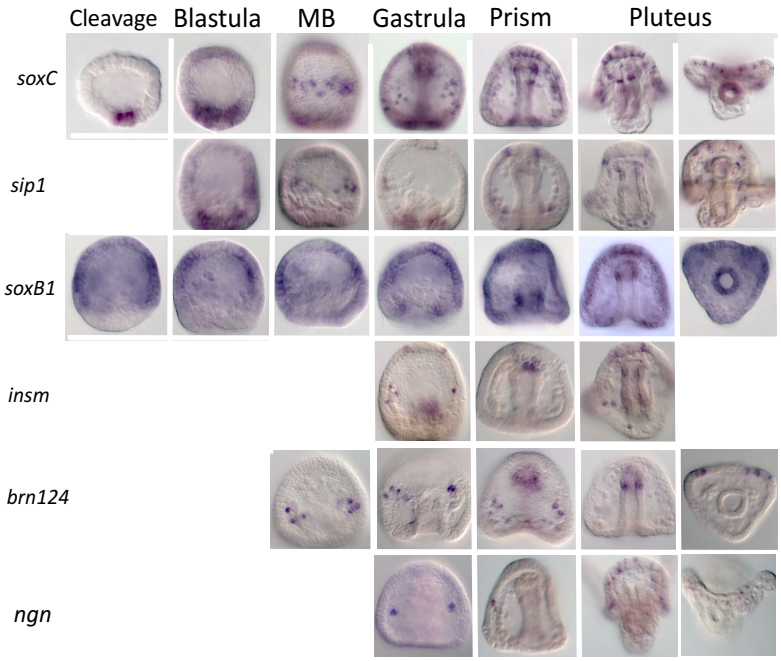

Fig. S1. Proneural transcription factors used. Shown are developmental expression profiles of the proneural transcription factors used in these experiments. The images from cleavage on the left to prism and early pluteus stages on the right reflect the timing of first expression of the genes and their patterns of expression over time. The three neural domains often were difficult to capture in the same focal plane so in some cases images reflect a midsaggital section with the CB domain of expression on one side, and in other cases the ANE domain, when lightly stained, is out of focus.

