



19 **Abstract**

20 Current evidence to support extensive use of probiotics in inflammatory bowel disease is  
21 limited and factors contribute to the inconsistent effectiveness of clinical probiotic therapy are  
22 not completely known. Here, as a proof-of-concept, we utilized *Bifidobacterium longum* JDM  
23 301, a widely used commercial probiotic strain in China, to study potential factors that may  
24 influence the beneficial effect of probiotics in experimental colitis. We found that the probiotic  
25 therapeutic effect was varied across individual mouse even with the same genetic background  
26 and consuming the same type of food. The different probiotic efficacy was highly correlated  
27 with different microbiome features in each mouse. Consumption of a diet rich in fat can change  
28 the host sensitivity to mucosal injury-induced colitis but did not change the host responsiveness  
29 to probiotic therapy. Finally, the host genetic factor TLR2 was required for a therapeutic effect  
30 of *B. longum* JDM 301. Together, our results suggest that personalized microbiome and genetic  
31 features may modify the probiotic therapeutic effect.

32

## 33 **Introduction**

34 It has long been recognized that the microbiota in the gut can impact many aspects of the host  
35 biology. Live microbes that confer health benefits to the host are often called probiotics.  
36 Consumption of probiotics in various forms like yogurt or other fermented dairy products, as  
37 dietary supplements and other functional foods, has become more and more popular. Probiotics  
38 are also claimed to have therapeutic benefits across a broad range of disorders including  
39 diseases in the gastrointestinal tract (1-3). Inflammatory bowel disease (IBD), including  
40 Crohn's disease (CD) and ulcerative colitis (UC), is a multi-factorial complex intestinal  
41 disorder with the highest prevalence in western countries (4-6). Probiotics are recommended by  
42 physicians as adjunctive therapy to treat IBD (7). Despite their popularity, the current evidence  
43 to support extensive use of probiotics in IBD is limited. Results from clinical trials are mixed,  
44 with some studies showing an improvement in maintenance of remission or induction of  
45 remission with probiotics while other trials have failed to show any benefit effect (8, 9). The  
46 reason behind the various outcomes of probiotic effectiveness in treating IBD is not clear.

47 It is now widely acknowledged that the gut microbiome together with the host genetic  
48 factors significantly contribute to the pathogenesis of IBD (10). Gut microbiota plays  
49 significant roles by preventing pathogen colonization (11), shaping the immune system (12,  
50 13), stimulating the production of gastrointestinal hormones (14), regulating brain behavior  
51 through the production of neuroactive substances and fermentation of non-digestible  
52 carbohydrates producing short chain fatty acids (SCFAs) (15, 16). Most recently, the  
53 microbiome is also emerging as contributing factor to interindividual variability in all aspects  
54 of a disease (17). However, whether the gut microbiota contributes to the person-to-person  
55 differences in response to probiotic therapy remains largely unknown.

56 *Bifidobacterium longum* JDM 301, isolated from healthy infants, is a widely used  
57 commercial probiotic strain in China (18). Our previous study demonstrated that *B. longum*  
58 JDM 301 can prevent *Clostridium difficile* infection (CDI) in mice (19). In the present study,  
59 we used *B. longum* JDM 301 as a proof-of-concept to test factors that could potentially  
60 influence the therapeutic effect of probiotics in experimental colitis. Our data demonstrate  
61 associations of the gut microbiome and host genetic factor to interindividual variability in  
62 probiotic biotherapeutic responses. Our results suggest personalized strategies are needed for  
63 the success of probiotics therapies.

64

## 65 **Materials and Methods**

### 66 **Animals**

67 5 to 6-week-old male C57Bl/6J mice were obtained from Shanghai Laboratory Animal  
68 Research Center, Shanghai or Beijing Vital River Laboratory Animal Technology Co., Ltd.,  
69 Beijing. *TLR2*<sup>-/-</sup> were originally purchased from Model Animal Research Center of Nanjing  
70 University and maintained under specific pathogen-free (SPF) conditions with a 12 hours light  
71 and 12 hours dark cycle and had free access to diet and drinking water. All animal procedures  
72 were done following the institutional guidelines and approved by the Animal Care and Use  
73 Committee of the University.

