

SUPPLEMENTARY MATERIALS

TABLE S1. piwi, vasa, ago and PL10 genes in *Helobdella robusta* genome

Gene name	Gene model	Protein ID (filtered model if different)	Scaffold location
<i>Hro-piwi1</i>	estExt_Genewise1Plus.C_160437	110366 (75625)	scaffold_16:3131614-3144934 (+)
<i>Hro-piwi2</i>	estExt_fgenesh4_pg.C_30678	188675 (65566)	scaffold_3:6235842-6244114(+)
<i>Hro-vasa1</i>	estExt_Genewise1Plus.C_80601	108526	scaffold_8:5337420-5340951(-)
<i>Hro-vasa2</i>	gw2.3.49.1	65423	scaffold_3:4512521-4516451(+)
<i>Hro-ago1</i>	e_gw1.3.302.1	65165	scaffold_3:2673663-2688060(-)
<i>Hro-ago2</i>	fgenesh4_pg.C_scaffold_79000057	182062	scaffold_79:528910-540709(-)
<i>Hro-PL10a</i>	estExt_Genewise1Plus.C_10717	105366	scaffold_1:5855406-5863348(+)
<i>Hro-PL10b</i>	estExt_fgenesh4_pg.C_1020014	194828	scaffold_102:152850-159086(-)
<i>Hro-PL10c</i>	estExt_Genewise1Plus.C_20327	105974	scaffold_2:2744284-2748920(-)
<i>Hro-PL10d</i>	fgenesh4_pm.C_scaffold_41000002	157479	scaffold_41:247727-249189(+)

Figure S1. Co-expression of *Hau-piwi1* and *Hau-vasal1* in developing ovaries.

(A) Two color fluorescent in situ showing *Hau-piwi1* (green, left) and *Hau-vasal1* (red, center) and merged views (right). Upper panels show ventral views of late stage 11 embryos, with paired spots of *Hau-piwi1* and *Hau-vasal1* expression (arrows), persisting from stage 9 in segment M6. (B) Lower panels show traverse sections (ventral down) through segment M6, counterstained with DAPI, revealing the dorsally projecting arms (arrowheads) of the developing ovaries and segmental ganglion (g). Scale bar, 200 μm in A; 100 μm in B.

Figure S2. The female germline originates from the ipsilateral M lineage.

Confocal micrographs of an embryo processed at early stage 11 by two color fluorescent in situ hybridization for *Hau-piwi1* (green, left) and *Hau-vasal1* (red, center). This embryo had also had its left mesodermal teloblast (M_L) injected with FarRed dextran amine (blue, right) at birth (stage 5); the righthand panels also show the merged views of the *Hau-piwi1* and *Hau-vasal1* signals in segment M6. (A) Upper panels show a ventral view including parts of about ten segments. (B) Lower panels show combined brightfield and fluorescence micrographs of a transverse section (ventral down) through segment M6. Labels indicate yolk (y) and ganglion (g). Scale bars, 100 μm .

Figure S1

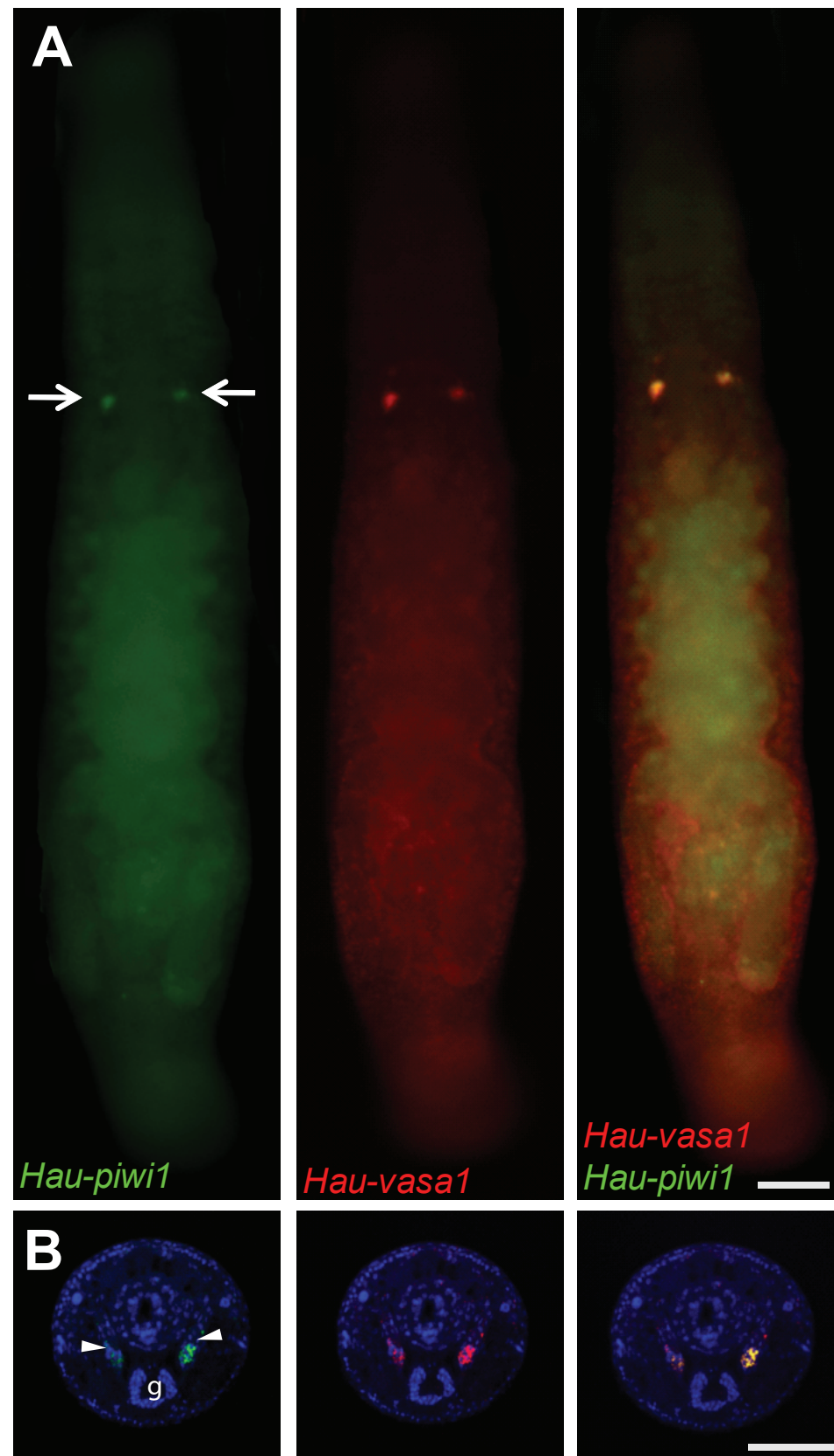


Figure S2

