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The Maf factor Traffic jam both enables and inhibits collective cell migration in *Drosophila* oogenesis

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SUMMARY

Border cell cluster (BCC) migration in the *Drosophila* ovary is an excellent system to study the gene regulatory network that enables collective cell migration. Here, we identify the large Maf transcription factor Traffic jam (Tj) as an important regulator of BCC migration. Tj has a multifaceted impact on the known core cascade that enables BCC motility, consisting of the Jak/Stat signaling pathway, the C/EBP factor Slow border cells (Slbo), and the downstream effector DE-cadherin (DEcad). The initiation of BCC migration coincides with a Slbo-dependent decrease in Tj expression. This reduction of Tj is required for normal BCC motility, as high Tj expression strongly impedes migration. At high concentration, Tj has a tripartite negative effect on the core pathway: a decrease in Slbo, an increase in the Jak/Stat inhibitor Socs36E, and a Slbo-independent reduction of DEcad. However, maintenance of a low expression level of Tj in the BCC during migration is equally important, as loss of *tj* function also results in a significant delay in migration concomitant with a reduction of Slbo and consequently of DEcad. Taken together, we conclude that the regulatory feedback loop between Tj and Slbo is necessary for achieving the correct activity levels of migration-regulating factors to ensure proper BCC motility.

KEY WORDS: Traffic jam (Tj), Slow border cells (Slbo), DE-cadherin, Jak/Stat, Border cell migration, *Drosophila* oogenesis, Large Maf transcription factor

INTRODUCTION

Cell migration is indispensable for metazoan development. The border cell cluster (BCC) in the *Drosophila* ovarian follicle is an important model system to study the regulation of collective cell migration (Rørth, 2009; Montell et al., 2012). The BCC is a small group of cells that segregate from an epithelium and collectively undergo guided migration on the surface of other cells. BCC migration has been extensively used to study the molecular mechanisms that control cell motility (Rørth, 2009; Montell et al., 2012).

In a *Drosophila* ovarian follicle, a layer of follicle cells surrounds 16 germline cells that include a posteriorly located oocyte, and 15 anteriorly located nurse cells. Each follicle goes through 14 stages of oogenesis (King, 1970). At stage 8, the anteriormost follicle cells develop into the BCC. The BCC consists of two nonmotile polar cells, surrounded by four to eight migratory border cells, which we refer to as rosette cells (Montell et al., 2012; Niewiadomska et al., 1999). At stage 9, the BCC segregates from the follicular epithelium and migrates between the nurse cells toward the oocyte. After 6 hours, at stage 10a, when most follicle cells, called main-body follicle cells (MBFCs) are arranged around the oocyte, the BCC completes migration. At stage 10b, another group of follicle cells, called centripetal cells, moves interiorly along the oocyte-nurse cell boundary and contacts the BCC (King, 1970).

A number of factors have been identified to regulate various aspects of BCC migration (Rørth, 2009; Montell et al., 2012). Among those, the Jak/Stat signaling pathway, transcription factor Slow border cells (Slbo), and adhesion molecule DE-cadherin (DEcad) form a core pathway that controls the motility of the BCC. The polar cells signal through the cytokine Unpaired (Upd) to the neighboring follicle cells, leading to phosphorylation of the Jak protein Hopscotch (Hop) and subsequently the Hop target Stat92E (Silver and Montell, 2001; Beccari et al., 2002; Ghiglione et al., 2002). Phosphorylated Stat92E activates expression of Slbo, the *Drosophila* homolog of CCAAT-enhancer binding proteins (C/EBP) (Montell et al., 1992). Slbo is crucial for regulating the expression of various factors involved in migration, including adhesion molecules, cytoskeletal regulators and endocytic factors (Borghese et al., 2006; Wang et al., 2006). An essential target of Slbo is *shotgun* (*shg*), which encodes DEcad (Niewiadomska et al., 1999; Mathieu et al., 2007). DEcad is enriched in the BCC compared with other follicle cells, and provides the necessary traction for rosette cells to migrate over the nurse cells (Niewiadomska et al., 1999).

Achieving a balanced expression level of these factors is important for proper motility of the BCC, because over- or under-expression of components of the Jak/Stat pathway, Slbo or DEcad negatively impacts migration. Reduction of Stat92E activity during the migration process (Silver et al., 2005), as well as its overactivation, interferes with BCC migration (Yoon et al., 2011). Loss of *slbo* function in rosette cells blocks the motility of the BCC (Montell et al., 1992; Rørth et al., 2000), whereas overexpression of *slbo* causes a delay in migration and frequently a fragmentation of the BCC (Rørth et al., 2000; Starz-Gaiano et al., 2008). Similarly, not only does lack of DEcad abolish BCC motility (Niewiadomska et al., 1999), but also higher-than-normal expression of DEcad in rosette cells delays migration (Schober et al., 2005).

It is not surprising therefore, that molecular checkpoints keep these factors in balance during BCC migration. A Stat92E target, Apontic, negatively regulates Jak/Stat signaling in rosette cells by activating a microRNA that causes *Stat92E* transcript degradation (Starz-Gaiano et al., 2008; Yoon et al., 2011) and upregulating Socs36E (Monahan and Starz-Gaiano, 2013). Socs36E, which is also a target of Stat92E, acts in a negative feedback loop to limit Jak/Stat signaling in many cell types (Callus and Mathey-Prevot, 2002; Rawlings et al., 2004; Baeg et al., 2005; Issigonis et al., 2009; Singh et al., 2010; Tarayrah et al., 2013), and its overexpression has

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been shown to impair BCC migration (Silver et al., 2005; Monahan and Starz-Gaiano, 2013). Although the molecular mechanism by which Socs36E regulates the Jak/Stat pathway is not well understood, it has been shown that loss of Socs36E leads to elevated Stat92E expression in the testis stem cell niche (Issigonis et al., 2009). The lifetime of Slbo is tightly controlled by antagonistic actions of two factors: the kinase Tribbles, which removes Slbo through ubiquitin-mediated proteolysis (Rørth et al., 2000; Masoner et al., 2013), and the ubiquitin hydrolase Ubp64E, which stabilizes Slbo by removing ubiquitin from the protein (Rørth et al., 2000). The transcription factor Yan (Anterior open) functions in the endocytosis of DEcad, regulating the local concentration of this adhesion molecule in rosette cells (Schober et al., 2005).

Here, we show that the large Maf transcription factor Traffic jam (Tj) is crucial for establishing the correct balance of the core components within the regulatory cascade that controls BCC motility. We previously reported that Tj is needed for *Drosophila* oogenesis (Li et al., 2003). Follicle cells that lack Tj display changes in the expression of adhesion molecules, undergo abnormal shape changes, and often leave the follicular epithelium (Li et al., 2003). To better understand the function of Tj in controlling cell behavior, we studied its function in the rosette cells, which naturally separate from an epithelium and undergo migration.

Our analysis revealed that knockdown of *tj* in rosette cells leads to a significant delay in BCC migration. This defect seems to be mediated by a reduction in Slbo and consequently DEcad. Interestingly, a natural decrease in Tj concentration, which seems to depend on Slbo, was observed in migrating BCCs. We also found that overexpression of Tj severely impedes migration. This suggests that too little or too much Tj has a negative impact on BCC motility. Tj overexpression caused a significant decrease in Slbo, which appears to be mediated by an increase in expression of the Jak/Stat inhibitor Socs36E. It also inhibited DEcad expression even in the presence of Slbo. Our data indicate that Tj is required at low, tightly regulated concentrations to allow proper expression levels of all three components within the core cascade, providing rosette cells with the necessary balance of factors that confers proper cell motility.

MATERIALS AND METHODS

Fly stocks

Unless noted otherwise, *Drosophila* crosses were done at 25°C and female flies were fed yeast for 2 days before dissection. We used *slbo-Gal4* [P{*Gal4-slbo2.6*}1206 (Rørth et al., 1998)] to drive expression in rosette cells, and *c306-Gal4* [P{*GawB*}c306; Bloomington Drosophila Stock Center (BDSC)] for expression in anterior follicle cells, including the BCC. The strains *UAS-tj^{RNAi}* (10034R-2; NIG-Fly Stock Center), *Df(2L)E55* (deletion of *tj*; BDSC), *tj^{eo2}* (null allele; Schüpbach and Wieschaus, 1991; Li et al., 2003), *tj^{39}* (hypomorphic *tj* allele; T. Panchal, E. Alchits and D.G., unpublished), *slbo^{1}* (Montell et al., 1992), *Stat92E^{F}* [*Stat92E^{ts}* (Baksa et al., 2002)], *Stat92E^{06346}* [*Stat92E-lacZ* (Hou et al., 1996)], *Socs36E^{PZ1647}* [*Socs36E-lacZ* (Issigonis et al., 2009)] and *Df(2L)Exel7070* (deletion of *Socs36E*; BDSC) were used for loss-of-function analysis, and *UAS-tj$^{6(3)}$* or *UAS-tj$^{1(2)}$* (see below), *UAS-slbo* (Starz-Gaiano et al., 2008), *UAS-shg* (Sarpal et al., 2012) and *UAS-hop3* (Harrison et al., 1995) for overexpression studies. *slbo2.6-lacZ* [*slbo-lacZ* (Rørth et al., 1998)], *shg1.3-lacZ* [*shg-lacZ* (Mathieu et al., 2007)], *10XStat-GFP* [*Socs36E-GFP* (Bach et al., 2007)], *Socs36E-lacZ* and *Stat92E-lacZ* were used as enhancer reporters. *Stat92E^{ts}/Stat92E^{06346}* flies, used for temperature-dependent reduction of Stat92E activity, were grown for 12 hours at restrictive temperature (29°C) before dissection. To generate Tj-overexpressing cell clones, *hsFlp1/Act5C>CD2>Gal4*; *UAS-tj$^{1(2)}$/UAS-lacZ* flies were heat shocked at 37°C for 15 minutes and dissected 24-48 hours later. Tj-overexpressing clones were identified by expression of *lacZ*. Unless noted otherwise, *slbo-Gal4*/+ served as the control genotype, and *slbo-Gal4* induced expression of *UAS* constructs.

