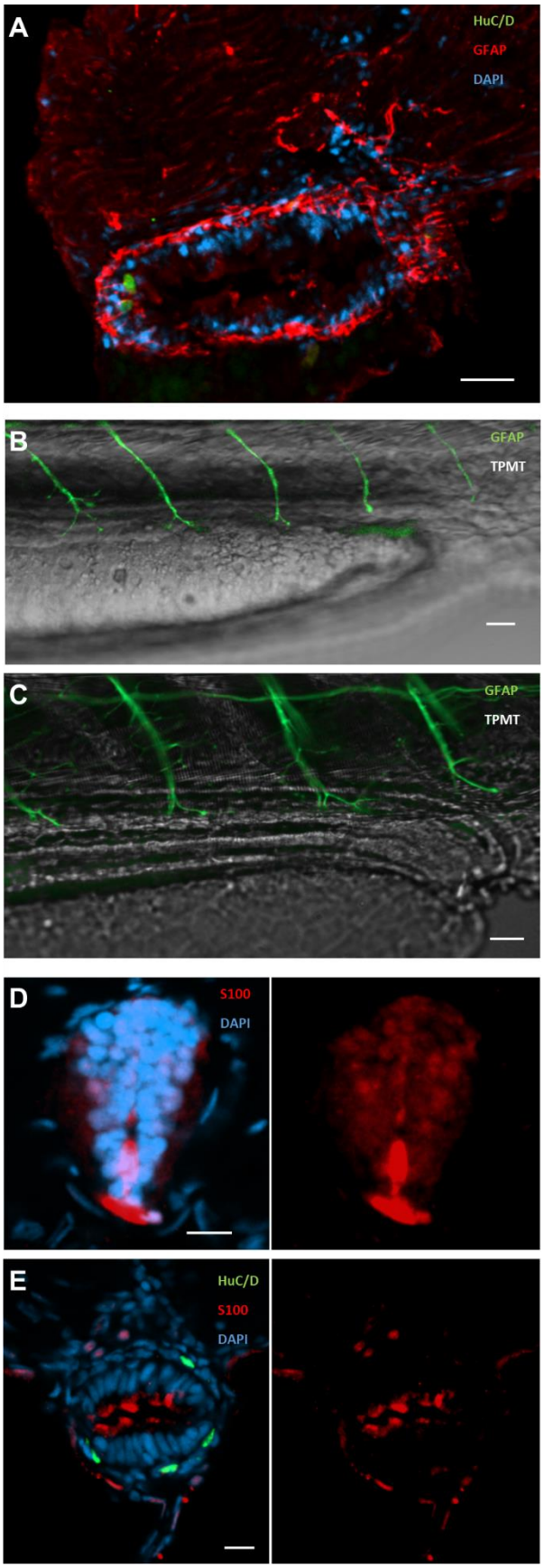


Fig S2. Expression pattern of glial markers in zebrafish intestine

(A) A 20 um section of a 5 dpf zebrafish immunostained for GFAP, imaged as a *z* stack and then projected in two dimensions, revealing immunoreactive extrinsic projections that extend to the intestine.

(B,C) GFAP-GFP transgenic line displays an expression pattern of GFAP that is absent in the intestine. $GFAP^{+}$ projections extend to the dorsal aspect of the hindgut at 2 dpf (B), prior to colonization by the enteric vagal neural crest. At 4.5 dpf (C), these projections have increased arborization, but signal within the intestine is limited to these projections.

(D,E) IHC of S100 in 5 dpf zebrafish reveals immunoreactive cells in the spinal cord (D) and serves as a positive control. In the intestine (E), no $S100^{+}$ nuclei are present within the ENS (though artifact is observed within the intestinal lumen).

