Supporting Information

Phosphorylation Reduces the Mechanical Stability of the α-Catenin/β-Catenin Complex
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Author Contributions

S.L., M.Y., and J.Y. conceived and designed the study; S.L. and M.Y. performed the experiments; S.L., M.Y., and J.Y. interpreted the data and wrote the paper.
Supporting Text S1: Details of single-molecule manipulation, plasmids constructs and protein expression

**Single-protein manipulation.** A vertical magnetic tweezers setup was combined with a disturbance-free, rapid solution-exchange flow channel for conducting in vitro protein stretching experiments \[1\]. Experiments were performed in standard buffer solution containing: 1X PBS, 1% BSA, 2 mM DTT, 10 mM sodium L-ascorbate at 23±1 °C, or in standard kinase buffer solution containing 20 mM HEPES pH 7.5, 100 mM NaCl, 10 mM MgCl2, 3 mM MnCl2, 1.5 mM Na3VO4, 1% BSA, 2 mM DTT, 10 mM sodium L-ascorbate with or without 10 nM FAK kinase and 1 mM ATP, at 23±1 °C. The bead-height determination has a ~3±1 nm standard deviation due to the thermal fluctuations of the molecule and tethered bead (Figure S4). The force calibration of the magnetic tweezers setup has a 10% uncertainty due to the heterogeneity of the diameter of paramagnetic beads. Details of theoretical force-dependent step size estimation and its conversion to contour length and the number of residues; kinetics parameters analysis based on Bell’s model (Figure S3); and the bead-height determination analysis can be found in supporting Text S2-S6.

**Plasmids constructs and protein expression.** The sequence of αN1N2 used in this study is the 55th-263rd of the α-catenin (CTNA1_MOUSE, uniprot ID: P26231); the sequence of βNt used in this study is the 119th-148th of β-catenin (CTNB1_MOUSE, Q02248); The FH1 sequence used in this study is the 583rd-764th of formin (DIAP1_HUMAN, O60610). The DNA fragments encodes these sequences were synthesized by IDT Gblock or GeneArt services. To prepare the plasmids of the wild-type (αN12/βNt) construct, the three DNA fragments (αN1N2, FH1 and βNt) were then ligated into a pET151 vector plasmid \[2\] that contains avi-tag, four repeats of titin I27 domain, and spy-tag by HiFi DNA Assembly (NEB). For the Y142E and the Y142E&T120E mutations of the construct, point mutations of the plasmids were performed by sub-cloning. All the three plasmids (αN12/βNt, (αN12/βNt)Y142E, and αN12/βNt)Y142E,T120E) were sequencing-confirmed by 1st Base sequencing services. Each plasmid was co-transformed with a BirA plasmid and expressed in Escherichia coli BL21 (DE3) cultured in LB-media with D-Biotin (Sigma Aldrich), and affinity purified through 6His-tag. The active FAK was purchased from Sigma.
The detailed sequences of the constructs are listed below. Here we note, between each main component, there is a short linker (such as GGGSG) to increase the flexibility of the domains/motifs.

