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# Histone demethylase dUTX antagonizes JAK-STAT signaling to maintain proper gene expression and architecture of the *Drosophila* testis niche

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## SUMMARY

Adult stem cells reside in microenvironments called niches, where they are regulated by both extrinsic cues, such as signaling from neighboring cells, and intrinsic factors, such as chromatin structure. Here we report that in the *Drosophila* testis niche an H3K27me3-specific histone demethylase encoded by *Ubiquitously transcribed tetratricopeptide repeat gene on the X chromosome* (*dUTX*) maintains active transcription of the *Suppressor of cytokine signaling at 36E* (*Socs36E*) gene by removing the repressive H3K27me3 modification near its transcription start site. *Socs36E* encodes an inhibitor of the Janus kinase signal transducer and activator of transcription (JAK-STAT) signaling pathway. Whereas much is known about niche-to-stem cell signaling, such as the JAK-STAT signaling that is crucial for stem cell identity and activity, comparatively little is known about signaling from stem cells to the niche. Our results reveal that stem cells send feedback to niche cells to maintain the proper gene expression and architecture of the niche. We found that dUTX acts in cyst stem cells to maintain gene expression in hub cells through activating *Socs36E* transcription and preventing hyperactivation of JAK-STAT signaling. dUTX also acts in germline stem cells to maintain hub structure through regulating DE-Cadherin levels. Therefore, our findings provide new insights into how an epigenetic factor regulates crosstalk among different cell types within an endogenous stem cell niche, and shed light on the biological functions of a histone demethylase *in vivo*.

**KEY WORDS: Germline, Cyst stem cell, Niche, Epigenetics, Histone demethylase, *Drosophila***

## INTRODUCTION

Extrinsic signals are important to maintain appropriate interaction between stem cells and their niches (Morrison and Spradling, 2008). In addition, epigenetic regulation that changes chromatin structure without altering the associated DNA sequence acts intrinsically to regulate proper gene expression in stem cells (Clapier and Cairns, 2009). Both mechanisms are essential for regulating stem cell identity and activity (Cherry and Matunis, 2010; Eliazer et al., 2011). However, the crosstalk between them is not fully understood.

The *Drosophila* male germline stem cell (GSC) lineage is a paradigmatic system with which to investigate the molecular mechanisms that govern adult stem cell activity in their physiological environment (Kiger et al., 2001; Tulina and Matunis, 2001; Yamashita et al., 2003; Yamashita et al., 2007). *Drosophila* male GSCs reside in a microenvironment composed of two types of somatic cells: postmitotic hub cells located at the tip of the testis and cyst stem cells (CySCs), two of which encapsulate each GSC (Fig. 1A). Hub cells and CySCs contribute to the niche of GSCs by providing crucial signals to preserve GSC identity and activity (Kiger et al., 2001; Leatherman and Dinardo, 2008; Leatherman and Dinardo, 2010; Tulina and Matunis, 2001; Yamashita et al., 2003; Yamashita et al., 2007; Lim and Fuller, 2012). The Janus kinase signal transducer and activator of transcription (JAK-STAT) and bone morphogenetic protein (BMP) signaling pathways are the two major pathways that maintain the activity of GSCs and CySCs. The JAK-STAT pathway is activated by the cytokine Unpaired (Upd; Outstretched – FlyBase) secreted from the hub cells, which initiates the downstream cascade to activate the Stat92E transcription factor in GSCs and CySCs (reviewed by de Cuevas and Matunis, 2011). Activation of Stat92E in CySCs initiates BMP signaling required for GSC self-renewal, and activation of Stat92E in GSCs enhances their adhesion to the hub cells (Leatherman and Dinardo, 2008; Leatherman and Dinardo, 2010). *Suppressor of cytokine signaling at 36E* (*Socs36E*), which is expressed in hub cells and CySCs, attenuates JAK-STAT signaling (Terry et al., 2006) to maintain an appropriate balance between CySCs and GSCs in the testis niche (Issigonis et al., 2009).

In addition to signaling pathways, epigenetic mechanisms can profoundly influence decisions of stem cell maintenance versus differentiation (Buszczak and Spradling, 2006; Li and Zhao, 2008). DNA wraps around four core histones (H3, H4, H2A and H2B) to form nucleosomes, the repeating basic units of chromatin. In *Drosophila*, there are two major epigenetic regulators: chromatin remodeling factors that use ATP hydrolysis to drive histone repositioning and histone-modifying enzymes that covalently modify histones (Becker and Hörz, 2002). Both mechanisms have been shown to act intrinsically to maintain GSCs in the testis niche (Buszczak et al., 2009; Cherry and Matunis, 2010).

Among the histone-modifying enzymes, histone demethylases have been identified as 'epigenetic erasers' that remove methyl groups from methylated lysine residues of histones (Klose et al., 2006). Among the 14 demethylases in *Drosophila* (Klose et al., 2006; Metzger et al., 2005; Shi et al., 2004), *Ubiquitously transcribed tetratricopeptide repeat gene on the X chromosome* (*dUTX*; also known as *Utx* – FlyBase) encodes the sole demethylase that specifically removes the repressive trimethylation on lysine 27 of histone H3 (H3K27me3) (Smith et al., 2008). H3K27me3 is generated by a member of the Polycomb group (PcG) family of

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proteins and has been shown to associate with silent regions of chromatin (Cao et al., 2002; Müller et al., 2002). Increased H3K27me3 levels have been reported to cause certain human cancers (Bracken et al., 2003; Kleer et al., 2003; Kondo et al., 2008; Varambally et al., 2002). Consistently, mutations that inactivate UTX (also known as KDM6A), the mammalian homolog of dUTX, cause an increase in H3K27me3 and lead to human cancers (van Haaften et al., 2009). In *Drosophila*, dUTX has been reported to act as a suppressor of Notch- and Retinoblastoma-dependent tumors (Herz et al., 2010).

Mammalian species have multiple H3K27me3-specific demethylases. Therefore, studying the functions of dUTX in *Drosophila* greatly reduces the complications that might result from gene redundancy. The UTX protein is evolutionarily conserved and contains several tetratricopeptide (TRP) repeats, as well as the catalytic Jumonji C (JmjC) domain (Klose et al., 2006). dUTX has been shown to physically associate with RNA polymerase II (Pol II) *in vivo*, suggesting its involvement in transcriptional activation (Smith et al., 2008). To date, much of the knowledge about the epigenetic regulation of histone demethylases comes from biochemical studies undertaken *in vitro* or in cell culture, and their *in vivo* functions are not well understood. Therefore, to better understand the biological roles of dUTX, we have examined its role in the *Drosophila* testis niche.

## MATERIALS AND METHODS

### Fly stocks

Flies were raised on standard yeast/molasses medium at 25°C. The following stocks were used: *dUTX$^{1}$ FRT40A* (from A. Shilatifard, Stowers Institute for Medical Research, Kansas City, MO, USA), *w$^{1118}$; Df(2L) BSC144* (Bloomington Stock Center, BL-9504), *UAS-dUTX shmiRNA* (TRiP.HMS00575 from Bloomington Stock Center), *upd-Gal4* (from D. Harrison, University of Kentucky, Lexington, KY, USA), *nanos-Gal4* (from M. Van Doren, Johns Hopkins University, Baltimore, MD, USA), *c587-Gal4* (from A. Spradling, Carnegie Institution Department of Embryology, Baltimore, MD, USA), *y,w; Ubi-GFP, Ubi-GFP, FRT40A* (Bloomington Stock Center, BL-5189), *hs-FLP$^{122}$* (Bloomington Stock Center, BL-33216), *Arm-lacZ, FRT40A* (Bloomington Stock Center, BL-7371), *UAS-dUTX* and *UAS-dUTX$^{JmjC}$* [from A. Shilatifard, refer to Materials and methods in Herz et al. (Herz et al., 2010)], *UAS-Socs36E-45* (from B. Callus, University of Western Australia, Perth, WA, Australia), *Stat92E$^{06346}$* (from N. Perrimon, Harvard Medical School, Boston, MA, USA), *UAS-DE-Cad$^{dCR4h}$* and *UAS-DE-Cad$^{DEFL}$* (from Y. Yamashita, University of Michigan, Ann Arbor, MI, USA), and *hs-FLP, UAS-GFP.nls, tub-Gal4/FM7; tub-Gal80 FRT40A/CyO* (from E. Bach, New York University School of Medicine, New York, NY, USA).

