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Lasp anchors the *Drosophila* male stem cell niche and mediates spermatid individualization

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ABSTRACT

Lasp family proteins contain an amino-terminal LIM domain, two actin-binding nebulin repeats and a carboxyl-terminal SH3 domain. Vertebrate Lasp-1 localizes to focal adhesions and the leading edge of migrating cells, and is required for cell migration. To assess the in vivo function of Lasp, we generated a null mutant in *Drosophila* Lasp. *Lasp*[1] is homozygous viable, but male sterile. In *Lasp* mutants the stem cell niche is no longer anchored to the apical tip of the testis, and actin cone migration is perturbed resulting in improper spermatid individualization. Hub cell mislocalization can by phenocopied by expressing Lasp or βPS integrin RNAi transgenes in somatic cells, and Lasp genetically interacts with βPS integrin, demonstrating that Lasp functions together with integrins in hub cells to anchor the stem cell niche. Finally, we show that the stem cell niche is maintained even if it is not properly localized.

1. Introduction

Drosophila spermatogenesis starts at the apical tip of the testis where the stem cell niche resides. The stem cell niche consists of three cell types, hub cells that are firmly attached to the basement membrane of the apical testis wall, and germ-line and somatic stem cells that are in turn attached to the hub cells (Hardy et al., 1979). Division of a somatic stem cell gives rise to a daughter cell that is displaced away from the hub and will differentiate into a cyst cell. Division of a germ-line stem cell gives rise to a daughter cell displaced away from the hub that undergoes four mitotic divisions followed by meiosis resulting in a syncitial cluster of 64 spermatids. Spermatids then undergo elongation, which is followed by individualization. During individualization, 64 actin cones move along the spermatid axoneme simultaneously pulling in plasma membranes around each spermatid and extruding the cytoplasm to generate fully differentiated individual spermatids (Tokuyasu et al., 1972). Throughout the entire differentiation process two cyst cells enclose the germ cells forming a cyst. Eventually, the tail cyst cell collapses and the bundle of mature sperm coils up before being released into the seminal vesicle (Fuller, 1993).

Here we investigate the in vivo function of Lasp (LIM and SH3 domain protein). Lasp was initially identified from a cDNA library of breast cancer metastases (Tomasetto et al., 1995). Lasp proteins contain an amino-terminal LIM domain, followed by two nebulin repeats and a carboxyl-terminal SH3 domain and are conserved from sponges to humans (Nichols et al., 2006). The Lasp nebulin repeats mediate binding to filamentous actin (Chew et al., 2002; Schreiber et al., 1998). The Lasp SH3 domain interacts with zyxin, which causes localization of Lasp to focal adhesions (Keicher et al., 2004; Li et al., 2004). RNAi-mediated depletion of Lasp inhibits cell migration and proliferation (Grunewald et al., 2006; Lin et al., 2004).

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2. Results and discussion

In *Drosophila*, CG3849 encodes the single member of the nebulin and the Lasp family, whereas in vertebrates the nebulin and Lasp families consist of five members, nebulin, nebulette, NRAP, Lasp-1, and LIM-nebulette/Lasp-2, (the last two members comprise the Lasp family (Grunewald and Butt, 2008; Moncman and Wang, 1995; Wang and Williamson, 1980). *Drosophila* Lasp exhibits very high homology to human Lasp-1 (Fig. 1). To analyze Lasp function in vivo, we generated a *Lasp* mutant by FRT-mediated recombination of piggyBac elements (Fig. 1). This deletes the second exon encompassing 31 amino acids of the LIM domain and results in a frameshift. We verified the deletion by PCR and refer to this mutant as $Lasp^1$. $Lasp^1$ mutants are homozygous viable, but sterile with only rare escapers. Females have reduced fertility, whereas males are almost completely sterile (Table 1). To check Lasp expression, we stained testes with anti-Lasp antibody. Lasp protein is strongly expressed in testes, but is undetectable in $Lasp^1$ mutants (Fig. 1). Our deletion also deletes *CG9692*, a gene without homologies sitting in the first intron of *Lasp*. The *Drosophila* gene disruption project (Bellen et al., 2004) recently generated a *minos* element (*MB03281*) that disrupts the predicted open reading frame of *CG9692*. This transposable element does not show male sterility both homozygously or as a transheterozygous over $Lasp^1$ (Table 1). Furthermore, $Lasp^1$ over the small deficiency *Df(3L)st7* is also homozygous viable and male-sterile and displays the same phenotypes. To unambiguously verify that a mutation in $Lasp^1$ is responsible for the observed sterility, we created a UAStLasp-GFP transgene with the short Lasp isoform from a testis cDNA library. Expressing UAStLasp-GFP with the germ-line driver nanos-Gal4 rescues slightly, while expression with the somatic driver ptc-Gal4 rescues fertility to a level about half that observed with wild-type (Table 1). Our combined data demonstrate that we most likely created a null mutant in *Lasp*.

The antibody staining indicates the presence of Lasp protein in both germ cells and somatic cells. To better define the expression of Lasp, we conducted co-immunostainings with well-characterized markers for different testis cell types. Vasa, a germ cell marker (Lasko and Ashburner, 1988), colocalizes with Lasp in early germ cells, but barely in later germ cells (Fig. 2A–C). We observe a gradient of Lasp, with its strongest expression observed in early germ cells. Lasp is also expressed in hub cells, as seen by its colocalization with the hub cell marker Fasciclin III, and is likely expressed in stem cells, because cells directly adjacent to hub cells express Lasp (Fig. 3). In mammalian cells, Lasp localizes to the leading edge of migrating cells and to focal adhesions (Grunewald et al., 2006; Lin et al., 2004). Focal adhesions are integrin adhesion sites in tissue culture, where heterodimeric integrin receptors connect cells to the extracellular matrix. We therefore tested colocalization of Lasp with βPS integrin, the major β integrin subunit in *Drosophila* (Devenport and Brown, 2004). Lasp fully colocalizes with βPS integrin in germ cells and cyst cells (Fig. 2D–F). Fax-GFP, another marker for somatic cyst cells

