1 **TITLE: INSIDE-OUT REGULATION OF E-CADHERIN CONFORMATION AND**
 2 **ADHESION**

3

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1 **ABSTRACT**

2 Cadherin cell-cell adhesion proteins play key roles in tissue morphogenesis and
3 wound healing. Cadherin ectodomains bind in two conformations, X-dimers and
4 strand-swap dimers, with different adhesive properties. However, the mechanisms
5 by which cells regulate ectodomain conformation are unknown. Cadherin
6 intracellular regions associate with several actin-binding proteins including
7 vinculin, which are believed to tune cell-cell adhesion by remodeling the actin
8 cytoskeleton. Here, we show at the single molecule level, that vinculin association
9 with the cadherin cytoplasmic region allosterically converts weak X-dimers into
10 strong strand-swap dimers, and that this process is mediated by myosin II
11 dependent changes in cytoskeletal tension. We also show that in epithelial cells,
12 75% of apical cadherins exist as strand-swap dimers while the remaining form X-
13 dimers, providing two cadherin pools with different adhesive properties. Our results
14 demonstrate, for the first time, the inside-out regulation of cadherin conformation
15 and establish a mechanistic role for vinculin in this process.

1 INTRODUCTION

2 E-cadherins (Ecads) are essential, calcium dependent cell-cell adhesion
3 proteins that play key roles in the formation of epithelial tissue and in the
4 maintenance of tissue integrity. Ecad adhesion is highly plastic and carefully
5 regulated to orchestrate complex movement of epithelial cells and dysregulation
6 of adhesion is a hallmark of numerous cancers ¹. However, little is known about
7 how cells dynamically regulate the biophysical properties of individual Ecads.

8 The extracellular region of Ecads from opposing cells bind in two distinct
9 *trans* orientations: strand-swap dimers and X-dimers (Figure 1A-B). Strand-swap
10 dimers are the stronger cadherin adhesive conformation and are formed by the
11 exchange of conserved Tryptophan (Trp) residues between the outermost
12 domains of opposing Ecads ²⁻⁴. In contrast, X-dimers, which are formed by
13 extensive surface interactions between opposing Ecads, are a weaker adhesive
14 structure and serve as an intermediate during the formation and rupture of strand
15 swap dimers ⁵⁻⁷. Using cell-free, single molecule experiments, we previously
16 showed that X-dimers and strand-swap dimers can be distinguished based on their
17 distinctly different response to mechanical force. When a strand-swap dimer is
18 pulled, its lifetime decreases with increasing force, resulting in the formation of a
19 slip bond ^{8,9} (Figure 1B). In contrast, an X-dimer responds to pulling force by
20 forming a catch bond, where bond lifetime initially increases up to a threshold
21 force, and then subsequently decreases ^{8,10} (Figure 1B). It has also been shown
22 that wild type Ecad ectodomains in solution can interconvert between X-dimer and

1 strand-swap dimer conformations ^{9,11}. However, the biophysical mechanisms by
2 which Ecad conformations (and adhesion) are regulated on the cell surface are
3 unknown.

4 The cytoplasmic region of Ecad associates with the catenin family of
5 proteins: namely p120-catenin, β -catenin and α -catenin. The Ecad-catenin
6 complex, in turn, links to filamentous actin (F-actin) either by the direct binding of
7 α -catenin and F-actin or by the indirect association of α -catenin and F-actin via
8 vinculin ¹² (Figure 1A). Adhesive forces transmitted across intercellular junctions
9 by Ecad induce conformational changes in α -catenin ^{13,14}, strengthen F-actin
10 binding ¹⁵ and recruit vinculin to the sites of force application ^{16,17}. However,
11 vinculin and α -catenin do not merely serve as passive cytoskeletal linkers; they
12 also dynamically modulate cytoskeletal rearrangement and recruit myosin to cell-
13 cell junctions ^{13,18-20}. Studies show that α -catenin and vinculin play important roles
14 in strengthening and stabilizing Ecad adhesion: bead twisting experiments show
15 force-induced stiffening of Ecad-based junctions and cell doublet stretching
16 experiments demonstrate reinforcement of cell-cell adhesion in vinculin and α -
17 catenin dependent manners ^{18,19,21}.

18 Currently, actin anchorage and cytoskeletal remodeling are assumed to be
19 the exclusive mechanisms by which α -catenin and vinculin strengthen Ecad
20 adhesion ²²⁻²⁴. Here, we directly map the allosteric effects of cytoplasmic proteins
21 on Ecad ectodomain conformation and demonstrate, at the single molecule level,
22 that vinculin association with the Ecad cytoplasmic region switches X-dimers to

1 strand-swap dimers. We show that cytoskeletal tension, due to vinculin mediated
2 recruitment of myosin II, regulates Ecad ectodomain structure and adhesion.
3 Finally, we demonstrate that only ~50% of Ecads are linked to the underlying
4 cytoskeleton and that while 75% of Ecads form strand-swap dimers, the remaining
5 form X-dimers, which provides cells with two Ecad pools with different adhesive
6 properties.

7

8 RESULTS

9 **Single molecule Atomic Force Microscope (AFM) measurements of specific**
10 **Ecad *trans* adhesion.** We measured interactions between recombinant Ecad
11 extracellular regions immobilized on AFM cantilevers and Ecad endogenously
12 expressed on the apical surface of confluent Madin-Darby Canine Kidney (MDCK)
13 cell monolayers (Figure 2A). To ensure that we measured single Ecad unbinding
14 events, we immobilized Ecad on the AFM tip at low density and pressed on the
15 cell surface with a low initial contact force (mean pressing force = 57 pN; Figure
16 S1). Poisson statistics predict that even the maximum event rate we measure
17 (19%; Figure 2B) corresponds to ~90% probability of measuring single molecule
18 events.

19 Our experiments were performed with the following MDCK cells (Figure 1C):
20 parental cells (*parental*), vinculin knockout cells (*vinc-KO*), α -catenin knockout
21 cells (*acat-KO*)²⁵, acat-KO cells rescued with α -catenin lacking the vinculin binding

site (ΔVBS)²⁶, and Ecad knockout cells (*Ecad-KO*). We also performed measurements with parental cells in the presence of free tryptophan (*parental-Trp*) in order to trap Ecad in an X-dimer conformation⁸. Additionally, we performed experiments in the presence of blebbistatin (*parental-Bleb*) in order to reduce myosin II dependent cytoskeletal contractility. We quantified the specific and nonspecific binding rates for every cell line by measuring single unbinding events using either AFM tips that were decorated with Ecad (specific binding) or AFM tips that lacked Ecad (nonspecific binding). All cell lines, except *Ecad-KO*, showed a significant increase in single unbinding events when the AFM tips were decorated with Ecad (Figure 2B). In contrast, in the *Ecad-KO* cells, event rates using AFM tips with/without Ecad were similar to each other and were comparable to the nonspecific event rates measured with the other cell lines (Figure 2B). Taken together, this confirmed that the measured specific events corresponded to Ecad-Ecad binding interactions.

