nature portfolio

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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	Single-cell sequencing data were downloaded from the DropViz and the Mousebrain datasets, as indicated in the Methods section.
Data analysis	In each dataset, read matrix from different clusters were re-clustered with the Seurat package (v4.3.0.1). Cell clusters were identified with the function of FindClusters by setting the resolution threshold as 1.0. The differential expression analysis of single-cell RNA sequencing data was done by Seurat using the function of FindAllMarkers (wilcox test, p value adjusted by FDR). The UMAPs and DEG heatmap were drawn using the plot functions of Seurat. GSEA analysis was done by clusterProfiler (v4.7.1.2) using Gene Ontology database.

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

Data supporting the findings of this study are available within the main text and supplementary materials. Source data are provided with this paper as a Source Data file. All data are available from the corresponding author 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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

Field-specific reporting

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

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

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	Sample size was chosen based on the standard practice in the field. The sample sizes for each experiment have been detailed in the figure legends.
Data exclusions	In the recognition memory test, mice with total exploration time less than 3 seconds were excluded from further analyses, in consistence with the standard practice in the field.
Replication	All experiments were independently replicated at least three times to obtain data for statistical analysis. The number of replicates for each experiment is shown in the figure legends.
Randomization	Age- and gender-matched mice were randomly allocated to different experimental groups based on their genotypes.
Blinding	Investigator was blinded to group allocation in all behavioral, photometry and electrophysiology experiments.

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	ChAT, AB144P, Millipore; NGFR, ab52987, Abcam; KCC2, 07-432, Millipore; Flag, 8146, CST; NKCC1, T4, Development Studies Hybridoma Bank.
Validation	ChAT, AB144P, Millipore, RRID:AB_2079751; NGFR, ab52987, Abcam, RRID:AB_881682; KCC2, 07-432, Millipore, RRID:AB_310611; Flag, 8146, CST, RRID:AB_10950495; NKCC1, T4, Development Studies Hybridoma Bank,RRID:AB_528406.

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	Ngfr knockout mice from Jackson Laboratories (stock number: 002213) were backcrossed into C57BL/6 genetic background over 15 generations. Adult Ngfr knockout (Ngfr-/-) and wild-type (Ngfr+/+) males were littermates derived from a heterozygous (Ngfr+/-) mating strategy. To conduct electrophysiology study of cholinergic neurons, Ngfr mice in C57BL/6 were crossed with ChAT-EGFP mice in Swiss-Webster (kindly provided by Dr. Hermes Yeh) for more than 5 generations. To perform behavioral studies, mice backcrossed to C57BL/6 background for over 5 generations were used.
Wild animals	This study did not include wild animals.
Reporting on sex	The behavioral and electrophysiological experiments were performed in male mice, owing to cyclic hormone effects of female mice. The molecular biology and photometry experiments were performed in both male and female mice.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All procedures were approved by the Animal Care and Use Committees of Tsinghua University.

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