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Control of germline stem cell differentiation by Polycomb and Trithorax group genes in the niche microenvironment

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ABSTRACT

Polycomb and Trithorax group (PcG and TrxG) genes function to regulate gene transcription by maintaining a repressive or active chromatin state, respectively. This antagonistic activity is important for body patterning during embryonic development, but whether this function module has a role in adult tissues is unclear. Here, we report that in the *Drosophila* ovary, disruption of the Polycomb repressive complex 1 (PRC1), specifically in the supporting escort cells, causes blockage of cystoblast differentiation and germline stem cell-like tumor formation. Tumors are caused by derepression of *decapentaplegic* (*dpp*), which prevents cystoblast differentiation. Interestingly, activation of *dpp* in escort cells requires the function of the TrxG gene *brahma* (*brm*), suggesting that loss of PRC1 in escort cells causes Brm-dependent *dpp* expression. Our study suggests a requirement for balanced activity between PcG and TrxG in an adult stem cell niche, and disruption of this balance could lead to the loss of tissue homeostasis and tumorigenesis.

KEY WORDS: Germline stem cell, *Drosophila* ovary, Escort cell, Differentiation niche, *Decapentaplegic*, Polycomb repressive complex 1 (PRC1), Brahma

INTRODUCTION

Tissue-specific adult stem cells are important for maintaining normal homeostasis for many tissues and organs. These stem cells commonly reside in a specialized niche microenvironment where they receive self-renewal signals to keep them in the undifferentiated state (Li and Xie, 2005; Morrison and Spradling, 2008). Outside the niche, these self-renewal signals are turned down to allow cell lineage differentiation. It is therefore important to understand how the self-renewal signals inside and outside the stem cell niche are regulated for balancing stem cell self-renewal and differentiation, whose dysregulation may lead to tissue degeneration or tumorigenesis.

The *Drosophila* ovary is one of the pioneer systems in which the niche regulation of stem cells has been extensively characterized (Xie, 2013). In each ovariole, two to three germline stem cells (GSCs) reside at the anterior tip where they directly contact a supporting niche composed of five to seven somatic cells, named cap cells (Xie and Spradling, 2000). The cap cell niche produces a Bone morphogenetic protein (BMP) signal, named Decapentaplegic (Dpp), which directly activates a canonical BMP signaling pathway in GSCs to suppress the expression of a differentiation-promoting gene, *bag of marbles* (*bam*), which is a necessary and sufficient factor for GSC differentiation (Chen and McKearin, 2003a; McKearin and Spradling, 1990; Ohlstein and McKearin, 1997; Song et al., 2004; Xie and Spradling, 1998). Outside the cap cell niche, the differentiating daughter cystoblasts and early dividing germline cysts intimately intermingle with somatic escort cells, which produce long protrusions and dynamically interact with the developing cysts and are considered to provide a differentiation microenvironment or niche for cystoblast differentiation (Kirilly et al., 2011; Morris and Spradling, 2011). High levels of BMP signaling are maintained in GSCs but not in differentiating cystoblasts to allow self-renewal of GSCs and differentiation of cystoblasts. This restricted BMP signaling activation is accomplished by multiple intrinsic and extrinsic mechanisms (Chen et al., 2011; Harris and Ashe, 2011). Transcriptional activation of *dpp* mediated by JAK/STAT signaling mainly occurs in cap cells and is limited in escort cells (Liu et al., 2015; Lopez-Onieva et al., 2008; Wang et al., 2008a); additionally, Hedgehog (Hh) signaling from the cap cells suppresses JAK/STAT activity in escort cells, thereby limiting the production of BMP signals outside of the cap cell niche (Liu et al., 2015; Lu et al., 2015). Both together provide a spatial limitation for the production of self-renewal signals. As a result, ectopic Dpp expression in escort cells is able to cause GSC-like cell accumulation in the germarium. Germline and soma interaction via EGFR signaling activates MAPK signaling in escort cells to suppress the expression of the proteoglycan Dally (Liu et al., 2010). As Dally binds to Dpp and facilitates Dpp spreading, the restricted distribution of Dally hampers Dpp spreading outside the cap cell niche (Guo and Wang, 2009; Hayashi et al., 2009). This effect is further enhanced by type IV collagen in the basement membrane, which can bind to Dpp and restrict its spreading (Wang et al., 2008b). Interestingly, the Dpp receptor Thickveins (Tkv), which is expressed on the membrane of escort cells, does not mainly function to transduce BMP signaling but, instead, has a sponge-like function for sequestering Dpp molecules to prevent excessive Dpp activity on the germ line outside the cap cell niche (Luo et al., 2015). These mechanisms together provide a spatial limitation of Dpp spreading and activity on the differentiating germline cysts.

Recent studies have revealed several epigenetic regulators that participate in transcriptional suppression of *dpp* in escort cells. Loss of Piwi in escort cells causes derepression of *dpp* and consequently, GSC-like tumor formation (Jin et al., 2013; Ma et al., 2014). A similar phenotype is also observed upon escort cell-specific depletion of Lsd1, a histone demethylase, Eggless, a H3K9 methyltransferase and dSet-1 (Set1), a H3K4 trimethylase (Eliazer et al., 2011; Wang et al., 2011; Xuan et al., 2013). These observations indicate that local chromatin structure is important for *dpp* suppression in escort cells. In addition, Lsd1 seems to regulate *dpp* expression indirectly by maintaining escort cell fate

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(Eliazer et al., 2014). Recent studies have revealed a role for Wnt signaling in suppressing *dpp* signaling in escort cells, but whether or not this occurs via regulation of Piwi expression is not yet clear (Hamada-Kawaguchi et al., 2014; Wang et al., 2015).

Polycomb group (PcG) and Trithorax group (TrxG) proteins were initially identified in *Drosophila* for their prominent roles in the regulation of Hox genes. They form evolutionarily conserved protein complexes to covalently modify histone tails and maintain the repressive (PcG) or active (TrxG) state of gene transcription (Grossniklaus and Paro, 2014; Schwartz and Pirrotta, 2007; Simon and Kingston, 2013). In addition to their roles during development, PcG proteins are also implicated in the regulation of stem cell self-renewal and differentiation in adult tissues and organs (Sparmann and van Lohuizen, 2006). During *Drosophila* oogenesis, specific PcG proteins function as tumor suppressors in follicle stem cells by autonomously regulating Wnt self-renewal signals (Li et al., 2010). In this study, we report a non cell-autonomous mechanism for PcG genes in regulating stem cell differentiation and tumor suppression in the germline stem cell lineage in the *Drosophila* ovary. In addition, a novel model of PcG and TrxG antagonism in the process is revealed.

RESULTS

Depletion of PcG proteins in escort cells causes GSC-like tumor formation

PcG proteins usually form multi-protein complexes to exert their function. The Polycomb repressive complex 1 (PRC1) generates monoubiquitylation of histone H2A (H2Aub), compacts polynucleosome chromatin and directly interferes with the transcription machinery to establish the repressive chromatin domains required for transcriptional repression (Geisler and Paro, 2015; Simon and Kingston, 2013). The *Drosophila* PRC1 contains four major components: Polycomb (Pc), Polyhomeotic (Ph), Posterior sex combs (Psc) and Sex combs extra (Sce). Our previous studies using mosaic analysis have demonstrated that PRC1 is not required for GSC maintenance or for their differentiation towards 16-cell cysts or egg chambers (Li et al., 2010). To study their potential function in escort cells, we utilized the binary GAL4/UAS system to perform tissue-specific RNAi gene knockdown by expressing dsRNAs or small hairpin RNAs (Brand and Perrimon, 1993). A temperature-sensitive GAL80 (*GAL80^{ts}*) was included to allow temperature-dependent control of RNAi (McGuire et al., 2004). We used the *c587-GAL4* driver that is specifically expressed in escort cells and follicle cell progenitors, to knock down PcG gene products specifically in these somatic cells (Song et al., 2004). In wild-type germarium, GSCs and immediate daughter cystoblasts can be identified by the spectrosome, a spherical-shaped fusome (Lin et al., 1994). During differentiation, the fusome becomes elongated and branched to connect all the cystocytes in a cyst (Fig. 1A,B). Therefore, fusome morphology can be used as a marker to distinguish progenitors from differentiating germline cysts. Two homologous PcG proteins, Psc and Suppressor of zeste 2 [Su(z)2], have been shown to function redundantly as tumor suppressors in follicle stem cells (FSCs). We found that knocking down either Psc or Su(z)2 did not produce any visible phenotype (data not shown and Fig. 1H). However, simultaneous knockdown of both Psc and Su(z)2 caused age-dependent accumulation of spectrosome-containing germ cells (which we refer to as GSC-like cells) in the germarium (Fig. 1D,E,H). Normally, there were ~2-5 spectrosome-containing germ cells in each germarium, but RNAi of both Psc and Su(z)2 caused each germarium to accumulate more than 20 GSC-like cells at day 7 and more than 40 at day 21, after shifting to the restrictive temperature.

