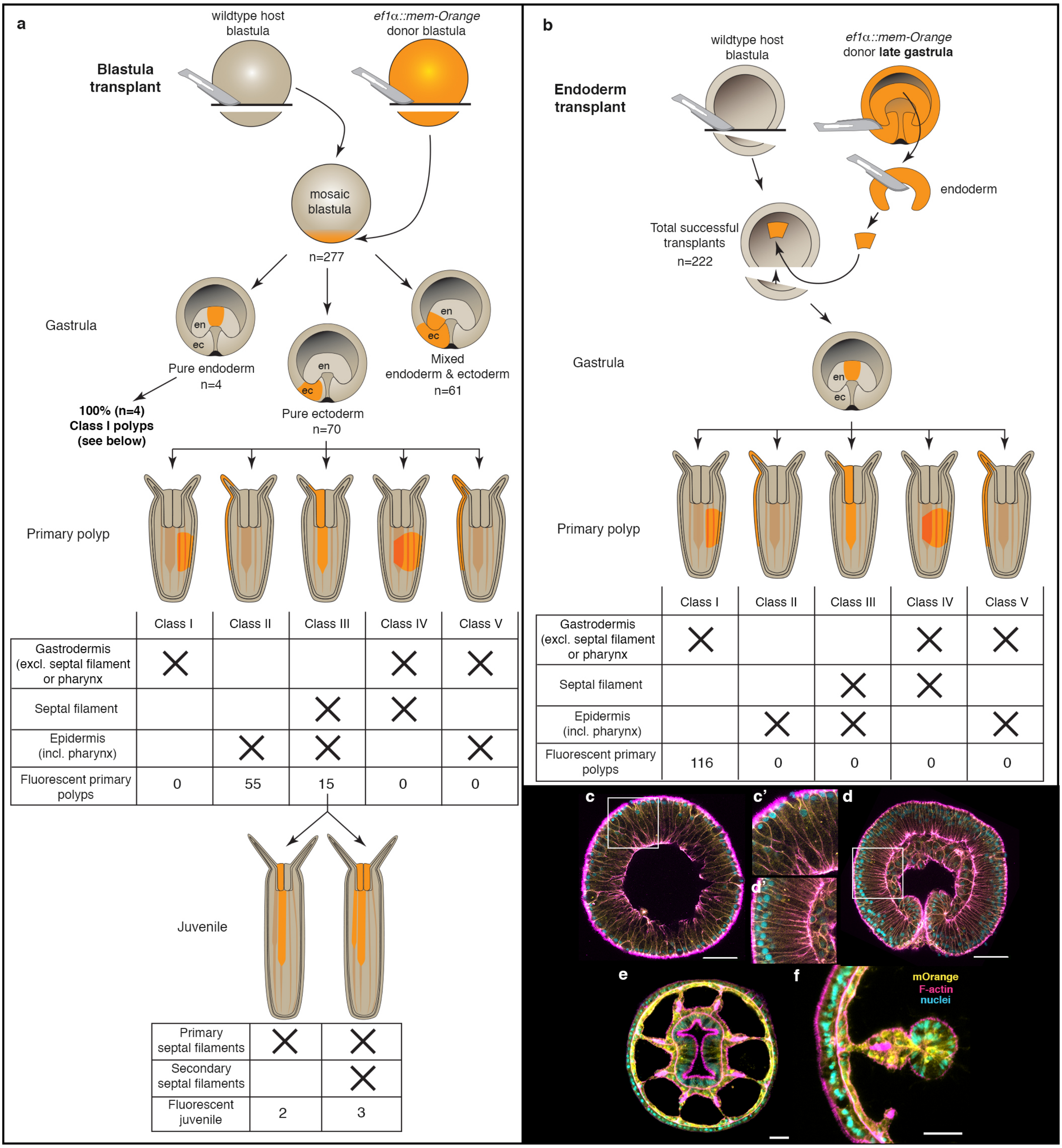
Gut-like ectodermal tissue in a sea anemone challenges germ layer homology

Supplemental Figures 1-11

Supplemental Tables 1-3

Supplemental References



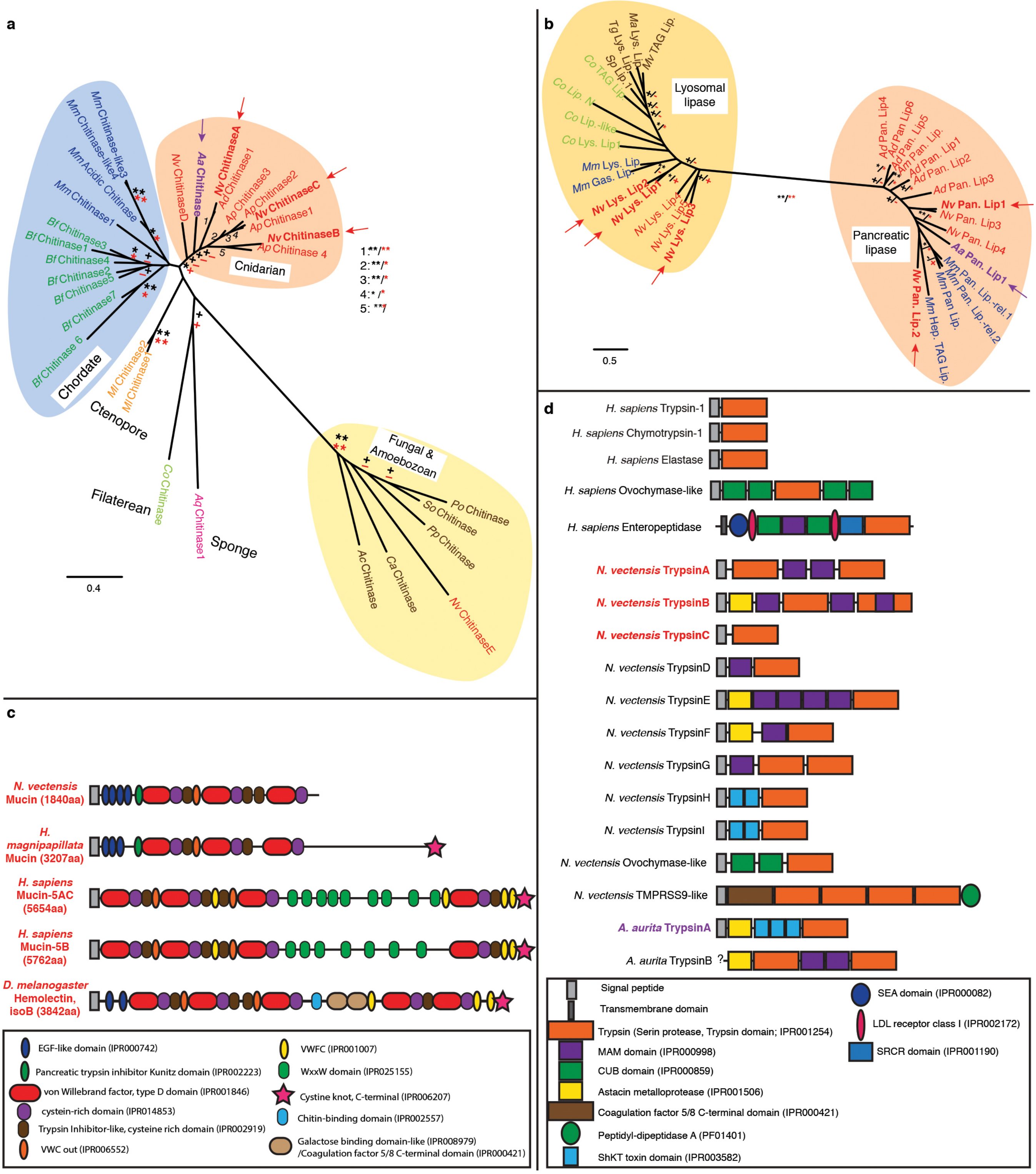
	Class I	Class II	Class III	Class IV	Class V
Gastrodermis (excl. septal filament or pharynx	X			X	X
Septal filament			X	X	
Epidermis (incl. pharynx)		X	X		X
Fluorescent primary polyps	0	55	15	0	0

Primary septal filaments	X	X
Secondary septal filaments		X
Fluorescent juvenile	2	3

