PP1-Mediated Dephosphorylation of Lgl Controls Apical-basal Polarity

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In Brief
Moreira et al. investigate the post-mitotic polarization of new daughter cells in Drosophila epithelia. They identify PP1 phosphatase as a regulator of the conserved basolateral determinant Lgl. Dephosphorylation of Lgl counteracts aPKC/AurA activity, being an essential mechanism to restore Lgl cortical localization and to maintain the architecture of proliferative tissue.

Highlights
- Cytoplasmic Lgl returns to the cortex at mitotic exit in Drosophila epithelia
- Lgl cortical localization depends on PP1-mediated dephosphorylation
- PP1 dephosphorylates Lgl to maintain epithelial architecture
- Apical polarization of new daughter cells is coupled with Lgl cortical reloading
PP1-Mediated Dephosphorylation of Lgl Controls Apical-basal Polarity

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SUMMARY

Apical-basal polarity is a common trait that underlies epithelial function. Although the asymmetric distribution of cortical polarity proteins works in a functioning equilibrium, it also retains plasticity to accommodate cell division, during which the basolateral determinant Lgl is released from the cortex. Here, we investigated how Lgl restores its cortical localization to maintain the integrity of dividing epithelia. We show that cytoplasmic Lgl is reloaded to the cortex at mitotic exit in Drosophila epithelia. Lgl cortical localization depends on protein phosphatase 1, which dephosphorylates Lgl on the serines phosphorylated by aPKC and Aurora A kinases through a mechanism that relies on the regulatory subunit Sds22 and a PP1-interacting RVxF motif of Lgl. This mechanism maintains epithelial polarity and is of particular importance at mitotic exit to couple Lgl cortical reloading with the polarization of the apical domain. Hence, PP1-mediated dephosphorylation of Lgl preserves the apical-basal organization of proliferative epithelia.

INTRODUCTION

Epithelial tissues compartmentalize multicellular organisms and have a variety of specialized roles that rely on asymmetries in protein and lipid composition and the precise positioning of intercellular junctions along its apical-basal axis (Rodriguez-Boulan and Macara, 2014). This polarity axis is defined by conserved apical-basal polarity proteins, which segregate into distinct domains along the epithelial cell cortex (Flores-Benitez and Knust, 2016). One such protein is Lethal giant larvae (Lgl), originally identified as a tumor suppressor in Drosophila and often misregulated in human cancer (Gateff, 1978; Halauwi and McCaffrey, 2015). In epithelia, Lgl cooperates with Discs Large (Dlg) and Scribbled as a basolateral determinant to restrict the localization and activity of the atypical protein kinase C (aPKC) and Crumbs complexes to the apical domain (Bilder et al., 2000, 2003; Tanentzapf and Tepass, 2003), possibly by inhibiting aPKC activity (Atwood and Prehoda, 2009; Wirtz-Peitz et al., 2008; Yamanaka et al., 2006) or regulating the trafficking of apical transmembrane proteins (Fletcher et al., 2012; Jossin et al., 2017).

Lgl associates with the actomyosin cytoskeleton (Betschinger et al., 2003; Dahan et al., 2012; Strand et al., 1994), and recent work revealed that its cortical localization is, however, primarily mediated by binding to plasma membrane phosphoinositides through a positively charged basic and hydrophobic (BH) motif (Bailey and Prehoda, 2015; Dong et al., 2015). This motif is phosphorylated by aPKC (Betschinger et al., 2003; Plant et al., 2003) to exclude Lgl from the apical cortex during interphase and by mitotic kinase Aurora A (AurA) to completely release Lgl from the cortex and promote proper mitotic spindle orientation in epithelial tissue (Bell et al., 2015; Carvalho et al., 2015). Interestingly, apical polarity proteins such as aPKC, Par-6, and Par-3 also adjust localization during epithelial mitosis, as shown in Drosophila and during early embryogenesis of Nematostella vectensis (Bergstrahl et al., 2013; Carvalho et al., 2015; Morais-de-Sá and Sunkel, 2013; Ragkousi et al., 2017; Rosa et al., 2015). However, it is unknown how Lgl cortical localization is restored at the end of cell division and how this is coordinated with daughter cell polarization. Moreover, although phosphorylation controls Lgl localization and function, how (and whether) counteracting protein phosphatases actively dephosphorylate Lgl remains undetermined.

Here we report the PP1-Sds22 complex as a critical regulator of Lgl dephosphorylation, promoting its localization at the cortex and plasma membrane. Because dividing cells must deal with the complete pool of Lgl in the cytoplasmic and hyperphosphorylated form, this mechanism has foremost significance to timely polarize daughter cells and maintain the architecture of proliferating epithelia.