Ecad conformation and cytoskeletal-linkage in each cell line were determined from previously reported ‘signatures’ in the measured force curves. Since force measurements have shown that pulling on a cell surface protein that is not linked to the cytoskeleton results in the formation of a *membrane-tether*^{27,28} (Figure 3A), we used membrane-tethers to identify Ecad that are uncoupled from the actin cytoskeleton (Figure 3B). Previous studies have also shown that pulling on a transmembrane protein that is firmly bound to the underlying cytoskeleton results in a linearly elastic response to pulling force^{29,30} (Figure 3C). Since these *jump* events occur when interactions between proteins on the AFM tip and cell

surface are weaker than the protein-cytoskeleton linkage, we interpreted jumps as a signature of actin-linked Ecads that form weak *trans* dimers, which rupture before failure of the Ecad-cytoskeletal linkage (Figure 3D). Finally, if the Ecad *trans* dimers adhere robustly compared to the Ecad-cytoskeletal linkage, an initial steep increase in force is measured which relaxes viscoelastically when the Ecad-cytoskeletal bond ruptures, resulting in the formation of a *cyto-tether* (Figure 3E-F). To further validate tether formation, we fit the membrane-tether and cyto-tether force-extension curves to the standard linear solid (SLS) model (SI Note 1) ³¹. The SLS fitting parameters we obtained for the tethers, were consistent with previous values obtained for tether formation on Jurkat cells and T-cells (Table S1) ^{31,32}.

About 50% of non-junctional Ecads are coupled to the actin cytoskeleton.

We first compared the fraction of Ecad uncoupled from the cytoskeleton (membrane-tethers) to the fraction of Ecads that are coupled to cortical actin (cyto-tethers and jumps). Our data showed that in parental cells, 53% $\pm$ 9% of Ecad on the apical surface of the cell are uncoupled from the actin cytoskeleton (Figure 4A). Since α -catenin is believed to be vital in mediating Ecad linkage to F-actin, we next measured the cytoskeletal linkage of Ecad in α cat-KO cells. As anticipated from previous biochemical and cell biological results ^{33,34}, our data showed that the vast majority of Ecad (89% $\pm$ 11%) in α cat-KO cells are uncoupled from the actin cytoskeleton (Figure 4A). In contrast, the fraction of uncoupled Ecads in both vinc-KO and Δ VBS cells, where Ecads are presumably weakly linked to F-actin by α -

1 catenin, but where this linkage is not reinforced by vinculin^{13,35}, was comparable
 2 to parental cells (64% ± 9% for vinc-KO and 46% ± 4% for ΔVBS cells; Figure 4A).
 3 Taken together, this data confirms the obligatory role of α-catenin in mediating
 4 Ecad linkage to F-actin. These direct, single molecule measurements of Ecad-actin
 5 coupling on the apical surface of live cells are in excellent agreement with previous
 6 single particle tracking of Ecad on the cell surface that inferred approximately 50%
 7 of Ecads on the apical surface of cells are coupled to the actin cytoskeleton³⁶.

8

9 **Absence of vinculin binding weakens adhesion of individual Ecad.** We next
 10 compared the fraction of weak (jumps) and strong (cyto-tethers) *trans* dimers,
 11 formed by cytoskeleton-coupled Ecad. Our data showed that in parental cells that
 12 contain both α-catenin and vinculin, 83% ± 15% of cytoskeleton-coupled Ecad bind
 13 robustly while the remaining 17% ± 12% of Ecad interact weakly (Figure 4B). In
 14 contrast, in vinc-KO cells, only 7% ± 4% of cytoskeleton-coupled Ecad bind
 15 strongly (Figure 4B). Similarly, in ΔVBS cells where vinculin cannot bind to α-
 16 catenin, only 3% ± 1% of cytoskeleton-linked Ecad bind in a robust binding
 17 conformation (Figure 4B). Taken together, this indicates that vinculin association
 18 with the Ecad cytoplasmic region is required for robust Ecad *trans* dimerization. It
 19 is important to point out that the strength of *trans* dimer adhesion in the absence
 20 of α-catenin could not be determined by comparing the fraction of cyto-tethers and
 21 jumps because these events were extremely rare in the αcat-KO cells.

Since Ecad *trans* dimer can exist in two different conformations with different adhesive strengths — weaker X-dimer and stronger strand-swap dimer — we asked whether the weak (jumps) and strong (cyto-tethers) adhesive states of cytoskeleton-coupled Ecad represent different Ecad conformations. Since we have previously shown that Ecad ectodomains can be trapped in an X-dimer conformation simply by incubating them in free Trp ⁸, we performed AFM measurements on parental-Trp cells to identify the signature of X-dimer formed by cytoskeleton-coupled Ecad. Interestingly, almost all ($99\% \pm 13\%$) cytoskeleton-bound Ecads in parental-Trp formed jump events (Figure 4B), suggesting that the jump events represent the Ecads coupled to the cytoskeleton that form X-dimers. This, along with exclusive presence of jumps in vinc-KO and Δ VBS (Figure 4B), indicates that the weakening of Ecad adhesion in the absence of vinculin binding is consistent with Ecad being trapped in an X-dimer conformation. Importantly, switching ectodomains into an X-dimer conformation did not decouple Ecad from the cytoskeleton and the fraction of cytoskeleton-coupled Ecad in the Trp treated cells ($52\% \pm 5\%$) remained the same as parental cells (Figure 4A).

We also inferred the *trans* dimer binding conformation of cytoskeleton-coupled Ecad in the parental-Trp, vinc-KO and Δ VBS cells using dynamic force spectroscopy analysis ³⁷. Since the number of jump events in vinc-KO cells were insufficient to obtain statistically reliable results, we combined the vinc-KO jumps with the jumps measured with Δ VBS cells. The measured k_{off} for jump events for vinc-KO and Δ VBS ($k_{\text{off}} = 2.17 \pm 0.54 \text{ s}^{-1}$; Figure 4C) was comparable to parental-Trp X-dimers ($k_{\text{off}} = 1.92 \pm 0.64 \text{ s}^{-1}$; Figure 4D), and higher than previously

1 published values for strand-swap dimers³⁸⁻⁴¹. Since a higher k_{off} corresponds to a
2 weaker binding, these data further suggest that in the absence of vinculin
3 association, cytoskeleton-coupled Ecad adopts a weaker X-dimer conformation.

