nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on 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.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Metamorph was used to acquire the images on a Leica Spinning Disk microscope as described in the Methods section. Simulation results were generated using a custom C++ code adapted from a previously published code available at https://github.com/torressancheza/ias .
Data analysis	FIJI was used to contrast and overlay images. Cell segmentation was performed with previously published FIJI plugin LimeSeg 0.4.2 (https://imagej.net/plugins/limeseg). All quantifications and data analysis were then performed using custom Python codes as described in the Methods section and Supplementary Information. Visualisation and rendering of 3D meshes were performed using Paraview Software 5.10.1. All plots were generated with Python (version 3.9.10).

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data are available upon request to the corresponding authors.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	All quantifications were performed for at least n=12 cell doublets, except for Fig. 5b,c (4 doublets), Fig.5e (9 doublets), Fig. 5g,h (9 doublets) and Ext. Fig. 1c (red curve, 6 doublets).
Data exclusions	No points were excluded from the data analysis.
Replication	All experiments were systematically performed at least 3 times and gave similar results.
Randomization	Cell doublets were chosen to be imaged randomly among a large population. The subset of doublets used for segmentation were chosen randomly among those with a rotation axis approximatively aligned with the microscope Z-axis (with random clockwise or counterwise rotation). This allowed better segmentation of cells.
Blinding	n/a

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

1. Rat monoclonal Anti-E-cadherin, Abcam, Cat# Ab11512.
2. Rabbit Polyclonal Anti-Phospho-Myosin Light Chain2 (Cell signaling technology, #3674).
3. Rabbit monoclonal Anti-Paxillin (Abcam, Ab32084).
4. Alexa FluorTM 357 Phalloidin 488 (Thermo Fisher, A12379).

Validation

Validated by the Companies.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

1. MDCK II VASP-GFP (see ref. 34).
2. MDCK II MRLC-KO1/E-cadherin-mNG and MDCK II MRLC-GFP (Riveline Lab.).
3. MDCK II E-cadherin-GFP and MDCK II E-cadherin-DsRed (from Nelson, see ref. 37).
4. MDCK II E-cadherin-GFP/Podocalyxin-mScarlett/Halo-CAAX (engineered in Honigmann Lab)
5. MDCK II iLID-LARG::mVenus - 2xrGBD-dTomato - MRLC-iRFP703 (optogenetic cell line, Riveline Lab.).
6. MDCK II Actin-GFP (Nelson Lab)
7. MDCK II Lifeact-iRFP (Riveline Lab)
8. MDCK II E-cadherin-KO (from Ladoux lab, ref. 16)

Authentication

From the sources.

Mycoplasma contamination

All cell lines were checked for the absence of mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in the study.