UAS-tj transgenic lines

A *tj* cDNA with the complete open reading frame and 3′ untranslated region, including a poly(A), and a partial 5′ untranslated region, was generated from two overlapping *tj* cDNAs (Li et al., 2003) and subcloned into pUAST vector to generate transgenic *UAS-tj* flies following standard procedures. *UAS-tj$^{1(2)}$* and *UAS-tj$^{6(3)}$* are inserted on the second and third chromosome, respectively. Exogenous Tj properly localized to the cell nucleus and rescued the *tj* mutant embryonic gonad phenotype (data not shown).

Immunostaining and tissue *in situ* hybridization

The following primary antibodies were used: polyclonal guinea-pig anti-Tj (G5 1:5000; the same Tj peptide used to immunize rats (Li et al., 2003) was injected into guinea pigs), rat anti-Slbo (1:4000) (Mathieu et al., 2007), rat anti-DEcad (DCad2; 1:25), mouse anti-Fasciclin III (FasIII, 7G10, 1:50), mouse anti-Armadillo (Arm, 7A1, 1:100) (Developmental Studies Hybridoma Bank), rabbit anti-β-galactosidase (β-gal) (1:100,000 for *slbo-lacZ*, 1:10,000 for *shg-lacZ*; 1:1500 for *Stat92E-lacZ*; 1:15,000 for *Socs36E-lacZ*; MP Biomedicals) and rabbit anti-Stat92E (1:1000) (Amoyel et al., 2013). Secondary antibodies (1:400) were conjugated to Cy3 (Jackson ImmunoResearch Laboratories) or Alexa Fluor 488, Alexa Fluor 555 or Alexa Fluor 647 (Molecular Probes, Life Technologies).

Tissue *in situ* hybridization was done as previously described (Niewiadomska et al., 1999) using cDNAs of *tj* (Li et al., 2003) and *slbo* (RE37385; *Drosophila* Genomics Resource Center) to generate digoxigenin-labeled (Roche) DNA probes.

Imaging

All imaging was done with a 40×/1.4 Plan-Apo objective using microscopes from Carl Zeiss MicroImaging. Confocal fluorescence images were acquired with an LSM510 microscope. Regular fluorescence images (not shown), used to calculate migration indices, were acquired with an Axioscope-2 microscope and Axiocam camera, and Axiovision 4.3 was used for length measurements. *In situ* hybridization images were generated with an Axioplan-2 microscope and a Canon EOS Rebel Digital SLR camera. Images were processed with Adobe Photoshop and Illustrator CS2 (Adobe Software).

Fluorescence signal quantification and migration index

Immunofluorescence signals of Tj and Slbo in confocal images were quantified using ImageJ (NIH), measuring a 7.16 μm^2 circular area per cell nucleus. For each BCC, a *z*-stack of confocal images was recorded, and for each nucleus, the focal plane with the brightest signal was chosen for measurement.

The BCC migration index [100% denotes complete migration; 0% no migration (Melani et al., 2008)] was determined for stages 9, 10a and 10b of oogenesis (King, 1970). We sub-categorized stage 9 follicles according to the position of the anteriormost MBFCs relative to the distance between the anterior follicle tip (0%) and the oocyte-nurse cell border (100%): 0-30% defined early stage 9, 30-60% mid stage 9 and 60-100% late stage 9. Unpaired, two-tailed Student's *t*-tests (for unequal sample sizes and equal variance) were used for all statistical analyses (Microsoft Excel), and graphs were created with Prism4 (GraphPad Software).

Border cell purification, RNA extraction and microarray

BCCs were isolated from follicles as previously described (Wang et al., 2006). Green fluorescent protein (GFP)-positive cells, composed of BCCs and centripetal cells, constituted 70% of the total pool of isolated cells. Total RNA extraction using TRIzol (Life Technologies) typically yielded 150 ng/μl per 150 ovary pairs. Microarray analysis was conducted by the Canadian *Drosophila* Microarray Center using *Drosophila* NimbleGen 4-plex (4x72k) arrays (Roche). Raw intensities were obtained using Nimblescan software, and were normalized and converted into fold changes using ArrayStar (DNASTAR).

RESULTS

Decrease or increase of Tj expression delays BCC migration

Our previous work showed that Tj is expressed in the BCC (Li et al., 2003). We examined the expression of Tj in more detail in stage 8 to 10 follicles, from the time of BCC formation to the end of its

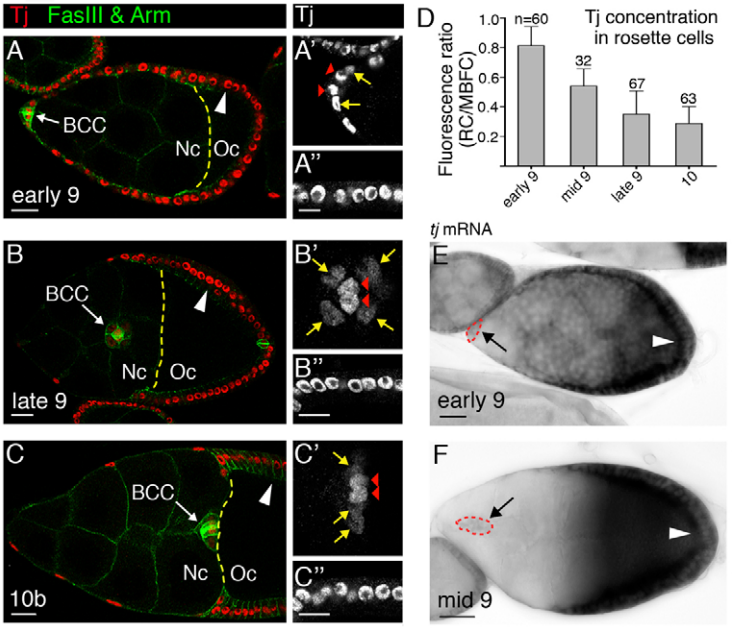

Fig. 1. Wild-type Tj expression profile during BCC migration. (**A-C″**) Follicles stained for Tj (red), and co-stained for FasIII and Arm (green) to highlight polar cells and plasma membranes. The white arrow points to the BCC, the white arrowheads to the MBFCs (magnified in A″-C″). The yellow line indicates the nurse cell-oocyte border. Numbers indicate follicle stages. (A′-C′) The yellow arrows point to rosette cells, the red arrowheads to polar cells. (A) Before migration, the amount of Tj is comparable between the BCC (A′) and MBFCs (A″), and between rosette and polar cells. (B,C) During (B) and after (C) BCC migration, the Tj signal is substantially weaker in the BCC (B′,C′) compared with MBFCs (B″,C″). There is less Tj in rosette cells than in polar cells. (**D**) Measurement of Tj signal intensity in nuclei of rosette cells compared to MBFCs. Each sample size (*n*) represents equal numbers of rosette cells and MBFCs. The histogram shows mean + s.d. of the fluorescence ratio. (**E**,**F**) *tj* mRNA signal is weaker in the BCC (arrows) compared with MBFCs (arrowheads) before (E, early stage 9) and during migration (F, mid stage 9). Anterior is to the left in all panels. Scale bars: 20 μm in A-C″; 25 μm in E,F″. Nc, nurse cells; Oc, oocyte; RC, rosette cells.

migration. At early stage 9 when the BCC segregates from the follicular epithelium, similar amounts of Tj protein were detected in the BCC and the neighboring follicle cells (Fig. 1A-A″). When the BCC had detached from the follicular epithelium and started to migrate toward the oocyte, Tj protein appeared significantly reduced in BCCs in comparison with MBFCs (Fig. 1B-C″). Within the BCC, the reduction of Tj protein seemed more prominent in the migratory rosette cells compared with the polar cells. Quantification of the Tj signal intensity at four different stages of oogenesis (early, mid and late stage 9, and stage 10) revealed a gradual reduction in rosette and polar cells during BCC migration (in comparison with MBFCs) (Fig. 1D; supplementary material Fig. S1F). When the BCC reached the oocyte at stage 10, the relative amount of Tj protein had dropped to ~30% of the original level (Fig. 1D). To ensure that the observed reduction of Tj in the migrating BCC was not caused by inaccessibility to the Tj antibody, we confirmed this result by immunostaining cryosections of ovarian follicles (supplementary material Fig. S1A-E). In contrast to the gradual reduction of Tj protein, *tj* mRNA appeared already strongly reduced in BCCs at early stage 9, but was maintained at a low level throughout migration (Fig. 1E,F), suggesting that the downregulation of *tj* expression occurs at the pre-translational level.

The presence of Tj throughout migration prompted us to ask whether it has a function in BCC migration. Generation of *tj* null mutant cells in the anterior region of the follicular epithelium prevented the recruitment of rosette cells, suggesting that *tj* is required for BCC formation (J. D. Alls and D.G., unpublished data). To determine whether Tj is needed for the actual migration process, we induced expression of *tj* double-stranded RNA (*UAS-tj*^{RNAi}) during migration using the *slbo-Gal4* line, which is strongly active in rosette cells (Rørth et al., 1998). *tj* RNAi was effective, as Tj protein was reduced to background levels even in early stage 9 rosette cells (compare Fig. 2A-A″ with 2B-B″). To examine the effect of *tj* knockdown on BCC migration, we determined the BCC migration index at four different stages (mid and late stage 9, stages 10a and 10b) (see Materials and methods) (Fig. 2G). The migration index was significantly lower after *tj* knockdown compared with the control at all four stages (Fig. 2E,G). In comparison with wild-type BCCs that would have reached the oocyte by stage 10a, the majority of BCCs with *tj* knockdown had reached the oocyte only by stage 10b. However, although delayed by several hours, most BCCs (66.3%, *n*=116) were eventually able to reach the oocyte. Removing one wild-type copy of *tj* only slightly enhanced the migration defect in response to *tj* RNAi (Fig. 2G), indicating the robustness of the RNAi effect. A significant delay in BCC migration was also observed in a hypomorphic *tj* mutant (tj^{eo2}/tj^{39}), in which BCCs could form (Fig. 2G). Our data indicate that a knockdown of *tj* in rosette cells causes a significant delay in BCC migration.

