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Supplementary Materials for

Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients

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This PDF file includes:

Materials and Methods
Figs. S1 to S28
Tables S1 to S9
References

Materials and Methods

Human studies

Patient cohort

An initial cohort of 112 patients with metastatic melanoma were included in this study (fig. S1 and table S1). All patients were treated with anti-PD-1 immune checkpoint blockade therapy at The University of Texas (UT) MD Anderson Cancer Center between April 2015 and May 2016 and signed voluntary informed consent for collection and analysis of tumor, blood and microbiome samples under Institutional Review Board (IRB)–approved protocols (IRB LAB00-063, 2012-0846, PA13-0291, PA15-0232, and PA12-0305). Patients who were diagnosed with uveal melanoma (n=10), or who received anti-PD-1 in combination with targeted agents or with adoptive T cell transfer therapy (n=7), or in whom response could not be determined (n=6) were excluded from this analysis. Electronic medical charts were reviewed independently by three investigators to assign clinical response group and document other clinical parameters. The primary outcome of clinical response (responder, R) was defined by radiographic evidence of complete response, partial response or stable disease per RECIST 1.1 criteria for at least 6 months. Lack of a clinical response (non-responder, NR) was defined by disease progression (PD) on serial CT scans or stable disease lasting less than 6 months.

Microbiome sample collection

Buccal samples were collected during routine pre-treatment clinic visits using the Catch-All Sample Collection Swab (Epicentre, Madison, WI). All patients were also provided an OMNIgene GUT kit (OMR-200) (DNA Genotek, Ottawa, Canada) for outpatient fecal sample collection. Importantly, this kit maintains microbial DNA stability at room temperature for up to 60 days. All samples were frozen at -80°C prior to DNA extraction and analysis.

Our final analysis cohort consisted of buccal samples collected from 86 patients, of whom 52 were R and 34 were NR, and fecal samples collected from 43 patients, of whom 30 were R and 13 were NR. All but 2 buccal and 2 fecal samples were collected at baseline. We included these as a baseline surrogate as a subset analysis on longitudinal samples showed no significant change after treatment intervention in this cohort.

Tumor and blood sample collection

Available tumor samples (n=21) at matched pre-treatment time points were obtained from the MD Anderson Cancer Center Department of Pathology archive and Institutional Tissue Bank. After samples underwent quality control checks for percent tumor viability by an MD Anderson pathologist, we included 15 samples from R and 6 from NR. Blood samples collected and stored for research (protocols previously listed) (n=11) were also queried for study inclusion yielding samples from 8R and 3NR.

DNA extraction and bacterial 16S rRNA sequencing

Sample preparation and sequencing was performed in collaboration with the Alkek Center for Metagenomics and Microbiome Research (CMMR) at Baylor College of Medicine. 16S rRNA gene sequencing methods were adapted from the methods developed for the NIH-Human Microbiome Project (*26, 39*). Briefly, bacterial genomic DNA was extracted using MO BIO PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA). The 16S rDNA V4 region was amplified by PCR and sequenced on the MiSeq platform (Illumina, Inc, San Diego, CA) using the 2x250 bp paired-end protocol yielding paired-end reads with near-complete overlap. The primers used for amplification contain adapters for MiSeq sequencing and single-end barcodes allowing pooling and direct sequencing of PCR products (*40*).

Quality filtered sequences with >97% identity were clustered into bins known as Operational Taxonomic Units (OTUs), using open-reference OTU picking, and classified at the species level against the NCBI 16S ribosomal RNA sequence database (release date February 11, 2017) using ncbi-blast+ package 2.5.0. Phylogenetic classification was obtained from the NCBI taxonomy database (release date February 16, 2017). The relative abundance of each OTU was determined for all samples. A step-by-step description of our analysis pipeline is included below:

Analysis pipeline

- Demultiplexing of fastq files from paired-end reads and barcodes was done by using the command ***split.libraries.fastq.py*** from QIIME.
- Merging of paired-end reads to create consensus sequences was done with the VSEARCH (*41*) command ***fastq_mergepairs***. A maximum of 10 mismatches were allowed in the alignment.
- Quality filtering and conversion of the .fastq file to .fasta format was done using the ***fastq_filter*** command in VSEARCH.
- The merged fasta file was then dereplicated in VSEARCH by full-length matching using the ***derep_fulllength*** command.
- All sequences were then sorted by decreasing length using the ***sortbylength*** command in VSEARCH. The minimum and maximum limits for this step were set at 200 and 350 base-pairs respectively.
- Next the ***sortbysize*** command was used to sort sequences by decreasing cluster size of representative sequences. The minimum cluster size allowed was 2, which resulted in the removal of singleton sequences.
- The ***cluster_otus*** command within USEARCH (*42*) was then used to perform 97% OTU-clustering using the UPARSE algorithm (*43*). The UPARSE algorithm allows for the construction of OTU sequences from pre-processed paired reads using greedy clustering, where input sequences are ordered by decreasing abundances such that the centroid of each OTU is selected from the most abundant reads. Further post-processing is still needed to map reads to OTUs in order to construct an OTU table. This step also allows for selection and exclusion of chimeras.
- Next the USEARCH algorithm was used (***usearch_global*** command) to search the database of merged reads for high identity hits and generate a .uc. file. It exploits the fact that similar sequences have several short words in common. If there is a tie, the tie is broken by choosing the first OTU in database file order. The reference database for this step is the previously generated representative OTU sequences. The identity threshold used for this step was 0.97.
- The mapped OTUs were converted into an OTU table using a series of python scripts summarized in ***uc2otutab.py***. The OTU table is a delimited text file that can be conveniently imported into a spreadsheet or parsed by a script.
- Representative OTU sequences were then classified at the species level from the NCBI 16S database using BLAST (Basic Local Alignment Search Tool). This step was done in R using the blastn UNIX executable and served as the database against which the original merged reads were mapped. Taxonomic assignment was validated using the mothur method with Greengenes (*44*), RDP (*45*), and SILVA (*46*).
- In order to estimate beta diversity, sequences were first aligned by the PyNAST method (*47*) using the ***align_seqs.py*** command in QIIME. ***filter_alignment.py*** was then used to filter the sequence alignment by removing the highly variable regions. The make_phylogeny.py script in QIIME was used to create the phylogenetic tree from multiple sequence alignment. Finally, the ***beta_diversity.py*** script was used to generate beta diversity distance matrices.
- In order to estimate alpha diversity the OTU table was first rarefied using the ***single_rarefaction.py*** command in QIIME. The rarefaction cutoff used was the total read count for the sample with the least number of reads. The ***alpha_diversity.py*** script in QIIME was then used to estimate alpha diversity.

Additionally, we performed validation of taxonomy assignment by classifying all identified OTUs within our fecal analysis cohort, using the mothur method (*48*), with the Greengenes (*44*), SILVA (*46*), and RDP databases (*45*), observing a high degree of concordance with NCBI taxonomies (table S9).

A phylogenetic tree was empirically constructed using the FastTree algorithm (*49*) in the QIIME software package (*50*), as described previously (*32*). Briefly, all nodes of the tree were considered as clusters of related OTU (crOTU), where the abundance of each crOTU was the sum of abundances of its member OTUs. The trees were constructed from a sequence alignment of all observed OTUs within both oral 1152 (97.5% of 1182 OTUs) and gut 1434 (98.6% of 1455 OTUs) microbiome samples. The resultant oral and gut microbiome crOTU trees contained 1152 and 1434 nodes respectively. We then performed simple hierarchical clustering of samples using Euclidean distances based on abundances of crOTUs within samples. This approach is based on the assumption that bacteria more closely related by evolutionary proximity, as determined by 16S sequence similarity, may share phenotypic traits (*51*), including traits that may be associated with clinical responses to cancer immunotherapy. It is analogous to examining effects at different phylogenetic levels (phylum, class, order, family, genus, species), but allows for a more comprehensive examination of possible bacterial groupings, including for example, subgenera, or subfamilies (fig. S28). The success of the clustering can be visualized in Fig. 3A (bottom) and fig. S14A, with notable taxonomical differences between patients in crOTU community type 1 vs crOTU community type 2. Furthermore, a distinct clustering effect was seen upon performing principal coordinate analysis on weighted UniFrac (*52*) distances by crOTU community type (fig. S14B).

Alpha diversity was estimated using the Inverse Simpson Index (D), which captures the variance of taxonomic abundance as $D=1/\sum_{k=1}^{S} \mathrm{pi}^2$, where pi is the proportion of the total species S that is comprised by the species i (*53*). Rarefaction limits were set based on the least number of reads in all oral (13,000) and fecal samples (8,000) that were analyzed. Results were also validated using other indices such as Chao1 (*54*), Simpson (*55*) and Shannon (*56, 57*).

Bipartite network to compare and contrast the oral and gut microbiota

The bipartite network was constructed using make_bipartite_network.py script in QIIME using default parameters (*50*) and then visualized in Cytoscape (*58*) using an edge-weighted spring-embedded layout. We generated 2 networks, using both aggregate and paired samples.

Enrichment Index to visualize differences in oral and gut microbiota between R and NR

To generate the heatmap we introduced a new parameter, the OTU enrichment index, "ei." The parameter is important to evaluate the enrichment when most OTUs in the dataset are rare, which is typical of sparse, compositional microbiome data. To calculate the index we first determined the representation of each OTU in each outcome (response) group. We refer to this value as the OTU representation index ("ri") and calculated it for each group, R (riR) and NR (riNR), as the proportion of samples that have non-zero abundance of the OTU. The values of ri range from 0 (OTU not found in any sample within a group) to 1 (OTU found in all samples within a group). The OTU enrichment index (ei) is then calculated from the ri values as:

$$\mathrm{ei} = (\mathrm{riR} - \mathrm{riNR}) / (\mathrm{riR} + \mathrm{riNR})$$

The ei index takes values from -1 to +1. An OTU found in all R samples but no NR sample takes ei=+1. Conversely, an OTU found in all NR samples but no R sample takes ei=-1.

We further used the ei to visualize differences between R and NR as a heatmap of OTU abundances where samples are shown in columns and OTUs in rows. We grouped samples according to response (R and NR), sorting within each group by alpha diversity, from high to low. OTUs (rows) were grouped into 3 sets according to their ei using thresholds determined from the overall ei distribution (fig. S9); Set 1 was comprised of OTUs enriched in R ($\mathrm{ei}>0.3$ for oral microbiome, $\mathrm{ei}>0.5$ for fecal microbiome), Set 2 included OTUs that were not differentially enriched in either response category ($-0.3 \leq \mathrm{ei} \leq 0.3$ for oral microbiome and $-0.5 \leq \mathrm{ei} \leq 0.5$ for fecal microbiome), and Set 3 was comprised of OTUs enriched in NR ($\mathrm{ei}<-0.3$ for oral microbiome, $\mathrm{ei}<-0.5$ for fecal microbiome). Within each set, OTUs were then sorted by average abundance prior to plotting. OTU abundance in each sample was designated by mapping abundance (low, medium, high) to color (green, yellow, red), using thresholds derived from the overall abundance distribution for all OTUs. We then explored the taxonomy of OTUs grouped together within each ei-derived set by summarizing taxa at the order level as shown in accompanying pie charts (Fig. 2B). A major utility of the enrichment index is that it allows visualization of both rare and common OTUs, and therefore allows for the appreciation of biologically-relevant, community-level effects (both cooperative and antagonistic).

Statistical assessment of biomarkers using LEfSe

The LEfSe method of analysis (*31*) first compares abundances of all bacterial clades (in this case between R and NR), using the Kruskal-Wallis test at a pre-defined α of 0.05. Significantly different vectors resulting from the comparison of abundances (e.g., *Faecalibacterium* relative abundance) between groups are used as input for linear discriminant analysis (LDA), which produces an effect size. The primary advantage of LEfSe over traditional statistical tests is that an effect size is produced in addition to a p-value. This allows sorting of results of multiple tests by the magnitude of the difference between groups. In the case of hierarchically-organized bacterial clades, there may be a lack of correlation between p-values and effect sizes due to differences in the number of hypotheses considered at different levels since a greater number of comparisons would need to be made at the genus and species levels when compared to the phylum and class levels.

Metagenomic whole genome shotgun (WGS) sequencing

Metagenomic sequencing provides species-level resolution of bacteria, and depending on sequence depth can quantify the near-complete genomic content of the collection of microbes in a particular sample, referred to as the sample's metagenome.

Whole genome shotgun (WGS) sequencing utilized the same extracted bacterial genomic DNA used for 16S rRNA gene compositional analysis and was done in collaboration with the CMMR and Metagenopolis (MGP). Briefly, individual libraries were constructed from each sample and loaded into the HiSeq platform (Illumina) and sequenced using the 2x100 bp paired-end read protocol. The process of quality filtering, trimming, and demultiplexing was carried out by an in-house pipeline developed by assembling publicly available tools such as Casava v1.8.3 (Illumina) for the generation of fastq files, bbduk for adapter and quality trimming, and PRINSEQ for sample demultiplexing.

Gut microbiota analysis was performed using the quantitative metagenomics pipeline developed at MGP. This approach allows the analysis of the microbiota at the gene and species level. High quality reads were selected and cleaned to eliminate possible contaminants such as human reads. These were mapped and counted using the 9.9M gene integrated reference catalog of the human microbiome (*59*). Filtered high-quality reads were mapped to the 9.9 million-gene catalogue using Bowtie 2 (*60*) included in METEOR software (*61*) using a >95% identity threshold to account for gene variability and for the non-redundant nature of the catalogue. The gene abundance profiling table was generated using a two-step procedure: first, the unique mapped reads (reads mapped to a unique gene in the catalogue) were attributed to their corresponding genes, then, the shared reads (reads that mapped with the same alignment score to multiple genes in the catalogue) were attributed according to the ratio of their unique mapping counts.

After a rarefaction step at 14M reads (to correct for the different sequencing depth) and normalization (RPKM), a gene frequency profile matrix was obtained which was used as the reference to perform the analyses using MetaOMineR, a suite of R packages developed at MGP and dedicated to the analysis of large quantitative metagenomic datasets (*62*).

The 9 million gene catalogue has been clustered into 1436 MGS (Metagenomic Species), groups of >500 genes that co-vary in abundance among hundreds of samples and thus belong to the same microbial species (*63*). The taxonomical annotation of the MGS was performed using the homology of its genes with previously sequenced organisms (using blastN against nt and wgs databanks, NCBI, November 2016 version). MGS signal among samples was calculated as the mean signal of the 50 genes defining a robust centroid of the cluster. A MGS frequency profile matrix was constructed using the MGS mean signals after normalization (sum of the MGS frequency of a sample =1).

Reads whose genomic coordinates overlap with known KEGG orthologs were tabulated, and KEGG modules were calculated step-wise and determined to be complete if 65% of the reaction steps were present per detected species and for the metagenome. Pathways were constructed for each taxa and metagenome by calculating the minimum set through MinPath resulting from the gene orthologs present.

Pathway Metagenome Databases (PGDB)

Databases were generated for each WGS sample using the PathoLogic program from the Pathway Tools software. Inputs for the program were produced using predicted gene functions based on KEGG orthology and their taxonomy assignment in the metagenomes. Thus, if the same function (KO group) had several taxonomic annotations, each annotation was considered as a separate gene. The definition of the KO group in KEGG was used as the gene function and the first name of the KO group, if available, was used as the gene name. EC numbers were assigned to the gene according to annotations of the KO group by the numbers in KEGG. The metabolic reconstructions were made in the automatic mode as described in the Pathway Tools manual with the option –tip to automatically predict transport reactions. We used the DOMAIN value 'TAX-2 (Bacteria)' as the organism class for the PGDB and the CODON-TABLE value to be equal to 1. The generated PGDB were summarized and compared using Pathway Tools (table S7).

Whole exome sequencing

Genomic DNA was extracted from 10 tumor samples and matched peripheral blood as germline controls. Exome capture was performed on 500ng of DNA per sample based on the Kapa Hyper Prep using the Agilent Human All Exon baits V3 kit according to the manufacturer's instructions. Whole exome sequencing was performed on the Illumina HiSeq 2500 sequencing platform. The average coverage for whole exome sequencing data was 247× in tumor samples and 111× in germline samples. Paired-end sequencing reads in fastq format were generated from BCL raw data using Illumina CASAVA (Consensus Assessment of Sequence and Variation) software. The reads were aligned to the hg19 human reference genome using BWA (*64*). Duplicate reads were removed using Picard tools (unpublished, http://broadinstitute.github.io/picard/), and local realignments were performed using GATK (*65*). The average mapping ratio was 95.8% in tumor samples and 96.8% in germline samples. The BAM files were then used for downstream analysis.

Mutation calling

MuTect (*66*) was used to identify somatic point mutations, and Pindel (*67*) was used to identify somatic insertions and deletions. A series of post-calling filters were applied including: (a) total read count in tumor sample ≥20, (b) total read count in germline sample ≥10, (c) VAF (Variant Allele Frequency) ≥0.02 in tumor sample and ≤0.02 in matched normal sample, (d) a population frequency threshold of 1% was used to filter out common variants in the databases of dbSNP129 (*68*), 1000 Genome Projects (*69*), Exome Aggregation Consortium (*70*) and ESP6500 (*71*). Driver mutations were identified by reference to a published list of driver genes and mutations of cutaneous melanoma from Zhang *et al* (*72*).

Immunohistochemistry

Immunohistochemistry was performed as described previously (*18*). Briefly, sections (4µm thickness) were prepared from formalin-fixed paraffin-embedded (FFPE) tissues. The presence of tumor was confirmed by a pathologist on hematoxylin & eosin-stained slides (H&E). Slides were then stained using a Leica Bond RX automated slide stainer (Leica Biosystems, Buffalo Grove, IL) for CD3 (n=17)(1:100, DAKO, Santa Clara, CA), CD8 (n=21)(1:100, Thermo Scientific, Waltham, MA), PD-1 (n=16)(1:250, Abcam, Cambridge, UK), PD-L1 (n=15)(1:100, Cell Signaling, Danvers, MA), GzmB (n=17; ready-to-use, Leica), RORγT (n=14)(1:800, EMD Millipore, Billerica, MA), FoxP3 (n=16)(1:50, BioLegend, San Diego, CA) and counter-stained with hematoxylin. Stained slides were then scanned using an automated Aperio Slide Scanner (Leica), and the density of the immune infiltrate was quantified in tumor regions using a modified version of the default "Nuclear v9" algorithm and expressed as positive counts/mm2 for CD3, CD8, PD-1, FoxP3, GzmB and RORγT and as an H-score for PD-L1 which takes into account a percentage of positive cells multiplied by their intensity on a scale of 1 to 3 for a score between 1-300.

Flow cytometry

Flow cytometry was performed as described previously (*73*). In short, peripheral blood mononuclear cells (PBMC) were stained with CD3 (UCHT1, BioLegend), CD4 (SK3, eBioscience, Thermo Scientific), CD8 (RPA- T8, BD Biosciences, Mississauga, Canada), FoxP3 (PCH101, eBioscience), CD127 (HIL-7R-M21, BD Biosciences), CD19 (HIB-19, BioLegend), CD14 (61D3, eBioscience), HLA-DR (L243, BD Biosciences), CD33 (WM53, BD Biosciences), CD56 (NCAM1, BD Biosciences), and CD11b (ICRF44, BD Biosciences) and acquisition was carried out on a Fortessa Flow Cytometer (BD Biosciences). Analysis was performed with FlowJo version 10 (Tree Star Inc., Ashland, OR).

Multiplex immunohistochemistry

Sequential 12-marker myeloid multiplex immunohistochemistry was performed as described previously (*38*). Briefly, FFPE sections underwent sequential staining, scanning and destaining cycles using AEC as chromagen and scanned with an Aperio Slide Scanner (Leica Biosystems). Staining was performed with hematoxylin (DAKO), CD68 (1:50, Abcam), Tryptase (1:20000, Abcam) CSF1R (1:150, Abcam), DC-SIGN (Santa Cruz, Dallas, TX, 1:100), CD66b (1:600, eBioscience), CD83 (1:40, Abcam), CD163 (Thermo Scientific, 1:100), HLA- DR (1:100, Novus Biological, Littleton, CO), PD-L1 (1:100, Cell Signaling), CD3 (1:150, Thermo Scientific), CD20 (1:1000, Santa Cruz), CD56 (1:25, Santa Cruz), and CD45 (1:100, Thermo Scientific). CD45-positive regions of all images were then extracted using ImageScope (Leica Biosystems), aligned, overlayed and segmented using CellProfiler (Cambridge, MA) and layers were pseudo-colored for analysis and quantification using FCS Express 6.0 Plus Research Edition (De Novo Software, Glendale, CA).

Cytokine multiplexing

41 plasma cytokine levels were assessed using multiplex bead assay (Bio-Rad, Hercules, CA). Cytokines, chemokines and soluble mediators quantified included IL-1β, IL-1Rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17, Eotaxin, FGF basic, G-CSF, GM-CSF, IFN-γ, IP-10, MIP-1α, PDGF-bb, MIP-1β, RANTES, TNF-α, VEGF, IL-2Rα, HGF, TRAIL, IL-17A, IL-17F, IL-23, SDF1/CXCL12, CCL22, MCP-1/CCL2, Gro-α/CXCL1, ENA78/CXCL5, EGF, TGF-β1, TGF-β2, and TGF-β3.

Statistical analyses

Alpha diversity was compared between R and NR using the Wilcoxon rank-sum or Mann-Whitney (MW) test. All patients were classified into high, intermediate or low diversity groups based on tertiles of distribution. Time to progression was defined as the interval (in days) from the date of microbiome sample collection to the date of progression. Patients who did not progress were censored at their date of last contact. Kaplan-Meier estimates of progression-free survival (PFS) were estimated for each group, and compared using the log-rank test. Hazard ratios and 95% confidence intervals were calculated using univariate and multivariate Cox proportional hazards models to assess associations between time to progression and features of the fecal microbiomes along with several clinical factors. Final models were selected using forward stepwise selection methods. All assumptions for the Cox models were met. The ability of clinical and significant bacterial variables in predicting PFS was assessed by computing receiver operating characteristic (ROC) curves using the "timeROC" package (*74*) in R. Area under the curve (AUC) values were calculated at t=50 days for these variables alone and in combinations with the "Cox" weighting option.

Pairwise comparisons of taxonomic abundances by both response and cluster were also conducted using the MW test. Within each level (phylum: n=3, class: n=3, order: n=3, family: n=7, genus: n=8, and species: n=9), we excluded the low abundance (<0.1%) and low variance taxa (<0.01). Adjustments for multiple comparisons were done using the false-discovery rate (FDR) method at an α level of 0.05. An effect size was estimated for each taxon as $U/\sqrt{n}$, where U is the test statistic for the MW test, and n is the total sample size for fecal samples (n=43) (*75*). Volcano plots were generated for $\log_{10}$(FDR-adjusted *p*-values) on the y-axis and median-adjusted effect sizes on the x-axis. In addition, patients were also classified as having high or low abundance of *Faecalibacterium* or Bacteroidales based on the median relative abundance of these taxa in the gut microbiome sample.

In general the MW test was used for comparisons between binary outcome variables (R vs NR), and the Spearman rank correlation test was used to compare continuous variables. Additionally, the Fisher's exact test was used when proportions were compared between binary variables. Hypothesis testing was done using both one-sided and two-sided tests as appropriate at a 95% significance level. All analyses were conducted in R (*76*), GraphPad Prism (La Jolla, CA) and SAS version 9.4 (Cary, NC).

Murine studies

Fecal microbiota transplantation (FMT)

All animal studies were approved by the Animal Care and Use Committee at The UT MD Anderson Cancer Center, in compliance with the *Guide for the Care and Use of Laboratory Animals* (*77*). B6 germ-free mice for murine studies were provided by the gnotobiotic facility of Baylor College of Medicine (Houston, TX). Mice were transported in specialized autoclaved shipping cages, and were housed at the MD Anderson Cancer Center mouse facility. All cages, bottles with stoppers and animal drinking water were autoclaved before being used. Food and bedding were double irradiated and tested to ensure sterility prior to being used in the experiment. Within each treatment category, a control group of mice received only pre-reduced PBS. All other mice from experimental groups received FMT from either R or NR donors, with each donor sample delivered to 3 mice. 200µl cleared supernatant from 0.1 g/µl human fecal suspension was obtained using a 100 µm strainer and gavaged into mice for 3 doses over 1 week, followed by a break of 1 week to allow microbiome establishment. Mice were then injected with the $BRAF^{V600E}/PTEN^{-/-}$ (BP) syngeneic tumor cell line (*78*) (Day 14), and animals were treated with anti-PD-L1 monoclonal antibody (purified low endotoxin, functional formulation, Leinco Technologies Inc., Fenton, MO) once tumors reached ~250-500 mm^3. Tumor growth and survival were assessed and fecal specimens, blood and tumors were harvested and processed for further analysis.

Immunofluorescence and immunohistochemistry on FFPE samples

Xenograft tumors, mouse gut and spleens were harvested, fixed in buffered 10% formalin (4 hours at room temperature), then switched to 70% ethanol and stored at 4°C. Tissues were embedded in paraffin and 5 µm sections were mounted on positively charged slides. Next, tissues were deparaffinized and antigen retrieval was performed in pH 6.0 Citrate buffer (Dako) using a microwave. Sections were then blocked in blocking buffer (5% Goat Serum/0.3% BSA/0.01%Triton in PBS) followed by primary antibody incubation overnight at 4°C. Finally, sections were washed and then incubated with Alexa-conjugated secondary antibody (1:500, Molecular Probes) for 1 hour at room temperature. Coverslips were washed 3 times in PBS/0.01% Triton and then incubated for 15 minutes at room temperature in Hoechst stain (1:5000, Invitrogen) then mounted in ProLog Diamond mounting media (Molecular Probes). Images where captured using a Nikon A1R+ confocal microscope equipped with a four solid state laser system and a 20× objective. IHC for FoxP3+ (FJK-16s, eBioscience) Treg and and RORγT+ (Q31-378, BD Pharmingen) Th17 cells was quantified by counting positive cells in five random 1 mm2 areas. The average total number of cells positive for each marker was expressed as density per mm2.

Cytometry by Time-Of-Flight (CyTOF)/Mass cytometry

Tumors were manually dissociated, digested using liberase TL (Roche) and DNase I for 30 minutes at 37°C, and passed through a 70 µm mesh filter. Samples were then centrifuged using a discontinuous gradient of Histopaque 1119 (Sigma-Aldrich) and RMPI media. Single cell suspensions of up to 2.5 x 10^6 cells per sample were Fc-receptor blocked and stained with a surface antibody mixture for 30 minutes at 4°C. Metal-conjugated antibodies were purchased from Fluidigm or conjugated using X8 polymer antibody labeling kits according to the manufacturer's protocol (Fluidigm). Samples were stained using 2.5 µM ^{194}Pt-cisplatin (Fluidigm) for 1 minute and washed twice with 2% FCS PBS. Cells were barcoded using a palladium mass tag barcoding approach according to the manufacturer's protocol (Fluidigm) and combined after two washes with 2% FCS PBS. Cells were then fixed and permeabilized using FoxP3 transcription factor staining kit according to the manufacturer's protocol (eBioscience). Samples were then stained using a mixture of antibodies against intracellular targets for 30 minutes at room temperature. Samples were washed twice with 2% FCS PBS and then incubated overnight in a 1.6% PFA/100 nM iridium/PBS solution prior to acquisition using a Helios mass cytometer (Fluidigm).

Mass cytometry data were bead normalized and debarcoded using Fluidigm software. Total live and CD45+ cells were manually gated using FlowJo. Dimension reduction (t-SNE) analyses were performed on total live and CD45+ cells using the Cyt package in Matlab (*79*). Data were arcsinh transformed using a coefficient of 4 and randomly down-sampled to 50,000 events per sample prior to t-SNE analysis. t-SNE plots for each experimental group were then generated by merging samples from each group and displaying an equal number of randomly down-sampled events (50,000) from each treatment group.

CyTOF panel:

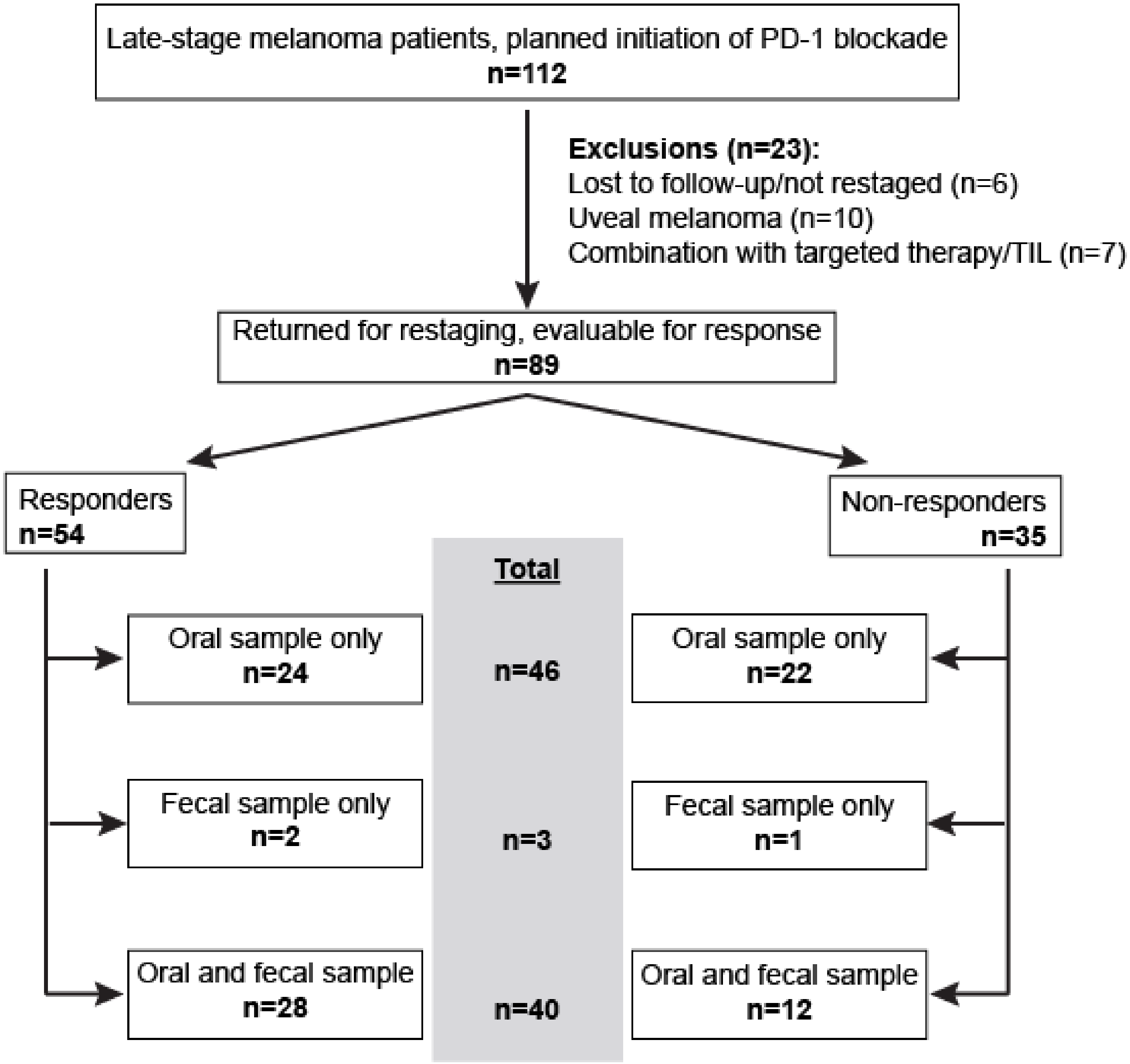
Target	**Clone**	**Metal Tag**	**Stain**	**Source**
CD45	30-F11	89Y	Surface	Fluidigm
c-MYC	9E10	115In	Intracellular	eBioscience
MHC-II	M5/114.15.2	139La	Surface	Biolegend
NK1.1	PK136	141Pr	Surface	Biolegend
CD11c	N418	142Nd	Surface	Biolegend
CD80	16-10A1	143Nd	Surface	Biolegend
MHC-I	28-14-8	144Nd	Surface	Fluidigm
CD4	RM4-5	145Nd	Surface	Fluidigm
CD8a	53-6.7	146Nd	Surface	Fluidigm
CD86	GL-1	147Sm	Surface	Biolegend
CD27	LG.3A10	148Nd	Surface	Biolegend
OX40	OX-86	149Sm	Surface	eBioscience
CD25	3C7	150Nd	Surface	Fluidigm
TIGIT	1G9	151Eu	Surface	Biolegend
CD3ε	145-2C11	152Sm	Intracellular	Fluidigm
PD-L1	10F.9G2	153Eu	Surface	Fluidigm
BATF	D7C5	154Sm	Intracellular	Fluidigm
ICOS	7E.17G9	155Gd	Surface	eBioscience
CD69	H1.2F3	156Gd	Surface	Biolegend
CXCR5	2G8	158Gd	Surface	BD
PD-1	29F.1A12	159Tb	Surface	Fluidigm
CD62L	MEL-14	160Gd	Surface	Fluidigm
CXCR3	CXCR3-173	161Dy	Surface	Biolegend
TIM3	RMT3-23	162Dy	Surface	Fluidigm
LAG3	C9B7W	163Dy	Surface	Biolegend
LAP-TGFβ	TW7-16B4	164Dy	Surface	Fluidigm
FoxP3	FJK-16s	165Ho	Intracellular	Fluidigm
BCL2	BCL/10C4	166Er	Intracellular	Biolegend
GATA3	L50-823	167Er	Intracellular	BD
BCL6	K112-91	168Er	Intracellular	BD
CD117	2B8	169Tm	Surface	Biolegend
CD127	A7R34	170Er	Surface	Biolegend
CTLA-4	UC10-4B9	171Yb	Intracellular	Biolegend
CD11b	M1/70	172Yb	Surface	Fluidigm
TBET	4B10	173Yb	Intracellular	Biolegend
RORγT	Q31-378	174Yb	Intracellular	BD

