

STEM CELLS AND REGENERATION

RESEARCH REPORT

SUMO regulates somatic cyst stem cell maintenance and directly targets the Hedgehog pathway in adult *Drosophila* testis

Xiangdong Lv^{1,*}, Chenyu Pan^{1,*}, Zhao Zhang¹, Yuanxin Xia¹, Hao Chen¹, Shuo Zhang¹, Tong Guo¹, Hui Han¹, Haiyun Song², Lei Zhang^{1,3} and Yun Zhao^{1,3,†}

ABSTRACT

SUMO (Small ubiquitin-related modifier) modification (SUMOylation) is a highly dynamic post-translational modification (PTM) that plays important roles in tissue development and disease progression. However, its function in adult stem cell maintenance is largely unknown. Here, we report the function of SUMOylation in somatic cyst stem cell (CySC) self-renewal in adult *Drosophila* testis. The SUMO pathway cell-autonomously regulates CySC maintenance. Reduction of SUMOylation promotes premature differentiation of CySCs and impedes the proliferation of CySCs, which leads to a reduction in the number of CySCs. Consistent with this, CySC clones carrying a mutation of the SUMO-conjugating enzyme are rapidly lost. Furthermore, inhibition of the SUMO pathway phenocopies disruption of the Hedgehog (Hh) pathway, and can block the proliferation of CySCs induced by Hh activation. Importantly, the SUMO pathway directly regulates the SUMOylation of Hh pathway transcription factor Cubitus interruptus (Ci), which is required for promoting CySC proliferation. Thus, we conclude that SUMO directly targets the Hh pathway and regulates CySC maintenance in adult *Drosophila* testis.

KEY WORDS: SUMOylation, Hedgehog, Adult stem cell, Testis, *Drosophila*

INTRODUCTION

PTMs regulate diverse cellular processes, including transcription, replication, and DNA repair (Flotho and Melchior, 2013). SUMOylation, first identified in the 1990s (Mahajan et al., 1997; Matunis et al., 1996), is a ubiquitin-like PTM (Hannoun et al., 2010; Smith et al., 2012). Through an enzymatic cascade involving E1-activating enzyme, E2-conjugating enzyme and E3 ligase, SUMO is finally attached to the substrate acceptor lysine (Lys, K) residue *in vivo* (Gareau and Lima, 2010; Hickey et al., 2012). Disruption of the SUMO pathway during embryogenesis is lethal in many species from *Drosophila* to mouse (Hickey et al., 2012). However, the function of SUMOylation in adults, especially in stem cell maintenance, is largely unknown.

Adult stem cells reside in a specific microenvironment called the niche to maintain their abilities of self-renewal and producing

daughter cells. In adult *Drosophila* testis, both germline stem cells (GSCs) and CySCs interact with a small group of somatic cells termed the hub, which produces major niche signals (de Cuevas and Matunis, 2011; Issigonis and Matunis, 2011; Losick et al., 2011; Voog et al., 2008) (Fig. 1A). Several signaling pathways have been reported to regulate CySCs, including the JAK-STAT pathway (Issigonis et al., 2009; Kiger et al., 2001; Leatherman and DiNardo, 2010; Singh et al., 2010; Tulina and Matunis, 2001), the EGFR pathway (Eun et al., 2014; Kiger et al., 2000; Tran et al., 2000), the Hpo pathway (Amoyel et al., 2014) and the Hh pathway (Amoyel et al., 2013; Michel et al., 2012; Zhang et al., 2013b). Although these pathways govern stem cell behavior, the function of PTMs during this process is rarely reported.

RESULTS AND DISCUSSION

The SUMO pathway is autonomously required for CySC maintenance

In order to identify novel factors that are autonomously required for CySC maintenance, we conducted a genetic screen for PTM regulators because PTMs regulate diverse cellular processes and might play important roles in CySCs. Genes were knocked down specifically in adult somatic cyst cells, by RNAi using *c587-Gal4* and a temperature-sensitive allele of Gal80 (*Gal80^{ts}*, a Gal4 inhibitor). Adult flies (0 to 3 days old) were shifted from 18°C to 29°C for 5 days to inactivate Gal80 and permit Gal4 to drive dsRNA expression. A zinc finger homeodomain 1 (Zfh1) antibody was used to mark CySCs and their direct daughters (Leatherman and DiNardo, 2008). As shown in Fig. 1A,C, cells with strong Zfh1 staining adjacent to the hub are CySCs, and those with lower Zfh1 staining level around the hub are newly formed cyst cells in wild-type (WT) testes. Interestingly, knocking down *Su(var)2-10* (*Suppressor of variegation 2-10*), a SUMO E3 ligase, significantly decreased the number of Zfh1⁺ cells (Fig. 1C,C',E,E') as early as 3 days after RNAi induction (Fig. 1B). To ascertain the function of the SUMO pathway, we knocked down *lwr* (*lesswright*, the only known SUMO E2 in *Drosophila*) in the same system, and observed a similar phenotype (Fig. 1B-D').

To confirm the phenotypes from the knockdown assays, we utilized the mosaic analysis with a repressible cell marker (MARCM) system (Lee and Luo, 2001) to generate clones for the hypomorphic allele *lwr⁴⁻³* (Apionishev et al., 2001). At 3 days after clone induction (ACI), GFP⁺ clones with strong Zfh1 staining (termed CySC clones) could be recovered in both control and *lwr⁴⁻³* testes (Fig. 1G-H"). At 5 days ACI, CySC clones could still be recovered in control testes (Fig. 1I-I"), whereas few *lwr⁴⁻³* testes contained CySC clones (Fig. 1J-J"). We then counted the percentage of testes with at least one CySC clone at 3, 5 or 7 days ACI to evaluate the stemness of clonal CySCs. Such fractions were comparable between control and *lwr⁴⁻³* mutants at 3 days ACI (Fig. 1F), suggesting comparable abilities to generate CySC clones.

