The Scribble Cell Polarity Module in the Regulation of Cell Signalling in Tissue Development and Tumourigenesis

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Summary

The Scribble cell polarity module, comprising Scribbled (Scrib), Discs-large (Dlg) and Lethal-2-giant larvae (Lgl), has a tumour suppressive role in mammalian epithelial cancers. The Scribble module proteins play key functions in the establishment and maintenance of different modes of cell polarity, as well as in the control of tissue growth, differentiation and directed cell migration, and therefore are major regulators of tissue development and homeostasis. Whilst molecular details are known regarding the roles of Scribble module proteins in cell polarity regulation, their precise mode of action in the regulation of other key cellular processes remains enigmatic. An accumulating body of evidence indicates that Scribble module proteins play scaffolding roles in the control of various signalling pathways, which are linked to the control of tissue growth, differentiation and cell migration. Multiple Scrib, Dlg and Lgl interacting proteins have been discovered, which are involved in diverse processes, however many function in the regulation of cellular signalling. Herein, we review the components of the Scrib, Dlg and Lgl protein interactomes, and focus on the mechanism by which they regulate cellular signalling pathways in metazoans, and how their disruption leads to cancer.

Introduction: Cell polarity regulation and tumourigenesis

Cell polarity involves the asymmetric distribution of macromolecules to specific membrane domains, which is essential for normal cellular function and morphogenesis during development in multicellular organisms [1-7]. There are four main types of cell polarity: 1) apico-basal cell polarity (ABCP, epithelial polarity) [1, 6-8], 2) planar cell polarity (PCP, polarity across the plane of an epithelium) [9-11], 3) asymmetric cell division (ACD, which is involved in the self-renewal of stem cells and differentiation of the daughter cells) [12, 13], and 4) front-rear cell polarity (FRCP, involved in directed cell migration) [14-16]. These polarity types are defined by the type of cells they regulate and the molecules and mechanisms involved. Additionally, specialised cells, such as lymphocytes and neurons, exhibit variations of the four main cell polarity mechanisms in order to establish and maintain specialized membrane domains for synapse formation, cell migration (eg T cell uropod formation) or asymmetric cell division [17-23]. Key to the establishment and maintenance of cell polarity in apico-basal, front-rear and ACD cell polarity types, are antagonistic interactions between two polarity modules. These are the Scribble module, comprising the scaffolding proteins, Scribbled (Scrib), Discs-large (Dlg) and Lethal-2-giant larvae (Lgl) [24, 25], and the Par module, containing two scaffolding proteins, Par3 and Par6, the protein kinase, atypical protein kinase C (aPKC) and the small GTPase, Cdc42 [8]. The Scribble module proteins are localized to the basolateral cortex in epithelial cells and each protein is required for the others proper localization [26, 27]. The Par complex proteins have well-defined direct physical interactions with each other [8, 28]. Although interactions have been observed between Dlg, Scrib and Lgl in mammalian systems [29, 30], this is less clear in other organisms, however in *Drosophila* an interaction between Scrib and Dlg1 (Dlg) has been observed in neural synapses through an adaptor protein, GUK-holder (Gukh) [31]. The antagonistic interaction between the Scribble and Par modules is mediated by aPKC binding to and phosphorylating Lgl, excluding Lgl from the plasma membrane, and conversely, Lgl binding to aPKC inhibits aPKC activity [32-39]. Lgl inhibits aPKC activity by binding to Par6 in the aPKC-Par6 complex and competing with the

binding of Par3 (Bazooka in *Drosophila*), as well as preventing membrane accessibility of the Par6-aPKC complex [32, 34, 35, 40]. The recruitment of Par3 to the Par6-aPKC complex enables aPKC-mediated phosophorylation of Numb (a cell fate determinant) in *Drosophila* neuroblast ACD [40], and presumably is required for aPKC phosphorylation of other substrates. E-cadherin-mediated cell-cell adhesion in apico-basal cell polarity in epithelial cells is also important in recruiting the Scribble module to the basolateral membrane, and the Scribble module is required for restricting the localization of apical proteins to the apical domain [26, 41-45]. Additionally, in apico-basal cell polarity in epithelial cells, the Par module interacts with another cell polarity module, the Crumbs (Crb) module, consisting of the transmembrane protein Crb, the scaffolding proteins, Pals and Patj [46], to regulate apical membrane identity [4, 47-49]. Here, a positive feedback loop is initiated, involving aPKC-mediated phosphorylation of Crb that promotes Crb apical clustering via its extracellular domains and prevents Crb endocytosis. In the basolateral domain, Lgl inhibits this positive feedback loop, thereby restricting apical determinants to the apical domain [47]. Additionally, in *Drosophila* organogenesis, the FERM-domain proteins, Coracle and Yurt, which form a complex with the membrane proteins, Neurexin IV and the Na^+/K^+-ATPase at basal lateral junctions, are involved in apico-basal establishment and exhibit antagonistic interactions with Crb [50, 51]. Yurt and aPKC also show antagonistic interactions, which depends on the phosphorylation of Yurt by aPKC [52]. Mammalian Yurt orthologs (Ymo1 and EHM2) also bind to Crb and Ymo1 is involved in lateral membrane formation in epithelial apico-basal cell polarity [50, 51], suggesting that this mode of cell polarity regulation might also be conserved in mammalian epithelial cells.

In addition to their role in cell polarity, the Scribble, Par and Crb modules also regulate cellular signalling pathways to control cell proliferation, survival and migration, and consistent with these roles, these cell polarity proteins are deregulated in cancer [2, 24, 53-60]. In human cancer, cell polarity gene expression/function can be perturbed by mutation or deregulated

gene expression [57], but also by alteration in protein localization and protein degradation [53, 54, 60-62]. Indeed, viral oncoprotein-mediated degradation of Dlg and Scrib proteins is associated with more aggressive cancers [63-67]. The tumour suppressive functions of the Scribble module genes are evolutionarily conserved. Indeed, Lgl, Dlg and Scrib were first discovered and characterized in the vinegar fly model organism, *Drosophila melanogaster,* where epithelial and brain tissues mutant in these genes, exhibit many hallmarks of cancer [26, 43, 68-76]. Loss of function of Scrib, Dlg or Lgl result in excessive cell proliferation and the formation of neoplastic tumours that show aberrant differentiation and cell morphology, leading to overgrown larvae unable to properly progress to the pupal stage of development. Subsequently, mammalian orthologs of Scribble module genes were shown to complement the corresponding *Drosophila* mutants, highlighting their role as tumour suppressors [77-79]. There are four mammalian orthologs of Dlg (Dlg1 (hDlg/SAP97), Dlg2 (PSD-93/Chapsyn-110), Dlg3 (NE-Dlg/SAP102) and Dlg4 (PSD-95/SAP90)), two of Lgl (Hugl/Llgl1 and Llgl2), and only one of Scrib [80], thus making the analysis of Scrib function in mammalian systems more amenable. Analysis of Scrib, Lgl1/2, Dlg1-4 in mammalian cell lines has supported their tumour suppressor role, in inhibiting cell proliferation and the epithelial-mesenchymal transition (EMT) [24, 25, 53, 80]. In mouse models, *scrib* knockout is embryonic lethal with neural closure defects, consistent with effects on PCP-regulated epithelial sheet migration [81]. Tissue specific tissue knockouts of *scrib* in the adult mouse leads to hyperproliferation and cell morphology changes, and predisposes cells to oncogenic-mediated tumourigenesis in the prostate, breast, lung and skin epithelial tissues [81-86]. Due to genetic redundancy, the analysis of Dlg and Lgl in epithelial tumourigenesis in mouse models has been less clear. However, analysis of Lgl1 knockout mice revealed that they develop neural-epithelial hyperplasia [87]. Conversely, Dlg1 (Dlgh1) knockout mice exhibited reduced cell proliferation and developmental defects in the urogenital system [88], and an independent Dlg1 mutation showed hypoplasia of the premaxilla and mandible and a cleft palate [89]. Another study, showed that Dlg1 null knockout mice exhibit open neural tube and open eyelids and other developmental defects [90], suggesting that Dlg1's function during development is more

important for the regulation of PCP-directed epithelial sheet migration and other PCP functions.

In this review, we will discuss the structures of Scribble module proteins, their molecular interactions with other proteins and how these are connected to signalling pathway regulation, primarily focusing on *Drosophila*, zebrafish and mammalian systems.

Scribble module protein structure and function

Although the Scrib, Dlg and Lgl proteins do not have intrinsic enzymatic activity, they all contain a number of well-characterized protein-protein interaction domains (described in more detail below) that enables them to bind to signalling proteins, such as kinases and phosphatases. Importantly, their function appears to be absolutely dependent on their cellular sublocalization. Hence, much of the currently known functions of the Scribble module proteins can be attributed to their role as signalling scaffold proteins that regulate site-specific signalling within the cell.

Scrib is a large multidomain scaffold protein and belongs to the LAP (LRR and PDZ) protein family [43, 91, 92]. It contains 16 Leucine-rich repeats (LRRs), two LAP specific domains (LAPSADa and LAPSADb) and four PDZ95, Dlg1, ZO1 (PDZ) domains (Figure 1, Supp Table 1). The N-terminal LRR domain and four PDZ domains (classified as type 1 PDZ domains) are important for proper localization of Scrib to the basolateral membrane of epithelial cells [42, 93, 94], with both domains key to allow Scrib to interact with a wide range of intracellular proteins (see below).

Dlg is a member of the membrane-associated guanylate kinase homolog (MAGUK) scaffolding protein family, and comprises three PDZ domains, a Src homology 3 (SH3) domain, a Hook domain and a guanylate kinase-like (GUK) domain at its C-terminus [69, 95] (Figure 2, Supp Table 2). In *Drosophila* epithelium, the Dlg PDZ2 domain is essential for correct localization to basolateral (septate) junctions, whilst the SH3 and Hook domains are critical mediators of Dlg localization to the cell membrane [96]. The C-terminal Guanylate Kinase (GUK) domain (residues 765-960) is catalytically inactive, however it is involved in protein-protein interactions (see below).

Similar to other key polarity regulators, Lgl is also a large multidomain protein comprising an N-terminal region featuring WD40 repeats, and a C-terminal region containing a polybasic region, harbouring aPKC and Aurora kinase phosphorylation sites [37, 38, 56, 97] (Figure 3, Supp Table 3). The structure of the WD40 repeat region of Lgl has not been determined, however the corresponding region in the yeast ortholog, Sro7, has been crystallized [98]. In Sro7, the WD40 region forms two seven-bladed WD40 β-propellers, with a 60-residue-long tail located C-terminally of the two β-propellers that is bound to the surface of the N-terminal propeller. Comparison of the Sro7 sequence with *Drosophila* and mammalian Lgl proteins, suggests that they also form the β-propeller structures [56].

Scribble module protein interactors

In addition to their roles in cell polarity, the Scribble module proteins interact with many proteins involved in different cellular processes, such as cell adhesion, membrane trafficking, cell migration and cellular signalling (see Figures 1-3, Supp Tables 1-3). Despite their common role in regulating cell polarity, Scrib, Dlg and Lgl interact with mostly distinct protein interactors. We will highlight below some examples of interacting proteins and the motifs within the Scrib, Dlg or Lgl proteins to which they bind.

Scrib: The Scrib N-terminal LRR domain has only been documented to interact with a few proteins, such as Llgl2, Sgt1/Hsp90, the phosphatase PHLPP1 (Pleckstrin homology and leucine rich repeat protein phosphatase) in mammalian cells and the BMP receptors (Tkv, Pnt), the BMP transcription factor, Mad, and the early endosomal protein, Rab5, in *Drosophila* [29, 99-101] (Figure 1, Supp Table 1). Conversely, the PDZ domains of Scrib are the major sites by which Scrib interacts with a plethora of proteins, including β-PIX and MCC involved in cell migration and β-catenin involved in cell adhesion [102-105] (Figure 1, Supp Table 1). How these many interactions are coordinated *in vivo* will be discussed in further detail below.

Dlg: Similar to Scrib, the PDZ domains of Dlg proteins interact with a plethora of proteins involved in diverse functions, including *Drosophila* Vang that regulates PCP [106] and human APC involved in cell adhesion [107] (Figure 2, Supp Table 2). By contrast, only a few proteins have been shown to interact with Dlg's SH3 domains, including human β-TrBP, an E3 ubiquitin ligase involved in cell adhesion, and human CASK, involved in cell polarity [108, 109]. Likewise, the HOOK domain only interacts with a few proteins, such as human band 4.1 protein involved in cell signalling [110] and *Drosophila* Dishevelled involved in spindle orientation in ACD [111]. The GUK domain interacts with several proteins, including Llgl2 in mammalian cells and the adaptor protein, Gukh in *Drosophila* [30, 31] (Figure 2, Supp Table 2).

Lgl: The greatest molecular insight on Lgl's interactions have come from the analysis of the yeast ortholog, Sro7. The C-terminal domain of Sro7 interacts with the t-SNARE protein, Sec9, via its N-terminal region, to regulate polarized exocytosis [112]. Furthermore, yeast-human Sro7-Lgl hybrid proteins can only rescue the yeast Sro7/ Sro77 deletion when the t-SNARE binding site is intact, suggesting that this interaction is an evolutionarily conserved function.

Interestingly, human Llgl2 also interacts with the SNARE protein, syntaxin-4, in mammalian cells [113] (Figure 3, Supp Table 3) and regulates polarized exocytosis, although the interaction domains have not been described. In Sro7, the tail segment acts to prevent the binding of Sro7 to the Qbc-SNARE region of Sec9, which inhibits the assembly of the SNARE complex to regulate polarized exocytosis. However, this tail region is not conserved in higher eukaryote Lgl proteins, but only in the Lgl-related protein, Tomosyn, which functions in exocytosis. In the regulation of t-SNARE activity, Sro7 is an effector of the Sec4 Rab-GTPase [114]. Structure-function analysis has revealed that Sec4 Rab-GTPase interacts with Sro7 via a binding site at the interface between the two β-propellers [115]. However, sequence comparison suggests that in higher eukaryotes this binding motif is only conserved in the Lgl-related protein Tomosyn, but not in Lgl [115].

Analysis of mammalian Lgl interacting proteins has identified the WD40 repeats, which are predicted to form the β-propeller structure, to mediate interaction with several proteins (Figure 3, Supp Table 3). These include *Drosophila* Par6, involved in cell polarity [35], the mammalian RanBPM and USP11 deubiquitining protein, involved in cell migration [116, 117], and the mammalian and *Drosophila* VprBP/Mahjong E3 ubiquitin ligase component, involved in cell proliferation [118, 119]. The C-terminal region of Lgl also interacts with several proteins, including *Drosophila* and mammalian Myosin II, which regulates actin-myosin contractility and cell migration [33, 120-122], and mammalian N-Cadherin, which regulates cell adhesion [123]. Moreover, Dlg4 binds to the aPKC phospho-site of Lgl in a phosphorylation-dependent manner [30].

General principles in Scribble module protein interactions

Given the multitude of potential interactions that occur between Scribble module proteins and other proteins, a critical question is, how are these interactions regulated *in vivo*? Molecular

analyses has revealed several key regulatory mechanisms that control binding of Scribble module proteins to interacting proteins, including affinity of protein binding to a particular domain, phosphorylation-dependent binding, and the involvement of multiple domains for protein binding. Moreover, the generation of large multi-protein complexes between Scribble module proteins and interacting proteins can occur to regulate protein function. Finally, the interaction of Scribble module proteins with other proteins can dictate their cellular localization. We will highlight these modes of regulation with some examples below.

Regulation of interactions through differential affinity of protein binding: The best characterized mode of interaction within the Scribble module is the PDZ domains interaction, and can be found in Scrib and Dlg ligand interaction. The PDZ domains consist of 80-90 amino acid residues that forms six β strands and two α-helices. The PDZ domains engage C-terminal PDZ binding motifs (PBM) in their target ligands through the consensus binding-groove consisting of β2, α2 and a GLGF motif embedded within the β1-β2 loop [124-126]. The binding of the ligand carboxyl group to the PDZ β1-β2 loop is core to the interaction as the ligand positions itself in an anti-parallel manner with the β2 strand. Early studies have classified the PDZ domains based on their ligand PBM motif, where Scrib and Dlg PDZ domains were classified as Class I that recognises the X-T/S-X-$Ø_{COOH}$ motif (where X can be any amino acid residue, and Ø is a hydrophobic residue), as opposed to Class II (X-Ø-X-$Ø_{COOH}$ motif) and Class III (X-D/E-X-$Ø_{COOH}$ motif) [127, 128].

As Scrib and Dlg have multiple PDZ domains, isolated and tandem PDZs have been studied through a number of biochemical techniques to identify their direct physical interactors, as well as associated proteins that are mediated through adaptor molecules. These include pull down assays, co-immunoprecipitation, ELISA, mass spectrometry and large peptide phage-display screens. The binding preferences of the PDZs were determined for Scrib's interactions with

the adherens junction protein β-catenin [104], the tight junction protein ZO-2 [129], the intermediate filament protein Vimentin [130], the zyxin-family protein TRIP6 [131], the hormone receptor TSHR [132], Influenza A virus NS1 [133] and TBEV NS5 [134], as well as the interactions between Dlg and Shaker K+ channel [135], HPV-16 and -18 E6 proteins [136, 137] and Gukh [31]. These studies show that it is not uncommon for each of the Scrib or Dlg PDZ domains to show overlapping preference against the same ligand, while maintaining their own unique binding profiles. For instance, recombinant Scrib PDZ1, 3 and 4 display affinities for HPV E6 PBM, whilst Scrib PDZ2 and 3 interacts with endogeneous Vangl2 protein. In Dlg, all PDZs can bind to HPV-18 E6 PBM [136], whilst only PDZ2 recognizes HPV-16 E6 oncoprotein [137]. Conversely, Scrib PDZ2 binds Gukh PBM, but Dlg PDZ1-2 and PDZ3 do not show any detectable affinities towards Gukh [31]. This demonstrates that, whilst Scrib and Dlg PDZs appear to be promiscuous binders, they still retain their ligand specificity. How can this be achieved? When the interactions between multiple PDZ domains that have affinities towards similar ligands were quantitatively examined, a hierarchy of ligand specificity was revealed. For instance, Scrib PDZ3 has the strongest affinity for the NMDA receptor subunit GluN2A PBM, followed by PDZ2 and PDZ4 with five-fold and twelve-fold weaker binding respectively [138]. A similar hierarchy of binding interactions has also been observed for Scrib's PDZ domains and the β-PIX PBM, with PDZ1 the strongest binder, followed by PDZ3 with four-fold weaker affinity, whilst PDZ2 represent the weakest with twenty-fold less affinity [139]. As there are limited studies on Dlg PDZ domains, the precise affinities and hierarchy of interactions remain to be deciphered. Thus, the PDZ domains share overlapping binding preferences, but are equipped with different affinities towards the same ligand, whilst on the other hand, the PDZ domains have distinct binding profiles and a vast range of affinities towards other ligands [139-141]. Nonetheless, quantitative studies of Scrib's and Dlg's PDZ domain-mediated interactions are still required, with competition studies between multiple ligands toward the same PDZ domain likely to provide invaluable information in understanding Scrib's and Dlg's ligand recognition.

The ligand binding preferences and affinities of PDZ domains represents at least one tier of regulatory mechanisms that controls Scrib and Dlg interactions. Hence, being able to identify, not only the hierarchy of PDZ affinities towards each ligand, but also the hierarchy of the ligand's affinities toward each PDZ domain, and the structural and sequence features determining affinity, is critical for deciphering PDZ-PDB specificity of interactions. Large-scale biochemical screening approaches have been very useful in this regard. In order to profile the PDZ domains, peptide libraries screens using protein microarray and phage-display with Scrib PDZ domains, have revealed that in addition to the well-recognised X-T/S-X-$Ø_{COOH}$ motif, the residues upstream of the motif are also crucial in determining the PDZ binding specificity [141-143]. Indeed, emerging structural studies have helped highlight some of the structural basis for this. For example, in the case of the Scrib-β-PIX complex, the interaction between β-PIX Trp residue located upstream of the four-residual PBM with Scrib PDZ1 and PDZ3 β2-β3 loop is critical, with the length and the residues of the β2-β3 loop responsible for the PDZ specificity [139]. Indeed, structure guided sequence alignment of human Scrib and Dlg PDZs supports this notion, as the length and residues varies in the β2-β3 loop, while the residues within the consensus binding groove (β2, α2, β3) is, unsurprisingly, highly conserved. Indeed, swapping of the Scrib PDZ1 into the PDZ3 β2-β3 loop could transfer high affinity for β-PIX ligand [139]. This infers that through proper manipulation of the PDZ β2-β3 loop, Scrib and Dlg complex formation may be controlled and specific interactions engineered.

Moreover, the phosphorylation state of the ligand PBM provides another layer of regulation with PDZ interaction. The phosphorylation state of MCC Serine-828 at the C-terminus PBM residue position -1 regulates its interaction with Scrib in actively migratory cells, since an impaired phosphomimetic mutation disrupted Scrib interaction and impaired lamellipodia formation [103, 144].