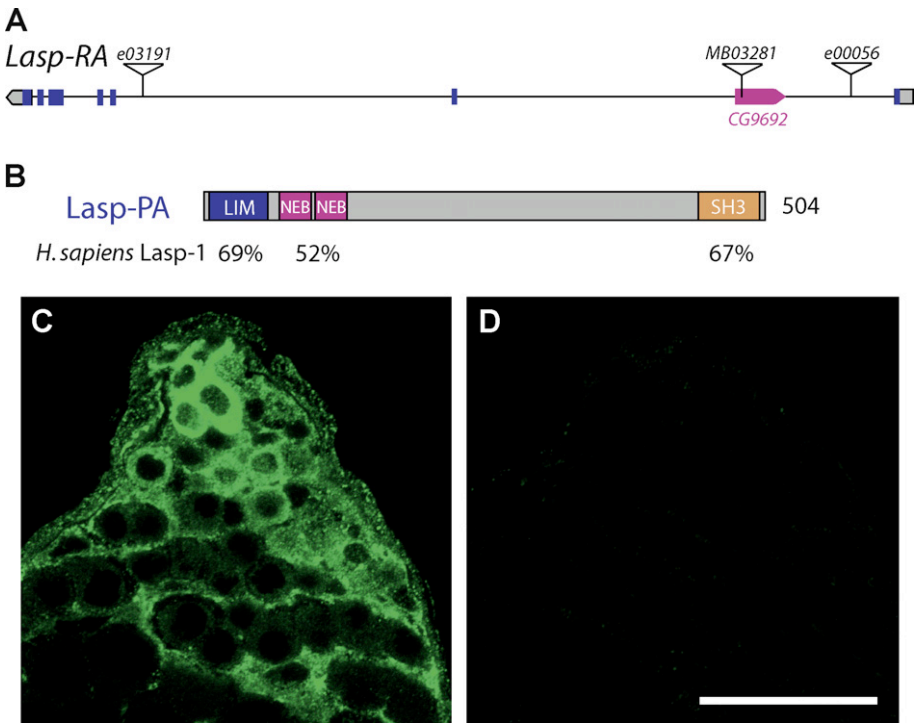

Fig. 1 – The *Lasp* gene. (A) Schematic presentation of the *Lasp* gene. Only the short isoform *Lasp-RA* is depicted. Translated exons are shown in blue and untranslated exons in gray. piggyBac insertions used to generate $Lasp^1$ and the minos element *MB03281* disrupting *CG9692* (in purple) are indicated. (B) The *D. melanogaster Lasp* gene encodes two isoforms containing the same domains. The short isoform Lasp-PA is depicted with the length in amino acids on the right. Below three conserved domains, we show the percent identity between Lasp and its human orthologue. (C and D) Immunostaining of the apical testis tip with rabbit anti-Lasp antibody in wild-type (C), and $Lasp^1$ mutant (D). Bar: 30 μm.

Table 1 – Male sterility

Genotype	Days of egglay	Average number of offspring	Number of single males tested
Oregon R	1	64	18
$Lasp^1/Lasp^1$	1	0	20
$Lasp^1/+$	1	84	20
MB03281/MB03281	1	61	26
$Lasp^1$/MB03281	1	113	28
$Lasp^1$/Df(3L)st7	1	0	20
$Lasp^1$ UASLasp-GFP/$Lasp^1$ UASLasp-GFP	2	0	21
ptcGal4; $Lasp^1$ UASLasp-GFP/$Lasp^1$ UASLasp-GFP	2	63	20
$Lasp^1$ nosGal4/$Lasp^1$ nosGal4	2	0	22
$Lasp^1$ nosGal4/$Lasp^1$ UASLasp-GFP	2	2	21
mys^{nj42}	1	57	19
mys^{nj42}; $Lasp^1/+$	1	81	14

(Decotto and Spradling, 2005), also colocalizes with Lasp demonstrating that Lasp is expressed in cyst cells (not depicted). Finally, Lasp very strongly localizes to actin cones of individualizing spermatids, where it partially colocalizes with actin in all stages of actin cone migration (Fig. 2G and H).

We next analyzed the phenotypes observed in *Lasp*^{1} mutants. In unsquashed wild-type testes hub cells always localize to the apical most tip of the testis. However, hub cells fail to localize to the apical tip of the testis in *Lasp*^{1} mutants (Fig. 4A and B, and Table 2). This phenotype is similar to a talin mutant that also shows a mislocalization of hub cells (Tanentzapf et al., 2007). However, the phenotype is considerably milder, and germ cells are still present in 14-day-old *Lasp*^{1} males (Fig. 4F and Table 2). We can phenocopy hub cell mislocalization by expressing *Lasp* RNAi transgenes targeting different regions of *Lasp* in somatic cells with *ptc-Gal4* and *c587-Gal4*, but not by expressing it with a germ-line driver (Table 2). This independently confirms that Lasp is responsible for the observed hub cell mislocalization in *Lasp*^{1} mutants, and it also demonstrates that Lasp acts in somatic cells, most likely the hub cells, to mediate their attachment to the testis wall at the apical tip. We did not analyze hub cell localization in the embryonic gonad, because the Lasp phenotype is already mild in the adult and *Lasp* mRNA expression is not detectable during embryogenesis (BDGP Gene Expression Report).

