Figure S1. The GRN model highlighting the new components and connections identified in this study. The model is essentially identical to that shown in Fig. 3; however, colored gene names and connections were assigned to the newly-introduced genes and interactions only. Previously documented genes or connections were shown in dark gray or black.

Figure S2. Temporal and spatial expression analysis of reporter constructs including various upstream regions of the *sip1* gene. A) Diagram illustrating the *sip1* upstream region and reporter constructs. Previously identified transcriptionally active regions (module B, basal, and module D, distal) are included in the 4.3 kb construct. B) Equivalence of 16kb and 4.3kb constructs. The reporter constructs were injected to the embryos and analyzed with QPCR to measure the transcription levels. C), Correct spatial expression of entire 16KB construct and of construct containing only modules B and D. D) Statistical results on spatial expression of GFP cis-regulatory reporter constructs. Both the 16kb construct and the "minimum" construct consisting of the D and B modules were able to drive correct ectodermal expression during the blastula stage.

Figure S3. Sequence of *sip1* distal module D. Predicted *otx*-binding sites and the 5' ends of m6.3 and m6.4 construct are marked.

Figure S4. Global evaluation of perturbation effects by NanoString transcript quantification following treatment with *sip1* MASO. RNA from treated embryos was extracted at 15h and 18h and quantified using the NanoString nCounter regulatory gene code set. Normalized counts from perturbed embryos were plotted against those of control embryos. Several ectodermal genes are highlighted in blue. Dotted lines indicate a 2-fold change threshold. Open gray circles indicate transcripts present at ≤25 transcripts or less per embryo; absolute transcript levels were estimated from prior measurements (Materna et al., 2010).

Figure S5. Expression territories of *nodal*, *gsc*, and *foxg* in the oral ectoderm. While *foxg* is initially expressed in the oral ectoderm as is *gsc* during blastula stage, it becomes a ciliary band gene as gastrulation occurs. lv—lateral view; vv—vegetal view; oev—oral ectoderm view. In the embryos shown in lv and vv, the oral ectoderm is on the left side. *one-cut* was used as a ciliary-band marker in the double WMISHs, as indicated.

Figure S6. Nodal signaling control of stomodeal *bra* expression. Blocking Nodal signaling with the receptor kinase inhibitor, SB-431542 resulted in loss of ectodermal *bra* expression. In contrast, elevating *nodal* expression by Lefty-MASO injection caused *bra* expression to expand to the whole ectoderm.

Figure S7. Spatial expression of lateral/CB genes A) Normal expression of *univin*; B)Expression of *one-cut*; left, control; right, *nodal* MASO.

Table S1. Graphic representation of ectodermal gene expression pattern.

Table S2. PCR primers to generate truncation, fusion, and mutation reporter constructs.

