An Atypical MAGUK GK Target Recognition Mode Revealed by the Interaction between DLG and KIF13B

Highlights
- KIF13B MBS interacts with DLG GK in a phosphorylation-independent manner
- KIF13B MBS forms a folded domain instead of a linear peptide when bound to DLG GK
- Bindings of KIF13B MBS and phosphor-LGN to DLG GK are mutually exclusive
- Phosphorylation-independent target binding is another general property of MAGUK GK

In Brief
The guanylate kinase (GK) domain of DLG, a membrane-associated guanylate kinase (MAGUK) scaffold protein, binds to the cargo binding tail of the KIF13B kinesin. Zhu et al. find that, unlike other known GK interactions, binding of DLG GK does not require KIF13B phosphorylation, revealing a unique binding mode for MAGUK GK.
An Atypical MAGUK GK Target Recognition Mode Revealed by the Interaction between DLG and KIF13B

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SUMMARY

The membrane-associated guanylate kinase (MAGUK) scaffold proteins share a signature guanylate kinase (GK) domain. Despite their diverse functional roles in cell polarity control and synaptic signaling, the currently known mode of action of MAGUK GK is via its binding to phosphorylated short peptides from target proteins. Here, we discover that the GK domain of DLG MAGUK binds to an unphosphorylated and autonomously folded domain within the stalk region (MAGUK binding stalk [MBS] domain) of a kinesin motor KIF13B with high specificity and affinity. The structure of DLG4 GK in complex with KIF13B MBS reveals the molecular mechanism governing this atypical GK/target recognition mode and provides insights into DLG/KIF13B complex-mediated regulation of diverse cellular processes such as asymmetric cell division. We further show that binding to non-phosphorylated targets is another general property of MAGUK GKs, thus expanding the mechanisms of action of the MAGUK family proteins.

INTRODUCTION

Membrane-associated guanylate kinases (MAGUKs) are a family of scaffold proteins concentrated at the sites of cell-cell junctions, playing critical roles in diverse cellular processes, including establishment and maintenance of cell polarity, cell proliferation and differentiation, and synaptic signal transduction (Elias and Nicoll, 2007; Funke et al., 2005; St Johnston and Ahrringer, 2010; Zhu et al., 2016). Discs large homologs (DLGs) are founding members of the MAGUK family proteins, with multiple protein-protein interaction domains capable of organizing many different protein complexes with broad cellular functions. For example, the DLG/LGL/SCRIB ternary complex, often acting cooperatively with the Crb/PALS1/PATJ and the PAR3/PAR6/ aPKC complexes, is essential for both the development and maintenance of polarities of cells in diverse tissues (Assemat et al., 2008; Chen and Zhang, 2013; Rodriguez-Boulan and Macara, 2014). Defects in DLG/LGL/SCRIB complex formation lead to loss of cell polarity and cancer formation (Bilder et al., 2000; Humbert et al., 2008). In addition to functioning as cell polarity regulators in epithelia, DLG-mediated protein complexes (e.g., the DLG/LGN complex) are essential for controlling cell-fate specification and epithelial architecture by regulating mitotic spindle orientations both in symmetric and asymmetric cell division, and thus are critical for stem cell development and tissue homeostasis in organs/tissues such as brain, muscle, skin, kidney, etc. (Albertson and Doe, 2003; Ben-Yair et al., 2011; Lu and Johnston, 2013; Morin and Bellaiche, 2011; Williams et al., 2014). Mechanistically, formation of these protein complexes is mediated by the guanylate kinase-like (GK) domain of DLG MAGUK, which evolved from the GK that catalyzes phospho-transfer from ATP to GMP (Johnston et al., 2012; Zhu et al., 2011, 2012). Binding of DLG GK to LGN and lethal giant larvae (LGL) strictly requires phosphorylation of short linear peptide sequences in LGN and LGL. Phosphorylated LGN and LGL peptides occupy the evolutionarily conserved binding sites on DLG GK. Structural biology studies indicated that binding to phosho-proteins is likely to be a common feature for most GK domains of MAGUKs including DLGs, calcium/calmodulin-dependent serine protein kinase (CASK), palmitoylated membrane proteins (MPPs), and membrane-associated guanylate kinase inverted (MAGIs) (Zhu et al., 2011, 2012).

