Supplementary Material

SUPPLEMENTARY METHODS

Definition of failure for prior clinical treatments

According to the standard operating procedures of our outpatient clinic treatment, failures are defined by Mayo-score as follows:

1. Patients are non-responders to thiopurines after treament with azathioprine 2-2.5mg/kg/day or 6-mercaptopurine 1-1.5mg/kg/day for at least 3-6 months despite 6-TGN levels > 235 pmol/8x10^8 red blood cells (RBC).

2. Primary lack of response to anti-TNF agents is determined within the first 12 weeks of therapy.

3. In patients with loss of response to anti-TNF agents, reduction in interval between doses, dose escalation or both (e.g. infliximab: up to 10mg/kg every 4 weeks, adalimumab: up to 40mg every week) is performed. If there is no response after reduction in interval and dose escalation patients are rated as secondary anti-TNF failures.

Evaluation of different methods for preparation of faecal suspensions

Because protocols for donor stool preparations differ between published faecal transplantation studies, prior to treating patients we assessed the quantitative and qualitative difference in the bacterial content between native stool and three different stool preparations: (A) 30 g of fresh stool were mixed with 450 ml of 0.9% sterile saline solution to an almost homogeneous suspension, (B) the suspension prepared from (A) was filtered several times through an increasing number of gauze pads to remove small particles, (C) the suspension prepared from (A) was centrifuged at 1912xg (3000 rpm) and the supernatant was used for further analysis. Native stool and each of the preparations were plated on five different solid culture media. Columbia CNA agar with 5% sheep blood (Becton Dickinson, Heidelberg, Germany), Mac Conkey agar (Becton Dickinson), and chromID™ CPS (bioMérieux, Marcy l'Etoile, France) were incubated at 37°C under aerobic conditions. Brucella Agar with 5% sheep blood, hemin and vitamin K1 (Becton Dickinson) and Schaedler kanamycin vancomycin agar with 5% sheep blood (Becton Dickinson) were incubated at 37°C under anaerobic conditions. Enumeration of cultivable microorganisms (colony forming units) was

performed semi-quantitatively for native stool and quantitatively for suspensions (which was possible because of their homogenized consistency). Growth was determined after 24 and 48 hours of incubation. Isolates were identified to the species level by conventional methods and MALDI-TOF mass spectrometry (MALDI Biotyper; Bruker Daltonics, Bremen, Germany). There was no significant difference in bacterial numbers between preparations (A) and (B), but there were reduced numbers in preparation (C) (data not shown).