Regulation of interactions by phosphorylation of the Scribble module: In addition to phosphorylation of the PDZ binding ligands, protein phosphorylation of the Scribble-module proteins themselves is a major regulatory mechanism in controlling ligand interactions, and can affect the cellular localization and function of Scribble module proteins, as well as their interacting protein. Indeed, phosphorylation appears to regulate the majority of Dlg and Lgl interactions, but to a much lesser extent the Scrib interactions. How phosphorylation regulates the interactions of each member of the Scribble module is further elaborated upon below.

The Scrib protein has multiple phosphorylation sites that controls the spatial-temporal localization of its ligands and consequently the signalling pathways they regulate. In particular, recent work has revealed two KIM docking sites within Scrib where the Ras signalling pathway protein kinase, ERK (MAPK), may bind and phosphorylate Scrib. This interaction has been proposed as a mechanism to prevent ERK translocation to the nucleus and thus regulate ERK signalling [145, 146]. Additionally, Scrib has a PKA protein kinase phosphorylation site close to one of the KIM binding site, suggesting that PKA phosphorylation could regulate ERK binding to Scrib [145]. Using mouse fibroblasts ectopically expressing E-cadherin, it has also been suggested that phosphorylation of Scrib at Serine-1601 could provide a mechanism for differential association to the E-cadherin-catenin complexes [147]. Scrib also has a conserved PP1γ motif that enables the binding and regulation of the localization of PP1γ, and is important for suppressing oncogenic-induced transformation of primary rodent cells [148]. Finally, CD74, a subunit of the MHC class II, can also phosphorylate the C-terminus of Scrib, can cause Scrib to translocate from the plasma membrane to the cytoplasm, and thus modify Scrib's function [149]. Altogether, these data demonstrate that Scrib phosphorylation can control ligand localization, and in turn can influence the formation of the downstream molecular hubs that contribute to the regulation of cell signalling pathways.

Dlg interactions with various proteins, including the kinases p85-PI3K and MEK2, is also regulated by phosphorylation. Phosphorylation of Serine/Threonine residues on Dlg1 prevents its ability to bind to the SH2 domain of p85-PI3K in subconfluent cells where Tyrosine phosphorylation of Dlg1 is essential for this interaction [150]. It is thought that Dlg1 Serine/Threonine phosphorylation places it in a closed confirmation where the phosphorylated Tyrosine is inaccessible for binding to the SH2 domain of p85-PI3K. In confluent cells, where E-cadherin engagement occurs, the dephosphorylation of Dlg1 on Serine/Threonine residues might result in a change of conformation that renders the phosphorylated Tyrosine residues accessible and promotes the binding of p85/PI3K via its SH2 domains, thereby enabling activation of PI3K. In the case of human Dlg1 binding to MEK2, the phosphorylation of MEK2 is required for the interaction to occur [151]. The Interaction occurs between the PDZ domains of Dlg1 and the C-terminal domain of phosphorylated MEK2. This interaction occurs at the midbody during cytokinesis, where Dlg1 is recruited by E-Cadherin [151].

Another example of Dlg regulation by phosphorylation occurs at *Drosophila* neuro-muscular junctions (NMJs), where the Par1 and CAMKII protein kinases phosphorylate Dlg at a conserved Serine-797 site, resulting in its dissociation from NMJs [152, 153]. This Par1 and CAMKII-mediated phosphorylation of Dlg is promoted by the Adducin protein, Hts, which via its myristoylated alanine-rich C-terminal kinase (MARCKS)-homology domain, binds to Dlg, and also the phospholipid, PIP2, at NMJs [154-156]. Hts regulates the protein levels of Par1 and CAMKII, thereby affecting the extent of Dlg phosphorylation and synaptic targeting [154, 156]. In turn, phosphorylation of the MARCKS-homology domain of Hts by Protein Kinase C (PKC) reduces the interaction of Hts with Dlg and PIP2, thereby modulating Dlg's interaction with the NMJ and restricting NMJ growth [154].

Drosophila and mammalian Lgl proteins are regulated by aPKC phosphorylation at any one of the three conserved Serine residues positioned in the Lgl central linker region [32, 33, 35]. For

example, this has been shown to be sufficient for the interaction of Llgl2 with Dlg4 GUK domain [30]. Additionally, Lgl is also phosphorylated at different Serine residues in a polybasic region by the Aurora protein kinases during mitosis, promoting its cytoplasmic re-localization and enabling the correct alignment of the mitotic spindle [97, 157, 158]. The polybasic region located at the C-terminus of Lgl overlaps with the aPKC and Aurora protein kinase phosphorylation sites [32-35, 40, 157, 158] and is required for the binding of Lgl to phosphatidylinositol phosphates at the cell membrane [159]. Phosphorylation of Serine amino acids in this region by aPKC or Aurora kinase is thought to neutralize the positive charge and therefore prevent membrane localization of Lgl [159].

The interaction of Lgl with several proteins is regulated by aPKC-mediated phosphorylation, including non-muscle Myosin II, the ubiquitin ligase regulator VprBP and cell adhesion protein N-Cadherin. In all three cases, Lgl binds to these proteins in the absence of aPKC, and aPKC-dependent phosphorylation of Lgl inhibits the binding of Lgl to these proteins. In the case of Myosin II, the phosphorylation of Lgl by aPKC has been shown to regulate the interaction of Lgl with Myosin II in *Drosophila* and human systems [33, 160-162]. This interaction has been molecularly defined for human Llgl1, where the residues 645-677 of Llgl1 were shown to directly interact with the Myosin IIA Rod domain (residues 1817–1842), thereby inhibiting Myosin IIA's ability to assemble into filaments [121, 122]. Llgl1 binding to Myosin IIA is inhibited upon phosphorylation by aPKC-iota, and Myosin IIA and aPKC-iota compete in their binding to the same domain in Llgl1 [121].

In mammalian cells, Llgl2 associates with the VprBP (DCaf1)-DDB1 ubiquitin ligase complex subunits independently of the PAR-aPKC complex and prevents the VprBP-DDB1 subunits from binding to Cul4A, an E3 ubiquitin ligase implicated in cell cycle regulation at the G1-S phase transition [119]. Llgl2 binds directly to the WD40 domain in the C-terminal region of VprBP, and aPKC-mediated phosphorylation of Llgl2 (at Serine residues 641, 645, 649, 653, and 660) negatively regulates the interaction between Lgl2 and VprBP-DDB1 complex [119].

Drosophila Lgl has also been shown to bind to the VprBP ortholog, Mahjong [118], however its regulation by aPKC has not been explored.

In the mouse brain, Llgl1 binds to N-cadherin mediated by the C-terminal region of Llgl1 and the β-catenin binding domain of N-cadherin [123]. The binding of Lgl to N-cadherin is important for the internalization of N-cadherin, which is inhibited by aPKC-mediated phosphorylation of Llgl1. This mechanism results in the internalization of N-cadherin in the basal-lateral regions thereby specifying the position of the apical junctional complexes.

Regulation of interactions by super-tertiary structure organization of multiple domains: Intramolecular interactions in Scribble module proteins can affect the binding of protein interactors. An example of this is the binding of Dlg1 to GKAP, a protein that interacts with Dlg proteins at neuronal synapses [163-165]. Analyses of crystal structures of PDZ, SH3, and GUK domains has enabled the modelling of Dlg1 protein and its interaction with GKAP [166]. In this instance, Dlg1 can exist in a compact U-shaped conformation in which the N-terminal domain folds back and interacts with the SH3 and GUK domains. The N terminal region facilitates the binding GKAP with the GUK domain of human Dlg1, but the SH3 domain interferes with this interaction [166].

For Scrib, intramolecular interaction has been shown for PDZ3-4 domains, where PDZ4 did not show any affinities towards the ligands tested, but acts in tandem with PDZ3 to form an extended PDZ3 binding groove, that enable PDZ3-4 to target different ligand specificities compared to the PDZ3 binding groove alone [167]. This mechanism of inter-PDZ interaction to define new ligand affinities is also observed in other PDZ domain-containing proteins, such as GRIP1 and INAD, where not all PDZ are involved in ligand recognition, but work in tandem to facilitate the formation and stability of other PDZ active sites for ligand recognition [168-171]. Given that Scrib-PDZ complex formation occurs in micromolar affinities, this would

enable transient Scrib complex formation between different signalling hubs, and thus facilitate Scrib's involvement in multiple cellular processes and signalling pathways. Nonetheless, more studies are in need to decipher Scrib's intramolecular interactions. In addition to Scrib's four PDZ domains, the loop region, together with the LRR, LAPSADs, phosphorylation sites and binding motifs, could all contribute to Scrib's dynamic conformation and the regulation of Scrib complex formation, as occurs with Dlg.

Similarly, intradomain interactions may regulate Lgl's interaction with its binding partners. In *Drosophila,* phosphorylation of Lgl was shown to induce a "closed" conformation, where the N- and C- terminus interacts intramolecularly resulting in auto-inhibition [33]. This prevents the tethering of Lgl C-terminal domain to the F-actin cytoskeleton [33]. In *C. elegans*, LGL-1 binds to the membrane through a membrane-binding site (MBS), which folds into an alpha-helix secondary structure upon binding [172]. The MBS consists of three regions, a positively charged interface, the aPKC Serine phosphorylation residues and a hydrophobic interface that is predicted to embed within the membrane during interaction. Phosphorylation of the MBS site by PKC-3 acts as a switch that decreases MBS affinity towards the membrane, consequently causing the MBS to detach from the membrane, and concurrently decreases Lgl association with Par6 [172].

Overall, super-tertiary structure organization allows the arrangement of multi-domain proteins in a spatial-temporal manner that expose and/or restrict access to specific binding sites, increase binding affinities, alter/expand ligand recognition, and function as an important mechanism for the regulation of protein-protein interaction. This emphasizes the importance in studying the Scribble module members in a multi-domain setting rather than their deconstructed forms.

Regulation of interactions via multi-protein complexes: Whilst proteins in the Scribble modules may adopt super-tertiary structures during ligand engagement, multi-protein complex formation via multiple protein interactions would presumably alter the dynamics of each protein and may be crucial for their function in many cellular processes. For example, Scrib LRR domain interacts with Lgl, whilst the Scrib PDZ domains interacts with Vangl2 PBM (involved in PCP), forming a Vangl2-Scrib-Lgl tripartite complex [29].

Interestingly, in *Drosophila,* Dlg-Scrib interactions are regulated through binding to the adaptor protein Gukh, which is important for the correct localization of Scrib at the neuromuscular junctions [31]. The function of Gukh is likely to be evolutionarily conserved in tethering Dlg and Scrib, since the Zebrafish Gukh ortholog, Nhs11b, physically interacts with Scrib and Dlg when transfected into human cultured cells [173]. The *Drosophila* Dlg GUK domain binds to Gukh via the GUK-holding domain in the C-terminus of Gukh, and Gukh binds to Scrib PDZ2 via its C-terminal PDZ interacting peptide [31, 111]. Additionally, another study showed that the binding of Gukh to the GUK domain of Dlg occurs in a mutually exclusive manner via Dlg's PDZ domains, only permitting Gukh interaction when the PDZ domains remain unbound [174]. However, others have reported that interactions between Gukh and Dlg require the SH3-GUK domain of Dlg [175, 176]. This interaction is regulated through inter-domain interactions of PDZ3-SH3-GUK via a PDZ3 binding motif in a linker region, thereby enabling dynamic regulation of ligand binding to Dlg PDZ3 [175, 176].

In *Drosophila* follicle cells, Dlg forms a complex with the ACD cell polarity proteins Pins and Mud, and is responsible for the planar spindle orientation in dividing cells, a function that is separable from Dlg apico-basal polarity role [177]. Pins recruits Dlg to the apical cortex of dividing cells through Dlg GUK domain, where Mud align the spindles through a pulling force through dynein attachment to the astral microtubules [178, 179]. The planar orientation of

epithelia spindles is thought to be attributed to Dlg lateral localization that is independent of Pins [177].

In addition, Dlg and Scrib can form multiprotein complexes with several key signalling proteins, for example, Dlg with PTEN (Phosphatase and Tensin homolog) and APC (Adenomatous Polyposis Coli) [180], and with p85-PI3K and E-Cadherin [181], and Scrib with PHLPP and AKT (PKB) [99] (see the signalling section for further details). This is consistent with Scrib and Dlg playing a scaffolding role in facilitating the formation of signalosomes at specific cellular localizations.

Together the above examples raise questions as to how multi-protein complexes interact with and discriminate against other complexes to form large signalling hubs in normal cellular homeostasis and disease state. As the mere formation of multi-protein complexes would impose limitations to the availability of binding sites, what factors are in play in this process? As discussed above, studies have shown that protein-protein interactions could be influenced by distal binding of allosteric sites, which cause changes in the binding affinity without need for any major conformational change, as well as gain of functional interfaces via protein dynamics and the development of alternative protein conformations from different proteins encounters. As we understand more about the internal interactions in CIS of the individual component domains for each member of the Scribble module, it is vital to start investigating the mechanism that governs the elaboration of highly organized structural assemblies of Scribble modules and how their localization may affect how they signal to the rest of the cell.

Regulation of interactions through protein localization: As Scrib and Dlg both act as scaffolding proteins and regulators of cellular signalling, their localization is crucial to their function. Recent studies have revealed the major determinants for Scrib localization. One key mechanism is through control of S-palmitoylation of Scrib by the two enzymes ZDHHC7 and

APT2 at two conserved N-terminal cysteine residues, Cys4 and Cys10 [182, 183]. Point mutations at these two palmitoylation sites result in mislocalization of Scrib and the cell's subsequent loss in polarity and failure to suppress oncogenic YAP, MAPK, and PI3K-AKT signalling [183]. Although palmytoylation appears critical, a number of other factors act in concert to establish correct localization. In the case of Erbin, a related LAP protein (containing LRRs and one PDZ domain), both palmitoylation and the LRR region are required for its membrane localization [184]. The same mechanism is likely for the regulation of Scrib's localization. In worms, flies and mammals, a single point mutation at the LRR region causes mislocalization of Scrib to the cytoplasm, which in mammals can cause perinatal death due to defects in neural tube closure [93, 94, 185]. Moreover, point mutations towards the C-terminal end of Scrib, similarly result in mislocalization and the development of severe neural tube defects [185, 186].

Although deregulation of Scrib function through mislocalization has been reported in several human cancers and is associated with aggressive tumours with poor patient survival, the manner by which Scrib mislocalization contributes to tumourigenesis is not fully understood [81, 85]. Similar to the palmitoylation mutants, the LRR mislocalization mutant (P305L) fails to suppress MAPK signalling and invasion, but continues to suppress Ras-dependent anchorage-independent cell growth [187]. This suggests that mislocalization of Scrib accounts for only part of its loss of tumour-suppressive functions, and that its full contribution to cancer is likely far more complex. One possible mechanism is that when Scrib is mislocalized to the cytoplasm, it loses its various interactions and may adopt a new set of interacting proteins and new function ("neomorph"), leading to altered signalling and expression. Such a mechanism is supported by studies in transgenic mice expressing the P305L mislocalization mutant in mammary tissue, whereby expression of PTEN is increased in the cytoplasm by binding to mislocalized Scrib, and thus activating PI3K-AKT signalling pathway [85].

Similar to Scrib, the exact mechanism of how mislocalization of Dlg contributes to its deregulation is unclear. However, Dlg is partially dependent on Scrib for correct localization at the plasma membrane and under conditions of osmotic stress this dependency is lost resulting in an accumulation of Dlg at regions of cell-cell contact [27, 188]. This independent localization follows an increase in JNK-dependent phosphorylation of Dlg, which similarly acts as a regulator for its localization. Interestingly, Dlg phosphorylation following osmotic stress renders it more susceptible to oncogenic HPV E6-induced protein degradation, an event that mislocalizes Dlg to the cytoplasm [27, 188].

In a neuronal setting, synaptic-targeting of Dlg4 and Dlg2 is dependent on palmitoylation [189, 190], similar to that of Scrib [182, 183]. Dlg1 lacks the N-terminal cysteine residues required for palmitoylation. Interestingly, Dlg3 contains three N-terminal cysteine residues, but it does not undergo palmitoylation. Rather, this cysteine-rich region, combined with several nearby histidine residues, confers a zinc-binding motif that localizes it to the pre- and post-synaptic sites, as well as allows a unique axonal localization [189]. Moreover, in the case of Dlg4, palmitoylation alone is not sufficient for its accumulation at the synapse, but in addition, it requires the first two PDZ domains and a C-terminal targeting motif for correct localization [190].

Thus, the localization of Scribble module proteins and therefore access to their binding partners, is regulated through a combination of post-translational modifications, such as phosphorylation and lipid modification of Scribble module proteins, as well as less well understood requirements for their structural domains. The fact that multiple mechanisms appear to contribute to membrane localization, both together and independently, in different cell and tissue types, further highlights how complex and extensive these regulations are.

Scribble module proteins in the regulation of cell signalling pathways

Since Scrib and Dlg are similar in structure and function, whilst Lgl is structurally and functionally different, with its function tightly linked to aPKC and the Par complex function, we will consider Scrib and Dlg separately to Lgl and aPKC/Par in discussing their regulation of cell signalling pathways.

Scrib and Dlg regulation of cell signalling pathways

From studies in various organisms and cell types, Scrib and Dlg have been linked to the regulation of Receptor Tyrosine Kinase (RTK)-Ras-MAPK, Tumour Necrosis Factor (TNF)-Jun Kinase (JNK)/P38, Phospho-Inositol-3-Kinase (PI3K)-AKT, Hippo, Wnt, Bone Morphogenetic Protein (BMP) receptor, Thyroid Hormone Receptor (THR), NMDA Receptors (NMDARs) and Dopamine Receptor (DR) signalling pathways. We will highlight below the current understanding of the control of these signalling pathways based on the physical interactions revealed between Scrib and/or Dlg and signalling pathway components (Figure 4, Supp Tables 1 and 2).

RTK-Ras-MAPK: The small GTPase, Ras, is the key effector of signalling downstream of mitogenic RTKs and acts via the MAPK protein kinase cascade to control cell proliferation and survival, and as such is a major oncogenic pathway in cancer [191]. In mammalian systems, Scrib negatively regulates the RTK-Ras–MAPK pathway *in vitro* and *in vivo* [81, 83, 84, 145, 146, 192-194]. Additionally, in *Drosophila dlg* depletion, or *scrib* depletion together with a mutant in *canoe* (an Afadin/AF6 ortholog, involved in adherens junction function) results in activation of Ras-MAPK signalling in the antennal epithelial tissue [195]. Moreover, overexpression of Scrib antagonizes ectopic Ras signalling [193]. However, whether the regulation of Ras-MAPK signalling by Scrib/Dlg involves similar mechanisms as occur in mammalian systems is currently unknown.

Several mechanisms have been revealed linking Scrib to the regulation of RTK-Ras–MAPK signalling. Firstly, human Scrib interacts with ERK [145, 146]. Scrib, via its PDZ1 domain, interacts with ERK via the kinase interaction motif (KIM) on ERK [145, 146] (Figure 4). This interaction prevents ERK phosphorylation and impedes the anchoring of ERK to membrane sites. Secondly, in response to NGF-TrkA signalling Scrib binds to Ha-Ras and pERK1/2, and inhibits sustained ERK activation in PC12 neural cells [196]. Scrib also affects the signalling of the RTK, EGFR, indirectly, by binding to the β-Pix (Pak-interactive exchange factor), GIT1, Arf-GAP complex and that acts as a MEK-ERK scaffold [102, 197]. The negative regulation of RTK-Ras-ERK signalling by Scrib, raises the possibility of treating Scrib impaired cancers with small molecule inhibitors of the Ras pathway. Indeed, the hyperproliferation associated with elevated Ras-ERK signalling observed in mouse Scrib conditional knockout prostate or mammary epithelial cells can be rescued by treatment with a MEK inhibitor [81, 83].

Dlg1-4 are involved in the control of the RTK-Ras-MAPK signalling pathway, however in contrast to Scrib, Dlgs appear to act by promoting Ras signalling. Dlg family members binds to the EGFR family member ErbB4 in neuronal cells, where they are thought to be involved in anchoring ErbB4 to the neuromuscular junctions and downstream signalling regulation [198, 199]. Dlg1 is also required for signalling via another RTK, FGFR2, and is required for FGFR2 stability/localization in the mouse lens, although whether this occurs via a direct interaction is unknown [200]. Additionally, Dlg1, via its PDZ domains, binds to the PDZ binding motif (RTAV) at the C terminus of MEK2 [201], and during mitosis this interaction at the midbody requires the activating phosphorylation of MEK2 [151]. As activated MEK2 is required for the completion of mitosis, the interaction between Dlg1 and MEK2 might be important for regulating the activity of MEK2.

Scrib and Dlg also interacts with other proteins that indirectly regulate RTK signalling, such as phosphatases (Figure 4). Mammalian Dlg2 and Dlg3 bind the catalytic subunit of the PP1

phosphatase, and Scrib via its PP1γ interaction motif in the C-terminal region, binds to protein phosphatase 1γ (PP1γ) [148]. Scrib's interaction with PP1γ is required for the downregulation of ERK phosphorylation and to prevent oncogenic transformation of primary rodent cells. Scrib also forms a complex with the PP1 phosphatase regulator, SHOC2/SUR-8, and with a protein of the RRas subgroup of Ras proteins MRAS [202]. Here, Scrib blocks SHOC2-mediated dephosphorylation of Ras downstream effector protein kinase, RAF, at the conserved inhibitory site (S259), thereby inhibiting RAF activation.