### Clonal induction

*dUTX$^{1}$* clones that are negative for the GFP or β-Gal marker were generated using the FLP/FRT recombination system. The flies used were of the following genotypes: *hs-FLP$^{122}$; Arm-lacZ, FRT40A/dUTX$^{1}$ FRT40A* or *hs-FLP$^{122}$; Ubi-GFP, Ubi-GFP, FRT40A/dUTX$^{1}$FRT40A*. The clones were induced by heat shocking pupae on days 8 and 9 for 2 hours at 37°C. After the second heat shock, flies were placed at 25°C and dissected and stained 3 days after clone induction. Mosaic analysis with a repressible cell marker (MARCM) clones were generated using flies of genotype *hs-FLP, UAS-GFP.nls, tub-Gal4/Y; tub-Gal80 FRT40A/dUTX$^{1}$ FRT40A*. The clones were induced by heat shocking pupae on days 8 and 9 for 2 hours at 37°C. After the second heat shock, flies were placed at 25°C and dissected and stained 1 day after clone induction.

### Immunofluorescence staining

Immunofluorescence staining was performed as previously described (Cheng et al., 2008). The primary antibodies used were: rabbit anti-Zfh1 (1:5000; from Ruth Lehmann, Skirball Institute of Biomolecular Medicine, NY, USA); mouse anti-Armadillo [1:100; developed by Eric Wieschaus (Princeton University, Princeton, NJ, USA) and obtained from Developmental Studies Hybridoma Bank (DSHB)]; rat anti-Vasa (1:100; developed by Allan Spradling and Dianne Williams and obtained from DSHB); rabbit anti-dUTX (1:2000; from Ali Shilatifard); rabbit anti-trimethyl-histone H3 (Lys27) (1:200; Millipore, #07-449); chicken anti-GFP (1:1000; Abcam, #13970); rabbit anti-Stat92E (1:800; from Denise Montell, Johns Hopkins School of Medicine, Baltimore, MD, USA); guinea pig anti-Traffic jam (1:3000; from Mark Van Doren); rabbit anti-phospho-histone H3 (Ser10) (1:2000; Millipore, #06-570); and rabbit anti-Caspase 3 (1:100; BD Biosciences, #610322).

### Isolation of total RNA and quantitative reverse transcription PCR (qRT-PCR)

Total RNA was isolated from wild-type (wt) and *dUTX* third instar larval testes using TRIzol reagent (Invitrogen, #15596-018) according to the manufacturer's instructions. Yield and quality of RNA were determined with a NanoDrop spectrometer (NanoDrop Technology, San Diego, CA, USA). Reverse transcription was performed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, #K1621). Transcript levels were measured using SYBR Green PCR Master Mix (Fermentas, #K0221) and normalized to *fringe*. Primers used for qRT-PCR are listed in supplementary material Table S1.

### Chromatin immunoprecipitation (ChIP)

ChIP was performed as described (Gan et al., 2010). For each biological replicate we dissected 200 pairs of *dUTX* testes and 200 pairs of wt testes. Primers used for qPCR are listed in supplementary material Table S1.

### Statistical analysis

Statistical significance was calculated using two-tailed Student's *t*-test or Fisher's test. *P*-values are indicated in figures or in figure legends. Error bars indicate s.d.

## RESULTS

### dUTX prevents overpopulation of Zfh1-expressing cells around the hub

*dUTX* encodes a histone demethylase that has been shown to remove H3K27me3 in somatic cells (Smith et al., 2008). To study the effect of *dUTX* loss on the level of H3K27me3 in testis, we used a strong loss-of-function allele of *dUTX* (*dUTX$^{1}$*) (Herz et al., 2010). The *dUTX$^{1}$*/Df hemizygous flies (referred to hereafter as *dUTX*) are adult lethal, but survive up to the early pupal stage. Because of the adult lethality, analysis of H3K27me3 levels in adult *dUTX* testes required the FLP/FRT recombination system (Xu and Rubin, 1993). Immunoreactivity with a dUTX-specific antibody raised against the N-terminal 153 residues (Smith et al., 2008) was absent in *dUTX$^{1}$* germline clones (supplementary material Fig. S1A,A″), suggesting that *dUTX$^{1}$* is a strong loss-of-function allele. Consistent with the H3K27me3-specific demethylase activity (Herz et al., 2010), *dUTX* homozygous germline clones showed an increase of the H3K27me3 signal using an H3K27me3-specific antibody (Chen et al., 2011) when compared with the neighboring heterozygous germ cells (supplementary material Fig. S1B,B″), demonstrating that dUTX acts as an H3K27me3 demethylase in germ cells. Using the MARCM system, we generated *dUTX* mutant cyst cell clones that are positively labeled by GFP (Lee and Luo, 1999), which showed increased H3K27me3 signal compared with a neighboring wild-type (wt) cyst cell (supplementary material Fig. S1C-C‴). These data demonstrate that dUTX also acts as an H3K27me3 demethylase in cyst cells in the testis.

To determine the function(s) of *dUTX* in the male GSC niche, we analyzed testes isolated from the third instar larvae of *dUTX* mutant males. Using antibodies against Armadillo (Arm) to label hub cells and zinc finger homeodomain 1 (Zfh1) to label CySCs and early cyst cells (Leatherman and Dinardo, 2008), we detected niche

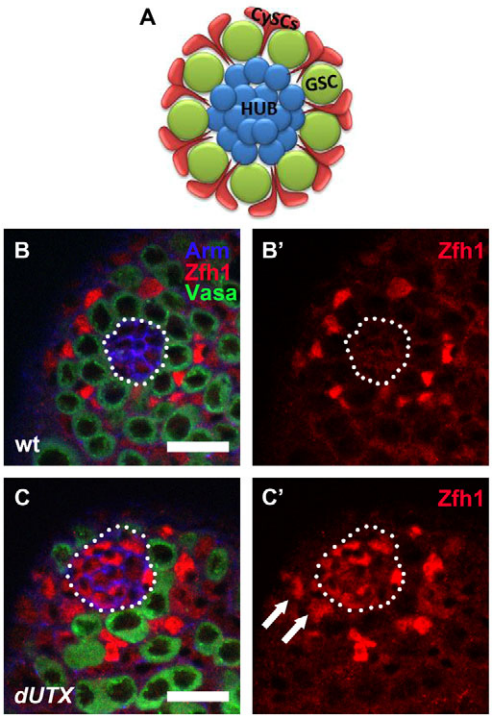


**Fig. 1. dUTX prevents Zfh1-expressing cells from overpopulating the niche and represses Zfh1 expression in the hub cells.** (**A**) Schematic of the *Drosophila* testis niche. CySCs, cyst stem cells; GSC, germline stem cell. (**B-C′**) Immunostaining using antibodies against Arm (blue), Vasa (green) and Zfh1 (red) in (B,B′) wt and (C,C′) *dUTX* testes. Arrows point to overpopulating Zfh1-expressing cells with nuclei that directly contact the hub (C′). Hub area is outlined (white dotted line). Scale bars: 10 μm.

architectural defects in *dUTX* testes. In wt testes, Zfh1-expressing CySCs surround GSCs and extend thin protrusions toward the hub, while their nuclei remain one cell diameter away from the hub (Fig. 1B,B′). However, 48% of *dUTX* testes had three or more Zfh1-expressing cells with their nuclei directly contacting the hub (Fig. 1C,C′, arrows; Fig. 2D, compare the first and second columns). These Zfh1-expressing cells with nuclei that directly contact hub cells stained positively for Traffic jam (TJ), a transcription factor expressed in early cyst cell nuclei (Li et al., 2003), suggesting that they retain their identity as early cyst cells (data not shown). Overpopulation of Zfh1-expressing cells was not, however, accompanied by an increase in the overall number of Zfh1-expressing cells surrounding the hub [30.6±6.6 in wt testes ($n$=27) versus 31±9.5 in *dUTX* testes ($n$=30), $P$>0.05]. These results suggest that loss of *dUTX* does not affect Zfh1-expressing cell number but rather their behavior, which causes the Zfh1-expressing cells to overpopulate around the hub area.

## dUTX acts in CySCs and early cyst cells to prevent overpopulation of Zfh1-expressing cells around the hub

To determine in which cell type dUTX is required to prevent overpopulation of Zfh1-expressing cells around the hub, different cell type-specific Gal4 drivers were used in combination with a *UAS-dUTX* small hairpin microRNA (shmiRNA) (Ni et al., 2011) to knockdown *dUTX* in a cell type-specific manner. Knockdown of *dUTX* exclusively in germ cells using *nanos* (*nos*)-*Gal4* (Van Doren et al., 1998) (supplementary material Fig. S2A,A′), or in hub cells using *upd-Gal4* (Boyle et al., 2007) (supplementary material Fig. S2B,B′), did not lead to overpopulation of Zfh1-expressing cells around the hub. By contrast, knockdown of *dUTX* using the cyst cell driver *c587-Gal4* (Manseau et al., 1997) led to a 45% increase in testes with an overpopulation of Zfh1-expressing cells around the hub (Fig. 2A,A′, arrows; 2D, compare the third and fourth columns). There was also an overpopulation of Zfh1-expressing cells around the hub in 30% of *c587-Gal4* control males, which was probably due to Gal4 expression in cyst cells, as a similar phenotype was observed in 35% of testes carrying another cyst cell-specific driver, *eya-Gal4* (Leatherman and Dinardo, 2008).