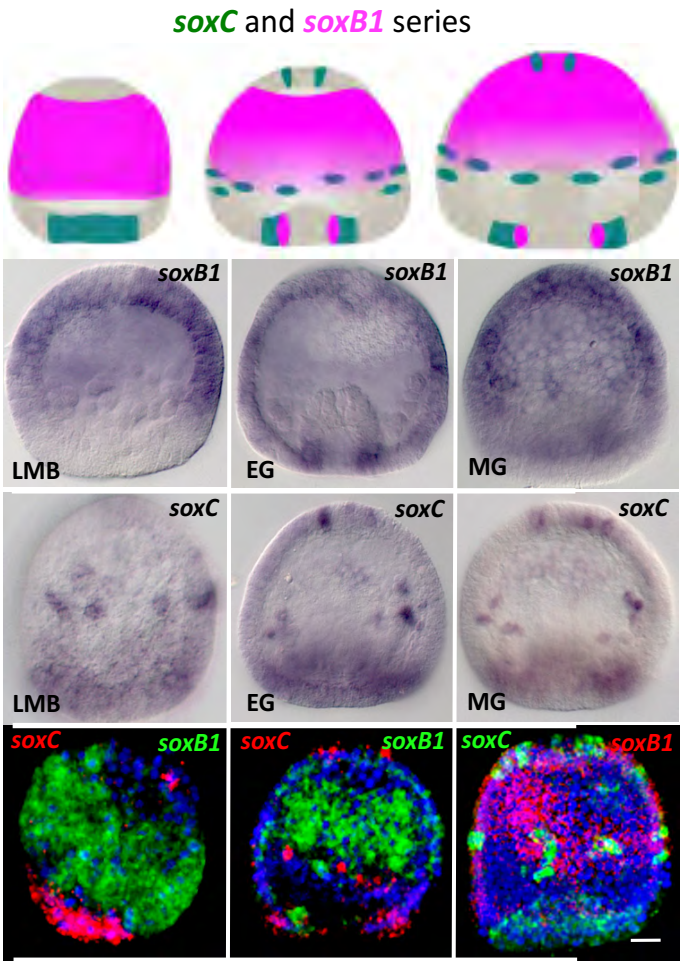

Fig. S2 Expression of soxC and soxb1 at the time neurogenesis is initiated. Just prior to invagination of the archenteron zygotic expression of soxb1 is seen as a light haze in an ectodermal band surrounding the middle of the embryo with little to no stain seen at the anterior end or at the posterior end of the embryo. SoxC then is expressed at the posterior edge and anterior to the soxb1 belt. At early gastrula soxb1 is expressed at higher levels in some cells and at that time Fig. 1 shows that at least some of those cells co-express soxC. As gastrulation continues soxb1 is expressed in cells at the blastopore and at least some of those cells also co-express soxC. The cartoons at the top show our interpretation of soxb1 and soxC expression over this interval. LMB, late mesenchyme blastula; EG, early gastrula; MG, mid gastrula. Scale Bar = 20 μm.

