Supporting Information

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SI Materials and Methods

Whole-Mount in Situ Hybridization. Using 1-d starved *Hydra* 105 animals, the experiment was performed using a protocol described previously (1). Fifty nanograms of digoxigenin (DIG)-labeled antisense probes was added per milliliter of hybridization solution. Double in situ hybridization of whole-mount *Hydra* was performed according to the method described by Hansen et al. (2). DIG-labeled nematogalectin B/FITC-labeled nematogalectin A antisense probes were used at concentrations of 50 and 100 ng/mL, respectively. DIG-labeled animals were subjected to nitro blue tetrazolium (NBT)/5-bromo-4-chloro-3-indolyl phosphate (BCIP) staining, and FITC-labeled animals were subjected to Fast Red staining. Medusae of *Clytia* were also used for in situ hybridization, as described by Chevalier et al. (3). Two *Clytia* EST clones, L0ABA6YM11 (accession no. AM753667) and IL0ABA24YB15 (accession no. AM757858) were used to synthesize DIG-labeled and FITC-labeled antisense probes, respectively. For hybridization, 100 ng/mL DIG-labeled probe or 200 ng/mL FITC-labeled probe was incubated with the medusae. Double whole-mount in situ hybridization of *Clytia* was modified as described by Hansen et al. (2).

Construction of Protein Expression Vector. The C-terminal half of nematogalectin A (residues 129–294) and the truncated nematogalectin B (residues 162–276) were separately cloned into the *Not*I and *Sal*I sites of the expression vector pET28a (Novagen) for the overexpression in *Escherichia coli* BL21 (DE3). His-tagged recombinant proteins were purified through a nickel resin and then isolated from 12% (wt/vol) SDS/PAGE. Desired fragments of nematogalectin A and B isolated from the gel were used to raise polyclonal antibodies in a rat and a chicken, respectively (OPERON Biotechnologies). Rat IgG was purified with protein G, and chicken antibody was affinity-purified with recombinant antigen.

Western Blot Analyses. For the Western blot analyses, *Hydra* cell lysate was obtained from dissociated whole-animal cells. Capsules were prepared by dissolving isolated tentacles in 0.1% SDS at room temperature (RT) for 15 min and then passing the solution through a 0.8-μm pore size polyethersulfone filter membrane (Vivascience AG). All protein samples were then mixed in SDS sample buffer and heated at 95 °C for 15 min before loading to 12% (wt/vol) SDS/PAGE. The Western blot procedure was performed as described previously (4), and 1:2,500 and 1:1,250 dilutions were subjected for the anti-nematogalectin A and anti-nematogalectin B polyclonal antibodies, respectively. For the DTT treatment, isolated capsules were incubated with 100 mM Tris HCl (pH 9.5) containing 0-, 0.05-, 0.1-, 0.5-, 1-, and 2-mM concentrations of DTT at RT for 1 h. To separate DTT-soluble and -insoluble proteins, samples were centrifuged at 10,000 × *g* for 5 min. The soluble supernatant and the insoluble pellet were boiled and separated on SDS/PAGE, as described above. To examine the oligomerization of nematogalectin A, isolated capsules were incubated with various concentrations of β-mercaptoethanol for 10 min either at RT or at 100 °C. All treated capsules were analyzed using nonreducing gel electrophoresis and Western blotting.

Immunofluorescence and Immunogold EM. Except for immunofluorescence of atrichous isorhiza, for which the holotrichous isorhiza-free strain nem-3 is used (4), all other staining experiments used the WT strain 105. Antibodies used in immunofluorescence staining were as follows: rat anti-nematogalectin A antibody (1:250 dilution), chicken anti-nematogalectin B antibody (1:125 dilution), rabbit anti-minicollagen-1 antiserum (1:500 dilution), and rabbit anti-minicollagen-15 antibody (1:500 dilution). Rat anti-nematogalectin A antibody and anti-rat–conjugated colloidal gold particles used in the immunogold EM were adjusted to dilutions of 1:100 and 1:200, respectively, to obtain a good signal-to-background ratio.

