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>.

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For	all statistical and	alyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.				
n/a	Confirmed					
\boxtimes	\Box The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement					
\boxtimes	A statement on 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 Only common tests should be described solely by name; describe more complex techniques in the Methods section.					
\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 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)					
\boxtimes	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 Give <i>P</i> values as exact values whenever suitable.					
\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	\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.				
So	ftware and	d code				
Polic	cy information a	about <u>availability of computer code</u>				
Da	ita collection	a collection SerialEM version 3.8, Tomography version 5				
Da	ita analysis	EMAN2 version 2.91, MotionCor2 version 1.1.0				

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

Density maps are deposited in EMDB with accession codes: EMD-29702 [https://www.ebi.ac.uk/emdb/EMD-29702] (GspDα on the inner membrane), EMD-29703 [https://www.ebi.ac.uk/emdb/EMD-29698] (GspDβ on the outer membrane), EMD-29698 [https://www.ebi.ac.uk/emdb/EMD-29698] (GspDβ on the inner membrane), EMD-29697 [https://www.ebi.ac.uk/emdb/EMD-29697] (GspDβ-GspS on the outer membrane, expressing only GspDβ), and EMD-29696 [https://www.ebi.ac.uk/emdb/EMD-29697] (GspDβ-GspS on the outer membrane, expressing only GspDβ).

www.ebi.ac.uk/emdl	b/EMD-29696] (0	GspDβ-GspS on the outer membrane, expressing GspDβ and GspS).		
Human rese	arch parti	cipants		
Policy information	about <u>studies i</u>	nvolving human research participants and Sex and Gender in Research.		
Reporting on sex and gender		Not applicable.		
Population characteristics		Not applicable		
Recruitment		Not applicable		
Ethics oversight		Not applicable		
	ation on the appr	oval of the study protocol must also be provided in the manuscript.		
Field-spe		porting s the best fit for your research. If you are not sure, read the appropriate sections before making your selection.		
X Life sciences	B	sehavioural & social sciences Ecological, evolutionary & environmental sciences		
For a reference copy of t	the document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>		
<u>Life scier</u>	nces stu	udy design		
All studies must dis	sclose on these	points even when the disclosure is negative.		
Sample size	Since our samp applicable.	le is not statistical sample, and we are not making inferences about a population based on a sampling, the sample size is not		
Data exclusions No data were excluded from the analyses.		xcluded from the analyses.		
Replication	All attempts at	replication were successful.		
Randomization Since our research does not contain the process of assigning individuals to different groups with different treatment, the applicable.		rch does not contain the process of assigning individuals to different groups with different treatment, the randomization is not		
Blinding Since our research does not contain t applicable.		rch does not contain the process of assigning individuals to different groups with different treatment, the blinding is not		
We require informati	on from authors	Decific materials, systems and methods about 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 & exp		·		
Antibodies Antibodies Lukaryotic Palaeontol Animals an Clinical dat	Antibodies ChIP-seq Lukaryotic cell lines Flow cytometry Palaeontology and archaeology MRI-based neuroimaging			
Antihodies				

Antibodies

Antibodies used

HRP-conjugated 6*His, His-tag mouse monoclonal antibody. Supplier: proteintech. Catalog number: HRP-66005. Clone Number: 18765.

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Recombinant protein were subjected to SDS PAGE followed by western blot with HRP-66005 (6*His, His-Tag antibody) at dilution of 1:10000 incubated at room temperature for 1.5 hours. Application: western blot. Positive western blot detected in recombinant protein.