### 74 **Diet**

75 WT and *TLR2*<sup>-/-</sup> mice were fed with standard chow diet for the first week of arrival to the  
76 laboratory. Then the mice were randomly divided into two groups. One group of mice was fed  
77 with standard chow diet (ND) and the other group of mice was fed with high-fat diet (HFD),  
78 60% energy from fat (Ke Ao Xie Li Co. Ltd., Beijing). HFD feeding was continued for a total

79 of 6 weeks.

## 80 **Colitis induction**

81 Colitis was induced using DSS (Molecular Weight = 36,000~50,000) (MP Biomedicals, Santa  
82 Ana, CA, USA). For mice under ND group (5-8 mice), DSS treatment was started when the  
83 mice became 8 weeks of age. For mice under HFD group (5-8 mice), DSS induced colitis was  
84 started after 6 weeks of HFD feeding. Both groups were given 3% DSS (w/v) in drinking water  
85 for 7 days followed by 3 days of recovery period during which sterilized drinking water was  
86 supplied. The control group (3-5 mice) fed with either ND or HFD received only sterilized  
87 drinking water throughout the experiment.

## 88 **Preparation of *B. longum* JDM 301 for inoculation to mice**

89 The *B. longum* JDM 301 was originally isolated from a commercial probiotic product from  
90 China (18). The frozen glycerol stock of *B. longum* JDM 301 was thawed and then plated on  
91 MRS agar plate. The plate was incubated anaerobically overnight. The next day, a single  
92 bacterial colony was inoculated in a tube containing 3-5 ml of MRS broth and was incubated  
93 for 16-24 hours anaerobically. The total number of bacteria for each mouse used each time was  
94  $1 \times 10^9$  colony forming unit (cfu).

## 95 **Colonic Tissue Collection and Processing**

96 The colons tissues were collected and fixed in 4% (w/v) paraformaldehyde (pH 7.0),  
97 dehydrated by increasing concentrations of ethanol, and embedded in paraffin for histological  
98 studies.

## 99 **Histological Examination**

100 For histological grading three different parameters were considered, severity of inflammation  
101 (based on polymorphonuclear neutrophil infiltration; 0-3: none, slight, moderate, and severe),

102 depth of injury (0–3: none, mucosal, mucosal and submucosal, and transmural), and crypt  
103 damage (0–4: none, basal one-third damaged, basal two-thirds damaged, only surface  
104 epithelium intact, entire crypt, and epithelium lost). The histological score for each mouse was  
105 a sum of the score of neutrophil infiltration, depth of injury and crypt damage.

### 106 **Gut Microbiota Analysis**

107 Fresh fecal pellets were collected in a clean sterile eppendorf tube, immediately frozen into  
108 liquid nitrogen, and then stored at -80° C. DNA was isolated using E.Z.N.A. Stool DNA Kit  
109 (Omega Bio-Tek) according to the manufacturer’s instructions. Fecal DNA samples were  
110 amplified by PCR using barcoded primer pairs targeting the V3-V4 region of 16S rRNA gene.  
111 PCR amplicons were sequenced using Illumia Mi-Seq sequencer. Bioinformatic analysis was  
112 done by Vazyme Biotech Co., Ltd., Nanjing, China. Briefly, the resulting bacterial sequence  
113 fragments were first clustered into Operational Taxonomic Units (OTUs) and aligned to  
114 microbial genes with 97% sequence similarity. Bacterial taxa summarization and rarefaction  
115 analyses of microbial diversity or compositional differences (dissimilarity value indicated by  
116 Unweighted UniFrac Distance) were then calculated and PCoA plots indicating compositional  
117 difference were generated accordingly with the Vegan package in R software.