Recently, a spindle capture pathway, which involves the Pins/ DLG/Khc73 complex, was identified to play critical roles in the telophase rescue process of asymmetric cell division (ACD) in Drosophila neuroblasts (Johnston et al., 2009; Lu and Prehoda, 2013; Siegrist and Doe, 2005). In this context, in addition to phosphorylated Pins, DLG GK also binds to Khc73 (KIF13B or GAKIN in mammals), a kinesin-3 family motor protein concentrated at the plus-end tips of astral microtubules. The Pins/ DLG/Khc73 complex, which serves as an adaptor bridging apical cell cortex and astral microtubules, was sufficient to promote the mitotic spindle orientation in Drosophila S2 cells engineered with an induced polarity assay system (Johnston et al., 2009). Loss of DLG or Khc73 activity led to oriented cell division defects in neuroblasts (Siegrist and Doe, 2005). In mammals, DLG and KIF13B are also known to interact directly with each other (Asaba
et al., 2003; Bolis et al., 2009; Hanada et al., 2000; Kanai et al., 2014). The interaction between DLG1 and KIF13B has been shown to be required for transporting DLG1 to paranodes of Schwann cells and for proper myelination of axons (Bolis et al., 2009). In neurons, KIF13B is enriched at the tips of growing axons and can promote axon formation (Yoshimura et al., 2010). Mutations of KIF13B have been implicated in autism (Li et al., 2015). The formation of the DLG1/KIF13B complex has also been indicated for polarized transport of DLG1 in epithelial cells and T lymphocytes (Asaba et al., 2003; Hanada et al., 2000). Moreover, DLG1 has been shown to function as the adaptor involved in KIF13B-mediated endocytosis of low-density lipoprotein receptor-related protein 1 (LRP1) (Kanai et al., 2014). However, little is known about the molecular basis governing DLG/KIF13B complex formation and the potential connections of this interaction with the reported phosphorylation-dependent binding of DLG GK to targets such as LGN, SAP90/PSD-95-associated proteins (SAPAPs), and LGL (Zhu et al., 2011, 2014), suggesting that the known phosphorylated targets of MAGUKs such as LGN, LID, and GMP-binding (GMP-BD) subdomains (Figure 2B and 3B). Amino acid sequence alignment analysis of the MBS domains from different members of the kinesin-3 motors shows the typical GK domain architecture, comprised of the CORE, LID, and GMP-binding (GMP-BD) subdomains (Figure 2B). Unexpectedly, KIF13B MBS forms a mixed α helix/β strand fold instead of a previously predicted coiled-coil conformation, and binds to the previously identified phospho-peptide binding site of DLG GK (see below for details). As such, although with totally different structures, the KIF13B binding pocket and the phospho-target binding pocket on DLG GK partially overlap (Figures 2B and 2C).

Overall Structure of the DLG4 GK/KIF13B MBS Complex
To elucidate the molecular details governing the DLG/KIF13B interaction, we solved the crystal structure of the DLG4 GK/KIF13B MBS complex at 2.7-Å resolution (Table 1). Consistent with our biochemical data shown in Figure 1C, each asymmetric unit of the crystal contains one complex with 1:1 stoichiometry (Figures 2A and 2B). The overall structure of the DLG4 GK/KIF13B MBS complex has a mushroom-like shape, with DLG4 GK and KIF13B MBS constituting the cap and stalk of the mushroom, respectively (Figure 2A). In the complex, DLG4 GK adopts the typical GK domain architecture, comprised of the CORE, LID, and GMP-binding (GMP-BD) subdomains (Figure 2B). Further truncations (Figures 2A and 3B). Amino acid sequence alignment analysis of the MBS domains from different members of the kinesin-3 motors shows that both the secondary structures (except for αD) and the key residues forming the folding core of KIF13B MBS are highly conserved (Figures 3A and S1), indicating that all other kinesin-3 family members likely contain a similar folded MBS domain observed in KIF13B.

