Appendix

Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models

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Supplementary Materials and Methods

Human fecal sample collection

Fecal samples were collected from 71 adult patients with relapsing-remitting multiple sclerosis that had not received treatment for at least 3 months prior to the time of collection and 71 controls without autoimmune disorders at the University of California, San Francisco (UCSF) and the Icahn School of Medicine at Mt Sinai (New York, NY). The inclusion criteria specified no use of antibiotics or cancer therapeutics in 3 months prior to the study. Detailed patient information is available in Supplementary Table 1. Samples were collected using culture swabs (BD #220135) and stored at -80C until DNA extraction or bacterial isolation.

16S rRNA amplicon sequencing and computational analysis of human and mouse microbiome samples

DNA was extracted from samples using MoBio Power Fecal DNA extraction kit (MoBio #12830) and amplicons of V4 region of the prokaryotic 16S rRNA gene were sequenced using the Earth Microbiome Project standard protocol (1). Analysis was performed using QIIME v1.9 as described (2). Essentially, amplicon sequences were quality-filtered and grouped to "species-level" OTUs using SortMeRNA method (3) using GreenGenes version 13.8 97% dataset for closed reference. Sequences that did not match reference sequences in the GreenGenes database were dropped from the analysis. Taxonomy was assigned to the retained OTUs based on the GreenGenes reference sequence, and the GreenGenes tree was used for all downstream phylogenetic community comparisons. Samples were filtered to at least 10000 sequences per sample, and OTUs were filtered to retain only OTUs present in at least 5% of samples and covering at least 100 total reads. After filtering samples were rarefied to 10000 sequences per sample. We identified 129 total genera and 1462 total operational taxonomic units (OTUs) in our samples. We systematically compared relative abundances of individual microbial taxa between MS patients and controls at the genus and OTU levels by negative binomial Wald test using Benjamini-Hochberg correction for multiple comparisons (4). Alpha diversity was calculated using the Chao1 method (5). For analysis of beta diversity, pairwise distance matrices were generated using the phylogenetic metric unweighted UniFrac (6) and used for principal coordinate analysis (PCoA). For comparison of individual taxa, samples were not rarefied. Instead, OTU abundances were normalized using variance-stabilizing transformation and taxa distributions were compared using Wald negative binomial test from R software package DESeq2 as described previously (4, 7) with Benjamini-Hochberg correction for multiple comparisons. All statistical analyses of differences between individual bacterial species were performed using QIIME v.1.9 or R (packages DESeq2 and phyloseq).

Comparison of functional pathways expressed by microbiota

$$KO_j = \sum_{i=1}^{m} \left(W_{otu\ i \to ko\ j} \times C_{otu\ i} \right) \quad (Equation\ 1)$$

$$Pathway_k = \sum_{j=1}^{n} KO_j \times B \text{ where } B = \begin{cases} 0, & if\ KO_j\ not\ in\ Pathway_k \\ 1, & if\ KO_j\ in\ Pathway_k \end{cases} \quad (Equation\ 2)$$

A matrix of containing samples and OTU counts was obtained for all datasets using QIIME (described above). We transformed the raw OTU counts (C_{otu}) into KEGG Ortholog (KO) values by taking the product of C_{otu} and pre-established OTU contribution weights ($W_{otu->ko}$) (8). This resulted in matrix of samples and KO values (*Equation 1*). The KO values were then transformed into pathway values by simply taking the sum of all KOs that contribute to a pathway (*Equation 2*). Once every sample had a pathway value for each pathway, we ran a logistic regression to determine which pathways were the most important in separating MS and control samples. The MS and controls samples separated very well in all three datasets (0.81 AUC (area under the curve) Human, 0.98 AUC Mouse 1, and 0.94 AUC Mouse 2). For each dataset, the pathway coefficients were used to calculate the odds ratio of each pathway. We then took the top 10% of pathways in each of the datasets and checked to see if there was any overlap between the human and mouse datasets. Next, we repeated the same analysis using only the fraction of OTUs that were differentially expressed in MS and control samples. Using this small subset of OTUs, the MS and control samples still separated (0.70 AUC Human and 1.0 AUC in both Mouse datasets).

Bacterial extract preparation for stimulation of human PBMCs

We used a combined experimental approach based on previously published studies to prepare bacterial extracts (9) and combine them with human PBMCs (10), followed by standard Th1 and Treg differentiation protocols (11, 12). All PBMC responses to bacterial extracts were compared to no-bacteria vehicle controls that contained the same protease and phosphatase inhibitors. PBMCs were immunostained and analyzed using standard flow cytometry protocols (described in detail in Supplementary Materials and Methods).

Total bacteria were isolated from patient or control stool samples by suspending ~0.5mg stool sample in 1.5ml PBS, passing it three times through a 40um cell strainer and washing twice with 1.5ml PBS by spinning at 8000rpm. Individual bacterial species were ordered directly from ATCC. *Parabacteroides distasonis* (ATCC #8503) was grown on pre-reduced OxyPRAS Brucella Blood Agar plates (Oxyrase #P-BRU-BA) or in MTGE broth (Anaerobe Systems #AS-778) for 2 days anaerobically. *Akkermansia muciniphila* (ATCC #BAA-835) was grown on Tryptic Soy Blood Agar plates (Anaerobe Systems, #AS-542) or in OxyPRAS BHI broth (Oxyrase #BHI-HK) for 3 days anaerobically. *Acinetobacter calcoaceticus* (ATCC #23055) was grown on OxyPRAS Brucella Blood Agar plates or in Brucella Broth (Anaerobe Systems #AS-105) for 2 days aerobically.

Human PBMC isolation and stimulation with bacterial extracts

Peripheral blood mononuclear cells were isolated from healthy controls or RRMS patients and stored at -80C in cryovials at 10^7 cells/ml concentration in FBS containing 10% DMSO. Before plating, cells were washed in PBS twice, re-counted, and plated at 10^6 cells/ml concentration in RPMI media supplemented with 10% FBS and 1% penicillin/streptomycin/glutamine.

After isolation bacteria were resuspended in PBS supplemented with protease inhibitor (Roche #4693159001) and phosphatase inhibitor (Roche #4906845001), heat-inactivated at 65C for 1h and sonicated for 10min as described previously (9). Protein concentration in the resulting suspension was measured using the Pierce BCA protein assay kit (Thermo Scientific #23227).

Bacterial extracts were added to PBMCs at 0.1µg/ml (*A. calcoaceticus*) or at 1µg/ml *(A. muciniphila, P. distasonis),* to represent their relative abundances, 1h after plating as described previously (10). Each experiment contained at least 6 independent donor samples and was repeated at least twice with no more than 50% overlap between donors. .

For human Treg differentiation, cells were stimulated with anti-human CD3 (BD #555336, 0.3 µg/ml), anti-human CD28 (BD #555725, 2 µg/ml) and recombinant human TGF-β1 (R&D #240B002, 2.5ng/ml) for 4 days (12), in presence of bacterial extracts. For human Th1 differentiation, cells were stimulated with anti-human CD3 (BD #555336, 2 µg/ml), anti-human CD28 (BD #555725, 2 µg/ml), anti-human IL-4 (BioLegend #500702, 5 µg/ml) and recombinant human IL-12 (Thermo Fisher Scientific #NBP143214, 20ng/ml) for 3 days (11), in presence of bacterial extracts. After 3 days the culture media was changed and Th1 cells were restimulated for 4 hours with 1µM ionomycin and 50ng/ml PMA in presence of 2 µM monensin, in absence of bacterial extracts.

Flow cytometry of human PBMCs

Live/dead cell gating was achieved using Live/Dead Fixable Aqua kit (ThermoFisher #L34957). BD Cytofix/Cytoperm kit (BD #554722) was used for staining of intracellular cytokines, and FoxP3/Transcription factor staining buffer set (eBioscience #00-5523-00) for transcription factors. The following antibodies were used for human PBMC staining: anti-CD3-PE.Cy7 (BD #563423), anti-CD4-PerCP.Cy5.5 (BioLegend #300530 or BD #560650) and anti-CD25-PE (BD #555432) or anti-CD25-BV421 (BD #562442) or anti-CD25-APC (BD #555434), anti-IFNγ-FITC (BioLegend #502506), anti-Tbet-PE (BD #561265), anti-FoxP3-AlexaFluor-488 (BD #560047) and anti-IL-10-PE (eBioscience #12-7108). Flow cytometry was performed on BD Fortessa cell analyzer and analyzed using FlowJo software (TreeStar). For both mouse and human PBMC flow cytometry, cells were gated to identify the lymphocyte population based on forward and side scatter, followed by gating for single cell and live cell populations. Unstained, single color and fluorescence-minus-one controls were used to identify stained populations.

Verification of germ-free or antibiotic-treated mouse status

Colonization level of GF mice was verified by quantification of colony forming units of bacteria in mouse feces, cultured anaerobically (Suppl. Fig. S11A) and colonization specificity was verified by PCR (Suppl. Fig. S11B). In bacterial monocolonization experiments, mice were euthanized and immunophenotyping was performed 3-4 weeks after colonization. GF mice remained in isolators for the duration of the experiment and transferred out when euthanized. GF mice that were subsequently associated with fecal microbes or single species of bacteria were taken out of isolators at 3-4 weeks of age and colonized with a single oral gavage. In experiments without EAE induction, GF control groups were kept in isolators until mice were euthanized for immunophenotyping. In experiments with EAE induction, GF mice were removed from the isolators in order to induce EAE, and maintained GF outside of the isolators by supplementing drinking water with erythromycin and gentamycin until the end of the experiment. In order to ensure that the GF animals remained GF both inside and outside the isolators, their stool samples were repeatedly plated on Brucella blood agar plates and cultured both aerobically and anaerobically. GF mice were determined to be clean after no colonies could be observed on the plates after a week of incubation.

To ensure that broad-spectrum antibiotic treatment depletes stool bacteria, total bacteria isolated from mouse stool was cultured on Brucella Blood Agar plates and no colonies were observed. Antibiotic-mediated depletion of microbiota was further verified by comparing community richness in antibiotic-treated mice and SPF controls (Suppl. Fig. S12; Chao1 metric of alpha diversity).

Mouse colonization with microbiota

Germ-free (GF) mice were bred and maintained in sterile isolators that were assayed every two weeks for GF status by bacterial plating and PCR. Fecal material from human donor samples or individual bacteria species at 10^8 CFU/mouse was diluted to a final solution of 1.5% sodium bicarbonate. For colonization experiments following antibiotic treatment, mice were treated with 1% solution of Amphotericin B in drinking water for 3 days, followed by 2 weeks of antibiotic solution composed of 1% Amphotericin B, 1mg/ml ampicillin, 1mg/ml neomycin, 1mg/ml metronidazole and 0.5mg/ml vancomycin in drinking water; 1% Amphotericin B solution was used as a control. After 2 weeks, we replaced the drinking solution by sterile water and mice were gavaged by specific bacteria of interest in a solution of 1.5% sodium bicarbonate at 10^8 CFU/mouse every 2-3 days for 2 weeks. SPF mice were gavaged with mock culture medium every 2-3 days for 2 weeks to mimic the esophageal trauma.

Induction of EAE

EAE was induced 6-7 weeks after colonization, corresponding to 9-10 weeks of age. Mice were subcutaneously immunized in the upper and lower back with 0.1ml MOG_{35-55} emulsion (1mg/ml) mixed with Complete Freud's Adjuvant and killed mycobacterium tuberculosis H37Ra (2-5mg/ml), followed by two 0.1ml intraperitoneal injections of pertussis toxin (4µg/ml) approximately 2 and 26 hours later (Hooke Laboratories #EK-2110). Mice were scored daily in a blinded fashion for motor deficits as follows: 0, no deficit; 1, limp tail only; 2, limp tail and hind limb weakness; 3, complete hind limb paralysis; 4, complete hind limb paralysis and partial/complete forelimb paralysis; 5, moribund.

Mouse immune cell isolation and intracellular cytokine staining

Mesenteric lymph nodes, cervical lymph nodes, and spleens were dissected and processed by grinding tissues through a 100um cell strainer. Single cell suspensions were incubated for 4-5 hours with 50ng/ml PMA, 2µg/ml ionomycin in presence of 2µg/ml protein transport inhibitor (GolgiPlug, BD #51-2301KZ). Cells were then incubated in 5% mouse serum for 15 min and stained for 20 min at 4ºC with Live/Dead Fixable Violet kit (Life Technologies #L34964) or Live/Dead Fixable Aqua kit (ThermoFisher #L34957) and anti-CD4-PE-Cy7 (eBioscience #25-0042-82). Cells were fixed and permeabilized with FoxP3/Transcription factor buffer set (eBioscience #00-5523-00), and stained with anti-Foxp3-APC (eBioscience, #17-5773-82) and anti-IL10-PE (eBioscience #12-7101-82), anti-IFNγ-FITC (eBioscience, #11-7311-82), anti-IL17-PerCP/Cy5.5 (eBioscience #45-7177-82).

