# 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.

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.

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

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n/a	Confirmed
	$\boxed{\mathbf{x}}$ The exact sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
	X A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
x	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.
x	A description of all covariates tested
x	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)
x	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 <i>Give P values as exact values whenever suitable.</i>
x	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
x	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	x Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
	Our way collection on statistics for highering articles on many of the points above

### Software and code

Policy information about availability of computer code

Epifluorescence microscopy was performed on DMi8 inverted microscope (Leica) and fluorescence correlation Data collection spectorscopy (FCS) was performed on LSM 780 inverted multiphoton laser scanning microscope (Zeiss).

Data analysis

Image analysis of epifluorescence microscopy was done in FIJI/ImageJ. Except for FCS, all statistical analysis and model fitting were done in R. For FCS, autocorrelation function was calculated with ZEN 2.3 SP1 FP3 310 (Black)

For manuscripts utilizing 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 reposition yield. Generally by Section 18 to 18 years and model fitting was done with 1900 Provenies on the storing published in the 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 authors declare that the data supporting the findings of this study are available within the paper and the Source Data file.

	Research involving	human participants,	their data,	or biological materia	al
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	it studies with <u>human participants or human data</u> . See also policy information about <u>sex, gender (identity/presentation),</u> and <u>race, ethnicity and racism</u> .
Reporting on sex and	gender n/a
Reporting on race, et other socially relevan groupings	I-
Population character	stics 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.
-iald apaci	ficesporting
<del></del>	fic reporting
_	elow that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.
x Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences
or a reference copy of the do	ocument with all sections, see <a href="mailto:nature.com/documents/nr-reporting-summary-flat.pdf">nature.com/documents/nr-reporting-summary-flat.pdf</a>
ife science	es study design
all studies must disclos	e on these points even when the disclosure is negative.
Sample Size	sample-size calculation was performed. Data were typically collected from at least three independent experiments. Multiple a points were typically generated from each independent experiment.
Data exclusions No	data were excluded from the analyses.
	a were typically collected from at least three independent experiments. All attempts were successful. FCS measurements re repeated at more than three independent positions.
	mples were collected from randomly picked Xenopus laevis frogs from a frog tank. In a typical experiment, eggs from only one givere collected. No grouping of frogs/eggs was performed.
Plinding	e investigators were not blinded to group allocation during data collection, as it would be impossible to perform correct
din	tions/treatment without knowing which well/tube a sample was in. Automatic analyses were done when possible.
Rehavioura	al & social sciences study design
	e on these points even when the disclosure is negative.
Study description	n/a
Research sample	n/a
Sampling strategy	n/a
Data collection	n/a
Timing	n/a
Data exclusions	n/a
Non-participation	n/a
Randomization	n/a

