

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 (<i>n</i>) 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), 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

In vivo injury procedures were performed using an electromagnetic Impact One stereotaxic controlled cortical impact system (Leica Biosystems), and sensorimotor performance was acquired with the Ugo Basile Rotarod. Fecal DNA concentration was measured on a DeNovix DS-11 spectrophotometer/fluorometer. Bacterial 16S rRNA gene copy number was acquired on a Bio-Rad CFX384 Touch Real-Time PCR Detection System using the associated instrument software. 16S rRNA amplicon sequencing libraries were generated and sequenced on an Illumina MiSeq (300 bp paired-end). Long-read shotgun metagenomic data were generated on Oxford Nanopore platforms using R9.4.1 chemistry with raw output in POD5 format. Serum short-chain fatty acids were acquired by LC-MS/MS on an Agilent 6495 triple quadrupole coupled to an Agilent HPLC/UPLC system. Histological and immunofluorescence imaging data were acquired using a Nikon Eclipse Ni-U fluorescence microscope and a Leica confocal imaging system, and experimental schematics were created with BioRender.

Data analysis

Statistical analyses and plotting were performed in GraphPad Prism 8, with additional multivariate metabolomics (oPLS-DA) conducted in R (v4.3.3) using MetaboAnalystR. Image-based quantification (lesion volume and immunostaining area/densitometry) was performed in ImageJ/Fiji (ImageJ64), and microglial morphometrics and Sholl analyses were performed using Neurolucida and NeuroExplorer (MBF Biosciences). For 16S rRNA sequencing, Illumina BCL files were converted/demultiplexed with bcl2fastq, reads were filtered and merged with BBMap v38.82 (bbduk.sh/bbmerge.sh), ASVs were inferred with DADA2 v1.32, and downstream microbiome analyses used phyloseq v1.48.0 with phylogenetic reconstruction via MAFFT v7.525 and FastTree v2.1.11; PERMANOVA used vegan::adonis2 v2.6-5. For long-read metagenomics, nanopore reads were basecalled with Dorado v0.7.0, processed with samtools v1.21, NanoPlot v1.43.0, hostile v1.1.0, and chopper v0.9.0, and assembled/binning/classified with the Snakemake pipeline Avary v0.10.0 with MAG quality assessed using CheckM2 per MIMAG criteria; functional annotation used SeqScreen v4.4 with custom R scripts, and Lemur/Magnet were used to verify taxa not recovered as bins. LC-MS/MS chromatograms were integrated in Agilent MassHunter Quantitative software and then normalized/transformed as described (sum-normalization, log transform, autoscaling) prior to statistical testing; custom R and bash scripts supported data wrangling, figure generation, and functional comparisons, with code available at villapollab.github.io/adult_abx_tbi/.

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](#)

Data availability.

All materials generated in this study are available from the lead contact upon request. Further information and requests for resources and reagents should be directed to the lead contact, Dr. Villapol (svillapol@houstonmethodist.org), who will fulfill them.

Code availability.

The 16S RNA sequencing and metagenomic profiling datasets generated in this study are available in the NCBI SRA database under BioProject numbers PRJNA1104663 (raw 16S sequencing data and MAG assemblies) and PRJNA1216228 (raw metagenomic sequencing data). The code used for the amplicon and metagenomic analysis can be found in the document on our GitHub page: https://villapollab.github.io/adult_abx_tbi/.

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

Sample sizes were selected a priori based on our prior experience with the CCI model and antibiotic (ABX) paradigms, expected effect sizes/variability for the primary endpoints (lesion volume and acute neuroinflammatory measures), and consistency with closely related CCI-microbiome studies; a formal prospective power analysis was not performed because this multi-endpoint design includes outcomes with different variance structures (histology, immunostaining, longitudinal microbiome metrics, and metabolites), so we instead used historical variance and pilot observations to choose group sizes that reliably detect biologically meaningful differences with the planned tests (t-tests).

and one-/two-way ANOVA with appropriate post hoc comparisons). This resulted in 7-10 mice/group for the main CCI/ABX experiments and 4-5 mice/group for the germ-free cohort. These sample sizes are sufficient because CCI typically produces robust, high-signal effects on lesion and glial outcomes, precision was increased by averaging technical replicates within each mouse (multiple sections/fields per brain region; qPCR technical triplicates) to yield one biological value per animal, and key findings were supported by concordant effects across independent readouts (neuropathology, cytokine signals, microbiome composition/diversity, and SCFAs).

