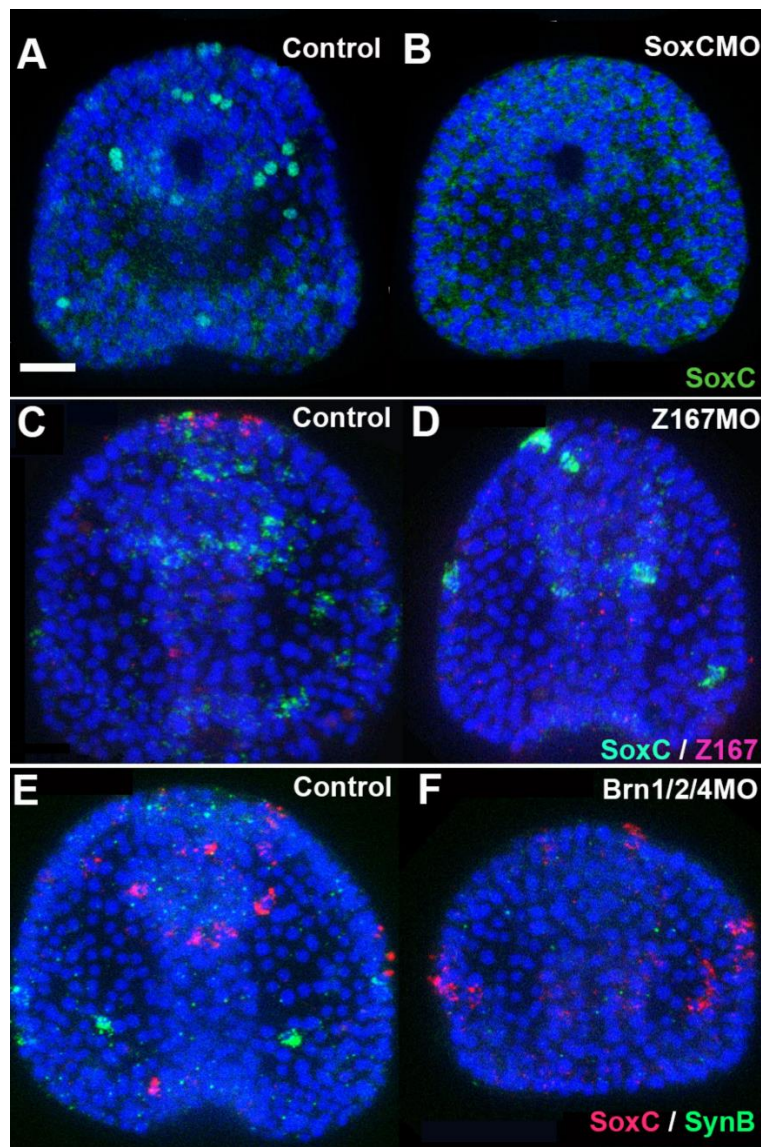
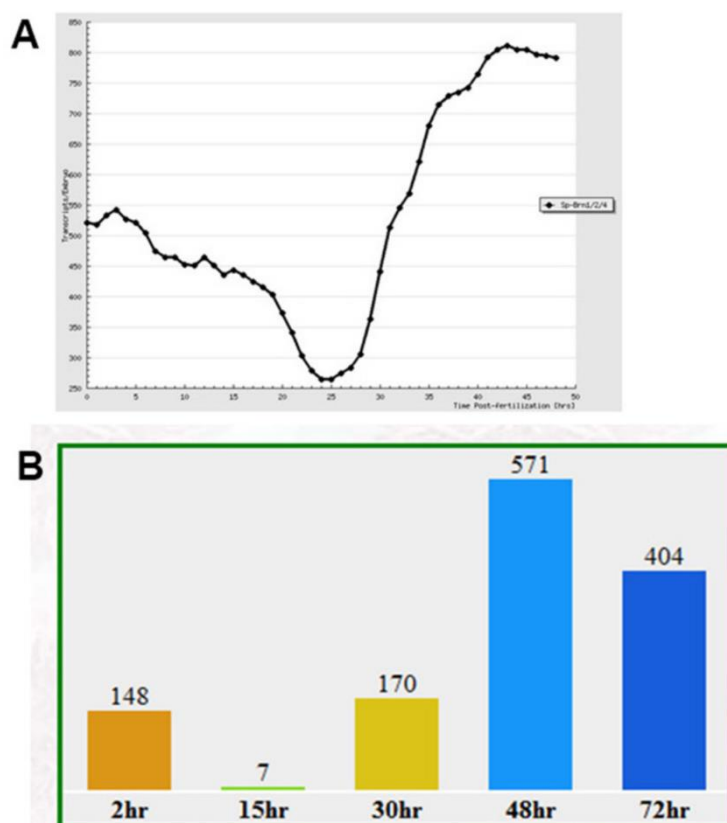


