Supporting Information

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SI Methods

Protein Expression and Purification. Constructs encoding EC1 and EC2 of E- and N-cadherins from mouse, chicken, and human, as well as chicken cadherin-6b, were expressed in the prokaryotic pSMT3 small ubiquitin-related modifier (Sumo)/His-tag fusion system [from modified pET 28b(+) expression plasmid].

The pSMT3 Sumo vector was linearized with 2 restriction enzymes. To prevent self-ligation, the vector was treated with 10 units of calf intestine alkaline phosphatase for 30 min at 37 °C. Constructs were amplified by using the 5′ ends of the PCR primers containing an overhang on either side of the vector's restriction site plus an extra Gly-Gly motif immediately before the first amino acid of each protein. Ulp1 protease recognizes the motif, releasing the protein with no extra amino acids at the N terminus site. PCR product and linearized vector were treated with 20 units of Exonuclease III (New England Biolabs), which has a 3′ to 5′ exonuclease activity, at 14 °C for exactly 1 min, resulting in complementary single-stranded stretches of DNA. The reaction was stopped with 80 μL of 50 mM EDTA, pH 8.0. After phenol/chloroform extract and EtOH precipitation, the DNA was resuspended with 10 μL of Tris/EDTA (TE) buffer, and ligase-free-annealed by incubation at 70 °C for 5 min, followed by incubation at 37 °C for 5 min. The DNA molecules were heat shock-transformed into XL10-Gold ultracompetent cells.

The amino acid boundaries of the 2 extracellular domains of each protein are as follows: E-cadherin EC1–2: 1–213 (mouse); 1–215 (chicken); and 1–215 (human). N-cadherin EC1–2: 1–216 (mouse); 1–217 (chicken); and 1–217 (human). Mouse cadherin-6: 1–207. The mouse, chicken, and human E- and N-cadherins also were expressed in a biotinylated form containing a C-terminal Avi tag fusion.

Proteins were expressed in *Escherichia coli* Rosetta II (DE3; Novagen) pLysS cells and grown at 37 °C until at OD_{600} of 0.6 was reached. The cells were induced by using 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and were subsequently grown at 18 °C for 16 h. For the biotinylated proteins, the plasmid containing the protein of interest was cotransformed with the pBirAcm plasmid, which contains an IPTG-inducible *birA* gene expressing biotin ligase, into the *E. coli* strain BL21(DE3). These cells were grown at 37 °C until at OD_{600} of 0.6 was reached, followed by simultaneous protein induction and biotinylation using 0.1 mM IPTG and 50 μM biotin for 3 h at 37 °C. At the end of the induction phase, cells were spun at 7,300 $\times$ *g* for 15 min at 4 °C. The pellets were resuspended in 10 mM Tris·HCl, pH 8.0; 500 mM NaCl; 20 mM imidazole; and 3 mM $CaCl_2$ and were sonicated for 3 min. The lysate was spun down for 30 min at 23,700 $\times$ *g*, and the supernatant was collected for affinity chromatography.

The proteins were purified by using ion metal affinity chromatography Sepharose 6 Fast Flow (GE Healthcare) resin charged with 0.2 column volume (CV) of a 0.2 M Ni^{2+} solution. The Ni^{2+} resins were then washed with 20 CV of wash buffer. The proteins were eluted with elution buffer (10 mM Tris·HCl, pH 8.0; 500 mM NaCl; 3 mM $CaCl_2$; and 250 mM imidazole). To cleave the His_6-Smt3 tag, 0.1 mg of ubiquitin-like protease 1 (Ulp1) was added to the protein sample and dialyzed in 50 mM NaCl; 20 mM Tris·Cl, pH 8.0; and 10 mM $CaCl_2$ for 10 h at 4 °C. After digestion, proteins were subjected to a second affinity chromatography step to remove the cleaved His_6-Smt3 fragment. The flow-throughs were collected and further purified by using ion exchange (MonoQ 10/100GL) and size exclusion (GE Healthcare) (S75 26/60) in a final buffer of 20 mM Tris, pH 8.0; 150 mM NaCl; and 10 mM $CaCl_2$.

