

Figure S1. Expression profile of *Ciona* signalling genes with predominant vegetal expression and related regulatory genes. Expression pattern of developmental regulatory ligands with predominant vegetal expression at the 32-, 64- and 112-cell stages (Imai *et al*, 2004; Yasuo *et al*, 2007; Hudson *et al*, 2005). Zygotic expression in red, maternal mRNA in blue, primordial endoderm cells in orange and presumptive endoderm cells in yellow.

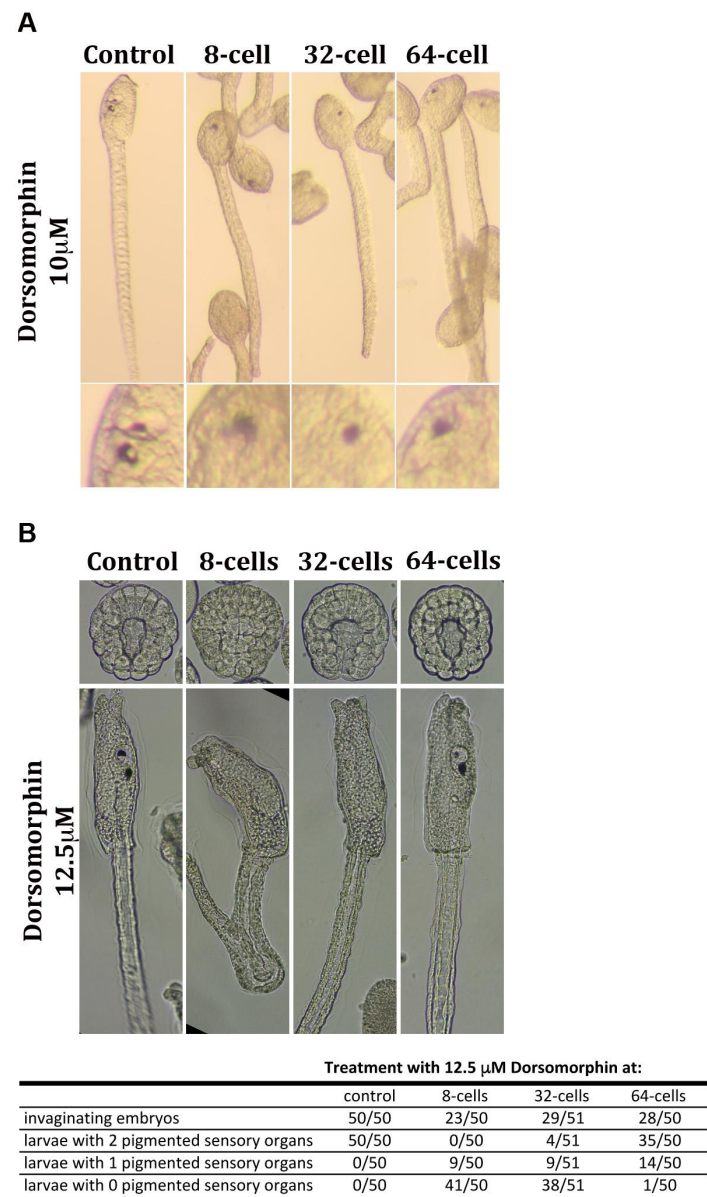


Figure S2. Dorsomorphin treatment prevents the formation of the otolith in *Phallusia mammillata*. (A) Pharmacological inhibition of BMP signalling with Dorsomorphin (10 μ M) from the 8-cell, 32-cell and 64-cell stages prevented the formation of the anterior-most pigmented sensory organ (the otolith) in *Phallusia mammillata*. (B) Pharmacological inhibition of BMP signalling with 12.5 μ M Dorsomorphin from the indicated stages abrogated both otolith and ocellus formation in *Phallusia mammillata*. This treatment also caused an invagination delay in about 50% of embryos (bottom table), from which treated embryos subsequently recovered to produce well-formed larvae.