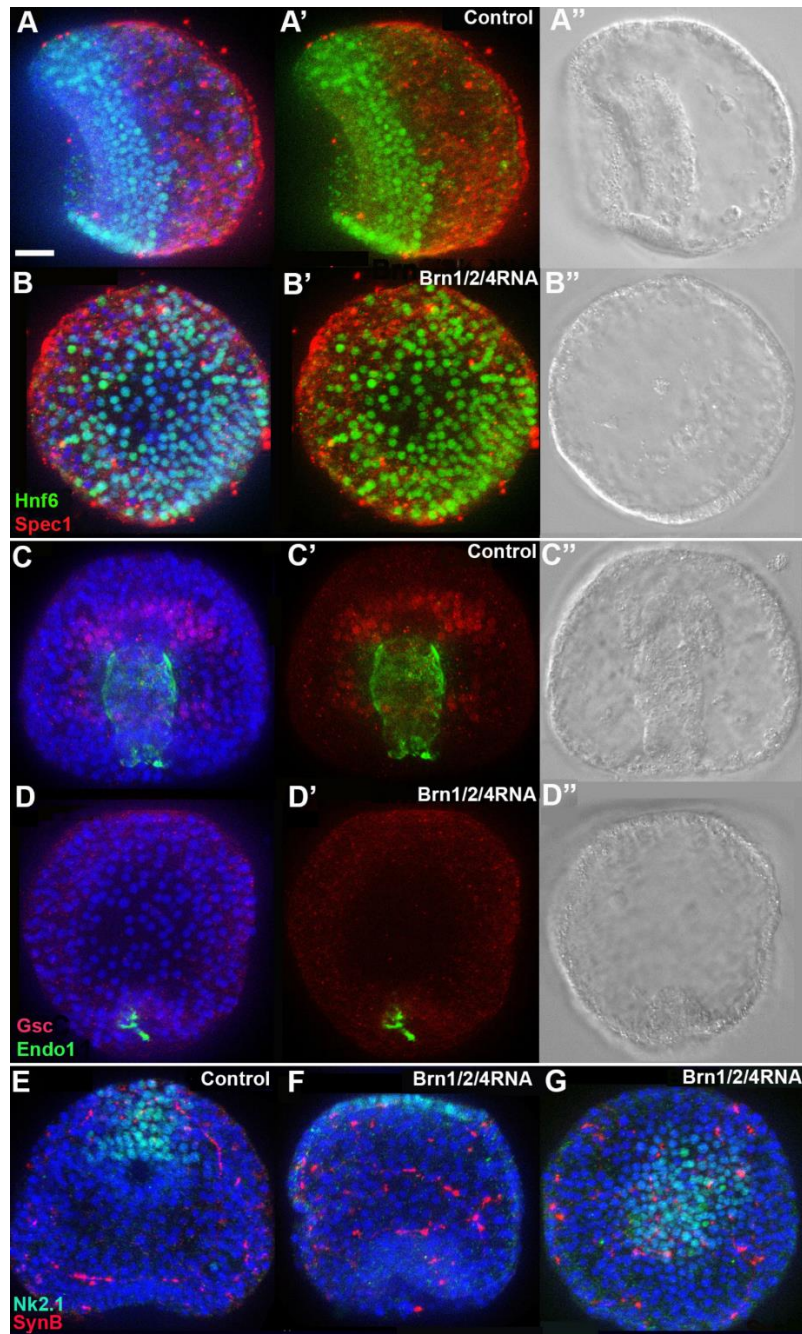
**Supplementary Figure S1. Diagram of neurogenic regions of a 3-day pluteus larva discussed in this work.** Left, a view from the ventral (oral) side, with the APD at top. Right, a lateral view. SynB neurons (yellow) differentiate in or adjacent to the APD (green) and ciliary band (magenta), while serotonergic neurons (dark blue) are restricted to the apical plate. Two additional neurons differentiated in the pharyngeal endoderm (brown).



**Supplementary Figure S2. *SoxC* morpholino knockdown prevented accumulation of SoxC protein and *Z167* or *Brn1/2/4* knockdown did not reduce *SoxC* expression.** (A and B) SoxC antibody immunostained (green) control (A) and *SoxC* morpholino-injected (B) embryos. SoxC protein signal was eliminated in the morphant. Embryos were at 52 hours. SoxC antibody courtesy of Dr. Robert Burke. (C and D) Control embryo (C) and *Z167* morphant hybridized with probes for *SoxC* (green) and *Z167* (red) transcripts. *Z167* knockdown does not affect SoxC expression, but *Z167* transcripts are down regulated, indicating its probable negative autoregulation. Control (E) and *Brn1/2/4* knockdown embryos (F) hybridized in situ for SoxC (red) and *SynB* (green). *Brn1/2/4* knockdown does not affect expression of *SoxC*, but prevents formation of *SynB*-positive neurons. Embryos were at 46 hours and are shown in lateral view with the APD at top. Nuclei were stained with DAPI (blue). The white bar in A represents 20 micrometers.



**Supplementary Figure S3. SoxC mRNA accumulates in two phases.** (A) Temporal expression pattern from a high-resolution transcription analysis (Materna et al., 2010). (B) Temporal expression pattern from a microarray experiment (Wei et al., 2006).



**Supplementary Figure S4. Further analysis of the *Brn1/2/4* mis/overexpression phenotype.** (A and B) Control (A) and *Brn1/2/4*-overexpressing embryos (B) immunostained for Hnf6 (ciliary band and APD, green) and Spec1 (aboral ectoderm, red). (C and D) Control (C) and *Brn1/2/4*-overexpressing (D) embryos immunostained for GSC (oral ectoderm, red) and Endo1 (midgut and hindgut, green). (A''-D'') are the corresponding DIC images and DAPI fluorescence (blue) is shown in (A-D). (E, F and G), Control (E) and *Brn1/2/4*-overexpressing (F and G) embryos immunostained for SynB (red) and Nk2.1 (green), a transcription factor operating in a gene regulatory pathway separate from that for serotonergic neurons, which leads to production of the ciliated apical tuft (Dunn et al., 2007). SynB staining encompasses most of the ectoderm and Nk2.1 staining is radialized, lacking dorsal-ventral polarity. Nuclei are stained with DAPI (blue). E and F are lateral views and G is toward the APD. The white bar in A represents 20 micrometers.