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

Afsoon Saadin and Michelle Starz-Gaiano[1]
Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore MD 21250
[1] Corresponding author, starz@umbc.edu, phone: 410-455-2217, ORCID ID: 0000-0002-0855-0715

Keywords: Drosophila oogenesis, JAK/STAT signaling, exocytosis, cell fate specification

JCS Advance Online Article. Posted on 7 November 2018

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 calcium may be 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.

Summary Statement

The vesicle trafficking regulator α-Snap acts upstream of the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway to induce motile cell fate during oogenesis.

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, specialized cells, including immune cells, neurons and pancreatic β-cells, need additional mechanisms to regulate secretion of histamines or cytokines, neurotransmitters and insulin, respectively (Burgoyne and Morgan, 2003, Südhof, 2012, Scheller, 2013, Xiong et al., 2017). In these cases, secretory cargo is separated, stored in vesicles, then rapidly released, independently from constitutive exocytosis. This is clearest in synaptic signaling, for which NSF and α-Snap are required.

While 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 phenocopies 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 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 called border cells (reviewed in (Saadin and Starz-Gaiano, 2016)).

Developing egg chambers are encased by a monolayer of follicle cells, which 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, 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, Borensztejn et al., 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, 2016). Border cell specification and migration requires multiple inputs that control transcription, signaling, cytoskeletal organization, adhesion, and protein trafficking. Here, we demonstrate a critical role for α-Snap, Syntaxin1A (Syx1A), and NSF/NSF2 in mediating trafficking event(s) required to induce JAK/STAT signaling.

Results and Discussion

α-Snap and Syx1A are required for border cell specification

To assay vesicle trafficking in developmental signaling, we disrupted trafficking regulators using 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 (using c306-Gal4, Fig. 1A-B).

Strikingly, depletion of *α-Snap* resulted in an unusual phenotype: egg chambers with no border cell cluster, as assayed by expression of border cell-enriched proteins (Fig. 1C, S3C; see *Materials and Methods*). We observed this phenotype in 20-50% of stage 10 egg chambers 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 likely 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 via *α-Snap* over-expression (>3 fold rescue compared to controls, Fig. S1A-B,J,M). Over-expression of α-Snap-HA alone caused no obvious defects (Fig.S1C). We verified the temporal requirement for *α-Snap* by reducing its expression specifically in adults using *tub*-Gal80^{ts} and also rescuing the mutant phenotype in adults (Fig.S1D-F and K). Using α-Snap antisera (Babcock et al., 2004), we detected the protein at/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 reveal 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, Miao et al., 2013, Naydenov et al., 2014). Depletion of *comt*/*NSF* or *NSF2* in follicle cells yielded wild-type phenotypes (Table S1, Table 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 may 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 over-expressed *NSF2* in follicle cells. While over-expression 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 independent of *α-Snap* at high concentrations (like overexpressed yeast Sec18 (Zick et al., 2015, Song et al., 2017)). Since the two NSF proteins are over 80% identical and both are detected in ovary, we suspect that *α-Snap* normally functions with either in follicle cells. These results, and the role for 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* using *upd*-Gal4 led to the absence of border cells in more than 80% of egg chambers (Fig. 1H,M), and a lack of polar cells by stage 10. This severe phenotype was rescued by *α-Snap* over-expression (Fig.S1G-I, L). Similarly, reduction of *Syx1A* in polar cells resulted in failed 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 reducing *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 are differentially sensitive to surviving α-Snap disruption (Babcock et al., 2004, Chae et al., 2004, Hong et al., 2004, Tomes et al., 2005, Batiz et al., 2009, Naydenov et al., 2014, Arcos et al., 2017) and may 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 (compare Fig. 2A,B to D,E). Interestingly, more than 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 sub-cellular trafficking

In mammalian cells, α-Snap is required for localization of E-Cadherin, 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/levels of E-Cad and Arm were normal in posterior cells upon *α-Snap* or *Syx1A* disruption (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*. *α-Snap* depletion did not result in fused egg chambers, stalk defects, or oocyte polarity defects (Fig. 1C,2B), as would disruption of Hedgehog, Hippo, and/or Notch (N) signaling (Lopez-Schier and St Johnston, 2001, Grammont and Irvine, 2002, Chen et al., 2011). Furthermore, although polar cell fate requires N activity, we found no genetic interaction between *N* (N^{ts2}) and *α-Snap* ($\alpha\text{-}Snap^{G8}$) 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, Batiz 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/NSF activity (Thorngren et al., 2004), or that NSF can function with an unidentified co-factor to promote exocytosis.

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

Border cell specification requires JAK/STAT signaling, so we tested if *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% penetrance, 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%, Fig. 3C-D), indicating that *α-Snap* and *Stat92E* likely function in the same genetic pathway.

Next we examined the secreted activator Upd. In epistasis tests, we found that over-expression 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), nor changes in stretch or centripetal cells, which require low levels of STAT signaling (Xi et al., 2003). We speculate that *α-Snap* was 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 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 if the α-Snap homolog in humans (NAPA, 60% identical to fly α-Snap (Lemons et al., 1997)) regulates STAT-mediated immune response.

Intracellular calcium increases transiently in polar cells prior to border cell specification

Some secreted molecules are sequestered and released in a calcium-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 calcium flux in egg chambers using multiple, verified calcium sensors (Chen et al., 2013). Interestingly, we observed periodic, transient increases in intracellular calcium signals in polar cells prior to border cell specification using three different Gal4 drivers (Fig. 4A-C; Supplementary movies 1-3). Consistent with a role for calcium, we observed significantly fewer border cells when free calcium was reduced in polar cells by overexpression of the human Parvalbumin protein (PV,(Harrisingh et al., 2007)) (Fig. 4D-G; 4.8 clustered migratory cells (average) in *upd*-Gal4;UAS-PV egg chambers(n=23), compared to 5.8 cells in *upd*-Gal4;UAS-GFP(n=28); $p<0.002$ in Mann-Whitney test). The number of border cells is known to drop with decreased 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, not constitutive manner, in response to calcium (Fig.4H). Notably, cation concentrations govern morphogen release during fly wing development (Dahal et al., 2012, Dahal et al., 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 Dr. Montell. UAS-NSF2 transgenic fly line was a generous gift from Dr. Pallanck. 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$^{l1N\text{-}ts2}$ (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).

Over-expression and knock down studies

Genetic expression of transgenes in follicle cells was performed as previously described (Saadin and Starz-Gaiano, 2016). All genetic crosses were established at 25°C. If 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 for Gal80^{ts}) to repress Gal4. Three to seven-day old female offspring were cultured on yeast supplemented food and maintained at either 25°C, 29°C or 31°C, depending on their genotype, for various amounts of time prior to dissection. Unless otherwise stated in the figure legend, flies bearing Gal4 were incubated at 29°C for 12-16 hours. Flies bearing Gal4 and Gal80^{ts} were incubated at 31°C (non-permissive temperature for Gal80^{ts}) for 54 hours, and flies that did not bear either Gal4 or Gal80^{ts}, were incubated at 25°C for 12-16 hours 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 to males from the indicated RNAi lines. Total RNA was isolated from 0-24 hour-old F1 embryos (25°C) 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:

α-snap ex1-2; for: 5'-TGGGTGACAACGAACAGAAG-3'; rev:5'-CCCAGCTTTGTCCAGTTTT-3'

α-snap ex2-3; for: 5'-GCTGCCAAACATCACCAAAG-3'; rev: 5'-CCACCTTCAACATGCACTTG-3'

comt set2; for: 5'-CCCGTGAAAATCAGCAAGAATC-3'; rev: 5'-ACCAACATTCACTATAGCCCG-3'

comt ex4-6; for: 5'-ACACGGATATCTTTAGCAAGGG-3'; rev: 5'-ACAGGAACTTTATGGCCCG-3'

NSF2 ex2-3; for: 5'-AGTTCCTCATGCAGTTCGC-3'; rev: 5'- GAGTCTTCGGTAGGGAATCG-3'

NSF2 ex3-4; for: 5'-AGTTGGAGGGTCTGGTTAGAG-3'; rev: GCTTGATGTCGTTGTCCAATG-3'

Immunofluorescence labeling and phenotypic analysis

The female fly ovaries were dissected to ovarioles in Schneider's media with 10% Fetal Bovine Serum and 0.6X penicillin-streptomycin (Prasad et al., 2007), and fixed in 4% paraformaldehyde in 0.1M 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 TrisHCl pH=7.4, 0.15 M NaCl, 1 mg/ml BSA, 0.5% Nonidet P-40 (Igepal CA-630, Sigma-Alderich), 0.02% Sodium Azide) (McDonald et al., 2006) and incubated in primary antibodies (listed below) diluted in NP40 wash buffer either overnight at 4°C or for 2-3 hours at room temperature. The egg chambers were then washed four times in NP40 wash buffer and incubated in the diluted secondary antibodies (1:400 Alexa Flour 488 and 568: Life technologies) at room temperature for 2-3 hours. The immuno-stained egg chambers were then nuclei-stained with 1:1000 diluted DAPI (Invitrogen: D1306) for 10 minutes, washed

and mounted in 70% glycerol. Stage 10 egg chambers were scored for border cell specification defects (characterized by the absence of border cell markers including: 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)), and migration defects, where the border cell cluster had not reached to 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 line in Fig. 1M is the average penetrance of four and two independent experiments respectively. To investigate the statistical significance, Mann-Whitney tests for border cell numbers or Fisher's exact test for proportions was performed using online tools, http://vassarstats.net.