4 Next, since cadherins trapped in an X-dimer conformation have delayed
5 junction formation⁴², we monitored the formation of cell-cell barriers in parental-
6 Trp and Δ VBS cells using Electric Cell-Substrate Impedance Sensing (ECIS), in
7 order to determine if the junction formation in these cell lines are consistent with
8 Ecad adopting an X-dimer conformation. Our experiments measuring the barrier
9 formation half time (T50), showed a significant delay in calcium-induced barrier
10 formation in parental-Trp and Δ VBS cells compared to parental cells (parental-Trp
11 1.23 ± 0.65 hr delay in T50, Δ VBS 3.66 ± 2.26 hr delay in T50; Figure 4F).
12 Importantly, delayed barrier formation with Δ VBS was consistent with previous
13 reports²⁶. Taken together, our ECIS results indicate that both Ecad conformation
14 and vinculin binding to α -catenin are important for the formation of cadherin
15 junctions, and further suggest that vinculin is responsible for regulating Ecad
16 conformation.

17

18 **Ecad ectodomain conformation is regulated by vinculin.** Next, we proceeded
19 to directly measure the binding conformation of Ecad ectodomains in the different
20 cell lines by measuring if the Ecads on the cell surface form catch bonds (X-dimers)
21 or slip bonds (strand-swap dimers). We measured the force vs. lifetimes of Ecad-
22 Ecad bonds, using a method described previously⁴³. Briefly, since the force

1 required to rupture membrane-tethers and cyto-tethers scales linearly with pulling
2 velocity ^{28,44} (Figure S2), and because these tethers extend at a constant force
3 (after any initial viscoelastic relaxation) when pulled at a constant velocity ^{27,31}
4 (Figure 3A, 3E), we determined bond lifetimes at a range of forces from the
5 persistence time of membrane-tethers and cyto-tethers.

6 We first measured the force vs. lifetime of Ecad endogenously expressed
7 on the apical surface of parental cells. Fits of our data to the composite of a slip
8 bond ⁴⁵ and catch bond ⁴⁶ model showed that 75% of Ecad in the parental cells
9 form strand-swap dimers while the remaining 25% of Ecad occupy an X-dimer
10 conformation (Figure 5A; Methods; Table S2). Upon Trp incubation, however,
11 Ecads in parental cells switched their conformation and formed catch bonds
12 characteristic of an X-dimer (Figure 5B).

13 Since the bond-lifetimes for parental cells (Figure 5A) were obtained from
14 all the tethers (a mix of cyto-tethers and membrane-tethers), we separately
15 analyzed the force-dependent lifetimes of cyto-tether and membrane-tether events
16 (Figure S3). Consistent with cyto-tethers corresponding to strong Ecad-Ecad
17 bonds, our analysis showed that cyto-tethers (which correspond to Ecad initially
18 coupled to the cytoskeleton) exclusively formed slip-bonds. Ecad that were
19 decoupled from the cytoskeleton (membrane-tether events) formed X-dimers and
20 strand-swap dimers with almost equal probabilities (49% catch bonds: 51% slip
21 bonds) (Figure S3). This provided further evidence that the association of vinculin

1 with the Ecad cytoplasmic domain is required for ectodomains to efficiently form
2 strand-swap dimers.

3 As anticipated from our DFS experiments, Ecads in the vinc-KO cells also
4 formed catch bonds characteristic of an X-dimer conformation (Figure 5C)
5 suggesting that vinculin association is necessary for Ecads to form robust strand-
6 swap dimers. Similarly, Ecads in α cat-KO cells, where vinculin cannot associate
7 with the Ecad cytoplasmic region ¹², also formed catch bonds (Figure 5D). Finally,
8 eliminating vinculin binding to α -catenin in Δ VBS cells also resulted in catch bonds
9 (Figure 5E), confirming that association of vinculin with the Ecad cytoplasmic
10 region via α -catenin is obligatory for transitioning from a weak X-dimer
11 conformation to a robust strand-swap dimer conformation.

12

13 **Regulation of Ecad ectodomain conformation is myosin II dependent.** Having
14 identified vinculin as a key protein involved in allosterically regulating Ecad
15 ectodomain conformation, we proceeded to identify the mechanism by which
16 vinculin regulates Ecad structure. Since vinculin localizes to force bearing sites in
17 the cell-cell junction and mediates the recruitment of myosin II ¹⁸, we hypothesized
18 that Ecad ectodomain conformation could be regulated by myosin II mediated
19 cytoskeletal contractile force. We therefore tested the effect of myosin II mediated
20 force on Ecad conformation in parental cells by reducing cytoskeleton contractility
21 using the myosin II inhibitor, blebbistatin (*parental-Bleb*). Strikingly, in parental-
22 Bleb cells, the fraction of Ecad in strand-swap dimer conformation (45%; Figure

5F) decreased compared to parental cells (75% strand-swap dimer; Figure 5A). This indicated that myosin II dependent cytoskeletal contractility plays a role in driving strand-swap dimerization of Ecad ectodomains.

Additionally, unlike parental cells where the majority of cytoskeleton-coupled Ecad existed in the robust binding conformation (cyto-tethers), most of the cytoskeleton-coupled Ecad in parental-Bleb formed weak binding structures (jumps) ($36\% \pm 8\%$ cyto-tethers, $64\% \pm 22\%$ jumps; Figure 4B). Importantly, the fraction of cytoskeleton-coupled Ecad in parental-Bleb ($44\% \pm 9\%$; Figure 4A) remained the same as in parental cells. Finally, the measured k_{off} for jump events in parental-Bleb ($2.50 \pm 0.55 \text{ s}^{-1}$; Figure 4E) was comparable to the values measured for X-dimers formed by parental-Trp, vinc-KO and Δ VBS cells (Figure 4C-D). Taken together, these data confirm that reduced myosin II dependent cytoskeletal contractility reduces the ability of Ecad to form robust strand-swap dimers.

Since forces generated by the actomyosin cytoskeleton can transmit to the Ecad cytoplasmic domain ⁴⁷, we used steered molecular dynamics (SMD) simulations to test if force could convert X-dimers to strand-swap dimers (Figure 6A-B). We modeled cytoskeletal forces by pulling on the C-terminus of the EC1-2 X-dimer crystal structure (PDB ID: 3LNH) at five angles (0° , $\pm 30^\circ$, $\pm 40^\circ$), chosen to account for different orientations of actin filaments (Figure 6B). Our simulations showed that regardless of the pulling angle, all X-dimers converted to strand-swap dimers after a period of force application (Figure 6B-C). Root mean squared

1 deviations (RMSDs) of the SMD structures, calculated relative to the strand-swap
2 dimer crystal structure (PDB ID: 2QVF), converged to an average of 0.67 ± 0.12
3 nm confirming that the structures closely resembled strand-swap dimers (Figure
4 6C). These simulations confirm that when Ecad dimers are firmly coupled to the
5 actin cytoskeleton by α -catenin and vinculin, myosin-II dependent force can
6 convert X-dimers to strand-swap dimers.

7

8 **DISCUSSION**

9 Using live-cell, single molecule AFM measurements, we directly
10 demonstrate inside-out regulation of cadherin conformation and show that
11 cytoplasmic proteins regulate Ecad adhesive properties, similar to inside-out
12 regulation of integrins⁴⁸. Our data establish a distinct, mechanistic role for vinculin
13 in allosterically regulating Ecad conformation and adhesion at the single-molecule
14 level. Our results also demonstrate that previous interpretations of how vinculin
15 strengthens Ecad adhesion are incomplete. While these studies solely focused on
16 vinculin mediated remodeling of the junctional actomyosin cytoskeleton²²⁻²⁴, we
17 demonstrate that vinculin also changes the adhesive properties of individual Ecad
18 ectodomains. Furthermore, our data suggests that vinculin mediates the
19 transmission of cytoskeletal contractile force to the Ecad ectodomain and drives
20 the conversion of X-dimers to strand-swap dimers. Consequently, when Ecads
21 decouple from vinculin and/or α -catenin, they are trapped in an X-dimer structure.

1 The idea that force induces change in Ecad dimer conformation has been
2 speculated on previously ⁷, but until now has not been directly measured.

3 While previous AFM measurements with α -catenin knockdown cells
4 suggest that reducing α -catenin levels decrease the unbinding force of Ecad
5 ectodomains, the molecular mechanism by which α -catenin influences Ecad
6 adhesion was not determined ^{49,50}. Our data shows that vinculin association and
7 myosin II dependent cytoskeletal contractility allosterically trigger strand-swap
8 dimerization of Ecad and suggests that α -catenin affects Ecad adhesion by serving
9 as the scaffold protein that binds vinculin to Ecad-catenin complex.

10 It is important to point out that our experiments measure the allosteric
11 conformational regulation of non-junctional Ecads present on the apical surface of
12 cells. While apical Ecads may be organized differently from Ecads localized to cell-
13 cell junctions ^{51,52}, the intracellular proteins that couple Ecad to the actin
14 cytoskeleton are similar for both Ecad pools. Previous studies show that both
15 apical and junctional Ecads are under similar levels of constitutive actomyosin
16 tension ⁴⁷, suggesting that both Ecad pools interact with the actin cytoskeleton in
17 similar manners. Moreover, magnetic twisting cytometry show force-induced,
18 vinculin-dependent stiffening of Ecad coated beads placed on the apical cell
19 surface, suggesting that vinculin also associates with the extra-junctional Ecad ¹⁸.
20 More recently, 3D super-resolution microscopy of MDCK cells attached to an
21 Ecad-coated substrate, show that the intracellular organization of many Ecad-
22 associating proteins including p-120 catenin, β -catenin, α -catenin and vinculin,

1 resemble native cell-cell junctions ⁵³. These studies suggest that the inside-out
2 regulation that we measure with apical Ecad, also occurs with junctional Ecad.

3 Despite the large number of proteins expressed on the cell surface, we were
4 able to measure single-molecule unbinding by limiting Ecad density on the AFM
5 tip and controlling the force with which the AFM tip contacts the cell. However, we
6 were also aided by a low density of Ecad expressed on the apical surface of
7 confluent MDCK cell monolayers. While the precise oligomeric state of Ecad on
8 apical cell surfaces is unclear, with super-resolution imaging suggesting that apical
9 Ecad in A431 cells are present as well separated monomers ⁵¹ while Ecad in
10 A431D cells organize into small nanoclusters ⁵², our low unbinding event rates
11 nonetheless suggest that only single Ecad on the AFM tip interact with Ecad on
12 the cell surface. Interestingly, the total Ecad event rate measured with α cat-KO
13 cells is higher than with parental, Δ VBS or vinc-KO cells (Figure 2B). This likely
14 occurs because the α cat-KO cells do not form stable cell-cell junctions ²⁵.
15 Consequently, Ecad that would otherwise be corralled within cell-cell junctions
16 are now redistributed on the apical cells surface and subsequently interact with
17 Ecad on the AFM tip with a higher probability. The same may be true for other
18 membrane proteins which contributes to an increase in non-specific event rate in
19 control experiments with α cat-KO cells (Figure 2B).

20 It is also worth noting that measurements in α cat-KO cells — where Ecad
21 cannot link to actin cytoskeleton — mainly resulted in membrane-tethers (Figure
22 4A), validating the use of membrane-tethers as a signature of cytoskeleton-

1 uncoupled Ecad. Furthermore, the very low occurrence of jumps and cyto-tethers
 2 in α cat-KO cells (Figure 4A) implies that jumps and cyto-tethers indeed correspond
 3 to the pulling of Ecad coupled to the underlying cytoskeleton. Additionally, the
 4 presence of only jumps in the parental-Trp, vinc-KO, and Δ VBS cells (Figure 4B),
 5 where Ecad exclusively formed weaker X-dimers (Figure 5B-C, E), supports that
 6 jumps represent X-dimers linked to the actin cytoskeleton. Finally, the decrease in
 7 fraction of cyto-tethers (Figure 4B) with the decreased probability of strand-swap
 8 dimer formation in parental-Bleb (Figure 5F) strongly suggests that cyto-tethers
 9 correspond to strand-swap dimers linked to the actin cytoskeleton.

10 While Ecad in parental cells, that are coupled to the cytoskeleton via α -
 11 catenin and vinculin (cyto-tethers) exclusively formed strand-swap dimers, we
 12 were surprised to observe that Ecad which are decoupled from the cytoskeleton
 13 (membrane-tethers) formed X-dimers and strand-swap dimers with coequal
 14 probabilities (Figure S3 A, B). A likely explanation for this result is that the Ecad-
 15 cytoskeletal linkage is dynamic and that even transient interactions with vinculin
 16 are sufficient to drive strand-swap dimer formation. Since the AFM-tip and the cell
 17 surface remain in contact for about 0.46s in our experiments (Figure S4), this may
 18 allow enough time from Ecads on the parental cells to convert to a strand-swap
 19 dimer conformation and detach from the cytoskeleton (thereby forming membrane-
 20 tethers), before the AFM-tip is withdrawn from the cell surface.

21 When parental cells were treated with blebbistatin, the reduced actomyosin
 22 tension resulted in a decrease in the fraction of cyto-tethers (Figure 4B). While

1 these cyto-tethers behaved like parental cyto-tethers and exclusively formed slip-
 2 bonds (Figure S3C), a larger fraction of parental-Bleb membrane-tethers formed
 3 X-dimers compared to parental cells (69% in parental-Bleb compared to 49% in
 4 parental cells), further demonstrating that myosin generated contractile force is
 5 important for cadherin strand-swap dimer formation (Figure S3D).

