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	Leica LAS X software (v3.5.6) was used to acquire confocal images. Proteomics raw data were collected on LTQ Velos Pro or LTQ Orbitrap Velos instrument with Thermo Xcalibur software (v2.2)
Data analysis	Graphing and statistical analysis: Image J software (v1.48),Graphpad Pism (v9.5), R (version 4.1.3) with packages Deseq2 (v1.34.0), pheatmap (v1.0.12),clusterProfiler (v4.2.2) and ggplot2 (v3.4.4), and Adobe Illustrator 2024(v28.0). The structural view was generated using PyMOL (v 3.1.0). Mass spectrometry data were processed with Mascot software (v2.3.02, Matrix Science) and MaxQuant (v2.4.10.0). P-values below 0.05 were termed as significant; P-values above 0.05 were termed as non-significant.

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

The mass spectrometry proteomics data generated in this study have been deposited to the ProteomeXchange Consortium (https://proteomecentral.proteomexchange.org/) via the iProX partner repository with the dataset identifier PXD070327. The link to access the data: https://www.iprox.cn//page/project.html?id=IPX0014063000. Previously published accession codes used in this study include the PDB entry 5CYP [http://doi.org/10.2210/pdb5CYP/pdb] (used for structural analysis of the septin NC-interface). The ADP-ribosylome and interactome search results are provided in Supplementary Data. Source data are provided with this paper.

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	No human research participant in this study.
Reporting on race, ethnicity, or other socially relevant groupings	No human research participant in this study.
Population characteristics	No human research participant in this study.
Recruitment	No human research participant in this study.
Ethics oversight	No human research participant in this study.

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	The sample sizes were chosen based on the previous similar studies. Experiments were performed with three or more replicates based on experience. For intracellular bacterial load assays in cells, n = 5.
Data exclusions	No samples were excluded from the analysis.
Replication	Experiments were independently repeated at least three times with similar results.
Randomization	Randomization was not relevant to this study because the experimental groups were predetermined by specific genotypes (e.g., wild-type S. flexneri vs. mutant strains) or specific transfection conditions (e.g., vectors expressing WT vs. mutant OspC). Sample allocation was defined by the biological nature of the materials rather than random assignment.
Blinding	Investigators were not blinded to group allocation during data collection and analysis. However, to minimize subjective bias, quantitative analyses of microscopy images (e.g., septin cage counting, plaque area measurement) were performed using standard software (ImageJ and LAS X) with defined intensity thresholds and objective criteria.

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

The following primary antibodies were used in this study: anti-poly/mono-ADP ribose (WB 1:2000, Cell Signaling Technology, 83732), anti-GFP (WB 1:2000, Proteintech, 66002-1-Ig), anti-GAPDH (WB 1:5000, Proteintech, 60004-1-Ig), anti-β-actin (WB 1:5000, Cell Signaling Technology, 4967), anti-FLAG for Western blot (WB 1:5000, Zen Bioscience, R24091), anti-FLAG for immunofluorescence (IF 1:300, Beyotime Biotechnology, AF519), anti-HA (WB 1:5000, Invitrogen, 26183), anti-GST (WB 1:1000, Zen Bioscience, 390028), anti-His (WB 1:1000, CWBIO, CW0286M), anti-S-tag (WB 1:2500, Sino Biological, 101290-T38), anti-SEPT2 (WB 1:2500, Proteintech, 11397-1-AP), anti-SEPT6 (WB 1:1000, Santa Cruz, sc-514781), anti-SEPT7 (WB 1:2000, Proteintech, 13818-1-AP), anti-SEPT9 (IF 1:500, WB 1:2500, Atlas Antibodies, HPA042564), and anti-Strep-Tag II (WB 1:2500, Abmart, M40014S). The secondary antibodies include HRP-conjugated anti-mouse IgG (WB 1:5000, Jackson ImmunoResearch Laboratories, 115-035-003), HRP-conjugated anti-rabbit IgG (WB 1:5000, Jackson ImmunoResearch Laboratories, 111-035-003), Alexa Fluor 488-labeled anti-mouse IgG (IF 1:500, Jackson ImmunoResearch Laboratories, 115-545-003), Cyanine3 (Cy3)-conjugated anti-rabbit IgG (IF 1:500, Jackson ImmunoResearch Laboratories, 111-165-003), and Alexa Fluor 647-labeled anti-mouse IgG (IF 1:500, Beyotime Biotechnology, A0473).