The primary antibodies and their working dilutions were: 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 (1:1000)(Liu et al., 2003)), guinea pig anti-Apt (1:5000) from GST-Apt (Pocono Rabbit Farm), rat anti-Slbo (provided by P. Rorth) (1:1000; (Beccari et al., 2002)), 1:250 anti-Rabbit GFP (Life Technologies), rat anti-Drosophila E-cad (DCAD2 , DSHB)(1:20;(Oda et al., 1997)), 0.5 µg/ml rat anti-HA (11867423001; Roche), mouse anti-FasIII (7G10, DSHB)(1:50; (Patel et al., 1987)), rabbit anti-NSF (a gift from J. Han) (1:50; (Li et al., 2015)) and α-Snap (a gift from L. Pallanck) (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 HA tag expression) 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 right upstream of the start codon. BP reaction was carried out using 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* (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 pDONR221 containing α-Snap and pUASg-HA.attB vector ((Bischof et al., 2007, Bischof et al., 2013), Gene Bank Accession: KC896837.1) by Gateway LR Clonase II Enzyme mix (Invitrogen: 11791020). The product of LR reaction was transformed into One Shot OmniMax 2T1 Competent E. coli cells. Successful cloning was confirmed by sequencing using the following primers: Forward- 5'-CGTCGCTAAGCGAAAGCTAAGC-3', Reverse 5'-AGCCTGCTGCTACACTTGCC-3'. The Destination vector (pUASg-HA.attB) containing α-Snap was used for PhiC31 injection through BestGene, Inc, plan H.

Live calcium imaging

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

Acknowledgements

The authors declare no competing interests.

Stocks obtained from the Bloomington Drosophila Stock Center were used in this study. We thank the Bloomington Stock Center for RNAi and other transgenic flies, the Vienna Drosophila RNAi Center for RNAi fly stocks, and the Developmental Studies Hybridoma Bank, Drs. D. Montell, L. Pallanck, J. Han, S. Hirose, and P. Rorth for Drosophila mutant stocks and antibodies. Further, we thank Flybase for providing database information (McQuilton et al., 2012). We thank Drs. Jocelyn McDonald and Fernando Vonhoff for helpful comments on the research and manuscript. We acknowledge technical assistance from Grant Wunderlin.

References:

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

Andreeva, A. V., M. A. Kutuzov, R. Vaiskunaite, J. Profirovic, T. E. Meigs, S. Predescu, A. B. Malik and T. Voyno-Yasenetskaya (2005). G alpha12 interaction with alphaSNAP induces VE-cadherin localization at endothelial junctions and regulates barrier function. *J Biol Chem* **280**(34): 30376-30383.

Arcos, A., M. Paola, D. Gianetti, D. Acuna, Z. D. Velasquez, M. P. Miro, G. Toro, B. Hinrichsen, R. I. Munoz, Y. Lin, G. A. Mardones, P. Ehrenfeld, F. J. Rivera, M. A. Michaut and L. F. Batiz (2017). alpha-SNAP is expressed in mouse ovarian granulosa cells and plays a key role in folliculogenesis and female fertility. *Sci Rep* **7**(1): 11765.

Assa-Kunik, E., I. L. Torres, E. D. Schejter, D. S. Johnston and B. Z. Shilo (2007). Drosophila follicle cells are patterned by multiple levels of Notch signaling and antagonism between the Notch and JAK/STAT pathways. *Development* **134**(6): 1161-1169.

Assaker, G., D. Ramel, S. K. Wculek, M. Gonzalez-Gaitan and G. Emery (2010). Spatial restriction of receptor tyrosine kinase activity through a polarized endocytic cycle controls border cell migration. *Proc Natl Acad Sci U S A* **107**(52): 22558-22563.

Babcock, M., G. T. Macleod, J. Leither and L. Pallanck (2004). Genetic analysis of soluble N-ethylmaleimide-sensitive factor attachment protein function in Drosophila reveals positive and negative secretory roles. *J Neurosci* **24**(16): 3964-3973.

Baeg, G. H., R. Zhou and N. Perrimon (2005). Genome-wide RNAi analysis of JAK/STAT signaling components in Drosophila. *Genes Dev* **19**(16): 1861-1870.

Bai, J. and D. Montell (2002). Eyes absent, a key repressor of polar cell fate during Drosophila oogenesis. *Development* **129**(23): 5377-5388.

Baksa, K., T. Parke, L. L. Dobens and C. R. Dearolf (2002). The Drosophila STAT protein, stat92E, regulates follicle cell differentiation during oogenesis. *Dev Biol* **243**(1): 166-175.

Batiz, L. F., G. A. De Blas, M. A. Michaut, A. R. Ramirez, F. Rodriguez, M. H. Ratto, C. Oliver, C. N. Tomes, E. M. Rodriguez and L. S. Mayorga (2009). Sperm from hyh mice carrying a point mutation in alphaSNAP have a defect in acrosome reaction. *PLoS One* **4**(3): e4963.

Beccari, S., L. Teixeira and P. Rørth (2002). The JAK/STAT pathway is required for border cell migration during Drosophila oogenesis. *Mech Dev* **111**(1-2): 115-123.

Bellen, H. J., R. W. Levis, Y. He, J. W. Carlson, M. Evans-Holm, E. Bae, J. Kim, A. Metaxakis, C. Savakis, K. L. Schulze, R. A. Hoskins and A. C. Spradling (2011). The Drosophila gene disruption project: progress using transposons with distinctive site specificities. *Genetics* **188**(3): 731-743.

Bischof, J., M. Björklund, E. Furger, C. Schertel, J. Taipale and K. Basler (2013). A versatile platform for creating a comprehensive UAS-ORFeome library in Drosophila. *Development* **140**(11): 2434-2442.

Bischof, J., R. K. Maeda, M. Hediger, F. Karch and K. Basler (2007). An optimized transgenesis system for Drosophila using germ-line-specific phiC31 integrases. *Proc Natl Acad Sci U S A* **104**(9): 3312-3317.

Bombardier, J. P. and M. Munson (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., W. M. Leiserson and S. Benzer (1993). The eyes absent gene: genetic control of cell survival and differentiation in the developing Drosophila eye. *Cell* **72**(3): 379-395.

Borensztejn, A., E. Boissoneau, G. Fernandez, F. Agnes and A. M. Pret (2013). JAK/STAT autocontrol of ligand-producing cell number through apoptosis. *Development* **140**(1): 195-204.

Borensztejn, A., A. Mascaro and K. A. Wharton (2018). JAK/STAT signaling prevents excessive apoptosis to ensure maintenance of the interfollicular stalk critical for Drosophila oogenesis. *Dev Biol* **438**(1): 1-9.

Boulianne, G. L. and W. S. Trimble (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 U S A* **92**(15): 7095-7099.
Burgoyne, R. D. and A. Morgan (2003). Secretory granule exocytosis. *Physiol Rev* **83**(2): 581-632.
Cai, D., S. C. Chen, M. Prasad, L. He, X. Wang, V. Choesmel-Cadamuro, J. K. Sawyer, G. Danuser and D. J. Montell (2014). Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. *Cell* **157**(5): 1146-1159.
Cavener, D. R. (1987). Comparison of the consensus sequence flanking translational start sites in Drosophila and vertebrates. *Nucleic Acids Res* **15**(4): 1353-1361.
Chae, T. H., S. Kim, K. E. Marz, P. I. Hanson and C. A. Walsh (2004). The hyh mutation uncovers roles for alpha Snap in apical protein localization and control of neural cell fate. *Nat Genet* **36**(3): 264-270.
Chen, H. J., C. M. Wang, T. W. Wang, G. J. Liaw, T. H. Hsu, T. H. Lin and J. Y. Yu (2011). The Hippo pathway controls polar cell fate through Notch signaling during Drosophila oogenesis. *Dev Biol* **357**(2): 370-379.
Chen, Q., M. Giedt, L. Tang and D. A. Harrison (2014). Tools and methods for studying the Drosophila JAK/STAT pathway. *Methods* **68**(1): 160-172.
Chen, T. W., T. J. Wardill, Y. Sun, S. R. Pulver, S. L. Renninger, A. Baohan, E. R. Schreiter, R. A. Kerr, M. B. Orger, V. Jayaraman, L. L. Looger, K. Svoboda and D. S. Kim (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**(7458): 295-300.
Dahal, G. R., S. J. Pradhan and E. A. Bates (2017). Inwardly rectifying potassium channels regulate Dpp release in the Drosophila wing disc. *Development*.
Dahal, G. R., J. Rawson, B. Gassaway, B. Kwok, Y. Tong, L. J. Ptácek and E. Bates (2012). An inwardly rectifying K+ channel is required for patterning. *Development* **139**(19): 3653-3664.
DasGupta, R., A. Kaykas, R. T. Moon and N. Perrimon (2005). Functional genomic analysis of the Wnt-wingless signaling pathway. *Science* **308**(5723): 826-833.
Dietzl, G., D. Chen, F. Schnorrer, K. C. Su, Y. Barinova, M. Fellner, B. Gasser, K. Kinsey, S. Oppel, S. Scheiblauer, A. Couto, V. Marra, K. Keleman and B. J. Dickson (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. *Nature* **448**(7150): 151-156.
Duhart, J. C., T. T. Parsons and L. A. Raftery (2017). The repertoire of epithelial morphogenesis on display: Progressive elaboration of Drosophila egg structure. *Mech Dev*.
Golby, J. A., L. A. Tolar and L. Pallanck (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**(1): 265-278.
Grammont, M. and K. D. Irvine (2002). Organizer activity of the polar cells during Drosophila oogenesis. *Development* **129**(22): 5131-5140.
Harrisingh, M. C., Y. Wu, G. A. Lnenicka and M. N. Nitabach (2007). Intracellular Ca2+ regulates free-running circadian clock oscillation in vivo. *J Neurosci* **27**(46): 12489-12499.
Hay, B. A., T. Wolff and G. M. Rubin (1994). Expression of baculovirus P35 prevents cell death in Drosophila. *Development* **120**(8): 2121-2129.
Hayashi, S., K. Ito, Y. Sado, M. Taniguchi, A. Akimoto, H. Takeuchi, T. Aigaki, F. Matsuzaki, H. Nakagoshi, T. Tanimura, R. Ueda, T. Uemura, M. Yoshihara and S. Goto (2002). GETDB, a database compiling expression patterns and molecular locations of a collection of Gal4 enhancer traps. *Genesis* **34**(1-2): 58-61.
Hayashi, Y., T. R. Sexton, K. Dejima, D. W. Perry, M. Takemura, S. Kobayashi, H. Nakato and D. A. Harrison (2012). Glypicans regulate JAK/STAT signaling and distribution of the Unpaired morphogen. *Development* **139**(22): 4162-4171.