Analytical Ultracentrifugation. Sedimentation equilibrium experiments were performed by using a Beckman XL-A/I ultracentrifuge equipped with Ti50An and Ti60An rotors. All proteins were dialyzed overnight at 4 °C in 10 mM Tris·HCl, pH 8.0; 150 mM NaCl; and 3 mM $CaCl_2$. E-cadherin and E-cadherin W2A were dialyzed in the same buffer supplemented with 3 mM TCEP. For each protein, 120 μL of each 0.24, 0.46, and 0.7 mg/mL was loaded onto a 12-mm, 6-channel cell centerpiece with sapphire windows. Data were collected at 25 °C and 37 °C at 280 and 660 nm. Samples were spun at 23,400 $\times$ *g* for 20 h, after which a scan was collected hourly for a total of 4 scans. The speed was increased to 35,200 $\times$ *g* for 10 h, followed by an hourly scan for a total of 4 scans. The speed was further increased to 49,100 $\times$ *g* for 10 h, followed by 4 additional hourly scans. This protocol results in 72 conditions that were scanned for each protein. Buffer density and v-bar were calculated by using SednTerp (Alliance Protein Laboratories). The scans were processed and analyzed by using HeteroAnalysis 1.1.0.28 (www.biotech/uconn.edu/auf/). The data were fit to a monomer/dimer equilibrium model to calculate the K_d for each homodimer. All values were determined from at least 2 independent experiments.

SPR Binding Analysis of Human and Chicken N- and E-Cadherins. Analysis of the human and chicken N- and E-cadherin interaction was performed in a manner similar to the experiments of the mouse N- and E-cadherins. Approximately 1,110 RU of biotinylated human N-cadherin and 308 RU of human E-cadherin (each corresponding to 70 μM monomer) were captured in adjacent flow cells for the experiments at 25 °C, whereas surfaces of 1,100 RU of human N-cadherin and 245 RU of human E-cadherin (66.3 μM monomer) were used for the analysis at 37 °C. Binding of human N- and E-cadherins was tested under the same conditions as described previously by using 121.0 μM N-cadherin and 15.2 μM E-cadherin (13.0 μM monomer for each) at 25 °C and 15.8 μM N-cadherin and 9.5 μM E-cadherin (8.8 μM monomer for each) at 37 °C. Biotinylated chicken N- and E-cadherins were captured at 1,085 RU and 445 RU, respectively, at 25 °C (63.0 μM monomer), and at 37 °C, 1,100 RU of N-cadherin and 326 RU of E-cadherin were captured over adjacent flow cells. Chicken N- and E-cadherins were tested at 33.9 and 20.3 μM, respectively (14.0 μM monomer each), at 25 °C and at 33.2 and 17.65 μM, respectively (14.0 μM monomer each), at 37 °C.

