

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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 Novel code for use with ImageJ to calculate capsule area is available at the following GitHub URL: <https://github.com/michelamarra/37-CE-paper.git>.

Data analysis Data from capsule areas were presented using GraphPad software (Prism)

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](#)

Data are contained within the manuscript files or Data Source File. Novel code for use with ImageJ to calculate capsule area is available at: Zenodo URL: DOI: 10.5281/zenodo.18216823. Genomes can be found at NCBI (Project ID PRJNA140182 and website <https://www.ncbi.nlm.nih.gov/bioproject/1401821>).

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	Not applicable
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

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](https://www.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 for bacterial studies were determined depending on when/if statistical significance could be reached. For example measuring capsule size with imaging. For animal infections numbers for these studies have been predetermined in our other papers, e.g. PLoS Pathog . 2018 May 11;14(5):e1007052. doi: 10.1371/journal.ppat.1007052.; PLoS Pathog . 2023 Jan 31;19(1):e1011035. doi: 10.1371/journal.ppat.1011035. Statistical significance can be achieved using 3 mice per bacterial strain for IVIS imaging, and 10 mice per strain for sepsis infection models, 8 mice per strain for the pneumonia model.
Data exclusions	There were no data exclusions in this work. All data points collected were used in these experiments except in cases where the model/assay deemed not established due to the technical issues, such as operator error.
Replication	When possible, technical replicates were performed in addition to (at least 3) biological replicates. Biological replicates were performed on different days.
Randomization	Studies were conducted with bacterial strains. There was no need to randomize. For animal work, we used Excel software to generate a column of random numbers which were use to randomly allocate animals to each group.
Blinding	Data was recorded as observed. The investigators were blinded to group allocation and data analysis.

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	Serotype 2 mAb and 19c serum were obtained from Cedarlane Labs, Burlington, ON, Canada. Serotype 6A and 6B mAbs were obtained from d provider of these antibodies to researchers in the pneumococcal field (Art. no. 16745 for serotype 2 and Art. no. 16964 which was raised against serotype 19F but crossreacts with 19C). Monoclonal antibodies or serum for serotypes 6A and 6B were obtained from the The WHO Pneumococcal Serology Reference Laboratory (Dr. Moon Nahm, University of Alabama at Birmingham).
Validation	Serum used to detect the Serotype 2 and 19C capsule was obtained from Cedarlane Labs which is the standard provider of these antibodies to researchers in the pneumococcal field. Monoclonal antibodies for serotypes 6A and 6B from the The WHO Pneumococcal Serology Reference Laboratory (Dr. Moon Nahm). Publications include J Biol Chem . 2013 Sep 6;288(36):25976-25985. doi: 10.1074/jbc.M113.480152; Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies

Animals and other research organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	In vivo studies were carried out using 8–10-week-old female CD1 outbred mice, bred in-house in the Division of Biomedical Services at the University of Leicester, UK. At UAB C57BL/6J mice, aged 6-8 weeks were used and housed in the Bevill Biomedical Sciences facility.
Wild animals	Wild animals were not used in this study.
Reporting on sex	Equal numbers of male and female mice were used in these studies.
Field-collected samples	There were no field-collected samples in this study.
Ethics oversight	Mouse studies were performed under the project (permit no. PP0757060) and personal (permit no. 80/10279) licenses according to the United Kingdom Home Office guidelines under the Animals Scientific Procedures Act 1986, and the University of Leicester ethics committee approval. The protocol used was approved by both the U.K. Home Office and the University of Leicester ethics committee. Where specified, the procedures were done under anesthetic with isoflurane. Animals were kept in individually ventilated cages in a controlled environment (temperature: 21±2 °C and relative humidity (RH): 55%rh ±10) with 12h dark and 12h light cycles, and were regularly monitored after infection to reduce suffering. Animal protocols for the pneumonia model were approved by UAB Institutional Animal Care and Use Committee (IACUC). (Protocol IACUC-21851).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable

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

BHI with 20% calf serum was used to culture pneumococcal cells to an OD of 0.4. RAW264.7 macrophages were seeded at 1×10^6 cells per well in 24-well plates with DMEM + 10% FBS and incubated (37 °C, 5% CO₂) for 12 h. Medium was replaced with serum-free DMEM prior to infection. Bacteria were added at MOI = 100 (1×10^8 CFU/well).

Instrument

BD FACSymphony™ A3

Software

Flow cytometry data were analyzed using FlowJo v10.10.0.

Cell population abundance

NA, no sorting was used.

Gating strategy

Raw 264.7 cells were gated as follows: Side scatter-area (SSC-A) vs Forward scatter area (SSC-A) -> FSC-A vs Forward scatter-height (FSC-H) to select single cells -> Live/dead marker (Aqua fluorophore) vs FSC-A to select live cells -> Cell count vs Streptococcus pneumoniae mKate2+ for histograms. Negative versus positive population was set up with FMO control (cells incubated with S. pneumoniae mKate2+ versus cells without S. pneumoniae mKate2+).

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