

Supplementary Information

Expression of segment polarity genes in brachiopods supports a non-segmental ancestral role of *engrailed* for bilaterians

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Supplementary discussion

Candidate genes are not iterated through all four partitions of *N. anomala* mesoderm

Expression of *en* borders the posterior boundary of the second and third coelomic sacs in the mesoderm of *N. anomala*. These domains initiate adjacent to the preceding ectodermal domains of *en*, suggesting the ectoderm might signal to the mesoderm during brachiopod development. None of the other genes expressed in the mesoderm of *N. anomala* (*pax2/5/8*, *ptc*, *smo* and *gli*) show iterated patterns throughout the four coelomic sacs. In addition, *en*, *ptc*, *smo* and *gli*, but not *pax2/5/8*, are also expressed in the unsegmented mesoderm of *T. transversa*, suggesting that their developmental role might not be related to the partitioned mesoderm morphology of *N. anomala*.

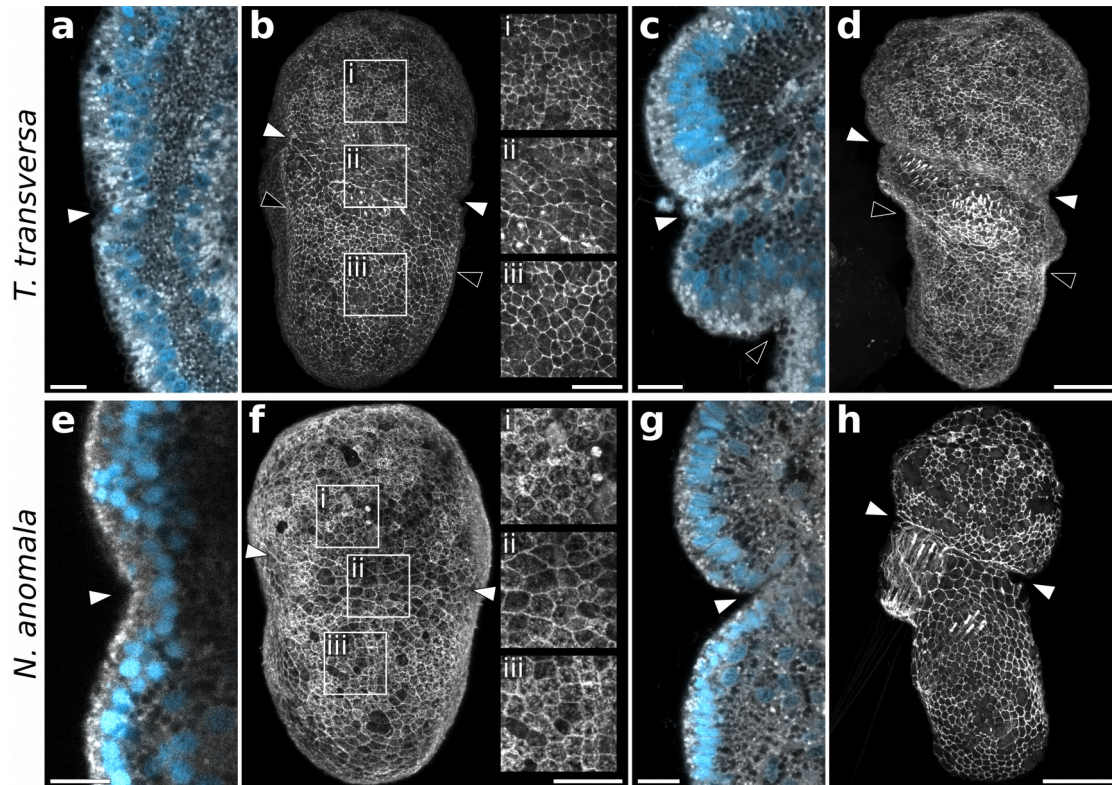
The connection between the ectodermal and the mesodermal expression of *en* was also found in annelids¹, even though the expression patterns do not resemble that of brachiopods^{1,2}. The mesodermal expression of *en* in *N. anomala* is similar to the more distantly related amphioxus³ and onychophorans^{4,5}, where *en* transcripts are localized to the posterior portion of the mesodermal somites. However, the actual role of a posterior *en* domain in the mesoderm is unknown, and requires further investigation in these animals.

Over-activation of the Wnt pathway abolishes *en* and *wnt1* but not *pax6* expression at the apical/mantle boundary

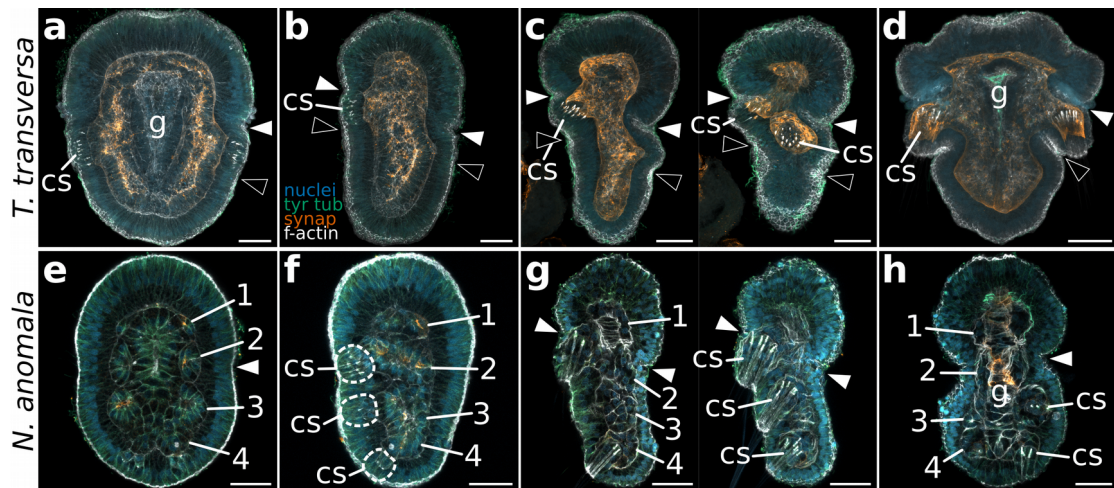
Over-activation of the Wnt pathway caused an expansion of the pedicle lobe and of the domains of gene expression therein, suppressed the formation of the mantle lobe and of the apical/mantle furrow, and shifted other expression domains anteriorly. Embryos treated at the mid-blastula stage do not show morphological or molecular traces of a mantle lobe. We thus suggest the boundary between the anterior and posterior portions of treated larvae does not correspond to any of the wild type boundaries, but to a unique apical/pedicle boundary defined by an ectodermal fold. Embryos treated at later stages (radial gastrula) show bilateral stubs of mantle tissue, suggesting

the specification of the mantle lobe of *T. transversa* might initiate between mid-blastula and radial gastrula stage. The period between mid-blastula and early gastrulation can also play a role in the positioning of the anterior domains of *en* and *wnt1*—but not in the establishment of *pax6* expression. Disturbance of *en* and *wnt1* expression in the mid-blastula treatments suggests these anterior domains are not yet established in the embryo, and their positioning is affected by the over-activation of the Wnt pathway. Differently, the consistent expression of *pax6* between mid-blastula and radial gastrula treatments indicates the anterior domain of *pax6* was not affected by the azaken-paullone, suggesting its specification must occur before the mid-blastula stage or be independent of the Wnt pathway. Thus, our experimental data suggests that disturbing *T. transversa* Wnt signaling affects the development of the mantle lobe and the morphology of the apical/mantle boundary, and that *pax6* might have an earlier upstream role in the embryonic body patterning of brachiopods.