CD28	37.51	175Lu	Surface	Biolegend
EOMES	Dan11mag	176Yb	Intracellular	eBioscience
Live/Dead	N/A	194Pt	Surface	Fluidigm
CD19	6D5	195Pt	Surface	Biolegend
TCRγδ	GL3	196Pt	Surface	Biolegend
KLRG1	2F1	198Pt	Surface	BD
CD44	IM7	209Bi	Surface	Fluidigm

Flow cytometry of mouse tumor and blood

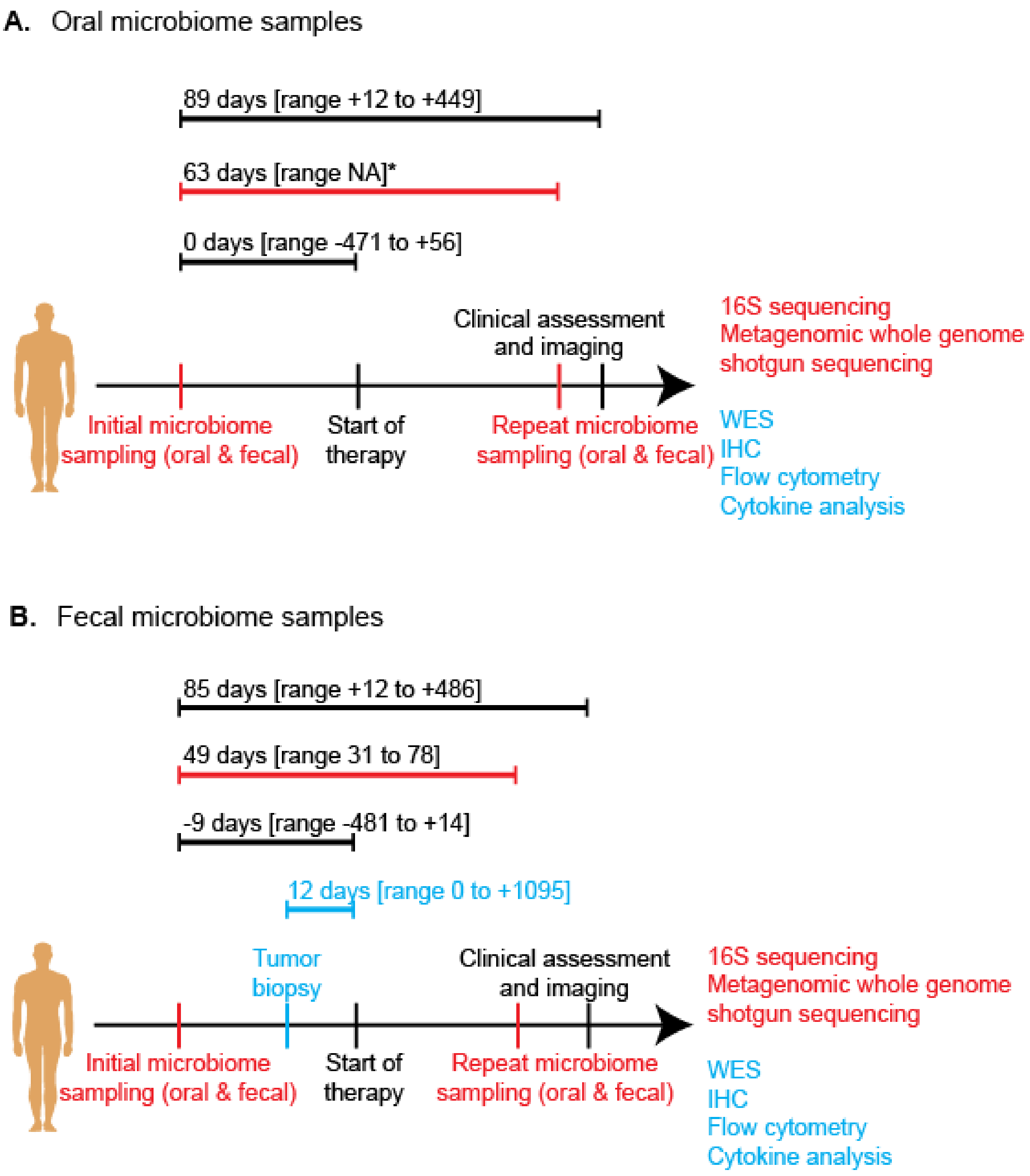
Tumors were isolated and minced into small pieces and digested for 1 hour in RPMI containing collagenase A (2 mg/mL; Roche) and DNase I (40 units/mL; Sigma-Aldrich) with agitation at 37°C. Cell suspensions were passed through a 70μm cell strainer, washed in 2% RPMI supplemented with 2 mmol/L EDTA and resuspended in FACS buffer (PBS containing 2% heat-inactivated FBS with 2 mM EDTA supplementation). For analysis of cell surface markers, the following antibodies were used: CD45 (30-F11, BD Biosciences), CD11b (M1/70, eBioscience), CD11c (HL3, BD Pharmigen), Ly6G (RB6-8C5, eBioscience), Ly6C (AL-21, BD Bioscience), and F4/80 (BM8, eBioscience). Cells were labeled with LIVE/DEAD viability stain (Life Technologies) and samples were acquired on a LSR Fortessa X20 flow cytometry (BD Biosciences). Doublets were distinguished and excluded by plotting FSC area versus FSC height. All data were analyzed using FlowJo software (Tree Star).

Supplementary Figure S1
Gopalakrishnan et al.



Supplementary figure S1: Microbiome sample acquisition schema. Oral and gut microbiome sample collection in melanoma patients initiating treatment with PD-1 blockade. Oral microbiome samples were collected at the time of clinic visits, whereas fecal microbiome samples were collected on an outpatient basis.

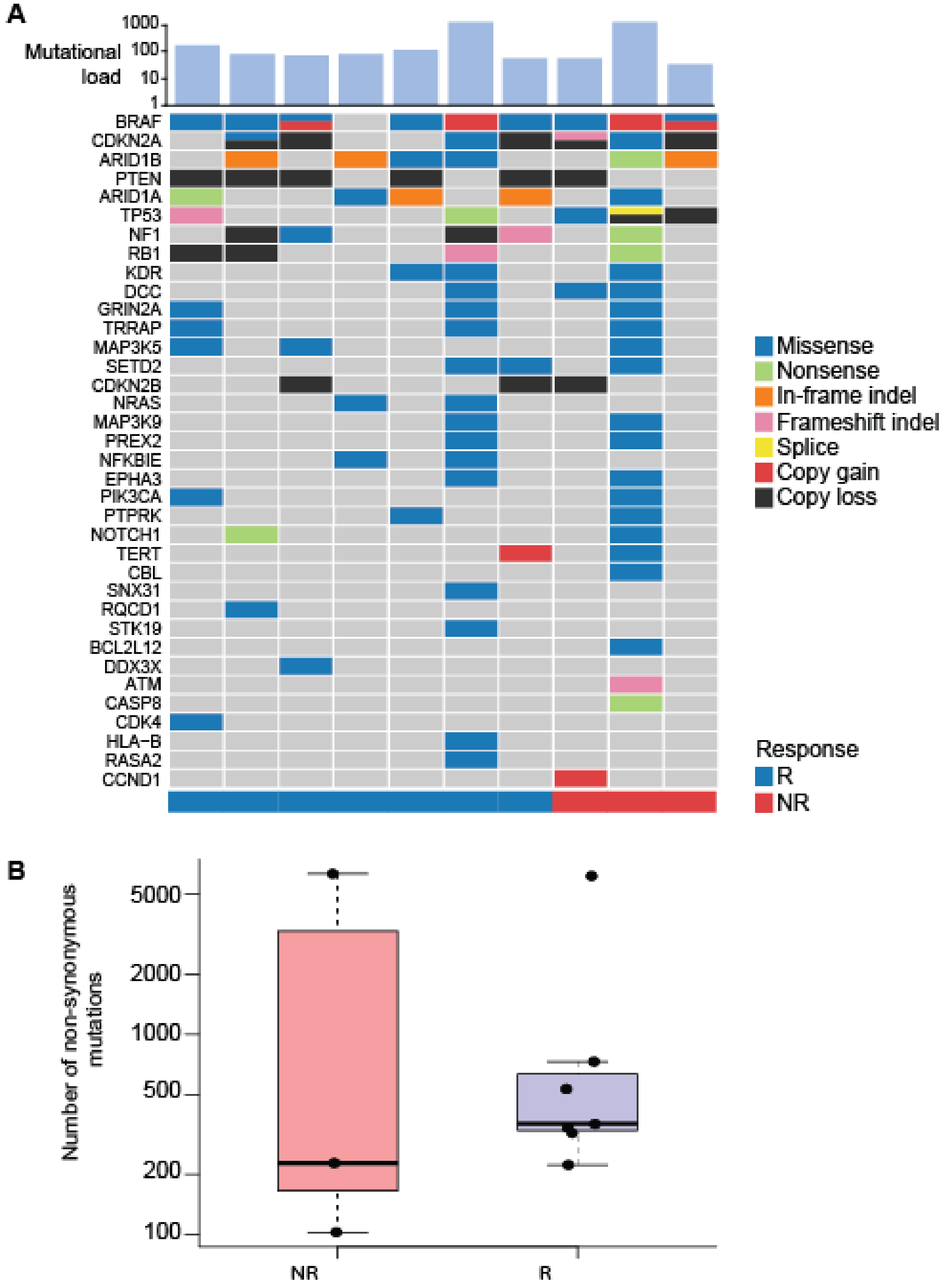
Supplemental Figure S2
Gopalakrishnan et al.



Supplementary figure S2: Sample collection timeline. Median time and range (parentheses) in days between important clinical events associated with patients that contributed (**A**) oral and (**B**) fecal microbiome samples. *Longitudinal oral microbiome samples were collected from only one patient.

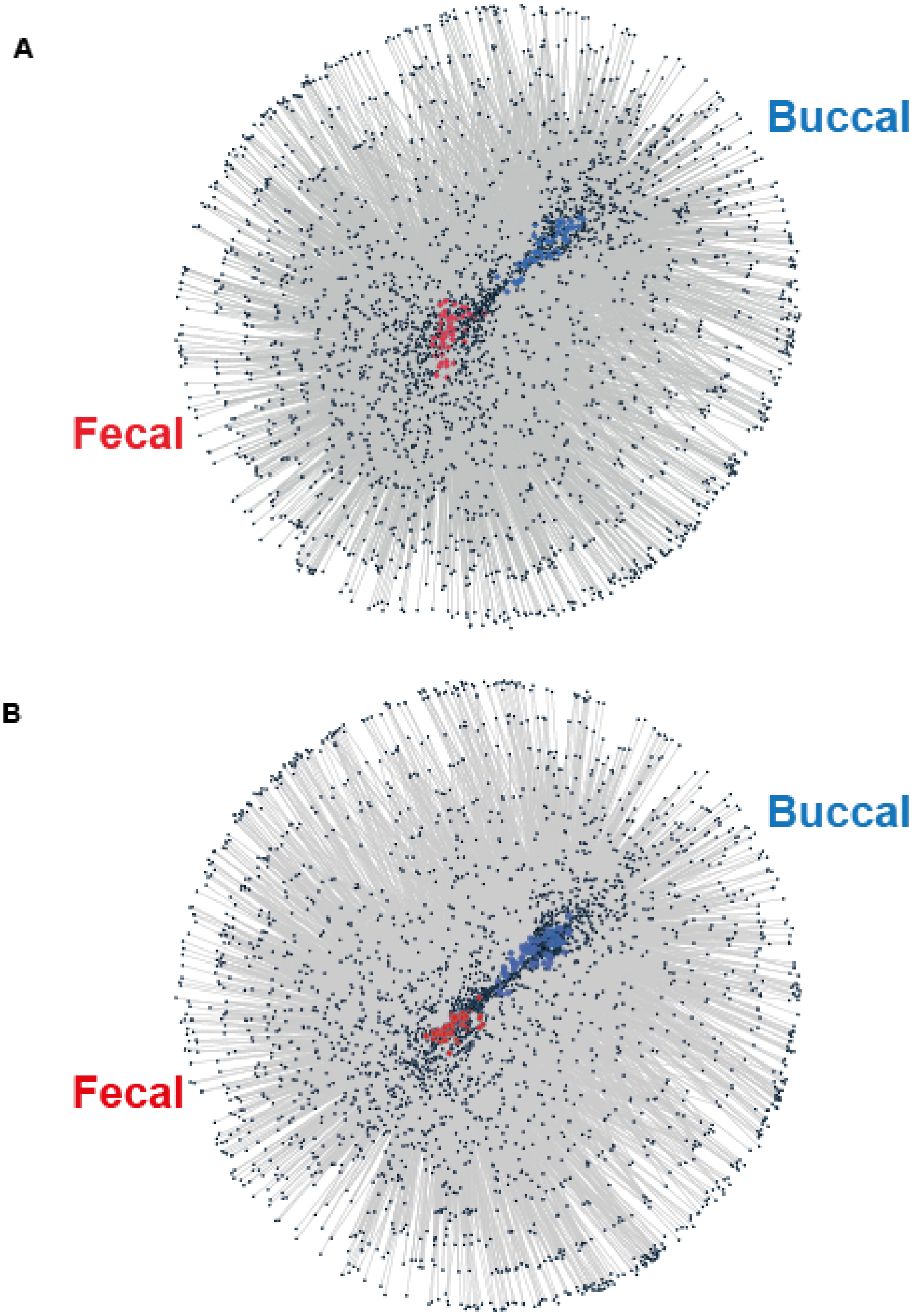
Supplemental Figure S3

Gopalakrishnan et al.



Supplementary figure S3: No differences were observed in the mutational landscape of R (n=7) and NR (n=3) to PD-1 blockade in a small subset of patients. (**A**) Number of mutations per megabase and landscape of driver mutations; blue=R, red=NR. (**B**) Total non-synonymous mutational burden in available tumors (p=0.52), in tumors of patients with matched fecal microbiome samples by two-sided Mann-Whitney (MW) test.

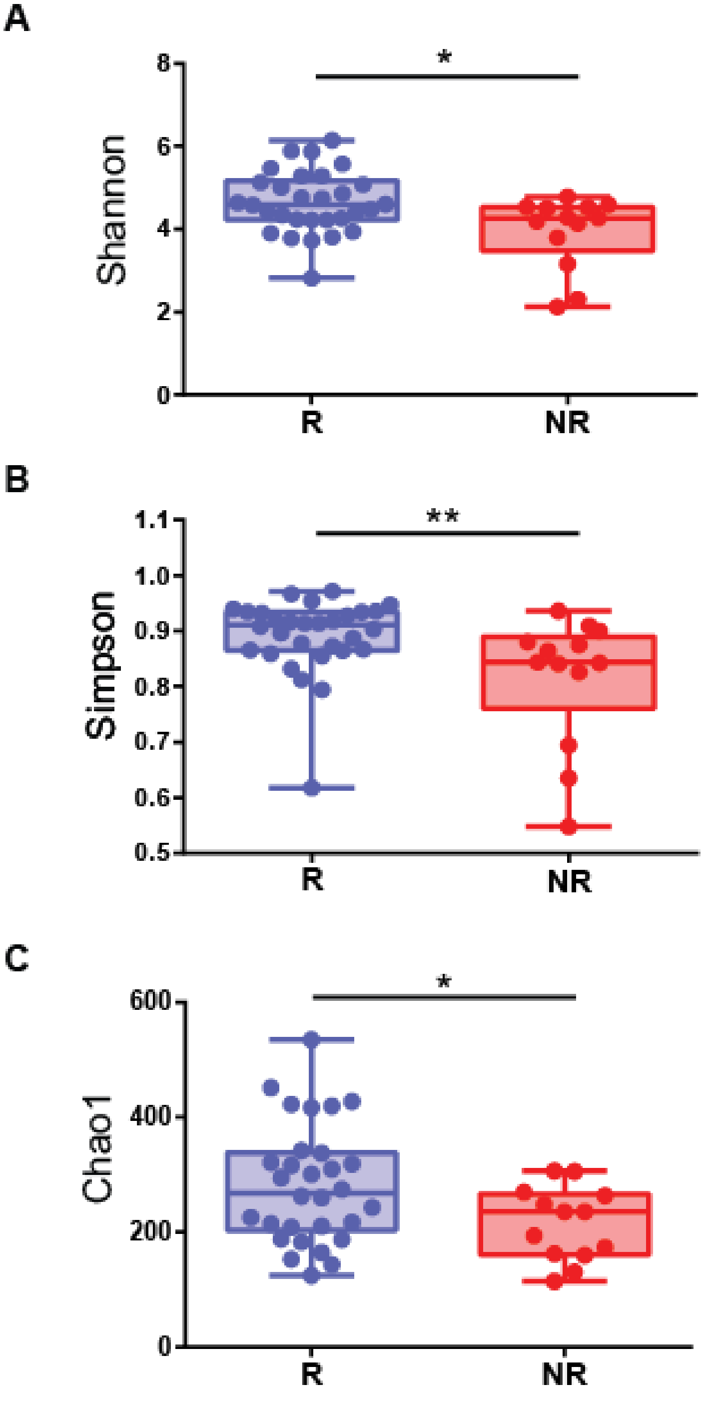
Supplementary Figure S4
Gopalakrishnan et al.



Supplementary figure S4: Differences in community structure between the oral and fecal microbiomes. (**A**) Bipartite network diagram of *matched* oral (blue) and fecal (red) samples from 48 anti-PD-1 treated patients. (**B**) Analogous bipartite network diagram of bacterial 16S rRNA derived operational taxonomic units (OTUs) from 109 buccal and 53 fecal samples. Edges connect species-level OTUs (blue diamonds) to sample nodes from oral (blue circles) and fecal (red circles) samples in which they are found.

Supplementary Figure S5

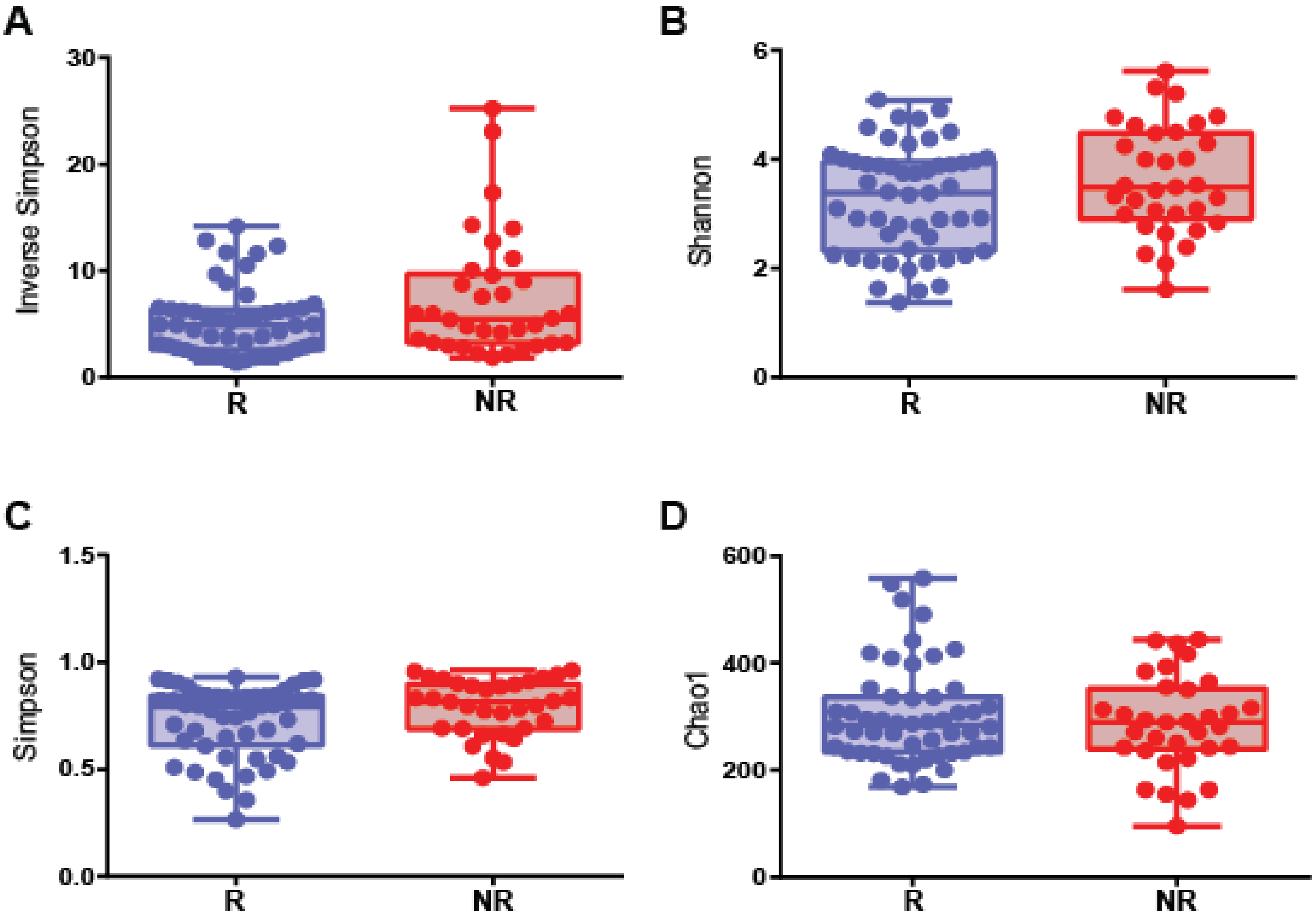
Gopalakrishnan et al.



Supplementary figure S5: Diversity of the fecal microbiome is increased in R to PD-1 blockade. Comparison of alpha diversity scores in R (n=30, blue) and NR (n=13, red) using the (**A**) Shannon, (**B**) Simpson and (**C**) Chao1 indices by MW test. * $p<0.05$, ** $p<0.01$.

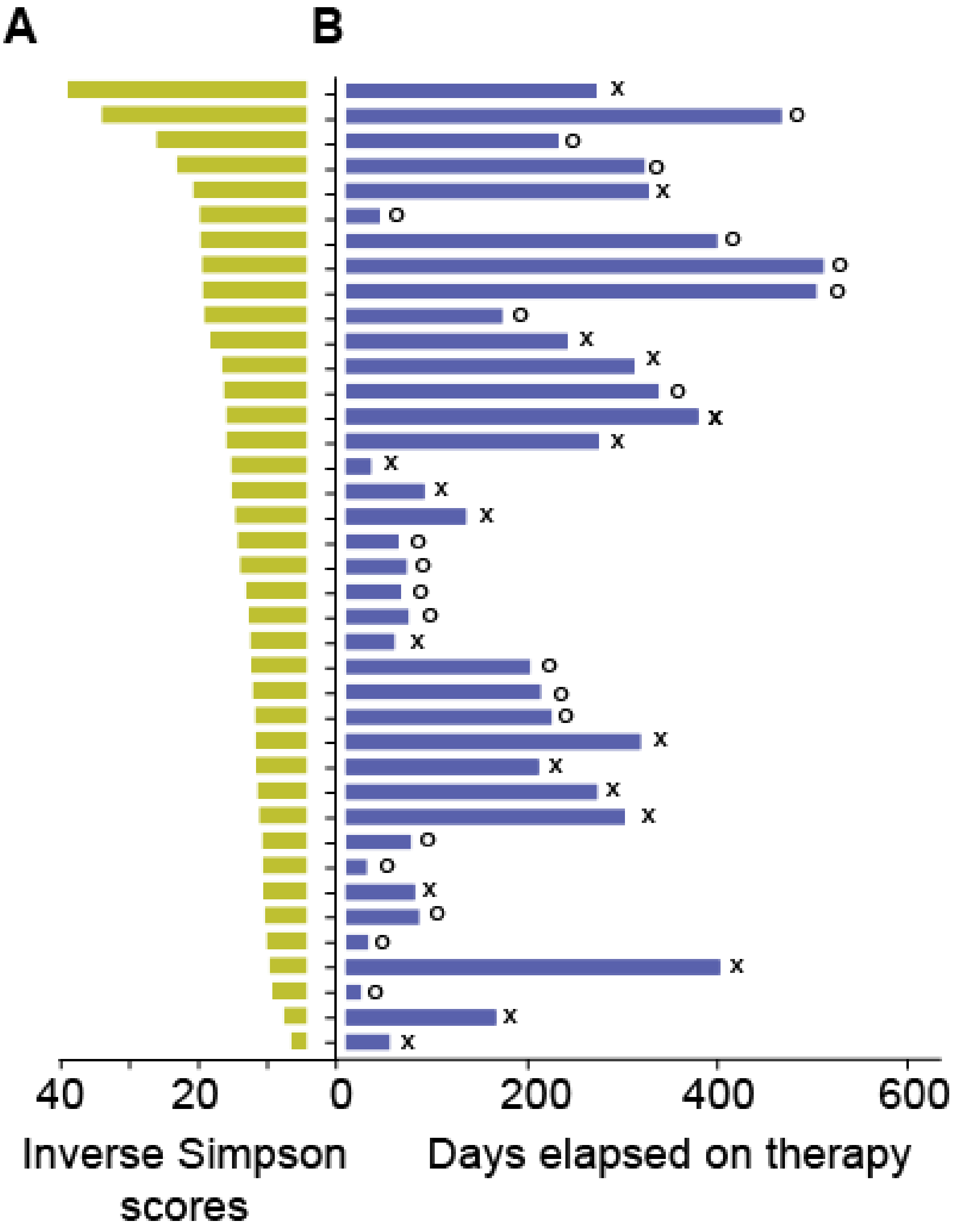
Supplementary Figure S6

Gopalakrishnan et al.



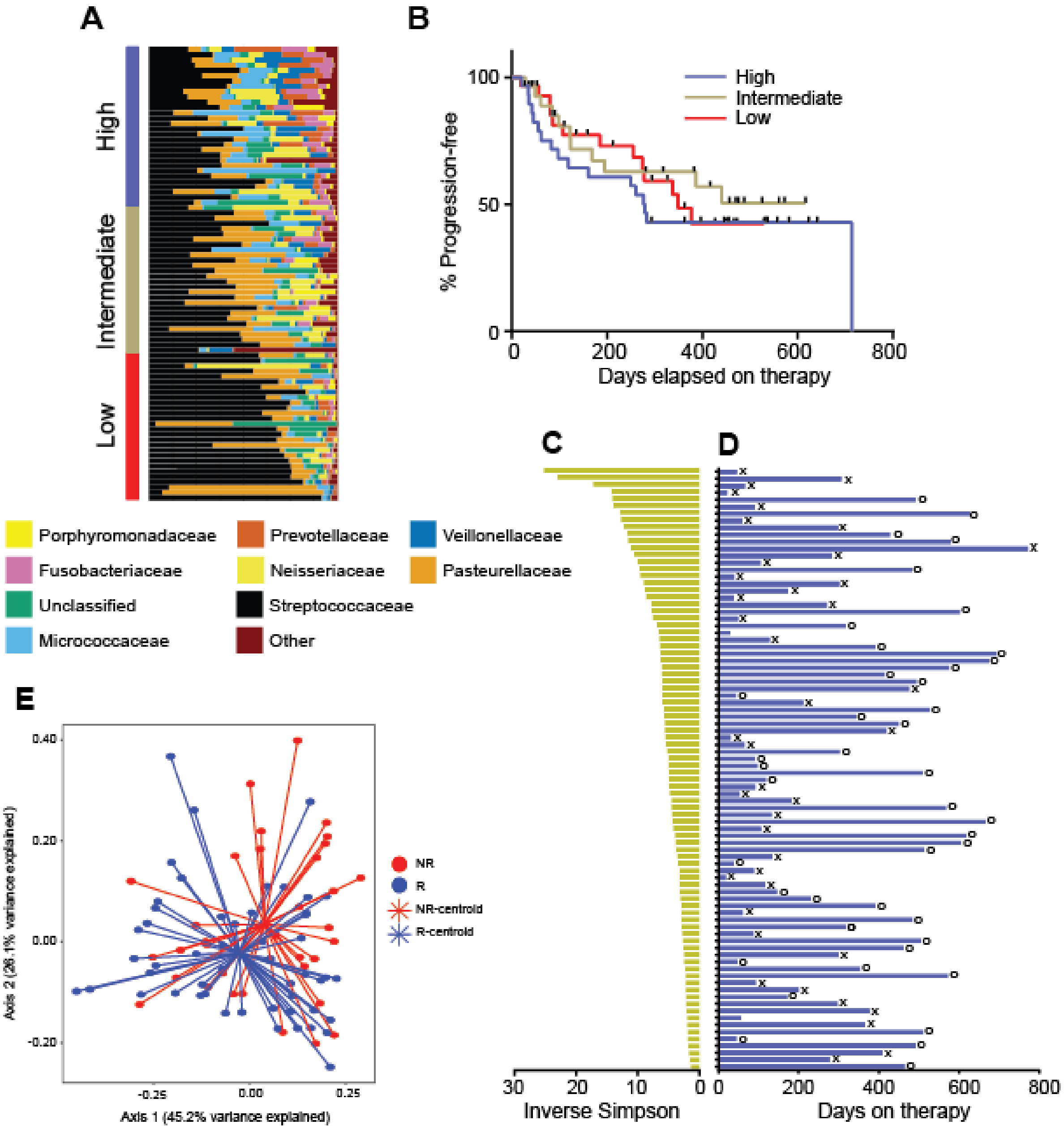
Supplementary figure S6: No differences are observed in the diversity of the oral microbiome between R and NR to PD-1 blockade. Comparison of alpha diversity scores in R (n=54, blue) and NR (n=32, red) using the (**A**) Inverse Simpson (*p*=0.11), (**B**) Shannon (*p*=0.14), (**C**) Simpson (*p*=0.14) and (**D**) Chao1 (p=0.83) indices, by MW test.

Supplementary Figure S7
Gopalakrishnan et al.



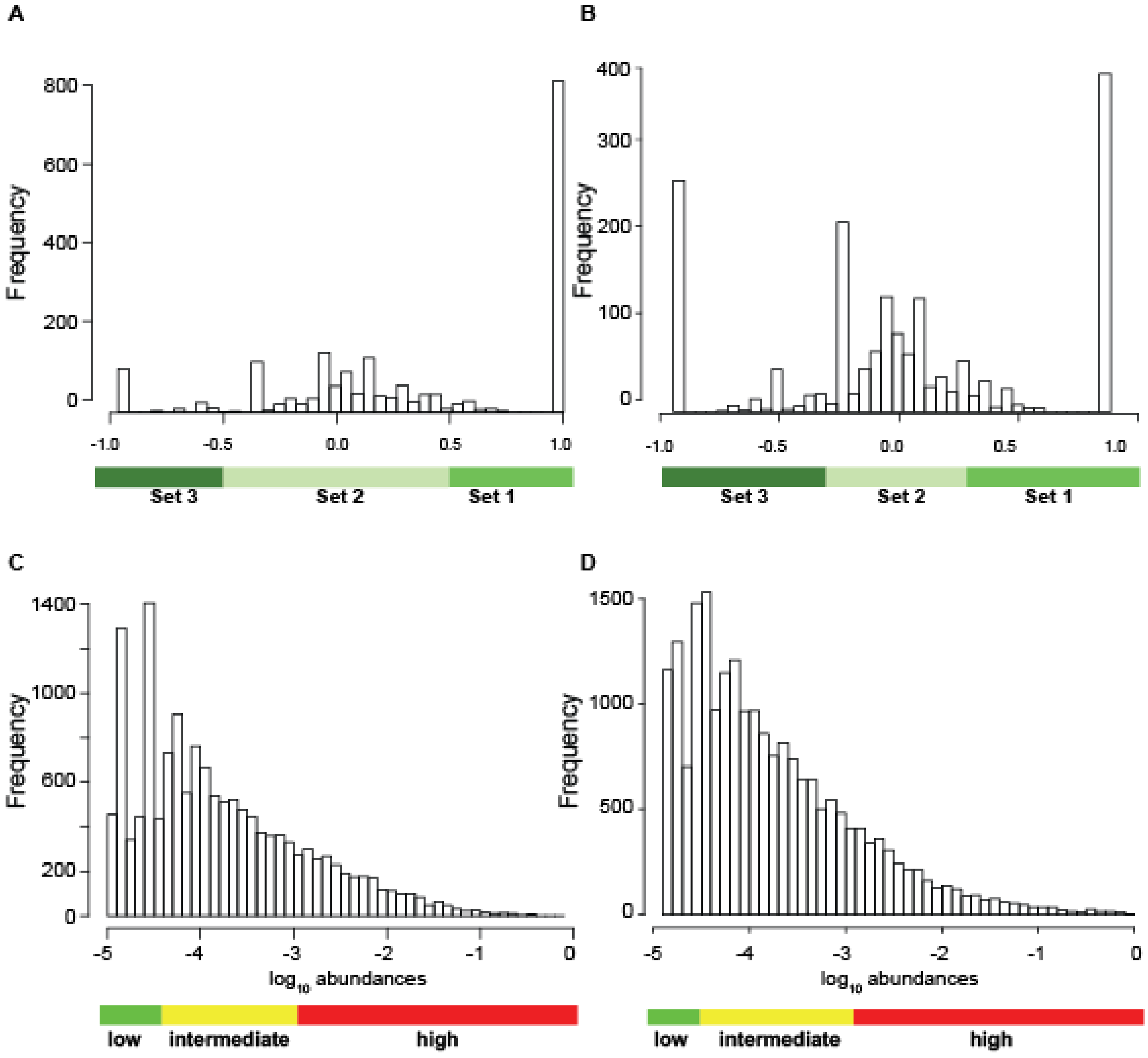
Supplementary figure S7: High diversity of the fecal microbiome is associated with longer PFS. Gut microbiota at baseline and subsequent treatment course by subject (n=39). (**A**) Horizontal bars represent alpha diversity scores measured by Inverse Simpson index in each patient. (**B**) Timeline plots showing days elapsed on therapy, x=progressed, o=not progressed at last follow-up.

