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Crystal structure of the human Scribble PDZ1 domain bound to the PDZ-binding motif of APC

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Running title: Crystal structure of Scribble PDZ1 with APC peptides

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ABSTRACT

Scribble (SCRIB) is an important adaptor protein that controls the establishment and maintenance of apico-basal cell polarity. To better understand how SCRIB controls cell polarity signalling via its PDZ domains, we investigated human SCRIB interactions with APC (adenomatous polyposis coli). We show that SCRIB PDZ1, PDZ2 and PDZ3 are the major interactors with the APC PDZ-binding motif (PBM), whereas SCRIB PDZ4 does not show detectable binding to APC. We then determined the crystal structure of SCRIB PDZ1 domain bound to the APC PBM. Our findings reveal a previously unreported pattern of interactions between the SCRIB PDZ domain region with the C-terminal PDZ binding motif of APC, where SCRIB PDZ1 domain is the highest affinity site.

Keywords: Scribble, APC, PDZ domain, cell polarity, X-ray crystallography

INTRODUCTION

Cell polarity is established via the asymmetric distribution into distinct cellular domains of cellular constituents such as proteins, lipids and carbohydrates [1]. This phenomenon is a crucial property of eukaryotic cells and pivotal for the correct establishment of tissue development and architecture. The proper distribution of cellular components leads to the establishment of apical-basal cell polarity in epithelial cells, and effects a range of important cellular processes and signalling pathways including apoptosis, vesicle trafficking, cell proliferation and migration [2]. Importantly, loss of cell polarity is recognized as an important hallmark of cancer development [3], underscoring the significance of correct cell polarity for healthy tissues. Epithelial apico-basal polarity is controlled by the antagonistic interaction of three multi-protein complexes, the Par, Crumbs and Scribble complexes [2][3]. In mammals, the Scribble complex comprises Scribble (SCRIB), one of 4 Dlg (Discs Large) homologues (DLG1-4) and 2 Lgl (Lethal Giant Larvae) homologues (LLGL1, LLGL2), which are highly conserved from the vinegar fly to humans [4]. Scribble was originally identified in *Drosophila melanogaster* as a tumour suppressor where loss of Scribble resulted in disrupted epithelial tissue organisation accompanied by aberrant growth in the imaginal discs of the larvae [5]. This tumour suppressing ability was subsequently shown to be conserved across species, with loss of Scribble promoting tumour initiation, and when coupled with oncogenic drivers including RAS, tumour progression in diverse epithelial tissues including mammary, prostate, skin and the lung [6-10].

Scribble is a large multi-domain scaffold protein comprising 16 Leucine Rich Repeats and 4 PSD-95/Disc-large/ZO-1 (PDZ) domains, and a member of the LRR And PDZ domain (LAP) family of proteins (Figure 1A). Via these domains Scribble is able to interact with a diverse set of interactors that play a role in a range of discrete signalling pathways [4]. The majority of interactions are modulated by Scribble's four PDZ domains, however the Scribble LRR domain also engages a specific subset of interactors, such as LLGL2, during the regulation

of cell polarity [11]. Similar to other PDZ domains, Scribble PDZ domains typically bind C-terminally located PDZ-binding motifs (PBMs) on specific interactors. Although these sequences are specific, numerous studies have now shown that whilst PBMs on Scribble interactors selectively engage Scribble, the Scribble PDZ domains appear to harbor overlapping specificities for particular ligands, with each PDZ domain able to bind multiple interaction partners [11-15]. Scribble PDZ domains are categorized as Class I PDZ domains, which recognises a consensus X-T/S-X-$Ø_{COOH}$ motif (where X can be any amino acid residue, and Ø is a hydrophobic residue) in the PBM of binding partners.

APC (Adenomatous Polyposis Coli) has been shown to be a Scribble interactor *in vitro* and *in vivo* [16]. APC is a large multi-domain protein found in epithelial cells that has been shown to exert influence on a number functions including control of the Wnt signal transduction, cell migration, cell-cell adhesion, and cell cycle control [17, 18]. Importantly, a large fraction of colorectal cancers harbor inactivating mutations in APC, and loss of APC was linked to the inhibition of ubiquitin-mediated degradation of β-catenin [19]. More recently, APC was also shown to play an essential role in inhibition of Wnt receptor activation [20]. In addition to Scribble, APC has also been reported to interact with mammalian Dlg, another member of the Scribble complex [21-24]. Both Scribble and Dlg1 colocalize with APC at cellular protrusions during migration events [20, 22]. Importantly, knockdown of human Scribble by RNAi in Caco-2 cells disrupted the proper localization of APC at adherens junction [16]. Intriguingly, APC was shown to engage Scribble through its C-terminal PDZ binding motif binding specifically to the Scribble PDZ1 and 4 domains [16], however a recent report using peptide-phage display proteomics approach indicates that the APC C-terminal PDZ binding motif binds directly to Scribble PDZ1 and 2 [15].

To understand the molecular and structural basis of this Scribble:APC interaction, we now report the outcomes of a systematic examination of the affinity of recombinant Scribble PDZ domains for peptides spanning the wild-type APC PBM domain together the crystal structure of Scribble PDZ1 bound to the APC PBM peptide.

