# nature portfolio

Corresponding author(s): Renhong Yan

Last updated by author(s): Jan 9, 2024

# **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>.

### Statistics

For	For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.			
n/a	Cor	firmed		
	×	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.		
×		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. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted Give P values as exact values whenever suitable.		
X		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		
X		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	EPU software		
Data analysis	cryoSPARC 3.3.1, ChimeraX 1.6.1, MotionCor 2 1.1.0, Phenix 1.11.1, Coot 0.8.2, GraphPad Prism 8.0		

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

Atomic coordinates and cryo-EM density maps of LayV-G protein (PDB: 8JZB, whole map: EMD-36741) have been deposited to the Protein Data Bank (http:// www.rcsb.org) and the Electron Microscopy Data Bank (https://www.ebi.ac.uk/pdbe/emdb/), respectively.

### 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, ethnicity and racism</u>.

Reporting on sex and gender	Human research participants were not involved in this study.
Reporting on race, ethnicity, or other socially relevant groupings	Human research participants were not involved in this study.
Population characteristics	Human research participants were not involved in this study.
Recruitment	Human research participants were not involved in this study.
Ethics oversight	Human research participants were not involved in this study.

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	Sample sizes are described in figure legends and metfnods. No statistical method predetermine sample size. Cryo-EM datasets were collected and generated interpretable map with sufficient resolution reported in this study. Due to the resolution range is 3.0-3.9 angstroms, we judged the sample size is enough.
Data exclusions	No data was excluded.
Replication	For the biochemical assay, each experiment was replicated at least 3 times on separate occasions. Experimental findings were reliably reproduced. For experiments of the LayV-G and NiV-G binding to ephrinB2 and ephrinB3 were pertormed four times, all attempts showed similar results. Multiple cryo-EM grids were prepared and frozen, datas were collected for two or three selected grids of the full-length LayV-G structures. Representative ones of protein purification and micrographs were shown in the supplementary information. Flow cytometry experiments were performed independently in duplicate. All ELISA assays were performed three times independently.
Randomization	Randomization is not relevant for this study, as there no groups allocated in any of the experiments. This study did not involve animals or humans research participants.
Blinding	Blinding is not applicable to this research as this is deemed not practically feasible.

# Behavioural & social sciences study design

All studies must disclose on 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.
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

### 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).
Disturbance	Describe any disturbance caused by the study and how it was minimized.

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

X Plants

### Antibodies

Antibodies used	Antibody name, Producer, Applicable, mAb or pAb, Cat. No., Lot. No., Clone No.
	SureLight® APC Anti-6X His tag, abcam, FC, mAb, ab72579, GR3296932-9, AD1.1.10
	HRP conjugated goat anti-rabbit IgG (H+L), ZSGB-BIO, ELISA, pAb, ZB-2301, 231400109
	HRP conjugated goat anti-human IgG (H+L), ZSGB-BIO, ELISA, pAb, ZB-2304, 228190725
	Anti-Mojiang Virus G [6E5-LY], absoluteantibody, ELISA, mAb, Ab02867-10.0, T2242A64, 6E5-LY
	Anti-Mojiang Virus G [10G2-LY], absoluteantibody, ELISA, mAb, Ab02868-10.0, T2313A20, 10G2-LY
	Anti-Langya virus/LayV G/Glycoprotein Polyclonal Antibody, AntibodySystem, ELISA, pAb, PVV18301, 98024
	Anti His-Tag Mouse Monoclonal Antibody, CWBIO, WB, mAb, CW0286M, 01249/18022, 2A11
	Goat Anti-Rabbit IgG, HRP Conjugated, CWBIO, WB, pAb, CW01035, 01325/11823
	HENV-117, expressed and puried in this study, ELISA
	HENV-151, expressed and puried in this study, ELISA
	HENV-165, expressed and puried in this study, ELISA
	HENV-45, expressed and puried in this study, ELISA
	HENV-242, expressed and puried in this study, ELISA
	HENV-103, expressed and puried in this study, ELISA
	HENV-78, expressed and puried in this study, ELISA
	HENV-72, expressed and puried in this study, ELISA
	HENV-160, expressed and puried in this study, ELISA
Validation	Commercially available antibodies were validated by suppliers.
	SureLight® APC Anti-6X His tag https://www.abcam.com/products/primary-antibodies/surelight-apc-6x-his-tag-antibody-ad1110- ab72579.html
	HRP conjugated goat anti-rabbit IgG (H+L) http://www.zsbio.com/product/ZB-2301
	HRP conjugated goat anti-human IgG (H+L) http://www.zsbio.com/product/ZB-2304
	Anti-Mojiang Virus G [6E5-LY] https://absoluteantibody.com/product/anti-mojiang-virus-g-6e5-ly/
	Anti-Mojiang Virus G [10G2-LY] https://absoluteantibody.com/product/anti-mojiang-virus-g-10g2-ly/
	Anti-Langya virus/LayV G/Glycoprotein Polyclonal Antibody (PVV18301) https://www.antibodysystem.com/product/11387.html

### Eukaryotic cell lines

Policy information about <u>cell lines and Sex and Gender in Research</u>			
Cell line source(s)	HEK293F (invitrogen), CHO(cellcook)		
Authentication	No further authentication was performed for commercially available cell lines.		
Mycoplasma contamination	Not tested for mycoplasma contamination.		
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.		

## 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.

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 confirm 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 research organisms

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

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

### Clinical data

### Policy information about <u>clinical studies</u>

All manuscripts should comply with 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 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:



### 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	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 was applied.
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 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.	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.
Files in database submission	Provide a list of all files available in the database submission.
Genome browser session (e.g. <u>UCSC</u> )	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 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:

**x** 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.

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

### Methodology

Sample preparation	Full length Ephrin B2 (EAX09085.1) or Ephrin B3 (NP_001397.1) was stably transfected into CHO cells using the lentivector with GFP as a marker to monitor gene expression. Briefly, pCDH-Ephrin B2 or B3 and envelope vectors were co-transfected into packaging cells. The cell culture supernatants containing the lentivirus were harvested 48 hours later and further incubated with CHO cells. Afterward, stable CHO-EphrinB2 or B3 cell lines were produced via puromycin selection. The CHO-Ephrin B2 or CHO-Ephrin B3 cells were incubated with various HNV G protein (20 µg/mL) at 4°C for 30 min. Afterwards, 50 µL of diluted SureLight <sup>®</sup> APC Anti-6X His tag (Abcam, Cat# ab72579) was added to the cells and incubated at 4°C for 30 min. Finally, cells were washed with PBS and resuspended after centrifuged.
Instrument	BD FACSymphony™ A3 Cell Analyzer - BD Biosciences
Software	FlowJo X
Cell population abundance	No cell sorting assay in this study
Gating strategy	The gating strategy is firstly gating the major population according to FSC-A and SSC-A, then exclude of doublets and dead cells(BV421 positive population), finally analyzing the mean fluorescence intensity of APC in GFP-positive population.

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

### Magnetic resonance imaging

### Experimental design Indicate task or resting state; event-related or block design. Design type Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial Design specifications or block (if trials are blocked) and interval between trials. State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used Behavioral performance measures to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across 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. 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. Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. Normalization template original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

pril 2023

Noise and artifact removal 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.

Statistical modeling & infe	erence	
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 ANOV 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 <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 Monte Carlo).	
Models & analysis		

n/a       Involved in the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the study       Image: State of the study         Image: State of the s		
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 metrics.	