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## Differentiation-Defective Stem Cells Outcompete Normal Stem Cells for Niche Occupancy in the *Drosophila* Ovary

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### Summary

Rapid progress has recently been made regarding how the niche controls stem cell function, but little is yet known about how stem cells in the same niche interact with one another. In this study, we show that differentiation-defective *Drosophila* ovarian germline stem cells (GSCs) can outcompete normal ones for niche occupancy in a cadherin-dependent manner. The differentiation-defective *bam* or *bgn* mutant GSCs invade the niche space of neighboring wild-type GSCs and gradually push them out of the niche by upregulating E-cadherin expression. Furthermore, the *bam/bgn*-mediated GSC competition requires E-cadherin and normal GSC division but not the self-renewal-promoting BMP niche signal, while different E-cadherin levels can sufficiently stimulate GSC competition. Therefore, we propose that GSCs have a competitive relationship for niche occupancy, which may serve as a quality control mechanism to ensure that accidentally differentiated stem cells are rapidly removed from the niche and replaced by functional ones.

### Introduction

Stem cells can self-renew to maintain a stable population and generate differentiated cells to replenish lost cells in adult animal tissues, and their self-renewal and proliferation are tightly controlled by signals from their niche (Li and Xie, 2005; Ohlstein et al., 2004). Normally, stem cells reside in the same niche or tissue site and are capable of repopulating the empty niche space left by a lost stem cell (Xie and Spradling, 2000). However, it remains unclear how the stem cells in the same or nearby niche quickly respond to the loss of stem cells and carry out subsequent repopulation of vacant niches. In this study, we provide experimental evidence that the stem cells in the same niche have a competitive relationship, which may provide a quality control mechanism for removing differentiated stem cells from the niche.

*Drosophila* germline stem cells (GSCs) have become an attractive system for studying stem cell biology, including the niche structure and function and self-renewal mechanisms. In a structure known as the germarium, at the tip of the *Drosophila* ovary, two or three GSCs can be reliably recognized by their size (the largest germ cells), location (in direct

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contact with cap cells) and anteriorly anchored spherical spectrosome (SS), and they can be effectively studied at the molecular and cellular level (Lin, 2002; Xie et al., 2005) (Figure 1A and 1B). At the tip of the germarium, somatic cap cells and possibly escort stem cells (ESCs), form the GSC niche (Figure 1A), where GSCs are anchored to the cap cells through E-cadherin-mediated cell adhesion (Song et al., 2002; Xie and Spradling, 2000). Such anchorage is essential for keeping GSCs in their niche for long-term self-renewal since the GSCs defective in E-cadherin-mediated cell adhesion are lost rapidly from the niche (Song et al., 2002). Cystoblasts, the immediate progeny of GSCs, divide synchronously four times with incomplete cytokinesis to form interconnected 16-cell cysts sharing a branched fusome (de Cuevas et al., 1997)(Figure 1B). Spectrosomes and fusomes are germ cell-specific organelles rich in membrane skeleton proteins such as adducin-like Hu li-tai shao (Hts) (Lin et al., 1994). In this study, we used this system to investigate how GSCs in the same niche interact with one another.

GSC self-renewal is primarily controlled by BMPs and *piwi*-mediated signals from the niche cells (Cox et al., 1998; Cox et al., 2000; Song et al., 2004; Xie and Spradling, 1998), and these signals maintain GSCs by repressing the expression of a differentiation-promoting gene, *bag of marbles* (*bam*) (Chen and McKearin, 2003a, 2005; Song et al., 2004; Szakmary et al., 2005). On the other hand, the differentiation of cystoblasts requires *bam* and *bgn* (*benign gonial cell neoplasm*), since mutations in either *bam* or *bgn* result in the accumulation of spectrosome-containing undifferentiated single germ cells (Lavoie et al., 1999; McKearin and Spradling, 1990; Ohlstein and al., 2000) (Figure 1C and 1D). These differentiation-defective single germ cells resemble GSCs based on their gene expression profiles (Kai et al., 2005). In this study, we show that *bam* and *bgn* also have important functions in GSCs in controlling their relative competitiveness.

In *Drosophila* imaginal discs, where cell competition is extensively studied, its *Drosophila* homolog of human proto-oncogene *myc* (*dmyc*) regulates the process (de la Cova et al., 2004; Moreno and Basler, 2004). Namely, *dmyc* mutant cells grow poorly and are outcompeted by their more vigorous wild-type neighboring cells (Johnston et al., 1999), while local *dmyc* overexpression causes cell competition by inducing the apoptosis of their neighboring wild-type cells (de la Cova et al., 2004). In the *Drosophila* ovary, dMyc protein is abundantly expressed in the nucleus of both somatic and germ cells throughout oogenesis (Maines et al., 2004). *dmyc* mutant follicle cells and germ cells exhibit defects in growth and endoreplication (Maines et al., 2004). Although *dmyc* is highly expressed in the nuclei of GSCs, its role in stem cells has not been established. In this study, we have developed an effective system for studying stem cell competition, and then have shown that stem cells in the same niche have a competitive relationship, which is regulated by *bam* and *bgn* but not *dmyc*.

## Results

### ***bam* and *bgn* Mutant GSCs Outcompete Wild-Type GSCs for Niche Occupancy**

To investigate whether *bgn* or *bam* mutant stem cells can have any competitive advantage over normal stem cells in occupying the niche, we chose *bam*<sup>86</sup>, a deletion mutant of *bam* (McKearin and Spradling, 1990), and two *bgn* mutants, *bgn*<sup>20093</sup> and *bgn*<sup>20915</sup>, which

bear a premature stop codon after residues 12 and 857, respectively (this study) to generate niches carrying a marked mutant *bam* or *bgn* GSC and an unmarked wild-type GSC using the FLP-mediated FRT mitotic recombination (Xie and Spradling, 1998). Among one week-old mutant *bam*<sup>86</sup>, *bgn*<sup>20093</sup> or *bgn*<sup>20915</sup> germaria labeled for Vasa (germ cells) and Hts (spectrosomes/fusomes), the *bgn*<sup>20093</sup> or *bgn*<sup>20915</sup> mutants contained only GSC-like single germ cells just like other previously characterized *bgn* mutants (Ohlstein and al., 2000), and the *bam*<sup>86</sup> mutant germaria consistently had more GSC-like cells than those *bgn* mutant germaria (Figure 1C and 1D), suggesting that the *bam* mutant GSC-like cells may proliferate faster than the *bgn* mutants (Figure 1C and 1D).

Following the FLP/FRT mediated mitotic recombination, the marked GSCs were identified by the absence of *arm-lacZ* expression, direct contact with cap cells and an anteriorly anchored spectrosome, whereas the unmarked GSCs were identified by the presence of *arm-lacZ* expression, and direct contact with cap cells and an anteriorly anchored spectrosome (Xie and Spradling, 1998)(Figure 1E–1K). In this study, a “partial clone” is defined as a germarium carrying a mixture of marked and unmarked GSCs (Figure 1E and 1F), whereas a marked “full clone” is a germarium containing only two or three marked GSCs (Figure 1G). In the control, most of the marked GSCs detected 3 days after clone induction (ACI) were still maintained in the germaria 3 weeks ACI (Figure 1E and 1F); 25.2% of them were lost due to natural turnover, which was consistent with previous results (Song et al., 2002; Xie and Spradling, 1998)(Table 1). Since a lost GSC can be efficiently repopulated by the progeny of a neighboring GSC in the same niche (Xie and Spradling, 2000), a partial clone in the germarium carrying only two GSCs will become a full clone after losing the unmarked GSC. Since marked wild-type GSCs should behave like unmarked wild-type GSCs, and a wild-type control germarium usually carries 2.5 GSCs (n=550), we would expect that the increased percentage of the germaria carrying a full clone is equal to about 40% of the percentage of the germaria losing a marked GSC. This agrees well with what we observed for wild-type control clones. From 3 days ACI to 3 weeks ACI, the percentage of the germaria carrying a partial clone decreased from 39.8% to 21.7% (18.1% net decrease), whereas the percentage of the germaria carrying a full clone increased from 1.8% to 9.4% (total 7.6% increase) (Table 1). Since natural GSC turnover is expected to be random, the lost marked wild-type GSCs should be roughly equal to the marked GSCs gained from replacement of the lost unmarked GSCs. Indeed, the percentage of marked GSCs versus total GSCs remained almost constant (without a net increase of the marked wild-type GSCs): 22.3% at 3 days ACI to 20.2% at 3 weeks ACI (Table 1). Together, this result shows that GSCs are an effective system to quantitatively study stem cell competition dynamics in the niche.

