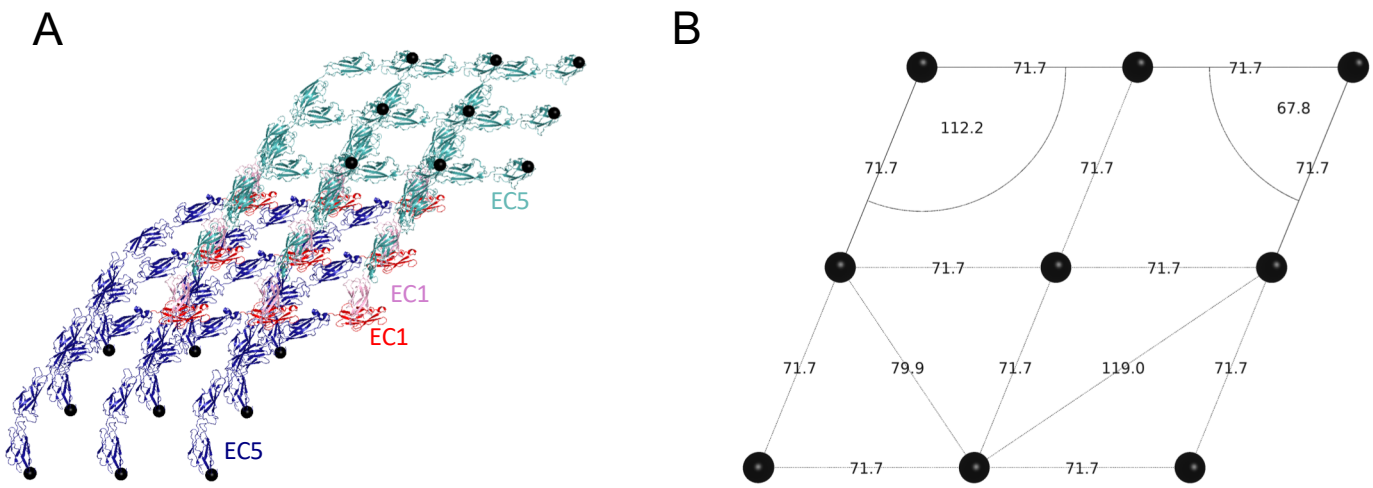


Figure S1

Extracellular cadherin lattice imposes constraints on cytoplasmic components. (**A**) 3x3 lattice formed by *cis*- and *trans*- interactions of E-cadherin ectodomains (blue or cyan) in ribbon representation. EC1 domains that interact in trans are highlighted in red or pink. The N-terminal (EC1) and the C-terminal (EC5) extracellular domains are marked for one cadherin *trans*-dimer. Ca atoms at the C-terminal of EC5 domains are shown as black dots. (**B**) Distances (in Å) and angles (in °) between Ca atoms denoted as dots define spatial constraints imposed by cadherin *cis*- and *trans*- interactions on cytoplasmic components.

