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# 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 Authors & Referees and the Editorial Policy Checklist.

## Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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 |
| ☐ | ☒ | An indication of 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<br>*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 statistics 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<br>*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 |
| ☐ | ☒ | Clearly defined error bars<br>*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 availability of computer code

| | |
|---|---|
| Data collection | UCSF TOMOGRAPHY |
| Data analysis | GraphPad Prism 6, IMOD, TOMO3D, PEET, CHIMERA, UniProt, Phyre, I-TASSER, Quark, TMHMM, Phobius |

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

*Provide your data availability statement here.*

# 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 ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Sample size | Sample sizes were chosen for the polarity experiments by selecting number of cells above saturation of medians and averages. Saturation was usually below 100 cells and sample size was roughly 200 cells. For the ECT experiments, number of tomograms and particles used for the subtomogram average are listed in Supplementary Information Table 1. |
| Data exclusions | No data was exluded. |
| Replication | All attempts at replication were successful. |
| Randomization | Data was collected randomly in each set of experiments. |
| Blinding | Researchers were blinded to sample identity prior to image collection. |

# Reporting for specific materials, systems and methods

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ Unique biological materials |
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Antibodies

| | |
|---|---|
| Antibodies used | Bacterial antibodies: DotU, IcmF, DotC, DotD, DotF, DotG, DotH, LepB, MomP, GFP and HA |
| Validation | 1. DotU and IcmF: validated in Sexton et. al. Infection and Immunity. Vol. 72:5983-5992. 2004<br>2. DotC, DotD, DotF, DotG, and DotH: validated in Vincent et al. Molecular Microbiology. Vol. 62:1278-1291. 2006<br>3. LepB and MomP: validated in Vincent et al. Molecular Microbiology. Vol. 62:1278-1291. 2006<br>4. Rabbit anti-GFP (Sigma G1544) was validated by comparing a sample that did not contain GFP to a sample that contained a GFP fusion.<br>5. Mouse anti-HA (Genscript A01244) was validated by comparing a sample that did not contain a HA fusion to a sample that did contain a fusion. |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | U937 macrophage-like cells were from ATCC. |
| Authentication | Identification was based by purchase from a verified source (ATCC), by displaying morphological and cultural characteristics that are typical of this cell line, and by being phagocytic. |
| Mycoplasma contamination | U937 cells were not tested for contamination. |

| | |
|---|---|
| Commonly misidentified lines<br>(See ICLAC register) | *Name any commonly misidentified cell lines used in the study and provide a rationale for their use.* |