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SHORT REPORT

Cytokine exocytosis and JAK/STAT activation in the *Drosophila* ovary requires the vesicle trafficking regulator α-Snap

Afsoon Saadin and Michelle Starz-Gaiano*

ABSTRACT

How vesicle trafficking components actively contribute to regulation of paracrine signaling is unclear. We genetically uncovered a requirement for α-soluble NSF attachment protein (α-Snap) in the activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway during *Drosophila* egg development. α-Snap, a well-conserved vesicle trafficking regulator, mediates association of N-ethylmaleimide-sensitive factor (NSF) and SNAREs to promote vesicle fusion. Depletion of *α-Snap* or the SNARE family member *Syntaxin1A* in epithelia blocks polar cells maintenance and prevents specification of motile border cells. Blocking apoptosis rescues polar cell maintenance in *α-Snap*-depleted egg chambers, indicating that the lack of border cells in mutants is due to impaired signaling. Genetic experiments implicate α-Snap and NSF in secretion of a STAT-activating cytokine. Live imaging suggests that changes in intracellular Ca^{2+} are linked to this event. Our data suggest a cell-type specific requirement for particular vesicle trafficking components in regulated exocytosis during development. Given the central role for STAT signaling in immunity, this work may shed light on regulation of cytokine release in humans.

KEY WORDS: *Drosophila* oogenesis, JAK/STAT signaling, Exocytosis, Cell fate specification

Introduction

Paracrine signaling relies on the proper spatiotemporal release of activators from signaling cells, typically by exocytosis, to elicit distant responses. During trafficking, fusion of a vesicle to the appropriate target membrane requires coordination through multiple conserved proteins, including Snap receptor (SNARE) superfamily members (reviewed in Rothman, 1996; Zhao et al., 2007; Bombardier and Munson, 2015; Zhao and Brunger, 2016; Wickner and Rizo, 2017). Generally, an R-Snare in the vesicle membrane tethers to three Q-Snares on the target membrane, bringing the membranes close and docking them for fusion. Different SNARE proteins have distinct subcellular localizations, mediating specific fusion events. α-soluble NSF attachment protein (α-Snap) binds to the *cis*-SNARE complex and recruits the ATPase N-ethylmaleimide-sensitive Factor (NSF). Upon ATP hydrolysis, NSF and α-Snap promote Snare complex disassembly, which is required for another round of fusion.

While much of vesicle trafficking occurs constitutively, additional specialized mechanisms are needed for certain cases, such as cytokine or histamine release from immune cells, neurotransmitter release from neurons, or insulin secretion from β-pancreatic cells. (Burgoyne and Morgan, 2003; Südhof, 2012; Scheller, 2013; Xiong et al., 2017). In these cases, secretory cargo is separated and stored in vesicles, followed by rapid release that is independent from constitutive exocytosis. This is clearest in synaptic signaling, for which NSF and α-Snap are required.

Although a few specific roles have been suggested for NSF and α-Snap, their distinct developmental functions remain largely unknown. *NSF* and *α-Snap* are required for *Drosophila* neurotransmission (Kawasaki et al., 1998; Babcock et al., 2004; Yu et al., 2011; Li et al., 2015), and in hypothalamus and neuron development in zebrafish and mice, respectively (Chae et al., 2004; Hong et al., 2004; Kurrasch et al., 2009). Two genes encode NSF proteins in *Drosophila* – *comatose* (*comt)* and *NSF2* – with some tissue-specific requirements (Siddiqi and Benzer, 1976; Boulianne and Trimble, 1995; Pallanck et al., 1995; Golby et al., 2001; Zhao et al., 2012). Notably, expression of a dominant-negative NSF2 mutant can phenocopy the loss of Wingless/WNT and Notch signaling, and can genetically interact with components of these pathways (Stewart et al., 2001) and other developmental signaling cascades (Laviolette et al., 2005). We found a specific requirement for α-Snap and an NSF component in ovaries to regulate Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling.

JAK/STAT signaling is well-conserved, required for development and immune function, and misregulated in disease (Amoyel et al., 2014; O'Shea et al., 2015; Villarino et al., 2015). In *Drosophila*, an Unpaired (Upd) protein binds to a cytokine receptor, which results in JAK activation, STAT phosphorylation and the regulation of target genes (Zeidler and Bausek, 2013; Chen et al., 2014). STAT signaling is required for the specification and migration of ovarian follicle cells that are called border cells (reviewed in Saadin and Starz-Gaiano, 2016a).

Developing egg chambers are encased by a monolayer of follicle cells that differentiate into multiple fates. The anterior and posterior-most follicle cells become polar cells, which are essential for patterning (reviewed in Wu et al., 2008; Duhart et al., 2017). Upd secreted from polar cells activates JAK/STAT signaling (Van de Bor et al., 2011; Hayashi et al., 2012). Early on, this signaling reduces the number of polar cells and specifies stalk cells (Baksa et al., 2002; McGregor et al., 2002; Assa-Kunik et al., 2007; Borensztejn et al., 2013, 2018). Later, follicle cells with the highest STAT activity become border cells and migrate to the oocyte (Fig. 1A,B) (Montell et al., 2012; Saadin and Starz-Gaiano, 2016a). Border cell specification and migration requires multiple inputs that control transcription, signaling, cytoskeletal organization, adhesion and protein trafficking. Here, we demonstrate a crucial role for α-Snap, Syntaxin1A (Syx1A) and NSF/NSF2 in mediating trafficking event(s) required to induce JAK/STAT signaling.

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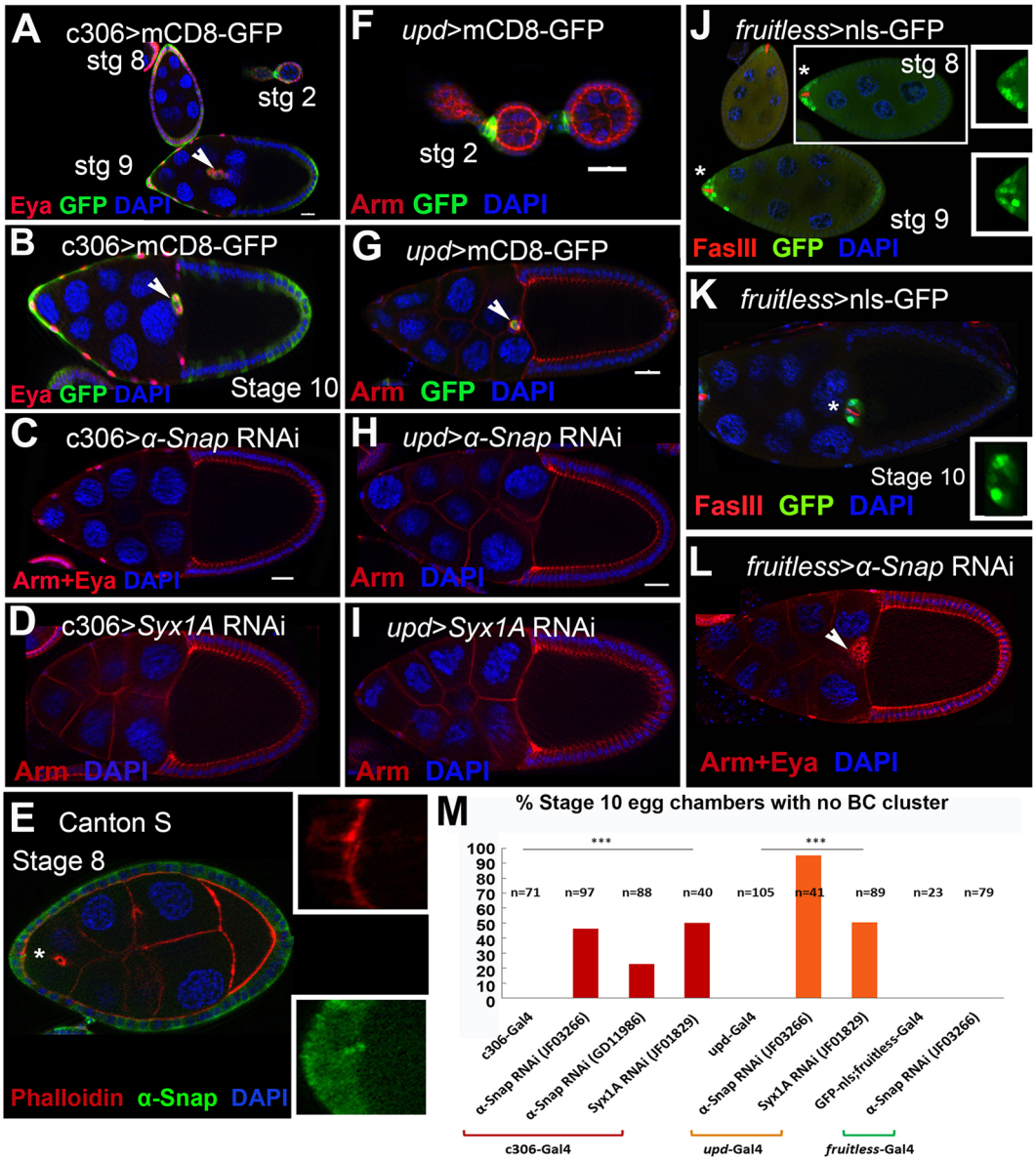