Supplementary Figure S8
Gopalakrishnan et al.



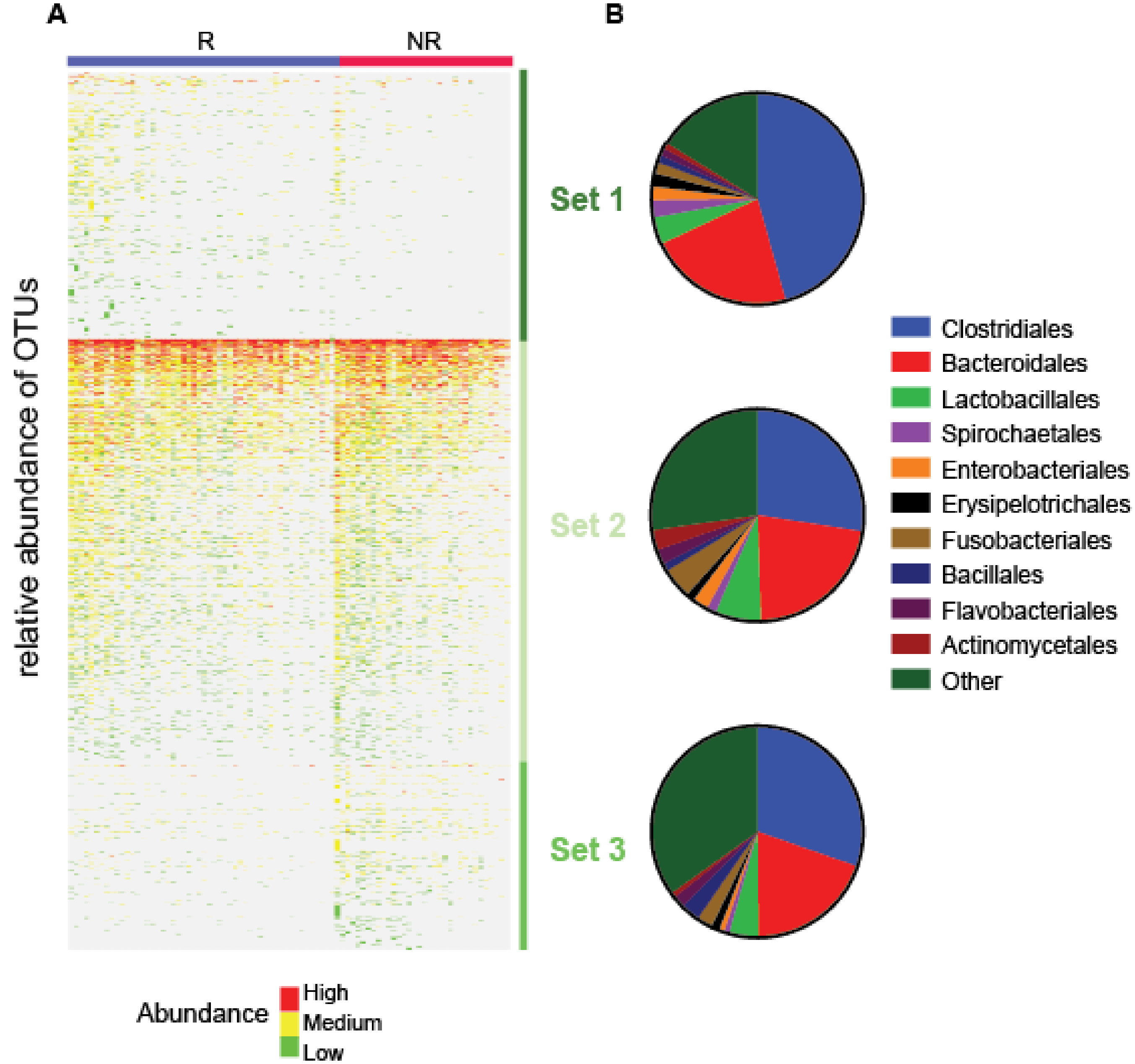
Supplementary figure S8: Diversity of the oral microbiome is not associated with PFS. (**A**) Stacked bars represent the phylogenetic composition of each sample at the family level at baseline. All patients were classified into high (>6.17, blue), intermediate (3.26-6.17, gold) and low (<3.26, red) diversity groups (indicated at left) based on tertiles of Inverse Simpson scores. Time to progression was defined as the interval between date of oral microbiome sample collection to the date of progression or censoring. (**B**) Kaplan-Meier plot of PFS by tertiles of oral diversity: blue=high (n=29, median PFS=279 days), gold=intermediate (n=28, median PFS undefined), red=low (n=29, median PFS=348 days). High vs intermediate, *p*=0.34; high vs low, *p*=0.54 by log-rank test. (**C**) Oral microbiota and subsequent treatment course by patient (n=86). Horizontal bars represent alpha diversity scores measured by Inverse Simpson index. (**D**) Timeline plots showing days elapsed on therapy, x=progressed, o=not progressed at last follow-up. (**E**) Principal coordinate analysis of buccal samples (n=86) by response using Weighted UniFrac distances.

Supplementary Figure S9
Gopalakrishnan et al.



Supplementary figure S9: Thresholds for enrichment index (*ei)* scores and relative abundances for OTUs in 86 oral and 43 fecal microbiome samples. Distribution of enrichment scores for bacterial OTUs at the species level in (**A**) fecal (n=43) and (**B**) oral microbiome samples (n=86) by set. The boundaries for each set are indicated. Distribution of $\log_{10}$ relative abundance of species in (**C**) fecal and (**D**) oral microbiome samples. The range for each abundance category is indicated.

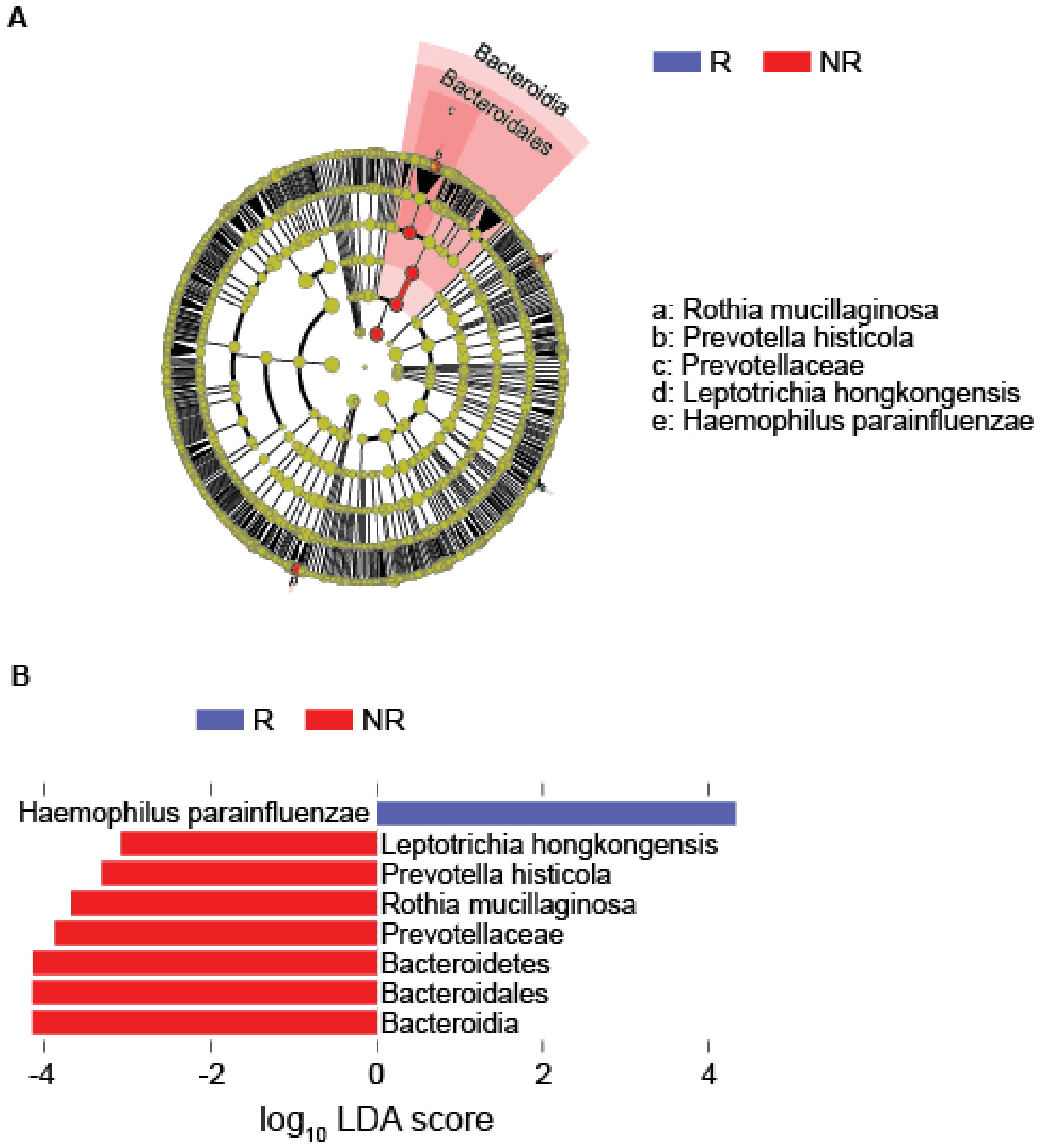
Supplementary Figure S10
Gopalakrishnan et al.



Supplementary figure S10: No significant differences in oral microbiome OTUs between R and NR to anti-PD-1 therapy by enrichment index *(ei)* score. (**A**) Heatmap of oral OTU abundances in R (n=52, blue) and NR (n=34, red). Columns denote patients grouped by response and sorted by diversity within R and NR groups; rows denote bacterial OTUs grouped into 3 sets according to their enrichment/depletion in R versus NR: Set 1 (enriched in R), Set 2 (unenriched), and Set 3 (enriched in NR), and then sorted by mean abundance within each set (**B**) Phylogenetic composition of bacterial OTUs within each set at the order level.

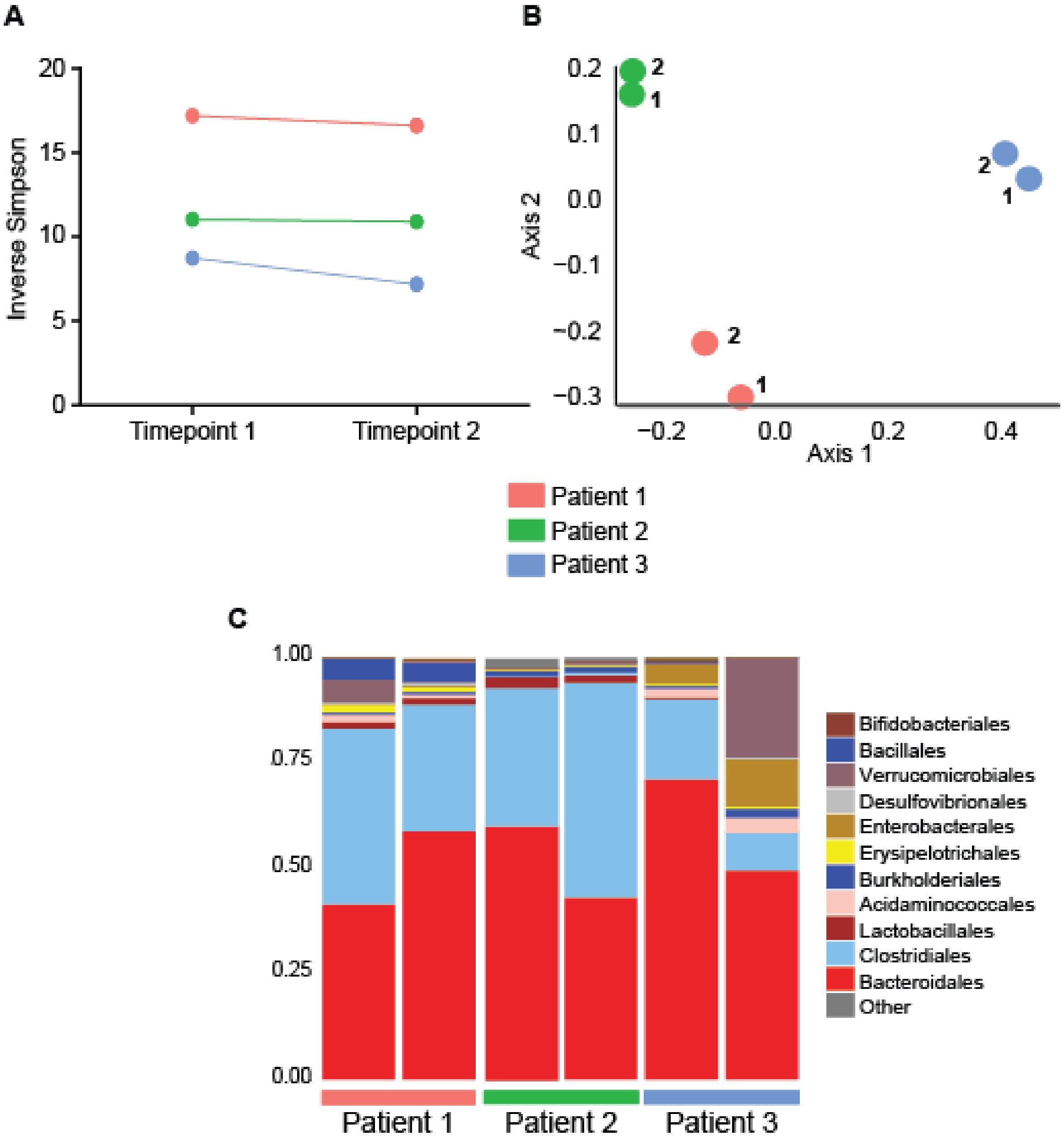
Supplementary Figure S11

Gopalakrishnan et al.



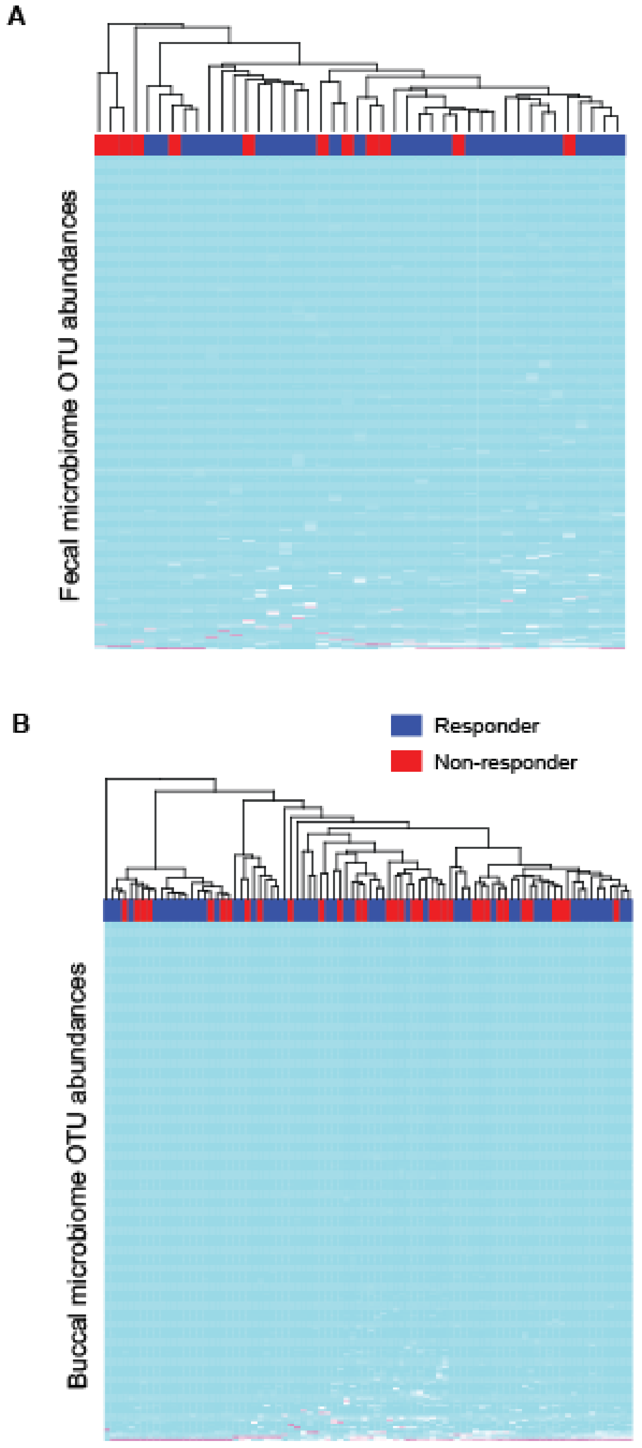
Supplementary figure S11: High-dimensional class comparisons using LEfSe reveal increased abundance of Bacteroidales in the oral microbiome of NR to PD-1 blockade. (**A**) Taxonomic Cladogram from LEfSe showing differences in the oral taxa. Blue and red show taxa enriched in R (n=52) and NR (n=34), respectively, with size of the dot proportional to abundance of the taxon. (**B**) Histogram of LDA scores computed for differentially abundant taxa between the oral microbiomes of R and NR, where the length of the bar indicates the effect size associated with a taxon. p=0.05 for Kruskal-Wallis test; LDA score >3.

Supplementary Figure S12
Gopalakrishnan et al.



Supplementary figure S12: The diversity and composition of the gut microbiome is stable over time. (**A**) Alpha diversity of the gut microbiome by Inverse Simpson over time in patients (n=3) with longitudinal collections. (**B**) Principal coordinate analysis using unweighted UniFrac distances; numbers (1, 2) indicate time point while color indicates patient. (**C**) Stacked bars showing the composition of the gut microbiome in patients over two longitudinal time points at the order level.

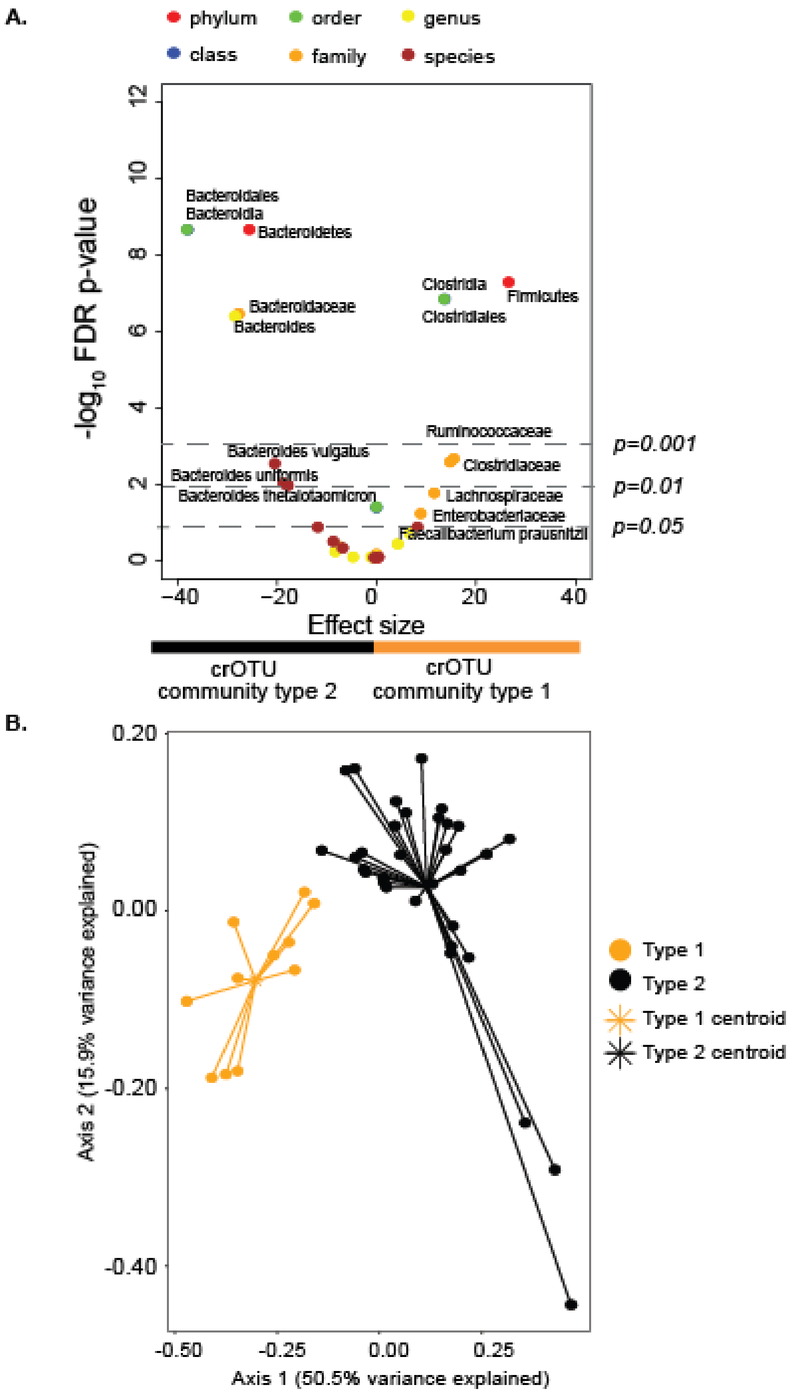
Supplementary Figure S13
Gopalakrishnan et al.



Supplementary figure S13: Clustering by relative OTU abundances shows no association with response to PD-1 blockade. Unsupervised hierarchical by complete linkage of Euclidean distances based on OTU abundance in (**A**) 43 fecal and (**B**) 86 oral microbiome samples. Each column represent a unique microbiome sample whereas each row represents a unique OTU. Sample annotation bar: blue=R, red=NR. OTU abundance within heatmap: light blue=low, white=intermediate, pink=high.

Supplementary Figure S14

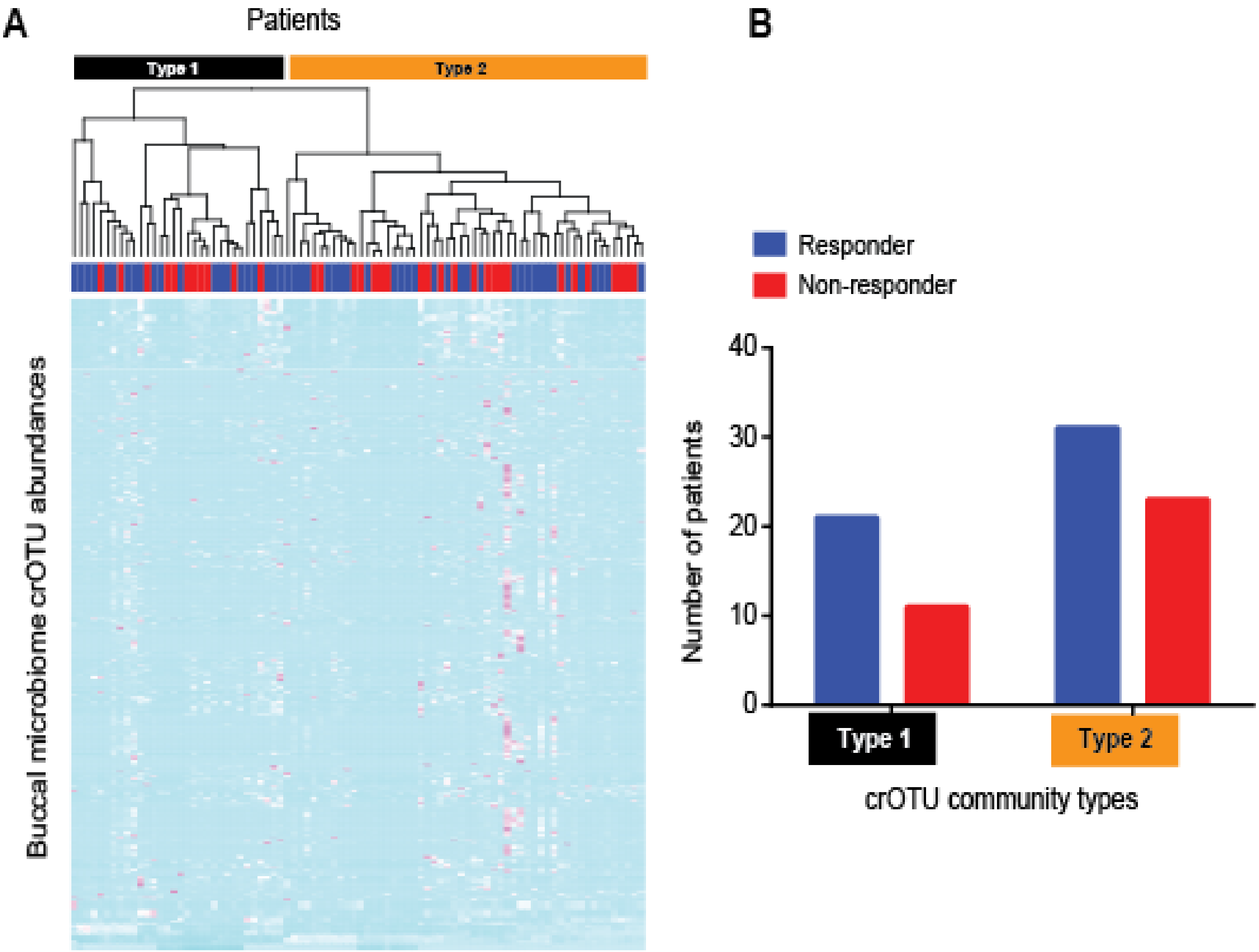
Gopalakrishnan et al.



Supplementary figure S14: Pairwise comparison of taxa abundances by cluster. Differentially abundant fecal taxa in crOTU community type 1 (n=11) vs crOTU community type 2 (n=32) by MW test (FDR-adjusted) within all taxonomic levels. **B)** Principal coordinate analysis of Weighted Unifrac distances between fecal samples (n=43) by crOTU community type.

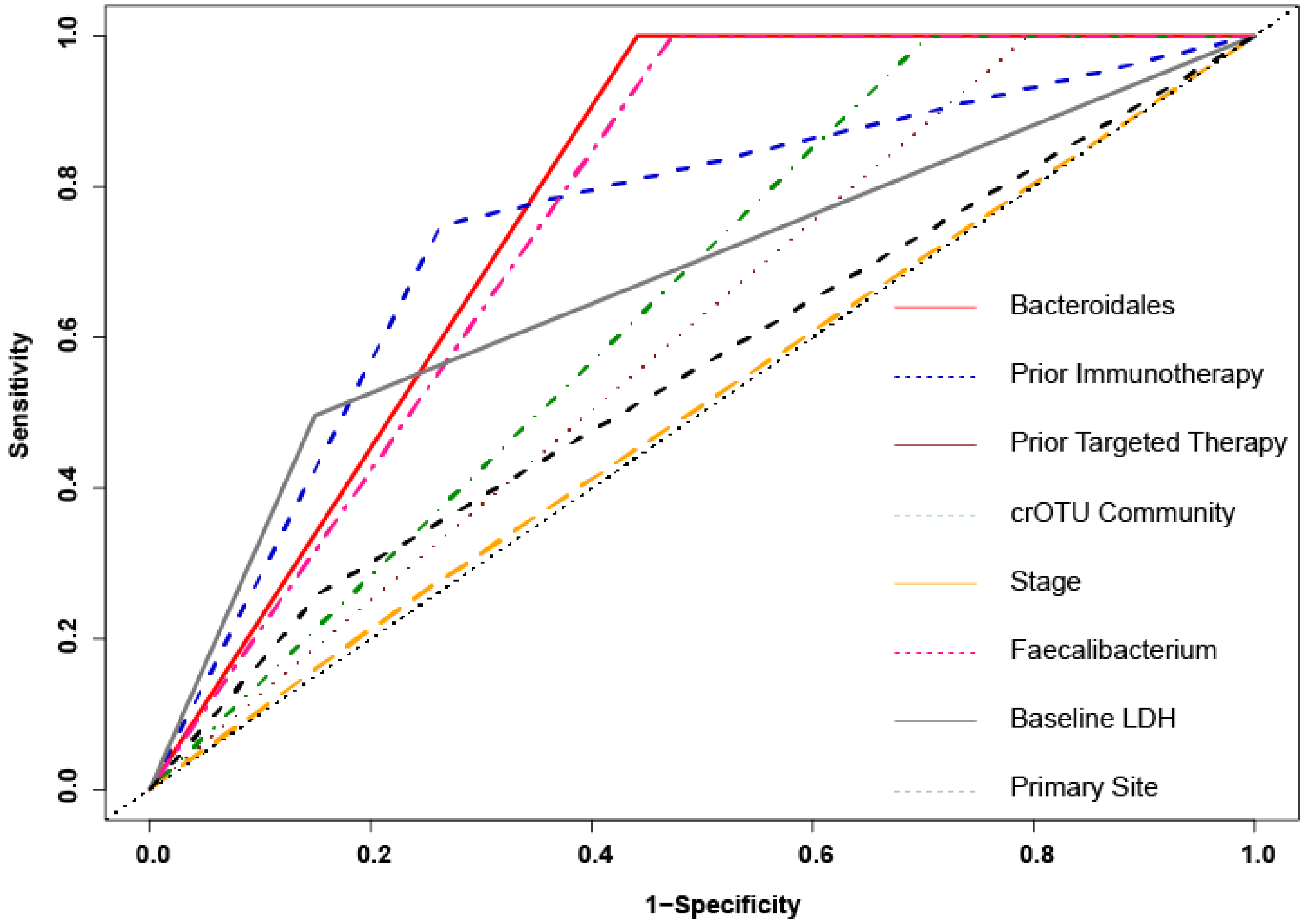
Supplementary Figure S15

Gopalakrishnan et al.



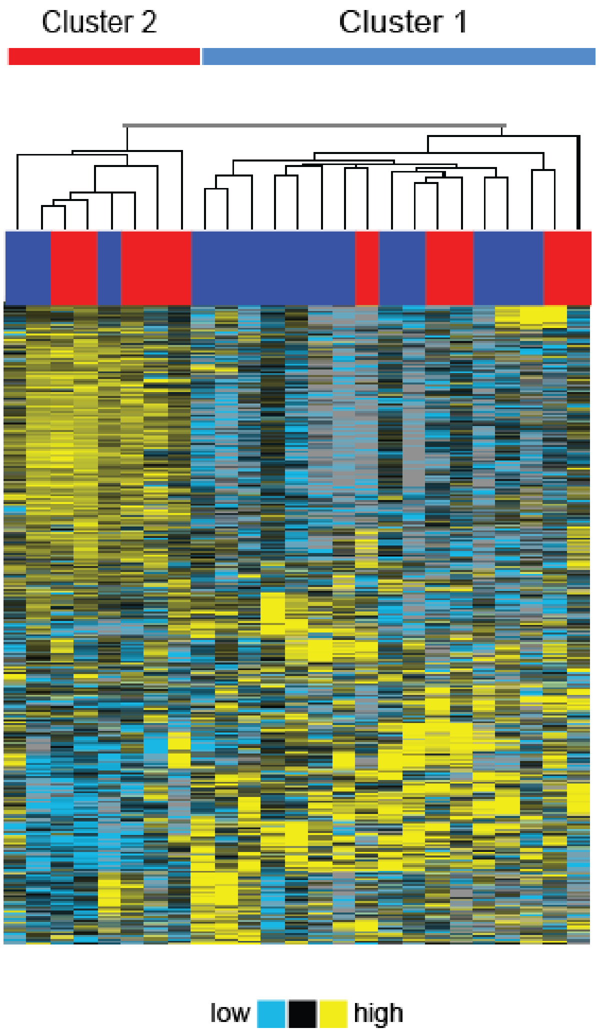
Supplementary figure S15: Oral crOTU communities are not associated with response to anti PD-1 immunotherapy. (**A**) Unsupervised hierarchical clustering of Euclidean distances by complete linkage of 86 oral microbiome samples based on crOTU abundances; light blue=low, white=intermediate, pink=high. (**B**) Comparison of clusters by response showing crOTU community type 1 (n=32, R=21 and NR=11) and community type 2 (n=54, R=31 and NR=23). *p*=0.20 by Fisher's exact test.

Supplementary Figure S16
Gopalakrishnan et. al.



Supplementary figure S16: Estimation of receiver operating characteristic curves (ROC) for clinical and microbial variables in predicting PFS. Plots display area under the curve estimation for microbial and relevant clinical variables at t=day 50 of follow up post anti PD-1 immunotherapy (n=39). AUCs are highest for *Faecalibacterium* (76.38) and Bacteroidales (77.93).