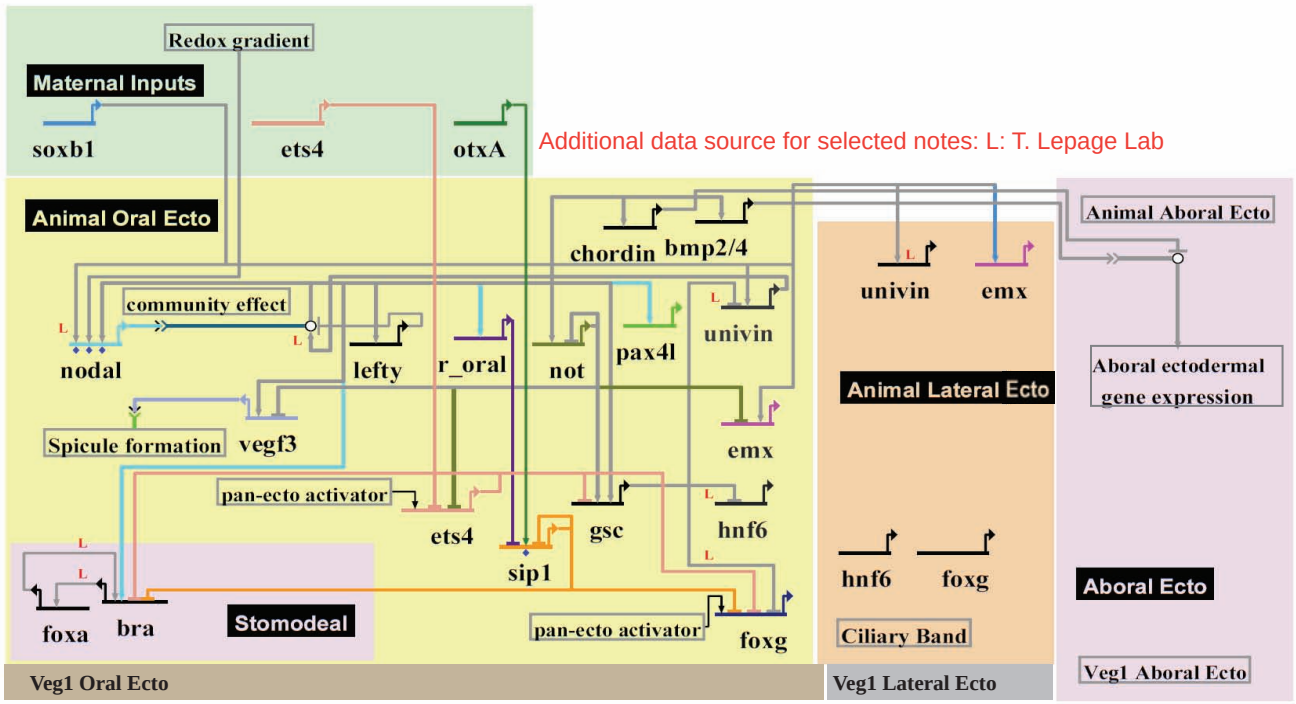

Redox gradient
Maternal Inputs
soxb1
ets4
otxA
Additional data source for selected notes: L: T. Lepage Lab
Animal Oral Ecto
chordin
bmp2/4
Animal Aboral Ecto
univin
emx
community effect
nodal
lefty
r_oral
not
pax4l
univin
Aboral ectodermal gene expression
Animal Lateral Ecto
Spicule formation
vegf3
emx
pan-ecto activator
ets4
gsc
hnf6
sip1
hnf6
foxg
Aboral Ecto
foxa
bra
Stomodeal
pan-ecto activator
foxg
Ciliary Band
Veg1 Oral Ecto
Veg1 Lateral Ecto
Veg1 Aboral Ecto

