Bianchini et al., http://www.jcb.org/cgi/content/full/jcb.201411080/DC1

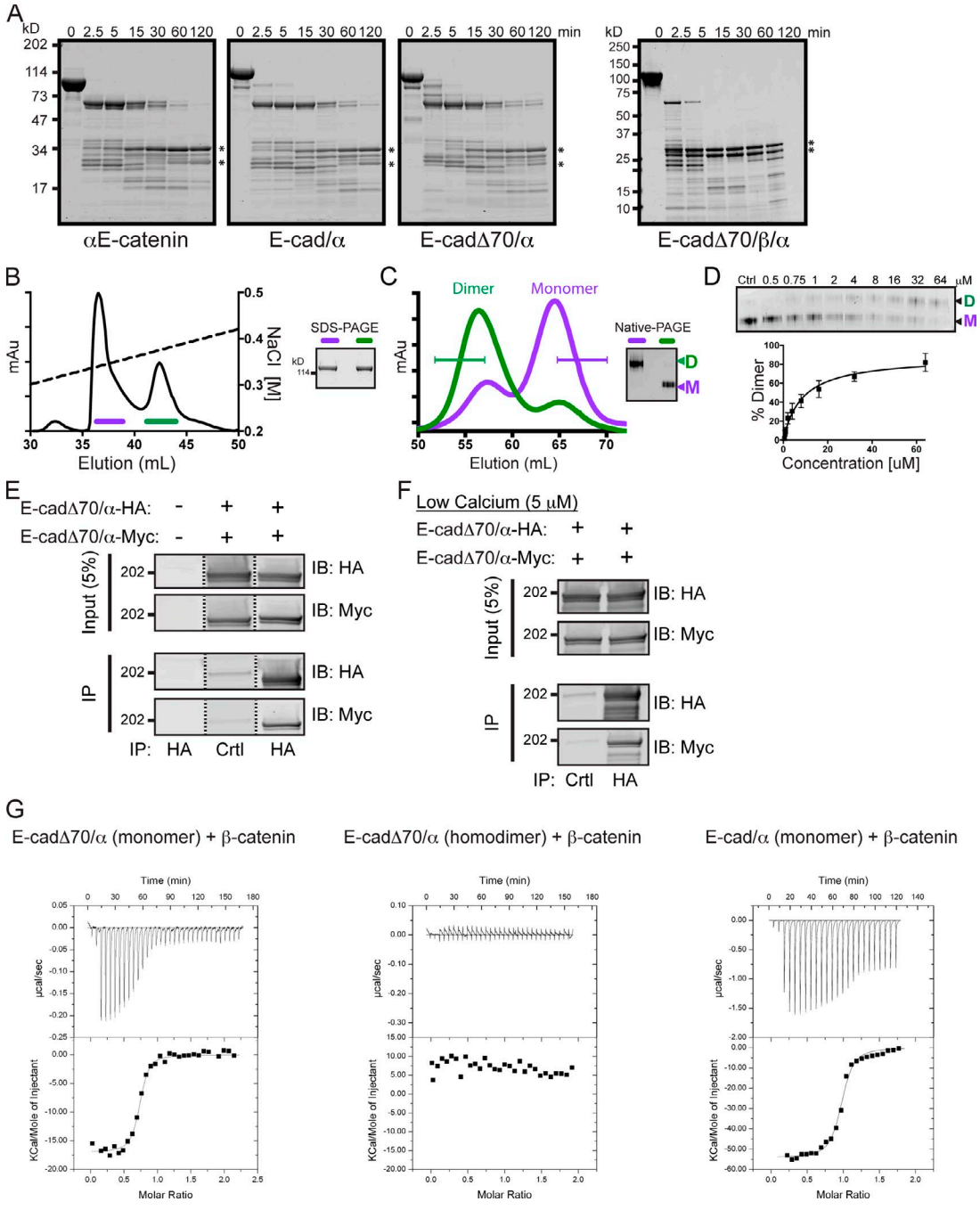

Figure S1. **In vitro characterization of E-cadherin/αE-catenin chimeras.** (A) SDS-PAGE and CBB staining of αE-catenin, E-cad/α, E-cadΔ70/α, and E-cadΔ70/β/α recombinant proteins incubated with 0.05 mg/ml trypsin. The E-cadherin cytoplasmic domain is unstructured and does not generate stable breakdown products. After 2 h, two major bands were resolved (*) and correspond to the previously published M-domain (385–651) and dimerization domain (82–287; Drees et al., 2005a; Kwiatkowski et al., 2010; Miller et al., 2013). (B) IEC of recombinant E-cad/α and SDS-PAGE and CBB staining of protein from the resulting two peaks (fractions indicated with purple and green lines). (C) SEC of E-cad/α from the two IEC peaks. Fractions (indicated with brackets) were pooled and analyzed by Native-PAGE stained with CBB. (D) Native-PAGE and CBB staining of increasing concentrations of monomeric E-cad/α chimera (0.5–64 μM) that had been incubated for 16 h at 37°C. Quantification of the percentage of dimerization with standard deviation from three independent experiments is shown. (Ctrl.) Purified monomeric E-cad/α that was kept at 4°C. (E) Coimmunoprecipitation of C-terminally HA- and Myc-tagged