nature portfolio

Corresponding author(s):	Edwin Chapman
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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>.

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FOI	an statistical analyses, commit that the following items are present in the figure regend, table regend, 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.
X	A description of all covariates tested
\boxtimes	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>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\times	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\times	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 <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>

Data collection

All data collection information are found within the Methods Section. Software includes Nikon Elements 5.20.01, Zen 2, Clampex 10 and Clampex 11.

Data analysis

All procedures for data analysis are found in the figure legends and Methods section. Analysis was performed using Graphpad Prism 7.04, Origin 2020, ImageJ (FIJI package), Chimera 1.13, Chimera 1.15, IMOD 4.11.11, pyCoAn 0.3, tomo3d 2.0, Clampfit 10 and Clampfit 11.

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 $\underline{\text{policy}}$

Add graphed raw data are provided as a supplemental document. With the exception of RCSB PBD to access PDB: 1KIL, no specific databases or third party data were used in this study.

Field-spe	ecific re	porting		
Please select the o	ne below that is	the best fit for your research. If you are not sure, read the appropriate sections before making your selection.		
∑ Life sciences	Ве	ehavioural & social sciences Ecological, evolutionary & environmental sciences		
For a reference copy of t	the document with a	Il sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>		
Life scier	nces stu	ıdy design		
All studies must dis	sclose on these	points even when the disclosure is negative.		
Sample size		s sample size calculation was performed. In each case, replications were performed to validate the reproducibility of the results. See legends for details.		
Data exclusions		single data point was excluded from Figure 5G, based on Z-score analysis. According to the Z-score analysis, this data point fell outside of e normal distribution.		
Replication	The replicated o	eplicated data were consistent between trials. In each case, the details are indicated in the figure legends.		
Randomization	Experimental groups are based solely on the protein composition of the samples.			
Blinding	Blinding was not relevant to this study because no group allocations were performed.			
We require informati	on from authors a	Decific materials, systems and methods bout some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.		
Materials & ex	perimental sy	ystems 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				
Human research participants				
Clinical data Dual use research of concern				
Z Dadi ase it	escareir or comeen			
Eukaryotic c	ell lines			
Policy information about <u>cell lines</u>				
Cell line source(s) HEK293T cells were sourced from the American Type Culture Collection (ATCC)		HEK293T cells were sourced from the American Type Culture Collection (ATCC)		

HEK293T cells were authenticated using Short Tandem Repeat profiling by ATCC (ATCC; 135-XV)

HEK293T cells were confimed free from mycoplasma by a Universal Mycoplasma Detection Kit (ATCC; 30–1012K).

Authentication

Mycoplasma contamination

Commonly misidentified lines (See ICLAC register)

none