

1,520 reference genomes from cultivated human gut bacteria enable functional microbiome analyses

Yuanqiang Zou^{1,2,3,13}, Wenbin Xue^{1,2,13}, Guangwen Luo^{1,2,4,13}, Ziqing Deng^{1,2,13}, Panpan Qin^{1,2,5,13}, Ruijin Guo^{1,2}, Haipeng Sun^{1,2}, Yan Xia^{1,2,5}, Suisha Liang^{1,2,6}, Ying Dai^{1,2}, Daiwei Wan^{1,2}, Rongrong Jiang^{1,2}, Lili Su^{1,2}, Qiang Feng^{1,2}, Zhuye Jie^{1,2}, Tongkun Guo^{1,2}, Zhongkui Xia^{1,2}, Chuan Liu^{1,2,6}, Jinghong Yu^{1,2}, Yuxiang Lin^{1,2}, Shanmei Tang^{1,2}, Guicheng Huo⁴, Xun Xu^{1,2}, Yong Hou^{1,2}, Xin Liu^{1,2,7}, Jian Wang^{1,8}, Huanming Yang^{1,8}, Karsten Kristiansen^{1,2,3,9}, Junhua Li^{1,2,10*}, Huijue Jia^{1,2,11*} and Liang Xiao^{1,2,6,9,12*}

Reference genomes are essential for metagenomic analyses and functional characterization of the human gut microbiota. We present the Culturable Genome Reference (CGR), a collection of 1,520 nonredundant, high-quality draft genomes generated from >6,000 bacteria cultivated from fecal samples of healthy humans. Of the 1,520 genomes, which were chosen to cover all major bacterial phyla and genera in the human gut, 264 are not represented in existing reference genome catalogs. We show that this increase in the number of reference bacterial genomes improves the rate of mapping metagenomic sequencing reads from 50% to >70%, enabling higher-resolution descriptions of the human gut microbiome. We use the CGR genomes to annotate functions of 338 bacterial species, showing the utility of this resource for functional studies. We also carry out a pan-genome analysis of 38 important human gut species, which reveals the diversity and specificity of functional enrichment between their core and dispensable genomes.

The human gut microbiota refers to all the microorganisms that inhabit the human gastrointestinal tract. Diverse roles of the gut microbiota in human health and disease have been recognized^{1,2}. Metagenomic studies have transformed our understanding of the taxonomic and functional diversity of human microbiota, but more than half of the sequencing reads from a typical human fecal metagenome cannot be mapped to existing bacterial reference genomes^{3,4}. The lack of high-quality reference genomes has become an obstacle for high-resolution analyses of the human gut microbiome.

Although the previously reported Integrated Gene Catalog (IGC) has enabled metagenomic, metatranscriptomic and metaproteomic analyses^{3,5,6}, the gap between compositional and functional analyses can only be filled by individual bacterial genomes. Genes that co-vary among samples can be clustered into metagenomic linkage groups⁷, metagenomic clusters⁸ and metagenomic species^{9,10}, whose annotation depends on alignment to the limited number of existing reference genomes. Other metagenomics-based analyses of the gut microbiome—for example, single nucleotide polymorphisms (SNPs), indels and copy number variations—rely on the coverage and quality of reference genomes^{11–13}.

Despite the rapid increase in the number of sequenced bacterial and archaeal genomes, reference genomes for gut bacteria are underrepresented. It is estimated that <4% of the bacterial genomes in the US National Center for Biotechnology Information (NCBI)

database belong to the human gut microbiota. Rather, the focus has been on clinically relevant pathogenic bacteria, which are over-represented in the microbial databases. The first catalog of 178 reference bacterial genomes for the human microbiota was reported by the Human Microbiome Project (HMP)¹⁴ in 2010. To date, the HMP has sequenced >2,000 microbial genomes cultivated from human body sites, 437 of which are gut microbiota (data accessed 8 September 2017). However, the number of reference gut bacterial genomes is still far from saturated.

We present a reference catalog of genomes of cultivated human gut bacteria (named the CGR), established by culture-based isolation of >6,000 bacterial isolates from fecal samples of healthy individuals. The CGR comprises 1,520 nonredundant, high-quality draft bacterial genomes, contributing at least 264 new reference genomes to the gut microbiome. After inclusion of CGR genomes, the mapping rate of selected metagenomic datasets improved from around 50% to over 70%. In addition to improving metagenomic analyses, the CGR will improve functional characterization and pan-genomic analyses of the gut microbiota at high resolution.

Results

Expanded catalog of gut bacterial genomes. We obtained 6,487 bacterial isolates from fresh fecal samples donated by 155 healthy volunteers by using 11 different media under anaerobic

¹BGI-Shenzhen, Shenzhen, China. ²China National Genebank, BGI-Shenzhen, Shenzhen, China. ³Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, Copenhagen, Denmark. ⁴Key Laboratory of Dairy Science, College of Food Sciences, Northeast Agricultural University, Harbin, Heilongjiang, China. ⁵BGI Education Center, University of Chinese Academy of Sciences, Shenzhen, China. ⁶Shenzhen Engineering Laboratory of Detection and Intervention of Human Intestinal Microbiome, Shenzhen, China. ⁷BGI-Qingdao, BGI-Shenzhen, Qingdao, China. ⁸James D. Watson Institute of Genome Sciences, Hangzhou, China. ⁹Qingdao-Europe Advanced Institute for Life Sciences, Qingdao, China. ¹⁰School of Bioscience and Biotechnology, South China University of Technology, Guangzhou, China. ¹¹Macau University of Science and Technology, Taipa, Macau, China. ¹²Department of Digestive Diseases, Huashan Hospital of Fudan University, Shanghai, China. ¹³These authors contributed equally: Yuanqiang Zou, Wenbin Xue, Guangwen Luo, Ziqing Deng, Panpan Qin. *e-mail: lijunhua@genomics.cn; jiahuijue@genomics.cn; xiaoliang@genomics.cn

