

SUPPLEMENTARY MATERIAL

Title: Characterization of *NvLWamide-like* neurons reveals stereotypy in *Nematostella* nerve net development

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Supplemental Figure Legends

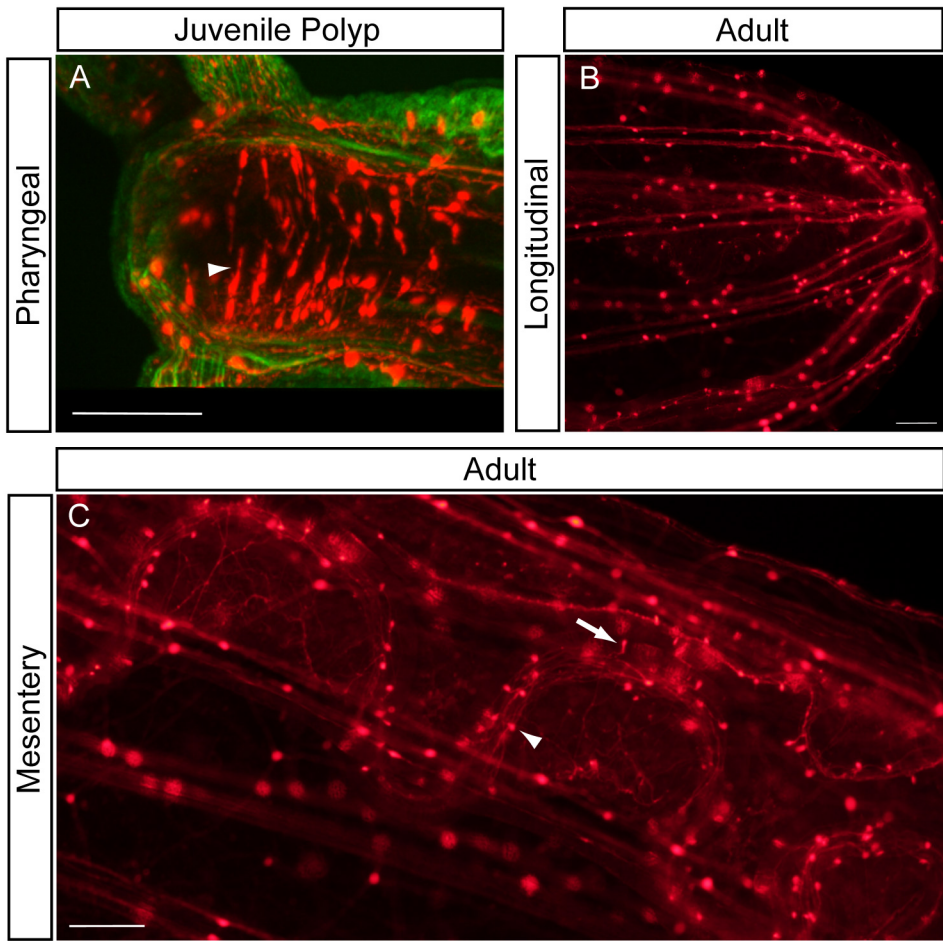
Supplemental Figure 1. Pharyngeal, longitudinal, and mesentery neurons in the juvenile polyp and adult *Nematostella*. (A) *NvLWamide-like::mCherry* positive soma (red) span the width of the pharyngeal ectoderm in the juvenile polyp (arrowhead). (B) *In vivo* image of an adult *NvLWamide-like::mCherry* positive animal highlights eight longitudinal tracts that converge at the aboral pole. (C) *NvLWamide::like::mCherry* expressing mesentery unipolar (arrow) and bipolar (arrowhead) neurons are visible in the adult *in vivo*. Animal in A was lightly fixed and co-stained with phalloidin (green). Oral end is to the left. Scale bars = 50 μ m (A) and 100 μ m (B and C).