To confirm that the *upd-Gal4* driving *dUTX shmiRNA* did reduce dUTX levels in hub cells, we stained testes from *upd>dUTX shmiRNA* males with the H3K27me3 antibody. As a control, the *c587>dUTX shmiRNA* testes were stained with the same antibody. The H3K27me3 signal in hub cells from *upd>dUTX shmiRNA* testes was higher than that in neighboring germ cells, which had normal levels of dUTX (supplementary material Fig. S2C,C′). By contrast, the H3K27me3 signal in hub cells from *c587>dUTX shmiRNA* testis was similar to that in the neighboring germ cells; in this genotype, both hub cells and germ cells have normal dUTX levels (supplementary material Fig. S2D,D′). These results demonstrate that normal function of dUTX is required in CySCs and/or early cyst cells, but not in hub cells, to prevent the overpopulation of Zfh1-expressing cells around the hub.

## The function of dUTX in CySCs and early cyst cells depends on its demethylase activity

dUTX was reported to demethylate H3K27me3 via its catalytic JmjC domain (Smith et al., 2008). To determine whether the demethylase activity of dUTX is required for its function in CySCs and early cyst cells, $dUTX^{JmjC}$ (Herz et al., 2010) was driven by the *c587-Gal4* driver in the *dUTX* mutant background. As a control, wild-type *dUTX* was expressed using the same driver. The overpopulation of Zfh1-expressing cells in *dUTX* testes was rescued significantly by the wild-type *dUTX* transgene (Fig. 2B,B′ and 2D, compare the fifth and sixth columns), but not by the $dUTX^{JmjC}$ transgene (Fig. 2C,C′ and 2D, compare the fifth and seventh columns). However, even the wild-type transgene did not completely rescue overpopulation of Zfh1-expressing cells around the hub. This could be due to insufficient expression or inappropriate expression timing using cDNA transgenes. In summary, these data demonstrate that the demethylase activity of dUTX is required to maintain proper niche architecture.

## dUTX demethylates H3K27me3 at the *Socs36E* genomic locus for its active transcription

Because the overpopulation of Zfh1-expressing cells around the hub in *dUTX* testes resembled the reported loss-of-function phenotype of the *Socs36E* gene (Issigonis et al., 2009), we used qRT-PCR to measure the *Socs36E* transcript level in *dUTX* testes. Since *Socs36E* is expressed specifically in hub cells and CySCs (Terry et al., 2006), we used the constitutively expressed somatic gene *fringe* as an internal control. Indeed, we found that the *Socs36E* transcript level in *dUTX* testes decreased to ~65% of the level in the wt control (Fig. 3A). However, using the entire testes might underestimate the change in *Socs36E* transcript level.

Previously, chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) data revealed that both the active histone modification H3K4me3 and RNA Pol II are enriched near the transcription start site (TSS) of *Socs36E* (Gan et al., 2010) (supplementary material Fig. S3A). By contrast, the repressive

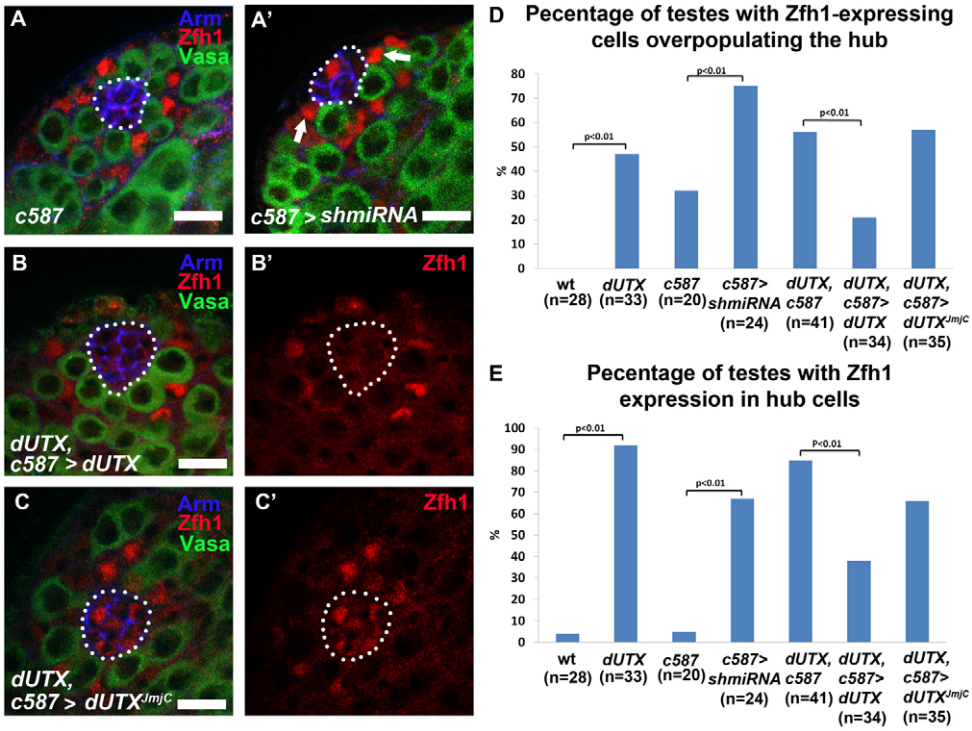


**Fig. 2. dUTX acts as a histone demethylase in CySCs and/or early cyst cells to repress overpopulation of Zfh1-expressing cells around the hub and ectopic Zfh1 expression in hub cells.** (**A-C′**) Immunostaining using antibodies against Arm (blue), Vasa (green) and Zfh1 (red). Hub area is outlined (white dotted line). (A) *c587-Gal4*. (A′) *c587-Gal4; UAS-dUTX shmiRNA*; arrows point to Zfh1-expressing cells with nuclei that directly contact the hub. (B,B′) *c587-Gal4; UAS-dUTX* and (C,C′) *c587-Gal4; UAS-dUTX*$^{JmjC}$, both in a *dUTX* background. (**D**) Percentage of testes with overpopulating Zfh1-expressing cells around the hub. (**E**) Percentage of testes with ectopic Zfh1 expression in hub cells. *P*-value calculated using Fisher's test. Scale bars: 10 μm.

histone modification H3K27me3 was depleted at the same region around the TSS of *Socs36E* (Gan et al., 2010) (supplementary material Fig. S3A). Because dUTX is an H3K27me3-specific demethylase (supplementary material Fig. S1B-C‴), we examined whether dUTX is required to remove H3K27me3 from the *Socs36E* TSS region, using anti-H3K27me3 ChIP followed by qPCR analysis. To generate high-resolution ChIP data, a 2 kb genomic region around the *Socs36E* TSS was divided into 400 bp intervals and tested for H3K27me3 binding using a series of primer sets (p1-p5 in Fig. 3B; supplementary material Fig. S3A). Control experiments were performed using two primer sets around the TSS of the control gene *fringe* (p7 and p8 in supplementary material Fig. S3B), as well as a primer set within the *Socs36E* gene body (p6 in supplementary material Fig. S3A). Consistent with decreased transcription of *Socs36E* in *dUTX* testes (Fig. 3A), there was a ~4-fold enrichment of the repressive H3K27me3 mark at the p2 region in the *dUTX* testes compared with the wt control (Fig. 3B). The H3K27me3 binding profile at the *Socs36E* locus was consistent with the published ChIP-seq results (Gan et al., 2010), which showed a peak enrichment of H3K27me3 at ~200-400 bp downstream of the TSSs of target genes. By contrast, the control regions showed similar H3K27me3 binding between *dUTX* and wt testes (supplementary material Fig. S3A,B, see numbers underneath control regions p6-p8). Furthermore, recently published ChIP-chip data using anti-dUTX antibody showed enrichment of dUTX around the *Socs36E* TSS region (supplementary material Fig. S3C) (Tie et al., 2012). Taken together, these results demonstrate that dUTX directly regulates *Socs36E* transcription by removing the repressive H3K27me3 histone modification from its TSS region.

We next examined whether overexpression of *Socs36E* independent of its genomic context is sufficient to rescue the niche architectural defects in *dUTX* testes. To achieve this, a *UAS-Socs36E* cDNA transgene (Callus and Mathey-Prevot, 2002) was driven by either the *upd-Gal4* or the *c587-Gal4* driver. Whereas *upd>Socs36E* failed to suppress the *dUTX* phenotype (Fig. 3C, arrows point to Zfh1-positive cells around the hub), *c587>Socs36E* reduced the overpopulation of Zfh1-expressing cells around the hub in 93% of *dUTX* testes (Fig. 3D), further suggesting that *Socs36E* is a critical target gene of dUTX in CySCs. In summary, dUTX acts in CySCs and/or early cyst cells to directly regulate the chromatin state of the *Socs36E* gene locus.