SUPPLEMENTARY FIGURES AND TABLES

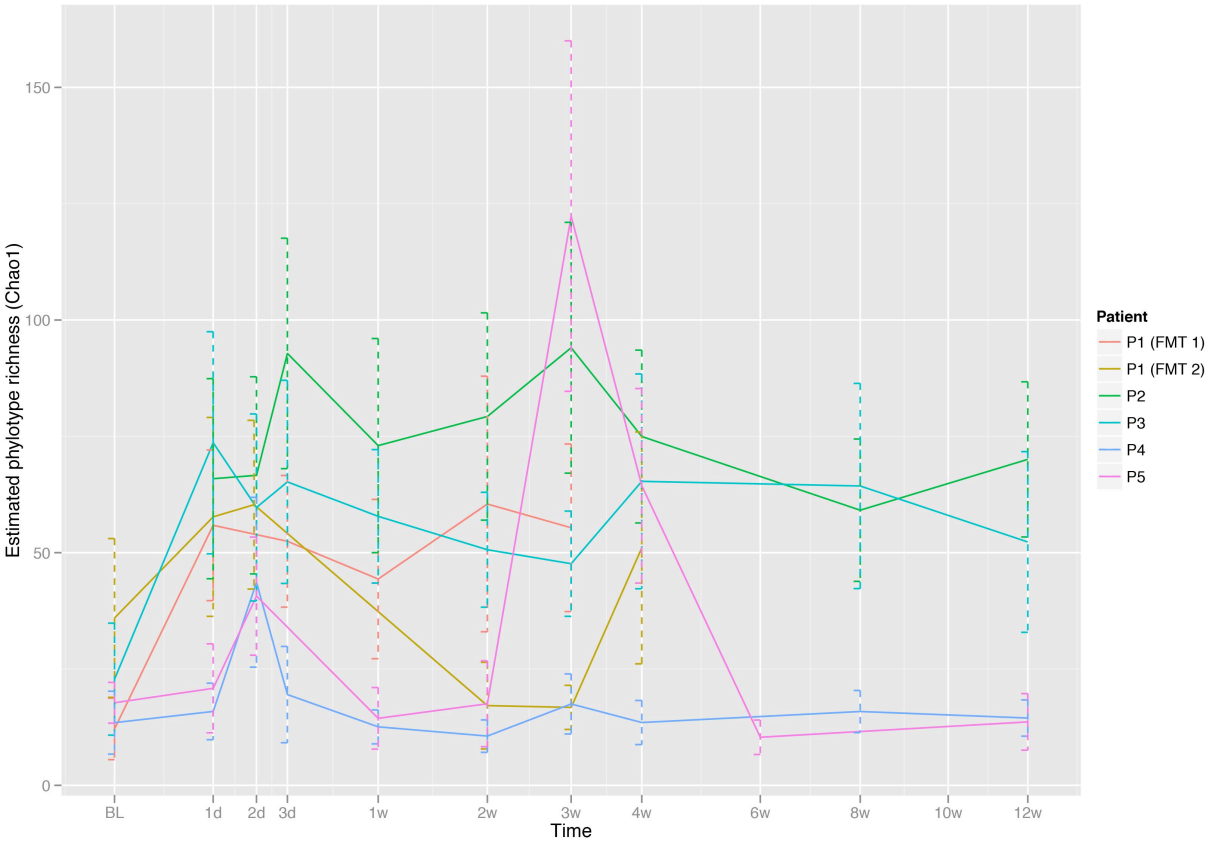

Figure S1. Estimated richness of bacterial, species-level phyloytpes (Chao 1). Estimated phylotype richness of patients over the course of the study. Donor microbiota had an average Chao 1 phylotype richness of 107 +/- 52 (s.d.). Patients are indicated by different colours and for Patient 1 the first (FMT 1) and second and third faecal microbiota transplantation (FMT 2) are shown separately. BL = baseline, which was preceded by an antibiotic treatment for all FMTs except FMT 1 of Patient 1 (see Figure 1).

Figure S2. Heatmap of relative abundance of abundant taxa in donor and patient faecal microbiota. Sequence reads were classified at the taxonomic level of "family" (or higher, when unclassified at the "family" level). The microbiota of donors is shown just to the left of their respective recipient patients. The color bar above the heatmap indicates which samples are from a donor (light brown) or a patient (light pink). Sample ID abbreviations indicate the participant (D = donor, P = patient) and time point (bl = baseline, which was preceded by an antibiotic treatment for all FMTs except FMT 1 of Patient 1 (see Figure 1), d = day, w = week). For donors the preparation of the sample is also indicated (n = native, s = suspended). Heatmap coloring is scaled using a square root normalization in order to visualize less abundant groups.

