

## 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

Data analysis

We provide information on publicly available software and settings in the Online Methods and the Supplementary Note. For custom data analysis, we describe the theoretical background as well as the models used in detail in the Supplementary Note. Custom code scripts are available on Zenodo (<https://doi.org/10.5281/zenodo.10804907>).

Publicly available software used in this study:

PLINK (v1.90b6.7): <https://www.cog-genomics.org/plink/1.9/>

SNPTTEST (v2.5.4) <https://www.chg.ox.ac.uk/~gav/snpctest/>

SAIGE (0.35.8.8): <https://github.com/saigegit/SAIGE>

N-GWAMA (v1.2.6) [https://github.com/baselmans/multivariate\\_GWAMA;](https://github.com/baselmans/multivariate_GWAMA;)

METAL (release 2011-03-25): <https://csg.sph.umich.edu/abecasis/metal/index.html>

METASOFT (v2.0.1): <https://web.cs.ucla.edu/~eeskin/>

GCTA (v1.93.0beta): <https://yanglab.westlake.edu.cn/software/gcta/#Overview>

LDSC (v1.0.1): <https://github.com/bulik/ldsc>

LDAC (v5.0): <https://dougsspeed.com/ldak/>

LHC-MR (v0.0.0.9000): <https://github.com/LizaDarrous/lhcMR>

DEPICT (v1 rel194): <https://github.com/perslab/depict>

FUMA (v1.3.6a): <https://fuma.ctglab.nl/>

MAGMA (v1.08) <https://cncr.nl/research/magma/>

MetaXcan (v0.7.4) <https://github.com/hakyimlab/MetaXcan>

eCAVIAR (v2.2): <https://github.com/fhormoz/caviar>

CAVIARBF (v0.2.1): <https://bitbucket.org/Wenan/caviarbf/src/master/>  
 CELLECT (v1.3.0) and CELLEX (v1.2.1): <https://github.com/perslab/CELLECT>  
 MAGMA\_celltyping (v2.0.0): [https://github.com/neurogenomics/MAGMA\\_Celltyping](https://github.com/neurogenomics/MAGMA_Celltyping)  
 pycox (v0.2.1): <https://github.com/havakv/pycox>  
 PyTorch (v1.6.0): <https://github.com/pytorch/pytorch>  
 H2O autoML (v3.36.0.2): <https://docs.h2o.ai/h2o/latest-stable/h2o-docs/automl.html>  
 Sanger imputation server: <https://imputation.sanger.ac.uk/>  
 EAGLE2 (v2.0.5): <https://alkesgroup.broadinstitute.org/Eagle/>  
 PBWT (v3.1): <https://github.com/richarddurbin/pbwt>  
 Minimac3: <https://genome.sph.umich.edu/wiki/Minimac3>  
 R (v4.0.4 and v4.0.2): <https://cran.r-project.org/>  
 Rrvgo package (v1.2.0): <https://bioconductor.org/packages/release/bioc/html/rrvgo.html>  
 WGCNA package (v1.69): <https://cran.r-project.org/web/packages/WGCNA/index.html>  
 TwoSampleMR package (v0.5.6): <https://mrcieu.github.io/TwoSampleMR/index.html>  
 Coloc package (v5.1.0) <https://chr1swallace.github.io/coloc/>  
 BayesianTools package (v0.0.10): <https://github.com/florianhartig/BayesianTools>  
 Bigsnpr package (v1.12.2), implements LDpred2: <https://privefl.github.io/bigsnpr/>  
 gwasvcf package (v0.1.0): <https://github.com/MRCIEU/gwasvcf>  
 randomForestSRC package (v3.0.1): <https://www.randomforestsrc.org/>

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](#)

Summary statistics of the meta-analysis are publicly available for the top 10,000 SNPs at Zenodo (<https://doi.org/10.5281/zenodo.10804907>). Summary statistics of the discovery stage International EU-RLS-GENE consortium GWAS and the INTERVAL GWAS are available at GWAS Catalog (<https://www.ebi.ac.uk/gwas/>) under accession codes GCST90399568, GCST90399569, GCST90399570, GCST90399571, GCST90399572, and GCST90399573. The full GWAS summary statistics for the 23andMe discovery data set will be made available through 23andMe to qualified researchers under an agreement with 23andMe that protects the privacy of the 23andMe participants. Datasets will be made available at no cost for academic use. Please visit <https://research.23andme.com/collaborate/#dataset-access/> for more information and to apply to access the data.