RNAseq analysis in gnotobiotic mouse spinal cords

RNA was isolated from whole spinal cord of transplanted mice, checked for RNA integrity and sequenced at the New York Genome Center. Samples were pair-end sequenced and each sample generated 100 million reads (50 million read-pairs). Raw reads were then aligned to the Ensemble reference genome (mapping rate = 80%). Differential expression of transcripts was analyzed using the DESeq2 package. We identified transcripts with baseMean (average count value normalized to library size) > 5 and a log2 fold change of at least 1.5. Since we were interested in determining changes in the spinal cord that occurred in GF mice transplanted with MS or control microbiota in response to EAE, we focused on transcripts with differential expression between pre-induction and at 35 days post-induction. A greater number of differentially expressed transcripts was identified in the spinal cord of GF mice transplanted with MS stools compared to controls. To begin understanding the cell type that could drive this transcriptional difference, we used this dataset to interrogate a cell-enrichment gene list that we generated using the data from Zhang et al. (J Neurosci. 2014 Sep 3;34(36):11929-47) and considering "cell-specific" those genes whose expression was at least 10 folds higher in one cell type than in any other. Six cell-specific lists were generated for astrocytes, myelinating oligodendrocytes, neurons, oligodendrocyte precursor cells, microglia and endothelial cells.

Statistical analysis of *in vitro* data

Statistical significance of expression changes in markers of T lymphocyte differentiation and proliferation was determined using two-tailed Student's *t* test to compare samples from different donors, two-tailed repeated measures *t* test to compare samples from the same donor, and one or two-way ANOVA to compare samples from different donors in different groups based on bacterial presence. When data was non-normally distributed, two-tailed nonparametric Mann-Whitney test was used instead. Benjamini-Hochberg adjustment was used to account for multiple comparisons. GraphPad Prism 6 software was used to analyze and plot the data. $P < 0.05$ was considered statistically significant.

Supplementary Discussion

While no major shifts in microbial diversity were identified, specific bacterial taxa were found to be significantly under- or over-represented in untreated MS patients compared to healthy controls. *In vitro* studies of MS-associated bacterial species revealed that even modest dysbiosis could drive a two-stage, immune deviation mechanism (either sequential or parallel), ultimately resulting in skewed T lymphocyte differentiation. *Acinetobacter* and *Akkermansia* species induces a pro-inflammatory environment, mediated by reduction in CD25+FoxP3+ Tregs and expansion of Th1 lymphocytes. On the other hand, a potential concomitant decrease in *P. distasonis* mediates a relative loss of CD25+IL-10+ T lymphocytes. Furthermore, we show that MS-associated microbiota inhibits IL-10+ T lymphocyte responses *in vivo* and increases disease severity in a mouse model of MS.

The interaction between microbiota and the immune system is likely bidirectional, as suggested by impaired lymphocyte responses of MS patients to self microbiota, but no difference in control lymphocyte responses to either control or MS non-self microbiota (SI Appendix, Fig. S10). The observed reduction in *Parabacteroides*, increase in *Acinetobacter* and increase in *Akkermansia* may together or separately contribute to inflammation and disease severity without being the only factors that account for the development of the disease. Thus, it is essential to view our findings not as an exclusive list of immunoregulatory microbiota, but rather as the first step in investigating the microbial species and pathways that contribute to the regulation of autoimmune inflammation in MS and other diseases.

In contrast to recent publications profiling the microbiome in MS and other chronic diseases (13-16), we focused exclusively on patients not receiving disease-modifying therapeutics at the time of sample collection to decouple the effects of the disease and therapy, a valuable strategy highlighted in a recent report on Crohn's disease (17). In concordance with prior studies in MS, we did not observe global changes in microbial community structures. However, the larger sample size in our study allowed us to identify individual bacterial taxa that are potentially associated with autoimmunity. In addition, in contrast to studies on MS microbiota that were limited to identifying correlations between microbial taxa and host phenotypes (18), we were able to investigate microbial immunoregulatory effects *in vitro* and *in vivo.*

Our taxa-specific investigation was focused on commensal microbiota, not infectious pathogens. *Akkermansia* and *Parabacteroides* are commensal and even *Acinetobacter*, even though it is typically acquired as an infection, is not considered a pathogen once it inhabits the human gut. We consciously chose to investigate the physiological contribution of commensals to systemic inflammation, as their ability to alter T lymphocyte differentiation is likely to create a systemic environment that may exacerbate inflammation in the host without overt infection, which constitutes a plausible model for MS etiology.

Our results suggest that both rare and common microbiota may play a role in the exacerbation of autoimmune inflammation in MS. Notably, the presence of the relatively common bacterial species *A. muciniphila* in total stool bacteria was sufficient to recapitulate its functions of skewing PBMC responses towards pro-inflammatory Th1 phenotype. Apart from their *A. muciniphila* content, the microbiomes of the subjects analyzed here were highly heterogeneous based on 16S sequencing, which further supports the hypothesis that *A. muciniphila* is one of the

keystone species sufficient to drive the microbiota towards Th1 induction. Despite this intriguing result, *A. muciniphila* is unlikely to be the only microbial species that is sufficient to induce Th1 differentiation. Instead, the discovery of *A. muciniphila* as a major pro-inflammatory regulator may be considered as the first step in identifying other microbes and microbial pathways that are sufficient for inducing Th1 differentiation.

Of note, the pro-inflammatory role of *A. muciniphila* observed in our *in vitro* experiments was not replicated in monocolonized mice. This difference may be caused by differences in mouse and human immune cell responses to *A. muciniphila.* In addition, the main *in vivo* role of *A. muciniphila* may have little to do with its direct effects on T cells: it may instead shape the rest of the microbial community towards more pro-inflammatory phenotype. This function would explain why *A. muciniphila* is more abundant in untreated MS patients both in our study and a previous investigation of an independent cohort (19), and is sufficient to induce pro-inflammatory functions of total microbial community.

Despite rarely detected in the human gut, *Acinetobacter* had a higher relative abundance in MS patients. Although it cannot be considered as a universal contributor to MS severity due to its rarity, we view it as one of multiple environmental contributors that are able to exacerbate the disease. Its role may be explained by drawing an analogy to genetics: same as a specific allele, a specific bacterial species may be rare overall, but significantly increased in MS patients compared to controls, and contribute to disease severity in those few MS patients in which it is present.

Furthermore, we observed a consistent association between healthy control microbiota and CD25+IL-10+ lymphocyte differentiation both *in vitro* and *in vivo.* A similar result is presented in the accompanying article where blocking IL-10 results in increased EAE incidence (Berer *et al.*). It has been previously reported that IL-10 deficient mice are susceptible to colitis induced by transplantation of normal mouse microbiota or simplified human microbiota (20, 21). Thus, CD25+IL-10+ lymphocyte differentiation in response to healthy microbiota, and especially to a common and highly abundant bacterial species like *P. distasonis*, may serve as a built-in control mechanism to dampen the pro-inflammatory characteristics of other microbial species. The loss of this mechanism due to MS-associated dysbiosis may then create a pro-inflammatory environment, which may further exacerbate the disease.

Supplementary Figure Legends

Figure S1. Microbial community variability within and between subjects.

A. Comparison of microbial community composition in the same subject between consecutive days. Each color indicates a different subject. Controls and MS patients have been combined. Unweighted Unifrac measure of beta diversity. **B.** Comparison of variability in microbial community composition within and between groups of subjects. Unweighted Unifrac distances. CTRL_CTRL, within healthy control group. MS_MS, within MS patient group. CTRL_MS, between healthy controls and MS patients. $^{**}P<0.01$, Mann-Whitney test, Bonferroni correction for multiple comparison.

Figure S2. Relative abundances of microbial genera in healthy controls.

X axis, genera (ranked by relative abundance). Y axis, log10 of mean relative abundance in healthy controls after rarefaction to 10,000 reads per sample. n = 71.

Figure S3. Examples of selected bacterial species showing no immunoregulatory effect on human PBMCs.

A. Quantification of CD25+FoxP3+ cell differentiation within CD3+CD4+ population in response to *Eggerthella lenta* (*E. len*). **B.** Quantification of IFNγ+ cell differentiation within CD3+CD4+ population in response to *Eggerthella lenta* (*E. len*). **C.** Quantification of CD25+IL10+ cell differentiation within CD3+CD4+ population in response to *Acinetobacter calcoaceticus* (*A. calc*). **D.** Quantification of IFNγ+ cell differentiation within CD3+CD4+ population in response to *Parabacteroides distasonis* (*P. dist*). **E.** Quantification of CD25+FoxP3+ cell differentiation within CD3+CD4+ population in response to *Akkermansia muciniphila* (*A. muci*). n=6 PBMC donors. Error bars, mean±SEM.

Figure S4. Monocolonization of antibiotic-treated mice with individual bacterial species recapitulates *in vitro* T lymphocyte differentiation patterns.

A. Quantification of FoxP3+ cell differentiation within CD4+ population in splenocytes of mice colonized with *A. calcoaceticus (A. calc)* after antibiotic treatment, compared to SPF control. **B.** Quantification of CD4+IL10+ cell differentiation within live cells in splenocytes of mice colonized with *P. distasonis (P. dist)* after antibiotic treatment, compared to SPF control. **C.** Quantification of CD4+IL10+ cell differentiation within live cells from splenocytes of mice colonized with *P. distasonis (P. dist)* or SPF control mice exposed to self bacterial extracts. $^{*}P<0.05$, $^{**}P<0.01$, two-tailed *t* test (**A, B**) or two-tailed repeated measures *t* test (**C**). n=5-7 mice per group. Error bars, mean ± SEM.

Figure S5. The effects of monocolonization of GF mice with Acinetobacter calcoaceticus, Akkermansia muciniphila and Parabacteroides distasonis on T lymphocyte differentiation.

A-C. Representative flow cytometry plots showing gating strategy (**A**) and quantification (**B, C**) of CD4+IFNγ+ Th1 lymphocytes (**B**) and CD4+IL-10+ lymphocytes (**C**) within live cell population in GF mice colonized with *Acinetobacter calcoaceticus, Akkermansia muciniphila* and *Parabacteroides distasonis*. GF mice and SPF mice used as controls. n=3-8 mice per group.

*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, 1-way ANOVA with Tukey adjustment for multiple comparisons. Error bars, mean±SEM.

Figure S6. Transfer of fecal microbiota from 3 different donor pairs (MS patient and healthy household control) into germ-free mice mediates EAE outcomes.

(A-C) Clinical EAE scores of mice that had been colonized at 3-4 weeks of age with healthy control or MS patient microbiota from 3 independent donor pairs for at least 6 weeks prior to induction of EAE. n=3-8 mice per group. Error bars, mean ± SEM. * = Control vs. MS, # = Germ-free vs. MS. */#, P < 0.05, **/##, P <0.01, ***/###, P < 0.0001, 2-way ANOVA with Tukey adjustment for multiple comparisons. Error bars, mean±SEM.

Figure S7. Immunophenotyping of mesenteric and cervical lymph nodes of mice colonized with MS and control microbiota from donor pair 1.

A. Flow cytometry gating strategy. **B-E.** Quantification of IFNγ+ Th1 and IL-17+ Th17 cell differentiation within CD4+FoxP3- population in mesenteric lymph nodes before induction of EAE (**B, C**) and at peak of disease (**D, E**). **F-K.** Quantification of IFNγ+ Th1, IL-17+ Th17 cell differentiation within CD4+FoxP3- population and IL-10+ cell differentiation within CD4+FoxP3+ population in cervical lymph nodes before induction of EAE (**F-H**) and at peak of disease (**I-K**). n=3-8 mice per group. Error bars, mean ± SEM.

Figure S8. Microglial gene expression in spinal cords of mice colonized with MS and control microbiota.

Log_2fold difference in microglia-enriched genes at peak EAE compared to pre-induction in gnotobiotic mice colonized with microbiota from human donor pair #1.

Figure S9. Differences in microbiome composition of mice colonized with MS and control microbiota.

