Human Gut Microbiome Transplantation in Ileitis Prone Mice: A Tool for the Functional Characterization of Microbiota in Inflammatory Bowel Disease

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SECTION A: Supplementary Methods

Animals and gnotobiotic facility. The fecal microbiota colonization experiments herein described was tested by housing age- and sex-matched inbred GF SAMP1/YitFC (SAMP) mice re-derived from Taconic Biosciences Inc. (Hudson, NY) and by inbred SPF-raised SAMP mice, routinely tested for SPF-level murine pathogens, including *Helicobacter spp.* Infection. The SAMP GF mouse colony (Cleveland Digestive Diseases Research Core Center; CDDRCC, Mouse Models Core) is maintained in high-efficiency particulate air-filtered pressurized isolators in the Animal Resource Center (ARC) ultra barrier facility, at Case Western Reserve University (CWRU) School of Medicine. All experiments were conducted in BSL-2 grade rooms (ARC, CWRU) dedicated to use for gnotobiotic animals. The SAMP GF mouse colony (Cleveland Digestive Diseases Research Core Center; CDDRCC, Mouse Models Core) is maintained in high-efficiency particulate air-filtered pressurized isolators in the Animal Resource Center (ARC) ultra barrier facility, at Case Western Reserve University (CWRU) School of Medicine. The GF status of our SAMP mouse colony is regularly tested by standard culture-based microbiological procedures and gram staining as described.[1]

Nested Isolation (*NesTiso*) Caging System. All mice were maintained following the same GF grade standards used for mice in our GF facility, including the housing of mice in our *NesTiso* caging system.[1] We controlled for bedding-dependent bacterial overgrowth using methods described by our group[1]: (1) housing all mice individually (reduce amount of organic matter produced), (2) systematically replaced all cages, irradiated/autoclaved food and autoclaved water, every 7 days (reduces permanent contact of mice with feces, which does not occur in humans).

Animal handling and disinfection. Strict disinfection protocols ensured aseptic environmental conditions using: *i)* quaternary ammonium-based soap (organic matter), *ii)* 70% ethanol (grease and dehydrate); *iii)* Spor-Klenz® (rust-sensitive equipment). Floors and other surfaces were disinfected with Spor-Klenz® and Clidox® (Pharmacal Research Laboratories, Inc., Waterbury, CT, 96120F, chlorine dioxide). Every cage was routinely replaced under biosafety cabinets. In all cases, animals were handled using Spor-Klenz® (Steris Corp., Groveport, OH, 6525; 1% hydrogen peroxide, 10% acetic acid, 0.08% paracetic acid) disinfected, or autoclaved and rubberized 12-inch long forceps. Biosafety HEPA filter hoods were sterilized with Spor-klenz vapors immediately after cages or animals were manipulated (e.g., feces collection, body weight measurements). Autoclaved sterile gowns and hairnets, masks (N95) and impermeable plastic sleeves were worn by personnel to prevent exposure of the *NesTiso* cage sets and animals to human dust or microorganisms, and to reduce personnel exposure to disinfectants.

Microbial monitoring of SAMP GF colony. Our GF facility performs routing testing of all GF SAMP mice inside both pressurized isolators and *NesTiso* sets using standard culture-based microbiological procedures (anaerobic, aerobic plating of feces and bedding) and culture-independent gram-staining of mouse feces to verify animals are not colonized in vivo by microorganisms that may be uncultivatable using in vitro methods.[2] Our data indicate that two consecutive negative results are optimal to prove GF status of mice were GF, and as such is an approach we use before enrolling any GF mouse cohort into experimentation.[1] Fungal contamination is monitored at 1-3 week intervals using fresh feces and direct plating onto potato dextrose agar, abouraud and Candida crohmID agars (Oxoid, BBL, bioMerieux SA, France; 30℃, 7 days). In addition, soiled cages are incubated after addition of 100ml from drinking water bottle (23℃, aerobically, 21 days) to allow for fungal spore germination.[3]

Fecal Microbiota Transplantation. Establishment of human gut microbiota in 7-week-old GF SAMP mice was performed using cryopreserved human or murine fecal microbiota communities (stored at -80℃; phosphate-buffered saline (PBS) /7% dimethyl sulfoxide (DMSO) mixture). All human donors were assigned 6 GF SAMP recipient mice and mice were randomly allocated (male/female 1:1 ratio) to experimental FMT donor groups for each experiment; Exp. A, B, C by a designated laboratory personnel and blinding was maintained

throughout experiments using a coding system. GF SAMP (littermates) administered a 'sham' gavage of 300µl PBS /7% DMSO were used as negative controls; untreated age/sex-matched SPF SAMP mice served as controls. The SPF donor groups were assigned 5-6 mice. De-identified fresh human stools were procured from age- and sex-matched donors after a normal bowel movement under an approved IRB protocol (IRB# NHR-11-22) through the CDDRCC Biorepository Core, at CWRU. All IBD donors were in complete remission (assessed by clinical, endoscopic radiologic and/or biochemical parameters) and were not taking biologics, or corticosteroids. No donors reported taking supplements/vitamins, antibiotics, or probiotics. Murine donor inocula (separate preparation for Exp. A, B, C) comprised a composite (pool) of feces from our SPF SAMP mouse colony. All fecal specimens were anaerobically prepared and stored within 2 hours of collection.

Fecal Transplantation. On the day of transplantation, donor inocula aliquots were thawed at room temperature (~6 min thaw/aliquot) and administered within <10 minutes after fully thawing. Fresh murine feces were collected routinely in the mornings (control for circadian effects on microbiome) on the same day each week for all animals (day 7 post cage changes), before inoculum administration.

16S Microbiome: 16S rRNA microbiome analyses of N=403 total samples included; Exp. A: 9 time points (day 1, 2, 4, 7, 14, 21, 28, 42, 60, inocula), Exp. B & C: 4 time points (day 4, 28, 42, 60, inocula), Exp. D (4 time points; day 7, 28, 42, 60, CD 'donor B' inocula). Donor inocula were analyzed as 2 or more technical aliquots (A and B) and interpreted 'in series' (i.e., sum of A+B reads). To ensure a static profile (i.e., freeze-thaw) across Experiments, all genomic DNA samples were preserved at -20°C after extraction. Based on hypotheses generated from Exp. A and to increase external validity, Exp. B and C employed new DNA extraction method, sequencing and bioinformatics pipelines (described below). Statistical analysis of OTU normalized 0.00017 + $\log_2$ transformed data tables was conducted using STATA v13.0 and R software v. 3.4.2 packages. Exp. A & D: fresh fecal samples (stored -80℃) prepared using Qiagen reagents (Tissue and blood kit), controlling for batch-dependent variability (i.e. technician, method, kit, reagent- dependent variables, DNA counts). Differences in fecal bacterial composition were assessed by V3-V4 16S rRNA gene sequences using the Illumina MiSeq platform to generate 25,000±178 reads per sample (30 mice & donors) and 20,000±168 reads per sample (10 mice & donor) average length 252bp passed quality control filter. Library preparation, sequencing, quality control, and primary bioinformatics analysis were performed by Beijing Genomics Institute in Shenzen, China. The raw data were filtered to eliminate the adapter pollution and low quality to obtain clean reads, then paired-end reads with overlap were merged to tags. Tags were clustered to OTU at 97% sequence similarity. Taxonomic ranks were assigned to OTU representative sequence using Ribosomal Database Project (RDP) Naive Bayesian Classifier v.2.2. At last, alpha diversity, beta diversity and the different species screening were analyzed based on OTU and taxonomic ranks. In order to obtain more accurate and reliable results in subsequent bioinformatics analysis the raw sequence data was pre-processed to get clean data by a Beijing Genomics Institute in-house procedure as following: 1) Truncation of sequence reads not having an average quality of 20 over a 30 bp sliding window based on the Phred algorithm, and trimmed reads having less than 75% of their original length, as well as its paired read, were removed; 2) Removal of reads contaminated by adapter (default parameter: 15 bases overlapped by reads and adapter with maximal 3 bases mismatch allowed); 3) Removal of reads with ambiguous base (N base), and its paired reads; 4) Removal of reads with low complexity (default: reads with 10 consecutive base). For pooling library with barcode samples mixed, the clean reads were assigned to corresponding samples by allowing 0 base mismatch to barcode sequences with in-house scripts. Paired-end reads were generated with Illumina HiSeq/MiSeq platform, then the reads with sequencing adapters, N base, poly base, low quality, etc., were filtered out with default parameters. If the two paired-end reads overlapped, the consensus sequence was generated by FLASH[4] (Fast Length Adjustment of Short reads, v1.2.11), and the detailed method is as follows: 1) Minimal overlapping length: 15 bp; 2) Mismatching ratio of overlapped region: <= 0.1. Removal of paired-end reads without overlaps. The high-quality paired-end reads were combined to tags based on overlaps, 5189519 tags were obtained in total with 24830 tags per sample on average, and the average length is 252 bp. The tags were clustered to OTU by scripts of software USEARCH(v7.0.1090),[5] detailed as follows: 1) The tags were clustered