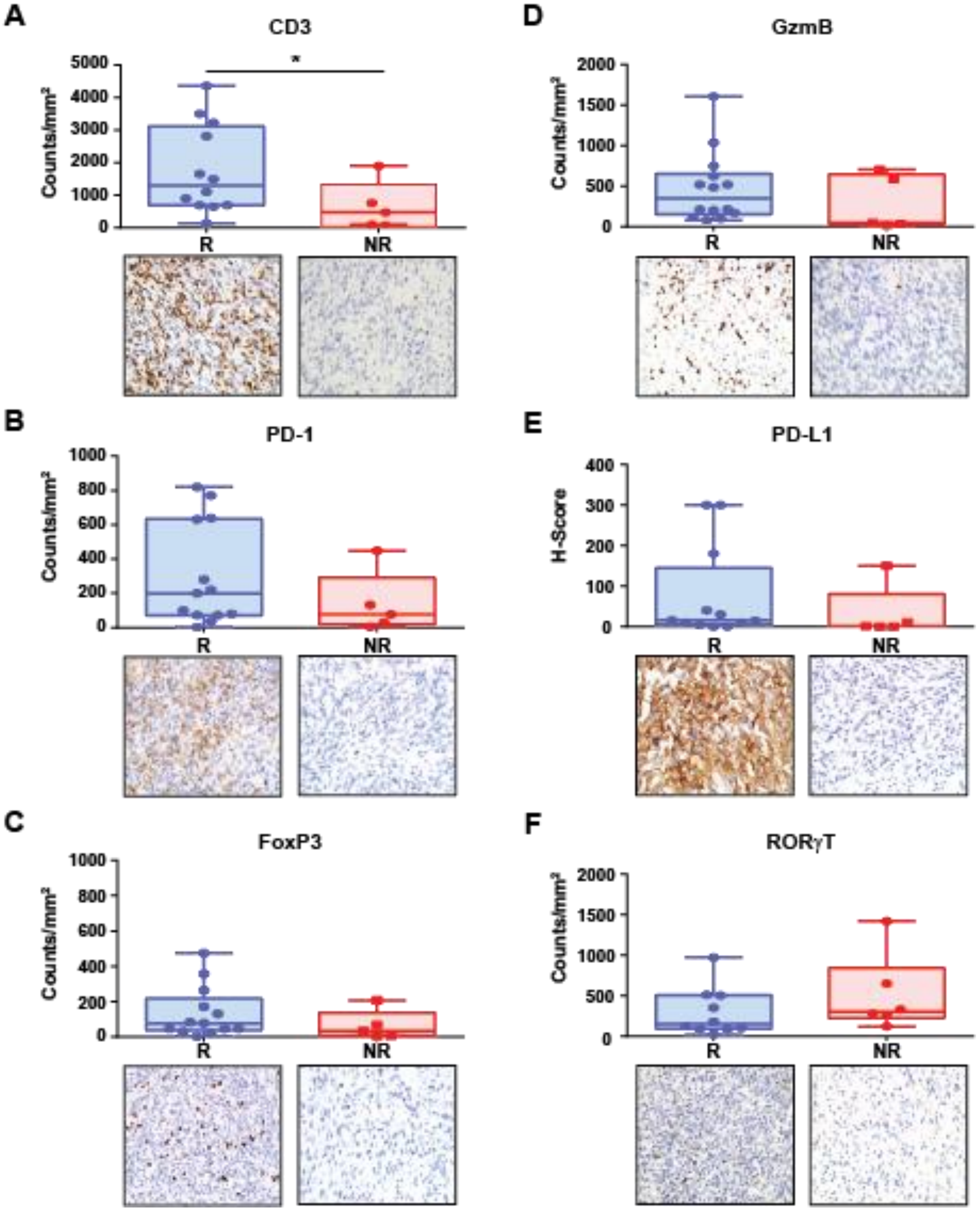
Supplementary Figure S17
Gopalakrishnan et al.



Supplementary figure S17. Differences in metabolic profiles in the gut microbiome of R vs NR to anti PD-1 immunotherapy using KO-abundances. Unsupervised hierarchical clustering of fecal samples (n=25) in terms of gene function abundances calculated as numbers of common (found in at least 20 samples) KEGG-orthologs. Columns represent patient samples (blue=R, n=14; red=NR, n=11) and rows represent KEGG-ortholog enrichment (blue=low enrichment, black=medium enrichment, yellow= high enrichment).

Supplementary Figure S18

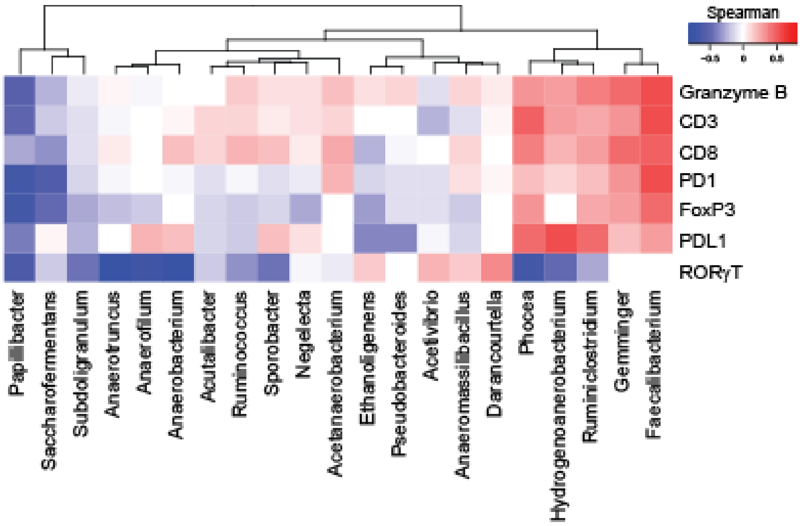
Gopalakrishnan et al.



Supplementary figure S18: Responders to anti PD-1 immunotherapy present an enriched tumor immune infiltrate at baseline. Immunohistochemical quantification and representative images at 40× magnification of (**A**) CD3, (**B**) PD-1 (p=0.13), (**C**) FoxP3 (p=0.13), (**D**) GzmB (p=0.11), (**E**) PD-L1 (p=0.13) and (**F**) RORγT (p=0.14) as counts/mm^2 or H-Score in R (blue) and NR (red) to anti PD-1 immunotherapy by one-sided MW test. * p<0.05.

Supplementary Figure S19

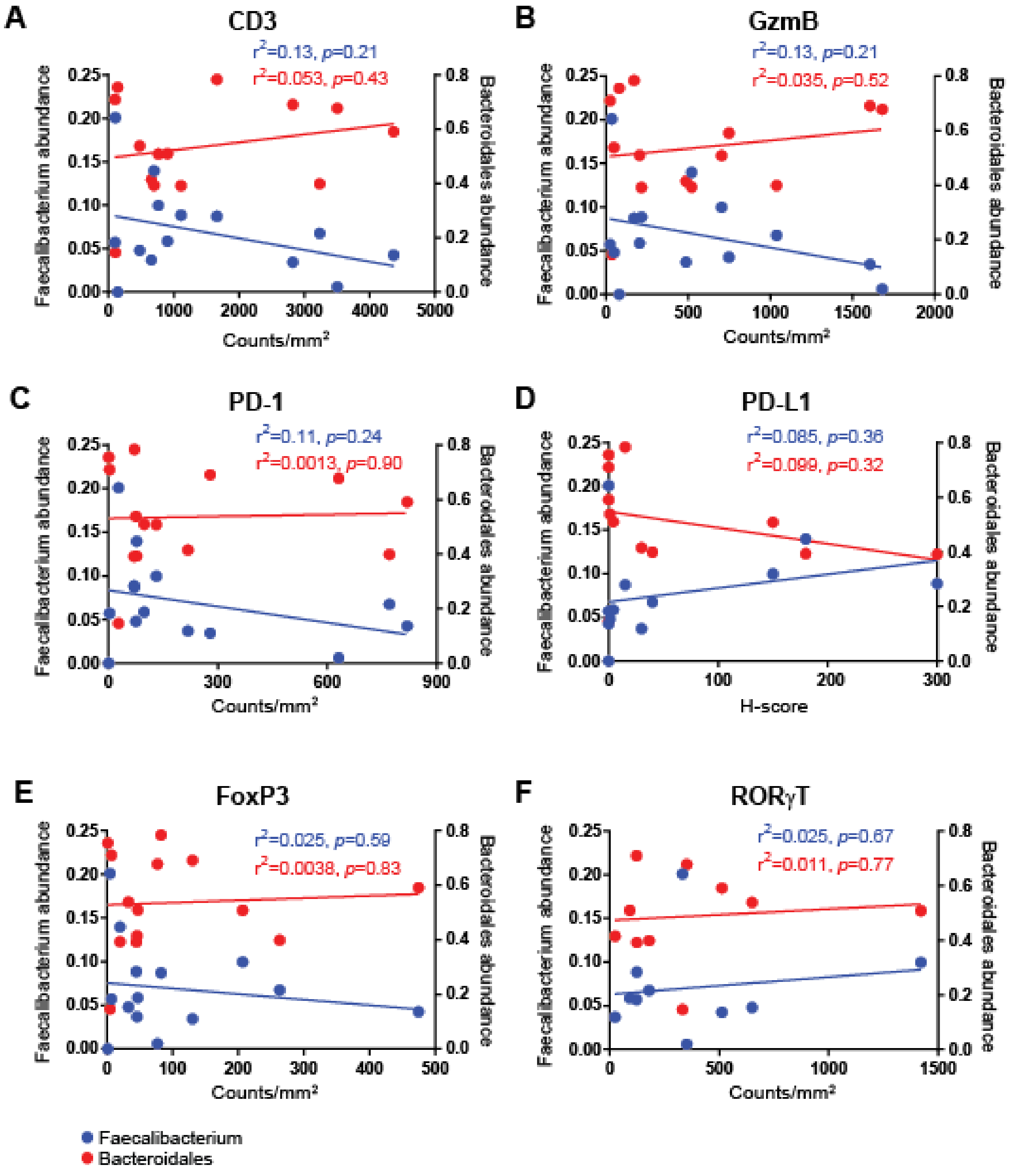
Gopalakrishnan et al.



Supplementary figure S19: Patients with a high abundance of *Faecalibacterium* present a favorable anti-tumor immune infiltrate prior to anti PD-1 immunotherapy. Spearman rank correlation heatmap of GzmB, CD3, CD8, PD-1, FoxP3, PD-L1 by H-Score, RORγT by counts/mm^2 by IHC and abundance of all genera within the Ruminococcaceae family in the fecal microbiome (n=15). Red=positive correlation, blue=negative correlation, white=no correlation.

Supplementary Figure S20

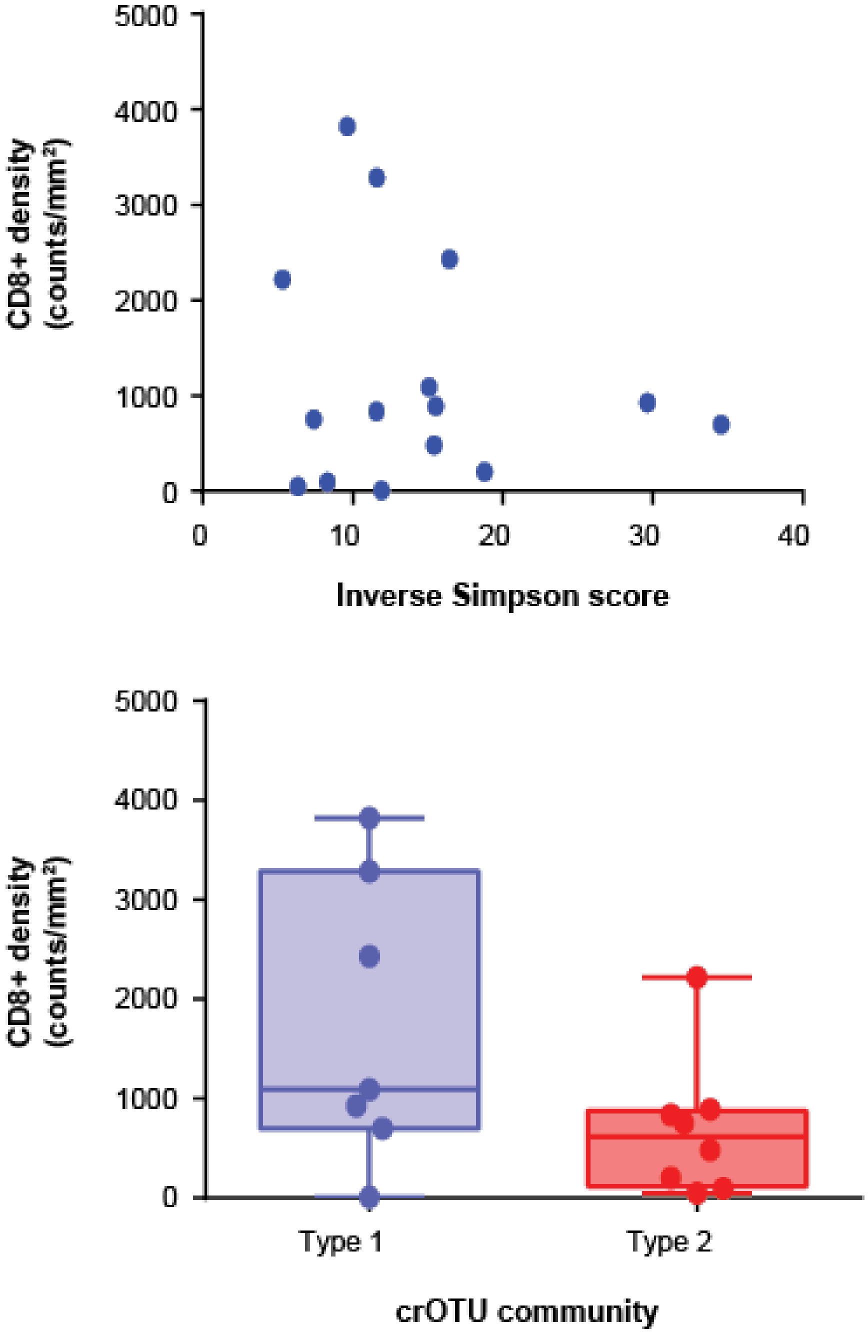
Gopalakrishnan et al.



Supplementary figure S20: *Faecalibacterium* and Bacteroidales abundance in the fecal microbiome have distinct associations with the tumor immune infiltrate prior to anti PD-1 immunotherapy. Univariate linear regression between *Faecalibacterium* abundance (blue), Bacteroidales abundance (red), and density by counts/mm^2 or H-score of (**A**) CD3, (**B**) GzmB, (**C**) PD-1, (**D**) PD-L1, (**E**) FoxP3, and (**F**) RORγT by IHC in tumors at baseline of patients treated with anti-PD-1. Lines show regression for *Faecalibacterium* (blue) and Bacteroidales (red) with the associated r^2 and *p*-values by Slope test.

Supplementary Figure S21

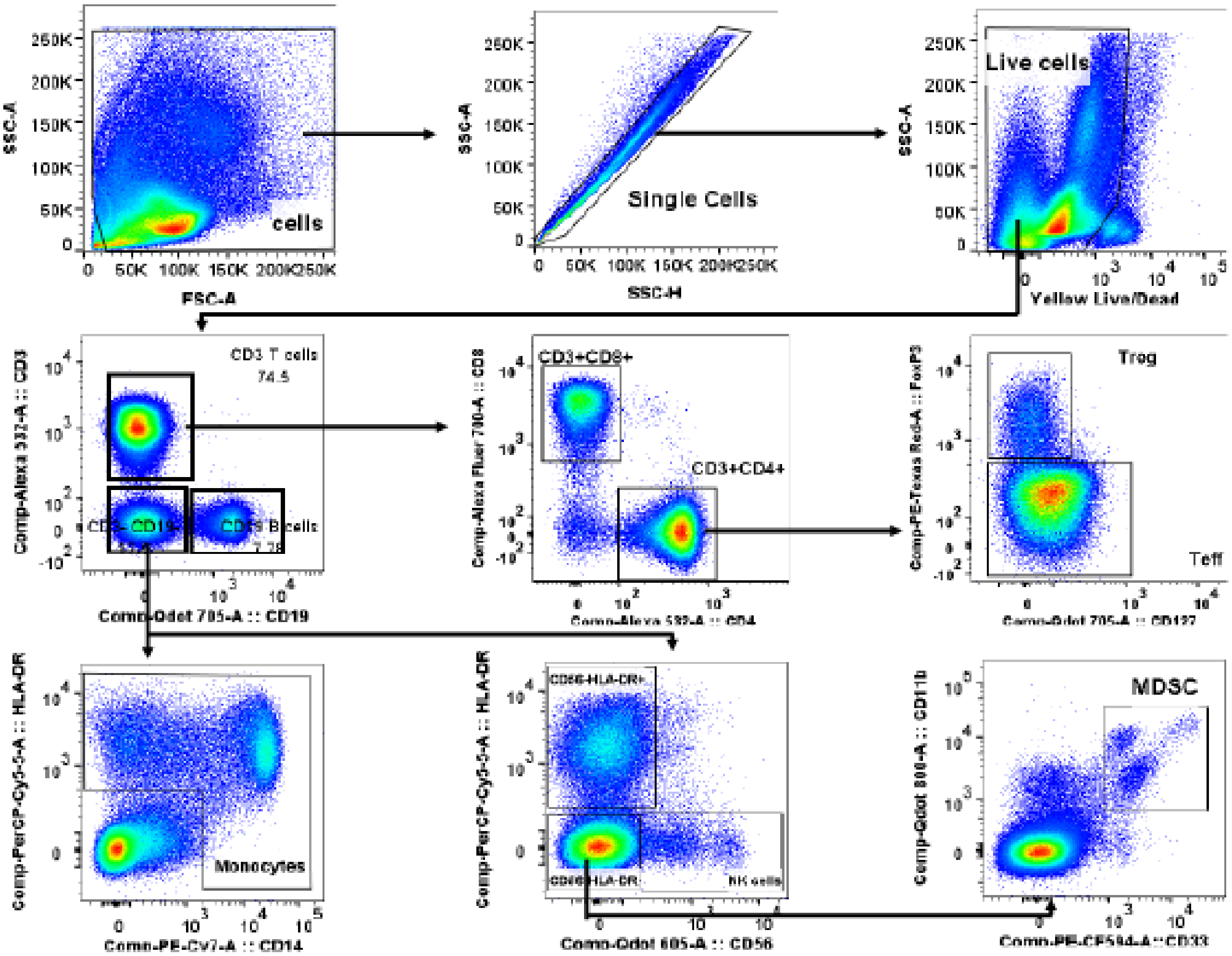
Gopalakrishnan et al.



Supplementary figure S21: No association between CD8+ T cell density and alpha diversity or crOTU community type. (**A**) Pairwise Spearman correlation between CD8+ T-cell density and Inverse Simpson score (n=15; r= 0.01, *p*=0.96). (**B**) MW test of CD8+ T-cell density between patients in crOTU community type 1 (n=5) vs crOTU community type 2 (n=11), (*p*= 0.58).

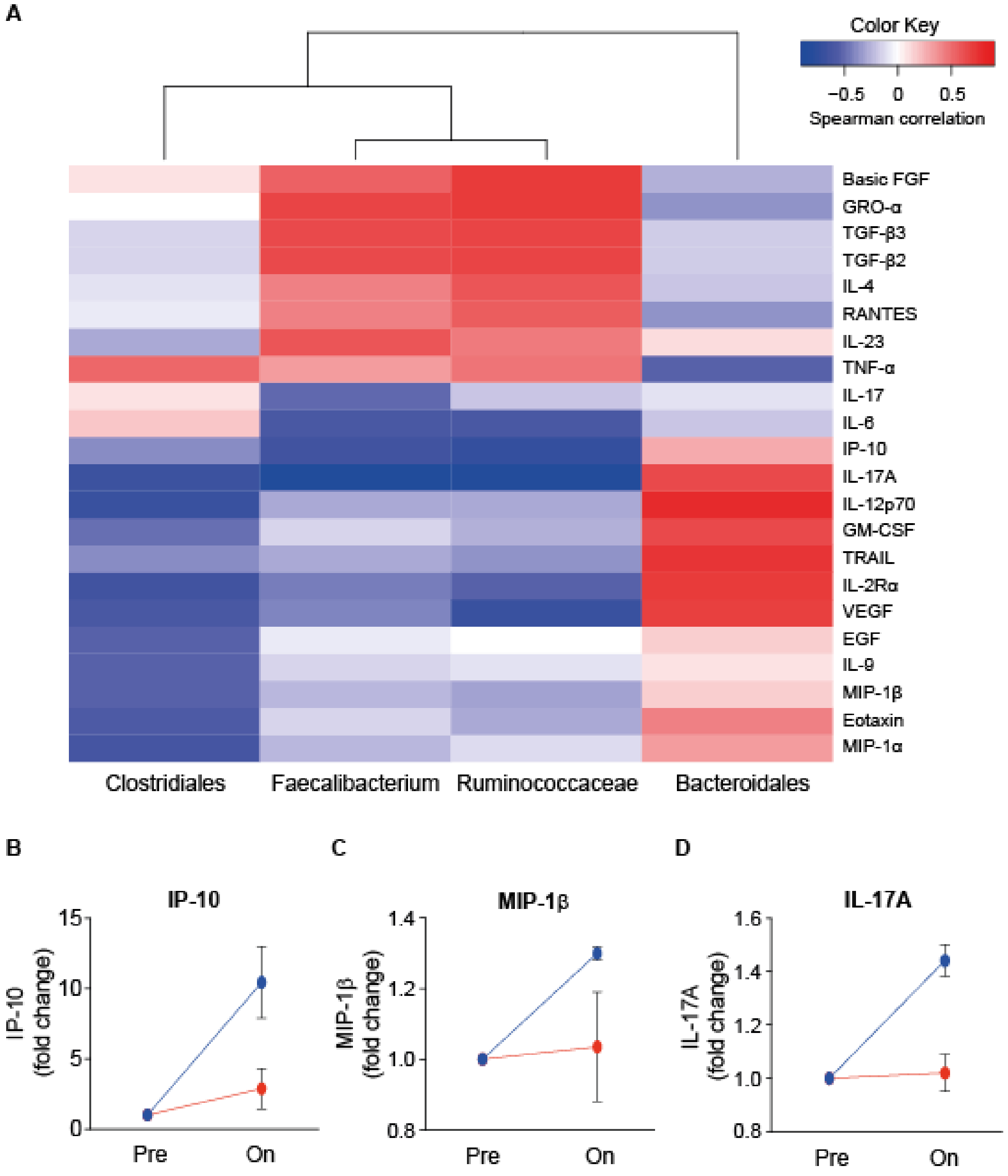
Supplementary Figure S22

Gopalakrishnan et al.



Supplementary figure S22: Gating strategy for flow cytometric analysis of peripheral blood in patients treated with anti PD-1 immunotherapy. PBMC at baseline in patients treated with anti PD-1 immunotherapy were analyzed by gating for CD19+ B cells, CD3+CD8+ T cells, CD3+CD4+ T cells (CD3+CD4+FoxP3+ regulatory and CD3+CD4+FoxP3- effector), monocytes (based on CD14/HLA-DR), and MDSC (CD3-CD19-HLADR- CD33+CD11b+).

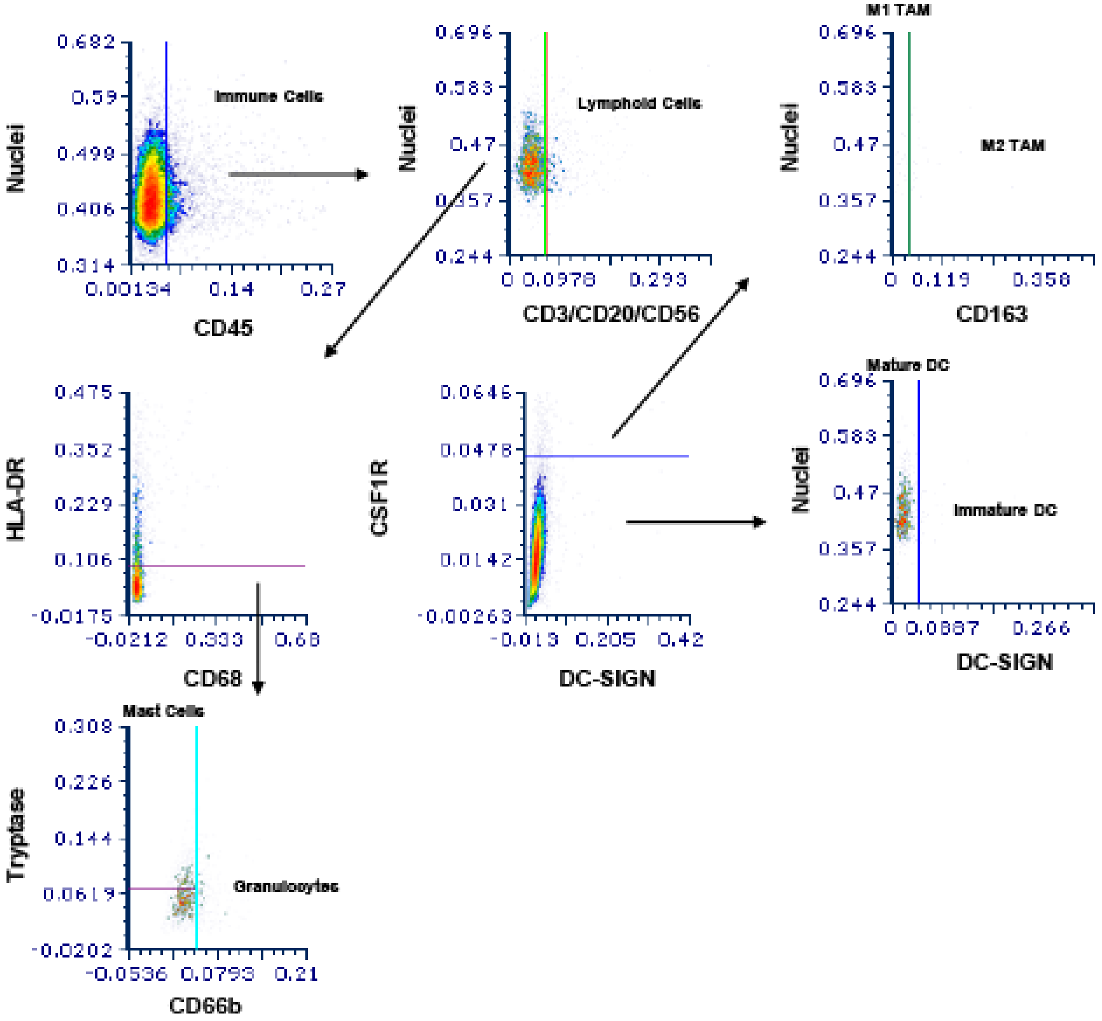
Supplementary Figure S23
Gopalakrishnan et al.



Supplementary figure S23. Patients with high *Faecalibacterium* abundance display a peripheral cytokine profile favorable for response to anti PD-1 immunotherapy at baseline and enhanced cytokine responses over the course of therapy. (**A**) Spearman rank correlation heatmap between Clostridiales, *Faecalibacterium*, Ruminococcaceae, and Bacteroidales abundance and peripheral concentration of cytokines in pg/mL at baseline by multiplex bead assay. Red=positive correlation, blue=negative correlation, white=no correlation. Change in production of cytokines in serum of responders (n=2, blue) and non-responders (n=2, red) to anti PD-1 immunotherapy for (**B**) IP-10 (*p*=0.04 and *p*=0.34, respectively), (**C**) MIP-1β (*p*=0.04 and *p*=0.90, respectively), and (**D**) IL-17A (*p*=0.07 and *p*=0.86, respectively) in fold-change from baseline by ratio paired t-test.

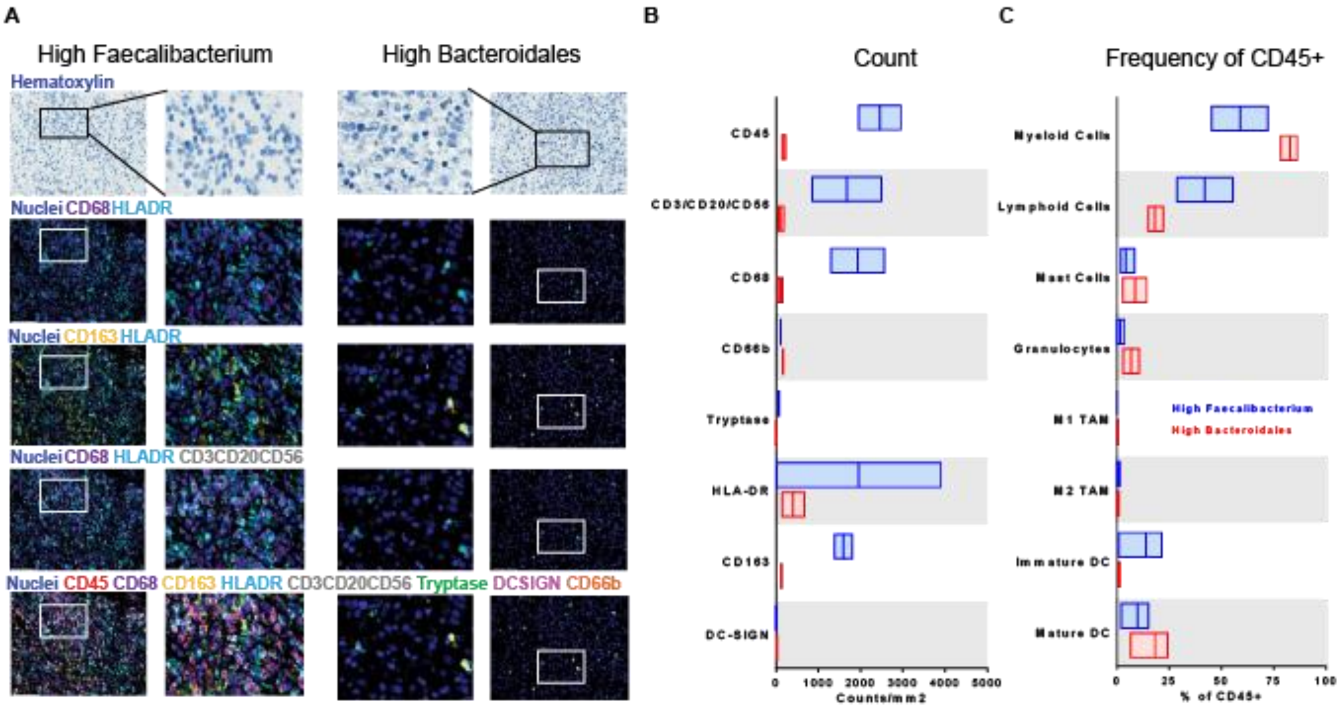
Supplementary Figure S24

Gopalakrishnan et al.

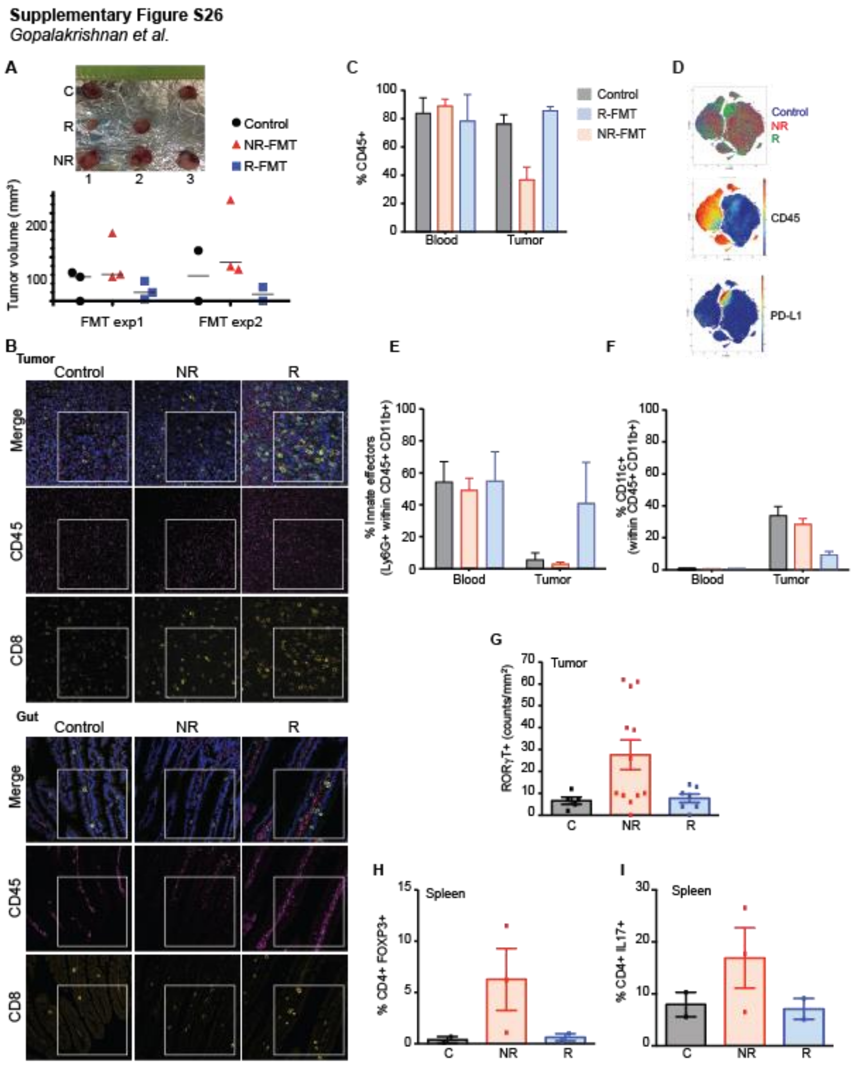


Supplementary figure S24: Gating strategy for myeloid multiplex IHC in the tumors of patients treated with anti PD-1 immunotherapy at baseline. Myeloid multiplex immunohistochemistry gating strategy showing immune cells (CD45+), lymphoid cells (CD45+CD3+CD20+CD56+), myeloid cells (CD45+CD3-CD20-CD56-), mast cells (CD45+CD3-CD20-CD56-HLADR-Tryptase+), granulocytes (CD45+CD3-CD20-CD56-HLADR-CD66b+), M1 tumor-associated macrophages (CD45+CD3-CD20-CD56-HLADR+CSF1R+CD163-), M2 tumor-associated macrophages (CD45+CD3-CD20-CD56-HLADR+CSF1R+CD163+), mature dendritic cells (CD45+CD3-CD20-CD56-HLADR+CSF1R-DCSIGN-) and immature dendritic cells (CD45+CD3-CD20- CD56-HLADR+CSF1R-DCSIGN+).

Supplementary Figure S25
Gopalakrishnan et al.

