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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	Metamorph Premier ver 7.8.12.0 (Molecular Devices, LLC, USA) for image acquisition, FluorEssence ver 6.5 for fluorescence measurements, Image Lab™ Software 6.0.0 for PAGE image acquisition
Data analysis	Origin 2018 SR1 b9.5.1.195 (OriginLab Corporation, Northampton, MA, USA) for data plotting, ImageJ/Fiji 2.0.0-rc-54/1.51h for image analysis

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

The raw data that supports all the figures in this paper and other findings of this study are available from the corresponding author upon reasonable request.

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 was not predetermined. Two ions measurement (2-IM) experiment were repeated two times independently. Other experiments described in this manuscript were performed three times independently. These sample sizes are widely adopted in the field of chemical biology.
Data exclusions	No data were excluded from the analyses.
Replication	In vitro fluorescence assays, steady state and kinetic fluorescence imaging were all performed independently in at least triplicates while 2 ions measurement (2-IM) imaging was performed in duplicate to ensure reproducibility. All replications were successful.
Randomization	Allocation was random. Cultured dishes containing various types of cells were randomized for selection in imaging experiments and biochemical assays.
Blinding	We used DNA based quantitative imaging technology to compare chloride ions and pH levels under different cellular conditions. Thus, it was not necessary to design a blinding group allocation.

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique biological materials are readily available from the authors or from IDT/IBALifesciences

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Mouse alveolar macrophage J774A.1 cells were a kind gift from Prof Deborah Nelson (Department of Pharmacological and Physiological Sciences, the University of Chicago) T47D cells were a kind gift from Prof. Geoffrey Greene (The Ben May Department for cancer research, the University of Chicago,). BHK-21 cells were a kind gift from Prof. Michaela Gack (Department of microbiology, the University of Chicago,).
Authentication	No further authentication was performed after receiving the cells as a gift.
Mycoplasma contamination	We routinely check our cell lines for Mycoplasma contamination using Hoechst staining and all cell lines used in this study turned out to be negative for Mycoplasma contamination.

Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Primary Human dermal fibroblasts (HDF) were a kind gift from Late Professor Janet Rowley's Lab at the University of Chicago. Primary fibroblasts GM08429, AG01518, GM00112, GM16195, GM13205, GM03252, GM11097, GM18414, GM23162 and GM17910 were purchased from a reliable public repository i.e., Coriell Institute (Camden, NJ)
Recruitment	Three patient samples corresponding to NP-A and NP-C disease and two for NP-B were studied based on sample availability, common mutations and characterization by enzyme activity