Dimer asymmetry defines α-catenin interactions

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The F-actin–binding cytoskeletal protein α-catenin interacts with β-catenin–cadherin complexes and stabilizes cell-cell junctions. The β-catenin–α-catenin complex cannot bind F-actin, whereas interactions of α-catenin with the cytoskeletal protein vinculin appear to be necessary to stabilize adherens junctions. Here we report the crystal structure of nearly full-length human α-catenin at 3.7-Å resolution. α-catenin forms an asymmetric dimer where the four-helix bundle domains of each subunit engage in distinct intermolecular interactions. This results in a left handshake–like dimer, wherein the two subunits have remarkably different conformations. The crystal structure explains why dimeric α-catenin has a higher affinity for F-actin than does monomeric α-catenin, why the β-catenin–α-catenin complex does not bind F-actin, how activated vinculin links the cadherin–catenin complex to the cytoskeleton and why α-catenin but not inactive vinculin can bind F-actin.

The formation and stabilization of cell-cell (adherens) junctions is essential for the development, architecture, maintenance and function of tissues in higher organisms. Adherens junctions are directed by the cadherin-receptor family of single-transmembrane-pass glycoproteins, which interact in a homotypic fashion through the agency of their calcium-binding ectodomains1–4. Clustering of these receptors stabilizes adherens junctions and remodels the actin cytoskeleton, and this response requires the interaction of the intracellular tail domains of cadherin receptors with the adaptor protein β-catenin. In turn, β-catenin binds the F-actin–binding cytoskeletal protein α-catenin to form a ternary cadherin–β-catenin–α-catenin complex5–7. Accordingly, α-catenin is necessary for mechanical connections between the E cadherin–β-catenin complex and the cortical actomyosin network8,9, and loss of α-catenin disrupts adherens junctions and enables connections of the cadherin–β-catenin complex to the actin cytoskeleton10–14.

α-catenin is a homodimer that binds F-actin, which suggests that the ternary cadherin–β-catenin–α-catenin complex forms direct links to the actin network. However, monomeric but not dimeric α-catenin binds the E cadherin–β-catenin complex, binding of β-catenin peptide disrupts the N-terminal α-catenin homodimer, and reconstituted cadherin–β-catenin–α-catenin complexes do not bind F-actin15–17. Thus, α-catenin stabilizes adherens junctions by other means, and its additional binding partners have been implicated in this response, in particular the cytoskeletal proteins vinculin18–20 and eplin21 that also bind F-actin. For example, vinculin is necessary to stabilize adherens junctions, and force-dependent unpurling of cadherin–β-catenin–α-catenin has been suggested to recruit vinculin to adherens junctions to stabilize these complexes19. Furthermore, β-catenin competes with α-catenin for binding to vinculin, which suggests that β-catenin also recruits vinculin to adherens junctions18,22.

α-catenin is a 906-residue polypeptide that has been reported to harbor four functional domains: an N-terminal homodimerization domain and β-catenin–binding domain23, an α-actinin– and vinculin-binding domain (VBD)22,24, an M fragment that can form cross-linked dimers and that can bind l-afadin25 and a C-terminal F-actin–binding domain26 that can also bind the tight-junction protein ZO-1 (refs. 27,28). The crystal structure of the N-terminal domain suggested that α-catenin was a symmetrical dimer whose dimerization occurs by two-fold related interactions of two α-helices from each subunit, and the structure of a chimera of this domain with β-catenin–binding peptide established that β-catenin binding disrupts this dimer29. The crystal structure of the isolated M fragment in the central portion of the protein revealed that this comprises two tandem four-helix bundles30,31.

To resolve its structure, regulation and function, here we determined the structure of nearly full-length human α-catenin (lacking its N-terminal residues 1–81) at 3.7-Å resolution. The structure revealed that α-catenin is an asymmetric dimer and suggests that asymmetry drives its functions in controlling binding to F-actin and in its interactions with activated vinculin. Further, our studies revealed that the activated vinculin–α-catenin complex is a 2:2 heterotetramer, thus explaining how vinculin stabilizes adherens junctions.