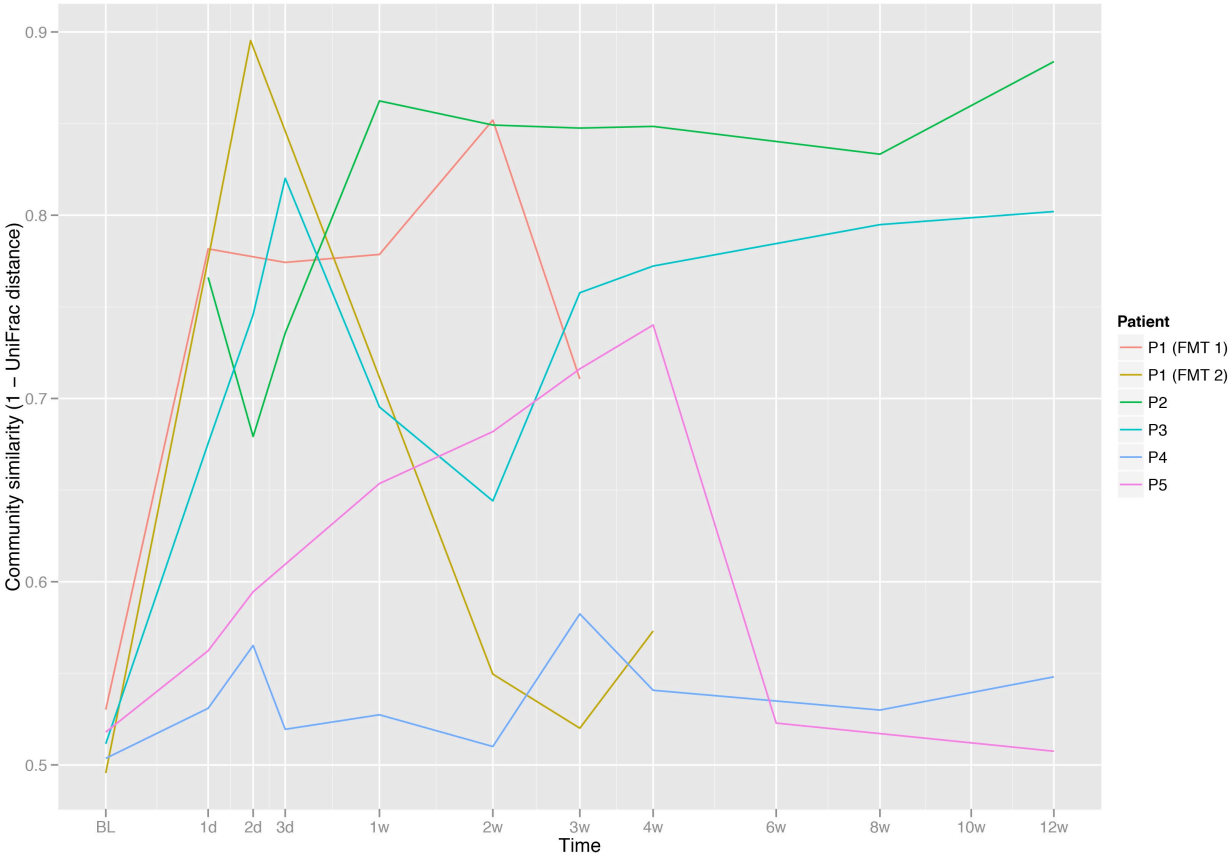

Figure S3. Bacterial community similarity to donor. The similarity of each patient's microbiota to his or her donor microbiota before, during and after FMT. Community similarity is calculated as 1 minus the weighted UniFrac distance metric. Patients are indicated by different colors and for Patient 1 the first (FMT 1) and second and third faecal microbiota transplantation (FMT 2) are shown separately. BL = baseline, which was preceded by an antibiotic treatment for all FMTs except FMT 1 of Patient 1 (see Figure 1).