Scale bars: 20 um (A-C); 10um (D-E)

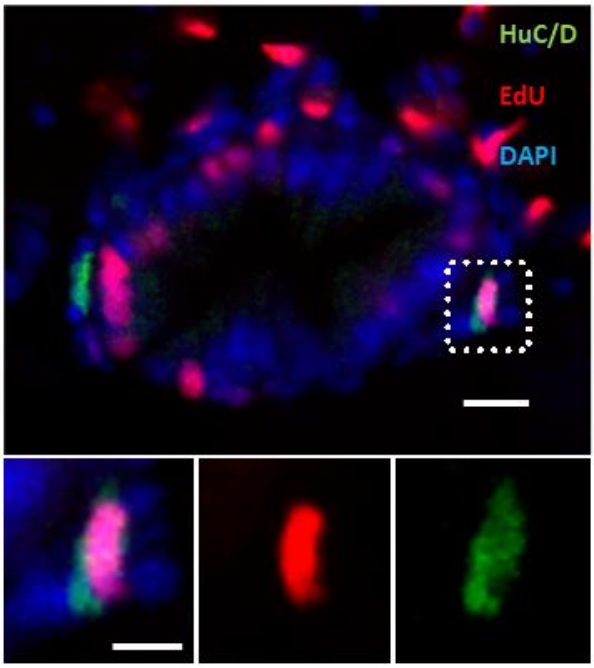


Fig S3. Post-embryonic enteric neurogenesis as evidenced by EdU^{+} enteric neurons.

Zebrafish larvae were pulsed with EdU from 4.5 -5 dpf, fixed and sectioned, and then assessed for EdU expression. While EdU labelling was observed diffusely throughout the larvae as expected, occasional enteric neurons were also labelled, indicating that these neurons arose in the post-embryonic period.

Scale bars: 10 um (inset: 5 um)

