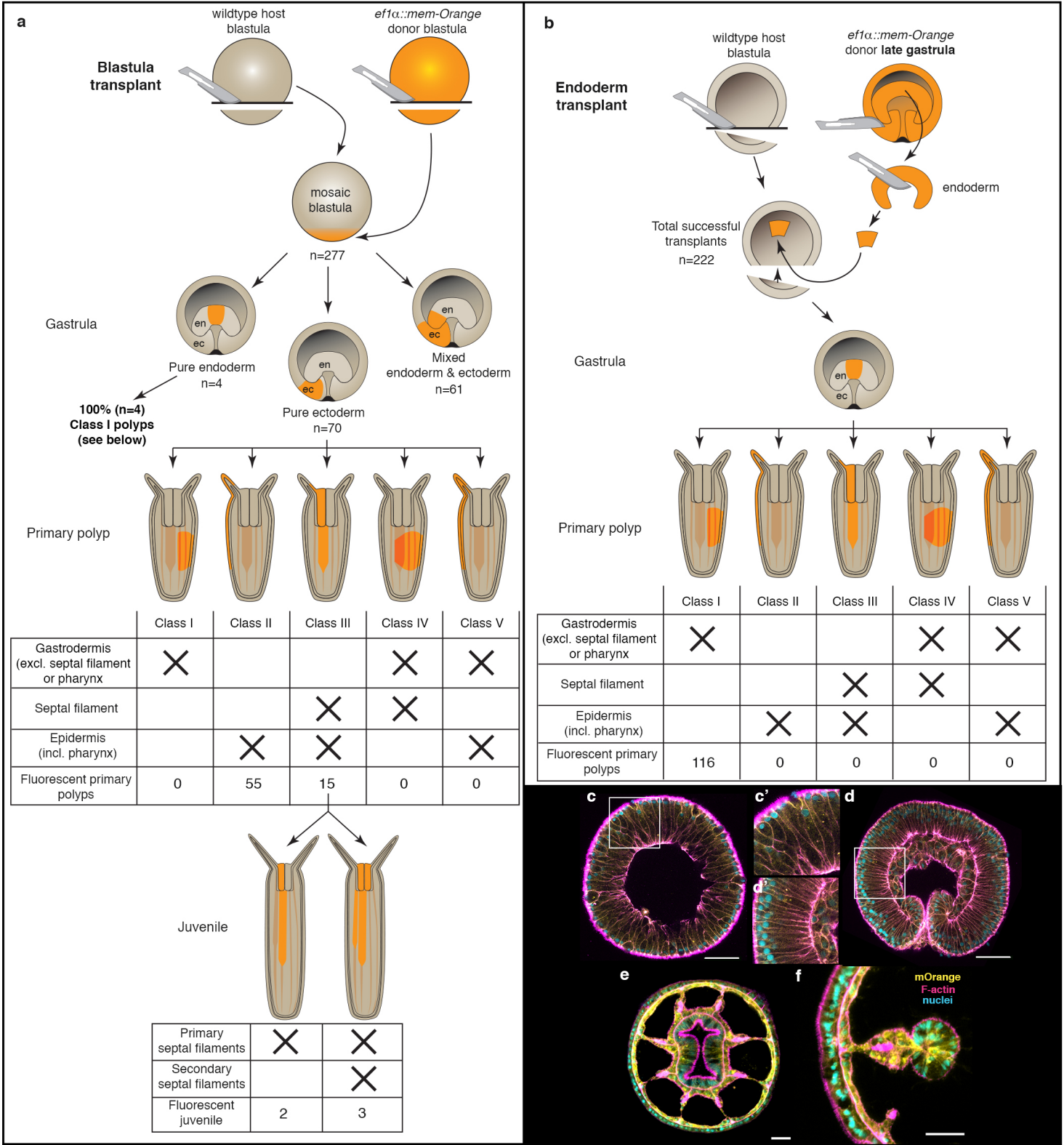


In the format provided by the authors and unedited.

Gut-like ectodermal tissue in a sea anemone challenges germ layer homology

Patrick R. H. Steinmetz ^{1,2*}, Andy Aman^{1,3}, Johanna E. M. Kraus^{1,2} and Ulrich Technau ^{1*}

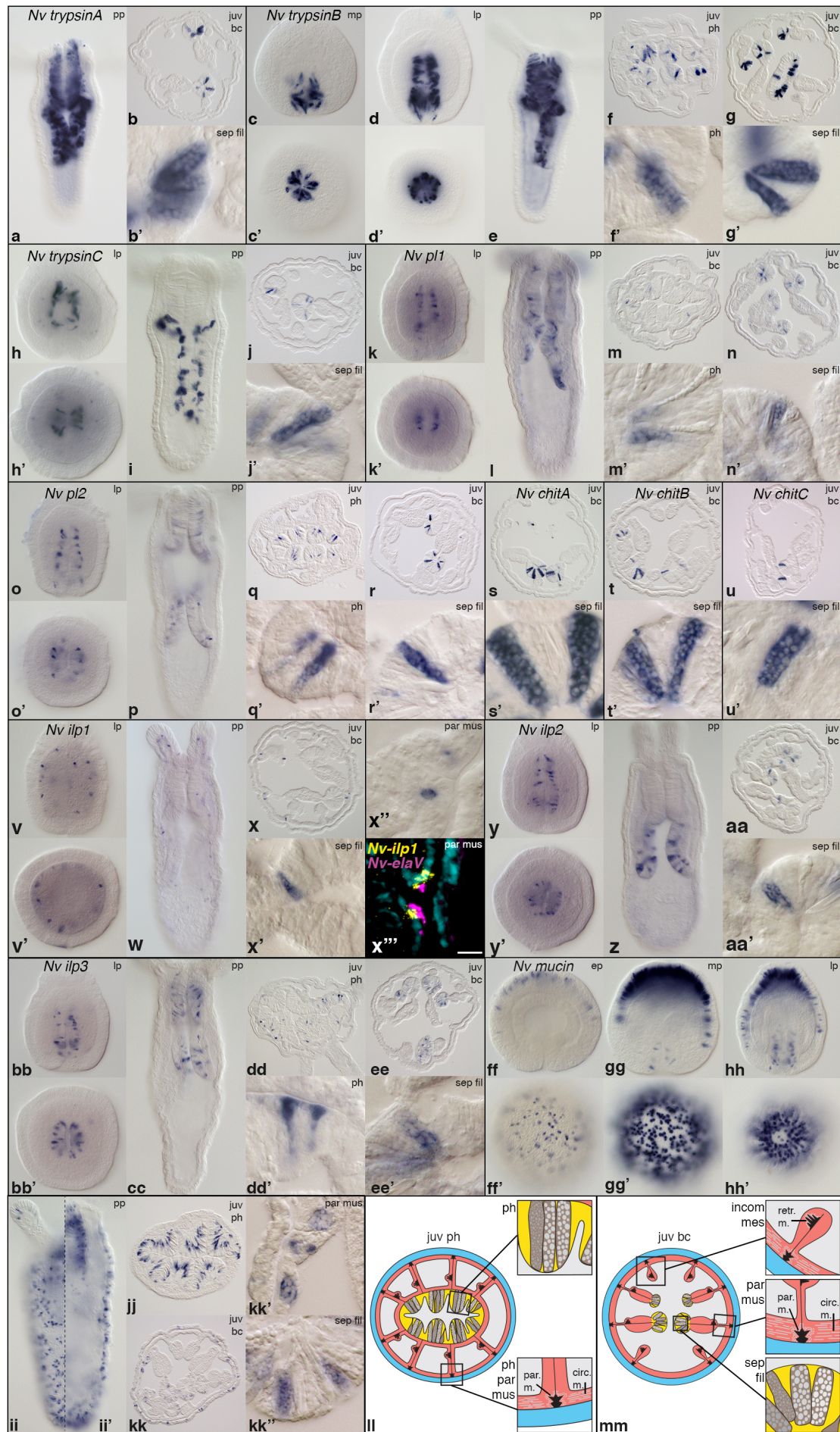
¹Department for Molecular Evolution and Development, Centre for Organismal Systems Biology, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria. ²Sars International Centre for Marine Molecular Biology, University of Bergen, Thormøhlensgate 55, N-5006 Bergen, Norway. ³Department of Biology, University of Virginia, Charlottesville, VA 22904, USA. *e-mail: patrick.steinmetz@uib.no; ulrich.technau@univie.ac.at



Supplementary Figure 1 Schematics of the experimental procedure, results, and control images of the fate mapping by transplantation experiment

(a) Transplantation of blastulae cells expressing CAAX-tagged mOrange (mem-Orange) fluorophore under the control of the ubiquitous *ef1 α* promotor on non-transgenic donor blastulae resulted predominantly in larvae with mixed origin, or showing predominantly ectodermal expression. The study of primary polyps and juveniles shows that the pharynx and both larval and juvenile septal filaments derive from ectodermal cells. (b) Introducing a piece of transgenic endoderm from *ef1 α ::mem-Orange* late gastrulae into the blastocoel of non-fluorescent donors resulted in the endodermal integration of the cells in all observed cases. Fate mapping of the tissue showed that endoderm develops into the gastrodermis excluding the septal filaments and pharynx. (c-d') Single confocal sections of blastula (c, c') or gastrula (d, d') stages showing ubiquitous membrane localisation of mem-Orange in embryos from transgenic *ef1 α ::mem-Orange* females as used for transplantation. Oral side in (d) oriented to the bottom. (c', d') Details of boxed parts in (c, d). (e, f) Cross-sections of immuno-stained fully transgenic *ef1 α ::mem-Orange* primary polyps show ubiquitous expression on the level of the pharynx (c) and the body column (d). yellow: mem-Orange localisation; magenta: F-actin staining; blue: nuclear staining (DAPI). Scale bars in c, d: 50 μ m; e, f: 20 μ m.

domain-containing proteins. Arrows indicate genes cloned and analysed from *N. vectensis* (red) or *A. aurita* (purple). Bootstraps in (a): **:100%, *:>90%, +:>50%; in (b): **:100%, *:>95%, +:>50%. Black signs: maximum likelihood bootstrap supports, reds signs: neighbour joining bootstrap supports. Lengths of proteins and their domain structures are not to scale. Scale bars represent number of amino acid changes per site. Species abbreviations, sequence accession and protein model numbers are available in Supplementary Table 3.



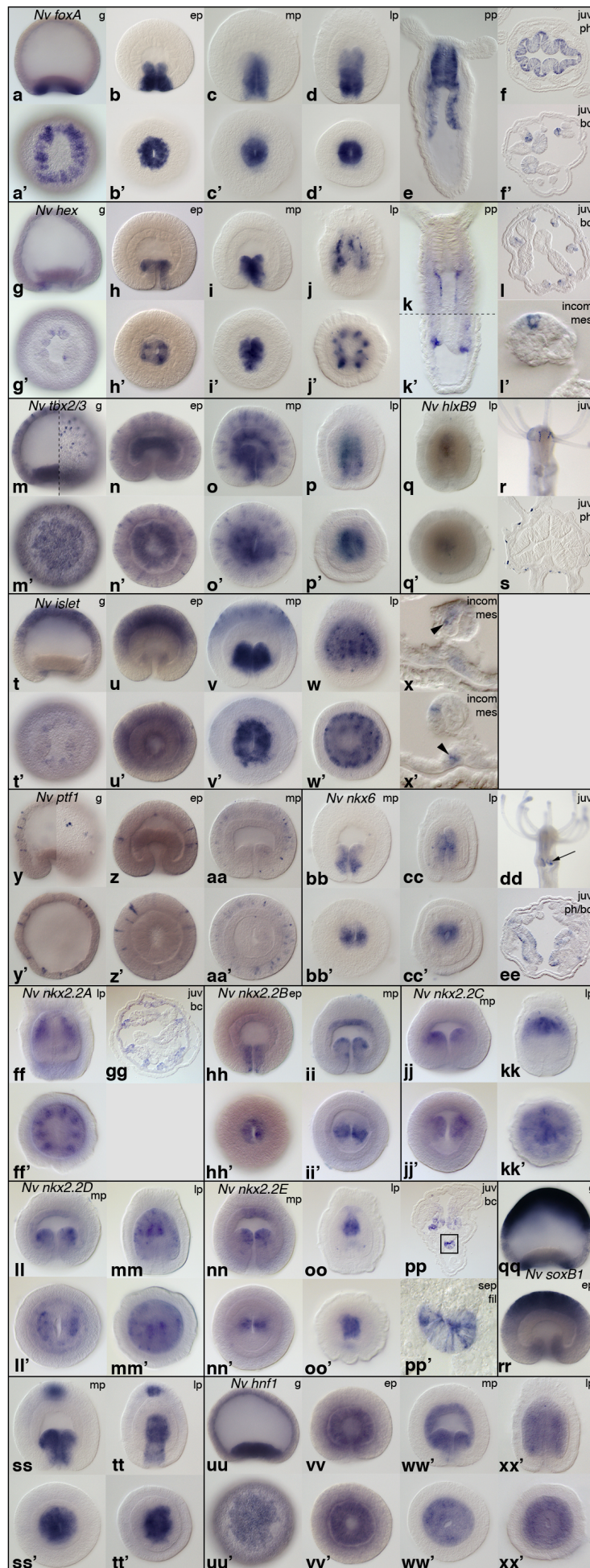
Supplementary Figure 3 Expression of exocrine digestive enzymes and the mucous component gene *mucin* during *N. vectensis* development and juvenile growth

Whole-mount *in situ* hybridisation of early planula ('ep'), mid-planula ('mp'), late planula ('lp'), primary polyps ('pp'), and cross-sections of stained juveniles at the level of the body column ('juv bc') or pharynx ('juv ph'). High magnification of the septal filament ('sep fil') to highlight glandular (b', f', g', j', m', n', q'-u', x', aa', dd', ee'), small uncharacterised cells (x'',x''') and mucous cells (kk'-kk''). Schematic overview of the pharynx (ll) and body column (mm) cross-sections show details of the areas magnified in this figure and Supplementary Figures 6, 8, 9 and 10. (x''') Maximal projection of confocal stack showing expression of *ilp1* and the neuronal marker *elav* in adjacent cells of the parietal muscle region. Scale bars in x''': 10µm. (ii') Deeper focus than in (ii) to highlight expression in the pharynx. (c', d', h', k', o', v', y', bb') Oral views of larvae in the respective panels above. (ff'-hh') Aboral views of larvae in the respective panels above. All other larvae and primary polyps: lateral views. See Material and methods for definition of stages.

protein sequences

Multiple sequence alignment (a) and maximum likelihood phylogenetic tree (b, c) of Preproinsulin-like peptides (ILPs) (a, b) and Insulin receptor (c) proteins. Lines above alignment indicate disulfide bridge formation between conserved cysteine residues (boxed). Blue block below alignment indicates the amino acid positions used for the phylogenetic analysis in (b) after trimming with GBlocks. Arrowheads indicate cleavage sites in mammalian Insulin. Both trees are unrooted. Bootstraps values in (a) above 50 as indicated; in (b): **:100%, *: >95%, +: >50%. Scale bars represent number of amino acid changes per site. Species abbreviations, sequence accession and protein model numbers in Supplementary Table 3.

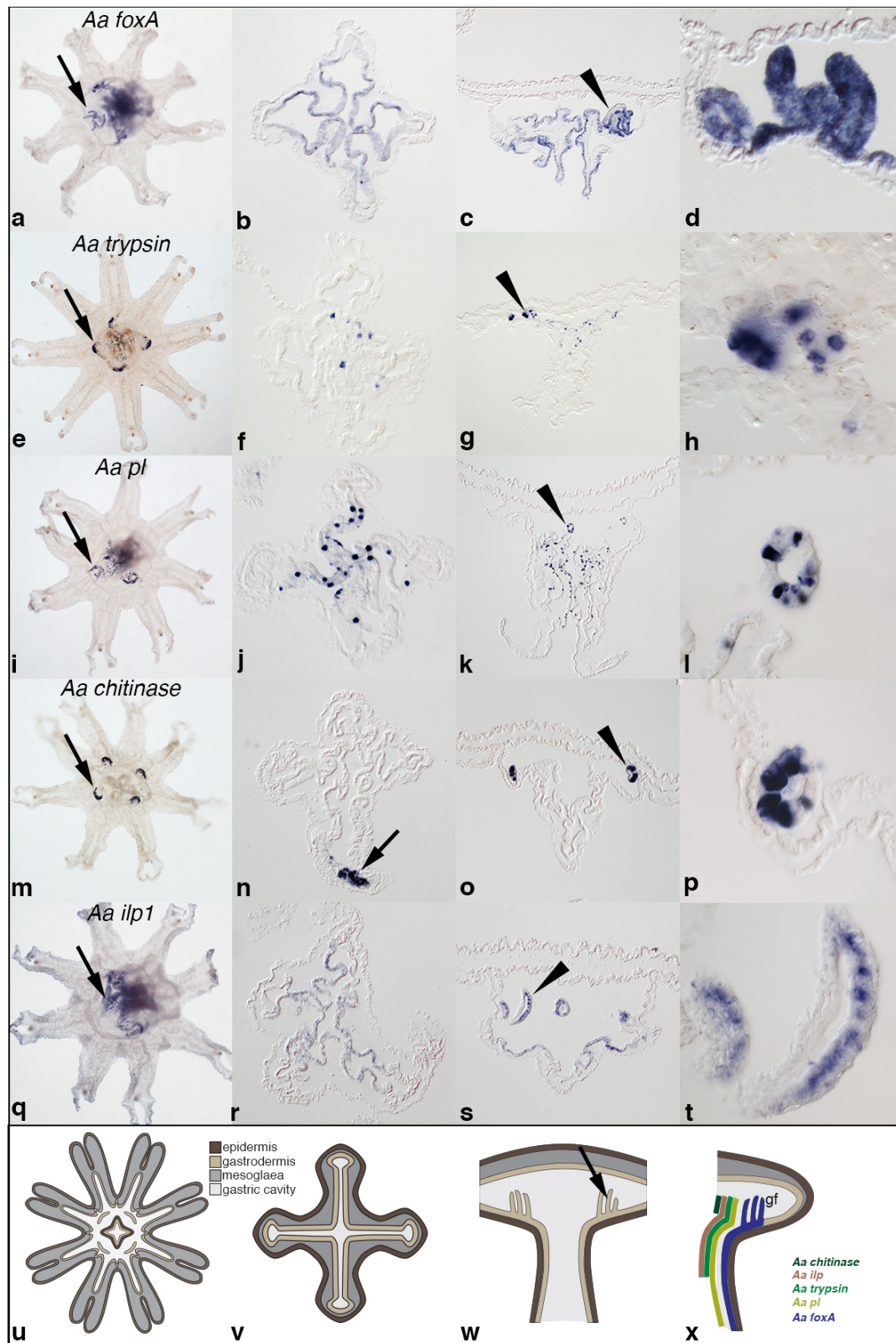
Double colour fluorescent *in situ* hybridisation between combinations of exocrine digestive enzymes or *insulin-like peptide* genes (a-rr). (ss) Summary of all studied combinations. Mixed orange/grey boxes indicate that a subset of the expressing cells show co-expression. White box: missing data. (a-n, p-s, u-z, bb-ee, gg, ii, jj, ll, mm, pp, rr): Maximal or average (qq) projection of a stack of confocal images. (o, t, aa, ff, hh, kk, nn, oo): Single confocal images. Scale bar in (a) and all other fluorescent images: 15µm.



**Supplementary Figure 6 Expression of ‘endodermal’ and
‘pancreatic’ transcription factor orthologs during *N. vectensis*
development and juvenile growth**

Whole-mount *in situ* hybridisation of gastrulae (‘g’), early planulae (‘ep’), late planulae (‘lp’), primary polyps (‘pp’) and cross-sections of juveniles at the level of the pharynx (‘juv ph’) and body column (‘juv bc’). For schematic overviews of magnifications of sections, see Supplementary Figure 3II and mm. (l’)

Higher magnification of *hex*-expressing cells in the retractor muscle region at the tip of an incomplete mesentery (‘incom mes’). (r) Juvenile polyp head with *hlxB9*-positive ectodermal cells in-between tentacle bases. (x, x’) Higher magnification of incomplete mesenteries with single *islet+* cells expressed in the retractor muscle (arrowhead in x) and parietal muscle region (arrowhead in x’). (dd, ee) Juvenile polyp head (dd) and section (ee) through cells with *nkx6* expression in the septal filament tissue directly below the pharynx (arrow). (pp’) Higher magnification of the *nkx2.2E*-expressing septal filament (‘sep fil’) boxed in (pp). (a’-d’, g’-j’, m’-q’, t’-w’, y’-cc’, ff’-oo’, ss’-xx’) Oral views of larvae in the respective panels above. (k’) Deeper focus than in (k) showing expression at the tip of the aboral tip of the septal filaments. In (m, y), left side is focussed on a deeper level than the right side. See Material and methods for definition of stages.