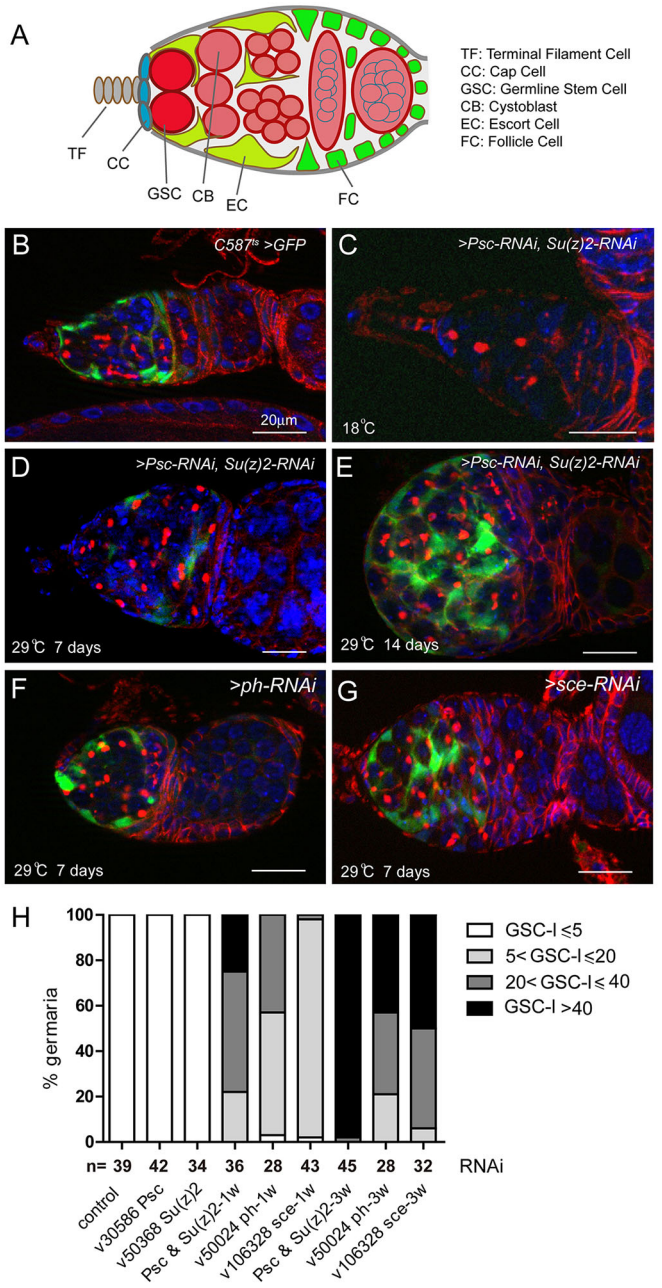

Fig. 1. Depletion of PcG proteins in escort cells causes GSC-like tumor formation. (A) Schematic drawing of the *Drosophila* germarium. GSCs are at the tip of the germarium and adjacent to the cap cells. (B-G) *c587-GAL4, uas-GFP;Tub-GAL80^{ts}* flies were crossed to *UAS-RNAi* transgenic flies at 18°C and 3- to 5-day-old females with desired genotype were shifted to 29°C and cultured on standard food with yeast paste for 7 days or 14 days before dissection. Flies were transferred every 2 days. A typical germarium has two to three GSCs containing spherical spectrosomes (anti-α-spectrin, red) and differentiating cystocytes containing branched fusomes (B). *Psc-RNAi*, *Su(z)2-RNAi* germarium appeared normal at 18°C (C). After shifting to 29°C, *Psc-RNAi*, *Su(z)2-RNAi* germarium accumulated GSC-like tumor at 7 days (D) and displayed a more severe phenotype at 14 days after shift (E). *ph-RNAi* (F) and *sce-RNAi* (G) caused a similar GSC-like tumor phenotype. (H) Quantification of GSC-like cells in germaria of indicated genotypes. Scale bars: 20 μm.

We also expressed truncated forms of Psc (Psc-N1) and Su(Z)2 [Su(Z)2-N1], which both had their large C-terminal high-similarity region removed (Fig. S1). Expression of these transgenes in imaginal disc cells caused tumor development, suggesting that the truncated

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forms have dominant-negative effects (Ansgar Klebes, Humboldt Universität, Berlin, personal communication). We found that expression of either Psc-N1 or Su(Z)2-N1 alone in escort cells for 1 week did not produce any visible phenotype. An enlarged germarium with an increased number of developing germline cysts was observed after 2 weeks. However, co-expression of Psc-N1 and Su(Z)2-N1 was sufficient to promote GSC-like cell accumulation in the germarium and this phenotype became much more dramatic with age (Fig. S1). These observations further support the conclusion that Psc and Su(z)2 function in escort cells to facilitate germline differentiation. The enlarged germarium phenotype produced by expressing either Psc-N1 or Su(Z)2-N1 alone indicates that Psc-N1 might also have a dominant negative effect on Su(z)2 and vice versa.

Depleting other PRC1 components, including Ph and Sce, produced similar GSC-like tumor phenotypes (Fig. 1F-H), indicating that the canonical PRC1 complex is required in escort cells to prevent non cell-autonomous GSC tumor formation. In these GSC-like tumors, the escort cells were still intermingled with the tumor cells, and these two cell populations displayed a largely proportional increase in tumor growth, indicating that disrupting PRC1 does not compromise the survival or morphology of escort cells – properties that are known to be important for supporting germline differentiation (Kirilly et al., 2011; Lu et al., 2015; Maimon et al., 2014; Wang et al., 2011). Consistent with this hypothesis, we did not observe any obvious increase in apoptosis of Psc- and Su(z)2-depleted escort cells (Fig. S2A,B). In addition, co-expression of p35, a potent cell death inhibitor, failed to suppress the GSC-like tumor phenotype caused by Psc and Su(z)2 depletion in escort cells (Fig. S2C,D).

Tumor cells have increased activity of Smad signaling and reduced expression of *bam*

The major regulatory pathway that controls GSC self-renewal and differentiation is the BMP-Bam pathway. Dpp signals produced from cap cells directly activate canonical SMAD signaling in GSCs to suppress transcription of *bam* to keep GSCs in the undifferentiated state (Chen and McKearin, 2003a; Song et al., 2004). Either ectopic *dpp* expression in escort cells or loss of *bam* in the germ line is sufficient to block GSC differentiation and consequently the formation of GSC-like tumors (McKearin and Ohlstein, 1995; Song et al., 2004). To determine whether alteration of this pathway underlies the tumor phenotype caused by escort cell-specific depletion of PRC1, we examined the expression of several activation markers for this pathway. SMAD signaling activity can be monitored by phosphorylated Mad (pMad), the active form of *Drosophila* SMAD, and by the expression of *Dad-lacZ*, an enhancer trap line for *Dad* expression, which encodes a negative SMAD protein and is transcriptionally regulated by SMAD signaling. Normally, high levels of pMad or *Dad-lacZ* expression is confined in GSCs in the anterior of the germarium, as its expression rapidly declines in the cystoblast (Zhao et al., 2008) (Fig. 2A,D). However, in germaria with escort cell-specific depletion of PRC1, the expression of pMad and *Dad-lacZ* was no longer confined to the anteriormost GSCs, but also expanded to the GSC-like cells outside of the cap cell niche (Fig. 2B,C,E,F). These observations indicate that depletion of PRC1 in escort cells causes ectopic activation of BMP signaling in the germ line.

To determine whether the ectopic activation of BMP signaling is able to suppress *bam* expression and consequently prevent GSC differentiation, we monitored expression of *bam* using *bam-GFP*, a GFP reporter driven by the *bam* promoter (Chen and McKearin, 2003b). Normally GFP expression is absent in GSCs, becomes detectable in cystoblast and reaches the highest levels in early cystocytes (Fig. 2G). We found that *bam-GFP* was undetectable in virtually all spectrosome-containing cells, including GSC-like cells outside the cap cell niche (Fig. 2H,I). These observations suggest that ectopic BMP signaling caused by the depletion of PRC1 in escort cells is sufficient to suppress *bam* expression in the germ line, which might be responsible for the blocked GSC differentiation phenotype.