### 118 **Accession number**

119 The 16S rRNA sequencing data has been deposited in NCBI SRA database. The accession  
120 number is: SRP149682.

### 121 **Statistical Analysis**

122 The data are shown as mean values  $\pm$  standard error of the mean (SEM). Differences between  
123 multiple groups were compared using one-way ANOVA with post-hoc Turkey’s Multiple  
124 Comparison Test and two-way ANOVA with post-hoc Bonferroni posttests. A Student’s t-test

125 was used for comparisons between two groups. Mantel-Cox test was used for survival analysis.  
126 Wilcoxon Signed Rank Test and Kruskal-Wallis (KW) sum-rank test were used as significance  
127 test in microbiota analysis. A  $P$ -value  $< 0.05$  was considered significant.

128

129

## 130 **Results**

### 131 **The therapeutic effect of the probiotic *B. longum* JDM 301 in IBD is correlated with host** 132 **microbiota**

133 As a proof-of-concept, we used *Bifidobacterium longum* JDM 301, a widely used commercial  
134 probiotic strain in China, to test whether the therapeutic effect of probiotics is correlated to the  
135 host microbiota. For this purpose, we actively monitored the constituents of gut microbiota in  
136 our mouse colonies by 16S rRNA sequencing. We found two different batches of C57Bl/6 wild-  
137 type (WT) mice purchased from outside resources had significant differences in their gut  
138 microbial communities (Fig. 1A). We treated them with *B. longum* JDM 301 ( $1 \times 10^9$  cfu/mouse)  
139 for 3 alternate days via oral gavage, and then induced colitis by providing 3% dextran sulfate  
140 sodium (DSS) in the drinking water for 7 consecutive days and then switched back to normal  
141 water (Fig. 1B). In the absence of probiotics treatment, DSS alone induced about 15% body  
142 weight loss on day 10 in the mice from cohort A, whereas it induced around 30% body weight  
143 loss in the mice from cohort B (Fig 1C). Furthermore, without probiotics, all the mice in cohort  
144 A survived the intestinal injury-induced wasting disease; while 7 out of 8 mice in cohort B died  
145 within the experimental period. Thus, mice with different microbiota can have different  
146 sensitivity to gut epithelial injury-induced colitis. These results are consistent with the notion  
147 that the intestinal bacterial flora contributes to the immunopathogenesis of IBD (10).

148 After the mice were pretreated with *B. longum* JDM 301, the body weight loss was  
149 minimized and the colon shrinking was reduced in cohort A compared to those treated only  
150 with DSS, while mice in cohort B did not show any sign of colitis improvement with probiotic  
151 treatment (Fig 1C and 1D). Microscopically, colonic epithelial damage and inflammatory cell  
152 infiltration were reduced in cohort A mice that were treated with *B. longum* JDM 301, while  
153 severe epithelial damage and inflammation remained in cohort B mice (Fig 1E and 1F). The  
154 data implied that the host microbiota not only influences IBD pathogenesis, it also influences  
155 the therapeutic effect of probiotics.

156

157 **The therapeutic effect of the probiotic *B. longum* JDM 301 in IBD is not correlated with**  
158 **high-fat diet**

159 The host microbiota can be easily modified by food. Consumption of high-fat diet (HFD) is  
160 regarded as one of the risk factors of IBD and several studies demonstrated that HFD  
161 exacerbates DSS induced colitis in animals (20, 21). We were wondering whether the effect of  
162 probiotics can be modified by HFD. To test this possibility, 5 to 6-week-old C57Bl/6 male mice  
163 from cohort A and B were fed with HFD for 6 weeks before DSS treatment. 6 weeks later, we  
164 collected their fecal pellets, extracted bacterial DNA and performed high throughput 16S rRNA  
165 gene DNA sequencing. Based upon unweighted UniFrac principal coordinate analysis (PCoA),  
166 HFD more or less changed mice microbiota as expected, but differences in the bacterial flora  
167 between batch A and B still remained (Fig 2A). We then challenged the mice with DSS (Fig  
168 2B). In consistency with the previous report, the loss of body weight after DSS challenge was  
169 more pronounced in HFD-fed versus normal chow diet (ND)-fed mice, especially in those from  
170 cohort A. Half of the mice under HFD in cohort A died after DSS challenge (Fig 2D), while



171 none of them died under ND condition. The body weight loss and intestinal inflammation  
172 became similar between batch A and B mice (Fig 2C, 2E, and 2F), However, the mice from  
173 batch B were still more sensitive to DSS-induced wasting disease compared to mice from batch  
174 A under HFD condition (100% death rate in batch B versus 50% death rate in batch A) (Fig  
175 2D). Overall, these data confirmed that HFD exacerbates experimental IBD as described  
176 previously (22).