Fig. 1. *α-Snap* or *Syx1A* depletion in polar cells prevents border cell specification. Shown are egg chambers of indicated genotypes and stages. Egg chambers in all panels are oriented with anterior to the left, posterior to the right; scale bars: 20 µm. Nuclei were stained using DAPI (blue). Arrowheads indicate border cell clusters. (A,B) c306-Gal4-driven expression of UAS-mCD8-GFP (green; the '>' symbol in labels denotes drives expression of) in polar cells of egg chambers at stage 2 (A), and in polar cells and border cells (BCs) of egg chambers at stages 8 and 9 (A) and stage 10 (B). Expression of Eya (red) marks follicle cells. (C,D) c306-Gal4-driven *α-Snap* RNAi (C) or *Syx1A* RNAi (D) results in no BC specification. Compare red staining of Arm+Eya (C) and Arm (D) with that shown in panel L. (E) Stage 8 wild-type anterior follicle cells, showing cytoplasmic α-Snap (green) apically enriched; cortical F-actin (red). The asterisk indicates the area shown magnified in insets. (F,G) *upd*-Gal4-driven GFP expression in polar cells at stages 2 (F) to 10 (G). (H,I) *upd*-Gal4-driven *α-Snap* RNAi (H) or *Syx1A* RNAi (I) results in no BC specification. (J,K) *fruitless*-Gal4 drives GFP (green) in anterior cells, excluding polar cells (FasIII, red), before (J) and after (K) BC specification (magnified in insets). (L) *fruitless*-Gal4-driven *α-Snap* RNAi produces wild-type BCs. (M) Penetrance of the BC phenotype for the indicated genotypes. A lack of bar (1st, 5th, 8th and 9th position) in the graph means that all egg chambers contained border cells. ***P<0.0005 (two-tailed Fisher's exact test).

RESULTS AND DISCUSSION

α-Snap and Syx1A are required for border cell specification

To assay vesicle trafficking in developmental signaling, we disrupted trafficking regulators by utilizing RNA interference (RNAi) (Table S1). We downregulated *α-Snap*, *γ-Snap*, *comt/NSF*, *NSF2* and ten syntaxins (Q-Snares) (Littleton, 2000; Lloyd et al., 2000; Zhao et al., 2012) in a subset of follicle cells, including polar cells and border cell precursors by using c306-Gal4 (Fig. 1A,B).

Strikingly, depletion of *α-Snap* resulted in an unusual phenotype, i.e. egg chambers without border cell clusters, as assayed by expression of border cell-enriched proteins (Fig. 1C; Fig. S3C; see Materials and Methods). We observed this phenotype in 20–50% of stage 10 egg chambers by using two *α-Snap* RNAi lines (Fig. 1M; Table S1). Depletion of *Syx1A* resulted in the same phenotype (Table S1; Fig. 1D). Downregulation of other vesicle trafficking regulators produced wild-type egg chambers or other defects (Table S1). Thus, different vesicle trafficking genes are likely to have different key roles in follicle cells.

We confirmed the on-target effect of *α-Snap* RNAi by qRT-PCR (Table S2) and by rescuing the no-border cell phenotype through *α-Snap* overexpression (>3-fold rescue compared to controls, Fig. S1A,B,J,M). Overexpression of α-Snap-HA alone caused no

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obvious defects (Fig.S1C). We verified the temporal requirement for *α-Snap* by reducing its expression specifically in adults by using a temperature-sensitive (ts) version of the transcriptional regulator Gal80 (*tub*-Gal80^{ts}) and also rescuing the mutant phenotype in adults (Fig.S1D–F,K). By using α-Snap antiserum (Babcock et al., 2004), we detected the protein at or near the plasma membrane of follicle cells, consistent with it functioning there (Fig. 1E).

α-Snap functions with NSF in border cell specification

α-Snap recruits NSF, which hydrolyzes ATP to provide the energy for conformational changes that disassemble the SNARE complex, thereby resetting the components for a later round of fusion. However, recent studies have revealed that α-Snap can act independently of NSF activity, for example, to promote yeast vacuolar fusion (Zick et al., 2015; Schwartz et al., 2017; Song et al., 2017) or to regulate other cellular behaviors (Naydenov et al., 2012, 2014; Miao et al., 2013). Depletion of *comt*/*NSF* or *NSF2* in follicle cells yielded wild-type phenotypes (Tables S1 and S2); thus, the function of α-Snap in these cells could be NSF-independent or the two NSFs might function redundantly (Golby et al., 2001). Consistent with the latter, depletion of both genes together caused lethality (Table S2). Different cell types show different levels of NSF/NSF2 expression (Ordway et al., 1994; Boulianne and Trimble, 1995; Pallanck et al., 1995; Golby et al., 2001; Mohtashami et al., 2001), indicating they have different demands for its function. We detected both genes by qRT-PCR from ovaries, and found high expression levels of NSF protein in the early germline and follicle cells, including polar cells, by antibody staining (Li et al., 2015) (not shown). Next, we overexpressed *NSF2* in follicle cells. While overexpression of *NSF2* alone yielded no mutant phenotype, it significantly rescued the phenotype caused by reduced *α-Snap* (Fig. S2). NSF2 overexpression may promote vesicle machinery resetting in the presence of a small amount of *α-Snap* through less favored interactions, or it may promote fusion independently of *α-Snap* at high concentrations – like overexpressed yeast Sec18 (Zick et al., 2015; Song et al., 2017). Since the two NSF proteins are more than 80% identical and both are detected in the ovary, we suspect that *α-Snap* normally functions with either in follicle cells. These results, and the role of Syx1A at the plasma membrane (Schulze et al., 1995), suggest that α-Snap, NSF and/or NSF2 and Syx1A are necessary for exocytosis during border cell specification.

α-Snap is required in polar cells for border cell specification

α-Snap could be required in signaling cells for signal release or in responding cells to traffic receptors to the cell surface. To distinguish between these, we depleted α-*Snap* in different cell types: polar cells early (*upd*-Gal4, Fig. 1F,G) (McGregor et al., 2002), border cell precursors (not polar cells) (*fruitless*-Gal4, Fig. 1J,K) (Borensztejn et al., 2013), or later in specified border cells (not polar cells) (stage 8/9, *slbo*-Gal4, not shown) (Rørth et al., 1998). *α-Snap* depletion in border cells or their precursors yielded wild-type phenotypes (Fig. 1L,M, and data not shown). In contrast, depletion of *α-Snap* by using *upd*-Gal4 led to the absence of border cells in >80% of egg chambers (Fig. 1H,M), and a lack of polar cells by stage 10. This severe phenotype was rescued by *α-Snap* overexpression (Fig. S1G–I,L). Similarly, reduction of *Syx1A* in polar cells resulted in impaired polar cell maintenance and no border cells (Fig. 1I,M).

These results suggest that α-Snap and Syx1A are required in polar cells to promote border cell specification but are largely dispensable in border cells themselves. This is unlike depletion of exocyst components, which affects cell migration but not specification (Assaker et al., 2010; Laflamme et al., 2012; Ramel et al., 2013). Together, these results indicate that different vesicle trafficking regulators have cell-type specific key functions.

α-Snap is required for polar cell maintenance and, separately, for border cell induction

The lack of border cells upon reduction of *α-Snap* expression could be due to cell death or failed specification. A primary marker for polar cells, fasciclin III (FasIII) (Ruohola et al., 1991), was present early but not detected later when *α-Snap* was depleted, indicating the cells are specified but not maintained (Fig. 2A). We do not believe that this reflects a general requirement for α-Snap in cell viability since depletion in other follicle cells does not result in obvious defects (Fig. 1L). Different cell types differ in their ability to survive α-Snap disruption (Babcock et al., 2004; Chae et al., 2004; Hong et al., 2004; Tomes et al., 2005; Bátiz et al., 2009; Naydenov et al., 2014; Arcos et al., 2017) and might require different amounts of this regulator. To overcome polar cell loss, we expressed the anti-apoptotic gene *p35* (Hay et al., 1994) in *α-Snap*-depleted polar cells. Polar cells were apparent at all stages in this genotype, indicating that *α-Snap* is essential for their maintenance (Fig. 2 compare panels A and B with panels D and E, respectively). Interestingly, >70% of these egg chambers still lacked border cells (Fig. 2F), revealing a separable, essential function for *α-Snap* to enable polar cells to specify border cells.

α-Snap is not required for all subcellular trafficking

In mammalian cells, α-Snap is required for localization of E-cadherin (E-Cad), β-catenin, and apical proteins (Chae et al., 2004; Hong et al., 2004; Andreeva et al., 2005; Naydenov et al., 2012). Since early depletion of *α-Snap* resulted in the absence of polar cells, we could not investigate protein localization there. However, the localization patterns and/or levels of E-Cad and Armadillo (Arm) were normal in posterior cells upon disruption of *α-Snap* or *Syx1A* (Fig. 1C,D,H,I; Fig. S3A–C) and normal upon *α-Snap* disruption in border cells (not targeting the polar cells, Fig. 1L). Additionally, reduction of *α-Snap* affects border cells differently than loss of adhesion and polarity molecules: reduction of E-Cad results in cluster separation (Cai et al., 2014) and disruption of apical proteins yields poor border cell migration (Niewiadomska et al., 1999; Pinheiro and Montell, 2004).