MATERIALS AND METHODS

Protein expression and purification

Synthetic cDNA codon optimized for *Escherichia coli* expression encoding the PDZ domains of human Scribble, SCRIB (Uniprot accession number: Q14160) PDZ1 (728–815); PDZ2 (833–965); PDZ3 (1005–1094); and PDZ4 (1099–1203)) were cloned into the pGil-MBP [25] and pGex-6P3 (GE Healthcare). Recombinant SCRIB PDZ domains were expressed using *Escherichia coli* BL21 (DE3) pLysS cells (BIOLINE) in super broth supplemented with 200 μg/mL ampicillin (AMRESCO) using auto-induction media (10 mM Tris-Cl pH7.6, 100 mM NaCl, 1 mM MgSO4, 0.2 % (w/v) D-lactose, 0.05 % (w/v) glucose, 0.5 % (v/v) glycerol) [26] at 37°C until the optical density at 600 nm (OD600) reached 1.0 before cooling cultures to 20°C for 24 hours for protein expression. Bacterial cells were harvested by centrifugation and lysed in the presence of deoxyribonuclease I (Sigma-Aldrich) from bovine pancreas using TS Series 0.75 kw model cabinet (Constant Systems Ltd.) at 25 kPsi, Qsonica Q700 sonicator. Cell lysates were subsequently clarified by centrifugation at 20,000 x g for 20 minutes using an Avanti® J-E (Beckman Coulter) and filtered using Millex-GP syringe filter unit 0.22 μM (Merck Millipore) prior to loading onto equilibrated columns for affinity purification.

Glutathione-S-transferase (GST) tagged recombinant SCRIB PDZ1 protein was captured using glutathione sepharose 4B (GE Healthcare) in buffer A (50 mM Tris-Cl pH 8.0, 150 mM NaCl and 1 mM EDTA) and was cleaved on-column with HRV 3C protease to liberate the PDZ1 domain, which was eluted with buffer. Hexahistidine maltose binding protein (His-MBP) tagged recombinant PDZ domains (PDZ2, PDZ3 and PDZ4) were purified using 5mL HisTrap HP

columns (GE Healthcare) in buffer B (50 mM Tris-Cl pH 8, 300 mM NaCl) and washed with buffer B supplemented with 20 mM Imidazole before eluting in buffer B supplemented with 300 mM Imidazole. Recombinant fusion proteins were cleaved with TEV protease in buffer B supplemented with 0.5 mM EDTA and 1 mM DTT before being subjected to a second round of affinity chromatography to remove cleaved HisMBP tag and uncleaved fusion protein. All cleaved target proteins were subjected to size exclusion chromatography using a HiLoad 16/600 Superdex 75 (GE Healthcare) equilibrated in 25 mM Tris pH8.0, 150 mM NaCl, where they eluted as single peaks.

Isothermal titration calorimetry

Purified human SCRIB PDZ domains were used in titration experiments against 8-mer peptides spanning the C terminus of human APC (Uniprot accession number: P25054; GSYLVTSV) or a non-binding mutant of APC (GSYLVASA) to determine the affinity for SCRIB PDZ domains. PDZ1 concentration was quantitated at 280 nm absorbance (A280nm) using a NanoDrop 2000/2000c UV-Vis Spectrophotometer (Thermo Scientific). Due to a lack of amino acids within the Scribble PDZ2, PDZ3 and PDZ4 domains that enable accurate concentration measurements at Abs280nm, protein concentrations were calculated using the Scopes method [27] by measuring absorbance at 205 and 280 nm using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific).

Titrations were performed at 25°C with a stirring speed of 750 rpm using the MicroCalTM iTC200 System (GE Healthcare). A total of 20 injections with 2 µL each and a spacing of 180 seconds were titrated into the 200 µL protein sample (25 mM Tris pH 8.0, 150 mM NaCl), except for the first injection which was only 0.4 µL. Protein concentration of 75 µM against peptide concentration of 0.9 mM were used. Peptides were purchased from Genscript (San Francisco, CA, USA). Raw thermograms were processed with MicroCal Origin® version 7.0 software (OriginLabTM Corporation) to obtain the binding parameters of each interaction. A

synthetic pan-PDZ binding peptide referred to as superpeptide (RSWFETWV) was used as a positive control [[14, 28].

Protein crystallisation, *data collection and refinement*

The complex of SCRIB PDZ1 with APC was reconstituted by mixing protein and peptide at a 1:10 molar ratio, with the dilute complex concentrated using a 3-kDa molecular mass cut-off centrifugal concentrator (Millipore). Concentrated Scribble PDZ1 and 3 complexes with APC were flash-cooled, and stored under liquid nitrogen for crystallization trials. Crystallization was performed using 96-well sitting-drop trays (Swissci) with the sitting drop vapor diffusion method at 20 °C either in-house or at the CSIRO C3 Collaborative Crystallization Centre, Melbourne, Australia. 0.15-µl of protein-peptide complexes were mixed with 0.15 µl of commercial sparse matrix screen crystallization conditions using a Phoenix nanodispenser robot (Art Robbins). Initial crystallization conditions were optimized using a 24-well limbro plate. Crystals of SCRIB PDZ1 bound to APC peptide were obtained at 30 mg/ml in 20% (w/v) polyethylene glycol 3350, 0.2 M potassium formate. The SCRIB PDZ1-APC crystals were cryo-protected using 20% ethylene glycol and flash-cooled at 100 K using liquid nitrogen. Rod shaped crystals were obtained belonging to space group C2.

All diffraction data were collected on either the MX1 beamline at the Australian Synchrotron equipped with an ADSC Quantum 210r CCD detector (Area Detector Systems Corporation, Poway, California, USA) and the MX2 beamline equipped with the EIGER 16M detector with an oscillation range of 1.0° and 0.1° per frame, respectively, using a wavelength of 0.9537 Å. Diffraction data were integrated using XDSme [29] and scaled using AIMLESS [30]. The structures was solved by molecular replacement using Phaser [31] with the structure of human Scribble PDZ1 (PDB code 5VWC [14]) as a search model. The final TFZ and LLG values were TFZ = 37.6 and LLG = 2014.734 for SCRIB PDZ1-APC. The initial solution produced by Phaser was manually rebuilt over multiple cycles using Coot [32] and refined using PHENIX

[33]. Data collection and refinement statistics details are summarized in Table 2. MolProbity scores were obtained from the MolProbity web server [34]. Coordinate files have been deposited in the Protein Data Bank under the accession code 6MS1. All images were generated using the PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC. All software was accessed using the SBGrid suite [35]. All raw diffraction images were deposited on the SBGrid Data Bank [36] using their PDB accession number 6MS1.

