# A cnidarian homologue of an insect gustatory receptor functions in developmental body patterning

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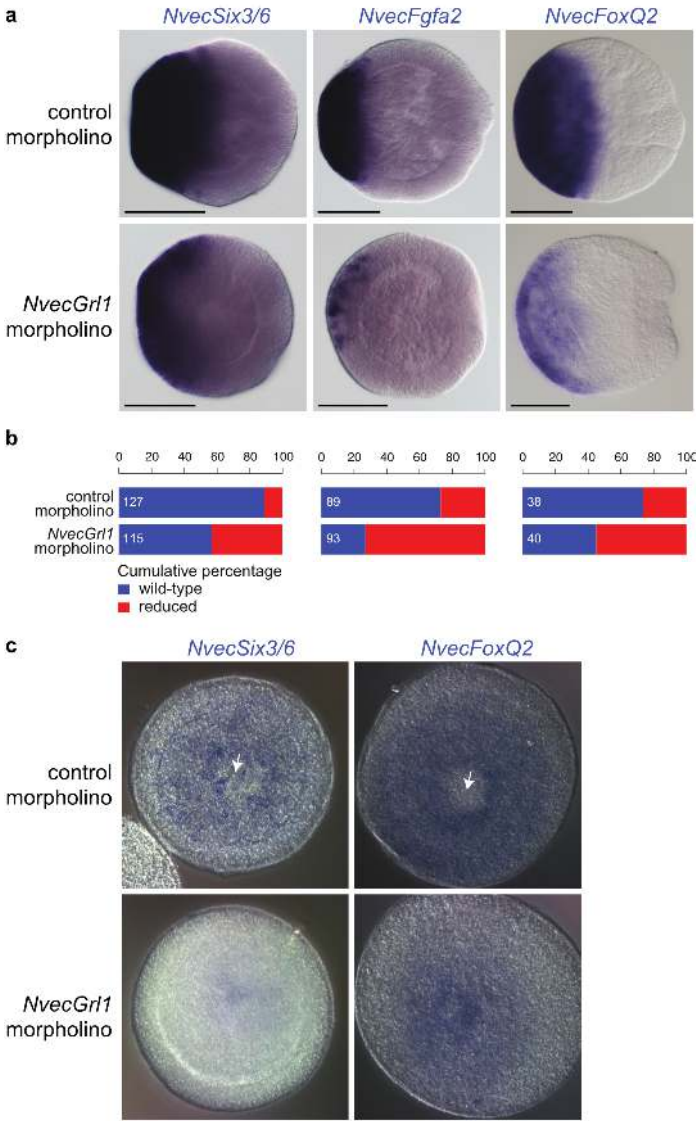
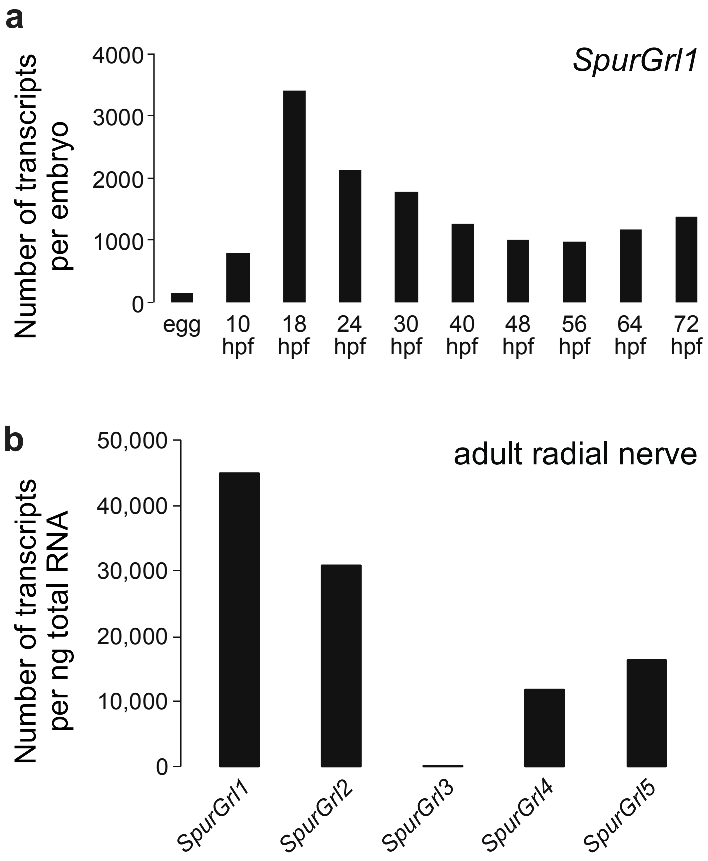
## Supplementary Information