Polar cells activate several signaling pathways but most do not appear to require *α-Snap*. Depletion of *α-Snap* did not result in fused egg chambers, stalk defects or oocyte polarity defects (Figs 1C and 2B), as would disruption of Hedgehog, Hippo and/or Notch signaling (Lopez-Schier and St Johnston, 2001; Grammont and Irvine, 2002; Chen et al., 2011). Furthermore, although polar cell fate requires Notch activity, we found no genetic interaction between *Notch* (*N*^{ts2}) and *α-Snap* (*α-Snap*^{G8}) in polar cell fate (Fig. S3D). These observations are consistent with cell culture studies linking *α-Snap* only to certain signaling cascades (Baeg et al., 2005; DasGupta et al., 2005; Nybakken et al., 2005). Notably, genetic disruptions of *α-Snap* dramatically compromise the functions of some cell types while not obviously affecting others (Babcock et al., 2004; Chae et al., 2004; Hong et al., 2004; Bátiz et al., 2009; Arcos et al., 2017), suggesting that some cells can overcome this defect. It is possible that less-sensitive cells synthesize SNAREs rapidly to reduce demand for recycling or have a 'bypass' mechanism that alleviates the need for α-Snap and/or NSF activity (Thorngren et al., 2004), or that NSF functions with a not-yet identified co-factor to promote exocytosis.

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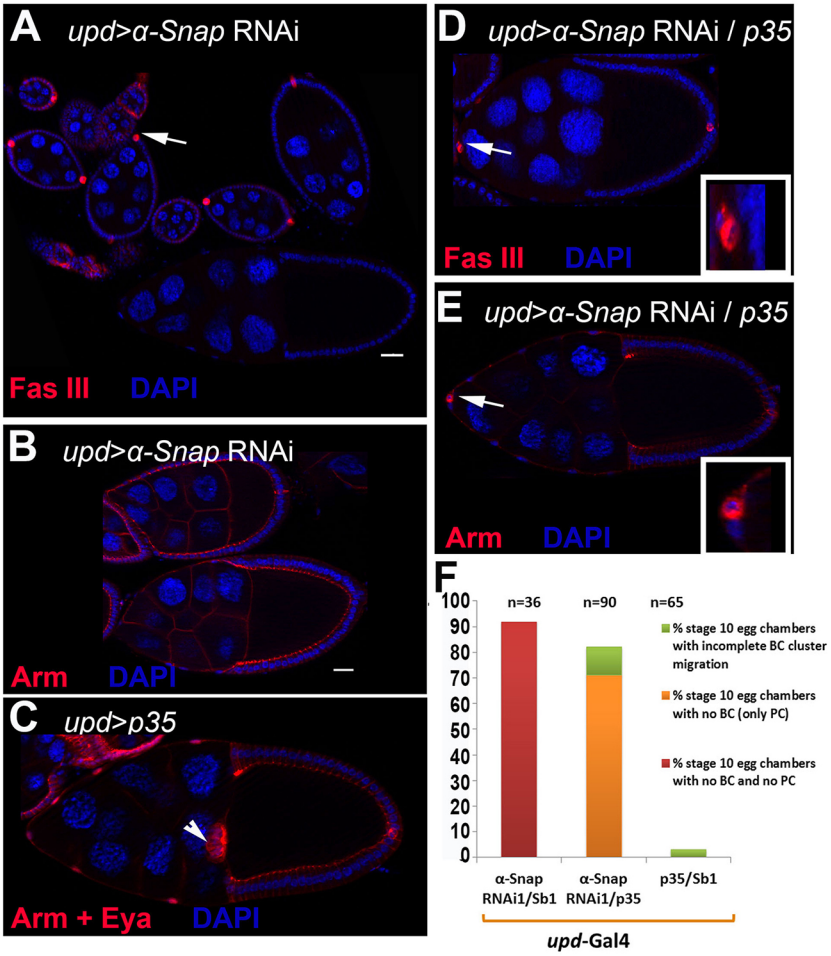

Fig. 2. Blocking apoptosis in α-Snap-depleted polar cells does not rescue border cell specification. (A,B) *upd*-Gal4 driving *α-Snap* RNAi results in egg chambers that have polar cells early (FasIII-positive, red; arrow in A), but lack polar and border cells later (B; Arm, red; DAPI, blue). (C) *p35* expression in polar cells yields normal border cells (arrowhead). (D,E) *p35* expression in *α-Snap*-depleted polar cells rescues polar cell maintenance but not border cell specification (arrow, magnified in insets). All scale bars: 20 µm. (F) Penetrance of the no-polar (PC) and/or no-border cells (BC) phenotypes by genotype (line JF03266).

α-Snap in polar cells regulates STAT activation in the border cells

Because border cell specification requires JAK/STAT signaling, we tested whether *α-Snap* could regulate components of this pathway. We assayed the recessive mutant alleles *Stat92E*^{397} (Silver and Montell, 2001) and *α-Snap*^{G8} (Babcock et al., 2004). Single heterozygotes have normal numbers of border cells but minor cell migration defects (<5% of stage 10 egg chambers had border cell migration defects; Fig. 3A, B,D) (Silver and Montell, 2001; Silver et al., 2005). Trans-heterozygous (*Stat92E*^{397}/*α-Snap*^{G8}) flies had a significantly enhanced mutant phenotype (>20% of stage 10 egg chambers had border cell migration defects; Fig. 3C,D), indicating that *α-Snap* and *Stat92E* are likely to function in the same genetic pathway.

Next we examined the secreted activator Upd. In epistasis tests, we found that overexpression of *upd* in the polar cells significantly rescued the phenotype caused by one *α-Snap* RNAi line (Fig. 3E) and to a lesser extent by another (not shown), resulting in normal border cells. These results are consistent with a model in which α-Snap and Syx1A are necessary in polar cells to regulate Upd release.

During early oogenesis, Upd in polar cells promotes stalk cell fate (Lopez-Schier and St Johnston, 2001; Baksa et al., 2002; McGregor et al., 2002; Assa-Kunik et al., 2007) and eliminates excess polar cells (Borensztejn et al., 2013; Torres et al., 2017). Although we disrupted *α-Snap* and *Syx1A* early, we did not see stalk defects or extra polar cells (Fig. 2B), or changes in stretch or centripetal cells, which require low levels of STAT signaling (Xi et al., 2003). We speculate that *α-Snap* is not depleted early enough to cause these defects, or that low levels of *α-Snap* function permit low levels of STAT activity. Upd is not required for polar cell fate, and the survival signal(s) that α-Snap regulates to maintain polar cells is unknown.

We propose that α-Snap mediates the controlled exocytosis of Upd separately from constitutive trafficking. Our results add another control node to JAK/STAT signaling. Given the strong conservation of this pathway, it will be interesting to see whether the human α-Snap homolog NAPA, which is 60% identical to fly α-Snap (Lemons et al., 1997), regulates STAT-mediated immune responses.

Intracellular Ca^{2+} increases transiently in polar cells prior to border cell specification

Some secreted molecules are sequestered and released in a Ca^{2+}-dependent, regulated manner (Südhof, 2012; Xiong et al., 2017), including secretory granules from neutrophils, neurotransmitter release and the sperm acrosome reaction (Littleton et al., 1993; Tomes et al., 2005; Stow et al., 2009; Scheller, 2013; Sheshachalam et al., 2014). The latter two events are known to be mediated by α-Snap, NSF and certain syntaxins. Since our data suggest that STAT activation depends on these regulators in polar cells, we examined intracellular Ca^{2+} flux in egg chambers using the verified Ca^{2+} sensors GCaMP6s and GCaMP6m, which fluoresce in response to high intracellular Ca^{2+} (Chen et al., 2013). Interestingly, we observed periodic, transient increases of intracellular Ca^{2+} signals in polar cells prior to border cell specification by using three different Gal4 drivers (Fig. 4A–C; Movies 1–3). Consistent with a role for Ca^{2+}, we observed significantly fewer border cells when free Ca^{2+} was reduced in polar cells by overexpression of the human