RESULTS

Cytoplasmic Lgl Returns to the Cortex at Mitotic Exit

To investigate how Lgl localization is restored in the daughter cells, we examined the dynamics of Lgl subcellular redistribution in the follicular epithelium, a monolayered tissue that envelops the germline in the Drosophila ovary. Lgl cytoplasmic levels decline from around 2 min until about 15 min after anaphase onset, with the concomitant accumulation of Lgl at the daughter-daughter (d-d) cell interface just as the new membrane ingresses from the basal side (Figures 1A and 1B; Video S1).
dynamic reallocation of Lgl-GFP at mitotic exit is also reproduced in the *Drosophila* embryonic epithelium (Figure S1A). To test whether Lgl returns from the cytoplasm to the cortex, we labeled cytoplasmic Lgl through photoconversion during mitosis in S2 cells (Figure 1C). Photoconverted Lgl-Dendra2 accumulates at the cortex when cells exit mitosis (Figure 1C). Moreover, the labeled pool of Lgl-Dendra2 is not degraded during mitotic exit because the total fluorescence of the photoconverted protein remains constant during the period of cytoplasmic signal decay (Figure 1D). Thus, at mitotic exit, Lgl is fully reloaded from the cytoplasm to the cortex. The kinetics of cytoplasmic decay are therefore an accurate readout for the sum of Lgl cortical reloading to the pre-existing cell membranes and to the newly formed daughter-daughter interface.

Lgl release from the cortex at mitotic entry is controlled by AurA (Bell et al., 2015; Carvalho et al., 2015), which is targeted for degradation by the Cdh1-activated anaphase-promoting complex (APC-Cdh1) at mitotic exit (Castro et al., 2002). To investigate whether AurA degradation determines Lgl cortical reloading, we overexpressed AurA-GFP and a stabilized version, AurA

![Figure 1](image-url)
regulation of AurA stability is not the timer controlling Lgl reloading to the cortex.

PP1 Antagonizes aPKC and AurA-Mediated Phosphorylation of Lgl

Lgl phosphorylation reduces its affinity to the plasma membrane and to the underlying cortex (Bailey and Prehoda, 2015; Dong et al., 2015; Betschinger et al., 2003). Thus, Lgl cortical reloading should require dephosphorylation, but a phosphatase for Lgl has yet to be described. Two good candidates are PP1 and PP2A, serine and threonine-specific phosphatases activated at mitotic exit (Barr et al., 2011). These phosphatases form multimeric holoenzyme complexes containing catalytic and regulatory subunits. There are four Drosophila genes encoding PP1 catalytic subunits: Pp1i9C (flw), Pp1-87B, Pp1α-96A, and Pp1α-13C, which is responsible for negligible PP1 activity, whereas the PP2A catalytic subunit is encoded by microtubule star (mts). RNAi-mediated depletion of Pp1α-96A, Flw, or Mts in the follicular epithelium does not significantly affect Lgl localization (Figures S2A–S2D), whereas PP1-87B depletion leads to Lgl mislocalization to the cytoplasm, as observed by clonal (Figure 2A) and global depletion (Figure 2C).

To confirm whether PP1-87B is the only essential PP1 catalytic subunit required for proper Lgl localization, we generated mutant clones using null alleles of flw and Pp1α-96A. Lgl localization in these mutant clones is indistinguishable from wild-type cells, whereas Lgl is fully cytoplasmic in double pp1-87B, pp1α-96A mutant clones (Figure 2B), indicating that PP1-87B enables cortical Lgl localization. This is unlikely to stem from a specific function of PP1-87B but, rather, from it being the most abundant PP1c isozyme because PP1 catalytic subunits have highly overlapping roles (Kirchner et al., 2007). Consistent with this, Lgl cortical localization in PP1-87B-depleted follicle cells is rescued by overexpression of human PPP1cc, whose closest ortholog is Pp1α-96A (Figure 2C). Moreover, overexpression of a global PP1 inhibitor, NiPp1, also disrupts Lgl localization (Figure 2D). Thus, overall PP1 activity is essential for proper Lgl localization to the lateral cortex in Drosophila epithelia, which is consistent with a role in Lgl dephosphorylation.

We used recombinant MBP-Lgl and Lgl-GFP immunoprecipitated from ovaries to examine whether PP1 could directly dephosphorylate Lgl on S656, an aPKC and AurA phosphorylation site. Incubation of recombinant PP1 with Lgl previously phosphorylated by AurA results in a concentration- and time-dependent decrease in S656 phosphorylation, indicating that Lgl is a substrate for PP1 (Figures 2E and 2F). Importantly, the activity of PP1 toward Lgl is specific compared with λ phosphatase, which fails to dephosphorylate Lgl as efficiently (Figure S2E). Furthermore, we used Drosophila S2 cells to dissect whether Lgl mislocalization upon PP1 inactivation results from hyperphosphorylation on the aPKC and AurA phosphorylation sites. Cytoplasmic localization of Lgl is reproduced upon PP1-87B depletion in S2 cells, whereas co-depletion of aPKC rescues Lgl cortical localization (Figures 2G, S2F, and S2G). Moreover, we simultaneously analyzed the localization of Lgl and LglS3A, which harbors mutations in the serines controlled by aPKC (S656, S660, and S664) and AurA (S656 and S664) (Bell et al., 2015; Betschinger et al., 2003; Carvalho et al., 2015). Strikingly, LglS3A is retained at the cortex, in contrast with the cytoplasmic accumulation of Lgl in the follicular epithelium and in S2 cells depleted of PP1-87B (Figure 2H). Hence, these results collectively suggest that PP1-mediated dephosphorylation of the aPKC and AurA phosphorylation sites promotes Lgl localization at the lateral cortex.