RESULTS

Overall fold of human α-catenin

We solved the human α-catenin crystal structure to 3.7-Å resolution (Table 1) by establishing a particular crystal-dehydration protocol (described in Online Methods) and by identifying a heavy-metal cluster that was compatible with the crystallization conditions. The crystal structure revealed that α-catenin is an all-helical asymmetric dimer that comprises four domains of helix bundles (Fig. 1a). The N-terminal domain (residues 82–262) of each subunit has two helix bundle domains that resembled the conformation seen in the crystal structure of this domain alone29, consisting of two antiparallel α-helices with the second α-helix shared by the following four-helix bundle.
The VBD (residues 277–393) was a four-helix bundle that harbored the two α-helical vinculin binding sites (VBS) of α-catenin, where the residues that direct the interaction with vinculin were buried within this four-helix bundle. The M fragment (residues 390–631) comprised tandem four-helix bundle domains as noted previously, yet they adopted a much more compact and vinculin-like open conformation for the B subunit of α-catenin. Finally, the F-actin–binding domain of α-catenin was a five-helix bundle that resembled the vinculin tail domain that also bound to F-actin (Fig. 1b).

### The α-catenin asymmetric dimer

The α-catenin dimer was about 130 Å in its longest dimension, and its architecture resembled that of an asymmetric left handshake (Fig. 2 and Supplementary Fig. 2), where the thumbs were the helix bundles of the N terminus, the palms were the M fragment, and the fingers were the F-actin–binding domain and the VBD. Superposition of both molecules within the dimer underscored the asymmetry and distinct orientations of the two subunits (subunit A and subunit B), where there was a large r.m.s. deviation of about 4.8 Å and of even 3.2 Å without the F-actin–binding domain (that is, residues 82–631). In contrast, the individual domains of the two subunits superimposed relatively well (0.8 Å for the VBD and M fragment (residues 277–631), 0.6 Å for the M fragment (residues 390–631) and 0.5 Å for the N-terminal domain (residues 82–262)), which indicated that there is intrinsically high flexibility within the polypeptide chain. This dynamic nature may account for the ability of α-catenin to switch between its two oligomeric states.

Notably, the structure of the N-terminal dimerization domain as found in the nearly full-length α-catenin dimer more closely resembled its conformation in the β-catenin–α-catenin chimera (PDB 1DOW) versus the isolated domain (PDB 1DOV) in its unbound state (r.m.s. deviation of 1.6 Å versus 2.1 Å). This was particularly the case for the subunit B conformation of α-catenin, which superimposed onto this chimera with an r.m.s. deviation of about 1.5 Å compared to superposition onto the unbound N-terminal dimerization domain (2.2 Å). In contrast, subunit A superimposed similarly onto either structure. Collectively, this architecture resulted in a more open conformation for the B subunit of α-catenin.

Except for the second four-helix bundle of the M fragment (residues 508–630), which stuck out in the α-catenin dimer, all helix bundles engaged in extensive interdomain interactions and contributed to the overall marked asymmetry of the molecule. For example, the first two α-helices of the VBD of subunit B bound the second and third C-terminal tail domains of α-catenin and vinculin were quite distinct. First, the N terminus of the vinculin tail domain folded back toward the end of α-helix H1, whereas the N terminus of the F-actin–binding domain of α-catenin interacted with and displaced the H2–H3 loop. Second, the C terminus of the α-catenin F-actin–binding domain adopted two distinct conformations in subunits A and B that were oriented in opposite directions, and only the conformation of subunit A was similar to that of vinculin (Fig. 1b). These differences could contribute, in part, to the distinct F-actin binding properties of the two proteins, wherein α-catenin can bind F-actin, whereas vinculin binding requires activation by severing of the head–tail clamp that keeps it in its inactive state.36–38

### Table 1 Data collection and refinement statistics

| Data collection | Nativea | 2Na2O·P2O5·12WO3b |
|-----------------|---------|------------------|
| Space group     | P32     | P32              |
| Cell dimensions |         |                  |
| a, b, c (Å)     | 145.6, 145.6, 139 | 144.7, 144.7, 139.9 |
| α, β, γ (°)     | 90, 90, 120 | 90, 90, 120      |
| Resolution (Å)  | 139.08–3.66 (3.86–3.66) | 46.68–5.58 (5.6–5.58) |
| Rmerge          | 0.077 (0.491) | 0.066 (0.537)   |
| I / aI          | 15.1 (3.6) | 24.7 (2.9)      |
| Completeness    | 0.99 (1)  | 0.99 (1)        |
| Redundancy      | 5.8 (5.8) | 5.2 (5)         |

aNative data were collected at a wavelength of 1 Å at APS/ANL beamline 22BM. bDerivative data were collected at a wavelength of 1.07 Å at SSRL beamline 11-1. The anomalous completeness was 0.98 overall and 1 in the highest-resolution shell with a multiplicity of 2.6 and 2.5, respectively. The final model, comprising 1,519 residues, has a correlation completeness was 0.98 overall and 1 in the highest-resolution shell.