RESULTS

Isolated Scribble PDZ1 and PDZ3 domains specifically interact with the APC PBM

SCRIB has previously been shown to directly interact with the APC C-terminal PDZ binding motif (PBM) via its PDZ1 and 4 domains using pull-down assays [16]. To quantify the interaction and understand the interplay between APC binding in the context of the extensive interaction network of Scribble PDZ domains, we examined the affinity of recombinant SCRIB PDZ1, 2, 3 and 4 domains for 8-mer peptide corresponding to the APC PBM (GSYLVTSV) (Figure 1, Table 1) using isothermal titration calorimetry (ITC). These analyses revealed that the APC PBM bound to SCRIB PDZ1, 2 and 3 domains with K_D values of 6.0, 36.0 and 18.3 μM, respectively, and showed no binding to SCRIB PDZ4. In contrast, a mutant APC PBM peptide (GSYLVASA) did not show any detectable binding (Figure S1). Analysis of the thermodynamic binding parameters of APC binding to SCRIB PDZ1, PDZ2 and 3 (Table 1) indicates that the tighter binding of APC to SCRIB PDZ1 compared to PDZ3 is largely driven by a more favourable entropic contribution (Table 1).

The crystal structure of SCRIB PDZ1:APC PBM peptide complex

We next examined the structural basis of SCRIB binding to the APC PBM by determining the crystal structure of the SCRIB PDZ1:APC complex. The PDZ1:APC structure was refined to a resolution of 1.35 Å with a final R_{work}/R_{free} of 17.0 and 20.5, respectively. Clear and continuous density is visible

for APC residues 2837-2843, with the remaining Gly presumed disordered. As shown previously [14] the SCRIB PDZ1 domain comprises a compact globular fold comprising six β-strands and two α-helices that adopt a β-sandwich structure, with the APC peptide bound in the canonical ligand binding groove located between the β2 strand and helix α2 (Figure 2A).

Examination of the PDZ1:APC structure reveals that binding of APC does not significantly change the PDZ1 domain structure when superimposed onto the previously determined structures of a SCRIB PDZ1:β-PIX complex [14] or ligand free SCRIB PDZ1 (PDB ID 5VWC) [14], with an rmsd of 0.9 Å over 93 Cα atoms between SCRIB PDZ1:β-PIX and APC complexes, and between PDZ1 alone and PDZ1:APC of 1.2 Å over 96 Cα atoms [37].

SCRIB PDZ1 utilizes a hydrophobic pocket to accommodate $V2843^{APC}$ that is formed by PDZ1 L738, I740, I742, V797 and L800. This hallmark interaction of PDZ domains with peptide ligands is supplemented by a number of hydrogen bonds with APC, including side chain mediated $H793^{PDZ1}$:$T2841^{APC}$, $T749^{PDZ1}$:$S2837^{APC}$ as well as $R801^{PDZ1}$:$V2843^{APC}$ (Figure 2B). In addition, there are main chain contacts from $L738^{PDZ1}$, $G739^{PDZ1}$ and $I740^{PDZ1}$ with the carboxyl group of $V2843^{APC}$ and inter main chain contacts between $I740^{PDZ1}$:$T2841^{APC}$.

To validate the interaction of SCRIB PDZ1 with APC, we performed structure-guided mutagenesis to selectively disrupt PDZ1 binding to APC. ITC analysis of a mutant of SCRIB PDZ1 revealed the PDZ1H793A bound with a 3-fold lower affinity with a K_D of 18.8 μM due to the loss of the hydrogen bond between $H793^{PDZ1}$:$T2841^{APC}$ (Figure 3C, Table 1).

DISCUSSION

Scribble is a crucial regulator of apico-basal cell polarity, and acts as central arbiter of a diverse set of signalling pathways that converge on Scribble via interactions with its four PDZ domains [4]. As part of this, Scribble has been shown to engage a diverse group of interactors including β-PIX [14], Vangl2 [11], β-catenin [38], MCC [12] and importantly for this study APC [16] to

ultimately control cell and tissue architecture. As a result, regulation of Scribble interactions is complex, with multiple interactors competing for Scribble binding at the same time. Considering the spatio-temporal regulation of expression of both Scribble and its interactors, it is likely that only a certain subset of potential interactors are able to compete for Scribble binding at any given time, however the molecular detail of how Scribble distinguishes and selects for particular interactors is currently unknown.

We reason that one important mechanism that impacts the Scribble interactor network is the affinity of the individual binding partners. However, quantitative measurements of affinities, and accompanying detailed examination of Scribble PDZ:ligand interactions are only available for a limited subset of Scribble ligands. Published data on these better studied interactors indicates that distinct patterns of interactions exist that impose one level of regulation for Scribble interactions. For instance, *Drosophila* Scribble interacts with Gukh (Gukholder) primarily via the PDZ1 domain, with the PDZ3 interaction being 40-fold weaker, and no detectable binding to PDZ2 and 4 [28]. In contrast, human Scribble (SCRIB) interacts with β-PIX via PDZ1,2 and 3 domains, with the interaction with PDZ1 being the strongest (3.3 μM) and PDZ2 the weakest (67.8 μM) [14]. Furthermore, IP, pull-down assays and affinity indicated that for other interactors such as PKP4, DNM1L and HPV E6, different SCRIB PDZ domains are able to discriminate between these ligands [13, 39]. However, a coherent analysis of the different interaction pattern is challenging due to a systematic absence of high quality affinity measurements for SCRIB's PDZ4 domain, with large scale analyses omitting SCRIB PDZ4 domain from their analyses [13]. Considering that SCRIB PDZ4 had been implicated to bind to APC[16], NOS1AP [40] and TBEV NS5 [41], matching affinity measurements for PDZ4 are needed to comprehensively examine the ability of Scribble PDZ domains to control polarity signalling.

