

Supplementary material

**AXONAL NERVE RING LOCALIZATION OF AN INSULIN-LIKE PEPTIDE mRNA
IN STRESS-RESISTANT *C. ELEGANS* LARVAE**

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. *ins-6* mRNA is expressed in the larval ASI and/or ASJ sensory neurons during reproductive growth. (A) Diagram of the 12 sensory neurons in the *C. elegans* amphid sensory organ [10]. The ASI and ASJ sensory neurons are highlighted in red and NR indicates the nerve ring axon bundle. (B-F) An overlay of *ins-6* mRNA (red), GFP protein (green), and its respective DIC image in the same z-plane at 20°C in L1 (B), L2 (C), L3 (D-E) and L4 (F). Yellow represents colocalization of *ins-6* mRNA and the GFP protein expressed specifically in ASI and ASJ (*daf-28p::gfp*; [3]). In this figure and later figures, the anterior of each animal is to the left of each panel. Scale bar is 10 μ m. (G) Mean fluorescence intensities ($\pm$ SEM) of *ins-6* mRNA in ASI vs ASJ are shown from 2 trials for larval stages L1-L4 and post-dauer L4 (PDL4) of *daf-28p::gfp* animals. The following indicates: a.u., arbitrary units; ****, $P < 0.0001$. (H) Distribution of larvae that express *ins-6* mRNA in ASI and/or ASJ in *daf-28p::gfp* animals from 2 trials.

Figure S2. Starvation alone is insufficient to localize *ins-6* mRNA to the nerve ring (NR) axon bundle. (A-D) Representative fluorescent images of *ins-6* mRNA in starved wild-type L1 (A), L2 (B), L3 (C) and L4 (D) at 20°C. Animals were starved for about 5 days. Scale bar, 10 μ m.

Figure S3. Fixation and hybridization treatment damage the dauer nerve ring. (A) Image of a live, untreated 5-day old dauer that expresses GFP protein from the ASJ neuron pair. (B) Ventral view of a fixed and hybridization buffer-treated dauer that expresses GFP from the ASJ neuron pair, similar to A. Note the diffuse and broader GFP protein fluorescence in the nerve ring (NR) in this panel compared to the NR fluorescence in A.





