Supporting Information:

Supporting Materials and Methods:

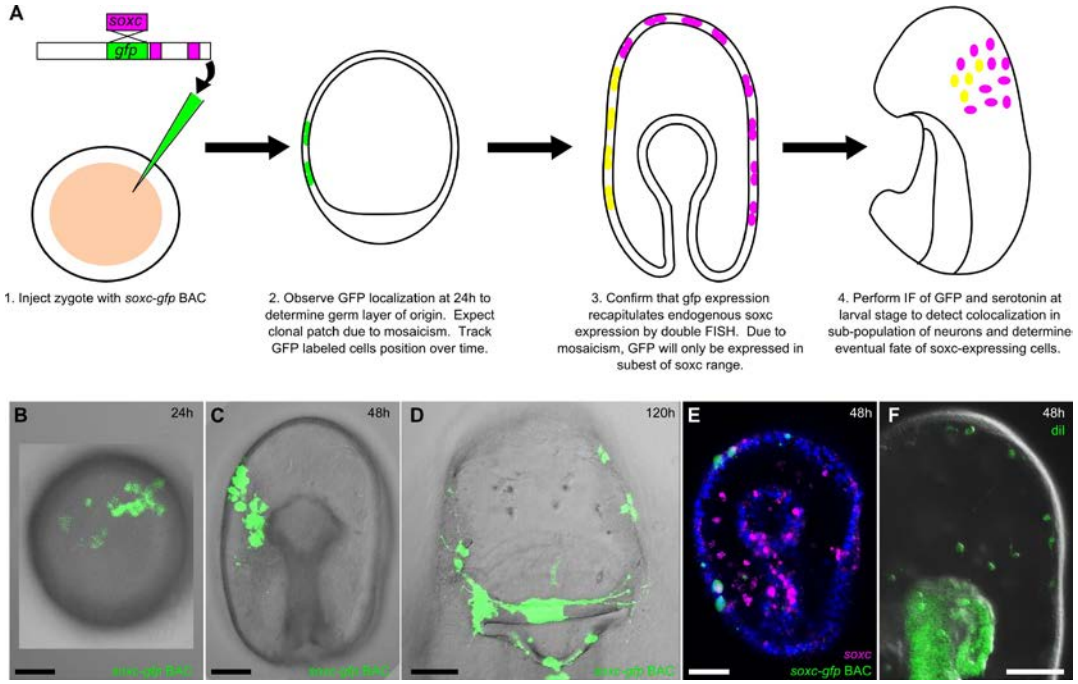
Morpholino Sequences:

*Pm*Lhx2/9: 5’ CCGGTTGCAAAGTGAAATACATTCA 3’
*Pm*Rx: 5’ GCACAGCTCCAACCCAGATAGCATC 3’
*Pm*FoxQ2_MASO2: 5’ TAAACGATGTTCTCTGCAAGACGGT 3’
*Pm*Lhx2/9_MASO2: 5’ AGTGTCCGAGCAAAACAAGAGTTGA 3’
*Pm*Soxc_MASO2: 5’ TCTGAAGATACGTGGATGAAACTGA 3’
*Pm*Rx_MASO2: 5’ TGGTCGCACGGTCCAGATACCAATT 3’
Other MASO sequences (i.e., *Pm*Foxq2, *Pm*Six3 1 and 2, *Pm*Soxc) can be found in our previous publication (1).

