METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Animal collections and sample fixations. Gravid adults were collected from the coasts near Friday Harbor Laboratories, San Juan Island, Washington, USA (*T. transversa*), Espeland Marine Biological Station, Norway (*M. stichopi* and *N. anomala*), Fanafjorden, Norway (*L. ruber*), Station Biologique de Roscoff, France (*O. fusiformis*), and Gullmarsfjord, Sweden (*N. westbladi* and *X. bocki*). P. Ladurner (University of Innsbruck) provided a stable culture of *I. pulchra*, which was maintained as previously described[43]. A stable laboratory culture of *E. senta* was maintained in glass bowls with 25 ml of Jaworski's medium in a controlled environment of 20 °C and a 14:10 h light:dark cycle. They were fed *ad libitum* with the algae *Rhodomonas* sp., *Cryptomonas* sp., and *Chlamydomonas reinhardtii*. Brachiopod, nemertean, and annelid adults were spawned as described elsewhere[44–47]. Acoelomorph eggs were collected year round (*I. pulchra*) and in September–October (*M. stichopi*)[28]. All samples were fixed in 4% paraformaldehyde in culture medium for 1 h at room temperature. After fixation, samples were washed in 0.1% Tween 20 phosphate buffer saline, dehydrated through a graded series of methanol, and stored at −20 °C in pure methanol. Samples used for immunohistochemistry were stored in Tween 20 phosphate buffer saline at 4 °C. Before fixation, larval and juvenile stages were relaxed in 7.4% magnesium chloride; *E. senta* were relaxed in 10% EtOH and 1% bupivacaine. The eggshells of *M. stichopi* and *I. pulchra* eggs were permeabilized with 1% sodium thioglycolate and 0.2 mg ml^{-1} protease for 20 min before fixation.

DMH1 treatments. *M. stichopi* and *I. pulchra* embryos were collected at the one- or two-cell stage and cultured with regular water changes in cell culture dishes until the desired developmental stage. Control embryos were treated with 0.1% dimethylsulfoxide and experimental embryos were treated with DMH1 (Sigma) up to 10 μM. Seawater containing the DMH1 was changed every day until fixation. Embryos and hatchlings were fixed as described above, and stored in Tween 20 phosphate buffer saline at 4 °C.

Gene identification and expression analyses. RNA sequencing data obtained from mixed developmental stages and juveniles/adults were used for gene identification. Gene orthology was based on reciprocal best BLAST hit. For particular gene families, maximum likelihood phylogenetic analyses were conducted with RAxML version 8.2.6 (ref. 48), after building multiple protein alignments with MAFFT version 7 (ref. 49) and trimming poorly aligned regions with gblocks version 0.91b (ref. 50) (Supplementary Fig. 1). Whole-mount colorimetric *in situ* hybridization on brachiopod embryos, *L. ruber*, *O. fusiformis*, and juvenile *E. senta* was performed following an already established protocol[30,44]. Probe concentrations ranged from 0.1 to 1 ng μl^{-1}, and permeabilization time was 15 min for *M. stichopi* and post-metamorphic brachiopod juveniles, 5 min for *I. pulchra*, and 10 min for the other species. Double fluorescent whole-mount *in situ* hybridization was performed as described elsewhere[30].

Immunohistochemistry. Samples were permeabilized in 0.1–0.5% Triton X-100 phosphate buffer saline (PTx), and blocked in 0.1–1% bovine serum albumin in PTx. The antibodies anti-tyrosinated tubulin (Sigma), anti-serotonin (Sigma), and anti-FMRFamide (Immunostar) were diluted in 5% normal goat serum in PTx at a concentration of 1:500, 1:200, and 1:200, respectively. Samples were incubated with the primary antibody solutions for 24–72 h at 4 °C. After several washes in 1% bovine serum albumin in PTx, samples were incubated overnight with Alexa-conjugated secondary antibodies at a 1:250 dilution in 5% normal goat serum in PTx. Before mounting and imaging, samples were washed several times in 1% bovine serum albumin in PTx. Nuclei and actin filaments were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; Molecular Probes) and BODIPY FL Phallacidin (Molecular Probes).

Imaging. Representative embryos from colorimetric *in situ* hybridization experiments were cleared in 70% glycerol and imaged with a Zeiss Axiocam HRc connected to a Zeiss Axioscope Ax10 using bright-field Nomarski optics. Fluorescently labelled samples were cleared and mounted in benzyl benzoate/benzyl alcohol (2:1) and scanned in a Leica SP5 confocal laser-scanning microscope. Images were analysed with Fiji and Photoshop CS6 (Adobe), and figure plates were assembled with Illustrator CS6 (Adobe). Brightness/contrast and colour balance adjustments were applied to the whole image, not parts.

Data availability. All newly determined sequences have been deposited in GenBank under accession numbers KY809717–KY809754, KY709718–KY709823, and MF988103–MF988108. Multiple protein alignments used for orthology assignment are available upon request from the corresponding author. Extended Data Fig. 6c has associated source data.

43. De Mulder, K. *et al.* Characterization of the stem cell system of the acoel *Isodiametra pulchra*. *BMC Dev. Biol.* **9,** 69 (2009).
44. Martín-Durán, J. M., Vellutini, B. C. & Hejnol, A. Evolution and development of the adelphophagic, intracapsular Schmidt's larva of the nemertean *Lineus ruber*. *Evodevo* **6,** 28 (2015).
45. Freeman, G. Regional specification during embryogenesis in the articulate brachiopod *Terebratalia*. *Dev. Biol.* **160,** 196–213 (1993).
46. Freeman, G. Regional specification during embryogenesis in the craniiform brachiopod *Crania anomala*. *Dev. Biol.* **227,** 219–238 (2000).
47. Smart, T. I. & Von Dassow, G. Unusual development of the mitraria larva in the polychaete *Owenia collaris*. *Biol. Bull.* **217,** 253–268 (2009).
48. Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30,** 1312–1313 (2014).
49. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30,** 772–780 (2013).
50. Talavera, G. & Castresana, J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* **56,** 564–577 (2007).