	Class I	Class II	Class III	Class IV	Class V
Gastrodermis (excl. septal filament or pharynx	X			X	X
Septal filament			X	X	
Epidermis (incl. pharynx)		X	X		X
Fluorescent primary polyps	116	0	0	0	0

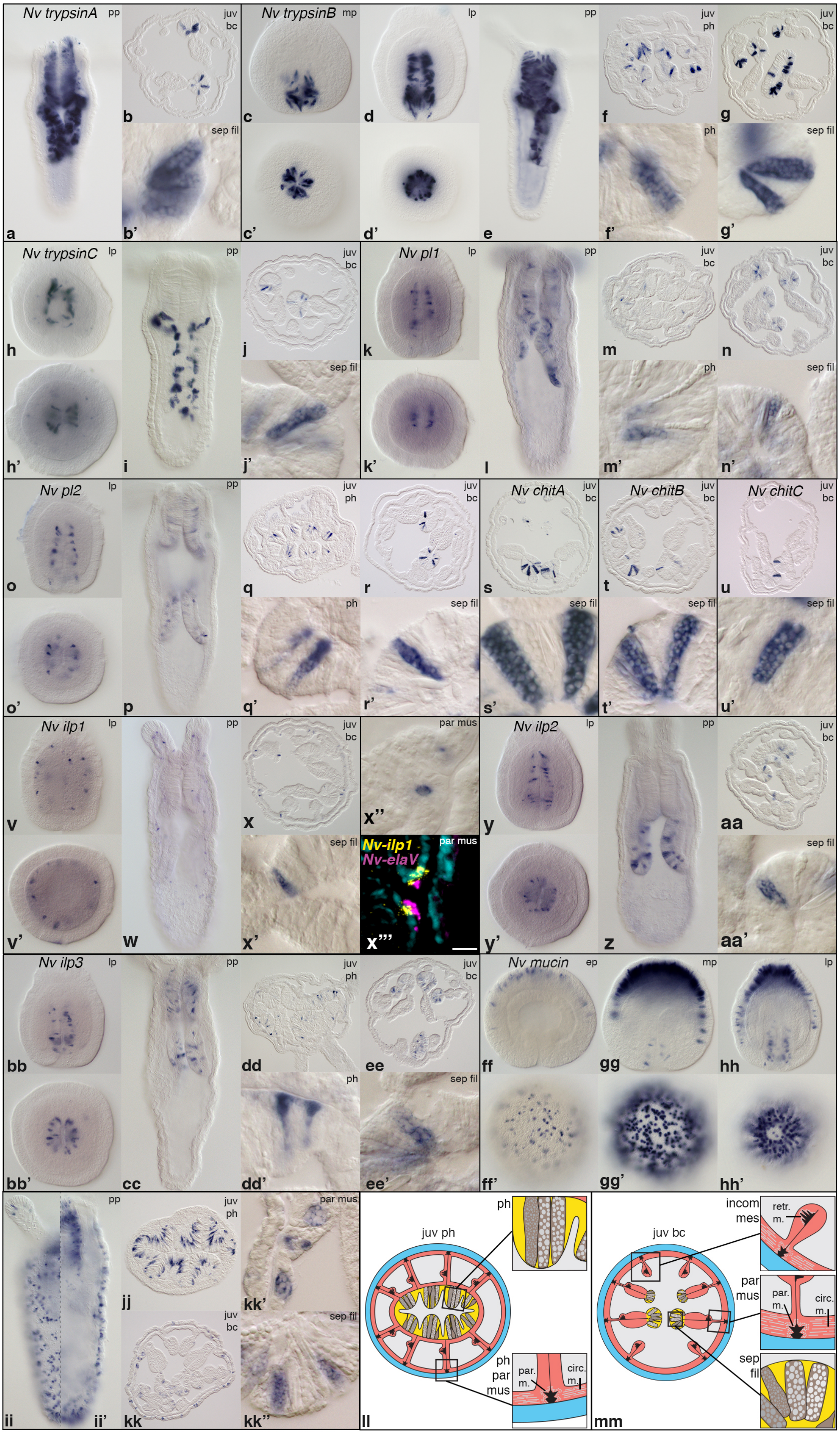
Supplementary Figure 1 Schematics of the experimental procedure, results, and control images of the fate mapping by transplantation experiment

(a) Transplantation of blastulae cells expressing CAAX-tagged mOrange (mem-Orange) fluorophore under the control of the ubiquitous *ef1α* 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 *ef1α::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 *ef1α::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 *ef1α::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 μm; e, f: 20μm.



Supplementary Figure 2 Phylogenetic or protein domain structure analysis of the cloned digestive enzymes and Mucin proteins

Maximum likelihood phylogenetic tree (a, b) or protein domain analysis (c, d) of Chitinase (a), Pancreatic and Lysosomal Lipase (b), Mucin (c) or Trypsin 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.



Nv trypsinA
Nv trypsinB
Nv trypsinC
Nv pl1
Nv pl2
Nv chitA
Nv chitB
Nv chitC
Nv ilp1
Nv ilp2
Nv ilp3
Nv mucin
Nv-ilp1
Nv-elaV
juv ph
juv bc
ph
par mus
incom mes
retr. m.
par. m.
circ. m.
sep fil

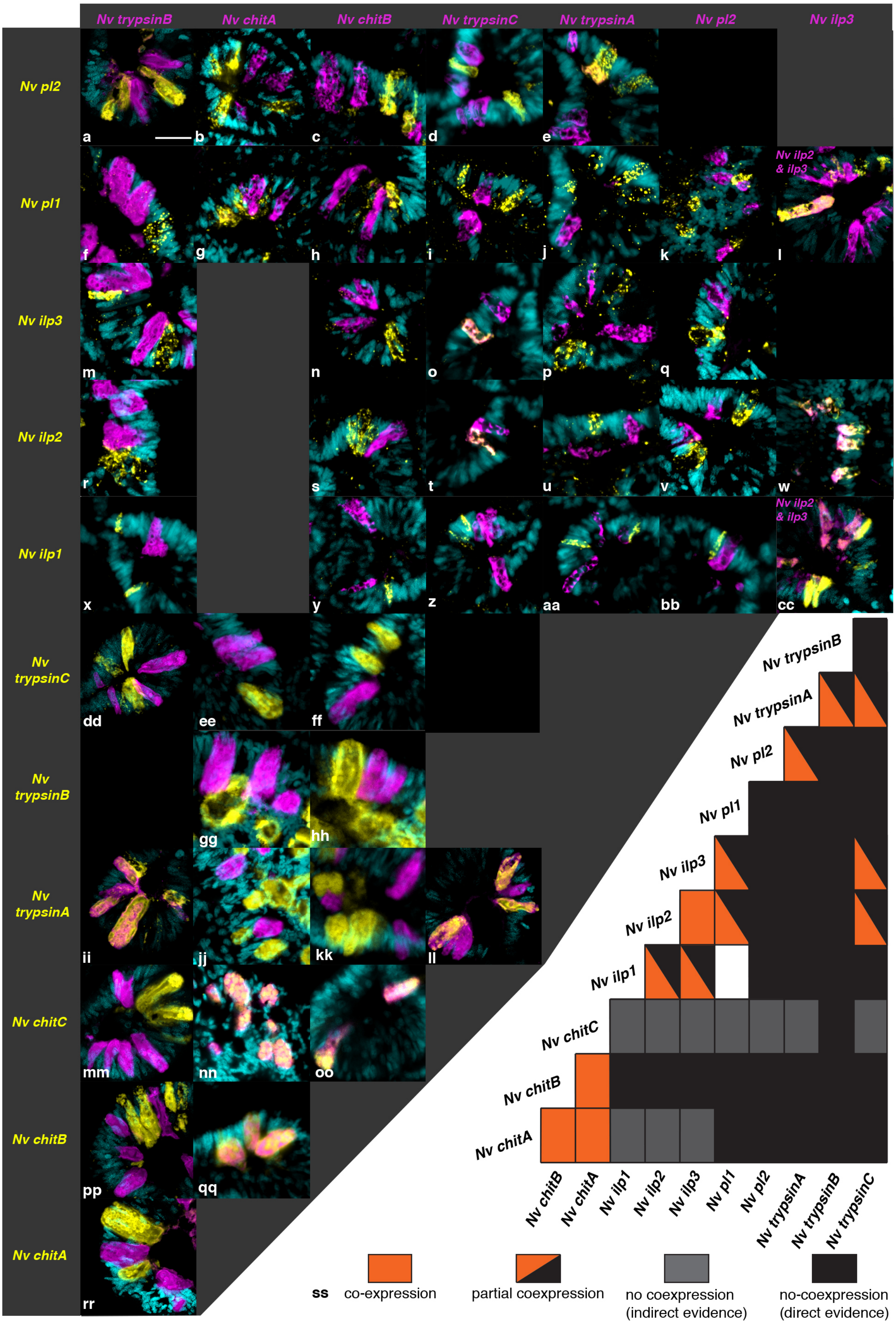
Supplementary Figure 3 Expression of exocrine digestive enzymes and the mucous component gene *mucin* during *N. vectensis* development and juvenile growth

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.

