Serial Fecal Microbiota Transplantation Alters Mucosal Gene Expression in Pediatric Ulcerative Colitis

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Fecal Transplant in Pediatric Ulcerative Colitis

Richard Kellermayer[1,3*], Dorottya Nagy-Szakal[1,3], R. Alan Harris[4], Ruth Ann Luna[2,5], Milena Pitashny[2,5], Deborah Schady[2,5], Sabina AV Mir[1,3], Monica E. Lopez[6], Mark A. Gilger[1#], John Belmont[4], Emily B. Hollister[2,5], and James Versalovic[2,5]

[1]Section of Pediatric Gastroenterology, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, United States of America; [2]Department of Pathology, Texas Children's Hospital, Houston, Texas, United States of America; [3]USDA/ARS Children's Nutrition Research Center, Houston, Texas, United States of America; [4]Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, Texas, United States of America; [5]Department of Pathology& Immunology, Baylor College of Medicine, Houston, Texas, United States of America; [6]Department of Surgery, Baylor College of Medicine, Houston, Texas, United States of America, [#]Current address: Children's Hospital of San Antonio, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, United States of America

Author contribution: study concept and design: RK, DNS, RAL, MP, MAG, JV; acquisition of data: RK, DNS, DS, SAVM, MEL; analysis and interpretation of data: RK, DNS, RAH, RAL, JB, EBH, JV; drafting of the manuscript: RK, DNS, EBH; critical revision of the manuscript for important intellectual content: RK, MEL, EBH, JB, JV; statistical analysis: RK, RAH, EBH; obtained funding: RK, JV; technical, or material support: MP, JV; study supervision: RK, JV

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[*]Correspondence:
Richard Kellermayer M.D., Ph.D.
Section of Pediatric Gastroenterology, Hepatology & Nutrition
Baylor College of Medicine
6621 Fannin St., CC1010.00
Houston, TX 77030-2399
Voice: 713-798-0319
Fax: 832-825-3633
Email: kellerma@bcm.edu

To the Editor: Complex bacteriotherapy, such as fecal microbiota transplantation (FMT) is an emerging therapeutic modality for ulcerative colitis (UC).(1) FMT has been implicated to allow for withdrawal of conventional therapies in select patients.(2) In our study, three immunotherapy (infliximab, 6-mercaptopurine, and steroid, respectively) dependent pediatric (14-16 year of age) UC patients **(Table 1)** received a tapering course (22-30 treatments) of FMT delivered by colonoscopy and enemas during a 6-12 week period. The phase 1, open label protocol was approved by the Institutional Review Board of Baylor College of Medicine (H-30591). The protocol is currently approved by the FDA (IND-15743; ClinicalTrials.gov number: NCT01947101). Filtered, frozen, and thawed stool specimen from a standardized single donor (37-year-old male) for all 3 patients was used, which provided unique opportunity to examine microbial changes. Patients were concomitantly withdrawn from their conventional medications. Mucosal disease activity was assessed before, and 2 weeks after the FMT series. Clinical disease activity was followed by the Pediatric Ulcerative Colitis Activity Index (PUCAI). The FMT series was well tolerated and transiently supported immunotherapy withdrawal (**Supplementary Figure 1**). FMT enabled all 3 patients to be symptom-free for at least 4 weeks following FMT and supported the withdrawal of immunotherapy (no treatment other than mesalamine) for more than 105 days in all. The number of FMT treatments significantly correlated with the time of being immunotherapy-free (r=0.998, p=0.04; **Supplementary Figure 2**). All patients were in endoscopic and histologic remission 2 weeks after the last FMT (**Supplementary Figure 3-4**).

Fecal microbiomes were analyzed by massively parallel pyrosequencing of the V3V5 regions within the bacterial *16S rRNA* gene. The nature of microbiota shifts differed, presumably due to differences in baseline composition of intestinal microbiota in each patient (detected by principal-coordinates-analysis, **Figure 1A**). Recipient microbiomes remained distinct from that of the anonymous donor. FMT series appeared to induce a transient engraftment of the donor microbiome in a recipient (**Supplementary Figure 5**). Microbiome richness (**Figure 1B**) and diversity (**Figure 1C**) increased secondary to FMT. Fifteen operational taxonomic units (OTUs

or bacterial taxa) consistently changed in relative abundance in all 3 patients following FMT ($p<0.25$; **Supplementary Table 1**). Six of 8 OTUs that increased in abundance were not detected in the donor or the recipient prior to FMT. Therefore, expansion of rare taxa may be functionally important in restoring colonic health, at least for short-term periods, following FMT. Of the OTUs that were increased in abundance 61.5% belonged to the *Lachnospiraceae* family. The abundance of *Lachnospiraceae* has been inversely correlated with UC disease activity,(3) and those were more abundant in healthy members of monozygotic twin pairs discordant for UC, compared to control.(4) At the genus level, only *Coprococcus* changed (increased) in abundance by more than 2 fold. The abundance of *Coprococcus* (a genus including butyrate producing bacteria) has been detected to be decreased in IBD patients.(5) Therefore, the increased abundance of *Coprococcus* and *Lachnospiraceae* upon the FMT series may have delivered beneficial effects to the colonic epithelium of the UC patients (recipients).

Colonic mucosal gene expression profiles in response to the FMT series were interrogated by RNA sequencing. The expression of 742 genes decreased and 12 increased (>1.5 fold change in expression, false discovery rate [FDR]<0.05) upon the FMT therapy (**Supplementary Table 2-3**). Importantly, the suppression of human gene expression relevant in leukocyte activation and mitotic cell cycle progression was observed (**Supplementary Figure 6-7**). These molecular findings associated with >50% decline in epithelial cell mitosis in 2 out of the 3 patients (**Supplementary Figure 8**).

In conclusion, this report describes high intensity FMT as a strategy to reset the intestinal microbiota in pediatric IBD. Serial FMT in pediatric UC may induce beneficial changes in patient microbiota and colonic mucosa. Randomized trials will be required in the future to answer many challenging questions (donor selection, patient selection, number and length of FMT therapy required, etc.) in respect to the clinical application of this treatment.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

TABLES

Patients (Age, Gender)	Disease Behavior	Mayo Score At Dx	Tx after Dx	Mayo Score Before FMT	FMT #	Remission during FMT (in Days)	Mayo Score After FMT	Remission after last Medication (Days)	Remission after last FMT (Days)	Tx following Flare
1 (16y M)	Pancolitis	2	IFX	0	30	65/70 (93%)	0	261	126	IFX
2 (15y M)	Pancolitis	2	6MP	1	25	58/58 (100%)	0	159	80	PRED FMT
3 (14y F)	Pancolitis	3	PRED	0	22	36/36 (100%)	0	105	79	PRED FMT Colectomy

Table 1. Patient characteristics and clinical outcomes after sequential FMT (IFX: infliximab; 6MP: 6-mercaptopurine; PRED; prednisone).

FIGURE LEGENDS

Figure 1. Fecal Microbiota Shifts Following FMT. Principle coordinates analysis of unweighted Unifrac distances (A.) revealed that microbial community changes during FMT (arrows connect pre-FMT [within 24 hours before first FMT] and post-FMT [2-3 weeks after last FMT] samples from each patient) were not consistent within each patient (did not shift in the same direction for each patient), and post-FMT fecal communities of recipients (patients) following FMT were dissimilar from that of the donor (▲: donor stool; ■: donor preparations from independent bowel movements on separate days). Microbial richness (B.) and diversity (C.) in terms of microbial taxa consistently increased following the FMT, although these trends did not reach statistical significance.

STUDY HIGHLIGHTS

1. WHAT IS CURRENT KNOWLEDGE
    - Fecal microbiota transplantation (FMT) is an emerging treatment for UC with limited number of clinical trials showing variable results.

2. WHAT IS NEW HERE
    - Three pediatric immunosuppression-dependent UC patients received an unprecedentedly intense FMT regimen during the cessation of their conventional medications.
    - This is the first study to examine genome-wide gene expression (RNA-sequencing) responses to FMT.
    - Our single donor strategy created an unprecedented opportunity to examine the effects of one source microbiome in different recipients.
    - FMT in the context of the 3 patients studied was safe and enabled the short-term withdrawal of immunotherapy.

Keywords: inflammatory bowel disease; fecal microbiota transplantation; gene expression; ulcerative colitis

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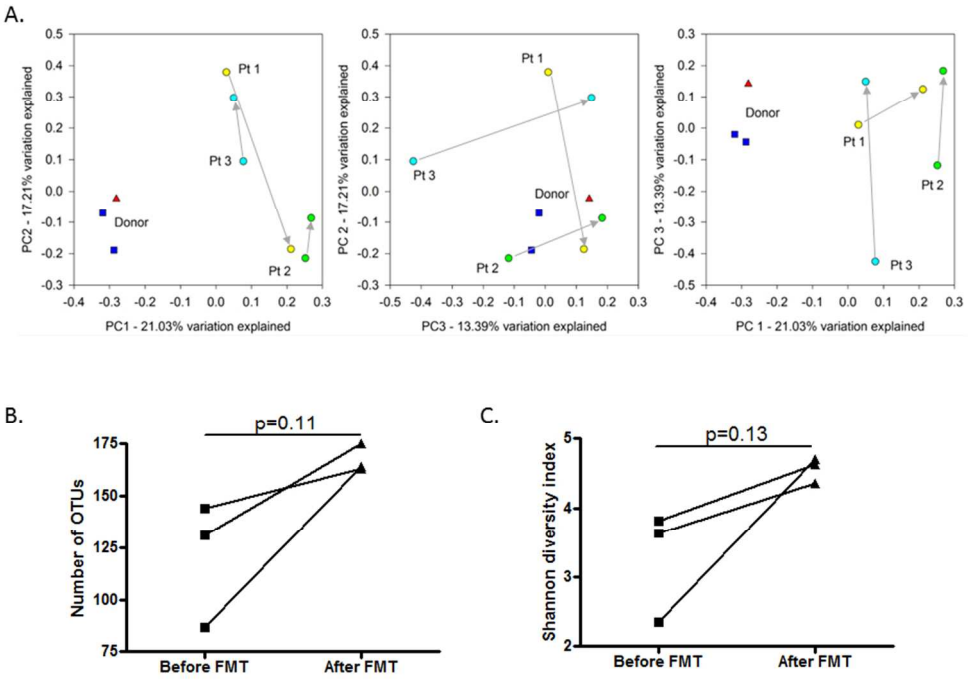

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Supplement to:
Serial Fecal Microbiota Transplantation Alters Mucosal Gene Expression in Pediatric Ulcerative Colitis

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SUPPLEMENTARY METHODS

FMT PROTOCOL

Subject and Donor Recruitment

Patients: Subjects were recruited from the patients treated by the Pediatric Gastroenterology, Hepatology, and Nutrition Section at Baylor College of Medicine/Texas Children's Hospital. Only patients whose clinical, endoscopic and histologic findings supported the diagnosis of UC were recruited. Only steroid, thiopurine, or biologic agent dependent patients were included following informed consent (i.e. "immunotherapy" dependent). Enrollees had to test negative for *Clostridium difficile* toxin by PCR, or enzyme immunoassay (EIA), and agree to withdraw all medications prior to and during the trial (see below). They also had to agree to a pre-treatment surgical consultation and acknowledge the potential need for colectomy, if disease exacerbations cannot be controlled by conventional medical therapy.

FMT Donors: Healthy adult stool donors (between 18 and 45 years of age) were recruited by the research staff following informed consent. Donors were asked to volunteer for the screening (pass a health questionnaire [Supplementary Table 4], serologic and stool tests [Supplementary Table 5]) and regularly supply stool samples according to the study protocol.

FMT preparation

The stool preparations were performed in the Texas Children's Microbiome Center (TCMC) in the Feigin Center, Texas Children's Hospital, Houston, Texas. This facility operates under Good Laboratory Practice (GLP) and is part of the clinical enterprise in the Department of Pathology, accredited by the College of American Pathologists (CAP) and certified by Clinical Laboratory Improvement Amendment (CLIA). Standard Operating Procedures on fecal specimen preparation and for decontamination procedures for biosafety cabinets and equipment were

followed before and after fecal preparation. Freshly collected stool specimens from the healthy adult donor (within 2 hours of passing) were delivered on ice for processing. Specimens were aliquoted to ~50 g aliquots, and cold sterile normal saline solution (NSS) was added prior to homogenization in a strainer bag with 500-µm pore size (Seward Laboratory Systems Inc., Port Saint Lucie, FL) using the Smasher Laboratory Blender/Homogenizer (AES CHEMUNEX Inc., Cranbury, NJ). Sterile glycerol was added to filtered homogenized stool specimens containing the fecal microbiome at a final concentration of 10% according to Hamilton et al.[1] Stool preparations were immediately stored at -80˚C until r transplantation or analysis. All stool preparations were labeled with an expiration date 8 weeks from the date of preparation. At each FMT treatment, fecal preparations were rapidly thawed at 35˚C in a water bath and used within 15 minutes. Sterile NSS was used as a diluent to reach the final volume of 250 ml from 50mg of original stool prior to delivery.

Steps before FMT treatment

Discontinuation or taper of UC therapy to initiation of FMTs: Subjects tapered their home medications for UC prior to the FMT protocol. Subjects did not have a history of antimicrobial therapy for at least 1 week prior to the initial pre-FMT colonoscopy. The corticosteroid dose was decreased to 50% of maintenance the day prior to the first pre-FMT colonoscopy. Thereafter, the dose was decreased by ~50% weekly over a maximum of 10 weeks following colonoscopy. The steroid taper was stopped once a patient's dose has been decreased to 5 mg/day for seven consecutive days. Immunomodulator and biologic treatments were discontinued for a minimum of 21 days prior to the FMT. Prebotic orprobiotic therapy were discontinued at least 1 week before the initiation of FMT. Oral or rectal treatments of mesalamine were stopped within a week of initiation of FMT. However, mesalamine preparations were allowed to be restarted in case of flares during the trial.

Surgical Consult: A surgical consultation was required prior to the initiation of FMT therapy. The subject and family were counseled with regard to possible worsening of symptoms and possible life-threatening conditions that may emerge, necessitating surgical and/or intensive care interventions.

Survey before the FMT therapy: Once a subject was enrolled in the trial, they completed a clinical symptoms survey. Only patients with remission or mild disease category (pediatric ulcerative colitis activity index;[2] PUCAI<35) were allowed to participate. We included patients with PUCAI <35 within 4 weeks of enrollment. However, all 3 patients in this report were in remission (PUCAI <10) at the start of the trial.

Initiation of FMT therapy and pre-colonoscopy preparation: One day prior to scheduled colonoscopy, subjects collected a stool sample and stored it at -20°C at home in an airtight container. The samples were brought to colonoscopy in a chilled container provided for collection and transport within 24 hours of collection and stored at -80°C. After the stool sample was obtained, the subjects started the institution's standard for colonoscopy preparation regimen with Miralax.

FMT treatment protocol

Initial colonoscopy and FMT treatment (Day 1): At the time of colonoscopy, an assessment for macroscopic colitis using the Mayo classification was performed. Biopsies were obtained from the rectosigmoid and cecum in an ascending fashion for routine histopathology and research purposes. Following mucosal sampling, subjects underwent FMT with 250 ml of thawed stool preparation, 1/3 of which was endoscopically administered into the terminal ileum and 2/3 into the right colon as targeted site as found feasible by Brandt and colleagues.[3]

Subsequent FMT Treatments: The duration of FMT therapy was planned to be 12 weeks.

Days 2 through 14: Subjects came to the ambulatory clinic daily for clinical symptom evaluation and fecal retention enema administration (60-250 ml rectally [as tolerated] with retention for at least 30 minutes).

Days 15 through 28: Enemas were given 3 times a week on weeks 3 and 4 of the protocol.

Days 29-84 (2 to 3 months): Enemas were given weekly for a total of 3-8 weeks (less than 8 secondary to the cessation of the protocol according to the FDA mandate).

As supportive care, patients were allowed to take 4 mg (2 tablets of over the counter Imodium) loperamide by mouth 15-30 minutes prior to enema treatments to help retain the preparation. This dose of loperamide is appropriate for the age group. Loperamide is over-the-counter and FDA approved for the treatment of inflammatory bowel disease associated diarrhea.

Response and progression was monitored by PUCAI during the protocol. The clinical symptoms survey was performed prior to each enema delivery and a disease progression table was recorded for each enrolled patient.

Follow-up

Follow-up colonoscopy/sigmoidoscopy, sample collection and evaluation

Two weeks after the last weekly enema, colonoscopy with FMT was performed for patient 1. For the consecutive patients, we decided to perform flexible sigmoidoscopy without prior colon cleansing and those patients did not receive additional FMT. Stool samples were collected within one day of sigmoidoscopy. At the time of sigmoidoscopy, an assessment for macroscopic colitis using the Mayo classification was performed. Biopsies were obtained from the rectosigmoid area for routine histopathology and research purposes.

DETAILED CASE REPORTS

The patients were enrolled into an Institutional Review Board (IRB) approved clinical trial prior to the Food and Drug Administration (FDA) mandating the need for an investigational drug application for FMT (that is not directed towards the treatment of recurrent CDI). The protocol was stopped after the FDA mandate was issued in May of 2013. Therefore, only 1 patient (patient 1) completed the full 12 week course (28 scheduled treatments + "rescue" enemas if clinically indicated) of FMT. The clinical course of patients is shown in Supplementary Figure 1.

Patient 1: A 16-year-old male was diagnosed with pancolitis (Mayo score[4]=2; Supplementary Figure 3) one year before enrollment. His prior therapy included infliximab, oral corticosteroids and mesalamine. He was weaned off oral steroids and continued on infliximab infusions with oral mesalamine. FMT was initiated when he was in clinical (pediatric ulcerative colitis activity index;[2] PUCAI=5), macroscopic (Supplementary Figure 3), and microscopic (Supplementary Figure 4) remission after 6 weeks without infliximab. Thirty (30) treatments were administered during 3 months. He received mesalamine enemas twice for mild exacerbations (PUCAI ≥15). The patient remained in clinical remission (PUCAI <10) for more than 90% (65/70 days) of the treatment course (Supplementary Figure 1A). No adverse events were noted. A follow-up endoscopy 3 weeks after the last FMT showed the entire colon to be grossly normal (Supplementary Figure 3). However, histological inflammation in his transverse colon was detected, and maintenance oral mesalamine therapy was started. The patient remained in clinical remission (symptom-free, lab values within normal ranges, including fecal calprotectin) for 8 months (261 days) after his last infliximab treatment, 4 months (126 days) after his last FMT. At this time, he developed a mild disease flare (PUCAI=25) and wished to go back on infliximab therapy. He has intermittent breakthrough symptoms on 7.5 mg/kg infliximab every 6 weeks and daily mesalamine treatments for over 1.5 years since re-institution of the biologic agent.

Patient 2: A 15-year-old male with a history of UC with pan-colitis (Mayo: 2; Supplementary Figure 3) was treated for more than one year with 6-mercaptopurine (6MP), allopurinol, and daily oral mesalamine. He tested positive for toxigenic *Clostridium difficile* infection (CDI) at enrollment. Metronidazole therapy successfully cleared the CDI prior to starting FMT. Allopurinol and 6MP were stopped 3 weeks prior to initiation of FMT. Initial colonoscopy showed mildly active colitis (Mayo: 1; Supplementary Figure 3) with histological inflammation (Supplementary Figure 4) throughout the colon. Mesalamine was stopped on day 6 after initiation of FMT. The patient remained in remission throughout FMT therapy (Supplementary Figure 1B). Twenty-five FMT treatments were administered to this patient, and no adverse events were noted. The colon was grossly normal by sigmoidoscopy performed 2 weeks after the final FMT (Mayo 0; Supplementary Figure 3). No evidence of mucosal inflammation in the colon was found by histology (Supplementary Figure 4). This patient remained in remission (PUCAI<10) without any therapy for more than 2 months (80 days after last FMT, 159 days after last 6MP dose). However, he became symptomatic (PUCAI=35) and did not respond to oral mesalamine therapy following this symptom-free period. Pulse steroid treatments were initiated, and he required intermittent low dose steroid therapy with 4-5 week steroid-free intervals. The patient re-enrolled into our FMT trial 1 year afterwards, and is currently in remission off of any therapy following a second course of FMTs for more than 2 months after last FMT with normal fecal calprotectin level.

Patient 3: A 14-year-old female was diagnosed with severely active pancolitis four months prior to enrollment (Mayo 3, Supplementary Figure 3). She could not be weaned off steroids for more than 4 week intervals, and she opted to enroll into the FMT trial. Steroids were tapered during the first 10 days of FMT treatment, and mesalamine was stopped on day 14. She remained in remission during the FMT period (Supplementary Figure 1C). She received 22 rounds of FMT

with only one episode of moderate, self-resolving, positional headache noted. Sigmoidoscopy 2 weeks after the last FMT showed a grossly normal (Mayo 0) colon. No evidence of colonic mucosal inflammation was found by histology. She developed a flare during travel 4 weeks following the last FMT (PUCAI=20). Oral mesalamine successfully induced remission. She repeatedly became symptomatic with bloody stools and cramping (PUCAI=50) 11 weeks (79 days) after the last FMT (105 days after last steroid dose), at which time pulse steroid therapy was initiated. She remained steroid dependent following re-initiation of steroid therapy. The patient re-enrolled into our FMT trial 1 year afterwards, but was withdrawn from the study for worsening symptoms and eventually opted for colectomy after 2 months of steroid therapy.

MICROBIOME ANALYSES

Fecal microbiome characterization: Stool samples were processed by the Texas Children's Microbiome Center (Texas Children's Hospital, Houston, TX, USA) for DNA extraction and sequencing. Community DNA was extracted from each specimen using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA), following manufacturer's instructions. The resulting DNA was quantified using both a NanoDrop-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA) and Qubit fluorometer (Life Technologies Corporation, Carlsbad, CA, USA). Barcoded universal primers 357F (5'-CCTACGGGAGGCAGCAG-3') and 926R (5'-CCGTCAATTCMTTTRAGT-3') were used to amplify the V3V5 region of the bacterial *16S rRNA* gene. Each library construct was then processed and purified for 454 sequencing. Sequencing was performed on the Roche GS FLX 454 sequencer (454 Life Sciences, Branford, CT, USA).

Data analysis: Sequence data was parsed by barcode and quality filtered using QIIME (version 1.3.0),[5] as implemented in the Genboree Microbiome Toolset.[6] Sequences shorter than 200 bp length, having average quality scores less than 20, harboring ambiguous base calls, or having

mismatches to their barcode or sequencing primer were excluded from further analysis. Both the barcodes and sequencing primers were trimmed away, and the remaining sequences from the donor and patients were pooled and assigned to operational taxonomic units (OTUs) at a similarity cut off of 97% using Cd-hit.[7] The data set was screened for potential chimeras using the ChimeraSlayer algorithm[8], and all potential chimeras were excluded from downstream analysis. Identities were assigned to each OTU using the Ribosomal Database Project Classifier.[9] Given variation in sequencing depth, the 16S rRNA gene libraries were sub-sampled to an equal depth (i.e., 2600 sequences per library) prior to the evaluation of richness or calculation of diversity indices, including the Shannon diversity index and unweighted UniFrac distance measures. The results from the microbiome characterization of the donor were compared to the microbiome of the patients pre-transplant and at 2-weeks following the end of FMT therapy.

The sequences generated for this project were deposited in the NCBI Sequence Read Archive (SRA) under project accession SRP034948

RNA SEQUENCING AND ANALYSES

Total RNA was isolated from rectal biopsy specimens (stored in RNALater™ [Ambion through Life Technologies, Carlsbad, California]) according to the manufacturer's recommendation, with Trizol (15596, Life Technologies) and the RNeasy Mini Kit (74106, Qiagen). RNA samples were stored at −80°C until processing for RNA sequencing.

RNA samples from colon biopsies were QCed by spectrophotometry (NanoDrop-1000 Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, U.S.A.) and microfluidic electrophoresis (Experion Automated Electrophoresis System, Bio-Rad Laboratories, Hercules, CA). PolyA-selected libraries were prepared from total RNA samples with TruSeq RNA Sample Preparation Kits (Illumina, San Diego, CA). Cluster generation was performed with Illumina

TruSeq SR Cluster Kits v3 - cBot – HS, in a cBot Cluster Generation System and 100 bp paired-end-sequencing using Illumina TruSeq SBS Kits on an Illumina HiSeq 2000 Sequencing System resulting in mean sequencing depth of 160 million (101-213 million) reads per sample. CASAVA software (Illumina) was used to convert raw read data to fastq format. Sequencing reads were trimmed for quality (q < 20) and adapters and then aligned to the human genome (GRCh37/hg19) using Tophat2[9]. Cufflinks[10] was used for estimation of transcript abundances based on Fragments Per Kilobase of exon per Million fragments mapped (FPKM). Differential expression analysis was carried out using Cuffdiff, which calculates a test statistic based on the log ratio of a gene's expression in two conditions against the log of one. Multiple testing correction at a false discovery rate < 0.05 was applied to identify differentially expressed genes. The raw data was made publically accessible in Bioproject: http://www.ncbi.nlm.nih.gov/bioproject/253048

HISTOLOGY

The biopsy specimens were examined by a board-certified pediatric pathologist with expertise in GI pathologywho was blinded to previously reported histology reports. The specimens were fixed in 10% neutral buffered formalin immediately following endoscopy. The tissue samples were routinely processed and paraffin embedded. Paraffin sections (3 micron) were cut and stained with hematoxylin and eosin (H&E) with eight tissue sections on one slide. Duplicated 3 micron sections were stained for histone (H3) via immunohistochemistry using a polyclonal rabbit anti-human histone (06-570; Millipore, Billerica, Massachusetts). After pretreatment with HIER1 (Bond Epitope Retrieval Solution 1) for 30 minutes at 100 degreesFahrenheit, the specimens were incubated with the primary antibody dilution 1:800 at room temperature for 15 minutes. The detection system used was the Novocastra Bond Polymer Refine Detection System (biotin-free, peroxide conjugated) from LEICA, Newcastle upon Tyne, United Kingdom

with diaminobenzidine tetrahydrochloride (DAB) as the chromogen and hematoxylin as the counterstain.

STATISTICAL ANALYSES

The two-tailed paired t-test was used for group comparisons. Significance was relaxed to an arbitrary $p<0.25$ for OTU comparisons; otherwise it was determined at $p<0.05$. The Pearson correlation coefficient was calculated in Excel (Microsoft Office Excel 2007).Statistical significance of correlations was calculated with public Statistics Calculator version 3.0 (http://www.danielsoper.com/statcalc3/calc.aspx?id=44) and determined at $p<0.05$. See Methods section in Supplementary Appendix for bioinformatic analyses applied to the RNA sequencing data.

