nature portfolio

Corresponding author(s): David Lara-Astiaso; Alexander A. Nevue; Inna Averbukh; George C. Hartoularos

Last updated by author(s): May 21, 2026

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	Sequencing was performed on NovaSeq 6000(Illumina). Microwell-array filling used a custom program on a Scienion sciFLEXARRAYER S3 piezo-driven non-contact liquid dispenser. FACS enrichment used a STEMCELL Technologies Highway1 Cell Sorter.
Data analysis	bcl2fastq2 (default parameters) for FASTQ demultiplexing; Cell Ranger multi v8.0.1 and v10.0.0 (10x Genomics) for read alignment and feature counting (Gene Expression and Antibody Capture modes); custom Python scripts for clonal-barcode feature-reference generation. Downstream analyses in Python using Jupyter notebooks with scanpy (Wolf et al., 2018), mudata (Bredikhin et al., 2022), and Harmony (Korsunsky et al., 2019) for batch correction. UMAP and Leiden community detection via scanpy. Geomux (Teyssier and Dobin, 2026) for guide and clonal-barcode calling. limma-trend for pseudobulk differential expression analysis. decoupler with the multivariate linear model (mlm) for MouSSE cytokine activity scoring (Javaid and Frost, 2025). MSigDB Hallmark, Canonical Pathways, and GO Biological Process gene sets (v7.5) for over-representation analysis. A Python package for Survey Genomics spatial data is available at https://github.com/survey-genomics/survey/. Compute on AWS EC2 (m5n.8xlarge). Custom analysis code will be released at publication.

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

Unprocessed sequencing data are available to reviewers as GEO: GSE332620 (reviewer access: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE332620; token: ozgrwkkoljejheb).

Processed data objects containing the transcriptomic, spatial, protein, and CRISPR-based data used for downstream analyses are available to reviewers at Zenodo: https://zenodo.org/records/20186639?preview=1&token=eyJhbGciOiJIUzUxMiJ9.eyJpZCI6IjVhMTE3NDJlLTFhZWUtNGZiOC1iMjZmLWM0N2IzNzdjNzQ2MyIsImRhdGEiOnt9LCJyYW5kb20iOiJhYjg3MzgxY2I0MjYyNDlhNzk5MjdiMjY2MmNmMjQ0ZSJ9.GLfU0eDUxTNMKl2X3ZgepjRDkk9AMkauORzwM1pBweCMd4Zb1oEAw2OEbLVt45nB0Yw5hXul3h4mb9xW5hywVA

Source data for all main and Extended Data figures are provided in Extended Data Tables 1-8. A Python package for analyzing Survey Genomics spatial data is publicly available at https://github.com/survey-genomics/survey/. Custom analysis code will be made publicly available upon publication.

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

Life sciences study design

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

Sample size	For the organ-scale spleen Perturb-seq experiment, three biological replicate spleens (each from an independently transplanted recipient mouse) were processed across 10 microwell arrays and 50 tissue sections to achieve organ-scale coverage of perturbed cells (479,649 single cells profiled). Sample size was not determined by a formal power analysis; instead, throughput was scaled to the practical capacity of the assay (one 10x 3' GEM reaction per array) to ensure adequate coverage per perturbation (median 6,597 cells per perturbation; minimum 50 colonies per perturbation required for downstream colony-size and diversity modeling, see Extended Data Tables 2-5). The liver IFNgamma paracrine experiment used two liver sections processed on PerturbSpace arrays, yielding 340 IFNgamma sender cells and 2,156 control sender

cells, with focal-receiver populations of n = 257 (IFNgamma neighborhoods) and n = 2,594 (control neighborhoods). For the tissue-compatibility benchmarking, 11 liver arrays (115,641 cells) and 23 kidney arrays (278,261 cells) were processed across varying microwell-array resolutions.

Data exclusions

All exclusion criteria were pre-established. Spatial barcode calling: cells with <5 total spatial-barcode UMIs were classified as ambiguous and excluded from spatial analyses; cells whose top-ranked barcode did not exceed a 2-fold ratio against the second-ranked barcode were further evaluated through rank- and distance-weighted scoring of valid combinatorial barcode pairs, with unresolvable cells assigned as ambiguous. Geomux guide and clonal-barcode calling: cells with <5 total UMIs and features with <10 total UMIs were excluded, with one count subtracted from every nonzero entry to control for ambient contamination; significant assignments below an adaptive log-odds-ratio threshold were revoked. Single-cell QC: genes were filtered to those with >=10 counts in >=20 cells. Colony analyses: doublets, high-mitochondrial cells, fibroblasts, and endothelial cells were excluded; colonies with <5 cells or no assigned perturbation were excluded from K-means clustering and enrichment testing; for colony-size vs proliferation analysis, only colonies with majority-cell-type purity >=75%, >=2 cells, a valid perturbation, and a non-missing proliferation score were retained, and cell-type x perturbation combinations represented by <5 colonies were removed. DGE pseudobulk analysis: genes with <1 count in <2 samples were excluded. NCA cytokine analysis: focal cells were restricted to the three most abundant non-construct cell types in IFNgamma neighborhoods (Kupffer cells, monocyte-derived macrophages, endothelial cells). Per-sample QC metrics are reported in Extended Data Table 2.

