

Supplementary Materials for

Nanos2* marks precursors of somatic lineages and is required for germline formation in the sea anemone *Nematostella vectensis

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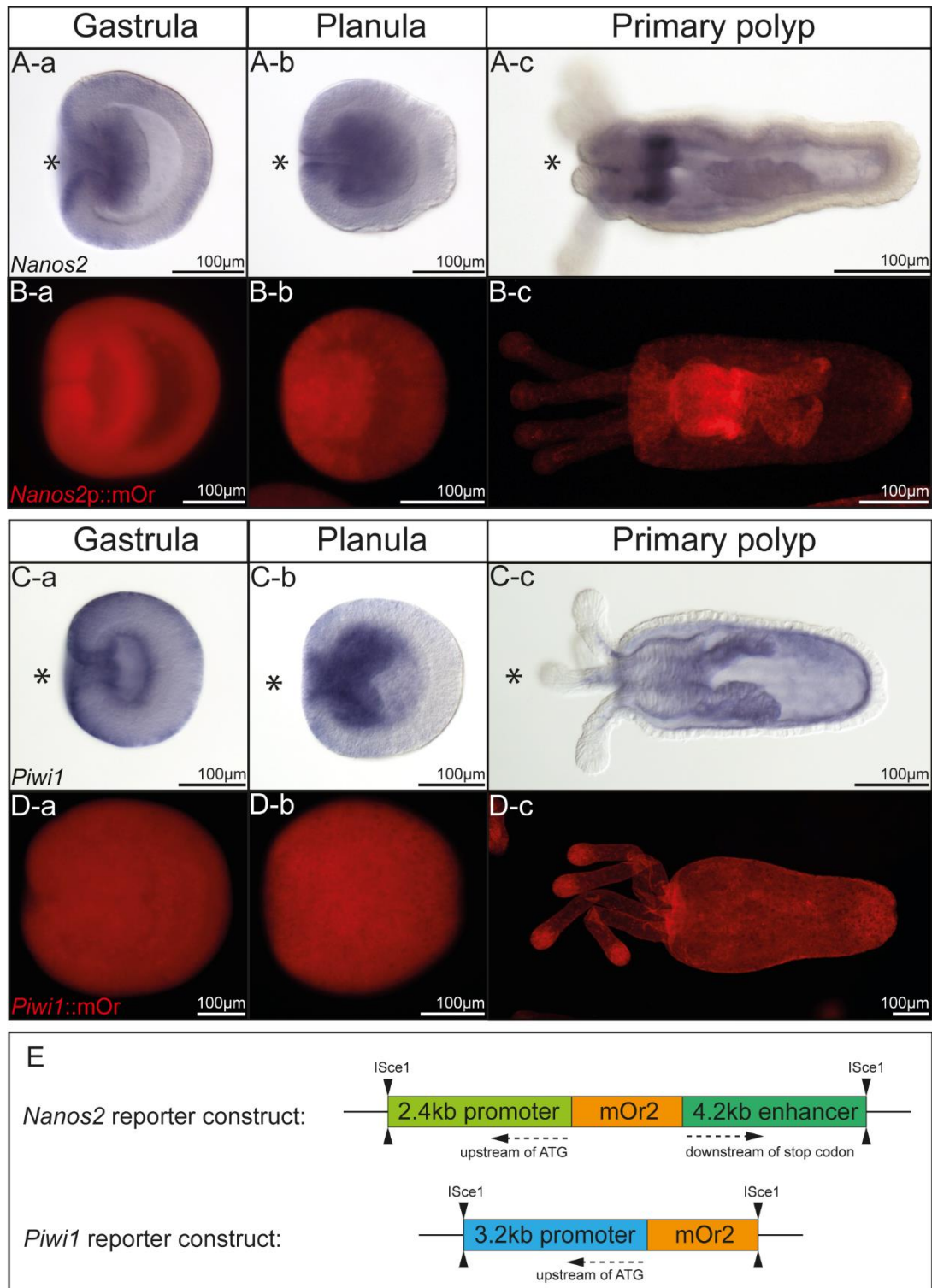
The PDF file includes:

