

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	Data were collected using Agilent MassHunter Workstation LC/MS Data Acquisition 10.1, Agilent Cary WinUV Kinetics Application V.5.0.0.999, Tecan i-control Version 3.9.1.0.
Data analysis	Data were analyzed using Microsoft Excel for Mac 2011 version 14.7.7, GraphPad Prism 8 for macOS version 8.4.0, Agilent MassHunter Workstation Quantitative Analysis (for Q-TOF & for QQQ) 10.0. MDF analysis used equilibrato _{api} v 0.4.5, equilibrato _{pathway} v 0.4.4. Sequencing data were analyzed using ONT Guppy base-calling software (v 6.0.1+652ffd179), breseq v0.35.7, minimap2 v2.24 (r1122), canu v2.2, ngmlr v0.2.7 and sniffles2 v2.0.3, Tablet v1.21.02.08.

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 genome sequence of MG1655 used in this study is available at GenBank under the accession number NC_000913. The genome sequence of JCL301 has been deposited to GenBank under CP107281. Raw reads of Illumina and Nanopore sequencing have been deposited at the NCBI Sequence Read Archive (SRA) and can be accessed under BioProject PRJNA884544. All other data are available in the main text or the supplementary materials. The source data that support the plots within this paper are available from the corresponding author upon request.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	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 statistical methods were used to predetermine the sample size. At least two or three independent experiments were performed in this work. Sample sizes were determined based on previous similar studies and considerations of biological and technical variabilities to ensure result confidence.
Data exclusions	No data were excluded in this study.
Replication	At least two or three independent experiments were performed to verify our results. All attempts of replication were successful.
Randomization	Randomization was not applicable in this study since it did not involve participants or samples requiring random allocation for statistical comparisons.
Blinding	Blinding was not relevant since the nature of the experiments in this study precludes any potential for subjective bias to influence the results.

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

Methods

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