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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	pClamp10
Data analysis	CRISPResso (command line), Guideseq pipeline v1.1b4, allele segregation: Python version 3.4.2, GraphPad Prism 7, TIDE (Desktop Genetics), Flash v1.2.11, Adobe Photoshop CC, ImageJ 1.50i. OriginPro 2015

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All sequencing .fastq files will be available through NCBI SRA Database. Data has been uploaded: https://www.ncbi.nlm.nih.gov/sra/PRJNA541170

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	Sample size for hearing rescue experiments was calculated based on power calculations. Sample size is given throughout the manuscript, and in Supplementary Table 3.
Data exclusions	Data was not excluded from the study.
Replication	All experiments (including fibroblast transfection, animal injections) were performed at least on two independent occasion using duplicates or triplicate experiments for fibroblasts and several ears (exact number of samples is given in Suppl. Table 3) for sequencing/hearing rescue experiments. All attempts of replication were successful. For SEM images one cochlear sample was process for each condition.
Randomization	Gender was randomized, but other randomization was not relevant to our study as mouse genotypes were identical.
Blinding	Sequencing experiments were not performed in a blinded way, as all samples were processed through the exact same analytical and computational pipeline, avoiding any potential bias.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	Myosin VIIa: Proteus Biosciences, PRODUCT #: 25-6790, 1:500 dilution
Validation	AAV constructs were validated by deep sequencing (MGH DNA Core). Supplementary Dataset has sequencing result from: pAAV-CMV-NLS(SV40)-SaCas9(E782K/N968K/R1015H)-NLS(nucleoplasmin)-3xHA-bGHpA0-U6-BsaI-sgRNA. gRNA constructs were validated by Sanger sequencing (MGH DNA Core). The MYOVIIa antibody was from a commercial source (Myosin VIIa: Proteus Biosciences, PRODUCT #: 25-6790), has been validated and is used routinely for staining hair cells. Hair cell morphology can be easily recognized in a confocal microscopy experiment.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	P1 to 1 year of age C57BL/6 Tmc1WT/WT and Tmc1Bth/WT mice were used. Both sexes were included in the study in a randomized way.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve field-collected samples.