177 To examine whether changes in diet can result in alteration of the therapeutic effects of the  
178 probiotics, the different batches of mice that fed on HFD were orally gavaged with *B. longum*  
179 JDM 301 one week before DSS challenge. Similar to the results obtained under the ND  
180 condition, the therapeutic effect of *B. longum* JDM 301 still was different between cohort A and  
181 B in HFD-fed condition. The body weight loss after DSS challenge was significantly relieved  
182 in mice from cohort A but not cohort B when treated with probiotics (Fig 2C). The colonic  
183 inflammation also became less severe in cohort A but not cohort B (Fig 2E and 2F). The result  
184 indicated that although HFD can exacerbate colitis, it may not be able to determine the  
185 outcomes of probiotic therapeutic effect in IBD. It is likely that certain preformed postnatal  
186 microbial members that are not disturbed by high-fat diet control the probiotic effectiveness.

### 187 **Ecological characteristics of the gut microbiota that are correlated with *B. longum* JDM** 188 **301 efficacy**

189 To look for the ecological characteristics of the gut microbiota that are correlated with the  
190 probiotic's effectiveness, we compared the overall community configurations in probiotic-  
191 sensitive cohort A and probiotic-insensitive cohort B mice at both ND and HFD-fed conditions.  
192 Significant differences in both species richness represented by Chao1 index and species  
193 evenness represented by Shannon's index were observed between cohort A and cohort B mice

194 (Fig 3A). Both indices were bigger in cohort B mice compared to those in cohort A mice,  
195 irrespective of the diet used. HFD feeding reduced total species richness (Chao1) in both  
196 cohorts, but species evenness (Shannon index) was not disturbed by HFD (Fig 3A). At phyla  
197 level, cohort A mice had more Firmicutes, Actinobacteria, Saccharibacteria and less  
198 Bacteroidetes compared to cohort B at both ND or HFD conditions (Fig 3B). Shifting diet from  
199 ND to HFD resulted in increase of the phylum Proteobacteria in both cohorts (from 1 and 5%  
200 to 14%, respectively) (Fig 3B). This is consistent with the notion that Proteobacteria expansion  
201 is an indicator of colon epithelial dysfunction and correlates to the increase of sensitivity to  
202 DSS-induced colitis at HFD condition (23). However, Proteobacteria was not a good indicator  
203 for the *B. longum* JDM 301's efficacy, as the abundance of Proteobacteria were not different  
204 between cohort A and B at both ND or HFD condition (Fig 3B). Furthermore, 18 different  
205 genera were found to be consistently different between the two cohorts of mice irrespective of  
206 the diet used (Fig 3C). Among them, *Alistipes* and *Parabacteroides*, two genera that have been  
207 implied participating in IBD pathogenesis (24), were increased significantly in mice from  
208 cohort B fed with either ND or HFD.

209 ***B. longum* JDM 301 has limited ability to change the taxonomic composition of the gut**  
210 **microbiota**

211 The mechanism of how probiotics work remain largely unknown. One possibility is that the  
212 probiotics change the host bacterial flora. To determine if probiotic *B. longum* JDM 301 alters  
213 the microbiome, we performed high-throughput gene-sequencing analysis of 16S rRNA in fecal  
214 bacterial DNA isolated from probiotic untreated and treated WT mice from batch A one day  
215 before DSS challenge. We used rarefaction analysis to compare bacterial diversity within  
216 individual mice of a group ( $\alpha$  diversity) in both ND- and HFD-fed conditions. *B. longum* JDM

217 301 treatment did not change species richness (Chao1) (Fig 4A and 4B) and species evenness  
218 (Shannon index) significantly (Fig 4C and 4D). PCoA analysis of the microbiota composition  
219 in probiotic treated mice did not show a different community composition relative to that of  
220 probiotic untreated mice in both ND- and HFD-fed conditions (Fig 4E). Thus, the impact of *B.*  
221 *longum* JDM 301 on the taxonomic composition of the fecal microbiota was very limited.