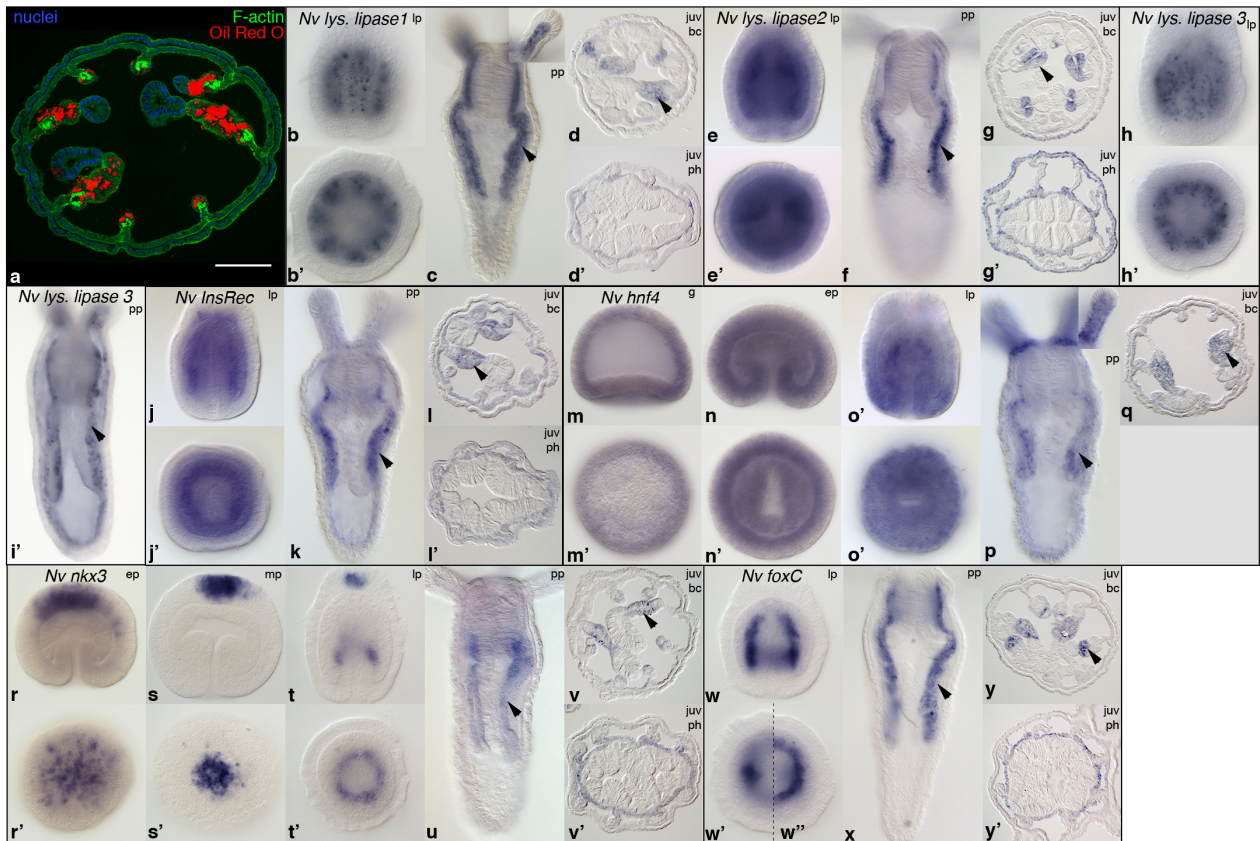


Supplementary Figure 7 Expression of *foxA*, secreted digestive enzyme and *preproinsulin-like peptide* genes in the *A. aurita* ephyra.

Whole-mount *in situ* hybridisation of *foxA* (a-d), *trypsin* (e-h), *pancreatic lipase* (i-l), *chitinase* (m-p), *preproinsulin-like peptide 1 (ilp1)* (q-t), and schematic summary of expression patterns (x) in ephyra of *Aurelia aurita*. (u-w)

Schematics represent the epithelial organisation of the ephyra in an oral view (u) as shown in (a, e, i, m, q), of cross-sections at the level of the mouth tube (q) as shown in (b, f, j, n, r), and of longitudinal sections through the mouth tube (c) as shown in (c, g, k, o, s). Arrows highlight gastric filaments.

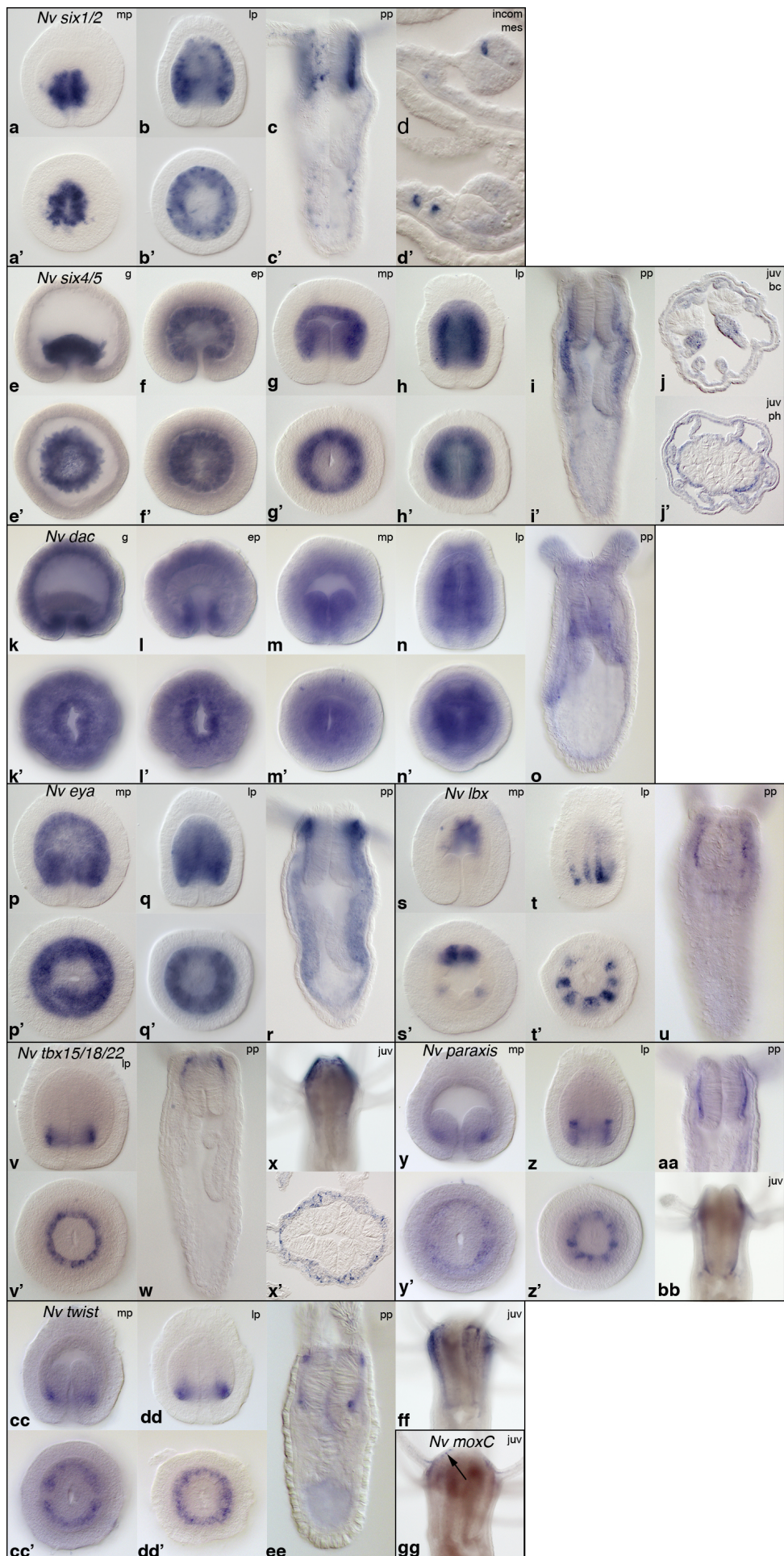
Arrowheads point out examples of cross-sectioned gastric filaments as shown magnified in (d, h, l, p, t). All ephyra in lateral views are oriented with the mouth tube pointing down.



Supplementary Figure 8 Lipid droplet localisation, and expression of *lysosomal lipase*, *insulin receptor*, *hnf4*, *nkx3* and *foxC* genes during *N. vectensis* development and juvenile growth

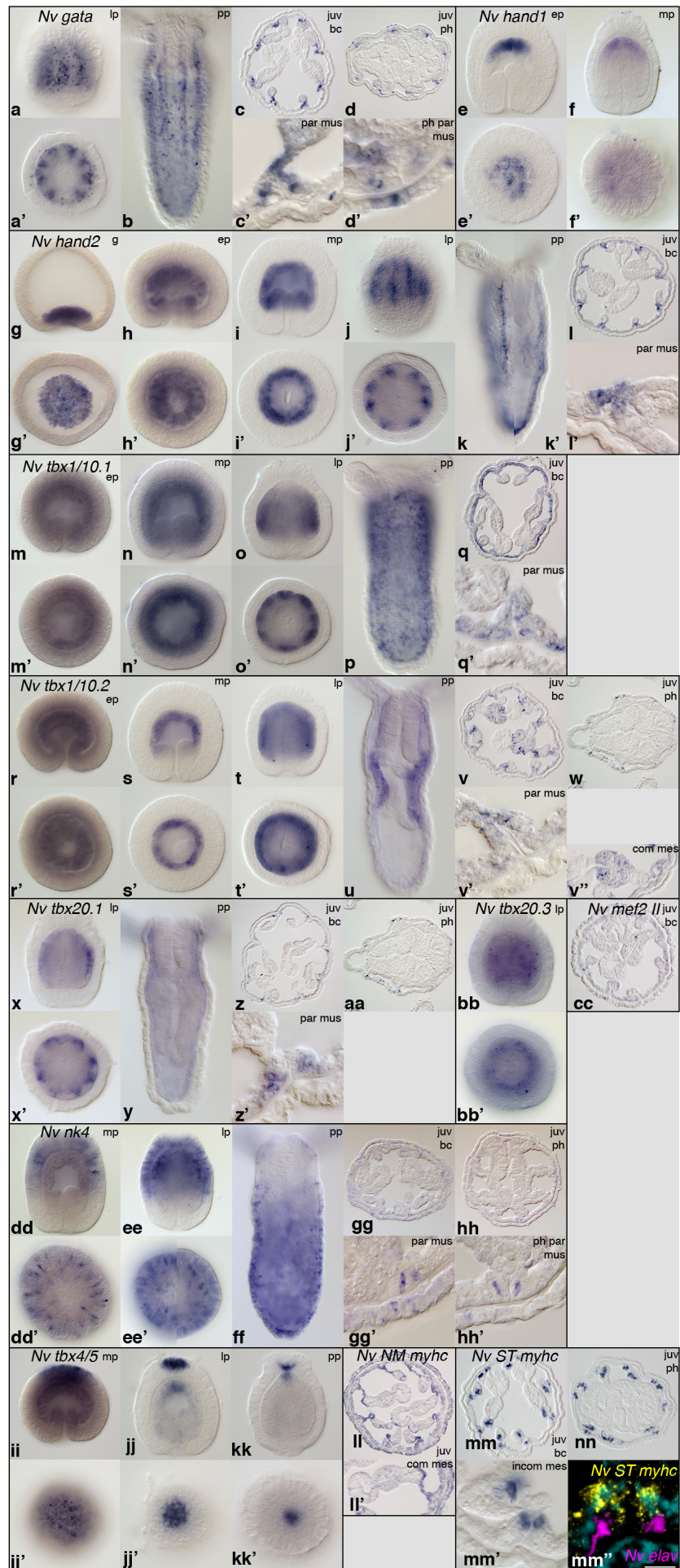
(a) Full picture of the detail presented in Fig. 4b. with Oil Red O fat vesicle (red), F-actin (green, phalloidin) and nuclear staining (blue, DAPI). (b-y) Whole mount *in situ* hybridisation of *lysosomal lipase 1* (b-d'), -2 (e-g'), -3 (h-i), *insulin receptor* (j-l'), *hnf4* (m-q), *nkx3* (r-v') and *foxC* (w-y') at early planula ('ep'), mid-planula ('mp'), late planula ('lp'), primary polyp ('pp') stages or cross-sections of juvenile body column ('juv bc') or pharynx ('juv ph'). Inlets in (c) and (p) show expression in tentacles. (b', e', h', j', m', n', o', t', w', w'') Oral views of larvae in the respective panels above. (r', s') Aboral views of larvae in the respective panels above. (w') Deeper focus level than (w''). Arrowheads

highlight the expression in the somatic gonad part of the mesentery. See
Material and methods for definition of stages.



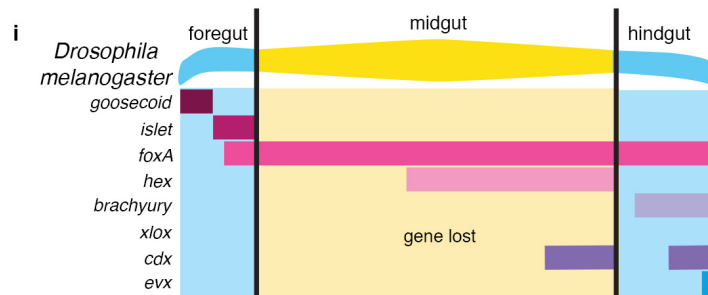
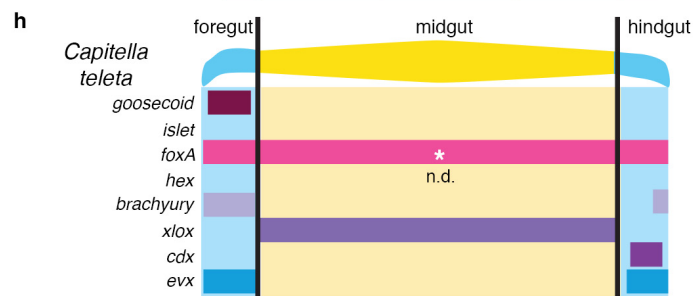
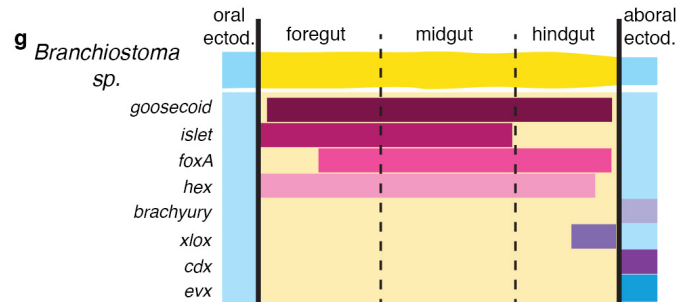
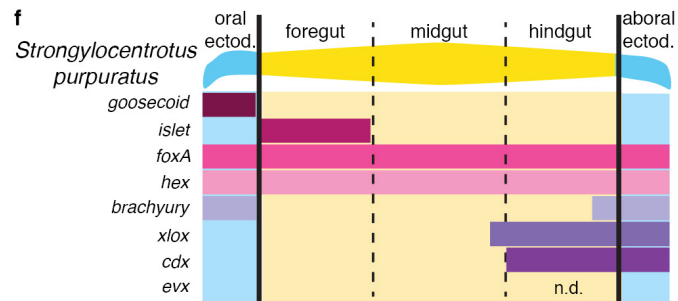
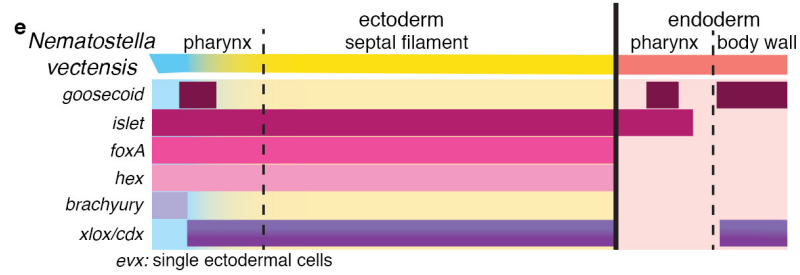
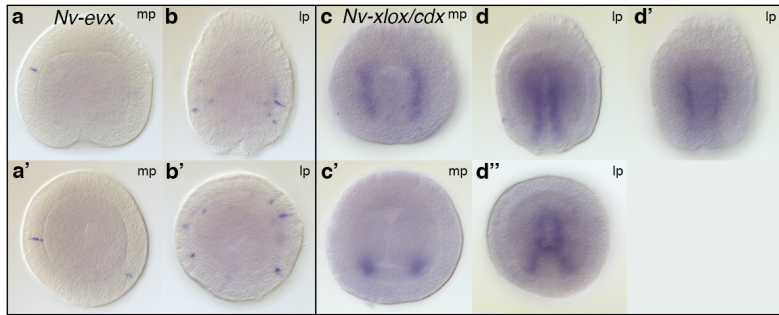
**Supplementary Figure 9 Expression of ‘skeletal muscle’ and
‘somite patterning’ gene orthologs during *N. vectensis*
development and growth**

Whole-mount *in situ* hybridisation of *six1/2* (a-d’), *six4/5* (e-j’), *dachshund* (k-o), *eyes absent* (p-r), *lbx* (s-u), *tbx15/18/22* (v-x’), *paraxis* (y-bb), *twist* (cc-ff’) and *moxC* (gg). (d, d’) Single *six1/2*-expressing cells in the retractor (d) or parietal muscle (d’) regions. See Supplementary Figure 3II and mm for schematic overviews of magnified regions. (x, bb, ff, gg) Whole-mount juvenile polyp heads with pharyngeal endoderm stainings before sectioning. The arrow in (gg) highlights the expression in the endodermal peristome.



Supplementary Figure 10 Expression of ‘cardiogenic’ transcription factor orthologs during *N. vectensis* development and juvenile growth

Whole-mount *in situ* hybridisation of *gata* (a-d’), *hand1* (e-f’), *hand2* (g-l’), *tbx1/10.1* (m-q’), *tbx1/10.2* (r-v’), *tbx20.1* (x-aa), *tbx20.3* (bb,bb’), *mef2* (cc), *nk4* (dd-hh’), *tbx4/5* (ii-kk’), *smooth/non-muscle-type myosin heavy chain* (ll, ll’), *striated-type myosin heavy chain* (mm-mm’’) at early planula (‘ep’), mid-planula (‘mp’), late planula (‘lp’), primary polyp (‘pp’) stages or cross-sections of body column (‘juv bc’, ‘com mes’, ‘incom mes’, ‘par mus’) or pharynx (‘juv ph’, ‘ph par mus’). See Supplementary Figure 3ll and mm for schematic overviews of magnified regions. Higher magnification of expression in the parietal muscle region of the body column (‘par mus’) or pharynx (‘ph par mus’), and in complete mesenteries (‘com mes’). (k’) Deeper focus as in (k). (mm’’) Maximal projection of confocal stack showing that *elaV*-expressing neurons locate just adjacent of the *striated-type myosin heavy chain*-expressing parietal muscle cells. (a’, g’-j’, m’-o’, r’-t’, x’, bb’) Oral views of larvae in the respective panels above. (e’, f’, dd’, ee’, ii’-kk’) Aboral views of larvae in the respective panels above. Scale bar in kk’’: 10µm



Supplementary Figure 11 Re-investigation of *Nv-evx* and *Nv-xlox/cdx*, and comparative analysis of fore-, mid- and hindgut marker genes between bilaterians and *N. vectensis*

Whole-mount *in situ* hybridisation of *evx* (a-b') and *xlox/cdx* (c-d'') at mid-planula ('mp') and late planula ('lp') stages. (e-i) Schematic representations and comparison of gut marker genes between the anthozoan *N. vectensis* (e), sea urchins (f), amphioxus (g), polychaetes (h) and insects (i). Represented stages are: *N. vectensis*: mid-late planula; *S. purpuratus*: prism/pluteus; *Branchiostoma sp.*: gastrula/early neurula except for *cdx*: 20h neurula; *C. teleta*: late stage 3/stage 4. *D. melanogaster*: stages 10-12. Asterisk: *foxA* is not expressed in the midgut of *C. teleta*, but in *Hydroides*, *Chaetopterus* (both Annelida) and *Themiste* (Sipuncula). References are found in the Supplementary Table 1. (a', b', c', d'') Oral views of larvae in the respective panels above, where lateral views are depicted. (d') Deeper focus than in (d) to highlight expression in mesenteries (in d'). Light blue: ectodermal derivatives; yellow: endodermal midgut; red: sea anemone endoderm.