A. Principal coordinate analysis (PCoA) of mouse microbiota at different time points after colonization with fecal microbiota from donor pair #1. Lines connect consecutive time points. **B.** Alpha diversity of microbiota of human donors and recipient mice from donor pair #1. **C.** Relative abundance of bacterial genera that were increased in mice colonized with either healthy control (*Sutterella*) or MS patient (*Ruminococcus*) microbiota in MS-Discordant Twin Study (Berer et al.).

Figure S10. CD25+ FoxP3+ Treg differentiation in response to non-self bacterial extracts.

Quantification of CD25+FoxP3+ cell differentiation within CD3+CD4+ population in response to non-self bacterial extracts from healthy controls or MS patients. Lines connect averaged responses of the same donor. n = 5 PBMC donors, bacterial extracts from 6 MS patients and 6 healthy controls.

Figure S11. Monocolonization of GF mice.

A. Colony forming units (CFUs) 2 days after the colonization with *A. calcoaceticus* (AC), *A. muciniphila* (AM) and *P. distasonis* (PD) after culturing fecal bacteria from monocolonized mice on Brucella BL plates anaerobically. **B.** Gel image indicating bands specific to *A. muciniphila (A. muci)* and *P. distasonis (P. dist)*.

Figure S12. Antibiotic-mediated reduction in alpha diversity of mouse microbial community.

Chao1 metric of alpha diversity in mice 2 weeks after treatment with broad-spectrum antibiotics (ABX) compared to SPF controls. n = 4 mice per group. Error bars, mean±SEM.

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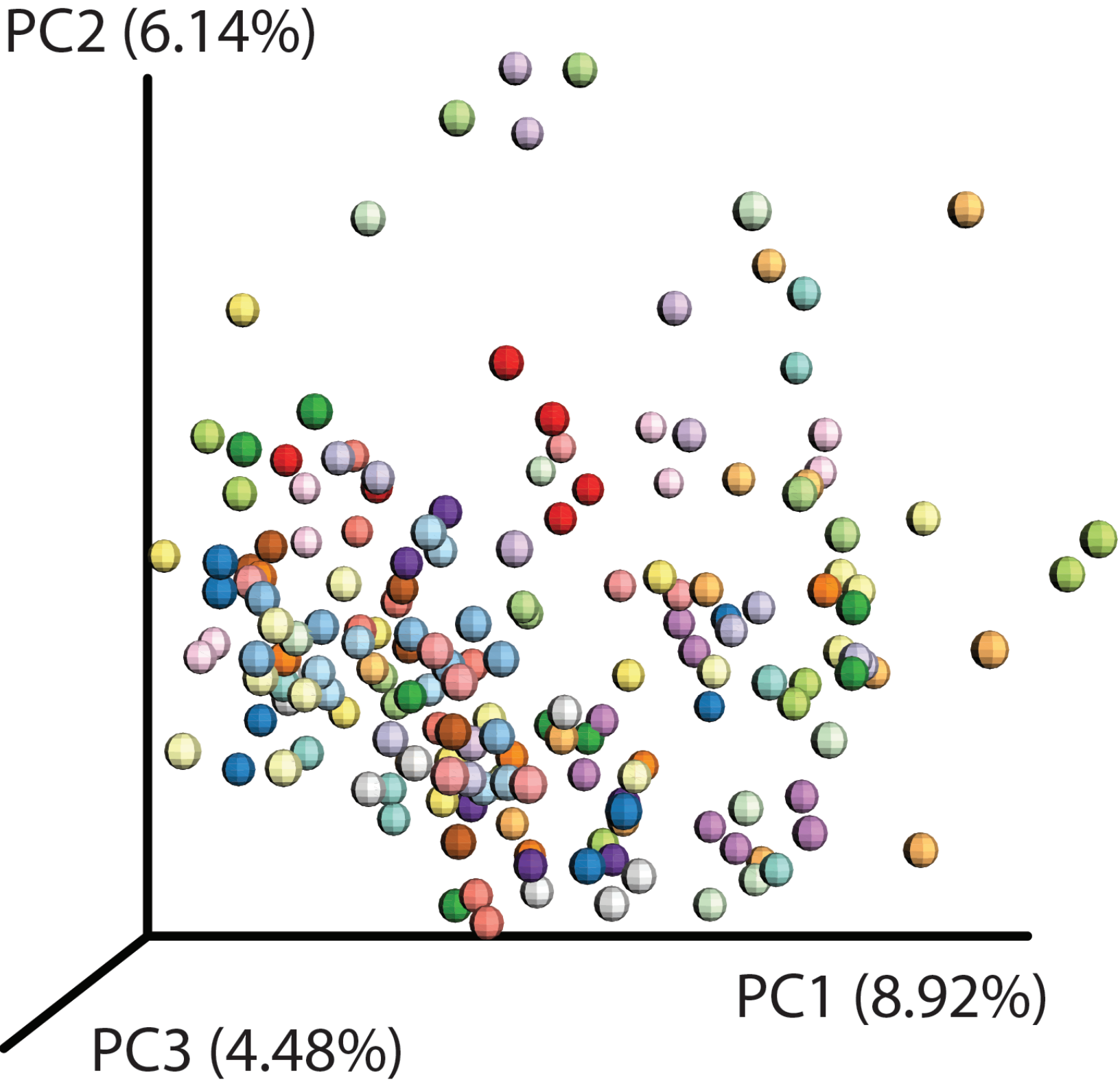
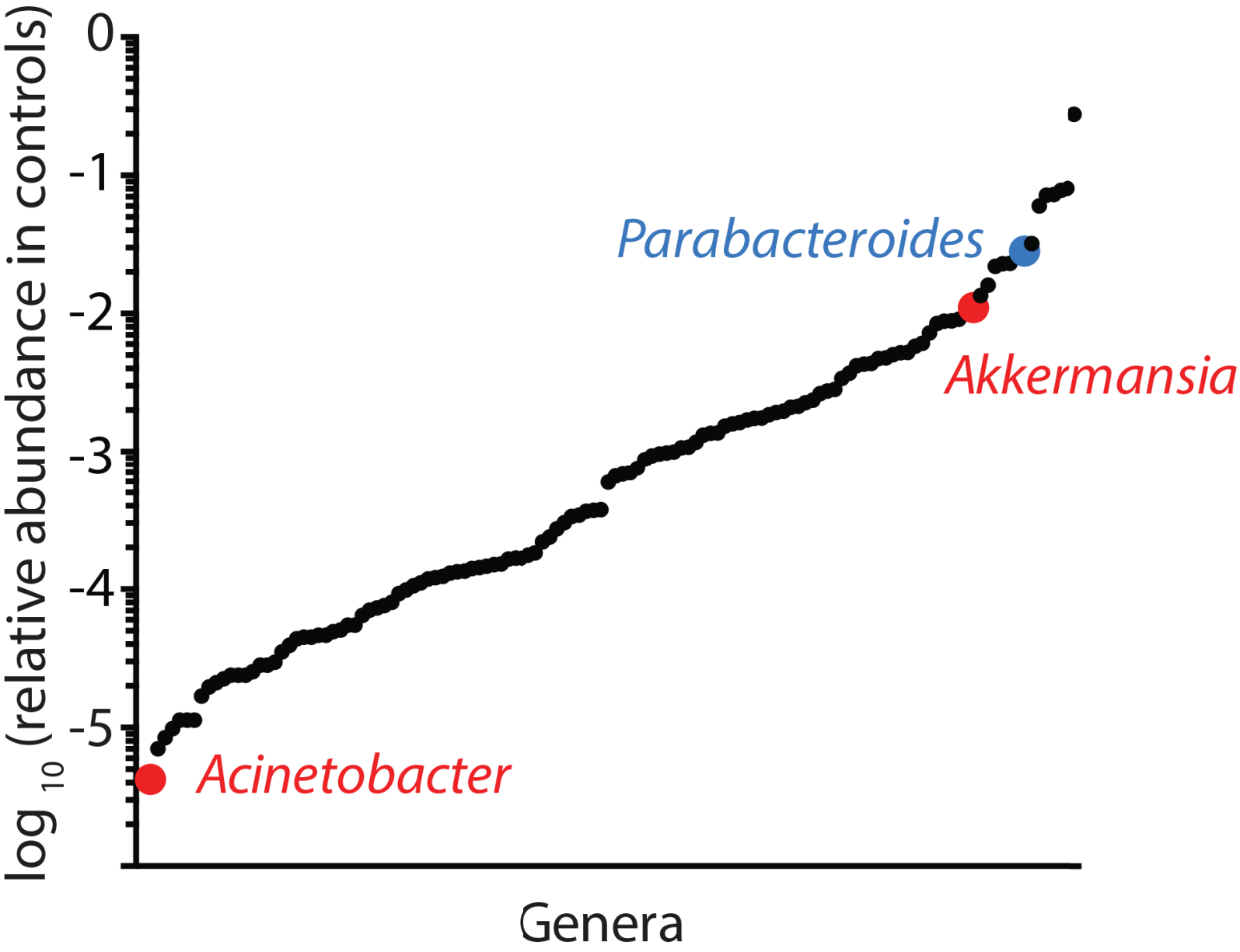
Supplementary Figures