## dUTX activates *Socs36E* transcription to control JAK-STAT signaling activity in the testis niche

Because Socs36E acts as a negative regulator of the JAK-STAT pathway (Terry et al., 2006), we assessed JAK-STAT signaling activity in the presence and absence of dUTX. In wt testes, Stat92E is enriched in GSCs and in some of their immediate daughter cells, called gonialblasts (GBs), but rapidly declines in further differentiated cells. Stat92E is also present in CySCs but is absent in hub cells (Leatherman and Dinardo, 2008) (Fig. 4A,A′). By contrast, Stat92E was ectopically turned on in *dUTX* testes (Fig. 4B,B′), including hub cells and further differentiated somatic cells. Using a *2×Stat-GFP* reporter, which reflects Stat92E activity in CySCs (Bach et al., 2007), we found that the GFP reporter was ectopically turned on in further differentiated cyst cells in *dUTX* mutant testes (supplementary material Fig. S4B,B′), but not in the heterozygous control (supplementary material Fig. S4A,A′).

By qRT-PCR, we detected a ~1.5-fold increase of the *Stat92E* transcript in *dUTX* testes compared with the wt control (Fig. 4C). However, because we used whole testes for this analysis, the change in *Stat92E* transcript levels might be underestimated. Furthermore, knockdown of *dUTX* using the cyst cell driver *c587-Gal4* (Fig. 4E,E′), but not the germ cell driver *nos-Gal4* (supplementary material Fig. S4C,C′ versus S4D,D′) nor the hub cell driver *upd-Gal4* (supplementary material Fig. S4E,E′ versus S4F,F′), led to ectopic Stat92E in further differentiated cells, similar to the phenotype observed in *dUTX* mutant testes. In addition, removing one copy of *Stat92E* using a strong loss-of-function allele (Hou et

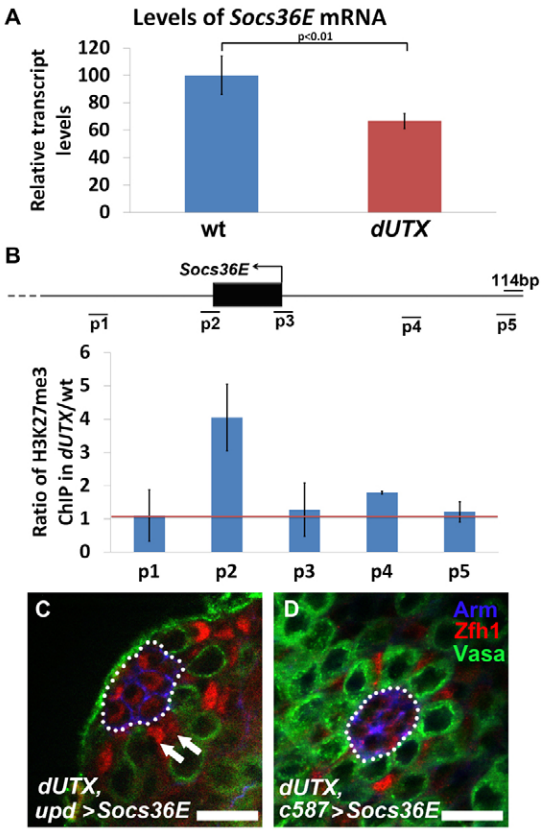


**Fig. 3. dUTX removes the repressive H3K27me3 histone modification at the *Socs36E* genomic locus and allows active transcription of *Socs36E*.** (**A**) *Socs36E* mRNA measured by qRT-PCR in three independent biological replicates, normalized by *fringe*. (**B**) Anti-H3K27me3 ChIPed DNA analyzed by qPCR, normalized to input (percentage input) and then compared between *dUTX* testes and wt controls, based on three independent biological replicates. *P*-value calculated using Student's *t*-test. Error bars represent s.d. (**C**,**D**) Immunostaining using antibodies against Arm (blue), Vasa (green) and Zfh1 (red). Hub area is outlined (white dotted line). (C) *upd-Gal4; UAS-Socs36E-cDNA* transgene in a *dUTX* background; arrows point to overpopulating Zfh1-expressing cells with nuclei that directly contact the hub. (D) *c587-Gal4; UAS-Socs36E-cDNA* transgene in a *dUTX* background. Scale bars: 10 μm.

al., 1996) suppressed the overpopulation of Zfh1-expressing cells around the hub in 90% of *dUTX* testes (Fig. 4F,F′), suggesting that hyperactivation of JAK-STAT signaling causes the niche architectural defects in *dUTX* testes. Together, these results indicate that dUTX acts in CySCs and early cyst cells to prevent ectopic JAK-STAT signaling activity.

## dUTX acts in CySCs to maintain proper gene expression in hub cells

Our results also revealed dynamic communication among different cell types within the testis niche, where CySCs can send feedback to hub cells to maintain proper gene expression. We found that *zfh1*, a target gene of the Stat92E transcription factor (Leatherman and Dinardo, 2008; Terry et al., 2006), was ectopically expressed in hub cells in 92% of *dUTX* testes (Fig. 1C,C′; Fig. 2E, compare the first and second columns). However, the total number of hub cells did not change in *dUTX* testes [13±2.3 hub cells for *dUTX* third instar testes ($n$=15) versus 13.6±2.0 hub cells for wt third instar testes ($n$=18), $P$>0.05]. In addition, no hub cells underwent cell death in *dUTX* testes as determined by immunostaining with anti-Caspase 3, an apoptotic marker ($n$=30), and none underwent mitosis as determined by immunostaining with anti-phospho-histone H3 (H3S10P; $n$=96), suggesting that hub cells maintain their number but turn on Zfh1 expression ectopically.

Because both the hub cells and cyst cells in adult testes originate from the same group of somatic gonadal precursors (SGPs) in embryonic testes (Le Bras and Van Doren, 2006), one possibility for ectopic Zfh1 expression in hub cells from adult testes is that Zfh1 becomes misexpressed in hub precursor cells in *dUTX* embryonic testes. In order to test this possibility, we induced *dUTX*$^{1}$ mutant mitotic clones in adult testes and found it to be sufficient to cause Zfh1 misexpression in hub cells (supplementary material Fig. S5). These results suggest that ectopic Zfh1 expression in hub cells is due to loss of *dUTX* in CySCs and/or GSCs, the two cell types capable of forming mitotic clones next to the hub cells. Furthermore, we found that shmiRNA knockdown of *dUTX* in CySCs and/or early cyst cells using *c587-Gal4* (Fig. 2A,A′ and 2E, compare the third and fourth columns) is sufficient to turn on Zfh1 expression ectopically in hub cells. By contrast, neither *nos-Gal4* (supplementary material Fig. S2A,A′) nor *upd-Gal4* (supplementary material Fig. S2B,B′) driving *dUTX shmiRNA* resulted in a similar phenotype. Together, these data demonstrate that loss of dUTX in CySCs leads to ectopic Zfh1 expression in hub cells.

In addition, we found that the catalytic domain of dUTX is required to prevent ectopic Zfh1 expression in hub cells, as expression of the wild-type *dUTX* transgene rescued this phenotype (Fig. 2B,B′ and 2E, compare the fifth and sixth columns). We also observed partial rescue upon expression of the *dUTX*$^{JmjC}$ transgene (Fig. 2C,C′ and 2E, compare the fifth and seventh columns), suggesting a demethylase-independent role of dUTX in regulating proper gene expression in hub cells. Finally, restoring *Socs36E* expression in CySCs and early cyst cells (Fig. 3D) or removing one copy of *Stat92E* (Fig. 4F,F′) reduced ectopic Zfh1 expression in the hub cells of *dUTX* testes from 92% to 43-45%. Together, these data demonstrate that dUTX acts primarily as a histone demethylase in CySCs to prevent ectopic Zfh1 expression in hub cells by maintaining proper JAK-STAT signaling activity.

## dUTX maintains hub architecture by regulating DE-Cadherin levels in GSCs

We found that dUTX also acts in germ cells to maintain proper hub size. Whereas dUTX directly regulates *Socs36E* transcription, unlike *Socs36E* testes (Issigonis et al., 2009) *dUTX* testes do not have decreased GSC numbers [12.8±3.0 for *dUTX* third instar testes ($n$=98) versus 12.6±3.0 for wt third instar testes ($n$=80), $P$>0.05]. This was due to a significant increase in hub area (Fig. 5A-C) in *dUTX* testes, which accommodated the overpopulation of Zfh1-expressing cells around the hub without affecting GSC number. As mentioned previously, the increase in hub area in *dUTX* testes could not be attributed to an increase in hub cell number. However, we did observe an increase in individual hub cell size in *dUTX* testes compared with the wt control (Fig. 5D). In addition, knockdown of *dUTX* using the germ cell driver *nos-Gal4*, but not the cyst cell driver *c587-Gal4* nor the hub cell driver *upd-Gal4*, led to an increased hub area (Fig. 5E).