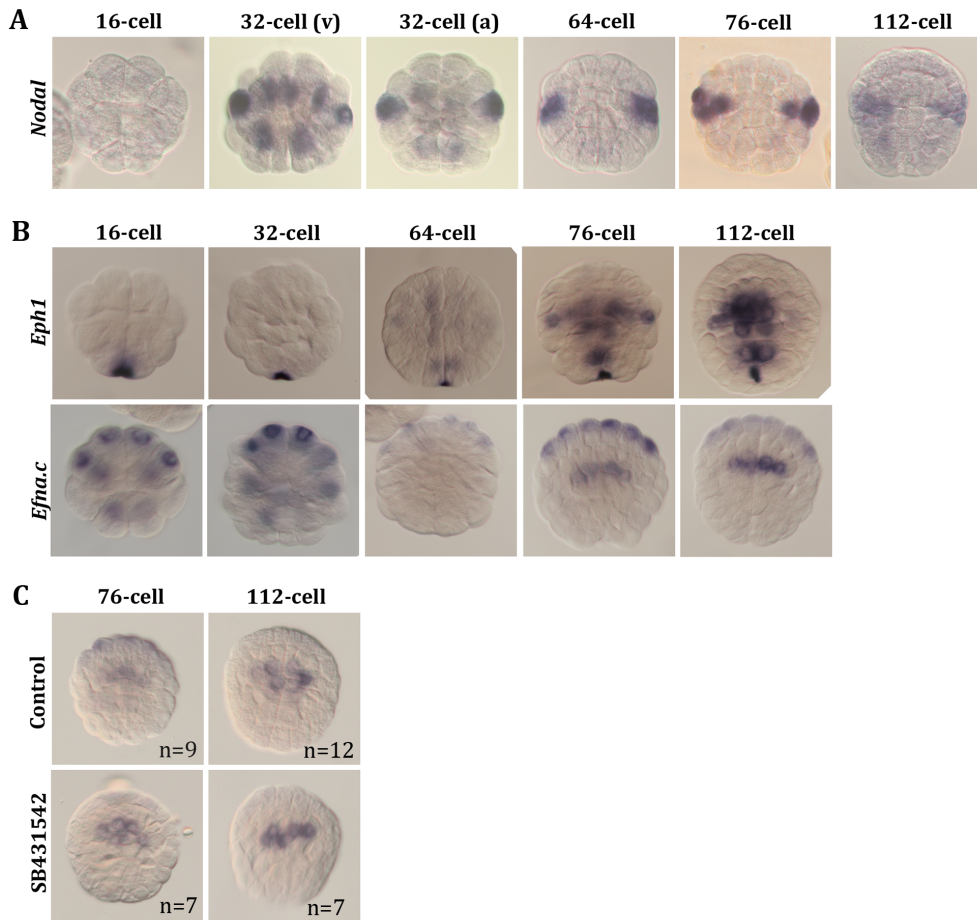


Figure S3. *Nodal*, *Eph1* and *Efna.c* gene expression patterns are conserved between *Phallusia mammillata* and *Ciona intestinalis*. (A) Pattern of expression of *Nodal* determined by whole mount *in situ* hybridization (WMISH) in *Phallusia mammillata*. (v) stands for vegetal view and (a) stands for animal view. (B) Pattern of expression of *Eph1* and *Efna.c* in *Phallusia mammillata* by WMISH. (C) Expression pattern of *Efna.c* detected by WMISH in *Phallusia mammillata* embryos under control (DMSO treated) and SB431542- treated (10 μ M from 16-cell stage) conditions at 76-cell and E112-cell stage. Data represents 3 independent experiments. (A-C) The developmental stage of each embryo is indicated above the corresponding picture.

