Supplemental Appendix

Specific functions of the Wnt signaling system in gene regulatory networks throughout the early sea urchin embryo

Miao Cui, Natnaree Siriwon, Enhu Li, Eric H. Davidson and Isabelle S. Peter*

* To whom correspondence may be addressed. Tel: +1 626 395 5887.
E-mail: ipeter@caltech.edu

Supplemental Materials and Methods

Gene cloning and Whole mount in situ hybridization (WMISH). cDNAs of *wnt*, *fzd*, *dkk*, and *sfrp* genes were amplified by PCR using primers listed in Table S2, and cloned into pGEM-T constructs. cDNA prepared from various developmental stages were used as templates for PCR reactions. Sequences used to prepare WMISH probes of regulatory genes, i.e. *alx*, *delta*, *foxa*, *hox11/13b*, *eve*, *lim1*, *emx*, and *foxq2*, were previously described (1-5). Cloned constructs were linearized for transcription of antisense RNA probes labeled with digoxigenin (DIG) or fluorescein (FL). WMISHs were performed according to the standard methods (6). In brief, embryos were fixed in 2.5% glutaraldehyde, 32.5% sea water, 32.5 mM MOPS (pH 7) and 162.5 mM NaCl at 4°C overnight. 25 ng/µl of proteinase K in TBST was used to treat embryos at room temperature for 5-10 minutes, followed by 30-minute fixation in 4% paraformaldehyde at room temperature. Hybridizations were performed overnight at 58-60°C using a probe concentration of 0.5-1ng/µl. Probes were detected using anti-DIG or anti-FL Fab fragments conjugated to alkaline phosphatase (Roche). Color was developed using NBT/BCIP or INT/BCIP reagents (Roche).

C59 treatment. The Porcupine inhibitor C59 was obtained from Cellagen Technology (C7641-2s). C59 was dissolved in DMSO at 10mM, and experiments were performed in the presence of C59 diluted in sea water to 0.5µM (see text), unless indicated otherwise. Except for the timing experiment in Fig. S6, embryos were treated with C59 at 1h post fertilization and were exposed until embryos were collected, at developmental times indicated in figure captions.

MASO injection and RNA isolation. MASOs were provided by GeneTools. Sequences of gene-specific MASOs are shown in Table S4. MASOs were diluted in injection solution including 0.12M KCl at 300µM and injected into fertilized eggs in a volume of 2-4 pl. Randomized control MASOs (N_{25}) were injected at same concentration. Experiments were performed on 2-5 independent embryonic batches. Embryos were cultured at 15°C, and approximately 300 MASO-injected embryos were collected at different time points. Total RNA was extracted using the RNeasy Micro Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions.

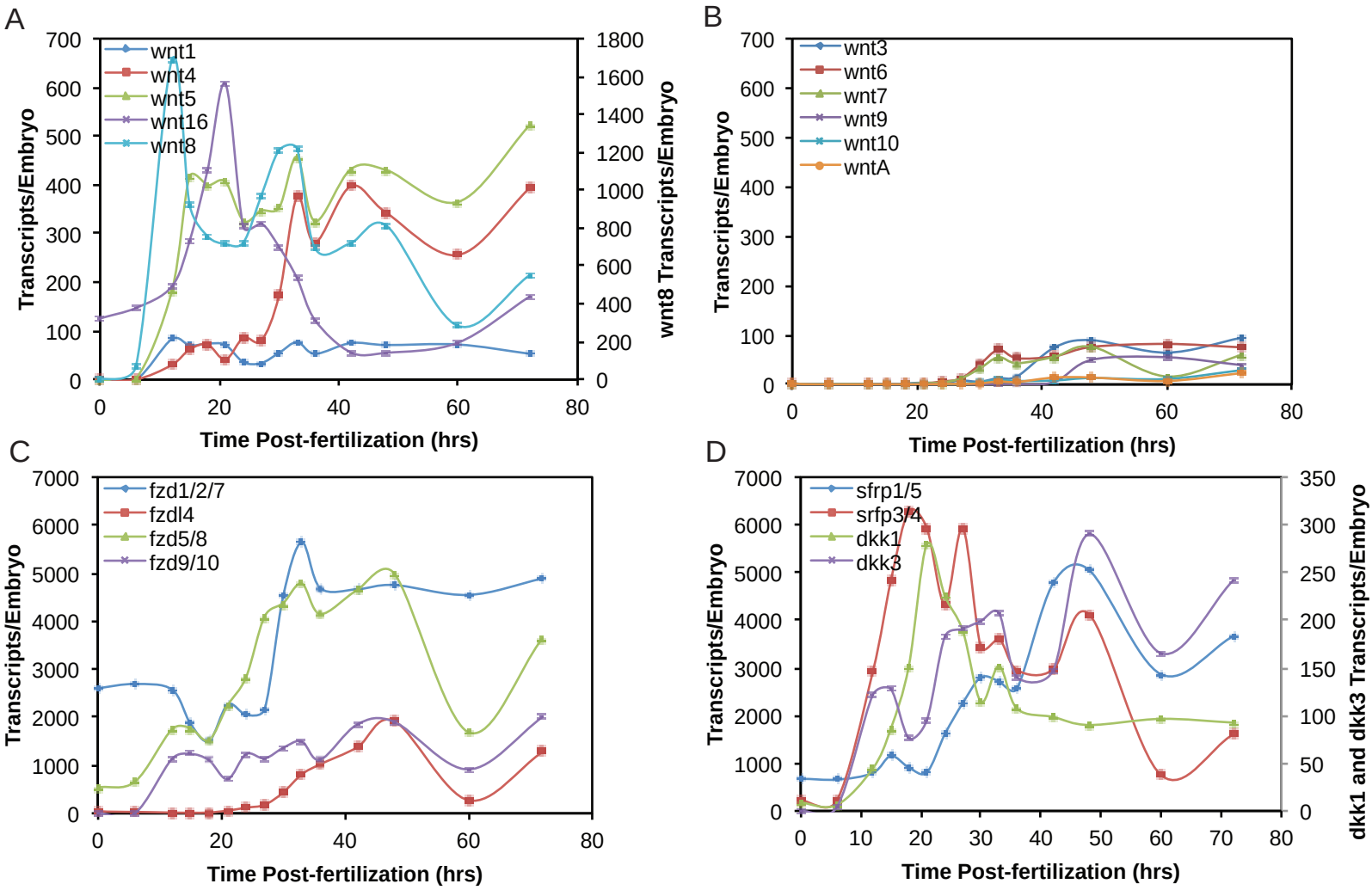
QPCR analysis. cDNA was synthesized using iScript cDNA synthesis kit (BioRad). QPCR was performed using Power SYBR Green Master Mix (Life Technology) with the primers listed in Table S3. Gene expression levels were normalized to those of poly-ubiquitin. Fold change was calculated by comparing normalized expression levels in the embryos injected with gene specific MASOs to expression levels in embryos injected with control MASOs.

NanoString nCounter. Approximately 300 embryos were collected at different time points and lysed in 15µl of RLT buffer (Qiagen, Hilden, Germany). Hybridization reactions were performed according to manufacturer's instructions in total 30µl solution with 10µl of detection probes, 10µl of hybridization buffer, 5µl of embryo lysate, and 5µl of capture probes. All hybridization reactions were incubated at 65 °C for a minimum of

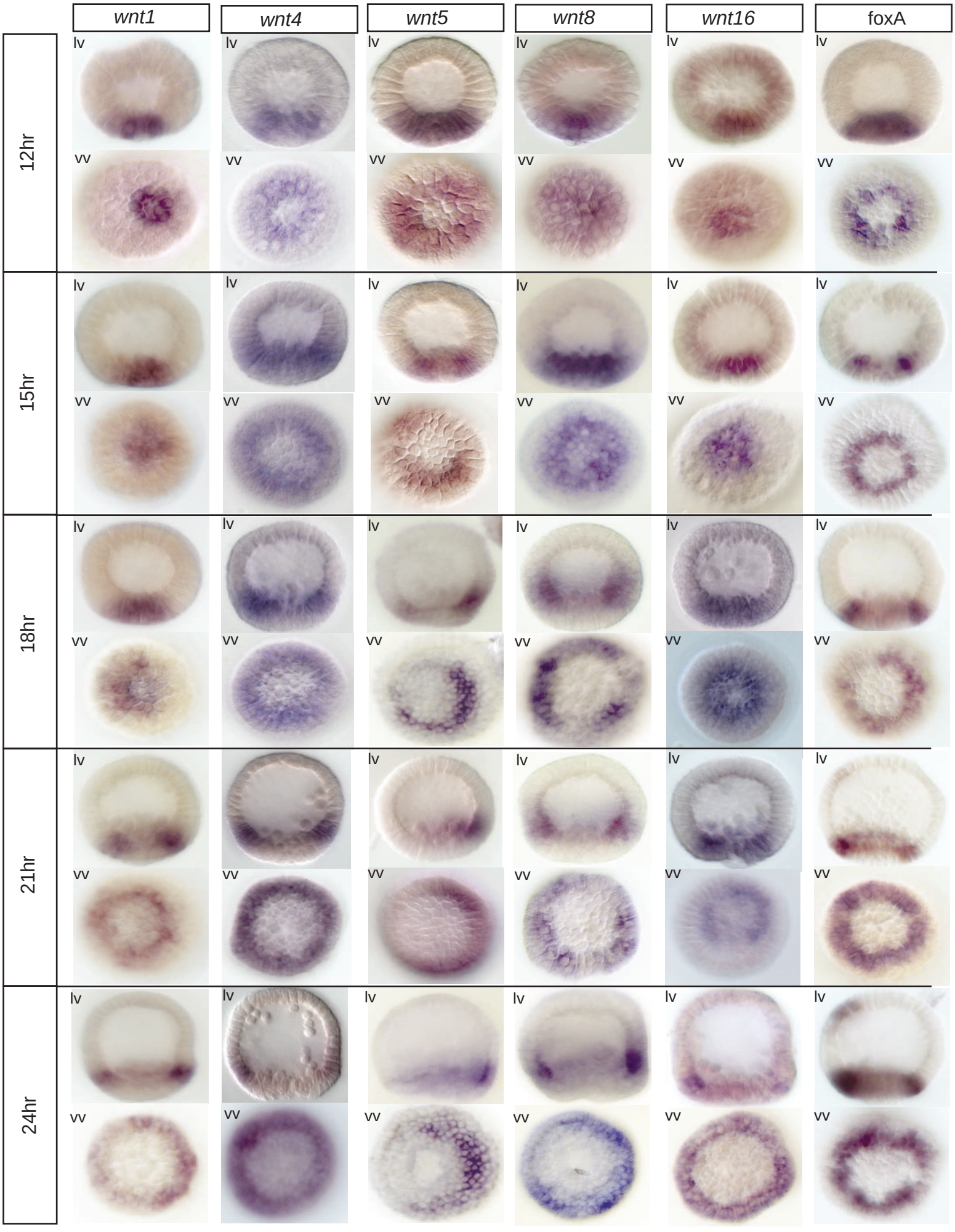
18 h. Hybridized probes were recovered with the NanoString Prep Station and immediately evaluated with the NanoString nCounter. The resulting count for each gene was subtracted by counts of negative spikes for background correction and then normalized using the sum of all corrected counts for all genes in the codeset. Fold changes of normalized counts in C59 treated versus untreated control embryos is shown for genes with counts >200, corresponding to approximately 100 transcripts. Experiments were performed on 3 independent embryonic batches. Probe sequences and accession numbers for the genes included in the codeset are as same as previously reported (1).

