Supporting Information

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SI Text

***Patiria miniata* (Sea Star) Sequences.** *P. miniata* cDNA sequences were deposited in GenBank [Accession nos.: JX844799 (*ephrin*), JX844800 (*ephrin receptor*), JX844801 (*elav*), JX844802 (*soxb1*), JX844804 (*soxc*), JX844803 (*wnt8*), KC669537 (*nodal*), and KC669538 (*bmp2/4*)]. Isolation of other *P. miniata* genes used in this study are described elsewhere (1–4).

Morpholinos. The Delta morpholino sequence is published in ref. 3.

Other morpholino sequences used in this study were as follows:

PmOnecut 5′- TAGCTCGCTTGAAAGCATCACAAAC-3′;
PmSoxc: 5′-CATGGTTCTTAACAGTGTCCCGTCT-3′;
PmFoxq2: 5′-CATGATGGTCGCCGAAACAGAGGAA-3′;
PmSix3-1: 5′-ACATTGAGCCGAGCATCTGGACCCG-3′;
PmSix3-2: 5′-TCTCAGCAGCGCAGTCGAGAGACAC-3′;
PmFoxg-1: 5′-CACTCCAACTTAACCATTTTTGGGT-3′;
PmFoxg-2: 5′-GTTTGTGGTCGAATAAACTCTTGCC-3′;
PmNodal: 5′-CTGGGTCAAGTTCTTGGGTCATTCT-3′;
PmBmp2/4-1: 5′-TGCTCATCGTAGGGACACCCACCAT-3′;
PmBmp2/4-2: 5′-GGATCTGTGAAACCAAACGAGAAAT-3′;
PmEphR: 5′-ACTTGTCCGGCATGAGCATCAGGCC-3′.

1. Hinman VF, Nguyen AT, Davidson EH (2003) Expression and function of a starfish Otx ortholog, AmOtx: A conserved role for Otx proteins in endoderm development that predates divergence of the eleutherozoa. *Mech Dev* 120(10):1165–1176.
2. Yankura KA, Martik ML, Jennings CK, Hinman VF (2010) Uncoupling of complex regulatory patterning during evolution of larval development in echinoderms. *BMC Biol* 8:143.
3. Hinman VF, Davidson EH (2007) Evolutionary plasticity of developmental gene regulatory network architecture. *Proc Natl Acad Sci USA* 104(49):19404–19409.
4. Otim O, Hinman VF, Davidson EH (2005) Expression of AmHNF6, a sea star orthologue of a transcription factor with multiple distinct roles in sea urchin development. *Gene Expr Patterns* 5(3):381–386.