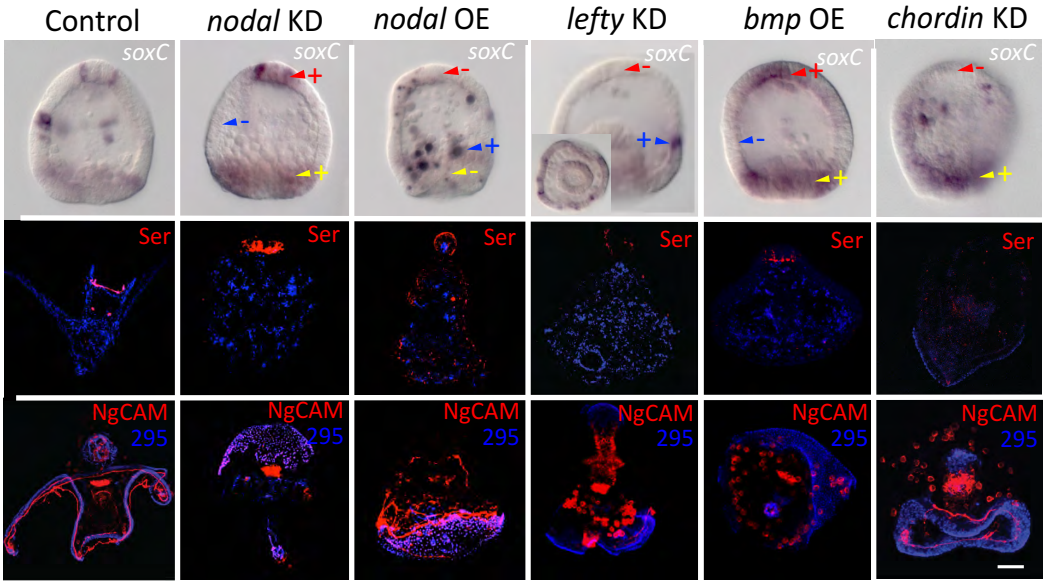

Fig. S3. Additional nodal and BMP perturbations. In addition to the nodal and BMP MO knockdowns shown in Fig. 3, other experiments perturbed nodal and BMP in different ways. Those perturbations were scored by noting proneural soxC expression, differentiation of serotonergic neurons (Ser), and of NgCAM staining of neurons. The 295 antibody stains the CB. The nodal knockdown eliminates CB neurons and expands serotonergic neurons as in Fig. 3. Overexpression of Nodal does the opposite as it results in clumps of soxC-expressing cells and a radialized embryo that contains a large number of CB neurons and no ANE neurons. Lefty knockdowns also radialize the embryo (insert of soxC shows expression all around the equator as viewed from the animal pole) and absence of Lefty allows Nodal to diffuse farther than normal thereby radializing the domain of CB neurons again at the edge of the vegetal plate. Compared to the nodal over-expression in the absence of Lefty there are very few CB neurons (The bright red cells above the blue CB are skeletogenic cells). BMP overexpression causes increased numbers of serotonergic neurons to differentiate in the ANE, and a relative loss of CB and EM neurons (skeletogenic cells only are stained). The chordin MO causes a loss of serotonergic neurons, and presence of CB and EM neurons in a partially radialized embryo. In each case the data are consistent with predictions of function based on effects of the nodal or BMP knockdowns. KD = knockdown, OE = overexpression. Scale bar = 20 μm.

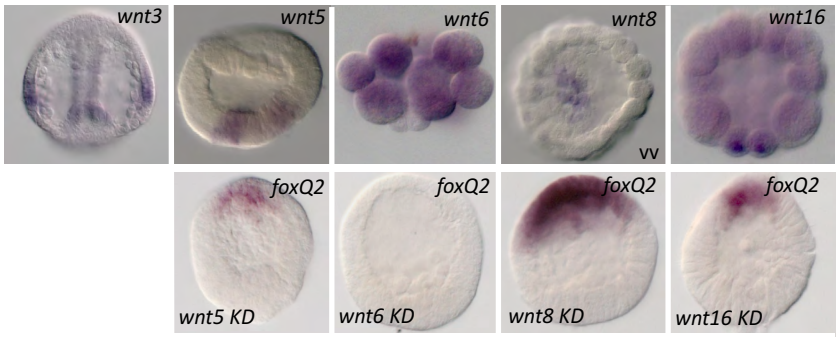

Fig. S4. Screen for Wnts that affect early neurogenesis. The top row shows the earliest expression of Wnt3, Wnt5, Wnt6, Wnt8 and Wnt16 in *L. variegatus.* The bottom row shows the expression of *foxQ2,* a gene necessary as an upstream protein for neurogenesis in the ANE. As shown, of the MO's to each of the Wnts tested only knockdown of Wnt6 eliminated expression of *foxQ2.* Wnt3 knockdowns were not tested because first expression of Wnt3 occurs after proneural specification has begun. Also, knockdown of Wnt8 causes an overexpression of *foxQ2*, an observation that was noted in an earlier publication (Range et al., 2013).

