Detailed Methods for Microbial Analysis:

Stool and donor FMT material sample collection and DNA extraction

Stool samples were collected by patients and shipped overnight to the lab where they were stored at -80 °C until processed. Samples were collected pre-FMT and 6 +/- 2 weeks post-FMT to coincide with the post-FMT patient survey, blood/stool collection for inflammation biomarker measurement, and endoscopic exam. Patients prescribed rifaximin were instructed to collect the pre-FMT stool sample prior to starting the antibiotic regimen; this occurred except for one patient (clinically responsive patient #3) who collected stool after completing the antibiotic regimen but before FMT. An aliquot of the FMT suspension was stored at -20 °C until processed for DNA extraction. The donor material was centrifuged at 16k g for 10 min to pellet the solids, all but ~150 uL supernatant was removed, pelleted material was resuspended in the remaining supernatant, and 100 µL of the pelleted material was used to extract DNA.

DNA was extracted from stool samples or donor material using an organic solvent method, derived from[1] with modifications as follows. Frozen stool (~0.3 g subsampled using 4mm sterile, disposable biopsy punches (Integra Miltex, Plainsboro, NJ)) or donor material was placed in Lysing Matrix E tubes (MP Biomedicals, Santa Ana, CA). Samples were suspended in 500 µl of cetyltrimethylammonium bromide (CTAB) extraction buffer (5% hexadecyltrimethylammonium bromide in 0.25 M phosphate buffer and 1M NaCl) by vortexing and incubated at 65 °C for 15 min and then homogenized by bead-beating at 5.5 m/s for 30 sec after addition of 500 µL phenol:chloroform:isoamyl alcohol (25:24:1). Sample tubes were centrifuged for 5 min at 16,000 x g at 4 °C, and approximately 400 µL aqueous phase were transferred to 96-deep-well plates. To improve extraction efficiency, an additional 500 µL CTAB were added to the extraction tubes and the heat incubation and bead-beating steps were repeated. Supernatants from both extractions were combined (total volume ~800 µL), and an equal volume of chloroform was added to each sample and mixed, followed by centrifugation at 3000 x g for 10 minutes to remove excess phenol. The aqueous phase (600 µl) was transferred to a deep-well 96-well plate, combined with 2 volume-equivalents of polyethylene glycol (PEG) and stored overnight at 4 °C to precipitate DNA. Plates were centrifuged for 60 min at 3000 x g to pellet DNA and the PEG solution was removed. DNA pellets were washed twice with 300 µl of 70% ethanol, air-dried for 10 minutes and suspended in 50 - 200 µl of sterile water. DNA concentrations were quantified using the Qubit dsDNA HS Assay Kit (ThermoFisher Scientific, MA) and diluted to 10 ng/µl.

PCR conditions and library preparation for sequencing

The variable region 4 (V4) of the 16S rRNA gene was amplified using primers and conditions previously described[2]. Samples were amplified in triplicate from a single mastermix per template, aliquoted into 384-well plates, using 100 µL (total volume for triplicate reactions and a no-template control per set) of 1x ExTaq HotStart buffer (TaKaRa), 2.5 U enzyme, 200 uM dNTPs, 0.56 µg/uL BSA (ThermoFisher Scientific), 0.4 µM each forward primer (f515) and barcoded reverse primer (r806), 10 ng template per triplicate reaction, and the following thermal cycling conditions: 98 °C for 2 min., 30 rounds of 98 °C 20 sec, 50 °C 30 sec, 72 °C 45 sec, with a final extension at 72 °C for 10 min. Amplicons were purified using the SequalPrep Normalization Plate Kit (ThermoFisher Scientific) according to the manufacturer's specifications, quantified using the Qubit dsDNA HS Assay Kit (ThermoFisher Scientific), and pooled at equimolar concentrations. The amplicon library was concentrated using the Agencourt

AMPure XP system (Beckman-Coulter), quantified using the KAPA Library Quantification Kit (KAPA Biosystems), and diluted to 2 nM. Equimolar PhiX was added at 40% final volume to the amplicon library and sequenced on the Illumina NextSeq 500 Platform on a 153bp x 153bp sequencing run.