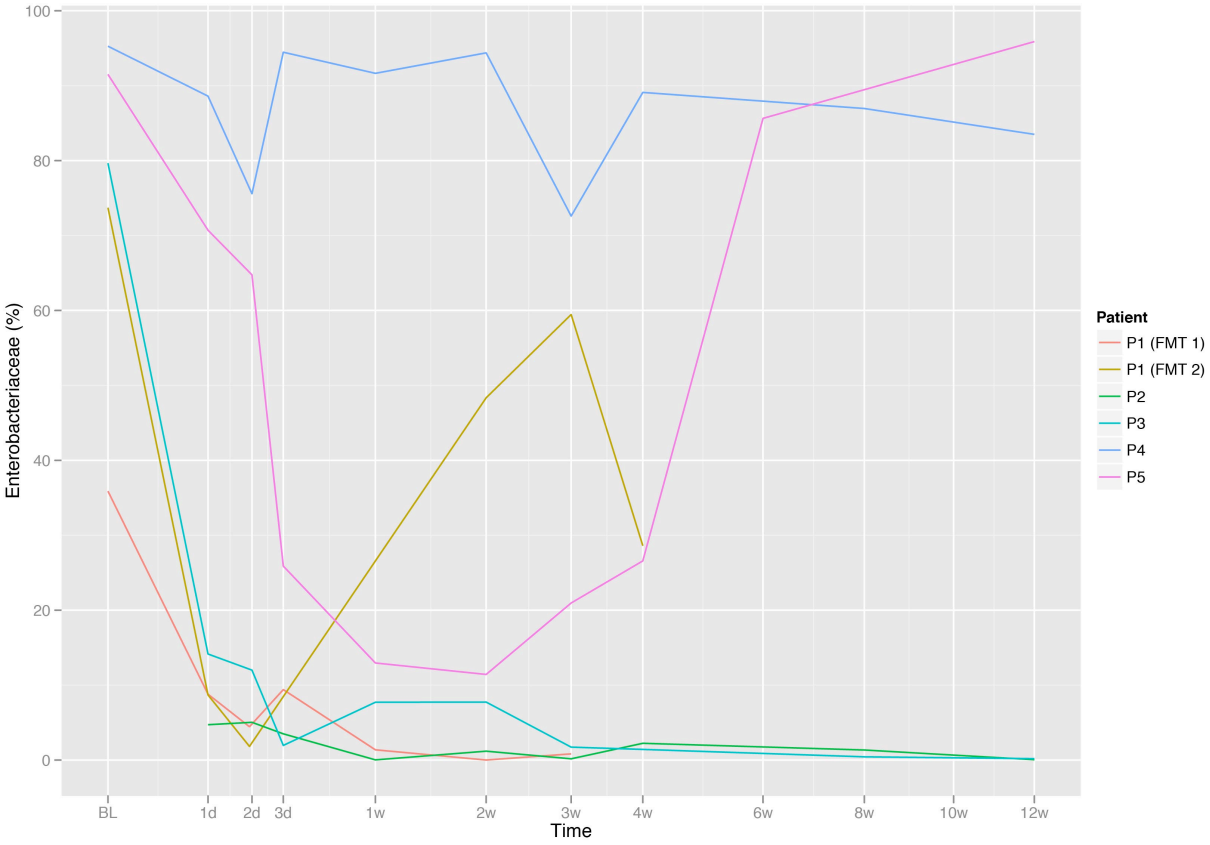

Figure S4. Abundance of *Enterobacteriaceae* in microbiota of patients. Relative abundance (as percentage) is shown. Low levels of *Enterobacteriaceae* were occasionally detected in donor microbiota (0.03 +/- 0.07 % mean and s.d.). Patients are indicated by different colors and for Patient 1 the first (FMT 1) and second and third faecal microbiota transplantation (FMT 2) are shown separately. BL = baseline, which was preceded by an antibiotic treatment for all FMTs except FMT 1 of Patient 1 (see Figure 1).

