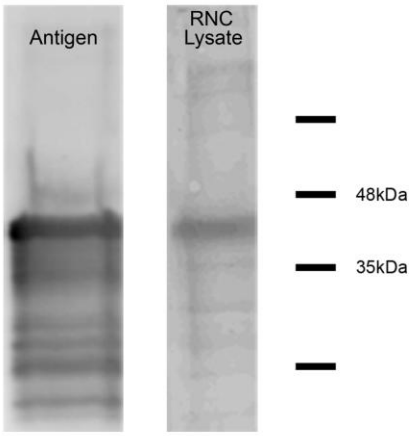
Supplemental Figure S1

SoxC

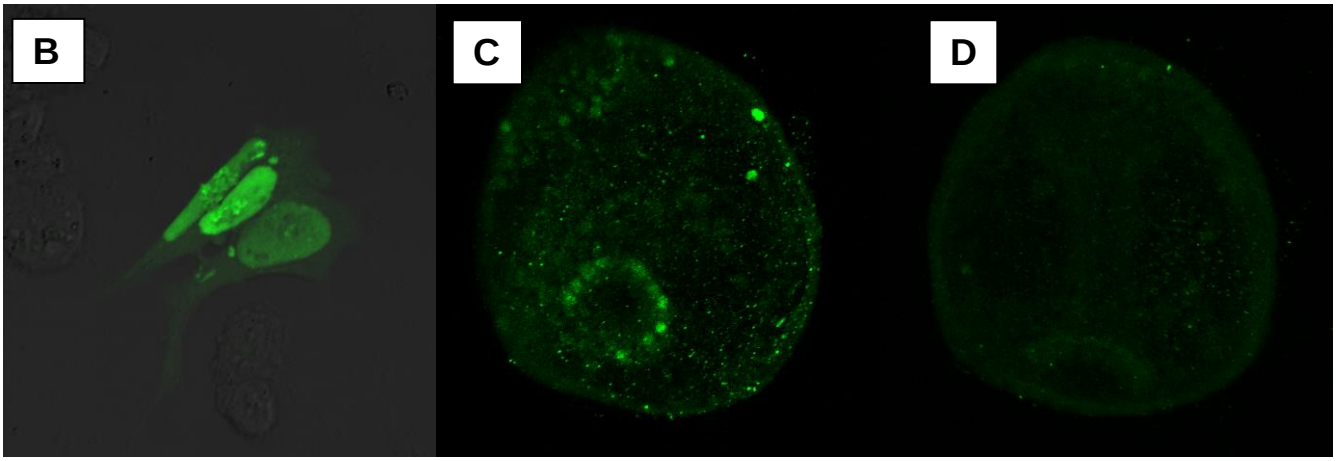
The predicted SoxC protein sequence is from the curated echinoderm genomic database, Echinobase (http://www.echinobase.org/Echinobase/). The bold sequence was amplified from cDNA, cloned, expressed and purified as described in Methods. A rat was immunized and a polyclonal serum prepared, as well, a mouse was immunized and a monoclonal antibody prepared. The two antibodies revealed identical immunolocalizations in doubly labelled preparations, and immunoreactivity was lost in embryos derived from eggs injected with a SoxC MASO. When HEK cells were transfected with a full length plasmid encoding urchin SoxC, they were immunoreactive to anti-SoxC.

>SPU_002603.3a Peptide Sequence
MVPQNLTSNGLITMQSQQIPNGHGSPGSTGSSEELRQSTLDIAEDICQTNWKGNNGHI
KRPMNAFMVWSQIERRRIMETTPDMHNAEISKRLGRRWKTLDEVAKSPYVEEAERLR
LLHMAQYPDYKY**RPRKKSKPTTKPEAAKTTSSKPKANKPKSSSKLTKMNGIVIDQMH**
PHQIVQSGRIEKIPKLKLTIDKKFRENMKASKIVELVPSQLTPPAKVPASPTGSNTDPC
NEQSLYEDYANIQHTYEMQRYEFGVPSGATSTTCSSPASSDVSQQSSMSTNSSVSS
MSTGSSYCAQQSIEDVVFPGGLTGSEFNNFNFGSVPDDLSPLDSVGSNGSHFEFPDY
TTPEVSELIDSDWLLSSMISAYN

