

**Supporting Information Appendix for:**

**A Premeiotic Function for *boule* in the Planarian *Schmidtea mediterranea***

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**This SI appendix includes:**

Detailed Supplementary Methods

Supplementary Figure Legends

Supplementary Figures S1-S10

Supplementary Tables S1-S4

## Detailed Supplementary Methods

### In situ hybridization

For colorimetric in situ hybridization (ISH) and fluorescent in situ hybridization (FISH), animals were killed with 10% N-acetyl cysteine (Sigma-Aldrich, St. Louis, MO), for 7.5 minutes, and then fixed in 4% formaldehyde in PBSTx (1X PBS+0.3% Triton X-100) for 20 minutes at room temperature.

Animals were dehydrated in 50% followed by 100% Methanol and stored in  $-20^{\circ}\text{C}$  until use. Animals were rehydrated in increasing concentrations of PBSTx, and bleached in freshly prepared Formamide bleaching solution (5% non-ionized Formamide, 0.5X SSC, and 1.2%  $\text{H}_2\text{O}_2$ ) for 3 hours. After bleaching, animals were treated with Proteinase K solution (100  $\mu\text{l}$  of 10% SDS and 5  $\mu\text{l}$  of 20 mg/ml Proteinase K (Invitrogen) in 9.9 ml of PBSTx) and post-fixed in 4% Formaldehyde. Following washes to remove the fixative, hybridization was carried out at  $56^{\circ}\text{C}$  for 16 hours at a riboprobe concentration of 0.1-0.5 ng/ $\mu\text{l}$ . After post-hybridization washes, samples were blocked in Blocking solution (5% horse serum and 0.5% Roche Western Blocking Reagent in MABT). Samples were incubated in primary antibody (anti-digoxigenin alkaline phosphatase (Roche) 1:1000, or anti-digoxigenin peroxidase (Roche) 1:1000) overnight at  $12^{\circ}\text{C}$ . For FISH, DAPI was added to the primary antibody solution (1:10,000 of 10 mg/ml stock). Samples were washed in MABT (100 mM Maleic acid, 150 mM NaCl, 0.1% Tween-20, pH 7.5). Colorimetric development was carried out in AP buffer (100 mM Tris, pH 9.5; 100 mM NaCl; 50 mM  $\text{MgCl}_2$ , 0.1% Tween-20 brought up to volume with 10% polyvinylalcohol (Sigma)) containing 4.5  $\mu\text{l}/\text{ml}$  NBT and 3.5  $\mu\text{l}/\text{ml}$  BCIP (Roche). For FISH, development was done in freshly made Tyramide solution (Fluor-tyramide (1:250-1:500), 4-IPBA (1:1000), and  $\text{H}_2\text{O}_2$  (0.003%) in TSA buffer (2 M NaCl, 0.1 M Boric acid, pH 8.5). 4-IPBA is 20 mg/ml of 4-iodophenylboronic acid in



dimethylformamide (DMF) stored at  $-20^{\circ}\text{C}$ . Samples were washed 6-8 times ( $\sim 20$  minutes each wash) in TNTx.

### **TUNEL on sections**

After two dsRNA feedings planarians were starved for a week and treated with 10% N-acetyl-L-cysteine for 7.5 minutes and fixed in 4% formaldehyde in PBSTx (0.3% Triton X-100) for 20 minutes at room temperature. Cryosectioning was done to generate 15-20  $\mu\text{m}$  sections. Sections were rehydrated and treated with pre-chilled ethanol:acetic acid (2:1) at  $-20^{\circ}\text{C}$  for 5 minutes. The slides were rinsed twice in DI water and equilibrated in equilibration buffer (100 mM Tris-HCl pH 7.5 + 1 mg/ml IgG-free BSA). Slides were covered with TdT solution (0.5  $\mu\text{l}$  NEB TdT (Cat. No. M0252L), 2  $\mu\text{l}$  NEB buffer 4, 2  $\mu\text{l}$  2.5 mM  $\text{CoCl}_2$ , 0.8  $\mu\text{l}$  1:50 DIG-dUTP in dATP, 14.7  $\mu\text{l}$  water) and incubated at  $37^{\circ}\text{C}$  in a dark humidified chamber for 1 hour. After rinsing 3X with PBSTx, the sections were blocked with 5% Horse Serum (Sigma H1138) in PBSTx for 2 hours. Block was replaced with 1:1000 anti-DIG-POD (Roche 11207733910) diluted in block solution. DAPI (1  $\mu\text{g}/\text{ml}$ ) was added at this step. Sections were covered with coverslips and incubated for overnight at  $4^{\circ}\text{C}$ . Slides were rinsed in PBSTx and signal was revealed using TAMRA-tyramide. Slides were rinsed in PBSTx and mounted in Vectashield.

### **Imaging**

Colorimetric in situ samples were mounted in 80% glycerol and images were captured with a Leica DFC420 camera mounted on a Leica M205A stereomicroscope (Leica, Wetzlar, Germany). Whole-mount FISH samples were mounted in Vectashield (Vector Laboratories, Burlingame, CA) and imaged on a Zeiss Stereo Lumar V12 (Carl Zeiss, Germany). For confocal imaging, FISH samples were mounted in Vectashield and images were obtained on a Zeiss LSM 710 confocal microscope (Carl

Zeiss). Images were processed (cropping, brightness and contrast adjustments to entire image) using Adobe Photoshop CS4/CS5 and/or Zen 2008/9/11.

### **Quantitative real-time PCR**

Total RNA was extracted using TRIzol (Invitrogen) according to manufacturer's instructions, DNase (Fisher Scientific) treated and cleaned using an RNA clean up kit (Zymo) before reverse transcription (iScript, Bio-Rad). Prior to RNA extraction, animals were starved for 7 days after the last RNAi feeding to ensure that any remnant dsRNA was cleared from the system. qRT-PCR was performed using GoTaq qPCR master mix (Promega) using Applied Biosystems StepOne Plus RT-PCR system. All experiments were done in biological and technical triplicates. Transcript levels were normalized to  *$\beta$ -tubulin*. Relative mRNA levels were calculated using  $\Delta\Delta CT$ . All primers are listed in SI Appendix, Table S4.

## Supplementary Figure Legends

**Figure S1. Planarian *boule* homologs are expressed in the ovaries.** Colorimetric in situ hybridization for *boule1* and *boule2* showing expression in the female reproductive system. *boule1* expression was seen in the ovaries of some animals (n=3/8) and possible *boule2* expression was detected in the ventral portion of the animals (n=7/7), where the ovaries are located. Scale bars, 100  $\mu$ m.

**Figure S2. *boule1* and *boule2* are expressed in spermatogonial stem cells (SSCs) and spermatogonia in the male germline.** Double fluorescence in situ hybridization (FISH) showing (A) *boule1* and (B) *boule2* coexpressed with *nanos*, which labels SSCs. Scale bars, 50  $\mu$ m. Magnified sections showing colocalization of (A') *boule1* and (B') *boule2* with *nanos* (arrows). *nanos*<sup>+</sup> cells show lower intensity of *boule1* signal than the surrounding spermatogonia. Scale bars, 10  $\mu$ m. (C) *boule1* and (D) *boule2* are coexpressed with *germinal histone H4 (gH4)* transcript in the male gonads. *gH4* labels SSCs and spermatogonia in the male germline. Scale bars, 50  $\mu$ m.

**Figure S3. Effect of *boule1* or *boule2* RNAi on SSCs and spermatids** (A) Distinct stages of planarian spermatogenesis and labels for individual testis cell types. *nanos* and *germinal histone H4* (in magenta) label SSCs and spermatogonia, respectively. *tektin-1* and *protein kinase A* (in cyan) label spermatocytes and spermatids respectively. (B) Following 2 feedings of *boule1* and *boule2* dsRNA (intermediate knockdown), *boule1*(RNAi) animals have SSCs (labeled by *nanos*) similar to controls (n=6/6). Half (n=3/6) the *boule2*(RNAi) animals show *nanos* expression and the remaining 3 animals have no *nanos*<sup>+</sup> SSCs. The spermatid population, labeled with *pka*, is slightly reduced in *boule1*(RNAi) animals, and the *gH4*<sup>+</sup> spermatogonial population is expanded (n=5/5). *boule2*(RNAi) animals show *pka* labeling

comparable to controls (n=6/6). **(C)** At later stages, following 4 feedings of dsRNA *boule1(RNAi)* animals have testes with clusters of SSCs and spermatogonia, and animals lack meiotic and postmeiotic cells (n=5/5). *boule2(RNAi)* animals show a complete loss of all male germ cells (n=6/6). Scale bars, 50  $\mu\text{m}$ .