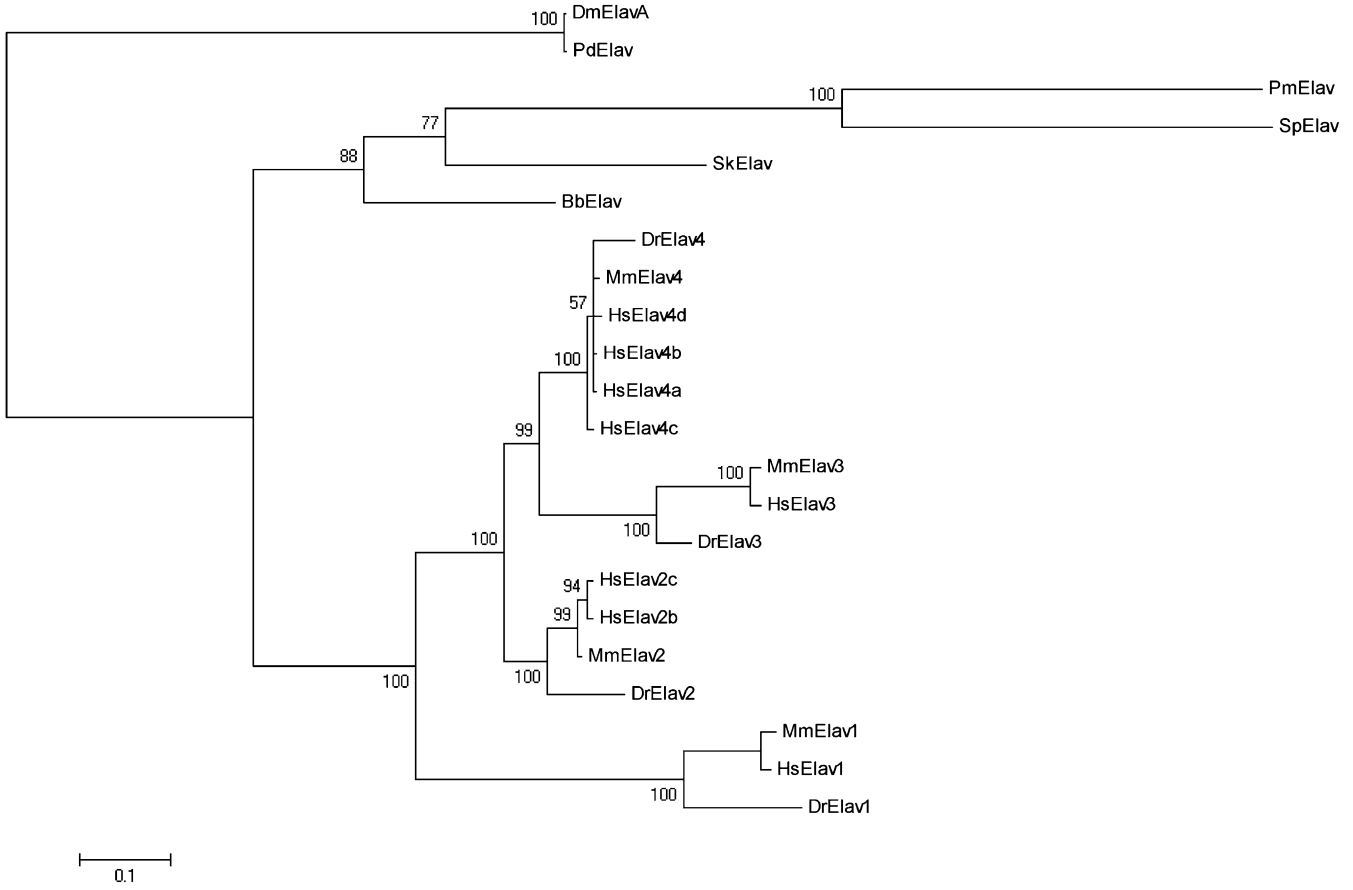

Fig. S1. Evolutionary relationships among *elav* orthologs. Phylogeny describes the evolutionary relationships of *P. minatata* (sea star) *elav* to other orthologs of *elav*. Tree topology was determined using maximum likelihood and Bayesian analysis. Like the hemichordate, *Saccoglossus kowalevskii*, the sea star, *P. miniata*, has only one transcribed *elav* gene that is likely to be the single ortholog of the four vertebrate *elav* genes. *Bb*, *Branchiostoma belcheri* (amphioxus); *Dm*, *Drosophila melanogaster*; *Dr*, *Danio rerio*; *Hs*, *Homo sapiens*; *Mm*, *Mus musculus* (mouse); *Pm*, *Patiria miniata* (sea star); *Sk*, *Saccoglossus kowalevskii* (hemichordate); *Sp*, *Strongylocentrotus purpuratus* (sea urchin).

