Slmb antagonises the aPKC/Par-6 complex to control oocyte and epithelial polarity

Eurico Morais-de-Sá*, Avik Mukherjee, Nick Lowe and Daniel St Johnston†

ABSTRACT
The Drosophila anterior-posterior axis is specified when the posterior follicle cells signal to polarise the oocyte, leading to the anterior/lateral localisation of the Par-6/aPKC complex and the posterior recruitment of Par-1, which induces a microtubule reorganisation that localises bicoid and oskar mRNAs. Here we show that oocyte polarity requires Slmb, the substrate specificity subunit of the SCF E3 ubiquitin ligase that targets proteins for degradation. The Par-6/aPKC complex is ectopically localised to the posterior of slmb mutant oocytes, and Par-1 and oskar mRNA are mislocalised. Slmb appears to play a related role in epithelial follicle cells, as large slmb mutant clones disrupt epithelial organisation, whereas small clones show an expansion of the apical domain, with increased accumulation of apical polarity factors at the apical cortex. The levels of aPKC and Par-6 are significantly increased in slmb mutants, whereas Baz is slightly reduced. Thus, Slmb may induce the polarisation of the anterior-posterior axis of the oocyte by targeting the Par-6/aPKC complex for degradation at the oocyte posterior. Consistent with this, overexpression of the aPKC antagonist Lgl strongly rescues the polarity defects of slmb mutant germine clones. The role of Slmb in oocyte polarity raises an intriguing parallel with C. elegans polarity defects of lgl [L(2)gl–FlyBase] might also play a redundant role in AP polarity in Drosophila, as it does in C. elegans, since it localises to a crescent at the posterior of the oocyte like Par-1, and lgl mutant germine clones show reduced Par-1 localisation although they show no obvious polarity phenotypes (Li et al., 2008; Tian and Deng, 2008). The cortical PAR proteins specify the AP axis by controlling the organisation of the oocyte microtubule cytoskeleton, so that...
microtubules are nucleated or anchored at the anterior and lateral cortex, but not at the posterior, where only plus ends are found (Cha et al., 2002; Clark et al., 1994, 1997; Theurkauf et al., 1992). This polarised microtubule network then directs the localisation of bicoid and oskar mRNAs to the anterior and posterior poles of the oocyte, respectively, where they define the AP axis of the embryo (Brendza et al., 2000; Weil et al., 2006; Zimyanin et al., 2008). How the PAR proteins organise the microtubules is unknown, but Par-1 seems to play a key role in this process by excluding microtubule minus ends from the posterior (Doerflinger et al., 2010). par-1 mutants therefore develop a symmetric microtubule cytoskeleton, in which minus ends are found all around the oocyte cortex with the plus ends and oskar mRNA concentrated in the centre (Shulman et al., 2000; Tomancak et al., 2000).

In order to identify missing components in Drosophila AP axis formation, we have performed a large-scale germline clone screen for mutants that disrupt the posterior localisation of GFP-Staufen, an RNA-binding protein that associates with oskar mRNA (Martin et al., 2003). Here, we report our analysis of a lethal complementation group from the screen that disrupts the earliest step in the AP polarisation of the oocyte.

RESULTS
slmb mutants disrupt oskar mRNA localisation

The wellman locus is a lethal complementation group of two alleles, wel8A6-5 and wel9H4-17, that was identified in the GFP-Staufen germline clone screen on chromosome 3R (Martin et al., 2003). In more than half of wel8A6-5 and wel9H4-17 homozygous mutant germline clones, GFP-Staun is mislocalised to the centre of the oocyte at stage 9, rather than to the posterior pole as in wild type (Fig. 1A,B,D). The remaining mutant oocytes display a milder phenotype, in which GFP-Staun localises at both the posterior and centre of the oocyte or extends from the posterior cortex into the cytoplasm (Fig. 1C). To confirm that this phenotype reflects a defect in oskar mRNA localisation, we performed in situ hybridisations on wel mutant clones in the absence of GFP-Staun, and observed a very similar range of phenotypes (Fig. 1E-G). By contrast, the localisation of bicoid and gurken mRNAs was largely normal in wel mutant oocytes, suggesting that the defects are focussed on the posterior of the oocyte (Fig. 2).

