

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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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	No software or code was used for data collection
Data analysis	####Bioinformatic analysis: Cell Ranger (v5.0.0, 10x Genomics); R (v4.2.0); R Studio(v1.1); Scapy (v1.9.8);Seurat (v4.3.0); biomaRT (2.52.0); sctransform(v0.3.5); g:Profiler (v0.2.2); scrattch.hicat(v1.0.0); MetaNeighbor (v1.9.1); ggplot2 (v3.4.1); corrplot(v0.92); eulerr (v7.0.0). ####Image analysis: Fiji Image J (v1.52p); Zen 2 (Carl Zeiss). ####Making figures: Adobe Illustrator CS6 (Adobe).

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 paper and the Supplementary Materials, or contact the authors for access. The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2025) in National Genomics Data Center (Nucleic Acids Res 2025), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA032615) that are publicly accessible at <https://ngdc.cncb.ac.cn/gsa>.

The custom codes for producing figure results are available at GitHub(<https://github.com/Shinnyee/Cell-atlas-of-non-human-primate-cochlea>).

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	For the purpose of single-nucleus RNA sequencing (snRNA-seq), cochleae from one 1-year-old male, two 5-year-old male and one 11-year-old female macaque were utilized. Immunostaining experiments were performed using cochleae from three 5-year-old male and one 11-year-old male macaque. Electromotility recordings were obtained from one 1-year-old male macaque. snRNA-seq was conducted using eight cochleae obtained from mice at P21-22.
Data exclusions	Cells expressing more than 5,000 genes and less than 500 counts were excluded from further analysis. We implemented pre-established quality control criteria for the single-cell data by filtering out cells with >5,000 genes or <500 UMI counts. These thresholds were set to exclude compromised cells (e.g., low-quality cells or multiplets) following established scRNA-seq QC standards [cite relevant literature]. These pre-defined exclusion criteria ensure subsequent analyses were based on high-quality single cells.
Replication	The main findings in the transcriptomic analysis were validated by using immunohistochemistry in cochlea cryosections. Staining experiments were independently repeated 2 to 6 times.
Randomization	Not relevant because there was no group allocation.
Blinding	Blinding was not relevant to these experiments due to species information essential for sequence alignment to correct reference genome, integration pipeline.

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
☒	☐ Plants

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

Antibodies

Antibodies used

Primary antibodies were as follows: anti-PBX3 (1:500 dilution, Thermo Fisher, PA5-40482); anti-TUJ1 (1:500 dilution, Biolegend, MMS-435P); anti-MBP (1:200 dilution, SIGMA, M3821); anti-GFAP (1:800 dilution, Abcam, ab7260); anti-CALB2 (1:200 dilution, Millipore, MAB1568); anti-CALB1 (1:100 dilution, New England Biolabs, 13176); Anti-PRESTIN (1:50000 dilution, abcam, ab242128); Anti-OTOF (1:200 dilution, abcam, ab53233); Anti-GATA3 (1:250 dilution, abcam, ab199428); Anti-PRPH (1:100 dilution, abcam, ab246502); Anti-MYO7A (1:500 dilution, Proteus Bioscience, 25-6790).

Secondary antibodies were as follows: donkey anti-mouse IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 555 (1:1000 dilution, Thermo Fisher, A-31570); donkey anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 488 (1:1000 dilution, Thermo Fisher, A-21206); goat anti-mouse IgG1 cross-adsorbed secondary antibody, Alexa Fluor 647 (1:1000 dilution, Thermo Fisher, A-21240); and phalloidin Alexa Fluor 488 (1:1000 dilution, Thermo Fisher, A12379).

Validation

All antibodies are commonly used in the field and have been validated in previous publications / by the manufacturer, as detailed here:

- 1) Rabbit anti-PBX3, Thermo Fisher, PA5-40482, <https://www.thermofisher.cn/cn/zh/antibody/product/PBX3-Antibody-Polyclonal/PA5-40482>
- 2) Mouse anti-TUJ1, Biolegend, MMS-435P, <https://www.biolegend.com/en-us/products/purified-anti-tubulin-beta-3-tubb3-antibody-11580?GroupID=GROUP686>
- 3) Rabbit anti-MBP, SIGMA, M3821, <https://www.sigmaaldrich.cn/CN/zh/product/sigma/m3821>
- 4) Rabbit anti-GFAP, Abcam, ab7260, <https://www.abcam.com/products/primary-antibodies/gfap-antibody-ab7260.html>
- 5) Mouse anti-CALB2, Millipore, MAB1568, https://www.merckmillipore.com/CN/zh/product/Anti-Calretinin-Antibody-clone-6B8.2,MM_NF-MAB1568?ReferrerURL=https%3A%2F%2Fwww4.bing.com%2F&bd=1
- 6) Rabbit anti-CALB1, New England Biolabs, 13176, <https://www.cellsignal.com/products/primary-antibodies/calbindin-d14q-xp-rabbit-mab/13176>
- 7) Rabbit anti-PRESTIN, Abcam, ab242128, <https://www.abcam.cn/products/primary-antibodies/slc26a5-antibody-epr22715-53-ab242128.html>
- 8) Mouse anti-OTOF, Abcam, ab53233, <https://www.abcam.cn/products/primary-antibodies/otoferlin-antibody-13a9-ab53233.html>
- 9) Rabbit anti-GATA3, Abcam, ab199428, <https://www.abcam.cn/products/primary-antibodies/gata3-antibody-epr16651-chip-grade-ab199428.html>
- 10) Rabbit anti-PRPH, Abcam, ab246502, <https://www.abcam.cn/products/primary-antibodies/peripherin-antibody-epr23445-28-ab246502.html>
- 11) Rabbit anti-MYO7A, Proteus Bioscience, 25-6790, <https://www.labome.com/product/Proteus-Biosciences/25-6790.html>

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

Macaca fascicularis
For the purpose of single-nucleus RNA sequencing (snRNA-seq), cochleae from one 1-year-old male, two 5-year-old male and one 11-year-old female macaque were utilized. Immunostaining experiments were performed using cochleae from three 5-year-old male and one 11-year-old male macaque. Electromotility recordings were obtained from one 1-year-old male macaque.

C57BL/6J mouse
snRNA-seq and immunostaining experiments were conducted using eight cochleae obtained from C57BL/6J mice at P21-22.

Wild animals

No wild animals were used in this study.

Reporting on sex

Data collected for RNA-seq contained male and female mice.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

Macaca fascicularis
Both young (1-5 years) and aged (11 years) Macaca fascicularis of both sexes used in this study were conducted in strict accordance with the guidelines set forth by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) for the ethical treatment of non-human primates. The study protocol was reviewed and approved by the ethical committee of the

State Key Laboratory of Primate Biomedical Research (LPBR). and housed in a controlled environment maintained at approximately 25°C, with a 12-hour light/dark cycle and 55% relative humidity. All monkeys were provided with enough meticulously formulated semi-purified, nutritionally rich diets and ad libitum access to drinking water.

C57BL/6J mouse

Mice in the C57BL/6J background at P1, P7, P14, and P21 were purchased from GenePharmtech Co., Ltd. Mice of both sexes were used for the validation experiments. All mice were housed in micro-isolator cages with standard housing conditions (ambient temperature of 20–23 °C and humidity of 40–60%), illuminated from 07:00 to 19:00 with ad libitum access to food and water (establishment license number SCXK2018-0008).

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

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A