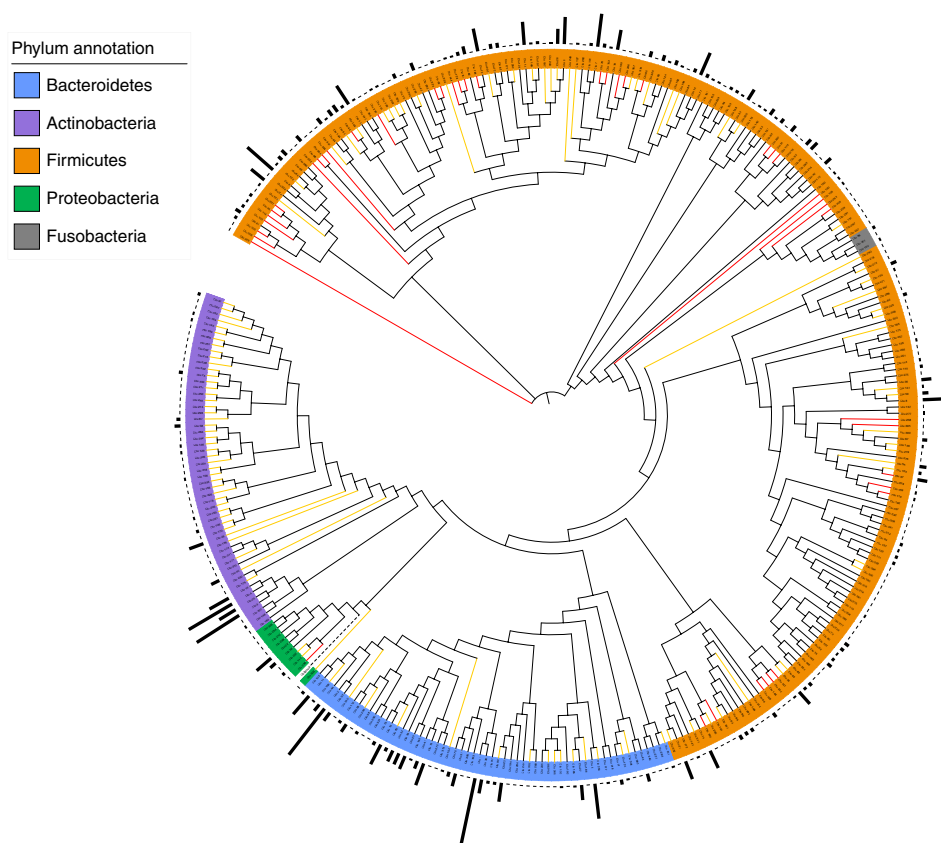


Fig. 1 | Phylogenetic tree of 1,520 isolated gut bacteria based on whole-genome sequences. The 1,520 high-quality genomes in CGR are classified into 338 species-level clusters (ANI \geq 95%) based on their whole-genome sequences. Bacterial species from Firmicutes are colored in orange; Bacteroidetes, blue; Proteobacteria, green; Actinobacteria, violet; Fusobacteria, gray. Novel genera and species are highlighted by red and orange branches, respectively. The bar on the outermost layer indicates the number of genomes archived in each cluster. *Rhizobium selenitireducens* ATCC BAA 1503 was used as an outgroup for phylogenetic analysis.

conditions (Supplementary Fig. 1a and Supplementary Table 1). Notably, more than half of the isolates were cultured from MPYG medium (Supplementary Fig. 1b). All the isolates were subjected to 16S rRNA gene amplicon sequencing analysis, and 1,759 nonredundant isolates that provided broad coverage of the phylogenetic tree were selected for whole-genome sequencing (Supplementary Fig. 1c and Supplementary Table 2). After de novo assembly of the next-generation sequencing reads, we identified 104 isolates that contained more than one genome. These assembled sequences were then parsed into 212 genomes using our in-house pipeline (Supplementary Table 3). Briefly, multi-genomes were split at scaffold level on the basis of G + C content versus sequencing depth. The closest reference genomes for the split scaffolds were determined on the basis of average nucleotide identity (ANI), and the mis-split scaffolds were mapped back to their closest reference genome to get the final split genome (see Methods). In total, we obtained a collection of 1,867 newly assembled genomes, 1,520 (81.4%) of which fulfilled the HMP's criteria for high-quality draft genomes and exceeded 95% genome completeness and less than 5% contamination as evaluated by CheckM. The genome sizes and G + C contents of CGR ranged from 0.2 to 7.9 Mbp and 26.56% to 64.28%, respectively. A total of 5,749,641 genes were predicted from the annotation data (Supplementary Table 4).

Taxonomic annotation of CGR was carried out using a self-constructed, efficient ANI-based pipeline (Supplementary Fig. 2). The 1,520 high-quality genomes were classified into 338 species-level clusters (ANI \geq 95%, a species delineation corresponding to 70% DNA–DNA hybridization), which covered all the major phyla of the human gut microbiota, including Firmicutes (211 clusters, 796

genomes), Bacteroidetes (60 clusters, 447 genomes), Actinobacteria (54 clusters, 235 genomes), Proteobacteria (10 clusters, 36 genomes) and Fusobacteria (3 clusters, 6 genomes) (Fig. 1a and Supplementary Table 5). Among these 338 clusters, 134 clusters (corresponding to 264 genomes) were not annotated to any present reference genomes in NCBI (Fig. 1a), and 50 clusters did not fall within any sequenced genera (Supplementary Table 5). To corroborate the presence of novel species in CGR, we carried out additional taxonomic identification using 16S rRNA gene analysis. A species was recognized as novel if its 16S rRNA gene sequence had < 98.7% similarity with known species in the EzBioCloud database (see Methods). Overall, we identified 350 distinct bacterial species (based on operational taxonomic units), including 149 candidate novel species, 42 of which represent candidate novel genera. These results underscore the value of the individual reference genomes provided by the CGR.

Despite the variation of individual microbiota at the genus level, the CGR identified bacterial populations with broad diversity, covering eight out of nine core genera in the Chinese gut microbiota¹⁵. More than 80 species were novel in comparison with the previously sequenced species from a reported 1,000 cultured bacterial species from the human gastrointestinal tract¹⁶ (Supplementary Fig. 3a). Moreover, the CGR successfully identified 38 genera that were of low relative abundance (< 1%) according to the IGC⁶, which is a large catalog of reference genes derived from a collection of ~1,250 metagenomic samples from individuals on three continents (Supplementary Fig. 3b). Among them, 7 genera were identified with more than 20 genomes (*Bifidobacterium*, *Collinsella*, *Coprobacillus*, *Dorea*, *Streptococcus*, *Prevotella* and *Parabacteroides*). The CGR also identified another 9 genera that were not detected

by IGC⁶ (*Butyricicoccus*, *Butyricimonas*, *Catenibacterium*, *Dielma*, *Erysipelatoclostridium*, *Megamonas*, *Melissococcus*, *Peptoclostridium* and *Vagococcus*) (Supplementary Fig. 3b). These results underscore the contribution of the CGR to the existing database of gut bacterial whole genomes.

Improvement in metagenomic and SNP analyses. The existing reference genomes for metagenomic sequence mapping are far from saturated. For example, the genomes or draft genomes of bacteria and archaea used in a recent study allowed mapping of less than half of the sequences in the fecal metagenome^{3,4}. To illustrate the value of the CGR to metagenomic analyses, we performed sequence mapping using previous metagenomic data⁶ with or without CGR. For Chinese samples, the read mapping rate in the original study that used the IGC dataset (3,449 reference genomes from IGC⁶) was 52.00%, which was significantly improved to 76.88% after the inclusion of the CGR dataset (Fig. 2a and Supplementary Table 6). Since all the samples in the CGR were from China, it is reasonable to assume that this genome dataset contributes substantially to the Chinese fecal metagenome. To evaluate the contribution of the CGR to the mapping of non-Chinese metagenomes, we carried out a similar analysis using metagenomic data from American, Spanish and Danish fecal samples. Notably, the metagenomic read mapping ratios of these samples all increased substantially (Fig. 2a), although to a lesser extent compared with that of Chinese samples (Supplementary Fig. 4a). The improvement of mapping rates in both Chinese and non-Chinese samples indicates that the CGR covers a considerable number of gut bacterial species shared by people between these countries. To reveal the improvement of gene and protein diversity enabled by the CGR, we compared the gene and protein cumulative curve based on genomes used in a previous IGC study and after addition of the CGR (Supplementary Fig. 4b,c). The number of gene and protein families increased with inclusion of the first 1,500 genomes, but more or less plateaued at around 3,000 genomes. The addition of our CGR genomes led to a substantial increase in the number of added gene and protein families as a function of genome number. A total of 373,555 gene clusters and 149,945 protein clusters were added by inclusion of the CGR, corresponding to a 22% and 16% increase in known gene and protein sequence diversity, respectively.