Scrib also regulates signalling via the Hepatocyte Growth Factor (HGF) RTK (Figure 4). In Madin Darby Canine Kidney epithelial cells (MDCK) cells, Scrib binds to Sgt1 (a co-chaperone-like protein that regulates Adenylate Cyclase activity in *S. cerevisiae*), which is facilitated by Hsp90 (a chaperone protein) [100]. Knockdown of Scrib, Sgt1 or Hsp90 decreased tubulogenesis, a form of collective cell migration, of MDCK cysts in response to HGF [100]. Thus, Scrib is necessary for HGF signalling, and the stabilization of Scrib by Sgt1-Hsp90 contributes to this function. Mechanistically, downstream of HGF signalling, Scrib binds to β-PIX (a Rho family GEF) and PAK (P21 activated kinase) and is required for the proper localization of phosphorylated active PAK during tubulogenesis of MDCK cysts. β-Pix and PAK are required for HGF-induced tubulogenesis, however, Scrib does not regulate other HGF downstream pathways, Ras, JNK or PI3K signalling, in this context. This finding that Scrib does not always negatively regulate Ras signalling downstream of RTKs, suggests that Scrib's role in signalling is context dependent.

TNF-JNK/P38: JNK and P38 are important stress response Serine/Threonine protein kinases that function in tissue homeostasis, development and are deregulated in cancer [203]. In *Drosophila, scrib* depletion leads to the activation of the JNK pathway [71, 204-206]. Interestingly, Scrib, via its PDZ domain, has been shown to bind to Traf4 [207], an upstream adaptor protein in the TNF-JNK signalling pathway, and it is possible that this interaction might mediate Scrib's regulation of JNK signalling (Figure 4). Current evidence suggests, however,

that cell polarity impairment is indirectly linked to JNK regulation through Rho1-GTPase activation of the JNK kinase kinase, Wallenda (Wnd) [208, 209]. Whether Traf4 is linked to Wnd activation remains to be determined. Additionally, TNF-JNK signalling can be triggered in polarity-impaired tissue by an extrinsic response due to the recruitment of the *Drosophila* macrophage-like cells (hemocytes) to the mutant tissue and their production of the *Drosophila* TNF, Eiger (Egr) , or by Egr production in the surrounding epithelial cells [210, 211]. In human MCF10A cells stimulated with EGF, Scrib knockdown also leads to JNK activation [193], which contributes to the invasive phenotype with oncogenic Ras, Ha-Ras^{V12} [193, 212], however the mechanism by which JNK is activated in these Scrib-deficient cells is unknown.

Conversely, Scrib has a positive role in activating JNK in mouse models of c-Myc driven breast cancer. Here, Scrib by its ability to promote assembly of the βPIX/GIT1 complex and activate Rac1, which in turn induces the JNK/c-Jun pathway leading to the expression of pro-apoptotic Bim, promotes c-Myc induced apoptosis [86] (Figure 4). Thus, downregulation of Scrib cooperates with c-Myc by preventing cell death and thereby leading to tumour overgrowth. In contrast, in Scrib depleted mosaic MDCK cultures, the JNK-related stress kinase, p38, is activated and contributes to apoptosis of the *Scrib* mutant cells [213] (Figure 4). Again, how Scrib depletion leads to p38 activation is unclear.

Dlg1 can also act as a positive regulator of JNK and the JNK-related p38-MAPK signalling. In GluR6 receptor signalling in neuronal cells, human Dlg4, via its SH3 domain, plays a scaffolding role in anchoring the JNK upstream regulatory kinases, MAP3K10 and MAP3K11, to the receptor to promote JNK signalling [214] (Figure 4). Likewise, human Dlg1 indirectly activates p38-MAPK in mitotic cells, by interacting with PBK/TOPK protein kinase, which phosphorylates and activates P38 [215, 216] (Figure 4). Interestingly, another study showed that human Dlg1 via its PDZ repeats interacts with SAPK3/p38γ MAPK, which phosphorylates Dlg1 and results in its dissociation from the cytoskeleton [217]. Thus, a positive feedback loop

might occur where altered localization of Dlg1 might trigger p38 activation that then reinforces Dlg1 cytoskeletal dissociation and p38 activity stimulation.

PI3K-AKT: The PI3K pathway is regulated by Insulin Receptor signalling and by Ras, and acts via a protein kinase cascade, AKT, mTOR (mechanistic Target Of Rapamycin) and p70-S6 kinases, to regulate cell growth, proliferation and survival and as such is a major deregulated pathway in overgrowth disorders and cancer [218-220]. In various mammalian systems, Dlg and Scrib act to regulate the PI3K-AKT pathway. Firstly, Scrib negatively regulates AKT activity by binding to the protein phosphatase, PHLPP (PH (pleckstrin homology) domain and LRR protein phosphatase) and anchoring it to the plasma membrane [99] (Figure 4). In its regulation of AKT, Scrib forms a tripartite complex with PHLPP and AKT, thereby inhibiting AKT activity. However, when Scrib is downregulated, PHLPP is released, and AKT activity is increased, resulting in increased cellular growth, proliferation, and survival. Relevant to the control of PI3K signalling, another study showed that high levels of mislocalized Scrib functions in a neomorphic manner to promote mammary tumourigenesis by altering subcellular localization of PTEN (phosphatase and tensin homologue deleted on chromosome 10), which normally acts to antagonise PI3K signalling [85]. This leads to the activation of AKT/mTOR/S6 kinase signaling pathway, thereby promoting mammary tumourigenesis. Scrib mislocalization is often observed in human cancer, and therefore this mechanism linking Scrib mislocalization to enhanced PI3K-AKT signalling might provide novel avenues for therapeutic intervention of these cancers.

In contrast to Scrib, there is evidence that Dlg positively regulates PI3K-AKT signalling at least in viral oncogene-mediated tumourigenesis. Human Dlg1 binds to p85-PI3K to recruit it to E-cadherin at adherens junctions in intestinal epithelial cells to promote PI3K activity and regulate adherens junctions stability and function [181] (Figure 4). Additionally, human Dlg1, via its 2nd PDZ domain binds to the Adenovirus 9 oncoprotein, E4-ORF1 (E4 region-encoded open reading frame 1) and is required to promote the constitutive activation of PI3K, leading

to the activation of PKB and p70-S6 kinase [221, 222] (Figure 4). The activation of PI3K signalling by E4-ORF1 is thought be due to plasma membrane translocalization of Dlg1, where PI3K is activated by Ras [223]. Thus in this setting, Dlg1 has an oncogenic role in promoting Adenovirus E4-ORF1 transformation. This finding suggests that inhibiting Ras or PI3K signalling might reduce Adenovirus 9-mediated tumourigenesis.

Similarly, in *Drosophila* there is evidence that Dlg positively regulates PI3K signalling [224]. Here Dlg-depleted epithelial cells resulted in PI3K signalling downregulation, and further RNAi-mediated knockdown of components of the PI3K pathway resulted in synthetic lethality of Dlg-depleted tissue in an oncogenic Ras background. This suggests that in Ras-driven polarity-impaired cancers that targeting the PI3K pathway might provide a novel therapeutic opportunity. However, whilst the above findings provide evidence that Dlg is required for PI3K signalling, conversely, there are also reports that human Dlg1 can inhibit PI3K signalling, by direct binding via the Dlg1 PDZ2 domain to PTEN, and promoting PTEN activity [180, 225] (Figure 1). Consistent with this, Dlg1 mutations in the PDZ2 domain that affect PTEN binding are tumourigenic [180]. Thus, whether Dlg1 acts in an oncogenic or tumour suppressor role appears to be dictated by its interacting proteins and context.

Hippo: The Hippo negative tissue growth control pathway responds to cell polarity and mechanical cues to control cell proliferation and survival [226, 227]. Central to the pathway are the Serine/Threonine protein kinases, Hippo (MST1/2) and Warts (LATS1/2), which act in a kinase cascade to phosphorylate the cotranscriptional factor, Yki (YAP/TAZ), sequestering it in the cytoplasm. Disruption to Hippo signalling, leads to Yki (YAP/TAZ) dephosphorylation, its nuclear entry and transcriptional upregulation of cell proliferation and survival genes. Scrib loss of function leads to Hippo pathway impairment in *Drosophila*, zebrafish and mammalian systems [194, 226, 228-233]. In mammalian cells, Scrib binds to TAZ and sequesters it to the cell cortex in breast cancer stem cells [232] (Figure 4). Upon Scrib depletion or induction of

an EMT (Epithelial to Mesenchymal Transition), TAZ is released from inhibition by MST1/2-LATS1/2 signalling at the cell cortex, thereby promoting tumourigenesis. However, whether the same regulation occurs in other organisms is unclear. Indeed in zebrafish pronephric cyst development, Scrib regulates Hippo signalling by another mechanism; Scrib binds to an upstream regulator of Hippo signalling, FAT1, and together with FAT1 inhibits YAP activity [229] (Figure 1). However, in *Drosophila*, no direct binding between Scrib or Dlg and Hippo pathway components have been described. Indeed, *scrib* and *dlg* mutants only inhibit Hippo signalling when cell polarity is lost and only do so robustly if cell death is also blocked [228, 230, 231, 234]. Mechanistically, *scrib* mutants deregulate the Hippo pathway by reducing Warts levels and activity [230], but other upstream signalling pathways appear to also contribute [228]. Interestingly, in *Drosophila scrib* mutant tissue, elevated Yki activity functions cooperatively with the Fos transcription factor (downstream of JNK signalling) to transcriptionally upregulate the Jak-STAT ligand, Upd (IL6 ortholog), which then ectopically activates Jak-Stat signalling and promotes tumour growth [235].

Wnt: Wnt (Wingless) signalling is mediated through the Frizzled (Fzd) Receptor and occurs via the canonical pathway involving the transcriptional activity of β-catenin, or via non-canonical pathways, such as in PCP, which signal through the adaptor protein, Dishevelled, and the Rho1-GTPase and JNK pathways [236, 237]. Canonical and non-canonical Wnt signalling is important in tissue growth and patterning during development and is deregulated in cancer [238]. Scrib was identified as a PCP gene by its mutant phenotype in mice and *Drosophila,* and physical interaction with the PCP gene, Vangl2 [185, 186, 239-243]. In addition to its well characterised interaction with Vangl2, Scribble can also interact with PCP regulator LPP, which is a zyxin-family actin cytoskeletal regulator involved in cell adhesion at focal adhesions and adherens junctions, but also may have a role in the nucleus in regulating transcription [244]. LPP, via its C-terminal domain, binds to the PDZ-domains of Scrib [245], as does another zyxin-family member, TRIP6 [131]. Scrib, LPP or TRIP6 colocalize at

adherens junctions in MDCKII and CV-1 mammalian epithelial cells, but are not required for each other's localization [131, 245]. Moreover, in zebrafish convergent-extension epithelial sheet migration during gastrulation, a process regulated by non-canonical Wnt-PCP signalling, Scrib and LPP play a cooperative role in the regulation of cell migration [240]. As LPP is emerging as an important invasion-metastasis driver in cancer [246], further research is needed to determine whether Scrib functions together with LPP in this process, and whether Wnt-PCP signalling is also involved.

Dlg also plays an important role in Wnt signalling. Human and *Xenopus* Dlg4, via its PDZ domain, interacts with FZD Receptors, which is thought to be important a scaffolding function in both canonical and non-canonical Wnt signalling [247, 248] (Figure 4). Moreover, the crystal structure of *Xenopus* Dlg4 PDZ2 domain with FZD7, reveals the phospho-lipid, PIP2, is also integral to the structure, and this interaction with PIP2 is important for FZD7 membrane targeting and PCP signalling [249]. There is also evidence that Scrib interacts with the canonical Wnt signalling pathway; in neural cell synaptic vesicle trafficking and recycling, Scrib, via its PDZ domains, interacts with β-catenin, which appears to be involved in Scrib protein localization at synapses in synaptic vesicle clustering [104] (Figure 4). Furthermore, mammalian Dlg4 and Scrib physically interact, via their PDZ domains, with the Wnt signalling component, Adenomatous Polyposis Coli (APC), which functions in the degradation of β-catenin, and through this interaction with APC, Dlg and Scrib play important roles in promoting cell cycle exit and in cell migration [107, 180, 250-253] (Figure 4). Thus, Scrib and Dlg have roles in Wnt signalling in the PCP pathway as well as in canonical Wnt signalling.

<u>BMP Receptors/TGFβ-Mad/Smad:</u> The TGFβ Receptor superfamily, including BMP, are Serine/Threonine receptor protein kinases that signal by phosphorylating the Smad (Mad) transcription factors and have roles in cell proliferation control, development and in the EMT [254-256]. In *Drosophila* wing posterior cross vein development, Scrib via its LRR domain,

has been shown to bind to the BMP Type I Receptor, Tkv, Type II Receptor, Pnt, the phosphorylated (active) Mad transcription factor and the early endosome marker, Rab5 [101] (Figure 4). This interaction occurred in early endosomes, and is thought to facilitate BMP Receptor signalling. This finding is in contrast with regulation of TGFβ signalling by Scrib in mouse lens development [257]. In *Scrib* mutant mouse ocular lens cells, the TGFβ pathway transcription factors, Smad3 and Smad4, accumulate in the nucleus, leading to upregulation of the TGFβ target, Snail, which is thought to contribute to the EMT in this tissue [257].

Thyroid Hormone Receptor (TSHR)-βPIX-GIT-ARF6: The TSHR is G protein-coupled receptor (GPCR) that is regulated after ligand binding by endocytosis and recycling to the plasma membrane. The direct binding of Scrib to TSHR is important to recruit the β-PIX-GIT1-ARF6 complex [102] and is required for TSHR recycling [132] (Figure 4). It will be interesting to determine if interaction of Scrib and the β-PIX-GIT1-ARF6 with other membrane proteins is important in the regulation of their signalling or recycling. Indeed, in proteomics analysis of another GPCR, ADRA1D (α1D-Adrenergic Receptor), Scrib was discovered as binding to ADRA1D's PDZ-binding domain, and ARHGEF6/7 (β-Pix), Git1 and the phosphatase 1 catalytic subunit, PPP1CC, were detected as Scrib's binding partners [258]. In this study, Scrib was shown to compete with the Syntrophin family of PDZ-domain containing proteins for binding, and the binding of Scrib conferred new signalling properties on ADRA1D in response to ligands, which is thought to be important for fine-tuning GPCR signalling. Whether, β-PIX-GIT1-ARF6 or Phosphatase 1 also contribute to ADRA1D signalling or recycling remains to be determined.

NMDA Receptor signalling: The *Scrib-circletail* mutant affects learning, memory and social behaviour [259]. In excitable synapses in the hippocampus, Scrib is a key mediator of the endocytic sorting of N-Methyl D-Aspartate (NMDA) Receptors (NMDARs), such as Glutamate

receptors GluN2A and GluN2B, which occurs through its selective interactions with the AP2 adaptor complex [138]. A specific YxxR motif between PDZ1 and PDZ2 on Scrib directly interacts with AP2 to control NMDAR endosomal sorting. Interestingly, Dlg4, via its PDZ2 domain, interacts with the C-terminal PDZ-binding motif in GluN2 subunits [260], and Dlg2 PDZ1,2 domains interact with the C-terminal octapeptide of GluN2 [261]. Whilst the Scrib PDZ3 domain has also been shown to interact with another NMDAR, GluD2 [261], whether Dlg4 and Scrib function together in regulation of the GluN2 receptors is not known.

Dopamine Receptor-Rac1-Pak3-Cofilin: In *Drosophila* neuronal signalling, during memory regulation, Scrib interacts physically and genetically with the Rac1, Pak3 and Cofilin proteins in the Mushroom body cells stimulating a forgetting signalosome downstream of the dopaminergic receptor [262].

In summary, whilst Dlg and Scrib are involved in the same genetic pathway in cell polarity and proliferation control in *Drosophila*, and are involved in regulating similar signalling pathways, they do so by different mechanisms and often in opposing ways. It appears that tissue context, localization and the availability of their specific interacting proteins dictates the outcome of their regulation of specific signalling pathways. Furthermore, the deregulation of several signalling pathways upon Scrib/Dlg depletion may result in cross-talk leading to unique outcomes. This is exemplified in *Drosophila scrib* mutant tissue, where deregulated JNK-Fos and Hippo-Yki signalling results in the coordinate transcriptional upregulation of Upd, which then induces Jak-Stat signalling [235].

Lgl Regulation of Cell Signalling Pathways

The validated Lgl interactors (Figure 5, Supp Table 3) reveal mostly proteins involved in the regulation of cell polarity, cytoskeleton, adhesion and membrane trafficking, which are not

directly connected to cell signalling processes. Since Lgl and aPKC are intimately connected, we will also discuss aPKC and the Par complex in describing how Lgl regulates various signalling pathways.

RTK-Ras-MAPK/JNK signalling: In lower organisms, there is evidence for Lgl/aPKC regulation RTK-Ras-MAPK and JNK pathways, however the mechanism by which this occurs is unknown. In the zebrafish epidermal cells, Lgl2 is implicated in the negative regulation of the ErbB2-Ras signalling pathway, since *lgl2* (*pen*) mutants showed increased ErbB2 signalling, which alters E-Cadherin localization and induces an EMT [263]. In *Drosophila* epithelial tissues, consistent with the inhibition of aPKC by Lgl, overexpression of a membrane-tethered constitutively-active version of aPKC (aPKC-CAAX) results in increased MAPK (ERK) activation [264]. Lgl depletion in the developing wing or antennal epithelium also leads to ERK activation [76, 195], and the elevation of EGFR-Ras signalling ligand genes [74]. Furthermore, in *Drosophila*, Lgl depletion activates JNK signalling in the developing wing epithelial tissue leading to cell death, proliferation or migration depending on the context [72, 76, 265-267]. Although JNK activation can be triggered extrinsically by *Drosophila* macrophage-like cells that secrete the Tumour Necrosis Factor, Egr, Lgl depletion activates JNK signalling independently of the Egr in the *Drosophila* wing epithelium [267], suggesting that a cell intrinsic mechanism is involved. Interestingly, in *Drosophila* and MDCK cells, the Lgl binding protein, VprBP (Mahjong) protein negatively regulates JNK signalling [118], and therefore might be involved downstream of Lgl depletion in triggering JNK activation. However, VprBP is also a component of the Cul4A ubiquitin ligase, and another study in MDCK cells, has revealed that Lgl by binding to VprBP, acts as a negative regulator of the Cul4 ubiquitin ligase, which is involved in G1-S phase cell cycle regulation [119]. Thus, JNK activation may be an indirect consequence of aberrant cell cycle entry in *lgl* and *VprBP* mutants. Alternatively, since a recent study has revealed that the Rho1-GTPase pathway regulates JNK activation, via the JNKKK

protein kinase, Wallenda, in polarity-impaired cell invasion in the wing epithelium [208, 209], Lgl/aPKC might regulate JNK signalling by regulating Rho1 or Wallenda activity.

In mammalian systems, the Lgl/Par axis has also been linked to Ras-MAPK and JNK signalling. Consistent with the Par complex' antagonistic role with Lgl, Par6 overexpression in mammalian mammary epithelial cells mammalian cells elevates Ras signalling [268]. Indeed, aPKCiota is an oncogene in colon, lung and ovarian tumours [269-274], and Par6 is overexpressed in breast cancer and induces cell proliferation [268]. In lung and ovarian cancers, Par6/aPKCiota binds to the guanine nucleotide exchange factor Ect2, an activator of Rac1-JNK and Pak1-Mek1/2-Erk1/2 signalling pathways to regulate tumour growth [269, 275, 276] (Figure 5). The PKCiota-Par6alpha-Rac1 signalling axis also drives anchorage-independent growth and invasion of non-small lung cancer cells through induction of MMP10 expression [277]. Conversely, the other mammalian aPKC ortholog, aPKCzeta, acts as a tumour suppressor in prostate cancer in cooperation with PTEN mutations, and acts to phosphorylate and inactivate c-Myc [278] (Figure 5).

Another component of the Par complex, Par3, has oncogenic or tumour suppressive roles depending on context. In chemically induced skin cancers, Par3 knockout protects mice from papilloma formation due to elevated Ras signaling, but increases the incidence of another type of skin cancer, keratoacanthomas [279]. Interestingly, Par3 conditional knockout in keratinocytes promotes melanoma progression non-cell autonomously, through elevating cell surface P-Cadherin expression in keratinocytes, which promotes melanocyte proliferation, dedifferentiation and motility [280]. However, in the keratinocytes, Par3 knockout reduces cell proliferation and survival, thereby suggesting it has an oncogenic function. Mechanistically, in Par3 knockout keratinocytes, Ras and Sos2 (a Ras-GEF) are delocalized from cell-cell junctions leading to reduced Ras activation, thereby impairing ERK1/2 (MAPK) and AKT signalling (Figure 5), resulting in reduced proliferation and increased apoptosis [279]. aPKC is

normally localized with Ras signalling components at cell-cell junctions, but in Par3 knockout cells, aPKC is mislocalized to the cytoplasm [279]. The impaired Ras signalling in Par3 knockout cells could be rescued by expression of a membrane tethered from of aPKC (aPKC-CAAX) [279], implying that correct junctional localization and activity of aPKC is important for Ras activation.