Structure of the KIF13B MBS Domain
All members of the kinesin-3 family motors (KIF1, KIF13, and KIF14) contain an MBS domain following the Forkhead-associated (FHA) domain. In the complex with DLG GK, KIF13B MBS adopts a compact folded structure, composed of four α helices (αA–D) and three antiparallel β strands (βA–C) (Figures 3A and 3B). The robust interaction between DLG4 GK and KIF13B MBS is mainly mediated by extensive polar and hydrophobic interactions, burying a total of 825 Å² of accessible surface area (Figures 4A and 4B). A large number of polar residues from the C-terminal half of αC and throughout the entire αD of KIF13B MBS contribute to the binding to DLG4 GK (Figures 4B and S2). Specifically, the side chain of Y783MBS from αD forms a
hydrogen-bonding interaction network with R571GK, Y573GK, and E769MBS from a C. The side chain of E769MBS also forms hydrogen bonds with Y604GK and N605GK (Figures 4B and S3). The side chain of S561GK forms hydrogen bonds with K785MBS and R786MBS, the latter in turn participates in the hydrogen bond network with the main chains of R578GK and D579GK. Several charge-charge interactions also contribute to the DLG4/KIF13B interaction (Figures 4B and S3). For example, R781MBS forms a salt bridge with D629GK, and R759MBS forms a salt bridge with E572GK. Consistent with these structural analyses, mutations that disrupt these polar interactions invariably weakened or even disrupted the DLG4/KIF13B interaction (Figure 4C). Notably, the R786E mutant that disrupted the interaction between the MBS domain and the GK domain also abolished the association between DLG4 GK and the full-length KIF13B, suggesting that the MBS domain is the necessary and sufficient region for KIF13B to bind to DLG4 GK (Figure 4D). In addition to these polar interactions, the hydrophobic residues from a D

Figure 1. KIF13B MBS Directly Interacts with DLG GK
(A) A schematic diagram showing the domain organization of LGN, DLG4, and KIF13B. Interactions between LGN, DLG4, and KIF13B are indicated by a two-way arrow.
(B) GST-pull-down-assay-based analyses of the interactions between various Flag-tagged KIF13B fragments and DLG4 GK expressed in heterologous cells.
(C) Pull-down assay showing that GST-DLG4 GK robustly binds to Trx-KIF13B MBS.
(D) Analytical gel filtration analysis showing that KIF13B MBS and DLG4 GK formed a 1:1 stoichiometric complex. The elution volumes of the molecular mass standards are indicated at the top of the panel.
(E) Isothermal titration calorimetry of purified Trx-KIF13B MBS and Trx-DLG4 GK, indicating a complex with a dissociation constant of 70.0 ± 0.6 nM.
of KIF13B MBS, such as C773MBS, V779MBS, S780MBS, and Y783MBS, insert into the hydrophobic pocket of DLG4 GK formed by P564GK, Y580GK, Y604GK, Y609GK, and T611GK, further enhancing their binding (Figures 4B and S3). Parallel to the above analysis, substitution of Y783MBS with Ala decreased binding of KIF13B MBS to DLG4 GK by ~200 fold. Substitution of Y604GK with Ala abolished the interaction (Figure 4C).

Since most of the binding sites on KIF13B MBS are located at φD and binding of GK to phospho-targets requires only a short peptide fragment, one might wonder whether the φD of MBS may provide the majority of the binding energy for the DLG4/KIF13B interaction (Figure 4B). To test this possibility, we purified the KIF13B fragment encompassing the φD of MBS (KIF13B-φD, aa 774–793; Figure S4A) and measured its interaction with DLG4 GK. KIF13B-φD did not bind to DLG4 GK (Figure S4B), suggesting that the integration of φD to the folded MBS domain structure is essential for KIF13B to bind to DLG4 GK. Consistent with this interpretation, substitution of F784MBS (a key residue for coupling φD to the folding core of MBS but not directly involved in DLG4 GK binding; Figure S4C) into Gly abolished the DLG4 GK/KIF13B MBS interaction (Figure S4D). We chose to substitute F784MBS with a Gly as the residue at the corresponding position of KIF13A MBS is a Gly (Figure S4A). Interestingly, key residues on φD of MBS involved in the DLG4/KIF13B interaction are not conserved among the kinesin-3 family proteins (Figure S4A), indicating that binding to DLG GK is likely to be highly specific for KIF13B. Indeed, no detectable binding could be observed between KIF13A MBS and DLG4 GK (Figure 4E), although the rest of their MBS regions are highly similar. The above observation also indicates that small amino acid sequence variation in the MBS regions can diversify the cargo binding capacities of the kinesin-3 motors.

