

Supplementary material

Time-course quantitative RT-PCR (qPCR) analysis

Primers used: *PmBlimp1*: F: 5'-CCAAACACGACGAAGCACTA-3', R: 5'-GGCGTTGAGGACAGGTTTAG-3'; *PmBra*: F: 5'-CGAGATGATCGTCACCAAGA-3', R: 5'-GGGGTGAAGTCCAGAAGGAT-3'; *PmDelta*: F: 5'-CGAAGGCTTCACGTGCTACTG-3', R: 5'-GCGCATGCGTAGCCATTCTC-3'; *PmErg*: F: 5'-AAGATGACCGACCCGGACGA-3', R: 5'-TGCCGTGGACCTTGGTCATA-3'; *PmEts1*: F: 5'-ATGCCTGTCTACGCGAGAGT-3', R: 5'-TCCAGGCTGAACTCCTTGAT-3'; *PmFoxa*: F: 5'-TGGCGCATATAATCCTCACC-3', R: 5'-GTCTCTCAGTGGCGCAAGAT-3'; *PmFoxg*: F: 5'-CAACGCTGCAGGTACAACCTT-3', R: 5'-AAGAGGCGATCCATGGAGAA-3'; *PmFoxn2/3*: F: 5'-TGAATGATCAGAGCCTGCCAAGA-3', R: 5'-TCTCGCTGGGCTTTGAGGAT-3'; *PmGata1/2/3*: F: 5'-GAGCCCACAGGCATTACAGA-3', R: 5'-CAACGGGCACTACCTGTGTA-3'; *PmGata4/5/6*: F: 5'-CGAGGTCTCTCCACTTGCAT-3', R: 5'-GACGGTTGGGAGTTTTTCAGA-3'; *PmHesc*: 5'-CCAGCAGCCAATCCTCCATC-3', R: 5'-TCCGAGGTGACATGGGTGAA-3'; *PmHex*: F: 5'-GGCCGTTGCATAAGCGGAAAG-3', R: 5'-TGCAGGAGTTTCGCCAGCTT-3'; *PmOtxα*: F: 5'-AGGCCGGCATGTACTCTATG-3', R: 5'-GGTTCTCTCCCGTCTGGTTT-3'; *PmOtxβb*: F: 5'-GAAAGGATGGATTGCGTCAT-3', R: 5'-ACCACTCATACTGCGGATTG-3'; *PmTbr*: 5'-CGACCGAAGACACAAGACAA-3', R: 5'-CAGGCTTTGGGTGCTGTAAT-3'; *PmTgif*: F: 5'-TGACCCTTCTGCAGGTCTGT-3', R: 5'-TCCCAGGAGACCGTCTACTG-3'; *PmWnt3*: 5'-CTGATCTACTACGACCCGTCACC, R: 5'-CTGGCACCCATCTATGGCGT; *PmWnt4*: F: 5'-TAGAGCAGGGTCCTACGGGAC-3', R: 5'-CTGCTCCGTGACCTCTGACA-3'; *PmWnt8*: F: 5'-GGG CAG AAA CCC AGA ACG AC-3'; R: 5'-TAC AGC TCC GTG CTT CCC AC-3'; *PmWnt16*: F: 5'-GGTCTCCGAACACTACTGCGTACG-3', R: 5'-TTGTATCCCCTCCCGCAGCA-3'; *PmLamin2breceptor*: F: 5'-GAGCATGCCTAAGCCAGACC-3', R: 5'-CTCCACCATGGGCTCCAGTA-3'.

Perturbation of gene expression

MASOs used: *PmEts1*: 5'-TCGAGCCCGTTGTGATACATTGTGG-3'; *PmFoxA*: 5'-CATGGGTTTCCCTCGACTCGTTGGG-3'; *PmGata4/5/6*: 5'-CTCCTAGAATCAAGTGGGTTGCAG-3'; *PmHex*: 5'-TGACATGGTGACACGAAATACGGTC-3'.

