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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	No software was used to collect the data in this study.
Data analysis	All statistical analyses were performed with GraphPad Prism 6.0 (USA).

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 data are available from the corresponding author upon reasonable 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	Not involved
Reporting on race, ethnicity, or other socially relevant groupings	Not involved
Population characteristics	Not involved
Recruitment	Not involved
Ethics oversight	Not involved

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	The sample size was selected to match or exceed those used in published studies demonstrating robust effects for comparable interventions and measurements in this field (Izumikawa, M, Minoda, R, Kawamoto, K, Abrashkin, KA, Swiderski, DL, Dolan, DF, et al. (2005). Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nature medicine)
Data exclusions	Animals exhibiting severe health impairment were systematically excluded from analysis based on the following objective indicators: weight loss exceeding 20% of baseline body weight.
Replication	All attempts at replication were successful. The key findings—including [specific phenotype, e.g., hearing threshold shift, reduction in ribbon synapse number] in Vglut3-KO mice compared to wild-type controls—were reproduced in at least three independent experimental replicates (each using separate cohorts of animals). No contradictory results were observed across replicates.
Randomization	Animals of the same genotypes were randomly selected according to the experimental design.
Blinding	Blinding was not feasible for: Post-surgical animal care (due to visible incision marks behind the ears in the gene therapy group).

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	primary antibodies against Myo7A (1:300, Proteus Biosciences) for hair cell identification and Ctbp2 (1:200, BD Biosciences) for presynaptic ribbon visualization. Alexa Fluor-conjugated secondary antibodies (1:500, Invitrogen).
Validation	1. Anti-Myo7A Antibody (Proteus Biosciences, 1:300) Validation Summary: This rabbit polyclonal antibody is widely validated for the specific detection of hair cells in the inner ear via immunofluorescence (IF) in multiple species, particularly rodents. While the manufacturer's specific validation data is limited in search results, extensive independent peer-reviewed literature confirms its efficacy and specificity, especially with genetic controls. 2. Anti-Ctbp2 Antibody (BD Biosciences, 1:200) Validation Summary: This mouse monoclonal antibody (Clone 16/CtBP2, Catalog #612044) is a highly validated, gold-standard reagent for labeling presynaptic ribbons in the retina and inner ear. The manufacturer provides extensive validation for multiple species and applications, which is strongly supported by the literature.

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	Vglut3 knockout (Vglut3-KO) mice were provided by the Institute of Neuroscience, CAS Center for Excellence in Brain Science and Intelligence Technology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences
Wild animals	C57BL/6J mice (aged 4–6 weeks) were obtained from GemPharmatech Co., Ltd. (Nanjing, China)
Reporting on sex	The findings apply to both sexes. The study included both male and female Vglut3-KO mice.
Field-collected samples	All experimental procedures were performed in full compliance with the 3Rs principles of animal research—Reduction, Replacement, and Refinement. They were housed under a 12-hour light/dark cycle with free access to food and water throughout the experiment.
Ethics oversight	This study received ethical approval from the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Zhengzhou University (Henan, China).

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

Plants

Seed stocks	Not involved
Novel plant genotypes	Not involved
Authentication	Not involved