**Figure S4. Demonstration of *boule1* or *boule2* RNAi specificity.** **(A)** Alignment of *boule1* and *boule2* nucleotide sequences showing no significant similarity between the sequences. **(B)** and **(C)** show qRT-PCR validation of *boule1* or *boule2* knockdown following a single dsRNA feeding. **(B)** Following *boule1(RNAi)* there is an increase in *boule2* transcript, most likely due to accumulation of spermatogonia, in which *boule2* is expressed. **(C)** The levels of *boule1* transcript are similar to controls in *boule2(RNAi)* animals. Two-tailed unpaired t-test with Welch's correction was performed for all samples,  $P < 0.05$ . **(D)** FISH for *boule1* or *boule2* was performed following 2 dsRNA feedings of either gene. Our experiments show that the knockdown of either *boule1* or *boule2* does not affect the expression of the other paralog. Scale bars, 50  $\mu\text{m}$ .

**Figure S5. *boule2(RNAi)* animals show increased apoptosis.** TUNEL was performed on cryosections following 2 feedings of *boule1* or *boule2* dsRNA. **(A)** *boule2(RNAi)* animals show a greater number of TUNEL<sup>+</sup> cells in the testes compared to control and *boule1* knockdown animals. Scatter plot shows mean with standard deviation. One-way ANOVA was performed using Dunnett's multiple comparisons test to determine significance at 95% confidence interval. **(B)** Representative images showing TUNEL<sup>+</sup> cells (arrows) in *boule2(RNAi)* animals. Scale bars, 20  $\mu\text{m}$ .

**Figure S6. Validation of *boule1* and *boule2* gene knockdowns in regenerates and hatchlings (A)** *dmd1(RNAi)* head fragments do not respecify their SSCs 14 days post amputation (n=4/4). Scale bars, 100  $\mu$ m. **(B)** qRT-PCR for samples corresponding to Figures 2A and B. Relative mRNA levels of *boule1* and *boule2* are low after 14 days of regeneration. Error bars represent 95% confidence intervals calculated based on standard error of the mean. Two-tailed unpaired t-test with Welch's correction was performed for all samples, P<0.05. **(C,D)** Knockdown phenotype of *boule1* or *boule2* in sexual hatchlings (<48 hours old) is similar to the RNAi phenotype in adults. **(C)** Following two feedings of *boule1* dsRNA, *nanos* and *gH4* expression appears comparable to control animals (n=6/6). Some *boule2(RNAi)* animals (n=2/6) show absence of male germ cells, and the remaining animals (n=4/6) show very small testis lobes with both *nanos*<sup>+</sup> and *gH4*<sup>+</sup> germ cells. **(D)** After 4 feedings of *boule1* dsRNA, animals show accumulation of spermatogonia compared to controls, and *boule2(RNAi)* animals lack all male germ cells. Scale bars, 50  $\mu$ m.

**Figure S7. Assay for determining the role of *boule1* and *boule2* in male germline regeneration and differentiation. (A)** Experimental schematic. When planarians are amputated posterior to their ovaries, the resulting tail fragments regress their testes approximately 7 days post-amputation, and contain clusters of early, undifferentiated male germ cells. Tail regenerates were fed *boule1* or *boule2* dsRNA (4 feedings, 4-5 days apart) after amputation and testes regression. **(B)** Testes were restored in control regenerates (n=6/6). *boule1(RNAi)* regenerates had small testis lobes containing only SSCs and spermatogonia (n=5/6). By contrast, in *boule2(RNAi)* regenerates, all the male germ cells were absent (n=6/6). **(C)** qRT-PCR validation. Amputated animals at the beginning of RNAi show low levels of *tkn-1* (spermatocytes) and *pka* (spermatids). Error bars represent 95% confidence intervals calculated based

on standard error of the mean. Two-tailed unpaired t-test with Welch's correction was performed for all samples,  $P < 0.05$ .

**Figure S8. Additional experiments with putative planarian DAZ family-associated proteins. (A)** Control (RNAi) animals show the expression of all four germ cell markers. **(B)** *DAZAP2* is not required for the maintenance of the male germ cells in homeostasis ( $n=6/6$ ). **(C)** *DAZAP1(RNAi)* and **(D)** *iguana(RNAi)* in homeostasis results in no change in SSC (*nanos*<sup>+</sup>) population and an accumulation of rounded spermatids (*pka*<sup>+</sup>) population ( $n=6/6$  for both). **(E-F)** *DAZAP1(RNAi)* in sexually immature regenerates corroborates the gene's homeostasis phenotype, with animals showing no mature sperm ( $n=4/6$ ). *iguana(RNAi)* animals undergo lysis upon amputation ( $n=6/6$ , Table S1). Scale bars, 50  $\mu\text{m}$ .

**Figure S9. Additional experiments on putative planarian DAZ family targets. (A)** Planarian homologs of DAZ/DAZL targets are expressed in the male germline. Scale bars, 1mm. **(B-D)** *SDADI(RNAi)*, *CDC25-1(RNAi)* and *CDC25-2(RNAi)* have SSCs (*nanos*) and spermatids (*pka*) ( $n=6/6$  for all except *SDADI*) at early stages of knockdown. Half ( $n=3/6$ ) of *SDADI(RNAi)* animals show no *nanos* labeling. *CDC25-1* knockdown results in enlarged SSCs possibly due to defects in cytokinesis (see insets in B and D). Sexually immature regenerates fed **(E)** control dsRNA regenerate their testes, whereas **(F)** in the absence of *CDC25-2*, regenerates cannot maintain the early male germ cells, similar to *boule2(RNAi)* animals ( $n=6/6$ ). *SDADI(RNAi)* and *CDC25-1(RNAi)* animals undergo lysis upon amputation ( $n=6/6$  for both, Table S2). Scale bars, 50  $\mu\text{m}$ ; inset scale bars, 10  $\mu\text{m}$ .

**Figure S10. Phylogenetic analyses of the DAZ family (A)** Alignment of Boule, DAZL, and DAZ RRM. **(B)** Branch length ratios of pre-meiotic (Smed-Boule2 or Dazl) to meiotic (Smed-Boule1 or

vertebrate Boule) terminal edge lengths in planarians and vertebrates. Note the markedly similar distribution of paralog branch length ratios in species with neofunctionalized Boule derivatives.