Hong, H. K., A. Chakravarti and J. S. Takahashi (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 U S A* **101**(6): 1748-1753.
Kawasaki, F., A. M. Mattiuz and R. W. Ordway (1998). Synaptic physiology and ultrastructure in comatose mutants define an in vivo role for NSF in neurotransmitter release. *J Neurosci* **18**(24): 10241-10249.
Khammari, A., F. Agnès, P. Gandille and A. M. Pret (2011). Physiological apoptosis of polar cells during Drosophila oogenesis is mediated by Hid-dependent regulation of Diap1. *Cell Death Differ* **18**(5): 793-805.
Kurrasch, D. M., L. M. Nevin, J. S. Wong, H. Baier and H. A. Ingraham (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., G. Assaker, D. Ramel, J. F. Dorn, D. She, P. S. Maddox and G. Emery (2012). Evi5 promotes collective cell migration through its Rab-GAP activity. *J Cell Biol* **198**(1): 57-67.
Laviolette, M. J., P. Nunes, J. B. Peyre, T. Aigaki and B. A. Stewart (2005). A genetic screen for suppressors of Drosophila NSF2 neuromuscular junction overgrowth. *Genetics* **170**(2): 779-792.
Lee, T. and L. Luo (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron* **22**(3): 451-461.
Lemons, P. P., D. Chen, A. M. Bernstein, M. K. Bennett and S. W. Whiteheart (1997). Regulated secretion in platelets: identification of elements of the platelet exocytosis machinery. *Blood* **90**(4): 1490-1500.
Li, T., Y. Tian, Q. Li, H. Chen, H. Lv, W. Xie and J. Han (2015). The Neurexin/N-Ethylmaleimide-sensitive Factor (NSF) Interaction Regulates Short Term Synaptic Depression. *J Biol Chem* **290**(29): 17656-17667.
Littleton, J. T. (2000). A genomic analysis of membrane trafficking and neurotransmitter release in Drosophila. *J Cell Biol* **150**(2): F77-82.
Littleton, J. T., M. Stern, K. Schulze, M. Perin and H. J. Bellen (1993). Mutational analysis of Drosophila synaptotagmin demonstrates its essential role in Ca(2+)-activated neurotransmitter release. *Cell* **74**(6): 1125-1134.
Liu, Q. X., M. Jindra, H. Ueda, Y. Hiromi and S. Hirose (2003). Drosophila MBF1 is a co-activator for Tracheae Defective and contributes to the formation of tracheal and nervous systems. *Development* **130**(4): 719-728.
Lloyd, T. E., P. Verstreken, E. J. Ostrin, A. Phillippi, O. Lichtarge and H. J. Bellen (2000). A genome-wide search for synaptic vesicle cycle proteins in Drosophila. *Neuron* **26**(1): 45-50.
Lopez-Schier, H. and D. St Johnston (2001). Delta signaling from the germ line controls the proliferation and differentiation of the somatic follicle cells during Drosophila oogenesis. *Genes Dev* **15**(11): 1393-1405.
Macleod, G. T. (2012). Imaging and analysis of nonratiometric calcium indicators at the Drosophila larval neuromuscular junction. *Cold Spring Harb Protoc* **2012**(7): 802-809.
Manning, L. and M. Starz-Gaiano (2015). Culturing Drosophila Egg Chambers and Investigating Developmental Processes Through Live Imaging. *Methods Mol Biol* **1328**: 73-88.
Manseau, L., A. Baradaran, D. Brower, A. Budhu, F. Elefant, H. Phan, A. V. Philp, M. Yang, D. Glover, K. Kaiser, K. Palter and S. Selleck (1997). GAL4 enhancer traps expressed in the embryo, larval brain, imaginal discs, and ovary of Drosophila. *Dev Dyn* **209**(3): 310-322.
McDonald, J. A., E. M. Pinheiro, L. Kadlec, T. Schupbach and D. J. Montell (2006). Multiple EGFR ligands participate in guiding migrating border cells. *Dev Biol* **296**(1): 94-103.
McGregor, J. R., R. Xi and D. A. Harrison (2002). JAK signaling is somatically required for follicle cell differentiation in Drosophila. *Development* **129**(3): 705-717.

McGuire, S. E., P. T. Le, A. J. Osborn, K. Matsumoto and R. L. Davis (2003). Spatiotemporal rescue of memory dysfunction in Drosophila. *Science* **302**(5651): 1765-1768.
Miao, Y., C. Miner, L. Zhang, P. I. Hanson, A. Dani and M. Vig (2013). An essential and NSF independent role for α-SNAP in store-operated calcium entry. *Elife* **2**: e00802.
Mohtashami, M., B. A. Stewart, G. L. Boulianne and W. S. Trimble (2001). Analysis of the mutant Drosophila N-ethylmaleimide sensitive fusion-1 protein in comatose reveals molecular correlates of the behavioural paralysis. *J Neurochem* **77**(5): 1407-1417.
Montell, D. J., P. Rorth and A. C. Spradling (1992). slow border cells, a locus required for a developmentally regulated cell migration during oogenesis, encodes Drosophila C/EBP. *Cell* **71**(1): 51-62.
Montell, D. J., W. H. Yoon and M. Starz-Gaiano (2012). Group choreography: mechanisms orchestrating the collective movement of border cells. *Nat Rev Mol Cell Biol* **13**(10): 631-645.
Naydenov, N. G., B. Brown, G. Harris, M. R. Dohn, V. M. Morales, S. Baranwal, A. B. Reynolds and A. I. Ivanov (2012). A membrane fusion protein αSNAP is a novel regulator of epithelial apical junctions. *PLoS One* **7**(4): e34320.
Naydenov, N. G., A. Feygin, L. Wang and A. I. Ivanov (2014). N-ethylmaleimide-sensitive factor attachment protein α (αSNAP) regulates matrix adhesion and integrin processing in human epithelial cells. *J Biol Chem* **289**(4): 2424-2439.
Neufeld, T. P., A. F. de la Cruz, L. A. Johnston and B. A. Edgar (1998). Coordination of growth and cell division in the Drosophila wing. *Cell* **93**(7): 1183-1193.
Ni, J. Q., M. Markstein, R. Binari, B. Pfeiffer, L. P. Liu, C. Villalta, M. Booker, L. Perkins and N. Perrimon (2008). Vector and parameters for targeted transgenic RNA interference in Drosophila melanogaster. *Nat Methods* **5**(1): 49-51.
Niewiadomska, P., D. Godt and U. Tepass (1999). DE-Cadherin is required for intercellular motility during Drosophila oogenesis. *J Cell Biol* **144**(3): 533-547.
Nybakken, K., S. A. Vokes, T. Y. Lin, A. P. McMahon and N. Perrimon (2005). A genome-wide RNA interference screen in Drosophila melanogaster cells for new components of the Hh signaling pathway. *Nat Genet* **37**(12): 1323-1332.
O'Shea, J. J., D. M. Schwartz, A. V. Villarino, M. Gadina, I. B. McInnes and A. Laurence (2015). The JAK-STAT pathway: impact on human disease and therapeutic intervention. *Annu Rev Med* **66**: 311-328.
Oda, H., T. Uemura and M. Takeichi (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**(1): 29-40.
Ordway, R. W., L. Pallanck and B. Ganetzky (1994). Neurally expressed Drosophila genes encoding homologs of the NSF and SNAP secretory proteins. *Proc Natl Acad Sci U S A* **91**(12): 5715-5719.
Pallanck, L., R. W. Ordway, M. Ramaswami, W. Y. Chi, K. S. Krishnan and B. Ganetzky (1995). Distinct roles for N-ethylmaleimide-sensitive fusion protein (NSF) suggested by the identification of a second Drosophila NSF homolog. *J Biol Chem* **270**(32): 18742-18744.
Patel, N. H., P. M. Snow and C. S. Goodman (1987). Characterization and cloning of fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in Drosophila. *Cell* **48**(6): 975-988.
Peifer, M., S. Orsulic, D. Sweeton and E. Wieschaus (1993). A role for the Drosophila segment polarity gene armadillo in cell adhesion and cytoskeletal integrity during oogenesis. *Development* **118**(4): 1191-1207.
Pinheiro, E. M. and D. J. Montell (2004). Requirement for Par-6 and Bazooka in Drosophila border cell migration. *Development* **131**(21): 5243-5251.
Prasad, M., A. C. Jang, M. Starz-Gaiano, M. Melani and D. J. Montell (2007). A protocol for culturing Drosophila melanogaster stage 9 egg chambers for live imaging. *Nat Protoc* **2**(10): 2467-2473.
Ramel, D., X. Wang, C. Laflamme, D. J. Montell and G. Emery (2013). Rab11 regulates cell-cell communication during collective cell movements. *Nat Cell Biol* **15**(3): 317-324.