PP1-Mediated Dephosphorylation of Lgl Maintains Epithelial Architecture and Polarity

PP1-87B RNAi leads to loss of epithelial polarity in the follicular epithelium, with consequent cell invasion and multilayering (Figure S3). This mimics Lgl loss of function phenotypes in the follicular epithelium (Bilder et al., 2000), but PP1 is involved in multiple pathways, which may indirectly contribute to the phenotype. Given that cortical Lgl antagonizes apical determinants, we tested whether reducing aPKC levels could attenuate this phenotype. aPKC heterozygosity strongly suppresses the multilayering phenotype of PP1-87B-depleted tissue (Figure 3A), suggesting that PP1 activity balances apical activity by promoting Lgl cortical localization.

Association of PP1 with regulatory subunits and substrates is often mediated by a RVxF docking motif, [KR][X]9,[V][P] [FW], which binds a PP1 hydrophobic pocket (Wakula et al., 2003). Interestingly, Lgl has a sequence that fits this motif and that is widely conserved in metazoans (Figure 3B). To define the functional significance of Lgl dephosphorylation by PP1, we mutated two critical residues of the PP1 docking motif (Meiselbach et al., 2006). In comparison to Lgl-GFP, LglK327A,F331A-GFP accumulates strongly in the cytoplasm of follicle cells and shows increased S656 phosphorylation (Figures 3C and 3D). More importantly, expression of LglK327A,F331A-GFP does not support epithelial apical-basal polarity in lgl-null mutant clones, leading to multilayered tissue where aPKC is mislocalized (Figures 3E and 3F). Thus, PP1-mediated dephosphorylation promotes Lgl localization to the cortex, where it can act as basolateral determinant to control epithelial organization.

PP1 Orchestrates Apical-basal Polarization at Mitotic Exit

Lgl must be relocated to the epithelial cortex of daughter cells at mitotic exit, making this period particularly challenging for PP1-mediated dephosphorylation of Lgl. Accordingly, Lgl cortical reloading is minor in PP1-87B depleted follicle cells (~70% of mitotic levels remain cytoplasmic 35 min after anaphase onset versus ~20% in the control; Figures 4A and 4B), even for cells that present Lgl largely cortical before mitotic entry (Video S2). Moreover, depletion of the PP1 regulatory subunit Sds22, which enhances PP1 activity during cytokinesis (Kundra et al., 2012; Ohkura and Yanagida, 1991), also delays Lgl reloading at mitotic exit (Figure 4B). Sds22 RNAi has, however, a milder effect than PP1-87B RNAi, culminating in lower levels of cytoplasmic Lgl during early interphase (~40% of mitotic levels). We obtained similar results in Drosophila S2 cells (Figure S4A), suggesting that Sds22 is particularly significant to boost PP1 activity to deal with the complete pool of phosphorylated and cytoplasmic Lgl at mitotic exit. LglK327A,F331A also shows disrupted cytoplasmic decay in both the embryonic and cell Reports 26, 293–301, January 8, 2019 295
folicular epithelium (Figures 4C, 4D, and S4B). Nevertheless, LglK327A,F331A-GFP cytoplasmic decay is much faster than Lgl-GFP in PP1-87B-depleted follicle cells. Because Sds22 RNAi induces strong cytoplasmic retention of LglK327A,F331A-GFP (Figures 4C and 4D), we conclude that this PP1-docking motif promotes robust dephosphorylation but is dispensable for partial PP1 activity when the PP1-Sds22 complex is available.

In many proliferative tissues, a new daughter-daughter interface is formed upon cell division to hold daughter cells together (Firmino et al., 2016; Herszterg et al., 2014; Olivier et al., 2010). We investigated the importance of Lgl cortical reloading for daughter-daughter interface polarization by examining the segregation of its mutual antagonist aPKC to the apical domain. Simultaneous imaging of Lgl-mCherry with aPKC-GFP shows that Lgl starts localizing to the lateral cortex before apical accumulation of aPKC (Figure 4E, kymographs). This likely results from the basal-to-apical ingression of the cytokinetic furrow, described previously in many epithelia (Herszterg et al., 2014; Morais-de-Sá and Sunkel, 2013). Moreover, Lgl accumulation...
is completed before aPKC reaches maximum apical levels (Figure 4E, kymographs). We therefore hypothesized that loading Lgl to the lateral cortex could be necessary for aPKC accumulation at the apical side. To test this idea, we used an lgl temperature-sensitive allele, lglts3, which reallocates to the cytoplasm at restrictive temperature (Manfruelli et al., 1996), and limited our analysis to the first 2 hr at 29°C, during which the tissue maintains its monolayered organization (Figure S4B). aPKC accumulation in the apical daughter-daughter interface is drastically impaired in lglts3/lgl4 mutant follicle cells (Figure 4F). Furthermore, aPKC localizes ectopically in the lateral domain at the daughter-daughter interface of lglts3/lgl4 mutant cells (Figure 4G; Video S3), suggesting that Lgl cortical reloading restricts aPKC to the apical side by limiting its lateral accumulation. Thus, by controlling the timing of Lgl cortical reloading, PP1 activation synchronizes the polarization of apical and basolateral domains at mitotic exit.

**DISCUSSION**

Apical-basal polarity relies on the asymmetric distribution of the polarity determinant Lgl to the lateral cortex of epithelial cells. Research regarding Lgl regulation has been focused on the modulation of phosphorylation via aPKC and Aurora kinases. Here we found that PP1 provides another layer of regulation, antagonizing Lgl phosphorylation by aPKC or AurA. We show that PP1-mediated dephosphorylation can control Lgl subcellular distribution in both epithelial and non-epithelial cells and is critical to maintain the monolayered organization and apical-basal polarity. Because Lgl is a general cell polarity regulator (Betschinger et al., 2003; Dahan et al., 2012; Raman et al., 2016), it is likely that the significance of Lgl regulation by PP1 extends to a range of processes, including asymmetric cell division, cell migration, fate specification, and growth control.
Figure 4. PP1-Mediated Dephosphorylation of Lgl Promotes Apical-basal Polarization at Mitotic Exit

(A) Disrupted cortical reloading of Lgl in PP1-87B- and Sds22-depleted follicle cells.

(B) Normalized mean intensity of cytoplasmic Lgl-GFP in control (n = 22, also in D), PP1-87B RNAi (n = 8), and Sds22 RNAi (n = 11).

(C) Defects in cortical reloading of LglK327A,F331A-GFP are enhanced by Sds22 depletion.

(D) Normalized mean intensity of cytoplasmic LglK327A,F331A-GFP in control (n = 11) and Sds22 RNAi (n = 12).

(E) Time-lapse images of follicle cells expressing aPKC-GFP and Lgl-mCherry at endogenous levels. The kymographs (pseudocolored to intensity) detail their accumulation at the daughter-daughter interface, projected in the selected apical and lateral planes, respectively.

(F) aPKC-GFP accumulation in the daughter-daughter interface (arrow, fluorescence intensity normalized to non-dividing cells) is strongly reduced in lgl4/lglts3 mutant cells imaged at restrictive temperature (n = 40) (~50% of lgl/+; n = 21).

(G) Time-lapse images of midsagittal egg chamber sections show that aPKC-GFP accumulates in the lateral side of the new daughter-daughter interface in lgl4/lglts3 mutant cells (arrows) imaged at restrictive temperature. Time is shown since anaphase. Data show mean ± SEM. Scale bars, 5 μm.

See also Figure S4.
PP1 directly dephosphorylates Lgl, and we show that Lgl localization is insensitive to PP1 depletion when the aPKC and AurA phosphorylation sites are mutated. Thus, PP1 dephosphorylation promotes the phosphorylation-inhibited binding between the BH motif of Lgl and plasma membrane phosphoinositides (Bailey and Prehoda, 2015; Dong et al., 2015). This mode of regulation parallels cell polarization in fission yeast, where the protein phosphatase 1 complex Tea4-Dis2 controls dephosphorylation of the polarity regulator Pom1 to expose a basic region with affinity for plasma membrane phospholipids (Hachet et al., 2011). Furthermore, PP1 has been shown to dephosphorylate Par-3 on aPKC phosphorylation sites (Traweger et al., 2008). PP1 is therefore a critical regulator of cell polarity and antagonizes aPKC activity over multiple substrates in epithelial tissues.

We also show that Sds22 promotes Lgl cortical localization. Sds22 has been identified previously as a regulator of epithelial organization, but this function has been mostly linked to the deleterious effect of myosin or moesin hyperphosphorylation (Gurscze et al., 2009; Jiang et al., 2011). Interestingly, similarly to loss of function of the basolateral polarity proteins Scrib, Dlg, and Lgl, sds22 mutation also promotes neoplastic tumor development in Drosophila imaginal discs (Jiang et al., 2011). Thus, the role of the PP1-Sds22 complex in Lgl cortical localization provides a possible mechanistic interpretation for the tumor-suppressive role of Sds22 and its human homolog PPP1R7 (Jiang et al., 2011; Narayan et al., 2003).

Lgl becomes fully cytoplasmic because of AurA phosphorylation at mitotic entry (Bell et al., 2015; Carvalho et al., 2015), and since preexisting phosphorylated Lgl is not degraded, PP1-mediated dephosphorylation is critical to reload cytoplasmic Lgl to the cortex of the daughter cells. Moreover, we show that cortically localized Lgl inhibits aPKC accumulation at the lateral domain of the newly formed interface between daughter cells, promoting its apical localization. Hence, although asymmetric furrow ingress and apical midbody positioning may assist with the establishment of apical-basal asymmetries at mitotic exit (Herszterg et al., 2014; Morais-de-Sa and Sunkel, 2013), the role of the PP1-Sds22 complex in Lgl cortical localization provides a possible mechanistic interpretation for the tumor-suppressive role of Sds22 and its human homolog PPP1R7.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **METHOD DETAILS**
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  - Preparation of ovary and S2 cell protein extracts
  - Immunofluorescence and F-actin staining
  - Cloning of Lgl variants
  - RT-PCR
  - Protein purification
  - *in vitro* dephosphorylation assays
  - Photoconversion experiments in S2 cells
  - Multiple sequence alignments
  - Imaging

- **QUANTIFICATION AND STATISTICAL ANALYSIS**
  - Cytoplasmic decay of Lgl
  - Lgl accumulation in daughter-daughter interface
  - Intensity of Lgl-Dendra2 upon photoconversion
  - aPKC accumulation in the apical interface formed between daughter cells
  - Interphase cortical/cytoplasmic intensity ratio of Lgl in S2 cells

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, one table, and three videos and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.12.060.

