

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 N/A

Data analysis N/A

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](#)

Source data are provided with this paper. Additional data 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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	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	No statistical methods were used to predetermine sample size. The number of biological replicates for each experiment was chosen based on standard practice in the field and our previous publications using similar assays. For most experiments, three independent biological replicates were performed, which we considered sufficient to detect reproducible and statistically significant differences between groups.
Data exclusions	No data were excluded from the analyses. All collected data points were included in the statistical evaluation.
Replication	All key experiments were independently replicated at least three times, and consistent results were obtained across replicates. All attempts at replication were successful.
Randomization	No formal randomization method was applied for sample allocation, as the experimental design did not involve treatment allocation in a way that would require randomization.
Blinding	Investigators were not blinded to group allocation during data collection or analysis, as the experimental procedures (e.g., Western blotting, qPCR, cell viability assays) were objective and not prone to observer bias. In all cases, quantitative data were generated from instrument-based readouts to minimize subjective 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
☐	☒ 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	<p>Primary antibodies:</p> <ul style="list-style-type: none"> • β-actin (clone C4, mouse monoclonal; sc-47778, Santa Cruz Biotechnology, Dallas, TX) • FLAG tag (clone M2, mouse monoclonal; F1084, Sigma-Aldrich) • HA tag (rabbit polyclonal; 51064-2-AP, Proteintech Group, Rosemont, IL) • NKCC1 (clone D13A9, rabbit monoclonal; #8351, Cell Signaling Technology, Danvers, MA) • p-NKCC1 (Thr212/Thr217) (rabbit polyclonal; ABS1104, Sigma-Aldrich) • EGFR (clone D38B1, rabbit monoclonal; #4267, Cell Signaling Technology) • LAMP1 (clone D2D11, rabbit monoclonal; #9091, Cell Signaling Technology) • Cleaved caspase-3 (polyclonal; #9661, Cell Signaling Technology) • Mouse IgG1 isotype control (clone G3A1, mouse monoclonal; #5415, Cell Signaling Technology) • Rabbit IgG isotype control (clone DA1E, rabbit monoclonal; #3900, Cell Signaling Technology) <p>Secondary antibodies (immunofluorescence):</p> <ul style="list-style-type: none"> • Goat anti-rabbit IgG (H+L), Alexa Fluor 568 (A-11011; Thermo Fisher Scientific) • Goat anti-mouse IgG (H+L), Alexa Fluor 488 (A-11001; Thermo Fisher Scientific) <p>Secondary antibodies (immunoblotting):</p> <ul style="list-style-type: none"> • Goat anti-mouse IgG (H+L), HRP (1031-05; SouthernBiotech, Birmingham, AL) • Goat anti-rabbit IgG (H+L), HRP (4050-05; SouthernBiotech) <p>Lot numbers: Specific lot numbers were recorded in laboratory records but are not reported in the manuscript; no lot-to-lot effects were observed. Exact lot identifiers can be provided to the editors upon request.</p>
Validation	<p>All primary antibodies are manufacturer-validated for the stated applications (WB, IP and/or IF) and species relevant to this study. In addition, specificity and expected patterns were supported by data in the manuscript:</p> <p>β-actin (C4, sc-47778) — Validated for WB; yielded a single band at the expected ~42 kDa and served as a loading control across immunoblots.</p> <p>FLAG (M2, F1084) — Validated for WB/IP; specifically detected 3$\times$FLAG-tagged RNF183 in induced cells and in anti-FLAG immunoprecipitates, with no signal in non-induced/mock controls.</p> <p>HA (51064-2-AP) — Validated for WB; detected HA-tagged ubiquitin in K63/K48 experiments, enabling visualization of RNF183-dependent NKCC1 ubiquitination.</p> <p>NKCC1 (D13A9, #8351) — Validated for WB/IP; produced a band at the expected size for NKCC1 and showed biologically coherent modulation: signal decreased upon RNF183 expression and was rescued by lysosomal inhibition (chloroquine/bafilomycin); NKCC1 ubiquitination increased in TUBE pulldowns and with K63-only ubiquitin, consistent with target specificity.</p> <p>p-NKCC1 (ABS1104) — Validated for WB; produce a band at the expected size for p-NKCC1.</p> <p>EGFR (D38B1, #4267) — Validated for WB/IP; used as a membrane-protein control in co-IP experiments. RNF183 did not co-immunoprecipitate with EGFR, supporting the selective interaction with NKCC1.</p> <p>LAMP1 (D2D11, #9091) — Validated for IF; generated the expected punctate lysosomal staining pattern that co-localized with RNF183 during NKCC1 trafficking to lysosomes.</p> <p>Cleaved caspase-3 (#9661) — Validated for WB/IF; IF signals increased under hypertonic stress and upon RNF183 overexpression in CaCo-2 cells, consistent with apoptosis markers and LDH cytotoxicity readouts.</p> <p>Isotype controls (G3A1 #5415; DA1E #3900) — Included to monitor non-specific binding in IF/IP; no specific staining or bands were observed under assay conditions.</p> <p>Together, manufacturer validation (datasheets) and our internal controls (induction vs. mock, lysosomal/proteasomal inhibition, isotype controls, co-localization, and co-IP/TUBE assays) support antibody specificity for the species and applications used in this study.</p>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	<p>The following cell lines were used in this study:</p> <ul style="list-style-type: none"> • HEK293T cells (female origin) obtained from the RIKEN BioResource Center (Tsukuba, Japan). • Caco-2 cells (male origin) obtained from the RIKEN BioResource Center. • mIMCD-3 cells (mouse inner medullary collecting duct cells; female origin) obtained from the American Type Culture Collection (ATCC, CRL-2123).
Authentication	All cell lines were obtained from authenticated repositories (RIKEN BRC or ATCC).
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination using a PCR-based detection method (MycoAlert™ Mycoplasma Detection Kit, Lonza) before experiments.
Commonly misidentified lines (See ICLAC register)	HEK293 and Caco-2 cells are listed as commonly misidentified according to the ICLAC database. Both were obtained from ATCC (or RIKEN BRC), which authenticates cell lines using STR profiling prior to distribution. mIMCD3 cells are not listed as commonly misidentified.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A