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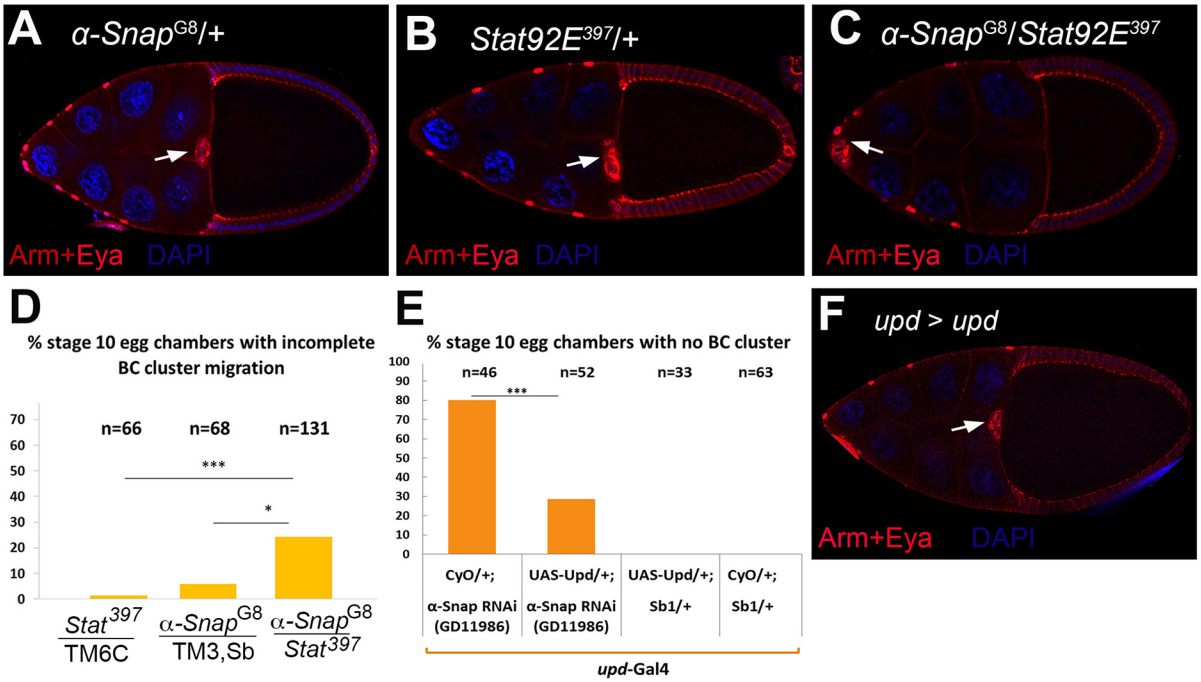

Fig. 3. α-Snap functions upstream of Upd to promote JAK/STAT signaling and cell specification. (A,B) Border cells (arrow; Eya- and Arm-positive, red) form and migrate correctly in *α-Snap*^{G8}/+ (A) or *Stat92E*^{397}/+ (B) heterozygotes. (C) An *α-Snap*^{G8}/*Stat*^{397} egg chamber that contains migratory-defective border cells. (D) Penetrance of border cell migration defects by genotype. (E) Penetrance of the no border cell phenotype by genotype. A lack of bar (4st and 5th position) in the graph indicates that all egg chambers contained border cells. *P<0.05, ***P<0.0005, two-tailed Fisher's exact test. (F) *upd* overexpression in polar cells yields a wild-type phenotype.

parvalbumin protein (PVALB, hereafter referred to as PV) (Harrisingh et al., 2007) (Fig. 4D–G). The number of border cells is known to drop with decreasing STAT activity (Silver et al., 2005; Starz-Gaiano et al., 2008; Van de Bor et al., 2011). Thus, we favor a model in which Upd is released in a regulated non-constitutive manner in response to Ca^{2+} (Fig. 4H). Notably, cation concentrations govern morphogen release during fly wing development (Dahal et al., 2012, 2017). Thus, ion-regulated exocytosis might represent a widespread means to mediate specificity of signal release during animal development.

MATERIALS AND METHODS

Fly stocks

The GD11986 and KK107910 transgenic *α-Snap* RNAi lines were from Vienna Drosophila Research Center (Dietzl et al., 2007). The polar cell driver (*upd*-Gal4) (Khammari et al., 2011), and UAS-*upd* (21.2) (Silver et al., 2005) transgenic and *stat92E*^{397} mutant flies were provided by D. J. Montell (University of California Santa Barbara, Santa Barbara, CA). UAS-NSF2 transgenic fly line was a generous gift from L. Pallanck (University of Washington, Seattle, WA). All other transgenic and mutant fly lines including UAS-mCD8-GFP/CyO (Lee and Luo, 1999), UAS-GFP.nls 14 (Neufeld et al., 1998), the anterior follicle cell drivers: c306-Gal4 (Manseau et al., 1997), *slbo*-Gal4 (Rørth et al., 1998) and *fruitless*-Gal4 (Hayashi et al., 2002), *tub*-Gal80^{ts}/TM6 (McGuire et al., 2003), UAS-*p35* (Hay et al., 1994), Ep-*Dome* (P(EPgy2)EY22614) (Bellen et al., 2011), *αSnap*^{M4} and *αSnap*^{G8} (Babcock et al., 2004), N$^{11N\text{-}ts2}$ (Shellenbarger and Mohler, 1975), UAS-GCaMP6m and UAS-GCaMP6s reporters (Chen et al., 2013), *tub*-Gal4/TM3 (Lee and Luo, 1999), UAS-PV-Myc (Harrisingh et al., 2007), JF03266 and HMS00872 RNAi lines for α-Snap, and the rest of RNAi lines were from the Bloomington Drosophila Stock Center (Ni et al., 2008).

Overexpression and knockdown studies

Genetic expression of transgenes in follicle cells was performed as previously described (Saadin and Starz-Gaiano, 2016b). All genetic crosses were established at 25°C. When offspring of a cross involving Gal4 were not viable at 25°C, the cross was maintained at 18°C or the temperature-sensitive allele of *tub*-Gal80 was introduced to the cross while the cross was maintained at 20–21°C (permissive temperature range for Gal80^{ts}) to repress Gal4. Female offspring (3–7 days old) were cultured on yeast-supplemented food and maintained at either 25°C, 29°C or 31°C, depending on their genotype, for different periods of time prior to dissection. Unless otherwise stated in the figure legends, flies bearing Gal4 were incubated at 29°C for 12–16 h. Flies bearing Gal4 and Gal80^{ts} were incubated at 31°C (non-permissive temperature for Gal80^{ts}) for 54 h, and flies that did not bear either Gal4 or Gal80^{ts}, were incubated at 25°C for 12–16 h before dissection.

RNAi validation and quantitative real time PCR

Genetic crosses were established and maintained at 25°C. Since follicle cell Gal4 drivers only impact a fraction of the cells in the ovary, we tested RNAi efficiency in embryos. *tub*-Gal4 females were crossed with males from the indicated RNAi lines. Total RNA was isolated from 0–24-hour-old F1 embryos (25°C) by using the Qiagen RNeasy Plus Micro Kit. BioRad iScript was used for cDNA synthesis. qRT-PCR was performed in triplicates, using BioRad iTaq SYBR Green Supermix and the BioRad CFX96 thermocycler. *Tubulin*-Gal4 embryos were used as the calibrating sample, and relative changes in gene expression were calculated using *rp49* as a reference gene. Fold-changes were determined using the Livak $2^{-\Delta\Delta CT}$ method.

Primers used, were α-snap ex1-2 forward: 5′-TGGGTGACAACGAA-CAGAAG-3′, reverse: 5′-CCCAGCTTTGTCCAGTTTT-3′; α-snap ex2-3 forward: 5′-GCTGCCAAACATCACCAAAG-3′′, reverse: 5′-CCACCTT-CAACATGCACTTG-3′; comt set2 forward: 5′-CCCGTGAAAATCAGC-AAGAATC-3′′, reverse: 5′-ACCAACATTCACTATAGCCCG-3′; comt ex4-6 forward: 5′-ACACGGATATCTTTAGCAAGGG-3′′, reverse: 5′-A-CAGGAACTTTATGGCCCG-3′; NSF2 ex2-3 forward: 5′-AGTTCCTC-ATGCAGTTCGC-3′′, reverse: 5′- GAGTCTTCGGTAGGGAATCG-3′; NSF2 ex3-4 forward: 5′-AGTTGGAGGGTCTGGTTAGAG-3′′, reverse: GCTTGATGTCGTTGTCCAATG-3′.

