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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.

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 data other than that supplied by the manufacturers for instrument operation (Tecan Infinite M1000 Pro microplate reader: Tecan i-control v3.3.10.0; Illumina MiSeq, MiSeq Control v2.6.2.1).
Data analysis	CRISPResso2 (v2.0.18b as well as previous v2.0 versions) was used to analyze data and is available from http://crispresso.pinellolab.partners.org. Graphpad Prism 7.0c was used for regression analysis and is available from https://www.graphpad.com/scientific-software/prism/. Other software and versions used were: IQ-Tree v1.6, MAFFT v7.427, T-Coffee v12.00_7fb08c2, I-Tasser_ZhanglabServer https://zhanglab.ccmb.med.umich.edu/I-TASSER/, FastML v3.11 and BLAST_NCBIserver_https://blast.ncbi.nlm.nih.gov/Blast.cgi.

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. 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

Key plasmids from this work will be available from Addgene (depositor: David R. Liu) and other plasmids are available upon request. All unmodified reads for sequencing-based data in the manuscript will be available from the NCBI Sequence Read Archive, accession number PRJNA511456. Figs. 4b, 5, 6, Supplementary Table 3, Supplementary Figs. 8-14, 16, and 18-22 are based on processing of sequencing data. Protein sequences used for Supplementary Fig. 17 are supplied as Supplementary Data 1.

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	Experiments were performed in biological triplicate n=3 unless otherwise noted. In previous studies we determined this sample size to be sufficient to ensure reproducibility. Statistical methods were not used to predetermine sample size.
Data exclusions	Out of >1,000 mammalian cell transfection replicates, four were excluded as presumed failed transfections because editing values across the entire experiment were much lower than other replicates using the same materials: pBT237 HEK3; pBT236 RNF2; pBT237 RNF2; pBT281 EMX1. A fourth replicate transfection was performed to replace each. Exclusion criteria were not pre-established.
Replication	Replicate experiments used distinct splits of cells (for mammalian cell transfection) or individual overnight cultures of bacteria (for luciferase assays) and when possible were conducted on different days. Results were comparable, with standard deviations in expected ranges, across these conditions.
Randomization	Different overnight cultures of bacteria or cell passages of mammalian cells were used for each biological replicate, with the exception of the TMC1 experiment (Fig. 6a), for which replicates originated from the same cell passage (replicates were separately nucleofected and cultivated after nucleofection). This is described in the Methods section.
Blinding	The experiments were not blinded. Blinding in our study would have required either a level of effort that we judged to be disproportionate to the potential for experimenter bias to affect the results (if sufficient steps were taken to track and verify blinding and unblinding of samples), or else introduced potential for experimental errors that would have been difficult to detect (if blinding and unblinding of samples was not extensively tracked and verified).

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T (ATCC), baringo fibroblasts (Harvard Medical School), hAPOE4 astrocyte (Eloise Hudry / Brad Hyman group)
Authentication	HEK293T cells were authenticated by the supplier (ATCC) using STR profiling. For baringo fibroblasts and hAPOE4 astrocytes, the presence of the relevant mutation(s)/genotype in the cell line at the locus to be edited was confirmed by sequencing of unmodified cells.
Mycoplasma contamination	HEK293T and baringo cells tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals	Baringo strain: The Baringo mouse carries a c.545AG (p.Y182C) missense mutation in Tmc1 gene (mutations were ENU induced). Baringo background: BALB/c (https://doi.org/10.1016/j.ajpath.2011.12.034). Species: Mus musculus. Sex: Baringo males 6-8 (mean 7 weeks) weeks old and females 4-5 (mean 4.5 weeks) weeks old. For the experiment 4 pregnant donor-Baringo females were used and sacrificed at day 13.5 after mating.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The baringo colony is maintained ad libitum and all animal procedures are approved by the Children's Hospital Boston IACUC in compliance with relevant ethical regulations.

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