Interestingly, the percentage of germaria carrying one or more marked *bgn*<sup>20093</sup> and *bgn*<sup>20915</sup> GSCs exhibited only a 4.9% and 10.7% reduction 3 weeks ACI, respectively, which is in contrast to a 25.2% reduction of marked wild-type GSCs, indicating that marked *bgn* mutant GSCs are lost at a slower rate than marked wild-types (Table 1). The observation that blocking differentiation by *bgn* mutations can prolong GSC lifespan also suggests that natural GSC turnover might be due to random differentiation. Moreover, we observed a dramatic increase in the percentage of germaria carrying a marked *bgn*<sup>20093</sup> or *bgn*<sup>20915</sup> full clone: from 6.6% at 3 days ACI to 32.4% at 3 weeks ACI for *bgn*<sup>20093</sup>,

and from 6.9% at 3 days ACI to 27.1% at 3 weeks ACI for *bgn*<sup>20915</sup>, indicating that *bgn* mutant GSCs can somehow replace their neighboring wild-type stem cells and occupy their niches (Figure 1H and 1I; Table 1). Similarly, we also observed a net increase in marked *bgn* mutant GSCs: from 25.1% (3 days ACI) to 37.5% (3 weeks ACI) for *bgn*<sup>20093</sup> mutant GSCs and from 26.0% (3 days ACI) to 34.9% (3 weeks ACI) for *bgn*<sup>20915</sup> mutant GSCs (Table 1), which is in contrast to no obvious net increase of total marked wild-type GSCs. These results provide direct evidence that *bgn* mutant GSCs can outcompete normal GSCs for their niches.

Since *bgn* and *bam* have been proposed to function in the same genetic pathway to control GSC differentiation (Lavoie et al., 1999; Ohlstein and al., 2000), we would expect that *bam* mutant GSCs behave in the same way as *bgn* mutant GSCs in stem cell competition. Surprisingly, the percentage of the germaria carrying a partial or full clone increased beyond the normal range of clone induction rate, from 44.1% at 3 days ACI to 86.1% at 2 weeks ACI (Table 1; Figure 1J and 1K), representing a three-fold net increase in total marked GSCs. The newly gained marked *bam* mutant GSCs must come from the mutant *bam* cystoblasts that occupied the niches, which were normally occupied by unmarked wild-type GSCs, indicating that *bam* mutant cystoblasts can successfully invade the niche occupied by wild-type GSCs and assume GSC identity. Interestingly, during the period from 2 weeks ACI to 3 weeks ACI, the percentage of the germaria carrying a partial marked GSC clone decreased, while the percentage of germaria carrying a full clone showed a dramatic increase, from 32.4% to 58.5%, indicating that *bam* mutant GSCs are able to outcompete wild-type GSCs for their niches like *bgn* mutant GSCs (Figure 1J and 1K; Table 1). As a consequence, there was a dramatic net increase of the marked GSCs (from 61.6% at 14 days ACI to 72.3% at 21 days ACI) (Table 1). All these data indicate that *bam* mutant GSCs are much more superior to wild-type GSCs in niche competition. However, it remains unclear why mutant *bgn* cystoblasts cannot invade the GSC niche as efficiently as the *bam* mutant cystoblasts.

### ***bam* and *bgn* Mutant GSCs Outcompete their Neighboring Wild-Type GSCs, but not through Inducing Differentiation and Apoptosis**

In the *Drosophila* wing imaginal disc, fast growing cells induced by higher *dmec* expression can outcompete and eliminate slow growing neighbors by promoting their apoptosis (de la Cova et al., 2004). To investigate whether *bgn* and *bam* mutant GSCs outcompete wild-type GSCs by the same mechanism, we performed the TUNEL assay to detect if there were apoptotic GSCs in the germaria carrying both marked *bam* or *bgn* mutant and unmarked wild-type GSCs. Interestingly, in the germaria with both one wild-type GSC and one marked mutant *bgn*<sup>20093</sup> or *bgn*<sup>20915</sup> GSC (total 163 germaria examined), neither the wild-type (LacZ-positive) GSCs nor the marked *bgn* (LacZ-negative) mutant GSCs were apoptotic (Figure 2A). The same is also true for those germaria harboring both wild-type and *bam* mutant GSCs (total 120 germaria examined) (Figure 2B). In addition, differentiating *lacZ*-positive wild-type cysts were seen within the germaria, intermingling with the *bgn* and *bam* mutant cell mass. These observations indicate that it is unlikely that the loss of the wild-type GSCs neighboring the marked mutant *bam* or *bgn* GSCs is due to cell death.

To test whether the loss of unmarked wild-type GSCs is due to differentiation, we investigated the expression of *bam-GFP* in the unmarked wild-type GSCs sharing their niches with a marked *bam* or *bgn* mutant GSC. As a GSC starts to differentiate, it immediately upregulates expression of *bam-GFP* (Chen and McKearin, 2003b). After examining 48 germaria carrying an 8-day-old *bgn*<sup>20093</sup> or *bgn*<sup>20915</sup> mutant GSC clone, we failed to observe *bam-GFP* expression in any of the unmarked wild-type GSCs (Figure 2C). Likewise, we did not observe any *bam* expression in any unmarked wild-type GSCs in the 45 germaria carrying an 8-day-old marked *bam* mutant GSC clone (Fig. 2D). We also noticed that the unmarked wild-type GSCs that had already moved out of their niches did not express *bam-GFP*, suggesting that the unmarked wild-type GSCs are pushed out of their niches before they differentiate (Figure 2E and 2F). Consistent with this idea, we observed a trend of gradual loss of the wild-type GSC sharing the niche with a marked mutant *bam* or *bgn* GSC over time (Figure 2G–G’'). Therefore, our results indicate that *bam* or *bgn* mutant GSCs outcompete their wild-type neighboring GSCs simply by forcing them out.

### E-Cadherin is Required for *bam/bgn*-Mediated GSC Competition

*shotgun* (*shg*) encodes E-cadherin, which is essential for anchoring GSCs in the niche (Song et al., 2002). To investigate whether E-cadherin participates in *bgn*-mediated GSC competition, we generated marked *bgn* and *shg* double mutant GSCs using two different *shg* alleles, *shg*<sup>10469</sup> and *shg*<sup>R69</sup>, which represent a hypomorphic allele and a null allele, respectively. The observed frequency of the germaria carrying a *bgn*<sup>20915</sup> *shg*<sup>10469</sup> mutant full GSC clone was similar to that of the germaria carrying a *bgn*<sup>20915</sup> mutant full GSC clone, indicating that the weak *shg*<sup>10469</sup> mutation only had a slight effect on *bgn*-mediated GSC competition (Figure 3A and 3B; Table 1). In contrast, the frequency of the germaria carrying a *bgn*<sup>20093</sup> *shg*<sup>R69</sup> mutant full GSC clone was significantly lower than that of the germaria carrying a *bgn*<sup>20093</sup> mutant full GSC clone, but was comparable to that of the germaria carrying a wild-type full GSC clone, indicating that the null *shg* mutation almost completely abolishes the competitive advantage of the *bgn* mutant GSCs over the wild-type ones (Figure 3C and 3D; Table 1). These results demonstrate that E-cadherin is involved in the *bgn*-mediated GSC competition. As reported previously, over 95% of the marked *shg*<sup>R69</sup> mutant GSCs detected at one week ACI are lost at three weeks ACI (Song et al., 2002). It is worth noting that over 50% of the marked *bgn*<sup>20093</sup> *shg*<sup>R69</sup> double mutant GSCs detected at three days ACI still remained in the niche at three weeks ACI, indicating that preventing *shg*<sup>R69</sup> mutant GSCs from differentiation stabilizes their interactions with cap cells. Due to their location on different chromosomes, we could not investigate whether E-cadherin also takes part in *bam*-mediated GSC competition.

### The *bam/bgn* Pathway Negatively Regulates E-Cadherin Accumulation in the GSC-Niche Junction

It is possible that *bam* or *bgn* mutant GSCs express higher levels of E-cadherin in the stem cell-niche junction and thus have a higher affinity for cap cells (niche cells) and a more competitive advantage for niche occupancy. We used the three-dimensional reconstruction of confocal sections (see Materials and Methods) to quantify E-cadherin in the stem cell-niche junction and the contact area with cap cells in the germaria carrying a wild-type GSC and a marked *bam* or *bgn* mutant GSC. In the control germaria, the marked and unmarked



wild-type GSCs in the same niche had similar contact areas in the stem cell-niche junction ( $38.2 \pm 9.9 \mu\text{m}^2$  for the unmarked GSC and  $37.4 \pm 9.0 \mu\text{m}^2$  for the marked one;  $p=0.70$ ;  $n=19$ ), and also had similar amounts of E-cadherin accumulation in the junction with cap cells (the ratio of the unmarked GSC to the marked one = 1.09;  $p=0.38$ ;  $n=19$ ) (Figure 3E–E'''). In contrast, the marked *bam*<sup>86</sup> mutant GSC had a significantly larger contact area with cap cells than the unmarked wild-type ( $35.1 \pm 18.5 \mu\text{m}^2$  for the *bam* mutant GSC and  $23.1 \pm 12.5 \mu\text{m}^2$  for the wild-type one;  $p=0.001$ ;  $n=20$ ) and also had significantly more E-cadherin accumulation in the stem cell-niche junction than the unmarked wild-type in the same niche (the ratio of the *bam* mutant GSC to the wild-type = 2.31;  $p=0.002$ ;  $n=20$ ) (Figure 3F–F'''). Similarly, the marked *bgcn*<sup>20093</sup> mutant GSC had a significantly larger contact area with cap cells than the unmarked wild-type ( $44.2 \pm 12.9 \mu\text{m}^2$  for the *bgcn* mutant GSC and  $32.3 \pm 11.6 \mu\text{m}^2$  for the wild-type;  $p=0.0009$ ;  $n=15$ ) and also had significantly more E-cadherin accumulation in the stem cell-niche junction than the unmarked marked wild-type in the same niche (the ratio of the *bgcn* mutant GSC to the wild-type = 1.73;  $p=0.01$ ;  $n=15$ ) (Figure 3G–G'''). The observation that the *bam* and *bgcn* mutant GSCs have larger contact areas with cap cells than their wild-type counterparts in the same niches further supports the notion that the wild-type GSCs are gradually dislodged from the niche as a result of the invasion of the mutant GSCs. Therefore, our results strongly suggest that the increase of E-cadherin in the junction between mutant GSCs and cap cells is likely one of the primary determining factors for their stronger competitiveness.