Fig. S1

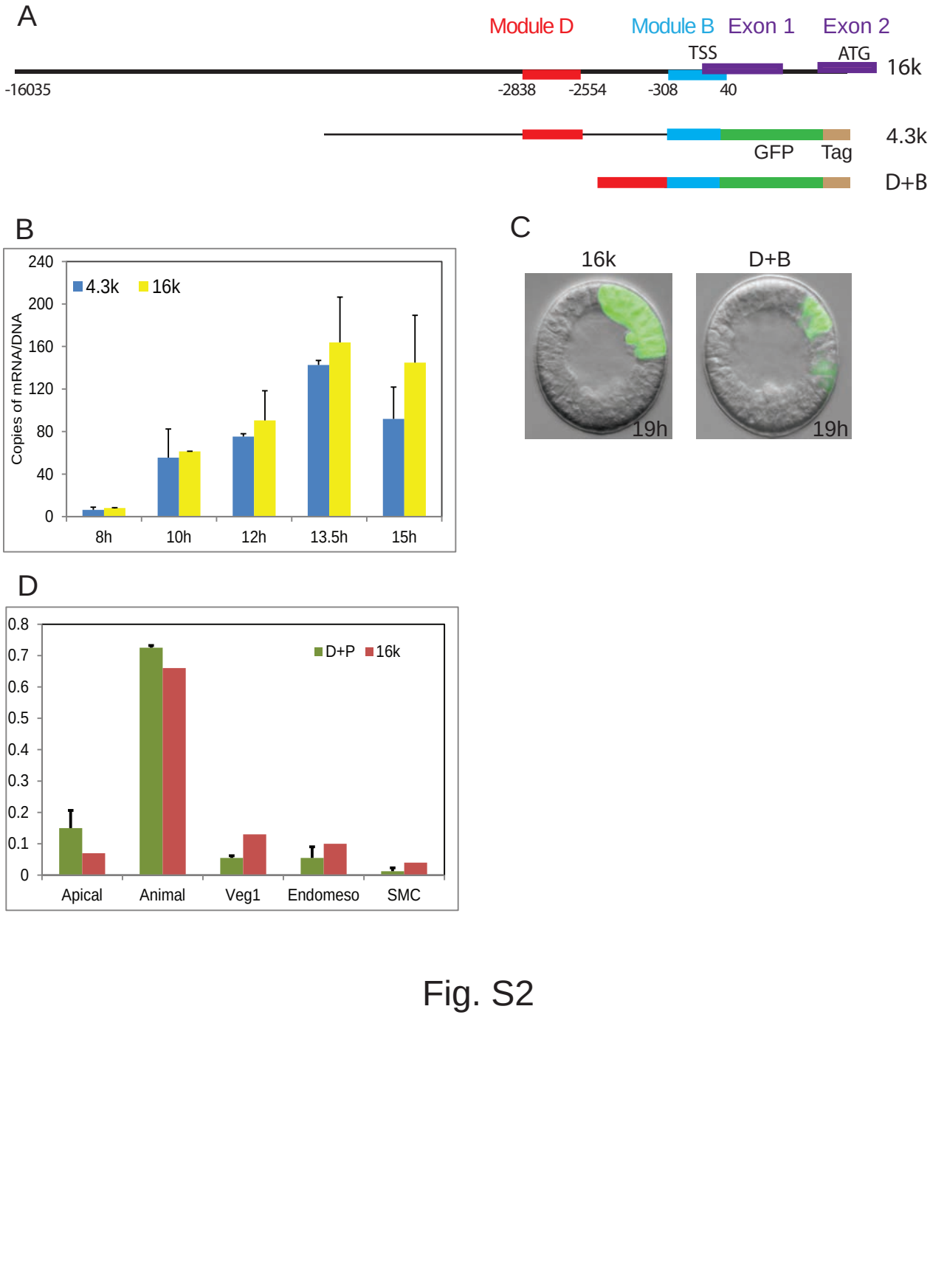

A
Module D
Module B
Exon 1
Exon 2
TSS
ATG
16k
-16035
-2838
-2554
-308
40
4.3k
GFP
Tag
D+B
B
Copies of mRNA/DNA
4.3k
16k
240
200
160
120
80
40
0
8h
10h
12h
13.5h
15h
C
16k
D+B
19h
19h
D
D+P
16k
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Apical
Animal
Veg1
Endomeso
SMC