¹State Key Laboratory of Cell Biology, CAS Center for Excellence in Molecular Cell Science, Innovation Center for Cell Signaling Network, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, P.R. China. ²Key Laboratory of Food Safety Research, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, P.R. China. ³School of Life Science and Technology, ShanghaiTech University, Shanghai 200031, P.R. China.
*These authors contributed equally to this work

[†]Author for correspondence (yunzhao@sibcb.ac.cn)

Received 9 September 2015; Accepted 16 March 2016

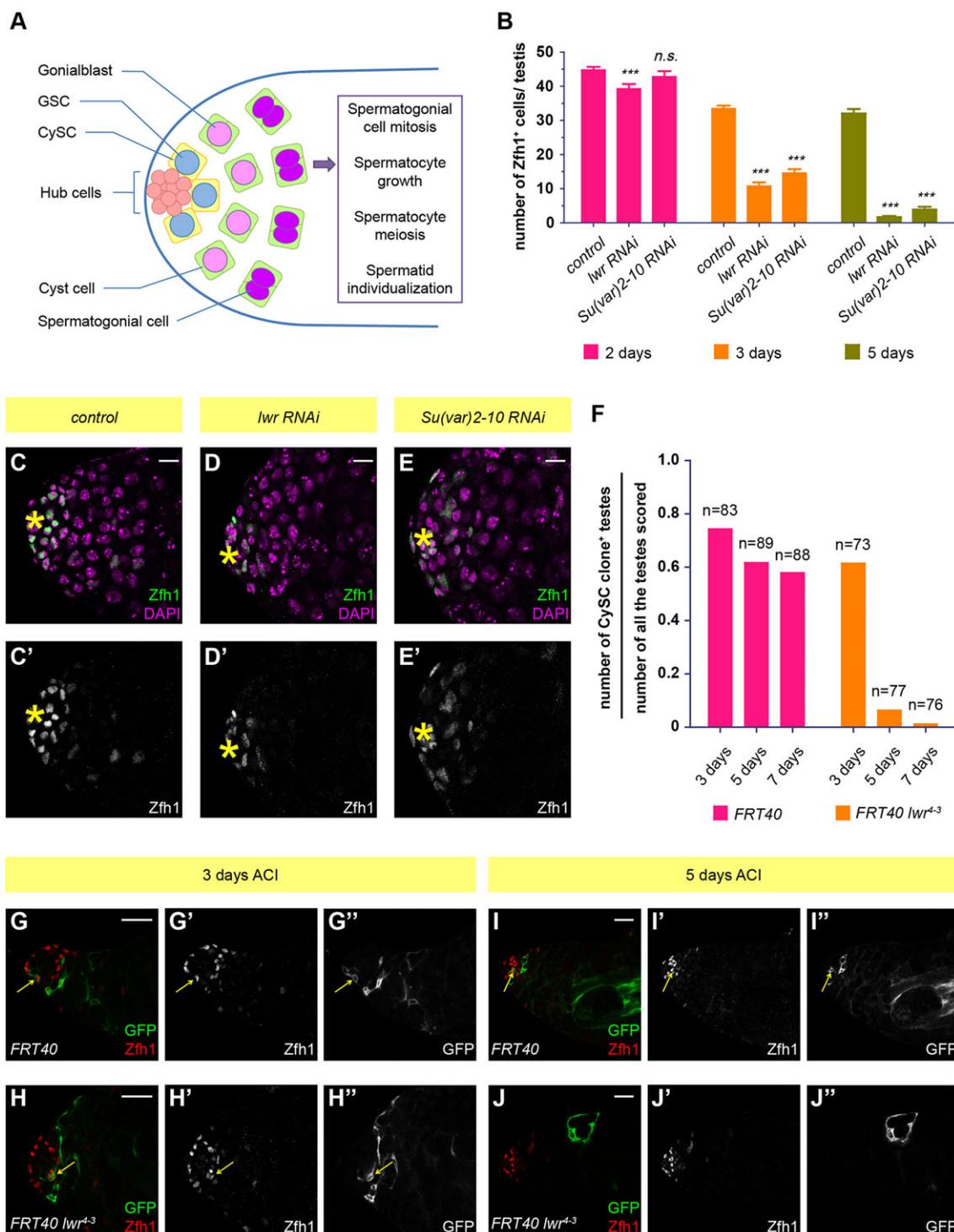


Fig. 1. The SUMO pathway is autonomously required for adult CySC maintenance. (A) Schematic of the *Drosophila* testis apex, showing different cell types: hub cells (red), germline stem cells (GSCs, blue), gonialblasts (pink), spermatogonial cells (purple), somatic cyst stem cells (CySCs, yellow), and differentiated cyst cells (green). (B) Numbers of *Zfh1*⁺ cells per testis with indicated genotypes at 2, 3 and 5 days after RNAi induction. Data are presented as mean±s.e.m. ***P<0.001; n.s., not significant; n>10. (C-E') Representative testes showing *Zfh1* (green) and DAPI (purple) staining, after RNAi induction for 3 days. Asterisks indicate the hub. Scale bars: 10 µm. (F) The fraction of testes with at least one CySC clone (*GFP*⁺ cell with strong *Zfh1* staining), at 3, 5 and 7 days after clone induction (ACI). (G-J') Representative testes showing clones with *Zfh1* (red) staining. Arrows indicate CySC clones. *FRT40*, control. Scale bars: 25 µm.

At 7 days, few testes contained detectable *lwr* mutant CySC clones, whereas CySC clones still existed in the control testes (Fig. 1F). Combining the results from RNAi assays with those from the MARCM assays, we conclude that SUMO pathway is autonomously required for maintaining the stemness of CySCs.

We also investigated the primary function of SUMOylation in GSCs maintenance. However, no obvious abnormality was observed even after *Su(var)2-10* or *lwr* was specifically knocked down in adult germline cells for 5 days (Fig. S1). Thus, we focused our further investigation on CySCs.