Replication

The organ-scale spleen Perturb-seq experiment used three independently transplanted recipient mice (biological replicates) processed across 10 microwell arrays, with every other 300-um section taken across the entire spleen (5 sections per array). Tissue compatibility for the universal antibody-based hashing cocktail was independently demonstrated in three tissue types (spleen, liver, kidney) across multiple arrays of varying resolution (11 liver arrays, 23 kidney arrays). The liver IFNgamma paracrine experiment was performed in one biological cohort, with two liver sections processed on PerturbSpace arrays. All replication attempts described in the manuscript were successful; no unsuccessful replication attempts are reported.

Randomization

Randomization of cells to experimental groups is not relevant to this study because experimental groups are defined by CRISPR perturbation identity, which is delivered as a pooled lentiviral library at low multiplicity of infection (MOI 0.2). Each cell receives a perturbation by stochastic transduction rather than by experimental assignment, and perturbation identity is read out post-hoc from sgRNA sequencing in the same cell. Non-targeting control sgRNAs were co-transduced in the same pool and the same recipient animals, providing an internal comparator matched for all biological and technical covariates (animal, transplantation procedure, tissue section, processing batch, sequencing batch).

Blinding

Blinding was not relevant to data collection because perturbation identity is encoded in the sequencing data (sgRNA barcode), not in any operator-applied label; tissue dissociation, staining, FACS enrichment, and library preparation were performed on pooled cells without operator knowledge of any individual cell's perturbation. For analysis, perturbation identity is required to define experimental groups, so analysts were necessarily unblinded; however, all primary statistical comparisons used automated pipelines (Geomux for perturbation calling, scanpy for clustering, limma-trend for differential expression, decoupler for cytokine scoring) that remove operator judgement from the test statistics.

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

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	TotalSeq-A Mouse Universal Cocktail (Biolegend, 199901), Totalseq-B Hashtag antibodies were used for spatial hashing.
Validation	validated with FMO

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Lentivirus production: HEK293T cells were used as packaging cells (transfected with pMD2.G (Addgene #12259), psPAX2 (Addgene #12260), and the CRISPR-seq transfer plasmid using Lipofectamine 3000). The HEK293T source (ATCC vs laboratory stock) and sex are not specified Takara, 632180 in the manuscript and should be added. Primary cells (not cell lines): hematopoietic progenitor cells (HPCs) were freshly isolated from the bone marrow of 8-12 week-old Rosa26-Cas9 mice (Jax #028555) and expanded ex vivo for 10 days in HemEx-Type9AO medium (Iwai #A5P10P01C); HPC sex is not reported.
Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*
Mycoplasma contamination	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines (per the ICLAC register) were used. HEK293T is a HEK293 derivative used here only for transient lentiviral packaging; this is the standard application of this line and is not problematic in this context.

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 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; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Mouse strains: C57BL/6J (Jackson Laboratory #000664; recipients for transplantation in all in vivo experiments); Rosa26-Cas9 (Jackson Laboratory #028555; HPC donors providing constitutive Cas9 expression); KH2 (Jackson Laboratory #029415; HPC donors for the doxycycline-inducible IFNgamma overexpression experiment, providing the rtTA allele). HPC donors were 8-12 weeks old. NOTE: Recipient mouse age at transplantation, 8-10 weeks. Sex of donors was mixed while recipients were all male.
Wild animals	No wild animals were used in this study.
Reporting on sex	Sex of donor and recipient mice is not reported in the manuscript. NOTE: The Methods should specify the sex of all donor and recipient mice and whether findings were assessed for sex-specific effects; if only one sex was used, justify.
Field-collected samples	No field-collected biological material was used in this study.
Ethics oversight	All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Arc Institute. Protocol number: ARC-024-003

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	*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:

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

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files 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 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	Tissues (spleen, liver, kidney) were harvested and sectioned at 300 um on a Compresstome (Precisionary Instruments). Sections were spatially hashed on PerturbSpace microwell arrays for 30 minutes at room temperature in a humidified chamber, then released and dissociated in collagenase at 10 C. Dissociated cells were stained with anti-rat PE secondary antibody (BioLegend #405406) to label spatial-hashing antibodies for FACS enrichment. In two arrays, an additional staining step with TotalSeq-A Mouse Universal Cocktail (BioLegend #199901) was added for CITE-seq. Cells were sorted on live (viability gate), PE-positive (spatial hash), and miRFP670nano3-positive (transplanted cells) markers, depending on the experiment.
Instrument	Highway1 Cell Sorter (STEMCELL Technologies).
Software	The FACS instrument-control software used was Highway1. FlowJo v10 was used for post-acquisition flow-cytometry analysis.
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	Complete gating strategy is in extended data fig 1e

☐ 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 (See Eklund et al. 2016)	*Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*
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.*