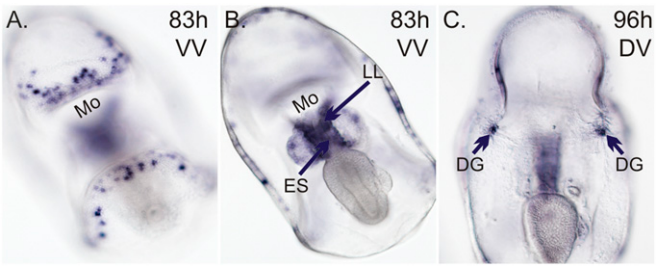

Fig. S2. Expression of *PmElav*. Sea star embryos are oriented with anterior pole up in ventral (VV) and dorsal (DV) views; h, hours postfertilization. (*A*–*C*) Whole-mount in situ hybridization (WMISH). (*A*) Expression of *elav* within two rows above and below the mouth (Mo) of 83-h-old larvae. (*B*) Transcripts of *elav* are detected in the lower lip (LL) and esophagus (ES), as well as in the mesodermally derived coeloms that are located on either side of the esophagus, which are not in the plane of focus in *A*. It is not yet known if mesodermal-derived *elav*-expressing cells are neurons. In vertebrates, *elav-like 1/huA* is expressed in the mesoderm, and *elav-like 2/huB*, *elav-like 3/huC* and *elav-like 4/huD* are expressed exclusively in neurons (1). It is likely that the different vertebrate orthologs of *elav* have partitioned these roles in concert with gene duplications. (*C*) Arrows point to transcripts of *elav* within the anterior dorsal ganglia (DG) of a 96-h-old larva. (Magnification: 200×.)

1. Pascale A, Govoni S (2012) The complex world of post-transcriptional mechanisms: is their deregulation a common link for diseases? Focus on ELAV-like RNA-binding proteins. *Cell Mol Life Sci* 69:501–517.

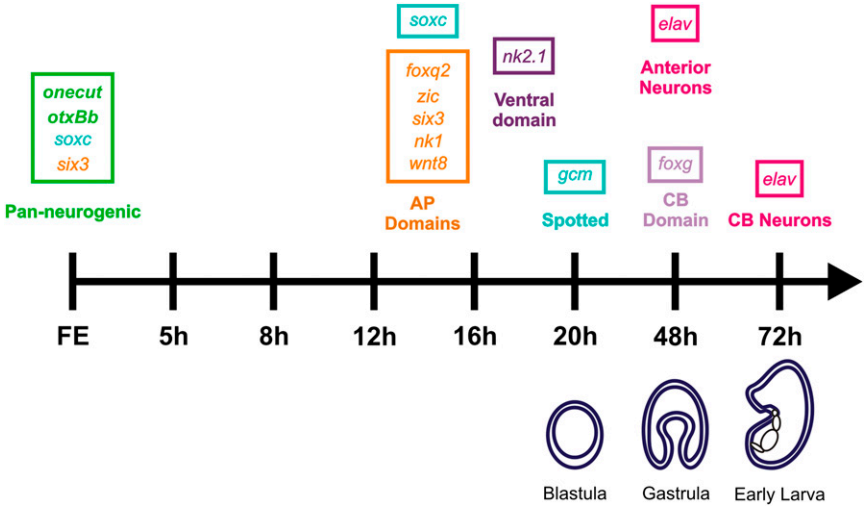

Fig. S3. Temporal expression of sea star genes. Schematic summarizes the onset of gene expression during sea star development, although expression of many of these genes continues following 24 h (see Fig. S4). Genes in *green* are abundant in fertilized eggs; transcripts of these genes are detected throughout the ectoderm of late blastulae using WMISH (1, 2, 4). Genes that are later expressed within ectodermal domains along the AP axis (orange) of late blastulae (2) are abundant between 12 and 16 h postfertilization (hpf) (i.e., early cleavage). Transcripts of *nk2.1* (*purple*), which are later detected within a ventral ectodermal domain of late blastulae (2), are abundant around 16–20 hpf. Transcripts of *gcm* (teal), which later have a spotted pattern of localization in late blastulae (3), are abundant around 20 hpf. Also included in this schematic is the onset of expression of *elav* (magenta) within anterior neurons and ciliated band (CB) neurons that surround the *foxg*-ciliated band domain (light purple). AP, anterior-posterior.