Supplemental Figures

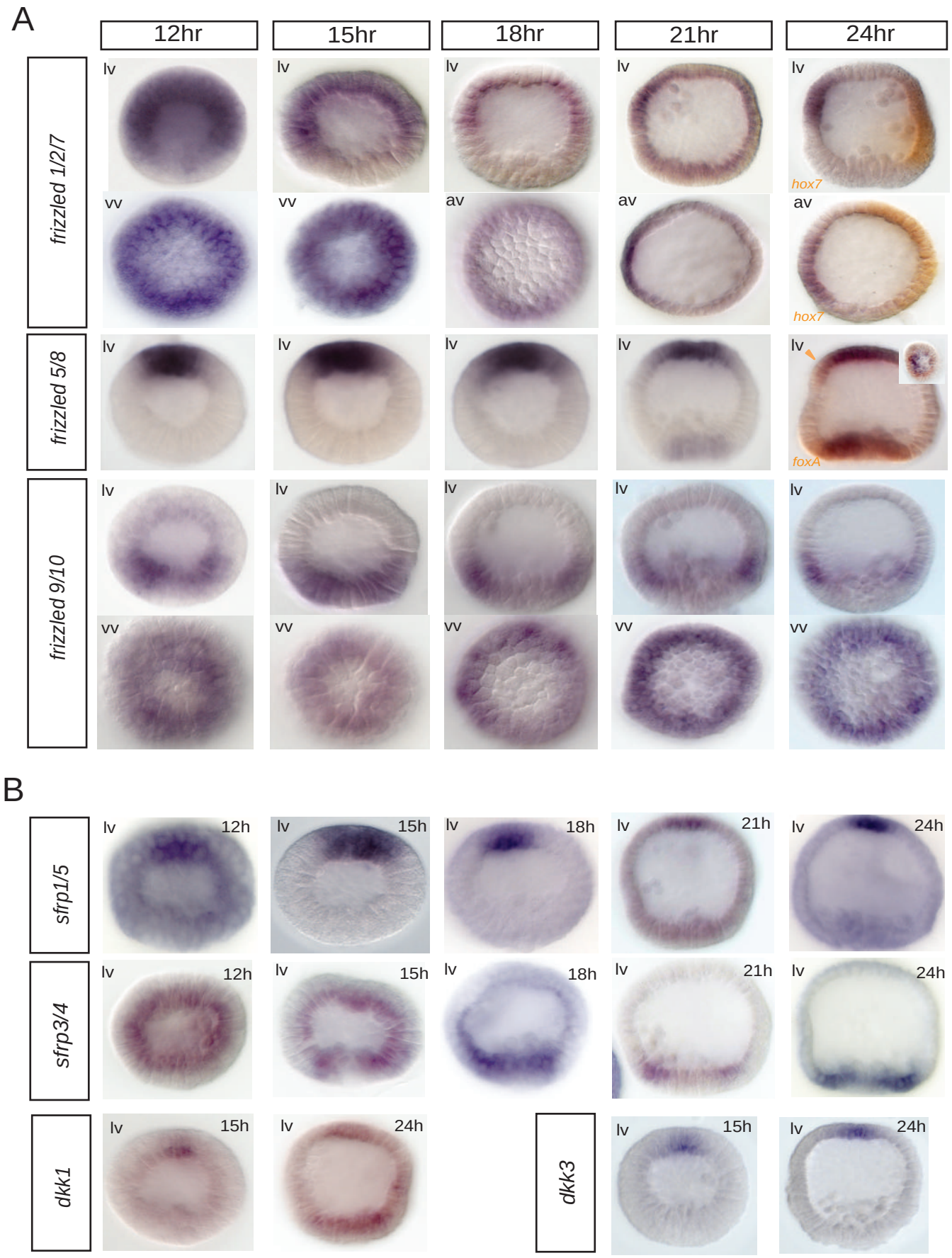
Supplemental Figure 1



Supplemental Figure 2

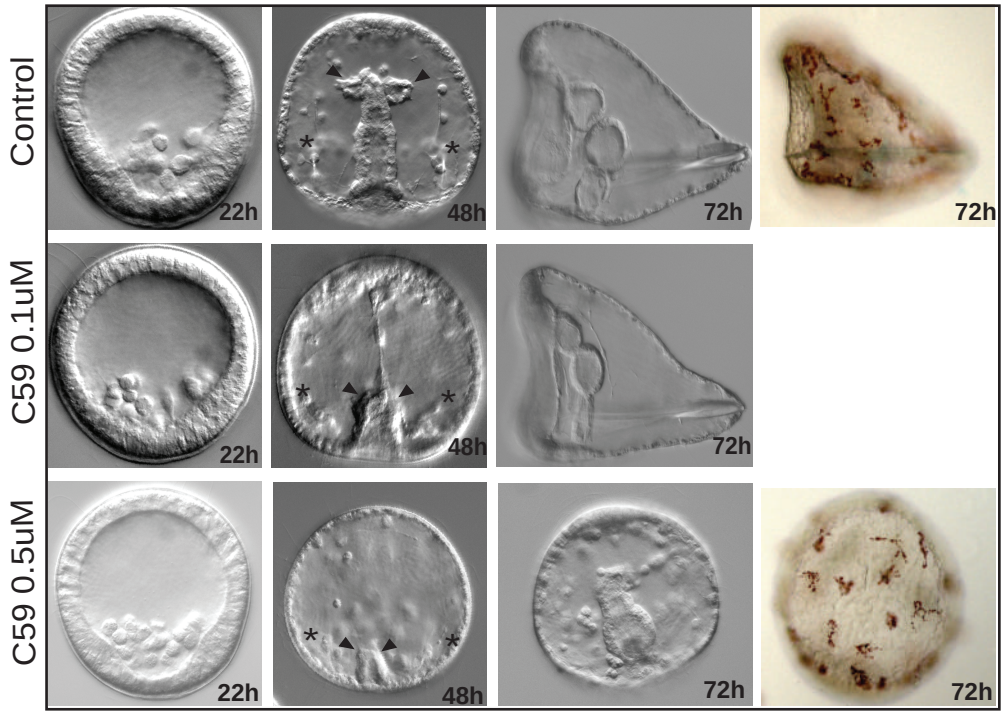


Supplemental Figure 3

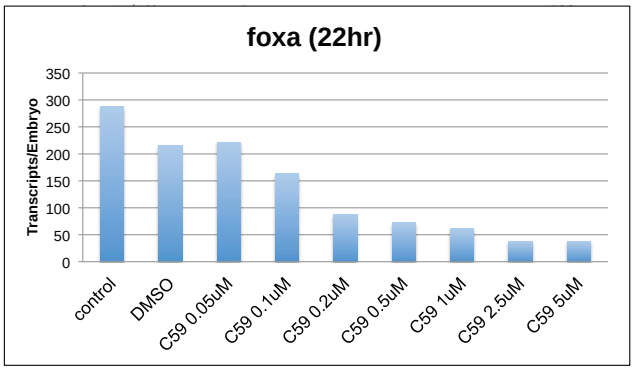


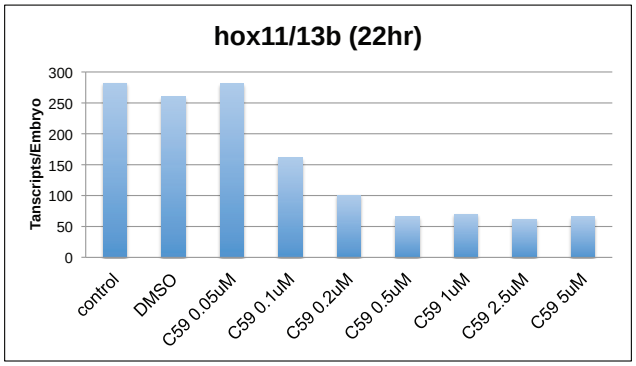
Supplemental Figure 4

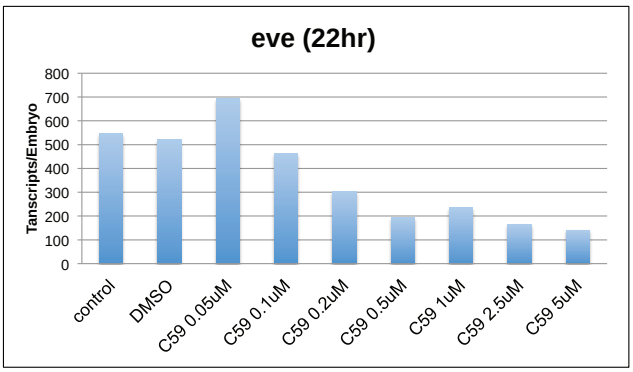
A

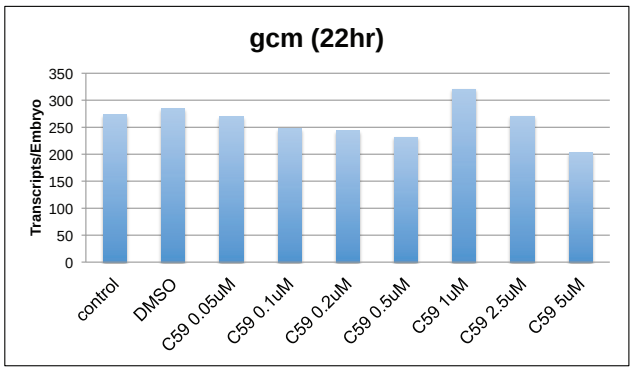


B

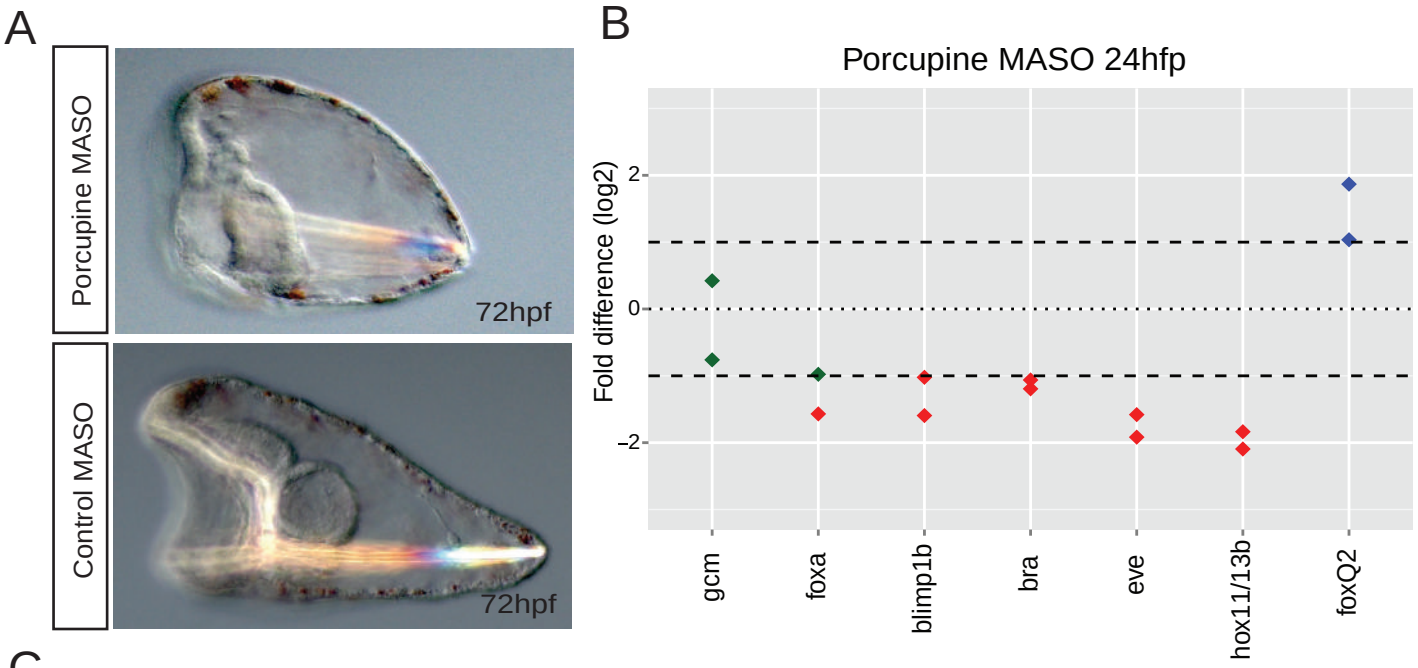








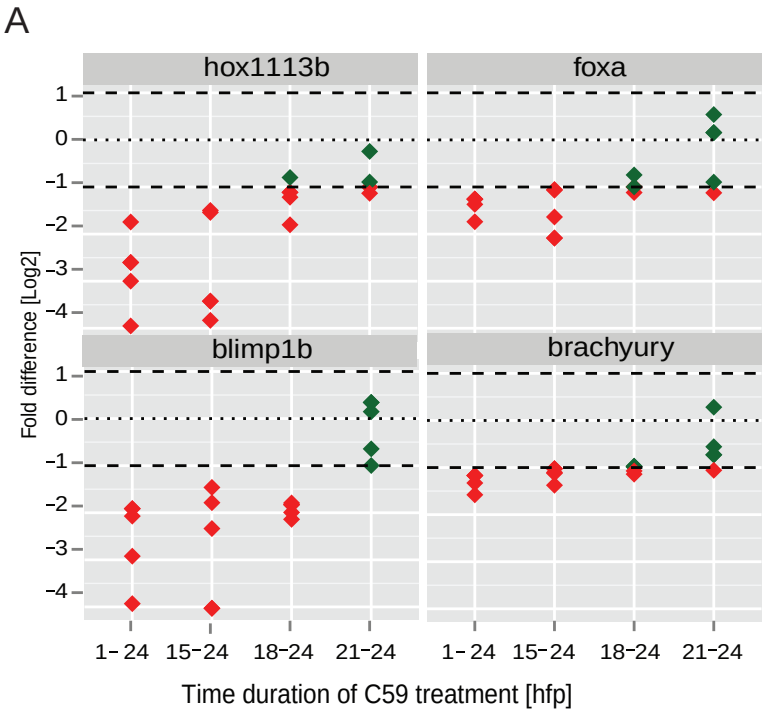
Supplemental Figure 5

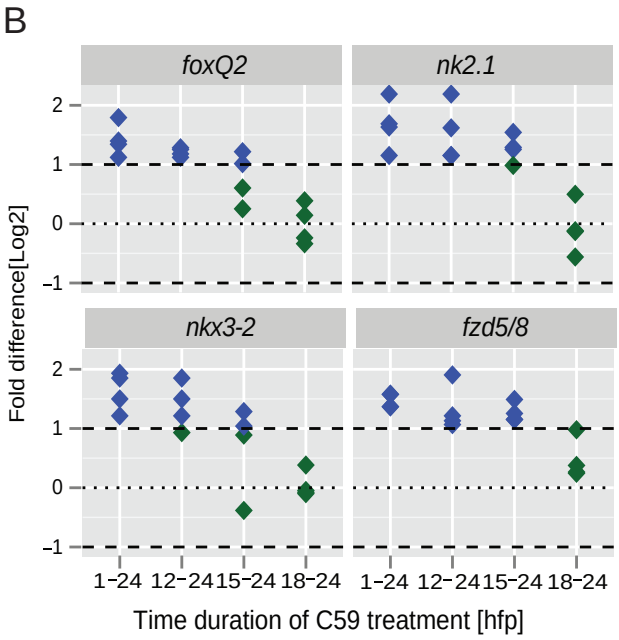


C

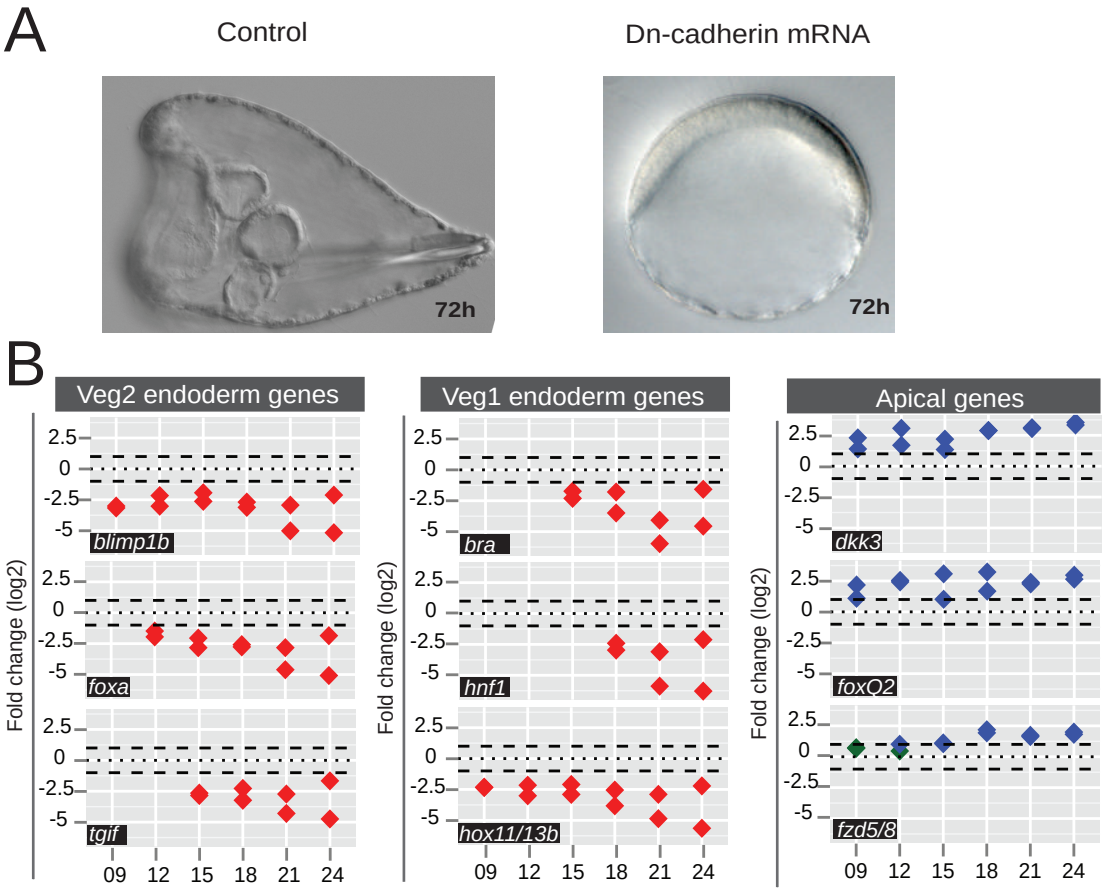
```
Wnt1/199-245     N L H N N E A G R K T V S S E M R R E C K C H G M S G S C T I E T C W M R L P T F R T V G E L
Wnt16/265-311    N L H N N E A G R Q L I K S G M K T L C R C H G V S A S C S L K T C W K A M P S F K E I G D L
Wnt4/15-61       N L H N N E A G R R T I E D N M R M E C K C H G V S G S C E M K T C W K S M P T F G D I G Q V
Wnt5/254-300     N L H N N E A G R K A V Y D N A G T E C K C H G V S G S C S L K T C W L Q L S P F N R V G T I
Wnt8/163-209     T L H N N E A G R K A V K Q T L Q R T C K C H G V S G S C S L Q T C W N H V A N F R V I G D E
```