Fig. S2

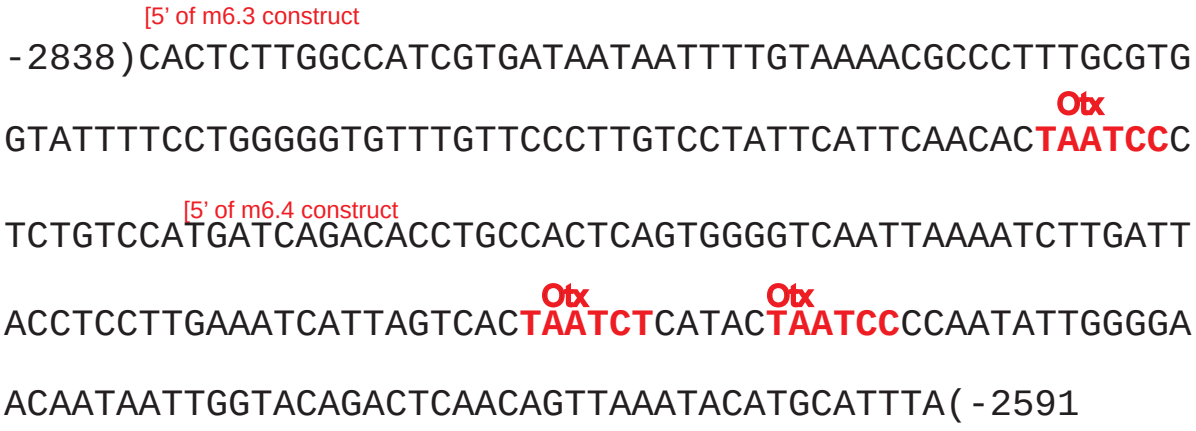
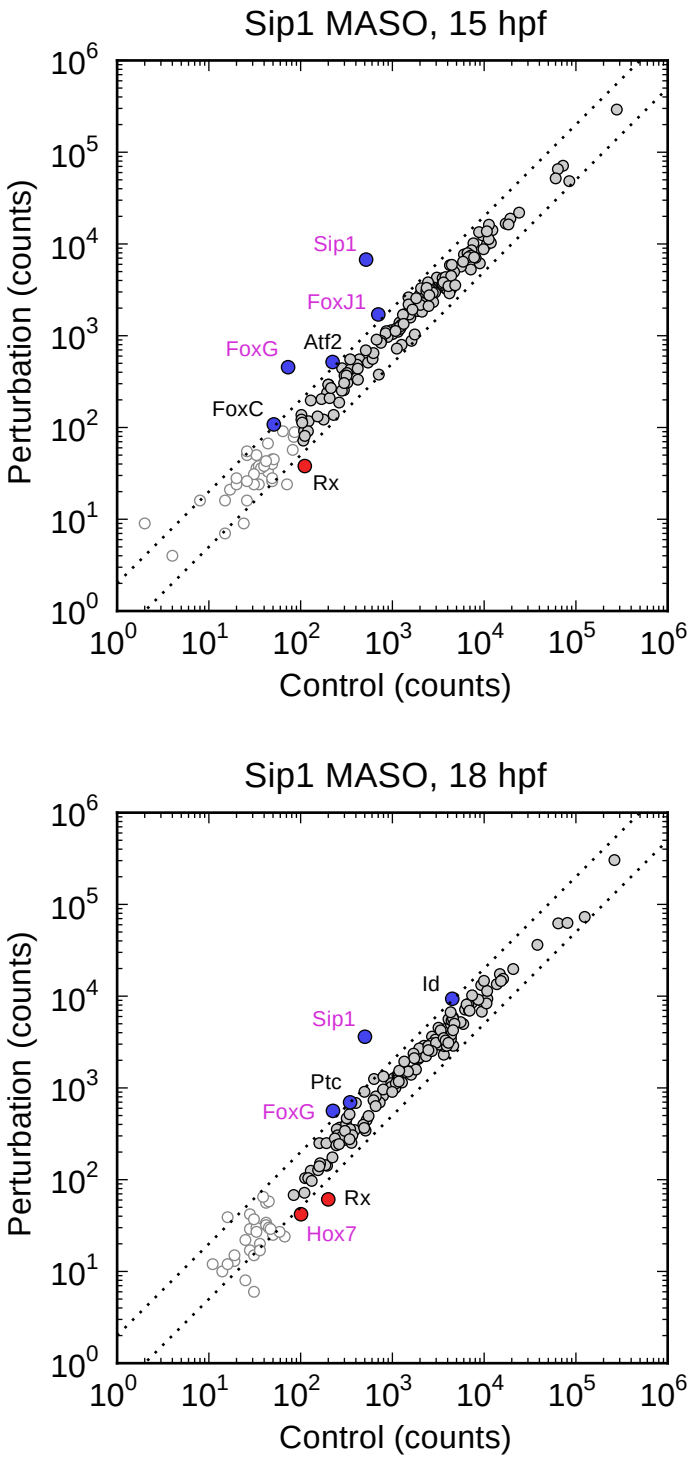

[5' of m6.3 construct
-2838)CACTCTTGGCCATCGTGATAATAATTTTGTAAAACGCCCTTTGCGTG
Otx
GTATTTTCCTGGGGGTGTTTGTTCCCTTGTCCTATTCATTCAACACTAATCCC
[5' of m6.4 construct
TCTGTCCATGATCAGACACCTGCCACTCAGTGGGGTCAATTAAAATCTTGATT
Otx
Otx
ACCTCCTTGAAATCATTAGTCACTAATCTCATACTAATCCCCAATATTGGGGA
ACAATAATTGGTACAGACTCAACAGTTAAATACATGCATTTA(-2591