Figs. S1 to S5
Legends tables S1 to S3

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S3

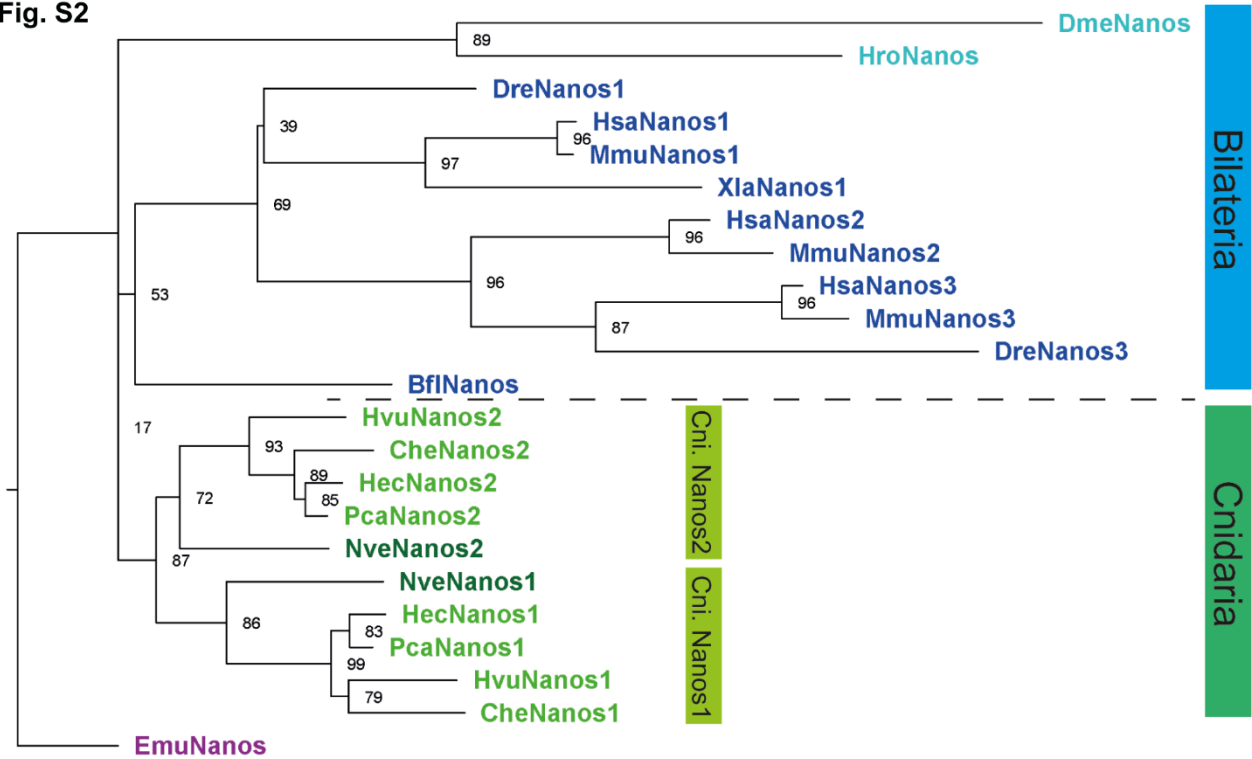
1 Supplementary Materials



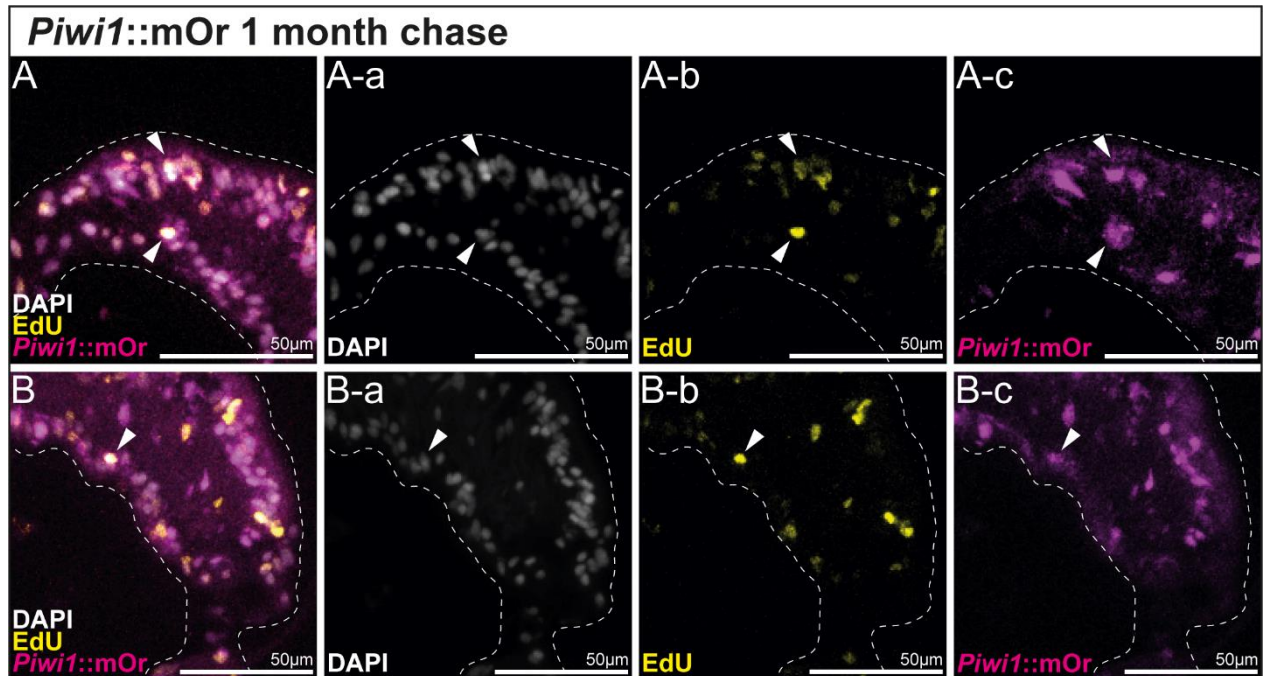
S1 Fig: *nanos2* and *piwi1* expression in developmental stages. a: 24h gastrula stage, b: 3d planula stage, c: 7d primary polyp; A: ISH visualizing *nanos2* expression throughout development;

B: *nanos2*::mOr expression; C: ISH visualizing *piwi1* expression throughout development; D: *piwi1*::mOr expression; E-F: *nanos2* and *piwi1* reporter gene constructs.