Supplementary Table 1

Comparison of expression patterns among animals

Genes expressed in early anterior endoderm of vertebrates (based on ⁷¹⁻⁷³)

Gene name	Vertebrate	Other deuterostomes	<i>Drosophila</i>	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
<i>hex, hhex</i>	<i>Xenopus</i>: anterior endoderm, foregut, liver ^{74,75}, vascular endothelium ⁷⁵ Mouse: endoderm, foregut, liver, thyroid, vascular endothelium precursor ⁷⁶ Chicken: anterior endoderm, foregut, liver thyroid, endothelium ⁷⁷ Zebrafish: Dorsal yolk syncytial layer endoderm, liver, thyroid, angioblasts ⁷⁸, pancreatic bud ⁷⁹	<i>Asterina miniata</i> (Sea star): endomesoderm, gut endoderm ⁸⁰ <i>Strongylocentrotus purpuratus</i> (sea urchin): skeletal mesoderm, gut endoderm ⁸¹ <i>Branchiostoma floridae</i> (Amphioxus): Anterior endoderm ⁸² <i>Saccoglossus kowalevskii</i>: endoderm, dorsal gut endoderm ⁸³	CG7056-PA: midgut ^{84,85} .	<i>C. elegans pha-2</i>: pharyngeal primordium, intestine, pharyngeal neurons, muscle, rectal cells ⁸⁶ <i>Crepidula fornicata</i>: foregut, hindgut rudiment ⁸⁷		<i>Nematostella vectensis</i> : pharyngeal ectoderm; single cells in endoderm (this paper)		<i>Sycon ciliatum</i> : inner cell mass ⁸⁸

<i>foxA</i>	Zebrafish <i>foxA2</i>, <i>foxA3</i>: pancreas ⁷¹ Mouse <i>foxA2</i>: <i>foxA3</i>: definitive endoderm, gut (incl. pancreas premordium), node, notochord, floor plate, midbrain ⁸⁹	<i>Branchiostoma floridae</i>: endoderm, notochord ^{90,91} <i>Stronglyocentrotus purpuratus</i>: mouth, gut ⁹² <i>Ptychodera flava</i>: endoderm, foregut ⁹³ <i>Saccoglossus kowalevskii</i>: endoderm, anterior collar groove ectoderm ^{94 95}	<i>forkhead</i>: foregut, midgut, hindgut, stomatogastric nervous system, CNS: interneurons, MP1, MP2 neuroblasts ^{96 97}	<i>Capitella capitata</i>: endoderm, foregut (pharynx&oesophagus), hindgut ⁹⁸ <i>Hydroides elegans</i> (Polychaeta): endoderm, precursors of foregut, midgut and hindgut ⁹⁹ <i>Chaetopterus variopedatus</i> (Polychaeta): endoderm, foregut, hindgut ¹⁰⁰ <i>Themiste lageniformis</i> (Sipunculida): endoderm, foregut, midgut, hindgut ¹⁰⁰ <i>Patella vulgata</i> (Mollusca): endoderm, foregut, midgut precursor ¹⁰¹ <i>Crepidula fornicata</i>: endoderm, foregut, hindgut rudiment ⁸⁷ <i>Priapulus caudatus</i>:	<i>Convolutiloba longifissura</i>: endoderm ¹⁰³, <i>Isodiametra pulchra</i>: digestive system, head myocytes, gonads, neoblasts ¹⁰⁴	<i>Nematostella vectensis</i>: pharyngeal ectoderm, septal filaments ^{105 106} & this paper <i>Hydra vulgaris</i>: hypostome endoderm ¹⁰⁷ <i>Aiptasia</i> sp.: pharyngeal ectoderm (larva) ¹⁰⁸ <i>Acropora millepora</i>: pharyngeal ectoderm, single ectod. cells (larva); pharynx & septal filament (post-metamorphosis) ¹⁰⁹, <i>Favia lizardensis</i>, <i>Ctenactis echinata</i>: Blastopore/pharyngeal ectoderm, single ectod. cells (gastrulation, early planula) ¹¹⁰ <i>Clytia hemisphaerica</i>: polyp head endoderm, medusal mouth tube		
-------------	---	---	---	--	--	--	--	--

				pharyngeal ectoderm, endodermal gut ¹⁰²		endoderm ¹¹¹		
<i>sox1/2/3</i> (B1 group)	Mouse: Neural ectoderm, gut endoderm, PNS ¹¹² Zebrafish: neural ectoderm ¹¹³ .	<i>Branchiostoma floridae</i> : neural ectoderm ¹¹⁴ Saccoglossus kowalevskii: neural ¹¹⁵ , sea urchin: neural apical pole; foregut ¹¹⁶	<i>Sox neuro</i> : neuroectoderm ¹¹⁷		<i>Convolutriloba longifissura</i> : soxB1 probably in neuroectoderm ¹¹⁸	<i>Nematostella vectensis</i> : aboral ectoderm, pharyngeal ectoderm (larva, primary polyp) ¹¹⁹		
<i>sox17/soxF</i>	Mouse: Definitive endoderm, restricts to mid- and hindgut ¹²⁰ Zebrafish <i>casanova/sox17</i> : Endoderm ¹²¹ , entire gut ¹²² <i>Xenopus</i> : Entire endoderm, posterior gut ¹²³	<i>Strongylocentrotus purpuratus</i> : secondary mesenchyme cells ¹²⁴	<i>sox15</i> : Peripheral nervous system ¹²⁵	<i>Apis mellifera</i> : ubiquitous in late larvae, ovaries ¹²⁶		<i>Acropora millepora</i> : planula endoderm ¹²⁷ <i>Nematostella vectensis</i> : Subset of planula endoderm ¹¹⁹ <i>Clytia hemisphaerica</i> : scatter endoderm (larva), radial and circular canal, somatic gonad, manubrium endoderm (all medusa) ¹²⁸	<i>Pleurobrachia pileus</i> : broadly endodermal ¹²⁹	<i>Sycon ciliatum</i> : absent in larvae and embryos, single cells in osculum, choanocytes & accessory cells,
<i>hnf4, HNF-4(D), dHNF4</i>	Zebrafish: yolk syncitial layer, liver bud, alimentary canal ^{122,130}		<i>dHNF4</i> : midgut, fat bodies, malpighian tubulues ¹³⁴	<i>Priapulus caudatus</i> : anterior midgut ¹³⁵		<i>Nematostella</i> : Broad endoderm, higher expression in the somatic gonad	<i>Mnemiopsis leidyi</i> : tentacle bulb ectoderm, apical organ, aboral pharynx,	<i>Amphimedon</i> : ubiquitous ¹³⁷

	<i>Xenopus</i> : pronephros, liver, gut ¹³¹ Mouse: visceral endoderm, liver, hindgut, mesonephros, pancreas, stomach, intestine ^{132,133}					(this paper)	broad endoderm ¹³⁶	
--	---	--	--	--	--	--------------	-------------------------------	--

Genes expressed during pancreatic cell differentiation (based on ⁷¹⁻⁷³)

Gene name	Vertebrate	Other deuterostomes	<i>Drosophila</i>	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
<i>islet</i>	Zebrafish: CNS (f.ex. motorneurons) ¹³⁸, pancreas ⁷¹, subset of cardiomyocytes ¹³⁹ Chicken: CNS (f.ex. motor neurons) ¹⁴⁰, pancreas ¹⁴¹, cardiogenic precursors ¹⁴² Rat: CNS (f.ex. motorneurons), all pancreatic islet types ¹⁴³ Mouse: CNS (f.ex. motorneurons), heart, ventral foregut endoderm ^{144 139}	Sea urchin: Ciliary band, foregut, anus ¹⁴⁵ <i>Branchiostoma floridae</i>: neural plate, anterior endoderm, pharyngeal endoderm, intestine ^{146,147}	<i>Isl1</i>: ventral nerve chord (f.ex. motor neurons, interneurons), pharynx, dorsal vessel ¹⁴⁸			<i>Nematostella vectensis</i>: pharynx, directive mesenteries ¹⁴⁹ & this paper (partly conflicting)	<i>Mnemiopsis leydi</i>: aboral ectodermal cells ¹⁵⁰	<i>Amphimedon queenslandica</i>: ubiquitous ¹⁴⁹
<i>nkx2.2</i>	Mouse: forebrain, pancreas, restricts to endocrine pancreas ¹⁵¹ Zebrafish: CNS (f.ex.	<i>Branchiostoma floridae</i>: ventral endoderm, pharynx, midgut, hindgut,	<i>Vnd</i>: ventral CNS (f.ex. MP2 neuroblast, progenitor of insulinergic neurons) ¹⁵⁵	<i>Platynereis dumerilii</i>: ventral neuroectodermal midline ¹⁵⁶		<i>Nematostella nkx2.2A-E</i>: pharyngeal ectoderm, septal		

	forebrain, ventral neural tube) pancreas ^{71,152}	anterior CNS ¹⁵³ <i>Stronglyocentrotus purpuratus</i> : oral and aboral ectoderm ¹⁵⁴				filaments, some paralogs low in endoderm		
<i>hnf6</i>	Zebrafish: CNS (f.ex. fore-, midbrain), liver, pancreas, gallbladder ¹⁵⁷ Mouse: CNS (fore-, mid-, hindbrain, motoneurons), fore-midgut junction, hindgut, liver, gall bladder, biliary system, pancreas precursor cells (endo-& exocrine) ^{158,159}	Stronglyocentrotus purpuratus: ciliary band (gastrula) ¹⁶⁰ Asterina miniata: ubiquitous except vegetal pole (blastula), restricts to ciliary bands ¹⁶¹ <i>Halocynthia roretzi</i> (Ascidia): neurons ¹⁶²	<i>onecut</i> : CNS, PNS ¹⁶³	<i>Platynereis dumerilii</i> : larval brain ¹⁶⁴ <i>Haliotis rufescens</i> : larval brain ¹⁶⁵		<i>Nematostella</i> : No expression detected		
<i>hlxB9/mnx</i>	Mouse: notochord, dorsal gut endoderm, ventral endoderm: pancreatic epithelium, β -cells ¹⁶⁶ Zebrafish: axial & lateral mesoderm, endoderm, pancreas (exo- & endocrine) ¹⁶⁷	<i>Branchiostoma floridae amphiMnx</i> : neural plate, dorsal endomesoderm, posterior gut ¹⁶⁸ <i>Saccoglossus kowalevskii</i> : ventral endoderm, ectodermal patches <i>Paracentrotus lividus</i> : anus (prism and	<i>hb9</i> : anterior & posterior midgut, motor neurons, interneurons, insulinergic MP2 neurons ^{96,170,171}	<i>Platynereis dumerilii</i> : Ventral nerve chord (motoneurons) ¹⁵⁶		<i>Nematostella vectensis</i> : pharyngeal ectoderm, single ectodermal cells ¹⁷² and this paper		

		pluteus) ¹⁶⁹						
<i>nkx6</i>	Zebrafish <i>nkx6.1</i>: spinal cord motor neurons, pancreas¹⁷³ Mouse: CNS (motoneuron, interneuron), pancreas^{174 175,176}		<i>nkx6</i>: CNS (brain, ventral & intermediate neuroblasts), hindgut,^{173,177}	<i>Platynereis dumerilii</i>: Ventral nerve chord (motoneurons¹⁵⁶		<i>Nematostella vectensis</i>: pharyngeal ectoderm, septal filament just below pharynx (this paper)		
<i>ptf1</i>	Mouse: exocrine pancreas¹⁷⁸ Zebrafish: hindbrain, retina, pancreas¹⁷⁹	<i>Stronglyocentrotus purpuratus</i>: ectoderm, midgut¹⁴⁵				<i>Nematostella</i>: single cells in ectoderm (this paper)		
<i>tbx2/3</i> <i>Bifid/optomotor-blind</i>	Mouse: extraembryonic mesoderm & endoderm, pharyngeal arch mesenchyme, pharynx (thyroid), otic & optic vesicle, trigeminal ganglia, dorsal root ganglia, diencephalum (infundibulum), hindbrain, limb buds, myotomes, smooth muscles, pancreas^{180,181}	<i>Branchiostoma floridae</i>: blastopore lip, invaginating mesendoderm, neural tube, ventral gut endoderm, surface ectoderm¹⁸⁵ <i>Lytechinus variegatus</i>: foregut, oral ectoderm, endoderm, skeletogenic mesoderm¹⁸⁶	<i>optomotor-blind</i>: optic lobe anlagen, large part of larval brain¹⁸⁸	<i>Hydroides elegans</i>: dorsal blastomeres, broad dorsal midline, dorsal sensory cells, dorsal endoderm, hindgut¹⁸⁹		<i>Nematostella</i>: endoderm, pharyngeal ectoderm, single cells in ectoderm (this paper)		

	Chicken: heart, branchial arches & pouches, pharynx floor, facial region, olfactory, optic and otic regions, dorsal root ganglia, limbs, somites^{182,183} Zebrafish: ventral prosencephalon, notochord, optic&otic placodes, Rohon-Beard sensory neurons, diencephalon (pineal), trigeminus, lateral line, pronephric ducts, branchial arches, heart, liver, fins,¹⁸⁴	<i>Paracentrotus lividus</i>: early presumptive endoderm & part of ectoderm, partly in primary mesenchyme¹⁸⁷						
<i>insulin-like peptides, insulin growth factor hormone</i>	Zebrafish: pancreatic island (insulin), ubiquitous with highest signal in anterior nervous system (IGF-I,-II)^{190,191} <i>Xenopus</i>: anterior&dorsal ectoderm & mesoderm,	<i>Stronglyocentrotus purpuratus</i>: Foregut, upper stomach, intestine¹⁹⁴ <i>Branchiostoma lanceolatum</i>: endoderm, paraxial mesoderm, gut,	<i>Ilp1-7</i>: midgut, mesoderm, imaginal discs, single brain neurons, salivary glands, ventral nerve chord¹⁹⁷			<i>Nematostella</i>: single cells in the pharyngeal ectoderm, and close to the parietal muscles (this paper)		

	liver, lung heart, kidney, peritoneal fat (IGF-1,- 2) ^{192,193}	hepatic caecum ^{195,196}						
--	--	-----------------------------------	--	--	--	--	--	--

Genes involved in cardiac development

Overall conservation in bilaterians ¹⁹⁸

Gene	Vertebrate	Other deuterostomes	<i>Drosophila</i>	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
<i>hand/dHand,/eHand/thing1/hxt</i>	Zebrafish, Chicken & <i>Xenopus</i>: lateral plate mesoderm, precardiogenic mesoderm, cardiac mesoderm, heart tube, branchial arches ^{199,200} Mouse: extra-embryonic, heart, neural crest (autonomous nervous system), gut, pharyngeal arches ²⁰¹	<i>Amphioxus</i> : ventral mesoderm ²⁰²	All heart progenitors (cardioblast & pericardium), circular visceral muscles, lymph gland, garland cells, neurons ²⁰³			<i>Nematostella</i> : larval endoderm, polyp: restricts to the parietal muscle (this paper)		
<i>tbx20/neuromancer-1/-2/H15/mid</i>	Zebrafish: lateral plate mesoderm, cardiac mesoderm & heart, hindbrain & midbrain motor	<i>Amphioxus</i> : ventral mesoderm, few ventral neurons ²⁰⁷	<i>Drosophila</i> : segment polarity pattern, CNS formation, cardiogenic	<i>Schmidtea polychroa</i>: ventral nerve chord (?) ²¹¹ <i>Cupiennius salei</i>: dorso-lateral edge		<i>Nematostella</i> : larval mesentery endoderm, parietal muscle region (this paper)		

	neurons, tailbud²⁰⁴ <i>Xenopus</i>: Cardiac tissue (myocardium & endocardium), cement gland, jugular vein, lung bud, cloacal aperture, hindbrain, motor neurons²⁰⁵ Mouse: Extraembryonic mesoderm, lateral plate, cardiogenic mesoderm & heart, retina, hindbrain²⁰⁶		region, entire myocardium²⁰⁸⁻²¹⁰	of embryo (heart precursor cells) & developing heart²¹² <i>Tribolium castaneum</i>: dorso-lateral edge of germ band (heart precursor) & developing heart²¹²				
<i>gata1/2/3</i>; <i>grain/dGATAc</i>	Zebrafish: ventral ectoderm, lateral mesoderm, erythroid progenitor cells, pronephric duct, CNS^{213,214} <i>Xenopus</i>:	Amphioxus: mesendoderm, forming somites, endoderm, coelomic diverticula, pharyngeal mesoderm,	<i>Drosophila</i>: CNS, midgut, lateral ectoderm, appendages²¹⁸	<i>Capitella sp.</i>: CNS (brain and ventral nerve chord), foregut⁹⁸; <i>Platynereis dumerilii</i>: neuroectoderm²¹⁹		<i>Nematostella</i>: single cells in ectoderm; mesenterial endoderm, parietal muscle¹⁰⁶ & this paper; <i>Hydra</i>: body column		<i>Amphimedon gata1/2/3/4/5/6</i> ortholog: inner layer of the larva²²¹

	hematopoietic mesoderm, erythroid cells ²¹⁵ Mouse: Brain & CNS, PNS, kidney, T-lymphocytes ²¹⁶	cerebral vesicle, floor plate of intestine ^{147,217}				epithelium ²²⁰ <i>Clytia hemisphaerica</i>: polyp body column ectoderm, sub- and exumbrellar ectoderm, bell rim neurons ¹¹¹ <i>Aurelia aurita</i>: aboral ectoderm of strobila, broadly ecto- and endoderm, neurons at mouth rim ¹¹¹		
<i>gata4/5/6</i>; <i>pannier/dGataa</i>; dGATAB/<i>serpent</i>; dGatad; dGATAe	<i>Xenopus</i>: marginal zone, antero-ventral mesoderm incl. cardiac mesoderm, developing heart (myo-, endo- and pericardium), blood anlagen ²²²⁻²²⁴ Mouse: pre-cardiac	<i>Strongylocentrotus purpuratus</i>, <i>Asterina miniata</i>: mesoderm progenitor cells, blastopore, hindgut & midgut, coelomic pouches ^{228,229}; Amphioxus: endoderm, central somites, hindgut	<i>Drosophila</i>: endoderm, midgut, fat body, hemocytes, dorsal mesoderm, cardial myocytes ^{230 231-233}	<i>Schmidtea polychroa</i>: dorsal parenchymal cells, blind gut epithelium ²¹¹; <i>Capitella sp.</i>: endoderm, visceral mesoderm, midgut ⁹⁸; <i>Chaetopterus sp.</i>: endoderm, mesoderm,	<i>Isodiametra pulchra</i>: around statocyst, cross muscles, gonads, neoblasts ¹⁰⁴	No homolog present in <i>Nematostella</i>		See <i>gata1/2/3</i> .

	mesoderm, embryonic & adult heart, primitive endoderm, intestine, gonads, lung, smooth muscle cells (bronchial, bladder) ^{225 226 227}	¹⁴⁷		midgut; <i>Themiste lageniformis</i> : endoderm, midgut ¹⁰⁰ , <i>Platynereis dumerilii</i> : mesoderm, muscles ²¹⁹ , <i>Priapul</i> <i>us caudatus</i> : anterior midgut ¹³⁵ <i>Terebratalia transversa</i> : gastrula mesoderm & endoderm, midgut, paired lateral mesoderm, chaetal sac mesoderm ²³⁴				
<i>tbx4/5</i>	Zebrafish: eye, fin buds, heart tube ^{235,236} <i>Xenopus</i> : dorsal retina, early heart field, heart tube,	Amphioxus: pharyngeal & ventral mesoderm (pre-mouth larva), posterior-ventral mesoderm from late larvae, Hesse	Absent.			Nematostella: aboral endoderm & ectoderm (this paper) <i>Clytia hemisphaerica</i> :		

	limbs ^{237, 238} Mouse: allantois, sinus venosus & ventricle of the developing heart, optic cup, genital papilla, mesenchyme of tail & mandibular arches, limb buds ¹⁸¹	organ neurons ^{147, 185}				polyp tentacle endoderm, medusal canals of gastro-vascular system ¹¹¹ <i>Aurelia aurita</i> : broad rhopalar and velar arm endoderm ¹¹¹		
<i>tbx1/10; org-1</i>	Mouse (ONLY <i>tbx1</i>): pharyngeal endoderm, otic vesicle, lung endoderm, sclerotome, hindbrain, secondary heart field ^{181, 239, 240} Zebrafish: head & lateral plate mesoderm, pharygeal endoderm, pharyngeal arch cardiac	<i>Amphioxus</i> : ventral somites, ventral branchial arch mesoderm & endoderm, pharynx, axial & paraxial mesoderm ²⁴³	<i>Drosophila</i> : visceral and somatic muscle progenitor cells, adult heart muscle ^{244, 245}	<i>Schmidtea polychroa</i> : dorsal parenchymal cells ²¹¹		<i>Nematostella</i> : larval endoderm, body wall endoderm, parietal muscle, somatic gonad (this paper)		

	precursors, heart , otic vesicle ²⁴¹ <i>Xenopus</i> : anterior ectoderm, pharyngeal region, branchial arches ²⁴²							
<i>Mef2</i>	Mouse: cardiac & skeletal muscle mesoderm, neural crest, vascular smooth muscle, limb buds, different regions of brain ^{246,247} Zebrafish: somitic and cardiac muscle progenitors ²⁴⁸	<i>Branchiostoma belcheri</i> <i>AmphiMef2</i> : presomitic mesoderm, somites, preoral pit ²⁴⁹	<i>Drosophila</i> : mesoderm, somatic, heart & visceral muscle progenitors, brain ²⁵⁰⁻²⁵²	<i>Terebratalia transversa</i> : apical ectoderm, lateral bands of mesoderm surrounding anterior endoderm, chaetal sac mesoderm ²³⁴ <i>Cupiennius salei</i> : somatic, visceral, and cardiac mesoderm, heart tube, CNS development ²¹²	<i>Isodiametra pulchra</i> : differentiating myocytes & copulatory organs, brain commissures, head myocytes ¹⁰⁴	<i>Nematostella</i> : endoderm, presumptive neurons ^{106,253} <i>Podocoryne</i> : larval endoderm, aboral ectoderm; polyp: oral epidermis, tentacles; medusal bud: distal ectoderm, endoderm, entocodon, subumbrellar plate & striated muscle layer ²⁵⁴		
<i>nk4</i> ; <i>Nkx2.3/2.5/2.7</i> ; <i>tinman</i> / <i>msh-2</i>	Zebrafish: cardiac precursor cells, developing heart,	<i>Amphioxus</i> : foregut endoderm, ventral mesoderm,	<i>Drosophila</i> : entire early mesoderm, developing	<i>Platynereis dumerilii</i> : Larva: segmental	<i>Nematostella</i> : broad endoderm & ectoderm, absent			

	pharyngeal endoderm ^{255,256} <i>Xenopus</i>: heart and visceral mesoderm, heart, foregut, pharynx ^{257,258} Mouse: precardiac mesoderm, heart, pharyngeal endoderm ^{259,260}	somite muscles, myocardial progenitors ^{202,261}	visceral & heart muscles ^{262,263}	ectodermal stripes, dorsal mesoderm (cardiogenic?); Posterior growth: ectodermal and mesodermal stripes ²⁶⁴ <i>Sepia officinalis</i>: cardiac & somatic muscles ²⁶⁵ <i>Terebratalia transversa</i>: no larval expression ²³⁴ <i>Cupiennius salei</i>: dorso-lateral edge of embryo (heart precursor cells) & developing heart ²¹² <i>Tribolium castaneum</i>: dorso-lateral edge of germ band (heart precursor) & developing heart	at oral pole (this paper)			
--	---	--	--	---	----------------------------------	--	--	--

[illegible]

Genes involved in visceral mesoderm development

Gene	Vertebrate	Other deuterostomes	<i>Drosophila</i>	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
<i>nkx3.2 / bapx1 / bapx / nk3</i>	Mouse: splanchnic lateral plate mesoderm, rostral somites (sclerotome), limb development ²⁶⁶ <i>Xenopus</i> : head cartilage, gut musculature ²⁶⁷	<i>Branchiostoma</i> : medial somites, right pharyngeal endoderm ²⁶⁸ <i>Strongylocentrotus purpuratus</i> : oral animal pole ectoderm, foregut endoderm (gastrulation)	<i>Drosophila</i> : dorsal-most mesoderm (cardiogenic), visceral mesoderm of midgut, procto- & stomodaeal mesoderm ²⁶³	<i>Platynereis</i> : apical organ mechanoreceptor cells, deep trunk mesoderm, head mesoderm ^{164,264}		<i>Nematostella</i> : apical organ ²⁶⁹ , pharyngeal endoderm & polyp somatic gonad (this paper)		
<i>foxC / foxC1, -C2 / foxC1a, -1b, -2a, -2b / mfh1 / mf1 / XFD-11</i>	Zebrafish: paraxial & lateral mesoderm, presomitic mesoderm, somites ²⁷⁰ Mouse: presomitic mesoderm, somites, heart, neural crest- and cephalic mesoderm derived mesenchyme, ²⁷¹⁻	<i>Branchiostoma</i> : dorsal mesoderm ²⁷⁵ <i>Strongylocentrotus purpuratus</i> : small micromere (mesodermal) lineage, coelomic pouch myoblasts ^{276 277,278} <i>Saccoglossus kowalevskii</i> :		<i>Terebratalia transversa</i> : Gastrula: Anterior mesoderm & ectoderm. Larva: anterior mesoderm, ring of anterior ectoderm adjacent to ciliary band, posterior-ventral mesoderm ²³⁴	<i>Isodiametra pulchra</i> : subepidermal, head myocytes, cross muscles, lateral domain incl. gonads, neoblasts ¹⁰⁴	<i>Nematostella</i> : larva: pharyngeal endoderm, primary mesenteries ¹¹⁹ & juvenile somatic gonad (this paper)		