Conversely, in other systems, Par3 behaves as a tumour suppressor. In Ras and Notch models of mammary cancer, Par3 functions as a tumour suppressor; loss of Par3 delocalizes and activates aPKC and potentiates tumour growth and invasion, and this occurs through elevated JAK/STAT signalling [281]. Mechanistically, Par3 knockout elevates Rac1-JNK pathway signalling, thereby promoting cell death (therefore alone it plays an oncogenic role), but in the presence of oncogenic Notch, apoptosis is reduced, thereby tumourigenesis is induced [282]. Par3 also acts as a tumour suppressor in ErbB2-induced breast cancer model; Par3 loss inhibits E-Cadherin junction stability and promotes cell invasion dependent on Tiam1 (Rac-GEF)-Rac signalling [283] (Figure 5). Thus, dependent on the activity of other signalling pathways in the cell, Par3 can act as an oncogene or a tumour suppressor.

PI3K: In *Drosophila lgl* mutant wing epithelial tissue, PI3K-AKT signalling is activated [76], however the mechanism by which this occurs has not been investigated. In mammalian MDCK cells and in *Drosophila*, Par3 binds to PTEN and is involved in cell polarity establishment [284, 285]. Analysis of *Drosophila* PTEN mutants, also suggest that the interaction between Par3 and PTEN is important for the organization of the actin cytoskeleton [286]. However, in this regulation, PTEN appears to be playing a role in the production of specific phospholipids required for the generation of membrane domains rather than regulating PI3K-AKT signalling.

Hippo: In *Drosophila* epithelial tissues, depletion of Lgl (or upregulation of aPKC) blocks Hippo pathway signalling, leading to the activation of Yki, which triggers cell proliferation and survival

[74, 76, 234]. Lgl/aPKC control the Hippo pathway by regulating the localization and therefore activity of Hippo [234, 264], which is activated when recruited to specific membrane regions where it forms a complex with Expanded, and with its downstream kinase, Warts [287, 288]. Precisely how Lgl/aPKC regulates Hippo localization in *Drosophila* is currently unclear, although in mammalian cells, aPKC has been shown to bind to the Hippo orthologs, MST1/2, and uncouple the binding of the Warts ortholog, LATS1/2, thereby preventing LATS1/2 phosphorylation and inactivation of YAP (Yki) [289] (Figure 5). Other inputs may also affect Hippo signalling upon Lgl depletion, as expression profiling has revealed that the upstream Hippo pathway regulator, Fat, is transcriptionally downregulated in *lgl* mutant brain and epithelial tissues, which may occur via effects of Lgl on micro-RNA levels . Additionally, in mammalian systems, the Hippo pathway component, Kibra, binds to the Par complex, which is required for LATS1/2 regulation [290, 291] (Figure 5).

Wnt: In *Drosophila* and *Xenopus* systems, the Wnt signalling component, Dishevelled, which is an adaptor protein involved in both PCP and canonical Wnt signalling, interacts with Lgl for the regulation of PCP [292, 293] (Figure 5). Here, Dishevelled regulates the localization of Lgl to the cortical membrane, whilst the Wnt receptor, Frizzled 7 (FZD7), induces dissociation of Lgl from the cortex [292]. Whether Lgl also regulates the canonical Wnt signalling pathway has not been explored, however a target of Wingless signalling in *Drosophila*, *naked*, is upregulated in *lgl* mutant tissue in the developing eye, and in a genetic screen of the *Drosophila* kinome, several protein kinase or phosphatase genes implicated in Wnt signalling regulation were revealed as *lgl* interacting genes [294]. Moreover, Wingless signalling ligand genes are elevated in *lgl* mutant wing epithelial tissue [74], which would be expected to lead to pathway activation.

Hedgehog: The Hedgehog pathway is a key developmental pathway, important in tissue patterning, and its deregulation contributes to developmental disorders and cancer [295, 296].

Hedgehog signals through the Smoothened receptor leading to the activation of the Hedgehog pathway transcription factor Gll1. In mammalian cells, the Par complex is tethered to the centrosome through binding to the scaffolding protein, Missing in Mitosis (MIM) [297], which is a regulator of Hedgehog signalling [298]. Gli1, is phosphorylated by aPKCiota, thereby increasing its DNA binding and transcriptional activity [297] (Figure 5). Additionally, aPKCiota is upregulated by Hedgehog signalling and Gli1-dependent transcriptional regulation, thereby generating a positive feedback loop amplifying Hedgehog signalling [297]. This study also showed that aPKCiota was elevated in Smoothened-inhibitor resistant basal cell carcinomas, the therefore might serve as novel target for therapeutic intervention in these cancers.

Notch: Lgl regulates Notch signalling in asymmetric cell division of the *Drosophila* neural stem cells, by dictating the localization of the Notch regulator, Numb (a membrane associated protein involved in the endocytosis of the Notch receptor), or the intracellular trafficking of Notch [299]. Mechanistically, Lgl's regulation of Numb localization, occurs by Lgl's inhibition of aPKC activity [40, 300]. When free from Lgl, aPKC binds Par3 and phosphorylates Numb, resulting in its exclusion from the cell cortex on one side of the cell (Figure 5). Similarly, the regulation of Notch signalling in the mouse neural epithelium by Lgl is associated with the asymmetric distribution of Numb [87]. In Llgl1 knockout mice, the brain neuro progenitor cells are unable to correctly segregate Numb and inhibit Notch signalling in the progeny, which is associated with continued cell proliferation and differentiation defects [87].

Lgl/aPKC also regulate Notch signalling more directly. In the Zebrafish retina, reduced neural differentiation also occurs upon depletion of Llgl1 in Zebrafish, which is associated with increased Notch signalling [301]. Here the mechanism for elevated Notch signalling appears to be due to the expansion of the apical domain resulting in the accumulation of more Notch receptors. Blocking Notch activity restores neurogenesis in the Llgl deficiency, indicating that the deregulation of this pathway was causative for the observed defects [301]. Whether, aPKC

is involved in this regulation was not examined. However, in *Drosophila* eye development, Lgl regulates ligand-activated Notch signalling in an aPKC-independent manner [302, 303]. Mechanistically, this occurred by Lgl depletion affecting endocytic vesicle acidification and gamma-secretase mediated cleavage activation of Notch [302, 303], which occurs in acidified vesicles [304, 305]. The precise way by which Lgl affect vesicular acidification is yet to be determined, however as Lgl binds to the t-Snare protein, Syntaxin 4, in mammalian cells [27, 113], and *lgl* mutants display endosomal trafficking defects [302, 303], it may relate to the role of Lgl in regulating vesicle trafficking.

A role for aPKC in Notch regulation, independent of Numb, has been revealed in the chick central nervous system [306]. Here, aPKCzeta phosphorylates ligand-activated Notch1 receptor on Serine-1791, which promotes trafficking of Notch1 to the nucleus, thereby promoting transcription of Notch1 targets (Figure 5). When Notch1 is not activated by engagement with its ligand, aPKC instead promotes the internalization of Notch1 from the cell surface and trafficking from the Golgi–ER to intracellular vesicles. The Notch1 Serine-1791 residue, which is phosphorylated by aPKC, however is not conserved in *Drosophila* and *C. elegans* Notch proteins nor vertebrate Notch3 and Notch4, therefore this mechanism might be specific for vertebrate Notch1.

In summary, Lgl/aPKC-Par are involved in the regulation of several signalling pathways, however the precise mechanism of pathway regulation and outcome appears to be context specific. Furthermore, different members of the Par complex regulate different signalling pathways or have different mechanisms in regulating signalling pathways. Additionally, there is also evidence in *Drosophila* wing epithelial tissue that other signalling pathways, such as Jak-STAT, Dpp (BMP) and hypoxia signalling are also elevated by Lgl depletion [74, 76], but currently it is unknown whether the regulatory mechanisms are direct or indirect.

Conclusions and Perspectives

We have highlighted in this review, the myriad of Scribble module interacting proteins and how they are involved in cell signalling pathways. It is clear that there is considerably complexity in the protein interactions that have been documented to occur with Scribble module proteins and in the regulation of signalling pathways. Although Scrib, Dlg and Lgl play a common function in cell polarity regulation, they interact with distinct proteins in the regulation of various signalling pathways, and these interactions are context dependent. Clearly, further molecular analysis is needed to understand the plethora of interactions that occur between Scribble module proteins and signalling regulators, and how cell and tissue types affect these interactions. Our knowledge in this area would benefit from a more complete understanding of Scrib, Dlg and Lgl/aPKC protein interactors in space and time in different cell types undergoing various physiological responses, such as response to growth factor/morphogen signalling, establishing apico-basal polarity, undergoing an epithelial-to-mesenchymal transition or cell migration. This analysis would be aided by new techniques in proteomics, such as BioID and Apex [307, 308], to identify at high sensitivity, protein interaction changes in response to stimuli. Additionally, state-of-the-art super-resolution microscopy approaches [309-311] would aid in the characterization of protein-protein interactions at specific localizations and over time in response to signalling stimulation. Moreover, a better understanding of Scribble complex protein-protein interactions is needed, which can be revealed by X-ray crystallography and cryo-EM [312-315], as well as the more routine biophysical measurements of protein domain-binding peptide interactions. The significance of these physical investigations can then be complemented and validated by the generation of mutations and functional analysis in model organisms, such as flies and mice.

As the Scribble module proteins are deregulated in human developmental disorders, such as neural tube closure defects [316-318], as well as in cancer [24, 25, 53, 54, 57, 319], greater knowledge of how Scrib, Dlg and Lgl/aPKC interact with other proteins is clearly important. Of

particular relevance to cancer, knowledge of the interacting proteins and signalling pathways deregulated upon Scribble module impairment during tumourigenesis, may provide new avenues for cancer therapy. For example, restoring correct Scribble module protein localization by enhancing or inhibiting particular protein interactions using small molecules might provide novel ways to restore cell polarity, inhibit the aberrant cell proliferation, and re-establish appropriate cell function. However, since there is evidence for context-dependent signalling effects conferred by Scrib, Dlg or Lgl/aPKC dysfunction (eg with Ras, PI3K or JNK signalling), this raises the need to profile tumours where the Scribble module is impaired for their signalling status before considering treatment options. Additionally, since the tumourigenic effect of apico-basal polarity dysfunction can be enhanced by the deregulation of various signalling pathways (eg Ras, PI3K, JNK, Notch or Hippo) [71, 74, 193, 204, 205, 224, 266, 281, 320-323], knowing the status of these pathways in polarity-impaired tumours will enable these cases to be triaged for treatment with pathway inhibitors to restore cell polarity or induce apoptosis, and thereby prevent tumour progression. In summary, whilst we have gained a large amount of knowledge on the Scribble module in cell polarity and signalling pathway regulation, a greater molecular level analysis of Scribble module protein interactions will provide greater understanding of the mechanism by which the Scribble module proteins function and reveal new avenues for the treatment of developmental disorders and cancer.

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Figure Legends

Figure 1: Venn diagram showing the interaction of domains of the Scrib protein with its interacting proteins

Venn diagram showing which Scrib interacting domains are responsible for specific proteins interactions, with overlapping regions depicting proteins that can bind to multiple domains. Proteins that interact with the LRR and four PDZ domains of Scrib are shown, with the venn diagram indicating binding proteins that interact with more than one PDZ domain. Scrib interacting proteins, where the Scrib interaction domain has not been determined, are listed separately under “Interacting Domain Unknown”. See Supp Table 1 for details.

Figure 2: Venn diagram showing the interaction of domains of the Dlg protein with its interacting proteins

Venn diagram showing which Dlg interacting domains are responsible for specific proteins interactions, with overlapping regions depicting proteins that can bind to multiple domains. Proteins that interact with the N-terminus, three PDZ, SH3, Hook or GUK domains of Dlg are shown, with the venn diagram indicating binding proteins that interact with more than one domain. Dlg interacting proteins, where the Dlg interaction domain has not been determined, are listed separately under, “Interacting Domain Unknown”. See Supp Table 2 for details.

Figure 3: Venn diagram showing the interaction of domains of the Lgl protein with its interacting proteins

Venn diagram showing which Lgl interacting domains are responsible for specific proteins interactions, with overlapping regions depicting proteins that can bind to multiple domains. Proteins that interact with the WD40 or C-terminal domains of Lgl are shown, with the venn diagram indicating binding proteins that interact with more than one domain. Lgl interacting

proteins, where the Lgl interaction domain has not been determined, are listed separately under, “Interacting Domain Unknown”. See Supp Table 3 for details.

Figure 4: Scrib and Dlg protein interactors in signalling pathway regulation.

The key Scrib and Dlg interacting proteins that are linked to the regulation of the BMP/TGFβ, TSHR, Wnt, Hippo, RTK-Ras-MAPK, TNF-JNK/p38 and PI3K-AKT pathways are depicted. In BMP/TGFβ signalling in *Drosophila* wing posterior cross vein development, Scrib interacts with the Serine/Threonine kinase receptors, Tkv and Pnt as well as the phosphorylated Mad transcription factor and Rab5 in early endosomes, where it to promotes BMP/TGFβ receptor signalling. In mammalian TSHR signalling, Scrib forms a complex with βPIX, GIT1 and ARF6 and is important for the recycling of the TSHR to the plasma membrane. In Wnt signalling, interactions between Dlg and the FZD receptor and phospholipid PIP2 are thought to be important in canonical and non-canonical PCP signalling via the adaptor protein, Dsh, which signals via the Rho1 and JNK pathways. Scrib and Dlg also interact with mammalian APC, which regulates β-catenin degradation. Scrib also interacts with Vangl2 in PCP. In Hippo pathway signalling in mammalian cells, Scrib binds to Taz (Yki ortholog) and sequesters it to the cortex, thereby preventing its pro-cell proliferation role. In zebrafish, Scrib binds to Fat1 and is required to inhibit Yap (Yki ortholog) activity, and in *Drosophila,* Scrib promotes Wts stabilization by an unknown mechanism. Scrib and Dlg are important for RTK signalling via different mechanisms. In HGF signalling, Scrib binds to Sgt1 and Hsp90, which stabilizes Scrib, which binds to phosphorylated PAK and βPix and promotes tubulogenesis. In ERB-Ras-MAPK signalling, Scrib interacts with βPIX, GIT1, ARFGAP, which acts as a scaffold to regulate Ras-MAPK signalling, and with PP1y, which dephosphorylates ERK. Binding of Scrib to the PP1 phosphatase regulator, SHOC2/SUR-8/MRAS, prevents dephosphorylation of RAF at its inhibitory phospho-site, therefore leading to RAF inactivation. In TNF-JNK/p38 signalling in mammalian cells, Scrib inhibits p38 signalling by an unknown mechanism. Mammalian Dlg

is also involved in p38 regulation by interacting with PBK protein kinase, which phosphorylates and activates p38, and conversely p38 can phosphorylate Dlg. JNK is also regulated by Dlg through its direct interaction with the upstream kinases, MAP3K10 and MAP3K11. In *Drosophila*, Scrib binds to Traf4, a downstream component of TNF signalling, and by an unknown mechanism, Scrib inhibits Rho1-Wnd regulated activation of JNK. The Scrib-Traf4 interaction might be involved in JNK regulation via Wnd (indicated by ?). In a mammalian Myc-driven cancer models, Scrib via its role in the assembly of the βPIX/GIT1 complex leads to Rac1 and JNK/c-Jun pathway activation, which induces the expression of the pro-apoptotic protein Bim. In PI3K-AKT signalling downstream of the InR, Scrib forms a tripartite complex with PHLPP and AKT leading to AKT inhibition. Mislocalized Scrib can act in a neomorphic role and bind to and inactivate PTEN, thereby promoting PI3K signalling. Human Dlg1 binds to p85-PI3K to recruit it to E-Cad at adherens junctions thereby promoting PI3K activity. Human Dlg1 binds to the Adenovirus 9 oncoprotein, E4-ORF1, which promotes PI3K-mediated activation of PKB and S6K. Conversely, human Dlg1 can inhibit PI3K signalling, through binding to and promoting PTEN activity. See the text for further details.

Figure 5: Lgl/aPKC interactions in signalling pathway regulation

In Notch signalling in *Drosophila*, aPKC phosphorylates Numb (an inhibitor of Notch) in ACD, whilst Lgl inhibits Notch activation by an unknown mechanism in retinal epithelial cells. In Hedgehog signalling, the aPKC-Par complex through its interaction with MIM, a scaffolding protein bound to centrosomes, phosphorylates and promotes Gli1 DNA binding and transcriptional activity. In mammalian cells, Hippo signalling is regulated by binding of aPKC the Hpo orthologs, Mst1/2, thereby uncoupling the binding of the Warts ortholog, Lats1/2, preventing Lats1/2 phosphorylation and inactivation of the Yki ortholog, YAP. The Par complex also binds to Kibra, an upstream regulator of Hpo. In Wnt (Wg) signalling in *Drosophila* and *Xenopus*, the adaptor protein Dsh is required for Lgl localization to the cortex, which is opposed by FZD8 signalling. In Ras-MAPK signalling in mammalian cancer models, the

aPKC-Par complex regulates Ect2-mediated activation of Pak1-ERK and Rac1-JNK signalling and expression of the metalloproteinase, MMP10. Lgl binds to the VprBP, which in *Drosophila* is a regulator of JNK signalling, however in mammalian cells, VprBP regulates the Cul4 ubiquitin ligase, a regulator of the G1-S phase transition. In keratinocytes, Par3 is required for the localization of Ras and its activator, Sos2 (a Ras-GEF) at cell-cell junctions, thereby promoting Ras-ERK1/2 (MAPK), as well as AKT signalling. In ErbB2-induced breast cancer models, Par3 is important for cadherin (ECad) stability, and for blocking Tiam1 (Rac-GEF)-Rac1 signalling, thereby preventing cell invasion. In Ras and Notch models of mammary cancer, Par3 acts to repress JAK-STAT signalling. In prostate cancer models, aPKCzeta, acts in cooperation with PTEN mutations to phosphorylate and inactivate Myc. In PI3K signalling, in *Drosophila* and mammalian cells, Par3 binds to PTEN and is involved in cell polarity establishment through regulating phospholipid membrane domains. See the text for further details.

References

[1] Tepass U. The apical polarity protein network in Drosophila epithelial cells: regulation of polarity, junctions, morphogenesis, cell growth, and survival. Annu Rev Cell Dev Biol. 2012;28:655-85.
[2] Martin-Belmonte F, Perez-Moreno M. Epithelial cell polarity, stem cells and cancer. Nat Rev Cancer. 2012;12:23-38.
[3] Neumuller RA, Knoblich JA. Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer. Genes Dev. 2009;23:2675-99.
[4] Thompson BJ. Cell polarity: models and mechanisms from yeast, worms and flies. Development. 2013;140:13-21.
[5] St Johnston D, Ahringer J. Cell polarity in eggs and epithelia: parallels and diversity. Cell. 2010;141:757-74.
[6] Macara IG, McCaffrey L. Cell polarity in morphogenesis and metastasis. Philos Trans R Soc Lond B Biol Sci. 2013;368:20130012.
[7] Roignot J, Peng X, Mostov K. Polarity in mammalian epithelial morphogenesis. Cold Spring Harb Perspect Biol. 2013;5.
[8] Chen J, Zhang M. The Par3/Par6/aPKC complex and epithelial cell polarity. Exp Cell Res. 2013;319:1357-64.
[9] Sebbagh M, Borg JP. Insight into planar cell polarity. Exp Cell Res. 2014;328:284-95.
[10] Carvajal-Gonzalez JM, Mlodzik M. Mechanisms of planar cell polarity establishment in Drosophila. F1000Prime Rep. 2014;6:98.
[11] Wansleeben C, Meijlink F. The planar cell polarity pathway in vertebrate development. Dev Dyn. 2011;240:616-26.
[12] Daynac M, Petritsch CK. Regulation of Asymmetric Cell Division in Mammalian Neural Stem and Cancer Precursor Cells. Results Probl Cell Differ. 2017;61:375-99.
[13] Lu MS, Johnston CA. Molecular pathways regulating mitotic spindle orientation in animal cells. Development. 2013;140:1843-56.
[14] Mayor R, Etienne-Manneville S. The front and rear of collective cell migration. Nat Rev Mol Cell Biol. 2016;17:97-109.
[15] Woodham EF, Machesky LM. Polarised cell migration: intrinsic and extrinsic drivers. Curr Opin Cell Biol. 2014;30:25-32.
[16] Venhuizen JH, Zegers MM. Making Heads or Tails of It: Cell-Cell Adhesion in Cellular and Supracellular Polarity in Collective Migration. Cold Spring Harb Perspect Biol. 2017.
[17] Zhang H. Polarity Determinants in Dendritic Spine Development and Plasticity. Neural Plast. 2016;2016:3145019.
[18] Bentley M, Banker G. The cellular mechanisms that maintain neuronal polarity. Nat Rev Neurosci. 2016;17:611-22.
[19] Dustin ML. Signaling at neuro/immune synapses. J Clin Invest. 2012;122:1149-55.
[20] Yassin M, Russell SM. Polarity and asymmetric cell division in the control of lymphocyte fate decisions and function. Curr Opin Immunol. 2016;39:143-9.
[21] Pham K, Sacirbegovic F, Russell SM. Polarized cells, polarized views: asymmetric cell division in hematopoietic cells. Front Immunol. 2014;5:26.
[22] Angus KL, Griffiths GM. Cell polarisation and the immunological synapse. Curr Opin Cell Biol. 2013;25:85-91.
[23] Lalli G. Regulation of neuronal polarity. Exp Cell Res. 2014;328:267-75.
[24] Elsum I, Yates L, Humbert PO, Richardson HE. The Scribble-Dlg-Lgl polarity module in development and cancer: from flies to man. Essays Biochem. 2012;53:141-68.
[25] Humbert PO, Russell SM, Smith L, Richardson HE. The Scribble–Dlg–Lgl Module in Cell Polarity Regulation. In: Ebnet K, editor. Cell Polarity. Switzerland Springer International Publishing 2015. p. 65-111.