As Lasp colocalizes with βPS integrin in somatic and germline cells, and mutants in both Lasp and talin cause hub cell mislocalization, we asked if Lasp acts together with βPS integrin. We therefore first tested if βPS integrin, which is required for hub cell localization in embryos (Tanentzapf et al., 2007), is also required in adult testes. We analyzed a *mys* (encoding βPS integrin) RNAi UAS transgene, which we expressed in hub cells with the somatic driver *c587-Gal4*. Similar to Lasp and in line with the talin phenotype, we observe a mislocalization of the stem cell niche (Table 2). Furthermore, mys^{nj42}, a hypomorphic viable βPS integrin mutant, displays a mislocalization of hub cells (Fig. 4C and Table 2). We checked βPS integrin expression in mys^{nj42} by antibody staining, but could not detect a difference compared to wild-type (data not shown), suggesting that complete absence of βPS integrin will result in a stronger phenotype. The mys^{nj42} phenotype is mildly progressive, as we observe a stronger mislocalization in 14-day-old males (Fig. 4G and Table 2). When we additionally remove one copy of *Lasp*^{1}, we observe a complete mislocalization of the stem cell niche in 50% of 3-day-old males (Fig. 4E and Table 2). Importantly, this does not affect fertility, because 13/14 (93%) individual mys^{nj42}; *Lasp*^{1}/+ males exhibit wild-type fertility (Table 1). The phenotype is also slightly progressive, as we observed one testis out of 18 without hub and germ cells and two testes with hub and germ cells halfway between the apical and basal end in 14-day-old males. To better analyze if the stem cell niche still functions when it is mislocalized, we stained 14-day-old males with anti-vasa and anti-Fas III antibody. Both germ cells and hub cells are still present in slightly (Fig. 4F) or completely mislocalized hubs (Fig. 4G and H), even when they are halfway between the apical and the basal end of the testis (Fig. 4I). Furthermore, we do not observe a disintegration of the hub cell cluster in mislocalized hub cells (Fig. 4B, C, E–I). Our data argue that localization of the stem cell niche is not important for stem cell proliferation and maintenance and that the occasional loss of germ cells occurs because the entire stem cell niche is swept into the seminal vesicle and lost. The stronger loss of germ cells previously observed with a talin RNAi transgene in 14-day-old cells (Tanentzapf et al., 2007) may be explained in two ways: the talin RNAi transgene likely acts as a stronger allele compared to the mild hypomorph mys^{nj42}, and additionally or alternatively, talin may not only function in integrin-dependent cell-matrix adhesion, but also in cell–cell adhesion (Becam et al., 2005). Intriguingly, Lasp is also specifically expressed in cap cells, the stem cell niche in the ovary, suggesting that Lasp may play a more general role in the attachment of stem cell niches (Fig. 5).

We also observe premature coiling of cysts and a premature disintegration of cysts into individual sperm at the coiling stage in *Lasp*^{1} mutants (Fig. 6A and B). This phenotype is most likely due to a function of Lasp in cyst cells, because the somatic driver ptc-Gal4 completely rescues premature coiling and disintegration, whereas the germ-line driver nos-Gal4 does not rescue this phenotype at all (Fig. 6C and D). Lasp may function in cyst cell maintenance or contribute to proper adhesion of cyst cells to the spermatid bundle, which could indirectly contribute to a breakdown of cyst cells after loss of adhesion.

Finally, we analyzed if Lasp plays a role in spermatid individualization. In wild-type actin cones, Lasp localizes to the actin cone colocalizing with filamentous actin. In *Lasp*^{1}

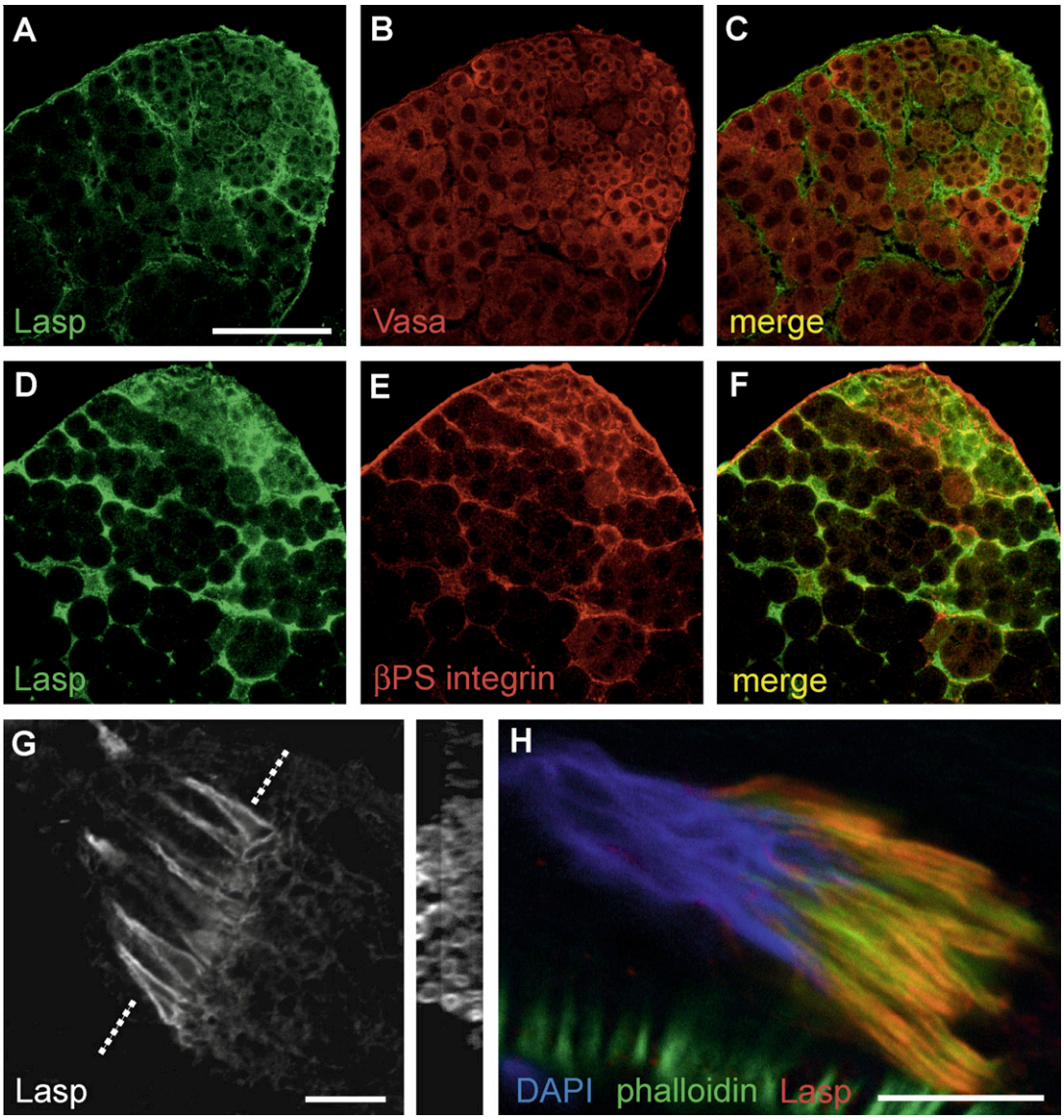

Fig. 2 – Lasp localizes to early germ cells, cyst cells and actin cones. (A) Anti-Lasp antibody staining, (B) anti-vasa antibody staining, (C) merge of the testis apical tip. (D) Anti-Lasp antibody staining, (E) anti-βPS integrin antibody staining, (F) merge of the testis apical tip. (G) Anti-Lasp antibody staining of actin cones. A z-section of 41 confocal planes at the position indicated by the dashed lines is shown on the right. (H) A triple staining for DAPI to visualize spermatid DNA, phalloidin and anti-Lasp antibody indicates colocalization of actin filaments and Lasp. All images are from squashed testis preparations. Bars: (A–F) 50 μm; (G and H) 10 μm.