**DISCUSSION**

The most important finding of this study is that the high-affinity binding of KIF13B MBS to DLG GK does not require phosphorylation of KIF13B. Our study suggests that, in addition to recognizing phosphorylated targets, MAGUK GKs can also recognize non-phosphorylated targets. Since the interaction between DLG GK and KIF13B MBS does not need the KIF13B to be phosphorylated, this interaction would be constitutively present as long as the two proteins can meet each other. In contrast, bindings of MAGUK GKs to targets such as LGN, SAPAP, and LGL require the two proteins can meet each other. In contrast, bindings of MAGUK GKs to targets such as LGN, SAPAP, and LGL require phosphorylation. For example, Tyr783 in KIF13B MBS plays a critical role in the formation of the GK/MBS complex (Figures 5B and 5C). In particular, Y783MBS occupies the residue at the corresponding position of KIF13A MBS is a Gly (Figure S4A). Interestingly, key residues on φD of KIF13B MBS occupy the same region in the DLG GK target binding pocket (Figure 5A). A close-up view of the GK domain in two complexes reveals that several residues from the GMP-BD subdomain of GK are critical for both binding to pLGN and MBS (Figures 5B and 5C). In particular, Y783MBS occupies the same position as the phosphoserine (S0) in pLGN, and R786MBS and R(−2)pLGN show the same binding mode in both complex structures (Figure 5B). The above analysis indicates that DLG GK cannot bind to pLGN and KIF13B MBS simultaneously. Indeed, pLGN can compete with KIF13B MBS in binding to DLG GK in a dose-dependent manner (Figure 5D). Thus, the bindings of KIF13B MBS and phospho-LGN to DLG GK are mutually exclusive.

**Binding of KIF13B MBS and Phospho-LGN to DLG GK Is Mutually Exclusive**

We next asked whether DLG GK can bind to pLGN and KIF13B simultaneously. Firstly, we compared the structures of DLG1/pLGN and DLG4/KIF13B complexes. The conformation of GK in the DLG1/pLGN and DLG4/KIF13B complexes is highly similar (root-mean-square deviation [RMSD] = 0.79 Å). The N-terminal half of the pLGN peptide (which also forms an α helix) and the φD of KIF13B MBS occupy the same region in the DLG GK target binding pocket (Figure 5A). A close-up view of the GK domain in two complexes reveals that several residues from the GMP-BD subdomain of GK are critical for both binding to pLGN and MBS (Figures 5B and 5C). In particular, Y783MBS occupies the same position as the phosphoserine (S0) in pLGN, and R786MBS and R(−2)pLGN show the same binding mode in both complex structures (Figure 5B). The above analysis indicates that DLG GK cannot bind to pLGN and KIF13B MBS simultaneously. Indeed, pLGN can compete with KIF13B MBS in binding to DLG GK in a dose-dependent manner (Figure 5D). Thus, the bindings of KIF13B MBS and phospho-LGN to DLG GK are mutually exclusive.
suggesting that phosphorylation of Tyr783 of KIF13B can disrupt its interaction with DLG.

The residues responsible for KIF13B MBS binding are essentially completely conserved among the members of the DLG family MAGUKs (Figure 5C). Therefore, all DLGs are expected to bind to KIF13B MBS with high affinity (data not shown). We noted that DLG3 has a Phe at the position corresponding to Tyr604 of DLG4. The hydroxyl group of Tyr604 of DLG4 GK forms a hydrogen-bonding network with E769MBS and N605GK, in addition to hydrophobic interactions (Figure 4B). Interestingly, although it does still bind, DLG3 GK binds to KIF13B MBS with an affinity ~400-folder weaker than that of DLG4 GK (data not shown), highlighting the importance of the key residues in the interaction interface for DLG4/KIF13B complex formation. We also notice that MPP5 (PALS1), another MAGUK member, shares essentially the same KIF13B MBS binding residues as DLGs. Satisfyingly, MPP5 (PALS1) GK was found to bind to KIF13B MBS with a strength comparable with that of DLG GK (Figure 5E). The residues corresponding to those in DLG GK for KIF13B MBS binding in CASK and MAGIs contain a small degree of variation. Interestingly, neither CASK GK nor MAGI3 GK showed detectable binding to KIF13B MBS (Figure 5E), indicating that binding of non-phosphorylated targets by MAGUK GKs may have higher specificity with respect to the mode observed in phosphorylation-dependent target binding. In conclusion, the findings described in this study significantly expand the target recognition mechanisms of the MAGUK GK domains. Given the extremely broad functional roles of MAGUKs in all multi-cellular eukaryotes, our results are likely to be helpful for future functional studies on this family of scaffold proteins in general.

