

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	Data collection was performed through the Microsetta Initiative platform (https://microsetta-rest.ucsd.edu) and country-specific food frequency questionnaires, including VioScreen version 5 for the US and UK cohorts, VioScreen version 5-Mex for the Mexico cohort, the PREDIMED FFQ for the Spain cohort, and a digitized JPHC-NEXT FFQ for the Japan cohort.
Data analysis	Shotgun metagenomic data were processed through Qiita using the Qiita admin processing workflow. Adapter trimming was performed with fastp, human-read filtering with minimap2 against GRCh38.p14, T2T-CHM13v2.0, and Human Pangenome Reference Consortium release 1, and metagenomic reads were mapped with Bowtie2 v2.5.4 against Web of Life release 2 using the SHOGUN parameter set. Feature tables were generated with woltk v0.1.7, and microbiome coverage was calculated with micov. Metagenome-assembled genomes were generated using MaxBin 2 v2.27, MetaBAT 2 v2.18, and CONCOCT v1.10, refined with DAS_Tool, assessed with CheckM, dereplicated with dRep v2.2.4, and classified with GTDB-Tk v2.4.3 against GTDB release 214. Statistical and machine-learning analyses were conducted using Python and R packages/tools including gemelli v0.0.9, scikit-learn v0.24.1, scikit-bio v0.5.7, cca-zoo v2.6.0, BIRDMAN v0.1.0, SciPy v1.10.1, vegan/adonis2 in R, panpiper-kit, Mash, pyseer v1.4.0, unitig-caller, Bakta, and iTOL version 7. Analyses included PCA/RPCA, PERMANOVA/adonis2, sparse canonical correlation analysis, Kruskal–Wallis tests, chi-squared tests, ANOVA, Pearson and Spearman correlations, Elastic Net regression with 5-fold cross-validation, Bayesian differential abundance modeling, unitig-based GWAS, and Benjamini–Hochberg false discovery rate correction.

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 reads are available through the European Bioinformatics Institute (EBI) (accession number: PRJEB11419). The per-sample-FASTQ files, processed tables, and metadata can be found in Qiita study ID #10317 (<https://qiita.ucsd.edu/study/description/10317>; Prep IDs: 16766, 16762, 16761, 16773, 16780, 16756, 16842, 16848, 16849, 16853, 16841, 16854, 16875). Dietary intake data used in this study are provided as Supplementary Data.

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

The present study included participants of both sexes, as determined by self-report. Gender identity was not collected. Differences in sex distribution across countries were tested and are reported in Table 1. Sex was included as a covariate in analyses where appropriate, including ADONIS, and sensitivity analyses stratified by sex were also conducted where appropriate.

Reporting on race, ethnicity, or other socially relevant groupings

Racial identity was collected through self-report but was not included in the analyses presented in this manuscript. Because the study focuses on cross-cultural differences across geographic regions, consideration of racial and ethnic identity was not feasible, as these measures were highly correlated with the region in which samples were collected. For example, 95% of participants in Japan reported Asian or Pacific Islander identity, and 93% of participants in Mexico reported Hispanic identity.

Population characteristics

Covariate-relevant population characteristics are described in Table 1 and in the Results section of the manuscript. The study included 1,976 participants, of whom 1,482 were female; the average age was 45.5 years. Briefly, demographic variables differed significantly across countries, with the most pronounced differences observed in cosmetics use, BMI, and age distributions.

Recruitment

Participants were recruited from a pool of prior American Gut Project/The Microsetta Initiative participants and individuals who had previously expressed interest in the project, as well as through social media outreach across all participating countries. In Mexico, in-person recruitment was additionally conducted through INCMNSZ. We acknowledge that these recruitment strategies are subject to self-selection bias, as participants were more likely to have a pre-existing interest in the study topic or related areas such as nutrition which limits the conclusions of the study.