**Table S1. Experimental details for planarian homologs of DAZ-associated proteins**

**Table S2. Experimental details for planarian homologs of DAZ family targets**

**Table S3. Accession numbers of sequences used for phylogenetic analyses**

**Table S4. Cloning and qRT-PCR primer sequences**

**Figure S1**

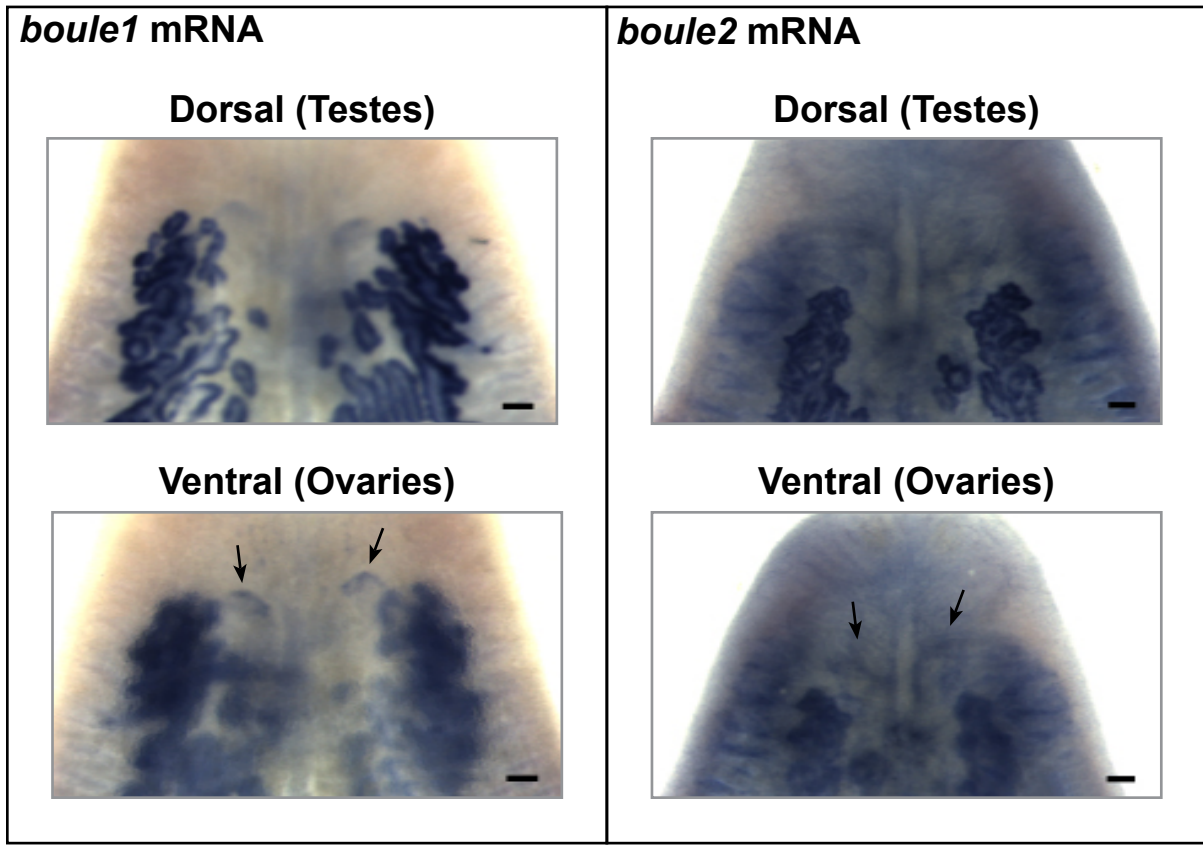