We used deficiencies to map wellman to a 180 kb region in 93C1-D4 based on the lethality of both wel alleles in trans to Df(3R)e-R1, but not Df(3R)Exel6272 and Df(3R)ED6658 (Parks et al., 2004; Ryder et al., 2007). Crossing wel alleles to available mutants in this region revealed that they failed to complement the lethality of a null allele of slmb, slmb8A6-5, leading us to rename the two wel alleles as slmb9H4-17 and slmb9H4-17 (Jiang and Struhl, 1998). Slmb is an F-box protein that functions as the substrate recognition subunit of the SCF (SKP1/Cullin/F-box protein) ubiquitin ligase, which targets proteins for degradation by the proteasome (Cardozo and Pagano, 2004). Slmb interacts with the SCF complex via its N-terminal F-box motif and binds to its substrates through seven WD40 repeats. Sequencing revealed that slmb9H4-17 contains a nonsense mutation at amino acid 167, and it is therefore presumably a null allele that encodes a truncated protein lacking the substrate interaction domains (Fig. 1I). The other allele, slmb8A6-5, has an alanine-to-valine substitution at amino acid 492 (Fig. 1I). Both alleles cause lethality during the first larval instar as homoygotes and produce a similar spectrum of phenotypes in germline clones, suggesting that slmb8A6-5 also behaves as a null. This might be because this allele introduces a bulkier side chain into a conserved amino acid adjacent to the key tyrosine residue that interacts with the substrates (Wu et al., 2003).

Germline clones of slmb null alleles have previously been found to cause a low frequency (17%) of morphological defects, but their effects on axis formation were not examined (Muzzopappa and Wapner, 2005). To confirm that the oskar mRNA localisation phenotype of slmb8A6-5 and slmb9H4-17 clones was due to the loss of Slmb, we took advantage of the fact that slmb transheterozygous adults can be generated by expressing Slmb under the control of a heat shock promoter (hs-Slmb) from the first instar larva until pupariation (Grima et al., 2002). Using this strategy, we generated slmb8A6-5;slmb9H4-17 transheterozygous ovaries, and observed a similar spectrum of Staun localisation defects to those of slmb9H4-17 germline clones, although these were somewhat less penetrant, presumably due to the perdurance of Slmb expressed from the heat
Fig. 2. slmb mutants do not affect Gurken and bicoid mRNA localisation. (A,B) Localisation of Gurken protein in stage 6 egg chambers. Gurken is expressed at the posterior of the oocyte at this stage to signal to the adjacent follicle cells. Gurken localisation is normal in slmb9H4-17 mutant clones. (C,D) Gurken accumulates at the dorsal-anterior corner of stage 9 oocytes in wild-type (C) and slmb9H4-17 (D) egg chambers. Note the ectopic GFP-Staufen localisation (green in D) in the middle of the mutant oocyte. (E,F) In situ hybridisations for bicoid (bcd) mRNA (red). bcd mRNA is localised to the anterior cortex of the oocyte in wild type (E) and in slmb9H4-17 mutants (F). DNA is marked with DAPI.

Fig. 3. Slmb is required for oocyte polarisation. (A,B) Kinesin-β-galactosidase (Kin-β-gal) visualised with an antibody against β-gal. Kin-β-gal accumulates at the posterior in wild-type (A) but is diffusely localised to the centre of slmb9H4-17 germline clone oocytes (B). (C) Microtubule organisation in wild-type (C) and slmb9H4-17 (D) stage 9 egg chambers visualised with Tau-GFP. (E,F) Egg chambers expressing matu4-GFP-Par-1N1S. GFP-Par-1 forms a posterior crescent in wild-type oocytes (E) but fails to localise to the posterior of most slmb9H4-17 germline clone oocytes (F). (G,H) Par-1-GFP (expressed from a protein trap insertion in the endogenous par-1 locus) forms a posterior crescent in wild-type oocytes (G) but is usually absent from the posterior of hs-Slmb, slmb8/slmbslmb9H4-17 oocytes (H).

**Slmb is required for the posterior recruitment of Par-1**

The oskar mRNA localisation phenotype of slmb germline clones resembles that of par-1 mutants, which fail to repolarise the oocyte microtubule cytoskeleton (Shulman et al., 2000). We examined the organisation of the microtubules by expressing a constitutively active form of the plus end-directed microtubule motor protein kinesin, fused to β-galactosidase (Kin-β-gal) (Clark et al., 1994). In wild type, Kin-β-gal localises to the posterior pole of the oocyte at stage 9 by moving along microtubules to the region with the highest ratio of plus ends to minus ends (Fig. 3A). By contrast, Kin-β-gal accumulates in the centre of most slmb mutant oocytes, indicating that the plus ends are enriched in this region (Fig. 3B). Microtubule organisation can also be visualised by directly labelling them with GFP fused to the microtubule-binding protein Tau (Micklem et al., 1997). Tau-GFP labels an anterior-to-posterior gradient of microtubules in wild-type oocytes, with the highest density at the oocyte anterior where most microtubules are nucleated or anchored, and with little or no signal at the posterior (Fig. 3C). Some slmb mutant oocytes, by contrast, show strong Tau-GFP signal at the posterior pole of the oocyte, indicating that the oskar mRNA phenotype is caused by defects in the AP polarisation of the microtubule cytoskeleton (Fig. 3D).