Controlled coordination between the mitotic spindle orientation and intrinsic cell polarity cue(s) is critical for ACD during tissue development (Gonczy, 2008; Knoblich, 2008, 2010; Morrison and Kimble, 2006; Siller and Doe, 2009; Williams and Fuchs, 2013). The NuMA/LGN/DLG/Gα pathway has been shown to be critical to link the mitotic spindle complex to the cell polarity complex at membrane cortices in many tissue types (Kulukian and Fuchs, 2013; Lu and Johnston, 2013; Siller and Doe, 2009; Zhu et al., 2012). Another pathway that involves the LGN/DLG/KIF13B complex (the Pins/DLG/Khc73 in Drosophila) has also been shown to be essential for coordinating cell polarity and mitotic spindle orientation (Johnston et al., 2009; Siegrist and Doe, 2005). DLG is involved in both pathways; one is via binding to phosphorylated LGN (Pins) and the other is via constitutive binding to KIF13B (Khc73). Although the two DLG targets bind to DLG GK with totally different molecular mechanisms, their binding sites on DLG GK largely overlap. Therefore, phospho-LGN and KIF13B bind to DLG GK in a mutually exclusive manner (Figure 5D). How might the above two pathways function together in orchestrating spindle orientation during ACD? One likely mechanism is via formation of DLG oligomers at the cell cortices, so that DLG molecules can simultaneously engage phospho-LGN and KIF13B (Figure 6). DLGs and other MAGUK members are known to be able to form oligomers (Tavares et al., 2001; Zhu et al., 2016). Mechanistically, the SH3 domain and GK domain of MAGUKs can form domain-swapped intermolecular dimers or high-order oligomers (Hara et al., 2015; Li et al., 2014; McGee et al., 2001; Tavares et al., 2001; Zhu et al., 2016). Alternatively, DLG may adopt a sequential target binding model. In this model, the Insc/Par/LGN/Gα pathway initiates DLG cortical polarization via the DLG/plLG/N interaction at prometaphase/metaphase. During metaphase, astral microtubule
plus-end-enriched KIF13B takes over to bind to DLG to facilitate cortical astral microtubule capture. This process may be accompanied by de-phosphorylation of LGN. Thus, DLG/KIF13B interaction may provide a physical connection between the astral microtubule and Ga/LGN/NuMA/dynein complex to orchestrate fully activated spindle orientation in ACD (Lu and Johnston, 2013; Lu and Prehoda, 2013; Siller and Doe, 2009) (Figure 6). It should be emphasized that the structural data in the current study, together with data already published, prompted us to suggest possible models on how DLG may function in interfacing the cortical polarity complex with the mitotic spindle orientation complex during ACD. Further studies are required to test the models hypothesized in Figure 6.

**EXPERIMENTAL PROCEDURES**

**Protein Preparation**

The MBS domain (aa 677–798) of mouse KIF13B was PCR-amplified from the mouse brain cDNA library and cloned into modified pET32a vectors. The rat DLG4 GK domain (aa 531–713) and various mutants (Figure 4C) were individually cloned into modified pET15b or pET32a vectors. All the mutations were created using the standard PCR-based method and confirmed by DNA sequencing. Recombinant proteins were expressed in *Escherichia coli* BL21 (DE3) host cells at 16°C and were purified by using Ni²⁺-NTA agarose affinity chromatography followed by size-exclusion chromatography.

**GST Pull-Down Assay**

GST-tagged DLG4 GK (2 nM) was incubated with fresh purified Trx-KIF13B MBS at a molar ratio of 1:5 for 1 hr at 4°C. The mixture was then loaded onto 30 μL GSH-Sepharose 4B slurry beads in assay buffer (50 mM Tris [pH 8.0], 100 mM NaCl, 1 mM DTT, and 1 mM EDTA) for 0.5 hr at 4°C. After washing three times, the proteins captured were eluted by boiling, resolved by 15% SDS-PAGE, and detected by Coomassie brilliant blue staining.

**Size-Exclusion Chromatography**

Size-exclusion chromatography experiments were carried out on an AKTA FPLC system (GE Healthcare). Proteins at concentrations of 10–20 μM in a volume of 100 μL were loaded on a Superose 12 10/300 GL column (GE Healthcare) equilibrated with buffer containing 50 mM Tris (pH 8.0), 100 mM NaCl, 1 mM DTT, and 1 mM EDTA. Protein elution was detected by absorbance at 280 nm.