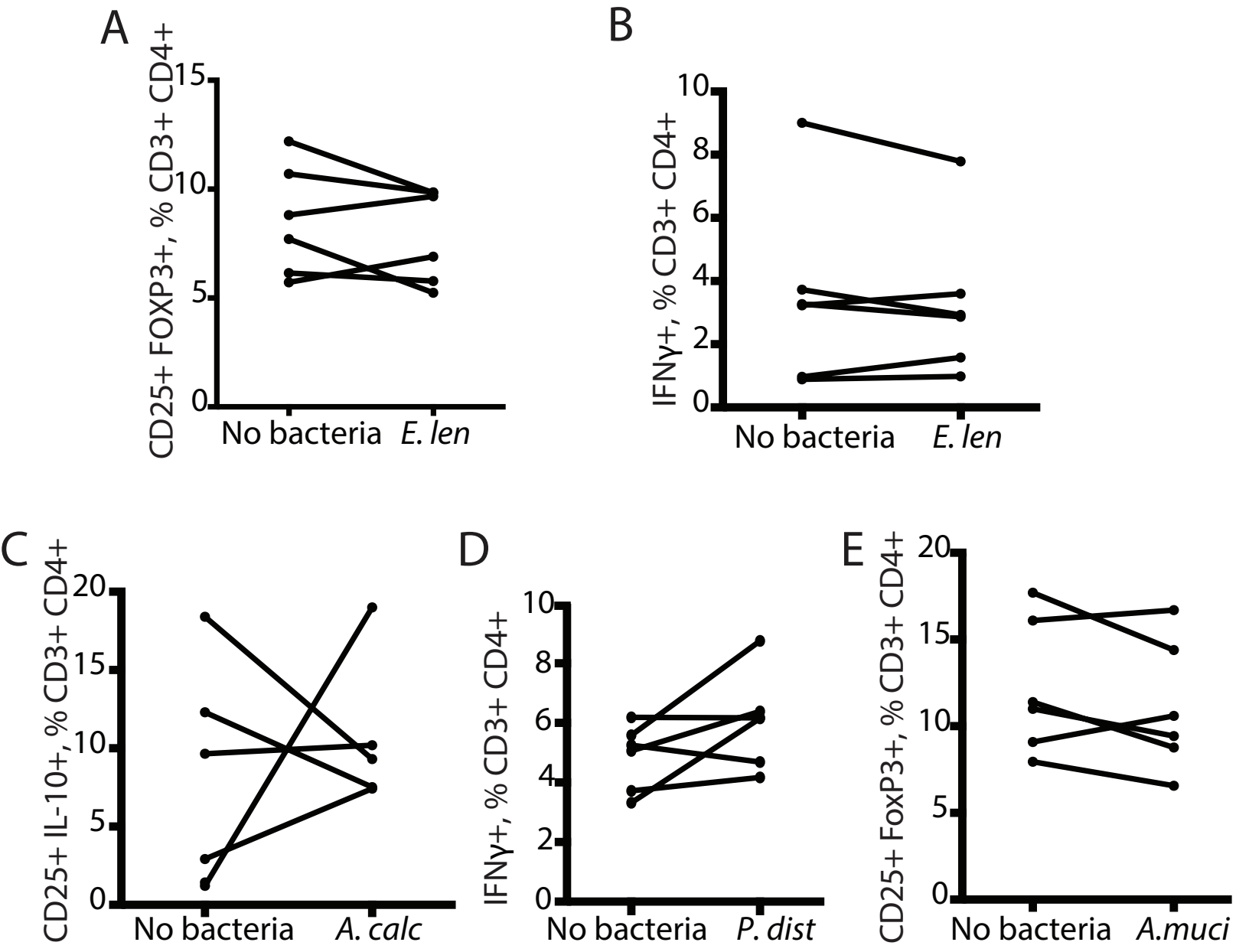
Supplementary Figure 1



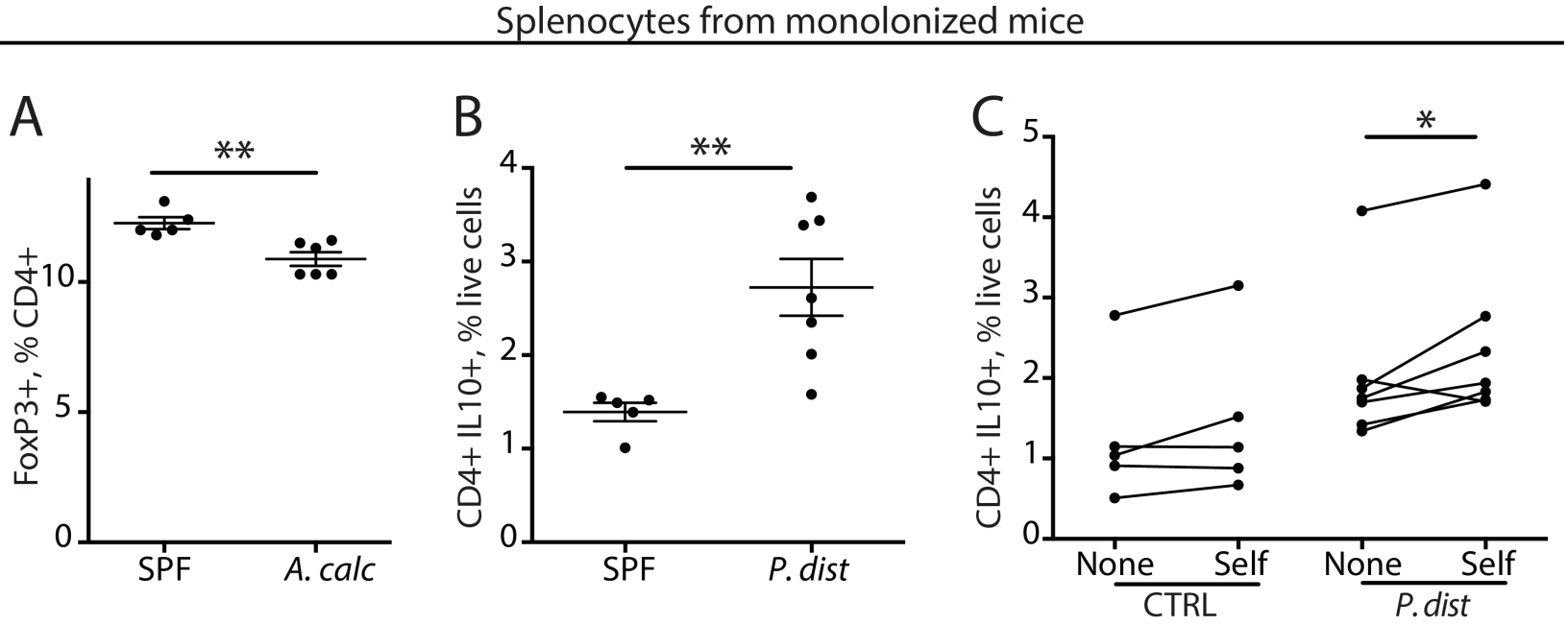
Supplementary Figure 2



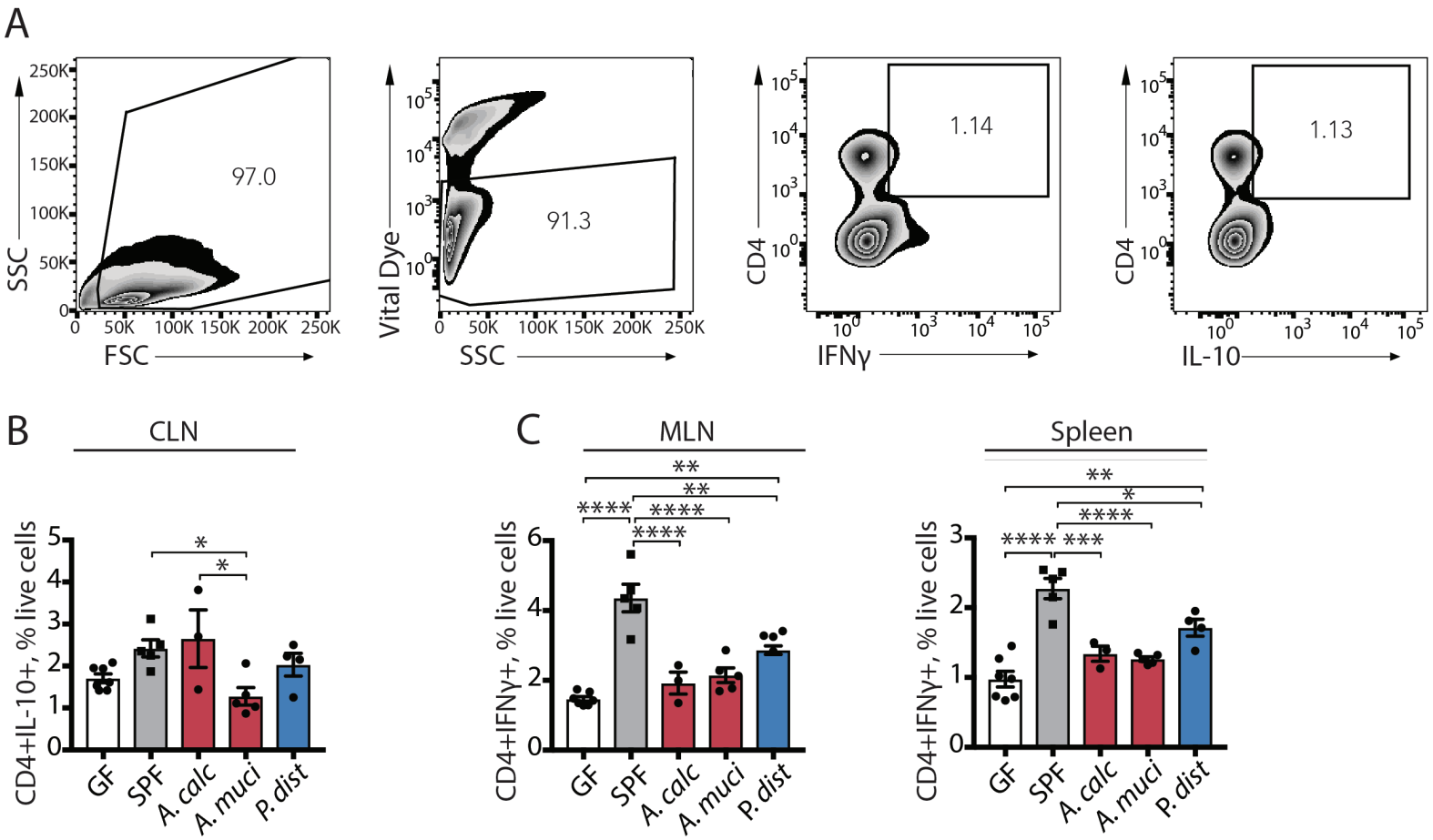
Supplementary Figure 3



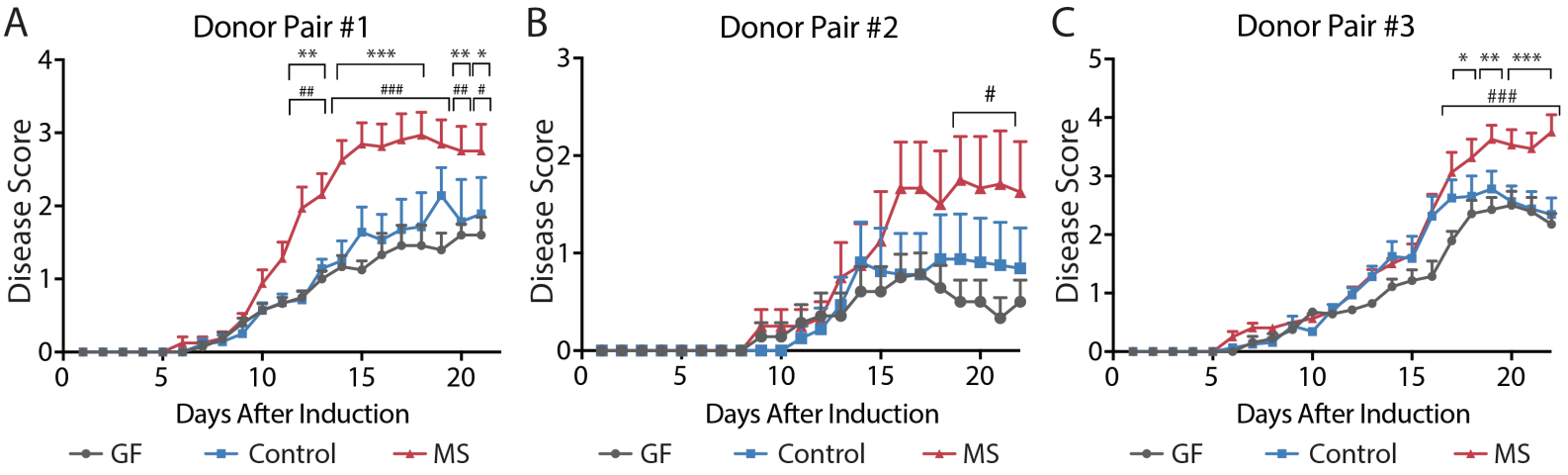
Supplementary Figure 4



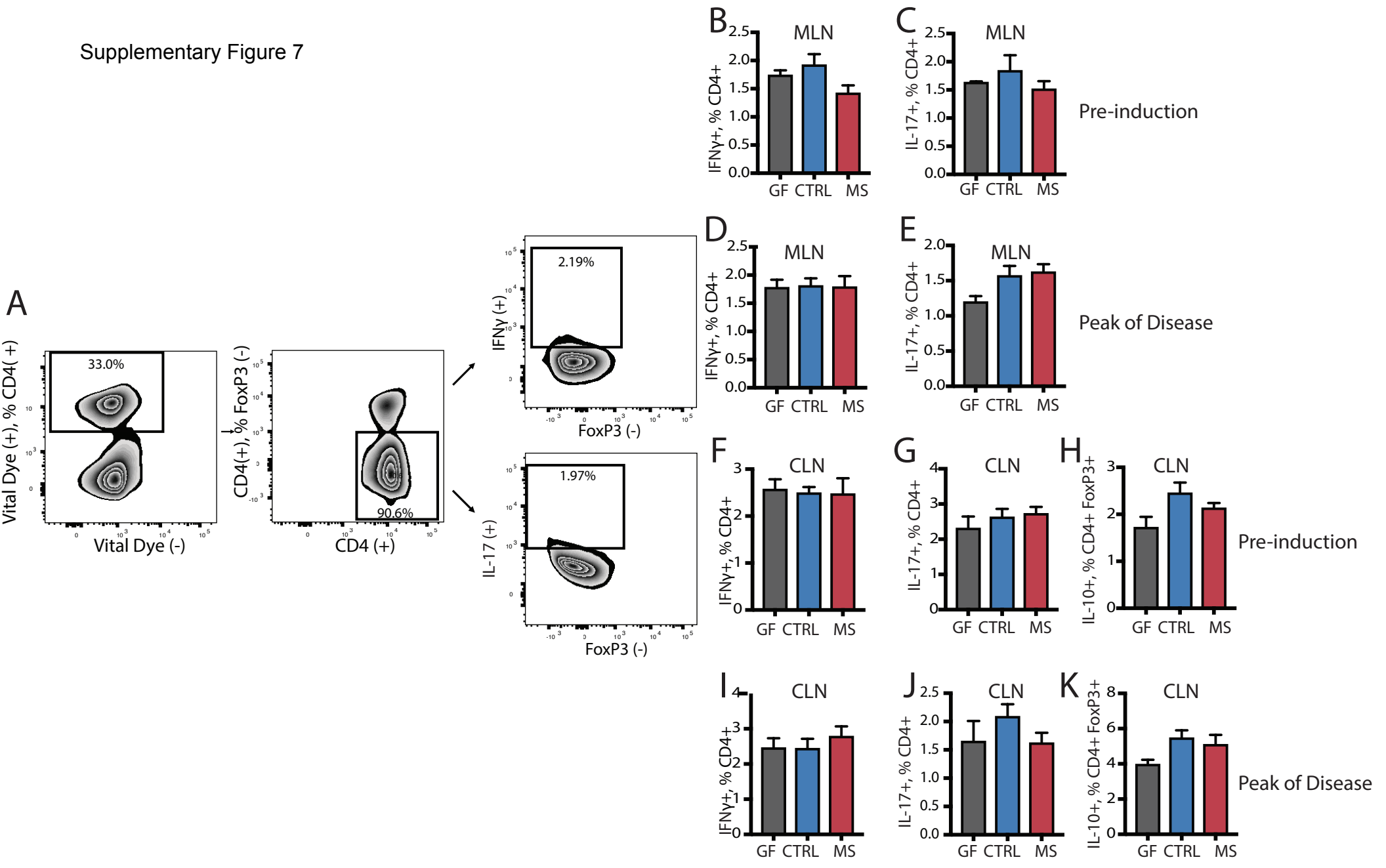
Supplementary Figure 5



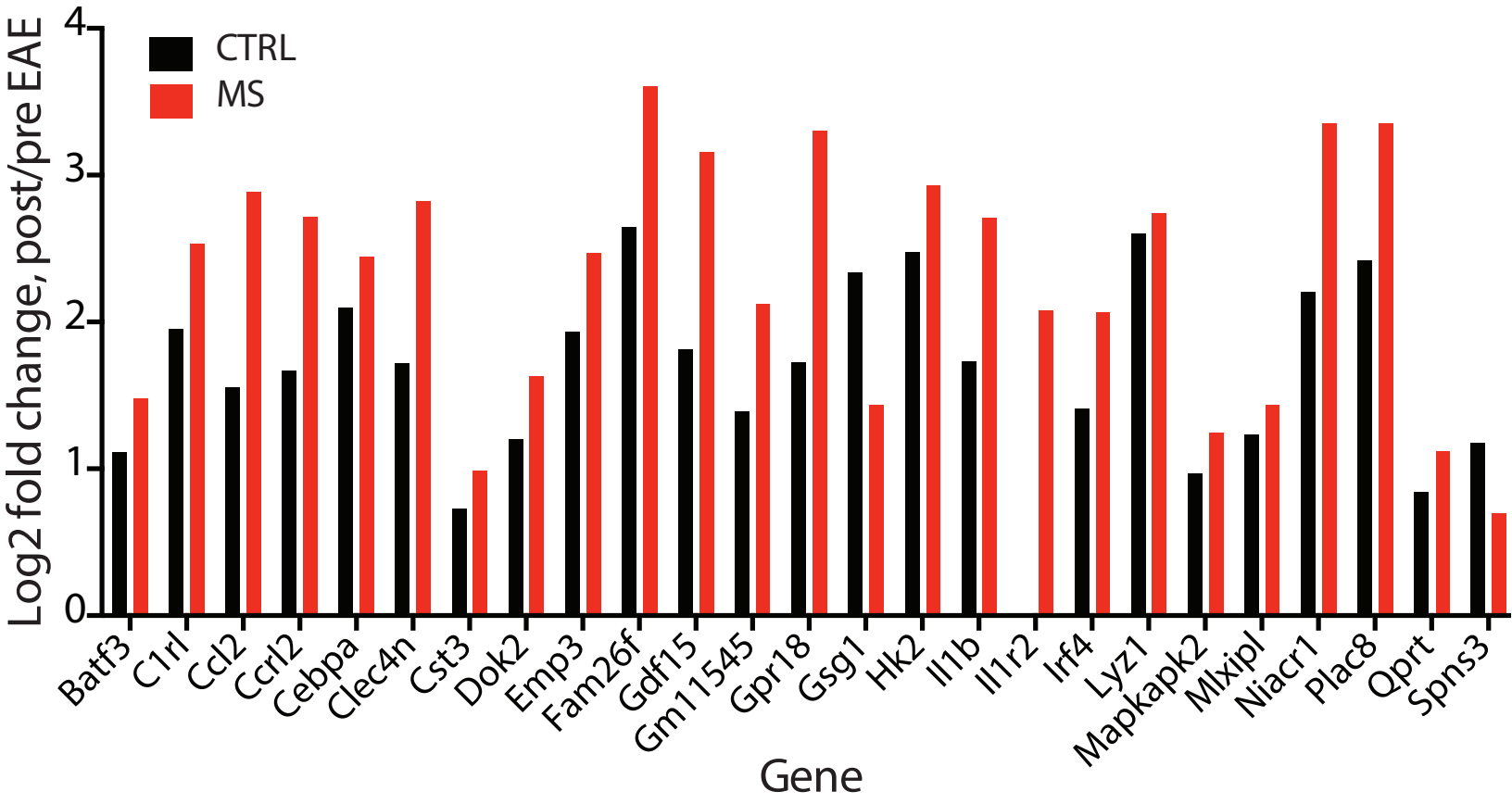
Supplementary Figure 6



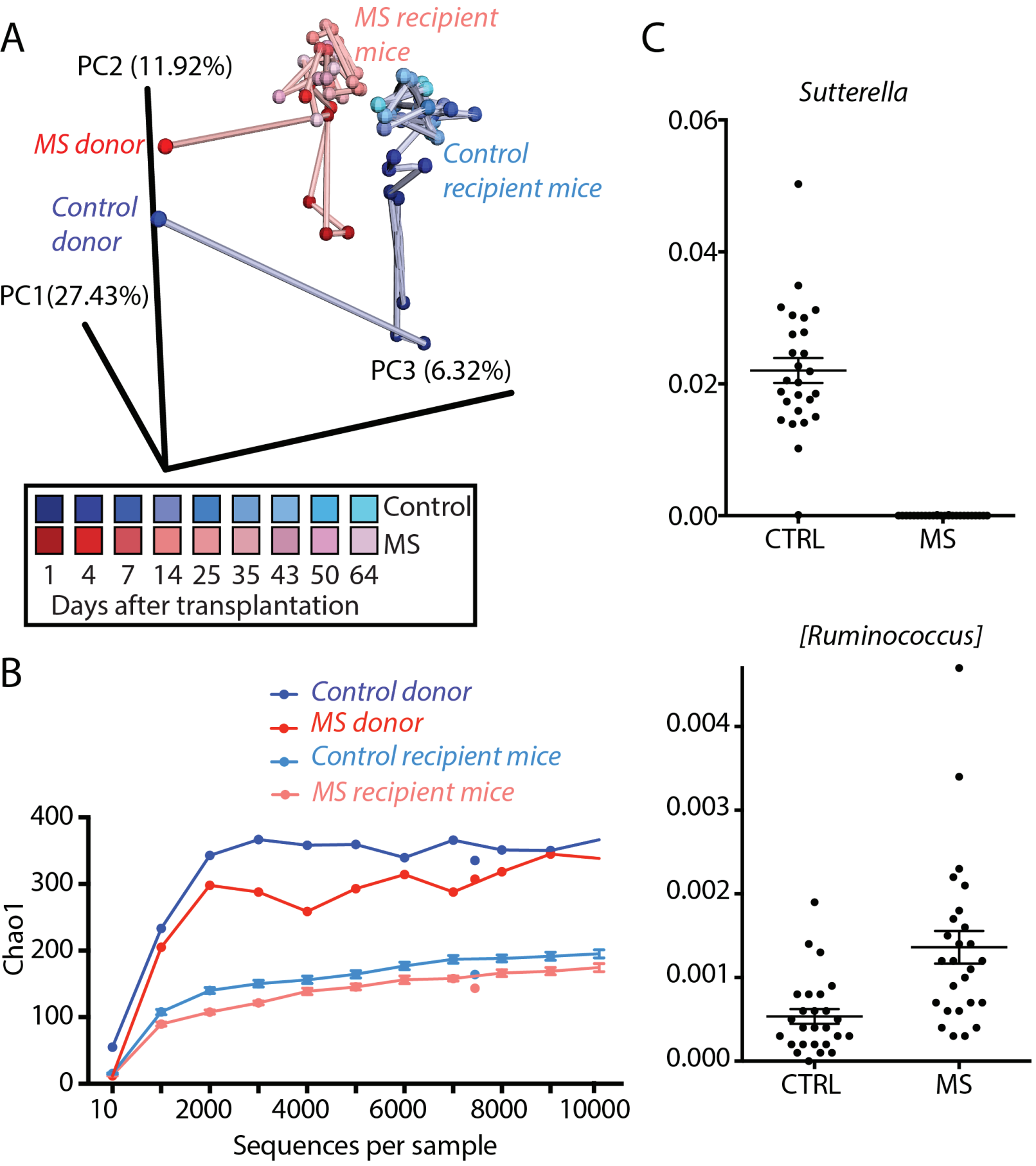
Supplementary Figure 7



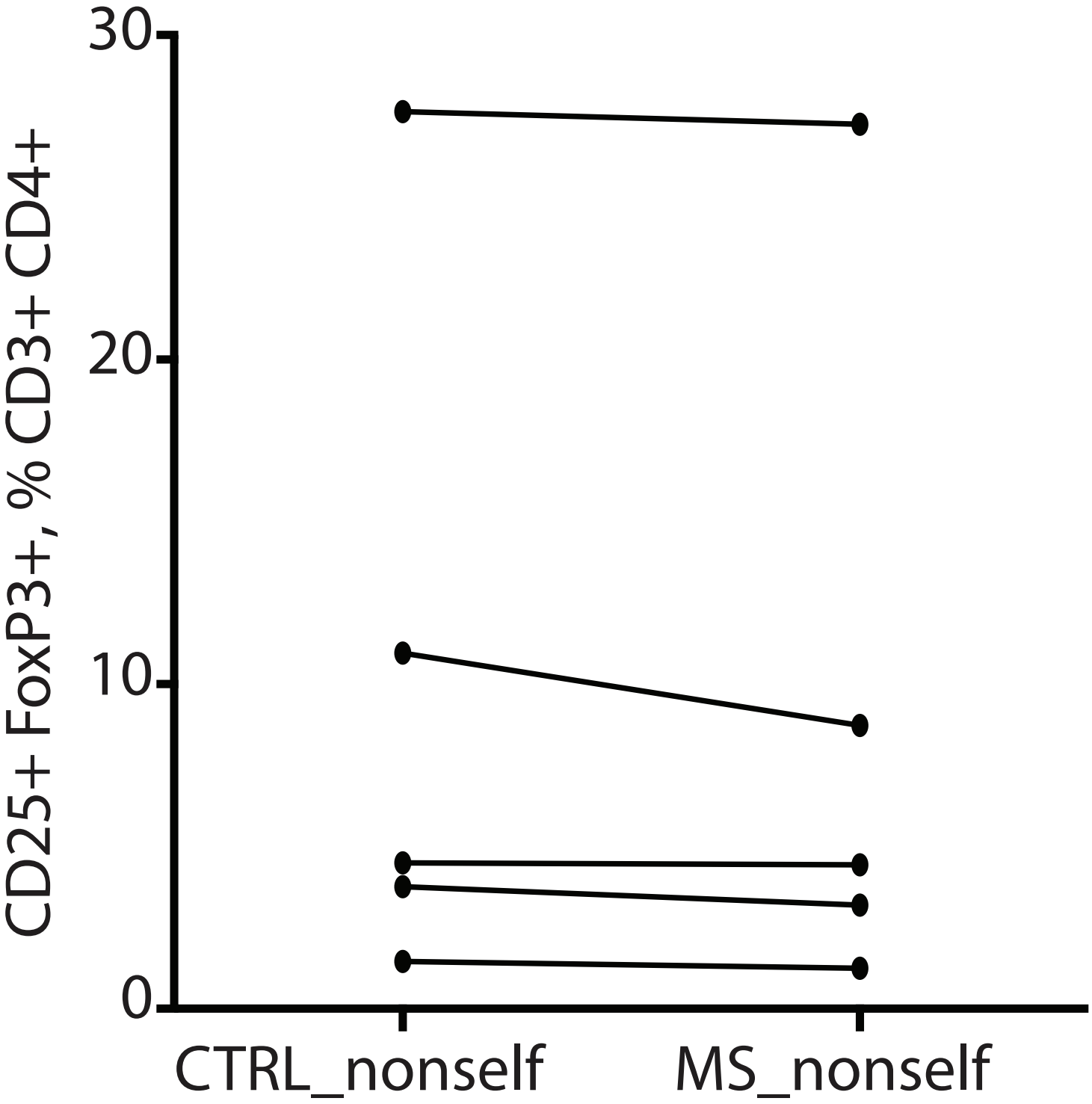
Supplementary Figure 8



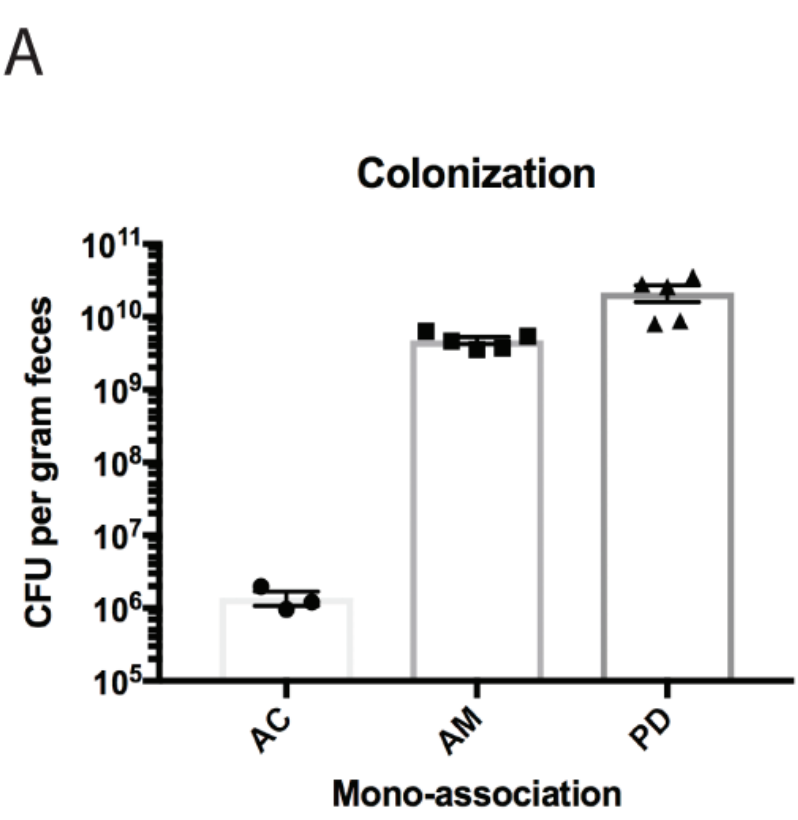
Supplementary Figure 9

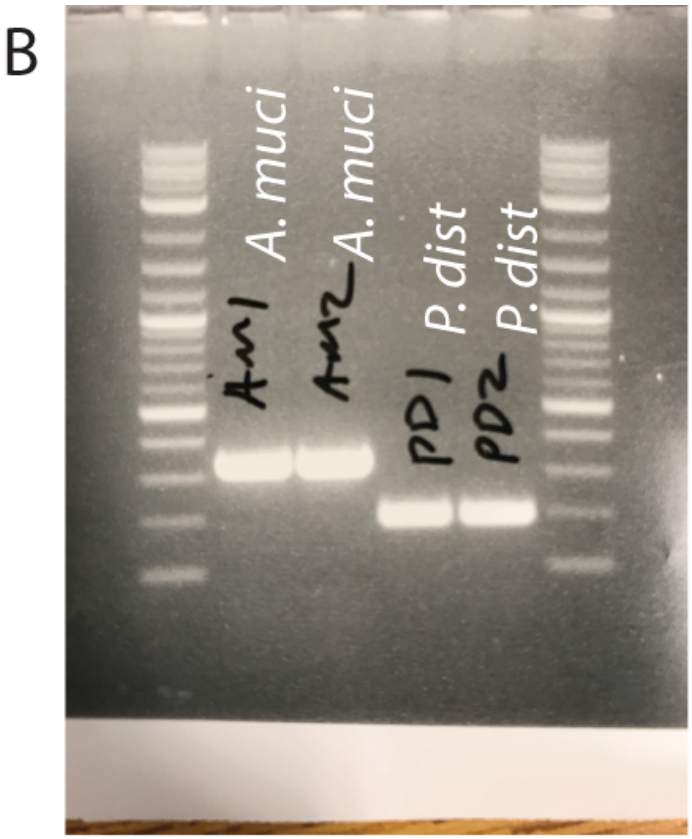


Supplementary Figure 10

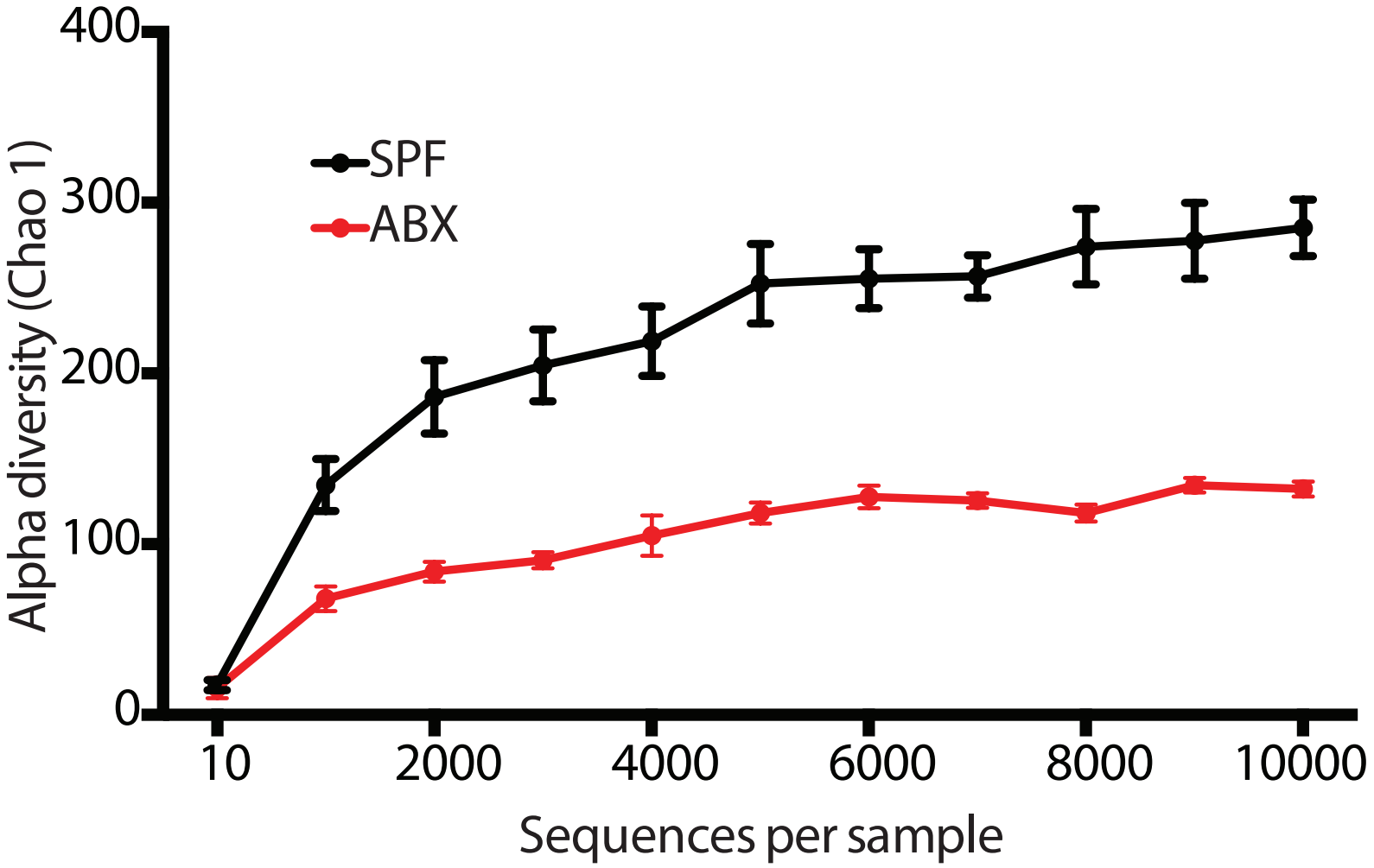


Supplementary Figure 11





Supplementary Figure 12



Supplementary Tables

Supplementary Table 1. Sample_metadata.

age	sex	collection_site	disease_state	treatment_status	year_onset	BMI
N/A	male	UCSF	Control	Control	Control	N/A
51	female	UCSF	Control	Control	Control	N/A
N/A	male	UCSF	Control	Control	Control	N/A
38	male	UCSF	Control	Control	Control	25.83
65	female	UCSF	Control	Control	Control	26.81
64	male	UCSF	Control	Control	Control	16.97
NA	NA	UCSF	Control	Control	Control	NA
62	male	UCSF	Control	Control	Control	NA
51	male	UCSF	Control	Control	Control	28.08
45	male	UCSF	Control	Control	Control	NA
39	male	UCSF	Control	Control	Control	28.8
63	female	UCSF	Control	Control	Control	NA
