nature portfolio

Corresponding author(s): Jamie Rossjohn
Dale I Godfrey
Adam P Uldrich

Last updated by author(s): 12 July 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 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	Clustering experiments - acquired using dSTORM
Data analysis	Clustering experiments - code available GitHub repository link (https://github.com/PRNicovich/ClusDoC) and MATLAB as per Pageon et al PNAS 2016

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

Protein databank (https://www.rcsb.org/) accession codes = PDB ID 7TSL and 7TSN

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	De-identified buffy coats from healthy individuals provided by the Australian Red cross, or from the Brigham and Women's Hospital Specimen Bank, Boston. Donors aged 18-70.
Reporting on race, ethnicity, or other socially relevant groupings	De-identified buffy coats from healthy individuals provided by the Australian Red cross - Melbourne, or from the Brigham and Women's Hospital Specimen Bank, Boston.
Population characteristics	Healthy Australian donors deemed fit to donate by the Australian Red Cross - Melbourne, or from the Brigham and Women's Hospital Specimen Bank, Boston.. This included such thing as, but not limited to, being aged between 18 and 70 years of age, having no recent tattoos or surgeries, and deemed healthy at the time of donation with negative serology for HIV, Hepatitis B and Hepatitis C.
Recruitment	Buffy coats derived from blood donors to the Australian Red Cross, or from the Brigham and Women's Hospital Specimen Bank, Boston, with written informed consent.
Ethics oversight	Buffy coat samples were obtained from the Australian Red Cross after approval from the University of Melbourne Human Research and Ethics Committee and donor informed consent provided (Ethics #1035100), or from the Brigham and Women's Hospital Specimen Bank, Boston (Ethics #2002P000057).

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	12 4D4 Retrogenic C57BL/6 mice acquired over 5 experiments, 7 WT mice acquired over 4 experiments (as per Fig 7 and source data Supp fig 10A)
Data exclusions	No data was excluded from the analysis of experiments shown within the manuscript and associated files, except where indicated in source data Figure 3D - 2 first injections of ITC (initial stabilization) - highlighed by *
Replication	All replication attempts were successful.
Randomization	Randomization not applicable to the techniques employed. The processes involved knowing and keeping track of cell lines/reagents/samples.
Blinding	Blinding was not applied and was not relevant for this study. The processes involved knowing and keeping track of cell lines/reagents/samples.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

As described in methods.
Specifically:

Flow cytometry:
Fluorochrome-conjugated mouse-specific antibodies were from BD Biosciences unless otherwise stated: anti-mouse TCRβ-AF700 (eBiosciences, #25-5961-82; 1:100) / BV421 (#562839; 1:200)/ APC (#553174; 1:100)/BV711 (#563135; 1:400)/ BV786 (#742484; 1:200) – all clone H57-597, CD69-APC/BUV797 (clone H1.2F3, #560016/ #612793; both 1:100), CD25 BV605 (clone PC61, #563061; 1:200), CD44-AF700 (clone IM7, #560567), CD4-BUV395 (clone RM4-5, #740208; 1:400), CD8-BUV797 (clone 53-6.7; 1:800), CD11c-APC-Cy7/BV421 (clone HL3, #561241, #562782; 1:400), CD1d-BV711 (clone 1B1, #740711; 1:200), CD11b-APC-Cy7 (clone M1/70, #557657; 1:200), B220-BUV496 (clone RA3-6B2, #612950, 1:400), CD19 APC-Cy7 (clone 6D5, #11530; 1:100), CD3 BUV395 (clone 17A2, #740268; 1:100), CD45.2 APC (clone 104, #109814; 1:100), CD45.1 BV786 (clone A20, #740889; 1:200). Anti-human CD25-BV605 (clone 2A3, #562661; 1:100), CD3-BV421 / BUV395 (clone UCHT1, #562426 / #563546; 1:200/ 1:100), CD44-AF700 (clone IM7, eBioscience #56-0441-82), CD69-BUV737 (clone FN50, #612817; 1:100), CD4-BV421 (clone RPA-T4, Biolegend #300532; 1:400), TCRγδ-PE-Cy7/ FITC (clone 11F2, #655410/ #347903; 1:50/ 1:25), TCR δ1-FITC (clone TS-1, Invitrogen #TCR2055; 1:50), 7-amino-actinomycin D (7AAD, Sigma #A9400; 1:300) or Near Infra-Red (NIR 780-Thermo Fisher, #L34992; 1:400) viability dyes were included in all flow cytometry-staining panels for dead cell exclusion. Streptavidin (SAV)-PE (#554061), SAV-APC (#554067), APC-Cy7 (#554063), SAV-PE-Cy7 (#557598) were purchased from BD Biosciences. SAV-BV421 (#405225) from Biolegend. SAV-PB (#S11222), SAV-PE (#S868, Supplementary Fig 1 D), SAV-APC (#S866, Supplementary Figure 1C-D) or Neutravidin (NAV)-PE (#A-2660) were purchased from Molecular Probes. All SAV-NAV conjugates were used at 1 ug/mL for BW58 cell line staining for flow cytometry, or 5 ug/mL for retrogenic/WT mice primary cell staining (Figure 8) flow cytometry analysis of cell lines, or 5 ug/mL for mouse primary cell staining (WT and retrogenic, . Non-conjugated R-PE was purchased from Prozyme (undisclosed biological source, #PB31-10MG) or Thermo Fisher Scientific (Porphyra Tenera, #46185), both also used at 1 ug/mL for cell staining for flow cytometry.