In wt testes, GSCs are attached to the hub via DE-Cadherin-mediated adherens junctions (Jenkins et al., 2003; Yamashita et al., 2003), resulting in a rosette-like structure (Fig. 5A,A′). The GSC-hub interface in wt testes averaged 4.3 μm (Fig. 6C, first column).

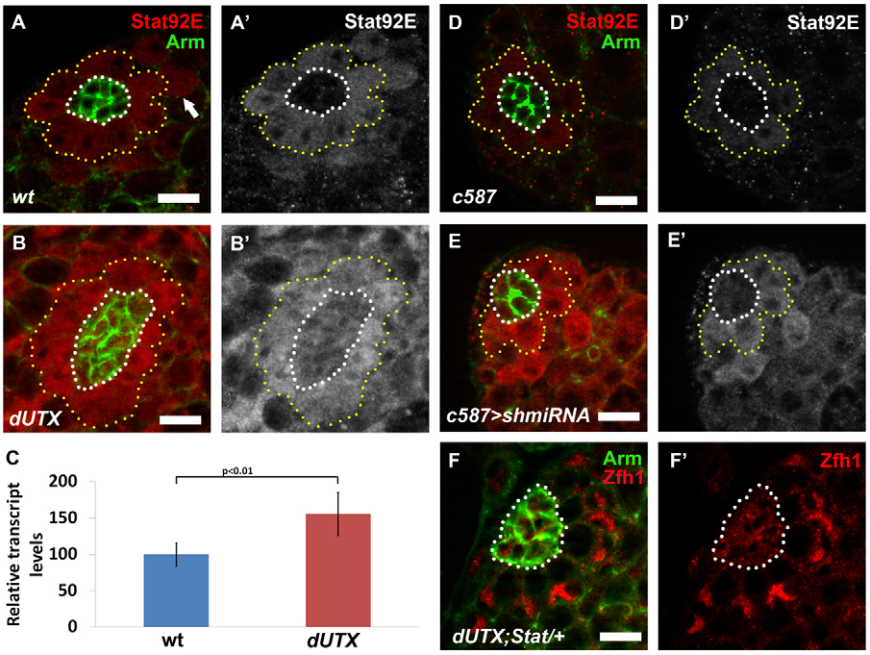


**Fig. 4. dUTX is required in CySCs and early cyst cells to prevent hyperactivation of the JAK-STAT signaling pathway.** (**A-B′**) Immunostaining using antibodies against Arm (green) and Stat92E (red). Hub area is outlined by white dotted line and stem cell zone by yellow dotted line. (A,A′) wt testis. Arrow points to a gonialblast that is positive for anti-Stat92E staining. (B,B′) *dUTX* testis. (**C**) *Stat92E* mRNA measured by qRT-PCR in five independent biological replicates, normalized by *fringe*. *P*-value calculated using Student's *t*-test. Error bars represent s.d. (**D-E′**) Immunostaining using anti-Arm (green) and anti-Stat92E (red) in (D,D′) *c587-Gal4* control and (E,E′) *c587-Gal4; UAS-dUTX shmiRNA* testes. (**F**,**F′**) Immunostaining using anti-Arm (green) and anti-Zfh1 (red) in *dUTX; Stat92E/+* testes. Scale bars: 10 µm.

By contrast, the GSC-hub interface in *dUTX* testes was disrupted (Fig. 5B,B′, arrows). GSCs appeared to intrude into the hub area, leading to an increase of the GSC-hub interface to an average of 5.9 µm (Fig. 6C, second column). We examined whether this defect in the *dUTX* mutant niche is due to misregulation of DE-Cadherin. Using qRT-PCR we detected a ~2-fold increase in the *DE-Cadherin* (*shotgun* – FlyBase) transcript level in *dUTX* testes compared with that in the wt control (Fig. 6D). Additionally, we found that expression of a dominant-negative form of DE-Cadherin (*UAS-DE-Cad*$^{dCR4h}$) (Inaba et al., 2010) in germ cells suppressed the *dUTX* hub size defect (Fig. 6A,A′,E) and resulted in a decrease of the GSC-hub interface (Fig. 6C, third column). By contrast, overexpression of the wild-type DE-Cadherin (*UAS-DE-Cad*$^{DEFL}$) (Inaba et al., 2010) in germ cells enhanced the *dUTX* hub size defect (Fig. 6B,B′,E) and led to an increase of the GSC-hub interface (Fig. 6B, arrows, and 6C, fourth column). As a control, when both forms of DE-Cadherin were expressed in germ cells in the wt background, no obvious defect was detected (Fig. 6C,E). *DE-Cadherin* is unlikely to be the only target gene of dUTX in germ cells. Therefore, although mutations in dUTX lead to upregulated *DE-Cadherin* transcript levels, overexpression of DE-Cadherin itself in germ cells is not sufficient to recapitulate the *dUTX* loss-of-function phenotype. In summary, our data demonstrate that dUTX acts in germ cells to maintain the proper GSC-hub interface and hub size by regulating *DE-Cadherin* transcription.

## DISCUSSION

In this study, we identify a new epigenetic mechanism that negatively regulates the JAK-STAT signaling pathway in the *Drosophila* testis niche (Fig. 6F): the H3K27me3-specific demethylase dUTX acts in CySCs to remove the repressive H3K27me3 histone modification near the TSS of *Socs36E* to allow its active transcription. Socs36E acts upstream to suppress Stat92E activity and to restrict CySCs from overpopulating the testis niche. In addition, dUTX acts in CySCs to prevent hyperactivation of Stat92E in hub cells, which would otherwise ectopically turn on Zfh1 expression. When we ectopically drove *zfh1* cDNA in hub cells using the *upd-Gal4* driver, no obvious defect could be identified. Therefore, the biological consequence of ectopic Zfh1 expression in hub cells remains unclear. However, ectopic Zfh1 expression in hub cells and the overpopulation of Zfh1-expressing cells around the hub are two connected phenomena because both phenotypes are caused by loss of *dUTX* in CySCs.

dUTX also acts in GSCs to regulate DE-Cadherin levels to maintain proper GSC-hub interaction and normal morphology of the hub. It has been reported that differential expression of different cadherins causes cells with similar cadherin types and levels to aggregate (Friedlander et al., 1989; Steinberg and Takeichi, 1994). In wt testes, hub cells express higher levels of DE-Cadherin and therefore tightly associate with each other (Le Bras and Van Doren, 2006). Loss of *dUTX* in germ cells leads to higher levels of DE-Cadherin in GSCs, which probably allows them to intermingle with hub cells and causes disrupted hub architecture. It has also been demonstrated that the major role of JAK-STAT in GSCs is for GSC-hub adhesion (Leatherman and Dinardo, 2010), suggesting that the expression and/or activity of cell-cell adhesion molecules, such as DE-Cadherin, depends on JAK-STAT signaling. Therefore, the abnormal DE-Cadherin activity in GSCs in *dUTX* testis could also result from misregulated JAK-STAT signaling in the testis niche.

### dUTX is a new negative epigenetic regulator of the JAK-STAT signaling pathway

The JAK-STAT signaling pathway plays crucial roles in stem cell maintenance in many different stem cell types across a wide range of species. Here, our studies identify the histone demethylase dUTX as a new upstream regulator of the JAK-STAT pathway, which directly controls the transcription of *Socs36E*. In addition to acting as an antagonist of JAK-STAT signaling, *Socs36E* has been reported to be a direct target gene of the Stat92E transcription factor (Terry et al., 2006). Therefore, increased Stat92E would be expected to upregulate *Socs36E* expression, but this was not observed in *dUTX* mutant testes. Instead, our data revealed that *Socs36E* expression decreased, whereas *Stat92E* expression increased, in *dUTX* testes, consistent with the hypothesis that *Socs36E* is a direct target gene of dUTX and acts upstream of *Stat92E*.