6 Consistent with a previous study that used optical tweezers and single
 7 particle tracking to infer the fraction of cytoskeleton-bound Ecad³⁶, our data shows
 8 that only ~50% of Ecads on the apical cell surface are coupled to the actin
 9 cytoskeleton. Studies also show that cells expressing Ecad/ α -catenin fusion
 10 constructs that constitutively bind to the actin cytoskeleton, exhibit higher cell-cell
 11 adhesion and reduced intercellular motility compared to cells expressing wild type
 12 Ecad^{36,54}. These findings are supported by our observation that two rapidly
 13 exchangeable pools of Ecad structures (X-dimers and strand-swap dimers) exist
 14 on the cell surface. It is likely that these different pools of Ecads are crucial in
 15 reorganizing cell-cell junctions during collective cell migration: as the cell-cell
 16 contacts rapidly rearrange, strand-swap dimers anchor the contact while X-dimers
 17 probe for formation of new contacts.

18 Based on the results presented in this manuscript, we propose a biophysical
 19 model for the inside-out regulation of Ecad conformation. Our data suggests that
 20 vinculin mediated myosin II recruitment generates a contractile force which
 21 propagates to the Ecad ectodomain and drives the conversion of X-dimers to
 22 strand-swap dimers. (Figure 6D). Consequently, treating parental cells with

blebbistatin, inhibits myosin II dependent cytoskeletal contractility and reduces the ability of Ecad to form robust strand-swap dimers (Figure 5F). All atom computer simulations also demonstrate that a force applied to the membrane proximal region of X-dimers results in formation of strand-swap dimers (Figure 6B, 6C). Previous studies suggest that Ecad adhesion is altered by changing the phosphorylation state of cytoplasmic p120-catenin ^{40,55,56}; however, the mechanism by which this occurs and exact conformational states Ecads adopt, are unknown. It is possible that p120-catenin also indirectly modulates vinculin association with the Ecad cytoplasmic region, which in turn regulates Ecad ectodomain conformation and adhesion.

Recent studies have suggested that regulation of Ecad adhesion plays an important role in cancer progression. For instance, it has been shown that up-regulation of Ecad adhesive activity, rather than its amount, reduces the number of cells metastasized from the mammary gland to the lung ⁵⁷. Additionally, several point mutations in Ecad ectodomains that are prevalent in hereditary diffuse gastric carcinoma selectively interfere with the inside-out regulation of Ecad adhesion, rather than the ability of Ecad to adhere ⁵⁷. Furthermore, studies have shown that while vinculin expression is significantly reduced in colorectal cancer (CRC), overexpressing vinculin reduces metastasis in CRC ⁵⁸ and other cancer cell lines ⁵⁹. Consequently, understanding how cytoplasmic proteins induce conformational changes of Ecad ectodomains could be crucial to understanding the role of Ecad in cancer progression and metastasis.

1

2 **METHODS**

3 **Generating mutant cell lines.** Generation of α cat-KO cells ($\Delta\alpha$ E-catenin MDCK
4 cells)²⁵ has been described previously. For generation of Δ VBS cells, α cat-KO
5 cells were stably rescued²⁶ with GFP- α -catenin- Δ VBS mutants³⁵ as described
6 previously. Vinc-KO and Ecad-KO cells were generated by stably expressing a
7 PiggyBac plasmid encoding Cas9 with a cumate inducible promoter, and gRNA
8 expression cassette for canine Ecad or vinculin specific gRNA, which resulted in
9 complete knockout cells (see Figure 1C). The mutations were sequence verified
10 by PCR amplifying the target sequences from genomic DNA of knockout cell lines
11 and inserted into TOPO vectors (Invitrogen) for sequencing.

12 Ecad:

13 gRNA: ACAGACCAGTAACTAACGA

14 KO: ACAGACCAGT----AACGA

15 Vinculin:

16 gRNA: GGA GCACCGAGTAATGTTGG

17 KO: GGA~~t~~GCACCGAGTgATGTTGG

18

19 **Western Blotting.** Cell lysates for western blotting were prepared in 1x SDS
20 sample buffer (10 mM Tris pH 6.8, 1% SDS, 10% glycerol, 0.005% Bromophenol
21 Blue, 2% β -mercaptoethanol). Primary antibodies against E-cadherin (clone 36;

1 BD Biosciences), α -catenin (clone 15D9; Enzo Life Sciences), vinculin (clone
2 hVIN-1; Milipore Sigma), and α -tubulin (clone DM1A; Cell Signaling) were used in
3 manufacturer recommended dilutions in Phosphate-Buffered Saline with 0.1%
4 Tween 20 (PBST). Horse Radish Peroxidase (HRP) conjugated secondary
5 antibody (catalog no. 1706516; Bio-Rad) was used in 1:1000 dilution in PBST.
6 Protein samples were detected with a WesternBright Chemiluminescence Kit
7 (catalog no. K-12045; Advansta).

8

9 **Purification of Ecad ectodomain.** Generation of Ecad monomer plasmids
10 containing a C-terminal Avi tag has been described previously ⁶⁰. The plasmids
11 were incorporated into pcDNA3.1(+) vectors and were transiently transfected into
12 HEK 293T cells using PEI (Milipore Sigma) as previously described ⁶¹. Three days
13 post transfection, conditioned media was collected for protein purification.
14 Purification of Ecad were performed using methods described previously ^{8,60}.
15 Media containing his-tagged Ecads was passed through a chromatography
16 column containing Ni-NTA agarose beads (Qiagen). Beads were then washed with
17 a pH 7.5 biotinylation buffer (25mM HEPES, 5mM NaCl, and 1mM CaCl₂). Ecads
18 bound to the Ni-NTA beads were biotinylated with BirA enzyme (BirA 500 kit;
19 Avidity) for 1hr at 30°C. Following biotinylation, free biotins were removed using
20 the Ni-NTA column and biotinylated Ecads bound to Ni-NTA beads were eluted
21 using a pH 7.5 buffer containing 200mM Imidazole, 20mM Na₂HPO₄, 500mM NaCl,
22 and 1mM CaCl₂.

1

2 **Epithelial barrier formation measurements.** Ecad-dependent formation of
3 epithelial barriers was assessed by real-time impedance sensing. To this end,
4 parental or Δ VBS cells were trypsinized, re-suspended in calcium-free DMEM
5 medium (Immunosource, D9800-10) supplemented with 10% dialyzed fetal calf
6 serum, and plated onto L-cysteine-reduced, collagen I-coated 8WE10 electrodes
7 (Applied Biophysics) at a density of 2×10^5 cells per well and in the presence or
8 absence of 2 mM Trp. Impedance measurements were performed at 37°C and 6%
9 CO₂ using a 1600R Electrical Cell Impedance Sensing (ECIS) system (Applied
10 Biophysics) at a frequency of 4000 Hz. CaCl₂ was added at a final concentration
11 of 2 mM to allow for adherens junction formation, and measurements were
12 continued until stable impedance levels were reached in all conditions. Within each
13 individual experiment, 4 technical replicates for each condition were averaged to
14 assess the time-dependent impedance for that condition. The half times of barrier
15 formation were calculated as the timepoint at which the impedance signal
16 surpassed 50% of the ultimate maximum impedance.