Fig. S3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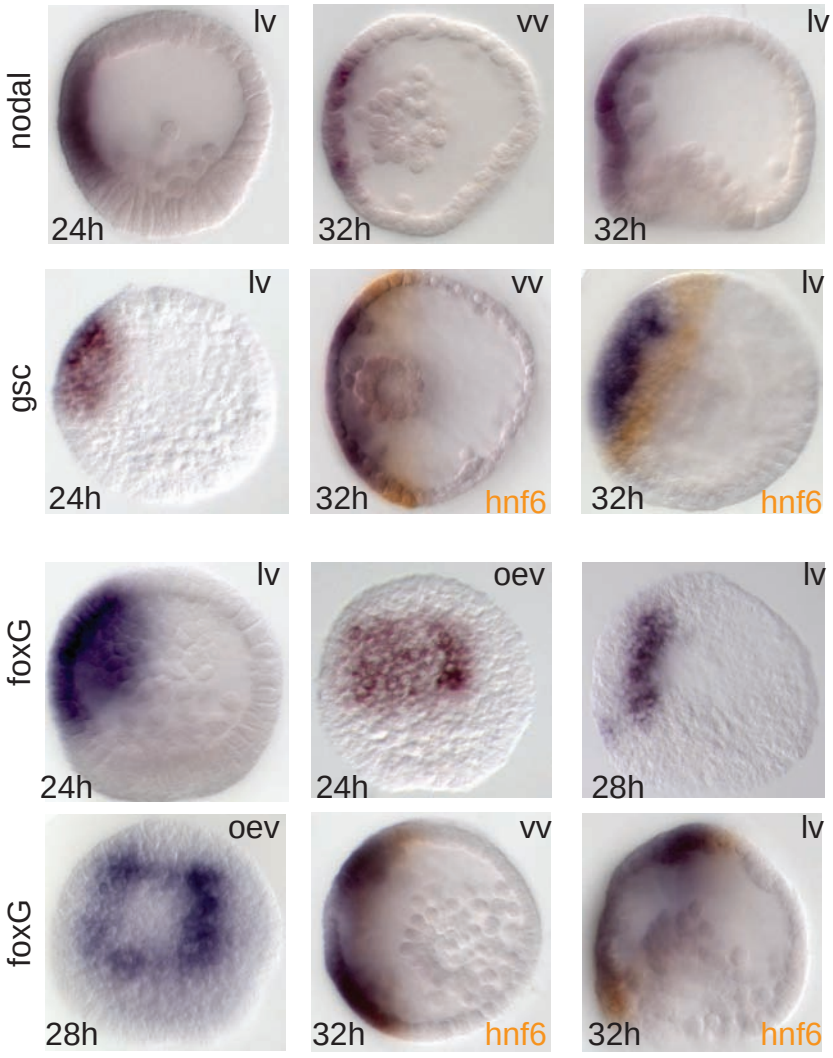
Perturbation (counts)
Control (counts)
Sip1
FoxJ1
FoxG
Atf2
FoxC
Rx
Sip1 MASO, 18 hpf
Perturbation (counts)
Control (counts)
Id
Sip1
Ptc
FoxG
Rx
Hox7

Fig. S4


nodal
lv
24h
vv
32h
lv
32h
gsc
lv
24h
vv
32h
hnf6
lv
32h
hnf6
foxG
lv
24h
oev
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lv
28h
foxG
oev
28h
vv
32h
hnf6
lv
32h
hnf6