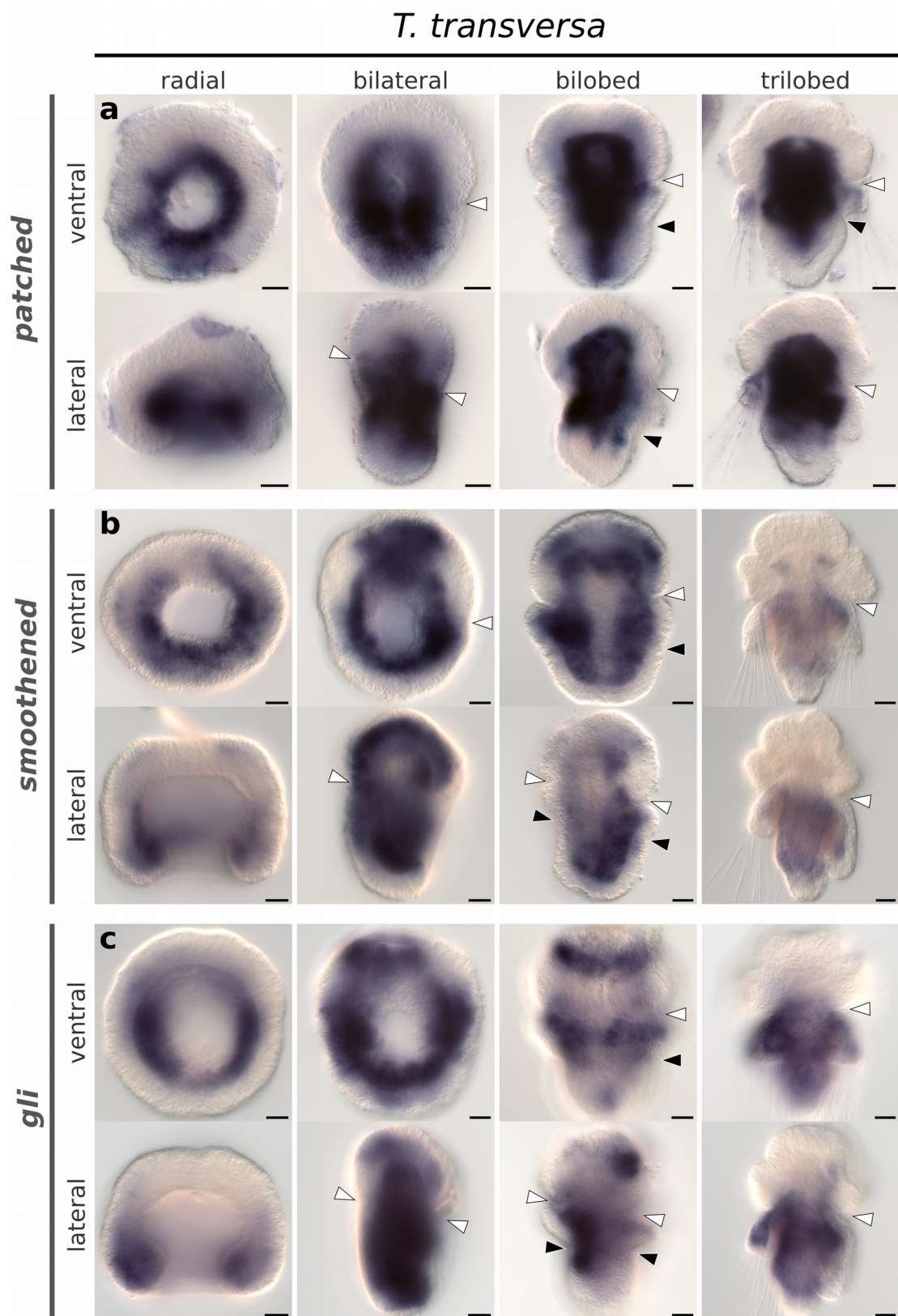
Supplementary figures



Supplementary Figure S1. Morphology of the ectodermal boundaries in the larvae of the brachiopods *T. transversa* and *N. anomala*. (a,e) Outline of the apical/mantle furrow in the epidermis of the bilateral gastrula in ventral view. (b,f) Lateral view of the bilateral gastrula epidermal surface showing the membrane outlines of epithelial cells. Cells abutting the posterior border of the furrow are more elongated compared to the isodiametric shape of anterior and posterior cell populations. Boxes mark the regions corresponding to the insets on the right side (i–iii). (c,g) Outline of the apical/mantle furrow in the epidermis of the bilobed larva in ventral view. (d,h) Lateral view showing the epidermal surface of the bilobed larva showing the membrane outlines of epithelial cells. Nuclei stained with DAPI (blue) and cytoplasmic staining (gray) obtained by mounting specimens stained with BODIPY FL in Thiodiethanol (a,c,e,g). Cell membrane outlines (F-Actin) stained with BODIPY FL mounted in Murray's Clear (b,d,f,h). White arrowheads mark the apical/mantle boundary and black arrowheads mark the mantle/pedicle boundary. Anterior is top and ventral is to the right in lateral views for all panels. Scale bars = 20 μm (b,d,f,h), 10 μm (a,c,e,g).

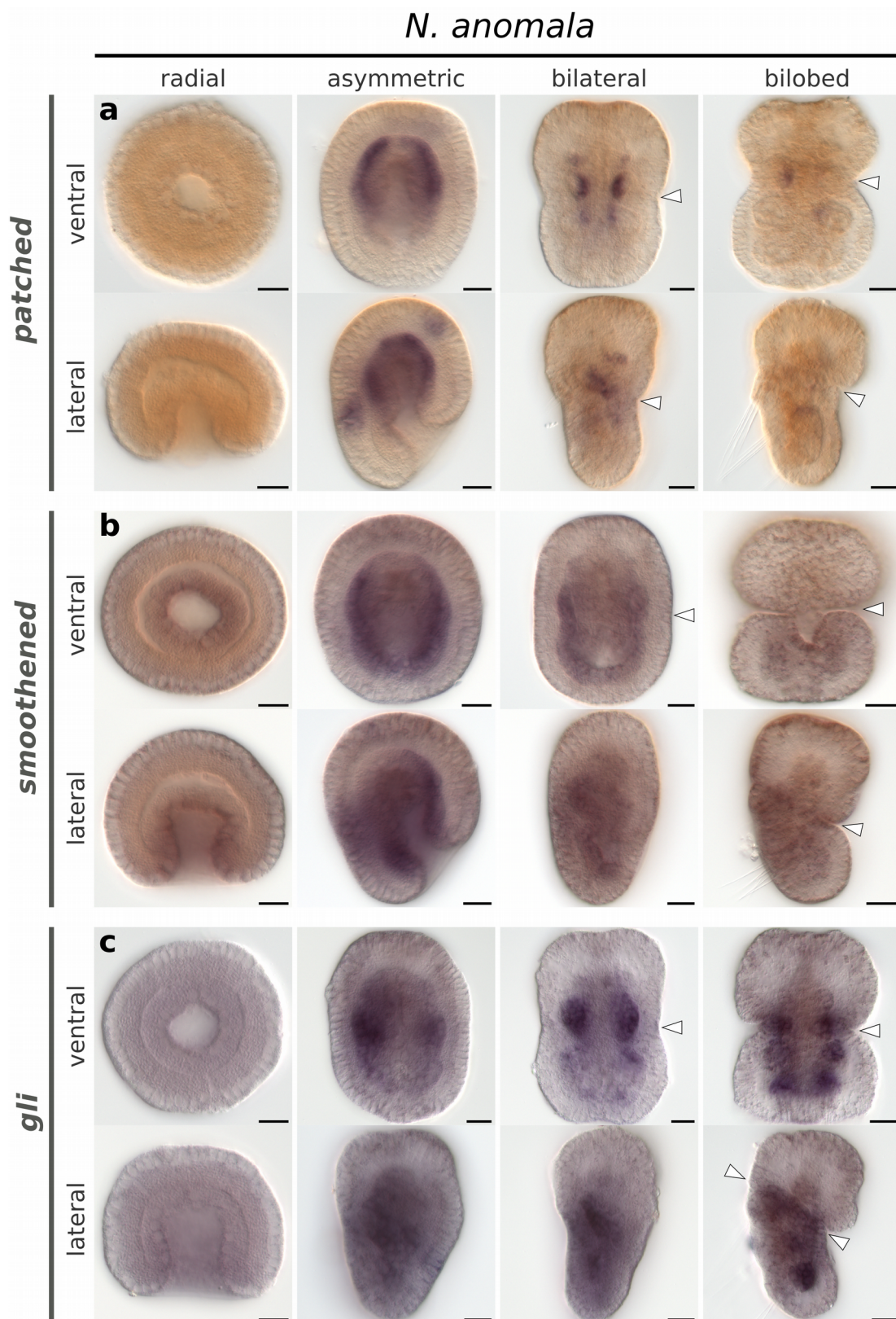


Supplementary Figure S2. Morphology of the mesodermal boundaries in the larvae of the brachiopods *T. transversa* and *N. anomala*. (a,e) Mesoderm morphology at the bilateral gastrula in a ventral view. While *T. transversa* mesoderm has no divisions (a), *N. anomala* mesoderm has lateral constrictions individualizing each of the four pairs of coelomic sacs (e). (b,f) Same stage, but lateral view, revealing that *N. anomala* mesoderm remains interconnected more medially in the ventral side (f). (c,g) Bilobed larva showing a more medial (left) and a more lateral (right) optical sections of an embryo in lateral view. Mesoderm is associated to the developing chaetae sacs (cs). (d) Ventral view of a *T. transversa* trilobed larva showing its unsegmented mesoderm with a distinct anterior portion in the apical lobe, an umbrella-like mesoderm in the mantle lobe connected to the chaetae sacs and an arrowhead-shaped pedicle portion. (h) Ventral view of a developed bilobed larva of *N. anomala* with reduced coelomic spaces. Mesoderm evidenced by the antibody Anti-Synapsin II (vermillion) with the counter staining of tyrosinated tubulin (green), F-Actin (white) and DAPI (blue). Anti-Synapsin II cross-react with an unknown component of *T. transversa* mesoderm and was used as a marker; this reaction is weaker and less specific in *N. anomala*. The four coelomic sacs of *N. anomala* are indicated by numerals 1–4 from anterior to posterior. cs: chaetae sac, g: gut. White arrowheads mark the apical/mantle boundary and black arrowheads mark the mantle/pedicle boundary. Anterior is top and ventral is to the right in lateral views for all panels. Scale bars = 20 µm.