1. Name: $\alpha N12/\beta Nt$:
   
   **Long Name: His-$$^{\text{avi}}$$I27-I27-$\alpha N12$-FH1-$\beta Nt$-I27-I27-spy:**
   
   MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGLNDIEAQKIEWEHGGGSLIEVEKPLYGVEVFVGETAHFEIELSEPDVHGQWKLKGQPLAASPDAAEIIEGDGKKHILHNAQLGMGTGEVSFAQANTKSAANLKVKELGGGSGLIEVEKPLYGVEVFVGETAHFEIELSEPDVHGQWKLKGQPLAASPDAAEIIEGDGKKHILHNAQLGMGTGEVSFAQANTKSAANLKVKELGGGSGKLSSKAHVLAASVEQATENFLERGDKIAKESQFLKEELVVAEDVVRKQGDLMLKSAAGEFADDPCSSVKRGNMVRAARRALLSAVEILILADMADVYKLVLQKEGILKLRNTGEQDLGIYQLYKALKPEVKLNIIMAAKRQQKELKIDVGNRDQMAAARGILQKNVPILYTASQACLQHPDVAAYKANRDLIYKQLQQAVTGISNAAQATAEFPAPPLPGDSGITIPPPPAIPDSTTPPPPPPPPPPPPLPGVCISPPSLPGGTVASIPPPPLSSGDAITIPPPPLPSPEGVIGISPSSSLPGGTAPIPPPLPGSARIPPPPLPGSAGIPPPPPPLLPGEAGMPPPPPPLPGLPGIPPPPPPGPGLPPPPSIPGMPPPFPFGVPAPVLPGSSGPNTVQR"LAEP"SMQLKHAVVNLIN"QDDAEL"GGGLEGSSSLIEVEKPLYGVEVFVGETAHFEIELSEPDVHGQWKLKGQPLAASPDAAEIIEGDGKKHILHNAQLGMGTGEVSFAQANTKSAANLKVKELGGGSGAHIVMVDAYKPTK

2. Name: $\alpha N12/\beta Nt^{Y142E}$:
   
   **Long Name: His-$$^{\text{avi}}$$I27-I27-$\alpha N12$-FH1-$\beta Nt^{Y142E}$-I27-I27-spy:**
   
   The $\beta Nt$: PTVQRLAEPSQMLKHAVVNLINQDDAEL in the first construct is replaced to be $\beta Nt^{Y142E}$: PTVQRLAEPSQMLKHAVVNLINQDDAEL. The rest of the sequence remains unchanged.

3. Name: $\alpha N12/\beta Nt^{Y142E,T120E}$:
   
   **Long Name: His-$$^{\text{avi}}$$I27-I27-$\alpha N12$-FH1-$\beta Nt^{Y142E,T120E}$-I27-I27-spy:**
   
   The $\beta Nt$: PTVQRLAEPSQMLKHAVVNLINQDDAEL in the first construct is replaced to be $\beta Nt^{Y142E,T120E}$: PENVQRLAEPSQMLKHAVVNLINQDDAEL. The rest of the sequence remains unchanged.

In addition, a 572-bp DNA fragment acting as a handle is prepared by PCR using Q5 polymerase and lambda-DNA template. One end of the DNA is labeled with thiol-group and another end of the DNA is labeled with Biotin by labeled PCR primers. The thiol-end of the DNA is specifically tethered to the Epoxy-M270 super-paramagnetic bead based on the product manual. The biotin-end of the DNA is linked to the biotin-end of the target protein via streptavidin.

Supporting Text S2: Theoretical force-dependent step-size of unfolding/rupturing transitions
A folded domain or complex can be considered as a rigid body. Hence, the force–extension curve of a folded domain or complex is determined by the rigid rotation fluctuation of a rigid-body with a characteristic length $b \sim 4.5$ nm, estimated from the PDB (ID:1DOW $^{[6]}$) file of the $\alpha$N1N2/$\beta$Nt complex, which is the distance between the two force-attaching points (i.e., the N- to C- terminal distance in the experiment). This force–extension curve can be described by the freely-jointed chain polymer model with a single segment: $x^{FJC}(f) = b(\coth(\frac{f_b}{k_BT}) - \frac{k_BT}{f_b})$, where $k_BT = 4.1$ pN·nm is the product of the Boltzmann constant and the temperature.

The unfolded state of a domain or the released unstructured FH1 linker can be considered as a flexible peptide chain, and its force–extension curve can be described by the worm-like chain (WLC) polymer model through the inverted Marko–Siggia formula $^{[4]}$ with a bending persistence length of $A \sim 0.8$ nm $^{[5]}$: $\frac{fA}{k_BT} = \frac{1}{4(1 - \frac{x^{WLC}(f)}{L})^2} - \frac{1}{4} + \frac{x^{WLC}(f)}{L}$, where $L = n \times L_0$ is the contour length of the unfolded state, $n$ is the number of residues of the domain and/or the released unstructured FH1 linker, $L_0 = 0.38$ nm is the contour length of per residue.