The correct organisation of the microtubules depends on the recruitment of Par-1 to the posterior cortex of the oocyte in response to the polarising follicle cell signal (Doerflinger et al., 2006). We expressed a GFP-Par-1N1S transgene in slmb mutant germline clones to determine whether Slmb functions upstream or downstream of Par-1 in microtubule organisation. In wild type, GFP-Par-1 becomes enriched at the posterior cortex of the oocyte from stage 7 onwards and provides the earliest marker for the repolarisation of the oocyte (Fig. 3E). This posterior GFP-Par-1 crescent fails to form in slmb9H4-17 oocytes (Fig. 3F). There is also no posterior GFP-Par-1 crescent in 38% (n=26) of rescued slmb8/slmbslmb9H4-17 adults, which correlates well with the penetrance of the Staufen localisation defect in this mutant combination. This result was confirmed by examining the localisation of a Par-1-GFP protein trap insertion that is expressed at endogenous levels, which also failed to form a posterior crescent in slmb8/slmbslmb9H4-17 (Fig. 3G,H). Thus, Slmb is required in the germline for the posterior recruitment of Par-1, suggesting that it acts downstream of the signal from the posterior follicle cells to polarise the oocyte.

Shock promoter (Fig. 1D,H). These phenotypes were largely rescued by expressing GFP-Slmb in the germline, confirming that mislocalisation of oskar mRNA and Staufen is caused by the specific loss of Slmb activity in the germline. A proportion of slmb mutant egg chambers escape the polarity defect and develop a later phenotype that we have already described elsewhere, in which short Oskar protein overaccumulates, leading to increased pole cell number and embryonic patterning defects (Morais-de-Sa et al., 2013).
Slmb limits the size of the apical domain in epithelial cells

slmb<sup>/----</sup>slmb<sup>9H4-17</sup> mutants rescued by the expression of hs-Slmb during larval development also show defects in the organisation of epithelial follicle cells. These cells normally form a simple epithelial monolayer with their apical surfaces facing the germline. In slmb<sup>/----</sup>slmb<sup>9H4-17</sup> egg chambers, the cells form multiple layers (Fig. 4A). This phenotype is reminiscent of that produced by mutants that affect epithelial polarity, and we therefore analysed the localisation of aPKC, a component of the apical polarity complex (St Johnston and Ahringer, 2010). aPKC localises normally to the apical surface of the mutant follicle cells that contact the germline, but shows a patchy distribution around the cortex of the cells that do not, which also lose their regular columnar arrangement (Fig. 4B,C). This suggests that Slmb is important for apicobasal polarity, but that an apical cue from the germline can compensate for reduced Slmb levels in most mutant cells (Tanentzapf et al., 2000).

Since the hs-Slmb-rescued slmb transheterozygotes show a weaker phenotype in the germline than slmb mutant clones, we also generated somatic clones of slmb<sup>/----</sup>slmb<sup>8A6-5</sup> and slmb<sup>9H4-17</sup>. The rare large clones that we recovered show a multilayered phenotype (Fig. 4D,L). Small clones also frequently show disruptions in epithelial polarity, even when the cells contact the germline (Fig. 4L). Clones comprising only a few mutant cells show a reduction of the basal domain and increased staining for the apical polarity proteins aPKC, Par-6 and Crumbs (Crb) (Fig. 4E,G-K). In larger clones, aPKC is also found throughout the cortex and cytoplasm, and the cells are often extruded from the epithelium (Fig. 4F). We also examined other polarity markers in slmb clones, including Baz, which marks adherens junctions, and Dlg (Dlg1 – FlyBase), which labels the lateral membrane (Moraïs-de-Sa et al., 2010; Woods et al., 1997). Both proteins show normal localisations in small clones with defective epithelial organisation, whereas aPKC and Par-6 levels are already elevated in small clones that still retain a normal shape (Fig. 4H-K). Thus, the increased levels of aPKC and Par-6 are not a consequence of disrupted epithelial organisation. This suggests that polarity defects in slmb mutants might result from overexpression of the Par-6/aPKC apical polarity complex, which disrupts the balance between the apical and basolateral domains. In support of this view, lowering aPKC levels by removing one copy of the gene partially rescues the multilayering phenotype of slmb transheterozygotes (Fig. 4M).