SUPPLEMENTARY TABLES

#OTU ID	Lowest taxonomic assignment	Before FMT (%)	After FMT (%)	Average change in abundance (%)	Average in donor preparations (%)
1179	Lachnospiraceae	1.538	6.205	4.667	0.404
1164	Lachnospiraceae	0.218	3.423	3.205	0.115
1406	Dorea	0.705	3.590	2.885	0.096
1886	Lachnospiraceae	0.205	1.885	1.679	0.000
1894	Lachnospiraceae	0.038	1.474	1.436	0.000
925	Ruminococcaceae	0.282	1.154	0.872	0.462
1929	Lachnospiraceae	0.013	0.410	0.397	0.058
577	Faecalibacterium	0.000	0.321	0.321	0.000
1094	Lachnospiraceae	0.000	0.295	0.295	0.000
1259	Ruminococcaceae	0.000	0.115	0.115	0.000
1135	Lachnospiraceae	0.000	0.064	0.064	0.000
423	Collinsella	0.000	0.051	0.051	0.000
1081	Lachnospiraceae	0.000	0.051	0.051	0.000
616	Ruminococcaceae	0.051	0.013	-0.038	0.000
1605	Streptococcus	1.244	0.192	-1.051	0.365

Supplementary Table 1: Consistent abundance (%) changes in Operational Taxonomic Units (OTUs) with lowest taxonomic assignment in the fecal microbiomes upon the FMT series in all 3 patients. Before and after values are average abundances from the 3 patients before and 2 weeks after the FMTs

Gene_id	Gene	Expression change	q_value
ENSG00000137757	FOLH1B	0.004	0.008
ENSG00000075856	CXCL5	0.010	0.000
ENSG00000064607	FSIP1	0.026	0.019
ENSG00000026508	SAA2	0.029	0.004
ENSG00000069493	SAA1	0.037	0.000
ENSG00000121152	RP11-124L5.7.1	0.052	0.012
ENSG00000126091	RC3H2	0.083	0.013
ENSG00000114742	TNIP3	0.084	0.002
ENSG00000065361	AC027323.1,LYSMD3	0.099	0.000
ENSG00000131236	PGLYRP4	0.110	0.024
ENSG00000075785	SLC6A20	0.169	0.001
ENSG00000106392	TNFSF15	0.171	0.031
ENSG00000198498	MTX3	0.179	0.000
ENSG00000111224	IL19	0.181	0.021
ENSG00000113593	TNFRSF6B	0.186	0.013
ENSG00000125885	FPR1	0.191	0.033

ENSG00000111711	ALPL	0.196	0.000
ENSG00000196517	FCGR3B	0.197	0.025
ENSG00000162747	AC131025.8.1,MIR143HG	0.204	0.007
ENSG00000141076	IL6STP1	0.206	0.032
ENSG00000136824	RP11-173P15.3.1	0.214	0.011
ENSG00000130881	RP11-622O11.2.1	0.224	0.040
ENSG00000122786	KCND3	0.229	0.001
ENSG00000261804	MCM10	0.229	0.020
ENSG00000108424	ANKRD36BP1	0.231	0.003
ENSG00000163482	AC110491.1	0.233	0.025
ENSG00000243955	TFPI2	0.236	0.020
ENSG00000173200	SKA3	0.237	0.003
ENSG00000117600	CREB3L3	0.238	0.000
ENSG00000184575	WASF3	0.239	0.001
ENSG00000172269	PHKA1P1	0.240	0.048
ENSG00000100934	GPR155	0.254	0.002
ENSG00000169398	PRSS21	0.258	0.042
ENSG00000138780	TPO	0.259	0.038
ENSG00000188529	THEMIS	0.259	0.006
ENSG00000214331	TRIM40	0.266	0.000
ENSG00000134851	OLFM4	0.266	0.019
ENSG00000149150	ST3GAL3	0.269	0.000
ENSG00000165118	HIN1L.1	0.272	0.038
ENSG00000104164	RP11-44F14.2.1	0.274	0.048
ENSG00000160949	CXCL1	0.277	0.019
ENSG00000109685	HS3ST2	0.281	0.000
ENSG00000197056	CDC7	0.285	0.001
ENSG00000165689	ADAMTS1	0.290	0.007
ENSG00000103197	RSAD2	0.292	0.003
ENSG00000115159	PADI1	0.294	0.046
ENSG00000090924	RP11-1220K2.2.1	0.297	0.010
ENSG00000241878	ERO1L	0.298	0.000
ENSG00000140575	CDCA2	0.304	0.046
ENSG00000160818	MTF1	0.308	0.019
ENSG00000147454	SHCBP1	0.309	0.019
ENSG00000188786	GVINP1	0.310	0.005
ENSG00000114770	DDX21	0.311	0.006
ENSG00000198373	RAD54L2	0.312	0.001
ENSG00000133816	CXCL3	0.315	0.001
ENSG00000221955	ZBTB41	0.318	0.002
ENSG00000157349	WDHD1	0.320	0.001
ENSG00000131149	TRAT1	0.321	0.046
ENSG00000183814	TET3	0.323	0.001
ENSG00000077809	RASGRF2	0.326	0.036
ENSG00000156990	ANLN	0.333	0.000
ENSG00000104388	PMAIP1	0.334	0.027
ENSG00000112812	XRN1	0.334	0.007

ENSG00000156931	ARPP19	0.336	0.041
ENSG00000156976	ATF7IP	0.341	0.000
ENSG00000113140	C6orf223	0.341	0.048
ENSG00000163820	CTPS	0.342	0.000
ENSG00000119950	RP11-356C4.4.1	0.344	0.034
ENSG00000120756	FAM111B	0.345	0.000
ENSG00000087586	SUV39H2	0.346	0.002
ENSG00000166912	CHI3L1	0.348	0.005
ENSG00000243943	HAS3	0.348	0.015
ENSG00000198431	ENAH	0.348	0.000
ENSG00000163655	CALD1	0.350	0.002
ENSG00000130653	ORC6	0.352	0.000
ENSG00000198964	CDC45	0.353	0.004
ENSG00000102901	CD28	0.353	0.020
ENSG00000107566	XRCC2	0.353	0.029
ENSG00000079246	NAV2	0.353	0.033
ENSG00000169251	SOCS3	0.355	0.033
ENSG00000113240	FSD1L	0.356	0.030
ENSG00000122188	SYNPO2	0.357	0.000
ENSG00000184220	RCAN3	0.357	0.007
ENSG00000021762	CHEK1	0.359	0.000
ENSG00000160072	CASP5	0.360	0.000
ENSG00000141682	PRDM1	0.361	0.000
ENSG00000133789	SCD	0.362	0.023
ENSG00000070269	PTPRC	0.365	0.000
ENSG00000135829	FSTL1	0.366	0.000
ENSG00000163714	MCM4	0.367	0.000
ENSG00000175216	C4BPB	0.368	0.003
ENSG00000143537	HELLS	0.368	0.004
ENSG00000143507	ACTG2	0.371	0.001
ENSG00000018699	DDX3Y	0.371	0.002
ENSG00000089280	RP11-343J24.1.1	0.373	0.015
ENSG00000165480	CDC25A	0.374	0.024
ENSG00000176945	GUCY1A3	0.375	0.047
ENSG00000164180	CHL1	0.377	0.044
ENSG00000101057	ATM	0.378	0.002
ENSG00000097046	ST3GAL1	0.378	0.006
ENSG00000177192	CLMP	0.379	0.040
ENSG00000142224	RTEL1.1	0.380	0.000
ENSG00000138074	CDC6	0.385	0.030
ENSG00000006652	RRM2	0.385	0.000
ENSG00000068438	ITGAL	0.387	0.000
ENSG00000154451	PHIP	0.390	0.015
ENSG00000100890	SLFN5	0.393	0.025
ENSG00000148926	FCRL1	0.393	0.000
ENSG00000103111	SMC4	0.394	0.000
ENSG00000148308	MS4A14	0.396	0.034

ENSG00000086015	PLEKHH2	0.397	0.050
ENSG00000055732	AHR	0.398	0.019
ENSG00000105993	PARP15	0.399	0.001
ENSG00000146670	ZFY	0.399	0.022
ENSG00000204394	FAM169A	0.400	0.014
ENSG00000168488	CSF3R	0.404	0.005
ENSG00000116539	RRM1	0.405	0.000
ENSG00000111912	RGS5	0.405	0.005
ENSG00000166923	CDCA5	0.406	0.007
ENSG00000137497	SLC7A6	0.406	0.000
ENSG00000170581	RP4-788L13.1.1	0.408	0.019
ENSG00000121281	CTDSPL2	0.409	0.012
ENSG00000094841	ATAD2	0.411	0.024
ENSG00000129422	NF1	0.411	0.000
ENSG00000114127	CENPK	0.412	0.000
ENSG00000160796	GREM1	0.412	0.000
ENSG00000091436	IL1RN	0.413	0.008
ENSG00000171051	KIT	0.413	0.042
ENSG00000177034	SLC38A7	0.416	0.027
ENSG00000105483	LMO7	0.417	0.000
ENSG00000174574	BIRC3	0.417	0.000
ENSG00000082153	MS4A1	0.419	0.000
ENSG00000164244	BLM	0.419	0.013
ENSG00000134369	SPAG9	0.420	0.004
ENSG00000152455	RTEL1,TNFRSF6B	0.423	0.014
ENSG00000117228	RP11-904M10.1.1	0.424	0.017
ENSG00000136560	GBP5	0.424	0.000
ENSG00000204120	ZNF169	0.424	0.011
ENSG00000168071	NEDD1	0.426	0.000
ENSG00000169679	KIF18B	0.426	0.003
ENSG00000157212	FAM55B	0.427	0.004
ENSG00000164649	CTA-286B10.7.1,MCM5	0.427	0.000
ENSG00000196873	MEF2C	0.427	0.010
ENSG00000123562	MEST	0.427	0.000
ENSG00000163017	TXNRD1	0.428	0.000
ENSG00000177888	PDP1	0.428	0.046
ENSG00000250251	REL	0.428	0.020
ENSG00000198121	C3orf26	0.428	0.001
ENSG00000112701	DHFR	0.431	0.012
ENSG00000013561	FCRL3	0.431	0.016
ENSG00000163519	MCM6	0.432	0.002
ENSG00000092439	B3GALT1	0.434	0.000
ENSG00000131504	NCOA7,RP11-73O6.4.1	0.437	0.000
ENSG00000132466	GBP1	0.439	0.001
ENSG00000148175	TRANK1	0.439	0.003
ENSG00000138398	DOCK2	0.440	0.006
ENSG00000118495	SMC1A	0.442	0.002

ENSG00000167522	PARP11	0.442	0.003
ENSG00000086666	SLFN13	0.442	0.002
ENSG00000140403	KYNU	0.443	0.025
ENSG00000166927	NCAPG	0.443	0.007
ENSG00000182866	ACSL4	0.443	0.036
ENSG00000215837	BANK1	0.444	0.013
ENSG00000138032	NFAT5	0.449	0.013
ENSG00000132142	CYR61	0.450	0.010
ENSG00000104205	TLE4	0.451	0.009
ENSG00000143297	RNASEH1	0.451	0.000
ENSG00000163635	PRKAA1	0.452	0.001
ENSG00000138764	GSTCD	0.452	0.030
ENSG00000099917	SCFD2	0.454	0.026
ENSG00000143811	HEG1	0.455	0.047
ENSG00000204713	SVEP1	0.456	0.025
ENSG00000159618	CEP135	0.456	0.006
ENSG00000175643	MCM8	0.456	0.000
ENSG00000135486	GART	0.456	0.000
ENSG00000124839	AKAP2	0.457	0.037
ENSG00000143337	S1PR1	0.459	0.021
ENSG00000166411	DEK	0.459	0.000
ENSG00000196159	LCP1	0.460	0.005
ENSG00000145041	ATXN7	0.460	0.022
ENSG00000214262	CIITA	0.461	0.001
ENSG00000213430	LONP2	0.461	0.000
ENSG00000079385	IL7R	0.461	0.035
ENSG00000168610	KIF24	0.462	0.041
ENSG00000140968	DUSP10	0.463	0.002
ENSG00000136699	SSX2IP	0.463	0.000
ENSG00000049130	PDE7A	0.464	0.000
ENSG00000140395	ACACA	0.465	0.001
ENSG00000100030	SAMD4A	0.466	0.010
ENSG00000106546	GTPBP1	0.466	0.002
ENSG00000110104	CFB	0.467	0.000
ENSG00000170144	CIRH1A	0.467	0.000
ENSG00000140263	MCM2	0.469	0.001
ENSG00000013503	CTTNBP2NL	0.469	0.003
ENSG00000113368	ITGA4	0.469	0.028
ENSG00000065328	IMPAD1	0.469	0.048
ENSG00000122420	MSH6	0.470	0.004
ENSG00000182271	SNHG3	0.470	0.020
ENSG00000154153	RFC5	0.471	0.000
ENSG00000077147	LMNB1	0.471	0.000
ENSG00000005483	DAPP1	0.472	0.000
ENSG00000172673	DIAPH3	0.473	0.004
ENSG00000138772	PCGF3	0.473	0.034
ENSG00000256364	GPRIN1	0.473	0.009

ENSG00000127603	KITLG	0.474	0.029
ENSG00000147649	STARD4	0.474	0.003
ENSG00000095564	WDR43	0.474	0.005
ENSG00000198901	NUP107	0.474	0.000
ENSG00000115282	FILIP1L	0.475	0.041
ENSG00000081026	GNL3L	0.475	0.001
ENSG00000116649	CNN1	0.475	0.000
ENSG00000024526	MAVS	0.475	0.006
ENSG00000134453	LIN9	0.477	0.000
ENSG00000232882	NT5C2	0.477	0.007
ENSG00000157827	ADCY7	0.478	0.019
ENSG00000114346	RP11-252A24.2.1	0.479	0.046
ENSG00000102837	FGD2	0.479	0.000
ENSG00000160688	TMEM97	0.479	0.000
ENSG00000140718	RAD51AP1	0.480	0.003
ENSG00000154237	WDFY1	0.482	0.034
ENSG00000198554	NBPF8	0.483	0.048
ENSG00000156804	CDK1	0.484	0.043
ENSG00000139641	TCF7	0.485	0.013
ENSG00000160703	LYN	0.485	0.025
ENSG00000105568	LEF1	0.485	0.027
ENSG00000162599	FCGR3A	0.486	0.000
ENSG00000172318	FAM55C	0.486	0.011
ENSG00000090316	EPB41L2	0.486	0.000
ENSG00000085449	MAP3K8	0.487	0.037
ENSG00000109919	TEAD4	0.487	0.002
ENSG00000145908	NPAS2	0.487	0.002
ENSG00000130826	RMI2	0.488	0.017
ENSG00000206053	NOLC1	0.488	0.027
ENSG00000197905	EHF	0.488	0.034
ENSG00000179978	RALGPS2	0.489	0.025
ENSG00000115239	USP45	0.489	0.021
ENSG00000139842	PHF20L1	0.489	0.002
ENSG00000125962	RAB5B	0.489	0.012
ENSG00000196584	SERPINE2	0.489	0.000
ENSG00000162692	AASS	0.489	0.034
ENSG00000181163	SGK3	0.490	0.004
ENSG00000172403	TNPO1	0.490	0.003
ENSG00000256667	PRRC2B	0.490	0.000
ENSG00000102606	FGR	0.491	0.000
ENSG00000136167	JAK3	0.491	0.022
ENSG00000168685	SMC2	0.491	0.000
ENSG00000079819	MSL2	0.492	0.045
ENSG00000109084	RPS6KA3	0.492	0.035
ENSG00000237683	BUB1	0.492	0.006
ENSG00000128928	RP11-261C10.3.1	0.493	0.023
ENSG00000168016	RAPGEF2	0.493	0.022

ENSG00000006744	ARHGAP11A	0.494	0.045
ENSG00000136286	RACGAP1	0.494	0.017
ENSG00000072501	MCOLN3	0.494	0.008
ENSG00000166197	MYBL2	0.494	0.041
ENSG00000116353	SEC23A	0.495	0.003
ENSG00000163735	PNPT1	0.495	0.000
ENSG00000088247	HAPLN3	0.496	0.031
ENSG00000093009	ECT2	0.496	0.000
ENSG00000146192	DYNC1LI2	0.496	0.005
ENSG00000196712	POLD3	0.496	0.005
ENSG00000138756	NAV1	0.497	0.027
ENSG00000137824	PLS1	0.497	0.001
ENSG00000143033	SLC5A6	0.497	0.000
ENSG00000137770	HERC3	0.498	0.012
ENSG00000132356	ARHGAP42	0.498	0.002
ENSG00000132780	COL12A1	0.498	0.048
ENSG00000029363	LILRB1	0.499	0.015
ENSG00000112096	AC013461.1.1	0.499	0.049
ENSG00000115919	PKD1P6	0.500	0.019
ENSG00000137504	SECISBP2L	0.500	0.011
ENSG00000126226	RFC4	0.501	0.000
ENSG00000203710	MGA	0.501	0.003
ENSG00000145725	GLIPR1	0.501	0.022
ENSG00000136319	BCLAF1	0.501	0.000
ENSG00000073417	PLDN	0.502	0.006
ENSG00000080189	PFAS	0.503	0.000
ENSG00000130723	FTO	0.503	0.027
ENSG00000164506	GEN1	0.504	0.000
ENSG00000177479	UBQLN1	0.504	0.004
ENSG00000166803	EXOC5	0.504	0.009
ENSG00000114857	SLAMF1	0.505	0.001
ENSG00000171385	STOM	0.505	0.015
ENSG00000049449	YIPF6	0.506	0.034
ENSG00000093183	FBXO32	0.506	0.000
ENSG00000259141	LMBR1	0.508	0.004
ENSG00000138795	GPD2	0.508	0.048
ENSG00000168918	PLEKHG2	0.508	0.007
ENSG00000197157	POLK	0.508	0.000
ENSG00000203879	TTC5	0.509	0.008
ENSG00000163734	ZNF268	0.509	0.000
ENSG00000135316	PRKD3	0.512	0.036
ENSG00000257743	WDR76	0.512	0.012
ENSG00000115232	TAF1A	0.513	0.019
ENSG00000116521	CD44	0.513	0.000
ENSG00000104331	PBK	0.513	0.008
ENSG00000145734	FAM126B	0.514	0.024
ENSG00000095739	DES	0.514	0.000

ENSG00000119408	C12orf48	0.514	0.001
ENSG00000198663	WDR62	0.514	0.034
ENSG00000175376	NR2C2	0.515	0.007
ENSG00000186998	ZEB2	0.515	0.031
ENSG00000155324	TTC27	0.515	0.042
ENSG00000179820	HPRT1	0.516	0.015
ENSG00000166012	STK38L	0.516	0.019
ENSG00000256269	AC093510.2	0.516	0.021
ENSG00000153395	CEP350	0.518	0.001
ENSG00000116062	IL10RA	0.518	0.033
ENSG00000160828	COL8A2	0.518	0.022
ENSG00000050426	HN1L	0.518	0.002
ENSG00000137801	GPRC5A	0.518	0.000
ENSG00000178295	CLEC2D	0.518	0.007
ENSG00000093167	HECTD2	0.519	0.000
ENSG00000197343	KDM2B	0.519	0.030
ENSG00000155545	CELF1	0.519	0.022
ENSG00000112297	LRRK2	0.520	0.019
ENSG00000139197	RAD54L	0.521	0.007
ENSG00000198826	FAT4	0.521	0.003
ENSG00000100376	CR1	0.522	0.002
ENSG00000177853	NAMPTL	0.522	0.028
ENSG00000112739	BMP2K	0.523	0.026
ENSG00000176619	MAN2A2	0.524	0.000
ENSG00000008513	GTF2I	0.524	0.006
ENSG00000221914	C1GALT1	0.524	0.000
ENSG00000163755	MYADM	0.524	0.000
ENSG00000137804	TRIB2	0.524	0.000
ENSG00000230724	STAT1	0.525	0.006
ENSG00000047457	SENP2	0.525	0.004
ENSG00000159131	KLHL29	0.526	0.000
ENSG00000167325	RASGRP4	0.526	0.019
ENSG00000186638	CAND1	0.526	0.000
ENSG00000134121	BZW1	0.526	0.006
ENSG00000005844	TONSL	0.527	0.008
ENSG00000071127	PALLD	0.528	0.006
ENSG00000003402	ETNK1	0.528	0.036
ENSG00000165792	INVS	0.528	0.003
ENSG00000104738	FAM118A	0.528	0.000
ENSG00000144580	PUS1	0.529	0.000
ENSG00000138185	NCAPD2	0.529	0.021
ENSG00000105639	HSPD1P1	0.529	0.007
ENSG00000122512	PRMT3	0.530	0.000
ENSG00000140829	MTR	0.532	0.001
ENSG00000090060	XPO4	0.532	0.032
ENSG00000197170	DDX11	0.533	0.000
ENSG00000118855	FGFR1	0.534	0.016

ENSG00000135968	HMG20A	0.534	0.001
ENSG00000143079	GCC2	0.535	0.001
ENSG00000114026	LPCAT1	0.535	0.000
ENSG00000188807	CAPRIN1	0.535	0.000
ENSG00000163918	NCAPH	0.535	0.031
ENSG00000159176	SLC25A37	0.536	0.004
ENSG00000198586	FMR1	0.536	0.000
ENSG00000205336	TYMS	0.536	0.013
ENSG00000163832	NFKBIZ	0.537	0.000
ENSG00000184863	TPD52L1	0.538	0.039
ENSG00000143375	IPO11	0.538	0.035
ENSG00000165732	PPP1R12A	0.538	0.000
ENSG00000182541	MTUS1	0.539	0.000
ENSG00000092201	WARS	0.539	0.001
ENSG00000111206	RPS6KB1	0.539	0.013
ENSG00000050730	NKTR	0.540	0.000
ENSG00000205583	MLLT6	0.540	0.009
ENSG00000113810	ZDHHC8	0.543	0.035
ENSG00000167775	ABCC5	0.544	0.002
ENSG00000107625	DCLRE1B	0.544	0.005
ENSG00000056097	CCP110	0.544	0.017
ENSG00000196547	UBE2T	0.545	0.036
ENSG00000144231	TOR1AIP1	0.545	0.000
ENSG00000013573	PPIP5K2	0.545	0.020
ENSG00000137267	MLL5	0.545	0.000
ENSG00000171681	SLC10A7	0.546	0.000
ENSG00000040341	SCLY	0.547	0.038
ENSG00000135919	HBS1L	0.547	0.000
ENSG00000173276	HYAL1	0.548	0.024
ENSG00000117155	GNAI1	0.548	0.044
ENSG00000135720	PPP2R1B	0.548	0.002
ENSG00000177189	MAPK1	0.548	0.036
ENSG00000102908	NFIX	0.548	0.022
ENSG00000073921	EIF1AY	0.549	0.034
ENSG00000105221	DPH2	0.549	0.000
ENSG00000132970	ABI3BP	0.550	0.014
ENSG00000082213	PARP14	0.550	0.023
ENSG00000043462	KBTBD2	0.550	0.007
ENSG00000144802	XRCC5	0.550	0.000
ENSG00000149269	BOP1	0.551	0.045
ENSG00000165124	ASH1L	0.551	0.044
ENSG00000117335	DKC1	0.551	0.009
ENSG00000119535	NMD3	0.552	0.001
ENSG00000165895	GLS	0.553	0.001
ENSG00000115364	KIAA1530	0.553	0.019
ENSG00000122008	MFN1	0.554	0.043
ENSG00000103202	CP	0.555	0.035

ENSG00000100852	MS4A7	0.555	0.011
ENSG00000111530	PTGFR	0.555	0.001
ENSG00000167491	CORO1C	0.555	0.032
ENSG00000138768	TCHP	0.555	0.032
ENSG00000196696	ANKRD17	0.555	0.050
ENSG00000144852	WEE1	0.555	0.004
ENSG00000129116	WDR1	0.557	0.004
ENSG00000227018	PDLIM5	0.557	0.002
ENSG00000132768	SLC6A9	0.558	0.037
ENSG00000164116	CLSTN3	0.558	0.015
ENSG00000154760	FAM86A	0.558	0.001
ENSG00000160593	MCM3	0.559	0.003
ENSG00000071575	KIAA0101	0.559	0.000
ENSG00000130227	PBX3	0.559	0.005
ENSG00000225241	FAM129C	0.559	0.000
ENSG00000129292	SOD2	0.559	0.014
ENSG00000111737	ATL2	0.559	0.000
ENSG00000203747	SORD	0.560	0.008
ENSG00000133835	PLXDC1	0.560	0.002
ENSG00000125730	DDX18	0.561	0.024
ENSG00000204614	SCAMP5	0.562	0.041
ENSG00000112282	RABL3	0.562	0.014
ENSG00000068308	NFATC2IP	0.562	0.000
ENSG00000138035	TMPO	0.563	0.014
ENSG00000111011	XPOT	0.564	0.045
ENSG00000162551	ADAM10	0.564	0.030
ENSG00000159140	SREK1	0.564	0.001
ENSG00000118894	LCP2	0.565	0.031
ENSG00000135387	SF3B3	0.565	0.000
ENSG00000103044	MTDH	0.566	0.001
ENSG00000120802	GOLT1B	0.568	0.028
ENSG00000117523	CDCA7L	0.568	0.000
ENSG00000121749	WHSC1	0.568	0.029
ENSG00000174799	SLC43A1	0.569	0.033
ENSG00000139190	STAT3	0.569	0.006
ENSG00000177426	GNA12	0.569	0.000
ENSG00000137752	SLC38A5	0.570	0.006
ENSG00000176142	DIAPH1	0.570	0.000
ENSG00000154175	VRK2	0.570	0.000
ENSG00000184163	FOXM1	0.571	0.032
ENSG00000125107	GNPDA2	0.571	0.004
ENSG00000154096	TMEM201	0.572	0.002
ENSG00000139163	BTN3A3	0.572	0.002
ENSG00000139182	KPNB1	0.572	0.005
ENSG00000163608	LIN54	0.573	0.000
ENSG00000057252	TMEM39A	0.573	0.004
ENSG00000163534	SMC6	0.574	0.003

ENSG00000163935	C9orf64	0.574	0.039
ENSG00000108039	NFIA	0.575	0.000
ENSG00000164045	POLR1B	0.575	0.041
ENSG00000086200	CALU	0.575	0.013
ENSG00000170485	VAMP1	0.576	0.032
ENSG00000135373	ACSL3	0.576	0.000
ENSG00000114867	GMPS	0.576	0.001
ENSG00000088833	MORF4L2	0.576	0.000
ENSG00000008294	MIER3	0.576	0.000
ENSG00000131018	KIF16B	0.577	0.046
ENSG00000139218	RAP1GDS1	0.577	0.000
ENSG00000144283	ZZZ3	0.578	0.001
ENSG00000134086	TTF2	0.578	0.041
ENSG00000154734	FAM111A	0.579	0.000
ENSG00000070367	MTF2	0.579	0.000
ENSG00000243716	VCAM1	0.579	0.030
ENSG00000010322	EXOSC2	0.580	0.000
ENSG00000151914	USP53	0.580	0.000
ENSG00000163386	SPN	0.580	0.043
ENSG00000114745	INPP5D	0.580	0.002
ENSG00000114650	RP11-368J21.2.1	0.581	0.008
ENSG00000155229	BTAF1	0.581	0.000
ENSG00000107968	EVL	0.581	0.001
ENSG00000168209	NPM1	0.581	0.003
ENSG00000163904	PEA15	0.581	0.025
ENSG00000119397	HSD17B12	0.582	0.034
ENSG00000198780	NUSAP1	0.582	0.028
ENSG00000172890	BDP1	0.582	0.000
ENSG00000178921	HPSE	0.582	0.001
ENSG00000217128	ATP2A2	0.582	0.000
ENSG00000101557	ZNF518A	0.583	0.020
ENSG00000150093	C12orf29	0.584	0.003
ENSG00000124508	ATAD3B	0.584	0.000
ENSG00000092470	RQCD1	0.584	0.002
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ENSG00000119777	TACC3	0.585	0.000
ENSG00000136653	SOAT1	0.585	0.000
ENSG00000139278	AKAP12	0.586	0.001
ENSG00000259131	HMBS	0.586	0.003
ENSG00000138434	STAG3L2	0.587	0.000
ENSG00000145675	ASRGL1	0.587	0.000
ENSG00000089335	ERLIN1	0.588	0.001
ENSG00000173706	C4orf43	0.588	0.027
ENSG00000166250	PIK3R1	0.589	0.010
ENSG00000171865	MICAL2	0.590	0.038
ENSG00000174720	USP14	0.590	0.006
ENSG00000146535	CEP164	0.590	0.021