Figure S2

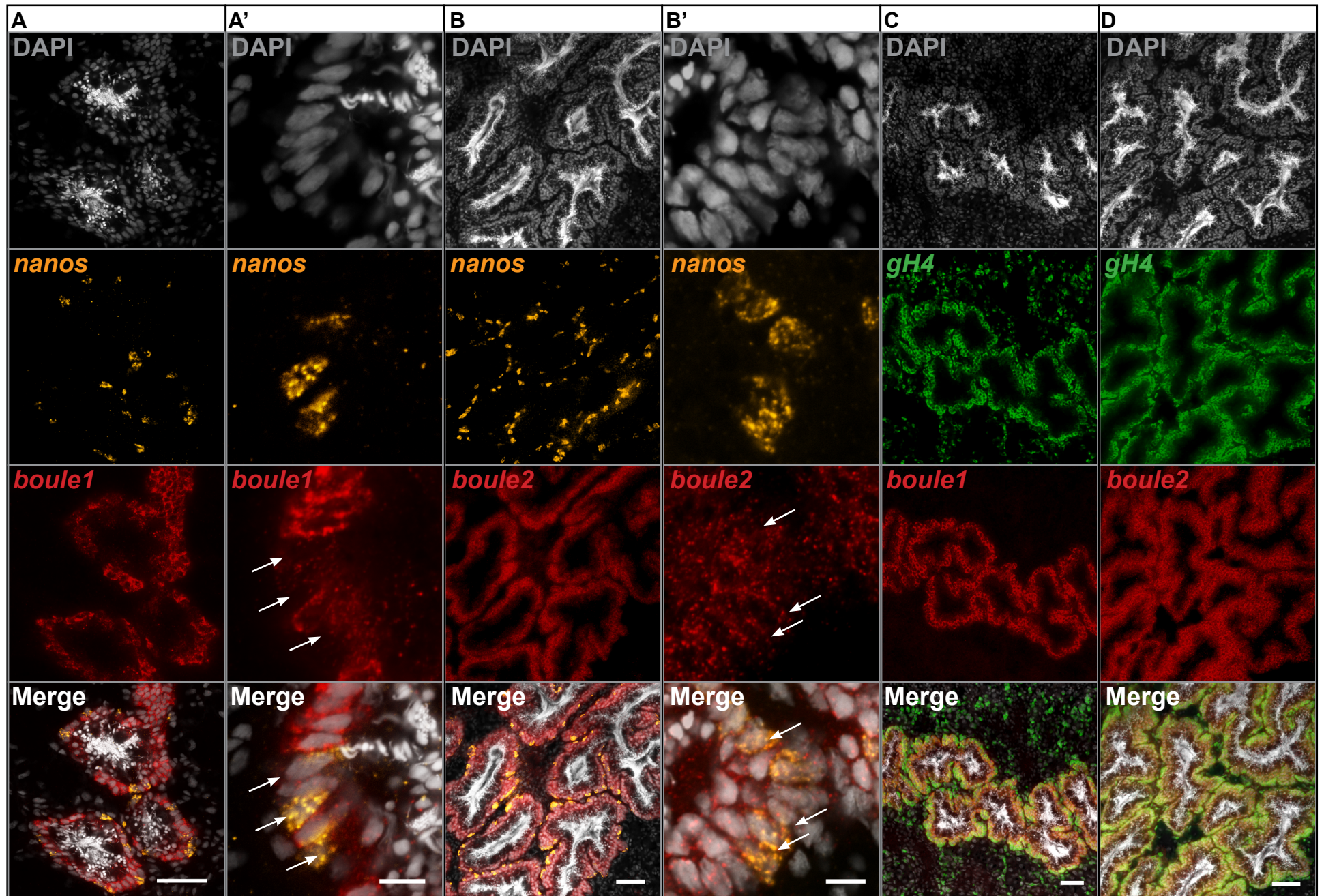




Figure S3

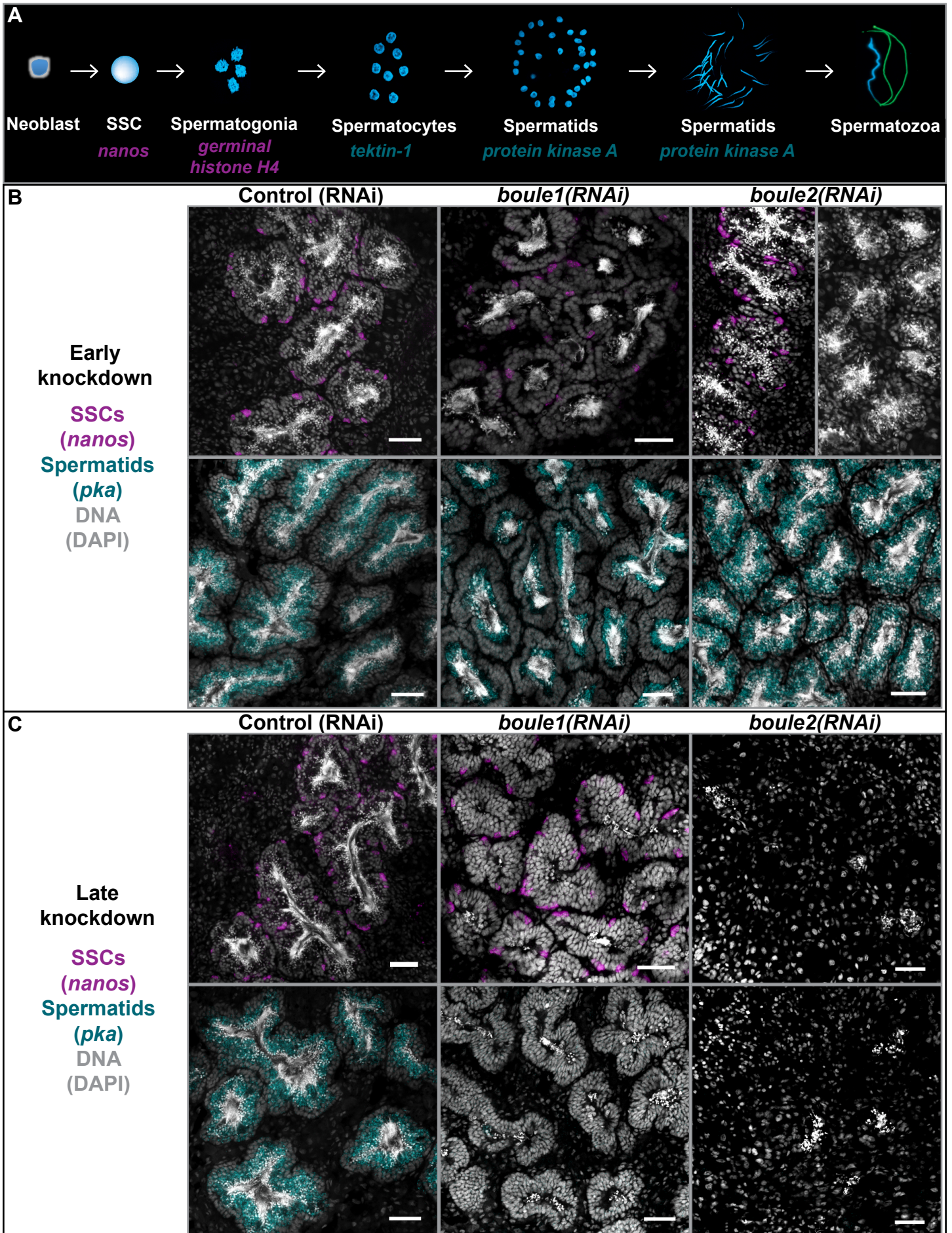




Figure S4

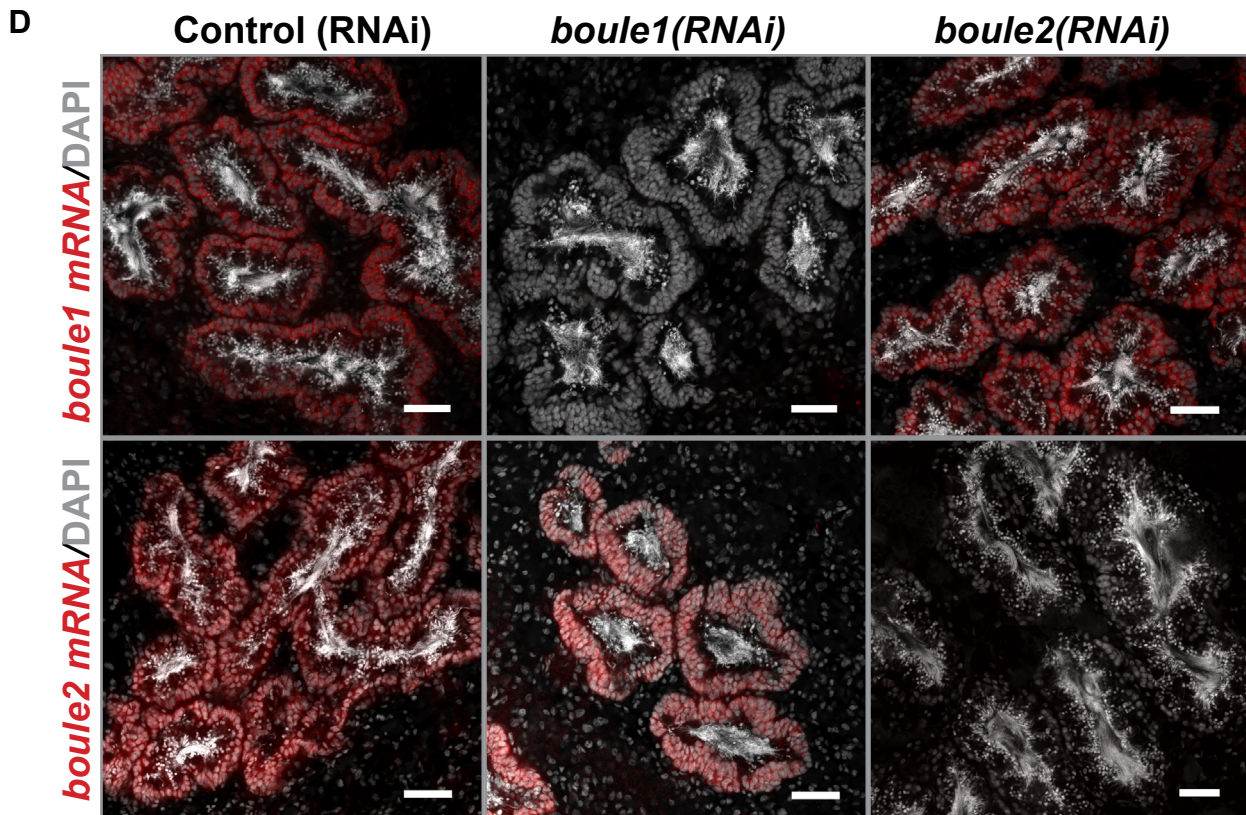
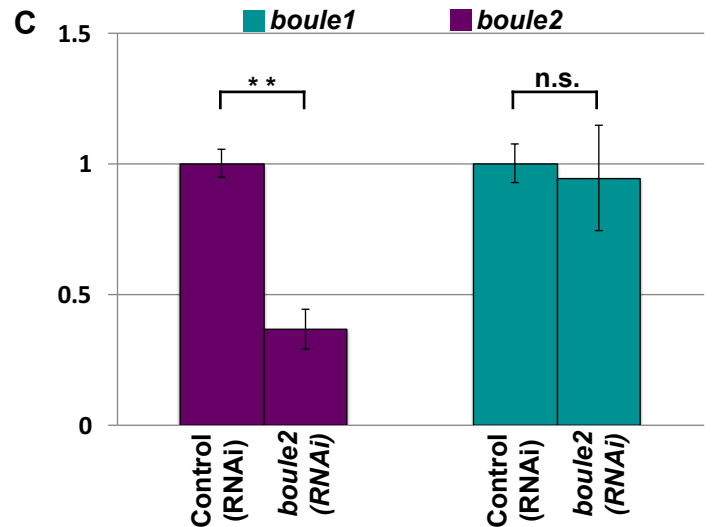
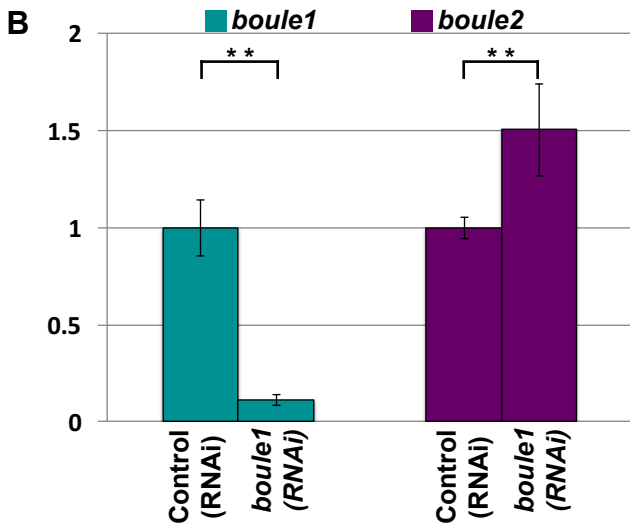
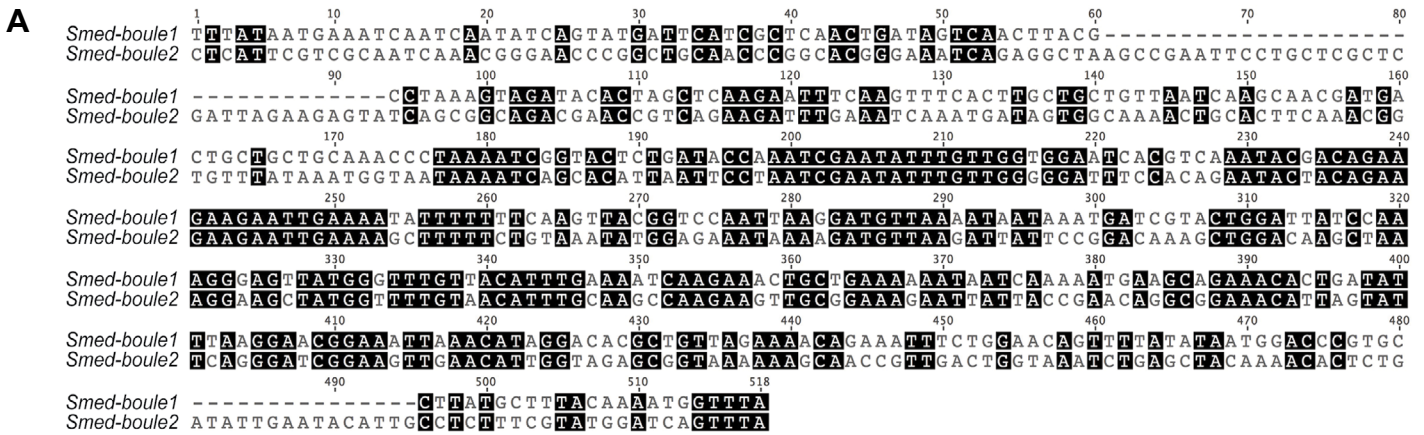






