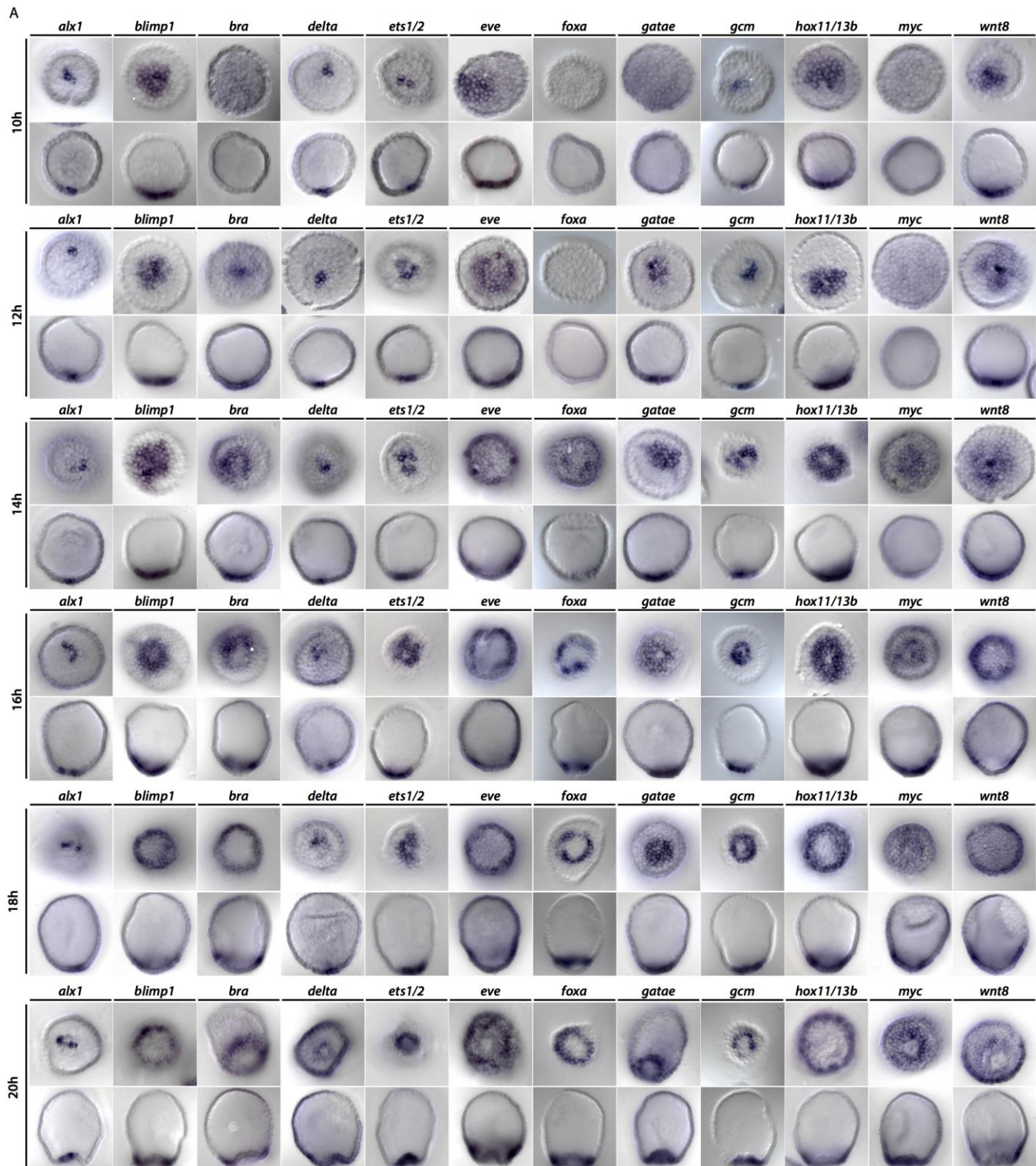
