Supplementary Text

Donor Selection:
To select a donor, we used 16S rRNA sequencing data from sample sequences collected from patients with HE and healthy controls(n=174) from Virginia Commonwealth University and McGuire VA Medical Center. These were used to train a Random Forest Classifier, a machine learning classification technique. This resulted in a classifier with AUC of 0.94 that was used to classify OpenBiome stool donors , giving each donor a classification score (formally, the percentage of trees in the Random Forest Classifier that classify the sample as 'healthy'). Furthermore, the relative abundances of *Lachnospiraceae* and *Ruminococcaceae* bacterial families in each donor, which were depleted in HE patients, were also studied. These data were combined to rank all 28 OpenBiome stool donors. From these rankings, the donor with the highest aggregate ranking was selected as the FMT donor f. This donor was a healthy 37-year-old male without serious adverse events (SAE) reported in >250 patients receiving his material for recurrent *C. difficile,* and had the highest relative abundances of *Lachnospiraceae*, and *Ruminococcaceae* among the stool donor registry. The same stool sample from this donor for each of the aliquots for the FMT-assigned groups.

OpenBiome Stool Donor Preparation:
FMT was prepared from a healthy volunteer screened by OpenBiome, in keeping with FMT best practices[1]. Stool donors underwent a rigorous 240-item donor health questionnaire and a laboratory assessment including comprehensive stool-based and serological assays for pathogenic organisms (supplementary table 1). Details are in supplementary table 1. Donors receive complete rescreening every 60 days, continuous health monitoring, and random health checks. All material was held in quarantine until the next rescreen was passed, and material donated 21 days prior to each rescreen was held in quarantine until the following rescreen was passed.
Donor stool was processed within six hours of passage in a UV-sterilized biosafety cabinet cleaned with a sporicidal agent. To produce FMT capsules, stool samples were transferred to a sterile filter bag and homogenized in a sterile glycerol-cocoa butter solution (20% glycerol). A ratio of 1 mL of diluent per gram of stool was used. The filter bag with the stool and buffer is sealed and introduced to secondary containment then homogenized for a minimum of 120 seconds. The solution is aliquoted (550 µL) with a multichannel pipette directly into empty size 0 gelatin capsules using sterile filter pipette tips. Filled capsules are capped and sealed using the capsule filler machine. These filled capsules are encapsulated in size 00 acid-resistant delayed release hypromellose capsule. Each capsule is visually inspected for integrity and contamination of the outer surface. Capsules passing inspection are placed into a high- density polyethylene plastic bottle for immediate storage at – 80^{o}C until administration.
Placebo treatments were prepared in the same manner except with an only a sterile solution of 80% cocoa butter, 20% glycerol and brown food coloring, without the addition of human stool.

Cognitive testing:
PHES is a battery consisting for five validated paper-pencil tests[2]. It consists of the number connection test-A, number connection test B, digit symbol test, serial dotting test and line tracing test (has two components; time and errors). Standard deviations are beyond population control values are calculated and the total is added to give the total PHES score; a low value indicates poor performance[3].

EncephalApp Stroop is a validated App version of the Stroop test available on Android and iOs devices[4]. This tests has an easier "Off" and harder "On" Stage. The Off stage requires subjects must identify the color of the pound-signs presented on the phone. The "On" stage in

which subjects must correctly identify the color of a discordant word presented. For example, the word “BLUE” will be presented in green colored letters and the correct response would be green and not blue. The presentation requires two practice runs and 5 correct runs in the Off and On Stage. The total time required for 5 correct On and 5 correct Off stage runs is the “OffTime+OnTime” which is the ultimate outcome in cirrhosis[3].

Human Biopsy specimen analysis:
Primers for genes of interest were designed using NCBI PrimerBlast. A 500 to 1,000 bp portion of the gene encompassing these primers was obtained from Life Technologies custom oligo GeneArt division and the gene fragment was cloned into pCR Blunt II TOPO vector (Invitrogen). The resulting plasmid is used to construct a standard curve for qPCR in the range of e8 to e3 copy number. Standard qPCR protocol is 95oC for 3 minutes, followed by 40 cycles of 10s at 95oC and 45s at 52oC, using BioRad SYBR Green Supermix as fluorophore.

Microbiota analysis
Interrogation of the Microbiome. We used the 16S rRNA to interrogate and characterize gut microbiome composition. We routinely use Length Heterogeneity PCR (LH-PCR) fingerprinting to rapidly survey our samples and standardize the community amplification. We then interrogated the microbial taxa associated with the gut mucosal microbiome using Multitag Sequencing (MTS) on the samples. This latter technique allows the rapid sequencing of multiple samples at one time yielding thousands of sequence reads per sample.