Reduction of SUMOylation induces differentiation and impedes proliferation of CySCs

Because CySCs were poorly maintained when the SUMO pathway was disrupted, we asked whether these phenotypes were caused by premature differentiation or impeded proliferation of CySCs. Eyes absent (Eya) was used to mark differentiated cyst cells, and it is normally undetectable in CySCs or their immediate daughter cyst

cells in WT testis (Fabrizio et al., 2003) (Fig. 2A). Yet we found several Eya⁺ cells locate very close, even adjacent to, the hub in *lwr* or *Su(var)2-10 RNAi* testis (Fig. 2B,C), suggesting that those cells around the hub initiated differentiation. We then measured the distance between the hub and the Eya⁺ cells, and found that when *lwr* was knocked down the percentage of testes with shorter distance was increased (Fig. 2D) and the distance between the hub and the

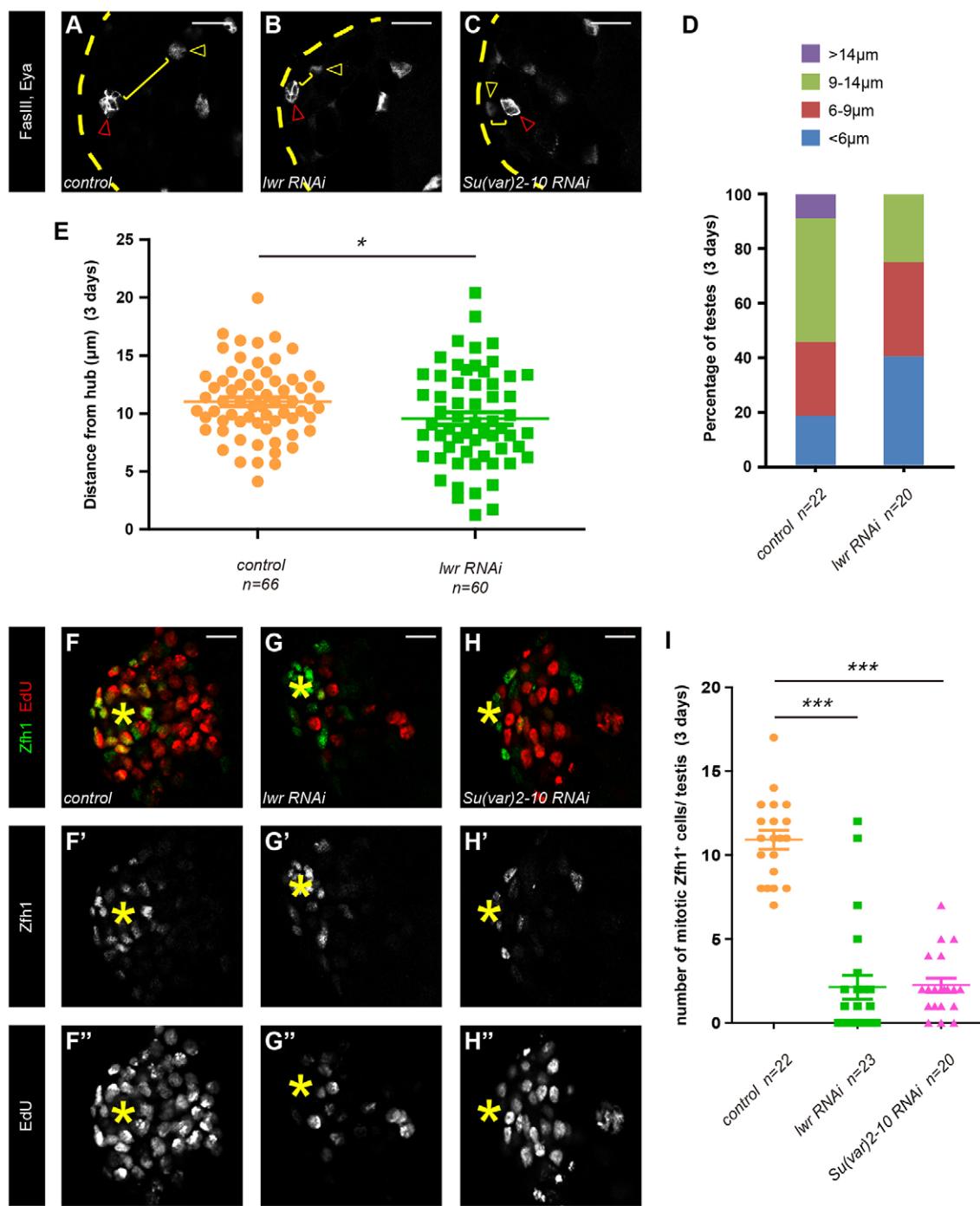


Fig. 2. The SUMO pathway inhibits CySC differentiation and promotes CySC proliferation. (A–C) Representative testes showing the distances (yellow brackets) between the closest differentiated cyst cell (Eya, yellow arrowhead) and the hub (FasIII, red arrowhead) after RNAi induction for 3 days. Scale bars: 25 μm. (D) The percentage of testes showing indicated distance between the hub and the closest Eya⁺ cell. (E) The distance between the hub and the three closest Eya⁺ cells. Data are presented as individual values and mean±s.e.m. *P<0.05. (F–H') Representative testes showing Zfh1 (green) and EdU (red) staining, after RNAi induction for 3 days. Asterisks indicate the hub. Scale bars: 10 μm. (I) The numbers of mitotic Zfh1⁺ cells (EdU⁺Zfh1⁺ cells). Data are presented as individual values and mean±s.e.m. ***P<0.001.

three nearest Eya⁺ cells became shorter (Fig. 2E). These results indicate that the SUMO pathway is required for inhibition of CySC differentiation.

Next, we conducted 5-ethynyl-2'-deoxyuridine (EdU) incorporation and it showed that dividing CySCs were detected with lower frequency in *lwr RNAi* and *Su(var)2-10 RNAi* testes than in controls (Fig. 2F-I; Fig. S2A), implying that the proliferation capacity of CySCs was also reduced.

The SUMO pathway genetically interacts with the Hh pathway

The JAK-STAT pathway, the EGFR pathway, the Hpo pathway and the Hh pathway are the major players currently identified in regulating CySCs (Amoyel et al., 2014; Kiger et al., 2000, 2001; Michel et al., 2012; Tran et al., 2000; Tulina and Matunis, 2001). Are the phenotypes induced by SUMO pathway suppression mediated by one of them? We monitored the activities of these pathways in CySCs upon *lwr* or *Su(var)2-10* suppression driven by *c587-Gal4*. Patched (Ptc) expression has been used as readout of the Hh pathway (Amoyel et al., 2013; Michel et al., 2012). We found that the level of Ptc was reduced in *lwr RNAi* testes compared with control (Fig. 3A-B'), implying that the SUMO pathway positively regulates Hh signaling activity in CySCs, although we cannot rule out the possibility that the change of Ptc staining might simply reflect a change in the number of CySCs, rather than a direct modification of Hh pathway activity.

