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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section

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n/a	Cor	nfirmed						
	\boxtimes	The exact	sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement					
	\boxtimes	A stateme	nt on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly					
	\boxtimes	The statist	istical test(s) used AND whether they are one- or two-sided mon tests should be described solely by name; describe more complex techniques in the Methods section.					
\boxtimes		A descripti	ion of all covariates tested					
X		A descripti	cription of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons					
	\boxtimes	A full desc AND variat	description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) 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>						
\boxtimes		For Bayesi	an analysis, information on the choice of priors and Markov chain Monte Carlo settings					
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes							
\boxtimes	Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated							
Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.								
Software and code								
Policy information about <u>availability of computer code</u>								
Da	ata co	ollection	Andor iQ;					
Da	ata ai	nalysis	MATLAB; Excel; Fiji; Bitplane Imaris.					
	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							

Data

Policy information about <u>availability of data</u>

All manuscripts must include a <u>data availability statement</u>. 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

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

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 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.

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 on	e below that is the best fit for your research. If	f you are not su	re, read the appropriate sections be	efore making your selection.
Life sciences	Behavioural & social sciences	Ecological	evolutionary & environmental scien	nces

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 size was not pre-determined. Averages over multiple liposomes were performed and the results were successfully reproduced. Sample sizes (n) are indicated at the relevant location in manuscript. Sample size calculation was not performed.

Data exclusions

Liposomes that did not show any actin signal in fluorescence microscopy were excluded since they were not affected by actin. Pore opening was analyzed for samples with fast pore opening at early stages of the observation (< 10 min), while the other minor population showing slow pore opening at later stages (> 1hr) were excluded as the quality of proteins and state of lipids could be altered for long time observation.

Replication

Experiments were repeated several times as indicated and displayed the same features

Randomization

The quantitative results were reproduced in different batches of purified proteins. Experiments were reproducible among two experimentalists (MM and RS)

Blinding

Blinding was used among authors for analysis. Data collection was randomized in taking random images.

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 Methods n/a Involved in the study n/a Involved in the study ☑ Antibodies ☑ ChIP-seq ☑ Eukaryotic cell lines ☑ Flow cytometry ☑ Palaeontology and archaeology ☑ MRI-based neuroimaging ☑ Animals and other organisms ☑ Clinical data

Dual use research of concern