Native protein predicted size: 41 kDa
Predicted expressed protein size: 30 kDa
A



Panel A. Lane 1 Immunoblot of antigen. The expressed SoxC protein used for immunization was probed with hybridoma supernatant that was derived from a mouse immunized with bacterially expressed SoxC protein. SoxC coding sequence was amplified from *S. pupuratus* cDNA and expressed in *E.coli* (see Methods).
Lane 2 Immunoblot of lysate of *S. pupuratus* radial nerve cord (RNC) probed with rat polyclonal antibodies directed against SoxC. Polyclonal antibodies were derived from serum of rats injected with bacterially expressed SoxC protein.



B. HEK cells transfected with pCS2+:SoxC, prepared with anti-SoxC antibody (rat serum) for immunofluorescence. Transfected cells were immunoreactive and indicate a nuclear localization of the SoxC protein.
C. Embryos (48h) from eggs injected with control MASO and prepared with anti-SoxC (mouse anti-SoxC hybridoma supernatant).
D. Embryos (48h) from eggs injected with SoxC MASO and prepared with anti-SoxC (mouse anti-SoxC hybridoma supernatant).

There are data from in situ hybridizations (Howard-Ashby et al. 2006; Poutska et al. 2007) with Sox C and the antibody localizations are consistent with the data and descriptions provided. In general there are more cells immunoreactive with the antisera than identified with in situ hybridization. However, this is typical of mRNA being less stable than protein and having a shorter half-life.

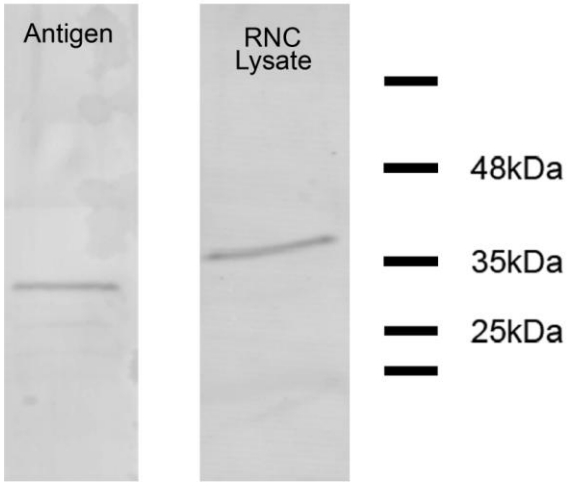
SoxB2

The predicted SoxB2 protein sequence is from the curated echinoderm genomic database, Echinobase (http://www.echinobase.org/Echinobase/). The bold sequence was amplified from cDNA, cloned, expressed and purified as described in Methods. A rat was immunized and a polyclonal serum prepared.

>SPU_025113.3a Peptide Sequence
MMMDSAMGKGTDHVKRPMNAFMVWSRGQRRKLAQENPKMHNSEISKRLGAEWKL LSEDDKRPFIDEAKRLRALHMKEHPDYKYRPRRKPKSLMKRDKYAFPIPCIPTSSPY QVATSQADIMNMASAEKARTYLSSHQHHAAAAAAAASQYSVLEHAQKLESPTSLIR DFPHHPALYPPPHMYPTSAGAVPGSAFGKLPGGSAAAAAAAAAAGYSAQPYMMPY PAWPGQDGVQRPVAYILVKPDMEPYGPTHPAIRPTMPLPTRPTAATAL