The JAK-STAT, EGFR and Hpo signaling activities in CySCs of *lwr* or *Su(var)2-10 RNAi* testes were indistinguishable from those in control testes as revealed by the intensity of staining for phosphorylated STAT (pSTAT; Stat92E – FlyBase) (Zhang et al., 2013a), the dual phosphorylated form of MAP kinase (dpERK; also known as Rolled) (Gabay et al., 1997; Kiger et al., 2000) and *expanded-lacZ* (*ex-lacZ*) (Amoyel et al., 2014), general reporters of these three pathways, respectively (Fig. S2B-G). Using a flip-out technique, we generated *lwr RNAi* clones and found that these readouts were also indistinguishable from nearby control cells (Fig. 3C-E'''), suggesting that the decreased number of Zfh1⁺ cells in 3-day-old *lwr/Su(var)2-10 RNAi* testis is unlikely to be mediated by blockage of the JAK-STAT, EGFR or Hpo pathways although we cannot entirely rule out this possibility.

To test further whether the Hh pathway mediates the function of the SUMO pathway on CySC maintenance, we performed genetic epistasis assays. Knockdown of *ptc* (an inhibitor of the Hh pathway; Beachy et al., 2004; Huang et al., 2013; Jiang and Hui, 2008; Jiang et al., 2016) increased the number of Zfh1⁺ cells (Fig. 3F,G,I). Importantly, this increase could be blocked by knockdown of *lwr* (Fig. 3F-I). To confirm this genetic relationship, we used MARCM assays to monitor three different aspects of stemness of CySCs: the clone size (number of clonal cells; Fig. 3J), the ratio of EdU incorporation (Fig. 3K), and the capacity for maintaining stemness at the niche (Fig. 3L). These data together showed that the effect of *ptc RNAi* on CySCs could be disrupted by mutating *lwr*. Taken together, we conclude that the SUMO pathway is indispensable for CySC maintenance promoted by Hh signaling.

The SUMO pathway directly modifies Ci

Based on the genetic interaction assays, we speculated that the SUMO pathway might directly modify some key component(s) of the Hh pathway to regulate CySC maintenance. Considering that SUMO substrates are highly enriched for transcription factors (Hendriks et al., 2014), a likely candidate is Ci, a transcription factor of the Hh pathway (Hui and Angers, 2011; Li et al., 2014; Motzny and Holmgren, 1995). Ci can interact directly with Lwr (Fig. 4A),

implying a potential for Ci to be SUMOylated. To test whether Ci could be SUMOylated, and to map the potential SUMOylation sites in Ci, we truncated full-length Ci into two fragments, named as Ci^N and Ci^C (Fig. S4A). We then generated an anti-SUMO antibody (Fig. S3) and performed a bacterial SUMOylation assay (Mencia and de Lorenzo, 2004; Nie et al., 2009; Uchimura et al., 2004). We did detect SUMOylated forms of both Ci^N and Ci^C (Fig. S4B).

To map further the SUMOylation sites in Ci, we truncated Ci^N and Ci^C into two smaller fragments: Ci^{N-1}, Ci^{N-2}, and Ci^{C-1}, Ci^{C-2} (Fig. S4A). Ci^{N-1}, Ci^{N-2} and Ci^{C-1} could be SUMOylated, whereas Ci^{C-2} could not (Fig. S4C), indicating multiple SUMOylation sites in Ci. To map the precise SUMOylation sites, we prepared SUMOylated Ci^N and Ci^{C-1} for liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Fig. S4D,E). LC-MS/MS recovered nine potential SUMOylation sites in Ci^N, and one potential SUMOylation site in Ci^{C-1} (Fig. S4A, asterisk). One representative LC-MS/MS map is shown in Fig. S4F.

We then mutated all nine K residues in Ci^N to generate Ci^{N-9KR}, and mutated K782 in Ci^C to generate Ci^{C-K782R}. A subsequent SUMOylation assay showed that SUMOylation levels of Ci^{N-9KR} and Ci^{C-K782R} were significantly reduced compared with Ci^N and Ci^C, respectively (Fig. 4B,C), indicating that these sites are real SUMOylation sites.

SUMO conjugation promotes Ci activity in terms of CySC proliferation

To investigate the role of Ci SUMOylation in CySCs, we generated transgenic flies for WT or different mutants of Ci. Mutating all ten K residues mentioned above to arginine (Arg; R) generated Ci^{10KR}. Overexpression of WT Ci, but not Ci^{10KR}, increased the number of Zfh1⁺ cells (Fig. 4D), implying that SUMO modification is indispensable for Ci activity in promoting CySC self-renewal. We then observed a crucial function of K782 as Ci^{K782R} did not increase the number of Zfh1⁺ cells (Fig. 4E-H'). Importantly, artificial fusion of SUMO protein to Ci^{K782R} can restore the function of Ci^{K782R} in promoting CySC self-renewal similar to wild-type Ci (Fig. 4E-I'). Taken together, we conclude that accurate SUMOylation of Ci is essential for CySC proliferation in adult *Drosophila* testis.

Considering that the conserved Hh signaling pathway (Briscoe and Thérond, 2013) has been reported to regulate the testis stem cell in mammals (Bitgood et al., 1996; Makela et al., 2011; Petrova and Joyner, 2014; Yao et al., 2002) and that the biological functions of the SUMO pathway in embryogenesis are highly conserved from *Drosophila* to mammals (Flotho and Melchior, 2013), it is intriguing to test whether the SUMO pathway is also required for mammalian adult testis by targeting the Hh signaling pathway. Actually, Gli proteins, the homologs of Ci in mammals, can be SUMOylated, as reported by two groups (Cox et al., 2010; Han et al., 2012), further supporting a conserved modification of the conserved transcription factors. However, their conclusions about the function of SUMOylation on Hh signaling activity are controversial. It is possible that different Gli proteins might be differently regulated by SUMOylation. Based on our study in adult CySCs, we favor the hypothesis that Ci SUMOylation promotes Hh signaling activity in maintaining CySCs, which might function in a context-dependent manner.

