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.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

## 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<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 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<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 |

*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 | Imaging data (live and fixed) were analyzed with Imaris 10.2 and IncuCyte S3 2020B |
| Data analysis | Mass spectrometry data analyzed using Mascot 2.4.1 and MSstats 3.16.0. NGS data were analyzed using DESeq2. Plots were generated with Prism v.9 & v.10.4.2 |

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

Raw data for Citrobacter whole-genome sequencing are deposited to Sequence Read Archive (PRJNA1150236), Proetomics deposted to MASSive (MSV000095663). Source data for Figs. 1-4 and Extended Data Figs. 1-5 are provided with the 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 | N/A |
| Reporting on race, ethnicity, or other socially relevant groupings | N/A |
| Population characteristics | N/A |
| Recruitment | N/A |
| Ethics oversight | N/A |

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 | No sample size calculations were performed. The *C. rodentium* infection model is well-established and highly reproducible (Nat Protoc. 2016 Oct;11(10): 1851-76. doi: 10.1038/nprot.2016.100). For our primary readout, we relied on bacterial burden, typically measured in feces or tissue samples through colony-forming unit (CFU) analysis, which is known for producing consistent and tightly grouped data. Based on previous studies, an average group size of 4 (Sci Rep. 2017 Sep 21;7(1):12099. doi: 10.1038/s41598-017-12256-z) to 5 (Nature. 2024 May;629(8012):669-678. doi: 10.1038/s41586-024-07288-1. Epub 2024 Apr 10) animals has been shown to yield reliable and statistically robust results for this outcome. |
| Data exclusions | No data were excluded from the analysis |
| Replication | For in vivo experiments, readouts were performed with 5 animals per genotype, and all attempts at replication were successful. For in vitro experiments, readouts were performed with 3 or greater biological replicates, and all attempts at replication were successful. In both cases, experiments were repeated independently at least 3 times. |
| Randomization | Groups were determined by genotype rather than treatment, therefore, randomization was not applicable. |
| Blinding | Imaging was performed blindly and automatically using a Leica Confocal or Incucyte system. For other experiments, mice and cell lines were picked and treated by the same individual, so blinding to genotype and treatment as well as during data collection and analysis was not possible. |

# 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 |
|---|---|
| [ ] | [X] Antibodies |
| [ ] | [X] Eukaryotic cell lines |
| [X] | [ ] Palaeontology and archaeology |
| [ ] | [X] Animals and other organisms |
| [X] | [ ] Clinical data |
| [X] | [ ] Dual use research of concern |
| [X] | [ ] Plants |

## Methods

| n/a | Involved in the study |
|---|---|
| [X] | [ ] ChIP-seq |
| [X] | [ ] Flow cytometry |
| [X] | [ ] MRI-based neuroimaging |

## Antibodies

Antibodies used

Antibodies used for western blots were from Genentech (anti-K11-linked; anti-K48-linked; and anti-63-linked polyubiquitin antibodies were used at 1 ug/mL), Cell Signaling Technology (anti-myc cat#2276, used at 1 ug/mL; anti-ROCK1 cat#4035 used at 1 ug/mL; anti-ROCK2 cat#8236 used at 1 ug/mL; anti-PARP cat#9542 used at 1 ug/mL; anti-phospho-MLC2 cat#3671 used at 1 ug/mL), Abcam (anti-beta tubulin cat#ab15568 used at 0.1 ug/mL), Bethyl (anti-P66beta cat#A301-281A-T used at 1 ug/mL), MPBio (anti-actin cat#0869100-CF used 0.1 ug/mL), Novus (anti-Rho1D4 cat#NBP1-47602 used at 1 ug/mL), SigmaAldrich (anti-FLAG M2 HRP cat#A8592 used at 1 ug/mL), and Lifesensors (anti-ubiquitin cat#VU101 used at 1 ug/mL).