Supplementary Figure 4 Alignment and phylogenetic analysis of *N. vectensis* and *A. aurita* Insulin-like peptide and Insulin receptor

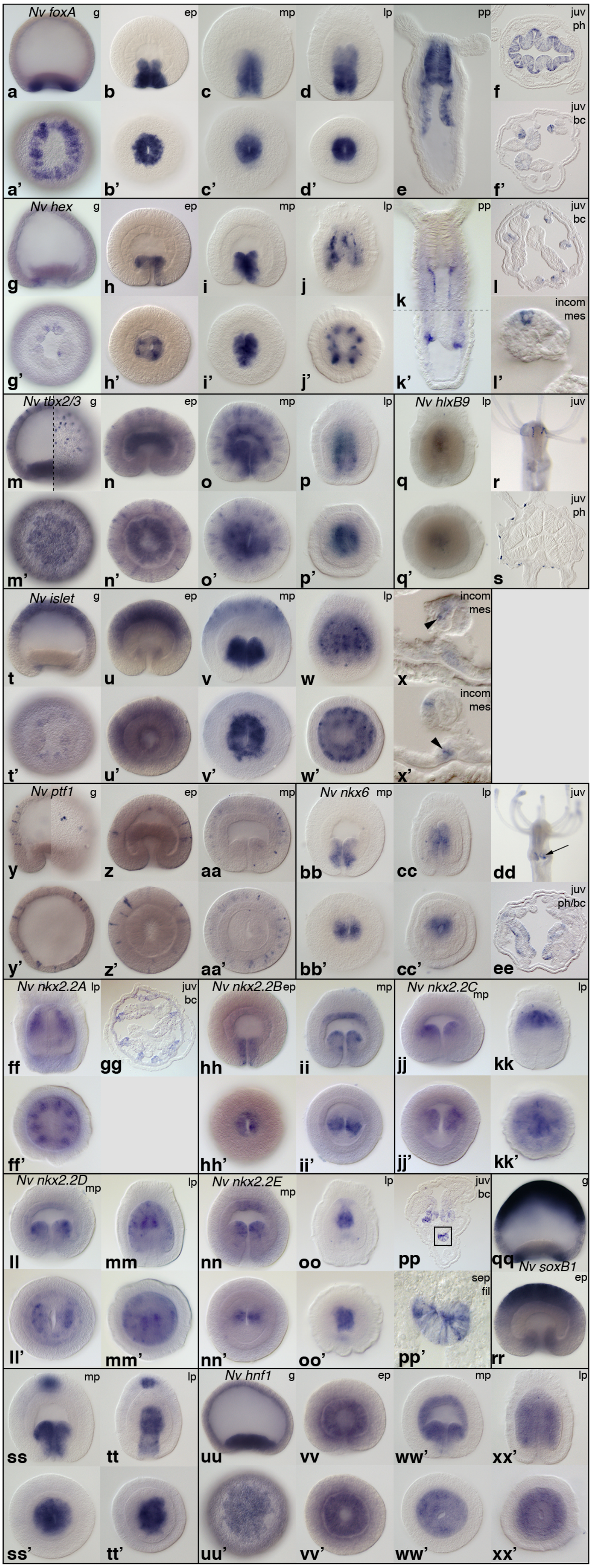
protein sequences

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 cystein 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.



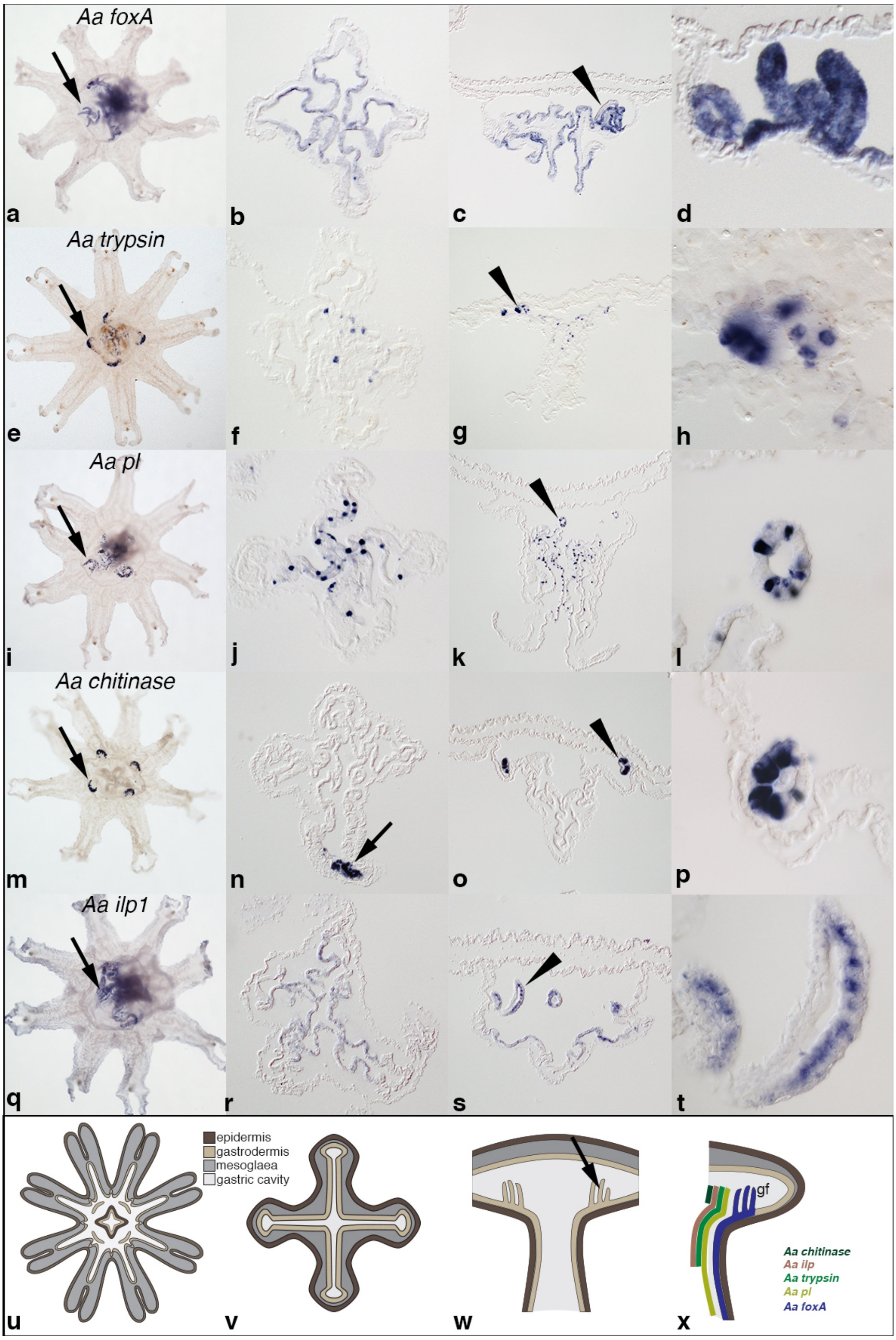
Supplementary Figure 5 Co-expression study of exocrine and insulinergic cell types in the septal filament of *N. vectensis*

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.


