

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ 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. <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> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Next-generation sequencing data utilized the AVITI Sequencing System (Element Biosciences), including automated de-multiplexing and adapter trimming.
Data analysis	Next-generation sequencing data were analyzed and visualized using custom scripts and IGV (version 2.8.13). Structural figures were generated with ChimeraX (v1.6.1). Protein structures were predicted with Alpha Fold 3. RNA sequencing data was processed with cutadapt (v4.2), aligned with bwa-mem2 (v2.2.1), filtered and converted to BAM with SAMtools (v1.17), and converted to bigwig files with deeptools (v3.5.1). For phylogenetic analysis of Gin proteins, MAFFT (v7.511) was used for alignments, FastTree (v2.1.11) was used to build phylogenies, and iTOL (online) was used to visualize trees. Sequence analyses in R utilized the Biostrings (v2.70.1) and ggplot2 (v3.4.4) packages. Loci maps were rendered in Geneious (v2024.0.3). Custom code used to analyze sequences has been deposited at Zenodo and is publicly available at 10.5281/zenodo.15307114.

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](#)

High-throughput sequencing data have been deposited at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (BioProject Accession: PRJNA1256936) and are publicly available.

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

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.

Ethics oversight

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.

☒ 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](https://www.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 reported in the figure legends and generally encompass three biological replicates. This sample size is sufficient to reflect the degree of uncertainty and experimental differences between measurements. For experiments involving mice, sample size has been calculated using a power analysis and appropriate statistical test in G*Power 3.1 software (Faul, Erdfelder, Land, & Buchner 2007). We determined based on previous studies and expected variability that 5 mice per group was required.

Data exclusions

No data were excluded

Replication

All data could be reproduced, and most experiments and analyses presented were the result of two to three independent biological replicates.

Randomization

Samples were not randomized as it was not applicable for the design of this study (this study did not involve selecting samples from a larger population).

Blinding

Investigators were not blinded as it was not applicable for the design of this study and experiments included necessary controls.

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 <i>Passer domesticus</i> , all <i>Stenocereus thurberi</i> 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

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	Monoclonal anti-FLAG M2 antibody produced in mouse, Sigma Aldrich, Catalogue number: F1804, clone M2
Validation	According to the manufacturer, the "monoclonal ANTI-FLAG M2 may be used in IP [immunoprecipitation] procedures when used in conjunction with an insoluble carrier matrix, such as a Protein G resin" (https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/175/747/f1804bul-ms.pdf). As suggested, the ANTI-FLAG M2 antibody was used together with Dynabeads Protein G resin (Thermo Fisher) in this study. Furthermore, ANTI-FLAG M2 antibody was used in a ChIP-seq study by Partridge et al., Nature (2020), titled "Occupancy maps of 208 chromatin-associated proteins in one human cell type."

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Human TLR5 Reporter HEK293 cells (Invivogen, HKB-HTLR5).
Authentication	Human TLR5 Reporter HEK293 cells were authenticated upon purchase from Invivogen.
Mycoplasma contamination	Human TLR5 Reporter HEK293 cells were tested negative for mycoplasma contamination. Stringent prophylactic measures were implemented to prevent potential mycoplasma contamination, including the use of antibiotics (Penicillin, Streptomycin, Blastidicin, and Zeocin) and conducting all experiments in a sterile environment.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Palaeontology and Archaeology

Specimen provenance	<i>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.</i>
Specimen deposition	<i>Indicate where the specimens have been deposited to permit free access by other researchers.</i>
Dating methods	<i>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.</i>
☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	<i>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.</i>

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	Mice used in this study, strain C57BL/6, provided by the Jackson Laboratory, were 6 weeks old at the beginning of the experiment. Mice were housed in a facility with a 12-hour light–dark cycle and provided ad libitum access to food and water. Housing conditions were maintained at 21–24°C and 40–60% humidity.
Wild animals	This study did not involve wild animals.
Reporting on sex	Mice used in this study were all females. Hence, sex was not considered a variable in the study.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All mouse experiments were performed at Columbia University and approved by the Institutional Animal Care and Use Committee (IACUC) of Columbia University (protocol AABQ5551).

