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Somatic regulation of female germ cell regeneration and development in planarians

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SUMMARY

Female germ cells develop into oocytes, with the capacity for totipotency. In most animals, these remarkable cells are specified during development and cannot be regenerated. By contrast, planarians, known for their regenerative prowess, can regenerate germ cells. To uncover mechanisms required for female germ cell development and regeneration, we generated gonad-specific transcriptomes and identified genes whose expression defines progressive stages of female germ cell development. Strikingly, early female germ cells share molecular signatures with the pluripotent stem cells driving planarian regeneration. We observe spatial heterogeneity within somatic ovarian cells and find that a regionally enriched *foxL* homolog is required for oocyte differentiation, but not specification, suggestive of functionally distinct somatic compartments. Unexpectedly, a neurotransmitter-biosynthetic enzyme, aromatic L-amino acid decarboxylase (AADC), is also expressed in somatic gonadal cells, and plays opposing roles in female and male germ cell development. Thus, somatic gonadal cells deploy conserved factors to regulate germ cell development and regeneration in planarians.

Graphical abstract

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AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and writing, U.W.K. and P.A.N.; investigation and visualization, U.W.K; funding acquisition, P.A.N.

SUPPLEMENTAL INFORMATION

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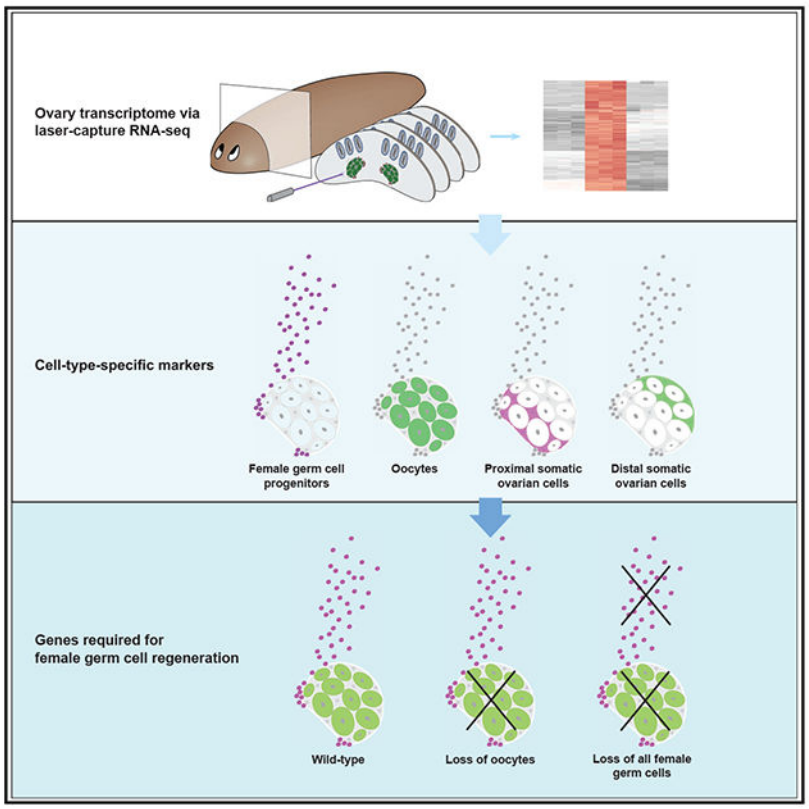
DECLARATION OF INTERESTS

The authors declare no competing interests.



In brief

Unlike most animals, planarians can regenerate germ cells. Here, Khan and Newmark characterize gene expression in the planarian ovary, identifying genes expressed at progressive stages of female germ cell development and in somatic ovarian cells. Functional characterization revealed somatically expressed genes required for specification or differentiation of female germ cells.

INTRODUCTION

Germ cells serve as a link between generations, producing gametes that propagate the genetic material from parent to offspring. During their development, germ cells undergo dramatic, sex-specific differentiation to generate highly specialized cell types, the sperm and egg, which ultimately yield a totipotent zygote. As the female germline differentiates into oocytes, it acquires the capacity for totipotency (Reik and Surani, 2015; Seydoux and Braun, 2006). This capacity is exemplified by somatic cell nuclear transfer, in which oocyte cytoplasm reprograms differentiated cell nuclei to produce viable clones (Campbell et al., 1996; Gurdon, 1962; Wakayama et al., 1998). Parthenogenesis, in which an unfertilized egg generates an entire new organism (Simon et al., 2003), provides another striking example of the oocyte's capacity for totipotency. Given the oocyte's critical roles in embryonic development, understanding the mechanisms underlying female germ cell development has enormous significance.

Somatic support cells within the gonads play critical roles in regulating germ cell development across the animal kingdom. Soma-germline communication is necessary throughout a germ cell's life, from regulating fate choices and survival, to proliferation and differentiation (Kiger et al., 2000; Kimble and White, 1981; Korta and Hubbard, 2010; Li and Albertini, 2013; Murray et al., 2010). The *in vitro* derivation of functional oocytes and spermatid-like cells from pluripotent stem cells requires co-culture with somatic gonadal cells (Hikabe et al., 2016; Zhou et al., 2016), further emphasizing the importance of soma-germ cell interactions in facilitating proper germ cell development.

Most animals, including model organisms typically used to study development, set aside germ cells early during embryonic development and cannot replace them if lost (Nieuwkoop and Sutasurya, 1979, 1981). By contrast, freshwater planarians demonstrate the striking ability to regenerate both male and female germ cells from tissues lacking any reproductive structures (Morgan, 1901; Sato et al., 2006; Wang et al., 2007). Planarians are best known for their remarkable whole-body regeneration, driven by stem cells called neoblasts (Baguñà et al., 1989), a subset of which are pluripotent (Wagner et al., 2011; Zeng et al., 2018). Planarian germ cells are specified inductively and are derived from neoblasts (Baguñà et al., 1989; Newmark et al., 2008; Sato et al., 2006; Wang et al., 2007). The vast majority of research on planarian regeneration focuses on asexual strains that reproduce by fission; however, planarians can also reproduce sexually as simultaneous hermaphrodites. Some planarians can even reproduce parthenogenetically: sperm serve to activate development, without contributing genetic information (a process known as gynogenesis), demonstrating the potency of the female germline (Benazzi and Benazzi, 1976). Germ cell development in sexual planarians is responsive to physiological cues: following prolonged starvation, gonads regress and accessory reproductive organs are resorbed (Berninger, 1911; Morgan, 1901; Schultz, 1904); when feeding is resumed, gonads again produce gametes and accessory reproductive organs are re-formed. Planarians' remarkable developmental plasticity provides a unique opportunity to investigate mechanisms regulating sex-specific germ cell specification and differentiation in the context of an adult organism.

In sexual planarians, ovarian tissue is scarce relative to testes; as such, previous transcriptomic analyses predominantly identified genes with enriched expression in the testes rather than ovaries (Rouhana et al., 2017; Wang et al., 2010; Zayas et al., 2005). Whole-animal single-cell sequencing approaches produced similar results (Fincher et al., 2018). Thus, little is known about the gene expression changes driving female germ cell development in planarians. An orphan G-protein-coupled receptor (GPCR), Ophis, is expressed in somatic gonadal cells of ovaries and testes and is required for differentiation of both oocytes and sperm (Saberi et al., 2016); however, gene expression differences distinguishing female from male somatic gonadal cells have yet to be identified, and the roles of somatic ovarian cells in various stages of female germ cell development remain to be determined. Here, to circumvent the limited quantity of ovarian tissue and identify genes with enriched expression in planarian ovaries, we generated gonad-specific transcriptomes using laser-capture microdissection followed by RNA sequencing (RNA-seq). Analysis and validation of these transcriptomic data enabled us to characterize the developmental progression of female germ cells, define somatic ovarian cell types, and identify somatically expressed genes required for proper regeneration and development of female germ cells.

RESULTS

LCM sequencing identifies transcripts that mark and distinguish ovaries from testes

The reproductive system of the sexual strain of the planarian *Schmidtea mediterranea* consists of two ovaries located ventrally under the posterior lobes of the brain, numerous dorsolaterally distributed testes, and accessory reproductive organs (yolk glands [vitellaria], oviducts, sperm ducts, copulatory apparatus, and gonopore) (Figure 1A) (Hyman, 1951; Issigonis and Newmark, 2019). To precisely excise ovarian, testis, and surrounding non-gonadal tissues for transcriptomic comparisons, we used laser-capture microdissection (LCM; Figure 1B) (Emmert-Buck et al., 1996; Espina et al., 2006; Forsthoefel et al., 2020). RNA-seq analysis (Mortazavi et al., 2008; Nagalakshmi et al., 2008) of the laser-captured tissues identified 36,036 non-redundant transcripts, of which 7,577 (21%) were upregulated (≥2-fold, false discovery rate p value ≤0.01) in either or both gonads: 1,491 (4%) in ovaries; 4,880 (14%) in testes; and 1,206 (3%) in both (Figures 1B, 1C, and S1A–S1D; Table S1). To validate the LCM-generated gonadal transcriptomes, we examined genes with previously reported gonadal expression (Wang et al., 2010). As expected, we found significant upregulation of the majority of these genes (82 out of 89) in the testis transcriptome (Figure S2A), including *nanos*, a conserved regulator of germ cell development (Handberg-Thorsager and Saló, 2007; Sato et al., 2006; Wang et al., 2007), which was also upregulated in the ovary transcriptome (Figure S2A).

To validate candidates and identify new genes expressed in the ovary, we performed whole-mount colorimetric RNA *in situ* hybridization (WISH) for ovary-enriched transcripts and detected diverse expression patterns in and around the ovaries (Figures 1D and S2B). Approximately 90% (204 out of 227) of genes tested showed enriched expression in the ovary or associated reproductive structures. These results demonstrate the successful application of LCM-RNA-seq for generating gonad-specific transcriptomes, providing a resource for identifying regulators of germ cell development and regeneration.

Female germ cell progenitors are specified outside the ovaries and express markers of pluripotency

Histological and ultrastructural studies have shown that planarian oocytes grow and mature in a progression from the margins of the ovary toward the tuba (fertilization duct) (Gremigni and Nigro, 1983; Harrath et al., 2011). Markers of the earliest stages of germ cell development (*nanos* and *krüppel-like factor 4* [*klf4*]) label oogonia at the ovary periphery (Issigonis et al., 2021; Wang et al., 2007); *nanos* and *klf4* are also expressed in two fields of cells, anterior to the ovary at the ventro-medial portion of each brain lobe (Issigonis et al., 2021). To help elucidate the origins and differentiation of the female germ cell lineage, we sought to identify ovary-enriched transcripts expressed at various stages of oogenesis. Fluorescent RNA *in situ* hybridization (FISH) analysis detected *galactose-binding lectin* (*lecg*) expression in differentiating cells throughout the ovary, but not in the anterior field of *klf4*-expressing cells (Figure 1E). Expression of *ubiquitin carboxyl-terminal hydrolase 8* (*ubp8*) was detected in larger oocytes residing within the ovary, proximal to the tuba, but not within smaller *lecg*$^{+}$ cells at the ovary periphery (Figure 1F). Thus, *ubp8* is upregulated later in oocyte differentiation than *lecg* (Figure 1F). The expression patterns of these oocyte

markers and the corresponding growth of oocytes reveal progressive stages of female germ cell differentiation, from the periphery of the ovary toward its interior.

Like the previously described patterns of *nanos* and *klf4* (Issigonis et al., 2021), we detected expression of a gene originally annotated as *polyketide synthase-1* (*pks1*) (Zeng et al., 2018) in fields of cells anterior to the ovary; its expression was also detected throughout the ovary (Figures 1D and 1G). This gene was identified via single-cell sequencing as a marker of a pluripotent subpopulation of neoblasts in asexual *S. mediterranea* (Zeng et al., 2018); because its predicted product shares no conserved domains with any reported proteins, including polyketide synthases, and based upon its expression dynamics (described below), we propose renaming it *germline-expressed*, *wound-induced in neoblasts* (*gwîn*; Sindarin for "youth," corresponding to its expression beginning in early stages of female germ cell development). *gwîn* is expressed in the *klf4*$^+$ fields anterior to (Figures 1G and 1Gi) and around the margins of the ovaries (Figures 1G and 1Gii). The *gwîn*$^+$ cells in the anterior fields and around the margins of the ovary were labeled by anti-phospho-Histone H3 (pHH3) antibodies (Figure S3Ai and ii), indicating that they are proliferative. *gwîn* is also expressed in *lecg*$^+$ oocytes within the ovary (Figures 1G and 1Giii), and, thus, serves as a marker spanning all stages of female germ cell development. These results suggest that the *nanos*$^+$ *klf4*$^+$ *gwîn*$^+$ cells located at the margin of the ovary are oogonia that give rise to *gwîn*$^+$*lecg*$^+$ oocytes within the ovary (Figure 1G). Finding proliferating cells expressing the earliest markers of germ cell development (*nanos*$^+$ *klf4*$^+$*gwîn*$^+$) anterior to the ovaries suggests that female germ cell progenitors (FGPs) can also be specified outside of the ovaries (Issigonis et al., 2021). Identifying markers for progressive stages of female germ cell development allows us to study early steps in female germ cell specification and differentiation.

A marker of pluripotent neoblasts is expressed in germ cells and only upregulated in neoblasts after wounding

In addition to *gwîn*, single-cell analysis of a pluripotent neoblast subpopulation identified other cluster-defining transcripts, including *tspan group-specific gene-1* (*tgs-1*) and *tetraspanin-1* (*tspan-1*) (Zeng et al., 2018). The expression of *gwîn* in FGPs of sexual planarians suggested affinities between pluripotent neoblasts and early germ cells; thus, we sought to further explore this relationship. First, we examined the expression of the neoblast marker *piwi-1* (Reddien et al., 2005), a planarian PIWI homolog that is also expressed in germ cells (Davies et al., 2017). We detected relatively high levels of *piwi-1* mRNA in *gwîn*$^+$ FGPs (Figures 2A and 2Ai and ii). Consistent with previous studies (Davies et al., 2017), we detected *piwi-1* expression in oocytes, suggesting that *piwi-1* expression is maintained through oocyte differentiation (Figures 2A and 2Aiii). Similar to *gwîn, tgs-1* is enriched in the ovarian transcriptome and expressed abundantly in FGPs (Figures 2B and S3C); however, *tgs-1* was also detected in a subset of neoblasts (Figure S3D). In the sexual strain, *gwîn* expression was most pronounced in germ cells in both ovaries and testes (Figures 1G, 2A, 2B, and S3B) and, aside from co-expression in the germ cells, its expression did not overlap with that of *piwi-1*.