Nv foxA
Nv hex
Nv tbx2/3
Nv hlxB9
Nv islet
Nv ptf1
Nv nkx6
Nv nkx2.2A
Nv nkx2.2B
Nv nkx2.2C
Nv nkx2.2D
Nv nkx2.2E
Nv soxB1
Nv hnf1
g
ep
mp
lp
pp
juv
ph
bc
incom
mes
sep
fil
ph/bc

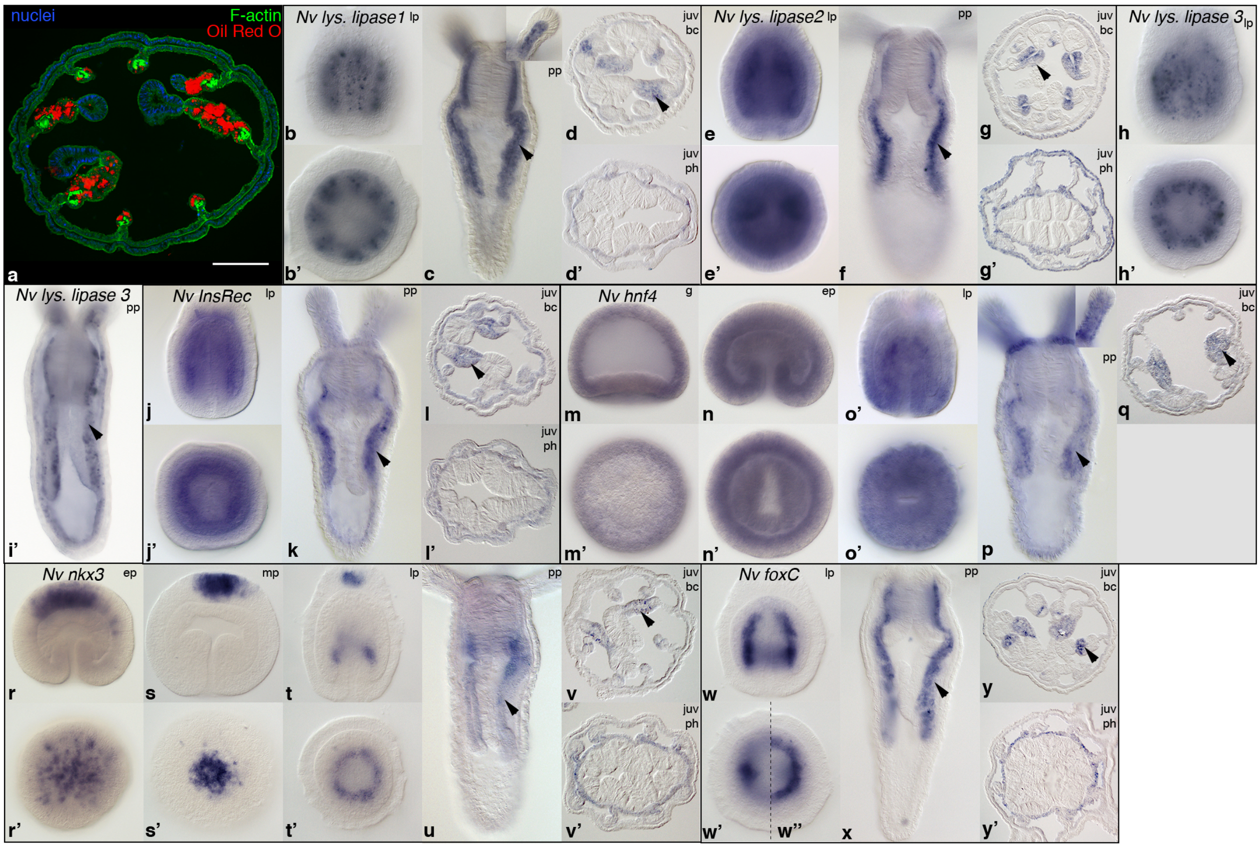
Supplementary Figure 6 Expression of 'endodermal' and 'pancreatic' transcription factor orthologs during *N. vectensis* development and juvenile growth

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 3ll 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.



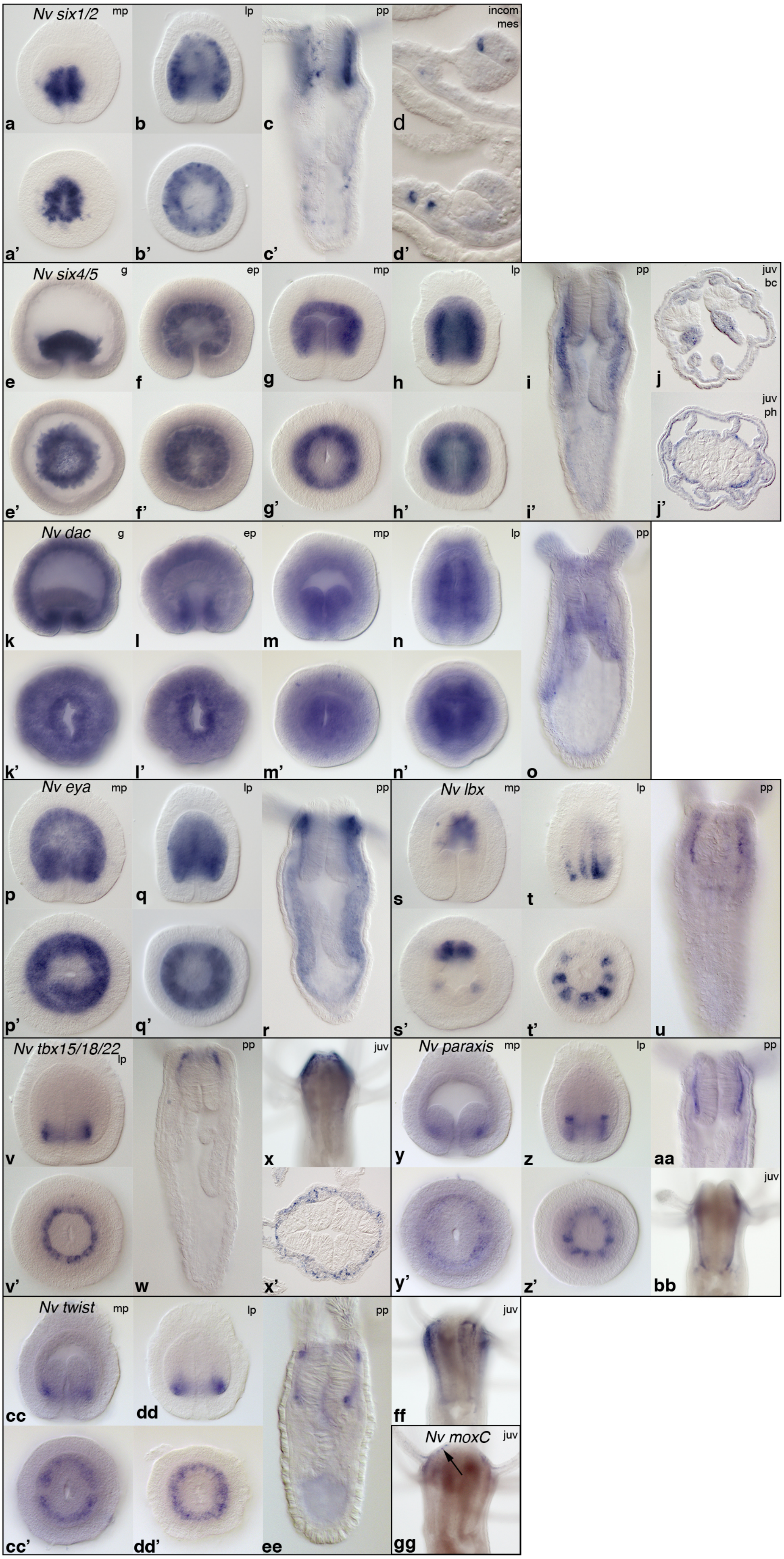
Supplementary Figure 7 Expression of *foxA*, secreted digestive enzyme and *preproinsulin-like peptide* genes in the *A. aurita* ephyra.

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.