Expression profiles of *Nodal*, *Eph1* and *Efna.c* in *Ciona* embryos at the corresponding stages can be found in the Aniseed database:

Nodal: https://www.aniseed.cnrs.fr/aniseed/gene/show_expression?unique_id=Cirobu.g00010576

Eph1: https://www.aniseed.cnrs.fr/aniseed/gene/show_expression?unique_id=Cirobu.g00000642

Efna.c: https://www.aniseed.cnrs.fr/aniseed/gene/show_expression?unique_id=Cirobu.g00005705

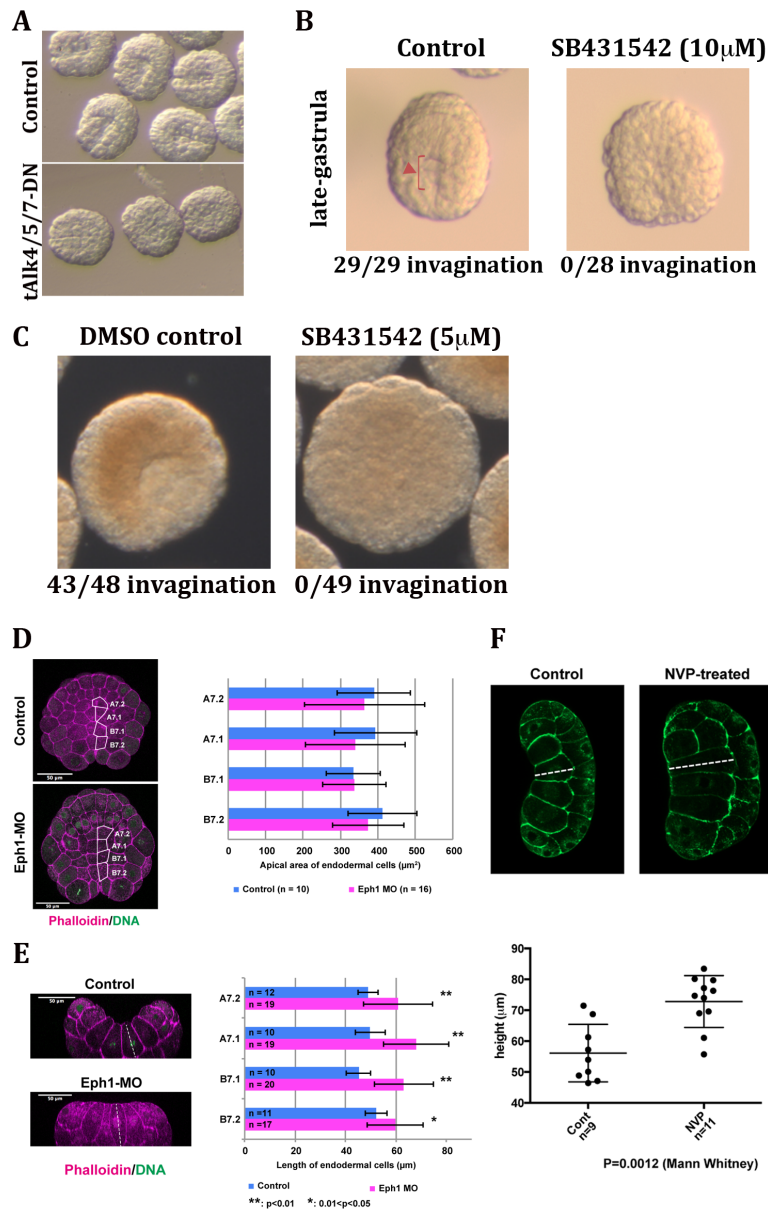


Figure S4. Inhibition of Nodal and Eph signalling prevents endoderm invagination in *Phallusia*

mammillata and *Ciona intestinalis*. (A) *Phallusia* early gastrulae microinjected with either FastGreen dye alone (Control) or with mRNA for a dominant negative Nodal receptor (tAlk4/5/7). 19/20 microinjected control embryos invaginated and 15/20 formed a normal larva. 1/17 embryos microinjected with tAlk4/5/7 mRNA invaginated and none formed a normal larva. (B) Analysis at the late gastrula stage of invagination in control and in SB431542-treated (10 μ M) embryos treated with from the 16-cell stage. Results are representative of 3 independent experiments. The blastopore (arrowhead in control embryos) is not present in treated embryos. (C) Analysis in early gastrulae of endoderm invagination in *Ciona* control and SB431542-treated (5 μ M from the 16-cell stage). Results representative of 2 independent experiments. (B, C) The fraction of invaginating embryos is indicated below each picture. (D) Apical area of endodermal cells at the 76-cell stage, in control and Eph1-MO-injected *Ciona* embryos. t-test analysis with $p > 0.05$ for all blastomeres. (E) Height of endodermal cells in control and Eph1-MO-injected *Ciona* embryos at the late 112-cell stage. The embryos analysed were fixed and stained for actin (Phalloidin) and DNA (DAPI). Statistically significant differences assessed by t-test analysis. Error bars indicate standard deviation values. (F) Endodermal cells height in control and in NVPBHG712-treated (8 μ M, from 8-cell stage) *Ciona* embryos at the late 112-cell stage.