64	male	UCSF	Control	Control	Control	NA
45	female	UCSF	Control	Control	Control	NA
43	male	UCSF	Control	Control	Control	NA
NA	male	UCSF	Control	Control	Control	NA
58	male	UCSF	Control	Control	Control	25.84
62	male	UCSF	Control	Control	Control	NA
64	male	UCSF	Control	Control	Control	NA
59	male	UCSF	Control	Control	Control	NA
38	male	UCSF	Control	Control	Control	NA
NA	male	UCSF	Control	Control	Control	NA
43	male	UCSF	Control	Control	Control	NA
56	male	UCSF	Control	Control	Control	NA
38	male	UCSF	Control	Control	Control	NA
32	male	UCSF	Control	Control	Control	24.39
68	male	UCSF	Control	Control	Control	31.07
NA	NA	UCSF	Control	Control	Control	NA
53	female	UCSF	Control	Control	Control	29.1
64	female	UCSF	Control	Control	Control	20.24
59	female	UCSF	Control	Control	Control	NA
64	male	UCSF	Control	Control	Control	NA
NA	male	UCSF	Control	Control	Control	NA
61	female	UCSF	Control	Control	Control	29.83
71	male	UCSF	Control	Control	Control	NA
53	male	UCSF	Control	Control	Control	NA
36	female	UCSF	Control	Control	Control	NA
31	male	UCSF	Control	Control	Control	NA
25	male	UCSF	Control	Control	Control	NA
25	female	UCSF	Control	Control	Control	NA

22	male	UCSF	Control	Control	Control	NA
22	male	UCSF	Control	Control	Control	NA
39	male	UCSF	Control	Control	Control	NA
33	female	UCSF	Control	Control	Control	NA
29	male	UCSF	Control	Control	Control	NA
23	male	UCSF	Control	Control	Control	NA
29	male	UCSF	Control	Control	Control	NA
