# 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.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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n/a	Con	firmed
	×	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.
x		A description of all covariates tested
	×	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	x	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)
	x	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>
x		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
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#### Software and code

Policy information about availability of computer code

Data collection

No software was used.

Data analysis

GeneMarkS-2 v1.10; CheckM v1.1.2; Prokka 1.14.6; fastANI v1.32; GTDB-Tk v2.1.0; Kraken v2.1.2; Bracken v2.5; iTOL v6.1.1; dbCAN v2.0; anti-SMASH v4.2.0; BiG-SCAPE/CORASON tool v1.0.1; Cytoscape v3.8.2; VirSorter v1.0.5; CheckV v0.7.0; Prodigal v2.6.3; VConTACT2 v0.9.19; MAFFT v7.407; FastTree v2.1.3; Mummer v3.22; lastz v1.03.73; TreeBeST v1.9.2; BLAST v2.2.26

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 <u>data availability statement</u>. 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

The genome data generated in this study have been deposited into CNSA of CNGBdb with accession number CNP000126 and CNP0001833, and NCBI under the projects PRJNA482748 and PRJNA903559. All the bacterial strains in CGR2 have been deposited in China National GeneBank (CNGB), a non-profit, public-service-oriented organization in China. The strain information, including taxonomy, donor, and culture conditions can be found and accessed through https://db.cngb.org/

codeplot/datasets/CGR2

The databases used in this study include GTDB database Release 07-RS207 (https://data.gtdb.ecogenomic.org/releases/release207/), dbCAN-PUL (https:// bcb.unl.edu/dbcan\_pul/Webserver/static/DBCAN-PUL/), KEGG database (https://www.genome.jp/kegg/), MIBiG version 1.4 (https:// mibig.secondarymetabolites.org/), checkv-db-v0.6(https://portal.nersc.gov/CheckV/), and Genebank (www.ncbi.nlm.nih.gov/genbank/);

The datasets used in this study include human gut metagenome sequencing data of a Chinese cohort (a part of 4D-SZ) (https://db.cngb.org/search/project/ CNP0000426/), HMP (https://portal.hmpdacc.org/), the Netherlands cohort (https://ega-archive.org/studies/EGAS00001005027), UHGG (http://ftp.ebi.ac.uk/pub/ databases/metagenomics/mgnify\_genomes), BIO-ML (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA544527), hGMB (https://www.ncbi.nlm.nih.gov/bioproject/ PRJNA656402), MGV (https://portal.nersc.gov/MGV/), and GPD (https://ftp.ebi.ac.uk/pub/databases/metagenomics/genome\_sets/gut\_phage\_database/).

#### Human research participants

Reporting on sex and gender

Policy information about studies involving human research participants and Sex and Gender in Research.

This study recruited 299 participants from China, including 4 infants (aged 0-1), 7 children (aged 1-10), 30 teenagers (aged Population characteristics

11-20), 194 adults (aged 21-50), and 64 elders (aged >50).

Sex / gender was not included in our study design and analysis.

Recruitment We publish recruitment information through posters, and participants sign up voluntarily. All participants were recruited in ShenZhen, China; Fecal samples were collected from 299 healthy donors not taking any drugs during the last months prior to

sampling. No additional volunteer selection criteria.

Ethics oversight The collection of the 299 samples was approved by the Institutional Review Board on Bioethics and Biosafety of BGI.

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 a	are not sure, read the appropriate sections before making your selection
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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.

This study collected 299 feces, isolated ~20,000 bacterial isolates, and generated 3324 high quality genomes. No sample size calculation was performed, but the sample size and amount of generated sequence data are larger than currently published studies of similar

Sample size

Replication

Data exclusions genomes with < 90% completeness or > 10% contamination were excluded. Quality controls used to exclude genomes were based on

previously published criteria.

Genomic data are publicly available, so analyzes can be reproduced using the data and software described in the Methods.

Randomization is applied when we select strains from the same cluster for sequencing based on a threshold of 98.7% identity of the 16S rRNA Randomization

gene sequence.

Blinding Blinding is not necessary for this study, because is not influenced by the subjective factors of the subjects or researchers.

# 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 Methods n/a Involved in the study x Antibodies x ChIP-seq x Flow cytometry x Animals and other organisms x Clinical data

Dual use research of concern