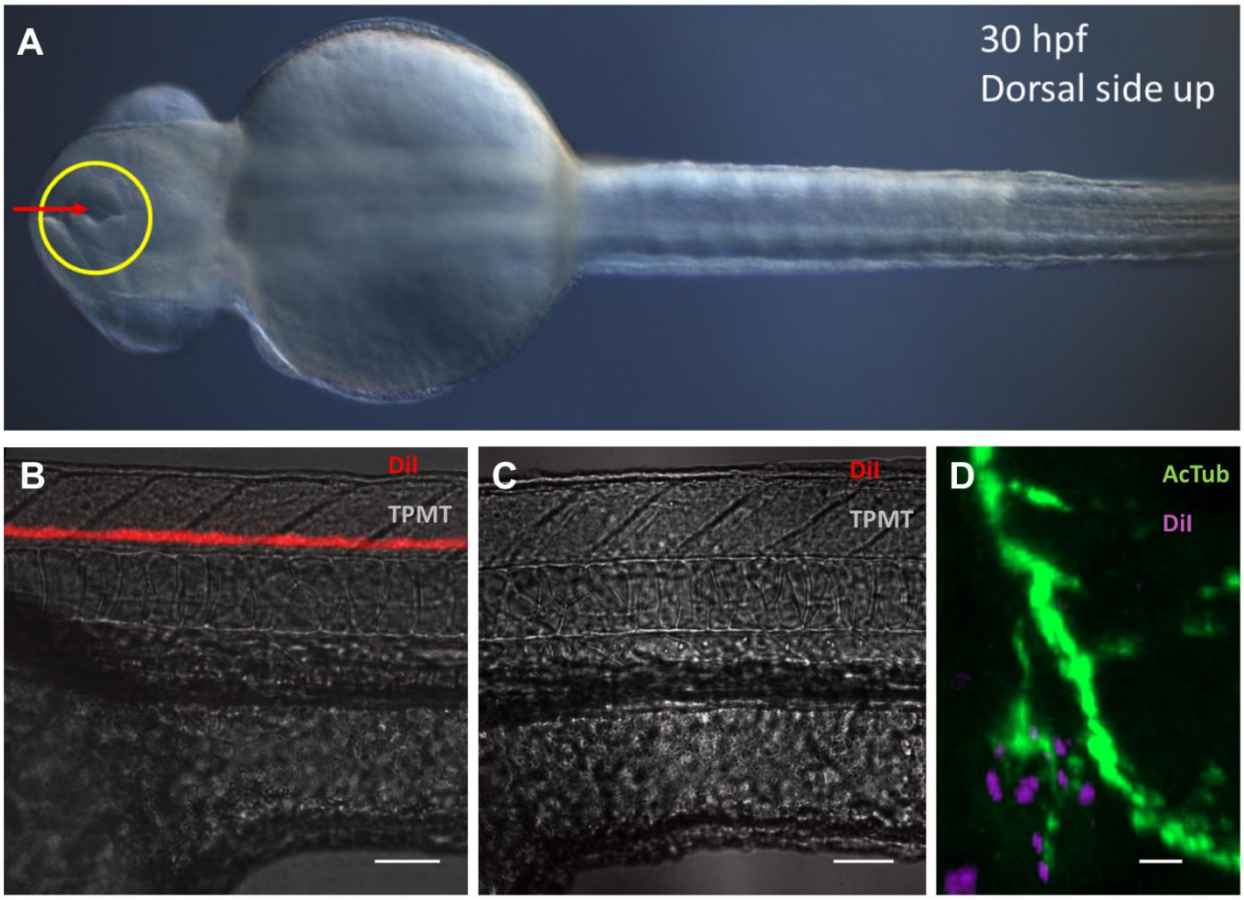


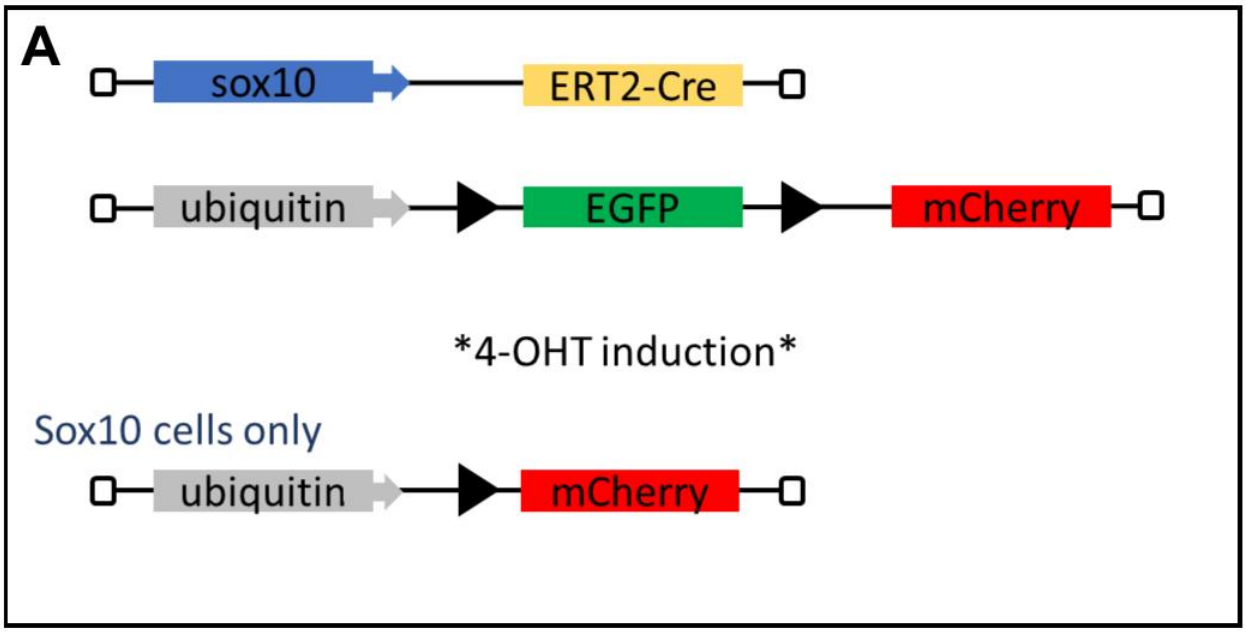
Fig S4.

