Supplementary information of https://doi.org/10.1631/jzus.B2400495

Methods

Metagenomic sequencing and analysis

Mouse fecal samples were collected in a clean cage inside the isolator and placed on dry ice for DNA extraction (cetyltrimethylammonium bromide (CTAB)) and sequencing. The input material for the DNA sample preparation was 1 μg of total DNA per sample (optical density (OD) value range: 1.8–2.0). The sequencing libraries were generated using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) according to the manufacturer's instructions, and index codes were added to match the sequences to each sample. The experiment was performed as described in our previous study y (Yang et al., 2022), with details provided in the supplementary methods.In brief, the DNA sample was first lysed into 350 bp fragments through sonication; then, these fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing, followed by further polymerase chain reaction (PCR) amplification. Finally, the PCR products were purified using the AMPure XP system, and the size distribution of the libraries was analyzed using an Agilent 2100 Bioanalyzer and quantified using real-time PCR. On a cBot cluster generation system, index-coded samples were clustered in accordance with the manufacturer's recommendations. Library preparations were sequenced on an Illumina HiSeq platform after cluster creation, and paired-end reads were produced. To determine the species abundance data for further investigation, the filtered host reads were compared to the Chocophlan (version MPa_V30_Chocophlan_201901) database using the MetaPhlAn3 (version 3.0.7) software. To obtain the UniRef database of gene family annotations and pathway abundance information from the gene family-based MetaCyc database (https://metacyc.org), the pan-gene database, ChocoPhlAn (version mpa_v30_CHOCOPhlAn_201901) was compared to the Uniref90 (version 201901) database using the HUMAnN (version v3.0.0. alpha.4) software. MetaCyc is a comprehensive reference database of metabolic pathways and enzymes in all domains of life (Caspi et al., 2020). Beta diversity was visualized using principal coordinate analysis (PCoA) or principal component analysis (PCA), and the data were imported into RStudio (4.1.1) and plotted using ggplot2 (3.3.5) for the mapping of PCoA, alpha diversity (Shannon's diversity index), and heatmap. In addition, the significance between groups and their degree of variance were examined using permutational multivariate analysis of variance (PERMANOVA, Adonis analysis). To analyze the degree of differences between groups by delivery route and their statistical significance using a permutation test, total variance was decomposed using a distance matrix based on Bray-Curtis distance. Wekemo Bioincloud (https://www.bioincloud.tech) was used to complete the PCA, Venn diagram, and linear discriminant analysis effect size (LEfSe, (Segata et al., 2011)). *P*-values were corrected for multiple testing using the Benjamini-Hochberg (BH) method.