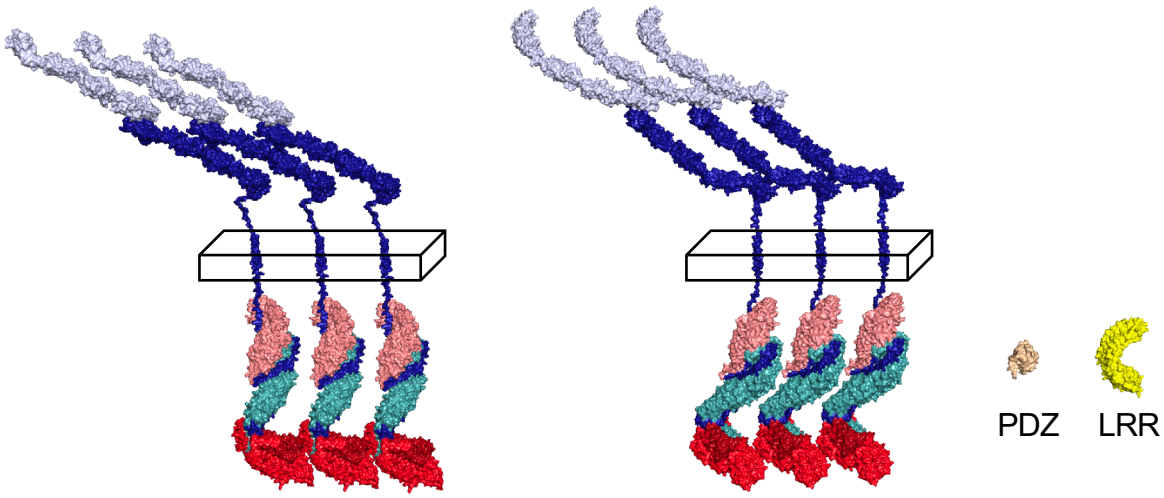


Figure S2

Visual size comparison of erbin and scribble protein domains with lattice spacings. Two 3x1 lattices along two distinct dimensions of the 3x3 lattice in Fig. 4 are shown alongside PDZ and LRR domains, all in surface representations and arranged at the same distance to the observer to demonstrate relative sizes of the proteins with respect to the lattice spacings. A PDZ domain (orange, PDBID 5VWC) would barely fit into the lattice, while LRR domain (yellow, PDBID 4U09) would not fit. All other notations and colors as in Fig. 4.