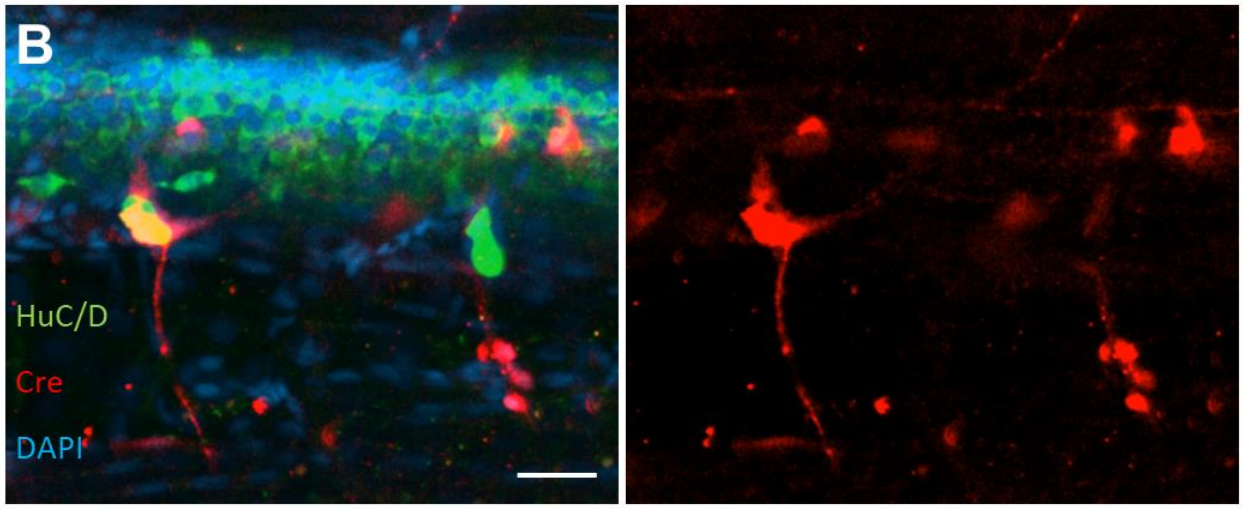
(A) Schematic of lipophilic dye injection at 30 hpf. The anterior neuropore (yellow circle) is open at this time point, allowing insertion of a dye-filled capillary in the trajectory depicted by the arrow.

(B,C) 1 h post injection, a dye-coloured stripe is present indicating successful neural tube fill (B). Control fish that were not injected did not exhibit far-red fluorescence (C).

(D) DiI-injected fish were fixed at 6 dpf, cross sectioned at 20 um thickness, and IHC was performed for acetylated tubulin, revealing DiI-labelled cells present along nerve projections. A *z* stack was collected and then projected in two dimensions, and the nerve presented here spans the space between the notochord and intestine.