**Supplementary Figure 1| *In situ* expression analysis of *NvecGrl2***
RNA in situ hybridisation using a riboprobe against *NvecGrl2* on a whole mount *N. vectensis* gastrula-stage embryo. No signal is detectable. Scale bar = 50 μm.



**Supplementary Figure 2 Gene expression defects in *NvecGrl1* morphants.**
(a) RNA in situ hybridisation using riboprobes against marker genes in animals injected with control (control#2) or *NvecGrl1* (*NvecGrl1#2*) morpholino oligonucleotides. The arboral side is on the left in all specimens. Quantification of gene expression phenotypes of the morphants is shown below. The n for each marker/morpholino combination is shown in white in each bar. Scale bars = 100 μm.
(b) Arboral view of animals injected, as in Fig. 5, with either control (control#1) or *NvecGrl1* (*NvecGrl1#1*) morpholino oligonucleotides subjected to RNA in situ hybridisation using probes against the indicated markers. The arrows mark the "apical spot", where expression of *NvecSix3/6* and *NvecFoxQ2* are excluded, which precedes the appearance of the apical organ in this region[1].



**Supplementary Figure 3| Gene expression analysis of *S. purpuratus Grls*.**
(a) Developmental analysis of transcripts levels by RNA-seq of *SpurGrl1* from www.spbase.org:3838/quantdev/[2].
(b) Quantitative RT-PCR analysis of the five *SpurGrl* genes in the adult radial nerve, a prominent structure in the sea urchin nervous system. *SpurGrl3* is expressed at low, but above-background, levels.

## Supplementary Table 1| Grl nomenclature and source data.

| Species | Protein name | Source accession number | Data source |
|---|---|---|---|
| *Capitella teleta* | *Ctel*Grl1 | ELT92320 | [3] |
| | *Ctel*Grl2 | ELT92788 | |
| | *Ctel*Grl3 | ELT90076 | |
| | *Ctel*Grl4 | ELU10797 | |
| | *Ctel*Grl5 | ELT90075 | |
| | *Ctel*Grl6 | ELU14052 | |
| | *Ctel*Grl7 | ELT97787 | |
| | *Ctel*Grl8 | ELU10343 | |
| | *Ctel*Grl9 | ELT92997 | |
| | *Ctel*Grl10 | ELT90557 | |
| | *Ctel*Grl11 | ELU06252 | |
| | *Ctel*Grl12 | ELU13825 | |
| *Helobdella robusta* | *Hrob*Grl1 | 163318 | [3] |
| | *Hrob*Grl2 | 163317 | |
| *Lottia gigantea* | *Lgig*Grl1 | 168741 | [3] |
| | *Lgig*Grl2 | 171766 | |
| | *Lgig*Grl3 | 152656 | |
| | *Lgig*Grl4 | 159388 | |
| | *Lgig*Grl5 | 172086 | |
| | *Lgig*Grl6 | 160675 | |
| | *Lgig*Grl7 | 159430 | |
| *Crassostrea gigas* | *Cgig*Grl1 | EKC21365 | [4] |
| | *Cgig*Grl2 | EKC32937 | |
| *Aplysia californica* | *Acal*Grl1 | XP_005105989.1 | Broad Institute, Harvard |
| | *Acal*Grl2 | XP_005105990.1 | |
| | *Acal*Grl3 | XP_005099561.1 | |
| *Saccoglossus kowalevskii* | *Skow*Grl1 | XM_002731392 | Baylor College of Medicine HGSC |
| *Strongylocentrotus purpuratus* | *Spur*Grl1 | XM_003730711 | [5] |
| | *Spur*Grl2 | Ctg127547 | |
| | *Spur*Grl3 | Ctg127545-2 | |
| | *Spur*Grl4 | Ctg127545-1 | |
| | *Spur*Grl5 | Ctg131000 | |
| *Lytechinus variegatus* | *Lvar*Grl1 | Contig203256 | Baylor College of Medicine HGSC |
| | *Lvar*Grl2 | Contig339833 | |
| | *Lvar*Grl3 | Contig203252 | |
| | *Lvar*Grl4 | Contig122808 | |
| | *Lvar*Grl5 | Contig122810 | |
| | *Lvar*Grl6 | Contig203253 | |
| | *Lvar*Grl7 | Contig17632 | |
| *Patiria miniata* | *Pmin*Grl1 | HP136897.1 | Baylor College of Medicine HGSC |
| *Acropora digitifera* | *Adig*Grl1 | adi_EST_assem_4631 | [6] |
| *Acropora millepora* | *Amil*Grl1 | EZ016165.1 | The Genome Institute at Washington University |
| *Nematostella vectensis* | *Nvec*Grl1 | KP294348 | [7] and this work |
| | *Nvec*Grl2 | KP294349 | |
| *Trichoplax adhaerens* | *Tadh*Grl1 | XM_002117218 | [8] |
| | *Tadh*Grl2 | XM_002110287 | |
| | *Tadh*Grl3 | XM_002110288 | |