37	male	UCSF	Control	Control	Control	NA
NA	NA	UCSF	Control	Control	Control	NA
NA	NA	UCSF	Control	Control	Control	NA
43	male	UCSF	MS	No_Treatment	2000	21.5
51	female	UCSF	MS	No_Treatment	1998	25.8
48	male	UCSF	MS	No_Treatment	2001	24
55	female	UCSF	MS	No_Treatment	2002	29.7
38	female	UCSF	MS	No_Treatment	2002	18.9
60	female	UCSF	MS	No_Treatment	2001	18.7
57	female	UCSF	MS	No_Treatment	2001	19.6
43	female	UCSF	MS	No_Treatment	2015	47.1
28	male	UCSF	MS	No_Treatment	2013	23.5
45	male	UCSF	MS	No_Treatment	2008	28.2
31	male	UCSF	MS	No_Treatment	2015	27.6
24	male	UCSF	MS	No_Treatment	2011	24.6
36	male	UCSF	MS	No_Treatment	2013	25.1
29	male	UCSF	MS	No_Treatment	2016	20.9
33	female	UCSF	MS	No_Treatment	2016	20.9
44	male	UCSF	MS	No_Treatment		26.1
44	male	UCSF	MS	No_Treatment	1993	30.36
56	male	UCSF	MS	No_Treatment	1993	26.54
61	female	UCSF	MS	No_Treatment	1989	24.13
52	female	UCSF	MS	No_Treatment	1998	21.11
61	male	UCSF	MS	No_Treatment	1984	NA
38	female	UCSF	MS	No_Treatment	2004	NA
44	female	UCSF	MS	No_Treatment	1990	30.65
51	female	UCSF	MS	No_Treatment	1995	19.63
39	female	UCSF	MS	No_Treatment	2004	NA
57	female	UCSF	MS	No_Treatment	1996	17.72
37	female	UCSF	MS	No_Treatment	1998	22.87
64	female	UCSF	MS	No_Treatment	1983	21.19
42	female	UCSF	MS	No_Treatment	2003	NA
47	female	UCSF	MS	No_Treatment	2003	NA
59	male	UCSF	MS	No_Treatment	1997	NA
62	female	UCSF	MS	No_Treatment	1985	22.96