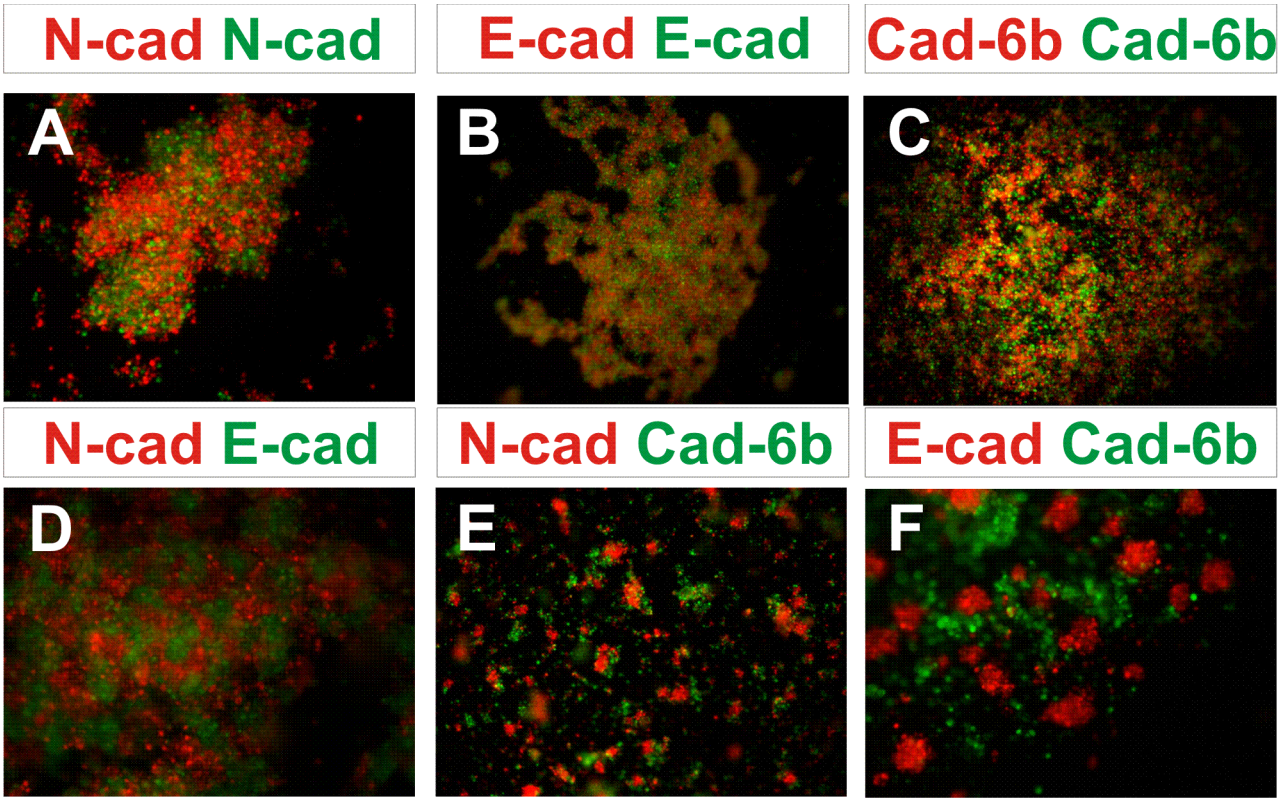

Fig. S1. Hanging-drop aggregation assays with cadherin-expressing CHO cells. Hanging-drop aggregation assays using 2 identical CHO cell lines expressing the same cadherin: (*A*) N-cadherin, (*B*) E-cadherin, or (*C*) cadherin-6b. The resulting aggregates also reveal an interspersed mixture of each cell line. (*D–F*) Hanging-drop aggregation assay using CHO cell lines expressing different cadherins. (*D*) E-cadherin and N-cadherin show separate ball-like aggregates of E-cadherin cells and N-cadherin cells that adhere to one another. (*E* and *F*) Type I cadherins E-cadherin and N-cadherin do not form such adherent aggregates with cells expressing the type II cadherin-6b. Thus, hanging assays give results consistent with those observed in mixing aggregation assays (Fig. 1). (Magnification: 20×.)

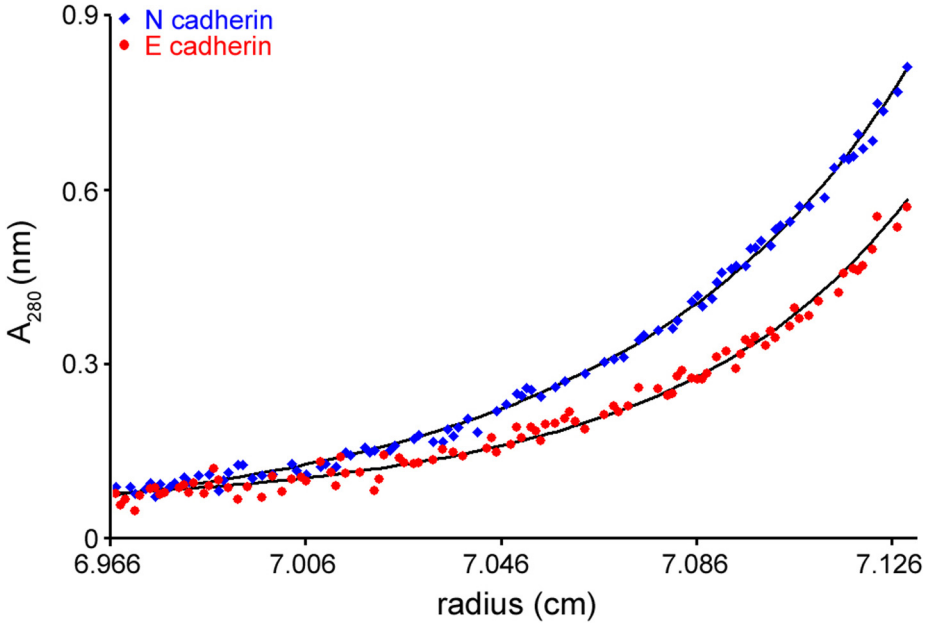

Fig. S2. AUC analysis of E- and N-cadherins. The data shown were collected at 25 °C by using a speed of 49,100 × *g*. The data points for N- and E-cadherins are shown in blue diamonds and red circles, respectively. The blank traces represent the fit to a monomer/dimer equilibrium model.