To validate the expression of neoblast markers in germ cells, we first assessed *gwîn* expression in asexual *S. mediterranea*. Although these animals reproduce by fission, they develop gonad primordia containing germ cells that fail to differentiate (Handberg-Thorsager and Saló, 2007; Wang et al., 2007). In small asexuals (worm length: 2.3 ± 0.2mm) we did not detect *gwîn* expression anywhere in the animal; larger asexuals (4.2 ± 0.5mm), however, displayed a small group of *gwîn*$^+$ cells ventral to the posterior brain lobes (Figures 2D and 2E), reminiscent of FGPs in the sexual strain and consistent with the increase of *nanos*$^+$ *klf4*$^+$ female germ cells in this region of larger asexual planarians (Issigonis et al., 2021). We did not detect *gwîn* expression in testis primordia of asexuals, suggesting that *gwîn* is upregulated during a later stage of male germ cell development (Figure 2D).

Considering the scarcity of FGPs in asexuals, it seems unlikely that these cells were captured during single-cell sequencing by Zeng et al.; rather, *gwîn* expression in pluripotent neoblasts could result from cell dissociation and the subsequent induction of wound-response transcriptional programs (Lacar et al., 2016; Wu et al., 2017). Indeed, wound responsiveness was one of the criteria used to define this neoblast subcluster (Zeng et al., 2018). To test this idea, we examined *gwîn* expression by WISH after amputating or injuring the worms and found that *gwîn* mRNA was upregulated at wound sites in both sexual and asexual strains (Figures 2C–2F). This dramatic upregulation at wound sites may account for the expression of *gwîn* in additional clusters beyond that expressing *tgs-1*, as well as its broad expression in neoblast colonies observed after partial irradiation (Zeng et al., 2018). We corroborated our ISH analysis by examining previous regeneration transcriptomes (Kao et al., 2013; Rozanski et al., 2019), which revealed that *gwîn* is upregulated as early as 4 to 6 h after injury, a time frame consistent with the possibility of cell-dissociation-induced upregulation in neoblasts (Figure S4A). In contrast, other early germ cell markers, such as *klf4* and *nanos*, are not upregulated at wound sites (Figures S4B and S4C). Next, we confirmed that *gwîn* was upregulated in neoblasts after amputation by performing double FISH with the neoblast marker *piwi-1*. In uninjured asexual planarians, *gwîin*$^+$*piwi-1*$^+$ cells were only detected in the FGP region (Figure 2E). However, after amputation, *gwîn*$^+$*piwi-1*$^+$ cells were detected at both anterior and posterior wound sites (Figure 2F). These data indicate that, in intact planarians, *gwîn* marks germ cells rather than neoblasts. Wound-inducible expression of this gene in neoblasts provides another link between germ cells and the planarian's pluripotent stem cells.

Gene-expression profiling reveals spatial heterogeneity in ovarian somatic cells

How are FGPs maintained and what regulates their differentiation within the ovary? Somatic gonadal cells play critical roles in regulating germ cell development throughout the animal kingdom. To date, studies of soma-germline interactions in the planarian ovary have been limited by the availability of appropriate markers. The first such marker, *ophis*, encodes an orphan GPCR expressed in somatic support cells of ovaries and testes and is required for both female and male germ cell differentiation (Saberi et al., 2016). Our ISH screen of ovary-enriched transcripts identified three genes with expression patterns distinct from those observed for germ-cell-enriched transcripts, and resembling a scaffold surrounding the germ cells: *delta3*; the forkhead-family transcription factor, *foxL*; and *endothelin converting enzyme 1 (ece1)* (Figures 1C, 1D, and S5A). We confirmed that *delta3, foxL*, and *ece1*

transcripts were not detected in female germ cells using double FISH with pooled probes for the germ cell markers *klf4* and *lecg* (Figures 3A, 3B, and 3C). Additionally, unlike *ophis* (Saberi et al., 2016), *delta3, foxL,* and *ece1* transcripts were not detectable in the testes, indicating sexually dimorphic expression (Figure S5A). *delta3*$^{+}$ cells are closely associated with oocytes, and co-express *ophis* within the ovary, confirming *delta3* as a marker of ovarian somatic cells (Figure 3D). Double FISH to detect *foxL* and *delta3* revealed higher expression of *foxL* in *delta3*$^{+}$ somatic cells situated proximal to the tuba, compared with *delta3*$^{+}$ cells at the periphery (Figure 3E). In contrast, *ece1* is expressed in the peripheral *delta3*$^{+}$ cells (Figure 3F). Double FISH to detect both *ece1* and *foxL* confirmed the presence of *ece1*^{high}*foxL*^{low} cells at the periphery of the ovary (Figures 3G, 3Gi, and 3H) and *ece1*^{low} *foxL*^{high} cells proximal to the tuba (Figures 3G, 3Gii, and 3H), indicating heterogeneity among ovarian somatic support cells. This heterogeneity could reflect distinct functional roles at different stages of oogenesis.

Ovarian somatic cells regulate female germ cell development via conserved factors

foxL encodes a planarian homolog of the forkhead-family transcription factor FoxL2 (Figure S5B), which is a critical regulator of granulosa cell differentiation in the mammalian ovary. Ovaries fail to develop properly in *FoxL2* knockout mice: the absence of functional granulosa cells in these mutants leads to oocyte atresia and infertility due to progressive follicular depletion (Schmidt et al., 2004; Uda et al., 2004). Human mutations in *FoxL2* can lead to premature ovarian failure or ovarian tumors (Crisponi et al., 2001; Georges et al., 2014; Schmidt et al., 2004; Shah et al., 2009; Uda et al., 2004; Uhlenhaut and Treier, 2011). *FoxL2* is also required to actively maintain female fate in the adult mouse ovary (Uhlenhaut et al., 2009). Although *FoxL2* genes have been identified in a range of invertebrates, its expression in ovarian somatic cells, but not germ cells, has been proposed to be a vertebrate innovation (Bertho et al., 2016). Furthermore, functional roles for *FoxL2* homologs in ovarian development have yet to be demonstrated in any invertebrate.

Thus, it was noteworthy to find planarian *foxL* similarly expressed in ovarian somatic cells, enriched in the tuba-proximal population surrounding late-stage oocytes (Figures 3B, 3E, 3G, and S5A). To examine whether *foxL* also plays a functional role in oogenesis, we used RNAi to knock down its expression in the context of decapitated worms that will regenerate new ovaries (Figure 4A). *foxL*(*RNAi*) worms regenerated their heads normally and displayed normal feeding behavior (Figure S5C). The distribution and numbers of *klf4*$^{+}$ FGPs in the anterior fields were not affected significantly in these animals (Figures 4B and 4C). However, *foxL* RNAi resulted in a dramatic and significant reduction in the number of *lecg*$^{+}$ oocytes (Figures 4B and 4C). These data suggest that *foxL* is required cell non-autonomously for oocyte differentiation and maintenance in planarians.

As reported above, ovarian somatic cells also express a *delta3* homolog (Figures 3A and 3B), which encodes a transmembrane Notch-signaling ligand (Figure S5D) (Bray, 2006). Notch signaling plays critical roles in various aspects of soma-germ cell interactions across species. For example, the *Caenorhabditis elegans* niche uses Notch signaling to regulate germline stem cell maintenance and proliferation (Byrd and Kimble, 2009), whereas, in the *Drosophila* ovary, Notch signaling controls the formation and maintenance of the niche (Xie

et al., 2008). To explore the role of Notch signaling in planarian oogenesis, we performed *delta3* RNAi experiments in the context of the ovarian regeneration paradigm (Figure 4A). *delta3*(*RNAi*) planarians displayed a significant increase of *klf4*$^+$ FGPs, the distribution of which was skewed toward the midline (Figures 4D, 4E, and 4F). Additionally, the ovaries of *delta3*(*RNAi*) planarians appeared disorganized, with *klf4*$^+$ FGPs intermingled with *lecg*$^+$ oocytes; oocyte numbers were significantly reduced (Figures 4D and 4E).

Because direct cell-cell interaction is necessary for Notch signaling (Bray, 2006), we asked whether Notch receptors were also expressed in the ovary. We found enriched expression of transcripts encoding both planarian Notch receptors in the ovarian transcriptome (Figures S5D and S5E). FISH revealed mRNAs of both *notch2* and *notch4* in the tuba and oviduct; *notch4* mRNA was also detected in the *delta3*$^+$ ovarian somatic cells (Figure 4G). Knockdown of *notch2* resulted in a significant expansion, midline-skewed distribution of *klf4*$^+$ FGPs, and disorganized ovaries with depleted oocytes, similar to *delta3*(*RNAi*) worms. By contrast, *klf4*$^+$ FGPs appeared unaffected after *notch4* RNAi, but oocyte numbers were reduced (Figures 4D, 4E, and 4F). Although *notch2*(*RNAi*) worms regenerated normally, they were smaller at the end of our assay, suggesting roles in growth or feeding, which could also influence oocyte development (Figure S5F). Altogether, Notch signaling plays several roles in female germ cell development in planarians, regulating the number and distribution of FGPs, as well as the spatial organization of the ovary, with effects upon oocyte production. The expression patterns of the Notch-signaling components reported here suggest that interactions between accessory reproductive organs (oviduct, tuba) and ovarian somatic cells may also help establish proper structure of the ovary.

A female-specific regulator of germ cell regeneration: bidirectional soma-germline communication

In addition to markers of ovarian somatic cells, the ovary transcriptome enabled us to identify *zfs1*, a female-specific early germ cell marker. This gene encodes a predicted RNA-binding protein (Figure S6A) and is expressed in ovaries as well as in cells anterior to the ovaries (Figures 5A and 5B). Double FISH with *gwîn* indicated that *zfs1* is co-expressed in FGPs and oocytes (Figure 5C); however, unlike other FGP markers (*klf4* and *nanos*) that are also expressed in early stages of male germ cell development, *zfs1* expression was restricted to female germ cells (Figures 5A and 5B). RNAi knockdown of *zfs1* using the previously described ovary-regeneration paradigm (Figure 4A) resulted in a dramatic reduction in the number of *klf4*$^+$ FGPs and ablation of *lecg*$^+$ oocytes (Figures 5D and 5E). By contrast, male germ cell development was unaffected by *zfs1* RNAi: *klf4*$^+$ early male germ cells at the testis periphery, and DAPI-stained sperm in the lumen (Issigonis et al., 2021) appeared normal (Figure 5F). Accessory reproductive structures and egg laying were also unaffected. However, *zfs1*(*RNAi*) animals were sterile: they failed to produce hatchlings (Figures S6B–S6D). These results indicate a germ-cell-intrinsic, female-specific role for *zfs1*, which belongs to a small, yet ancient, family of RNA-binding proteins that regulate mRNA turnover (Cuthbertson et al., 2008; Wells et al., 2017). The yeast ortholog is a regulator of sexual differentiation (Kanoh et al., 1995; Navarro et al., 2017), and the mammalian homolog, ZFP36L2, is critical for oocyte maturation, targeting transcriptional regulators and chromatin modifiers for degradation (Dumdie et al.,2018). Understanding how *zfs1*

expression is restricted to female germ cells will provide insight into the modulation of germ cell sex in a simultaneous hermaphrodite.

Although *zfs1*(*RNAi*) animals lack female germ cells, DAPI staining suggested that ovaries were still present (Figure 5D). To examine whether the somatic ovary was affected, we used *delta3* as a marker, revealing increased somatic cells in *zfs1*(*RNAi*) worms (Figures 5G and 5H). We recently reported that *klf4* RNAi resulted in loss of female germ cells and concomitant expansion of the somatic ovary; however, *klf4* RNAi also leads to loss of testes and vitellaria (Issigonis et al., 2021). The ovary-specific effects of *zfs1* RNAi suggest that the absence of female germ cells, rather than altered systemic cues resulting from lack of testes and vitellaria, triggers expansion of ovarian somatic cells in *klf4*(*RNAi*) worms (Figures 5G and 5H). Thus, germ cells and somatic support cells communicate bidirectionally during ovary regeneration.

Unexpected roles of AADC in germ cell development and regeneration

The laser-capture transcriptomes also revealed unanticipated gonadal expression of conserved genes not previously implicated in gonadal function. Aromatic L-amino acid decarboxylase (AADC) is a broadly conserved enzyme (Figure S7A) that catalyzes a key reaction in the production of monoamine neurotransmitters, such as serotonin and dopamine. Previous studies of asexual planarians showed that *aadc* is expressed in serotonergic neurons within the central nervous system, as well as in pigment cups of the photoreceptors and secretory cells around the pharynx (Currie and Pearson, 2013; März et al., 2013). Thus, it was surprising to find enriched expression of *aadc* mRNA in the planarian ovary (Figure S7B). We confirmed ovary-enriched *aadc* expression using *in situ* hybridization (Figure 6Ai), and also detected *aadc* expression in the testes and vitellaria (Figure 6Aii, iii). Double FISH to detect *aadc* and somatic support cell markers in ovaries (*delta3, foxL*, and *ece1*) and testes (*ophis*) revealed that *aadc* is expressed in both female and male somatic gonadal cells (Figures 6B and 6C). In ovaries, *aadc* expression was enriched in the $ece1^{+}foxL^{low}$ cells at the periphery (Figure 6B). To confirm this expression in somatic gonadal cells, we generated and validated polyclonal antibodies against AADC (Figures S7C, S7D; STAR Methods). Immunofluorescence labeling with anti-AADC antibodies detected AADC within the somatic support cells, which form scaffolds surrounding the germ cells in ovaries and testes (Figures 6D and 6E, and S7D). These data suggest that AADC could act locally within somatic gonadal cells to produce monoamines. In support of this idea, we find differential expression of various serotonin synthetic enzymes and other pathway components in the ovary and testis transcriptomes (Figure S7E).