To further illustrate the utility of the CGR, we used it to analyze gut microbiome SNPs in a cohort of 250 samples from the TwinsUK registry¹⁷. We generated a new set of 282 nonredundant representative genomes from the CGR (see Methods, Supplementary Fig. 5 and Supplementary Table 7), which number nearly doubled the 152 reference genomes used in the original TwinsUK analysis¹⁷. To highlight the new reference genomes identified by analysis with the existing genomes and the CGR genomes, we performed an ANI-based alignment of the 282 genomes with the previously reported 152 genomes. Among the 192 newly added reference genomes, 85 were classified species while 107 were unclassified species (Fig. 2b). A high SNP density was found in *Ruminococcus* sp. CAG:108 (Clu 21), unclassified Firmicutes (Clu 157), *Eubacterium rectale* (Clu 6), *Escherichia coli* (Clu 22), and *Ruminococcus* sp. CAG:57 (Clu 19), suggesting a high degree of variations in the genomes of these species, while *Lactobacillus gasseri* (Clu 241), *Enterococcus faecalis* (Clu 316), *Enterococcus durans* (Clu 274) and *Streptococcus mutans* (Clu 217) showed lower SNP density. A total of 9.14 million SNPs were identified. The number of SNPs was increased for some species due to the newly added high-quality reference genomes in the CGR. We conclude that the CGR is a valuable resource for metagenomic studies because of the significant improvement in metagenomic resolution it enables.

Functions of gut microbiome bacteria. To better elucidate functions of the gut microbiota, we annotated gene functions in 1,520

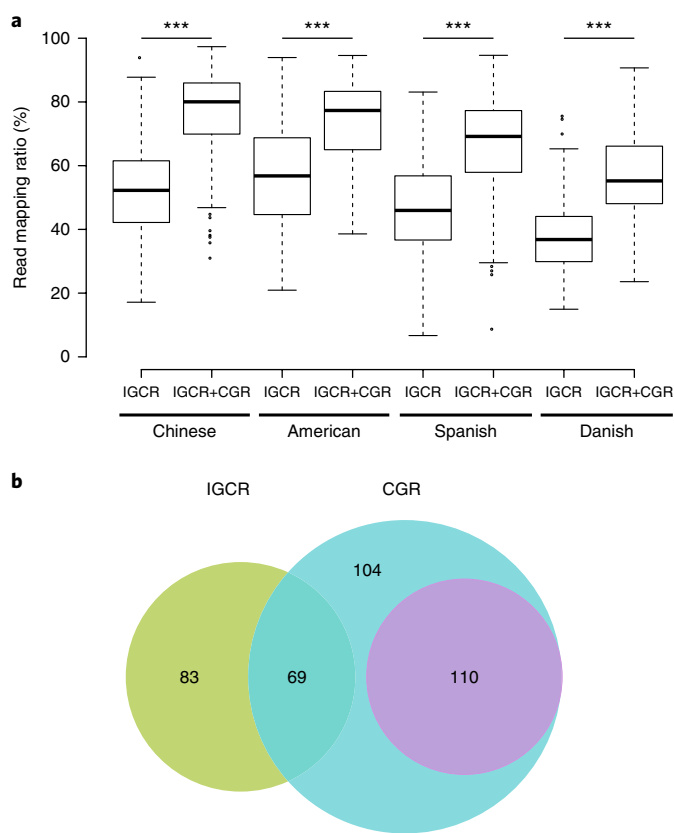
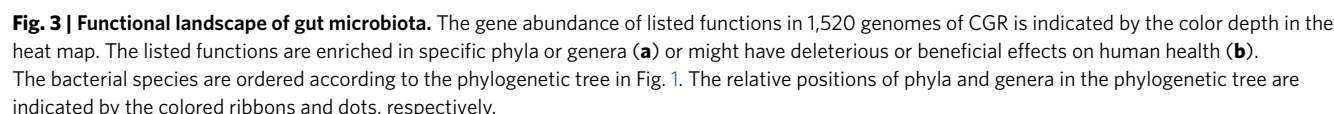


Fig. 2 | Contribution of CGR to metagenomic and SNP analyses. **a**, The read mapping ratio of a previous metagenomic analysis (IGCR) was significantly improved by CGR (IGCR + CGR) in fecal samples from Chinese ($n = 368$, $P = 6 \times 10^{-78}$), American ($n = 139$, $P = 2 \times 10^{-17}$), Spanish ($n = 320$, $P = 4 \times 10^{-50}$) and Danish ($n = 109$, $P = 4 \times 10^{-17}$) individuals. The significance of improvement was determined by two-side Wilcoxon rank-sum test. IGC, 3,449 reference genomes used in the IGC study⁶; CGR, 1,520 reference genomes generated in this study. Each box plot illustrates the estimated median (center line), upper and lower quartiles (box limits), $1.5 \times$ interquartile range (whiskers), and outliers (points) of the read mapping ratio. **b**, Reference genomes for SNP analysis generated in previous study¹⁷ (IGCR, green) and current study (CGR, blue). The unclassified species of reference genomes in CGR are highlighted in violet.

CGR genomes using KEGG (the Kyoto Encyclopedia of Genes and Genomes)¹⁸. Functional pathways at KEGG level 2 showed that pathways involved in carbohydrate and amino acid metabolism are abundant in all isolated strains, suggesting that these are core functions of the gut microbiota (Supplementary Fig. 6). We also analyzed KEGG level 3 pathways and focused on those enriched at the phylum or genus level (Fig. 3a). We found that lipopolysaccharide biosynthesis (ko00540) genes were widely distributed in the phyla Fusobacteria, Bacteroidetes and Proteobacteria, the main phyla of gram-negative bacteria. Genes involved in glycan degradation (ko00531 and ko00511) were abundant in the genomes of the Bacteroidetes phylum. This observation is consistent with the notion that members of Bacteroidetes are prominent human gut symbionts that help degrade glycans in the diet and the gut mucosa¹⁹. The members of the Bacteroidetes also possess a high proportion of genes involved in sphingolipid metabolism (ko00600), glycosphingolipid biosynthesis (ko00601, ko00603 and ko00604) and steroid hormone biosynthesis (ko00140). Sphingolipids and hormone biosynthesis are ubiquitous in eukaryotic cells but not present in most bacteria. These results suggest that members of the Bacteroidetes



Core and pan-genomes of underrepresented gut bacteria. We carried out a pan-genome analysis of 36 species or clusters that contain more than ten genomes, as well as two other species enriched in healthy controls compared with patients with type 2 diabetes in previous studies^{7,23,24}, *Fecalibacterium prausnitzii* (cluster 63, seven genomes) and butyrate-producing bacterium SS3_4 (cluster 45, nine genomes). These clusters covered the phyla Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria (Supplementary Fig. 7a and Supplementary Table 8a). The pan-genome of a cluster can be defined as the sum of the core genes and dispensable genes (including unique genes and accessory genes) of all the members within that cluster²⁵. Our pan-genome analysis showed that *Eubacterium rectale* (cluster 37) contained the lowest proportion of core genes (12%); the remaining genes fell into accessory and unique genomes (38% and 40%, respectively). In contrast, *Eubacterium 3_1* (cluster 6) contained the largest proportion of core genes (53%) (Supplementary Fig. 7b). The pan-genome fitting curves showed that most clusters in Bacteroidetes displayed an 'open' pan-genome and had a relatively large pan-genome size, with *Bacteroides vulgatus*