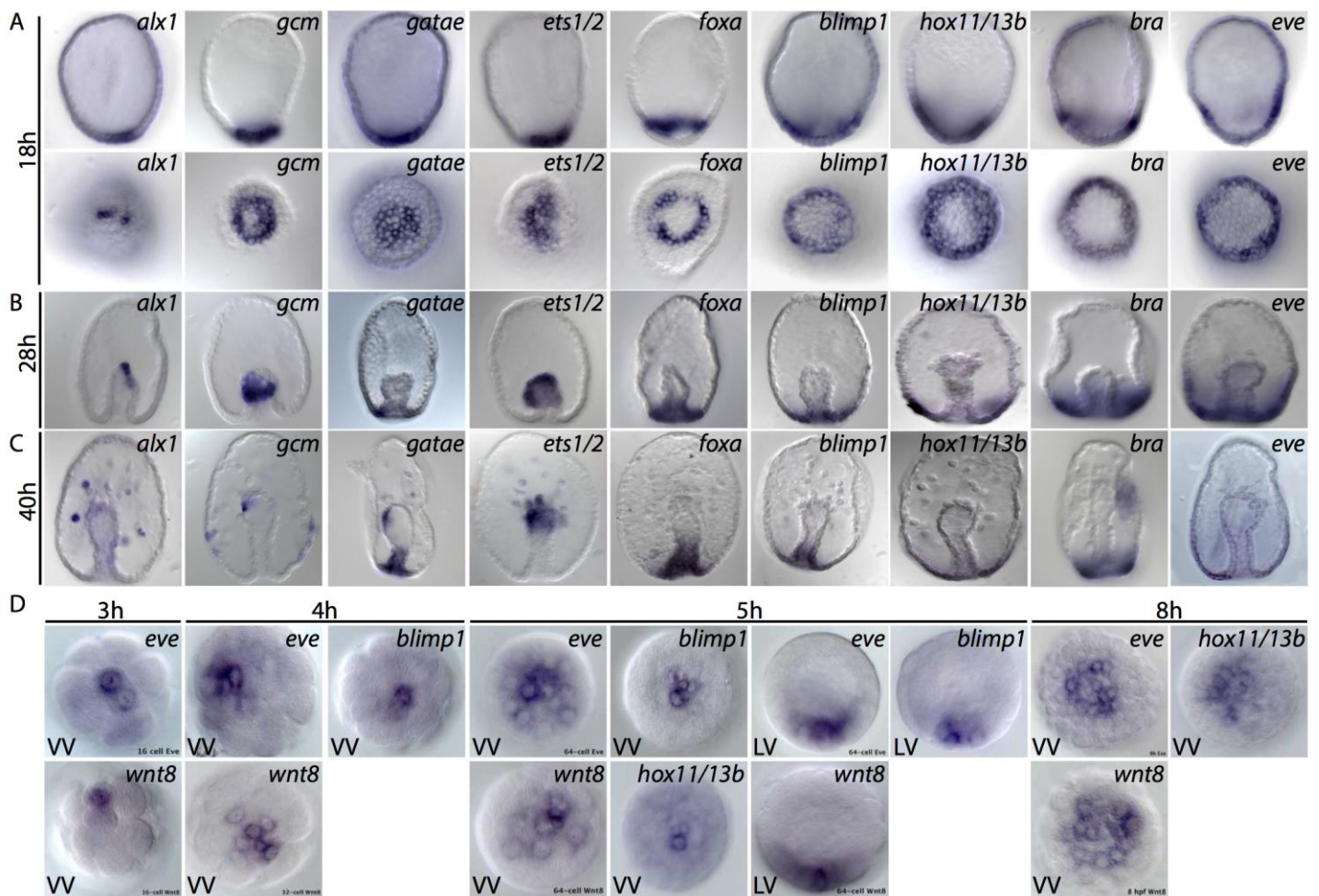


