

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	For RNA-seq, libraries were sequenced using an Illumina Novaseq 6000. For scRNA-seq, libraries were sequenced on an Illumina HiSeq X Ten system.
Data analysis	fastp (v0.23): Open-source software used for quality control and preprocessing of RNA-seq data, including read filtering and trimming. hisat2 (v2.2.1): Open-source tool used for aligning RNA-seq reads to the rat genome. Samtools (v1.19.2): Open-source software used to process and manipulate sequencing alignments, including calculating read coverage and depth. Stringtie (v2.2.1): Open-source tool used for transcript assembly and quantification, generating transcript counts for gene expression analysis. DESeq2 (v1.42.1): Open-source package used for differential gene expression analysis. Variance-stabilized transformed data were used for Spearman's correlation calculation. EnhancedVolcano (v1.20.0): Open-source R package used to visualize differentially expressed genes in a volcano plot. pheatmap (v1.0.12): Open-source R package used for visualizing gene expression data in heatmaps. Cellranger (v7.2.0): Commercial software from 10x Genomics used for processing scRNA-seq data, including converting raw sequencing data to FASTQ format and performing alignment and gene quantification. Seurat (v5.0.2): Open-source R package used for scRNA-seq data analysis, including quality control, normalization, clustering, and differential gene expression analysis. CellChat (v1.6.0): Open-source R package for analyzing cell-cell communication in scRNA-seq data. It models ligand-receptor interactions, computes communication probabilities, and visualizes signaling pathways to explore cellular interactions in various biological contexts. TwoSampleMR (4.3.1): Open-source R package used for Mendelian randomization analysis to infer causality between sleep disorders and male infertility. GraphPad Prism (8.0): Commercial software used for statistical analysis and generating visualizations, including graphs and charts for data

presentation.

All analyses were conducted using the standard analysis strategies provided by these tools, and no custom algorithms or code were developed.

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](#)

All data needed to evaluate the conclusions in the paper are present in the article and/or the supplementary Material. Sequence data were submitted to NCBI and are accessible with BioProject ID: PRJNA1107966.

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

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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

Data exclusions

Replication

Randomization

Blinding

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

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	Ten-week-old specific pathogen-free male Sprague-Dawley rats were purchased from the animal research center at Zhejiang University.
Wild animals	No wild animals were involved in this study.
Reporting on sex	This study investigated the effects of sleep deprivation on the male reproductive system, so only male Sprague-Dawley rats were used.
Field-collected samples	This study did not involved the samples collected from the field.
Ethics oversight	All animal procedures were approved by the animal research ethics committee of Zhejiang University (No. ZJU20240220).

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

Plants

Seed stocks	No relevant study were involved.
Novel plant genotypes	No relevant study were involved.
Authentication	No relevant study were involved.