The aforementioned findings also raise an interesting possibility that *bam/bgcn* might negatively regulate E-cadherin accumulation in the GSC. To test the possibility, we performed three-dimensional reconstruction to determine E-cadherin expression in the stem cell-niche junction 9 hours after *bam* overexpression using the *hs-bam* transgene, when the GSCs still remained in the niches and contained an anteriorly anchored spectrosome. Since the germaria carrying three stem cells could have a larger contact area and possibly more E-cadherin between the stem cells and cap cells (the niche) than the ones containing two stem cells, we focused our analyses only on the germaria carrying two stem cells. In the control experiments, the germaria from the control (*yw*) females (not carrying *hs-bam*) with heatshock treatments and the ones without heatshock treatments had similar E-cadherin accumulation in the stem cell-niche junction (the ratio = 1.00;  $p=0.49$ ;  $n=14$ ). In contrast, in the germaria of females carrying *hs-bam*, there was significantly less E-cadherin accumulation in the stem cell-niche junction after heatshock treatments, compared with those without heatshock treatments (the ratio = 0.68;  $p=0.001$ ;  $n=15$ ) (Figure 3H–3I'). This result demonstrates that *bam* upregulation in the GSC can sufficiently downregulate E-cadherin expression, which sheds light on why differentiated stem cells are detached from the niche. In conjunction with the fact that E-cadherin expression is increased in *bam* and *bgcn* mutant GSCs, this result leads us to conclude that *bam/bgcn* normally negatively regulate E-cadherin accumulation in the stem cell-niche junction.

### Different Expression Levels of E-Cadherin can Induce GSC Competition

Our earlier results also imply that the mutant GSCs defective in adhesion would have reduced their contact area with cap cells, and thus are gradually competed out by their wild-type neighbors. To directly test this, we quantified the contact areas of the marked

mutant *shg* GSC and the unmarked wild-type GSC in the mosaic germlaria, which only host a *shg* mutant GSC marked by loss of *arm-lacZ* expression and an unmarked wild-type one with the expression of *arm-lacZ*. As shown in our previous study, most marked *shg* mutant GSCs are lost two weeks after clone induction (Song et al., 2002). Thus, we only examined the one-week-old germlaria carrying a mutant *shg* GSC and a wild-type GSC. As predicted, the unmarked wild-type GSCs indeed had a larger contact area with cap cells ( $52.2 \pm 12.9 \mu\text{m}^2$ ) than their companion mutant *shg* GSCs ( $26.1 \pm 7.7 \mu\text{m}^2$ ) in the same niche ( $n=11$ ;  $p=0.0004$ ; Figure 4A and 4B), indicating that the mutant *shg* GSCs are indeed gradually competed out by their neighboring wild-type GSCs.

We also further tested whether different E-cadherin expression levels in the GSCs in the same niche are sufficient to determine their relative competitiveness for niche occupancy. To generate a marked GSC that expresses different levels of E-cadherin from its neighboring GSCs, we utilized a combination of the FLP-mediated FRT recombination and the binary UAS-Gal4 expression system. In these experiments, *Drosophila* females carry a *nanos (nos)-gal4VP16* located distal to FRT<sub>19A</sub> on one X chromosome and the *ubi-GFP* (a *ubiquitin* promoter driven GFP) on the other X chromosome distal to FRT<sub>19A</sub> (Figure 4C). The *nos-gal4VP16* was used to drive a *UASp-E-cad-FLAG* (a C-terminal FLAG-tagged E-cadherin controlled by a *UASp* promoter) to overexpress E-cadherin specifically in germ cells (Van Doren et al., 1998); (Pan et al., 2007), while *ubi-GFP* is ubiquitously expressed in all the cells of the germlarium. After clone induction, the marked GFP-negative GSCs would carry two copies of *nos-gal4VP16*, while the GFP-positive GSCs carry only one copy of *nos-gal4VP16*. In order to test if the GSCs carrying two copies of *nos-gal4* can indeed express more E-cadherin than the ones carrying only one copy of *nos-gal4*, we used the 3-D reconstruction to measure expression levels of E-cadherin in the GFP<sup>-</sup> marked GSCs and their neighboring GFP<sup>+</sup> unmarked GSCs. Indeed, the GFP-negative marked GSCs carrying two copies of *nos-gal4* had significantly higher levels of E-cadherin than the GFP-positive unmarked GSCs carrying only one copy of *nos-gal4* (the ratio of the former to the latter: 2.15;  $p=0.0001$ ;  $n=13$ ) (Figure 4D). Consequently, the GSCs expressing more E-cadherin ( $54.8 \pm 17.8 \mu\text{m}^2$ ) had larger contact areas with cap cells than the GSCs expressing less E-cadherin ( $37.6 \pm 9.8 \mu\text{m}^2$ ) ( $p=0.001$ ;  $n=13$ ; Figure 4E).

We then determined if the E-cadherin-overexpressing GSCs can expel their neighboring stem cells out of their niche. In this set of experiments, the initial clone induction rates at one week ACI for the control and E-cadherin overexpression were 16.3% and 20.0%, respectively, both of which were lower than earlier experiments. At three weeks ACI, the percentage of the germlaria carrying a marked full clone in the control was 4.1%, which was much lower than that of the germlaria carrying a partial clone (7.1%) (Table 1). In contrast, the germlaria carrying a GFP-negative marked full clone carrying two copies of *nos-gal4VP16* continued to increase from one week to three weeks ACI, and they became more prevalent than the germlaria carrying a partial clone three weeks ACI (11.8% of the germlaria carrying a full clone versus 5.6% of the germlaria carrying a partial clone) (Figure 4F and 4G; Table 1). This result indicates that the GSCs expressing more E-cadherin are consistently more competitive than their neighbors expressing less E-cadherin. Together, our loss-of-function and gain-of-function experiments demonstrate that different levels of E-cadherin expression in GSCs within the same niche can stimulate stem cell competition.

## Slow Proliferation of GSCs Compromises their Competitiveness

As reported above, *bam* GSCs are more competitive than *bgn* mutant GSCs and *bam* mutant tumors are larger than *bgn* mutant tumors, suggesting that the stronger competitiveness of *bam* mutant stem cells may result from their faster proliferation rate. We therefore used BrdU labeling to determine if the *bam* mutant GSCs indeed divide faster than *bgn* mutant GSCs. In the 320 germaria carrying a *lacZ*-negative marked *bam* mutant GSC and a *lacZ*-positive unmarked wild-type GSC, there were 23 of the *lacZ*-negative *bam* mutant GSCs that were BrdU positive, while only 11 of the *lacZ*-positive wild-type GSCs were labeled by BrdU (the  $\chi^2$  test:  $p=0.04$ ), indicating that *bam* mutant GSCs divide significantly faster than wild-type neighbors. In contrast, in the 258 germaria carrying a *lacZ*-negative marked *bgn* mutant GSC and a *lacZ*-positive unmarked wild-type GSC, 11 of the *lacZ*-negative *bgn* mutant GSCs and 15 of the *lacZ*-positive wild-type GSCs were positive for BrdU (the  $\chi^2$  test:  $p=0.433$ ), indicating that *bgn* mutant GSCs and wild-type neighbors divide at similar rates. These results suggest that *bam* mutant GSCs divide faster than *bgn* mutant GSCs.

Based on the finding that *bam* mutant GSCs divide faster and are more competitive than *bgn* mutant GSCs, we hypothesized that the rate of cell proliferation contributes to stem cell competitiveness. To test this hypothesis, we used the same strategy described earlier to generate marked GSCs mutant for both *bam* and *dE2F1*. *dE2F1* is known to be important for controlling cell cycle progression (Korenjak and Brehm, 2005), and two moderate *dE2F1* mutants, *dE2F1<sup>7172</sup>* and *dE2F1<sup>M729</sup>*, were used to disrupt *dE2F1* function (Asano et al., 1996). In comparison with the control, the marked GSCs mutant for *dE2F1<sup>7172</sup>* and *dE2F1<sup>M729</sup>* were maintained for three weeks just like or close to the wild-type GSCs, and the marked mutant *dE2F1*GSC clones detected at one week ACI could be frequently observed at three weeks ACI, indicating that these two *dE2F1* mutations do not dramatically affect stem cell maintenance (Table 2; Figure 5A and 5B). The relative division rate for a marked GSC is determined by the number of cysts generated by a marked mutant GSC divided by the number of cysts generated by a marked wild-type GSC (Xi and Xie, 2005); and the marked mutant *dE2F1<sup>7172</sup>* and *dE2F1<sup>M729</sup>* GSCs had relative division rates of 0.45 (17 mutant GSCs examined) and 0.47 (16 mutant GSCs examined), respectively. These results indicate that different division rates are not sufficient for inducing stem cell loss, and that the two *dE2F1* mutants are suitable for studying the effect of proliferation on stem cell competition. Interestingly, the marked GSC mutant for both *bam* and *dE2F1* became much less competitive than the marked GSCs only mutant for *bam*. For example, at three weeks ACI, only 22.5% and 23.5% of the germaria carried *bam<sup>86</sup> dE2F1<sup>7172</sup>* and *bam<sup>86</sup> dE2F1<sup>M729</sup>* full clones in comparison with 58.5% of the germaria carrying marked *bam<sup>86</sup>* GSC full clones (Tables 1 and 2); the germaria carrying a partial *bam dE2F1* mutant GSC clone were frequently observed (Figure 5C). These results indicate that different cell proliferation potentials contribute to, but do not sufficiently change, the relative competitiveness of stem cells for niche occupancy.