Supplemental Figure 6

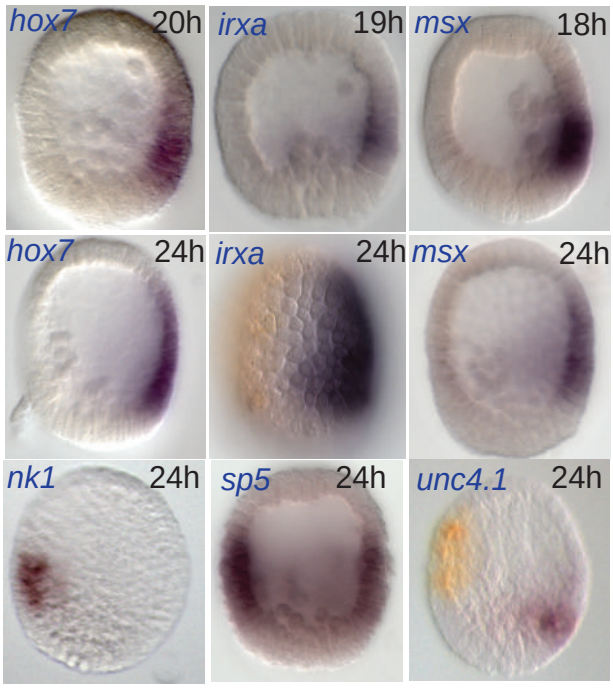




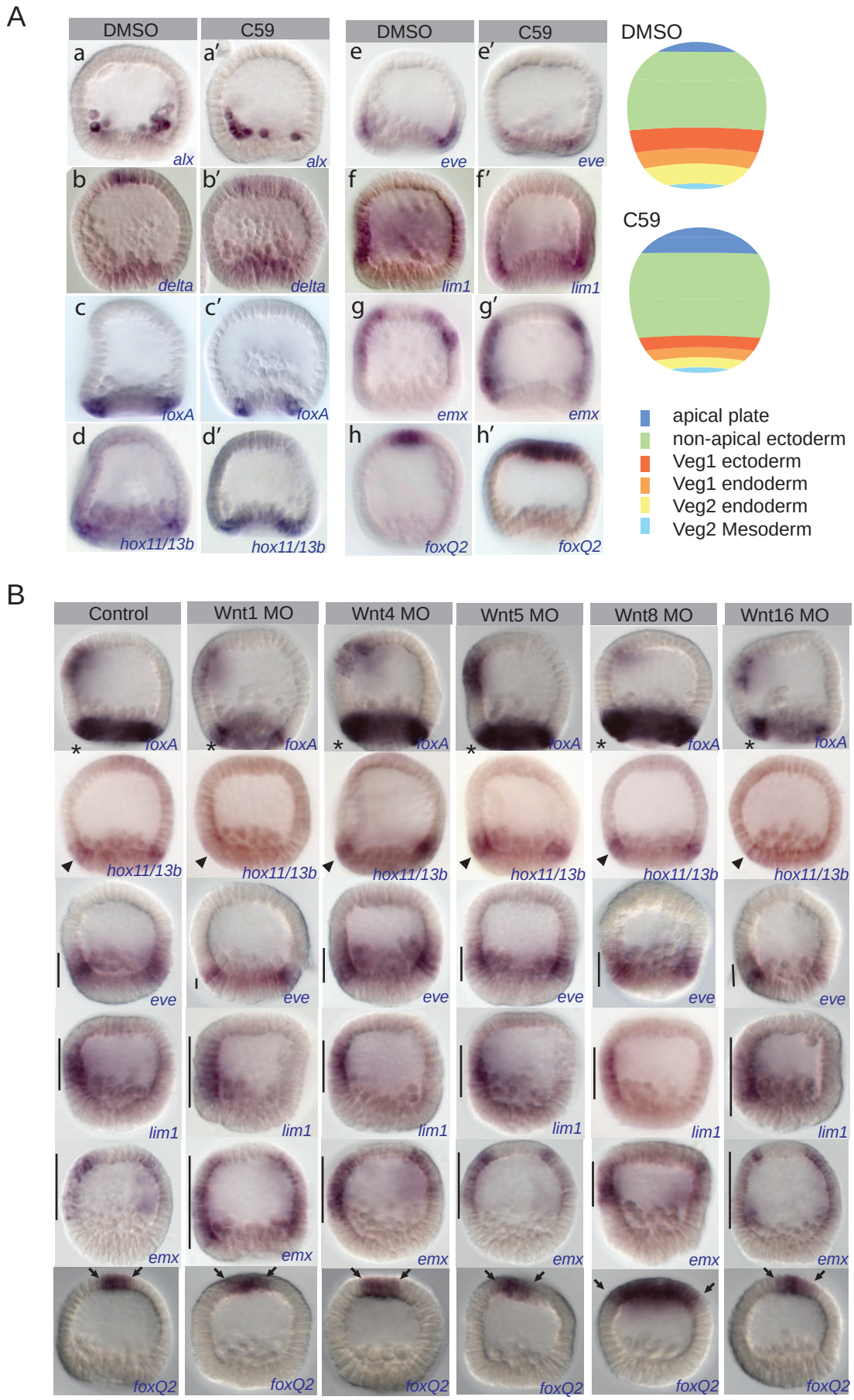
Supplemental Figure 7



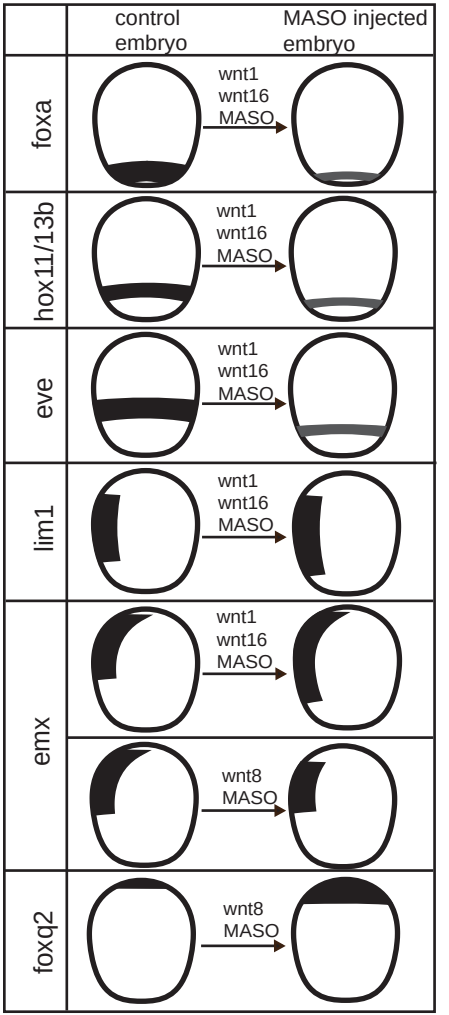
Supplemental Figure 8



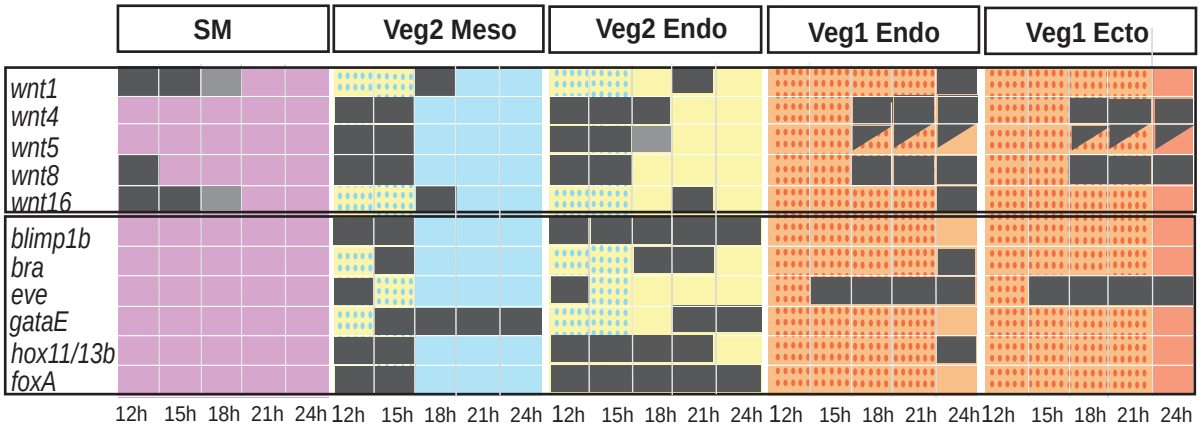
Supplemental Figure 9



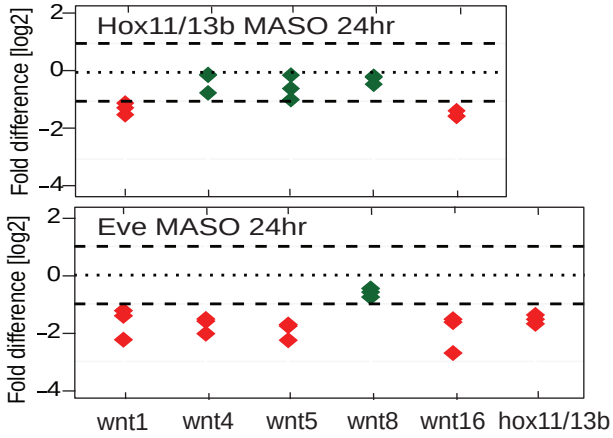
Supplemental Figure 10



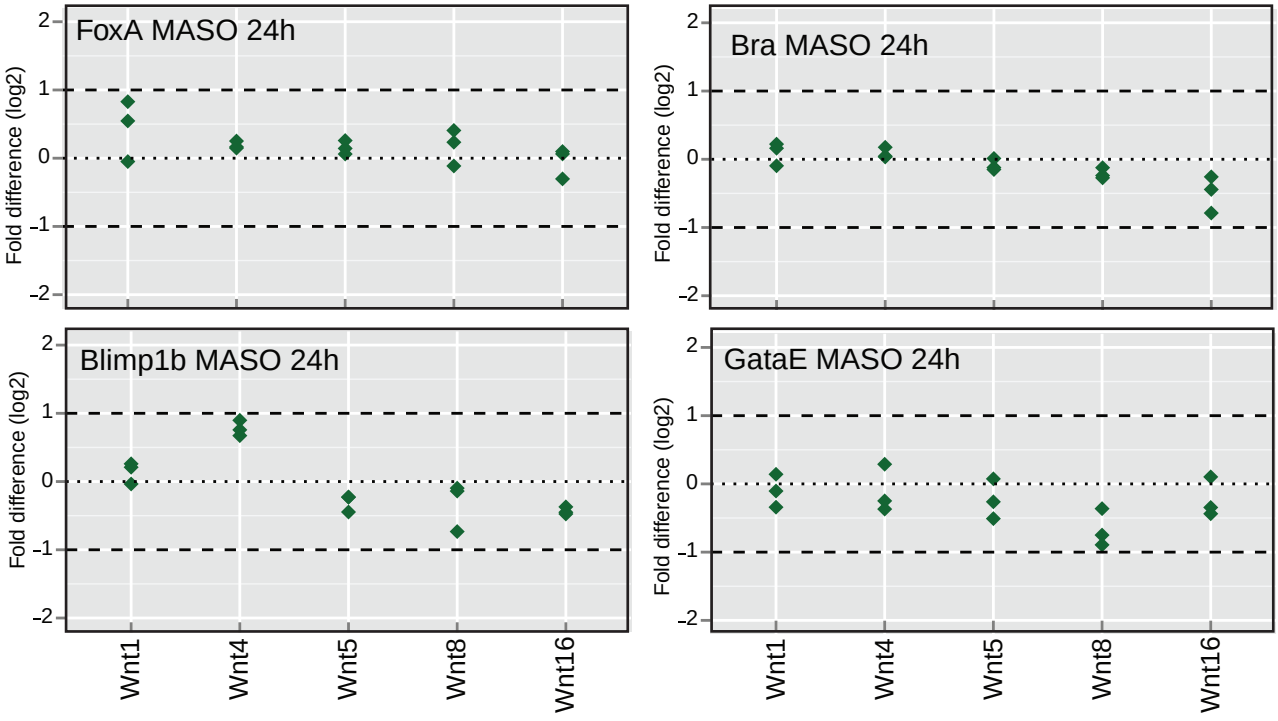
Supplemental Figure 11



Supplemental Figure 12



Supplemental Figure 13



Supplemental Figure Legends

Supplemental Figure 1. Temporal expression profiles of Wnt signaling components from 0 to 72h. (A) Early expressed *wnt* genes: *wnt1*, *wnt4*, *wnt5*, *wnt8*, and *wn16*; transcript levels for *wnt8* are plotted on a different scale shown at right. (B) Late expressed *wnt* genes: *wnt3*, *wnt6*, *wnt7*, *wnt9*, *wnt10*, and *wntA*. (C) *frizzled* genes: *fzd1/2/7*, *fzdl4*, *fzd5/8*, and *fzd9/10*. (D) Genes encoding soluble frizzled related proteins, and Dickkopf proteins: *sfrp1/5*, *sfrp3/4*, *dkk*1, and *dkk3*.

Supplemental Figure 2. Spatial expression of *wnt* genes, 12-24h. Representative WMISH images for all early expressed wnt genes at 12h, 15h, 18h, 21h, and 24h, using probes against all early expressed *wnt* genes. Expression of *foxa* in veg2 endoderm at the same stages is shown to provide a spatial reference in mapping *wnt* expression domains.

Supplemental Figure 3. Spatial expression of *fzd*, *sfrp*, and *dkk* genes, 12-24h. (A) Representative WMISH images for *fzd* genes. (B) Representative WMISH images for *sfrp* and *dkk* genes (*dkk* genes shown only at 15 and 24h).

Supplemental Figure 4. Effects of C59 porcupine inhibitor on morphology and gene expression. (A) Embryonic morphology at 22h, 48h, and 72h in the presence of 0.1% DMSO (control), 0.1μM C59 or 0.5μM C59. Inhibition of Wnt signaling affects the formation of the gut, the development of coelomic pouches (arrow head), and the arrangement and production of spicules by skeletogenic cells (asterisk) in a dose-dependent manner. Specification and patterning of pigment cells are not