Sequence data processing

Raw 16S rRNA sequence data were converted from bcl to fastq format using bcl2fastq v2.16.0.10. Paired sequencing reads with a minimum overlap of 25 bp were merged using FLASH v1.2.11[3]. Successfully merged reads were identified, had index sequences extracted and were demultiplexed in the absence of quality filtering using QIIME[4] (Quantitative Insights Into Microbial Ecology, v1.9.1). Reads were then quality filtered using USEARCH's fastq filter (v7.0.1001[5]) to remove reads having >2 expected errors. Quality filtered reads were dereplicated at 100% identity, clustered at 97% sequence identity into operational taxonomic units (OTUs) and had chimeras removed, and mapped back to the resulting OTUs using USEARCH v8.0.1623. The Greengenes database (May 2013) was used to assign taxonomy to OTUs[6]. OTUs were filtered by removing any remaining OTU that had a total read count across all samples less than 2/1000th of a percent of the total read counts across all samples (QIIME). Finally, sequencing reads were normalized by multiply-rarefying to 39,000 reads for each sample as described previously[7]. The process of multiply rarefying the sequences was employed to assure reduced data were representative of the fuller data for each sample[8].

Statistical Analysis

Fecal microbiota alpha diversity measures were generated using R[9] (richness, evenness) or QIIME[5] (Faith's diversity). Bacterial beta diversity (QIIME) was evaluated using weighted and unweighted UniFrac distance metrics[10]. Alpha diversity metrics were compared using linear mixed effects (lme) model (Individual ID was used as a random effect) followed by Tukey multiple comparisons test for each pairwise comparison (R packages ImerTest[11] and multcomp[12]). Group-wise p values of <0.05 were considered significant, values <0.1 and >0.05 were considered trending. Weighted and unweighted UniFrac distance comparisons were performed using Mann-Whitney U test (Prism, v. 7). PERMANOVA and PERMDISP analyses were performed using Primer-E software[13,14]. To identify bacterial OTU that differed in relative abundance between responders and non-responders (pre- and post-FMT, separately), a 3-model comparative approach was used: Poisson, negative-binomial, and zero-inflated negative-binomial models were applied to each taxon individually, and the model that minimized the Akaike information criterion value (AIC) was selected for each taxon[15]. To adjust for multiple-comparisons, the false-discovery rate was calculated for each taxon; a q-value of ≤ 0.2 was considered significant. The same 3-model approach was used to identify genera containing significantly different numbers of OTU (binary data) between groups of interest (Responders pre- vs post-FMT or Donors vs Responders post-FMT). Piphillin[16] was used to predict the functional capacity of the significantly differentially abundant OTU that had a mean difference of ≥ 50 sequences.