mutants, the actin cytoskeleton in the cone is affected: filamentous actin is only weakly visible at the assembly stage of actin cones (Fig. 7A and B). We calculated the average mean gray value of pixels in actin cones stained and imaged under identical conditions and observe that filamentous actin staining is approximately twice as strong in wild-type (76 on a scale of 0–255; $n = 53$) than in *Lasp*^{1} mutants (31; $n = 30$). At the migrating stage filamentous actin is barely or not visible at all (not depicted). This phenotype is similar to the myosin VI mutant (Noguchi et al., 2006). Weaker F-actin staining is not enhanced in a *mys*^{nj42} background, consistent with the absence of integrin staining in actin cones (data not shown). Given Lasp's dual function in binding to filamentous actin and localizing to the leading edge of migrating cells in vertebrates (Chew et al., 2002; Lin et al., 2004), we suggest that Lasp may tether the actin cytoskeleton to the plasma membrane of the actin cone. To support this notion, we investigated cross sections of cysts by transmission electron microscopy. Apart from incomplete and slower spermatid individualization (Fig. 7C), we always observe incomplete extrusion of the cytoplasm indicated by a loose membrane surrounding individual spermatids and additional membrane fragments in between spermatids, indicating a failure to properly pull in the membrane around individual spermatids (Fig. 7D and E).

Finally, we wanted to determine the phenotype responsible for the observed sterility. To this end, we compared the rescue of actin cone defects and cyst disintegration in ptc-

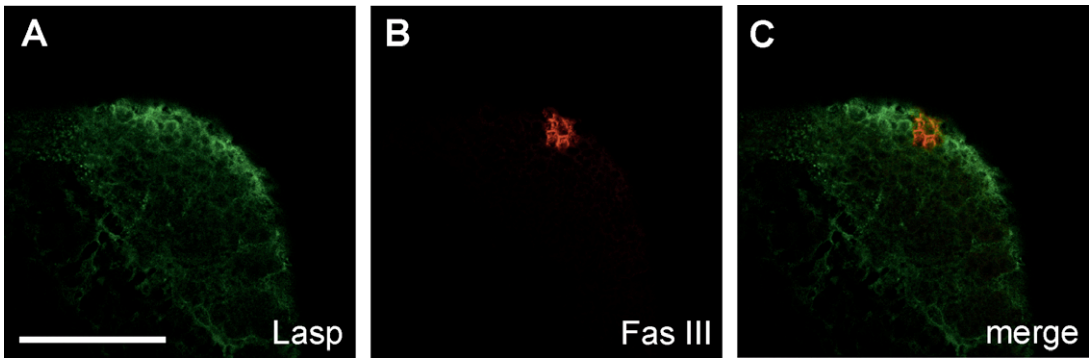

Fig. 3 – Lasp is expressed in hub cells. (A) Anti-Lasp antibody staining, (B) anti-fasciclin III antibody staining outlining hub cells. (C) Merge. Also note that cells directly adjacent to hub cells, which are likely stem cells, strongly express Lasp. Bar: 50 μm.