To determine whether the natural reduction of Tj protein is necessary for BCC migration, we overexpressed full-length Tj [*UAS-tj*$^{6(3)}$] in rosette cells (Fig. 2C-C″). Analysis of the migration index indicates that *tj* overexpression (Fig. 2F,H) impairs migration more severely than *tj* knockdown (Fig. 2E,G). At mid or late stage 9 when most control BCCs were undergoing migration, almost all *tj*-overexpressing BCCs failed to migrate (Fig. 2H). Several BCCs appeared to have moved away from the anterior tip of the follicle but were unable to detach from the epithelium. Even at stage 10b, very few BCCs (5.3%, *n*=113) had reached the oocyte. Moreover, in mosaic BCCs, Tj-overexpressing rosette cells were usually found at the trailing end of the BCC (94%, *n*=32; Fig. 3G-H; Fig. 5G-H″). Our data suggest that the endogenous downregulation of *tj* in rosette cells is necessary for BCC migration.

Tj functions upstream of Slbo to control BCC migration

Based on the importance of Slbo (Montell et al., 1992) and Tj as regulators of BCC migration, we asked whether they have a functional relationship. In control BCCs, Slbo is expressed at similar levels in rosette and polar cells throughout migration (Fig. 3A,A′,D,D′,J). Both knockdown and overexpression of *tj* (using *slbo-Gal4*) caused a significant reduction of Slbo in rosette cells even by early stage 9 (Fig. 3B-C′,E-F′). Signal quantification indicates a reduction of Slbo by 50% in both genotypes compared with the control (Fig. 3J). Inducing Tj overexpression in cell clones before BCC formation caused Slbo expression to be undetectable in rosette cells (Fig. 3G-I). *In situ* hybridization suggested that altered *tj* expression affects *slbo* at the mRNA level (supplementary material Fig. S2A-C). This was corroborated by microarray analysis

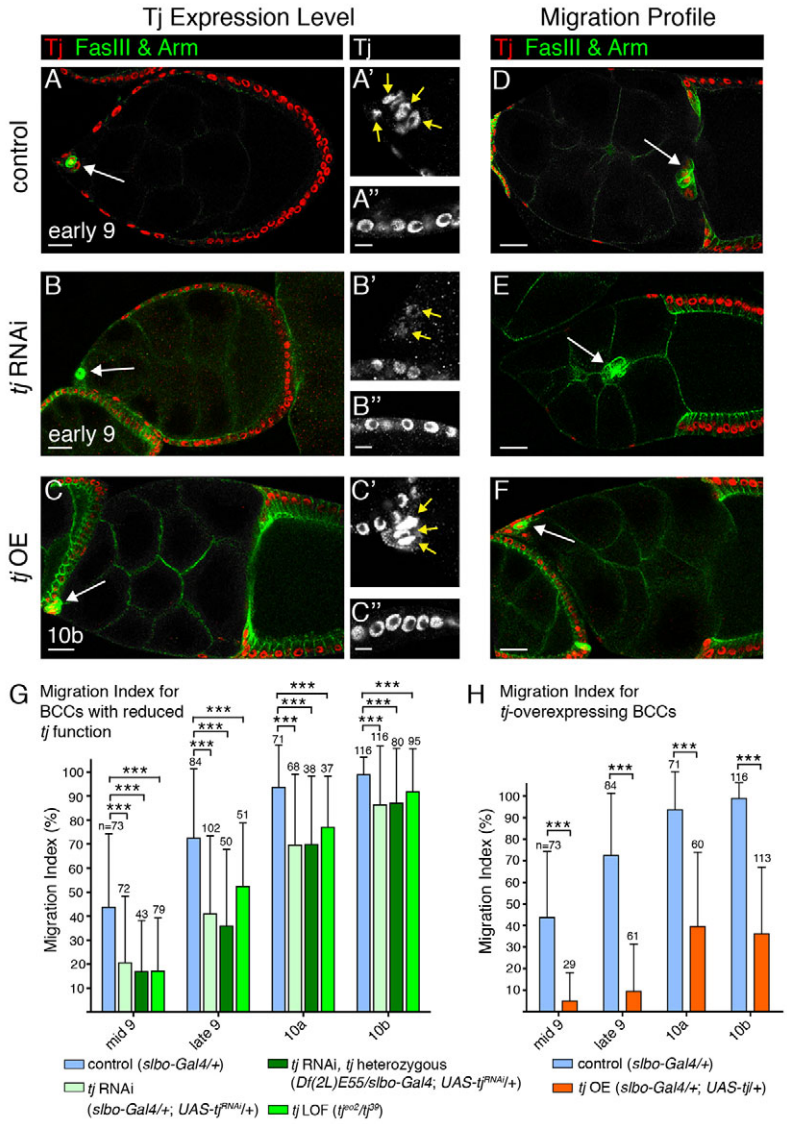

Fig. 2. *tj* knockdown and *tj* overexpression cause significant delays in BCC migration. (A-F) The white arrows point to BCCs. (**A-C″**) Tj expression level in BCCs (magnified in A′-C′) and MBFCs (magnified in A″-C″). Compared with the control (A′), Tj signal intensity in rosette cells (yellow arrows) is reduced in response to *tj* RNAi (B′), and increased after *tj* overexpression (OE) (C′). Tj signal intensity in MBFCs remains unchanged (A″-C″). (**D-F**) BCC migration profiles at stage 10. Compared with the control (D), *tj* RNAi (E) and *tj* OE (F) cause a significant delay in BCC migration. (**G**,**H**) Migration indices of BCCs, comparing the control to three genotypes with reduced Tj function (G) or increased amount of Tj (H) at stages 9 and 10. Graphs show mean + s.d. of the migration index; *n*, number of BCCs evaluated. ***P<0.001. Scale bars: 20 μm in A-F; 10 μm in A′-C″. LOF, loss of function.

using RNA isolated from border cells, showing a reduction of *slbo* mRNA by 28% when *tj* was knocked down and 46% when *tj* was overexpressed (Fig. 3K, data not shown). These data suggest that the endogenous low level of Tj is important for normal transcriptional activity of *slbo*.

The *slbo* enhancer reporter *slbo2.6-lacZ* is active in rosette and centripetal cells (Rørth et al., 1998) (supplementary material Fig. S2D,D′). Interestingly, *slbo-lacZ* activity in rosette cells seemed noticeably reduced when *tj* was knocked down (supplementary material Fig. S2E,E′), but did not appear to change compared to the control when *tj* was overexpressed (supplementary material Fig. S2F,F′). This suggests that Tj may activate *slbo* expression through this 2.6 kb enhancer element but that Tj inhibits *slbo* expression through a different molecular mechanism.

To confirm that Tj acts upstream of Slbo, we tested whether *slbo* overexpression can rescue the BCC migration defect caused by altered *tj* expression. Expression of exogenous Slbo (*UAS-slbo*) in rosette cells fully rescued the migration defect caused by *tj* knockdown (Fig. 4A,E) and partially restored the ability of Tj-overexpressing BCCs to migrate (Fig. 4B,F). Our results indicate that the regulation of *slbo* by Tj in rosette cells is important for BCC migration. Strikingly, combined *tj* knockdown and *slbo* overexpression could induce precocious BCC migration. This was not observed for BCCs that only overexpress Slbo (Jang et al., 2009; Rørth et al., 2000; Starz-Gaiano et al., 2008) (our own observations). Some of those BCCs with reduced Tj and elevated Slbo were able to complete migration at early-to-mid stage 9, when BCCs have normally only begun migration (Fig. 4G,H). This suggests that a combination of *tj* downregulation and *slbo* activation can induce BCCs to migrate earlier and/or faster than normal.

To test whether *tj* downregulation can enable BCC migration independently of Slbo, we examined whether altering *tj* expression modified the BCC migration defect observed in a hypomorphic *slbo* mutant. *slbo*^{1} mutant BCCs display a severe delay in BCC migration (Montell et al., 1992), but 30% and 70% of BCCs had moved away from the anterior tip of the follicle by stage 10b and stage 11/12, respectively (supplementary material Fig. S3A,D). When *tj* was knocked down in *slbo*^{1} mutant BCCs, the percentage of BCCs that showed signs of movement at stage 10b and stage 11/12 was reduced to 10% and 30%, respectively (supplementary material Fig. S3B,D). Overexpression of *tj* in the *slbo*^{1} mutant background caused complete blockage of BCC migration, even by stage 12 (supplementary material Fig. S3C,D). Thus, both changes in *tj* expression exacerbated the BCC migration delay in the *slbo* mutant,

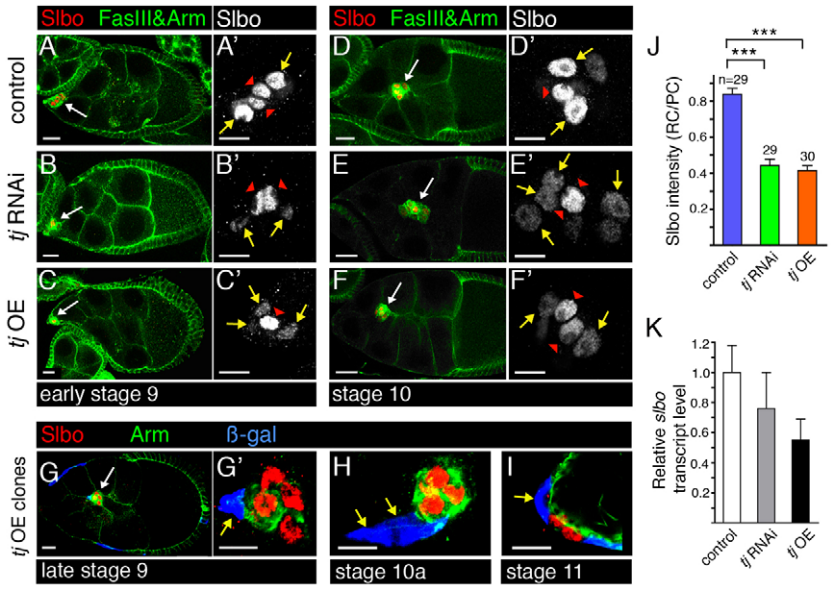

Fig. 3. Loss or increase of Tj causes a reduction in Slbo expression. (**A-F′**) Slbo expression in BCCs (white arrows) at early stage 9 (A-C) and stage 10 (D-F). (A′-F′) Close-ups of BCCs. The red arrowheads highlight polar cells, the yellow arrows mark rosette cells. (A,D) In control BCCs, the Slbo signal is comparable between polar and rosette cells. In *tj*-RNAi-treated (B,E) or *tj*-overexpressing (OE) BCCs (C,F), the Slbo signal is strongly reduced in rosette cells compared with polar cells. (**G-I**) Mosaic BCCs contain *tj*-OE cells that are labeled with β-gal (yellow arrows). Slbo is undetectable in *tj*-OE rosette cells, but present in neighboring wild-type cells. (**J**) Average Slbo signal intensity in the nuclei of rosette cells relative to that of polar cells per BCC. The histogram shows mean + s.e.m.; *n*, number of BCCs examined. ***P<0.001. (**K**) Relative *slbo* transcript levels based on three biological replicas per genotype, as quantified by microarray analysis (mean + s.e.m.). Scale bars: 20 μm in A-G; 10 μm in A′-G′,H,I. PC, polar cells; RC, rosette cells.

possibly due to further reduction of the already low amount of Slbo. This result shows that reduction of Tj expression is not sufficient to drive BCC migration in the absence of Slbo.