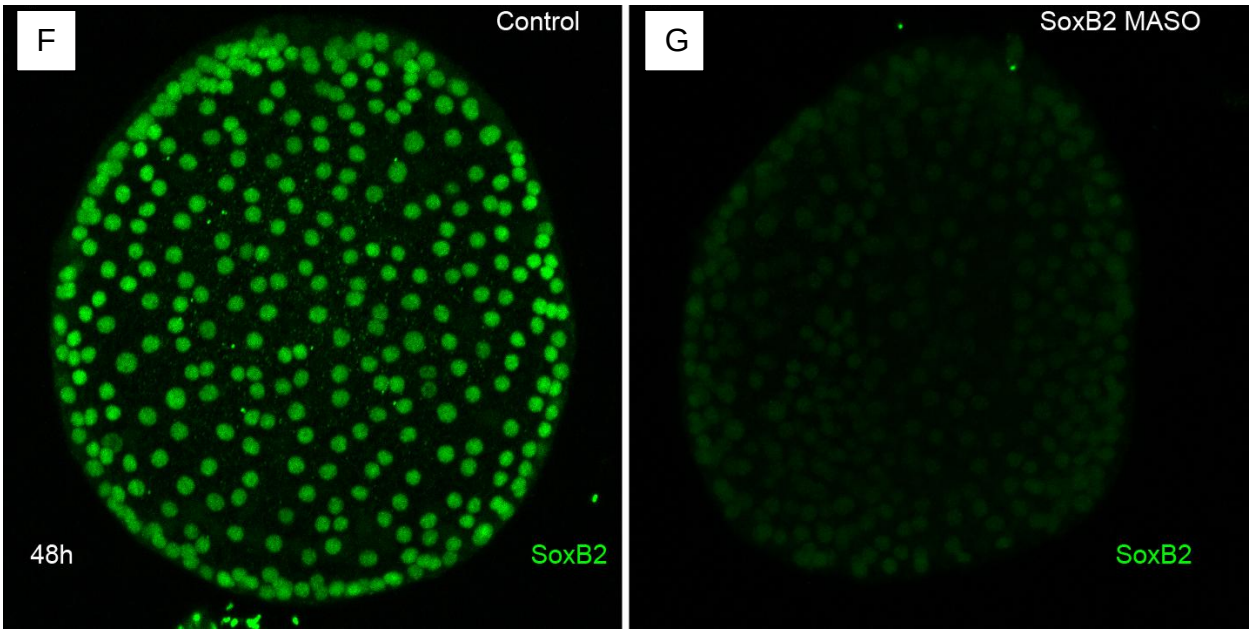
Native protein predicted size: 30 kDa
Predicted expressed protein size: 25 kDa

E



Panel E Lane 1 Immunoblot of SoxB2 antigen. The expressed SoxB2 protein used for immunization was separated by PAGE, transferred, and the membrane probed with serum of a rat immunized with bacterially expressed SoxB2 protein. SoxB2 coding sequence was amplified from *S. pupuratus* cDNA and expressed in *E.coli* (see Methods).

Lane 2 Immunoblot of lysate of *S. pupuratus* radial nerve cord (RNC) probed with rat serum immunized with SoxB2..



Maximum intensity projections of embryos prepared with anti-SoxB2 serum. F. Control, 48h embryo showing the normal distribution of SoxB2. G. Embryo derived from egg injected with 400 µM SoxB2 MASO, prepared as in panel F and imaged with identical settings. There is only a very weak signal indicating that there is reduced antibody binding and that the MASO has suppressed expression of the SoxB2 protein.

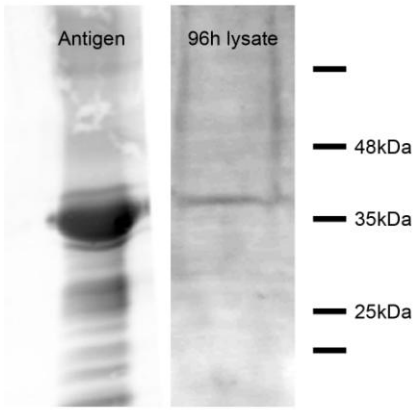
ELAV

The predicted ELAV protein sequence is from the curated echinoderm genomic database, Echinobase (http://www.echinobase.org/Echinobase/). The bold sequence was amplified from cDNA, cloned, expressed and purified as described in Methods. A rat was immunized and a polyclonal serum prepared, as well, a rabbit was immunized and a polyclonal serum prepared. The two antibodies revealed identical immunolocalizations in doubly labelled preparations.

