Supplementary methods:

DNA was extracted for microbiome analysis from saliva and stool samples using either MP Biomedicals FastDNA Spin Kit for Soil (for Saliva samples) or QIAGEN kit (for stool samples) (1). We first used Length Heterogeneity PCR (LH-PCR) fingerprinting of the 16S rRNA to rapidly survey our samples and standardize the protocols for community amplification method. We then interrogated the microbial taxa associated with the gut fecal microbiome using Multitag Pyrosequencing (MTPS) (2). This technique allows the high throughput sequencing of multiple samples at one time. Microbiome Community Fingerprinting: 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. 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 an 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 and operational taxonomic units (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.

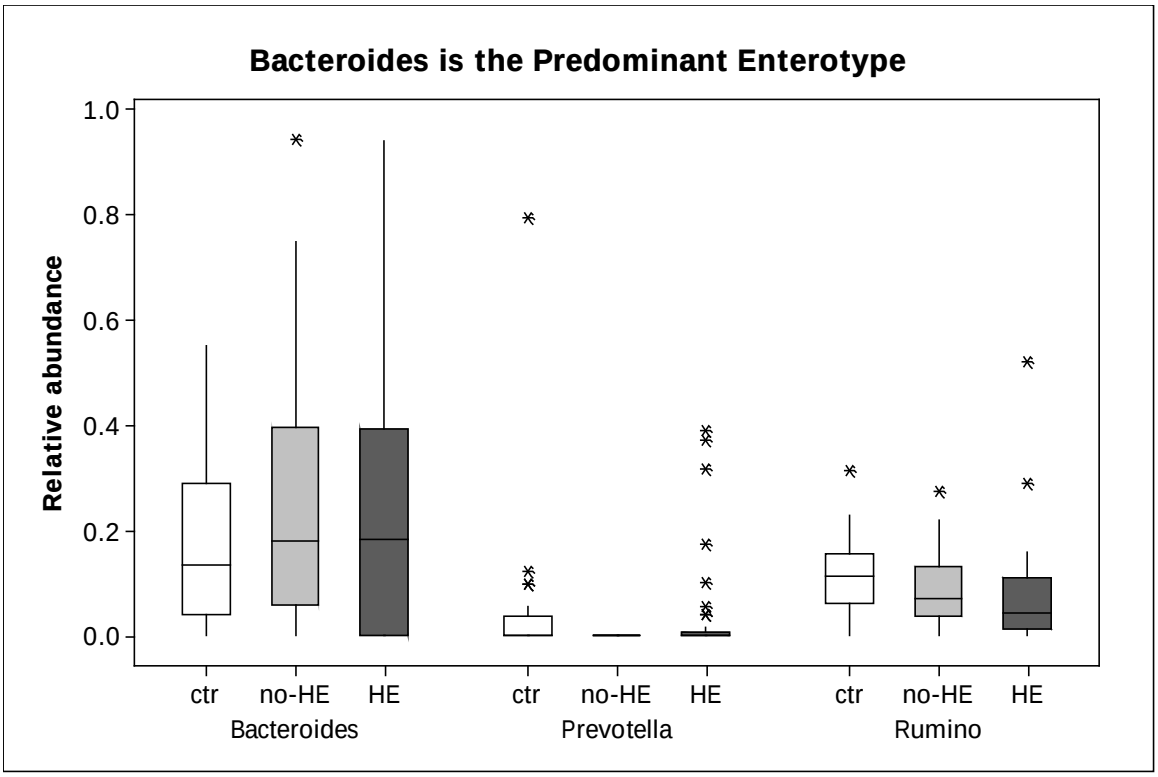
MTPS (2): We employed the MTPS process to characterize the microbiome from the stool and saliva samples. Specifically, we have generated a set of 96 emulsion PCR fusion primers that contain the 454 emulsion PCR adapters on the 27F and 355R primers and a different 8 base "barcode" between the A adapter and 27F primer. 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. Thus, each sample (either fecal or saliva) was amplified with unique bar-coded forward 16S rRNA primers and then up to 96 samples were

pooled and subjected to emulsion PCR and pyrosequenced using a GS-FLX pyrosequencer (Roche). Data from each pooled sample were "demultiplexed" by sorting the sequences into bins based on the barcodes using custom PERL scripts.

Microbiome Community Analysis: We identified the taxa present in each sample using the Bayesian analysis tool in Version 10 of the Ribosomal Database Project (RDP10). The abundances of the bacterial identifications were then normalized for each sample by the total number of reads from each barcode using a custom PERL script and genera present at >1% of the community were tabulated. We chose this cutoff because of our *a priori* assumption that genera 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.

Supplementary figure and legend

Supplementary Figure 1: Enterotypes in the stool microbiota: Using enterotypes postulated by Arumugam et al, the figure below shows that *Bacteroides* was the predominant phenotype (not significantly different between groups) while *Ruminococcus* was less likely to be predominant in any of the groups. This reduction in *Ruminococcus* worsened with advancing cirrhosis. The figures shows the median, IQR and range of relative abundance (from 0% to 100% range) while the asterisk symbols signify outliers. Ctr=control, no-HE: cirrhotics without prior hepatic encephalopathy, HE=cirrhotics with prior hepatic encephalopathy, Rumino=*Ruminococcae*



Supplementary Information References

1. Bajaj JS, Hylemon PB, Ridlon JM, Heuman DM, Daita K, White MB, Monteith P, et al. Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. Am J Physiol Gastrointest Liver Physiol 2012;303:G675-685.
2. Gillevet P, Sikaroodi M, Keshavarzian A, Mutlu EA. Quantitative assessment of the human gut microbiome using multitag pyrosequencing. Chem Biodivers 2010;7:1065-1075.