Fig. S5

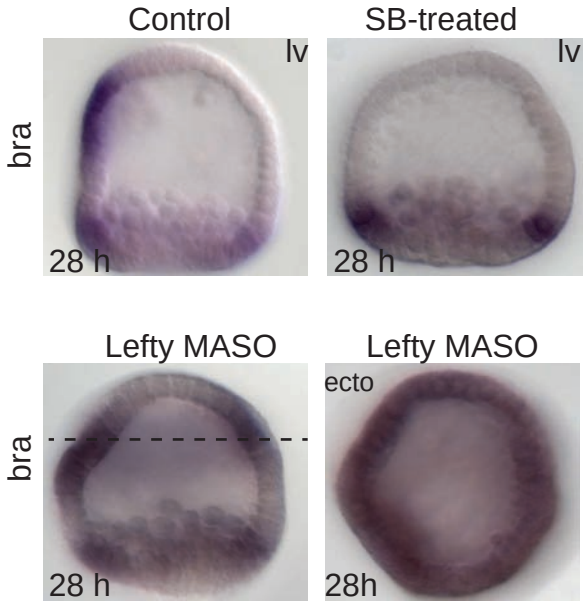

Control
SB-treated
lv
lv
bra
28 h
28 h
Lefty MASO
Lefty MASO
ecto
bra
28 h
28h

Fig. S6

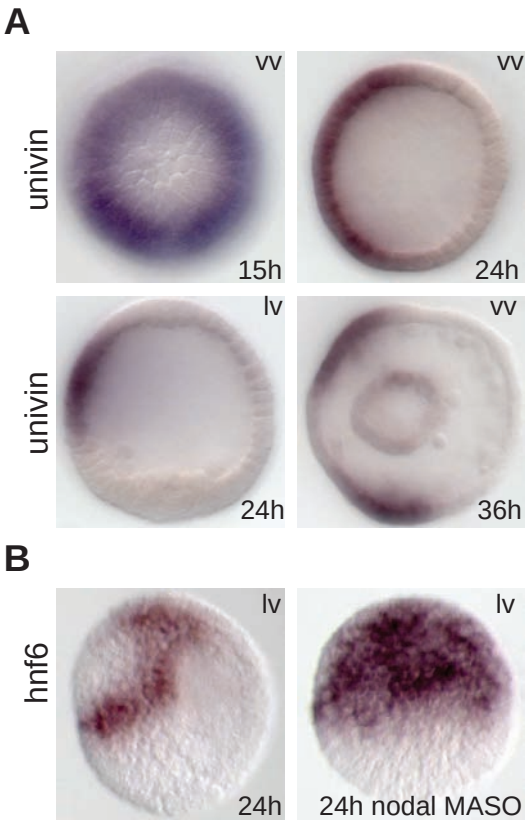

A
univin
vv
15h
vv
24h
univin
lv
24h
vv
36h
B
hnf6
lv
24h
lv
24h nodal MASO

Fig. S7

Table S1 Graphic representation of ectodermal gene expression pattern

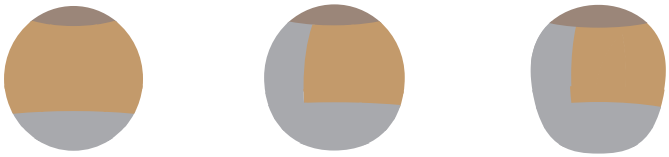
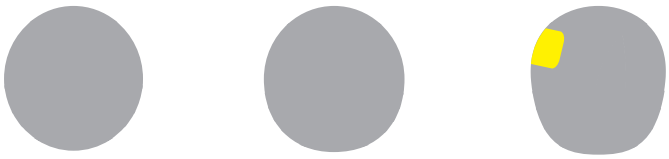
Gene	Spatial expression pattern		
	12h	18h	24h
ets4			
sip1			
emx			
nodal			
not			
pax4l			
gsc			
foxg			
bra			

Table S2. PCR primers to generate truncation, fusion, and mutation reporter constructs

Primer	Sequence
Sip1-m6.1-5’	CCTGTTCTTTATGGGAGTGTGA
Sip1-m6.2-5’	TCTCTATCAAGGATGTGTTACAGGA
Sip1-m6.3-5’	CACTCTTGGCCATCGTGATA
Sip1-m6.4-5’	GATCAGACACCTGCCACTCA
Sip1-m6.5-5’	CCTGAAATGACAAAGGTCCA
Sip1-m6.6-5’	CGTGCATGGTGAACTTTGAA
Sip1-m6.7-5’	CTCCACCGATAGGCAATCAT
Sip1-m7.5(Module B)-5’	GGTGGGACACAGAGAAAGGA
Module D-5’	GGTGTTTGTTCCCTTGTCCT
Sip1/GFP-fusion	TTCCTCGCCCTTGCTCATTCCACAGCTTCAAATCCAAA
Module-D/B-fusion	AACTCCTTTCTCTGTGTCCCACCCCTGTATCTCCGATTGTCTAACG
Sip1-Otx-M1-5’	CTACTTTAATGGCCGCCGAGTTCAAGGTGCGTG
Sip1-Otx-M1-3’	CACGCACCTTGAACTCGGCGGCCATTAAAGTAG
Sip1-Otx-23del-5’	CTTGAAATCATTAGTCACCCAATATTGGGGAAC
Sip1-Otx-23del-3’	GTTCCCCAATATTGGGTGACTAATGATTTCAAG