affected, as shown in light photographic images at right. (B) C59 effects on endomesodermal regulatory genes at various concentrations. QPCR measurements are shown for the veg2 endodermal regulator *foxa*, the veg1 posterior endoderm regulators *eve* and *hox11/13b* and aboral mesodermal regulator *gcm*, in control embryos and in embryos exposed to C59 at the concentrations between 0.1uM and 5uM.

Supplemental Figure 5. Porcupine in sea urchin embryos. (A) Morphological effects of Porcupine knock-down by morpholino, seen at 72h. (B) Endomesodemal genes expression of which is significantly decreased by Porcupine MASO are the same as those responding to inhibition of Wnt signaling by C59; QPCR analysis, symbolism as in Fig.2 of text. (C) Porcupine target sequences shared among all early expressed the *wnt* genes.

Supplemental Figure 6. Efficacy windows for C59 inhibition of regulatory genes expression. Embryos were incubated with 0.5uM of C59 for different developmental intervals, as indicated on the abscissae. Transcripts of genes were measured by QPCR at 24h. (A) Endodermal genes, *hox11/13b*, *foxa*, *blimp1b*, *brachyury*; as above red symbols indicate significant decreases. Maximal effects were obtained in C59 treatment between 15 to 24h. (B) Apical genes, *foxq2*, *nk2.1*, *nkx3-2*, *fzd5/8*; here as discussed in text, C59 causes up-regulation is due to expansion of expression domain, and maximal effects were obtained at the earliest exposure interval.

Supplemental Figure 7. Effects of maternal β-catenin depletion by Dn-cadherin mRNA injection. A) Complete animalization phenotype of embryos shown at 72hpf. B) Timing of effects on veg1 and veg2 endodermal gene, *blimp1b*, *foxa*, *hox11/13b*; veg1 endodermal genes, *eve*, *bra*, *hnf1*; apical genes, *dkk3*, *foxq2* and *fzd5/8*. Significant depression of veg1 and veg2 endodermal genes is observed from the earliest time these genes are expressed. Similarly up-regulation of apical genes is observed at the earliest time of their expression; symbolism as in Fig.2 of the text.