We now show that human Scribble binds the APC PBM with low micromolar affinities, with SCRIB PDZ1 domain being the highest affinity site with a K_D of 6.0 μM, whereas SCRIB PDZ3 binds with 18.3 μM and the SCRIB PDZ2 an affinity of 36.0 μM. These findings are in disagreement with previous data indicating that APC interacts with SCRIB PDZ1 and 4 domains [16] or with SCRIB PDZ1 and 2 domain only [15]. Instead, the pattern in interactions and affinities we observed for Scribble APC interactions resembles the binding pattern for SCRIB:β-PIX [14], which also binds SCRIB PDZ domains 1,2 and 3, with PDZ1 being the highest affinity interactor and PDZ2 the weakest binder.

Previous studies revealed that the PDZ domains of DLG1 interact with the C-terminal region of APC [21]. Isothermal titration calorimetry revealed that the DLG1 PDZ1 domain bound APC with K_D of 18.2 μM, whereas the PDZ2 domain bound with significantly higher affinity at a K_D of 1.05 μM [24]. Considering the affinities measured for DLG1 PDZ domain interactions it seems likely that both SCRIB and DLG1 will compete with each other for APC binding, whilst APC also being under competition from other ligands such as β-PIX. Thus, the precise spatio-temporal location of APC, SCRIB and DLG1 are likely to have a substantial impact on the type of interactions that APC will be engaged in with Scribble module components.

Interestingly, the affinity of APC with SCRIB PDZ1 is comparable to that of SCRIB PDZ1:β-PIX. However, analysis of the detailed interactions in both complexes reveals that a key hydrophobic interaction by a Trp in the -5 position with SCRIB PDZ1 Y751 is not replicated in the APC complex, where a Tyr in the -6 position occupies a similar position to the Trp in β-PIX, but does not engage with the β2-3 loop (Figure 3 B). This suggests that to achieve similar affinities, interactions by hydrophobic PBM resides at the -4,-5 or -6 position such as those observed in the Gukh [28], ZO1 [43] or a high affinity artificial peptide [42] complexes are not required. Similarly, the high affinity interaction of APC with the DLG1 PDZ2 domain does not involve specific engagement of the β2-3 loop by Tyr at the -5 position in the APC PBM (Figure 3C) [23, 24]. Evidently, the high affinity interaction of APC with DLG1 PDZ2 is achieved without

involvement of aromatic ring stacked interactions. A comparison of the thermodynamic parameters of DLG1 PDZ1 and PDZ2 binding to APC with binding to SCRIB PDZ1, 2 and 3 domains reveals that SCRIB PDZ3 binding to APC features a similar profile of -ΔH and TΔS contributions as DLG1 PDZ1 and PDZ2, with both displaying very favourable -ΔH that is off-set by a less favourable TΔS contribution [24].

Using site directed mutagenesis we specifically targeted the $H793^{PDZ1}$:$T2841^{APC}$, and affinity measurements using ITC indicated an ~3-fold loss of affinity. Previous studies on the drosophila Scribble PDZ1:Gukh complex indicated that mutation of the equivalent His to Ala (H796A) completely abolished Gukh binding [28]. This suggests that targeted mutagenesis would be a feasible approach to selectively disrupt specific Scribble PDZ:ligand interactions to examine more precisely how particular interaction contribute to the vast signalling network that is anchored by Scribble and its PDZ domains.

In summary, we show that human Scribble PDZ1, 2 and 3 domains bind APC with micromolar affinity, with PDZ1 being the highest affinity site and PDZ2 the lowest affinity site. Furthermore, the crystal structure of the human Scribble PDZ1:APC complex indicates that APC makes a limited number of specific contacts with the PDZ1 binding groove, thus enabling selective engagement of APC as part of the wider Scribble interactor network. Our findings provide a structural basis for Scribble PDZ1:APC interactions, and will enable more detailed structure-guided investigations including increased ligand specificity via protein engineering to understand the role of APC during the control of cell polarity and cell adhesion.

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CONFLICT OF INTERESTS

The authors have no conflicts of interest to report.

AUTHOR CONTRIBUTIONS

JH: Acquisition of data; Analysis and interpretation of data; Drafting and revising the article

SC: Acquisition of data; Analysis and interpretation of data.

POH: Conception and design; Analysis and interpretation of data; Drafting and revising the article

MK: Conception and design; Acquisition of data; Analysis and interpretation of data; Drafting and revising the article

REFERENCES

1. Nelson, W. J. (2003) Adaptation of core mechanisms to generate cell polarity, *Nature*. **422**, 766-74.
2. McCaffrey, L. M. & Macara, I. G. (2012) Signaling pathways in cell polarity, *Cold Spring Harb Perspect Biol*. **4**.
3. Halaoui, R. & McCaffrey, L. (2015) Rewiring cell polarity signaling in cancer, *Oncogene*. **34**, 939-50.

