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The binding interface of kindlin-2 and ILK involves Asp344/Asp352/Thr356 in kindlin-2 and Arg243/Arg334 in ILK

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Kindlins are four-point-one, ezrin, radixin, moesin (FERM)-containing cytosolic proteins that regulate integrin-mediated cell adhesion and signaling [1]. Kindlin-1, -2, and -3 share high sequence homology, but they have different tissue distributions [2]. Mutations in kindlin-1 cause the skin atrophy disease Kindler syndrome [3,4]. Loss of kindlin-2 expression leads to peri-implantation defect and embryonic lethality [5]. Mutations in kindlin-3 cause the disease leukocyte adhesion deficiency III (LAD III), characterized by recurrent infections and bleeding [6–8]. In addition, an abundance of evidence shows the importance of kindlins in cancer progression [9]. Kindlins contain F0, F1, F2, pleckstrin homology (PH), and F3 subdomains (Fig. 1A) [10]. The F0 subdomain adopts an ubiquitin-like fold and it promotes the localization of kindlins to the plasma membrane [11,12]. The F1 subdomain contains a lysine-rich loop that mediates binding to negatively charged lipid head groups [13,14]. The PH domain binds with different affinities to phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3,4,5-triphosphate [15–17]. The F3 subdomain interacts with the second Nxx(Y/F) motif in the integrin β cytoplasmic tails [18,19]. Kindlins cooperate with talin to activate integrins, and they mediate integrin outside-in signaling [1,20–24].

Focal adhesion (FA) proteins, kindlin-2 and integrin-linked kinase (ILK), regulate cell adhesion and migration. ILK interacts with and promotes kindlin-2 targeting to FAs. Leu353 and Leu357 in kindlin-2 have been reported to be important for the interaction between kindlin-2 and ILK. However, the binding interface between kindlin-2 and ILK remains unclear. Using molecular modeling and molecular dynamics simulations, we show that Asp344, Asp352, and Thr356 in kindlin-2 and Arg243 and Arg334 in ILK kinase domain (KD) are important in kindlin-2/ILK complex formation. Mutations that disrupt these interactions abrogate kindlin-2 and ILK colocalization in HeLa cells. The interactions are direct based on data from pull-down assays using purified recombinant kindlin-2 F2-pleckstrin homology and ILK KDS. These data provide additional insights into the binding interface between kindlin-2 and ILK.

Keywords: cell adhesion; integrin-linked kinase; kindlins

Abbreviations
EV, empty vector; FA, focal adhesion; FERM, four-point-one, ezrin, radixin, moesin; ILK, integrin-linked kinase; KD, kinase domain; LAD, leukocyte adhesion deficiency; MD, molecular dynamics; MOC, Manders’ overlap coefficient; PH, pleckstrin homology; PINCH, particularly interesting new cysteine–histidine; PME, particle mesh Ewald; ROI, region-of-interest; SEC, size exclusion chromatography.
Interaction between kindlin-2 and integrin-linked kinase (ILK) involves the F2 subdomain of kindlin-2 and the kinase domain (KD) of ILK [25]. ILK forms a complex with α-parvin and particularly interesting new cysteine–histidine-rich protein (PINCH) [26], and its KD has been shown to interact with the C-terminal calponin homology domain (CH2) of α-parvin [27]. Unlike humans, Caenorhabditis elegans has only one kindlin (UNC-112) [28]. It has been shown that C. elegans UNC-112 (kindlin), PAT-4 (ILK), PAT-6 (α-parvin), and UNC-97 (PINCH) form a complex that is required for the attachment of striated muscle cells to the basement membrane [29]. Furthermore, a number of point mutations in UNC-112 and PAT-4 have been shown to disrupt UNC-112 and PAT-4 interaction [29,30].

Based on NMR analysis, it has been shown that the sequence Ser339-Glu358 between the F2’ and PH domains of kindlin-2 adopts a helical conformation (Fig. 1B) [31], and Leu353 and Leu357 in kindlin-2 that are involved in ILK KD binding are shown [31], and they project into a nonpolar region in ILK KD. By contrast, kindlin-2 Asp344, Asp352, and Thr356 project into a positively charged region in ILK KD. Illustrations were generated using PyMOL.