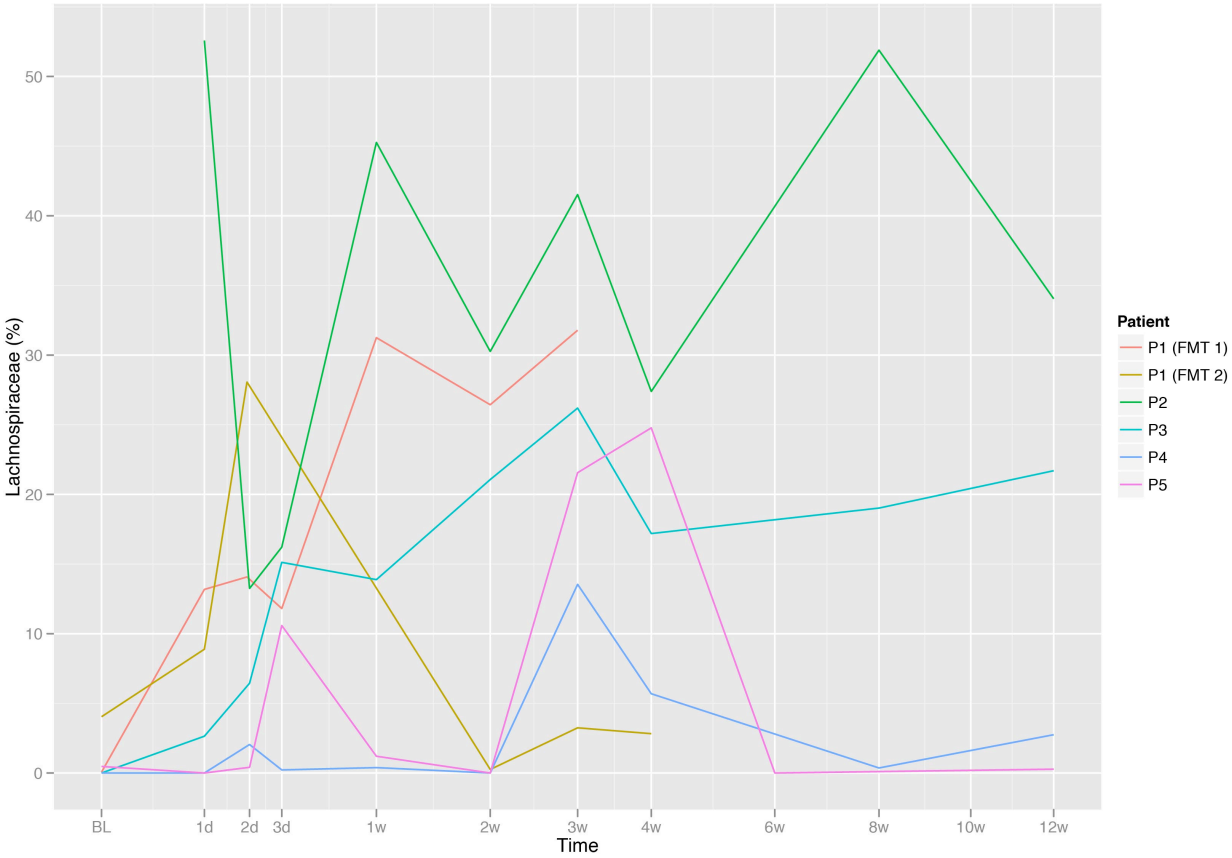

Figure S5. Abundance of *Lachnospiraceae* in microbiota of patients. Relative abundance (as percentage) is shown. Consistently high levels of *Lachnospiraceae* were detected in donor microbiota (44.68 +/- 13.38 % mean and s.d.). Patients are indicated by different colors and for Patient 1 the first (FMT 1) and second and third faecal microbiota transplantation (FMT 2) are shown separately. BL = baseline, which was preceded by an antibiotic treatment for all FMTs except FMT 1 of Patient 1 (see Figure 1).

Table S1. Study activities.

	Screening	Baseline[+]	1d	2d	3d	1w	2w	4w	8w	12w	Early termination
Inclusion/Exclusion	X										
Informed Consent	X										
Medical/Surgical History	X										
Previous /concomitant medication	X	X	X	X	X	X	X	X	X	X	X
X-ray		X									
Physical Examination		X									
General Lab*	X	X	X	X	X	X	X	X	X	X	X
General stool samples[#]	X										
Calprotectin		X								X	X
Partial Mayo Score		X				X	X	X	X	X	X
Mayo Score		X						X		X	
Nasojejunal tube		X									
Endoscopy		X						X		X	
Biopsy for CMV		X									
Stool samples for microbiota analysis		X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X
Faecal transplanation		X	X	X							
H2-glucose breath test								X		X	X

* Blood analysis includes: complete blood count and differential blood picture, chemistry (C-Reactive Protein, Na^+, K^+, Cl^-, Ca^+, Mg^+, Ph^+, iron profile, blood urea nitrogen, creatinine, total bilirubin, serum glutamic-pyruvic transaminase, serum glutamic-oxaloacetic transaminase, alkaline phosphatase, uric acid, cholesterol, triglycerides, total protein, albumin, glucose, amylase, lipase).

[#] *Yersinia, Campylobacter jejuni, Salmonella, Shigella*, *Clostridium difficile* toxin, Helminths, ova and parasites including *Cryptosporidium* spp.

[+] Baseline was preceded by an antibiotic treatment for all FMTs except FMT 1 of Patient 1 (see Figure 1).

Table S2. Demographic and clinical information of faecal donors.

Donor ID	Sex	Age	Smoker	Concomitant therapy	Eating habits	Relationship to patient
1	m	25	ex-smoker	no	vegetarian	Partner of Patient 1
2	f	21	yes	no	-	Friend of Patient 1
3	f	31	no	levothyroxine	-	Sister-in-law of Patient 2
4	m	23	yes	no	-	Son of a work colleague of Patient 4

Table S3. Summary statistics for each bacterial 16S rRNA gene sequencing library. For each library, the number of reads, estimated coverage of the library, observed phylotype richness and Chao1 estimated phylotype richness were calculated. To facilitate comparisons between libraries of unequal sizes, the libraries were bootstrapped resampled at a level of 200 reads and the observed and estimated richness is shown as mean and standard deviation of the resampling (n=1000 resamplings). Donor samples were either processed in their native state (n) or after preparation for transplantation (s). Baseline preceded by an antibiotic treatment for all FMTs except FMT 1 of Patient 1 (see Figure 1).