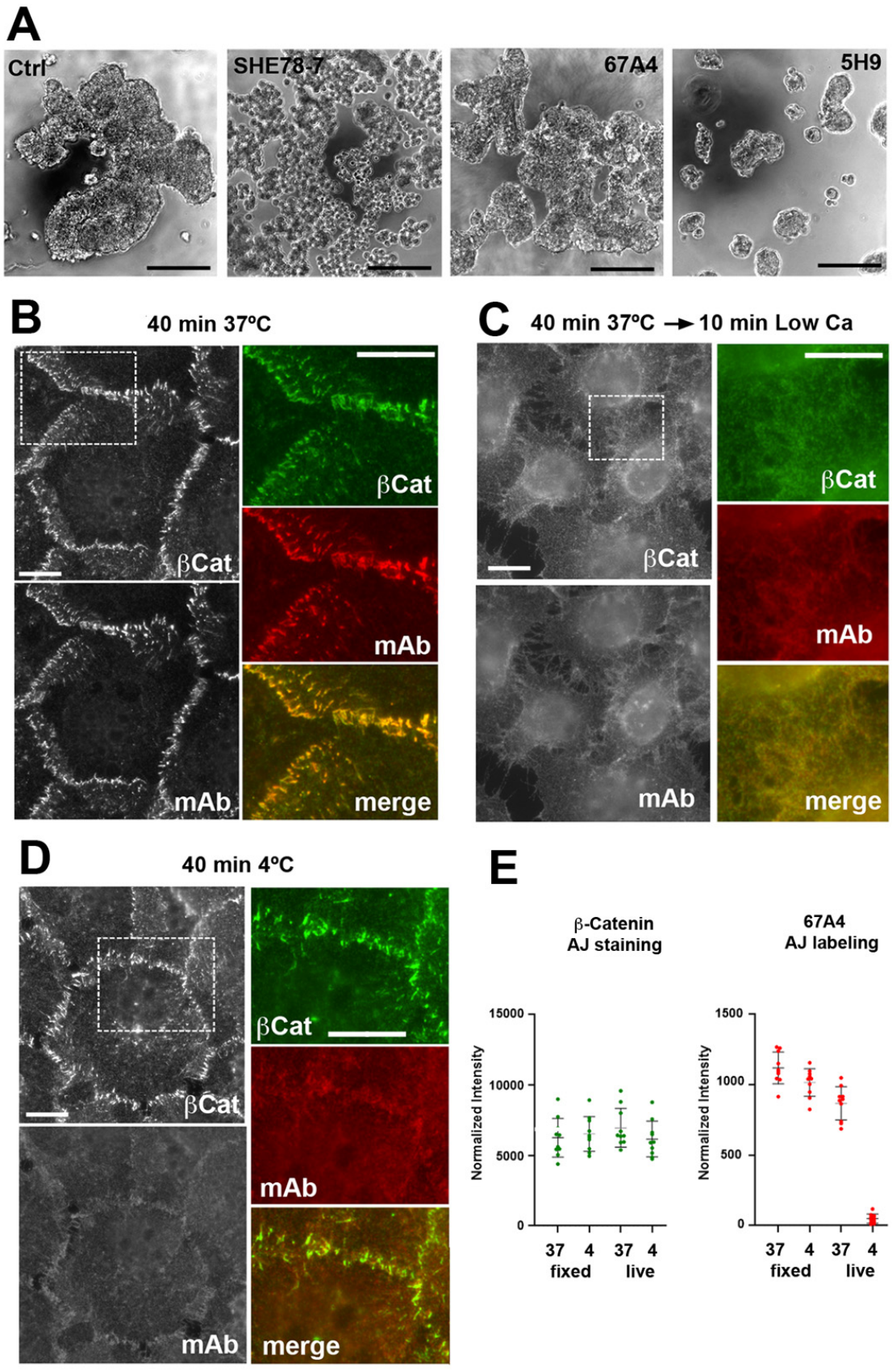


Figure S3

mAbs 67A4 and 5H9 do not block cadherin adhesion. See full caption on the next page

Figure S3

mAbs 67A4 and 5H9 do not block cadherin adhesion. (**A**) Hanging drop assay with A431 cells in standard media (Ctrl), in the presence of adhesion-blocking SHE78-7 (SHE78-7) or adhesion-neutral 67A4 (67A4) or 5H9 (5H9) mAbs. Note that the "neutral" mAbs failed to inhibit formation of compact cell aggregates. Bars, 0.5 μm. (**B**) Immunofluorescence microscopy of A431 cells cultured for 40 min with 67A4 mAb at 37 ºC. Cells were then stained with rabbit β-catenin antibody to reveal AJs (bCat) and with mouse IgG antibody to detect 67A4-bound E-cadherin (mAb). Bar, 10 μm. (**C**) The parallel cell culture, after 40 min with the mAb as in B, was incubated for additional 10 min with 67A4 mAb in low calcium media. Complete dissolution of mAb-bound AJs indicates that the mAb does not cross-link cadherin through adjacent cells. Bar, 10 μm. (**D**) The same experiment as in B but performed at 4ºC. Note that AJs remain unlabeled in metabolically inactive cells. The dash line boxed regions (B-D) are magnified on the right of each panel. To show the distribution of mAb on the cell surface, the exposure time for mAb staining in D was twice longer than that in B and C. Bar, 10 μm. (**E**) Quantification of the 67A4 mAb incorporation into AJs at 4°C and 37°C in fixed and live cells. Fixed and permeabilized A431 cells (fixed) were stained with rabbit β-catenin antibody (β-catenin AJ staining) or with mouse 67A4 mAb (67A4 AJ labeling) at 4°C or 37°C and then stained with the corresponding secondary antibodies at RT. Live cells (live) were cultured for 40 min with the mAb before fixation, and then stained for β-catenin and for the mouse IgG. Note, the staining of AJs in fixed cells is temperature-independent. In live cells, by contrast, the mAb incorporates into AJs only at 37°C.