[26] Bilder D, Li M, Perrimon N. Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science. 2000;289:113-6.
[27] Massimi P, Narayan N, Thomas M, Gammoh N, Strand S, Strand D, et al. Regulation of the hDlg/hScrib/Hugl-1 tumour suppressor complex. Exp Cell Res. 2008;314:3306-17.
[28] Goldstein B, Macara IG. The PAR proteins: fundamental players in animal cell polarization. Dev Cell. 2007;13:609-22.
[29] Kallay LM, McNickle A, Brennwald PJ, Hubbard AL, Braiterman LT. Scribble associates with two polarity proteins, Lgl2 and Vangl2, via distinct molecular domains. J Cell Biochem. 2006;99:647-64.
[30] Zhu J, Shang Y, Wan Q, Xia Y, Chen J, Du Q, et al. Phosphorylation-dependent interaction between tumor suppressors Dlg and Lgl. Cell Res. 2014;24:451-63.
[31] Mathew D, Gramates LS, Packard M, Thomas U, Bilder D, Perrimon N, et al. Recruitment of scribble to the synaptic scaffolding complex requires GUK-holder, a novel DLG binding protein. Curr Biol. 2002;12:531-9.
[32] Plant PJ, Fawcett JP, Lin DC, Holdorf AD, Binns K, Kulkarni S, et al. A polarity complex of mPar-6 and atypical PKC binds, phosphorylates and regulates mammalian Lgl. Nat Cell Biol. 2003;5:301-8.
[33] Betschinger J, Eisenhaber F, Knoblich JA. Phosphorylation-induced autoinhibition regulates the cytoskeletal protein Lethal (2) giant larvae. Curr Biol. 2005;15:276-82.
[34] Hutterer A, Betschinger J, Petronczki M, Knoblich JA. Sequential roles of Cdc42, Par-6, aPKC, and Lgl in the establishment of epithelial polarity during Drosophila embryogenesis. Dev Cell. 2004;6:845-54.
[35] Betschinger J, Mechtler K, Knoblich JA. The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. Nature. 2003;422:326-30.
[36] Rolls MM, Albertson R, Shih HP, Lee CY, Doe CQ. Drosophila aPKC regulates cell polarity and cell proliferation in neuroblasts and epithelia. J Cell Biol. 2003;163:1089-98.
[37] Vasioukhin V. Lethal giant puzzle of Lgl. Dev Neurosci. 2006;28:13-24.
[38] Wirtz-Peitz F, Knoblich JA. Lethal giant larvae take on a life of their own. Trends Cell Biol. 2006;16:234-41.
[39] Moreira S, Morais-de-Sa E. Spatiotemporal phosphoregulation of Lgl: Finding meaning in multiple on/off buttons. Bioarchitecture. 2016;6:29-38.
[40] Wirtz-Peitz F, Nishimura T, Knoblich JA. Linking cell cycle to asymmetric division: Aurora-A phosphorylates the Par complex to regulate Numb localization. Cell. 2008;135:161-73.
[41] Qin Y, Capaldo C, Gumbiner BM, Macara IG. The mammalian Scribble polarity protein regulates epithelial cell adhesion and migration through E-cadherin. J Cell Biol. 2005;171:1061-71.
[42] Navarro C, Nola S, Audebert S, Santoni MJ, Arsanto JP, Ginestier C, et al. Junctional recruitment of mammalian Scribble relies on E-cadherin engagement. Oncogene. 2005.
[43] Bilder D, Perrimon N. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature. 2000;403:676-80.
[44] Bilder D, Schober M, Perrimon N. Integrated activity of PDZ protein complexes regulates epithelial polarity. Nat Cell Biol. 2003;5:53-8.
[45] Tanentzapf G, Tepass U. Interactions between the crumbs, lethal giant larvae and bazooka pathways in epithelial polarization. Nat Cell Biol. 2003;5:46-52.
[46] Pocha SM, Knust E. Complexities of Crumbs function and regulation in tissue morphogenesis. Curr Biol. 2013;23:R289-93.
[47] Fletcher GC, Lucas EP, Brain R, Tournier A, Thompson BJ. Positive feedback and mutual antagonism combine to polarize Crumbs in the Drosophila follicle cell epithelium. Curr Biol. 2012;22:1116-22.
[48] Sotillos S, Diaz-Meco MT, Caminero E, Moscat J, Campuzano S. DaPKC-dependent phosphorylation of Crumbs is required for epithelial cell polarity in Drosophila. J Cell Biol. 2004.
[49] Thompson BJ, Pichaud F, Roper K. Sticking together the Crumbs - an unexpected function for an old friend. Nat Rev Mol Cell Biol. 2013;14:307-14.

[50] Laprise P, Lau KM, Harris KP, Silva-Gagliardi NF, Paul SM, Beronja S, et al. Yurt, Coracle, Neurexin IV and the Na(+),K(+)-ATPase form a novel group of epithelial polarity proteins. Nature. 2009;459:1141-5.
[51] Laprise P, Beronja S, Silva-Gagliardi NF, Pellikka M, Jensen AM, McGlade CJ, et al. The FERM protein Yurt is a negative regulatory component of the Crumbs complex that controls epithelial polarity and apical membrane size. Dev Cell. 2006;11:363-74.
[52] Gamblin CL, Hardy EJ, Chartier FJ, Bisson N, Laprise P. A bidirectional antagonism between aPKC and Yurt regulates epithelial cell polarity. J Cell Biol. 2014;204:487-95.
[53] Halaoui R, McCaffrey L. Rewiring cell polarity signaling in cancer. Oncogene. 2015;34:939-50.
[54] Khursheed M, Bashyam MD. Apico-basal polarity complex and cancer. J Biosci. 2014;39:145-55.
[55] Ellenbroek SI, Iden S, Collard JG. Cell polarity proteins and cancer. Semin Cancer Biol. 2012;22:208-15.
[56] Cao F, Miao Y, Xu K, Liu P. Lethal (2) giant larvae: an indispensable regulator of cell polarity and cancer development. Int J Biol Sci. 2015;11:380-9.
[57] Lin WH, Asmann YW, Anastasiadis PZ. Expression of polarity genes in human cancer. Cancer Inform. 2015;14:15-28.
[58] Ruan L, Shen Y, Lu Z, Shang D, Zhao Z, Lu Y, et al. Roles of partitioning-defective protein 6 (Par6) and its complexes in the proliferation, migration and invasion of cancer cells. Clin Exp Pharmacol Physiol. 2017.
[59] Li P, Mao X, Ren Y, Liu P. Epithelial cell polarity determinant CRB3 in cancer development. Int J Biol Sci. 2015;11:31-7.
[60] Tervonen TA, Partanen JI, Saarikoski ST, Myllynen M, Marques E, Paasonen K, et al. Faulty epithelial polarity genes and cancer. Adv Cancer Res. 2011;111:97-161.
[61] Banks L, Pim D, Thomas M. Human tumour viruses and the deregulation of cell polarity in cancer. Nat Rev Cancer. 2012;12:877-86.
[62] Ganti K, Broniarczyk J, Manoubi W, Massimi P, Mittal S, Pim D, et al. The Human Papillomavirus E6 PDZ Binding Motif: From Life Cycle to Malignancy. Viruses. 2015;7:3530-51.
[63] Tomaic V, Gardiol D, Massimi P, Ozbun M, Myers M, Banks L. Human and primate tumour viruses use PDZ binding as an evolutionarily conserved mechanism of targeting cell polarity regulators. Oncogene. 2009;28:1-8.
[64] Cavatorta AL, Fumero G, Chouhy D, Aguirre R, Nocito AL, Giri AA, et al. Differential expression of the human homologue of drosophila discs large oncosuppressor in histologic samples from human papillomavirus-associated lesions as a marker for progression to malignancy. Int J Cancer. 2004;111:373-80.
[65] Mantovani F, Massimi P, Banks L. Proteasome-mediated regulation of the hDlg tumour suppressor protein. J Cell Sci. 2001;114:4285-92.
[66] Thomas M, Narayan N, Pim D, Tomaic V, Massimi P, Nagasaka K, et al. Human papillomaviruses, cervical cancer and cell polarity. Oncogene. 2008;27:7018-30.
[67] Guccione E, Pim D, Banks L. HPV-18 E6*I modulates HPV-18 full-length E6 functions in a cell cycle dependent manner. Int J Cancer. 2004;110:928-33.
[68] Mechler BM, McGinnis W, Gehring WJ. Molecular cloning of lethal(2)giant larvae, a recessive oncogene of Drosophila melanogaster. Embo J. 1985;4:1551-7.
[69] Woods DF, Bryant PJ. Molecular cloning of the lethal(1)discs large-1 oncogene of Drosophila. Dev Biol. 1989;134:222-35.
[70] Brumby A, Secombe J, Horsfield J, Coombe M, Amin N, Coates D, et al. A genetic screen for dominant modifiers of a cyclin E hypomorphic mutation identifies novel regulators of S-phase entry in Drosophila. Genetics. 2004;168:227-51.
[71] Brumby AM, Richardson HE. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. Embo J. 2003;22:5769-79.
[72] Calleja M, Morata G, Casanova J. Tumorigenic Properties of Drosophila Epithelial Cells Mutant for lethal giant larvae. Dev Dyn. 2016;245:834-43.

[73] Froldi F, Ziosi M, Tomba G, Parisi F, Garoia F, Pession A, et al. Drosophila lethal giant larvae neoplastic mutant as a genetic tool for cancer modeling. Curr Genomics. 2008;9:147-54.
[74] Khan SJ, Bajpai A, Alam MA, Gupta RP, Harsh S, Pandey RK, et al. Epithelial neoplasia in Drosophila entails switch to primitive cell states. Proc Natl Acad Sci U S A. 2013;110:E2163-72.
[75] Agrawal N, Kango M, Mishra A, Sinha P. Neoplastic transformation and aberrant cell-cell interactions in genetic mosaics of lethal(2)giant larvae (lgl), a tumor suppressor gene of Drosophila. Dev Biol. 1995;172:218-29.
[76] Grifoni D, Sollazzo M, Fontana E, Froldi F, Pession A. Multiple strategies of oxygen supply in Drosophila malignancies identify tracheogenesis as a novel cancer hallmark. Sci Rep. 2015;5:9061.
[77] Grifoni D, Garoia F, Schimanski CC, Schmitz G, Laurenti E, Galle PR, et al. The human protein Hugl-1 substitutes for Drosophila lethal giant larvae tumour suppressor function in vivo. Oncogene. 2004;23:8688-94.
[78] Thomas U, Phannavong B, Muller B, Garner CC, Gundelfinger ED. Functional expression of rat synapse-associated proteins SAP97 and SAP102 in Drosophila dlg-1 mutants: effects on tumor suppression and synaptic bouton structure. Mech Dev. 1997;62:161-74.
[79] Dow LE, Brumby AM, Muratore R, Coombe ML, Sedelies KA, Trapani JA, et al. hScrib is a functional homologue of the Drosophila tumour suppressor Scribble. Oncogene. 2003;22:9225-30.
[80] Humbert PO, Grzeschik NA, Brumby AM, Galea R, Elsum I, Richardson HE. Control of tumourigenesis by the Scribble/Dlg/Lgl polarity module. Oncogene. 2008;27:6888-907.
[81] Pearson HB, Perez-Mancera PA, Dow LE, Ryan A, Tennstedt P, Bogani D, et al. SCRIB expression is deregulated in human prostate cancer, and its deficiency in mice promotes prostate neoplasia. J Clin Invest. 2011;121:4257-67.
[82] Pearson HB, McGlinn E, Phesse TJ, Schluter H, Srikumar A, Godde NJ, et al. The polarity protein Scrib mediates epidermal development and exerts a tumor suppressive function during skin carcinogenesis. Mol Cancer. 2015;14:169.
[83] Godde NJ, Sheridan JM, Smith LK, Pearson HB, Britt KL, Galea RC, et al. Scribble modulates the MAPK/Fra1 pathway to disrupt luminal and ductal integrity and suppress tumour formation in the mammary gland. PLoS Genet. 2014;10:e1004323.
[84] Elsum IA, Yates LL, Pearson HB, Phesse TJ, Long F, O'Donoghue R, et al. Scrib heterozygosity predisposes to lung cancer and cooperates with KRas hyperactivation to accelerate lung cancer progression in vivo. Oncogene. 2013.
[85] Feigin ME, Akshinthala SD, Araki K, Rosenberg AZ, Muthuswamy LB, Martin B, et al. Mislocalization of the cell polarity protein scribble promotes mammary tumorigenesis and is associated with basal breast cancer. Cancer Res. 2014;74:3180-94.
[86] Zhan L, Rosenberg A, Bergami KC, Yu M, Xuan Z, Jaffe AB, et al. Deregulation of scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma. Cell. 2008;135:865-78.
[87] Klezovitch O, Fernandez TE, Tapscott SJ, Vasioukhin V. Loss of cell polarity causes severe brain dysplasia in Lgl1 knockout mice. Genes Dev. 2004;18:559-71.
[88] Iizuka-Kogo A, Ishidao T, Akiyama T, Senda T. Abnormal development of urogenital organs in Dlgh1-deficient mice. Development. 2007;134:1799-807.
[89] Caruana G, Bernstein A. Craniofacial dysmorphogenesis including cleft palate in mice with an insertional mutation in the discs large gene. Mol Cell Biol. 2001;21:1475-83.
[90] Rivera C, Simonson SJ, Yamben IF, Shatadal S, Nguyen MM, Beurg M, et al. Requirement for Dlgh-1 in planar cell polarity and skeletogenesis during vertebrate development. PLoS One. 2013;8:e54410.
[91] Bilder D, Birnbaum D, Borg JP, Bryant P, Huigbretse J, Jansen E, et al. Collective nomenclature for LAP proteins. Nat Cell Biol. 2000;2:E114.
taf/DynaPage.taf?file=/ncb/journal/v2/n7/full/ncb0700_E114a.html
taf/DynaPage.taf?file=/ncb/journal/v2/n7/abs/ncb_E114a.html.

[92] Santoni MJ, Pontarotti P, Birnbaum D, Borg JP. The LAP family: a phylogenetic point of view. Trends Genet. 2002;18:494-7.
[93] Zeitler J, Hsu CP, Dionne H, Bilder D. Domains controlling cell polarity and proliferation in the Drosophila tumor suppressor Scribble. J Cell Biol. 2004;167:1137-46.
[94] Legouis R, Jaulin-Bastard F, Schott S, Navarro C, Borg JP, Labouesse M. Basolateral targeting by leucine-rich repeat domains in epithelial cells. EMBO Rep. 2003;4:1096-102.
[95] Woods DF, Bryant PJ. The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions. Cell. 1991;66:451-64.
[96] Hough CD, Woods DF, Park S, Bryant PJ. Organizing a functional junctional complex requires specific domains of the Drosophila MAGUK Discs large. Genes Dev. 1997;11:3242-53.
[97] Nakajima Y, Gibson MC. Epithelial cell division: Aurora kicks Lgl to the cytoplasmic curb. Curr Biol. 2015;25:R43-5.
[98] Hattendorf DA, Andreeva A, Gangar A, Brennwald PJ, Weis WI. Structure of the yeast polarity protein Sro7 reveals a SNARE regulatory mechanism. Nature. 2007;446:567-71.
[99] Li X, Yang H, Liu J, Schmidt MD, Gao T. Scribble-mediated membrane targeting of PHLPP1 is required for its negative regulation of Akt. EMBO Rep. 2011.
[100] Eastburn DJ, Zegers MM, Mostov KE. Scrib regulates HGF-mediated epithelial morphogenesis and is stabilized by Sgt1-HSP90. J Cell Sci. 2012;125:4147-57.
[101] Gui J, Huang Y, Shimmi O. Scribbled Optimizes BMP Signaling through Its Receptor Internalization to the Rab5 Endosome and Promote Robust Epithelial Morphogenesis. PLoS Genet. 2016;12:e1006424.
[102] Audebert S, Navarro C, Nourry C, Chasserot-Golaz S, Lecine P, Bellaiche Y, et al. Mammalian Scribble forms a tight complex with the betaPIX exchange factor. Curr Biol. 2004;14:987-95.
[103] Arnaud C, Sebbagh M, Nola S, Audebert S, Bidaut G, Hermant A, et al. MCC, a new interacting protein for Scrib, is required for cell migration in epithelial cells. FEBS Lett. 2009;583:2326-32.
[104] Sun Y, Aiga M, Yoshida E, Humbert PO, Bamji SX. Scribble interacts with beta-catenin to localize synaptic vesicles to synapses. Mol Biol Cell. 2009;20:3390-400.
[105] Nola S, Sebbagh M, Marchetto S, Osmani N, Nourry C, Audebert S, et al. Scrib regulates PAK activity during the cell migration process. Hum Mol Genet. 2008;17:3552-65.
[106] Bellaiche Y, Beaudoin-Massiani O, Stuttem I, Schweisguth F. The planar cell polarity protein Strabismus promotes Pins anterior localization during asymmetric division of sensory organ precursor cells in Drosophila. Development. 2004;131:469-78.
[107] Matsumine A, Ogai A, Senda T, Okumura N, Satoh K, Baeg GH, et al. Binding of APC to the human homolog of the Drosophila discs large tumor suppressor protein. Science. 1996;272:1020-3.
[108] Leonoudakis D, Conti LR, Radeke CM, McGuire LM, Vandenberg CA. A multiprotein trafficking complex composed of SAP97, CASK, Veli, and Mint1 is associated with inward rectifier Kir2 potassium channels. J Biol Chem. 2004;279:19051-63.
[109] Nix SL, Chishti AH, Anderson JM, Walther Z. hCASK and hDlg associate in epithelia, and their src homology 3 and guanylate kinase domains participate in both intramolecular and intermolecular interactions. J Biol Chem. 2000;275:41192-200.
[110] Lue RA, Marfatia SM, Branton D, Chishti AH. Cloning and characterization of hdlg: the human homologue of the Drosophila discs large tumor suppressor binds to protein 4.1. Proc Natl Acad Sci U S A. 1994;91:9818-22.
[111] Garcia JD, Dewey EB, Johnston CA. Dishevelled binds the Discs large 'Hook' domain to activate GukHolder-dependent spindle positioning in Drosophila. PLoS One. 2014;9:e114235.
[112] Gangar A, Rossi G, Andreeva A, Hales R, Brennwald P. Structurally conserved interaction of Lgl family with SNAREs is critical to their cellular function. Curr Biol. 2005;15:1136-42.
[113] Musch A, Cohen D, Yeaman C, Nelson WJ, Rodriguez-Boulan E, Brennwald PJ. Mammalian homolog of Drosophila tumor suppressor lethal (2) giant larvae interacts with basolateral exocytic machinery in Madin-Darby canine kidney cells. Mol Biol Cell. 2002;13:158-68.

