Structure

Structure of the Dual-Mode Wnt Regulator Kremen1 and Insight into Ternary Complex Formation with LRP6 and Dickkopf

Graphical Abstract

Highlights

- The structure of the KREMEN 1 ectodomain is solved from three crystal forms
- Kringle, WSC, and CUB subdomains interact tightly to form a single structural unit
- The interface to DKKs is formed from the Kringle and WSC domains
- The CUB domain is found to interact directly with LRP6_{PE1PE2}

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In Brief
Zebisch et al. describe the ectodomain structure of KREMEN 1, a receptor for Wnt antagonists of the DKK family. Apo structures and a complex with functional fragments of DKK1 and LRP6 shed light on the function of this dual-mode regulator of Wnt signaling.

Accession Numbers
5FWS
5FWT
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5FWV
5FWW
Structure of the Dual-Mode Wnt Regulator Kremen1 and Insight into Ternary Complex Formation with LRP6 and Dickkopf

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http://dx.doi.org/10.1016/j.str.2016.06.020

SUMMARY

Kremen 1 and 2 have been identified as co-receptors for Dickkopf (Dkk) proteins, hallmark secreted antagonists of canonical Wnt signaling. We present here three crystal structures of the ectodomain of human Kremen1 (KRM1ECD) at resolutions between 1.9 and 3.2 Å. KRM1ECD emerges as a rigid molecule with tight interactions stabilizing a triangular arrangement of its Kringle, WSC, and CUB structural domains. The structures reveal an unpredicted homology of the WSC domain to hepatocyte growth factor. We further report the general architecture of the ternary complex formed by the Wnt co-receptor Lrp5/6, Dkk, and Krm, determined from a low-resolution complex formed by the Wnt co-receptor LRP6 (LRP6PE3PE4), the cysteine-rich domain 2 (CRD2) of DKK1, and KRMECD. DKK1CRD2 is sandwiched between LRP6PE3 and KRM1Kringle-WSC. Modeling studies supported by surface plasmon resonance suggest a direct interaction site between Krm1CUB and Lrp6PE2.

INTRODUCTION

Signaling by Wnt morphogens is renowned for its fundamental roles in embryonic development, tissue homeostasis, and stem cell maintenance (Holstein, 2012). Due to these functions, generation, delivery, and interpretation of Wnt signals are all heavily regulated in the animal body (Clevers and Nusse, 2012; Niehrs, 2007). Vertebrate Dickkopf proteins (Dkk1, 2, and 4) are one of many secreted antagonists of Wnt and function by blocking activation of the Wnt co-receptor LRP5/6 (Semenov et al., 2001). Kremen1 and 2 change their function from inhibition to enhancement of Wnt signaling, as well as growth of ectopic forelimb digits. Growth of ectopic digits is further enhanced upon additional loss of dkk expression (Ellwanger et al., 2008; Schulze et al., 2010). The Wnt antagonistic activity of Krm1 is also linked to its importance for correct thymus epithelium formation in mice (Osada et al., 2006). The importance of intact KRM1 for normal human development and health is highlighted by the recent finding that a homozygous mutation in the ectodomain of KRM1 leads to severe ectodermal dysplasia including oligodontia (Issa et al., 2016). Interestingly, the Wnt antagonistic activity of Krm is context dependent, and Krm proteins are actually dual-mode Wnt regulators. In the absence of Dkk, Krm1 and 2 change their function from inhibition to enhancement of Lrp6-mediated signaling. By direct binding to Lrp6 via the ectodomains, Krm proteins promote Lrp6 cell-surface localization and hence increase receptor availability (Hassler et al., 2007; Cselemyi and Lee, 2008). Further increasing the complexity of Krm functionality, it was recently found that Krm1 (but not Krm2) can also act independently of LRP5/6 and Wnt as a dependence receptor, triggering apoptosis unless bound to Dkk (Caurser et al., 2015).

