Tip60 complex promotes expression of a differentiation factor to regulate germline differentiation in female *Drosophila*

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ABSTRACT **Germline stem cells (GSCs) self-renew and differentiate to sustain a continuous production of gametes. In the female *Drosophila* germ line, two differentiation factors, *bag of marbles* (*bam*) and *benign gonial cell neoplasm* (*bgcn*), work in concert in the stem cell daughter to promote the generation of eggs. In GSCs, *bam* transcription is repressed by signaling from the niche and is activated in stem cell daughters. In contrast, *bgcn* is transcribed in both the GSCs and stem cell daughters, but little is known about how *bgcn* is transcriptionally modulated. Here we find that the conserved protein Nipped-A acts through the Tat interactive protein 60-kDa (Tip60) histone acetyl transferase complex in the germ line to promote GSC daughter differentiation. We find that *Nipped-A* is required for efficient exit from the gap phase 2 (G2) of cell cycle of the GSC daughter and for expression of a differentiation factor, *bgcn*. Loss of *Nipped-A* results in accumulation of GSC daughters. Forced expression of *bgcn* in *Nipped-A* germline-depleted ovaries rescues this differentiation defect. Together, our results indicate that Tip60 complex coordinates cell cycle progression and expression of *bgcn* to help drive GSC daughters toward a differentiation program.**

Monitoring Editor
Denise Montell
University of California, Santa Barbara

Received: Jun 22, 2018
Revised: Sep 6, 2018
Accepted: Sep 13, 2018

INTRODUCTION

Germ line gives rise to sperm and eggs that function as links between generations by passing information from parent to offspring. In the adult gonads, germ cells can acquire germline stem cell (GSC) fate that allows them to both self-renew and differentiate to ensure a steady supply of gametes. Failure to regulate GSC self-renewal and differentiation programs leads to infertility. Thus, understanding how GSCs divide and differentiate is critical to understanding the biological basis of reproductive success (Spradling *et al.*, 1997, 2008, 2011; Boyle *et al.*, 2007; Morrison and Spradling, 2008; Lehmann, 2012).

Drosophila female GSCs are an excellent model system to study stem cell dynamics due to precise characterization of early events in GSC differentiation and availability of mutants and markers (Dansereau and Lasko, 2008; Spradling *et al.*, 2011; Flora *et al.*, 2017). *Drosophila* female GSCs are housed in a structure called the germarium (Figure 1A). The germarium consists of both germ line and somatic cells. The somatic cells constitute the niche for the GSCs that divide asymmetrically, giving rise to self-renewed GSCs and daughters called precystoblasts (pre-CBs) (Chen and McKearin, 2003a). Both GSCs and their daughters are marked by endoplasmic reticulum (ER)-rich structures called spectrosomes (de Cuevas *et al.*, 1996). The pre-CB will then differentiate to become a CB by expressing the differentiation factor Bag of marbles (Bam) that acts in concert with Benign gonial cell neoplasm (Bgcn) (McKearin and Spradling, 1990; Ohlstein and McKearin, 1997). The CB then undergoes four incomplete divisions and gives rise to cysts, marked by branched structures called fusomes (de Cuevas and Spradling, 1998). Following the fourth division, a 16-cell cyst is made, where one cell will develop into the oocyte and the other 15 will become support cells called nurse cells. The 16-cell cyst

This article was published online ahead of print in MBoC in Press (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E18-06-0385) on September 19, 2018.

Author contributions: A.M., E.T.M., and P.R. designed experiments, analyzed and interpreted data. A.M., A.D., and M.U. performed experiments. A.M. and P.R. wrote the manuscript, which all authors edited and approved.

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Abbreviations used: ac, acetylation; Act87E, Actin 87E; Bam, bag of marbles; Bgcn, benign gonial cell neoplasm; CB, cystoblast; chk1, grapes; chk2, loki; CycA, Cyclin A; CycB, Cyclin B; DDR, DNA damage response; DN, dominant negative; Dpp, Decapentaplegic; DSB, double-stranded break; ER, endoplasmic reticulum; FC, fold change; FDR, false discovery rate; G2, gap phase 2; GNAT, Gcn5 (general control of nuclear-5)-related N-acetyltransferases; GO, Gene Ontology; GSC, germline stem cell; H2Av, histone H2A variant; HAT, histone acetyltransferase; hs, heat shock; MYST, MOZ, Ybf2/Sas3, Sas2, and Tip60; nos, nanos; Pgc, polar granule component; pH3, phospho-histone 3; pH2Av, phosphorylated H2Av; pMAD, Mothers-against-Dpp; rept, reptin; RNAseq, RNA sequencing; SAGA, Spt-Ada-Gcn5-acetyltransferase; Sax, Saxophone; Tip60, Tat interactive protein 60-kDa; tj, traffic jam; TKV, Thick veins; YTHDC2, YTH Domain Containing 2.