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Supplementary Figure 1

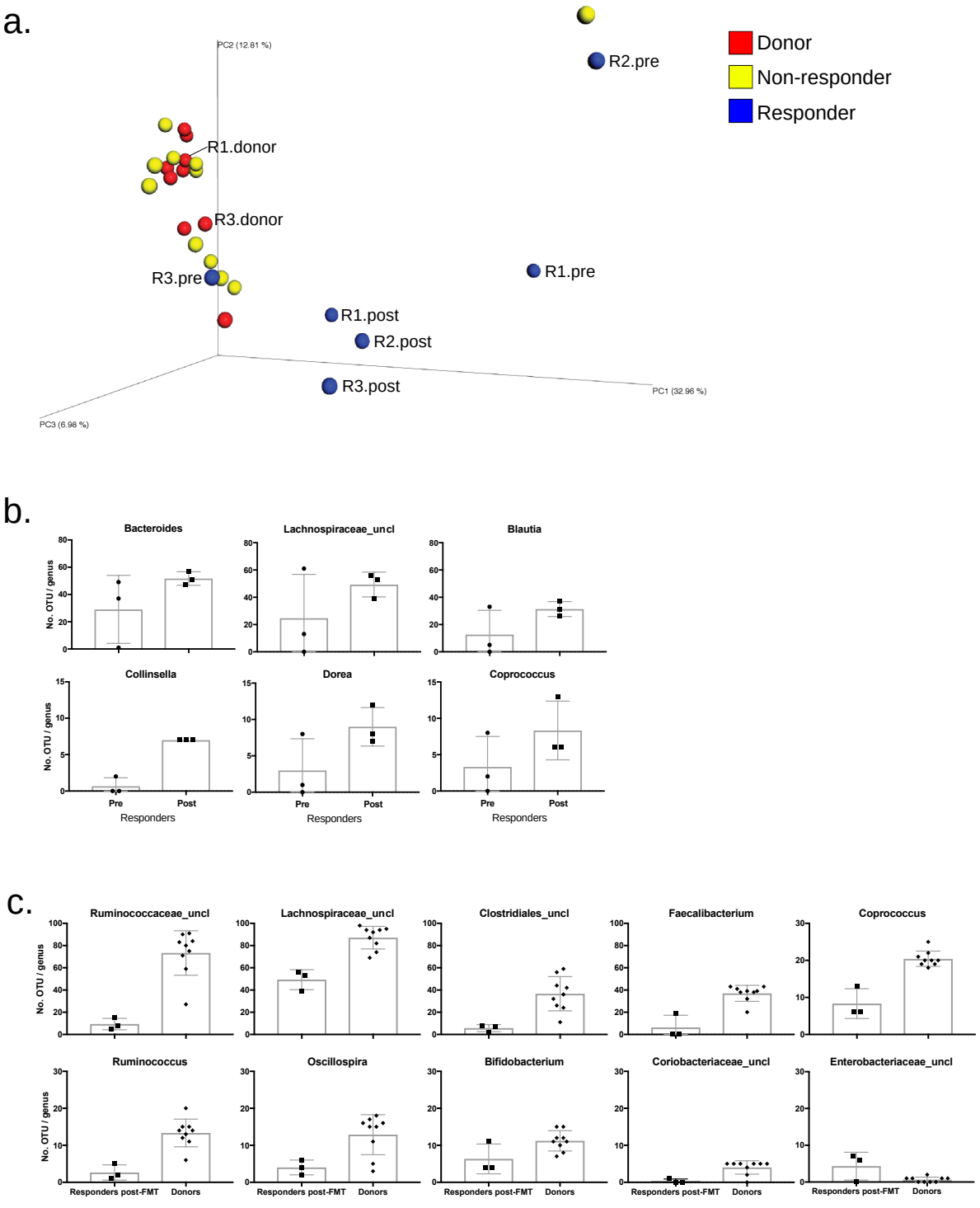

Figure S1: Fecal bacterial community composition of clinically responsive patients converged following FMT. a) Principle Coordinate Analysis plot showing relative (dis)similarity of individual samples using unweighted UniFrac distances with response group

denoted by color (pre, pre-FMT sample; post, post-FMT sample; R, clinical responder). b) Genera with significantly (3-model comparative approach, $q<0.1$) more representatives (OTU) post-FMT than pre-FMT in responders. Of note, the pre-FMT patient sample with the greatest numbers of Lachnospiraceae OTU (all genera) was from the patient who had completed a course of antimicrobial (rifaximin) before collecting the pre-FMT stool sample. c) Genera with significantly ($q<0.1$) different numbers of representatives (OTU) in FMT donors compared to FMT-responsive patients.

Supplemental Table 1: Detailed Baseline Patient Characteristics

Variable	Patient 1	Patient 2	Patient 3†	Patient 4*	Patient 5	Patient 6	Patient 7*	Patient 8†	Patient 9*	Patient 10
Age (years)	56	26	37	44	62	34	28	32	70	31
Sex	Male	Male	Female	Male	Male	Male	Female	Female	Female	Female
BMI (mm/kg^2)	25	24.4	21.8	23	25	24	22.8	22	23	19
Disease duration (years)	4	6	10	33	1	18	18	16	46	7
Prior steroid use	Yes (Prednisone, Budesonide)	Yes (Prednisone)	Yes (Prednisone, Budesonide)	Yes (Prednisone, Budesonide)	Yes (Prednisone)	No	Yes (Prednisone, Budesonide)	Yes (Prednisone, Budesonide)	Yes (Prednisone, Budesonide)	No
Steroid use at time of FMT	No	No	No	Yes (Prednsone)	No	No	No	No	Yes (Budesonide)	No
Prior biologic use	Yes	Yes	No	Yes	No	No	Yes	Yes	No	Yes
Biologic use at time of FMT	Yes (Certolizumab)	Yes (Adalimumab)	No	No	No	No	Yes (Certolizumab)	Yes (Certolizumab)	No	Yes (Adalimumab)
Biologics used, duration	Adalimumab, 1 year Certolizumab, 5 months	Adalimumab, 5 years	-	Inflixamab, 1 year	-	-	Adalimumab, 5 years Infliximab, 1 year Certolizumab, 2 years	Infliximab, 7 years Adalimumab, 8 years Certolizumab, 1 year	-	Adalimumab, 7 years
Location	Colonic	Ileocolonic	Colonic	Ileocolonic	Colonic	Colonic	Ileocolonic	Colonic	Colonic	Colonic
Behavior - B1 (non-stricturing, non-penetrating) - B2 (stricturing) - B3 (penetrating) - p (perianal disease modifier)	B1	B1	B1	B2p	B1	B1	B2p	B1	B2	B1
Received Rifaximin	No	No	No	No	No	No	No	Yes	Yes	Yes

*Responder

†Flare

Supplemental Table 2: Detailed Clinical Outcomes Pre and Post-Fecal Microbiota Transplant