Tj negatively regulates DEcad expression in the BCC

Slbo upregulates DEcad expression in the BCC, which was found to be essential for migration (Niewiadomska et al., 1999; Mathieu et al., 2007). As Tj modulates *slbo* expression, one would expect Tj also to have an effect on DEcad. We examined the activation of a *shg* enhancer reporter (*shg1.3-lacZ* that contains putative Slbo-binding sites; Mathieu et al., 2007) in the background of *tj* knockdown and overexpression. In control follicles, this *shg-lacZ* is strongly active in rosette cells, weakly in centripetal cells, and not detected in MBFCs (Mathieu et al., 2007) (Fig. 5D,D′, Fig. 6E,E′). *shg-lacZ* expression was reduced moderately when *tj* was knocked down and more strongly when *tj* was overexpressed (Fig. 5E-F′). Consistent with these data, DEcad protein levels were visibly reduced under both conditions (Fig. 5A-C′). This reduction is particularly evident in Tj-overexpressing rosette cells of mosaic BCCs (Fig. 5G-H″). These findings are consistent with a Slbo-dependent function of Tj in the regulation of DEcad.

To confirm that the Slbo-dependent increase in DEcad is an important downstream process of Tj activity, we asked whether an induced increase of DEcad could rescue the *tj* mutant effects on BCC

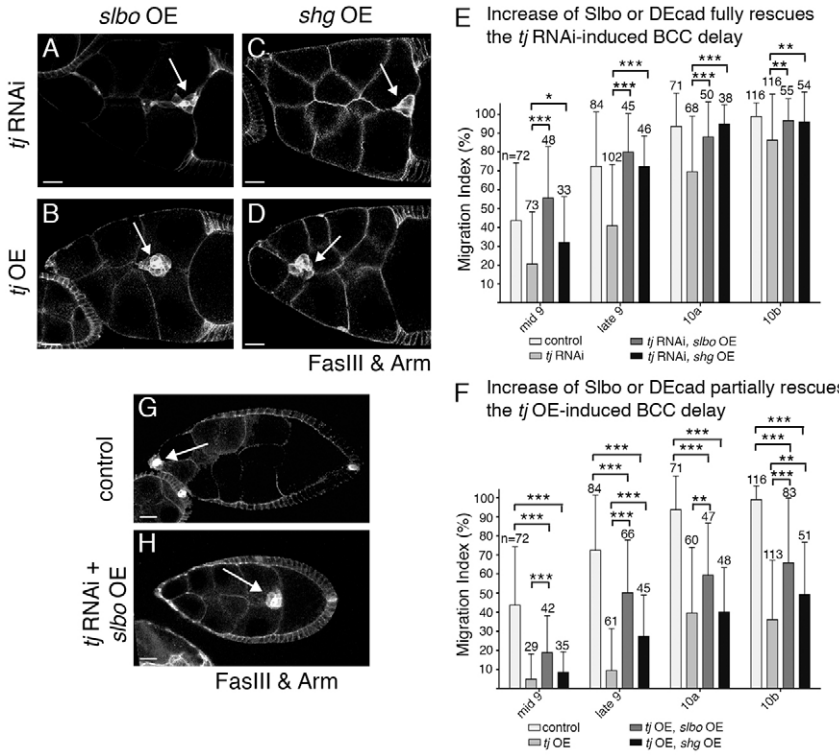

Fig. 4. The BCC migration delay caused by *tj* knockdown or overexpression is rescued by restoration of Slbo or DEcad expression. (**A-D**) Migration profiles of BCCs at stage 10b. The BCC expressing *tj*-RNAi and either exogenous *slbo* (*slbo* OE) (A) or *shg* (*shg* OE) (C) has completed migration. The BCC overexpressing *tj* (*tj* OE) and expressing either exogenous *slbo* (B) or *shg* (D) has undergone partial migration. (**E**,**F**) Migration indices of BCCs that have exogenous *slbo* or *shg* expression combined with either *tj* RNAi (E) or *tj* OE (F). Graphs show means + s.d.; *n*, number of BCCs examined. *P<0.05; **P<0.01; ***P<0.001. (**G**,**H**) BCCs with reduced Tj and increased Slbo expression show precocious migration. In comparison to the control BCC that is still at the anterior tip of the follicle (G), the BCC expressing *tj*-RNAi and exogenous *slbo* has already completed migration at early-to-mid stage 9 (H). Scale bars: 20 μm.

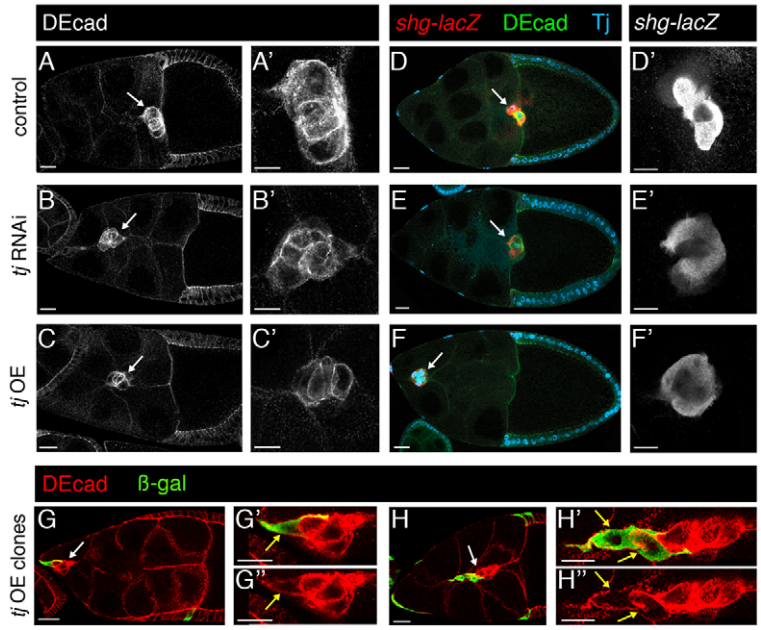

Fig. 5. Abnormally low or high Tj expression causes a decrease in DEcad expression and *shg* enhancer activity. (A-F) Arrows point to BCCs (magnified in A′-F′). (**A-C′**) Contrary to the control (A), DEcad does not appear enriched in the BCC when Tj is reduced (*tj* RNAi) (B) and appears very weak when Tj is overexpressed (OE) (C). (**D-F′**) Similarly, contrary to the control, *shg-lacZ* signal is weak when Tj is reduced (E) or overexpressed (F). (**G-H″**) Mosaic BCCs (white arrows, magnified in G′-H″). Tj-overexpressing rosette cells (yellow arrows), identified by β-gal (G′,H′), display significantly lower DEcad expression compared with their wild-type neighbors (G″,H″), and are located at the trailing edge of the BCC at stages 9 (G) and 10 (H). Scale bars: 20 μm in A-H; 10 μm in A′-H″.

migration. Exogenous DEcad expression (using *UAS-shg*) fully rescued the moderate migration delay caused by *tj* knockdown (Fig. 4C,E) and partially rescued the severe delay induced by *tj* overexpression (Fig. 4D,F). Although DEcad upregulation completely rescued the *tj* knockdown phenotype, it did not induce precocious BCC migration, in contrast to *slbo* overexpression. In addition, DEcad upregulation was not as effective as *slbo* overexpression in rescuing the *tj* overexpression phenotype (Fig. 4F). Our data suggest that the upregulation of DEcad is not the only motility-enabling process that is dependent on Slbo, and in turn on Tj.

We previously showed that Tj modulates DEcad expression in MBFCs that do not contain Slbo (Li et al., 2003), raising the question of whether Tj might also regulate *shg* expression independently of Slbo in the BCC. We tested whether *slbo* overexpression can rescue the Tj-mediated effect on *shg*. We first looked at *shg-lacZ* expression in *slbo*-overexpressing MBFCs and BCCs (excluding rosette cells that were left behind) without changing Tj expression. Surprisingly, although Slbo expression is crucial for *shg* upregulation, excessive amounts of Slbo attenuated *shg-lacZ* expression in the BCC (Fig. 6F,F′). However, MBFCs that normally contain minimal Slbo began to express *shg-lacZ* in response to *slbo* overexpression (Fig. 6F,F″). This indicates that Slbo needs to be present at the proper concentration to activate the *shg* enhancer. Interestingly, *shg-lacZ* expression was substantially