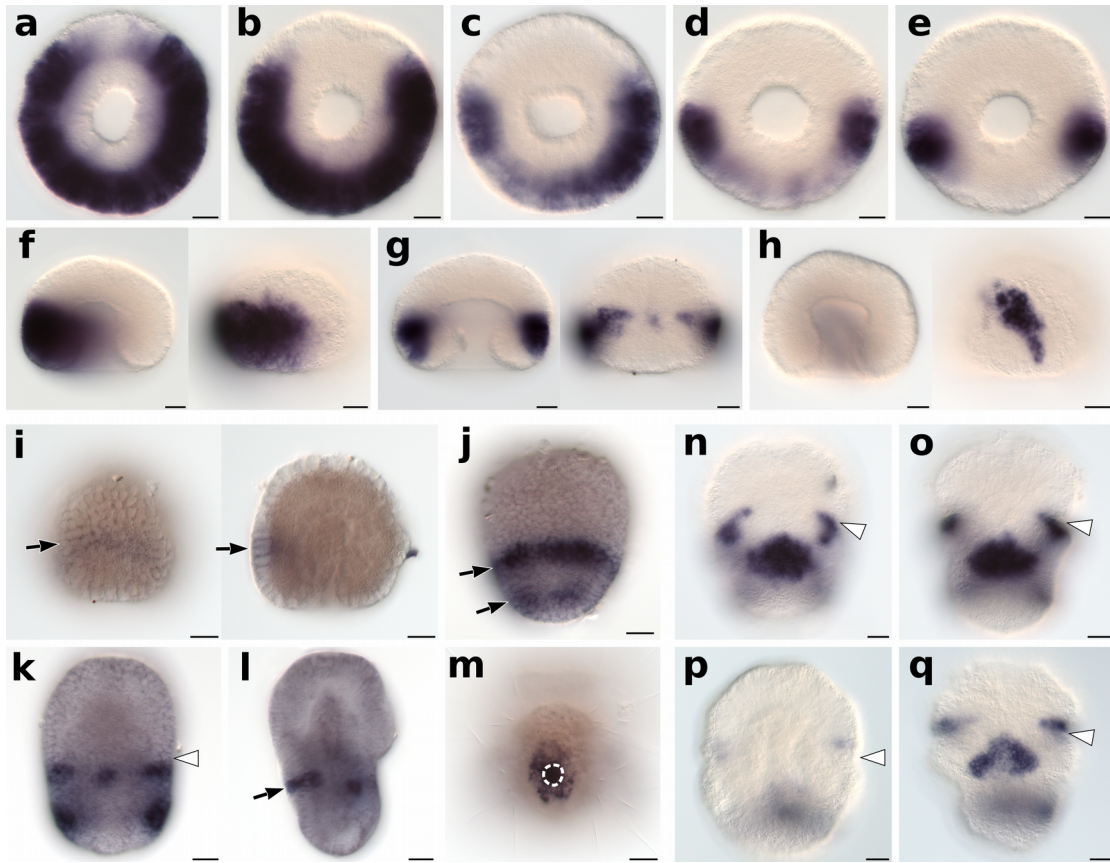
Supplementary Figure S3. Whole mount in situ hybridization of the Hedgehog pathway orthologs in representative developmental stages of the brachiopod *T. transversa*. (a) Expression of *ptc*. (b) Expression of *smo*. (c) Expression of *gli*. In the radial gastrula, transcripts of *ptc*, *smo* and *gli* occur in the mesoderm. Transcripts of *ptc* are expressed in the mesoderm and endoderm of subsequent stages, except at the posterior pedicle mesoderm. The gene *smo* is ubiquitously expressed in the mesoderm dur-

ing gastrulation and, in the asymmetric and bilateral gastrula, in a conspicuous anterior ectodermal domain. Expression of *smo* cleared from the anterior mesoderm in the trilobed larva. Expression of *gli* covers the whole mesoderm of the radial and asymmetric gastrula, and in two additional ectodermal domains at the anterior region of the apical lobe and mantle lobe. Expression becomes restricted to patches in the mantle mesoderm and the ectodermal domains resolve into stripes in the anterior portion of the apical and mantle lobes in the bilobed larva. Finally, in the trilobed larva transcripts of *gli* are restricted to the mantle and pedicle mesoderm. Anterior is top in all panels and ventral is to the right in all lateral views. White arrowheads mark the apical/mantle boundary and black arrowheads mark the mantle/pedicle boundary. Scale bars = 20 μm .

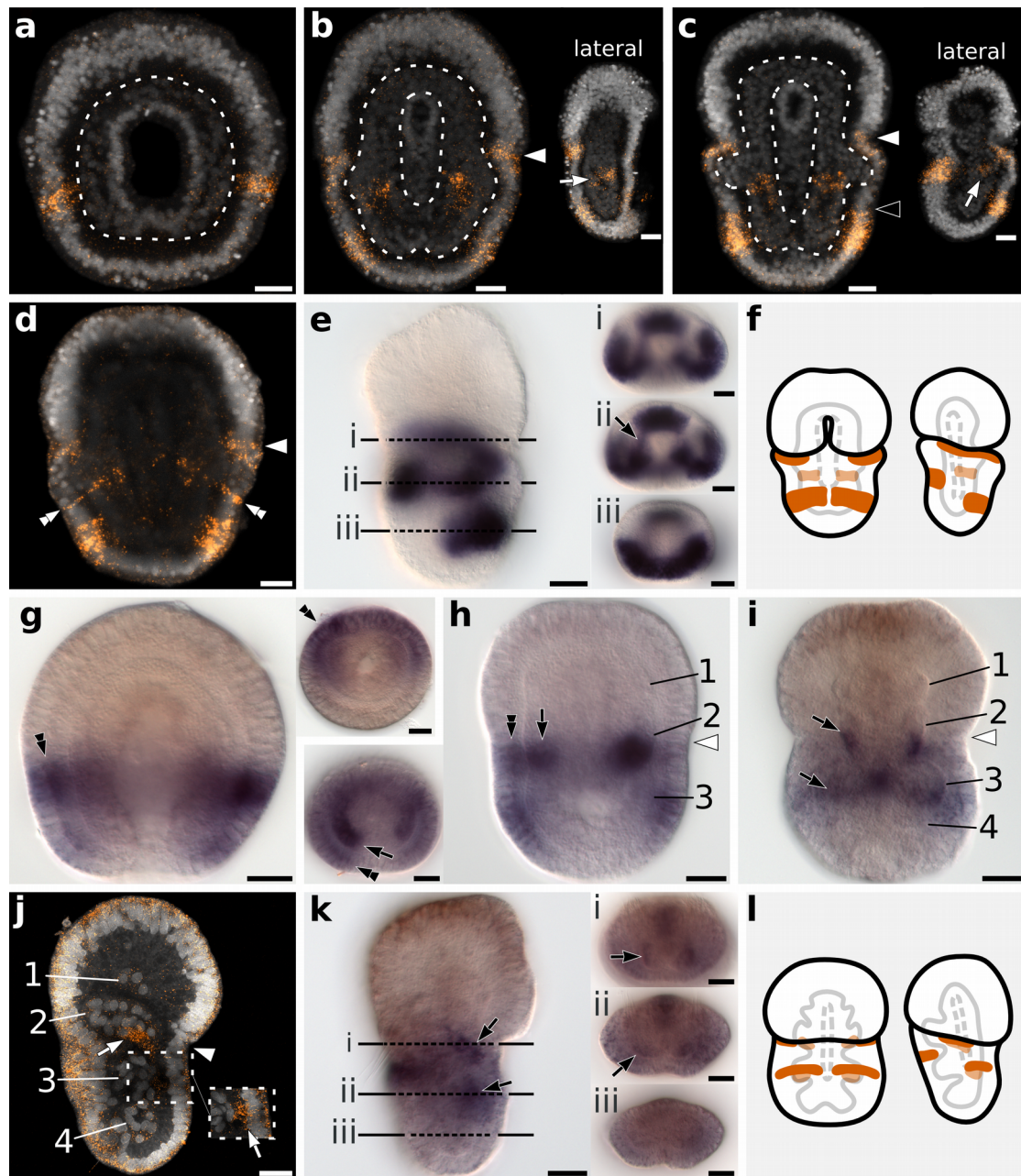


Supplementary Figure S4. Whole mount in situ hybridization of the Hedgehog pathway orthologs in representative developmental stages of the brachiopod *N. anomala*. (a) Expression of *ptc1*. (b) Expression of *smo*. (c) Expression of *gli*. In the radial gastrula, *ptc1* is not expressed, whereas in the asymmetric gastrula it is restricted to the anterior mesoderm and anterior-dorsal domains. Transcripts of *ptc1* remain in the ventral mesoderm associated with the first, second and third coelomic sacs and fade in the