## The GSC Competition Does not Require BMP Signaling and dMyc Function

Since *bam* is proposed to negatively regulate BMP signaling (Casanueva and Ferguson, 2004), one explanation for the *bam/bgn*-mediated GSC competition is that BMP signaling



activities may be upregulated in the *bam* or *bgn* mutant GSCs. The *Dad-lacZ* line has been extensively used to monitor BMP signaling activities in different tissue types, including the ovary (Casanueva and Ferguson, 2004; Kai and Spradling, 2003; Song et al., 2004). Interestingly, the marked *bgn* mutant GSCs and their neighboring unmarked wild-type GSCs expressed *Dad-lacZ* at similar levels, indicating that BMP signaling activity does not change in *bgn* mutant GSCs (Figure 5D and 5E). To directly test whether blocking BMP signaling could compromise *bam*-mediated stem cell competition, we used weak (*punt*<sup>10460</sup>) and strong (*punt*<sup>135</sup>) mutations of *punt*, which encodes the type II receptor essential for BMP signaling (Letsou et al., 1995; Ruberte et al., 1995), to block BMP signaling in *bam* mutant GSCs. The marked double *punt*<sup>10460</sup> *bam*<sup>86</sup> or *punt*<sup>135</sup> *bam*<sup>86</sup> GSCs still retained a similar ability to those marked *bam* mutant GSCs to outcompete wild-type GSCs in the same niches based on the percentages of germaria carrying a marked GSC full clone 3 weeks ACI, indicating that BMP signaling is not required for *bam*-mediated GSC competition (Figure 5F–5I; Tables 1 and 2). Corroborating the *Dad-lacZ* expression result, these results demonstrate that BMP signaling is not involved in the *bam/bgn*-mediated GSC competition.

Since *dmyc* is capable of inducing cell competition in the *Drosophila* wing imaginal disc (de la Cova et al., 2004; Moreno and Basler, 2004), we sought to determine if *dmyc* is also involved in GSC competition by generating the marked GSCs homozygous for strong *dmyc* mutations, *dm*<sup>2</sup> and *dm*<sup>4</sup> (Maines et al., 2004; Pierce et al., 2004). Surprisingly, 69.7% and 73.5% of the marked *dm*<sup>2</sup> and *dm*<sup>4</sup> mutant GSCs detected at one week ACI were maintained at three weeks ACI, respectively, indicating that *dmyc* mutant GSCs are as stable as the marked controls (Table S1 and Figure S1A–1D). Furthermore, the marked *dm*<sup>2</sup> and *dm*<sup>4</sup> mutant GSCs had normal division rates, which are 1.0 (n=16) and 0.91 (n=39), respectively. To further investigate if overexpressing *dmyc* in the marked GSCs can strengthen their competitiveness, we used the same strategy for overexpressing E-cadherin to overexpress *dmyc* in marked GSCs. Interestingly, the percentages of the germaria carrying a GFP-negative marked GSC full clone, which carried two copies of *nos-gal4* and should express more *dmyc*, decreased over time indicating that *dmyc* overexpression does not make GSCs more competitive. Furthermore, the germaria carrying a marked GSC clone overexpressing *dmyc* appeared to be lost slightly faster than the controls (Figure S1E–1H). Together, our results show that *dmyc* is not essential for GSC competition.

## Discussion

In this study, we have shown that the differentiation-defective *bam* or *bgn* mutant GSCs can drive wild-type stem cells away from their niche in an E-cadherin-dependent manner in the *Drosophila* ovary (Figure 5J). Our genetic and cell biological analyses indicate that different levels of E-cadherin expression in GSCs determine their competency in occupying the niche and that the *bam/bgn* pathway controls E-cadherin protein expression levels in the GSC. Furthermore, we demonstrate that cell proliferation, but not BMP signaling or dMyc, modulates *bam/bgn*-mediated GSC competition. This study also offers new insight into how a differentiated GSC is forced out of its niche by its neighboring stem cells. Its departure from the niche is probably caused by the upregulation of *bam* and consequent downregulation of E-cadherin. Such competition may serve as a quality control mechanism to ensure that the niche is always occupied by functional stem cells. Finally, the knowledge

gained from studying stem cell competition may make it possible to deliver stem cells to diseased tissues by replacing nonfunctional stem cells in the future.

### ***bam/bgcn* Controls GSC Competition for Niche Occupancy by a Novel Mechanism**

*bam*, encoding a novel protein with no previously known functional domain, is primarily expressed in a fraction of cystoblasts and proliferating cysts (McKearin and Ohlstein, 1995; McKearin and Spradling, 1990), while *bgcn*, encoding a putative RNA binding protein, is expressed in GSCs, cystoblasts, and proliferating cysts (Ohlstein et al., 2000). Mutations in *bam* and *bgcn* lead to accumulation of cystoblast-like or GSC-like single germ cells, further supporting the view that their functions are restricted to cystoblasts and proliferating cysts. In this study, however, we have shown that both *bam* and *bgcn* are required in GSCs to control their competitiveness in niche occupancy. This study also suggests that natural GSC turnover might be caused by the fluctuation of *bam/bgcn* function in GSCs. The competition mechanism we have discovered in this study could account for stem cell quality control and efficient replacement of lost GSCs due to their upregulated *bam* expression. Although *bam* is transcriptionally repressed by BMP signaling, low levels of *bam* transcription in the GSC can still be detected using the *bam-GFP* transgenic line (Figure S2). It is, therefore, possible that low levels of *bam* transcription detected in GSCs may provide a function for controlling GSC competitiveness.

Although mutations in *bam* and *bgcn* generate similar germ cell tumor phenotypes, there are two obvious differences in their mutant phenotypes. One difference is that *bam* mutant ovaries have more single germ cells than *bgcn* mutant ovaries. Our results show that *bam* mutant GSCs are more mitotically active than *bgcn* mutants, suggesting that *bam* has a *bgcn*-independent function in controlling GSC and/or cystoblast proliferation rate. The second difference is that mutant *bam* GSCs are more competitive than mutant *bgcn* GSCs in niche occupancy. This could be due to their difference in mitotic activities since we have shown in this study that the competitiveness of GSCs is modulated by mitotic activity. Taken together, we conclude that *bam* can function in *bgcn*-dependent and *bgcn*-independent manners to control the competitiveness of GSCs.

In the *Drosophila* imaginal disc, *dmyc* mediates cell competition by inducing apoptosis in disadvantaged cells (expressing less *dmyc*). This competitive behavior correlates with, and can be corrected by, the activation of the BMP/Dpp survival signaling pathway (Moreno and Basler, 2004). As shown in this study, however, *dmyc* and BMP signaling are dispensable for GSC competition, suggesting that GSCs use a distinct mechanism to control their competition. Indeed, we have shown that *bam* or *bgcn* mutant stem cells do not promote apoptosis or differentiation of their wild-type counterparts in the same niche. Instead, *bam/bgcn* mutant GSCs push their wild-type counterparts out of the niche through their adhesive advantage, which is supported by our observation that the mutant GSCs gradually increase E-cadherin accumulation at the GSC/cap cell junction. In addition, unlike in the imaginal disc, slowly dividing *E2F1* mutant GSCs do not exhibit any obvious competitive disadvantages over wild-type neighbors. However, slow GSC division does compromise *bam/bgcn*-mediated GSC competition since we observed that mutant *bam dE2F1* GSCs are less competitive than *bam* mutant GSCs. The proliferation of the advantaged GSC may help

produce a physical force to push the disadvantaged GSC out of its niche and occupy the space of the doomed GSC. Therefore, our study has revealed a novel function of *bam/bgcn* in stem cell competition and a novel mechanism for cell competition.

### The *bam/bgcn* Pathway Controls GSC Competition by Regulating E-Cadherin Accumulation in the Stem Cell-Niche Junction

Our previous study showed that the mutant GSCs lacking E-cadherin are lost rapidly from their niche, but the underlying mechanism is not determined (Song et al., 2002). In this study, we provide an important insight into the mechanism by showing that the GSCs lacking E-cadherin lose their competition for the niche to their neighboring wild-type GSCs. This observation suggests that different levels of E-cadherin can induce competition among the GSCs in the same niche. Indeed, we have further shown that the GSCs expressing more E-cadherin become more competitive than the neighboring GSCs expressing less E-cadherin. Therefore, this study has demonstrated that different levels of E-cadherin in GSCs can sufficiently stimulate GSC competition for niche occupancy.