**Fig. 1.** Molecular modeling of kindlin-2 Ser339-Glu358 in complex with ILK KD. (A) Domain organization of kindlin-2. (B) Structure of kindlin-2 Ser339-Glu358 based on PDB (2MSU) [31]. (C) Different interacting sites in ILK KD. Structure of ILK KD in complex with α-parvin CH2 domain is based on PDB (3KMU) [27]. (D) Molecular model of kindlin-2 Ser339-Glu358 in complex with ILK KD (Model 1). (E) Electrostatic surface potential of ILK KD in complex with kindlin-2 Ser339-Glu358. Leu353 and Leu357 in kindlin-2 that are involved in ILK KD binding are shown [31], and they project into a nonpolar region in ILK KD. By contrast, kindlin-2 Asp344, Asp352, and Thr356 project into a positively charged region in ILK KD. Illustrations were generated using PyMOL.
this study, we characterize the binding interface between kindlin-2 and ILK KD.

Materials and methods

Cell culture and transfection

HeLa cell line (American Type Culture Collection, Manassas, VA, USA) was maintained in RPMI1640 medium containing 10% heat-inactivated fetal bovine serum, penicillin (100 IU·mL⁻¹), and streptomycin (100 µg·mL⁻¹; Hyclone, Logan, UT, USA). Transfection was performed using either PolyFect (Qiagen, Valencia, CA, USA) or TransitIT-×2 (Mirus Bio LLC, WI, USA) transfection reagent according to manufacturers’ protocols.

Expression plasmids

pcDNA3.1 zeo (−) containing GFP-kindlin-2 (human, full-length) and GFP were only constructed by standard procedures of molecular cloning. mCherry-ILK was a gift from Michael W. Davidson, Florida State University, Tallahassee, FL, USA (Adgene plasmid). Kindlin-2 (Ser278-Ile577) F2-PH was subcloned into expression vector pET24a (+) to create 6xHis tagged recombinant protein. ILK KD (Gln213-Lys452) was subcloned into pGEX-KG expression vector to generate GST-ILK KD. Mutations were generated using the site-directed mutagenesis kit (Stratagene, San Diego, CA, USA) and relevant primer pairs. All constructs were verified by sequencing (Axil Scientific, Singapore).

Confocal fluorescence microscopy and colocalization analysis

Transfected cells were plated onto fibronectin-coated (2 µg·cm⁻²) glass-bottom culture dish (MatTek, Ashland, MA, USA) and incubated for 1 h under culture conditions. Bound cells were fixed, permeabilized and blocked with BSA [32], stained with 1 µg of anti-paxillin mAb (Merck-Millipore, Billerica, MA, USA) at RT for 1 h followed by staining with Alexa Fluor 647-conjugated anti-mouse IgG (1:400; Molecular Probes, Eugene, OR, USA) at RT for 1 h. Cells were washed and stained with DAPI before visualizing by confocal laser scanning microscopy LSM710 (Zeiss, Oberkochen, Germany) at a magnification of ×63 under oil immersion. Images were analyzed by ZEN and IMAGE J softwares. Three different region-of-interests (ROIs) were analyzed per cell. Colocalization analysis was performed using IMAGE J JACoP. Mander’s overlap coefficient was calculated at each focal adhesion (FA) site within each ROI. Data from each group of cells were collected from three independent experiments. Cell spread area was measured using the IMAGE J Measure function. Overall brightness and contrast of whole-cell images were adjusted for presentations using IMAGE J for clarity.