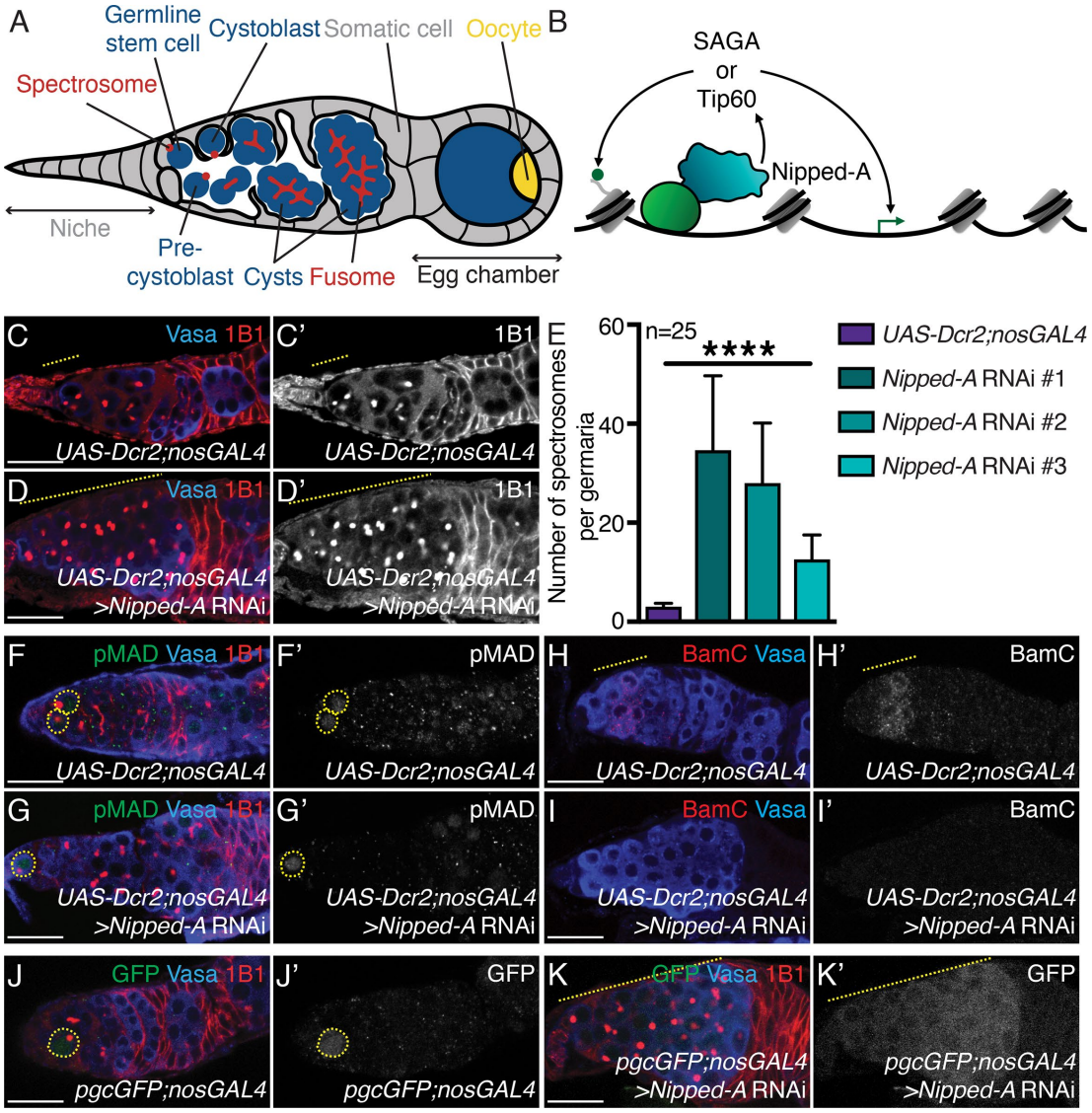


FIGURE 1: Nipped-A is required in the germ line for differentiation of the germline stem cell daughter. (A) Schematic representation of a *Drosophila* germarium where germ cells (blue) are surrounded by somatic cells (gray). The germline stem cells (GSCs) reside in the germarium and are maintained by a somatic niche. The GSC divides to give rise to a daughter, called a precystoblast (pre-CB). The pre-CB turns on differentiation factors and is then called the cystoblast (CB). The CB undergoes incomplete mitotic divisions to give rise to 2-, 4-, 8-, and 16-cell cysts. Single cells are marked by punctate structures called spectrosomes (red), and cysts are marked by the elongated, branched spectrosomes called fusomes (red). The 16-cell cyst migrates, buds off from the germarium, and is encapsulated by the soma (gray) to generate egg chambers. Developing egg chambers will have one germ cell that becomes the oocyte (yellow), and the other germ cells will be support cells (blue). (B) Schematic of Nipped-A function. Nipped-A (teal) can associate with transcriptional activators (light green) to recruit SAGA and Tip60 complexes. These complexes can acetylate lysines on histones (dark green circle) to regulate transcription (dark green arrow). Nipped-A cartoon is based on the Cryo-EM structure of Tra1 in SAGA complex in *Pichia pastoris* yeast (Sharov *et al.*, 2017). (C, C′) Control and (D, D′) germline-depleted *Nipped-A* (RNAi line #1) germaria stained with Vasa (blue) and 1B1 (red). Germaria depleted of *Nipped-A* show accumulation of single cells (yellow dashed line). 1B1 channel is shown in C′ and D′. (E) Quantitation of the number of single cells in germaria of control and germline-depleted *Nipped-A* using three RNAi lines (34.64 ± 15.04 in *Nipped-A* RNAi #1, 27.96 ± 12.17 in *Nipped-A* RNAi #2, and 12.56 ± 4.94 in *Nipped-A* RNAi #3 compared with 3.04 ± 0.68 in *UAS-Dcr2;nosGAL4* control; $n = 25$ for all, ****$P < 0.0001$). (F, F′) Control and (G, G′) germline-depleted *Nipped-A* germaria stained with pMAD (green), Vasa (blue), and 1B1 (red). Germaria depleted of *Nipped-A* do not accumulate pMAD-positive germ cells (yellow dashed circle) ($n = 20$ for both, $P < 0.0001$). pMAD channel is shown in F′ and G′. (H, H′) Control and (I, I′) germline-depleted *Nipped-A* germaria stained with BamC (red) and Vasa (blue). Germaria depleted of *Nipped-A* do not accumulate BamC-positive germ cells (yellow dashed line in control) ($n = 25$ for both). BamC channel is shown in H′ and I′. (J, J′) Control and (K, K′) *pgcGFP* with *Nipped-A* germline-depletion germaria stained with GFP (green), Vasa (blue), and 1B1 (red). Germaria depleted of *Nipped-A* accumulate a higher number of Pgc-positive germ cells ($n = 25$ for both, $P < 0.0001$). Pgc expression is marked by GFP (yellow dashed circle/line in control and knockdown, respectively). GFP channel is shown in J′ and K′. Statistical analysis performed with Student's *t* test for all except for Chi-square for H–I′. Scale bar for all images is 20 μm.

migrates, buds off from the germarium to form egg chambers, and eventually generates mature eggs (Huynh and St Johnston, 2000; Narbonne-Reveau *et al*., 2006).

GSC self-renewal and differentiation is exquisitely balanced by both extrinsic and intrinsic factors (Xie and Spradling, 2000; Pan *et al*., 2007; Slaidina and Lehmann, 2014; Upadhyay *et al*., 2016; Flora *et al*., 2017). One such extrinsic factor is the signaling from the somatic niche, which promotes GSC self-renewal. GSCs remain in contact with the somatic niche and receive Decapentaplegic (Dpp) signaling, while the pre-CB will be displaced from the niche after asymmetric division (Xie and Spradling, 1998, 2000; Kai and Spradling, 2003; Casanueva, 2004). The Dpp ligand binds to Thick veins (TKV) and Saxophone (Sax) receptors on GSCs, leading to the phosphorylation of Mothers-against-Dpp (pMAD) (Brummel *et al*., 1994; Penton *et al*., 1994; Xie *et al*., 1994; Letsou *et al*., 1995; Sekelsky *et al*., 1995). pMAD translocates to the nucleus to directly suppress the expression of the differentiation factor, *bam*, to promote GSC maintenance (Kai and Spradling, 2003; Chen and McKearin, 2003a,b). Once the pre-CB is displaced from the niche it is removed away from the source of Dpp signaling and is now able to express *bam* (Chen and McKearin, 2003b; Xia *et al*., 2010). Additionally, transient transcriptional silencing in the pre-CB helps reprogram the cell cycle of the pre-CB to promote the expression of Bam (Flora *et al*., 2018). TKV receptors are turned over, and Bam complexes with Bgcn, which is expressed from GSC stage onwards, to down-regulate GSC maintenance factors (Li *et al*., 2009; Kim *et al*., 2010a). Like Bgcn in *Drosophila*, its homologue in mice, YTH Domain Containing 2 (YTHDC2) is required for a meiotic cell fate and gamete production (Bailey *et al*., 2017; Soh *et al*., 2017; Jain *et al*., 2018). However, it is not yet known what regulates *bgcn* expression in the GSCs and pre-CBs.

Extensive remodeling of the GSC epigenome occurs during differentiation (Chen *et al*., 2008; Rangan *et al*., 2011; Flora *et al*., 2017). While several repressive mechanisms have been identified in GSCs, that down-regulate the differentiation program, little is known about how differentiation-promoting genes are positively regulated. One class of enzymes that can activate transcription is histone acetyltransferases (HATs). There are five classes of HATs, two of which are general control of nuclear-5–(Gcn5)-related *N*-acetyltransferases (GNATs) and "MOZ, Ybf2/Sas3, Sas2, and Tip60" (MYST)-related HATs (Roth *et al*., 2001; Carrozza *et al*., 2003; Lee and Workman, 2007). GNATs and MYST HATs are members of Spt-Ada-Gcn5-acetyltransferase (SAGA) and Tat interactive protein 60-kDa (Tip60) complexes, respectively (Grant *et al*., 1998; Saleh *et al*., 1998; Allard *et al*., 1999). Nipped-A is a conserved member of both Tip60 and SAGA complexes (Grant *et al*., 1998; Allard *et al*., 1999; Ikura *et al*., 2000; Kusch *et al*., 2004). In yeast and humans, Nipped-A interacts with transcriptional activators to recruit Tip60 and SAGA complexes (Grant *et al*., 1998; Brown *et al*., 2001; Carrozza *et al*., 2003) (Figure 1B). Thus, Nipped-A plays an important role in targeting those HAT-containing complexes to promoters (Vassilev *et al*., 1998; Allard *et al*., 1999). SAGA complex's HAT module includes Gcn5 that in *Drosophila* is required for oogenesis but does not seem to affect the earliest stages of GSC daughter differentiation (Li *et al*., 2017). And while Tip60 complex HAT activity has been shown to regulate GSC maintenance and differentiation in *Drosophila* testis (Feng *et al*., 2017, 2018), its role in promoting female germline differentiation both intrinsically and extrinsically is not known.

Tip60 complex acetylates (ac) lysines (K) 5, 8, 12, and 16 on histone H4, among other targets, to maintain cellular homeostasis (Steunou *et al*., 2013; Jacquet *et al*., 2016). By acetylating histones and transcription factors, Tip60 complex regulates expression of proto-oncogenes, cell cycle regulators, and stress responses (Patel *et al*., 2004; Avvakumov and Côté, 2007a,b; Ikeda *et al*., 2017) (Figure 1B). Furthermore, human Tip60 complex has been implicated in several diseases, most notably cancer (Avvakumov and Côté, 2007b; Voss and Thomas, 2009). But how Tip60 complex regulates normal development of stem cells and how this can become misregulated to lead to cancer has not been well characterized.

Here we have shown that Nipped-A is a crucial regulator of germline differentiation in female *Drosophila*. We find that Nipped-A, in conjunction with Tip60 complex members, is required for oogenesis. We report that Tip60 HAT activity is required for proper differentiation and loss of Tip60 complex members or its HAT activity leads to pre-CB accumulation and failure to generate mature eggs. We find Nipped-A to be required for expression of *bgcn*, a crucial component for differentiation. We propose that Tip60 complex coordinates cell cycle progression and expression of differentiation factors to drive germ cells into a differentiated cell fate.

RESULTS

Nipped-A is required in the germ line for proper germline stem cell daughter differentiation

To identify critical regulators of germline differentiation, we depleted epigenetic modifiers in the germ line utilizing a germline driver, *nanosGAL4* (*nosGAL4*) and RNA interference (RNAi), then assayed for defects by staining for Vasa and 1B1 (Navarro-Costa, McCarthy, *et al*., 2016). Vasa marks the germ line and 1B1 marks specrosomes, fusomes, and somatic cell membranes (Figure 1A). We found depletion of *Nipped-A* in the germ line, leads to an accumulation of single germ cells with spectrosomes compared with control (Figure 1, C–E). Furthermore, we used two other RNAi lines targeting *Nipped-A*, and similarly observed accumulation of single cells on depletion (Figure 1E; Supplemental Figure 1, A–C′). To validate the RNAi line, we made use of a GFP-trap inserted into the *Nipped-A* locus (*Nipped-A*^{MI10513}) and observed GFP intensity in control and *Nipped-A*–depleted germaria. We found *Nipped-A*–depleted germ cells had diminished levels of GFP intensity compared with control germaria (Supplemental Figure 1, D–E′). To determine whether *Nipped-A* has a role in somatic cells, we drove *Nipped-A* RNAi in the soma by using a *traffic jamGAL4* (*tjGAL4*) driver and then stained for Vasa and 1B1. On depletion of somatic *Nipped-A,* we found that ovaries failed to form properly (100% in *Nipped-A* RNAi compared with 0% in *tjGAL4* control; $n = 10$ ovary pairs for both, $P < 0.0001$) (Supplemental Figure 1, F and G). Thus, Nipped-A is required both in the germ line and the surrounding soma for germline development. Here, we focus on the germline role of Nipped-A that promotes GSC daughter differentiation.