To determine whether monoamines play a role in female germ cell development, we disrupted AADC function using the RNAi paradigm described above (Figure 4A). Knockdown of *aadc* resulted in the failure to regenerate ovaries after amputation: we observed a dramatic reduction of $klf4^{+}$ FGPs and loss of oocytes (Figures 6F and 6K). Furthermore, *aadc*(*RNAi*) worms failed to regenerate the somatic compartment of the ovary (Figures 6G and 6L). These results suggest that AADC activity is necessary for the specification of FGPs and implicates ovarian somatic cells in this process. More broadly, accessory reproductive organs (vitellaria, oviducts, sperm ducts, and the gonopore) failed to

regenerate or be maintained, and egg laying was abolished in *aadc*(*RNAi*) worms (Figures S7F–S7H). No obvious *aadc* expression was detected in oviducts or sperm ducts (Figure 6A), suggesting that AADC function within neurons, somatic gonadal cells, and/or vitellaria could act extrinsically in regeneration of these organs.

In contrast to the lack of ovaries after *aadc* knockdown, *aadc*(*RNAi*)animals displayed hyperplastic testes, full of early male germ cells (Figure 6H). Testes of control worms consist of peripherally located, immature (*klf4*$^{+}$*nanos*$^{+}$ and *klf4*$^{-}$*nanos*$^{+}$) germ cells, with luminal differentiating germ cells (spermatids and sperm) (Issigonis et al., 2021; Wang et al., 2007). Testes of *aadc*(*RNAi*) worms, however, consisted mainly of *klf4*$^{+}$*nanos*$^{+}$ early male germ cells and lacked differentiated cells (Figures 6H, 6I, and 6M). Unlike the ovaries, somatic cells in the testes appeared unaffected after *aadc* knockdown (Figures 6J and 6N). Taken together, these results implicate somatic gonadal cells as a potential source of monoamines, which may be acting differentially upon male and female germ cells in planarians.

DISCUSSION

Planarians exhibit extraordinary plasticity in reproductive development, including the ability to regenerate germ cells *de novo*; the mechanisms underlying this plasticity remain poorly understood. To uncover mechanisms underlying female germ cell development and regeneration, we generated gonad-specific transcriptomes. These studies identified genes defining progressive stages of female germ cell development and revealed heterogeneity of somatic ovarian cells. We uncovered intriguing similarities between female germ cells and neoblasts, the pluripotent stem cells that drive regeneration. We identified conserved, somatically expressed regulators, which have sex-specific functions in germ cell development and regeneration. Our findings underscore the key role played by somatic support cells in both development and regeneration of germ cells.

Germ cell sex in a simultaneous hermaphrodite and the relationship of FGPs to pluripotent stem cells

Planarians are simultaneous hermaphrodites, developing ovaries and testes in different regions of the body. Because the genes implicated thus far in the earliest stages of germ cell development (*nanos* and *klf4*) are expressed in both male and female germ cells (Handberg-Thorsager and Saló, 2007; Issigonis et al., 2021; Sato et al., 2006; Wang et al., 2007), it was unclear whether the sex of these early germ cells was already determined. The identification of *zfs1* as a female-specific, germcell-intrinsic factor indicates that planarian germ cells “know” their sex early in their development. Presumably, inputs from global-patterning signals that define the animal’s major body axes (Reddien, 2018) are integrated such that germ cells born antero-ventrally toward the base of the brain adopt female fates, whereas those born dorso-laterally adopt male fates. Exploring the impact of global-patterning signals on sex-specific germ cell specification will be an important future direction.

The distribution of FGPs in fields anterior to the ovaries suggests two plausible scenarios. These fields could represent streams of migratory female germ cells that will later be incorporated into the ovary, similar to the example of planarian eye progenitors that are

found in streams outside of the photoreceptors (Atabay et al., 2018; Lapan and Reddien, 2012). Alternatively, early female germ cells may be specified in a relatively broad antero-ventral region, but only those cells interacting with somatic gonadal cells (or other anatomical landmarks) will be incorporated into the ovary and produce oocytes. Whichever of these models best reflects reality, the gene expression changes we identified throughout female germ cell development suggest that FGPs/oogonia found around the margin of the ovary differentiate peripherally into oocytes that mature and move internally toward the tuba, where they exit from the ovary for fertilization (Figure 7).

Previous studies have noted the similarities between neoblasts and germ cells, from the presence of ribonucleoprotein granules called chromatoid bodies, to the expression of several genes associated with germline development in other animals, including *vasa, piwi, tudor, pumilio*, and *bruno* (Guo et al., 2006; Reddien et al., 2005; Salvetti et al., 2005; Shibata et al., 1999; Solana et al., 2009). Single-cell transplantation into lethally irradiated hosts revealed a subpopulation of neoblasts capable of giving rise to all cell types in the animal; these pluripotent neoblasts are functionally defined as clonogenic neoblasts (cNeoblasts) (Wagner et al., 2011). Single-cell sequencing efforts to characterize a pluripotent neoblast subpopulation identified TSPAN-1 as a cell-surface marker that can be used to enrich for cNeoblasts (Zeng et al., 2018). These authors identified *tgs-1* and *pks1* (here renamed *gwîn*) as signature transcripts associated with this *tspan-1*$^{+}$ cluster in the asexual strain of S. *mediterranea.* We have shown that, in the sexual strain, these two genes are expressed abundantly in FGPs. Although *gwîn* is expressed at high levels constitutively in germ cells during homeostasis, it is only upregulated in neoblasts in response to wounding. This wound-responsive, neoblast-specific upregulation of genes associated with early stages of female germ cell development is particularly intriguing given the recent demonstration that neoblast fates are far more plastic than generally appreciated: specialized neoblast subclasses that express transcription factors associated with specific cell types are capable of generating daughters that adopt other fates (Raz et al., 2021). These authors also found that *tgs-1* was not expressed in all early neoblast colonies; rather, their single-cell sequencing analysis detected *tgs-1* expression in clusters displaying features of neural specification (Raz et al., 2021). Whether *tgs-1* expression in female germ cells reflects additional similarities between FGPs and neural fates (e.g., reliance on post-transcriptional control of gene expression; Kulkarni et al., 2020) will be a fascinating avenue for future study. Nonetheless, our results suggest that neoblast plasticity may involve transient activation of some germ-cell-associated genes. Similarities in gene expression between germ cells and pluripotent neoblasts during regeneration are consistent with previous observations that planarian germ cells are capable of contributing to the regeneration of somatic tissues (Gremigni et al., 1980a; 1980b).

Ovarian somatic support cells: Key players in germ cell regeneration

Somatic support cells of planarian ovaries were observed ultrastructurally decades ago (Fischlschweiger, 1991, 1994; Gremigni and Falleni, 1998; Gremigni and Nigro, 1983; Harrath et al., 2011), but their functional characterization began with the discovery of *ophis*, a GPCR-encoding gene that is expressed in somatic gonadal cells and required for germ cell differentiation (Saberi et al., 2016). The paucity of these cells had thus far hindered

their discovery by bulk and single-cell sequencing approaches (Fincher et al., 2018; Zayas et al., 2005). We used LCM to overcome this limitation, and the resulting gonad-specific transcriptomes enabled us to identify a set of sexually dimorphic ovarian support cell markers (*delta3*, *foxL,* and *ece1*). Their expression patterns revealed heterogeneity within ovarian somatic cells: enriched expression of *ece1* and *aadc* was observed in cells distal to the tuba, whereas enriched expression of *foxL* was detected in cells proximal to the tuba, associated with later-stage oocytes. We found that *aadc* was required for FGP specification and maintenance, whereas *foxL* was required for oocyte differentiation and maintenance, without affecting FGPs (Figure 7). These disparate effects suggest that the tuba-distal and tuba-proximal somatic cell populations may control distinct stages of female germ cell development (Figure 7). Such distinct functional domains would be consistent with the recently described functional compartmentalization of the somatic niche in the germarium of the *Drosophila* ovary (Shi et al., 2021; Tu et al., 2021) and the distinct populations of granulosa cells regulating reproductive onset versus duration in the mouse ovary (Niu and Spradling, 2020).

The expression patterns and knockdown phenotypes of the Notch-signaling components reported here suggest that interactions between ovarian somatic cells (which express *delta3* and *notch4*) and accessory reproductive organs, the oviduct and tuba (which express *notch2* and *notch4*), help establish (and/or maintain) proper ovarian structure. Notch signaling also affects the germ cells: RNAi knockdown of *delta3* or *notch2* resulted in both the skewed distribution of FGPs toward the midline and an increase in the number of FGPs. The midline skewing could reflect the role of *delta3* in midline patterning: it is expressed at the midline and knockdown in the asexual strain leads to cyclopia (Sasidharan et al., 2017). We did not observe cyclopic worms in our RNAi regeneration assays, so it remains to be determined if FGPs are more sensitive to the disruption of midline cues than the photoreceptors, or if the altered distribution reflects altered signaling from the somatic ovary. Nonetheless, potential alterations in midline patterning seem unlikely to affect the number of FGPs; thus, the somatic ovary may be capable of communicating with FGPs and regulating their specification and/or proliferation at a distance.

FoxL2 is a critical regulator of granulosa cell fate in mammals; homologs of this gene had yet to be characterized functionally in any invertebrate. Our observations that a planarian homolog (*foxL*) is expressed robustly in a subpopulation of somatic ovarian cells and is required for oocyte differentiation and maintenance suggest that the female-specific role of FoxL family members in the somatic gonad may well predate the emergence of vertebrates. The sexually dimorphic expression of planarian *foxL* is particularly intriguing in the context of this simultaneous hermaphrodite. In mice, female-specific FoxL2 and male-specific Dmrt1 transcription factors act in a mutually antagonistic manner to maintain gonadal sex: post-natal conditional knockout of *foxL2* results in granulosa cell transdifferentiation into Sertoli cells (Uhlenhaut et al., 2009); by contrast, post-natal conditional knockout of *dmrt1* results in Sertoli cell transdifferentiation into granulosa cells (Matson et al., 2011). In addition to *foxL*, planarians also have a *dmrt1* homolog (*dmd-1*), which is expressed specifically in male reproductive organs (including somatic cells of the testes) and is required for the specification, differentiation, and maintenance of male germ cells as well as accessory reproductive organs (Chong et al., 2013). Whether functional antagonism, like that

observed in mice, between female-specific *foxL* and male-specific *dmd-1* also plays a role in maintaining gonadal sex in this simultaneous hermaphrodite will be an important avenue for future studies.

Potential non-neuronal roles of monoamines in germ cell development

Biogenic monoamines, like serotonin and dopamine, act as neurotransmitters across the animal kingdom (Weiger, 1997; Yamamoto and Vernier, 2011). It is perhaps less widely appreciated that many of these molecules predate the evolution of nervous systems (Roshchina, 2016) and play other, non-neuronal roles (Bellono et al., 2017; Lv and Liu, 2017; Matsuda et al., 2004). Our finding that AADC, an enzyme required for monoamine synthesis, plays sex-specific roles in planarian germ cell regeneration implicates monoamines in the regulation of crucial stages of germ cell development, from specification to differentiation. AADC is involved in the synthesis of many different monoamines (including serotonin, tyramine, tryptamine, histamine, dopamine, and other catecholamines); thus, it is unclear whether the observed sex-specific effects reflect sex-specific activities of different monoamines or whether male and female tissues respond differentially to the same monoamine.

Although we do not yet know the identity of the monoamine(s) mediating the *aadc*(*RNAi*) phenotype, planarian gonads possess the machinery to respond differentially to the same monoamine. For example, we queried the gonad transcriptomes and found evidence for serotonergic pathways in both testes and ovaries (Figure S7E). Tryptophan hydroxylase (TPH) catalyzes the conversion of L-tryptophan to 5-hydroxy-L-tryptophan, which is decarboxylated by AADC to produce serotonin. Vesicular monoamine transporters (VMAT) then package serotonin into vesicles for release, where it can bind serotonin receptors (5HTR). Serotonin transporters (SERTs) terminate signaling by taking up extracellular serotonin. A homolog of the *Drosophila* peripheral tryptophan hydroxylase (TPH), *henna*, is highly expressed in both testes and ovaries, relative to the neuronally expressed *tph1* (Currie and Pearson, 2013; März et al., 2013; Sarkar et al., 2019). Planarian gonads also express *vmat* and *sert* homologs, suggesting the ability to release and recycle serotonin. Notably, ovaries and testes appear to differentially express different *vmats, serts,* and serotonin receptors. Thus, gonadal cells are capable of producing and responding locally to monoamines such as serotonin; the expression of distinct receptors in ovaries and testes suggests the possibility of differential responses. The expression of AADC by somatic support cells and the presence of specific monoamine receptors in both ovaries and testes supports the idea that these molecules can be produced and sensed locally within the gonads. Moreover, a recent report showed that serotonin could induce ovary development in another planarian species (*Dugesia ryukyuensis*), further supporting the role of monoamines in planarian reproductive system development (Sekii et al., 2019).

Might monoamines act in germ cell development in other animals? Genes encoding an entire serotonergic network, consisting of synthetic enzymes, receptors, and transporters, are expressed in mammalian ovaries (Dubé and Amireault, 2007). In mice and humans, two paralogous genes encode tryptophan hydroxylase, the rate-limiting enzyme in serotonin synthesis. Expression of one paralog (*Tph1*) is found in a wide range of non-neuronal tissues

and is responsible for synthesizing ~95% of the body's serotonin, whereas expression of the other (*Tph2*) is detected largely in neural cells (reviewed in Amireault et al., 2013). The embryonic defects observed in offspring of $Tph1^{-/-}$ knockout females (Côté et al., 2007) are confounded by potential indirect effects from the compromised physiological states of these mice, which have diabetes and anemia (Amireault et al., 2011; Paulmann et al., 2009). These complications of interpreting $Tph1^{-/-}$ phenotypes and the finding that the placenta serves as the source of serotonin that acts in the fetal forebrain (Bonnin et al., 2011) seem to have quelled investigations into potential roles of serotonin in oogenesis and early embryonic development. Furthermore, because *Tph2* is among the genes showing the greatest degree of upregulation in the female somatic gonad at the time of sex determination (Beverdam and Koopman, 2006), it seems quite likely that it may act redundantly with *Tph1* in the ovaries. Similar expression of multiple serotonin receptors in the gonads would also likely compensate for loss of any single gonadally enriched gene. Thus, exploring potential roles of these deeply conserved molecules in the reproductive organs of a simpler animal, like the planarian, may overcome such concerns about redundancy and guide future tissue-specific knockout studies.