17

18 **Ecad functionalization on AFM cantilever.** Purified Ecad monomers were
19 immobilized on Si tip of AFM cantilevers (Hydra 2R-100N; AppNano) as described
20 previously^{8,9,60,62}. Briefly, the AFM cantilevers were cleaned by immersing in 25%
21 H₂O₂/ 75% H₂SO₄ solution overnight. The cantilevers were then sequentially
22 washed with deionized water and acetone. The cantilevers were functionalized

with amine groups by immersing in 2% (vol/vol) 3-aminopropyltriethoxysilane (Millipore Sigma) solution dissolved in acetone. Polyethylene glycol (PEG) (MW 5000, Lysan Bio) spacers containing amine-reactive N-hydroxysuccinimide ester group at one end were covalently attached to the cantilever (100 mg/ml in 100mM NaHCO₃ dissolved in 600mM K₂SO₄, for 4 hrs); 20% of the PEG spacers presented biotin molecules at the other end. Unreacted amine groups on the silane molecules were capped using NHS-sulfo acetate (10 mg/ml for 30 mins; Thermo Fisher). PEG-functionalized AFM cantilevers were incubated in 1 mg/ml Bovine Serum Albumin⁶² overnight. The cantilevers were then sequentially incubated with streptavidin (0.1 mg/ml for 30 mins) and biotinylated Ecads (200 nM for 90 mins). Finally, free biotin-binding sites of streptavidin were blocked by using 0.02 mg/ml free biotin for 20 mins. All steps, except the incubation with Ecad, were kept the same for cantilever functionalization in control experiments.

Cell culture for AFM experiment. Cells were cultured in low glucose Dulbecco's modified Eagle's medium (DMEM) (Milipore Sigma) supplemented with 10% FBS (Milipore Sigma) and 1% antibiotics solution (penicillin + streptomycin + kanamycin) at 37°C and 5% CO₂. Cells were trypsinized 48-72 hrs prior to the experiment and plated on an ethanol-cleaned glass coverslip at a low density. The confluent monolayer of cells was washed 5 times with a pH 7 DMEM and antibiotics solution before loading onto the AFM setup with the same medium.

1 AFM force measurements. Force measurements were performed using two
 2 Agilent 5500 AFMs with closed loop scanners. An AFM cantilever functionalized
 3 with purified Ecad was brought in contact with a confluent monolayer of cells,
 4 pressed against the cell surface with a low force (typical pressing force was about
 5 50 pN) for 0.1s, and then retracted at one of five constant velocities (3, 5, 7, 9, 11
 6 $\mu\text{m/s}$). Control experiments used cantilevers decorated with streptavidin but
 7 lacking Ecad. Cantilever spring constants were measured using the thermal
 8 fluctuation method ⁶³. All the experiments were performed in a pH 7 DMEM and
 9 antibiotics solution in a custom-built environmental chamber supplied with 5% CO₂
 10 at room temperature. Parental-Bleb and parental-Trp experiments were performed
 11 with 100 μM ($\pm$) -Blebbistatin (Milipore Sigma) and 2mM L-Tryptophan (Alfa Aesar)
 12 in the solution respectively. A typical experiment lasted for ~10 hours yielding
 13 ~1200 force-distance traces.

14

15 Analysis of AFM data. AFM-generated force-distance traces were analyzed using
 16 custom MATLAB scripts. Traces containing single rupture events were classified
 17 into membrane-tether, jump, and cyto-tether categories based on the following
 18 criteria:

- 19 • Membrane-tethers: force plateau at least 100nm long and slope less than
- 20 25 pN/ μm .
- 21 • Cyto-tethers: have a difference of at least 5 pN between the peak force and
- 22 the plateau force.

- 1 • Jumps: have a linear force-distance relationship with a slope of more than
2 25 pN/μm.

3 Rupture events having unbinding force <5 pN and >150 pN were not
4 considered for analysis. Tethers were further fit to the SLS model as described in
5 ³¹ while jumps were fit to a Hookean spring model. We only retained events that fit
6 either the SLS or spring model with a Root Mean Squared Error (RMSE) of less
7 than the mean plus two standard deviations of all fits for each respective model.

8 To obtain the fractions of membrane-tether, cyto-tether, and jump events
9 for each cell line, we first measured the binding probability of each type of event
10 for both the Ecad experiment and the control. The binding probabilities obtained
11 from the control experiment were subtracted from the corresponding event rate of
12 the Ecad experiment.

13 Loading rates for the jump events used for dynamic force spectroscopy
14 analysis (Figure 4C-E) were calculated by multiplying the slope of the jump by the
15 pulling velocity. K-means clustering was used to group the loading rates as
16 described previously ⁶⁴. Mean unbinding force and mean loading rate for the
17 groups were fit using a weighted non-linear least square fit to the Bell-Evans model
18 ³⁷. Raw data was bootstrapped with replacement, grouped using k-means, and the
19 mean values were fit to the Bell-Evans model 1000 times; the fitting parameters
20 and errors were determined from the mean and standard deviations of the resulting
21 distributions. The data used in each fit was weighted by the fraction of data points
22 in a particular bin relative to the total number of data points. Force-dependent

1 Ecad-Ecad bond mechanics were studied by analyzing the forces and lifetimes of
 2 membrane-tethers and cyto-tethers. Bond lifetimes were determined from the
 3 force curves starting at the beginning of the plateau region to the bond rupture.
 4 The unbinding force was calculated as the mean of the force values spanning that
 5 same region. This data was used to create the plots shown in Figure 5. The
 6 unbinding forces were fit to a Gaussian mixture model (GMM) using an expectation
 7 maximization algorithm to determine the bins; the means and standard deviations
 8 used in the GMM fit were initialized using the k-means clustering algorithm. We
 9 determined the characteristic lifetime of each bin using maximum likelihood
 10 estimation (MLE) on bootstrapped samples. The likelihood function, $\mathcal{L}(\theta; X) =$
 11 $\prod_{i=1}^N f_i(x_i; \theta)$, was formulated using an exponential distribution f .