## 222 **The therapeutic effect of the probiotic *B. longum* JDM 301 in IBD requires TLR2 signals**

223 Another possibility of how probiotics work in IBD pathogenesis is to engage the host cells,  
224 particularly the host immune system to maintain intestinal homeostasis. Toll-like receptors  
225 (TLRs) are critical host sensors for microbes. In a rat necrotizing enterocolitis model, the effect  
226 of *Bifidobacterium bifidum* to reduce mucosal injury and to preserve intestinal layer was  
227 reported to be through the TLR2 pathway (25). We posited that the effectiveness of the  
228 probiotic *B. longum* JDM 301 in treating IBD might also depend on TLR2 signals. To test this  
229 hypothesis, colitis was induced in *TLR2*<sup>-/-</sup> mice fed with either ND or HFD. In consistency with  
230 previous report that TLR2 plays critical role in maintaining gut epithelial homeostasis (26, 27),  
231 *TLR2*<sup>-/-</sup> mice raised in our facility also developed severe DSS-induced colitis indicated by  
232 severe body weight loss, high histologic scores, and 100% mortality rate in both ND- and HFD-  
233 fed conditions (Fig 5A-5D). To determine if probiotic therapy could ameliorate DSS-induced  
234 colitis in *TLR2*<sup>-/-</sup> mice, the mice were pretreated with *B. longum* JDM 301 and challenged with  
235 3% DSS. Unlike the WT mice in cohort A, *TLR2*<sup>-/-</sup> mice pretreated with *B. longum* JDM 301  
236 did not show improvement in body weight loss (Fig 5A). No difference was obtained on the  
237 survival rate with or without *B. longum* JDM 301 treatment (Fig 5B). All the *TLR2*<sup>-/-</sup> mice  
238 challenged by DSS died before the end of the experiment. Severe colonic inflammation  
239 evaluated by H&E stained samples remained the same with or without *B. longum* JDM 301

240 treatment (Fig 5C and 5D). The data imply a requirement of intact TLR2 signals in establishing  
241 the protective effect by *B. longum* JDM 301.

242

## 243 **Discussion**

244 In this work, we measured the ability of gut microbiota, high-fat diet and host genetic factor  
245 (e.g. TLR2) to influence the host response to a model probiotic, *B. longum* JDM 301, in a DSS-  
246 induced mouse colitis model. We demonstrated that the probiotic therapeutic effect can be  
247 varied across individual mouse even when the mice have the same genetic background and  
248 consume the same type of food. We further showed different microbiome features were highly  
249 correlated with different probiotic response. Consumption of diet rich in fat can change the host  
250 sensitivity to mucosal injury-induced colitis, but may not necessarily change the host  
251 responsiveness to probiotic therapy. Finally, the host genetic factor TLR2 was also required for  
252 a therapeutic effect of *B. longum* JDM 301.

253 Although probiotics are defined as beneficial microorganisms to the host, exact  
254 mechanisms of how probiotics function between the host and the gut microbiome remain  
255 incompletely understood. The bacterial species that can be called probiotics are still expanding  
256 (28), but whether one type of probiotic fits for all people at the same or different disease  
257 conditions is currently not clear. Our data suggested that the individual host gut microbiome  
258 can probably influence whether a given probiotic can have beneficial effects on the specific  
259 host or not. Possible pathways that have been suggested for how probiotics works include: (i)  
260 restoring microbial imbalances, (ii) enhancing the epithelial barrier function and/or (iii)  
261 modulating the immune responses (29). It remains to be determined which pathways can be  
262 modified by the host microbiome.

263       How personalized microbiome influence probiotic effect requires further investigation. One  
264 possible influence of the host microbiome is to influence the probiotic engraftment efficacy  
265 (30-32). Another possible influence of the microbiome to the probiotics is to influence their  
266 functions. One earlier study indicated that when the gut microbes translocated to the internal  
267 tissue, they can induce disease tolerance (33). Many other more possible mechanisms remain to  
268 be determined.

269       Our data further suggested that it might be possible to predict probiotic efficacy via analysis  
270 of the host microbial and genetic features. Personalized measurements including gut  
271 microbiome have been shown to be able to more accurately predict postprandial glycemic  
272 response for each unique person (34), it might also help for personalized probiotic therapies.

273       In aggregate, this study demonstrated correlation of individual host microbiome and  
274 genetics to the protective effects of probiotic therapy in colitis. Therefore, carefully monitoring  
275 personal and microbiome features might be needed for a success of probiotic therapy for IBD  
276 patients.

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287

288 **Author Contributions**

289 Conceived and designed the experiments: Y. Wang, K. Zheng; Performed the experiments: S.  
290 Suwal, Q. Wu, W. Liu, Q. Liu, H. Sun, M. Liang, J. Gao, Y. Kou, Z. Liu, Y. Wei; Analyzed the  
291 data: Y. Wang, S. Suwal; Wrote the paper: Y. Wang, S. Suwal.

292

293 **Conflict of Interest**

294 The authors have declared no conflict of interests.

295

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408 **Figure Legends**

409 **Figure 1. The therapeutic effect of the probiotic *B. longum* JDM 301 is correlated with**

410 **host microbiota. (A)** principal co-ordinate analysis (PCoA) based on OTU abundance of each

411 mouse via 16S rRNA sequencing, which indicates the overall microbiota similarities between

412 different groups. Each symbol represents one individual mouse. **(B)** Schematic diagram of

413 experimental design. **(C)** The body weight changes during DSS treatment. **(D)** Mean colon

414 length in cm. Colons were collected on day 11 post DSS initiation. **(E)** Representative images

415 of H&E stained distal colon tissues from indicated mice (magnification: 200x). **(F)** Histologic

416 scores. All data are given as means±SEMs. ns, no statistic significance; \* $P<0.05$ ; \*\*\* $P<0.001$ .

417

418 **Figure 2. The therapeutic effect of the probiotic *B. longum* JDM 301 is not correlated with**

419 **high-fat diet. (A)** PCoA analysis illustrating the presence of different microbial community

420 between HFD-fed mice from cohort A and B. Each symbol represents one individual mouse.

421 **(B)** Schematic diagram for experimental design. **(C)** Body weight changes during DSS

422 treatment in indicated mice. **(D)** Survival curve. **(E)** Representative images of H&E stained

423 distal colon tissues from indicated mice (magnification: 200x). **(F)** Histologic scores. All data

424 are given as means±SEMs. \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$ . The number of mice per group

425 was 3~6.

426

427 **Figure 3. Ecological characteristics of the gut microbiota that are correlated with *B.***

428 ***longum* JDM 301 efficacy. (A)**  $\alpha$ -diversity indicated by Chao1 (species richness) and Shannon

429 index (species evenness). The line drawn in the middle of the box represents the median value

430 and the box represents the range of values. **(B)** Taxonomic composition at the phyla level in the

431 indicated mice. **(C)** Taxonomic composition at genera level in the indicated mice under ND and  
432 HFD. The top 18 genera that were significantly different ( $P<0.05$ ) between the two cohorts in  
433 both ND and HFD conditions were shown. \* $P<0.05$ ; \*\* $P<0.01$ .

434

435 **Figure 4. *B. longum* JDM 301 has limited ability to change the taxonomic composition of**  
436 **the gut microbiota. (A to D)** High-throughput sequencing of 16S rRNA in fecal bacterial DNA  
437 from WT mice in batch A fed with ND or HFD. Chao1, indicative of bacterial species richness  
438 (A and B), Shannon, indicative of bacterial species evenness (C and D). **(E)** PCoA analysis of  
439 the microbiota composition in indicated mice. Each symbol represents one individual mouse.

440

441 **Figure 5. The therapeutic effect of the probiotic *B. longum* JDM 301 in IBD requires**  
442 **TLR2 signals. (A)** Body weight changes during DSS treatment in *TLR2*<sup>-/-</sup> mice that feed on  
443 ND or HFD. **(B)** Survival curve. **(C)** Representative images of H&E stained distal colon tissues  
444 from indicated mice (magnification: 200x. **(D)** Histologic scores. All data are given as  
445 means±SEMs. \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$ . The number of mice per group was 3~6.