Supplemental Figure 8. Normal spatial expression of Veg1 genes. Changing expression patterns of *hox7*, *irxa*, and *msx*, 4-6 hours before 24h. All three of these genes are initially activated in the veg1 cells whereupon the transcription expands to include the aboral animal ectoderm. In the lower tier are shown the expression of *nk1* and *sp5* in the oral veg1 ectoderm, and of *unc4.1* in the aboral veg1 ectoderm.

Supplemental Figure 9. Wnt signaling functions in the spatial patterning of regulatory territories along the animal-vegetal axis. (A) WMISH displays of regulatory genes expression in C59 treated embryos, and in controls exposed only to DMSO. Genes studied are normally expressed in the following domains: *alx*, skeletogenic mesoderm; *delta*, veg2 mesoderm; *foxa*, anterior endoderm; *hox11/13b*, posterior endoderm; *eve*, posterior endoderm plus vegetal ectoderm; *lim1* and *emx*, animal ectoderm; and *foxq2*, apical neurogenic domain. (B) Schematic, summarizing overall alterations in spatial disposition of regulatory state domains indicated in the key below. In general, it can be seen that the endomesodermal domains contain fewer cells and the apical domain expands in C59 treated embryos. (C) Effects on expression patterns of same genes resulting from morpholino knockdown of each of the early expressed wnt genes. Symbols: asterisks, arrowheads, vertical lines, arrows, mark the expression domains in normal and perturbed embryos.

Supplemental Figure 10. Schematic summary of changes in spatial gene expression upon Wnt morpholino injection. Embryos were injected with morpholinos targeting Wnt1, Wnt4, Wnt5, Wnt8 or Wnt16 and analyzed by WMISH at 24h. Spatial expression of regulatory genes representing regulatory state domains throughout the embryo are shown in Fig. S9. Here the expression domains of regulatory genes are schematically represented for control embryos (left schemes) and Wnt morpholino injected embryos (right schemes). Wnt morpholinos responsible for observed effects are indicated. Expression domains represented by regulatory genes at 24h are as follows: *foxa* (veg2 endoderm), *hox11/13b* (veg1 endoderm), *eve* (veg1 endoderm and veg1 ectoderm), *lim1* (veg1 ectoderm, oral animal ectoderm), *emx* (oral animal ectoderm), and *foxq2* (apical neurogenic ectoderm).

Supplemental Figure 11. Expression matrix for candidate *wnt* activators in the vegetal lineages. Expression matrix for the five early expressed *wnt* genes is shown as in Fig.1, and below for comparison, similar expression matrixes are shown for candidate endomesodermal regulatory genes which were tested as candidate drivers of the *wnt* genes.

Supplemental Figure 12. Roles of Hox11/13b and Eve in the control of *wnt* gene expression. Expression levels of *wnt* genes were analyzed by qPCR at 24h in embryos injected with morpholinos blocking expression of Hox11/13b or Eve, or with randomized control morpholinos. Results are shown as ratios (log2) of expression levels of *wnt* genes in embryos injected with gene-specific morpholinos versus control morpholinos. Results of three independent experiments are shown, with symbolism as in Fig. 2.

Supplemental Figure 13. Lack of effect of *foxa*, *brachyury*, *blimp1b*, and *gatae* morpholinos on transcript levels of the five early expressed *wnt* genes. QPCR data are shown, symbolism as in Fig.2 of text.

Supplemental Tables

Table S1. Complete list of genes examined by nCounter and their fold-change upon inhibition of Wnt signaling by C59 treatment. Gene counts between samples are normalized by total reads and are corrected for background using negative spikes. Only gene counts that are great than 200 after normalization and correction are thus calculated for fold changes. Numbers shown are average of three independent experiment.

Genes	12h	15h	18h	21h	24h	Genes	12h	15h	18h	21h	24h
acsc	N/E	N/E	N/E	2.72	1.84	ngn	N/E	N/E	N/E	N/E	N/E
activinb	N/E	N/E	N/E	N/E	N/E	nk1	N/E	N/E	0.33	0.28	0.26
alx1	0.91	1.02	1.01	1.03	1.15	nk2.1	N/E	2.06	2.77	3.46	2.81
arnt	N/E	N/E	N/E	1.06	1.08	nk2.2	0.80	0.76	0.65	0.66	0.66
atbf1	1.07	0.95	1.18	1.23	1.11	nkx3-2	N/E	2.43	3.89	4.65	4.27
atf2	1.06	1.12	1.18	1.03	1.09	nlk	0.94	1.01	1.02	0.98	0.92
blimp1a	N/E	N/E	N/E	N/E	N/E	nodal	1.06	0.91	0.73	0.77	0.85
blimp1b	0.81	0.50	0.24	0.23	0.18	not	1.00	1.06	0.80	0.81	1.04
bmp2/4	1.12	0.98	0.93	0.99	1.12	notch	0.99	1.09	1.02	0.95	0.97
bmp5/8	0.88	0.94	1.07	1.10	1.11	nr1h6b	1.10	N/E	N/E	N/E	1.06
bra	0.81	0.38	0.41	0.36	0.52	otp	1.06	N/E	N/E	N/E	N/E
brn1/2/4	1.07	0.98	1.21	1.02	0.99	otx alpha	1.15	1.25	0.80	0.92	1.21
cdx	N/E	N/E	N/E	N/E	N/E	otx beta1/2	1.21	0.87	0.79	0.90	1.94
cebpa	1.00	1.19	1.43	1.31	1.47	pax2/5/8	N/E	N/E	N/E	0.80	0.88
chd	N/E	0.95	0.82	0.78	0.87	pax4	0.84	0.91	1.03	0.99	1.10
coe	N/E	N/E	N/E	N/E	N/E	pax6	N/E	N/E	N/E	N/E	N/E
cyclophilin	0.89	0.93	1.07	0.98	1.01	paxb	0.92	1.00	1.00	1.01	0.97
dach	0.93	1.01	1.07	1.08	1.05	paxc	N/E	N/E	N/E	N/E	N/E
delta	0.90	0.87	1.15	1.43	1.49	pea	0.85	0.89	1.14	1.02	0.95
dlx	N/E	N/E	0.72	0.70	0.77	pitx2	N/E	N/E	N/E	N/E	N/E
dmrt	N/E	N/E	N/E	N/E	N/E	pks	N/E	0.70	0.91	0.87	0.88
dpc4	1.05	1.05	1.05	1.04	0.96	pmar	0.99	1.10	1.11	0.96	1.02
dri	0.77	0.88	1.11	1.16	0.99	prox1	1.09	0.94	1.03	0.92	0.94
e2a	0.95	0.90	1.11	1.13	1.10	ptc1	1.11	0.98	1.17	1.30	1.31
e2f3	0.93	0.96	0.99	1.09	1.03	ptf1a	N/E	N/E	N/E	N/E	0.55
e2f4	1.05	0.97	1.11	1.03	1.06	rel	N/E	N/E	N/E	1.12	1.09
ecr	1.28	0.95	1.06	1.04	0.84	reverb	N/E	N/E	N/E	0.99	1.05
egf2	0.86	0.90	0.97	0.98	0.98	rfp	N/E	N/E	N/E	N/E	N/E
elfb	N/E	0.96	1.03	1.02	0.97	runt1	1.03	1.25	1.35	1.26	1.30
elk	0.90	1.09	1.03	0.92	1.00	rx	N/E	N/E	N/E	3.00	2.39
emx	0.82	0.66	0.83	1.36	1.32	scl	0.91	1.07	0.99	0.86	0.95
endo16	N/E	0.56	0.64	0.59	0.56	scratchx	N/E	N/E	N/E	N/E	N/E
erf	1.09	0.97	0.93	0.81	1.02	shr2/tf2.4	0.99	1.03	1.13	1.02	1.03