Study description Research sample N/a  Sampling strategy N/a  Data collection N/a  Timing and spatial scale Data exclusions N/a  Reproducibility N/a  Randomization N/a  Randomization N/a  Blinding N/a  Did the study involve field work?  \ yes \ \ \ No  Field work, collection and transport  Field work, collection and transport  Field conditions N/a  Location N/a  Access & import/export Disturbance N/a  Reporting for specific materials, systems and methods  We require information from authors about some types of materials, 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	All studies must disclose or	these points even when the disclosure is negative.
Sampling strategy  Data collection  I'ming and spatial scale  N/a  Data exclusions  N/a  Reproducibility  N/a  Randomization  Blinding  N/a  Did the study involve field work?    yes   x   No  Field work, collection and transport  Field conditions  N/a  Location  N/a  Access & import/export  N/a  Disturbance  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  Materials & experimental systems  Not involved in the study	Study description	n/a
Data collection   n/a    Timing and spatial scale   n/a    Data exclusions   n/a    Reproducibility   n/a    Randomization   n/a    Blinding   n/a    Did the study involve field work?   yes   x   No    Field work, collection and transport  Field conditions   n/a    Location   n/a    Access & import/export   n/a    Disturbance   n/a    Reporting for specific materials, systems and methods were 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	Research sample	n/a
Timing and spatial scale  Data exclusions  n/a  Reproducibility  n/a  Randomization  n/a  Blinding  n/a  Did the study involve field work?  Yes  No  Field work, collection and transport  Field conditions  n/a  Location  n/a  Access & import/export  Disturbance  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	Sampling strategy	n/a
Data exclusions  Reproducibility  Randomization  Blinding  n/a  Did the study involve field work?	Data collection	n/a
Reproducibility   n/a   Randomization   n/a   Blinding   n/a   Did the study involve field work?   Yes   X No  Field work, collection and transport  Field conditions   n/a   Location   n/a   Access & import/export   n/a   Disturbance   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   Methods   n/a   Involved in the study   n/a   Involved in the study   ChIP-seq   X   ChIP-seq   Flow cytometry	Timing and spatial scale	n/a
Randomization  Blinding  n/a  Did the study involve field work?  ves  No  Field work, collection and transport  Field conditions  n/a  Location  n/a  Access & import/export  Disturbance  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  Methods  n/a Involved in the study  Antibodies  Methods  N/a Involved in the study  Antibodies  Discreption of the study  ChIP-seq  Flow cytometry	Data exclusions	n/a
Did the study involve field work?  \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	Reproducibility	n/a
Field work, collection and transport  Field conditions  n/a  Location  n/a  Access & import/export  Disturbance  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     ChIP-seq       ChIP-seq	Randomization	n/a
Field work, collection and transport  Field conditions  n/a  Location  n/a  Access & import/export  Disturbance  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     ChIP-seq       ChIP-seq	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  Methods  n/a Involved in the study  Involved in the study  Antibodies  Discreption of the study  Methods  n/a Involved in the study  Discreption of the study  Discreption	Field work, collec	
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		tion and transport
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 materials 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	Field conditions	tion and transport
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       Involved in the study       X	Field conditions  Location	tion and transport  n/a  n/a
n/a Involved in the study  x	Field conditions  Location  Access & import/export	n/a n/a
X Antibodies   X ChIP-seq   X Eukaryotic cell lines      X Flow cytometry	Field conditions  Location  Access & import/export  Disturbance  Reporting fo	n/a  n/a  n/a  n/a  n/a  r specific materials, systems and methods  nuthors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material
	Field conditions  Location  Access & import/export  Disturbance  Reporting fo  We require information from a system or method listed is released.  Materials & experime	n/a  n/a  n/a  n/a  r specific materials, systems and methods  nuthors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material want to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.  ntal systems  Methods
	Field conditions  Location  Access & import/export  Disturbance  Reporting fo  We require information from a system or method listed is relevant in the study in	tion and transport  n/a  n/a  r specific materials, systems and methods  n/a  n/a  r specific materials, experimental systems and methods used in many studies. Here, indicate whether each material want to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.    Methods
Clinical data  Dual use research of concern	Field conditions  Location  Access & import/export  Disturbance  Reporting fo  We require information from a system or method listed is relevant in the study in	n/a  n/a  r specific materials, systems and methods  n/a  n/a  r specific materials, systems and methods  nuthors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material want to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.    Nethods

# Antibodies

Antibodies used	n/a
Validation	n/a

Eukaryotic cell lin	es	
Policy information about <u>ce</u>	ell lines	and Sex and Gender in Research
Cell line source(s)		n/a
Authentication		n/a
Mycoplasma contaminat	ion	n/a
Commonly misidentified (See ICLAC register)	lines	n/a
Palaeontology an	d Arc	chaeology
Specimen provenance	n/a	
Specimen deposition	n/a	
Dating methods	n/a	
Tick this box to confir	m that	the raw and calibrated dates are available in the paper or in Supplementary Information.
Ethics oversight	n/a	
Note that full information on t	he appro	oval of the study protocol must also be provided in the manuscript.
Animals and othe	r res	earch organisms
Policy information about st Research	udies ir	nvolving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in
Laboratory animals	Xenop	ous laevis > 3 yr
Wild animals	n/a	
Reporting on sex	female	es and males
Field-collected samples	This st	audy did not involve field-collected samples
Ethics oversight		nopus experiments and animal care adhered to protocols (ALPAC-13307) approved by Institutional Animmal Care see Committee (IACUC) of Stanford University.
Note that full information on t	he appro	oval of the study protocol must also be provided in the manuscript.
Clinical data		
Policy information about <u>cl</u> All manuscripts should comply		udies  CONSORT checklist must be included with all submissions.
Clinical trial registration	n/a	
Study protocol	n/a	
Data collection	n/a	
Outcomes	n/a	

## Dual use research of concern

Policy information about <u>dual use research of concern</u>

### 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		
x Public health		
X National security		
x Crops and/or livest	tock	
x Ecosystems		
X Any other significar	nt area	
Experiments of concer	n	
Does the work involve any	y of these experiments of concern:	
No Yes		
X Demonstrate how t	to render a vaccine ineffective	
X Confer resistance to	to therapeutically useful antibiotics or antiviral agents	
X Enhance the viruler	ence of a pathogen or render a nonpathogen virulent	
X Increase transmissi	ibility of a pathogen	
X Alter the host range	ge of a pathogen	
<b>x</b> Enable evasion of d	diagnostic/detection modalities	
<b>x</b> Enable the weapon	nization of a biological agent or toxin	
X Any other potentia	ally harmful combination of experiments and agents	
Plants		
Seed stocks	n/a	$\overline{}$
	n/a	
Novel plant genotypes		
Authentication	n/a	
ChIP-seq		
Data deposition		
·	v and final processed data have been deposited in a public database such as GEO.	
Confirm that you have	e deposited or provided access to graph files (e.g. BED files) for the called peaks.	
Data access links May remain private before public	cation. n/a	
Files in database submissi	ion n/a	
Genome browser session (e.g. <u>UCSC</u> )	n/a	
Methodology		
Replicates	n/a	
Sequencing depth	n/a	
Antibodies	n/a	
Peak calling parameters	n/a	
Data quality	n/a	

n/a

Software

Flow Cytometry	
Plots  Confirm that:  The axis labels state the mark	ker and fluorochrome used (e.g. CD4-FITC).
	ible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
	th outliers or pseudocolor plots.
A numerical value for numbe	r of cells or percentage (with statistics) is provided.
Methodology	
Sample preparation	n/a
Instrument	(n/a
Software	n/a
Cell population abundance	n/a
Gating strategy	n/a
Tick this box to confirm that	a figure exemplifying the gating strategy is provided in the Supplementary Information.
Magnetic resonance in	maging
Experimental design	
Design type	n/a
Design specifications	n/a
Behavioral performance measur	es n/a
Imaging type(s)	n/a
Field strength	n/a
Sequence & imaging parameters	n/a
Area of acquisition	n/a
Diffusion MRI Used	☐ Not used
Preprocessing	
Preprocessing software	n/a
Normalization	n/a
Normalization template	n/a
Noise and artifact removal	n/a
Volume censoring	n/a
Statistical modeling & infere	
Model type and settings	n/a
woder type and settings	IIVQ

Model type and settings	n/a
Effect(s) tested	n/a
Specify type of analysis: W	/hole brain ROI-based Both

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Statistic type for inference	n/a
(See Eklund et al. 2016)	
Correction	/a
Models & analysis	
n/a   Involved in the study   Functional and/or effective connectivity	
X       Graph analysis         X       Multivariate modeling or predictive analysis	
Functional and/or effective connec	ctivity n/a
Graph analysis	n/a

Multivariate modeling and predictive analysis n/a