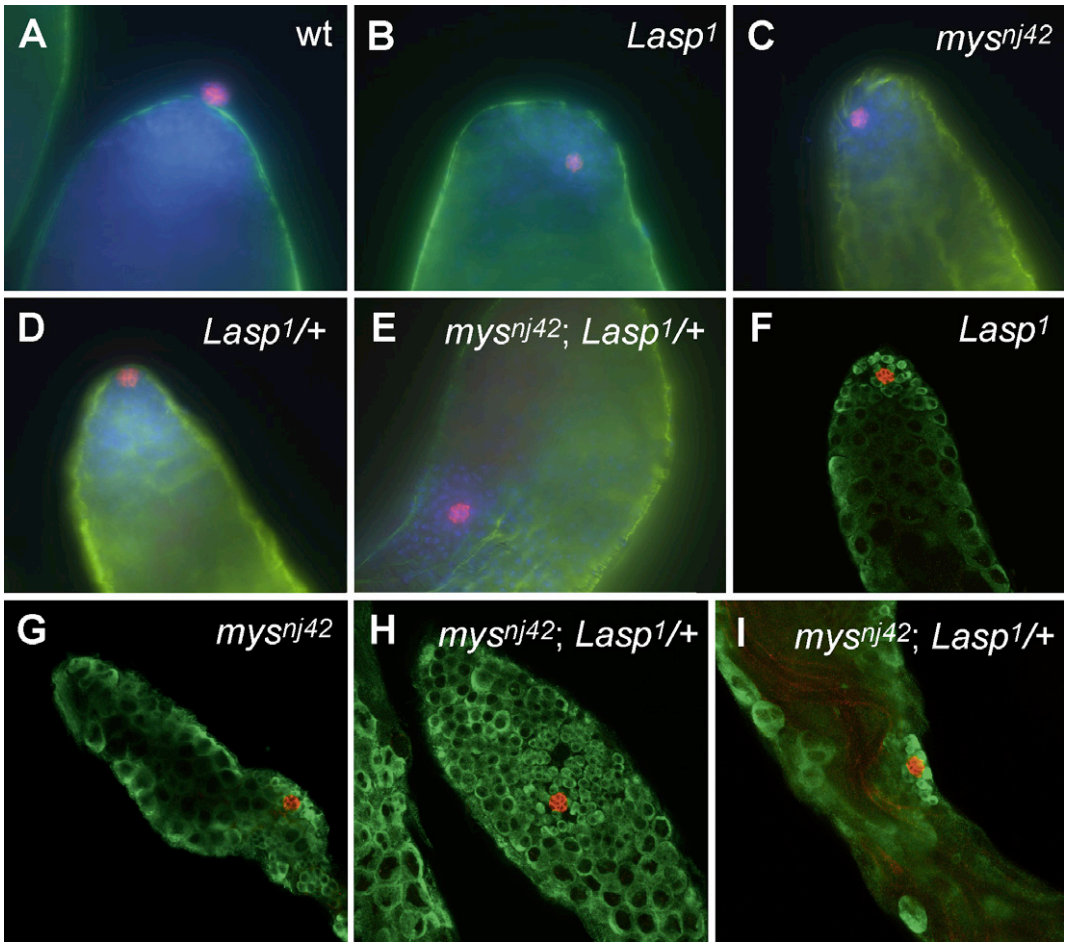

Fig. 4 – In *Lasp*^{1} testes the stem cell niche is not anchored to the apical tip. (A–E) 3-day-old unsquashed testes stained with anti-Fas III antibody (red) to visualize hub cells, phalloidin-Alexa 488 to visualize filamentous actin, and DAPI to visualize DNA. (A) While the hub cell in wild-type is anchored to the tip, (B) hub cells are no longer attached to the apical tip in *Lasp*^{1} mutants. (C) Hub cells are also mislocalized in *mys*^{nj42}. (D) Hub cells localize normally in a *Lasp*^{1} heterozygous mutant. (E) Hub cells completely mislocalize in *mys*^{nj42}; *Lasp*^{1}/+. (F–I) 14-day-old unsquashed testes stained with anti-vasa to visualize germ cells and anti-Fas III to visualize hub cells. (F) *Lasp*^{1}. (G) *mys*^{nj42}. (H and I) *mys*^{nj42}; *Lasp*^{1}/+. In (I) the stem cell niche is located halfway between the apical and the basal end.

Gal4 rescued flies and nos-Gal4 rescued flies. We could not evaluate the role of hub cell localization with these driver lines, but we already know that mislocalization of hub cells does not cause sterility. The actin cone defects are rescued with nos-Gal4 (data not shown), but nos-Gal4 barely rescues sterility (Table 1), indicating that the actin cone defect con-

Table 2 – Hub cell mislocalization*

Genotype	Mislocalized hub cells 3-day-old (n)	Mislocalized hub cells 14-day-old (n)
Oregon R	0% (20)	ND
$Lasp^1/Lasp^1$ §	85% (20)	60% (20)
UASiLasp1; c587Gal4§	25% (20)	25% (20)
UASiLasp2; c587Gal4§	30% (20)	30% (20)
UASiLasp1; ptcGal4§	25% (20)	20% (20)
UASiLasp1; nosGal4::VP16§	0% (20)	ND
UASimys; c587Gal4§	30% (20)	30% (20)
mys^{nj42} §	95% (19)	85% (20)
$Lasp^1/+$#	0% (16)	0% (20)
mys^{nj42} #	0% (19)	20% (20)
mys^{nj42}; $Lasp^1/+$#	50% (18)	39% (18)

* Hub cell localization was analyzed in unsquashed testes.
§ The percentages for 14-day-old males are most likely an underestimate because of the generally perturbed morphology of testes at that age.
# Only hubs completely mislocalized at least 100 μm from the apical tip or absent were counted.

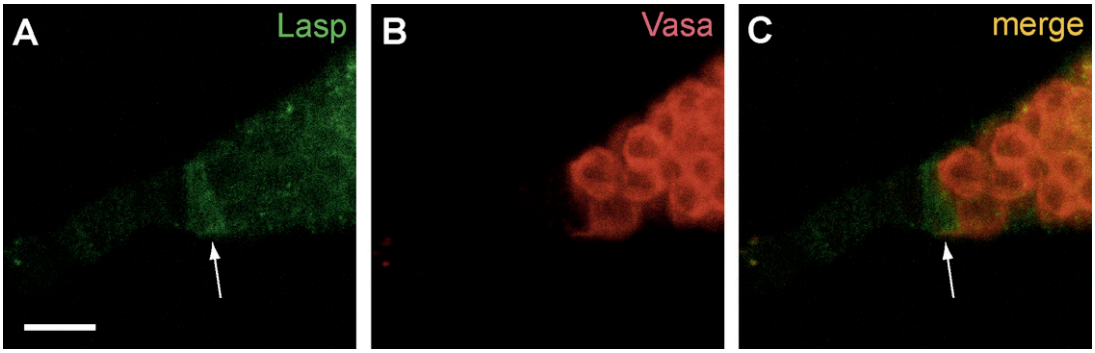

Fig. 5 – Lasp is expressed in cap cells in the ovary. (A) Anti-Lasp antibody staining, (B) anti-vasa antibody staining, and (C), merge of the germarium. Lasp is expressed anterior and adjacent to germ cells (arrows). Bar: 10 μm.

tributes only slightly to the observed sterility of $Lasp^1$. Even though actin cones are rescued as judged by phalloidin staining, it is possible that using UASp or the long isoform of Lasp may result in a better rescue. In contrast, actin cone defects are not rescued with the ptc-Gal4 driver, while premature cyst cell coiling and disintegration is completely rescued and sterility is substantially rescued suggesting that this phenotype is largely responsible for the observed sterility (Fig. 6, and Table 1).

In conclusion, Lasp has an integrin-dependent function in anchoring the stem cell niche, where Lasp likely functions at the periphery of the integrin adhesion site, given its weak phenotype compared to βPS integrin. Lasp also exhibits an integrin-independent function in actin cone migration. Intriguingly, human Lasp-1 similarly localizes to focal adhesions and to the cortical actin cytoskeleton in the leading edge of migrating cells, and it is the leading edge localization that appears to mediate cell migration (Lin et al., 2004). Lasp function in whole animals is restricted to highly specialized tissues, suggesting that integrin adhesion sites and actin organization differ depending on tissue and function, which may be one reason for the large number of biochemically defined components of integrin adhesion sites (Zaidel-Bar et al., 2007) and actin-binding proteins (Winder and Ayscough, 2005). Furthermore, our data show that the stem cell niche does not require a specific location to function properly, and by extension, that the male stem cell niche in *Drosophila* requires only one type of support cells, the hub cells.