1. Grens A, Gee L, Fisher DA, Bode HR (1996) CnNK-2, an NK-2 homeobox gene, has a role in patterning the basal end of the axis in *hydra*. *Dev Biol* 180:473–488.
2. Hansen GN, Williamson M, Grimmelikhuijzen CJP (2000) Two-color double-labeling in situ hybridization of whole-mount Hydra using RNA probes for five different Hydra neuropeptide preprohormones: Evidence for colocalization. *Cell Tissue Res* 301: 245–253.
3. Chevalier S, Martin A, Leclère L, Amiel A, Houliston E (2006) Polarised expression of FoxB and FoxQ2 genes during development of the hydrozoan *Clytia hemisphaerica*. *Dev Genes Evol* 216:709–720.
4. Sugiyama T, Fujisawa T (1977) Genetic analysis of developmental mechanisms in hydra. I. Sexual reproduction of *Hydra magnipapillata* and isolation of mutant. *Dev Growth Differ* 19:187–200.

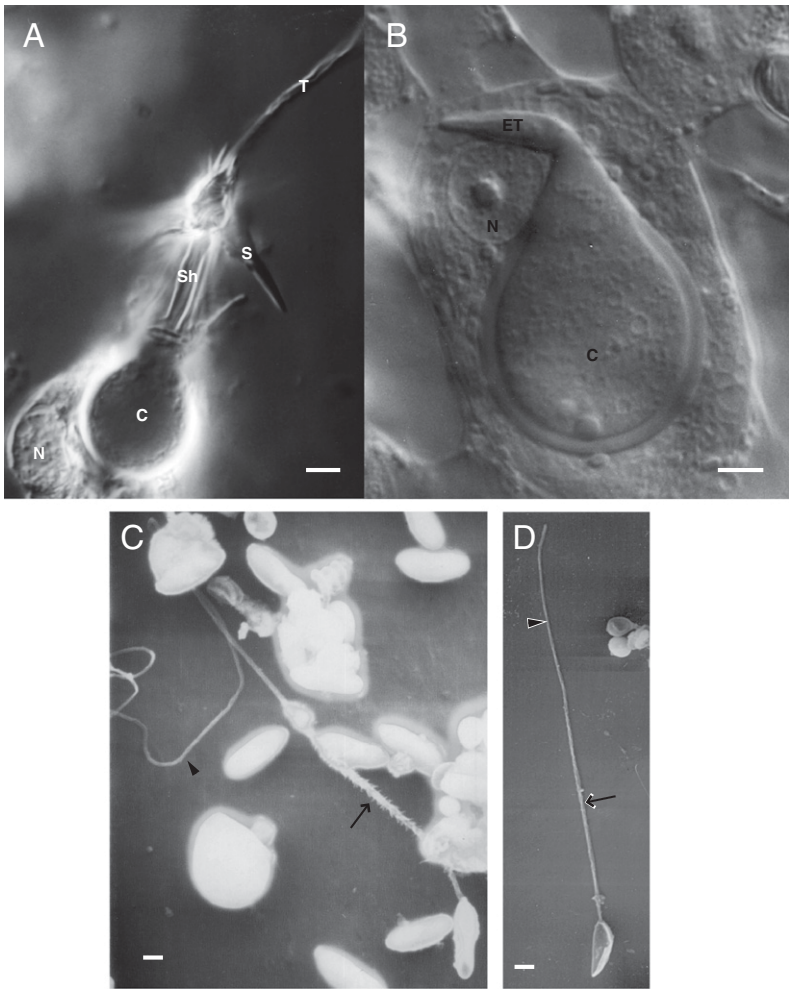

Fig. S1. Nomarski images of a discharged stenotele showing the proximal shaft armed with spines and the proximal end of the long thin distal tubule (*A*) and a differentiating nematocyte showing an early stage in the elongation of the external tubule (*B*). C, capsule; ET, external tubule; N, nucleus; S, spine; Sh, shaft; T, tubule. SEM images of a discharged holotrichous isorhiza with spines anchoring at the proximal tubule (arrow) and a smooth surface at the distal tubule (arrowhead) (*C*), and a discharged atrichous isorhiza having twisted ridges at the proximal tubule, which may correspond to the attachment sides for spines (arrow) as compared with the distal end (arrowhead) (*D*). (Scale bar: 2 μm.)