We next asked at which step during the transition from GSCs to cysts does Nipped-A promote in the germ line. Tumors of single germ cells with spectrosomes can be either GSCs or GSC daughters. To test whether these accumulating germ cells are GSCs, we stained for the GSC marker, pMAD, and found no significant difference in the number of pMAD-positive germ cells in *Nipped-A*–depleted germaria compared with control (1.90 ± 0.85 in *Nipped-A* RNAi compared with 2.25 ± 0.44 in *UAS-Dcr2;nosGAL4* control; $n = 20$ for both, $P = 0.1117$) (Figure 1, F–G′). We then asked whether these accumulating germ cells are GSC daughters. Immediate GSC daughters that have yet to express the differentiation factor Bam are pre-CBs. On Bam expression, the GSC daughter is then referred to as a CB. To identify whether these accumulating cells are either pre-CBs or CBs, we stained for BamC and found that only 4% of *Nipped-A*–depleted germaria were BamC-positive, while 100% of control germaria stained positive for BamC ($n = 25$ for both, $P < 0.0001$)

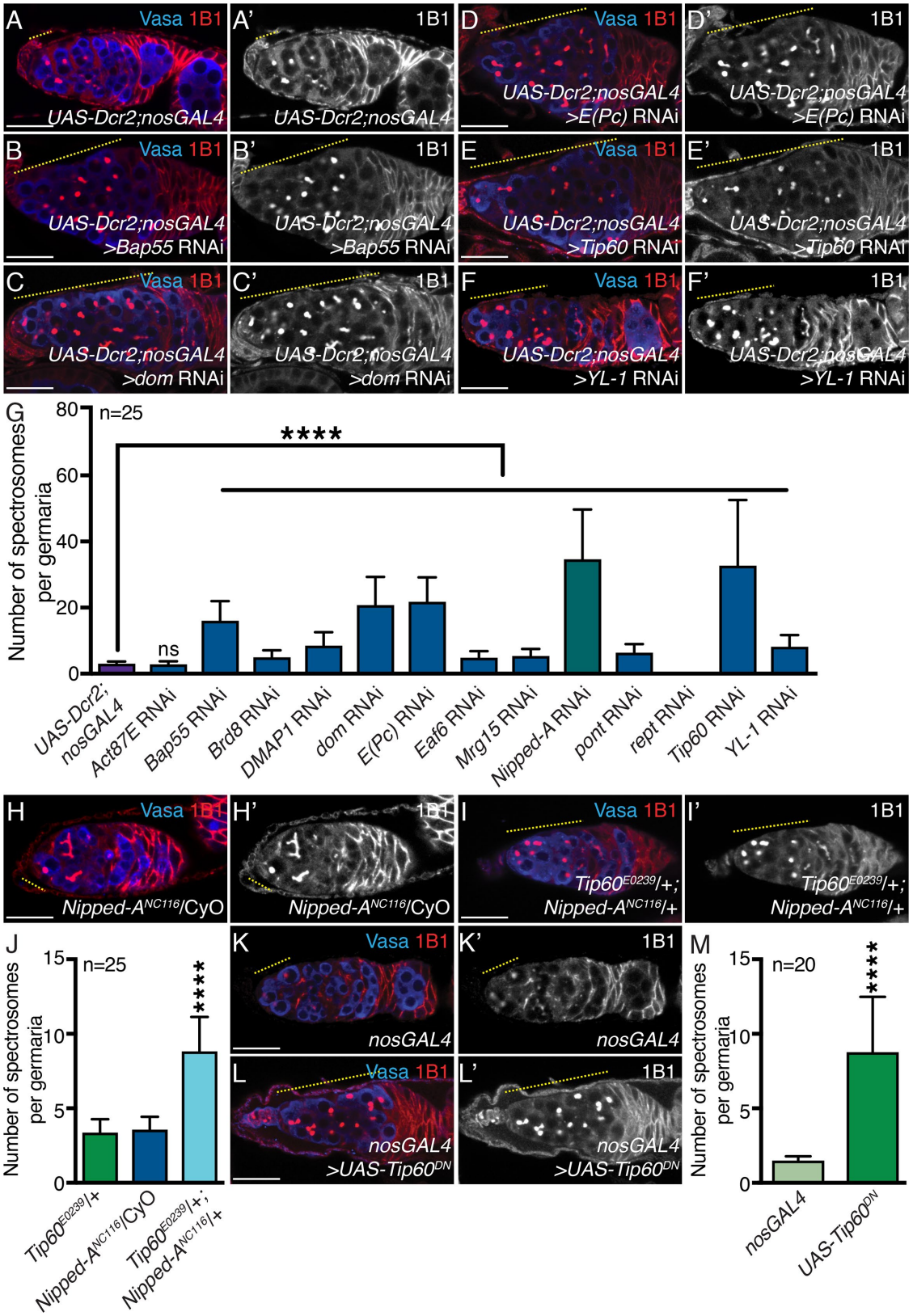


FIGURE 2: Tip60 complex members and HAT activity are required for timely differentiation. (A, A′) Control and germline-depleted Tip60 complex members (B,B′) *Bap55*, (C, C′) *dom*, (D, D′) *E(Pc)*, (E, E′) *Tip60*, and (F, F′) *YL-1* germaria stained with Vasa (blue) and 1B1 (red). Tip60 complex member–depleted germaria show accumulation of single cells (yellow dashed line). 1B1 channel is shown in A′, B′, C′, D′, E′, and F′. (G) Quantitation of the number of single cells in control and germline-depleted Tip60 complex members *Act87E*, *Bap55*, *Brd8*, *DMAP1*, *dom*, *E(Pc)*, *Eaf6*, *Mrg15*, *Nipped-A*, *pont*, *rept*, *Tip60*, and *YL-1* germaria (3.20 ± 1.50 in *Act87E*, 16.04 ± 5.93 in *Bap55* RNAi, 4.96 ± 2.15 in *Brd8* RNAi, 8.52 ± 4.02 in *DMAP1* RNAi, 20.76 ± 8.54 in *dom* RNAi, 21.76 ± 7.42 in *E(Pc)* RNAi, 4.80 ± 2 in

(Figure 1, H–I′). These accumulating germ cells were neither pMAD nor BamC positive, suggesting that these cells are pre-CBs. Polar granule component (Pgc) is a transcriptional silencer expressed in gap phase 2 (G2) of the pre-CB cell cycle (Flora *et al.*, 2018). To test whether the accumulating GSC daughters express Pgc, we utilized a fly line that expresses a *pgcGFP* reporter and *nosGAL4* element (Flora *et al.*, 2018). We found an average of 28.4 ± 7.11 germ cells of *Nipped-A*–depleted germaria were GFP positive compared with only an average of 0.05 ± 0.22 GFP-positive germ cells in control germaria (n = 20 for both, $P < 0.0001$) (Figure 1, J–K′). Thus, we conclude that Nipped-A in the germ line promotes differentiation of Pgc-positive pre-CBs.

Loss of Nipped-A in the germ line leads to accumulation of germ cells expressing Pgc that fail to differentiate. Pgc is required to promote expression of Cyclin B (CycB), which then promotes expression of Bam (Flora *et al.*, 2018). We asked whether Nipped-A is required for up-regulation of CycB post-Pgc expression by staining for both Cyclin A (CycA) and CycB, markers of early and late G2, respectively (Jia *et al.*, 2015). If *Nipped-A*–depleted germ cells fail to properly express CycB, then as a consequence they would not differentiate in a timely manner. To study the transient developmental pre-CB stage, we and others used genetic tools to enrich for pre-CBs by arresting development prior to differentiation (Kai and Spradling, 2003; Tastan *et al.*, 2010; Flora *et al.*, 2018). When we compared *Nipped-A* RNAi to *bam* RNAi germaria, we found that like *bam*-depleted germaria, *Nipped-A*–depleted germaria also accumulated cells in G2 phase of the cell cycle. However, *Nipped-A*–depleted germaria had a significantly higher number of CycB-positive cells compared *bam*-depleted germaria (Supplemental Figure 2, A–E). These cells are not arrested, as they eventually enter the cell cycle and divide (2.36 ± 3.34% phosphohistone– 3 (pH3) positive germ cells germ cells in *Nipped-A* RNAi compared with 3.92 ± 4.77% pH 3–positive germ cells in *bam* RNAi control; n = 25 for both, P = 0.2366). Taken together, we conclude that Nipped-A is required in the germ line after both Pgc and CycB expression to drive undifferentiated pre-CBs into a CB differentiation program.