Validation

-anti-K11-linked; anti-K48-linked; and anti-63-linked polyubiquitin antibodies was validated previously by Matsumoto, M. L. et al. (2010) and Newton, K. et al (2008).
-9B11 anti-myc (Cell Signaling Technology, cat#2276, lot 24). The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 2422 publications.
-C8F7 anti-ROCK1 (Cell Signaling Technology, cat#4035, lot 3). The antibody guarantee covers the use of the antibody for WB applications. The antibody was verified in our study showing WB depletion in ROCK1/2 deficient mice Figure 4l and Extended Data Figure 5f.
-anti-ROCK2 (Cell Signaling Technology, cat#8236, lot 2). The antibody guarantee covers the use of the antibody for WB applications. The antibody was verified in our study showing WB depletion in ROCK1/2 deficient mice Figure 4l and Extended Data Figure 5f.
-anti-PARP (Cell Signaling Technology, cat #9542, lot 15). The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 4458 publications.
-anti-phospho-MLC2 (Cell Signaling Technology, cat#3671, lot 6). The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 828 publications.
-anti-beta Tubulin (Abcam, cat#ab15568, lot GR3237077-10). The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 101 publications.
-C4 anti-actin (MPbio, cat#0869100-CF, lot QR14180). The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 1215 publications.
-anti-P66-beta (Bethyl, cat#A301-281A-T, lot 1). The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 5 publications.
-anti-Rho1D4 (Novus, cat#NBP1-47602, lot 1006). The antibody has been validated previously by Molday, R.S. et al (2014).
-anti-FLAG M2 HRP (SigmaAldrich, cat#A8592, lot 1006). The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 1904 publications.
-VU-1 anti-ubiquitin (Lifesensors, cat#VU101, lot not available). The antibody guarantee covers the use of the antibody for WB applications. The antibody has been verified with di-ubiquitin standards by the vendor. Reactivity is independent of species due to the high conservation of ubiquitin across eukaryotes.

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | 293T (ATCC CRL-3216), Caco-2 (ATCC HTB-37), Ea.hy926 (ATCC CRL-922), HT-29 (ATCC HTB-38) |
| Authentication | Cells not authenticated |
| Mycoplasma contamination | Cells are negative for mycoplasma |
| Commonly misidentified lines (See ICLAC register) | not used |

## Palaeontology and Archaeology

| | |
|---|---|
| Specimen provenance | |
| Specimen deposition | |
| Dating methods | |

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

| | |
|---|---|
| Ethics oversight | |

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

## 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 | All mice (Mus musculus) were maintained on a C57BL/6N genetic background. Strains included Villen.CreERT2 Rock1 wt/wt Rock2 wt/wt and VillenCreERT2 Rock1 fl/fl Rock2 fl/fl (Sambandam, A. et al 2023. heliyon.2023.e14238). For C. rodentium studies, female mice were aged 12-14 weeks (Extendend Data Fig. 5h) or 5-8 weeks (Fig. 4 i-k and Extended Data Fig. 5j).<br><br>Mice were housed in individually ventilated cages within animal rooms maintained on a 14:10-hour, light-dark cycle. Animal rooms were temperature and humidity-controlled, between 68-79°F and 30-70% respectively, with 10 to 15 room air exchanges per hour. |
| Wild animals | The study did not use wild animals |
| Reporting on sex | Female mice were used in all experiments. |
| Field-collected samples | The study did not involve samples collected from the field. |
| Ethics oversight | All mouse studies complied with relevant ethics regulations and were approved by either the Genentech Institutional Animal Care and Use Committee (IACUC) in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility or by the Oregon Health and Science University IACUC. |

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

| No | Yes | |
|---|---|---|
| ☐ | ☐ | Public health |
| ☐ | ☐ | National security |
| ☐ | ☐ | Crops and/or livestock |
| ☐ | ☐ | Ecosystems |
| ☐ | ☐ | Any other significant area |

### Experiments of concern

Does the work involve any of these experiments of concern:

| No | Yes | |
|---|---|---|
| ☐ | ☐ | Demonstrate how to render a vaccine ineffective |
| ☐ | ☐ | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| ☐ | ☐ | Enhance the virulence of a pathogen or render a nonpathogen virulent |
| ☐ | ☐ | Increase transmissibility of a pathogen |
| ☐ | ☐ | Alter the host range of a pathogen |
| ☐ | ☐ | Enable evasion of diagnostic/detection modalities |
| ☐ | ☐ | Enable the weaponization of a biological agent or toxin |
| ☐ | ☐ | Any other potentially harmful combination of experiments and agents |

## Plants

Seed stocks

Novel plant genotypes

Authentication

## ChIP-seq

### Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
*May remain private before publication.*

Files in database submission

Genome browser session
(e.g. UCSC)

### Methodology

Replicates

Sequencing depth

Antibodies

Peak calling parameters

Data quality

Software

## 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

Instrument

Software

Cell population abundance

Gating strategy

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

## Magnetic resonance imaging

### Experimental design

Design type

Design specifications

Behavioral performance measures

Imaging type(s)

Field strength

Sequence & imaging parameters

Area of acquisition

Diffusion MRI ☐ Used ☐ Not used

### Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

### Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

(See Eklund et al. 2016)

Correction

## Models & analysis

| n/a | Involved in the study |
|---|---|
| ☐ | ☐ Functional and/or effective connectivity |
| ☐ | ☐ Graph analysis |
| ☐ | ☐ Multivariate modeling or predictive analysis |

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis

09/08/2025

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