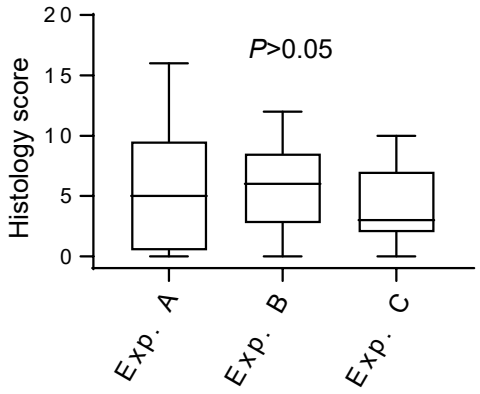
into OTU with a 97% threshold by using UPARSE, and the OTU unique representative sequences were obtained; 2) Chimeras were filtered out by using UCHIME (v4.2.40); The 16S rDNA and ITS sequences were screened for chimeras by mapping to gold database (v20110519), UNITE (v20140703) separately, 3) All tags were mapped to each OTU representative sequences using USEARCH GLOBAL, then the tags number of each OTU in each sample were summarized to an OTU abundance table. OTU representative sequences were taxonomically classified using RDP Classifier v.2.2 trained on the Greengenes database, using 1 confidence values as cutoff. Databases used for species annotation: 16S rDNA is used for bacterial and archaea community: Greengene (default): V201305[6] using 1 confidence values as cutoff. RDP: (Release9 201203)[7] OTUs were Filtered as follow: 1) Unassigned OTUs were removed; 2) OTUs not assigned to the target species were removed. For example, the OTUs assigned to archeae were removed because project was about 16S rDNA for bacterial community study. The filtered OTUs were used for downstream processing. Filtered tags were clustered into OTU at 97% similarity, OTU number per sample primarily represented the degree of sample diversity. Based on the OTU abundance information, the relative abundance of each OTU in each sample was calculated, and the PCA of OTU was done with the relative abundance value. The software used in this step was package 'ade4' of software R(v3.1.1). Downstream analyses of OTU tables used principles based on QIIME1[8] (Beijing Genomics Institute) and the Rhea pipeline. Exp. B & C: fresh fecal samples (stored -80℃) prepared using Illumina *TruSeq DNA*. Only qualified DNA (quality, quantity) was used to construct 16S libraries, verified by Invitrogen Qubit Fluorometer (Life Technologies), OD260/280 & OD260/230 NanoDrop (Thermo Scientific) and by Agarose gel electrophoresis (Agarose Gel: 1%, Voltage: 150V, Time: 40min). Processing of frozen fecal specimens, DNA extraction and DNA QC was performed by the Genomics Core, CWRU.16S rRNA Sequencing. Differences in fecal bacterial composition were assessed by V3-V4 16S rRNA gene sequences using the Illumina MiSeq platform to generate~100,000 reads per sample (mice & donors) average length 460bp passed quality control filter. Library preparation and library QC (Agilent Bioanalyzer High Sensitivity DNA chip), pooling, quantification (NEBNext Library Quantification Kit for Illumina) and sequencing/data QC (Illumina software, FastQC) were performed by the Genomics Core, CWRU. In brief, a modified version of the Illumina Microbiome 2-step PCR protocol was used, which amplifies the V3/V4 regions of the 16S rRNA gene with overhang adapters attached. Secondary PCR attaches unique dual indices and Illumina sequencing adapters using the Nextera XT index kit (Illumina). Final libraries were pooled and sequenced on the Illumina MiSeq system using a v3 paired-end flowcell, following the Illumina user-guide instructions. 16S Sequence Data Workflow. The IMNGS (Integrated Microbial Next Generation Sequencing) platform[9] used to build OTU profiles and all downstream bioinformatics analysis was carried out using Rhea pipeline, as previously described in detail.[10]

Culture-Based Methods: We adapted the “parallel lanes plating” method (described by our group).[11] For plating of fecal specimens to isolate cultivable members of *Enterobacteriaceae* in McConkey agar, focusing on lactobacilli and *Streptococcus* spp. counts as comparators using selective agar media (MRS, and *Streptococcus* Azide Blood agar, respectively). In brief, 19-20µl of 10-fold serial fecal dilutions in chilled PBS were plated simultaneously using a multichannel pipette on one side of the selected agar, tilted at >60-80° angle (creates ‘parallel lanes). Once dry, 48-hr incubation of agar plates (aerobic, anaerobic) at 37°C permits enumeration of single colonies relative to total CFUs of cultivable bacteria in sample. Representative colonies encompassing all possible coliform morphologies were selected for purification and were then Sanger sequenced following standard protocols.

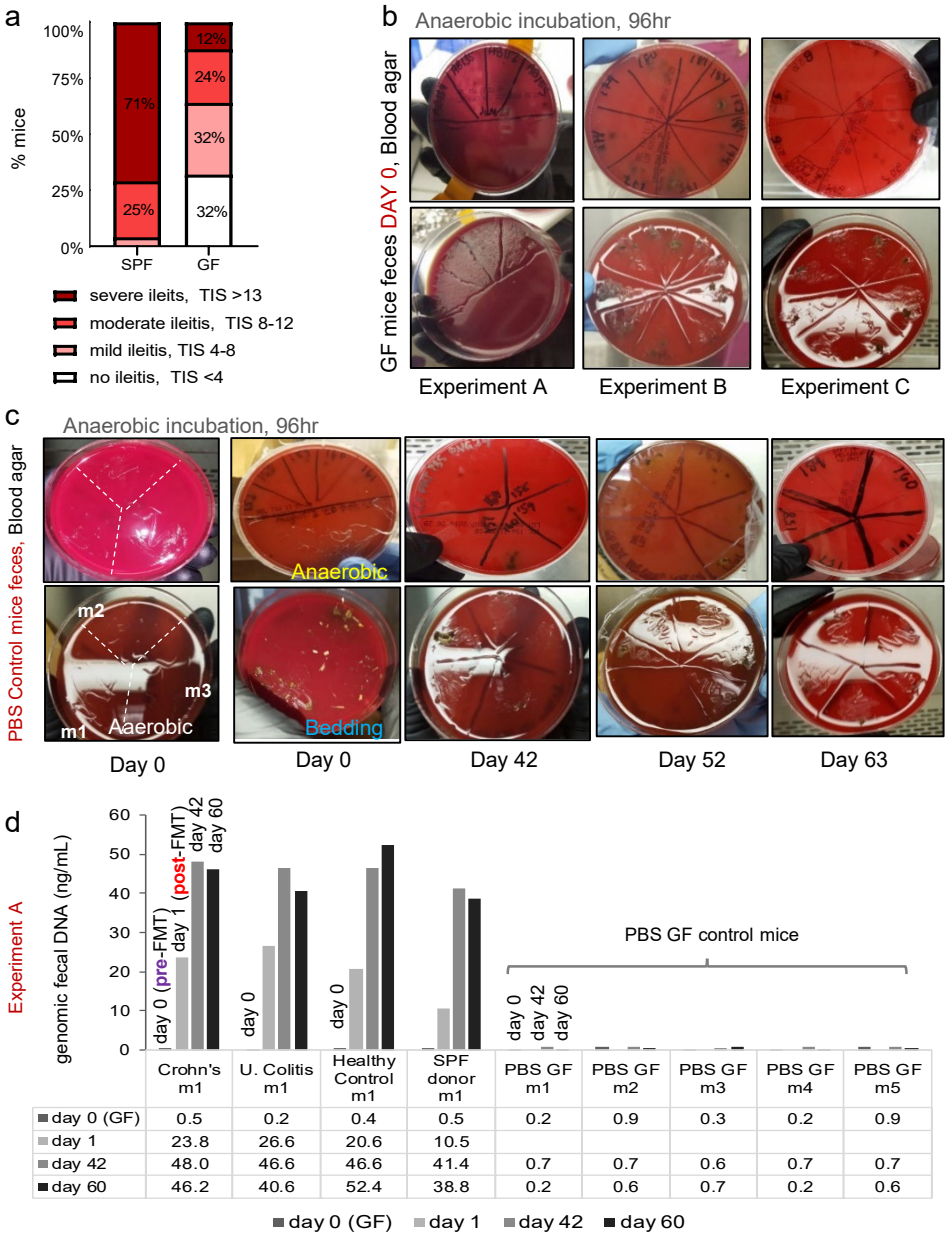
Real-time qPCR. qPCR amplification of DNA samples was performed using white 384-well plates (Roche, foil sealing 03197567) and the Roche 480 LightCycler SYBR Green run template settings (hot start 95℃, 10 min and 54 amplification cycles; 95℃, 15s; 60℃, 30s; 72℃, 30s). Samples were run as singlets in each plate to allow the inclusion of all samples from a given mouse in a group for the 2-time points (day 2, day 60), but experiments were repeated twice to determine variability and reproducibility. Each plate included samples from experimental negative controls (GF PBS control mice) and GF mice at baseline (before initial FMT gavage),

and at least one positive (SPF SAMP) and one negative control (GF SAMP), as well as a H20 negative control.

Murine intestinal disease and survival analysis. Measurement of body weight and diarrhea score (adopted from previous publication)[12] was used as an indicator of animal health. Diarrhea score system is as follows; 0: No diarrhea; solid stool, 1: Very mild diarrhea; formed stool that appears moist on outside, 2: Mild diarrhea; formed stools that easily submit to pressure applied by forceps, 3: Diarrhea; no fully formed stools, with a mucous-like appearance, 4: Severe watery diarrhea; mostly clear or mucous-like liquid stool with very minimal solid presence. Murine colonoscopy was performed across groups (day 0, 12, 28, 60) using a flexible digital ureteroscope (URF-V; Olympus America) and inflammation quantified using a validated endoscopic scoring system.[13] Post-mortem terminal ilea (last 10cm length) were submitted for blinded histological evaluation (Bouin's fixative, hematoxylin and eosin-stained) of intestinal inflammation by a board-certified pathologist and for 3D-stereomicroscopic (SM) pattern profiling to determine and quantify presence of abnormal mucosa and lesion severity using a validated scoring system and criteria, described elsewhere.[14] Areas with abnormal 3D mucosa (involved) and areas exhibiting normal villous morphology (uninvolved) were isolated for quantification of MPO activity. Inflammatory indices for histological villous distortion, active and chronic inflammation, and total inflammation was assessed in a semi-quantitative fashion. *Mortality:* in Exp. A; a total of 2 mice developed dermatitis and were sacrificed (day 14, day 21). For Exp. B; a total of 3 mice died as a result of *i*) colonoscopy procedure (n=2), *ii*) gavage administration (n=1). For Exp. C; a total of 4 mice died as a result of; *i*) colonoscopy procedure (n=2), *ii*) gavage administration (n=1), and iii) unknown reason (n=1); however, this was a dwarf mouse. For Exp. D: one mouse developed dermatitis (excluded from analyses). One mouse died for unknown reasons (day 7).