Nipped-A acts as part of the Tip60 complex to promote differentiation

How does Nipped-A promote the transition from pre-CB to Bam-expressing CB state? Nipped-A is a member of both Tip60 and SAGA complexes that can activate transcription (Grant *et al.*, 1998; Allard *et al.*, 1999; Ikura *et al.*, 2000; Kusch *et al.*, 2004). To determine through which complex Nipped-A regulates early germline development, we systematically depleted Tip60 and SAGA complex members in the germ line. After depletion of 13 of the 16 Tip60 complex members in the germ line, we found loss of 11 of the members leads to an accumulation of germ cells with spectrosomes, depletion of *reptin* (*rept*) leads to complete loss of germ line, and depletion of *Actin 87E* (*Act87E*) failed to give any phenotype (Figure 2, A–G; Supplemental Figure 3, A–H′). When we depleted six of the 21 SAGA complex members in the germ line, including the HAT Gcn5 and SAGA-specific Ada2b, we found no significant difference in the number of spectrosome-containing cells compared with the control; consistent with what has been previously reported (Supplemental Figure 3I) (Li *et al.*, 2017). Altogether, these data suggest that Nipped-A and members of the Tip60 complex in the germ line promote differentiation. Depletion-of-Tip60-complex members phenocopy loss of *Nipped-A* in the germ line. But accumulation of spectrosome-containing germ cells could also arise if Nipped-A and Tip60 complex work in parallel pathways. To test whether Nipped-A works in the same pathway as Tip60 complex, we genetically reduced one copy of *Nipped-A* and *Tip60* in the same fly and stained for Vasa and 1B1. $Tip60^{E0239}$;$Nipped\text{-}A^{NC116}$ trans-heterozygote germaria accumulated more spectrosome-containing germ cells as compared with $Nipped\text{-}A^{NC116}$heterozygotes and $Tip60^{E0239}$heterozygotes alone (Figure 2, H–J). These results suggest that Nipped-A works as a part of the Tip60 complex to promote proper differentiation.

One of Tip60 complex's major functions is its HAT activity, so we next asked whether HAT activity is required for timely differentiation. To test this, we expressed a dominant negative HAT-defective Tip60 mutant (*UAS-*$Tip60^{DN}$; Lorbeck *et al.*, 2011) in the germ line and found a significantly higher number of spectrosome-containing germ cells compared with the control (Figure 2, K–M). To determine whether the accumulated cells are also pre-CBs, we used the *pgcGFP;nosGAL4* reporter fly in conjunction with the Tip60 dominant negative conditional mutant. Upon expression of $Tip60^{DN}$ in the germ line, in conjunction with the *pgcGFP* reporter, we found an average of 0.97 ± 1.40 GFP-positive germ cells per germaria compared with 0.07 ± 0.25 GFP-germ cells in control germaria (n = 30 for both, P = 0.001) (Supplemental Figure 3, J–K′). Thus, we conclude that the HAT activity of the Tip60 complex is required for GSC daughter differentiation.

Tip60 complex localizes to promoters of highly transcribed genes and acetylates histone H4, including lysine 16 (H4K16ac), to promote decompaction (Sánchez-Molina *et al.*, 2014; Lau *et al.*, 2016). We find that loss of Tip60 complex HAT activity alone is sufficient to cause a differentiation defect, so we next asked whether global levels of H4K16ac decrease on depletion of *Nipped-A* or expression of $Tip60^{DN}$ in the germ line. To test for this, we stained for H4K16ac in *UAS-Dcr2;nosGAL4*, *bam* RNAi, *Nipped-A* RNAi, and *UAS-*$Tip60^{DN}$ germaria. We quantified the intensity of fluorescence per nuclei of single germ cells one cell diameter away from the niche and normalized this to somatic levels. We found that, while H4K16ac somatic levels were not significantly different between conditions (Figure 3E), *Nipped-A* RNAi and single cells of *UAS-*$Tip60^{DN}$germaria have significantly lower intensity of normalized H4K16ac levels compared with *bam* RNAi and single cells

Eaf6 RNAi, 5.36 ± 2.16 in *Mrg15* RNAi, 34.64 ± 15.04 in *Nipped-A* RNAi, 6.36 ± 2.58 in *pont* RNAi, 0 ± 0 in *rept* RNAi, 32.72 ± 19.86 in *Tip60* RNAi, and 8.16 ± 3.57 in *YL-1* RNAi compared with 3.04 ± 0.68 in *UAS-Dcr2;nosGAL4* control; n = 25 for all, P = 0.3110 for *Act87E*; ****$P < 0.0001$). (H, H′) *Nipped-A* heterozygote and (I, I′) *Tip60;Nipped-A* trans-heterozygote germaria stained with Vasa (blue) and 1B1 (red). Trans-heterozygote germaria show accumulation of single cells in (yellow dashed line). 1B1 channel is shown in H′ and I′. (J) Quantitation of the number of single cells in germaria of *Tip60* heterozygote, *Nipped-A* heterozygote, and *Tip60;Nipped-A* trans-heterozygote (8.80 ± 2.33 in *Tip60;Nipped-A* trans-heterozygote compared with 3.36 ± 0.91 in *Tip60* heterozygote control and 3.56±0.87 in *Nipped-A* heterozygote control; n = 25 for both; ****$P < 0.0001$). (K, K′) Control and (L, L′) *nosGAL4*-driven *UAS-*$Tip60^{DN}$ germaria stained with Vasa (blue) and 1B1 (red). Germaria with overexpression of $Tip60^{DN}$ show accumulation of single cells (yellow dashed line). 1B1 channel is shown in K′ and L′. (M) Quantitation of the number of single cells in control and *nosGAL4*-driven *UAS-*$Tip60^{DN}$germaria (17.5 ± 7.47 in *UAS-*$Tip60^{DN}$ compared with 2.95 ± 0.60 in *nosGAL4* control; n = 20 for both; ****P = 0.0009). Statistical analysis performed with Student's t test. Scale bar for all images is 20 μm.

in *UAS-Dcr2;nosGAL4* control (Figure 3, A–D′ and E′). Thus, we conclude that Nipped-A works in the same pathway as the Tip60 complex and that Tip60 complex's HAT activity is required to modulate H4K16ac to support normal germline differentiation.

Tip60 complex–mediated differentiation is independent of DNA damage and nucleolar stress

Tip60 complex is known to regulate tumor suppressors, such as p53, and DNA damage response (DDR) proteins to promote DNA double strand break (DSB) repair (Avvakumov and Côté, 2007a; Rossetto *et al.*, 2010; Kim *et al.*, 2010b; Jeong *et al.*, 2011; Ravens *et al.*, 2015). Upon DSB formation in *Drosophila*, the histone variant, H2Av, is phosphorylated (pH2Av) and initiates a signaling cascade to recruit DDR proteins (Sibon *et al.*, 1999; Laurençon *et al.*, 2003; Jaklevic and Su, 2004; Shiloh, 2006; Joyce and McKim, 2009; Joyce *et al.*, 2011). Tip60 complex remodels chromatin by acetylating near the lesion and exchanging pH2Av with unphosphorylated H2Av, thus resolving DDR (Kusch *et al.*, 2004; Joyce *et al.*, 2011). To ensure that damaged DNA is repaired, DDR is activated and p53 orchestrates quality control mechanisms, such as cell cycle arrest or apoptosis (Levine, 1997; Vousden and Lu, 2002). Cell cycle progression is also influenced by levels of ribosomal proteins, as ribosome biogenesis defects can lead to cell cycle arrest or apoptosis (Pestov *et al.*, 2001; Deisenroth and Zhang, 2010; James *et al.*, 2014; Russo and Russo, 2017). In human cell lines TRRAP (Nipped-A) can modulate ribosomal transcription, implicating Nipped-A in regulating nucleolar size and stress response (Arabi *et al.*, 2005; Sanchez *et al.*, 2016). Altogether, this suggests that HAT complexes play a role in sensing and regulating ribosomal biogenesis.

We find that loss of Tip60 complex leads to accumulation of cells in late G2 phase. During cell cycle transitions, cells with DNA damage fail to proceed through cell cycle in a timely manner as a DDR checkpoint is activated (Tang *et al.*, 2006; Sun *et al.*, 2010; Kaidi and Jackson, 2013). We asked whether Tip60 complex–depleted germ cells accumulate pre-CBs that cannot differentiate because of cell cycle checkpoint activation. We hypothesize that if loss of Tip60 complex leads to accumulation of DSBs in early oogenesis, then there would be a higher number of pH2Av-positive germ cells in Tip60 complex member–depleted germaria as compared with control. To determine the frequency of pre-CBs that are pH2Av-positive, we stained for pH2Av in *UAS-Dcr2;nosGAL4* ovaries. Only 8% of observed single cells one cell diameter removed from the niche are positive for pH2Av, suggesting that DDR in these cells is a rare event ($n = 25$) (Supplemental Figure 4, A and A′). We then stained for pH2Av in *Nipped-A*–depleted germaria and quantitated the percentage of germ cells that are pH2Av-positive compared with single germ cells in *bam*-depleted germaria (15.90 ± 8.76% germ cells per germaria in *Nipped-A* RNAi compared with 14.20 ± 6.27% germ cells per germaria in *bam* RNAi control; $n = 20$ for both, $P = 0.4380$). We found no significant difference between the percentage of H2Av-positive germ cells in *Nipped-A*–depleted germaria compared with *bam*-depleted germaria (Supplemental Figure 4, B–C′). Thus, perturbed differentiation is not due to the persistence of DDR pathway signaling.