ENSG00000162174	ATG4C	0.591	0.004
ENSG00000237289	MLLT4	0.591	0.000
ENSG00000080603	RBBP8	0.591	0.000
ENSG00000087266	ANKRD11	0.592	0.017
ENSG00000119402	KHSRP	0.592	0.001
ENSG00000119318	C4orf46	0.592	0.007
ENSG00000113328	GPAM	0.593	0.012
ENSG00000067646	RP11-304M2.2.1	0.593	0.007
ENSG00000134283	MYO19	0.593	0.013
ENSG00000153914	GPCPD1	0.594	0.000
ENSG00000142623	STK36	0.594	0.010
ENSG00000152527	CD8A	0.594	0.002
ENSG00000118655	ITGB1	0.594	0.045
ENSG00000137845	TMEM62	0.595	0.002
ENSG00000100897	G3BP1	0.595	0.028
ENSG00000198794	TM9SF3	0.595	0.034
ENSG00000133884	KLHDC4	0.595	0.010
ENSG00000111445	TMEM165	0.596	0.007
ENSG00000102854	MACF1	0.596	0.000
ENSG00000028116	MAP4	0.597	0.000
ENSG00000118976	SYNCRIP	0.597	0.012
ENSG00000008311	STAU2	0.597	0.025
ENSG00000146247	VARS	0.598	0.000
ENSG00000099904	IFNAR1	0.598	0.022
ENSG00000170275	SRRM1	0.598	0.000
ENSG00000135624	IARS	0.598	0.006
ENSG00000165055	LGALS8	0.598	0.000
ENSG00000156738	ADM	0.599	0.000
ENSG00000160856	PAXIP1	0.599	0.033
ENSG00000138593	EIF5	0.599	0.000
ENSG00000142949	UEVLD	0.599	0.001
ENSG00000149743	U2SURP	0.599	0.000
ENSG00000144848	HSD17B4	0.600	0.004
ENSG00000043514	SEC24D	0.600	0.000
ENSG00000145781	STXBP5	0.601	0.049
ENSG00000095787	HPS3	0.601	0.001
ENSG00000184661	SENP6	0.602	0.000
ENSG00000184557	GPATCH4	0.602	0.001
ENSG00000117481	PLAA	0.602	0.013
ENSG00000076685	TNFAIP3	0.603	0.029
ENSG00000121067	KDM5D	0.603	0.030
ENSG00000173083	LAX1	0.603	0.000
ENSG00000149187	FMNL2	0.604	0.000
ENSG00000100226	SGMS1	0.604	0.001
ENSG00000149182	CCNT2	0.604	0.000
ENSG00000164080	SPARC	0.605	0.000
ENSG00000143753	ANKRD49	0.605	0.000

ENSG00000117090	SMG7	0.606	0.000
ENSG00000111581	SNX19	0.606	0.008
ENSG00000214135	DNAJA4	0.606	0.001
ENSG00000052126	ZAP70	0.608	0.000
ENSG00000135164	FERMT1	0.608	0.002
ENSG00000245164	VPS8	0.609	0.034
ENSG00000150961	PAK1	0.610	0.000
ENSG00000204256	GTF2H2	0.610	0.012
ENSG00000163328	SP4	0.610	0.017
ENSG00000109920	DDIT4	0.610	0.003
ENSG00000170266	PMS2	0.611	0.000
ENSG00000138698	MYO1G	0.611	0.000
ENSG00000184162	SSFA2	0.611	0.031
ENSG00000112699	EI24	0.611	0.000
ENSG00000091651	MSTO1	0.612	0.001
ENSG00000013588	ZNF567	0.612	0.025
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ENSG00000174827	CLK4	0.612	0.003
ENSG00000108443	ESYT1	0.612	0.005
ENSG00000127955	TRPM7	0.613	0.012
ENSG00000187147	ITGA6	0.613	0.004
ENSG00000211455	CDCA7	0.614	0.001
ENSG00000157404	ATAD3A	0.614	0.000
ENSG00000081059	DNM1L	0.614	0.004
ENSG00000180771	PISD	0.614	0.000
ENSG00000148187	CARD8	0.615	0.005
ENSG00000149636	FBXW2	0.615	0.000
ENSG00000100664	PLCB2	0.615	0.044
ENSG00000133048	CSNK1G3	0.615	0.006
ENSG00000134255	AC138035.1	0.616	0.006
ENSG00000082458	DFFA	0.617	0.016
ENSG00000205268	SEC22C	0.618	0.041
ENSG00000168958	PRC1	0.618	0.020
ENSG00000099194	CCDC86	0.618	0.000
ENSG00000198692	ZNF92	0.618	0.000
ENSG00000089177	CGGBP1	0.618	0.026
ENSG00000083099	DHX9	0.618	0.000
ENSG00000078668	NOP58	0.619	0.014
ENSG00000189042	TFDP1	0.620	0.005
ENSG00000115705	DDX50	0.620	0.041
ENSG00000114378	PPHLN1	0.620	0.000
ENSG00000110274	ABCB8	0.620	0.000
ENSG00000198951	PRRC2C	0.620	0.003
ENSG00000101773	SCAF11	0.621	0.006
ENSG00000139734	SWAP70	0.621	0.004
ENSG00000189091	DEPDC1	0.621	0.019
ENSG00000112339	AEBP2	0.622	0.001

ENSG00000006194	IVD	0.622	0.048
ENSG00000125630	POLR3B	0.622	0.038
ENSG00000154814	CBWD3	0.623	0.048
ENSG00000139546	LCK	0.623	0.026
ENSG00000145736	EIF1AD	0.623	0.025
ENSG00000142910	VEZT	0.623	0.000
ENSG00000138081	POLR3E	0.623	0.032
ENSG00000153064	PAPOLA	0.624	0.009
ENSG00000110696	SET	0.624	0.021
ENSG00000183010	CNTRL	0.624	0.003
ENSG00000169032	HNRNPA3	0.625	0.000
ENSG00000136689	PPIG	0.625	0.000
ENSG00000138964	SDCCAG3	0.626	0.008
ENSG00000249669	OGG1	0.626	0.027
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ENSG00000100813	AVL9	0.627	0.004
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ENSG00000166483	NBPF3	0.627	0.004
ENSG00000160223	NQO1	0.628	0.006
ENSG00000165862	TAGLN	0.628	0.000
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ENSG00000122515	STAG3L3	0.629	0.001
ENSG00000167965	TBC1D15	0.629	0.000
ENSG00000114416	DEGS1	0.629	0.018
ENSG00000157800	CCNG2	0.629	0.010
ENSG00000131373	SNRNP40	0.629	0.026
ENSG00000151116	CKAP5	0.630	0.000
ENSG00000008517	PARVG	0.630	0.007
ENSG00000181704	NASP	0.630	0.006
ENSG00000169554	SLC12A8	0.630	0.004
ENSG00000068724	MAGI3	0.630	0.017
ENSG00000204361	HNRPDL	0.630	0.006
ENSG00000116984	METTL2B	0.630	0.022
ENSG00000116337	IRF1	0.630	0.001
ENSG00000123219	CEACAM1	0.631	0.002
ENSG00000131016	TP53BP2	0.632	0.046
ENSG00000138750	SEC63	0.634	0.025
ENSG00000008441	GPR114	0.634	0.025
ENSG00000110324	CCAR1	0.634	0.030
ENSG00000134108	CAP1	0.634	0.004
ENSG00000148688	UBE2W	0.635	0.029
ENSG00000119969	STAG3L1	0.635	0.046
ENSG00000104731	NEK6	0.636	0.000
ENSG00000100714	MAPK9	0.636	0.010
ENSG00000140511	SKIV2L2	0.636	0.017
ENSG00000109756	RSPH3	0.636	0.030

ENSG00000104472	ARL8B	0.637	0.010
ENSG00000116191	BTN2A2	0.637	0.032
ENSG00000013810	GABARAPL1	0.638	0.000
ENSG00000104972	CHRAC1	0.639	0.042
ENSG00000204392	MYO1E	0.639	0.014
ENSG00000144840	TRIT1	0.639	0.003
ENSG00000130176	FAM82A2	0.639	0.030
ENSG00000128989	SRP72	0.639	0.000
ENSG00000229404	SRM	0.641	0.021
ENSG00000116698	AMPD2	0.642	0.000
ENSG00000106484	SH3BP2	0.642	0.016
ENSG00000170312	RBBP7	0.642	0.017
ENSG00000140382	TUBB6	0.642	0.000
ENSG00000103381	ZMIZ2	0.642	0.000
ENSG00000132341	MATR3	0.643	0.001
ENSG00000000938	XPO7	0.643	0.019
ENSG00000185215	KIAA1598	0.643	0.022
ENSG00000150667	SLMAP	0.643	0.006
ENSG00000094804	THBS1	0.644	0.010
ENSG00000125772	ANKRD28	0.644	0.046
ENSG00000105983	RASSF5	0.644	0.001
ENSG00000122482	ELAC2	0.644	0.020
ENSG00000166833	C3orf17	0.645	0.020
ENSG00000175787	ARHGAP5	0.646	0.000
ENSG00000102054	CFLAR	0.646	0.000
ENSG00000066136	TRIO	0.646	0.045
ENSG00000056586	RP11-1319K7.1.1	0.646	0.048
ENSG00000164062	EPAS1	0.646	0.000
ENSG00000164930	ZNF300	0.646	0.014
ENSG00000170852	SART3	0.647	0.000
ENSG00000132676	C5orf22	0.647	0.000
ENSG00000168386	ASPM	0.648	0.013
ENSG00000111247	ARHGEF19	0.649	0.029
ENSG00000160049	FUT8	0.649	0.037
ENSG00000108292	HNRNPH1	0.649	0.000
ENSG00000050748	TLK1	0.649	0.000
ENSG00000038382	RAN	0.649	0.021
ENSG00000168876	C3	0.649	0.042
ENSG00000171777	CPPED1	0.649	0.000
ENSG00000116977	NR2C2AP	0.650	0.020
ENSG00000156802	DLG3	0.650	0.042
ENSG00000133706	FNIP1	0.650	0.002
ENSG00000085999	MAP3K7	0.650	0.003
ENSG00000162924	GATAD2A	0.650	0.000
ENSG00000070756	TRAF3IP3	0.650	0.005
ENSG00000106638	MCFD2	0.650	0.000
ENSG00000111726	IDH3A	0.651	0.000

ENSG00000143870	SLC37A3	0.651	0.000
ENSG00000174579	SLC25A46	0.651	0.000
ENSG00000214837	CUL2	0.651	0.002
ENSG00000101311	WDR48	0.651	0.027
ENSG00000170727	PWP1	0.652	0.000
ENSG00000119707	RPS6KA4	0.652	0.047
ENSG00000111679	ZMYM1	0.652	0.003
ENSG00000119927	AIM1	0.652	0.024
ENSG00000140400	RCOR3	0.653	0.024
ENSG00000174353	ATXN2L	0.654	0.000
ENSG00000145390	LYRM2	0.654	0.007
ENSG00000092148	ICOSLG	0.654	0.018
ENSG00000114850	YME1L1	0.654	0.001
ENSG00000119335	SUN2	0.654	0.011
ENSG00000010292	SFMBT1	0.654	0.006
ENSG00000110619	ITGB3BP	0.654	0.002
ENSG00000132640	COMMD10	0.654	0.042
ENSG00000119509	CAMK2D	0.655	0.000
ENSG00000086102	TCERG1	0.655	0.026
ENSG00000149311	CCDC25	0.655	0.008
ENSG00000163430	SNX13	0.655	0.008
ENSG00000197299	FZD6	0.655	0.000
ENSG00000175084	UBR3	0.656	0.000
ENSG00000142794	MARCH7	0.656	0.023
ENSG00000196923	TMED2	0.656	0.000
ENSG00000149554	FAM134B	0.656	0.005
ENSG00000163945	NSUN4	0.656	0.046
ENSG00000139350	NME4	0.657	0.000
ENSG00000067048	PA2G4	0.657	0.001
ENSG00000086475	MTHFD1	0.657	0.018
ENSG00000144354	TP53	0.657	0.000
ENSG00000116560	B4GALT2	0.657	0.000
ENSG00000139579	VPRBP	0.658	0.043
ENSG00000198176	MON1B	0.658	0.010
ENSG00000066279	EIF4G1	0.658	0.000
ENSG00000011465	THY1	0.659	0.038
ENSG00000110090	SDHAP2	0.659	0.005
ENSG00000197822	DSN1	0.660	0.000
ENSG00000151690	PGP	0.660	0.032
ENSG00000171793	SND1	0.660	0.000
ENSG00000120708	MAP2K1	0.661	0.048
ENSG00000099282	KIAA0182	0.662	0.000
ENSG00000161048	NBEAL2	0.663	0.000
ENSG00000153563	PKP4	0.663	0.038
ENSG00000120519	CUL4A	0.663	0.000
ENSG00000130363	RNF2	0.663	0.005
ENSG00000164733	NFKB1	0.664	0.044

ENSG00000198642	AC024560.3.1	0.664	0.025
ENSG00000117625	SRSF10	0.665	0.000
ENSG00000141140	RBM25	0.665	0.009
ENSG00000188938	FUS	0.665	0.000
ENSG00000179583	CASD1	0.665	0.001
ENSG00000111540	OBFC2B	0.665	0.002
ENSG00000060642	ODF2	0.666	0.039
ENSG00000241978	TNFAIP2	0.666	0.036
ENSG00000137825	ZNF644	0.666	0.001
ENSG00000028203	ARMCX5	0.668	0.034
ENSG00000055044	CARD16,CARD17,CASP1	0.668	0.018
ENSG00000162302	SEH1L	0.668	0.036
ENSG00000113319	FYN	0.668	0.010
ENSG00000260528	KIAA0895L	0.668	0.034
ENSG00000039123	PPWD1	0.668	0.005
ENSG00000068366	RCN1	0.669	0.002
ENSG00000258366	THEM4	0.669	0.011
ENSG00000213213	MEF2D	0.669	0.003
ENSG00000023445	LPAR1	0.669	0.000
ENSG00000178462	LMNB2	0.669	0.000
ENSG00000145860	BTBD3	0.670	0.023

Supplementary Table 2: Genes with significantly decreased (>1.5 fold decrease in expression, q value = false discovery rate [FDR] <0.05) expression by RNA sequencing according to Cuffdiff bioinformatic analysis (see Supplementary Methods)

Gene_id	Gene	Fold change	q_value
ENSG00000133226	AMIGO3	undetected before	0.000
ENSG00000127314	LGSN	17.620	0.000
ENSG00000130396	AC027323.1	4.763	0.013
ENSG00000139154	PDZK1	3.812	0.001
ENSG00000100297	GSTA1	3.368	0.007
ENSG00000111799	MSLN	3.278	0.001
ENSG00000197785	PNLIPRP2	2.509	0.004
ENSG00000157954	KIAA1984	1.706	0.017
ENSG00000115419	SLC37A2	1.665	0.002
ENSG00000116406	BAMBI	1.634	0.000
ENSG00000166928	PIGZ	1.607	0.008
ENSG00000163110	FAM132A	1.589	0.034

Supplementary Table 3: Genes with significantly increased (>1.5 fold increase in expression, q value = false discovery rate [FDR] <0.05) expression by RNA sequencing according to Cuffdiff bioinformatic analysis (see Supplementary Methods)

MEDICAL HISTORY

Please check conditions **YOU** have now, or have had in the past or list below.

☐ Alcohol/drug Abuse	☐ Cataracts	☐ Herpes	☐ Rheumatic Fever
☐ Anemia	☐ Depression	☐ High Blood Pressure	☐ Stroke
☐ Anxiety	☐ Diabetes	☐ High Cholesterol	☐ Thyroid Problem
☐ Arthritis	☐ Emphysema	☐ HIV/AIDS	☐ Tuberculosis
☐ Asthma	☐ Epilepsy	☐ Kidney Disease	☐ Sexually Transmitted Diseases
☐ Bleeding Disorder	☐ Glaucoma	☐ Liver Disease	
☐ Breast Lump	☐ Heart Disease	☐ Migraine Headaches	
☐ Cancer: _____	☐ Hepatitis	☐ Prostate Problem	

Other (please list): _____

_____

SURGERIES

Please check or list the surgeries **YOU** have had.

☐ Appendectomy	☐ Heart Bypass/Stent	☐ Hysterectomy	**Other**:_____ _____
☐ Gallbladder Removed	☐ Tonsilectomy	☐ Tubal Ligation	_____ _____

HOSPITALIZATIONS List all of your hospital stays for illness or surgery beginning with the most recent.

Date	**Reason**	**Hospital**	**Physician**

LIFESTYLE CHOICES

Smoking Status ☐ Never ☐ Currently ☐ Quit: YEAR _____

Type: ☐ Cigarettes (Packs/day _____) ☐ Cigars ☐ Pipes ☐ Second-hand Smoke

Smokeless Tobacco ☐ Never ☐ Currently ☐ Snuff ☐ Chew ☐ Quit: YEAR_____

Alcohol Use *Type:* ☐ Liquor ☐ Wine ☐ Beer

☐ 0 drinks/week ☐ 1-6 /week ☐ 7-14 /week ☐ Over 14/week

On any single occasion during the past **3 months**, have you had more than **5 drinks** containing alcohol? ☐ Yes ☐ No

Weight Now _____ 1 year ago_____ Desired_____

Caffeine Drinks per day _____ Types: ☐ Cola ☐ Coffee ☐ Tea

Special diet? ☐ Vegetarian ☐ Vegan ☐ Other:_____

Exercise Type: _____Frequency per week_____ Duration:_____

Donor Health Information Sheet		
Chronic diseases:		
	YES	NO
Antibiotics in past 6 months:		
If yes, when: _____		
Have you been exposed to HIV or viral hepatitis at any time?		
Have you ever had sex for drugs or money?		
Are you a man who has had sex with men?		
Have you had more than one sexual partner in the last 12 months?		
Have you ever been incarcerated?		
Have you ever used intravenous drugs or intranasal cocaine?		
Did you have a tattoo or body piercing within 12 months?		
Have you traveled to areas of the world with increased risk of traveler's diarrhea in the past 3 months?		
Are you currently ill (flu symptoms, fevers, runny nose, etc.)?		

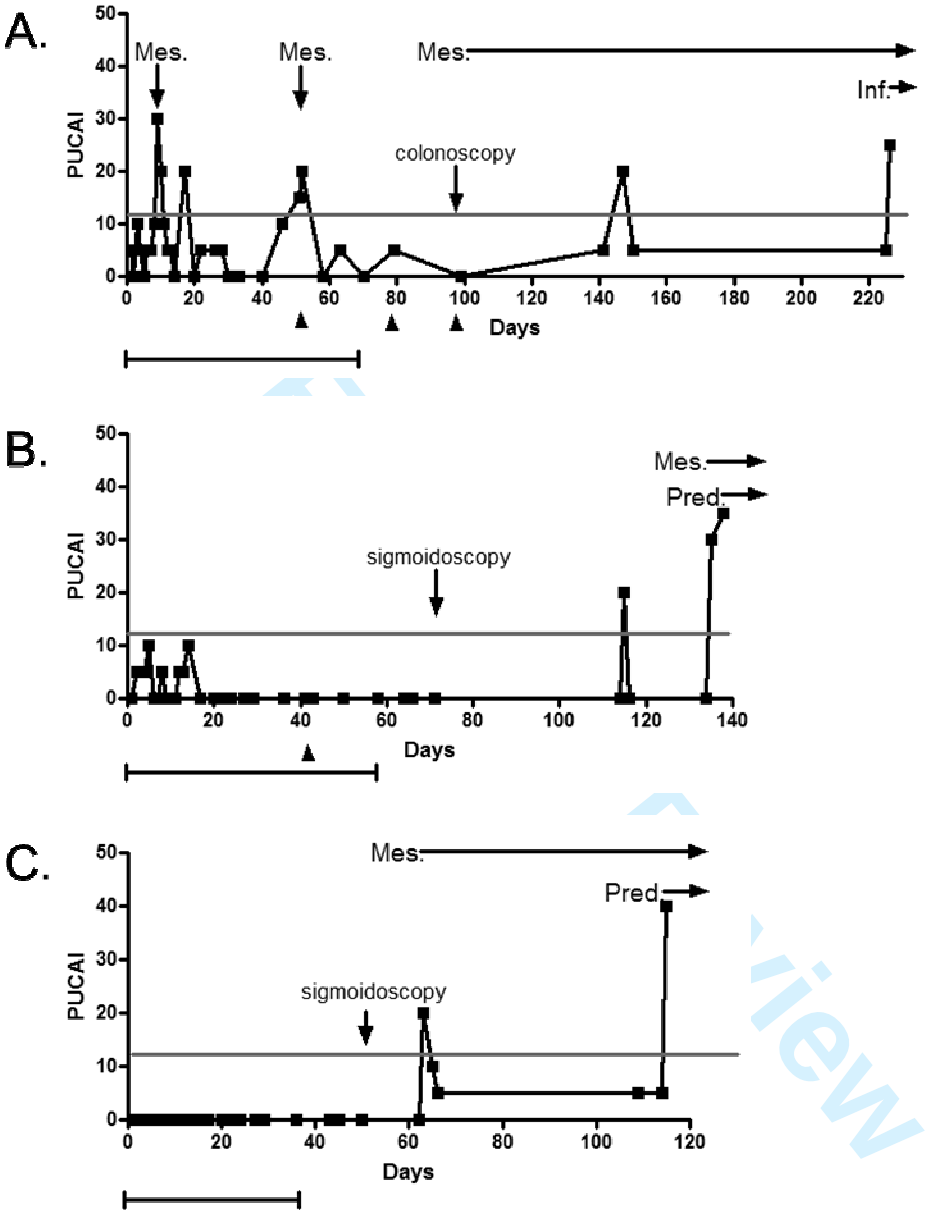
Do you have a history of irritable bowel syndrome, or any of the associated symptoms (frequent abdominal cramps, excessive gas, bloating, abdominal distension, fecal urgency, diarrhea or constipation)?		
Do you have a history of inflammatory bowel disease such as Crohn's disease, ulcerative colitis, lymphocytic colitis?		
Do you have chronic diarrhea?		
Do you have chronic constipation or use laxatives regularly?		
Do you have a history of gastrointestinal malignancy (cancer) or known colon polyposis?		
Have you ever had abdominal surgery (for example: gastric bypass, intestinal resection, appendectomy, cholecystectomy, etc.)		
Do you use probiotics or any other over the counter aids for specific purposes of regulating digestion?		

