

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 No computer code requiring software was used to collect data

Data analysis Graph Pad Prism software version 10.6.0 was used to plot and statistically analyze data. Chem Draw v 22.0 was used to draw chemical structures. MAVEN 2.10.21 and Freestyle 1.8.65.0 software were used for LC/MS data analysis. Identification of folates without available standards was performed using SIRIUS version 6.3.3 and comparisons to previously published results.

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](#)

All data generated or analyzed during this study are included in this article and in supplementary information files. The folate atlas data can be accessed and

visualized using an interactive website: <https://chaudharilab.com/folate-atlas/>. Raw LC/MS data can be accessed through Metabolights (<https://www.ebi.ac.uk/metabolights/MTBLS13484>). Source data for all figures are provided with this paper. Requests for additional information can be made to the corresponding author.

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](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	A power calculation was used to determine number of animals (https://clincalc.com/stats/samplesize.aspx). Briefly, a continuous endpoint (average endpoint outcome for concentration means) for 2 independent study groups (conventional vs. germ-free animals) with an expected error in the mean of 10%, and a difference of at least 20% was considered. A false positive rate of 5% and a false negative rate of 10% (power) identified an n=5 for each study group. All data shown in the manuscript were analyzed using the appropriate parametric statistical tests. All data that met normality assumptions by the Shapiro-Wilk test for small sample sizes ($\alpha > 0.05$) were subjected to parametric Welch's or Student's ttest, or two-way ANOVA followed by Šidák's multiple comparisons test as appropriate. Non-normal data were subjected to Mann-Whitney test. All statistical tests are indicated in figure legends.
Data exclusions	No data were excluded from the analyses.
Replication	In vivo mouse studies: Metabolomic analyses in germ-free animals were reproduced twice and the data was replicable. Data from both batches are presented in the manuscript. In vitro qPCR was performed in at least 3 biological replicates. Each experiment was reproduced in triplicate at least twice with similar results.
Randomization	No randomization was performed, as conventional and germ-free animals were housed separately.
Blinding	In vivo mouse studies: Due to the need for separate housing for conventional and germ-free animals, blinding was not possible for organ collection. Data from in vitro experiments were acquired from equipment capable of batch-analysis and were analyzed using computational softwares. This eliminated human error and bias, and thus we did not blind the data from investigators.

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

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	Conventional and germ-free C57BL/6 male and female mice (age 6-8 weeks). All animals were maintained in climate-controlled animal facilities with a 12 h light-dark cycle, 30-70% humidity and 20-24 °C, with ad libitum access to food and water.
Wild animals	No wild animals were used in this study.
Reporting on sex	Metabolomics analyses were performed in 35 tissues from male and female mice. Data in the study is segregated by sex. Sex organs from male (prostate and testicle) and female (uterus and ovary) were performed in conventional and germ-free animals. Male mice were used for folate deficient diet study.
Field-collected samples	n/a
Ethics oversight	Experiments were approved by the IACUC at the University of Wisconsin-Madison.

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