3. Materials and methods

3.1. *Fly stocks and genetics*

The following fly stocks were used: *e00056* and *e03191* from the Exelixis *Drosophila* stock collection, *47127* (*UASiLasp1*), *21500* (*UASiLasp2*), and *29619* (*UASimys*) from the Vienna *Drosophila* RNAi center (Dietzl et al., 2007), mys^{nj42} from Nick Brown, *MB03281* from Hugo Bellen, *Df(3L)st7* from the Bloomington *Drosophila* stock center, *nos-Gal4::VP16* from David Dansereau, *ptc-Gal4* from Leanne Jones, and *c587-Gal4* from Allan Spradling. The following recombinants and stocks were made by standard genetic crosses: *UASLasp-GFP* $Lasp^1$, *ptc-Gal4*; *UASLasp-GFP* $Lasp^1$ and *nosGal4::VP16* $Lasp^1$.

To generate the UAStLasp-GFP construct, the short isoform of *Lasp* encoding a 504 amino acid protein isolated from an adult testes cDNA library (AT23571) was amplified by primers CACCATGAATAAAACCTGTGCCCCGT and TATAACCGCCTGCT CCACGTA and cloned into the Gateway pENTR/D-TOPO vector (Invitrogen), then recombined into pTWG from the *Drosophila* Gateway vector collection (http://www.ciwemb.edu/labs/murphy/Gateway%20vectors.html).

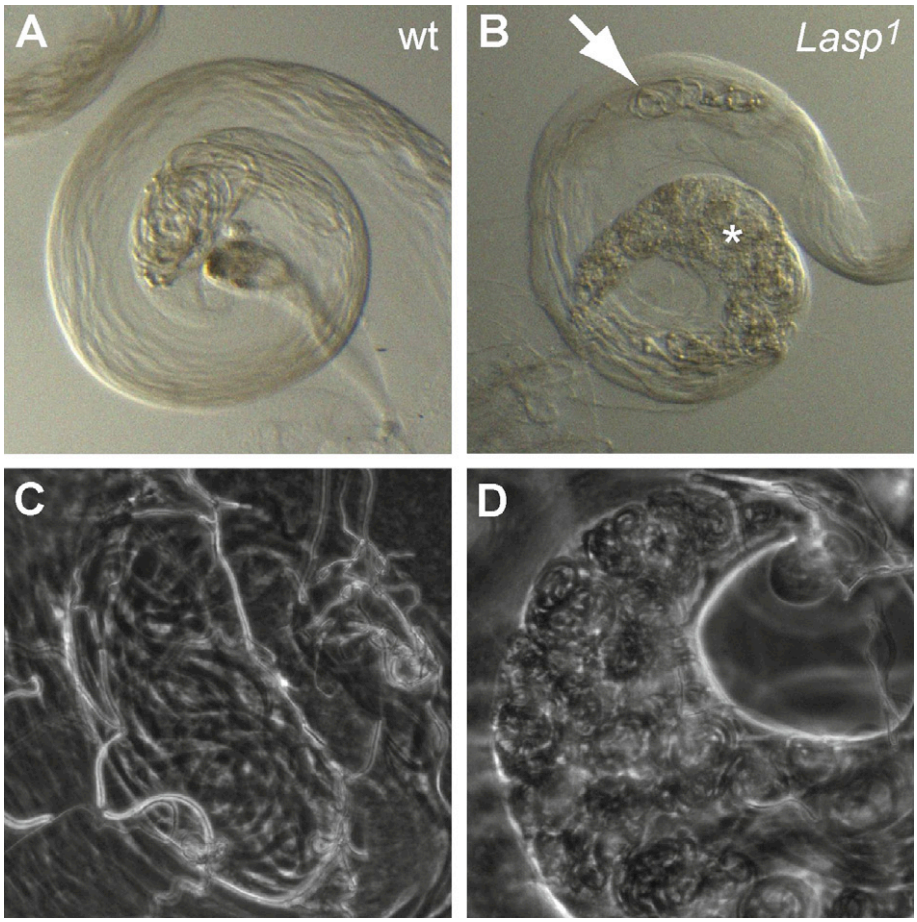

Fig. 6 – Cyst cells disintegrate prematurely in *Lasp*^{1} mutants. (A) Bright-field image of a wild-type testis showing the coiling stage of cysts in the basal end. (B) Cysts coil prematurely (arrow) and disintegrate into individual sperm at the coiling stage (asterisk) in *Lasp*^{1} mutant. (C) Differential interference contrast image of the basal end of a *Lasp*^{1} mutant rescued by ptcGal4-driven expression of UASLasp-GFP. Coiling defects are completely rescued. (D) Differential interference contrast image of the basal end of a *Lasp*^{1} mutant. Defects are not rescued by nosGal4-driven expression of UASLasp-GFP.

A *Lasp* mutant was generated by using piggyBac elements *e00056* and *e03191* for heat shock-mediated FLP/FRT recombination (Parks et al., 2004). The *Lasp* mutant fly line was verified by genomic PCRs for the presence of *e03191* (with primers TCCAAGCGGCGACTGAGATG and CTGACGAGCATTT TCATTTATATA), absence of exon 2 (with primers ATGGCAC AAAACGTG and TGCTGCACTTTTTGC), absence of *CG9692* (with primers ATTAGGGTTCAGCCACATGC and GATTCCAAC GCCTATCTGGA) and presence of exon 1 (with primers CGTTT TAAGCCGCTCTTTTG and AACACCGAAGCAGAGC).

To test male sterility, one male fly and four OreR female flies (1–2 days of age) were mated in a vial for two days at 25 °C. Then the flies were transferred to a fresh vial and allowed to lay eggs for 24 or 48 h at 25 °C. Only vials with all flies alive were analyzed.