Variable	**Patient 1**		**Patient 2**		**Patient 3**[†]		**Patient 4***		**Patient 5**		**Patient 6**[††]		**Patient 7***		**Patient 8**[†]		**Patient 9***		**Patient 10**	
Pre/Post-FMT	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
HBI	8	7	3	4	3	16	14	11	9	8	7	16	14	10	11	13	6	3	6	6
Number of stool/day	7	6	0	2	0	10	9	6	8	7	4	12	7	5	8.5	8.1	3	3	3	4
Pain (0-3)	1	1	1	1	2	3	2	2	1	1	1	2	2	1	2	3	1	0	1	0
CRP (mg/L)	16	20.2	1	1	2	15.5	16.3	-	5.3	4.1	2.4	2	68.5	51.4	18.3	33.1	1.5	3.8	2.8	1.2
ESR (mm/h)	43	61	2	1	75	100	11	-	25	15	10	8	100	100	25	37	31	35	14	4
Fecal calprotectin (mcg/g)	-	1645.2	23.2	-	475.6	2000	-	-	429.2	789.6	16	244.2	851	1157.7	748.1	222.8	269.7	90.2	184	307
SES CD score	14	12	3	5	6	-	8	9	13	6	1	-	20	19	8	-	0	0	9	-

*Responder

[†]Flare

[††]Scores worsened post-FMT but patient did not require escalation of therapy

Of note for patient 8, HBI and fecal calprotectin measured after initiaton of steroids

Supplemental Table 3. Taxa differing significantly between clinically responsive and non-responsive patients pre- or post-FMT where the difference in mean value was ≥ 50 sequences. A 3-model comparative approach was used to identify differentially abundant OTU. Models considered were Poisson, negative binomial, and zero-inflated negative-binomial. Best model, p-value, and false discovery rate-corrected p-values (q.best) are presented for each OTU.

		Responder	Non-responder					
Timepoint	**OTUID**	**mean**	**mean**	**mean difference**	**Taxonomy (Phylum_Family_Genus)**	**best.mod**	**best.pval**	**qval.best**
Pre-FMT	OTU_5	4463	317	4146	*Proteobacteria_Enterobacteriaceae*	ZI-NegBin	1.16E-03	1.63E-02
	OTU_1142	925	33	892	*Actinobacteria_Bifidobacteriaceae_Bifidobacterium*	ZI-NegBin	1.45E-08	5.43E-07
	OTU_20	685	84	602	*Actinobacteria_Bifidobacteriaceae_Bifidobacterium*	ZI-NegBin	5.56E-03	4.29E-02
	OTU_69	116	3	113	*Firmicutes_Veillonellaceae_Veillonella*	NegBin	1.30E-04	2.65E-03
	OTU_324	60	5	55	*Firmicutes_Lachnospiraceae_Blautia*	NegBin	7.73E-03	5.48E-02
	OTU_85	1	56	-55	*Firmicutes_Lachnospiraceae*	ZI-NegBin	6.72E-04	1.08E-02
	OTU_2630	4	67	-63	*Firmicutes_Ruminococcaceae*	ZI-NegBin	1.30E-02	7.09E-02
	OTU_1514	1	71	-70	*Firmicutes_Lachnospiraceae*	ZI-NegBin	1.47E-05	3.29E-04
	OTU_565	0	74	-74	*Firmicutes_Lachnospiraceae_Dorea*	NegBin	3.39E-03	2.92E-02
	OTU_95	3	108	-105	*Firmicutes_Turicibacteraceae_Turicibacter*	NegBin	3.92E-03	3.25E-02
	OTU_1129	1	144	-143	*Firmicutes_Ruminococcaceae*	ZI-NegBin	2.39E-06	6.68E-05
	OTU_2459	5	225	-221	*Firmicutes_Lachnospiraceae*	ZI-NegBin	1.02E-10	5.72E-09
	OTU_16	2	340	-338	*Firmicutes_Lachnospiraceae_Blautia*	ZI-NegBin	8.93E-06	2.22E-04
	OTU_4	42	670	-628	*Firmicutes_Clostridiales*	ZI-NegBin	2.24E-03	2.18E-02
	OTU_27	5	657	-652	*Proteobacteria_Pasteurellaceae_Haemophilus*	NegBin	1.10E-03	1.63E-02