12 Where, $f(x; \lambda) = \begin{cases} \lambda e^{-\lambda x} & x \geq 0, \\ 0 & x < 0. \end{cases}$

13 The MLE lifetimes were fit by Chi-squared minimization to one of two models: a
 14 composite slip-catch bond model or a catch bond model ⁴⁶. Assuming the errors in
 15 the measurements are Gaussian distributed, fitting by minimizing Chi-squared is
 16 the same as maximizing the log likelihood ⁴⁶. The composite slip-catch model used
 17 to fit the parental and parental-Bleb data was the sum of a slip bond model and a
 18 catch bond model. The slip bond model ⁴⁵, $\tau(F) = \frac{1}{k_{off}(F)} = \tau(0)e^{-\frac{Fx_{\beta}}{k_B T}}$, was used
 19 as the first component of the composite slip-catch model to fit the parental and
 20 parental-Bleb data (Figure 5A, F), where τ is the characteristic bond lifetime in the
 21 absence of force and x_{β} is the reaction coordinate distance. The catch bond model
 22 used in the composite model and to fit all other cell lines is given by,

$$\tau(F) = \frac{\sqrt{\pi} r_0 (E_1 - 2F(d + r_0)) e^{\beta(E_0 + dF)} (e^{\beta F r_0} - 1)}{4D(\beta E_0)^{\frac{3}{2}} F \left(1 + \frac{r_0}{d}\right)^2 (1 - e^{\beta(2F(d+r_0) - E_1)})}$$

where r_0 is the natural bond length, d is the reaction coordinate (same as x_β for the slip bond model), E_0 and E_1 are components of the bond energy, $\beta = \frac{1}{k_B T}$ (k_B is the Boltzmann constant), and D is a diffusion constant ($D = \frac{k_B T}{6\pi\eta r_0}$). The number of bins used was established by fitting the force and lifetime data to the GMM using 5, 6, 7, or 8 bins (only 7 and 8 for parental cell data) for each cell line. The composite slip-catch and catch bond model fitted parameters are shown in Table S2. The Chi-squared fitting function was acquired from the Mathworks File Exchange website (Generalized Nonlinear Non-analytic Chi-Square Fitting by Nathaniel Brahms, May 26, 2006).

The fraction of strand-swap and X-dimers calculated from Figure 5A and 5F were determined by integrating each component of the composite model (slip/catch models) in 5 pN bins from 5 to 150 pN to determine the slip/catch area of that bin. The number of data points in each bin that corresponded to the slip bond model, for example, was determined by,

$$\Delta n_{slip} = \left(\frac{\Delta A_{slip}}{\Delta A_{slip} + \Delta A_{catch}} \right) \Delta n$$

where ΔA_{slip} and ΔA_{catch} are the area under slip and catch bond fit respectively for the 5pN bin, and Δn is the total number of data points in the 5pN bin. As expected, the sum of the slip and catch bond fits—and therefore the sum of their areas—is equal to the composite fit. The total fraction of data corresponding to the slip bond (strand-swap dimers) is then,

$$Fraction_{slip} = \frac{\sum \Delta n_{slip}}{\sum \Delta n}$$

Steered Molecular Dynamics Simulations and Structural Analysis. Steered molecular dynamics simulations were performed with GROMACS 2020.1 using the FARM high performance computing cluster at UC Davis. The X-dimer crystal structure (PDB ID: 3LNH) was used in all simulations. Missing residues were added using PDBFixer and the N-terminal β -strand with Trp2 was positioned near the strand-swap interface using the sculpting tool in PyMOL. All simulations were performed with the OPLS-AA/L force field and the TIP4P water model.

The X-dimer crystal structure was equilibrated by performing a 20 ns molecular dynamics (MD) simulation. To setup the simulation the X-dimer was placed in the center of a dodecahedral box such that no atom of the X-dimer was closer than 1 nm to any boundary. The box was solvated by adding water molecules and charge-neutralized by adding Na^+ ions. Additionally, NaCl, KCl, and CaCl_2 were added to the box at concentrations of 150 mM, 4 mM, and 2 mM, respectively. Energy minimization, using the steepest decent algorithm, was performed until the force on any atom in the system was less than 1000 kJ/mol/nm. The temperature, pressure, and density of the system were stabilized by equilibrating under *isothermal-isochoric* and *isothermal-isobaric* conditions using a modified Berendsen thermostat and Berendsen barostat. Following equilibration, a 20 ns MD simulation was performed with 2 fs integration steps, LINCS constraints on bond length, and long-range interactions were evaluated using the

1 particle mesh Ewald method. The stability of the X-dimer was monitored by
2 calculating the root mean squared deviation (RMSD) of the X-dimer relative to the
3 initial structure.

4 Conformational switching was modeled using Steered molecular dynamics
5 (SMD) simulations performed on the fully equilibrated X-dimer by pulling on a
6 group of residues at the C-terminal end of one Ecad monomer in five directions
7 (0° , $\pm 30^\circ$, $\pm 40^\circ$; see *Figure 6A*). The C-terminal of the opposing Ecad was
8 constrained to simulate a robust linkage to the actin cytoskeleton. The X-dimer
9 from the final frame of the MD simulation was placed at the center of a 12 x 30 x 8
10 nm³ box, and solvated and equilibrated as described above. After centering the X-
11 dimer in the box, the pulling angle was achieved by rotating the X-dimer about the
12 z-axis (short axis) by the corresponding pulling angle. For each simulation, the X-
13 dimer was pulled with a constant force (force constant = 498.2 pN/nm; 300
14 kJ/mol/nm²) along the long axis of the box.

15 The conversion of the X-dimer to strand-swap dimer was determined by
16 aligning the simulated structures to the X-dimer (PDB ID: 3LNH) and strand-swap
17 dimer (PDB ID: 2QVF) crystal structures and calculating the RMSD of the alpha
18 carbon atoms before and after pulling, respectively. The strand-swap dimer was
19 formed from 2QVF by crystallographic symmetry operations.

20

1 **Acknowledgments:** Research in the SS lab was supported in part by the National
2 Science Foundation (PHY-1607550) and National Institute of General Medical
3 Sciences of the National Institutes of Health (R01GM121885). Research in the SY
4 lab was supported by NIH R03 EB021636, NSF 1562095 (SY, REU supplement
5 to JC), and UC Davis Provost's Undergraduate Fellowship (to JC). JC is a recipient
6 of the Beckman Scholars Award. Research in the MG lab was supported by the
7 Netherlands Organization for Scientific Research (NWO; 016.Vidi.189.166 and
8 NWO gravitational program CancerGenomiCs.nl).

9

10 **Data availability:** All data produced by this research are made available in the
11 Supporting Information.

12

13 **Author contributions:** R.K., A.V.P. and S.S. designed research; S.S. directed the
14 research; R.K., A.V.P. and C.F.Y. performed AFM experiments; A.V.P. performed
15 computer simulations; W.J.P and M.G. performed ECIS experiments; J.S.C., M.G.,
16 and S.Y., generated cell lines; R.K., A.V.P., and S.S. wrote the paper.

17

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1 **Figures**

2 **Figure 1. Overview of experiment. (A)** The extracellular region of Ecad from
 3 opposing cells mediate adhesion. The cytoplasmic region of Ecad associates
 4 either directly or indirectly with p120 catenin, β -catenin, α -catenin, vinculin, and F-
 5 actin. **(B)** Strand-swap dimers form slip bonds (blue) while X-dimers form catch
 6 bonds (red). Ecads interconvert between these two dimer conformations.
 7 Structures were generated from the crystal structure of mouse Ecad (PDB: 3Q2V);
 8 the X-dimer was formed by alignment to an X-dimer crystal structure (PDB: 3LNH).
 9 **(C)** Graphics showing the cell lines used in experiments and western blot analysis
 10 of corresponding cell lysates.

