nature portfolio

Corresponding author(s): Ulrich Mueller

Last updated by author(s): Nov 11, 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	Zeiss Zen version 2.3 Microscopy Software; Illumina NovaSeq 6000; Illumina's BaseSpace software.
Data analysis	Imaris versions 8 to10 (Bitplane - OXFORD Instruments); ImageJ (Fiji), version 2.14.0/1.54f (National Institutes of Health); GraphPad Prism 10 (Dotmatics); Adobe Photoshop version 25.4 (Adobe); Adobe Illustrator version 28(Adobe); Snapgene version 5.0.8 (Biotech LLC); Cell Ranger (10X Genomics); Python Programming Language (Python Software Foundation); Enrichr version 3.2 (Mount Sinai Center for Bioinformatics); Ensembl genome browser 111.

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 scRNAseq data (GSE281207) has been deposited at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE281207 and code used has been deposited at https://doi.org/10.5281/zenodo.14052608. Source Data are provided within this paper.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

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 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.*
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	No statistics-based calculations were performed to determine sample size within the study. Sample size was determined based on prior publications in studies using mice in auditory system development.
Data exclusions	Two samples from single cell RNA sequencing were excluded in the study due to likely experimental failure. Specifically, these samples contained a majority of cells that contained low numbers of unique molecular identifiers (UMI), indicative of low complexity. Exclusion criteria were established prior to analysis, using standard analysis of quality control metrics in single cell RNA sequencing data including cell counts, UMI per cell, genes detected, mitochondrial counts, and red blood cell contamination.
Replication	All experiments were done in replicate of at least 2 biologically discrete samples. All replications were successful.
Randomization	Mice were assigned experimental groups randomly.
Blinding	Investigators were blinded to group allocation. In mouse lines where genotype is apparent due to balance phenotype (e.g. Tmie-/-), samples were collected and data was coded to prevent bias during analysis.

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

Methods

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

Antibodies

Antibodies used

1. Rabbit anti-NGFR (Weskam and Reichardt, 1991. Neuron), 1:10,000, Custom made antibody
2. Rabbit anti-RFP (Abcam, ab62341), 1:200, Lot#GR2431812-3
3. Goat anti-mCherry (SicGen, AB0040-020), 1:100, Lot#0081030119
4. Mouse anti-PV (Swant, PV235), 1:100, Lot# unavailable
5. Rabbit anti-MYO7A (Proteus Biosciences, 25-6790), 1:500, Lot# not provided
6. Rabbit anti-CALB1 (Cell Signaling Technology, 13176), 1:200, Lot#3
7. Mouse anti-CALB2 (Millipore, MAB1568), 1:1000, Lot#3519796
8. Mouse anti-GLUR2 (Millipore, MAB397), 1:250, Lot#4015134
9. Rabbit anti-CTBP2 (Bioworld Technology, BS2287), 1:250, Lot#CN89330
10. Mouse anti-CTBP2 (BD Biosciences, 612044), 1:200, Lot#8314624
11. Chicken anti-GFP (Aves Labs, GFP1020), 1:100, Lot#GFP3717982

Validation

1. Antibody was shown to bind to a 64 kD protein identified as the low affinity nerve growth factor receptor (NGFR i.e. p75). Mouse L Cells which do not express NGFR showed no immunoreactivity. (Weskamp and Reichardt, 1991. Neuron)
2. Antibody binds 32 kDa protein in cells expressing recombinant RFP. Used in at least 290 publications. (https://www.abcam.com/products/primary-antibodies/rfp-antibody-ab62341.html)
3. Antibody binds 29 kDa protein in cells expressing recombinant RFP. Used in at least 81 publications. (https://store.sicgen.pt/catalog/product/AB0040)
4. Antibody binds 12 kDa prrotein in mouse brain cells. Used in at least 190 publications. (https://scicrunch.org/resolver/AB_10000343).
5. Antibody binds protein expressed in mouse hair cells within the organ of Corti. Used in at least 21 publications. (https://www.proteus-biosciences.com/product/antibodies/organellemarkers/myosin-viia.html)
6. Antibody binds 28 kDa protein in mouse brain cells and various cells line expressing recombinant Calbindin. Used in at least 48 citations (https://www.cellsignal.com/products/primary-antibodies/calbindin-d1i4q-xp-rabbit-mab/13176).
7. Antibody binds 31 kDa protein expressed in mouse brain cells. Used in at least 61 publications. (https://www.emdmillipore.com/us/en/product/anti-calretinin-antibody-clone-6b8.2,mm_nf-mab1568)
8. Antibody binds 102 kDa protein expressed in rat brain cells. Used in at least 336 publications (https://www.sigmaaldrich.com/US/en/product/mm/mab397).
9. Antibody binds 48 kDa protein in cells expressing recombinant CtBP2. Used in at least 2 publications (https://www.bioworlde.com/pdf/BS2287.pdf).
10. Antibody binds 48 kDa protein in B3CH1 cells expressing CtBP2. Used in at least 446 publications. (https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-ctbp2.612044)
11. Antibody binds 27 kDa protein in cells of transgenic mice expressing GFP. Used in at least 2,178 publications. (https://www.aveslabs.com/products/anti-green-fluorescent-protein-antibody-gfp)

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

Within the study, 6 different strains of mice that have been previously described were used:
1. Tmie -/- (Zhao et al., 2014. Nature)
2. Vglut3 -/- (Seal et al., 2008. Neuron)
3. Pdch15 av3j/av3j (Algramam et al., 2001. Hum Mol Genet)
4. Lypd1-CreERT2 (Siebald et al., 2023. PNAS)
5. TH-CreER (Vyas et al., 2019. Sci Rep)
6. Ai14 (Madisen et al., 2010. Nat Neuro)

Additionally, 1 strain which has not been previously described was used: Clrn2 -/-. Clrn2 -/- mice were generated by CRISPR-Cas9 technology to delete 48 basepairs spanning the 5'UTR and exon 1 of the Clrn2 gene, including the start codon.

All strains were maintained on the C57BL6/J background.

	For all strains, mice were used for experimental endpoints at various timepoints based on the postnatal day (P) of birth being designated as 0. Specifics for all strains and ages used per experiment are found in the Results and/or Figure Legends.
Wild animals	N/A
Reporting on sex	Single cell RNA sequencing was performed on only male mice to limit the potential effects of sex on transcriptomic-level analysis. Confirmation of single cell RNA sequencing results using Fluorescent In Situ Hybridization was similarly performed in male mice for consistency. For all other experiments, both female and male mice were used in approximately equal proportions by random assignment into experimental groups. In experiments with both female and male mice, no analysis on the basis of sex was performed due to lack of evidence for sexual dimorphism in auditory system development in published literature.
Field-collected samples	N/A
Ethics oversight	All animal experiments adhered to the guidelines of the National Institute of Health and were approved by the Institutional Animal Care and Use Committee at Johns Hopkins University School of Medicine (Protocol #MO22M215).

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