# 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.

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	Coi	nfirmed
	x	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	×	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
	ı	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.
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#### Software and code

Policy information about availability of computer code

Data collection Raw microscopy data was acquired using μManager (version 2.0, DOI: 10.1002/0471142727.mb1420s92)

Data analysis dSTORM data were analyzed using Picasso (version 0.3.1, DOI: 10.1038/nprot.2017.024) and Fiji (DOI: 10.1038/nmeth.2019);

FRET data were analyzed using the open source software iSMS (version 2.01, DOI: 10.1038/nmeth.3435);

MD simulation data and parameter files as well as analysis code are freely available on Zenodo (DOI: 10.5281/zenodo.13913751).

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

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Single-molecule imaging have been deposited in the EMBL Biolmaging Archive under accession code S-BIAD1347 (https://www.ebi.ac.uk/biostudies/bioimages/studies/S-BIAD1347, DOI: 10.6019/S-

BIAD1347). The MD simulation data and parameter files and analysis code are freely available on Zenodo (DOI: 10.5281/zenodo.13913751). Source data are provided with this paper.

### 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), and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.

Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.

Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).

Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)

Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ecological, evolutionary & environmental sciences

Ethics oversight

**X** Life sciences

Data exclusions

Identify the organization(s) that approved the study protocol.

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 <a href="mailto:nature.com/documents/nr-reporting-summary-flat.pdf">nature.com/documents/nr-reporting-summary-flat.pdf</a>

Behavioural & social sciences

### 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 sample sizes, but all experiments were repeated several times yielding similar results.

dSTORM measurements that were of low quality due to strong drift, high background signals, or artifacts at the cell borders were excluded from the analysis. For smFRET, only time traces that showed anti-correlated behavior of donor and acceptor intensity and single-step

photobleaching were included in the analysis.

Replication dSTORM and smFRET experiments as well as western blots were replicated at least in three independent experiments. All replications were successful.

Randomization Randomization was not necessary as our experiments did not include any experimental group allocation.

Blinding Blinding was not necessary as our experiments did not include any experimental group allocation.

## Behavioural & social sciences study design

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

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

Study description

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.

Non-participation

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

Randomization

If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if 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.

Study description

Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.

Research sample

Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National 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.

Sampling strategy

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.

Data collection

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

Data exclusions

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.

Reproducibility

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

Randomization

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.

Blinding

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?

Yes		No
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### 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).

Access & import/export

Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in 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).

### 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	<b>✗</b> ☐ ChIP-seq
Eukaryotic cell lines	Flow cytometry
Palaeontology and archaeology	MRI-based neuroimaging
Animals and other organisms	
X Clinical data	
Dual use research of concern	
Plants	

#### **Antibodies**

Antibodies used

- 1) goat@MET, #AF276, lot CMQ0720032, R&D Systems
- 2) Alexa Fluor 647 rabbit@goat, #A-21446, Invitrogen, Thermo Scientific
- 3) rabbit anti-MET, #4560, lot 2, Cell Signaling Technology
- 4) rabbit anti-pMET, #3077, lot 9, Cell Signaling Technology
- 5) rabbit anti-actin, #ab14130, lot 487755, abcam
- 6) goat anti-rabbit HRP, #111-035-003, Jackson ImmunoResearch

Validation

- 1) Specificity of the goat@MET primary antibody was tested by R&D Systems in ELISAs and western blots.
- 2) Specificity of Alexa Fluor 647 rabbit@goat antibody was validated by negative controls where cells were incubated with this secondary antibody without addition of primary antibody (Figure 3 in the manuscript).
- 3) Specificity of rabbit anti-MET primary antibody was tested by Cell Signaling Technology in western blots.
- 4) Specificity of rabbit anti-pMET primary antibody was tested by Cell Signaling Technology in western blots.
- 5) Specificity of rabbit anti-actin primary antibody was tested by Abcam in western blots.
- 6) Specificity of goat anti-rabbit HRP secondary antibody was validated by Jackson ImmunoResearch based on immunoelectrophoresis and/or ELISA, the antibody reacts with whole molecule rabbit IgG.

#### Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

U-2 OS (# 300364, CLS Cell Lines Service GmbH), HeLa (# ACC 57, DSMZ), Huh 7.5 (DKFZ Heidelberg), U-251 (# 300385, CLS Cell Lines Service GmbH), 23132/87 (# ACC 201, DSMZ).

Authentication

None of the cell lines was authenticated.

Mycoplasma contamination

All cell lines were tested negative for mycoplasm using the Mycoplasma Detection Kit Venor (R) GeM Classic (minerva biolabs).

Commonly misidentified lines (See ICLAC register)

### Palaeontology and 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 confi	rm that the raw and calibrated dates are available in the paper or in Supplementary Information.
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.
Note that full information on	the approval of the study protocol must also be provided in the manuscript.
Animals and other	er research organisms
	tudies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in
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.
	the approval of the study protocol must also be provided in the manuscript.
Clinical data	
Policy information about <u>c</u> All manuscripts should comply	<u>llinical studies</u> ywith 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.
Dual use research	n of concern
Policy information about d	dual use research of concern
Hazards	
Could the accidental, de in the manuscript, pose	liberate or reckless misuse of agents or technologies generated in the work, or the application of information presented a threat to:

Vo	Yes	
x		Public health
×		National security
X		Crops and/or livestock
x		Ecosystems
x		Any other significant are

#### Experiments of concern

Doe	is the work involve any of these experiments of concern:
No	Yes
x	Demonstrate how to render a vaccine ineffective
×	Confer resistance to therapeutically useful antibiotics or antiviral agent
x	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
×	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

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

May remain private before publication.

Files in database submission

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

Genome browser session

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

(e.g. UCSC)

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

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

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 used.

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 mark	er and fluorochrome used (e.g. CD4-FITC).
The axis scales are clearly visib	ole. 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	h 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.
	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.
	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.
	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 in	naging
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 measure	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 parameters	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.
Area of acquisition	State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.
Diffusion MRI Used	☐ Not used
Preprocessing	
,	Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).
	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.
	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.
	Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

otatistical modeling a line	chec
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:	Whole brain ROI-based Both
Statistic type for inference	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.
(See Eklund et al. 2016)	
Correction	Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).
Models & analysis	
n/a Involved in the study  Functional and/or effect  Graph analysis	tive connectivity
Multivariate modeling of	or predictive analysis

mutual information).

etc.).

Multivariate modeling and predictive analysis

Functional and/or effective connectivity

Graph analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation,

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,