SUPPLEMENTARY METHOD

Bacterial 16S rRNA gene amplification and Illumina MiSeq sequencing.

The 16S ribosomal RNA gene of iDNA and eDNA were amplified in 50-μL triplicates with the 515F (5'- GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') primers specific for the V4-V5 hyper variable regions of the 16S rRNA gene, where barcode is an eight-base sequence unique to each sample (Caporaso et al., 2012). PCR condition was 95 ^{o}C for 2 min, followed by 25 cycles at 95 ^{o}C for 30 s, 55 ^{o}C for 30 s, and 72 ^{o}C for 30 s and a final extension at 72 ^{o}C for 5 min. PCRs were performed in triplicate 20 mL mixture containing 4 mL of 5 × FastPfu Buffer, 2 mL of 2.5 mmol/L dNTPs, 0.8 mL of each primer (5 mmol/L), 0.4 mL of FastPfu Polymerase, and 10 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, United States). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP072153).

Processing and analyzing of sequencing data

Raw fastq files were demultiplexed, quality-filtered using QIIME (version 1.17) with the following criteria: (1) The 300bp reads were truncated at any site receiving an average quality score < 20 over a 50bp sliding window, discarding the truncated reads that were shorter than 50bp. (2) exact barcode matching, 2 nucleotide mismatch in primer matching, reads containing ambiguous characters were removed. (3) only sequences that overlap longer than 10bp were assembled according to their overlap sequence. Reads which could not be assembled were discarded.

Operational units (OTUs) were clustered with 97% similarity cutoff using

UPARSE（version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU115)16S rRNA database using confidence threshold of 70% (Amato et al., 2013). In addition to community structure analysis, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was also used to infer the functional content, based on 16S OTU BIOM generated from QIIME (Langille et al., 2013).

Fluorescence in situ hybridization

According to the results of Illumina MiSeq sequencing, oligonucleotide probes were designed to match the specific species, *Bacteroidates S24-7* (5′-GCACTTAAGCCGACACCT-3′) (G+) and *Staphylococcus* (5′-GAAGCAAGCTTCTCGTCCG-3′) (G-), with fluorescence in situ hybridization (FISH). The *Eub*338 (5′-GCTGCCTCCCGTAGGAGT-3′) probe binds the bacteria 16S rRNA region of a broad range of bacteria genera. All probes were labeled at the N terminus with FAM. The procedure of hybridization was referred without lysozyme incubation to specific probe eDNA. We compared the distribution of *Bacteroidates S24-7* and *Staphylococcus* from ileum and colon mucus respectively.