61	female	UCSF	MS	No_Treatment	1993	NA
37	female	UCSF	MS	No_Treatment	1999	NA
41	female	UCSF	MS	No_Treatment	1996	24.28
21	female	UCSF	MS	No_Treatment	2013	NA
41	female	UCSF	MS	No_Treatment	2011	21.61
21	female	UCSF	MS	No_Treatment	2005	NA
36	female	UCSF	MS	No_Treatment	2014	NA
32	female	UCSF	MS	No_Treatment	2013	NA
30	female	UCSF	MS	No_Treatment	2014	21.97
39	male	UCSF	MS	No_Treatment	2013	NA
38	male	UCSF	MS	No_Treatment	2011	19.37
26	female	UCSF	MS	No_Treatment	2013	NA
32	male	UCSF	MS	No_Treatment	2014	NA
45	female	UCSF	MS	No_Treatment	NA	NA
47	female	UCSF	MS	No_Treatment	2015	21.66
33	male	UCSF	MS	No_Treatment	2015	NA
37	female	UCSF	MS	No_Treatment	2015	NA
49	female	Sinai	Control	Control	Control	23
30	female	Sinai	Control	Control	Control	20.1
28	female	Sinai	Control	Control	Control	24.4
27	female	Sinai	Control	Control	Control	24
29	female	Sinai	Control	Control	Control	20.6
37	female	Sinai	Control	Control	Control	24.4
37	female	Sinai	Control	Control	Control	21.3
43	female	Sinai	Control	Control	Control	18.3
55	female	Sinai	Control	Control	Control	23.8
57	male	Sinai	Control	Control	Control	20.2
25	female	Sinai	Control	Control	Control	19.5
62	male	Sinai	Control	Control	Control	33.2
28	female	Sinai	Control	Control	Control	24.1
32	female	Sinai	Control	Control	Control	20.9
35	male	Sinai	Control	Control	Control	25
51	female	Sinai	Control	Control	Control	21.9
30	male	Sinai	Control	Control	Control	42.3
50	female	Sinai	Control	Control	Control	24.7
55	female	Sinai	Control	Control	Control	46.9
53	female	Sinai	Control	Control	Control	22.1
30	female	Sinai	Control	Control	Control	26.6
48	male	Sinai	MS	No_Treatment	1997	22.69
22	female	Sinai	MS	No_Treatment	2014	45
29	male	Sinai	MS	No_Treatment	2011	27.17
44	female	Sinai	MS	No_Treatment	2009	21.66

38	female	Sinai	MS	No_Treatment	2013	34.2
51	female	Sinai	MS	No_Treatment	2006	30.04
27	female	Sinai	MS	No_Treatment	2006	23.17
26	female	Sinai	MS	No_Treatment	2012	20.66
20	male	Sinai	MS	No_Treatment	2013	27.89
28	female	Sinai	MS	No_Treatment	2013	28.34
60	male	Sinai	MS	No_Treatment	2009	26
42	female	Sinai	MS	No_Treatment	2013	35.58
19	male	Sinai	MS	No_Treatment	2014	24.37
37	female	Sinai	MS	No_Treatment	2011	21.42
20	male	Sinai	MS	No_Treatment	2013	26.4
30	female	Sinai	MS	No_Treatment	2010	19.7
52	female	Sinai	MS	No_Treatment	2009	31.89
44	male	Sinai	MS	No_Treatment	2005	32.28
41	female	Sinai	MS	No_Treatment	2014	22.31
44	male	Sinai	MS	No_Treatment	2014	28
36	male	Sinai	MS	No_Treatment	2014	24.3
34	male	Sinai	MS	No_Treatment	2015	20.54

Supplementary Table 2. Differential abundance of microbial genera in MS patients and healthy controls.

	log2 fold change (MS/CTRL)	Adjusted p-value
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	1.97	9.15E-08
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Varibaculum	1.66	3.08E-05
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Corynebacteriaceae;g__Corynebacterium	1.64	3.08E-05
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae];g__	-1.47	3.08E-05
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	1.64	1.05E-04
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae];g__[Prevotella]	-1.25	2.23E-03
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megamonas	1.06	3.38E-03
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Serratia	-1.15	3.38E-03
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];g__	9.89	6.68E-03
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	-9.85	7.85E-03
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megasphaera	1.05	8.06E-03
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Klebsiella	1.29	8.50E-03
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__	1.10	1.35E-02
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Acidaminococcus	-1.02	2.79E-02
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Clostridium	-8.08	2.90E-02
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__ph2	8.52	2.94E-02
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces	8.16	2.94E-02
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];g__Mogibacterium	7.99	3.55E-02
k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Akkermansia	1.09	3.78E-02
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Aquamonas	-7.36	3.78E-02
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Parabacteroides	-8.56	3.78E-02
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Phascolarctobacterium	-1.10	3.78E-02
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__	-6.65	3.78E-02
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__SMB53	8.22	3.86E-02
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Bulleidia	8.84	3.86E-02

Supplementary Table 3. Results of differential abundance analysis of MS and control microbiota, OTU level, Wald test.

OTU	log2 fold change (MS/Control)	p value	FDR adjusted p value	taxonomy
851323	-2.709	7.08E-15	9.83E-12	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__
4443143	2.437	1.18E-12	6.77E-10	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__
3910247	-2.468	1.46E-12	6.77E-10	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]; s__
365385	2.299	1.40E-11	4.86E-09	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium; s__bifidum
2990918	-2.120	3.33E-11	9.23E-09	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__Collinsella; s__stercoris
4442459	-2.003	4.62E-11	1.07E-08	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__
437137	-2.082	1.37E-10	2.72E-08	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__Sutterella; s__
4306262	2.664	1.89E-10	3.29E-08	k__Bacteria; p__Verrucomicrobia; c__Verrucomicrobiae; o__Verrucomicrobiales; f__Verrucomicrobiaceae; g__Akkermansia; s__muciniphila
4435982	1.990	2.71E-10	3.90E-08	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__veronii
211720	-2.045	2.81E-10	3.90E-08	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__RF32; f__; g__; s__
4476065	-2.177	1.20E-09	1.51E-07	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__
184678	2.050	4.35E-09	5.04E-07	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii
197698	-1.650	6.77E-09	7.22E-07	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
197517	-1.652	1.17E-08	1.16E-06	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Barnesiellaceae]; g__; s__
1039584	1.858	1.41E-08	1.31E-06	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Tissierellaceae]; g__Anaerococcus; s__
2403301	2.076	2.00E-08	1.67E-06	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
520657	-1.857	2.04E-08	1.67E-06	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__stercorea
215097	-1.573	1.29E-07	9.98E-06	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__Sutterella; s__
172163	-1.585	1.39E-07	1.02E-05	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
198190	-1.634	2.34E-07	1.62E-05	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__distasonis
4305923	1.652	2.52E-07	1.65E-05	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__
182044	1.527	2.73E-07	1.65E-05	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
128300	-1.727	2.68E-07	1.65E-05	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__
187248	-1.532	3.48E-07	2.01E-05	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
328472	1.677	5.23E-07	2.79E-05	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Actinomycetaceae; g__Varibaculum; s__
4319785	-1.469	5.05E-07	2.79E-05	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__; s__
196381	1.437	5.87E-07	3.02E-05	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
296394	1.541	7.37E-07	3.65E-05	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4425368	1.470	7.69E-07	3.68E-05	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Megasphaera; s__
4404187	1.471	1.07E-06	4.92E-05	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
186233	-1.426	1.17E-06	4.92E-05	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__distasonis
178713	-1.552	1.10E-06	4.92E-05	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
190913	-1.738	1.15E-06	4.92E-05	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__
4428714	-1.619	1.74E-06	7.10E-05	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__
562244	-1.611	2.10E-06	8.34E-05	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__Sutterella; s__
703635	1.345	2.70E-06	1.04E-04	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Moraxellaceae; g__Acinetobacter; s__
1082607	1.340	3.71E-06	1.39E-04	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Corynebacteriaceae; g__Corynebacterium; s__
1028632	-1.275	3.89E-06	1.42E-04	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
345951	-1.249	4.10E-06	1.46E-04	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__

4339144	-1.594	4.54E-06	1.58E-04	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Odoribacteraceae]; g__Butyricimonas; s__
299770	-1.338	4.96E-06	1.68E-04	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__stercorea
195029	-1.278	5.16E-06	1.70E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
4227110	1.383	1.18E-05	3.48E-04	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Citrobacter; s__freundii
300829	1.287	1.08E-05	3.48E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__
587933	-1.258	1.25E-05	3.48E-04	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Coprobacillus; s__
847711	-1.274	1.19E-05	3.48E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__
4333943	-1.306	1.23E-05	3.48E-04	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__RF32; f__; g__; s__
354574	-1.522	1.11E-05	3.48E-04	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__Bilophila; s__
4365130	-1.581	1.23E-05	3.48E-04	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__distasonis
179159	-1.748	1.20E-05	3.48E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
2368865	-1.431	1.28E-05	3.48E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
4461030	-1.518	1.31E-05	3.50E-04	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
3531225	-1.222	1.35E-05	3.54E-04	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
4415390	-1.513	1.46E-05	3.76E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
4356080	-1.551	1.80E-05	4.54E-04	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Barnesiellaceae]; g__; s__
4426298	-1.317	1.99E-05	4.93E-04	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium; s__animalis
173810	-1.275	2.07E-05	5.05E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
319275	-1.295	2.28E-05	5.47E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii
4436046	-1.263	2.37E-05	5.57E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Dorea; s__
4299126	1.290	2.52E-05	5.81E-04	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__RF32; f__; g__; s__
4413619	1.153	2.64E-05	5.81E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
566243	-1.190	2.61E-05	5.81E-04	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
4442899	-1.614	2.57E-05	5.81E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
4449851	-1.687	3.52E-05	7.64E-04	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
363389	-1.130	3.68E-05	7.73E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__
524661	-1.206	3.62E-05	7.73E-04	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__
191251	-1.309	4.09E-05	8.47E-04	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__
4470870	1.679	4.21E-05	8.60E-04	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Barnesiellaceae]; g__; s__
554163	1.118	4.49E-05	9.02E-04	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
324882	1.052	4.79E-05	9.50E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__
326626	-1.203	4.92E-05	9.60E-04	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__
4402605	-1.336	4.98E-05	9.60E-04	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__
4378683	-1.109	5.41E-05	1.03E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
317315	-1.162	5.48E-05	1.03E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
367946	-1.149	5.86E-05	1.08E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
195998	0.974	7.19E-05	1.31E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
1071450	-1.172	1.02E-04	1.84E-03	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__fragi
4449244	-1.010	1.12E-04	2.00E-03	k__Bacteria; p__Fusobacteria; c__Fusobacteriia; o__Fusobacteriales; f__Fusobacteriaceae; g__Fusobacterium; s__
234443	1.073	1.15E-04	2.02E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__; s__
650171	1.301	1.26E-04	2.18E-03	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Corynebacteriaceae; g__Corynebacterium; s__
110192	-1.139	1.27E-04	2.18E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__
505565	1.183	1.33E-04	2.23E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Tissierellaceae]; g__Anaerococcus; s__
839282	-1.084	1.34E-04	2.23E-03	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Leuconostocaceae; g__; s__