bilobed larva. The expression of *smo* in the asymmetric gastrula covers most of the mesoderm except for the anterior portion correspondent to the first coelomic sac; a dorsal ectodermal domain is also present. The expression fades in the bilateral gastrula and *smo* is not detected in the bilobed larva. The gene *gli* is expressed in the forming second to fourth coelomic pouches, but not the in the first, and the expression covers most of the mesoderm in the bilobed larva. Anterior is top in all panels and ventral is to the right in all lateral views. White arrowheads mark the apical/mantle boundary. Scale bars = 20 μ m.

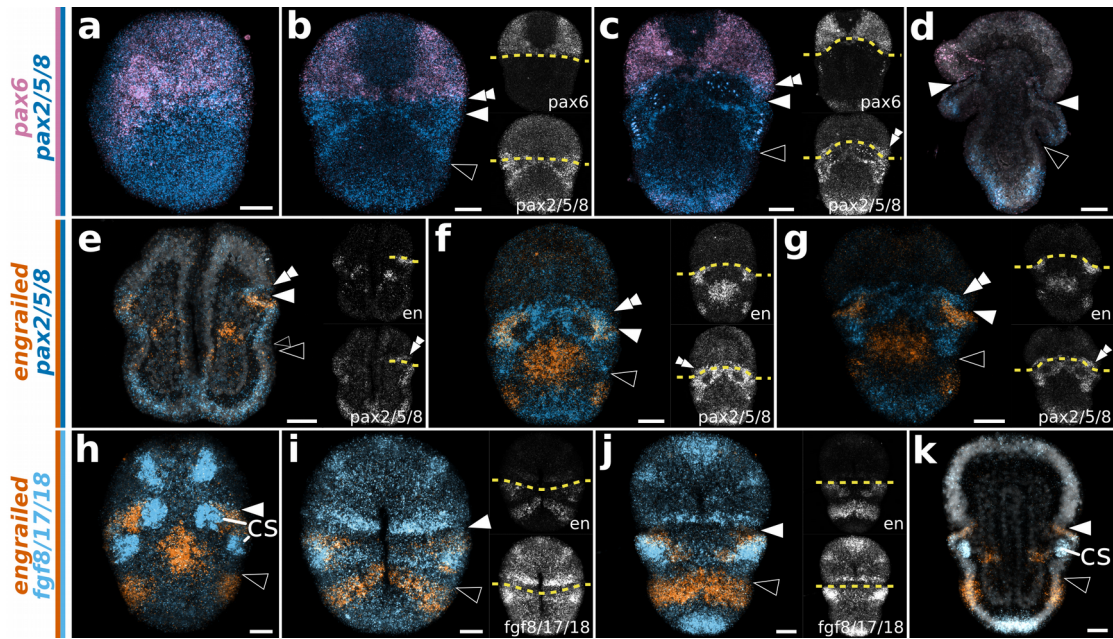


Supplementary Figure S5. Additional details about the embryonic expression of *en* and *wnt1* in the brachiopods *T. transversa* and *N. anomala*. (a–e) Dynamic progression of *en* transcripts at the radial gastrula of *T. transversa* in blastoporal view. (f,g,h) Lateral views correspondent to (b,d,e), respectively, with anterior to the right side. (i) Dorsal (left) and lateral view (right) of *N. anomala* radial gastrula showing faint *en* stripe (arrow). (j) Dorsal view of *N. anomala* asymmetric gastrula with two stripes of *en* (arrows). (k) Dorsal view of *N. anomala* bilateral gastrula. (l) Conspicuous dorsal domain of *en* associated with the shell rudiment in the bilobed larva of *N. anomala* in lateral view (arrow). (m) Developed larva of *N. anomala* with shell primordium. Original area with *en* signal is outlined by white dashed line. Surrounding area is likely unspecific staining related to the shell deposition. (n) Dorsal view of a *T. transversa* bilateral larva showing *en* expression. (o) Same view as (n) showing *en* expression at the subsequent bilobed larva stage. (p) Absence of *wnt1* expression on the dorsal side of *T. transversa* bilateral gastrula. (q) Same view as (p) showing dorsal *wnt1* domain of *T. transversa* bilobed larva. Scale bars = 20 μ m.