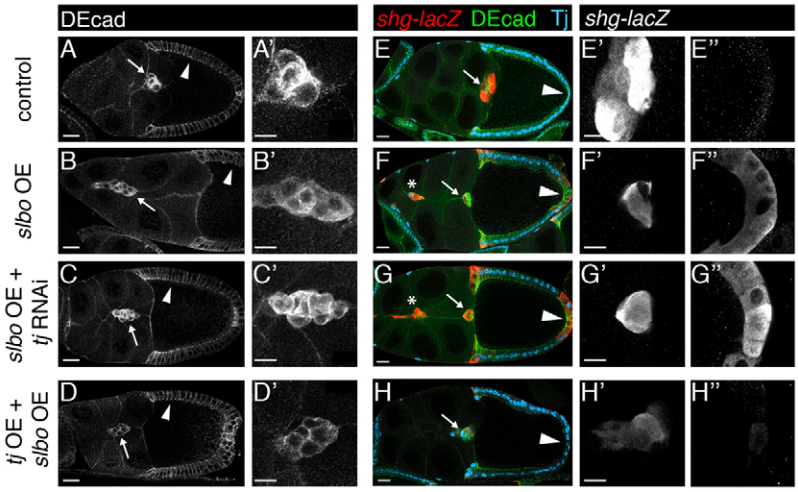

Fig. 6. High Tj expression inhibits DEcad upregulation even in the presence of Slbo. (**A-D′**) DEcad expression in BCCs (arrows, magnified in A′-D′). (A) In a control follicle, DEcad expression is considerably higher in the BCC than in MBFCs (arrowheads). Enrichment of DEcad in the BCC is less prominent when Slbo is overexpressed (OE) (B), is normal when Slbo is overexpressed and Tj is reduced (*tj* RNAi) (C), and is abolished when both Slbo and Tj are overexpressed (D). (**E-H″**) *shg* enhancer (*shg-lacZ*) activity in BCCs (arrows, magnified in E′-H′) and MBFCs (arrowheads, magnified in E″-H″). In the control, *shg-lacZ* is strong in the BCC, but undetectable in MBFCs (E-E″). Note that *shg-lacZ* signal is variable between cells. On average, when *slbo* is overexpressed alone, *shg-lacZ* appears weak in the BCC, but is ectopically present in MBFCs (F-F″), whereas *slbo* overexpression together with *tj* reduction causes a strong *shg-lacZ* signal in both the BCC and MBFCs (G-G″). For reasons unknown, rosette cells that are not part of the main BCC due to fragmentation (asterisks) display a stronger *shg-lacZ* signal compared with the cells within the cluster (F,G). When *tj* and *slbo* are both overexpressed, *shg-lacZ* is very weak in the BCC and undetectable in MBFCs (H-H″). Scale bars: 20 μm in A-H; 10 μm in A′-H″.

stronger in both the BCC and MBFCs when *tj* was knocked down in the background of *slbo* overexpression (Fig. 6G-G″). By contrast, *shg-lacZ* expression was strongly reduced in the BCC (Fig. 6H,H′) and undetectable in MBFCs (Fig. 6H,H″) when both *tj* and *slbo* were co-overexpressed. Analysis of DEcad protein levels yielded similar results (Fig. 6A-D′). Our data suggest that high amounts of Tj suppress activation of the *shg* enhancer. In summary, the analysis of DEcad expression in BCCs with varying Tj and/or Slbo concentrations indicates that Tj has both Slbo-dependent and -independent effects on DEcad.

The natural decrease in Tj expression in BCCs is mediated through Slbo

Our analysis indicates that the downregulation of Tj starts at the onset of BCC migration when Slbo becomes highly active, raising the question of whether Tj downregulation depends on Slbo. In contrast to the control, Tj protein was found not to be properly reduced in *slbo*^{1} mutant rosette cells, when BCCs were analyzed either according to stage (stage 10b; Fig. 7A-B″,E) or migration index (Fig. 7F). A similar effect was observed for *tj* mRNA (data not shown). *slbo* overexpression in rosette cells had the opposite effect, causing a stronger than normal reduction of Tj at early stage 9 (Fig. 7C-D″,G). These observations suggest that Slbo is needed for the downregulation of *tj* at the onset of BCC migration.

Because the initial reduction of Tj seems to also coincide with the activation of Jak/Stat signaling in BCCs, we studied whether this pathway played a role in regulating Tj expression either through or independently of Slbo. We induced ectopic activity of Hop in anterior follicle cells (*c306-Gal4 UAS-hop3*), which causes the formation and migration of additional border cells (Silver and Montell, 2001). Similar to endogenous border cells, these extra migratory cells showed progressive decrease in the amount of Tj protein (supplementary material Fig. S4A). This suggests that the Jak/Stat pathway at least indirectly acts upstream of Tj. To test whether it downregulates Tj independently of Slbo, we overexpressed Hop in *slbo*^{1} mutant rosette cells and examined Tj expression levels. Tj protein did not appear reduced in these cells (supplementary material Fig. S4B-B″), suggesting that overactivation of the Jak/Stat pathway is not sufficient to cause a decrease of Tj in the absence of Slbo. We conclude that the Jak/Stat pathway regulates *tj* through Slbo.

Tj appears to act through Socs36E to inhibit *slbo* expression

As the Jak/Stat pathway is important not only during BCC formation but also migration (Silver et al., 2005), we examined whether changing Tj expression affected the Jak/Stat pathway. We utilized a *Socs36E-GFP* enhancer reporter that had previously been used to monitor Jak/Stat activity (Bach et al., 2007). In control follicles (stages 9 to 10), *Socs36E-GFP* expression was strong in rosette cells and weak in centripetal cells and posterior MBFCs (Fig. 8A,A′; supplementary material Fig. S5A,G,G′). Inducing *tj* overexpression in these three cell types led to a striking overactivation of *Socs36E-GFP* (Fig. 8B,B′; supplementary material Fig. S5B). The increase in *Socs36E* expression seen in Tj-overexpressing rosette cells was confirmed with another *Socs36E* enhancer reporter line (*Socs36E-lacZ*) (supplementary material Fig. S5E,F). Although *Socs36E-GFP* expression did not noticeably change when *tj* was knocked down during migration (with *slbo-Gal4*; Fig. 8C,C′), it did appear to be weaker in rosette cells when *tj* was reduced in follicle cells before stage 8 (with *c306-Gal4*; supplementary material Fig. S5G-H′). Taken together, these observations suggest that Tj can enhance *Socs36E* expression.

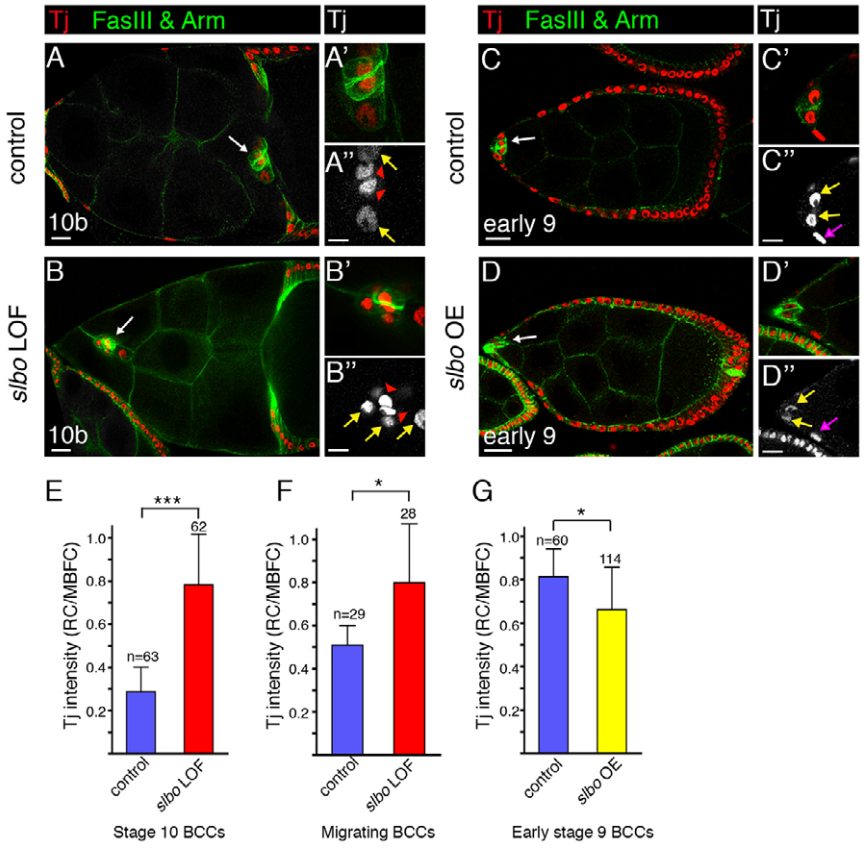

Fig. 7. Reduction of Tj expression is mediated by Slbo. (**A-D″**) Tj expression in BCCs (white arrows). Yellow arrows point to rosette cells (A″-D″), FasIII (A′-D′) or red arrowheads (A″,B″) to polar cells, and pink arrows to neighboring anterior follicle cells (C″,D″). (A-B) Migrating BCCs show weaker Tj expression in rosette than in polar cells in the control (A-A″), but similar Tj expression in both cell types in a *slbo*^{1} loss-of-function mutant (B-B″). (C,D) In pre-migratory BCCs, Tj expression is similar in rosette and anterior follicle cells in the control (C-C″), but reduced in rosette cells when *slbo* is overexpressed (OE) (D-D″). (**E-G**) Measurement of Tj signal intensity in nuclei of *slbo* mutant or *slbo*-overexpressing rosette cells, compared to MBFCs. For *slbo* mutant BCCs, Tj signal intensity was measured in stage 10 follicles (E) or in BCCs with a migration index of 25-50% (F). For *slbo*-overexpressing BCCs, Tj intensity was measured in early stage 9 follicles (G). Graphs show mean + s.d. Each sample size (*n*) represents equal numbers of rosette cells and MBFCs. **P*<0.05; ****P*<0.001. Scale bars: 20 μm in A-D; 10 μm in A′-D″. LOF, loss of function; RC, rosette cells.

