

## 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

*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*

Data analysis

Prism 6 from GraphPad was used for statistic analysis. ImageJ (NIH image) was used for IHC and OHC counting. Canvas X GIS 2019 was used to composite images showing the whole cochlea.

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 authors declare that the relevant data supporting the findings of this study are available within the paper and its supplementary information files

# 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://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     | In experimental groups to show reproducible and robust hearing, we used a minimum of 3 animals (from 3 to 18) for each of the frequency studied. For auditory startle evaluation, we used 5 injected animals to better measure the response and variations, and used 7 uninjected control animals. We used a minimum of 4 animals in all control studies. |
| Data exclusions | For ABR measurements, a small fraction of frequencies for which thresholds were not apparent were excluded to ensure that only ABR thresholds scored accurately were used.  |
| Replication     | All the experimental findings were replicated with the number of replicates, animals and variations shown by n, SD (in vitro assays), and SEM (in vivo experiments).  |
| Randomization   | The animals of the same genotypes (Atp2b2 Obl/+; C3H or Atp2b2 Obl/+; Tmc1 Bth/+) were randomly chosen to be in experimental or control groups according to experimental design.  |
| Blinding        | All data from in vivo experiments were collected and analyzed by at least two researchers who were both blinded to experiment.  |

## 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   | n/a                                 | Involved in the study                           |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  | <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines       | <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology          | <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |                                     |   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants            |                                     |   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |                                     |   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern           |                                     |   |

## Antibodies

|                 |  |
|-----------------|--|
| Antibodies used | Immunofluorescence: mouse anti-Parvalbumin (Sigma P3088), rabbit anti-pmc2 (PA1-915 ThermoFisher scientific), donkey anti-rabbit Alex488 (A21206), donkey anti- mouse Alex594 (A32744). All of them have been used by others and by us in previously published studies on the adult mouse cochlea. |
| Validation      | <i>Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.</i>  |

## Eukaryotic cell lines

|   |  |
|---|--|
| Policy information about <a href="#">cell lines</a> |  |
| Cell line source(s)                                 | Atp2b2Obl/+ and Atp2b2Obl/Obl mouse primary fibroblasts were obtained from P4 pups. HEI-OC1 cells were a gift from Dr. Albert Edge, Mass Eye & Ear/Harvard Medical School. Obl-OC1 cells were created in the lab |
| Authentication                                      | Primary fibroblasts and Obl-OC1 cells were genotyped in the lab. HEI-OC1 is a commercial cell line.  |
| Mycoplasma contamination                            | All cell lines tested negative for mycoplasma contamination  |

## Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Mouse strains used in this study:  
C3HeB/FeJ (C3H),  
heterozygous Atp2b2 Obl/+ , in C3H background  
homozygous Atp2b2 Obl/Obl, in C3H background  
Pmca2Obl/+Tmc1Bth/+ , in C3H background  
Mice of either sex at P0-P5 were used for culture and injection. Acoustic tests were performed 1-4 months after the injection

Wild animals

The study did not use wild animals

Field-collected samples

The study did not involve samples collected from the field

Ethics oversight

All in vivo experiments were carried out in accordance with NIH guidelines for the care and use of laboratory animals and were approved by the Massachusetts Eye & Ear Infirmary IACUC committee.

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