Nv six1/2
mp
lp
pp
incom
mes
a
b
c
d
a'
b'
c'
d'
Nv six4/5
g
ep
mp
lp
pp
juv
bc
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i
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Nv lbx
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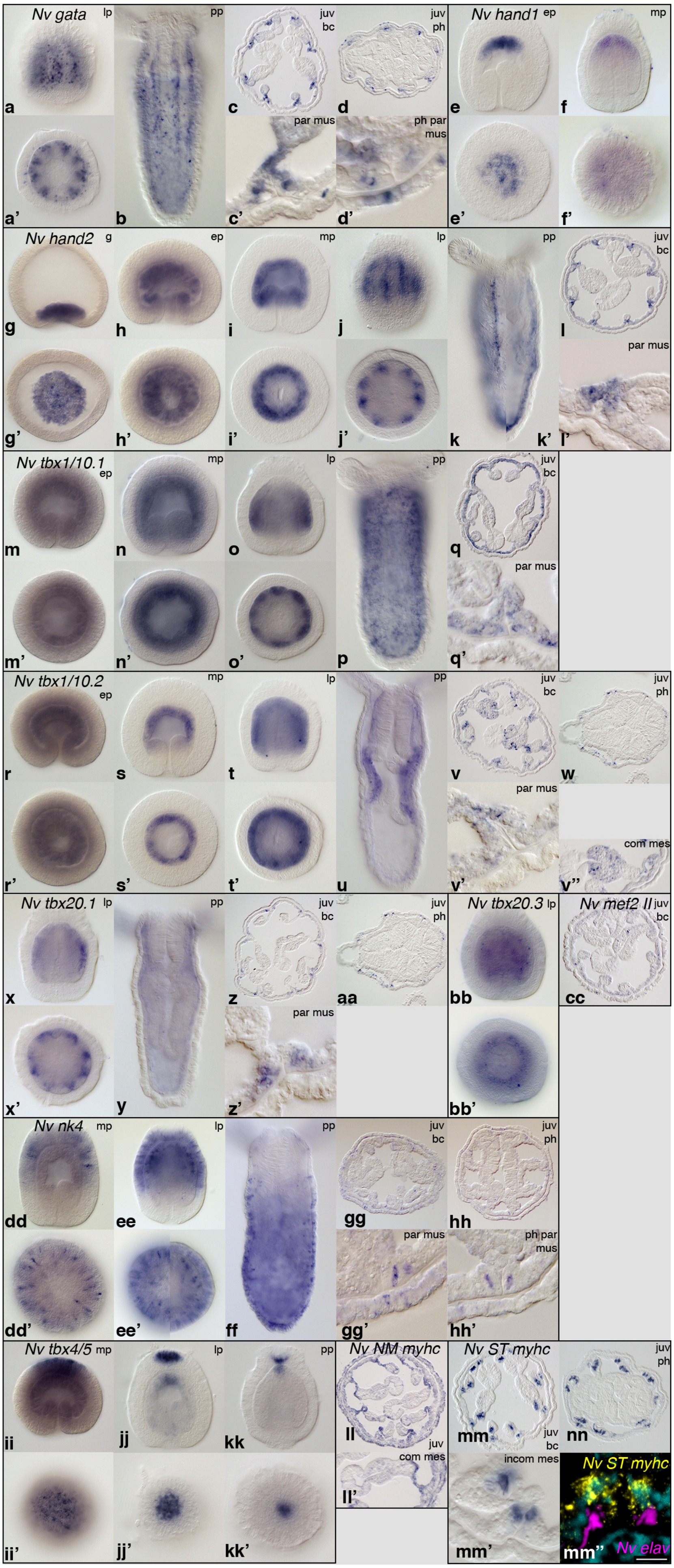
Nv tbx15/18/22
lp
pp
juv
Nv paraxis
mp
lp
pp
v
x
y
z
aa
juv
v'
w
x'
y'
z'
bb
Nv twist
mp
lp
pp
juv
cc
dd
ff
Nv moxC
juv
cc'
dd'
ee
gg

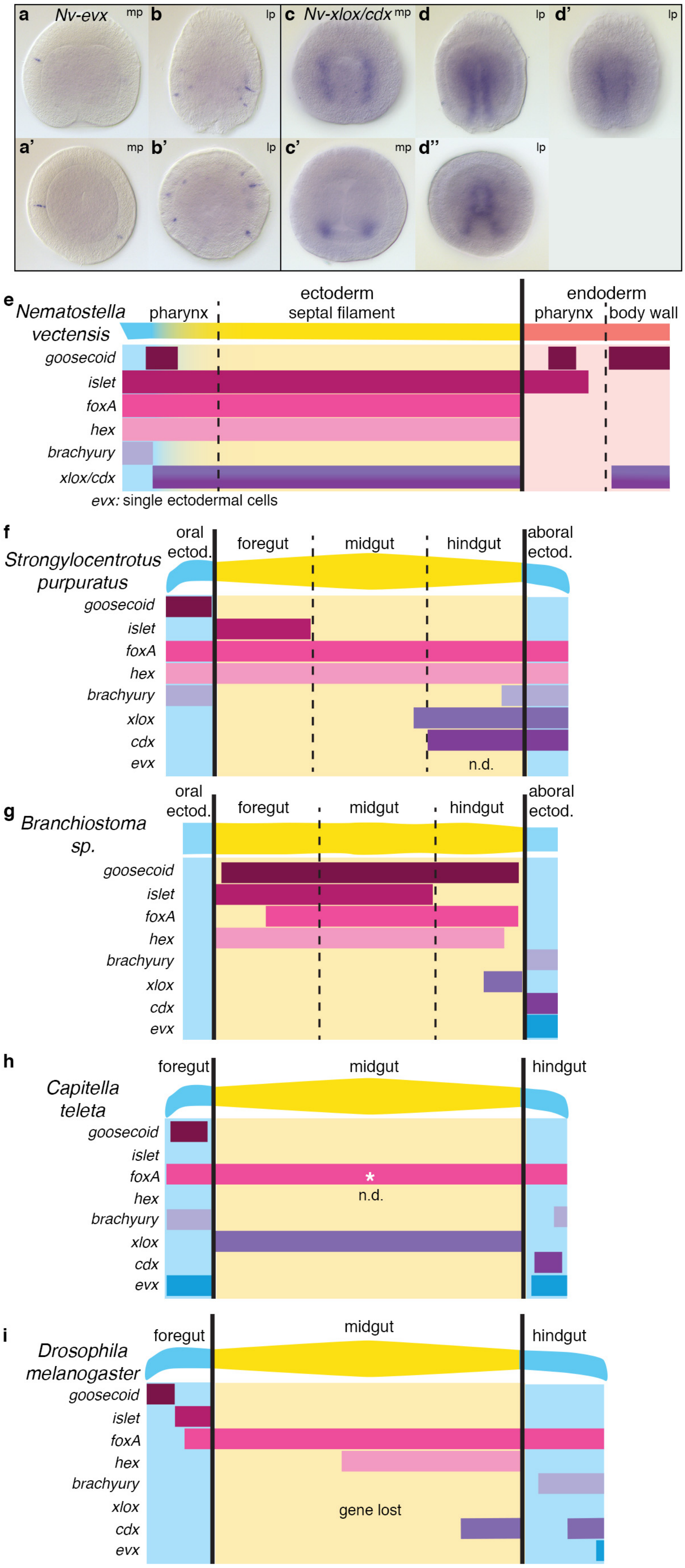
Supplementary Figure 9 Expression of 'skeletal muscle' and 'somite patterning' gene orthologs during *N. vectensis* development and growth

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 3ll 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.


Nv gata
Nv hand1
Nv hand2
Nv tbx1/10.1
Nv tbx1/10.2
Nv tbx20.1
Nv tbx20.3
Nv mef2 II
Nv nk4
Nv tbx4/5
Nv NM myhc
Nv ST myhc
Nv elav
juv bc
juv ph
par mus
ph par mus
com mes
incom mes

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



a Nv-evx mp
b lp
c Nv-xlox/cdx mp
d lp
d' lp
a' mp
b' lp
c' mp
d'' lp
e Nematostella vectensis
ectoderm
pharynx
septal filament
endoderm
pharynx
body wall
goosecoid
islet
foxA
hex
brachyury
xlox/cdx
evx: single ectodermal cells
f Strongylocentrotus purpuratus
oral ectod.
foregut
midgut
hindgut
aboral ectod.
goosecoid
islet
foxA
hex
brachyury
xlox
cdx
evx
n.d.
g Branchiostoma sp.
oral ectod.
foregut
midgut
hindgut
aboral ectod.
goosecoid
islet
foxA
hex
brachyury
xlox
cdx
evx
h Capitella teleta
foregut
midgut
hindgut
goosecoid
islet
foxA
hex
brachyury
xlox
cdx
evx
*
n.d.
i Drosophila melanogaster
foregut
midgut
hindgut
goosecoid
islet
foxA
hex
brachyury
xlox
cdx
evx
gene lost