[114] Grosshans BL, Andreeva A, Gangar A, Niessen S, Yates JR, 3rd, Brennwald P, et al. The yeast lgl family member Sro7p is an effector of the secretory Rab GTPase Sec4p. J Cell Biol. 2006;172:55-66.
[115] Watson K, Rossi G, Temple B, Brennwald P. Structural basis for recognition of the Sec4 Rab GTPase by its effector, the Lgl/tomosyn homologue, Sro7. Mol Biol Cell. 2015;26:3289-300.
[116] Suresh B, Ramakrishna S, Kim YS, Kim SM, Kim MS, Baek KH. Stability and function of mammalian lethal giant larvae-1 oncoprotein are regulated by the scaffolding protein RanBPM. J Biol Chem. 2010;285:35340-9.
[117] Lim KH, Suresh B, Park JH, Kim YS, Ramakrishna S, Baek KH. Ubiquitin-specific protease 11 functions as a tumor suppressor by modulating Mgl-1 protein to regulate cancer cell growth. Oncotarget. 2016;7:14441-57.
[118] Tamori Y, Bialucha CU, Tian AG, Kajita M, Huang YC, Norman M, et al. Involvement of Lgl and Mahjong/VprBP in cell competition. PLoS Biol. 2010;8:e1000422.
[119] Yamashita K, Ide M, Furukawa KT, Suzuki A, Hirano H, Ohno S. Tumor suppressor protein Lgl mediates G1 cell cycle arrest at high cell density by forming an Lgl-VprBP-DDB1 complex. Mol Biol Cell. 2015;26:2426-38.
[120] Strand D, Unger S, Corvi R, Hartenstein K, Schenkel H, Kalmes A, et al. A human homologue of the Drosophila tumour suppressor gene l(2)gl maps to 17p11.2-12 and codes for a cytoskeletal protein that associates with nonmuscle myosin II heavy chain. Oncogene. 1995;11:291-301.
[121] Dahan I, Petrov D, Cohen-Kfir E, Ravid S. The tumor suppressor Lgl1 forms discrete complexes with NMII-A and Par6alpha-aPKCzeta that are affected by Lgl1 phosphorylation. J Cell Sci. 2014;127:295-304.
[122] Dahan I, Yearim A, Touboul Y, Ravid S. The tumor suppressor Lgl1 regulates NMII-A cellular distribution and focal adhesion morphology to optimize cell migration. Mol Biol Cell. 2012;23:591-601.
[123] Jossin Y, Lee M, Klezovitch O, Kon E, Cossard A, Lien WH, et al. Llgl1 Connects Cell Polarity with Cell-Cell Adhesion in Embryonic Neural Stem Cells. Dev Cell. 2017;41:481-95 e5.
[124] Doyle DA, Lee A, Lewis J, Kim E, Sheng M, MacKinnon R. Crystal structures of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by PDZ. Cell. 1996;85:1067-76.
[125] Cho KO, Hunt CA, Kennedy MB. The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. Neuron. 1992;9:929-42.
[126] Saras J, Heldin CH. PDZ domains bind carboxy-terminal sequences of target proteins. Trends Biochem Sci. 1996;21:455-8.
[127] Songyang Z, Fanning AS, Fu C, Xu J, Marfatia SM, Chishti AH, et al. Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. Science. 1997;275:73-7.
[128] Stricker NL, Christopherson KS, Yi BA, Schatz PJ, Raab RW, Dawes G, et al. PDZ domain of neuronal nitric oxide synthase recognizes novel C-terminal peptide sequences. Nat Biotechnol. 1997;15:336-42.
[129] Metais JY, Navarro C, Santoni MJ, Audebert S, Borg JP. hScrib interacts with ZO-2 at the cell-cell junctions of epithelial cells. FEBS Lett. 2005;579:3725-30.
[130] Phua DC, Humbert PO, Hunziker W. Vimentin regulates scribble activity by protecting it from proteasomal degradation. Mol Biol Cell. 2009;20:2841-55.
[131] Petit MM, Crombez KR, Vervenne HB, Weyns N, Van de Ven WJ. The tumor suppressor Scrib selectively interacts with specific members of the zyxin family of proteins. FEBS Lett. 2005;579:5061-8.
[132] Lahuna O, Quellari M, Achard C, Nola S, Meduri G, Navarro C, et al. Thyrotropin receptor trafficking relies on the hScrib-betaPIX-GIT1-ARF6 pathway. Embo J. 2005;24:1364-74.
[133] Liu H, Golebiewski L, Dow EC, Krug RM, Javier RT, Rice AP. The ESEV PDZ-binding motif of the avian influenza A virus NS1 protein protects infected cells from apoptosis by directly targeting Scribble. J Virol. 2010;84.

[134] Werme K, Wigerius M, Johansson M. Tick-borne encephalitis virus NS5 associates with membrane protein scribble and impairs interferon-stimulated JAK-STAT signalling. Cell Microbiol. 2008;10:696-712.
[135] Tejedor FJ, Bokhari A, Rogero O, Gorczyca M, Zhang J, Kim E, et al. Essential role for dlg in synaptic clustering of Shaker K+ channels in vivo. J Neurosci. 1997;17:152-9.
[136] Gardiol D, Kuhne C, Glaunsinger B, Lee SS, Javier R, Banks L. Oncogenic human papillomavirus E6 proteins target the discs large tumour suppressor for proteasome-mediated degradation. Oncogene. 1999;18:5487-96.
[137] Kiyono T, Hiraiwa A, Fujita M, Hayashi Y, Akiyama T, Ishibashi M. Binding of high-risk human papillomavirus E6 oncoproteins to the human homologue of the Drosophila discs large tumor suppressor protein. Proc Natl Acad Sci U S A. 1997;94:11612-6.
[138] Piguel NH, Fievre S, Blanc JM, Carta M, Moreau MM, Moutin E, et al. Scribble1/AP2 complex coordinates NMDA receptor endocytic recycling. Cell Rep. 2014;9:712-27.
[139] Lim KYB, Godde NJ, Humbert PO, Kvansakul M. Structural basis for the differential interaction of Scribble PDZ domains with the guanine nucleotide exchange factor beta-PIX. J Biol Chem. 2017.
[140] Tonikian R, Zhang Y, Sazinsky SL, Currell B, Yeh JH, Reva B, et al. A specificity map for the PDZ domain family. PLoS Biol. 2008;6:e239.
[141] Ivarsson Y, Arnold R, McLaughlin M, Nim S, Joshi R, Ray D, et al. Large-scale interaction profiling of PDZ domains through proteomic peptide-phage display using human and viral phage peptidomes. Proc Natl Acad Sci U S A. 2014;111:2542-7.
[142] Appleton BA, Zhang Y, Wu P, Yin JP, Hunziker W, Skelton NJ, et al. Comparative structural analysis of the Erbin PDZ domain and the first PDZ domain of ZO-1. Insights into determinants of PDZ domain specificity. J Biol Chem. 2006;281:22312-20.
[143] Stiffler MA, Chen JR, Grantcharova VP, Lei Y, Fuchs D, Allen JE, et al. PDZ domain binding selectivity is optimized across the mouse proteome. Science. 2007;317:364-9.
[144] Pangon L, Van Kralingen C, Abas M, Daly RJ, Musgrove EA, Kohonen-Corish MR. The PDZ-binding motif of MCC is phosphorylated at position -1 and controls lamellipodia formation in colon epithelial cells. Biochim Biophys Acta. 2012;1823:1058-67.
[145] Nagasaka K, Pim D, Massimi P, Thomas M, Tomaic V, Subbaiah VK, et al. The cell polarity regulator hScrib controls ERK activation through a KIM site-dependent interaction. Oncogene. 2010;29:5311-21.
[146] Nagasaka K, Massimi P, Pim D, Subbaiah VK, Kranjec C, Nakagawa S, et al. The mechanism and implications of hScrib regulation of ERK. Small Gtpases. 2010;1:108-12.
[147] Yoshihara K, Ikenouchi J, Izumi Y, Akashi M, Tsukita S, Furuse M. Phosphorylation state regulates the localization of Scribble at adherens junctions and its association with E-cadherin-catenin complexes. Exp Cell Res. 2011;317:413-22.
[148] Nagasaka K, Seiki T, Yamashita A, Massimi P, Subbaiah VK, Thomas M, et al. A novel interaction between hScrib and PP1gamma downregulates ERK signaling and suppresses oncogene-induced cell transformation. PLoS One. 2013;8:e53752.
[149] Metodieva G, Nogueira-de-Souza NC, Greenwood C, Al-Janabi K, Leng L, Bucala R, et al. CD74-dependent deregulation of the tumor suppressor scribble in human epithelial and breast cancer cells. Neoplasia. 2013;15:660-8.
[150] Laprise P, Langlois MJ, Boucher MJ, Jobin C, Rivard N. Down-regulation of MEK/ERK signaling by E-cadherin-dependent PI3K/Akt pathway in differentiating intestinal epithelial cells. J Cell Physiol. 2004;199:32-9.
[151] Gaudet S, Langlois MJ, Lue RA, Rivard N, Viel A. The MEK2-binding tumor suppressor hDlg is recruited by E-cadherin to the midbody ring. BMC Cell Biol. 2011;12:55.
[152] Zhang Y, Guo H, Kwan H, Wang JW, Kosek J, Lu B. PAR-1 kinase phosphorylates Dlg and regulates its postsynaptic targeting at the Drosophila neuromuscular junction. Neuron. 2007;53:201-15.

[153] Koh YH, Popova E, Thomas U, Griffith LC, Budnik V. Regulation of DLG localization at synapses by CaMKII-dependent phosphorylation. Cell. 1999;98:353-63.
[154] Wang SJ, Tsai A, Wang M, Yoo S, Kim HY, Yoo B, et al. Phospho-regulated Drosophila adducin is a determinant of synaptic plasticity in a complex with Dlg and PIP2 at the larval neuromuscular junction. Biol Open. 2014;3:1196-206.
[155] Wang S, Yoo S, Kim HY, Wang M, Zheng C, Parkhouse W, et al. Detection of in situ protein-protein complexes at the Drosophila larval neuromuscular junction using proximity ligation assay. J Vis Exp. 2015:52139.
[156] Wang S, Yang J, Tsai A, Kuca T, Sanny J, Lee J, et al. Drosophila adducin regulates Dlg phosphorylation and targeting of Dlg to the synapse and epithelial membrane. Dev Biol. 2011;357:392-403.
[157] Carvalho CA, Moreira S, Ventura G, Sunkel CE, Morais-de-Sa E. Aurora A triggers Lgl cortical release during symmetric division to control planar spindle orientation. Curr Biol. 2015;25:53-60.
[158] Bell GP, Fletcher GC, Brain R, Thompson BJ. Aurora kinases phosphorylate Lgl to induce mitotic spindle orientation in Drosophila epithelia. Curr Biol. 2015;25:61-8.
[159] Dong W, Zhang X, Liu W, Chen YJ, Huang J, Austin E, et al. A conserved polybasic domain mediates plasma membrane targeting of Lgl and its regulation by hypoxia. J Cell Biol. 2015;211:273-86.
[160] Kalmes A, Merdes G, Neumann B, Strand D, Mechler BM. A serine-kinase associated with the p127-l(2)gl tumour suppressor of Drosophila may regulate the binding of p127 to nonmuscle myosin II heavy chain and the attachment of p127 to the plasma membrane. J Cell Sci. 1996;109 (Pt 6):1359-68.
[161] Strand D, Jakobs R, Merdes G, Neumann B, Kalmes A, Heid HW, et al. The Drosophila lethal(2)giant larvae tumor suppressor protein forms homo-oligomers and is associated with nonmuscle myosin II heavy chain. J Cell Biol. 1994;127:1361-73.
[162] Strand D, Raska I, Mechler BM. The Drosophila lethal(2)giant larvae tumor suppressor protein is a component of the cytoskeleton. J Cell Biol. 1994;127:1345-60.
[163] Kim E, Naisbitt S, Hsueh YP, Rao A, Rothschild A, Craig AM, et al. GKAP, a novel synaptic protein that interacts with the guanylate kinase-like domain of the PSD-95/SAP90 family of channel clustering molecules. J Cell Biol. 1997;136:669-78.
[164] Takeuchi M, Hata Y, Hirao K, Toyoda A, Irie M, Takai Y. SAPAPs. A family of PSD-95/SAP90-associated proteins localized at postsynaptic density. J Biol Chem. 1997;272:11943-51.
[165] Satoh K, Yanai H, Senda T, Kohu K, Nakamura T, Okumura N, et al. DAP-1, a novel protein that interacts with the guanylate kinase-like domains of hDLG and PSD-95. Genes Cells. 1997;2:415-24.
[166] Wu H, Reissner C, Kuhlendahl S, Coblentz B, Reuver S, Kindler S, et al. Intramolecular interactions regulate SAP97 binding to GKAP. Embo J. 2000;19:5740-51.
[167] Ren J, Feng L, Bai Y, Pei H, Yuan Z, Feng W. Interdomain interface-mediated target recognition by the Scribble PDZ34 supramodule. Biochem J. 2015;468:133-44.
[168] Long JF, Tochio H, Wang P, Fan JS, Sala C, Niethammer M, et al. Supramodular structure and synergistic target binding of the N-terminal tandem PDZ domains of PSD-95. J Mol Biol. 2003;327:203-14.
[169] Liu W, Wen W, Wei Z, Yu J, Ye F, Liu CH, et al. The INAD scaffold is a dynamic, redox-regulated modulator of signaling in the Drosophila eye. Cell. 2011;145:1088-101.
[170] Feng W, Shi Y, Li M, Zhang M. Tandem PDZ repeats in glutamate receptor-interacting proteins have a novel mode of PDZ domain-mediated target binding. Nat Struct Biol. 2003;10:972-8.
[171] Long J, Wei Z, Feng W, Yu C, Zhao YX, Zhang M. Supramodular nature of GRIP1 revealed by the structure of its PDZ12 tandem in complex with the carboxyl tail of Fras1. J Mol Biol. 2008;375:1457-68.
[172] Visco I, Hoege C, Hyman AA, Schwille P. In vitro Reconstitution of a Membrane Switch Mechanism for the Polarity Protein LGL. J Mol Biol. 2016;428:4828-42.

[173] Walsh GS, Grant PK, Morgan JA, Moens CB. Planar polarity pathway and Nance-Horan syndrome-like 1b have essential cell-autonomous functions in neuronal migration. Development. 2011;138:3033-42.
[174] Qian Y, Prehoda KE. Interdomain interactions in the tumor suppressor discs large regulate binding to the synaptic protein GukHolder. J Biol Chem. 2006;281:35757-63.
[175] Zhang J, Lewis SM, Kuhlman B, Lee AL. Supertertiary structure of the MAGUK core from PSD-95. Structure. 2013;21:402-13.
[176] McCann JJ, Zheng L, Rohrbeck D, Felekyan S, Kuhnemuth R, Sutton RB, et al. Supertertiary structure of the synaptic MAGuK scaffold proteins is conserved. Proc Natl Acad Sci U S A. 2012;109:15775-80.
[177] Bergstralh DT, Lovegrove HE, St Johnston D. Discs large links spindle orientation to apical-basal polarity in Drosophila epithelia. Curr Biol. 2013;23:1707-12.
[178] Pecreaux J, Roper JC, Kruse K, Julicher F, Hyman AA, Grill SW, et al. Spindle oscillations during asymmetric cell division require a threshold number of active cortical force generators. Curr Biol. 2006;16:2111-22.
[179] Kotak S, Busso C, Gonczy P. Cortical dynein is critical for proper spindle positioning in human cells. J Cell Biol. 2012;199:97-110.
[180] Sotelo NS, Valiente M, Gil A, Pulido R. A functional network of the tumor suppressors APC, hDlg, and PTEN, that relies on recognition of specific PDZ-domains. J Cell Biochem. 2012;113:2661-70.
[181] Laprise P, Viel A, Rivard N. Human homolog of disc-large is required for adherens junction assembly and differentiation of human intestinal epithelial cells. J Biol Chem. 2004;279:10157-66.
[182] Hernandez JL, Davda D, Cheung See Kit M, Majmudar JD, Won SJ, Gang M, et al. APT2 Inhibition Restores Scribble Localization and S-Palmitoylation in Snail-Transformed Cells. Cell Chem Biol. 2017;24:87-97.
[183] Chen B, Zheng B, DeRan M, Jarugumilli GK, Fu J, Brooks YS, et al. ZDHHC7-mediated S-palmitoylation of Scribble regulates cell polarity. Nat Chem Biol. 2016;12:686-93.
[184] Izawa I, Nishizawa M, Hayashi Y, Inagaki M. Palmitoylation of ERBIN is required for its plasma membrane localization. Genes Cells. 2008;13:691-701.
[185] Robinson A, Escuin S, Doudney K, Vekemans M, Stevenson RE, Greene ND, et al. Mutations in the planar cell polarity genes CELSR1 and SCRIB are associated with the severe neural tube defect craniorachischisis. Hum Mutat. 2012;33:440-7.
[186] Lei Y, Zhu H, Duhon C, Yang W, Ross ME, Shaw GM, et al. Mutations in planar cell polarity gene SCRIB are associated with spina bifida. PLoS One. 2013;8:e69262.
[187] Elsum IA, Humbert PO. Localization, not important in all tumor-suppressing properties: a lesson learnt from scribble. Cells Tissues Organs. 2013;198:1-11.
[188] Massimi P, Narayan N, Cuenda A, Banks L. Phosphorylation of the discs large tumour suppressor protein controls its membrane localisation and enhances its susceptibility to HPV E6-induced degradation. Oncogene. 2006;25:4276-85.
[189] El-Husseini AE, Topinka JR, Lehrer-Graiwer JE, Firestein BL, Craven SE, Aoki C, et al. Ion channel clustering by membrane-associated guanylate kinases. Differential regulation by N-terminal lipid and metal binding motifs. J Biol Chem. 2000;275:23904-10.
[190] Craven SE, El-Husseini AE, Bredt DS. Synaptic targeting of the postsynaptic density protein PSD-95 mediated by lipid and protein motifs. Neuron. 1999;22:497-509.
[191] Fey D, Matallanas D, Rauch J, Rukhlenko OS, Kholodenko BN. The complexities and versatility of the RAS-to-ERK signalling system in normal and cancer cells. Semin Cell Dev Biol. 2016;58:96-107.
[192] Elsum IA, Martin C, Humbert PO. Scribble regulates an EMT-polarity pathway through modulation of MAPK-ERK signaling to mediate junction formation. J Cell Sci. 2013.
[193] Dow LE, Elsum IA, King CL, Kinross KM, Richardson HE, Humbert PO. Loss of human Scribble cooperates with H-Ras to promote cell invasion through deregulation of MAPK signalling. Oncogene. 2008;27:5988-6001.

[194] Kapil S, Sharma BK, Patil M, Elattar S, Yuan J, Hou SX, et al. The cell polarity protein Scrib functions as a tumor suppressor in liver cancer. Oncotarget. 2017;8:26515-31.
[195] Rives-Quinto N, Franco M, de Torres-Jurado A, Carmena A. Synergism between canoe and scribble mutations causes tumor-like overgrowth via Ras activation in neural stem cells and epithelia. Development. 2017;144:2570-83.
[196] Wigerius M, Asghar N, Melik W, Johansson M. Scribble controls NGF-mediated neurite outgrowth in PC12 cells. Eur J Cell Biol. 2013;92:213-21.
[197] Yin G, Haendeler J, Yan C, Berk BC. GIT1 functions as a scaffold for MEK1-extracellular signal-regulated kinase 1 and 2 activation by angiotensin II and epidermal growth factor. Mol Cell Biol. 2004;24:875-85.
[198] Garcia RA, Vasudevan K, Buonanno A. The neuregulin receptor ErbB-4 interacts with PDZ-containing proteins at neuronal synapses. Proc Natl Acad Sci U S A. 2000;97:3596-601.
[199] Huang YZ, Wang Q, Won S, Luo ZG, Xiong WC, Mei L. Compartmentalized NRG signaling and PDZ domain-containing proteins in synapse structure and function. Int J Dev Neurosci. 2002;20:173-85.
[200] Lee S, Griep AE. Loss of Dlg-1 in the mouse lens impairs fibroblast growth factor receptor signaling. PLoS One. 2014;9:e97470.
[201] Maiga O, Philippe M, Kotelevets L, Chastre E, Benadda S, Pidard D, et al. Identification of mitogen-activated protein/extracellular signal-responsive kinase kinase 2 as a novel partner of the scaffolding protein human homolog of disc-large. FEBS J. 2011;278:2655-65.
[202] Young LC, Hartig N, Munoz-Alegre M, Oses-Prieto JA, Durdu S, Bender S, et al. An MRAS, SHOC2, and SCRIB complex coordinates ERK pathway activation with polarity and tumorigenic growth. Mol Cell. 2013;52:679-92.
[203] Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. Nat Rev Cancer. 2009;9:537-49.
[204] Uhlirova M, Bohmann D. JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumors in Drosophila. Embo J. 2006;25:5294-304.
[205] Igaki T, Pagliarini RA, Xu T. Loss of cell polarity drives tumor growth and invasion through JNK activation in Drosophila. Curr Biol. 2006;16:1139-46.
[206] Uhlirova M, Jasper H, Bohmann D. Non-cell-autonomous induction of tissue overgrowth by JNK/Ras cooperation in a Drosophila tumor model. Proc Natl Acad Sci U S A. 2005;102:13123-8.
[207] Mathew SJ, Rembold M, Leptin M. Role for Traf4 in polarizing adherens junctions as a prerequisite for efficient cell shape changes. Mol Cell Biol. 2011;31:4978-93.
[208] Ma X, Chen Y, Zhang S, Xu W, Shao Y, Yang Y, et al. Rho1-Wnd signaling regulates loss-of-cell polarity-induced cell invasion in Drosophila. Oncogene. 2016;35:846-55.
[209] Ma X, Xu W, Zhang D, Yang Y, Li W, Xue L. Wallenda regulates JNK-mediated cell death in Drosophila. Cell Death Dis. 2015;6:e1737.
[210] Ohsawa S, Sugimura K, Takino K, Xu T, Miyawaki A, Igaki T. Elimination of oncogenic neighbors by JNK-mediated engulfment in Drosophila. Dev Cell. 2011;20:315-28.
[211] Igaki T, Pastor-Pareja JC, Aonuma H, Miura M, Xu T. Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. Dev Cell. 2009;16:458-65.
[212] Brumby AM, Goulding KR, Schlosser T, Loi S, Galea R, Khoo P, et al. Identification of novel Ras-cooperating oncogenes in Drosophila melanogaster: a RhoGEF/Rho-family/JNK pathway is a central driver of tumorigenesis. Genetics. 2011;188:105-25.
[213] Norman M, Wisniewska KA, Lawrenson K, Garcia-Miranda P, Tada M, Kajita M, et al. Loss of Scribble causes cell competition in mammalian cells. J Cell Sci. 2012;125:59-66.
[214] Savinainen A, Garcia EP, Dorow D, Marshall J, Liu YF. Kainate receptor activation induces mixed lineage kinase-mediated cellular signaling cascades via post-synaptic density protein 95. J Biol Chem. 2001;276:11382-6.
[215] Gaudet S, Branton D, Lue RA. Characterization of PDZ-binding kinase, a mitotic kinase. Proc Natl Acad Sci U S A. 2000;97:5167-72.