>SPU_002324.3b Peptide Sequence
MINVIDNMEAQTVQPA**MQNGGLMKPNVVGVGGDEDSKTNLIVNYLPQNMAQDEMKS LFGKFGEIESCKLVRDKLTGQSLGYGFVNYLKPADALKAVKTLNGLRLQCKTIKVSFA RPSSQAIKDANLYISGIPKHYGQLDLDNLFNAFGRIICSRLLLDHECGRPRGVGFVRY DRRCEAEKAIEGLNGNIPHGGKDPLIVKFANNPGQHYQKCLQQMYQQMPIISPTLSP RRVGGPVSAGGSQNFIGPMRHMAHCFRWQKMGSKMQGLIGKLLPKNFMFNPMTSS DVISHMNLQAMTNNGQGWCIFVYNLPADCEDGLLWQLFGPYGAVTNVKVVRDQPN QRCKGYGFVNMLNYDEALSAINTLNGYQLNGKRTLQVSFKSSKQKS**
385 AA

Native protein predicted size:
SPU_002324.3b: 43 kDa
SPU_002324.3a: 41 kDa
SPU_002324.3b: 41 kDa
SPU_002324.3a: 40 kDa

H



Panel H. Lane 1 Immunoblot of ELAV antigen. The expressed ELAV protein used for immunization was separated by PAGE, transferred, and the membrane probed with serum of a rabbit immunized with bacterially expressed ELAV protein. ELAV coding sequence was amplified from *S. pupuratus* cDNA and expressed in *E.coli* (see Methods).

Lane 2 Immunoblot of lysate of *S. pupuratus* plutei (96h) probed with serum of a rabbit immunized with ELAV..

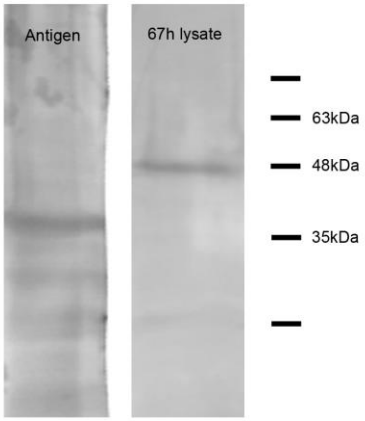
Brn1/2/4

The predicted Brn1/2/4 protein sequence is from the curated echinoderm genomic database, Echinobase (http://www.echinobase.org/Echinobase/). The bold sequence was amplified from cDNA, cloned, expressed and purified as described in Methods. A rat was immunized and a polyclonal serum prepared.

>SPU_016443.3a Peptide Sequence
METVISTPYSLSLSQADITPSSNSLILSSATDHIHHNITSLSDNSMQSGQVNVMYQKLGN
EFLQQQGGNGLPLAHHGAQWVTGLSAPPHADPTSHWAGVPAHLLGHGQDIKPNLGQ
TRDEINELHRSGHSHVQSAATWNTGNA**HMAMPMSMTMPMTTSSGGGPLGHTPTSA**
HPMYTYGAMNGMMSCAQQFGQNGPMRGVLGPGGGQLPSHNGSETVIEDDAPSSD
DLEQFAKTFKQRRIKLGYTQADVGLALGTLYGNVFSQTTICRFEALQLSFKNMCKLK
PLLAKWLEEADSTSGSPTSLDKIAAQGRKRKKRTSIEVTIKGALENAFLKQPKPSAQ
EISALADGLQLEKEVVRVWFCNRRQKEKRMTPPLNGIGPGGMQSADSPPPGQAATG
EHVLPHSTASGLHHHHHHHHPVITSLSSHAHHIGPGSSPIHGPPASVSPPAVHSPISSA
LTPHSQHQAQSVQ