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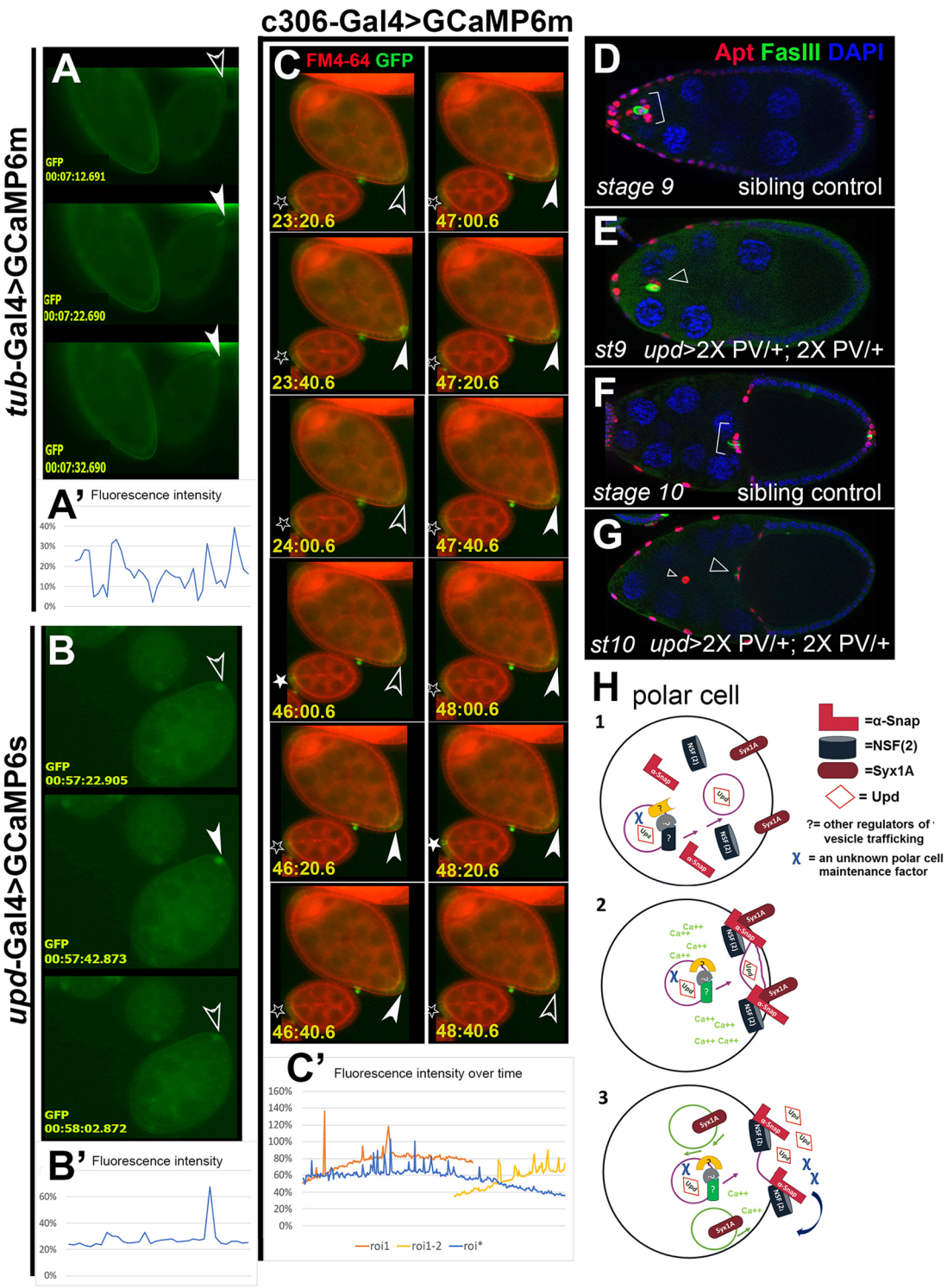

Fig. 4. See next page for legend.

Immunofluorescence labeling and phenotypic analysis

Fly ovaries were dissected to ovarioles in Schneider's medium supplemented with 10% fetal bovine serum (FBS) and 0.6× penicillin–streptomycin (Pen–Strep) (Prasad et al., 2007), and fixed in 4% paraformaldehyde diluted in 0.1 M potassium phosphate buffer. The egg chambers were immunostained following a previously established protocol (McDonald et al., 2006). Briefly, fixed egg chambers were washed in NP40 wash buffer (0.05 M Tris-HCl pH 7.4, 0.15 M NaCl, 1 mg/ml BSA, 0.5% Nonidet P-40 (Igepal CA-630, Sigma-Aldrich), 0.02% sodium azide) (McDonald et al., 2006) and incubated with primary antibodies (see below) diluted in NP40 wash buffer either overnight at 4°C or for 2–3 h at room temperature. The egg chambers were then washed four times in NP40 wash buffer and incubated with diluted (1:400) secondary antibodies (Alexa Flour 488 and Alexa Flour 568; Life Technologies) at room temperature for 2–3 h. Nuclei of the immuno-stained egg chambers were then stained with DAPI (1:1000; D1306, Invitrogen,) for 10 min, washed and mounted in 70%

Fig. 4. Ca^{2+} signaling in polar cells prior to border cell specification. (A,B,C) Movie stills of Movies 1–3, showing transient Ca^{2+}-dependent GFP fluorescence in polar cells of egg chambers. The transgenes used are indicated. White arrowheads mark fluorescence in polar cells; outlined arrowheads indicate the same cells at a different time without fluorescence. In C, white stars indicate fluorescence in polar cells of a younger egg chamber; outlined stars indicate the same cell at a different time without fluorescence. GCaMP6-GFP (green; the '>' symbol after Gal4 in labels denotes 'drives expression of'); FM4-64 lipophilic dye, red. (A',B',C') Quantifications of the changes in fluorescence intensity (ΔF/F) of the respective cells shown in Movies 1–3 over the timeframe of each movie. C' shows the fluorescence intensity of the starred cell (ROI*, blue) and the cell marked by the arrow at two different positions due to shifting of the egg chamber (ROIs orange and yellow). (D–G) Egg chambers at stages 9 and 10 of the indicated genotypes; Apt (red) marks anterior follicle cells, FasIII (green) polar cells and DAPI (blue) nuclei. *upd*-Gal4-driven expression of UAS-GFP (green) in polar cells results in at least six migratory cells (see square bracket in D). *upd*-Gal4-driven expression of UAS-PV (green) in polar cells to sequesters free Ca^{2+} results in fewer migratory cells (three cells, triangles), and poor migration (E,G). The average number of clustered migratory cells in *upd*-Gal4; UAS-PV, 4.8 (n=23), was significantly smaller than the average of 5.8 cells observed in *upd*-Gal4; UAS-GFP, (n=28); P<0.002; Mann-Whitney U test. (H) A proposed model of Upd cytokine secretion from polar cells. (1) Upd is loaded into apically targeted vesicles that then associate with Syx1A at the plasma membrane and dock. (2) α-Snap and NSF/NSF2 associate with Syx1 and the vesicle SNARE complex. In the presence of Ca^{2+}, membranes fuse and Upd is released (3). α-Snap and NSF are required to reset the vesicle fusion machinery for another round; Syx1A is recycled back to the plasma membrane. α-Snap also mediates the release of a polar cell maintenance factor(s) (X).

glycerol. Stage-10 egg chambers were scored for defects in border cell specification characterized by the absence of border cell markers, including the *Drosophila* β-catenin, Armadillo (Arm) (Peifer et al., 1993) (Fig. 1C), Eyes Absent (Eya) (Bai and Montell, 2002) (Fig. 1C), Apontic (Apt) (Starz-Gaiano et al., 2008), Slow border cells (Slbo) (Montell et al., 1992) and E-cadherin (E-Cad) (Niewiadomska et al., 1999) (Fig. S3C and data not shown), as well as defects in migration, where the border cell clusters had not reached the oocyte. The absence of the border cells was further confirmed using optical sections to examine the whole depth of the tissue. Data for c306-driven and *fruitless*-driven JF03266 RNAi lines in Fig. 1M are the average penetrance of four and two independent experiments, respectively. Statistical significance was established using the Mann–Whitney U test for border cell numbers and Fisher's exact test for proportions by using online tools (http://vassarstats.net).

The following primary antibodies were used: mouse anti-Eya (10H6, DSHB; 1:100) (Bonini et al., 1993), mouse anti-Armadillo (N27A1, DSHB; 1:40) (Riggleman et al., 1990), rabbit anti-Apt (provided by S. Hirose, National Institute of Genetics, Mishima, Japan; 1:1000) (Liu et al., 2003), guinea pig anti-Apt from GST-Apt (Pocono Rabbit Farm, 1:5000), rat anti-Slbo (provided by P. Rorth, Institute of Molecular and Cell Biology A-STAR, Proteos, Singapore; 1:1000) (Beccari et al., 2002), anti-rabbit GFP (Life Technologies; 1:250), rat anti-*Drosophila* E-Cad (DCAD2, DSHB;1:20) (Oda et al., 1997), rat anti-human influenza hemagglutinin (HA) (11867423001, Roche; at 0.5 µg/ml), mouse anti-FasIII (7G10, DSHB;1:50) (Patel et al., 1987), rabbit anti-NSF (provided by J. Han, Southeast University, Nanjing, China; 1:50; Li et al., 2015) and α-Snap (provided by L. Pallanck, University of Washington, Seattle, WA; 1:600; Babcock et al., 2004). Images were acquired with a Carl Zeiss AxioImager Z1 and Apotome optical sectioning with Axiovision acquisition software. Image size adjustments and figure assembly were completed using Photoshop CS6 Adobe.

Generation of UAS–α-Snap–HA transgenic flies

Drosophila melanogaster α-Snap cDNA clone (LD21601) was obtained from Drosophila Genomic Research Center in pOT2 vector. The coding sequence – excluding the stop codon – to allow expression of the HA tag was amplified using primers that contained attB sites following the GateWay cloning protocol by Invitrogen/ThermoFisher. The Kozak sequence (CAAC) (Cavener, 1987) was also added to the forward primer just upstream of the start codon. The BP reaction was carried out using a pDNOR221 vector (Invitrogen, 12536-017) and Gateway BP Clonase II enzyme mix (Invitrogen, 11789020) followed by heat shock-induced transformation into One Shot OmniMax 2T1 competent *E. coli* cells (Invitrogen, C854003). Successful cloning of α-Snap into pDONR221 was confirmed by sequencing using M13F(-21) and M13R primers (Genewiz). The LR reaction was then carried out using a pDONR221 vector containing α-Snap and pUASg-HA.attB (Bischof et al., 2007, 2013) (Gene Bank Accession number KC896837.1) with a Gateway LR Clonase II enzyme mix (Invitrogen, 11791020). The product of the LR reaction was transformed into One Shot OmniMax 2T1 competent E. coli cells. Successful cloning was confirmed by sequencing, using the primers Forward 5′-CGTCGCTAAGCGAAAGCTAAGC-3′ and Reverse 5′-AGCCTGCTGCTACACTTGCC-3′. The Destination vector (pUASg-HA.attB) containing α-Snap was used for PhiC31 injection through BestGene, Inc, plan H.