E-cadΔ70/α from HEK293T. *n* = 3. (F). Coimmunoprecipitation of C-terminally HA- and Myc-tagged E-cadΔ70/α was also observed when cells were cultured in low calcium medium (5 μM). *n* = 3. (G) Isothermal titration calorimetry measurements conducted at 25°C in a HEPES buffer with a 10-fold molar excess of β-catenin titrated into a 6 μM solution of either monomeric E-cadΔ70/α, homodimeric E-cadΔ70/α, or monomeric E-cad/α by a series of 9 μl injections with a 240 s delay between each injection. The thermodynamic parameters observed for the reactions are listed in Table 1.

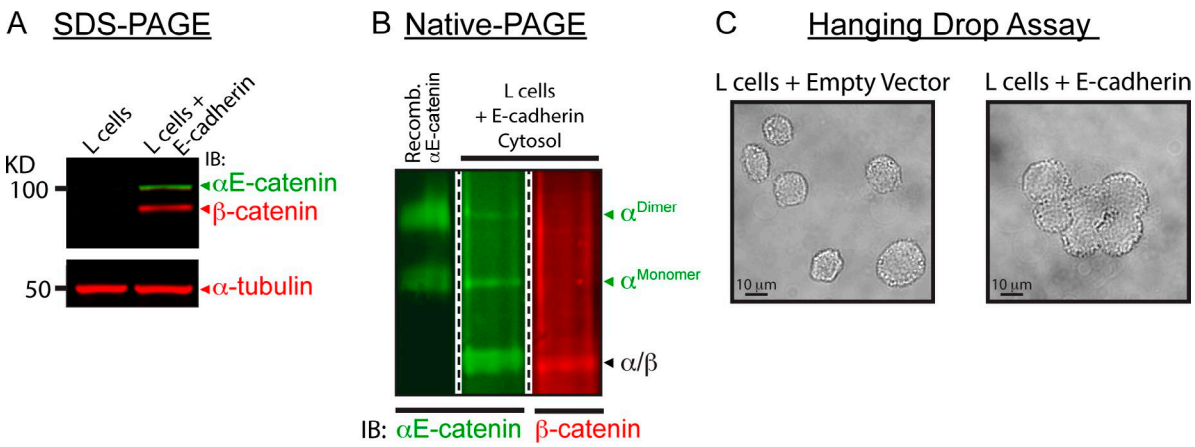

Figure S2. **Cytosolic expression of αE-catenin in L cells, and hanging drop cell–cell adhesion assay.** (A) Total cell lysate of L cells and L cells upon dexamethasone-induced E-cadherin expression were run on SDS-PAGE and blotted for αE-catenin and β-catenin. α-Tubulin serves as a loading control. (B) Recombinant αE-catenin homodimer and monomer and cytosol of L cells upon dexamethasone-induced E-cadherin expression were run on Native-PAGE and blotted for αE-catenin and β-catenin. (C) Hanging drop assay of L cells expressing E-cadherin or an empty vector control are shown. Clusters with four or more cells with compacted membranes were counted in the hanging drop assay.