In this study, we identified the involvement of a SUMO E3 ligase in CySC maintenance through a genetic screen. Combining a targeted gene knockdown approach with a MARCM system generating a homozygous mutant allele, we demonstrated that SUMOylation inhibits differentiation as well as promotes proliferation of CySCs under physiological conditions.

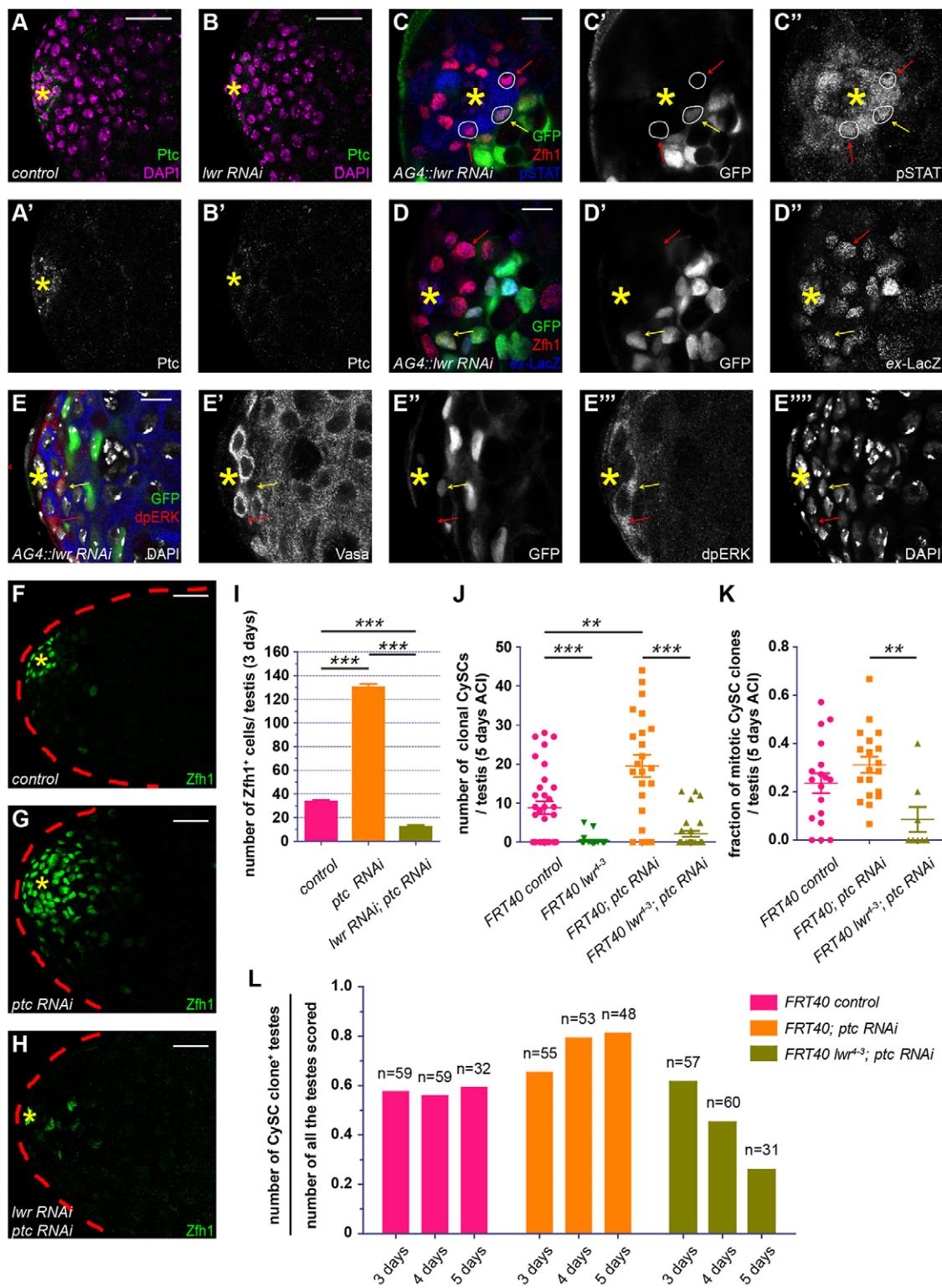


Fig. 3. The SUMO pathway genetically interacts with the Hh pathway. (A-B') Representative testes showing Ptc (green) and DAPI (purple) staining after RNAi induction for 3 days. Asterisks indicate the hub. Scale bars: 10 µm. (C-D'') Representative testes showing Zfh1 (red), and pSTAT (C) or ex-lacZ (detected by staining with anti-β-galactosidase antibody; D) (blue) staining after flip-out clone induction for 3 days. Yellow arrows indicate Iwr RNAi CySC clones, and red arrows indicate control CySCs. Asterisks indicate the hub. White circles outline the cells. Scale bars: 10 µm. (E-E''') Representative testes showing dpERK (red), Vasa (blue) and DAPI (white) staining, after RNAi induction for 3 days. Yellow arrows indicate an Iwr RNAi CySC clone, and red arrows indicate a control CySC. Asterisks indicate the hub. Scale bar: 10 µm. (F-H) Representative testes showing Zfh1 (green) staining after RNAi induction for 3 days. Asterisks indicate the hub. Scale bars: 25 µm. (I) The numbers of Zfh1⁺ cells per testis after RNAi induction for 3 days. Data are presented as mean±s.e.m., ***P<0.001, n>15. (J) The numbers of clonal CySCs (GFP⁺ cells with strong Zfh1 staining) per testis after clone induction (ACI) for 5 days. Data are presented as individual values and mean±s.e.m. ***P<0.001; **P<0.01. (K) The fraction of mitotic CySC clones. Each point indicates the ratio of EdU⁺ CySC clones to all CySC clones in each testis. Data are presented as individual values and mean±s.e.m. **P<0.01. (L) The fraction of testes with at least one CySC clone, at 3, 4 and 5 days ACI.