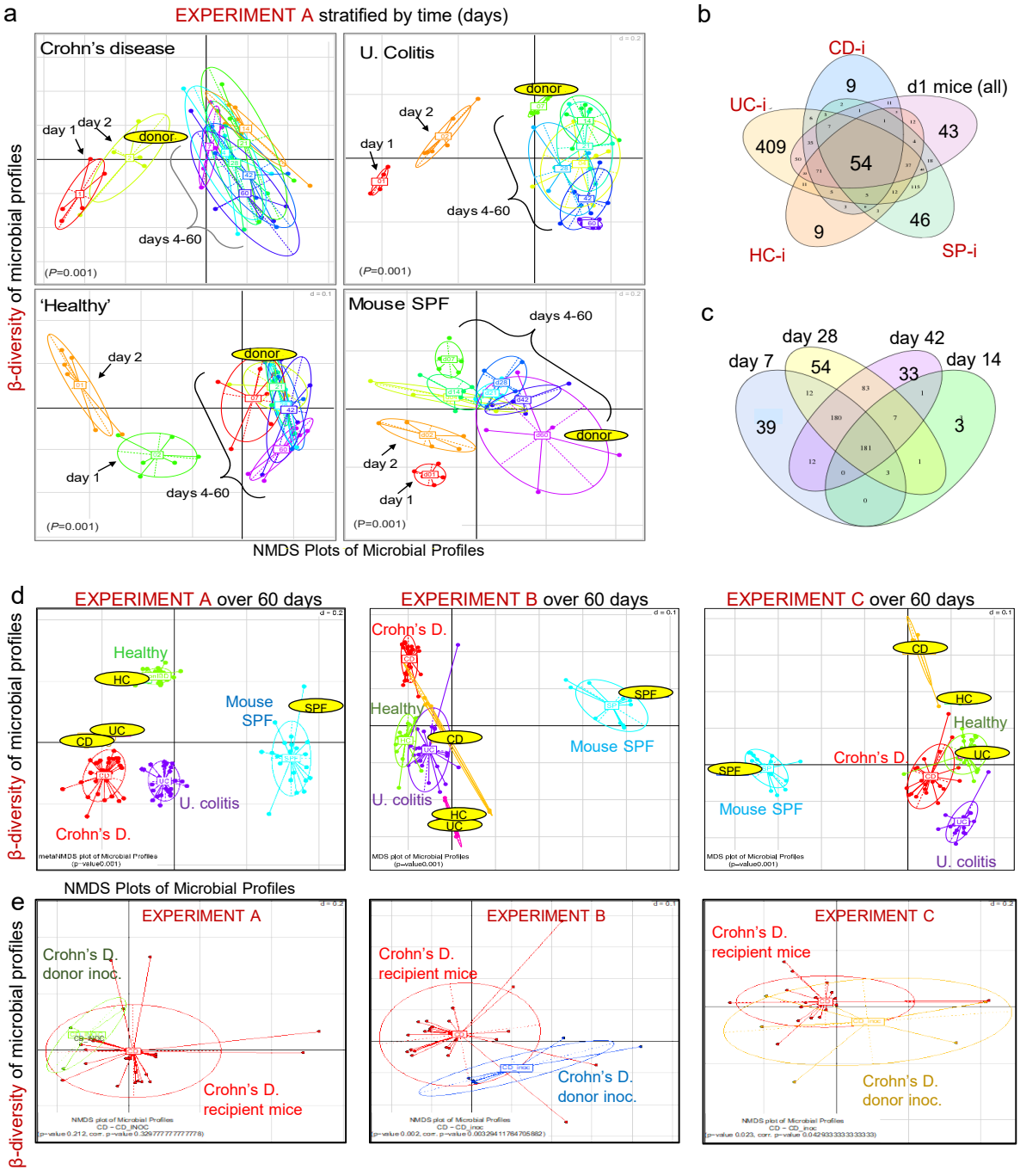


Supplementary Figure 1. Comparative analyses of groups show reproducibility of histological data. Histological score (ileum tissue) data pooled for each Exp. A, B, C. Comparative analyses (Kruskal-Wallis) showed no difference between experiments indicating within, but not between group variability, and reproducibility of histological data. All transplanted mice had histology score for colon tissue of zero (not shown).

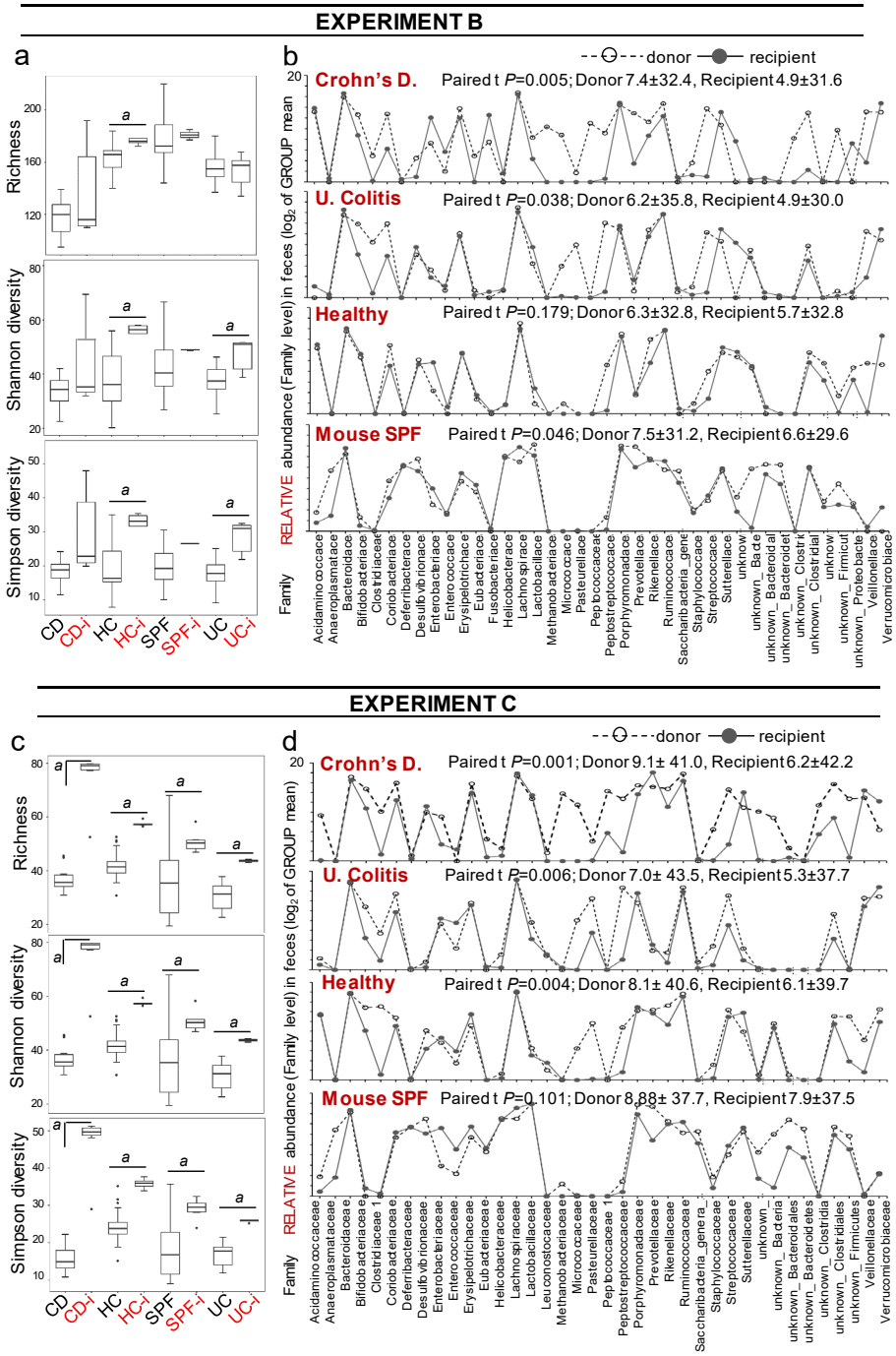


	Crohn's m1	U. Colitis m1	Healthy Control m1	SPF donor m1	PBS GF m1	PBS GF m2	PBS GF m3	PBS GF m4	PBS GF m5
day 0 (GF)	0.5	0.2	0.4	0.5	0.2	0.9	0.3	0.2	0.9
day 1	23.8	26.6	20.6	10.5					
day 42	48.0	46.6	46.6	41.4	0.7	0.7	0.6	0.7	0.7
day 60	46.2	40.6	52.4	38.8	0.2	0.6	0.7	0.2	0.6