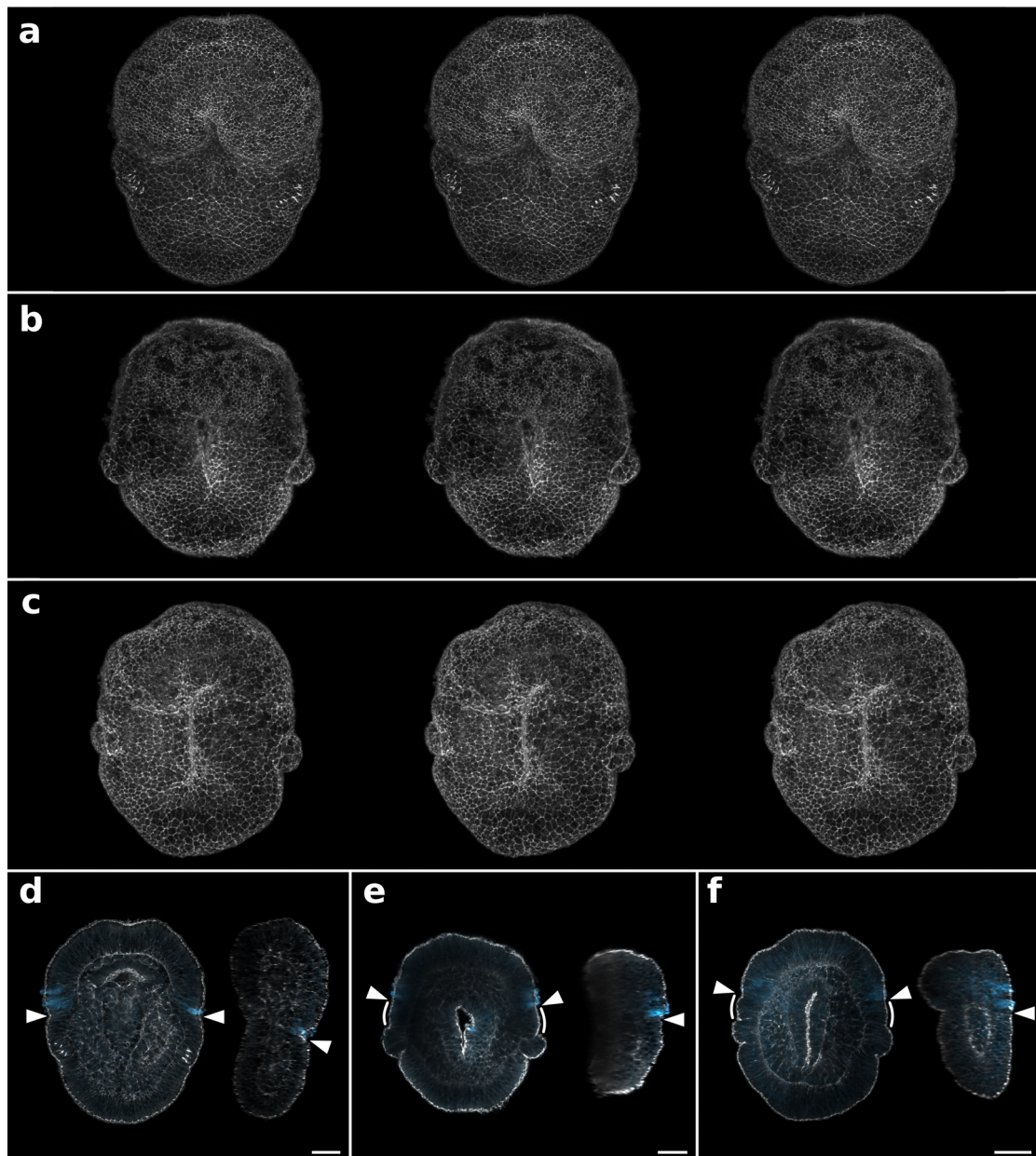


Supplementary Figure S6. Whole mount in situ hybridization *en* orthologs in representative developmental stages of the brachiopods *T. transversa* and *N. anomala*. Anterior is top in all panels and ventral is to the right in all lateral views. (a–f) Expression of *en* in the mesoderm of *T. transversa*. White dashed lines are highlighting the mesoderm. (a) Asymmetric gastrula with no mesodermal expression of *en*. (b) Onset of mesodermal *en* in the bilateral gastrula in a ventral and lateral view (inset). Specimens are the same of Figure 2c. (c) Resolved bands of *en* in the mesoderm of the bilobed larva in a ventral and lateral view (inset). (d) Connection between ectoderm and mesoderm cells expressing *en* in a bilateral gastrula. (e) Lateral view of bilobed larva. Black dashed lines mark the position of the optical sections of a posterior view of the same embryo (insets i–iii). (f) Line drawings represent expression at the bilobed stage of *T. transversa*. (g–l) Expression of *en* in the mesoderm of *N. anomala*. (g) Asymmetric gastrula with weak mesodermal staining of *en* in a ventral and posterior view (inset). (h) Strong *en* domains in the mesoderm of the bilateral gastrula adjacent to the ectodermal domains at the posterior region of coelomic sac “2”. Inset shows the mesoderm in a posterior view. (i) Two pairs of mesodermal domains of *en* in the pos-

terior portion of the coeloms “2” and “3” of the bilobed larva. (j) Detail of *en* expression in the bilobed larva in the coeloms “2” and “3” (inset). (k) Lateral view and optical sections of a posterior view of a bilobed larva showing the expression in the coelomic sacs (insets). (l) Line drawings represent expression at the bilobed stage of *N. anomala*. White arrowheads mark the apical/mantle boundary and black arrowheads mark the mantle/pedicle boundary. Double arrowheads mark the ectodermal expression of *en* while arrows point to the mesodermal domains. The four coelomic sacs of *N. anomala* are indicated by numerals 1–4 from anterior to posterior. Scale bars = 20 μm .

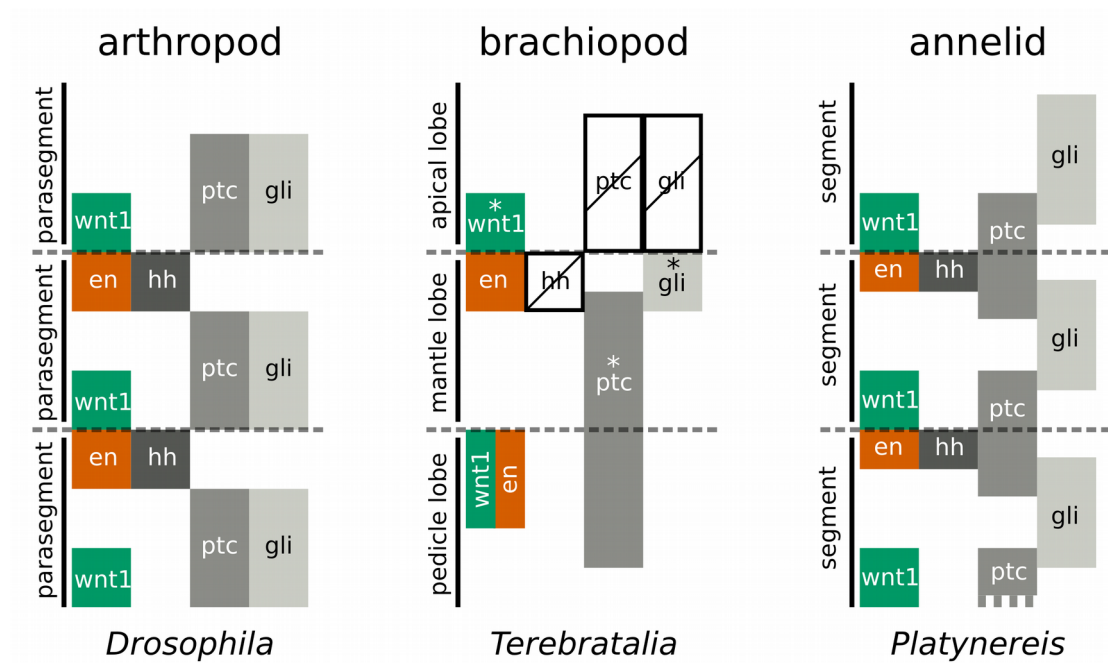


Supplementary Figure S7. Whole mount double fluorescent in situ hybridization of *pax6* + *pax2/5/8* (a–d), *en* + *pax2/5/8* (e–g) and *en* + *fgf8/17/18* (h–k) in the brachiopod *T. transversa*. (a) Radial gastrula with overlapping domains of *pax6* and *pax2/5/8*. (b,c) Dorsals views of a bilateral and a bilobed stage highlighting that the posterior limit of *pax6* expression borders the apical/mantle boundary while the *pax2/5/8* domain crosses the furrow anteriorly. (d) Trilobed larva. (e) Detail of the apical/mantle boundary. (f,g) Dorsal views of a bilateral and a bilobed stage showing the expression of *en* and *pax2/5/8*. (h) Asymmetric gastrula exhibiting *fgf8/17/18* expression in the chaetae sac primordia (cs) with non-overlapping *en* domains. (i,j) Ventral views of a bilateral and a bilobed stage highlighting that *fgf8/17/18* does not directly border the apical/mantle boundary. (k) Section of (j) showing the *fgf8/17/18* expression in the chaetae sacs (cs). Anterior is top in all panels and ventral is to the right in all lateral views. Side panels show the relation between gene expression and the apical/mantle boundary (striped yellow line). White arrowheads mark the apical/mantle boundary and black arrowheads mark the mantle/pedicle boundary. Scale bars = 20 μ m.