Ethics oversight

The study was approved by the University of California San Diego Institutional Review Board, under the University of California San Diego Human Research Protections Program (Protocol #141853). The Mexico cohort was additionally approved by the Comité de Ética en Investigación of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) (Protocol #3853). All participants provided written informed consent.

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 size was determined by the availability of eligible participants with paired stool samples and harmonized dietary metadata across the five participating cohorts. No a priori power calculation was performed, as this study leverages an observational, multi-cohort design with pre-existing data rather than a hypothesis-driven experimental intervention. The final sample size (N = 1,976 across five countries) is comparable to or exceeds those of prior population-scale metagenomic studies examining diet-microbiome associations and provides sufficient variability within and across cohorts to detect robust, cross-cultural patterns.

Data exclusions

Only samples with complete information, including FFQ data, were used for sequencing and analysis. Further, quality control steps included removing blank and non-THDMI samples. Samples with invalid entries in any of the following metadata fields were excluded: thdmi_cohort, host_age, sex, antibiotic_history, covid_level_of_wellbeing, and host_body_mass_index. We further removed samples missing dietary information. An additional 58 samples from Mexico were excluded due to technical bias introduced by re-sequencing following low read quality. Finally, samples with feature sums less than or equal to 1,000,000 reads were filtered out.

Replication	All analyses were conducted using version-controlled code, with fully specified software environments and package versions provided to enable exact reproduction of results. Analyses were repeated across multiple cohorts and countries, allowing assessment of consistency of observed patterns across independent populations. Where applicable, models were re-run with alternative preprocessing choices and covariate adjustments to confirm that primary findings were not driven by specific analytic decisions. All key results reported in the manuscript were reproducible using the provided code and data inputs. No analyses central to the main conclusions failed replication.
Randomization	This study is observational, and participants were not randomly allocated to experimental groups. Groups were defined by country of residence and associated dietary characteristics. Potential confounding was addressed through covariate adjustment and, where appropriate, stratified or country-specific analyses. Randomization was not applicable to the study design.
Blinding	Blinding was not applicable, as this study is observational and did not involve experimental interventions or subjective outcome assessments requiring blinding.

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	<i>Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.</i>
Validation	<i>Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.</i>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	<i>State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.</i>
Authentication	<i>Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.</i>
Mycoplasma contamination	<i>Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.</i>
Commonly misidentified lines (See ICLAC register)	<i>Name any commonly misidentified cell lines used in the study and provide a rationale for their use.</i>

Palaeontology and Archaeology

Specimen provenance	<i>Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.</i>
Specimen deposition	<i>Indicate where the specimens have been deposited to permit free access by other researchers.</i>
Dating methods	<i>If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.</i>
☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance</i>

was required and explain why not.

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	<i>For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.</i>
Wild animals	<i>Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.</i>
Reporting on sex	<i>Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.</i>
Field-collected samples	<i>For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.</i>
Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	<i>Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.</i>
Study protocol	<i>Note where the full trial protocol can be accessed OR if not available, explain why.</i>
Data collection	<i>Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.</i>
Outcomes	<i>Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.</i>

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

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	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

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 <i>May remain private before publication.</i>	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.
Files in database submission	Provide a list of all files available in the database submission.
Genome browser session (e.g. UCSC)	Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

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 *Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
- Instrument *Identify the instrument used for data collection, specifying make and model number.*
- Software *Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
- Cell population abundance *Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
- Gating strategy *Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*
- ☐ 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 *Indicate task or resting state; event-related or block design.*
- Design specifications *Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
- Behavioral performance measures *State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

- Imaging type(s) *Specify: functional, structural, diffusion, perfusion.*
- Field strength *Specify in Tesla*
- Sequence & imaging parameters *Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
- Area of acquisition *State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*
- Diffusion MRI ☐ Used ☐ Not used

Preprocessing

- Preprocessing software *Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
- Normalization *If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
- Normalization template *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
- Noise and artifact removal *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*
- Volume censoring *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

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

Statistic type for inference

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See [Eklund et al. 2016](#))

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

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

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.