Inature portfolio | reporting summary

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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Statistics

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	firmed
	\square	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\square	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.
	\square	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.
\checkmark		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\checkmark		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\checkmark		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 FEM models were developed by means of ABAQUS/CAE/ABAQUS Standard (SIMULIA, Dassault Systems). For the estimation of iMeSH nominal stiffness, Scilab (version 6.1.0, Esi Group, Paris, France) was used. Immunostaining and live imaging data were acquired using ZEN 2.3 software.

Data analysis

All quantifications reported have been performed with ImageJ software (1.54f). All statistical analyses were performed with Origin 2020. We expressed data as mean ± s.d. or mean ± 95% CI of multiple biological replicates (as indicated in the figure legends).

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

The source data used for the graphs are available as Supplementary information. The raw microscopy data supporting the findings are available on Zenodo.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race</u>, <u>ethnicity and racism</u>.

Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	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.

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

Life sciences study design

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

Sample size	N numbers defined by the expected magnitude of difference between treatment groups and anticipated variance to detect biologically relevant differences at p value< 0.05, power 0.8.
Data exclusions	No data were excluded.
Replication	Specified in each Figure legend.
Randomization	Embryos needed to be stage-matched to receive vehicle or ROCK inhibitor treatment within each experiment.
Blinding	The effect of ROCK inhibitor was obvious so it was not possible to do the analysis blindly.

Behavioural & social sciences study design

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

Study description	
Research sample	
Sampling strategy	
Data collection	
Timing	
Data exclusions	
Non-participation	
Randomization	

Ecological, evolutionary & environmental sciences study design

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

Study description		
Research sample		
Sampling strategy		
Data collection		
Timing and spatial scale		
Data exclusions		
Reproducibility		
Randomization		
Blinding		
Did the study involve field work? Yes No		

Field work, collection and transport

Field conditions	
Location	
Access & import/export	
Disturbance	

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	Methods		
n/a Involved in the study	n/a Involved in the study		
Antibodies	ChIP-seq		
Eukaryotic cell lines	Flow cytometry		
Palaeontology and archaeology	MRI-based neuroimaging		
Animals and other organisms			
Clinical data			
Dual use research of concern			
Plants			
Antibodies			

Antibodies usedThe primary antibody against Myosin IIb (CMII 23s) was purchased from Developmental Studies Hybridoma Bank stock
concentration of 44 g/mL. The secondary goat anti-mouse Alexa 568 antibody was from Life Technologies, #A-11004.ValidationCM1123 produced and validated in: 10.1002/cm.970190307

Eukaryotic cell lines

Policy information	about o	cell lines	and Sex and	Gender in	Research

Cell line source(s)	iPSC line HO-193b was differentiated into neuroepithelial cells over eight days using dual-SMAD inhibition as previously reported.
Authentication	The iPSC line used had been previously published
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See <u>ICLAC</u> register)	

Palaeontology and Archaeology

Specimen provenance				
Specimen deposition				
Dating methods				
Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.				
Ethics oversight				

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

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and <u>Sex and Gender in</u> <u>Research</u>

Laboratory animals	Fertilised chicken eggs (Gallus gallus) were supplied by a commercial lab supplier, ensuring welfare of adult chickens.
Wild animals	
Reporting on sex	
Field-collected samples	
Ethics oversight	The chicken embryos were dissected from the eggs after 34 hours incubation and cultured in vitro for up to 20 hours after dissection. No procedures were carried out on sentient animals.

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

Clinical data

Policy information about clin	nical studies
All manuscripts should comply	with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.
Clinical trial registration	
Study protocol	
Data collection	
Outcomes	

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
	Public health
	National security
	Crops and/or livestock
	Ecosystems
	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
	Demonstrate how to render a vaccine ineffective
	Confer resistance to therapeutically useful antibiotics or antiviral agents
	Enhance the virulence of a pathogen or render a nonpathogen virulent
	Increase transmissibility of a pathogen
	Alter the host range of a pathogen
	Enable evasion of diagnostic/detection modalities
	Enable the weaponization of a biological agent or toxin
	Any other potentially harmful combination of experiments and agents

		Any other	potentially	harmful	combination	of expe	eriments	and	agei
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Plants

Seed stocks	
Novel plant genotypes	
Authentication	

ChIP-seq

Data deposition

	Confirm that both raw and final processed data have been deposited in a public database such as g	GE	(2
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Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links May remain private before publication.	
Files in database submission	
Genome browser session (e.g. <u>UCSC</u>)	

Methodology

Replicates	
Sequencing depth	
Antibodies	
Peak calling parameters	
Data quality	

Flow Cytometry

Plots

Confirm that:

The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

All plots are contour plots with outliers or pseudocolor plots.

A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Effect(s) tested

Sample preparation	
Instrument	
Software	
Cell population abundance	
Gating strategy	

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design	
Design type	
Design specifications	
Behavioral performance measures	
Imaging type(s)	
Field strength	
Sequence & imaging parameters	
Area of acquisition	
Diffusion MRI Used	□ Not used
Preprocessing	
Preprocessing software	
Normalization	
Normalization template	
Noise and artifact removal	
Volume censoring	
Statistical modeling & inferenc	e
Model type and settings	

Specify type of analysis: Whole brain ROI-based Both
Statistic type for inference
(See Eklund et al. 2016)
Correction
Models & analysis
n/a Involved in the study Functional and/or effective connectivity Graph analysis Multivariate modeling or predictive analysis
Functional and/or effective connectivity
Graph analysis
Multivariate modeling and predictive analysis

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