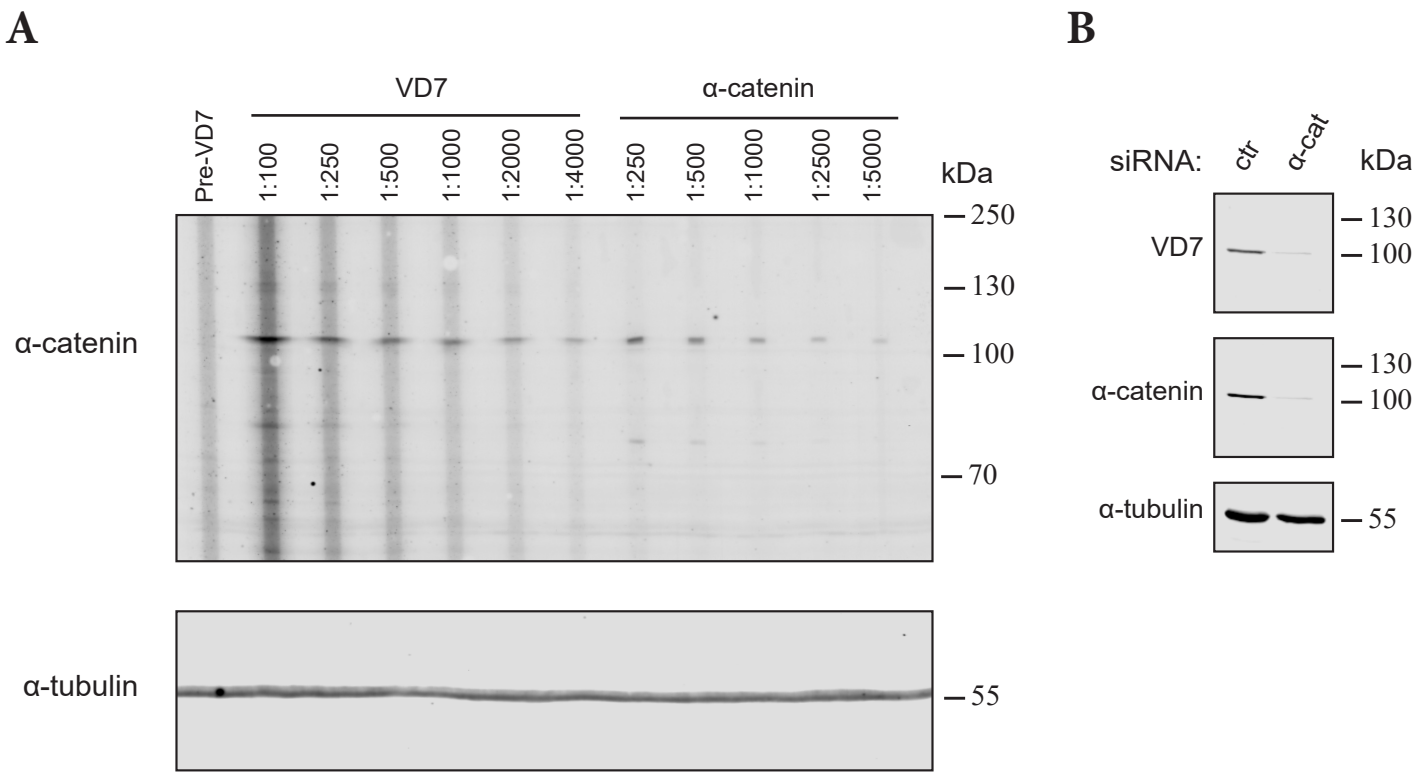


**Fig. S1. VD7 antibody validation.**
(A) Specificity of the VD7 antibody was tested on HUVEC lysates by western blot analysis. Preimmune-serum, VD7 immune serum or a commercial α-catenin antibody were tested at the indicated dilutions. (B) HUVECs were transfected with control siRNA or α-catenin–targeting siRNA for 72h. Total cell lysates were immunoblotted with affinity purified VD7 antibodies or commercial antibodies against α-catenin and α-tubulin (as indicated).

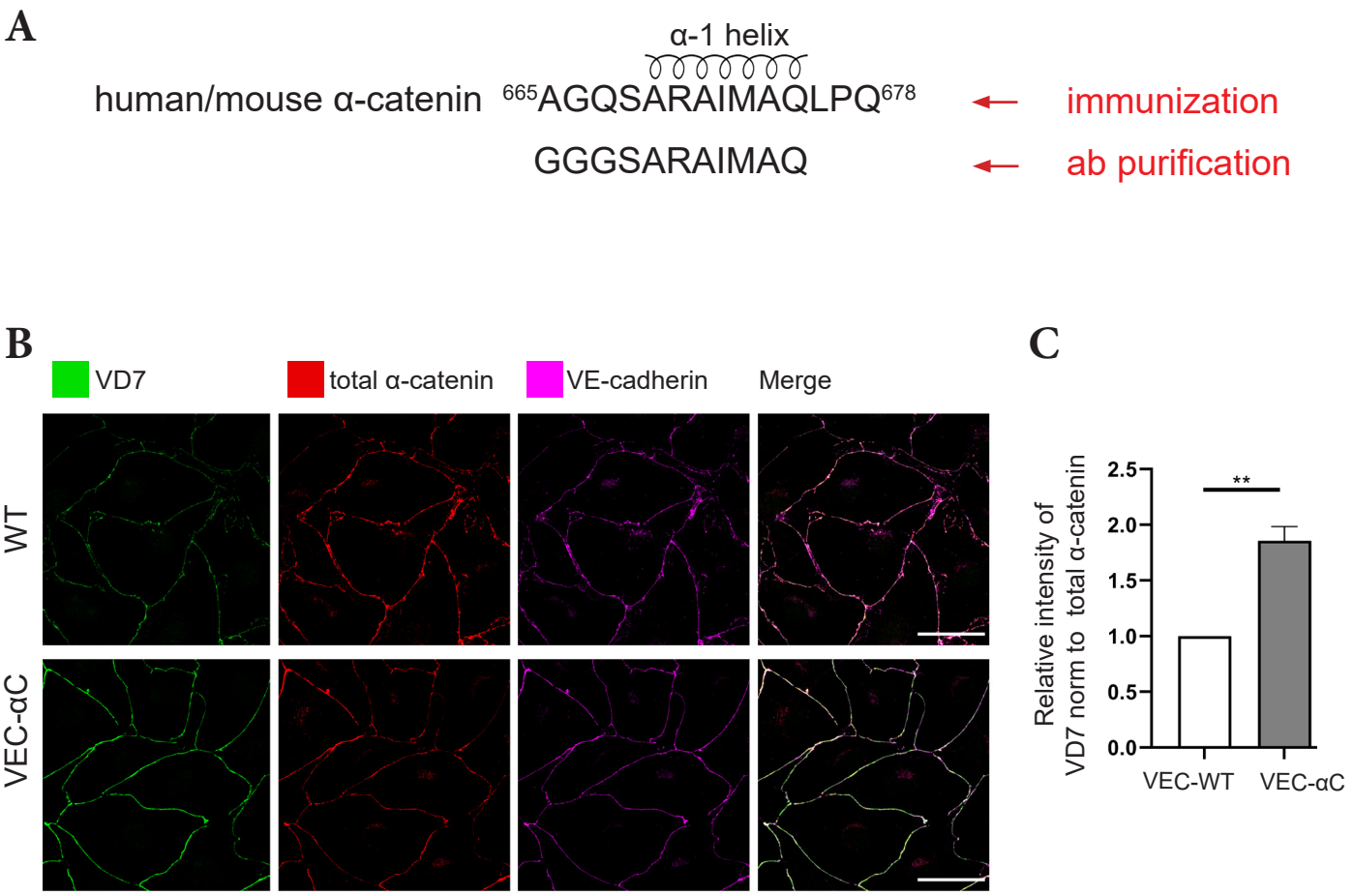


**Fig. S2. Unfolding of the 7 aa α1-helix of α-catenin in junctional VEC-αC, as documented by antibodies restricted to this stretch of amino acids.**
(A) Peptide sequence of α-catenin used for rabbit immunization (top) and for antibody purification (bottom). Note that both peptides share the 7 amino acids that form the α1-helix in α-catenin. (B) Confluent MDMVECs were fixed, permeabilized, and stained with the VD7 antibody restricted to the α1-helix, total α-catenin and VE-cadherin antibodies. (C) Quantification of the signal intensities of staining with the 7aa motif-specific VD7 antibodies relative