

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	Software used: • Phenochart v1.1 (Akoya Biosciences, Inc.) – for image acquisition and region selection on the Vectra Polaris™ platform. • inForm v2.6.0 (Akoya Biosciences, Inc.) – for spectral unmixing of multiplex IHC images. • HALO v3.5 (Indica Labs) – for tissue and cell segmentation and cell coordinate extraction. • FlowJo v10.8.1 (BD Biosciences) – for flow cytometry data analysis and gating. • Cell Ranger (10x Genomics) – for processing scRNA-seq and TCR-seq raw data, including alignment, gene quantification, and clonotype calling. Statement: Commercial software was used for mIHC image acquisition and processing (Phenochart, inForm, HALO), flow cytometry gating and quantification (FlowJo), and single-cell data processing (Cell Ranger). No custom code was used during data collection stages.
Data analysis	Open-source and custom software used: • R (v4.x) – Core platform for statistical, spatial, and transcriptomic analysis. • SPIAT – spatial analysis of tissue sections, cell distances, neighborhood detection (via DBScan). • AMMIT – R package developed by the Neeson Lab to streamline the processing of multiplex immunohistochemistry (mIHC) data. It utilizes a mixture of skew-normal distributions to model marker intensity distributions, enabling automated and probabilistic thresholding for cell phenotyping. This approach reduces the subjectivity and labor associated with manual thresholding, particularly in large-scale studies. (https://github.com/neeson-lab/AMMIT). • Seurat – preprocessing, filtering, integration (via Harmony), UMAP visualization, clustering, differential gene expression. • Harmony – batch correction and integration for scRNA-seq datasets.

- scds – doublet detection.
- SingleR and Azimuth – reference-based cell annotation.
- GSVA – module scoring and z-score calculation.
- edgeR and limma – differential gene expression and abundance analysis (for scRNA-seq, NanoString, and bulk RNA-seq).
- ruv - Subsequent normalization and gene quantification
- fgsea – gene set enrichment analysis using MSigDB pathways.
- uwot – UMAP and nearest-neighbour network generation for neighborhood clustering.
- CellPhoneDB (v2.1.7) – inference of ligand-receptor interactions from scRNA-seq.
- Leiden clustering of the adjacency graph for this network was used to identify clusters of similar neighborhoods

Statement:

All data analysis was performed using R and established open-source packages for transcriptomic, spatial, and statistical analysis. Custom code was used to implement AMMIT for probabilistic mIHC thresholding. Code for AMMIT is publicly available on GitHub (<https://github.com/neeson-lab/AMMIT>).

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](#)

The processed sequencing data have been deposited in the Gene Expression Omnibus (GEO) database: series GSE294272 for bulk RNA-Seq, series GSE294441 for Nanostring, series GSE294273 for scRNA-Seq, and series GSE294274 for TCR-Seq. Given the human origin of the samples, the raw data are available on the European Genome-Phenome Archive (EGA; accession number EGAS50000001722) and will be released from the lead contact upon request. Raw primary imaging data and any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Research involving human participants, their data, or biological material

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

Reporting on sex and gender

Sex was recorded for all participants based on clinical records and defined as a binary biological variable (male/female). Gender identity was not recorded. Sex was not used as a stratification factor in study design or downstream analysis, as the primary aim was to investigate tumour-resident T cell phenotypes and immune responses across treatment groups. No sex-based analysis was performed, and the omission is justified by limited sample size and absence of prior data suggesting sex-specific differences in TRM-mediated ICI response in melanoma. Consent for sharing de-identified clinical metadata was obtained via Melanoma Research Victoria (MRV).

Reporting on race, ethnicity, or other socially relevant groupings

Race and ethnicity data were not collected for this study and were not used in study design or analysis. The study was conducted on a clinically annotated cohort from Melanoma Research Victoria (MRV), where samples were de-identified prior to analysis. As such, socially constructed variables were not analysed nor used as proxies for biological or socioeconomic factors. No socially relevant group stratification was applied.

Population characteristics

The study included 32 adult patients (stage III/IV melanoma) who underwent lymph node biopsy at Peter MacCallum Cancer Centre between 2015 and 2023. Clinical characteristics including age, sex, histopathological diagnosis, treatment history, and response status were obtained through Melanoma Research Victoria (MRV) (see Supplementary Table 1). Patients were classified into three treatment groups: untreated (n=13), ICI-resistant (n=13), and ICI-responsive (n=6). The majority received anti-PD-1 monotherapy or combination therapy with anti-CTLA-4. No genetic stratification or tumour mutational burden analysis was performed.