Figure S6

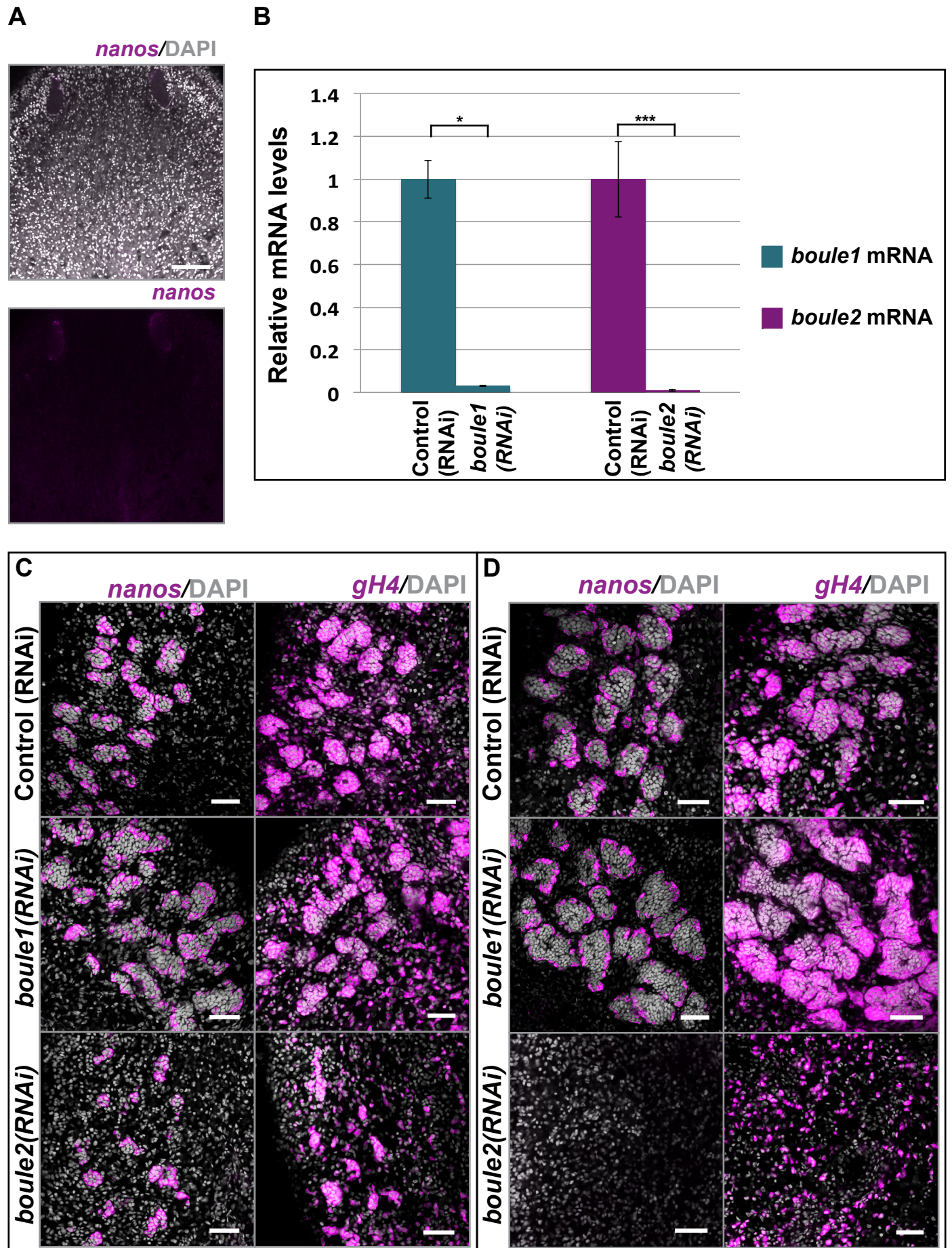


Figure S7

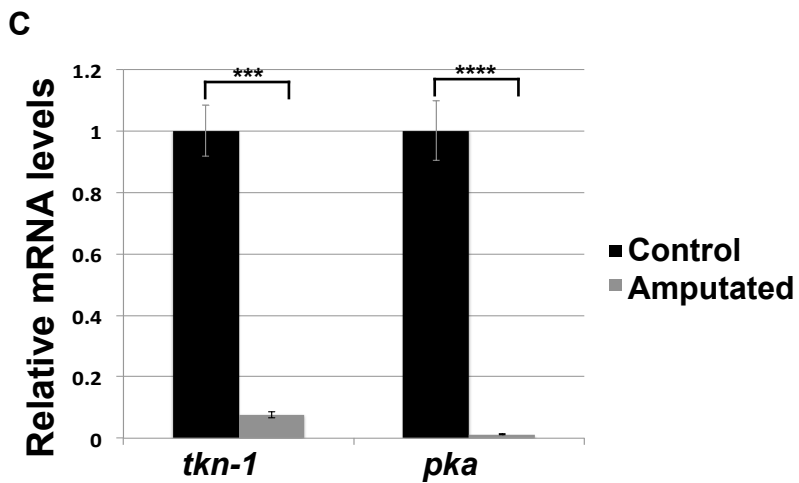
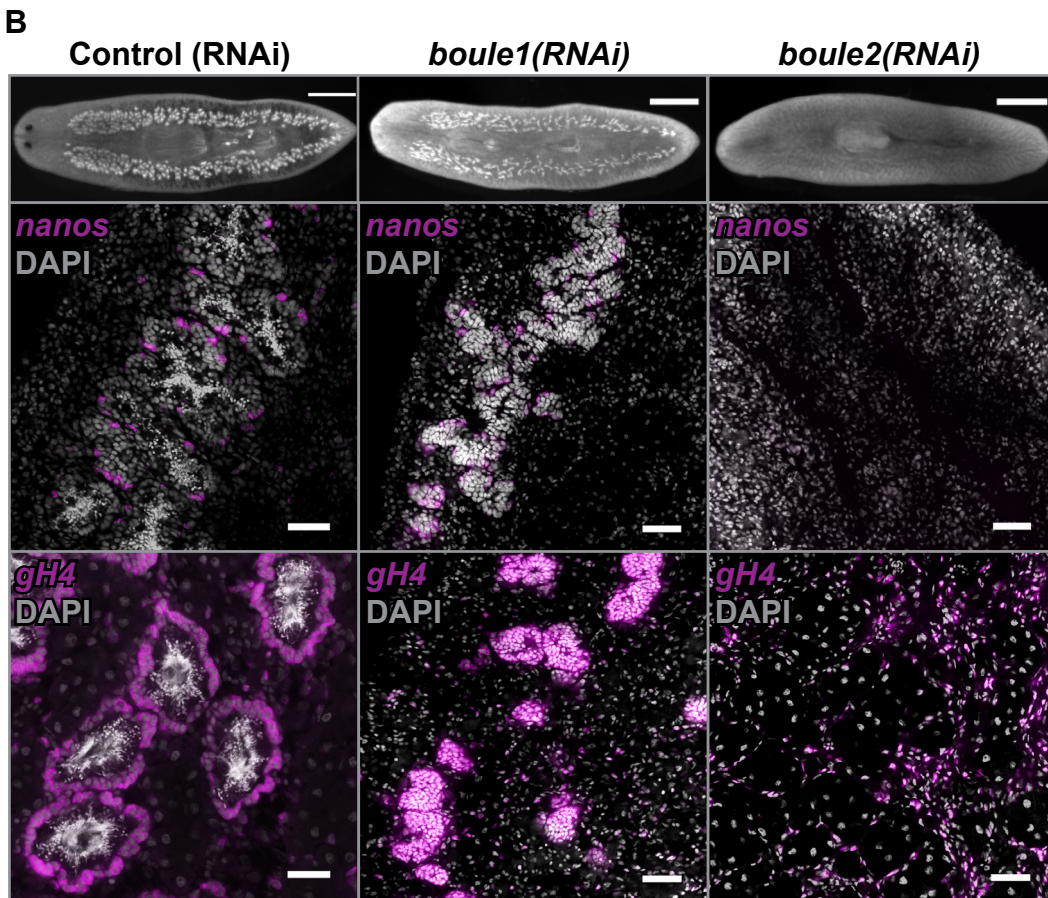
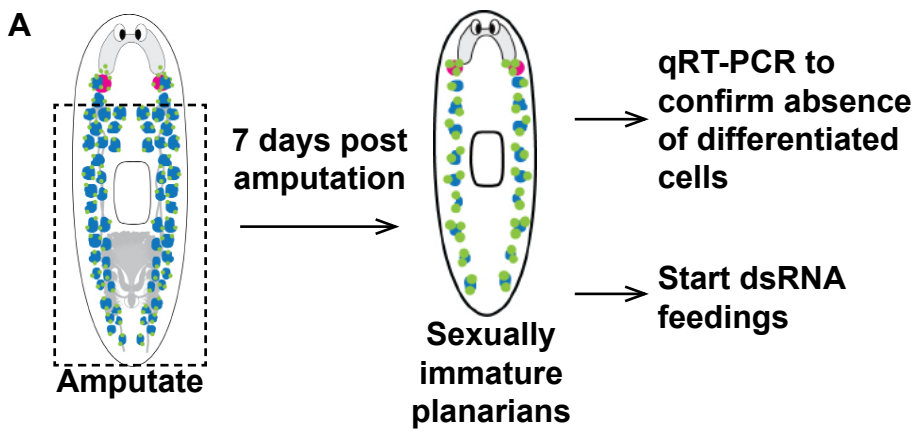