Live Ca^{2+} imaging

Live imaging was performed following the previously established protocol by Manning and Starz-Gaiano, 2015. Briefly, fly ovaries were dissected in Schneider's medium containing 0.2 mg/ml insulin, 10% FBS and 0.6× Pen–Strep. Egg chambers were cultured in insulin-containing medium supplemented with 9 µM FM4-64 dye (Invitrogen) during imaging. The dissected ovarioles were imaged every 10–20 s at 1–2 s exposure time for 0.5–2 h using a Carl Zeiss AxioImager Z1 microscope with AxioVision acquisition software. Changes in fluorescence intensity were measured using ImageJ, if needed after background correction, by defining regions of interest (ROIs) around polar cells and by using Multi Measure on a stack of time-lapse images. The ROI of a nearby cell was defined as background. Change over background (ΔF) was normalized against fluorescence intensity (F) at the start of the imaging procedure (Macleod, 2012).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.S.-G.; Methodology: A.S., M.S.-G.; Formal analysis: A.S., M.S.-G.; Investigation: A.S.; Resources: M.S.-G.; Writing - original draft: A.S.; Writing - review & editing: M.S.-G.; Visualization: A.S.; Supervision: M.S.-G.; Project administration: M.S.-G.; Funding acquisition: M.S.-G.

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Supplementary information

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References

Amoyel, M., Anderson, A. M. and Bach, E. A. (2014). JAK/STAT pathway dysregulation in tumors: a Drosophila perspective. *Semin. Cell Dev. Biol.* **28**, 96-103.

Andreeva, A. V., Kutuzov, M. A., Vaiskunaite, R., Profirovic, J., Meigs, T. E., Predescu, S., Malik, A. B. and Voyno-Yasenetskaya, T. (2005). G alpha12 interaction with alphaSNAP induces VE-cadherin localization at endothelial junctions and regulates barrier function. *J. Biol. Chem.* **280**, 30376-30383.

Arcos, A., Paola, M., Gianetti, D., Acuña, D., Velásquez, Z. D., Miró, M. P., Toro, G., Hinrichsen, B., Munoz, R. I., Lin, Y. et al. (2017). alpha-SNAP is expressed in mouse ovarian granulosa cells and plays a key role in folliculogenesis and female fertility. *Sci. Rep.* **7**, 11765.

Assaker, G., Ramel, D., Wculek, S. K., Gonzalez-Gaitan, M. and Emery, G. (2010). Spatial restriction of receptor tyrosine kinase activity through a polarized