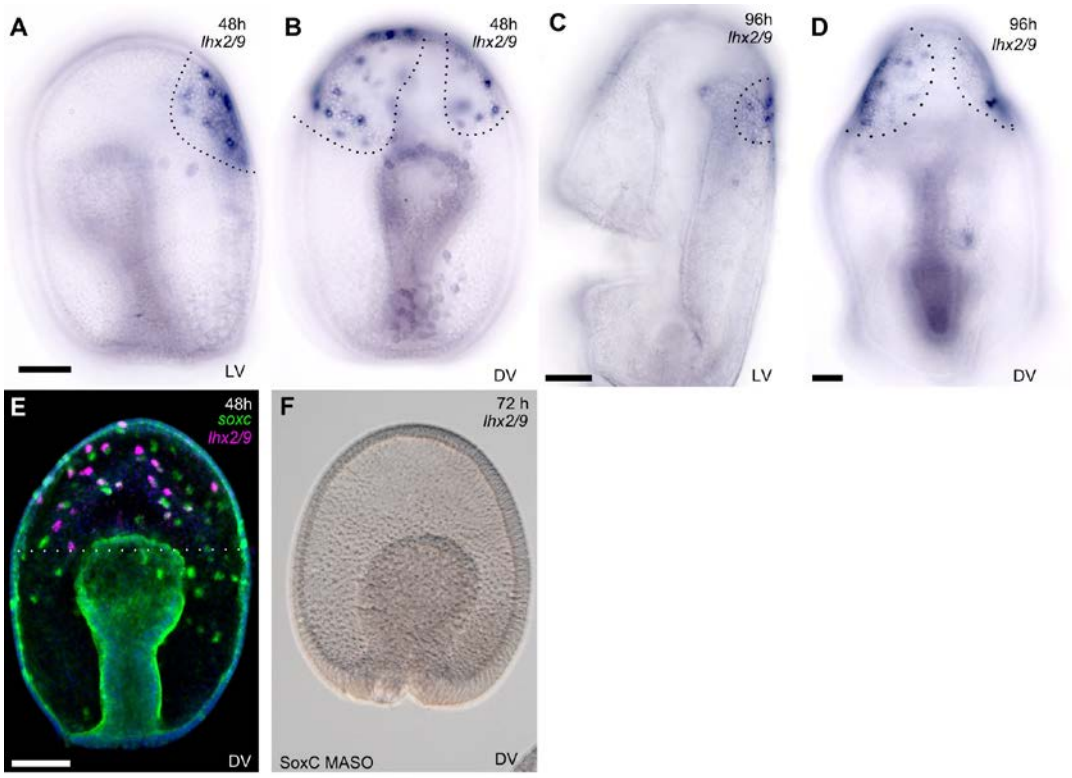
Immunofluorescence: Embryos were fixed in 4% paraformaldehyde/MOPS (100mM MOPS, 2mM MgSO4, 1mM EGTA, 80mM NaCl, 0.1% Triton x-100) at room temperature for 90 minutes. They were subsequently stored in 70% EtOH at -20°C until use. Rehydration was accomplished by stepping embryos into MAB/0.1% Triton x-100 (100mM maelic acid, 150mM NaCl), followed by four washes in MAB/0.1% Triton x-100, and blocked in 2% BSA/MAB/0.1% Triton x-100. Embryos were next incubated with 1:250 rabbit anti-serotonin (Sigma), and in some cases also mouse anti-GFP (Pierce), diluted in 2% BSA block, overnight at 4°C. Antibodies were removed by washing four times with MAB/0.1% Triton x-100, and the embryos were next incubated with 1:1000 anti-rabbit alexa-fluor 568 or 1:1000 anti-mouse alexa-fluor 488 as appropriate, diluted in 2% BSA block. Finally, embryos were rinsed twice, incubated with 1:10,000 DAPI for 20 minutes, and rinsed four more times in MAB/0.1% Triton x-100. Imaging was performed as described for FISH.

EdU Labeling: Briefly, EdU was added to embryo culture at a final concentration of 10 µM and incubated for 15 minutes at 15°C. For pulse-chase experiment, EdU was subsequently flushed out with artificial sea water, and embryos were incubated an additional 30 minutes. Embryos were fixed and processed for FISH as previously described. After completion of the FISH protocol, embryos were washed three times in PBS, permeabilized in 0.5% Triton-x100/PBS, and then washed three times in 3% BSA/PBS. Embryos were incubated for 30 minutes in Click-It cocktail (prepared according to manufacturer’s instructions, purchased from Thermo Fisher). After three additional PBS washes, nuclei were stained with DAPI, and embryos were imaged by confocal microscopy as described above.