Figure S8

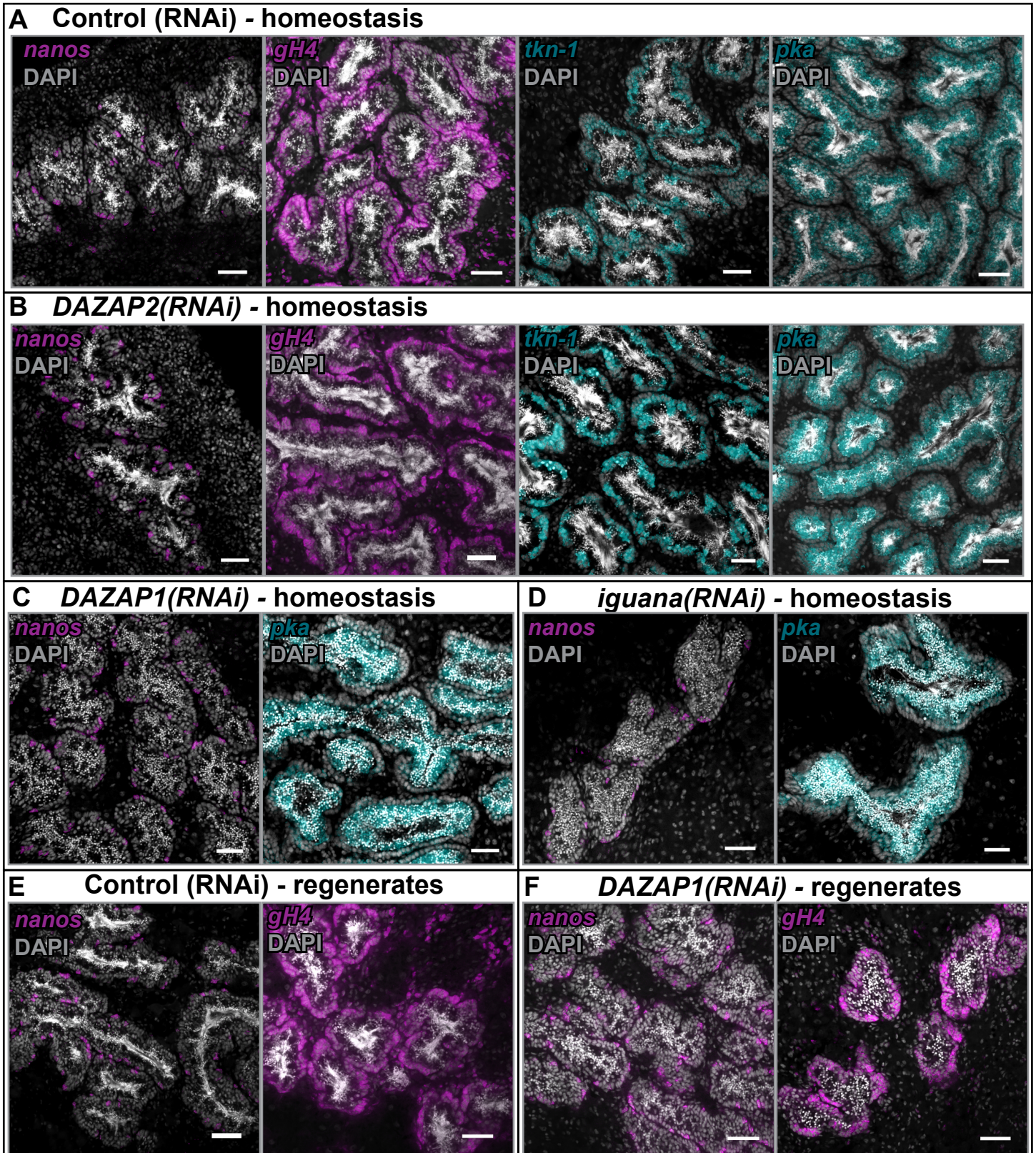
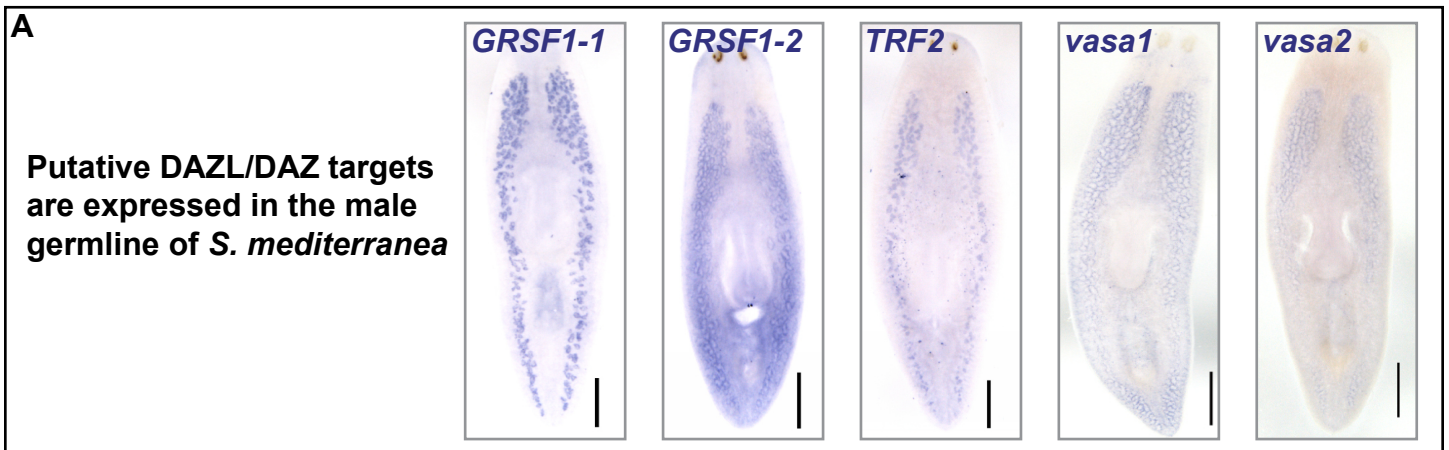
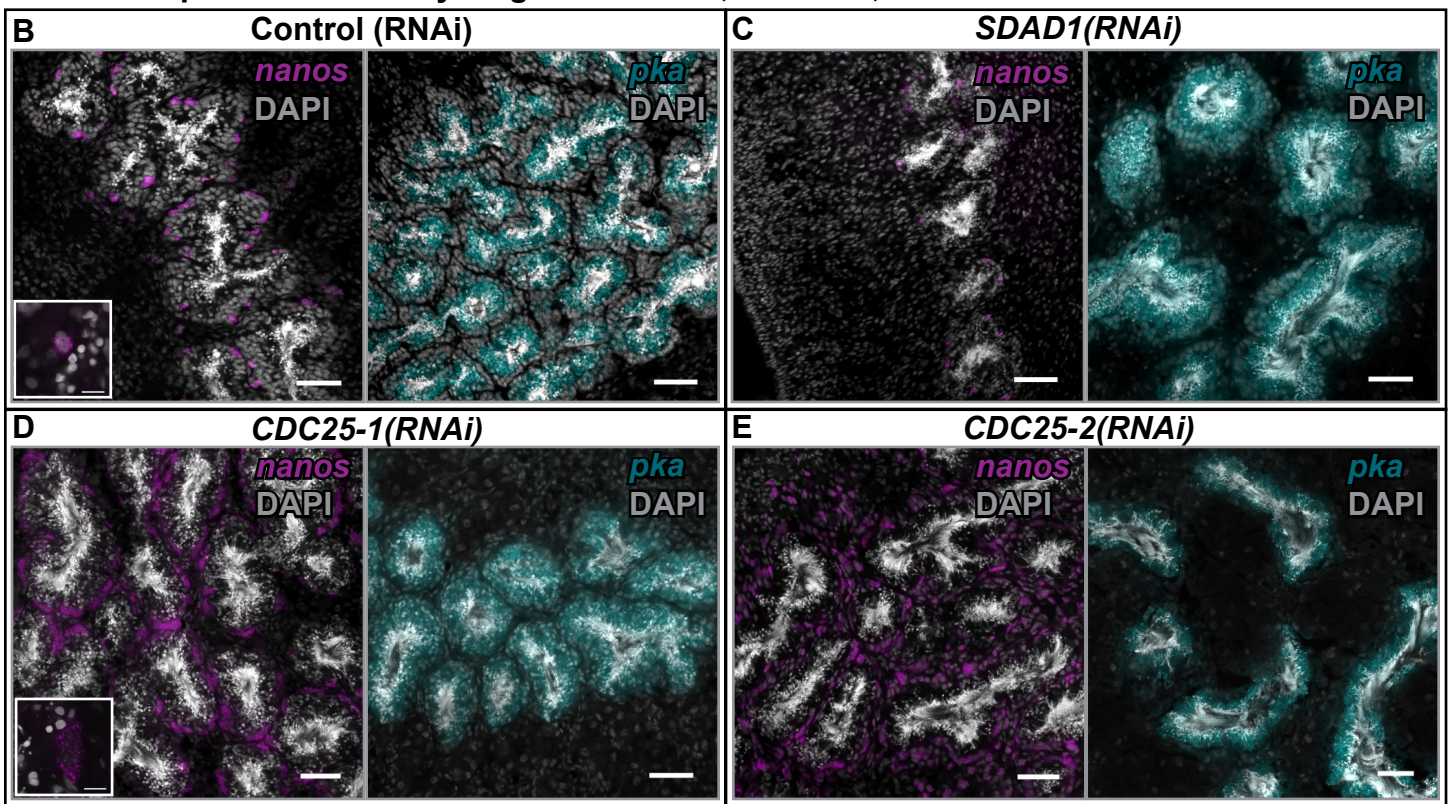




Figure S9



SSCs and spermatids in early stages of *SDAD1*, *CDC25-1*, and *CDC25-2* knockdown



*CDC25-2*(RNAi) and *boule2*(RNAi) in sexually immature regenerates phenocopy each other

