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Corresponding author(s): Bechara Kachar

Reporting Summary

Nature Research 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 Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main

Statistical parameters

text,	r Methods section).	
n/a	onfirmed	
	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement	
	$rac{3}{3}$ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly	/
\boxtimes	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>	
\boxtimes	A description of all covariates tested	
\boxtimes	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons	
\boxtimes	A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals))
\boxtimes	For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value not Give P values as exact values whenever suitable.	ted
\boxtimes	For Bayesian 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 <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated	
\boxtimes	Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)	

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about <u>availability of computer code</u>
Data collection Digital Micrograph (Gatan)

Data analysis Digital Micrograph (Gatan), Bsoft (referenced), FIJI (referenced), Chimera (referenced)

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All data in support of the findings of this study are available from the corresponding author by reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

es Behavioural & social sciences

Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.					
Sample size	Freeze-fracture and freeze-etching intrinsically view semi-random biological surfaces. We examined at least two independent replicas for each condition and in each replica examined many areas containing the proteins of interest.				
	For single particle analysis the n was determined by how many particles were visible in a 40 kX micrograph at the same time.				
Data exclusions	For single particle analysis, connexin hemichannels that were not perpendicular to the beam were examined				
Replication	All freeze fracture replicas were performed at least twice, from new starting materials				
Randomization	no randomization				
Blinding	no blinding				

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study	r
\boxtimes	Unique biological materials	
\boxtimes	Antibodies	
	Eukaryotic cell lines	
\boxtimes	Palaeontology	
	Animals and other organisms	
\boxtimes	Human research participants	

Methods

n/a	Involved in the study
\boxtimes	ChIP-seq
\boxtimes	Flow cytometry
\boxtimes	MRI-based neuroimaging

Eukaryotic cell lines

Policy information about <u>cell lines</u>				
Cell line source(s)	HEK293T (ATCC)			
Authentication	Acquired directly from ATCC			
Mycoplasma contamination	Not examined			
Commonly misidentified lines (See <u>ICLAC</u> register)	HEK cells, these cells were directly acquired from ATCC and we do not culture the known contaminating line, HELA			

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Mouse C57BL/6
Wild animals	NA
Field-collected samples	NA