endocytic cycle controls border cell migration. *Proc. Natl. Acad. Sci. USA* **107**, 22558-22563.
Assa-Kunik, E., Torres, I. L., Schejter, E. D., Johnston, D. S. and Shilo, B.-Z. (2007). Drosophila follicle cells are patterned by multiple levels of Notch signaling and antagonism between the Notch and JAK/STAT pathways. *Development* **134**, 1161-1169.
Babcock, M., Macleod, G. T., Leither, J. and Pallanck, L. (2004). Genetic analysis of soluble N-ethylmaleimide-sensitive factor attachment protein function in Drosophila reveals positive and negative secretory roles. *J. Neurosci.* **24**, 3964-3973.
Baeg, G. H., Zhou, R. and Perrimon, N. (2005). Genome-wide RNAi analysis of JAK/STAT signaling components in Drosophila. *Genes Dev.* **19**, 1861-1870.
Bai, J. and Montell, D. (2002). Eyes absent, a key repressor of polar cell fate during Drosophila oogenesis. *Development* **129**, 5377-5388.
Baksa, K., Parke, T., Dobens, L. L. and Dearolf, C. R. (2002). The Drosophila STAT protein, stat92E, regulates follicle cell differentiation during oogenesis. *Dev. Biol.* **243**, 166-175.
Bátiz, L. F., De Blas, G. A., Michaut, M. A., Ramírez, A. R., Rodriguez, F., Ratto, M. H., Oliver, C., Tomes, C. N., Rodriguez, E. M. and Mayorga, L. S. (2009). Sperm from hyh mice carrying a point mutation in alphaSNAP have a defect in acrosome reaction. *PLoS ONE* **4**, e4963.
Beccari, S., Teixeira, L. and Rørth, P. (2002). The JAK/STAT pathway is required for border cell migration during Drosophila oogenesis. *Mech. Dev.* **111**, 115-123.
Bellen, H. J., Levis, R. W., He, Y., Carlson, J. W., Evans-Holm, M., Bae, E., Kim, J., Metaxakis, A., Savakis, C., Schulze, K. L. et al. (2011). The Drosophila gene disruption project: progress using transposons with distinctive site specificities. *Genetics* **188**, 731-743.
Bischof, J., Maeda, R. K., Hediger, M., Karch, F. and Basler, K. (2007). An optimized transgenesis system for Drosophila using germ-line-specific phiC31 integrases. *Proc. Natl. Acad. Sci. USA* **104**, 3312-3317.
Bischof, J., Björklund, M., Furger, E., Schertel, C., Taipale, J. and Basler, K. (2013). A versatile platform for creating a comprehensive UAS-ORFeome library in Drosophila. *Development* **140**, 2434-2442.
Bombardier, J. P. and Munson, M. (2015). Three steps forward, two steps back: mechanistic insights into the assembly and disassembly of the SNARE complex. *Curr. Opin. Chem. Biol.* **29**, 66-71.
Bonini, N. M., Leiserson, W. M. and Benzer, S. (1993). The eyes absent gene: genetic control of cell survival and differentiation in the developing Drosophila eye. *Cell* **72**, 379-395.
Borensztejn, A., Boissoneau, E., Fernandez, G., Agnes, F. and Pret, A.-M. (2013). JAK/STAT autocontrol of ligand-producing cell number through apoptosis. *Development* **140**, 195-204.
Borensztejn, A., Mascaro, A. and Wharton, K. A. (2018). JAK/STAT signaling prevents excessive apoptosis to ensure maintenance of the interfollicular stalk critical for Drosophila oogenesis. *Dev. Biol.* **438**, 1-9.
Boulianne, G. L. and Trimble, W. S. (1995). Identification of a second homolog of N-ethylmaleimide-sensitive fusion protein that is expressed in the nervous system and secretory tissues of Drosophila. *Proc. Natl. Acad. Sci. USA* **92**, 7095-7099.
Burgoyne, R. D. and Morgan, A. (2003). Secretory granule exocytosis. *Physiol. Rev.* **83**, 581-632.
Cai, D., Chen, S. C., Prasad, M., He, L., Wang, X., Choesmel-Cadamuro, V., Sawyer, J. K., Danuser, G. and Montell, D. J. (2014). Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. *Cell* **157**, 1146-1159.
Cavener, D. R. (1987). Comparison of the consensus sequence flanking translational start sites in Drosophila and vertebrates. *Nucleic Acids Res.* **15**, 1353-1361.
Chae, T. H., Kim, S., Marz, K. E., Hanson, P. I. and Walsh, C. A. (2004). The hyh mutation uncovers roles for alpha Snap in apical protein localization and control of neural cell fate. *Nat. Genet.* **36**, 264-270.
Chen, H.-J., Wang, C.-M., Wang, T.-W., Liaw, G.-J., Hsu, T.-H., Lin, T.-H. and Yu, J.-Y. (2011). The Hippo pathway controls polar cell fate through Notch signaling during Drosophila oogenesis. *Dev. Biol.* **357**, 370-379.
Chen, T.-W., Wardill, T. J., Sun, Y., Pulver, S. R., Renninger, S. L., Baohan, A., Schreiter, E. R., Kerr, R. A., Orger, M. B., Jayaraman, V. et al. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**, 295-300.
Chen, Q., Giedt, M., Tang, L. and Harrison, D. A. (2014). Tools and methods for studying the Drosophila JAK/STAT pathway. *Methods* **68**, 160-172.
Dahal, G. R., Rawson, J., Gassaway, B., Kwok, B., Tong, Y., Ptácek, L. J. and Bates, E. (2012). An inwardly rectifying K+ channel is required for patterning. *Development* **139**, 3653-3664.
Dahal, G. R., Pradhan, S. J. and Bates, E. A. (2017). Inwardly rectifying potassium channels regulate Dpp release in the Drosophila wing disc. *Development* **144**, 2771-2783.
DasGupta, R., Kaykas, A., Moon, R. T. and Perrimon, N. (2005). Functional genomic analysis of the Wnt-wingless signaling pathway. *Science* **308**, 826-833.
Dietzl, G., Chen, D., Schnorrer, F., Su, K. C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S. et al. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. *Nature* **448**, 151-156.
Duhart, J. C., Parsons, T. T. and Raftery, L. A. (2017). The repertoire of epithelial morphogenesis on display: progressive elaboration of Drosophila egg structure. *Mech. Dev.* **148**, 18-39.
Golby, J. A., Tolar, L. A. and Pallanck, L. (2001). Partitioning of N-ethylmaleimide-sensitive fusion (NSF) protein function in Drosophila melanogaster: dNSF1 is required in the nervous system, and dNSF2 is required in mesoderm. *Genetics* **158**, 265-278.
Gramates, L. S., Marygold, S. J., dos Santos, G., Urbano, J. -M., Antonazzo, G., Matthews, B. B., Rey, A. J., Tabone, C. J., Crosby, M. A. et al. (2017). FlyBase at 25: looking to the future. *Nucleic Acids Res.* **45**, D663-D671.
Grammont, M. and Irvine, K. D. (2002). Organizer activity of the polar cells during Drosophila oogenesis. *Development* **129**, 5131-5140.
Harrisingh, M. C., Wu, Y., Lnenicka, G. A. and Nitabach, M. N. (2007). Intracellular Ca2+ regulates free-running circadian clock oscillation in vivo. *J. Neurosci.* **27**, 12489-12499.
Hay, B. A., Wolff, T. and Rubin, G. M. (1994). Expression of baculovirus P35 prevents cell death in Drosophila. *Development* **120**, 2121-2129.
Hayashi, S., Ito, K., Sado, Y., Taniguchi, M., Akimoto, A., Takeuchi, H., Aigaki, T., Matsuzaki, F., Nakagoshi, H., Tanimura, T. et al. (2002). GETDB, a database compiling expression patterns and molecular locations of a collection of Gal4 enhancer traps. *Genesis* **34**, 58-61.
Hayashi, Y., Sexton, T. R., Dejima, K., Perry, D. W., Takemura, M., Kobayashi, S., Nakato, H. and Harrison, D. A. (2012). Glypicans regulate JAK/STAT signaling and distribution of the Unpaired morphogen. *Development* **139**, 4162-4171.
Hong, H.-K., Chakravarti, A. and Takahashi, J. S. (2004). The gene for soluble N-ethylmaleimide sensitive factor attachment protein alpha is mutated in hydrocephaly with hop gait (hyh) mice. *Proc. Natl. Acad. Sci. USA* **101**, 1748-1753.
Kawasaki, F., Mattiuz, A. M. and Ordway, R. W. (1998). Synaptic physiology and ultrastructure in comatose mutants define an in vivo role for NSF in neurotransmitter release. *J. Neurosci.* **18**, 10241-10249.
Khammari, A., Agnès, F., Gandille, P. and Pret, A.-M. (2011). Physiological apoptosis of polar cells during Drosophila oogenesis is mediated by Hid-dependent regulation of Diap1. *Cell Death Differ.* **18**, 793-805.
Kurrasch, D. M., Nevin, L. M., Wong, J. S., Baier, H. and Ingraham, H. A. (2009). Neuroendocrine transcriptional programs adapt dynamically to the supply and demand for neuropeptides as revealed in NSF mutant zebrafish. *Neural Dev* **4**, 22.
Laflamme, C., Assaker, G., Ramel, D., Dorn, J. F., She, D., Maddox, P. S. and Emery, G. (2012). Evi5 promotes collective cell migration through its Rab-GAP activity. *J. Cell Biol.* **198**, 57-67.
Laviolette, M. J., Nunes, P., Peyre, J. B., Aigaki, T. and Stewart, B. A. (2005). A genetic screen for suppressors of Drosophila NSF2 neuromuscular junction overgrowth. *Genetics* **170**, 779-792.
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.
Lemons, P. P., Chen, D., Bernstein, A. M., Bennett, M. K. and Whiteheart, S. W. (1997). Regulated secretion in platelets: identification of elements of the platelet exocytosis machinery. *Blood* **90**, 1490-1500.
Li, T., Tian, Y., Li, Q., Chen, H., Lv, H., Xie, W. and Han, J. (2015). The neurexin/N-ethylmaleimide-sensitive factor (NSF) interaction regulates short term synaptic depression. *J. Biol. Chem.* **290**, 17656-17667.
Littleton, J. T. (2000). A genomic analysis of membrane trafficking and neurotransmitter release in Drosophila. *J. Cell Biol.* **150**, F77-F82.
Littleton, J. T., Stern, M., Schulze, K., Perin, M. and Bellen, H. J. (1993). Mutational analysis of Drosophila synaptotagmin demonstrates its essential role in Ca(2+)-activated neurotransmitter release. *Cell* **74**, 1125-1134.
Liu, Q. X., Jindra, M., Ueda, H., Hiromi, Y. and Hirose, S. (2003). Drosophila MBF1 is a co-activator for Tracheae Defective and contributes to the formation of tracheal and nervous systems. *Development* **130**, 719-728.
Lloyd, T. E., Verstreken, P., Ostrin, E. J., Phillippi, A., Lichtarge, O. and Bellen, H. J. (2000). A genome-wide search for synaptic vesicle cycle proteins in Drosophila. *Neuron* **26**, 45-50.
Lopez-Schier, H. and St Johnston, D. (2001). Delta signaling from the germ line controls the proliferation and differentiation of the somatic follicle cells during Drosophila oogenesis. *Genes Dev.* **15**, 1393-1405.
Macleod, G. T. (2012). Imaging and analysis of nonratiometric calcium indicators at the Drosophila larval neuromuscular junction. *Cold Spring Harb. Protoc.* **2012**, 802-809.
Manning, L. and Starz-Gaiano, M. (2015). Culturing Drosophila egg chambers and investigating developmental processes through live imaging. *Methods Mol. Biol.* **1328**, 73-88.
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.
McDonald, J. A., Pinheiro, E. M., Kadlec, L., Schupbach, T. and Montell, D. J. (2006). Multiple EGFR ligands participate in guiding migrating border cells. *Dev. Biol.* **296**, 94-103.