to total α-catenin signal intensities as shown in (B) (n=3 independent experiments). Bars (B): 25μm. Statistical significance was analyzed using the unpaired two-tailed Student's t-test (C). Results are shown as means ±SEM. **, P ≤ 0.01.

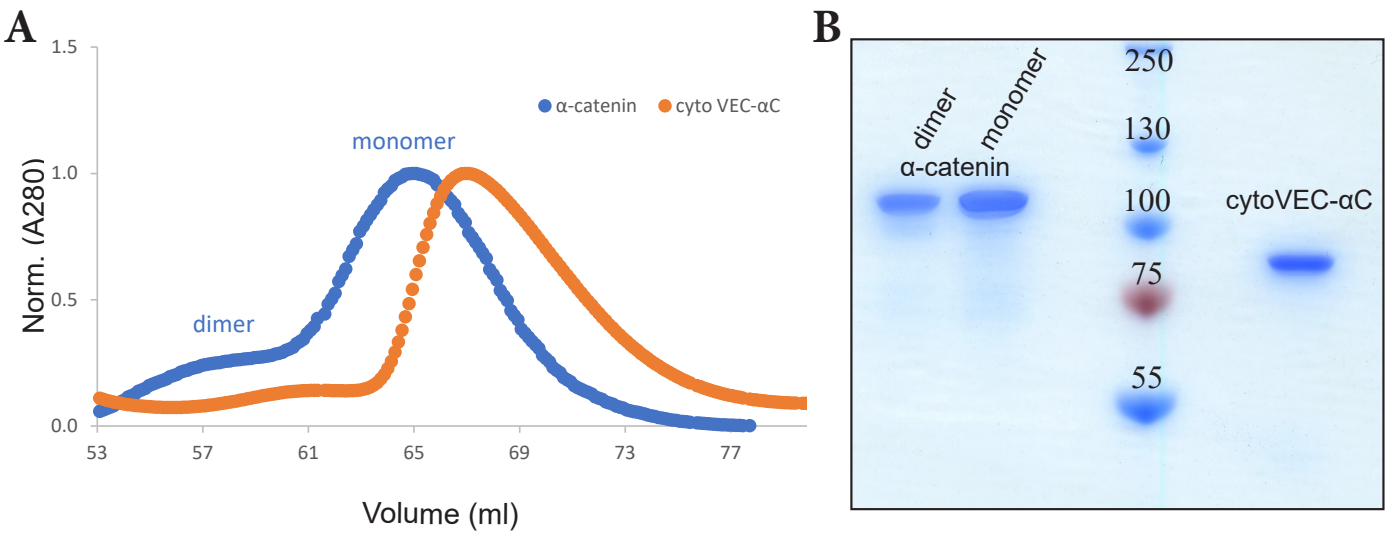


**Fig. S3. Purification of recombinant α-catenin and the cytoplasmic tail of VEC-αC.** (A) Size exclusion chromatography of recombinant α-catenin and the cytoplasmic tail of VEC-αC (cytoVEC-αC). (B) Coomassie-stained SDS-PAGE of isolated dimeric and monomeric α-catenin and cytoVEC-αC (data from one representative experiment of three independent experiments).