Validation

anti-poly/mono-ADP ribose (Cell Signaling Technology, 83732): https://www.cellsignal.com/products/primary-antibodies/poly-mono-adp-ribose-e6f6a-rabbit-mab/83732
anti-GFP (Proteintech, 66002-1-Ig): https://www.ptgcn.com/products/eGFP-Antibody-66002-1-Ig.htm
anti-GAPDH (Proteintech, 60004-1-Ig): https://www.ptgcn.com/products/GAPDH-Antibody-60004-1-Ig.htm
anti-β-actin (Cell Signaling Technology, 4967): https://www.cellsignal.com/products/primary-antibodies/b-actin-antibody/4967
anti-FLAG for Western blot (Zen Bioscience, R24091): http://www.zen-bioscience.com/goodsDetail?productNo=R24091
anti-FLAG for immunofluorescence (Beyotime Biotechnology, AF519): https://www.beyotime.com/product/AF519.htm
anti-HA (Invitrogen, 26183): https://www.thermofisher.cn/cn/zh/antibody/product/HA-Tag-Antibody-clone-2-2-2-14-Monoclonal/26183
anti-GST (Zen Bioscience, 390028): http://www.zen-bioscience.com/goodsDetail?productNo=390028
anti-His (CWBIO, CW0286M): https://www.cwbio.com/product/detail/id/10177
anti-S-tag (Sino Biological, 101290-T38): https://www.sinobiological.com/antibodies/s-tag-101290-t38
anti-SEPT2 (Proteintech, 11397-1-AP): https://www.ptgcn.com/products/SEPT2-Antibody-11397-1-AP.htm
anti-SEPT6 (Santa Cruz, sc-514781): https://www.scbt.com/zh/p/septin-6-antibody-b-8
anti-SEPT7 (Proteintech, 13818-1-AP): https://www.ptgcn.com/products/SEPT7-Antibody-13818-1-AP.htm
anti-SEPT9 (Atlas Antibodies, HPA042564): https://www.atlasantibodies.com/products/primary-antibodies/triple-a-polyclonals/anti-sept9-antibody-hpa042564/
and anti-Strep-Tag II (Abmart, M40014S): https://www.ab-mart.com.cn/page.aspx?node=59&id=18049
HRP-conjugated anti-mouse IgG (Jackson ImmunoResearch Laboratories, 115-035-003): https://www.jacksonimmuno.com/catalog/products/115-035-003
HRP-conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories, 111-035-003): https://www.jacksonimmuno.com/catalog/products/111-035-003
Alexa Fluor 488-labeled anti-mouse IgG (Jackson ImmunoResearch Laboratories, 115-545-003): https://www.jacksonimmuno.com/catalog/products/115-545-003
Cyanine3 (Cy3)-conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories, 111-165-003): https://www.jacksonimmuno.com/catalog/products/111-165-003
Alexa Fluor 647-labeled anti-mouse IgG (Beyotime Biotechnology, A0473): https://www.beyotime.com/product/A0473.htm

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	293T (Cat No. CRL-3216) and HeLa cells (Cat No. CRM-CCL-2) were obtained from American Type Culture Collection (ATCC). Both cell lines are of female origin.
Authentication	Cells were purchased and received directly from their respective manufacturer. Authentication was performed by the manufacturer, no additional in-house authentication was performed.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study.

Plants

Seed stocks	No plants were used in this study.
Novel plant genotypes	No plants were used in this study.
Authentication	No plants were used in this study.