	²⁷³ <i>Xenopus</i> : ventral & lateral mesoderm (gastrula); anterior neural plate, neuroectoderm borders, posterior mesoderm, lateral mesoderm pronephros (neurula); heart, cranial neural crest ²⁷⁴	vegetal plate (endomesoderm), anterior mesoderm, coelomic pouches of trunk and collar, anterior and posterior collar groove ectodermal rings, pharyngeal endoderm ⁹⁵						
six4/5	Zebrafish: head mesoderm, presomitic mesoderm, somites, cranial sensory placodes, ^{279,280} <i>Xenopus</i> : cranial sensory placodes, somites, head mesenchyme, abdominal muscle precursors ²⁸¹ Mouse: Brain,	<i>Branchiostoma</i> : anterodorsal mesendoderm, notochord, somites, neural plate, pharyngeal endoderm ²⁸⁵	<i>Drosophila</i> : procephalic lobes, ventral nerve chord, trunk mesoderm, restricts to ventral and lateral mesoderm, then to somatic gonadal precursors ²⁸⁶⁻²⁸⁸			<i>Nematostella</i> : overall endoderm, stronger in somatic gonad (this paper)		

	CNS, cranial sensory placodes, somites, limb buds, branchial arches, mesonephros ²⁸²⁻ 284							
--	--	--	--	--	--	--	--	--

Genes involved in 'skeletal' muscles development

Gene	Vertebrate	Other deuterostomes	<i>Drosophila</i>	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
<i>eyes absent / eya / clift</i>	Zebrafish: cranial placodes, somites, fins, branchial arches ²⁸⁹ <i>Xenopus</i>: somites, hypaxial muscle precursors, cranial placodes ²⁹⁰ Mouse: cranial placodes, branchial arches, CNS, eye, head mesenchyme, somites ^{291,292}	<i>Branchiostoma</i>: anterodorsal mesendoderm, pharyngeal endoderm, notochord, posterior somites, neural plate ²⁸⁵ <i>Strongylocentrotus purpuratus</i>: mesoderm, coelomic pouch, non-myoblast mesoderm ^{278,293} <i>Strongylocentrotus purpuratus</i>: secondary mesenchyme cells, coelomic pouch ²⁹³ <i>Patiria miniata</i>: secondary mesenchyme	<i>Drosophila</i>: broad mesoderm, somatic gonadal precursors, eye ^{294,295}	<i>Terebratalia transversa</i>: animal cap ectoderm, dorso-lateral anterior ectoderm, anterior mesoderm ²³⁴ <i>Dugesia japonica</i>: eyes, CNS, parenchyma ²⁹⁶		<i>Nematostella</i>: broad endoderm (this paper)		<i>Sycon ciliatum</i>: choanocytes ²⁹⁷

		cells, coelomic pouch ²⁹³						
<i>six1/2 / sine oculis</i>	Zebrafish: cranial sensory placode, brain, somites, ventral abdomen & cranial muscles, fins²⁹⁸ <i>Xenopus</i>: sensory placode anlagen, head mesenchyme, somites, abdominal muscle precursors, intermediate & lateral plate mesoderm derivatives,²⁸¹ Mouse: head mesoderm & pharyngeal pouches, notochord, somites, skeletal & visceral muscles, CNS, limbs^{284,299}	<i>Branchiostoma</i>: anterior mesendoderm, somites, pharyngeal endoderm²⁸⁵ <i>Strongylocentrotus purpuratus</i>: secondary mesenchyme cells, coelomic pouch, non-myoblast mesoderm^{278,293}	<i>Drosophila</i>: optic lobe, eye, ventral head mesoderm, ectodermal segment boundaries^{300,301}	<i>Terebratalia transversa</i>: gastrula: anterior mesoderm, spots of lateral ectoderm. Larva: broadly in mesoderm, ectoderm of mantle lobe²³⁴	<i>Isodiametra pulchra</i>: brain (?), female genital organ, anterior myocytes, neoblasts¹⁰⁴	<i>Nematostella</i>: pharyngeal ectoderm & endoderm, single cells in retractor muscle and parietal muscle regions (this paper)		

<i>six4/5</i>	See above	See above	See above	See above	See above	See above	See above	See above
<i>dachshund / dac / dach</i>	Zebrafish: sensory organs, CNS, fin buds, somites, neural crest, pronephros ³⁰² Mouse: lateral mesoderm, neural crest, CNS, limbs, brain, eyes, somites ³⁰³⁻³⁰⁵	<i>Amphioxus</i>: paraxial mesoderm, somites, anterior endoderm, cerebral ganglia, pharynx endoderm, endostyle ³⁰⁶ <i>Strongylocentrotus purpuratus</i>: veg1 endoderm precursors, gut ⁸¹ <i>Saccoglossus</i>: neuroectodermal stripe anterior to collar, low ubiquitous ³⁰⁷	<i>Drosophila</i> : optic lobe, ventral nerve chord, eye disc ³⁰⁸	<i>Neanthes arenaceodentata</i>: ventral nerve chord, posterior and newly differentiated mesoderm ³⁰⁹ <i>Glomeris marginata</i>: visual centre, brain, ventral nerve chord, appendages, proctodaeum, heart ³¹⁰ <i>Terebratalia transversa</i>: gastrula mesoderm, dorsal & lateral ectoderm, nearly all mesodermal derivatives, ectodermal eye		<i>Nematostella</i> : ubiquitous (this paper)		

				spots, ganglia, pedicle lobe ²³⁴				
<i>lbx1 / lbx2 / ladybird early / ladybird late</i>	Mouse: spinal chord, hindbrain, eye, limb myogenic cells, urogenital ridge ^{311,312} <i>Xenopus</i>: hypaxial myoblasts contributing to head and all body wall muscles ³¹³ Zebrafish: paraxial mesoderm, adaxial muscle myoblasts, fin muscle myoblasts, hindbrain, spinal chord ^{314,315}		<i>Drosophila</i>: neuroblasts, cardioblasts, pericardial cell precursors, somatic myoblasts, terminal regions ³¹⁶⁻³¹⁸	<i>Platynereis</i>: stripes of putative neuroblasts in ventro-lateral ectoderm and putative myoblasts in lateral mesoderm ²⁶⁴		<i>Nematostella</i>: larva: aboral endoderm, mesenteries; polyp: pharyngeal endoderm (this paper)		

Genes involved in somite development

Gene	Vertebrate	Other deuterostomes	<i>Drosophila</i>	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
<i>paraxis/scleraxis</i>	Zebrafish: presomitic mesoderm, somite³¹⁹ <i>Xenopus</i>: presomitic mesoderm, somites, head mesoderm, neural tube³²⁰ Mouse: presomitic, paraxial mesoderm, somite^{321,322}	<i>Branchiostoma</i>: posterior mesendoderm, tailbud, anterior endoderm³²³	–	<i>Platynereis</i>: ventrol-lateral mesoderm³²⁴ <i>Terebratalia transversa</i>: two ventro-medial stripes & posterior mesoderm,²³⁴		<i>Nematostella</i>: pharyngeal endoderm (this paper)		
<i>tbx15/18/22</i>	Zebrafish: developing presomitic & somitic mesoderm, heart mesenchyme, paraxial head mesenchyme, ventral neuroectoderm	<i>Branchiostoma</i>: pair of mesendoderm stripes, somites^{323,329}	–			<i>Nematostella</i>: pharyngeal endoderm (this paper)		

	^{325,326} Mouse: cranial paraxial mesoderm, presomitic mesoderm, anterior somite half, genital ridge, limb buds, pharyngeal region ^{327 328}							
<i>twist /X-twi / M-twi</i>	Zebrafish: neural crest, somite (sclerotome), lateral plate mesoderm, notochord, hypochord, dorsal aorta ³³⁰ <i>Xenopus</i> : blastocoel roof, prechordal plate, notochord, somites, lateral mesoderm, neural crest, neural plate ³³¹	<i>Branchiostoma</i> : developing somites, notochord, head coelom ³³³ <i>Lytechinus</i> <i>variegatus</i> : primary and secondary mesenchyme cells ³³⁴	<i>Drosophila</i> : mesoderm anlagen, entire mesoderm ^{335,336}	<i>Terebratalia</i> <i>transversa</i> : gastrula mesoderm, anterior & chaetal sac mesoderm ²³⁴ <i>Alitta (Nereis)</i> <i>virens</i> : blastopore margin, mesodermal bands, pharynx ectomesoderm ³³⁷ <i>Platynereis</i> : stomodaeal ectomesoderm, trunk mesoderm,	<i>Isodiametra</i> <i>pulchra</i> : only adult: myoblasts, gonads & male copulatory organ, few neoblasts ¹⁰⁴	<i>Nematostella</i> : pharyngeal endoderm ¹⁰⁶ & this paper <i>Podocoryne</i> : broad in larva, medusa bud, subumbrellar plate cells, tentacle bud anlagen, manubrium ³⁴² <i>Clytia</i> <i>hemisphaerica</i> : polyp tentacle endoderm, medusal umbrellar		

	Mouse: lateral plate mesoderm (somatopleura), notochord, somite, neural crest ³³²			developing muscles ³³⁸ <i>Capitella</i>: trunk mesoderm, stomodaeal and proctodaeal muscles, foregut ³³⁹ <i>Tribolium</i>: mesodermal anlagen, invaginating mesoderm, mesodermal growth zone ³⁴⁰ <i>Parhyale</i>: segmental mesoderm ³⁴¹		plate endoderm ¹¹¹ <i>Aurelia aurita</i>: endodermal cells of the gastro-vascular system ¹¹¹		
<i>mox / mox1 / mox2 / meox1 / moxA-D</i>	Mouse: presomitic mesoderm, somites, lateral plate mesoderm ³⁴³ <i>Xenopus</i>: undifferentiated ventral, lateral & dorsal mesoderm,	<i>Branchiostoma</i>: nascent somites ³⁴⁶	–	<i>Terebratalia transversa</i>: two ventro-medial stripes & posterior mesoderm, ²³⁴ <i>Alitta (Nereis) virens</i>: ventral mesoderm,		<i>Nematostella</i>: pharyngeal endoderm ¹⁷² & this paper		

	somites, tailbud ³⁴⁴ Zebrafish: early somite, appendicular muscles, vascular- associated cells ³⁴⁵			pharynx ectomesoderm ³³⁷ <i>Halotis</i> : paraxial, myogenic mesoderm bands ³⁴⁷				
--	---	--	--	---	--	--	--	--

References in Supplementary Figure 11 not found in the tables above:

gooseoid: *Nematostella vectensis*³⁴⁸, *Drosophila melanogaster*³⁴⁹, *Branchiostoma sp.*⁸², *Strongylocentrotus purpuratus*³⁵⁰, *Capitella teleta*³⁵¹

brachyury: *Nematostella vectensis*³⁵², *Drosophila melanogaster*^{84,85,353}, *Branchiostoma sp.*⁹¹, *Strongylocentrotus purpuratus*³⁵⁴, *Capitella teleta*³⁵¹

xlox/cdx: *Nematostella vectensis*¹⁷²&this paper

xlox: *Branchiostoma sp.*^{355,356}, *Strongylocentrotus purpuratus*^{145,357,358}, *Capitella teleta*³⁵⁹

cdx: *Drosophila melanogaster*^{360,361}, *Branchiostoma sp.*^{355,356}, *Strongylocentrotus purpuratus*^{145,357}, *Capitella teleta*³⁵⁹

evx: *Nematostella vectensis*¹⁷²&this paper, *Drosophila melanogaster*^{84,85}, *Branchiostoma sp.*⁸², *Capitella teleta*³⁶²

Supplementary Table 2: Gene orthologies and accession numbers of cloned genes

N. vectensis transcription factors

Gene name	Alternative name(s)	Reference for gene orthology	Notes	Genbank number
<i>Nv foxA</i>	<i>Forkhead; HNF3</i>	105,106		AY457634.1
<i>Nv hex</i>	<i>HHex</i>	363,364		LT795553
<i>Nv islet</i>	<i>ISL</i>	363,364		LT795561
<i>Nv soxB1</i>		119		LT795573
<i>Nv nkx6</i>	<i>NK6</i>	363-365		LT795566
<i>Nv hlxB9</i>	<i>mnx, hb9</i>	363-365		LT795554
<i>Nv tbx2/3</i>	<i>Nv_e_gw.65.117.1</i>	366		LT795540
<i>Nv hnf4</i>	<i>NvNR4</i>	367		LT795556
<i>Nv foxC</i>		119		LT795581
<i>Nv nkx3</i>	<i>NK3; bap; bagpipe</i>	363,364		LT795535
<i>Nv six4/5</i>	<i>six4/5a; six45-related1</i>	363,364	A second six4/5b gene is not expressed based on transcriptome data of diff. larval and adult stages (unpublished)	LT795572
<i>Nv six1/2</i>	Six12A	363,364	A second <i>Nv six1/2-2</i> paralog (SIX12B after Chourrout et al.) could be cloned, but not detected at any stage of	LT795571

			development or in juveniles.	
<i>Nv eyes absent</i>	<i>eya</i>	297		LT795552
<i>Nv dachshund</i>	<i>dac</i>	Determined by BLAST (this paper)		LT795576
<i>Nv lbx</i>	<i>ladybird</i>	363-365		LT795538
<i>Nv ptf</i>	<i>PTFb</i> (Nem12)	368		LT795570
<i>Nv hnf1</i>		363		LT795555
<i>Nv tbx15/18/22</i>	<i>Nv_e_gw.19.61.1</i>	366		LT795542
<i>Nv paraxis-1</i>	<i>paraxis</i> (Nem2)	368		LT795539
<i>Nv paraxis-2</i>		Determined by BLAST (this paper)		LT795569
<i>Nv twist</i>	(Nem14)	106,368		LT795591
<i>Nv moxC</i>		363,364		LT795546
<i>Nv gata</i>	<i>gata1/2/3</i>	106		AY496948.1
<i>Nv hand1</i>	(Nem6)	368		LT795536
<i>Nv hand2</i>	(Nem21)	368		LT795537
<i>Nv tbx1/10.1</i>	<i>Tbx1</i>	366		LT795587
<i>Nv tbx1/10.2</i>	<i>Nv_fgenesh1_pg.scaffold_203000031</i>	366		LT795574
<i>Nv tbx20.1</i>	<i>Nv_e_gw.146.31.1</i>	366		LT795543
<i>Nv tbx20.3</i>	<i>Nv_e_gw.80.47.1</i>	366		LT795544
<i>Nv mef2-II</i>		253		HQ634795.1

<i>Nv nkx2.5</i>	<i>NK2-tinman</i>	364		LT795545
<i>Nv nkx2.2a</i>	<i>NK2-VndA</i>	364		LT795582
<i>Nv nkx2.2b</i>	<i>NK2-VndB</i>	364		LT795583
<i>Nv nkx2.2c</i>	<i>NK2-VndC</i>	364		LT795584
<i>Nv nkx2.2d</i>	<i>NK2-VndD</i>	364		LT795585
<i>Nv nkx2.2e</i>	<i>NK2-VndE</i>	364		LT795586
<i>Nv tbx4/5</i>	<i>Nv_estExt_gwp.C_650150</i>	366		LT795541
<i>Nv evx</i>	<i>even-skipped</i>			LT795589
<i>Nv xlox/cdx</i>				LT795590

***N. vectensis* non-transcription factor genes**

Gene name	Alternative name(s)	Reference for gene orthology	GenBank number
<i>Nv trypsin A</i>		This paper (Ext. Data Figure 2)	LT795575
<i>Nv trypsin B</i>		This paper (Ext. Data Figure 2)	LT795550
<i>Nv trypsin C</i>		This paper (Ext. Data Figure 2)	LT795551
<i>Nv chitinase A</i>		This paper (Ext. Data Figure 2)	LT795547
<i>Nv chitinase B</i>		This paper (Ext. Data Figure 2)	LT795548
<i>Nv chitinase C</i>		This paper (Ext. Data Figure 2)	LT795549

<i>Nv pancreatic lipase 1</i>		This paper (Ext. Data Figure 2)	LT795567
<i>Nv pancreatic lipase 2</i>		This paper (Ext. Data Figure 2)	LT795568
<i>Nv lysosomal lipase 1</i>		This paper (Ext. Data Figure 2)	LT795562
<i>Nv lysosomal lipase 2</i>		This paper (Ext. Data Figure 2)	LT795563
<i>Nv lysosomal lipase 3</i>		This paper (Ext. Data Figure 2)	LT795564
<i>Nv insulin-like prepropeptide 1</i>	<i>ilp1</i>	This paper (Ext. Data Figure 4)	LT795557
<i>Nv insulin-like prepropeptide 2</i>	<i>ilp2</i>	This paper (Ext. Data Figure 4)	LT795558
<i>Nv insulin-like prepropeptide 3</i>	<i>ilp3</i>	This paper (Ext. Data Figure 4)	LT795559
<i>Nv Insulin receptor</i>	<i>insR</i>	This paper (Ext. Data Figure 4)	LT795560
<i>Nv mucin</i>		This paper (Ext. Data Figure 2)	LT795565
<i>Nv elav</i>	<i>NvELAV1</i>	³⁶⁹	LT795588

A. aurita

<i>Aa foxA</i>		¹¹¹	LN611630.1
<i>Aa trypsin</i>		This paper (Ext. Data Figure 2)	LT795580
<i>Aa chitinase</i>		This paper (Ext. Data Figure 2)	LT795577
<i>Aa pancreatic lipase</i>		This paper (Ext. Data Figure 2)	LT795579
<i>Aa insulin-like prepropeptide 1</i>	<i>Ilp1</i>	This paper (Ext. Data Figure 4)	LT795578

Supplementary Table 3

Gene accession numbers of genes used for phylogenetic analyses, and list of oligo sequences

Important Note: 'NVE' gene models do NOT refer to Uni.Gene models, but to models published in Fredman D. et al.:
<https://dx.doi.org/10.6084/m9.figshare.807696.v1>

Mucin

Species name	Gene name	Accession number	Gene model name	Scaffold coordinates
<i>Homo sapiens</i>	Mucin-5AC	NP_001291288		
<i>Homo sapiens</i>	Mucin-5B	NP_002449.2		
<i>Hydra magnipapillata</i>	Mucin	Hma2.225482		
<i>Drosophila melanogaster</i>	Hemolymph, isoform B	NP_001261809.1		
<i>Nematostella vectensis</i>	Mucin	NVE16870 & NVE16871 & NVE16872		

Chitinase

Species name	Gene name	Accession number	Gene model name	Scaffold coordinates
<i>Amphimedon queenslandica</i>	Chitinase1		Aqu1.229503	
<i>Branchiostoma floridae</i>	Chitinase1		fgenes2_pg.scaffold_94000128	
<i>Branchiostoma floridae</i>	Chitinase2		estExt_fgenes2_pg.C_800022	
<i>Branchiostoma floridae</i>	Chitinase3		fgenes2_pg.scaffold_23000018	
<i>Branchiostoma floridae</i>	Chitinase4		estExt_gwp.C_800027	
<i>Branchiostoma floridae</i>	Chitinase5		e_gw.80.73.1	
<i>Branchiostoma floridae</i>	Chitinase6		fgenes2_pg.scaffold_148000024	
<i>Branchiostoma floridae</i>	Chitinase7		fgenes2_pg.scaffold_119000091	
<i>Mus musculus</i>	Acidic Chitinase	AAG60018.1		

<i>Mus musculus</i>	Chitinase1	EDL39627.1		
<i>Mus musculus</i>	Chitinase-like 4	NP_660108.2		
<i>Mus musculus</i>	Chitinase-like 3	NP_034022.2		
<i>Nematostella vectensis</i>	ChitinaseC		estExt_GenewiseH_1.C_20251	scaffold_2:948712-950468
<i>Nematostella vectensis</i>	ChitinaseD		NVE18677	scaffold_49:39174-40490
<i>Nematostella vectensis</i>	ChitinaseA			scaffold_85:549244-550918
<i>Nematostella vectensis</i>	ChitinaseB			scaffold_2:922307-924202
<i>Aurelia aurita</i>	Chitinase		Sequence available upon request.	
<i>Acropora digitifera</i>	Chitinase1		aug_v2a.00687.t1	scaf293:1539-17805(-)
<i>Aiptasia pallida</i>	Chitinase1		AIPGENE15455	
<i>Aiptasia pallida</i>	Chitinase2		AIPGENE13251	
<i>Aiptasia pallida</i>	Chitinase3		AIPGENE23293	
<i>Aiptasia pallida</i>	Chitinase4		AIPGENE15503	
<i>Mnemiopsis leidyi</i>	Chitinase1		ML368913a	
<i>Mnemiopsis leidyi</i>	Chitinase2		ML07445a	
<i>Capsaspora owczarzaki</i>	Chitinase		CAOG_07823.4	Supercontig 16: 276880-281438 -
<i>Pleurotus ostreatus</i>	Chitinase	AFM30903.1		
<i>Candida albicans SC5314</i>	Chitinase	XP_719348.1		
<i>Polysphondylium pallidum PN500</i>	Chitinase	EFA81365.1		
<i>Schizophyllum commune H4-8</i>	Chitinase	XP_003038495.1		
<i>Acanthamoeba castellanii str. Neff</i>	Chitinase	XP_004345976.1		