Regardless of whether or not the role of monoamines uncovered here reflects deeply conserved mechanisms for regulating germ cell development across animal phylogeny, the observed effects upon the planarian's reproductive system have other important implications. Planarians are free-living representatives of the phylum Platyhelminthes (the flatworms) and their reproductive system shares several important features with those of parasitic flatworms (flukes and tapeworms), which have major impacts on global public health (Collins and Newmark, 2013). All of these flatworms have ectolecithal eggs (i.e., yolk on the outside); yolk cells are produced by specialized accessory reproductive organs known as vitellaria and they are essential for embryonic development. Furthermore, transmission of parasitic flatworms requires prolific egg and yolk cell production. Because inhibition of *aadc* results in the loss of ovaries and other female accessory reproductive organs (including vitellaria), unraveling the role of monoamines in the female flatworm reproductive system may lead to new approaches for preventing parasite transmission.

Limitations of the study

This work represents a first step in characterizing global gene expression in the planarian ovary and identifying genes that play critical roles in various stages of female germ cell development in this animal. The use of LCM to identify genes with enriched expression in ovarian tissues was quite successful, as indicated by the ~90% validation using *in situ* hybridization as well as by our ability to define successive stages of female germ cell development and distinct somatic ovarian cell types. However, this initial expression-based screen likely missed genes that are also expressed abundantly in non-gonadal tissues. Future single-cell sequencing efforts will provide a more comprehensive picture of gene expression in all ovarian cell types. Moreover, the functional analysis presented here, using RNAi in the context of an ovary-regeneration assay, only allowed us to examine germ cell phenotypes in knockdown animals that could properly regenerate their heads (including cephalic ganglia) and their feeding circuitry. Thus, any genes required for proper female germ cell development that are also required more broadly for proper neoblast function,

neural activity, or viability would be missed by our assay, because knockdown planarians would fail to regenerate, feed, or survive. Despite such limitations, the genes identified here will facilitate future studies in which subtler experimental perturbations (e.g., localized ovarian ablation) are used to dissect more precisely the processes by which planarian neoblasts give rise to functional female germ cells.

STAR★METHODS

RESOURCE AVAILABILITY

Lead contact

- Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Phillip A. Newmark (pnewmark@morgridge.org).

Materials availability

- Newly generated materials generated in this study will be made available upon request.

Data and code availability

- All next-generation sequencing data have been deposited at the GEO repository under the accession number GSE191229 and are publicly available as of the date of publication.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Planarian maintenance and care

Hermaphroditic, sexual *S. mediterranea* were maintained at 18°C in 0.75X Montjuïc salts (Cebrià and Newmark, 2005) supplemented with 50 μg/mL Gentamicin (Gemini Bio-Products) and fed beef liver paste. Asexual *S. mediterranea* (clone CIW4; Sánchez Alvarado et al., 2002) were maintained at 22°C in 1X Montjuïc salts and fed beef liver paste. Planarians were maintained in Ziploc containers and kept in Petri dishes for RNAi experiments. Planarians were starved at least one week before initiating RNAi experiments, *in situ* hybridizations, or immunostainings.

METHOD DETAILS

Laser-capture microdissection and RNA extraction—Planarians were killed in chilled 2% HCl in PBS (RNAse-Free) and then fixed in 100% Acetone for 1 hour at −20°C. Planarians were then incubated in 10, 20, 30% sucrose in PBS (RNAse-Free) for 20 minutes each before embedding in Tissue Freezing Medium (TFM) blocks (Ted Pella). The samples were then cryosectioned at 16 mm thickness onto PEN membrane slides (Thermo Fisher). The slides were stained with 1% cresyl violet and 1% eosin Y (Sigma) in 75% ethanol

(Clément-Ziza et al., 2008). The tissue samples were dissected using Veritas Laser Capture Microdissection System (Arcturus) and captured on to Capsure HS LCM Caps (Thermo Fisher). After capture, RNA was isolated using the Arcturus PicoPure RNA isolation kit (Thermo Fisher). Ovarian tissue samples were collected from 14 worms (10 worms used for cross-sections and 4 worms used for sagittal sections) for each replicate (3 replicates total). Testis- and non-gonadal-tissue samples were collected from 6 worms for each replicate (4 worms used for cross-sections and 2 worms used for sagittal sections). RNA concentrations were determined using Qubit Fluorometer (Thermo Fisher), and RNA integrity was analyzed using 2100 Bioanalyzer Instrument (Agilent).

RNA sequencing and gene expression analysis—Libraries were generated using Trueseq RNA stranded kit (Illumina), and 100 nt single-end sequencing reads were generated on Hi-Seq 2500 (Illumina). Trimming of adapters and low-quality reads as well as subsequent read mapping and differential gene expression analyses were performed using CLC Genomics Workbench (Qiagen). Reads were mapped to a de novo assembled *S. mediterranea* transcriptome (smed_20140614) (Robb et al., 2015) with the *Smed-zfs1* transcript added. The transcriptome was annotated in OmicsBox (BioBam) using the SwissProt Protein database. Heatmaps were generated in R using the ComplexHeatmaps package (Gu et al., 2016).

Molecular biology methods—200–1000 bp fragments of the gene of interest (Table S2) were amplified using Platinum Taq DNA Polymerase (Invitrogen) from cDNA. Amplified fragments were cloned into pJC53.2 (Collins et al., 2010) via TA cloning. Riboprobes and dsRNA were synthesized as previously described (King and Newmark, 2013; Rouhana et al., 2013).

***In situ* hybridization and immunohistochemistry—**Whole-mount ISH was performed as previously described (King and Newmark, 2013) with modifications for larger sexual worms: formaldehyde fixation was increased to 30 min, proteinase K treatment was increased to 20-25 min, and post-proteinase K fixation was increased to 30 min. pHH3 was labeled using anti-phospho-Histone H3 (Ser10) (Millipore Sigma). AADC polyclonal antibody was generated by injecting a synthetic peptide (DVYTPKMDAEEFRKRGKE) into rabbits (Pierce Biotechnology, Rockford, IL). The serum was affinity purified with the peptide antigen and used at a dilution of 1:2000 for immunofluorescence. Colorimetric and FISH/immunofluorescence samples were imaged on Axio Zoom.V16 (Carl Zeiss) and LSM 710 or 880 confocal microscope (Carl Zeiss), respectively. Cell counts were performed using the spot detection tool in Imaris (Bitplane).

RNA interference—Knockdowns were performed by feeding *in vitro*-transcribed dsRNA, as previously described (Rouhana et al., 2013). 6-8 mature sexual animals were fed ~10-20 ug dsRNA mixed with 90 uL liver puree: water mixture 5:1 (with food coloring) in a petri dish. For all RNAi experiments, dsRNA corresponding to bacterial *ccdB* gene was used for negative controls. Animals were fed dsRNA 4 times, cut posterior to the ovaries, and tail fragments were allowed to regenerate for two weeks, followed by 8 dsRNA feedings.

Protein domain \ analysis—Conserved protein domains were analyzed using InterProScan, SMART and Phobius protein domain analysis tools. Predicted protein sequences were aligned using MUSCLE.

QUANTIFICATION AND STATISTICAL ANALYSIS

All two-sample and three-sample comparisons were analyzed using Welch's t-test or one-way ANOVA (Dunnett's test), respectively, in Prism (GraphPad). Differential expression for RNAseq was analyzed in CLC Genomics Workbench (Qiagen) using the Wald test.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

Amireault P, Hatia S, Bayard E, Bernex F, Collet C, Callebert J, Launay J-M, Hermine O, Schneider E, Mallet J, et al. (2011). Ineffective erythropoiesis with reduced red blood cell survival in serotonin-deficient mice. Proc. Natl. Acad. Sci. U S A 108, 13141–13146. [PubMed: 21788492]

Amireault P, Sibon D, and Côté F (2013). Life without peripheral serotonin: insights from tryptophan hydroxylase 1 knockout mice reveal the existence of paracrine/autocrine serotonergic networks. ACS Chem. Neurosci 4, 64–71. [PubMed: 23336045]

Atabay KD, LoCascio SA, de Hoog T, and Reddien PW (2018). Self-organization and progenitor targeting generate stable patterns in planarian regeneration. Science 360, 404–409. [PubMed: 29545509]

Baguñà J, Saló E, and Auladell C (1989). Regeneration and pattern formation in planarians. III. that neoblasts are totipotent stem cells and the cells. Development 107, 77–86.

Bellono NW, Bayrer JR, Leitch DB, Castro J, Zhang C, O'Donnell TA, Brierley SM, Ingraham HA, and Julius D (2017). Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. Cell 170, 185–198.e16. [PubMed: 28648659]

Benazzi M, and Benazzi LG (1976). Platyhelminthes. In Animal Cytogenetics, Bernard J, ed. (Gebrüder Borntraeger), pp. 1–182.

Berninger J (1911). Über die Einwirkung des Hungers auf Planarien. Zool. Jahrb 30, 181–216.

Bertho S, Pasquier J, Pan Q, Le Trionnaire G, Bobe J, Postlethwait JH, Pailhoux E, Schartl M, Herpin A, and Guiguen Y (2016). Foxl2 and its relatives are evolutionary conserved players in gonadal sex differentiation. Sex Dev. 10, 111–129. [PubMed: 27441599]

Beverdam A, and Koopman P (2006). Expression profiling of purified mouse gonadal somatic cells during the criticial time window of sex determination reveals novel candidate genes for human sexual dysgenesis syndromes. Hum. Mol. Genet 15, 417–431. [PubMed: 16399799]

Bonnin A, Goeden N, Chen K, Wilson ML, King J, Shih JC, Blakely RD, Deneris ES, and Levitt P (2011). A transient placental source of serotonin for the fetal forebrain. Nature 472, 347–350. [PubMed: 21512572]

Bray SJ (2006). Notch signalling: a simple pathway becomes complex. Nat. Rev. Mol. Cell Biol 7, 678–689. [PubMed: 16921404]

Byrd DT, and Kimble J (2009). Scratching the niche that controls *Caenorhabditis elegans* germline stem cells. Semin. Cell Dev. Biol 20, 1107–1113. [PubMed: 19765664]

Campbell KH, McWhir J, Ritchie WA, and Wilmut I (1996). Sheep cloned by nuclear transfer from a cultured cell line. Nature 380, 64–66. [PubMed: 8598906]

Cebrià F, and Newmark PA (2005). Planarian homologs of netrin and netrin receptor are required for proper regeneration of the central nervous system and the maintenance of nervous system architecture. Development 132, 3691–3703. [PubMed: 16033796]

Chong T, Collins JJ 3rd, Brubacher JL, Zarkower D, and Newmark PA (2013). A sex-specific transcription factor controls male identity in a simultaneous hermaphrodite. Nat. Commun 4, 1814. [PubMed: 23652002]

Clément-Ziza M, Munnich A, Lyonnet S, Jaubert F, and Besmond C (2008). Stabilization of RNA during laser capture microdissection by performing experiments under argon atmosphere or using ethanol as a solvent in staining solutions. RNA 14, 2698–2704. [PubMed: 18945804]

Collins JJ 3rd, and Newmark PA (2013). It's no fluke: the planarian as a model for understanding schistosomes. PLOS Pathog. 9, e1003396. [PubMed: 23874195]

Collins JJ 3rd, Hou X, Romanova EV, Lambrus BG, Miller CM, Saberi A, Sweedler JV, and Newmark PA (2010). Genome-wide analyses reveal a role for peptide hormones in planarian germline development. PLOS Biol. 8, e1000509. [PubMed: 20967238]

Côté F, Fligny C, Bayard E, Launay J-M, Gershon MD, Mallet J, and Vodjdani G (2007). Maternal serotonin is crucial for murine embryonic development. Proc. Natl. Acad. Sci. U S A 104, 329–334. [PubMed: 17182745]

Crisponi L, Deiana M, Loi A, Chiappe F, Uda M, Amati P, Bisceglia L, Zelante L, Nagaraja R, Porcu S, et al. (2001). The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. Nat. Genet 27, 159–166. [PubMed: 11175783]

Currie KW, and Pearson BJ (2013). Transcription factors *lhx1/5-1* and *pitx* are required for the maintenance and regeneration of serotonergic neurons in planarians. Development 140, 3577–3588. [PubMed: 23903188]

Cuthbertson BJ, Liao Y, Birnbaumer L, and Blackshear PJ (2008). Characterization of zfs1 as an mRNA-binding and -destabilizing protein in *Schizo-saccharomyces pombe*. J. Biol. Chem 283, 2586–2594. [PubMed: 18042546]

Davies EL, Lei K, Seidel CW, Kroesen AE, McKinney SA, Guo L, Robb SM, Ross EJ, Gotting K, and Sánchez Alvarado A (2017). Embryonic origin of adult stem cells required for tissue homeostasis and regeneration. eLife 6, e21052. [PubMed: 28072387]

Dubé F, and Amireault P (2007). Local serotonergic signaling in mammalian follicles, oocytes and early embryos. Life Sci. 81, 1627–1637. [PubMed: 18023821]

Dumdie JN, Cho K, Ramaiah M, Skarbrevik D, Mora-Castilla S, Stumpo DJ, Lykke-Andersen J, Laurent LC, Blackshear PJ, Wilkinson MF, et al. (2018). Chromatin modification and global transcriptional silencing in the oocyte mediated by the mRNA decay activator ZFP36L2. Dev. Cell 44, 392–402.e7. [PubMed: 29408237]

Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, Weiss RA, and Liotta LA (1996). Laser capture microdissection. Science 274, 998–1001. [PubMed: 8875945]

Espina V, Wulfkuhle JD, Calvert VS, VanMeter A, Zhou W, Coukos G, Geho DH, Petricoin EF 3rd, and Liotta LA (2006). Laser-capture microdissection. Nat. Protoc 1, 586–603. [PubMed: 17406286]

Fincher CT, Wurtzel O, de Hoog T, Kravarik KM, and Reddien PW (2018). Cell type transcriptome atlas for the planarian *Schmidtea mediterranea*. Science 360, eaaq1736. [PubMed: 29674431]

Fischlschweiger W (1991). Ultrastructure of the seminal receptacle, ovovitelline duct, and yolk-funnel cell of *Dugesia tigrina* (Platyhelminthes: Tricladida). Trans. Am. Microscopical Soc 110, 300–314.