Structurally, Krm1 and 2 are type I transmembrane proteins with a 40 kDa ectodomain and a flexible cytoplasmic tail consisting of 60–75 residues. The ectodomain consists of three similarly sized structural domains of around 10 kDa each: the N-terminal Kringle domain (KR), the middle武CUB domain (CUB), and the C-terminal transmembrane domain (TM) spanned by a GPI linker (Verna et al., 1997). The third structural domain is a CUB fold (Verna et al., 1997). The third structural domain is a CUB domain (Romero et al., 1997). An approximately 70-residue linker connects the CUB domain to the transmembrane span. An intact KR-WSC-CUB domain triplet and membrane attachment is required for Wnt antagonism (Mao et al., 2002). The transmembrane span and cytoplasmic tail can be replaced with a GPI linker without impact on Wnt antagonism (Mao et al., 2002; Caurser et al., 2015).

We sought to provide structural insights into the multi-functionality of this cell-surface receptor. The structures presented here reveal the unknown fold of the WSC domain and the tight interactions of all three domains. We further...
succeeded in determination of a low-resolution LRP6\textsubscript{PE3PE4}\textsubscript{DKK1CRD2-KRM1ECD} complex, defining the architecture of the Wnt inhibitory complex that leads to Lrp6 cell-surface depletion.

**RESULTS**

The recombinant production of the extracellular domain of Krm for structural studies proved challenging (see Experimental Procedures). We succeeded in purifying KRM1\textsubscript{ECD} complexes with DKK1\textsubscript{Linker-CRD2} and DKK1\textsubscript{CRD2} that were monodisperse and stable in gel filtration, hence indicating at least micromolar affinity (data not shown). Several crystal forms were obtained from these complexes, however, crystals always contained only KRM1 protein.

We solved the structure of KRM1\textsubscript{ECD} in three crystal forms at 1.9, 2.8, and 3.2 Å resolution (Table 1). The high-resolution structure is a near full-length model (Figure 1). The small, flexible, and charged\textsuperscript{98}AEHED\textsuperscript{102} loop could only be modeled in a slightly lower resolution structure and in crystal form III. The KR, WSC, and CUB are arranged in a roughly triangular fashion with tight interactions between all three domains. The KR domain, and

| Crystal form | KRM1\textsubscript{ECD} | KRM1\textsubscript{ECD} | KRM1\textsubscript{ECD} | KRM1\textsubscript{ECD} | LRP6\textsubscript{PE3PE4-DKK1CRD2-KRM1ECD} |
|--------------|------------------------|------------------------|------------------------|------------------------|---------------------------------|
| X-ray source | Diamond i04            | Diamond i03            | Diamond i03            | Diamond i04            | Diamond i04                    |
| Wavelength (Å) | 0.9793                | 0.9700                | 0.9700                | 0.9795                | 0.9795                          |
| Space group | P,3,21                | P,3,21                | P,4\textsubscript{3}   | P,4,2,2               | C,222                           |
| Unit cell a/Å | 50.9/90               | 50.5/90               | 65.8/90               | 67.8/90               | 68.9/90                         |
| b/Å | 50.9/90               | 50.5/90               | 65.8/90               | 67.8/90               | 100.1/90                        |
| c/Å | 188.4/120             | 187.4/120             | 75.0/90               | 198.2/90              | 270.7/90                        |
| Wilson B factor (Å\textsuperscript{2}) | 31                    | 41                    | 76                    | 77                    | NA                              |
| Resolution range (Å) | 47.10–1.90            | 62.47–2.10            | 75.00–2.80            | 67.80–3.20            | 67.68–3.50                      |
| Unique reflections | 23,300 (1,524)        | 17,089 (1,428)        | 7,964 (1,448)         | 8,171 (1,343)         | 8,070 (723, 645)                |
| Average multiplicity | 9.1 (9.2)             | 5.2 (5.3)             | 3.7 (3.7)             | 22.7 (12.6)           | 3.8 (3.5, 4.4)                  |
| Completeness (%) | 99.8 (98.5)           | 100 (100)             | 99.8 (100)            | 98.8 (93.4)           | 51.6 (98.5, 14.1)               |
| R<sub>merge</sub> (%) | 11.4 (1.7)            | 12.0 (1.7)            | 14.9 (1.5)            | 13.1 (1.9)            | 4.6 (1.1, 2.2)                  |
| R<sub>pim</sub> (%) | 14.8 (158.3)          | 9.3 (98.0)            | 6.2 (98.9)            | 29.8 (142.2)          | 44.9 (40.5, 114.2)              |
| R<sub>w</sub> (%) | 15.7 (55.3)           | 10.3 (109.0)          | 3.7 (53.8)            | 6.3 (40.0)            | 24.7 (23.9, 59.9)               |