### Figure 1 α-catenin structure

(a) α-catenin harbors four distinct domains: the N-terminal dimerization domain (DD), the vinculin-binding domain (VBD), the M fragment (M) and the F-actin–binding domain (FABD). F-actin–binding domain α-helices are labeled H1 through H5, as are the termini. Subunit B is shown. HO is the additional N-terminal α-helix that is not part of the five-helix bundle. (b) Superposition of the F-actin–binding domain of the two subunits in the asymmetric unit onto the vinculin tail domain. α-catenin terminal residues are labeled, and ‘N’ and ‘C’ indicate vinculin termini.
α-helices of the VBD in subunit A (Fig. 2b). Further, unlike the structure of the M fragment alone, where the two four-helix bundles were purported to interact, neither of these bundles interacted in the asymmetric α-catenin dimer.

Notably, the orientation of the F-actin–binding domain markedly differed in the two subunits of the α-catenin dimer. Specifically, although the orientation of the F-actin–binding domain in the A subunit generally resembled that seen in vinculin, the F-actin–binding domain in subunit B was rotated by about 166° compared to its orientation in subunit A (Supplementary Fig. 3). Specifically, in subunit A the F-actin–binding domain α-helices H4 and H5 interacted with the VBD, α-helix H3 interacted with the M fragment, and its N terminus interacted with the N-terminal four-helix bundle of the N-terminal dimerization domain of subunit B (residues 146–262). Further, the C terminus of the F-actin–binding domain of subunit A interacted with the second four-helix bundle of the M fragment of subunit B (Fig. 2a). In contrast, in the more open subunit B, the N terminus of the F-actin–binding domain interacted with the second four-helix bundle of the M fragment of subunit A, α-helix H4 was in contact with the VBD, and the C terminus bound the second four-helix bundle of the dimerization domain (Fig. 2b). Finally, asymmetry did not seem to be driven by crystallization and crystal contacts, as the F-actin–binding domains, in particular those of subunit B, did not engage in any crystal contacts, and those present in subunit A seemed too minor to affect its conformation.

The closely related vinculin protein harbored five domains that also comprised four- or five-helix bundles (Vh1, Vh2, Vh3, Vt2 and the F-actin–binding domain)\(^{39,40}\). A comparison of the full-length structures of α-catenin and vinculin indicated that their helix bundle domains were conserved, with the exception that α-catenin lacked an equivalent Vh2 domain (Supplementary Fig. 3). Furthermore, in the α-catenin structure, the F-actin–binding domain was oriented much differently, in particular for the flipped conformation in subunit B. These features probably explain the distinct F-actin–binding properties of the two proteins, wherein α-catenin but not inactive vinculin can bind F-actin.

\(\alpha\)-catenin binding disrupts dimerization and F-actin binding

\(\alpha\)-catenin interacts with the E cadherin–β-catenin complex at adherens junctions by binding the N-terminal α-helix of β-catenin. Superposition of the α-catenin structure onto the β-catenin–α-catenin chimera\(^{29}\) and onto the full-length β-catenin structure\(^{41}\) revealed the consequences of the β-catenin interaction on α-catenin structure and function (Fig. 3). As shown experimentally, β-catenin and α-catenin bound as a 1:1 complex, in which β-catenin binding displaced the two N-terminal α-catenin α-helices, thus disrupting the α-catenin dimer, which had a much higher affinity for F-actin\(^{15}\). The exact F-actin–binding site of α-catenin has, however, not been defined other than that residues 864–906 were necessary for the interaction\(^{42}\). Notably, the β-catenin–α-catenin model clearly showed that β-catenin sterically hinders F-actin binding by the α-catenin dimer. Specifically, the C terminus of α-catenin that is essential for F-actin binding with the E cadherin–β-catenin complex at adherens junctions by binding the N-terminal α-helix of β-catenin.
binding was too close to β-catenin in subunit A (about 25 Å, Fig. 3a) to accommodate F-actin and indeed was in direct contact, through at least one electrostatic interaction, with β-catenin in subunit B (Fig. 3b). Indeed, a portion of the 864–906 F-actin–binding site of α-catenin (residues 865–869) was positioned to facilitate interactions with β-catenin in subunit B (Fig. 3b). Thus, our structure explains how α-catenin can bind either F-actin or β-catenin but not both at the same time.