Fischlschweiger W (1994). Ultrastructure of the reproductive system of *Cura foremanii* (Platyhelminthes: Tricladida). Trans. Am. Microsc. Soc 113, 1–14.

Forsthoefel DJ, Cejda NI, Khan UW, and Newmark PA (2020). Cell-type diversity and regionalized gene expression in the planarian intestine. eLife 9, e52613. [PubMed: 32240093]

Georges A, Auguste A, Bessière L, Vanet A, Todeschini A-L, and Veitia RA (2014). FOXL2: a central transcription factor of the ovary. J. Mol. Endocrinol 52, R17–R33. [PubMed: 24049064]

Gremigni V, and Falleni A (1998). Characters of the female gonad and the phylogeny of Platyhelminthes. Hydrobiologia 383, 235–242.

Gremigni V, and Nigro M (1983). An ultrastructural study of oogenesis in a marine triclad. Tissue Cell 15, 405–415. [PubMed: 6684334]

Gremigni V, Miceli C, and Puccinelli I (1980a). On the role of germ cells in planarian regeneration. I. A karyological investigation. J. Embryol. Exp. Morphol 55, 53–63. [PubMed: 7373204]

Gremigni V, Miceli C, and Picano E (1980b). On the role of germ cells in planarian regeneration. II. Cytophotometric analysis of the nuclear Feulgen-DNA content in cells of regenerated somatic tissues. J. Embryol. Exp. Morphol 55, 65–76. [PubMed: 6989946]

Gu Z, Eils R, and Schlesner M (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 32, 2847–2849. [PubMed: 27207943]

Guo T, Peters AHFM, and Newmark PA (2006). A Bruno-like gene is required for stem cell maintenance in planarians. Dev. Cell 11, 159–169. [PubMed: 16890156]

Gurdon JB (1962). Adult frogs derived from the nuclei of single somatic cells. Dev. Biol 4, 256–273. [PubMed: 13903027]

Handberg-Thorsager M, and Saló E (2007). The planarian *nanos*-like gene Smednos is expressed in germline and eye precursor cells during development and regeneration. Dev. Genes Evol 217, 403–411. [PubMed: 17390146]

Harrath AH, Alwasal SH, Alhazza I, Zghal F, and Tekaya S (2011). [An ultrastructural study of oogenesis in the planarian *Schmidtea mediterranea* (Platyhelminthe, Paludicola)]. C. R. Biol 334, 516–525. [PubMed: 21784361]

Hikabe O, Hamazaki N, Nagamatsu G, Obata Y, Hirao Y, Hamada N, Shimamoto S, Imamura T, Nakashima K, Saitou M, et al. (2016). Reconstitution *in vitro* of the entire cycle of the mouse female germ line. Nature 539, 299–303. [PubMed: 27750280]

Hyman LH (1951). The Invertebrates, Vol II: Platyhelminthes and Rhynchocoela (McGraw-Hill Book Company Inc.).

Issigonis M, and Newmark PA (2019). From worm to germ: germ cell development and regeneration in planarians. Curr. Top. Dev. Biol 135, 127–153. [PubMed: 31155357]

Issigonis M, Redkar AB, Rozario T, Khan UW, Mejia-Sanchez R, Lapan SW, Reddien PW, and Newmark PA (2021). Krüppel-like factor 4 is required for development and regeneration of germline and yolk cells from somatic stem cells in planarians. Preprint at bioRxiv. 10.1101/2021.11.08.467675.

Kanoh J, Sugimoto A, and Yamamoto M (1995). *Schizosaccharomyces pombe zfs1+* encoding a zinc-finger protein functions in the mating pheromone recognition pathway. Mol. Biol. Cell 6, 1185–1195. [PubMed: 8534915]

Kao D, Felix D, and Aboobaker A (2013). The planarian regeneration transcriptome reveals a shared but temporally shifted regulatory program between opposing head and tail scenarios. BMC Genom. 14, 797.

Kiger AA, White-Cooper H, and Fuller MT (2000). Somatic support cells restrict germline stem cell self-renewal and promote differentiation. Nature 407, 750–754. [PubMed: 11048722]

Kimble JE, and White JG (1981). On the control of germ cell development in *Caenorhabditis elegans*. Dev. Biol 81, 208–219. [PubMed: 7202837]

King RS, and Newmark PA (2013). In situ hybridization protocol for enhanced detection of gene expression in the planarian *Schmidtea mediterranea*. BMC Dev. Biol 13, 8. [PubMed: 23497040]

Korta DZ, and Hubbard EJA (2010). Soma-germline interactions that influence germline proliferation in *Caenorhabditis elegans*. Dev. Dyn 239, 1449–1459. [PubMed: 20225254]

Kulkarni A, Lopez DH, and Extavour CG (2020). Shared cell biological functions may underlie pleiotropy of molecular interactions in the germ lines and nervous systems of animals. Front. Ecol. Evol 8, 215.

Lacar B, Linker SB, Jaeger BN, Krishnaswami SR, Barron JJ, Kelder MJE, Parylak SL, Paquola ACM,Venepally P, Novotny M, et al. (2016). Nuclear RNA-seq of single neurons reveals molecular signatures of activation. Nat. Commun 7, 11022. [PubMed: 27090946]

Lapan SW, and Reddien PW (2012). Transcriptome analysis of the planarian eye identifies ovo as a specific regulator of eye regeneration. Cell Rep. 2, 294–307. [PubMed: 22884275]

Li R, and Albertini DF (2013). The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. Nat. Rev. Mol. Cell Biol 14, 141–152. [PubMed: 23429793]

Lv J, and Liu F (2017). The role of serotonin beyond the central nervous system during embryogenesis. Front. Cell. Neurosci 11, 74. [PubMed: 28348520]

März M, Seebeck F, and Bartscherer K (2013). A Pitx transcription factor controls the establishment and maintenance of the serotonergic lineage in planarians. Development 140, 4499–4509. [PubMed: 24131630]

Matson CK, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, and Zarkower D (2011). DMRT1 prevents female reprogramming in the postnatal mammalian testis. Nature 476, 101–104. [PubMed: 21775990]

Matsuda M, Imaoka T, Vomachka AJ, Gudelsky GA, Hou Z, Mistry M, Bailey JP, Nieport KM, Walther DJ, Bader M, et al. (2004). Serotonin regulates mammary gland development via an autocrine-paracrine loop. Dev. Cell 6, 193–203. [PubMed: 14960274]

Morgan TH (1901). Growth and regeneration in *Planaria lugubris*. Arch. Entw. Mech. Org 13, 179–212.

Mortazavi A, Williams BA, McCue K, Schaeffer L, and Wold B (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat. Methods 5, 621–628. [PubMed: 18516045]

Murray SM, Yang SY, and Van Doren M (2010). Germ cell sex determination: a collaboration between soma and germline. Curr. Opin. Cell Biol 22, 722–729. [PubMed: 21030233]

Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, and Snyder M (2008). The transcriptional landscape of the yeast genome defined by RNA sequencing. Science 320, 1344–1349. [PubMed: 18451266]

Navarro FJ, Chakravarty P, and Nurse P (2017). Phosphorylation of the RNA-binding protein Zfs1 modulates sexual differentiation in fission yeast. J. Cell Sci 130, 4144–4154. [PubMed: 29084823]

Newmark PA, Wang Y, and Chong T (2008). Germ cell specification and regeneration in planarians. Cold Spring Harb. Symp. Quant. Biol 73, 573–581. [PubMed: 19022767]

Nieuwkoop PD, and Sutasurya LA (1979). Primordial Germ Cells in the Chordates: Embryogenesis and Phylogenesis (Cambridge University Press).

Nieuwkoop PD, and Sutasurya LA (1981). Primordial Germ Cells in the Invertebrates: From Epigenesis to Preformation (Cambridge University Press).

Niu W, and Spradling AC (2020). Two distinct pathways of pregranulosa cell differentiation support follicle formation in the mouse ovary. Proc. Natl. Acad. Sci. U S A 117, 20015–20026. [PubMed: 32759216]

Paulmann N, Grohmann M, Voigt J-P, Bert B, Vowinckel J, Bader M, Skelin M, Jevsek M, Fink H, Rupnik M, et al. (2009). Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. Plos Biol. 7, e1000229. [PubMed: 19859528]

Raz AA, Wurtzel O, and Reddien PW (2021). Planarian stem cells specify fate yet retain potency during the cell cycle. Cell Stem Cell 28, 1307–1322.e5. [PubMed: 33882291]

Reddien PW (2018). The cellular and molecular basis for planarian regeneration. Cell 175, 327–345. [PubMed: 30290140]

Reddien PW, Oviedo NJ, Jennings JR, Jenkin JC, and Sánchez Alvarado A (2005). SMEDWI-2 is a PIWI-like protein that regulates planarian stem cells. Science 310, 1327–1330. [PubMed: 16311336]

Reik W, and Surani MA (2015). Germline and pluripotent stem cells. Cold Spring Harb. Perspect. Bio 7, a019422. [PubMed: 26525151]

Robb SMC, Gotting K, Ross E, and Sánchez Alvarado A (2015). SmedGD 2.0: the *Schmidtea mediterranea* genome database. Genesis 53, 535–546. [PubMed: 26138588]

Roshchina VV (2016). New trends and perspectives in the evolution of neurotransmitters in microbial, plant, and animal cells. Adv. Exp. Med. Biol 874, 25–77. [PubMed: 26589213]

Rouhana L, Weiss JA, Forsthoefel DJ, Lee H, King RS, Inoue T, Shibata N, Agata K, and Newmark PA (2013). RNA interference by feeding *in vitro*-synthesized double-stranded RNA to planarians: methodology and dynamics. Dev. Dyn 242, 718–730. [PubMed: 23441014]

Rouhana L, Tasaki J, Saberi A, and Newmark PA (2017). Genetic dissection of the planarian reproductive system through characterization of *Schmidtea mediterranea* CPEB homologs. Dev. Biol 426, 43–55. [PubMed: 28434803]

Rozanski A, Moon H, Brandl H, Martín-Durán JM, Grohme MA, Hüttner K, Bartscherer K, Henry I, and Rink JC (2019). PlanMine 3.0-improvements to a mineable resource of flatworm biology and biodiversity. Nucleic Acids Res. 47, D812–D820. [PubMed: 30496475]

Saberi A, Jamal A, Beets I, Schoofs L, and Newmark PA (2016). GPCRs direct germline development and somatic gonad function in planarians. PLOS Biol. 14, e1002457. [PubMed: 27163480]

Sánchez Alvarado A, Newmark PA, Robb S, and Juste R (2002). The *Schmidtea mediterranea* database as a molecular resource for studying platyhelminthes, stem cells and regeneration. Development 129, 5659–5665. [PubMed: 12421706]

Salvetti A, Rossi L, Lena A, Batistoni R, Deri P, Rainaldi G, Locci MT, Evangelista M, and Gremigni V (2005). *DjPum*, a homologue of *Drosophila Pumilio*, is essential to planarian stem cell maintenance. Development 132, 1863–1874. [PubMed: 15772127]

Sarkar A, Mukundan N, Sowndarya S, Dubey VK, Babu R, Lakshmanan V, Rangiah K, Panicker MM, Palakodeti D, Subramanian SP, et al. (2019). Serotonin is essential for eye regeneration in planaria *Schmidtea mediterranea*. FEBS Lett. 593, 3198–3209. [PubMed: 31529697]

Sasidharan V, Marepally S, Elliott SA, Baid S, Lakshmanan V, Nayyar N, Bansal D, Sánchez Alvarado A, Vemula PK, and Palakodeti D (2017).The *miR-124* family of microRNAs is crucial for regeneration of the brain and visual system in the planarian Schmidtea mediterranea. Development 144, 3211–3223. [PubMed: 28807895]

Sato K, Shibata N, Orii H, Amikura R, Sakurai T, Agata K, Kobayashi S, and Watanabe K (2006). Identification and origin of the germline stem cells as revealed by the expression of *nanos*-related gene in planarians. Dev. Growth Differ 48, 615–628. [PubMed: 17118016]

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. [PubMed: 22743772]

Schmidt D, Ovitt CE, Anlag K, Fehsenfeld S, Gredsted L, Treier A-C, and Treier M (2004). The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. Development 131, 933–942. [PubMed: 14736745]

Schultz E (1904). Uber reduktionen. I. Uber Hungerserscheinungen bei *Planaria lactea*. Arch. Entwm 18, 555–577.

Sekii K, Yorimoto S, Okamoto H, Nagao N, Maezawa T, Matsui Y, Yamaguchi K, Furukawa R, Shigenobu S, and Kobayashi K (2019). Transcriptomic analysis reveals differences in the regulation of amino acid metabolism in asexual and sexual planarians. Sci. Rep 9, 6132. [PubMed: 30992461]

Seydoux G, and Braun RE (2006). Pathway to totipotency: lessons from germ cells. Cell 127, 891–904. [PubMed: 17129777]

Shah SP, Köbel M, Senz J, Morin RD, Clarke BA, Wiegand KC, Leung G, Zayed A, Mehl E, Kalloger SE, et al. (2009). Mutation of FOXL2 in granulosa-cell tumors of the ovary. N. Engl. J. Med 360, 2719–2729. [PubMed: 19516027]

Shi J, Jin Z, Yu Y, Zhang Y, Yang F, Huang H, Cai T, and Xi R (2021). A progressive somatic cell niche regulates germline cyst differentiation in the *Drosophila* ovary. Curr. Biol 31, 840–852.e5. [PubMed: 33340458]

Shibata N, Umesono Y, Orii H, Sakurai T, Watanabe K, and Agata K (1999). Expression of *vasa(vas)*-related genes in germline cells and totipotent somatic stem cells of planarians. Dev. Biol 206, 73–87. [PubMed: 9918696]

Simon J-C, Delmotte F, Rispe C, and Crease T (2003). Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. Biol. J. Linn. Soc. Lond 79, 151–163.