216111	-1.123	1.38E-04	2.28E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
2749126	-0.977	1.43E-04	2.34E-03	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
7366	-1.164	1.46E-04	2.35E-03	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Oxalobacter; s__formigenes
361966	-1.284	1.50E-04	2.39E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii
4338624	-1.035	1.69E-04	2.67E-03	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__Sutterella; s__
210262	-1.092	1.80E-04	2.80E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
3235048	1.607	1.84E-04	2.82E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__
573061	-1.032	1.85E-04	2.82E-03	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__Desulfovibrio; s__
516553	-0.923	1.89E-04	2.85E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
348642	1.056	1.93E-04	2.88E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__gnavus
1097961	0.980	2.00E-04	2.95E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Porphyromonas; s__
289883	-1.048	2.07E-04	3.02E-03	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Bacillus; s__
9846	0.991	2.19E-04	3.17E-03	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Citrobacter; s__
194654	-0.957	2.28E-04	3.26E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
2017729	-0.916	2.38E-04	3.38E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
25695	-1.040	2.48E-04	3.47E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
188735	-1.149	2.73E-04	3.79E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__
4375000	-1.017	2.97E-04	4.09E-03	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
691423	-0.934	3.39E-04	4.56E-03	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
208479	-1.112	3.36E-04	4.56E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Odoribacteraceae]; g__Butyricimonas; s__
2530636	0.993	3.52E-04	4.69E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Megamonas; s__
187780	-1.222	3.67E-04	4.85E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
424038	0.957	3.80E-04	4.94E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Tissierellaceae]; g__Anaerococcus; s__
188333	-0.901	3.81E-04	4.94E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__gnavus
308760	-1.145	3.91E-04	5.02E-03	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__
180414	-1.050	3.97E-04	5.06E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__
4020502	-0.873	4.03E-04	5.09E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__
184534	-1.048	4.12E-04	5.15E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__
176062	1.313	4.36E-04	5.26E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
4428313	-0.917	4.34E-04	5.26E-03	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__
1599042	-0.980	4.31E-04	5.26E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4402645	-1.099	4.26E-04	5.26E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
195556	-0.997	4.57E-04	5.46E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
730906	-1.059	4.60E-04	5.46E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
178759	-0.978	4.80E-04	5.60E-03	k__Bacteria; p__Tenericutes; c__RF3; o__ML615J-28; f__; g__; s__
178845	-1.256	4.79E-04	5.60E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
153075	0.896	4.88E-04	5.65E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
179989	0.898	6.17E-04	7.08E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__
360890	1.102	6.25E-04	7.11E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
187385	0.833	6.36E-04	7.18E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__
232030	-0.913	6.65E-04	7.45E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__
4434579	1.329	6.82E-04	7.58E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
2730944	-0.928	7.09E-04	7.81E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__coprophilus
4473788	-0.984	8.00E-04	8.69E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__

366147	-1.073	8.03E-04	8.69E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__Paraprevotella; s__
2740950	-1.243	8.08E-04	8.69E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__
4476877	1.088	8.28E-04	8.72E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Odoribacteraceae]; g__Odoribacter; s__
178859	0.861	8.18E-04	8.72E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__
4374084	-0.754	8.32E-04	8.72E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__distasonis
1566691	-0.920	8.35E-04	8.72E-03	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
4347159	1.205	8.59E-04	8.89E-03	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium; s__adolescentis
269937	-1.088	8.99E-04	9.24E-03	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__stercorea
191913	-0.872	9.55E-04	9.70E-03	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
810831	-1.010	9.57E-04	9.70E-03	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
329820	0.946	9.68E-04	9.74E-03	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__[Eubacterium]; s__biforme
329096	-0.790	9.79E-04	9.77E-03	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
842596	0.832	1.03E-03	1.02E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__
216010	0.821	1.09E-03	1.06E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__
185558	0.814	1.08E-03	1.06E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
13986	-0.857	1.09E-03	1.06E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
4476780	1.161	1.21E-03	1.16E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__; s__
4318470	-0.760	1.21E-03	1.16E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__
2835813	-0.943	1.46E-03	1.38E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
188881	0.867	1.51E-03	1.43E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__
4471245	-1.074	1.56E-03	1.45E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Dorea; s__
297414	-1.110	1.55E-03	1.45E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__copri
4457872	-1.089	1.59E-03	1.47E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__
552376	0.816	1.61E-03	1.47E-02	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Enterobacter; s__
4473883	-0.876	1.61E-03	1.47E-02	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__alactolyticus
325850	1.122	1.66E-03	1.50E-02	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__RF32; f__; g__; s__
146586	-0.906	1.67E-03	1.50E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__
4381553	-1.097	1.68E-03	1.51E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__
2751958	0.892	1.75E-03	1.55E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Tissierellaceae]; g__Anaerococcus; s__
180121	-0.833	1.74E-03	1.55E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4379449	-0.836	1.77E-03	1.56E-02	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Clostridium; s__saccharogumia
2957436	-0.775	1.80E-03	1.56E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Dorea; s__
174901	-0.823	1.80E-03	1.56E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
163243	-1.159	1.81E-03	1.56E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__
214331	0.807	1.84E-03	1.58E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__Mogibacterium; s__
194626	-0.739	1.85E-03	1.58E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4448331	-0.851	1.86E-03	1.58E-02	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
4393466	0.944	1.96E-03	1.65E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__SMB53; s__
4474593	-0.741	1.99E-03	1.65E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__fragilis
294246	-0.900	1.99E-03	1.65E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4476604	-1.118	2.02E-03	1.67E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__gnavus
320915	-0.789	2.05E-03	1.69E-02	k__Bacteria; p__Cyanobacteria; c__4C0d-2; o__YS2; f__; g__; s__
2814830	-0.755	2.08E-03	1.70E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4353913	-0.677	2.10E-03	1.71E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__

13994	0.722	2.12E-03	1.71E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
323135	-1.169	2.18E-03	1.75E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__
175761	1.227	2.20E-03	1.75E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4439487	-0.863	2.24E-03	1.78E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4458306	0.870	2.28E-03	1.79E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Veillonella; s__dispar
218710	0.738	2.28E-03	1.79E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
182073	-1.051	2.31E-03	1.80E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
3090117	-0.834	2.38E-03	1.85E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Barnesiellaceae]; g__; s__
4396298	-0.884	2.46E-03	1.90E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
190864	-0.728	2.49E-03	1.91E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
358639	0.735	2.55E-03	1.94E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4447188	-1.000	2.67E-03	2.02E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__uniformis
1028501	0.807	2.69E-03	2.03E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Dialister; s__
296052	0.730	2.74E-03	2.05E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
4336939	-0.839	2.73E-03	2.05E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
514257	0.954	2.77E-03	2.05E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
10180	-0.775	2.78E-03	2.05E-02	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Aquamonas; s__haywardensis
510295	0.762	2.84E-03	2.09E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
125624	-1.032	2.87E-03	2.10E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
198221	0.817	2.99E-03	2.17E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
544996	-0.900	3.00E-03	2.17E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__
120281	-1.063	3.04E-03	2.19E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__producta
199490	-0.852	3.08E-03	2.20E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
593868	-0.904	3.10E-03	2.21E-02	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__
4349519	0.832	3.17E-03	2.25E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Tissierellaceae]; g__Anaerococcus; s__
215468	0.757	3.28E-03	2.31E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
563572	0.707	3.35E-03	2.35E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
111135	-0.764	3.43E-03	2.39E-02	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__Sutterella; s__
196742	0.745	3.49E-03	2.42E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
311950	-1.030	3.53E-03	2.44E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__plebeius
4473763	-0.722	3.59E-03	2.47E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__fragilis
187468	0.668	3.65E-03	2.49E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__
232828	-0.717	3.74E-03	2.54E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
176306	-0.922	3.77E-03	2.55E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
179663	-0.766	3.80E-03	2.56E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4477861	-1.078	3.97E-03	2.66E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__
4378081	-0.950	4.01E-03	2.68E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__
332241	0.778	4.13E-03	2.74E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4419459	1.001	4.32E-03	2.85E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
728119	-0.936	4.33E-03	2.85E-02	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
4454586	-0.959	4.44E-03	2.91E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Odoribacteraceae]; g__Odoribacter; s__
851634	0.808	4.47E-03	2.91E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
561607	-0.769	4.48E-03	2.91E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
180352	-0.656	4.54E-03	2.92E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__

192983	-0.792	4.55E-03	2.92E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
1102370	0.986	4.61E-03	2.95E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
176726	0.695	4.69E-03	2.98E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
4413347	0.659	4.84E-03	3.07E-02	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium; s__
194297	1.088	4.91E-03	3.09E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__
4409730	0.958	4.95E-03	3.11E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptostreptococcaceae; g__; s__
2829179	-0.948	4.97E-03	3.11E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Acidaminococcus; s__
195015	-0.637	5.13E-03	3.19E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospira; s__
193763	-0.700	5.29E-03	3.28E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
364289	0.861	5.64E-03	3.46E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptococcaceae; g__rc4-4; s__
585914	-0.607	5.63E-03	3.46E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__distasonis
809658	0.793	5.84E-03	3.57E-02	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__Desulfovibrio; s__
195186	0.696	5.91E-03	3.60E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__
134265	-0.831	6.18E-03	3.75E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
797229	-0.815	6.39E-03	3.85E-02	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
185141	0.612	6.53E-03	3.92E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
4425669	-0.965	6.65E-03	3.98E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__
262095	-0.684	6.77E-03	4.03E-02	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__; s__
175180	0.842	6.89E-03	4.09E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
157424	-0.764	7.00E-03	4.14E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__; s__
292921	-0.796	7.06E-03	4.15E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__copri
281015	0.623	7.18E-03	4.20E-02	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
158771	-0.661	7.37E-03	4.28E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
192210	-0.677	7.36E-03	4.28E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
362793	0.701	7.44E-03	4.30E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__
721569	-0.703	7.48E-03	4.31E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
182483	-0.989	7.52E-03	4.32E-02	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__[Eubacterium]; s__biforme
175844	-0.894	7.92E-03	4.52E-02	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Barnesiellaceae]; g__; s__
174840	-0.790	8.07E-03	4.59E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
259604	0.650	8.17E-03	4.63E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Tissierellaceae]; g__WAL_1855D; s__
357930	0.802	8.54E-03	4.82E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Dialister; s__
145401	0.616	8.72E-03	4.90E-02	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__

Supplementary Table S4. Top pathways that separate MS and control samples in the human dataset and at least 1 mouse dataset.

Pathway	Human	Mouse 1	Mouse 2
Cellular Processes;Cell Motility;Bacterial chemotaxis			
Cellular Processes;Cell Motility;Flagellar assembly			
Cellular Processes;Transport and Catabolism;Lysosome			
Environmental Information Processing;Membrane Transport;ABC transporters			
Metabolism;Amino Acid Metabolism;Histidine metabolism			
Metabolism;Biosynthesis of Other Secondary Metabolites;Streptomycin biosynthesis			
Metabolism;Carbohydrate Metabolism;Fructose and mannose metabolism			
Metabolism;Carbohydrate Metabolism;Starch and sucrose metabolism			
Metabolism;Enzyme Families;Peptidases			
Metabolism;Glycan Biosynthesis and Metabolism;Lipopolysaccharide biosynthesis proteins			
Metabolism;Lipid Metabolism;Sphingolipid metabolism			
Metabolism;Metabolism of Terpenoids and Polyketides;Polyketide sugar unit biosynthesis			
Unclassified;Metabolism;Carbohydrate metabolism			

Supplementary Table S5. Top pathways that separate MS and control samples in the human dataset and at least 1 mouse dataset, calculated only the differentially expressed OTUs.

Pathway	Human	Mouse 1	Mouse 2
Cellular Processes;Transport and Catabolism;Lysosome			
Environmental Information Processing;Membrane Transport;Transporters			
Metabolism;Biosynthesis of Other Secondary Metabolites;Streptomycin biosynthesis			
Metabolism;Carbohydrate Metabolism;Fructose and mannose metabolism			
Metabolism;Carbohydrate Metabolism;Pentose phosphate pathway			
Metabolism;Glycan Biosynthesis and Metabolism;Other glycan degradation			
Metabolism;Metabolism of Terpenoids and Polyketides;Polyketide sugar unit biosynthesis			
Metabolism;Xenobiotics Biodegradation and Metabolism;Chloroalkane and chloroalkene degradation			
Metabolism;Xenobiotics Biodegradation and Metabolism;Drug metabolism - other enzymes			
Unclassified;Cellular Processes and Signaling;Other ion-coupled transporters			
Unclassified;Cellular Processes and Signaling;Pores ion channels			
Unclassified;Cellular Processes and Signaling;Sporulation			
Unclassified;Genetic Information Processing;Restriction enzyme			
Unclassified;Metabolism;Energy metabolism			
Unclassified;Metabolism;Others			
Unclassified;Poorly Characterized;Function unknown			