	FE	5h	8h	12h	16h	20h	24h	48h	72h
hnf6/onecut	**22**	23	22	22	21	20	20	23	23
otxBb	**22/36717**	21/27586	21/48056	21/58648	21/53567	20/53745	21/61343	21	22
bmp2/4	**1104**	879	1365	2584	5182	6214	6832	25	26
six3	**28/1121**	28/983	28/1554	27/3765	**25/20671**	**22/38538**	**23/39022**	**23**	**22**
soxc	**28**	28	28	27	**24**	**22**	**23**	**20**	**23**
zic	35/1	32/1	UD/0	**29/1498**	**25/7304**	24/8671	25/3404	25	24
foxq2	33/194	32/129	32/296	**28/16876**	**25/46692**	24/18172	26/7732	26	26
nk1	34	35	35	**28**	**27**	**25**	25		
wnt8	1	1	1	**1539**	**3837**	2859	6352	25	25
nk2.1	33/147	35/60	31/126	31/186	**29/205**	**25/2577**	25/3374	22	24
gcm	34	34	34	33	30	**28**	**29**	**23**	26
foxg								**23**	**24**
elav								27	27

Fig. S4. Abundance of *P. miniata* orthologs as determined by quantitative RT-PCR (qPCR) and NanoString. Temporal expression of sea star, *P. miniata*, orthologs was determined using a time-course cDNA from fertilization (FE, fertilized egg) to 72 h postfertilization and qPCR. Cycle threshold (Ct) values at each time-point are provided for each gene. Ct values are inversely related to transcript abundance. Based on our analyses of reverse-transcriptase minus samples, we consider Ct values of less than 30 as not expressed or expressed at low enough levels to not be biologically relevant. Alternatively, or in addition to qPCR, Nanostring nCounter assays provided transcript prevalence at the same developmental time points. NanoString counts (in italics) are provided for *otxβb*, bone morphogenetic protein 2/4 (*bmp2/4*), *six3*, *zic*, *foxq2*, *nk2.1*, and *wnt8*. Here, values less than 200 counts are considered as not expressed or expressed at low enough levels to not be biologically relevant. UD, undetected. Colors indicate when corresponding genes are highly expressed.

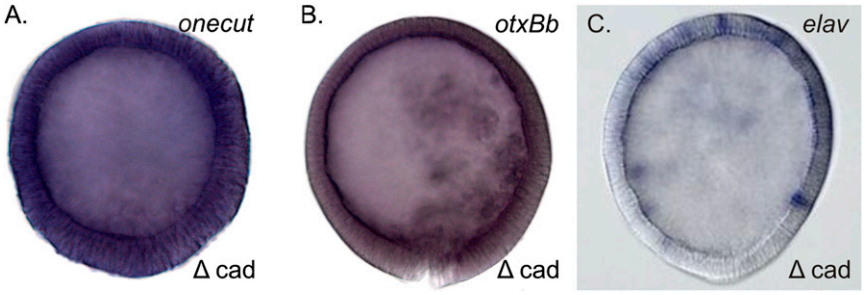

Fig. S5. Canonical Wnt (cWnt) signaling establishes a neuroectoderm from endomesoderm. Embryos are oriented with the anterior pole up. Transcripts of *onecut* (*A*), *otxßb* (*B*), and *elav* (*C*) are found throughout the ectoderm of embryos in which cWnt signaling is blocked via injection of Δ-cadherin RNA. All panels are WMISH. (Magnification: 200×.)

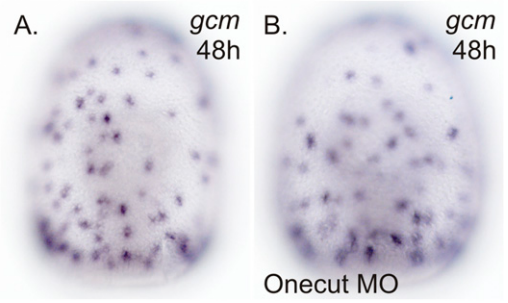

Fig. S6. Expression of *glial cells missing* (*gcm*) within the ectoderm does not change in Onecut morphants. Embryos are oriented with the anterior pole up. Both panels are WMISH. (*A*) Transcripts of *glial cells missing* (*gcm*) are detected within distinct ectodermal cells throughout the ectoderm in ~48-h-old gastrulae. (*B*) Expression of *gcm* remains unchanged in Onecut morphants (MO).