Riggleman, B., P. Schedl and E. Wieschaus (1990). Spatial expression of the Drosophila segment polarity gene armadillo is posttranscriptionally regulated by wingless. *Cell* **63**(3): 549-560.
Rørth, P., K. Szabo, A. Bailey, T. Laverty, J. Rehm, G. M. Rubin, K. Weigmann, M. Milán, V. Benes, W. Ansorge and S. M. Cohen (1998). Systematic gain-of-function genetics in Drosophila. *Development* **125**(6): 1049-1057.
Rothman, J. E. (1996). The protein machinery of vesicle budding and fusion. *Protein Sci* **5**(2): 185-194.
Ruohola, H., K. A. Bremer, D. Baker, J. R. Swedlow, L. Y. Jan and Y. N. Jan (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in Drosophila. *Cell* **66**(3): 433-449.
Saadin, A. and M. Starz-Gaiano (2016). Circuitous Genetic Regulation Governs a Straightforward Cell Migration. *Trends Genet* **32**(10): 660-673.
Saadin, A. and M. Starz-Gaiano (2016). Identification of Novel Regulators of the JAK/STAT Signaling Pathway that Control Border Cell Migration in the Drosophila Ovary. *G3 (Bethesda)* **6**(7): 1991-2002.
Scheller, R. H. (2013). In search of the molecular mechanism of intracellular membrane fusion and neurotransmitter release. *Nat Med* **19**(10): 1232-1235.
Schulze, K. L., K. Broadie, M. S. Perin and H. J. Bellen (1995). Genetic and electrophysiological studies of Drosophila syntaxin-1A demonstrate its role in nonneuronal secretion and neurotransmission. *Cell* **80**(2): 311-320.
Schwartz, M. L., D. P. Nickerson, B. T. Lobingier, R. L. Plemel, M. Duan, C. G. Angers, M. Zick and A. J. Merz (2017). Sec17 (α-SNAP) and an SM-tethering complex regulate the outcome of SNARE zippering in vitro and in vivo. *Elife* **6**.
Shellenbarger, D. L. and J. D. Mohler (1975). Temperature-sensitive mutations of the notch locus in Drosophila melanogaster. *Genetics* **81**(1): 143-162.
Sheshachalam, A., N. Srivastava, T. Mitchell, P. Lacy and G. Eitzen (2014). Granule protein processing and regulated secretion in neutrophils. *Front Immunol* **5**: 448.
Siddiqi, O. and S. Benzer (1976). Neurophysiological defects in temperature-sensitive paralytic mutants of Drosophila melanogaster. *Proc Natl Acad Sci U S A* **73**(9): 3253-3257.
Silver, D. L., E. R. Geisbrecht and D. J. Montell (2005). Requirement for JAK/STAT signaling throughout border cell migration in Drosophila. *Development* **132**(15): 3483-3492.
Silver, D. L. and D. J. Montell (2001). Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in Drosophila. *Cell* **107**(7): 831-841.
Song, H., A. Orr, M. Duan, A. J. Merz and W. Wickner (2017). Sec17/Sec18 act twice, enhancing membrane fusion and then disassembling cis-SNARE complexes. *Elife* **6**.
Starz-Gaiano, M., M. Melani, X. Wang, H. Meinhardt and D. J. Montell (2008). Feedback inhibition of Jak/STAT signaling by apontic is required to limit an invasive cell population. *Dev Cell* **14**(5): 726-738.
Stewart, B. A., M. Mohtashami, L. Zhou, W. S. Trimble and G. L. Boulianne (2001). SNARE-dependent signaling at the Drosophila wing margin. *Dev Biol* **234**(1): 13-23.
Stow, J. L., P. C. Low, C. Offenhäuser and D. Sangermani (2009). Cytokine secretion in macrophages and other cells: pathways and mediators. *Immunobiology* **214**(7): 601-612.
Südhof, T. C. (2012). Calcium control of neurotransmitter release. *Cold Spring Harb Perspect Biol* **4**(1): a011353.
Thorngren, N., K. M. Collins, R. A. Fratti, W. Wickner and A. J. Merz (2004). A soluble SNARE drives rapid docking, bypassing ATP and Sec17/18p for vacuole fusion. *EMBO J* **23**(14): 2765-2776.
Tomes, C. N., G. A. De Blas, M. A. Michaut, E. V. Farré, O. Cherhitin, P. E. Visconti and L. S. Mayorga (2005). alpha-SNAP and NSF are required in a priming step during the human sperm acrosome reaction. *Mol Hum Reprod* **11**(1): 43-51.
Torres, A. Y., M. Malartre, A. M. Pret and F. Agnès (2017). JAK/STAT signaling is necessary for cell monosis prior to epithelial cell apoptotic extrusion. *Cell Death Dis* **8**(5): e2814.

Van de Bor, V., G. Zimniak, D. Cerezo, S. Schaub and S. Noselli (2011). Asymmetric localisation of cytokine mRNA is essential for JAK/STAT activation during cell invasiveness. *Development* **138**(7): 1383-1393.
Villarino, A. V., Y. Kanno, J. R. Ferdinand and J. J. O'Shea (2015). Mechanisms of Jak/STAT signaling in immunity and disease. *J Immunol* **194**(1): 21-27.
Wickner, W. and J. Rizo (2017). A cascade of multiple proteins and lipids catalyzes membrane fusion. *Mol Biol Cell* **28**(6): 707-711.
Wu, X., P. S. Tanwar and L. A. Raftery (2008). Drosophila follicle cells: morphogenesis in an eggshell. *Semin Cell Dev Biol* **19**(3): 271-282.
Xi, R., J. R. McGregor and D. A. Harrison (2003). A gradient of JAK pathway activity patterns the anterior-posterior axis of the follicular epithelium. *Dev Cell* **4**(2): 167-177.
Xiong, Q. Y., C. Yu, Y. Zhang, L. Ling, L. Wang and J. L. Gao (2017). Key proteins involved in insulin vesicle exocytosis and secretion. *Biomed Rep* **6**(2): 134-139.
Yu, W., F. Kawasaki and R. W. Ordway (2011). Activity-dependent interactions of NSF and SNAP at living synapses. *Mol Cell Neurosci* **47**(1): 19-27.
Zeidler, M. P. and N. Bausek (2013). The Drosophila JAK-STAT pathway. *JAKSTAT* **2**(3): e25353.
Zhao, C., J. T. Slevin and S. W. Whiteheart (2007). Cellular functions of NSF: not just SNAPs and SNAREs. *FEBS Lett* **581**(11): 2140-2149.
Zhao, C., E. C. Smith and S. W. Whiteheart (2012). Requirements for the catalytic cycle of the N-ethylmaleimide-Sensitive Factor (NSF). *Biochim Biophys Acta* **1823**(1): 159-171.
Zhao, M. and A. T. Brunger (2016). Recent Advances in Deciphering the Structure and Molecular Mechanism of the AAA+ ATPase N-Ethylmaleimide-Sensitive Factor (NSF). *J Mol Biol* **428**(9 Pt B): 1912-1926.
Zick, M., A. Orr, M. L. Schwartz, A. J. Merz and W. T. Wickner (2015). Sec17 can trigger fusion of trans-SNARE paired membranes without Sec18. *Proc Natl Acad Sci U S A* **112**(18): E2290-2297.

Figures

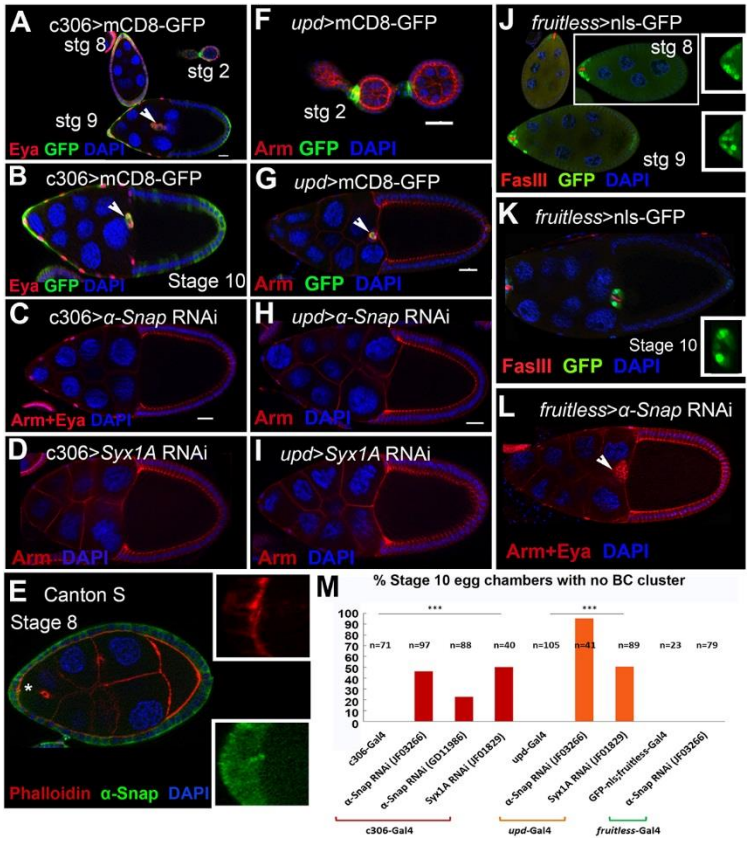


Figure 1. *α-Snap* or *Syx1A* depletion in polar cells prevents border cell specification. (A-L) Egg chambers from indicated genotypes and stages; anterior, left; scale bars =20µm; DAPI (blue) marks nuclei. (A-B) c306-Gal4-driven expression of UAS-mCD8-GFP (green) in polar cells in some stage 2 egg chambers, and in all polar and border cells (arrowhead) from stages 8-10; Eya expression (red) marks follicle cells. (C-D) c306-Gal4-driven *α-Snap* RNAi (C) or *Syx1A* RNAi (D) results in no border cells (Arm, red, with Eya in C, compare to L). (E) Cytoplasmic α-Snap protein (green), apically enriched in wild-type anterior follicle cells; cortical F-actin, red (asterisk, magnified in insets). (F-G) *upd*-Gal4-driven GFP expression in polar cells, stages 2-10. (H-I) *upd*-Gal4-driven *α-Snap* RNAi (H) or *Syx1A* RNAi (I) results in no border cells. (J-K) *fruitless*-Gal4 drives GFP (green) in anterior cells, excluding polar cells (FasIII-positive, red), before border cell specification(J) and later(K), magnified in insets. (L) *fruitless*-Gal4-driven *α-Snap* RNAi produces

wild-type border cells. (M) Penetrance of the no border cell (BC) phenotype for indicated genotypes. No bar means all egg chambers contained border cells. ***indicates $p < 0.0005$ by two-tailed Fisher's exact test.