4. Stephens, R., Lim, K. Y. B., Portela, M., Kvansakul, M., Humbert, P. O. & Richardson, H. E. (2018) The Scribble Cell Polarity Module in the Regulation of Cell Signalling in Tissue Development and Tumourigenesis, *J Mol Biol*.
5. Bilder, D., Li, M. & Perrimon, N. (2000) Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors, *Science.* **289**, 113-6.
6. Zhan, L., Rosenberg, A., Bergami, K. C., Yu, M., Xuan, Z., Jaffe, A. B., Allred, C. & Muthuswamy, S. K. (2008) Deregulation of scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma, *Cell.* **135**, 865-78.
7. Pearson, H. B., McGlinn, E., Phesse, T. J., Schluter, H., Srikumar, A., Godde, N. J., Woelwer, C. B., Ryan, A., Phillips, W. A., Ernst, M., Kaur, P. & Humbert, P. (2015) The polarity protein Scrib mediates epidermal development and exerts a tumor suppressive function during skin carcinogenesis, *Mol Cancer.* **14**, 169.
8. Pearson, H. B., Perez-Mancera, P. A., Dow, L. E., Ryan, A., Tennstedt, P., Bogani, D., Elsum, I., Greenfield, A., Tuveson, D. A., Simon, R. & Humbert, P. O. (2011) SCRIB expression is deregulated in human prostate cancer, and its deficiency in mice promotes prostate neoplasia, *J Clin Invest.* **121**, 4257-67.
9. Godde, N. J., Sheridan, J. M., Smith, L. K., Pearson, H. B., Britt, K. L., Galea, R. C., Yates, L. L., Visvader, J. E. & Humbert, P. O. (2014) Scribble modulates the MAPK/Fra1 pathway to disrupt luminal and ductal integrity and suppress tumour formation in the mammary gland, *PLoS Genet.* **10**, e1004323.
10. Elsum, I. A., Yates, L. L., Pearson, H. B., Phesse, T. J., Long, F., O'Donoghue, R., Ernst, M., Cullinane, C. & Humbert, P. O. (2014) Scrib heterozygosity predisposes to lung cancer and cooperates with KRas hyperactivation to accelerate lung cancer progression in vivo, *Oncogene.* **33**, 5523-33.
11. Kallay, L. M., McNickle, A., Brennwald, P. J., Hubbard, A. L. & Braiterman, L. T. (2006) Scribble associates with two polarity proteins, Lgl2 and Vangl2, via distinct molecular domains, *J Cell Biochem.* **99**, 647-64.
12. Arnaud, C., Sebbagh, M., Nola, S., Audebert, S., Bidaut, G., Hermant, A., Gayet, O., Dusetti, N. J., Ollendorff, V., Santoni, M. J., Borg, J. P. & Lecine, P. (2009) MCC, a new interacting protein for Scrib, is required for cell migration in epithelial cells, *FEBS Lett.* **583**, 2326-32.
13. Ivarsson, Y., Arnold, R., McLaughlin, M., Nim, S., Joshi, R., Ray, D., Liu, B., Teyra, J., Pawson, T., Moffat, J., Li, S. S., Sidhu, S. S. & Kim, P. M. (2014) 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.* **111**, 2542-7.
14. Lim, K. Y. B., Godde, N. J., Humbert, P. O. & Kvansakul, M. (2017) Structural basis for the differential interaction of Scribble PDZ domains with the guanine nucleotide exchange factor beta-PIX, *J Biol Chem.* **292**, 20425-20436.
15. Sundell, G. N., Arnold, R., Ali, M., Naksukpaiboon, P., Orts, J., Guntert, P., Chi, C. N. & Ivarsson, Y. (2018) Proteome-wide analysis of phospho-regulated PDZ domain interactions, *Mol Syst Biol.* **14**, e8129.
16. Takizawa, S., Nagasaka, K., Nakagawa, S., Yano, T., Nakagawa, K., Yasugi, T., Takeuchi, T., Kanda, T., Huibregtse, J. M., Akiyama, T. & Taketani, Y. (2006) 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.* **11**, 453-64.
17. Fodde, R., Kuipers, J., Rosenberg, C., Smits, R., Kielman, M., Gaspar, C., van Es, J. H., Breukel, C., Wiegant, J., Giles, R. H. & Clevers, H. (2001) Mutations in the APC tumour suppressor gene cause chromosomal instability, *Nat Cell Biol.* **3**, 433-8.