Recombinant protein expression and purification

Recombinant proteins were expressed and purified from transformed Escherichia coli strain BL-21 (DE3). Expression of GST, GST-ILK KD, and GST-ILK KD mutants was induced with the addition of 0.2 mM IPTG. Bacteria were subsequently left to grow overnight at 16 °C. On the following day, bacteria were centrifuged and the pellet was resuspended in lysis buffer (150 mM NaCl, 0.5 mM TCEP, 50 mM Tris, pH 7.4) containing 0.5 mM PMSF and other protease inhibitors (Merck Millipore) followed by sonication. Affinity purification of recombinant proteins was performed by incubating the cell lysate with glutathione Sepharose 4 Fast Flow beads (GE Healthcare, Buckinghamshire, UK) overnight. Beads were washed in lysis buffer, and bound proteins were eluted with elution buffer (50 mM Tris, 150 mM NaCl, 20 mM reduced glutathione, 0.5 mM TCEP, pH 8.0). Eluted proteins were subjected to size exclusion chromatography (SEC) in 50 mM Tris buffer (pH 7.4) containing 150 mM NaCl and 0.5 mM TCEP on a HiLoad 16/600 superdex 200 pg column FPLC system (GE Healthcare). GST-ILK KD was purified as a soluble oligomer based on SEC calibration. Expression of kindlin-2-F2-PH-6His and its mutants was induced with the addition of 0.2 mM IPTG followed by incubation overnight at 16 °C. Bacteria were centrifuged and the pellet was resuspended in IMAC buffer (500 mM NaCl, 10 mM imidazole, 10% glycerol, 0.5 mM TCEP, 2.5 mM iodoacetamide, 100 mM HEPES, pH 8.0) containing protease inhibitors followed by sonication. The lysate was incubated with Ni-NTA agarose beads (Qiagen) for 1 h at 4 °C. Bound proteins were eluted with elution buffer (500 mM NaCl, 500 mM imidazole, 10% glycerol, 0.5 mM TCEP, 20 mM HEPES, pH 7.4). Purified His-tagged recombinant proteins in elution buffer were dialyzed against 50 mM Tris buffer (pH 7.4) containing 150 mM NaCl and 0.5 mM TCEP.

GST pull-down assay

Purified GST-ILK KD (52.5 µg) in binding buffer (150 mM NaCl, 50 mM Tris, pH 7.4) was incubated with 7.5 µL glutathione Sepharose 4 fast flow bead for overnight at 4 °C. GST-ILK KD bound beads were washed once in binding buffer and incubated with purified WT or mutant kindlin-2 F2-PH-6His proteins at a 1 : 1 molar ratio to that of GST-ILK KD for 3 h at 4 °C. Beads were washed three times in binding buffer and bound proteins were eluted by boiling in SDS loading buffer containing 40 mM DTT followed by SDS/PAGE. Immunoblottings were performed using the following antibodies: rabbit anti-His tag (Genway, San Diego, CA, USA), rabbit anti-GST (Molecular Probes), and HRP-conjugated goat-anti-rabbit IgG (H + L; Advanta, Menlo Park, CA, USA).
Molecular dynamics (MD) simulations

Molecular models of ILK KD and α-parvin CH2 domain were obtained from PDB (3KMU) [27]. The molecular model of kindlin-2 Ser339-Glu358 that was shown to interact with ILK KD was obtained from PDB (2MSU) [31]. Protein–protein docking was performed using CLUSPRO 2.0 webserver with default settings [33–36]. Molecular dynamics (MD) simulations were performed for model 1 of the docked kindlin-2-ILK complex using GROMACS 4.6.3 [37,38]. The docked complex was placed at the center of a periodic, rectangular box of size $8.3 \times 8.05 \times 8.5$ Å. Pre-equilibrated water molecules at 300 K were then added based on the TIP3P water model [39]. Sodium and chloride ions (150 mM each) were added to electrically neutralize the system. The AMBER99SB-ILDN force field was used to describe interatomic interactions [40]. The system was then energy minimized using 100 steps of steepest descent, with positional restraints placed on the protein heavy atoms.

Next, equilibration MD simulations were performed with restraints in place for 100 ps under constant temperature of 100 K. This was followed by another 100 ps of simulation at 100 K but with additional control on pressure at 1 atm. The desired system temperature was then increased every 100 ps by 100 K till 300 K is reached. For the 300 K simulation, 100 ps, positional restraints were only imposed on backbone C-α atoms and at 1/10 the strength. This was repeated for another 100 ps with positional restraints again reduced by 10 times. The above procedure allows for refinement of the kindlin-2-ILK KD side chain interactions while keeping the complex intact. All positional restraints were then removed and each production run was performed for 30 000 ps. Multiple, independent production runs are required to adequately sample the possible interaction behaviors between ILK KD and kindlin-2 peptide. In view of resource limitations, only three independent production runs were performed, each starting from a different set of particle velocities with the same mean speed. The velocity-rescaling thermostat with a time constant of 0.1 ps was used [41]. Berendsen barostat was used for pressure control with a time constant of 1 ps and compressibility of $4.6 \times 10^{-5}$ bar$^{-1}$ [42]. The particle mesh Ewald (PME) sum method [43] was employed for all electrostatic calculations with a cut-off distance of 1.2 nm for the real-space component. van der Waals interactions were calculated with a Lennard–Jones potential with a cut-off distance of 1.2 nm. The Verlet cut-off scheme was used in order to take advantage of the GPU hardware. The simulation time step was 2 fs, with bonds constrained by the LINear Constraint solver [44,45].