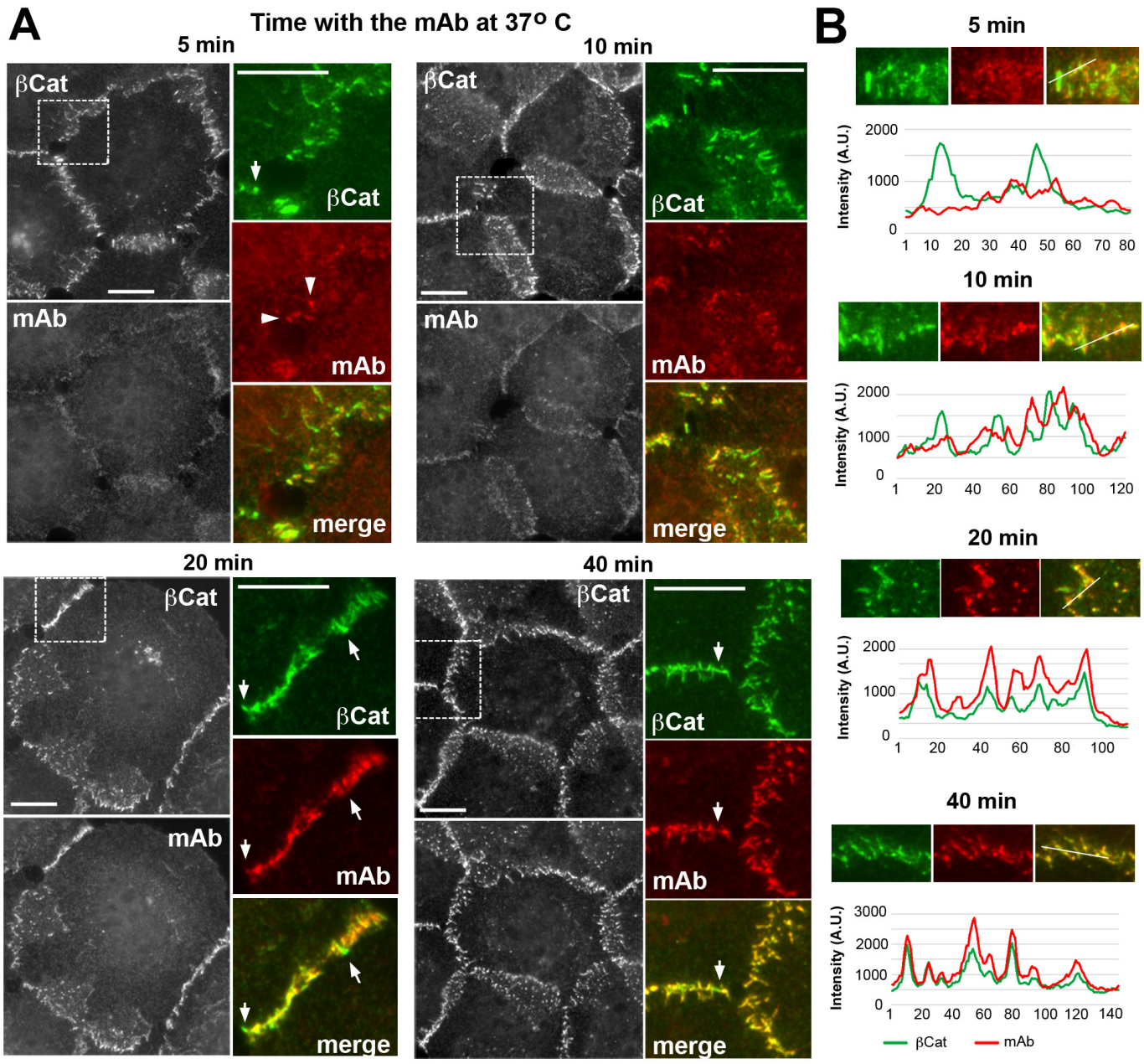
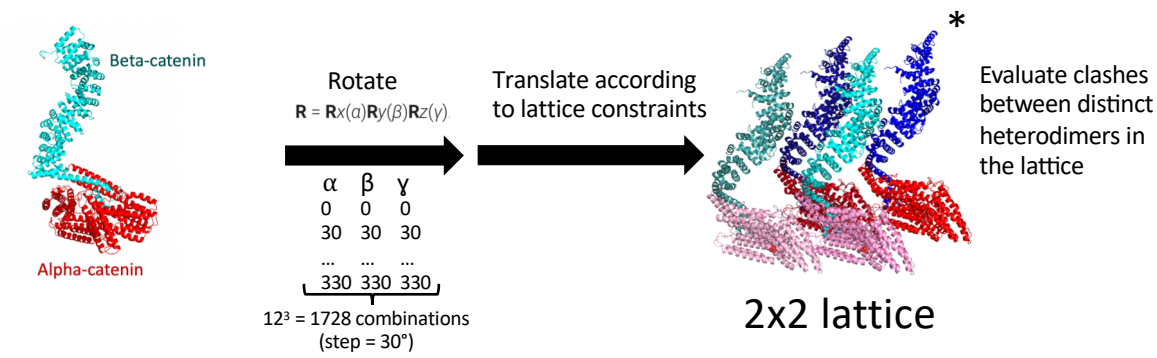