Additional data used for tissue and cell-type enrichment analysis are available here: developmental (<http://mousebrain.org/development/downloads.html>) and adult single cell RNAseq datasets (<http://mousebrain.org/adult/downloads.html>) from the Mouse Brain Atlas (<http://mousebrain.org/>), the Human Gene Expression During Development dataset from the BBI-Allen Single Cell atlases (<https://descartes.brotmanbaty.org/>), the BrainSpan Developmental Transcriptome RNA-Seq dataset from the BrainSpan Atlas of the Developing Human Brain (<https://www.brainspan.org/static/home>), the V8 RNA-Seq dataset (GTEx\_Analysis\_2017-06-05\_v8\_RNASeQCv1.1.9\_gene\_reads.gct.gz) from GTEx (<https://gtexportal.org/home/datasets>), and the human C8 collection from MSigDb v7.4 (<http://software.broadinstitute.org/gsea/msigdb/>, with the legacy versions available at [https://www.gsea-msigdb.org/gsea/downloads\\_archive.jsp](https://www.gsea-msigdb.org/gsea/downloads_archive.jsp) after creating a user account with GSEA/MSigDB).

Summary statistics of GWAS for genetic correlation and MR analyses are available at the University of Bristol Integrative Epidemiology Unit OpenGWAS server (<https://gwas.mrcieu.ac.uk>) and GWAS atlas (<https://atlas.ctglab.nl/>). Additional GWAS summary statistics for iron-related traits are available at [https://www.fmrrib.ox.ac.uk/ukbiobank/gwas\\_resources/index.html](https://www.fmrrib.ox.ac.uk/ukbiobank/gwas_resources/index.html), <https://open.win.ox.ac.uk/ukbiobank/big40/BIGv2/>, and <https://www.decode.com/summarydata/>. A complete list of sources used for annotation with FUMA is available at <https://fuma.ctglab.nl/links> and <https://fuma.ctglab.nl/tutorial>. Auxiliary files for use with MAGMA are available at <https://ctg.cncr.nl/software/magma>. Additional files for use with LDSC and LDK are available at <https://alkesgroup.broadinstitute.org/LDScore/>.

Information about drug targets is available at the free-to-access database DrugBank Online (<https://go.drugbank.com/>).

## Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

### Reporting on sex and gender

The study included both female and male individuals. A pooled analysis of all individuals was performed as well as analyses stratified by sex. Sex was determined by self-reporting and by genotyping. During genotyping data quality control, individuals with non-matching genotype-determined sex and self-reported sex were excluded and were not included in any further analyses. Sample sizes for the sex-specific discovery analyses were 78,333 cases and 844,872 controls in women and 38,314 cases and 701,594 controls in men.

### Population characteristics

The discovery dataset for the pooled analysis included 116,647 cases and 1,546,466 controls. 78,333 cases and 844,872 controls were women and 38,314 cases and 701,594 controls were men. All individuals were of European ancestry, determined by running PCA or MDS analysis on the genetic data. Individuals of non-European ancestry were excluded to avoid spurious associations due to population stratification. Age and sex were used as covariates. A total of 9,196,648 common variants with minor allele frequency (MAF)  $\geq 1\%$  were available for statistical analysis in the discovery stage data. The case phenotype "restless legs syndrome" (RLS) was determined by either clinical face-to-face interviews or using validated questionnaires for RLS cases, implementing the IRLSSG diagnostic criteria for RLS. In the 23andMe dataset, RLS

cases were defined by asking a single question to customers about having received an RLS diagnosis or therapy.

## Recruitment

Participants were recruited

1) in a clinical setting: Cases were recruited in specialized outpatient clinics for movement disorders and in sleep clinics by conducting face-to-face diagnostic interviews to assess the IRLSSG diagnostic criteria for RLS  
 2) in cohorts of whole blood donors by self-report based on validated questionnaires for RLS (Cambridge-Hopkins Restless Legs Questionnaire)  
 3) in a direct-to-consumer genetic testing company customer database by self-report based on survey questions which assessed whether someone has ever been diagnosed with RLS or has ever received treatment for RLS.  
 Genetic correlations between the three GWAS with different case recruitment strategies were strong but indicated some degree of heterogeneity, therefore, a multivariate genome-wide-association meta-analysis approach was used (N-GWAMA).

## Ethics oversight

All studies were approved by the respective local ethical committees and all participants provided informed consent. The EU-RLS-GENE study was approved by an institutional review board at the University Hospital of the Technical University Munich (2488/09). The INTERVAL dataset was approved by the National Research Ethics Service Committee East of England - Cambridge East (REC: 11/EE/0538). 23andMe Participants provided informed consent under a protocol approved by the external AAHRPP-accredited IRB, Ethical & Independent (E&I) Review Services. As of 2022, E&I Review Services is part of Salus IRB (<https://www.versitclinicaltrials.org/salusirb>). The deCODE dataset was approved by the National Bioethics Committee of Iceland. The DBDS dataset was approved by The Scientific Ethical Committee of Central Denmark (M-20090237) and by the Danish Data Protection agency (30-0444). GWAS studies in DBDS were approved by the National Ethical Committee (NVK-1700407). The Emory dataset was approved by an institutional review board at Emory University, Atlanta, Georgia, US (HIC ID 133-98).

