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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main

Statistical parameters

text	text, or Methods section).			
n/a	Co	nfirmed		
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement		
		An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly		
		The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.		
\boxtimes		A description of all covariates tested		
\boxtimes		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons		
		A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)		
		For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> values as exact values whenever suitable.		
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings		
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes		
\boxtimes		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated		
		Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)		

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	All software used in this study for data collection are either commercially available or open source.	
Data analysis	All software used in this study for data analysis are either commercially available or open source.	

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Crystal structure coordinates and structure factors of α Ncat-ABD-H1 form A, α Ncat-ABD-H1 form B and α Ecat-ABD-WT are deposited in the Protein Data Bank under accession codes: 6DUW, 6DUY and 6DV1, respectively. Backbone chemical shift assignments of α Ncat-ABD-H1 are deposited in the Biological Magnetic Resonance Data Bank under accession code 27526. All reagents and experimental data are available from the authors upon request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Eco

avioural & social sciences 🛛 🗌 Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.						
Sample size	Sufficient sample sizes were chosen for each experiment to determine whether the outcome was statistically significant.					
Data exclusions	No data were excluded from this study.					
Replication	We confirmed that all attempts to replicate experiments were successful.					
Randomization	Randomly selected samples and organisms were allocated into experimental groups.					
Blinding	Blinding was not implemented in this study.					

Reporting for specific materials, systems and methods

Materials & experimental s	ystems Me	thods			
n/a Involved in the study	n/a	Involved in the study			
Unique biological mater	ials 🛛 🕅	ChIP-seq			
Antibodies	\boxtimes	Flow cytometry			
Eukaryotic cell lines	\boxtimes	MRI-based neuroimaging			
Palaeontology					
Animals and other organ					
Human research participants					
Unique biological materials					
Policy information about <u>availability of materials</u>					
Obtaining unique materials all unique biological materials are readily available from the authors					

Antibodies

Antibodies used	hybridoma mouse anti-αCatenin (5B11; undiluted), polyclonal rabbit anti-phospho-serine 641 αCat (21330; 1:300, Signalway Antibody), monoclonal mouse anti-GAPDH (9484; 1:5000, Abcam), fluorescently labeled donkey anti-mouse and -rabbit antibodies (680RD or 800RD; 1:5000, LiCor Biosciences), secondary abs IgG Alexa Fluor 488 or 568-conjugated goat anti-mouse or -rabbit antibodies (1:200, Invitrogen), anti-DDDDK tag rabbit polyclonal antibody, which recognizes the FLAG tag (1:200, MBL), anti-ZO-1 mouse monoclonal antibody (T8-754; undiluted, a gift from Sa. Tsukita, Osaka University, Japan), anti-E-cadherin mouse monoclonal antibody (clone36/E-cadherin; 1:100, BD) and anti-E-cadherin rat monoclonal antibody (ECCD2; 1:200, a gift from M. Takeichi, Riken CDB, Japan), Alexa Fluor 488- or 555-conjugated secondary antibodies (1:200) were purchased from Invitrogen, and Alexa Fluor 647-conjugated phalloidin (1:50, Invitrogen) was used for staining actin filaments.
Validation	all primary antibodies were validated by the suppliers

Eukaryotic cell lines

Policy information about <u>cell lines</u>

Cell line source(s)

DLD1 R2/7 from Dr. Fran van Roy (Ghent University, Belgium)

Authentication	none of the cell line used were authenticated	
Mycoplasma contamination	cell lines were not tested for mycoplasma contamination	
Commonly misidentified lines (See <u>ICLAC</u> register)	n/a	

Animals and other organisms							
Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research							
Laboratory animals	Drosophila melanogaster						
Wild animals	n/a						
Field-collected samples	n/a						