11

12 **Figure 2. Measuring specific and non-specific interaction using AFM. (A)**
 13 Biotinylated Ecad monomers were immobilized on AFM cantilevers functionalized
 14 with Polyethylene glycol (PEG) and Streptavidin as described in the methods
 15 section. Interactions between Ecads on the cantilever and Ecads expressed on the
 16 apical surface of MDCK cells were measured. **(B)** Binding probabilities were
 17 measured using AFM cantilevers lacking Ecad (control experiment, light gray) or
 18 AFM cantilevers decorated with Ecad (Ecad, dark blue). Total number of
 19 Ecad/control measurements performed on each cell line were 6793/3361
 20 measurements on parental cells, 8593/3719 measurements on parental-Bleb,
 21 7268/4858 measurements on parental-Trp, 4291/3666 measurements on vinc-KO
 22 cells, 4518/3381 measurements on α cat-KO cells, 6124/6070 measurements on

1 Δ VBS cells, and 3672/2449 measurements on Ecad-KO cells. Error bars are
2 bootstrapped standard deviations.

3

4 **Figure 3. Force ‘signatures’ used to identify cytoskeletal coupling and**
5 **strength of *trans* adhesion. (A)** Typical force curve showing a membrane-tether
6 which extends at a constant force. **(B)** Membrane-tethers are measured when an
7 Ecad, which is not linked to the actin cytoskeleton is pulled. **(C)** Jump events
8 exhibit a linear increase in force and are measured upon pulling **(D)** an Ecad *trans*
9 dimer that is weaker than its cytoskeleton coupling. **(E)** Typical force curve showing
10 a cyto-tether with an initial increase in force that viscoelastically relaxes as a
11 membrane-tether is formed. **(F)** Cyto-tethers are measured when a robust Ecad
12 *trans* dimer that is linked to the actin cytoskeleton is pulled. Raw data (gray) was
13 acquired at 25 kHz and smoothed (blue) to 111 Hz. Green curve is the Standard
14 Linear Solid model fit to membrane-tethers and cyto-tethers. Black bars indicate
15 bond lifetimes determined from membrane-tethers and cyto-tethers.

16

17 **Figure 4. Role of α -catenin and vinculin in Ecad-cytoskeleton linkage and**
18 **adhesion strength. (A)** Fraction of uncoupled (membrane-tether) and coupled
19 (cyto-tether + jump) events. Fractions were calculated after subtracting the binding
20 probability of the control experiment for each cell line. **(B)** Fractions of strong (cyto-
21 tether) and weak (jump) Ecad *trans* dimer events. Error bars are the propagated

errors obtained from bootstrapped standard deviations. Dynamic force spectroscopy of jump events in **(C)** vinc-KO + Δ VBS, **(D)** parental-Trp, and **(E)** parental-Bleb cells. Loading rates and peak forces in jump events were bootstrapped 1000 times then clustered using k-means and fit to the Bell-Evans model (green line) using weighted nonlinear least-square fitting. Data points represent single unbinding events, and colors correspond to data points from the same k-means cluster. The data from Δ VBS (filled circles) and vinc-KO (circles) cells in (C) were combined because the number of data points were insufficient for a statistically reliable calculation. Intrinsic off rate (k_{off}) and distance to the transition energy barrier (x_{β}) were obtained from the mean of the fitted parameters acquired by fitting the k-means clusters that were calculated from the bootstrapped samples. Errors in the fitted parameters correspond to standard deviations of the bootstrapped means. **(F)** Trans-epithelial resistance measurement of parental cells in the absence (Parental; $n = 9$) and presence (Parental-Trp; $n = 8$) of 2 mM tryptophan, or Δ VBS cells (Δ VBS; $n = 5$), following addition of 2 mM $CaCl_2$ to induce Ecad dependent formation of the epithelial barrier. Barrier formation half times (T50) were calculated as the time-point at which the impedance signal surpassed 50% of the ultimate maximum impedance. * $p = 0.0025$, ** $p = 0.0011$; Paired t-test.

Figure 5. Measuring conformation of Ecad *trans* dimers using an AFM. Force vs lifetime profile for **(A)** parental cells show that 75% of Ecad form slip bonds

(strand-swap dimer structure shown in blue) while the remaining 25% form catch bonds (X-dimer structure shown in red). **(B)** Parental-Trp, **(C)** vinc-KO, **(D)** α cat-KO, and **(E)** Δ VBS cells form catch bonds. **(F)** Inhibition of Myosin II using Blebbistatin reduces the fraction of slip bond forming Ecad. Parental-Bleb shows that 45% of Ecad form slip bonds while the remaining 55% form catch bonds. Solid lines represent fits to the corresponding models. Data was binned using a Gaussian mixture model and the lifetimes were determined using maximum likelihood estimation. The colored data points correspond to the data points in each respective bin. The data was fit by χ^2 minimization and errors were obtained by bootstrapping. The y-axes were limited to 1s for clarity.

11

Figure 6. Cytoskeletal tension converts X-dimers to strand-swap dimers. (A) X-dimer formed by Ecad on opposing cells (left). X-dimer crystal structure (PDB ID: 3LNH) used in SMD simulations (right). The pulling directions are indicated by colored arrows. The full X-dimer (left) was simulated by aligning 3Q2V to 3LNH. **(B)** X-dimer conversion to strand-swap dimer. Simulated structures remain in an X-dimer conformation after stabilizing MD simulations (left). X-dimers become strand-swap dimers after pulling on the C-terminal of one Ecad at a range of angles (right). The transparent blue structure is the strand-swap crystal structure (PDB ID: 2QVF). The transparent red structure is the X-dimer crystal structure (PDB ID: 3LNH). The remaining, opaque structures are the simulated structures. **(C)** Root mean squared deviations (RMSD) of the pulled structures relative to the strand-

1 swap dimer crystal structure as a function of the time it took to converge to the
 2 strand-swap crystal structure (minimum RMSD). RMSDs were determined by
 3 least-squares fitting to the strand-swap dimer crystal structure and calculating the
 4 distance between alpha carbons of the two structures. **(D)** Model for vinculin-
 5 dependent, force-induced conversion of X-dimers to strand-swap dimers. In the
 6 absence of vinculin, force applied is insufficient to convert the X-dimer to a strand-
 7 swap dimer (left). Cytoskeletal tension is transmitted in a vinculin dependent
 8 manner to the Ecad extracellular bond to promote conversion to a strand-swap
 9 dimer (right).

10

Figure 1

A

E-cadherin

p120-catenin

β-catenin

α-catenin

Vinculin

F-actin

B Strand-swap dimer

X-dimer

Slip Bond

Catch Bond

C

Parental

αcat-KO

Vinc-KO

ΔVBS

Ecad-KO

Figure 2

A

B

Figure 3

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A

B

C

D

E

F

Figure 4

A **B** **C** **D** **E** **F**

Figure 5

Figure 6