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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.

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	Confocal microscopy images were captured using Zeiss ZEN system 2011 software.
Data analysis	DeconvolutionLab2 (2017) and ImageJ (Version: 2.0.0-rc-69/1.52p Build: 269a0ad53f) were used to deconvolve the images.

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. 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

Additional data that support the findings of this study are available from the corresponding author, Brian P. Conlon, upon request (brian_conlon@med.unc.edu).

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	For all in vitro and tissue culture experiments, experiments and statistical analysis was performed using 3 biological replicates. Based on previous experience with in vivo persister analysis (Conlon et al. Nature. 2013) groups of 5 mice are sufficient to achieve statistical power. With this in mind, we ran an initial experiment with 6 mice per group (an extra mouse was included to mitigate potential loss of animals during the experiment). Statistically significant changes were observed between wild type and mutant mice in the initial experiment (P<0.0001) and a second experiment with 5 mice was performed to verify reproducibility.
Data exclusions	Bacterial burden was not measured in mice that died before the experiment was completed as per our pre-established criteria
Replication	In vitro and tissue culture experiments depicted in this manuscript show data from a minimum of 3 independent biological samples. In addition, experiments were repeated at least 3 times on different days to ensure reproducibility. Animal experiments were performed twice independently to ensure reproducibility.
Randomization	Mice were randomly assigned to groups
Blinding	Blinding was not necessary as all outputs (cfu/g tissue) are objective. No subjective measurements such as clinical symptoms were measured.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Rabbit polyclonal anti-Staphylococcus aureus: Abcam; Cat# ab20920; Lot# GR32095552; used at 1:400. Rat monoclonal anti-mouse F4/80 (clone BM8): eBioscience; Cat# 14-4801-82; Lot# 1948594; used at 1:100. Rat monoclonal anti-mouse Ly6G (clone 1A8): eBioscience; Cat#16-9668-82; Lot# 2017446; used at 1:100. Goat polyclonal anti-Rabbit IgG (H+L) conjugated to Alexa Fluor Plus 488: Invitrogen; Cat# A3273; Lot# TH266005; used at 1:1000. Goat polyclonal anti-Rat IgG (H+L) conjugated to Alexa Fluor 594: Invitrogen; Cat# A11007; Lot# 2026149; used at 1:1000.
Validation	The BM8 monoclonal antibody reacts with mouse F4/80 antigen which is expressed by a majority of mature macrophages and is one of the best markers for this population of cells. It is expressed on a wide range of mature tissue macrophages including Kupffer cells, Langerhans, microglia, macrophages located in the gut lamina propria, peritoneal cavity, lung, thymus, bone marrow stroma and macrophages in the red pulp of the spleen. The BM8 antibody has been reported for use in flow cytometric analysis, immunohistochemical staining of frozen tissue sections, and immunohistochemical staining of paraffin embedded tissue sections. Mogami et al. 2018. Sci Rep. The monoclonal antibody 1A8-Ly6g reacts with mouse Ly-6G, which is also known as Gr-1. Ly-6G is a 25-kDa GPI protein expressed exclusively by neutrophils. Tian et al. Basic Res Cardiol. 2016. The anti-staphylococcal antibody was developed against S. aureus strain ATCC 27660 (mixture of intact organisms and lysed cells). Antiserum is not absorbed for related microorganisms. It has been validated for immunofluorescence. Hussain et al. Nat Biomed Eng. 2018.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	J774.1 ATCC Cat# TIB--67, THP-1 ATCC cat# TIB-202
Authentication	cell line was not authenticated
Mycoplasma contamination	not tested for Mycoplasma contamination

Commonly misidentified lines (See ICLAC register)	n/a

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	C57BL/6J Jackson# 000664, C57BL/6J Ncf1–/– Jackson# 027331. All mice used were 8-10 week old female mice
Wild animals	no wild animals were used in this study
Field-collected samples	no field samples were collected in this study
Ethics oversight	All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill and met guidelines of the US National Institutes of Health for the humane care of animals.

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