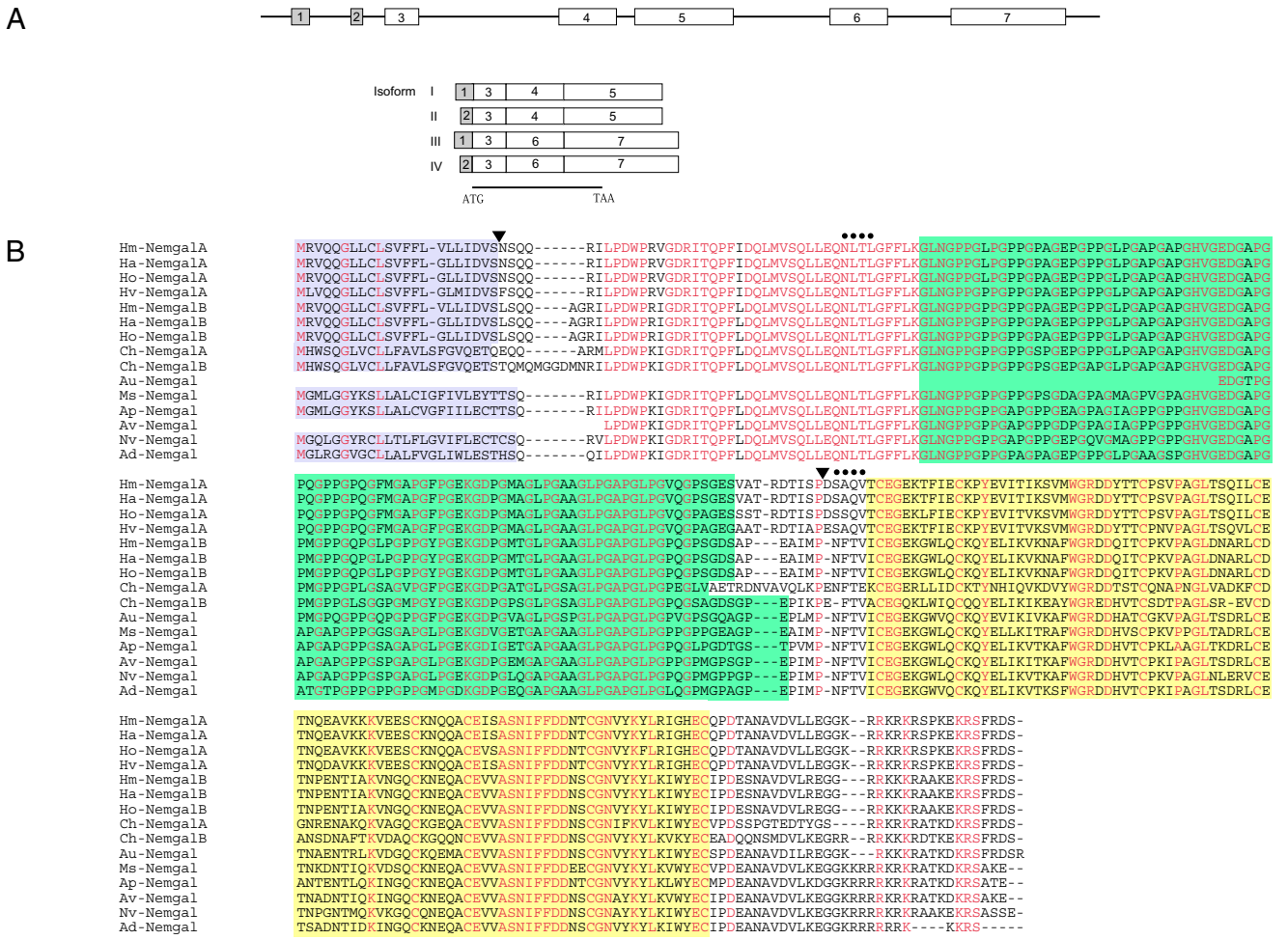

Fig. S2. *Hydra* and cnidarian nematogalectins. (*A*) Schematic drawing of four nematogalectin transcripts (I–IV) generated by alternative splicing. Exons are represented as boxes, and introns are represented as thin lines. Exons 1 and 2 are noncoding exons (gray-shaded boxes). A start codon is found in exon 3, and a stop codon is found in both exons 5 and 7. Transcripts I and II generate nematogalectin B, whereas transcripts III and IV generate nematogalectin A. (*B*) Multiple sequence alignment was done using the program ClustalX. Nematogalectin proteins contain a signal peptide (blue-shaded box) at the N terminus, repeats of the tripeptide GlyXY (green-shaded box) in the center, and a sugar-binding galectin domain (yellow-shaded box) at the C terminus. Identical amino acids are colored in red. (●●●●) N-glycosylation site predicted by the NetNGlyc 1.0 server (Center for Biological Sequence Analysis, Technical