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Reporting Summary

Nature Research 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 Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main

Statistical parameters

text	, or N	Aethods section).
n/a	Cor	firmed
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
\boxtimes		An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
\boxtimes		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.
\boxtimes		A description of all covariates tested
\boxtimes		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	\boxtimes	A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)
\boxtimes		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>
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes		Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated
	\boxtimes	Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

 Policy information about availability of computer code

 Data collection
 Nanopore signal data was basecalled using Metrichor, Albacore or Guppy and fastq files were generated using Albacore or poretools.

 Data analysis
 Sequencing data was assembled using metaSPAdes, IDBA-UD, MEGAHIT, Canu, and OPERA-MS (https://github.com/CSB5/OPERA-MS). The assemblies were further processed and analyzed using the following software: Pilon, Racon, MetaQUAST, CheckM, BLASR KAAS, MUSCLE, PhyML, blastn and MUMmer.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Behavioural & social sciences

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 list of figures that have associated raw data
- A description of any restrictions on data availability

The GIS20 mock community sequencing data can be obtained from the European Nucleotide Archive (ENA) under project ID PRJEB29139 (Illumina, PacBio and ONT) and sequencing data for the 28 gut metagenomes can be found under project ID PRJEB29152 (Illumina and ONT).

Ecological, evolutionary & environmental sciences

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

X Life sciences

Data

For a reference copy of the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	(n/a
Data exclusions	No sequencing data was excluded from any analysis.
Replication	No technical replicates were generated, but abundance profiles derived from different sequencing technologies (Illumina, PacBio and Nanopore) were compared and showed strong correlation.
Randomization	n/a
Blinding	n/a

Reporting for specific materials, systems and methods

Materials & experimental systems		Methods		
n/a Involved in the study	n/a	Involved in the study		
Unique biological materials	\ge	ChIP-seq		
Antibodies	\ge	Flow cytometry		
Eukaryotic cell lines	\times	MRI-based neuroimaging		
Palaeontology				
Animals and other organisms				
Human research participants				
Unique biological materials				

Policy information about availability of materials

Obtaining unique materials

The GIS20 mock community was constructed using publicly available strains from ATCC and DSM as described in Supplementary Table 3

Human research participants

Policy information about studies involving human research participants

Population characteristics n/a Recruitment

Any potential biases in recruitment are unlikely to affect any of the conclusions presented in this study.