### Refinement

| Protein | 2,260 | 2,301 | 2,102 | 2,305 | 7,730 |
| N-glycans | 42 | 42 | 28 | 28 | 0 |
| Water | 79 | 54 | 0 | 2 | 0 |
| Ligands | 6 | 6 | 2 | 5 | 0 |
| Average B factor (Å\textsuperscript{2}) | 63 | 65 | 108 | 84 | – |
| N-glycans | 35 | 46 | 102 | 18 | – |
| Water | 68 | 85 | – | 75 | – |
| Ligands | 36 | 47 | 91 | 75 | 66 |
| RMSD from Ideality | 0.020 | 0.016 | 0.019 | 0.016 | 0.004 |
| Bond lengths (Å) | 2.050 | 1.748 | 1.952 | 1.796 | 0.770 |
| Ramachandran Plot | 96.8 | 95.5 | 96.9 | 94.9 | 92.3 |
| Allowed (%) | 99.7 | 100.0 | 100.0 | 99.7 | 99.8 |
| Number of outliers | 1 | 0 | 0 | 1 | 2 |
| PDB code | 5FWS | 5FWT | 5FWU | 5FWV | 5FWV |

Values in parentheses refer to the highest-resolution shell. An additional shell given for the ternary complex corresponds to the last shell with near-complete diffraction data. NA, not announced.
which bears two of the four glycosylation sites, contains the canonical three disulfide bridges (C32-C114, C55-C95, C84-C109) and, like other Kringle domains, is in secondary structure elements. The structurally most similar Kringle domain is that of human plasminogen (PDB: 1PKR; Wu et al., 1994) with an root-mean-square deviation (RMSD) of 1.7 Å for 73 aligned Ca (Figure 1B). The KRM1 structure reveals the fold of the WSC domain for the first time. The structure is best described as a sandwich of a α1-β5-α3-β4-β2 antiparallel β sheet and a single α helix. The structure is also rich in loops and is stabilized by four disulfide bridges (C122-C186, C147-C167, C151-C169, C190-C198). Using the PDBeFold server, we detected a surprising yet significant homology to PAN module domains (Issa et al., 2016) maps to the hydrophobic core of the protein at the interface of the three subdomains (Figure 1A). Such a mutation is bound to severely destabilize the protein structure of KRM1, leading to disturbance of its Wnt antagonistic, Wnt stimulatory, and Wnt independent activity.

**Low-Resolution Insight into Ternary Complex Formation**

Co-crystallization of LRP6PE3PE4 with DKK1CRD2, and LRP6PE1 with an N-terminal peptide of DKK1 has provided valuable structural insight into direct Wnt inhibition by Dkk ligands (Cheng et al., 2011; Ahn et al., 2011; Bourhis et al., 2011; Bao et al., 2012). One face of the rather flat DKK1CRD2 fragment binds to the third β propeller of LRP6. Mutational analyses further implied that the LRP6PE3 PE3-averted face of DKK1CRD2 bears the Krm binding site, hence suggesting how Dkk can recruit both receptors into a ternary complex (Wang et al., 2008).