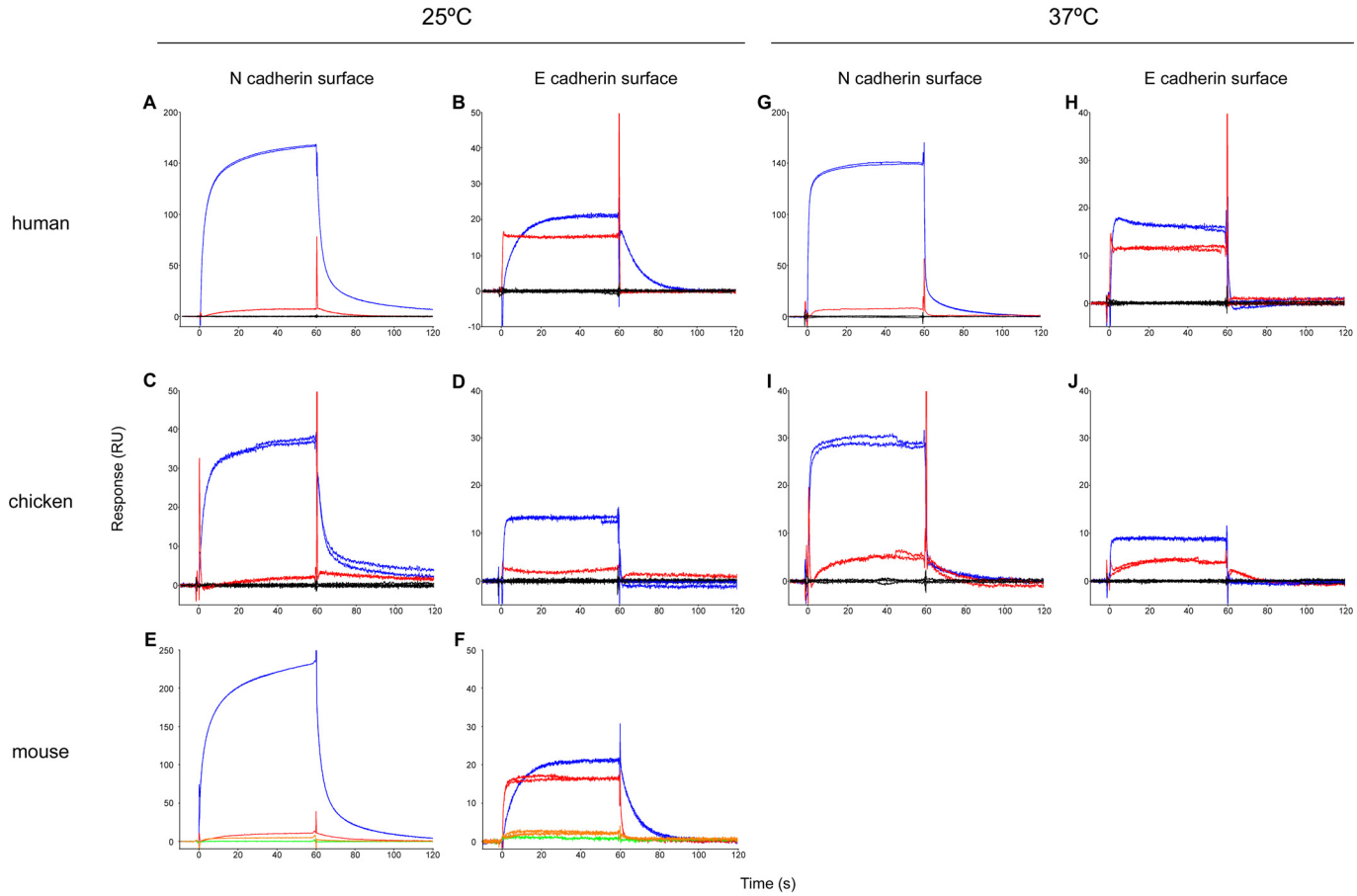

Fig. S3. SPR binding analysis for human, chicken, and mouse N- and E-cadherins. The interaction of human N- and E-cadherins was tested over surfaces containing human N-cadherin, as shown in *A* and *G*, and surfaces containing human E-cadherin (*B* and *H*). The analysis over each surface was performed at both 25 °C (*A* and *B*) and 37 °C (*G* and *H*). Similarly, binding of chicken N- and E-cadherins was examined over a surface containing N-cadherin at 25 °C and 37 °C (*C* and *I*) and over an E-cadherin surface at the same temperatures (*D* and *J*). The data for mouse N- and E-cadherin interaction at 25 °C are shown in *E* and *F* (the 37 °C data are included in Fig. 3). The binding traces for N-cadherin are colored blue, and the traces for E-cadherin are in red for all species (*A*–*J*). The black traces represent buffer injections that were performed throughout the experiment for appropriate referencing of the data. Mouse N-cadherin W2A (orange) and mouse E-cadherin W2A (green) in *E* and *F* did not interact with wild-type N- or E-cadherins as expected based on crystallographic studies. The capture levels of E- and N-cadherins and the concentrations of N- and E-cadherins injected are described in *Methods*.