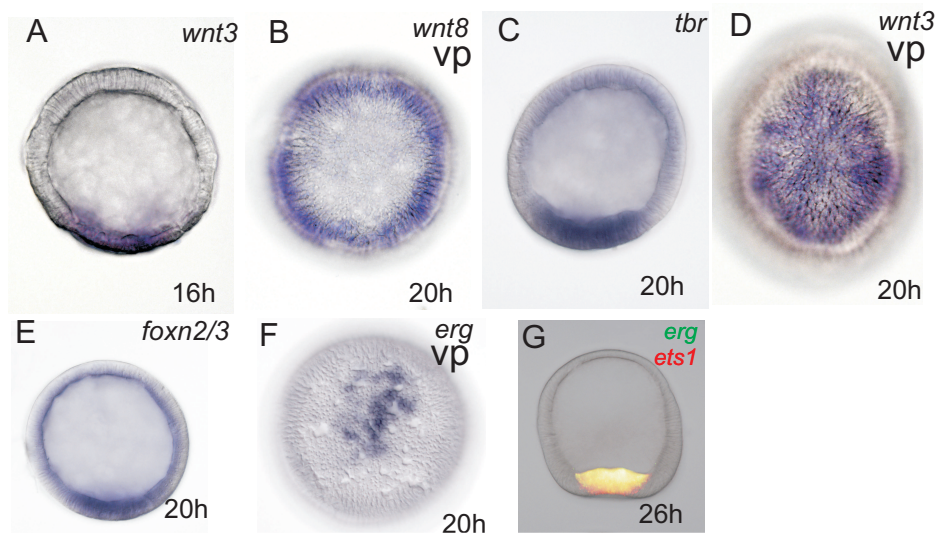


Figure S1. Characterization of additional gene expression patterns in early through late blastulae

(A-G) Whole mount *in situ* hybridization (WMISH) of the indicated genes in early (16h), mid (20h) and late blastulae (26h). Embryos are shown in a lateral view with vegetal towards the bottom unless indicated as vp for vegetal pole view. (G) Two color fluorescent *in situ* hybridization (FISH) of the indicated genes.

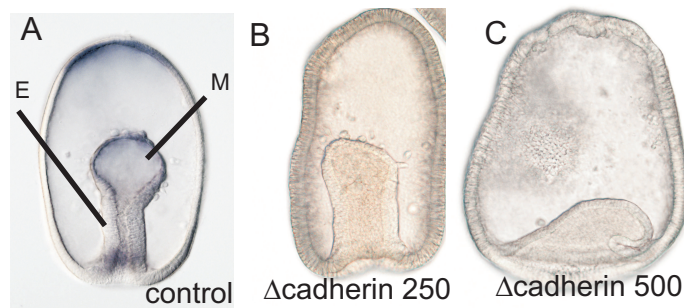


Figure S2. Phenotype of 2 day embryos injected with various concentrations of Δ -cadherin at one cell stage

(A) Control showing normal gastrulation and formation of endoderm (E) and mesodermal bulb (M). (B) Embryo injected with 250ng/ μ l (low) Δ -cadherin showing a reduction in the size of the mesodermal bulb but extended endodermal archenteron. (C) Embryo injected with 500ng/ μ l (medium) Δ -cadherin showing reduction of the entire archenteron. All embryos are shown in a lateral view.