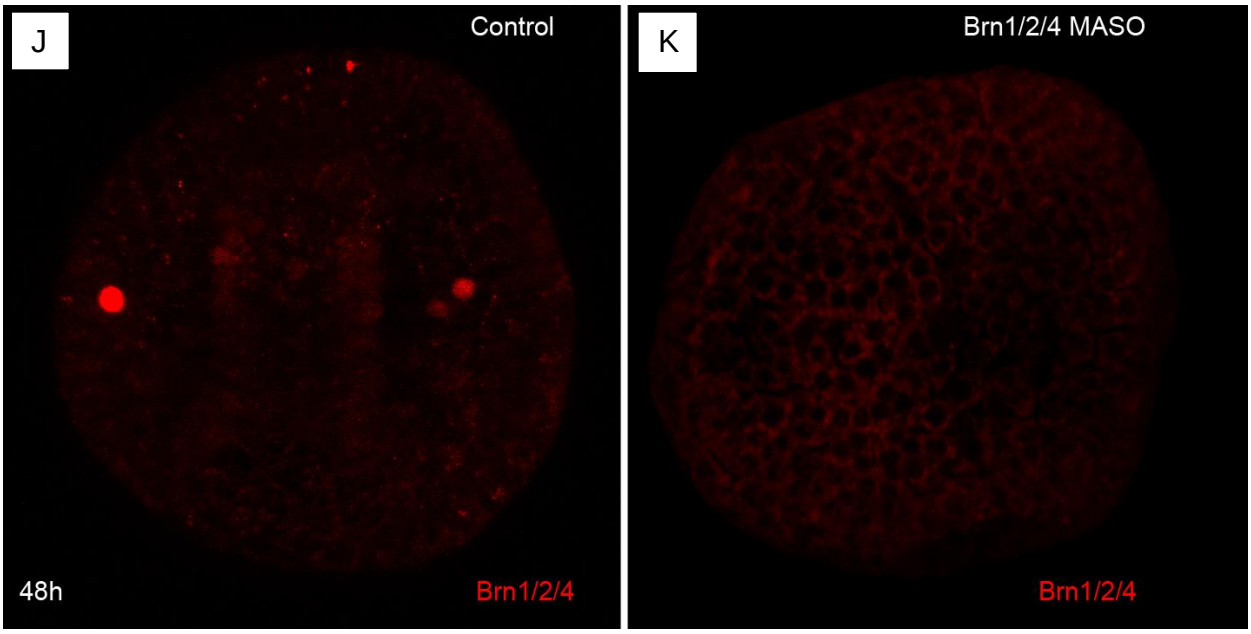
Native protein predicted size: 50 kDa
Predicted expressed protein size: 33 kDa

I



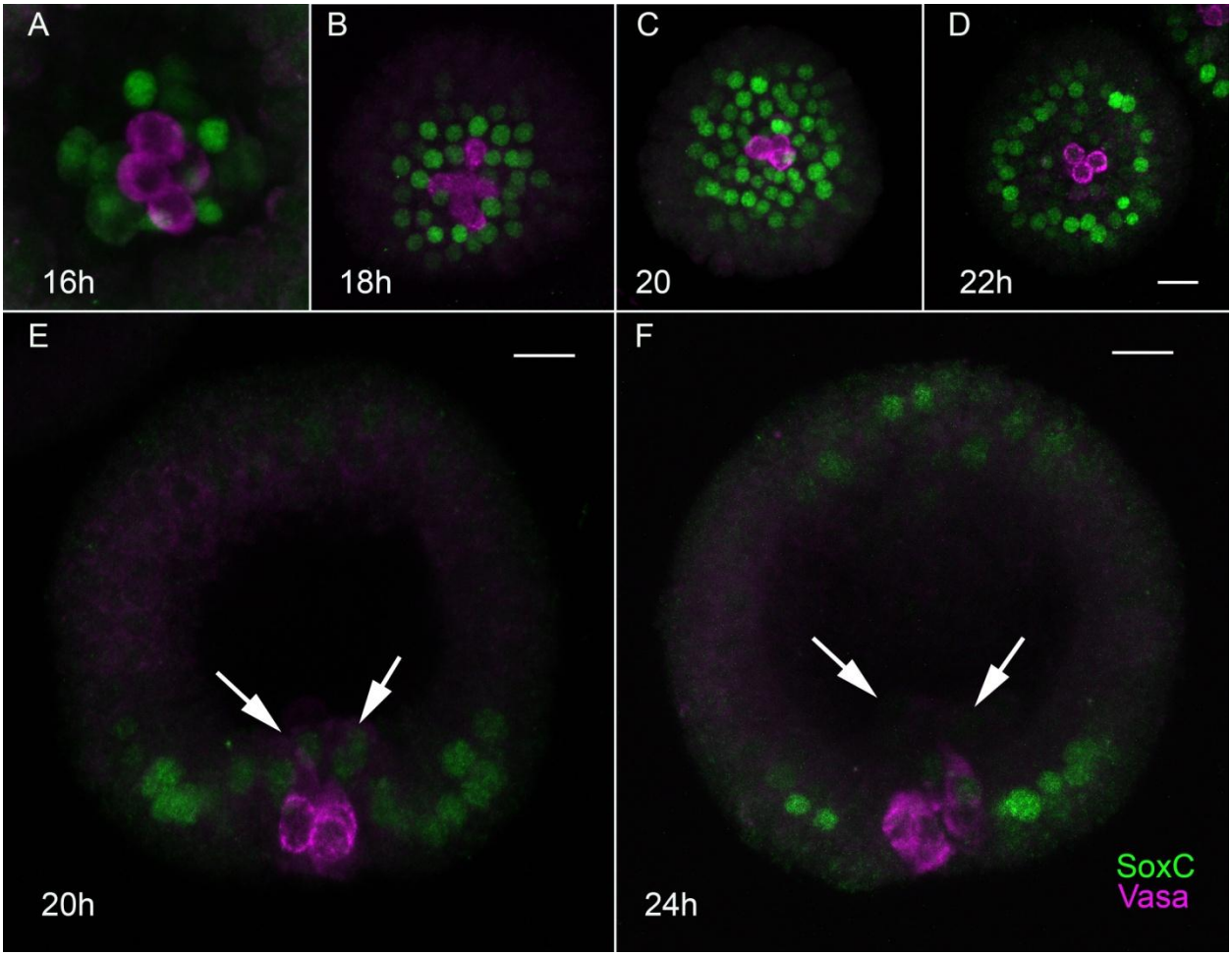
Panel I. Lane 1 Immunoblot of Brn1/2/4 antigen. The expressed Brn1/2/4 protein used for immunization was separated by PAGE, transferred, and the membrane probed with serum of a rat immunized with bacterially expressed Brn1/2/4 protein. Brn1/2/4 coding sequence was amplified from *S. pupuratus* cDNA and expressed in *E.coli* (see Methods).