F-actin binding

The F-actin–binding site in the closely related tail domain of vinculin was masked in its closed-clamp, inactive conformer but was released and was fully accessible to F-actin following severing of the vinculin head-tail interactions43. Superposition of the F-actin–binding domain of vinculin onto our α-catenin structure revealed that different surfaces were buried and exposed in these two cytoskeletal proteins (Fig. 4). For example, the N terminus of α-helix H4 and the C terminus of α-helix H5 were buried in inactive vinculin through interactions with its Vt2 domain, whereas these regions were largely solvent exposed in the F-actin–binding domain of subunit A of α-catenin. Further, the N terminus of α-helix H3 and the C terminus of α-helix H4 were buried in inactive vinculin by interactions with its head domain yet were solvent exposed in subunit B of α-catenin (Fig. 4). Thus, the α-catenin structure also explains how full-length α-catenin can bind F-actin whereas vinculin cannot.

The α-catenin–vinculin interactions

Vinculin was also necessary for stabilizing adherens junctions19, and force-activated α-catenin had been suggested to bind and recruit vinculin to adherens junctions18–20. However, our studies have established that only preactivated vinculin was capable of binding α-catenin, as the Vh1 domain that binds both α-catenin and the vinculin tail domain to hold vinculin in its closed clamp conformation had a higher affinity for the vinculin tail domain than for the VBD of α-catenin20. The structure of the VBD four-helix bundle within nearly full-length α-catenin presented herein, and that of VBD in complex with the vinculin Vh1 domain20, confirmed that, as proposed19,20, the VBD unfurled when bound to activated vinculin. On a sizing column, the vinculin head (residues 1–840) in complex with α-catenin eluted well before dimeric α-catenin, which indicated that the α-catenin–vinculin formed a 2:2 complex (Fig. 5a). Notably, as shown by native gel-shift assays and immunoblotting, the asymmetric nature of the α-catenin dimer was also manifest in its interaction with vinculin, wherein the α-catenin dimer first bound one vinculin molecule before then forming the 2:2 complex (Fig. 5b). Thus, activated vinculin unfurls dimeric α-catenin, and this 2:2 heterotetrameric complex is fully competent to bind F-actin20.
studies with purified recombinant proteins, as well as measurements of protein dynamics in cells, have clearly established that α-catenin cannot simultaneously bind β-catenin and F-actin and that the oligomeric state of α-catenin dictates which partner it binds\textsuperscript{13}. These findings were difficult to reconcile with earlier work\textsuperscript{28,48–50}, but a plausible explanation was provided by the fact that E-cadherin–α-catenin fusions were used in earlier studies\textsuperscript{16}. Our structural data now provide mechanistic evidence that explains why α-catenin binding to F-actin and β-catenin is indeed mutually exclusive. Specifically, the structure shows that binding of β-catenin disrupts the intermolecular interactions of the four-helix bundle of the N terminus of one subunit of α-catenin with a region of the C terminus of the other subunit that holds the asymmetric dimer together—interactions that are necessary for binding F-actin.

The mechanism by which α-catenin binds F-actin has been a conundrum for the field, as extensive maturation of the C-terminal F-actin–binding domain has failed to define the F-actin–binding motif\textsuperscript{42}. Although in cells there are likely contributions from other partners, such as vinculin, that also bind F-actin, the fact remains that recombinant dimeric α-catenin alone binds avidly to F-actin. Notably, asymmetry also explains the F-actin binding functions of the α-catenin dimer and why monomeric α-catenin binds F-actin rather poorly\textsuperscript{15}. Specifically, our structure reveals that the F-actin–binding surface is probably created by intermolecular interactions of the tail of α-catenin with a four-helix bundle of its N terminus, which is lost in monomeric or β-catenin–bound α-catenin. This finding also reconciles reports of F-actin binding by both the N terminus and C terminus of α-catenin\textsuperscript{44}. Only the structure of the α-catenin dimer in complex with F-actin will allow for the full definition of the mechanism of F-actin binding.