## Supplementary Table 2| qPCR data for *N. vectensis Grl*s.

| **Samples** | ***EF1* CNRQ** | ***EF1* SE(CNRQ)** | ***NvecGrl1* CNRQ** | ***NvecGrl1* SE(CNRQ)** | ***NvecGrl2* CNRQ** | ***NvecGrl2* SE(CNRQ)** | ***RibL* CNRQ** | ***RibL* SE(CNRQ)** |
|---|---|---|---|---|---|---|---|---|
| *unfertilised egg #1* | 1.3501 | 0.0430 | 3.3211 | 0.0623 | 9.0450 | 0.3981 | 0.7407 | 0.0193 |
| *unfertilised egg #2* | 1.2540 | 0.0433 | 3.4095 | 0.1432 | 7.5778 | 0.3533 | 0.7975 | 0.0199 |
| *unfertilised egg #3* | 1.2817 | 0.0187 | 4.2815 | 0.2560 | 7.6899 | 0.1144 | 0.7802 | 0.0164 |
| *early cleavage 6 hpf #1* | 1.2307 | 0.0296 | 5.0873 | 0.2114 | 8.6304 | 0.6532 | 0.8125 | 0.0162 |
| *early cleavage 6 hpf #2* | 1.5165 | 0.1245 | 3.6316 | 0.1626 | 7.3053 | 0.3620 | 0.6594 | 0.0475 |
| *early cleavage 6 hpf #3* | 1.7952 | 0.0413 | 4.3050 | 0.2548 | 9.8793 | 0.2502 | 0.5570 | 0.0122 |
| *early blastula 12 hpf #1* | 1.1916 | 0.0060 | 1.7518 | 0.1207 | 4.1646 | 0.2536 | 0.8392 | 0.0031 |
| *early blastula 12 hpf #2* | 1.1731 | 0.0276 | 1.3881 | 0.0297 | 3.3569 | 0.1833 | 0.8524 | 0.0227 |
| *early blastula 12 hpf #3* | 1.2705 | 0.0569 | 2.1652 | 0.1103 | 4.6190 | 0.2472 | 0.7871 | 0.0363 |
| *late blastula 18 hpf #1* | 0.9684 | 0.0020 | 1.8517 | 0.1059 | 0.6966 | 0.0480 | 1.0326 | 0.0032 |
| *late blastula 18 hpf #2* | 1.0161 | 0.0122 | 1.7343 | 0.0448 | 0.8839 | 0.0467 | 0.9841 | 0.0144 |
| *late blastula 18 hpf #3* | 0.9265 | 0.0155 | 1.7744 | 0.1525 | 1.2228 | 0.0285 | 1.0794 | 0.0179 |
| *gastrula 24 hpf #1* | 0.8781 | 0.0531 | 2.6723 | 0.1012 | 0.5965 | 0.0315 | 1.1388 | 0.0525 |
| *gastrula 24 hpf #2* | 0.9129 | 0.0300 | 2.2372 | 0.0372 | 0.5175 | 0.0345 | 1.0954 | 0.0183 |
| *gastrula 24 hpf #3* | 0.8815 | 0.0030 | 2.9678 | 0.1172 | 0.7310 | 0.0284 | 1.1344 | 0.0034 |
| *early planula 36 hpf #1* | 0.7614 | 0.0125 | 1.1005 | 0.0232 | 0.4279 | 0.0132 | 1.3134 | 0.0322 |
| *early planula 36 hpf #2* | 0.7371 | 0.0462 | 0.9164 | 0.0419 | 0.2371 | 0.0088 | 1.3566 | 0.0607 |
| *early planula 36 hpf #3* | 0.7746 | 0.0161 | 1.3221 | 0.0248 | 0.4478 | 0.0230 | 1.2910 | 0.0291 |
| *planula 48 hpf #1* | 0.7764 | 0.0162 | 0.4054 | 0.0082 | 0.3360 | 0.0176 | 1.2881 | 0.0374 |
| *planula 48 hpf #2* | 0.7013 | 0.0203 | 0.2370 | 0.0057 | 0.1839 | 0.0044 | 1.4259 | 0.0526 |
| *planula 48 hpf #3* | 0.6485 | 0.0419 | 0.5915 | 0.0281 | 0.4001 | 0.0253 | 1.5419 | 0.0819 |
| *metamorphosis #1* | 0.9094 | 0.0392 | 0.1581 | 0.0039 | 0.2448 | 0.0071 | 1.0996 | 0.0369 |
| *metamorphosis #2* | 1.0456 | 0.0306 | 0.1599 | 0.0066 | 0.2789 | 0.0210 | 0.9564 | 0.0440 |
| *metamorphosis #3* | 0.9873 | 0.0345 | 0.1737 | 0.0060 | 0.2718 | 0.0145 | 1.0128 | 0.0238 |
| *primary polyp #1* | 0.9410 | 0.0388 | 0.1319 | 0.0040 | 0.2769 | 0.0118 | 1.0627 | 0.0426 |
| **Reference Target** | **M** | **CV** | | | | | | |
| *EF1* | 0.7060 | 0.258 | | | | | | |
| *RibL* | 0.706 | 0.235 | | | | | | |
| *Average* | 0.706 | 0.247 | | | | | | |