The polarity defects of slmb mutants are not caused by overexpression of Arm or α-spectrin

Since Slmb ubiquitylates proteins to target them for degradation, loss of Slmb should lead to the overexpression of its substrates. This suggests that the epithelial phenotype results from the overexpression of one or more Slmb targets that regulate polarity. Although many Slmb substrates have been identified, these are mainly involved in signal transduction or cell cycle control (Fuchs et al., 2004). One possible candidate is Armadillo (Arm), which links E-cadherin to the actin cytoskeleton at adherens junctions as well as functioning in the Wingless pathway (Bienz, 2005). As expected, Arm is strongly overexpressed in slmb mutant follicle cells and germline clones (supplementary material Fig. S1A-D). This does not appear to be the cause of the polarity phenotype, however, as the overexpression of a stabilised form of Arm has no effect on epithelial organisation (supplementary material Fig. S1E-G). Furthermore, the strength of the germline polarity phenotype of slmb null mutant clones is not altered by removing one copy of arm (supplementary material Fig. S1I).

We noticed that the levels of α-spectrin are also elevated in slmb mutant clones. α<sub>1</sub>/β<sub>3</sub> spectrin tetramers are a major component of the cortical cytoskeleton that cross-links the plasma membrane to actin.

![Fig. 4. Slmb regulates follicle cell organisation and polarity.](image)
and tethers proteins in specific membrane domains (Knust, 2000). The Drosophila spectrin cytoskeleton is polarised with αβ-tetramers marking the basolateral membrane and αβH1, spectrin localising apically, where βH-spectrin is recruited by Crb (Lee et al., 1997; Medina et al., 2002; Thomas and Kiehart, 1994). slmb mutant clones show a dramatic increase in the levels of α-spectrin, whereas βH-spectrin is not significantly increased (Fig. 5A,B). This suggests that α-spectrin is normally ubiquitylated by the SCF complex and targeted for degradation. Since α-spectrin mutants disrupt the organisation of the follicular epithelium, we tested whether its overexpression could explain the polarity defects of slmb mutants. However, the expression of very high levels of α-spectrin in the follicle cells using the UAS-Gal4 system had no effect on epithelial polarity or the levels and apical localisation of aPKC or βH-spectrin (Fig. 5C-E). In addition, α-spectrin overexpression in the germline did not disrupt oocyte polarity (Fig. 5F). Thus, the upregulation of α-spectrin appears to be unrelated to the increased localisation of apical polarity factors and the expansion of the apical domain in slmb mutant clones.

**Slmb regulates the levels of Par-6 and aPKC**

Since none of the obvious Slmb substrates could account for its polarity phenotype, we next considered the possibility that it targets the Par-6/aPKC complex for degradation. Western blots of adult tissue showed that the levels of aPKC and Par-6 increase significantly compared with wild type, whereas the levels of Baz and Crb are slightly reduced (Fig. 6A). Quantification indicates that Par-6 levels approximately double and aPKC levels are ~60% higher than normal in slmb mutant egg chambers (Fig. 6A,B). This increase is almost completely suppressed by expressing GFP-Slmb in the germline. Thus, Slmb is required to limit the levels of the Par-6/aPKC complex, suggesting that it acts directly or indirectly to stimulate Par-6 or aPKC turnover. Since the levels of Baz and Crb are reduced in slmb mutants, it does not appear that the Baz/Par-6/aPKC complex as a whole is being regulated or that increased levels of Crb are recruiting more Par-6/aPKC to the cortex. Slmb must therefore target the Par-6/aPKC complex by a different mechanism.

Par-1 has also been reported to be a target of Slmb in neurons (Lee et al., 2012), and we therefore examined whether Par-1 levels are altered in hs-Slmb, slmb<sup>9H4-17</sup> egg chambers. Because the available antibodies against Par-1 do not work well on western blots, we examined whether loss of Slmb affected the amount of Par-1-GFP expressed from a protein trap line in which a GFP exon is inserted into the middle of the endogenous par-1 locus (Lighthouse et al., 2008). Although one would expect the levels of a target of Slmb-dependent degradation to be elevated in an slmb mutant, Par-1-GFP levels are slightly reduced, indicating that it is not a substrate of the SCF<sub>slmb</sub> complex in the ovary (Fig. 6A).