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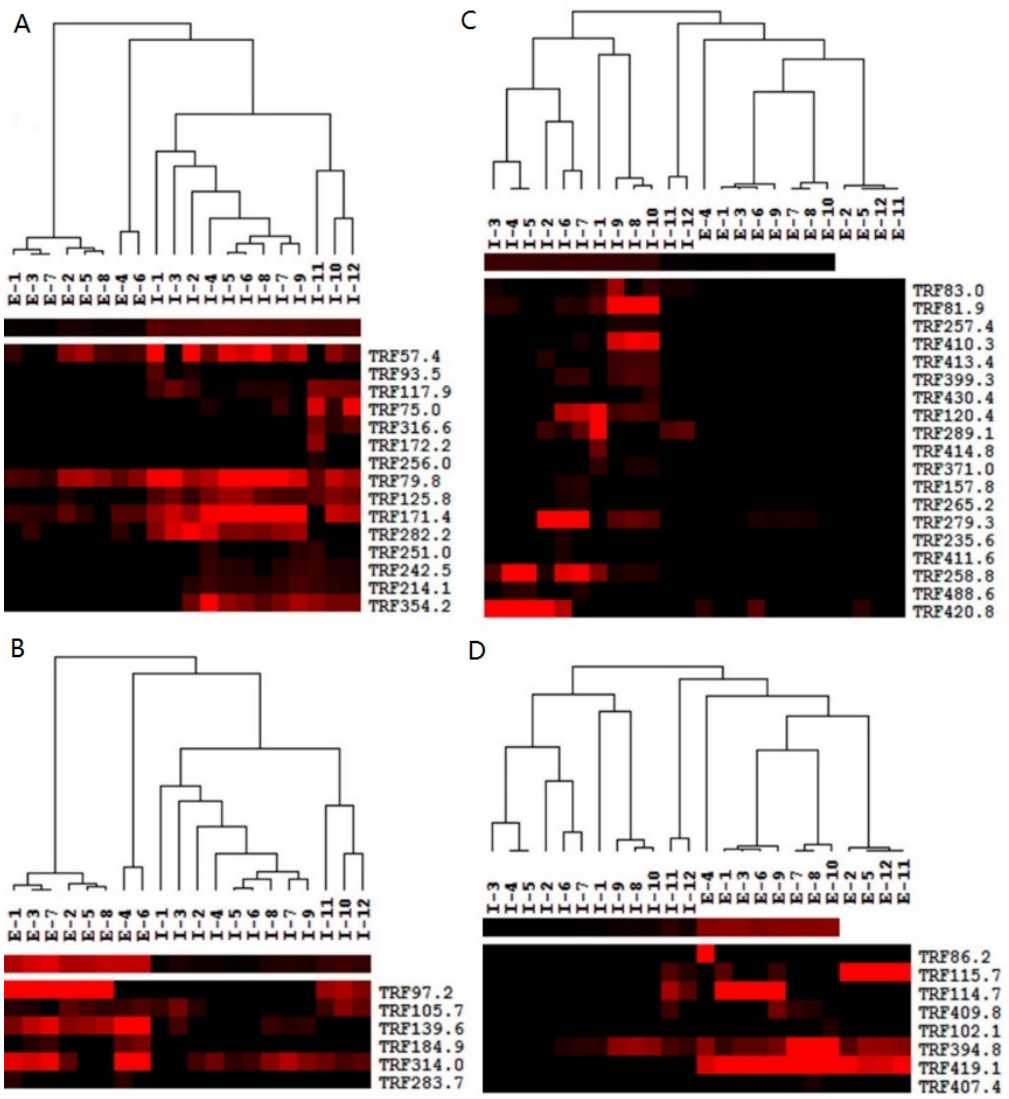
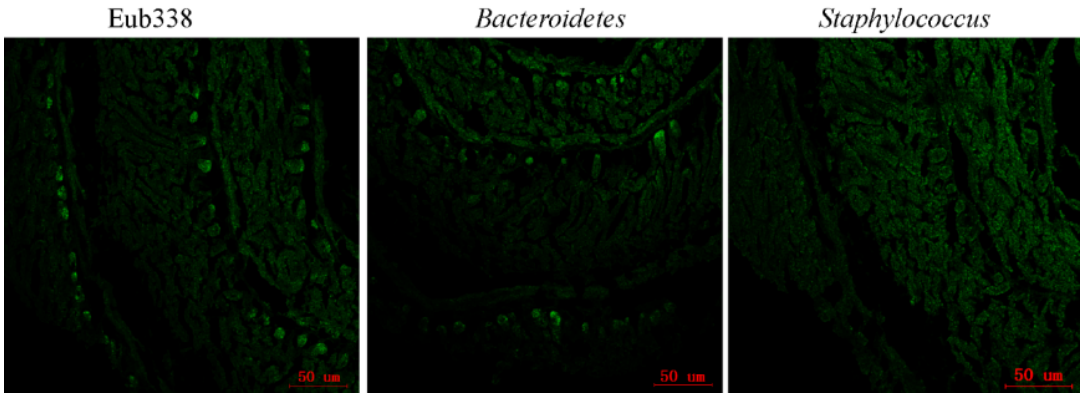
Supplementary Table 1 Total percentage of gram negative and positive bacteria and Genus of percentage higher than 0.5% in extracellular and intracellular bacterial DNA of mice small intestine mucus layer

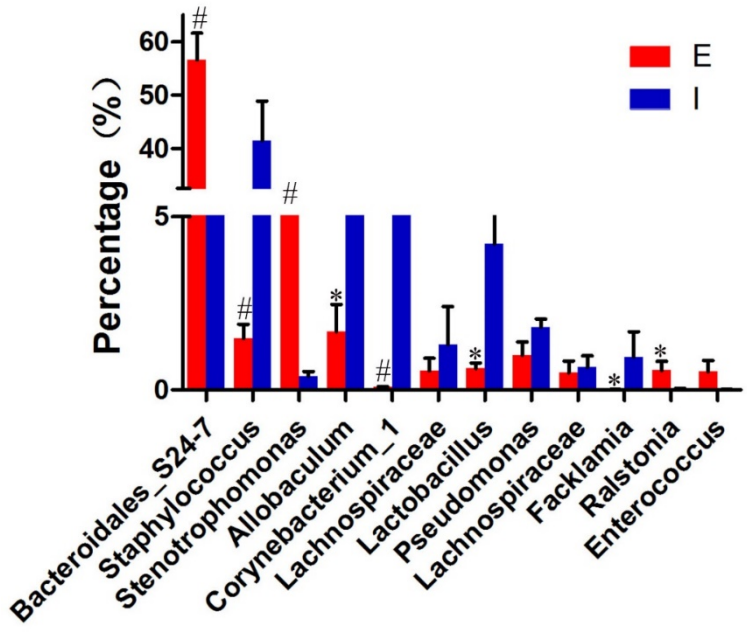
Extracellular DNA		Intracellular DNA	
Taxon	**Percentage, %**	**Taxon**	**Percentage, %**
Gram negative bacteria (92.3%)		**Gram negative bacteria (14.2%)**	
Bacteroidales_S24-7_ group_norank	46.4	Bacteroidales_S24-7_ group_norank	9.4
Stenotrophomonas	32.3	Stenotrophomonas	2
Prevotellaceae_UCG-001	1.4	Pseudomonas	1.8
Helicobacter	1.3	**Gram-positive bacteria （86.1%）**	
Pseudomonas	1.0	Staphylococcus	42.8
Ralstonia	0.5	Allobaculum	12
Acinetobacter	0.5	Corynebacterium_1	8.5
Gram-positive bacteria (1%)		Lachnospiraceae_ unclassified	4.8
Allobaculum	1.7	Lactobacillus	4.2
Staphylococcus	1.5	Jeotgalicoccus	1.5
Ruminococcaceae_UCG-014	1.1	Lachnoclostridium	1
Methylobacterium	0.6	Facklamia	0.9
Lactobacillus	0.6	Atopostipes	0.8
Lachnospiraceae_ uncultured	0.5	Turicibacter	0.7
Enterococcus	0.5	Mollicutes_RF9_norank	0.6
Roseburia	0.5	Ruminococcaceae_UCG-005	0.6
		Ruminococcaceae_UCG-014	0.6
		Lachnospiraceae_incertae _sedis	0.5



