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☐ Initial submission ☒ Revised version ☐ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   Sample sizes were calculated with a power analysis before experiments were conducted, except in the case of germfree studies where we were limited by the availability of age and sex matched mice.

   415-416 pg 19; 441-442 pg 20; 508-509 pg23; 539-540 pg 24; 571-572 pg 26

2. Data exclusions

   Describe any data exclusions.

   No data were excluded

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   Replication attempts were not made.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   For antibiotic experiments, all dosing was conducted by veterinary technicians who randomly assigned half of the mice to be treated and half to be controls at the beginning of the experiment. (439-441 pg 20)
   For diet manipulation experiments, mice were randomly assigned to treatment group by researchers carrying out the experiments. (414-415 pg 19)
   For tracer experiments, cages were randomly assigned to treatment by the researcher. (507-508 pg 23)

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Researchers collecting data were blinded to mouse treatment status throughout the antibiotic treatment period. All dosing was conducted by veterinary technicians who were not blinded but did not participate in sample collection or measurement. (439-441 pg 20)
   Researchers collecting weight data and fecal samples during diet manipulation experiments were not blinded to treatment, but processing of fecal samples was done by researchers who were blinded to the treatment group until the time of analysis.
   Blinding was not performed for stable isotope tracer experiments. (507-508 pg 23)

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	All data were used with R and standard packages available there within.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	n/a

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	n/a

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	n/a
b. Describe the method of cell line authentication used.	n/a
c. Report whether the cell lines were tested for mycoplasma contamination.	n/a
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	n/a

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	All animal experiments were performed with adult (8-10 week old) male C57BL/6 mice sourced from Charles River Labs except for germfree mice which were sourced from the Duke Gnotobiotic facility.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	Human fecal samples were used in observational analyses. They were sourced from healthy human adults with no use of antibiotics within the past month.