Fig. S2



S2 Fig: Phylogenetic analysis of Nanos proteins. Bayesian consensus tree of metazoan Nanos proteins; Numbers at the branches indicate bootstrap support (1000 iterations). *Nanos* genes have duplicated in the cnidarian lineage and triplicated at the base of the teleost lineage independently. Dme: *Drosophila melanogaster*; Hro: *Hirundo robusta*; Dre: *Danio rerio*; Hsa: *Homo sapiens*; Mmu: *Mus musculus*; Xla: *Xenopus laevis*; Bfl: *Branchiostoma floridae*; Hvu: *Hydra vulgaris*; Che: *Clytia hemisphaerica*; Hec: *Hydractinia echinata*; Pca: *Podocoryne carnea*; Nve: *Nematostella vectensis*; Emu: *Ephydatia muelleri*.



S3 Fig: Long term EdU label-retention assay in *piwi1::mOr* transgenic animals. A-C: EdU signal is retained in few mOr+ cells in the mesentery epithelia over 1 month, marking a subpopulation of slowly cycling cells. A, B: 1 month label retention in mesenteries. A-a, B-a: DAPI only. A-b, B-b: EdU only. A-c, B-c: mOrange only.

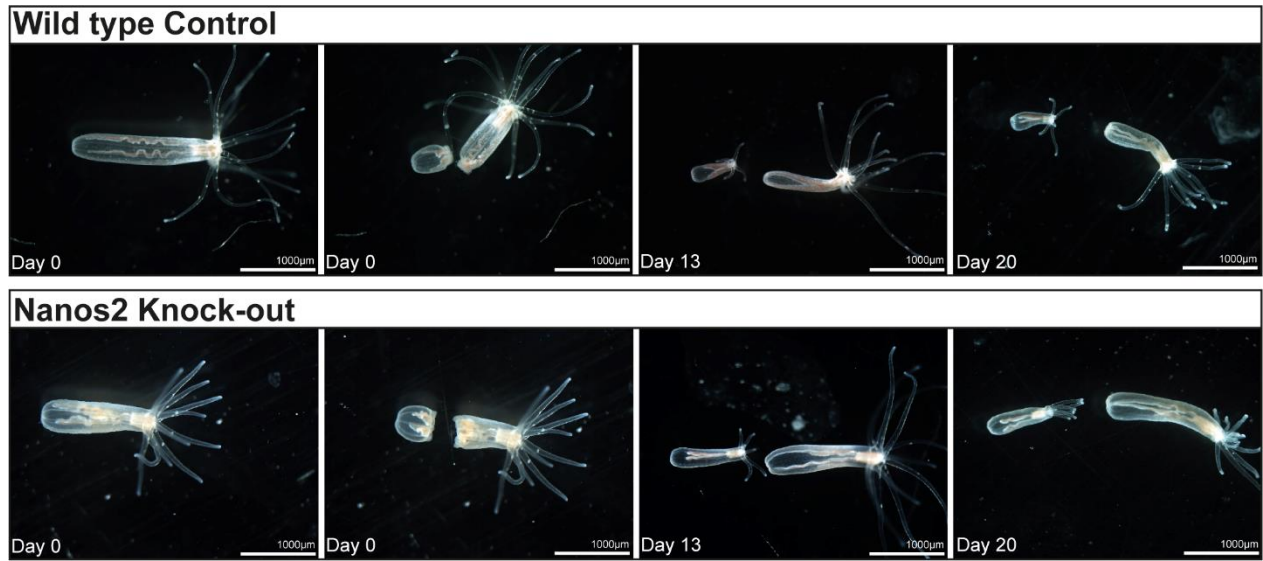
Fig. S4

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S4 Fig: *nanos2* genomic coding sequence of *nanos2* KO mutants. Nanos Zinc-finger sequence is highlighted in turquoise, 1bp insertion is highlighted in red.



S5 Fig: Regeneration of Wild type (top row) and *nanos2* KO animals (bottom row). When regenerating from a mid-body bisection, *nanos2* KO animals regenerate in a similar fashion and speed to WT animals, resulting in wound closure and the reformation of a functional head (n=3).

Table 1: EdU counts of dissociated *nanos2* and *piwi1* Transgenic tissue

Table 2: Primers

Table 3: List of differentially expressed genes