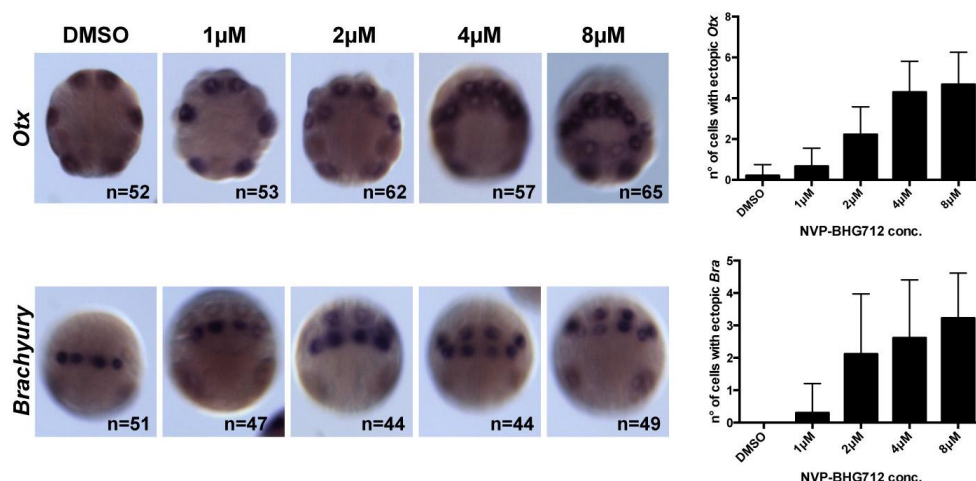


Figure S5. NVPBHG712 treatment mimics the inhibition of *Efna.d*/Eph3 signals. Treatment of *Ciona* embryos from the 16-cell stage with NVPBHG712 leads to ectopic expression of *Otx* and *Brachyury*, mimicking the effects of *Efna.d*/Eph3 inhibition obtained following the microinjection of an *Efna.d* Morpholino or of a dominant-negative form of the *Eph3* receptor (Picco *et al*, 2007; Ohta and Satou, 2013). The severity of the phenotype is concentration-dependent. NVPBHG712 thus efficiently blocks Eph signals in *Ciona*. Error bars indicate standard deviation values.