This study has also offered new mechanistic insights into why *bam/bgcn* mutant GSCs are more competitive than their neighboring wild-type GSCs. First, we show that *bam* and *bgcn* mutant GSCs accumulate more E-cadherin in the stem cell-niche junction than their neighboring wild-type counterparts. Second, *bam* overexpression is sufficient to downregulate E-cadherin accumulation in the junction. Together, these observations demonstrate that the *bam/bgcn* pathway is necessary and sufficient for controlling E-cadherin accumulation in the stem cell-niche junction. This *bam/bgcn*-mediated E-cadherin regulation may represent a quality control mechanism to ensure that a differentiated GSC triggered by a spontaneous mutation or abnormal upregulation of *bam* can be efficiently removed from the niche and then be replaced with a functional GSC generated by its neighboring GSC. Consistent with the notion that E-cadherin is involved in *bam/bgcn*-mediated cell competition, we showed that the removal of E-cadherin can abolish the competitive advantage of GSCs gained from *bgcn* mutations. However, it remains unclear why *bam/bgcn* mutant GSCs contain more E-cadherin in the stem cell-niche junction than their neighboring wild-type GSCs. Since *bam/bgcn* have been proposed to regulate translation (Ohlstein et al., 2000), it is possible that *bam/bgcn* normally control E-cadherin expression in GSCs by repressing its translation. Elucidating biochemical functions of Bam and Bgcn proteins will be essential for understanding how the *bam/bgcn* pathway controls GSC competitiveness.

## Materials and Methods

### Generation of marked GSC clones

To determine the roles of *bgcn* and *bam* in GSC competition, we used the FLP-mediated mitotic recombination technique to generate and analyze mutant GSC clones of the following genotypes: (1) *hs-FLP; FRT<sub>42D</sub>/FRT<sub>42D</sub> arm-lacZ*; (2) *hs-FLP; FRT<sub>42D</sub> bgcn<sup>20093</sup>/FRT<sub>42D</sub> arm-lacZ*; (3) *hs-FLP; FRT<sub>42D</sub> bgcn<sup>20915</sup>/FRT<sub>42D</sub> arm-lacZ*; (4) *hs-FLP; FRT<sub>82B</sub> bam<sup>86</sup>/FRT<sub>82B</sub> arm-lacZ*. To determine the potential role of E-cadherin or BMP signaling in regulating the *bgcn/bam*-mediated GSC competition, we performed analysis on double

mutant GSC clones of the following genotypes: (1) *hs-FLP; FRT<sub>42D</sub> bgn<sup>20093</sup> shg<sup>R69</sup>/FRT<sub>42D</sub> arm-lacZ*; (2) *hs-FLP; FRT<sub>42D</sub> bgn<sup>20915</sup> shg<sup>10469</sup>/FRT<sub>42D</sub> arm-lacZ*; (3) *hs-FLP; FRT<sub>82B</sub> bam<sup>86</sup> punt<sup>135</sup>/FRT<sub>82B</sub> arm-lacZ*; (4) *hs-FLP; FRT<sub>82B</sub> bam<sup>86</sup> punt<sup>10460</sup>/FRT<sub>82B</sub> arm-lacZ*.

To determine the role of GSC division in regulating the *bgn/bam*-mediated GSC competition, we performed analysis on *dE2F1* single or *dE2F1 bam* double mutant GSC clones of the following genotypes: (1) *hs-FLP; FRT<sub>82B</sub>/FRT<sub>82B</sub> arm-lacZ*; (2) *hs-FLP; FRT<sub>82B</sub> dE2F1<sup>7172</sup>/FRT<sub>82B</sub> arm-lacZ*; (3) *hs-FLP; FRT<sub>82B</sub> dE2F1<sup>rM729</sup>/FRT<sub>82B</sub> arm-lacZ*; (4) *hs-FLP; FRT<sub>82B</sub> bam<sup>86</sup> dE2F1<sup>7172</sup>/FRT<sub>82B</sub> arm-lacZ*; (5) *hs-FLP; FRT<sub>82B</sub> bam<sup>86</sup> dE2F1<sup>rM729</sup>/FRT<sub>82B</sub> arm-lacZ*. To determine if *dmyc* is involved in regulating GSC competition, we analyzed marked *dmyc* mutant GSC clones of the following genotypes: (1) *FRT<sub>19A</sub>/Ubi-GFP FRT<sub>19A</sub>; hs-FLP*; (2) *dmyc<sup>dm2</sup> FRT<sub>19A</sub>/Ubi-GFP FRT<sub>19A</sub>; hs-FLP*; (3) *dmyc<sup>dm4</sup> FRT<sub>19A</sub>/Ubi-GFP FRT<sub>19A</sub>; hs-FLP*.

To assay whether mutations in *bgn* or *bam* affect BMP signaling in GSCs, flies of the following genotypes were used: (1) *hs-FLP; FRT<sub>42D</sub> bgn<sup>20093</sup>/FRT<sub>42D</sub> ubi-GFP; Dad-lacZ*; (2) *hs-FLP; FRT<sub>42D</sub> bgn<sup>20915</sup>/FRT<sub>42D</sub> ubi-GFP; Dad-lacZ*. *bam-GFP*, which is under the control of the endogenous *bam* promoter (Chen and McKearin, 2003b), was used in this study to monitor *bam* expression in both *bam* and *bgn* mutant GSCs. To carry out this experiment, flies of the following genotypes were generated: (1) *hs-FLP; FRT<sub>42D</sub> bgn<sup>20093</sup>/FRT<sub>42D</sub> arm-lacZ; bam-GFP*; (2) *hs-FLP; FRT<sub>42D</sub> bgn<sup>20915</sup>/FRT<sub>42D</sub> arm-lacZ; bam-GFP*; (3) *hs-FLP; bam-GFP; FRT<sub>82B</sub> bam<sup>86</sup>/FRT<sub>82B</sub> arm-lacZ*.

To determine if E-cadherin and dMyc overexpression can induce GSC competition, we used the following genotypes: (1) *nos-gal4VP16 FRT<sub>19A</sub>/Ubi-GFP FRT<sub>19A</sub>; hs-FLP*; (2) *nos-gal4VP16 FRT<sub>19A</sub>/Ubi-GFP FRT<sub>19A</sub>; UAS-E-cadherin-Flag/hs-FLP*; (3) *nos-gal4VP16 FRT<sub>19A</sub>/Ubi-GFP FRT<sub>19A</sub>; UAS-dmyc/hs-FLP*.

Adult females, 3- to 5-days old, were heat-shocked twice daily at 37°C for 1 hour with a 7- to 8-hour interval, for three consecutive days. Unless otherwise specified, fly ovaries were removed at 3 days, 1 week, 2 weeks and 3 weeks after the last heat-shock, and were then fixed for immunostaining as previously described (Xie and Spradling, 1998). For BrdU labeling of one-week-old marked *bam* and *bgn* GSC clones, the ovaries from the females carrying appropriate genotypes for *bam* or *bgn* were processed one week after heatshock treatments, and BrdU detection was performed as described previously (Zhu and Xie, 2003).

## Immunostaining

The following antisera were used: monoclonal mouse anti-Hts antibody 1B1 (1:3, DSHB), monoclonal mouse anti-Arm N2 7A1 (1:3, DSHB), monoclonal rat anti-DE-Cadherin DCAD2 (1:3, DSHB), polyclonal rabbit anti-β-galactosidase antibody (1:300, Cappel), monoclonal mouse anti-β-galactosidase antibody (1:100, Promega), polyclonal rabbit anti-GFP antibody (1:200, Molecular Probes), rat anti-Vasa antibody (1:200, kindly provided by P. Lasko), Alexa 488 and Alexa 568-conjugated goat anti-mouse, anti-rabbit IgG and anti-rat (1:300, Molecular Probes), and Cy5-conjugated goat anti-mouse IgG (1:200, Jackson ImmunoResearch). The immunostaining protocol and the TUNEL assay using an ApopTag

kit from Chemion have been described previously (Kawase et al., 2004). All micrographs were taken using a Leica TCS SP2 confocal microscope.