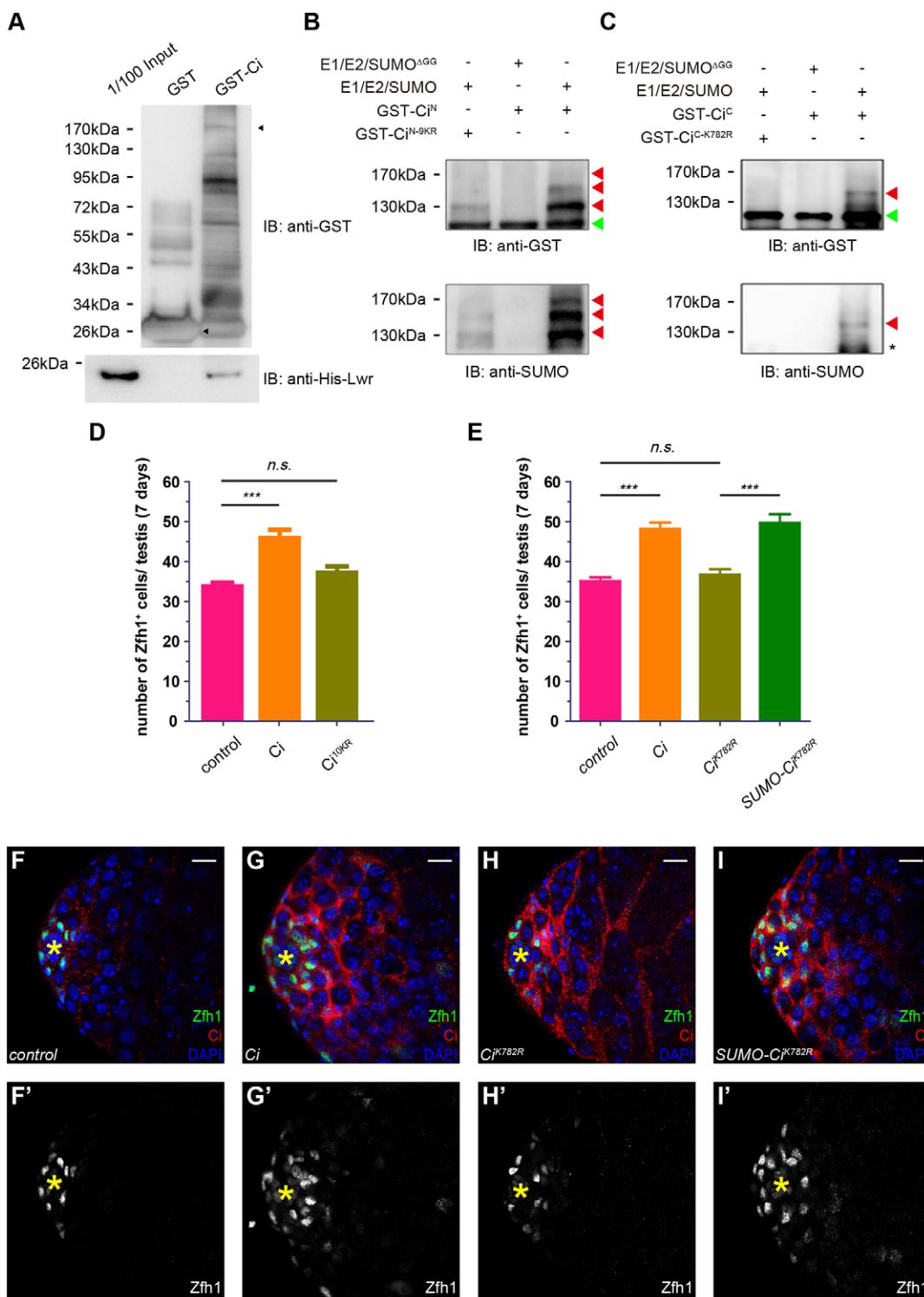


Fig. 4. SUMOylation directly targets Ci and promotes Ci activity in terms of CySC proliferation. (A) Western blot assay showing that GST-Ci can directly interact with His-Lwr in a GST-fusion protein pull-down assay. Black arrowheads point to the GST or GST-Ci in lanes 1 and 2, respectively. (B,C) Western blot assay showing the SUMOylation status of different Ci fragments. Red arrowheads point to the SUMOylated Ci fragments and green arrowheads point to the un-SUMOylated Ci fragments. Asterisk indicates a non-specific band. (D,E) The numbers of Zfh1⁺ cells per testis, after transgene induction for 7 days. Data are presented as mean±s.e.m. ***P<0.001; n.s., not significant. n>20. (F–I') Representative testes showing Zfh1 (green), Ci (red) and DAPI (blue) staining, after transgene induction for 7 days. Asterisks indicate the hub. Scale bars: 10 μm.

Mechanistically, we found that the SUMO pathway genetically interacts with the Hh pathway, and directly catalyzes Ci SUMOylation, which is required for promoting CySC proliferation, although the function of SUMOylation in keeping

CySCs stemness might involve other unknown factor(s). In summary, we identified that the SUMO pathway/SUMOylation directly targets the Hh pathway and regulates CySC maintenance in adult *Drosophila* testis (Fig. S4G).

MATERIALS AND METHODS

Fly stocks

Flies were raised on standard yeast/molasses medium at 25°C unless otherwise stated. Fly stocks used are detailed in supplementary Materials and Methods.

RNAi and overexpression studies

All RNAi and overexpression-related experiments were performed in the *Gal4-Gal80^{ts}* system (Kawase et al., 2004; Melcher and Xu, 2001; Suster et al., 2004). Except where specifically mentioned, all the assays were carried out using *c587-Gal4*.

Generation of clones using the MARCM system

Adult male flies were collected 0–3 days after eclosion and heat shocked for two rounds of 1 h heat shock at 37°C and 1 h rest at 25°C. After the final heat shock, they were returned to 25°C.

Immunostaining of testes, quantification of GSCs and EdU incorporation

Immunostaining of testes and quantification of GSCs were carried out as previously described (Zhang et al., 2013b). Antibodies used in this study: mouse anti-FasIII (DSHB, 7G10, 1:1000), mouse anti-Ptc (DSHB, Apa1, 1:100), mouse anti-Eya (DSHB, 10H6, 1:100), mouse anti-Hts (DSHB, 1B1-C, 1:1000), rabbit anti-Vasa (Santa Cruz, sc-26877, 1:200), rabbit anti-Zfh1 [a gift from Dr. Ruth Lehmann (School of Medicine, New York University), 1:5000], rat anti-Ci (DSHB, 2A1, 1:200), mouse anti-pSTAT [a gift from Dr. Xinhua Lin (Cincinnati Children's Hospital Medical Center), 1:500], rabbit anti-dpERK (Cell Signaling, 4370P, 1:100). EdU incorporation was performed with standard protocol following the manufacturer's instructions (Invitrogen). See supplementary Materials and Methods for details.