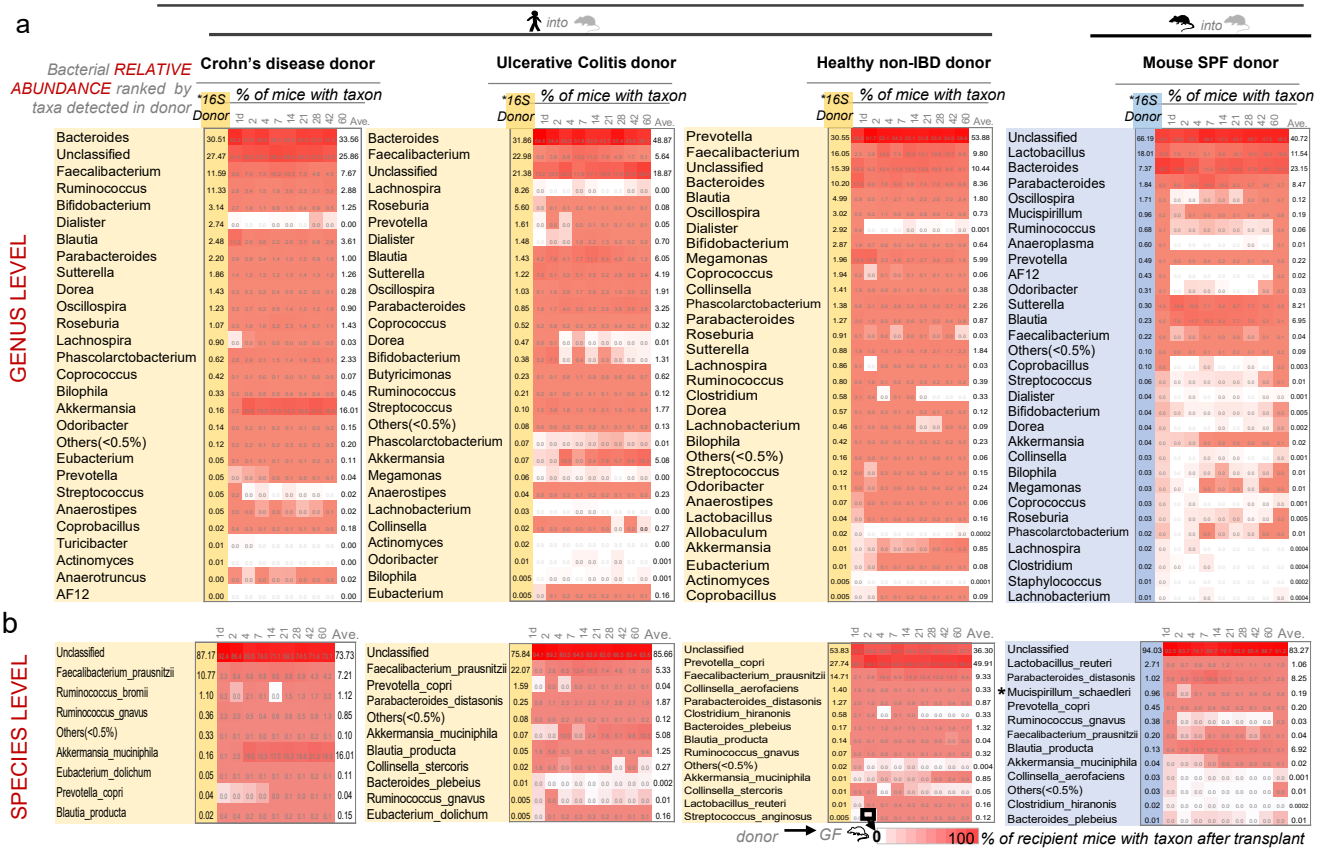
Supplementary Figure 2. **Microbial screening and monitoring of germ-free mice.** In all experiments, standard culture-based microbial procedures were used for screening and monitoring of GF mice. **a)** Impact of bacterial flora on the development of SAMP ileitis. Distribution of ileitis in GF vs SPF SAMP mice (>13 weeks of age); TIS, total inflammatory score. Data originally published in *Inflamm Bowel Dis*. 2011;17(12):2566-2586. **b)** Photographs illustrate fresh feces from GF mice prior to colonization plated on blood agar (anaerobic, 37℃, 7 days) prior to colonization for Exp. A, B, C. **c)** Photographs illustrate culture-based microbial monitoring of PBS control mice over 60-day experiment by plating fresh feces on Blood agar (anaerobically, anaerobically, 37℃, 7 days) for Exp. A. **d)** Bar plot shows genomic DNA concentration (ng/mL) of mouse feces before initial colonization [one mouse per group; CD, UC, HC, SPF (day 0, 1, 2, 21, 60) and all PBS control mice; (day 0, 42, 60)].



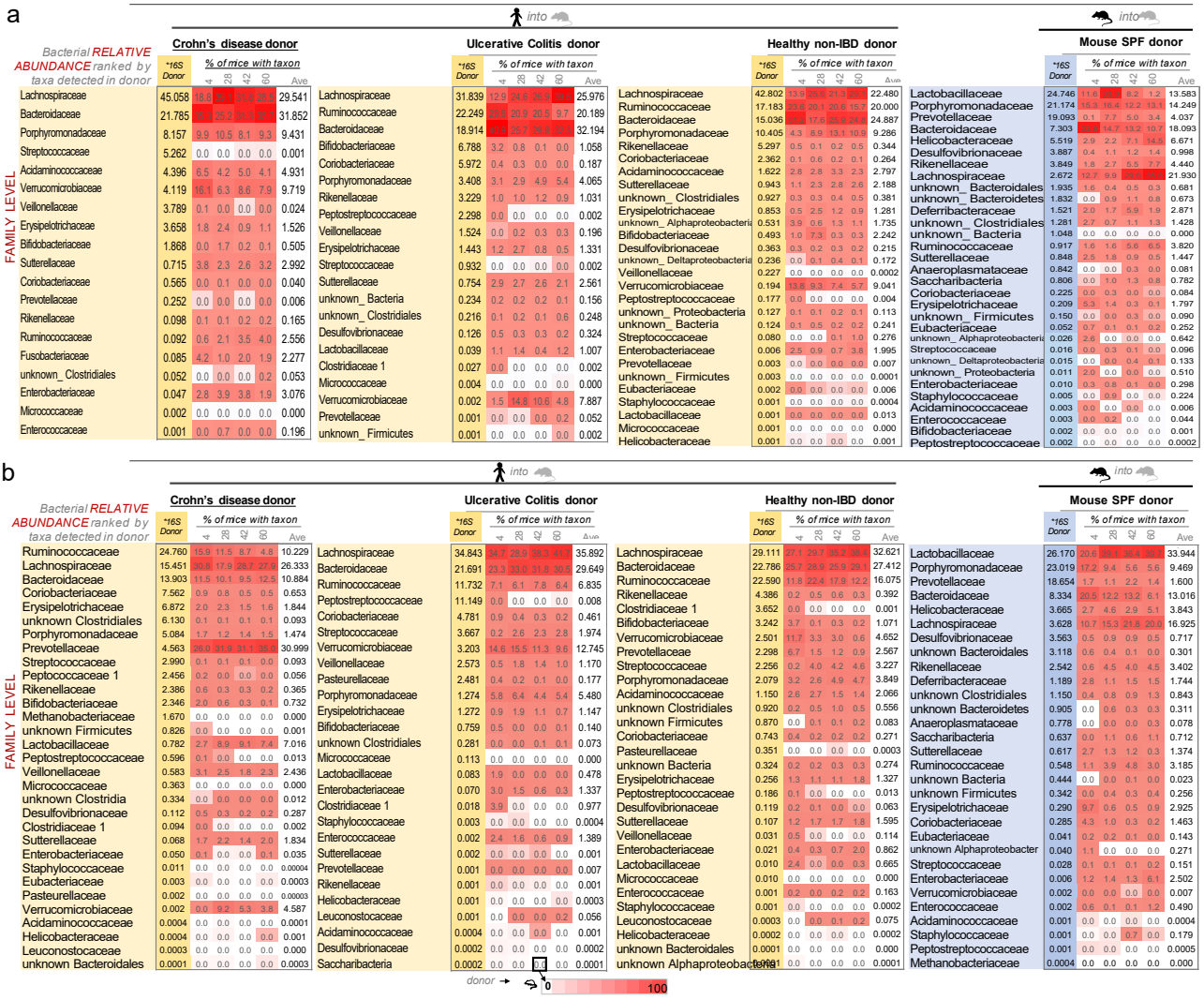
Supplementary Figure 3. PCA Plots for all donor experiments. Relative abundance 16S data from donor and recipient mice for Exp. A, B, Cover 60 days. PCA plots show β-diversity profiles for donor (yellow ovals) and recipient mice. **a)** PCA plots Exp. A. for donor and recipient mice. **b)** Venn diagram shows the number of shared OTUS between donor inocula and that of the collective of mice one day after initial inoculation. **c)** Venn diagram shows the number of shared and unique OTUs in mice from Exp. A as a collective over time. CD-i; *Crohn's inoculum*, UC-i; *ulcerative colitis inoculum*, HC-i; *healthy control inoculum*, SPF-i; *SPF mouse inoculum*. **d)** Pairwise analysis between Crohn's donor inoculum and recipient mice for Exp. A, B & C illustrate reproducibility of engraftment. Collectively, pairwise analyses suggested 'stability' of microbial community structure started from day 4 in human donor-recipient mice groups following initial transplantation.



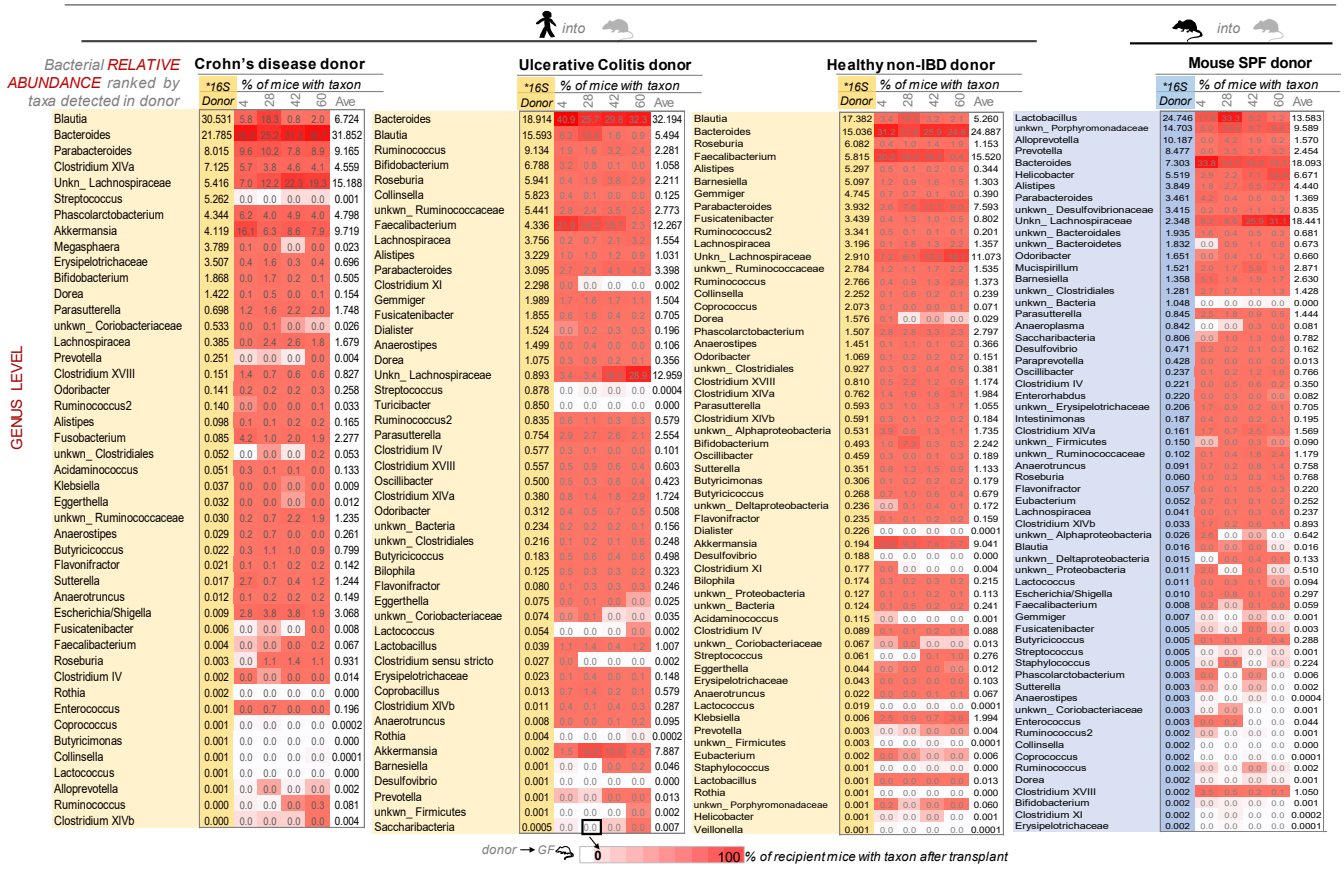
Supplementary Figure 4. Signature plot analyses of second set of donors at Family-level illustrate quantitative parallel between donor and recipient (Exp. B & C). Alpha diversity measures and line plots of mean relative abundance (Family-level) 16S rRNA sequencing data (log_2) comparing donor inocula to that of the mean abundance for 4 fecal samples (day 4, 28, 42, 60) for each recipient mouse from Exp. B & C. **a)** Box plots and statistical analyses (Wilcoxon Rank Sum test, Pairwise analysis; annotation 'a' indicates $P<0.05$) show differences in alpha diversity measures between mice and donor (Exp. B). **b)** Line plots for donor and recipient mice [data accounts for 40 bacterial families; including 9 unassigned], Exp. B. **c)** Box plots and statistical analyses (Wilcoxon Rank Sum test, Pairwise analysis; annotation 'a' indicates $P<0.05$) show differences in alpha diversity measures between mice and donor (Exp. C) and **d)** Line plots for donor and recipient mice [data accounts for 38 bacterial families, including 7 unassigned], Exp. C.