To test whether a p53-dependent DNA damage checkpoint is activated in Tip60 complex–depleted germ cells, we utilized a *p53* bio-sensor fly expressing *nosGAL4* (Wylie *et al.*, 2014). Expectedly, the bio-sensor was active in 16-cell cyst stages of the positive control, during meiotic recombination that induces DSBs (Brodsky *et al.*, 2000; Lu *et al.*, 2010; Wylie *et al.*, 2014) (Supplemental Figure 4, D and D′). As *Nipped-A*–depleted germ cells fail to differentiate and

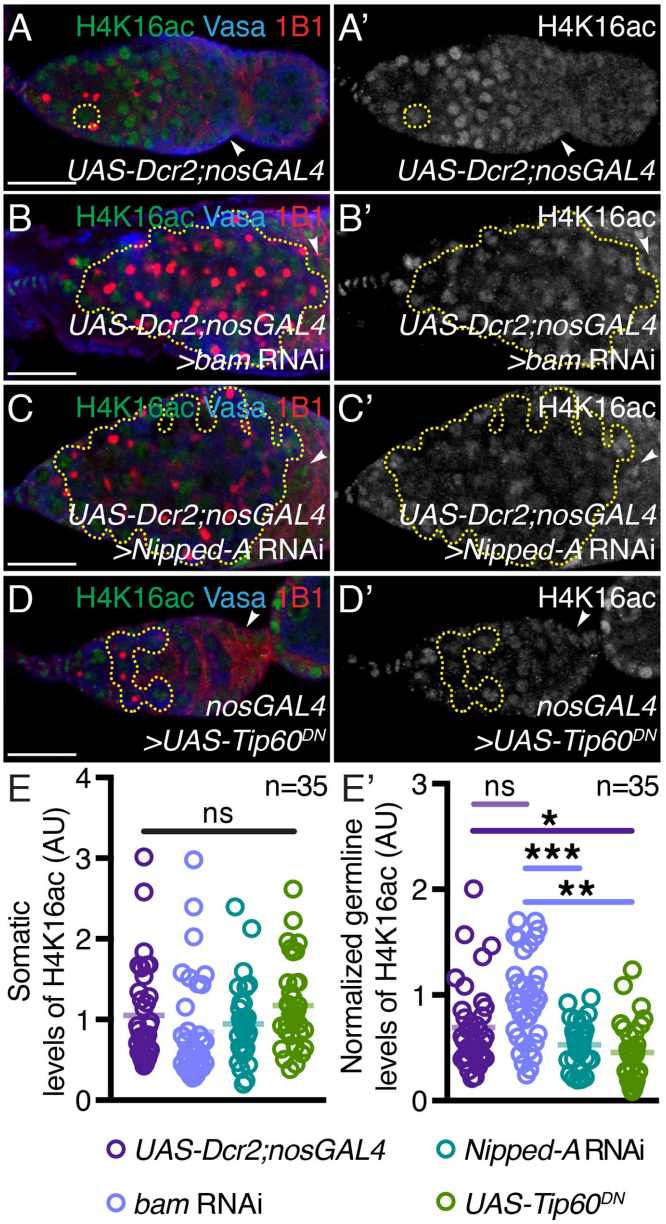


FIGURE 3: Tip60 complex members are required for acetylation of H4K16. (A, A′) *UAS-Dcr2;nosGAL4*, (B, B′) germline-depleted *bam*, (C, C′) germline-depleted *Nipped-A*, and (D, D′) *nosGAL4*-driven *UAS-Tip60^{DN}* germaria stained with H4K16ac (green), Vasa (blue), and 1B1 (red). Germaria with depletion of *Nipped-A* or Tip60 HAT activity show lower levels of H4K16ac levels in the germ line. Stem cell daughters are outlined with yellow dashed lines in all images. H4K16ac channel is shown in A′, B′, C′, and D′. (E) Quantitation of somatic H4K16ac intensity in *UAS-Dcr2;nosGAL4*, germline-depleted *bam* and *Nipped-A*, and *nosGAL4*-driven *UAS-Tip60^{DN}*(1.05 ± 0.60 in *UAS-Dcr2;nosGAL4*, 0.85 ± 0.65 in *bam* RNAi, 0.94 ± 0.47 in *Nipped-A* RNAi, and 1.18 ± 0.09 in *UAS-Tip60^{DN}*; $n = 35$ for all; $P = 0.0924$). (E′) Quantitation of normalized germline H4K16ac intensity in *UAS-Dcr2;nosGAL4*, germline-depleted *bam* and *Nipped-A*, and *nosGAL4*-driven *UAS-Tip60^{DN}*germ cells (0.52 ± 0.20 in *Nipped-A* RNAi and 0.45 ± 0.27 in *UAS-Tip60^{DN}*compared with 0.69 ± 0.41 in *UAS-Dcr2;nosGAL4* and 0.96 ± 0.43 in *bam* RNAi controls; $n = 35$ for all; $^*P < 0.03$, $^{**}P = 0.0093$, and $^{***}P = 0.004$). Statistical analysis performed with Student's *t* test for all except for one-way analysis of ANOVA for E. Scale bar for all images is 20 µm.

thus enter meiosis, any GFP expression will be a consequence of recombination-independent p53 activation. To test this, we stained for GFP, Vasa, and 1B1 in *Nipped-A*- and *bam*-depleted germaria that also carry the *p53* bio-sensor. We found no significant difference in the percentage of germ cells with active p53 in *Nipped-A*–depleted germaria compared with *bam*-depleted germaria (3.47 ± 6.73% germ cells in *Nipped-A* RNAi compared with 2.73 ± 4.28% germ cells in *bam* RNAi control; $n = 20$ for both, $P = 0.6464$) (Supplemental Figure 4, E–F′). Altogether, this suggests that the accumulation of undifferentiated cells in *Nipped-A*–depleted germaria is independent of DDR.

Differentiation can also be impeded by nucleolar stress. *Nipped-A* has been implicated in regulating nucleolar size, stress response, and cell cycle progression (Arabi *et al.*, 2005; Sanchez *et al.*, 2016; Russo and Russo, 2017). We hypothesized that if loss of *Nipped-A* leads to nucleolar stress response, inhibiting differentiation, then *Nipped-A*–depleted germ cells would have altered nucleolar volume (James *et al.*, 2013; Falahati *et al.*, 2016). To test for this, we utilized a nucleolar marker, Fibrillarin (Supplemental Figure 4, G and G′). When we compared *Nipped-A*- and *bam*-depleted germ cells' nucleolar-to-nuclear area ratios, we found no significant difference (0.19 ± 0.82 in *Nipped-A* RNAi compared with 0.22 ± 0.79 in *bam* RNAi control; $n = 20$ for both, $P = 0.2383$) (Supplemental Figure 4, H–I′). Altogether, these data suggest that loss of *Nipped-A* does not lead to nucleolar stress, suggesting that Tip60 complex regulates differentiation independent of DDR and nucleolar stress.

Tip60 complex regulates expression of the differentiation factor, *bgcn*

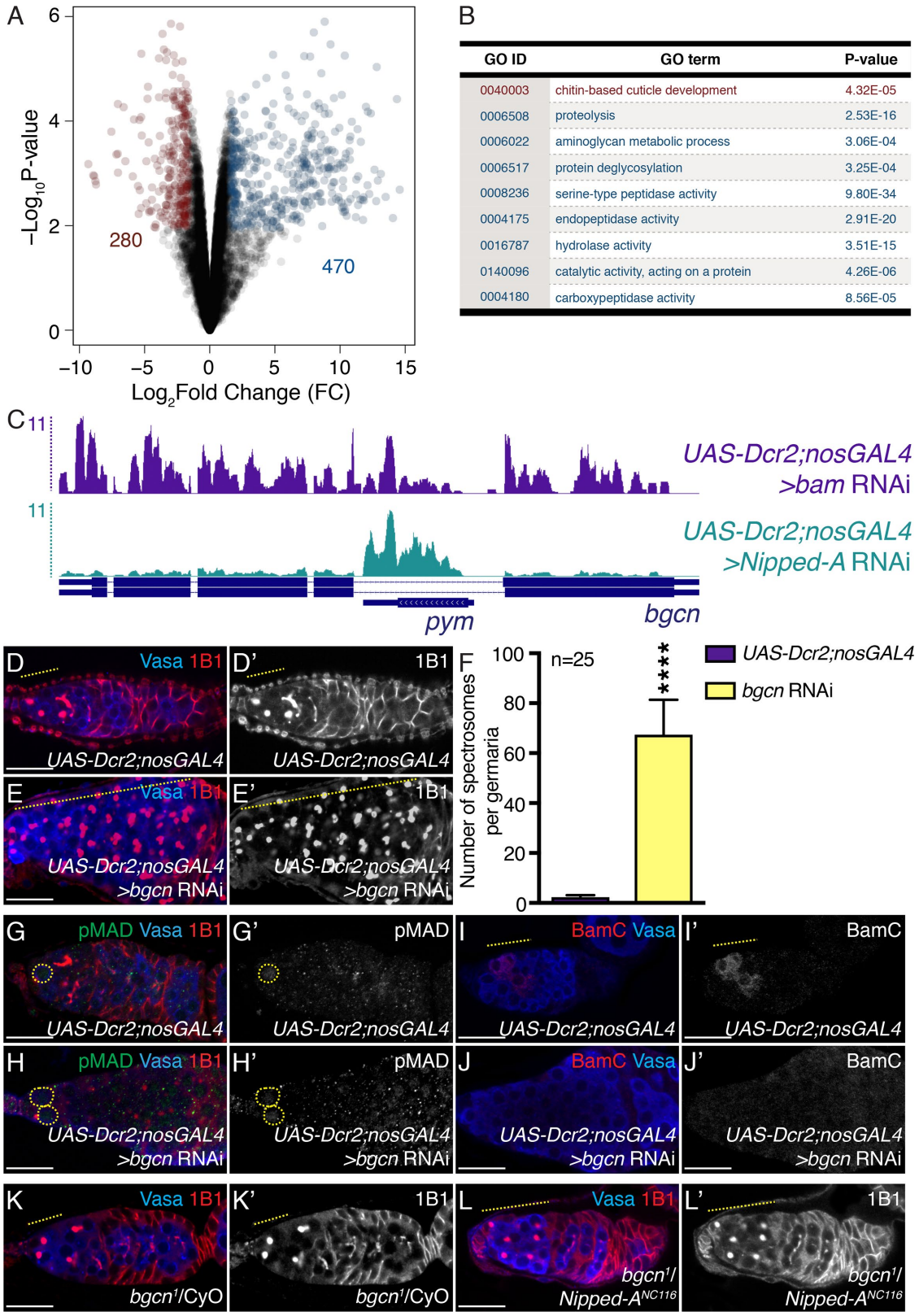
To identify the regulators of Tip60 complex-mediated differentiation in an unbiased manner, we utilized high-throughput RNA sequencing (RNAseq). Comparing transcriptomes of *Nipped-A*–depleted ovaries to *bam*-depleted ovaries, both of which accumulate pre-CBs, we identified 750 differentially expressed genes (Figure 4A). Of those genes, 470 genes were up-regulated and 280 were down-regulated in *Nipped-A* RNAi compared with *bam* RNAi. Among those significantly up-regulated were genes related to cell cycle regulation, such as *string*, *twine*, *CycA*, and *CycB* (false discovery rates [FDRs] = 1.38×10^{-2}, 1.51×10^{-2}, 1.63×10^{-2}, and 1.87×10^{-2}, respectively). Furthermore, there was an enrichment for genes involved in chitin-based cuticle development among the down-regulated data set (Gene Ontology [GO]: 0040003) and endopeptidase activity among the up-regulated data set (GO: 0004175), suggestive of stunted development and protein turnover, respectively (Figure 4B). Interestingly, we found that *bgcn* was among the significantly down-regulated genes (sixfold change, FDR = 7.65×10^{-3}) (Figure 4C). Collectively, our transcriptome analysis is consistent with our immunohistochemistry data, which showed loss of Tip60 complex leads to accumulation of G2 phase cells that fail to differentiate.