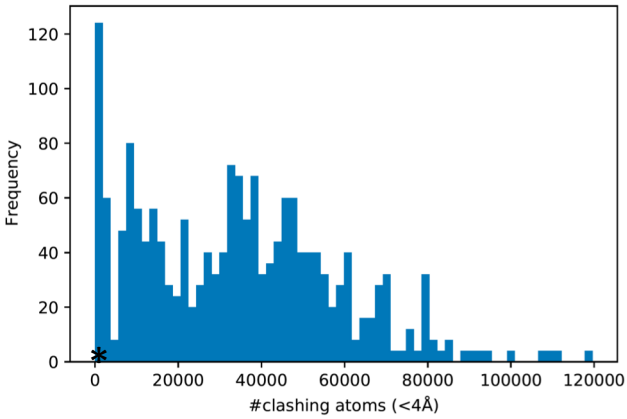
Figure S4

67A4 mAb integrates into the junctions through junction reassembly. (**A**) A431 cells were cultured with 67A4 mAb at 37ºC for 5, 10, 20, or 40 minutes. Cells were then stained for β-catenin (bCat) and for mouse IgG (mAb) as indicated in Fig. 5. Note that mAb-containing clusters gradually replace the unlabeled AJs. Arrowheads show two of the mAb-bound cadherin clusters formed 5 min after addition of the mAb. Arrows show mAb-free AJs after 20 and 40 min in the mAb-containing media. Dashed line boxed regions are magnified on the right. Bars, 10 μm. (**B**) A line scan analysis of the mAb-bound clusters performed along the lines (their lengths are shown in pixels in the graphs) shown in the merge images. The time after the addition of the mAb is indicated. Note that the similarities between mAb and E-cadherin distribution is gradually increasing over time.

A



B



C

atom NH1 (ARG137, chain A) clashes with atom NZ (LYS577, chain C), d =3.9 Å
atom NH1 (ARG137, chain B) clashes with atom NZ (LYS577, chain D), d =3.9 Å
atom NZ (LYS577, chain C) clashes with atom NH1 (ARG137, chain A), d =3.9 Å
atom NZ (LYS577, chain D) clashes with atom NH1 (ARG137, chain B), d =3.9 Å

Figure S5

Finding orientation of a full-length α-catenin/β-catenin with no clashes in the lattice. (**A**) Model of full-length α-catenin/β-catenin heterodimer built from crystal structure fragments in ribbon representation followed by a flow chart to generate 2x2 lattices using rotations by different Euler angle combinations and subsequent evaluation of clashes. (**B**) Distribution of clashes found in 1728 2x2 lattices. (**C**) List of closest atoms in the lattice with minimal number of clashes. This lattice, denoted with asterisk, was used in the final model.