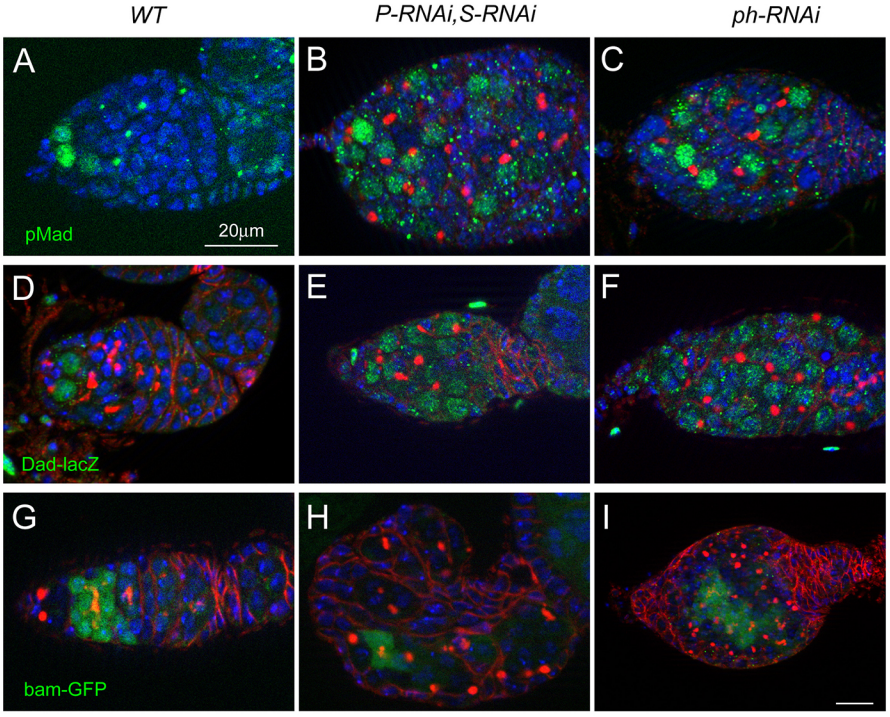

Fig. 2. Tumor cells have increased activity of Smad signaling and reduced expression of *bam*. (A,D,G) In the wild-type germarium, strong pMad signal (green) was confined to GSCs (A), similar to *Dad-lacZ* expression (detected by anti-β-galactosidase; green in D); *bam-GFP* expression was absent in GSCs, but expressed in cystoblasts and differentiating germline cysts (G). (B,E,H) In *c587^{ts}>Psc-RNAi*, *Su(z)2-RNAi* germaria (at 10-14 days after shift), the accumulated GSC-like cells showed strong pMad (B) and *Dad-lacZ* (E) expression even when they were distant from the cap cells. *bam-GFP* expression in these GSC-like cells (with spherical fusomes) was undetectable (H). (C,F,I) Similar results were observed in *c587^{ts}>ph-RNAi* germaria. Scale bars: 20 μm.

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Bam activation induces differentiation of tumor cells

Blockage in GSC differentiation could also occur independent of the BMP-Bam regulatory axis. For example, escort cells exhibit dynamic membrane protrusions to interact with the developing cyst and alteration of the membrane dynamics also causes defects in GSC differentiation (Kirilly et al., 2011). Therefore, it remains possible that loss of PcG proteins in escort cells compromise a certain aspect of escort cell function that is important for GSC differentiation. However, if the altered activity of the BMP-Bam pathway is indeed responsible for the GSC-like tumor development in PcG mutants, we would expect to see the rescue of the differentiation blockage if Bam expression was provided in the tumor cells. We therefore induced *bam* expression in the tumor cells using a *hs-bam* transgene. Induction of *bam* expression was indeed sufficient to induce differentiation of these GSC-like cells into germline cysts [100% (51/51)], which were distinguished by their branched fusome morphology (Fig. 3). Taken together, these data suggest that depletion of PcG proteins in escort cells causes the expansion of BMP signaling into the differentiation niche, which is sufficient to prevent *bam* activation. Consequently, cystoblast differentiation is blocked, leading to GSC-like tumor development.

Transcriptional activation of *dpp* in escort cells following depletion of PRC1

Non cell-autonomous activation of BMP signaling indicates that the secreted ligands, Dpp in particular, could be involved. *dpp* is also known as a Polycomb target in *Drosophila* (Schwartz and Pirrotta, 2007) and it has several mRNA transcripts via alternative promoter usage and splicing, although all transcripts encode identical protein products (Fig. 4A). We found that *dpp-RB* was specifically and strongly upregulated in *c587>Psc-RNAi*, *Su(z)2 RNAi* ovaries (Fig. 4B). As a positive control, the HOX gene *AbdB* was also significantly upregulated (Fig. 4B). By contrast, other *dpp* isoforms remained unchanged (Fig. 4B). In addition, transcription of other signaling molecules, including another BMP ligand Gbb, JAK/STAT signaling ligands, Upd, Upd2 and Upd3, and glypicans Dally and Dlp remained unchanged (Fig. 4B). To further determine whether derepression of *dpp* occurs in escort cells, we screened several existing *lacZ* enhancer trap and enhancer-GAL4 lines for *dpp* and identified *GMR19E03-GAL4*, which was able to drive *UAS-GFP* expression specifically in cap cells and terminal filament cells, a pattern that is largely consistent with endogenous *dpp* expression pattern. A *lacZ* reporter driven by a portion of this enhancer element (1494 bp in length) was then generated (Fig. 4C). Similarly, β-galactosidase (*P4-lacZ*) was only expressed in cap cells and terminal filament cells (Fig. 4D). By using *P4-lacZ* as a reporter, we observed ectopic β-galactosidase expression in escort cells of *c587>Psc-RNAi*, *Su(z)2 RNAi* germarium, indicative of transcriptional activation of *dpp* (Fig. 4E). Taken together, these observations suggest that disruption of PRC1 in escort cells causes transcriptional derepression of *dpp*.

Ectopic Dpp expression mediates GSC-like tumor formation following PRC1 disruption

To determine whether ectopic *dpp* expression from escort cells is responsible for tumor development, we examined whether knocking down *dpp* in escort cells could suppress the GSC-like tumor phenotype. Indeed, *dpp* RNAi (TRiP lines JF01090, JF01091 or JF01677) in escort cells significantly reduced the number of GSC-like cells in the germarium. Interestingly, removing one functional copy of *dpp* gene (dpp^{hr56} or dpp^{e90}) also significantly reduced the number of GSC-like cells in the germarium (Fig. 5). Taken together, these results suggest a model in which PRC1 functions to suppress *dpp* expression in escort cells, and disruption of PRC1 in escort cells causes activation of Dpp, which leads to the expansion of Dpp signaling from the self-renewal niche to the differentiation niche and consequently blocks cystoblast differentiation and GSC-like tumor development.

PRC1 depletion does not transform escort cells into cap cells

PcG genes are also implicated in maintaining cellular fate during development. Because *dpp* expression is normally restricted in cap cells, regaining *dpp* expression in PRC1-depleted escort cells could be indicative of cell fate transformation. We therefore examined several cellular markers to test this possibility. Cap cells, but not escort cells, normally have a high level of laminin C expression at the nuclear envelope (Fig. 6A). Interestingly, high levels of Lamin C expression were observed in PRC1-depleted escort cells (Fig. 6B), suggesting that they display some characteristics of cap cells. *P1444* is a β-galactosidase enhancer trap line, which normally is specifically expressed in cap cells and escort cells (Xie and Spradling, 2000) (Fig. 6C). However, depletion of PRC1 in escort cells caused the loss of β-galactosidase expression (Fig. 6D), suggesting that PRC1-depleted escort cells do not simply switch to

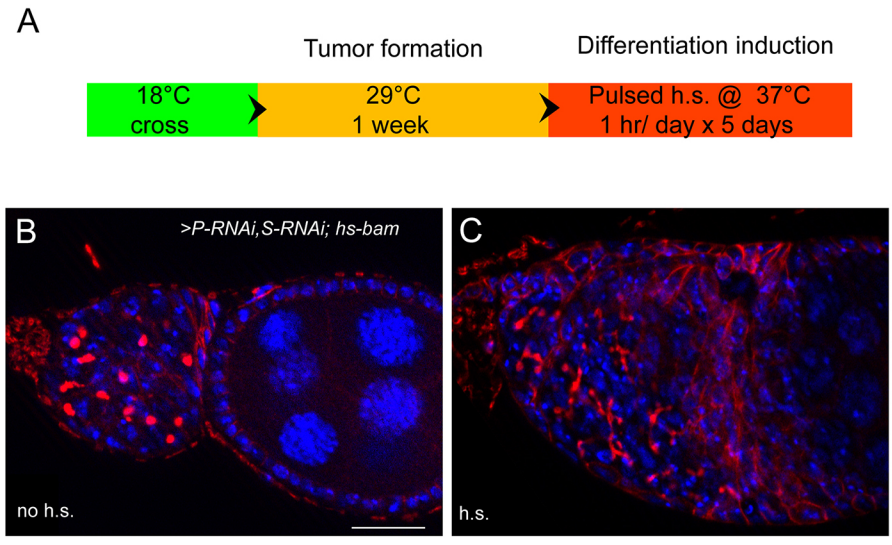

Fig. 3. Bam activation induces differentiation of tumor cells. (A) Schematic of the strategy for testing the ability of Bam to induce tumor cell differentiation. *c587-GAL4;Tub-GAL80^{ts}* flies were crossed to *Psc-RNAi*, *Su(z)2-RNAi; hs-bam* flies at 18°C. Females (3-5 days old) with desired genotype were shifted to 29°C for 1 week. Next, flies were heat shocked for 1 h per day in a 37°C water bath for 5 consecutive days and were then dissected and examined. Females without heat shock served as a control. (B) Without heat shock, GSC-like tumors accumulate in *Psc-RNAi*, *Su(z)2-RNAi* germarium. Scale bar: 20 µm. (C) With Bam induction, the tumor cells were able to undergo differentiation, indicated by the appearance of branched fusomes.