18. Henderson, B. R. (2000) Nuclear-cytoplasmic shuttling of APC regulates beta-catenin subcellular localization and turnover, *Nat Cell Biol.* **2**, 653-60.
19. Segditsas, S. & Tomlinson, I. (2006) Colorectal cancer and genetic alterations in the Wnt pathway, *Oncogene.* **25**, 7531-7.
20. Saito-Diaz, K., Benchabane, H., Tiwari, A., Tian, A., Li, B., Thompson, J. J., Hyde, A. S., Sawyer, L. M., Jodoin, J. N., Santos, E., Lee, L. A., Coffey, R. J., Beauchamp, R. D., Williams, C. S., Kenworthy, A. K., Robbins, D. J., Ahmed, Y. & Lee, E. (2018) APC Inhibits Ligand-Independent Wnt Signaling by the Clathrin Endocytic Pathway, *Dev Cell.* **44**, 566-581 e8.
21. Matsumine, A., Ogai, A., Senda, T., Okumura, N., Satoh, K., Baeg, G. H., Kawahara, T., Kobayashi, S., Okada, M., Toyoshima, K. & Akiyama, T. (1996) Binding of APC to the human homolog of the Drosophila discs large tumor suppressor protein, *Science.* **272**, 1020-3.
22. Etienne-Manneville, S., Manneville, J. B., Nicholls, S., Ferenczi, M. A. & Hall, A. (2005) Cdc42 and Par6-PKCzeta regulate the spatially localized association of Dlg1 and APC to control cell polarization, *J Cell Biol.* **170**, 895-901.
23. Slep, K. C. (2012) Structure of the human discs large 1 PDZ2- adenomatous polyposis coli cytoskeletal polarity complex: insight into peptide engagement and PDZ clustering, *PLoS One.* **7**, e50097.
24. Zhang, Z., Li, H., Chen, L., Lu, X., Zhang, J., Xu, P., Lin, K. & Wu, G. (2011) Molecular basis for the recognition of adenomatous polyposis coli by the Discs Large 1 protein, *PLoS One.* **6**, e23507.
25. Rautureau, G. J., Yabal, M., Yang, H., Huang, D. C., Kvansakul, M. & Hinds, M. G. (2012) The restricted binding repertoire of Bcl-B leaves Bim as the universal BH3-only prosurvival Bcl-2 protein antagonist, *Cell Death Dis.* **3**, e443.
26. Studier, F. W. (2005) Protein production by auto-induction in high density shaking cultures, *Protein Expr Purif.* **41**, 207-34.
27. Scopes, R. K. (1974) Measurement of protein by spectrophotometry at 205 nm, *Anal Biochem.* **59**, 277-82.
28. Caria, S., Magtoto, C. M., Samiei, T., Portela, M., Lim, K. Y. B., How, J. Y., Stewart, B. Z., Humbert, P. O., Richardson, H. E. & Kvansakul, M. (2018) Drosophila melanogaster Guk-holder interacts with the Scribbled PDZ1 domain and regulates epithelial development with Scribbled and Discs Large, *J Biol Chem.* **293**, 4519-4531.
29. Kabsch, W. (2010) Xds, *Acta Crystallogr D Biol Crystallogr.* **66**, 125-32.
30. Winn, M. D., Ballard, C. C., Cowtan, K. D., Dodson, E. J., Emsley, P., Evans, P. R., Keegan, R. M., Krissinel, E. B., Leslie, A. G., McCoy, A., McNicholas, S. J., Murshudov, G. N., Pannu, N. S., Potterton, E. A., Powell, H. R., Read, R. J., Vagin, A. & Wilson, K. S. (2011) Overview of the CCP4 suite and current developments, *Acta Crystallogr D Biol Crystallogr.* **67**, 235-42.
31. Storoni, L. C., McCoy, A. J. & Read, R. J. (2004) Likelihood-enhanced fast rotation functions, *Acta Crystallogr D Biol Crystallogr.* **60**, 432-8.
32. Emsley, P. & Cowtan, K. (2004) Coot: model-building tools for molecular graphics, *Acta Crystallogr D Biol Crystallogr.* **60**, 2126-32.
33. Adams, P. D., Afonine, P. V., Bunkoczi, G., Chen, V. B., Davis, I. W., Echols, N., Headd, J. J., Hung, L. W., Kapral, G. J., Grosse-Kunstleve, R. W., McCoy, A. J., Moriarty, N. W., Oeffner, R., Read, R. J., Richardson, D. C., Richardson, J. S., Terwilliger, T. C. & Zwart, P. H. (2010) PHENIX: a comprehensive Python-based system for macromolecular structure solution, *Acta Crystallogr D Biol Crystallogr.* **66**, 213-21.
34. Chen, V. B., Arendall, W. B., 3rd, Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S. & Richardson, D. C. (2010) MolProbity: all-atom structure validation for macromolecular crystallography, *Acta Crystallogr D Biol Crystallogr.* **66**, 12-21.

35. Morin, A., Eisenbraun, B., Key, J., Sanschagrin, P. C., Timony, M. A., Ottaviano, M. & Sliz, P. (2013) Collaboration gets the most out of software, *Elife.* **2**, e01456.
36. Meyer, P. A., Socias, S., Key, J., Ransey, E., Tjon, E. C., Buschiazzo, A., Lei, M., Botka, C., Withrow, J., Neau, D., Rajashankar, K., Anderson, K. S., Baxter, R. H., Blacklow, S. C., Boggon, T. J., Bonvin, A. M., Borek, D., Brett, T. J., Caflisch, A., Chang, C. I., Chazin, W. J., Corbett, K. D., Cosgrove, M. S., Crosson, S., Dhe-Paganon, S., Di Cera, E., Drennan, C. L., Eck, M. J., Eichman, B. F., Fan, Q. R., Ferre-D'Amare, A. R., Fromme, J. C., Garcia, K. C., Gaudet, R., Gong, P., Harrison, S. C., Heldwein, E. E., Jia, Z., Keenan, R. J., Kruse, A. C., Kvansakul, M., McLellan, J. S., Modis, Y., Nam, Y., Otwinowski, Z., Pai, E. F., Pereira, P. J., Petosa, C., Raman, C. S., Rapoport, T. A., Roll-Mecak, A., Rosen, M. K., Rudenko, G., Schlessinger, J., Schwartz, T. U., Shamoo, Y., Sondermann, H., Tao, Y. J., Tolia, N. H., Tsodikov, O. V., Westover, K. D., Wu, H., Foster, I., Fraser, J. S., Maia, F. R., Gonen, T., Kirchhausen, T., Diederichs, K., Crosas, M. & Sliz, P. (2016) Data publication with the structural biology data grid supports live analysis, *Nat Commun.* **7**, 10882.
37. Holm, L. & Laakso, L. M. (2016) Dali server update, *Nucleic Acids Res.* **44**, W351-5.
38. Sun, Y., Aiga, M., Yoshida, E., Humbert, P. O. & Bamji, S. X. (2009) Scribble interacts with beta-catenin to localize synaptic vesicles to synapses, *Mol Biol Cell.* **20**, 3390-400.
39. Thomas, M., Myers, M. P., Massimi, P., Guarnaccia, C. & Banks, L. (2016) Analysis of Multiple HPV E6 PDZ Interactions Defines Type-Specific PDZ Fingerprints That Predict Oncogenic Potential, *PLoS Pathog.* **12**, e1005766.
40. Richier, L., Williton, K., Clattenburg, L., Colwill, K., O'Brien, M., Tsang, C., Kolar, A., Zinck, N., Metalnikov, P., Trimble, W. S., Krueger, S. R., Pawson, T. & Fawcett, J. P. (2010) NOS1AP associates with Scribble and regulates dendritic spine development, *J Neurosci.* **30**, 4796-805.
41. Werme, K., Wigerius, M. & Johansson, M. (2008) Tick-borne encephalitis virus NS5 associates with membrane protein scribble and impairs interferon-stimulated JAK-STAT signalling, *Cell Microbiol.* **10**, 696-712.
42. Appleton, B. A., Zhang, Y., Wu, P., Yin, J. P., Hunziker, W., Skelton, N. J., Sidhu, S. S. & Wiesmann, C. (2006) 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.* **281**, 22312-20.
43. Zhang, Y., Yeh, S., Appleton, B. A., Held, H. A., Kausalya, P. J., Phua, D. C., Wong, W. L., Lasky, L. A., Wiesmann, C., Hunziker, W. & Sidhu, S. S. (2006) Convergent and divergent ligand specificity among PDZ domains of the LAP and zonula occludens (ZO) families, *J Biol Chem.* **281**, 22299-311.