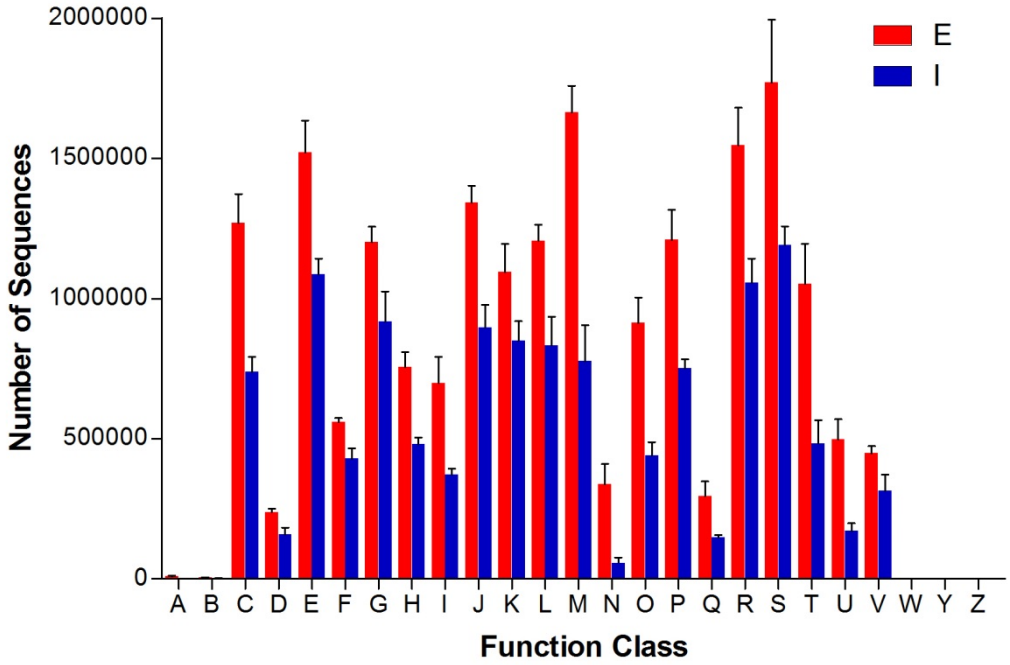
Supplementary Figure 1 Cluster analysis of TRFs profile. A and C referred to specific TRFs clustered in iDNA with AluI and DdeI respectively. B and D referred to specific TRFs clustered in eDNA with AluI and DdeI, respectively. E: Extracellular bacterial DNA; I: Intracellular bacterial DNA.



Supplementary Figure 2 Fluorescence *in situ* hybridization from small interstine with specific probes, Eub338, Bacteroidetes and Staphylococcus from left to right. Scale bar was 50 μm.



Supplementary Figure 3 Abundance of top 12 genera bacteria on mucus eDNA and iDNA. $^{*}P < 0.05$, $^{\#}P < 0.01$, compared with group of iDNA. E: Extracellular bacterial DNA; I: Intracellular bacterial DNA.



Supplementary Figure 4 Function abundance profile of predicted 16S gene COG from eDNA and iDNA. Note: A: RNA processing and modification; B: Chromatin structure and dynamics; C: Energy production and conversion; D: Cell cycle control, cell division, chromosome partitioning; E: Amino acid transport and metabolism; F: Nucleotide transport and metabolism; G: Carbohydrate transport and metabolism; H: Coenzyme transport and metabolism; I: Lipid transport and metabolism; J: Translation, ribosomal structure and biogenesis; K: Transcription; L: Replication, recombination and repair; M: Cell wall/membrane/envelope biogenesis; N: Cell motility; O: Posttranslational modification, protein turnover, chaperones; P: Inorganic ion transport and metabolism; Q: Secondary metabolites biosynthesis, transport and catabolism; R: General function prediction only; S: Function unknown; T: Signal transduction mechanisms; U: Intracellular trafficking, secretion, and vesicular transport; V: Defense mechanisms; W: Extracellular structures; Y: Nuclear structure; Z: Cytoskeleton. E: Extracellular bacterial DNA; I: Intracellular bacterial DNA.