Supplementary Table 4: Donor Health Information Sheet

Stool testing
Clostridium difficile toxin A and B by PCR; if unavailable, then toxins A and B by EIA
Routine bacterial culture for enteric pathogens
Fecal Giardia antigen
Fecal Cryptosporidium antigen
Ova and parasites
Serologic testing
HIV type 1 and 2
Hepatitis A virus (HAV) immunoglobulin (Ig) M
Hepatitis B virus (HBV) (surface antigen/antibody; core antibody)
Hepatitis C virus (HCV) antibody
Syphilis serology (Trep-sure ELISA; RPR done if treponemal test is positive)

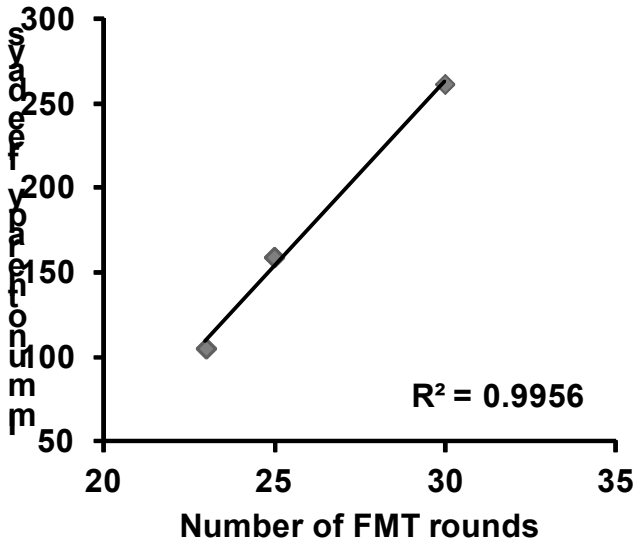
Supplementary Table 5: Donor Screening Test

SUPPLEMENTARY FIGURES

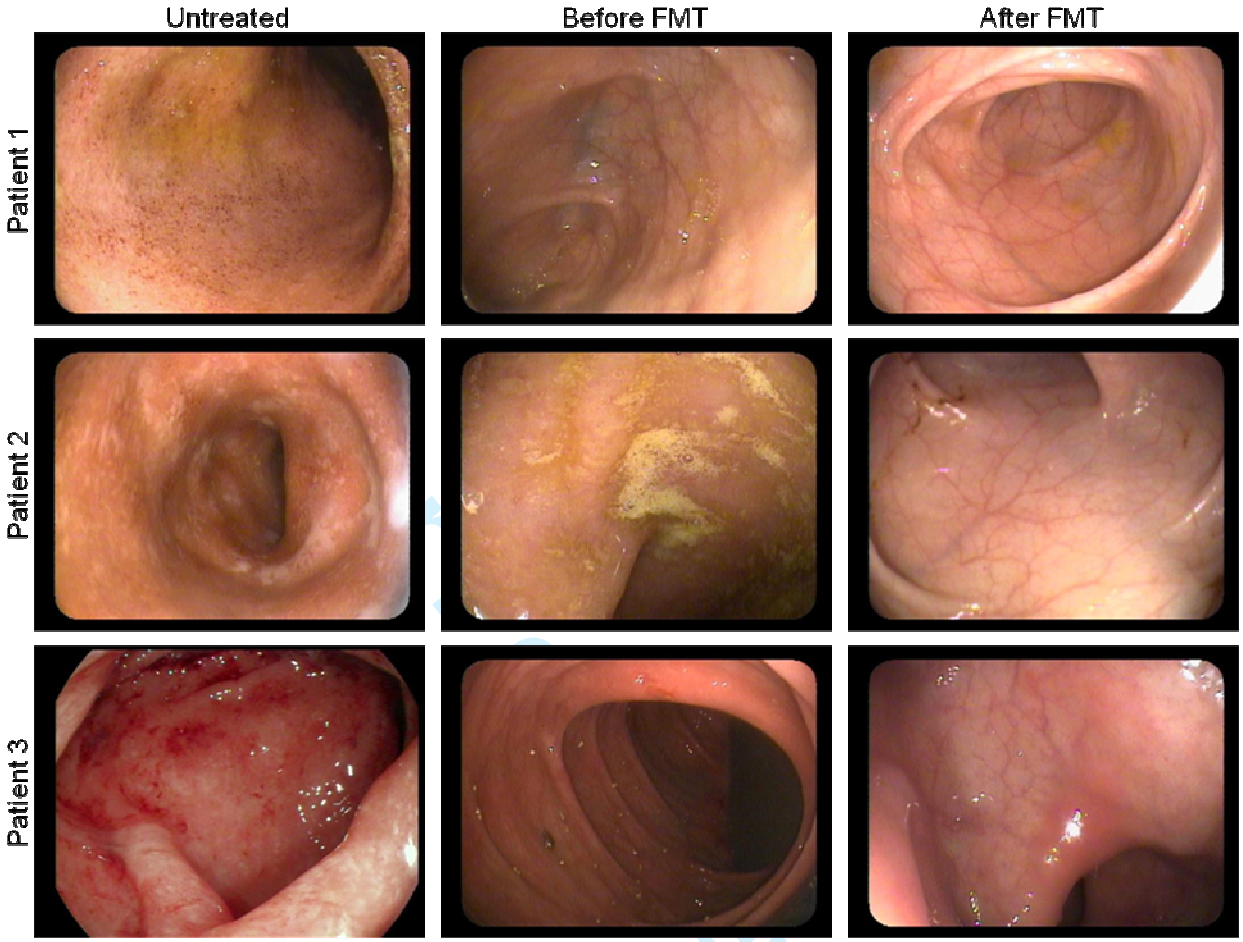


Supplementary Figure 1. Clinical Disease Activity during the Trial. Patient 1 (A.) received 30 rounds of FMT and experienced the longest clinical remission (pediatric ulcerative colitis activity index [PUCAI] ≤10; grey line). Patient 2 (B.; 25 treatments) and 3 (C.; 22 treatments) were in remission while receiving the FMT course (— below the x axes designates the time of active FMT therapy). All patients remained in remission following FMT for more than 11 weeks,

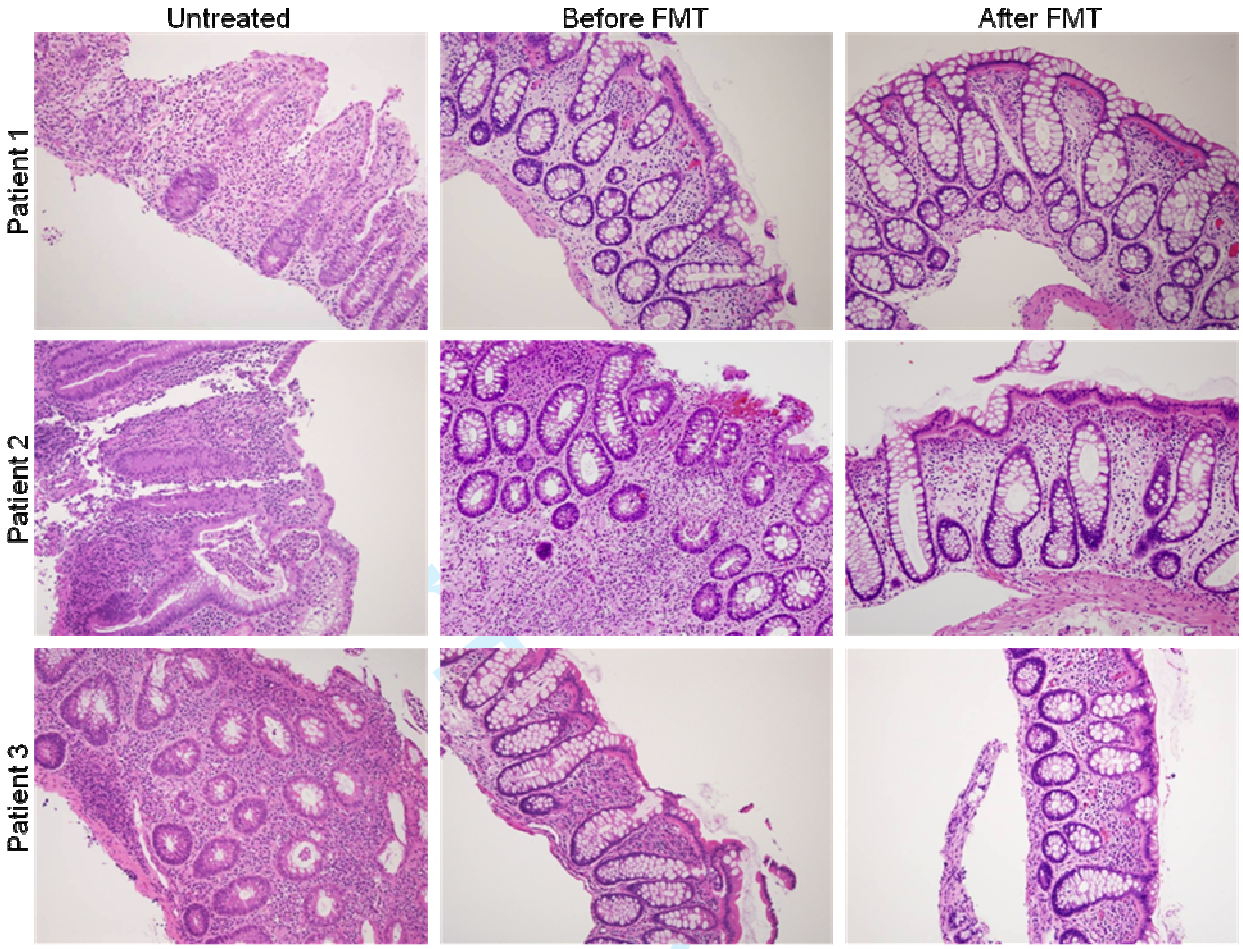
but eventually experienced a relapse requiring immunotherapy. The length of the immunotherapy free period correlated with the number of treatments received. Oral and/or rectal mesalamine (Mes.) was allowed during the trial, depending on clinical disease activity. Additional ("rescue") enemas (▲) were allowed during the first 12 weeks of the trial (i.e. during the weaning course of FMTs). Inf.: infliximab; Pred.: prednisone.



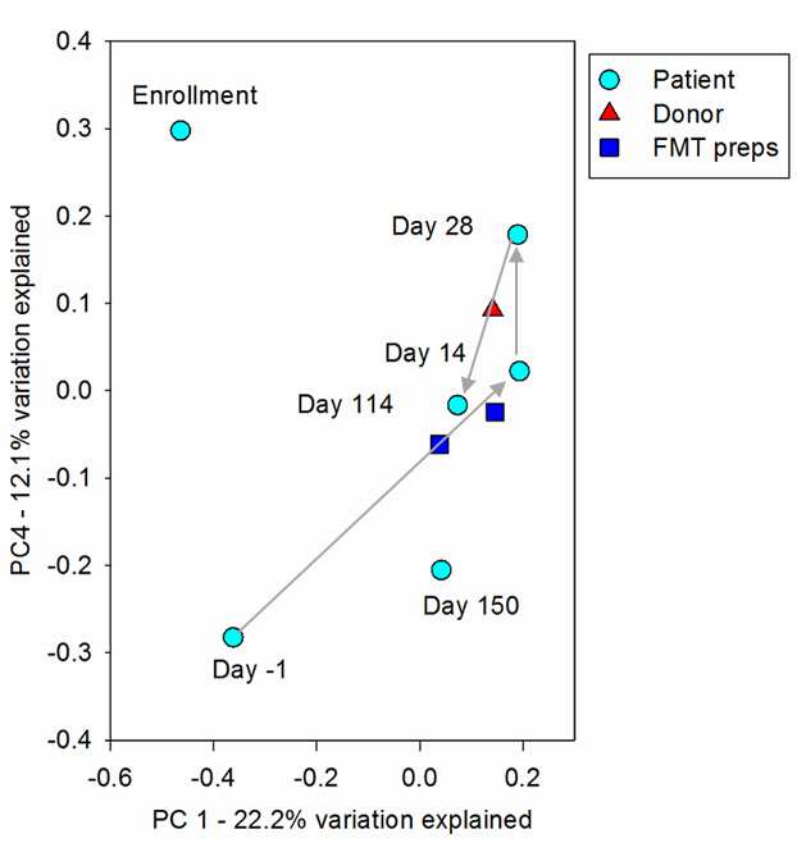
Supplementary Figure 2. Correlation between Number of FMTs and the Immunotherapy Free Period. There was a significant (r=0.998, two tailed p=0.04) correlation between the number of FMT rounds received, and the length of the immunotherapy free period (in days) in the 3 patients studied.



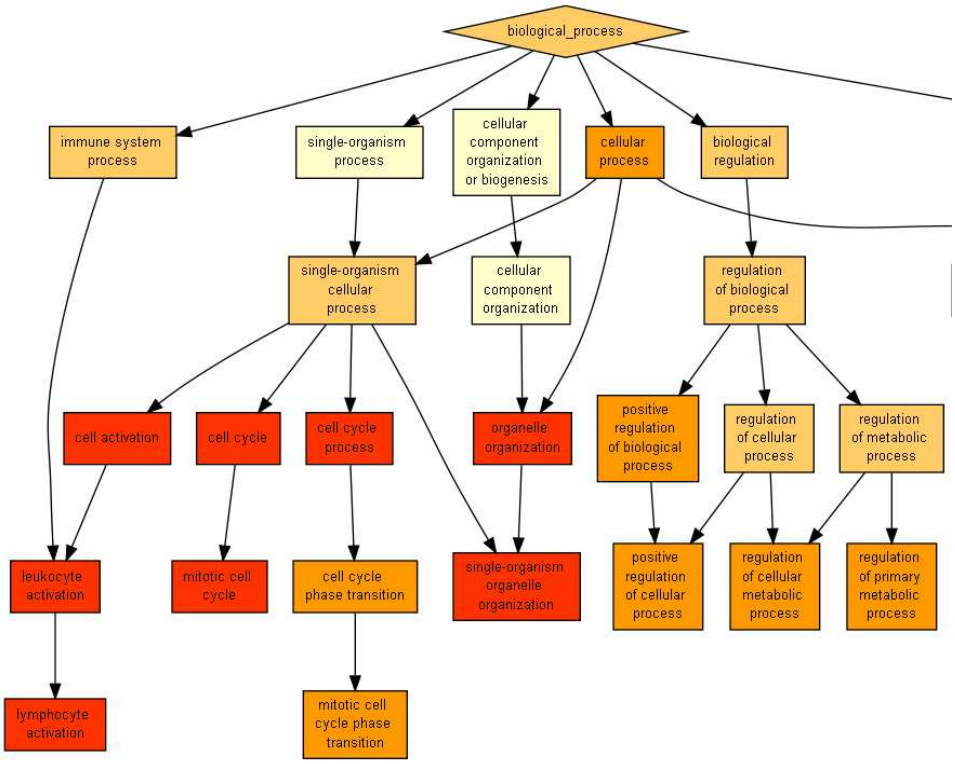
Supplementary Figure 3. Endoscopic images of the colonic mucosa at diagnosis (untreated), prior to FMT therapy and 2 weeks following last FMT in the patients. Patient 1 had Mayo 2 level mucosal pancolitis at diagnosis that turned into grossly normal (Mayo 0) picture with infliximab therapy and remained normal following the FMT series. These numbers for patient 2 were: Mayo 2, 1, and 0 at diagnosis, before, and following the FMTs, respectively. Patient 3 had severe (Mayo 3) pancolitis at diagnosis, enrolled in remission on steroids into the trial, then remained in gross mucosal remission (Mayo 0) following the FMTs.



Supplementary Figure 4. Histologic (hematoxylin and eosin) images of the colonic mucosa at diagnosis (untreated), prior to FMT therapy and 2 weeks following last FMT in the patients. The histologic severity of inflammation largely mirrored the gross endoscopic findings (see Supplementary Figure 1) in the rectosigmoid colon. All three patients had normal mucosal architecture following the FMT series. Magnification 200x



Supplementary Figure 5. Temporal Microbiome Shifts in Patient 1. Principle coordinates analysis of unweighted Unifrac distances between patient 1 and the donor (stool and fecal preparations) showed that the recipient microbiomes became similar to that of the donor by 14, 28, and even 114 (2 weeks after last FMT received) days into the study. By day 150, the patient microbiome started to shift back to that before the start of the trial (Day-1). Therefore, the FMT series appeared to induce a transient engraftment of the donor microbiome in the recipient.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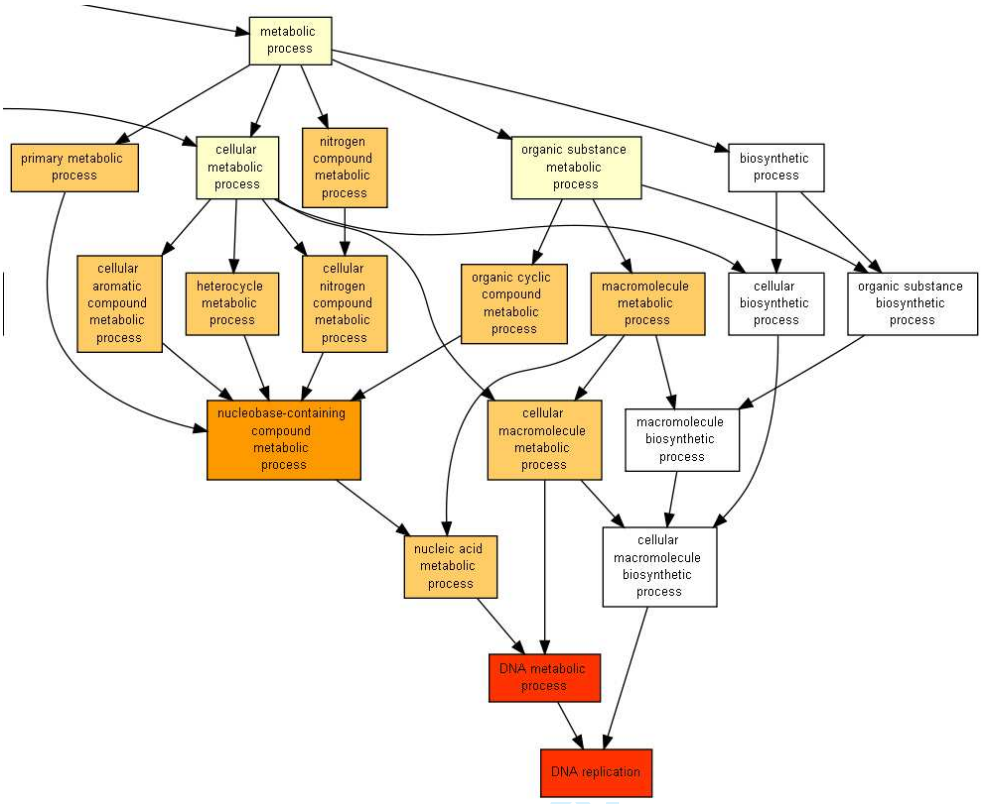
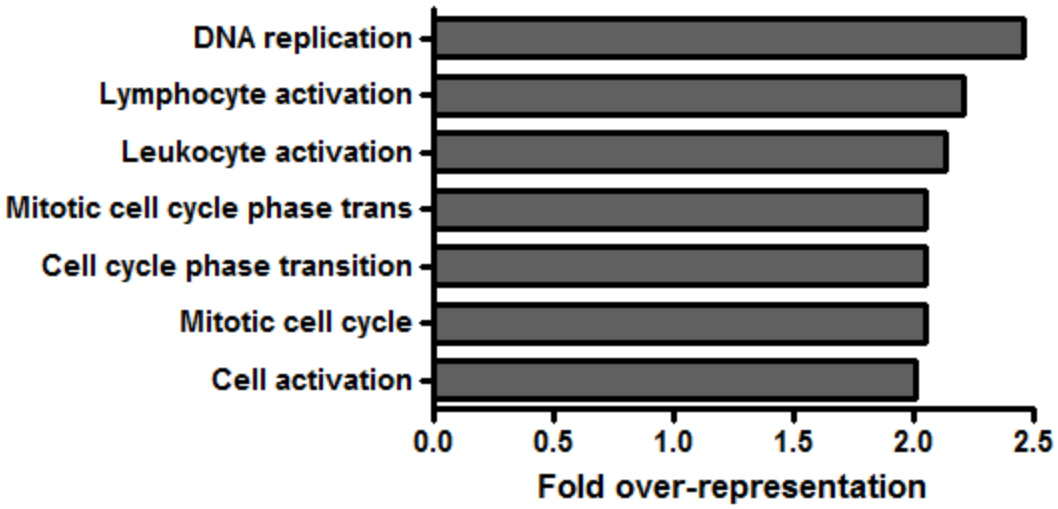
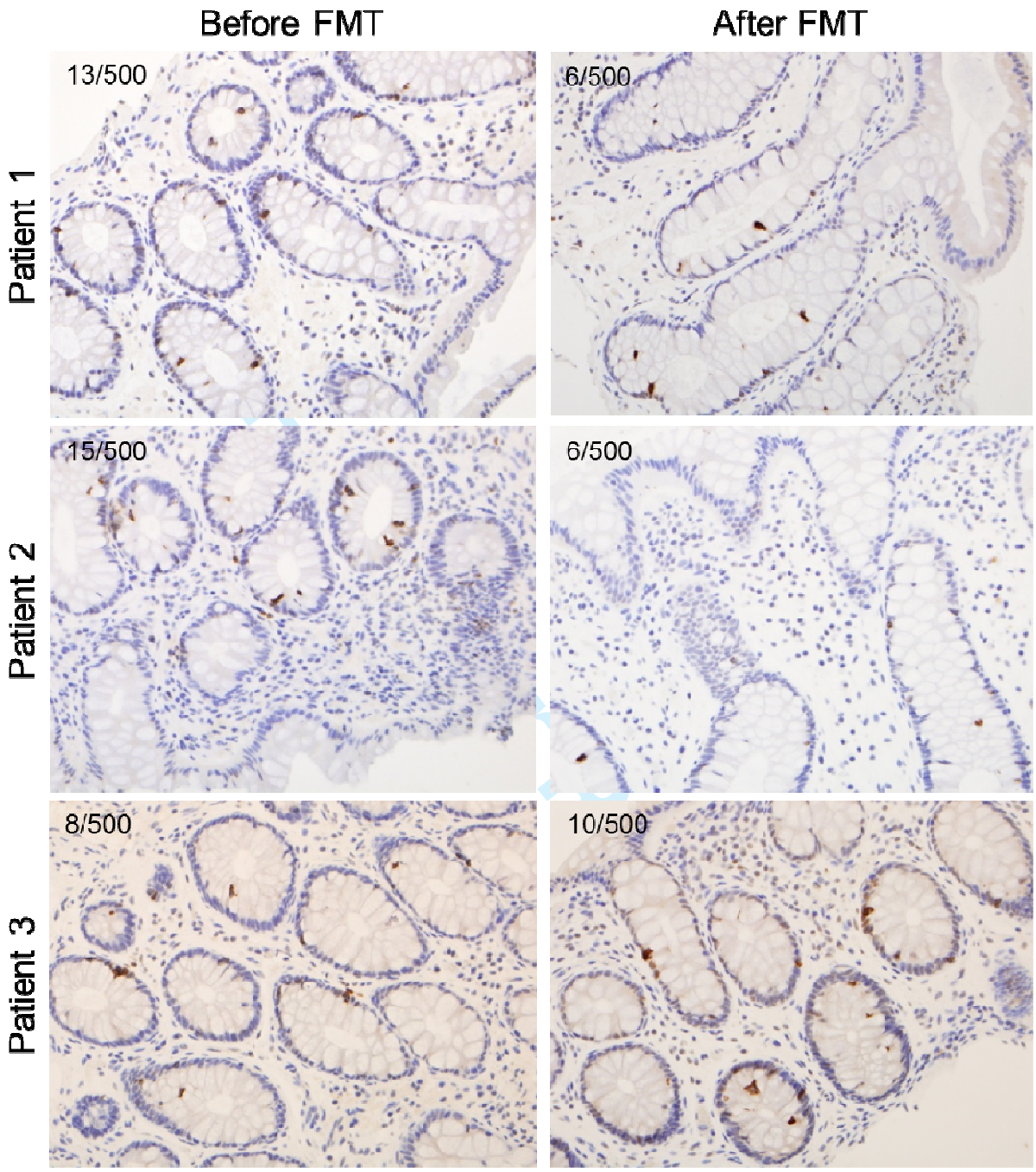


Supplementary Figure 6. Enrichment of genes in relationship to biological processes in the down-regulated transcripts upon the series of FMTs in our 3 UC patients (>1.5 fold suppression; FDR<0.05) by Gene Ontology enRIchment anaLysis and visuaLizAtion tool (Gorilla: http://cbl-gorilla.cs.technion.ac.il/). The down-regulated transcripts were compared to a control set without expression change (FDR>0.97) upon the FMTs in the rectosigmoid mucosa. light yellow: p= 10^{-3}-10^{-5}; dark yellow: p= 10^{-5}-10^{-7}; orange: p= 10^{-7}-10^{-9}; red: p<10^{-9}



Supplementary Figure 7. Biological processes with more than 2 fold over-representation (FDR $<10^{-5}$) in the down-regulated genes compared to control following serial FMTs in UC patients.



Supplementary Figure 8. Epithelial Cell Mitosis Suppression Following FMT. In 2 out of 3 patients, the numbers of epithelial mitoses (blinded examination of 500 epithelial cells) decreased by more than 50% following high intensity FMT. Mitoses were highlighted by histone (H3) immunohistochemistry. Magnification 40x

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Serial Fecal Microbiota Transplantation ~~May Transiently Support Immunotherapy Withdrawal~~ Alters Mucosal Gene Expression in Pediatric Ulcerative Colitis

Fecal Transplant in Pediatric Ulcerative Colitis

Richard Kellermayer[1,3*], Dorottya Nagy-Szakal[1,3], R. Alan Harris[4], Ruth Ann Luna[2,5], Milena Pitashny[2,5], Deborah Schady[2,5], Sabina AV Mir[1,3], Monica E. Lopez[6], Mark A. Gilger[1#], John Belmont[4], Emily B. Hollister[2,5], and James Versalovic[2,5]

[1]Section of Pediatric Gastroenterology, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, United States of America; [2]Department of Pathology, Texas Children's Hospital, Houston, Texas, United States of America; [3]USDA/ARS Children's Nutrition Research Center, Houston, Texas, United States of America; [4]Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, Texas, United States of America; [5]Department of Pathology& Immunology, Baylor College of Medicine, Houston, Texas, United States of America; [6]Department of Surgery, Baylor College of Medicine, Houston, Texas, United States of America, [#]Current address: Children's Hospital of San Antonio, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, United States of America

Author contribution: study concept and design: RK, DNS, RAL, MP, MAG, JV; acquisition of data: RK, DNS, DS, SAVM, MEL; analysis and interpretation of data: RK, DNS, RAH, RAL, JB, EBH, JV; drafting of the manuscript: RK, DNS, EBH; critical revision of the manuscript for important intellectual content: RK, MEL, EBH, JB, JV; statistical analysis: RK, RAH, EBH; obtained funding: RK, JV; technical, or material support: MP, JV; study supervision: RK, JV

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Word count: 732

[*]**Correspondence:**
Richard Kellermayer M.D., Ph.D.
Section of Pediatric Gastroenterology, Hepatology & Nutrition
Baylor College of Medicine
6621 Fannin St., CC1010.00
Houston, TX 77030-2399
Voice: 713-798-0319
Fax: 832-825-3633
Email: kellerma@bcm.edu

~~**ABSTRACT**~~

~~**OBJECTIVES:**~~ **To the Editor:** Complex bacteriotherapy, such as fecal microbiota transplantation (FMT) is an emerging therapeutic modality for ulcerative colitis (UC).(1) FMT has been implicated to allow for withdrawal of conventional therapies in select patients.(2) ~~Recent trials showed variable efficacy of limited (1-6) FMT series in treating UC. We wished to explore the clinical responses, metagenomic modifications, and mucosal gene expression changes induced by a larger series of FMT in pediatric UC patients during withdrawal of their immunosuppressive treatment.~~ ~~**METHODS:**~~ In our study, ~~T~~three immunotherapy (infliximab, 6-mercaptopurine, and steroid, respectively) dependent pediatric (14-16 year of age) UC patients **(Table 1)** received a tapering course (22-30 treatments) of FMT delivered by colonoscopy and enemas during a 6-12 week period. The phase 1, open label protocol was approved by the Institutional Review Board of Baylor College of Medicine (H-30591). The protocol is currently approved by the FDA (IND-15743; ClinicalTrials.gov number: NCT01947101). Filtered, frozen, and thawed stool specimen from a standardized single donor (37-year-old male) for all 3 patients was used, which provided unique opportunity to examine microbial changes. ~~They~~ Patients were concomitantly withdrawn from their conventional medications. Mucosal disease activity was assessed before, and 2 weeks after the FMT series. Clinical disease activity was followed by the Pediatric Ulcerative Colitis Activity Index (PUCAI). The FMT series was well tolerated and transiently supported immunotherapy withdrawal (**Supplementary Figure 1**). FMT enabled all 3 patients to be symptom-free for at least 4 weeks following FMT and supported the withdrawal of immunotherapy (no treatment other than mesalamine) for more than 105 days in all. The number of FMT treatments significantly correlated with the time of being immunotherapy-free (r=0.998, p=0.04; **Supplementary Figure 2**). All patients were in endoscopic and histologic remission 2 weeks after the last FMT (**Supplementary Figure 3-4**).

Fecal microbiomes were analyzed by massively parallel pyrosequencing of the V3V5 regions within the bacterial *16S rRNA* gene. The nature of microbiota shifts differed, presumably due to differences in baseline composition of intestinal microbiota in each patient (detected by principal-coordinates-analysis, **Figure 1A**). Recipient microbiomes remained distinct from that of the anonymous donor. FMT series appeared to induce a transient engraftment of the donor microbiome in a recipient (**Supplementary Figure 5**). Microbiome richness (**Figure 1B**) and diversity (**Figure 1C**) increased secondary to FMT. Fifteen operational taxonomic units (OTUs or bacterial taxa) consistently changed in relative abundance in all 3 patients following FMT ($p<0.25$; **Supplementary Table 1**). Six of 8 OTUs that increased in abundance were not detected in the donor or the recipient prior to FMT. Therefore, expansion of rare taxa may be functionally important in restoring colonic health, at least for short-term periods, following FMT. Of the OTUs that were increased in abundance 61.5% belonged to the *Lachnospiraceae* family. The abundance of *Lachnospiraceae* has been inversely correlated with UC disease activity,(3) and those were more abundant in healthy members of monozygotic twin pairs discordant for UC, compared to control.(4) At the genus level, only *Coprococcus* changed (increased) in abundance by more than 2 fold. The abundance of *Coprococcus* (a genus including butyrate producing bacteria) has been detected to be decreased in IBD patients.(5) Therefore, the increased abundance of *Coprococcus* and *Lachnospiraceae* upon the FMT series may have delivered beneficial effects to the colonic epithelium of the UC patients (recipients).