Scale bars: 50 um





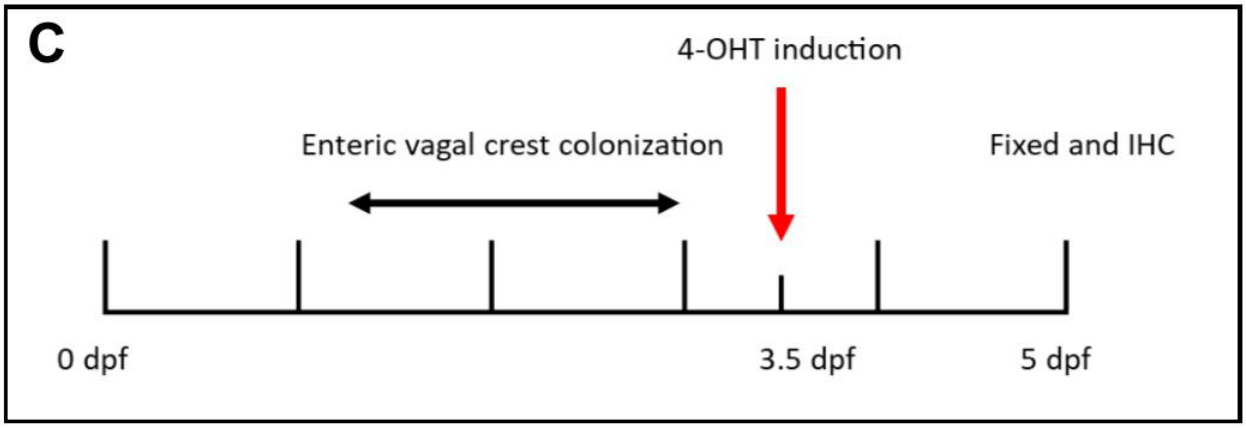


Fig S5.

(A) The inducible Sox10-Cre line was crossed with the reporter, ubi:switch. After exposure to the induction agent, 4-OHT, Cre is activated, cleaves the loxP sites specifically in cells expressing Sox10 at the time of induction, leading to those cells permanently being labelled by mCherry.

(B) Fish from the inducible Sox10-Cre line were induced with tamoxifen at 30 hpf and collected at 72 hpf, fixed and whole-mount immunostained. Imaging was collected as a *z* stack and presented as a two-dimensional projection. Early induction of this Cre line notably revealed Cre-labelled cells consistent with dorsal root ganglia. Cre^+ projections emanated from these ganglia, and clusters of Cre^+ cells can be seen at the ventral ends of these projections. Scale bar: 20 µm.

(C) Schematic of the induction protocol for Fig. 5C-D. Fish were exposed to 4-OHT at 3.5 dpf for 16 hours, when Sox10 is no longer expressed in the intestine. Cells labelled during this time are thus gut-extrinsic Sox10-expressing cells.