Recruitment

Patients were recruited through Melanoma Research Victoria (MRV) and provided informed consent for tissue donation and data use. Samples were obtained as part of standard surgical procedures. There is potential for self-selection bias as patients who consented to research participation may differ from those who did not. However, selection was based on availability of annotated clinical response data and sufficient tissue material for multi-omic analyses. Potential biases were addressed through careful cohort curation and cross-platform validation.

Ethics oversight

Melanoma Research Victoria (MRV)

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

Life sciences study design

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

Sample size	Sample size was not predetermined by statistical calculation, as this study was exploratory in nature and based on the availability of biospecimens from the Melanoma Research Victoria (MRV) biobank. Sample size was selected to provide a representative distribution of immune checkpoint inhibitor (ICI) responders and non-responders, sufficient for downstream single-cell and functional analyses.
Data exclusions	Samples were excluded if cell viability was below 70% post-thaw, or if cell recovery was insufficient for flow cytometry or functional assays. These criteria were pre-established to ensure data quality. No exclusions were made based on outcome or phenotype.
Replication	Key findings were validated across independent biological replicates from multiple patients. Where possible, technical replicates were included for flow cytometry and functional assays. Results were consistent across replicates, supporting reproducibility.
Randomization	Randomisation was not applicable as this was an observational study using human samples. However, experimental batches were balanced for responder status and tissue type to control for batch effects.
Blinding	Blinding was not performed during experimental processing due to the logistical constraints of working with limited patient-derived material. However, data analysis was conducted using automated or unbiased computational pipelines, and clinical outcomes were masked until after initial data interpretation.

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	Antibodies used for multiplex IHC: SOX10 BioCare Medical ACI3099A BC34 1:1000 CD3 Abcam ab16669 SP7 1:150 CD4 Abcam ab133616 EPR6855 1:150 CD8 Invitrogen MA1-80231 4B11 1:50 CD69 Abcam ab233396 EPR21814 1:750 CD103 Abcam ab129202 EPR4166 1:1000 TCF1 Cell Signalling Technology 2203 C63D9 1:100 CD11c Abcam ab52632 EP1347Y 1:250 Antibodies used for flow cytometry: CD3 BD Biosciences 564001 BUV 395 SK7 CD4 Biolegend 344622 Alexa Fluor 700 SK3 CD8 BD Biosciences 612889 BUV805 SK1 CD69 BD Biosciences 557745 PE-cy7 FN50 CD45Ro Biolegend 304206 PE UCHL1 CCR7 Biolegend 353228 BV711 G043H7 CD39 Biolegend 328210 APC A1 CD103 BD Biosciences 563882 BV421 BerACT8 CD45 Biolegend 304036 BV510 HI30 GZMB BD Biosciences 570498 RB744 GB11 CD107a BD Biosciences 561343 APC-H7 H4A3
Validation	Antibodies Used for Multiplex IHC SOX10 – BioCare Medical ACI3099A (clone BC34), 1:1000

- Application: IHC
- Species: Human
- Validation: Manufacturer states this clone is validated for IHC on formalin-fixed paraffin-embedded (FFPE) human tissues. Widely cited in melanoma studies (e.g., Appl Immunohistochem Mol Morphol. 2013 Dec; 21(6):506-10). Detected nuclear SOX10 expression in melanocytes and melanoma cells, consistent with expected pattern.

CD3 – Abcam ab16669 (clone SP7), 1:150

- Application: IHC
- Species: Human
- Validation: Manufacturer validates for IHC-P in human with strong positive staining in T-cell rich tissues (tonsil, lymph node). Clone SP7 is extensively cited for CD3 detection (e.g., Nat Commun. 2023 Dec 9;14(1):8172. doi: 10.1038/s41467-023-44053-w.). Listed on the Human Protein Atlas with consistent staining.

CD4 – Abcam ab133616 (clone EPR6855), 1:150

- Application: IHC
- Species: Human
- Validation: Manufacturer validated in IHC-P on human FFPE tissues. Clone EPR6855 is widely used and appears in >100 publications (e.g Clin Transl Med 2023 Dec;13(12):e1515. doi: 10.1002/ctm2.1515.). Specific staining of helper T cells in lymphoid organs.

