

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	Sequencing reads were collected on an Illumina NextSeq 550 sequencer with default base calling software.
Data analysis	Cutadapt (4.6) was used for removing adapter sequences from FASTQ reads and barcode demultiplexing. UMI-tools (1.1.4) was used to extract UMI sequences from read 2 and collapse PCR duplicates. STAR (2.7.10a) was used for alignment of read 1 to the annotated <i>S. aureus</i> USA300 FPR3757 genome. featureCounts (2.0.3) was used for annotating and enumerating transcript features. Python (3.10.12) was used for compiling count matrices. For downstream analysis after count matrix generation, a Jupyter notebook with IPython interface was set up. Scanpy (1.9.6) was used for preprocessing, filtering, normalization, and clustering. MAST (1.30.0) was used for differential expression. The iModulonDB database was used for transcriptional regulatory studies. Palantir (1.3.3) was used for trajectory analysis, with integrated MAGIC imputation. GraphPad Prism (10.0.2) was used for select statistical and correlation tests.

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](#)

Sequencing data and processed count matrices have been deposited in the GEO database under accession code GSE270986 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE270986>]. Source data are provided as a Source Data file.

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 (no human subjects involved)
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable (no human subjects involved)
Population characteristics	Not applicable (no human subjects involved)
Recruitment	Not applicable (no human subjects involved)
Ethics oversight	Not applicable (no human subjects involved)

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	No sample-size calculations were performed. The number of cells used for analysis within each sample/condition was determined by reads/cell cutoff, as described in the methods.
Data exclusions	No data was excluded.
Replication	Two independent sequencing runs were performed as described in the text: One comparing biofilm to planktonic growth, and the other comparison biofilm co-cultured with different leukocytes. Each sample/conditions within the two sequencing runs contained thousands of individual cells. Reproducibility was verified through integrated clustering analyses across multiple samples. Additionally, clusters were determined such that each contained adequate cell numbers for statistical analysis.
Randomization	Bacterial cells were collected in equal numbers across each growth or co-culture condition and pooled prior to barcoding. The barcoding process involves mixing and random splitting of cells to ensure unbiased distribution across the 96-well plates.
Blinding	No blinding was performed during sample processing and sequencing, as sequencing would not have been altered by blinding. All steps after the first barcoding step were performed on combined samples/conditions. Clustering and visualization was performed in an unbiased manner with established methods (UMAP, Leiden).

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	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	All primary cells were isolated from the bone marrow of both male and female 8-10 wk old WT C57BL/6J mice (RRID:IMSR_JAX:000664), as described in the methods.
Authentication	Each primary cell type was authenticated via flow cytometry in previously published reports from our laboratory.
Mycoplasma contamination	Primary cells were not tested for mycoplasma as they were used immediately following isolation.
Commonly misidentified lines (See ICLAC register)	Not applicable

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	Primary cells were isolated from the bone marrow of 8-10 wk old WT C57BL/6J mice (RRID:IMSR_JAX:000664). All mice were group-housed at 21-23°C (22°C average) and 30-70% humidity (55% average) under a 12 h light/dark cycle with free access to food (2019S Teklad Global 19% Protein Extruded Rodent Diet; Inotiv, West Lafayette, IN) and water.
Wild animals	The study did not involve wild animals.
Reporting on sex	Both male and female mice were used for primary cell isolation.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The animal use protocol was approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee (#18-013-03).

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

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

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

S. aureus cells were fixed and/or processed to varying stages of permeabilization as described in Supplementary Figure 2.

Instrument

A Becton Dickinson (BD) six laser (355-nm, 405-nm, 532-nm, 561-nm, 488-nm, and 633-nm) LSR II Y/G

Software

DiVa and FlowJo

Cell population abundance

All cells within the FSC-A/SSC-A were processed for analysis, with numbers provided in Supplementary Figure 2.

Gating strategy

All cells within the FSC-A/SSC-A were processed for analysis with no further gating.

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