	OTU_2	65	1578	-1513	*Firmicutes_Ruminococcaceae_Faecalibacterium*	ZI-NegBin	9.64E-03	5.83E-02
Post-FMT	OTU_25	1535	14	1521	*Firmicutes_Lachnospiraceae_[Ruminococcus]*	NegBin	1.27E-07	3.78E-06
	OTU_67	976	21	955	*Firmicutes_Lachnospiraceae_Blautia*	ZI-NegBin	6.62E-03	3.85E-02
	OTU_324	642	1	642	*Firmicutes_Lachnospiraceae_Blautia*	NegBin	7.30E-05	8.50E-04
	OTU_83	616	5	612	*Firmicutes_Lachnospiraceae_[Ruminococcus]*	NegBin	3.95E-08	1.29E-06
	OTU_582	454	1	454	*Firmicutes_Lachnospiraceae_Blautia*	ZI-NegBin	1.38E-05	1.95E-04
	OTU_108	308	20	288	*Firmicutes_Erysipelotrichaceae_[Eubacterium]*	NegBin	3.11E-03	2.16E-02
	OTU_2243	289	3	286	*Firmicutes_Lachnospiraceae*	NegBin	8.53E-07	1.54E-05
	OTU_215	195	1	194	*Firmicutes_Lachnospiraceae_Coprococcus*	NegBin	4.55E-07	9.27E-06
	OTU_46	406	226	180	*Firmicutes_Veillonellaceae_Phascolarctobacterium*	Poisson	1.28E-40	2.08E-38
	OTU_62	170	43	127	*Firmicutes_Lachnospiraceae*	NegBin	6.73E-06	1.04E-04
	OTU_52	173	63	110	*Bacteroidetes_Porphyromonadaceae_Parabacteroides*	ZI-NegBin	1.02E-02	5.27E-02
	OTU_2237	59	1	59	*Firmicutes_Lachnospiraceae_[Ruminococcus]*	NegBin	2.10E-07	4.55E-06
	OTU_142	2	69	-67	*Firmicutes_Ruminococcaceae*	NegBin	2.16E-02	9.15E-02
	OTU_2459	2	70	-68	*Firmicutes_Lachnospiraceae*	NegBin	1.14E-02	5.64E-02
	OTU_820	0	69	-68	*Firmicutes_Ruminococcaceae_Faecalibacterium*	NegBin	1.44E-07	3.90E-06
	OTU_2630	3	74	-72	*Firmicutes_Clostridia_Clostridiales_Ruminococcaceae*	ZI-NegBin	5.27E-05	6.36E-04
	OTU_214	2	87	-85	*Firmicutes_Ruminococcaceae_Faecalibacterium*	NegBin	1.02E-06	1.75E-05
	OTU_2868	9	97	-88	*Firmicutes_Clostridiales*	ZI-NegBin	4.24E-04	3.84E-03
	OTU_1951	2	119	-117	*Firmicutes_Ruminococcaceae_Faecalibacterium*	NegBin	1.80E-07	4.44E-06
	OTU_1531	1	120	-119	*Bacteroidetes_Bacteroidaceae_Bacteroides*	NegBin	1.65E-03	1.22E-02
	OTU_565	1	129	-128	*Firmicutes_Lachnospiraceae_Dorea*	NegBin	1.88E-04	1.97E-03
	OTU_16	104	262	-157	*Firmicutes_Lachnospiraceae_Blautia*	Poisson	3.00E-46	9.77E-44
	OTU_2144	2	160	-158	*Bacteroidetes_Bacteroidaceae_Bacteroides*	NegBin	1.23E-02	5.89E-02
	OTU_597	3	262	-259	*Firmicutes_Ruminococcaceae*	NegBin	1.91E-07	4.44E-06
	OTU_1530	4	282	-278	*Bacteroidetes_Bacteroidaceae_Bacteroides*	ZI-NegBin	4.63E-04	3.91E-03
	OTU_1399	5	286	-281	*Firmicutes_Lachnospiraceae_Blautia*	ZI-NegBin	1.64E-02	7.28E-02
	OTU_32	26	317	-291	*Bacteroidetes_Porphyromonadaceae_Parabacteroides*	ZI-NegBin	8.58E-06	1.27E-04
	OTU_28	17	453	-435	*Firmicutes_Lachnospiraceae_Coprococcus*	ZI-NegBin	3.00E-04	2.97E-03

	OTU_34	8	448	-440	*Bacteroidetes_Bacteroidaceae_Bacteroides*	ZI-NegBin	5.59E-04	4.44E-03
	OTU_2	67	982	-914	*Firmicutes_Ruminococcaceae_Faecalibacterium*	ZI-NegBin	1.72E-14	9.35E-13
	OTU_2363	7	1459	-1452	*Firmicutes_Ruminococcaceae*	ZI-NegBin	2.72E-34	2.22E-32
	OTU_17	15	1754	-1738	*Bacteroidetes_Bacteroidaceae_Bacteroides*	ZI-NegBin	5.97E-03	3.54E-02