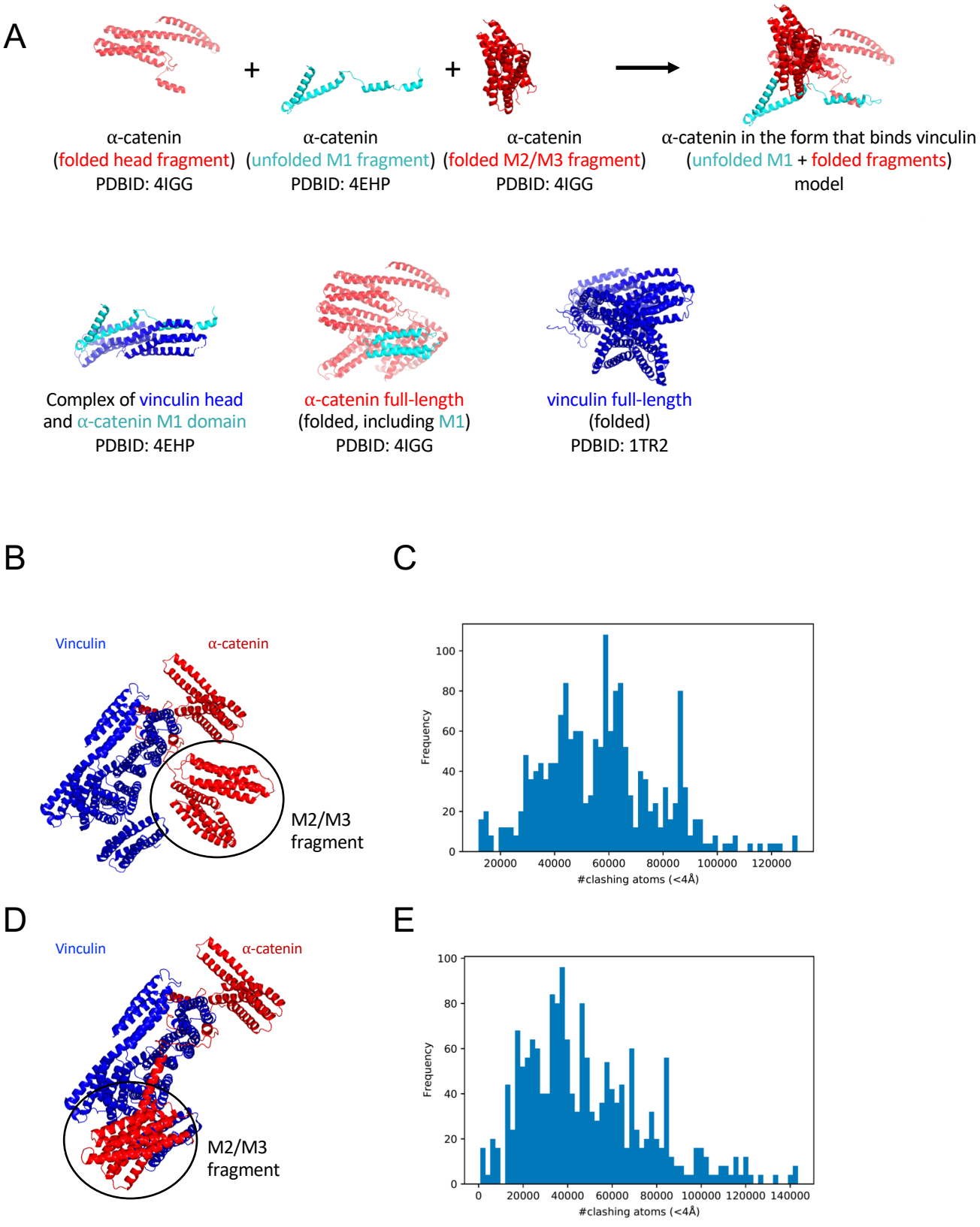
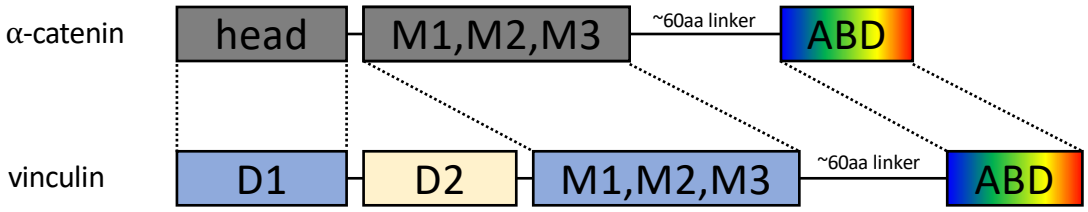


Figure S6

Vinculin is unlikely to form an interspersed lattice with α-catenin in E-clusters. (**A**) Model of α-catenin in the form to bind vinculin and crystal structure fragments (with PDBIDs given in parenthesis) used to build the model, all in ribbon representation. Head domain of full-length α-catenin was stitched to an unfurled M1-domain of α-catenin from the complex with vinculin, and further to the M2/M3 fragment of full-length α-catenin (top panel). The reference crystal structures used to build the model are shown in the bottom panel. (**B**) α-catenin/vinculin heterodimer model in ribbon representation obtained by merging full-length vinculin structure and α-catenin in the form to bind vinculin from (A). (**C**) Distribution of clashes found in 1728 2x2 lattices built by rotating the heterodimer in (B) by different combinations of Euler angles (from a,β,ɣ=0° to a,β,ɣ =330° with a stepsize of 30°. (**D**) same as B but with reoriented M2/M3 fragment - a more compact model. (**E**) Distribution of clashes found in 1728 2x2 lattices built by rotating heterodimer in (D).