### Measuring contact areas and E-cadherin between GSCs and cap cells

Using a Leica SP2 confocal microscope, z-stacks were taken along the Z axis (0.5  $\mu\text{m}$  each step) from those germaria that only bear one mutant and one control GSC or two GSCs in *bam* over-expression experiments. All images were acquired within the dynamic range of the detector at 12 bit depth. A contour plot for E-cadherin was generated with Imaris Bitplane (Saint Paul MN). The contour was drawn along the boundary of a single GSC (or two GSCs in *bam* over-expressing germaria) and cap cells on each focal plane. After 3-D reconstruction, a contour surface should represent the contact area between a single GSC (or two GSCs in *bam* over-expressing germaria) and cap cells. The intensity of E-cadherin staining on the contour surface was measured with Imaris Bitplane. All the *p* values were generated from the paired *t*-test using Microsoft Excel unless otherwise specified.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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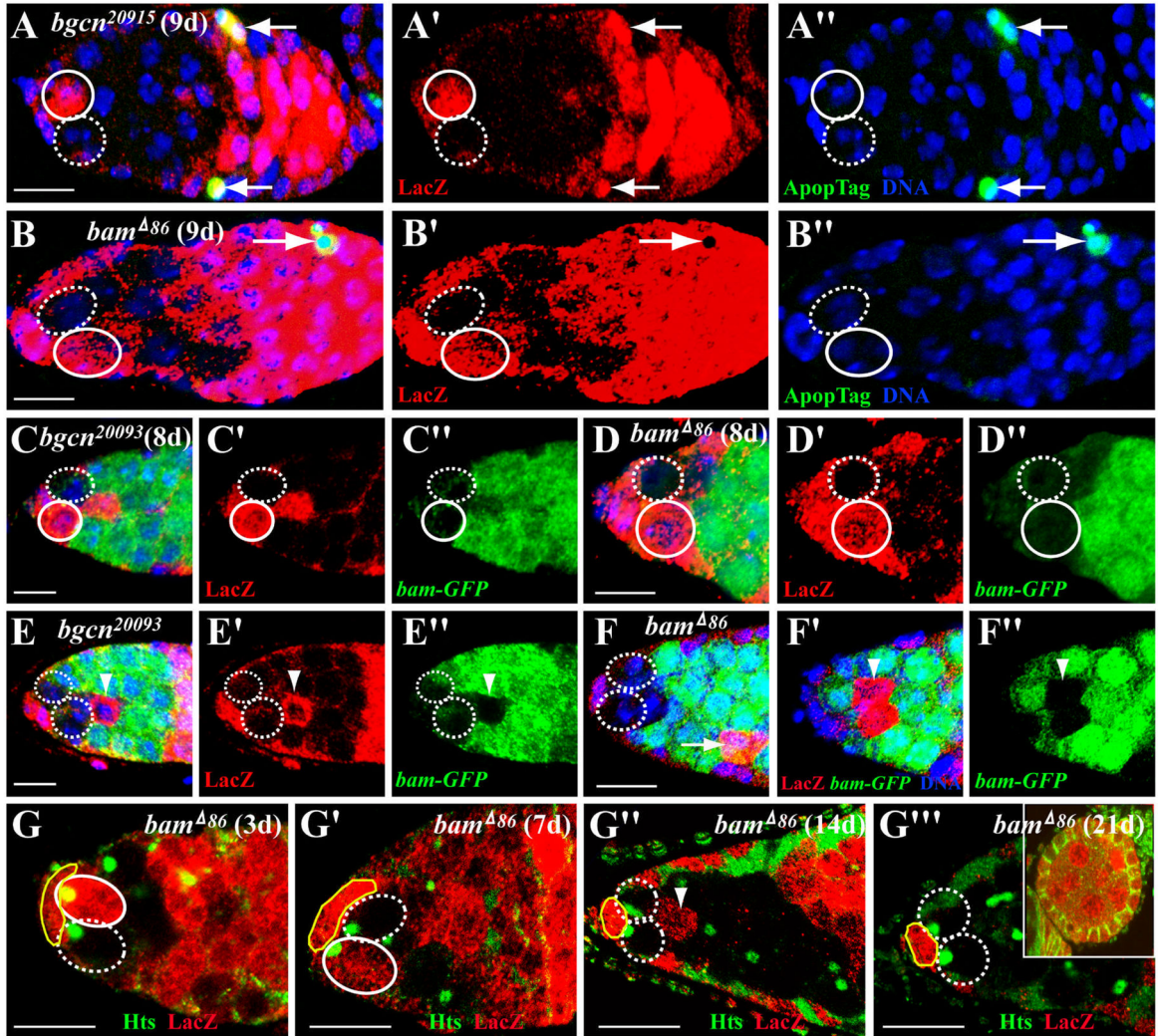


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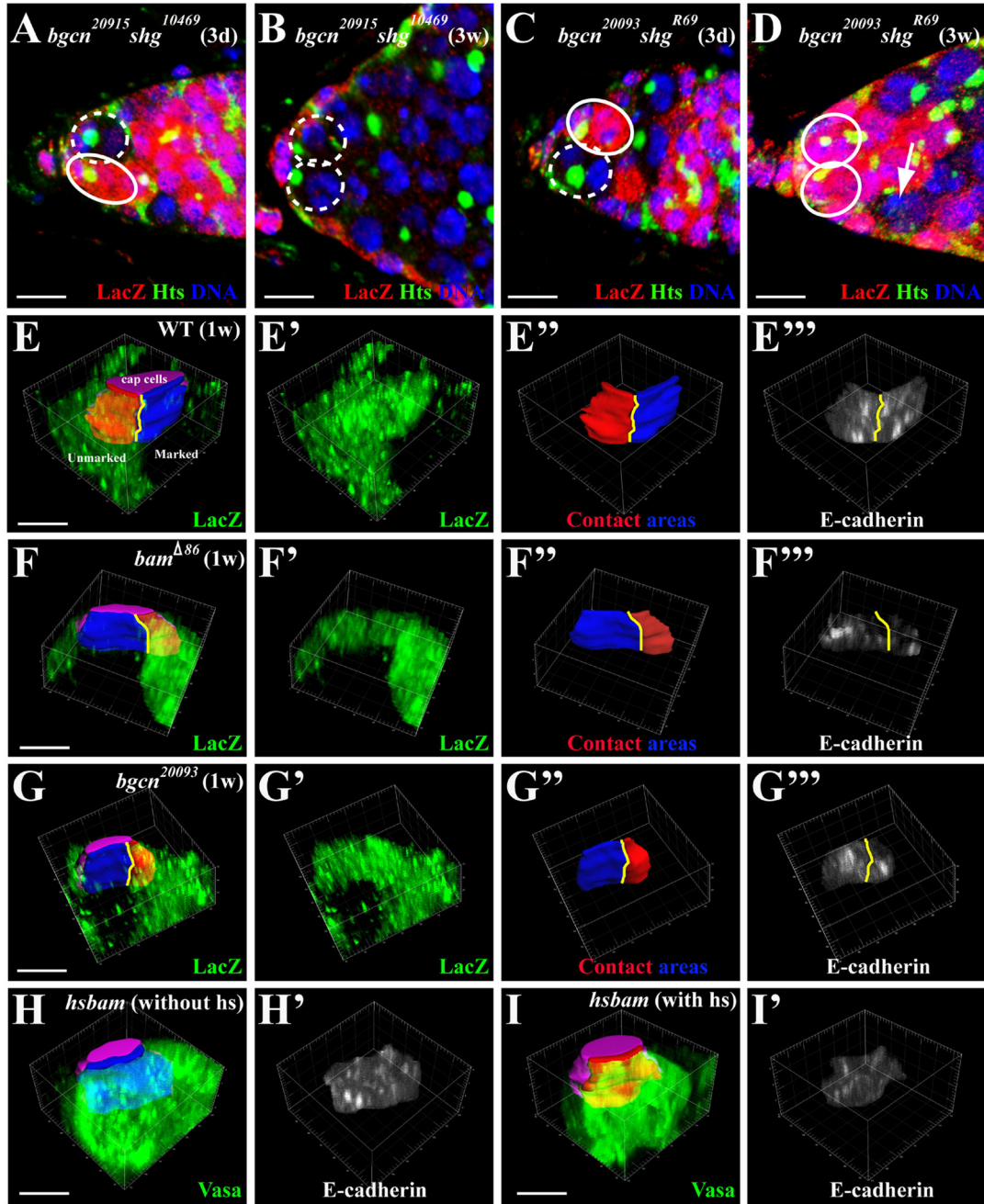


**Figure 2.**

*bgn* or *bam* mutant GSCs push their neighboring wild-type GSC out of its niche space. The LacZ-negative marked GSCs and the LacZ-positive unmarked GSCs are highlighted by broken and solid circles, respectively. (A, B) Germarial tips showing no apoptosis for the 9-day-old marked *bgn*<sup>20915</sup> (A–A'') or *bam*<sup>Δ86</sup> (B–B'') GSC and its neighboring unmarked GSC and the dying somatic cells in the midway of the germarium (arrows). (C, D) Germarial tips labeled showing no *bam-GFP* upregulation in the 8-day-old marked *bgn*<sup>20093</sup> (C–C'') or *bam*<sup>Δ86</sup> (D–D'') GSC and its neighboring unmarked GSC. (E–E'') A germarial tip showing that a recently displaced wild-type GSC (arrowhead) lying posteriorly to the two marked *bgn*<sup>20093</sup> GSCs still does not upregulate *bam-GFP*. (F–F'') A germarial tip labeled for LacZ (red, F'), *bam-GFP* (green, F'') and DNA (blue, F') showing that the recently evacuated wild-type still connected GSC daughters (arrowhead; F' and F'', on a different confocal section) lying posteriorly to the two marked *bam*<sup>Δ86</sup> GSCs still do not upregulate *bam-GFP*. A LacZ-positive differentiated germ cell cyst (arrow, F) is positive for *bam-GFP*. (G–G'') Different germarial tips showing a time course of forcing out a wild-type unmarked GSC by its marked *bam* mutant neighbor GSC. The three-day-old

*bam*<sup>86</sup> mutant GSC and its neighboring wild-type GSC in (**G**) have similar contact areas with cap cells (yellow lines), whereas the one-week-old *bam*<sup>86</sup> mutant GSC has a larger contact area with cap cells than its neighboring wild-type GSC as shown in (**G'**). In the germarium carrying a two-week-old GSC clone (**G''**), a wild type GSC (arrowhead) has just been displaced from the niche, resulting in a full clone with two mutant GSCs. In the germarium carrying a three-week-old GSC full clone (**G'''**), the displaced wild-type GSC has differentiated into a normal egg chamber (inset). The bars represent 10µm.