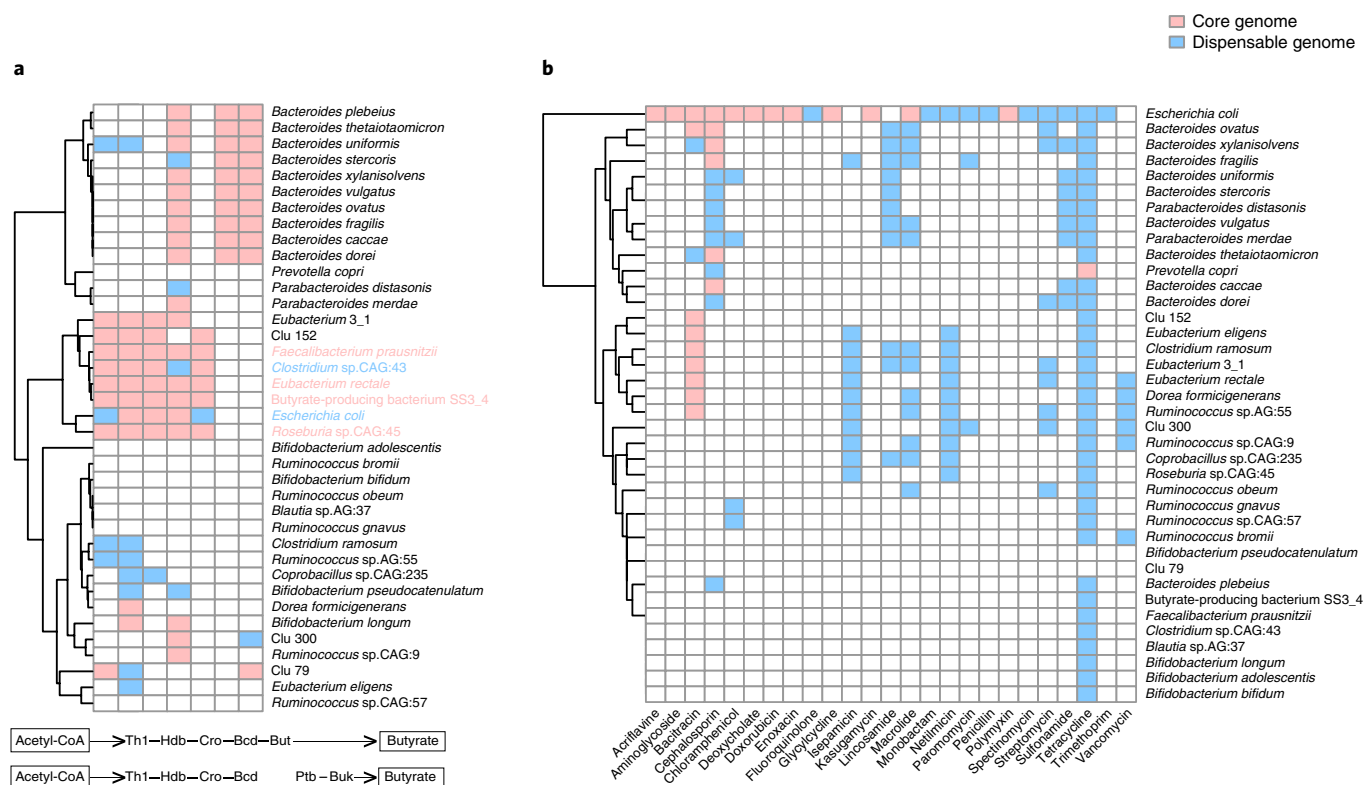


Fig. 4 | Pan-genome analysis of 38 representative clusters. a, The distribution of genes involved in butyrate biosynthesis pathway in the core genomes (pink) and dispensable genomes (cyan). The two pathways for butyrate biosynthesis from acetyl-CoA are shown below. The species with a complete butyrate biosynthesis pathway in the core genome and pan-genome are highlighted in pink and cyan, respectively. Th1, thiolase; Hdb, β -hydroxybutyryl-CoA dehydrogenase; Cro, crotonase; Bcd, butyryl-CoA dehydrogenase (including electron transfer protein α and β subunits); But, butyryl-CoA:acetate CoA transferase; Ptb, phosphate butyryltransferase; Buk, butyrate kinase. **b**, The distribution of ARGs in the core genomes (pink) and dispensable genomes (cyan).

having the largest pan-genome size at 14,970 genes (Supplementary Figs. 8 and 9 and Supplementary Table 8b). In contrast, members in the phylum Actinobacteria tend to represent a relatively ‘closed’ pan-genome, which was only slightly expanded by the addition of CGR genomes. These results suggest that gut bacterial genomes are variable in the Bacteroidetes phylum, less variable in the Firmicutes and Proteobacteria, and fairly conserved in the Actinobacteria.

We also explored the distribution of genes involved in butyrate synthesis and antibiotic resistance in the pan-genomes of gut bacteria. Functional annotation showed that six clusters contained the complete acetyl-CoA to butyrate biosynthesis pathway (Fig. 4a). Among them, *F. prausnitzii*, *E. rectale*, butyrate-producing bacterium SS3_4 and *Roseburia* sp. CAG:45 harbored the complete pathway in their core genome, suggesting that the butyrate-producing function was highly conserved in these species. This result is consistent with the reported butyrate-producing capacity of these species^{26–28}. To explore the distribution of antibiotic resistance within the established pan-genomes, we annotated 25 antibiotic resistance genes (ARGs) in each pan-genome. Consistent with a previous report²⁹, the tetracycline resistance gene was widely present in the dispensable genome of these clusters (Fig. 4b). Notably, *Escherichia coli* contained almost all ARGs (23 of 25) in its pan-genome, with half of these present in the core genome (Fig. 4b). In contrast, *Bifidobacterium* species, including *B. bifidum*, *B. adolescentis*, *B. longum* and *B. pseudocatenulatum*, rarely contained ARGs in their pan-genomes.

To obtain a better understanding of the distribution of bacterial functions in the core and dispensable genomes, we annotated the genomes using the Clusters of Orthologous Groups (COG)

database³⁰. This revealed that several housekeeping functions were significantly enriched in the core genome, including post-translational modification, protein turnover and chaperones (O, $P = 7.28 \times 10^{-12}$); translation, ribosomal structure and biogenesis (J, $P = 7.28 \times 10^{-12}$); energy production and conversion (C, $P = 7.28 \times 10^{-12}$); amino acid transport and metabolism (E, $P = 7.28 \times 10^{-12}$); nucleotide transport and metabolism (F, $P = 7.28 \times 10^{-12}$); coenzyme transport and metabolism (H, $P = 1.46 \times 10^{-11}$); lipid transport and metabolism (I, $P = 2.40 \times 10^{-10}$); and inorganic ion transport and metabolism (P, $P = 2.40 \times 10^{-10}$) (Supplementary Fig. 10). By contrast, COG categories enriched in the dispensable genome included cell wall-membrane-envelope biogenesis (M, $P = 2.70 \times 10^{-9}$); cell motility (N, $P = 3.11 \times 10^{-5}$); signal transduction mechanisms (T, $P = 0.00039$); intracellular trafficking secretion and vesicular transport (U, $P = 1.22 \times 10^{-7}$); defense mechanisms (V, $P = 7.28 \times 10^{-12}$); transcription (K, $P = 3.64 \times 10^{-11}$); replication recombination and repair (L, $P = 7.28 \times 10^{-12}$); and function unknown (S, $P = 0.03111$). The remaining COG categories showed no significant differences in core and dispensable genome.