**Fig. S1. Developmental stages and gene expression timecourses in *Eucidaris*.** (A) Morphology and timing of *Eucidaris tribuloides* and *Strongylocentrotus purpuratus* development over the first 40 hours post fertilization (hpf). *E. tribuloides* embryos exhibit blastocoels devoid of mesenchyme until early-gastrula stage. *S. purpuratus* embryos possess a precociously-ingressing pregastrular mesenchyme lineage that later synthesizes embryonic skeleton. By 40 hpf, both embryos are gastrulating, but only *S. purpuratus* exhibits mesodermal skeleton and pigment cells in the ectoderm. (B) High-density temporal timecourse of RNA transcripts for 12 regulatory genes in *E. tribuloides*. For *alx1*, transcript abundance is listed on the red-colored y-ordinate at the right.



**Fig. S2. High-density timecourse of spatial expression for endomesoderm regulatory genes in *Eucidaris tribuloides*.** (A) WMISH was carried out every 2 hours from 10 hpf to 20 hpf (just prior to gastrulation) as described above. At each timepoint, ventral views (top panels) and lateral views (bottom panels) are shown.





**Fig. S3. Spatial expression of endomesodermal regulatory genes at early and mid-gastrula and prior to hatching in *Eucidaris tribuloides*.** (A-C) Early and mid-gastrula stage WMISH detection at 18, 28 and 40 hpf shows the spatial expression of regulatory genes at the onset of gastrulation. Timepoints at 18 hpf are shown in both lateral view (top panels) and ventral views (bottom panels). Timepoints at 28 and 40 hpf are shown in lateral views. (D) Early cleavage stage dynamics of regulatory genes at 3, 4, 5 and 8 hpf in *E. tribuloides*. LV, lateral view; VV, ventral view.

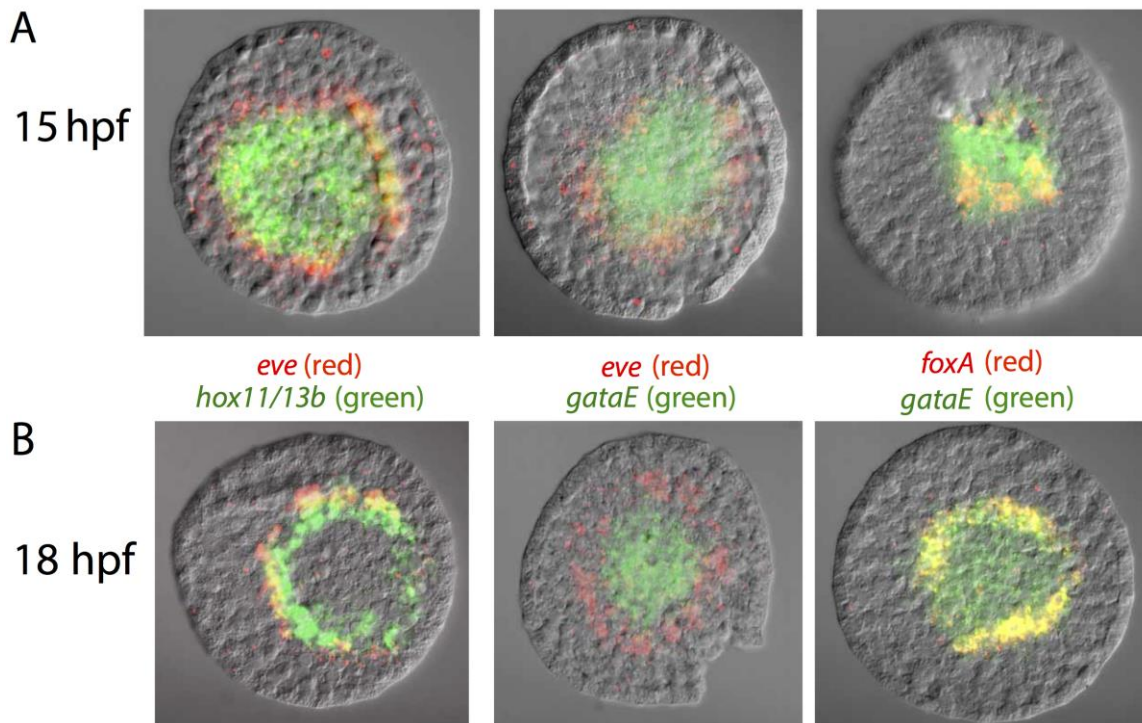
A *Eucidaris tribuloides* gene expression matrix

gene	10h					12h					14h					16h					18h					20h				
	SM	NSM	AE	PE	PAE	SM	NSM	AE	PE	PAE	SM	NSM	AE	PE	PAE	SM	NSM	AE	PE	PAE	SM	NSM	AE	PE	PAE	SM	NSM	AE	PE	PAE
<i>alx1</i>																														
<i>delta</i>																														
<i>ets1/2</i>																														
<i>gcm</i>																														
<i>gatae</i>																														
<i>blimp1</i>																														
<i>foxa</i>																														
<i>myc</i>																														
<i>bra</i>																														
<i>hox11/13b</i>																														
<i>eve</i>																														
<i>wnt8</i>																														