Colonic mucosal gene expression profiles in response to the FMT series were interrogated by RNA sequencing. ~~The FMT series was well tolerated and transiently supported immunotherapy withdrawal.~~ The expression of 742 genes decreased and 12 increased (>1.5 fold change in expression, false discovery rate [FDR]<0.05) upon the FMT therapy (**Supplementary Table 2-3**). Importantly, the ~~Therapeutic effects were associated with~~ suppression of human gene expression relevant in leukocyte activation and mitotic cell cycle

progression was observed (Supplementary Figure 6-7). These molecular findings associated with >50% decline in epithelial cell mitosis in 2 out of the 3 patients (Supplementary Figure 8).

In conclusion, this report describes high intensity FMT as a strategy to reset the intestinal microbiota in pediatric IBD. Serial FMT in pediatric UC may induce beneficial changes in patient microbiota and colonic mucosa. ~~be well tolerated and potentially therapeutic for select patients.~~ Randomized trials will be required in the future to answer many challenging questions (donor selection, patient selection, number and length of FMT therapy required, etc.) in respect to the clinical application of this treatment.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

TABLES

Patients (Age, Gender)	Disease Behavior	Mayo Score At Dx	Tx after Dx	Mayo Score Before FMT	FMT #	Remission during FMT (in Days)	Mayo Score After FMT	Remission after last Medication (Days)	Remission after last FMT (Days)	Tx following Flare
1 (16y M)	Pancolitis	2	IFX	0	30	65/70 (93%)	0	261	126	IFX
2 (15y M)	Pancolitis	2	6MP	1	25	58/58 (100%)	0	159	80	PRED FMT
3 (14y F)	Pancolitis	3	PRED	0	22	36/36 (100%)	0	105	79	PRED FMT Colectomy

Table 1. Patient characteristics and clinical outcomes after sequential FMT (IFX: infliximab; 6MP: 6-mercaptopurine; PRED; prednisone).

FIGURE LEGENDS

Figure 1. Fecal Microbiota Shifts Following FMT. Principle coordinates analysis of unweighted Unifrac distances (A.) revealed that microbial community changes during FMT (arrows connect pre-FMT [within 24 hours before first FMT] and post-FMT [2-3 weeks after last FMT] samples from each patient) were not consistent within each patient (did not shift in the same direction for each patient), and post-FMT fecal communities of recipients (patients) following FMT were dissimilar from that of the donor (▲: donor stool; ■: donor preparations from independent bowel movements on separate days). Microbial richness (B.) and diversity (C.) in terms of microbial taxa consistently increased following the FMT, although these trends did not reach statistical significance.

STUDY HIGHLIGHTS

1. WHAT IS CURRENT KNOWLEDGE

- Fecal microbiota transplantation (FMT) is an emerging treatment for UC with limited number of clinical trials showing variable results.

2. WHAT IS NEW HERE

- Three pediatric immunosuppression-dependent UC patients received an unprecedentedly intense FMT regimen during the cessation of their conventional medications.
- This is the first study to examine genome-wide gene expression (RNA-sequencing) responses to FMT.
- Our single donor strategy created an unprecedented opportunity to examine the effects of one source microbiome in different recipients.
- FMT in the context of the 3 patients studied was safe and enabled the short-term withdrawal of immunotherapy.

Keywords: inflammatory bowel disease; fecal microbiota transplantation; gene expression; ulcerative colitis

INTRODUCTION

Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD) affecting up to 1/500 people in the developed world.(6) No medical cure exists for the disorder, and it is frequently resistant to conventional therapies. Approximately 20% of patients with UC are diagnosed before age 18.(7, 8) Up to 40.9% of children with UC require surgery (colectomy) within 10 years of diagnosis compared to 19.9% of adults.(9) UC is a precancerous condition(10) with increased risk of colorectal cancer for patients diagnosed before 30 years of age.(11) Therefore, the development of novel therapeutic approaches for pediatric UC is an important medical need.

The procedure of transferring stool from healthy donors into patients has been commonly designated as fecal microbiota transplantation (FMT).(12) FMT has been found superior to conventional antimicrobial therapy in treating recurrent *Clostridium difficile* infection (CDI).(13) CDI affects IBD patients (including children) more commonly than the general population.(14) FMT has been also explored for treating IBD, including UC.(1, 15) According to the current disease paradigm, UC develops secondary to an uncontrolled immune response against the gut microbiome that is transmitted by the intestinal mucosa.(16, 17) Therefore, the disruption of healthy microbiota composition (dysbiosis) may be an important element in disease development and it is hypothesized that the restoration of normal microbiota structure may be therapeutic for this condition.(12, 18) In spite of encouraging outcomes in small cohorts,(19) including children,(20) recent adult FMT (uncontrolled and controlled) trials incorporating metagenomic analyses provided discouraging results.(3, 21, 22) Similarly, 4 pediatric patients with mild to moderate disease activity received no benefit (according to clinical or laboratory parameters) from a single FMT.(23) Importantly, these latter trials involved patients with active UC indicating that individuals with milder disease, or who are in medically induced remission may be better candidates for this unconventional treatment modality.(24) In the meantime, an early onset (10 months of age), conventional treatment refractory IBD case was successfully treated with 7 rounds of FMT from two different donors.(25) However, none of the published

case series utilized more than 6 consecutive FMT rounds at baseline. Interestingly, 9 out of 16 patients who did not achieve clinical remission after 6 weekly FMTs did so following an additional 6-12 weekly treatments.(22) Therefore, based on the current literature, patients in remission may receive the most benefit from an extended course (more than 5 treatments) of FMT.

We present our findings in 3 pediatric patients with immunosuppression-dependent UC, who enrolled into a phase 1, uncontrolled clinical study delivering a serial, highly intense FMT regimen during clinical remission.

CASE REPORTS

The patients were enrolled into an Institutional Review Board (IRB) approved clinical trial (see Supplementary Appendix) prior to the Food and Drug Administration (FDA) mandating the need for an investigational drug application for FMT (that is not directed towards the treatment of recurrent CDI). The protocol was stopped after the FDA mandate was issued in May of 2013. Therefore, only 1 patient (patient 1) completed the full 12 week course (28 scheduled treatments + "rescue" enemas if clinically indicated) of FMT.

Patient 1: A 16-year-old male was diagnosed with pancolitis (Mayo score(26)=2; Supplementary Figure 1) one year before enrollment. His prior therapy included infliximab, oral corticosteroids and mesalamine. He was weaned off oral steroids and continued on infliximab infusions with oral mesalamine. FMT was initiated when he was in clinical (pediatric ulcerative colitis activity index;(27) PUCAI=5), macroscopic (Supplementary Figure 1), and microscopic (Supplementary Figure 2) remission after 6 weeks without infliximab. Thirty (30) treatments were administered during 3 months. He received mesalamine enemas twice for mild exacerbations (PUCAI ≥15).

The patient remained in clinical remission (PUCAI <10) for more than 90% (65/70 days) of the treatment course (Figure 1A). No adverse events were noted. A follow-up endoscopy 3 weeks after the last FMT showed the entire colon to be grossly normal (Supplementary Figure 1). However, histological inflammation in his transverse colon was detected, and maintenance oral mesalamine therapy was started. The patient remained in clinical remission (symptom-free, lab values within normal ranges, including fecal calprotectin) for 8 months (261 days) after his last infliximab treatment, 4 months (126 days) after his last FMT. At this time, he developed a mild disease flare (PUCAI=25) and wished to go back on infliximab therapy. He had intermittent breakthrough symptoms on 7.5 mg/kg infliximab every 6 weeks and daily mesalamine treatments.

Patient 2: A 15-year-old male with a history of UC with pan-colitis (Mayo: 2; Supplementary Figure 1) was treated for more than one year with 6-mercaptopurine (6MP), allopurinol, and daily oral mesalamine. He tested positive for toxigenic *Clostridium difficile* infection (CDI) at enrollment. Metronidazole therapy successfully cleared the CDI prior to starting FMT. Allopurinol and 6MP were stopped 3 weeks prior to initiation of FMT. Initial colonoscopy showed mildly active colitis (Mayo: 1; Supplementary Figure 1) with histological inflammation (Supplementary Figure 2) throughout the colon. Mesalamine was stopped on day 6 after initiation of FMT. The patient remained in remission throughout FMT therapy (Figure 1B). Twenty-five FMT treatments were administered to this patient, and no adverse events were noted. The colon was grossly normal by sigmoidoscopy performed 2 weeks after the final FMT (Mayo 0; Supplementary Figure 1). No evidence of mucosal inflammation in the colon was found by histology (Supplementary Figure 2). This patient remained in remission (PUCAI<10) without any therapy for more than 2 months (80 days after last FMT, 159 days after last 6MP dose). However, he became symptomatic (PUCAI=35) and did not respond to oral mesalamine therapy following

this symptom-free period. Pulse steroid treatments were initiated, and he required intermittent low-dose steroid therapy with 4-5 week steroid-free intervals.

Patient 3: A 14-year-old female was diagnosed with severely active pancolitis four months prior to enrollment (Mayo 3, Supplementary Figure 1). She could not be weaned off steroids for more than 4 week intervals, and she opted to enroll into the FMT trial. Steroids were tapered during the first 10 days of FMT treatment, and mesalamine was stopped on day 14. She remained in remission during the FMT period (Figure 1C). She received 22 rounds of FMT with only one episode of moderate, self-resolving, positional headache noted. Sigmoidoscopy 2 weeks after the last FMT showed a grossly normal (Mayo 0) colon. No evidence of colonic mucosal inflammation was found by histology. She developed a flare during travel 4 weeks following the last FMT (PUCAI=20). Oral mesalamine successfully induced remission. She repeatedly became symptomatic with bloody stools and cramping (PUCAI=50) 11 weeks (79 days) after the last FMT (105 days after last steroid dose), at which time pulse steroid therapy was initiated. She remained steroid dependent following re-initiation of steroid therapy.

METHODS

FMT protocol

The patients received a tapering course of FMT following informed consent according to a phase 1, open-label protocol approved by the Institutional Review Board of Baylor College of Medicine (H-30591). The protocol is currently approved by the FDA (IND-15743). Stool was collected for analysis one day prior to the initial FMT by colonoscopy, and 2 weeks after the last FMT. Rectal biopsy samples were formalin-fixed for histologic analysis, flash frozen on dry ice, or stored in RNALater™. The first FMT utilizing filtered, frozen, and thawed stool specimen from a standardized single donor (37-year-old male) for all 3 patients was administered via endoscopic delivery. Thereafter, a tapering course of rectal FMT enemas were given to all

patients, culminating in a range of 22-30 rounds of FMT (for further details see Methods section in the Supplementary Appendix). The corresponding author and co-authors had access to the study data and had reviewed and approved the final manuscript.

Microbiome analyses

The fecal microbiota was analyzed according to previous studies(28) by massively parallel pyrosequencing of the V3V5 regions of the bacterial *16S rRNA* gene (see Methods section in the Supplementary Appendix).

RNA sequencing and analyses

Total RNA was isolated from rectal biopsy specimens (stored in RNALater™ [Ambion through Life Technologies, Carlsbad, California]) according to the manufacturer's recommendation, with Trizol (15596, Life Technologies) and the RNeasy Mini Kit (74106, Qiagen). RNA samples were stored at −80°C until processing for RNA sequencing (see Methods section in the Supplementary Appendix).

Statistical analyses

The two-tailed paired t-test was used for group comparisons. Significance was relaxed to an arbitrary $p<0.25$ for OTU comparisons; otherwise it was determined at $p<0.05$. The Pearson correlation coefficient was calculated in Excel (Microsoft Office Excel 2007).Statistical significance of correlations was calculated with public Statistics Calculator version 3.0 (http://www.danielsoper.com/statcalc3/calc.aspx?id=44) and determined at $p<0.05$. See Methods section in Supplementary Appendix for bioinformatic analyses applied to the RNA sequencing data.

RESULTS

Serial FMT transiently supported immunotherapy withdrawal

FMT enabled all 3 patients to be symptom-free for at least 4 weeks following FMT and supported the withdrawal of immunotherapy (no treatment other than mesalamine) for more than 105 days in all. The number of FMT treatments significantly correlated with the time of being immunotherapy-free (r=0.998; p=0.04, Figure 2).

Fecal microbiome shifts upon serial FMTs

Fecal microbiomes were examined before and 2 weeks after the FMT series. Shifts in recipient fecal bacterial compositions were detected by principal-coordinates analysis (PCoA) (Figure 3A). The nature of microbiota shifts differed, presumably due to differences in baseline composition of intestinal microbiota in each patient. Recipient microbiomes remained distinct from that of the anonymous donor. In the meantime, microbiome richness (Figure 3B) and diversity (Figure 3C) increased secondary to FMT. Fifteen operational taxonomic units (OTUs or bacterial taxa) consistently changed in relative abundance in all 3 patients following FMT ($p<0.25$) (Supplementary Table 1). The relative abundance of each of these fifteen OTUs was originally <2% in the recipients, and averaged <1% in two independent donor preparations. Two OTUs decreased, and 13 taxa increased in relative abundance following FMT. Eight of the 13 (61.5%) OTUs that increased in relative abundance were undetected in the examined donor preparations. Presumably these species pre-existed at low levels (perhaps below the lower limits of detection) in the donor or recipients, and were able to proliferate in the recipient intestine following FMT. Six of 8 OTUs that increased in relative abundance in recipient patients were not detected in the donor or the recipient prior to FMT. Therefore, expansion of rare taxa may be functionally important in restoring colonic health, at least for short-term periods, following FMT. At the genus level, the abundance of *Coprococcus* increased more than 3-fold after the FMT series, and no other genera changed in abundance by more than 2 fold following FMT.

We also examined temporal changes in the fecal microbiome of Patient 1, who received the full 12-week course of the planned FMT series from the same donor (Donor 2). The patient's microbiome shifted in composition after cessation of immunotherapy, independent of FMT. The enrollment sample was obtained within 3 weeks (19 days) after an infliximab treatment, while the consecutive (Day-1) sample was collected 6 weeks (43 days) following the receipt of the biologic agent, which can significantly influence microbiome composition.(29) By the end of the multi-FMT treatment course (daily in days 1-14) and thrice weekly (days 15-28), the fecal microbiomes of the recipient became more similar to that of the donor (Figure 3A), and this shift towards the donor's microbial community profile continued following 2 weeks after the last FMT received (Day 114). Seven weeks after the last FMT (Day 150), the fecal microbiome of the recipient started drifting back to its original state before the FMT series. Therefore, the FMT series appeared to induce a transient engraftment of the donor microbiome in the recipient.

Mucosal transcriptome and epithelial cell proliferation changes after the FMT series

RNA sequencing demonstrated that 742 genes decreased in expression (>1.5 fold decrease in expression, false discovery rate [FDR]<0.05) in the rectal mucosa of the patients 2 weeks following FMT (Supplementary Table 2). Only 12 genes increased significantly in terms of relative expression. Down-regulated genes were compared by gene ontology enrichment analysis to a control set of human genes lacking evidence of changes in gene expression following FMT (FDR>0.97). Genes linked to leukocyte activation and mitotic cell cycle progression were down-regulated (Supplementary Figure 4). More specifically, 7 biological processes, were highly significantly (FDR<10^{-5}) enriched by more than 2 fold in association with the down-regulated genes, compared to the control genes (Supplementary Figure 4). Based on these findings, we decided to functionally assess the consequences of FMT in the colonic mucosa of the patients in respect to mitotic activity changes. We found that 2 out of the 3

patients showed evidence that epithelial mitoses had been reduced by more than 50 percent (Figure 5).

DISCUSSION

This report describes high intensity FMT as strategy to reset the intestinal microbiota in pediatric IBD. A single anonymous donor was used as the microbiota source for all 3 patients. This strategy created an unprecedented opportunity to examine the effects of single source FMT in different recipients. Furthermore, this is the first study to interrogate human gene expression responses in the intestinal mucosa following FMT by an unbiased genome-wide methodology. Treatment of pediatric UC patients in medically induced remission, with relatively short disease duration, and lacking other medical complications may have created a fertile opportunity for FMT to alter human biology. Pediatric FMT in the context of UC was well tolerated and enabled the short-term withdrawal of immunotherapy. However, all 3 patients eventually relapsed, requiring the re-institution of immunotherapy after a "washout" period. Interestingly, the length of the "washout" correlated with the number of FMT rounds. This result indicates that high intensity FMT may provide an alternative to immunotherapy in pediatric UC.

The microbiome shifts following multi-FMT regimens in pediatric UC have yielded interesting results regarding microbial compositional changes with intensive microbial manipulation strategies. Grehan et al.,(30) found that durable alterations of fecal microbiomes were observed following 4-15 rounds of FMT in patients with variable indications. This study differed from ours by employing 5-10 days of antibiotic treatment prior to FMT and using different methods such as denaturing gradient gel electrophoresis for microbial composition studies. Our findings share conclusions from a recent publication utilizing similar methodologies,(21) whereby no obvious association was found between clinical responses and whole bacterial community based metagenomic changes in UC patients after FMT. The recipient (patient) intestinal microbiomes did become more similar to that of the donor, but

retained distinctive features compared to changes seen in FMT for CDI. Possibly, these differences are due to richer and more diverse microbiomes in IBD (relative to CDI) without antibiotic pre-treatment prior to FMT. Microbial richness and diversity increased secondary to the FMT series, although the donor's microbiome was neither richer, nor more diverse than that of the recipients. The donor microbiome was compositionally different than that of patients, and yielded some common effects in all 3 recipients. Of the OTUs that were increased in abundance 61.5% belonged to the *Lachnospiraceae* family. The abundance of *Lachnospiraceae* has been inversely correlated with UC disease activity,(3) and those were more abundant in healthy members of monozygotic twin pairs discordant for UC, compared to control.(4) At the genus level, only *Coprococcus* changed (increased) in abundance by more than 2 fold. The abundance of *Coprococcus* (a genus including butyrate producing bacteria) has been detected to be decreased in IBD patients(5). Therefore, the increased abundance of *Coprococcus* upon the FMT series may have delivered beneficial effects to the colonic epithelium of the UC patients (recipients).

The temporal changes during the FMT series, in one of our patients who received the entirety of the treatments planned, supported a transient engraftment of the donor microbiome consistent with some longitudinal observations of CDI patients after FMT.(31)

Altogether, our metagenomic findings argue that high intensity FMT can change the composition and presumably function of the intestinal microbiota in pediatric patients with UC. The resulting patient microbiomes reflected new combinations of microbes that could not simply be explained by addition of the donor fecal microbiota.

Changes in the UC gut microbiomes following the FMT series associated with suppression of mucosal gene expression, especially genes involved in leukocyte activation and cell proliferation (mitosis). Histology showed evidence of decreased mitoses in the intestinal mucosa, suggesting that microbial composition and function is likely to influence mucosal

immune responses and intestinal epithelial cell proliferation. Indeed, single probiotics have already been shown to affect intestinal epithelial cell differentiation in mammals.(32)

We acknowledge the limitations of this study:

1. This is an uncontrolled trial on a very small patient population. Correlation between number of rounds of FMT and clinical remission without major immunotherapy may have been a coincidence in the setting of a more sustained and durable remission in Patient 1 than in the other patients. However, Patient 1 had persistent breakthrough symptoms on infliximab necessitating treatments every 6 weeks prior to enrollment. He also had mild disease flares during the trial that responded to mesalamine. This clinical course argues against deeper remission in this patient compared to the other 2 cases. Additionally, the mucosal histologic and gene expression changes indicate that the patients were in deeper remission (dampened leukocyte activation) two weeks after the last FMT (17 weeks after last infliximab, 12 weeks after last 6MP, and 6 weeks after last prednisone) than at the initiation of the protocol. Therefore, we argue that the correlation between the number of FMTs and length of immunotherapy free disease may be clinically relevant in spite of the low number of cases studied.

2. Secondary to the FDA mandate on IND requirements the patients did not receive the same number of FMT rounds and had variable length of treatment. This unplanned variation allowed for us to draw correlation between the number of FMTs received and the length of immunotherapy free disease.

3. Although FMT was well tolerated by our patients, we can only make limited conclusions about the safety of this treatment in pediatric UC. Adverse events and even worsening of disease have been certainly noted in both pediatric and adult UC patients following FMT.(23, 33-35) However, those patients had more active disease when receiving FMT than the subjects in this study. Our findings support the conclusion(24) that UC patients in clinical

and endoscopic remission, or those with only mild disease activity, may benefit the most from FMT.

Along with the limitations, this work possesses multiple strengths:

1. This is the first study on FMT that attempts to address mechanisms by linking clinical effects with changes in fecal metagenomics and mucosal gene expression.

2. Standardized measures of clinical (PUCAI) and endoscopic (Mayo score) disease activity were employed.

3. The patients studied were of similar age, disease extent, and disease duration.

4. The same donor was used to treat all 3 patients.

In conclusion, this study provides the rationale to extend clinical research in IBD beyond simple bacteriotherapy towards the optimization of complex bacteriotherapy for the treatment of UC.

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~~FIGURE LEGENDS~~

~~Figure 1. Clinical Disease Activity during the Trial.~~ ~~Patient 1 (A.) received 30 rounds of FMT and experienced the longest clinical remission (pediatric ulcerative colitis activity index [PUCAI] ≤10; grey line). Patient 2 (B.; 25 treatments) and 3 (C.; 22 treatments) were in remission while receiving the FMT course (— below the x axes designates the time of active FMT therapy). All patients remained in remission following FMT for more than 11 weeks, but eventually experienced a relapse requiring immunotherapy. The length of the immunotherapy free period correlated with the number of treatments received. Oral and/or rectal mesalamine (Mes.) was allowed during the trial, depending on clinical disease activity. Additional ("rescue") enemas (▲)~~

were allowed during the first 12 weeks of the trial (i.e. during the weaning course of FMTs). Inf.: infliximab; Pred.: prednisone.

Figure 2. Correlation between Number of FMTs and the Immunotherapy Free Period. There was a significant (r=0.998, two tailed p=0.04) correlation between the number of FMT rounds received, and the length of the immunotherapy free period (in days) in the 3 patients studied.

Figure 3. Fecal Microbiota Shifts Following FMT. Principle coordinates analysis of unweighted Unifrac distances (A.) revealed that microbial community changes during FMT (arrows connect pre-FMT [within 24 hours before first FMT] and post-FMT [2-3 weeks after last FMT] samples from each patient) were not consistent within each patient (did not shift in the same direction for each patient), and post-FMT fecal communities of recipients (patients) following FMT were dissimilar from that of the donor (▲: donor stool; ■: donor preparations from independent bowel movements on separate days). Microbial richness (B.) and diversity (C.) in terms of microbial taxa consistently increased following the FMT, although these trends did not reach statistical significance.

Figure 4. Temporal Microbiome Shifts in Patient 1. Principle coordinates analysis of unweighted Unifrac distances between patient 1 and the donor (stool and fecal preparations) showed that the recipient microbiomes became similar to that of the donor by 14, 28, and even 114 (2 weeks after last FMT received) days into the study. By day 150, the patient microbiome started to shift back to that before the start of the trial (Day -1).

Figure 5. Epithelial Cell Mitosis Suppression Following FMT. In 2 out of 3 patients, the numbers of epithelial mitoses (blinded examination of 500 epithelial cells) decreased by more than 50% following high intensity FMT. Mitoses were highlighted by histone (H3) immunohistochemistry (See Supplementary Appendix for details). Magnification 40x

Supplement to:
Serial Fecal Microbiota Transplantation Alters Mucosal Gene Expression in Pediatric Ulcerative Colitis

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SUPPLEMENTARY METHODS

FMT PROTOCOL

Subject and Donor Recruitment

Patients: Subjects were recruited from the patients treated by the Pediatric Gastroenterology, Hepatology, and Nutrition Section at Baylor College of Medicine/Texas Children's Hospital. Only patients whose clinical, endoscopic and histologic findings supported the diagnosis of UC were recruited. Only steroid, thiopurine, or biologic agent dependent patients were included following informed consent (i.e. "immunotherapy" dependent). Enrollees had to test negative for *Clostridium difficile* toxin by PCR, or enzyme immunoassay (EIA), and agree to withdraw all medications prior to and during the trial (see below). They also had to agree to a pre-treatment surgical consultation and acknowledge the potential need for colectomy, if disease exacerbations cannot be controlled by conventional medical therapy.

FMT Donors: Healthy adult stool donors (between 18 and 45 years of age) were recruited by the research staff following informed consent. Donors were asked to volunteer for the screening (pass a health questionnaire [Supplementary Table 4], serologic and stool tests [Supplementary Table 5]) and regularly supply stool samples according to the study protocol.

FMT preparation

The stool preparations were performed in the Texas Children's Microbiome Center (TCMC) in the Feigin Center, Texas Children's Hospital, Houston, Texas. This facility operates under Good Laboratory Practice (GLP) and is part of the clinical enterprise in the Department of Pathology, accredited by the College of American Pathologists (CAP) and certified by Clinical Laboratory Improvement Amendment (CLIA). Standard Operating Procedures on fecal specimen preparation and for decontamination procedures for biosafety cabinets and equipment were

followed before and after fecal preparation. Freshly collected stool specimens from the healthy adult donor (within 2 hours of passing) were delivered on ice for processing. Specimens were aliquoted to ~50 g aliquots, and cold sterile normal saline solution (NSS) was added prior to homogenization in a strainer bag with 500-µm pore size (Seward Laboratory Systems Inc., Port Saint Lucie, FL) using the Smasher Laboratory Blender/Homogenizer (AES CHEMUNEX Inc., Cranbury, NJ). Sterile glycerol was added to filtered homogenized stool specimens containing the fecal microbiome at a final concentration of 10% according to Hamilton et al.[1] Stool preparations were immediately stored at -80˚C until r transplantation or analysis. All stool preparations were labeled with an expiration date 8 weeks from the date of preparation. At each FMT treatment, fecal preparations were rapidly thawed at 35˚C in a water bath and used within 15 minutes. Sterile NSS was used as a diluent to reach the final volume of 250 ml from 50mg of original stool prior to delivery.

Steps before FMT treatment

Discontinuation or taper of UC therapy to initiation of FMTs: Subjects tapered their home medications for UC prior to the FMT protocol. Subjects did not have a history of antimicrobial therapy for at least 1 week prior to the initial pre-FMT colonoscopy. The corticosteroid dose was decreased to 50% of maintenance the day prior to the first pre-FMT colonoscopy. Thereafter, the dose was decreased by ~50% weekly over a maximum of 10 weeks following colonoscopy. The steroid taper was stopped once a patient's dose has been decreased to 5 mg/day for seven consecutive days. Immunomodulator and biologic treatments were discontinued for a minimum of 21 days prior to the FMT. Prebotic orprobiotic therapy were discontinued at least 1 week before the initiation of FMT. Oral or rectal treatments of mesalamine were stopped within a week of initiation of FMT. However, mesalamine preparations were allowed to be restarted in case of flares during the trial.

Surgical Consult: A surgical consultation was required prior to the initiation of FMT therapy. The subject and family were counseled with regard to possible worsening of symptoms and possible life-threatening conditions that may emerge, necessitating surgical and/or intensive care interventions.