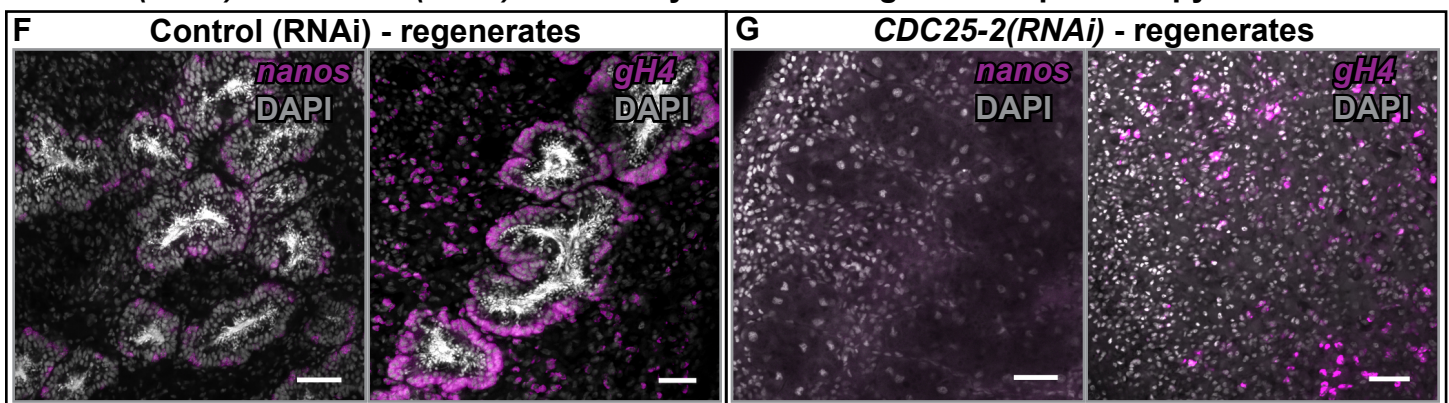
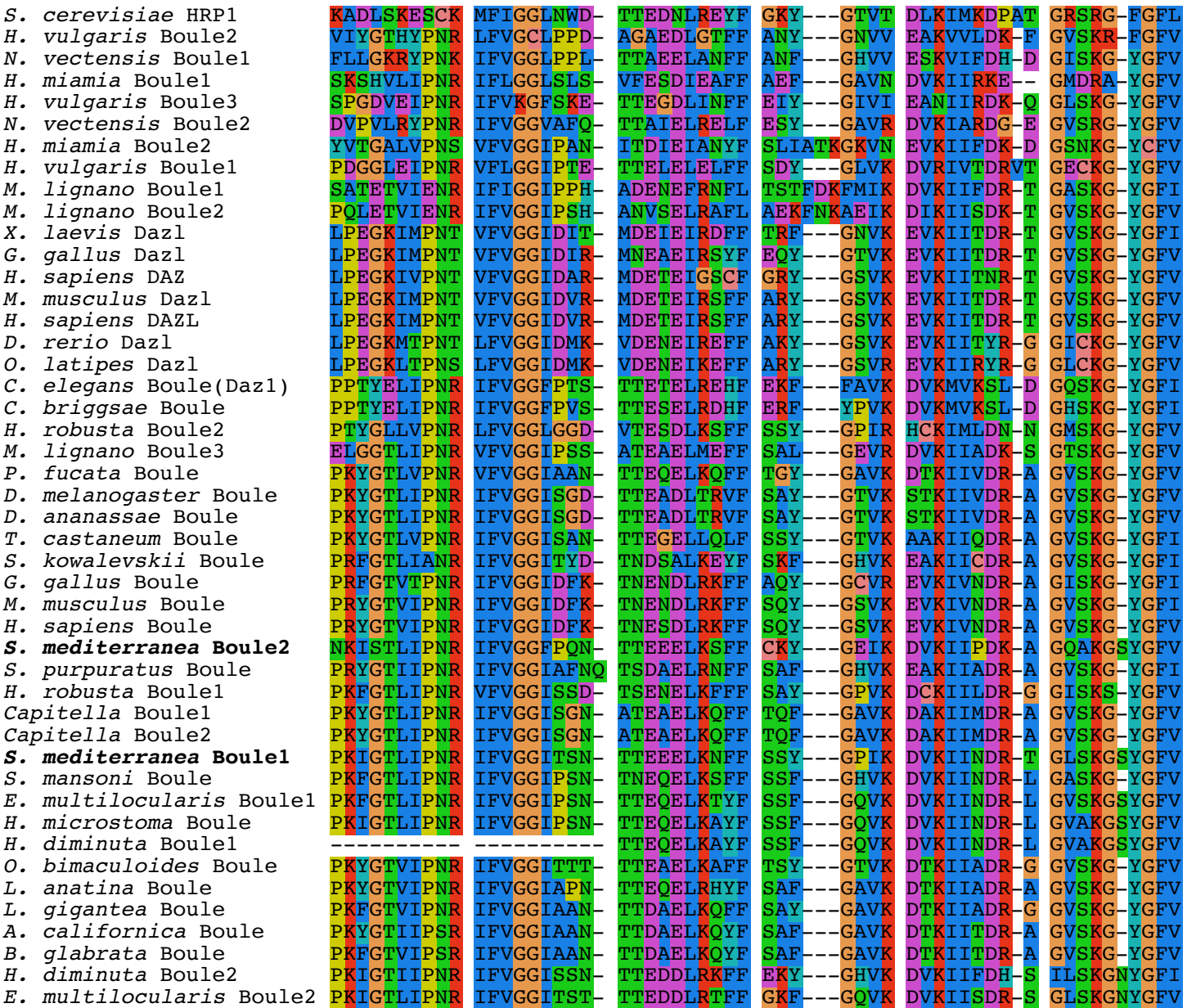


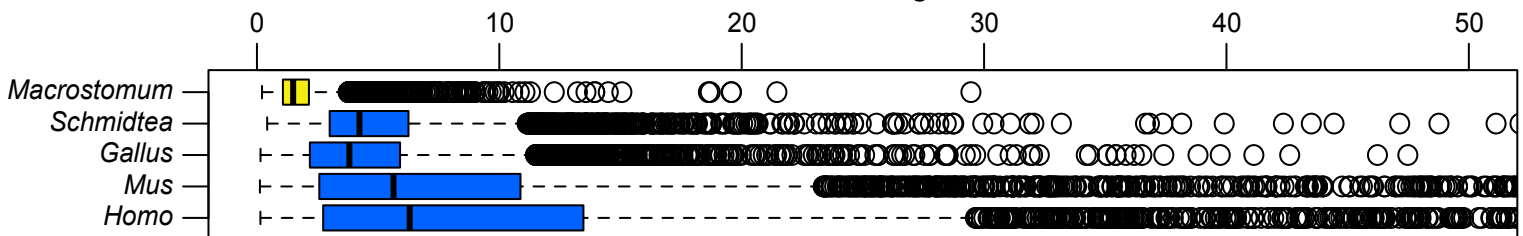


Figure S10

A



B Branch length ratio



**Table S1. Experimental details for planarian homologs of DAZ-associated proteins**

<b>Associated protein name</b>	<b>Required for regeneration</b>	<b>Expressed in male germ cells?</b>	<b>Germ cell RNAi phenotype in adult sexually mature worms</b>	<b>Germ cell RNAi phenotype in hatchlings</b>	<b>Required for <i>de novo</i> specification of germ cells?</b>	<b>RNAi phenotype in sexually immature regenerates</b>
<i>DAZAP1</i>	No	Yes	No mature sperm	No mature sperm	No	No mature sperm
<i>DAZAP2</i>	No	Yes	No phenotype	No phenotype	No	Small testes with only SSCs and spermatogonia
<i>DAZAP1+2</i>	No	-	No mature sperm	Not tested	Not tested	Not tested
<i>DZIP/Smed-iguana</i>	Yes (bloating observed in homeostasis)	Yes	No mature sperm	Not tested	Lysis	Lysis, no testes in remaining fragments
<i>pumilio</i>	Yes	Yes	Small testes, no loss of specific cell type marker	Not tested	Lysis	Lysis, no testes in remaining fragments

**Table S2. Experimental details for planarian homologs of DAZ family targets**

Target name	Required for regeneration (neoblast maintenance)?	Expressed in the male germ cells?	Germ cell RNAi phenotype in adult sexually mature worms	Germ cell RNAi phenotype in hatchlings	Required for <i>de novo</i> germ cell specification?	RNAi phenotype in sexually immature regenerates
<i>CDC25-1</i>	Yes	Yes	Loss of early germ cells followed by more differentiated cells	No male germ cells	Lysis	Lysis, no testes in remaining fragments
<i>CDC25-2</i>	No	Yes	Loss of early germ cells followed by more differentiated cells	No male germ cells	No	No testes
<i>SDAD1</i>	Yes	Yes	Loss of early germ cells followed by more differentiated cells	Not tested	Lysis	Lysis
<i>CDC25-3</i>	No	Not tested	No	Not tested	No	No
<i>vasa1</i>	Yes	Yes	Small testes, no loss of specific cell type marker	Not tested	Lysis	Lysis
<i>vasa2</i>	No	Yes	No	Not tested	No	Small testes with only SSCs and spermatogonia
<i>Ringo/SPY</i>	No	Yes	No mature sperm	Not tested	No	Small testes with only SSCs and spermatogonia
<i>TPX-1</i>	No	No	No	Not tested	No	No
<i>TRF2-1</i>	No	Yes	No	Not tested	No	No
<i>TRF2-2</i>	No	No	No	Not tested	No	No
<i>TRF2-3</i>	No	No	No	Not tested	No	No
<i>GRSF1-1</i>	Yes	Yes	Early lysis	Not tested	Lysis	Lysis
<i>GRSF1-2</i>	No	Yes	No	Not tested	No	No
<i>PAM</i>	No	Not tested	No	Not tested	No	No
<i>TSSK</i>	No	Not tested	No	Not tested	No	No