To obtain direct insight into ternary complex formation by Lrp5/6, Dkk, and Krnn, we subjected an LRP6PE3PE4-DKK1CRD2-KRM1ECD complex to crystallization trials. Diffraction data collected from the resulting crystals were highly anisotropic with diffraction extending in the best directions to 3.5 Å and 3.7 Å but only to 6.4 Å in the third direction. Despite the lack of high-resolution diffraction, the general architecture of the ternary complex is revealed (Figure 2A). DKK1CRD2 binds to the top face of the LRP6 PE3 β propeller as described earlier for the binary complex (Cheng et al., 2011; Ahn et al., 2011). KRM1ECD does indeed bind on the opposite side of DKK1CRD2 with only its KR and WSC domains engaged in binding (Figure 2A). Although present in the complex subjected to crystallization, we observe no density that could correspond to CRD1 or the domain linker (L). We confirm that the CRD2 of DKK1 is required and sufficient for binding to KRM1 (Mao and Niehrs, 2003). In surface plasmon resonance (SPR), we measured low micromolar affinity between full-length DKK1 and immobilized KRM1ECD (Figure 2B). A SUMO fusion of DKK1L-CRD2 displayed a similar (slightly higher) affinity. In contrast, a SUMO fusion of DKK1CRD1L did not display binding for concentrations tested up to 325 µM (Figure 2B).
Overall, the DKK1-KRM1 interface is characterized by a large number of polar interactions but only few hydrophobic contacts (Figure 2C). The crystal structure gives an explanation for DKK1 loss-of-binding mutations identified previously (Wang et al., 2008): R191 of DKK1 forms a double salt bridge to D125 and E162 of KRM1 (Figure 2C). A charge reversal as in the mouse Dkk1 (mDkk1) R197E variant would severely disrupt the binding. Similarly, the K226 side chain of DKK1, which points to a small hydrophobic pocket on the surface of KRM1 formed by Y108, W94, and W106, forms salt bridges with the side chains of KRM1 D88 and D90. Again, a charge reversal as shown for mDkk1 K232E would be incompatible with binding. The side chain of DKK1 S192 was also predicted to be involved in Krn binding (Wang et al., 2008). Indeed, we found (Figure 2C) that the side chain of D201 of KRM1 forms two hydrogen bonds to the side-chain hydroxyl and the backbone amide of S192 (mouse, S198). Additional polar interactions are formed between the N140, S163, and Y165 side chains of KRM1 and DKK1 backbone carbonyls of W206, L190, and C189, respectively. The carboxyl of DKK1 R224 is hydrogen bonded to Y105 and W106 of KRM1. We suspect that the Dkk charge reversal hypothesis that KRM1CUB binds to LRP6PE2, we used SPR and compared binding of the wild-type and the GlycoCUB mutant of KRM1ECB (bearing an N-glycosylation site at N309) with a purified LR6PEP2 fragment. Indeed, we found that in the absence of Dkk, KRM1ECB bound with considerable affinity to LR6PEP2 (Figure 3C). In contrast, no saturable binding was observed between KRM1 and LR6PEP4. Introduction of an N-glycosylation site at N309 in KRM1ECB abolished LR6PEP2 binding (Figure 3C), while binding to DKK1 was unaffected (Figure 2D).

We conclude that the predicted binding site between KRM1CUB and LR6PEP2 is a strong candidate for mediating the direct Lp6-Krm interaction, which is thought to increase Wnt responsiveness by stabilizing Lp6 at the cell surface (Hassler et al., 2007; Cseleenyi and Lee, 2008). Further experiments are required to pinpoint the exact binding site. Although LR6PEP2 appears somewhat out of reach in the modeled ternary complex, it cannot
KrmECD fragments were cloned into pHLsec or variants thereof (Seiradake Large-Scale Mammalian Expression and Protein Purification EXPERIMENTAL PROCEDURES linked to LRP6PE3 via DKK1CRD2 (Figure 3D). Low-resolution interface is expected to be higher once Krm is also cross-binding. which propeller (PE1 versus PE2) of LRP6 is available for Krm LRP6-Krm binary complex. The presence of DKK may govern be excluded as the Krm binding site in the ternary complex and LRP6-Krm binary complex. The presence of DKK may govern which propeller (PE1 versus PE2) of LRP6 is available for Krm binding.