Discussion

We used 11 culturing conditions for isolation of gut bacteria and archived more than 6,000 isolates. From this collection of isolates, we generated 1,520 high-quality draft reference genomes. The high coverage of the resulting CGR at the genus and species levels (including low-abundance species) demonstrates the value of culture-based methods for strain isolation from the gut microbiota. In line with this, a large number of gut bacterial species that were previously considered as unculturable have been successfully cultivated

in two recent studies^{31,32}. Although there was some overlap between the novel species archived by CGR and in these two studies, the CGR contains 659 additional genomes (representing 209 clusters or species). Our cultivation methods can be applied to expand the CGR until it is saturated with the genomes of culturable gut bacteria. After that, single-cell sequencing can be used to investigate genomes of unculturable bacteria, with an overall aim of defining a saturated set of reference genomes of gut microbiota to underpin a better understanding of gut microbiome biology.

We applied our CGR genome dataset to assign functions to gut bacteria. For example, we found that virulence factors and antibiotic resistance genes are enriched in *Klebsiella*, *Escherichia*, *Citrobacter* and *Enterobacter*, which are opportunistic pathogens frequently isolated in clinical samples³³. The abundance of signal transduction and cell motility genes in these bacteria could further contribute to their pathogenicity^{34,35}. Notably, the Proteobacteria also possess abundant genes for degradation of xenobiotics, which might affect drug metabolism of patients in drug therapy. In line with this, a recent study reported that intratumor Proteobacteria can metabolize chemotherapeutic drugs into inactive forms and thus attenuate the efficacy of cancer therapies³⁶. The genes involved in beneficial functions such as glycan degradation and vitamin B synthesis are enriched in the *Bacteroides* genus, consistent with its mutualistic role in the human gut. Notably, we found that *Bacteroides* species contain a considerable number of genes involved in sphingolipid and steroid hormone synthesis, suggesting their potential for modulating signaling in mammalian cells. In support of this, a recent study reported that *Bacteroides fragilis* can take advantage of sphingolipid signaling to enable symbiosis in the intestine³⁷. It is noteworthy that genes involved in glycan degradation and sphingolipid metabolism were also enriched in the genus *Bifidobacterium*, another well-known gut commensal microbe. However, genes involved in both pathways were not abundant in the *Prevotella* genus, suggesting a distinct function of *Prevotella* compared with other members of the Bacteroidetes phylum. This might account for observed negative correlations between the relative abundances of *Prevotella* and *Bacteroides* in the gut microbiota³⁸. The potential role of gut bacteria in metabolism of estrogens has long been recognized³⁹, but detailed mechanistic studies are still lacking. It will be interesting to explore the implication of this unique function of gut bacteria in hormone-related health or disease. The CGR also enabled the identification of several potential bacteriocin-producing bacteria strains, which merit further verification.

The CGR will improve metagenomic analyses, genome variation analyses, functional characterization and pan-genome analyses. The isolated gut bacteria strains have been deposited in the China National GeneBank (CNGB) and may be useful for studies that aim to alter microbiota functions, as novel probiotics, or for verification of disease-associated bacterial markers.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability, and associated accession codes are available at <https://doi.org/10.1038/s41587-018-0008-8>.

Received: 26 October 2017; Accepted: 13 December 2018;

Published online: 4 February 2019

References

- Wang, J. & Jia, H. Metagenome-wide association studies: fine-mining the microbiome. *Nat. Rev. Microbiol.* **14**, 508–522 (2016).
- Lynch, S. V. & Pedersen, O. The human intestinal microbiome in health and disease. *N. Engl. J. Med.* **375**, 2369–2379 (2016).
- Qin, J. et al. A human gut microbial gene catalog established by metagenomic sequencing. *Nature* **464**, 59–65 (2010).

- Sunagawa, S. et al. Metagenomic species profiling using universal phylogenetic marker genes. *Nat. Methods* **10**, 1196–1199 (2013).
- Methé, B. A. et al. A framework for human microbiome research. *Nature* **486**, 215–221 (2012).
- Li, J. et al. An integrated catalog of reference genes in the human gut microbiome. *Nat. Biotechnol.* **32**, 834–841 (2014).
- Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **490**, 55–60 (2012).
- Karlsson, F. H. et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* **498**, 99–103 (2013).
- Le Chatelier, E. et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* **500**, 541–546 (2013).
- Nielsen, H. B. et al. Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. *Nat. Biotechnol.* **32**, 822–828 (2014).
- Schloissnig, S. et al. Genomic variation landscape of the human gut microbiome. *Nature* **493**, 45–50 (2013).
- Hu, Y. et al. Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. *Nat. Commun.* **4**, 2151 (2013).
- Greenblum, S., Carr, R. & Borenstein, E. Extensive strain-level copy-number variation across human gut microbiome species. *Cell* **160**, 583–594 (2015).
- Nelson, K. E. et al. A catalog of reference genomes from the human microbiome. *Science* **328**, 994–999 (2010).
- Zhang, J. et al. A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. *ISME J.* **9**, 1979–1990 (2015).
- Rajilic-Stojanovic, M. & de Vos, W. M. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiol. Rev.* **38**, 996–1047 (2014).
- Xie, H. et al. Shotgun metagenomics of 250 adult twins reveals genetic and environmental impacts on the gut microbiome. *Cell Systems* **3**, 572–584.e573 (2016).
- Kanehisa, M. & Goto, S. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* **28**, 27–30 (2000).
- Koropatkin, N. M., Cameron, E. A. & Martens, E. C. How glycan metabolism shapes the human gut microbiota. *Nat. Rev. Microbiol.* **10**, 323–335 (2012).
- Chen, L. et al. VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res.* **33**, D325–D328 (2005).
- Jia, B. et al. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res.* **45**(D1), D566–D573 (2017).
- Sund, C. J. et al. The *Bacteroides fragilis* transcriptome response to oxygen and H₂O₂: the role of OxyR and its effect on survival and virulence. *Mol. Microbiol.* **67**, 129–142 (2008).
- Aw, W. & Fukuda, S. Understanding the role of the gut ecosystem in diabetes mellitus. *J. Diabetes Investig.* **9**, 5–12 (2018).
- Aw, W. & Fukuda, S. Toward the comprehensive understanding of the gut ecosystem via metabolomics-based integrated omics approach. *Semin. Immunopathol.* **37**, 5–16 (2015).
- Medini, D., Donati, C., Tettelin, H., Masignani, V. & Rappuoli, R. The microbial pan-genome. *Curr. Opin. Genet. Dev.* **15**, 589–594 (2005).
- Louis, P. & Flint, H. J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol. Lett.* **294**, 1–8 (2009).
- Van den Abbeele, P. et al. Butyrate-producing *Clostridium* cluster XIVa species specifically colonize mucins in an in vitro gut model. *ISME J.* **7**, 949–961 (2013).
- Louis, P., Young, P., Holtrop, G. & Flint, H. J. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. *Environ. Microbiol.* **12**, 304–314 (2010).
- de Vries, L. E. et al. The gut as reservoir of antibiotic resistance: microbial diversity of tetracycline resistance in mother and infant. *PLoS One* **6**, e21644 (2011).
- Tatusov, R. L. et al. The COG database: an updated version includes eukaryotes. *BMC Bioinformatics* **4**, 41 (2003).
- Lagier, J.-C. et al. Culture of previously uncultured members of the human gut microbiota by culturomics. *Nat. Microbiol.* **1**, 16203 (2016).
- Browne, H. P. et al. Culturing of ‘unculturable’ human microbiota reveals novel taxa and extensive sporulation. *Nature* **533**, 543–546 (2016).
- Guentzel, M.N. *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter*, and *Proteus*. *Medical Microbiology* Ch. 25 (University of Texas Medical Branch, Galveston, Texas, USA, 1996).
- Josenshans, C. & Suerbaum, S. The role of motility as a virulence factor in bacteria. *Int. J. Med. Microbiol.* **291**, 605–614 (2002).
- Gotoh, Y. et al. Two-component signal transduction as potential drug targets in pathogenic bacteria. *Curr. Opin. Microbiol.* **13**, 232–239 (2010).
- Geller, L. T. et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science* **357**, 1156–1160 (2017).