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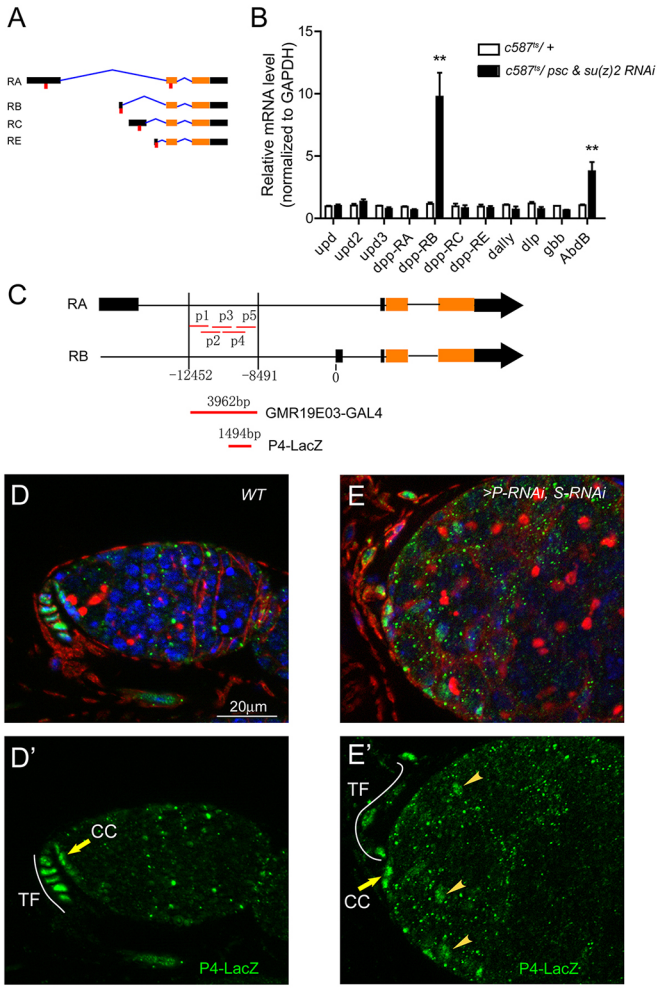

Fig. 4. Transcriptional activation of *dpp* in escort cells following the depletion of PRC1. (A) A schematic drawing of *dpp* isoforms. Black boxes represent 5′ UTR and 3′ UTR; orange boxes represent exons; blue lines represent introns; primer positions used for qPCR are indicated by red dots. Generally, different *dpp* isoforms are detected by individual specific forward primers in the 5′UTR and the same reverse primer in the first exon. (B) Quantitative RT-PCR results of mRNAs from ovaries of indicated genotypes. *dpp-RB* isoform and a positive control HOX gene *AbdB* were significantly upregulated in *Psc-RNAi*, *Su(z)2-RNAi* ovaries (at 14 days after shift) compared with control. Values are mean±s.e.m.; n=3. $^{**}P$<0.01, Student's t-test. (C) A schematic drawing of the *dpp-RB* gene region. The GMR19E03 enhancer region is indicated by the 3962 bp red line, which is localized in the first intron of *dpp-RB*. A subfragment of 1494 bp in length was used for the *P4-lacZ* reporter. (D,D′) In wild-type germarium, *P4-lacZ* (detected by anti-β-galactosidase; green) showed specific TF and cap cell expression. (E,E′) In *c587^{ts}>Psc-RNAi, Su(z)2-RNAi* germarium (at 14 days after shift), *P4-lacZ* expression was also detected in the escort cells. CC, cap cell; TF, terminal filament cell.

cap cell fate. Previous studies have revealed a role of Lsd1, a histone demethylase, in regulating niche cell fate during development. Loss of Lsd1 causes all escort cells to adopt cap cell fate, as all mutant escort cells express Engrailed (En) and Hedgehog (Hh), whereas both are normally confined to cap cells (Eliazer et al., 2014, 2011). Interestingly, ectopic expression of En in escort cells is also sufficient to induce ectopic *dpp* expression (Eliazer et al., 2014). Using immunocytochemistry, we found that the En expression pattern remained normal in the germarium after escort cell-specific disruption of PRC1, as En expression was only detected in the cap cells but not in the mutant escort cells (Fig. 6E,F). Similarly, Hh expression, which was reflected by a *lacZ* enhancer trap (*Hh-lacZ*) and was expressed at high levels in terminal filament cells and cap cells, and low levels in escort cells in the wild-type germarium, also remained at low or undetectable levels in PRC1-depleted escort cells (Fig. 6G,H). Therefore, conditional disruption of PRC1 in escort cells does not fully convert escort cells into cap cells.

Another possible but less likely explanation for the change of cell fate markers in escort cells following PRC1 depletion is that these cells are lost and replaced by pre-follicle cells, as it has been reported that loss of escort cells allows pre-follicle cells to be recruited to the GSC niche (Kai and Spradling, 2003). To test this possibility, we examined several cellular markers, including cell adhesion molecule Fasciclin III (Fas III) and apical polarity marker aPKC, both are normally expressed in follicle cells but not in escort cells. The expression patterns for Fas III and aPKC remained unchanged in *c587>Psc-RNAi*, *Su(z)2-RNAi* germaria, as they were not detectable in the mutant escort cells and were only just beginning to be expressed in pre-follicle cells (Fig. S3). These observations further support the notion that conditional disruption of PRC1 in escort cells does not compromise their survival, but causes changes in the gene expression profile.

Tumor development requires the Trithorax gene *brm*

During development, PcG genes function in concert with TrxG genes and their antagonistic activities determine the repression or activation state of chromatin required for gene repression or activation (Geisler and Paro, 2015). If this function module is also utilized in escort cells for *dpp* repression, we would expect that activation of *dpp* would require TrxG function. We therefore performed RNAi screens in a pool of TrxG genes for those that are required for GSC-like tumor development following the disruption of PRC1 in escort cells. Interestingly, we identified a single gene, *brahma* (*brm*), whose RNAi led to significant suppression of GSC-like tumor growth in *c587>Psc-RNAi*, *Su(z)2-RNAi* or *c587>ph RNAi* ovaries. With *brm* RNAi, although many germaria had still accumulated germline cells, these cells began to show branched fusomes [28.9% (13/45) germaria examined, compared with 6.8% (3/44) in the control], indicative of cyst differentiation (Fig. 7A-E). *brm* encodes a chromatin remodeling factor that promotes transcription by RNA polymerase II by facilitating the establishment of active chromatin (Armstrong et al., 2002; Tamkun et al., 1992; Tie et al., 2012). Expression of a dominant negative form of Brm (Brm-DN) which carries a mutation in the ATP binding site (K804R) (Elfring et al., 1998), produced a similar tumor suppressive effect. Many enlarged germaria no longer contained GSC-like cells, but instead they contained germline cysts with a branched fusome [40.8% (20/49) germaria, compared with 6.8% (3/44) in the control], indicative of cyst differentiation (Fig. 7A-E). Interestingly, inhibiting Brm function in escort cells alone (*c587>brm-RNAi* or *c587>brm-DN*) did not produce any visible phenotype (data not shown). To determine whether this tumor suppressive effect is due to the inhibition of *dpp* expression, we performed quantitative RT-PCR analysis to determine whether Brm inhibition could dampen the levels of *dpp* expression in *c587>Psc-RNAi*, *Su(z)2-RNAi; bam$^{\Delta 86}$/bam$^{EP(3)667}$* ovaries. The purpose of introducing them in a *bam* mutant background is to minimize the effect of tumor size (and therefore the number of escort cells) on the levels of *dpp* expression. Consistent with previous observations, *Psc* and *Su(z)2* RNAi also caused specific derepression of *dpp-RB* in *bam* mutant ovaries (Fig. 7F). Strikingly, this derepression was completely inhibited when Brm-DN was co-expressed (Fig. 7F). Consistent with the strong effect on *dpp* signaling, the ectopic pMad expression pattern found in *Psc-RNAi*, *Su(z)2-RNAi* ovaries was

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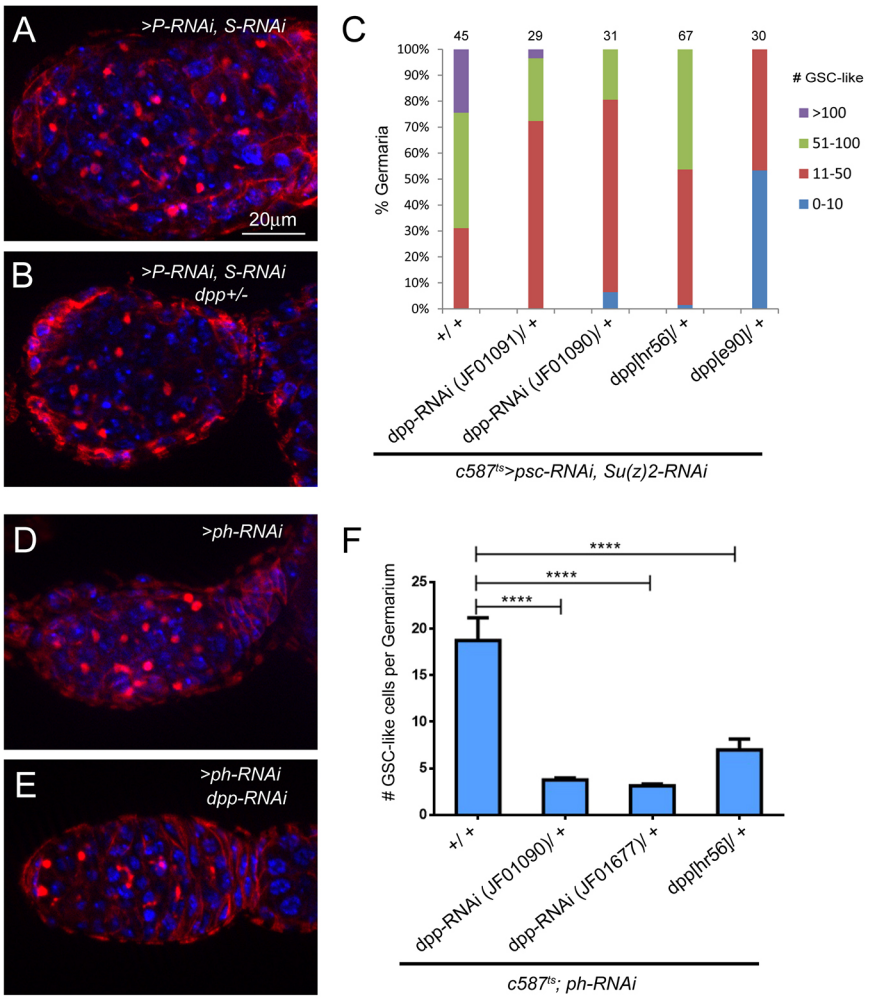