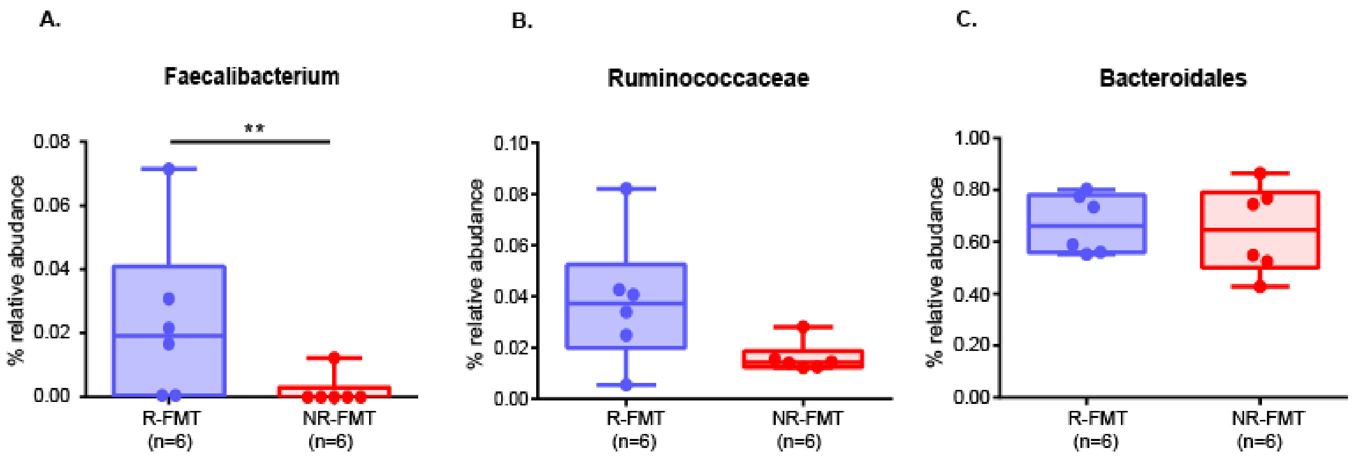


Supplementary figure S25: High *Faecalibacterium* abundance at baseline is associated with an increased immune infiltrate prior to anti PD-1 immunotherapy. (**A**) Multiplex immunohistochemistry showing representative myeloid immune cell staining at 40× magnification. (**B**) Quantification of CD45, CD3/CD20/CD56, CD68, CD66b, Tryptase, HLA-DR, CD163, and DC-SIGN as counts/mm^2. (**C**) Quantification of myeloid cells, lymphoid cells, mast cells, granulocytes, M1 and M2 tumor-associated macrophages, immature dendritic cells, and mature dendritic cells as a percentage of total CD45+ immune cells in patients with a high *Faecalibacterium* (n=2, blue) or high Bacteroidales (n=2, red) abundance.



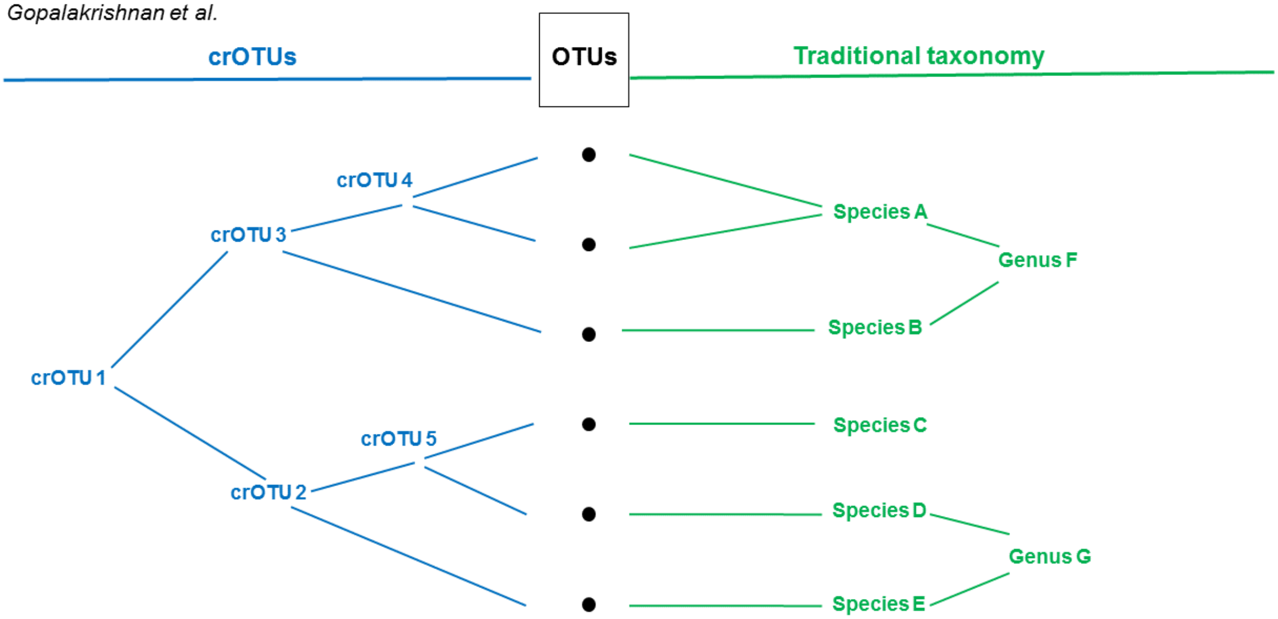
Supplementary figure S26: A favorable gut microbiome is associated with systemic anti-tumor immunity. (A, top) Snapshot of explanted xenograft tumors from FMT exp2, showing differences in tumor size between the indicated experimental groups. **(A, bottom)** Difference in size of tumors implanted in R-FMT (blue) and NR-FMT mice (red), or control mice (black). Tumor volumes on day 14 post-tumor implantation are plotted, each value representing a single mouse. Data from two independent experiments. Also reported in Figure 4H as fold change. (B) Representative immunofluorescent staining of tumors (top) and gut (bottom) from Control (left), NR-FMT (middle), and R-FMT (right) in GF mice post-FMT for CD45 (purple), CD8 (yellow), and nuclei (DAPI, blue). **(C)** Flow cytometry quantification showing the frequency of CD45+ immune cells in R-FMT (blue), NR-FMT (red), and control mice (black) in blood and tumor. **(D)** t-SNE plot of total live cells (top) isolated from tumors derived from control (n=2, Blue), NR (n=3, Red), and R- (n=2,Green) colonized mice by mass cytometry. t-SNE plot of total live cells (top) overlaid with the expression of CD45 (middle) and PD-L1 (bottom). Equal numbers of cells are displayed from each group. **(E)** Flow cytometry quantification showing the frequency of CD45+CD11b+Ly6G+ innate effector cells in R-FMT (n=2, blue), NR-FMT (n=3, red), and control mice (n=2, black) in blood and tumor. **(F)** Flow cytometry quantification showing the frequency of CD45+CD11b+CD11c+ suppressive cells in R-FMT (blue, n=2), NR-FMT (red, n=3), and control mice (black, n=2) in blood and tumor (n=3). **(G)** IHC quantification showing the number of RORγT+ Th17 cells in R-FMT (n=2, blue), NR-FMT (n=3, red), and control mice (n=2, black) in tumor as counts/mm^2. **(H)** Flow cytometry quantification showing the frequency of CD4+FoxP3+ regulatory T cells in R-FMT (blue, n=2), NR-FMT (red, n=3), and control mice (black, n=2) in spleen. **(I)** Flow cytometry quantification showing the frequency of CD4+IL17+ Th17 cells in R-FMT (blue), NR-FMT (red), and control mice (black) in spleen.

Supplementary figure S27
Gopalakrishnan et al.



Supplementary figure S27: 16S analysis of fecal samples from R and NR donors and germ-free recipient mice. Relative abundance comparisons of **(A)** *Faecalibacterium*, **(B)** Ruminococcaceae and **(C)** Bacteroidales by MW test on day 14 post tumor injection. Data from 2 independent experiments are presented. ** $p<0.01$.

Supplementary fig. S28
Gopalakrishnan et al.



Supplementary figure S28: Schematic representation of crOTUs compared to traditional taxonomy. 5 hypothetical OTUs are represented as black dots in the middle of the panel. Traditional taxonomy is depicted on the right and the crOTU methodology is depicted on the left. In this figure, crOTU4 recapitulates species level classification by grouping the two OTUs that both belonged to species A. However, crOTU5 is made up of OTUs from species C and species D. Higher up in the tree, crOTU2 and crOTU3 recapitulate genus level classification. crOTU3 recapitulates genus F, whereas crOTU2 contains OTUs from genus G as well as species C. Such *de novo* clusters also occur at higher taxonomic levels and provide more systematic bacterial groupings when compared to standard taxonomy.

List of Supplementary Tables

Table S1: Availability of biospecimens (oral and gut microbiome samples, tumor biopsies, blood draws) by analyses.

Table S2. Baseline characteristics of patients included in final fecal and oral microbiome analyses cohorts.

Table S3. Pairwise comparisons of 16S-derived bacterial taxa between responders and non-responders by two-sided Mann-Whitney test within each level of taxonomy.

Table S4. Differences in WGS-derived fecal bacterial species by treatment response status.

Table S5. Pairwise comparisons of 16S-derived bacterial taxa between crOTU community type 1 and crOTU community type 2 by two- sided Mann-Whitney test within each level of taxonomy.

Table S6. Univariate and multivariate Cox regression model results.

Table S7. Pairwise comparison of MetaCyc pathway class by response.

Table S8. Individual values and summary statistics of metabolic reconstructions.

Table S9. Taxonomic classification by NCBI, SILVA, RDP and Greengenes 16S Databases of top 50 most abundant OTUs.

Supplementary Tables

Table S1: Availability of biospecimens (oral and gut microbiome samples, tumor biopsies, blood draws) by analyses.

Subset	Corresponding figures	n (R/NR)	Sample Type	Inclusion Criteria*
A	Fig. 1B, S4B	109**	Oral	Banked baseline oral sample available
B	Fig. 1B	53	Fecal	Banked baseline fecal sample available
C	S4A	48	Fecal, Oral	Matched fecal and oral samples
D	Fig. 1C, S5, S9A, Fig. 2A-E, S13A, Fig. 3A,B, S14	43 (30/13)	Fecal	Banked baseline fecal sample available, clinical response information known
E	Fig. 1D,E, S7, Fig. 3C,D	39	Fecal	Banked baseline fecal sample available, sufficient follow up time
F	Fig. 2F, Fig. 3E, S16	25 (14/11)	Fecal	Banked baseline fecal sample available, clinical response information known
G	Fig. 4A, S17	21 (15/6)	Tissue	Banked baseline FFPE tissue
H	Fig. 4B,C, S18, S19	15 (11/4)	Tissue, Fecal	Banked baseline FFPE tissue and fecal sample available, clinical response information known
I	Fig 4E-F, S24	4 (2/2)	Tissue, Fecal	Multiplex IHC
K	S3	10 (7/3)	Tissue	Banked baseline tumor sample available, passed QC for WES, matched fecal sample available
L	S6, S8, S10A, B, S13B, S15A,B	86 (54/32)	Oral	Banked baseline oral sample available, clinical response information known
M	S3	3 (3/0)	Fecal	Banked baseline and on treatment fecal samples available (all 4 patients included in subset D)
N	Fig. 4D, S22A	11 (8/3)	Blood	Banked baseline PBMC and fecal samples available, clinical response information known
O	S22	4 (2/2)	Blood	Banked baseline serum and fecal samples available, clinical response information known

*all patients with samples included went onto PD-1 based therapy

**112 patients total- 3 did not give oral sample, includes longitudinal sample from 1 patient

Table S2. Baseline characteristics of patients included in final fecal and oral microbiome analyses cohorts.

	Oral Microbiome			Fecal Microbiome		
	R	NR	P[a]	R	NR	P[a]
	n=52 (%)	n=34 (%)		n=30 (%)	n=13 (%)	
Age			0.57			0.60
Median	66.5	64.5		64	70	
Range	21-88	32-87		21-88	42-80	
Gender			0.14			0.74
Male	41 (79)	22 (65)		20 (67)	8 (62)	
Female	11 (21)	12 (35)		10 (33)	5 (38)	
Ethnicity			0.47			0.57
White	48 (92)	29 (85)		28 (94)	11 (85)	
Other	4 (6)	5 (6)		2 (6)	2 (15)	
Primary Type			0.032			0.68
Cutaneous	44 (85)	22 (65)		25 (83)	10 (77)	
Other[b]	8 (15)	12 (35)		5 (17)	3 (23)	
Prior Targeted Therapy			0.76			0.76
Yes	8 (15)	4 (12)		5 (17)	6 (46)	
No	44 (85)	30 (88)		25 (83)	7 (54)	
Prior Checkpoint Therapy			0.81			0.46
Yes	17 (33)	12 (35)		9 (30)	2 (15)	
No	35 (67)	22 (65)		21 (70)	11 (85)	
Disease Stage			0.05			<0.01
III	14 (27)	3 (9)		20 (67)	1 (8)	
IV	38 (73)	31 (91)		10 (33)	12 (92)	
Lactate Dehydrogenase[c]			0.11			0.21
Normal	44 (85)	23 (68)		25 (86)	9 (69)	
Elevated[d]	8 (15)	10 (30)		4 (14)	4 (31)	
Treatment Type			0.05			0.22
PD-1 Monotherapy	50 (96)	28 (82)		29 (97)	11 (85)	
PD-1 Combination[e]	2 (4)	6 (18)		1 (3)	2 (15)	

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[a] *p*-values calculated by Wilcoxon rank sum (age), Chi-squared (gender, prior checkpoint) and Fisher's exact (all others)

[b] other category includes acral, mucosal and unknown primaries

[c] LDH not available on one (R) patient in the fecal cohort and one (NR) patient in the oral cohort.

[d] elevated LDH: exceeding the upper limit of normal (618 IU/mL), all samples assayed in a common laboratory.

[e] combinations included: Abraxane, Urelumab, Aldara cream

Table S3. Pairwise comparisons of 16S-derived bacterial taxa between responders and non-responders by two-sided Mann-Whitney test within each level of taxonomy.

Taxon	Effect size	Unadjusted *p*-value	FDR-adjusted *p*-value	Taxonomy level
Bacteroidetes	19.0623213	0.000939189	0.002664296	Phylum
Firmicutes	-16.927341	0.001776197	0.002664296	Phylum
Proteobacteria	0	0.927224012	0.927224012	Phylum
Bacteroidia	21.3497999	0.000939189	0.002165015	Class
Clostridia	-14.94486	0.001443343	0.002165015	Class
Gammaproteobacteria	0	0.628910832	0.628910832	Class
Bacteroidales	19.6723156	0.000939189	0.002165015	Order
Clostridiales	-16.622344	0.001443343	0.002165015	Order
Enterobacterales	0	0.842779296	0.842779296	Order
Bacteroidaceae	9.72178386	0.159028057	0.307087386	Family
Clostridiaceae	-6.4430646	0.175478507	0.307087386	Family
Enterobacteriaceae	0.41937107	0.863520547	0.863520547	Family
Lachnospiraceae	-2.6306003	0.488319512	0.683647316	Family
Porphyromonadaceae	-9.3405374	0.061699411	0.21594794	Family
Rikenellaceae	-0.4193711	0.750982964	0.863520547	Family
Ruminococcaceae	-19.557942	0.000118163	0.000827139	Family
Alistipes	-3.4312178	0.750982964	0.844805974	Genus
Bacteroides	6.7099371	0.159028057	0.424074818	Genus
Blautia	-0.3049971	0.844805974	0.844805974	Genus
Escherichia	1.98248141	0.551319278	0.844805974	Genus
Faecalibacterium	-17.384837	0.005128301	0.041026409	Genus
Lachnoclostridium	2.13497999	0.539045677	0.844805974	Genus
Parabacteroides	-11.132396	0.098795392	0.395181568	Genus
Roseburia	0	0.804153902	0.844805974	Genus
Alistipes onderdonkii	0.03812464	0.577459319	0.577459319	Species
Bacteroides caccae	-0.0381246	0.569645414	0.577459319	Species
Bacteroides ovatus	-1.6393596	0.397413648	0.577459319	Species
Bacteroides thetaiotaomicron	8.12054887	0.412327809	0.577459319	Species
Bacteroides uniformis	7.51055459	0.475213018	0.577459319	Species
Bacteroides vulgatus	-2.7830989	0.300376456	0.577459319	Species
Escherichia coli	6.82431102	0.551319278	0.577459319	Species
Faecalibacterium prausnitzii	-12.543007	0.005128301	0.04615471	Species
Parabacteroides merdae	-1.1818639	0.440288136	0.577459319	Species

[a] FDR *p*-values are adjusted within each level of taxonomy level

[b] Table excludes low variance and low abundance taxa

Table S4. Differences in WGS-derived fecal bacterial species by treatment response status.

CAG Number	Unadjusted p-value	Response status	Taxonomy Level	Species	Genus	Family
CAG00720	0.02	NR	Species	Anaerotruncus colihominis	Anaerotruncus	Ruminococcaceae
CAG00124	0.02	NR	Species	Klebsiella variicola	Klebsiella	Enterobacteriaceae
CAG00011	0.04	NR	Species	Escherichia coli	Escherichia	Enterobacteriaceae
CAG00050	0.04	NR	Species	Bacteroides thetaiotaomicron	Bacteroides	Bacteroidaceae
CAG00834	0.05	NR	Species	Oxalobacter formigenes	Oxalobacter	Oxalobacteraceae
CAG00426	0.06	NR	Species	Paraprevotella clara	Paraprevotella	Prevotellaceae
CAG01272	0.06	NR	Species	Adlercreutzia equolifaciens	Adlercreutzia	Eggerthellaceae
CAG01320	0.07	NR	Species	Clostridium bolteae	Lachnoclostridium	Lachnospiraceae
CAG00012	0.07	NR	Species	Klebsiella pneumoniae	Klebsiella	Enterobacteriaceae
CAG00826	0.09	NR	Genus	unclassified Clostridium	Clostridium	Clostridiaceae
CAG00117	0.09	NR	Species	Parabacteroides merdae	Parabacteroides	Porphyromonadaceae
CAG00093	0.12	NR	Species	Klebsiella quasipneumoniae	Klebsiella	Enterobacteriaceae
CAG00114	0.12	NR	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG00161	0.12	NR	Species	Bacteroides coprocola	Bacteroides	Bacteroidaceae
CAG00163	0.12	NR	Species	Prevotella sp. CAG:255	Prevotella	Prevotellaceae
CAG00256	0.12	NR	Family	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG00462	0.12	NR	Species	Streptococcus pasteurianus	Streptococcus	Streptococcaceae
CAG00815	0.12	NR	Species	Lactococcus lactis	Lactococcus	Streptococcaceae
CAG00817	0.12	NR	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00010_2	0.12	NR	NA	unclassified	unclassified	unclassified
CAG01203	0.12	NR	Species	Streptococcus mutans	Streptococcus	Streptococcaceae
CAG00949	0.12	NR	Species	Ruminococcaceae bacterium D16	unclassified Ruminococcaceae	Ruminococcaceae
CAG00775	0.13	NR	Species	Firmicutes bacterium CAG:102	unclassified Firmicutes	unclassified Firmicutes
CAG00931	0.13	NR	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG01263	0.15	NR	Species	Clostridium clostridioforme	Lachnoclostridium	Lachnospiraceae
CAG00086	0.15	NR	Species	Bacteroides massiliensis	Bacteroides	Bacteroidaceae
CAG00113	0.16	NR	Species	Clostridium scindens	Lachnoclostridium	Lachnospiraceae
CAG01323	0.17	NR	Species	Parabacteroides merdae	Parabacteroides	Porphyromonadaceae
CAG00502	0.18	NR	Species	Eubacterium sp. CAG:161	Eubacterium	Eubacteriaceae
CAG00254	0.19	NR	Species	Ruminococcus gnavus	Blautia	Lachnospiraceae
CAG01264	0.20	NR	Species	Clostridium clostridioforme	Lachnoclostridium	Lachnospiraceae
CAG00764	0.20	NR	Genus	unclassified Hungatella	Hungatella	Clostridiaceae
CAG00020	0.22	NR	Species	Clostridium sp. KLE 1755 & Clostridiales bacterium VE202-27	unclassified Clostridiales	unclassified Clostridiales
CAG00243	0.23	NR	Species	Ruminococcus torques 1	Blautia	Lachnospiraceae

CAG00808	0.23	NR	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00176	0.23	NR	Species	Coprococcus sp.x5	Coprococcus	Lachnospiraceae
CAG00008	0.23	NR	Species	Clostridium bolteae	Lachnoclostridium	Lachnospiraceae
CAG00061	0.24	NR	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00271	0.25	NR	Genus	unclassified Ruminococcus	Ruminococcus	Ruminococcaceae
CAG01377	0.25	NR	Species	Streptococcus vestibularis	Streptococcus	Streptococcaceae
CAG00212	0.26	NR	Species	Bilophila wadsworthia	Bilophila	Desulfovibrionaceae
CAG01238	0.26	NR	Species	butyrate-producing bacterium SS3/4 - Clostridium sp. CAG:81	unclassified Clostridiales	unclassified Clostridiales
CAG00975	0.27	NR	Species	Veillonella atypica	Veillonella	Veillonellaceae
CAG00034	0.30	NR	Species	Enterobacter cloacae 2	Enterobacter	Enterobacteriaceae
CAG00043	0.30	NR	Species	Enterobacter cloacae complex 'Hoffmann cluster IV'	Enterobacter	Enterobacteriaceae
CAG00064	0.30	NR	NA	unclassified	unclassified	unclassified
CAG00081	0.30	NR	Species	Bacteroides fragilis 2	Bacteroides	Bacteroidaceae
CAG00083	0.30	NR	Species	Odoribacter sp. CAG:788	Odoribacter	Odoribacteraceae
CAG00111	0.30	NR	Genus	unclassified Alistipes	Alistipes	Rikenellaceae
CAG00153	0.30	NR	Species	Hafnia paralvei	Hafnia	Enterobacteriaceae
CAG00171	0.30	NR	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG00189	0.30	NR	Species	Roseburia sp. CAG:100	Roseburia	Lachnospiraceae
CAG00263	0.30	NR	Species	Klebsiella oxytoca 1	Klebsiella	Enterobacteriaceae
CAG00299	0.30	NR	Species	Burkholderiales bacterium YL45	unclassified Burkholderiales	unclassified Burkholderiales
CAG00300	0.30	NR	Genus	unclassified Prevotella	Prevotella	Prevotellaceae
CAG00317	0.30	NR	Species	Clostridium sp. CAG:230	unclassified Clostridiales	unclassified Clostridiales
CAG00326	0.30	NR	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00347	0.30	NR	Species	Enterococcus faecium	Enterococcus	Enterococcaceae
CAG00380	0.30	NR	Genus	unclassified Prevotella	Prevotella	Prevotellaceae
CAG00415	0.30	NR	Genus	unclassified Desulfovibrio	Desulfovibrio	Desulfovibrionaceae
CAG00430	0.30	NR	Species	Lactobacillus salivarius	Lactobacillus	Lactobacillaceae
CAG00432	0.30	NR	Genus	unclassified Alistipes	Alistipes	Rikenellaceae
CAG00456	0.30	NR	Species	Bifidobacterium dentium	Bifidobacterium	Bifidobacteriaceae
CAG00449	0.30	NR	Species	Clostridium sp. CAG:253	unclassified Clostridiales	unclassified Clostridiales
CAG00500	0.30	NR	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00507	0.30	NR	Species	Clostridium hylemonae	Lachnoclostridium	Lachnospiraceae
CAG00546	0.30	NR	Species	Prevotella sp. CAG:5226	Prevotella	Prevotellaceae
CAG00567	0.30	NR	Species	Clostridium perfringens	Clostridium	Clostridiaceae
CAG00579	0.30	NR	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00616	0.30	NR	Genus	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00662	0.30	NR	Species	Bacteroides sp. CAG:875	Bacteroides	Bacteroidaceae
CAG00693	0.30	NR	Species	Parabacteroides sp. CAG:409	Parabacteroides	Porphyromonadaceae

CAG00998	0.30	NR	Species	Cryptobacterium sp. CAG:338	Cryptobacterium	Eggerthellaceae
CAG00768	0.30	NR	Genus	unclassified Coprobacter	Coprobacter	Porphyromonadaceae
CAG00787	0.30	NR	Species	Dialister sp. CAG:486	Dialister	Veillonellaceae
CAG00816	0.30	NR	Genus	unclassified Candidatus Stoquefichus	Candidatus Stoquefichus	Erysipelotrichaceae
CAG00886	0.30	NR	Species	Sutterella sp. CAG:351	Sutterella	Sutterellaceae
CAG00912	0.30	NR	Species	Roseburia sp. CAG:471	Roseburia	Lachnospiraceae
CAG00918	0.30	NR	Species	Collinsella sp. CAG:289	Collinsella	Coriobacteriaceae
CAG00933	0.30	NR	Species	Mitsuokella jalaludinii	Mitsuokella	Selenomonadaceae
CAG00968	0.30	NR	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00986	0.30	NR	Species	Collinsella intestinalis	Collinsella	Coriobacteriaceae
CAG00987	0.30	NR	Species	Megasphaera micronuciformis	Megasphaera	Veillonellaceae
CAG00989	0.30	NR	Species	Bacillus sp. CAG:988	Bacillus	Bacillaceae
CAG01002	0.30	NR	Genus	unclassified Coprobacillus	Coprobacillus	Erysipelotrichaceae
CAG01004	0.30	NR	Genus	unclassified Prevotella	Prevotella	Prevotellaceae
CAG01016	0.30	NR	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG01042	0.30	NR	Species	Enterococcus faecium	Enterococcus	Enterococcaceae
CAG01073	0.30	NR	Genus	unclassified Bacteroides	Bacteroides	Bacteroidaceae
CAG01105	0.30	NR	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01126	0.30	NR	Genus	unclassified Bacteroides	Bacteroides	Bacteroidaceae
CAG01139	0.30	NR	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01179	0.30	NR	Order	unclassified Bacteroidales	unclassified Bacteroidales	unclassified Bacteroidales
CAG01212	0.30	NR	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01244	0.30	NR	Species	Parabacteroides merdae	Parabacteroides	Porphyromonadaceae
CAG01246	0.30	NR	Genus	unclassified Butyricimonas	Butyricimonas	Odoribacteraceae
CAG01247	0.30	NR	Species	Clostridium sp. D5	Clostridium	Clostridiaceae
CAG01262	0.30	NR	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG01297	0.30	NR	Genus	unclassified Tyzzerella	Tyzzerella	Lachnospiraceae
CAG01316	0.30	NR	Genus	unclassified Bacteroides	Bacteroides	Bacteroidaceae
CAG01359	0.30	NR	Species	Clostridium sp. CAG:221 - Clostridium sp. CAG:265	unclassified Clostridiales	unclassified Clostridiales
CAG00005_4	0.30	NR	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00674	0.30	NR	Species	Dorea sp. CAG:317	Dorea	Lachnospiraceae
CAG00015	0.32	NR	Species	Hungatella hathewayi 2	Hungatella	Clostridiaceae
CAG00044	0.32	NR	Species	Clostridium symbiosum	Lachnoclostridium	Lachnospiraceae
CAG00301	0.33	NR	Species	Akkermansia muciniphila	Akkermansia	Akkermansiaceae
CAG00686	0.33	NR	Species	Prevotella copri	Prevotella	Prevotellaceae
CAG01155	0.33	NR	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00745	0.33	NR	Species	Dialister invisus	Dialister	Veillonellaceae
CAG00862	0.34	NR	Species	Firmicutes bacterium	unclassified Firmicutes	unclassified Firmicutes

				CAG:129 & uncultured Collinsella sp.		
CAG00038	0.37	NR	Family	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01192	0.37	NR	Genus	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00994	0.37	NR	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00223	0.38	NR	Genus	unclassified Clostridium	Clostridium	Clostridiaceae
CAG00270	0.38	NR	Species	Oscillibacter sp. KLE 1728 / KLE 1745 / VE202-24	Oscillibacter	Oscillospiraceae
CAG00054	0.38	NR	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00839	0.38	NR	Genus	unclassified Candidatus Stoquefichus	Candidatus Stoquefichus	Erysipelotrichaceae
CAG00844	0.38	NR	Genus	unclassified Akkermansia	Akkermansia	Akkermansiaceae
CAG00938	0.38	NR	Genus	unclassified Bacteroides	Bacteroides	Bacteroidaceae
CAG00942	0.38	NR	Species	Prevotella disiens	Prevotella	Prevotellaceae
CAG01239	0.38	NR	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00945	0.40	NR	Species	Bacteroides xylanisolvens	Bacteroides	Bacteroidaceae
CAG00268	0.40	NR	Species	Firmicutes bacterium CAG:24 & uncultured Ruminococcus sp.	unclassified Firmicutes	unclassified Firmicutes
new_4	0.40	NR	Species	Holdemania filiformis	Holdemania	Erysipelotrichaceae
CAG00275	0.41	NR	Species	Roseburia sp. CAG:18	Roseburia	Lachnospiraceae
CAG00718	0.41	NR	Species	Eubacterium ventriosum	Eubacterium	Eubacteriaceae
CAG00651	0.42	NR	Species	Lachnospiraceae bacterium 1_4_56FAA & CAG:215	unclassified Lachnospiraceae	Lachnospiraceae
CAG00805	0.42	NR	Genus	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG50002	0.43	NR	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00194	0.43	NR	Species	Eubacterium hallii	Eubacterium	Eubacteriaceae
CAG00515	0.44	NR	Species	Eubacterium eligens	Eubacterium	Eubacteriaceae
CAG00315	0.44	NR	Species	Citrobacter freundii	Citrobacter	Enterobacteriaceae
CAG00362	0.44	NR	Genus	unclassified Sutterella	Sutterella	Sutterellaceae
CAG00675	0.44	NR	Species	Clostridium sp. CAG:217	unclassified Clostridiales	unclassified Clostridiales
CAG00731	0.44	NR	Species	Proteobacteria bacterium CAG:139 (Parasutterella)	Parasutterella	Sutterellaceae
CAG00360	0.44	NR	Species	Alistipes finegoldii	Alistipes	Rikenellaceae
CAG00284	0.44	NR	Genus	unclassified Ruminococcus	Ruminococcus	Ruminococcaceae
CAG00311	0.45	NR	Species	Tyzzerella nexilis	Tyzzerella	Lachnospiraceae
CAG01230	0.46	NR	Species	Clostridiales bacterium 1_7_47FAA & VE202-28	unclassified Clostridiales	unclassified Clostridiales
CAG00149	0.46	NR	Genus	unclassified Hungatella	Hungatella	Clostridiaceae
CAG00082	0.46	NR	Species	Erysipelatoclostridium ramosum	Erysipelatoclostridium	Erysipelotrichaceae
CAG00025	0.47	NR	Species	Hungatella hathewayi 1	Hungatella	Clostridiaceae
CAG00493	0.51	NR	Species	Clostridium sp. CAG:127	unclassified Clostridiales	unclassified Clostridiales
CAG00170	0.52	NR	Species	Victivallis vadensis	Victivallis	Victivallaceae

CAG00165	0.52	NR	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG00479	0.52	NR	Genus	unclassified Alistipes	Alistipes	Rikenellaceae
CAG00873	0.52	NR	Genus	unclassified Butyricimonas	Butyricimonas	Odoribacteraceae
CAG00984	0.52	NR	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG01162	0.52	NR	Genus	unclassified Roseburia	Roseburia	Lachnospiraceae
CAG00638	0.53	NR	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG01379	0.54	NR	Genus	unclassified Hungatella	Hungatella	Clostridiaceae
CAG00897	0.55	NR	Species	Oscillibacter sp.	Oscillibacter	Oscillospiraceae
CAG01245	0.57	NR	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00159	0.57	NR	Species	Bacteroides uniformis	Bacteroides	Bacteroidaceae
CAG00267	0.58	NR	Species	Clostridium sp. CAG:58	unclassified Clostridiales	unclassified Clostridiales
CAG01276	0.58	NR	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00482	0.59	NR	Species	Blautia sp. KLE 1732	Blautia	Lachnospiraceae
CAG00618	0.60	NR	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00291	0.60	NR	Species	Roseburia intestinalis	Roseburia	Lachnospiraceae
CAG00168	0.62	NR	Species	Clostridiales bacterium VE202-14	unclassified Clostridiales	unclassified Clostridiales
CAG00582	0.62	NR	Species	Roseburia inulinivorans	Roseburia	Lachnospiraceae
CAG01380	0.62	NR	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00260	0.64	NR	Species	Acidaminococcus intestini	Acidaminococcus	Acidaminococcaceae
CAG01207	0.66	NR	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00022	0.69	NR	Species	Clostridium clostridioforme	Lachnoclostridium	Lachnospiraceae
CAG00424	0.69	NR	Species	Firmicutes bacterium CAG:137	unclassified Firmicutes	unclassified Firmicutes
CAG00649	0.69	NR	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG00228	0.70	NR	Family	unclassified Selenomonadaceae	unclassified Selenomonadaceae	Selenomonadaceae
CAG01268	0.70	NR	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00414	0.71	NR	Species	Eubacterium sp. CAG:76	Eubacterium	Eubacteriaceae
CAG00293	0.72	NR	Species	Firmicutes bacterium CAG:145	unclassified Firmicutes	unclassified Firmicutes
CAG01369	0.72	NR	Species	Roseburia intestinalis	Roseburia	Lachnospiraceae
CAG00603	0.73	NR	Species	Firmicutes bacterium CAG:114	unclassified Firmicutes	unclassified Firmicutes
CAG00578	0.74	NR	Species	Alistipes putredinis	Alistipes	Rikenellaceae
CAG00036	0.76	NR	Species	Clostridium innocuum	Erysipelatoclostridium	Erysipelotrichaceae
CAG01386	0.76	NR	Species	Blautia sp. KLE 1732	Blautia	Lachnospiraceae
CAG00186	0.76	NR	Species	Streptococcus salivarius	Streptococcus	Streptococcaceae
CAG00014	0.76	NR	Species	Lachnospiraceae bacterium x4	unclassified Lachnospiraceae	Lachnospiraceae
CAG00909	0.78	NR	Species	Parasutterella excrementihominis	Parasutterella	Sutterellaceae
CAG01321	0.78	NR	Species	Faecalibacterium prausnitzii 1 (SL3/3 - M21/2)	Faecalibacterium	Ruminococcaceae
CAG00142	0.79	NR	Species	Bacteroides stercoris	Bacteroides	Bacteroidaceae
CAG00407	0.80	NR	Genus	unclassified Hungatella	Hungatella	Clostridiaceae