University of Denmark). (▼) Exon 3/4 and exon 4/5 boundaries of *Hydra* nematogalectin B and exon 3/6 and exon 6/7 boundaries of *Hydra* nematogalectin A. Ad, *Acropora millepora*, Ap, *Aiptasia pallida*; Au, *Aurelia aurita*; Av, *Anemonia viridis*, Ch, *Clytia hemisphaerica*, Ha, *Hydra vulgaris*; Hm, *Hydra magnipapillata*, Ho, *Hydra oligactis*; Hv, *Hydra viridis*; Ms, *Metridium senile*, Nv, *Nematostella vectensis*. The missing sequences at the N terminus of *Anemonia* and *Aurelia* are attributable to the incomplete EST sequences.

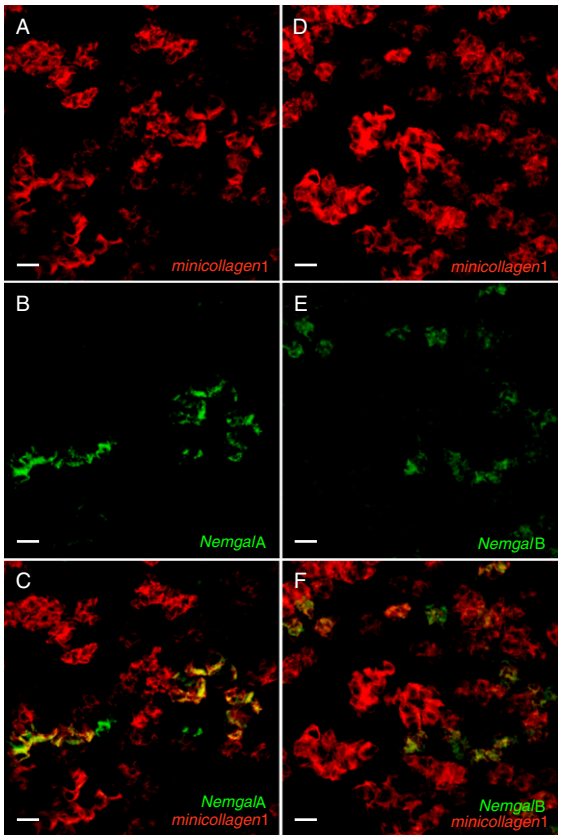

Fig. S3. Double whole-mount in situ hybridization of *Hydra*. Fluorescent signal is observed in the differentiating nematocytes of the gastric region. (*A–C*) Coexpression analysis of minicollagen-1 transcript (red) and nematogalectin A transcript (green). (*D–F*) Coexpression analysis of minicollagen-1 transcript (red) and nematogalectin B transcript (green). (Scale bar: 10 μm.)

