

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](#).

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.

For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	(HDX-MS): Deuterated proteins were proteolyzed and separated using an LC valve system from Trajan LEAP HDX Base automation platform coupled with ThermoFisher UltiMate-3000 pumps. Peptide mass analysis was done by a ThermoFisher Q Exactive Orbitrap mass spectrometer.	(Cryo-EM): Screening was carried out on either a Glacios TEM or Talos Arctica TEM using SerialEM. CryoEM data collection was carried out on a Titan Krios using SerialEM.	(Ratiometric calcium imaging): For ratiometric calcium imaging, an inverted microscope equipped with a Grasshopper 3 (FLIR) and Sutter Lambda LS illuminator was controlled using MicroManager 2.0.
Data analysis	(HDX-MS): For HDX-MS data analysis, peptides were identified using SearchGUI (v4.0.25) and Byonic (Protein Metrics). Deuteration calculation for each peptide was performed with HDExaminer 3.4 (Trajan) and HX-Express3. Free energy calculation from HDX data was performed with Python 3.12.1.	(Cryo-EM): For cryoEM data analysis and representation, MotionCor2, Relion5.0.0, cryoSPARC4.7, Phenix1.2.1, Coot0.9.6, and ChimeraX1.8 were used.	(Ratiometric calcium imaging): For ratiometric calcium imaging data analysis, FIJI software (NIH, v2.14) was used during analysis and to generate ratiometric or deltaF/F images. Statistical analyses were done using GraphPad Prism (v10.5.0).

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](#)

For cryo-EM data, EM volumes and fitted atomic coordinates shall be accessible through the EMDB and PDB, respectively. For HDX-MS data, raw mass spectrometry files, peptide identification results, and HDX calculation results for all available peptides have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD064468.

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](https://www.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	(HDX-MS): Sample sizes: For HDX-MS, all TRPM8 peptides identified were used for analysis.	(Cryo-EM): Sample sizes: Sample sizes were not predetermined for cryo-EM datasets	Ratiometric calcium imaging: Sample sizes: For statistical comparison of responses, we collected data from 40-86 cells. Sample sizes were not predetermined in these experiments.
Data exclusions	Data exclusions: When peptides identified by search algorithms show kinetics of deuterium exchange that are inconsistent with at least three overlapping peptides, we defined them as mis-identified peptides and manually excluded them from analysis.	Data exclusions: Images were excluded based on CTF estimations provided from cryoSPARC (<4Å). Particles were discarded based on standard practices in the single-particle cryoEM field, and exclusions were determined based on 2D or 3D classification. Details are provided as Extended Data Figures 1 and 3.	Data exclusions: For analysis of ratiometric calcium imaging data, cells showing substantial movement during imaging that cannot be further analyzed, and/or cells with abnormally high baseline calcium levels/responses (indicative of unhealthy cells) are routinely excluded from analysis. In these datasets, we did not analyze these cells.
Replication	Replication: One-two biological replicates were performed. HDX experiments were conducted with multiple labeling times. Peptides with different charge states were analyzed. Detailed replication information is provided in Extended Data Table	Replication: Sample purifications used in EM were done with at least two to three independent instances.	Replication: Temperature stimuli were repeated twice for a given imaging experiment.
Randomization	Randomization: HDX reactions were injected in random order.	Randomization: GSFSC calculations were done using random subsets of finalized particle sets in either cryoSPARC or RELION.	Randomization: Randomization was not applied during imaging experiments.
Blinding	Blinding: Blinding is not required because HDX-MS studies is mechanism focused.	Blinding: Blinding is not required because the studies are mechanism focused.	Blinding: Blinding is not required because the studies are mechanism focused.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description N/A

Research sample N/A

Sampling strategy N/A

Data collection N/A

Timing N/A

Data exclusions N/A

Non-participation N/A

Randomization N/A

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	N/A
Research sample	N/A
Sampling strategy	N/A
Data collection	N/A
Timing and spatial scale	N/A
Data exclusions	N/A
Reproducibility	N/A
Randomization	N/A
Blinding	N/A

Did the study involve field work? ☐ Yes ☒ No

Field work, collection and transport

Field conditions	N/A
Location	N/A
Access & import/export	N/A
Disturbance	N/A

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	N/A
Validation	N/A

Eukaryotic cell lines

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

Cell line source(s)	HEK293T cell line was purchased from ATCC (CRL-3216)
Authentication	No authentication was performed on the purchased cell line.
Mycoplasma contamination	No mycoplasma contamination test was conducted on the purchased cell line.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were utilized in this study.

Palaeontology and Archaeology

Specimen provenance	N/A
Specimen deposition	N/A
Dating methods	N/A
☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	N/A

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

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	N/A
Wild animals	N/A
Reporting on sex	N/A
Field-collected samples	N/A
Ethics oversight	N/A

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	N/A
Study protocol	N/A
Data collection	N/A
Outcomes	N/A

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
☒	☐ Public health
☒	☐ National security
☒	☐ Crops and/or livestock
☒	☐ Ecosystems
☒	☐ Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
☒	☐ Demonstrate how to render a vaccine ineffective
☒	☐ Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐ Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐ Increase transmissibility of a pathogen
☒	☐ Alter the host range of a pathogen
☒	☐ Enable evasion of diagnostic/detection modalities
☒	☐ Enable the weaponization of a biological agent or toxin
☒	☐ Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

ChIP-seq

Data deposition

- ☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links <i>May remain private before publication.</i>	N/A
Files in database submission	N/A
Genome browser session (e.g. UCSC)	N/A

Methodology

Replicates	N/A
Sequencing depth	N/A
Antibodies	N/A
Peak calling parameters	N/A
Data quality	N/A

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

N/A

Instrument

N/A

Software

N/A

Cell population abundance

N/A

Gating strategy

N/A

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

Magnetic resonance imaging

Experimental design

Design type

N/A

Design specifications

N/A

Behavioral performance measures

N/A

Imaging type(s)

N/A

Field strength

N/A

Sequence & imaging parameters

N/A

Area of acquisition

N/A

Diffusion MRI

☐ Used

☐ Not used

Preprocessing

Preprocessing software

N/A

Normalization

N/A

Normalization template

N/A

Noise and artifact removal

N/A

Volume censoring

N/A

Statistical modeling & inference

Model type and settings

N/A

Effect(s) tested

N/A

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

N/A

(See [Eklund et al. 2016](#))

Correction

N/A

Models & analysis

n/a | Involved in the study

☐ Functional and/or effective connectivity☐ Graph analysis☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

N/A

Graph analysis

N/A

Multivariate modeling and predictive analysis

N/A