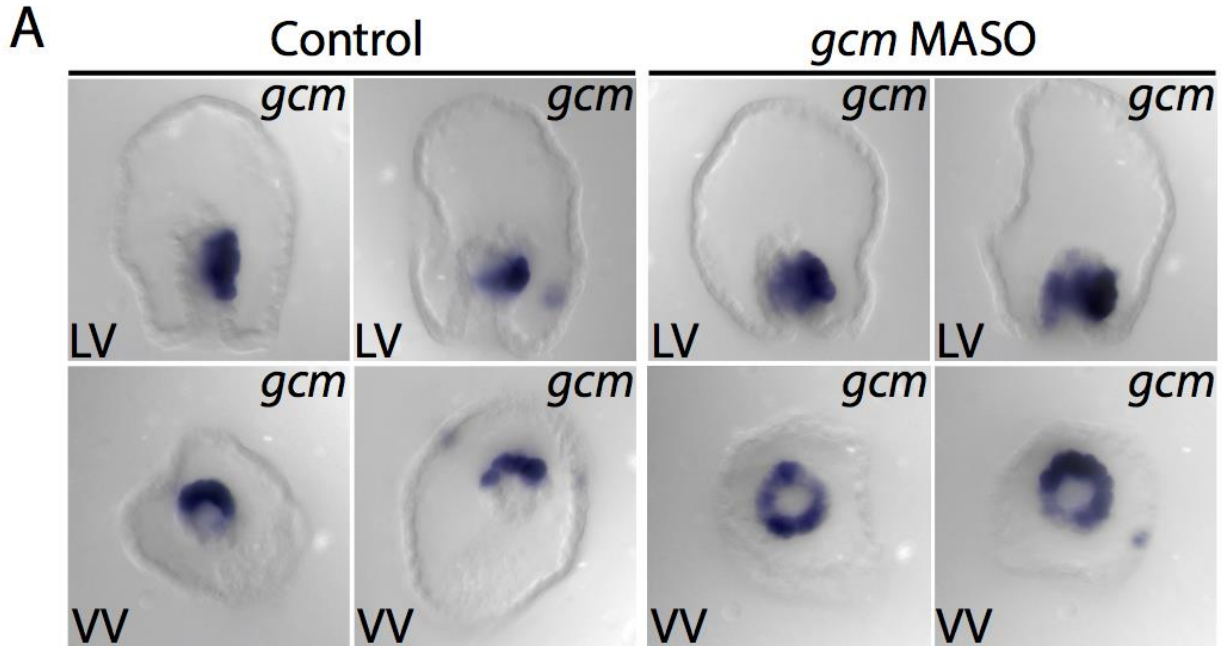
B *Strongylocentrotus purpuratus* gene expression matrix

gene	6h					12h					18h					24h					30h				
	SM	NSM	AE	PE	PAE	SM	NSM	AE	PE	PAE	SM	NSM	AE	PE	PAE	SM	NSM	AE	PE	PAE	SM	NSM	AE	PE	PAE
<i>alx1</i>																									
<i>delta</i>																									
<i>ets1/2</i>																									
<i>gcm</i>																									
<i>gatae</i>																									
<i>blimp1</i>																									
<i>foxa</i>																									
<i>myc</i>																									
<i>bra</i>																									
<i>hox11/13b</i>																									
<i>eve</i>																									
<i>wnt8</i>																									

**Fig. S4. Spatial gene expression matrices for *Strongylocentrotus purpuratus* and *Eucidaris tribuloides* showing expression of regulatory genes during pre-gastrular development.** Columns represent embryonic domains. Gray squares indicate expression, white squares absence of expression in the corresponding embryonic domain. (A) Matrix for *E. tribuloides* derived from observations in this study. (B) Data for *S. purpuratus* and are derived from Peter and Davidson (2011).