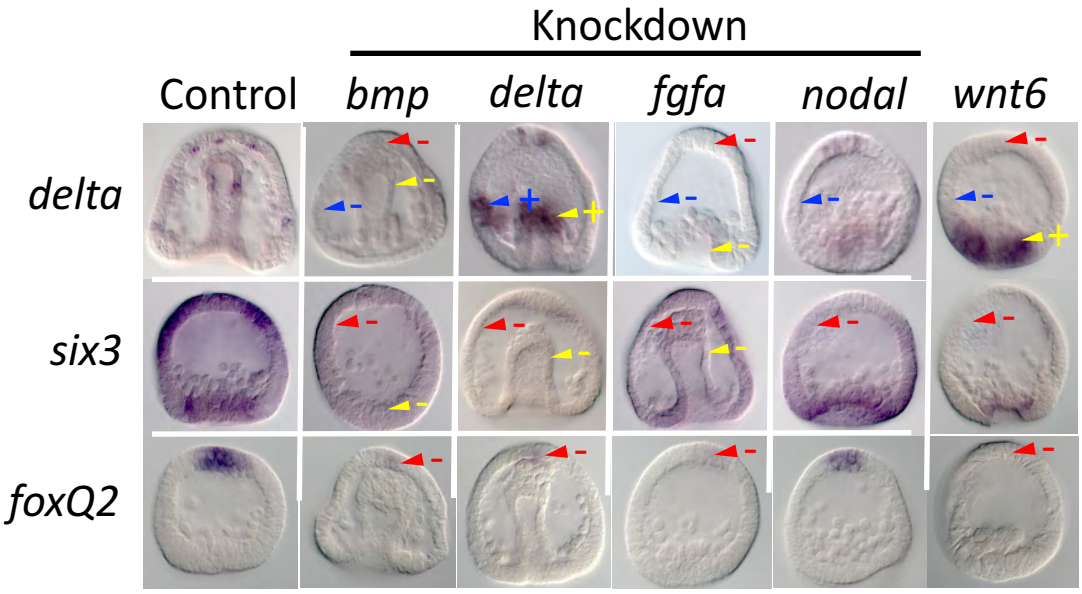

Fig. S5. Additional perturbations of genes included in the GRNs shown in Fig. 6. As with all perturbations shown, these data reflect expression seen in at least 80% of more than 100 embryos in each of three trials for each perturbation. Arrows indicate interpretation.