[216] Abe Y, Matsumoto S, Kito K, Ueda N. Cloning and expression of a novel MAPKK-like protein kinase, lymphokine-activated killer T-cell-originated protein kinase, specifically expressed in the testis and activated lymphoid cells. J Biol Chem. 2000;275:21525-31.
[217] Sabio G, Arthur JS, Kuma Y, Peggie M, Carr J, Murray-Tait V, et al. p38gamma regulates the localisation of SAP97 in the cytoskeleton by modulating its interaction with GKAP. Embo J. 2005;24:1134-45.
[218] Keppler-Noreuil KM, Parker VE, Darling TN, Martinez-Agosto JA. Somatic overgrowth disorders of the PI3K/AKT/mTOR pathway & therapeutic strategies. Am J Med Genet C Semin Med Genet. 2016;172:402-21.
[219] Dibble CC, Cantley LC. Regulation of mTORC1 by PI3K signaling. Trends Cell Biol. 2015;25:545-55.
[220] Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K Pathway in Human Disease. Cell. 2017;170:605-35.
[221] Lee SS, Weiss RS, Javier RT. Binding of human virus oncoproteins to hDlg/SAP97, a mammalian homolog of the Drosophila discs large tumor suppressor protein. Proc Natl Acad Sci U S A. 1997;94:6670-5.
[222] Frese KK, Lee SS, Thomas DL, Latorre IJ, Weiss RS, Glaunsinger BA, et al. Selective PDZ protein-dependent stimulation of phosphatidylinositol 3-kinase by the adenovirus E4-ORF1 oncoprotein. Oncogene. 2003;22:710-21.
[223] Frese KK, Latorre IJ, Chung SH, Caruana G, Bernstein A, Jones SN, et al. Oncogenic function for the Dlg1 mammalian homolog of the Drosophila discs-large tumor suppressor. Embo J. 2006;25:1406-17.
[224] Willecke M, Toggweiler J, Basler K. Loss of PI3K blocks cell-cycle progression in a Drosophila tumor model. Oncogene. 2011;30:4067-74.
[225] Adey NB, Huang L, Ormonde PA, Baumgard ML, Pero R, Byreddy DV, et al. Threonine phosphorylation of the MMAC1/PTEN PDZ binding domain both inhibits and stimulates PDZ binding. Cancer Res. 2000;60:35-7.
[226] Richardson HE, Portela M. Tissue growth and tumorigenesis in Drosophila: cell polarity and the Hippo pathway. Curr Opin Cell Biol. 2017;48:1-9.
[227] Meng Z, Moroishi T, Guan KL. Mechanisms of Hippo pathway regulation. Genes Dev. 2016;30:1-17.
[228] Doggett K, Grusche FA, Richardson HE, Brumby AM. Loss of the Drosophila cell polarity regulator Scribbled promotes epithelial tissue overgrowth and cooperation with oncogenic Ras-Raf through impaired Hippo pathway signaling. BMC Dev Biol. 2011;11:57.
[229] Skouloudaki K, Puetz M, Simons M, Courbard JR, Boehlke C, Hartleben B, et al. Scribble participates in Hippo signaling and is required for normal zebrafish pronephros development. Proc Natl Acad Sci U S A. 2009;106:8579-84.
[230] Verghese S, Waghmare I, Kwon H, Hanes K, Kango-Singh M. Scribble acts in the Drosophila fat-hippo pathway to regulate warts activity. PLoS One. 2012;7:e47173.
[231] Chen CL, Schroeder MC, Kango-Singh M, Tao C, Halder G. Tumor suppression by cell competition through regulation of the Hippo pathway. Proc Natl Acad Sci U S A. 2012;109:484-9.
[232] Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, et al. The Hippo Transducer TAZ Confers Cancer Stem Cell-Related Traits on Breast Cancer Cells. Cell. 2011;147:759-72.
[233] Yang CC, Graves HK, Moya IM, Tao C, Hamaratoglu F, Gladden AB, et al. Differential regulation of the Hippo pathway by adherens junctions and apical-basal cell polarity modules. Proc Natl Acad Sci U S A. 2015;112:1785-90.
[234] Grzeschik NA, Parsons LM, Allott ML, Harvey KF, Richardson HE. Lgl, aPKC, and Crumbs regulate the Salvador/Warts/Hippo pathway through two distinct mechanisms. Curr Biol. 2010;20:573-81.
[235] Bunker BD, Nellimoottil TT, Boileau RM, Classen AK, Bilder D. The transcriptional response to tumorigenic polarity loss in Drosophila. Elife. 2015;4.

[236] Yang Y, Mlodzik M. Wnt-Frizzled/planar cell polarity signaling: cellular orientation by facing the wind (Wnt). Annu Rev Cell Dev Biol. 2015;31:623-46.
[237] Nusse R. Wnt signaling. Cold Spring Harb Perspect Biol. 2012;4.
[238] Daulat AM, Borg JP. Wnt/Planar Cell Polarity Signaling: New Opportunities for Cancer Treatment. Trends Cancer. 2017;3:113-25.
[239] Anastas JN, Biechele TL, Robitaille M, Muster J, Allison KH, Angers S, et al. A protein complex of SCRIB, NOS1AP and VANGL1 regulates cell polarity and migration, and is associated with breast cancer progression. Oncogene. 2012;31:3696-708.
[240] Vervenne HB, Crombez KR, Lambaerts K, Carvalho L, Koppen M, Heisenberg CP, et al. Lpp is involved in Wnt/PCP signaling and acts together with Scrib to mediate convergence and extension movements during zebrafish gastrulation. Dev Biol. 2008;320:267-77.
[241] Montcouquiol M, Rachel RA, Lanford PJ, Copeland NG, Jenkins NA, Kelley MW. Identification of Vangl2 and Scrb1 as planar polarity genes in mammals. Nature. 2003;423:173-7.
[242] Courbard JR, Djiane A, Wu J, Mlodzik M. The apical/basal-polarity determinant Scribble cooperates with the PCP core factor Stbm/Vang and functions as one of its effectors. Dev Biol. 2009;333:67-77.
[243] Milgrom-Hoffman M, Humbert PO. Regulation of cellular and PCP signalling by the Scribble polarity module. Semin Cell Dev Biol. 2017.
[244] Petit MM, Fradelizi J, Golsteyn RM, Ayoubi TA, Menichi B, Louvard D, et al. LPP, an actin cytoskeleton protein related to zyxin, harbors a nuclear export signal and transcriptional activation capacity. Mol Biol Cell. 2000;11:117-29.
[245] Petit MM, Meulemans SM, Alen P, Ayoubi TA, Jansen E, Van de Ven WJ. The tumor suppressor Scrib interacts with the zyxin-related protein LPP, which shuttles between cell adhesion sites and the nucleus. BMC Cell Biol. 2005;6:1.
[246] Ngan E, Kiepas A, Brown CM, Siegel PM. Emerging roles for LPP in metastatic cancer progression. J Cell Commun Signal. 2017.
[247] Hering H, Sheng M. Direct interaction of Frizzled-1, -2, -4, and -7 with PDZ domains of PSD-95. FEBS Lett. 2002;521:185-9.
[248] Luyten A, Mortier E, Van Campenhout C, Taelman V, Degeest G, Wuytens G, et al. The postsynaptic density 95/disc-large/zona occludens protein syntenin directly interacts with frizzled 7 and supports noncanonical Wnt signaling. Mol Biol Cell. 2008;19:1594-604.
[249] Egea-Jimenez AL, Gallardo R, Garcia-Pino A, Ivarsson Y, Wawrzyniak AM, Kashyap R, et al. Frizzled 7 and PIP2 binding by syntenin PDZ2 domain supports Frizzled 7 trafficking and signalling. Nat Commun. 2016;7:12101.
[250] Ishidate T, Matsumine A, Toyoshima K, Akiyama T. The APC-hDLG complex negatively regulates cell cycle progression from the G0/G1 to S phase. Oncogene. 2000;19:365-72.
[251] Takizawa S, Nagasaka K, Nakagawa S, Yano T, Nakagawa K, Yasugi T, et al. Human scribble, a novel tumor suppressor identified as a target of high-risk HPV E6 for ubiquitin-mediated degradation, interacts with adenomatous polyposis coli. Genes Cells. 2006;11:453-64.
[252] Etienne-Manneville S, Manneville JB, Nicholls S, Ferenczi MA, Hall A. Cdc42 and Par6-PKCzeta regulate the spatially localized association of Dlg1 and APC to control cell polarization. J Cell Biol. 2005;170:895-901.
[253] Etienne-Manneville S. APC in cell migration. Adv Exp Med Biol. 2009;656:30-40.
[254] Davis H, Raja E, Miyazono K, Tsubakihara Y, Moustakas A. Mechanisms of action of bone morphogenetic proteins in cancer. Cytokine Growth Factor Rev. 2016;27:81-92.
[255] Itoh F, Watabe T, Miyazono K. Roles of TGF-beta family signals in the fate determination of pluripotent stem cells. Semin Cell Dev Biol. 2014;32:98-106.
[256] Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. Cell Res. 2009;19:156-72.

[257] Yamben IF, Rachel RA, Shatadal S, Copeland NG, Jenkins NA, Warming S, et al. Scrib is required for epithelial cell identity and prevents epithelial to mesenchymal transition in the mouse. Dev Biol. 2013;384:41-52.
[258] Camp ND, Lee KS, Wacker-Mhyre JL, Kountz TS, Park JM, Harris DA, et al. Individual protomers of a G protein-coupled receptor dimer integrate distinct functional modules. Cell Discov. 2015;1.
[259] Moreau MM, Piguel N, Papouin T, Koehl M, Durand CM, Rubio ME, et al. The planar polarity protein Scribble1 is essential for neuronal plasticity and brain function. J Neurosci. 2010;30:9738-52.
[260] Kornau HC, Schenker LT, Kennedy MB, Seeburg PH. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science. 1995;269:1737-40.
[261] Fiorentini M, Bach A, Stromgaard K, Kastrup JS, Gajhede M. Interaction partners of PSD-93 studied by X-ray crystallography and fluorescence polarization spectroscopy. Acta Crystallogr D Biol Crystallogr. 2013;69:587-94.
[262] Cervantes-Sandoval I, Chakraborty M, MacMullen C, Davis RL. Scribble Scaffolds a Signalosome for Active Forgetting. Neuron. 2016;90:1230-42.
[263] Reischauer S, Levesque MP, Nusslein-Volhard C, Sonawane M. Lgl2 executes its function as a tumor suppressor by regulating ErbB signaling in the zebrafish epidermis. PLoS Genet. 2009;5:e1000720.
[264] Parsons LM, Grzeschik NA, Richardson HE. lgl Regulates the Hippo Pathway Independently of Fat/Dachs, Kibra/Expanded/Merlin and dRASSF/dSTRIPAK. Cancers (Basel). 2014;6:879-96.
[265] Sun G, Irvine KD. Regulation of Hippo signaling by Jun kinase signaling during compensatory cell proliferation and regeneration, and in neoplastic tumors. Dev Biol. 2011;350:139-51.
[266] Zhu M, Xin T, Weng S, Gao Y, Zhang Y, Li Q, et al. Activation of JNK signaling links lgl mutations to disruption of the cell polarity and epithelial organization in Drosophila imaginal discs. Cell Res. 2010;20:242-5.
[267] Froldi F, Ziosi M, Garoia F, Pession A, Grzeschik NA, Bellosta P, et al. The lethal giant larvae tumour suppressor mutation requires dMyc oncoprotein to promote clonal malignancy. BMC Biol. 2010;8:33.
[268] Nolan ME, Aranda V, Lee S, Lakshmi B, Basu S, Allred DC, et al. The polarity protein Par6 induces cell proliferation and is overexpressed in breast cancer. Cancer Res. 2008;68:8201-9.
[269] Wang Y, Hill KS, Fields AP. PKCiota maintains a tumor-initiating cell phenotype that is required for ovarian tumorigenesis. Mol Cancer Res. 2013;11:1624-35.
[270] Regala RP, Davis RK, Kunz A, Khoor A, Leitges M, Fields AP. Atypical protein kinase C{iota} is required for bronchioalveolar stem cell expansion and lung tumorigenesis. Cancer Res. 2009;69:7603-11.
[271] Stallings-Mann M, Jamieson L, Regala RP, Weems C, Murray NR, Fields AP. A novel small-molecule inhibitor of protein kinase Ciota blocks transformed growth of non-small-cell lung cancer cells. Cancer Res. 2006;66:1767-74.
[272] Regala RP, Weems C, Jamieson L, Khoor A, Edell ES, Lohse CM, et al. Atypical protein kinase C iota is an oncogene in human non-small cell lung cancer. Cancer Res. 2005;65:8905-11.
[273] Regala RP, Weems C, Jamieson L, Copland JA, Thompson EA, Fields AP. Atypical protein kinase Ciota plays a critical role in human lung cancer cell growth and tumorigenicity. J Biol Chem. 2005;280:31109-15.
[274] Murray NR, Jamieson L, Yu W, Zhang J, Gokmen-Polar Y, Sier D, et al. Protein kinase Ciota is required for Ras transformation and colon carcinogenesis in vivo. J Cell Biol. 2004;164:797-802.
[275] Justilien V, Jameison L, Der CJ, Rossman KL, Fields AP. Oncogenic activity of Ect2 is regulated through protein kinase C iota-mediated phosphorylation. J Biol Chem. 2011;286:8149-57.
[276] Justilien V, Fields AP. Ect2 links the PKCiota-Par6alpha complex to Rac1 activation and cellular transformation. Oncogene. 2009;28:3597-607.
[277] Frederick LA, Matthews JA, Jamieson L, Justilien V, Thompson EA, Radisky DC, et al. Matrix metalloproteinase-10 is a critical effector of protein kinase Ciota-Par6alpha-mediated lung cancer. Oncogene. 2008;27:4841-53.

[278] Kim JY, Valencia T, Abu-Baker S, Linares J, Lee SJ, Yajima T, et al. c-Myc phosphorylation by PKCzeta represses prostate tumorigenesis. Proc Natl Acad Sci U S A. 2013;110:6418-23.
[279] Iden S, van Riel WE, Schafer R, Song JY, Hirose T, Ohno S, et al. Tumor type-dependent function of the par3 polarity protein in skin tumorigenesis. Cancer Cell. 2012;22:389-403.
[280] Mescher M, Jeong P, Knapp SK, Rubsam M, Saynisch M, Kranen M, et al. The epidermal polarity protein Par3 is a non-cell autonomous suppressor of malignant melanoma. J Exp Med. 2017;214:339-58.
[281] McCaffrey LM, Montalbano J, Mihai C, Macara IG. Loss of the Par3 polarity protein promotes breast tumorigenesis and metastasis. Cancer Cell. 2012;22:601-14.
[282] Archibald A, Mihai C, Macara IG, McCaffrey L. Oncogenic suppression of apoptosis uncovers a Rac1/JNK proliferation pathway activated by loss of Par3. Oncogene. 2015;34:3199-206.
[283] Xue B, Krishnamurthy K, Allred DC, Muthuswamy SK. Loss of Par3 promotes breast cancer metastasis by compromising cell-cell cohesion. Nat Cell Biol. 2013;15:189-200.
[284] Feng W, Wu H, Chan LN, Zhang M. Par-3-mediated junctional localization of the lipid phosphatase PTEN is required for cell polarity establishment. J Biol Chem. 2008;283:23440-9.
[285] Pinal N, Goberdhan DC, Collinson L, Fujita Y, Cox IM, Wilson C, et al. Regulated and polarized PtdIns(3,4,5)P3 accumulation is essential for apical membrane morphogenesis in photoreceptor epithelial cells. Curr Biol. 2006;16:140-9.
[286] von Stein W, Ramrath A, Grimm A, Muller-Borg M, Wodarz A. Direct association of Bazooka/PAR-3 with the lipid phosphatase PTEN reveals a link between the PAR/aPKC complex and phosphoinositide signaling. Development. 2005;132:1675-86.
[287] Sun S, Irvine KD. Cellular Organization and Cytoskeletal Regulation of the Hippo Signaling Network. Trends Cell Biol. 2016;26:694-704.
[288] Sun S, Reddy BV, Irvine KD. Localization of Hippo signalling complexes and Warts activation in vivo. Nat Commun. 2015;6:8402.
[289] Archibald A, Al-Masri M, Liew-Spilger A, McCaffrey L. Atypical protein kinase C induces cell transformation by disrupting Hippo/Yap signaling. Mol Biol Cell. 2015;26:3578-95.
[290] Moleirinho S, Chang N, Sims AH, Tilston-Lunel AM, Angus L, Steele A, et al. KIBRA exhibits MST-independent functional regulation of the Hippo signaling pathway in mammals. Oncogene. 2013;32:1821-30.
[291] Xiao L, Chen Y, Ji M, Dong J. KIBRA regulates Hippo signaling activity via interactions with large tumor suppressor kinases. J Biol Chem. 2011;286:7788-96.
[292] Dollar GL, Weber U, Mlodzik M, Sokol SY. Regulation of Lethal giant larvae by Dishevelled. Nature. 2005;437:1376-80.
[293] Kaplan NA, Tolwinski NS. Spatially defined Dsh-Lgl interaction contributes to directional tissue morphogenesis. J Cell Sci. 2010.
[294] Parsons LM, Grzeschik NA, Amaratunga K, Burke P, Quinn LM, Richardson HE. A Kinome RNAi Screen in Drosophila Identifies Novel Genes Interacting with Lgl, aPKC and Crb Cell Polarity Genes in Epithelial Tissues. G3 (Bethesda). 2017.
[295] Lee RT, Zhao Z, Ingham PW. Hedgehog signalling. Development. 2016;143:367-72.
[296] Katoh Y, Katoh M. Hedgehog target genes: mechanisms of carcinogenesis induced by aberrant hedgehog signaling activation. Curr Mol Med. 2009;9:873-86.
[297] Atwood SX, Li M, Lee A, Tang JY, Oro AE. GLI activation by atypical protein kinase C iota/lambda regulates the growth of basal cell carcinomas. Nature. 2013;494:484-8.
[298] Callahan CA, Ofstad T, Horng L, Wang JK, Zhen HH, Coulombe PA, et al. MIM/BEG4, a Sonic hedgehog-responsive gene that potentiates Gli-dependent transcription. Genes Dev. 2004;18:2724-9.
[299] Ohshiro T, Yagami T, Zhang C, Matsuzaki F. Role of cortical tumour-suppressor proteins in asymmetric division of Drosophila neuroblast. Nature. 2000;408:593-6.
[300] Haenfler JM, Kuang C, Lee CY. Cortical aPKC kinase activity distinguishes neural stem cells from progenitor cells by ensuring asymmetric segregation of Numb. Dev Biol. 2012;365:219-28.

[301] Clark BS, Cui S, Miesfeld JB, Klezovitch O, Vasioukhin V, Link BA. Loss of Llgl1 in retinal neuroepithelia reveals links between apical domain size, Notch activity and neurogenesis. Development. 2012;139:1599-610.
[302] Portela M, Parsons LM, Grzeschik NA, Richardson HE. Regulation of Notch signaling and endocytosis by the Lgl neoplastic tumor suppressor. Cell Cycle. 2015;14:1496-506.
[303] Parsons L, Portela M, Grzeschik N, Richardson HE. Lgl regulates Notch signaling via endocytosis, independently of aPKC, in the Drosophila developing eye epithelia. Curr Biol. 2014;In Press.
[304] Vaccari T, Duchi S, Cortese K, Tacchetti C, Bilder D. The vacuolar ATPase is required for physiological as well as pathological activation of the Notch receptor. Development. 2010;137:1825-32.
[305] Vaccari T, Lu H, Kanwar R, Fortini ME, Bilder D. Endosomal entry regulates Notch receptor activation in Drosophila melanogaster. J Cell Biol. 2008;180:755-62.
[306] Sjoqvist M, Antfolk D, Ferraris S, Rraklli V, Haga C, Antila C, et al. PKCzeta regulates Notch receptor routing and activity in a Notch signaling-dependent manner. Cell Res. 2014;24:433-50.
[307] Kim DI, Roux KJ. Filling the Void: Proximity-Based Labeling of Proteins in Living Cells. Trends Cell Biol. 2016;26:804-17.
[308] Ramberger E, Dittmar G. Tissue Specific Labeling in Proteomics. Proteomes. 2017;5.
[309] Hauser M, Wojcik M, Kim D, Mahmoudi M, Li W, Xu K. Correlative Super-Resolution Microscopy: New Dimensions and New Opportunities. Chem Rev. 2017;117:7428-56.
[310] Tam J, Merino D. Stochastic optical reconstruction microscopy (STORM) in comparison with stimulated emission depletion (STED) and other imaging methods. J Neurochem. 2015;135:643-58.
[311] Sydor AM, Czymmek KJ, Puchner EM, Mennella V. Super-Resolution Microscopy: From Single Molecules to Supramolecular Assemblies. Trends Cell Biol. 2015;25:730-48.
[312] Wang HW, Wang JW. How cryo-electron microscopy and X-ray crystallography complement each other. Protein Sci. 2017;26:32-9.
[313] Orlov I, Myasnikov AG, Andronov L, Natchiar SK, Khatter H, Beinsteiner B, et al. The integrative role of cryo electron microscopy in molecular and cellular structural biology. Biol Cell. 2017;109:81-93.
[314] Chen B, Frank J. Two promising future developments of cryo-EM: capturing short-lived states and mapping a continuum of states of a macromolecule. Microscopy (Oxf). 2016;65:69-79.
[315] Bai XC, McMullan G, Scheres SH. How cryo-EM is revolutionizing structural biology. Trends Biochem Sci. 2015;40:49-57.
[316] Juriloff DM, Harris MJ. A consideration of the evidence that genetic defects in planar cell polarity contribute to the etiology of human neural tube defects. Birth Defects Res A Clin Mol Teratol. 2012;94:824-40.
[317] Nikolopoulou E, Galea GL, Rolo A, Greene ND, Copp AJ. Neural tube closure: cellular, molecular and biomechanical mechanisms. Development. 2017;144:552-66.
[318] Eom DS, Amarnath S, Agarwala S. Apicobasal polarity and neural tube closure. Dev Growth Differ. 2013;55:164-72.
[319] Rejon C, Al-Masri M, McCaffrey L. Cell Polarity Proteins in Breast Cancer Progression. J Cell Biochem. 2016;117:2215-23.
[320] Sasaki AT, Firtel RA. Regulation of chemotaxis by the orchestrated activation of Ras, PI3K, and TOR. Eur J Cell Biol. 2006;85:873-95.
[321] Leong GR, Goulding KR, Amin N, Richardson HE, Brumby AM. scribble mutants promote aPKC and JNK-dependent epithelial neoplasia independently of Crumbs. BMC Biol. 2009;7:62.
[322] Menendez J, Perez-Garijo A, Calleja M, Morata G. A tumor-suppressing mechanism in Drosophila involving cell competition and the Hippo pathway. Proc Natl Acad Sci U S A. 2010;107:14651-6.
[323] Pagliarini RA, Xu T. A genetic screen in Drosophila for metastatic behavior. Science. 2003;302:1227-31.