Solana J, Lasko P, and Romero R (2009). *Spoltud-1* is a chromatoid body component required for planarian long-term stem cell self-renewal. Dev. Biol 328,410–421. [PubMed: 19389344]

Tu R, Duan B, Song X, Chen S, Scott A, Hall K, Blanck J, DeGraffenreid D, Li H, Perera A, et al. (2021). Multiple niche compartments orchestrate stepwise germline stem cell progeny differentiation. Curr. Biol 31, 827–839.e3. [PubMed: 33357404]

Uda M, Ottolenghi C, Crisponi L, Garcia JE, Deiana M, Kimber W, Forabosco A, Cao A, Schlessinger D, and Pilia G (2004). Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. Hum. Mol. Genet 13, 1171–1181. [PubMed: 15056605]

Uhlenhaut NH, and Treier M (2011). Forkhead transcription factors in ovarian function. Reproduction 142, 489–495. [PubMed: 21810859]

Uhlenhaut NH, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, Treier A-C, Klugmann C, Klasen C, Holter NI, et al. (2009). Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. Cell 139, 1130–1142. [PubMed: 20005806]

Wagner DE, Wang IE, and Reddien PW (2011). Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. Science 332, 811–816. [PubMed: 21566185]

Wakayama T, Perry AC, Zuccotti M, Johnson KR, and Yanagimachi R (1998). Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature 394, 369–374. [PubMed: 9690471]

Wang Y, Zayas RM, Guo T, and Newmark PA (2007). *Nanos* function is essential for development and regeneration of planarian germ cells. Proc. Natl. Acad. Sci. U S A 104, 5901–5906. [PubMed: 17376870]

Wang Y, Stary JM, Wilhelm JE, and Newmark PA (2010). A functional genomic screen in planarians identifies novel regulators of germ cell development. Genes Dev. 24, 2081–2092. [PubMed: 20844018]

Weiger WA (1997). Serotonergic modulation of behaviour: a phylogenetic overview. Biol. Rev. Camb. Philos. Soc 72, 61–95. [PubMed: 9116165]

Wells ML, Perera L, and Blackshear PJ (2017). An ancient family of RNA-binding proteins: still important!. Trends Biochem. Sci 42, 285–296. [PubMed: 28096055]

Wu YE, Pan L, Zuo Y, Li X, and Hong W (2017). Detecting activated cell populations using single-cell RNA-seq. Neuron 96, 313–329.e6. [PubMed: 29024657]

Xie T, Song X, Jin Z, Pan L, Weng C, Chen S, and Zhang N (2008). Interactions between stem cells and their niche in the *Drosophila* ovary. Cold Spring Harb. Symp. Quant. Biol 73, 39–47. [PubMed: 19022749]

Yamamoto K, and Vernier P (2011). The evolution of dopamine systems in chordates. Front. Neuroanat 5, 21. [PubMed: 21483723]

Zayas RM, Hernández A, Habermann B, Wang Y, Stary JM, and Newmark PA (2005). The planarian *Schmidtea mediterranea* as a model for epigenetic germ cell specification: analysis of ESTs from the hermaphroditic strain. Proc. Natl. Acad. Sci. U S A 102, 18491–18496. [PubMed: 16344473]

Zeng A, Li H, Guo L, Gao X, McKinney S, Wang Y, Yu Z, Park J, Semerad C, Ross E, et al. (2018). Prospectively isolated Tetraspanin+ neoblasts are adult pluripotent stem cells underlying planaria regeneration. Cell 173, 1593–1608.e20. [PubMed: 29906446]

Zhou Q, Wang M, Yuan Y, Wang X, Fu R, Wan H, Xie M, Liu M, Guo X, Zheng Y, et al. (2016). Complete meiosis from embryonic stem cell-derived germ cells *in vitro*. Cell Stem Cell 18, 330–340. [PubMed: 26923202]

Highlights

- Laser-capture microdissection generated planarian gonad-specific transcriptomes
- Identified transcripts defining female germ cells and somatic ovarian cells
- Defined functional roles for conserved somatic gonadal genes in ovary regeneration
- Implicated somatically derived monoamines in male and female germ cell development

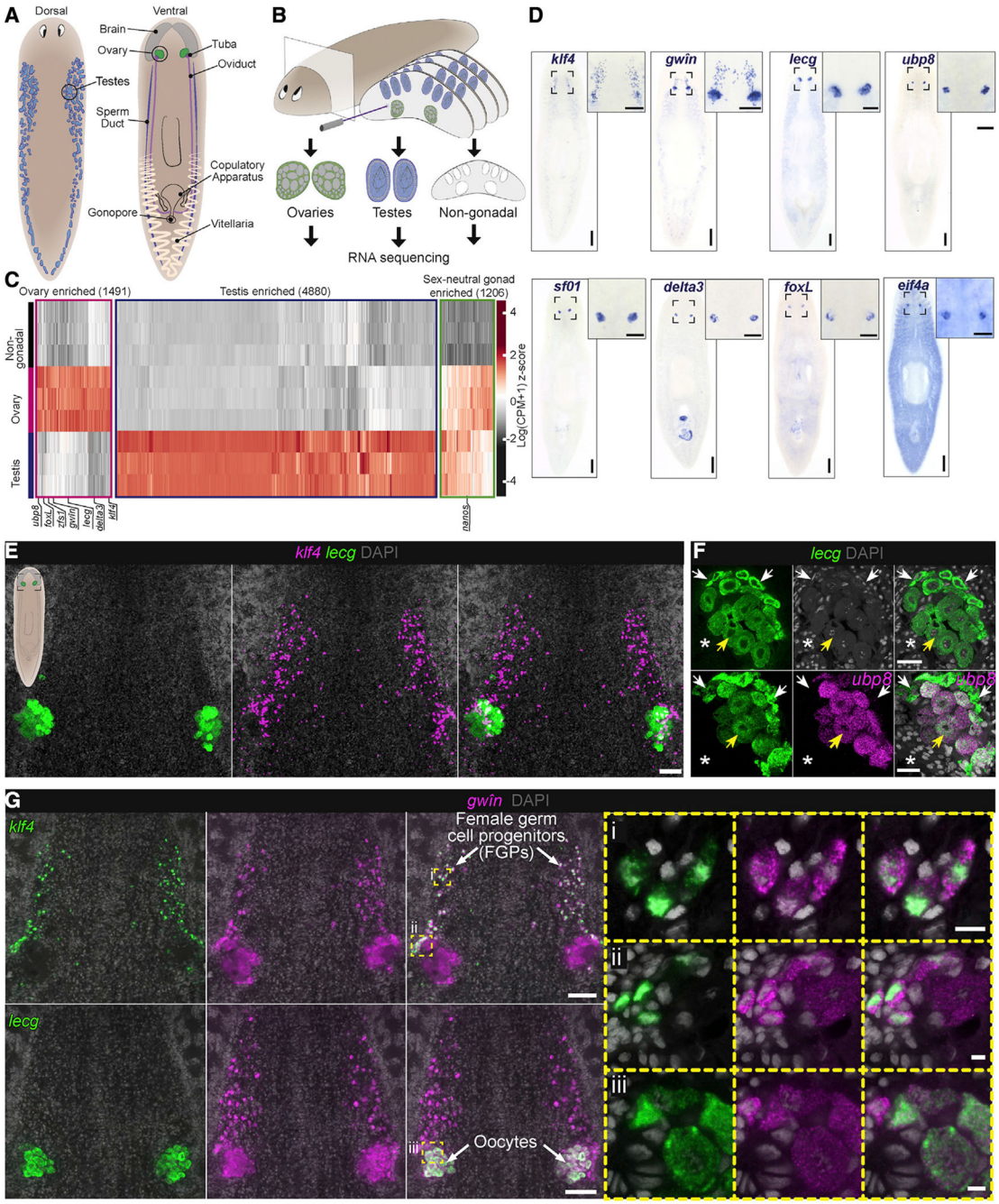

Figure 1. Ovary transcriptome reveals progressive stages of female germ cell development

(A) Reproductive system of the sexual strain of *S. mediterranea*.

(B) LCM-RNA-seq approach to generate gonadal transcriptomes.

(C) Hierarchical clustering of genes with significantly enriched expression in ovaries, testes, or both.

(D) Representative whole-mount RNA *in situ* hybridizations (WISH) to detect candidate ovary-enriched transcripts.

(E) Double fluorescent RNA *in situ* hybridization (FISH) to detect *klf4* and *lecg* reveals a field of *klf4*$^{+}$ cells anterior to the ovary.
(F) FISH to detect *lecg* and *ubp8*, revealing expression in oocytes. Smaller, immature oocytes (white arrows) are observed at the periphery of the ovary; larger, mature oocytes (yellow arrows) reside internally, proximal to the tuba (fertilization duct; asterisks).
(G) FISH to detect *gwîn* and *klf4* or *lecg* shows *gwîn* expression in *klf4*$^{+}$ cells anterior to the ovary (i) and at the margin of the ovary (ii). Co-expression of *gwîn* with *lecg* is observed in oocytes (iii). *klf4*$^{+}$ *gwîn*$^{+}$ cells are presumptive female germ cell progenitors (FGPs) that differentiate into *gwîn*$^{+}$ *lecg*$^{+}$ oocytes within the ovary. Nuclei are labeled with DAPI. Scale bars: (D) 500 μm, insets 200 μm; (E) 100 μm; (F) 50 μm; (G) 100 μm, insets 10 μm. See also Figures S1 and S2.

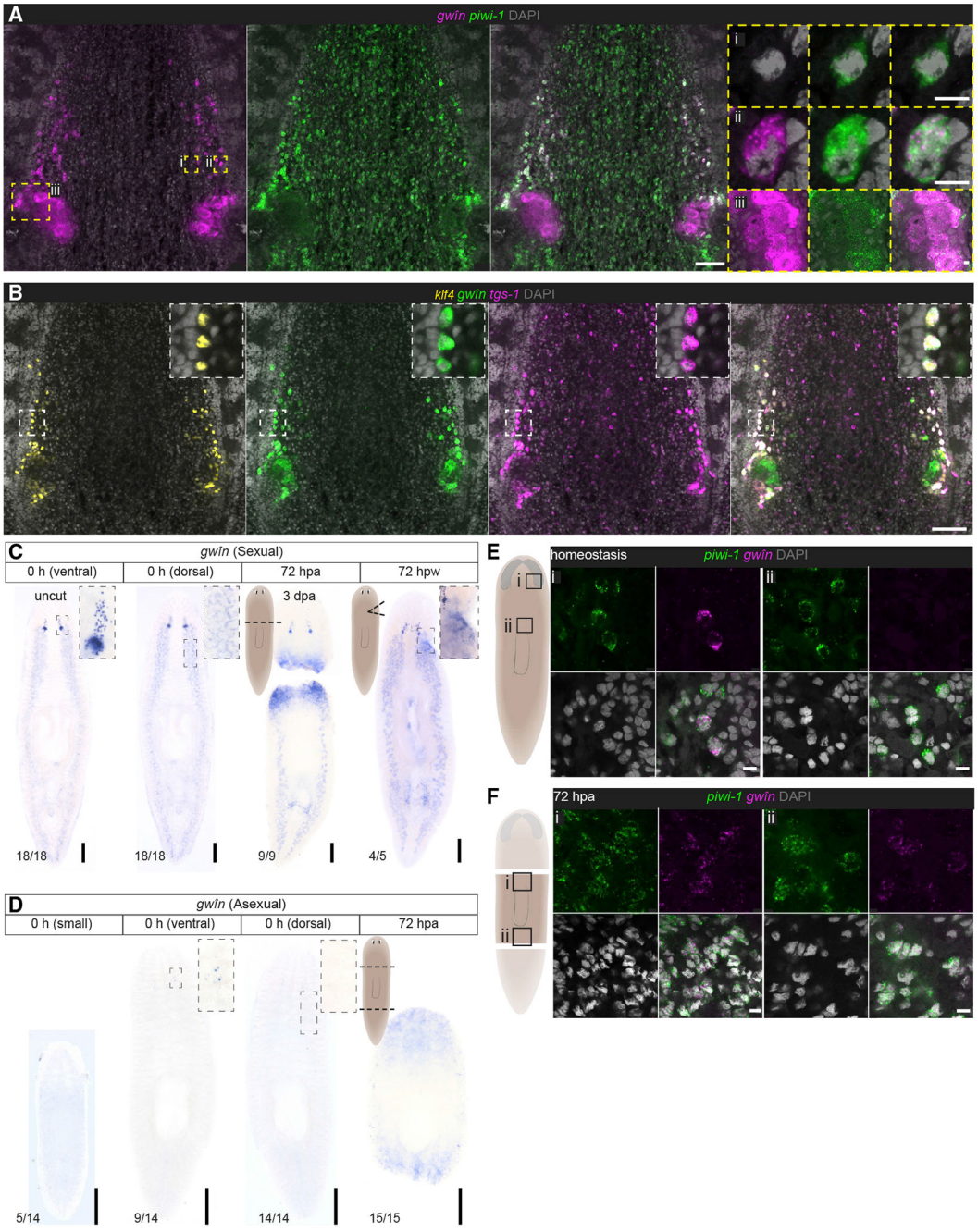

Figure 2. FGPs express markers associated with a pluripotent neoblast subpopulation

(A) FISH to detect *gwîn* and the neoblast marker, *piwi-1.* Expression of *piwi-1* is maintained from neoblasts (i) into FGPs (ii) and oocytes (iii).

(B) FISH to detect *tgs-1* with *klf4* and *gwîn. tgs-1* marks a pluripotent subset of neoblasts and is enriched in FGPs.