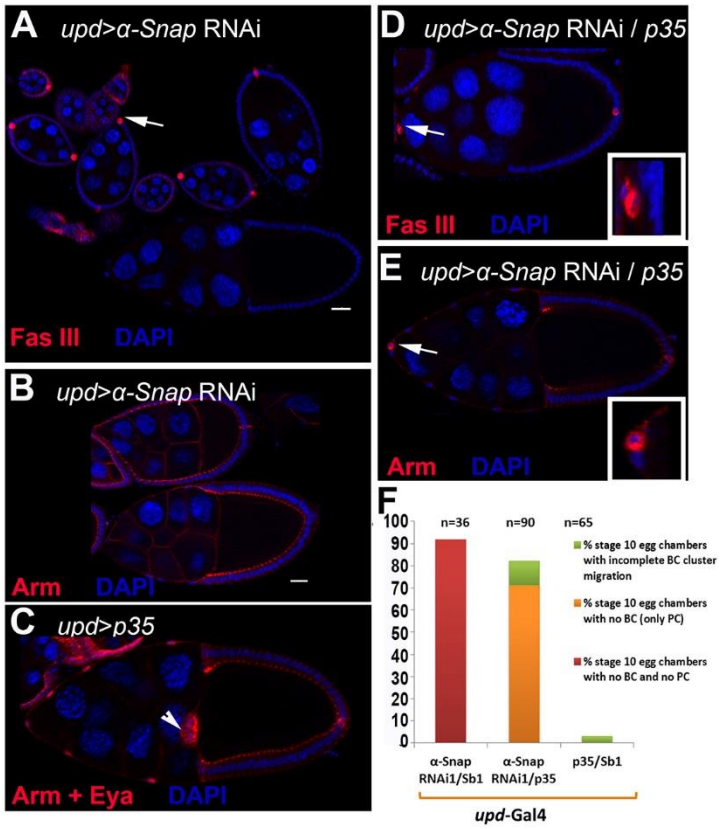


Figure 2. Blocking apoptosis in α-Snap-depleted polar cells does not rescue border cell specification. (A-B) *upd*-Gal4 driving *α-Snap* RNAi results in egg chambers that have polar cells early (FasIII-positive, red; arrow in A), but lack polar and border cells later (B; Arm, red; DAPI, blue). (C) *p35* 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). (F) Penetrance of the no-polar (PC) and/or no-border cells (BC) phenotypes by genotype (line JF03266).

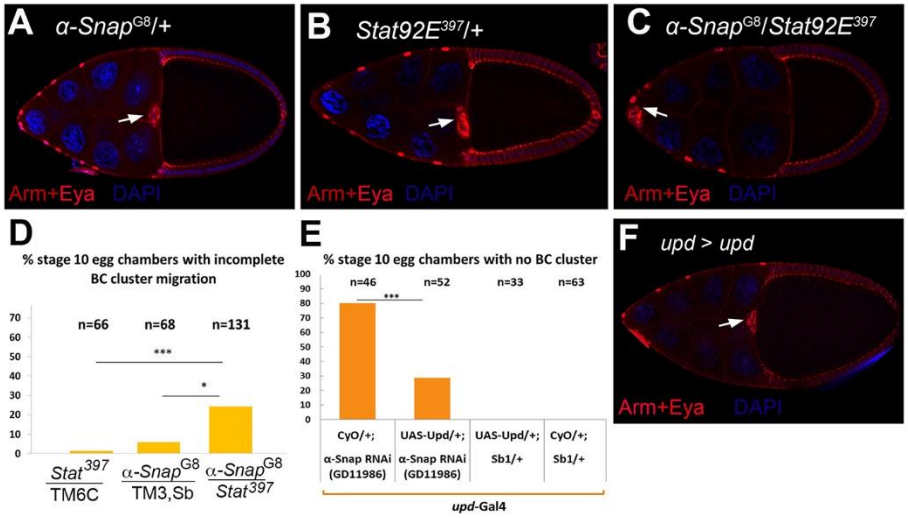


Figure 3. α-Snap functions upstream of Upd to promote JAK/STAT signaling and cell specification. (A-B) Border cells (arrow; Eya and Arm-positive, red) form and migrate correctly in *α-Snap^{G8}*/+ (A) or *Stat92E^{397}*/+ (B) heterozygotes. (C) An *α-Snap^{G8}/Stat^{397}* egg chamber that contains migratory-defective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

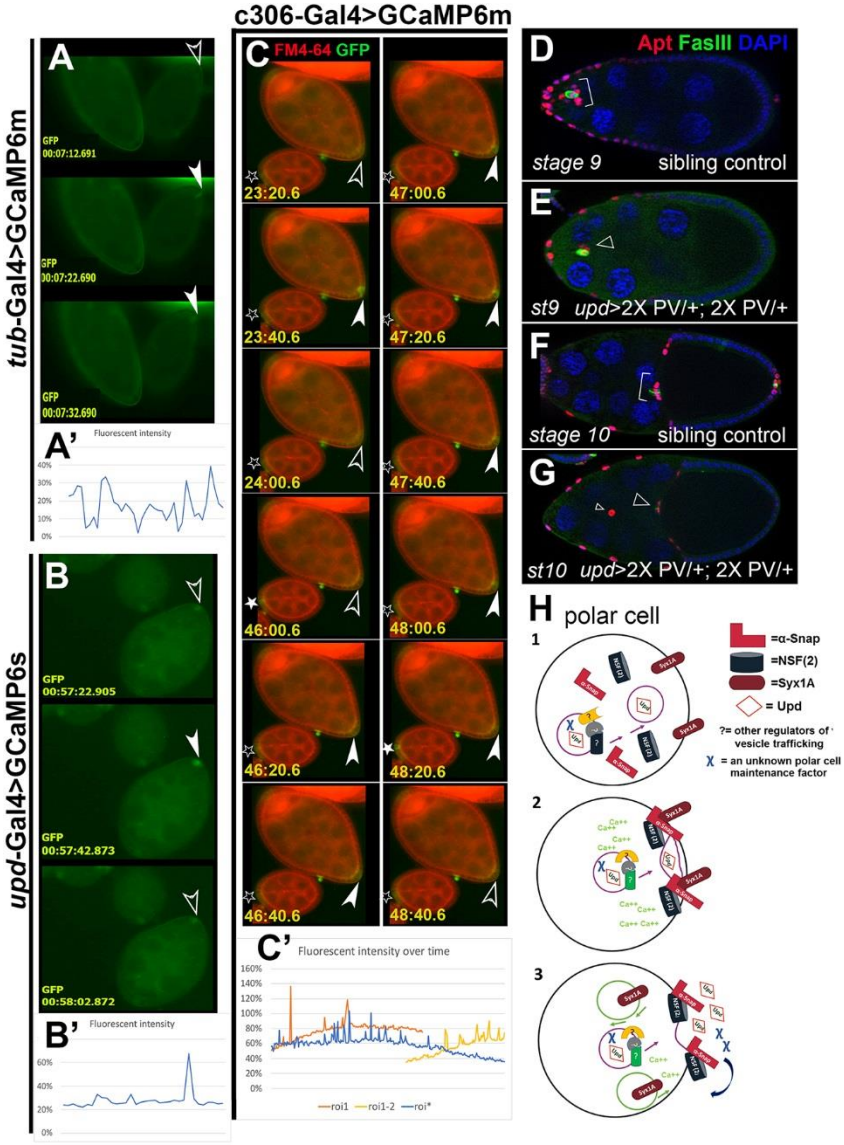


Figure 4. Calcium signaling in polar cells prior to border cell specification. Transient calcium-dependent GFP fluorescence in polar cells in egg chambers; transgenes indicated. White arrowheads mark polar cells fluorescing; empty arrowheads indicate the same cells at a different time not fluorescing. (A-C) Green, GCaMP6-GFP expression; (C) red, FM4-64 lipophilic dye. See supplementary movies 1-3. (A-C') Changes in fluorescence intensity, ΔF/F, for the indicated cells over the timeframe of each movie. In C', yellow and orange traces indicate the same cell at different positions due to the egg chamber shifting. (D-G) Egg chambers from

indicated genotypes; Apt (red) marks anterior follicle cells, FasIII (green) polar cells, and DAPI (blue) nuclei. (D,F) *upd*-Gal4-driven expression of UAS-GFP (green) in polar cells results in at least six migratory cells (bracket). (E,G) *upd*-Gal4-driven expression of UAS-PV (green) in polar cells to sequesters free calcium results in fewer migratory cells (three cells, triangles), and poor migration. (H) A model for Upd cytokine secretion from polar cells. (1) Upd is loaded into apically-targeted vesicles, which will associate with Syx1A at the plasma membrane and dock. (2) α-Snap and NSF/NSF2 associate with Syx1 and the vesicular SNARE complex. In the presence of Ca^{++}, the membranes fuse and Upd is released (3). α-Snap and NSF are required to reset vesicle fusion machinery for another round; Syx1A is recycled back to the plasma membrane. α-Snap also mediates release of a polar cell maintenance factor(X).