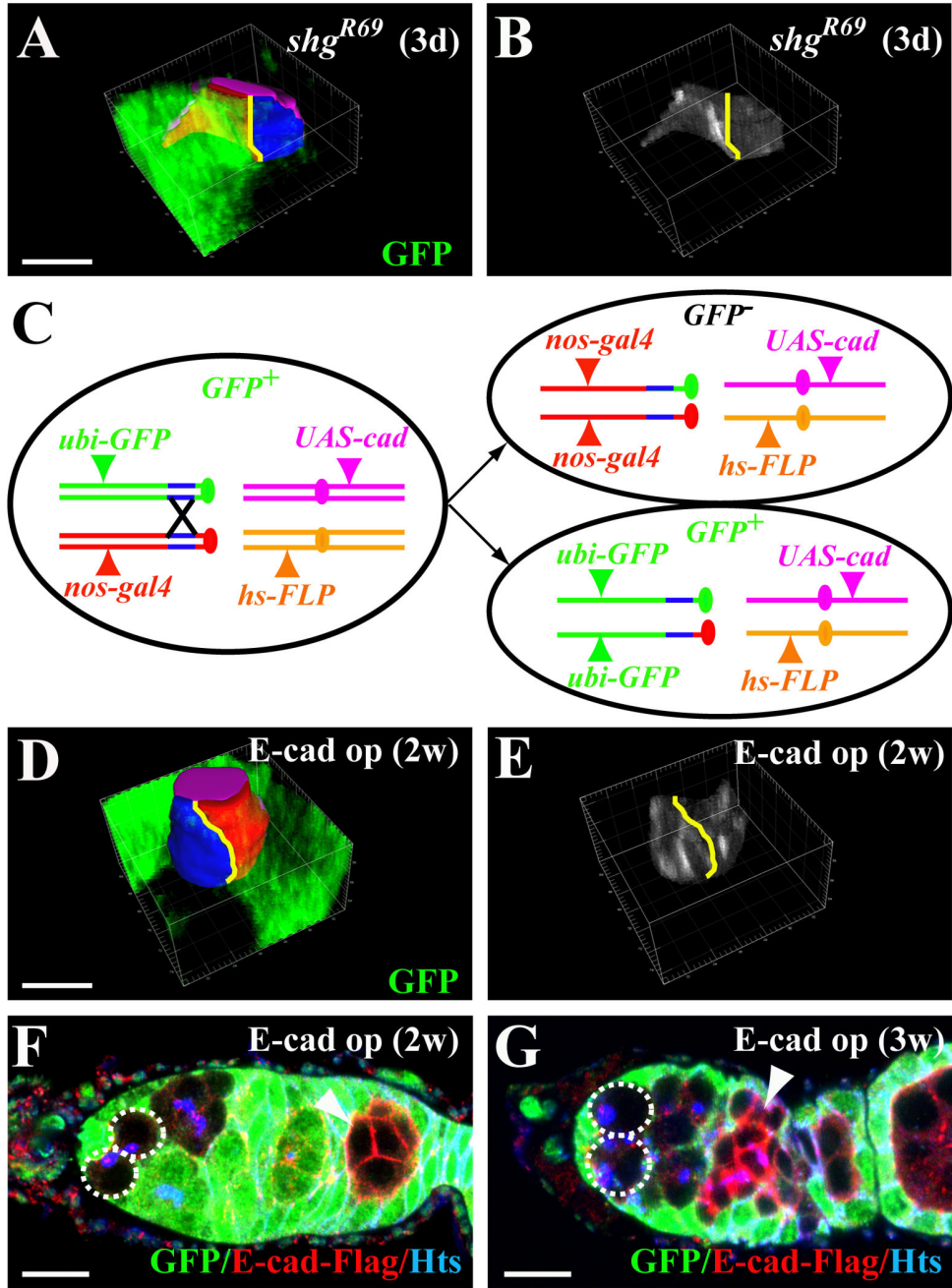




**Figure 3.**

The *bgcn/bam*-mediated GSC competition requires E-cadherin. In the germline tips (A–D), the marked GSCs and the unmarked GSCs are highlighted by broken and solid circles, respectively. (A, B) Germline tips harboring a three-day-old partial *bgcn<sup>20915</sup> shg<sup>10469</sup>* mutant GSC clone (A), and a three-week-old full mutant *bgcn<sup>20915</sup> shg<sup>10469</sup>* clone (B). (C, D) The germline tips showing a three-day-old partial *bgcn<sup>20093</sup> shg<sup>R69</sup>* mutant GSC clone (C), and a recently lost three-week-old partial *bgcn<sup>20093</sup> shg<sup>R69</sup>* clone (arrow, D). (E–G) 3-D projections of germline tips (pointing into the page) carrying control (E), *bam<sup>86</sup>* (F) and *bgcn<sup>20093</sup>* (G) partial GSC clones, showing that the *lacZ*-negative (black) marked GSC

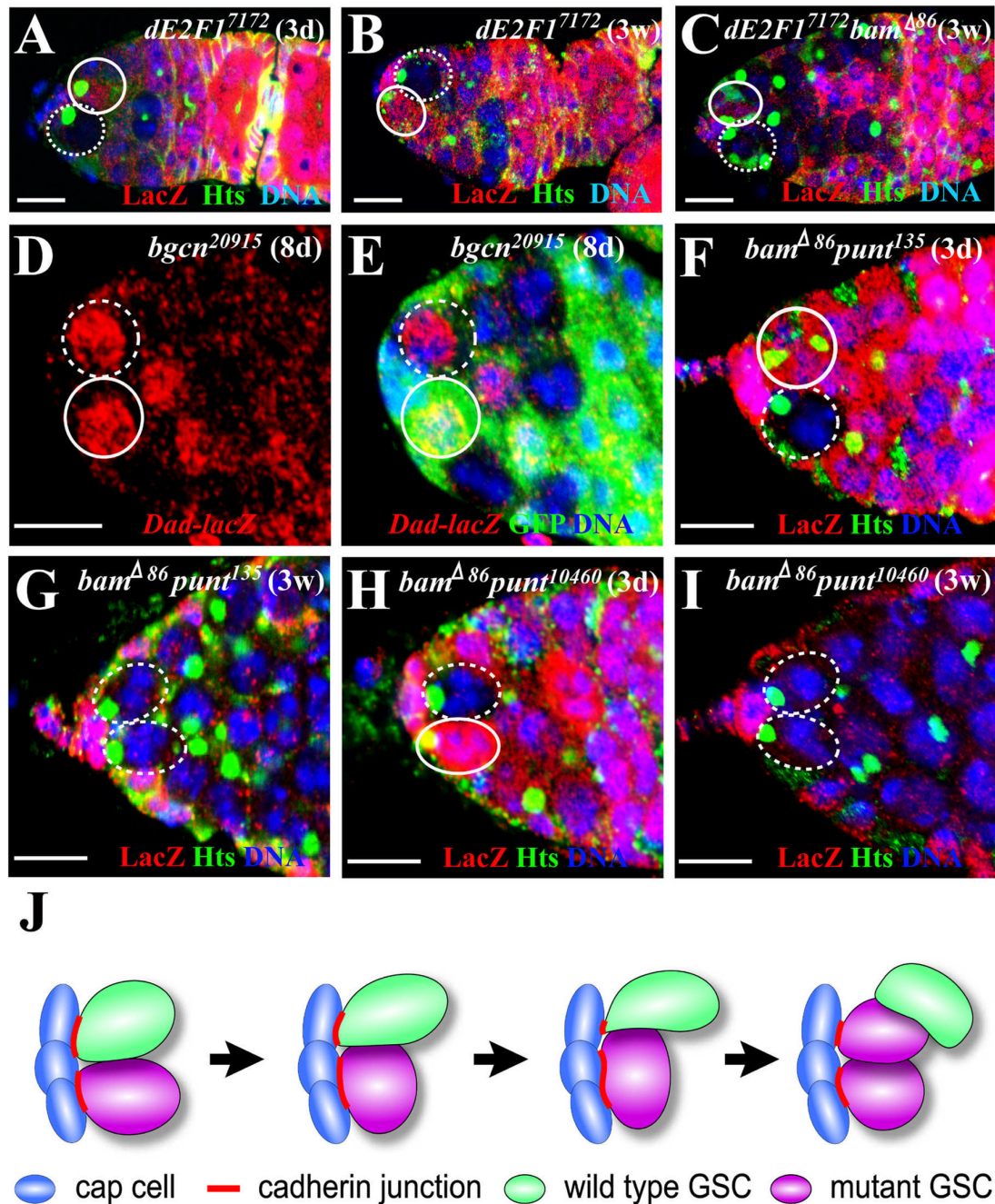
(blue-contact area with cap cells colored purple) and the *lacZ*-positive (green) unmarked GSC (red-contact area) are separated by yellow lines. **E'**–**G'** and **E''**–**G''** show LacZ staining and the contact areas with cap cells for the marked GSC (red) and the unmarked GSC (blue), respectively. **E'''**–**G'''** represent E-cadherin staining intensity in stem cell-niche junction. (**H**, **I**) The 3-D projections of a *hs-bam* germarial tip without (**H**) or with (**I**) heatshock treatments showing the stem cell-niche junction (**H**, blue; **I**, red) and E-cadherin accumulation in the junction (**H'** and **I'**) between cap cells (purple) and GSCs (green, Vasa). The bars in **A–D** and in **E–I** represent 10 $\mu$ m and 5  $\mu$ m, respectively.