TABLES

Table 1: Summary of thermodynamic binding parameters for SCRIB PDZ domain interactions with APC peptide measured at pH 7.5 and 25 °C. NB denotes no binding, n.d. denotes not determined. Each of the value was calculated from at least three independent experiments.

	K_D (nM)	-ΔH (kcal/mol)	TΔS (cal/mol/K)	N
PDZ1	5970±1100	-5.0±0.7	-2.2±0.5	1.1±0.04
PDZ2	35940±1100	-2.2±0.5	-3.9±0.6	1.1±0.08
PDZ3	18280±3330	-10.8.3±1.3	4.3±1.3	0.9±0.03
PDZ4	NB	NB	NB	NB
PDZ1 H793A	18800±6160	n.d.	n.d.	1.0±0.04

Table 2: Data collection and refinement statistics.

	SCRIB PDZ1:APC
Data collection	
Space group	C 2
No of molecules in AU	2+2
Cell dimensions	
a, *b*, *c* (Å)	62.34, 51.10, 57.05
α, β, γ (°)	90.00, 92.69, 90.00
Wavelength (Å)	0.9537
Resolution (Å)*	39.5-1.35 (1.398-1.35)
R_{sym} or R_{merge}*	0.031 (0.27)
$I / \sigma I$*	6.8 (1.5)
CC(1/2)	0.998 (0.863)
Completeness (%)*	97.2 (92.1)
Redundancy*	4.6 (4.1)
Wilson B-factor	16.4
Refinement	
Resolution (Å)	39.5-1.35
No. reflections	38341
R_{work} / R_{free}	0.170/0.205
No. non-hydrogen atoms	
Protein	1507
Ligand/ion	24
Water	214
B-factors	
Protein	28.1
Ligand/ion	47.7
Water	35.5
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angle (°)	1.26
Ramachandran plot (%)	
Favored	99.48
Allowed	0.52
Disallowed	0

* Data in parentheses are for highest resolution shell

FIGURE LEGENDS

FIGURE 1: Interaction profiles of SCRIB PDZ domains with APC peptide. Binding isotherms of isolated SCRIB PDZ domains with APC peptide. Each isotherm is represented by a raw thermogram (top panel) and a binding isotherm fitted with a one-site binding model (bottom panels). K_D: dissociation constant; ±: standard deviation; NB: no binding. Each of the value was calculated from at least three independent experiments.

FIGURE 2: The crystal structures of SCRIB PDZ1 bound to APC peptide. The APC peptide binds to the SCRIB PDZ1 domain via the shallow canonical ligand binding groove located between the β2 strand and α2 helix. (A) PDZ1 (magenta) is shown as a cartoon with wild type APC peptide (cyan) represented as sticks. (B) PDZ1 (magenta) is shown as a cartoon with the APC peptide (cyan) represented as sticks. Hydrogen bonds are indicated as dashed black lines.

FIGURE 3: Analysis of SCRIB PDZ1 complex with APC. (A) Electron density map encompassing the binding groove of SCRIB PDZ1 in complex with APC peptide. PDZ1 is shown as pink sticks whereas APC peptide is shown as magenta sticks. The electron density map is shown as a blue mesh contoured at 1.5 σ. (B) Superimposition of SCRIB PDZ1 bound to APC (pink and cyan) complexes with β-PIX (yellow and light gray, PDB ID 5VWK) and Gukh (purple and orange, PDB ID 5WOU) complexes. (C) Binding isotherm of isolated SCRIB PDZ1 H793A domain mutant with APC peptide. Raw thermogram (top panel) and a binding isotherm fitted with a one-site binding model (bottom panels) are shown. K_D: dissociation constant; ± standard deviation. K_D was calculated from three independent experiments. (D) Superimposition of SCRIB PDZ1 bound to APC (pink and cyan) with DLG1 PDZ2:APC complexes (blue and gray, PDB ID 3RL8; salmon and green, PDB ID 4G69). PDZ domains are shown as tubes, APC PBM peptides are shown as sticks.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Interaction profiles of SCRIB PDZ domains with mutant APC peptide. Binding isotherms of isolated SCRIB PDZ domains with mutant APC peptide (GSYLVASA). Each isotherm is represented by a raw thermogram (top panel) and a binding isotherm fitted with a one-site binding model (bottom panels). NB denotes no binding.

