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	IncuCyte ZOOM (Essen Bioscience) Tonometer (Icare TonoLab, TV02) FUSION SOLO.7S.EDGE (Vilber-Lourmat) Multi-mode micro plate reader Nivo S (PerkinElmer) The Signaling Pathways Project (http://www.signalingpathways.org/ominer/query.jsf) RaNA-seq (https://ranaseq.eu/index.php) Enrichr (https://maayanlab.cloud/Enrichr/) Quant Studio1 (ABI)
Data analysis	IncuCyte ZOOM 2015A software (Essen Bioscience) GraphPad Prism 10, 11 software (GraphPad Software Inc.) Image J (NIH) Microsoft Office 365 Excel (Microsoft) SRplot (https://www.bioinformatics.com.cn/en) Venn diagram web tool (https://bioinformatics.psb.ugent.be/webtools/Venn/) Quant Studio1 (ABI) Transcriptome Analysis Console□TAC□software (Thermo)

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

Gene expression data in human AH outflow-related TM cells were deposited under the Gene Expression Omnibus (GEO) accession number GSE14618832. Microarray data and RNA-seq data generated in this study were deposited under GEO accession number GSE299452 and GSE336386, respectively. cAMP-related five gene sets obtained from GEO datasets (GSE21727, GSE12056, and GSE5041) and Open TG-GATEs (https://dbarchive.biosciencedbc.jp/en/open-tggates/download.html).
Source data underlying all graphs and quantitative analyses are provided in Supplementary Data 1. Plasmid sequences, coding sequences (CDSs), and sgRNA information used in this study are provided in Supplementary Data 2. Primer sequences used for quantitative PCR analyses are provided in Supplementary Table 1. Supplementary Methods, Supplementary Figure 1–17, and Supplementary Table 1 are provided in the Supplementary Information. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

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	We used the human data for reanalysis of human AH outflow-related cells (GSE146188)).
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	No ocular disease was reported in any of the human donors, and no abnormalities were noted during microdissection.
Recruitment	We do not know.
Ethics oversight	Human AH outflow-related cells; immortalized human trabecular meshwork cells (iHTMCs)

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	No statistical methods were used to predetermine sample size. The number of mice used was set to N = 5 or more because individual differences are likely to occur in the IOP data. Sample sizes of at least 3 independent experimental replicates were used based on previous experience and the standard practices of the field.
Data exclusions	No data were excluded from the analysis
Replication	All experiments were performed in at least biological triplicate with similar results. Where only one biological repeat was performed for screening, this is noted in the figure legends. In the animal experiments of intraocular pressure measurement, positive control was included in the independent measurement.
Randomization	Inbread mice of the same age and sex were bred in the same environment and used in the experiment. Intraocular pressure measurements were performed randomly. Randomization was not required for the type of in vitro data we have reported in this study
Blinding	No blinding was used in this study

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	HRP-conjugated goat polyclonal antibody against rabbit IgG Cell Signalling Technology Cat# 7074 Rabbit anti-RhoB primary antibody (14326-1-AP, ProteinTech) goat polyclonal anti-ADBR1 (1:2,000; Novus Biologicals, #NB600-978) HRP-conjugated donkey polyclonal antibodies against goat IgG (1: 20,000; Thermo Fisher, #A15999)
Validation	All antibodies were validated by commercial source. No homemade or previously unpublished antibodies were used in this study. All antibodies were validated using western blotting and/or immunofluorescence staining.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Immortalized human trabecular meshwork cell (T0371-C) (Applied Biological Materials Inc.) human primary trabecular meshwork cell (#6590, ScienCell Research Laboratories, Carlsbad, CA, USA)
Authentication	This iHTMC identity used in this study was confirmed by our previous report (Ikegami et al., 2022 Commun Biol)
Mycoplasma contamination	These cell line were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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	Five-week-old male C57BL/6JJmsSlc.
Wild animals	This study did not involve wild animals.
Reporting on sex	We used male mice because female has clear estrous cycle to change physiological function and behavior.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All animal experiments were conducted in accordance with the Guidelines for Animal Experiments of Aichi Medical University and the Faculty of Agriculture at Kyushu University. All experiments were approved by the Animal Care and Use Committee of Aichi Medical University (2022-63) and Kyushu University (A23-223-2).

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

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.*