Statistical analysis

Statistics were calculated based on unpaired two-tailed Student’s t-test (GraphPad Prism, GraphPad Software, Inc. La Jolla, CA, USA).

Results and Discussion

Molecular modeling and molecular dynamics (MD) simulations of kindlin-2/ILK KD interaction

The crystal structure of ILK KD in complex with parvin CH2 domain has been solved [27]. A positively charged surface in ILK KD that is remote from the parvin CH2 interaction interface could allow membrane docking of ILK KD (Fig. 1C). Furthermore, an integrin β tail interaction site on ILK KD that is distinct from the parvin interaction site could facilitate the formation of a ternary complex involving integrin β tail, ILK KD, and parvin CH2 [27,46]. We therefore made use of the ILK KD structure to perform molecular docking of kindlin-2 Ser339-Glu358. This sequence is highly exposed and flexible based on the recently reported kindlin-2 structure [47]. The top five docked models (in terms of interaction energy) favoring hydrophobic interactions are shown: Model 1 (Fig. 1D) and Models 2–5 (Fig. S1). Parvin-CH2 domain was not included in the docking process but was incorporated in the illustration with reference to the ILK KD-parvin CH2 structure [27]. Model 1 was selected for follow-on analyses because it is least likely to disrupt ILK KD membrane docking as compared to models 2 and 3 in which kindlin-2 Ser339-Glu358 interacts with residues near to or on the positively charged membrane interacting surface of ILK KD. Model 1 is also least likely to have steric clashes between kindlin-2 and the parvin CH2 domain compared with models 4 and 5. In model 1, the favored kindlin-2 Ser339-Glu358 docking site on ILK KD is distinct from the integrin β tail-binding site. This would permit multiprotein interactions involving kindlin-2 and ILK with the integrin β tail. In model 1, the helical segment Ser339-Glu358 of kindlin-2 snugs into a groove in ILK KD. The side chains of Leu353 and Leu357 in kindlin-2 are facing a relatively uncharged surface in ILK KD (Fig. 1E). Importantly, the side chains of Thr356, Asp352, and Asp344 in kindlin-2 are oriented toward a charged surface in ILK KD. Furthermore, interactions between these residues and ILK KD Arg243 and Arg334 were predicted.

To examine the dynamics of these interactions, we performed three independent MD production runs based on model 1, each starting from a different set of particle velocities with the same mean speed (MD1, 2, and 3, 30 ns duration; Fig. 2A). In MD1 and MD3, WT kindlin-2 Thr356 forms H-bond with ILK KD Arg334 through time as shown in the distance (nm) against time (ns) plot. We observed a similar profile in
MD2 except that the distance separation of the two residues increased after 20 ns. Snapshots of MD1 and MD3 are included to illustrate H-bond formation between the two residues (Fig. 2A, four inset panels). In all three MD runs, kindlin-2 Asp344 formed stable H-bond with ILK KD Arg243 through time (Fig. 2B). Kindlin-2 Asp352 also formed stable H-bond with ILK KD Arg243 in two of the three MD runs. A simplified illustration depicting these interactions is shown (Fig. 2C).
Asp344/Asp352/Thr356 in kindlin-2 and Arg243/Arg334 in ILK contribute to kindlin-2/ILK complex formation