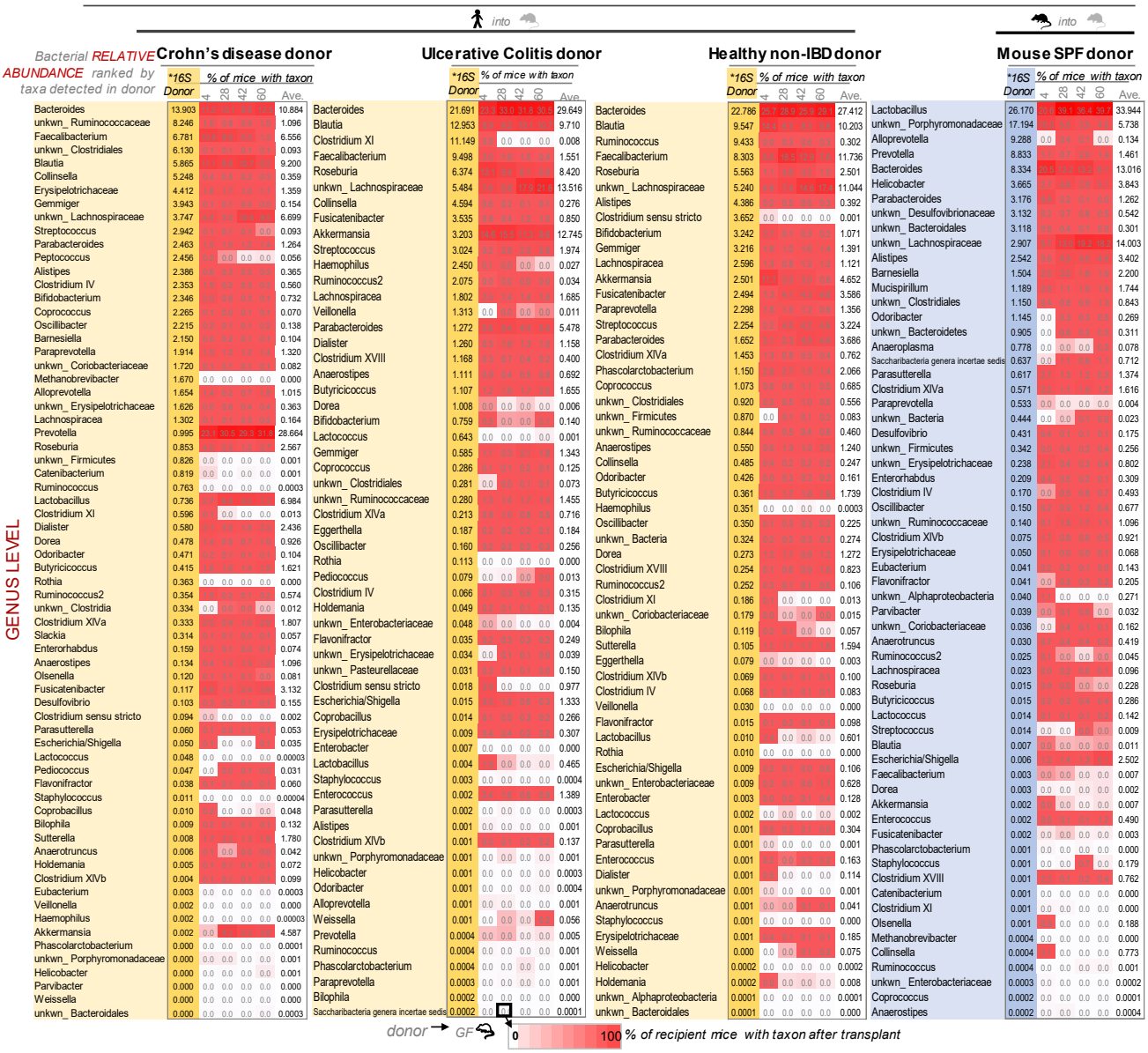
Supplementary Figure 5. Human engraftment of gut microbiota in mice is reproducible when analysis is conducted at the genus and species-level. Exp. A. Heat maps depict normalized relative abundance 16S rRNA data for donor inoculum and the mean recovery in recipient mice colonized over time (9 consecutive fecal samples for days 1 through 60) at the genus and species level. **a)** Genus level; 41 total genera were detected (absolute abundance; 61 detected) [% taxa recovery (CD;26/28, 92.8%), (UC; 27/28, 96.4%), (HC; 31/31, 100%), (SPF; 31/31; 100%)]. **b**) Species-level; 18 total species were detected (absolute abundance; 129 detected) 17 were assigned for donor inoculum and the % of mice colonized over time [% taxa recovery (CD; 9/9, 100%), (UC; 11/11, 100%), (HC; 14/14, 100%), (SPF; 13/13, 100%)]. Note presence of *Mucispirillum schaedleri* (asteric), a mucus-dwelling 'pathobiont' occasionally detected in humans[15] that can cause disease by initiating IgA response,[16] shows predominance in SPF SAMP donor-recipient group.



Supplementary Figure 6. Analysis of the second set of donors at Family level shows reproducible findings (Exp. B & C). Heat maps depict normalized relative abundance 16S rRNA detected taxa for donor inocula and the mean recovery in recipient mice colonized over time (fecal samples for 4 time points; day 4, 28, 42, 60) and the group average % ('Ave') at the family-level for Exp. B & C. **a)** Exp. B**;** 40 total Families were detected, 9 were not assigned ('unknown') for donor inoculum [% taxa recovery (CD; 19/19, 100%), (UC 20/21, 95.2%), (HC 27/28; 96.4%), (SPF 30/31, 96.7%)]. **b)** Exp. C**;** 38 total Families were detected, 5 were not assigned ('unknown') for donor inoculum [% taxa recovery (CD; 29/31, 93.5%), (UC 27/27, 100%), (HC 28/30; 93.3%), (SPF 29/30, 96.6%)].



Supplementary Figure 7. Analysis of the second set of donors at Genus level shows reproducible findings (Exp. B). Heat map depicts normalized relative abundance 16S rRNA detected taxa for donor inocula and the mean recovery in recipient mice colonized over time (fecal samples for 4 time points; day 4, 28, 42, 60) and the group average % ('Ave') at the genus-level for Exp. B. A total of 82 genera were detected, 15 were not assigned ('unknown') for donor inoculum [% taxa recovery (CD; 42/45, 93.3%), (UC 46/48, 95.8%), (HC 56/58; 96.5%), (SPF 61/62, 98.3%)].



Supplementary Figure 8. Analysis of second set of donors at Genus level shows reproducible findings (Exp. C). Heat map depicts normalized relative abundance 16S rRNA detected taxa for donor inocula and the mean recovery in recipient mice colonized over time (fecal samples for 4 time points; day 4, 28, 42, 60) and the group average % ('Ave') at the genus-level for Exp. C. A total of 82 genera were detected, 15 were not assigned ('unknown') for donor inoculum [% taxa recovery (CD; 63/68, 92.6%), (UC 56/59, 94.9%), (HC 57/60; 95%), (SPF 60/62, 96.7%)].