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AUTHORS CONTRIBUTIONS


DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES


# STAR METHODS

## KEY RESOURCES TABLE

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### Oligonucleotides

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(Continued on next page)
CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for reagents may be directed to and will be fulfilled by the Lead Contact, Eurico Morais-de-Sá (eurico.sa@ibmc.up.pt).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Drosophila stocks and genetics

Drosophila melanogaster flies were cultured on standard cornmeal/agar/molasses/yeast media at 18°C or 25°C, unless otherwise noted. tj-GAL4 and GR1-GAL4 drivers were used to induce expression of UAS transgenes in the follicular epithelium, whereas matα4-GAL4-VP16 was used to induce the expression in embryos. The FLP/FRT-mediated mitotic recombination system was used to generate clones, which were induced by heat shock at 37°C. The GAL80 repressor was used together with FLP/FRT recombination system to control the induction of Pp1-87B RNAi in clonal analysis (Figure 2A) and to express UAS-LglK327A,F331A-GFP in lgl mutant cells (Figure 3E). Crosses were maintained at 18°C and then flies were incubated at 29°C to boost the efficiency of RNAi depletion in experiments with UAS-driven RNAi. Flies were also incubated at 29°C during 1 day for the rescue experiment in Figures 3E and 3F. Both UAS-LglK327A,F331A-GFP (this study) and UAS-Lgl-GFP (unpublished, gift of Daniel St Johnston, Gurdon Institute, Cambridge, UK) were inserted into the same genomic landing site, attP-VK18. Detailed genotypes are depicted below.

Figure 1

A) hs-FLP nls-RFP FRT19/hs-FLP tub-Gal80 FRT19; tj-GAL4/+; UAS-Lgl-GFP/+ (clonal expression to evaluate local Lgl-GFP signal specifically in dividing cell) 
B) w; tj-GAL4/+; UAS-Lgl-GFP, HisRFP/+ 
E, F) Control: w; UAS-Lgl-RFP/ tj-GAL4

REAGENT or RESOURCE SOURCE IDENTIFIER

Cloning of LglKA,FA Antisense (5’ to 3’: AGACGT AAAGTCAAGACACACTTTGTGTCATCGCTGG) This paper N/A

Recombinant DNA

pENTR-Lgl Carvalho et al., 2015 N/A
pENTR-Lgl3A Carvalho et al., 2015 N/A
pMTW-Dendra Gilles Hickson, University Montréal, Canada N/A
pHWG (Drosophila Gateway Vector Collection) Drosophila Genomics Resource Center Gateway Collection
pHWR (Drosophila Gateway Vector Collection) Drosophila Genomics Resource Center Gateway Collection
pUAst.attb.WG Brogna. S. N/A
pMT-Lgl-Dendra2 This paper N/A
pHW-Lgl-GFP Carvalho et al., 2015 N/A
pHW-Lgl3A-RFP This paper N/A
pUAst.attb.LglK327A,F331A-GFP This paper N/A
pMAL-LGL402-802 Knoblich, J.A. (Betschinger et al., 2003) N/A

Software and Algorithms

ImageJ/FUI Schindelin et al., 2012 https://fiji.sc/
Icy Quantitative Image Analysis Unit at Institut Pasteur http://icy.bioimageanalysis.org
EpiTools Heller et al., 2016 N/A
MATLAB version R2016a MathWorks RRID: SCR_001622
GraphPad Prism GraphPad Software https://www.graphpad.com/scientific-software/prism/
PROMALS3D N/A http://prodata.swmed.edu
Jalview 2.10.5 N/A http://www.jalview.org

Other

Confocal microscope Leica Microsystems, Germany TCS SP5 II
Spinning Disk Confocal Microscope Olympus, UK Andor Revolution XD
**UAS-AurA**: w; UAS-Lgl-RFP/ tj-GAL4; UAS-AurA-GFP/+  
**UAS-AurAΔAb**: w; UAS-Lgl-RFP/ tj-GAL4; UAS-AurAΔAb-GFP/+  

**Figure 2**

A) hs-FLP nls-RFP FRT19/hs-FLP tub-Gal80 FRT19; tj-GAL4/+; UAS-Pp1-87B RNAi/+  
B) hs-FLP ubi-RFP-nls FRT19/FLP; tj-GAL4/+; +/+  
hs-FLP/+; FRT82 RFP/ FRT82 pp1α-96A2  
hs-FLP/+; FRT82 RFP/ FRT82 pp1-87B87Bg-3 e pp1α-96A2  
C) w; tj-GAL4/+; UAS-Pp1-87B RNAi/+  
w; tj-GAL4/+; UAS-Pp1-87B RNAi/+  
D) w; tj-GAL4 Lgl-GFP/+; UAS-NiPp1-HA/+  
**Figure 3**