CAG00824	0.80	NR	NA	unclassified	unclassified	unclassified
CAG00101	0.81	NR	Species	Ruminococcus sp. CAG:17	Blautia	Lachnospiraceae
CAG00119	0.81	NR	Genus	unclassified Clostridium	Clostridium	Clostridiaceae
CAG00122	0.81	NR	Family	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG00645	0.81	NR	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00941	0.81	NR	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00943	0.81	NR	Species	Firmicutes bacterium CAG:212	unclassified Firmicutes	unclassified Firmicutes
CAG01079	0.81	NR	NA	unclassified	unclassified	unclassified
CAG01237	0.81	NR	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00420	0.83	NR	NA	unclassified	unclassified	unclassified
CAG00818	0.83	NR	Genus	unclassified Coprobacillus	Coprobacillus	Erysipelotrichaceae
CAG00736	0.83	NR	Species	Ruminococcus torques 2	Blautia	Lachnospiraceae
CAG00178	0.84	NR	Species	Eubacterium sp. CAG:38	Eubacterium	Eubacteriaceae
CAG00650	0.84	NR	Species	Dorea formicigenerans	Dorea	Lachnospiraceae
CAG00140	0.85	NR	Species	Subdoligranulum sp. 4_3_54A2FAA	Subdoligranulum	Ruminococcaceae
CAG00558	0.86	NR	Species	Ruminococcus lactaris	Ruminococcus	Ruminococcaceae
CAG00029	0.86	NR	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG00072	0.86	NR	Species	Odoribacter laneus	Odoribacter	Odoribacteraceae
CAG00100	0.86	NR	Species	Clostridium sp. JCC	unclassified Clostridiales	unclassified Clostridiales
CAG00135	0.86	NR	Species	Bacteroides eggerthii	Bacteroides	Bacteroidaceae
CAG00143	0.86	NR	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00226	0.86	NR	Family	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG00242	0.86	NR	Species	Roseburia sp. CAG:197	Roseburia	Lachnospiraceae
CAG00278	0.86	NR	Species	Roseburia sp. CAG:380	Roseburia	Lachnospiraceae
CAG00375	0.86	NR	Genus	unclassified Prevotella	Prevotella	Prevotellaceae
CAG00448	0.86	NR	Species	Clostridium sp. CAG:122	unclassified Clostridiales	unclassified Clostridiales
CAG00982	0.86	NR	Genus	unclassified Slackia	Slackia	Eggerthellaceae
CAG00557	0.86	NR	Species	Ruminococcus callidus	Ruminococcus	Ruminococcaceae
CAG00626	0.86	NR	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00711	0.86	NR	Species	Bacteroides plebeius	Bacteroides	Bacteroidaceae
CAG00961	0.86	NR	Genus	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00814	0.86	NR	Species	Eubacterium sp. CAG:192	Eubacterium	Eubacteriaceae
CAG00871	0.86	NR	Family	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG00936	0.86	NR	Species	Eggerthella sp. CAG:209	Eggerthella	Eggerthellaceae
CAG01037	0.86	NR	Genus	unclassified Roseburia	Roseburia	Lachnospiraceae
CAG01223	0.86	NR	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01282	0.86	NR	Species	Bacteroides plebeius	Bacteroides	Bacteroidaceae
CAG01304	0.86	NR	Genus	unclassified Megasphaera	Megasphaera	Veillonellaceae
CAG00274	0.86	NR	Species	Erysipelotrichaceae bacterium 5_2_54FAA	unclassified Erysipelotrichaceae	Erysipelotrichaceae

				- Eubacterium sp. 3_1_31		
CAG00672	0.87	NR	Species	Clostridium sp. CAG:169	unclassified Clostridiales	unclassified Clostridiales
CAG00950	0.87	NR	Genus	unclassified Haemophilus	Haemophilus	Pasteurellaceae
CAG01132	0.87	NR	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01358	0.88	NR	Species	Lachnospiraceae bacterium x4	unclassified Lachnospiraceae	Lachnospiraceae
CAG00580	0.88	NR	Species	Clostridium sp. CAG:62	unclassified Clostridiales	unclassified Clostridiales
CAG00780	0.91	NR	Species	Firmicutes bacterium CAG:41	unclassified Firmicutes	unclassified Firmicutes
CAG00610	0.91	NR	Species	Hungatella hathewayi 2	Hungatella	Clostridiaceae
CAG00481	0.94	NR	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG01387	0.94	NR	Genus	unclassified Dorea	Dorea	Lachnospiraceae
CAG00554	0.95	NR	Species	Eubacterium ramulus	Eubacterium	Eubacteriaceae
CAG01115	0.95	NR	Species	Eggerthella sp. CAG:298	Eggerthella	Eggerthellaceae
CAG00829	0.95	NR	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00018_2	0.95	NR	Species	Klebsiella michiganensis	Klebsiella	Enterobacteriaceae
CAG01270	0.95	NR	NA	unclassified	unclassified	unclassified
CAG00160	0.97	NR	NA	unclassified	unclassified	unclassified
CAG00962	0.97	NR	Species	Dorea longicatena 2	Dorea	Lachnospiraceae
CAG00049	0.98	NR	Species	Bacteroides caccae	Bacteroides	Bacteroidaceae
CAG00692	0.98	NR	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00164	0.98	NR	Species	Blautia wexlerae	Blautia	Lachnospiraceae
CAG00027_1	1.00	NR	Genus	unclassified Akkermansia	Akkermansia	Akkermansiaceae
CAG00187	1.00	NR	Species	Sutterella wadsworthensis 1	Sutterella	Sutterellaceae
CAG00253	1.00	NR	Species	Lactococcus lactis	Lactococcus	Streptococcaceae
CAG01056	1.00	NR	Species	Haemophilus parainfluenzae	Haemophilus	Pasteurellaceae
CAG01190	1.00	NR	Species	Butyricimonas virosa	Butyricimonas	Odoribacteraceae
CAG00327	0.01	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00659	0.02	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00492	0.03	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae
CAG00518	0.03	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae
CAG01146	0.03	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00079	0.04	R	Species	Clostridium sp. CAG:7	unclassified Clostridiales	unclassified Clostridiales
CAG00393	0.05	R	Species	Eubacterium sp. CAG:86	Eubacterium	Eubacteriaceae
CAG00766	0.07	R	Species	Firmicutes bacterium CAG:176	unclassified Firmicutes	unclassified Firmicutes
CAG00095	0.07	R	Species	Akkermansia sp. CAG:344	Akkermansia	Akkermansiaceae
CAG00010_1	0.07	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00342	0.07	R	Species	Bifidobacterium pseudocatenulatum	Bifidobacterium	Bifidobacteriaceae
CAG00303	0.07	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00337	0.07	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae

CAG00381	0.07	R	Species	Clostridium sp. CAG:242	unclassified Clostridiales	unclassified Clostridiales
CAG00559	0.07	R	Family	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00570	0.07	R	NA	unclassified	unclassified	unclassified
CAG00635	0.07	R	Species	Bifidobacterium bifidum	Bifidobacterium	Bifidobacteriaceae
CAG00636	0.07	R	Genus	unclassified Roseburia	Roseburia	Lachnospiraceae
CAG00660	0.07	R	Species	Alistipes timonensis	Alistipes	Rikenellaceae
CAG00669	0.07	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00708	0.07	R	Species	Alistipes senegalensis	Alistipes	Rikenellaceae
CAG00773	0.07	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00807	0.07	R	Genus	unclassified Holdemanella	Holdemanella	Erysipelotrichaceae
CAG00880	0.07	R	Species	Subdoligranulum sp. CAG:314	Subdoligranulum	Ruminococcaceae
CAG00907	0.07	R	Family	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01086	0.07	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01215	0.07	R	Family	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01277	0.07	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01308	0.07	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00577	0.07	R	Species	Faecalibacterium prausnitzii 3 (L2-6)	Faecalibacterium	Ruminococcaceae
CAG00506	0.08	R	Genus	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00852	0.09	R	Species	Clostridium spiroforme	Erysipelatoclostridium	Erysipelotrichaceae
CAG01046	0.09	R	Genus	unclassified Intestinimonas	Intestinimonas	unclassified Clostridiales
CAG00320	0.09	R	Species	Phascolarctobacterium sp. CAG:207	Phascolarctobacterium	Acidaminococcaceae
CAG00619	0.10	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae
CAG01366	0.10	R	Species	Streptococcus parasanguinis	Streptococcus	Streptococcaceae
CAG00509	0.10	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00441	0.10	R	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00249	0.11	R	Species	Clostridium leptum	Ruminiclostridium	Ruminococcaceae
CAG50003	0.12	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00039	0.12	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00127	0.12	R	Family	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG00854	0.12	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00166	0.12	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00272	0.12	R	Species	Faecalibacterium 5 (sp. CAG:74)	Faecalibacterium	Ruminococcaceae
CAG00294	0.12	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00367	0.12	R	Species	Firmicutes bacterium CAG:170	unclassified Firmicutes	unclassified Firmicutes
CAG00445	0.12	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00452	0.12	R	Species	Clostridium sp. CAG:167	unclassified Clostridiales	unclassified Clostridiales
CAG00497	0.12	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00505	0.12	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes

CAG00721	0.12	R	Species	Methanobrevibacter smithii 1	Methanobrevibacter	Methanobacteriaceae
CAG00624	0.12	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00648	0.12	R	Genus	unclassified Eubacterium	Eubacterium	Eubacteriaceae
CAG00735	0.12	R	Genus	unclassified Eubacterium	Eubacterium	Eubacteriaceae
CAG00770	0.12	R	Family	unclassified Eggerthellaceae	unclassified Eggerthellaceae	Eggerthellaceae
CAG00812	0.12	R	Species	Catenibacterium sp. CAG:290	Catenibacterium	Erysipelotrichaceae
CAG00861	0.12	R	Species	Oscillibacter sp. CAG:241	Oscillibacter	Oscillospiraceae
CAG00863	0.12	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00925	0.12	R	NA	unclassified	unclassified	unclassified
CAG00934	0.12	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01003	0.12	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG01325	0.12	R	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG02021	0.12	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01349	0.12	R	NA	unclassified	unclassified	unclassified
CAG01350	0.12	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01402	0.12	R	Species	Turicibacter sp. H121	Turicibacter	Erysipelotrichaceae
CAG01403	0.12	R	Species	Bacteroides stercorirosoris	Bacteroides	Bacteroidaceae
CAG01551	0.12	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG01028	0.13	R	Species	Ruminococcaceae bacterium LM158	unclassified Ruminococcaceae	Ruminococcaceae
CAG00121	0.13	R	Species	Bacteroides finegoldii	Bacteroides	Bacteroidaceae
CAG00670	0.13	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00324	0.14	R	Species	Firmicutes bacterium CAG:94	unclassified Firmicutes	unclassified Firmicutes
CAG00218	0.14	R	Species	Barnesiella intestinihominis	Barnesiella	Porphyromonadaceae
CAG00755	0.14	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae
CAG00560	0.18	R	Genus	unclassified Subdoligranulum	Subdoligranulum	Ruminococcaceae
CAG00259	0.18	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01039	0.18	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae
CAG00239	0.18	R	Species	Flavonifractor plautii	Flavonifractor	unclassified Clostridiales
CAG00112	0.18	R	Species	Blautia sp. CAG:52	Blautia	Lachnospiraceae
CAG00697	0.20	R	Genus	unclassified Hungatella	Hungatella	Clostridiaceae
CAG00595	0.20	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00629	0.21	R	Species	Firmicutes bacterium CAG:124	unclassified Firmicutes	unclassified Firmicutes
CAG00549	0.22	R	Species	Bifidobacterium longum	Bifidobacterium	Bifidobacteriaceae
CAG00328	0.22	R	Species	Alistipes indistinctus	Alistipes	Rikenellaceae
CAG00760	0.22	R	Species	Ruminococcus sp. CAG:177	Ruminococcus	Ruminococcaceae
CAG00031	0.22	R	NA	unclassified	unclassified	unclassified
CAG00102	0.22	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00130	0.22	R	Species	Weissella confusa	Weissella	Leuconostocaceae
CAG00134	0.22	R	Species	Cloacibacillus	Cloacibacillus	Synergistaceae

				porcorum		
CAG00145	0.22	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00200	0.22	R	Genus	unclassified Flavonifractor	Flavonifractor	unclassified Clostridiales
CAG00179	0.22	R	Species	Blautia sp. CAG:237	Blautia	Lachnospiraceae
CAG00183	0.22	R	Species	Ruminococcus sp. CAG:60	Ruminococcus	Ruminococcaceae
CAG00198	0.22	R	NA	unclassified	unclassified	unclassified
CAG00214	0.22	R	Species	Prevotella corporis	Prevotella	Prevotellaceae
CAG00261	0.22	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00241	0.22	R	Species	Anaerotruncus sp. CAG:390	Anaerotruncus	Ruminococcaceae
CAG00363	0.22	R	Genus	unclassified Intestinimonas	Intestinimonas	unclassified Clostridiales
CAG00373	0.22	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00436	0.22	R	Species	Clostridium sp. CAG:299	unclassified Clostridiales	unclassified Clostridiales
CAG00470	0.22	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00541	0.22	R	Species	Dorea sp. CAG:105	Dorea	Lachnospiraceae
CAG00542	0.22	R	Species	Butyrivibrio crossotus	Butyrivibrio	Lachnospiraceae
CAG00644	0.22	R	Species	Clostridium sp. CAG:226	unclassified Clostridiales	unclassified Clostridiales
CAG00658	0.22	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00676	0.22	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00698	0.22	R	Genus	unclassified Ruminococcus	Ruminococcus	Ruminococcaceae
CAG00703	0.22	R	Species	Candidatus Methanomassiliicoccus intestinalis	Methanomassiliicoccus	Methanomassiliicoccaceae
CAG00831	0.22	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00841	0.22	R	Species	Firmicutes bacterium CAG:345	unclassified Firmicutes	unclassified Firmicutes
CAG00850	0.22	R	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00851	0.22	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00048_1	0.22	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00866	0.22	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00892	0.22	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00959	0.22	R	Genus	unclassified Alistipes	Alistipes	Rikenellaceae
CAG00965	0.22	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00988	0.22	R	Species	Clostridium sp. CAG:349	unclassified Clostridiales	unclassified Clostridiales
CAG01047	0.22	R	Family	unclassified Clostridiales Family XIII. Incertae Sedis	unclassified Clostridiales Family XIII. Incertae Sedis	Clostridiales Family XIII. Incertae Sedis
CAG01075	0.22	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01099	0.22	R	Species	Raoultella ornithinolytica	Raoultella	Enterobacteriaceae
CAG01108	0.22	R	Species	Clostridium sp. CAG:798	unclassified Clostridiales	unclassified Clostridiales
CAG01145	0.22	R	Genus	unclassified Bacteroides	Bacteroides	Bacteroidaceae
CAG01156	0.22	R	Genus	unclassified Eubacterium	Eubacterium	Eubacteriaceae
CAG01169	0.22	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG01240	0.22	R	Species	Parabacteroides gordonii	Parabacteroides	Porphyromonadaceae

CAG00068_2	0.22	R	Genus	unclassified Porphyromonas	Porphyromonas	Porphyromonadaceae
CAG01372	0.22	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG01394	0.22	R	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00052	0.23	R	Species	Parabacteroides goldsteinii	Parabacteroides	Porphyromonadaceae
CAG00116	0.23	R	Species	Bacteroides nordii	Bacteroides	Bacteroidaceae
CAG00429	0.23	R	Species	Eubacterium sp. CAG:248	Eubacterium	Eubacteriaceae
CAG00702	0.24	R	Species	Bifidobacterium adolescentis	Bifidobacterium	Bifidobacteriaceae
CAG00309	0.24	R	Species	Alistipes onderdonkii	Alistipes	Rikenellaceae
CAG01637	0.26	R	Species	Firmicutes bacterium CAG:65	unclassified Firmicutes	unclassified Firmicutes
CAG01051	0.27	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00792	0.27	R	Species	Firmicutes bacterium CAG:65	unclassified Firmicutes	unclassified Firmicutes
CAG00208	0.27	R	Species	Faecalibacterium 8	Faecalibacterium	Ruminococcaceae
CAG01700	0.28	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG01371	0.28	R	Species	Escherichia coli	Escherichia	Enterobacteriaceae
CAG00653	0.29	R	Species	Eubacterium siraeum	Ruminiclostridium	Ruminococcaceae
CAG00520	0.29	R	Species	Firmicutes bacterium CAG:56	unclassified Firmicutes	unclassified Firmicutes
CAG00273	0.31	R	Species	Blautia sp. CAG:37	Blautia	Lachnospiraceae
CAG00563	0.32	R	Species	Clostridium sp. CAG:91	unclassified Clostridiales	unclassified Clostridiales
CAG01202	0.32	R	Species	Bacteroides cellulosilyticus	Bacteroides	Bacteroidaceae
CAG00601	0.32	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae
CAG01030	0.32	R	Species	Coprobacillus sp. CAG:235	Coprobacillus	Erysipelotrichaceae
CAG00366	0.32	R	Species	Coprococcus comes	Coprococcus	Lachnospiraceae
CAG00937	0.33	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01025	0.33	R	Species	Clostridium citroniae	Lachnoclostridium	Lachnospiraceae
CAG00508	0.33	R	Species	Alistipes obesi	Alistipes	Rikenellaceae
CAG01228	0.33	R	Genus	unclassified Hungatella	Hungatella	Clostridiaceae
CAG01214	0.34	R	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00611	0.34	R	Species	Ruminococcus bromii	Ruminococcus	Ruminococcaceae
CAG01208	0.36	R	Species	Coprococcus catus	Coprococcus	Lachnospiraceae
CAG00377	0.36	R	Species	Desulfovibrio sp. 6_1_46AFAA & sp. 3_1_syn3	Desulfovibrio	Desulfovibrionaceae
CAG00460	0.37	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00042	0.37	R	Species	uncultured Faecalibacterium sp.	Faecalibacterium	Ruminococcaceae
CAG00265	0.37	R	Species	Firmicutes bacterium CAG:194	unclassified Firmicutes	unclassified Firmicutes
CAG00321	0.37	R	Species	Clostridium sp. CAG:138	unclassified Clostridiales	unclassified Clostridiales
CAG00402	0.37	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00474	0.37	R	Species	Sutterella wadsworthensis 2	Sutterella	Sutterellaceae
CAG01008	0.37	R	Species	Clostridium sp. CAG:343	unclassified Clostridiales	unclassified Clostridiales
CAG01090	0.37	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes

CAG01024	0.37	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01011	0.37	R	Genus	unclassified Dorea	Dorea	Lachnospiraceae
CAG00090	0.39	R	Species	Anaerostipes caccae	Anaerostipes	Lachnospiraceae
CAG00620	0.39	R	Species	Blautia obeum	Blautia	Lachnospiraceae
CAG00296	0.42	R	Species	Turicibacter sanguinis	Turicibacter	Erysipelotrichaceae
CAG01401	0.42	R	Family	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG01408	0.42	R	Genus	unclassified Butyricimonas	Butyricimonas	Odoribacteraceae
CAG00009	0.42	R	Genus	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG00017	0.42	R	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG00035	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00051	0.42	R	Species	Clostridium clostridioforme	Lachnoclostridium	Lachnospiraceae
CAG00055	0.42	R	Species	Roseburia sp. CAG:182	Roseburia	Lachnospiraceae
CAG00056	0.42	R	Genus	unclassified Bilophila	Bilophila	Desulfovibrionaceae
CAG00060	0.42	R	NA	unclassified	unclassified	unclassified
CAG00490	0.42	R	Species	Coprococcus sp. CAG:131 & Coprococcus sp. ART55/1	Coprococcus	Lachnospiraceae
CAG00084	0.42	R	NA	unclassified	unclassified	unclassified
CAG00089	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00126	0.42	R	Species	Parabacteroides gordonii	Parabacteroides	Porphyromonadaceae
CAG00333	0.42	R	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG00131	0.42	R	Species	Bacteroides sp. CAG:754	Bacteroides	Bacteroidaceae
CAG00133	0.42	R	Genus	unclassified Bacteroides	Bacteroides	Bacteroidaceae
CAG00151	0.42	R	Family	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG00155	0.42	R	Species	Firmicutes bacterium CAG:424	unclassified Firmicutes	unclassified Firmicutes
CAG00157	0.42	R	NA	unclassified	unclassified	unclassified
CAG00175	0.42	R	Species	Bacteroides clarus	Bacteroides	Bacteroidaceae
CAG00180	0.42	R	Genus	unclassified Bacteroides	Bacteroides	Bacteroidaceae
CAG00190	0.42	R	Genus	unclassified Victivallis	Victivallis	Victivallaceae
CAG00199	0.42	R	Species	uncultured Alistipes sp.	Alistipes	Rikenellaceae
CAG00203	0.42	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00207	0.42	R	Species	Clostridium sp. CAG:411	unclassified Clostridiales	unclassified Clostridiales
CAG00217	0.42	R	Species	Dialister sp. CAG:357	Dialister	Veillonellaceae
CAG00227	0.42	R	Species	Firmicutes bacterium CAG:646	unclassified Firmicutes	unclassified Firmicutes
CAG00232	0.42	R	Genus	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00245	0.42	R	NA	unclassified	unclassified	unclassified
CAG00246	0.42	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00251	0.42	R	Species	Alistipes sp. CAG:157	Alistipes	Rikenellaceae
CAG00264	0.42	R	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG00266	0.42	R	Species	Streptococcus infantarius	Streptococcus	Streptococcaceae

CAG00282	0.42	R	Species	Clostridium sp. CAG:277	unclassified Clostridiales	unclassified Clostridiales
CAG00297	0.42	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae
CAG00350	0.42	R	NA	unclassified	unclassified	unclassified
CAG00356	0.42	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00365	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00388	0.42	R	NA	unclassified	unclassified	unclassified
CAG00390	0.42	R	Species	Butyrivibrio sp. CAG:318	Butyrivibrio	Lachnospiraceae
CAG00391	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00394	0.42	R	NA	unclassified	unclassified	unclassified
CAG00404	0.42	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00406	0.42	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00410	0.42	R	Genus	unclassified Erysipelotrichaceae	unclassified Erysipelotrichaceae	Erysipelotrichaceae
CAG00413	0.42	R	Species	Bacteroides sp. CAG:144	Bacteroides	Bacteroidaceae
CAG00421	0.42	R	Genus	unclassified Alistipes	Alistipes	Rikenellaceae
CAG00422	0.42	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00447	0.42	R	Species	Clostridium sp. CAG:413	unclassified Clostridiales	unclassified Clostridiales
CAG00453	0.42	R	NA	unclassified	unclassified	unclassified
CAG00458	0.42	R	Species	Fusobacterium nucleatum	Fusobacterium	Fusobacteriaceae
CAG00459	0.42	R	NA	unclassified	unclassified	unclassified
CAG00464	0.42	R	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00465	0.42	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00480	0.42	R	Species	Lactobacillus casei == paracasei	Lactobacillus	Lactobacillaceae
CAG00483	0.42	R	Species	Clostridium sp. CAG:352	unclassified Clostridiales	unclassified Clostridiales
CAG00489	0.42	R	NA	unclassified	unclassified	unclassified
CAG00495	0.42	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae
CAG00499	0.42	R	Species	Prevotella sp. CAG:891	Prevotella	Prevotellaceae
CAG00512	0.42	R	Species	Bacteroides sp. CAG:927	Bacteroides	Bacteroidaceae
CAG00513	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00533	0.42	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00571	0.42	R	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00573	0.42	R	Genus	unclassified Brachyspira	Brachyspira	Brachyspiraceae
CAG00583	0.42	R	NA	unclassified	unclassified	unclassified
CAG00584	0.42	R	Genus	unclassified Ruminococcus	Ruminococcus	Ruminococcaceae
CAG00598	0.42	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00600	0.42	R	Species	Prevotella sp. CAG:1031	Prevotella	Prevotellaceae
CAG00602	0.42	R	Genus	unclassified Prevotella	Prevotella	Prevotellaceae
CAG00606	0.42	R	Phylum	unclassified Euryarchaeota	unclassified Euryarchaeota	unclassified Euryarchaeota
CAG00614	0.42	R	NA	unclassified	unclassified	unclassified
CAG00621	0.42	R	Species	Firmicutes bacterium	unclassified Firmicutes	unclassified Firmicutes

				CAG:272		
CAG00622	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00783	0.42	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00625	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00631	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00632	0.42	R	Species	Weissella cibaria	Weissella	Leuconostocaceae
CAG00637	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00643	0.42	R	NA	unclassified	unclassified	unclassified
CAG00656	0.42	R	Species	Ruminococcus sp. CAG:488	Ruminococcus	Ruminococcaceae
CAG00661	0.42	R	Species	Clostridium sp. CAG:715	unclassified Clostridiales	unclassified Clostridiales
CAG00664	0.42	R	Species	Alistipes sp. CAG:268	Alistipes	Rikenellaceae
CAG00678	0.42	R	NA	unclassified	unclassified	unclassified
CAG00679	0.42	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00682	0.42	R	Genus	unclassified Fusobacterium	Fusobacterium	Fusobacteriaceae
CAG00983	0.42	R	Species	Clostridium sp. CAG:269	unclassified Clostridiales	unclassified Clostridiales
CAG00695	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00707	0.42	R	Species	Ruminococcus sp. CAG:382	Ruminococcus	Ruminococcaceae
CAG00710	0.42	R	Species	Enorma massiliensis	Enorma	Coriobacteriaceae
CAG00719	0.42	R	Genus	unclassified Ruminococcus	Ruminococcus	Ruminococcaceae
CAG00725	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00737	0.42	R	Genus	unclassified Alistipes	Alistipes	Rikenellaceae
CAG00750	0.42	R	NA	unclassified	unclassified	unclassified
CAG00751	0.42	R	Species	Firmicutes bacterium CAG:238	unclassified Firmicutes	unclassified Firmicutes
CAG00754	0.42	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00756	0.42	R	Species	Coprobacter sp. 177	Coprobacter	Porphyromonadaceae
CAG00759	0.42	R	NA	unclassified	unclassified	unclassified
CAG00761	0.42	R	Species	Acinetobacter sp. CAG:196	Acinetobacter	Moraxellaceae
CAG00763	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00778	0.42	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00784	0.42	R	Genus	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00801	0.42	R	Genus	unclassified Sutterella	Sutterella	Sutterellaceae
CAG00806	0.42	R	NA	unclassified	unclassified	unclassified
CAG00811	0.42	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00830	0.42	R	Species	Ruminococcus sp. CAG:254	Ruminococcus	Ruminococcaceae
CAG00837	0.42	R	Species	Clostridium sp. CAG:302	unclassified Clostridiales	unclassified Clostridiales
CAG00840	0.42	R	NA	unclassified	unclassified	unclassified
CAG00846	0.42	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00849	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00859	0.42	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00864	0.42	R	Species	Solobacterium moorei	Solobacterium	Erysipelotrichaceae
CAG00872	0.42	R	Species	Firmicutes bacterium CAG:240	unclassified Firmicutes	unclassified Firmicutes

CAG00882	0.42	R	Species	Clostridium sp. CAG:288	unclassified Clostridiales	unclassified Clostridiales
CAG00887	0.42	R	Species	Alistipes sp. CAG:435	Alistipes	Rikenellaceae
CAG00895	0.42	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00898	0.42	R	Genus	unclassified Eggerthella	Eggerthella	Eggerthellaceae
CAG00906	0.42	R	Genus	unclassified Acidiphilium	Acidiphilium	Acetobacteraceae
CAG00913	0.42	R	NA	unclassified	unclassified	unclassified
CAG00167_1	0.42	R	Species	Clostridium sp. CAG:417 - uncultured Clostridium sp.	unclassified Clostridiales	unclassified Clostridiales
CAG00985	0.42	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00167_2	0.42	R	Species	Clostridium sp. CAG:492	unclassified Clostridiales	unclassified Clostridiales
CAG00939	0.42	R	Species	Firmicutes bacterium CAG:313	unclassified Firmicutes	unclassified Firmicutes
CAG00947	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00951	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00954	0.42	R	Genus	unclassified Mycoplasma	Mycoplasma	Mycoplasmataceae
CAG00970	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00981	0.42	R	Family	unclassified Erysipelotrichaceae	unclassified Erysipelotrichaceae	Erysipelotrichaceae
CAG00999	0.42	R	Species	Mycoplasma sp. CAG:956	Mycoplasma	Mycoplasmataceae
CAG01000	0.42	R	Species	Firmicutes bacterium CAG:631	unclassified Firmicutes	unclassified Firmicutes
CAG01006	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01019	0.42	R	Genus	unclassified Senegalimassilia	Senegalimassilia	Coriobacteriaceae
CAG01029	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01043	0.42	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01049	0.42	R	Species	Methanosphaera stadtmanae	Methanosphaera	Methanobacteriaceae
CAG01057	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00007_3	0.42	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01072	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01083	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01085	0.42	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG01091	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01106	0.42	R	Species	Clostridium sp. CAG:451	unclassified Clostridiales	unclassified Clostridiales
CAG01119	0.42	R	Species	Bacteroides intestinalis	Bacteroides	Bacteroidaceae
CAG01123	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01124	0.42	R	Species	Parvimonas micra	Parvimonas	Peptoniphilaceae
CAG01130	0.42	R	Species	Prevotella bivia	Prevotella	Prevotellaceae
CAG01131	0.42	R	Species	Sutterella sp. CAG:521	Sutterella	Sutterellaceae
CAG01133	0.42	R	Species	Senegalimassilia anaerobia	Senegalimassilia	Coriobacteriaceae
CAG01134	0.42	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01137	0.42	R	Genus	unclassified Acholeplasma	Acholeplasma	Acholeplasmataceae
CAG01148	0.42	R	Species	Clostridium scindens	Lachnoclostridium	Lachnospiraceae

CAG01151	0.42	R	Species	Mycoplasma sp. CAG:472	Mycoplasma	Mycoplasmataceae
CAG01181	0.42	R	Species	Firmicutes bacterium CAG:460	unclassified Firmicutes	unclassified Firmicutes
CAG01182	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01183	0.42	R	Family	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01198	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01204	0.42	R	Species	Slackia piriformis	Slackia	Eggerthellaceae
CAG01217	0.42	R	Family	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG01235	0.42	R	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG01241	0.42	R	Species	Clostridium sp. CAG:433	unclassified Clostridiales	unclassified Clostridiales
CAG01261	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01284	0.42	R	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG01292	0.42	R	Species	Mycoplasma sp. CAG:611	Mycoplasma	Mycoplasmataceae
CAG01298	0.42	R	Species	Oxalobacter formigenes	Oxalobacter	Oxalobacteraceae
CAG01336	0.42	R	NA	unclassified	unclassified	unclassified
CAG01339	0.42	R	Species	Peptoniphilus lacrimalis	Peptoniphilus	Peptoniphilaceae
CAG01347	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01357	0.42	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01360	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01364	0.42	R	Genus	unclassified Bacteroides	Bacteroides	Bacteroidaceae
CAG01365	0.42	R	Phylum	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01367	0.42	R	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG01370	0.42	R	Genus	unclassified Bacteroides	Bacteroides	Bacteroidaceae
CAG01382	0.42	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01385	0.42	R	Genus	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG01388	0.42	R	Genus	unclassified Alistipes	Alistipes	Rikenellaceae
CAG01392	0.42	R	Family	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00799	0.43	R	Species	Dorea longicatena 1	Dorea	Lachnospiraceae
CAG01040	0.43	R	Species	Clostridium asparagiforme	Lachnoclostridium	Lachnospiraceae
CAG00612	0.43	R	Species	Faecalibacterium prausnitzii 2 (A2-165)	Faecalibacterium	Ruminococcaceae
CAG00858	0.44	R	Species	Streptococcus thermophilus	Streptococcus	Streptococcaceae
CAG00210	0.44	R	Species	Ruminococcus bicirculans	Ruminococcus	Ruminococcaceae
CAG00013	0.44	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00463	0.44	R	Species	Faecalibacterium 6 (sp. CAG:82)	Faecalibacterium	Ruminococcaceae
CAG01020	0.45	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG01299	0.46	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00288	0.47	R	Species	Firmicutes bacterium CAG:95	unclassified Firmicutes	unclassified Firmicutes
CAG00298	0.47	R	Species	Bacteroides intestinalis	Bacteroides	Bacteroidaceae
CAG00732	0.47	R	Genus	unclassified Eubacterium	Eubacterium	Eubacteriaceae