Apparent binding across the proposed KRM1CUB-LRP6PE2 interface is expected to be higher once Krm is also cross-linked to LRP6PE3 via DKK1CRD2 (Figure 3D). Low-resolution negative-stain EM and small-angle X-ray scattering studies of LRP6PE1PE2PE3PE4, in isolation and in complex with Dkk1 (Cheng et al., 2011; Ahn et al., 2011), plus negative-stain EM of full-length LRP6 ectodomain (Chen et al., 2011), have indicated curved, platform-like conformations but also potential flexibility between PE2 and PE3. It is therefore possible that the interplay of Krm and Dkk binding can promote changes in LRP6 ectodomain conformation with functional consequences; however, such ideas await investigation.

Taken together, the structural and biophysical studies we report here extend our mechanistic understanding of Wnt signal regulation. We describe the ectodomain structure of the dual Wnt regulator Krm1, providing an explanation for the detrimental effect on health and development of a homozygous KRM1 mutation. We also reveal the interaction mode of Krm-Dkk and the architecture of the ternary complex formed by Lrp5/6, Dkk, and Krm. Furthermore, the ternary crystal structure has guided the N-terminal flexible region and CRD1 were not secreted from HEK cells. Similar way as described (Chen et al., 2011). Shorter constructs of DKK1 lack- ing the N-terminal flexible region and CRD1 were not secreted from HEK cells. However, using the approach of an N-terminal fusion to a modified SUMO protein as described earlier (Peroutka et al., 2008; Chang et al., 2015), we succeeded in secretory expression of a SUMO-DKK1Linker-CRD2 construct encompassing residues S141-H266. A variant of this containing a TEV protease cleavage site just before T181, SUMO-DKK1Linker-TEV-CRD2, was also well expressed and allowed removal of the flexible linker region.

To obtain complexes of KRM1ECD-TEV, we (co-)transfected the stable cell line with DKK and LRP6PE3PE4 constructs described earlier (Chen et al., 2011). Binary and ternary KRM1ECD-DKK1fl and KRM1ECD-DKK1fl-LRP6PE3PE4 complexes were well secreted into the conditioned medium (CM) of HEK293T cells, but exhibited extensive O-glycosylation (as judged from smeary bands in western blot), which would be detrimental to crystallization. Fragments truncated to the KR-WSC-CUB core gave sharp bands but were barely secreted. We therefore engineered an A23-G373 (isof orm 1 numbering used throughout the article) full ectodomain construct (KRM1ECD-TEV) with a C-terminal His10 tag that contained a TEV protease cleavage site after E324. The expected sequence of the secreted protein was ETG320AVKEE324-GSENLYFQGGS325LPGVPG373-THHHHHHHHHH (the isoform-2-specific PG insertion and the TEV site are underlined). This construct was well secreted and could be processed using TEV protease. However, 80%–90% of the protein eluted as aggregates from a size-exclusion column even before TEV treatment. The same applied to analog constructs for Krm1 expressing stable GntI-deficient HEK293S cell line was generated by excision of an EcoRI-Xhol fragment, sub-cloning into pNeo-Seq-1, and selection of neomycin-resistant cells (Seiradake et al., 2015). The stable cell line showed expression levels superior to transiently transfected cells (not shown).