Trypsin

Species name	Gene name	Accession number	Gene model name	Scaffold coordinates
<i>Homo sapiens</i>	Ovocymase-like	XP_011518941.1		
<i>Homo sapiens</i>	Trypsin-1	AAI28227.1		
<i>Homo sapiens</i>	Chymotrypsin-1	NP_001897.4		

<i>Homo sapiens</i>	Elastase	S70439		
<i>Homo sapiens</i>	Enteropeptidase	AAB37317.1		
<i>Nematostella vectensis</i>	TrypsinA		NVE4603	scaffold_149:423833-4331735
<i>Nematostella vectensis</i>	TrypsinB		scaffold_212.26 (GeneScan Model)	scaffold_212:345517-357008
<i>Nematostella vectensis</i>	TrypsinC		NVE23587	scaffold_77:595511-597869
<i>Nematostella vectensis</i>	TrypsinD		NVE26085	scaffold_98:347697-358091
<i>Nematostella vectensis</i>	TrypsinE		NVE26086	scaffold_98:358618-374311
<i>Nematostella vectensis</i>	TrypsinF		fgenes1_pg.scaffold_59000100	scaffold_59:1043846-1050902
<i>Nematostella vectensis</i>	TrypsinG		NVE16289	scaffold_4:77656-84822
<i>Nematostella vectensis</i>	TrypsinH		NVE20133	scaffold_55:481055-484207
<i>Nematostella vectensis</i>	TrypsinI		NVE15608	scaffold_373:66006-71643
<i>Nematostella vectensis</i>	Ovochymase-like		NVE1216	scaffold_11:180697-187994
<i>Nematostella vectensis</i>	TPRSS9-like		fgenes1_pg.scaffold_239000008	scaffold_239:117045-137009
<i>Aurelia aurita</i>	TrypsinA			CL311Contig4, comp107629_c7_seq3
<i>Aurelia aurita</i>	TrypsinB			CL1Contig7036

Pancreatic & lysosomal lipase

Species name	Gene name	Accession number	Gene model name	Scaffold coordinates
<i>Mus musculus</i>	Gastric triacylglycerol lipase (Gas.Lip.)		NP_080610.1	
<i>Mus musculus</i>	lysosomal acid lipase/cholesteryl ester hydrolase (Lys. Lip.)		NP_001104570.1	
<i>Nematostella vectensis</i>	lysosomal lipase 1		NVE7404	scaffold_189:217383-224328
<i>Nematostella vectensis</i>	lysosomal lipase 2		NVE22145	scaffold_68:569445-579437
<i>Nematostella vectensis</i>	lysosomal lipase 3		NVE24309	scaffold_81:684289-691080

<i>Nematostella vectensis</i>	lysosomal lipase 4		estExt_GenewiseH_1.C_1200028	scaffold_120:159376-173772
<i>Nematostella vectensis</i>	lysosomal lipase 5		fgenes1_pg.scaffold_339000003	scaffold_339:9333-15083
<i>Mus musculus</i>	hepatic triacylglycerol lipase (Hep. TAG Lip.)		NP_032306.2	
<i>Mus musculus</i>	pancreatic triacylglycerol lipase (Pan. Lip.)		NP_081201.2	
<i>Mus musculus</i>	pancreatic lipase-related protein 1 (Pan. Lip.-rel.1)		NP_061362.1	
<i>Mus musculus</i>	pancreatic lipase-related protein 2 (Pan. Lip.-rel.2)		NP_035258.2	
<i>Nematostella vectensis</i>	Pancreatic Lipase 1		NVE22447	scaffold_7:917415-930038
<i>Nematostella vectensis</i>	Pancreatic Lipase 2		NVE1082	scaffold_108:281416-288384
<i>Nematostella vectensis</i>	Pancreatic Lipase 3		NVE6324	scaffold_171:227079-237363
<i>Nematostella vectensis</i>	Pancreatic Lipase 4		fgenes1_pg.scaffold_32000021	scaffold_32:263826-272292
<i>Acropora digitifera</i>	Pancreatic Lipase 1		aug_v2a.09961.t1	
<i>Acropora digitifera</i>	Pancreatic Lipase 2		aug_v2a.09322.t1	
<i>Acropora digitifera</i>	Pancreatic Lipase 3		aug_v2a.12434.t1	
<i>Acropora digitifera</i>	Pancreatic Lipase 4		aug_v2a.00019.t1	
<i>Acropora digitifera</i>	Pancreatic Lipase 5		aug_v2a.00020.t1	
<i>Acropora digitifera</i>	Pancreatic Lipase 6		aug_v2a.13757.t1	
<i>Acropora millepora</i>	Pancreatic Lipase		ACF05269.1	
<i>Capsaspora owczarzaki</i>	Lysosomal Acid Lipase/Cholesteryl Ester Hydrolase (Lys. Lip.)		CAOG_04798.2	
<i>Capsaspora owczarzaki</i>	Lysosomal Lipase-like (Lip.-like)		CAOG_05206.2	
<i>Capsaspora owczarzaki</i>	Triacylglycerol Lipase (TAG Lip.)		CAOG_06974.2	
<i>Capsaspora owczarzaki</i>	Lipase, family N (Lip. N)		CAOG_06135.2 lipase	
<i>Mortierella verticillata</i>	Triacylglyceride lipase (TAG Lip.)		MVEG_01507.1	
<i>Spizellomyces punctatus</i>	Lipase 1		SPPG_01944.3	
<i>Mucor ambiguus</i>	Lysosomal Acid Lipase Cholesteryl Ester Hydrolase (Lys. Lip.)		GAN00734.1	

<i>Trichoderma gamsii</i>	Lysosomal Acid Lipase Cholesteryl Ester Hydrolase (Lys. Lip.)		KUE95802.1	
<i>Aurelia aurita</i>	Pancreatic Lipase		Sequence available upon request.	

Insulin-like peptides

Species name	Gene name	Accession number	Gene model name	Scaffold coordinates
<i>Mus musculus</i>	Insulin-1	ABF48502.1		
<i>Mus musculus</i>	Insulin-2	NP_001172013.1		
<i>Mus musculus</i>	Insulin-like growth factor	NP_001104745.1		
<i>Branchiostoma floridae</i>	Insulin-like peptide 1		estExt_fgenes2_pg.C_410021	scaffold_41:718784-735750
<i>Branchiostoma floridae</i>	Insulin-like peptide 2		fgenes2_pg.scaffold_73000034	scaffold_73:350818-355411
<i>Nematostella vectensis</i>	Insulin-like peptide 1		fgenes1_pg.scaffold_81000010	scaffold_81:111202-111979
<i>Nematostella vectensis</i>	Insulin-like peptide 2		fgenes1_pg.scaffold_14000031	scaffold_14:341281-344623
<i>Nematostella vectensis</i>	Insulin-like peptide 3		NVE3796	scaffold_14:339662-340816
<i>Nematostella vectensis</i>	Insulin-like peptide 4		fgenes1_pg.scaffold_11000068	scaffold_11:592691-609219
<i>Nematostella vectensis</i>	Insulin-like peptide 6		NVE1259	scaffold_11:604757-609261
<i>Nematostella vectensis</i>	Insulin-like peptide 7		NVE1257	scaffold_11:586257-592951
<i>Nematostella vectensis</i>	Insulin-like peptide 8		NVE2432	scaffold_123:358532-363296
<i>Nematostella vectensis</i>	Insulin-like peptide 9		NVE20730	scaffold_588:27016-31966
<i>Aurelia aurita</i>	Insulin-like peptide 1		Sequence available upon request.	
<i>Aurelia aurita</i>	Insulin-like peptide 2		Sequence available upon request.	
<i>Aurelia aurita</i>	Insulin-like peptide 3		Sequence available upon request.	

Insulin receptor

Species name	Gene name	Accession number	Gene model name	Scaffold coordinates
<i>Nematostella vectensis</i>	Fibroblast growth factor receptor A (FGFR-A)	ABO92763.1		
<i>Nematostella vectensis</i>	Fibroblast growth factor receptor B (FGFR-B)	ABO92762.1		
<i>Nematostella vectensis</i>	Insulin Receptor		NVE2224	scaffold_12:1639644-1656298
<i>Nematostella vectensis</i>	Receptor tyrosine kinase-like orphan receptor (ROR)		fgenes1_pg.scaffold_9000144	scaffold_9:1406531-1412439
<i>Mus musculus</i>	Insulin receptor	P15208.1		
<i>Mus musculus</i>	Insulin-like growth factor 1 receptor	P24062.2		
<i>Mus musculus</i>	Receptor tyrosine kinase-like orphan receptor 1 (ROR1)	Q9Z139.2		
<i>Mus musculus</i>	Receptor tyrosine kinase-like orphan receptor 2 (ROR2)	Q9Z138.2		
<i>Mus musculus</i>	Fibroblast growth factor receptor 1	NP_034336.2		
<i>Mus musculus</i>	Fibroblast growth factor receptor 2	NP_034337.2		
<i>Mus musculus</i>	Fibroblast growth factor receptor 3	NP_001156689.1		
<i>Mus musculus</i>	Fibroblast growth factor receptor 4	NP_032037.2		
<i>Drosophila melanogaster</i>	Insulin receptor	AAC47458.1		
<i>Drosophila melanogaster</i>	Receptor tyrosine kinase-like orphan receptor 1 (ROR1)	AAF52885.1		
<i>Drosophila melanogaster</i>	Receptor tyrosine kinase-like orphan receptor 2 (ROR2)	Q9V6K3.2		
<i>Drosophila melanogaster</i>	FGF receptor breathless (Btl)	AAX52746.1		
<i>Drosophila melanogaster</i>	FGF receptor heartless (Htl)	NP_732287.1		

**Sequences of oligos
used for cloning**

<i>Nematostella vectensis</i> genes	Forward primer	Reverse primer	Comment
<i>chitinaseA</i>	GCCGTTCTAGGCTGCGCCCTGTC	CAGTGCTCACCGCTAAAGTCGTCC	
<i>chitinaseB</i>	ATGCGCTCCCTTGCGTTCTTG	GTAACCGTAGGGAGCCTTAGCTGG	
<i>chitinaseC</i>	TCCCAGTATCGTCAGGGCAGG	GAGCCATCGAAGTCATCCAGGTCC	
<i>dachshund</i>	TGTA CTGCCTCCTCCTCACC	GAGAGGTTCCGCTCATTTCTGTC	
<i>eyes absent</i>	AGCCCCAATGACAAGGATAGCA	TTCAAGCCCCAAAGGCAAACATC	
<i>hand1</i>	- (EST SEQUENCE)	- (EST SEQUENCE)	
<i>hand2</i>	CTGACTTGATCAGCGCTGCTGAAT	GGGTCGATTGTTAACATTTATTA ACTTCT	OligodT worked as reverse primer
<i>hhex</i>	GAGAACATCAAAGACACAGCTAC	GAATAAGAACCATTTAATCCTGACC	
<i>hlxB9</i>	AACAAACATGCAGGCCACAGCG	CTTCGTCGTCTGTGTCGTCTGTGC	
<i>hlxB9</i>	AACAAACATGCAGGCCACAGCG	oligodT	3'RACE fragment
<i>hnf1</i>	CAACCGAGCTACAGAGAGAGC	GCCACGCACATATAGTAGCCAC	
<i>hnf1</i>	CCTCAAGCCTACGTCAGCACACC	oligodT	3'RACE fragment
<i>hnf4</i>	ATGACCGCCAACTTAGTGAATG	GAATGTCCATATCAGGGCTTGC	
<i>hnf4</i>	ATGACCGCCAACTTAGTGAATG	GAATGTCCATATCAGGGCTTGC	3'RACE fragment
<i>ilp1</i>	TTCGGCTTATTTGATGTCTGTGGC	TACGTTGATAAAGATGACGACGACG	
<i>ilp2</i>	ATAGTTTATTACTGTCCCTCTGTTATC	TAAGATACTAGAAGTTTATAGATAGCC	
<i>ilp3</i>	GATTAGCTTTCTTATTGCTTGGC	TTGATACGTTTAATAGATTTAGATTCAGC	OligodT worked as reverse primer
<i>insulin receptor</i>	GTTGGTACAGAGCTCTCGTGTTGG	CTGATTGGACAGTCTCTGCTCGC	
<i>islet</i>	AAGCGGCGTGTGTCCATGTG	CCGTCGATACTATCCCATCAGAGGAG	
<i>lbx</i>	- (EST SEQUENCE)	- (EST SEQUENCE)	
<i>lysosomal lipase1</i>	TGGAACACTGGCACGAGCTTCC	CTTGGACTTGATGTGCCTGATGACG	
<i>lysosomal lipase2</i>	TGATGATGTTTGCCGAATCATCCTGC	CCAATACAAATCATCACAGCTTCAATCAG	
<i>lysosomal lipase3</i>	GACGTGTGGCTTGGAACATCCG	CGTTTCCATCGATCGGCTGTGG	OligodT worked as reverse primer
<i>moxC</i>	CGCTCAAACATGTACGACTTGTACT	GCGTTACAATGCATAGAAATAATATCCA	
<i>mucin</i>	GACTTCAAGGCTCAGTGTATCGC	ACTCGTACTCTGGCTGGGC	
<i>nkx2.2A</i>	CGTGTGATAGTATCAAAGCC	GTCACACATCTCTTTACAAAGC	
<i>nkx2.2B</i>	GAGTCTGTTGGACCGTCTTGC	CGAATTAGAGACTTTGCTCGG	
<i>nkx2.2C</i>	GCAGAAATGGAAC TACTTAGAGGGC	TCCCTTATTCGTTTGACAGTCGC	

<i>nkx2.2D</i>	GCAGACGAGCCATGACTTCG	AACCCTACCAAGTCCAATACGGG	
<i>nkx2.2E</i>	CAATCGGTTTACACTGAGCAC	CTTACAGGGATCTTTCAATGC	
<i>nkx2.5</i>	ATCCCTAGAGTTACTTATCTAGTGGTGT	AAATACCTTTTATTAGACTGTCTAACA	
<i>nkx3.2</i>	- (EST SEQUENCE)	- (EST SEQUENCE)	
<i>nkx6</i>	TGTAAGCAACGAGCTGCGAGCC	GTGGGCTCAGGACTTGAGGAATCG	
<i>pancreatic lipase1</i>	CAGGTCTGTTATGGAAGTACGGC	GCTCTTCACAGCGTTGTAGCC	
<i>pancreatic lipase2</i>	GTCTTGCAATCGGCAGGTCAAGG	GACAGGGATACCATTATGTCGCACG	
<i>paraxis</i>	- (EST SEQUENCE)	- (EST SEQUENCE)	
<i>ptf</i>	AACGTGTTAACCCTGTCTGGTCTTG	CAGTGTTTATAATGGCGTGTGTGG	
<i>six1/2</i>	TTCCCTCCTTCAGTTTCACTCCA	AACTCTCCCTGCTGAGGTCCA	
<i>six1/2-2</i>	GCCTCACACATGCCCTTCTCG	ATCGCTTAGAGCTCACACTTTCCC	
<i>six4/5</i>	CGGACTTGATTCACTAAGGGC	AGTGTAGGTACGACTCCTCAGG	
<i>soxB1</i>	GACCCTCAAACAGACGCTGTGC	CGTATGCTAGACTACTTCGGCAGTCG	
<i>tbx1/10.1</i>	GGGGAGATTTAGTTTACGCTCATG	GTCTTCGCAGCGTGTATCC	
<i>tbx1/10.2</i>	TCTGCGCGATTTAATGGACCCTGC	CGAAGCCACAGGCGAGGTAGG	
<i>tbx15/18/22</i>	ACAGCGGTGCAGGCCACATAA	CATGTCAGGGTACATAAGCTGTGGC	
<i>tbx2/3</i>	- (EST SEQUENCE)	- (EST SEQUENCE)	
<i>tbx20.1</i>	AGCATTGATTTACAGAACATCTCGGT	CCTTTCTACGGGCATCAAGC	
<i>tbx20.3</i>	AGCGAGCTGACACGGCCTGA	GTTGTATCCCTGAAGCCCTTAGCG	
<i>tbx4/5</i>	AATCGATGTGATAAATCCATACACGC	CACCGTTTTAGACCGAGGTGGTGA	
<i>trypsinA</i>	TCTTGCTCCATCCCGAGGTTTGC	CAACTTCGACACCGACATGTGTGG	
<i>trypsinB</i>	TTTCTACCACATGCAAGGTGCC	ACCTGGGCGGCTCTTATCC	
<i>trypsinC</i>	TTCTAAGTTCACTGGTTGCTGAAGCG	CTACCGTGTGGCCATCTGTG	

Aurelia aurita genes

<i>chitinase</i>	CGATTCTTCTACTCGGCCTGGC	TGGTGTTACATCCCTCCCTAAAGC
<i>ilp1</i>	CCTAACTAAAGGTCACCATGCCTCG	CTTATATGAACCCACACACTGGCAG
<i>pancreatic lipase</i>	GGTGTTGCTTGCATATTTTCAGAGG	ATGGTGCTACCCAGCATCG
<i>trypsin</i>	ATCCAGTGATGAAGCATTGCGTTCC	GCAGAGTTCCTCCAACATTGACAGC

Supplementary References

- 71 Biemar, F. *et al.* Pancreas development in zebrafish: early dispersed appearance of endocrine hormone expressing cells and their convergence to form the definitive islet. *Developmental biology* **230**, 189-203 (2001).
- 72 Gittes, G. K. Developmental biology of the pancreas: a comprehensive review. *Dev Biol* **326**, 4-35, doi:10.1016/j.ydbio.2008.10.024 (2009).
- 73 Zorn, A. M. & Wells, J. M. Vertebrate endoderm development and organ formation. *Annu Rev Cell Dev Biol* **25**, 221-251, doi:10.1146/annurev.cellbio.042308.113344 (2009).
- 74 Zorn, A. M., Butler, K. & Gurdon, J. B. Anterior Endomesoderm Specification in *Xenopus* by Wnt/ β -catenin and TGF- β Signalling Pathways. *Developmental biology* **209**, 282-297 (1999).
- 75 Newman, C. S., Chia, F. & Krieg, P. A. The *XHex* homeobox gene is expressed during development of the vascular endothelium: overexpression leads to an increase in vascular endothelial cell number. *Mechanisms of development* **66**, 83-93 (1997).
- 76 Thomas, P., Brown, A. & Beddington, R. Hex: a homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors. *Development* **125**, 85-94 (1998).
- 77 Yatskievych, T. A., Pascoe, S. & Antin, P. B. Expression of the homeobox gene Hex during early stages of chick embryo development. *Mechanisms of development* **80**, 107-109 (1999).
- 78 Ho, C.-Y., Houart, C., Wilson, S. W. & Stainier, D. Y. A role for the extraembryonic yolk syncytial layer in patterning the zebrafish embryo suggested by properties of the hex gene. *Current biology* **9**, 1131-S1134 (1999).
- 79 Sun, Z. & Hopkins, N. *vhnf1*, the MODY5 and familial GCKD-associated gene, regulates regional specification of the zebrafish gut, pronephros, and hindbrain. *Genes & development* **15**, 3217-3229 (2001).
- 80 McCauley, B. S., Weideman, E. P. & Hinman, V. F. A conserved gene regulatory network subcircuit drives different developmental fates in the vegetal pole of highly divergent echinoderm embryos. *Developmental biology* **340**, 200-208 (2010).
- 81 Howard-Ashby, M. *et al.* Identification and characterization of homeobox transcription factor genes in *Strongylocentrotus purpuratus*, and their expression in embryonic development. *Developmental biology* **300**, 74-89 (2006).
- 82 Yu, J.-K. *et al.* Axial patterning in cephalochordates and the evolution of the organizer. *Nature* **445**, 613-617 (2007).
- 83 Lowe, C. J. *et al.* Dorsoventral patterning in hemichordates: insights into early chordate evolution. (2006).

- 84 Tomancak, P. *et al.* Systematic determination of patterns of gene expression during *Drosophila* embryogenesis. *Genome Biol* **3**, 0081-0088 (2002).
- 85 Tomancak, P. *et al.* Global analysis of patterns of gene expression during *Drosophila* embryogenesis. *Genome biology* **8**, R145 (2007).
- 86 Mörck, C., Rauthan, M., Wågberg, F. & Pilon, M. pha-2 encodes the *C. elegans* ortholog of the homeodomain protein HEX and is required for the formation of the pharyngeal isthmus. *Developmental biology* **272**, 403-418 (2004).
- 87 Perry, K. J. *et al.* Deployment of regulatory genes during gastrulation and germ layer specification in a model spiralian mollusc *Crepidula*. *Developmental Dynamics* (2015).
- 88 Fortunato, S. A. *et al.* Calcisponges have a ParaHox gene and dynamic expression of dispersed NK homeobox genes. *Nature* **514**, 620-623 (2014).
- 89 Sasaki, H. & Hogan, B. L. M. Differential Expression of Multiple Fork Head Related Genes During Gastrulation and Axial Pattern Formation in the Mouse Embryo. *Development* **118**, 47-59 (1993).
- 90 Shimeld, S. M. Characterisation of amphioxus HNF-3 genes: conserved expression in the notochord and floor plate. *Developmental biology* **183**, 74-85 (1997).
- 91 Terazawa, K. & Satoh, N. Formation of the chordamesoderm in the amphioxus embryo: Analysis with Brachyury and fork head/HNF-3 genes. *Dev Genes Evol* **207**, 1-11, doi:10.1007/s004270050086 (1997).
- 92 Oliveri, P., Walton, K. D., Davidson, E. H. & McClay, D. R. Repression of mesodermal fate by foxa, a key endoderm regulator of the sea urchin embryo. *Development* **133**, 4173-4181 (2006).
- 93 Taguchi, S. *et al.* Characterization of a hemichordate fork head/HNF-3 gene expression. *Dev Genes Evol* **210**, 11-17 (2000).
- 94 Pani, A. M. *et al.* Ancient deuterostome origins of vertebrate brain signalling centres. *Nature* **483**, 289-294 (2012).
- 95 Fritzenwanker, J. H., Gerhart, J., Freeman Jr, R. M. & Lowe, C. J. The Fox/Forkhead transcription factor family of the hemichordate *Saccoglossus kowalevskii*. *EvoDevo* **5**, 1-26 (2014).
- 96 Miguel-Aliaga, I., Thor, S. & Gould, A. P. Postmitotic specification of *Drosophila* insulinergic neurons from pioneer neurons. *PLoS Biol* **6**, e58 (2008).
- 97 Weigel, D., Bellen, H. J., Jürgens, G. & Jäckle, H. Primordium specific requirement of the homeotic gene fork head in the developing gut of the *Drosophila* embryo. *Roux's archives of developmental biology* **198**, 201-210 (1989).
- 98 Boyle, M. J. & Seaver, E. C. Developmental expression of foxA and gata genes during gut formation in the polychaete annelid, *Capitella* sp. I. *Evolution & development* **10**, 89-105 (2008).
- 99 Arenas-Mena, C. Embryonic expression of HeFoxA1 and HeFoxA2 in an indirectly developing polychaete. *Development genes and evolution* **216**, 727-736 (2006).