CAG00450	0.47	R	Family	unclassified Erysipelotrichaceae	unclassified Erysipelotrichaceae	Erysipelotrichaceae
CAG00696	0.47	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00879	0.47	R	Genus	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00996	0.47	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01061	0.47	R	Species	Clostridium sp. CAG:245	unclassified Clostridiales	unclassified Clostridiales
CAG01065	0.47	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01197	0.47	R	Genus	unclassified Dialister	Dialister	Veillonellaceae
CAG00078	0.50	R	Species	Proteus mirabilis	Proteus	Enterobacteriaceae
CAG00341	0.50	R	Species	Eubacterium sp. CAG:115	Eubacterium	Eubacteriaceae
CAG00843	0.50	R	Species	Citrobacter freundii	Citrobacter	Enterobacteriaceae
CAG00439	0.50	R	Genus	unclassified Flavonifractor	Flavonifractor	unclassified Clostridiales
CAG01293	0.51	R	Species	Blautia sp. CAG:257	Blautia	Lachnospiraceae
CAG00071	0.51	R	Species	Bacteroides cellulosilyticus	Bacteroides	Bacteroidaceae
CAG00088	0.51	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG50001	0.52	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00148	0.53	R	Species	Coprobacillus sp. D6 & sp. 29_1	Coprobacillus	Erysipelotrichaceae
CAG00604	0.54	R	Species	Firmicutes bacterium CAG:110	unclassified Firmicutes	unclassified Firmicutes
CAG01373	0.54	R	Species	Clostridium sp. CAG:505 - Firmicutes bacterium CAG:466	unclassified Clostridiales	unclassified Clostridiales
CAG00673	0.55	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00869	0.55	R	Genus	unclassified Roseburia	Roseburia	Lachnospiraceae
CAG00231	0.55	R	Species	Odoribacter splanchnicus	Odoribacter	Odoribacteraceae
CAG00070	0.56	R	Species	Blautia hydrogenotrophica	Blautia	Lachnospiraceae
CAG00919	0.56	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00639	0.57	R	Species	Eubacterium sp. CAG:156	Eubacterium	Eubacteriaceae
CAG01052	0.58	R	Species	Firmicutes bacterium CAG:103	unclassified Firmicutes	unclassified Firmicutes
CAG00455	0.58	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00519	0.58	R	Genus	unclassified Lachnoclostridium	Lachnoclostridium	Lachnospiraceae
CAG00666	0.59	R	Species	Clostridium sp. CAG:43	unclassified Clostridiales	unclassified Clostridiales
CAG00454	0.59	R	Species	uncultured Ruminococcaceae bacterium	unclassified Ruminococcaceae	Ruminococcaceae
CAG00369	0.61	R	Species	Blautia sp. CAG:257	Blautia	Lachnospiraceae
CAG00137	0.62	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00727	0.62	R	Species	Eggerthella lenta	Eggerthella	Eggerthellaceae
CAG00238	0.62	R	Species	Clostridium saccharolyticum	Lachnoclostridium	Lachnospiraceae
CAG00408	0.62	R	Species	Clostridium sp. CAG:75	unclassified Clostridiales	unclassified Clostridiales
CAG00024	0.63	R	Species	Bacteroides fragilis 1	Bacteroides	Bacteroidaceae
CAG00568	0.64	R	Species	uncultured Faecalibacterium sp.	Faecalibacterium	Ruminococcaceae
CAG00304	0.64	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales

CAG00329	0.64	R	Species	Roseburia hominis	Roseburia	Lachnospiraceae
CAG00118	0.65	R	Species	Bacteroides vulgatus	Bacteroides	Bacteroidaceae
CAG00409	0.66	R	Genus	unclassified Ruminococcus	Ruminococcus	Ruminococcaceae
CAG00461	0.66	R	Species	Azospirillum sp. CAG:260	Azospirillum	Rhodospirillaceae
CAG00796	0.66	R	Species	Eubacterium sp. CAG:202	Eubacterium	Eubacteriaceae
CAG00893	0.66	R	Genus	unclassified Eubacterium	Eubacterium	Eubacteriaceae
CAG01302	0.66	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00782	0.68	R	Species	Eubacterium rectale	Eubacterium	Eubacteriaceae
CAG00037	0.69	R	Species	Bacteroides faecis	Bacteroides	Bacteroidaceae
CAG00357	0.70	R	Species	Bacteroides ovatus	Bacteroides	Bacteroidaceae
CAG00371	0.70	R	Species	Bacteroides dorei	Bacteroides	Bacteroidaceae
CAG00305	0.72	R	Species	Intestinimonas butyriciproducens	Intestinimonas	unclassified Clostridiales
CAG00110	0.73	R	Species	uncultured Clostridium sp.	unclassified Clostridiales	unclassified Clostridiales
CAG00384	0.73	R	Species	Alistipes shahii	Alistipes	Rikenellaceae
CAG00062	0.73	R	Species	Bacteroides salyersiae	Bacteroides	Bacteroidaceae
CAG00372	0.73	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00387	0.73	R	Genus	unclassified Roseburia	Roseburia	Lachnospiraceae
CAG00553	0.73	R	Genus	unclassified Ruminococcus	Ruminococcus	Ruminococcaceae
CAG00588	0.73	R	Species	Clostridium sp. CAG:505 - Firmicutes bacterium CAG:466	unclassified Clostridiales	unclassified Clostridiales
CAG00596	0.73	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00690	0.73	R	Genus	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00713	0.73	R	Species	Firmicutes bacterium CAG:270	unclassified Firmicutes	unclassified Firmicutes
CAG00746	0.73	R	Genus	unclassified Eggerthella	Eggerthella	Eggerthellaceae
CAG01001	0.73	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG01035	0.73	R	Genus	unclassified Coprococcus	Coprococcus	Lachnospiraceae
CAG00974	0.74	R	Genus	unclassified Eubacterium	Eubacterium	Eubacteriaceae
CAG00215	0.74	R	Genus	unclassified Anaerotruncus	Anaerotruncus	Ruminococcaceae
CAG00628	0.75	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae
CAG01005	0.75	R	NA	unclassified	unclassified	unclassified
CAG01153	0.75	R	Family	unclassified Ruminococcaceae	unclassified Ruminococcaceae	Ruminococcaceae
CAG00128	0.76	R	Genus	unclassified Blautia	Blautia	Lachnospiraceae
CAG00667	0.76	R	Species	Faecalibacterium prausnitzii 4 (cf. KLE1255)	Faecalibacterium	Ruminococcaceae
CAG00469	0.78	R	Species	Eubacterium sp. CAG:146	Eubacterium	Eubacteriaceae
CAG01222	0.78	R	Species	Blautia obeum	Blautia	Lachnospiraceae
CAG00523	0.81	R	Species	Eubacterium sp. CAG:180	Eubacterium	Eubacteriaceae
CAG00597	0.81	R	Species	Candidatus Alistipes marseilloanorexicus	Alistipes	Rikenellaceae
CAG01165	0.83	R	Species	Bacteroides ovatus	Bacteroides	Bacteroidaceae
CAG00957	0.83	R	Genus	unclassified Subdoligranulum	Subdoligranulum	Ruminococcaceae

CAG01281	0.83	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01225	0.83	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00019_1	0.84	R	Species	Intestinibacter bartlettii	Intestinibacter	Peptostreptococcaceae
CAG00141	0.85	R	Species	Parabacteroides distasonis	Parabacteroides	Porphyromonadaceae
CAG01159	0.87	R	Species	Veillonella sp. oral taxon 158	Veillonella	Veillonellaceae
CAG00006_1	0.88	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae
CAG00364	0.90	R	Species	Blautia hansenii	Blautia	Lachnospiraceae
CAG00562	0.90	R	Species	Eubacterium dolichum	unclassified Erysipelotrichaceae	Erysipelotrichaceae
CAG00681	0.90	R	Species	Faecalitalea cylindroides	Faecalitalea	Erysipelotrichaceae
CAG01356	0.90	R	Species	Butyricimonas virosa	Butyricimonas	Odoribacteraceae
CAG01301	0.90	R	Species	Blautia obeum	Blautia	Lachnospiraceae
CAG00248	0.91	R	Species	Roseburia sp. CAG:45	Roseburia	Lachnospiraceae
CAG00335	0.91	R	Species	Anaerostipes hadrus	Anaerostipes	Lachnospiraceae
CAG00211	0.92	R	Species	Firmicutes bacterium CAG:227	unclassified Firmicutes	unclassified Firmicutes
CAG00555	0.93	R	Genus	unclassified Flavonifractor	Flavonifractor	unclassified Clostridiales
CAG00209	0.95	R	Species	Coprobacter fastidiosus	Coprobacter	Porphyromonadaceae
CAG00076	0.95	R	Genus	unclassified Firmicutes	unclassified Firmicutes	unclassified Firmicutes
CAG00132	0.95	R	Species	Bacteroides coprophilus	Bacteroides	Bacteroidaceae
CAG00306	0.95	R	Species	Clostridium sp. CAG:221 - Clostridium sp. CAG:265	unclassified Clostridiales	unclassified Clostridiales
CAG00419	0.95	R	Species	Butyricicoccus pullicaecorum	Butyricicoccus	Clostridiaceae
CAG00476	0.95	R	Species	Ruminococcus sp. CAG:403	Ruminococcus	Ruminococcaceae
CAG00496	0.95	R	Species	Fusobacterium mortiferum	Fusobacterium	Fusobacteriaceae
CAG00552	0.95	R	Family	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00607	0.95	R	Species	Eubacterium sp. CAG:251	Eubacterium	Eubacteriaceae
CAG01319	0.95	R	Species	Clostridium sp. CAG:354	unclassified Clostridiales	unclassified Clostridiales
CAG00640	0.95	R	Species	Bifidobacterium breve	Bifidobacterium	Bifidobacteriaceae
CAG00875	0.95	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG00883	0.95	R	Species	Escherichia coli	Escherichia	Enterobacteriaceae
CAG00908	0.95	R	Genus	unclassified Intestinibacter	Intestinibacter	Peptostreptococcaceae
CAG00969	0.95	R	Species	Clostridium sp. L2-50	unclassified Clostridiales	unclassified Clostridiales
CAG01017	0.95	R	Order	unclassified Clostridiales	unclassified Clostridiales	unclassified Clostridiales
CAG01059	0.95	R	Family	unclassified Lachnospiraceae	unclassified Lachnospiraceae	Lachnospiraceae
CAG01194	0.95	R	Species	Clostridium sp. L2-50	unclassified Clostridiales	unclassified Clostridiales
CAG00202	0.97	R	Species	Parabacteroides johnsonii	Parabacteroides	Porphyromonadaceae
CAG00572	0.97	R	Genus	unclassified Faecalibacterium	Faecalibacterium	Ruminococcaceae
CAG01094	0.97	R	Species	Clostridium sp. CAG:273	unclassified Clostridiales	unclassified Clostridiales
CAG01399	0.97	R	Genus	unclassified Oscillibacter	Oscillibacter	Oscillospiraceae

Table S5. Pairwise comparisons of 16S-derived bacterial taxa between crOTU community type 1 and crOTU community type 2 by 2 sided Mann-Whitney test within each level of taxonomy.

Taxon	p-value	Effect Size	FDR-adjusted p-value[a]	Taxonomic Level
Bacteroidetes	6.95e-10	-25.62	2.09e-09	Phylum
Firmicutes	3.37e-08	26.53	5.06e-08	Phylum
Proteobacteria	0.86	0.00	0.86	Phylum
Bacteroidia	6.95e-10	-37.97	2.09e-09	Class
Clostridia	9.42e-08	13.72	1.41e-07	Class
Gammaproteobacteria	0.04	0.00	0.04	Class
Bacteroidales	6.95e-10	-38.12	2.09e-09	Order
Clostridiales	9.42e-08	13.57	1.41e-07	Order
Enterobacterales	0.04	0.00	0.04	Order
Bacteroidaceae	4.83e-08	-27.75	3.38e-07	Family
Ruminococcaceae	0.00	15.55	0.00	Family
Clostridiaceae	0.0011	14.79	0.00	Family
Lachnospiraceae	0.01	11.59	0.02	Family
Enterobacteriaceae	0.04	8.84	0.06	Family
Rikenellaceae	0.36	-7.55	0.42	Family
Porphyromonadaceae	0.67	0.00	0.67	Family
Bacteroides	4.83e-08	-28.52	3.87e-07	Genus
Blautia	0.07	6.86	0.19	Genus
Faecalibacterium	0.07	6.71	0.19	Genus
Roseburia	0.18	4.27	0.36	Genus
Alistipes	0.36	-8.31	0.57	Genus
Lachnoclostridium	0.57	0.00	0.76	Genus
Escherichia	0.71	-1.07	0.79	Genus
Parabacteroides	0.79	-4.73	0.79	Genus
Bacteroides.vulgatus	0.00	-20.47	0.00	Species
Bacteroides.uniformis	0.00	-18.72	0.01	Species
Bacteroides.thetaiotaomicron	0.00	-17.80	0.01	Species
Bacteroides.ovatus	0.07	-11.86	0.13	Species
Faecalibacterium.prausnitzii	0.07	8.20	0.13	Species
Bacteroides.caccae	0.21	-8.73	0.31	Species
Alistipes.onderdonkii	0.36	-6.82	0.46	Species
Escherichia.coli	0.71	0.42	0.80	Species
Parabacteroides.merdae	0.82	-0.42	0.82	Species

[a] FDR *p*-values are adjusted within each level of taxonomy level

Table S6. Univariate and multivariate Cox regression model results.

Variable	Univariate HR[a] (95% C.I.)	*p*-value	HR[a]- Final model (95% C.I.)	*p*-value
alpha-diversity (Inverse Simpson)[c]				
High (ref)				
Intermediate	3.60 (1.02-12.74)	**0.05**		
Low	3.57 (1.02-12.52)	**0.05**		
***Faecalibacterium* abundance[d]**				
High (ref)				
Low	2.92 (1.08-7.89)	**0.03**	2.95 (1.31-7.29)	**0.03**
Bacteroidales abundance				
High (ref)				
Low	0.39 (0.15-1.03)	0.06		
crOTU community type				
Community type 1 (ref)				
Community type 2	2.55 (0.74-8.86)	0.14		
Sex				
Male (ref)				
Female	0.84 (0.30-2.35)	0.74		
Race				
White (ref)				
Other	3.23 (0.86-12.09)	0.08		
Age	1.01 (0.98-1.03)	0.76		
Primary Site				
Cutaneous (ref)				
Other	1.05 (0.24-4.63)	0.95		
Stage				
III (ref)				
IV	1.20 (0.34-4.16)	0.78		
Baseline LDH[b]				
Normal (ref)				
Elevated[c]	2.24 (0.79-6.32)	0.13		
Prior Immunotherapy				
No (ref)				
Yes	2.28 (1.11-7.18)	**0.03**	2.87 (1.10-7.89)	**0.03**
Prior Targeted therapy				
No (ref)				
Yes	1.88 (0.66-5.31)	0.24		
Anti-PD-1 combination vs monotherapy				
Monotherapy (ref)				
Combinations	1.40 (0.23-6.13)	0.65		
CD8+ density in baseline tumor[c]	1.0 (0.99-1.00)	0.11		

a HRs represent 4 patients excluded from analysis due to insufficient follow up data
b elevated LDH: exceeding the upper limit of normal (618 IU/mL), all samples assayed in a common laboratory. LDH not available on one (R) patient
c CD8+ density based on samples from 15 patients with baseline tumor available

Table S7. Pairwise comparison of MetaCyc pathway class by response.

Pathway	Estimate[a]	p-value	Enrichment Index	Enriched In
glycolate and glyoxylate degradation I	0.05	0.00	-0.52	NR
oxalate degradation II	0.08	0.03	-0.36	NR
formate to trimethylamine N-oxide electron transfer	0.11	0.04	-0.29	NR
D-sorbitol degradation II	0.00	0.05	-0.22	NR
ketogluconate metabolism	0.00	0.05	-0.22	NR
phenylacetate degradation I (aerobic)	0.14	0.09	-0.23	NR
pyridoxal 5'-phosphate biosynthesis I	0.18	0.10	-0.31	NR
superpathway of pyridoxal 5'-phosphate biosynthesis and salvage	0.18	0.10	-0.31	NR
4-aminobutanoate degradation III	0.00	0.11	-0.17	NR
hydrogen to trimethylamine N-oxide electron transfer	0.00	0.11	-0.17	NR
4-hydroxybenzoate biosynthesis II (microbes)	0.22	0.11	-0.34	NR
glycocholate metabolism (bacteria)	0.24	0.12	-0.38	NR
malonate decarboxylase activation	0.21	0.18	-0.52	NR
4-aminobutanoate degradation II	0.19	0.18	-0.17	NR
aspartate superpathway	0.19	0.18	-0.17	NR
D-malate degradation	0.19	0.18	-0.17	NR
formate to dimethyl sulfoxide electron transfer	0.19	0.18	-0.17	NR
L-lysine degradation I	0.19	0.18	-0.17	NR
NAD biosynthesis I (from aspartate)	0.19	0.18	-0.17	NR
nitrate reduction III (dissimilatory)	0.19	0.18	-0.17	NR
2-methylcitrate cycle I	0.24	0.21	-0.24	NR
GDP-D-glycero-alpha-D-manno-heptose biosynthesis	0.24	0.21	-0.24	NR
sulfoquinovose degradation I	0.24	0.21	-0.24	NR
superpathway of 4-aminobutanoate degradation	0.24	0.21	-0.24	NR
tetrahydromonapterin biosynthesis	0.24	0.21	-0.24	NR
mevalonate pathway I	0.30	0.23	-0.26	NR
superpathway of geranylgeranyldiphosphate biosynthesis I (via mevalonate)	0.30	0.23	-0.26	NR
D-galactonate degradation	0.00	0.23	-0.12	NR
trehalose degradation I (low osmolarity)	0.00	0.23	-0.12	NR
4-aminobutanoate degradation I	0.31	0.23	-0.18	NR
adenine and adenosine salvage V	0.31	0.23	-0.18	NR
guanine and guanosine salvage III	0.31	0.23	-0.18	NR
superpathway of guanine and guanosine salvage	0.31	0.23	-0.18	NR
L-ascorbate degradation II (bacterial, aerobic)	0.33	0.24	-0.28	NR
fatty acid beta-oxidation I	0.26	0.34	-0.12	NR
glucose and glucose-1-phosphate degradation	0.26	0.34	-0.12	NR
glyoxylate cycle	0.26	0.34	-0.12	NR
L-gulonate degradation	0.26	0.34	-0.12	NR

superpathway of glyoxylate bypass and TCA	0.26	0.34	-0.12	NR
3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate	0.34	0.39	-0.36	NR
L-phenylalanine biosynthesis II	0.34	0.39	-0.36	NR
tetrathionate reduction I (to thiosulfate)	0.34	0.39	-0.36	NR
urate degradation to allantoin II	0.34	0.39	-0.36	NR
cob(II)yrinate a,c-diamide biosynthesis I (early cobalt insertion)	0.41	0.41	-0.12	NR
glycerol-3-phosphate to cytochrome bo oxidase electron transfer	0.41	0.41	-0.12	NR
NADH to cytochrome bo oxidase electron transfer I	0.41	0.41	-0.12	NR
proline to cytochrome bo oxidase electron transfer	0.41	0.41	-0.12	NR
pyruvate to cytochrome bo oxidase electron transfer	0.41	0.41	-0.12	NR
succinate to cytochrome bo oxidase electron transfer	0.41	0.41	-0.12	NR
cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate	0.39	0.41	-0.19	NR
Entner-Doudoroff pathway I	0.39	0.41	-0.19	NR
superpathway of glycolysis and Entner-Doudoroff	0.39	0.41	-0.19	NR
glycerol degradation II	0.44	0.43	-0.20	NR
L-threonine degradation III (to methylglyoxal)	0.44	0.43	-0.20	NR
neurosporene biosynthesis	0.44	0.43	-0.20	NR
phenylethylamine degradation I	0.44	0.43	-0.20	NR
trans-lycopene biosynthesis I (bacteria)	0.44	0.43	-0.20	NR
acetate formation from acetyl-CoA II	0.49	0.43	-0.23	NR
crotonate fermentation (to acetate and cyclohexane carboxylate)	0.49	0.43	-0.23	NR
D-allose degradation	0.49	0.43	-0.23	NR
2-O-alpha-mannosyl-D-glycerate degradation	0.48	0.43	-0.21	NR
galactitol degradation	0.48	0.43	-0.21	NR
malonate degradation I (biotin-independent)	0.48	0.43	-0.21	NR
(R)-acetoin biosynthesis I	0.48	0.43	-0.21	NR
(R,R)-butanediol biosynthesis	0.48	0.43	-0.21	NR
(R,R)-butanediol degradation	0.48	0.43	-0.21	NR
aerobactin biosynthesis	0.00	0.44	-1.00	NR
benzoyl-CoA degradation I (aerobic)	0.00	0.44	-1.00	NR
glutaryl-CoA degradation	0.00	0.44	-1.00	NR
nitrate reduction I (denitrification)	0.00	0.44	-1.00	NR
pectin degradation II	0.00	0.44	-1.00	NR
fluoroacetate and fluorothreonine biosynthesis	0.00	0.49	-0.08	NR
catechol degradation to beta-ketoadipate	0.36	0.56	-0.44	NR
chondroitin sulfate degradation I (bacterial)	0.36	0.56	-0.44	NR
dermatan sulfate degradation I (bacterial)	0.36	0.56	-0.44	NR
L-methionine salvage cycle III	0.36	0.56	-0.44	NR
phenylmercury acetate degradation	0.36	0.56	-0.44	NR
urea degradation I	0.36	0.56	-0.44	NR
4-hydroxyphenylpyruvate biosynthesis	0.38	0.60	-0.07	NR
glutathionylspermidine biosynthesis	0.38	0.60	-0.07	NR

L-phenylalanine biosynthesis I	0.38	0.60	-0.07	NR
methylerythritol phosphate pathway II	0.38	0.60	-0.07	NR
nitrate reduction IX (dissimilatory)	0.38	0.60	-0.07	NR
nitrate reduction X (periplasmic, dissimilatory)	0.38	0.60	-0.07	NR
paraoxon degradation	0.38	0.60	-0.07	NR
parathion degradation	0.38	0.60	-0.07	NR
superpathway of aromatic amino acid biosynthesis	0.38	0.60	-0.07	NR
3-methylthiopropanoate biosynthesis	0.49	0.66	-0.26	NR
allantoin degradation to glyoxylate III	0.49	0.66	-0.26	NR
gallate degradation I	0.49	0.66	-0.26	NR
sulfoacetaldehyde degradation III	0.49	0.66	-0.26	NR
syringate degradation	0.49	0.66	-0.26	NR
(5Z)-dodec-5-enoate biosynthesis	0.57	0.66	-0.07	NR
fatty acid beta-oxidation III (unsaturated, odd number)	0.57	0.66	-0.07	NR
formaldehyde oxidation II (glutathione-dependent)	0.57	0.66	-0.07	NR
oleate biosynthesis IV (anaerobic)	0.57	0.66	-0.07	NR
superpathway of fatty acids biosynthesis (E. coli)	0.57	0.66	-0.07	NR
superpathway of unsaturated fatty acids biosynthesis (E. coli)	0.57	0.66	-0.07	NR
NAD salvage pathway III	0.51	0.68	-0.12	NR
NAD salvage pathway IV	0.51	0.68	-0.12	NR
superpathway of ubiquinol-8 biosynthesis (prokaryotic)	0.51	0.68	-0.12	NR
ubiquinol-7 biosynthesis (prokaryotic)	0.51	0.68	-0.12	NR
ubiquinol-8 biosynthesis (prokaryotic)	0.51	0.68	-0.12	NR
ubiquinol-9 biosynthesis (prokaryotic)	0.51	0.68	-0.12	NR
dTDP-N-acetylthomosamine biosynthesis	0.58	0.69	-0.12	NR
putrescine degradation II	0.58	0.69	-0.12	NR
taurine degradation IV	0.58	0.69	-0.12	NR
bile acids degradation	0.64	0.70	-0.12	NR
guanine and guanosine salvage II	0.64	0.70	-0.12	NR
guanosine nucleotides degradation II	0.64	0.70	-0.12	NR
superpathway of phenylethylamine degradation	0.64	0.70	-0.12	NR
formaldehyde assimilation I (serine pathway)	0.68	0.70	-0.12	NR
L-arginine degradation II (AST pathway)	0.68	0.70	-0.12	NR
S-methyl-5'-thioadenosine degradation II	0.68	0.70	-0.12	NR
superpathway of (R,R)-butanediol biosynthesis	0.68	0.70	-0.12	NR
2,3-dihydroxybenzoate biosynthesis	0.94	1.00	-0.01	NR
2-oxoisovalerate decarboxylation to isobutanoyl-CoA	0.00	1.00	-0.04	NR
acyl carrier protein metabolism	0.77	1.00	-0.05	NR
allantoin degradation IV (anaerobic)	0.61	1.00	-0.03	NR
allantoin degradation to ureidoglycolate II (ammonia producing)	0.61	1.00	-0.03	NR
allantoin degradation to ureidoglycolate I (urea producing)	0.78	1.00	-0.12	NR
anthranilate degradation I (aerobic)	0.78	1.00	-0.12	NR

Bifidobacterium shunt	0.69	1.00	-0.06	NR
cyanate degradation	0.71	1.00	-0.12	NR
D-glucarate degradation II	0.00	1.00	-0.04	NR
D-gluconate degradation	0.00	1.00	-0.04	NR
D-glucosaminate degradation	0.76	1.00	-0.12	NR
D-serine degradation	0.00	1.00	-0.04	NR
glucose degradation (oxidative)	0.84	1.00	-0.04	NR
glutathione-glutaredoxin redox reactions	0.00	1.00	-0.04	NR
heme biosynthesis I (aerobic)	0.94	1.00	-0.01	NR
heptadecane biosynthesis	0.61	1.00	-0.03	NR
lactose and galactose degradation I	0.71	1.00	-0.12	NR
L-methionine degradation I (to L-homocysteine)	0.74	1.00	-0.12	NR
L-methionine salvage cycle I (bacteria and plants)	0.76	1.00	-0.12	NR
maltose degradation	0.00	1.00	-0.04	NR
mannitol degradation I	0.00	1.00	-0.04	NR
mannosylglycerate biosynthesis I	0.90	1.00	-0.03	NR
PRPP biosynthesis II	0.77	1.00	-0.05	NR
pseudouridine degradation	0.69	1.00	-0.06	NR
purine nucleotides degradation II (aerobic)	0.00	1.00	-0.04	NR
pyrimidine deoxyribonucleotides de novo biosynthesis III	0.94	1.00	-0.01	NR
pyrimidine deoxyribonucleotides dephosphorylation	0.90	1.00	-0.03	NR
pyrimidine nucleobases salvage II	0.00	1.00	-0.04	NR
pyrroloquinoline quinone biosynthesis	0.76	1.00	-0.12	NR
pyruvate decarboxylation to acetyl CoA	0.00	1.00	-0.04	NR
quinate degradation I	0.90	1.00	-0.03	NR
(R)-acetoin biosynthesis II	0.82	1.00	-0.02	NR
(S)-acetoin biosynthesis	0.77	1.00	-0.05	NR
salicylate biosynthesis I	0.90	1.00	-0.03	NR
shikimate degradation I	0.90	1.00	-0.03	NR
S-methyl-5'-thioadenosine degradation I	0.90	1.00	-0.03	NR
S-methyl-5-thio-alpha-D-ribose 1-phosphate degradation	0.76	1.00	-0.12	NR
(S,S)-butanediol biosynthesis	0.77	1.00	-0.05	NR
(S,S)-butanediol degradation	0.77	1.00	-0.05	NR
sulfate reduction I (assimilatory)	0.00	1.00	-0.04	NR
sulfate reduction III (assimilatory)	0.00	1.00	-0.04	NR
sulfite oxidation III	0.00	1.00	-0.04	NR
sulfur reduction II (via polysulfide)	0.78	1.00	-0.12	NR
superpathway of L-arginine, putrescine, and 4-aminobutanoate degradation	0.90	1.00	-0.03	NR
superpathway of ornithine degradation	0.90	1.00	-0.03	NR
TCA cycle I (prokaryotic)	0.00	1.00	-0.04	NR
trehalose biosynthesis IV	0.00	1.00	-0.04	NR
trehalose biosynthesis V	0.78	1.00	-0.12	NR

trehalose degradation II (trehalase)	0.61	1.00	-0.03	NR
trehalose degradation VI (periplasmic)	0.61	1.00	-0.03	NR
two-component alkanesulfonate monooxygenase	0.94	1.00	-0.01	NR
UDP-2,3-diacetamido-2,3-dideoxy-alpha-D-mannuronate biosynthesis	0.61	1.00	-0.03	NR
urate biosynthesis/inosine 5'-phosphate degradation	0.00	1.00	-0.04	NR
vanillin and vanillate degradation II	0.90	1.00	-0.03	NR
vancomycin resistance II	Inf	0.02	1.00	R
inosine-5'-phosphate biosynthesis III	Inf	0.11	1.00	R
fructan biosynthesis	4.48	0.11	0.40	R
putrescine degradation I	6.84	0.13	0.19	R
L-cysteine degradation II	4.66	0.18	0.22	R
D-serine metabolism	5.21	0.18	0.59	R
bis(guanylyl molybdenum cofactor) biosynthesis	Inf	0.18	0.10	R
gentisate degradation I	Inf	0.23	1.00	R
kojibiose degradation	Inf	0.23	1.00	R
methanogenesis from H2 and CO2	Inf	0.23	1.00	R
phosphopantothenate biosynthesis III	Inf	0.23	1.00	R
reductive acetyl coenzyme A pathway II (autotrophic methanogens)	Inf	0.23	1.00	R
(R)-cysteate degradation	3.00	0.24	0.28	R
L-cysteine biosynthesis III (from L-homocysteine)	2.86	0.24	0.22	R
glycolysis IV (plant cytosol)	3.26	0.35	0.15	R
UDP-N-acetyl-D-galactosamine biosynthesis I	2.91	0.39	0.18	R
formaldehyde oxidation I	2.41	0.41	0.33	R
trehalose degradation IV	2.41	0.41	0.33	R
heme biosynthesis II (anaerobic)	2.56	0.41	0.29	R
superpathway of heme biosynthesis from uroporphyrinogen-III	2.56	0.41	0.29	R
8-amino-7-oxononanoate biosynthesis III	2.25	0.43	0.22	R
2-oxoglutarate decarboxylation to succinyl-CoA	Inf	0.44	0.05	R
CMP-N-acetylneuraminate biosynthesis II (bacteria)	Inf	0.44	0.05	R
cob(II)yrinate a,c-diamide biosynthesis II (late cobalt incorporation)	Inf	0.44	0.05	R
cyclopropane fatty acid (CFA) biosynthesis	Inf	0.44	0.05	R
guanylyl molybdenum cofactor biosynthesis	Inf	0.44	0.05	R
hydrogen production III	Inf	0.44	0.05	R
hydrogen production VI	Inf	0.44	0.05	R
L-arginine degradation I (arginase pathway)	Inf	0.44	0.05	R
L-arginine degradation VI (arginase 2 pathway)	Inf	0.44	0.05	R
L-citrulline biosynthesis	Inf	0.44	0.05	R
L-ornithine biosynthesis II	Inf	0.44	0.05	R
L-proline biosynthesis III	Inf	0.44	0.05	R
pectin degradation III	Inf	0.44	0.05	R
putrescine biosynthesis IV	Inf	0.44	0.05	R
adenine and adenosine salvage VI	Inf	0.49	1.00	R

alginate degradation	Inf	0.49	1.00	R
glycine biosynthesis III	Inf	0.49	1.00	R
sulfolactate degradation I	Inf	0.49	1.00	R
superpathway of L-cysteine biosynthesis (mammalian)	Inf	0.49	1.00	R
L-histidine degradation III	2.77	0.56	0.06	R
nitrogen fixation I (ferredoxin)	2.77	0.56	0.06	R
chlorosalicylate degradation	2.63	0.60	0.40	R
methylsalicylate degradation	2.63	0.60	0.40	R
salicylate degradation I	2.63	0.60	0.40	R
acrylate degradation	2.18	0.62	0.08	R
L-methionine degradation II	2.18	0.62	0.08	R
cyanophycin metabolism	1.76	0.66	0.22	R
TCA cycle VII (acetate-producers)	1.76	0.66	0.22	R
Kdo transfer to lipid IVA II	1.95	0.68	0.22	R
aminopropanol phosphate biosynthesis I	1.71	0.69	0.16	R
hopanoid biosynthesis (bacteria)	1.57	0.70	0.11	R
D-galactarate degradation I	1.48	0.70	0.08	R
D-glucarate degradation I	1.48	0.70	0.08	R
formaldehyde assimilation II (RuMP Cycle)	1.48	0.70	0.08	R
ribose degradation	1.48	0.70	0.08	R
superpathway of D-glucarate and D-galactarate degradation	1.48	0.70	0.08	R
1,2-dichloroethane degradation	Inf	1.00	1.00	R
1,4-dihydroxy-2-naphthoate biosynthesis	0.00	1.00	0.00	R
2-aminoethylphosphonate degradation I	0.00	1.00	0.00	R
2'-deoxy-alpha-D-ribose 1-phosphate degradation	0.00	1.00	0.00	R
[2Fe-2S] iron-sulfur cluster biosynthesis	0.00	1.00	0.00	R
2-heptyl-3-hydroxy-4(1H)-quinolone biosynthesis	Inf	1.00	1.00	R
3-dehydroquinate biosynthesis I	0.00	1.00	0.00	R
4-amino-2-methyl-5-diphosphomethylpyrimidine biosynthesis	0.00	1.00	0.00	R
4-aminobenzoate biosynthesis	0.00	1.00	0.00	R
4-deoxy-L-threo-hex-4-enopyranuronate degradation	0.00	1.00	0.00	R
5-aminoimidazole ribonucleotide biosynthesis I	0.00	1.00	0.00	R
6-hydroxymethyl-dihydropterin diphosphate biosynthesis I	0.00	1.00	0.00	R
8-amino-7-oxononanoate biosynthesis I	0.00	1.00	0.00	R
acetate conversion to acetyl-CoA	0.00	1.00	0.00	R
acetate formation from acetyl-CoA I	0.00	1.00	0.00	R
adenine and adenosine salvage I	0.00	1.00	0.00	R
adenine and adenosine salvage III	0.00	1.00	0.00	R
adenine salvage	0.00	1.00	0.00	R
adenosine deoxyribonucleotides de novo biosynthesis	0.00	1.00	0.00	R
adenosine deoxyribonucleotides de novo biosynthesis II	0.00	1.00	0.00	R
adenosine nucleotides degradation II	0.00	1.00	0.00	R

adenosine nucleotides degradation III	0.00	1.00	0.00	R
adenosine ribonucleotides de novo biosynthesis	0.00	1.00	0.00	R
adenosylcobalamin biosynthesis from cobyrinate a,c-diamide I	0.00	1.00	0.00	R
adenosylcobalamin biosynthesis from cobyrinate a,c-diamide II	0.00	1.00	0.00	R
adenosylcobalamin salvage from cobalamin	0.00	1.00	0.00	R
adenosylcobalamin salvage from cobinamide I	0.00	1.00	0.00	R
ADP-L-glycero-beta-D-manno-heptose biosynthesis	1.32	1.00	0.02	R
aerobic respiration I (cytochrome c)	1.63	1.00	0.22	R
allantoin degradation to glyoxylate I	Inf	1.00	1.00	R
ammonia assimilation cycle III	0.00	1.00	0.00	R
androstenedione degradation	Inf	1.00	1.00	R
anhydromuropeptides recycling	0.00	1.00	0.00	R
arginine dependent acid resistance	0.00	1.00	0.00	R
arsenate detoxification II (glutaredoxin)	0.00	1.00	0.00	R
autoinducer AI-2 biosynthesis I	0.00	1.00	0.00	R
autoinducer AI-2 degradation	0.00	1.00	0.00	R
base-degraded thiamine salvage	0.00	1.00	0.00	R
beta-alanine biosynthesis II	Inf	1.00	1.00	R
beta-alanine biosynthesis III	0.00	1.00	0.00	R
beta-D-glucuronide and D-glucuronate degradation	0.00	1.00	0.00	R
biotin biosynthesis from 8-amino-7-oxononanoate I	0.00	1.00	0.00	R
biotin biosynthesis I	0.00	1.00	0.00	R
biotin-carboxyl carrier protein assembly	0.00	1.00	0.00	R
C4 photosynthetic carbon assimilation cycle, NAD-ME type	0.00	1.00	0.00	R
Calvin-Benson-Bassham cycle	Inf	1.00	1.00	R
CDP-diacylglycerol biosynthesis I	0.00	1.00	0.00	R
CDP-diacylglycerol biosynthesis II	0.00	1.00	0.00	R
cellulose and hemicellulose degradation (cellulolosome)	1.29	1.00	0.01	R
cellulose biosynthesis	1.32	1.00	0.02	R
chitin degradation II	0.00	1.00	0.00	R
chitobiose degradation	0.00	1.00	0.00	R
chorismate biosynthesis from 3-dehydroquinate	0.00	1.00	0.00	R
chorismate biosynthesis I	0.00	1.00	0.00	R
cis-genanyl-CoA degradation	1.63	1.00	0.22	R
cis-vaccenate biosynthesis	0.00	1.00	0.00	R
citrate degradation	0.00	1.00	0.00	R
citrate lyase activation	0.00	1.00	0.00	R
CMP-3-deoxy-D-manno-octulosonate biosynthesis	0.00	1.00	0.00	R
CMP phosphorylation	0.00	1.00	0.00	R
coenzyme A biosynthesis I	0.00	1.00	0.00	R
creatinine degradation I	0.00	1.00	0.00	R
cytidylyl molybdenum cofactor biosynthesis	0.00	1.00	0.00	R