3.2. *Histochemistry and microscopy*

Testes dissected from 3-day-old flies were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl) for 15 min, and permeabilized in 0.1% Triton X-100 in PBS for 1 min after fixation. After preincubation with blocking solution (3% goat serum, 0.05% Tween 20 in PBS) for 30 min, testes were incubated at 4 °C overnight with the following primary antibodies: rabbit anti-Lasp-C terminal (1:4000 provided by Anne Ephrussi), rat anti-vasa (1:10,000 provided by Paul Lasko) (Styhler et al., 1998), mouse anti-βPS integrin (1:10, CF.6G11 obtained from Developmental Studies Hybridoma Bank) (Brower et al., 1984), and mouse anti-fasciclin III (1:50, 7G10 obtained from DSHB) (Patel et al., 1987). After washing for 1 h in PBT (0.05% Tween 20 in PBS) next day, the testes were incubated in secondary antibodies of the Alexa-Fluor series (1:400, Invitrogen) for 2 h at room temperature. The testes were mounted in Prolong Gold Antifade solution (Invitrogen) after 1 h of washing in PBT. Some testes were squashed prior to fixation. Testes were placed on a poly-L-lysine-coated slide in Testis Buffer (TB; 15 mM potassium phosphate pH 6.7, 80 mM KCl, 16 mM NaCl, 5 mM $MgCl_2$, 1% PEG 8000). A siliconized cover slip was dropped onto the sample and pressed softly. Then the slide was frozen in liquid nitrogen for 3 min, and washed in 95% ethanol for 10 min. Finally, testes were fixed and stained in a wet chamber like the unsquashed samples.

Images were obtained on an upright microscope (DM6000B, Leica) with a digital camera (Orca-ER, Hamamatsu)

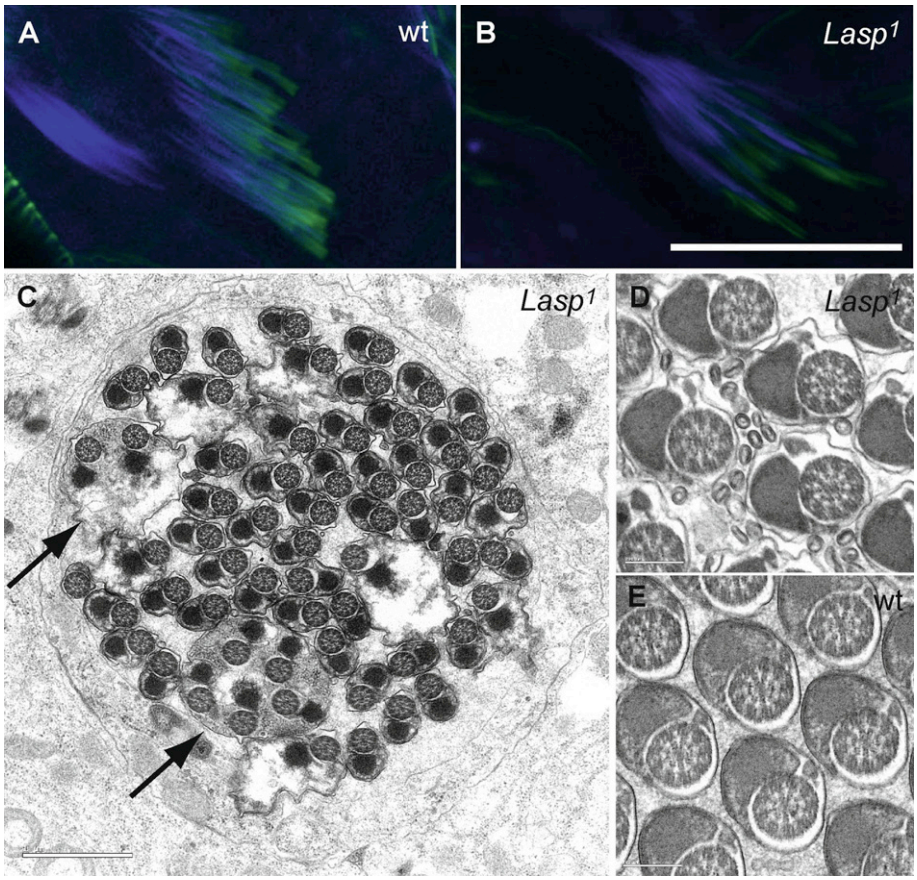

Fig. 7 – Actin cones are perturbed causing individualization defects in *Lasp*^{1} mutant testis. (A and B) Phalloidin and DAPI double staining. Compared to actin cones in wild-type (A), *Lasp*^{1} mutant actin cones are weakly visible (B). (C–E) Transmission electron micrographs of testes cross-sections. (C) Spermatids are not completely individualized in *Lasp*^{1} (arrows indicate membranes surrounding two and five spermatids, respectively). (D) Individualized spermatids are surrounded by a loose membrane and membrane fragments are found in between spermatids in *Lasp*^{1}. (E) In wild-type, membranes are tightly apposed and spermatids are closely spaced. Bars: (A and B) 25 μm; (C) 1 μm; (D and E) 200 nm.

and OpenLab software or a confocal microscope (LSM510 Meta, Zeiss), and processed with ImageJ and Adobe Photoshop software. To measure actin cone density, images were taken at the confocal microscope with a 63× C-Apochromat NA 1.2 water objective at three times zoom, and ImageJ was used to measure the mean pixel values in a 150 × 150 pixel square, which covered the basal ends of multiple actin cones.

3.3. *Electron microscopy*

To obtain cross-section images of cysts, testes were dissected from 3-day-old adult flies and fixed in 1.5% glutaraldehyde for 2 h on ice. After fixation, testes were washed for three times in PBT for 10 min. Then they were postfixed in 1% osmium tetroxide for 2 h. After washing, testes were dehydrated in acetone and embedded in epon-815 (Electron Microscopy Sciences). Samples were cut with a Reichert Ultracut AV microtome. EM grids were stained in 4% aqueous uranyl acetate for 8 min, and in Reynold's Lead for 5 min. Images were obtained on a transmission electron microscope (Tecnai-12, FEI) with a wide-angle multiscan CCD camera (792 Bioscan 1k × 1k, Gatan).

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REFERENCES

Becam, I.E., Tanentzapf, G., Lepesant, J.A., Brown, N.H., Huynh, J.R., 2005. Integrin-independent repression of cadherin transcription by talin during axis formation in *Drosophila*. Nat. Cell Biol. 7, 510–516.

Bellen, H.J., Levis, R.W., Liao, G., He, Y., Carlson, J.W., Tsang, G., Evans-Holm, M., Hiesinger, P.R., Schulze, K.L., Rubin, G.M., Hoskins, R.A., Spradling, A.C., 2004. The BDGP gene disruption project: single transposon insertions associated with 40% of *Drosophila* genes. Genetics 167, 761–781.