Values are corrected, average, relative quantities of three technical replicates.

## Supplementary Table 3 qPCR data for *S. purpuratus Grl*s.

| **Samples** | ***SpurGrl1*** | ***SpurGrl2*** | ***SpurGrl3*** | ***SpurGrl4*** | ***SpurGrl5*** |
|---|---|---|---|---|---|
| *unfertilised egg #1* | 97 | 0 | 0 | 1 | 1 |
| *unfertilised egg #2* | 93 | 0 | 1 | 0 | 1 |
| *mid-cleavage 7 hpf #1* | 881 | 0 | 0 | 10 | 0 |
| *mid-cleavage 7 hpf #2* | 215 | 0 | 0 | 2 | 3 |
| *late cleavage 12 hpf #1* | 612 | 16 | 1 | 14 | 39 |
| *late cleavage 12 hpf #2* | 1302 | 54 | 7 | 4 | 21 |
| *blastula 18 hpf #1* | 2450 | 0 | 0 | 2 | 26 |
| *blastula 18 hpf #2* | 1959 | 0 | 1 | 0 | 10 |
| *mesenchyme blastula 24 hpf #1* | 1824 | 0 | 0 | 7 | 20 |
| *mesenchyme blastula 24 hpf #2* | 1363 | 4 | 0 | 0 | 12 |
| *early gastrula 30 hpf #1* | 2074 | 0 | 0 | 7 | 19 |
| *early gastrula 30 hpf #2* | 1099 | 0 | 0 | 0 | 14 |
| *mid gastrula 40 hpf #1* | 2139 | 10 | 14 | 23 | 14 |
| *mid gastrula 40 hpf #2* | 1132 | 0 | 1 | 0 | 18 |
| *late gastrula 48 hpf #1* | 1505 | 0 | 0 | 6 | 36 |
| *late gastrula 48 hpf #2* | 1016 | 7 | 8 | 1 | 7 |
| *pluteus 72 hpf #1* | 1454 | 29 | 2 | 22 | 25 |
| *pluteus 72 hpf #2* | 1359 | 28 | 5 | 3 | 20 |

Values are averages of transcripts per embryo in 4 technical replicates.

## Supplementary References

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8. Srivastava M*, et al.* The *Trichoplax* genome and the nature of placozoans. *Nature.* **454**, 955-960 (2008).