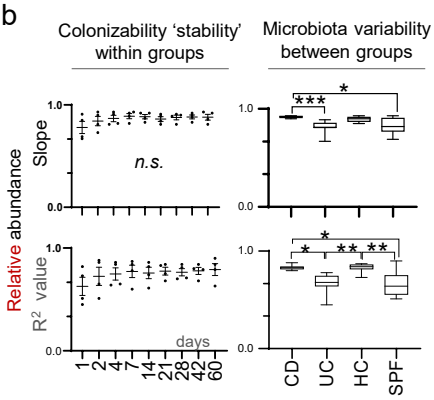
a

RELATIVE abundance 16s data

	Crohn's disease		U. Colitis		Healthy		Mouse SPF	
Days	slope	R^2	slope	R^2	slope	R^2	slope	R^2
1	0.91	0.82	0.67	0.45	0.85	0.73	0.69	0.51
2	0.93	0.88	0.81	0.65	0.90	0.86	0.73	0.51
4	0.93	0.84	0.81	0.65	0.91	0.86	0.82	0.64
7	0.92	0.84	0.89	0.78	0.93	0.87	0.82	0.60
14	0.92	0.82	0.87	0.74	0.92	0.86	0.83	0.61
21	0.91	0.83	0.84	0.75	0.89	0.84	0.81	0.68
28	0.91	0.81	0.81	0.70	0.90	0.84	0.89	0.71
42	0.90	0.80	0.83	0.68	0.88	0.84	0.92	0.79
60	0.92	0.84	0.81	0.63	0.86	0.79	0.93	0.90
AVE[a]	**0.92**	**0.83**	**0.83**	**69.00**	**0.89**	**0.83**	**0.87**	**0.73**

[a] average for day 4-60.

b



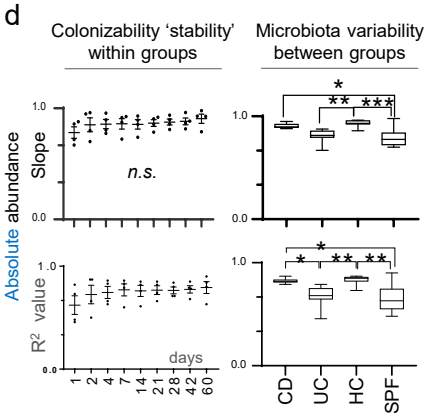
c

ABSOLUTE read count abundance 16s data (log_2)

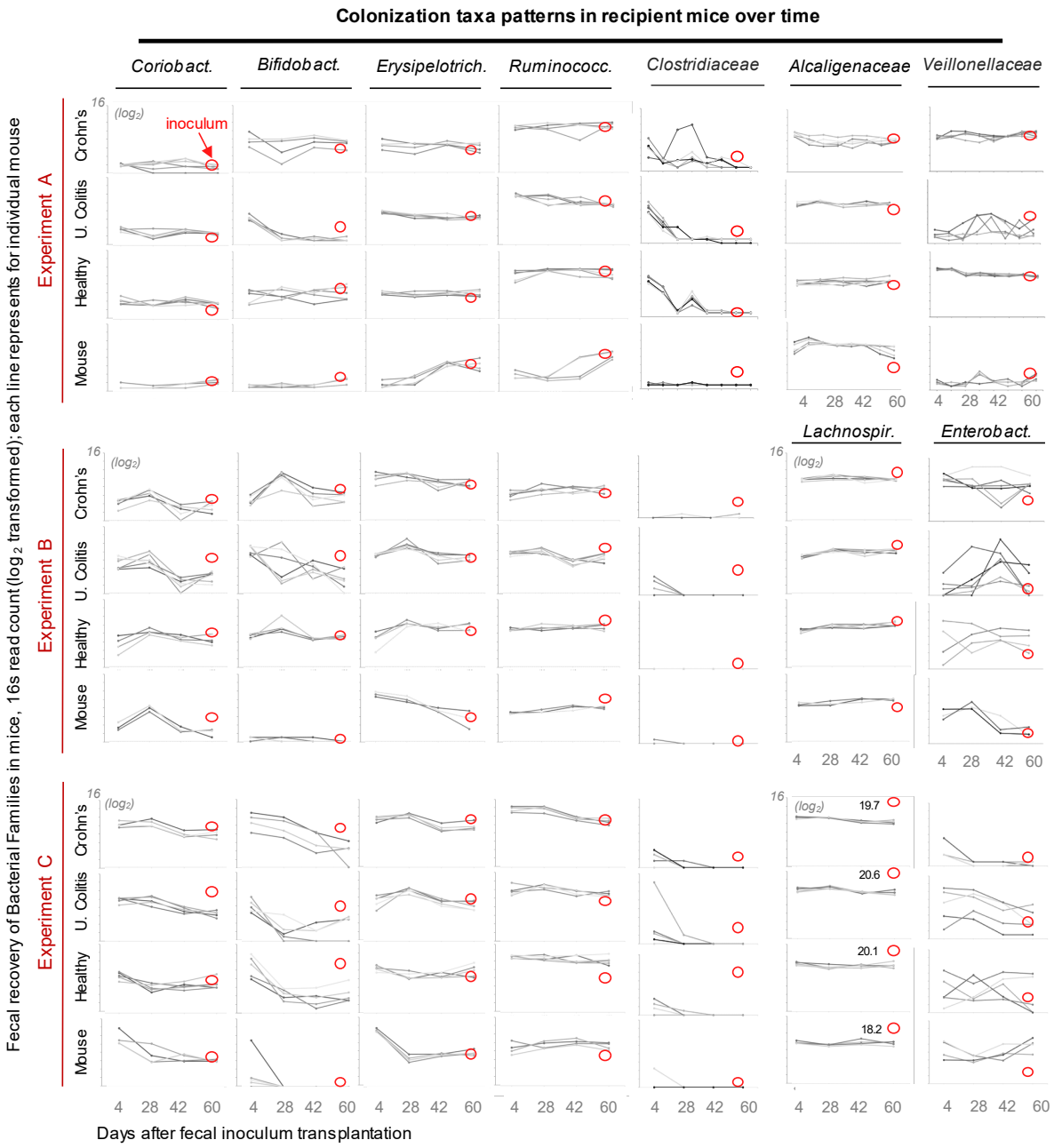
	Crohn's disease		U. Colitis		Healthy		Mouse SPF	
Days	slope	R^2	slope	R^2	slope	R^2	slope	R^2
1	0.88	0.82	0.67	0.46	0.86	0.73	0.72	0.48
2	0.95	0.87	0.80	0.66	0.96	0.87	0.70	0.50
4	0.90	0.83	0.80	0.66	0.96	0.86	0.78	0.63
7	0.92	0.83	0.88	0.79	0.93	0.86	0.72	0.60
14	0.90	0.82	0.86	0.76	0.93	0.85	0.74	0.61
21	0.91	0.82	0.86	0.74	0.92	0.85	0.79	0.66
28	0.90	0.80	0.86	0.71	0.94	0.84	0.81	0.71
42	0.88	0.79	0.82	0.68	0.96	0.85	0.87	0.78
60	0.93	0.84	0.79	0.63	0.93	0.80	0.98	0.90
AVE[a]	**0.90**	**0.82**	**0.84**	**0.71**	**0.94**	**0.84**	**0.81**	**0.70**

[a] average for day 4-60.