erg	0.87	0.82	0.93	0.96	1.01	sim	N/E	N/E	N/E	N/E	N/E
ese	1.03	1.19	1.05	1.01	1.02	sip/smadip	0.97	1.34	1.33	1.10	1.19
ets1/2	1.19	1.12	1.15	1.01	1.11	six1/2	N/E	N/E	N/E	1.03	0.78
ets4	1.17	0.95	0.91	1.23	1.14	six3	1.12	1.27	1.57	1.54	1.31
eve	0.66	0.51	0.34	0.43	0.54	sm50	0.82	1.22	1.07	0.99	1.01
fgf	N/E	N/E	N/E	1.05	1.07	smad2/3	0.94	0.98	1.05	0.99	1.03
fgfr3	0.83	1.11	1.37	1.28	1.09	smad4	N/E	N/E	N/E	N/E	N/E
fic	0.81	0.88	1.04	1.18	1.30	smad5	0.94	1.00	1.00	0.93	0.95
follistatin	N/E	N/E	N/E	N/E	N/E	smo	1.01	1.11	1.01	0.95	1.11
foxa	0.66	0.61	0.32	0.35	0.46	snail	N/E	N/E	N/E	N/E	0.68
foxb	N/E	N/E	N/E	0.84	1.26	soxb1	0.95	1.04	1.03	1.31	1.37
foxc	N/E	N/E	N/E	N/E	N/E	soxb2	0.87	0.73	1.07	1.14	1.01
foxd	N/E	N/E	N/E	N/E	N/E	soxc	0.91	0.82	0.98	1.04	1.05
foxf	N/E	N/E	N/E	N/E	N/E	soxd1	1.21	1.25	1.56	0.86	1.11
foxg	N/E	1.10	1.15	1.17	1.24	soxe	N/E	N/E	N/E	N/E	N/E
foxi	N/E	N/E	N/E	N/E	N/E	soxf	1.15	N/E	N/E	N/E	1.31
foxj1	0.92	1.16	2.12	2.12	1.87	spec1	0.85	0.87	0.94	0.94	0.94
foxj2	1.02	1.05	1.12	1.19	1.15	spz12	0.92	1.02	1.01	1.09	1.02
foxk	0.92	0.99	0.99	1.06	0.99	srf	0.99	1.05	1.02	0.91	0.95
foxm	0.97	0.97	1.05	1.04	1.01	su(h)	0.95	1.11	1.22	0.97	1.05
foxn2/3	0.84	0.79	0.71	0.55	0.67	tbr	1.05	0.90	0.81	0.83	1.04
foxo	N/E	N/E	N/E	1.19	1.06	tbx2/3	N/E	N/E	0.98	0.92	0.79
foxp	0.91	0.99	1.11	1.05	0.96	tbx6	1.13	0.96	1.04	1.24	1.08
foxq2	1.24	2.95	3.12	3.67	3.63	tcf	1.09	0.98	1.40	1.04	1.09
foxy	1.12	1.20	1.20	1.35	1.12	tead4	0.94	0.96	1.19	1.10	1.07
fzd4	1.22	1.10	0.96	1.04	0.78	tel	0.92	0.92	0.97	0.97	1.05
fxr	N/E	N/E	N/E	N/E	N/E	tgif	N/E	0.90	0.84	0.51	0.41
gatac	N/E	N/E	N/E	0.85	1.04	thr	1.06	1.15	1.24	1.12	1.01
gatae	N/E	0.56	0.63	0.45	0.33	tll1	N/E	N/E	N/E	0.55	0.86
gbx	N/E	N/E	N/E	N/E	0.44	tulp4l	1.05	1.01	1.08	1.04	1.02
gcm	0.76	0.78	0.86	0.85	0.82	ubq	1.08	1.05	1.00	1.04	1.00
gfp	N/E	N/E	N/E	N/E	N/E	unc4.1	N/E	N/E	N/E	0.62	0.44
gsc	N/E	1.31	1.13	1.19	1.21	univin	0.89	0.79	0.76	0.94	1.09
hbn	0.96	2.42	2.87	2.72	2.30	usf	0.88	0.86	1.06	0.93	0.96
hes	1.17	1.50	1.42	1.19	1.25	vegf3	0.90	0.76	0.82	0.78	0.78
hesc	0.84	0.78	0.71	0.81	0.88	vegfr	N/E	N/E	1.01	1.03	1.06
hex	0.84	0.93	0.94	0.84	0.85	vitellogenin2	N/E	1.52	2.22	2.51	2.50
hey4	0.89	0.63	0.56	0.50	0.51	wnt1	0.80	0.67	0.60	0.57	0.58
hh	N/E	N/E	N/E	N/E	N/E	wnt16	0.92	0.86	0.88	0.68	0.43
hlf	0.86	0.93	1.18	1.19	1.17	wnt3	N/E	N/E	N/E	N/E	N/E
hlx	N/E	N/E	N/E	N/E	N/E	wnt4	0.88	0.88	0.67	0.62	0.68

hmg1	0.93	0.95	1.05	1.05	1.04	wnt5	0.76	0.83	0.58	0.45	0.46
hmg2	0.86	0.83	1.10	1.04	1.02	wnt6	N/E	N/E	N/E	N/E	N/E
hmx1	0.73	0.51	0.65	1.01	0.93	wnt8	0.89	0.65	0.38	0.47	0.64
hnf1	N/E	N/E	0.23	0.17	0.16	wnta	N/E	N/E	N/E	N/E	N/E
hnf4	N/E	N/E	N/E	N/E	N/E	z108	0.91	1.08	1.00	1.00	1.01
hnf6	1.07	1.05	1.15	1.23	1.24	z115	0.93	1.00	1.08	0.98	0.94
hox11/13b	0.79	0.79	0.47	0.28	0.33	z121	N/E	N/E	N/E	N/E	N/E
hox7	N/E	N/E	N/E	0.55	0.56	z13/krl	0.80	0.60	0.37	0.45	0.89
id	0.90	0.76	1.20	0.83	0.85	z133/fez	N/E	N/E	N/E	N/E	N/E
irf4	N/E	N/E	N/E	N/E	N/E	z141	0.97	1.02	1.01	1.04	0.95
irxa	N/E	N/E	0.49	0.76	0.82	z157/ovo	0.96	1.03	1.06	1.08	1.31
jun	1.11	1.10	1.15	1.21	1.18	z166	N/E	0.74	0.87	0.89	0.97
lefty	1.09	0.96	0.75	0.74	0.89	z188/klf13	0.99	1.15	1.63	1.41	1.30
lhx2.9	N/E	N/E	N/E	3.24	2.69	z199/sp5	0.58	0.25	0.07	0.18	0.30
lim1	0.73	0.78	0.58	0.61	0.70	z204	N/E	N/E	N/E	N/E	1.07
lmo4	N/E	N/E	N/E	N/E	0.82	z214	0.88	0.96	1.09	1.03	0.96
lmx1	N/E	N/E	N/E	N/E	N/E	z22/gli1	1.07	0.93	0.98	0.91	0.97
lox	N/E	N/E	N/E	N/E	N/E	z244/zic	1.04	2.15	2.97	3.26	2.30
mad	0.94	1.02	1.11	1.02	0.97	z30	0.97	1.06	1.20	1.28	1.05
max	0.98	0.97	1.04	0.98	0.97	z400	0.86	0.97	1.10	1.02	0.96
mbx1	N/E	N/E	N/E	N/E	N/E	z48	1.02	0.84	1.19	0.84	0.83
mef2	0.91	0.91	1.03	1.06	1.13	z487	0.98	1.07	1.12	1.06	1.09
mitf/tfe3	1.02	0.95	1.15	0.92	0.99	z54/spalt	0.92	0.98	1.05	1.02	0.86
mlx	1.05	1.05	1.04	1.00	0.99	z55	0.96	0.96	1.11	1.05	1.01
Msx	N/E	N/E	0.43	0.61	0.85	z57	0.85	0.91	1.01	1.11	1.04
myb	0.97	0.99	1.04	1.03	1.03	z60	0.92	0.92	0.87	1.07	1.25
myc	0.85	0.70	0.83	0.77	0.79	z62	0.89	0.96	1.06	1.04	1.02
myor2	N/E	N/E	N/E	N/E	N/E	z85/klf2/4	0.91	0.90	1.09	0.99	0.87
nfe2	0.93	0.97	0.96	0.95	0.99	z86	0.99	0.95	0.99	1.05	0.91
nfkb	0.95	0.97	1.07	1.08	1.07	z92	0.92	0.95	1.00	1.01	0.97

N/E: counts ≤ 200

Table S2. Summary of experimental evidence for regulatory genes affected by Wnt signaling.

Regulatory genes affected by Wnt signaling	Evidence[1]	Effect of C59 on gene expression	Tcf target [2]	Domain of expression at 24h	Source
blimp1b	C59, Wnt1 MASO, Wnt16 MASO, CRA	Decreased	Y	veg2 Endo	This study, (7), (8)
bra	C59, Wnt1 MASO, CRA	Decreased	Y	veg1 Endo	This study, (8)
eve	C59, Wnt1 MASO, Wnt16 MASO, CRA	Decreased	Y	veg1 Endo, veg1 Ecto	This study, (7), (8)
foxa	C59, Wnt1 MASO, CRA	Decreased	Y	veg2 Endo	This study, (9), (8)
gatae	C59, Wnt1 MASO, CRA	Decreased	NA	veg2 Meso, veg2 Endo	This study, (8)
gbx	C59	Decreased	NA	NA	This study
hey4	C59, Wnt1 MASO	Decreased	NA	veg2 Endo	This study
hnf1	C59, Wnt1 MASO	Decreased	NA	veg1 Endo	This study
hox7	Wnt4 MASO	Decreased	NA	Aboral veg1, Aboral animal Ecto	This study
hox11/13b	C59, Wnt1 MASO, Wnt16 MASO, CRA	Decreased	NA	veg1 Endo	This study, (8)
irxa	C59	Decreased	NA	Aboral veg1, Aboral animal Ecto	This study, (10)
lim1	Wnt5 MASO	Decreased	NA	Lateral and Oral veg1, Lateral and Oral animal Ecto	(10)
msx	C59, Wnt4 MASO	Decreased	NA	Aboral veg1, Aboral animal Ecto	This study
nk1	C59, Wnt1 MASO	Decreased	NA	Oral veg1	This study, (10)
pax2/5/8	Wnt5 MASO	Decreased	NA	Lateral veg1 ectoderm	(10)
tgif	C59, Wnt1 MASO	Decreased	NA	veg2 Endo	This study
unc4.1	C59, Wnt1 MASO	Decreased	NA	Aboral veg1	This study
wnt1	C59, Wnt1 MASO	Decreased	NA	veg1 Endo	This study
wnt4	C59, Wnt1 MASO	Decreased	NA	veg1 Endo, veg1 Ecto	This study
wnt5	C59, Wnt1 MASO, Wnt5 MASO	Decreased	NA	veg1 Endo, veg1 Ecto	This study, (10)
wnt8	C59, Wnt1 MASO, CRA	Decreased	Y	veg1 Endo, veg1 Ecto	This study, (11)
wnt16	C59, Wnt1 MASO	Decreased	NA	veg1 Endo	This study
z13/krl	C59, Wnt1 MASO	Decreased	NA	veg2 Endo	This study
z199/sp5	C59, Wnt1 MASO	Decreased	NA	Oral veg1, Oral animal Ecto	This study
acsc	C59, Wnt8 MASO	Increased	N	NA	This study
foxj1	C59, Wnt8 MASO	Increased	N	Apical plate	This study
foxq2	C59, Wnt8 MASO	Increased	N	Apical plate	This study, (12)
hbn	C59, Wnt8 MASO	Increased	N	Apical plate	This study
ihx2.9	C59, Wnt8 MASO	Increased	N	Apical plate	This study
nk2.1	C59, Wnt8 MASO	Increased	N	Apical plate	This study
nkx3-2	C59, Wnt8 MASO	Increased	N	Apical plate	This study
rx	C59, Wnt8 MASO	Increased	N	Apical plate	This study
z244	C59, Wnt8 MASO	Increased	N	Apical plate	This study

[1] CRA: Cis-Regulatory Analysis
[2] Regulatory genes directly regulated by TCF as shown by cis regulatory studies; Y, direct target genes; N, genes affected by C59 but are not direct TCF targets; NA, potential TCF target genes, but not analyzed at the cis-regulatory level.