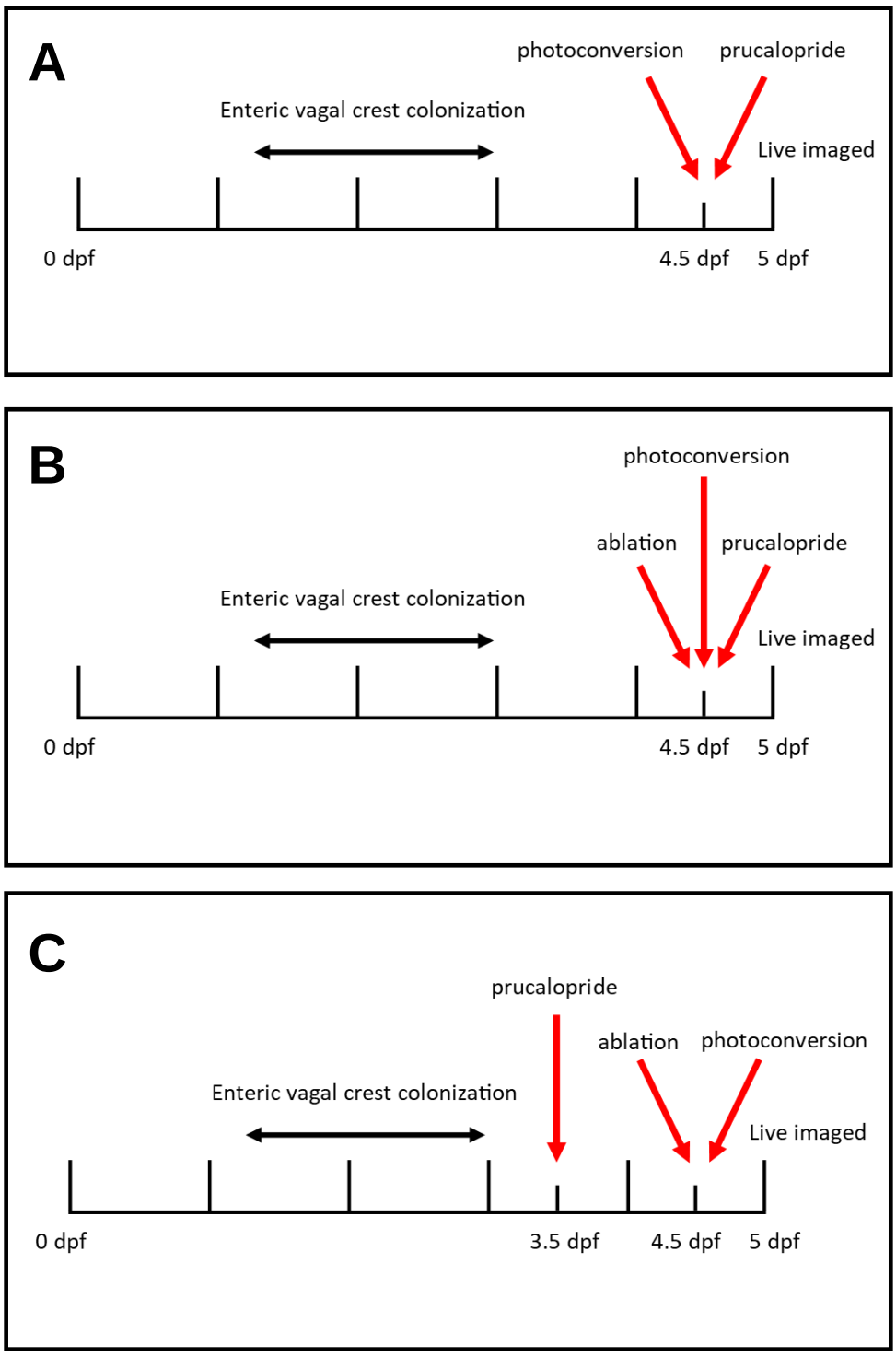
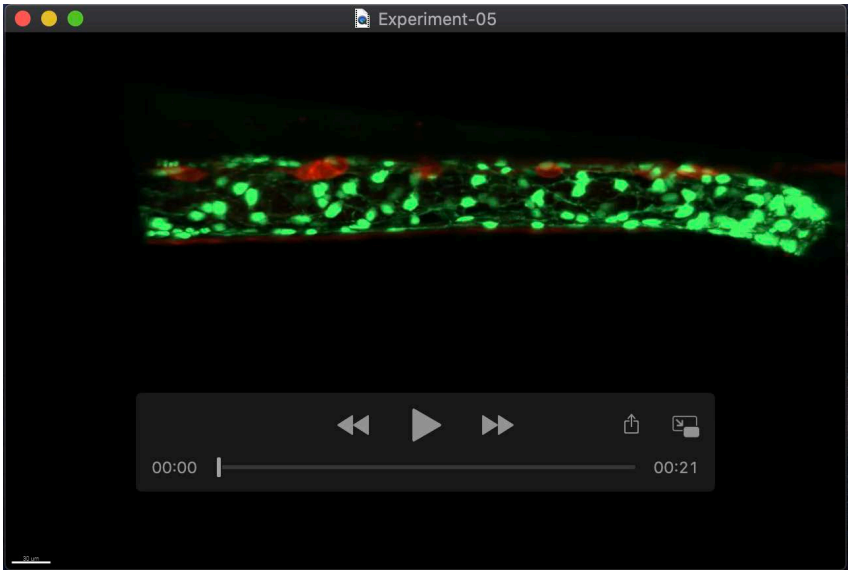


Fig S6.

