

## 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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)
<input checked="" type="checkbox"/>	<input type="checkbox"/> For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted <i>Give <math>P</math> values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input checked="" type="checkbox"/>	<input type="checkbox"/> 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	Not applicable
Data analysis	Not applicable

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](#)

Source data are provided with this paper. The raw images from immunofluorescence experiments generated in this study have been deposited in the FigShare database under the accession code: <https://doi.org/10.6084/m9.figshare.28295240>.

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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](https://www.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	No statistical method was used to predetermine the sample size. However, for the immunofluorescence (IF) experiments, we captured as many images as possible in each experiment. To ensure statistical confidence, we obtained a minimum of 5 biological replicates for each independent experiment, and we analyzed around 30 junctions per biological replicates. With the exception of a few specific experiments mentioned in the manuscript, we conducted each experiment 3 times independently and pooled the data from the three replicates for the final graphs. Specific quantification numbers are provided in the figure legend. Finally, although the IF results were provided only for 1 clone in the manuscript, we performed all these experiments in 3 different clonal cell lines which gave essentially similar results. This approach is in agreement with standard practices in the field, ensuring the reproducibility and robustness of our findings.
Data exclusions	We used the nested analysis from Graphpad Prism to identify the outliers. Any detected outliers (only few data points) were excluded from the statistical analysis.
Replication	All experiments were performed at least 3 times except in very few cases (specified into the manuscript). For each experiment, the number of biological replicates is indicated in the figure legend.
Randomization	This is not relevant to this study as we selected representative images for every experiments. We did not have any experiments where randomization would be pertinent.
Blinding	This is not relevant for our study as cited above.

## 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

### Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

### Antibodies used

- Mouse IgG2b monoclonal anti-gamma-actin #2A368E2
- Mouse IgG1 monoclonal anti-beta-actin #4C259H12
- Rat polyclonal anti-PLEKHA6 #RtSZR127
- Guinea pig polyclonal anti-PLEKHA7 #GP2737
- Rabbit polyclonal anti-NM2A #909801
- Rabbit polyclonal anti-NM2B #909901
- Mouse monoclonal anti-pan-actin #mab1501
- FITC-phalloidin # P5282
- Mouse monoclonal anti-GFP #11814460001
- Mouse monoclonal anti-beta-tubulin #32-2600
- Rat monoclonal anti-ZO-1 #R40.76
- Mouse monoclonal anti-ZO-1 #33-9100
- Rabbit polyclonal anti-cingulin #C532
- Rabbit polyclonal anti-beta-catenin #C2206
- Rabbit polyclonal anti-E-cadherin #7870
- Mouse monoclonal anti-E-cadherin #BD610181
- Rabbit polyclonal anti-Claudin-1 #51-9000
- Mouse monoclonal anti-Claudin-2 #32-5600
- Rabbit polyclonal anti-Claudin-3 #34-1700
- Mouse monoclonal anti-Claudin-4 #32-9400
- Rabbit polyclonal anti-Claudin-7 #34-9100
- Rabbit polyclonal anti-Claudin-8 #40-0700Z
- Rabbit polyclonal anti-Claudin-10 #38-8400

### Validation

- Validation 2A368E2 and 4C259H12: Dugina et al 2009, DOI: 10.1242/jcs.041970
- Validation RtSZR127 and GP2737: Sluysmans et al 2021, DOI: 10.1091/mbc.E21-07-0355
- Validation 909801 and 909901: Weissenbruch et al 2021, DOI: 10.7554/eLife.71888
- Validation mab1501: [https://www.merckmillipore.com/CH/fr/product/Anti-Actin-Antibody-clone-C4,MM\\_NF-MAB1501](https://www.merckmillipore.com/CH/fr/product/Anti-Actin-Antibody-clone-C4,MM_NF-MAB1501)
- Validation P5282: [https://www.sigmaaldrich.com/CH/fr/product/sigma/p5282?srsltid=AfmBOopeNmsOY1FRyv9KDNRW70Ye8iO\\_JZJRNfMecl3OJl3LKM\\_xJsx](https://www.sigmaaldrich.com/CH/fr/product/sigma/p5282?srsltid=AfmBOopeNmsOY1FRyv9KDNRW70Ye8iO_JZJRNfMecl3OJl3LKM_xJsx)
- Validation 11814460001: <https://www.citeab.com/antibodies/8906830-11814460001-anti-gfp>
- Validation 32-2600: <https://www.citeab.com/antibodies/2399654-32-2600-beta-tubulin-monoclonal-antibody-2-28-33>
- Validation R40.76: Itoh et al 1993, DOI: 10.1083/jcb.121.3.491
- Validation 33-9100: Spadaro et al 2017, DOI: 10.1016/j.cub.2017.11.014
- Validation C532: Cordenonsi et al 1999, DOI: 10.1083/jcb.147.7.1569; Rouaud et al 2023, DOI: 10.1083/jcb.202208065
- Validation C2206: <https://www.citeab.com/antibodies/1201469-c2206-anti-catenin-antibody-produced-in-rabbit>
- Validation 7870: [https://www.scbt.com/fr/p/e-cadherin-antibody-h-108?srsltid=AfmBOoqsXPmlMMq1msOqCXiafCype\\_4QRvDQ9JjTt8lJtSrkkOW2NM-S](https://www.scbt.com/fr/p/e-cadherin-antibody-h-108?srsltid=AfmBOoqsXPmlMMq1msOqCXiafCype_4QRvDQ9JjTt8lJtSrkkOW2NM-S)
- Validation BD610181: [https://www.bdbiosciences.com/en-ie/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-e-cadherin.610181?tab=citations\\_references](https://www.bdbiosciences.com/en-ie/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-e-cadherin.610181?tab=citations_references); Capaldo et al 2007, DOI:10.1091/mbc.E06-05-0471
- Validation 51-9000: Arnold et al 2024, DOI: 10.1111/exd.15084
- Validation 32-5600: Raju et al 2020, DOI: 10.1172/JCI138697
- Validation 24-1700, 32-9400 and 34-9100: Furuse et al 2022, DOI: 10.1247/csf.22068
- Validation 40-0700Z: Sassi et al 2020, DOI: 10.1681/ASN.2019080790
- Validation 38-8400: Prot-Bertoye et al 2021, DOI: 10.1152/ajprenal.00579.2020

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

### Cell line source(s)

MDCKII (Madin-Darby Canine Kidney type II, female, tet-off) cell lines were kindly provided by A. Fanning from the University of North Carolina.  
EpH4 (mouse mammary epithelial, female) cell lines were kindly provided by E. Reichmann from the Hebrew University of Jerusalem: Fialka I et al 1996, DOI: 10.1083/jcb.132.6.1115.  
mCCD (Mouse Cortical Collecting Duct) cell lines were kindly provided by E: Féraillé from the University of Geneva: Wang Y.B. et al 2014, DOI: 10.1681/ASN.2013040429.

### Authentication

Cell lines were not authenticated.

### Mycoplasma contamination

Cells were regularly tested for mycoplasma contamination and were negative for mycoplasma.

### Commonly misidentified lines (See [ICLAC](#) register)

No commonly misidentified cell lines were used.

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable