1   **Mouse Adaptation of Human Inflammatory Bowel Diseases Microbiota Enhances Colonization**

2   **Efficiency and Alters Microbiome Aggressiveness Depending on Recipient Colonic**

3   **Inflammatory Environment**

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21   **Abstract**

22   Understanding the cause vs consequence relationship of gut inflammation and microbial

23   dysbiosis in inflammatory bowel diseases (IBD) requires a reproducible mouse model of human-

24   microbiota-driven experimental colitis. Our study demonstrated that human fecal microbiota transplant

25   (FMT) transfer efficiency is an underappreciated source of experimental variability in human microbiota

26   associated (HMA) mice. Pooled human IBD patient fecal microbiota engrafted germ-free (GF) mice with

27   low amplicon sequence variant (ASV)-level transfer efficiency, resulting in high recipient-to-recipient

28   variation of microbiota composition and colitis severity in HMA Il-$10^{-/-}$ mice. In contrast, mouse-to-

29   mouse transfer of mouse-adapted human IBD patient microbiota transferred with high efficiency and

30   low compositional variability resulting in highly consistent and reproducible colitis phenotypes in

31   recipient Il-$10^{-/-}$ mice. Human-to-mouse FMT caused a population bottleneck with reassembly of

32   microbiota composition that was host inflammatory environment specific. Mouse-adaptation in the

33   inflamed Il-$10^{-/-}$ host reassembled a more aggressive microbiota that induced more severe colitis in

34   serial transplant to Il-$10^{-/-}$ mice than the distinct microbiota reassembled in non-inflamed WT hosts. Our

35   findings support a model of IBD pathogenesis in which host inflammation promotes aggressive resident

36   bacteria, which further drives a feed-forward process of dysbiosis exacerbated gut inflammation. This

37   model implies that effective management of IBD requires treating both the dysregulated host immune

38   response and aggressive inflammation-driven microbiota. We propose that our mouse-adapted human

39   microbiota model is an optimized, reproducible, and rigorous system to study human microbiome-driven

40   disease phenotypes, which may be generalized to mouse models of other human microbiota-modulated

41   diseases, including metabolic syndrome/obesity, diabetes, autoimmune diseases, and cancer.

45

Main Body

Introduction

Human inflammatory bowel diseases (IBD) are heterogeneous chronic inflammatory conditions driven by microbial activation of dysregulated intestinal immune responses in genetically susceptible hosts[1]. Host genetic susceptibility loci, such as polymorphisms in *Nod2*, *Il23r*, *Il-10r*, and *Il-10*, explain <20% of IBD variance[2-4] and disease incidence is rising globally[5], suggesting that environmental factors (diet, microbiome) are important drivers of IBD. IBD patients have altered intestinal microbiota composition (dysbiosis), functionally characterized by reduced diversity, unstable community structure over time and following perturbation, and expanded aggressive (*Gammaproteobacteria, Enterococcaceae*, sulfur-reducing bacteria) but reduced beneficial (short-chain fatty acid [SCFA]-producing *Clostridiales*, *Blautia*) resident bacteria[6-10]. Viable microbes are required to develop chronic T-cell mediated intestinal inflammation in most experimental colitis models (i.e. $Il\text{-}10^{-/-}$, $Il2^{-/-}$, $Tcrab^{-/-}$, Naïve CD4$^+$ T cell transfer to $Rag1/2^{-/-}$, $Tlr5^{-/-}$, $Tnf^{\Delta ARE}$ mice) in which GF mice have no inflammation but develop progressive intestinal inflammation after colonization with complex microbiota[11-16]. Aggressive resident bacteria (pathobionts) within the complex gut microbiota are the key drivers of intestinal inflammation[17-22]; however, whether dysbiotic expansion of pathobionts is a cause or consequence of intestinal inflammation and how the host environment shapes microbial ecology in IBD remain poorly understood.

Colonization of GF animals with defined human bacterial consortia or human fecal microbiota transplant (FMT) are the gold-standard methods to demonstrate causality and investigate mechanisms of human microbiome-driven disease phenotypes[23-29]. Defined consortia enable strict control of microbiota composition, which facilitates mechanistic studies using genetically modified consortium members but requires selection of bacterial strains by variable criteria[28, 30-32]. Strain-level genetic and functional variation are human disease-state specific, strongly impact host-microbe interaction, and alter disease severity in experimental colitis models[22, 33-38]. Because defined consortia may omit strain-specific genetic and functional attributes responsible for human disease phenotypes, direct transplant

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72   of human disease-associated feces to GF rodents is an appealing method to study human microbiome-

73   driven diseases.

74   Human IBD patient FMT to colitis-prone, GF mice (*Il-10^{-/-}* and *Rag1^{-/-}* T-cell transfer models)

75   transfers enhanced colitis severity compared to healthy patient FMT and induces a T_H17- and T_H2-

76   dominant immune phenotype that is characteristic of human IBD[26, 39-42]. These fecal transplant studies

77   clearly transfer disease phenotype to susceptible mice by human IBD-associated microbes.

78   Importantly, human-to-mouse fecal transplant causes a microbial population bottleneck that engrafts a

79   compositionally distinct microbiome in recipient mice compared to human donor stool, likely due to low

80   human-to-mouse strain-level transfer efficiency (~40%) and host-specific microbe preferences[43-46]. We

81   took advantage of the microbiota reassembly associated with human-to-mouse FMT to ask if 1) the

82   host environment controls microbiota assembly and inflammatory potential, and 2) mouse-adaptation of

83   human fecal microbiota forms a microbial community that is stable in serial transplant to GF mice and

84   leads to more reproducible experimental phenotypes.

85   To evaluate the impact of the host inflammatory environment on gut microbiota assembly we

86   transferred pooled feces from human IBD patients with active disease to wild-type (WT) or *Il-10^{-/-}* mice.

87   Human microbiota-associated (HMA) *Il-10^{-/-}* mice had lower microbial alpha diversity, higher

88   compositional variability, and expansion of pathobionts compared to HMA WT mice, illustrating the

89   influence of an inflammatory colonic environment on dysbiosis. Serial transfer of non-inflamed (WT)

90   mouse-adapted human microbiota to GF *Il-10^{-/-}* mice induced less severe colitis than inflamed (*Il-10^{-/-}*)

91   mouse-adapted human microbiota. Transplant of human fecal microbiota to GF mice resulted in low

92   human-to-mouse transfer efficiency at the strain level, while mouse-adapted human microbiota yielded

93   high strain level transfer efficiency. High microbiota compositional variability in HMA *Il-10^{-/-}* mice was

94   associated with variable colitis severity, but recipient mice colonized with mouse-adapted human

95   microbiota exhibited low compositional variability and more consistent colitis phenotypes. Our findings

96   suggest that the reproducibility and rigor of HMA animal studies are impacted by the variability of

97   human-to-mouse FMT; however, experimental design can be improved by first adapting the human

98   microbiota to the mouse host followed by transfer of mouse-adapted human microbiota for subsequent

99   highly reproducible mechanistic studies.

Methods

Mouse Lines

GF 129S6/SvEv background wildtype (WT) and *Il-10*-deficient (*Il-10$^{-/-}$*) mice[13] were obtained from the

National Gnotobiotic Rodent Resource Center (NGRRC) at the University of North Carolina at Chapel

Hill. All animal experiments were conducted under approved Institutional Animal Care and Use

Committee protocols.

Human fecal samples

Human fecal samples from 5 adult patients with active Crohn's disease (CD) (4 donors) or ulcerative

colitis (UC) (1 donor) without prior intestinal surgery or antibiotic exposure within 3 months were

collected under an Institutional Review Board approved protocol (Fig S1A). De-identified stool samples

were aliquoted immediately after collection in an anaerobic chamber and stored without preservatives

at -80°C until use.

Human fecal microbiota and mouse-adapted fecal microbiota colonization of GF mice

Human fecal material from two sets of 3 human donors with active IBD (HM1: Donors 1, 2, 3; HM2:

Donors 3, 4, 5) was thawed and pooled in equal proportions by weight under anaerobic conditions

(N_2:H_2:CO_2 = 80:10:10), diluted with anaerobically reduced phosphate-buffered saline (PBS) to

generate a fecal slurry, and administered by 150μl oral gavage to recipient GF 129 WT or 129 *Il-10$^{-/-}$*

mice at 2mg pooled human donor stool per mouse. Mouse-adapted fecal pellets from HMA 129 WT or

129 *Il-10$^{-/-}$* mice were freshly collected and pooled daily between 14- and 21-days post-colonization and

frozen at -80°C without preservatives. To generate a standardized slurry of mouse-adapted microbiota,

mass collected fecal pellets from all mice in a group were pooled, homogenized, and diluted to

100mg/ml under anaerobic conditions in sterile anaerobically reduced lysogeny broth (LB) with 20%

glycerol. Solid particulate matter was pelleted by brief slow centrifugation and slurry supernatant was

aliquoted to cryovials for storage at -80°C. Slurry supernatant contains the same microbial community

124 composition as whole fecal material[43, 47]. Mouse-adapted microbiota slurry generated as above from

125 fecal pellets of HMA 129 WT mice is called non-inflamed mouse adapted microbiota (NIMM), while

126 slurry generated from fecal pellets of HMA colitis-prone 129 *Il-10$^{-/-}$* mice is called inflamed mouse

127 adapted microbiota (IMM) (Fig 1A). To colonize GF mice with mouse-adapted microbiota, standardized

128 aliquots of 100mg/ml fecal slurry were thawed under anaerobic conditions, diluted with anaerobically

129 reduced PBS, and administered by oral gavage to recipient GF 129 WT or 129 *Il-10$^{-/-}$* mice at 2mg per

130 mouse in 150μl. Fecal pellets from IMM or NIMM associated 129 WT or 129 *Il-10$^{-/-}$* mice were collected

131 daily between 14- and 21-days post-colonization when the *Il-10$^{-/-}$* recipient microbiota has stabilized and

132 before cage effects are reported to develop[48, 49], processed and frozen in aliquots as above to generate

133 standardized slurries of serial passages (-g1, -g2, and -g3) of mouse-adapted microbiota (Fig 1A). All

134 experiments were performed using aliquots from a single production batch of mouse-adapted

135 microbiota. All mouse fecal transplant experiments were performed in BSL-2 isolation cubicles with

136 HEPA-filtered air on a 12-hour dark/light cycle with ad libitum access to autoclaved water and mouse

137 chow (Purina Advanced Protocol Select Rodent 50 IF/6F Auto Diet) using the sterile out-of-isolator

138 gnotobiotic cage technique (Complete cage GM500, Green Line, Tecniplast)[50]. Cage changes and all

139 animal handling were performed in a laminar flow biosafety cabinet under sterile technique following

140 ultraviolet light treatment and 10-minute Peroxigard sterilization of all equipment and surfaces. We

141 maintained strict GF conditions with the out-of-isolator gnotobiotic technique for at least 2 weeks. We

142 consider the complex microbiota fecal transplant experiments reported here to be 'near-gnotobiotic'

143 with low risk of environmental contamination, but not strictly gnotobiotic since they are performed with

144 out-of-isolator gnotobiotic cage technique for durations >2 weeks and sterility could not be monitored

145 due to complex microbiota transplants.

146 **Gene expression by qRT-PCR, Intestine histopathology score, and Fecal lipocalin-2**

147 **quantification**

148   Standard molecular assays and histopathology scoring were performed as previously described[51-53].

149   Details of these procedures and a list of qPCR primers are found in the Supplemental Experimental

150   Procedures.

151   **Statistical Analyses**

152   Non-sequencing based statistical analyses were performed with Prism 10 (GraphPad) with statistical

153   tests and significance thresholds indicated in figure legends.

154   **16S rRNA Amplicon Metagenomic Sequencing and Analysis**

155   16S rRNA amplicon (variable regions 3-4) sequencing was performed on the Illumina NextSeq 2000

156   platform, processed, and taxonomically classified through QIIME2 by the UNC Microbiome Core[54].

157   Additional details of these procedures are found in the Supplemental Experimental Procedures.

158   Sequence counts data at both the genus and phylum level were extracted from the respective QIIME2

159   artifact files. The amplicon sequence variant (ASV)-level counts table was generated with forward reads

160   using the following parameters with single-end DADA2 on the QIIME2 (version 2021.2) platform: the

161   first 10 base pairs of each sequence were trimmed, and the sequences were truncated to 180 base

162   pairs as determined by sequence quality using FastQC (version 0.11.9)[54, 55]. Statistical analysis was

163   conducted with the *vegan* package (ver.2.6-2) in R (ver. 4.2.2) and visualized with the Shiny application

164   *Plotmicrobiome* and custom R code (Sun et al. GitHub https://github.com/ssun6/plotmicrobiome,

165   Supplemental File 1). To ensure reproducibility and rigor, the results of our analyses were

166   independently reproduced with custom Python code by a second bioinformatician (JBY) with replicated

167   key figures and reproducible tested code available in a Jupyter Notebook file (Supplemental File 1). R

168   and Python code used in our analyses are available at https://github.com/anhmoss/Mouse-Adaptation-

169   of-Human-Inflammatory-Bowel-Disease-Microbiota-Enhances-Colonization-Efficiency and in

170   Supplemental File 1. 16S rRNA amplicon sequencing data are available at

171   https://github.com/anhmoss/Mouse-Adaptation-of-Human-Inflammatory-Bowel-Disease-Microbiota-

172   Enhances-Colonization-Efficiency.

173   To account for varying sequencing depth, all counts data were normalized according to the

174   following formula prior to downstream statistical analyses:

175 $$log10 \left(\frac{raw\ OTU\ count\ for\ sample_i}{total\ sequences\ for\ sample_i} \times average\ sequence\ depth\ +\ 1 \right)$$

176   This formula adjusts the pseudo-count to have a similar effect across samples by scaling all

177   samples to the average sequencing depth. ASV transfer efficiency was measured as Pearson

178   correlation coefficient (r) for pairs of samples within a given group or between two groups.

179   **Results**

180   **Mouse-adapted human microbiota induces more consistent and reproducible colitis than**

181   **directly transplanted human microbiota.**

182   Human fecal microbiota transplantation into GF mice can transfer microbe-dependent

183   pathological phenotypes to recipient animals, allowing investigation of microbial mechanisms of human

184   diseases such as IBD[23, 26, 56]. The large interpersonal variation of human gut microbiota, host-specificity

185   of gut microbial ecology, and variable engraftment of human gut microbes into GF mice pose

186   challenges to transplanted phenotype reproducibility and interpretation[43, 45, 46, 57]. To understand the

187   impact of recipient host environment on human fecal microbiota engraftment and phenotype transfer in

188   a mouse model of experimental colitis, we transplanted pooled feces from 3 humans with active IBD (2

189   CD, 1UC) to non-inflamed WT or colitis-susceptible *Il-10*^{-/-} GF mice (Fig 1A, S1A). We then

190   transplanted these mouse-adapted microbiota to sequential cohorts of non-inflamed WT or colitis-

191   susceptible *Il-10*^{-/-} GF recipient mice, generating serial transfers of mouse-adapted human microbiota

192   identified as -g1, -g2, and -g3 (Fig 1A). In our nomenclature, different human IBD patient fecal pools are

193   called Human Microbiota (HM1 or HM2) (shown in S1A), feces from HMA WT mice are called Non-

194   Inflamed Mouse-adapted Microbiota (NIMM), and feces from HMA *Il-10*^{-/-} mice are called Inflamed

195   Mouse-adapted Microbiota (IMM) (Fig 1A). Serial mouse-adapted fecal transplant experiments were

196   only conducted with HM1-dervied HMA mouse stool due to resource constraints; HM1 was selected

197   because the cohort contained both UC and CD donors (Fig 1A; S1A). Because colonic immune

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198 stimulation of GF mice is equivalent following transplant of human or mouse microbiota, HMA mice are

199 a clinically relevant model of experimental colitis[44, 45].

200 GF 129 WT mice receiving HM1, NIMM-g1, or NIMM-g2 fecal transplant did not develop colitis

201 as assessed by colon histology, non-invasive fecal lipocalin-2 (f-LCN2), and tissue inflammatory

202 cytokine levels (Fig 1B-D; S1B-C). Transplantation of both human microbiota HM1 and mouse-adapted

203 microbiota IMM-g1 or IMM-g2 to GF 129 $Il-10^{-/-}$ mice induced severe colitis as assessed by colon

204 histology, non-invasive f-LCN2, and inflammatory cytokine levels (Fig 1C-F; S1B,D). IMM-g1 and IMM-

205 g2 induced cecal predominant colitis that was equivalent in severity and kinetics to colitis induced by

206 HM1 (Fig 1E; S1B,D). However, HM1 induced colitis was more variable than IMM-g1 or IMM-g2

207 induced colitis as quantified by segment and total histology score variance and interquartile range (Fig

208 1E; Fig S1G). The high phenotypic variance of human microbiome-induced colitis was replicated by a

209 separate cohort (HM2) of pooled feces from 3 humans with active CD transplanted to $Il-10^{-/-}$ GF mice

210 (Fig S1E,F). In contrast to the highly variable phenotype of human microbiome-induced colitis, mouse-

211 adapted microbiome IMM-g1 induced colitis had little variation in severity or distribution within or across

212 independent experiments (Fig 1E,F; S1G,H). To evaluate whether variability in colitis phenotype was

213 related to microbiome composition, we performed 16S amplicon sequencing of input donor microbiota

214 and fecal samples collected from ex-GF 129 WT and 129 $Il-10^{-/-}$ mice colonized for 28 days with human

215 microbiota or mouse-adapted microbiotas (Fig 1A). As we show later in the results (Fig 3), human

216 microbiota transplant to GF mice was associated with significantly lower microbiota engraftment

217 consistency than mouse-adapted microbiota transplant, suggesting that variability in engrafted human

218 microbiota composition may cause variability in colitis phenotypes.

219 **Human microbiome restructures with transplant to GF mice.**

220 To investigate how the recipient host intestinal environment shapes human microbiota

221 engraftment in GF mice, we assessed microbiome compositional variation by calculating the average

222 relative abundance of genera across all samples for each fecal transplant condition. Fig 2 shows

223 taxonomic barplots of the 8 most abundant genera across groups with the remaining lower abundance

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224　taxa grouped as "Other" (Fig 2, S2A). The 30 most abundant genera across groups and the relative

225　abundance of genera for individual mice are visualized in barplots in Fig S2. We performed pairwise t-

226　tests to assess differential abundance between groups, excluding genera present in less than 10% of

227　the samples (Table S1).

228　　　Pooled human microbiome composition (HM1 input and HM2 input) was compositionally distinct

229　from all colonized mouse groups as visualized by taxonomic barplots and principal coordinates analysis

230　(PCoA) clustering (aka multidimensional scaling), with the strongest separation existing between

231　human and mouse-adapted microbiotas along the first MDS axis (Fig 2, 3A). Compared to human

232　microbiomes, HMA mouse and MA-FMT mouse microbiomes had increased relative abundance of

233　*Akkermansia*, *Lachnoclostridium*, *Ruminococcus gnavus* group, and *Hungatella*, a low-abundance

234　member of the human gut that was not detectable by 16S in HM1 or HM2 inputs (Fig 2). *Bacteroides*, a

235　major constituent of the human gut microbiome, was present in HM1 input and HM2 input, and

236　expanded in HM2-associated *Il-10*$^{-/-}$ mice but reduced in HM1-associated WT and *Il-10*$^{-/-}$ mice (Fig 2,

237　Table S1). The expansion of *Bacteroides* in HM2- but reduction in HM1-associated *Il-10*$^{-/-}$ mice was

238　surprising because *Bacteroides* was more abundant in HM1 input compared to HM2 input, suggesting

239　stochastic factors influence engraftment of human microbiota in GF mice (Fig 2). These data

240　demonstrate that human microbiota association of GF mice results in major compositional restructuring

241　of the engrafted microbiome that may be partially stochastic.

242　**Recipient host environment influences engraftment composition of human-microbiota**

243　**associated mice.**

244　　　The recipient host environment shapes the engrafted microbiome composition of HMA mice (Fig

245　2, S2, 3A, B). After removing HM1 and HM2 inputs, PCoA showed separation of inflamed mouse-

246　adapted microbiota (IMM) and non-inflamed mouse-adapted microbiota (NIMM) along the first MDS

247　axis (Fig 3B). PERMANOVA test with all mouse recipient groups as the model term demonstrated that

248　approximately 43% of the variation in the data is explained by the recipient host environment

249　(coefficient of determination, R^2 = 0.43, p=0.001). Mouse adaptation in the inflamed *Il-10*$^{-/-}$ host (IMM)

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250 enriched for significantly higher relative abundance of *Escherichia-Shigella, Parasutterella,*

251 *Enterococcus, Clostridium_sensu_stricto_1, Ruminococcus gnavus group,* and *Bifidobacterium* but

252 significantly lower relative abundance of *Clostridium innocuum, Blautia, Lachnoclostridium,* and multiple

253 other genera within *Lachnospiraceae* and *Ruminococaceae* when compared to mouse adaptation in the

254 non-inflamed WT host (NIMM) (Fig 2, S2, Table S1). PCoA of serial microbiota passage within the non-

255 inflamed WT host environment (NIMM-g1, -g2) showed that the global microbiome structure remained

256 stable with no distinct clustering of groups (Fig 3D) and only 7 operational taxonomic units (OTUs)

257 demonstrated significantly differential abundance between NIMM-g1 and -g2 using a cutoff of FDR<0.1

258 (Fig 2, S2, Table S1). PCoA of serial microbiota passage within the inflamed *Il-10$^{-/-}$* host environment

259 (IMM-g1, -g2) similarly showed globally stable microbiome structure with no distinct clustering of groups

260 (Fig 3C, S3A), while no OTUs were differentially abundant between IMM-g1 and -g2 (Fig 2, S2, Table

261 S1). Mouse adaptation in the inflamed *Il-10$^{-/-}$* host (IMM) was associated with significantly lower alpha

262 diversity at the amplicon sequence variant (ASV) level compared to the non-inflamed WT host (NIMM),

263 consistent with observations that human IBD patients have lower alpha diversity than healthy humans

264 (Fig 3E, S3B,C)[8]. Together, these data demonstrate that the composition of the human microbiome is

265 fundamentally restructured with transplant to GF mice and that the recipient host environment strongly

266 shapes the relative abundance of engrafted strains with the inflamed *Il-10$^{-/-}$* host (IMM) driving a

267 dysbiotic microbiome defined by lower alpha-diversity, enrichment of pathobionts, and reduction of

268 protective SCFA-producing bacteria relative to the non-inflamed WT host (NIMM).

269 **Human microbiota engrafts with variable composition compared to more consistent**

270 **engraftment by mouse-adapted microbiota.**

271 Since the human microbiome restructures with transplant to GF mice, we speculated that

272 variability in engrafted microbiota composition may explain the colitis phenotype variability of HMA *Il-10^{-}*

273 *$^{/-}$* mice (Fig 1E, S1E-F). Variability of microbiota composition was quantified by pairwise calculation of

274 Pearson correlation coefficient for all samples within the same group (i.e., all mice within HM1->KO). A

275 high Pearson correlation coefficient indicates compositional similarity between samples in a group,

276   while a low coefficient indicates compositional variability between samples in a group. Human

277   microbiota transplant to 129 $Il-10^{-/-}$ mice (HM1->KO) was associated with significantly lower Pearson

278   correlation coefficients than mouse-adapted microbiota transplants to 129 $Il-10^{-/-}$ mice (IMM-g1->KO,

279   IMM-g2->KO) (Fig 3F, S3D). A similar trend was seen with human microbiota or mouse-adapted

280   microbiota transplant to 129 WT mice (Fig 3F). Pearson correlation coefficients for 129 $Il-10^{-/-}$ recipient

281   mice were consistently lower than 129 WT recipients at each stage of serial passage (i.e., HM1->WT vs

282   HM1->KO or NIMM-g1->WT vs IMM-g1->KO), demonstrating that inflammation promotes variability of

283   microbiome composition while health is associated with microbiome stability (Fig 3F). These results are

284   consistent with observations in humans that the composition of IBD patient microbiomes fluctuate more

285   than healthy controls over time[28, 58]. Together, these data suggest that 1) inflammation promotes

286   microbiome variability and 2) variability in colitis phenotype with human microbiota transplant may be

287   due to variability in engrafted human microbiota composition, while the more consistent colitis induced

288   by mouse-adapted microbiota may be due to homogeneity of engraftment of mouse-adapted

289   microbiota.

290   **Mouse-adapted human IBD microbiota transfers with higher efficiency than human fecal**

291   **transplant.**

292   Since HMA mice had significantly different microbiome composition than human donor stool but

293   mouse-adapted FMT mice had highly consistent microbiomes between serial transfer, we evaluated

294   whether mouse-adapted microbiota transfers to GF mice more efficiently than human fecal transplant

295   (Fig 4A-D). To quantify transfer efficiency, we detected all ASVs across all samples and compared ASV

296   abundance between human stool, HMA mice, and mouse-adapted FMT mice across serial transfers

297   (Fig 4A-D). We visualized these data using scatter plots where each dot represents a unique ASV

298   plotted by log_{10} relative abundance in the input microbiome (x-axis) vs recipient mouse microbiome (y-

299   axis). We quantified transfer efficiency using Pearson correlation coefficient (r), where high Pearson r

300   indicates consistent ASV abundances between samples and high transfer efficiency. We used deep

301   16S amplicon sequencing rather than whole genomic shot gun sequencing (WGS) because repeat

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302 sequencing of the same region allows for exact identification of ASVs in a database-independent

303 manner without reliance on classification algorithms.

304 Human fecal transplant to WT or *Il-10*$^{-/-}$ mice was associated with low transfer efficiency and

305 poor transfer of relative composition to recipient mice (Fig 4A-D, S5D-E). Very similar results were seen

306 in WT and *Il-10*$^{-/-}$ mice. A large proportion of ASVs present in human stool did not transfer to recipient

307 mice, which is illustrated by ASVs falling on the x-axis (Fig 4A,C). The relative abundance (log$_{10}$

308 normalized) of non-transferring ASVs demonstrated that even moderately to highly abundant ASVs in

309 human stool did not transfer efficiently to GF mice (Fig 4E,H). ASV relative abundance in human stool

310 had little correlation with relative abundance in recipient mice (Fig 4A,C), leading to a very low ASV

311 level transfer efficiency for human fecal transplant to WT (r=0.34±0.03) or *Il-10*$^{-/-}$ (r=0.33±0.03) mice

312 (Fig 4B,D). For mouse-adapted FMT, however, ASV relative abundance in MA-FMT input (IMM or

313 NIMM) was highly correlated with relative abundance in recipient mice (Fig 4A,C). Only a small

314 proportion of ASVs present in mouse-adapted microbiota did not transfer to recipient mice, and those

315 non-transferring ASVs were primarily low-abundance strains (Fig 4A,C,F-G,I-J). Serial transfer of

316 mouse-adapted microbiota further improved the correlation between input and recipient microbiomes

317 and reduced non-transferring ASV numbers, leading to very high ASV level transfer efficiency for

318 mouse-adapted FMT to WT (r=0.84±0.02) or *Il-10*$^{-/-}$ mice (r=0.85±0.05) (Fig 4 A-D). Similar results were

319 found when comparing human microbiome input to mouse-adapted microbiome inputs (Fig S4A-F).

320 Analysis of transfer efficiency at the genus level also demonstrated low transfer efficiency for human

321 fecal transplant but high transfer efficiency for mouse-adapted FMT; however, phylum level analysis

322 showed high transfer efficiency for all conditions, giving a misleading perception of transfer efficiency

323 (Fig 5A-H, S5F).

324 Some ASVs (falling on the y-axis) in HMA mice were not detected in human stool, representing

325 either mutation of the V3-V4 sequence, *in vivo* expansion of very low abundance strains undetected at

326 the depth of 16S sequencing utilized, or environmental contamination (Fig 4A,C). To rule out

327 environmental contamination, we performed human FMT to GF mice in strictly gnotobiotic isolators and

328 still detected many ASVs in HMA mice that were not detected in human input stool by 16S Seq (Fig

329 S5D-E). We analyzed an independently published 16S Seq dataset of HMA WT mice colonized and

330 then bred in a gnotobiotic isolator and found similar results of low human-to-mouse but high mouse-

331 adapted-to-offspring mouse transfer efficiency at the ASV level (Fig S5A-C)[44]. These data demonstrate

332 that a large fraction of the human microbiome does not efficiently engraft GF mice; however, once

333 engrafting strains adapt to the mouse gut they transfer with very high efficiency in serial fecal

334 transplant.

335 **Transfer efficiency varies between taxa**.

336   To assess the transfer efficiency of different taxa from transplant of human microbiota or

337 mouse-adapted microbiota to GF mice, we compared Pearson correlation coefficients (r) between phyla

338 (Fig 5I-N). Unclassified bacteria had the lowest transfer efficiency in all groups, consistent with prior

339 reports (Fig 5I-N)[43]. *Verrucomicrobiota* and *Fusobacteriota* consistently had very high transfer

340 efficiency, which likely reflected that a single species from each phylum was present in donor stool (Fig

341 5I-N). *Akkermansia muciniphila*, a known keystone species, is the only human gut member of

342 *Verrucomicrobiota* and transferred highly efficiently across all transplant conditions and recipients.

343 Transfer efficiencies trended lower for *Firmicutes, Bacteroidota,* and *Actinobacteriota* and trended

344 somewhat lower in *Proteobacteria* in all *Il-10*$^{-/-}$ mice compared to WT mice (Fig 5I-N).

345 **Inflamed mouse-adapted microbiome induces faster onset colitis than non-inflamed mouse**

346 **adapted microbiome.**

347   Since mouse-adaptation of human microbiota in the non-inflamed (WT) host reduced the

348 frequency of pathobionts while expanding putatively protective bacteria, we investigated whether

349 NIMM-g1 induces less severe colitis than IMM-g1 when transplanted to *Il-10*$^{-/-}$ GF mice (Fig 6A). At 14

350 days post-colonization, NIMM-g1 colonized *Il-10*$^{-/-}$ mice had significantly lower f-LCN2 levels, cecum-

351 and total colon histologic inflammation than IMM-g1 colonized *Il-10*$^{-/-}$ mice (Fig 6B, S6A-B). At 28 days

352 post-colonization, NIMM-g1 colonized *Il-10*$^{-/-}$ mice continued to have significantly reduced cecal

353   inflammation scores and trend toward lower cecal inflammatory cytokine levels but had developed

354   increased rectal inflammation compared to IMM-g1 colonized *Il-10*^{-/-} mice (Fig 6C). NIMM-g1 colonized

355   *Il-10*^{-/-} mice had significantly lower maximum segment inflammation on a per-mouse basis compared to

356   IMM-g1 colonized *Il-10*^{-/-} mice (Fig 6C). However, the increase in rectal inflammation resulted in a non-

357   significant trend toward lower f-LCN2 levels and no difference in total colon histology scores between

358   NIMM-g1 and IMM-g1 colonized *Il-10*^{-/-} mice at 28 days post-colonization (Fig 6C; S6C). PCoA

359   demonstrated that the microbiome of NIMM-g1 colonized *Il-10*^{-/-} mice (NIMM-g1->KO) clustered with

360   WT HMA and MA-FMT mice, rather than *Il-10*^{-/-} HMA or MA-FMT mice (Fig 6E). Alpha diversity of

361   NIMM-g1 colonized *Il-10*^{-/-} mice was equal to NIMM-g1 colonized WT mice and non-significantly higher

362   than IMM-g1 colonized *Il-10*^{-/-} mice (Fig 6G). These data suggest that major changes in community

363   restructuring occur during initial adaptation of human microbiota to the non-inflamed mouse host, but

364   that once a stable mouse-adapted community forms it transfers with stable global structure in serial

365   transplant to subsequently inflamed GF host mice. Although PCoA demonstrated that global

366   microbiome structure of NIMM was stable between the inflamed and non-inflamed environments,

367   taxonomic bar plots and differential abundance analysis demonstrate that several taxa undergo

368   changes in frequency (Fig 6F; S6D; Table S1). Putatively protective *Blautia* and *Lachnospiraceae*

369   *NK4A136* group were significantly reduced while the pathobiont containing genera *Ruminococcus*

370   *gnavus* group and *Hungatella* were significantly expanded in *Il-10*^{-/-} compared to WT mice colonized

371   with NIMM-g1, suggesting that these genera may be particularly responsive to the inflammatory

372   environment – consistent with observations in human IBD microbiome profiling studies (Fig 6F, S6E-

373   H)[7, 9, 59, 60]. Together, these data demonstrate that human microbiome adaptation is dependent on the

374   host environment, but once a stable mouse-adapted microbiome has been established it remains

375   remarkably stable in composition despite an altered host environment.

376

377   **Discussion**

15

378   The role of gut microbiota dysbiosis as cause or consequence of intestinal inflammation is an

379   area of active investigation and debate with clinical importance for the management of IBD[1]. Transplant

380   of human disease-associated feces to GF rodents is an approach that captures strain-specific

381   functional and genetic variation responsible for human-microbiome driven disease phenotypes without

382   biased selection of defined input strains. Although widely accepted, this approach is complicated by

383   phenotypic and experimental variability of unclear etiology. Our study identified that FMT transfer

384   efficiency is an underappreciated source of experimental variability. Using high depth, low-error rate

385   Illumina 16S amplicon sequencing (16S Seq), we showed that pooled human IBD patient fecal

386   microbiota engrafts GF mice with low ASV-level transfer efficiency, resulting in high recipient-to-

387   recipient variation of microbiota composition and colitis severity in HMA *Il-10$^{-/-}$* mice. Human-to-mouse

388   FMT caused a population bottleneck with reassembly of microbiota composition that was host

389   inflammatory environment specific. In the inflamed environment of HMA *Il-10$^{-/-}$* mice, the microbiota

390   reassembled with lower microbial alpha diversity, higher recipient-to-recipient microbiota compositional

391   variability, and expansion of pathobionts compared to the distinct microbiota reassembled in the non-

392   inflamed environment of HMA WT mice. Following the initial human-to-mouse population bottleneck

393   and microbiota reassembly, the mouse-adapted human IBD patient microbiota transferred with high

394   efficiency and low compositional variability to GF recipients, which correlated with highly consistent and

395   reproducible colitis phenotypes in *Il-10$^{-/-}$* recipient mice. The mouse-adapted microbiota composition

396   was remarkably stable in serial transplant to both inflamed and non-inflamed host environments. We

397   replicated the key finding of low human-to-mouse but high mouse-adapted-to-mouse transfer efficiency

398   at the ASV level by analysis of an independently published 16S Seq dataset of HMA WT mice bred in a

399   gnotobiotic isolator[44]. Microbiota adaptation in the inflamed environment assembled a more aggressive

400   microbiota than adaptation in the non-inflamed environment, demonstrating that the genetically

401   determined host inflammatory environment shapes dysbiosis that subsequently drives more severe

402   inflammation. Our data demonstrate that host gut inflammation is both a cause and consequence of

403   microbial dysbiosis.

404   Our data support recent criticism that stochastic ecological processes and donor heterogeneity

405   influence phenotypes in HMA murine models[61]. We found that OTU based metrics, especially at higher

406   taxonomic levels, over-estimated transfer efficiency compared to ASV analysis[23, 44, 61]. The low transfer

407   efficiency and population bottleneck of human-to-mouse FMT led to high variability in engrafted

408   microbiota composition between individual recipients of the same human input stool, which correlated

409   with significant variability in colitis severity in recipient $Il\text{-}10^{-/-}$ mice. We speculate that stochastic

410   differences in engraftment were accentuated by the bottleneck of human-to-mouse FMT and drove

411   phenotypic variability[61]. Large interindividual variability of human donor microbiota likely exacerbates

412   this phenomenon in HMA murine studies[8, 57, 61]. We used pooled human IBD donor stool to mitigate the

413   impact of individual human donor microbiota heterogeneity and replicated our results with 2 pooled

414   human donor pools. Our pooling approach is suitable for experimental designs that require a

415   representative human disease associated microbiome to interrogate mechanistic questions (i.e., the

416   impact of diet or host genetic background) or test therapeutics (i.e., live biotherapeutics or novel

417   biologics); however, studies evaluating microbiome-driven phenotype transfer require an appropriately

418   powered number of individual human donors to establish causality and avoid bias from pseudo-

419   replication[61]. In our study, both pooled human fecal cohorts HM1 and HM2 contained *Bacteroides*

420   genus at high abundance. However, following human-to-mouse transplant, *Bacteroides* abundance

421   dramatically decreased in HM1 recipients but expanded in HM2 recipients. Although our study was not

422   powered to distinguish whether this divergent engraftment arose from microbial ecology of the donor

423   microbiota or stochastic processes, our data suggest that low human-to-mouse transfer efficiency in the

424   setting of donor heterogeneity and stochastic ecological processes is an underappreciated source of

425   variability in HMA animal models. In contrast, transplant of mouse-adapted human microbiota yielded

426   highly reproducible and consistent microbiota composition and colitis phenotypes – an improved model

427   for studying human microbiota driven diseases.

428   Our data demonstrated that mouse-adaptation of human fecal microbiota was shaped by the

429   host inflammatory environment to form stable microbial communities that reproducibly engrafted GF

17

430 mice with high efficiency to drive distinct colitis phenotypes. Mouse-adaptation in the inflamed

431 genetically susceptible host assembled an aggressive microbiota with low alpha-diversity and high

432 pathobiont abundance (*Enterobacteriaceae, R. gnavus*) that drove more severe colitis in serial

433 transplant to *Il-10*$^{-/-}$ mice than microbiota adapted in the non-inflamed host. Gut inflammation induces

434 host-derived metabolites, such as nitrate, lactate, and ethanolamine, that enhance fitness, abundance,

435 and virulence of aggressive *E. coli* and promote ectopic gut colonization of inflammation-associated

436 *Veillonella* species[62-66]. Adherent and invasive *E. coli* and other inflammation-associated aggressive

437 resident bacteria drive intestinal inflammation in murine colitis models[1, 18, 19, 21]. Together with the

438 literature, our data support a model of IBD pathogenesis in which host inflammation in genetically

439 susceptible hosts promotes the expansion, fitness, and virulence of aggressive resident bacteria, which

440 further drive a feed-forward process of dysbiosis exacerbated gut inflammation. This model implies that

441 effective management of IBD requires treating both the dysregulated host immune response and

442 aggressive inflammation-associated microbiota.

443   Our study benefitted from several strengths including an experimental design that incorporated

444 multiple serial FMT, high recipient mouse numbers, and application of high-depth low error rate

445 sequencing for accurate ASV tracking; however, there were some limitations. First, most experiments

446 were conducted in out-of-isolator gnotobiotic cages, where contamination risk is extremely low but

447 could not be monitored due to the complex FMT inputs. To address this, we replicated key experiments

448 in strict gnotobiotic isolators, confirming our findings of low human-to-mouse ASV-level transfer

449 efficiency and the emergence of ASVs in HMA mice not detected in human input stool. Second, we did

450 not analyze WGS data to compare transfer efficiency of microbial functions vs taxonomic composition.

451 We used 16S Seq rather than WGS because repeat sequencing of the same region allows for exact

452 identification of ASVs in a database-independent manner without reliance on classification algorithms.

453 Future WGS studies are needed to evaluate the impact of taxonomic transfer efficiency on transfer of

454 microbial functions. Third, we did not evaluate the impact of mouse diet on the initial human-to-mouse

455 engraftment bottleneck – an important topic for follow up studies.

456   Our mouse-adapted human microbiota model is an optimized, reproducible, and rigorous

457   system to study human microbiome-driven disease phenotypes. Multiple approaches (human

458   microbiome profiling, defined consortia animal studies, HMA animal models) can investigate causality

459   and identify mechanisms of microbiota-driven diseases[1, 29, 67]. Mono-association and defined

460   consortium studies are reductionist approaches where a single variable (i.e., single-gene mutations)

461   can interrogate bacterial mechanisms[1, 29]. Representative synthetic microbiota, such as hCOM2,

462   PedsCom, and SIHUMI, provide a more ecologically complex system with known input strain identity

463   and the ability to easily track relative abundance by simplified metagenomic sequencing approaches[28,

464   31, 32, 67, 68]. However, even large complex defined consortia do not capture the understudied strain level

465   variation that exists in heterogeneous human resident microbiota and contributes to important

466   differences in strain dependent microbiota aggressiveness[22, 36, 37, 66]. The high transfer efficiency of

467   mouse-adapted human microbiota transplant to GF mice improves phenotype consistency, experiment

468   reproducibility and rigor of mouse models of human microbiota-driven disease. Homogenous

469   repositories of mouse-adapted human microbiota provide an identical microbial starting point for every

470   experiment that can be replicated over time and between institutions/collaborators without transfer of

471   human host genetic material present in human feces to collaborators[69, 70]. Because of high transfer

472   efficiency and reproducible engraftment, mouse-adapted human microbiota repositories can be

473   expanded *in vivo* when stocks run low, mitigating limitations of finite human fecal samples. While this

474   study focused on colitis, our mouse-adapted human microbiota approach is a framework that may be

475   generalized to mouse FMT models of other human microbiota-modulated diseases, such as metabolic

476   syndrome/obesity, diabetes, autoimmune diseases, and cancer.

477

478   **Acknowledgements**

Competing Interests

488   None relevant to this study. RBS receives grant support from Gusto Global LLC, Biomica, and

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Data Availability Statement

491   The datasets, R code, and Python code are publicly available at https://github.com/anhmoss/Mouse-

492   Adaptation-of-Human-Inflammatory-Bowel-Disease-Microbiota-Enhances-Colonization-Efficiency and

493   from the corresponding authors upon reasonable request.

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687

688 **Tables**

689 **Supplementary Table S1:** Differential abundance analysis between groups excluding genera present

690 in less than 10% of the samples.

691

692 **Figure Legends**

693

694 **Figure 1. Mouse-adapted human microbiota induces more consistent and reproducible colitis**

695 **than directly transplanted human microbiota.** A) Experimental design. Pooled feces from 3 humans

696 with active IBD (2 CD, 1UC) were transplanted to non-inflamed WT or colitis-susceptible $Il\text{-}10^{-/-}$ (IL-

697 10KO, KO) GF recipient mice. Mouse-adapted microbiotas were serial transplanted to non-inflamed WT

698 or colitis-susceptible $Il\text{-}10^{-/-}$ GF recipient mice. B) Total colon and ileum histology score for WT mice at

699 day 28 post-colonization. C) f-LCN2 level at day 28 post-colonization. D) TNFα mRNA levels in cecal

700 tissue at day 28 post-colonization. E) Segment, total colon and ileum, and max segment histology score

701 for $Il\text{-}10^{-/-}$ mice at day 28 post-colonization. F) Segment, total colon and ileum, and max segment

702 histology score for IMM-g1 colonized $Il\text{-}10^{-/-}$ mice at day 28 post-colonization from 4 independent

703 experiments. Data shown are representative of (C-D) or cumulative (B, E-F) from 2-4 independent

704 experiments. n=7-9 (B-D), n=15-26 (E), n=5-8 (F) mice per group. Data are expressed as mean±SD or

705 geometric mean ± geometric SD (C). Statistical significance calculated by unpaired t-test or Mann-

706 Whitney test (C) with *p<0.05, **p<0.01, ***p<0.001.

707

708 **Supplemental Figure S1 (related to Figure 1). Mouse-adapted human microbiota induces more**

709 **consistent and reproducible colitis than directly transplanted human microbiota.** A)

710 Demographics and cohort membership of human IBD donors. B-D) f-LCN2 time-course of (B) HM1

711 colonized WT and KO mice, (C) NIMM-g1 colonized WT mice, (D) IMM-g1 or IMM-g2 colonized KO

712 mice. E-F) Bar plot (E) and Box-and-whisker (F) plot of segment histology score for HM1 and HM2

713 colonized KO mice at day 28 post-colonization. G) Box-and-whisker plot of segment histology score for

714 KO mice at day 28 post-colonization. H) Box-and-whisker plot of segment histology score for IMM-g1

715 colonized KO mice at day 28 post-colonization from 4 independent experiments. Data shown are

716 representative of (B-D, H) or cumulative (E-G) from 2-4 independent experiments. n=7-9 (B-D), n=11-

717 15 (E-F), n=15-26 (G), n=5-8 (H) mice per group. Data are expressed as mean±SD (E) or geometric

718 mean ± geometric SD (B-D). In box-and-whisker plots, box represents lower, median, and upper

719 quartiles; whiskers are min to max. Statistical significance calculated by Mann-Whitney test (B-D) or

720 unpaired t-test (E-H) with *p<0.05, **p<0.01, ***p<0.001.

721

722 **Figure 2. Recipient host environment influences engraftment composition of human-**

723 **microbiome associated mice.** A) 16S Seq taxonomic bar plots show top 8 most abundant genera in

724 FMT inputs and recipient mouse feces at day 28 post-colonization. For mouse recipient groups, bar

725 plots are average of 16S seq data from n=7-18 mice/group.

726

727 **Supplemental Figure S2 (related to Figure 2). Recipient host environment influences**

728 **engraftment composition of human-microbiome associated mice.** A) 16S Seq taxonomic bar plots

729 show top 8 most abundant genera in human and mouse-adapted FMT inputs and individual recipient

730 mouse feces at day 28 post-colonization. B) 16S Seq taxonomic bar plots show top 30 most abundant

731 genera in human and mouse-adapted FMT inputs and recipient mouse feces at day 28 post-

732 colonization. For mouse recipient groups, bar plots are average of 16S Seq data from n=7-18

733 mice/group.

734

735 **Figure 3. Human microbiome restructuring with transplant to GF mice is host inflammatory**

736 **environment specific.** A) Principal coordinates analysis, PCoA, of 16S Seq data for human and

737 mouse-adapted FMT inputs and FMT recipient WT and KO mouse groups. B) PCoA of FMT recipient

738 WT and KO mouse groups. C) PCoA of FMT recipient KO mouse groups. D) PCoA of FMT recipient

739 WT mouse groups. E) Shannon index at ASV level for FMT recipient WT and KO mouse groups. F)

740 Pearson correlation coefficients (r) within group for FMT recipient WT and KO mouse groups quantify

741 variability of microbiota composition between mice in the same group (microbiota engraftment

742 consistency). Dots in PCoA plots represent individual mice for FMT recipient WT and Il-$10^{-/-}$ (KO)

743 mouse groups. For FMT inputs, a single input slurry was used in each experiment and input dots

744 represent sequencing data from three 16S amplicon PCR technical replicates. Analysis conclusions did

745 not change when using average input vs individual technical replicates, so technical replicates are

746 displayed to demonstrate the high consistency of 16S amplicon PCR in our dataset.

747

748 **Supplemental Figure S3 (related to Figure 3). Human microbiome restructuring with transplant**

749 **to GF mice is host inflammatory environment specific.** A) PCoA of 16S Seq data for HM1, HM2,

750 and mouse-adapted FMT inputs and FMT recipient KO mouse groups. B) Shannon index at genus level

751 for FMT recipient WT and KO mouse groups. C) Shannon index at ASV and genus level for HM1 and

752 HM2 FMT inputs. D) Pearson correlation coefficient (r) within group for HM1->KO and HM2->KO

753 recipient mouse groups quantifies variability of microbiota composition between mice in the same group

754 (microbiota engraftment consistency).

755

756 **Figure 4. Mouse-adapted human IBD microbiota transfers with higher efficiency than human**

757 **fecal transplant.** A) ASV level $\log_{10}$-normalized relative abundance correlations for FMT input and WT

758 recipient mice where each dot represents a unique ASV plotted in the input microbiome (x-axis) vs

759 recipient mouse microbiome (y-axis). B) Transfer efficiency quantified by Pearson correlation coefficient

760 (r) between FMT input and WT recipient mouse groups at the ASV level. C) ASV level $\log_{10}$-normalized

761 relative abundance correlations for FMT input and KO recipient mice. D) Transfer efficiency quantified

762 by Pearson correlation coefficient (r) between FMT input and KO recipient mouse groups at the ASV

763 level. E-J) Representative histograms of non-transferring ASVs (red, representing y=0 ASVs in above

764 dot plots) and newly detected *in vivo* ASVs (blue, representing x=0 ASVs in above dot plots) binned by

765 $\log_{10}$-normalized relative abundance for (E) HM1->WT, (F) NIMM-g1->WT, (G) NIMM-g2->WT, (H)

766 HM1->KO, (I) IMM-g1->KO, and (J) IMM-g2->KO FMT recipient mouse groups.

767

768 **Supplemental Figure S4 (related to Figure 4). Mouse-adapted human IBD microbiota transfers**

769 **with higher efficiency than human fecal transplant.** A-B) ASV level $\log_{10}$-normalized relative

770 abundance correlations comparing (A) HM1 input to NIMM-g1 input and (B) NIMM-g1 input to NIMM-g2

771 input. C) Pearson correlation coefficient (r) between HM1 input, NIMM-g1 input, and NIMM-g2 inputs at

772 the ASV level. D-E) ASV level $\log_{10}$-normalized relative abundance correlations comparing (D) HM1

773 input to IMM-g1 input and (E) IMM-g1 input to IMM-g2 input. F) Pearson correlation coefficient (r)

774 between HM1 input, IMM-g1 input, and IMM-g2 inputs at the ASV level. G) Pearson correlation

775 coefficient (r) between WT recipient and KO recipient groups at the ASV level.

776

777 **Figure 5. Transfer efficiency varies between taxa**. A) Genus-level and B) phylum-level $\log_{10}$-

778 normalized relative abundance correlations comparing HM1 input to HM1->KO, C-D) Pearson

779   correlation coefficient (r) between input and inflamed (KO) recipient at the (C) genus- and (D) phylum-

780   level. E) Genus-level and F) phylum-level $\log_{10}$-normalized relative abundance correlations comparing

781   HM1 input to HM1->WT, G-H) Pearson correlation coefficient (r) between input and non-inflamed (WT)

782   recipient at the (G) genus- and (H) phylum-level. I-K) Pearson correlation coefficient (r) between input

783   and non-inflamed (WT) recipients by phylum. L-N) Pearson correlation coefficient (r) between input and

784   inflamed (KO) recipients by phylum.

785

786   **Supplemental Figure S5 (related to Figure 5). Transfer efficiency varies between taxa**. A-C)

787   Analysis of published 16S Seq data from Lundberg *et al.*[44] (A) Experimental design. Feces from a

788   single healthy human donor were transplanted to GF adult WT mice (Parent, P) in a gnotobiotic

789   isolator. HMA WT mice were bred in-isolator to generate F1 pups. B) ASV level $\log_{10}$-normalized

790   relative abundance correlations for FMT input and recipient mice. For F1 pups, the input was natural

791   colonization by vertical transmission in-isolator from Parent (P). C) Transfer efficiency quantified by

792   Pearson correlation coefficient (r) between FMT input and recipient mouse groups at the ASV level. D)

793   Experimental design. Pooled feces from 3 humans with active IBD (3 CD, HM2) were transplanted to

794   colitis-susceptible *Il-10^{-/-}* (KO) GF recipient mice in a gnotobiotic isolator. E) ASV level $\log_{10}$-normalized

795   relative abundance correlations for HM2 input and HM2->KO recipient mice. F) Table comparing

796   percentage shared OTU between input and recipient group at the ASV, Genus, and Phylum level.

797

798   **Figure 6. Inflamed mouse-adapted microbiome more rapidly induces severe colitis than non-**

799   **inflamed mouse adapted microbiome.** A). Experimental design. Human IBD patient microbiota (HM1)

800   was adapted in the inflamed (IMM-g1) or non-inflamed (NIMM-g1) host, then transplanted to *Il-10^{-/-}*

801   (KO) GF recipient mice. B) Segment and total colon + ileum histology score for KO mice at day 14 post-

802   colonization. C) Segment, total colon + ileum, and max segment histology score for KO mice at day 28

803   post-colonization. D) TNFα mRNA levels in cecal tissue at day 28 post-colonization. E) PCoA of FMT

804   recipient WT and KO mouse groups, including NIMM-g1->KO group. F) 16S Seq taxonomic barplots

805 show top 8 most abundant genera in FMT inputs and recipient mouse feces at day 28 post-

806 colonization. For mouse recipient groups, barplots are average of 16S seq data from n=7-18

807 mice/group. G) Shannon diversity index at ASV level for IMM-g1->WT, NIMM-g1->KO and NIMM-g1-

808 >WT groups. Data shown are representative of (D) or cumulative (B-C, E-F) from 2-4 independent

809 experiments. n=15-16 (B-C), n=5-8 (D), n=7-16 (E-G) mice per group. Data are expressed as

810 mean±SD. Statistical significance calculated by unpaired t-test (B-D, G) with *p<0.05, **p<0.01,

811 ***p<0.001, ****p<0.0001.

812

813 **Supplemental Figure S6 (related to Figure 6). Inflamed mouse-adapted microbiome more rapidly**

814 **induces severe colitis than non-inflamed mouse adapted microbiome.** A-C) f-LCN2 level of IMM-

815 g1->KO and NIMM-g1->KO mice at (A) day 14, (B) time-course from day 0 to day 14, and (C) day 28

816 post-colonization. D) 16S Seq taxonomic bar plots show top 30 most abundant genera in human and

817 mouse-adapted FMT inputs and recipient mouse feces at day 28 post-colonization. E-G) $\log_{10}$-

818 normalized relative abundance of (E) *Blautia,* (F) *Lachnospiraceae NK4A136* group, (G) *R gnavus*

819 group, (H) *Hungatella* in NIMM-g1->KO vs NIMM-g1->WT mice. For mouse recipient groups, barplots

820 are average of 16S Seq data from n=7-18 mice/group. Data shown are cumulative (A) or representative

821 (B-C) of 2 independent experiments. n=15-16 (A), n=8-9 (B), n=7-8 (C) mice per group. Data are

822 expressed as geometric mean ± geometric SD (A-C). Statistical significance calculated by Mann-

823 Whitney test (A-C) or unpaired t-test (E-H) with *p<0.05, **p<0.01, ***p<0.001.

824

825 **Supplemental Information:**

826

827 **Supplemental File 1**: RMarkdown notebook of R Code Analysis and Jupyter Notebook of Python Code

828 Analysis. Also publicly available at https://github.com/anhmoss/Mouse-Adaptation-of-Human-

829 Inflammatory-Bowel-Disease-Microbiota-Enhances-Colonization-Efficiency.

830

Supplemental Experimental Procedures

Gene expression by qRT-PCR

Tissues were immediately placed in RNAprotect cell reagent to stabilize RNA. Total RNA extraction

from tissues (AllPrep PowerViral DNA/RNA Kit, Qiagen) and cDNA generation (iScript cDNA Synthesis

Kit, Bio-Rad) were performed according to the manufacturer's protocols. Quantitative RT-PCR was

performed on cDNA in duplicate or triplicate with a QuantStudio3 machine (ThermoFisher) using iTaq™

Universal SYBR Green Supermix (Bio-Rad). Target gene expression was quantified relative to internal

control b-actin and expressed using the comparative Ct method ($2^{-\Delta\Delta Ct}$). qPCR primer sequences are:

Tnfa-F 5'-ACCCTCACACTCAGATCATCTTCTC-3', Tnfa-R 5'-TGAGATCCATGCCGTTGG-3'. Actb-F

5'-AGCCATGTACGTAGCCATCCAG-3'; Actb-R 5'-TGGCGTGAGGGAGAGCATAG-3'

Intestine histopathology scoring

Small bowel and colon tissue sections were fixed in 10% phosphate-buffered formalin. Fixed tissue was

paraffin-embedded, sectioned at 5μm thickness, and stained with hematoxylin and eosin by the UNC

Center for Gastrointestinal Biology and Disease Histology Core. Histologic tissue inflammation was

quantified by blinded scoring as previously described on a scale of 0-4 for 5 tissue segments (terminal

ileum, cecum, proximal colon, distal colon, rectum)[1]. Total inflammatory score was the summation of

the 5 segments. Max segment score was the single highest score from the 5 segments.

Fecal lipocalin-2 quantification:

Fecal samples (10-30mg) were homogenized in PBS with 0.1% Tween 20 and incubated at 4°C

overnight, followed by centrifugation to pellet solid debris. Lipocalin-2 ELISA was performed on clear

fecal supernatant according to manufacturer's instructions (DY1857, R&D Systems)[2].

Microbial and Statistical Analyses

16S rRNA amplicon (variable regions 3-4) PCR and sequencing were performed by the UNC

Microbiome Core. Sequencing was performed on an Illumina MiSeq platform. Sequencing outputs were

855 converted to fastq format and demultiplexed using Illumina Bcl2Fastq 2.20.0. The resulting paired-end

856 reads were processed with the QIIME2 2022-2 wrapper for DADA2 including merging paired ends,

857 quality filtering, error correction, and chimera detection[3, 4]. Amplicon sequencing variants from DADA2

858 were assigned taxonomy with respect to the Silva databases, their sequences were aligned using

859 maFFT in QIIME2, and a phylogenetic tree was built with FastTree in QIIME2[5-7].

860

861 **Supplemental References:**

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Figure 1

A

Donor Input FMT		GF Mouse Recipient	Recipient Group Label	Recipient Microbiome
HM1	→	WT	HM1 -> WT	NIMM-g1
NIMM-g1	→	WT	NIMM-g1 -> WT	NIMM-g2
NIMM-g2	→	WT	NIMM-g2 -> WT	NIMM-g3
HM1	→	KO	HM1 -> KO	IMM-g1
IMM-g1	→	KO	IMM-g1 -> KO	IMM-g2
IMM-g2	→	KO	IMM-g2 -> KO	IMM-g3
HM2	→	KO	HM2 -> KO	~

B Total Histology Score (0-20)

C fecal lipocalin-2 (ng/g)

D TNFα Fold Expression Relative to Germ-Free

E

● HM1->KO   ■ IMM-g1->KO   ▲ IMM-g2->KO

Segment Histology Score (0-4): Terminal Ileum, Cecum, Proximal Colon, Distal Colon, Rectum

Total Histology Score (0-20): Total

Max Segment Histology Score (0-4): Max

	Terminal Ileum	Cecum	Proximal Colon	Distal Colon	Rectum		Total		Max
IQR	0.0 0.0 0.1	1.6 0.4 0.6	0.6 0.4 0.8	1.8 0.2 0.5	1.9 0.4 0.5	IQR	5.2 1.0 1.1	IQR	1.6 0.4 0.6
VAR	0.0 0.1 0.0	0.9 0.2 0.1	0.3 0.1 0.3	1.1 0.0 0.6	1.1 0.7 1.5	VAR	8.2 1.7 4.8	VAR	0.8 0.2 0.2

F

IMM-g1->KO

● Replicate 1   ■ Replicate 2   ▲ Replicate 3   ◆ Replicate 4

Segment Histology Score (0-4): Terminal Ileum, Cecum, Proximal Colon, Distal Colon, Rectum

Total Histology Score (0-20): Total

Max Segment Histology Score (0-4): Max

Figure S1, related to Figure 1

A

Human Donor ID	IBD Phenotype	Sex	Age (Decade)	Pooled Cohort	
1	CD	M	50	HM1	
2	UC	F	40		
3	CD	F	40		HM2
4	CD	M	40		
5	CD	F	60		

B

C

D

E

IQR	0.0 0.0	1.6 1.5	0.6 0.8	1.8 1.0	1.9 1.9
VAR	0.0 0.0	0.9 1.0	0.3 0.2	1.1 0.9	1.1 1.4

F

IQR	0.0 0.0	1.6 1.5	0.6 0.8	1.8 1.0	1.9 1.9
VAR	0.0 0.0	0.9 1.0	0.3 0.2	1.1 0.9	1.1 1.4

G

IQR	0.0 0.0 0.1	1.6 0.4 0.6	0.6 0.4 0.8	1.8 0.2 0.5	1.9 0.4 0.5
VAR	0.0 0.1 0.0	0.9 0.2 0.1	0.3 0.1 0.3	1.1 0.0 0.6	1.1 0.7 1.5

H

Figure 2

Legend:
- Other
- d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Hungatella
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia
- d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Escherichia−Shigella
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__[Ruminococcus]_gnavus_group
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnoclostridium
- d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia

X-axis categories: HM2input, HM2->KO, HM1input, HM1->KO, HM1->WT, IMM-g1input, IMM-g1->KO, IMM-g2input, IMM-g2->KO, NIMM-g1input, NIMM-g1->WT, NIMM-g2input, NIMM-g2->WT

Y-axis: Percentage (%)

Figure S2, related to Figure 2

A

Legend (A):
- Other
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;__
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Hungatella
- d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Escherichia-Shigella
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnoclostridium
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__[Ruminococcus]_gnavus_group
- d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia

B

Legend (B):
- Other
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Agathobacter
- d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Dielma
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Flavonifractor
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Faecalibacterium
- d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Collinsella
- d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae;g__Parasutterella
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Incertae_Sedis
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__[Ruminococcus]_torques_group
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Epulopiscium
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Sellimonas
- d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium
- d__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__UBA1819
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Anaerostipes
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Eisenbergiella
- d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelatoclostridiaceae;g__Erysipelatoclostridium
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Clostridioides
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_13
- d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__[Clostridium]_innocuum_group
- d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;__
- d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Hungatella
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia
- d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Escherichia-Shigella
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__[Ruminococcus]_gnavus_group
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnoclostridium
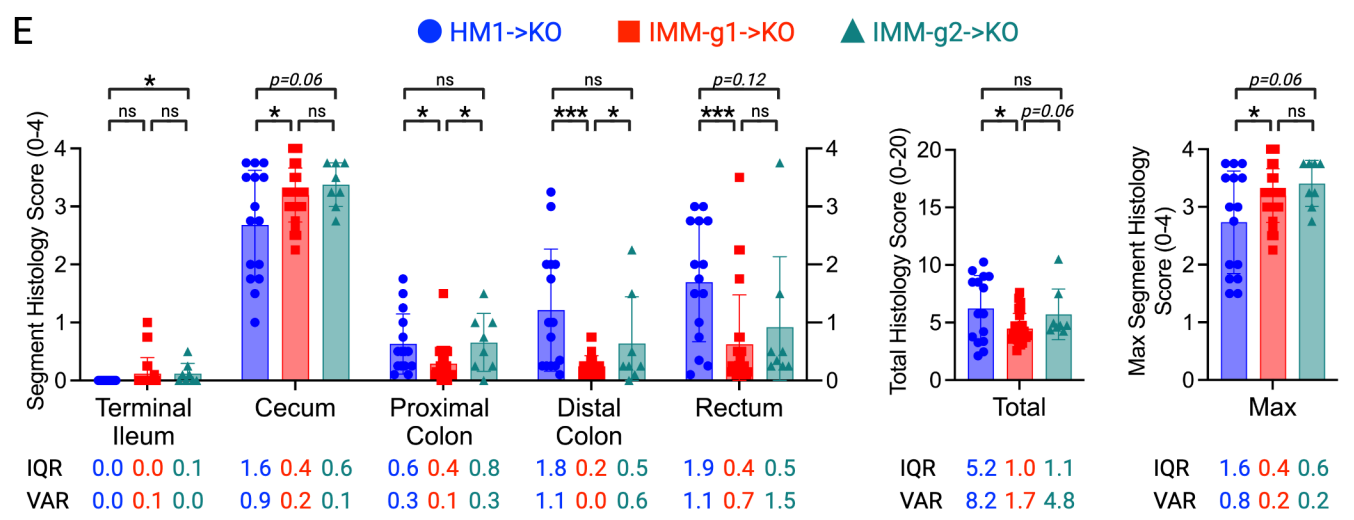- d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia

Figure 3

Figure S3, related to Figure 3

A — Inflamed Recipient Groups
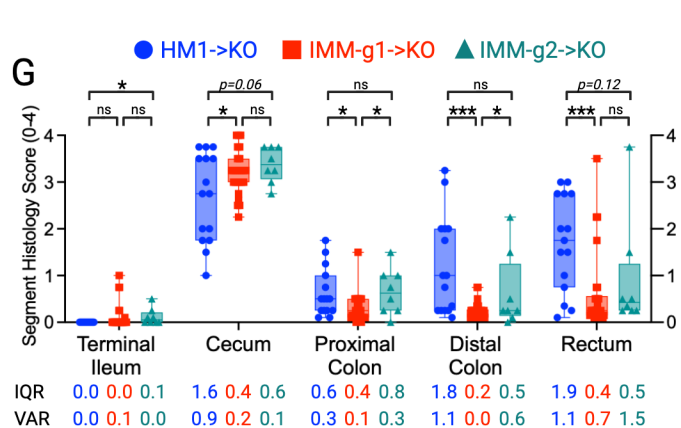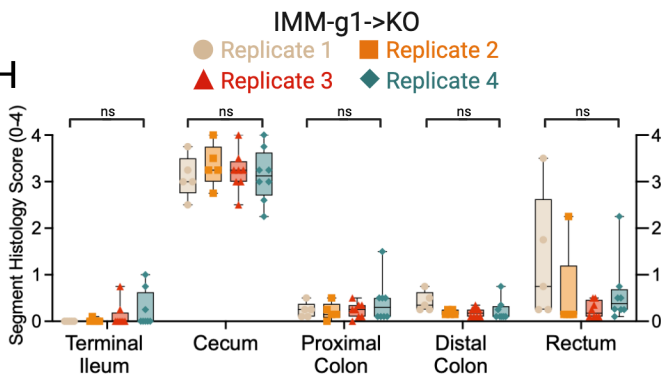
Legend:
- HM1→KO
- HM2→KO
- IMM−g1→KO
- IMM−g1input
- IMM−g2→KO
- IMM−g2input

MDS2 (16.5%) vs MDS1 (29.9%)

B — Genus-level Alpha Diversity

Shannon Diversity Index

Groups: HM1→WT, NIMM−g1→WT, NIMM−g2→WT, HM1→KO, IMM−g1→KO, IMM−g2→KO

C — ASV- and Genus-level Alpha Diversity

Shannon Diversity Index

Groups: HM1input (ASV), HM2input (ASV), HM1input (Genus), HM2input (Genus)
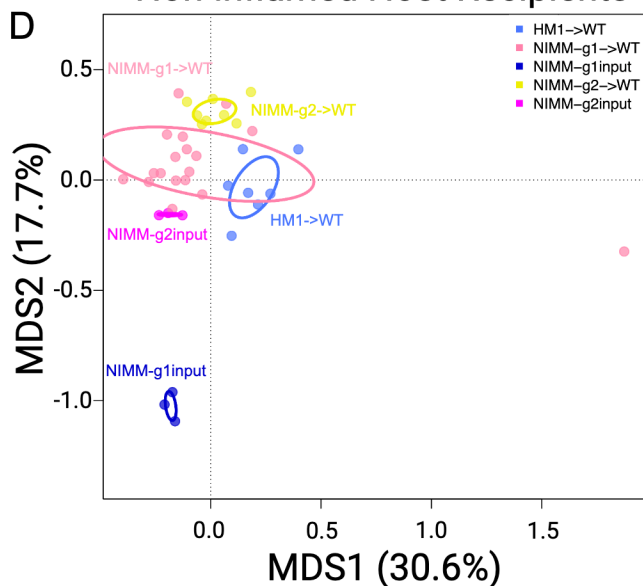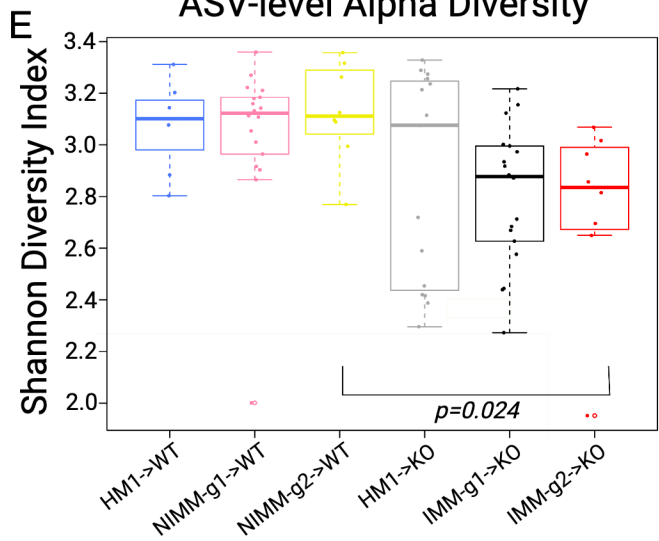
D — Microbiota correlation within group (Microbiota engraftment consistency)
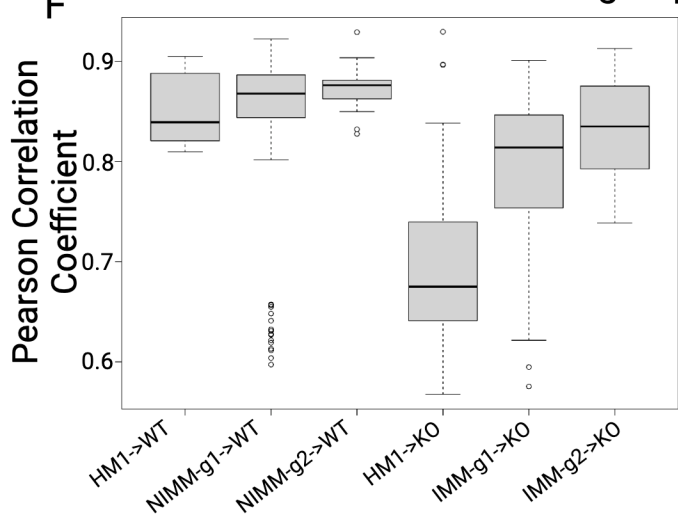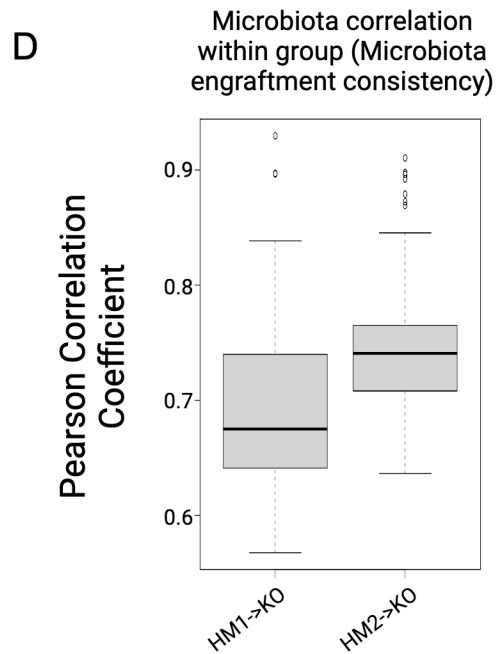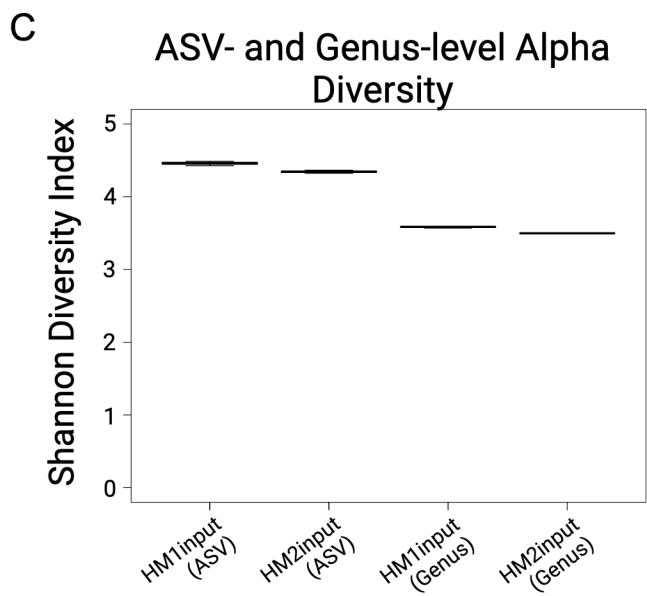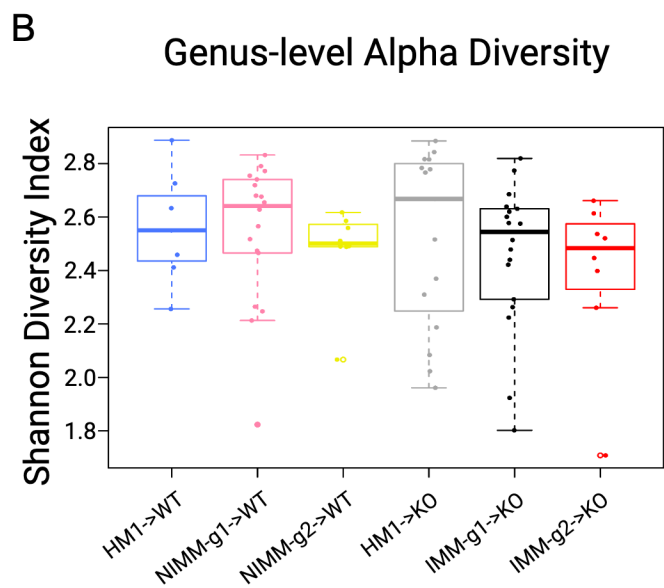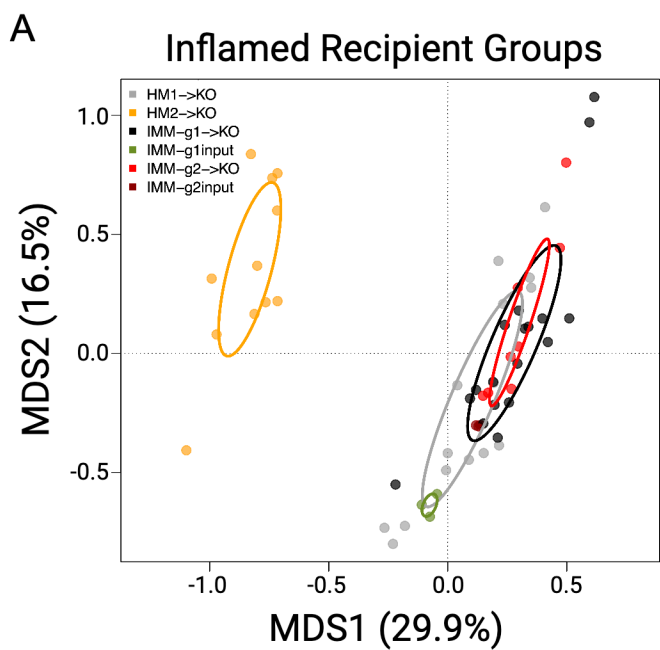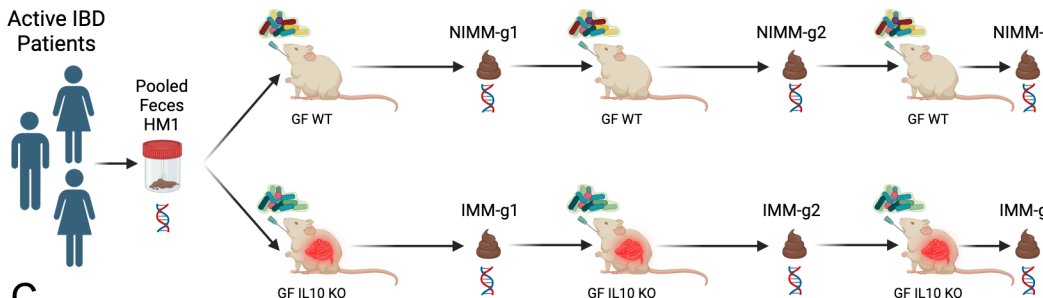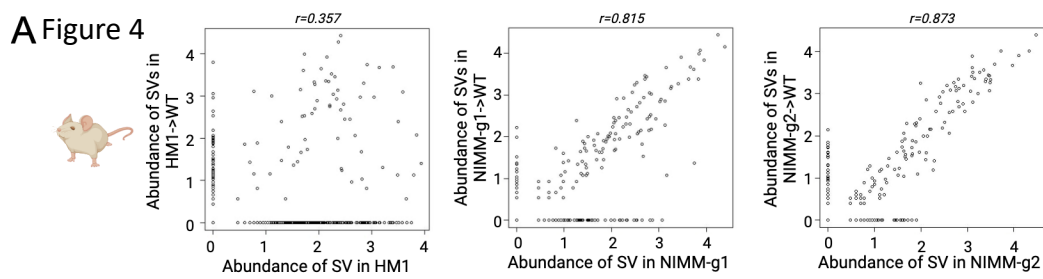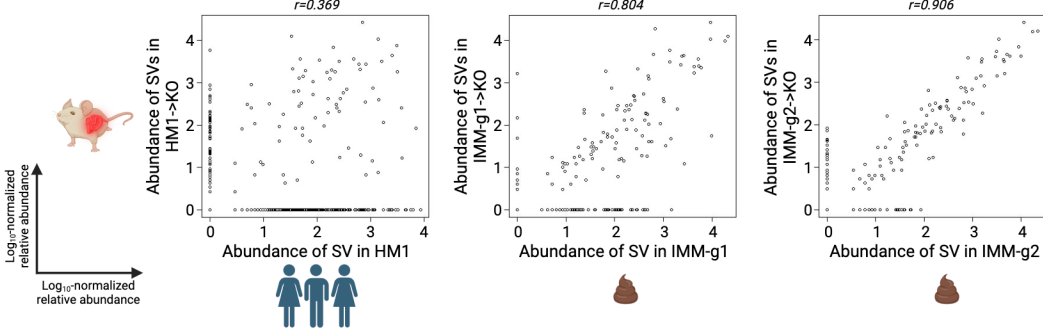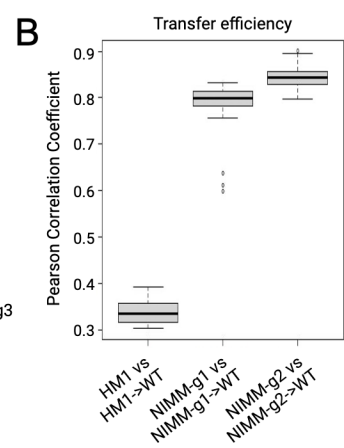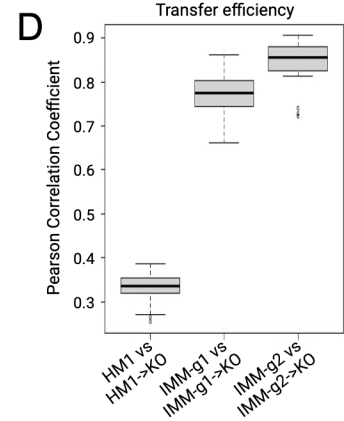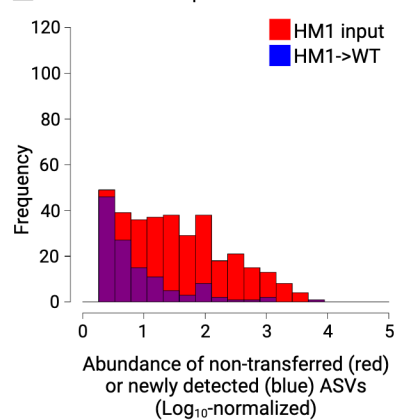
Pearson Correlation Coefficient

Groups: HM1→KO, HM2→KO
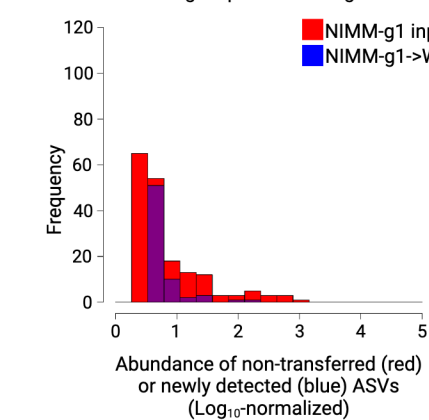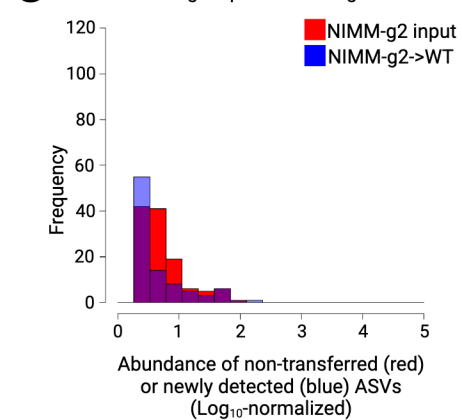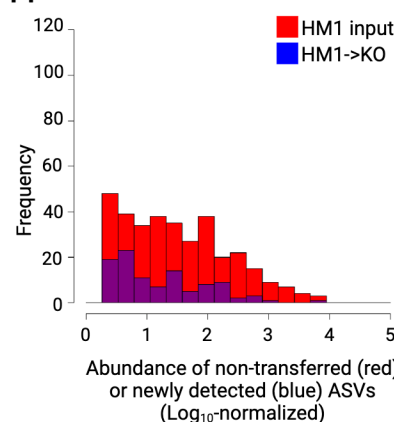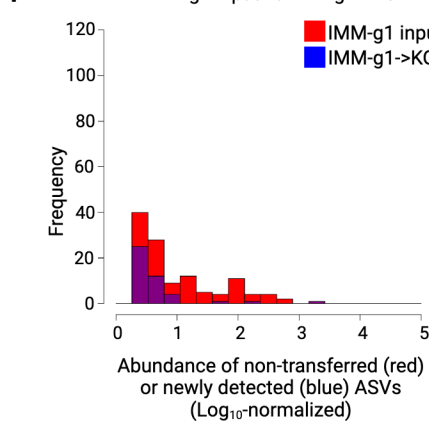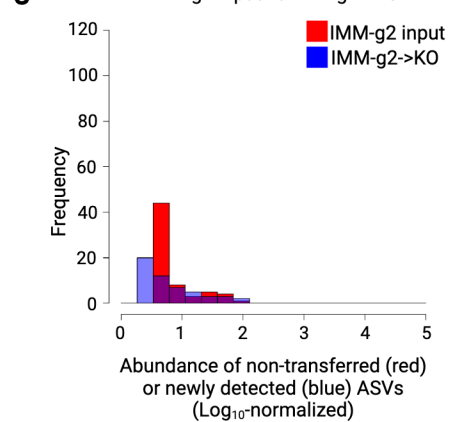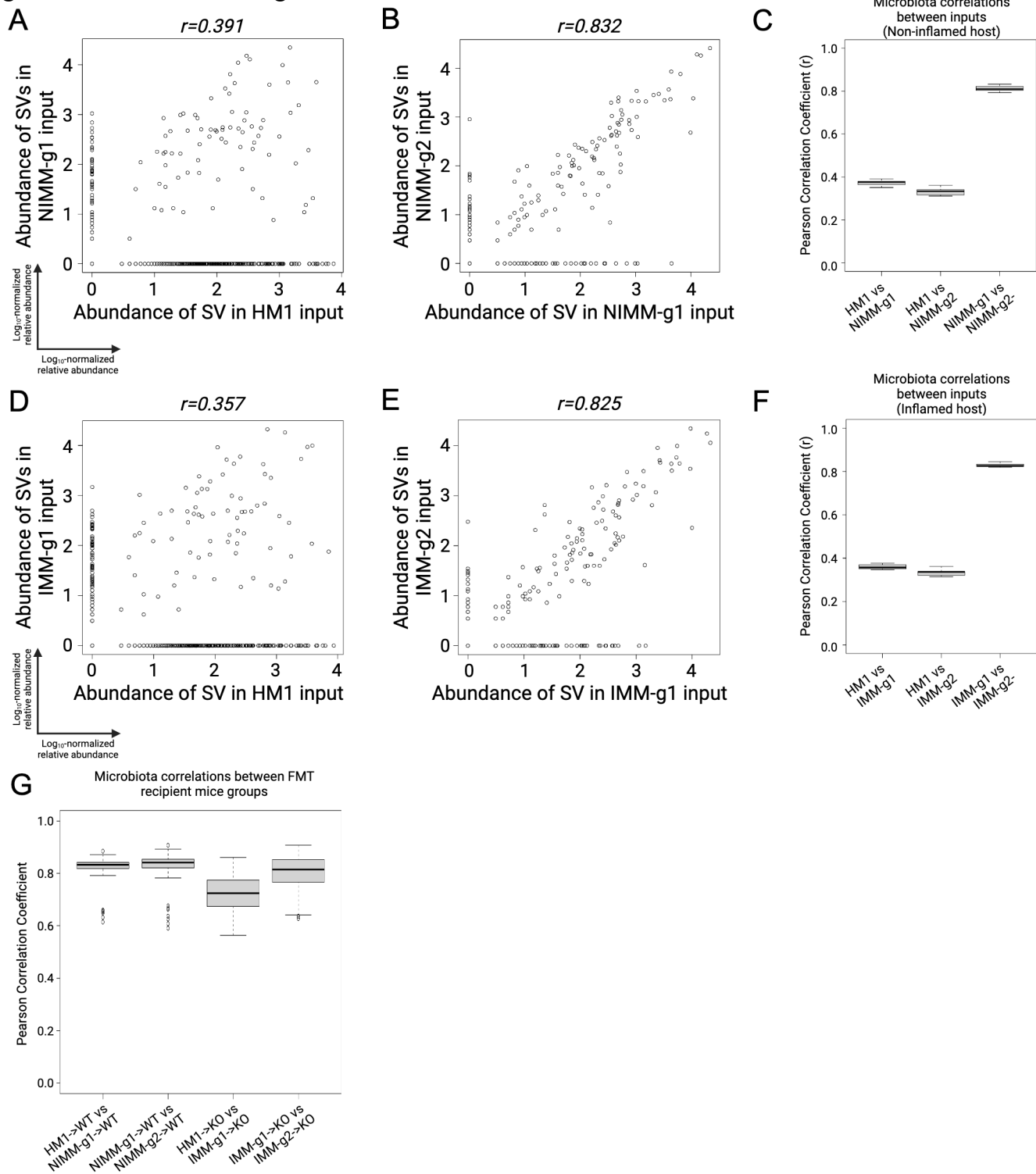
Figure 4

Figure S4, related to Figure 4
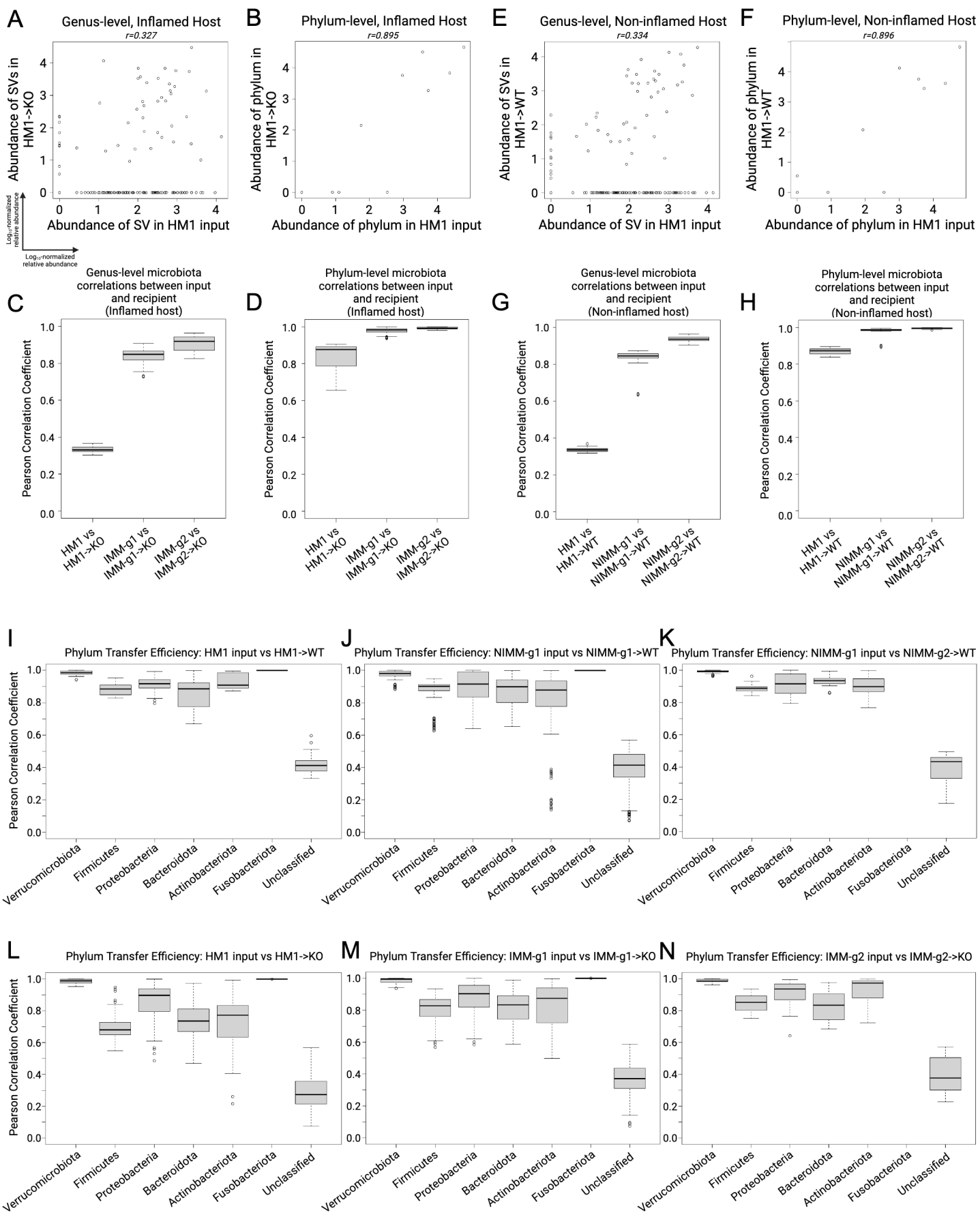
Figure 5

A

In Gnotobiotic Isolator

Healthy human

Feces

Germ-free recipient

Parent (P)

Natural colonization by vertical transmission

F1 pup

B

r=0.257

Abundance of SV in Parent (P)

Abundance of SV in Human Feces

Log₁₀-normalized relative abundance

r=0.791

Abundance of SV in F1

Abundance of SV in Parent (P)

C

Transfer efficiency

Pearson Correlation Coefficient

Human stool vs Parent (P)

Parent (P) vs F1 pup

D

Active IBD Patients

Pooled Feces HM2

Metagenomic Sequencing Timepoint

Experiment Performed in Gnotobiotic Isolator

GF IL10 KO

Day 28 HM2->KO Feces

E

Cage 1

r=0.21

Abundance of SV in HM2->KO

Abundance of SV in HM2 input

Log₁₀-normalized relative abundance

Cage 2

r=0.238

Abundance of SV in HM2->KO

Abundance of SV in HM2 input

F

Percentage OTU Shared Between Donor Input and Recipient Group

Donor Input FMT	Recipient Group	% Shared ASV OTU	% Shared Genus OTU	% Shared Phylum OTU
HM1	HM1->WT	14.95%	31.36%	68.18%
HM1	HM1->KO	14.53%	30.73%	65.20%
HM2	HM2->KO	18.69%	32.25%	73.20%
IMM-g1	IMM-g1->KO	47.19%	69.63%	96.30%
IMM-g2	IMM-g2->KO	52.04%	80.91%	100%
NIMM-g1	NIMM-g1->WT	44.97%	71.93%	92.59%
NIMM-g2	NIMM-g2->WT	44.76%	84.74%	100%

Figure 6

A — Active IBD Patients → Pooled Feces HM1 → HM1->WT (GF WT), NIMM-g1, NIMM-g1->KO (GF IL10 KO); HM1->KO (GF IL10 KO), IMM-g1, IMM-g1->KO (GF IL10 KO). Metagenomic Sequencing Timepoint

B day 14

IMM-g1->KO ● NIMM-g1->KO ■

Segment Histology Score (0-4): Terminal Ileum, Cecum (***), Proximal Colon (**), Distal Colon (ns), Rectum (ns)

Total Histology Score (0-20): Total (***)

C day 28

IMM-g1->KO ● NIMM-g1->KO ■

Segment Histology Score (0-4): Terminal Ileum (ns), Cecum (***), Proximal Colon (ns), Distal Colon (ns), Rectum (**)

Total Histology Score (0-20): Total (ns)

Max Segment Histology Score (0-4): Max (****)

D TNFα Fold Expression Relative to Germ-Free: GF, IMM-g1->KO (****), NIMM-g1->KO (p=0.08), (**)

E Inflamed Host Recipients

MDS2 (10%) vs MDS1 (42.5%)

IMM-g2->KO, HM1->WT, NIMM-g2->WT, IMM-g1->KO, NIMM-g1->WT, NIMM-g1->KO, HM1->KO

Legend: HM1->KO, HM1->WT, IMM-g1->KO, IMM-g2->KO, NIMM-g1->KO, NIMM-g1->WT, NIMM-g2->WT

F Percentage (%): HM1->KO, IMM-g1->KO, IMM-g2->KO, NIMM-g1->KO, HM1->WT, NIMM-g1->WT, NIMM-g2->WT

Legend:
- Other
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;__
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Hungatella
- d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Escherichia−Shigella
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__[Ruminococcus]_gnavus_group
- d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnoclostridium
- d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia

G ASV-level Alpha Diversity

Shannon Diversity Index: IMM-g1->WT, NIMM-g1->KO (P=0.16), NIMM-g1->WT (P=0.75), p=0.015

Figure S6

A day 14

f-Lcn (ng/g feces)

IMM-g1->KO   NIMM-g1->KO

p = 0.0012

B

● IMM-g1->KO   ■ NIMM-g1->KO

f-Lcn (ng/g feces)

ns   *p=0.14*   *   ns   *

Germ-free   day 3   day 7   day 11   day 14

C day 28

f-Lcn (ng/g feces)

IMM-g1->KO   NIMM-g1->KO

p = 0.0717

D

Percentage (%)

HM1->KO   IMM-g1->KO   IMM-g2->KO   NIMM-g1->KO   HM1->WT   NIMM-g1->WT   NIMM-g2->WT

■ Other
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__uncultured
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Monoglobales;f__Monoglobaceae;g__Monoglobus
■ d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__Eggerthella
■ d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Dielma
■ d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Collinsella
■ d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae;g__Parasutterella
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Incertae_Sedis
■ d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__[Ruminococcus]_torques_group
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Flavonifractor
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Sellimonas
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Epulopiscium
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Anaerostipes
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__UBA1819
■ d__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_13
■ d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelatoclostridiaceae;g__Erysipelatoclostridium
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Eisenbergiella
■ d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__[Clostridium]_innocuum_group
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales−Tissierellales;f__Peptostreptococcaceae;g__Clostridioides
■ d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Hungatella
■ d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Escherichia−Shigella
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__[Ruminococcus]_gnavus_group
■ d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnoclostridium
■ d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia

E *Blautia*

Relative Abundance (Log$_{10}$ Normalized)

**

NIMM-g1->KO   NIMM-g1->WT

F *Lachnospiraceae NK4A136*

Relative Abundance (Log$_{10}$ Normalized)

*

NIMM-g1->KO   NIMM-g1->WT

G *R gnavus* group

Relative Abundance (Log$_{10}$ Normalized)

***

NIMM-g1->KO   NIMM-g1->WT

H *Hungatella*

Relative Abundance (Log$_{10}$ Normalized)

*

NIMM-g1->KO   NIMM-g1->WT