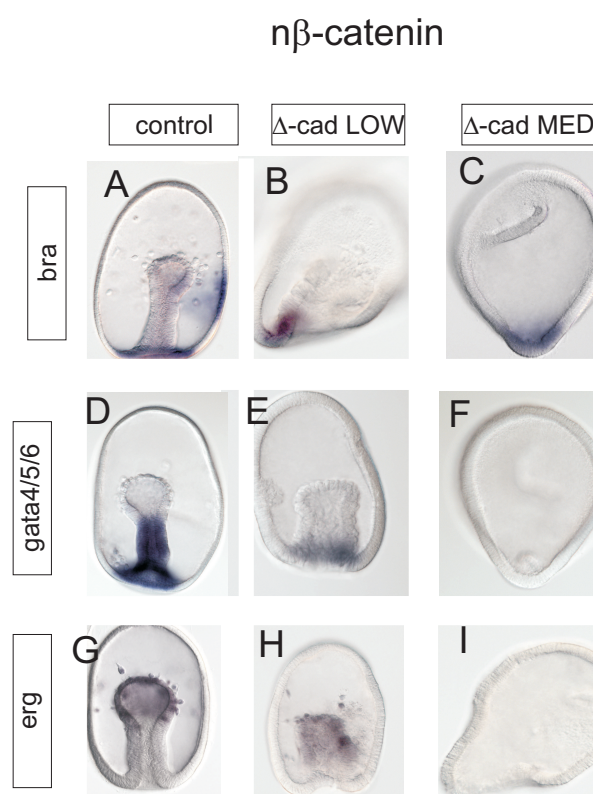


Figure S3. Gene expression in 2 day embryos injected with various concentrations of Δ -cadherin at one cell stage determined using whole mount in situ hybridization
WMISH of *bra* expression (A-C), *gata4/5/6* expression (D-F) and *erg* expression (G-I) in control embryos or embryos expressing low or medium doses of Δ -cadherin as indicated. Concentration of Δ -cadherin injected was determined as in Figure S2. Embryos are shown in the lateral view with vegetal towards the bottom.

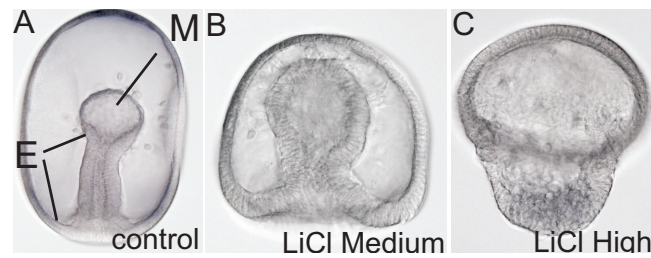


Figure S4. Phenotype of 2 day embryos treated within medium and high doses of LiCl from two cell stage

(A) Control embryo showing normal gastrulation and formation of endoderm (E) and mesodermal bulb (M). (B) Embryo treated with 30mM LiCl showing expansion of archenteron and a greatly reduced ectoderm. (C) Embryo bathed in 50mM LiCl. These embryos are unable to gastrulate and frequently form exogastrulae, a phenotype classically associated with expanded endoderm and mesoderm territories, as shown here. Embryos are shown in the lateral view with vegetal towards the bottom.

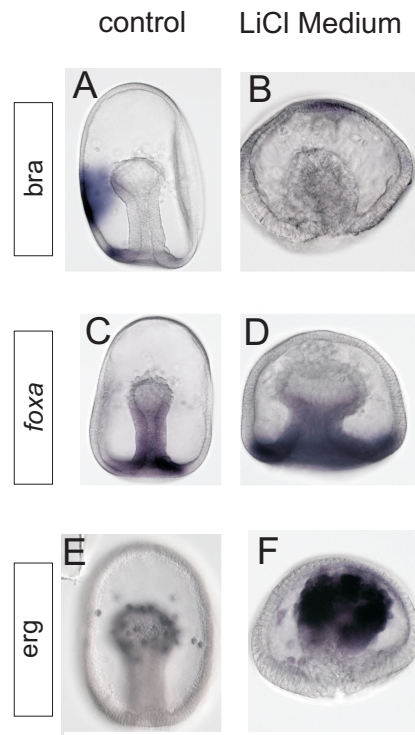


Figure S5. Gene expression in 2 day embryos treated within medium doses of LiCl from two cell stage

WMISH of *bra* (A, B), *foxa* (C, D) and *erg* (E, F) expression in gastrulae. (A, C, E) are control embryos and (B, D, F) depict embryos treated with a medium dose of LiCl. Expression in embryos treated with a high Li^+ dose was not examined as these embryos fail to gastrulate. Embryos are shown in the lateral view with vegetal towards the bottom.