Survey before the FMT therapy: Once a subject was enrolled in the trial, they completed a clinical symptoms survey. Only patients with remission or mild disease category (pediatric ulcerative colitis activity index;[2] PUCAI<35) were allowed to participate. We included patients with PUCAI <35 within 4 weeks of enrollment. However, all 3 patients in this report were in remission (PUCAI <10) at the start of the trial.

Initiation of FMT therapy and pre-colonoscopy preparation: One day prior to scheduled colonoscopy, subjects collected a stool sample and stored it at -20°C at home in an airtight container. The samples were brought to colonoscopy in a chilled container provided for collection and transport within 24 hours of collection and stored at -80°C. After the stool sample was obtained, the subjects started the institution's standard for colonoscopy preparation regimen with Miralax.

FMT treatment protocol

Initial colonoscopy and FMT treatment (Day 1): At the time of colonoscopy, an assessment for macroscopic colitis using the Mayo classification was performed. Biopsies were obtained from the rectosigmoid and cecum in an ascending fashion for routine histopathology and research purposes. Following mucosal sampling, subjects underwent FMT with 250 ml of thawed stool preparation, 1/3 of which was endoscopically administered into the terminal ileum and 2/3 into the right colon as targeted site as found feasible by Brandt and colleagues.[3]

Subsequent FMT Treatments: The duration of FMT therapy was planned to be 12 weeks.

Days 2 through 14: Subjects came to the ambulatory clinic daily for clinical symptom evaluation and fecal retention enema administration (60-250 ml rectally [as tolerated] with retention for at least 30 minutes).

Days 15 through 28: Enemas were given 3 times a week on weeks 3 and 4 of the protocol.

Days 29-84 (2 to 3 months): Enemas were given weekly for a total of 3-8 weeks (less than 8 secondary to the cessation of the protocol according to the FDA mandate).

As supportive care, patients were allowed to take 4 mg (2 tablets of over the counter Imodium) loperamide by mouth 15-30 minutes prior to enema treatments to help retain the preparation. This dose of loperamide is appropriate for the age group. Loperamide is over-the-counter and FDA approved for the treatment of inflammatory bowel disease associated diarrhea.

Response and progression was monitored by PUCAI during the protocol. The clinical symptoms survey was performed prior to each enema delivery and a disease progression table was recorded for each enrolled patient.

Follow-up

Follow-up colonoscopy/sigmoidoscopy, sample collection and evaluation

Two weeks after the last weekly enema, colonoscopy with FMT was performed for patient 1. For the consecutive patients, we decided to perform flexible sigmoidoscopy without prior colon cleansing and those patients did not receive additional FMT. Stool samples were collected within one day of sigmoidoscopy. At the time of sigmoidoscopy, an assessment for macroscopic colitis using the Mayo classification was performed. Biopsies were obtained from the rectosigmoid area for routine histopathology and research purposes.

DETAILED CASE REPORTS

The patients were enrolled into an Institutional Review Board (IRB) approved clinical trial prior to the Food and Drug Administration (FDA) mandating the need for an investigational drug application for FMT (that is not directed towards the treatment of recurrent CDI). The protocol was stopped after the FDA mandate was issued in May of 2013. Therefore, only 1 patient (patient 1) completed the full 12 week course (28 scheduled treatments + "rescue" enemas if clinically indicated) of FMT. The clinical course of patients is shown in Supplementary Figure 1.

Patient 1: A 16-year-old male was diagnosed with pancolitis (Mayo score[4]=2; Supplementary Figure 3) one year before enrollment. His prior therapy included infliximab, oral corticosteroids and mesalamine. He was weaned off oral steroids and continued on infliximab infusions with oral mesalamine. FMT was initiated when he was in clinical (pediatric ulcerative colitis activity index;[2] PUCAI=5), macroscopic (Supplementary Figure 3), and microscopic (Supplementary Figure 4) remission after 6 weeks without infliximab. Thirty (30) treatments were administered during 3 months. He received mesalamine enemas twice for mild exacerbations (PUCAI ≥15). The patient remained in clinical remission (PUCAI <10) for more than 90% (65/70 days) of the treatment course (Supplementary Figure 1A). No adverse events were noted. A follow-up endoscopy 3 weeks after the last FMT showed the entire colon to be grossly normal (Supplementary Figure 3). However, histological inflammation in his transverse colon was detected, and maintenance oral mesalamine therapy was started. The patient remained in clinical remission (symptom-free, lab values within normal ranges, including fecal calprotectin) for 8 months (261 days) after his last infliximab treatment, 4 months (126 days) after his last FMT. At this time, he developed a mild disease flare (PUCAI=25) and wished to go back on infliximab therapy. He has intermittent breakthrough symptoms on 7.5 mg/kg infliximab every 6 weeks and daily mesalamine treatments for over 1.5 years since re-institution of the biologic agent.

Patient 2: A 15-year-old male with a history of UC with pan-colitis (Mayo: 2; Supplementary Figure 3) was treated for more than one year with 6-mercaptopurine (6MP), allopurinol, and daily oral mesalamine. He tested positive for toxigenic *Clostridium difficile* infection (CDI) at enrollment. Metronidazole therapy successfully cleared the CDI prior to starting FMT. Allopurinol and 6MP were stopped 3 weeks prior to initiation of FMT. Initial colonoscopy showed mildly active colitis (Mayo: 1; Supplementary Figure 3) with histological inflammation (Supplementary Figure 4) throughout the colon. Mesalamine was stopped on day 6 after initiation of FMT. The patient remained in remission throughout FMT therapy (Supplementary Figure 1B). Twenty-five FMT treatments were administered to this patient, and no adverse events were noted. The colon was grossly normal by sigmoidoscopy performed 2 weeks after the final FMT (Mayo 0; Supplementary Figure 3). No evidence of mucosal inflammation in the colon was found by histology (Supplementary Figure 4). This patient remained in remission (PUCAI<10) without any therapy for more than 2 months (80 days after last FMT, 159 days after last 6MP dose). However, he became symptomatic (PUCAI=35) and did not respond to oral mesalamine therapy following this symptom-free period. Pulse steroid treatments were initiated, and he required intermittent low dose steroid therapy with 4-5 week steroid-free intervals. The patient re-enrolled into our FMT trial 1 year afterwards, and is currently in remission off of any therapy following a second course of FMTs for more than 2 months after last FMT with normal fecal calprotectin level.

Patient 3: A 14-year-old female was diagnosed with severely active pancolitis four months prior to enrollment (Mayo 3, Supplementary Figure 3). She could not be weaned off steroids for more than 4 week intervals, and she opted to enroll into the FMT trial. Steroids were tapered during the first 10 days of FMT treatment, and mesalamine was stopped on day 14. She remained in remission during the FMT period (Supplementary Figure 1C). She received 22 rounds of FMT

with only one episode of moderate, self-resolving, positional headache noted. Sigmoidoscopy 2 weeks after the last FMT showed a grossly normal (Mayo 0) colon. No evidence of colonic mucosal inflammation was found by histology. She developed a flare during travel 4 weeks following the last FMT (PUCAI=20). Oral mesalamine successfully induced remission. She repeatedly became symptomatic with bloody stools and cramping (PUCAI=50) 11 weeks (79 days) after the last FMT (105 days after last steroid dose), at which time pulse steroid therapy was initiated. She remained steroid dependent following re-initiation of steroid therapy. The patient re-enrolled into our FMT trial 1 year afterwards, but was withdrawn from the study for worsening symptoms and eventually opted for colectomy after 2 months of steroid therapy.

MICROBIOME ANALYSES

Fecal microbiome characterization: Stool samples were processed by the Texas Children's Microbiome Center (Texas Children's Hospital, Houston, TX, USA) for DNA extraction and sequencing. Community DNA was extracted from each specimen using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA), following manufacturer's instructions. The resulting DNA was quantified using both a NanoDrop-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA) and Qubit fluorometer (Life Technologies Corporation, Carlsbad, CA, USA). Barcoded universal primers 357F (5'-CCTACGGGAGGCAGCAG-3') and 926R (5'-CCGTCAATTCMTTTRAGT-3') were used to amplify the V3V5 region of the bacterial *16S rRNA* gene. Each library construct was then processed and purified for 454 sequencing. Sequencing was performed on the Roche GS FLX 454 sequencer (454 Life Sciences, Branford, CT, USA).

Data analysis: Sequence data was parsed by barcode and quality filtered using QIIME (version 1.3.0),[5] as implemented in the Genboree Microbiome Toolset.[6] Sequences shorter than 200 bp length, having average quality scores less than 20, harboring ambiguous base calls, or having

mismatches to their barcode or sequencing primer were excluded from further analysis. Both the barcodes and sequencing primers were trimmed away, and the remaining sequences from the donor and patients were pooled and assigned to operational taxonomic units (OTUs) at a similarity cut off of 97% using Cd-hit.[7] The data set was screened for potential chimeras using the ChimeraSlayer algorithm[8], and all potential chimeras were excluded from downstream analysis. Identities were assigned to each OTU using the Ribosomal Database Project Classifier.[9] Given variation in sequencing depth, the 16S rRNA gene libraries were sub-sampled to an equal depth (i.e., 2600 sequences per library) prior to the evaluation of richness or calculation of diversity indices, including the Shannon diversity index and unweighted UniFrac distance measures. The results from the microbiome characterization of the donor were compared to the microbiome of the patients pre-transplant and at 2-weeks following the end of FMT therapy.

The sequences generated for this project were deposited in the NCBI Sequence Read Archive (SRA) under project accession SRP034948

RNA SEQUENCING AND ANALYSES

Total RNA was isolated from rectal biopsy specimens (stored in RNALater™ [Ambion through Life Technologies, Carlsbad, California]) according to the manufacturer's recommendation, with Trizol (15596, Life Technologies) and the RNeasy Mini Kit (74106, Qiagen). RNA samples were stored at −80°C until processing for RNA sequencing.

RNA samples from colon biopsies were QCed by spectrophotometry (NanoDrop-1000 Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, U.S.A.) and microfluidic electrophoresis (Experion Automated Electrophoresis System, Bio-Rad Laboratories, Hercules, CA). PolyA-selected libraries were prepared from total RNA samples with TruSeq RNA Sample Preparation Kits (Illumina, San Diego, CA). Cluster generation was performed with Illumina

TruSeq SR Cluster Kits v3 - cBot – HS, in a cBot Cluster Generation System and 100 bp paired-end-sequencing using Illumina TruSeq SBS Kits on an Illumina HiSeq 2000 Sequencing System resulting in mean sequencing depth of 160 million (101-213 million) reads per sample. CASAVA software (Illumina) was used to convert raw read data to fastq format. Sequencing reads were trimmed for quality ($q < 20$) and adapters and then aligned to the human genome (GRCh37/hg19) using Tophat2[9]. Cufflinks[10] was used for estimation of transcript abundances based on Fragments Per Kilobase of exon per Million fragments mapped (FPKM). Differential expression analysis was carried out using Cuffdiff, which calculates a test statistic based on the log ratio of a gene's expression in two conditions against the log of one. Multiple testing correction at a false discovery rate < 0.05 was applied to identify differentially expressed genes. The raw data was made publically accessible in Bioproject: http://www.ncbi.nlm.nih.gov/bioproject/253048

HISTOLOGY

The biopsy specimens were examined by a board-certified pediatric pathologist with expertise in GI pathologywho was blinded to previously reported histology reports. The specimens were fixed in 10% neutral buffered formalin immediately following endoscopy. The tissue samples were routinely processed and paraffin embedded. Paraffin sections (3 micron) were cut and stained with hematoxylin and eosin (H&E) with eight tissue sections on one slide. Duplicated 3 micron sections were stained for histone (H3) via immunohistochemistry using a polyclonal rabbit anti-human histone (06-570; Millipore, Billerica, Massachusetts). After pretreatment with HIER1 (Bond Epitope Retrieval Solution 1) for 30 minutes at 100 degreesFahrenheit, the specimens were incubated with the primary antibody dilution 1:800 at room temperature for 15 minutes. The detection system used was the Novocastra Bond Polymer Refine Detection System (biotin-free, peroxide conjugated) from LEICA, Newcastle upon Tyne, United Kingdom

with diaminobenzidine tetrahydrochloride (DAB) as the chromogen and hematoxylin as the counterstain.

STATISTICAL ANALYSES

The two-tailed paired t-test was used for group comparisons. Significance was relaxed to an arbitrary $p<0.25$ for OTU comparisons; otherwise it was determined at $p<0.05$. The Pearson correlation coefficient was calculated in Excel (Microsoft Office Excel 2007).Statistical significance of correlations was calculated with public Statistics Calculator version 3.0 (http://www.danielsoper.com/statcalc3/calc.aspx?id=44) and determined at $p<0.05$. See Methods section in Supplementary Appendix for bioinformatic analyses applied to the RNA sequencing data.

SUPPLEMENTARY TABLES

#OTU ID	Lowest taxonomic assignment	Before FMT (%)	After FMT (%)	Average change in abundance (%)	Average in donor preparations (%)
1179	Lachnospiraceae	1.538	6.205	4.667	0.404
1164	Lachnospiraceae	0.218	3.423	3.205	0.115
1406	Dorea	0.705	3.590	2.885	0.096
1886	Lachnospiraceae	0.205	1.885	1.679	0.000
1894	Lachnospiraceae	0.038	1.474	1.436	0.000
925	Ruminococcaceae	0.282	1.154	0.872	0.462
1929	Lachnospiraceae	0.013	0.410	0.397	0.058
577	Faecalibacterium	0.000	0.321	0.321	0.000
1094	Lachnospiraceae	0.000	0.295	0.295	0.000
1259	Ruminococcaceae	0.000	0.115	0.115	0.000
1135	Lachnospiraceae	0.000	0.064	0.064	0.000
423	Collinsella	0.000	0.051	0.051	0.000
1081	Lachnospiraceae	0.000	0.051	0.051	0.000
616	Ruminococcaceae	0.051	0.013	-0.038	0.000
1605	Streptococcus	1.244	0.192	-1.051	0.365

Supplementary Table 1: Consistent abundance (%) changes in Operational Taxonomic Units (OTUs) with lowest taxonomic assignment in the fecal microbiomes upon the FMT series in all 3 patients. Before and after values are average abundances from the 3 patients before and 2 weeks after the FMTs

Gene_id	Gene	Expression change	q_value
ENSG00000137757	FOLH1B	0.004	0.008
ENSG00000075856	CXCL5	0.010	0.000
ENSG00000064607	FSIP1	0.026	0.019
ENSG00000026508	SAA2	0.029	0.004
ENSG00000069493	SAA1	0.037	0.000
ENSG00000121152	RP11-124L5.7.1	0.052	0.012
ENSG00000126091	RC3H2	0.083	0.013
ENSG00000114742	TNIP3	0.084	0.002
ENSG00000065361	AC027323.1,LYSMD3	0.099	0.000
ENSG00000131236	PGLYRP4	0.110	0.024
ENSG00000075785	SLC6A20	0.169	0.001
ENSG00000106392	TNFSF15	0.171	0.031
ENSG00000198498	MTX3	0.179	0.000
ENSG00000111224	IL19	0.181	0.021
ENSG00000113593	TNFRSF6B	0.186	0.013
ENSG00000125885	FPR1	0.191	0.033

ENSG00000111711	ALPL	0.196	0.000
ENSG00000196517	FCGR3B	0.197	0.025
ENSG00000162747	AC131025.8.1,MIR143HG	0.204	0.007
ENSG00000141076	IL6STP1	0.206	0.032
ENSG00000136824	RP11-173P15.3.1	0.214	0.011
ENSG00000130881	RP11-622O11.2.1	0.224	0.040
ENSG00000122786	KCND3	0.229	0.001
ENSG00000261804	MCM10	0.229	0.020
ENSG00000108424	ANKRD36BP1	0.231	0.003
ENSG00000163482	AC110491.1	0.233	0.025
ENSG00000243955	TFPI2	0.236	0.020
ENSG00000173200	SKA3	0.237	0.003
ENSG00000117600	CREB3L3	0.238	0.000
ENSG00000184575	WASF3	0.239	0.001
ENSG00000172269	PHKA1P1	0.240	0.048
ENSG00000100934	GPR155	0.254	0.002
ENSG00000169398	PRSS21	0.258	0.042
ENSG00000138780	TPO	0.259	0.038
ENSG00000188529	THEMIS	0.259	0.006
ENSG00000214331	TRIM40	0.266	0.000
ENSG00000134851	OLFM4	0.266	0.019
ENSG00000149150	ST3GAL3	0.269	0.000
ENSG00000165118	HIN1L.1	0.272	0.038
ENSG00000104164	RP11-44F14.2.1	0.274	0.048
ENSG00000160949	CXCL1	0.277	0.019
ENSG00000109685	HS3ST2	0.281	0.000
ENSG00000197056	CDC7	0.285	0.001
ENSG00000165689	ADAMTS1	0.290	0.007
ENSG00000103197	RSAD2	0.292	0.003
ENSG00000115159	PADI1	0.294	0.046
ENSG00000090924	RP11-1220K2.2.1	0.297	0.010
ENSG00000241878	ERO1L	0.298	0.000
ENSG00000140575	CDCA2	0.304	0.046
ENSG00000160818	MTF1	0.308	0.019
ENSG00000147454	SHCBP1	0.309	0.019
ENSG00000188786	GVINP1	0.310	0.005
ENSG00000114770	DDX21	0.311	0.006
ENSG00000198373	RAD54L2	0.312	0.001
ENSG00000133816	CXCL3	0.315	0.001
ENSG00000221955	ZBTB41	0.318	0.002
ENSG00000157349	WDHD1	0.320	0.001
ENSG00000131149	TRAT1	0.321	0.046
ENSG00000183814	TET3	0.323	0.001
ENSG00000077809	RASGRF2	0.326	0.036
ENSG00000156990	ANLN	0.333	0.000
ENSG00000104388	PMAIP1	0.334	0.027
ENSG00000112812	XRN1	0.334	0.007

ENSG00000156931	ARPP19	0.336	0.041
ENSG00000156976	ATF7IP	0.341	0.000
ENSG00000113140	C6orf223	0.341	0.048
ENSG00000163820	CTPS	0.342	0.000
ENSG00000119950	RP11-356C4.4.1	0.344	0.034
ENSG00000120756	FAM111B	0.345	0.000
ENSG00000087586	SUV39H2	0.346	0.002
ENSG00000166912	CHI3L1	0.348	0.005
ENSG00000243943	HAS3	0.348	0.015
ENSG00000198431	ENAH	0.348	0.000
ENSG00000163655	CALD1	0.350	0.002
ENSG00000130653	ORC6	0.352	0.000
ENSG00000198964	CDC45	0.353	0.004
ENSG00000102901	CD28	0.353	0.020
ENSG00000107566	XRCC2	0.353	0.029
ENSG00000079246	NAV2	0.353	0.033
ENSG00000169251	SOCS3	0.355	0.033
ENSG00000113240	FSD1L	0.356	0.030
ENSG00000122188	SYNPO2	0.357	0.000
ENSG00000184220	RCAN3	0.357	0.007
ENSG00000021762	CHEK1	0.359	0.000
ENSG00000160072	CASP5	0.360	0.000
ENSG00000141682	PRDM1	0.361	0.000
ENSG00000133789	SCD	0.362	0.023
ENSG00000070269	PTPRC	0.365	0.000
ENSG00000135829	FSTL1	0.366	0.000
ENSG00000163714	MCM4	0.367	0.000
ENSG00000175216	C4BPB	0.368	0.003
ENSG00000143537	HELLS	0.368	0.004
ENSG00000143507	ACTG2	0.371	0.001
ENSG00000018699	DDX3Y	0.371	0.002
ENSG00000089280	RP11-343J24.1.1	0.373	0.015
ENSG00000165480	CDC25A	0.374	0.024
ENSG00000176945	GUCY1A3	0.375	0.047
ENSG00000164180	CHL1	0.377	0.044
ENSG00000101057	ATM	0.378	0.002
ENSG00000097046	ST3GAL1	0.378	0.006
ENSG00000177192	CLMP	0.379	0.040
ENSG00000142224	RTEL1.1	0.380	0.000
ENSG00000138074	CDC6	0.385	0.030
ENSG00000006652	RRM2	0.385	0.000
ENSG00000068438	ITGAL	0.387	0.000
ENSG00000154451	PHIP	0.390	0.015
ENSG00000100890	SLFN5	0.393	0.025
ENSG00000148926	FCRL1	0.393	0.000
ENSG00000103111	SMC4	0.394	0.000
ENSG00000148308	MS4A14	0.396	0.034

ENSG00000086015	PLEKHH2	0.397	0.050
ENSG00000055732	AHR	0.398	0.019
ENSG00000105993	PARP15	0.399	0.001
ENSG00000146670	ZFY	0.399	0.022
ENSG00000204394	FAM169A	0.400	0.014
ENSG00000168488	CSF3R	0.404	0.005
ENSG00000116539	RRM1	0.405	0.000
ENSG00000111912	RGS5	0.405	0.005
ENSG00000166923	CDCA5	0.406	0.007
ENSG00000137497	SLC7A6	0.406	0.000
ENSG00000170581	RP4-788L13.1.1	0.408	0.019
ENSG00000121281	CTDSPL2	0.409	0.012
ENSG00000094841	ATAD2	0.411	0.024
ENSG00000129422	NF1	0.411	0.000
ENSG00000114127	CENPK	0.412	0.000
ENSG00000160796	GREM1	0.412	0.000
ENSG00000091436	IL1RN	0.413	0.008
ENSG00000171051	KIT	0.413	0.042
ENSG00000177034	SLC38A7	0.416	0.027
ENSG00000105483	LMO7	0.417	0.000
ENSG00000174574	BIRC3	0.417	0.000
ENSG00000082153	MS4A1	0.419	0.000
ENSG00000164244	BLM	0.419	0.013
ENSG00000134369	SPAG9	0.420	0.004
ENSG00000152455	RTEL1,TNFRSF6B	0.423	0.014
ENSG00000117228	RP11-904M10.1.1	0.424	0.017
ENSG00000136560	GBP5	0.424	0.000
ENSG00000204120	ZNF169	0.424	0.011
ENSG00000168071	NEDD1	0.426	0.000
ENSG00000169679	KIF18B	0.426	0.003
ENSG00000157212	FAM55B	0.427	0.004
ENSG00000164649	CTA-286B10.7.1,MCM5	0.427	0.000
ENSG00000196873	MEF2C	0.427	0.010
ENSG00000123562	MEST	0.427	0.000
ENSG00000163017	TXNRD1	0.428	0.000
ENSG00000177888	PDP1	0.428	0.046
ENSG00000250251	REL	0.428	0.020
ENSG00000198121	C3orf26	0.428	0.001
ENSG00000112701	DHFR	0.431	0.012
ENSG00000013561	FCRL3	0.431	0.016
ENSG00000163519	MCM6	0.432	0.002
ENSG00000092439	B3GALT1	0.434	0.000
ENSG00000131504	NCOA7,RP11-73O6.4.1	0.437	0.000
ENSG00000132466	GBP1	0.439	0.001
ENSG00000148175	TRANK1	0.439	0.003
ENSG00000138398	DOCK2	0.440	0.006
ENSG00000118495	SMC1A	0.442	0.002

ENSG00000167522	PARP11	0.442	0.003
ENSG00000086666	SLFN13	0.442	0.002
ENSG00000140403	KYNU	0.443	0.025
ENSG00000166927	NCAPG	0.443	0.007
ENSG00000182866	ACSL4	0.443	0.036
ENSG00000215837	BANK1	0.444	0.013
ENSG00000138032	NFAT5	0.449	0.013
ENSG00000132142	CYR61	0.450	0.010
ENSG00000104205	TLE4	0.451	0.009
ENSG00000143297	RNASEH1	0.451	0.000
ENSG00000163635	PRKAA1	0.452	0.001
ENSG00000138764	GSTCD	0.452	0.030
ENSG00000099917	SCFD2	0.454	0.026
ENSG00000143811	HEG1	0.455	0.047
ENSG00000204713	SVEP1	0.456	0.025
ENSG00000159618	CEP135	0.456	0.006
ENSG00000175643	MCM8	0.456	0.000
ENSG00000135486	GART	0.456	0.000
ENSG00000124839	AKAP2	0.457	0.037
ENSG00000143337	S1PR1	0.459	0.021
ENSG00000166411	DEK	0.459	0.000
ENSG00000196159	LCP1	0.460	0.005
ENSG00000145041	ATXN7	0.460	0.022
ENSG00000214262	CIITA	0.461	0.001
ENSG00000213430	LONP2	0.461	0.000
ENSG00000079385	IL7R	0.461	0.035
ENSG00000168610	KIF24	0.462	0.041
ENSG00000140968	DUSP10	0.463	0.002
ENSG00000136699	SSX2IP	0.463	0.000
ENSG00000049130	PDE7A	0.464	0.000
ENSG00000140395	ACACA	0.465	0.001
ENSG00000100030	SAMD4A	0.466	0.010
ENSG00000106546	GTPBP1	0.466	0.002
ENSG00000110104	CFB	0.467	0.000
ENSG00000170144	CIRH1A	0.467	0.000
ENSG00000140263	MCM2	0.469	0.001
ENSG00000013503	CTTNBP2NL	0.469	0.003
ENSG00000113368	ITGA4	0.469	0.028
ENSG00000065328	IMPAD1	0.469	0.048
ENSG00000122420	MSH6	0.470	0.004
ENSG00000182271	SNHG3	0.470	0.020
ENSG00000154153	RFC5	0.471	0.000
ENSG00000077147	LMNB1	0.471	0.000
ENSG00000005483	DAPP1	0.472	0.000
ENSG00000172673	DIAPH3	0.473	0.004
ENSG00000138772	PCGF3	0.473	0.034
ENSG00000256364	GPRIN1	0.473	0.009

ENSG00000127603	KITLG	0.474	0.029
ENSG00000147649	STARD4	0.474	0.003
ENSG00000095564	WDR43	0.474	0.005
ENSG00000198901	NUP107	0.474	0.000
ENSG00000115282	FILIP1L	0.475	0.041
ENSG00000081026	GNL3L	0.475	0.001
ENSG00000116649	CNN1	0.475	0.000
ENSG00000024526	MAVS	0.475	0.006
ENSG00000134453	LIN9	0.477	0.000
ENSG00000232882	NT5C2	0.477	0.007
ENSG00000157827	ADCY7	0.478	0.019
ENSG00000114346	RP11-252A24.2.1	0.479	0.046
ENSG00000102837	FGD2	0.479	0.000
ENSG00000160688	TMEM97	0.479	0.000
ENSG00000140718	RAD51AP1	0.480	0.003
ENSG00000154237	WDFY1	0.482	0.034
ENSG00000198554	NBPF8	0.483	0.048
ENSG00000156804	CDK1	0.484	0.043
ENSG00000139641	TCF7	0.485	0.013
ENSG00000160703	LYN	0.485	0.025
ENSG00000105568	LEF1	0.485	0.027
ENSG00000162599	FCGR3A	0.486	0.000
ENSG00000172318	FAM55C	0.486	0.011
ENSG00000090316	EPB41L2	0.486	0.000
ENSG00000085449	MAP3K8	0.487	0.037
ENSG00000109919	TEAD4	0.487	0.002
ENSG00000145908	NPAS2	0.487	0.002
ENSG00000130826	RMI2	0.488	0.017
ENSG00000206053	NOLC1	0.488	0.027
ENSG00000197905	EHF	0.488	0.034
ENSG00000179978	RALGPS2	0.489	0.025
ENSG00000115239	USP45	0.489	0.021
ENSG00000139842	PHF20L1	0.489	0.002
ENSG00000125962	RAB5B	0.489	0.012
ENSG00000196584	SERPINE2	0.489	0.000
ENSG00000162692	AASS	0.489	0.034
ENSG00000181163	SGK3	0.490	0.004
ENSG00000172403	TNPO1	0.490	0.003
ENSG00000256667	PRRC2B	0.490	0.000
ENSG00000102606	FGR	0.491	0.000
ENSG00000136167	JAK3	0.491	0.022
ENSG00000168685	SMC2	0.491	0.000
ENSG00000079819	MSL2	0.492	0.045
ENSG00000109084	RPS6KA3	0.492	0.035
ENSG00000237683	BUB1	0.492	0.006
ENSG00000128928	RP11-261C10.3.1	0.493	0.023
ENSG00000168016	RAPGEF2	0.493	0.022