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	*Drosophila*	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
hex, hhex	*Xenopus*: 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]	*Asterina miniata* (Sea star): endomesoderm, gut endoderm [80] *Strongylocentrotus purpuratus* (sea urchin): skeletal mesoderm, gut endoderm [81] *Branchiostoma floridae* (Amphioxus): Anterior endoderm [82] *Saccoglossus kowalevskii*: endoderm, dorsal gut endoderm [83]	CG7056-PA: midgut [84,85].	*C. elegans pha-2*: pharyngeal primordium, intestine, pharyngeal neurons, muscle, rectal cells [86] *Crepidula fornicata*: foregut, hindgut rudiment [87]		*Nematostella vectensis*: pharyngeal ectoderm; single cells in endoderm (this paper)		*Sycon ciliatum*: inner cell mass [88]

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

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

	Xenopus: 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	*Drosophila*	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
islet	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] *Branchiostoma floridae*: neural plate, anterior endoderm, pharyngeal endoderm, intestine [146,147]	*Isl1*: ventral nerve chord (f.ex. motor neurons, interneurons), pharynx, dorsal vessel [148]			*Nematostella vectensis*: pharynx, directive mesenteries [149] & this paper (partly conflicting)	*Mnemiopsis leydii*: aboral ectodermal cells [150]	*Amphimedon queenslandica* : ubiquitous [149]
nkx2.2	Mouse: forebrain, pancreas, restricts to endocrine pancreas [151] Zebrafish: CNS (f.ex.	*Branchiostoma floridae*: ventral endoderm, pharynx, midgut, hindgut,	*Vnd*: ventral CNS (f.ex. MP2 neuroblast, progenitor of insulinergic neurons) [155]	*Platynereis dumerilii*: ventral neuroectodermal midline [156]		*Nematostella nkx2.2A-E*: pharyngeal ectoderm, septal		

	forebrain, ventral neural tube) pancreas [71,152]	anterior CNS [153] *Stronglyocentrotus purpuratus*: oral and aboral ectoderm [154]				filaments, some paralogs low in endoderm		
hnf6	Zebrafish: CNS (f.ex. fore-, midbrain), liver, pancreas, gallbladder [157] Mouse: CNS (fore-, mid-, hindbrain, motorneurons), 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] *Halocynthia roretzi* (Ascidia): neurons [162]	*onecut*: CNS, PNS [163]	*Platynereis dumerilii*: larval brain [164] *Haliotis rufescens*: larval brain [165]		*Nematostella*: No expression detected		
hlxB9/mnx	Mouse: notochord, dorsal gut endoderm, ventral endoderm: pancreatic epithelium, β-cells [166] Zebrafish: axial & lateral mesoderm, endoderm, pancreas (exo- & endocrine) [167]	*Branchiostoma floridae amphiMnx*: neural plate, dorsal endomesoderm, posterior gut [168] *Saccoglossus kowalevskii*: ventral endoderm, ectodermal patches *Paracentrotus lividus*: anus (prism and	*hb9*: anterior & posterior midgut, motor neurons, interneurons, insulinergic MP2 neurons [96,170,171]	*Platynereis dumerilii*: Ventral nerve chord (motorneurons) [156]		*Nematostella vectensis*: pharyngeal ectoderm, single ectodermal cells [172] and this paper		

		pluteus) [169]						
nkx6	Zebrafish *nkx6.1*: spinal cord motor neurons, pancreas [173] Mouse: CNS (motorneuron, interneuron), pancreas [174] [175,176]		*nkx6*: CNS (brain, ventral & intermediate neuroblasts), hindgut, [173,177]	*Platynereis dumerilii*: Ventral nerve chord (motorneurons [156]		*Nematostella vectensis*: pharyngeal ectoderm, septal filament just below pharynx (this paper)		
ptf1	Mouse: exocrine pancreas [178] Zebrafish: hindbrain, retina, pancreas [179]	*Stronglyocentrotus purpuratus*: ectoderm, midgut [145]				*Nematostella*: single cells in ectoderm (this paper)		
tbx2/3 *Bifid/optomotor-blind*	Mouse: extraembryonic mesoderm & endoderm, pharyngeal arch mesenchyme, pharynx (thyroid), otic & optic vesicle, trigeminal ganglia, dorsal root ganglia, diencephalum (infudibulum), hindbrain, limb buds, myotomes, smooth muscles, pancreas [180,181]	*Branchiostoma floridae*: blastopore lip, invaginating mesendoderm, neural tube, ventral gut endoderm, surface ectoderm[185] *Lytechnius variegatus*: foregur, oral ectoderm, endoderm, skeletogenic mesoderm[186]	*optomotor-blind:* optic lobe anlagen, large part of larval brain [188]	*Hydroides elegans*: dorsal blastomeres, broad dorsal midline, dorsal sensory cells, dorsal endoderm, hindgut [189]		*Nematostella*: 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 beurons, diencphalon (pineal), trigeminus, lateral line, pronephric ducts, branchial arches, heart, liver, fins, [184]	*Paracentrotus lividus*: early presumptive endoderm & part of ectoderm, partly in primary mesenchyme[187]						
insulin-like peptides, *insulin growth factor hormone*	Zebrafish: pancreatic island (insulin), ubiquitous with highest signal in anterior nervous system (IGF-I,-II) [190,191] *Xenopus*: anterior&dorsal ectoderm & mesoderm,	*Stronglyocentrotus purpuratus*: Foregut, upper stomach, intestine[194] *Branchiostoma lanceolatum*: endoderm, paraxial mesoderm, gut,	*Ilp1-7*: midgut, mesoderm, imaginal discs, single brain neurons, salivary glands, ventral nerve chord [197]			*Nematostella*: 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	*Drosophila*	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
hand/dHand,/eHand/ thing1/hxt	Zebrafish, Chicken & *Xenopus*: 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]	*Amphioxus*: ventral mesoderm [202]	All heart progenitors (cardioblast & pericardium), circular visceral muscles, lymph gland, garland cells, neurons [203]			*Nematostella*: larval endoderm, polyp: restricts to the parietal muscle (this paper)		
tbx20/neuromancer-1/-2/H15/mid	Zebrafish: lateral plate mesoderm, cardiac mesoderm & heart, hindbrain & midbrain motor	*Amphioxus*: ventral mesoderm, few ventral neurons [207]	*Drosophila*: segment polarity pattern, CNS formation, cardiogenic	*Schmidtea polychroa*: ventral nerve chord (?) [211] *Cupiennius salei*: dorso-lateral edge		*Nematostella*: larval mesentery endoderm, parietal muscle region (this paper)		

	neurons, tailbud [204] *Xenopus*: Cardiac tissue (myocardium & endocardium), cement gland, jugular vein, lung bud, cloacal aperture, hindbrain, motor neurons [205] Mouse: Extraembryonic mesoderm, lateral plate, cardiogenic mesoderm & heart, retina, hindbrain [206]		region, entire myocardium [208-210]	of embryo (heart precursor cells) & developing heart [212] *Tribolium casteneum*: dorso-lateral edge of germ band (heart precursor) & developing heart [212]				
gata1/2/3; grain/dGATAc	Zebrafish: ventral ectoderm, lateral mesoderm, erythroid progenitor cells, pronephric duct, CNS [213,214] *Xenopus*:	Amphioxus: mesendoderm, forming somites, endoderm, coelomic diverticula, pharyngeal mesoderm,	*Drosophila*: CNS, midgut, lateral ectoderm, appendages [218]	*Capitella sp.*: CNS (brain and ventral nerve chord), foregut [98]; *Platynereis dumerilii*: neuroectoderm [219]		*Nematostella*: single cells in ectoderm; mesenterial endoderm, parietal muscle [106] & this paper; *Hydra*: body column		*Amphimedon gata1/2/3/4/5/6* ortholog: inner layer of the larva [221]

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

	mesoderm, embryonic & adult heart, primitive endoderm, intestine, gonads, lung, smooth muscle cellls (bronchial, bladder)[225 226 227]	[147]		midgut; *Themiste lageniformis*: endoderm, midgut [100]; *Platynereis dumerilii*: mesoderm, muscles [219]; *Priapulus caudatus*: anterior midgut [135] *Terebratalia transversa*: gastrula mesoderm & endoderm, midgut, paired lateral mesoderm, chaetal sac mesoderm [234]				
tbx4/5	Zebrafish: eye, fin buds, heart tube [235,236] *Xenopus*: 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) *Clytia hemisphaerica*:		