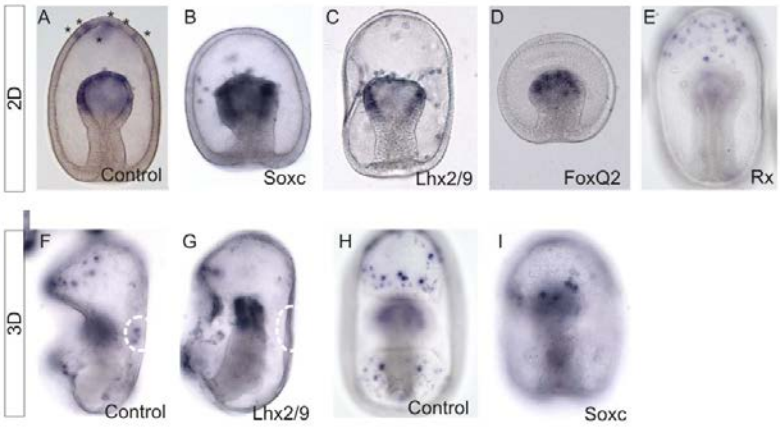
Supplemental Figures:



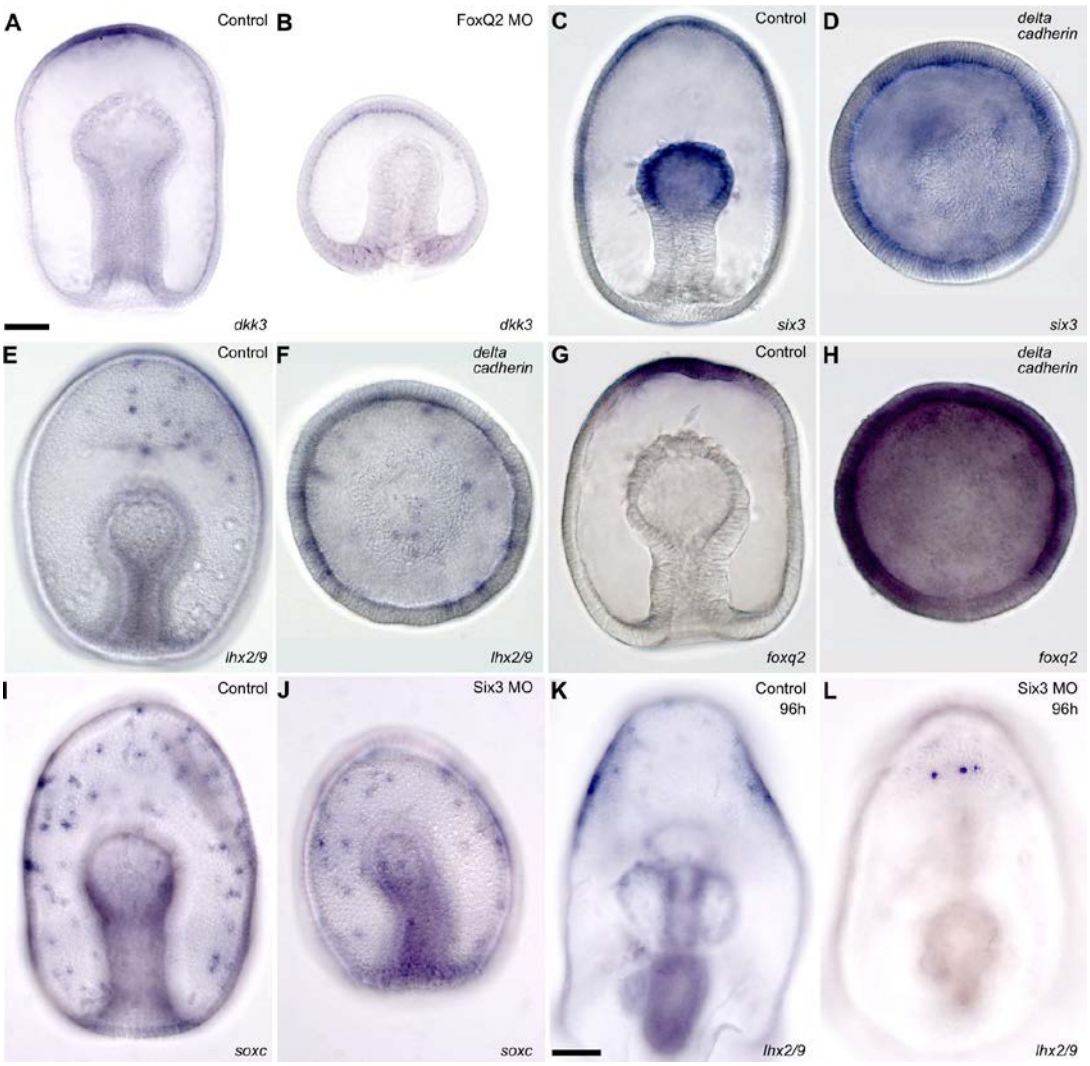
Supplemental Figure 1: Soxc-GFP knock-in BAC recapitulates endogenous ectodermal *soxc* expression. A. Schematic depicting GFP BAC injection and subsequent expression. B. BAC-directed GFP expression in a 24h embryo. C. BAC-directed GFP expression in a 48h embryo. D. BAC-directed GFP expression in a sub-population of mature neurons in the apical organ and mouth. E. *gfp* transcripts (green) resulting from BAC expression overlap in clonal patches with endogenous *soxc* expression (magenta) as determined by double FISH. This indicates that the BAC construct faithfully directs *gfp* expression in a pattern that mimics *soxc*'s expression. F. A lineage of mesodermal cells was labeled with diI by injection at the 8-cell stage. At 48h, some of these cells ingress into the blastocoel, but do not embed themselves within the ectoderm. This indicates that *elav*+ cells found in the ectoderm are of ectodermal, rather than mesodermal origin. Scale bars indicate 50 µm.