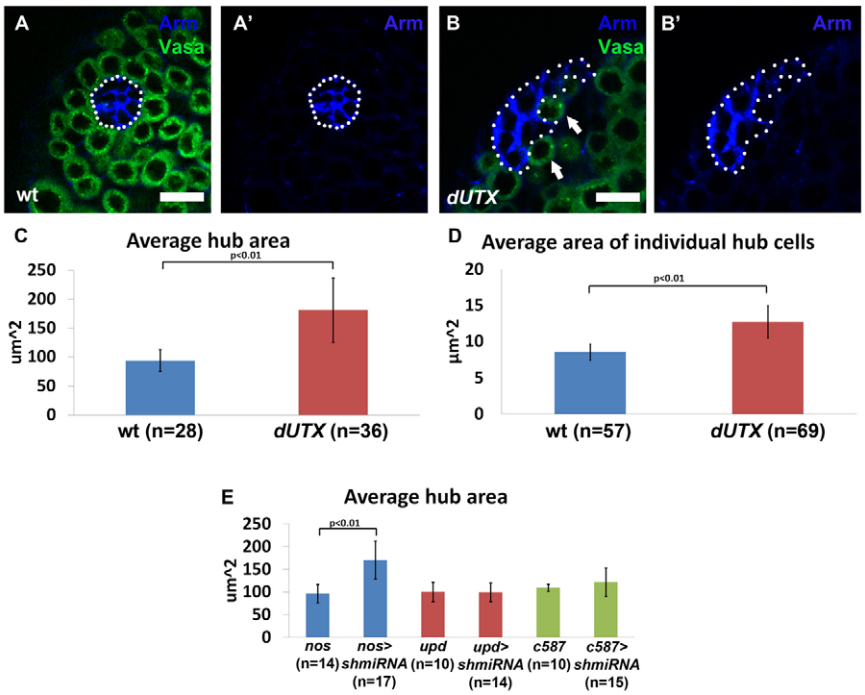


**Fig. 5. dUTX acts in germ cells to maintain proper hub size.** (**A-B′**) Immunostaining using antibodies against Arm (blue) and Vasa (green). Hub area is outlined (white dotted line). (A,A′) wt testis. (B,B′) *dUTX* testis displays enlarged hub. Arrows indicate GSCs with disrupted GSC-hub interface. (**C**) Quantification of average hub area: 94±18.65 μm$^2$ in wt testes versus 181±55.5 μm$^2$ in *dUTX* testes. (**D**) Quantification of average area of individual hub cells: 8.5±1.1 μm$^2$ in wt testes versus 12.7±2.2 μm$^2$ in *dUTX* testes. (**E**) Quantification of average hub area in testes from males of the following genotypes: *nos-Gal4* control (96±20.35 μm$^2$); *nos-Gal4; UAS-dUTX shmiRNA* (170±41.7 μm$^2$, $P<0.01$); *upd-Gal4* control (100±21.4 μm$^2$); *upd-Gal4; UAS-dUTX shmiRNA* (99±20.3 μm$^2$, $P>0.05$); *c587-Gal4* control (109±7.7 μm$^2$); *c587-Gal4; UAS-dUTX shmiRNA* (121±31.4 μm$^2$, $P>0.05$). *P*-value calculated using Student's *t*-test. Error bars represent s.d. Scale bars: 10 μm.

Sustained activity of the JAK-STAT pathway in cyst cells has been reported to activate BMP signaling, which leads to GSC self-renewal outside the niche and gives rise to a tumor-like phenotype in testis (Leatherman and Dinardo, 2010). To examine BMP pathway activity, we performed immunostaining experiments using antibodies against phospho-SMAD (pSMAD), a downstream target of BMP signaling. We did not detect any obvious difference in the pSMAD signal between the *dUTX* testes and wt control (data not shown), nor did we detect any germline tumors in *dUTX* testes. We speculate that germline tumor formation upon activation of the JAK-STAT pathway is secondary to the overproliferation of Zfh1-expressing cells, which was not observed in *dUTX* testes.

## dUTX coordinates crosstalk among different cell types within the testis niche

Our study also provides an example of the multidimensional cell-cell communication that takes place within a stem cell niche. Many studies of the stem cell niche have focused on understanding niche-to-stem cell signaling in controlling stem cell identity and activity. For example, in the *Drosophila* female GSC niche, Upd secreted from terminal filaments activates the JAK-STAT pathway in cap cells and escort cells, which subsequently produce the BMP pathway ligand Decapentaplegic (Dpp) to activate BMP signaling and prevent transcription of the differentiation factor *bag-of-marbles* (*bam*) in GSCs (Chen and McKearin, 2003; López-Onieva et al., 2008). In the *Drosophila* intestinal stem cell (ISC) niche, the visceral muscle cells underlying the intestine secrete Wingless to activate Wnt signaling and Upd to activate JAK-STAT signaling in ISCs, which are required to maintain ISC identity and activity (Beebe et al., 2010; Jiang et al., 2009; Lin et al., 2008; Lin et al., 2010; Xu et al., 2011).

More studies have now revealed the multidirectionality of signaling within the stem cell niche. For example, in the *Drosophila* female GSC niche, GSCs activate Epidermal growth factor receptor (Egfr) signaling in the neighboring somatic cells, which subsequently represses expression of the glypican Dally, a protein required for the stabilization and mobilization of the BMP pathway ligand Dpp. Through this communication between GSCs and the surrounding somatic cells, only GSCs maintain high BMP signaling (Liu et al., 2010). Here, our studies establish another example of the multidimensional cell-cell communications that occur within the testis stem cell niche, where CySCs and GSCs have distinct roles in regulating hub cell identity and morphology.

## Distinct biological functions of histone demethylases

Our data identified new roles of a histone demethylase in regulating endogenous stem cell niche architecture and proper gene expression. Previous studies have reported *in vivo* functions of histone demethylases in several model organisms. For example, mammalian UTX has been shown to associate with the H3K4me3 histone methyltransferase MLL2 (Issaeva et al., 2007), suggesting its potential antagonistic role to the PcG proteins. The PcG proteins play a crucial role in Hox gene silencing in both *Drosophila* and mammals (Beuchle et al., 2001; Ringrose and Paro, 2007; Schuettengruber et al., 2007; Schwartz and Pirrotta, 2007). Consistently, mammalian UTX has been reported to directly bind and activate the *HOXB1* gene locus (Agger et al., 2007). In addition to antagonizing PcG function, H3K27me3 demethylases play crucial roles during development. For example, in zebrafish, inactivating the *UTX* homolog (*kdm6al*) using morpholino oligonucleotides leads to defects in posterior development (Lan et al., 2007), and in *C. elegans* the dUTX homolog (UTX-1) is required for embryonic and postembryonic development (Vandamme et al., 2012), including gonad development (Agger et al., 2007). Furthermore, loss of UTX function in embryonic stem cells leads to defects in mesoderm differentiation (Wang et al., 2012), and somatic cells derived from *UTX* loss-of-function human or mouse tissue fail to return to the ground state of pluripotency (Mansour et al., 2012). These reports demonstrate that UTX is not only required for proper cellular differentiation but also for successful reprogramming. However,