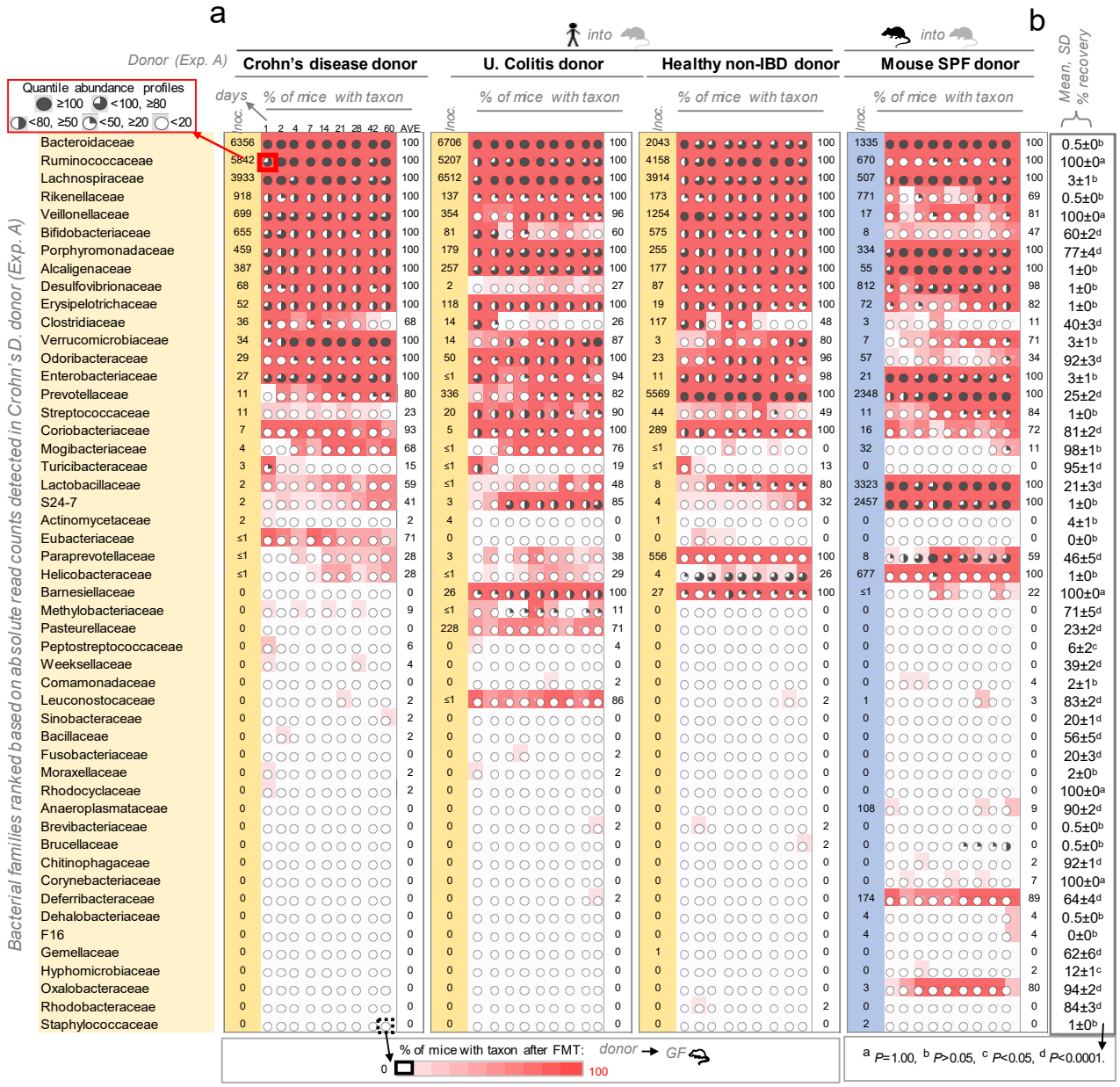
d



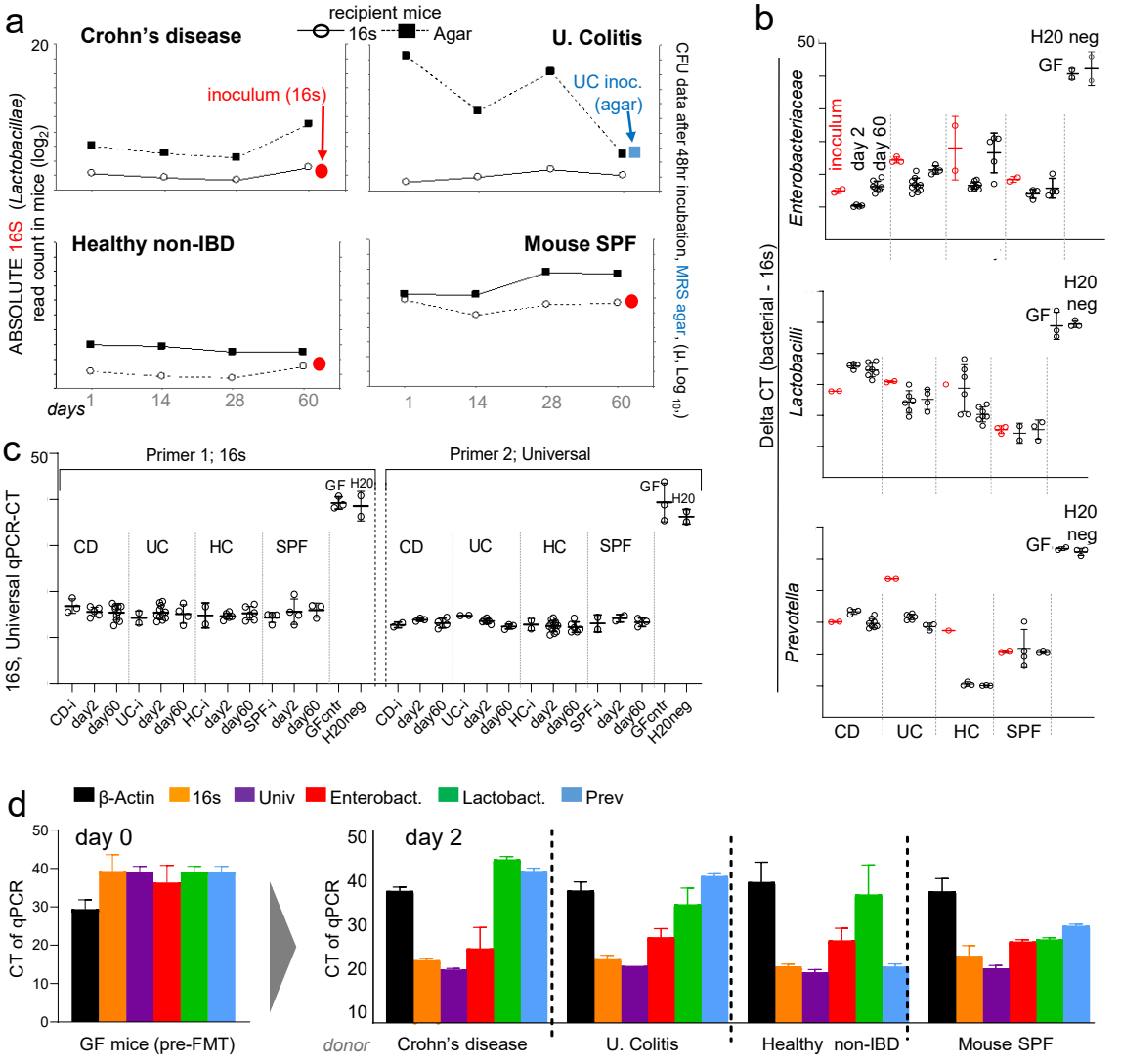
Supplementary Figure 9. Logarithmic slope and R^2 values illustrate reproducibility between donor inoculum and mouse profiles over time. Summary statistics for relative **Figure 4D** and absolute abundance for Exp. A over time. **a)** Logarithmic slope and R^2 values (9 time points) show that transplantation of human microbiota into a mouse prone to CD is reproducible over 60 days. [Summary statistics (day 21-60): Crohn's disease (y = 0.87x + 0.01 R^2 = 0.82; $F_{(1,40)}$, *P*=1.23e-16), U. colitis (y = 0.81x + 0.61 R^2 = 0.69; $F_{(1,40)}$, *P*=6.6e-11), Healthy (y = 0.89x + 2e-05 R^2 = 0.84; $F_{(1,40)}$, *P*=1.23e-16), Mouse SPF (y = 0.89x - 0.48 R^2 = 0.78; $F_{(1,40)}$, *P*=3.26e-18)]. **b)** Corresponding relative abundance summary statistics (Kruskal-Wallis) for slope/R^2 values [R^2: (CD vs UC; *P*=0.036, CD vs SPF; *P*=0.028, UC vs HC *P*=0.003, HC vs SPF; *P*=0.002), slope: (CD vs SPF; *P*=0.033, UC vs HC; *P*=0.002, HC vs SPF; *P*=0.001)]. **C)** Corresponding logarithmic values (panel A, B) using absolute abundance [slope, R^2 (day 4-28): (CD; 0.90±0.01, 0.82±0.01), (UC; 0.84±0.71, 0.71±0.05), (HC; 0.94±0.01, 0.84±0.02); (SPF; 0.65±0.13, 0.70±0.11)], and **d)** comparative summary statistics (Kruskal-Wallis) of slope/R^2 values [R^2: (CD vs UC; *P*=0.017, CD vs SPF; *P*=0.023, UC vs HC *P*=0.004, HC vs SPF; *P*=0.006), slope: (CD vs UC; *P*=0.0006, UC vs HC; *P*=0.017)]. Notice that there are no significant differences in taxa variability of abundance within groups but that significant differences exist between donor groups.



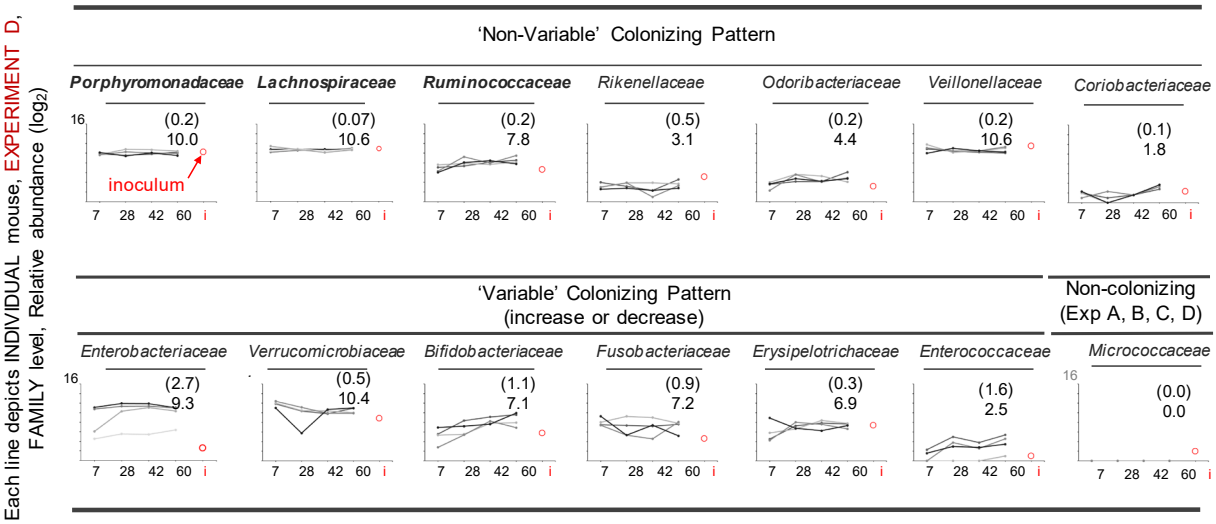
Supplementary Figure 10. Correlation line plots for the second/third set of donors shows reproducible colonization patterns with comparable taxa abundance to donor inoculum. Relative abundance (log_2) profiles at the Family level of donor inocula (open red circles) and recipient mice groups for Exp. A, B, C over time (day 4, 28, 42, 60). Plots illustrate that taxon colonization patterns are reproducible irrespective of donor microbiome. Note that taxon abundance profiles in mice over time are remarkably comparable to that of their donor inoculum.