Vesicle Trafficking Regulator Genes	RNAi lines	c306-Gal4 driven RNAi		*upd*-Gal4 driven RNAi	
		No border cell cluster	Incomplete BC cluster migration	No border cell cluster	Incomplete BC cluster migration
α-Snap (soluble NSF attachment protein)	KK107910	lethal	-		
	JF03266	**46.4%** (n=97)	ns	**88.3%** (n=120)	ns
	HMS00872	0% (n=38)	0%		
	GD11986 *	**22.7%** (n=88)	31.8%	**80.4%** (n=46)	ns
Rab5	JF03335	0% (n=22)	91%	0% (n=104)	ns
	HMS00147	0% (n=64)	ns		
Syntaxin 1A	JF01829 *	**50%** (n=40)	35%	**50.6%** (n=89)	14.6%
Syntaxin 4	JF01714 *	0% (n=136)	ns		
	JF01460	0% (n=93)	ns		
	HMS02771	0% (n=86)	ns		
Syntaxin 5	JF03330 *	ns (n=67)	20.9%	0% (n=72)	12.5%
Syntaxin 6	JF03125	0% (n=148)	ns		
Syntaxin 7	JF02436 *	0% (n=65)	40%	0% (n=48)	0%
Syntaxin 8	JF02038	0% (n=122)	ns		
Syntaxin 13	HMS01723	0% (n=48)	ns		
	JF01920	0% (n=98)	ns		
Syntaxin 16	JF01924	0% (n=174)	ns	0% (n=132)	ns
	HMC03430	0% (n=104)	ns		
Syntaxin 17	JF01937 *	0% (n=52)	0%		
Syntaxin 18	JF02263 *	0% (n=87)	ns		
NSF 1 (*comatose*)	HMS01261	0% (n=56)	ns		
	JF01459	0% (n=89)	0%		
	JF01233	0% (n=65)	ns	0% (n=27)	0%
NSF2	JF02765	0% (n=48)	ns	lethal	
	HMS01262	lethal	-	lethal	
γ-Snap	JF03124	0% (n=90)	ns		

Table S1. Cell-type specific depletion of *α-Snap* or *Syx1A* leads to a failure of border cell specification, while depletion of other vesicle trafficking regulators leads to defects in border cell migration or normal, migrating border cells. The percent penetrance of

the phenotype was scored in stage 10 egg chambers. Penetrance of the phenotype caused by c306- and *upd*-Gal4 driven JF03266 *α-Snap* RNAi is the average of four and two independent experiments, respectively. Penetrance of the phenotype caused by *Syx1A* RNAi is the average of two independent experiments. While some *α-Snap* RNAi lines and *Syx1A* RNAi occasionally led to cell migration defects, the no-border-cells phenotype was more unusual; thus, this was our focus for further characterization. If the border cell migration defect was observed in less than 10% of stage 10 egg chambers, it was deemed not significant (ns) since control (c306-Gal4) egg chambers sometimes displayed defects to this extent. Rab5 disruption was used as a positive control, which resulted in incomplete border cell migration (Assaker et al., 2010); however, border cell fate was not affected and *Rab5* depletion in polar cells yielded a wild-type phenotype. In all experiments flies were incubated overnight at 29^{o}C. RNAi lines with an asterisk have been shown to cause mutant phenotypes in other tissues, which indicates they are functional (Mummery-Widmer et al., 2009, Schnorrer et al., 2010, Khuong et al., 2013, Meehan et al., 2015, Peng et al., 2015, Harris et al., 2016, Mauvezin et al., 2016).

Assaker, G., D. Ramel, S. K. Wculek, M. Gonzalez-Gaitan and G. Emery (2010). Spatial restriction of receptor tyrosine kinase activity through a polarized endocytic cycle controls border cell migration. *Proc Natl Acad Sci U S A* **107**(52): 22558-22563.

Harris, K. P., Y. V. Zhang, Z. D. Piccioli, N. Perrimon and J. T. Littleton (2016). The postsynaptic t-SNARE Syntaxin 4 controls traffic of Neuroligin 1 and Synaptotagmin 4 to regulate retrograde signaling. *Elife* **5**.

Khuong, T. M., R. L. Habets, S. Kuenen, A. Witkowska, J. Kasprowicz, J. Swerts, R. Jahn, G. van den Bogaart and P. Verstreken (2013). Synaptic PI(3,4,5)P3 is required for Syntaxin1A clustering and neurotransmitter release. *Neuron* **77**(6): 1097-1108.

Mauvezin, C., A. L. Neisch, C. I. Ayala, J. Kim, A. Beltrame, C. R. Braden, M. K. Gardner, T. S. Hays and T. P. Neufeld (2016). Coordination of autophagosome-lysosome fusion and transport by a Klp98A-Rab14 complex in Drosophila. *J Cell Sci* **129**(5): 971-982.

Meehan, T. L., S. E. Kleinsorge, A. K. Timmons, J. D. Taylor and K. McCall (2015). Polarization of the epithelial layer and apical localization of integrins are required for engulfment of apoptotic cells in the Drosophila ovary. *Dis Model Mech* **8**(12): 1603-1614.

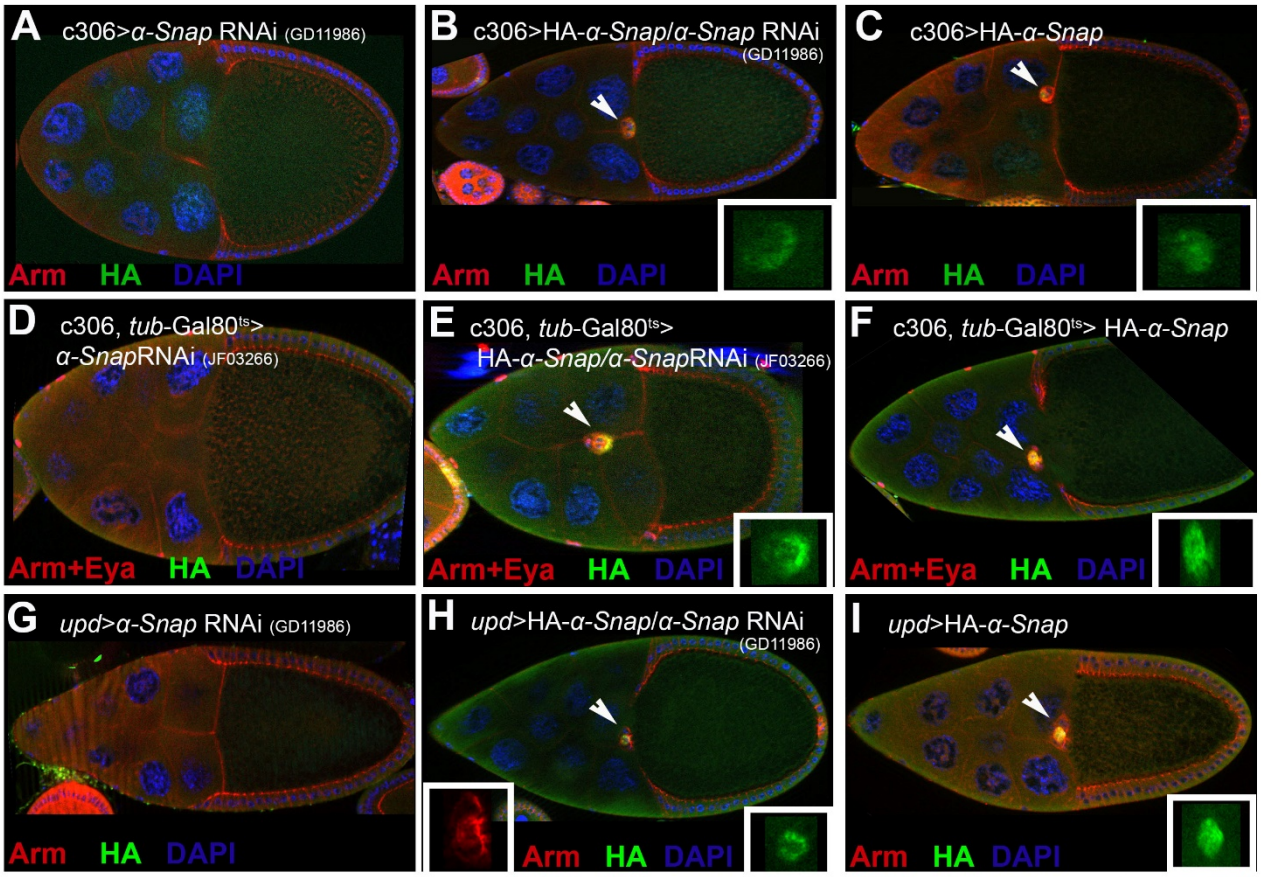
Mummery-Widmer, J. L., M. Yamazaki, T. Stoeger, M. Novatchkova, S. Bhalerao, D. Chen, G. Dietzl, B. J. Dickson and J. A. Knoblich (2009). Genome-wide analysis of Notch signalling in Drosophila by transgenic RNAi. *Nature* **458**(7241): 987-992.

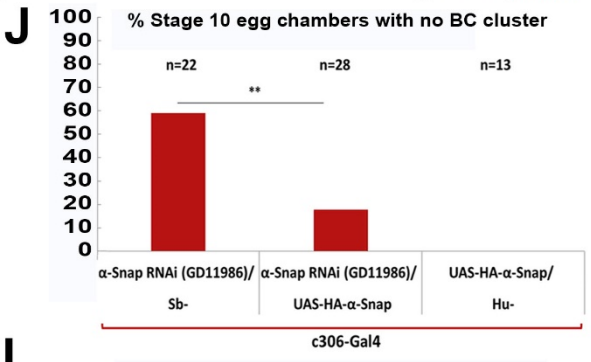
Peng, Y., J. Lee, K. Rowland, Y. Wen, H. Hua, N. Carlson, S. Lavania, J. Z. Parrish and M. D. Kim (2015). Regulation of dendrite growth and maintenance by exocytosis. *J Cell Sci* **128**(23): 4279-4292.

