SUPPLEMENTARY MATERIAL

The Gut Microbiota Modulates Innate Immunity and Lymph Node Remodeling, And Affects Cardiac Allograft Inflammation, Fibrosis, and Survival

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Supplementary Methods

Murine Intestinal Gene Expression

Total RNA Extraction from Gastro-Intestinal Tissues and Associated Microbiotas. On day of graft rejection or other endpoint, mice were euthanized via CO2 inhalation followed by cervical dislocation. Mouse large intestine, cecum, and small intestine were collected in RNALater (Ambion) and stored at 4°C overnight before processing. Total RNA was then extracted from tissue samples using an acid-phenol protocol. Briefly, cell lysis was achieved using bead-beating (FastPrep instrument with 0.1 mm silica spheres) in the presence of acid phenol. After chloroform extraction, total RNA was precipitated using isopropanol and resuspended in DEPC-treated water. After DNase treatment to remove any residual DNA, total RNA was further processed using the RNEasy kit (Qiagen).

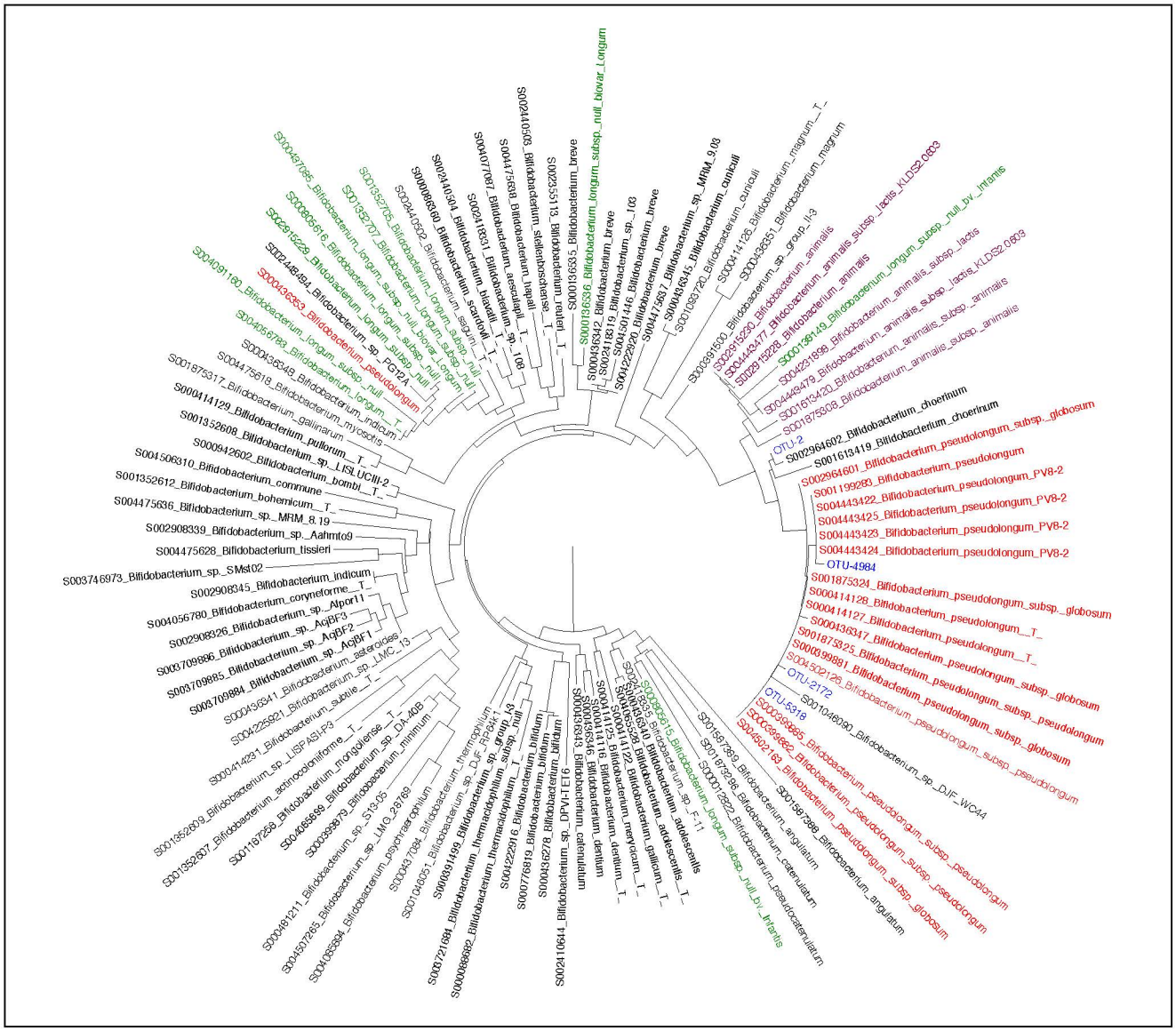
rRNA Depletion, mRNA Amplification and Library Preparation for Metatranscriptomic Sequencing using Illumina RNASeq. Ribosomal RNA depletion from the total RNA samples was performed using the Ribo-Zero Meta-Bacteria (Gram-negative and Gram- positive) rRNA Removal Kit and the Ribo-Zero rRNA Removal Kit (Human/Mouse/Rat) from Illumina, according to the manufacturer's recommendations. Following rRNA depletion, RNAseq libraries were constructed using the Nextera XT DNA Library Preparation Kit (Illumina). A total of 12 RNA samples were multiplexed and sequenced on a single lane of Illumina HiSeq 4000 at the IGS Genome Resource Center, generating on average 13.89 Gbp and 92 million reads (read length: 150-bp) per sample.