(C and D) WISH to detect gwîn sexuals (C) and asexuals (D) during homeostasis (uncut) and 72 h post-amputation (hpa) or-wounding (hpw). *gwîn* is upregulated at wound sites in both sexuals and asexuals.

(E and F) FISH to detect *gwîn* and *piwi-1* in asexuals during homeostasis (E) and 72 hpa (F), shows upregulation of *gwîn* in neoblasts at wound sites after amputation. Scale bars: (A and B) 100 μm, insets 10 μm; (C and D) 500 μm; (E and F) 10 μm. See also Figures S3 and S4.

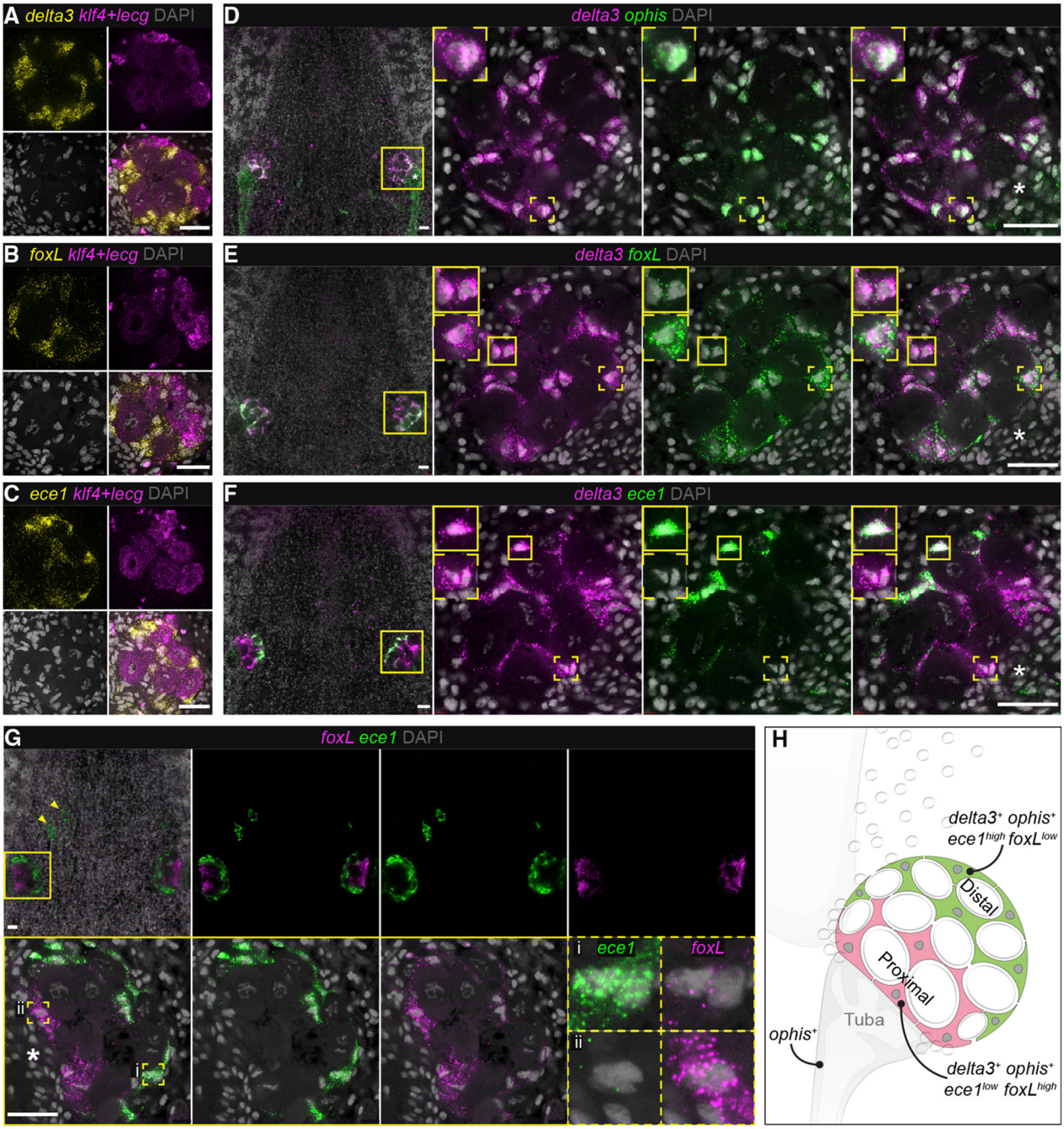

Figure 3. Characterization of somatic ovarian cells

(A–C) FISH to detect *delta3* (A), *foxL* (B), *ece1* (C), and pooled probes for *klf4* (FGPs) and *lecg* (oocytes) shows expression of these transcripts in non-germ-cell populations of the ovary.

(D) *ophis*$^{+}$ ovarian somatic cells co-express *delta3*.

(E and F) *delta3*$^{+}$ somatic support cells co-express *foxL* (E) and *ece1* (F).

(G) FISH to detect *ece1* and *foxL* reveals heterogeneity in gene expression within somatic ovarian cells: (i) *ece1*^{low} *foxL*^{high} cells are located proximal to the tuba and (ii) *ece1*^{high}

foxL^{low} cells are located distal to the tuba. Arrowheads indicate ectopic ovaries often found in large sexual worms.

(H) Schematic depicting marker gene expression in somatic ovarian cells and associated reproductive structures (tuba and oviduct). Asterisks indicate tuba. Scale bars: 50 μm. See also Figure S5.

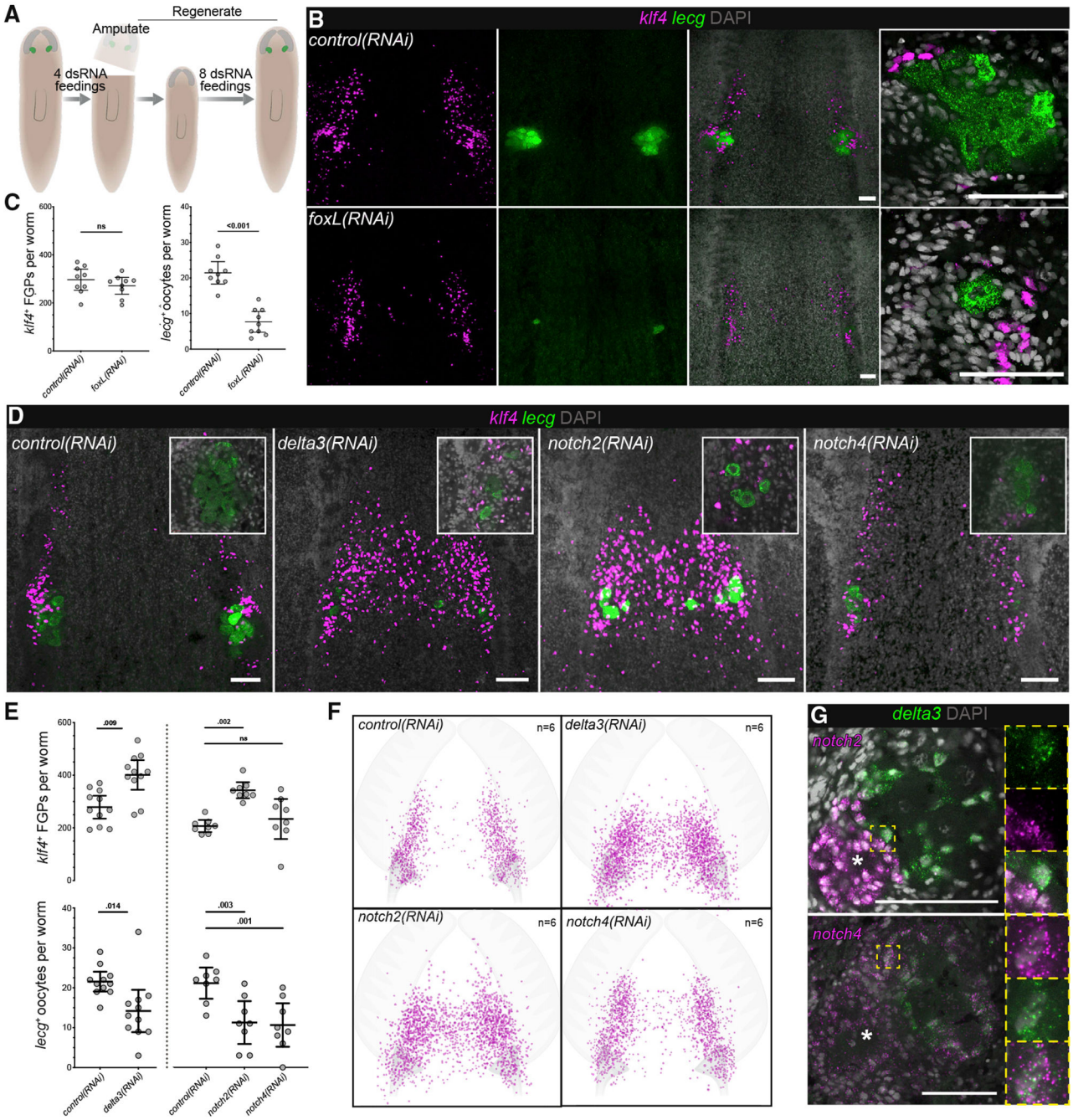

Figure 4. Ovarian somatic cells are essential for female germ cell development

(A) Regeneration assay for RNAi-mediated gene knockdown: sexually mature planarians were fed double-stranded RNA (dsRNA) corresponding to the target gene four times over 2 weeks. Then, the heads were amputated posterior to the ovaries and discarded; after head regeneration in tail fragments, eight dsRNA feedings were performed for 4 weeks before fixing the animals for further analysis.

(B) FISH to detect *klf4* and *lecg* in *control(RNAi)* and *foxL(RNAi)* worms.

(C) Quantification of *klf4*$^{+}$ FGPs and *lecg*$^{+}$ oocytes in control(RNAi) and *foxL(RNAi)* worms (mean with 95% confidence interval; ns, not significant).
(D) FISH to detect *klf4* and *lecg* in control(RNAi), *delta3(RNAi)*, *notch2(RNAi)*, and *notch4(RNAi)* worms.
(E) Quantification of *klf4*$^{+}$ FGPs and *lecg*$^{+}$ oocytes in control(RNAi), *delta3(RNAi), notch2(RNAi),* and *notch4(RNAi)* worms (mean with 95% confidence interval; ns, not significant).
(F) Density plot of *klf4*$^{+}$ FGPs in *control(RNAi), delta3(RNAi), notch2(RNAi)*, and *notch4(RNAi)* worms. The brain lobe and tuba were used to define the relative position of cells.
(G) Double FISH for *notch2* or *notch4* with the somatic marker *delta3*. Asterisks indicate tuba. Scale bars: 100 μm. See also Figure S5.

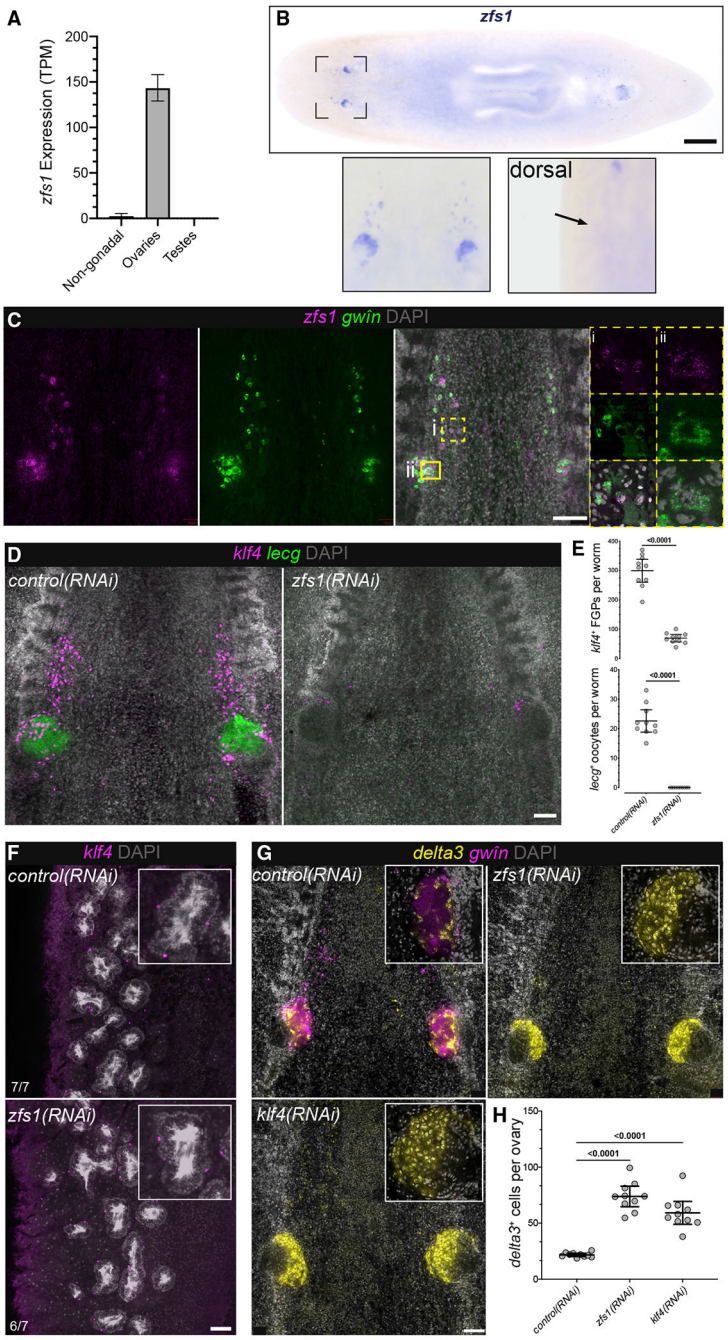

Figure 5. Female-specific role of *zfs1* in germ cell regeneration

(A) *zfs1* expression is enriched in the ovary versus testis and non-gonadal transcriptomes (mean ± SD).

(B) WISH to detect *zfs1* reveals expression in the ovary and cells anterior to the ovary (ventral view). Dorsal view shows no detectable *zfs1* expression in the testes.