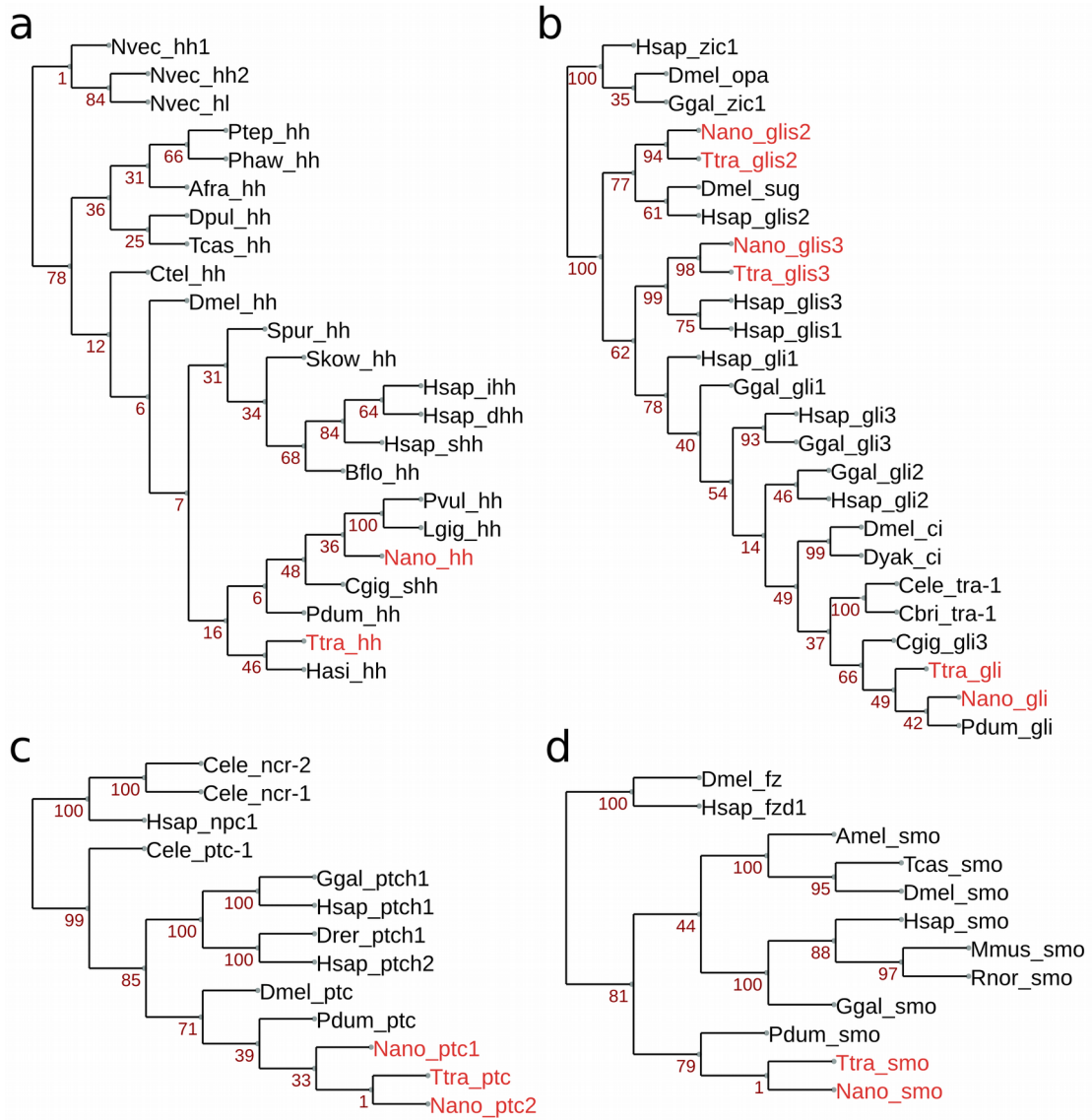


Supplementary Figure S8. Perturbation of the apical/mantle boundary during over-activation of Wnt pathway in *T. transversa*. (a–c) Stereo images showing a maximum intensity projection of the epithelial surface of embryos stained for F-actin (cell membranes in white). (d–f) Maximum intensity projections of five inner optical sections (left) and orthogonal views (right) of the same embryos as above, but including nuclei staining (blue) to highlight the position of corpuscular bodies. Control sample incubated with DMSO shows the wild type ectodermal furrow at the apical/mantle boundary (a,d). Embryos treated with 1 μ M (b,e) and 10 μ M (c,f) 1-azakenpaullone do not form a furrow at the apical/mantle boundary. Arrowheads mark the posterior end of the apical lobe. White line marks the area between the expected furrow position and the mantle lobe stub in treated embryos (E, F). Scale bars = 20 μ m.

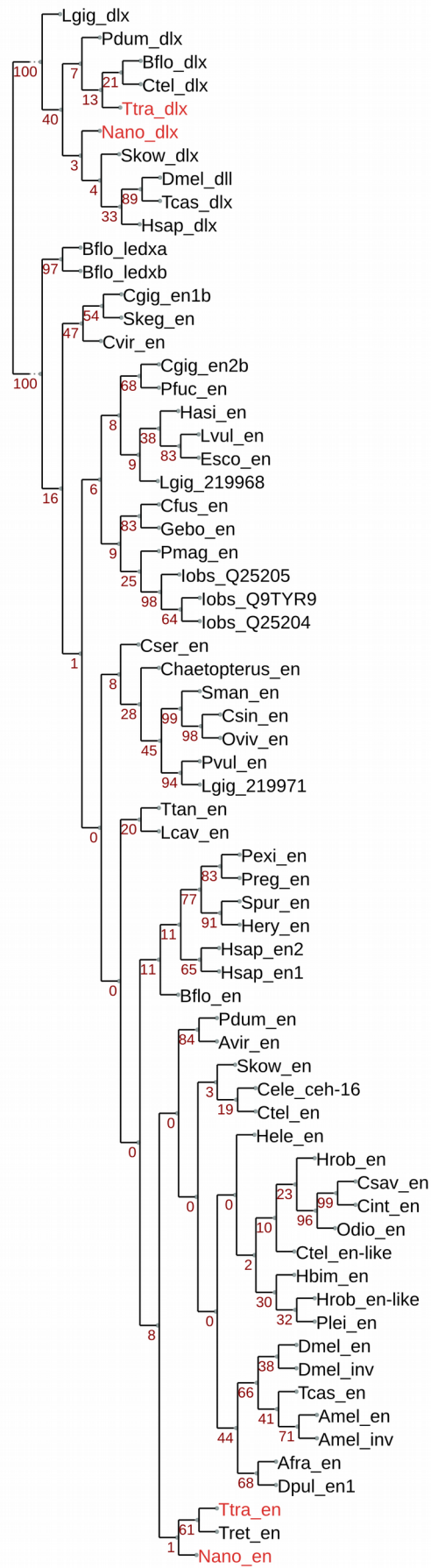
correspondent to incipient mantle lobe tissue. Treated embryos show no sign of chaetae formation. (b,b',f,f') Expression of *en* in control and treated embryos. Control embryos have an apical/mantle stripe, a ventral and a dorsal domain of *en*. Early (mid-blastula) and late (radial gastrula) treatments show a posterior laterodorsal *en* domain, but only in the latter we detected an anterior *en* domain consisting of scattered ectodermal cells, and a broad mesodermal domain in the anterior end (arrowhead). (c,c',g,g') Expression of *wnt1* in control and treated embryos. Control embryos have *wnt1* expressed around the posterior lip of the blastopore. In the treated samples there is an expansion of this posterior domain, occupying half of the blastopore length. Only embryos treated at the radial gastrula stage show an anterior expression domain of *wnt1* at the apical plate (arrowhead), separated from the posterior domain by a sub-terminal band clear of expression. (d,d',h,h') Expression of *pax6* in control and treated embryos. Control embryos have *pax6* transcripts contained in the apical lobe. In all treated embryos the expression of *pax6* shifts anteriorly localizing to the apical plate (arrowheads). (l) Morphology of control larvae incubated with DMSO. The apical lobe has a row of corpuscular bodies at its posterior portion, just above the apical/mantle furrow. The mantle lobe has outgrown with the extensive underlying mesoderm and musculature, and the pedicle lobe shows dorsal putative gland cells (gc). (l',l'') Morphology of the treated embryos reveals an expansion of posterior structures. (l') The larval body has a distinct anterior portion covered by corpuscular bodies and an unclosed blastoporal opening at its center. (l'') Higher concentrations of 1-azakenpaullone (10 μ M) resulted in a diminished anterior portion and the morphology of the posterior portion resembles an over-developed pedicle lobe with putative gland cells on the dorsal side. An ectodermal fold divides the anterior and posterior regions and there were neither chaetae sacs nor evidence of a mantle lobe. (i,m,m',m'') Expression of *en* in wild type, control and treated embryos. (i) Wild type expression of *en* consists of a ventral and a dorsal domain in the pedicle lobe (arrowheads; same specimen as in Figure 3). (m) Control embryos show the same pattern, although regions of unspecific staining in the pedicle lobe (*) obfuscate the signal. (m',m'') Treated embryos show a broader expression domain of *en* encircling the embryo at the anterior portion of the pedicle lobe, similar to wild type and controls. At 10 μ M, *en* is also expressed in the mesoderm at the anterior end (arrowhead). (j,n,n',n'') Expression of *wnt1* in wild type, control and treated embryos. (j) Wild type expression of *wnt1* colocalizes with *en* expression, except for an additional posterior domain at the pedicle lobe (same specimen as in Figure 3). (n) Control embryos display shell background (*), but signal at the dorsal side is visible (arrowhead). (n',n'') Transcripts of *wnt1* in treated embryos occupy the same territories as *en*, except for the absence of *wnt1* in the anterior mesoderm. (k,o,o',o'') Expression of *pax6* in wild type, control and treated embryos. (k) Wild type expression of *pax6* occurs in the dorsal portion of the apical lobe (arrowhead). We did not detect transcripts of *pax6* in control or treated embryos (o,o',o''). Scores represent the number of embryos with the phenotype shown in the panel against the total number of embryos analyzed: (b) 34/48, (b') 36/41, (c) 25/26, (c') 27/33, (d) 25/26, (d') 13/18, (f) 28/36, (f') 58/88 (21/88 without anterior mesodermal domain), (g) 56/59, (g') 57/72 (12/72 without anterior domain), (h) 28/31, (h') 51/53, (m) 30/33, (m') 38/43, (m'') 47/66 (11/66 without anterior mesodermal domain), (n) 13/24, (n') 34/38, (n'') 37/41, (o) 24/24, (o') 36/36, (o'') 29/29. Asterisks (*) mark unspecific staining due to the secretion of the larval shell in *T. transversa*. cb: corpuscular bodies, gc: putative gland cells, al: apical lobe, ml: mantle lobe, pl: pedicle lobe, cs: chaetae sacs. Scale bars = 20 μ m.