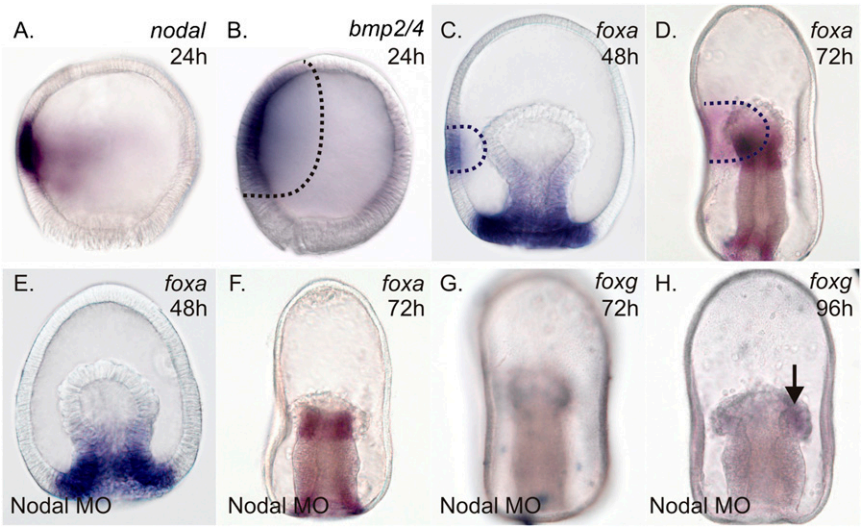

Fig. S7. Nodal signaling establishes *foxa* and *foxg* expression on the ventral side of gastrulae. Embryos are oriented with the anterior pole up. Ventral is to the left, except when indicated otherwise. All panels are WMISH. Transcripts of *nodal* (*A*) and *bmp2/4* (*B*) are localized to the ventral side of blastulae. Transcripts of *foxa* are localized within the endoderm and in a single ventral ectodermal domain (dotted lines) in 48-h (*C*) and 72-h (*D*) gastrulae. Expression of *foxa* is lost from the ectoderm of Nodal morphants (MO) at around 48 h (*E*) and 72 h (*F*, ventral view); *foxa* expression remains within the endoderm as in normal development. In Nodal morphants (MO) at around 72 h (*G*) and 96 h (*H*, ventral view), expression of *foxg* is lost from the ectoderm; *foxg* expression remains within a mesodermally derived coelom (not in plane of focus in *G*; arrow in *H*) as in normal development. (Magnification: 200×.)