A



B

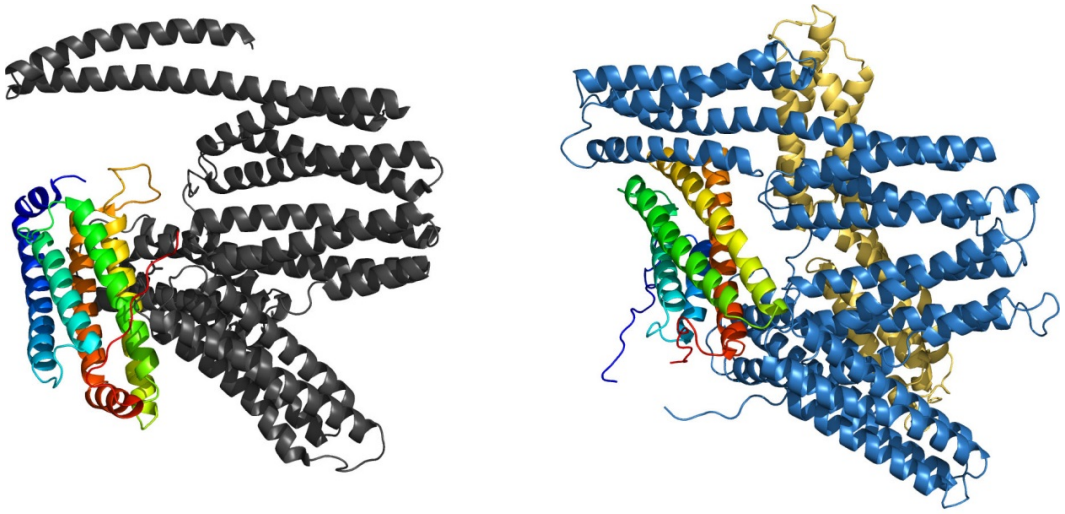


Figure S7

Structural similarity of α-catenin and vinculin. (A) Schematic representation and similarities of domain composition between α-catenin and vinculin. Structurally similar domains are connected by dotted lines. Both proteins can bind actin via ABD (rainbow), which is connected via long and flexible linker to M domain. Vinculin has an extra domain, D2 in yellow, not present in α-catenin. (B) Ribbon representation of aligned full-length α-catenin (PDBID: 4IGG) and vinculin (PDBID: 1TR2) structures. Color code of protein domains as in (A).