McGregor, J. R., Xi, R. and Harrison, D. A. (2002). JAK signaling is somatically required for follicle cell differentiation in Drosophila. *Development* **129**, 705-717.
McGuire, S. E., Le, P. T., Osborn, A. J., Matsumoto, K. and Davis, R. L. (2003). Spatiotemporal rescue of memory dysfunction in Drosophila. *Science* **302**, 1765-1768.
Miao, Y., Miner, C., Zhang, L., Hanson, P. I., Dani, A. and Vig, M. (2013). An essential and NSF independent role for α-SNAP in store-operated calcium entry. *Elife* **2**, e00802.
Mohtashami, M., Stewart, B. A., Boulianne, G. L. and Trimble, W. S. (2001). Analysis of the mutant Drosophila N-ethylmaleimide sensitive fusion-1 protein in comatose reveals molecular correlates of the behavioural paralysis. *J. Neurochem.* **77**, 1407-1417.
Montell, D. J., Rorth, P. and Spradling, A. C. (1992). slow border cells, a locus required for a developmentally regulated cell migration during oogenesis, encodes Drosophila C/EBP. *Cell* **71**, 51-62.
Montell, D. J., Yoon, W. H. and Starz-Gaiano, M. (2012). Group choreography: mechanisms orchestrating the collective movement of border cells. *Nat. Rev. Mol. Cell Biol.* **13**, 631-645.
Naydenov, N. G., Brown, B., Harris, G., Dohn, M. R., Morales, V. M., Baranwal, S., Reynolds, A. B. and Ivanov, A. I. (2012). A membrane fusion protein αSNAP is a novel regulator of epithelial apical junctions. *PLoS One* **7**, e34320.
Naydenov, N. G., Feygin, A., Wang, L. and Ivanov, A. I. (2014). N-ethylmaleimide-sensitive factor attachment protein α (αSNAP) regulates matrix adhesion and integrin processing in human epithelial cells. *J. Biol. Chem.* **289**, 2424-2439.
Neufeld, T. P., de la Cruz, A. F., Johnston, L. A. and Edgar, B. A. (1998). Coordination of growth and cell division in the Drosophila wing. *Cell* **93**, 1183-1193.
Ni, J. Q., Markstein, M., Binari, R., Pfeiffer, B., Liu, L. P., Villalta, C., Booker, M., Perkins, L. and Perrimon, N. (2008). Vector and parameters for targeted transgenic RNA interference in Drosophila melanogaster. *Nat. Methods* **5**, 49-51.
Niewiadomska, P., Godt, D. and Tepass, U. (1999). DE-Cadherin is required for intercellular motility during Drosophila oogenesis. *J. Cell Biol.* **144**, 533-547.
Nybakken, K., Vokes, S. A., Lin, T. Y., McMahon, A. P. and Perrimon, N. (2005). A genome-wide RNA interference screen in Drosophila melanogaster cells for new components of the Hh signaling pathway. *Nat. Genet.* **37**, 1323-1332.
Oda, H., Uemura, T. and Takeichi, M. (1997). Phenotypic analysis of null mutants for DE-cadherin and Armadillo in Drosophila ovaries reveals distinct aspects of their functions in cell adhesion and cytoskeletal organization. *Genes Cells* **2**, 29-40.
Ordway, R. W., Pallanck, L. and Ganetzky, B. (1994). Neurally expressed Drosophila genes encoding homologs of the NSF and SNAP secretory proteins. *Proc. Natl. Acad. Sci. USA* **91**, 5715-5719.
O'Shea, J. J., Schwartz, D. M., Villarino, A. V., Gadina, M., McInnes, I. B. and Laurence, A. (2015). The JAK-STAT pathway: impact on human disease and therapeutic intervention. *Annu. Rev. Med.* **66**, 311-328.
Pallanck, L., Ordway, R. W., Ramaswami, M., Chi, W. Y., Krishnan, K. S. and Ganetzky, B. (1995). Distinct roles for N-ethylmaleimide-sensitive fusion protein (NSF) suggested by the identification of a second Drosophila NSF homolog. *J. Biol. Chem.* **270**, 18742-18744.
Patel, N. H., Snow, P. M. and 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.
Peifer, M., Orsulic, S., Sweeton, D. and Wieschaus, E. (1993). A role for the Drosophila segment polarity gene armadillo in cell adhesion and cytoskeletal integrity during oogenesis. *Development* **118**, 1191-1207.
Pinheiro, E. M. and Montell, D. J. (2004). Requirement for Par-6 and Bazooka in Drosophila border cell migration. *Development* **131**, 5243-5251.
Prasad, M., Jang, A. C., Starz-Gaiano, M., Melani, M. and Montell, D. J. (2007). A protocol for culturing Drosophila melanogaster stage 9 egg chambers for live imaging. *Nat. Protoc.* **2**, 2467-2473.
Ramel, D., Wang, X., Laflamme, C., Montell, D. J. and Emery, G. (2013). Rab11 regulates cell-cell communication during collective cell movements. *Nat. Cell Biol.* **15**, 317-324.
Riggleman, B., Schedl, P. and Wieschaus, E. (1990). Spatial expression of the Drosophila segment polarity gene armadillo is posttranscriptionally regulated by wingless. *Cell* **63**, 549-560.
Rørth, P., Szabo, K., Bailey, A., Laverty, T., Rehm, J., Rubin, G. M., Weigmann, K., Milán, M., Benes, V., Ansorge, W. et al. (1998). Systematic gain-of-function genetics in Drosophila. *Development* **125**, 1049-1057.
Rothman, J. E. (1996). The protein machinery of vesicle budding and fusion. *Protein Sci.* **5**, 185-194.
Ruohola, H., Bremer, K. A., Baker, D., Swedlow, J. R., Jan, L. Y. and Jan, Y. N. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in Drosophila. *Cell* **66**, 433-449.
Saadin, A. and Starz-Gaiano, M. (2016a). Circuitous genetic regulation governs a straightforward cell migration. *Trends Genet.* **32**, 660-673.
Saadin, A. and Starz-Gaiano, M. (2016b). Identification of novel regulators of the JAK/STAT signaling pathway that control border cell migration in the drosophila ovary. *G3 (Bethesda)*, 1991-2002.
Scheller, R. H. (2013). In search of the molecular mechanism of intracellular membrane fusion and neurotransmitter release. *Nat. Med.* **19**, 1232-1235.
Schulze, K. L., Broadie, K., Perin, M. S. and Bellen, H. J. (1995). Genetic and electrophysiological studies of Drosophila syntaxin-1A demonstrate its role in nonneuronal secretion and neurotransmission. *Cell* **80**, 311-320.
Schwartz, M. L., Nickerson, D. P., Lobingier, B. T., Plemel, R. L., Duan, M., Angers, C. G., Zick, M. and Merz, A. J. (2017). Sec17 (α-SNAP) and an SM-tethering complex regulate the outcome of SNARE zippering in vitro and in vivo. *Elife* **6**, e27396.
Shellenbarger, D. L. and Mohler, J. D. (1975). Temperature-sensitive mutations of the notch locus in Drosophila melanogaster. *Genetics* **81**, 143-162.
Sheshachalam, A., Srivastava, N., Mitchell, T., Lacy, P. and Eitzen, G. (2014). Granule protein processing and regulated secretion in neutrophils. *Front Immunol* **5**, 448.
Siddiqi, O. and Benzer, S. (1976). Neurophysiological defects in temperature-sensitive paralytic mutants of Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA* **73**, 3253-3257.
Silver, D. L. and Montell, D. J. (2001). Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in Drosophila. *Cell* **107**, 831-841.
Silver, D. L., Geisbrecht, E. R. and Montell, D. J. (2005). Requirement for JAK/STAT signaling throughout border cell migration in Drosophila. *Development* **132**, 3483-3492.
Song, H., Orr, A., Duan, M., Merz, A. J. and Wickner, W. (2017). Sec17/Sec18 act twice, enhancing membrane fusion and then disassembling cis-SNARE complexes. *Elife* **6**, e26646.
Starz-Gaiano, M., Melani, M., Wang, X., Meinhardt, H. and Montell, D. J. (2008). Feedback inhibition of Jak/STAT signaling by apontic is required to limit an invasive cell population. *Dev. Cell* **14**, 726-738.
Stewart, B. A., Mohtashami, M., Zhou, L., Trimble, W. S. and Boulianne, G. L. (2001). SNARE-dependent signaling at the Drosophila wing margin. *Dev. Biol.* **234**, 13-23.
Stow, J. L., Low, P. C., Offenhäuser, C. and Sangermani, D. (2009). Cytokine secretion in macrophages and other cells: pathways and mediators. *Immunobiology* **214**, 601-612.
Südhof, T. C. (2012). Calcium control of neurotransmitter release. *Cold Spring Harb. Perspect Biol.* **4**, a011353.
Thorngren, N., Collins, K. M., Fratti, R. A., Wickner, W. and Merz, A. J. (2004). A soluble SNARE drives rapid docking, bypassing ATP and Sec17/18p for vacuole fusion. *EMBO J.* **23**, 2765-2776.
Tomes, C. N., De Blas, G. A., Michaut, M. A., Farré, E. V., Cherhitin, O., Visconti, P. E. and Mayorga, L. S. (2005). alpha-SNAP and NSF are required in a priming step during the human sperm acrosome reaction. *Mol. Hum. Reprod.* **11**, 43-51.
Torres, A. Y., Malartre, M., Pret, A. M. and Agnès, F. (2017). JAK/STAT signaling is necessary for cell monosis prior to epithelial cell apoptotic extrusion. *Cell Death Dis.* **8**, e2814.
Van de Bor, V., Zimniak, G., Cérézo, D., Schaub, S. and Noselli, S. (2011). Asymmetric localisation of cytokine mRNA is essential for JAK/STAT activation during cell invasiveness. *Development* **138**, 1383-1393.
Villarino, A. V., Kanno, Y., Ferdinand, J. R. and O'Shea, J. J. (2015). Mechanisms of Jak/STAT signaling in immunity and disease. *J. Immunol.* **194**, 21-27.
Wickner, W. and Rizo, J. (2017). A cascade of multiple proteins and lipids catalyzes membrane fusion. *Mol. Biol. Cell* **28**, 707-711.
Wu, X., Tanwar, P. S. and Raftery, L. A. (2008). Drosophila follicle cells: morphogenesis in an eggshell. *Semin. Cell Dev. Biol.* **19**, 271-282.
Xi, R., McGregor, J. R. and Harrison, D. A. (2003). A gradient of JAK pathway activity patterns the anterior-posterior axis of the follicular epithelium. *Dev. Cell* **4**, 167-177.
Xiong, Q. Y., Yu, C., Zhang, Y., Ling, L., Wang, L. and Gao, J. L. (2017). Key proteins involved in insulin vesicle exocytosis and secretion. *Biomed. Rep.* **6**, 134-139.
Yu, W., Kawasaki, F. and Ordway, R. W. (2011). Activity-dependent interactions of NSF and SNAP at living synapses. *Mol. Cell. Neurosci.* **47**, 19-27.
Zeidler, M. P. and Bausek, N. (2013). The Drosophila JAK-STAT pathway. *JAKSTAT* **2**, e25353.
Zhao, M. and Brunger, A. T. (2016). Recent advances in deciphering the structure and molecular mechanism of the AAA+ ATPase N-ethylmaleimide-sensitive factor (NSF). *J. Mol. Biol.* **428**, 1912-1926.
Zhao, C., Slevin, J. T. and Whiteheart, S. W. (2007). Cellular functions of NSF: not just SNAPs and SNAREs. *FEBS Lett.* **581**, 2140-2149.
Zhao, C., Smith, E. C. and Whiteheart, S. W. (2012). Requirements for the catalytic cycle of the N-ethylmaleimide-sensitive factor (NSF). *Biochim. Biophys. Acta* **1823**, 159-171.
Zick, M., Orr, A., Schwartz, M. L., Merz, A. J. and Wickner, W. T. (2015). Sec17 can trigger fusion of trans-SNARE paired membranes without Sec18. *Proc. Natl. Acad. Sci. USA* **112**, E2290-E2297.