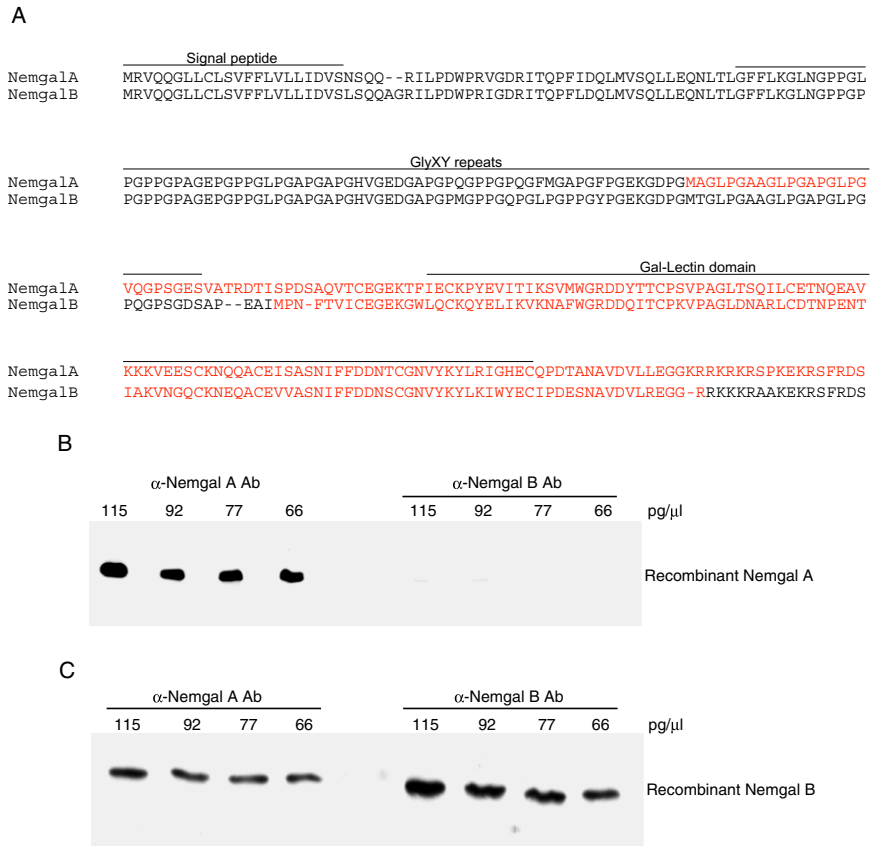

Fig. S4. Determination of specificity of anti-nematogalectin A and B antibodies. (*A*) Truncated fragments of nematogalectin A and B (highlighted in red) were expressed in *Escherichia coli* to produce recombinant proteins, which were then used to raise anti-nematogalectin polyclonal antibodies. (*B* and *C*) Mature nematogalectin A and B proteins (excluding the signal peptide) were expressed in *E. coli*, and an equal amount of bacterial lysates was loaded into each lane. Serial dilutions of anti-nematogalectin A and B antibodies (115, 92, 77, and 66 pg/μL) were used for Western blots. (*B*) Recombinant nematogalectin A. (*C*) Recombinant nematogalectin B.

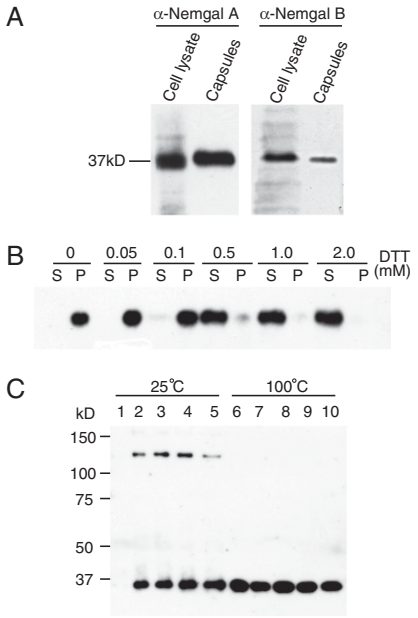

Fig. S5. Western blot analysis using antibodies against *Hydra* nematogalectin A and B. (*A*) A 37-kDa band was detected in total cell lysates and capsules. (*B*) Isolated capsules were dissolved in different concentrations of DTT (0–2 mM). Dissolved [supernatant (S)] and undissolved [pellet (P)] proteins were detected using nematogalectin A antibody. (*C*) Isolated capsules were treated with different concentrations of β-mercaptoethanol at 25 °C and 100 °C. Lanes 1 and 6, 0 mM; lanes 2 and 7, 25 mM; lanes 3 and 8, 100 mM; lanes 4 and 9, 250 mM; lanes 5 and 10, 500 mM.