Recombinant full-length vinculin cannot link preexisting cadherin–catenin complexes and actin filaments as determined by actin pelletting assays in the presence of all four proteins\textsuperscript{13,15}. However, this is the expected outcome because vinculin is in its closed conformation, which cannot bind either F-actin or α-catenin. However, at adherens junctions, vinculin is in its activated, open conformation\textsuperscript{51}, a scenario that would allow it to bind α-catenin at adherens junctions and to facilitate interactions of α-catenin with the actin network. The fact that the vinculin tail domain readily displaced α-catenin from preexisting complexes comprising α-catenin and the vinculin head domain (that is, vinculin lacking its F-actin–binding domain)\textsuperscript{13,20,24} establishes that vinculin must be preactivated at adherens junctions to interact with dimeric α-catenin and to stabilize adherens junctions (Supplementary Fig. 4). Finally, activated vinculin also appears to directly bind cadherin receptors in cells\textsuperscript{22} and, because the α-catenin dimer is competent to bind activated vinculin, vinculin may serve as a scaffold that tethers both α-catenin and cadherin receptors as well as F-actin.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Accession codes.** α-Catenin coordinates have been deposited in the Protein Data Bank, with accession code 4IGG.

*Note: Supplementary information is available in the online version of the paper.*

**ACKNOWLEDGMENTS**

We are indebted to our colleagues at Scripps Florida; J. Cleveland for discussions and critical review of the manuscript; Z. Wu and P. Bois for sequencing and P. Bois for fruitful discussions. We thank C. Vonrhein and G. Bricegon (Global Phasing Ltd.) for analyses and helpful discussions. We are grateful to the stafl at the SER-CAT (BM22) and SSRL (11-1) for synchrotron support. T.I. is supported by grants from the US National Institute of General Medical Sciences from the US National Institutes of Health (GM071996 and GM094483) and by start-up funds provided to Scripps Florida from the State of Florida. This publication is no. 21863 from The Scripps Research Institute.

**AUTHOR CONTRIBUTIONS**

Both authors contributed to the design and interpretation of all aspects of this work. E.S.R. performed all of the experiments. T.I. wrote the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

Published online at http://www.nature.com/doifinder/10.1038/nsmb.2479. Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

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ONLINE METHODS

Crystalization. Human α-catenin (residues 82–906) was expressed in E. coli and purified as described and dialyzed into 20 mM Tris-HCl, pH 8, 150 mM NaCl and 5 mM DTT and concentrated to 25 mg/ml. Initial trigonal crystals were obtained, from either 0.9 M (NH₄)₂SO₄, 0.25 M NaCl and 0.1 M Tris-HCl, pH 7, or 0.9 M Na/K phosphate, pH 6.8 and 0.3 M sodium formate, that diffracted X-rays at various synchrotron beamlines (11-1 at Stanford Synchrotron Radiation Laboratory, SSRL, or 22ID and 22BM at the Advanced Photon Source at Argonne National Laboratory, APS/ANL) to about 6 Å Bragg spacings. Conventional strategies failed to improve the diffraction, but ultimately systematic dehydra-
tion of human α-catenin crystals grown from 0.9 M Na/K phosphate and 0.2 M sodium formate in 2–3.5 M Na/K phosphate, pH 6.8, in the presence of glycero-
ly polyethylene glycol 3350 resulted in diffraction beyond 4 Å. Dehydration was successful only for crystals that were harvested within 1 week and were 0.15 to 0.3 mm in size, as dehydration did not improve diffraction of larger or smaller crystals. The best diffraction of up to 3.2-Å Bragg spacings was obtained from crystals that were dehydrated with 3 M Na/K phosphate and 5% polyethylene glycol 3350 for 1 week. However, significant anisotropy and sensitivity to X-rays limited data collection beyond 3.7-Å Bragg spacings.