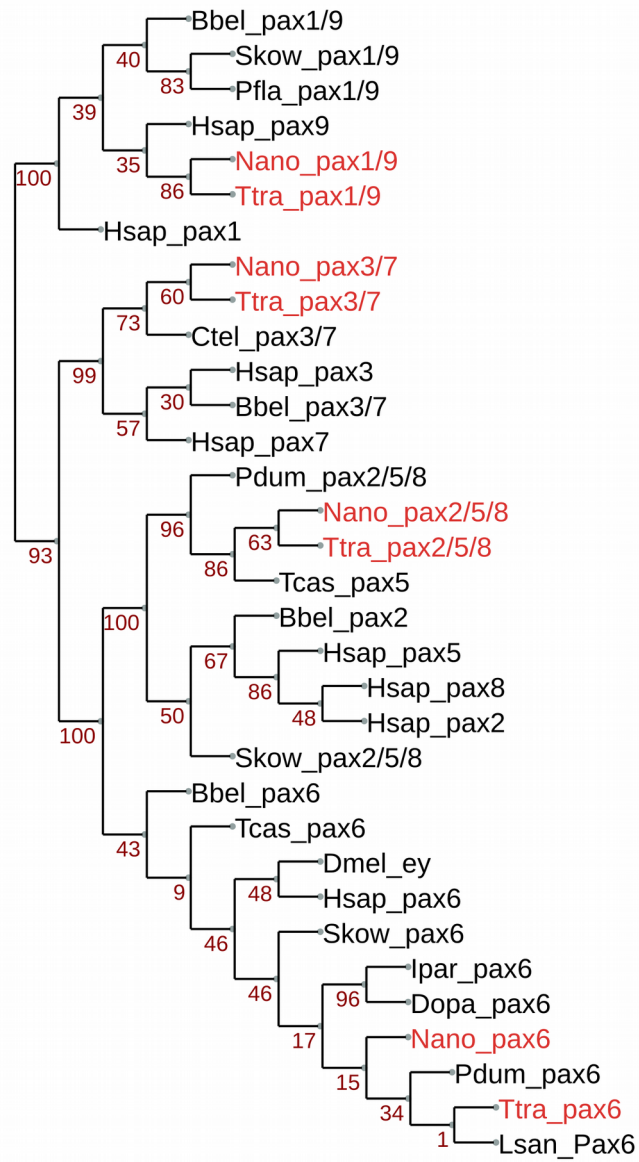
Supplementary Figure S10. Comparative expression of the segment polarity genes and components of the Hedgehog pathway. Expression patterns for *en*, *wnt1*, *hh*, *ptc* and *gli* in relation to the parasegments of an arthropod (*D. melanogaster*), the larval lobes of a brachiopod (*T. transversa*) and the body segments of an annelid (*P. dumerilii*). White boxes with black border indicate the expected localization of transcripts, if a segment polarity role was occurring in *T. transversa*. Asterisks mark expression patterns that are variable between *T. transversa* and *N. anomala*.



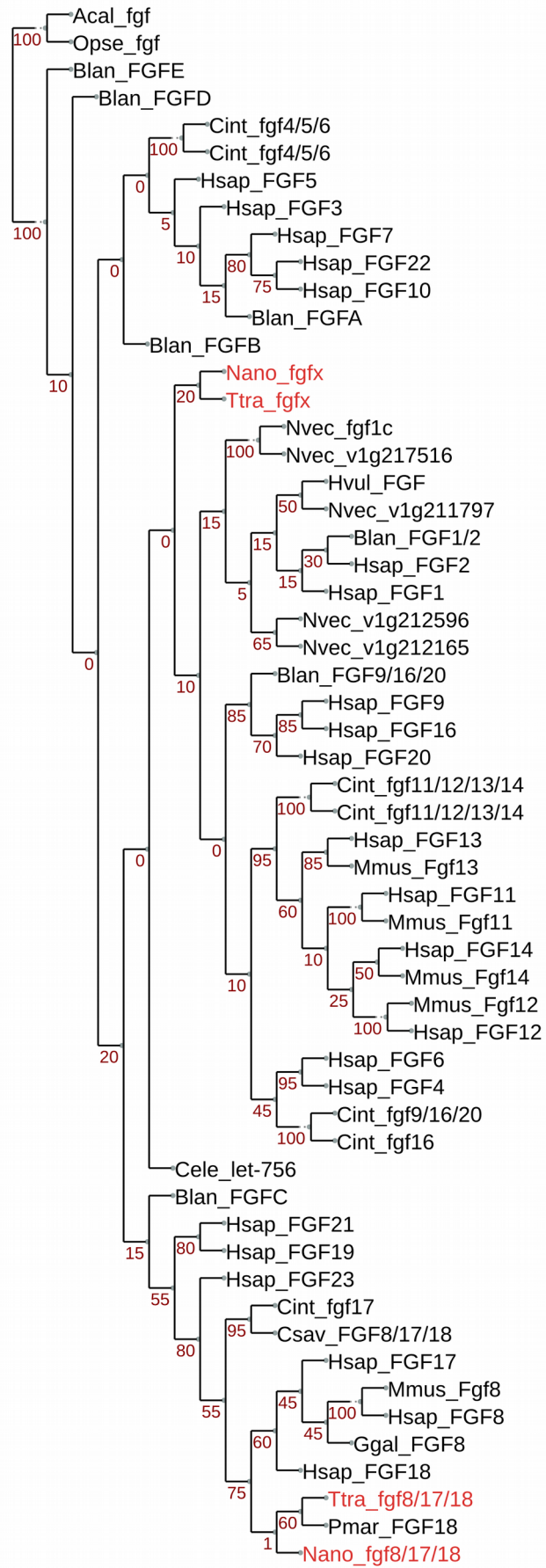
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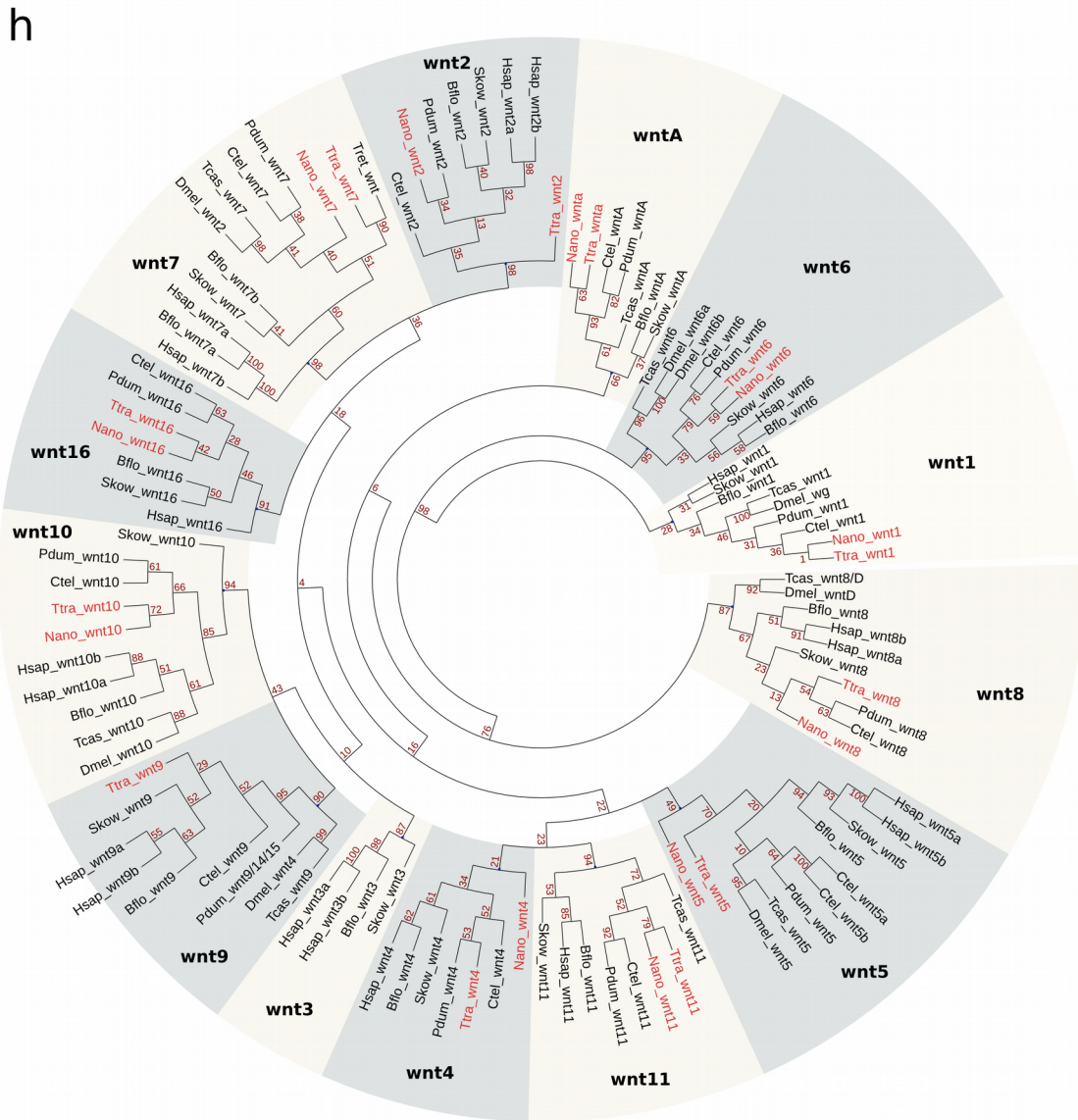