RNASeq Reads Processing and Analyses. Reads were initially pre-processed by removing low-quality reads (mean quality value of less than 25), trimming low-quality positions (quality less than 15), removing reads less than 90 nucleotides in length using FastqMcf and removing reads from the Illumina spike-in (bacteriophage Phi-X174). Ribosomal RNAs were also removed by mapping against the SILVA 16S and 23S rRNA gene databases and to remove contaminant adapters using trim_galore (http://www.bioinformatics.babraham.ac.uk/ projects/trim_galore/). To characterize the murine intestinal

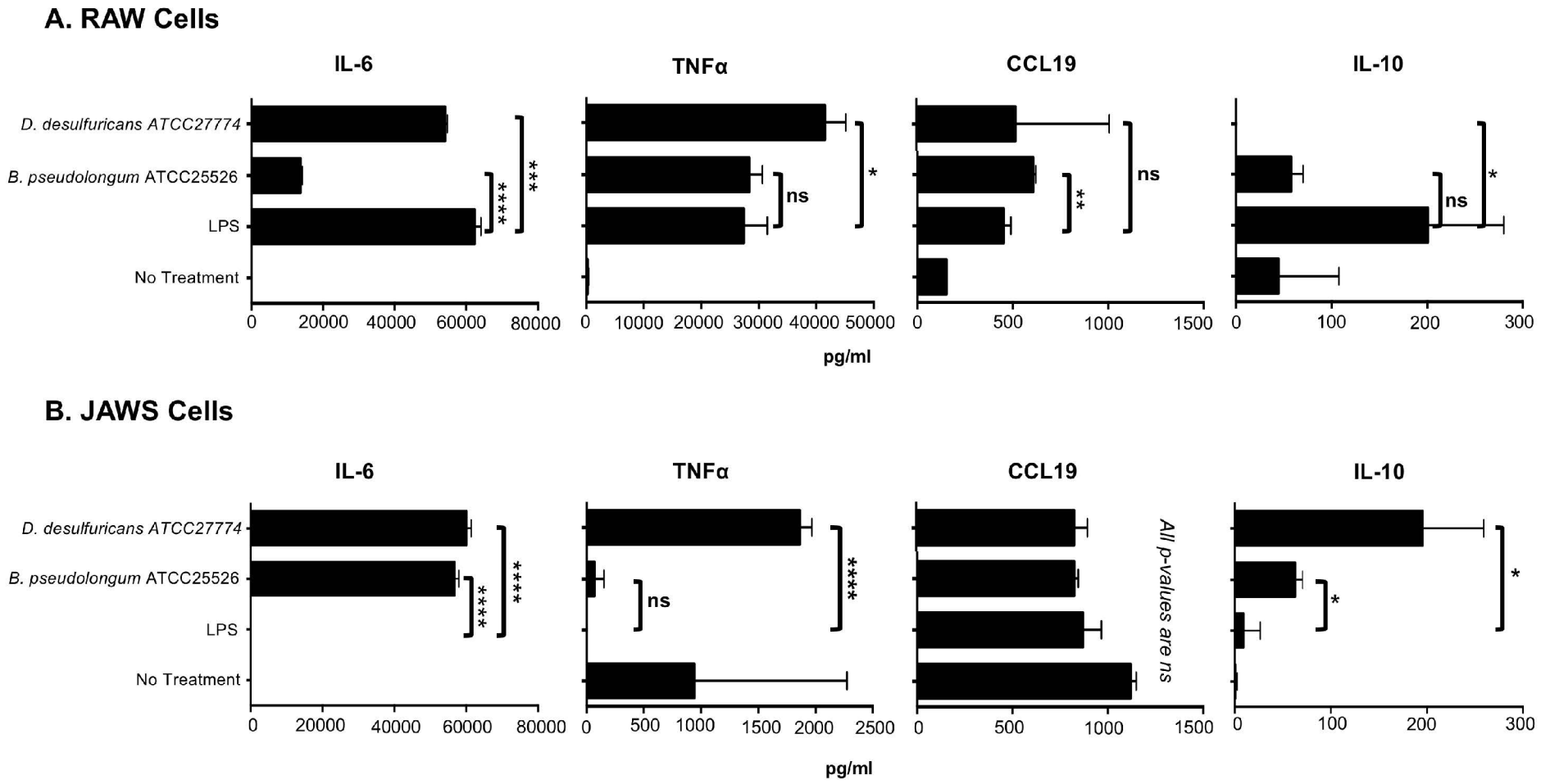
gene expression profile, alignment of the filtered sequencing dataset was performed against the mouse genome (release 91, downloaded from Ensembl [1]) using HISAT2 [2] after which the python wrapper script "htseq-count" was used to perform gene-level counts. The resulting count files (one for each samples) were then merged, and the combined file was then imported into the PIVOT (Platform for Interactive Analysis and Visualization of Transcriptomics Data ; v1.0.0) program [3]. Within the PIVOT platform, the dataset was normalized using DeSeq2 [4], low-expressing genes (<5 reads) were filtered out, and differentially-expressed transcripts identified using edgeR [5] with a False Discovery Rate (FDR) cutoff of 0.1.