**Fig. S5. Double-fluorescent WMISH of endodermal regulatory genes in *Eucidaris tribuloides*.** At 15 and 18 hpf, *eve* and *hox11/13b* are expressed in overlapping cells in the posterior endoderm, with *eve* being exclusively expressed in perianal ectoderm and *hox11/13b* in anterior endoderm. On the other hand, *eve* and *gatae* are not co-expressed at 15h nor at 18 h, but are expressed exclusively in either mesoderm and anterior endoderm (*gatae*) or posterior endoderm and perianal ectoderm (*eve*). *Foxa* expression at 15h occurs in a ring of cells within the domain of *gatae*, the anterior endoderm, similar to 18 hpf where the ring of *foxa* expression in the anterior endoderm overlaps with *gatae* expression. *Gatae* continues to also be expressed in mesodermal domains. All embryos are shown in ventral views.



**Fig. S6. Effect of perturbing expression of Gcm by injection of morpholinos on spatial expression of *gcm* in *Eucidaris tribuloides*.** Two uninjected control embryos are shown in lateral and ventral views in the left panels. Two *gcm* injected embryos are shown in the same views in the right panels. LV, lateral view; VV, ventral view.

**Table S1. QPCR data for perturbation experiments in *Eucidaris tribuloides*.**

Change in gene expression levels is indicated as difference in QPCR cycles (ddCt) compared to uninjected control embryos. A negative value indicates decreased gene expression upon perturbation. The p-value was determined by two-tailed t-test on dCt values of control and treatment experiments. Data of individual experiments are indicated as repeats 1-4. Genes shown in red are significantly affected ( $p < 0.05$ ) by at least 2-fold.

[Click here to Download Table S1](#)

**Table S2. GenBank accession numbers for cDNA sequences.**

BankIt1988370	alx1	MF990311
BankIt1988370	blimp1	MF990312
BankIt1988370	brachyury	MF990313
BankIt1988370	ets1/2	MF990314
BankIt1988370	even-skipped	MF990315
BankIt1988370	foxa	MF990316
BankIt1988370	gatae	MF990317
BankIt1988370	gcm	MF990318
BankIt1988370	hox11/13b	MF990319
BankIt1988370	myc	MF990320
BankIt1988370	wnt8	MF990321

**Table S3. Sequences of QPCR primers.**

Gene	qPCR forward primer	qPCR reverse primer
<i>alx1</i>	ATCCGGGTATGAAATGCCCA	TTCTGCAGATGCGGAGCATA
<i>blimp1</i>	AAGAGCCACCAAGTCCTCCA	TGTAAGTGCTGGATCTCACGTGGT
<i>bra</i>	GATAGGGTGACGTACGGTGACTT	CACATGCTGCCGTATTGGTT
<i>delta</i>	AAATGTAACGTGCCGTGTGAGCCA	TACAGCTCACATTGGTCGCACCT
<i>dri</i>	AGCAGAGACGAATCATTGAGG	GGAGACTGATGCGACAAGAG
<i>ets1/2</i>	TGAGTCATCACCGAACTCGAACCA	GGTGTCCGTCAAACGTGTCAAA
<i>eve</i>	AACAGATCGGTCGTCTGGAGAA	AAGCGCCAACGAATGTTCGATGT
<i>foxa</i>	ATGGGTATGAATGCAGGGATGGGA	ATCCTGCTGCTATGTTCGATGAGGT
<i>gatae</i>	GCAACATAACGCGACGACCAAAACA	AATGAACGGGTACAACAGACCGCT
<i>gcm</i>	ATCGATGCACGGAAACATCTCAGC	TCCAACAACTGTCAAACGAGTGCG
<i>foxq2</i>	TACGCCTATCCTTCCACCATC	GTGAAGGCAGCGACGAATATG
<i>hox11/13b</i>	ATGGCCCACCAGCAACAATACA	GCGACCGTACTCGAAACTGCAAT
<i>myc</i>	CCGACTCTGAGGAGGAAATC	ACAGAGCACACAGGTCATTA
<i>wnt8</i>	TGTCCAAACTCTTCGTGGATGCTG	TTGTAAATGCCATGGTGTCTCCGG