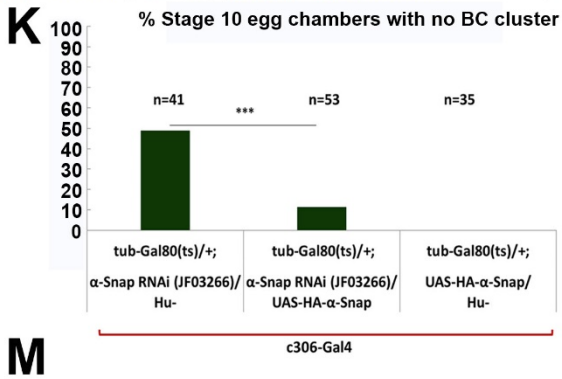
Schnorrer, F., C. Schonbauer, C. C. Langer, G. Dietzl, M. Novatchkova, K. Schernhuber, M. Fellner, A. Azaryan, M. Radolf, A. Stark, K. Keleman and B. J. Dickson (2010). Systematic genetic analysis of muscle morphogenesis and function in Drosophila. *Nature* **464**(7286): 287-291.

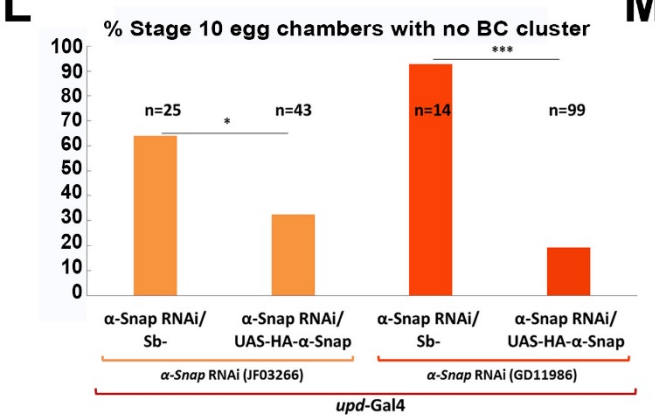
Gene	RNAi line	*tub*-Gal4 driven RNAi in embryos		c306-Gal4 driven RNAi	
		Fold change	Primers	Single RNAi	Interaction tests
α-Snap (soluble NSF attachment protein)	KK107910			lethal	
	JF03266	**0.19**	α-snap ex1-2; α-snap ex2-3; α-snap FRP		Lethal with JF01233 HMS01261, (NSF RNAi), JF02765 (NSF2 RNAi)
	GD11986				Lethal with JF02765, HMS01262 (NSF2 RNAi)
NSF (*comatose*)	HMS01261	**0.31**	comt set2; comt ex4-6		Lethal with JF02765 (NSF2 RNAi)
	JF01459				
	JF01233				
NSF2	JF02765	**0.02**	NSF2 ex2-3 NSF2 ex3-4		Lethal with HMS01261 (NSF RNAi)
	HMS01262	**0.09**	NSF2 ex2-3 NSF2 ex3-4	lethal at 25°	

Table S2. Verification of functional RNA interference. To test knockdown efficiency, *tub*-Gal4 females were crossed to males from the indicated RNAi lines. Total RNA was collected from F1 embryos and assayed by qRT-PCR in triplicates. Fold changes are represented as averages of two or four experiments. Endogenous ovarian expression of *α-Snap, NSF,* and *NSF2* mRNAs was verified using the same primer sets. For interaction tests, RNAi lines were balanced and combined as indicated, then males carrying two RNAi insertions were crossed to c306-Gal4 females.









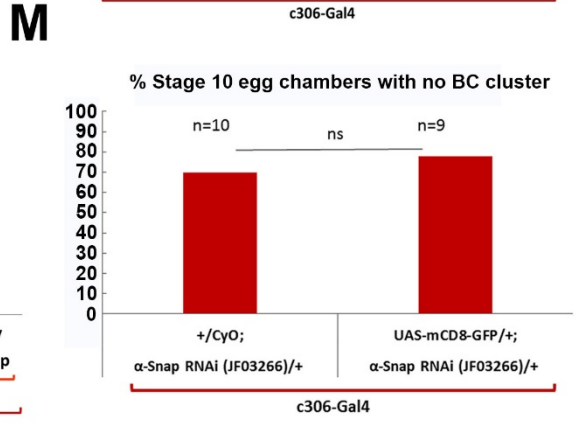


Figure S1. α-Snap is required in the anterior follicle cells to induce border cell fate.

Depletion of *α-Snap* in the anterior follicle cells (A&D) and in polar cells (G) leads to egg chambers that lack the border cell cluster. c306- and *upd*-Gal4-driven expression of HA-tagged *α-Snap* in the depleted egg chambers rescues the lack of a border cell cluster phenotype caused by the RNAi (B, E&H, arrowheads, insets; border cells are marked by HA expression (green) and Arm (red), and DAPI stain (blue). Over-expression of *α-Snap* alone in the control egg chambers (c306-Gal4, c306-Gal4; *tub*-Gal80 (ts) and *upd*-Gal4) does not affect border cell specification (C, F&I). (J-M) Comparison of the penetrance of the no border cell (BC) cluster phenotype caused by *α-Snap* RNAi to that caused by the RNAi plus expression of HA-tagged *α-Snap* in the anterior follicle cells (J&K) and polar cells (L). The presence of a second UAS in the genome (UAS-GFP), which might dilute out the concentration of Gal4, does not lead to suppression of the phenotype caused by *α-Snap* RNAi (M). *indicates $p< 0.05$, **$p<0.005$, *** $p< 0.0005$ significance value by two-tailed Fisher's exact test. Not significant change is shown as ns. Genotypes with no bar did not have any cases of the no border cell cluster phenotype. (A-C) and (J) flies were incubated at 29°C for 20 hours prior to dissection. (D-F) and (K) flies were incubated at 31°C (non-permissive temperature for $Gal80^{ts}$) for 54 hours prior to dissection. (G-I) and (L) flies were incubated at 29°C for 45 hours (for the JF03266 RNAi line (L)) and for 26 hours (for the GD11986 RNAi line (G-I & L)) prior to dissection. (M) flies were incubated at 29°C for 48 hours prior to dissection.