ENSG00000006744	ARHGAP11A	0.494	0.045
ENSG00000136286	RACGAP1	0.494	0.017
ENSG00000072501	MCOLN3	0.494	0.008
ENSG00000166197	MYBL2	0.494	0.041
ENSG00000116353	SEC23A	0.495	0.003
ENSG00000163735	PNPT1	0.495	0.000
ENSG00000088247	HAPLN3	0.496	0.031
ENSG00000093009	ECT2	0.496	0.000
ENSG00000146192	DYNC1LI2	0.496	0.005
ENSG00000196712	POLD3	0.496	0.005
ENSG00000138756	NAV1	0.497	0.027
ENSG00000137824	PLS1	0.497	0.001
ENSG00000143033	SLC5A6	0.497	0.000
ENSG00000137770	HERC3	0.498	0.012
ENSG00000132356	ARHGAP42	0.498	0.002
ENSG00000132780	COL12A1	0.498	0.048
ENSG00000029363	LILRB1	0.499	0.015
ENSG00000112096	AC013461.1.1	0.499	0.049
ENSG00000115919	PKD1P6	0.500	0.019
ENSG00000137504	SECISBP2L	0.500	0.011
ENSG00000126226	RFC4	0.501	0.000
ENSG00000203710	MGA	0.501	0.003
ENSG00000145725	GLIPR1	0.501	0.022
ENSG00000136319	BCLAF1	0.501	0.000
ENSG00000073417	PLDN	0.502	0.006
ENSG00000080189	PFAS	0.503	0.000
ENSG00000130723	FTO	0.503	0.027
ENSG00000164506	GEN1	0.504	0.000
ENSG00000177479	UBQLN1	0.504	0.004
ENSG00000166803	EXOC5	0.504	0.009
ENSG00000114857	SLAMF1	0.505	0.001
ENSG00000171385	STOM	0.505	0.015
ENSG00000049449	YIPF6	0.506	0.034
ENSG00000093183	FBXO32	0.506	0.000
ENSG00000259141	LMBR1	0.508	0.004
ENSG00000138795	GPD2	0.508	0.048
ENSG00000168918	PLEKHG2	0.508	0.007
ENSG00000197157	POLK	0.508	0.000
ENSG00000203879	TTC5	0.509	0.008
ENSG00000163734	ZNF268	0.509	0.000
ENSG00000135316	PRKD3	0.512	0.036
ENSG00000257743	WDR76	0.512	0.012
ENSG00000115232	TAF1A	0.513	0.019
ENSG00000116521	CD44	0.513	0.000
ENSG00000104331	PBK	0.513	0.008
ENSG00000145734	FAM126B	0.514	0.024
ENSG00000095739	DES	0.514	0.000

ENSG00000119408	C12orf48	0.514	0.001
ENSG00000198663	WDR62	0.514	0.034
ENSG00000175376	NR2C2	0.515	0.007
ENSG00000186998	ZEB2	0.515	0.031
ENSG00000155324	TTC27	0.515	0.042
ENSG00000179820	HPRT1	0.516	0.015
ENSG00000166012	STK38L	0.516	0.019
ENSG00000256269	AC093510.2	0.516	0.021
ENSG00000153395	CEP350	0.518	0.001
ENSG00000116062	IL10RA	0.518	0.033
ENSG00000160828	COL8A2	0.518	0.022
ENSG00000050426	HN1L	0.518	0.002
ENSG00000137801	GPRC5A	0.518	0.000
ENSG00000178295	CLEC2D	0.518	0.007
ENSG00000093167	HECTD2	0.519	0.000
ENSG00000197343	KDM2B	0.519	0.030
ENSG00000155545	CELF1	0.519	0.022
ENSG00000112297	LRRK2	0.520	0.019
ENSG00000139197	RAD54L	0.521	0.007
ENSG00000198826	FAT4	0.521	0.003
ENSG00000100376	CR1	0.522	0.002
ENSG00000177853	NAMPTL	0.522	0.028
ENSG00000112739	BMP2K	0.523	0.026
ENSG00000176619	MAN2A2	0.524	0.000
ENSG00000008513	GTF2I	0.524	0.006
ENSG00000221914	C1GALT1	0.524	0.000
ENSG00000163755	MYADM	0.524	0.000
ENSG00000137804	TRIB2	0.524	0.000
ENSG00000230724	STAT1	0.525	0.006
ENSG00000047457	SENP2	0.525	0.004
ENSG00000159131	KLHL29	0.526	0.000
ENSG00000167325	RASGRP4	0.526	0.019
ENSG00000186638	CAND1	0.526	0.000
ENSG00000134121	BZW1	0.526	0.006
ENSG00000005844	TONSL	0.527	0.008
ENSG00000071127	PALLD	0.528	0.006
ENSG00000003402	ETNK1	0.528	0.036
ENSG00000165792	INVS	0.528	0.003
ENSG00000104738	FAM118A	0.528	0.000
ENSG00000144580	PUS1	0.529	0.000
ENSG00000138185	NCAPD2	0.529	0.021
ENSG00000105639	HSPD1P1	0.529	0.007
ENSG00000122512	PRMT3	0.530	0.000
ENSG00000140829	MTR	0.532	0.001
ENSG00000090060	XPO4	0.532	0.032
ENSG00000197170	DDX11	0.533	0.000
ENSG00000118855	FGFR1	0.534	0.016

ENSG00000135968	HMG20A	0.534	0.001
ENSG00000143079	GCC2	0.535	0.001
ENSG00000114026	LPCAT1	0.535	0.000
ENSG00000188807	CAPRIN1	0.535	0.000
ENSG00000163918	NCAPH	0.535	0.031
ENSG00000159176	SLC25A37	0.536	0.004
ENSG00000198586	FMR1	0.536	0.000
ENSG00000205336	TYMS	0.536	0.013
ENSG00000163832	NFKBIZ	0.537	0.000
ENSG00000184863	TPD52L1	0.538	0.039
ENSG00000143375	IPO11	0.538	0.035
ENSG00000165732	PPP1R12A	0.538	0.000
ENSG00000182541	MTUS1	0.539	0.000
ENSG00000092201	WARS	0.539	0.001
ENSG00000111206	RPS6KB1	0.539	0.013
ENSG00000050730	NKTR	0.540	0.000
ENSG00000205583	MLLT6	0.540	0.009
ENSG00000113810	ZDHHC8	0.543	0.035
ENSG00000167775	ABCC5	0.544	0.002
ENSG00000107625	DCLRE1B	0.544	0.005
ENSG00000056097	CCP110	0.544	0.017
ENSG00000196547	UBE2T	0.545	0.036
ENSG00000144231	TOR1AIP1	0.545	0.000
ENSG00000013573	PPIP5K2	0.545	0.020
ENSG00000137267	MLL5	0.545	0.000
ENSG00000171681	SLC10A7	0.546	0.000
ENSG00000040341	SCLY	0.547	0.038
ENSG00000135919	HBS1L	0.547	0.000
ENSG00000173276	HYAL1	0.548	0.024
ENSG00000117155	GNAI1	0.548	0.044
ENSG00000135720	PPP2R1B	0.548	0.002
ENSG00000177189	MAPK1	0.548	0.036
ENSG00000102908	NFIX	0.548	0.022
ENSG00000073921	EIF1AY	0.549	0.034
ENSG00000105221	DPH2	0.549	0.000
ENSG00000132970	ABI3BP	0.550	0.014
ENSG00000082213	PARP14	0.550	0.023
ENSG00000043462	KBTBD2	0.550	0.007
ENSG00000144802	XRCC5	0.550	0.000
ENSG00000149269	BOP1	0.551	0.045
ENSG00000165124	ASH1L	0.551	0.044
ENSG00000117335	DKC1	0.551	0.009
ENSG00000119535	NMD3	0.552	0.001
ENSG00000165895	GLS	0.553	0.001
ENSG00000115364	KIAA1530	0.553	0.019
ENSG00000122008	MFN1	0.554	0.043
ENSG00000103202	CP	0.555	0.035

ENSG00000100852	MS4A7	0.555	0.011
ENSG00000111530	PTGFR	0.555	0.001
ENSG00000167491	CORO1C	0.555	0.032
ENSG00000138768	TCHP	0.555	0.032
ENSG00000196696	ANKRD17	0.555	0.050
ENSG00000144852	WEE1	0.555	0.004
ENSG00000129116	WDR1	0.557	0.004
ENSG00000227018	PDLIM5	0.557	0.002
ENSG00000132768	SLC6A9	0.558	0.037
ENSG00000164116	CLSTN3	0.558	0.015
ENSG00000154760	FAM86A	0.558	0.001
ENSG00000160593	MCM3	0.559	0.003
ENSG00000071575	KIAA0101	0.559	0.000
ENSG00000130227	PBX3	0.559	0.005
ENSG00000225241	FAM129C	0.559	0.000
ENSG00000129292	SOD2	0.559	0.014
ENSG00000111737	ATL2	0.559	0.000
ENSG00000203747	SORD	0.560	0.008
ENSG00000133835	PLXDC1	0.560	0.002
ENSG00000125730	DDX18	0.561	0.024
ENSG00000204614	SCAMP5	0.562	0.041
ENSG00000112282	RABL3	0.562	0.014
ENSG00000068308	NFATC2IP	0.562	0.000
ENSG00000138035	TMPO	0.563	0.014
ENSG00000111011	XPOT	0.564	0.045
ENSG00000162551	ADAM10	0.564	0.030
ENSG00000159140	SREK1	0.564	0.001
ENSG00000118894	LCP2	0.565	0.031
ENSG00000135387	SF3B3	0.565	0.000
ENSG00000103044	MTDH	0.566	0.001
ENSG00000120802	GOLT1B	0.568	0.028
ENSG00000117523	CDCA7L	0.568	0.000
ENSG00000121749	WHSC1	0.568	0.029
ENSG00000174799	SLC43A1	0.569	0.033
ENSG00000139190	STAT3	0.569	0.006
ENSG00000177426	GNA12	0.569	0.000
ENSG00000137752	SLC38A5	0.570	0.006
ENSG00000176142	DIAPH1	0.570	0.000
ENSG00000154175	VRK2	0.570	0.000
ENSG00000184163	FOXM1	0.571	0.032
ENSG00000125107	GNPDA2	0.571	0.004
ENSG00000154096	TMEM201	0.572	0.002
ENSG00000139163	BTN3A3	0.572	0.002
ENSG00000139182	KPNB1	0.572	0.005
ENSG00000163608	LIN54	0.573	0.000
ENSG00000057252	TMEM39A	0.573	0.004
ENSG00000163534	SMC6	0.574	0.003

ENSG00000163935	C9orf64	0.574	0.039
ENSG00000108039	NFIA	0.575	0.000
ENSG00000164045	POLR1B	0.575	0.041
ENSG00000086200	CALU	0.575	0.013
ENSG00000170485	VAMP1	0.576	0.032
ENSG00000135373	ACSL3	0.576	0.000
ENSG00000114867	GMPS	0.576	0.001
ENSG00000088833	MORF4L2	0.576	0.000
ENSG00000008294	MIER3	0.576	0.000
ENSG00000131018	KIF16B	0.577	0.046
ENSG00000139218	RAP1GDS1	0.577	0.000
ENSG00000144283	ZZZ3	0.578	0.001
ENSG00000134086	TTF2	0.578	0.041
ENSG00000154734	FAM111A	0.579	0.000
ENSG00000070367	MTF2	0.579	0.000
ENSG00000243716	VCAM1	0.579	0.030
ENSG00000010322	EXOSC2	0.580	0.000
ENSG00000151914	USP53	0.580	0.000
ENSG00000163386	SPN	0.580	0.043
ENSG00000114745	INPP5D	0.580	0.002
ENSG00000114650	RP11-368J21.2.1	0.581	0.008
ENSG00000155229	BTAF1	0.581	0.000
ENSG00000107968	EVL	0.581	0.001
ENSG00000168209	NPM1	0.581	0.003
ENSG00000163904	PEA15	0.581	0.025
ENSG00000119397	HSD17B12	0.582	0.034
ENSG00000198780	NUSAP1	0.582	0.028
ENSG00000172890	BDP1	0.582	0.000
ENSG00000178921	HPSE	0.582	0.001
ENSG00000217128	ATP2A2	0.582	0.000
ENSG00000101557	ZNF518A	0.583	0.020
ENSG00000150093	C12orf29	0.584	0.003
ENSG00000124508	ATAD3B	0.584	0.000
ENSG00000092470	RQCD1	0.584	0.002
ENSG00000076003	ARIH2	0.584	0.000
ENSG00000119777	TACC3	0.585	0.000
ENSG00000136653	SOAT1	0.585	0.000
ENSG00000139278	AKAP12	0.586	0.001
ENSG00000259131	HMBS	0.586	0.003
ENSG00000138434	STAG3L2	0.587	0.000
ENSG00000145675	ASRGL1	0.587	0.000
ENSG00000089335	ERLIN1	0.588	0.001
ENSG00000173706	C4orf43	0.588	0.027
ENSG00000166250	PIK3R1	0.589	0.010
ENSG00000171865	MICAL2	0.590	0.038
ENSG00000174720	USP14	0.590	0.006
ENSG00000146535	CEP164	0.590	0.021

ENSG00000162174	ATG4C	0.591	0.004
ENSG00000237289	MLLT4	0.591	0.000
ENSG00000080603	RBBP8	0.591	0.000
ENSG00000087266	ANKRD11	0.592	0.017
ENSG00000119402	KHSRP	0.592	0.001
ENSG00000119318	C4orf46	0.592	0.007
ENSG00000113328	GPAM	0.593	0.012
ENSG00000067646	RP11-304M2.2.1	0.593	0.007
ENSG00000134283	MYO19	0.593	0.013
ENSG00000153914	GPCPD1	0.594	0.000
ENSG00000142623	STK36	0.594	0.010
ENSG00000152527	CD8A	0.594	0.002
ENSG00000118655	ITGB1	0.594	0.045
ENSG00000137845	TMEM62	0.595	0.002
ENSG00000100897	G3BP1	0.595	0.028
ENSG00000198794	TM9SF3	0.595	0.034
ENSG00000133884	KLHDC4	0.595	0.010
ENSG00000111445	TMEM165	0.596	0.007
ENSG00000102854	MACF1	0.596	0.000
ENSG00000028116	MAP4	0.597	0.000
ENSG00000118976	SYNCRIP	0.597	0.012
ENSG00000008311	STAU2	0.597	0.025
ENSG00000146247	VARS	0.598	0.000
ENSG00000099904	IFNAR1	0.598	0.022
ENSG00000170275	SRRM1	0.598	0.000
ENSG00000135624	IARS	0.598	0.006
ENSG00000165055	LGALS8	0.598	0.000
ENSG00000156738	ADM	0.599	0.000
ENSG00000160856	PAXIP1	0.599	0.033
ENSG00000138593	EIF5	0.599	0.000
ENSG00000142949	UEVLD	0.599	0.001
ENSG00000149743	U2SURP	0.599	0.000
ENSG00000144848	HSD17B4	0.600	0.004
ENSG00000043514	SEC24D	0.600	0.000
ENSG00000145781	STXBP5	0.601	0.049
ENSG00000095787	HPS3	0.601	0.001
ENSG00000184661	SENP6	0.602	0.000
ENSG00000184557	GPATCH4	0.602	0.001
ENSG00000117481	PLAA	0.602	0.013
ENSG00000076685	TNFAIP3	0.603	0.029
ENSG00000121067	KDM5D	0.603	0.030
ENSG00000173083	LAX1	0.603	0.000
ENSG00000149187	FMNL2	0.604	0.000
ENSG00000100226	SGMS1	0.604	0.001
ENSG00000149182	CCNT2	0.604	0.000
ENSG00000164080	SPARC	0.605	0.000
ENSG00000143753	ANKRD49	0.605	0.000

ENSG00000117090	SMG7	0.606	0.000
ENSG00000111581	SNX19	0.606	0.008
ENSG00000214135	DNAJA4	0.606	0.001
ENSG00000052126	ZAP70	0.608	0.000
ENSG00000135164	FERMT1	0.608	0.002
ENSG00000245164	VPS8	0.609	0.034
ENSG00000150961	PAK1	0.610	0.000
ENSG00000204256	GTF2H2	0.610	0.012
ENSG00000163328	SP4	0.610	0.017
ENSG00000109920	DDIT4	0.610	0.003
ENSG00000170266	PMS2	0.611	0.000
ENSG00000138698	MYO1G	0.611	0.000
ENSG00000184162	SSFA2	0.611	0.031
ENSG00000112699	EI24	0.611	0.000
ENSG00000091651	MSTO1	0.612	0.001
ENSG00000013588	ZNF567	0.612	0.025
ENSG00000133641	HLA-DQB1	0.612	0.018
ENSG00000174827	CLK4	0.612	0.003
ENSG00000108443	ESYT1	0.612	0.005
ENSG00000127955	TRPM7	0.613	0.012
ENSG00000187147	ITGA6	0.613	0.004
ENSG00000211455	CDCA7	0.614	0.001
ENSG00000157404	ATAD3A	0.614	0.000
ENSG00000081059	DNM1L	0.614	0.004
ENSG00000180771	PISD	0.614	0.000
ENSG00000148187	CARD8	0.615	0.005
ENSG00000149636	FBXW2	0.615	0.000
ENSG00000100664	PLCB2	0.615	0.044
ENSG00000133048	CSNK1G3	0.615	0.006
ENSG00000134255	AC138035.1	0.616	0.006
ENSG00000082458	DFFA	0.617	0.016
ENSG00000205268	SEC22C	0.618	0.041
ENSG00000168958	PRC1	0.618	0.020
ENSG00000099194	CCDC86	0.618	0.000
ENSG00000198692	ZNF92	0.618	0.000
ENSG00000089177	CGGBP1	0.618	0.026
ENSG00000083099	DHX9	0.618	0.000
ENSG00000078668	NOP58	0.619	0.014
ENSG00000189042	TFDP1	0.620	0.005
ENSG00000115705	DDX50	0.620	0.041
ENSG00000114378	PPHLN1	0.620	0.000
ENSG00000110274	ABCB8	0.620	0.000
ENSG00000198951	PRRC2C	0.620	0.003
ENSG00000101773	SCAF11	0.621	0.006
ENSG00000139734	SWAP70	0.621	0.004
ENSG00000189091	DEPDC1	0.621	0.019
ENSG00000112339	AEBP2	0.622	0.001

ENSG00000006194	IVD	0.622	0.048
ENSG00000125630	POLR3B	0.622	0.038
ENSG00000154814	CBWD3	0.623	0.048
ENSG00000139546	LCK	0.623	0.026
ENSG00000145736	EIF1AD	0.623	0.025
ENSG00000142910	VEZT	0.623	0.000
ENSG00000138081	POLR3E	0.623	0.032
ENSG00000153064	PAPOLA	0.624	0.009
ENSG00000110696	SET	0.624	0.021
ENSG00000183010	CNTRL	0.624	0.003
ENSG00000169032	HNRNPA3	0.625	0.000
ENSG00000136689	PPIG	0.625	0.000
ENSG00000138964	SDCCAG3	0.626	0.008
ENSG00000249669	OGG1	0.626	0.027
ENSG00000176014	MARS	0.627	0.000
ENSG00000100813	AVL9	0.627	0.004
ENSG00000149591	GIGYF2	0.627	0.013
ENSG00000166483	NBPF3	0.627	0.004
ENSG00000160223	NQO1	0.628	0.006
ENSG00000165862	TAGLN	0.628	0.000
ENSG00000197930	ENTPD1	0.629	0.050
ENSG00000149547	CHD1	0.629	0.004
ENSG00000122515	STAG3L3	0.629	0.001
ENSG00000167965	TBC1D15	0.629	0.000
ENSG00000114416	DEGS1	0.629	0.018
ENSG00000157800	CCNG2	0.629	0.010
ENSG00000131373	SNRNP40	0.629	0.026
ENSG00000151116	CKAP5	0.630	0.000
ENSG00000008517	PARVG	0.630	0.007
ENSG00000181704	NASP	0.630	0.006
ENSG00000169554	SLC12A8	0.630	0.004
ENSG00000068724	MAGI3	0.630	0.017
ENSG00000204361	HNRPDL	0.630	0.006
ENSG00000116984	METTL2B	0.630	0.022
ENSG00000116337	IRF1	0.630	0.001
ENSG00000123219	CEACAM1	0.631	0.002
ENSG00000131016	TP53BP2	0.632	0.046
ENSG00000138750	SEC63	0.634	0.025
ENSG00000008441	GPR114	0.634	0.025
ENSG00000110324	CCAR1	0.634	0.030
ENSG00000134108	CAP1	0.634	0.004
ENSG00000148688	UBE2W	0.635	0.029
ENSG00000119969	STAG3L1	0.635	0.046
ENSG00000104731	NEK6	0.636	0.000
ENSG00000100714	MAPK9	0.636	0.010
ENSG00000140511	SKIV2L2	0.636	0.017
ENSG00000109756	RSPH3	0.636	0.030

ENSG00000104472	ARL8B	0.637	0.010
ENSG00000116191	BTN2A2	0.637	0.032
ENSG00000013810	GABARAPL1	0.638	0.000
ENSG00000104972	CHRAC1	0.639	0.042
ENSG00000204392	MYO1E	0.639	0.014
ENSG00000144840	TRIT1	0.639	0.003
ENSG00000130176	FAM82A2	0.639	0.030
ENSG00000128989	SRP72	0.639	0.000
ENSG00000229404	SRM	0.641	0.021
ENSG00000116698	AMPD2	0.642	0.000
ENSG00000106484	SH3BP2	0.642	0.016
ENSG00000170312	RBBP7	0.642	0.017
ENSG00000140382	TUBB6	0.642	0.000
ENSG00000103381	ZMIZ2	0.642	0.000
ENSG00000132341	MATR3	0.643	0.001
ENSG00000000938	XPO7	0.643	0.019
ENSG00000185215	KIAA1598	0.643	0.022
ENSG00000150667	SLMAP	0.643	0.006
ENSG00000094804	THBS1	0.644	0.010
ENSG00000125772	ANKRD28	0.644	0.046
ENSG00000105983	RASSF5	0.644	0.001
ENSG00000122482	ELAC2	0.644	0.020
ENSG00000166833	C3orf17	0.645	0.020
ENSG00000175787	ARHGAP5	0.646	0.000
ENSG00000102054	CFLAR	0.646	0.000
ENSG00000066136	TRIO	0.646	0.045
ENSG00000056586	RP11-1319K7.1.1	0.646	0.048
ENSG00000164062	EPAS1	0.646	0.000
ENSG00000164930	ZNF300	0.646	0.014
ENSG00000170852	SART3	0.647	0.000
ENSG00000132676	C5orf22	0.647	0.000
ENSG00000168386	ASPM	0.648	0.013
ENSG00000111247	ARHGEF19	0.649	0.029
ENSG00000160049	FUT8	0.649	0.037
ENSG00000108292	HNRNPH1	0.649	0.000
ENSG00000050748	TLK1	0.649	0.000
ENSG00000038382	RAN	0.649	0.021
ENSG00000168876	C3	0.649	0.042
ENSG00000171777	CPPED1	0.649	0.000
ENSG00000116977	NR2C2AP	0.650	0.020
ENSG00000156802	DLG3	0.650	0.042
ENSG00000133706	FNIP1	0.650	0.002
ENSG00000085999	MAP3K7	0.650	0.003
ENSG00000162924	GATAD2A	0.650	0.000
ENSG00000070756	TRAF3IP3	0.650	0.005
ENSG00000106638	MCFD2	0.650	0.000
ENSG00000111726	IDH3A	0.651	0.000

ENSG00000143870	SLC37A3	0.651	0.000
ENSG00000174579	SLC25A46	0.651	0.000
ENSG00000214837	CUL2	0.651	0.002
ENSG00000101311	WDR48	0.651	0.027
ENSG00000170727	PWP1	0.652	0.000
ENSG00000119707	RPS6KA4	0.652	0.047
ENSG00000111679	ZMYM1	0.652	0.003
ENSG00000119927	AIM1	0.652	0.024
ENSG00000140400	RCOR3	0.653	0.024
ENSG00000174353	ATXN2L	0.654	0.000
ENSG00000145390	LYRM2	0.654	0.007
ENSG00000092148	ICOSLG	0.654	0.018
ENSG00000114850	YME1L1	0.654	0.001
ENSG00000119335	SUN2	0.654	0.011
ENSG00000010292	SFMBT1	0.654	0.006
ENSG00000110619	ITGB3BP	0.654	0.002
ENSG00000132640	COMMD10	0.654	0.042
ENSG00000119509	CAMK2D	0.655	0.000
ENSG00000086102	TCERG1	0.655	0.026
ENSG00000149311	CCDC25	0.655	0.008
ENSG00000163430	SNX13	0.655	0.008
ENSG00000197299	FZD6	0.655	0.000
ENSG00000175084	UBR3	0.656	0.000
ENSG00000142794	MARCH7	0.656	0.023
ENSG00000196923	TMED2	0.656	0.000
ENSG00000149554	FAM134B	0.656	0.005
ENSG00000163945	NSUN4	0.656	0.046
ENSG00000139350	NME4	0.657	0.000
ENSG00000067048	PA2G4	0.657	0.001
ENSG00000086475	MTHFD1	0.657	0.018
ENSG00000144354	TP53	0.657	0.000
ENSG00000116560	B4GALT2	0.657	0.000
ENSG00000139579	VPRBP	0.658	0.043
ENSG00000198176	MON1B	0.658	0.010
ENSG00000066279	EIF4G1	0.658	0.000
ENSG00000011465	THY1	0.659	0.038
ENSG00000110090	SDHAP2	0.659	0.005
ENSG00000197822	DSN1	0.660	0.000
ENSG00000151690	PGP	0.660	0.032
ENSG00000171793	SND1	0.660	0.000
ENSG00000120708	MAP2K1	0.661	0.048
ENSG00000099282	KIAA0182	0.662	0.000
ENSG00000161048	NBEAL2	0.663	0.000
ENSG00000153563	PKP4	0.663	0.038
ENSG00000120519	CUL4A	0.663	0.000
ENSG00000130363	RNF2	0.663	0.005
ENSG00000164733	NFKB1	0.664	0.044

ENSG00000198642	AC024560.3.1	0.664	0.025
ENSG00000117625	SRSF10	0.665	0.000
ENSG00000141140	RBM25	0.665	0.009
ENSG00000188938	FUS	0.665	0.000
ENSG00000179583	CASD1	0.665	0.001
ENSG00000111540	OBFC2B	0.665	0.002
ENSG00000060642	ODF2	0.666	0.039
ENSG00000241978	TNFAIP2	0.666	0.036
ENSG00000137825	ZNF644	0.666	0.001
ENSG00000028203	ARMCX5	0.668	0.034
ENSG00000055044	CARD16,CARD17,CASP1	0.668	0.018
ENSG00000162302	SEH1L	0.668	0.036
ENSG00000113319	FYN	0.668	0.010
ENSG00000260528	KIAA0895L	0.668	0.034
ENSG00000039123	PPWD1	0.668	0.005
ENSG00000068366	RCN1	0.669	0.002
ENSG00000258366	THEM4	0.669	0.011
ENSG00000213213	MEF2D	0.669	0.003
ENSG00000023445	LPAR1	0.669	0.000
ENSG00000178462	LMNB2	0.669	0.000
ENSG00000145860	BTBD3	0.670	0.023

Supplementary Table 2: Genes with significantly decreased (>1.5 fold decrease in expression, q value = false discovery rate [FDR] <0.05) expression by RNA sequencing according to Cuffdiff bioinformatic analysis (see Supplementary Methods)

Gene_id	Gene	Fold change	q_value
ENSG00000133226	AMIGO3	undetected before	0.000
ENSG00000127314	LGSN	17.620	0.000
ENSG00000130396	AC027323.1	4.763	0.013
ENSG00000139154	PDZK1	3.812	0.001
ENSG00000100297	GSTA1	3.368	0.007
ENSG00000111799	MSLN	3.278	0.001
ENSG00000197785	PNLIPRP2	2.509	0.004
ENSG00000157954	KIAA1984	1.706	0.017
ENSG00000115419	SLC37A2	1.665	0.002
ENSG00000116406	BAMBI	1.634	0.000
ENSG00000166928	PIGZ	1.607	0.008
ENSG00000163110	FAM132A	1.589	0.034

Supplementary Table 3: Genes with significantly increased (>1.5 fold increase in expression, q value = false discovery rate [FDR] <0.05) expression by RNA sequencing according to Cuffdiff bioinformatic analysis (see Supplementary Methods)

MEDICAL HISTORY

Please check conditions **YOU** have now, or have had in the past or list below.