37. An, D., Na, C., Bielawski, J., Hannun, Y. A. & Kasper, D. L. Membrane sphingolipids as essential molecular signals for *Bacteroides* survival in the intestine. *Proc. Natl Acad. Sci. USA* **108**(Suppl. 1), 4666–4671 (2011).
38. Ley, R. E. Gut microbiota in 2015: *Prevotella* in the gut: choose carefully. *Nat. Rev. Gastroenterol. Hepatol.* **13**, 69–70 (2016).
39. Rowland, I., Wiseman, H., Sanders, T., Adlercreutz, H. & Bowey, E. Metabolism of oestrogens and phytoestrogens: role of the gut microflora. *Biochem. Soc. Trans.* **27**, 304–308 (1999).

Acknowledgements

We gratefully acknowledge colleagues at BGI-Shenzhen for DNA extraction, library construction, sequencing and discussions. This research was supported by the National Natural Science Foundation of China (grants 81670606 and 81673850), the Shenzhen Municipal Government of China (JCYJ20160229172757249 and JCYJ20170818111103886).

Author contributions

H.J., J.L., L.X., Y.Z. and W.X. conceived and designed the project. H.J., J.L. and L.X. monitored the project. Y.Z., W.X., Y.D., D.W. and R.J. collected samples and performed experiments. Y.Z., W.X., G.L., P.Q., Z.D., R.G., H.S., Y.X., S.L., Q.F., Z.J., L.S., T.G., X.X., Y.H., X.L., J.W., H.Y., Y.L., S.T., G.H., C.L., Z.X., J.Y., K.K., L.X., J.L. and H.J. analyzed and interpreted the data. Y.Z. and Z.D. wrote the paper. K.K., H.J. and L.X. revised the paper. All authors commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41587-018-0008-8>.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to J.L., H.J. or L.X.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2019



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

Methods

Anaerobic cultivation of fecal bacteria. Fecal samples were collected from 155 healthy human donors not taking any drugs during the last month before sampling. Detailed information is given in Supplementary Table 2. The samples were immediately transferred to an anaerobic chamber (Bactron Anaerobic Chamber, Bactron IV-2, Shellab, USA), homogenized in pre-reduced phosphate buffered saline (PBS) supplemented with 0.1% cysteine, and then diluted and spread on agar plates with different growth media (Supplementary Table 1). Plates were incubated under anaerobic condition in an atmosphere of 90% N₂, 5% CO₂ and 5% H₂ at 37 °C for 2–3 d. Single colonies were picked and streaked onto new plates to obtain single clones. All the strains were stored in a glycerol suspension (20%, v/v) containing 0.1% cysteine at –80 °C. The collection of the 155 samples was approved by the Institutional Review Board on Bioethics and Biosafety of BGI under number BGI-IRB17005-T1. All protocols were in compliance with the Declaration of Helsinki and explicit informed consent was obtained from all participants. Bacteria in the CGR (Culturable Genome Reference) are deposited in and are available from the E-BioBank (EBB) of the China National GeneBank (http://ebiobank.cnbg.org/index.php?g=Content&m=Hql&a=sample_5&id=393).

Whole-genome sequencing and de novo assembly. *DNA extraction.* Isolates cultivated to stationary phase were centrifuged at 7,227g at 4 °C for 10 min, and the resulting pellets were resuspended in 1 ml of Tris-EDTA. For bacterial cell lysis, 50 µl of 10% SDS and 10 µl of proteinase K (20 mg/ml) were added, and the solution was incubated at 55 °C in a water bath for 2 h. The released genomic DNA was extracted using the phenol-chloroform method⁴⁰.

Genome sequencing and assembly. Paired-end libraries with an insert size of 500 bp were constructed and sequenced on Illumina HiSeq 2000 platform to obtain about 100 × clean data for each sample. The reads were assembled using SOAPdenovo 2.0⁴¹ to form scaffolds from which the rRNA genes were extracted by RNAMmer 1.2⁴². An in-house pipeline was used to obtain the best assembly containing an orthogonal design to investigate L,M,d,D,L,u,G (arguments of SOAPdenovo) and a single-factor design to investigate K (argument of SOAPdenovo) by comprehensively considering contig average length, longest scaffold and rRNA score. Libraries with an insert size of 240 bp were constructed and sequenced on the ionProton platform, which produced about 100 × clean data for each sample. The reads were assembled through SPAdes (version 3.1.0)⁴³ to form scaffolds.

Assessment of genome quality. Six high-quality draft assembly criteria from the Human Microbiome Project (HMP)¹⁴ and rRNA (5 s, 16 s and 23 s) completeness were adopted to ensure the assembly quality. The criteria are (i) 90% of the genome assembly must be included in contigs > 500 bp, (ii) 90% of the assembled bases must be at > 5 × read coverage, (iii) the contig N50 must be > 5 kb, (iv) scaffold N50 must be > 20 kb, (v) average contig length must be > 5 kb, and (vi) > 90% of the core genes^{44,45} must be present in the assembly.

Splitting for multi-genome isolates. The multi-genomes in isolates were initially identified using CheckM⁴⁶ (contamination > 5%) and confirmed by manual inspection of the plot of G + C percentage vs. sequencing depth. An in-house pipeline was developed to split the scaffolds of multi-genomes into single genomes. Briefly, scaffolds in multi-genomes were first split on the basis of G + C percentage vs. sequencing depth values using the dbscan function of R (package “fpc”). The “complete” and “contamination” of split genomes were checked using CheckM. For split genomes with “complete” > 100% or “contamination” > 15%, an additional species-designating pipeline was used to obtain their closest reference (with ANI value > 90%). Finally, the mis-split scaffolds in each split genome were mapping back to the closest reference genome using BLASTn (–e 1e-5 –F F –m 8, blastn hits’ length > 90 nt, query scaffold coverage ≥ 50%) to obtain the final split genomes.

Massive species and genus assignment process. *NCBI-retrieved prokaryotic genomes.* All complete genomes (update time 19 November 2014) and draft genomes (update time 8 August 2014) on the NCBI ftp site were downloaded to a local server. Items with more than one NCBI taxonomy identifier (taxid) or genome sequence not available or of non-prokaryotic source were removed, and of redundant items, only one was kept. As a result, 24,552 genomes, 19,116 genome-scale amino acid sequences, and their taxonomic information were obtained.