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

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences  Behavioural & social sciences  Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

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

### Sample size

No sample-size calculation was performed. Restless legs syndrome is a polygenic trait for which heritability is not yet fully explained. Therefore, we collected all available (at the time of initiating the study) GWAS datasets for this phenotype into a discovery dataset. This dataset is about 8 times larger than the datasets used in previous GWAS on RLS, therefore could be expected to provide a reasonable increase in study power.

### Data exclusions

Phenotypic data: only RLS patients fulfilling the diagnostic criteria based on either a face-to-face interview, validated questionnaires, or a single question about RLS diagnosis/treatment were included in the study.  
 Genetic data: Standard SNP and sample GWAS quality control procedures were applied to exclude low quality data.

### Replication

Data for all independent lead SNPs of the discovery stage was obtained in independent replication dataset consisting of 29,028 RLS cases and 398,815 controls. 71% of the lead SNPs from the pooled discovery meta-analysis were nominally significant in the replication ( $p < 0.05$ ) and there was a high positive correlation between the effect size estimates of the discovery stage and the replication dataset. A joint analysis of discovery and replication datasets revealed that all lead SNPs of the discovery pooled and sex-specific meta-analyses reached Bonferroni-corrected significance.

### Randomization

GWAS are observational genetic studies and not randomized experiments. For a GWAS, individuals are assigned to either the case group (individuals affected by RLS) or the control group (unaffected individuals).

### Blinding

Meta-analysis of GWAS and functional GWAS interpretation do not require blinding because GWAS are observational genetic studies and not randomized experiments.

## 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,

|                   |  |
|-------------------|--|
| Study description | <i>quantitative experimental, mixed-methods case study).</i>   |
| Research sample   | <i>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.</i>  |
| Sampling strategy | <i>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.</i> |
| Data collection   | <i>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.</i>  |
| Timing            | <i>Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.</i>   |
| Data exclusions   | <i>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.</i>  |
| Non-participation | <i>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.</i>   |
| Randomization     | <i>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.</i>   |

## Ecological, evolutionary & environmental sciences study design

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

|                          |   |
|--------------------------|---|
| Study description        | <i>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.</i>   |
| Research sample          | <i>Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i>, all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.</i> |
| Sampling strategy        | <i>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.</i>  |
| Data collection          | <i>Describe the data collection procedure, including who recorded the data and how.</i>   |
| Timing and spatial scale | <i>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</i>  |
| Data exclusions          | <i>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.</i>  |
| Reproducibility          | <i>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.</i>  |
| Randomization            | <i>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.</i>   |
| Blinding                 | <i>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.</i>  |

Did the study involve field work?  Yes  No

## Field work, collection and transport

|                  |   |
|------------------|---|
| Field conditions | <i>Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).</i>                              |
| Location         | <i>State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).</i> |

## Access &amp; 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

### Methods

- n/a Involved in the study
- Antibodies
- Eukaryotic cell lines
- Palaeontology and archaeology
- Animals and other organisms
- Clinical data
- Dual use research of concern

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

### Antibodies

## Antibodies used

Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.

## Validation

Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.

### Eukaryotic cell lines

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

## Cell line source(s)

State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.

## 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)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

### 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      | <i>For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.</i>  |
| Wild animals            | <i>Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.</i>   |
| Reporting on sex        | <i>Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.</i> |
| Field-collected samples | <i>For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.</i>  |
| Ethics oversight        | <i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>  |

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

## Clinical data

Policy information about [clinical studies](#)

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

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

## Dual use research of concern

Policy information about [dual use research of concern](#)

### Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

| No                       | Yes                      |                            |
|--------------------------|--------------------------|----------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | Public health              |
| <input type="checkbox"/> | <input type="checkbox"/> | National security          |
| <input type="checkbox"/> | <input type="checkbox"/> | Crops and/or livestock     |
| <input type="checkbox"/> | <input type="checkbox"/> | Ecosystems                 |
| <input type="checkbox"/> | <input type="checkbox"/> | Any other significant area |

## Experiments of concern

Does the work involve any of these experiments of concern:

- | No                       | Yes                      |   |
|--------------------------|--------------------------|---|
| <input type="checkbox"/> | <input type="checkbox"/> | Demonstrate how to render a vaccine ineffective                             |
| <input type="checkbox"/> | <input type="checkbox"/> | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| <input type="checkbox"/> | <input type="checkbox"/> | Enhance the virulence of a pathogen or render a nonpathogen virulent        |
| <input type="checkbox"/> | <input type="checkbox"/> | Increase transmissibility of a pathogen                                     |
| <input type="checkbox"/> | <input type="checkbox"/> | Alter the host range of a pathogen  |
| <input type="checkbox"/> | <input type="checkbox"/> | Enable evasion of diagnostic/detection modalities                           |
| <input type="checkbox"/> | <input type="checkbox"/> | Enable the weaponization of a biological agent or toxin                     |
| <input type="checkbox"/> | <input type="checkbox"/> | Any other potentially harmful combination of experiments and agents         |

## 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

Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument

Identify the instrument used for data collection, specifying make and model number.

Software  Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

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  Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

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.*