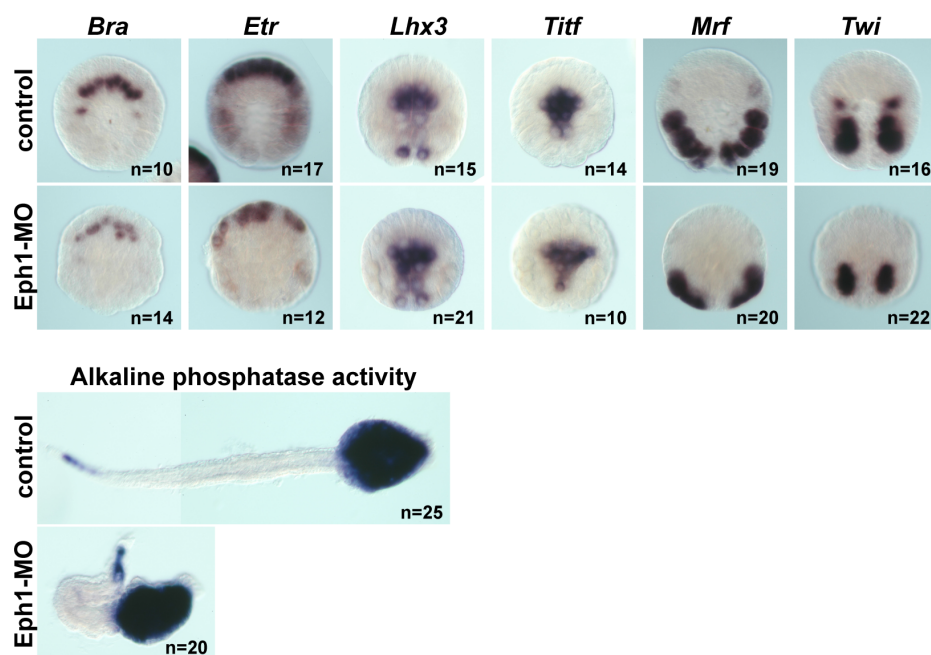


Figure S6. Effects of MO-mediated gene knockdown of *Eph1* on major lineage specification events in *Ciona* embryos. (A) Results of *in situ* hybridization experiments at the early gastrula stage for the following genes: *Bra* (notochord), *Etr* (neural), *Lhx3* (endoderm), *Titf1* (endoderm), *Mrf* (muscle) and *Twist-like-1* (*Tw*, mesenchyme). (B) Effects of *Eph1*-MO microinjection on alkaline phosphatase activity (late endoderm marker) in *Ciona* larvae.

Table S1. Gene identities

Gene name	<i>Ciona</i>	<i>Phallusia</i>
	U nique gene identity	U nique gene identity
<i>Alkaline phosphatase</i>	<i>Cirobu.g00011480</i>	<i>Phmamm.g00011476</i>
<i>Alk4/5/7</i>	<i>Cirobu.g00012156</i>	<i>Phmamm.g00004838</i>
<i>Beta-catenin</i>	<i>Cirobu.g00010084</i>	<i>Phmamm.g00012274</i>
<i>Bmp1</i>	<i>Cirobu.g00002684</i>	<i>Phmamm.g00000600</i>
<i>Bmp3</i>	<i>Cirobu.g00003050</i>	<i>Phmamm.g00001174</i>
<i>Brachyury</i>	<i>Cirobu.g00013860</i>	<i>Phmamm.g00007005</i>
<i>Eph1</i>	<i>Cirobu.g00000642</i>	<i>Phmamm.g00004451</i>
<i>Eph3</i>	<i>Cirobu.g00008427</i>	<i>Phmamm.g00005695</i>
<i>Efna.c</i>	<i>Cirobu.g00005705</i>	<i>Phmamm.g00000939</i>
<i>Efna.d</i>	<i>Cirobu.g00005918</i>	<i>Unclear identity</i>
<i>Etr</i>	<i>Cirobu.g00007645</i>	<i>Phmamm.g00007762</i>
<i>Fgf 8/17/18</i>	<i>Cirobu.g00007390</i>	<i>Phmamm.g00011773</i>
<i>Fgf 9/16/20</i>	<i>Cirobu.g00004295</i>	<i>Phmamm.g00003805</i>
<i>FoxA.a</i>	<i>Cirobu.g00002136</i>	<i>Phmamm.g00001891</i>
<i>FoxD</i>	<i>Cirobu.g00009025</i>	<i>Phmamm.g00006179</i>
<i>Lhx3</i>	<i>Cirobu.g00014215</i>	<i>Phmamm.g00016546</i>
<i>Mrf</i>	<i>Cirobu.g00003985</i>	<i>Phmamm.g00010708</i>
<i>Mycn</i>	<i>Cirobu.g00012221</i>	<i>Phmamm.g00007048</i>
<i>Nodal</i>	<i>Cirobu.g00010576</i>	<i>Phmamm.g00015500</i>
<i>Perlecan</i>	<i>Cirobu.g00005372</i>	<i>Phmamm.g00005761</i>
<i>Ttf1</i>	<i>Cirobu.g00001550</i>	<i>Phmamm.g00010419</i>
<i>Twist-like-1</i>	<i>Cirobu.g00007069</i>	<i>Phmamm.g00000523</i>