DNA constructs and transgenes

DNA constructs and transgenes are described in supplementary Materials and Methods.

Bacterial SUMOylation assay, GST protein pull-down assay and western blot analysis

Bacterial SUMOylation assay, GST protein pull-down assay and western blot analysis were carried out as previously described (Nie et al., 2009; Shi et al., 2013). See supplementary Materials and Methods for details.

Statistical analysis

Data are presented as mean±s.e.m. Statistical significance was calculated using two-tailed Student's *t*-tests. *P*-values are indicated in figures or in figure legends.

Acknowledgements

We are grateful to Drs Albert J. Courey, Ruth Lehmann, Gary Karpen, Xinhua Lin, Jinke Cheng, the Developmental Studies Hybridoma Bank, the Vienna *Drosophila* RNAi Center, the National Institute of Genetics and the Bloomington Stock Center for fly stocks and reagents. We also thank Dr Jinqiu Zhou for discussions and comments on the manuscript. We thank Drs Rong Zeng and Chen Li for helping with mass spectrometry assay. Some of our confocal images were captured at the National Center for Protein Science Shanghai.

Competing interests

The authors declare no competing or financial interests.

Author contributions

X.L. and C.P. conceived the study, designed and performed experiments, analyzed data and prepared the manuscript; Z.Z., Y.X., H.C., S.Z., T.G. and H.H. contributed reagents/materials/analysis tools; H.S. and L.Z. analyzed the data; Y.Z. supervised and conceived the study, analyzed data and edited the manuscript.

Funding

This work was supported by grants from the 'Strategic Priority Research Program' of the Chinese Academy of Sciences [XDA01010405 to Y.Z.]; and from the National Natural Science Foundation of China [31171414, 31371492 to Y.Z.].

Supplementary information

Supplementary information available online at <http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.130773/-DC1>