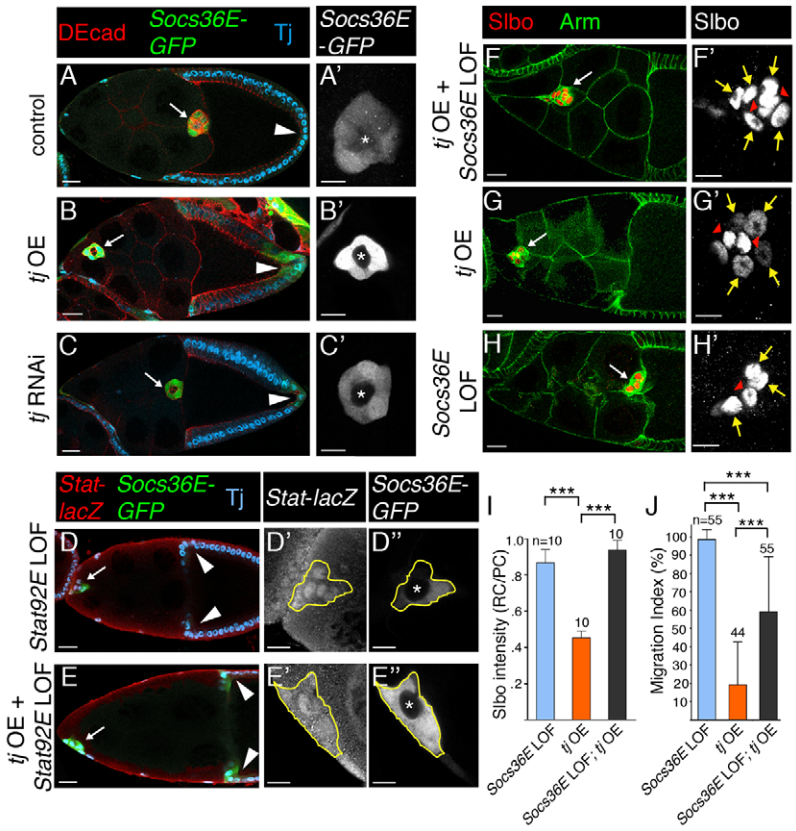

Fig. 8. Tj overexpression causes reduction of Slbo through a Stat92E-independent increase of *Socs36E* expression. (A-H) White arrows point to BCCs. (**A-C′**) *Socs36E* enhancer activity (*Socs36E-GFP*) is weak in rosette cells (magnified in A′-C′) of a control (A) or *tj*-RNAi treated BCC (C), but is present prominently in rosette cells and ectopically in posterior MBFCs (arrowheads) in a *tj*-overexpressing (OE) BCC (B). (**D-E″**) *Stat92E-lacZ* expression is similar in *Stat92E* LOF ($Stat92E^{st}/Stat92E^{06346}$ at restrictive temperature) (D,D′) and *Stat92E* LOF in combination with *tj* OE (E,E′). By contrast, *Socs36E-GFP* signal is strongly increased in the BCC and ectopically present in centripetal cells (arrowheads) in *Stat92E* LOF with *tj* OE (E-E″) compared with *Stat92E* LOF (D-D″). Note that *Socs36E-GFP* is absent in polar cells (asterisks). (**F-H′**) In BCCs with Tj OE and *Socs36E* LOF [$Socs36E^{PZ1647}/Df(2L)Exel7070$], Slbo expression is equally strong in polar (red arrowheads) and rosette cells (yellow arrows) (F,F′), in contrast to BCCs with Tj OE alone (G,G′). Slbo expression is normal in *Socs36E* LOF (H,H′). (**I**,**J**) Significantly higher Slbo expression in rosette cells compared with polar cells (I) and a higher migration index in stage 10 follicles (J) are observed when Tj OE is combined with loss of Socs36E function compared with Tj OE alone. Graphs show mean + s.d.; *n*, number of BCCs evaluated. ***$P<0.001$. Scale bars: 20 μm in A-H; 10 μm in A′-H″. LOF, loss of function.

To test whether Tj might increase *Socs36E* levels by enhancing *Stat92E* expression, we examined the activity of the *Socs36E* enhancer reporter in a *Stat92E* mutant background ($Stat92E^{ts}/Stat92E^{06346}$ at the restrictive temperature). As expected, when Tj was unaltered, the expression of *Socs36E-GFP* was weak in the *Stat92E* mutant BCC (Fig. 8D,D″). Interestingly, when Tj was overexpressed in the *Stat92E* mutant, *Socs36E-GFP* expression appeared considerably enhanced in rosette and centripetal cells, and posterior MBFCs (Fig. 8E,E″), similar to the effect of Tj overexpression in a wild-type background (Fig. 8B,B′; supplementary material Fig. S5B). However, we did not observe an obvious increase in the activity of a *Stat92E* enhancer reporter (Fig. 8D′,E′) in any of these cell types. In addition, Tj overexpression did not cause a detectable increase in Stat92E protein level in the rosette or centripetal cells (supplementary material Fig. S5C-D′). Taken together, these results suggest that Tj can enhance *Socs36E* expression independently of Stat92E.

As Socs36E antagonizes the Jak/Stat pathway (Rawlings et al., 2004; Baeg et al., 2005), which normally activates *slbo* (Silver and Montell, 2001), we asked whether Tj overexpression might reduce Slbo levels through a Socs36E-mediated inhibition of Jak/Stat activity. If increased *Socs36E* expression is responsible for the observed decrease in Slbo, a reduction of *Socs36E* function [$Socs36E^{PZ1647}/Df(2L)Exel7070$] in the background of Tj overexpression would be expected to restore Slbo. Indeed, Slbo expression returned to control levels under this condition (Fig. 8F-I), and the motility of the BCCs was rescued to a degree similar to that seen when Slbo was directly co-overexpressed with Tj (compare Fig. 8J with Fig. 4F). Our data suggest that high amounts of Tj interfere with BCC migration by enhancing Socs36E expression, which in turn reduces Slbo.

DISCUSSION

Our findings indicate that Tj is an important component of the molecular network that controls BCC migration. Tj interacts with at least three regulators of BCC motility to coordinate proper migratory behavior of rosette cells (Fig. 9). Tj functions in: (1) maintaining proper Slbo expression; (2) limiting expression of DEcad; and (3) enhancing expression of Socs36E, which in turn presumably restricts the Jak/Stat pathway. In addition to regulating cell motility, Tj influences the correct temporal initiation of BCC migration.

The bZip transcription factors Tj and Slbo seem to act in a feedback loop to keep each other's expression in balance during migration. The endogenous low amount of Tj is needed to sustain Slbo, as too much or too little Tj leads to Slbo reduction. Tj seems to activate *slbo* transcription either directly or indirectly, and to reduce Slbo by enhancing expression of the Jak/Stat antagonist Socs36E. Previous analysis revealed that Slbo is regulated at the post-translational level through ubiquitin-mediated proteolysis (Rørth et al., 2000). Our results here indicate an additional layer of Slbo regulation at the transcriptional level, emphasizing the importance of having the appropriate amount of Slbo to enable normal BCC migration. Interestingly, Slbo expression appears not to be completely dependent on Jak/Stat activity once the BCC has formed and initiated migration (Beccari et al., 2002; Silver et al., 2005). Our results suggest that Tj is a key factor that maintains Slbo expression during BCC migration, probably in conjunction with the Jak/Stat signaling pathway.

Similar to Slbo, Tj is needed at a particular expression level to enable cell motility. Not only loss but also excess of Tj has a negative impact on BCC migration. This suggests that limiting Tj expression is necessary for migration, a process that appears to be mediated by Slbo. Whether Slbo inhibits Tj expression through

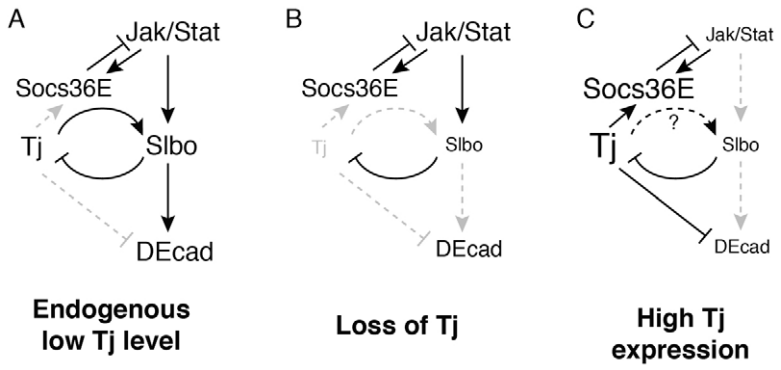

Fig. 9. Model of Tj function in conferring cell motility. (**A**) Normally, in migrating border cells, Tj and Slbo engage in a feedback loop, which leads to a relatively low expression of Tj that keeps Slbo and subsequently DEcad expression at the proper levels. At this expression level, Tj seems not to upregulate the Jak/Stat inhibitor Socs36E or inhibit DEcad expression. This regulatory network promotes cell motility. (**B**) Loss of Tj leads to reduced Slbo expression, which in turn prevents DEcad upregulation. This reduces cell motility. (**C**) High Tj expression enhances the expression of Socs36E, which probably inhibits the Jak/Stat pathway and consequently Slbo. High Tj also prevents the upregulation of DEcad expression. This severely impairs motility. (A-C) Black solid lines indicate active interactions; gray broken lines indicate inactive interactions.

transcriptional repression or a more indirect mechanism awaits further investigation. We conclude that the mutual regulation of Tj and Slbo is essential to confer normal BCC motility.

Our study here uncovers a feedback loop between a large Maf (Tj) and a C/EBP factor (Slbo), expanding our insight into the interactions between these types of transcription factors. It was previously shown that C/EBPβ regulates the expression of MafB in mouse osteoclasts (Smink et al., 2009). In several mammalian tissues, such as ovaries (Pall et al., 1997), liver (Sakai et al., 1997; Akira et al., 1990) and kidney (Sadl et al., 2002; Alam et al., 1992), C/EBP and large Maf factors have similar expression patterns, raising the possibility that the observed cross-regulatory interactions between Tj and Slbo are conserved.

A substantial increase in DEcad in rosette cells is essential for BCC migration (Niewiadomska et al., 1999). Our findings indicate that Tj has both positive and negative effects on DEcad expression. The rise in DEcad levels is dependent on Slbo (Niewiadomska et al., 1999; Mathieu et al., 2007), and therefore indirectly on Tj. The Tj-mediated negative impact on DEcad became apparent when high Tj expression still prevented the upregulation of *shg* (which encodes DEcad), even in the presence of exogenous Slbo. Given that both Tj and Slbo are bZip proteins, Tj may dimerize with Slbo, preventing it from binding to the *shg* enhancer. Notably, Tj homologs c-Maf and MafB have been shown to prevent other transcription factors from activating their targets by physically binding to them (Sieweke et al., 1996; Hegde et al., 1998). Alternatively, Tj may inhibit *shg* upregulation through transcriptional repression. We previously found that loss of Tj function leads to stronger expression of *shg* in follicle cells (Li et al., 2003). The absence of Slbo in these cells suggests that Tj can repress *shg* independently of Slbo.