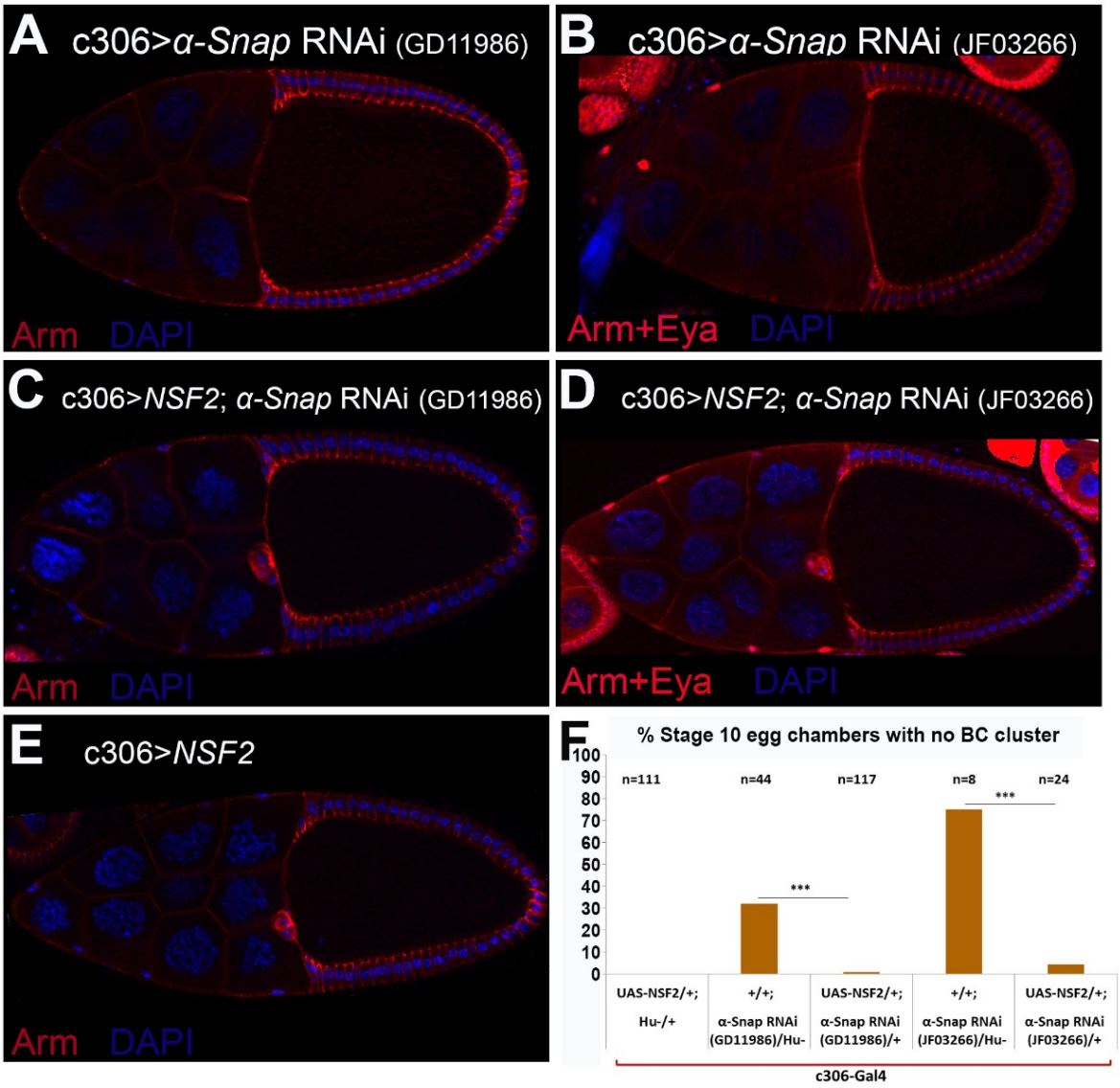


Figure S2. **NSF2 and α-Snap function together to regulate border cell specification.** (A-B) c306-Gal4-driven depletion of *α-Snap* using either the GD11986 (A) or JF03266 (B) RNAi line results in egg chambers that lack a border cell cluster. (C-E) Over-expression of *NSF2* using c306-Gal4 rescues the phenotype caused by *α-Snap* RNAi (C-D), while overexpression of *NSF2* alone yields a wild-type phenotype (E). (F) The penetrance of the phenotype caused by *α-Snap* RNAi is significantly reduced upon over-expression of *NSF2*. ***indicates p< 0.0005 significance value by two-tailed Fisher's exact test. Flies bearing JF03266 RNAi and the ones additionally expressing *NSF2* (c306-Gal4; UAS-*NSF2*; *α-Snap* RNAi (JF03266) flies) were incubated at 29°C for 24-48 hours prior to dissection.

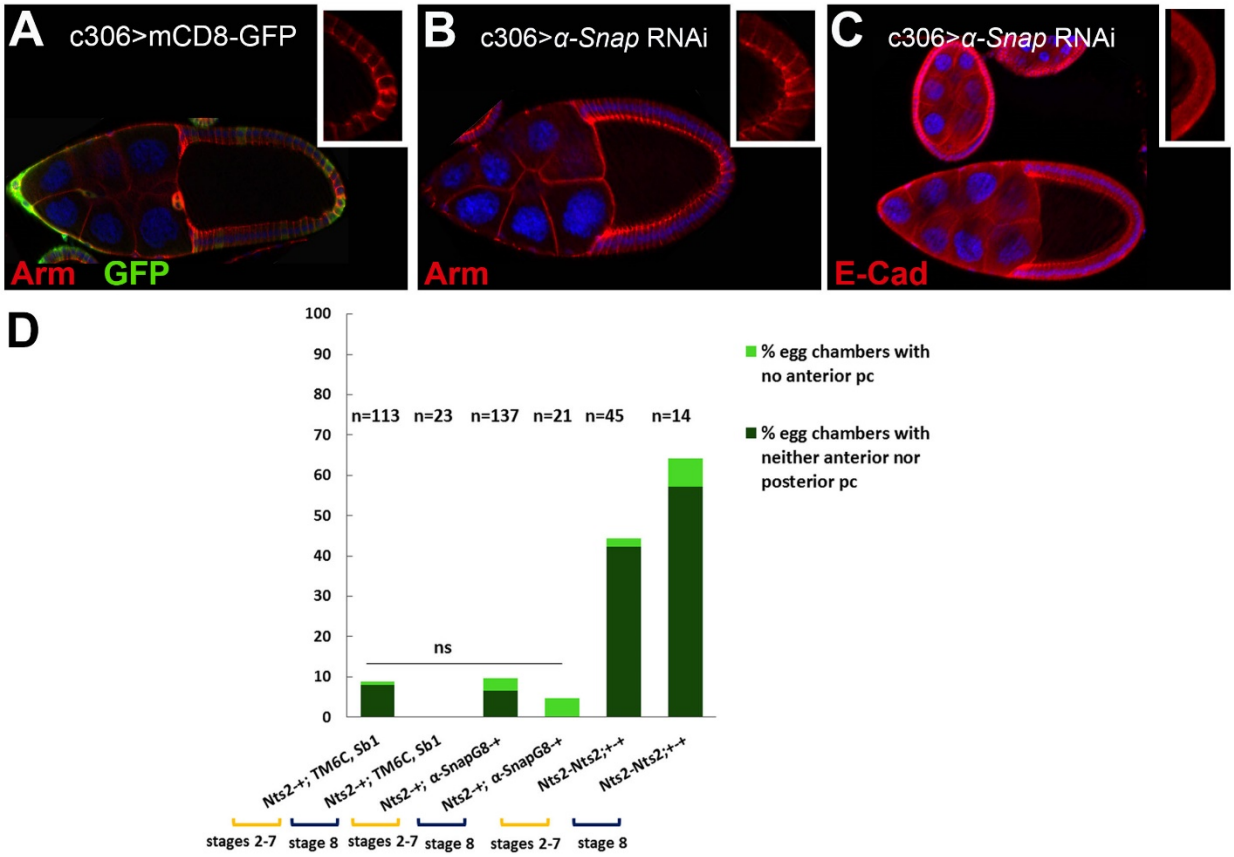
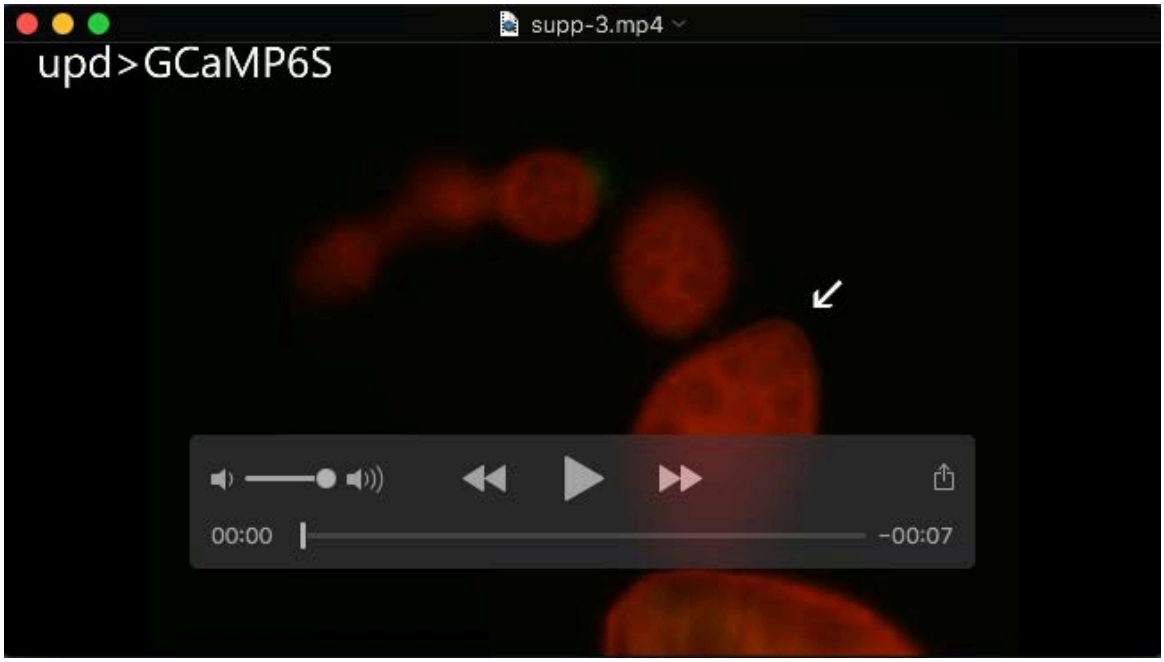


Figure S3. α-Snap does not affect trafficking of all cell surface molecules. (A-C) In egg chambers with *α-Snap* depletion, cell-surface adhesion molecule complexes are properly localized. c306-Gal4 drives membrane-tethered GFP expression (green) in the anterior and posterior follicle cells (A). Arm (A,B, red) and E-Cad (C, red) localization patterns in the posterior follicle cells appear normal in a c306-Gal4-driven *α-Snap* RNAi egg chamber. Insets in (A-C) are magnified views of posterior follicle in the control and in the *α-Snap* depleted egg chambers. (D) *α-Snap* and *Notch* (*N*) do not genetically interact during polar cell specification. Penetrance of stages 2-8 egg chambers that lack either only the anterior or both the anterior and the posterior polar cells (pc) in the N^{ts2}/+ heterozygous, N^{ts2}/+; α-Snap^{G8}/+ double heterozygous or N^{ts2}/N^{ts2} homozygous flies. Two-tailed Fisher's exact test was run to calculate the statistical significance. All flies were incubated at 29°C (non-permissive temperature for *ts2* allele of *N*) for 116 hours.

Movie 1: Transient GFP fluorescence in a polar cell prior to border cell specification in a *tub*-Gal4>UAS-GCaMP6m egg chamber. A 7:20 (seven minute, twenty second) portion of a 40 minute-long time lapse movie. Images were captured every 10 seconds with a 1 second exposure time. Pulses are seen in the stage 6/7 egg chamber.



Movie 2: Transient GFP fluorescence in polar cells in a stage seven *upd*-Gal4>UAS-GCaMP6s egg chamber. An 11 minute portion of an hour-long time lapse movie. Images were capture every 20 seconds, with a 1.24second exposure time; red shows FM4-64 marking the cell membranes. A pulse is seen in the stage 8 egg chamber.

Movie 3: Periodic GFP fluorescence in polar cells prior to border cell specification in a c306-Gal4-UAS-GCaMP6m egg chamber. A 1 h, 37 min, 20 s (01:37:20) portion of a two hour-long time lapse movie. Images were captured every 20 second with a 1 second exposure time in the green channel. Pulses are seen in the anterior of the stage 7/8 egg chamber and the posterior of the younger egg chamber.