☐ Alcohol/drug Abuse	☐ Cataracts	☐ Herpes	☐ Rheumatic Fever
☐ Anemia	☐ Depression	☐ High Blood Pressure	☐ Stroke
☐ Anxiety	☐ Diabetes	☐ High Cholesterol	☐ Thyroid Problem
☐ Arthritis	☐ Emphysema	☐ HIV/AIDS	☐ Tuberculosis
☐ Asthma	☐ Epilepsy	☐ Kidney Disease	☐ Sexually Transmitted Diseases
☐ Bleeding Disorder	☐ Glaucoma	☐ Liver Disease	
☐ Breast Lump	☐ Heart Disease	☐ Migraine Headaches	
☐ Cancer: _____	☐ Hepatitis	☐ Prostate Problem	

Other (please list): _____

SURGERIES

Please check or list the surgeries **YOU** have had.

☐ Appendectomy ☐ Heart Bypass/Stent ☐ Hysterectomy **Other:**_____ _____

☐ Gallbladder Removed ☐ Tonsilectomy ☐ Tubal Ligation _____ _____

HOSPITALIZATIONS List all of your hospital stays for illness or surgery beginning with the most recent.

Date	**Reason**	**Hospital**	**Physician**

LIFESTYLE CHOICES

Smoking Status ☐ Never ☐ Currently ☐ Quit: YEAR _____

Type: ☐ Cigarettes (Packs/day _____) ☐ Cigars ☐ Pipes ☐ Second-hand Smoke

Smokeless Tobacco ☐ Never ☐ Currently ☐ Snuff ☐ Chew ☐ Quit: YEAR_____

Alcohol Use *Type:* ☐ Liquor ☐ Wine ☐ Beer

☐ 0 drinks/week ☐ 1-6 /week ☐ 7-14 /week ☐ Over 14/week

On any single occasion during the past **3 months**, have you had more than **5 drinks** containing alcohol? ☐ Yes ☐ No

Weight Now _____ 1 year ago_____ Desired_____

Caffeine Drinks per day _____ Types: ☐ Cola ☐ Coffee ☐ Tea

Special diet? ☐ Vegetarian ☐ Vegan ☐ Other:_____

Exercise Type: _____Frequency per week_____ Duration:_____

Donor Health Information Sheet		
Chronic diseases:		
	YES	NO
Antibiotics in past 6 months:		
If yes, when: _____		
Have you been exposed to HIV or viral hepatitis at any time?		
Have you ever had sex for drugs or money?		
Are you a man who has had sex with men?		
Have you had more than one sexual partner in the last 12 months?		
Have you ever been incarcerated?		
Have you ever used intravenous drugs or intranasal cocaine?		
Did you have a tattoo or body piercing within 12 months?		
Have you traveled to areas of the world with increased risk of traveler's diarrhea in the past 3 months?		
Are you currently ill (flu symptoms, fevers, runny nose, etc.)?		

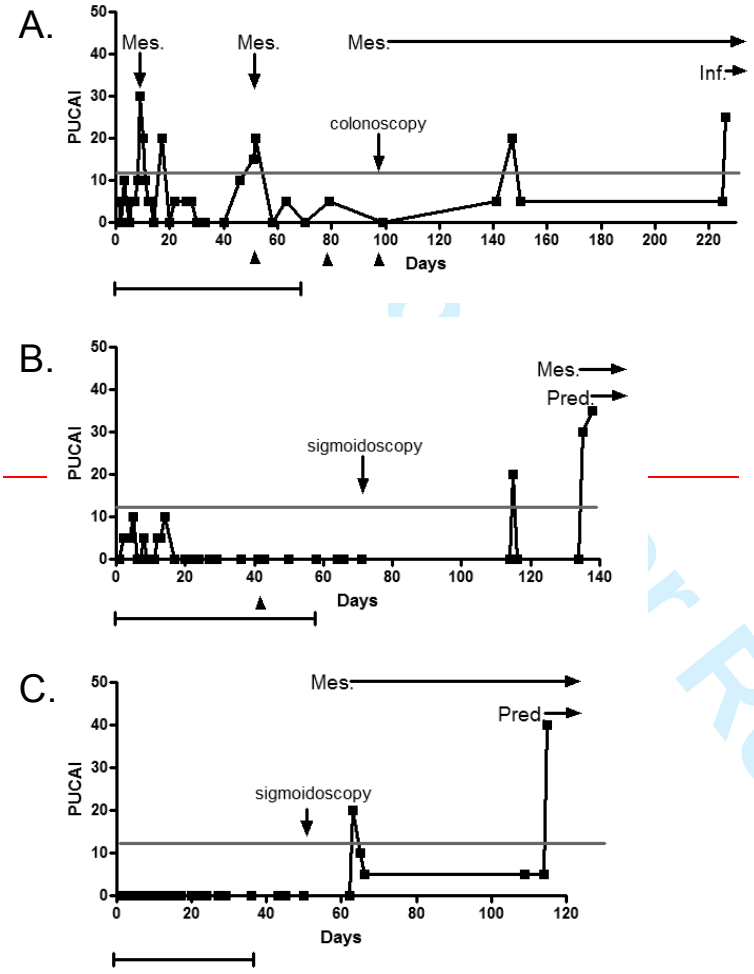
Do you have a history of irritable bowel syndrome, or any of the associated symptoms (frequent abdominal cramps, excessive gas, bloating, abdominal distension, fecal urgency, diarrhea or constipation)?		
Do you have a history of inflammatory bowel disease such as Crohn's disease, ulcerative colitis, lymphocytic colitis?		
Do you have chronic diarrhea?		
Do you have chronic constipation or use laxatives regularly?		
Do you have a history of gastrointestinal malignancy (cancer) or known colon polyposis?		
Have you ever had abdominal surgery (for example: gastric bypass, intestinal resection, appendectomy, cholecystectomy, etc.)		
Do you use probiotics or any other over the counter aids for specific purposes of regulating digestion?		

Supplementary Table 4: Donor Health Information Sheet

Stool testing
Clostridium difficile toxin A and B by PCR; if unavailable, then toxins A and B by EIA
Routine bacterial culture for enteric pathogens
Fecal Giardia antigen
Fecal Cryptosporidium antigen
Ova and parasites
Serologic testing
HIV type 1 and 2
Hepatitis A virus (HAV) immunoglobulin (Ig) M
Hepatitis B virus (HBV) (surface antigen/antibody; core antibody)
Hepatitis C virus (HCV) antibody
Syphilis serology (Trep-sure ELISA; RPR done if treponemal test is positive)

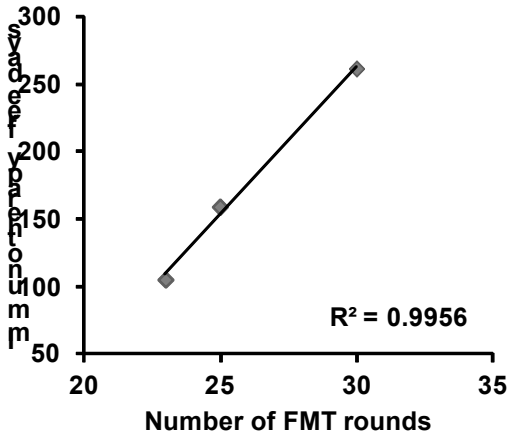
Supplementary Table 5: Donor Screening Test

SUPPLEMENTARY FIGURES

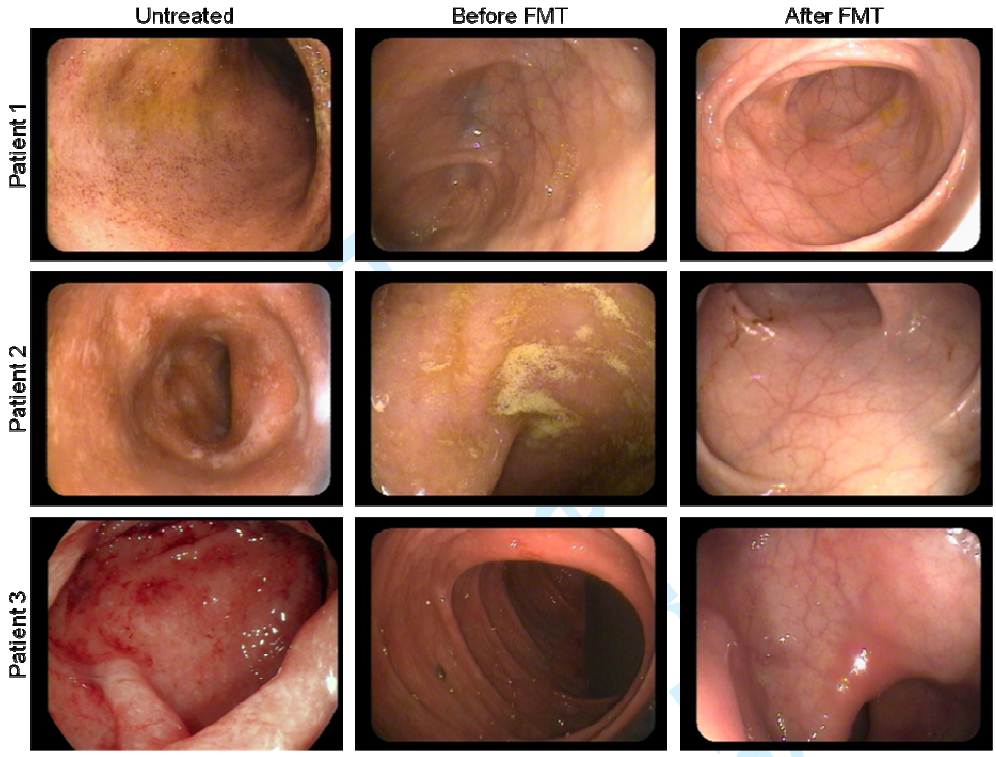


Supplementary Figure 1. Clinical Disease Activity during the Trial. Patient 1 (A.) received 30 rounds of FMT and experienced the longest clinical remission (pediatric ulcerative colitis activity index [PUCAI] ≤10; grey line). Patient 2 (B.; 25 treatments) and 3 (C.; 22 treatments) were in remission while receiving the FMT course (— below the x axes designates the time of active FMT therapy). All patients remained in remission following FMT for more than 11 weeks,

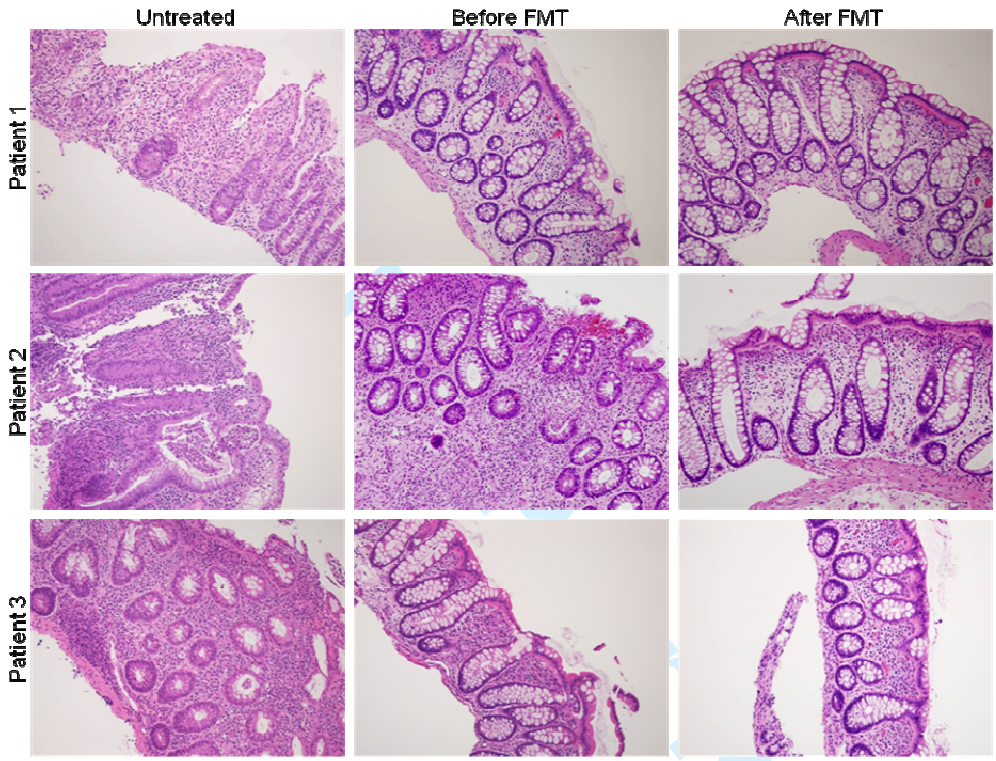
but eventually experienced a relapse requiring immunotherapy. The length of the immunotherapy free period correlated with the number of treatments received. Oral and/or rectal mesalamine (Mes.) was allowed during the trial, depending on clinical disease activity. Additional ("rescue") enemas (▲) were allowed during the first 12 weeks of the trial (i.e. during the weaning course of FMTs). Inf.: infliximab; Pred.: prednisone.



Supplementary Figure 2. Correlation between Number of FMTs and the Immunotherapy Free Period. There was a significant (r=0.998, two tailed p=0.04) correlation between the number of FMT rounds received, and the length of the immunotherapy free period (in days) in the 3 patients studied.



Supplementary Figure ~~1~~3. Endoscopic images of the colonic mucosa at diagnosis (untreated), prior to FMT therapy and 2 weeks following last FMT in the patients. Patient 1 had Mayo 2 level mucosal pancolitis at diagnosis that turned into grossly normal (Mayo 0) picture with infliximab therapy and remained normal following the FMT series. These numbers for patient 2 were: Mayo 2, 1, and 0 at diagnosis, before, and following the FMTs, respectively. Patient 3 had severe (Mayo 3) pancolitis at diagnosis, enrolled in remission on steroids into the trial, then remained in gross mucosal remission (Mayo 0) following the FMTs.



Supplementary Figure ~~2~~4. Histologic (hematoxylin and eosin) images of the colonic mucosa at diagnosis (untreated), prior to FMT therapy and 2 weeks following last FMT in the patients. The histologic severity of inflammation largely mirrored the gross endoscopic findings (see Supplementary Figure 1) in the rectosigmoid colon. All three patients had normal mucosal architecture following the FMT series. Magnification 200x

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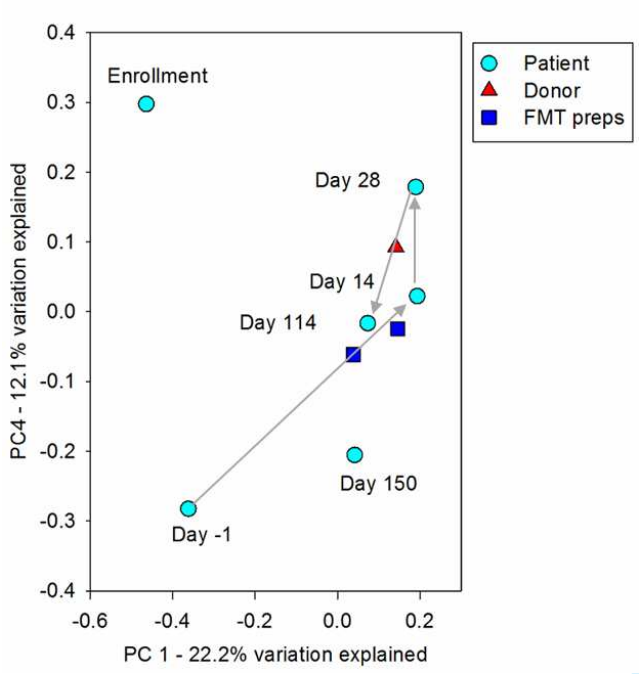
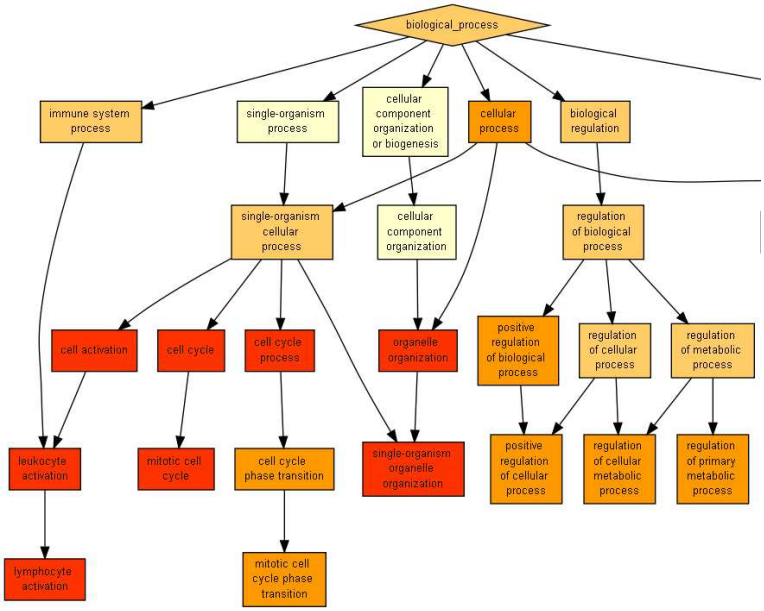


Supplementary Figure 5. Temporal Microbiome Shifts in Patient 1. Principle coordinates analysis of unweighted Unifrac distances between patient 1 and the donor (stool and fecal preparations) showed that the recipient microbiomes became similar to that of the donor by 14, 28, and even 114 (2 weeks after last FMT received) days into the study. By day 150, the patient microbiome started to shift back to that before the start of the trial (Day-1). Therefore, the FMT series appeared to induce a transient engraftment of the donor microbiome in the recipient.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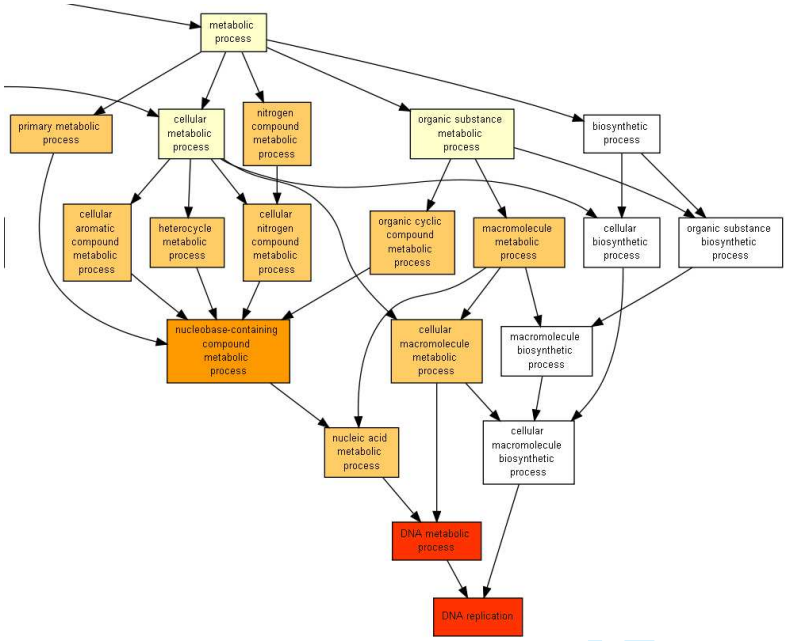
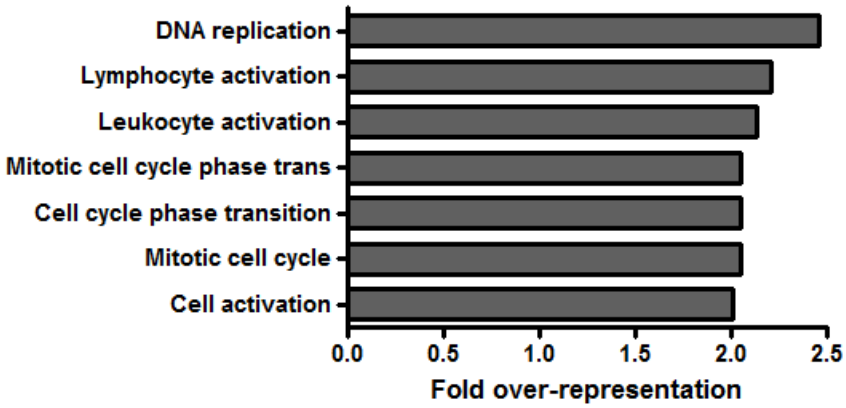
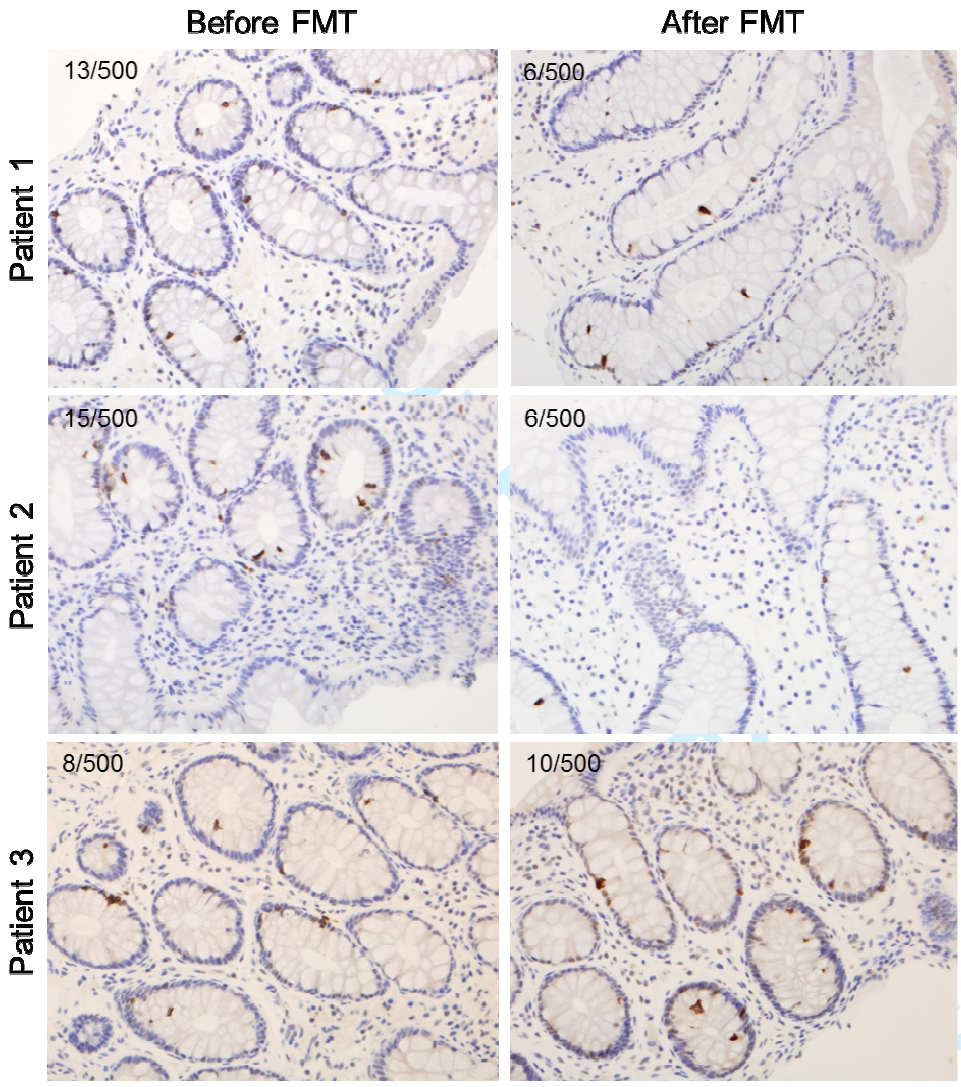


Supplementary Figure ~~3~~6. Enrichment of genes in relationship to biological processes in the down-regulated transcripts upon the series of FMTs in our 3 UC patients (>1.5 fold suppression; FDR<0.05) by Gene Ontology enRIchment anaLysis and visuaLizAtion tool (Gorilla: http://cbl-gorilla.cs.technion.ac.il/). The down-regulated transcripts were compared to a control set without expression change (FDR>0.97) upon the FMTs in the rectosigmoid mucosa. light yellow: p= 10^{-3}-10^{-5}; dark yellow: p= 10^{-5}-10^{-7}; orange: p= 10^{-7}-10^{-9}; red: p<10^{-9}



Supplementary Figure 47. Biological processes with more than 2 fold over-representation (FDR $<10^{-5}$) in the down-regulated genes compared to control following serial FMTs in UC patients.



Supplementary Figure 8. Epithelial Cell Mitosis Suppression Following FMT. In 2 out of 3 patients, the numbers of epithelial mitoses (blinded examination of 500 epithelial cells) decreased by more than 50% following high intensity FMT. Mitoses were highlighted by histone (H3) immunohistochemistry. Magnification 40x

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