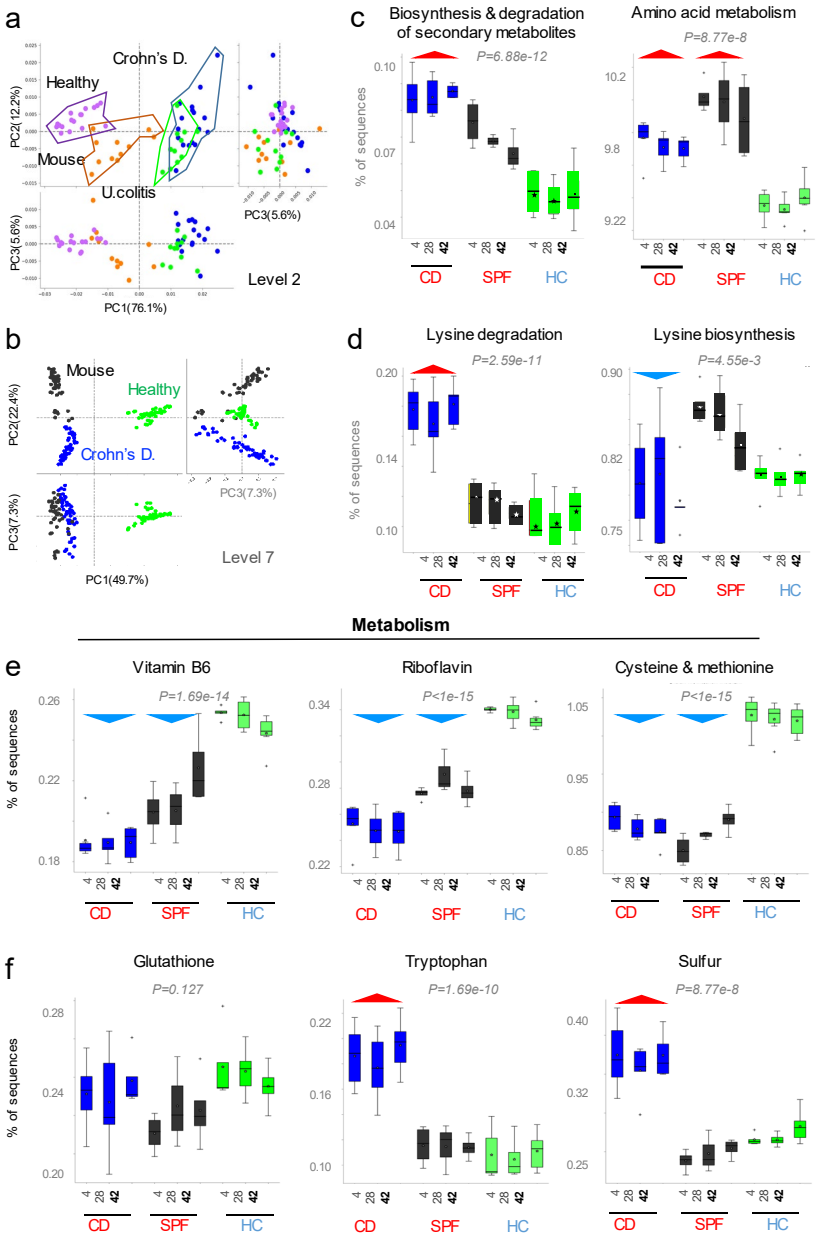
Supplementary Figure 11. Integrated abundance profiles in taxa colonization patterns that are both time and donor-dependent. Heat map depicts raw 16S rRNA read count data for donor inoculum and the % of mice colonized over time (9 consecutive fecal samples, 5-6 mice/group, on days 1 through 60) and the group average % ('Ave') at the family-level for Exp. A. Piecharts superimposed on heat map for each transplanted mice group represent the quantile distribution of the mean 16S read abundance in mice for each time point ranked across the entire dataset. **a)** Integrated abundance profiles (% of mean recovered reads for each time point / total 16S reads, circle icons) show patterns of colonization abundance over time. Notice that the read count quantile allocation in mice parallels that of the human donor. **b)** Mean+SD values for absolute read count recovered for each taxon.



Supplementary Figure 12. Quantitative culture-based and qPCR validation of fecal microbiome analyses. Quantitative validation of the DNA-and taxa-based 16S rRNA microbiome analysis for donor inoculum and fecal samples collected on day 2 and 60 from recipient mice groups using internal controls (β-actin, Universal bacterial primers), Exp. A. **a)** Comparative summary for 16S *Lactobacillaceae* read count data and CFU data (MRS agar) using Parallel Lanes plating method confirmed 16S microbiome profiles in mice and donor inoculum (solid red circles) and presence of non-16S detected species in UC donor inoculum (solid blue square). **b)** Box plots of *Enterobacteriaceae*, *Lactobacilli* and *Prevotella* qPCR-CT values for recipient mice (day 2, 60) and donor inocula after subtraction of (ΔCT) 16S (i.e. panel D; orange bar) confirms culture enumeration data. **c)** Box plots of qPCR-CT values for 16S bacterial families in feces from recipient mouse (day 2, 60) and donor inocula indicated comparable total bacterial count across mouse groups. **d)** Bar plots show DNA qPCR quantification of 16S bacterial families and host gut cells using β-Actin as a referent in feces from recipient mice for day 0 (GF mice before initial FMT) and day 2 following initial transplantation



Supplementary Figure 13. Correlation line plots for single-dose transplanted mice shows reproducible colonization patterns with comparable taxa abundance to donor inoculum. Experiment D; Crohn's 'donor B'. Line plots depict absolute abundance profiles (log2) for donor inoculum (open red circles) and individual recipient mice in single-dose transplanted mice groups over time (4 data points 7, 28, 42, 60) at the family-level. Plots illustrate various colonization patterns of the human microbiota that are reproducible in SAMP mice (see Supplementary Figure 10 for analysis in single donor intensive FMT mice, Exp. A, B, C), and that endpoint taxon abundance in recipient mice groups is remarkably comparable to that of their donor, irrespective of inoculation protocol (see **Figure 4**, **Supplementary Figure 10**). Non-variable colonizing taxa not shown; Alcaligenaceae, Bacteriodaceae Abbrev: i; inoculum.



Supplementary Figure 14. Chronically 'dysfunctional' amino acid pathways in the gut microbiota of mice transplanted with feces from human donors. Statistical analyses of metagenomic profiles (STAMP)17 of relative abundance values for various KEGG metabolic pathways encoded in fecal samples from mice for Exp. A. **a)** PCA plot of Level 2 KEGG pathways (days 4, 14, 28, 60), and **b)** for Level 7 pathways (over 60 days), from transplanted mice. Notice that Crohn's and Mouse SPF transplanted groups (i.e. 'pro-inflammatory' microbiota) cluster differently to that of the HC ('anti-inflammatory' microbiota) (**Figure 4A**), suggesting potential pro- and anti-inflammatory drivers of phenotype in mice. **c & d)** Boxplots of sequence abundances illustrates the chronic differences in microbiota functionality between the CD and SPF compared to HC over time (day 2, 28, 42). Notice the potential for a lysine deficient microbiome in the CD group. **e & f)** Boxplots of sequence abundances illustrates the chronic differences in microbiota functionality compared to HC over time (day 4, 28, and 42) suggest that microbiota have a preceding role in inflammation development. Note the consistency of profiles over time, suggesting that repeated, weekly inoculation ('replenishing' the donor microbiome in recipient) had minimal influence on the ecology of existing microbiome in recipient mice.

SECTION C: Supplementary Tables

Supplementary Table 1: Human donor characteristics

Donor	Donor Study ID	Age (years)	Gender	Race	Disease duration (years)
Crohn's disease					
	Donor #1, Exp. A	58	M	Caucasian	14
	Donor #2, Exp. B	36	M	Caucasian	18
	Donor #3, Exp. C	42	M	Caucasian	5
Ulcerative colitis					
	Donor #4, Exp. A	39	F	African American	5
	Donor #5, Exp. B	43	F	Caucasian	8
	Donor #6, Exp. C	48	M	Caucasian	20
Healthy non-IBD					
	Donor #4, Exp. A	38	F	unreported	N/A
	Donor #5, Exp. B	45	M	Caucasian	N/A
	Donor #6, Exp. C	56	F	Asian	N/A

Supplementary Table 2. Recovery rate of taxa from donor in recipient mice for Exp. A, B & C

Experiment A

Analysis at	Donor type	N of observed taxa		Recovery rate	
		in donor	in mice	Observed	Average
Family level	Crohn's disease	22	22	100%	96%
n=42	Ulcerative colitis	21	21	100%	
	Healthy control	22	22	100%	
	Mouse SPF	28	28	100%	
Genus level	Crohn's disease	28	26	93%	97%
n=40	Ulcerative colitis	28	27	96%	
	Healthy control	31	31	100%	
	Mouse SPF	31	31	100%	
Species level	Crohn's disease	9	9	100%	100%
n=18	Ulcerative colitis	11	11	100%	
	Healthy control	14	14	100%	
	Mouse SPF	13	13	100%	
	Overall average	22	21	99.1%	

Experiment B

Analysis at	Donor type	N of observed taxa		Recovery rate	
		in donor	in mice	Observed	Average
Family level	Crohn's disease	19	18	95%	98%
n=40	Ulcerative colitis	21	21	100%	
	Healthy control	28	27	96%	
	Mouse SPF	31	31	100%	
Genus level	Crohn's disease	45	43	96%	97%
n=82	Ulcerative colitis	48	46	96%	
	Healthy control	58	56	97%	
	Mouse SPF	62	61	98%	
	Overall average	39	38	97.2%	

Experiment C

Analysis at	Donor type	N of observed taxa		Recovery rate	
		in donor	in mice	Observed	Average
Family level	Crohn's disease	31	29	94%	95%
n=38	Ulcerative colitis	27	26	96%	
	Healthy control	30	28	93%	
	Mouse SPF	30	29	97%	
Genus level	Crohn's disease	52	49	94%	96%
n=82	Ulcerative colitis	46	45	98%	
	Healthy control	45	42	93%	
	Mouse SPF	42	41	98%	
	Overall average	38	36	95%	

Cumulative mean, SD	
Family-level	95% ± 0.04
Genus-level	95% ± 0.03

Normalized Relative abundance of assigned 16S rRNA gene sequencing data.

[a] Observed recovery rate refers to the % of taxa present in the fecal inoculum of the donor that are detected in the inoculated mice = 100 x (total taxa of donor – taxa of donor detected in mice) / total taxa of donor. Recovery rate represents taxa in mice at the end of the 60-day experiment.

[b] 16S data interpreted as taxa positive in 4-6 analytical replicas of donor inocula samples and evaluated 'in series'.[18]

Supplementary Table 2. Host and 16S bacterial primers used in this study

GENE	Forward (5'-3")	Reverse (5'-3")
β-actin (mouse)	CAGGGTGTGATGGTGGGAATG	GTAGAAGGTGTGGTGCCAGATC
β-actin (human)	GCACAATGAAGATCAAGGTG	AGTACTTGCGCTCAGGAGGA
16S	TCCTACGGGAGGCAGCAGT	GACTACCAGGGTATCTAATCCTGTT
16S Universal	AGAGTTTGATCMTGGCTCAG	CTGCTGCCTYCCGTA
Enterobacteriaceae	CATTGACGTTACCCGCAGAAGAAGC	CTCTACGAGACTCAAGCTTGC
Lactobacilli	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG
Prevotella	CACCAAGGCGACGATCA	GGATAACGCCYGGACCT

References supporting the bacterial primers have been previously validated for qPCR quantification.[19-21]
Validated mouse primers were obtained from http://pga.mgh.harvard.edu/primerbank/.

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