nature portfolio

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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	Cell sorter BD FACS Melody (BD, Germany) equipped with BD FACSChorus software (BD) were used to generate and collect data for FACS sorting. Raman microspectroscope (LabRAM HR800, Horiba Scientific, France) equipped with a 532 nm neodymium-yttrium aluminum garnet (Nd:YAG) laser and 300 grooves/mm diffraction grating was used to collect data after heavy water incubatios. Spectra were acquired with the software LabSpec 6 in the range of 400–3,200 cm−1 for 30 s and for each sample 35-40 individual cells were measured. Cells of interest were identified and sorted using a platform that combines the Raman microspectroscope (532 nm at 90 mW), optical tweezers (1,064 nm Nd:YAG laser at 500 mW), and a polydimethylsiloxane (PDMS) microfluidic device. Anaerobic incubations were performed in an anaerobic tent (Coy, 85% N2, 10% CO2, 5% H2). To measure Od600, a plate reader (MultiskanTM GO Microplate Spectrophotometer, Thermo Fisher Scientific) placed in an anaerobic tent using the SkanIt software (Thermo Fisher Scientific) was used. The OD600 was recorded automatically every 30 min for a total of 120 h with a shaking of 5 s between readings. Data for sugars utlilization were measured by HPTLC silica gel 60 plates (Merck KGaA, Germany) using a Linomat 5 (Camag AG, Switzerland). and by HPAEC (Dionex ICS 3000, ThermoFisher, Germany). Microscopy images were collected with Confocal LSM Zeiss 710 equipped with ELYRA PS, Leica TCS SP8X, Mannheim, Germany and SEM images were obtained using a FEI Verios 460 field emission scanning electron microscope Matlab code for Raman microspectroscopy has been uploaded in the revised submission.
Data analysis	All software used for analysis has been properly cited in the Methods. Statistical analysis was performed using R statistical software (https://www.r-project.org/) Version 1.4.1106 and GraphPad Prims version 7 (www.graphpad.com). Statistical analysis to compare samples groups

was performed using ANOVA, Student's t-test, and the R package DESeq2 (v.1.30.1) that estimate variance-mean dependence in count data from high-throughput sequencing assays and test for differential expression based on a model using the negative binomial distribution. The statistical significance of factors affecting microbiota composition and differences between time points was evaluated using non-parametric permutational multivariate analysis of variance (PerMANOVA), significant clustering of groups was evaluated with analysis of similarities (ANOSIM), ordination was performed using principal component analysis (PCoA) and non-metric multidimensional scaling (NMDS) in the vegan package (v.2.5-7) (https://cran.r-project.org/web/packages/vegan/vegan.pdf). Jackknife diversity index and Bray-Curtis distances were also calculated in the vegan package.

MATLAB GUI (graphical user interface, version 4.2) software for the operation of the RACS platform is provided in Supplementary Files 1 and 2.

SILVA database SSU Ref NR 99 release 138 (https://doi.org/10.5281/zenodo.3986799) was used for sequence classification and IQtree86 was used for phylogenetic reconstruction.

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

16S rRNA gene amplicon sequence data from FACS and RACS experiments has been deposited in the NCBI Short Read Archive under PRJNA718139 (https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA718139). Eggerthella lenta 6.2 and Gordonibacter urolithinfaeciens AL-11 genomes were deposited at NCBI under accession JAIPUQ000000000 and JAIPUP000000000 respectively and under PRJNA718139. 16S rRNA gene sequences data of the strains isolated with RACS after inulin supplementation have been deposited in GenBank with accession numbers OK067598-OK067669 and RACS strains sequences isolated after XOS supplementation have been deposited in GenBank with accession numbers OP183499-OP183547 (https://www.ncbi.nlm.nih.gov/genbank/). MATLAB GUI (graphical user interface, version 4.2) software for the operation of the RACS platform is provided in Supplementary Files 1 and 2.

Source data are provided with this paper. All data submitted are publicly available.

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	Sex and gender were not considered for inclusion in the present study. We report in the manuscript that we had roughly equal number of females (13) and males (10).
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, and other socially-relevant groupings were not considered in the present study, and there was no reporting in the manuscript about them.
Population characteristics	23 healthy subjects (13 females and 10 males, age mean ± sd :29.3 ± 5.6; BMI:mean ± sd: 23.2 ± 3.0) were enrolled in this study. Donors that consumed antibiotic or food supplements containing probiotic/prebiotic in the previous six months as well as individuals suffering from chronic or acute intestinal disease were excluded from the study.
Recruitment	Participants were recruited based on public advertisement of the study. Written informed consent was signed by all enrolled participants. All stool samples were self-collected using a fecal collection tube, transported anaerobically and immediately used for experiment. No bias were present in our enrollment and sample collection.
Ethics oversight	The study was approved by, and conducted in accordance, with the University of Vienna ethics committee (reference number 00161) and written informed consent was signed by all enrolled participants.

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	Fecals samples were collected from 23 healthy subjects. The number was chosen based on feasibility, as suitable sample size calculations were not available.

Data exclusions	Data was excluded from amplicon sequencing datasets if ASVs were more abundant in negative controls, as described in the Methods
Replication	Technical replicates (ie, duplicates or tripplicates) were included in the study to ensure reproducibility. All replicates were successful.
Randomization	Allocation was random.
Blinding	Blinding was not relevant to the study and investigator knowledge would not be expected to bias the outcome.

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

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, mosiacism, off-target gene editing) were examined.*

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	For binding experiments, 6 fecal samples were incubated anaerobically in the presence of fluorescently labeled mesoporous silica nanoparticles (MSNs) grafted with inulin. Fecal samples were incubated in the presence of MSNs at 37 °C for 1 h under anaerobic conditions. After the incubation time, samples were counter-stained with 4, 6-diamidino-2-phenylindole (DAPI) and used immediately for FACS. For activity based experiments, 11 fecal samples were incubated in the presence of inulin and 6 fecal samples in the presence of XOS and the activity marker L-azidohomoalanine (AHA). Samples were incubated in an anaerobic tent at 37°C for 6 h. After incubation samples were fixed in ethanol : PBS (1:1). Cu(I)-catalyzed click labelling of chemically fixed microbial cells was performed in solution immeditely before FACS.
Instrument	BD FACS Melody (BD, Germany).
Software	BD FACSChorus software (BD, Germany) and the R package flowCore (doi:10.1186/1471-2105-10-106).

Cell population abundance	500,000 events were sorted for each sample. Analysis of the samples showed a purity of >99%.
Gating strategy	Background noise of the machine and of PBS was detected using the parameters forward scatter (FSC) and side scatter (SSC). Bacteria were then displayed using the same settings in a scatter plot using the forward scatter (FSC) and side scatter (SSC) and pre-gated. Singlets discrimination was performed. For binding experimts, MSNs resuspend in PBS and a sample incubated in the same conditions without MSNs but stained with DAPI, were used to set the gate for rhodamine positive signal. Rhodamine-DAPI positive signal corresponding to the MSNs with bacteria attached, were then sorted out into tubes. For BONCAT experiments, the same settings were applied as described above. Cy5 negative control bacteria were displayed to set the gate threshold. Gated Cy5 positive bacteria were then sorted.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.