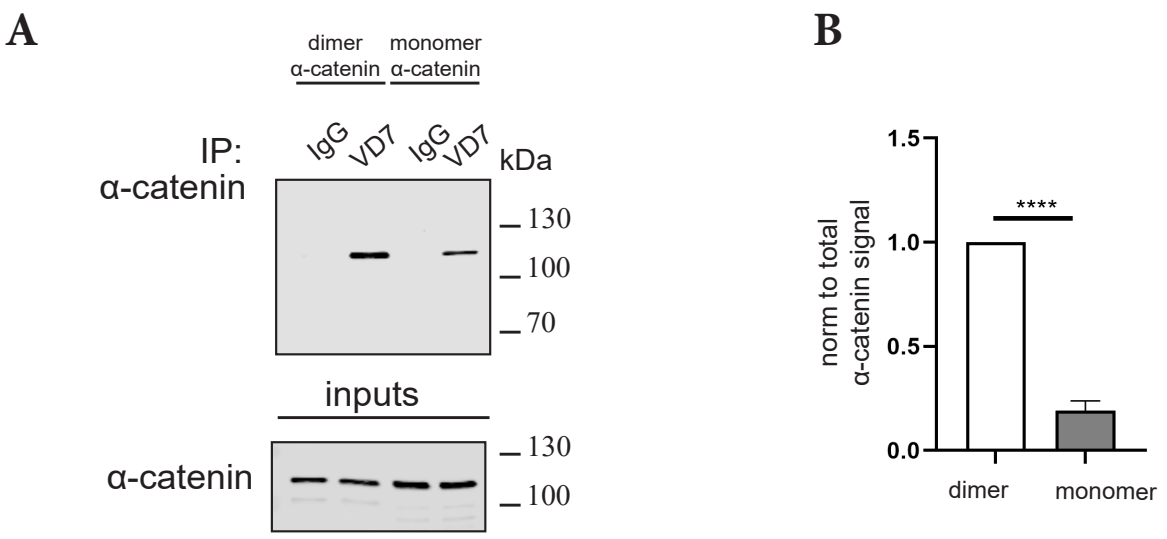


**Fig. S4. The α1-helix is unfolded in dimeric α-catenin.**
(A) α-catenin was precipitated from purified dimeric and monomeric α-catenin preparations using the VD7 antibody. Isotype-matched antibodies were used as a control. The immunoprecipitates were analyzed by SDS-PAGE and immunoblotted for total α-catenin. (B) Quantification of immunoprecipitated α-catenin dimers and monomers relative to total α-catenin input as shown in (A) (n=3 independent experiments). Statistical significance was analyzed using the unpaired two-tailed Student's t-test. Results are shown as means ±SEM. **** P ≤ 0.0001.

**Table S1. Genotypes from intercrosses of VEC-αC_ΔVBD and VEC-αC_swapVBD mice**

| Mating | no. of litters | no. of offspring | +/VEC-αC_ΔVBD | VEC-αC_ΔVBD /VEC-αC_ΔVBD | % of VEC-αC_ΔVBD /VEC-αC_ΔVBD (% of expected) |
|---|---|---|---|---|---|
| +/VEC-αC_ΔVBD x VEC-αC_ΔVBD/ VEC-αC_ΔVBD | 97 | 239 | 199 | 40 | 17 (34) |

| Mating | no. of litters | no. of offspring | +/VEC-αC_swapVBD | VEC-αC_swapVBD /VEC-αC_swapVBD | % of VEC-αC_swapVBD /VEC-αC_swapVBD (% of expected) |
|---|---|---|---|---|---|
| +/VEC-αC_swapVBD x VEC-αC_swapVBD/VEC-αC_swapVBD | 39 | 152 | 104 | 48 | 32 (64) |