Bam and its partner, Bgcn, play a critical role in germline differentiation as differentiation fails to occur if either is absent in GSC daughters (McKearin and Ohlstein, 1995; Ohlstein and McKearin, 1997; Ohlstein *et al.*, 2000; Lavoie *et al.*, 1999). We hypothesized that if significantly lower levels of *bgcn* in *Nipped-A*–depleted germaria are impeding differentiation, then ectopic expression of *bam* in *Nipped-A*–depleted germaria should not rescue the defect. To test whether *bam* is limiting, we induced *bam* expression under control of a heat shock (hs) promoter (Ohlstein and McKearin, 1997). Ectopic expression of *bam* in *nosGAL4* or in *bam* RNAi background alone lead to a conversion to cysts post–heat shock (Supplemental Figure 5, A–D′ and G). However, this is not observed post–heat shock in *Nipped-A*–depleted germaria (Supplemental Figure 5, E–G). As ectopic expression of *bam* alone cannot rescue *Nipped-A*, we then asked whether *bgcn* levels limit pre-CB differentiation in *Nipped-A* RNAi. If this is the case, then loss of *bgcn* should phenocopy loss of Tip60 complex members in the germ line. As previously reported, we found *bgcn*-depleted germaria accumulated a significantly higher number of germ cells with spectrosomes compared with the control (Figure 4, D–F) (Gateff, 1982; Lavoie *et al.*, 1999). These accumulated cells are also negative for pMAD (1.76 ± 0.44 in *bgcn* RNAi compared with 1.96 ± 0.45 in *UAS-Dcr2;nosGAL4* control; $n =$ 25 for both, $P = 0.1189$) (Figure 4, G–H′) and BamC (0% germaria BamC-positive in *bgcn* RNAi compared with 100% germaria BamC-positive in *UAS-Dcr2;nosGAL4* control; $n = 25$ for both, $P < 0.0001$) (Figure 4, I–J′). Next, we asked whether Nipped-A works in the same pathway as Bgcn. To test for this, we genetically reduced one copy of Nipped-A and Bgcn in the same fly and stained for Vasa and 1B1. Trans-heterozygote germaria accumulated more spectrosome-containing germ cells compared with $bgcn^{1}$ and $Nipped\text{-}A^{NC116}$ heterozygotes alone (6.76 ± 1.45 in $bgcn^{1};Nipped\text{-}A^{NC116}$ transheterozygotes compared with 3.92 ± 1.26 in $bgcn^{1}$ heterozygotes and 3.44 ± 1.00 in $Nipped\text{-}A^{NC116}$heterozygotes; $n = 25$ for all, $P < 0.0001$ for both) (Figure 4, K–L′). Taken together, these data demonstrate that Nipped-A regulates expression of *bgcn*.

We hypothesized that if Nipped-A regulates *bgcn* expression, then overexpression of *bgcn* could rescue the differentiation defect in germline-depleted *Nipped-A* ovaries. To determine this, we made use of an EP line ($bgcn^{EY00974}$), which carries a *UAS* element and basal core promoter upstream of the *bgcn* gene. We found that overexpression of *bgcn* in the germ line did not lead to an increase in the number of single cells per germaria or later-stage defects (Figure 5, A, A′, and D; Supplemental Figure 6, A and A′). Depletion of *Nipped-A* alone in the germ line leads to accumulation of single cells, whereas overexpression of *bgcn* in *Nipped-A*–depleted germaria resulted in a significant reduction in the number of single cells and the appearance of fusomelike structures (Figure 5, B–D). Furthermore, we found that 78% of rescue germaria had subsequent egg chambers compared with only 10% of *Nipped-A*–depleted germaria ($n = 50$ for both; $P < 0.05$) (Supplemental Figure 6, B–C′). However, rescue egg chambers died mid-oogenesis (Supplemental Figure 6, A, A′, C, and C′). Thus, we conclude that Tip60 complex controls GSC daughter differentiation via expression of the differentiation factor Bgcn (Figure 5E).

DISCUSSION

Here we find that Nipped-A regulates female *Drosophila* germline differentiation through the Tip60 complex. We observe that Nipped-A plays a role in both the germ line and soma of the gonad to regulate oogenesis (Figure 1; Supplemental Figure 1, F and G). In the germ line, we show that Tip60 complex members and Tip60 HAT activity is required for timely differentiation and loss of either leads to accumulation of pre-CBs (Figure 2; Supplemental Figure 3, A–H′ and J–K′). Furthermore, we demonstrate that Tip60 complex regulates differentiation downstream of Pgc-mediated CycB accumulation (Figure 1, J–K′; Supplemental Figure 2, C–E; Supplemental Figure 3, J–K′) (Flora *et al.*, 2018). We find that *Nipped-A*–depleted germ cells fail to differentiate independently of DDR (Supplemental Figure 4, A–F′) and is also separate from nucleolar stress (Supplemental Figure 4, H–I′) (Sanchez *et al.*, 2016; Feng *et al.*, 2018). Consistent with these findings, we saw no significant difference in mRNA levels of DDR genes *p53*, *reaper*, *hid*, *grim*, or *sickle* between *Nipped-A*- and *bam*-depleted germaria (Song *et al.*, 2004b; Bi *et al.*, 2005; Klattenhoff *et al.*, 2007). Additionally, there was no significant difference in cell cycle arrest regulators *grapes* (*chk1*) and



GO ID	GO term	P-value
0040003	chitin-based cuticle development	4.32E-05
0006508	proteolysis	2.53E-16
0006022	aminoglycan metabolic process	3.06E-04
0006517	protein deglycosylation	3.25E-04
0008236	serine-type peptidase activity	9.80E-34
0004175	endopeptidase activity	2.91E-20
0016787	hydrolase activity	3.51E-15
0140096	catalytic activity, acting on a protein	4.26E-06
0004180	carboxypeptidase activity	8.56E-05

FIGURE 4: Nipped-A regulates *bgcn* levels. (A) Volcano plot of $\log_2$ fold change (FC) vs. $-\log_{10}$ *P* value. Red dots represent significantly down-regulated transcripts, and blue dots represent significantly up-regulated transcripts in *Nipped-A* RNAi ovaries compared with *bam* RNAi ovaries (FDR = 0.05). Genes with threefold or higher change were considered significant. Two-hundred eighty genes were significantly down-regulated while 470 genes were significantly up-regulated. (B) Table representing the Gene Ontology analysis carried out on differentially expressed genes in *Nipped-A*–depleted ovaries compared with *bam*-depleted ovaries. GO: 0040003 was identified from significantly down-regulated genes (red), while GO: 0006508, GO: 0006022, GO: 0006517, GO: 0008236, GO: 0004175,