Figure 1

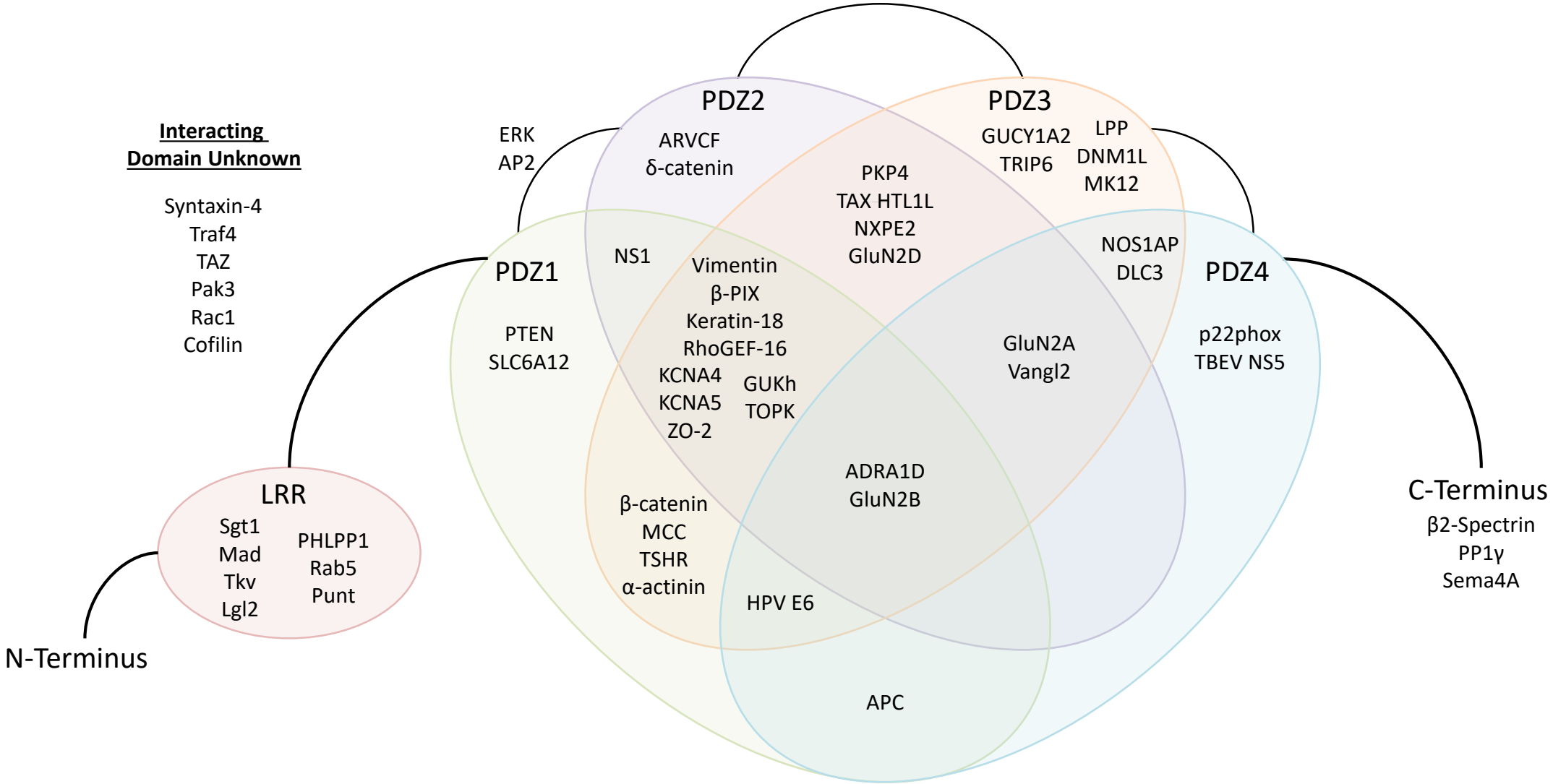
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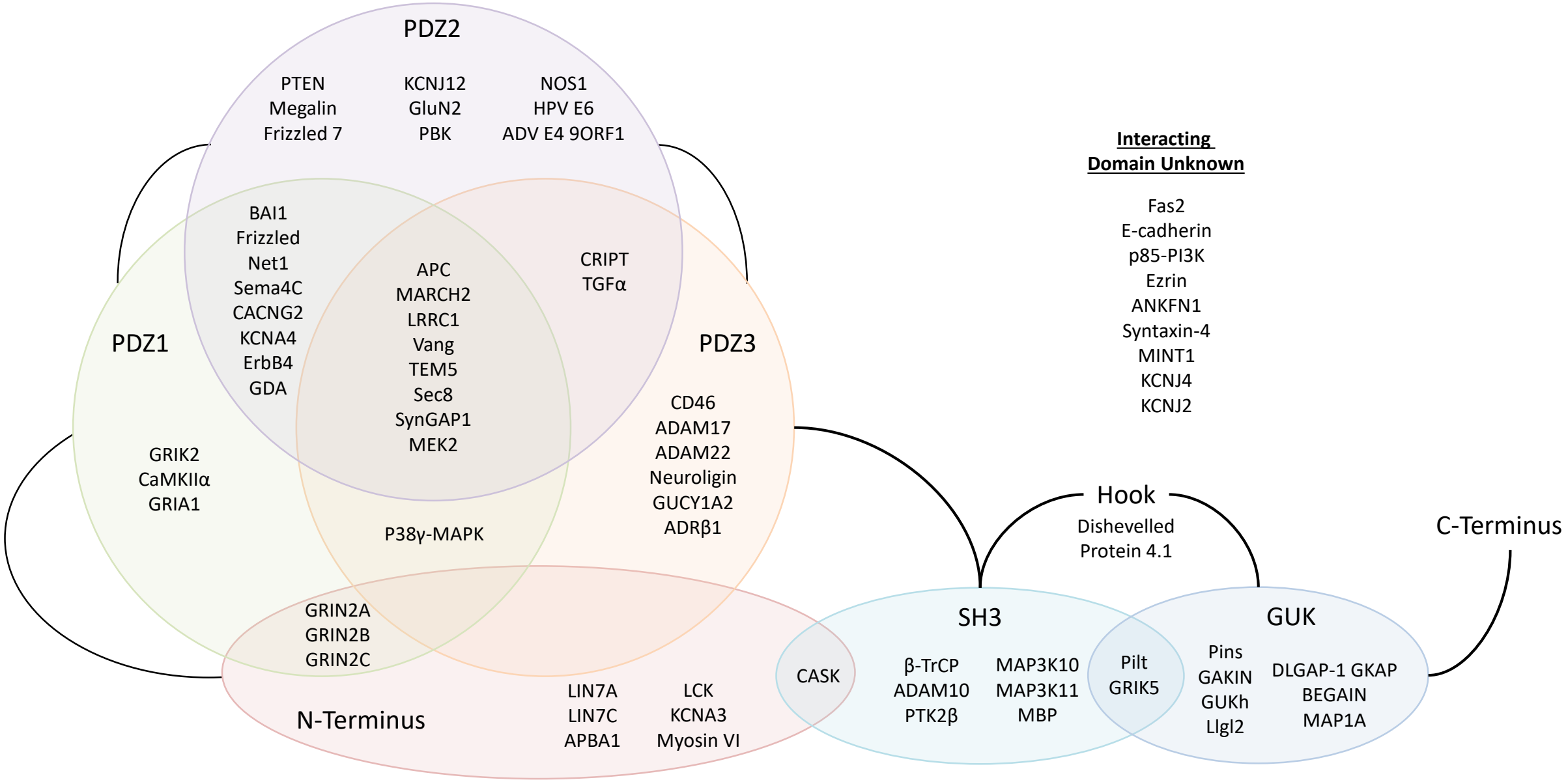
Figure 2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GRIK5
Pins
GAKIN
GUKh
Llgl2
DLGAP-1 GKAP
BEGAIN
MAP1A
LIN7A
LIN7C
APBA1
LCK
KCNA3
Myosin VI
N-Terminus
Interacting
Domain Unknown
Fas2
E-cadherin
p85-PI3K
Ezrin
ANKFN1
Syntaxin-4
MINT1
KCNJ4
KCNJ2

Figure 3

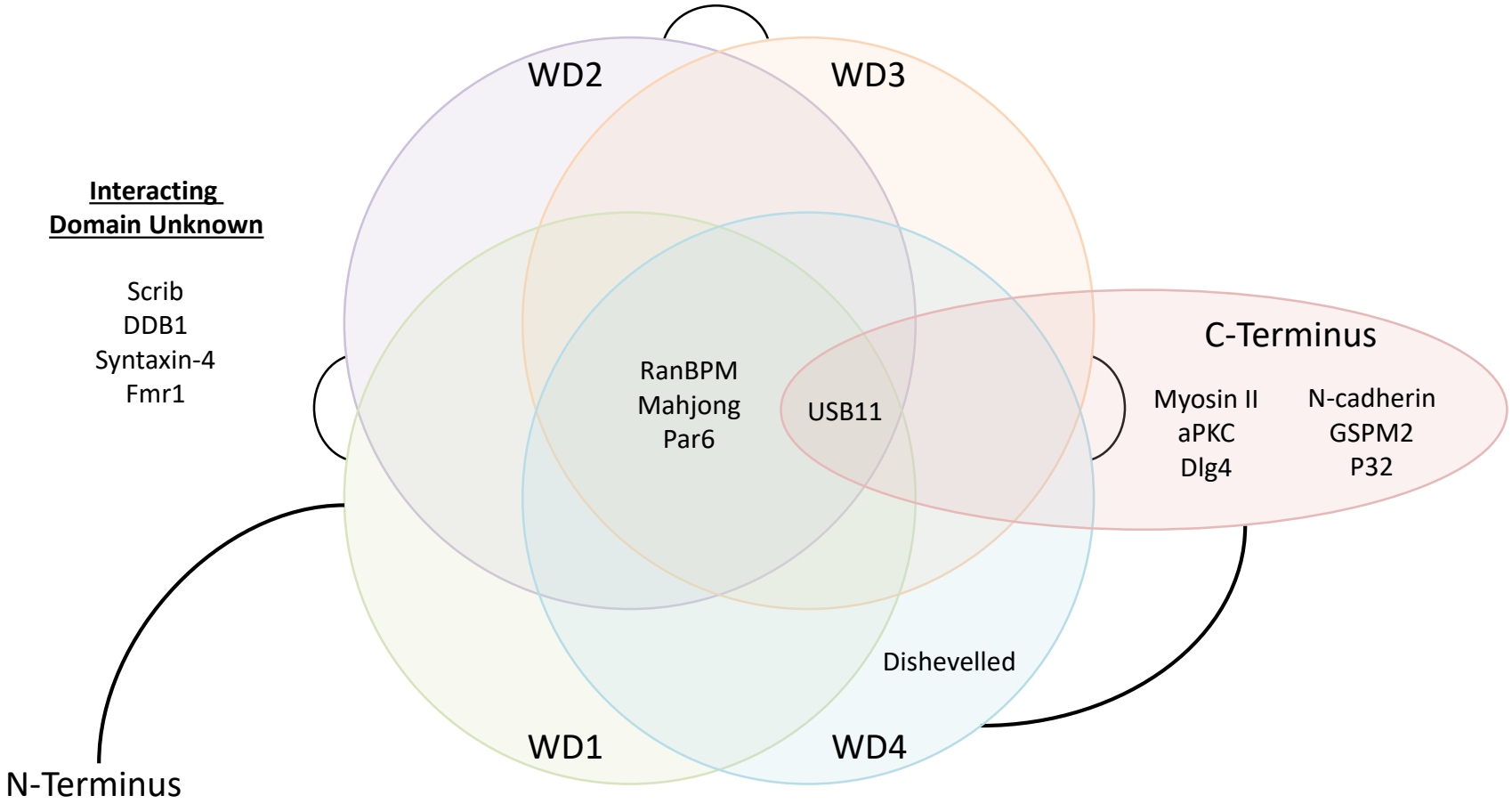

WD2
WD3
Interacting
Domain Unknown
Scrib
DDB1
Syntaxin-4
Fmr1
RanBPM
Mahjong
Par6
USB11
C-Terminus
Myosin II
aPKC
Dlg4
N-cadherin
GSPM2
P32
Dishevelled
WD1
WD4
N-Terminus

Figure 4

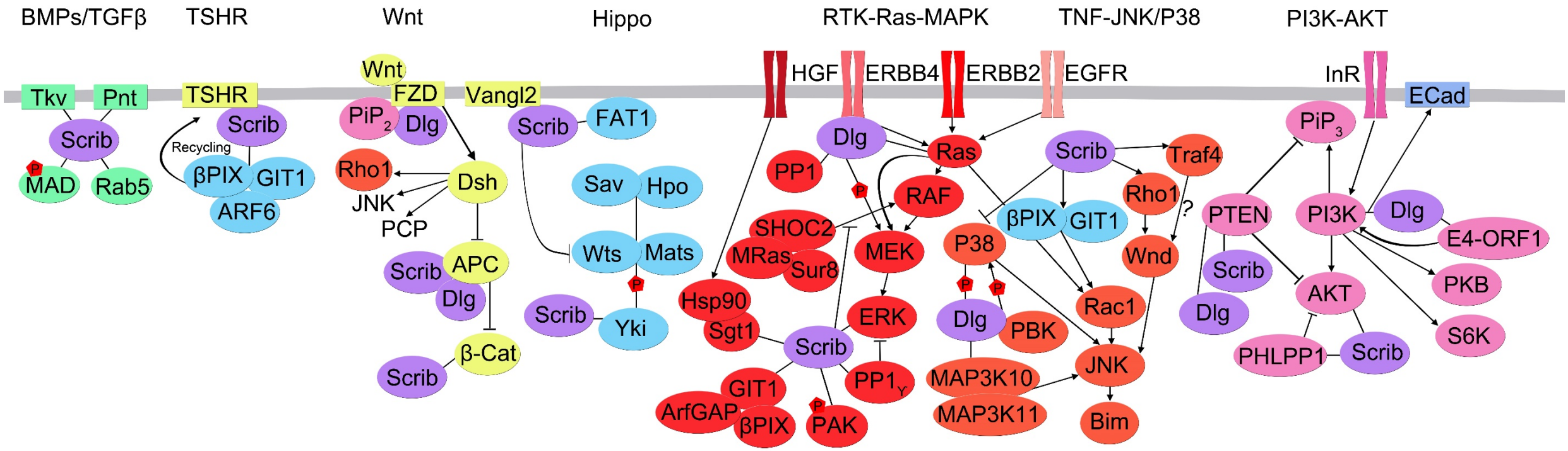
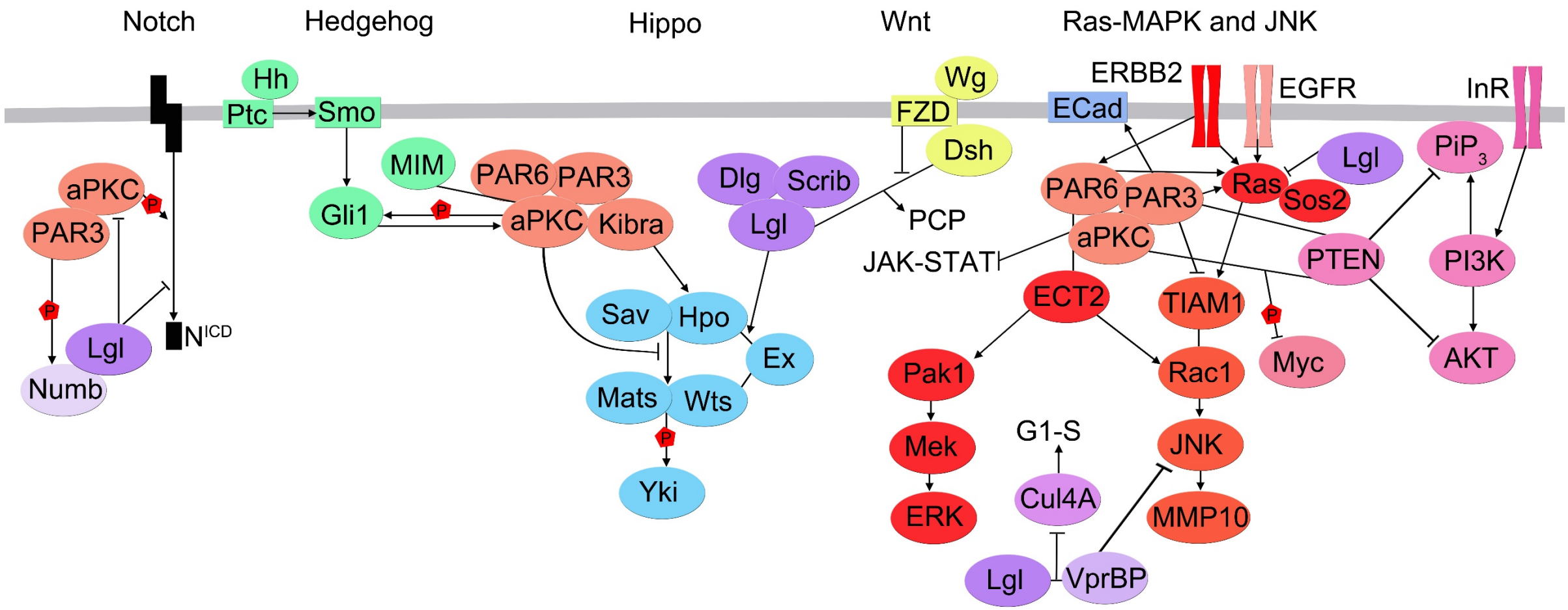

BMPs/TGFβ
TSHR
Wnt
Hippo
RTK-Ras-MAPK
TNF-JNK/P38
PI3K-AKT
Tkv
Pnt
Scrib
MAD
Rab5
TSHR
Recycling
Scrib
βPIX
GIT1
ARF6
Wnt
FZD
Vangl2
PiP2
Dlg
Rho1
Dsh
JNK
PCP
Scrib
APC
Dlg
β-Cat
Scrib
Scrib
FAT1
Sav
Hpo
Wts
Mats
Scrib
Yki
HGF
ERBB4
ERBB2
EGFR
Dlg
PP1
Ras
RAF
SHOC2
MRas
Sur8
MEK
Hsp90
Sgt1
Scrib
ERK
GIT1
ArfGAP
βPIX
PAK
PP1Y
Scrib
Traf4
Rho1
?
βPIX
GIT1
Wnd
P38
Rac1
Dlg
PBK
MAP3K10
MAP3K11
JNK
Bim
InR
ECad
PiP3
PTEN
PI3K
Dlg
E4-ORF1
Scrib
AKT
PKB
Dlg
PHLPP1
Scrib
S6K

Figure 5


Notch
Hedgehog
Hippo
Wnt
Ras-MAPK and JNK
Hh
Ptc
Smo
Gli1
MIM
PAR6
PAR3
aPKC
Kibra
Dlg
Scrib
Lgl
Sav
Hpo
Ex
Mats
Wts
Yki
aPKC
PAR3
Lgl
Numb
N^ICD
Wg
FZD
Dsh
PCP
JAK-STAT
ERBB2
EGFR
InR
ECad
PAR6
PAR3
aPKC
Ras
Sos2
Lgl
PiP3
PTEN
PI3K
AKT
ECT2
TIAM1
Rac1
Myc
Pak1
Mek
ERK
G1-S
Cul4A
Lgl
VprBP
JNK
MMP10