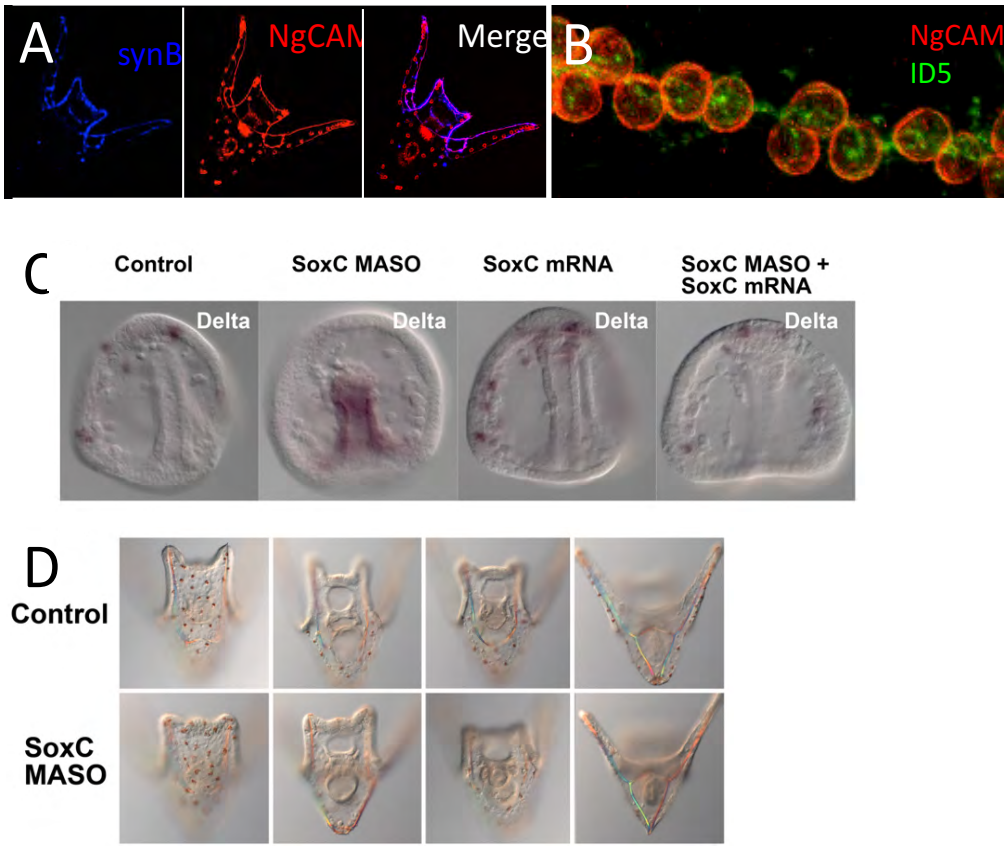

Fig. S6. Controls. A. SynB and NgCAM stain the same SynB-positive neurons. NgCAM also stains neurons of the gut sphincters and the posterior mouth ganglion. NgCAM also stains non-neural skeletogenic cells and a patch of non-neural cells in the pharyngeal sphincter as well. The non-neural staining allowed us to use this antibody in perturbations to show specific effects on neural without affecting the skeletogenic lineage. **B.** NgCAM (red) stains membranes of skeletogenic cells near the cell bodies of the syncitium. 1D5 (green) is a monoclonal antibody that stains all skeletogenic cells. **C.** A MO to soxC causes delta expression in the gut to increase while at the same time the soxC MO causes a loss of delta expression in the ANE and CB domains. Addition of soxC mRNA augments delta expression in the ANE and CB domains and also rescues the soxC MO phenotype. **D.** Under conditions where inhibition of proneural transcription factors block differentiation of serotonergic and synaptotagmin B-expressing neurons, the embryos nevertheless appear to be normal larvae. The soxC MO used in the perturbed embryos is the same concentration as shown in Fig. 3, and both control and soxC-MO panels show different focal planes of two larvae each. Both soxC morpholinos eliminate neurons but do not affect the overt appearance of pluteus larva.

Table S1. Genes screened for neural markers in early sea urchin neurogenesis

Gene	Type	Reference	Gene	Type	Reference
acSc	bHLH	1,2	nacha	acetylcholine receptor	29
ato	bHLH	1	netrin	axon guidance protein	1,18
bace	beta secretase	3	neurod	bHLH	1,11
brn124	Pou	1,4	nfe2	basic zipper	11
delta	signal ligand	5	ngn	bHLH	1,2,19
dlx	h-box	1	nos1	enzyme	30
ebf3	HLH	1	nt	NGF	31
emx	Hox	1,4	otp	homeobox	2,20
ese	ets-related	6	prox	homeobox	16,21
eya	coactivator	7	rx	homeobox	1,16
foxg	forkhead	1,8	sip1	zinc finger	33
foxM	forkhead	1,8	six3	homeobox	1,16
glass	zn finger	1,9	soxb1	sox/hmg	22
glia	zn finger	28	soxc	sox/hmg	11,23
hbn	hbox-paired	1,10	synb	synaptotagmin b	1
hey	bHLH	1,11	th	tyrosine hydroxylase	1,2
hey4	bHLH	11	tlx	nuclear receptor	24
hnf4	nuclear receptor	12	trak	mitochondrial protein	25
hox7	homeobox	16	trim	neural ubiquitin ligase	26
insm	Snag zn finger	13	vacht	transporter	27
islet	lim homeobox	1,14	zic	zn finger	32
lox	parahox	15			
mbx	homeobox	17			

1 (Burke et al., 2006a)
2 (Slota and McClay, 2018)
3 (Vassar et al., 2009)
4 (Yuh et al., 2005)
5 (Sweet et al., 2002)
6 (Rizzo et al., 2006)
7 (Pignoni et al., 1997)
8 (Tu et al., 2006)
9 (Moses et al., 1989)
10 (Walldorf et al., 2000)
11 (Howard-Ashby et al., 2006b)
12 (Duncan et al., 1994)
13 (Chiang and Ayyanathan, 2013)
14 (Lee et al., 2015)
15 (Arnone et al., 2006)
16 (Howard-Ashby et al., 2006a)
17 (Kawahara et al., 2002)
18 (Serafini et al., 1994)
19 (Sun et al., 2001)
20 (Di Bernardo et al., 1999)
21 (Torii et al., 1999)
22 (Kenny et al., 1999)
23 (Garner et al., 2016)
24 (Yu et al., 2000)
25 (van Spronsen et al., 2013)
26 (Khazaei et al., 2011)
27 (Prado et al., 2006)
28 (Vokes et al., 2007)
29 (Miyazawa et al., 2003)
30 (Bredt and Snyder, 1989)
31 (Patruno et al., 2001)
32 (Materna et al., 2006)
33 (Sheng et a., 2003)