Data exclusions	Data exclusions were predefined and limited to technical/quality-control reasons. Animals were excluded if they did not meet the a priori CCI inclusion criteria (righting reflex time >5 min and appropriate post-injury responsiveness) or had procedural complications. For histology, brains were excluded from specific analyses (e.g., TUNEL) when tissue integrity was compromised by section drying or loss during mounting, preventing reliable quantification (typically 1–3 animals per group for that endpoint). For microbiome assays, fecal samples were excluded when DNA extraction yielded undetectable bacterial DNA, precluding valid downstream quantification or sequencing. Exclusions were applied uniformly across groups and were not based on the direction or magnitude of results.
Replication	Reproducibility was ensured through rigorous design and transparent analysis: mice were randomly assigned to groups and experimenters were blinded during data collection and quantification; protocols were standardized (fixed CCI and ABX regimens, defined sampling timepoints, and consistent region-of-interest/field selection). Technical replicates (e.g., qPCR triplicates and multiple sections/fields per mouse) were averaged to one value per biological replicate (one mouse) before statistics. Key findings were confirmed across independent, orthogonal endpoints (lesion and cell death, glial activation, immune infiltration, microbiome diversity/composition, and serum SCFAs), and germ-free status was verified by 16S qPCR. Finally, reproducible, versioned computational pipelines were used, with raw sequencing data deposited in NCBI SRA and analysis code shared via GitHub to allow independent reanalysis.
Randomization	Mice were acclimated after arrival and then randomly assigned to experimental conditions. Conventional C57BL/6J males were allocated to receive either the 3-day oral antibiotic cocktail (ABX) or vehicle (water), and then to injury conditions (sham, single CCI [1xTBI], or repeated CCI [2xTBI] with the second injury 38 days after the first); an additional control group consisted of 2xTBI mice treated with vehicle for direct comparison with 2xTBI-ABX. Germ-free and matched wild-type mice were allocated to the acute CCI cohort using the same injury parameters. Across all groups, investigators were blinded to treatment during outcome acquisition and quantification.
Blinding	Investigators were blinded to group allocation throughout data collection and outcome assessment: mice were randomly assigned to experimental conditions, and experimenters remained blinded to treatment/injury groups during behavioral testing, sample collection, imaging acquisition, and all quantitative analyses until after datasets were finalized for statistical testing.

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

Antibodies

Antibodies used	Please see Supplemental Table 1. Primary antibodies used for immunofluorescence included rabbit anti-Iba-1 (Wako; 1:500), mouse anti-CD68 (Bio-Rad; 1:200), rat anti-F4/80 (R&D Systems; 1:200), rabbit anti-P2Y12 (AnaSpec; 1:500), rabbit anti-GFAP (Millipore; 1:500), and rat anti-Ly6B2 (Bio-Rad; 1:500); corresponding Alexa Fluor 488- and 568-conjugated IgG secondary antibodies were from Thermo Fisher Scientific (1:1000). The Methods state that complete reagent identifiers, including supplier, catalog numbers, lot numbers, host species, and working dilutions, are provided in Supplementary Table 1.
Validation	Primary antibodies were validated for mouse brain immunofluorescence using a combination of vendor-supported use in mouse tissue, expected cell-type-specific localization patterns (Iba-1/CD68/P2Y12 for microglia, GFAP for astrocytes, F4/80 and Ly6B2 for infiltrating myeloid cells), and in-study specificity controls. Negative controls, including omission of primary antibody and/or species- and isotype-matched controls, produced no specific signal, supporting antigen-dependent staining rather than nonspecific background.

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	Laboratory animals used in this study were mice (<i>Mus musculus</i>). Conventional animals were C57BL/6J male mice aged 14 weeks (Jackson Laboratories). Germ-free animals were C57BL/6 germ-free (C57BL/6-GF) male mice aged 12 weeks (Baylor College of Medicine Gnotobiotic Rodent Facility).
Wild animals	N/A
Reporting on sex	Findings in this study apply only to males, because the experiments were conducted exclusively in young adult male C57BL/6 mice (including the germ-free cohort). Sex was considered at the study-design level by intentionally restricting the work to males to reduce variability and because prior evidence indicates males can exhibit a more pronounced acute neuroinflammatory response after CCI (e.g., greater microglial activation and peripheral myeloid recruitment); however, the design did not include sex as a biological variable, no female groups were enrolled, and no sex-by-treatment or sex-by-injury analyses were planned. Accordingly, sex-disaggregated data cannot be provided, because all source data are male-only. The manuscript appropriately notes this as a limitation and indicates that future studies should test whether the observed ABX–microbiome–neuroinflammation relationships generalize to females and across the lifespan.
Field-collected samples	These were not field-collected samples; all samples were obtained from laboratory-housed mice. Conventional C57BL/6J male mice were maintained at the Houston Methodist Research Institute animal facility under a standard 12-hour light/dark cycle with ad libitum access to food and water, and were allowed 1 week of acclimatization after arrival prior to any procedures. Germ-free C57BL/6-GF male mice were sourced from the Baylor College of Medicine Gnotobiotic Rodent Facility and maintained under gnotobiotic conditions, with germ-free status verified by 16S qPCR on fecal material from transfer crates upon arrival. Specific environmental parameters such as room temperature, humidity, cage type/bedding, enrichment, and diet formulation were not reported in the text provided.
Ethics oversight	The Animal Care and Use Committee of Houston Methodist Research Institute, Houston, TX, USA, approved all animal experiments in the study.

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