Average nucleotide identity (ANI)⁴⁷ for species level taxonomic assignment. The taxonomic assignment of each query genome was determined by the taxonomic information of all the NCBI-available prokaryotic genomes. The tetra-base signature profiles of all the genomes and each query genome were acquired. A Pearson correlation test was performed between each query genome and all the genomes, resulting in a reference list sorted by decreasing correlation coefficient for each query genome. **Then pairwise ANI alignment was performed between query and reference genomes one by one according to the reference list (tetra-base profile’s Pearson correlation test: correlation coefficient > 0 and P < 0.001) until the ANI value was larger than 95% in the top 500 items (defined as assigned in this case) or reference item number exceeded 500 without any ANI value being larger than 95% (defined as not assigned in this case).**

Percentage of conserved proteins (POCP)⁴⁸ for genus-level taxonomic assignment. The taxonomic assignment of each query genome was determined by the taxonomic information of all the NCBI-available prokaryotic genomes. The tetra-base signature profiles of all the reference genomes and the query genomes with no species assignment based on ANI were acquired. A Pearson correlation test was performed between each query genome and all the reference genomes, resulting in a reference list sorted by decreasing correlation coefficient for each query genome. Then the POCP calculation was performed between query and reference genomes one by one according to the reference list until the POCP value was larger than 50% in the top 500 items (defined as “assigned” in this case) or reference items number exceeded 500 without any POCP value being larger than 50% (defined as “not assigned” in this case).

16S rRNA sequence analysis and novel species determination. 16S rRNA gene sequences were extracted from the isolate genomes using RNAMmer⁴², except for 16 genomes where extraction failed. The sequences were quality-control processed in EzBioCloud (<http://www.ezbiocloud.net>)⁴⁹. The species-level operational taxonomic units (OTUs) were classified using mothur⁵⁰ with an identity of 98.7% as a species-level cut-off, and cut-offs of 94.5% and 86.5% were used for genera and families⁵¹, respectively.

Comparison of CGR with genome datasets from other studies. To compare the new genomes and novel species archived in CGR with those identified in two recent studies, we downloaded 215 genomes reported by Browne et al.³² and 169 genomes reported by Lagier et al.³¹. We adopted a similar ANI pipeline as described above for species-level comparison by replacing the NCBI references with these newly downloaded genomes. “Map” was defined if the pairwise ANI value between a query genome in our 1,520 high-quality genomes and any one of references genomes (tetra-base profile’s Pearson correlation test: correlation coefficient > 0 and P < 0.001) was larger than 95%; if not, the species was defined as “unmap.”

Construction of species clusters. Pairwise ANI alignment was performed among the 1,520 high-quality genomes, and then hclust from the R package was used for hierarchical clustering with distance of 0.05 (equivalent to 95% ANI). A set of 40 universally conserved single-copy genes encoding proteins in bacteria and archaea was used for construction of a phylogenetic tree. Marker genes were detected and aligned using spec⁵² and prank⁵³. Alignments were trimmed by trimal⁵⁴ and concatenated with in-house scripts. A phylogenetic tree was inferred using the maximum likelihood method with RAXML (version 8.2.8)⁵⁵ for the clusters’ representative genomes (N50 longest among cluster) with *Rhizobium selenitireducens* ATCC BAA 1503 (taxoid:1336235) as an outgroup, and was visualized in iTOL (<http://itol.embl.de/>)⁵⁶ online.

Genome function annotation. The 1,520 high-quality genomes were functionally annotated. Genes were identified using Genemark⁵⁷. The translated amino acid sequences of coding genes were aligned with RAPSearch (–s f –e 1e-2 –v 100 –u 2)⁵⁸ against the Kyoto Encyclopedia of Genes and Genomes (KEGG version 76)^{18,59} (query match length higher than 50%) or with BLASTp (–e 1e-2 –F T –b 100 –K 1 –a 1 –m 8) against the Antibiotic Resistance Genes Database (ARDB) (both query and subject match length higher than 40%, with identity higher than the ARDB-recommended thresholds)⁶⁰, the Virulence Factor Database (VFDB)^{20,61} (query match length higher than 50%, with identity higher than 60%), and the bacteriocin database (downloaded from BAGEL3⁶², with identity higher than 60%). Annotation of genes against the Comprehensive Antibiotic Resistance Database (CARD)²¹ was performed using Resistance Gene Identifier available as a downloadable command-line tool in the download section of the CARD website using default parameters.

Mapping ratio of metagenomic samples. The metagenomic reads⁶ were first aligned to the reference genomes of IGCR (3,449 sequenced prokaryotic genomes from IGC⁶) using SOAP2⁶³ (default parameters, except –m 100 –x 1000 –r 1 –l 30 –v 5 –c 0.95 –u). The unmapped reads were then aligned to the newly sequenced genomes of CGR. The read mapping ratio of different samples was calculated, and the difference between samples was determined by Wilcoxon test in R.

Analysis of gene and protein diversity. *Gene clusters.* 5,749,641 genes in the 1,759 CGR genomes and 11,330,042 genes in 3,449 IGCR genomes were clustered using CD-HIT⁶⁴ with default parameters, except –G 0 –aS 0.9 –c 0.95 –M 0 –d 0 –r 1 –g 1, which amounts to 95% local sequence identity over 90% alignment coverage for the shorter sequence. A cluster is composed of two or more genes. An accumulative curve of gene clusters was drawn according to the sample name alphabetically with IGCR at the front part and CGR at the latter part.

Protein clusters. 5,749,641 protein sequences translated from genes in the 1,759 CGR genomes and 11,330,042 protein sequences translated from genes in 3,449 IGCR genomes were clustered using the kClust algorithm⁶⁵ with default parameters, which amounts to 20–30% maximum pairwise sequence identity over 80% alignment length with the longest sequence or seed of the cluster. A cluster is composed of two or more protein sequences. An accumulative curve of protein

clusters was drawn according to the sample name alphabetically with IGCR at the front part and CGR at the latter part.

SNP identification and similarity score. 1,520 genomes from the CGR were aligned with the sequenced reads from the 250 TwinsUK samples using SOAP2 with identity $\geq 90\%$. Representative genomes used for SNP analysis were identified according to three criteria described previously¹⁷. The resulting 282 genomes (Supplementary Table 7) that fulfilled these criteria were used as references for SNP calling using SAMtools (frequency $> 1\%$ and supported by ≥ 4 reads) as previously described^{10,17,20}. The reference genomes used in a previous study¹⁸ (152 genomes) were compared with that from CGR of this study (282 genomes) to identify shared and new reference genomes using ANI $\geq 95\%$ as a threshold (species level).

Pan genome analysis for 38 cluster. Clusters containing more than ten genomes (from CGR and NCBI), as well as *Fecalibacterium prausnitzii* (seven genomes) and butyrate-producing bacterium SS3_4 (nine genomes), were used for pan-genome analysis using the Bacterial Pan Genome Analysis tool (BPGA) pipeline⁶⁶. The set of genes shared by all the members of cluster was defined as core genes, while genes partially shared in members (accessory genes) and unique to single members (unique genes) in a cluster were defined as dispensable gene⁶⁷. The pan-genome fitting curves of 38 clusters were generated by the BPGA workflow and plotted in R (v.3.3.3). The functions of genes in the pan-genomes of 38 clusters were annotated by KEGG and ARDB, using arguments identical to those used for functional annotation of genomes. The acetyl-CoA-to-butyrate biosynthesis pathway was generated according to a previous study⁶⁸, and the associated enzymes were identified according to the functional annotation and BLAST to the NCBI protein database (cut-off 1e-5, identity $\geq 70\%$, coverage $\geq 70\%$). The COG database³⁰ was also used to identify the functional distribution in the core and dispensable sections via the BPGA pipeline. The significance of the difference between COG distribution in core and dispensable genomes was examined using Wilcoxon test as implemented in R (v.3.3.3).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The assembly draft genomes and annotation information for the 1,520 CGR strains are deposited in the NCBI under accession code [PRJNA482748](https://www.ncbi.nlm.nih.gov/PRJNA482748), and these data are also available in the China National GeneBank (CNCB) Nucleotide Sequence Archive (CNSA; accession code CNP0000126). All bacterial strains in the CGR have been deposited in the CNGB, a nonprofit, public-service-oriented organization in China. The accession code for each strain is given in Supplementary Table 5 (Genebank_id). Researchers can explore strain information and request strains via http://ebiobank.cnbg.org/index.php?g=Content&m=Hql&a=sample_5&id=393#.