- 100 Boyle, M. J. & Seaver, E. C. Expression of FoxA and GATA transcription factors correlates with regionalized gut development in two lophotrochozoan marine worms: Chaetopterus (Annelida) and Themiste lageniformis (Sipuncula). *EvoDevo* **1**, 2 (2010).
- 101 Lartillot, N., Le Gouar, M. & Adoutte, A. Expression patterns of fork head and goosecoid homologues in the mollusc Patella vulgata supports the ancestry of the anterior mesendoderm across Bilateria. *Dev Genes Evol* **212**, 551-561. (2002).
- 102 Martin-Duran, J. M., Janssen, R., Wennberg, S., Budd, G. E. & Hejnol, A. Deuterostomic development in the protostome Priapulus caudatus. *Curr Biol* **22**, 2161-2166, doi:10.1016/j.cub.2012.09.037 (2012).
- 103 Hejnol, A. & Martindale, M. Q. Acoel development indicates the independent evolution of the bilaterian mouth and anus. *Nature* **456**, 382-386 (2008).
- 104 Chiodin, M. *et al.* Mesodermal gene expression in the acoel Isodiametra pulchra indicates a low number of mesodermal cell types and the endomesodermal origin of the gonads. *PLoS One* **8**, e55499, doi:10.1371/journal.pone.0055499 (2013).
- 105 Fritzenwanker, J. H., Saina, M. & Technau, U. Analysis of *forkhead* and *snail* expression reveals epithelial-mesenchymal transitions during embryonic and larval development of *Nematostella vectensis*. *Dev Biol* **275**, 389-402 (2004).
- 106 Martindale, M. Q., Pang, K. & Finnerty, J. R. Investigating the origins of triploblasty: 'mesodermal' gene expression in a diploblastic animal, the sea anemone *Nematostella vectensis* (Phylum Cnidaria; Class Anthozoa). *Development* **131**, 2463-2474 (2004).
- 107 Martinez, D. E. *et al.* Budhead, a fork head/HNF-3 homologue, is expressed during axis formation and head specification in hydra. *Developmental biology* **192**, 523-536 (1997).
- 108 Bucher, M., Wolfowicz, I., Voss, P. A., Hambleton, E. A. & Guse, A. Development and Symbiosis Establishment in the Cnidarian Endosymbiosis Model Aiptasia sp. *Sci Rep* **6**, 19867, doi:10.1038/srep19867 (2016).
- 109 Hayward, D. C., Grasso, L. C., Saint, R., Miller, D. J. & Ball, E. E. The organizer in evolution-gastrulation and organizer gene expression highlight the importance of Brachyury during development of the coral, Acropora millepora. *Dev Biol* **399**, 337-347, doi:10.1016/j.ydbio.2015.01.006 (2015).
- 110 Okubo, N., Hayward, D. C., Foret, S. & Ball, E. E. A comparative view of early development in the corals Favia lizardensis, Ctenactis echinata, and Acropora millepora - morphology, transcriptome, and developmental gene expression. *BMC Evol Biol* **16**, 48, doi:10.1186/s12862-016-0615-2 (2016).
- 111 Kraus, J. E., Fredman, D., Wang, W., Khalturin, K. & Technau, U. Adoption of conserved developmental genes in development and origin of the medusa body plan. *Evodevo* **6**, 23, doi:10.1186/s13227-015-0017-3 (2015).

- 112 Wood, H. B. & Episkopou, V. Comparative expression of the mouse Sox1, Sox2 and Sox3 genes from pre-gastrulation to early somite stages. *Mechanisms of development* **86**, 197-201 (1999).
- 113 Okuda, Y. *et al.* Comparative genomic and expression analysis of group B1 sox genes in zebrafish indicates their diversification during vertebrate evolution. *Developmental dynamics* **235**, 811-825 (2006).
- 114 Cattell, M. V., Garnett, A. T., Klymkowsky, M. W. & Medeiros, D. M. A maternally established SoxB1/SoxF axis is a conserved feature of chordate germ layer patterning. *Evolution & development* **14**, 104-115 (2012).
- 115 Cunningham, D. & Casey, E. S. Spatiotemporal development of the embryonic nervous system of *Saccoglossus kowalevskii*. *Developmental biology* **386**, 252-263 (2014).
- 116 Wei, Z., Angerer, R. C. & Angerer, L. M. Direct development of neurons within foregut endoderm of sea urchin embryos. *Proceedings of the National Academy of Sciences* **108**, 9143-9147 (2011).
- 117 Crémazy, F., Berta, P. & Girard, F. Sox neuro, a new *Drosophila* Sox gene expressed in the developing central nervous system. *Mechanisms of development* **93**, 215-219 (2000).
- 118 Hejnal, A. & Martindale, M. Q. Coordinated spatial and temporal expression of Hox genes during embryogenesis in the acoel *Convolutriloba longifissura*. *Bmc Biology* **7**, 65 (2009).
- 119 Magie, C. R., Pang, K. & Martindale, M. Q. Genomic inventory and expression of Sox and Fox genes in the cnidarian *Nematostella vectensis*. *Dev Genes Evol* **215**, 618-630 (2005).
- 120 Kanai-Azuma, M. *et al.* Depletion of definitive gut endoderm in Sox17-null mutant mice. *Development* **129**, 2367-2379 (2002).
- 121 Alexander, J. & Stainier, D. Y. A molecular pathway leading to endoderm formation in zebrafish. *Current biology* **9**, 1147-1157 (1999).
- 122 Field, H. A., Ober, E. A., Roeser, T. & Stainier, D. Y. Formation of the digestive system in zebrafish. I. Liver morphogenesis. *Developmental biology* **253**, 279-290 (2003).
- 123 Hudson, C., Clements, D., Friday, R. V., Stott, D. & Woodland, H. R. Xsox17alpha and -beta mediate endoderm formation in *Xenopus*. *Cell* **91**, 397-405 (1997).
- 124 Poustka, A. J. *et al.* A global view of gene expression in lithium and zinc treated sea urchin embryos: new components of gene regulatory networks. *Genome biology* **8**, R85 (2007).
- 125 Crémazy, F., Berta, P. & Girard, F. Genome-wide analysis of Sox genes in *Drosophila melanogaster*. *Mechanisms of development* **109**, 371-375 (2001).
- 126 Wilson, M. J. & Dearden, P. K. Evolution of the insect Sox genes. *BMC evolutionary biology* **8**, 120 (2008).
- 127 Shinzato, C. *et al.* Sox genes in the coral *Acropora millepora*: divergent expression patterns reflect differences in developmental mechanisms within the Anthozoa. *BMC evolutionary biology* **8**, 311 (2008).

- 128 Jager, M., Quéinnec, E., Le Guyader, H. & Manuel, M. Multiple Sox genes are expressed in stem cells or in differentiating neuro-sensory cells in the hydrozoan *Clytia hemisphaerica*. *EvoDevo* **2**, 12 (2011).
- 129 Jager, M., Quéinnec, E., Chiori, R., Le Guyader, H. & Manuel, M. Insights into the early evolution of SOX genes from expression analyses in a ctenophore. *J Exp Zool B Mol Dev Evol* **310**, 650-667 (2008).
- 130 Kudoh, T. *et al.* A gene expression screen in zebrafish embryogenesis. *Genome research* **11**, 1979-1987 (2001).
- 131 Holewa, B., Strandmann, E. P. v., Zapp, D., Lorenz, P. & Ryffel, G. U. Transcriptional hierarchy in *Xenopus* embryogenesis: HNF4 a maternal factor involved in the developmental activation of the gene encoding the tissue specific transcription factor HNF1 α (LFB1). *Mechanisms of development* **54**, 45-57 (1996).
- 132 Duncan, S. A. *et al.* Expression of transcription factor HNF-4 in the extraembryonic endoderm, gut, and nephrogenic tissue of the developing mouse embryo: HNF-4 is a marker for primary endoderm in the implanting blastocyst. *Proceedings of the National Academy of Sciences USA* **91**, 7598-7602 (1994).
- 133 Chen, W. S. *et al.* Disruption of the HNF-4 gene, expressed in visceral endoderm, leads to cell death in embryonic ectoderm and impaired gastrulation of mouse embryos. *Genes & development* **8**, 2466-2477 (1994).
- 134 Zhong, W., Sladek, F. M. & Darnell Jr, J. The expression pattern of a *Drosophila* homolog to the mouse transcription factor HNF-4 suggests a determinative role in gut formation. *The EMBO Journal* **12**, 537 (1993).
- 135 Martin-Duran, J. M. & Hejnol, A. The study of *Priapulus caudatus* reveals conserved molecular patterning underlying different gut morphogenesis in the Ecdysozoa. *BMC Biol* **13**, 29, doi:10.1186/s12915-015-0139-z (2015).
- 136 Reitzel, A. M. *et al.* Nuclear receptors from the ctenophore *Mnemiopsis leidyi* lack a zinc-finger DNA-binding domain: lineage-specific loss or ancestral condition in the emergence of the nuclear receptor superfamily? *EvoDevo* **2**, 1 (2011).
- 137 Bridgham, J. T. *et al.* Protein evolution by molecular tinkering: diversification of the nuclear receptor superfamily from a ligand-dependent ancestor. *PLoS Biol* **8**, e1000497 (2010).
- 138 Korzh, V., Edlund, T. & Thor, S. Zebrafish primary neurons initiate expression of the LIM homeodomain protein *Isl-1* at the end of gastrulation. *Development* **118**, 417-425 (1993).
- 139 Prall, O. W. *et al.* An *Nkx2-5/Bmp2/Smad1* negative feedback loop controls heart progenitor specification and proliferation. *Cell* **128**, 947-959 (2007).
- 140 Ericson, J., Thor, S., Edlund, T., Jessell, T. M. & Yamada, T. Early stages of motor neuron differentiation revealed by expression of homeobox gene *Isl-1*. *Science* **256**, 1555-1560 (1992).

- 141 Kim, S. K., Hebrok, M. & Melton, D. A. Notochord to endoderm
signaling is required for pancreas development. *Development* **124**,
4243-4252 (1997).
- 142 Yuan, S. & Schoenwolf, G. C. Islet - 1 marks the early heart rudiments
and is asymmetrically expressed during early rotation of the foregut in
the chick embryo. *The Anatomical Record* **260**, 204-207 (2000).
- 143 Thor, S., Ericson, J., Brännström, T. & Edlund, T. The homeodomain
LIM protein Isl-1 is expressed in subsets of neurons and endocrine
cells in the adult rat. *Neuron* **7**, 881-889 (1991).
- 144 Cai, C.-L. *et al.* Isl1 identifies a cardiac progenitor population that
proliferates prior to differentiation and contributes a majority of cells to
the heart. *Developmental cell* **5**, 877-889 (2003).
- 145 Annunziata, R. *et al.* Pattern and process during sea urchin gut
morphogenesis: the regulatory landscape. *genesis* **52**, 251-268 (2014).
- 146 Jackman, W. R., Langeland, J. A. & Kimmel, C. B. islet reveals
segmentation in the Amphioxus hindbrain homolog. *Developmental
biology* **220**, 16-26 (2000).
- 147 Pascual-Anaya, J. *et al.* The evolutionary origins of chordate
hematopoiesis and vertebrate endothelia. *Dev Biol* **375**, 182-192,
doi:10.1016/j.ydbio.2012.11.015 (2013).
- 148 Thor, S. & Thomas, J. B. The Drosophila islet gene governs axon
pathfinding and neurotransmitter identity. *Neuron* **18**, 397-409 (1997).
- 149 Srivastava, M. *et al.* Early evolution of the LIM homeobox gene family.
BMC biology **8**, 4 (2010).
- 150 Simmons, D. K., Pang, K. & Martindale, M. Q. Lim homeobox genes in
the Ctenophore Mnemiopsis leidyi: the evolution of neural cell type
specification. *EvoDevo* **3**, 2-2 (2012).
- 151 Sussel, L. *et al.* Mice lacking the homeodomain transcription factor
Nkx2. 2 have diabetes due to arrested differentiation of pancreatic beta
cells. *Development* **125**, 2213-2221 (1998).
- 152 Barth, K. A. & Wilson, S. W. Expression of zebrafish nk2.2 is influenced
by sonic hedgehog/vertebrate hedgehog-1 and demarcates a zone of
neuronal differentiation in the embryonic forebrain. *Development* **121**,
1755-1768 (1995).
- 153 Holland, L. Z., Venkatesh, T. V., Gorlin, A., Bodmer, R. & Holland, N.
Characterization and developmental expression of AmphiNk2-2, an
NK2 class homeobox gene from amphioxus (Phylum Chordata;
Subphylum Cephalochordata). *Development genes and evolution* **208**,
100-105 (1998).
- 154 Saudemont, A. *et al.* Ancestral regulatory circuits governing ectoderm
patterning downstream of Nodal and BMP2/4 revealed by gene
regulatory network analysis in an echinoderm. *PLoS Genet* **6**,
e1001259-e1001259 (2010).
- 155 McDonald, J. A. *et al.* Dorsoventral patterning in the Drosophila central
nervous system: the vnd homeobox gene specifies ventral column
identity. *Genes & development* **12**, 3603-3612 (1998).

- 156 Denes, A. S. *et al.* Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in Bilateria. *Cell* **129**, 277-288 (2007).
- 157 Matthews, R. P., Lorent, K., Russo, P. & Pack, M. The zebrafish onecut gene *hnf-6* functions in an evolutionarily conserved genetic pathway that regulates vertebrate biliary development. *Developmental biology* **274**, 245-259 (2004).
- 158 Landry, C. *et al.* HNF-6 is expressed in endoderm derivatives and nervous system of the mouse embryo and participates to the cross-regulatory network of liver-enriched transcription factors. *Developmental biology* **192**, 247-257 (1997).
- 159 Francius, C. & Clotman, F. Dynamic expression of the Onecut transcription factors HNF-6, OC-2 and OC-3 during spinal motor neuron development. *Neuroscience* **165**, 116-129 (2010).
- 160 Otim, O., Amore, G., Minokawa, T., McClay, D. R. & Davidson, E. H. SpHnf6, a transcription factor that executes multiple functions in sea urchin embryogenesis. *Developmental biology* **273**, 226-243 (2004).
- 161 Otim, O., Hinman, V. F. & Davidson, E. H. Expression of AmHNF6, a sea star orthologue of a transcription factor with multiple distinct roles in sea urchin development. *Gene expression patterns* **5**, 381-386 (2005).
- 162 Sasakura, Y. & Makabe, K. W. A gene encoding a new ONECUT class homeodomain protein in the ascidian *Halocynthia roretzi* functions in the differentiation and specification of neural cells in ascidian embryogenesis. *Mechanisms of development* **104**, 37-48 (2001).
- 163 Nguyen, D. N., Rohrbaugh, M. & Lai, Z.-C. The *Drosophila* homolog of Onecut homeodomain proteins is a neural-specific transcriptional activator with a potential role in regulating neural differentiation. *Mechanisms of development* **97**, 57-72 (2000).
- 164 Marlow, H. *et al.* Larval body patterning and apical organs are conserved in animal evolution. *BMC biology* **12**, 7 (2014).
- 165 Dunn, E. F. *et al.* Molecular paleoecology: using gene regulatory analysis to address the origins of complex life cycles in the late Precambrian. *Evolution & development* **9**, 10-24 (2007).
- 166 Li, H., Arber, S., Jessell, T. M. & Edlund, H. Selective agenesis of the dorsal pancreas in mice lacking homeobox gene *Hlxb9*. *Nature genetics* **23**, 67-70 (1999).
- 167 Wendik, B., Maier, E. & Meyer, D. Zebrafish *mnx* genes in endocrine and exocrine pancreas formation. *Developmental biology* **268**, 372-383 (2004).
- 168 Ferrier, D., Brooke, N. M., Panopoulou, G. & Holland, P. The *Mnx* homeobox gene class defined by *HB9*, *MNR2* and *amphioxus* *AmphiMnx*. *Development genes and evolution* **211**, 103-107 (2001).
- 169 Di Bernardo, M. *et al.* Homeobox genes and sea urchin development. *International journal of developmental biology* **44**, 637-644 (2000).
- 170 Broihier, H. T. & Skeath, J. B. *Drosophila* homeodomain protein dHb9 directs neuronal fate via crossrepressive and cell-nonautonomous mechanisms. *Neuron* **35**, 39-50 (2002).

- 171 Odden, J. P., Holbrook, S. & Doe, C. Q. Drosophila HB9 is expressed in a subset of motoneurons and interneurons, where it regulates gene expression and axon pathfinding. *The Journal of neuroscience* **22**, 9143-9149 (2002).
- 172 Ryan, J. F. *et al.* Pre-bilaterian origins of the Hox cluster and the Hox code: evidence from the sea anemone, *Nematostella vectensis*. *PLoS ONE* **2**, e153 (2007).
- 173 Cheesman, S. E., Layden, M. J., Von Ohlen, T., Doe, C. Q. & Eisen, J. S. Zebrafish and fly Nkx6 proteins have similar CNS expression patterns and regulate motoneuron formation. *Development* **131**, 5221-5232 (2004).
- 174 Sander, M. *et al.* Ventral neural patterning by Nkx homeobox genes: Nkx6. 1 controls somatic motor neuron and ventral interneuron fates. *Genes & development* **14**, 2134-2139 (2000).
- 175 Nelson, S. B., Janiesch, C. & Sander, M. Expression of Nkx6 genes in the hindbrain and gut of the developing mouse. *Journal of Histochemistry & Cytochemistry* **53**, 787-790 (2005).
- 176 Sander, M. *et al.* Homeobox gene Nkx6. 1 lies downstream of Nkx2. 2 in the major pathway of beta-cell formation in the pancreas. *Development* **127**, 5533-5540 (2000).
- 177 Uhler, J., Garbern, J., Yang, L., Kamholz, J. & Mellerick, D. M. Nk6, a novel Drosophila homeobox gene regulated by vnd. *Mechanisms of development* **116**, 105-116 (2002).
- 178 Krapp, A. *et al.* The p48 DNA-binding subunit of transcription factor PTF1 is a new exocrine pancreas-specific basic helix-loop-helix protein. *EMBO J* **15**, 4317-4329 (1996).
- 179 Lin, J. W. *et al.* Differential requirement for ptf1a in endocrine and exocrine lineages of developing zebrafish pancreas. *Developmental biology* **274**, 491-503 (2004).
- 180 Begum, S. & Papaioannou, V. E. Dynamic expression of Tbx2 and Tbx3 in developing mouse pancreas. *Gene Expr Patterns* **11**, 476-483, doi:10.1016/j.gep.2011.08.003 (2011).
- 181 Chapman, D. L. *et al.* Expression of the T-box family genes, Tbx1-Tbx5, during early mouse development. *Developmental Dynamics* **206**, 379-390 (1996).
- 182 Yamada, M., Revelli, J. P., Eichele, G., Barron, M. & Schwartz, R. J. Expression of chick Tbx-2, Tbx-3, and Tbx-5 genes during early heart development: evidence for BMP2 induction of Tbx2. *Dev Biol* **228**, 95-105, doi:10.1006/dbio.2000.9927 (2000).
- 183 Gibson-Brown, J. J., S, I. A., Silver, L. M. & Papaioannou, V. E. Expression of T-box genes Tbx2-Tbx5 during chick organogenesis. *Mech Dev* **74**, 165-169 (1998).
- 184 Ruvinsky, I., Oates, A. C., Silver, L. M. & Ho, R. K. The evolution of paired appendages in vertebrates: T-box genes in the zebrafish. *Dev Genes Evol* **210**, 82-91 (2000).
- 185 Horton, A. C. *et al.* Conservation of linkage and evolution of developmental function within the Tbx2/3/4/5 subfamily of T-box genes:

- implications for the origin of vertebrate limbs. *Dev Genes Evol* **218**, 613-628, doi:10.1007/s00427-008-0249-5 (2008).
- 186 Gross, J. M., Peterson, R. E., Wu, S. Y. & McClay, D. R. LvTbx2/3: a T-box family transcription factor involved in formation of the oral/aboral axis of the sea urchin embryo. *Development* **130**, 1989-1999 (2003).
- 187 Croce, J., Lhomond, G. & Gache, C. Coquillotte, a sea urchin T-box gene of the Tbx2 subfamily, is expressed asymmetrically along the oral-aboral axis of the embryo and is involved in skeletogenesis. *Mech Dev* **120**, 561-572 (2003).
- 188 Poeck, B., Hofbauer, A. & Pflugfelder, G. O. Expression of the *Drosophila* optomotor-blind gene transcript in neuronal and glial cells of the developing nervous system. *Development* **117**, 1017-1029. (1993).
- 189 Arenas-Mena, C. Brachyury, Tbx2/3 and sall expression during embryogenesis of the indirectly developing polychaete *Hydroides elegans*. *Int J Dev Biol* **57**, 73-83, doi:10.1387/ijdb.120056ca (2013).
- 190 Milewski, W., Duguay, S., Chan, S. & Steiner, D. Conservation of PDX-1 Structure, Function, and Expression in Zebrafish 1. *Endocrinology* **139**, 1440-1449 (1998).
- 191 Maures, T. *et al.* Structural, biochemical, and expression analysis of two distinct insulin-like growth factor I receptors and their ligands in zebrafish*. *Endocrinology* **143**, 1858-1871 (2002).
- 192 Kajimoto, Y. & Rotwein, P. Evolution of insulin-like growth factor I (IGF-I): structure and expression of an IGF-I precursor from *Xenopus laevis*. *Molecular Endocrinology* **4**, 217-226 (1990).
- 193 Richard-Parpaillon, L., Héligon, C., Chesnel, F., Boujard, D. & Philpott, A. The IGF pathway regulates head formation by inhibiting Wnt signaling in *Xenopus*. *Developmental biology* **244**, 407-417 (2002).
- 194 Perillo, M. & Arnone, M. I. Characterization of insulin-like peptides (ILPs) in the sea urchin *Strongylocentrotus purpuratus*: Insights on the evolution of the insulin family. *General and comparative endocrinology* **205**, 68-79 (2014).
- 195 Guo, B., Zhang, S., Wang, S. & Liang, Y. Expression, mitogenic activity and regulation by growth hormone of growth hormone/insulin-like growth factor in *Branchiostoma belcheri*. *Cell Tissue Res* **338**, 67-77, doi:10.1007/s00441-009-0824-8 (2009).
- 196 Lecroisey, C., Le Petillon, Y., Escriva, H., Lammert, E. & Laudet, V. Identification, evolution and expression of an insulin-like peptide in the cephalochordate *Branchiostoma lanceolatum*. *PLoS One* **10**, e0119461, doi:10.1371/journal.pone.0119461 (2015).
- 197 Brogiolo, W., Stocker, H., Rintelen, F., Fernandez, R. & Hafen, E. An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr Biol* **11**, 213-221 (2001).
- 198 Davidson, E. H. & Erwin, D. H. Gene regulatory networks and the evolution of animal body plans. *Science* **311**, 796-800, doi:10.1126/science.1113832 (2006).
- 199 Srivastava, D., Cserjesi, P. & Olson, E. N. A subclass of bHLH proteins required for cardiac morphogenesis. *Science* **270**, 1995-1999 (1995).