Our findings further indicate that Tj enhances the expression of the Jak/Stat antagonist Socs36E, which in turn reduces Slbo. This effect on Slbo is probably mediated through the Jak/Stat pathway, as Socs36E is a well-known inhibitor of Jak/Stat signaling in multiple tissues, including the follicular epithelium (Callus and Mathey-Prevot, 2002; Rawlings et al., 2004; Issigonis et al., 2009; Singh et al., 2010; Tarayrah et al., 2013). Socs36E acts not only upstream but also downstream of Stat92E (Rawlings et al., 2004; Bach et al., 2007). Our data suggest that Tj affects *Socs36E* expression in a Stat92E-independent manner. In addition to Tj and Stat92E, the histone demethylase dUTX and the transcriptional regulator Apontic were recently found to upregulate *Socs36E* (Tarayrah et al., 2013; Monahan and Starz-Gaiano, 2013). It seems that *Socs36E* has various upstream activators but operates consistently to limit Jak/Stat pathway activity.

We propose the following model for achieving a balance between the factors that regulate BCC motility (Fig. 9). Before BCC formation, follicle cells have a high Tj expression level. This could prevent Slbo expression, block an increase of DEcad, and inhibit Stat92E activity by enhancing Socs36E expression (Fig. 9C). Through a not-yet-fully-understood mechanism, which involves the activation of ecdysone signaling (Jang et al., 2009), the Jak/Stat pathway becomes active, and induces the expression of Slbo in the developing BCC. We speculate that this initiation of Slbo expression triggers a shift in the balance of the factors that leads to a homeostatic feedback loop between Tj and Slbo. This feedback loop also keeps Socs36E activity and DEcad expression at levels that enable migration (Fig. 9A). Tj appears to function as a mediator that balances the activities within the motility regulating core pathway. In addition, Tj is involved in the temporal control of BCC migration, as too much Tj blocks initiation of migration, whereas too little Tj, in the presence of Slbo, drives precocious migration. We propose that the natural reduction of Tj is one of the important temporal cues needed to initiate BCC migration.

The BCC is a system that displays both the pro- and anti-migratory attributes of the large Maf transcription factor Tj, a contradiction that renders it necessary for Tj expression to be maintained at a balanced level. In vertebrates, large Maf factors have not been directly implicated in regulating cell migration during normal development. However, they were shown to be involved in oncogenesis (Eychène et al., 2008). Overactivation of large Mafs can lead to increase in metastasis-inducing factors and tumorous growth in certain tissues (Nishizawa et al., 1989; Hurt et al., 2004; Pouponnot et al., 2006; Morito et al., 2006). However, in other tissues, large Mafs have been shown to repress invasive cell behavior (Pouponnot et al., 2006; Watson et al., 2004). These findings support the view that large Mafs could have pro- and anti-metastatic properties. The role of Tj in fine-tuning cell motility might therefore be a conserved function of large Maf transcription factors.

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Competing interests statement
The authors declare no competing financial interests.

Author contributions
F.G. conducted the majority of the experiments; M.A. carried out the clonal analysis for *tj* overexpression; F.G., M.A. and D.G. designed and analyzed the

experiments; D.G. supervised the project and generated new reagents in collaboration with M.A. and technical staff; and F.G. and D.G. wrote the manuscript.

Supplementary material

Supplementary material available online at
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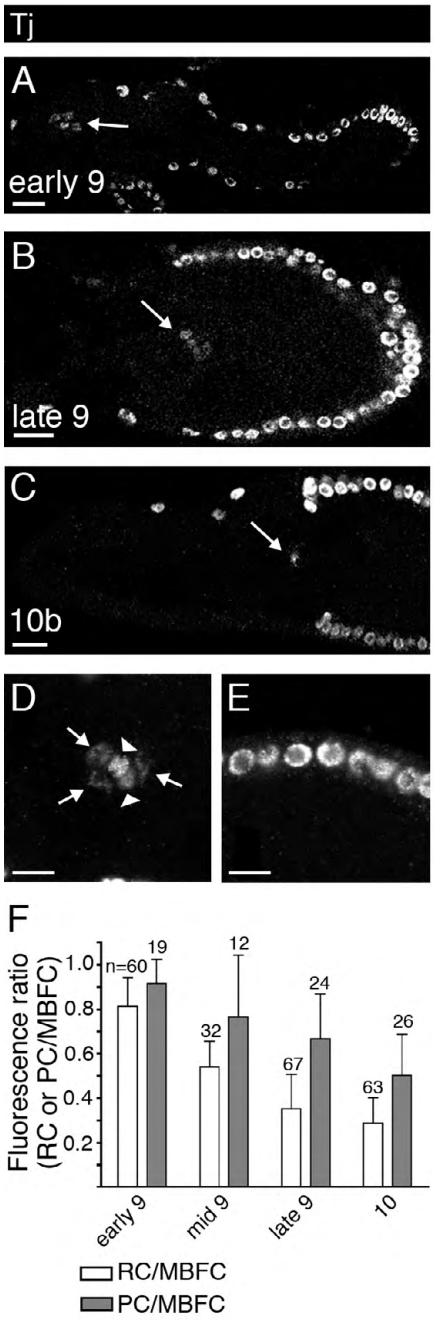

Fig. S1. Tj expression decreases in the BCC during migration. (**A-E**) Cryosections confirm the decrease of Tj expression during BCC migration. The long arrows arrows point to the BCC in A-C, the short arrows point to rosette cells and the arrowheads point to polar cells in D. Tj signal is considerably weaker in BCCs (A-D) than in MBFCs (A-C,E) throughout and after migration. (D) Tj expression appears weaker in rosette than in polar cells. Cryosections 15 µm thick were generated using a Leica CM3050S cryostat. (**F**) Measurement of Tj signal intensity shows a gradual decrease of Tj in both rosette and polar cells compared with MBFCs. The graph shows mean + s.d. Each sample size (*n*) represents equal numbers of rosette or polar cells and MBFCs. Scale bars: 20 µm in A-C; 10 µm in D,E. PC, polar cells; RC, rosette cells.

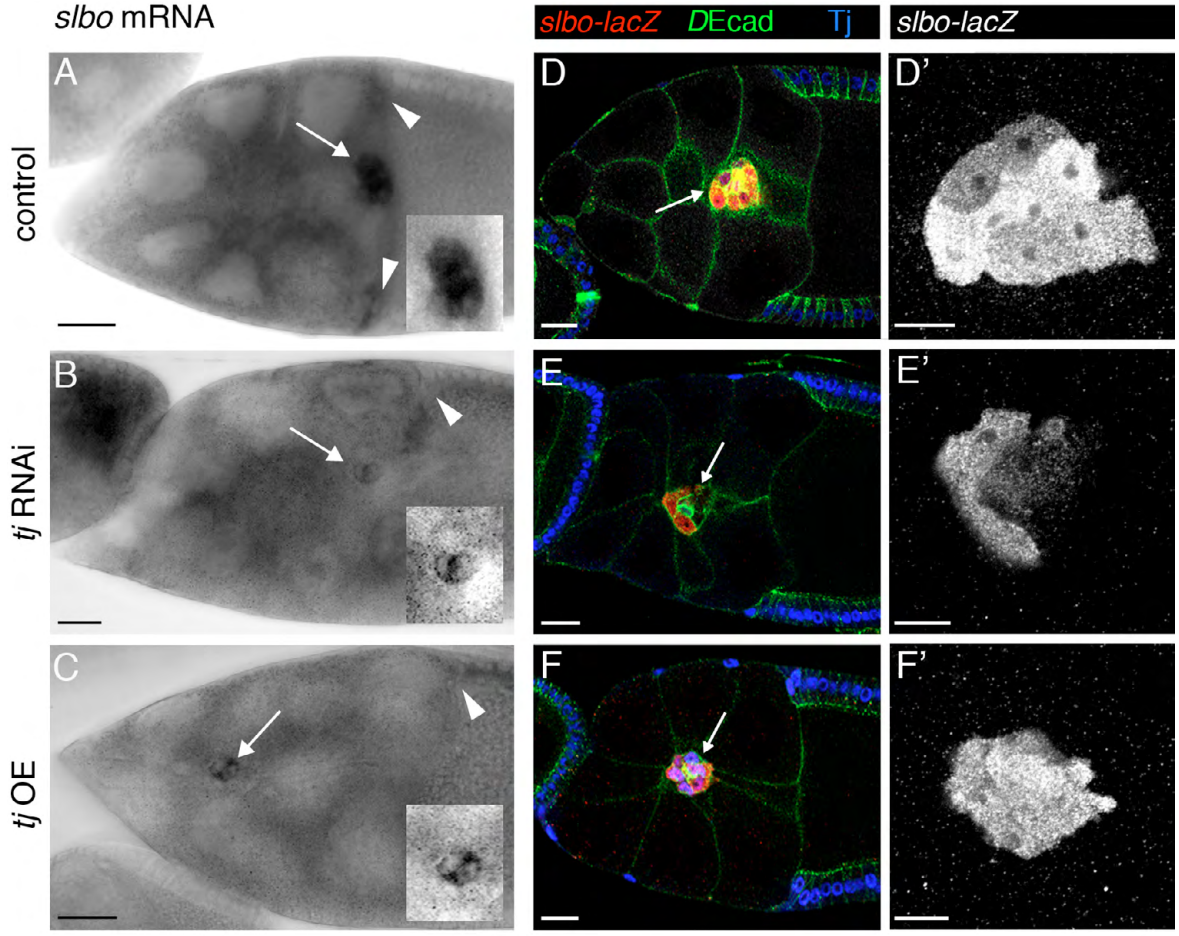

Fig. S2. Tj reduction or overexpression causes reduction of *slbo* mRNA, but only Tj reduction affects *slbo-lacZ* activity. (A-F) Stage 10 follicles. (**A-C**) *slbo* mRNA expression in BCCs (arrows, magnified and contrast-enhanced in insets) and centripetal cells (arrowheads). In comparison to the control follicle, where *slbo* mRNA is found in rosette, polar, and centripetal cells (A), *slbo* mRNA is only detected in polar cells, but not in rosette or centripetal cells in follicles with *tj* RNAi (B) or *tj* overexpression (OE) (C). (**D-F′**) *slbo* enhancer (*slbo-lacZ*) activity in BCCs (arrows; magnified in D′-F′). Although *slbo-lacZ* expression varies from cell to cell, compared to the control BCC (D), *slbo* enhancer expression appears reduced in BCCs with *tj* RNAi (E), but remains unchanged in BCCs with *tj* OE (F). Scale bars: 25 µm in A-C; 20 µm in D-F; 10 µm in D′-F′.