Fig. 5. Ectopic Dpp expression mediates GSC-like tumor formation following PRC1 disruption. (A,B) Representative images of germaria of indicated genotypes. The size of the GSC-like tumor in *Psc-RNAi, Su(z)2-RNAi* germarium (A) could be significantly reduced by removing one functional copy of *dpp* (B). (C) Quantification of GSC/cystoblast cells (identified by spherical spectrosomes) in germaria of indicated genotypes. (D,E) Representative images of germaria of indicated genotypes. The GSC-like tumor phenotype caused by *ph-RNAi* (D) could be efficiently suppressed by co-depleting *dpp* (E). (F) Quantification of GSC-like cells in germaria of indicated genotypes. *dpp* RNAi or removing one functional copy of *dpp* could significantly reduce the number of accumulated GSC-like cells. All germaria were analyzed at 12 days after shifting to restrictive temperature. Values are mean±s.e.m.; n=3. ****P<0.0001, Student's t-test.

also suppressed when Brm-DN was expressed, as pMad-positive cells were only observed in GSCs at the anterior tip of the germarium (Fig. 7G,H). Taken together, these data indicate that Brm-dependent activation of *dpp* drives GSC-like tumor development in *c587>Psc-RNAi*, *Su(z)2 RNAi* ovaries.

DISCUSSION

PRC1 maintains the repressive state of *dpp* transcription in the differentiation niche

In the GSC niche, BMP signaling activity is mainly restricted in GSCs, and quickly declined in daughter cystoblasts. Previous studies have suggested that this regionally restricted BMP activity is achieved by multiple intrinsic and extrinsic mechanisms. One key mechanism is the restricted expression of *dpp* in cap cells, but not in escort cells. Cap cell-specific expression of *dpp* is thought to be established in the pupal gonad when cap cells are initially specified from an anterior population of IGS precursor cells through the induction of Dl-Notch signaling (Song et al., 2007; Ward et al., 2006). In adult ovary, Notch activity in the anterior germarium is limited to cap cells and terminal filament cells, but not escort cells. However, although Notch activity is required in cap cells for cell survival, ectopic Notch activation in adult escort cells failed to induce either cap cell fate or *dpp* expression, suggesting that Notch signaling regulates cap cell maintenance but does not directly regulate *dpp* expression (Song et al., 2007). Instead, JAK/STAT signaling seems to be the primary driver for *dpp* expression in cap cells after eclosion and a low level of *dpp* expression in escort cells is also correlated with the low JAK/STAT signaling activity (Liu et al., 2015; Lopez-Onieva et al., 2008; Wang et al., 2008a). Loss of Lsd1, a histone demethylase, also causes expansion of *dpp* signaling, but this is because the escort cells have adopted a cap cell-like fate, as they display strong En and Hh expression, which is normally found only in cap cells (Eliazer et al., 2014). Although depletion of PRC1 in escort cells produces a similar GSC-like tumor phenotype, it is likely to be caused by different molecular mechanisms to any of the above. PRC1-depleted escort cells do not have increased expression of JAK/STAT signaling ligands and do not regain En and Hh expression. Taking into account that *dpp* is known as a direct Polycomb target in *Drosophila* (Schwartz and Pirrotta, 2007), these observations suggest that PRC1 could serve as a major mechanism to specifically maintain the repressive state of *dpp* in escort cells, which is important for restricting the self-renewal niche from expanding to the differentiation niche, and thereby facilitating proper germline differentiation.

Mechanisms underlying PRC1-mediated transcriptional silencing are not well understood. It has been proposed that H2Aub generated by PRC1 is essential for the establishment of repressive chromatin required for transcriptional repression. Because knockdown of *sce*, which encodes the E3 ligase required for H2Aub formation, also causes derepression of *dpp* and GSC-like tumor formation, whether H2Aub is essential for PRC1-mediated transcriptional silencing of *dpp* in escort remains to be determined.

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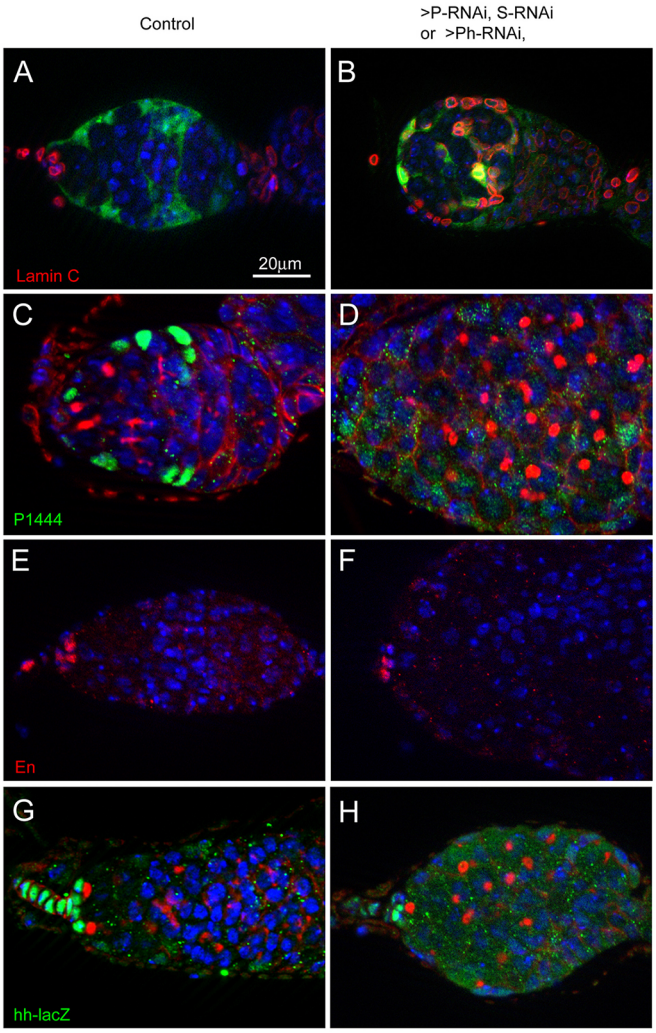

Fig. 6. PRC1 depletion does not transform escort cells into cap cells. (A,C,E,G) Wild-type germaria. (A) Escort cells were labeled by *c587>UAS-GFP* (green) and Lamin C (red) expression was specifically enriched in TFs and cap cells. (C) *P1444-lacZ* (green) was specifically expressed in escort cells. (E,G) En (E, red) and *Hh-lacZ* detected by anti-β-galactosidase (G, green) were both expressed in TFs and cap cells. (B) In *Psc-RNAi*, *Su(z)2-RNAi* germarium, escort cells displayed enriched expression of lamin C (red). (D) In *ph-RNAi* germarium, *P1444-lacZ* expression (green) was dampened in escort cells. (F,H) In *Psc-RNAi*, *Su(z)2-RNAi* germaria, En (F, red) and *Hh-lacZ* (H, green) expression was not altered in escort cells. The germaria were analyzed at 10-14 days after shifting to restrictive temperature. α-spectrin to highlight spectrosomes is red in C,D,G,H.

However, a recent study reveals that during development of the *Drosophila* embryo and larva, the repressive state of many PRC1 target genes is still properly maintained in H2Aub-deficient animals (Pengelly et al., 2015), indicating that other mechanisms, such as polynucleosome compaction, or direct interference with transcription machinery, could have more important roles in PRC1-mediated transcriptional silencing in *Drosophila* (Simon and Kingston, 2013).

Potential epigenetic regulation of *dpp* expression in escort cells has been suggested by a number of previous studies. Depletion of Eggless (Egg), a histone H3K9 trimethylase, in escort cells, causes defects in heterochromatin formation, derepression of transposable elements and derepression of *dpp* signaling (Wang et al., 2011). A similar mechanism may explain the GSC-like tumor phenotype caused by mutation of Piwi, which regulates heterochromatin formation and transposon silencing (Jin et al., 2013; Ma et al., 2014). The involvement of both heterochromatin formation and PcG proteins in suppressing *dpp* transcription in escort cells indicates an intriguing link among PcG, Piwi function and heterochromatin formation in the regulation of chromatin structure and gene expression, at least at the *dpp* locus. Of note, a recent study reveals that Piwi interacts with PRC2 to regulate GSC maintenance (Peng et al., 2016). It would be of great interest to understand whether and how these pathways interact to control global and regional chromatin structure and gene expression.