- 200 Angelo, S. *et al.* Conservation of sequence and expression of *Xenopus*
and zebrafish dHAND during cardiac, branchial arch and lateral
mesoderm development. *Mech Dev* **95**, 231-237 (2000).
- 201 Hollenberg, S. M., Sternglanz, R., Cheng, P. F. & Weintraub, H.
Identification of a new family of tissue-specific basic helix-loop-helix
proteins with a two-hybrid system. *Mol Cell Biol* **15**, 3813-3822 (1995).
- 202 Onimaru, K., Shoguchi, E., Kuratani, S. & Tanaka, M. Development
and evolution of the lateral plate mesoderm: comparative analysis of
amphioxus and lamprey with implications for the acquisition of paired
fins. *Dev Biol* **359**, 124-136, doi:10.1016/j.ydbio.2011.08.003 (2011).
- 203 Kölsch, V. & Paululat, A. The highly conserved cardiogenic bHLH
factor Hand is specifically expressed in circular visceral muscle
progenitor cells and in all cell types of the dorsal vessel during
Drosophila embryogenesis. *Development genes and evolution* **212**,
473-485 (2002).
- 204 Ahn, D. G., Ruvinsky, I., Oates, A. C., Silver, L. M. & Ho, R. K. *tbx20*, a
new vertebrate T-box gene expressed in the cranial motor neurons and
developing cardiovascular structures in zebrafish. *Mech Dev* **95**, 253-
258 (2000).
- 205 Brown, D. D., Binder, O., Pagratis, M., Parr, B. A. & Conlon, F. L.
Developmental expression of the *Xenopus laevis* Tbx20 orthologue.
Dev Genes Evol **212**, 604-607, doi:10.1007/s00427-002-0276-6
(2003).
- 206 Kraus, F., Haenig, B. & Kispert, A. Cloning and expression analysis of
the mouse T-box gene *tbx20*. *Mech Dev* **100**, 87-91 (2001).
- 207 Belgacem, M. R., Escande, M. L., Escriva, H. & Bertrand, S.
Amphioxus Tbx6/16 and Tbx20 embryonic expression patterns reveal
ancestral functions in chordates. *Gene Expr Patterns* **11**, 239-243,
doi:10.1016/j.gep.2010.12.006 (2011).
- 208 Buescher, M. *et al.* *Drosophila* T box proteins break the symmetry of
hedgehog-dependent activation of wingless. *Curr Biol* **14**, 1694-1702,
doi:10.1016/j.cub.2004.09.048 (2004).
- 209 Qian, L., Liu, J. & Bodmer, R. Neuromancer Tbx20-related genes
(H15/midline) promote cell fate specification and morphogenesis of the
Drosophila heart. *Dev Biol* **279**, 509-524,
doi:10.1016/j.ydbio.2005.01.013 (2005).
- 210 Buescher, M. *et al.* Functions of the segment polarity genes midline
and H15 in *Drosophila melanogaster* neurogenesis. *Dev Biol* **292**, 418-
429, doi:10.1016/j.ydbio.2006.01.016 (2006).
- 211 Martin-Duran, J. M. & Romero, R. Evolutionary implications of
morphogenesis and molecular patterning of the blind gut in the
planarian *Schmidtea polychroa*. *Dev Biol* **352**, 164-176,
doi:10.1016/j.ydbio.2011.01.032 (2011).
- 212 Janssen, R. & Damen, W. G. Diverged and conserved aspects of heart
formation in a spider. *Evol Dev* **10**, 155-165, doi:10.1111/j.1525-
142X.2008.00223.x (2008).

- 213 Detrich, H. W., 3rd *et al.* Intraembryonic hematopoietic cell migration during vertebrate development. *Proc Natl Acad Sci U S A* **92**, 10713-10717 (1995).
- 214 Neave, B., Rodaway, A., Wilson, S. W., Patient, R. & Holder, N. Expression of zebrafish GATA 3 (*gta3*) during gastrulation and neurulation suggests a role in the specification of cell fate. *Mech Dev* **51**, 169-182 (1995).
- 215 Kelley, C., Yee, K., Harland, R. & Zon, L. I. Ventral expression of GATA-1 and GATA-2 in the *Xenopus* embryo defines induction of hematopoietic mesoderm. *Developmental Biology* **165**, 193-205 (1994).
- 216 George, K. M. *et al.* Embryonic expression and cloning of the murine GATA-3 gene. *Development* **120**, 2673-2686 (1994).
- 217 Zhang, Y.-J. & Mao, B.-Y. Developmental expression of an amphioxus (*Branchiostoma belcheri*) gene encoding a GATA transcription factor. *Zoological Research* **30**, 137-143 (2009).
- 218 Brown, S. & Castelli-Gair Hombria, J. *Drosophila* grain encodes a GATA transcription factor required for cell rearrangement during morphogenesis. *Development* **127**, 4867-4876 (2000).
- 219 Gillis, W. J., Bowerman, B. & Schneider, S. Q. Ectoderm- and endomesoderm-specific GATA transcription factors in the marine annelid *Platynereis dumerilli*. *Evol Dev* **9**, 39-50, doi:10.1111/j.1525-142X.2006.00136.x (2007).
- 220 Nakamura, Y., Tsiairis, C. D., Ozbek, S. & Holstein, T. W. Autoregulatory and repressive inputs localize Hydra Wnt3 to the head organizer. *Proc Natl Acad Sci U S A* **108**, 9137-9142, doi:10.1073/pnas.1018109108 (2011).
- 221 Nakanishi, N., Sogabe, S. & Degnan, B. M. Evolutionary origin of gastrulation: insights from sponge development. *BMC Biol* **12**, 26, doi:10.1186/1741-7007-12-26 (2014).
- 222 Gove, C. *et al.* Over-expression of GATA-6 in *Xenopus* embryos blocks differentiation of heart precursors. *EMBO J* **16**, 355-368, doi:10.1093/emboj/16.2.355 (1997).
- 223 Jiang, Y. & Evans, T. The *Xenopus* GATA-4/5/6 genes are associated with cardiac specification and can regulate cardiac-specific transcription during embryogenesis. *Dev Biol* **174**, 258-270, doi:10.1006/dbio.1996.0071 (1996).
- 224 Kelley, C., Blumberg, H., Zon, L. I. & Evans, T. GATA-4 is a novel transcription factor expressed in endocardium of the developing heart. *Development* **118**, 817-827 (1993).
- 225 Morrissey, E. E., Ip, H. S., Tang, Z., Lu, M. M. & Parmacek, M. S. GATA-5: a transcriptional activator expressed in a novel temporally and spatially-restricted pattern during embryonic development. *Dev Biol* **183**, 21-36, doi:10.1006/dbio.1996.8485 (1997).
- 226 Arceci, R. J., King, A. A., Simon, M. C., Orkin, S. H. & Wilson, D. B. Mouse GATA-4: a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart. *Mol Cell Biol* **13**, 2235-2246 (1993).

- 227 Morrissey, E. E., Ip, H. S., Lu, M. M. & Parmacek, M. S. GATA-6: a zinc finger transcription factor that is expressed in multiple cell lineages derived from lateral mesoderm. *Dev Biol* **177**, 309-322, doi:10.1006/dbio.1996.0165 (1996).
- 228 Lee, P. Y. & Davidson, E. H. Expression of Spgatae, the Strongylocentrotus purpuratus ortholog of vertebrate GATA4/5/6 factors. *Gene Expr Patterns* **5**, 161-165, doi:10.1016/j.modgep.2004.08.010 (2004).
- 229 Hinman, V. F. & Davidson, E. H. Expression of a gene encoding a Gata transcription factor during embryogenesis of the starfish *Asterina miniata*. *Gene Expr Patterns* **3**, 419-422 (2003).
- 230 Okumura, T., Matsumoto, A., Tanimura, T. & Murakami, R. An endoderm-specific GATA factor gene, dGATAe, is required for the terminal differentiation of the Drosophila endoderm. *Dev Biol* **278**, 576-586, doi:10.1016/j.ydbio.2004.11.021 (2005).
- 231 Rehorn, K. P., Thelen, H., Michelson, A. M. & Reuter, R. A molecular aspect of hematopoiesis and endoderm development common to vertebrates and Drosophila. *Development* **122**, 4023-4031 (1996).
- 232 Gajewski, K., Fossett, N., Molkentin, J. D. & Schulz, R. A. The zinc finger proteins Pannier and GATA4 function as cardiogenic factors in Drosophila. *Development* **126**, 5679-5688 (1999).
- 233 Murakami, R., Okumura, T. & Uchiyama, H. GATA factors as key regulatory molecules in the development of Drosophila endoderm. *Dev Growth Differ* **47**, 581-589, doi:10.1111/j.1440-169X.2005.00836.x (2005).
- 234 Passamaneck, Y. J., Hejnal, A. & Martindale, M. Q. Mesodermal gene expression during the embryonic and larval development of the articulate brachiopod *Terebratalia transversa*. *Evodevo* **6**, 10, doi:10.1186/s13227-015-0004-8 (2015).
- 235 Tamura, K., Yonei-Tamura, S. & Izpisua Belmonte, J. C. Differential expression of Tbx4 and Tbx5 in Zebrafish fin buds. *Mech Dev* **87**, 181-184 (1999).
- 236 Garritty, D. M., Childs, S. & Fishman, M. C. The heartstrings mutation in zebrafish causes heart/fin Tbx5 deficiency syndrome. *Development* **129**, 4635-4645 (2002).
- 237 Takabatake, Y., Takabatake, T. & Takeshima, K. Conserved and divergent expression of T-box genes Tbx2-Tbx5 in *Xenopus*. *Mech Dev* **91**, 433-437 (2000).
- 238 Horb, M. E. & Thomsen, G. H. Tbx5 is essential for heart development. *Development* **126**, 1739-1751 (1999).
- 239 Yamagishi, H. *et al.* Tbx1 is regulated by tissue-specific forkhead proteins through a common Sonic hedgehog-responsive enhancer. *Genes Dev* **17**, 269-281, doi:10.1101/gad.1048903 (2003).
- 240 Bush, J. O., Maltby, K. M., Cho, E. S. & Jiang, R. The T-box gene Tbx10 exhibits a uniquely restricted expression pattern during mouse embryogenesis. *Gene Expr Patterns* **3**, 533-538 (2003).

- 241 Kochilas, L. K., Potluri, V., Gitler, A., Balasubramanian, K. & Chin, A. J. Cloning and characterization of zebrafish *tbx1*. *Gene Expr Patterns* **3**, 645-651 (2003).
- 242 Showell, C., Christine, K. S., Mandel, E. M. & Conlon, F. L. Developmental expression patterns of *Tbx1*, *Tbx2*, *Tbx5*, and *Tbx20* in *Xenopus tropicalis*. *Dev Dyn* **235**, 1623-1630, doi:10.1002/dvdy.20714 (2006).
- 243 Mahadevan, N. R., Horton, A. C. & Gibson-Brown, J. J. Developmental expression of the amphioxus *Tbx1/10* gene illuminates the evolution of vertebrate branchial arches and sclerotome. *Dev Genes Evol* **214**, 559-566, doi:10.1007/s00427-004-0433-1 (2004).
- 244 Schaub, C., Nagaso, H., Jin, H. & Frasch, M. *Org-1*, the *Drosophila* ortholog of *Tbx1*, is a direct activator of known identity genes during muscle specification. *Development* **139**, 1001-1012, doi:10.1242/dev.073890 (2012).
- 245 Schaub, C., Marz, J., Reim, I. & Frasch, M. *Org-1*-dependent lineage reprogramming generates the ventral longitudinal musculature of the *Drosophila* heart. *Curr Biol* **25**, 488-494, doi:10.1016/j.cub.2014.12.029 (2015).
- 246 Edmondson, D. G., Lyons, G. E., Martin, J. F. & Olson, E. N. *Mef2* gene expression marks the cardiac and skeletal muscle lineages during mouse embryogenesis. *Development* **120**, 1251-1263 (1994).
- 247 Lyons, G. E., Micales, B. K., Schwarz, J., Martin, J. F. & Olson, E. N. Expression of *mef2* genes in the mouse central nervous system suggests a role in neuronal maturation. *J Neurosci* **15**, 5727-5738 (1995).
- 248 Ticho, B. S., Stainier, D. Y., Fishman, M. C. & Breitbart, R. E. Three zebrafish *MEF2* genes delineate somitic and cardiac muscle development in wild-type and mutant embryos. *Mech Dev* **59**, 205-218 (1996).
- 249 Zhang, Y., Wang, L., Shao, M. & Zhang, H. Characterization and developmental expression of *AmphiMef2* gene in amphioxus. *Sci China C Life Sci* **50**, 637-641, doi:10.1007/s11427-007-0082-5 (2007).
- 250 Schulz, R. A., Chromey, C., Lu, M. F., Zhao, B. & Olson, E. N. Expression of the *D-MEF2* transcription in the *Drosophila* brain suggests a role in neuronal cell differentiation. *Oncogene* **12**, 1827-1831 (1996).
- 251 Lilly, B., Galewsky, S., Firulli, A. B., Schulz, R. A. & Olson, E. N. *D-MEF2*: a MADS box transcription factor expressed in differentiating mesoderm and muscle cell lineages during *Drosophila* embryogenesis. *Proc Natl Acad Sci U S A* **91**, 5662-5666 (1994).
- 252 Nguyen, H. T., Bodmer, R., Abmayr, S. M., McDermott, J. C. & Spoerel, N. A. *D-mef2*: a *Drosophila* mesoderm-specific MADS box-containing gene with a biphasic expression profile during embryogenesis. *Proc Natl Acad Sci U S A* **91**, 7520-7524 (1994).
- 253 Genikhovich, G. & Technau, U. Complex functions of *Mef2* splice variants in the differentiation of endoderm and of a neuronal cell type in

- a sea anemone. *Development* **138**, 4911-4919, doi:10.1242/dev.068122 (2011).
- 254 Spring, J. *et al.* Conservation of Brachyury, Mef2, and Snail in the myogenic lineage of jellyfish: a connection to the mesoderm of bilateria. *Dev Biol* **244**, 372-384. (2002).
- 255 Chen, J. N. & Fishman, M. C. Zebrafish tinman homolog demarcates the heart field and initiates myocardial differentiation. *Development* **122**, 3809-3816 (1996).
- 256 Lee, K. H., Xu, Q. & Breitbart, R. E. A new tinman-related gene, nkx2.7, anticipates the expression of nkx2.5 and nkx2.3 in zebrafish heart and pharyngeal endoderm. *Dev Biol* **180**, 722-731, doi:10.1006/dbio.1996.0341 (1996).
- 257 Tonissen, K. F., Drysdale, T. A., Lints, T. J., Harvey, R. P. & Krieg, P. A. XNkx-2.5, a Xenopus gene related to Nkx-2.5 and tinman: evidence for a conserved role in cardiac development. *Developmental Biology* **162**, 325-328 (1994).
- 258 Evans, S. M., Yan, W., Murillo, M. P., Ponce, J. & Papalopulu, N. tinman, a Drosophila homeobox gene required for heart and visceral mesoderm specification, may be represented by a family of genes in vertebrates: XNkx-2.3, a second vertebrate homologue of tinman. *Development* **121**, 3889-3899 (1995).
- 259 Lints, T. J., Parsons, L. M., Hartley, L., Lyons, I. & Harvey, R. P. Nkx-2.5: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants [published erratum appears in Development 1993 Nov;119(3):969]. *Development* **119**, 419-431 (1993).
- 260 Lyons, I. *et al.* Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2-5. *Genes Dev* **9**, 1654-1666 (1995).
- 261 Holland, N. D., Venkatesh, T. V., Holland, L. Z., Jacobs, D. K. & Bodmer, R. AmphiNk2-tin, an amphioxus homeobox gene expressed in myocardial progenitors: insights into evolution of the vertebrate heart. *Dev Biol* **255**, 128-137 (2003).
- 262 Bodmer, R., Jan, L. Y. & Jan, Y. N. A new homeobox-containing gene, msh-2, is transiently expressed early during mesoderm formation of Drosophila. *Development* **110**, 661-669 (1990).
- 263 Azpiazu, N. & Frasch, M. tinman and bagpipe: two homeo box genes that determine cell fates in the dorsal mesoderm of Drosophila. *Genes Dev* **7**, 1325-1340 (1993).
- 264 Saudemont, A. *et al.* Complementary striped expression patterns of NK homeobox genes during segment formation in the annelid Platynereis. *Dev Biol* **317**, 430-443, doi:S0012-1606(08)00109-7 [pii] 10.1016/j.ydbio.2008.02.013 (2008).
- 265 Navet, S., Bassaglia, Y., Baratte, S., Martin, M. & Bonnaud, L. Somatic muscle development in Sepia officinalis (cephalopoda - mollusca): a new role for NK4. *Dev Dyn* **237**, 1944-1951, doi:10.1002/dvdy.21614 (2008).

- 266 Tribioli, C., Frasch, M. & Lufkin, T. Bapx1: an evolutionary conserved homologue of the *Drosophila* bagpipe homeobox gene is expressed in splanchnic mesoderm and the embryonic skeleton. *Mech Dev* **65**, 145-162 (1997).
- 267 Newman, C. S., Grow, M. W., Cleaver, O., Chia, F. & Krieg, P. Xbp, a vertebrate gene related to bagpipe, is expressed in developing craniofacial structures and in anterior gut muscle. *Dev Biol* **181**, 223-233, doi:10.1006/dbio.1996.8416 (1997).
- 268 Meulemans, D. & Bronner-Fraser, M. Insights from amphioxus into the evolution of vertebrate cartilage. *PLoS One* **2**, e787, doi:10.1371/journal.pone.0000787 (2007).
- 269 Marlow, H., Matus, D. Q. & Martindale, M. Q. Ectopic activation of the canonical wnt signaling pathway affects ectodermal patterning along the primary axis during larval development in the anthozoan *Nematostella vectensis*. *Dev Biol* **380**, 324-334, doi:10.1016/j.ydbio.2013.05.022 (2013).
- 270 Topczewska, J. M., Topczewski, J., Solnica-Krezel, L. & Hogan, B. L. Sequence and expression of zebrafish foxc1a and foxc1b, encoding conserved forkhead/winged helix transcription factors. *Mech Dev* **100**, 343-347 (2001).
- 271 Kume, T., Jiang, H., Topczewska, J. M. & Hogan, B. L. The murine winged helix transcription factors, Foxc1 and Foxc2, are both required for cardiovascular development and somitogenesis. *Genes Dev* **15**, 2470-2482, doi:10.1101/gad.907301 (2001).
- 272 Iida, K. *et al.* Essential roles of the winged helix transcription factor MFH-1 in aortic arch patterning and skeletogenesis. *Development* **124**, 4627-4638 (1997).
- 273 Swiderski, R. E. *et al.* Expression of the Mf1 gene in developing mouse hearts: implication in the development of human congenital heart defects. *Dev Dyn* **216**, 16-27, doi:10.1002/(SICI)1097-0177(199909)216:1<16::AID-DVDY4>3.0.CO;2-1 (1999).
- 274 Koster, M., Dillinger, K. & Knochel, W. Expression pattern of the winged helix factor XFD-11 during *Xenopus* embryogenesis. *Mech Dev* **76**, 169-173 (1998).
- 275 Mazet, F., Amemiya, C. T. & Shimeld, S. M. An ancient Fox gene cluster in bilaterian animals. *Curr Biol* **16**, R314-316, doi:10.1016/j.cub.2006.03.088 (2006).
- 276 Ransick, A., Rast, J. P., Minokawa, T., Calestani, C. & Davidson, E. H. New early zygotic regulators expressed in endomesoderm of sea urchin embryos discovered by differential array hybridization. *Dev Biol* **246**, 132-147, doi:10.1006/dbio.2002.0607 (2002).
- 277 Tu, Q., Brown, C. T., Davidson, E. H. & Oliveri, P. Sea urchin Forkhead gene family: phylogeny and embryonic expression. *Dev Biol* **300**, 49-62, doi:10.1016/j.ydbio.2006.09.031 (2006).
- 278 Andrikou, C., Iovene, E., Rizzo, F., Oliveri, P. & Arnone, M. I. Myogenesis in the sea urchin embryo: the molecular fingerprint of the myoblast precursors. *Evodevo* **4**, 33, doi:10.1186/2041-9139-4-33 (2013).