**Slmb is required to exclude aPKC and Par-6 from the oocyte posterior cortex**

In wild type, aPKC localises around the lateral cortex of the oocyte, but is excluded from the posterior cortex (Fig. 6C). By contrast, slmb mutant oocytes show a uniform cortical localisation of aPKC that spans the posterior pole (Fig. 6D). A Par-6-Cherry transgene can also be used as a marker for the Par-6/aPKC complex, as Par-6 binds to aPKC directly and colocalises with it in all other tissues (St Johnston and Ahringer, 2010). When expressed specifically in the germline, Par-6-Cherry is excluded from the posterior cortex of the oocyte at stages 8-9 in wild type, coincident with the posterior recruitment of Par-1 (Doerflinger et al., 2010). By contrast, Par-6-Cherry shows an almost uniform localisation around the cortex in slmb mutant germline clones (Fig. 6E). Thus, Slmb is required to remove Par-6 and aPKC from the oocyte posterior. This function of Slmb in controlling the asymmetric distribution of PAR-6 and aPKC in the oocyte differs from its role in the follicle cells, where it appears to simply restrict the levels of two polarity proteins to limit the size of the apical domain. Consistent with this, reducing the levels of aPKC by removing one copy of the gene does not

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**Fig. 5. α-spectrin overaccumulates in slmb mutant cells.** (A) Surface view of a large slmb<sup>9H4-17</sup> clone marked by the absence of GFP (green), showing the strong increase in α-spectrin levels (red and as separate channel). (B) An slmb<sup>9H4-17</sup> clone marked by the absence of nuclear RFP (red) stained for α-spectrin (green) and βH-spectrin (white, bottom panel). (C-E) Overexpression of α-spectrin in FLPout clones marked with GFP. Overexpressed α-spectrin (red) accumulates along the cell cortex and also in the cytoplasm (C). Overexpression of α-spectrin does not disrupt epithelial organisation, aPKC (red) localisation and stability (D) or βH-spectrin localisation and stability (E). (F) A wild-type egg chamber overexpressing α-spectrin in the germline driven by matα4-GAL4:VP16. α-spectrin (green) overexpression does not affect the posterior localisation of Stau (red).
significantly rescue the oocyte polarity phenotype of an \textit{slmb} mutant (supplementary material Fig. S2).

**Overexpression of Lgl partially rescues the \textit{slmb} polarity phenotype**

Lgl might play a redundant role in the polarisation of the oocyte, since it localises to the posterior cortex and is a known antagonist of aPKC (Tian and Deng, 2008; Wirtz-Peitz et al., 2008). We examined the effects of overexpressing UAS-Lgl-GFP in \textit{slmb} mutants. Expression of Lgl-GFP in the germline strongly suppresses the polarity phenotype of \textit{slmb} \textit{9H4-17} germline clones, and the majority of oocytes show a posterior crescent of Lgl-GFP and normal posterior localisation of Staufen protein (Fig. 7A-C). Thus, Lgl appears to function independently of Slmb in the inactivation of the Par-6/aPKC complex at the posterior cortex of the oocyte, and can compensate for the loss of Slmb when overexpressed.

**DISCUSSION**

Very little is known about how the posterior follicle cells signal to polarise the AP axis of the oocyte, except that signalling is disrupted when the germline is mutant for components of the exon junction complex, such as Mago nashi (Micklem et al., 1997; Newmark et al., 1997). Our results reveal that Slmb also plays an essential role in this asymmetry, the observation that it regulates the expression of Baz/Par-6/aPKC and Par-1. Although Slmb might act in a variety of pathways to establish this asymmetry, the observation that it regulates the normal posterior localisation of Staufen protein (Fig. 7A-C). Thus, Lgl appears to function independently of Slmb in the inactivation of the Par-6/aPKC complex at the posterior cortex of the oocyte, and can compensate for the loss of Slmb when overexpressed.