Hence the force-dependent unfolding/rupturing step size is the extension differences of the domain before and after unfolding at the transition (unfolding/refolding) force, i.e., $\Delta x(f) = x^{WLC}(f) - x^{FJC}(f)$. If the number of residues involved in a transition is known, then the $\Delta x(f)$ can be theoretically estimated based on these polymer models.

For the $\alpha$N12/$\beta$Nt construct used in this study, at low forces when the $\alpha$N12/$\beta$Nt complex is formed, the force response of the construct can be described by $x^{FJC}(f)$ During the force-increase scan, there are several possible force-induced transitions: 1) the $\alpha$N12/$\beta$Nt interface disengaged and the $\alpha$N12 domains unfolded. In this scenario, the total released number of residue in the unstructured peptide chain state is $n = 427$ a.a., the sum of $\alpha$N12, $\beta$Nt and the long linker 188 a.a. (as detailed in the Supporting Text S1). The force-extension curve can be described by $x^{WLC}(f)$ with $n = 427$. 2) the $\alpha$N12/$\beta$Nt interface disengaged and one of the $\alpha$N12 domains concurrently unfolded, while another domain remains folded. To simplify the denotation in Figure S1, we assume the concurrently unfolded domain is the $\alpha$N1 domain since it is the interacting region with $\beta$Nt. In this scenario, the total released number of residue in the unstructured peptide chain state is $n = 313$ a.a., the sum of $\alpha$N1, $\beta$Nt and the long linker. The $\alpha$N2 remains as a folded rigid body. The force-extension curve can be described by the summation of $x^{WLC}(f)$ with $n = 313$ a.a., and $x^{FJC}(f)$~4 nm of the $\alpha$N2. 3) the $\alpha$N12/$\beta$Nt interface disengaged, while the $\alpha$N12 remain folded. In this scenario, the total released number of residue in the unstructured peptide chain state is $n = 218$ a.a. (the sum of $\beta$Nt and the long linker). The $\alpha$N12 remains as a folded rigid body. The force-extension curve can be described by the summation of $x^{WLC}(f)$ with $n = 218$ a.a., and $x^{FJC}(f)$~5 nm of the $\alpha$N12. 4-5) if the $\alpha$N1 and/or $\alpha$N2 was folded during interface disengagement transition, there will be additional unfolding step or steps. The unfolded force-extension curves can be described by $x^{WLC}(f)$ with $n = 95$ for unfolding $\alpha$N1 or $n = 114$ for unfolding $\alpha$N2.

For the above five possible transitions, we theoretically estimated the force-dependent step sizes based on the state of construct before and after the transition, and plotted them as supporting Figure S1. The denotation is as following: $\alpha$N12-L-$\beta$Nt for scenario 1; $\alpha$N1-L-$\beta$Nt for scenario 2; L(FH1&$\beta$Nt) for scenario 3; $\alpha$N1 or $\alpha$N2 for scenario 4 or 5, respectively.
By comparing the experimental measured force-dependent step sizes with the theoretically estimations (the main Figure 2&3 in the main text), we can tell that the first transition step is mainly the scenario 2 that the αN12/βNt interface disengaged and one of the αN12 domains concurrently unfolded, while the other domain remain folded, and the second transition step is mainly the scenario 5 that unfolding of one of the αN12 domains (assumed to be αN2). To simplify the main figures, we only plotted the theoretically estimated curves of αN12-L-βNt, αN1-L-βNt and αN2.