Table S3. Sequences of primer sets used for temporal and spatial characterization of Wnt signaling components.

Gene	ID	Q-PCR Primer fwd	Q-PCR Primer rev	WMISH primer fwd	WMISH primer rev
wnt1	WHL22.596782	TGCGATCTTATGTGCTGCTC	GAAACGACGTGCACTCTTCA	TTAATCCGCAGCAAATAGCC	ACAACTTCCTCAGCGTTTCC
wnt3	WHL22.87121	GAGAGCGAGGACAAAGATGG	CCACCAGCAGGTCTTGAGTT		
wnt4	WHL22.587606	GCGGACTTTAAGCCTCACAC	GTCGTCCATGAGTTCCCAGT	CCTTTGAGCCAATTTGTTTG	TCTCGGACATTACGGAAACA
wnt5	WHL22.52081	TGCTGTGGAAGAGGCTACAA	TTCTGCACTTCCGACACTTG	AGGAAGGACTGTGCTCGAA	AGGAAAACACTCCCGAAGAC
wnt6	WHL22.596784	CGGGCTCCTGTACTCTCAAG	GGTGGGCTGTTTGACAGTTT		
wnt7	WHL22.475027	AGGCATGTATGCAGGGAAAC	GAAAAGCCGTGAAAACCTGA		
wnt8	WHL22.8923	TGTCGTTCATTCAAGCCATC	TATCACTCGCCATTCGTTCA	GTCACCAGCAAGCAACGTTC	AACACCAAACGAAGTTGCAG
wnt9	WHL22.596775	TGACCTTGGAATAAGGACCG	TGACCTGAACACTTCGTTCG		
wnt10	WHL22.23530	TGTCACACTACGCCGAAAAG	CATGAGACGGTTTCCAACCT		
wnt16	WHL22.735232	CGATCCCGAGACTCTCTGTC	CGATTTCCCGGTTAGTACGG	TCTCTCATTTTCTGTGTCAG	GTCCATGGTTTAAGCAGACC
wntA	WHL22.540942	ATGGGTCACTCGTGGAACTC	CAGTTCCATCGTTCGTTCCT		
frizzled 1/2/7	WHL22.45238	TTGCCACCACTACAGCTTTG	AACTGGGTCCACGATCTCAC	AGAGGGAAGTTACGGCAACA	CCCATGGAAATAGCACACCT
frizzled 4	SPU_008022	AGGAGGGGTTGGAGAACACT	TGATGACGGTTTTGACGAAG		
frizzled 5/8	WHL22.42488	TCCTATCTGTTTGGCGGACT	CACTCGTTCCTGCATTCGTA	GTGGAACAATCCATCAGTTG	CGTGGTTGCCTACGTAACAG
frizzled 9/10	WHL22.60681	ACGATCCTGACGTTCTCACC	GTGGCAGGCACTGTGTAGAG	TCCTTCGTGTTTTCTTGATG	GTTTCACTGATAACAC
sfrp 1/5	WHL22.146031	CATGTGCGAGAACTTGGAGA	CTTTCCCGTCTTGTGTTGGT	TCACCTTGCTCGATCACTCA	GTTTCCTCCCGTTTGTCAGA
sfrp 3/4	WHL22.62451	TTGGATCTAGGGGCTTTCCT	TCTTGCCGACTTCTGATCCT	GCAGTTTGCTCCTCTCATCC	AGCTCCGAACACGGTAAGAC
dkk1	WHL22.342226	TGCTATGTGAGGCAGACAGG	GTTTCCCTGGCAACACATCT	CGGAGCAACTGGGTATTTGT	GCGACAGACAGGGAGAGTTC
dkk3	WHL22.685463	ATGGTTCGGATTATGGACACC	CTGGGATGTTCTCTTTCCAG	CGAAACCAGCATAGGCTCTC	TAGTTGCTTCGGCTTGGTCT

Table S4. Morpholino antisense oligonucleotide (MASO) sequences.

gene	MASO sequence	MASO interfering with
wnt1	ACGCTACAAACCACTCAAGTTTCAT	Translation (13)
wnt4	GATATAAATTCCTTACTCTTCTTCC	Splicing
wnt4	GAGCAGTTCCATCTCCTGTTTTGGA	Translation
wnt5	TCATGGGACAGAATGATCTTCGTCA	Translation
wnt5	GAGTTCGTACACGTTTCCATTTTGC	Translation
wnt8	AGACATCCATGATGTACACTCCAAT	Translation
wnt8	GTAAAGTGTTTTTCTTACCTTGGAT	Translation(12)
wnt16	TCTCAACAAACTCGATAGTTCAACC	Translation
porcupine	CGCACCTGCATAAACAAAGAGAGTA	Splicing
blimp1b	CTCCCTTTCGCTTGAAAAACACCGC	Translation (14)
brachyury	CGCTCATTGCAGGCATAGTGGCG	Translation (4)
eve	CAGAAACCACTCGATCAATGTTTGC	Translation (4)
foxa	TGGGTTCCTCTTTGAAATCCACGAT	Translation (15)
gatae	GACTTACACCGACCTGATGTGGCAT	Translation (4)
hox11/13b	AAGCCTGTTCCATGCCGATCTGCAT	Translation (16)

Supplemental References:

1. Li E, Materna SC, & Davidson EH (2012) Direct and indirect control of oral ectoderm regulatory gene expression by Nodal signaling in the sea urchin embryo. *Developmental biology* 369(2):377-385.
2. Li E, Cui M, Peter IS, & Davidson EH (2014) Encoding regulatory state boundaries in the pregastrular oral ectoderm of the sea urchin embryo. *Proc Natl Acad Sci U S A*.
3. Li E, Materna SC, & Davidson EH (2013) New regulatory circuit controlling spatial and temporal gene expression in the sea urchin embryo oral ectoderm GRN. *Dev Biol* 382(1):268-279.
4. Peter IS & Davidson EH (2011) A gene regulatory network controlling the embryonic specification of endoderm. *Nature* 474(7353):635-639.
5. Peter IS & Davidson EH (2010) The endoderm gene regulatory network in sea urchin embryos up to mid-blastula stage. *Dev. Biol.* 340(2):188-199.
6. Ransick A & Davidson EH (2012) Cis-regulatory logic driving glial cells missing: self-sustaining circuitry in later embryogenesis. *Developmental biology* 364(2):259-267.
7. Joel Smitha EK, Hongdau Liua, Christina Theodorisa, Eric Davidsona (2008) A spatially dynamic cohort of regulatory genes in the endomesodermal gene network of the sea urchin embryo. *Developmental biology* 313(2):863-875.
8. Peter IS & Davidson EH (2010) The endoderm gene regulatory network in sea urchin embryos up to mid-blastula stage. *Developmental biology* 340(2):188-199.
9. de-Leon SB & Davidson EH (2010) Information processing at the foxa node of the sea urchin endomesoderm specification network. *Proceedings of the National Academy of Sciences of the United States of America* 107(22):10103-10108.
10. McIntyre DC, Seay NW, Croce JC, & McClay DR (2013) Short-range Wnt5 signaling initiates specification of sea urchin posterior ectoderm. *Development (Cambridge, England)* 140(24):4881-4889.
11. Minokawa T, Wikramanayake AH, & Davidson EH (2005) cis-Regulatory inputs of the wnt8 gene in the sea urchin endomesoderm network. *Developmental biology* 288(2):545-558.
12. Range RC, Angerer RC, & Angerer LM (2013) Integration of canonical and noncanonical Wnt signaling pathways patterns the neuroectoderm along the anterior-posterior axis of sea urchin embryos. *PLoS biology* 11(1):e1001467.
13. Sethi AJ WR, Angerer RC, Range RC, Angerer LM. (2012) Sequential signaling crosstalk regulates endomesoderm segregation in sea urchin embryos. *sciene* 335(6068):590-593.
14. Livi CB & Davidson EH (2006) Expression and function of blimp1/krox, an alternatively transcribed regulatory gene of the sea urchin endomesoderm network. *Developmental biology* 293(2):513-525.
15. Oliveri P, Walton KD, Davidson EH, & McClay DR (2006) Repression of mesodermal fate by foxa, a key endoderm regulator of the sea urchin embryo. *Development (Cambridge, England)* 133(21):4173-4181.
16. Arenas-Mena C, Cameron RA, & Davidson EH (2006) Hindgut specification and cell- adhesion functions of Sphox11/13b in the endoderm of the sea urchin embryo. *Dev. Growth Differ* 48(7):463-472