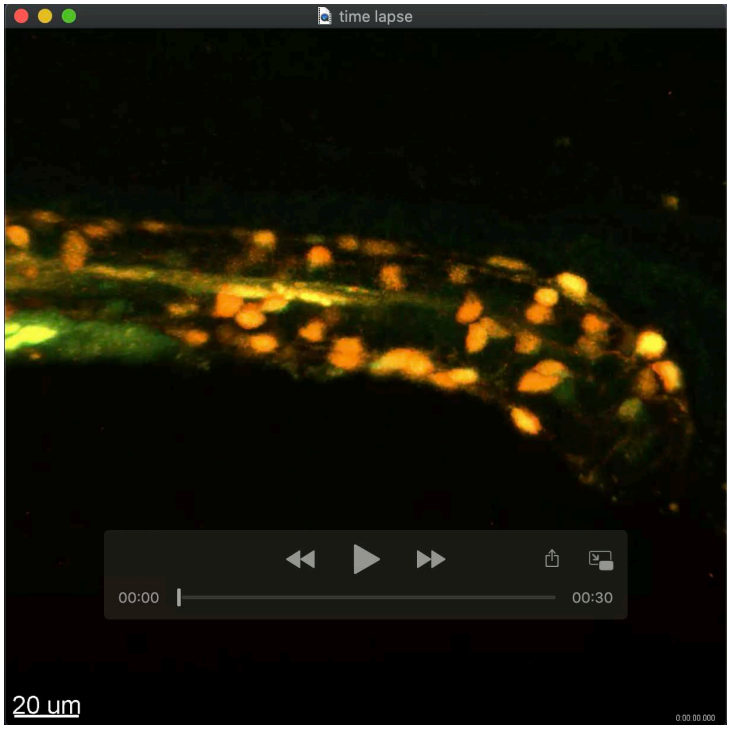
(A-C) Schematic of the protocols for Figures 7A, B, and C depicting the timing of drug exposure, photoconversion, and laser ablation. Corresponding experiments with the $5HT_4R$ antagonist GR 113808 followed analogous protocols.



Movie 1: Sox10 expression at 5 dpf likely corresponds to melanocytes

A two-dimensional projection of a *z* stack collected at 5 dpf of a Phox2b-kaede x Sox10-mRFP fish hindgut reveals a linearly arrange collection of Sox10-expressing cells. However, three-dimensional assessment indicates that these cells are located dorsolateral to the intestine and likely correspond to melanocytes which reside in this location.