	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] *Aurelia aurita*: broad rhopalar and velar arm endoderm [111]		
tbx1/10; org-1	Mouse (ONLY tbx1): pharyngeal endoderm, otic vesicle, lung endoderm, sclerotome, hindbrain, secondary heart field [181,239] [240] Zebrafish: head & lateral plate mesoderm, pharygeal endoderm, pharyngeal arch cardiac	*Amphioxus*: ventral somites, ventral branchial arch mesoderm & endoderm, pharynx, axial & paraxial mesoderm [243]	*Drosophila*: visceral and somatic muscle progenitor cells, adult heart muscle [244,245]	*Schmidtea polychroa*: dorsal parenchymal cells [211]		*Nematostella*: larval endoderm, body wall endoderm, parietal muscle, somatic gonad (this paper)		

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

	pharyngeal endoderm [255,256] *Xenopus*: 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] *Sepia officinalis*: cardiac & somatic muscles [265] *Terebratalia transversa*: no larval expression [234] *Cupiennius salei*: dorso-lateral edge of embryo (heart precursor cells) & developing heart [212] *Tribolium casteneum*: dorso-lateral edge of germ band (heart precursor) & developing heart	at oral pole (this paper)			

islet	See above	See above	See above	See above	See above	See above	See above	See above

Genes involved in visceral mesoderm development

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

	[273] *Xenopus*: 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] *Xenopus*: cranial sensory placodes, somites, head mesenchyme, abdominal muscle precursors [281] Mouse: Brain,	*Branchiostoma*: anterodorsal mesendoderm, notochord, somites, neural plate, pharyngeal endoderm [285]	*Drosophila*: procephalic lobes, ventral nerve chord, trunk mesoderm, restricts to ventral and lateral mesoderm, then to somatic gonadal precursors [286-288]			*Nematostella*: 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	*Drosophila*	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
eyes absent / eya / clift	Zebrafish: cranial placodes, somites, fins, branchial arches [289] *Xenopus*: somites, hypaxial muscle precursors, cranial placodes [290] Mouse: cranial placodes, branchial arches, CNS, eye, head mesenchyme, somites [291,292]	*Branchiostoma*: anterodorsal mesendoderm, pharyngeal endoderm, notochord, posterior somites, neural plate [285] *Strongylocentrotus purpuratus*: mesoderm, coelomic pouch, non-myoblast mesoderm [278,293] *Strongylocentrotus purpuratus*: secondary mesenchyme cells, coelomic pouch [293] *Patiria miniata*: secondary mesenchyme	*Drosophila*: broad mesoderm, somatic gonadal precursors, eye [294,295]	*Terebratalia transversa*: animal cap ectoderm, dorso-lateral anterior ectoderm, anterior mesoderm [234] *Dugesia japonica*: eyes, CNS, parenchyma [296]		*Nematostella*: broad endoderm (this paper)		*Sycon ciliatum*: choanocytes [297]

		cells, coelomic pouch [293]						
six1/2 / sine oculis	Zebrafish: cranial sensory placode, brain, somites, ventral abdomen & cranial muscles, fins [298] *Xenopus*: 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]	*Branchiostoma*: anterior mesendoderm, somites, pharyngeal endoderm [285] *Strongylocentrotus purpuratus*: secondary mesenchyme cells, coelomic pouch, non-myoblast mesoderm [278,293]	*Drosophila*: optic lobe, eye, ventral head mesoderm, ectodermal segment boundaries [300,301]	*Terebratalia transversa*: gastrula: anterior mesoderm, spots of lateral ectoderm. Larva: broadly in mesoderm, ectoderm of mantle lobe [234]	*Isodiametra pulchra*: brain (?), female genital organ, anterior myocytes, neoblasts [104]	*Nematostella*: pharyngeal ectoderm & endoderm, single cells in retractor muscle and parietal muscle regions (this paper)		

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

				spots, ganglia, pedicle lobe [234]				
lbx1 / lbx2 / ladybird early / ladybird late	Mouse: spinal chord, hindbrain, eye, limb myogenic cells, urogenital ridge [311,312] *Xenopus*: 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]		*Drosophila*: neuroblasts, cardioblasts, pericardial cell precursors, somatic myoblasts, terminal regions [316-318]	*Platynereis*: stripes of putative neuroblasts in ventro-lateral ectoderm and putative myoblsts in lateral mesoderm [264]		*Nematostella*: larva: aboral endoderm, mesenteries; polyp: pharyngeal endoderm (this paper)		

Genes involved in somite development

Gene	Vertebrate	Other deuterostomes	*Drosophila*	Other protostomes	Acoela	Cnidarians	Ctenophores	Sponges
paraxis/scleraxis	Zebrafish: presomitic mesoderm, somite [319] *Xenopus*: presomitic mesoderm, somites, head mesoderm, neural tube [320] Mouse: presomitic, paraxial mesoderm, somite [321,322]	*Branchiostoma*: posterior mesendoderm, tailbud, anterior endoderm [323]	–	*Platynereis*: ventrol-lateral mesoderm [324] *Terebratalia transversa*: two ventro-medial stripes & posterior mesoderm, [234]		*Nematostella*: pharyngeal endoderm (this paper)		
tbx15/18/22	Zebrafish: developing presomitic & somitic mesoderm, heart mesenhyme, paraxial head mesenchyme, ventral neuroectoderm	*Branchiostoma*: pair of mesendoderm stripes, somites [323,329]	–			*Nematostella*: pharyngeal endoderm (this paper)		

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

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

	somites, tailbud [344] Zebrafish: early somite, appendicular muscles, vascular-associated cells [345]			pharynx ectomesoderm [337] *Haliotis*: paraxial, myogenic mesoderm bands [347]				

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

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

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

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

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

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

Nv pancreatic lipase 1		This paper (Ext. Data Figure 2)	LT795567
Nv pancreatic lipase 2		This paper (Ext. Data Figure 2)	LT795568
Nv lysosomal lipase 1		This paper (Ext. Data Figure 2)	LT795562
Nv lysosomal lipase 2		This paper (Ext. Data Figure 2)	LT795563
Nv lysosomal lipase 3		This paper (Ext. Data Figure 2)	LT795564
Nv insulin-like prepropeptide 1	*ilp1*	This paper (Ext. Data Figure 4)	LT795557
Nv insulin-like prepropeptide 2	*Ilp2*	This paper (Ext. Data Figure 4)	LT795558
Nv insulin-like prepropeptide 3	*Ilp3*	This paper (Ext. Data Figure 4)	LT795559
Nv Insulin receptor	*insR*	This paper (Ext. Data Figure 4)	LT795560
Nv mucin		This paper (Ext. Data Figure 2)	LT795565
Nv elaV	*NvELAV1*	[369]	LT795588

A. aurita

Aa foxA		[111]	LN611630.1
Aa trypsin		This paper (Ext. Data Figure 2)	LT795580
Aa chitinase		This paper (Ext. Data Figure 2)	LT795577
Aa pancreatic lipase		This paper (Ext. Data Figure 2)	LT795579
Aa insulin-like prepropeptide 1	*Ilp1*	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
Homo sapiens	Mucin-5AC	NP_001291288		
Homo sapiens	Mucin-5B	NP_002449.2		
Hydra magnipapillata	Mucin	Hma2.225482		
Drosophila melanogaster	Hemolectin, isoform B	NP_001261809.1		
Nematostella vectensis	Mucin	NVE16870 & NVE16871 & NVE16872		

Chitinase

Species name	Gene name	Accession number	Gene model name	Scaffold coordinates
Amphimedon queenslandica	Chitinase1		Aqu1.229503	
Branchiostoma floridae	Chitinase1		fgenesh2_pg.scaffold_94000128	
Branchiostoma floridae	Chitinase2		estExt_fgenesh2_pg.C_800022	
Branchiostoma floridae	Chitinase3		fgenesh2_pg.scaffold_23000018	
Branchiostoma floridae	Chitinase4		estExt_gwp.C_800027	
Branchiostoma floridae	Chitinase5		e_gw.80.73.1	
Branchiostoma floridae	Chitinase6		fgenesh2_pg.scaffold_148000024	
Branchiostoma floridae	Chitinase7		fgenesh2_pg.scaffold_119000091	
Mus musculus	Acidic Chitinase	AAG60018.1		