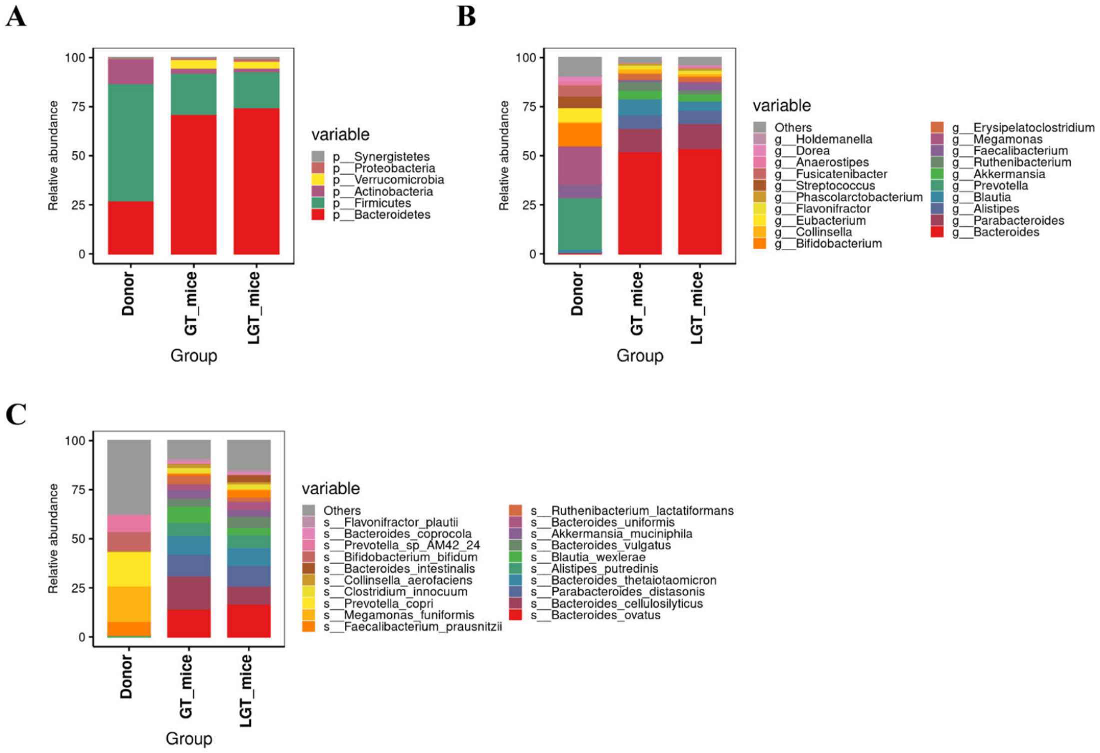

Fig. S1 Analysis of the dominant bacteria among the donor and the two groups of recipient mice. (A) Phylum level; (B) Genus level; (C) Species level.

Table S1 Analysis of differential colonization in recipients under different delivery routes

LGT_mice-Features	GT_mice-Features	Common--Features
s__Eggerthella_lenta	*s__Enterococcus_hirae*	*s__Bifidobacterium_adolescentis*
s__Bacteroides_eggerthii	*s__Clostridium_scindens*	*s__Collinsella_aerofaciens*
s__Bacteroides_intestinalis	*s__Erysipelatoclostridium_ramosum*	*s__Collinsella_stercoris*
s__Coprobacter_fastidiosus	*s__Parasutterella_excrementihominis*	*s__Adlercreutzia_equolifaciens*
s__Parabacteroides_gordonii	*s__Turicimonas_muris*	*s__Asaccharobacter_celatus*
s__Enterococcus_faecalis	*s__Proteobacteria_bacterium_CAG_139*	*s__Bacteroides_caccae*
s__Catabacter_hongkongensis		*s__Bacteroides_coprocola*
s__Blautia_hydrogenotrophica		*s__Bacteroides_coprophilus*
s__Holdemania_filiformis		*s__Bacteroides_ovatus*
s__Phascolarctobacterium_sp_CAG_266		*s__Bacteroides_stercoris*
s__Cloacibacillus_evryensis		*s__Bacteroides_thetaiotaomicron*
s__Cloacibacillus_porcorum		*s__Bacteroides_uniformis*
		s__Bacteroides_vulgatus
		s__Odoribacter_splanchnicus
		s__Alistipes_putredinis
		s__Parabacteroides_distasonis
		s__Parabacteroides_merdae
		s__Eubacterium_ramulus
		s__Eubacterium_ventriosum
		s__Anaerostipes_hadrus
		s__Blautia_wexlerae
		s__Roseburia_inulinivorans
		s__Faecalibacterium_prausnitzii
		s__Gemmiger_formicilis
		s__Clostridium_leptum
		s__Holdemanella_biformis
		s__Bilophila_wadsworthia
		s__Desulfovibrio_piger
		s__Enterobacter_cloacae_complex
		s__Escherichia_coli
		s__Klebsiella_pneumoniae
		s__Klebsiella_quasipneumoniae
		s__Klebsiella_variicola

Table S2 Dominant metabolic pathway among three groups[b]

Pathway	Donor	GT_mice	LGT_mice
PWY0-1586	0.007 216 61	0.010 208 695	0.010 002 328
PWY-7237	0.009 627 804	0.009 633 768	0.010 085 548
PWY-6121	0.010 359 667	0.009 958 044	0.009 780 127
BRANCHED-CHAIN-AA-SYN-PWY	0.010 487 4	0.009 541 224	0.009 397 586
PWY-7221	0.010 798 267	0.011 662 85	0.011 316 928
PWY-6122	0.011 190 034	0.009 840 308	0.009 585 083
COA-PWY-1	0.011 656 067	0.010 040 271	0.009 940 971
DTDPRHAMSYN-PWY	0.011 869 634	0.015 191 11	0.014 584 389
PWY-6385	0.012 025 034	0.010 576 09	0.010 216 442
PWY-6163	0.012 343 034	0.010 033 095	0.009 948 433
PEPTIDOGLYCANSYN-PWY	0.012 386 2	0.010 498 95	0.010 028 11
PWY-6387	0.012 507 6	0.010 540 41	0.010 057 436
PWY-6386	0.012 902 867	0.010 516 36	0.010 079 768
PWY-5686	0.013 710 267	0.010 422 71	0.010 342 064
PWY-7219	0.013 801 167	0.009 432 199	0.009 478 499
PWY-6737	0.013 986 101	0.010 022 165	0.009 808 742
PWY-7111	0.014 996 234	0.010 231 52	0.010 23 154
Other	0.798 136 013	0.821 650 228	0.825 116 005

[b] Data represent relative abundance. Pathway information from the gene family-based MetaCyc database.