A) w; tj-GAL4/CyO; UAS-Pp1-87B RNAi/+  
w; tj-GAL4/ aPKC-GFP; UAS-Pp1-87B RNAi/+  
C) w; tj-GAL4/UAS-Lgl-GFP  
w; tj-GAL4/UAS-LglΔF331A-GFP  
D) w; tj-GAL4/+; UAS-LglΔF331A-GFP  
w; tj-GAL4/UAS-LglΔF331A-GFP  
E) hs-FLP/+; IgF733 FRT40 UAS-LglK327A,F331A-GFP/ tub-Gal80 FRT40; GR1-GAL4/+  
(lgl homozygous mutant cells are the only ones expressing UAS-LglK327A,F331A-GFP)  
F) hs-FLP/+; IgF733 FRT40/ RFP FRT40  
hs-FLP/+; IgF733 FRT40 UAS-LglK327A,F331A-GFP/ RFP FRT40; GR1-GAL4/+  
(lgl homozygous mutant cells are marked by absence of RFP in a tissue with general expression of UAS-Lgl transgenes)  

**Figure 4**

A,B) w; tj-GAL4/+; UAS-Lgl-GFP, HisRFP/+  
w; tj-GAL4/+; UAS-Pp1-87B RNAi/UAS-Lgl-GFP, HisRFP  
w; tj-GAL4/UAS-sds22 RNAi; UAS-Lgl-GFP, HisRFP/+  
C,D) w; tj-GAL4/UAS-LglK327A,F331A-GFP/+  
w; tj-GAL4/UAS-LglK327A,F331A-GFP; UAS-sds22 RNAi;  
E) aPKC-GFP; Lgl-mCherry  
F,G) aPKC-GFP/+; lgl4 or ts3 /+; HisRFP/+ (control)  
aPKC-GFP/+; lgl4 or ts3 /+; HisRFP/+ (lgl temperature sensitive)  

**Figure S1**

A) embryos from females: matu4-GAL4 crossed with males: UAS-Lgl-GFP  

**Figure S2**

A) w (control)  
B) w; tj-GAL4/+; UAS-mts RNAi/+  
C) w; tj-GAL4/UAS-flw RNAi; +/+  
D) w; pp1-87B87Bg-3 e pp1α-96A2 / Df(3R)Exel7357  

**Figure S3**

aPKC-GFP/+; CyO/+; UAS-Pp1-87B RNAi/+ (Control)  
aPKC-GFP/+; tj-GAL4/+; UAS-Pp1-87B RNAi/+ (PP1-87B RNAi)  

**Figure S4**

B) Embryos from females: matu4-GAL4 crossed with males: UAS-Lgl-GFP or UAS-LglKAPA-GFP  
C) aPKC-GFP/+; lgl4 or lglts3/++ (Control)  
aPKC-GFP/+; lglts3/lglts3 (lgl temperature sensitive)  

**Drosophila cell culture**

Drosophila Schneider 2 (S2) cells obtained from Drosophila Genomics Resource Center, Indiana University (DGRC) were cultured in Schneider’s Drosophila media supplemented with 10% FBS (fetal bovine serum), at 25°C.
METHOD DETAILS

S2 cell stable lines and RNA interference
Stable S2 cell lines co-expressing Lgl-GFP and mCherry-Tubulin (Carvalho et al., 2015), co-expressing Lgl3A–RFP and Lgl-GFP, or expressing Lgl-Dendra2 were generated using either Cellfectin® II reagent or Effecten Transfection Reagent, according to manufactures’ instructions. Gene expression was induced at 37°C for 1 hour in cells expressing Lgl under heat-shock promoter (plasmids: pHW-Lgl-GFP and pHW-Lgl3A–RFP). For double-strand RNAi production, genomic DNA was amplified using primers containing a T7 RNA polymerase-binding site flanked by gene specific sequences (see Table S1). The length of sequences amplified was: 362 bp of aurA, 311 bp of aPKC, 511 bp of sds22 and 614 bp of Pp1-87B. Megascript® T7 kit was used to transcribe dsRNA from PCR amplified sequences. For RNAi treatment, S2 cells in an exponential growth phase were seeded in a serum-free Schneider media (at a concentration of 1 x 10^6 cells/ml) and incubated with 30 μg dsRNAi (15 μg in case of Pp1-87B) for 1 hour. After that, 2 mL of Schneider’s growth media supplemented with 10% of FBS was added and cells analyzed after 96h or 120h.

Preparation of ovary and S2 cell protein extracts
To prepare ovary protein extracts, Drosophila ovaries were disrupted through sonication in Lysis Buffer (150 mM KCl, 75 mM HEPES, pH 7.5, 1.5 mM EGTA, 1.5 mM MgCl₂, 15% glycerol, 0.1% NP-40) containing 1x protease inhibitors cocktail (Roche) and 1x phosphatase inhibitors cocktail 3 (Sigma). For protein extracts from S2 cells, harvested cells were washed with PBS 1x supplemented with Protease Inhibitors Cocktail (Roche). Lysis buffer was then added and cells were disrupted by sonication. Samples were then centrifuged and supernatant was collected for analyses.

Immunofluorescence and F-actin staining
Drosophila ovaries were fixed using a 4% paraformaldehyde solution (in PBS), followed by washing steps with 0.05% PBT (PBS with 0.05% Tween® 20 (Sigma), 1 hour-blocking with 10% BSA in 0.2% PBT, and overnight incubation at room temperature with primary antibodies diluted in 0.05% PBT (supplemented with 1% BSA). Ovaries were then washed with 0.05% PBT containing 1% BSA, incubated 2 hours with the secondary antibody and washed with 0.05% PBT before mounting in Vectashield with DAPI. The primary antibodies used are indicated in the key resources table. F-actin staining was performed by adding Phalloidin-TRITC at 1 μg/mL to the paraformaldehyde solution during the fixation.