Individual	Time Point	No. Reads	Good's Coverage	Observed richness	Chao1 richness	Observed richness (200 reads)	Chao1 richness (200 reads)
Patient 1 (FMT 1)	Baseline	6275	1	41	80	8 (2)	12 (7)
Patient 1 (FMT 1)	D 1	387	0.95	45	66	33 (2)	56 (16)
Patient 1 (FMT 1)	D 2	135	0.91	25	36	N.A.	N.A.
Patient 1 (FMT 1)	D 3	415	0.96	43	57	31 (2)	52 (14)
Patient 1 (FMT 1)	W 1	1248	0.98	58	139	25 (3)	44 (17)
Patient 1 (FMT 1)	W 2	923	0.96	67	134	30 (3)	60 (27)
Patient 1 (FMT 1)	W 3	365	0.95	43	74	34 (2)	55 (18)
Patient 1 (FMT 2)	Baseline	6807	0.99	110	184	20 (3)	36 (17)
Patient 1 (FMT 2)	D 1	979	0.97	65	88	32 (3)	58 (21)
Patient 1 (FMT 2)	D 2	880	0.97	69	120	36 (3)	60 (18)
Patient 1 (FMT 2)	W 2	786	0.98	20	66	8 (2)	17 (9)
Patient 1 (FMT 2)	W 3	370	0.99	16	26	13 (1)	17 (5)
Patient 1 (FMT 2)	W 4	2514	0.98	98	288	25 (3)	51 (25)

Patient 2	D 1	2206	0.97	116	281	40 (4)	66 (22)
Patient 2	D 2	4370	0.99	141	286	41 (3)	67 (21)
Patient 2	D 3	2917	0.98	170	272	51 (4)	93 (25)
Patient 2	W 1	4553	0.98	157	312	41 (4)	73 (23)
Patient 2	W 2	846	0.96	83	153	41 (3)	79 (22)
Patient 2	W 3	1221	0.96	118	184	47 (4)	94 (27)
Patient 2	W 4	3852	0.98	159	293	48 (4)	75 (19)
Patient 2	W 8	2170	0.98	101	166	40 (3)	59 (15)
Patient 2	W 12	2209	0.98	115	240	42 (4)	70 (17)
Patient 3	Baseline	536	0.98	20	28	11 (2)	23 (12)
Patient 3	D 1	530	0.95	61	102	37 (3)	74 (24)
Patient 3	D 2	450	0.95	49	72	33 (3)	60 (20)
Patient 3	D 3	615	0.95	61	116	34 (3)	65 (22)
Patient 3	W 1	389	0.95	48	83	36 (2)	58 (14)
Patient 3	W 2	750	0.98	54	64	36 (3)	51 (12)
Patient 3	W 3	523	0.98	47	56	34 (2)	48 (11)
Patient 3	W 4	989	0.97	74	109	35 (3)	65 (23)
Patient 3	W 8	468	0.95	55	86	37 (3)	64 (22)
Patient 3	W 12	553	0.96	47	93	30 (3)	52 (19)
Patient 4	Baseline	592	0.99	14	25	8 (2)	13 (7)
Patient 4	D 1	667	1	17	18	12 (2)	16 (6)
Patient 4	D 2	733	0.97	45	64	24 (3)	44 (18)
Patient 4	D 3	452	0.98	17	40	11 (2)	20 (10)
Patient 4	W 1	515	0.99	14	24	10 (1)	13 (4)
Patient 4	W 2	285	0.99	9	12	8 (1)	11 (3)
Patient 4	W 3	635	0.99	18	19	13 (2)	17 (6)
Patient 4	W 4	826	0.99	17	28	10 (2)	13 (5)
Patient 4	W 8	276	0.98	13	18	12 (1)	16 (5)
Patient 4	W 12	291	0.99	13	14	12 (1)	14 (4)
Patient 5	Baseline	212	0.97	10	18	10 (1)	18 (4)
Patient 5	D 1	577	0.98	20	48	12 (2)	21 (10)