We performed functional assays to verify the MD simulations data. HeLa cells were cotransfected with mCherry-ILK (full-length; WT) and GFP-kindlin-2 (full-length; WT) or mutants that contained single, double or triple mutation(s). Cells were also cotransfected with mCherry-ILK (WT) and GFP (empty vector; EV) or mCherry (EV) and GFP (EV) as controls (Fig. 3A). Cells were seeded onto fibronectin and stained with anti-paxillin mAb followed by fluorescence microscopy analysis. FAs were identified based on paxillin staining and the colocalization of mCherry-ILK and GFP-kindlin-2 at FAs was determined based on Manders’ overlap coefficient (MOC). Three ROIs (R1, R2, R3) per cell and 30 cells per group were analyzed, and MOCs were plotted (Fig. 3B). Consistent with previous studies, colocalization of mCherry-ILK (WT) with GFP-kindlin-2 (WT) but not with GFP-kindlin-2 L353L357/AA mutant. Importantly, GFP-kindlin-2 containing T356/G, D344D352/T356/GGG substitutions exhibited diminished colocalization with mCherry-ILK (WT). We then analyzed cells transfected with mCherry-ILK (WT) and GFP-kindlin-2 D344D352T356/AAA mutants as controls to maintain the side chain length of the mutated residues. GFP-kindlin-2 D344D352T356/AAA also exhibited diminished colocalization with mCherry-ILK (WT; Fig. S2A,C). Immunoblotting of GFP-kindlin-2 and its mutants was also performed (Fig. S2D). We then measured the cell spread area of different groups of transfected cells. Cells expressing mCherry-ILK (WT) and GFP-kindlin-2 (WT) showed larger cell spread area compared with cells expressing mCherry-ILK (WT) and GFP (EV) or mCherry (EV) and GFP (EV), which corroborates well with the established role of kindlin-2 in promoting cell adhesion and spreading [23]. By contrast, enhanced cell spreading was not observed in cells expressing GFP-kindlin-2 mutants.

To demonstrate that Asp344, Asp352, and Thr356 in kindlin-2 are directly involved in kindlin-2 and ILK interaction, we performed GST pull-down assays using purified recombinant GST-ILK KD (WT) and kindlin-2 F2-PH-6His domain (WT) and its mutants (Fig. 3D). In line with previous study [31], GST-ILK KD (WT) interacted with kindlin-2 F2-PH-6His domain (WT) but not L353L357/AA mutant. Importantly, GST-ILK KD (WT) also interacted minimally with kindlin-2 mutants T356/G, D344D352/GG, and D344D352T356/GGG, suggesting that these residues are directly involved in kindlin-2/ILK KD interaction.

We noted that kindlin-2 mutants D344D352/GG and D344D352T356/GGG showed lower MWs compared with WT protein. This may be attributed to the double Asp substitutions for the neutral and small Gly.

We then examined whether Arg243 and Arg334 in ILK KD are crucial for ILK KD/kindlin-2 interaction as predicted in the MD simulations. HeLa cells were cotransfected with GFP-kindlin-2 (WT) and mCherry (EV), mCherry-ILK (WT), or mCherry-ILK R243R334/GG. Colocalization of these proteins was analyzed by fluorescence microscopy followed by MOC determination. R243R334/GG substitutions significantly abrogated mCherry-ILK colocalization with GFP-kindlin-2 (WT; Fig. 4A,B). Immunoblotting of mCherry-ILK WT and its mutant was also performed (Fig. S2D). We also observed diminished colocalization of mCherry-ILK R243R334/AA with GFP-kindlin-2 (WT; Fig. S2B,C). Furthermore, ILK KD Arg243 and Arg334 are directly involved in kindlin-2 interaction as demonstrated in pull-down assays (Fig. 4C).

Taken together, we have demonstrated for the first time that Asp344, Asp352, and Thr356 in kindlin-2 and Arg243 and Arg334 in ILK are important molecular determinants in the binding interface of kindlin-2 and ILK. Our data are consistent with two previous studies that examined UNC-112 (kindlin) and PAT-4 (ILK) in C. elegans [29,30]. In the first study, the nonsense mutation D382V in UNC-112 abrogated its interaction with PAT-4 and its localization to FAs in vivo [30]. Based on sequence alignment, UNC-112 Asp382 is located in a region that corresponds to kindlin-2 Ser339-Glu359 containing the three key residues Asp344, Asp352, and Thr356 investigated herein (Fig. S3). In the second study, nine suppressor mutations in PAT-4 that restore interaction of UNC-112 D382V with PAT-4 and its localization to FAs were identified [29]. Among these mutations, three (P257L, I261N, F262L) involved residues that are conserved and located very close to ILK Arg243 (Fig. S4). Residues in kindlin-2 and ILK that correspond to those found to be important for UNC-112 and PAT-4 interaction are shown in our kindlin-2/ILK KD model for visualization in 3D (Fig. S5). Except ILK Ser418 (PAT-4 Ala433), Met425 (PAT-4 Met440), and Lys438 (PAT-4 Asn453), the relative positions of other residues important for UNC-112 and PAT-4 interaction aforementioned are in the proximity of the kindlin-2/ILK KD binding interface characterized herein.