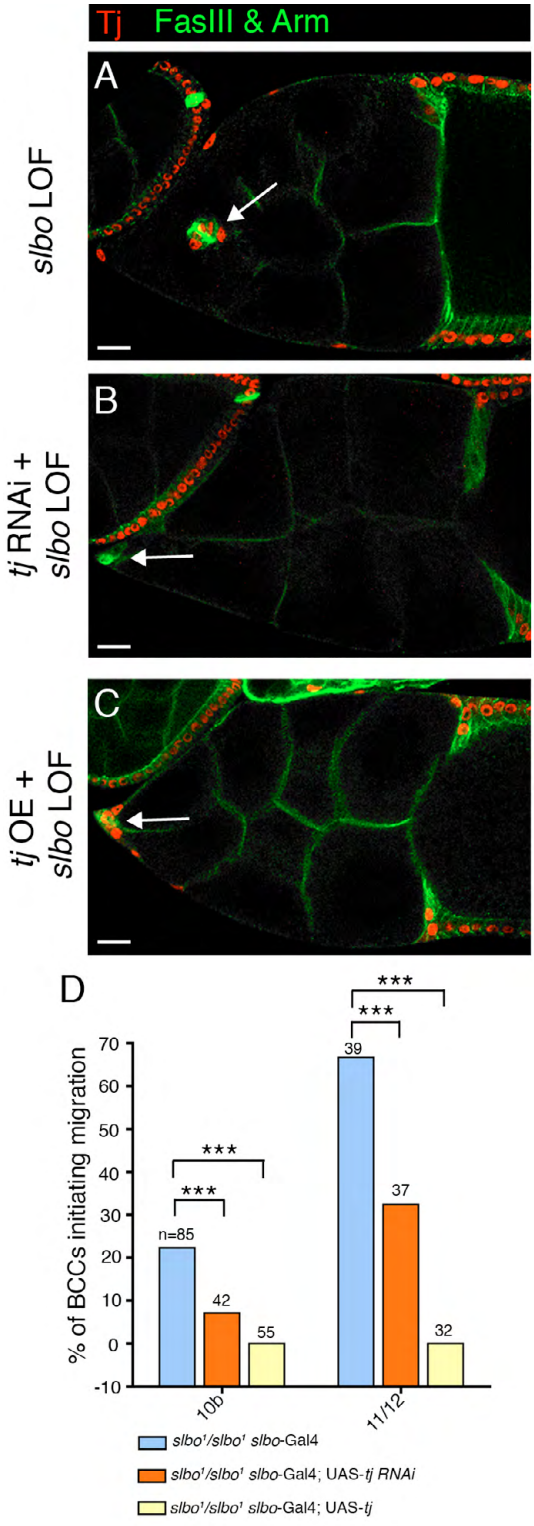

Fig. S3. Tj reduction or overexpression enhances the BCC migration defect of *slbo¹* mutants. (**A-C**) Late stage 10b follicles. Arrows points to BCCs. A *slbo¹* mutant BCC initiated migration (A). *slbo¹* mutant BCCs expressing *tj*-RNAi (B) or overexpressing Tj (C) failed to migrate. Note lack of Tj in B and strong Tj signal in C. (**D**) Quantification of the ability of BCCs to initiate migration in response to altered Tj expression in *slbo¹* mutant BCCs at indicated stages. The graph shows mean + s.d.; *n*, number of BCCs evaluated. ***P<0.001. Scale bars: 20 µm.

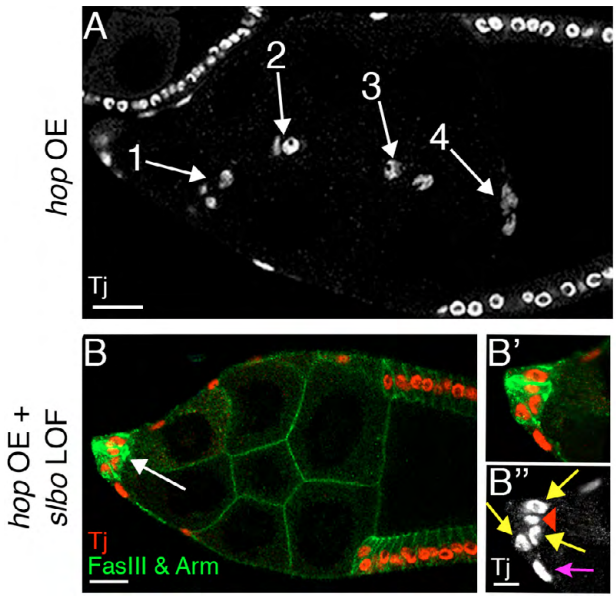

Fig. S4. The Jak/Stat pathway mediates Tj reduction in the BCC through Slbo. (**A**) In a Slbo-positive background, ectopic expression of Hop in anterior follicle cells (*c306-Gal4*/+; *UAS-hop3*/+) induces formation of extra migratory rosette cells (1-3), each of which has a reduced Tj level similar to the main BCC (4). (**B-B″**) By contrast, in a *slbo*^{1} mutant background, Tj signal in the BCC (B, arrow; magnified in B′,B″) does not appear reduced in rosette cells (yellow arrows) compared to polar (red arrowhead) and anterior follicle cells (pink arrow) even with Hop overexpression (*slbo*^{1}/*slbo*^{1} *slbo-Gal4*; *UAS-hop3*/+). Scale bars: 20 μm in A,B; 10 μm in B′,B″.

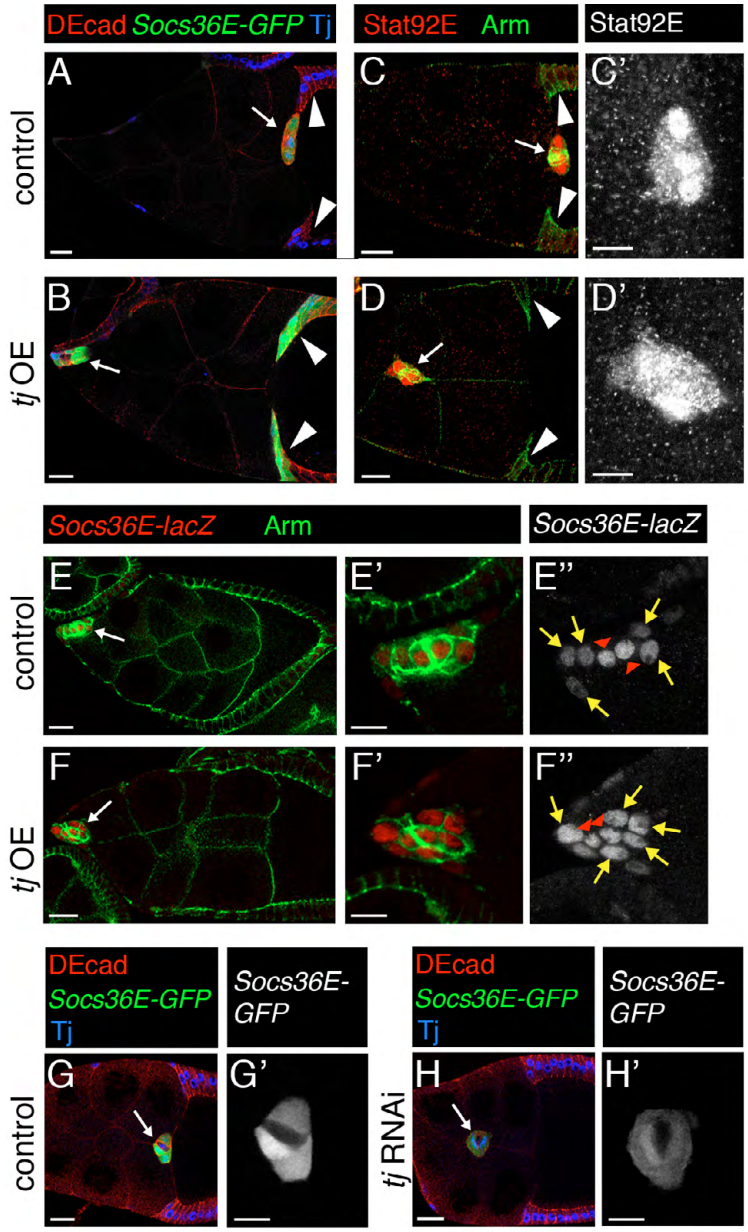

Fig. S5. Tj enhances expression of *Socs36E*, but not Stat92E. (A-H′) White arrows point to BCCs in stage 10 follicles (A-D,G-H) and in mid stage 9 follicles (E-F). (**A**,**B**) *Socs36E-GFP* expression is considerably stronger in the BCC and centripetal cells of a *tj*-overexpressing follicle (B) than the control (A). (**C-D**′) Stat92E expression appears similar in control (C,C′) and *tj*-overexpressing BCCs (D,D′). (**E-F**″) In contrast to the control, where polar cells (red arrowheads) show higher *Socs36E-lacZ* (*Socs36E*^{PZ1647}) expression than rosette cells (yellow arrows) (E-E″), Tj-overexpressing rosette cells express *Socs36E-lacZ* at a level comparable to polar cells (F-F″). (**G-H**′) *Socs36E-GFP* signal is considerably weaker in a BCC, in which Tj had been knocked down before BCC formation (*c306-Gal4*/+; *Socs36E-GFP*/+; *UAS-tj*^{RNAi}/+) (H,H′), than in a control BCC (*c306-Gal4/+; Socs36E-GFP/+*) (G,G′). Scale bars: 20 µm in A-H; 10 µm in C′-H′.