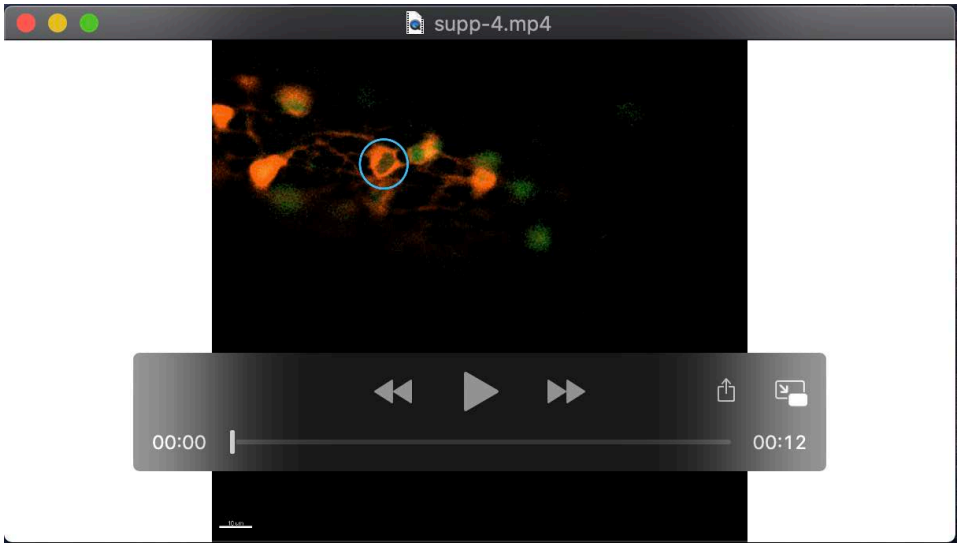
Scale bar: 30 um



Movie 2

Video of the live time-lapse experiment from Fig. 3D depicts the gradual appearance of a *de novo* enteric neuron in a portion of the hindgut that initially did not contain an enteric neuron. The *de novo* enteric neuron appears to make contact with neighbouring cells.

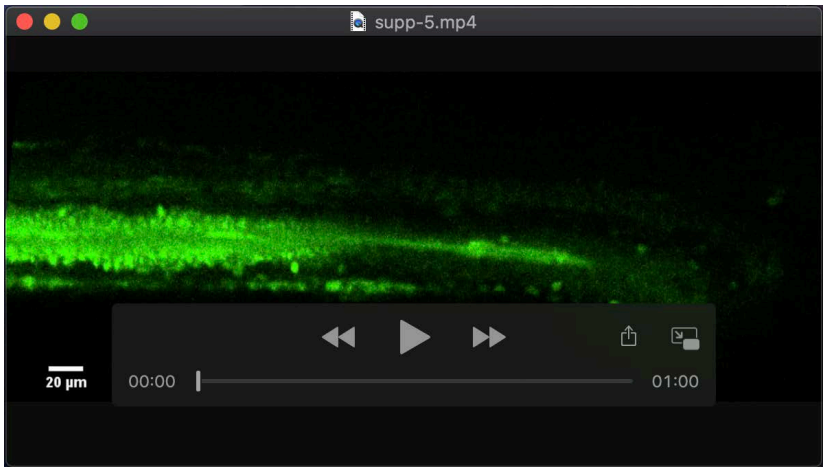
Scale bar: 20 um



Movie 3

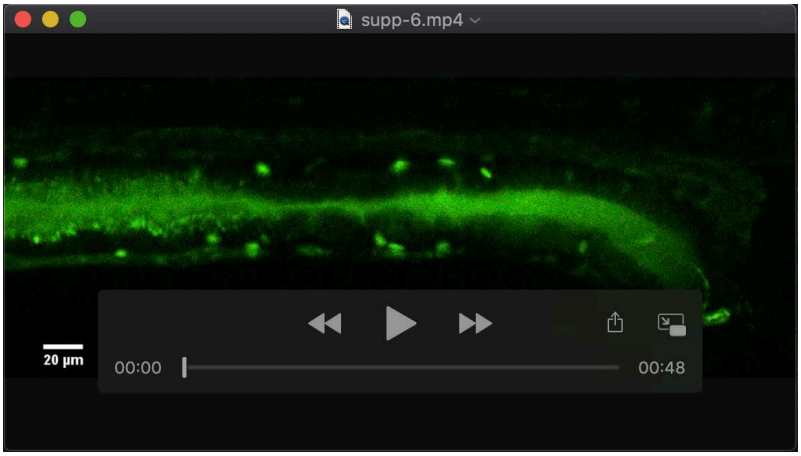
Video of the live time-lapse experiment from Fig. 4D depicts the appearance of a *de novo* enteric neuron that initially appears very faintly in the dorsal periphery of the intestine. As an injured enteric neuron involutes, it is replaced by the migrating *de novo* enteric neuron, which gradually increases Phox2b-kaede expression and extends projections to nearby enteric neurons.

Scale bar: 10 um



Movie 4.

Video of a 5 dpf HuC-H2B GCaMP6 fish exposed to DMSO reveals low baseline motility over a 15 min time frame.



Movie 5.

Multiple expulsive contractions are observed with exposure to 10 µM prucalopride.