Cloning of Lgl variants
Site-directed mutagenesis in the PP1 - docking site of Lgl was performed using primers containing the desired mutations (see Key Resources Table) and pENTR-Lgl as template (Carvalho et al., 2015). PCRs were performed using Phusion High Fidelity DNA polymerase, followed by a digestion of the template plasmid with DpnI, and transformation of competent bacteria. The Gateway Cloning System (Life Technologies) was used to generate the following plasmids: pMT-Lgl-Dendra2 - recombination of pENTR-Lgl with pMT-W-Dendra2 (gift of Gilles Hickson, University Montréal, Canada); pHW-Lgl3A–RFP - recombination of pENTR-Lgl3A (Carvalho et al., 2015) with pHWR; pUASt.attb.LglK327A,F331A-GFP, upon recombination of pEntr-LglK327A,F331A with pUASt.attb.WG or expressing Lgl-Dendra2 were generated using either Cellfectin® II reagent or Effecten Transfection Reagent, according to

RT-PCR
Total RNA was isolated using RNeasy Mini Kit from RNAi depleted and control S2 cells (3x10^6 cells) following the manufacturer’s protocol. RNA samples were then used to synthetize cDNA using SuperScript™ II Reverse Transcriptase and then PCRs for the required genes were performed using DreamTaq DNA polymerase and primers listed in the Key Resources Table. Amplified PCR products were resolved in 1% agarose gel electrophoresis.

Protein purification
MBP–Lgl402-802 protein was expressed in BL21 Star E.coli transformed with pMAL-Lgl402-802 ((Betschinger et al., 2003), Gift of Jurgen Knoblich (IMBA, Vienna, Austria)) and purified using amylose magnetic beads according to the manufacturer’s instructions (New England Biolabs).

in vitro dephosphorylation assays
For in vitro dephosphorylation assays using Lgl from ovary extracts (Figure 2F), Lgl-GFP was immunoprecipitated from ovary extracts using GFP-Trap_MA system according to the manufacturer’s instructions. Lgl bound to the beads was then incubated with 200 ng of AurA in kinase reaction buffer (5 mM MOPS, pH 7.2, 2.5 mM β-glycerol-phosphate, 5 mM MgCl₂, 1 mM EGTA, 0.4 mM EDTA, 0.25 mM DTT, 100 μM ATP) during 30 min at 30°C to saturate Lgl phosphorylation status. The kinase reaction mix was removed and the phosphatase reaction mix was added, containing 2 μM MLN8237 for inhibition of the AurA kinase, PMP phosphatase buffer (New England Biolabs) containing 50mM HEPES pH 7.5, 100 mM NaCl, 2 mM DTT, 0.01% Brij 35, 1 mM MnCl₂ and respective amounts of recombinant human GST-PP1γ. For in vitro dephosphorylation assays using recombinant MBP-Lgl (Figure 2E and Figure S2E), the purified protein was first phosphorylated using the kinase assay performed as described above. Dephosphorylation
was then performed in a reaction solution containing 2 μM MLN8237, PMP phosphatase buffer containing 1 mM MnCl₂ (New England Biolabs), and 1U of either Protein Phosphatase 1 or Lambda PP (Figure S2E) or GST-PP1γ (Figure 2E). Dephosphorylation reactions were carried at 30 °C for 30 min and stopped by addition of SDS Laemmli buffer. p-Lgl656 antibody was used to detect phosphorylation in western blot, whereas anti-GFP, anti-MBP-HRP conjugated or anti-GST were performed to control for the amount of Lgl-GFP, MBP-Lgl or PP1-GST, respectively.

**Photoconversion experiments in S2 cells**
For photoconversion experiments, expression of Lgl-Dendra2 in S2 cells was induced with 500 μM CuSO₄ for at least 12h before live imaging, and cells were plated in glass bottom microwell dishes (MaTek) coated with Poly-L-Lysine. We used Leica TCS SP5 II (Leica Microsystems) confocal microscope and a 405 nm UV laser to perform photoconversion of Lgl-Dendra2. Initial frames were taken using 488 nm and 561 nm lasers to validate photoconversion. Time-lapse stacks covering all the dividing cell were then taken using the 561 nm laser every 2 min, for at least 30 min after anaphase onset.

**Multiple sequence alignments**
Multiple sequence alignment of Lgl proteins from several species was performed using CLUSTAL format by PROMALS3D (http://prodata.swmed.edu) and the aligned amino acid sequences of the putative PP1-docking site motif were visualized using Jalview 2.10.5 (http://www.jalview.org). Protein sequences used were the following: *H. sapiens* LLGL1 (UniProt:Q15334); *H. sapiens* LLGL2 (UniProt:Q6P1M3); *D. rerio* LLGL1 (UniProt:E7FD67); *D. rerio* LLGL2 (UniProt:Q7SZE3); *S. purpuratus* predicted L2GL2 (NCBI: XP_011678406.1); *D. melanogaster* L2GL (UniProt:P08111); *N. vectensis* predicted protein (UniProt:A7RKR7); *A. queenslandica* uncharacterized protein (UniProt:A0A1X7U8H8); *S. cerevisiae* SRO7 (UniProt:Q12038).