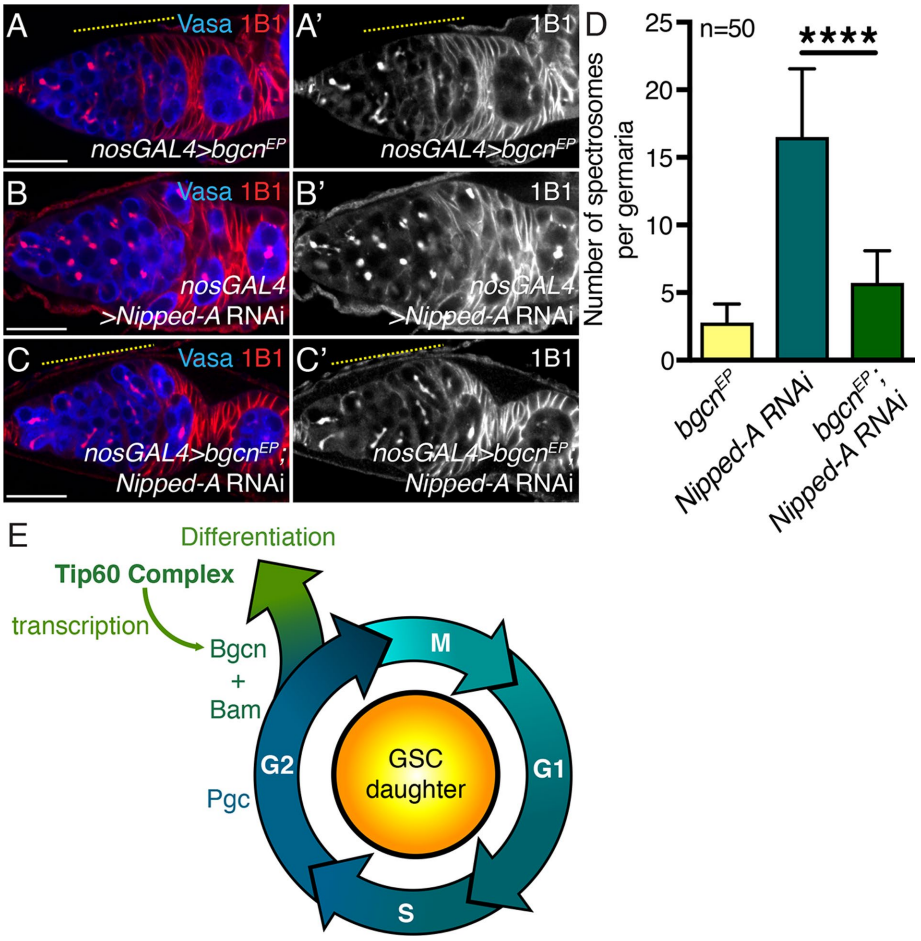


FIGURE 5: Ectopic expression of *bgcn* rescues *Nipped-A* RNAi early defect. (A, A′) Germline-driven $bgcn^{EP}$, (B, B′) depletion of *Nipped-A*, and (C, C′) rescue germaria stained with Vasa (blue) and 1B1 (red). Germline-driven $bgcn^{EP}$and rescue germaria show fusome formation (yellow dashed line). Germaria depleted of *Nipped-A* accumulate only spectrosome-containing cells (white arrow). 1B1 channel is shown in A′, B′, and C′. (D) Quantitation of the number of single cells in germline-driven $bgcn^{EP}$, depletion of *Nipped-A*, and rescue germaria (2.78 ± 1.38 in $bgcn^{EP}$; 5.72 ± 2.37 in rescue compared with 16.52 ± 5.02 in *Nipped-A* RNAi; $n = 50$ for all ****$P < 0.0001$). (E) Schematic showing Tip60 complex regulates differentiation in the precystoblast (GSC daughter). In G2 phase, Pgc is expressed prior to differentiation, we postulate that Tip60 complex modulates *bgcn* mRNA levels to promote differentiation. Bgcn and Bam proteins then complex to stimulate differentiation in the GSC daughter. Statistical analysis performed with Student's *t* test. Scale bar for all images is 20 μm.

loki (*chk2*) (Fogarty *et al.*, 1997; Abdu *et al.*, 2002). Instead, we find that Nipped-A is required for expression of the differentiation factor *bgcn* (Figure 4, C and K–L′). Thus, Tip60 complex drives germ cells into a differentiation program by regulating *bgcn*, a critical component of differentiation.

While Bam and Bgcn are both required for differentiation, previous studies have primarily focused on elucidating the molecular mechanism of Bam and its role in germline maintenance and differentiation (Chen and McKearin, 2003a,b; Song *et al.*, 2004a). Bgcn has been well characterized posttranslationally and predominantly in the context of protein–protein interactions, but its transcriptional modulators had yet to be identified (Ohlstein and McKearin, 1997; Ohlstein *et al.*, 2000; Lavoie *et al.*, 1999; Li *et al.*, 2009; Kim *et al.*, 2010a). We have identified Tip60 complex as a regulator of *bgcn* in the female germ line. We find that Nipped-A is required for expression of the differentiation factor *bgcn*, and overexpression of *bgcn*, but not *bam*, suppresses the early differentiation defect we observe in *Nipped-A*–depleted germaria (Figure 5, A–D; Supplemental Figures 5 and 6). While overexpression of *bgcn* resulted in egg chamber formation in *Nipped-A* RNAi, these egg chambers did not give rise to fertile eggs, suggesting that *Nipped-A* is also required at late stages of oogenesis to regulate yet-unknown targets (Supplemental Figure 6, C and C′). Intriguingly, *bgcn* mRNA is expressed only in the undifferentiated stages. While our data show that *Nipped-A* is required for activation of *bgcn* expression, it does not explain what restricts *bgcn* expression postdifferentiation. Feng *et al.* has described Tip60 complex's role in *Drosophila* testis to promote differentiation via *bam* (Feng *et al.*, 2018). They find that on depletion of *Enhancer of Polycomb (E(Pc))*, germ cells fail to properly differentiate

GO: 0016787, GO: 0140096, and GO: 0004180 were identified from significantly up-regulated genes (blue). (C) UCSC genome browser view of *bgcn* locus and *pym* intervening locus. Germline-depleted *bam* and *Nipped-A* reads shown on top (purple and teal, respectively). RefSeq annotations (bottom, blue) indicate that germline-depleted *Nipped-A* has reads mapping to *Partner of Y14 and Mago* (*pym*); *pym* reads do not change but reads mapping to *bgcn* are lower in germline-depleted *Nipped-A*. (D, D′) Control and (E, E′) germline-depleted *bgcn* germaria stained with Vasa (blue) and 1B1 (red). Germaria depleted of *bgcn* show accumulation of single cells (yellow dashed line). 1B1 channel is shown in D′ and E′. (F) Quantitation of the number of single cells in control and germline-depleted *bgcn* germaria (67.36 ± 13.99 in *bgcn* RNAi compared with 2.28 ± 0.84 in *UAS-Dcr2;nosGAL4* control; $n = 25$ for both, ****$P < 0.0001$). (G, G′) Control and (H, H′) germline-depleted *bgcn* germaria stained with pMAD (green), Vasa (blue), and 1B1 (red). Germaria depleted of *bgcn* do not accumulate pMAD-positive germ cells (yellow dashed circle) ($n = 25$, $P = 0.1189$). pMAD channel is shown in G′ and H′. (I, I′) Control and (J, J′) germline-depleted *bgcn* germaria stained with BamC (red) and Vasa (blue). Germaria depleted of *bgcn* do not accumulate BamC-positive germ cells (yellow dashed line in control) ($n = 25$ for both). BamC channel is shown in I′ and J′. (K, K′) *bgcn* heterozygote and (L, L′) *bgcn/Nipped-A* trans-heterozygote germaria stained with Vasa (blue) and 1B1 (red). Trans-heterozygote shows accumulation of single cells (yellow dashed line) ($n = 25$). 1B1 channel is shown in K′ and L′. Statistical analysis performed with Student's *t* test for all except for Chi-square for I–J′. Scale bar for all images is 20 μm.

and that while *bam* mRNA levels are unperturbed, they suggest E(Pc) is required for posttranscriptional regulation of this differentiation factor (Feng *et al.*, 2018). Together with Feng *et al.*'s findings, we suggest that Tip60 complex may have developed different strategies to promote differentiation in the male and female germ line.

The Bgcn mammalian homologue YTHDC2 is required for meiotic progression in mice (Bailey *et al.*, 2017; Soh *et al.*, 2017; Jain *et al.*, 2018). YTHDC2 partners with a protein similar to Bam, MEIOC, to regulate this developmental switch. Loss of either YTHDC2 or MEIOC lead to a failure to execute a more specialized program, strikingly reminiscent to what has been observed in *Drosophila* development. Prior work in other stem cell systems suggests that Tip60 complex regulation of differentiation factors at the transcriptional level may not be unique to just *Drosophila* GSCs (Fazzio *et al.*, 2008a,b; Acharya *et al.*, 2017). To investigate this, we made use of publically available Tip60 ChIPseq data (Ravens *et al.*, 2015). When we interrogated whether Tip60 was found enriched at promoters of YTH family genes (*YTHDC1*, *YTHDC2*, *YTHDF1*, *YTHDF2*, and *YTHDF3*) in mouse embryonic stem cells, we found that indeed there was enrichment at their promoter regions (Fold changes = 17.6, 12.46, 19.33, 17.09, and 9.60, respectively; FDR = 0.05). However, we do not know whether Tip60 complex regulates *YTHDC2* or *bgcn* directly in the mouse and *Drosophila* gonads, respectively.

We, and others, have identified Nipped-A as being a regulator of differentiation (Tapias *et al.*, 2014; Flegel *et al.*, 2016; Sanchez *et al.*, 2016; Tauc *et al.*, 2017). Flegel *et al.* show that loss of Tip60 complex does not affect terminal differentiation of *Drosophila* wing cells but rather the cells ectopically express cell cycle markers and lose cell identity (Flegel *et al.*, 2016). Across many species, Tip60 complex regulates cell cycle genes, presumably directly by histone acetylation (Fazzio *et al.*, 2008a,b; Tapias *et al.*, 2014; Feng *et al.*, 2018). We find that loss of Nipped-A leads to an up-regulation of G2 phase regulators (*string*, *twine*, *CycA*, and *CycB*) (Supplemental Figure 2). These results are consistent with Feng *et al.*'s data, where they similarly showed that germline Tip60 complex is required for proper levels of CycB (Feng *et al.*, 2017, 2018). As we can rescue the differentiation defects by expressing *bgcn*, this suggests that the cell cycle defects we observe could be a consequence of lack of differentiation factor. Altogether, we show that Tip60 complex mediates a critical decision in germline differentiation, and we suggest that this is coordinated at the level of chromatin structure and transcriptional modulation of differentiation factors (Figure 5E).