Patient 5	D 2	244	0.95	25	45	23 (1)	41 (13)
Patient 5	D 3	85	0.94	15	25	N.A.	N.A.
Patient 5	W 1	46748	1	113	215	10 (2)	14 (7)
Patient 5	W 2	674	0.99	18	33	10 (2)	18 (9)
Patient 5	W 3	835	0.92	127	257	60 (4)	122 (38)
Patient 5	W 4	444	0.95	52	75	36 (3)	64 (21)
Patient 5	W 6	390	0.99	10	13	8 (1)	10 (4)
Patient 5	W 12	365	0.98	11	16	8 (1)	14 (6)
Donor 1	D 1 (s)	8246	0.91	1366	2806	105 (6)	409 (136)
Donor 1	D 2 (s)	529	0.96	55	81	36 (3)	59 (15)
Donor 2	D 1 (n)	880	0.96	88	132	44 (3)	80 (22)
Donor 2	D 1 (s)	11737	0.96	876	1539	69 (6)	250 (108)
Donor 2	D 2 (n)	1219	0.97	95	133	47 (3)	74 (18)
Donor 3	D 1 (n)	407	0.89	77	167	52 (3)	108 (30)
Donor 3	D 1 (s)	453	0.92	73	156	48 (3)	89 (24)
Donor 3	D 3 (n)	803	0.92	120	250	55 (4)	116 (34)
Donor 4	D 3 (s)	1198	0.96	114	200	48 (4)	87 (24)
Donor 4	D 1 (s)	456	0.94	59	109	41 (3)	68 (22)
Donor 4	D3 (n)	876	0.94	110	193	51 (4)	103 (31)
Donor 4	D3 (s)	424	0.92	78	131	54 (3)	98 (22)

Table S4. Representative sequences of stably transferred phylotypes in Patient 3.

>OTU_91
CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATGGTCAGTTACTACGCAAGAGGA
CTCTGGCCAGACTGCCGTTGACAAAACGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTTATGACT
TGGGCTACACACGTACTACAATGGCGTTAAACAAAGAGAAGCAAGACCGCGAGGTGGAGCAAAACTCAGAA
ACAACGTCCCAGTTCGGACTGCAGGCTGCAACTCGCCTGCACGAAGTCGGAATTGCTAGTAATCGTGGATCA
GCATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGCCGGGGGGACC
CGAAGTCGGTAGTCTAACCGCAAGGAGGACGCCGCCGAAGGTAAAACTGGTGATTGGGGTGA

>OTU_110
AACGAGCGCAACCCCTGTTGCCAGTTGCCAGCATTAAGTTGGGGACTCTGGCGAGACTGCCGGTGACAAGC
CGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGA
CAGAGCAGAGGGAAGCGAAGCCGCGAGGTGGAGCGAAACCCATAAAACTGTTCTCAGTTCGGACTGCAGTC
TGCAACTCGACTGCACGAAGATGGAATCGCTAGTAATCGCGAATCAGCATGTCGCGGTGAATACGTTCTCGG
GCCTTGTACACACCGCCCGTCACACCATGAGAGTCGGTAACACCCGAAGCCGGTGGCCTAACCGCAAGGAA
GGAGCTGTCTAAGGTGGGACTGATGATTGGGGTGA

>OTU_1766
GTGCCATAACGAGCGCAACCCTTATCTTTAGTTACTAACAGGTCATGCTGAGGACTCTAGAGAGACTGCCGT
CGTAAGATGTGAGGAAGGTGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCTACACACGTGTT
ACAATGGGGGGTACAGAAGGCGGCTACCTGGTGACAGGATGCTAATCCCAAAAACCTCTCTCAGTTCGGAT
CGAAGTCTGCAACCCGACTTCGTGAAGCTGGATTCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAATA
CGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGAAAGCCGGGGGTACCTGAAGTACGTAACCGCAA
GGAGCGTCCTAGGGTAAAACTGGTAATTGGGGCTA

>OTU_2499
AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTGTTCTTAGTAGCCAGCGGTTCGGCCGGGCACTCT
AGGGAGACTGCCAGGGATAACCTGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTTATGACTTGG
GCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGGAGCCGTGAGGCCGAGCAAATCTCAAAAATAA
CGTCTCAGTTCGGACTGTAGTCTGCAACCCGACTACACGAAGCTGGAATCGCTAGTAATCGCAGATCAGAAT
GCTGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTTGGAAATGCCCGAAG
TCAGTGACCCAACCGCAAGGAGGGAGCTGCCGAAGGCAGGTTCGATAACTGGGGTGA