X-ray data collection and processing. Native and phosphotungstate-derivate X-ray diffraction data were collected on beamlines 22BM at APS/ANL and 11-1 at SSRL, respectively, and integrated and scaled by using autoProc, which uses XDS and SCALAPACK as the data-reduction engine. Data-reduction statistics are provided in Table 1.

Structure determination and crystallographic refinement. Molecular replace-
ment was unsuccessful when using crystal structures of the dimerization domain (residues 82–262; PDB ID 1DOV or the M fragment (residues 377–631; PDB ID H6GC) or homology models for the VBD or F-actin–binding domain as search models. Selenomethionine-labeled α-catenin crystals did not grow beyond 0.05 mm, and their diffraction was limited to 8-Å Bragg spacings. Derivatization was also limited due to the high concentration of phosphate in the crystallization reservoir, which caused standard heavy atoms, such as Pt, Hg and Au, to precipitate. This precipitation was overcome to some degree by short (10-min) soaking times with high concentrations (10 mM) of heavy metals such as K₂PtCl₄. However, this significantly affected the diffraction and anomalous signal detection. Sodium phosphotungstate was eventually identified as a suitable heavy atom, owing to its solubility in phosphate conditions.

Crystals were incubated for 24 h in a low-phosphate conditions (0.2 M Na/K phosphate, 2.5 mM sodium formate and 0.7 M sodium malonate, pH 7) to avoid competition of phosphate from the reservoir. Effective heavy-atom incorporation was accomplished by short soaking times (15 min) in a 10 mM phosphotungstate solution containing 0.2 M Na/K phosphate, 2.5 mM sodium formate and 0.7 M sodium malonate (pH 7), then back soaked for 10 min in 0.2 M Na/K phosphate, 2.5 mM sodium formate and 0.7 M sodium malonate, pH 7, and mounted without including any additional cryoprotectant. X-ray diffraction data were obtained at SSRL beamline 11-1 near the L-II absorption edge of tungsten (1.07 Å) to 5.6-Å Bragg spacings.

Determination of the heavy-atom substructure was performed by using autoSHARP. Two tungsten cluster sites with peak heights of 1 and 0.44 and a correlation of 0.207 were located, from which phases (with a figure of merit of 0.15) were obtained to 5.6 Å (Supplementary Table 1). The resulting electron den-
sity map provided clear definition of the various α-catenin domains but did not allow chain tracing. SIRAS using autoSHARP allowed phase extension to 4.3-Å resolution and manual building of α-helices and placement of the high-resolution dimerization domain and M-fragment structures into the experimental 4.3-Å SIRAS electron density map. The resulting model was used as a search model for molecular replacement with MOLREP to position the dimer and further refine to 3.7 Å by using the native X-ray diffraction data. Iterative cycles of model building were performed by using Coot, and maximum-likelihood crystallographic refinement by using autoBUSTER, by imposing target restraints using the high-resolution structures. The model was improved by local noncrystallo-
graphic symmetry through MORPH. Model bias was minimized by model building into composite omit maps. Map sharpening was performed in Coot to ensure the directionality and identity of the α-helices. The quality of the final model assessment using MolProbity resulted in no outliers and over 95% of the amino acid residues in the favored region of the Ramachandran plot. Refinement statistics are provided in Table 1.

Size-exclusion chromatography. α-catenin (residues 82–906), VH (residues 1–840) and α-catenin plus a 2.5 molar excess of VH were loaded onto a Superdex 200 10/300 GL (GE Healthcare) analytical-chromatography column equilibrated in 20 mM Tris-HCl, pH 8, 150 mM NaCl and 5 mM DTT. Fractions were analyzed on a 10–15% gradient PHAST gel with native buffer strips.

Native gel-shift assays and immunoblotting. Samples (in 20 mM Tris-HCl, pH 8, 150 mM NaCl, 5 mM DTT) were incubated for 1 h at 4 °C. Increasing concentrations of VH (0 µM, 2.5 µM, 5 µM, 10 µM, 20 µM, 30 µM and 50 µM) were titrated to purified histidine-tagged α-catenin (~10 µM), and the resultant complex was analyzed by running a 10–15% gradient PHAST gel with native buffer strips. The bands were visualized by Coomassie-blue staining. α-catenin was detected with an anti-histidine antibody.

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