# 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>.

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FUI	dII S	latistical analyses, commit that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Со	nfirmed
	×	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
	×	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)
	×	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 web collection on <b>statistics for biologists</b> contains articles on many of the points above.

#### Software and code

Policy information about availability of computer code

Data collection

Leica Application Suite X (LASX) for confocal microscopy, bright-field imaging, hyperspectral imaging and FRAP; Abberior STED setup (Abberior Instruments GmbH) for super-resolution images; OneMP (Refeyn Ltd) for mass photometry.

Data analysis

ImageJ v.1.53t for image analysis, OriginLab 2021 for making plots and statistical analysis, Microsoft Excel (2019), SimFCS 4 (2021) for the phasor plots.

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 <u>data availability statement</u>. 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 underlying Figs. 1a,c,e, 2c,e, 3b-f, 4b-d, 5c-e, 6e,f,h, 7d-f, 8a-c as well as Supplementary Figs. 1c-e, 2c, 3a,d, 5a, 6c, 7b, 8a-c, 9b, 10b,c, and 11b,c are provided in a separate Excel file labeled 'Source Data'. Source data are provided with this paper.

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•	race, ethnicity and racism.
Reporting on sex and ge	nder n/a
Reporting on race, ethniother socially relevant groupings	city, or n/a
Population characteristic	s n/a
Recruitment	n/a
Ethics oversight	n/a
lote that full information on	the approval of the study protocol must also be provided in the manuscript.
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lease select the one belo	w 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
or a reference copy of the docun	nent 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 sciences	s study design
ll studies must disclose o	n these points even when the disclosure is negative.
Sample size Sample	sizes number were not predetermined but all vesicles/condensates suitable for a measurement were considered in each sample
Data exclusions Vesicle	s exhibing defects (internal or external structures) were excluded.
Replication All resu	lts were obtained in three independent experiments, obtaining successful replicates in all cases.
Randomization No rele	vant since the work involves in vitro characterization and samples were not allocated into experimental groups
not be	our in vitro biophysical studies. Vesicle-condensate pairs within a given sample were randomly chosen for experiments and could visually distinguished from each other. For the protein purification, blinding is typically not used; the proteins had to be separately d with different protocols and the buffer conditions of different groups need to be carefully controlled by the experimentalists.
	& social sciences study design  these points even when the disclosure is negative.
Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.
Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.

Data exclusions If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no Non-participation participants dropped out/declined participation.

If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if Randomization allocation was not random, describe how covariates were controlled.

## Ecological, evolutionary & environmental sciences study design

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

Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g., factorial, nested, Study description hierarchical), nature and number of experimental units and replicates. Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Research sample

Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.

Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size

calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. Describe the data collection procedure, including who recorded the data and how.

Timing and spatial scale Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken

> If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to Reproducibility repeat the experiment failed OR state that all attempts to repeat the experiment were successful.

> Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.

Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

Did the study involve field work?

Sampling strategy

Data collection

Data exclusions

Randomization

Blinding

### Field work, collection and transport

Field conditions Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).

Location State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).

 $Describe \ the \ efforts \ you \ have \ made \ to \ access \ habitats \ and \ to \ collect \ and \ import/export \ your \ samples \ in \ a \ responsible \ manner \ and \ in$ Access & import/export compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).

Describe any disturbance caused by the study and how it was minimized. 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 & experime	ental systems Methods		
n/a Involved in the study	n/a Involved in the study		
Antibodies	ChiP-seq		
<b>x</b> Eukaryotic cell lines	Flow cytometry		
Palaeontology and a	archaeology MRI-based neuroimaging		
Animals and other of	5, 2,12		
	nganisnis		
Clinical data			
Dual use research o	f concern		
Plants			
Antibodies			
Antibodies used	Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.		
Validation	Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.		
Eukaryotic cell lin	es		
Policy information about <u>ce</u>	ell lines and Sex and Gender in Research		
Cell line source(s)	Expi293F™ cells were purchased from Thermo Fisher Scientific.		
Authentication	Authentication done by the source supplier.		
Mycoplasma contaminat	ion Cell lines tested negative for mycoplasma.		
Commonly misidentified (See <u>ICLAC</u> register)	lines Cell line used is not on the list.		
Palaeontology an	d Archaeology		
Specimen provenance	Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.		
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.		
Dating methods	If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.		
Tick this box to confir	m that the raw and calibrated dates are available in the paper or in Supplementary Information.		
Ethics oversight	Ethics oversight		
Note that full information on t	he approval of the study protocol must also be provided in the manuscript.		
Animals and othe	r research organisms		
Policy information about <u>st</u> <u>Research</u>	<u>sudies involving animals</u> ; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in</u>		
Laboratory animals	For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.		
Wild animals	Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.		
Reporting on sex	Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide		

data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify

reasons for lack of sex-based analysis.

Field-collected samples	For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.
Ethics oversight	Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.
ote that full information on t	the approval of the study protocol must also be provided in the manuscript.
Clinical data	
olicy information about <u>cl</u> II manuscripts should comply	linical studies vith the ICMJEguidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions
Clinical trial registration	Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.
Study protocol	Note where the full trial protocol can be accessed OR if not available, explain why.
Data collection	Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.
Outcomes	Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.
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olicy information about <u>d</u>	ual use research of concern
lazards	
Could the accidental, del	iberate 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 livestock
×	Ecosystems
x	Any other significant area

#### Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	5
x		Demonstrate how to render a vaccine ineffective
x		Confer resistance to the rapeutically useful antibiotics or antiviral agents
×		Enhance the virulence of a pathogen or render a nonpathogen virulent
x		Increase transmissibility of a pathogen
x		Alter the host range of a pathogen
x		Enable evasion of diagnostic/detection modalities
x		Enable the weaponization of a biological agent or toxin
x		Any other potentially harmful combination of experiments and agents

#### Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

#### ChIP-seq

#### Data deposition

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks. Data access links For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document,

May remain private before publication.

provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Confirm that both raw and final processed data have been deposited in a public database such as GEO.

Genome browser session (e.g. UCSC)

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

#### Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

**Antibodies** 

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

### 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

Sample preparation

Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument

Identify the instrument used for data collection, specifying make and model number.

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance	Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.		
Gating strategy	Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.		
Tick this box to confirm that	a figure exemplifying the gating strategy is provided in the Supplementary Information.		
Magnetic resonance i	maging		
Experimental design			
Design type	Indicate task or resting state; event-related or block design.		
Design specifications	Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.		
Behavioral performance measu	State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).		
Acquisition			
Imaging type(s)	Specify: functional, structural, diffusion, perfusion.		
Field strength	Specify in Tesla		
Sequence & imaging parameter	Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.  State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.		
Area of acquisition			
Diffusion MRI Used	☐ Not used		
Preprocessing			
Preprocessing software	Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).		
Normalization	If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.		
Normalization template	Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.		
Noise and artifact removal	scribe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and usiological signals (heart rate, respiration).		
Volume censoring	fine your software and/or method and criteria for volume censoring, and state the extent of such censoring.		
Statistical modeling & infer	ence		
Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).		
Effect(s) tested	Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.		
Specify type of analysis: $\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \$	Vhole brain ROI-based Both		
Statistic type for inference	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.		
(See <u>Eklund et al. 2016</u> )			
Correction Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Mod			

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	Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).			
Graph analysis	Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).			
Multivariate modeling and predictive analysis	Specify independent variables, features extraction and dimension reduction, model, training and evaluation			