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 ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	<i>Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.</i>
Study protocol	<i>Note where the full trial protocol can be accessed OR if not available, explain why.</i>
Data collection	<i>Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.</i>
Outcomes	<i>Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.</i>

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	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, mosaicism, 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	High-throughput sequencing data have been deposited at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (BioProject Accession: PRJNA1256936) and are publicly available. <i>May remain private before publication.</i>
Files in database submission	Raw ChIP-seq files: A_R1.fastq.gz A_R2.fastq.gz B_R1.fastq.gz B_R2.fastq.gz C_R1.fastq.gz C_R2.fastq.gz D_R1.fastq.gz D_R2.fastq.gz E_R1.fastq.gz E_R2.fastq.gz F_R1.fastq.gz F_R2.fastq.gz G_R1.fastq.gz G_R2.fastq.gz H_R1.fastq.gz H_R2.fastq.gz MG-input_R1.fastq.gz MG-input_R2.fastq.gz WT_R1.fastq.gz WT_R2.fastq.gz R_R1.fastq.gz R_R2.fastq.gz R-input_R1.fastq.gz R-input_R2.fastq.gz Processed ChIP-seq files (bigWig): A_coverage_CPM.bw B_coverage_CPM.bw C_coverage_CPM.bw D_coverage_CPM.bw E_coverage_CPM.bw F_coverage_CPM.bw G_coverage_CPM.bw H_coverage_CPM.bw MG-input_coverage_CPM.bw WT_coverage_CPM.bw R_coverage_CPM.bw R-input_coverage_CPM.bw
Genome browser session (e.g. UCSC)	Modified reference genomes (provided at 10.5281/zenodo.15307114) were used. Normalized bigWig files can be visualized in the Integrative Genomics Viewer (IGV) using the bigWig (.bw) file of choice (normalized using bamCoverage).

Methodology

Replicates	One biological replicate was used for ChIP-seq samples, consistent with exploratory mapping analyses.
Sequencing depth	Number of raw reads, uniquely mapped reads, length of reads and paired- or single-end nature are provided in Supplementary Table 1.
Antibodies	Monoclonal anti-FLAG M2 antibody produced in mouse, Sigma Aldrich, Catalogue number: F1804, clone M2
Peak calling parameters	<pre>#Trim reads using fastp fastp -i "input_read1.fastq.gz" -l "input_read2.fastq.gz" -o "trimmed_output_read1.fastq.gz" -O "trimmed_output_read2.fastq.gz" -j "log".json -h "log".html #Map reads using bowtie2 (creates a .sam output file) #Reads were mapped to a modified E. coli K12 reference genome (derivative of GenBank: NC_000913.3) bowtie2 -x "directory_to_E-coli_reference_genome_file" -1 "trimmed_output_read1.fastq.gz" -2 "trimmed_output_read2.fastq.gz" -S "output.sam" #Convert .sam into .bam file using samtools samtools view -b "input.sam" > "output_directory" #Sort the .bam files using samtools samtools sort -o output_directory "input.bam" #Index the aligned and sorted .bam files using samtools samtools index -b "input.bam" "output.bam.bai" #Eliminate multi-mapping reads using samtools (retains only uniquely mapping reads); #uses a MAPQ score of 10 as a cutoff samtools view -bq 10 "input.bam" > "output_directory" #Create index files for the trimmed, aligned, sorted and uniquely mapping reads using samtools samtools index -b "input.bam" "output.bam.bai" #Normalize reads using deepTools2 bam Coverage with the option "RPKM" or "CPM" bamCoverage --normalize Using CPM -bs 1 -b "not_normalized.bam" -o "normalized.bw"</pre>
Data quality	Bowtie2 was used to map reads using default parameters. SAMtools was used to filter for uniquely mapping reads using a MAPQ score threshold of 10.
Software	The AVITI Sequencing System (Element Biosciences) was used for automated de-multiplexing and adapter trimming. All custom code for analysis is deposited at Zenodo (10.5281/zenodo.15307114).

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	<i>Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.</i>
Instrument	<i>Identify the instrument used for data collection, specifying make and model number.</i>
Software	<i>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.</i>
Cell population abundance	<i>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.</i>
Gating strategy	<i>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.</i>

- ☐ 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	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 measures	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

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	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 & inference

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 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,

Graph analysis

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.