Supporting Text S3: Theoretical conversion from force-dependent step-size into contour length and number of residues of the transition

Based on the above equations in Supporting Text S2, the contour length $L$ and the number of residues $n$ involved in the transition can be obtained from the measured step-sizes of the transition at given force. In supplementary Figure S2, the experimentally measured force-dependent step sizes $\Delta x(f)$ for each construct was first converted to be the contour length $L$ associated with the transition by solving the $\Delta x(f) = x^{WLC}(f) - x^{FJC}(f)$. The histograms of the contour length distribution estimated by the theoretical conversion are plotted in the figure. Then, the number of released residues associated with the transition was obtained by $n = L/L_0$, and the histograms of the number of residues distribution estimated by the theoretical conversion are plotted in the Figure S2. Here we note that the theoretical estimations of the contour length and number of residues are affected by the accuracy the determination of the step size $\Delta x$ and the transition forces $f$, which will be detailed in Supporting Text S6.

Supporting Text S4: Kinetics parameters analysis based on Bell’s model

The rupturing (un-pairing) force distributions of the αN12/βNt complex obtained at given loading rates carry the information of the kinetics of the transition. The force-dependent rupturing kinetics predicts a rupturing force distribution, $\rho_r(f)$, at a given loading $r$ as:

$$
\rho_r(f) = \frac{k(f)}{r} e^{-\frac{f}{f_0} k(f) \frac{df}{r}},
$$

where the $f_0$ is the initial force, $f$ is the force that transitions occurs. $k(f)$ is the force-dependent rupturing rate, it can be described by Bell model as $k_u^{Bell}(f) = k_0^{Bell} \exp(\beta f \Delta)$, where $\beta = \frac{1}{k_B T}$, and $\Delta$ is the transition distance, which is assumed to be a constant, and $k_0^{Bell}$ is the extrapolated zero-force un-pairing rate. Based on above equation, the kinetics parameters of rupturing can be estimated to be transition distances to be $4.1 \pm 0.8$ nm and $2.6 \pm 0.5$ nm for the αN12/βNt and αN12/βNt $\gamma^{142E}$, respectively.

The transition distance $\Delta$ and extrapolated zero-force un-pairing rate $k_0^{Bell}$ can also be more directly estimated by fitting the force-dependent lifetime with the Bell’s model. The fitting gives the estimated transition distances to be $3.5 \pm 1.5$ nm and $3.1 \pm 1.2$ nm for the two constructs respectively. These fittings overall give a transition distance estimation in 2-4 nm. On the other hand, the extrapolated zero-force rates estimated by the rupture force-histogram fitting or by force-dependent lifetime fitting differs with ~5 folds for both constructs ($k_0^{Bell}$ estimated by rup-
ture force histogram fitting: 1.8±3.1 \times 10^{-4} \text{ s}^{-1} and 4±3 \times 10^{-2} \text{ s}^{-1} for \alpha N12/\beta Nt and \alpha N12/\beta Nt^{Y142E}, respectively; \kappa^0_{\text{Bell}} estimated by force-dependent lifetime: 3±4 \times 10^{-5} \text{ s}^{-1} and 1±0.4 \times 10^{-2} \text{ s}^{-1} for \alpha N12/\beta Nt and \alpha N12/\beta Nt^{Y142E}, respectively, supporting Figure S3). This difference can be explained by the different susceptibility of the fitting to the two types of data. In the fitting to the transition force histogram, the fitting is predominated by the central regions of the data point. While the fitting to the force-dependent lifetime are dependent on data obtained over the entire tested force range. Since the Bell’s predicts force-dependent lifetime, the fitting to force-dependent lifetime data should be a more direct way to estimate the kinetic parameters in Bell’s model.

**Supporting Text S5: Bootstrap analysis on the rupture forces.**

To obtain the mean, standard error of the kinetic parameters of rupturing transitions, we employed the bootstrap analysis to the experimental data of the rupturing forces. Briefly, in single-molecule stretching experiments for each loading rates, an original data pool with N (>100) number of data points of the rupturing forces is obtained experimentally. We then randomly pick one data point from the original data pool, and repeat this procedure for N times to establish a new data set with a number of N data points included. By repeating such procedure for M=50 times, we obtained M sets of data pool. For each pool, there are a number of N data points. Then, for each set of data, we performed the analysis (described in Text S4), and then obtained the errors from analysis on these M set of data. Here we note, for each construct, the number N of original data pool differs and is indicated in the corresponding figure caption.

**Supporting Text S6: The step-size and force determinations in magnetic tweezers experiments.**

Our measurements of the force-dependent step size of the transitions were done based on measurement of the bead height from surface during linear force-increase scans. Over the force range of ~2-14 pN that the transitions were observed, the molecule undergoes significant extension fluctuation and the bead undergoes significant rotation fluctuation around the tethering point. Together, they result in bead height fluctuations at these low forces. The camera of our instrument has a sampling frequency of 100-200 Hz. The transition step size was determined by 10-point average before and after the transition. In the example shown in Supporting Figure S4, the standard deviations of the raw data before and after transition at ~10 pN are ~3±1 nm, respectively. The 10-point average results in a standard error in determining the step size of ~± 4 nm in the force range of ~2-14 pN, which can lead to uncertainty in the estimated number of residues in ~± 20 a.a. assuming WLC polymer model of the unfolded peptide with a bending persistence length of ~0.8 nm. In addition, due to the heterogeneity of the super-paramagnetic bead, the force-calibration of the magnetic tweezers setup has an intrinsic relative uncertainty of ~10%.
Supporting Figure S1. Theoretical estimations of the force-dependent step-size of unfolding/rupturing transitions in αN12/βNt construct. The denotation is as following: αN12-L-βNt for scenario 1: the αN12/βNt interface disengaged and the αN12 domains unfolded.; αN1-L-βNt for scenario 2: the αN12/βNt interface disengaged and one of the αN12 domains concurrently unfolded, while the another domain remain folded. ; L(FH1&βNt) for scenario 3: the αN12/βNt interface disengaged, while the αN12 remain folded. ; αN1 and/or αN2 for scenario 4 or 5: if the αN1 or αN2 was folded during interface disengagement transition, there will be additional unfolding step or steps.
Supporting Figure S2. Theoretical estimations of contour length and number of residues associated with the complex-rupture and domain-unfold transitions. The Residues that involved in the rupturing or unfolding steps converted from the force-dependent step sizes (details described in Supplementary Text S2). The red data is the normalized histogram of estimated residue number of the rupturing of the $\alpha$N12/$\beta$Nt complex with concurrent unfolding of one of the $\alpha$-catenin N domains. The orange data is the normalized histogram of estimated residue number of the second unfolding of the remaining $\alpha$-catenin N domain. Here we note that the whole complex involves 425 a.a., including 209 a.a. of the N1 and N2 of the $\alpha$-catenin ($\alpha$N12), 182 a.a. of the FH1 linker, 30 a.a. of the N-tail of $\beta$-catenin ($\beta$Nt) and additional short linkers (GGGSG) between domains.
Supporting Figure S3. Bell’s model fitting on the force-dependent rupture rates of the complex. The colored solid circles are the average value of the experimentally measured force-dependent rupture rates. The colored dash lines are representative curves of the Bell’s model fitting. To obtain the standard deviations of parameters, for each data set, we repeated the fitting with one data point skipped each time. The average values and the standard deviations of the parameters obtained from multiple such fitting procedures are indicated in the panel.
Supporting Figure S4. Standard deviations of the force bead-height determination. Top panel: the typical force-bead height curves of the αN12/βNt construct during linear force-increase scans (1pN s⁻¹ in the example). Middle-top panel: the standard deviations of the bead height determination obtained by 10-point window sliding of the raw data. The green-dashed box indicates the data points where transitions were detected; Middle-bottom panel: zoom-in of the force-dependent standard deviations of the bead height. Bottom panel: The corresponding standard deviations of the number of the residues.

Supporting References:

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