nature portfolio

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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	Sequencing data were collected using Illumina's Miseq, Nextseq 2000, and NovaSeq 6000. Illumina's Bcl2Fastq software v2.20 was used to generate sequencing reads in the FASTQ format.
Data analysis	Cell Ranger v6, Cell Ranger ARC v2, Seurat v4.3.0.1, ArchR v1.0.2, Epiregulon v1.0.34, Scanpy 1.9.1, PROGENy v1.18.0, dcoupleR v1.8.0, https://github.com/bingwu2017/OAK_manuscript.git

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

Sequencing data generated in this study have been deposited to NCBI Sequence Read Archive under accession number PRJNA1046517 [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1046517]. External datasets are publicly available from GEO: scifi-RNA-seq GSE168620 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE168620], sci-RNA-seq GSE98561 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE98561], SPLiT-seq GSE110823 [https://

www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE110823], Paired-seq GSE130399 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE130399], sci-CAR GSE117089 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE117089]. Source data are provided with this paper.

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	Male
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	Detailed description of donor recruitment and consent is included in Owen et al., 2019 (doi: 10.1167/iovs.18-24254). We follow Eye Bank Association of America guidelines. Following Utah Code, Title 26 Chapter 28 (Uniform Anatomical Gift Act or UAGA), our coordination department uses the Utah donor registry to obtain authorization (Section 114), but will also reach out to authorized decision makers such as families when the individual is not found on a donor registry.
Ethics oversight	Institutional approval and the consent of patients to donate their eyes for research purposes was obtained from the University of Utah, and conformed to the tenets of the Declaration of Helsinki. All tissue was de-identified in accordance with HIPPA privacy rules.

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	Sample sizes were determined based on assay throughput and published work on similar subjects.
Data exclusions	No data exclusion.
Replication	4 different sample types were used in the manuscript to confirm assay performance. Library preparation for bronchial epithelial cell sample was performed for 4 aliquots (n=4), for retinal tissue sample with n=13 , and for IPC298 cells with n=20 at Day0 and n=12 at Day 10. All attempts at replication were successful.
Randomization	Not relevant. There is no assignment of test subjects or therapies in this study.
Blinding	Not relevant. There is no assignment of test subjects or therapies in this study. The Investigators were not blinded during experiments and outcome assessment.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Each sample well of cells were stained with 1 µL of one of the following antibodies in 50uL suspension.
TotalSeq™-A0251 anti-human Hashtag 1 Antibody
Supplier: Biolegend, Catalog number: 394601, Clone names: LNH-94; 2M2;
TotalSeq™-A0252 anti-human Hashtag 2 Antibody
Supplier: Biolegend, Catalog number: 394603, Clone names: LNH-94; 2M2;
TotalSeq™-A0253 anti-human Hashtag 3 Antibody
Supplier: Biolegend, Catalog number: 394605, Clone names: LNH-94; 2M2;
TotalSeq™-A0254 anti-human Hashtag 4 Antibody
Supplier: Biolegend, Catalog number: 394607, Clone names: LNH-94; 2M2;
TotalSeq™-A0256 anti-human Hashtag 6 Antibody
Supplier: Biolegend, Catalog number: 394611, Clone names: LNH-94; 2M2;
TotalSeq™-A0257 anti-human Hashtag 7 Antibody
Supplier: Biolegend, Catalog number: 394613, Clone names: LNH-94; 2M2;
TotalSeq™-A0259 anti-human Hashtag 9 Antibody
Supplier: Biolegend, Catalog number: 394617, Clone names: LNH-94; 2M2;
TotalSeq™-A0262 anti-human Hashtag 12 Antibody
Supplier: Biolegend, Catalog number: 394623, Clone names: LNH-94; 2M2;
TotalSeq™-A0263 anti-human Hashtag 13 Antibody
Supplier: Biolegend, Catalog number: 394625, Clone names: LNH-94; 2M2;

Validation

Validation of antibodies is performed by the manufacturer. According to BioLegend website, the products are tested on endogenous cells, and each lot is compared to an internally established gold standard to maintain lot-to-lot consistency.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

K562 ATCC number CCL-243 Female
NIH/3T3 ATCC number CRL-1658 Male
IPC-298 DSMZ number ACC 251 Female

Authentication

Authenticated by suppliers using STR method

Mycoplasma contamination

Not tested for mycoplasma contamination

Commonly misidentified lines
(See ICLAC register)

None

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.