Figure 1

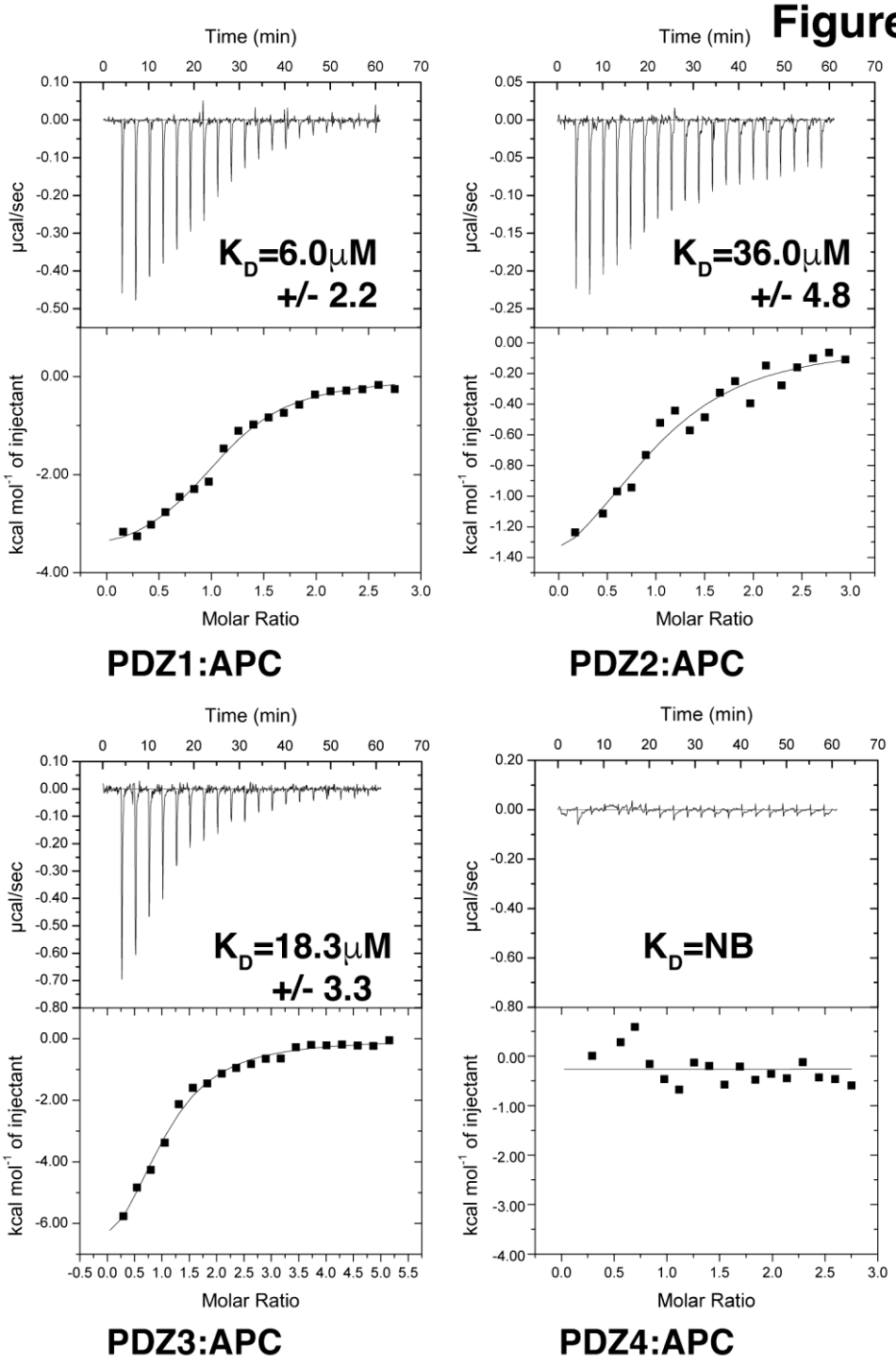

Figure 2

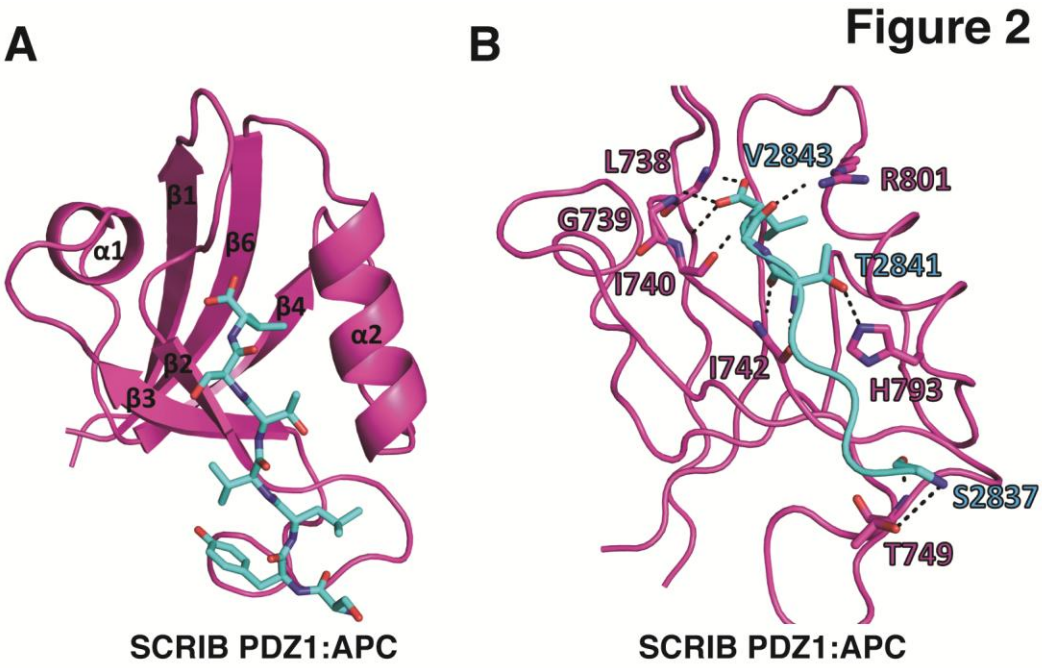

Figure 3