Supplementary Figures & Tables

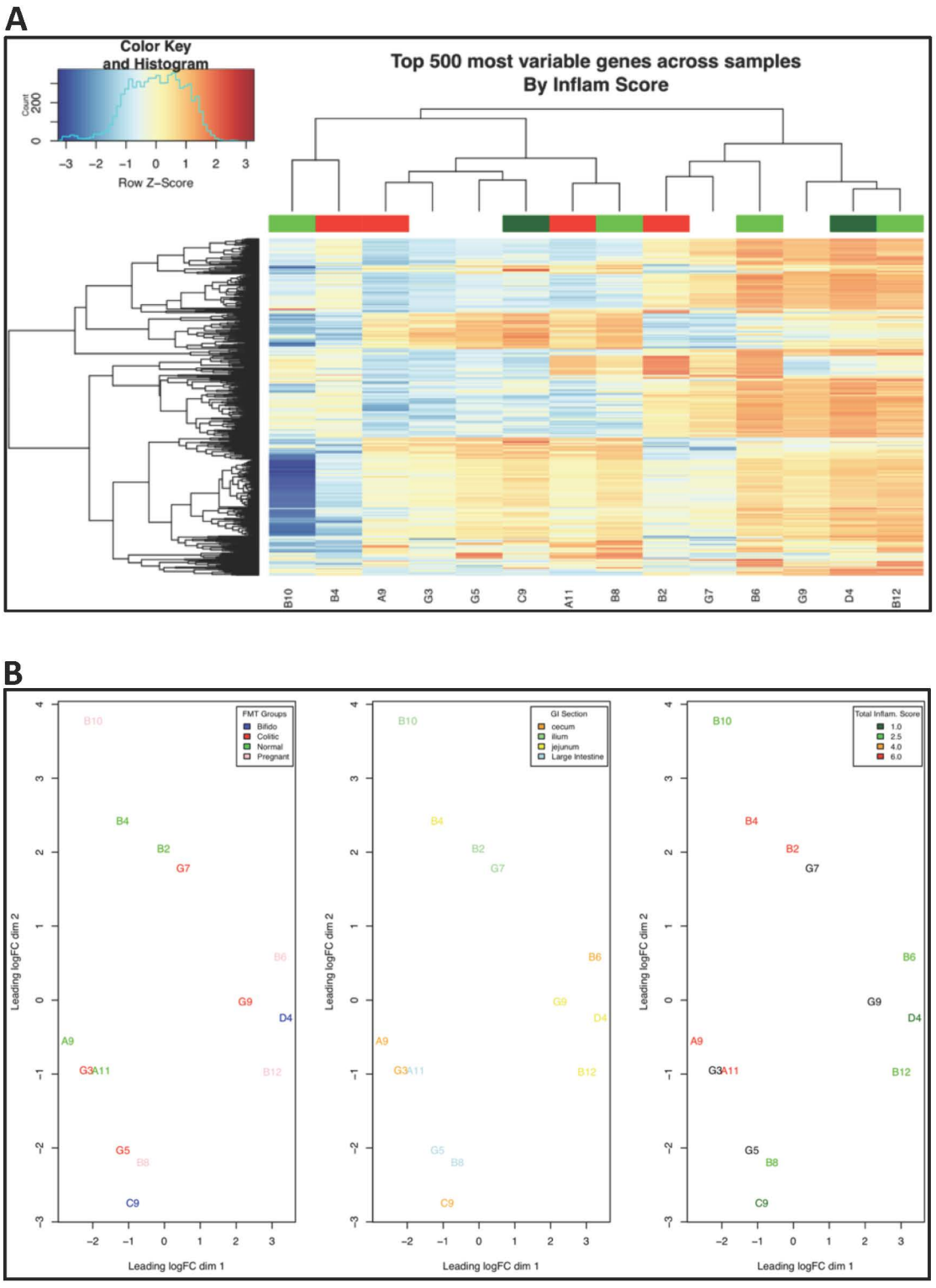
Supplementary Figure 1. Phylogenetic tree of 16S rRNA V3V4 sequences from *Bifidobacterium* reference sequences and *Bifidobacterium* OTUs significantly associated with the pregnant FMT transplant outcome.



Supplementary Figure 2. Pro-inflammatory and homeostatic cytokine secretion by macrophage (RAW264.7) and dendritic (JAWSII) cell lines stimulated by *B. pseudolongum* or *D. desulfuricans* bacteria. Supernatants from RAW and JAWS cells stimulated for 24 hours with indicated bacterial strains at an MOI of 2, or with 10 ng/ml LPS, and assayed by ELISA. Results expressed as means ± SEM of triplicate cultures. Representative of 3 independent experiments. ns: *p*-value > 0.05; * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001; **** *p* -value < 0.0001 by unpaired t-test.



Supplementary Figure 3. Intestinal gene expression analysis. A. Hierarchical clustering dendogram of the top 500 most variable genes across all samples. B. Multi-dimensional scaling (MDS) plot of distances between gene expression profiles of intestinal samples from *B. pseudolongum*, colitic, normal and pregnant FMT donor groups. For both plots, sample groups are: blue, *B. pseudolongum*; red, colitic FMT donor; green, normal FMT donor; pink, pregnant FMT donor.



Supplementary Table 1: Primary antibodies for immunohistochemistry

In vitro	Source	Isotype	Clone	Reactivity	Target Molecule	Dilution	Company
Primary	Rat	IgG2a		Mouse	ER-TR7	1:100	Santa Cruz
	Rabbit	IgG		Mouse, Human	LAMA 4	1:100	Novus Biologicals
	Rat	IgG2a	MAB3837	Mouse, Human	LAMA 5	1:200	R&D
	Rabbit	IgG			Isotype control	1:100	BD
	Rat	IgG2a	MAB3837		Isotype control	1:200	R&D

Supplementary Table 2: Secondary antibodies for immunohistochemistry

In vitro	Source	Isotype	Clone	Reactivity	Target Molecule	Flourophore	Dilution	Company
Secondary	Donkey	IgG	***	Rabbit	IgG(H+L)	Alexa Fluor 647	1:400	Jackson ImmunoResearch
	Goat	IgG	***	Rat	IgG(H+L)	Alexa Fluor 594	1:400	Jackson ImmunoResearch

Supplementary Table 3: Differentially expressed genes between high vs. low inflammation intestinal samples

Ensembl	logFC	logCPM	PValue	FDR	Symbol	Name
ENSMUSG00000095280	7.4943	4.7669	1.89E-06	0.06933	Gm21738	predicted gene, 21738
ENSMUSG00000069917	5.0831	2.5171	4.90E-06	0.07534	Hba-a2	hemoglobin alpha, adult chain 2
ENSMUSG00000097312	6.5731	3.1172	8.01E-06	0.07534	Gm26870	predicted gene, 26870
ENSMUSG00000037974	6.7033	2.1893	8.26E-06	0.07534	Muc5ac	mucin 5, subtypes A and C, tracheobronchial/gastric
ENSMUSG00000095866	5.0660	0.0213	1.02E-05	0.07534	Ighv2-4	immunoglobulin heavy variable V2-4
ENSMUSG00000047935	4.3820	-1.7412	1.82E-05	0.08358	Sox1ot	Sox1 overlapping transcript
ENSMUSG00000076655	3.7771	1.3790	1.92E-05	0.08358	Ighv4-1	immunoglobulin heavy variable 4-1
ENSMUSG00000091028	7.4109	2.0927	2.02E-05	0.08358	Gm10722	predicted gene 10722
ENSMUSG00000075015	7.5084	3.0110	2.05E-05	0.08358	Gm10801	predicted gene 10801

LogFC: log2 fold changes ; logCPM: log2 counts-per-million ; FDR: false discovery rate

Supplementary References

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