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Last updated by author(s): 2025/11/28

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

Image acquisition was performed in total internal reflection fluorescence (TIRF) illumination using an inverted Olympus IX-83 microscope. The setup included an sCMOS camera (ORCA-Flash 4.0, Hamamatsu), a 4-line TIRF condenser (Olympus) fiber-connected to a 488 nm laser diode (200 mW, LuxX+), a 561 nm fiber laser (50 mW, MBP communications), and a 642 nm fiber laser (50 mW, MBP communications), as well as a 405 nm laser diode for FRAP (60 mW, LuxX+). A UPLAPO 100 x HR objective with a NA of 1.5 and oil immersion was used for TIRF illumination and image capturing. Precise positioning was ensured by a motorized ultrasonic xy-stage (IX3-SSU, Olympus). Shifts in the z-plane were prevented by a hardware autofocus (IX3-ZDC2, 830 nm version, Olympus). For FRAP measurements, the cellFRAP (Olympus) unit was used with the following bleaching set up: for bleaching of lipid-associated (OG488) or VDAC1-associated (DY-547P1) fluorescence, the 405 nm laser was set to 50% laser power and the sample was bleached for 200 ms followed by 10 cycles of image acquisition with a 30 s interval between each image acquisition. For FRAP measurements in the DY-647P1 channel (642 nm), the 405 nm laser was set to 50% laser power and the sample was bleached for 200 ms followed by 150 cycles of image acquisition with a 0.5 s interval between each image acquisition. To increase the fluorescence intensity, a 2x2 binning was applied.

Data analysis	Data analysis and processing was done with FIJI (ImageJ NIH, Bethesda, MD). For FRAP curves, analysis was done with a bleaching correction using the following equation: I_corrected=(I_in/(I_out/I_(out t0))))/I_(in max) For quantifying I_in, in a circular ROI was drawn into the region of bleaching. Intensity out was determined by a circular ROI outside of the bleaching region to measure the overall fluorescence decrease. For analysis, the FIJI plugin Time Series Analyzer v3 was used. For intensity plots, the offset of the 2x2 binning (400 counts) was subtracted. To determine the lifetime τ of the interaction, a mono exponential decay was fitted from 1-25 s (for pH 6.5 0.5-10s) of the curves using the equation: I=I_0+A_1 (1-e^(-t/τ)) using Origin 9.0 (OriginLab, USA).

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

All data generated or analyzed in this study are included in the manuscript and supporting files. Source data with sample sizes, number of technical and/or biological replicates, means and standard deviations are provided in the Source Data file.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
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	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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 the author's experience of what is necessary to generate a convincing and compelling result.

Data exclusions	No data were excluded from the analyses.
Replication	Each experiment was repeated at least twice with similar results using independent experimental samples as specified in the figure legends. Source data with sample sizes, number of technical and/or biological replicates, means, standard deviations are provided in the Source Data file.
Randomization	N/A
Blinding	No blinding was done in this study. Virtually all the data are quantitative. Most measurements were made using a machine and not easily subject to operator bias.

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	A monoclonal rabbit α-VDAC1 antibody was purchased from Cell Signaling (cat. no. 4661, IB 1:1000) and a monoclonal mouse α-MBP antibody was purchased from Biolabs (cat. no. E8030-s, IB 1:5000). A goat α-mouse IgG-HRP conjugate (cat. no. 172-1011, IB 1:5000) and a goat α-rabbit IgG-HRP (cat. no. 170-6515, IB 1:5000) were purchased from BioRad.
Validation	Specificity of the α-VDAC1 antibody was verified by immunoblot analysis of total lysates from VDAC1-KO cells. All other commercial antibodies were validated by the suppliers.

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.*