PRC1-Brm antagonism in regulating *dpp* expression in escort cells

Our studies demonstrate that loss of PcG genes could cause TrxG-dependent activation of niche signals and tumor development. Because antagonistic functions of PcG and TrxG protein complexes are important for the establishment of repressive or active chromatin domains, which are required for HOX gene repression or activation, respectively (Geisler and Paro, 2015), our observations here indicate that some aspects of the antagonistic function module of PcG and TrxG are also utilized in adult tissues for maintaining tissue homeostasis. But it is worthwhile to note that the way this module is utilized is different. For the regulation of HOX genes during development, loss of PcG function is sufficient to switch the balance to TrxG dominance and cause derepression of HOX genes. Conversely, loss of TrxG function is sufficient to switch the balance and cause repression of HOX genes. However, our observations here suggest that in escort cells, PRC1-mediated silencing of *dpp* expression seems to be the default state, as inhibiting the function of the TrxG protein Brm or overexpression of Brm in escort cells does not produce any obvious phenotype (not shown) and the function of Brm manifests only if PcG function is disrupted, in which activation of *dpp* transcription and tumor development require Brm. Therefore, some, but not all aspects of the antagonistic function module of PcG and TrxG are utilized in the niche cells to control GSC differentiation in the *Drosophila* ovary. It is unclear why overexpression of Brm alone is not sufficient to turn on *dpp* transcription. It is possible that Brm must function in a protein complex to promote transcriptional activation, or Brm must function in concert with other chromatin regulators, such as chromatin remodeling factors, to activate transcription. We also could not exclude the possibility that Brm regulates *dpp* indirectly by influencing the transcription of other genes.

Tumor suppressive function of PcG genes

Polycomb group proteins are generally regarded as proto-oncogenes in mammals, and many proteins are upregulated in cancers and are considered to have a causative role in promoting tumorigenesis (Sparmann and van Lohuizen, 2006). Emerging evidence suggests that PcG proteins could also be tumor suppressors (Grossniklaus and Paro, 2014; Koppens and van Lohuizen, 2015; Su et al., 2011). For example, loss of functional mutation of PRC2 rather than overexpression has been implicated in malignant myeloid diseases. In addition, loss of EZH2 in hematopoietic stem cells is sufficient to cause T-acute lymphoblastic leukemia (Hock, 2012; Koppens and van Lohuizen, 2015). In *Drosophila*, loss of PcG genes in larval imaginal disc cells causes derepression of Notch and JAK/STAT signaling and consequently leads to tumorigenesis. Psc and Su(z)2 also have tumor suppressive roles in somatic gonad cells by

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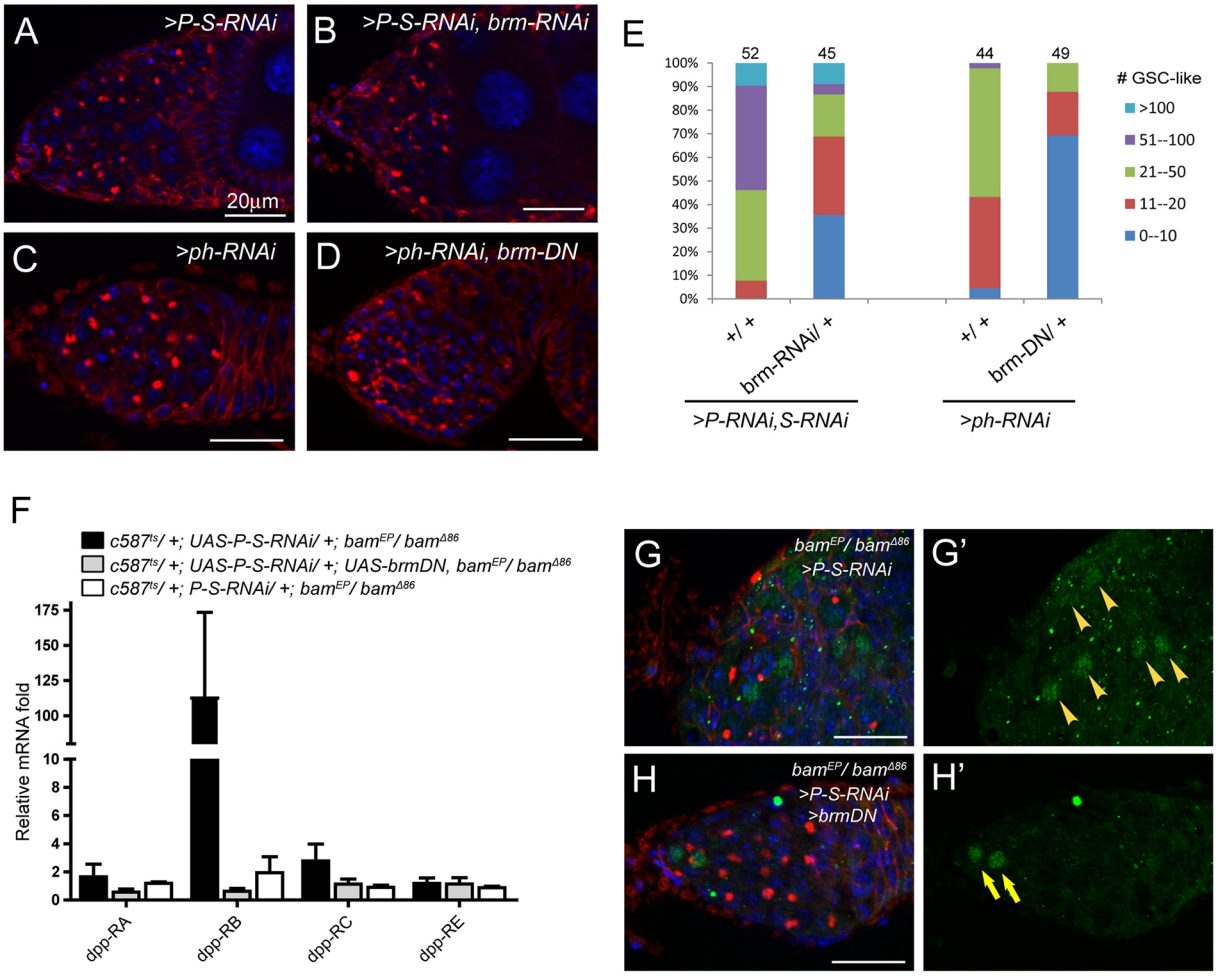

Fig. 7. Tumor development requires the Trithorax gene *brm*. (A-D) Representative images of germaria of indicated genotypes using *c587-GAL4; Tub-GAL80^{ts}* crossed with UAS transgenic flies at 12 days after shifting to restrictive temperature. Spectrosomes are highlighted by α-spectrin staining (red). (A) *Psc-RNAi*, *Su(z)2-RNAi* GSC-like tumor accumulation (B) In *brm-RNAi*, the number of GSC-like cells was significantly reduced and many underwent differentiation evidenced by appearance of the branched fusomes. (C) GSC-like tumor accumulation in *ph-RNAi*. (D) Co-expression of *brm-DN* significantly reduced the number of GSC-like cells and promoted differentiation into germline cysts. (E) Quantitative analysis of GSC-like cells in each germarium of indicated genotypes (flies were crossed with *c587-GAL4; Tub-GAL80^{ts}*). (F) Quantitative RT-PCR of *dpp* mRNAs from ovaries of indicated genotypes. *dpp-RB* isoform was significant upregulated by *Psc-RNAi*, *Su(z)2-RNAi* in *bam$^{Δ86/EP(3)667}$* mutant background ovaries compared with *bam$^{Δ86/EP(3)667}$* mutant alone. Co-expression of *brm-DN* resulted in an effective inhibition of *dpp-RB* derepression. Values are mean±s.e.m., *n*=3. (G,G′) In *Psc-RNAi*, *Su(z)2-RNAi* and *bam$^{Δ86/EP(3)667}$* germarium, pMad (green) is expressed not only in the anterior GSCs but also in GSC-like cells (arrowheads) distant from the cap niche. (H,H′) GSC-like cells distant from the cap cell niche no longer express pMad upon co-expression of *brm-DN* (green). Enriched pMad expression is only observed in the GSCs in the cap cell niche (arrows). Scale bars: 20 μm.

facilitating differentiation (Classen et al., 2009; Li et al., 2010; Martinez et al., 2009; Morillo Prado et al., 2012). Therefore, PcG proteins can act as either oncogenes or tumor suppressors, which probably depends on tissue context and the identity and function of their target genes in the designated cell types. In all cases previously reported, the tumor suppressive function of PcG is mainly cell autonomous. Our study here demonstrates that PcG can also function non cell-autonomously to suppress tumorigenesis by regulating the niche environment required for progenitor cell differentiation. Given the evolutionary conservation of PcG/TrxG functions, we speculate that this non cell-autonomous, tumor suppressive function of PcG genes could be conserved in adult tissues of mammals and humans. The requirement of TrxG for tumorigenesis following the loss of PcG could also have important implications on the therapeutic intervention of cancers caused by mutations in PcG/TrxG genes. Rebalancing PcG/TrxG gene activity could be an effective strategy for treating cancers caused by imbalances of PcG/TrxG proteins.