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

Trypsin

Species name	Gene name	Accession number	Gene model name	Scaffold coordinates
Homo sapiens	Ovocyhmase-like	XP_011518941.1		
Homo sapiens	Trypsin-1	AAI28227.1		
Homo sapiens	Chymotrypsin-1	NP_001897.4		

Homo sapiens	Elastase	S70439		
Homo sapiens	Enteropeptidase	AAB37317.1		
Nematostella vectensis	TrypsinA		NVE4603	scaffold_149:423833-4331735
Nematostella vectensis	TrypsinB		scaffold_212.26 (GeneScan Model)	scaffold_212:345517-357008
Nematostella vectensis	TrypsinC		NVE23587	scaffold_77:595511-597869
Nematostella vectensis	TrypsinD		NVE26085	scaffold_98:347697-358091
Nematostella vectensis	TrypsinE		NVE26086	scaffold_98:358618-374311
Nematostella vectensis	TrypsinF		fgenesh1_pg.scaffold_59000100	scaffold_59:1043846-1050902
Nematostella vectensis	TrypsinG		NVE16289	scaffold_4:77656-84822
Nematostella vectensis	TrypsinH		NVE20133	scaffold_55:481055-484207
Nematostella vectensis	TrypsinI		NVE15608	scaffold_373:66006-71643
Nematostella vectensis	Ovochymase-like		NVE1216	scaffold_11:180697-187994
Nematostella vectensis	TMPRSS9-like		fgenesh1_pg.scaffold_239000008	scaffold_239:117045-137009
Aurelia aurita	TrypsinA			CL311Contig4, comp107629_c7_seq3
Aurelia aurita	TrypsinB			CL1Contig7036

Pancreatic & lysosomal lipase

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

Nematostella vectensis	lysosomal lipase 4		estExt_GenewiseH_1.C_1200028	scaffold_120:159376-173772
Nematostella vectensis	lysosomal lipase 5		fgenesh1_pg.scaffold_339000003	scaffold_339:9333-15083
Mus musculus	hepatic triacylglycerol lipase (Hep. TAG Lip.)		NP_032306.2	
Mus musculus	pancreatic triacylglycerol lipase (Pan. Lip.)		NP_081201.2	
Mus musculus	pancreatic lipase-related protein 1 (Pan. Lip.-rel.1)		NP_061362.1	
Mus musculus	pancreatic lipase-related protein 2 (Pan. Lip.-rel.2)		NP_035258.2	
Nematostella vectensis	Pancreatic Lipase 1		NVE22447	scaffold_7:917415-930038
Nematostella vectensis	Pancreatic Lipase 2		NVE1082	scaffold_108:281416-288384
Nematostella vectensis	Pancreatic Lipase 3		NVE6324	scaffold_171:227079-237363
Nematostella vectensis	Pancreatic Lipase 4		fgenesh1_pg.scaffold_32000021	scaffold_32:263826-272292
Acropora digitifera	Pancreatic Lipase 1		aug_v2a.09961.t1	
Acropora digitifera	Pancreatic Lipase 2		aug_v2a.09322.t1	
Acropora digitifera	Pancreatic Lipase 3		aug_v2a.12434.t1	
Acropora digitifera	Pancreatic Lipase 4		aug_v2a.00019.t1	
Acropora digitifera	Pancreatic Lipase 5		aug_v2a.00020.t1	
Acropora digitifera	Pancreatic Lipase 6		aug_v2a.13757.t1	
Acropora millepora	Pancreatic Lipase		ACF05269.1	
Capsaspora owczarzaki	Lysosomal Acid Lipase/Cholesteryl Ester Hydrolase (Lys. Lip.)		CAOG_04798.2	
Capsaspora owczarzaki	Lysosomal Lipase-like (Lip.-like)		CAOG_05206.2	
Capsaspora owczarzaki	Triacylglycerol Lipase (TAG Lip.)		CAOG_06974.2	
Capsaspora owczarzaki	Lipase, family N (Lip. N)		CAOG_06135.2 lipase	
Mortierella verticillata	Triacylglyceride lipase (TAG Lip.)		MVEG_01507.1	
Spizellomyces punctatus	Lipase 1		SPPG_01944.3	
Mucor ambiguus	Lysosomal Acid Lipase Cholesteryl Ester Hydrolase (Lys. Lip.)		GAN00734.1	

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

Insulin-like peptides

Species name	Gene name	Accession number	Gene model name	Scaffold coordinates
Mus musculus	Insulin-1	ABF48502.1		
Mus musculus	Insulin-2	NP_001172013.1		
Mus musculus	Insulin-like growth factor	NP_001104745.1		
Branchiostoma floridae	Insulin-like peptide 1		estExt_fgenesh2_pg.C_410021	scaffold_41:718784-735750
Branchiostoma floridae	Insulin-like peptide 2		fgenesh2_pg.scaffold_73000034	scaffold_73:350818-355411
Nematostella vectensis	Insulin-like peptide 1		fgenesh1_pg.scaffold_81000010	scaffold_81:111202-111979
Nematostella vectensis	Insulin-like peptide 2		fgenesh1_pg.scaffold_14000031	scaffold_14:341281-344623
Nematostella vectensis	Insulin-like peptide 3		NVE3796	scaffold_14:339662-340816
Nematostella vectensis	Insulin-like peptide 4		fgenesh1_pg.scaffold_11000068	scaffold_11:592691-609219
Nematostella vectensis	Insulin-like peptide 6		NVE1259	scaffold_11:604757-609261
Nematostella vectensis	Insulin-like peptide 7		NVE1257	scaffold_11:586257-592951
Nematostella vectensis	Insulin-like peptide 8		NVE2432	scaffold_123:358532-363296
Nematostella vectensis	Insulin-like peptide 9		NVE20730	scaffold_588:27016-31966
Aurelia aurita	Insulin-like peptide 1		Sequence available upon request.	
Aurelia aurita	Insulin-like peptide 2		Sequence available upon request.	
Aurelia aurita	Insulin-like peptide 3		Sequence available upon request.	

Insulin receptor

Species name	Gene name	Accession number	Gene model name	Scaffold coordinates
Nematostella vectensis	Fibroblast growth factor receptor A (FGFR-A)	ABO92763.1		
Nematostella vectensis	Fibroblast growth factor receptor B (FGFR-B)	ABO92762.1		
Nematostella vectensis	Insulin Receptor		NVE2224	scaffold_12:1639644-1656298
Nematostella vectensis	Receptor tyrosine kinase-like orphan receptor (ROR)		fgenesh1_pg.scaffold_9000144	scaffold_9:1406531-1412439
Mus musculus	Insulin receptor	P15208.1		
Mus musculus	Insulin-like growth factor 1 receptor	P24062.2		
Mus musculus	Receptor tyrosine kinase-like orphan receptor 1 (ROR1)	Q9Z139.2		
Mus musculus	Receptor tyrosine kinase-like orphan receptor 2 (ROR2)	Q9Z138.2		
Mus musculus	Fibroblast growth factor receptor 1	NP_034336.2		
Mus musculus	Fibroblast growth factor receptor 2	NP_034337.2		
Mus musculus	Fibroblast growth factor receptor 3	NP_001156689.1		
Mus musculus	Fibroblast growth factor receptor 4	NP_032037.2		
Drosophila melanogaster	Insulin receptor	AAC47458.1		
Drosophila melanogaster	Receptor tyrosine kinase-like orphan receptor 1 (ROR1)	AAF52885.1		
Drosophila melanogaster	Receptor tyrosine kinase-like orphan receptor 2 (ROR2)	Q9V6K3.2		
Drosophila melanogaster	FGF receptor breathless (Btl)	AAX52746.1		
Drosophila melanogaster	FGF receptor heartless (Htl)	NP_732287.1		

Sequences of oligos used for cloning

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

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

Aurelia aurita genes

chitinase	CGATTCTTCTACTCGGCCTGGC	TGGTGTTACATCCCTCCCTAAAGC
ilp1	CCTAACTAAAGGTCACCATGCCTCG	CTTATATGAACCCCACACACTGGCAG
pancreatic lipase	GGTGTTGCTTGCATATTTTGCAGAGG	ATGGTGCTACCCCAGCATCG
trypsin	ATCCAGTGATGAAGCATTGCGTTCC	GCAGAGTTCCTCCAACATTGACAGC

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