Supported Lipid Bilayer TCR Clustering assays:
anti-mouse CD3 (Invitrogen, #13-0031-82, clone 145-2C11) and anti-mouse CD28 mAbs (Invitrogen, #13-0281-82, clone 37.51), 100 µg/mL of streptavidin with no fluorochrome (Invitrogen, #434301), 200 ng/mL of His-tagged mouse ICAM-1 (Sino Biological, #50440-MO8H) .

Immunostaining:
TCRβ subunit and conjugated to Alexa Fluor 647 fluorophore (clone H57-597, BioLegend, #109218)
pCD3ζ-Alexa Fluor 647 (#558402, BD Biosciences)

Validation

All antibodies used in this study are commercially available, validated by manufacturers and widely accepted in the field for their intended use. Antibodies were validated to be working by staining of relevant control samples and titrated in house.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293T, Jurkat(76), K562 and BW58 cells have been in stocked in the laboratory for approx. 20 years. Original source is unknown.
HEK293S are from ATCC

Authentication

HEK293T and HEK293S cells have not been validated, though they morphologically and functionally resemble appropriately. BW58 and Jurkat cell lines have been validated by our collaborators.

Mycoplasma contamination

HEK293t, HEK293S and BW58 cell lines tested negative for mycoplasma contamination. Jurkat lines have not been specifically tested, although random testing of parental/sister lines often yields negative results.

Commonly misidentified lines
(See ICLAC register)

n/a

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	C57BL/6 WT, C57BL/6 LY5.1, C57BL/6 CD1d-/- or C57BL/6 Ragl-/- mice (7-10 weeks old, age and gender matched per experiment)
Wild animals	No wild animals used
Reporting on sex	Animals were gender matched for the experiment
Field-collected samples	No field-collected samples.
Ethics oversight	Animals were bred and maintained in specific pathogen free conditions in the Monash Animal Research Platform, or Department of microbiology and Immunology. Animal experimentation and sacrifice by CO2 inhalation were conducted following the Australian National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Scientific Purposes guidelines for housing and care of laboratory animals and under approval from the Monash University Animal Ethics Committee (MARP/2016/118 and 2023-40644), or the University of Melbourne (UoM) Animal Ethics Committee (#1513734).

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	*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	As described in methods. Specifically: Mouse thymocytes were isolated as described 31 using complement-mediated enrichment of mature thymocytes with anti-CD24 (clone J11d; produced in house) on ice for 30 min, followed by the addition of rabbit complement (GTI Diagnostics, #RC-100) and 70 µg/mL deoxyribonuclease I (DNase1, Merck, #10104159001) for another 30 min at 37°C. Thymocytes were washed with 10 % FBS/RPMI and further purified using a gradient enrichment (Histopaque-1083; Sigma, #10831). Livers were perfused using cold PBS and liver lymphocytes isolated following an 33 % V/V isotonic Percoll (Cytiva, # 17-0891-01) gradient. Lymphocytes from the spleen, liver, peripheral lymph nodes, bone marrow and lungs were then incubated with red blood cell lysis buffer (Sigma, #R7757-100ML). Staining: Cell suspensions were first incubated for 10 min on ice with Fc-receptor block (anti-mouse CD16/CD32, clone 2.4G2; produced in house, or anti-human Milteny #130-059-901) in PBS supplemented with 2% FCS, prior to antibody staining. Fluorochrome conjugated antibodies were added at working concentrations without washing for another 25 min. Cells were washed twice (400g) in PBS supplemented with 2% FCS prior to acquisition.
Instrument	BD LSRFortessa II™ with or BD FACSAria III™ (BD)
Software	DIVA, FlowJo version 8 or 10, FCAP v3, Graph Pad Prism 10
Cell population abundance	Stable TCR transduced lines were selected for having high but variable TCR expression as shown in Fig 1A. As shown in plots. Cells from 4D4 retrogenic mice or from human gdTCR-expanded cells were single sorted for TCR sequencing (only one TCR amplified per well validating 100% purity). BMDCs were >95% pure after differentiation with GMCS-F and IL-4. TCR-transduced BW58 lines were sorted based on GFP/TCR expression (100% purity -Fig 1, displaying a range of TCR intensities)
Gating strategy	As detailed in figure legend and supplementary Figure 10: An FCS versus SSC gate is applied to gate on lymphocytes, followed by FCS-H versus FSC-A to gate out doublets, followed by FCS-A Vs 7AAD (or Live dead NIR-Fig 7). In cell lines this was followed by a gate on cells with similar CD3 Vs GFP, followed by analysis of activation markers. As shown in Supplementary Figure 10A In mouse primary cells this was proceeded by a time Vs SSC-A gate, and followed by a gate applied exclude B cells (B220 Vs CD3). This was followed by GFP versus TCRbeta gating for phenotypic analysis, as displayed in Fig 7 - as shown in Supplementary Figure 10B. BMDCs were analysed post GMCSF-IL4 differentiation, by assessing CD1d expression after gating on CD11c+ cells as Per Supplementary Fig 10C

☒ 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, 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.*