**Table S4. Sequences of WMISH primers.**

Gene	WMISH forward primer	WMISH reverse primer
<i>alx1</i>	TGAAATGCCCATAGCTCCACGA	ATGCCCATGACTGAACTGTGCT
<i>blimp1</i>	TTGACCTCGTAGATGCATCG	TGTCTGCCATCGTGAATTTG
<i>bra</i>	TGGACACGTGGCTCGTATTTGT	AAACGGGCTATCAGGACAGT
<i>delta</i>	ACGGTGATACTAATCCTTCACTGG	AGACAGGTGTACCCGTCAGC
<i>ets1/2</i>	AATGAGGTTGGACGAGTGCTGTCA	GTCCGTCAAACGTGTCAAAGGGT
<i>eve</i>	CGTGACCAGCAACAGTAATCCCAA	TACGCCAGGCCATTCCCGA
<i>foxa</i>	AAAGTACCGAGAACGCCAGA	CAGCACAAACAAATCACGCG
<i>gatae</i>	AACCCACAACGGTCTGACGGGCTA	TGCCGTAGCCGTTTCCGTAGATAA
<i>gcm</i>	GGCCATGCGAAACACCAACAATCA	AGACGCACACGACAACGTTACTGA
<i>hox11/13b</i>	ATGCAGATAGGCATGGAGCA	TCGTCACAACCACATCACCACA
<i>myc</i>	AGGAGGTCAAGCGAATGT	GATTACGACATGACACTGCC
<i>wnt8</i>	AATGAATCGAGCCATCGAGGAGTG	AAGTTGTCGTGACCTCTAGCTGCA

**Table S5. Sequences of morpholino antisense oligonucleotides.**

Gene	MASO sequence	Interferes with
<i>delta</i>	ATAACATATAGCACGCCGAGAAGGC	Translation
<i>eve</i>	ATGGTGAAACCTCTTTCCATGTTAC	Translation
<i>gcm</i>	TGTCTTCTGGACCATGTTGACCGTC	Translation
<i>hox11/13b</i>	GATTATGGATGTTGGCTTACCTGTC	Splicing



## Sequence of dnOtx-Engrailed construct

>EtOtx\_homeodomain\_fused\_to\_Engrailed\_repressor\_domain

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CACCATCAGCAACAACAACAGCAGCAACAACAACAGCAGATGCAACATCTCCATCAACTCCAGCA
ACTGCAACAACACTACATCAGCAGCAGCTCGCAGCTGGTGTCTTTTACCACCCGGCTATGGCCTTTG
ACGCCGCCGCTGCTGCCGCTGCAGCTGCCGCTGCTGCCGCTGCTCATGCCCATGCTGCTGCCCTGC
AACAGAGACTCTCTGGTTCCGGCTCCCCTGCTAGCTGCAGCACGCCAGCATCTTCAACCCCACTCA
CCATCAAGGAAGAAGAAAGCGATTCCGTCATCGGAGATATGTCTTTCCACAACCAGACACATACA
ACCAATGAAGAGGAAGAGGCTGAGGAAGACGACGATATCGATGTGGATGTCGATGATACCAGCG
CCGGAGGTCGACTCCCTCCCCCTGCTCATCAACAGCAATCTACAGCCAAGCCTTCTCTTGCATTCT
CAATTTCAAACATTCTCTCAGACCGTTTTTGGTGATGTTTCAGAAACCCGGAAAATCAATTGAAAAC
CAGGCCTCAATTTTCCGACCGTTTCGAGGCAAATCGTAGCCAGACGGCTACACCTTCCGCATTAC
ACGTGTTGACCTCCTCGAGTTCAGTCGTCAGCAACAGGCTGCAGCTGCCGCAGCTACTGCTGCAA
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GAACCACTTTCACTCGCGCTCAACTGGATGTGCTCGAAACACTGTTTTTACGTA CTGTTATCCA
GATATCTTCATGCGTGAAGAAGTCGCTATGAAGATCAATCTGCCAGAGTCGAGAGTACAAGTCT
GGTTTAAGAATCGTCGAGCTAAGTG TAGGCAACAGCAGCAAATGGCTCCCAAGAAGAAGAGGAA
GGTC
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