**Isothermal Titration Calorimetry**

Isothermal titration calorimetry measurements were performed on a VP-ITC calorimeter (MicroCal) at 25°C. All protein samples were in 50 mM Tris (pH 8.0), 100 mM NaCl, 1 mM DTT, and 1 mM EDTA buffer. The titration process was performed by injecting 10 μL aliquots of the MBS fragments into GK proteins at time intervals of 2 min to ensure that the titration peak returned to the baseline. The titration data were analyzed using the Origin7.0 program from MicroCal.

**Crystallography**

Crystals of the DLG4 GK/KIF13B MBS complex (10 mg/mL) were obtained by the hanging-drop vapor-diffusion method at 18°C. The GK/MBS complex crystals were grown in 1.0 M ammonium sulfate, 0.1 M HEPES (pH 7.0), 0.5% w/v polyethylene glycol 8000. Crystals were soaked in crystallization solution containing 25% glycerol for cryo-protection. The diffraction data were collected at 0.97915 Å from a single crystal from Shanghai Synchrotron Radiation Facility. The data were integrated and scaled using HKL2000 (Otwinowski and Minor, 1997). Molecular replacement was used to solve the complex structure using
Figure 4. The Interface of the DLG4 GK/KIF13B MBS Complex
(A) Semitransparent surface representation of the GK/MBS complex with the underlying backbone structure showing (left). The flipped open interaction interface of the GK/MBS complex is also shown (right). GK and MBS have been rotated and separated as indicated by the arrows. Residues involved in binding to MBS on GK are shown in pale green. Residues involved in binding to GK on MBS are shown in purple.
(B) Detailed interface of the GK/MBS complex in stereo view. Dotted lines denote hydrogen bonds and salt bridge interactions.
(C) Summary of the binding between the wild-type and various DLG4 GK and KIF13B MBS mutants derived from isothermal titration calorimetry analyses.
(D) GST pull-down assay showed that DLG4 GK binds to full-length KIF13B but not the R786E mutant.
(E) GST pull-down assay showed that DLG4 GK specifically binds to KIF13B MBS but not KIF13A MBS. See also Figures S2–S4.
Figure 5. Competitive Binding of KIF13B and pLGN to DLG GK
(A) Ribbon diagram representation showing the superposition of the GKDLG1/pLGN (PDB: 3UAT) and GKDLG4/MBS complexes. The pLGN peptide is shown in blue with the phosphate group shown in red using the stick-and-ball model. The MBS domain of KIF13B is shown in purple. The side chain of Y783MBS is also shown in the stick-and-ball model.
(B) Comparison of the GKDLG1/pLGN and GKDLG4/MBS interfaces. A selected set of residues of GK, which are involved in binding both pLGN and MBS, are drawn in the stick model. pLGN and MBS are shown in blue and purple, respectively.
(C) Structure-based sequence alignment of the GMP-BD subdomains of GKs of MAGUK family proteins. Key residues involved in binding to KIF13B MBS and pLGN are indicated with purple dots and blue triangles, respectively. Residues coordinating the phosphate group of pLGN are highlighted in yellow. Highly conserved and conserved residues are colored in red and green, respectively.
(D) In vitro competition binding assay shows that pLGN dose-dependently inhibits the interaction between KIF13B MBS and DLG4 GK.
(E) GK domains of DLG4 and MPP5 (PALS1) but not CASK and MAGI3 bind to KIF13B MBS.
Figure 6. Proposed Model for the DLG/KIF13B Complex in the Spindle Orientation of ACD

During prometaphase/metaphase, cell intrinsic polarity cue(s) initiates cortical enrichment of the Insc/PAR/LGN/Gα complex, which in turn induces polarization of DLG at the apical cortex (via phosphorylation-dependent binding of LGN to DLG Gκ). Later, astral microtubule (astral MT) plus-end localized KIF13B facilitates the cortical microtubule capture pathway via direct interaction with DLG Gκ. Given the competitive binding of phospho-LGN and KIF13B to DLG described in this study, DLG cannot simultaneously bind to LGN and KIF13B. DLGs may form high-order oligomers via the intermolecular interactions between the SH3 domain and Gκ domain, thus linking the phospho-LGN and KIF13B. Alternatively, DLG may adopt a sequential target binding model. Insc/PAR/LGN/Gκ first recruits DLG to the apical cortex. Later, KIF13B takes over to interact with DLG, which provides an anchor site for astral MT at the cell cortex.

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Statistical Methods

This study does not involve statistical methods.

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