D-arabinose degradation I	0.00	1.00	0.00	R
demethylmenaquinol-6 biosynthesis I	0.00	1.00	0.00	R
demethylmenaquinol-8 biosynthesis I	0.00	1.00	0.00	R
demethylmenaquinol-9 biosynthesis	0.00	1.00	0.00	R
D-fructuronate degradation	0.00	1.00	0.00	R
D-galactarate degradation II	0.00	1.00	0.00	R
D-galactose degradation I (Leloir pathway)	0.00	1.00	0.00	R
D-galactose degradation V (Leloir pathway)	0.00	1.00	0.00	R
D-galacturonate degradation I	0.00	1.00	0.00	R
di-trans,poly-cis-undecaprenyl phosphate biosynthesis	0.00	1.00	0.00	R
D-mannose degradation	0.00	1.00	0.00	R
D-sorbitol degradation I	0.00	1.00	0.00	R
dTDP-L-rhamnose biosynthesis I	0.00	1.00	0.00	R
ethanolamine utilization	0.00	1.00	0.00	R
ethanol degradation I	0.00	1.00	0.00	R
ethanol degradation II	0.00	1.00	0.00	R
fatty acid biosynthesis initiation I	0.00	1.00	0.00	R
fatty acid biosynthesis initiation III	Inf	1.00	1.00	R
fatty acid elongation -- saturated	0.00	1.00	0.00	R
fatty acid salvage	Inf	1.00	1.00	R
flavin biosynthesis I (bacteria and plants)	0.00	1.00	0.00	R
fluoroacetate degradation	Inf	1.00	1.00	R
folate polyglutamylation	0.00	1.00	0.00	R
folate transformations I	0.00	1.00	0.00	R
formate assimilation into 5,10-methylenetetrahydrofolate	0.00	1.00	0.00	R
formate oxidation to CO2	0.00	1.00	0.00	R
fructose degradation	0.00	1.00	0.00	R
fucose degradation	0.00	1.00	0.00	R
GABA shunt	Inf	1.00	1.00	R
GDP-L-fucose biosynthesis I (from GDP-D-mannose)	0.00	1.00	0.00	R
GDP-mannose biosynthesis	0.00	1.00	0.00	R
geranyl diphosphate biosynthesis	0.00	1.00	0.00	R
geranylgeranyl diphosphate biosynthesis	0.00	1.00	0.00	R
gluconeogenesis I	0.00	1.00	0.00	R
glutaminyl-tRNAgln biosynthesis via transamidation	0.00	1.00	0.00	R
glutathione biosynthesis	0.00	1.00	0.00	R
glutathione-peroxide redox reactions	0.00	1.00	0.00	R
glycerol-3-phosphate to fumarate electron transfer	0.00	1.00	0.00	R
glycerol and glycerophosphodiester degradation	0.00	1.00	0.00	R
glycerol degradation I	0.00	1.00	0.00	R
glycerophosphodiester degradation	0.00	1.00	0.00	R
glycine biosynthesis I	0.00	1.00	0.00	R

glycine cleavage	0.00	1.00	0.00	R
glycogen biosynthesis I (from ADP-D-Glucose)	0.00	1.00	0.00	R
glycolysis I (from glucose 6-phosphate)	0.00	1.00	0.00	R
glycolysis III (from glucose)	0.00	1.00	0.00	R
gondoate biosynthesis (anaerobic)	0.00	1.00	0.00	R
guanine and guanosine salvage	0.00	1.00	0.00	R
guanosine deoxyribonucleotides de novo biosynthesis I	0.00	1.00	0.00	R
guanosine deoxyribonucleotides de novo biosynthesis II	0.00	1.00	0.00	R
guanosine nucleotides degradation III	0.00	1.00	0.00	R
guanosine ribonucleotides de novo biosynthesis	0.00	1.00	0.00	R
heptaprenyl diphosphate biosynthesis	0.00	1.00	0.00	R
histamine biosynthesis	1.36	1.00	0.04	R
homolactic fermentation	0.00	1.00	0.00	R
hyaluronan degradation	0.00	1.00	0.00	R
hydrogen oxidation I (aerobic)	0.00	1.00	0.00	R
hydrogen oxidation III (anaerobic, NADP)	0.00	1.00	0.00	R
hydrogen to dimethyl sulfoxide electron transfer	0.00	1.00	0.00	R
hydrogen to fumarate electron transfer	0.00	1.00	0.00	R
hydroxymethylpyrimidine salvage	0.00	1.00	0.00	R
hypotaurine degradation	Inf	1.00	1.00	R
incomplete reductive TCA cycle	0.00	1.00	0.00	R
inosine-5'-phosphate biosynthesis I	0.00	1.00	0.00	R
L-1,2-propanediol degradation	1.03	1.00	0.01	R
lactose degradation III	0.00	1.00	0.00	R
L-alanine biosynthesis I	0.00	1.00	0.00	R
L-alanine biosynthesis II	0.00	1.00	0.00	R
L-alanine biosynthesis III	0.00	1.00	0.00	R
L-alanine degradation IV	0.00	1.00	0.00	R
L-arabinose degradation I	0.00	1.00	0.00	R
L-arginine biosynthesis II (acetyl cycle)	0.00	1.00	0.00	R
L-arginine biosynthesis I (via L-ornithine)	0.00	1.00	0.00	R
L-arginine degradation III (arginine decarboxylase/agmatinase pathway)	0.00	1.00	0.00	R
L-arginine degradation V (arginine deiminase pathway)	0.00	1.00	0.00	R
L-asparagine biosynthesis I	0.00	1.00	0.00	R
L-asparagine biosynthesis II	0.00	1.00	0.00	R
L-asparagine biosynthesis III (tRNA-dependent)	0.00	1.00	0.00	R
L-asparagine degradation I	0.00	1.00	0.00	R
L-aspartate biosynthesis	0.00	1.00	0.00	R
L-aspartate degradation I	0.00	1.00	0.00	R
L-citrulline degradation	0.00	1.00	0.00	R
L-cysteine biosynthesis I	0.00	1.00	0.00	R
leucine degradation IV	Inf	1.00	1.00	R

L-glutamate biosynthesis I	0.00	1.00	0.00	R
L-glutamate biosynthesis II	0.00	1.00	0.00	R
L-glutamate biosynthesis III	0.00	1.00	0.00	R
L-glutamate degradation I	0.00	1.00	0.00	R
L-glutamate degradation II	0.00	1.00	0.00	R
L-glutamate degradation IX (via 4-aminobutanoate)	0.00	1.00	0.00	R
L-glutamate degradation X	0.00	1.00	0.00	R
L-glutamine biosynthesis I	0.00	1.00	0.00	R
L-glutamine biosynthesis III	0.00	1.00	0.00	R
L-glutamine degradation I	0.00	1.00	0.00	R
L-glutamine degradation II	0.00	1.00	0.00	R
L-histidine biosynthesis	0.00	1.00	0.00	R
L-histidine degradation I	0.00	1.00	0.00	R
L-homocysteine biosynthesis	0.00	1.00	0.00	R
L-homoserine and L-methionine biosynthesis	0.00	1.00	0.00	R
L-homoserine biosynthesis	0.00	1.00	0.00	R
L-idonate degradation	0.00	1.00	0.00	R
lipid IVA biosynthesis	0.00	1.00	0.00	R
lipoate biosynthesis and incorporation I	0.00	1.00	0.00	R
lipoate biosynthesis and incorporation II	0.00	1.00	0.00	R
lipoate salvage I	0.00	1.00	0.00	R
L-isoleucine biosynthesis I (from threonine)	0.00	1.00	0.00	R
L-isoleucine degradation I	0.00	1.00	0.00	R
L-lactaldehyde degradation (anaerobic)	0.00	1.00	0.00	R
L-leucine biosynthesis	0.00	1.00	0.00	R
L-leucine degradation I	1.63	1.00	0.22	R
L-lysine biosynthesis I	0.00	1.00	0.00	R
L-lysine biosynthesis III	0.00	1.00	0.00	R
L-lysine biosynthesis VI	0.00	1.00	0.00	R
L-lysine fermentation to acetate and butanoate	0.00	1.00	0.00	R
L-malate degradation II	0.00	1.00	0.00	R
L-methionine biosynthesis I	0.00	1.00	0.00	R
L-methionine biosynthesis III	0.00	1.00	0.00	R
long-chain fatty acid activation	0.00	1.00	0.00	R
L-ornithine biosynthesis I	0.00	1.00	0.00	R
L-phenylalanine degradation I (aerobic)	Inf	1.00	1.00	R
L-proline biosynthesis I	0.00	1.00	0.00	R
L-proline degradation	0.00	1.00	0.00	R
L-rhamnose degradation I	0.00	1.00	0.00	R
L-selenocysteine biosynthesis I (bacteria)	0.00	1.00	0.00	R
L-serine biosynthesis	0.00	1.00	0.00	R
L-serine degradation	0.00	1.00	0.00	R

L-threonine biosynthesis	0.00	1.00	0.00	R
L-threonine degradation II	0.00	1.00	0.00	R
L-threonine degradation IV	0.00	1.00	0.00	R
L-tryptophan biosynthesis	0.00	1.00	0.00	R
L-tryptophan degradation II (via pyruvate)	0.00	1.00	0.00	R
L-tryptophan degradation I (via anthranilate)	Inf	1.00	1.00	R
L-tryptophan degradation to 2-amino-3-carboxymuconate semialdehyde	Inf	1.00	1.00	R
L-tyrosine biosynthesis I	0.00	1.00	0.00	R
L-valine biosynthesis	0.00	1.00	0.00	R
mannan degradation	0.00	1.00	0.00	R
melibiose degradation	0.00	1.00	0.00	R
menaquinol-6 biosynthesis	0.00	1.00	0.00	R
menaquinol-7 biosynthesis	0.00	1.00	0.00	R
menaquinol-8 biosynthesis	0.00	1.00	0.00	R
menaquinol-9 biosynthesis	0.00	1.00	0.00	R
methylerythritol phosphate pathway I	0.00	1.00	0.00	R
methylglyoxal degradation I	0.00	1.00	0.00	R
methylphosphonate degradation I	0.00	1.00	0.00	R
mevalonate degradation	1.32	1.00	0.02	R
mixed acid fermentation	0.00	1.00	0.00	R
molybdenum cofactor biosynthesis	0.00	1.00	0.00	R
myo-, chiro- and scillo-inositol degradation	0.00	1.00	0.00	R
myo-inositol biosynthesis	0.00	1.00	0.00	R
myo-inositol degradation I	0.00	1.00	0.00	R
N10-formyl-tetrahydrofolate biosynthesis	0.00	1.00	0.00	R
N6-L-threonylcarbamoyladenosine37-modified tRNA biosynthesis	0.00	1.00	0.00	R
N-acetylglucosamine degradation I	0.00	1.00	0.00	R
N-acetylglucosamine degradation II	1.03	1.00	0.01	R
N-acetylneuraminate and N-acetylmannosamine degradation I	0.00	1.00	0.00	R
NAD biosynthesis from 2-amino-3-carboxymuconate semialdehyde	0.00	1.00	0.00	R
NAD biosynthesis II (from tryptophan)	Inf	1.00	1.00	R
NAD biosynthesis III	1.22	1.00	0.08	R
NADH repair	0.00	1.00	0.00	R
NADH to cytochrome bd oxidase electron transfer I	0.00	1.00	0.00	R
NAD phosphorylation and dephosphorylation	0.00	1.00	0.00	R
NAD salvage pathway II	0.00	1.00	0.00	R
octane oxidation	Inf	1.00	1.00	R
octaprenyl diphosphate biosynthesis	0.00	1.00	0.00	R
oleate beta-oxidation	0.00	1.00	0.00	R
oxidized GTP and dGTP detoxification	0.00	1.00	0.00	R
palmitate biosynthesis II (bacteria and plants)	0.00	1.00	0.00	R
palmitoleate biosynthesis I (from (5Z)-dodec-5-enoate)	0.00	1.00	0.00	R

pantothenate and coenzyme A biosynthesis I	0.00	1.00	0.00	R
partial TCA cycle (obligate autotrophs)	0.00	1.00	0.00	R
pentose phosphate pathway	0.00	1.00	0.00	R
pentose phosphate pathway (non-oxidative branch)	0.00	1.00	0.00	R
pentose phosphate pathway (oxidative branch)	0.00	1.00	0.00	R
peptidoglycan biosynthesis I (meso-diaminopimelate containing)	0.00	1.00	0.00	R
peptidoglycan maturation (meso-diaminopimelate containing)	0.00	1.00	0.00	R
phosphate acquisition	0.00	1.00	0.00	R
phosphatidylethanolamine biosynthesis I	0.00	1.00	0.00	R
phosphatidylglycerol biosynthesis II (non-plastidic)	0.00	1.00	0.00	R
phosphatidylglycerol biosynthesis I (plastidic)	0.00	1.00	0.00	R
phospholipases	0.00	1.00	0.00	R
phosphopantothenate biosynthesis I	0.00	1.00	0.00	R
polyisoprenoid biosynthesis (E. coli)	0.00	1.00	0.00	R
ppGpp biosynthesis	0.00	1.00	0.00	R
preQ0 biosynthesis	0.00	1.00	0.00	R
propanoyl CoA degradation I	0.00	1.00	0.00	R
protocatechuate degradation II (ortho-cleavage pathway)	0.00	1.00	0.00	R
PRPP biosynthesis I	0.00	1.00	0.00	R
purine deoxyribonucleosides degradation I	0.00	1.00	0.00	R
purine ribonucleosides degradation	0.00	1.00	0.00	R
putrescine biosynthesis I	0.00	1.00	0.00	R
putrescine biosynthesis III	0.00	1.00	0.00	R
pyridoxal 5'-phosphate biosynthesis II	0.00	1.00	0.00	R
pyridoxal 5'-phosphate salvage I	0.00	1.00	0.00	R
pyrimidine deoxyribonucleosides degradation	0.00	1.00	0.00	R
pyrimidine deoxyribonucleosides salvage	0.00	1.00	0.00	R
pyrimidine deoxyribonucleotide phosphorylation	0.00	1.00	0.00	R
pyrimidine deoxyribonucleotides de novo biosynthesis I	0.00	1.00	0.00	R
pyrimidine deoxyribonucleotides de novo biosynthesis II	0.00	1.00	0.00	R
pyrimidine nucleobases salvage I	0.00	1.00	0.00	R
pyrimidine ribonucleosides degradation	0.00	1.00	0.00	R
pyrimidine ribonucleosides salvage I	0.00	1.00	0.00	R
pyruvate fermentation to acetate and lactate II	0.00	1.00	0.00	R
pyruvate fermentation to acetate I	0.00	1.00	0.00	R
pyruvate fermentation to acetate IV	0.00	1.00	0.00	R
pyruvate fermentation to ethanol I	0.00	1.00	0.00	R
pyruvate fermentation to ethanol III	0.00	1.00	0.00	R
pyruvate fermentation to lactate	0.00	1.00	0.00	R
pyruvate to cytochrome bd terminal oxidase electron transfer	0.00	1.00	0.00	R
queuosine biosynthesis	0.00	1.00	0.00	R
reactive oxygen species degradation	0.00	1.00	0.00	R

reductive acetyl coenzyme A pathway I (homoacetogenic bacteria)	0.00	1.00	0.00	R
reductive monocarboxylic acid cycle	0.00	1.00	0.00	R
rhamnogalacturonan type I degradation II (bacteria)	0.00	1.00	0.00	R
Rubisco shunt	Inf	1.00	1.00	R
S-adenosyl-L-methionine biosynthesis	0.00	1.00	0.00	R
S-adenosyl-L-methionine cycle I	0.00	1.00	0.00	R
S-adenosyl-L-methionine cycle II	0.00	1.00	0.00	R
salicylate degradation II	Inf	1.00	1.00	R
selenate reduction	0.00	1.00	0.00	R
siroheme biosynthesis	0.00	1.00	0.00	R
S-methyl-5'-thioadenosine degradation III	1.06	1.00	0.02	R
spermidine biosynthesis I	0.00	1.00	0.00	R
stearate biosynthesis II (bacteria and plants)	0.00	1.00	0.00	R
succinate to cytochrome bd oxidase electron transfer	0.00	1.00	0.00	R
sucrose degradation III (sucrose invertase)	0.00	1.00	0.00	R
sucrose degradation IV (sucrose phosphorylase)	0.00	1.00	0.00	R
sulfate activation for sulfonation	0.00	1.00	0.00	R
sulfate reduction IV (dissimilatory)	Inf	1.00	1.00	R
sulfoacetaldehyde degradation I	Inf	1.00	1.00	R
sulfolactate degradation III	Inf	1.00	1.00	R
superoxide radicals degradation	0.00	1.00	0.00	R
superpathway of acetate utilization and formation	0.00	1.00	0.00	R
superpathway of adenosine nucleotides de novo biosynthesis I	0.00	1.00	0.00	R
superpathway of adenosine nucleotides de novo biosynthesis II	0.00	1.00	0.00	R
superpathway of beta-D-glucuronide and D-glucuronate degradation	0.00	1.00	0.00	R
superpathway of branched chain amino acid biosynthesis	0.00	1.00	0.00	R
superpathway of demethylmenaquinol-8 biosynthesis	0.00	1.00	0.00	R
superpathway of geranylgeranyl diphosphate biosynthesis II (via MEP)	0.00	1.00	0.00	R
superpathway of glucose and xylose degradation	0.00	1.00	0.00	R
superpathway of guanosine nucleotides de novo biosynthesis I	0.00	1.00	0.00	R
superpathway of guanosine nucleotides de novo biosynthesis II	0.00	1.00	0.00	R
superpathway of L-alanine biosynthesis	0.00	1.00	0.00	R
superpathway of L-asparagine biosynthesis	0.00	1.00	0.00	R
superpathway of L-aspartate and L-asparagine biosynthesis	0.00	1.00	0.00	R
superpathway of L-isoleucine biosynthesis I	0.00	1.00	0.00	R
superpathway of L-lysine, L-threonine and L-methionine biosynthesis I	0.00	1.00	0.00	R
superpathway of L-methionine biosynthesis (by sulfhydrylation)	0.00	1.00	0.00	R
superpathway of L-methionine biosynthesis (transsulfuration)	0.00	1.00	0.00	R
superpathway of L-serine and glycine biosynthesis I	0.00	1.00	0.00	R
superpathway of L-threonine biosynthesis	0.00	1.00	0.00	R
superpathway of menaquinol-7 biosynthesis	0.00	1.00	0.00	R
superpathway of menaquinol-8 biosynthesis I	0.00	1.00	0.00	R

superpathway of N-acetylglucosamine, N-acetylmannosamine and N-acetylneuraminate degradation	0.00	1.00	0.00	R
superpathway of purine deoxyribonucleosides degradation	0.00	1.00	0.00	R
superpathway of pyrimidine deoxyribonucleoside salvage	0.00	1.00	0.00	R
superpathway of pyrimidine deoxyribonucleosides degradation	0.00	1.00	0.00	R
superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis	0.00	1.00	0.00	R
superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (E. coli)	0.00	1.00	0.00	R
superpathway of pyrimidine nucleobases salvage	0.00	1.00	0.00	R
superpathway of pyrimidine ribonucleotides de novo biosynthesis	0.00	1.00	0.00	R
superpathway of sulfate assimilation and cysteine biosynthesis	0.00	1.00	0.00	R
superpathway of tetrahydrofolate biosynthesis	0.00	1.00	0.00	R
superpathway of thiamine diphosphate biosynthesis I	0.00	1.00	0.00	R
superpathway of UDP-glucose-derived O-antigen building blocks biosynthesis	0.00	1.00	0.00	R
taurine degradation I	Inf	1.00	1.00	R
taurine degradation III	1.32	1.00	0.02	R
TCA cycle VIII (helicobacter)	1.36	1.00	0.04	R
tetrahydrofolate biosynthesis	0.00	1.00	0.00	R
tetrahydrofolate salvage from 5,10-methenyltetrahydrofolate	0.00	1.00	0.00	R
tetrapyrrole biosynthesis I (from glutamate)	0.00	1.00	0.00	R
thiamine diphosphate biosynthesis I (E. coli)	0.00	1.00	0.00	R
thiamine diphosphate biosynthesis II (Bacillus)	0.00	1.00	0.00	R
thiamine salvage II	0.00	1.00	0.00	R
thiamine salvage III	0.00	1.00	0.00	R
thiamine salvage IV (yeast)	0.00	1.00	0.00	R
thiazole biosynthesis I (facultative anaerobic bacteria)	0.00	1.00	0.00	R
thioredoxin pathway	0.00	1.00	0.00	R
thiosulfate disproportionation III (rhodanese)	1.29	1.00	0.01	R
thymine degradation	0.00	1.00	0.00	R
trans, trans-farnesyl diphosphate biosynthesis	0.00	1.00	0.00	R
trehalose biosynthesis I	0.00	1.00	0.00	R
triacylglycerol degradation	1.22	1.00	0.08	R
tRNA charging	0.00	1.00	0.00	R
tRNA processing	0.00	1.00	0.00	R
UDP-alpha-D-glucuronate biosynthesis (from UDP-glucose)	0.00	1.00	0.00	R
UDP-D-galactose biosynthesis	0.00	1.00	0.00	R
UDP-D-galacturonate biosynthesis I (from UDP-D-glucuronate)	0.00	1.00	0.00	R
UDP-galactofuranose biosynthesis	0.00	1.00	0.00	R
UDP-glucose biosynthesis	0.00	1.00	0.00	R
UDP-N-acetyl-alpha-D-galactosaminuronate biosynthesis	Inf	1.00	1.00	R
UDP-N-acetyl-alpha-D-mannosaminouronate biosynthesis	0.00	1.00	0.00	R
UDP-N-acetyl-D-glucosamine biosynthesis I	0.00	1.00	0.00	R
UDP-N-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing)	1.32	1.00	0.02	R

UDP-N-acetylmuramoyl-pentapeptide biosynthesis I (meso-diaminopimelate containing)	0.00	1.00	0.00	R
UMP biosynthesis	0.00	1.00	0.00	R
uracil degradation III	1.63	1.00	0.22	R
urea cycle	0.00	1.00	0.00	R
urea degradation II	0.00	1.00	0.00	R
UTP and CTP de novo biosynthesis	0.00	1.00	0.00	R
UTP and CTP dephosphorylation I	0.00	1.00	0.00	R
xanthine and xanthosine salvage	0.00	1.00	0.00	R
xylose degradation I	0.00	1.00	0.00	R

[a] Estimates apply to a subset of 25 patients who had Metagenomic WGS data available

Table S8. Individual values and summary statistics of metabolic reconstructions.

Sample	Genes	Genes of known or predicted molecular function	Pathways	Metabolic Reactions	Transport Reactions	Compounds
1	36475	23417	451	2016	276	1669
2	36914	23811	459	2089	281	1751
3	44948	28820	498	2312	323	1895
4	31710	20446	397	1861	234	1583
5	25541	15702	500	2272	339	1877
6	41761	26459	507	2372	324	1943
7	27071	17620	404	1851	245	1559
8	29091	18566	481	2163	328	1793
9	27740	17946	486	2255	328	1854
10	44204	27899	523	2471	344	2009
11	26897	17433	400	1814	240	1547
12	22879	14385	490	2248	334	1857
13	37311	24090	435	2017	274	1708
14	35781	22589	444	2094	287	1770
15	23732	15066	491	2253	331	1886
16	19793	12789	395	1899	240	1619
17	23184	14702	436	2020	276	1695
18	31203	19760	517	2329	354	1919
19	24237	15593	458	2117	311	1785
20	35021	22007	498	2251	336	1853
21	32373	21032	492	2218	314	1825
22	30518	19678	463	2114	277	1792
23	29535	19148	467	2165	316	1793
24	24410	15301	506	2305	349	1894
25	38701	24639	515	2325	358	1928
26	28840	18767	399	1879	245	1601
27	26919	17486	439	2012	306	1684
28	28250	18066	449	2004	282	1715
Mean	30894	19758	464	2133	302	1779

S9. Taxonomic classification by NCBI, SILVA, RDP and Greengenes 16S databases of top 50 most abundant OTUs.

NCBI				SILVA		RDP		Greengenes		
OTU Id	Genus	Species	% identity	Genus	% identity	Genus	% identity	Genus	Species	% identity
OTU_1	Bacteroides	vulgatus	100	Bacteroides	100	Bacteroides	100	Bacteroides	Unclassified	100
OTU_2	Bacteroides	uniformis	100	Bacteroides	100	Bacteroides	99	Bacteroides	uniformis	100
OTU_3	Prevotella	copri	100	Prevotella_9	100	Prevotella	100	Prevotella	copri	100
OTU_4	Bacteroides	thetaiotaomicron	100	Bacteroides	100	Bacteroides	100	Bacteroides	Unclassified	65
OTU_7	Faecalibacterium	prausnitzii	99	Faecalibacterium	100	Faecalibacterium	99	Faecalibacterium	prausnitzii	100
OTU_9	Bacteroides	stercoris	100	Bacteroides	100	Bacteroides	100	Bacteroides	Unclassified	92
OTU_26	Bacteroides	ovatus	99	Bacteroides	100	Bacteroides	99	Bacteroides	ovatus	100
OTU_5	Parabacteroides	merdae	100	Parabacteroides	100	Parabacteroides	100	Parabacteroides	Unclassified	100
OTU_8	Escherichia	coli	100	Escherichia-Shigella	100	Escherichia/Shigella	100	Unclassified Enterobacteriaceae	Unclassified	94
OTU_6	Akkermansia	muciniphila	98	Akkermansia	100	Akkermansia	98	Akkermansia	muciniphila	100
OTU_10	Alistipes	putredinis	100	Alistipes	100	Alistipes	100	Unclassified Rikenellaceae	Unclassified	100
OTU_460	Prevotella	copri	99	Prevotella_9	100	Prevotella	99	Prevotella	copri	100
OTU_13	Bacteroides	caccae	100	Bacteroides	100	Bacteroides	100	Bacteroides	caccae	100
OTU_11	Parasutterella	excrementihominis	100	Parasutterella	100	Parasutterella	100	Sutterella	Unclassified	100
OTU_542	Bacteroides	xylanisolvens	100	Bacteroides	100	Bacteroides	100	Bacteroides	ovatus	81
OTU_12	Alistipes	onderdonkii	100	Alistipes	100	Alistipes	100	Unclassified Rikenellaceae	Unclassified	100
OTU_14	Faecalibacterium	prausnitzii	96	Subdoligranulum	100	Faecalibacterium	96	Unclassified Ruminococcaceae	Unclassified	52
OTU_19	Ruminococcus	bromii	100	Ruminococcus_2	100	Ruminococcus	99	Ruminococcus	bromii	100
OTU_15	Bacteroides	cellulosilyticus	100	Bacteroides	100	Bacteroides	100	Bacteroides	Unclassified	87
OTU_23	Roseburia	intestinalis	100	Roseburia	94	Roseburia	100	Roseburia	Unclassified	98
OTU_17	Eubacterium	siraeum	100	Ruminiclostridium_6	100	Clostridium_IV	99	Ruminococcus	Unclassified	97
OTU_602	Faecalibacterium	prausnitzii	98	Faecalibacterium	100	Faecalibacterium	97	Faecalibacterium	prausnitzii	97
OTU_20	Bacteroides	massiliensis	100	Bacteroides	100	Bacteroides	100	Bacteroides	Unclassified	97
OTU_22	Blautia	wexlerae	100	Blautia	100	Blautia	100	Blautia	Unclassified	99
OTU_27	[Clostridium]	clostridioforme	100	Lachnoclostridium	96	Clostridium_XlVa	100	Unclassified Lachnospiraceae	Unclassified	88
OTU_21	Dialister	invisus	100	Dialister	100	Dialister	100	Dialister	Unclassified	100
OTU_366	Eubacterium	rectale	100	Roseburia	91	Roseburia	100	Roseburia	faecis	86
OTU_18	Flavonifractor	plautii	100	Flavonifractor	100	Flavonifractor	100	Oscillospira	Unclassified	100
OTU_500	Akkermansia	muciniphila	97	Akkermansia	100	Akkermansia	97	Akkermansia	muciniphila	100
OTU_24	Prevotella	shahii	91	Prevotellaceae_NK3B31_group	100	Prevotella	91	Prevotella	Unclassified	100

OTU_33	Parabacteroides	distasonis	99	Parabacteroides	100	Parabacteroides	99	Parabacteroides	distasonis	100
OTU_58	Bacteroides	acidifaciens	97	Bacteroides	100	Bacteroides	97	Bacteroides	Unclassified	60
OTU_290	Bacteroides	uniformis	97	Bacteroides	100	Bacteroides	97	Bacteroides	Unclassified	71
OTU_25	Butyrivibrio	crossotus	100	Butyrivibrio	100	Butyrivibrio	100	Unclassified Lachnospiraceae	Unclassified	50
OTU_28	Mobilitalea	sibirica	98	Mobilitalea	100	Mobilitalea	98	Coprococcus	Unclassified	72
OTU_31	Gemmiger	formicilis	100	Subdoligranulum	100	Gemmiger	100	Unclassified Ruminococcaceae	Unclassified	87
OTU_210	Bacteroides	ovatus	98	Bacteroides	100	Bacteroides	98	Bacteroides	ovatus	45
OTU_50	Bacteroides	plebeius	99	Bacteroides	100	Bacteroides	99	Bacteroides	plebeius	100
OTU_29	Pseudoflavonifractor	capillosus	91	Ruminococcaceae_UCG-002	100	Flavonifractor	90	Unclassified Ruminococcaceae	Unclassified	80
OTU_32	Sporobacter	termitidis	93	Ruminococcaceae_UCG-005	100	Sporobacter	93	Unclassified Ruminococcaceae	Unclassified	96
OTU_494	Alistipes	finegoldii	98	Alistipes	100	Alistipes	98	Unclassified Rikenellaceae	Unclassified	100
OTU_37	Alistipes	shahii	97	Alistipes	100	Alistipes	97	Unclassified Rikenellaceae	Unclassified	100
OTU_61	Barnesiella	intestinihominis	99	Barnesiella	100	Barnesiella	99	Unclassified Barnesiellaceae	Unclassified	100
OTU_36	Blautia	luti	100	Blautia	99	Blautia	99	Blautia	Unclassified	94
OTU_40	Roseburia	inulinivorans	100	Roseburia	100	Roseburia	100	Roseburia	Unclassified	94
OTU_30	Phascolarctobacterium	faecium	100	Phascolarctobacterium	100	Phascolarctobacterium	100	Phascolarctobacterium	Unclassified	100
OTU_54	[Clostridium]	amygdalinum	98	Lachnoclostridium	94	Clostridium_XlVa	98	Unclassified Lachnospiraceae	Unclassified	87
OTU_38	Fusicatenibacter	saccharivorans	100	Fusicatenibacter	100	Fusicatenibacter	100	Unclassified Lachnospiraceae	Unclassified	93
OTU_16	Kiloniella	laminariae	86	uncultured	68	Aestuariispira	86	Unclassified RF32	Unclassified	100
OTU_46	Bacteroides	xylanolyticus	98	Lachnospiraceae_NK4A136_group	90	Clostridium_XlVa	98	Unclassified Lachnospiraceae	Unclassified	97

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