MATERIALS AND METHODS

Fly lines

The following RNAi stocks were used in this study; if more than one line is listed, then only the first was used for quantitation and shown in figures, unless otherwise stated in the text: *Act87E* RNAi (Bloomington #42652), *Bap55* RNAi (VDRC #v24704), *Brd8* RNAi (Bloomington #42658 and VDRC #v104879), *DMAP1* RNAi (Bloomington #63666), *dom* RNAi (Bloomington #31054 and #40914), *Eaf6* RNAi (Bloomington #33905 and VDRC #101457), *E(Pc)* RNAi (Bloomington #28686 and VDRC #v35271), *Mrg15* RNAi (Bloomington #35241), *Nipped-A* RNAi (Bloomington #34849 [line #1], VDRC #v52486 [line #2], and Bloomington #31255 [line #3]), *pont* RNAi (Bloomington #50972 and VDRC #v105408), *rept* RNAi (Bloomington #32415 and VDRC #v103483), *Tip60* RNAi (Bloomington #35243), *YL-1* RNAi (Bloomington #31938), *Ada2b* RNAi (Bloomington #31347 and #35334), *Ada3* RNAi (Bloomington #28905 and #32451), *Ataxin7* RNAi (VDRC #v102078), *Gcn5* RNAi (Bloomington #33981 and #35601), *Sgf29* RNAi (Bloomington #36636 and #39000), *Spt3* RNAi (Bloomington #35148 and #57733), *bam* RNAi (Bloomington #58178), and *bgcn* RNAi (VDRC #v25591).

The following tissue-specific drivers were used in this study: *UAS-Dcr2;nosGAL4* (Bloomington #25751), *nosGAL4;MKRS*/TM6 (Bloomington #4442), *pgcGFP;nosGAL4* (Rangan Lab; Flora *et al.* [2018]), *tjGAL4* (Lehmann Lab), and *nosGAL4;p53R-GFP* (Abrams Lab).

The following mutant and overexpression stocks were used in this study: *Nipped-A*^{NC116}*cn*^{1}*bw*^{1}/CyO (Bloomington #7188), *w*^{1118},*Tip60*^{e02395} (Bloomington #18052), *bgcn*^{1}/CyO (Bloomington #6054), *bgcn*^{EY00974} (Bloomington #20106), *Nipped-A*^{MI10513}/SM6a (Bloomington #54566), *hs-bam* (Bloomington #24637), and *UAS-Tip60*^{DN} (*UAS-Tip60*^{E431Q}, Elefant Lab; Lorbeck *et al.* [2011]).

Dissection and immunostaining

Two- to 3-d-old ovaries were dissected in 1x phosphate-buffered saline (PBS), fixed for 15 min in PBS plus 5% methanol-free formaldehyde. The ovaries were washed in PBT (1x PBS, 0.5% Triton X-100, and 0.3% BSA) three times for 10 min each. Primary antibodies were added in PBT and incubated at 4°C overnight. The ovaries were washed three times for 10 min each in PBT and then washed once in PBT supplemented with 2% donkey serum (Sigma) for 10 min. Secondary antibodies were added in PBT supplemented with 4% donkey serum and incubated at room temperature for 4 h. Ovaries were washed four times for 10 min each in 1x PBST (1x PBS with 0.2% Tween 20) and mounted using Vectashield with 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories). The following primary antibodies were used: mouse anti-1B1 (1:20, DSHB), Rabbit anti-Vasa (1:1000, Rangan Lab), Chicken anti-Vasa (1:1000, Rangan Lab (Upadhyay *et al.*, 2016)), Rabbit anti-GFP (1:2000, abcam, ab6556), Rabbit anti-pMAD (1:200, abcam, ab52903), Mouse anti-BamC (1:200, DSHB, Supernatant), Mouse anti-CycA (1:20, DSHB), Rabbit anti-CycB (1:200, Santa Cruz Biotechnology, 25764), Rabbit anti-pH2Av (1:500, Rockland, 600-401-914), Rabbit anti-H4K16ac (1:500, Millipore, 07-329), and Mouse anti-Fibrillarin (1:50, Fuchs Lab). The following secondary antibodies were used: Alexa 488 (Molecular Probes), Cy3 and Cy5 (Jackson Labs) were used at a dilution of 1:500. All experiments were conducted at least three times for each experimental condition.

Fluorescence imaging

The tissues were visualized under 10× dry and 40× oil objective lenses, and images were acquired using a Zeiss LSM-710 confocal microscope under the 20×, 40×, and 63× oil objectives. Images of whole ovaries were visualized and acquired under 4× and 10× objectives using the EVOS FL Cell Imaging System.

RNA extraction and RNAseq library preparation

Ovaries from *UAS-Dcr2;nosGAL4>bam* RNAi and *UAS-Dcr2;nosGAL4>Nipped-A* RNAi flies were dissected in 1x PBS. RNA was isolated using TRIzol (Invitrogen, 15596026), treated with DNase (TURBO DNA-free Kit, Life Technologies, AM1907), and then run on a 1% agarose gel to check integrity of the RNA. To generate mRNA-enriched libraries, total RNA was treated with poly(A)tail selection beads and then following the manufacturer's instructions of the NEXTflex Rapid Directional RNAseq Kit (Bioo Scientific Corp., NOVA-5138-08), except that RNA was fragmented for 13 min. Single-end mRNA sequencing (75 base pair) was performed on biological duplicates from each genotype on an Illumina NextSeq500 by the Center for Functional Genomics (CFG). After quality assessment, the sequenced reads were aligned to the *Drosophila melanogaster* genome (UCSCdm6) using HISAT2 (version 2.1.0) with the RefSeq-annotated transcripts as a guide (Kim *et al.*, 2015). Raw counts were

generated using featureCounts (version 1.6.0.4) (Liao *et al.*, 2014). Differential gene expression was assayed by edgeR (version 3.16.5), using a false discovery rate (FDR) of 0.05, and genes with threefold or higher were considered significant. GO term enrichment on differentially expressed genes was performed using Panther (Robinson *et al.*, 2010; Mi *et al.*, 2017). Raw and processed data for *UAS-Dcr2;nosGAL4>bam* RNAi and *UAS-Dcr2;nosGAL4>Nipped-A* RNAi RNAseq is deposited in the Gene Expression Omnibus (GEO) databank under accession number GSE119328.

ChIPseq analysis

To analyze Tip60 ChIPseq data, we aligned Tip60 reads to the *Mus musculus* genome (UCSCmm9) (Input [GSM798320]) [Karmodiya *et al.*, 2012] and Experimental [GSE69671] [Ravens *et al.*, 2015]) using Bowtie2. ChIPseq peaks were called by using MACS2 (narrow peaks), using default parameters, and then visualized using UCSC genome browser.

Quantification analysis

Statistical analysis. The *P* values were determined by using Student's *t* test, one-way analysis of variance (ANOVA), or chi-square analysis (see figure legends). All analyses were performed using Prism 7 software (GraphPad).

A.U. of protein levels. To calculate intensities for H4K16ac *UAS-Dcr2;nosGAL4*, *bam* RNAi, *Nipped-A* RNAi, and *UAS-Tip60^{DN}*, mutant germaria were taken under the same confocal settings with Z stacks. For quantification, nuclei of single cells approximately one cell diameter away from the niche were outlined, and the intensity of the selected region in the H4K16ac channel was analyzed using Fiji. Nuclei of a somatic cell in the same germarium was outlined, and the intensity of H4K16ac fluorescence in the selected region was also analyzed using Fiji. The ratio between mean intensity and area was measured for germ cells and somatic cells. Mean intensity of H4K16ac per area in germ cells were normalized to mean intensity of H4K16ac per area in somatic cells. The average of all ratios, per genotype was calculated and comparisons were made between *UAS-Dcr2;nosGAL4* and *bam* RNAi controls and *Nipped-A* RNAi and *UAS-Tip60^{DN}* experimentals. The *P* value was determined by Student's *t* test by comparing the average mean H4K16ac intensities per area in germ cells normalized to average mean H4K16ac intensities per area in somatic cells of controls and experimentals. A minimum of five germaria were chosen for each quantification.

Materials and reagents

Fly food was made by using the procedures from the Ruth Lehmann lab at NYU (summer/winter mix) and filled narrow vials (Fisherbrand Drosophila Vials; Fischer Scientific) to approximately 12 ml.

ACKNOWLEDGMENTS

We are particularly grateful to all members of the Rangan laboratory for discussion and comments on the manuscript, especially E.T.M. for Bioinformatic support. Additionally, we thank Felice Elefant, Ruth Lehmann, Gabriele Fuchs, the Bloomington Drosophila Stock Center, the Vienna Drosophila Resource Center, the Transgenic RNAi Project (National Institutes of Health/National Institute of General Medical Sciences [NIH/NIGMS] R01-GM084947), The BDGP Gene Disruption Project, and FlyBase for fly stocks and reagents. Furthermore, we thank the CFG Core Facility at the University at Albany for performing RNAseq analysis and Faraz Ahmed at UAlbany for discussion. P.R. acknowledges funding from NIH/NIGMS RO11119484-1-68857 and The Pew Biomedical Scholars Program.

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