Human LRP6E1PE2E3, LRP6E2PE3PE4, and full-length DKK1 were produced in a similar way as described (Chen et al., 2011). Shorter constructs of DKK1 lacking the N-terminal flexible region and CRD1 were not secreted from HEK cells. However, using the approach of an N-terminal fusion to a modified SUMO protein as described earlier (Peroutka et al., 2008; Chang et al., 2015), we succeeded in secretory expression of a SUMO-DKK1Linker-CRD2 construct encompassing residues S141-H266. A variant of this containing a TEV cleavage site just before T181, SUMO-DKK1Linker-TEV-CRD2, was also well expressed and allowed removal of the flexible linker region.

To obtain complexes of KRM1ECD-TEV, we (co-)transfected the stable cell line with DKK and LRP6PE3PE4 constructs described earlier (Chen et al., 2011). Binary and ternary KRM1ECD-DKK11 and KRM1ECD-DKK11-LRP6PE3PE4 complexes were stable in gel-filtration eluting as distinct monodisperse peaks.

Crystallization and Data Collection

All samples subjected to crystallization were purified from CM by affinity and size-exclusion chromatography (Zebisch et al., 2013; Kakugawa et al., 2015). After treatment with TEV protease and endoglycosidase F1 overnight using mass equivalents of 1%, samples were subjected to size-exclusion chromatography in 10 mM HEPES/NaOH (pH 7.5), 150 mM NaCl. The crystals giving rise to the 1.9 Å dataset for KRM1 in crystal form I were obtained from a KRM1ECD-DKK1Linker-CRD2 complex concentrated to 12 mg/mL. Out of this complex, KRM1ECD crystallized alone in 2.0 M ammonium sulfate, 5% (v/v) isopropanol. For cryoprotection, crystals were transferred to mother liquor

**Figure 3. LRP6-KRM1 Direct Interaction and Summary**

(A) In a construction of a ternary complex with all four β propellers of LRP6 intact, the CUB domain points via its Ca2+-binding region toward the top face of the second β propeller.

(B) Close-up view of the potential interaction site. In addition, LRP6PE2 has been superimposed with DKK1 (yellow) and SOST (pink) peptide complexes of LRP6β1.

(C) SPR measurements comparing LRP6FLPE2PE3 binding with wild-type KRM1ECD and the GlycoCUB mutant bearing an N-glycan at N309.

(D) Schematic representation of structural and biophysical findings and their implications for Wnt-dependent (left, middle) and independent (right) signaling. Conformational differences in the de- pictions of LRP6 are included purely for ease of representation.
mixed 1:1 with 3.4 M sodium malonate (pH 7.0). The slightly less well-ordered crystal of crystal form I and crystals of form II were obtained from a KRM1ECOD-DKK1ECOD complex using the SUMO-DKK1LAPEN-TEV-CRD2 construct and releasing SUMO and the DKK linker region by TEV and 3C protease treatment. Crystals of form I (2.1 Å) appeared from protein at 12 mg/mL in 1.0 M (NH₄)₂PO₄, 0.10 M sodium citrate (pH 5.8) and were cryoprotected by transfer to 2.9 M sodium malonate (pH 5.0). Crystals of form II grew from protein concentrated to 17 mg/mL in 1.0 M MgSO₄, 0.1 M trisodium citrate (final pH 5.6). For cryoprotection, crystals were transferred to mother liquor mixed 1:3 with 3.0 M ammonium sulfate, 18% glycerol. Crystal form III appeared after 11 months in a dried-out drop of condition H5 of the Morpheus screen. The protein concentration had been 9 mg/mL. For cryoprotection, fresh liquid from Morpheus/H5 was added. The ternary complex structure was obtained from an LRPEP3PE4-DKK1L1-KRM1ECOD complex at 9 mg/mL that grew in condition E10 of the PACTpremier screen (pH approximately 6.8) over the course of 2–11 months. For cryoprotection, 10% PEG2000 was added. By mistake, the crystals were incubated for 1 hr with 1 mM platinum compound in this cryosolution before cryocooling.