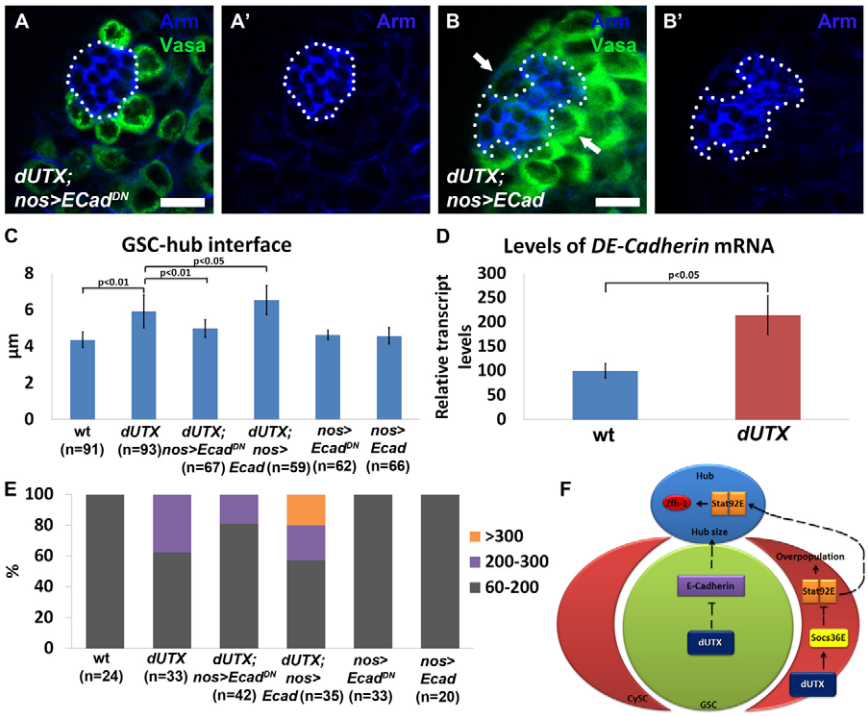


**Fig. 6. dUTX controls hub size through regulating DE-Cadherin levels in GSCs and model of dUTX function in the testis niche.** (**A-B'**) Immunostaining for Arm (blue) and Vasa (green). Hub area is outlined (white dotted line). (A,A') *dUTX; nos>DE-Cad*$^{dCR4h}$ testis. (B,B') *dUTX; nos>DE-Cad*$^{DEFL}$. Arrows indicate GSCs with disrupted GSC-hub interface. (**C**) Quantification of the average GSC-hub interface in testes from males of the following genotypes: wt (4.3±0.4 µm); *dUTX* (5.9±0.9 µm); *dUTX; nos>DE-Cad*$^{dCR4h}$ (4.9±0.5 µm); *dUTX; nos>DE-Cad*$^{DEFL}$ (6.5±0.8 µm); *dUTX/+; nos>DE-Cad*$^{dCR4h}$ control (4.6±0.3 µm); *dUTX/+; nos>DE-Cad*$^{DEFL}$ control (4.5±0.4 µm). (**D**) *DE-Cadherin* mRNA measured by qRT-PCR in three independent biological replicates, normalized by *RpL32*. (**E**) Quantification of percentage of testes with average hub area of 60-200 µm$^2$, 200-300 µm$^2$ or exceeding 300 µm$^2$, from the following males (left to right): wt; *dUTX* mutant; *dUTX; nos>DE-Cad*$^{dCR4h}$; *dUTX; nos>DE-Cad*$^{DEFL}$; *dUTX/+; nos>DE-Cad*$^{dCR4h}$ control; *dUTX/+; nos>DE-Cad*$^{DEFL}$ control. (**F**) Outline of dUTX functions in the *Drosophila* testis niche. dUTX negatively regulates the JAK-STAT signaling pathway in CySCs and hub cells. dUTX also regulates DE-Cadherin levels in GSCs to maintain hub architecture (see Discussion for details). *P*-value by Student's *t*-test. Error bars represent s.d. Scale bars: 10 µm.

despite multiple reports on the *in vivo* roles of H3K27me3-specific demethylases, little is known about their functions in any endogenous adult stem cell system.

Whereas mammals have multiple H3K27me3 demethylases, dUTX is the sole H3K27me3-specific demethylase in *Drosophila*. This unique feature, plus the well-characterized nature of *Drosophila* adult stem cell systems, make interpretation of the endogenous functions of histone demethylases in *Drosophila* unambiguous. Because mammalian UTX has been reported as a tumor suppressor (van Haaften et al., 2009), understanding the endogenous functions of dUTX in an adult stem cell system might facilitate the use of histone demethylases for cancer treatment.

In summary, our results demonstrate that stem cells send feedback to the niche cells to maintain their proper gene expression and morphology. Furthermore, this feedback is regulated through the JAK-STAT signaling pathway, the activity of which is controlled by a chromatin factor, providing an example of crosstalk between these two regulatory pathways.

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**Competing interests statement**
The authors declare no competing financial interests.

**Supplementary material**
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**References**

**Agger, K., Cloos, P. A., Christensen, J., Pasini, D., Rose, S., Rappsilber, J., Issaeva, I., Canaani, E., Salcini, A. E. and Helin, K.** (2007). UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature* **449**, 731-734.
**Bach, E. A., Ekas, L. A., Ayala-Camargo, A., Flaherty, M. S., Lee, H., Perrimon, N. and Baeg, G. H.** (2007). GFP reporters detect the activation of the Drosophila JAK/STAT pathway in vivo. *Gene Expr. Patterns* **7**, 323-331.
**Becker, P. B. and Hörz, W.** (2002). ATP-dependent nucleosome remodeling. *Annu. Rev. Biochem.* **71**, 247-273.
**Beebe, K., Lee, W. C. and Micchelli, C. A.** (2010). JAK/STAT signaling coordinates stem cell proliferation and multilineage differentiation in the Drosophila intestinal stem cell lineage. *Dev. Biol.* **338**, 28-37.
**Beuchle, D., Struhl, G. and Müller, J.** (2001). Polycomb group proteins and heritable silencing of Drosophila Hox genes. *Development* **128**, 993-1004.
**Boyle, M., Wong, C., Rocha, M. and Jones, D. L.** (2007). Decline in self-renewal factors contributes to aging of the stem cell niche in the Drosophila testis. *Cell Stem Cell* **1**, 470-478.