**Figure 4.** Different levels of E-cadherin can induce GSC competition. (**A**, **B**) 3-D projections of a germarial tip showing that the *GFP*-negative (black) marked *shg* mutant GSC (blue-contact area, **A**) has a smaller contact area with cap cells and much less E-cadherin (**B**) in the stem cell-niche junction than the *GFP*-positive (green) unmarked wild-type GSC (red-contact area, **A**). Solid yellow lines indicate the boundary between the marked GSC and the unmarked GSC. (**C**) A diagram explaining the genetic strategy for overexpressing different levels of E-cadherin in a marked GSC (carrying two copies of *nos-gal4VP16*; *GFP*<sup>-</sup>) and

its neighboring unmarked GSC (carrying one copy of *nos-gal4VP16; GFP<sup>+</sup>*). (**D, E**) 3-D projections of a germarial tip showing that the *GFP*-negative (black) marked E-cadherin-overexpressing GSC (blue-contact area, **D**) has a larger contact area with cap cells and more E-cadherin in the stem cell-niche junction (**E**) than the *GFP*-positive (green) unmarked wild-type GSC (red-contact area, **D**). Solid yellow lines indicate the boundary between the marked GSC and the unmarked GSC. (**F, G**) Germaria carrying two-week-old (**F**) and three-week-old (**G**) marked full GSC clones, in which both the GSCs are indicated by broken lines. The bars in **A** and **D** and the ones in **F** and **G** represent 5 and 10  $\mu\text{m}$ , respectively.





**Figure 5.**

The *bam/bgn*-mediated GSC competition is affected by the ability to proliferate but not by BMP signaling. Marked mutant GSCs and unmarked wild-type GSCs are highlighted by broken and solid circles, respectively. (A, B) Germaria carrying a three-day-old (A) or three-week-old (B) partial *dE2F1*<sup>7172</sup> mutant GSC clone. (C) A germarium carrying a three-week-old partial *dE2F1*<sup>7172</sup> *bam*<sup>Δ86</sup> double mutant GSC clone. (D, E) A germarial tip showing that Dad expression does not change in an 8-day-old GFP-negative marked *bgn*<sup>20915</sup> GSC in comparison with its neighboring GFP-positive unmarked wild-type GSC.



(F, G) Germarial tips showing a marked *punt<sup>135</sup> bam<sup>86</sup>* partial clone (F) and a marked *punt<sup>135</sup> bam<sup>86</sup>* full clone (G). (H, I) Germarial tips showing a marked *punt<sup>10460</sup> bam<sup>86</sup>* partial clone (H) and a marked *punt<sup>10460</sup> bam<sup>86</sup>* full clone (I). The bars represent 10 $\mu$ m. (J) A working model explaining *bam/bgcn*-mediated GSC competition. At the beginning, the newly generated marked *bgcn* or *bam* mutant GSC (purple) has a similar contact area (similar amount E-cadherin accumulation, red) with cap cells (blue) to that of the wild-type GSC (green). Over time, the mutant GSC expands its interface with cap cells, and consequently more E-cadherin accumulates in the interface between the mutant GSC and cap cells. Eventually, the mutant GSC divides to generate two daughters that both contact cap cells and push out the wild-type GSC.

**Table 1**

Competition between *bgc1* or *bam* mutant GSCs and their neighboring wild-type GSCs in the same niche requires E-cadherin-mediated cell adhesion.

Genotypes	Ages	Percentage <sup>a</sup> of the germlia carrying a marked full GSC clone	Percentage <sup>b</sup> of the germlia carrying a marked partial GSC clone	Percentage <sup>c</sup> of the germlia carrying a marked GSC clone	Percentage <sup>d</sup> of the GSCs marked	Number of the germlia examined
Control ( <i>FRT<sub>42D</sub></i> )	3 d	1.8%	39.8%	41.6%	22.3%	113
	1w	7.6%	33.8%	41.4%	23.6%	225
	2w	5.0%	29.0%	34.0%	19.5%	221
	3w	9.4%	21.7%	31.1%	20.2%	212
<i>bgc1<sup>2003</sup></i>	3 d	6.6%	38.3%	44.9%	25.1%	183
	1w	13.1%	24.9%	38.0%	26.3%	221
	2w	27.6%	20.8%	48.4%	37.6%	221
	3w	32.4%	10.3%	42.7%	37.5%	213
<i>bgc1<sup>2015</sup></i>	3 d	6.9%	38.9%	45.8%	26.0%	175
	1w	12.4%	37.6%	50.0%	33.6%	226
	2w	19.8%	25.6%	45.4%	33.3%	227
	3w	27.1%	13.8%	40.9%	34.9%	225
<i>bam<sup>86</sup></i>	3 d	2.2%	41.9%	44.1%	20.8%	143
	1w	10.0%	58.1%	68.1%	36.4%	171
	2w	32.4%	53.7%	86.1%	61.6%	205
	3w	58.5%	26.8%	85.3%	72.3%	231
<i>bgc1<sup>2003</sup>;Shg<sup>R609</sup></i>	3 d	6.5%	20.4%	26.9%	15.5%	186
	1w	2.3%	12.3%	14.6%	9.4%	130
	2w	9.8%	5.8%	15.6%	12.6%	173
	3w	11.1%	4.3%	15.5%	13.7%	207
<i>bgc1<sup>2015</sup>;Shg<sup>R609</sup></i>	3 d	1.9%	36.7%	38.6%	20.0%	215
	1w	12.1%	35.9%	48.0%	31.1%	231
	2w	19.8%	19.4%	39.2%	29.7%	217
	3w	16.1%	16.1%	32.2%	24.5%	218
Control ( <i>FRT<sub>19A nos-gal4</sub></i> )	1w	2.9%	13.3%	16.3%	8.4%	173

Genotypes	Ages	Percentage <sup>a</sup> of the germaria carrying a marked full GSC clone	Percentage <sup>b</sup> of the germaria carrying a marked partial GSC clone	Percentage <sup>c</sup> of the germaria carrying a marked GSC clone	Percentage <sup>d</sup> of the GSCs marked	Number of the germaria examined
	2w	4.3%	9.8%	14.1%	7.6%	163
	3w	4.1%	7.1%	11.2%	7.4%	169
<i>FRT<sup>19A</sup>nos-gal4; UAS-shg</i>	1w	6.1%	13.9%	20.0%	12.3%	165
	2w	10.4%	10.4%	20.8%	15.2%	182
	3w	11.8%	5.6%	17.4%	14.2%	195

<sup>a</sup>The percentage of the germaria carrying a marked full GSC clone at a given time point = the number of germaria in which all GSCs are marked/total germaria examined.

<sup>b</sup>The percentage of germaria carrying a partial marked GSC clone at a given time point = the number of germaria in which at least one GSC is not marked and also at least one GSC is marked/total germaria examined.

<sup>c</sup>The percentage of germaria carrying a marked GSC clone at a given time point = the number of germaria carrying a marked full or partial GSC clone/total germaria examined.

<sup>d</sup>The percentage of the GSCs marked = the number of marked GSCs/total GSCs examined.

Table 2

Compromised stem cell proliferation but not BMP signaling weakens its competition capacity.

Genotypes	Ages	Percentage <sup>d</sup> of the germaria carrying a marked full GSC clone	Percentage <sup>b</sup> of the germaria carrying a marked partial GSC clone	Percentage <sup>c</sup> of the germaria carrying a marked GSC clone	Number of the germaria examined
Control ( <i>FRT<sub>82B</sub></i> )	1w	1.6%	31.3%	33.0%	182
	2w	3.2%	23.9%	27.1%	218
	3w	4.3%	22.2%	26.5%	185
<i>dE2F1<sup>7/172</sup></i>	1w	4.6%	38.3%	32.2%	174
	2w	6.0%	27.6%	27.7%	184
	3w	10.0%	21.7%	24.2%	190
<i>dE2F1<sup>7/172</sup>bam<sup>86</sup></i>	1w	11.4%	38.9%	50.3%	167
	2w	23.2%	31.9%	55.1%	185
	3w	30.6%	31.8%	62.4%	173
<i>dE2F1<sup>ΔM729</sup></i>	1w	4.6%	27.6%	33.3%	171
	2w	4.8%	20.2%	25.0%	168
	3w	5.5%	10.9%	16.4%	183
<i>dE2F1<sup>ΔM729</sup>bam<sup>86</sup></i>	1w	9.1%	46.3%	55.4%	175
	2w	19.7%	41.0%	60.7%	173
	3w	28.7%	38.9%	67.7%	167
<i>bam<sup>86</sup>punt<sup>10460</sup></i>	3 d	0.9%	50.2%	51.1%	209
	1w	10.5%	65.0%	75.5%	161
	2w	32.2%	50.7%	83.9%	217
<i>bam<sup>86</sup>punt<sup>135</sup></i>	3w	60.2%	28.0%	88.2%	211
	3d	0.6%	37.6%	38.2%	157
	1w	6.3%	58.0%	64.3%	207
	2w	24.2%	38.9%	63.1%	198
	3w	54.6%	28.2%	82.8%	227

<sup>a</sup>The percentage of the germaria carrying a marked full GSC clone at a given time point = the number of germaria in which all GSCs are marked/total germaria examined.

<sup>b</sup>The percentage of germaria carrying a partial marked GSC clone at a given time point = the number of germaria in which at least one GSC is not marked and also at least one GSC is marked/total germaria examined.

The percentage of germlaria carrying a marked GSC clone at a given time point = the number of germlaria carrying a marked full or partial GSC clone/total germlaria examined.

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