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proteins act to remove the Par-6/aPKC complex from the posterior cortex to allow the posterior recruitment of Par-1, which then reinforces polarity by excluding Baz/PAR-3 by phosphorylation (Fig. 7D). Furthermore, the polarity phenotypes of both slmb and par-2 mutants can be rescued by the overexpression of Lgl (Beatty et al., 2010; Hoeger et al., 2010). Slmb and PAR-2 act by different mechanisms, since the former is a subunit of the SCF ubiquitin ligase complex and promotes the degradation of the Par-6/aPKC complex, whereas the latter functions by recruiting PAR-1 (Motejí et al., 2011). Nevertheless, it is intriguing that PAR-2 contains a RING finger domain that is typically found in ubiquitin ligases (Joazeiro and Weissman, 2000; Lorick et al., 1999), suggesting that it might have lost this activity during evolution.

MATERIALS AND METHODS

Drosophila strains and genetics

wellman was genetically mapped using a set of 3R deficiency stocks obtained from the Bloomington and Szeged Drosophila stock centres. Clonal analysis was performed with the FLP/FRT system using nuclear GFP as a marker of wild-type cells (Xu and Rubin, 1993) or using the dominant female sterile technique with the hsSlmb construct (Linsley et al., 2008). The construct was remobilised from its original location on the third chromosome onto the second chromosome to allow recombination with stock centres.

Immunological techniques and in situ hybridisation

Immunofluorescence (IF) and western blots (WB) were performed using standard procedures with the following primary antibodies: rabbit anti-Stau [1/100 (St Johnston et al., 1991)], rabbit anti-PKcz [1/50 IF, 1/2000 WB (C-20, Santa Cruz Biotechnology)], mouse anti-Gurken [1/30; Developmental Studies Hybridoma Bank (DSHB)], mouse anti-Dlg [1/200; DSHB], rabbit anti-Par-6 [1/2000 WB (Petronczki and Knoblich, 2001)], rabbit anti-Baz [1/1000 IF, 1/5000 WB (Wodarz et al., 1999)], mouse anti-Crb [1/50; DSHB], mouse anti-Arm [1/100 IF, 1/2000 WB; DSHB], mouse anti-α-spectrin [1/100; DSHB], rabbit anti-α-H-spectrin [1/500 (Thomas and Kiehart, 1994)] and mouse anti-α-tubulin [1/2000 WB; DMIA, Sigma]. Actin was visualised with Rhodamine-conjugated phalloidin (Invitrogen). In situ hybridisations for oskar and bicaudal mRNA were performed as previously described (Doerflinger et al., 2006; Schnorrer et al., 2002). Imaging was performed with a Zeiss LSM510 confocal microscope with a 40× oil immersion lens (Plan-NeoFluor; NA 1.3). Images were processed using ImageJ (NIH) and Adobe Photoshop.

For quantitative western blots, the ovaries of 15 females of each genotype were lysed in 225 μl ice-cold RIPA buffer (Sigma) with protease inhibitors and both can be rescued by overexpressing Lgl. Border cells are shown in yellow.

Fig. 7. Expression of Lgl-GFP in the germline.

(A) A slmb9H4-17 germline clone egg chamber showing the mislocalisation of Staufen (Stau, red) in a cloud in the centre of the oocyte. (B) A typical slmb9H4-17 germline clone egg chamber expressing Lgl-GFP. Lgl-GFP (green) localises normally to the posterior cortex of the oocyte and rescues the posterior localisation of Stau. (C) The frequency of Stau localisation phenotypes in slmb9H4-17 germline clones with and without expression of Lgl-GFP. (D) Model presenting Slmb as the functional counterpart of C. elegans PAR-2. Both proteins are required for the posterior exclusion of the aPKC complex and for the recruitment of Par-1, and both can be rescued by overexpressing Lgl. Border cells are shown in yellow.

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RESEARCH ARTICLE

DEVELOPMENT
Molecular biology
To sequence the slmb alleles, exons were individually amplified from genomic DNA and sequenced by Geneservice (Cambridge, UK). To clone UASp-a-spectrin, the α-Spectrin genomic region including all the coding sequences was amplified from Drosophila genomic DNA with the primer pair 5′-TATAGTGACCATGAGAATCTTACC-3′ and 5′-TATAGC-GGCCTAATTGTCGAGACCAGGTTCT-3′, and cloned into the KpnI/NotI sites of the pUASp-Pl vector (Rørth, 1998). This transgene was introduced into flies by standard germline transformation.

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Competing interests
The authors declare no competing financial interests.

Author contributions
E.M. and D. StJ. designed the study and prepared the manuscript. E.M. and A.M. performed all the experiments except for the analysis of Lgl suppression of the slmb phenotype in Fig. 7, which was carried out by N.L.

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