Bacterial Community Fingerprinting. LH-PCR was done as previously published. Briefly, total genomic DNA was extracted from tissue using Bio101 kit from MP Biomedicals Inc., Montreal, Quebec as per the manufacturer’s instructions. About 10 ng of extracted DNA was amplified by PCR using a fluorescently labeled forward primer 27F (5’-(6FAM) AGAGTTTGATCCTGGCTCA G-3’) and unlabeled reverse primer 355R’ (5’-GCTGCCTCCCGTAGGAGT-3’). Both primers are universal primers for Bacteria [5]. The LH-PCR products were diluted according to their intensity on agarose gel electrophoresis and mixed with ILS-600 size standards (Promega) and HiDi Formamide (Applied Biosystems, Foster City, CA). The diluted samples were then separated on a ABI 3130xl fluorescent capillary sequencer (Applied Biosystems, Foster City, CA) and processed using the Genemapper™ software package (Applied Biosystems, Foster City, CA). Normalized peak areas were calculated using a custom PERL script by and OTUs constituting less than 1% of the total community from each sample were eliminated from the analysis to remove the variable low abundance components within the communities.

MTS**:** We employed Multitag Sequencing to characterize the microbiome from a subset of the mucosal samples that were used in the LH-PCR analysis. Specifically, we have generated a set of 96 emulsion PCR fusion primers that contain the Ion Torrent emulsion PCR linkers and different 8 base “barcode” on either of the 27F or 355R universal 16S rRNA primers. Thus, each mucosal sample was amplified with a uniquely barcoded set of forward and reverse 16S rRNA primers and then up to 48 samples were pooled and subjected to emulsion PCR and sequenced using a Ion Torrent PGM. Data from each pooled sample were “deconvoluted” by sorting the sequences into bins based on the barcodes using custom PERL scripts. Thus, we were able to normalize each sample by the total number of reads from each barcode. We have noted that ligating tagged primers to PCR amplicons distorts the abundances of the communities and thus it is critical to incorporate the tags during the original amplification step. Several groups have employed various barcoding strategies to analyze multiple samples and this strategy is now well accepted [6].

Bacterial Taxon Analysis**:** We identified the taxa present in each sample by blasting the sequences against the Ribosomal Database Project (bacteria). We filter the raw data using a 180 base pair cutoff. The abundances of the bacterial identifications were then normalized using a custom PERL script and taxa present at >1% of the community were tabulated. For this approach, a 1% cut off was chosen because of our *a priori* assumption that taxa present in < 1% of the community vary between individuals and have minimal contribution to the functionality of that community and 2,000 reads per sample will only reliably identify community components that are greater than 1% in abundance [7]. The underlying *a priori* assumption for this filtering is that the low abundance components of the community vary between individual subjects and will not contribute significantly to the functionality of the gut mucosal microbiome [8]. Additionally, we use a Bayesian classifier that uses a posterior probability to identify query sequence based on the occurrence of seven-length base pair subsequences in the rRNA database and have implemented the Quantitative Insights Into Microbial Ecology (QIIME) package for UNIFRAC analysis. UPARSE was used to define diversity indices and clustering.

Supplementary Table 1: Donor selection procedures at OpenBiome

<table>
<tr><th colspan="2">Health Questionnaire</th></tr>
<tr><td colspan="2">

Gastrointestinal disease history
Autoimmune disease history
Asthma history
Allergy history
Chronic fatigue and chronic pain history
Cardiovascular and metabolic disease history
Neurological disease history
Mental health and wellbeing history
Cancer history
Infectious disease history
Surgical history
Medication history
Family history
Travel history
Sexual history
Social history

</td></tr>
<tr><th>Stool tests</th><th>Blood tests</th></tr>
<tr><td>

Clostridium difficile
Isospora and Cyclospora
Ova and parasites
Salmonella
Shigella
Escherichia coli (EHEC, O157:H7)
Campylobacter
Vibrio
Vancomycin-resistant Enterococci
CRE
ESBL
Giardia
Cryptosporidium
Helicobacter pylori
Norovirus
Rotavirus
Microsporidia
Adenovirus

</td><td>

Complete blood count and differential
Liver function panel (ALP, ALT, AST, Bilirubin, Albumin)
HIV (antibodies type 1+2)
Hepatitis A (anti-HAV IgM antibody)
Hepatitis B (HBsAg, anti-HBs IgM+IgG antibodies)
Hepatitis C (anti-HCV antibodies)
Syphilis
HTLV (HTLV 1+2 antibodies)
Strongyloides

Nasal Swab test

Methicillin-resistant Staphylococcus aureus

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Supplementary Table 2: Eligibility Criteria for the Study