**Imaging**
Fixed samples were analyzed using a Leica TCS SP5 II (Leica Microsystems) confocal microscope with HC PL APO CS 40x/NA 1.10 objective or HC PL APO CS 63x/1.30 Glycerine and a LAS 2.6 software. Live imaging was performed using a spinning disc confocal system (Andor Revolution XD) equipped with an electron multiplying charge coupled device camera (iXonEM++; Andor) and a CSU-22 unit (Yokogawa) based on an inverted microscope (IX81; Olympus) with a PLAPON 60x/NA 1.42 objective using iQ software (Andor). Z stacks were collected with serial optical sections separated by 0.5-1 μm. For live imaging of the *Drosophila* follicular epithelia, ovarioles were dissected and incubated in Schneider’s media supplemented with 10% FBS and 200 μg/ml insulin (Sigma-Aldrich). Egg chambers with low expression levels of Lgl-RFP (Figures 1E and 1F) were imaged in 10S VOLTALEF® (VWR chemicals) oil for optimization of the fluorescence signal to noise. Live imaging of *Drosophila* embryos (5 to 7 hours of development; stage 10 to stage 11) was performed by immobilizing the embryos in glass bottom microwell dishes (MaTek) containing heptane glue and filled with Halocarbon oil (700) (Sigma-Aldrich). S2 cells were plated on poly-L-lysine-coated culture dishes for live imaging (MaTek). Live imaging experiments were performed at 25 °C, except in experiments using the Lgl temperature sensitive mutants (lgts3), which were filmed at 29 °C.

**QUANTIFICATION AND STATISTICAL ANALYSIS**
Image processing and quantifications were largely performed using FIJI (Schindelin et al., 2012). All the statistical analysis and graphs were performed using GraphPad Prism 6.

**Cytoplasmic decay of Lgl**
Cytoplasmic decay of Lgl at mitotic exit in follicular epithelia, S2 cells and embryonic epithelia was quantified by measuring the mean pixel intensity of a manually tracked ROI in the cytoplasm (12x 12 ROI in S2 cells, 8x8 ROI in the follicular epithelia and 6x6 ROI in embryonic epithelia; 1 px = 0.13 μm) in sum-intensity Z projections. To overcome large signal fluctuation that arise from low signal intensity, we averaged 3 consecutive time points in epithelial cells (separated by 30 s) and 2 consecutive time points in S2 cells (separated by 90 s). Background subtracted mean intensity values were presented as a percentage of the anaphase value [average of the first two (in S2 cells) and three (in epithelia) frames after anaphase onset]. Since Lgl invariably reaches a steady-state with negligible cytoplasmic intensity value in control or upon AurA overexpression/AurA RNAi, the average of the cytoplasmic intensity of the first two frames since anaphase was set to 0% and the average intensities in the plateau of accumulation was set to 100%.

**Lgl accumulation in daughter-daughter interface**
To quantify the kinetics of Lgl accumulation in the daughter-daughter interface in epithelia, time-lapse images were aligned using the “StackReg” plugin and we manually tracked the interface to quantify mean intensity values since anaphase. The background subtracted mean intensity value of Lgl in the daughter-daughter interface or dividing cell equator (before cytokinesis) was normalized to the background subtracted mean intensity in the cytoplasm. The resulting anaphase value (average of the first three frames since anaphase) was set to 0% and the average intensities in the plateau of accumulation was set to 100%.
Intensity of Lgl-Dendra2 upon photoconversion

To quantify the overall intensity of Lgl-Dendra2 at mitotic exit after photoconversion (Figure 1D) we used FIJI to perform a Z sum-projection of all optical sections covering the cell. We then used the active contour protocol of Icy (http://icy.bioimageanalysis.org) for automated cell segmentation. For each time-point, we calculated the background subtracted integrated density (cell area x mean intensity value), which is normalized to the value at anaphase onset (100%).

aPKC accumulation in the apical interface formed between daughter cells

To quantify the accumulation of aPKC in the apical daughter-daughter interface (Figure 4F), we used the image analysis toolkit EpiTools (Heller et al., 2016) to generate selective plane projections of the apical area of the follicular epithelium. The aPKC-GFP signal was then manually tracked in: 1) the equator of dividing cell/daughter-daughter interface (10 pixel width line); 2) four non-dividing neighboring cell interfaces (10 pixel width line); 3) three circular ROI with 8 pixel diameter in the cytoplasm, which is used for background subtraction. We generated a custom FIJI script to measure the background subtracted mean intensities of the new daughter-daughter interface and of the non-dividing interfaces, and to normalize the signal of aPKC in the new daughter-daughter interface to the average of non-dividing ones.

Interphase cortical/cytoplasmic intensity ratio of Lgl in S2 cells

To measure the ratio of cortical to cytoplasmic mean intensity of Lgl in S2 cells during interphase (Figure 2G), we used a FIJI macro for automated segmentation of each cell contour (CC) and measured the mean intensity of the region between CC - 1 pixel and CC – 5 pixel (1 px = 0.13 μm) as the cortical intensity. Cytoplasmic intensity was measured using a circular ROI in the cytoplasm. Ratio was obtained after background subtraction.