CD8 – Invitrogen MA1-80231 (clone 4B11), 1:50

- Application: IHC
- Species: Human
- Validation: Manufacturer validated in human IHC-P. Clone 4B11 is widely cited and used in melanoma T-cell infiltration studies (e.g., Clin Cancer Res. 2007;13(7):2055–2061). Provides expected membranous staining of cytotoxic T cells.

CD69 – Abcam ab233396 (clone EPR21814), 1:750

- Application: IHC
- Species: Human
- Validation:
- Validated by Abcam for IHC-P in human.
- Cited in studies of tissue-resident T cells in FFPE samples (BMC Cancer . 2023 Oct 20;23(1):1011. doi: 10.1186/s12885-023-11438-5.) Consistent with expected membrane/cytoplasmic staining on activated T cells.

CD103 – Abcam ab129202 (clone EPR4166), 1:1000

- Application: IHC
- Species: Human
- Validation:
- Manufacturer validated in human IHC-P.
- Widely used to detect tissue-resident CD8⁺ T cells in FFPE tissues.
- Confirmed membranous expression in intraepithelial lymphocytes.

TCF1 – Cell Signaling Technology 2203 (clone C63D9), 1:100

- Application: IHC
- Species: Human
- Validation: CST reports validation in IHC-P on human samples. Clone C63D9 has been used in peer-reviewed studies characterising TCF1⁺ T cells (e.g., Nature. 2019; 565(7739): 265–269). Nuclear staining in T cells consistent with transcription factor localisation.

CD11c – Abcam ab52632 (clone EP1347Y), 1:250

- Application: IHC
- Species: Human
- Validation: Manufacturer validated for IHC-P on human tissues. EP1347Y is cited in multiple DC-related publications (e.g. Cancer Res Treat. 2024 Apr;56(2):624-633. doi: 10.4143/crt.2023.1076. Epub 2023 Nov 29). Stains dendritic cells and monocytes with appropriate distribution.

Antibodies Used for Flow Cytometry

All antibodies listed below are conjugated monoclonal antibodies validated by the manufacturer for human flow cytometry and sourced from reputable commercial suppliers (BD, BioLegend).

CD3 – BD Biosciences 564001 (clone SK7, BUV395)

- Application: Flow cytometry
- Species: Human
- Validation: Manufacturer validation for human flow cytometry. Clone SK7 is a gold-standard clone used in clinical and research flow cytometry panels (Nat Commun. 2025 May 10;16(1):4362. doi: 10.1038/s41467-025-59370-5. PMID: 40348752; PMCID: PMC12065855.)

CD4 – BioLegend 344622 (clone SK3, Alexa Fluor 700)

- Application: Flow cytometry
- Species: Human
- Validation: SK3 is a well-established clone for CD4. Validated by BioLegend and used in >23 publications (e.g. iScience. 2025 Apr 2;28(5):112343. doi: 10.1016/j.isci.2025.112343. PMID: 40276759; PMCID: PMC12020857.).

CD8 – BD Biosciences 612889 (clone SK1, BUV805)

- Application: Flow cytometry
- Species: Human
- Validation: SK1 is a validated clone used in clinical cytometry. BD Biosciences confirms use in human samples. Cited in 44 publications (e.g. Nat Cancer. 2025 Apr;6(4):647-665. doi: 10.1038/s43018-025-00927-0. Epub 2025 Mar 17.)

CD69 – BD Biosciences 557745 (clone FN50, PE-Cy7)

- Application: Flow cytometry
- Species: Human
- Validation: Clone FN50 is widely used for early activation marker detection. Validated and cited in 44 publications (citeAb: <https://www.citeab.com/antibodies/2412860-557745-bd-pharmingen-pe-cy-7-mouse-anti-human-cd69?des=5b85d27f1bef4e53>).

CD45RO – BioLegend 304206 (clone UCHL1, PE)

- Application: Flow cytometry
- Species: Human
- Validation: Clone UCHL1 is the canonical marker for memory T cells. Validated and cited in 46 publications (citeAb: <https://www.citeab.com/antibodies/521256-304206-pe-anti-human-cd45ro-antibody?des=f24d20e3ae462fce>).

CCR7 – BioLegend 353228 (clone G043H7, BV711)

- Application: Flow cytometry
- Species: Human
- Validation: Clone G043H7 is a standard reagent for T-cell memory phenotyping. Confirmed use in human blood and tissue. Validated and cited in 16 publications (citeAb: <https://www.citeab.com/antibodies/2398711-353228-brilliant-violet-711-anti-human-cd197-ccr7?des=a9dd03e555ed00ee>).