Our data also provide additional supporting evidence for the importance of region Ser339-Glu359 in kindlin-2 to bind ILK that was reported earlier [31]. Apart from Leu353 and Leu357, there were...
perturbations of residues Asp344, Asp352, and Thr356 in kindlin-2 peptide when titrated with ILK KD/Parvin CH2 based on two-dimensional 1H NOESY NMR [31]. However, in the same study, substitution of Asp352 for Ala in kindlin-2 did not disrupt its interaction with ILK KD/Parvin CH2 based on recombinant protein pull-down assay. Functional analysis of kindlin-2 Asp344 and Thr356 was not examined, and the interaction site on ILK KD was not determined. The lack of significant effect of D352A substitution alone in that study may be explained by our current model in which both Asp344 and Asp352 in kindlin-2 interact with Arg243 in ILK KD (Fig. 2C). Single D352A substitution may not be sufficient to disrupt the interaction. In this study, both Asp residues in kindlin-2 were mutated, and the kindlin-2/ILK interaction was

![Functional analyses of kindlin-2 Asp344, Asp352, and Thr356. (A) Representative images of transfected HeLa cells on fibronectin. Three ROIs per cell were selected and FAs were identified based on paxillin staining. Colocalization of GFP-kindlin-2 and mCherry-ILK at FA was determined by calculating the MOC and plotted in (B). Each datum point represents one cell. Data were collated from three independent experiments. (C) Cell spread area of these cells was determined and plotted. 
P < 0.05 is considered significant. (D) Pull-down assay using an equimolar of GST-ILK KD (or GST) and kindlin-2 F2-PH-6His recombinant proteins. Numbers below immunoblots are means (± SD) of protein band intensities relative to the K2 F2-PH WT/GST-ILK KD sample (normalized to 1.0) from three independent experiments.](image-url)
effectively abrogated. We also demonstrated the importance of Thr356 in kindlin-2 and Arg243 and Arg334 in ILK KD for their binding.

There are differences in the expression, localization and interacting partners among human kindlins [2]. In endothelial cells, kindlin-2, but not kindlin-3, localizes to mature FAs [48]. The molecular basis of this difference lies in the binding of kindlin-2, but not kindlin-3, to ILK [25]. The kindlin-2 sequence Ser339-Glu359 investigated herein shares high homology with the corresponding sequence in kindlin-3. However, the residues that correspond to kindlin-2 Asp352 and Thr356 in kindlin-3 are Asn329 and Lys333, respectively. Whether different properties of these side chains contribute to the differential binding of these kindlins to ILK KD remains to be determined.

Finally, both kindlin-2 and ILK are associated with cancer progression [9,49]. Our findings not only provide novel insights into the binding interface of kindlin-2 and ILK, they could be useful in the design and development of small molecule cancer therapeutics.

Acknowledgements

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Author contributions

CP performed the molecular modeling and MD simulations. SYG, LTO, and HFT performed the wet laboratory experiments. CPC, SYG, HFT, SKA, and SMT analyzed the data. CPC, SYG, and SMT wrote the manuscript.

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**Supporting information**

Additional Supporting Information may be found online in the supporting information tab for this article:

**Fig. S1.** Five models of ILK-KD (gray), kindlin-2 Ser339-Glu358 (orange) and parvin-CH2 (green) association.

**Fig. S2.** (A,B) Colocalization analysis of mCherry-ILK WT with GFP-kindlin-2 WT or D344D352T356/AAA, and GFP-kindlin-2 WT with mCherry-ILK WT or R243R334/AA. Each datum point represents one cell. Statistics were calculated based on unpaired two-tailed Student’s *t*-test. (C) Representative images of transfected cells are shown. For clarity, overall brightness and contrast of whole-cell images were adjusted using image j. Scale bar: 10 μm. (D) Immunoblotting of GFP-kindlin-2 WT/mutants and mCherry-ILK WT/mutant using anti-kindlin-2 antibody (Sigma Cat#K3269, dil-L: 1000) and anti-ILK antibody (Sigma Cat#I0783, dil-L: 1000), respectively.

**Fig. S3.** Sequence alignment of UNC-112 and kindlin-2.

**Fig. S4.** Sequence alignment of PAT-4 and ILK.

**Fig. S5.** Residues in kindlin-2 and ILK that correspond to those involved in UNC-112 and PAT-4 interaction are shown (red sticks.)