Inclusion Criteria	Exclusion Criteria
-21-75 years of age -Cirrhosis diagnosed by any one of the following in a patient with chronic liver disease (a) Liver Biopsy (b) Radiologic evidence of varices, cirrhosis or portal hypertension (c) Laboratory evidence of platelet count <100,000 or AST/ALT ratio>1 (d) Endoscopic evidence of varices or portal gastropathy -At least two HE episodes, one within the last year but not within the last month - Adherent on HE medications (patient can be on lactulose and rifaximin) - Able to give written, informed consent (mini-mental status exam>25 at the time of consenting) -Women of child bearing potential must agree to use effective contraception for the duration of the study and for 10 days prior and 30 days after the study -Negative pregnancy test in women of childbearing age	*Disease-related:* (1) MELD score>17 (2) WBC count<1000 (3) TIPS, non-elective hospitalization or HE within last month (4) on dialysis (5) known untreated, in-situ luminal GI cancers (6) chronic intrinsic GI diseases (ulcerative colitis, Crohn's disease or microscopic colitis, eosinophilic gastroenteritis and celiac disease) (7) major gastrointestinal or intra-abdominal surgery within three months *Endoscopy-related*: (1) Platelet count<50,000 (2) adverse reactions to sedation (3) lack of driver or other contra-indications (4) unwilling to undergo endoscopic procedures *Safety-related*: (1) Dysphagia (2) History of aspiration, gastroparesis, intestinal obstruction (3) Ongoing antibiotic use (except for Rifaximin) (4) Severe anaphylactic food allergy (5) allergy to ingredients Generally Recognized As Safe in the FMT capsules (glycerol, sodium chloride, hypromellose, gellan gum, titanium dioxide, theobroma oil) (6) Adverse event attributable to prior FMT (7) ASA Class IV or V (8) Pregnant or nursing patients (9) acute illness or fever within 48 hours of the day of planned FMT (10) immunocompromised due to medical conditions or immunosuppressive therapies (11) Probiotic use within the last 48 hours of the day of planned FMT (12) Any condition that the physician investigators deems unsafe, including other conditions or medications that the investigator determines puts the participant at greater risk from FMT

Supplementary Table 3: Symptom severity changes

PARAMETER COMPARED TO BASELINE	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
ESTIMATING SEVERITY GRADE				
	Symptoms with minimal change from baseline causing no or minimal interference with usual social & functional activities	Symptoms with moderate change from baseline causing greater than minimal interference with usual social & functional activities	Symptoms with severe change from baseline causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
SYMPTOM SPECIFIC SEVERITY GRADE				
Fever (oral)	(99.9-100.5°F)	(100.6-101.5°F)	(101.6. -104°F)	> 104°F
Diarrhea	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Distension/bloating, abdominal discomfort	Asymptomatic	Symptomatic, but not interfering with GI function	Symptomatic, interfering with GI function	---
Abdominal Pain	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Life threatening consequences (i.e. acute peritonitis)
Dehydration	Increased oral fluids indicated; dry mucous membranes; diminished skin turgor	IV fluids indicated <24 hours	IV fluids indicated >24 hours	Life-threatening consequences (e.g. hemodynamic collapse)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)

PARAMETER COMPARED TO BASELINE	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Procedural Enema	Rectal discomfort; minor bleeding <24 hours on toilet paper	Rectal discomfort; minor bleeding >24 hours on toilet paper	Gross hematochezia	Bowel perforation
Change in mental status (West-Haven Criteria)	No change in orientation and no asterixis	Disorientation to time, asterixis	Disorientation to place or person, asterixis, lethargy and stupor	Coma
MELD score increase (includes INR, bilirubin and creatinine)	<3	3-7	>8	NA
Serum WBC	NA	NA	>12,000/ml or <1000/ml	NA
AST	NA	≥ 5.0 to < 10.0 x ULN	≥ 10.0 x ULN	NA
ALT	NA	≥ 5.0 to < 10.0 x ULN	≥ 10.0 x ULN	NA
Alkaline phosphatase	NA	≥ 5.0 to < 10.0 x ULN	≥ 10.0 x ULN	NA

Supplementary Table 4: Primers used for the study

GENE	FWD Primer	Sense Sequence	REV Primer	Anti-Sense Sequence
DEFA5	DEFA5_qFWD	CACTCCAGGAAAGAGCTGATGAGG	DEFA5_qREV	GGTTCGGCAATAGCAGGTGGC
DEFA6	DEFA6_qFWD	TCACTGCTGTTCTCCTCGTG	DEFA6_qREV	GTTGAGCCCAAAGCTCTAAGAC
IL6	IL6_qFWD	AGTGAGGAACAAGCCAGAGC	IL6_qREV	AGCTGCGCAGAATGAGATGA
CDH1	CDH1_qFWD	GCTGGACCGAGAGAGTTTCC	CDH1_qREV	GGTGTATACAGCCTCCCACG

Supplementary References

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