(C) Double FISH to detect *zfs1* and *gwîn* shows *zfs1* expression in *gwîn*$^{+}$ FGPs anterior to the ovary (i) and oocytes within the ovary (ii).

(D) FISH to detect *klf4*$^{+}$ FGPs and *lecg*$^{+}$ oocytes in *control(RNAi)* and *zfs1(RNAi)* worms.

(E) Quantification of *klf4*$^{+}$ FGPs and *lecg*$^{+}$ oocytes in *control(RNAi)* and *zfs1(RNAi)* worms.
(F) FISH to detect *klf4* (early male germ cells) in testes of *control(RNAi)* and *zfs1(RNAi)* worms.
(G) FISH to detect *delta3* (somatic ovarian cells) and *gwîn* (female germline) in *control(RNAi), zfs1(RNAi),* and *klf4(RNAi)* worms.
(H) Quantification of *delta3*$^{+}$ support cells in *control(RNAi), zfs1(RNAi)*, and *klf4(RNAi)* worms. (E and H): means with 95% confidence intervals. Scale bars: (B) 500 μm; (C–F) 100 μm. See also Figure S6.

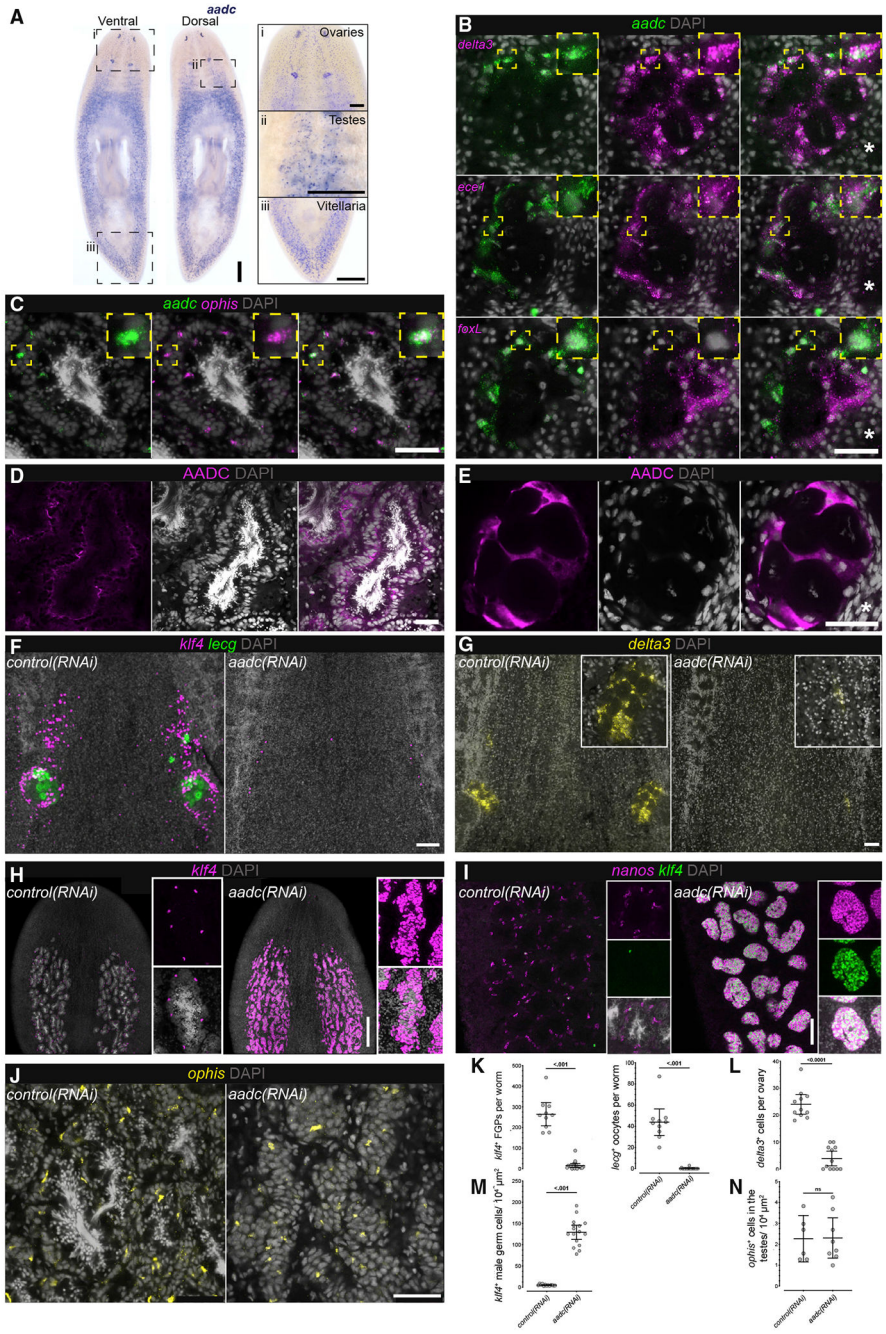

Figure 6. Opposing roles of AADC in female and male germ cell regeneration

(A) Expression of *aadc* (aromatic L-amino acid decarboxylase) is detected in the planarian reproductive system: (i) ovaries, (ii) testes, and (iii) vitellaria.

(B) FISH to detect *aadc* and ovarian somatic markers (*delta3, ece1,* and *foxL*): *aadc* transcripts are enriched in the distal *ece1*^{high} *foxL*^{low} ovarian somatic cells.

(C) FISH to detect *aadc* and testis somatic marker (*ophis*) indicates *aadc* expression in testis somatic support cells.

(D and E) Immunostaining for AADC protein in testes (D) and ovaries (E).

(F) FISH to detect *klf4*$^{+}$ FGPs and *lecg*$^{+}$ oocytes in *control(RNAi)* and *aadc(RNAi)* worms.
(G) FISH for *delta3* to mark somatic ovarian cells in *control* and *aadc(RNAi)* worms.
(H and I) FISH to detect *klf4* (H) and *nanos* (I), labeling early male germ cells in testes of *control(RNAi)* and *aadc(RNAi)* worms.
(J) FISH for *ophis* to mark testis somatic support cells in *control* and *aadc*(RNAi) worms.
(K–N) Quantification of *klf4*$^{+}$ FGPs and *lecg*$^{+}$ oocytes (K); *delta3*$^{+}$ ovarian support cells (L); *klf4*$^{+}$ early male germ cells (M); and *ophis*$^{+}$ male somatic cells (N), in *control(RNAi)* and *aadc(RNAi)* worms. Means with 95% confidence intervals; ns, not significant. Asterisks in (B) and (E) indicate tuba. Scale bars: (A and H) 500 μm; (B–E, J) 50 μm; (F, G, and I) 100 μm. See also Figure S7.

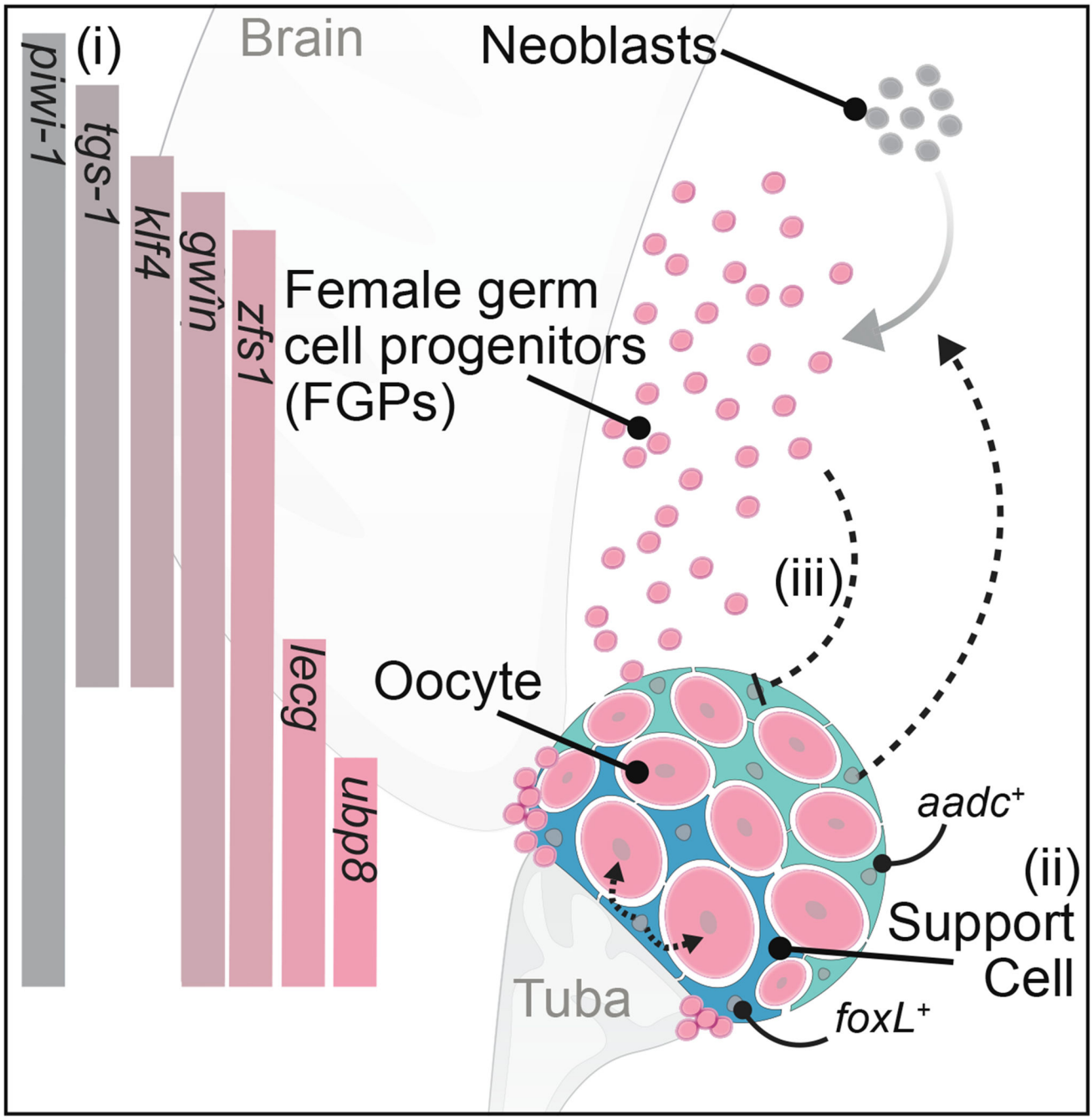

Figure 7. Schematic of planarian female germ cell differentiation and the roles of somatic gonadal cells

(i) Neoblasts specialize into FGPs antero-ventrally toward the base of the brain. FGPs around the margin of the ovary differentiate peripherally into oocytes that mature and move internally toward the tuba. Gene expression at distinct stages of female germ cell differentiation is indicated to the left. Expression of female-specific *zfs1* reveals early and spatially restricted specification of germ cell sex in a simultaneous hermaphrodite. (ii) Somatic ovarian cells are closely associated with germ cells and display spatial heterogeneity in gene expression. Tuba-distal *aadc*$^{+}$ somatic cells are required

for regeneration of the female germ cells, whereas *foxL*$^{+}$ tuba-proximal cells regulate oocyte development, suggesting distinct functional domains within the ovarian somatic compartment. (iii) Loss of female germ cells triggers an expansion of ovarian somatic cells, suggesting that feedback from female germ cells regulates support cell numbers.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-Digoxigenin-POD, Fab fragments (sheep polyclonal)	Roche	Cat: 11207733910 RRID: AB_514500
Anti-Dinitrophenyl-HRP (rabbit polyclonal)	Vector Laboratories	Custom made
Anti-Fluorescein-POD, Fab fragments (sheep polyclonal)	Roche	Cat: 11426346910 RRID: AB_840257
Anti-phospho-Histone H3 (Ser10) (rabbit monoclonal)	Millipore Sigma	Cat: 05-817R-I; RRID: AB_11215621)
Anti-SmedAADC (rabbit polyclonal)	This paper	N/A
Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch Laboratories	Cat: 111-035-003 RRID: AB_2313567
Alkaline Phosphatase AffiniPure Goat Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch Laboratories	Cat: 111-055-144 RRID: AB_2337953
Bacterial and virus strains		
DH5-alpha	Thermo Fisher	Cat: EC0112
Chemicals, peptides, and recombinant proteins		
Alcoholic Eosin Y	Sigma Aldrich	Cat: HT110116
Cresyl Violet Acetate	Sigma Aldrich	Cat: C5042
Critical commercial assays		
PEN membrane glass slides	Thermo Fisher	Cat: LCM0522
Capsure HS LCM caps	Thermo Fisher	Cat: LCM0214
PicoPure RNA Isolation Kit	Thermo Fisher	Cat: KIT0204
Deposited data		
Raw and analyzed data	This paper	GEO: GSE191229
Experimental models: Organisms/strains		
Schmidtea mediterranea, Sexual strain	Zayas et al., 2005	N/A
Schmidtea mediterranea, Asexual clonal strain CIW4	Sánchez Alvarado et al., 2002	N/A
Oligonucleotides		
Sequences used for probes and dsRNA	This Paper	Table S2
Recombinant DNA		
Plasmid: pJC53.2	Collins et al., 2010	Cat: 26536 RRID: Addgene_26536
Software and algorithms		
CLC Genomics Workbench	Qiagen	RRID: SCR_011853
R Studio	RStudio	RRID: SCR_000432
Complex Heatmaps	Gu et al., 2016	N/A
Zen	ZEISS	RRID: SCR_013672
GraphPad Prism	GraphPad Software	RRID: SCR_002798
Fiji	Schindelin et al., 2012	RRID: SCR_002285
Imaris	Oxford Instruments	RRID: SCR_007370
Other		
Differential gene expression analysis	This paper	Table S1
Transcriptome (smed_20140614)	Robb et al., 2015	https://planosphere.stowers.org

REAGENT or RESOURCE	SOURCE	IDENTIFIER
PlanMine	Rozanski et al., 2019	https://planmine.mpibpc.mpg.de