f



g





Supplementary Figure S11. Orthology assignment for the brachiopod candidate genes used in this study. (a) *hh*. (b) *gli*. (c) *ptc*. (d) *smo*. (e) *en*. (f) *pax*. (g) *fgf*. (h) *wnt*. Cladograms show branch support values and brachiopod orthologs in red.

Supplementary tables

Supplementary Table S1. Summary of embryonic expression of *en*, *pax6* and *pax2/5/8* across bilaterians. Dashes represent absence of expression and question marks represent missing data. Expression information based on Cephalochordata *en*³, *pax6*⁶ and *pax2/5/8*⁷; Urochordata *en*^{8–10}, *pax6*¹¹ and *pax2/5/8*^{10,12,13}; Vertebrata *en*^{14–22}, *pax6*^{23–25} and *pax2/5/8*^{26–28}; Hemichordata *en*, *pax6* and *pax2/5/8*^{29,30}; Echinodermata *en*^{31–34} and *pax6*³⁵; Nematoda *en*³⁶, *pax6*³⁷ and *pax2/5/8*³⁸; Tardigrada *en*³⁹; Onychophora *en*^{5,40}, *pax6*^{41,42} and *pax2/5/8*⁴²; Arthropoda *en*^{15,43–56}, *pax6*⁵⁷ and *pax2/5/8*⁵⁸; Rotifera *pax6*⁵⁹; Platyhelminthes *pax6*⁶⁰; Mollusca *en*^{61–66}, *pax6*^{67–69} and *pax2/5/8*^{70,71}; Annelida *en*^{1,2,72–75}, *pax6*^{76–78} and *pax2/5/8*⁷⁷; Nemertea *pax6*⁷⁹.

taxon	<i>en</i> (ectoderm)	<i>en</i> (mesoderm)	<i>pax6</i>	<i>pax2/5/8</i>
Cephalochordata	Bilateral domains in the brain.	Transverse stripes at posterior region of first eight somites.	Anterior third of neural plate.	Bilateral clusters at the neural plate around fifth somite.
Urochordata	Anterior neuroectoderm.	-	Animal hemisphere in two bilaterally arranged domains.	Bilateral pair of neural tube cells (coexpressed or intercalated with <i>en</i>).
Vertebrata	Mid/hindbrain boundary.	Posterior portion of somites after morphology (muscle pioneers).	Anterior domain bordering the di/mesencephalon border and developing eyes.	Mid/hindbrain boundary. Pronephros, otic vesicle, endostyle.
Hemichordata	Transverse stripe anterior to collar/trunk boundary.	-	Anterior domain bordering the collar/trunk boundary, overlap with <i>en</i> domain.	Posterior expression (trunk) adjacent to <i>en</i> .
Echinodermata	Pair of serotonin positive cells around the mouth. Adult nervous system.	Lining coelomic sacs.	Anterior wall of the archenteron in the late gastrula.	?
Chaetognatha	?	?	?	?
Nematoda	Bilateral row of epidermal cells (seam cells) and anterior neurons.	-	Anterior head cells.	Egg-laying system during organogenesis.
Tardigrada	Transverse stripes corresponding to posterior region of underlying endomesodermal pouches before ectodermal segmentation.	-	?	?
Onychophora	Segmental transverse stripes at posterior region of segment with no precise boundaries.	Pair of domains in presomitic mesoderm before segments, but sequentially after morphology.	Cephalic lobe expression posterior of the protocerebral/ocular segment.	Segmental expression matching nephridial openings.
Arthropoda	Segmental transverse stripes at parasegmental boundary and posterior region of segment before	Transient segmental expression.	Forming brain, eye primordia, ventral nerve cord and lateral sense organs.	Few cells per segment.

	morphology. Posterior of imaginal discs, adult abdominal segments and nervous system.			
Scalidophora	?	?	?	?
Rotifera	?	?	Anterior bilateral patches.	?
Micrognathozoa	?	?	?	?
Gnathostomulida	?	?	?	?
Platyhelminthes	?	?	Eyes.	?
Gastrotricha	?	?	?	?
Mollusca	Delimiting shell compartment.	-	Optic region, brain and arms.	Apical pole during embryogenesis, anterior region of the mantle, sensory systems and cephalopod brain.
Annelida	Variable. Segmental expression mainly associated to neurons but can occur before morphology.	Mesodermal derivatives (nephrostome).	Anterior bilateral patches abutting the prototroch and eyes. Nervous system before segmentation.	Trunk nervous system.
Nemertea	?	?	Anterior dorsolateral patches in regenerating eyes.	?
Brachiopoda	Two lateral pairs with anterior domain bordering the apical/mantle boundary.	Posterior portion of two coelomic sacs.	Broad anterior domain bordering the apical/mantle boundary.	Expressed in the mantle lobe.
Phoronida	?	?	?	?
Bryozoa	?	?	?	?

Supplementary Table S2. Gene specific primer pairs used for molecular cloning in *T. transversa* (Ttra) and *N. anomala* (Nano), and gene accession numbers in NCBI.

gene	primers	NCBI accession
Ttra en	R1 CCATCAGATGAAGAGCCAGACCATTTCG	KT253953
	R2 AGTTTTGCTCGTTTTGTTTTGGAACC	
Ttra fgf8	F CCGATAGCTTTTGGGAAGCAAG	KT253954
	R CAATGCCAACAATTCAACCA	
Ttra gli	F CCAGTGAAGCATCTCAAGTGTCTG	KT253955
	R CGGTTGCGTGCCATACTATTTTG	
Ttra hh	F AGTGGGAGTTCAGATGGACG	KT253956
	R ATGCTCTCACACGGTCTCAA	
Ttra pax6	F1 GGATAGTGGAAGTGGCACACAGCGGAG	KT253957
	F2 CGGATGTGTGAGCAAGATCCTCGGGAG	
Ttra pax258	F TTGCCTGGGAAATACGGGAC	KT253958
	R ATCCAGCTGGTTGACTGGTG	
Ttra ptc	F TTACAGCAGTCAAGAAAGTGGTCTG	KT253959
	R TGGGGTTGGATGGATGTTAGC	
Ttra smo	F TGGTCACATTCATTGTCCACCTG	KT253960
	R ATCCTCGGTTTCCTCCTTCTCG	
Ttra wnt1	F TAGCACACACAGGCAAGATAGTCC	KT253961
	R GGAGTAGCAAGTGGAAATGGGG	
Nano en	F ATGTACTCACAGGAGGAGCCACTC	KT253962
	R AGTTGCAGAGCCAATCCGTG	
Nano fgf8	F CAACACGGACACTCCAGAAA	KT253963
	R TATACGGGCTGTTGGTGTCA	
Nano gli	F TCAACAAGGCTACAGTCAGC	KT253964
	R TGCCTTTTATCCTCGTGACG	
Nano hh	F ACCTGAGCAGACGACCAGTT	KT253965
	R AAAATCCCCCAGTTCAAACC	
Nano pax6	F GCAACAGAAGTCAACATGCC	KT253966
	R GACAGGCTGATGGATTGAGG	
Nano pax258	F TGGCTGTGTGAGCAAGATACTCG	KT253967
	R CTGTGAGGAGAGGAGGCATTGTAG	
Nano ptc1	F GGACCCATCCAGCTTTTAGG	KT253968
	R ATCCTGTGTCCAGTCAATGC	
Nano smo	F GCATCCTCTGTACGTGAAGC	KT253969
	R CTCGTACCCAGTGGTTTACG	
Nano wnt1	F CGAGGAAGTAAAGTGGTGGACATTAG	KT253970
	R TTTCCATCAAGCCCCCTTGG	

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