MATERIALS AND METHODS

Fly stocks

Flies were cultured on standard food medium with yeast paste added to the food surface. For temperature shift experiments, crosses were made at 18°C and 2- to 5-day-old eclosed flies were then shifted to 29°C until dissection. Strains used in this study: *c587-GAL4* (Song et al., 2004); *bam-GFP* (Chen and McKearin, 2003a); *Dad-lacZ* (Tsuneizumi et al., 1997); *bam^{Δ86}* (McKearin and Ohlstein, 1995); *bam$^{EP(3)667}$* (gift from Jean Maines, University of Texas Southwestern Medical Center); *dpp^{e90}* and *dpp^{hr56}* (Xie and Spradling, 1998); *P1444-lacZ* (Xie and Spradling, 2000), *UAS-brm^{K804R}* (Elfring et al., 1998) (gift from Jessica Treisman, Skirball Institute of Biomolecular Medicine, New York); *UAS-PSC-N1* and *UAS-Su(z)2-N1* (gift from Ansgar Klebes, Humboldt-Universität zu Berlin); *dpp-GAL4* (Janelia Farm, GMR19E03); from Bloomington *Drosophila* Stock Center (BDSC):

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UAS-GAL80^{ts} (#7016, #7018), *UAS-p35* (#5072); *hs-bam* (#24638), *hh-lacZ* (#101646); TRiP: *psc-RNAi* (#31611), *sce-RNAi* (#31612), *dpp-RNAi* (#31172, #31530), *brm-RNAi* (#31712); from Vienna *Drosophila* RNAi Center (VDRC): *psc-RNAi* (v30586), *su(z)2-RNAi* (v50368), *ph-RNAi* (v50024), *sce-RNAi* (v106328), *E(z)-RNAi* (v27645).

Immunostaining

Drosophila ovaries were dissected and immunostained as described previously (Li et al., 2010). Briefly, ovaries were fixed in 4% paraformaldehyde for 15 min, and blocked in 5% normal goat serum in PBT (10 mM NaH_2PO_4/Na_2HPO_4, 175 mM NaCl, pH 7.4, 0.1% Triton X-100). The following primary antibodies were used: rabbit anti-pMad (1:1000; gift from Ed Laufer, Columbia University Medical Center, New York), rabbit anti-β-galactosidase (1:3000; MP Biologicals, 0855976), mouse anti-α-spectrin (1:50, DSHB), anti-laminin C (1:50, DSHB), anti-En (1:50, DSHB), anti-β-AbdB (1:50, DSHB), anti-FasIII (1:10, DSHB), anti-aPKC (Santa Cruz Biotechnology, sc-216, 1:100). Secondary antibodies, including goat anti-rabbit, anti-mouse IgGs, conjugated to Alexa (488 or 568) (Molecular Probes) were used at a dilution of 1:300 and tissues were also stained with 0.1 mg/ml DAPI (4′,6′-diamidino-2-phenylindole; Sigma) for 5 min. TUNEL staining was as previously described (Zhao et al., 2008). Images were collected using either a Zeiss Meta 510 confocal microscope system or a Zeiss Imager Z1 equipped with an ApoTome. All acquired images were processed in Adobe Photoshop and Illustrator.

Generation of transgenic flies

The GMR19E03-GAL4 line contains an enhancer region of about 4.0 kb in length, which is downstream of the transcript start site at 9370 to 13331 bp of RA, and is upstream of transcript start site −12452 to −8491 bp of RB. This 4.0 kb fragment was divided into five fragments: P1 fragment (corresponding to 9115-10540 bp of *dpp* gene), P2 (9961-11063), P3 (10521 to 11939), P4 (11134-12627) and P5 (12222 to 13359). These fragments were cloned into the C4PLZ vector. Plasmids were purified using a Qiagen Plasmid Midi Kit (#12145) and introduced into *w^{1118}* embryos to generate transgenic flies using standard procedures. *P4-lacZ* showed TF/cap cell-specific expression, a pattern that is similar with *GMR19E03-GAL4>GFP* and therefore was used in the follow-up study.

RNA isolation and qPCR

Total RNA from 10-20 ovaries was extracted using TRIzol reagent (TaKaRa). After DNase treatment, complementary DNA (cDNA) was synthesized using oligo dT primers and PrimeScript Reverse Transcriptase (TaKaRa, #2680A). RT-qPCR was performed in three duplicates using ChamQ SYBR qPCR master Mix (Vazyme, #Q331) on an ABI PRISM 7500 fast real-time PCR system (Applied Biosystems). Endogenous *Actin5c* mRNA levels were measured for normalization. Fold changes for mRNA were calculated using the ΔΔCt method (Livak and Schmittgen, 2001). Primer sequences used were: *Gapdh* F, GTGAAGCTGATCTCTTGGTACGAC; *Gapdh* R, CCGCGCCCTAATCTTTAACTTTTAC; *AbdB* F, GGAGATGTCGGGGAGGAGTA; *AbdB* R, TATCAGGATCAAGCGGCGTC; *gbb* F, AGTGGCTGGTCAAGTCGAAG; *gbb* R, CCGATCATGAAGGGCTGGAA; *dpp-RA* F, TTGGAGCGTAACTGAGCGG; *dpp-RB* F, CACTCTGCTGCTCGAAGGAA; *dpp-RC* F, GGGCGATCCATCCATCAAAC; *dpp-RE* F, TGCCAGATACGAAGAGTTGGG; *dpp* R, CGTTTGAAAAGTCGCCAGCA; *upd* F, GAAAGCGGAACAGCAACTGG; *upd* R, TGTTTAGGCTGCGGTACTCC; *upd2* F, CCACAACCTGCGACTCTTCT; *upd2* R, AAGTTTTCGAGGTGCTTGCG; *upd3* F, TACGCATCTGGACTGGGAGA; *upd3* R, GCAGGATCCTTTGGCGTTTC; *dally* F, TTGTGCTACGGCTACTGCAA; *dally* R, TAATGATGCCGGTGTCCGAG; *dlp* F, AATCACCACAGAACACGGCT; *dlp* R, AACATCCTGGCCGTTGTAGG.

Statistical analysis

Data are presented as mean±s.e.m. *P*-values were calculated using one-way ANOVA or unpaired two-tailed Student's *t*-test using GraphPad Prism 5.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Xuewen Li, F.Y., B.D. and R.X. conceived and designed the experiments and analyzed the data; Xuewen Li, F .Y., H.C., B.D. and Xinghua Li performed the experiments; R.X. wrote the manuscript.

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Supplementary information

Supplementary information available online at
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References

Armstrong, J. A., Papoulas, O., Daubresse, G., Sperling, A. S., Lis, J. T., Scott, M. P. and Tamkun, J. W. (2002). The Drosophila BRM complex facilitates global transcription by RNA polymerase II. *EMBO J.* **21**, 5245-5254.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Chen, D. and McKearin, D. (2003a). Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. *Curr. Biol.* **13**, 1786-1791.

Chen, D. and McKearin, D. M. (2003b). A discrete transcriptional silencer in the bam gene determines asymmetric division of the Drosophila germline stem cell. *Development* **130**, 1159-1170.

Chen, S., Wang, S. and Xie, T. (2011). Restricting self-renewal signals within the stem cell niche: multiple levels of control. *Curr. Opin. Genet. Dev.* **21**, 684-689.

Classen, A.-K., Bunker, B. D., Harvey, K. F., Vaccari, T. and Bilder, D. (2009). A tumor suppressor activity of Drosophila Polycomb genes mediated by JAK-STAT signaling. *Nat. Genet.* **41**, 1150-1155.

Elfring, L. K., Daniel, C., Papoulas, O., Deuring, R., Sarte, M., Moseley, S., Beek, S. J., Waldrip, W. R., Daubresse, G., DePace, A. et al. (1998). Genetic analysis of brahma: the Drosophila homolog of the yeast chromatin remodeling factor SWI2/SNF2. *Genetics* **148**, 251-265.

Eliazer, S., Shalaby, N. A. and Buszczak, M. (2011). Loss of lysine-specific demethylase 1 nonautonomously causes stem cell tumors in the Drosophila ovary. *Proc. Natl. Acad. Sci. USA* **108**, 7064-7069.

Eliazer, S., Palacios, V., Wang, Z., Kollipara, R. K., Kittler, R. and Buszczak, M. (2014). Lsd1 restricts the number of germline stem cells by regulating multiple targets in escort cells. *PLoS Genet.* **10**, e1004200.

Geisler, S. J. and Paro, R. (2015). Trithorax and Polycomb group-dependent regulation: a tale of opposing activities. *Development* **142**, 2876-2887.

Grossniklaus, U. and Paro, R. (2014). Transcriptional silencing by polycomb-group proteins. *Cold Spring Harb. Perspect. Biol.* **6**, a019331.

Guo, Z. and Wang, Z. (2009). The glypican Dally is required in the niche for the maintenance of germline stem cells and short-range BMP signaling in the Drosophila ovary. *Development* **136**, 3627-3635.

Hamada-Kawaguchi, N., Nore, B. F., Kuwada, Y., Smith, C. I. E. and Yamamoto, D. (2014). Btk29A promotes Wnt4 signaling in the niche to terminate germ cell proliferation in Drosophila. *Science* **343**, 294-297.

Harris, R. E. and Ashe, H. L. (2011). Cease and desist: modulating short-range Dpp signalling in the stem-cell niche. *EMBO Rep.* **12**, 519-526.

Hayashi, Y., Kobayashi, S. and Nakato, H. (2009). *Drosophila* glypicans regulate the germline stem cell niche. *J. Cell Biol.* **187**, 473-480.