References

- Giraffa, G., Rossetti, L. & Neviani, E. An evaluation of chelex-based DNA purification protocols for the typing of lactic acid bacteria. *J. Microbiol. Methods* **42**, 175–184 (2000).
- Luo, R. et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *Gigascience* **1**, 18 (2012).
- Lagesen, K. et al. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* **35**, 3100–3108 (2007).
- Bankevich, A. et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **19**, 455–477 (2012).
- Callister, S. J. et al. Comparative bacterial proteomics: analysis of the core genome concept. *PLoS One* **3**, e1542 (2008).
- Li, L., Stoeckert, C. J. Jr & Roos, D. S. OrthoMCL: identification of ortholog groups for eukaryotic genomes. *Genome Res.* **13**, 2178–2189 (2003).
- Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P. & Tyson, G. W. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* **25**, 1043–1055 (2015).
- Richter, M. & Rosselló-Móra, R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl Acad. Sci. USA* **106**, 19126–19131 (2009).
- Qin, Q. L. et al. A proposed genus boundary for the prokaryotes based on genomic insights. *J. Bacteriol.* **196**, 2210–2215 (2014).
- Kim, O. S. et al. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* **62**, 716–721 (2012).
- Schloss, P. D. et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**, 7537–7541 (2009).
- Yarza, P. et al. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat. Rev. Microbiol.* **12**, 635–645 (2014).
- Mende, D. R., Sunagawa, S., Zeller, G. & Bork, P. Accurate and universal delineation of prokaryotic species. *Nat. Methods* **10**, 881–884 (2013).
- Löytynoja, A. Phylogeny-aware alignment with PRANK. *Methods Mol. Biol.* **1079**, 155–170 (2014).
- Capella-Gutiérrez, S., Silla-Martínez, J. M. & Gabaldón, T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972–1973 (2009).
- Stamatakis, A. RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313 (2014).
- Letunic, I. & Bork, P. Interactive tree of life (iTOL)v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.* **44**(W1), W242–W245 (2016).
- Besemer, J. & Borodovsky, M. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. *Nucleic Acids Res.* **33**, W451–W454 (2005).
- Ye, Y., Choi, J. H. & Tang, H. RAPSearch: a fast protein similarity search tool for short reads. *BMC Bioinformatics* **12**, 159 (2011).
- Du, J. et al. KEGG-PATH: Kyoto encyclopedia of genes and genomes-based pathway analysis using a path analysis model. *Mol. Biosyst.* **10**, 2441–2447 (2014).
- Liu, B. & Pop, M. ARDB—Antibiotic Resistance Genes Database. *Nucleic Acids Res.* **37**, D443–D447 (2009).
- Chen, L., Zheng, D., Liu, B., Yang, J. & Jin, Q. VFDB 2016: hierarchical and refined dataset for big data analysis—10 years on. *Nucleic Acids Res.* **44**(D1), D694–D697 (2016).
- van Heel, A. J., de Jong, A., Montalbán-López, M., Kok, J. & Kuipers, O. P. BAGEL3: Automated identification of genes encoding bacteriocins and (non-) bactericidal posttranslationally modified peptides. *Nucleic Acids Res.* **41**, W448–W453 (2013).
- Li, R. et al. SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics* **25**, 1966–1967 (2009).
- Li, W. & Godzik, A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**, 1658–1659 (2006).
- Hauser, M., Mayer, C. E. & Söding, J. kClust: fast and sensitive clustering of large protein sequence databases. *BMC Bioinformatics* **14**, 248 (2013).
- Chaudhari, N. M., Gupta, V. K. & Dutta, C. BPGA—an ultra-fast pan-genome analysis pipeline. *Sci. Rep.* **6**, 24373 (2016).
- Bosi, E. et al. Comparative genome-scale modelling of *Staphylococcus aureus* strains identifies strain-specific metabolic capabilities linked to pathogenicity. *Proc. Natl Acad. Sci. USA* **113**, E3801–E3809 (2016).
- Vital, M., Howe, A. C. & Tiedje, J. M. Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *MBio* **5**, e00889 (2014).

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 [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- ☒ ☐ An indication of 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 statistics 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
- ☐ ☒ 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

NCBI (19 Nov, 2014) , Ezbiocloud (23 Oct, 2017),KEGG (version 76), ARDB (Version 1.1), VFDB (2012),bacteriocin database (2013), CARD (2013)

Data analysis

SOAPdenovo2(v r240), RNAMMer (v1.2) , SPAdes (v3.1.0), CheckM (v1.0.12), spec1 (v1.0), prank (v.150803), trimal (v1.4.1), RAxML (v8.2.9), Genemark (v2.6r), RAPSearch (v2.23), BLASTp (v2.2.26), BPGA (v.1.3), RStudio (v1.0.153), R (v.3.3.3).

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.

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

The assembly draft genome and annotation information of the 1,520 strains have been deposited into the NCBI under accession number PRJNA482748 and the data are also available in the CNGB Nucleotide Sequence Archive (CNSA: <https://db.cngb.org/cnsa>; accession number CNP0000126).

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	This study collected 155 feces, isolated 6,487 bacterial clones, and generated 1520 high quality genomes, which is the largest dataset to date for gut bacterial reference genomes.
Data exclusions	No data were excluded from analyses.
Replication	not applicable; no biological experiments were involved.
Randomization	not applicable; no biological experiments were involved.
Blinding	not applicable; no biological experiments were involved.

Behavioural & social sciences study design

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

Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.
Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Non-participation	State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.
Randomization	If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

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

Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.
Research sample	Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i> , all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.
Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.
Did the study involve field work? <input type="checkbox"/> Yes <input type="checkbox"/> No	

Field work, collection and transport

Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).
Access and import/export	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).
Disturbance	Describe any disturbance caused by the study and how it was minimized.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Unique biological materials
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Unique biological materials

Policy information about [availability of materials](#)

Obtaining unique materials

Describe any restrictions on the availability of unique materials OR confirm that all unique materials used are readily available from the authors or from standard commercial sources (and specify these sources).

Antibodies

Antibodies used

Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.

Validation

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.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

State the source of each cell line used.

Authentication

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.

Mycoplasma contamination

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.

Commonly misidentified lines (See [ICLAC](#) register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology

Specimen provenance

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).

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

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.

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.

Wild animals

Provide details on animals observed in or captured in the field; report species, sex 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.

Field-collected samples

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.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

This study recruited 155 participants from China, including 79 male and 76 females; Among them 2 were age 1-10, 21 were age 11-20, 109 were age 21-50, and 23 were age >50; Please also see population characteristics in Supplementary Figure 1a.

Recruitment

All participants were recruited in ShenZhen, China; Fecal samples were collected from 155 healthy donors not taking any drugs during the last months prior to sampling;

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

May remain private before publication.

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

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

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.