Supplemental Figure 2: *Lhx2/9* expression is specific to the presumptive apical organ. A. Lateral view (LV) of a 48 h embryo. The dorsal face is on the right. *Lhx2/9* expression occurs in spots in the anterior dorsal ectoderm, outlined by dashes. B. Dorsal view of a 48 h embryo reveals lhx2/9 expression in two anterior ectoderm clusters. C. A lateral view of a 96 h larvae, with the dorsal face positioned to the right. *Lhx2/9* expression is localized to the dorsal ectoderm. D. A dorsal view of a 96 h larvae. *Lhx2/9* is expressed in two clusters in the anterior ectoderm. E. *Soxc* and *lhx2/9* are co-expressed in the anterior ectoderm, but pairs of *soxc*+ cells without *lhx2/9* are also present more posteriorly (below the dotted line). F. 72 h SoxC morphant embryo still does not express *lhx2/9* in spite of additional time to develop. SoxC morphants do not achieve a normal 72 h phenotype, most likely because early ectodermal progenitors require this gene product. Scale bars indicate 50 µm.



Supplemental Figure 3: A-G Confirmation of morpholino phenotypes with use of second independent MASO sequence. All staining is for the expression of Elav. Second morpholinos to *soxc*, *lhx2/9*, *foxq2*, and *rx* were injected into embryos at 600 micro molar and expression of *elav* was analyzed to confirm knock-down phenotypes match those described in the main text and figures. In all cases, phenotypes correspond to previous results; i.e. loss of apical ectodermal *elav* expression in Soxc, Lhx2/9 and FoxQ2 morphants, and expansion of *elav* cells in Rx morphants. H-I Older stage soxc morphants (3d) injected with lower doses of MASO (400 micromolar) are able to develop to larval stage, but still show a very reduced number of *elav*+ neurons. Stars in A show location of *elav* positive cells in control 48h embryo. Dashed circles in F and G show location of dorsal ganglion in this stage, which has no elav+ cells in Lhx2/9 morphants.



Supplemental Figure 4: Roles of AP patterning genes in establishing unique neurogenic domains. All embryos are 48 h unless otherwise indicated. A. *dkk3* is expressed in domain 1, the apical pole domain. B. *dkk3* expression is lost when Foxq2 is knocked-down. C-H. Wnt signaling limits expression of AP domains to their proper boundaries. Canonical wnt signaling is perturbed by the introduction of *delta-cadherin* mRNA. C. *six3* is expressed in the mesodermal bulb, and also in domains 1-3 of the ectoderm. D. *Delta-cadherin* injected embryos ubiquitously express *six3*. E. *lhx2/9* is normally expressed in domains 1-2, but expands throughout the ectoderm in *delta-cadherin* embryos (F). G. *foxq2* expression is limited to domain 1 in control embryos. H. *Delta-cadherin* embryos exhibit ubiquitous *foxq2* expression. I. *soxc* is expressed in pairs of cells throughout the ectoderm. J. Six3 morphants express *soxc* normally. K. *lhx2/9* is expressed in bilateral clusters in the anterior dorsal ectoderm at 96 h. L. 96 h Six3 morphants produce notably reduced numbers of *lhx2/9*-expressing cells. Scale bars indicate 50 µm.