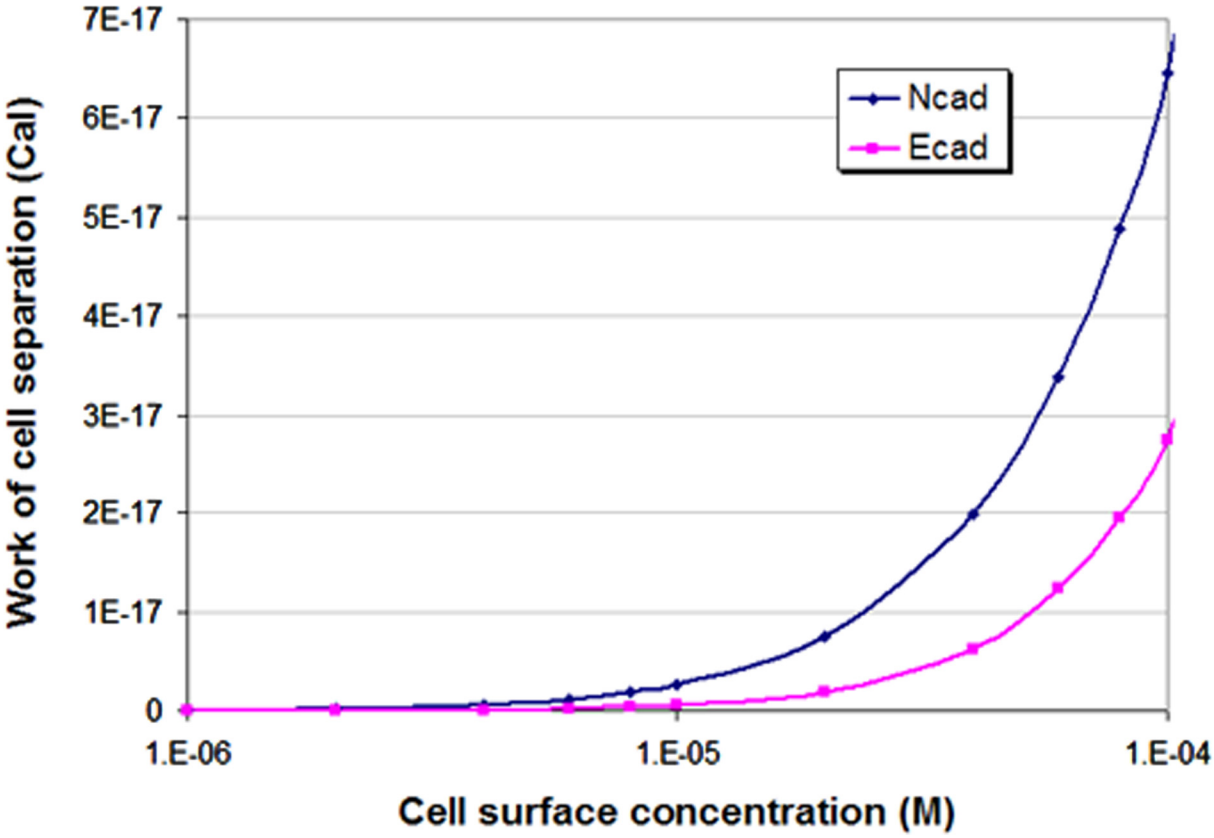

Fig. S4. Plot of work of cell separation vs. surface concentration for cells expressing N- and E-cadherins. The calculations are based on a model published previously [Chen CP, et al. (2005) *Proc Natl Acad Sci USA* 102:8531–8536] and assume that the interface between 2 cells occupies 4% of the total cell surface. The absolute numbers reported in the figure depend on this assumption, but the relative values of $W(N,N)$ and $W(E,E)$ do not. At the same concentration, $W(N,N)$ is always greater than $W(E,E)$ because the binding affinity of N-cadherin is greater than that of E-cadherin, and below saturating levels there will be more N-cadherin dimers formed. Even when expression levels of E-cadherin are higher than for N-cadherin, there is a range of concentrations where $W(N,N) > W(E,E)$. For example, when the N-cadherin concentration is equal to its K_d, 23 μM, $W(N,N) > W(E,E)$ for E-cadherin expression levels up to 50 μM (i.e., a range of 27 μM). However, at higher concentrations (e.g., when the N-cadherin concentration is 88 μM), $W(N,N) > W(E,E)$ for E-cadherin expression levels up to its K_d of 160 μM, a range of 72 μM. Thus, cell separation mediated by affinity differences between N- and E-cadherins is robust over a significant range of concentrations.