References

- Amoyel, M., Sanny, J., Burel, M. and Bach, E. A. (2013). Hedgehog is required for CySC self-renewal but does not contribute to the GSC niche in the Drosophila testis. *Development* **140**, 56–65.
- Amoyel, M., Simons, B. D. and Bach, E. A. (2014). Neutral competition of stem cells is skewed by proliferative changes downstream of Hh and Hpo. *EMBO J.* **33**, 2295–2313.
- Apionishev, S., Malhotra, D., Raghavachari, S., Tanda, S. and Rasooly, R. S. (2001). The Drosophila UBC9 homologue lesswright mediates the disjunction of homologues in meiosis I. *Genes Cells* **6**, 215–224.
- Beachy, P. A., Karhadkar, S. S. and Berman, D. M. (2004). Tissue repair and stem cell renewal in carcinogenesis. *Nature* **432**, 324–331.
- Bitgood, M. J., Shen, L. and McMahon, A. P. (1996). Sertoli cell signaling by Desert hedgehog regulates the male germline. *Curr. Biol.* **6**, 298–304.
- Briscoe, J. and Thérond, P. P. (2013). The mechanisms of Hedgehog signalling and its roles in development and disease. *Nat. Rev. Mol. Cell Biol.* **14**, 418–431.
- Cox, B., Briscoe, J. and Ulloa, F. (2010). SUMOylation by Pias1 regulates the activity of the Hedgehog dependent Gli transcription factors. *PLoS ONE* **5**, e11996.
- de Cuevas, M. and Matunis, E. L. (2011). The stem cell niche: lessons from the Drosophila testis. *Development* **138**, 2861–2869.
- Eun, S. H., Shi, Z., Cui, K., Zhao, K. and Chen, X. (2014). A non-cell autonomous role of E(z) to prevent germ cells from turning on a somatic cell marker. *Science* **343**, 1513–1516.
- Fabrizio, J. J., Boyle, M. and DiNardo, S. (2003). A somatic role for eyes absent (eya) and sine oculis (so) in Drosophila spermatocyte development. *Dev. Biol.* **258**, 117–128.
- Flotho, A. and Melchior, F. (2013). Sumoylation: a regulatory protein modification in health and disease. *Annu. Rev. Biochem.* **82**, 357–385.
- Gabay, L., Seger, R. and Shilo, B. Z. (1997). MAP kinase in situ activation atlas during Drosophila embryogenesis. *Development* **124**, 3535–3541.
- Gareau, J. R. and Lima, C. D. (2010). The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. *Nat. Rev. Mol. Cell Biol.* **11**, 861–871.
- Han, L., Pan, Y. and Wang, B. (2012). Small ubiquitin-like Modifier (SUMO) modification inhibits GLI2 protein transcriptional activity in vitro and in vivo. *J. Biol. Chem.* **287**, 20483–20489.
- Hannoun, Z., Greenhough, S., Jaffray, E., Hay, R. T. and Hay, D. C. (2010). Post-translational modification by SUMO. *Toxicology* **278**, 288–293.
- Hendriks, I. A., D'Souza, R. C. J., Yang, B., Verlaan-de Vries, M., Mann, M. and Vertegaal, A. C. O. (2014). Uncovering global SUMOylation signaling networks in a site-specific manner. *Nat. Struct. Mol. Biol.* **21**, 927–936.
- Hickey, C. M., Wilson, N. R. and Hochstrasser, M. (2012). Function and regulation of SUMO proteases. *Nat. Rev. Mol. Cell Biol.* **13**, 755–766.
- Huang, S., Zhang, Z., Zhang, C., Lv, X., Zheng, X., Chen, Z., Sun, L., Wang, H., Zhu, Y., Zhang, J. et al. (2013). Activation of Smurf E3 ligase promoted by smoothened regulates hedgehog signaling through targeting patched turnover. *PLoS Biol.* **11**, e1001721.
- Hui, C.-c. and Angers, S. (2011). Gli proteins in development and disease. *Annu. Rev. Cell Dev. Biol.* **27**, 513–537.
- Issigonis, M. and Matunis, E. (2011). SnapShot: stem cell niches of the Drosophila testis and ovary. *Cell* **145**, 994–994.e2.
- 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.
- Jiang, J. and Hui, C.-c. (2008). Hedgehog signaling in development and cancer. *Dev. Cell* **15**, 801–812.
- Jiang, K., Liu, Y., Fan, J., Zhang, J., Li, X.-A., Evers, B. M., Zhu, H. and Jia, J. (2016). PI(4)P promotes phosphorylation and conformational change of smoothened through interaction with its C-terminal tail. *PLoS Biol.* **14**, e1002375.
- Kawase, E., Wong, M. D., Ding, B. C. and Xie, T. (2004). Gbb/Bmp signaling is essential for maintaining germline stem cells and for repressing bam transcription in the Drosophila testis. *Development* **131**, 1365–1375.
- Kiger, A. A., White-Cooper, H. and Fuller, M. T. (2000). Somatic support cells restrict germline stem cell self-renewal and promote differentiation. *Nature* **407**, 750–754.
- 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.
- 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.** (2001). Mosaic analysis with a repressible cell marker (MARCM) for *Drosophila* neural development. *Trends Neurosci.* **24**, 251-254.
- Li, Z., Guo, Y., Han, L., Zhang, Y., Shi, L., Huang, X. and Lin, X.** (2014). Debra-mediated Ci degradation controls tissue homeostasis in *Drosophila* adult midgut. *Stem Cell Rep.* **2**, 135-144.
- Losick, V. P., Morris, L. X., Fox, D. T. and Spradling, A.** (2011). *Drosophila* stem cell niches: a decade of discovery suggests a unified view of stem cell regulation. *Dev. Cell* **21**, 159-171.
- Mahajan, R., Delphin, C., Guan, T., Gerace, L. and Melchior, F.** (1997). A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* **88**, 97-107.
- Makela, J.-A., Saario, V., Bourguiba-Hachemi, S., Nurmio, M., Jahnukainen, K., Parvinen, M. and Toppuri, J.** (2011). Hedgehog signalling promotes germ cell survival in the rat testis. *Reproduction* **142**, 711-721.
- Matunis, M. J., Coutavas, E. and Blobel, G.** (1996). A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J. Cell Biol.* **135**, 1457-1470.
- Melcher, K. and Xu, H. E.** (2001). Gal80-Gal80 interaction on adjacent Gal4 binding sites is required for complete GAL gene repression. *EMBO J.* **20**, 841-851.
- Mencía, M. and de Lorenzo, V.** (2004). Functional transplantation of the sumoylation machinery into *Escherichia coli*. *Protein Expr. Purif.* **37**, 409-418.
- Michel, M., Kupinski, A. P., Raabe, I. and Bokel, C.** (2012). Hh signalling is essential for somatic stem cell maintenance in the *Drosophila* testis niche. *Development* **139**, 2663-2669.
- Motzny, C. K. and Holmgren, R.** (1995). The *Drosophila* cubitus interruptus protein and its role in the wingless and hedgehog signal transduction pathways. *Mech. Dev.* **52**, 137-150.
- Nie, M., Xie, Y., Loo, J. A. and Courey, A. J.** (2009). Genetic and proteomic evidence for roles of *Drosophila* SUMO in cell cycle control, Ras signalling, and early pattern formation. *PLoS ONE* **4**, e5905.
- Petrova, R. and Joyner, A. L.** (2014). Roles for Hedgehog signaling in adult organ homeostasis and repair. *Development* **141**, 3445-3457.
- Shi, D., Lv, X., Zhang, Z., Yang, X., Zhou, Z., Zhang, L. and Zhao, Y.** (2013). Smoothened oligomerization/higher order clustering in lipid rafts is essential for high Hedgehog activity transduction. *J. Biol. Chem.* **288**, 12605-12614.
- Singh, S. R., Zheng, Z., Wang, H., Oh, S. W., Chen, X. and Hou, S. X.** (2010). Competitiveness for the niche and mutual dependence of the germline and somatic stem cells in the *Drosophila* testis are regulated by the JAK/STAT signalling. *J. Cell Physiol.* **223**, 500-510.
- Smith, M., Turki-Judeh, W. and Courey, A. J.** (2012). SUMOylation in *Drosophila* development. *Biomolecules* **2**, 331-349.
- Suster, M. L., Seugnet, L., Bate, M. and Sokolowski, M. B.** (2004). Refining GAL4-driven transgene expression in *Drosophila* with a GAL80 enhancer-trap. *Genesis* **39**, 240-245.
- Tran, J., Brenner, T. J. and DiNardo, S.** (2000). Somatic control over the germline stem cell lineage during *Drosophila* spermatogenesis. *Nature* **407**, 754-757.
- Tulina, N. and Matunis, E.** (2001). Control of stem cell self-renewal in *Drosophila* spermatogenesis by JAK-STAT signalling. *Science* **294**, 2546-2549.
- Uchimura, Y., Nakamura, M., Sugawara, K., Nakao, M. and Saitoh, H.** (2004). Overproduction of eukaryotic SUMO-1- and SUMO-2-conjugated proteins in *Escherichia coli*. *Anal. Biochem.* **331**, 204-206.
- Voog, J., D'Alterio, C. and Jones, D. L.** (2008). Multipotent somatic stem cells contribute to the stem cell niche in the *Drosophila* testis. *Nature* **454**, 1132-1136.
- Yao, H. H.-C., Whoriskey, W. and Capel, B.** (2002). Desert Hedgehog/Patched 1 signalling specifies fetal Leydig cell fate in testis organogenesis. *Genes Dev.* **16**, 1433-1440.
- Zhang, Y., You, J., Ren, W. and Lin, X.** (2013a). *Drosophila* glycanins Daily and Daily-like are essential regulators for JAK/STAT signalling and Unpaired distribution in eye development. *Dev. Biol.* **375**, 23-32.
- Zhang, Z., Lv, X., Jiang, J., Zhang, L. and Zhao, Y.** (2013b). Dual roles of Hh signalling in the regulation of somatic stem cell self-renewal and germline stem cell maintenance in *Drosophila* testis. *Cell Res.* **23**, 573-576.