Brower, D.L., Wilcox, M., Piovant, M., Smith, R.J., Reger, L.A., 1984. Related cell-surface antigens expressed with positional specificity in *Drosophila* imaginal discs. Proc. Natl. Acad. Sci. USA 81, 7485–7489.

Chew, C.S., Chen, X., Parente Jr., J.A., Tarrer, S., Okamoto, C., Qin, H.Y., 2002. Lasp-1 binds to non-muscle F-actin in vitro and is localized within multiple sites of dynamic actin assembly in vivo. J. Cell Sci. 115, 4787–4799.

Decotto, E., Spradling, A.C., 2005. The *Drosophila* ovarian and testis stem cell niches: similar somatic stem cells and signals. Dev. Cell 9, 501–510.

Devenport, D., Brown, N.H., 2004. Morphogenesis in the absence of integrins: mutation of both *Drosophila* beta subunits prevents midgut migration. Development 131, 5405–5415.

Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., Couto, A., Marra, V., Keleman, K., Dickson, B.J., 2007. A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. Nature 448, 151–156.

Fuller, M.T., 1993. Spermatogenesis. In: Bate, M., Martinez Arias, A. (Eds.), The Development of *Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 71–147.

Grunewald, T.G., Butt, E., 2008. The LIM and SH3 domain protein family: structural proteins or signal transducers or both? Mol. Cancer 7, 31.

Grunewald, T.G., Kammerer, U., Schulze, E., Schindler, D., Honig, A., Zimmer, M., Butt, E., 2006. Silencing of LASP-1 influences zyxin localization, inhibits proliferation and reduces migration in breast cancer cells. Exp. Cell Res. 312, 974–982.

Hardy, R.W., Tokuyasu, K.T., Lindsley, D.L., Garavito, M., 1979. The germinal proliferation center in the testis of *Drosophila melanogaster*. J. Ultrastruct. Res. 69, 180–190.

Keicher, C., Gambaryan, S., Schulze, E., Marcus, K., Meyer, H.E., Butt, E., 2004. Phosphorylation of mouse LASP-1 on threonine 156 by cAMP- and cGMP-dependent protein kinase. Biochem. Biophys. Res. Commun. 324, 308–316.

Lasko, P.F., Ashburner, M., 1988. The product of the *Drosophila* gene vasa is very similar to eukaryotic initiation factor-4A. Nature 335, 611–617.

Li, B., Zhuang, L., Trueb, B., 2004. Zyxin interacts with the SH3 domains of the cytoskeletal proteins LIM-nebulette and Lasp-1. J. Biol. Chem. 279, 20401–20410.

Lin, Y.H., Park, Z.Y., Lin, D., Brahmbhatt, A.A., Rio, M.C., Yates 3rd., J.R., Klemke, R.L., 2004. Regulation of cell migration and survival by focal adhesion targeting of Lasp-1. J. Cell Biol. 165, 421–432.

Moncman, C.L., Wang, K., 1995. Nebulette: a 107 kDa nebulin-like protein in cardiac muscle. Cell Motil. Cytoskeleton 32, 205–225.

Nichols, S.A., Dirks, W., Pearse, J.S., King, N., 2006. Early evolution of animal cell signaling and adhesion genes. Proc. Natl. Acad. Sci. USA 103, 12451–12456.

Noguchi, T., Lenartowska, M., Miller, K.G., 2006. Myosin VI stabilizes an actin network during *Drosophila* spermatid individualization. Mol. Biol. Cell 17, 2559–2571.

Parks, A.L., Cook, K.R., Belvin, M., Dompe, N.A., Fawcett, R., Huppert, K., Tan, L.R., Winter, C.G., Bogart, K.P., Deal, J.E., Deal-Herr, M.E., Grant, D., Marcinko, M., Miyazaki, W.Y., Robertson, S., Shaw, K.J., Tabios, M., Vysotskaia, V., Zhao, L., Andrade, R.S., Edgar, K.A., Howie, E., Killpack, K., Milash, B., Norton, A., Thao, D., Whittaker, K., Winner, M.A., Friedman, L., Margolis, J., Singer, M.A., Kopczynski, C., Curtis, D., Kaufman, T.C., Plowman, G.D., Duyk, G., Francis-Lang, H.L., 2004. Systematic generation of high-resolution deletion coverage of the *Drosophila melanogaster* genome. Nat. Genet. 36, 288–292.

Patel, N.H., Snow, P.M., Goodman, C.S., 1987. Characterization and cloning of fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in *Drosophila*. Cell 48, 975–988.

Schreiber, V., Moog-Lutz, C., Regnier, C.H., Chenard, M.P., Boeuf, H., Vonesch, J.L., Tomasetto, C., Rio, M.C., 1998. Lasp-1, a novel type of actin-binding protein accumulating in cell membrane extensions. Mol. Med. 4, 675–687.

Styhler, S., Nakamura, A., Swan, A., Suter, B., Lasko, P., 1998. Vasa is required for GURKEN accumulation in the oocyte, and is involved in oocyte differentiation and germline cyst development. Development 125, 1569–1578.

Tanentzapf, G., Devenport, D., Godt, D., Brown, N.H., 2007. Integrin-dependent anchoring of a stem-cell niche. Nat. Cell Biol. 9, 1413–1418.

Tokuyasu, K.T., Peacock, W.J., Hardy, R.W., 1972. Dynamics of spermiogenesis in *Drosophila melanogaster*. *I. Individualization process. Z. Zellforsch. 124, 479–506.*

Tomasetto, C., Regnier, C., Moog-Lutz, C., Mattei, M.G., Chenard, M.P., Lidereau, R., Basset, P., Rio, M.C., 1995. Identification of four novel human genes amplified and overexpressed in breast carcinoma and localized to the q11-q21.3 region of chromosome 17. Genomics 28, 367–376.

Wang, K., Williamson, C.L., 1980. Identification of an N2 line protein of striated muscle. Proc. Natl. Acad. Sci. USA 77, 3254–3258.

Winder, S.J., Ayscough, K.R., 2005. Actin-binding proteins. J. Cell Sci. 118, 651–654.

Zaidel-Bar, R., Itzkovitz, S., Ma'ayan, A., Iyengar, R., Geiger, B., 2007. Functional atlas of the integrin adhesome. Nat. Cell Biol. 9, 858–867.