Supplemental Figure 2. *NvLWamide-like::mCherry* expressing neurons in unfed juvenile polyps *in vivo*. (A) The number of *NvLWamide-like* positive neurons does not increase in unfed animals 18 days post fertilization (dpf), (B) and 23 dpf. Confocal images show *mCherry* expression in live animals relaxed in 7.14% MgCl₂ in 1/3X ASW. Oral end is to the left. Scale bars = 50 μ m

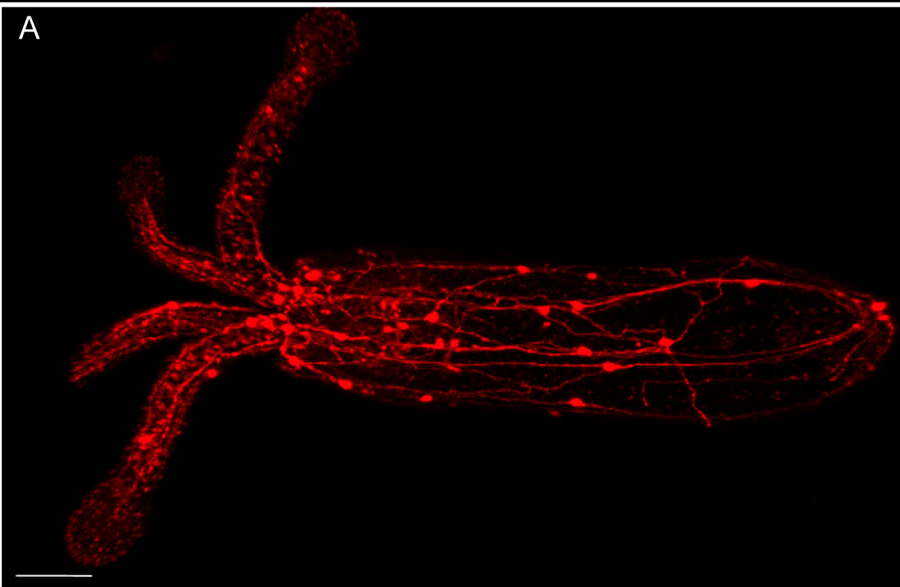
Supplemental Figure 3. Filet prep of adult *Nvelav1::mOrange* transgenic animal. (A) An endodermal focal plane shows the longitudinal tracts (arrows) are connected by commissural like structures (arrowheads). (B) A more superficial focal plane approximately at the depth of the mesoglea shows nerve net structure. Images depict *in vivo* *mCherry* expression.

Supplemental Movie 1: Composite of sequential z-stacks showing *NvLWamide-like* positive pharyngeal neurons in the juvenile polyp. *NvLWamide-like* positive pharyngeal neural soma are observed first, each with two neurite projections, visualized by *mCherry* expression (red). Deeper into the embryo additional soma that span the pharyngeal ectoderm appear on either side of the pharynx. Embryo was lightly fixed and co-stained with phalloidin (green). Oral end is located to the right.

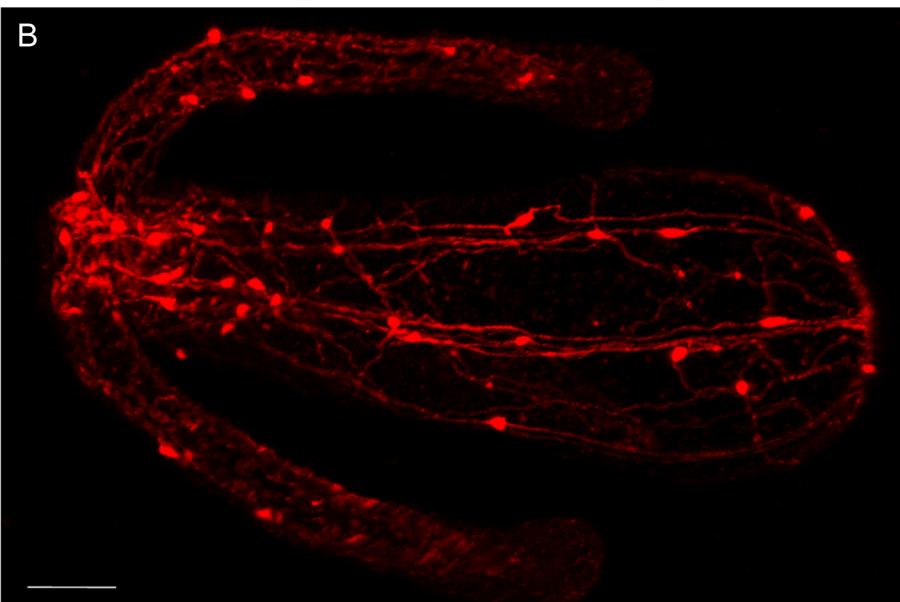
Supplemental Figure 1
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18 Day Old Unfed Juvenile Polyp



23 Day Old Unfed Juvenile Polyp



Supplemental Figure 3

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