Lane 2 Immunoblot of lysate of *S. pupuratus* prisms (67h) probed with serum of a rat immunized with Brn1/2/4..



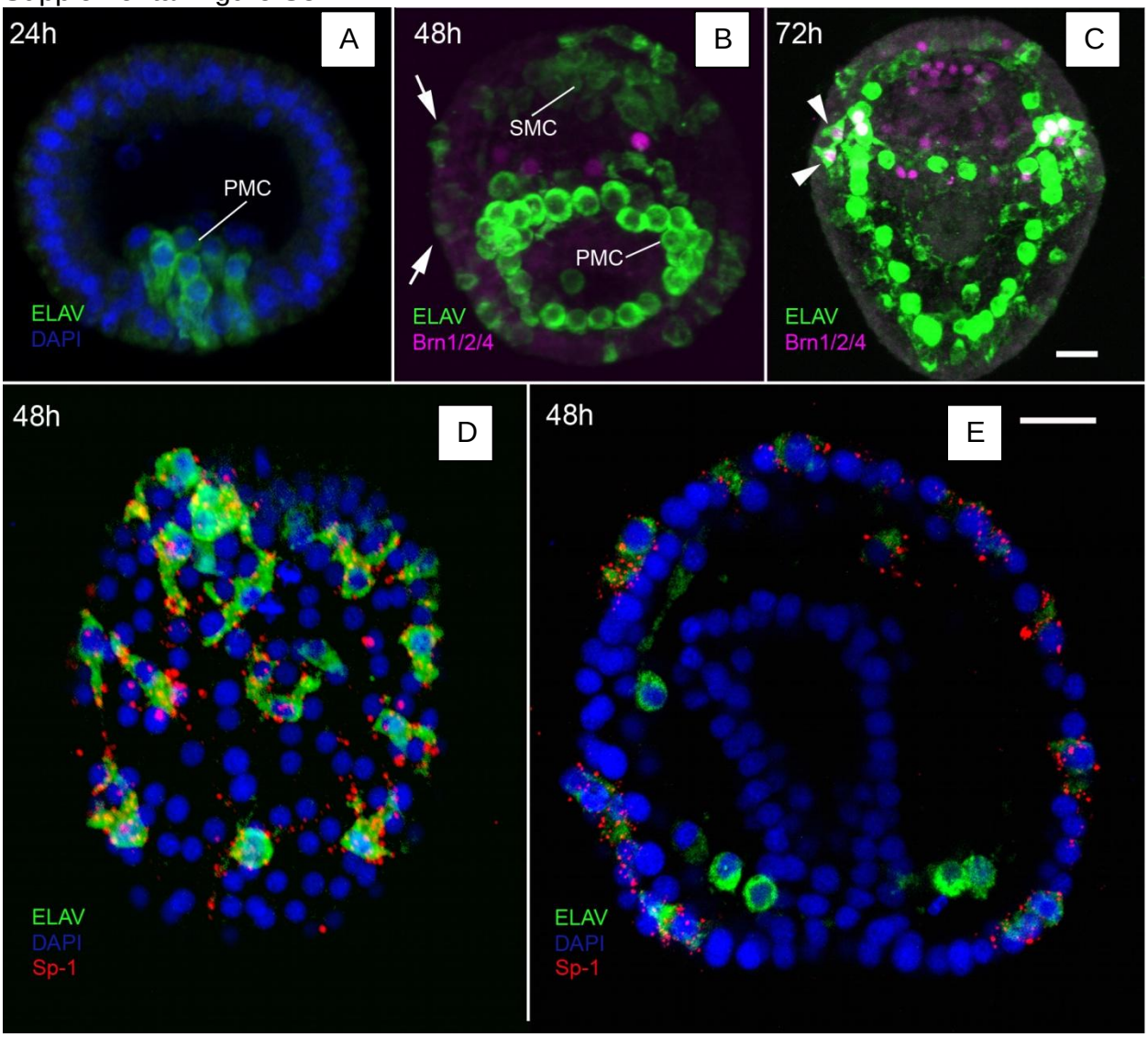
Maximum intensity projections of embryos prepared with anti-Brn1/2/4 serum. J Control, 48h embryo showing the normal distribution of Brn1/2/4. K Embryo derived from egg injected with 100 µM Brn1/2/4 MASO, prepared as in panel J and imaged with identical settings. There is only a very weak signal indicating that there is reduced antibody binding and that the MASO has suppressed expression of the Brn1/2/4 protein.