- 279 Kobayashi, M., Osanai, H., Kawakami, K. & Yamamoto, M. Expression of three zebrafish Six4 genes in the cranial sensory placodes and the developing somites. *Mech Dev* **98**, 151-155 (2000).
- 280 Seo, H. C., Drivenes, O. & Fjose, A. A zebrafish Six4 homologue with early expression in head mesoderm. *Biochim Biophys Acta* **1442**, 427-431 (1998).
- 281 Ghanbari, H., Seo, H. C., Fjose, A. & Brandli, A. W. Molecular cloning and embryonic expression of Xenopus Six homeobox genes. *Mech Dev* **101**, 271-277. (2001).
- 282 Ozaki, H. *et al.* Six4, a putative myogenin gene regulator, is not essential for mouse embryonal development. *Mol Cell Biol* **21**, 3343-3350, doi:10.1128/MCB.21.10.3343-3350.2001 (2001).
- 283 Ohto, H. *et al.* Tissue and developmental distribution of six family gene products. *International Journal of Developmental Biology* **42**, 141-148 (1998).
- 284 Grifone, R. *et al.* Six1 and Six4 homeoproteins are required for Pax3 and Mrf expression during myogenesis in the mouse embryo. *Development* **132**, 2235-2249, doi:10.1242/dev.01773 (2005).
- 285 Kozmik, Z. *et al.* Pax-Six-Eya-Dach network during amphioxus development: conservation in vitro but context specificity in vivo. *Dev Biol* **306**, 143-159, doi:10.1016/j.ydbio.2007.03.009 (2007).
- 286 Kirby, R. J., Hamilton, G. M., Finnegan, D. J., Johnson, K. J. & Jarman, A. P. Drosophila homolog of the myotonic dystrophy-associated gene, SIX5, is required for muscle and gonad development. *Curr Biol* **11**, 1044-1049 (2001).
- 287 Clark, I. B., Boyd, J., Hamilton, G., Finnegan, D. J. & Jarman, A. P. D-six4 plays a key role in patterning cell identities deriving from the Drosophila mesoderm. *Dev Biol* **294**, 220-231, doi:10.1016/j.ydbio.2006.02.044 (2006).
- 288 Seo, H. C., Curtiss, J., Mlodzik, M. & Fjose, A. Six class homeobox genes in drosophila belong to three distinct families and are involved in head development. *Mech Dev* **83**, 127-139 (1999).
- 289 Sahly, I., Andermann, P. & Petit, C. The zebrafish eya1 gene and its expression pattern during embryogenesis. *Dev Genes Evol* **209**, 399-410 (1999).
- 290 David, R., Ahrens, K., Wedlich, D. & Schlosser, G. Xenopus Eya1 demarcates all neurogenic placodes as well as migrating hypaxial muscle precursors. *Mech Dev* **103**, 189-192 (2001).
- 291 Xu, P. X., Woo, I., Her, H., Beier, D. R. & Maas, R. L. Mouse eya homologues of the Drosophila eyes absent gene require Pax6 for expression in lens and nasal placode. *Development* **124**, 219-231 (1997).
- 292 Zimmerman, J. E. *et al.* Cloning and characterization of two vertebrate homologs of the Drosophila eyes absent gene. *Genome Research* **7**, 128-141 (1997).
- 293 Yankura, K. A., Martik, M. L., Jennings, C. K. & Hinman, V. F. Uncoupling of complex regulatory patterning during evolution of larval

- development in echinoderms. *BMC Biol* **8**, 143, doi:10.1186/1741-7007-8-143 (2010).
- 294 Boyle, M., Bonini, N. & DiNardo, S. Expression and function of *clift* in the development of somatic gonadal precursors within the *Drosophila* mesoderm. *Development* **124**, 971-982 (1997).
- 295 Bonini, N. M., Leiserson, W. M. & Benzer, S. The Eyes Absent Gene Genetic Control Of Cell Survival and Differentiation In the Developing *Drosophila* Eye. *Cell* **72**, 379-395 (1993).
- 296 Mannini, L. *et al.* *Djeyes absent* (*Djeya*) controls prototypic planarian eye regeneration by cooperating with the transcription factor *Djsix-1*. *Dev Biol* **269**, 346-359, doi:10.1016/j.ydbio.2004.01.042 (2004).
- 297 Fortunato, S. A., Leininger, S. & Adamska, M. Evolution of the Pax-Six-Eya-Dach network: the calcisponge case study. *Evodevo* **5**, 23, doi:10.1186/2041-9139-5-23 (2014).
- 298 Bessarab, D. A., Chong, S. W. & Korzh, V. Expression of zebrafish *six1* during sensory organ development and myogenesis. *Dev Dyn* **230**, 781-786, doi:10.1002/dvdy.20093 (2004).
- 299 Oliver, G. *et al.* Homeobox genes and connective tissue patterning. *Development* **121**, 693-705 (1995).
- 300 Serikaku, M. A. & O'Tousa, J. E. *sine oculis* is a homeobox gene required for *Drosophila* visual system development. *Genetics* **138**, 1137-1150. (1994).
- 301 Cheyette, B. N. R. *et al.* The *Drosophila sine oculis* locus encodes a homeodomain-containing protein required for the development of the entire visual system. *Neuron* **12**, 977-996 (1994).
- 302 Hammond, K. L., Hill, R. E., Whitfield, T. T. & Currie, P. D. Isolation of three zebrafish *dachshund* homologues and their expression in sensory organs, the central nervous system and pectoral fin buds. *Mech Dev* **112**, 183-189 (2002).
- 303 Caubit, X. *et al.* Mouse *Dac*, a novel nuclear factor with homology to *Drosophila dachshund* shows a dynamic expression in the neural crest, the eye, the neocortex, and the limb bud. *Developmental Dynamics* **214**, 66-80 (1999).
- 304 Hammond, K. L., Hanson, I. M., Brown, A. G., Lettice, L. A. & Hill, R. E. Mammalian and *Drosophila dachshund* genes are related to the *Ski* proto-oncogene and are expressed in eye and limb. *Mechanisms of Development* **74**, 121-131 (1998).
- 305 Heanue, T. A. *et al.* Synergistic regulation of vertebrate muscle development by *Dach2*, *Eya2*, and *Six1*, homologs of genes required for *Drosophila* eye formation. *Genes & Development* **13**, 3231-3243 (1999).
- 306 Candiani, S. *et al.* Cloning and developmental expression of amphioxus *Dachshund*. *Gene Expr Patterns* **3**, 65-69 (2003).
- 307 Lemons, D., Fritzenwanker, J. H., Gerhart, J., Lowe, C. J. & McGinnis, W. Co-option of an anteroposterior head axis patterning system for proximodistal patterning of appendages in early bilaterian evolution. *Dev Biol* **344**, 358-362, doi:10.1016/j.ydbio.2010.04.022 (2010).

- 308 Mardon, G., Solomon, N. M. & Rubin, G. M. dachshund encodes a nuclear protein required for normal eye and leg development in *Drosophila*. *Development* **120**, 3473-3486 (1994).
- 309 Winchell, C. J., Valencia, J. E. & Jacobs, D. K. Expression of Distal-less, dachshund, and optomotor blind in *Neanthes arenaceodentata* (Annelida, Nereididae) does not support homology of appendage-forming mechanisms across the Bilateria. *Dev Genes Evol* **220**, 275-295, doi:10.1007/s00427-010-0346-0 (2010).
- 310 Prpic, N. M. & Tautz, D. The expression of the proximodistal axis patterning genes Distal-less and dachshund in the appendages of *Glomeris marginata* (Myriapoda: Diplopoda) suggests a special role of these genes in patterning the head appendages. *Dev Biol* **260**, 97-112 (2003).
- 311 Jagla, K. *et al.* Mouse Lbx1 and human LBX1 define a novel mammalian homeobox gene family related to the *Drosophila* lady bird genes. *Mech Dev* **53**, 345-356 (1995).
- 312 Chen, F., Liu, K. C. & Epstein, J. A. Lbx2, a novel murine homeobox gene related to the *Drosophila* ladybird genes is expressed in the developing urogenital system, eye and brain. *Mech Dev* **84**, 181-184 (1999).
- 313 Martin, B. L. & Harland, R. M. A novel role for lbx1 in *Xenopus* hypaxial myogenesis. *Development* **133**, 195-208, doi:10.1242/dev.02183 (2006).
- 314 Ochi, H. & Westerfield, M. Lbx2 regulates formation of myofibrils. *BMC Dev Biol* **9**, 13, doi:10.1186/1471-213X-9-13 (2009).
- 315 Lukowski, C. M., Drummond, D. L. & Waskiewicz, A. J. Pbx-dependent regulation of lbx gene expression in developing zebrafish embryos. *Genome* **54**, 973-985, doi:10.1139/g11-061 (2011).
- 316 Jagla, K. *et al.* ladybird, a new component of the cardiogenic pathway in *Drosophila* required for diversification of heart precursors. *Development* **124**, 3471-3479 (1997).
- 317 Jagla, K. *et al.* ladybird, a tandem of homeobox genes that maintain late wingless expression in terminal and dorsal epidermis of the *Drosophila* embryo. *Development* **124**, 91-100 (1997).
- 318 Jagla, T. *et al.* ladybird determines cell fate decisions during diversification of *Drosophila* somatic muscles. *Development* **125**, 3699-3708 (1998).
- 319 Shanmugalingam, S. & Wilson, S. W. Isolation, expression and regulation of a zebrafish paraxis homologue. *Mech Dev* **78**, 85-89 (1998).
- 320 Carpio, R., Honore, S. M., Araya, C. & Mayor, R. *Xenopus* paraxis homologue shows novel domains of expression. *Dev Dyn* **231**, 609-613, doi:10.1002/dvdy.20147 (2004).
- 321 Burgess, R., Cserjesi, P., Ligon, K. L. & Olson, E. N. Paraxis: a basic helix-loop-helix protein expressed in paraxial mesoderm and developing somites. *Dev Biol* **168**, 296-306, doi:10.1006/dbio.1995.1081 (1995).

- 322 Cserjesi, P. *et al.* Scleraxis: a basic helix-loop-helix protein that prefigures skeletal formation during mouse embryogenesis. *Development* **121**, 1099-1110 (1995).
- 323 Beaster-Jones, L. *et al.* Expression of somite segmentation genes in amphioxus: a clock without a wavefront? *Dev Genes Evol* **218**, 599-611, doi:10.1007/s00427-008-0257-5 (2008).
- 324 Lauri, A. *et al.* Development of the annelid axochord: insights into notochord evolution. *Science* **345**, 1365-1368, doi:10.1126/science.1253396 (2014).
- 325 Begemann, G., Gibert, Y., Meyer, A. & Ingham, P. W. Cloning of zebrafish T-box genes *tbx15* and *tbx18* and their expression during embryonic development. *Mech Dev* **114**, 137-141 (2002).
- 326 Jezewski, P. A., Fang, P. K., Payne-Ferreira, T. L. & Yelick, P. C. Alternative splicing, phylogenetic analysis, and craniofacial expression of zebrafish *tbx22*. *Dev Dyn* **238**, 1605-1612, doi:10.1002/dvdy.21962 (2009).
- 327 Agulnik, S. I., Papaioannou, V. E. & Silver, L. M. Cloning, mapping, and expression analysis of *TBX15*, a new member of the T-Box gene family. *Genomics* **51**, 68-75, doi:10.1006/geno.1998.5278 (1998).
- 328 Kraus, F., Haenig, B. & Kispert, A. Cloning and expression analysis of the mouse T-box gene *Tbx18*. *Mech Dev* **100**, 83-86 (2001).
- 329 Beaster-Jones, L., Horton, A. C., Gibson-Brown, J. J., Holland, N. D. & Holland, L. Z. The amphioxus T-box gene, *AmphiTbx15/18/22*, illuminates the origins of chordate segmentation. *Evol Dev* **8**, 119-129, doi:10.1111/j.1525-142X.2006.00083.x (2006).
- 330 Germanguz, I., Lev, D., Waisman, T., Kim, C. H. & Gitelman, I. Four twist genes in zebrafish, four expression patterns. *Dev Dyn* **236**, 2615-2626, doi:10.1002/dvdy.21267 (2007).
- 331 Stoetzel, C. *et al.* X-twi is expressed prior to gastrulation in presumptive neurectodermal and mesodermal cells in dorsalized and ventralized *Xenopus laevis* embryos. *Int J Dev Biol* **42**, 747-756 (1998).
- 332 Wolf, C. *et al.* The M-twist gene of *Mus* is expressed in subsets of mesodermal cells and is closely related to the *Xenopus* X-twi and the *Drosophila* twist genes. *Dev Biol* **143**, 363-373. (1991).
- 333 Yasui, K., Zhang, S. C., Uemura, M., Aizawa, S. & Ueki, T. Expression of a twist-related gene, *Bbtwist*, during the development of a lancelet species and its relation to cephalochordate anterior structures. *Dev Biol* **195**, 49-59, doi:10.1006/dbio.1997.8834 (1998).
- 334 Wu, S. Y., Yang, Y. P. & McClay, D. R. Twist is an essential regulator of the skeletogenic gene regulatory network in the sea urchin embryo. *Dev Biol* **319**, 406-415, doi:10.1016/j.ydbio.2008.04.003 (2008).
- 335 Thisse, B., Stoetzel, C., Gorostiza-Thisse, C. & Perrin-Schmitt, F. Sequence of the twist gene and nuclear localization of its protein in endomesodermal cells of early *Drosophila* embryos. *EMBO J* **7**, 2175-2183 (1988).
- 336 Leptin, M. & Grunewald, B. Cell shape changes during gastrulation in *Drosophila*. *Development* **110**, 73-84 (1990).

- 337 Kozin, V. V., Filimonova, D. A., Kupriashova, E. E. & Kostyuchenko, R. P. Mesoderm patterning and morphogenesis in the polychaete *Alitta virens* (Spiralia, Annelida): Expression of mesodermal markers Twist, Mox, Evx and functional role for MAP kinase signaling. *Mech Dev*, doi:10.1016/j.mod.2016.03.003 (2016).
- 338 Pfeifer, K., Schaub, C., Wolfstetter, G. & Dorresteyn, A. Identification and characterization of a twist ortholog in the polychaete annelid *Platynereis dumerilii* reveals mesodermal expression of Pdu-twist. *Dev Genes Evol* **223**, 319-328, doi:10.1007/s00427-013-0448-6 (2013).
- 339 Dill, K. K., Thamm, K. & Seaver, E. C. Characterization of twist and snail gene expression during mesoderm and nervous system development in the polychaete annelid *Capitella* sp. I. *Dev Genes Evol* **217**, 435-447, doi:10.1007/s00427-007-0153-4 (2007).
- 340 Sommer, R. J. & Tautz, D. Expression patterns of twist and snail in *Tribolium* (Coleoptera) suggest a homologous formation of mesoderm in long and short germ band insects. *Dev Genet* **15**, 32-37, doi:10.1002/dvg.1020150105 (1994).
- 341 Price, A. L. & Patel, N. H. Investigating divergent mechanisms of mesoderm development in arthropods: the expression of Ph-twist and Ph-mef2 in *Parhyale hawaiiensis*. *J Exp Zool B Mol Dev Evol* **310**, 24-40, doi:10.1002/jez.b.21135 (2008).
- 342 Spring, J. *et al.* The mesoderm specification factor twist in the life cycle of jelly fish. *Dev Biol* **228**, 363-375 (2000).
- 343 Candia, A. F. *et al.* Mox-1 and Mox-2 define a novel homeobox gene subfamily and are differentially expressed during early mesodermal patterning in mouse embryos. *Development* **116**, 1123-1136 (1992).
- 344 Candia, A. F. & Wright, C. V. The expression pattern of *Xenopus* Mox-2 implies a role in initial mesodermal differentiation. *Mech Dev* **52**, 27-36 (1995).
- 345 Nguyen, P. D. *et al.* Haematopoietic stem cell induction by somite-derived endothelial cells controlled by meox1. *Nature* **512**, 314-318, doi:10.1038/nature13678 (2014).
- 346 Minguillon, C. & Garcia-Fernandez, J. The single amphioxus Mox gene: insights into the functional evolution of Mox genes, somites, and the asymmetry of amphioxus somitogenesis. *Dev Biol* **246**, 455-465, doi:10.1006/dbio.2002.0660 (2002).
- 347 Hinman, V. F. & Degnan, B. M. Mox homeobox expression in muscle lineage of the gastropod *Haliotis asinina*: evidence for a conserved role in bilaterian myogenesis. *Dev Genes Evol* **212**, 141-144, doi:10.1007/s00427-002-0223-6 (2002).
- 348 Matus, D. Q. *et al.* Molecular evidence for deep evolutionary roots of bilaterality in animal development. *Proc Natl Acad Sci U S A* **103**, 11195-11200 (2006).
- 349 Goriely, A. *et al.* A functional homologue of goosecoid in *Drosophila*. *Development* **122**, 1641-1650. (1996).
- 350 Angerer, L. M. *et al.* Sea urchin goosecoid function links fate specification along the animal-vegetal and oral-aboral embryonic axes. *Development* **128**, 4393-4404 (2001).

- 351 Boyle, M. J., Yamaguchi, E. & Seaver, E. C. Molecular conservation of metazoan gut formation: evidence from expression of endomesoderm genes in *Capitella teleta* (Annelida). *Evodevo* **5**, 39, doi:10.1186/2041-9139-5-39 (2014).
- 352 Scholz, C. B. & Technau, U. The ancestral role of Brachyury: expression of *NemBra1* in the basal cnidarian *Nematostella vectensis* (Anthozoa). *Dev Genes Evol* **212**, 563-570. (2003).
- 353 Kusch, T. & Reuter, R. Functions for *Drosophila brachyenteron* and *forkhead* in mesoderm specification and cell signalling. *Development* **126**, 3991-4003 (1999).
- 354 Rast, J. P., Cameron, R. A., Poustka, A. J. & Davidson, E. H. brachyury Target genes in the early sea urchin embryo isolated by differential macroarray screening. *Dev Biol* **246**, 191-208. (2002).
- 355 Osborne, P. W., Benoit, G., Laudet, V., Schubert, M. & Ferrier, D. E. Differential regulation of ParaHox genes by retinoic acid in the invertebrate chordate amphioxus (*Branchiostoma floridae*). *Dev Biol* **327**, 252-262, doi:10.1016/j.ydbio.2008.11.027 (2009).
- 356 Brooke, N. M., Garcia-Fernandez, J. & Holland, P. W. The ParaHox gene cluster is an evolutionary sister of the Hox gene cluster. *Nature* **392**, 920-922. (1998).
- 357 Arnone, M. I. *et al.* Genetic organization and embryonic expression of the ParaHox genes in the sea urchin *S. purpuratus*: insights into the relationship between clustering and colinearity. *Dev Biol* **300**, 63-73, doi:10.1016/j.ydbio.2006.07.037 (2006).
- 358 Cole, A. G., Rizzo, F., Martinez, P., Fernandez-Serra, M. & Arnone, M. I. Two ParaHox genes, SpLox and SpCdx, interact to partition the posterior endoderm in the formation of a functional gut. *Development* **136**, 541-549, doi:10.1242/dev.029959 (2009).
- 359 Frobius, A. C. & Seaver, E. C. ParaHox gene expression in the polychaete annelid *Capitella* sp. I. *Dev Genes Evol* **216**, 81-88, doi:10.1007/s00427-005-0049-0 (2006).
- 360 Mlodzik, M., Fjose, A. & Gehring, W. J. Isolation of caudal, a *Drosophila* homeo box-containing gene with maternal expression, whose transcripts form a concentration gradient at the pre-blastoderm stage. *EMBO J* **4**, 2961-2969 (1985).
- 361 Mlodzik, M. & Gehring, W. J. Expression of the caudal gene in the germ line of *Drosophila*: formation of an RNA and protein gradient during early embryogenesis. *Cell* **48**, 465-478 (1987).
- 362 Seaver, E. C., Yamaguchi, E., Richards, G. S. & Meyer, N. P. Expression of the pair-rule gene homologs runt, Pax3/7, even-skipped-1 and even-skipped-2 during larval and juvenile development of the polychaete annelid *Capitella teleta* does not support a role in segmentation. *Evodevo* **3**, 8, doi:10.1186/2041-9139-3-8 (2012).
- 363 Ryan, J. F. *et al.* The cnidarian-bilaterian ancestor possessed at least 56 homeoboxes: evidence from the starlet sea anemone, *Nematostella vectensis*. *Genome Biol* **7**, R64 (2006).
- 364 Chourrout, D. *et al.* Minimal ProtoHox cluster inferred from bilaterian and cnidarian Hox complements. *Nature* **442**, 684-687 (2006).

- 365 Kamm, K. & Schierwater, B. Ancient complexity of the non-Hox ANTP gene complement in the anthozoan *Nematostella vectensis*: implications for the evolution of the ANTP superclass. *J Exp Zool B Mol Dev Evol* **306**, 589-596 (2006).
- 366 Yamada, A., Pang, K., Martindale, M. Q. & Tochinai, S. Surprisingly complex T-box gene complement in diploblastic metazoans. *Evol Dev* **9**, 220-230 (2007).
- 367 Reitzel, A. M. & Tarrant, A. M. Nuclear receptor complement of the cnidarian *Nematostella vectensis*: phylogenetic relationships and developmental expression patterns. *BMC Evol Biol* **9**, 230, doi:10.1186/1471-2148-9-230 (2009).
- 368 Simionato, E. *et al.* Origin and diversification of the basic helix-loop-helix gene family in metazoans: insights from comparative genomics. *BMC Evol Biol* **7**, 33 (2007).
- 369 Marlow, H. Q., Srivastava, M., Matus, D. Q., Rokhsar, D. & Martindale, M. Q. Anatomy and development of the nervous system of *Nematostella vectensis*, an anthozoan cnidarian. *Dev Neurobiol* **69**, 235-254, doi:10.1002/dneu.20698 (2009).