CD39 – BioLegend 328210 (clone A1, APC)

- Application: Flow cytometry
- Species: Human
- Validation: Clone A1 is a validated and widely cited marker for Tregs and exhausted T cells. Specific for human ENTPD1. Validated and cited in 17 publications (citeAb: <https://www.citeab.com/antibodies/523583-328210-apc-anti-human-cd39-antibody?des=3a0a2979d97dbb82>).

CD103 – BD Biosciences 563882 (clone Ber-ACT8, BV421)

- Application: Flow cytometry
- Species: Human
- Validation: Ber-ACT8 is the gold-standard clone for integrin α E. Used in flow: <https://www.citeab.com/antibodies/3289009-563882-bd-horizon-bv421-mouse-anti-human-cd103?des=9fd42b84d3b75173>

CD45 – BioLegend 304036 (clone HI30, BV510)

- Application: Flow cytometry
- Species: Human
- Validation: HI30 is the most widely used anti-CD45 clone for flow cytometry. Validated for broad leukocyte staining.

GZMB – BD Biosciences 570498 (clone GB11, RB744)

- Application: Flow cytometry
- Species: Human
- Validation: Clone GB11 is validated for intracellular staining of granzyme B. Routinely used in cytotoxicity and effector profiling studies (e.g. J Exp Med. 1997 Nov 3;186(9):1407-18. doi: 10.1084/jem.186.9.1407.

Eukaryotic cell lines

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

Cell line source(s)	NA
Authentication	NA
Mycoplasma contamination	NA
Commonly misidentified lines (See ICLAC register)	NA

Palaeontology and Archaeology

Specimen provenance	NA
Specimen deposition	NA
Dating methods	NA

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

Ethics oversight

NA

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

NA

Wild animals

NA

Reporting on sex

NA

Field-collected samples

NA

Ethics oversight

NA

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

NA

Study protocol

Melanoma Research Victoria. HREC MRV69.

Data collection

Clinical data and biospecimens were collected from patients enrolled in the Melanoma Research Victoria (MRV) biobank at Peter MacCallum Cancer Centre. Recruitment occurred between 2015 and 2023. Fresh tumour samples were collected on the day of surgery, dissociated into single-cell suspensions, and cryopreserved in liquid nitrogen. Formalin-fixed paraffin-embedded (FFPE) samples were also obtained from MRV. Patient-matched clinical annotations were prospectively curated by MRV in accordance with ethical protocol MRV69.

Outcomes

Primary outcome measures were immune checkpoint inhibitor (ICI) response status (clinical response vs non-response), defined using RECIST criteria or clinical assessment. Secondary outcomes included survival (overall and progression-free) and clinicopathological variables (e.g., tumour stage, mutation status). Outcome measures were assessed retrospectively through curated clinical annotations and linked to biospecimens for multi-omics analysis.

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	NA
Novel plant genotypes	NA
Authentication	NA

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 <i>May remain private before publication.</i>	<i>For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.</i>
Files in database submission	<i>Provide a list of all files available in the database submission.</i>
Genome browser session (e.g. UCSC)	<i>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.</i>

Methodology

Replicates	<i>Describe the experimental replicates, specifying number, type and replicate agreement.</i>
Sequencing depth	<i>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.</i>
Antibodies	<i>Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.</i>
Peak calling parameters	<i>Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.</i>
Data quality	<i>Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.</i>
Software	<i>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.</i>

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	Single-cell suspensions were prepared from fresh tumour samples collected at the time of surgical resection. Tissues were enzymatically dissociated using a cocktail of collagenase IV and DNase I, followed by filtration through a 70µm mesh. Red blood cells were lysed, and viable cells were stained directly for surface or intracellular markers, depending on the panel.
Instrument	Flow cytometry was performed on a BD FACSymphony A3 or A5 cytometer equipped with five lasers (355, 405, 488, 561, and 640nm).
Software	Data acquisition was performed using BD FACSDiva v9.0 software. Downstream analysis and gating were conducted in FlowJo v10.9.
Cell population abundance	The frequency of relevant immune cell subsets was quantified as a percentage of CD45 ⁺ live single cells.
Gating strategy	Gating was performed using FSC-A/SSC-A to exclude debris, followed by FSC-H/FSC-A for doublet discrimination and Zombie Aqua exclusion for live/dead discrimination. CD45 ⁺ events were gated first, followed by lineage-specific markers. Marker-positive and -negative gates were set using FMO controls where applicable. A representative gating strategy is included in Supplementary Figure 4 and 5.

- ☒ 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, 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.