nature portfolio

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Last updated by author(s): Jan 6, 2023

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	FACS DIVA, Living Image v 4.5.5,
Data analysis	Graph pad Prism v9, Chemdraw v20, 500MHx NMR, ImageJ, ImageScope 12.1, Living Image v 4.5.5, bcl2fastq conversion software v2.18, FlowJo 10.8.1

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

Illumina Sequencing data have been submitted to the Sequence Read Archive. These datasets are available under BioProject Accession number PRJNA918691 . The authors declare that all other data supporting the findings of this study are available within the paper and its Supplementary Information files or upon reasonable request. Plasmids are available from Addgene.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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	All cell samples were evaluated in at least biological triplicates (n=3) to ensure the reproducibility. Our previous editing studies have shown that this sample size and replications are sufficient to ensure reproducibility (Jiang et al, Nature Biotechnology, 2022, 40, 227). For animal experiment, we described the size in the specific figure legend. The size is determined based on the availability of the mice and previous reports (Jiang et al, Nature Biotechnology, 2022, 40, 227, Liang et al, Molecular Therapy, 2022, Vol. 30 No 1).
Data exclusions	No data were excluded
Replication	Three or more independent experiments were performed. All attempts at replication were successful, and standard deviations were in the expected ranges.
Randomization	Cells from the same passages were randomly used for experiment group or control group. The results were confirmed by different cells passages. Group allocation for mouse study was performed randomly.
Blinding	It is not applied to molecular and cell experiments. All mouse work are blind.

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

Methods

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

Antibodies

Antibodies used	anti-GFP (CST, Cat. #2956, 1:200) or anti-tdTomato (Rockland, Cat. #600-401-379, 1:300); anti-CCSP antibody (Santa Cruz, Cat. # SC-25555, 1:2000) and an anti-tdTomato antibody (ThermoFisher, Cat. #MA5-15257, 1:300). acetylated tubulin double staining,

	Alexa Fluor 647 anti-acetylated tubulin antibody (Santa Cruz, Cat. # SC-23950, 1:200), Alexa Fluor 647 Donkey anti-mouse IgG (ThermoFisher, Cat. #A-31571, 1:200).
Validation	The antibodies have been previously validated by the authors (Liang et al, Molecular Therapy, 2022, Vol. 30 No 1).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T (ATCC), A549 (ATCC)
Authentication	Cell lines were authenticated by using PCR assays with species-specific primers
Mycoplasma contamination	Cell lines were tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	The cell lines used in this article are not in the list of misidentified lines.

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	Six-week-old female C57BL/6J mice and eight-week-old Ai9 mice from Jackson laboratory were used; A 14-hour light/10-hour dark cycle and a temperature of 65-75°F (~18-23°C) with 40-60% humidity are used.
Wild animals	No wild animals were used in the study.
Reporting on sex	Female.
Field-collected samples	No field-collected samples were used in the study.
Ethics oversight	All animal experiments were authorized by the Division of Comparative Medicine at the Massachusetts Institute of Technology or the Institutional Animal Care and Use Committee (IACUC) at UMass medical school.

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

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Mouse Lung was dissociated into single cells for Flow cytometry analysis.
Instrument	BD LSRII
Software	All data were analyzed by FlowJo 10.8.1 software
Cell population abundance	No purfication
Gating strategy	The cells were first gated based on FSC-A/SSC-A and FSC-A/FSC-H to select for live single cells. For in vitro CIRSPR-Cas9 knockout assay, unedited GFP-expressing HEK cells were employed as the negative control for gating GFP signal. For in vivo gene editing experiments, lung cells from untreated Ai9 mice were employed as the negative control for gating tdTomato signal.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.