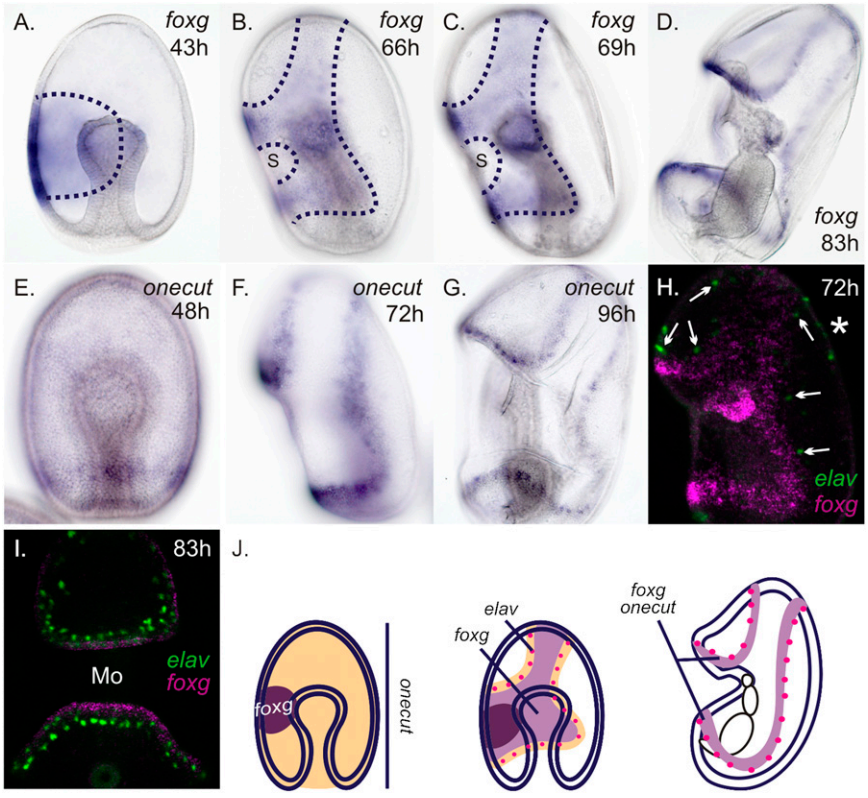

Fig. S8. Spatial relationships among *foxg*-expressing ciliary band domain (CBD), *onecut*, and *elav* expression. Embryos are oriented with the anterior pole up. Ventral is to the left, except when indicated otherwise. Panels *A*–*G* are WMISH; panels *H* and *I* are FISH. (*A*) Ectodermal expression of *foxg* is initially localized to a single ventral domain. (*B*) Expression of *foxg* clears from the stomodeal (S) ectoderm and begins to extend into the anterior pole domain. (*C*) By 69 h, the expression of *foxg* is observed in a single CBD that forms around the mouth and loops over the anterior pole. (*E*) By 83 h, *foxg* is clearly expressed within the ectoderm of the ciliary bands, at what we think is the edge of its former domain. (*F*) During the time when *foxg* expression is restricted to the ventral ectoderm, the expression of *onecut* is pan-ectodermal. (*G*) Around 72 h, the expression of *onecut* is localized to the ectoderm of the ciliary bands, which form at the edge of the *foxg*-CBD. (*H*) By 96 h, expression of *onecut* within the ciliary bands is similar to that of *foxg* (compare with *D*). *elav*-expressing cells (*green*, see arrows) do not colocalize with *foxg* expression (pink) in the CBD (*H*) or in the ciliary bands (*I*, ventral view); anterior neurons (asterisk in *H*). (*J*) Schematic summarizes the spatial relationships among domains of *foxg*, *onecut*, and *elav* expression during development. (Magnification: 200×.)

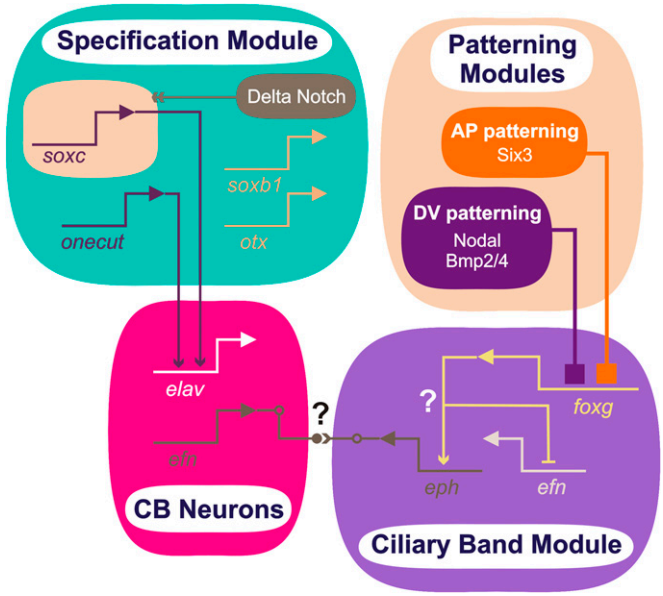

Fig. S9. Schematic of gene regulatory network interactions linking broad neural specification and localized patterning. A suite of pan-ectodermal regulatory genes that includes *onecut*, *otxßb*, and *soxb1* establishes a broad ectodermal territory in which *soxc*-expressing neural precursors are patterned by Delta Notch signaling (specification module, teal). Inputs from Onecut and Soxc are required for the specification of *elav*-expressing ciliary band neurons (magenta) that are patterned across the AP and DV axes. An ectodermal *foxg*-expressing CBD (purple) is patterned along the AP and DV axes by conserved patterning mechanisms (patterning module, tan). The *foxg*-CBD, possibly along with Ephrin receptor (Eph)/Ephrin ligand (Efn)-mediated directed cell migration, organizes the formation of ciliary band neurons along the edge of the CBD within the broad neurogenic ectoderm.