Structure Determination
Diffraction data were collected at DIAMOND synchrotron light source at the beamlines detailed in Table 1. The structure was initially solved from crystal form III by molecular replacement (MR) with PHASER (McCoy et al., 2007), placing models for the CUB domain (PDB: 2WNO, CUB_C domain of Tsg-6 (Briggs et al., 2015), 37% sequence identity), and the KR domain (PDB: 1PKR, Kringle 1 of plasminogen; Wu et al., 1994; 39% sequence identity). Traceable density for the WSC domain became immediately evident. The KRM1 structure was then built and refined by cycling between the various crystal forms.

For the ternary complex, we obtained only a low-resolution, highly anisotropic dataset extending to Bragg spacings of 3.5 Å, 6.4 Å, and 3.7 Å along the three principle directions (c-axis = 2). All data to 3.5 Å were used during structure determination by MR. LRPEP3PE4 (PDB: 4A0P; Chen et al., 2011) and KRM1ECOD (both stripped of glycosylation sites) could be placed independently by PHASER, giving Z scores of +10 and log likelihood gains (LLG) of >200. The combined LLG was 673, increasing to 901 after rigid-body refinement. Strong electron density became apparent at glycosylation sites and close to methionines (see platinum soak above), further supporting the MR solution. Additional strong density was evident between LRPE and KRM1, suggesting the presence of DKK1. A model of the DKK1ECOD (PDB: 3S2K and 3S8V (Cheng et al., 2011; Ahn et al., 2011)) could then be placed with PHASER by testing all rotation function peaks. This increased the LLG from 901 to 973 indicating a correct solution. The individually placed LRPE6 and DKK models were then replaced with chains B and C from the LRPE-DKK complex in PDB: 3S2K. The structure was subjected to rigid-body refinement using single structural domains as individually positioned bodies.

We then performed restrained refinement of the coordinates against the ellipsoidally truncated and anisotropically scaled (Strong et al., 2006) diffraction data as obtained from the diffraction anisotropy server at UCLA. The resolution cutoffs were 3.5 Å, 6.4 Å, and 3.7 Å. Strong geometric restraints generated by PROSMART from the available high-resolution reference structures were used during refinement. No manual model building was attempted. Restrained refinement was followed by ten cycles of structure idealization. The final model had Rwork/Rfree errors of 32.5%/36.1% against the anisotropy-corrected data and 32.1%/35.5% against the unmodified but ellipsoidally truncated (Zebisch et al., 2012) diffraction data.

Surface Plasmon Resonance
Equilibrium experiments were performed as described before (Zebisch et al., 2013; Kakugawa et al., 2015) with the addition of 2 mM CaCl₂ for experiments investigating the direct LRPEP3PE4-KRM1ECOD interaction.

ACCESSION NUMBERS
Coordinates and structure factors have been deposited in the PDB with successions PDB: 5FWS, 5FWT, 5FWU, 5FWV, and 5FWW.

SUPPLEMENTAL INFORMATION
Supplemental Information includes one figure and can be found with this article online at http://dx.doi.org/10.1016/j.str.2016.06.020.

AUTHOR CONTRIBUTIONS
M.Z. and V.A.J. performed experiments with support from Y.Z., who generated the stable cell line. M.Z. and E.Y.J. designed the research. M.Z. wrote the paper with input from all other authors.

ACKNOWLEDGMENTS
We thank the staff of Diamond beamlines i03 and i04 for assistance with X-ray diffraction data collection (proposal mx6423), W. Lu for help with tissue culture, and K. Harlos and T. Walter for assistance with crystalization. This work was funded by Cancer Research UK and the UK Medical Research Council (to E.Y.J., A10976 and G9900061) and an IEF Marie Curie fellowship awarded to M.Z. The Wellcome Trust Center for Human Genetics is supported by Wellcome Trust Centre grant 090532/Z/09/Z.

Received: February 23, 2016
Revised: June 12, 2016
Accepted: June 13, 2016
Published: August 11, 2016

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