Supplemental Figure S2

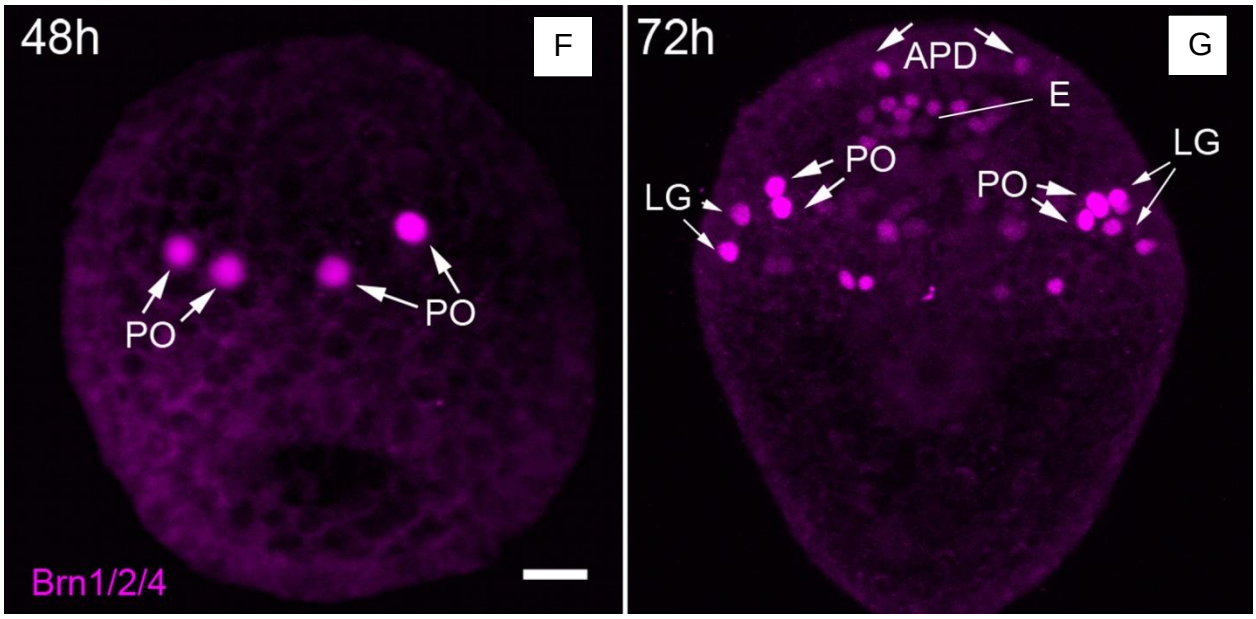


SoxC is initially expressed in late cleavage stages of *S. purpuratus* embryos in vegetal lineages. A-D Vegetal views. A. Expression in large micromeres, identified by their number and position relative to the small micromeres (Vasa) at 16 h. At subsequent stages (B –D) the number of cells expression SoxC increases as the expression domain expands outward. By 22 h, immediately prior to ingression of primary mesenchyme cells, SoxC is downregulated in micromere lineages. E. Lateral view of 20 h embryo in which expression of SoxC is throughout the cells of the endomesodermal lineages (arrows). F. Lateral view of 24 h embryo in which primary mesenchyme has begun to ingress (arrows) and expression of SoxC has returned to background levels. Note the initiation of expression in the animal pole domain at this stage. Bars = 10 µM.

Supplemental Figure S3



Ontogeny of expression of ELAV in mesodermal lineages. A. In mesenchyme blastulae, primary mesenchyme (PMC) express ELAV as a cytoplasmic protein as they ingress. B. In late gastrulae, the primary mesenchyme have formed a ring and continue to express ELAV, in addition, secondary mesenchyme (SMC) at the tip of the archenteron express ELAV. Note the cells within the plane of the ectoderm that express ELAV (arrows) C. In early plutei ELAV is expressed in skeletogenic mesenchyme and pigment cells. Presumptive neurons can be distinguished (arrowheads) by co-expression of Brn1/2/4. D, E. Pigment cells, identified by expression of the Sp1 antigen, co-express ELAV and have inserted in the aboral ectoderm of late gastrulae. Bars = 10 µM.



Embryonic expression of the transcription factor Brn1/2/4. F. Initial expression of Brn1/2/4 is at 48 h in late gastrulae. The protein localizes to the nucleus of 2-4 cells located in 2 bilateral clusters in the oral ectoderm; the presumptive PO neurons. G. Over the next 24 h expression in PO neural progenitors persists and cells in the aboral ectoderm adjacent to the ciliary band, which will form the lateral ganglia LG, begin expression. As well there is expression in cell along the dorsal periphery of the animal pole domain (APD). Expression in esophagus (E) and in neural progenitors was first reported by Cole and Arnone (2009).

Table S1: Antibodies and Dilutions

	Antibody	Species	Dilution	Reference
Primary	SoxC	Mouse (monoclonal)	1:10	This Study
		Rat (polyclonal)	1:600	This Study
	Hnf6	Rat	1:600	Yaguchi *et al.* 2010 Dev. Biol. 347: 71–81
	Nk2.1	Rabbit	1:500	Takacs *et al.* 2004 Dev. Biol., 269: 152–164
	Brn1/2/4	Rat	1:200	This Study
	ELAV	Rabbit	1:200	This Study
	SoxB2	Rat	1:500	This Study
	SynB (1E11)	Mouse (monoclonal)	1:5	Nakajima *et al.* 2004 Evol. Dev., 6: 95–104
Secondary	Alexa Fluor 488 IgG (H+L)	goat anti-mouse goat anti-rat goat anti-rabbit	1:12000	Invitrogen
	Alexa Fluor 568 IgG (H+L)	goat anti-mouse goat anti-rat goat anti-rabbit	1:12000	Invitrogen
	Alexa Fluor 633 IgG (H+L)	goat anti-rat	1:12000	Invitrogen
	Alexa Fluor 635 IgG (H+L)	goat anti-rabbit goat anti-mouse	1:12000	Invitrogen