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 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	Serial EM version 3.7
Data analysis	MotionCor2 (v 1.1.0); CTFFIND4 (v 4.1.5); SAMUEL (v 17.05); RELION (v 3.0); cryoSPARC (v 4.4.1); Phenix (v 1.19.2); Coot (v 0.8.9); Chimera (v 1.17.3); ChimeraX (v 1.5); AMBER22; LigPlot (v 2.2); COFACTOR; Moleonline (v 2.5).

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

Three cryo-EM maps have been deposited into the Electron Microscopy Data Bank under accession numbers EMD-37641 (MtEfpA with PE and cardiolipin), EMD-42204 (MtEfpA with PE and BRD8000.3) and EMD-42205 (MsEfpA with PE). The atomic coordinates are deposited in the Protein Data Bank under accession codes PDB 8WM5 (MtEfpA with PE and cardiolipin), 8UFD (MtEfpA with PE and BRD8000.3) and 8UFE (MsEfpA with PE).

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	This is not relevant to our studies, because our research did not involve human research participants.
Reporting on race, ethnicity, or other socially relevant groupings	This is not relevant to our studies, because our research did not involve human research participants.
Population characteristics	This is not relevant to our studies, because our research did not involve human research participants.
Recruitment	This is not relevant to our studies, because our research did not involve human research participants.
Ethics oversight	This is not relevant to our studies, because our research did not involve human research participants.

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 previous reports and best practices. Sufficient EM data were collected to achieve adequate single-particle EM analysis and 3D cryo-EM reconstructions.
Data exclusions	Poor-quality micrographs and particles were excluded during the cryo-EM data analysis.
Replication	Biochemical experiments were performed at least three times with similar results.
Randomization	This is not relevant to our study, because no grouping was needed.
Blinding	Investigators were not blinded to group allocation, because no grouping was needed for this study.

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	APC anti-GFP antibody (# 338010, BioLegend, Cat. No. 338010, Clone FM264G)
Validation	Manufacturer website lists one application References using this product [Stephen LA, et al. 2018. Dev. Cell. 47(1):122-132.e4.]

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Expi293F™ Cells (Cat. No.: A14527, ThermoFisher Scientific); Mycobacterium smegmatis mc(2)155 (ATCC).
Authentication	No further authentications were performed for this study.
Mycoplasma contamination	Mycoplasma contamination test was performed and shown to be negative.
Commonly misidentified lines (See ICLAC register)	None of the cell lines used are listed in the ICLAC database.

Plants

Seed stocks	No Plant seed was used in this study.
Novel plant genotypes	No Plant was used in this study.
Authentication	No further authentications were performed for this study.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cells with MtEfpA constitutive expression, with empty Expi293F™ Cells as control, were collected, resuspended in PBS and aliquoted into three. One aliquot was fixed with 1% Paraformaldehyde (BioLegend). Another was incubated with APC anti-GFP antibody (# 338010, BioLegend, Cat. No. 338010, Clone FM264G) followed by fixation. The third one was incubated with APC anti-GFP antibody, fixed, and permeabilized followed by incubating with APC anti-GFP antibody.
Instrument	The SH800S Cell Sorter (Sony) was employed for isolating cells exhibiting a high expression level of EfpA. The CytoFLEX flow cytometer (Beckman) was utilized to analyze the cellular membrane topology of EfpA.
Software	FlowJo Software (BD Biosciences) was used for analysis.
Cell population abundance	Prior to analyzing the cellular membrane topology of EfpA, cells with constitutive expression of EfpA were sorted. The sorted cells exhibited a purity level generally exceeding 95%.
Gating strategy	We appied forward/side scatter parameters to exclude cell debri (FSC/SSC). GFP and APC intensity were analyzed based on the gating cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.