Hock, H. (2012). A complex Polycomb issue: the two faces of EZH2 in cancer. *Genes Dev.* **26**, 751-755.

Jin, Z., Flynt, A. S. and Lai, E. C. (2013). Drosophila piwi mutants exhibit germline stem cell tumors that are sustained by elevated Dpp signaling. *Curr. Biol.* **23**, 1442-1448.

Kai, T. and Spradling, A. (2003). An empty Drosophila stem cell niche reactivates the proliferation of ectopic cells. *Proc. Natl. Acad. Sci. USA* **100**, 4633-4638.

Kirilly, D., Wang, S. and Xie, T. (2011). Self-maintained escort cells form a germline stem cell differentiation niche. *Development* **138**, 5087-5097.

Koppens, M. and van Lohuizen, M. (2015). Context-dependent actions of Polycomb repressors in cancer. *Oncogene* **35**, 1341-1352.

Li, L. and Xie, T. (2005). Stem cell niche: structure and function. *Annu. Rev. Cell Dev. Biol.* **21**, 605-631.

Li, X., Han, Y. and Xi, R. (2010). Polycomb group genes Psc and Su(z)2 restrict follicle stem cell self-renewal and extrusion by controlling canonical and noncanonical Wnt signaling. *Genes Dev.* **24**, 933-946.
Lin, H., Yue, L. and Spradling, A. C. (1994). The Drosophila fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. *Development* **120**, 947-956.
Liu, M., Lim, T. M. and Cai, Y. (2010). The Drosophila female germline stem cell lineage acts to spatially restrict DPP function within the niche. *Sci. Signal.* **3**, ra57.
Liu, Z., Zhong, G., Chai, P. C., Luo, L., Liu, S., Yang, Y., Baeg, G.-H. and Cai, Y. (2015). Coordinated niche-associated signals promote germline homeostasis in the Drosophila ovary. *J. Cell Biol.* **211**, 469-484.
Livak, K. J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408.
Lopez-Onieva, L., Fernandez-Minan, A. and Gonzalez-Reyes, A. (2008). Jak/Stat signalling in niche support cells regulates dpp transcription to control germline stem cell maintenance in the Drosophila ovary. *Development* **135**, 533-540.
Lu, T., Wang, S., Gao, Y., Mao, Y., Yang, Z., Liu, L., Song, X., Ni, J. and Xie, T. (2015). COP9-Hedgehog axis regulates the function of the germline stem cell progeny differentiation niche in the Drosophila ovary. *Development* **142**, 4242-4252.
Luo, L., Wang, H., Fan, C., Liu, S. and Cai, Y. (2015). Wnt ligands regulate Tkv expression to constrain Dpp activity in the Drosophila ovarian stem cell niche. *J. Cell Biol.* **209**, 595-608.
Ma, X., Wang, S., Do, T., Song, X., Inaba, M., Nishimoto, Y., Liu, L. P., Gao, Y.-p., Mao, Y., Li, H. et al. (2014). Piwi is required in multiple cell types to control germline stem cell lineage development in the Drosophila ovary. *PLoS ONE* **9**, e90267.
Maimon, I., Popliker, M. and Gilboa, L. (2014). Without children is required for Stat-mediated zfh1 transcription and for germline stem cell differentiation. *Development* **141**, 2602-2610.
Martinez, A.-M., Schuettengruber, B., Sakr, S., Janic, A., Gonzalez, C. and Cavalli, G. (2009). Polyhomeotic has a tumor suppressor activity mediated by repression of Notch signaling. *Nat. Genet.* **41**, 1076-1082.
McGuire, S. E., Mao, Z. and Davis, R. L. (2004). Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in Drosophila. *Sci. STKE* **2004**, pl6.
McKearin, D. and Ohlstein, B. (1995). A role for the Drosophila bag-of-marbles protein in the differentiation of cystoblasts from germline stem cells. *Development* **121**, 2937-2947.
McKearin, D. M. and Spradling, A. C. (1990). bag-of-marbles: a Drosophila gene required to initiate both male and female gametogenesis. *Genes Dev* **4**, 2242-2251.
Morillo Prado, J. R., Chen, X. and Fuller, M. T. (2012). Polycomb group genes Psc and Su(z)2 maintain somatic stem cell identity and activity in Drosophila. *PLoS ONE* **7**, e52892.
Morris, L. X. and Spradling, A. C. (2011). Long-term live imaging provides new insight into stem cell regulation and germline-soma coordination in the Drosophila ovary. *Development* **138**, 2207-2215.
Morrison, S. J. and Spradling, A. C. (2008). Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* **132**, 598-611.
Ohlstein, B. and McKearin, D. (1997). Ectopic expression of the Drosophila Bam protein eliminates oogenic germline stem cells. *Development* **124**, 3651-3662.
Peng, J. C., Valouev, A., Liu, N. and Lin, H. (2016). Piwi maintains germline stem cells and oogenesis in Drosophila through negative regulation of Polycomb group proteins. *Nat. Genet.* **48**, 283-291.
Pengelly, A. R., Kalb, R., Finkl, K. and Müller, J. (2015). Transcriptional repression by PRC1 in the absence of H2A monoubiquitylation. *Genes Dev.* **29**, 1487-1492.
Schwartz, Y. B. and Pirrotta, V. (2007). Polycomb silencing mechanisms and the management of genomic programmes. *Nat. Rev. Genet.* **8**, 9-22.
Simon, J. A. and Kingston, R. E. (2013). Occupying chromatin: Polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put. *Mol. Cell* **49**, 808-824.
Song, X., Wong, M. D., Kawase, E., Xi, R., Ding, B. C., McCarthy, J. J. and Xie, T. (2004). Bmp signals from niche cells directly repress transcription of a differentiation-promoting gene, bag of marbles, in germline stem cells in the Drosophila ovary. *Development* **131**, 1353-1364.
Song, X., Call, G. B., Kirilly, D. and Xie, T. (2007). Notch signaling controls germline stem cell niche formation in the Drosophila ovary. *Development* **134**, 1071-1080.
Sparmann, A. and van Lohuizen, M. (2006). Polycomb silencers control cell fate, development and cancer. *Nat. Rev. Cancer* **6**, 846-856.
Su, Y., Deng, B. and Xi, R. (2011). Polycomb group genes in stem cell self-renewal: a double-edged sword. *Epigenetics* **6**, 16-19.
Tamkun, J. W., Deuring, R., Scott, M. P., Kissinger, M., Pattatucci, A. M., Kaufman, T. C. and Kennison, J. A. (1992). brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* **68**, 561-572.
Tie, F., Banerjee, R., Conrad, P. A., Scacheri, P. C. and Harte, P. J. (2012). Histone demethylase UTX and chromatin remodeler BRM bind directly to CBP and modulate acetylation of histone H3 lysine 27. *Mol. Cell. Biol.* **32**, 2323-2334.
Tsuneizumi, K., Nakayama, T., Kamoshida, Y., Kornberg, T. B., Christian, J. L. and Tabata, T. (1997). Daughters against dpp modulates dpp organizing activity in Drosophila wing development. *Nature* **389**, 627-631.
Wang, L., Li, Z. and Cai, Y. (2008a). The JAK/STAT pathway positively regulates DPP signaling in the Drosophila germline stem cell niche. *J. Cell Biol.* **180**, 721-728.
Wang, X., Harris, R. E., Bayston, L. J. and Ashe, H. L. (2008b). Type IV collagens regulate BMP signalling in Drosophila. *Nature* **455**, 72-77.
Wang, X., Pan, L., Wang, S., Zhou, J., McDowell, W., Park, J., Haug, J., Staehling, K., Tang, H. and Xie, T. (2011). Histone H3K9 trimethylase Eggless controls germline stem cell maintenance and differentiation. *PLoS Genet.* **7**, e1002426.
Wang, S., Gao, Y., Song, X., Ma, X., Zhu, X., Mao, Y., Yang, Z., Ni, J., Li, H., Malanowski, K. E. et al. (2015). Wnt signaling-mediated redox regulation maintains the germ line stem cell differentiation niche. *eLife* **4**, e08174.
Ward, E. J., Shcherbata, H. R., Reynolds, S. H., Fischer, K. A., Hatfield, S. D. and Ruohola-Baker, H. (2006). Stem cells signal to the niche through the Notch pathway in the Drosophila ovary. *Curr. Biol.* **16**, 2352-2358.
Xie, T. (2013). Control of germline stem cell self-renewal and differentiation in the Drosophila ovary: concerted actions of niche signals and intrinsic factors. *Wiley Interdiscip. Rev. Dev. Biol.* **2**, 261-273.
Xie, T. and Spradling, A. C. (1998). decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. *Cell* **94**, 251-260.
Xie, T. and Spradling, A. C. (2000). A niche maintaining germ line stem cells in the Drosophila ovary. *Science* **290**, 328-330.
Xuan, T., Xin, T., He, J., Tan, J., Gao, Y., Feng, S., He, L., Zhao, G. and Li, M. (2013). dBre1/dSet1-dependent pathway for histone H3K4 trimethylation has essential roles in controlling germline stem cell maintenance and germ cell differentiation in the Drosophila ovary. *Dev. Biol.* **379**, 167-181.
Zhao, R., Xuan, Y., Li, X. and Xi, R. (2008). Age-related changes of germline stem cell activity, niche signaling activity and egg production in Drosophila. *Aging Cell* **7**, 344-354.