**Table S3. Accession numbers of sequences used for phylogenetic analyses**

<b>Organism</b>	<b>Common name/taxon</b>	<b>Gene name</b>	<b>NCBI Accession number/source</b>
<i>Aplysia californica</i>	Mollusc	Boule	XP_005103136
<i>Caenorhabditis elegans</i>	Nematode	Boule	NM_062635.4
<i>Caenorhabditis briggsae</i>	Nematode	Boule	XP_002630720
<i>Drosophila ananassae</i>	Arthropod (Diptera)	Boule	XP_001958310
<i>Drosophila melanogaster</i>	Arthropod (Diptera)	Boule	Q24207
<i>Gallus gallus</i>	Aves	Boule	XP_421917
<i>Hofstenia miamia</i>	Acoelomorpha	Boule1	Not available
<i>Hofstenia miamia</i>	Acoelomorpha	Boule2	Not available
<i>Homo sapiens</i>		Boule	NP_001271291.1
<i>Hydra vulgaris</i>	Cnidaria	Boule1	JN379588
<i>Hydra vulgaris</i>	Cnidaria	Boule2	JN379589
<i>Hydra vulgaris</i>	Cnidaria	Boule3	JN379590
<i>Macrostomum lignano</i>	Flatworm (Turbulera)	Boule1	HM222645
<i>Macrostomum lignano</i>	Flatworm (Turbulera)	Boule2	JF911416
<i>Macrostomum lignano</i>	Flatworm (Turbulera)	Boule3	JF911417
<i>Mus musculus</i>	Mouse	Boule	NM_029267.3
<i>Nematostella vectensis</i>	Cnidaria	Boule1	XM_001635170
<i>Nematostella vectensis</i>	Cnidaria	Boule2	XM_001637198
<i>Saccoglossus kowalevskii</i>	Hemichordate	Boule	XM_011683988.1
<i>Schistosoma mansoni</i>	Flatworm (Trematode)	Boule	XM_002575473
<i>Schmidtea mediterranea</i>	Flatworm (Turbulera)	Boule1	KU519616
<i>Schmidtea mediterranea</i>	Flatworm (Turbulera)	Boule2	KU519617
<i>Strongylocentrotus purpuratus</i>	Echinoderm	Boule	XM_011683988.1

<i>Tribolium castaneum</i>	Arthropod (Coleoptera)	Boule	EFA05679
<i>Lingula anatina</i>	Brachiopod	Boule	<a href="http://marinegenomics.oist.jp/">http://marinegenomics.oist.jp/</a>
<i>Pinctada fucata</i>	Pearl Oyster	Boule	<a href="http://marinegenomics.oist.jp/">http://marinegenomics.oist.jp/</a>
<i>Biomphalaria glabrata</i>	Snail	Boule	XR_001216766.1
<i>Lottia gigantea</i>	Sea snail	Boule	<a href="http://genome.jgi.doe.gov/">http://genome.jgi.doe.gov/</a>
<i>Capitella sp. I ESC-2004</i>	Polychaete	Boule1	<a href="http://genome.jgi.doe.gov/">http://genome.jgi.doe.gov/</a>
<i>Capitella sp. I ESC-2004</i>	Polychaete	Boule2	<a href="http://genome.jgi.doe.gov/">http://genome.jgi.doe.gov/</a>
<i>Octopus bimaculoides</i>		Boule	XM_014929311.1
<i>Helobdella robusta</i>	Leech	Boule1	<a href="http://genome.jgi.doe.gov/">http://genome.jgi.doe.gov/</a>
<i>Helobdella robusta</i>	Leech	Boule2	<a href="http://genome.jgi.doe.gov/">http://genome.jgi.doe.gov/</a>
<i>Echinococcus multilocularis</i>	Flatworm (Cestode)	Boule1	parasite.wormbase.org
<i>Echinococcus multilocularis</i>	Flatworm (Cestode)	Boule2	parasite.wormbase.org
<i>Hymenolepis diminuta</i>	Flatworm (Cestode)	Boule1	parasite.wormbase.org
<i>Hymenolepis diminuta</i>	Flatworm (Cestode)	Boule2	parasite.wormbase.org
<i>Hymenolepis microstoma</i>	Flatworm (Cestode)	Boule	parasite.wormbase.org
<i>Taenia asiatica</i>	Flatworm (Cestode)	Boule	parasite.wormbase.org
<i>Danio rerio</i>	Zebrafish	Dazl	AB018191.1
<i>Gallus gallus</i>	Aves	Dazl	NM_204218.1
<i>Mus musculus</i>	Mouse	Dazl	NM_010021.5
<i>Homo sapiens</i>		DAZL	NM_001190811.1
<i>Oryzias latipes</i>	Japenese killfish	Dazl	NP_001098269.1
<i>Xenopus laevis</i>	Frog	Dazl	AF017778.1
<i>Homo sapiens</i>		DAZ	U21663.1

**Table S4. Cloning and qRT-PCR primer sequences****Cloning primers**

<b>Gene name</b>	<b>Forward cloning primer</b>	<b>Reverse cloning primer</b>	<b>Genbank accession number</b>
<i>boule1</i>	TGCAAACAAAATGTCAACTGAT	CATAAGGCACGGGTCCAT	KU519616
<i>boule2</i>	TATTTGTTGGGGGATTTCCA	CTTTGAGGTGTTGCCATTGA	KU519617
<i>CDC25-1</i>	TCACAACACTCCTGAAACACCA	TTCTGGTCCACGAACCGATG	KU852687
<i>CDC25-2</i>	ATGCAATATTTCTGTCAGTC	AAGACGCTTAATATCACATC	KU852688
<i>CDC25-3</i>	TGGCCACCTGTTTATTCCTC	CGACTTGACAATTCCCATCA	KU852689
<i>DAZAP1</i>	GATGGTAACGAGATTGGAAA	TATGACGTTGTTTGGTTTGA	KU852669
<i>DAZAP2</i>	TGACGGTGTCATAAAAAGTCA	AGCTCCTTGATCCCATAAAT	KU852670
<i>DZIP/iguana</i>	ATCACCGTTGTCCATATTGT	GTCATCCTCCAAATTTTCA	KU852671
<i>GRSF1-1</i>	TACAGGGGAGGCATTTGTTC	TCCGTCTGGGCCTATTTGTA	KU852676
<i>GRSF1-2</i>	GAGAAAGGCCACGAAGAGAA	AAAACATCATCTGGGCGTGT	KU852677
<i>PAM</i>	ACCGTCAGACCAAACGAAC	TGCTTGAGCCACATCTGAAC	KU852680
<i>pumilio</i>	GGCAGGATTGTGCAACTCAG	CCAAGATCCTGATTGTTTTTCA	KU852681
<i>Ringo/SPY</i>	GCTCACGATGTGGAAGAAGA	ATCTGACTCGTCGCTGTCAT	KU852682
<i>SDAD1</i>	TGGAGTTGCTGTCGAGATTG	CTTTCGGTTTTTGCTTGCTC	KU852686
<i>TPX1</i>	TTCTAACCGCCCATAACACC	CAGAGTCCGTCATTGCATGT	KU852672
<i>TRF2-1</i>	CACTTTTTCCAGTGGTCATGATT	CATTGGACGCGAGTTCATAA	KU852673
<i>TRF2-2</i>	TTGTGATGCCTACACCTCAGTT	TTTTCCCGAAACGAAAATCA	KU852674
<i>TRF2-3</i>	TATCGCCTGCTTTTTTCGACT	TCTTTTCTCCGGTCAAAT	KU852675
<i>TSSK</i>	TTGCTGAAAATCGAGAACAG	ACTCGTCAGACTCGTTGCAC	KU852683
<i>vasa1</i>	TTGACCCAGTGCAAAAATA	GCCAACAACCTCAACAGCTA	KU852684
<i>vasa2</i>	TTCCAACGCGTGAATTATGT	GTCGCCATGGATTGTAGTTG	KU852685

**qRT-PCR primers**

<b>Gene name</b>	<b>Forward qRT-PCR primer</b>	<b>Reverse qRT-PCR primer</b>
<i>boule1</i>	TCAAGCAACGATGACTGCTG	TTTGACGTGATTCCACCAAC
<i>boule2</i>	CCACTATCAATGGCAACACC	TCAACGGTTCTACTGGCATC
<i>nanos</i>	CAAGGACAAATGTTGCCTGTA	CAACCCATCGATCCAACCTCT
<i>pka</i>	CATAGTCCAAGGCGATGATG	GGCGTTGTACATCAGTGCTAGT
<i>tkn-1</i>	CTGACATGCTTGGCACTTCT	GCGGTCTTCCCTATTCACTT
<i><math>\beta</math>-tubulin</i>	TGGCTGCTTGTGATCCAAGA	AAATTGCCGCAACAGTCAAATA