**Bracken, A. P., Pasini, D., Capra, M., Prosperini, E., Colli, E. and Helin, K.** (2003). EZH2 is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer. *EMBO J.* **22**, 5323-5335.
**Buszczak, M. and Spradling, A. C.** (2006). Searching chromatin for stem cell identity. *Cell* **125**, 233-236.
**Buszczak, M., Paterno, S. and Spradling, A. C.** (2009). Drosophila stem cells share a common requirement for the histone H2B ubiquitin protease scrawny. *Science* **323**, 248-251.
**Callus, B. A. and Mathey-Prevot, B.** (2002). SOCS36E, a novel Drosophila SOCS protein, suppresses JAK/STAT and EGF-R signalling in the imaginal wing disc. *Oncogene* **21**, 4812-4821.
**Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R. S. and Zhang, Y.** (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* **298**, 1039-1043.
**Chen, D. and McKearin, D.** (2003). Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. *Curr. Biol.* **13**, 1786-1791.
**Chen, X., Lu, C., Prado, J. R., Eun, S. H. and Fuller, M. T.** (2011). Sequential changes at differentiation gene promoters as they become active in a stem cell lineage. *Development* **138**, 2441-2450.
**Cheng, J., Türkel, N., Hemati, N., Fuller, M. T., Hunt, A. J. and Yamashita, Y. M.** (2008). Centrosome misorientation reduces stem cell division during ageing. *Nature* **456**, 599-604.
**Cherry, C. M. and Matunis, E. L.** (2010). Epigenetic regulation of stem cell maintenance in the Drosophila testis via the nucleosome-remodeling factor NURF. *Cell Stem Cell* **6**, 557-567.
**Clapier, C. R. and Cairns, B. R.** (2009). The biology of chromatin remodeling complexes. *Annu. Rev. Biochem.* **78**, 273-304.
**de Cuevas, M. and Matunis, E. L.** (2011). The stem cell niche: lessons from the Drosophila testis. *Development* **138**, 2861-2869.
**Eliazer, S., Shalaby, N. A. and Buszczak, M.** (2011). Loss of lysine-specific demethylase 1 nonautonomously causes stem cell tumors in the Drosophila ovary. *Proc. Natl. Acad. Sci. USA* **108**, 7064-7069.
**Friedlander, D. R., Mège, R. M., Cunningham, B. A. and Edelman, G. M.** (1989). Cell sorting-out is modulated by both the specificity and amount of different cell adhesion molecules (CAMs) expressed on cell surfaces. *Proc. Natl. Acad. Sci. USA* **86**, 7043-7047.
**Gan, Q., Schones, D. E., Ho Eun, S., Wei, G., Cui, K., Zhao, K. and Chen, X.** (2010). Monovalent and unpoised status of most genes in undifferentiated cell-enriched Drosophila testis. *Genome Biol.* **11**, R42.
**Herz, H. M., Madden, L. D., Chen, Z., Bolduc, C., Buff, E., Gupta, R., Davuluri, R., Shilatifard, A., Hariharan, I. K. and Bergmann, A.** (2010). The H3K27me3 demethylase dUTX is a suppressor of Notch- and Rb-dependent tumors in Drosophila. *Mol. Cell. Biol.* **30**, 2485-2497.
**Hou, X. S., Melnick, M. B. and Perrimon, N.** (1996). Marelle acts downstream of the Drosophila HOP/JAK kinase and encodes a protein similar to the mammalian STATs. *Cell* **84**, 411-419.
**Inaba, M., Yuan, H., Salzmann, V., Fuller, M. T. and Yamashita, Y. M.** (2010). E-cadherin is required for centrosome and spindle orientation in Drosophila male germline stem cells. *PLoS ONE* **5**, e12473.
**Issaeva, I., Zonis, Y., Rozovskaia, T., Orlovsky, K., Croce, C. M., Nakamura, T., Mazo, A., Eisenbach, L. and Canaani, E.** (2007). Knockdown of ALR (MLL2) reveals ALR target genes and leads to alterations in cell adhesion and growth. *Mol. Cell. Biol.* **27**, 1889-1903.
**Issigonis, M., Tulina, N., de Cuevas, M., Brawley, C., Sandler, L. and Matunis, E.** (2009). JAK-STAT signal inhibition regulates competition in the Drosophila testis stem cell niche. *Science* **326**, 153-156.
**Jenkins, A. B., McCaffery, J. M. and Van Doren, M.** (2003). Drosophila E-cadherin is essential for proper germ cell-soma interaction during gonad morphogenesis. *Development* **130**, 4417-4426.
**Jiang, H., Patel, P. H., Kohlmaier, A., Grenley, M. O., McEwen, D. G. and Edgar, B. A.** (2009). Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. *Cell* **137**, 1343-1355.
**Kiger, A. A., Jones, D. L., Schulz, C., Rogers, M. B. and Fuller, M. T.** (2001). Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. *Science* **294**, 2542-2545.
**Kleer, C. G., Cao, Q., Varambally, S., Shen, R., Ota, I., Tomlins, S. A., Ghosh, D., Sewalt, R. G., Otte, A. P., Hayes, D. F. et al.** (2003). EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc. Natl. Acad. Sci. USA* **100**, 11606-11611.
**Klose, R. J., Kallin, E. M. and Zhang, Y.** (2006). JmjC-domain-containing proteins and histone demethylation. *Nat. Rev. Genet.* **7**, 715-727.
**Kondo, Y., Shen, L., Cheng, A. S., Ahmed, S., Boumber, Y., Charo, C., Yamochi, T., Urano, T., Furukawa, K., Kwabi-Addo, B. et al.** (2008). Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. *Nat. Genet.* **40**, 741-750.
**Lan, F., Bayliss, P. E., Rinn, J. L., Whetstine, J. R., Wang, J. K., Chen, S., Iwase, S., Alpatov, R., Issaeva, I., Canaani, E. et al.** (2007). A histone H3 lysine 27 demethylase regulates animal posterior development. *Nature* **449**, 689-694.
**Le Bras, S. and Van Doren, M.** (2006). Development of the male germline stem cell niche in Drosophila. *Dev. Biol.* **294**, 92-103.
**Leatherman, J. L. and Dinardo, S.** (2008). Zfh-1 controls somatic stem cell self-renewal in the Drosophila testis and nonautonomously influences germline stem cell self-renewal. *Cell Stem Cell* **3**, 44-54.
**Leatherman, J. L. and Dinardo, S.** (2010). Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in Drosophila testes. *Nat. Cell Biol.* **12**, 806-811.
**Lee, T. and Luo, L.** (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron* **22**, 451-461.
**Li, X. and Zhao, X.** (2008). Epigenetic regulation of mammalian stem cells. *Stem Cells Dev.* **17**, 1043-1052.
**Li, M. A., Alls, J. D., Avancini, R. M., Koo, K. and Godt, D.** (2003). The large Maf factor Traffic Jam controls gonad morphogenesis in Drosophila. *Nat. Cell Biol.* **5**, 994-1000.
**Lim, J. G. and Fuller, M. T.** (2012). Somatic cell lineage is required for differentiation and not maintenance of germline stem cells in Drosophila testes. *Proc. Natl. Acad. Sci. USA* **109**, 18477-18481.
**Lin, G., Xu, N. and Xi, R.** (2008). Paracrine Wingless signalling controls self-renewal of Drosophila intestinal stem cells. *Nature* **455**, 1119-1123.
**Lin, G., Xu, N. and Xi, R.** (2010). Paracrine unpaired signaling through the JAK/STAT pathway controls self-renewal and lineage differentiation of Drosophila intestinal stem cells. *J. Mol. Cell Biol.* **2**, 37-49.
**Liu, M., Lim, T. M. and Cai, Y.** (2010). The Drosophila female germline stem cell lineage acts to spatially restrict DPP function within the niche. *Sci. Signal.* **3**, ra57.
**López-Onieva, L., Fernández-Miñán, A. and González-Reyes, A.** (2008). Jak/Stat signalling in niche support cells regulates dpp transcription to control germline stem cell maintenance in the Drosophila ovary. *Development* **135**, 533-540.
**Manseau, L., Baradaran, A., Brower, D., Budhu, A., Elefant, F., Phan, H., Philp, A. V., Yang, M., Glover, D., Kaiser, K. et al.** (1997). GAL4 enhancer traps expressed in the embryo, larval brain, imaginal discs, and ovary of Drosophila. *Dev. Dyn.* **209**, 310-322.
**Mansour, A. A., Gafni, O., Weinberger, L., Zviran, A., Ayyash, M., Rais, Y., Krupalnik, V., Zerbib, M., Amann-Zalcenstein, D., Maza, I. et al.** (2012). The H3K27 demethylase Utx regulates somatic and germ cell epigenetic reprogramming. *Nature* **488**, 409-413.
**Metzger, E., Wissmann, M., Yin, N., Müller, J. M., Schneider, R., Peters, A. H., Günther, T., Buettner, R. and Schüle, R.** (2005). LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* **437**, 436-439.
**Morrison, S. J. and Spradling, A. C.** (2008). Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* **132**, 598-611.
**Müller, J., Hart, C. M., Francis, N. J., Vargas, M. L., Sengupta, A., Wild, B., Miller, E. L., O'Connor, M. B., Kingston, R. E. and Simon, J. A.** (2002). Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. *Cell* **111**, 197-208.
**Ni, J. Q., Zhou, R., Czech, B., Liu, L. P., Holderbaum, L., Yang-Zhou, D., Shim, H. S., Tao, R., Handler, D., Karpowicz, P. et al.** (2011). A genome-scale shRNA resource for transgenic RNAi in Drosophila. *Nat. Methods* **8**, 405-407.
**Ringrose, L. and Paro, R.** (2007). Polycomb/Trithorax response elements and epigenetic memory of cell identity. *Development* **134**, 223-232.
**Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B. and Cavalli, G.** (2007). Genome regulation by polycomb and trithorax proteins. *Cell* **128**, 735-745.
**Schwartz, Y. B. and Pirrotta, V.** (2007). Polycomb silencing mechanisms and the management of genomic programmes. *Nat. Rev. Genet.* **8**, 9-22.
**Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., Casero, R. A. and Shi, Y.** (2004). Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* **119**, 941-953.
**Smith, E. R., Lee, M. G., Winter, B., Droz, N. M., Eissenberg, J. C., Shiekhattar, R. and Shilatifard, A.** (2008). Drosophila UTX is a histone H3 Lys27 demethylase that colocalizes with the elongating form of RNA polymerase II. *Mol. Cell. Biol.* **28**, 1041-1046.
**Steinberg, M. S. and Takeichi, M.** (1994). Experimental specification of cell sorting, tissue spreading, and specific spatial patterning by quantitative differences in cadherin expression. *Proc. Natl. Acad. Sci. USA* **91**, 206-209.
**Terry, N. A., Tulina, N., Matunis, E. and DiNardo, S.** (2006). Novel regulators revealed by profiling Drosophila testis stem cells within their niche. *Dev. Biol.* **294**, 246-257.
**Tie, F., Banerjee, R., Conrad, P. A., Scacheri, P. C. and Harte, P. J.** (2012). The histone demethylase UTX and the chromatin remodeler BRM bind directly to Drosophila CBP and modulate its acetylation of histone H3 lysine 27. *Mol. Cell. Biol.* **32**, 2323-2334.
**Tulina, N. and Matunis, E.** (2001). Control of stem cell self-renewal in Drosophila spermatogenesis by JAK-STAT signaling. *Science* **294**, 2546-2549.

**Van Doren, M., Williamson, A. L. and Lehmann, R.** (1998). Regulation of zygotic gene expression in Drosophila primordial germ cells. *Curr. Biol.* **8**, 243-246.
**van Haaften, G., Dalgliesh, G. L., Davies, H., Chen, L., Bignell, G., Greenman, C., Edkins, S., Hardy, C., O'Meara, S., Teague, J. et al.** (2009). Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. *Nat. Genet.* **41**, 521-523.
**Vandamme, J., Lettier, G., Sidoli, S., Di Schiavi, E., Nørregaard Jensen, O. and Salcini, A. E.** (2012). The C. elegans H3K27 demethylase UTX-1 is essential for normal development, independent of its enzymatic activity. *PLoS Genet.* **8**, e1002647.
**Varambally, S., Dhanasekaran, S. M., Zhou, M., Barrette, T. R., Kumar-Sinha, C., Sanda, M. G., Ghosh, D., Pienta, K. J., Sewalt, R. G., Otte, A. P. et al.** (2002). The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* **419**, 624-629.
**Wang, C., Lee, J. E., Cho, Y. W., Xiao, Y., Jin, Q., Liu, C. and Ge, K.** (2012). UTX regulates mesoderm differentiation of embryonic stem cells independent of H3K27 demethylase activity. *Proc. Natl. Acad. Sci. USA* **109**, 15324-15329.
**Xu, T. and Rubin, G. M.** (1993). Analysis of genetic mosaics in developing and adult Drosophila tissues. *Development* **117**, 1223-1237.
**Xu, N., Wang, S. Q., Tan, D., Gao, Y., Lin, G. and Xi, R.** (2011). EGFR, Wingless and JAK/STAT signaling cooperatively maintain Drosophila intestinal stem cells. *Dev. Biol.* **354**, 31-43.
**Yamashita, Y. M., Jones, D. L. and Fuller, M. T.** (2003). Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. *Science* **301**, 1547-1550.
**Yamashita, Y. M., Mahowald, A. P., Perlin, J. R. and Fuller, M. T.** (2007). Asymmetric inheritance of mother versus daughter centrosome in stem cell division. *Science* **315**, 518-521.