			Mean protein spectra counts for cells:					Statistics for EcGFP	
#	Gene	Protein name	EcGFP	WK-EcGFP	αCat-KO	p120-KO	Pg/βCat-KO	stand dev	p value
1	CTNNA1	Catenin Alpha 1	390	388	0	313	0	39.42805383	0.00000010
2	CTNNB1	Catenin Beta 1	372	315	305	184	0	98.23441352	0.00002865
3	CTNND1	Catenin Delta 1	244	235	232	0	61	28.91201760	0.00000026
4	JUP	Plakoglobin	239	343	276	210	0	136.91359806	0.00181053
5	CDH1	E-Cadherin	151	245	410	181	287	51.13000000	0.00018769
6	TLN1	Talin 1	127	136	94	35	3	27.78403104	0.00000952
7	ITGB4	Integrin Subunit Beta 4	61	82	67	30	1	12.30756639	0.00000598
8	ANK3	Ankyrin 3	58	35	39	0	0	13.91470616	0.00001725
9	MYO1C	Myosin IC	49	59	31	5	0	9.87782509	0.00000583
10	PKP4	Plakophilin 4	49	52	51	76	9	18.07260489	0.00017676
11	ARVCF	ARVCF Delta Catenin Family Member	48	48	65	61	45	18.59979519	0.00023093
12	SCRIB	Scribble	41	46	9	27	6	11.88436348	0.00005057
13	PLEKHA5	Pleckstrin Homology Domain Containing A5	41	31	25	8	0	15.69349274	0.00024000
14	CDH3	P-Cadherin	34	0	22	8	14	11.20799035	0.00009320
15	ITGA6	Integrin Subunit Alpha 6	30	31	26	11	0	9.29925752	0.00007282
16	GNAI3	G Protein Subunit Alpha I3	26	30	22	12	8	5.53774924	0.00000831
17	PTPRF	Protein Tyrosine Phosphatase Receptor Type F	26	33	22	4	0	12.15181742	0.00065298
18	ERBIN	Erbb2 Interacting Protein (erbin)	23	19	19	11	0	8.36660027	0.00017189
19	YWHAH	Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Eta	22	26	13	4	2	6.28300808	0.00004638
20	VCL	Vinculin	21	28	29	11	0	9.74435123	0.00056641
21	TAGLN2	Transgelin 2	21	19	17	14	4	4.82059076	0.00001387
22	PPFIA1	PTPRF Interacting Protein Alpha 1	18	18	7	5	0	5.22812905	0.00004918
23	AFDN	Afadin	17	25	7	7	0	5.95618926	0.00013363
24	SRC	Non-Receptor Tyrosine Kinase	17	19	15	4	0	4.64962876	0.00003036
25	SLC9A3R1	SLC9A3 Regulator 1	14	12	8	4	0	2.91138978	0.00000768
26	CYFIP1	Cytoplasmic FMR1 Interacting Protein 1	14	14	2	4	0	7.15807902	0.00093239
27	SPTAN1	Spectrin Alpha	13	2	1	5	0	6.39940473	0.00072180
28	YES1	YES Proto-Oncogene 1	12	13	8	3	0	4.94734176	0.00031723
29	PPP2R1A	Protein Phosphatase 2 Scaffold Subunit Aalpha	12	12	4	5	0	5.09434794	0.00032760
30	EHD4	EH Domain Containing 4	10	9	10	3	0	5.06152621	0.00079280
31	SPTBN1	Spectrin Beta	9	1	0	0	0	10.88467687	0.03740228
32	PPP1CA	Protein Phosphatase 1 Catalytic Subunit Alpha	8	10	5	4	0	5.74041644	0.00649435
33	LPP	LIM Domain Containing Preferred Translocation Partner In Lipoma	7	7	0	0	0	2.30940108	0.00010037
34	CSNK1D	Casein Kinase 1 Delta	7	7	0	0	0	2.47847880	0.00013266
35	PLEKHA6	Pleckstrin Homology Domain Containing A6	7	8	10	0	0	3.81725406	0.00116710
36	TLDC1	LysM-associated domain containing 1	6	10	4	2	0	1.67616342	0.00003452
37	MAPK1	Mitogen-Activated Protein Kinase 1	6	3	2	0	0	3.81725406	0.00372336
38	S100A11	S100 Calcium Binding Protein A11	6	6	4	3	4	1.27241802	0.00001245
39	STAT3	Signal Transducer And Activator Of Transcription 3	6	6	3	0	0	3.95209408	0.00486935
40	DSG2	Desmoglein 2	6	5	4	0	0	4.59813627	0.00614967
41	RAP1A	RAP1A	6	7	7	4	2	2.03540098	0.00013376
42	UBASH3B	Ubiquitin Associated And SH3 Domain Containing B	6	11	5	0	0	4.52506248	0.00574050
43	FAM110A	Family With Sequence Similarity 110 Member A	6	4	0	0	0	2.23606798	0.00019614
44	CPNE3	Copine 3	5	5	2	0	0	2.57275098	0.00070159
45	LRRC1	Leucine Rich Repeat Containing 1	5	5	2	0	0	2.99205297	0.00339240
46	SEPTIN9	Septin 9	5	0	0	0	0	3.38765265	0.00349303
47	DLG1	Discs Large MAGUK Scaffold Protein 1	5	5	3	0	0	3.65148372	0.00552972
48	PSEN1	Presenilin 1	5	5	4	0	0	1.60356745	0.00006284
49	PDLIM1	PDZ And LIM Domain 1	5	6	0	3	0	2.91138978	0.00170927
50	CTTN	Cortactin	4	4	2	0	0	2.54483604	0.00351881
51	CAP1	Cyclase Associated Actin Cytoskeleton Regulatory Protein 1	4	0	0	0	0	3.40168026	0.00787071
52	PKP3	Plakophilin 3	4	12	15	0	0	1.34518542	0.00009186
53	DSTN	Destrin	4	2	1	0	0	1.61834719	0.00017624
54	VASP	Vasodilator Stimulated Phosphoprotein	4	5	4	0	0	2.58198890	0.00318304
55	PLEKHA4	Pleckstrin Homology Domain Containing A4	4	10	6	4	0	2.88675135	0.00525169
56	AP1B1	Adaptor Related Protein Complex 1 Subunit Beta 1	3	5	5	1	3	1.39727626	0.00139858
57	PXN	Paxillin	3	4	0	0	0	1.34518542	0.00067816
58	PTK7	Protein Tyrosine Kinase 7	2	3	4	0	0	2.62769136	0.03049066
59	S100A2	S100 Calcium Binding Protein A2	2	1	1	0	0	1.61834719	0.00368203

Core Components of Cadherin-Catenin Complex
Lateral Membrane Receptors and their Adaptors
Actin Cytoskeleton
Signaling Intermediates
Traffic
Others

Table S1

Proteins identified in anti-GFP precipitates.

Movie S1.

Dynamics of the mAb-bound E-cadherin clusters. A431 cells expressing EcDn were briefly stained with the Alexa Fluor 594-conjugated 67A4 mAb and then immediately imaged simultaneously in green and red channels. Images were acquired at 10 sec intervals.