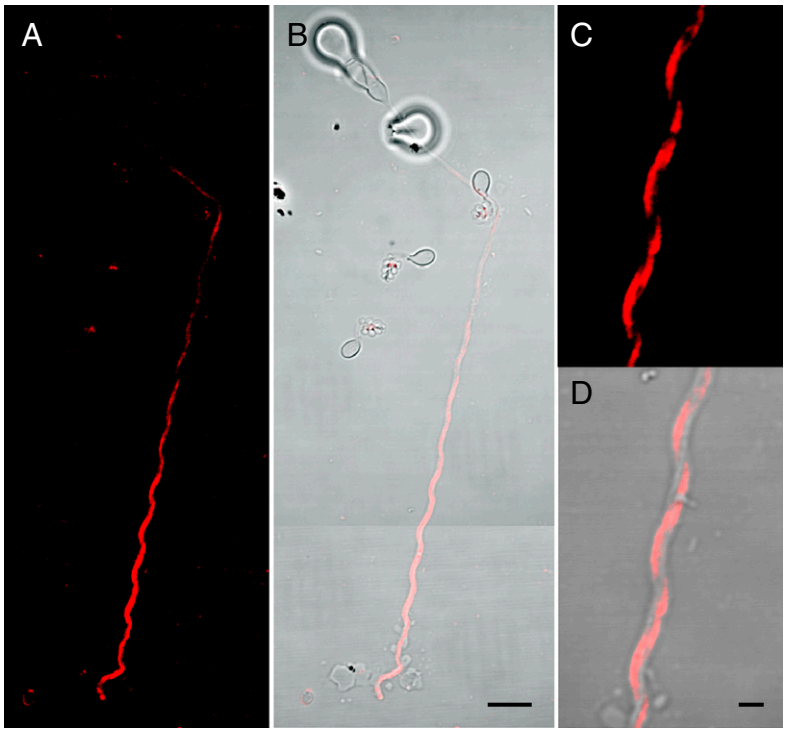

Fig. S6. Immunofluorescence of discharged stenotele showing the localization of nematogalectin A staining on the tubule. Fluorescent staining (*A*), merged fluorescence and phase contrast image (*B*), and high-magnification images of the distal part of the tubule shown in *A* and *B* (*C* and *D*). (*B*) Two exposures of the same image were combined to improve clarity. (Scale bars: *B*, 10 μm; *D*, 2 μm.)

