nature portfolio

Corresponding author(s): Weishe Zhang, Xiangyu Liu

Last updated by author(s): May 22, 2025

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.

Statistics

For all statistical analyses, confirm that the following items are present in the 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
☐	☒	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.*
☒	☐	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.*
☒	☐	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

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

In vitro biological experiments:
- Cyclic adenosine monophosphate (cAMP) assay: Ensight (PerkinElmer)
- Flow cytometry assay: Accuri C6 (BD Biosciences), FACS AriaII (BD Biosciences)

Structural biology experiments:
- Cryo-EM: Batch data collection was performed with AutoEMation in FEI Titan Krios 300KV (D3786) microscope.

Sequence bioinformatics:
- RefSeq at NCBI, GPCRdb, AlphaFold2 database.

Data analysis

- In vitro biological experiments: Graphpad Prism v10, FlowJo 10.4.0
- Sequence bioinformatics: Snapgene, AlphaFold2
- Structural biology experiments: cryoSPARC v4.5.1, PHENIX v1.20.1, ChimeraX v1.3, COOT v0.9.8.7

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

• Cryo-EM structures were deposited in the Protein Data Bank (https://www.rcsb.org), with the corresponding PDB accession codes: 9UOT,9UTO, 9UTP and 9UVQ. All the other relevant structures referenced in this work are available under the accession codes 6TPK and 7RYC.
• The source data is available from the corresponding authors upon reasonable request.

Research involving human participants, their data, or biological material

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

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.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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

Life sciences study design

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

Sample size	•For cryo-EM data, the sample size was chosen based on the sufficient number of particles to achieve high-resolution reconstructions. •For the in vitro assay, a sample size of n=3 is commonly used in biological studies. For the mice experiment, a sample size of n=6 is used in LPS-induced preterm birth study. All experiments were performed in at least three independent biologial replicates.
Data exclusions	No data exclusion
Replication	In vitro experiments were replicated in n independent runs as described in the methods section, figure legends or tables.
Randomization	Cells placed in different positions on 96-well signaling assay plate and randomly allocated into control and treatment groups.
Blinding	Blinding is not relevant to this study, all experiments were performed based on standardized protocols and readouts and are not influenced by the investigator.

Behavioural & social sciences study design

All studies must disclose 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

Ecological, evolutionary & environmental sciences study design

All studies must disclose 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 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 ☒ No

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
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	•Alexa-488 conjugated anti-Flag antibody (Thermo Fisher, Cat # MA1-142-A488),Alexa-647 conjugated anti-Flag antibody(Thermo Fisher, Cat # MA1-142-A647) •Alexa-488 conjugated anti-HA tag(Cell Signaling Technology, cat#2350S),Alexa-647 conjugated anti-HA tag(Cell Signaling Technology, cat#3444S) •Yeast surface displayed synthetic nanobody library was obtained from Dr. A.C. Kruse (Harvard University) and Dr. A. Manglik (University of California, San Francisco)(McMahon, C. et al. Nat. Struct. Mol. Biol. 25, 289–296).
Validation	•The antibody was commercially available and validated by the manufacturer. There are the websites: https://www.thermofisher.cn/cn/zh/antibody/product/DYKDDDDK-Tag-Antibody-clone-L5-Monoclonal/MA1-142-A488. https://www.thermofisher.cn/cn/zh/antibody/product/DYKDDDDK-Tag-Antibody-clone-L5-Monoclonal/MA1-142-A647.https://

www.cellsignal.cn/products/antibody-conjugates/ha-tag-6e2-mouse-mab-alexa-fluor-488-conjugate/2350. https://www.cellsignal.cn/products/antibody-conjugates/ha-tag-6e2-mouse-mab-alexa-fluor-647-conjugate/3444.
•Synthetic nanobody characterization was performed by flow-cytometry.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	• Sf9 cells were purchased from Expression Systems • Primary uterine smooth muscle cells (USMCs) were purchased from TongPai(Shanghai) Biotechnology Co., Ltd • HEK293T cells and EXPI293F cells were purchased from Thermo Fisher Scientific. • Yeast cells were obtained from Dr. A.C. Kruse (Harvard University).
Authentication	None of the used cell lines were authenticated by us.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Palaeontology and Archaeology

Specimen provenance	N/A
Specimen deposition	N/A
Dating methods	N/A

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	N/A

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 Sex and Gender in Research

Laboratory animals	Female and male C57BL/6J mice were purchased from Hunan Yuantai Biotechnology Co., Ltd.
Wild animals	N/A
Reporting on sex	N/A
Field-collected samples	N/A
Ethics oversight	All procedures complied with the ARRIVE Guidelines and the Chinese Ministry of Health Guidelines for Laboratory Animal Care and Use, approved by the Ethics Committee of Xiangya Hospital, Central South University (Approval No.2023030192).

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completedCONSORT 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 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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

ChIP-seq

Data deposition

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

☐ 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.*	N/A
Files in database submission	N/A
Genome browser session (e.g. UCSC)	N/A

Methodology

Replicates	N/A

Sequencing depth	N/A
Antibodies	N/A
Peak calling parameters	N/A
Data quality	N/A
Software	N/A

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	• Two rounds of FACS were performed using a FACSAria II instrument (BD Biosciences), for FACS round 1, yeast cells were stained with Alexa Fluor-FITC conjugated anti-HA antibody (Cell Signaling Technology) and 100 nM OTR_Clip3 labeled with anti-FLAG M1-647. In FACS round 2, the staining conditions were similar, except that 50 nM OTR_Clip3 was used. • Single clone yeast cells displaying nanobodies were stained in selection buffer with Alexa Fluor-FITC conjugated anti-HA antibody (Cell Signaling Technology) and 50 nM purified OTR_5mut or OTR_Clip3 pre-labeled with anti-FLAG M1-647.
Instrument	Accuri C6 (BD Biosciences) for analysis and FACS AriaII (BD Biosciences) for sorting.
Software	FlowJo 10.4.0
Cell population abundance	Not applicable to the flow analysis and sorting.
Gating strategy	Standard gating strategy was used based on the size and granularity of the cells and gating strategy was shown in the supplementary information.

☒ 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	N/A
Design specifications	N/A
Behavioral performance measures	N/A

Acquisition

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 & inference

Model type and settings: N/A

Effect(s) tested: N/A

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference: N/A

(See Eklund et al. 2016)

Correction: N/A

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis