

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 [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- 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.
- 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 statistics 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
- 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

Data collection was carried out using Leica Application Suite v4.5 and Nikon ACT-1 v2.62, for imaging in epifluorescence and brightfield, and Zeiss' Zen 2012 SP1 Black edition for confocal and multiphoton imaging.

Data analysis

Data analysis was performed using commercial software packages, including Bitplane Imares 8.0 for cell tracking, ImageJ 1.5.1U for image analysis, GraphPad Prism 6 for statistical analyses and graphing, Microsoft Excel 2010 was used for data entry and simple calculation, MATLAB R2015a for coding of various computational tasks, including the mathematical model and postprocessing of data, and Adobe Illustrator CS6 was used for final assembly of figures and schematics.

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

Source data for all figures are provided with the paper.

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 Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Life sciences study design

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

Sample size	Sample sizes were chosen by taking into account the variability of each measure, such that addition of further samples would not alter the interpretation of results, and to keep consistent with standards in the field of study. We deemed the n sufficient owing to small subsequent changes in mean relative to measurement error by addition of n.
Data exclusions	Embryos deemed as abnormal or damaged with respect to previously established criteria and due to either electroporation or subsequent experimentation were omitted from analyses.
Replication	All experiments were repeated as described within the manuscript. Consistency across replicates is captured in each experiment by means and standard deviations calculated across multiple replicates. All attempts at replication were successful to the extent reflected in means, data distributions, and statistical tests described in the manuscript.
Randomization	Samples were randomly allocated to distinct experimental groups in each experiment.
Blinding	In data collection, blinding was performed when possible. However blinding was not always possible owing to the nature of the experiment (e.g. small molecule inhibitor that tints the media yellow). Quantitative, and whenever possible automated approaches were used to further eliminate observer bias during analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Unique biological materials
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants

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

chicken anti-GFP (1:1,000, ab13970, abcam), rabbit anti-phospho histone H3 (pHH3, 1:300, 06-570, Millipore), mouse anti-laminin (1:100, 3H11, Developmental Studies Hybridoma Bank), mouse anti-Ecadherin (1:100, 610182, BD Biosciences), mouse anti-aPKC Zeta (1:100, sc17781, Santa Cruz), mouse anti-ZO-1 (1:100, 33-9100, Thermo Fisher), rabbit anti-RhoA (1:300, 2117, Cell Signaling), mouse anti-RhoA-GTP (1:100, 26904, New East Biosciences), rabbit anti phospho-myosin light chain (1:300, 3671, Cell Signaling), rabbit anti di-phospho (dp) ERK 1/2 (1:300, 4376, Cell Signaling), horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody (1:500 7074, Cell Signaling), biotinylated anti-rabbit antibody, HRP-conjugated streptavidin (each 1:500 for 1 hour at room temperature, 111065003 and 016030084, respectively, Jackson Immuno).

Validation

All primary antibodies were validated by manufacturer, as per statements included below, and were validated using positive controls in the brain and neural tube, where epithelial markers, RhoA activity, and myosin phosphorylation are well characterized, and in particular in the midbrain hindbrain boundary, a site of well characterized and specific FGF activity. chicken anti-GFP was validated in our system by immunostaining embryos with and without electroporation of a plasmid encoding GFP. Validation by manufacturer employed transgenic mouse spinal cords in western blot and GFP-transfected NIH/3T3 cells by immunofluorescence: <https://www.abcam.com/gfp-antibody-ab13970.html>

pHH3 was validated by our group in confirming the mitogenic activity of Noggin in the embryonic small intestine mesenchyme, and by the manufacturer for immunofluorescent staining applications: http://www.emdmillipore.com/US/en/product/Anti-phospho-Histone-H3-Ser10-Antibody-Mitosis-Marker,MM_NF-06-570

laminin validated in chick by "Axonal Growth on Solubilized and Reconstituted Matrix from the Embryonic Chicken Retina Inner Limiting Membrane." by Von Boxberg Y. in The European journal of neuroscience 4.9 (1992): 840-852. http://dshb.biology.uiowa.edu/laminin_3

E-Cadherin validated by manufacturer in immunofluorescent staining of WIDR cells at 1:50 dilution and by Western blot: <http://www.bdbiosciences.com/eu/applications/research/stem-cell-research/cancer-research/human/purified-mouse-anti-e-cadherin-36e-cadherin/p/610182>

PKC validated by manufacturer in western blot and immunofluorescence staining of formalin-fixed

SW480 cells showing membrane localization: <https://www.scbt.com/scbt/product/pkc-zeta-antibody-h-1>

ZO-1 manufacturer validated in iPSC-derived forebrain organoids derived at Day 40: <https://www.thermofisher.com/antibody/product/ZO-1-Antibody-clone-ZO1-1A12-Monoclonal/33-9100>

RhoA manufacturer validated by western blot analysis of extracts from various cell lines, and using tagged RhoA expression in cell lines followed by western blot: <https://www.cellsignal.com/products/primary-antibodies/rhoa-67b9-rabbit-mab/2117>

RhoA-GTP validated by western blotting of MEF cells were treated with PDGF.: <http://www.neweastbio.com/RhoAssay.do>

pMLC manufacturer validated by immunofluorescence in phosphate treated HeLa cells, and by western blot of m HEK293 cells stimulated with ionophore A23187 : <https://www.cellsignal.com/products/primary-antibodies/phospho-myosin-light-chain-2-ser19-antibody/3671>

ERK manufacturer validated by immunofluorescence of human breast carcinoma cells and by western in extracts from serum-induced PC12 cells: https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-antibody/9102?gclid=Cj0KCQjw_vfcBRDJARIsAJafEnFolvxQQyQlxwVkhoSKyaMQwVZxmD1MGD6Lx12-z3i3jT-XqwBzrEwaAgUzEALw_wcB

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Fertilized White Leghorn chicken eggs were used in this study, and all experiments were carried out according to Hamburger Hamilton staging, between HH10 (E1.5) and HH 18 (E3)

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve field collected animals.