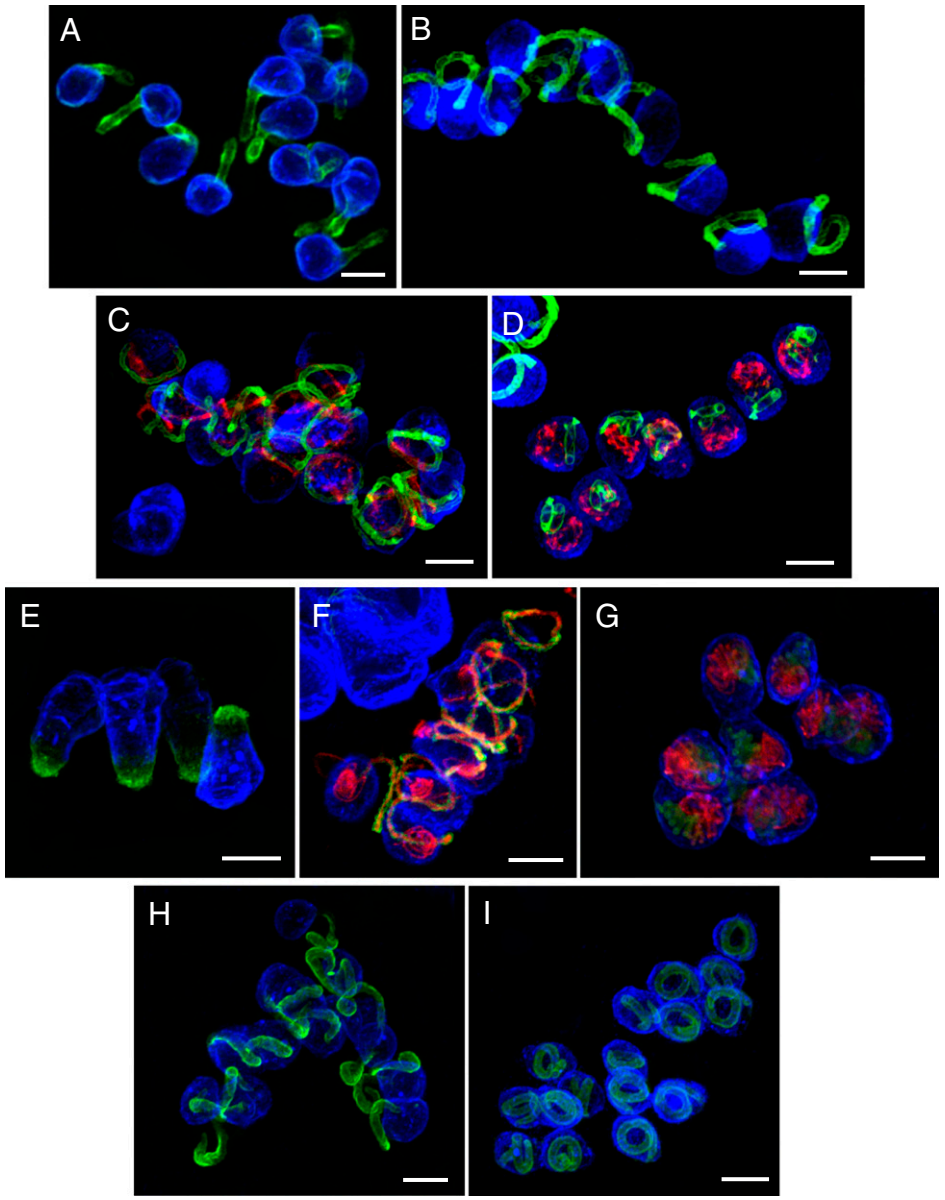

Fig. S7. Immunostaining of isorhizas and desmoneme with nematogalectin A and B antibodies. (*A*–*D*) Confocal images of differentiating atrichous isorhizas. (*A*) Only nematogalectin B appears at the beginning of tubule protrusion. The nematogalectin B-stained tubules continue to elongate (*B*), and very soon after this stage, the capsule starts to create a nematogalectin A-stained tubule (*C*). The invaginated tubule shows nematogalectin A and B at the distal and the proximal ends, respectively (*D*). (*E*–*G*) Confocal images of differentiating holotrichous isorhizas. (*H*–*I*) Confocal images of differentiating desmonemes. Only nematogalectin B antibody stains the tubule. Red signal, nematogalectin A; green signal, nematogalectin B; blue signal, minicollagen-1. (Scale bar: 5 μm.)

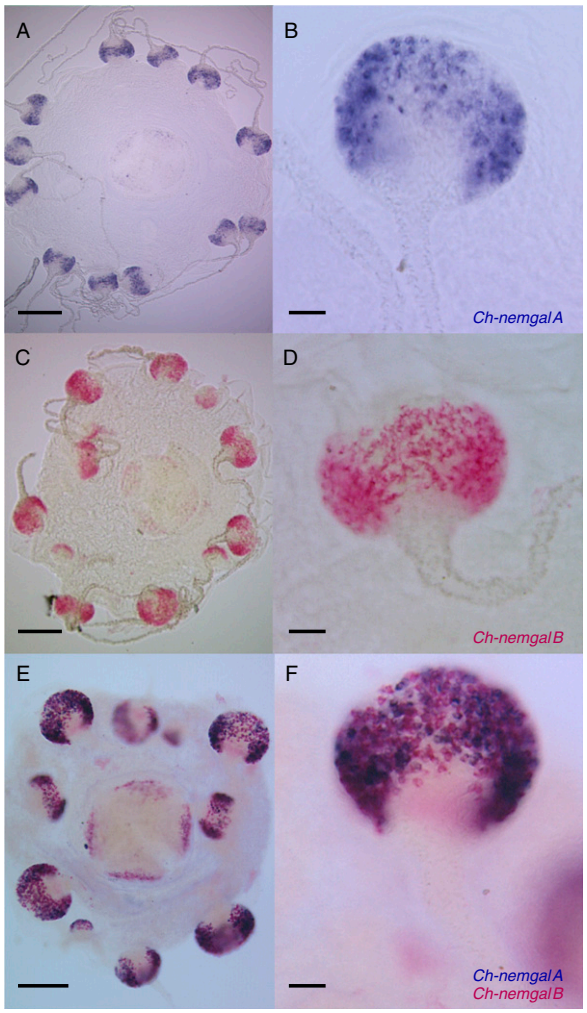

Fig. S8. Whole-mount in situ hybridization of *Clytia* medusa. (*A* and *B*) DIG-conjugated RNA probe of Ch-nemgalA (blue) stains differentiating nematocytes in the tentacle bulb. (*C* and *D*) FITC-conjugated RNA probe of Ch-nemgalB (red) also stains differentiating nematocytes in the tentacle bulb. (*E* and *F*) Double staining with Ch-nemgalA and Ch-nemgalB RNA probes shows that most nests express only one nematogalectin gene. (*A, C,* and *E*) Bottom view of medusa. (*B, D,* and *F*) Magnified view of tentacle bulb from the outside. (Scale bars: *A, C,* and *E*, 250 μm; *B, D,* and *F*, 25 μm.)

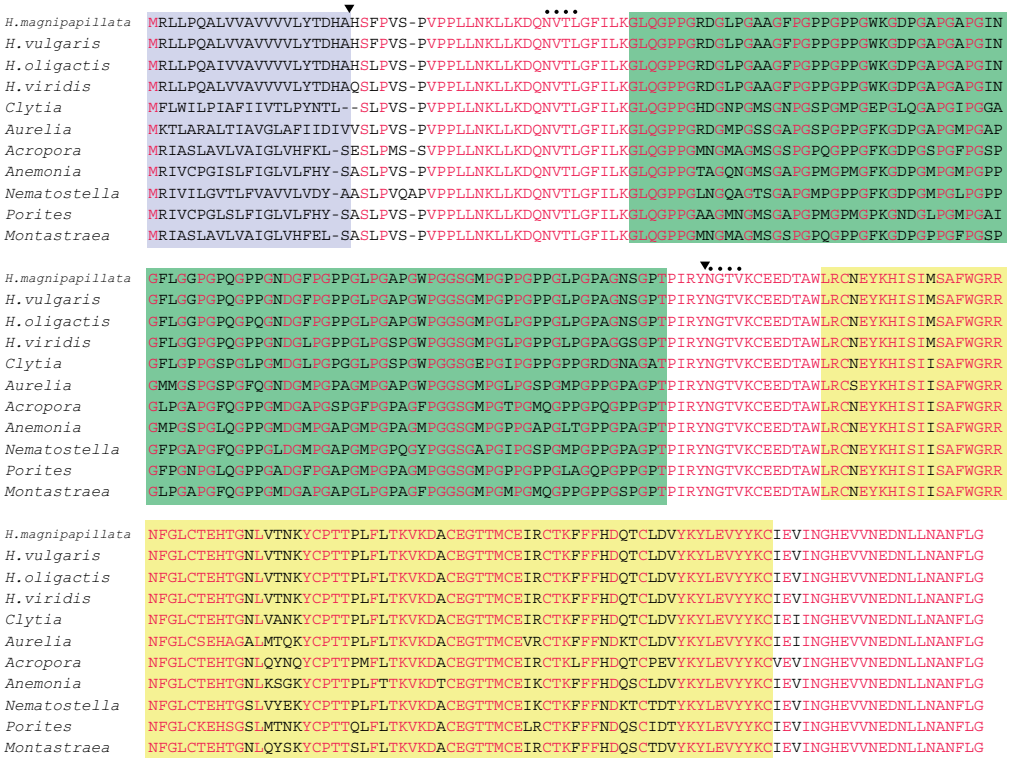

Fig. S9. Multiple sequence alignment of nematogalectin-related proteins derived from 11 cnidarian species. Identical residues are highlighted in red. (●●●●) Putative N-glycosylation sites are marked. (▼) Boundaries between exons in nematogalectin-related sequences of *Hydra* and *Nematostella*. Signal peptide is shaded in blue, GlyXY repeats are shaded in green, and galectin domain is shaded in yellow.

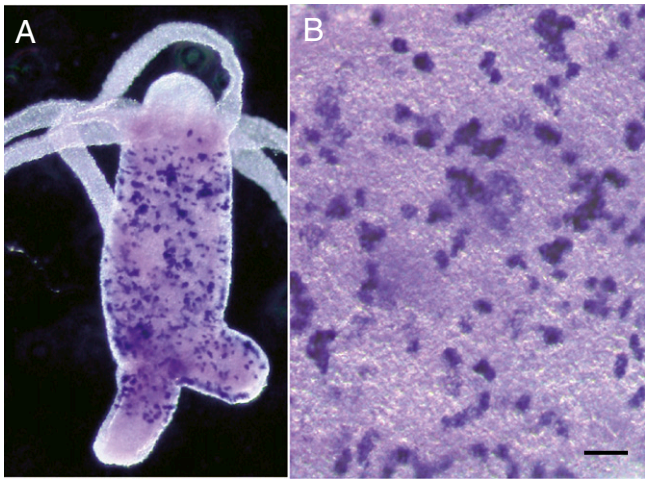

Fig. S10. Whole-mount in situ hybridization using antisense RNA probe of *Hydra* nematogalectin-related. Polyp (*A*) and higher magnification at the body column (*B*). (Scale bar: 50 μm.)