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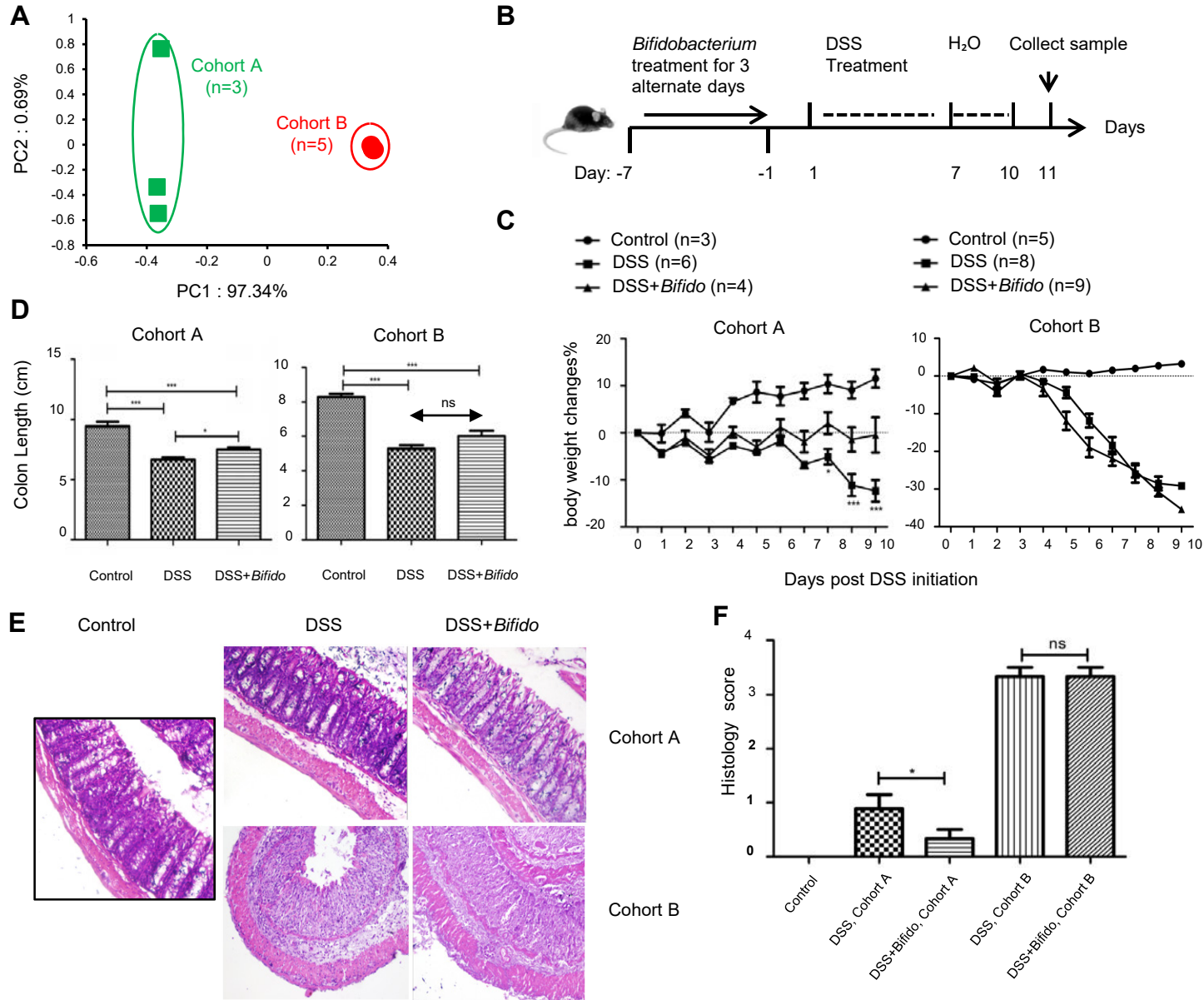


Fig 1

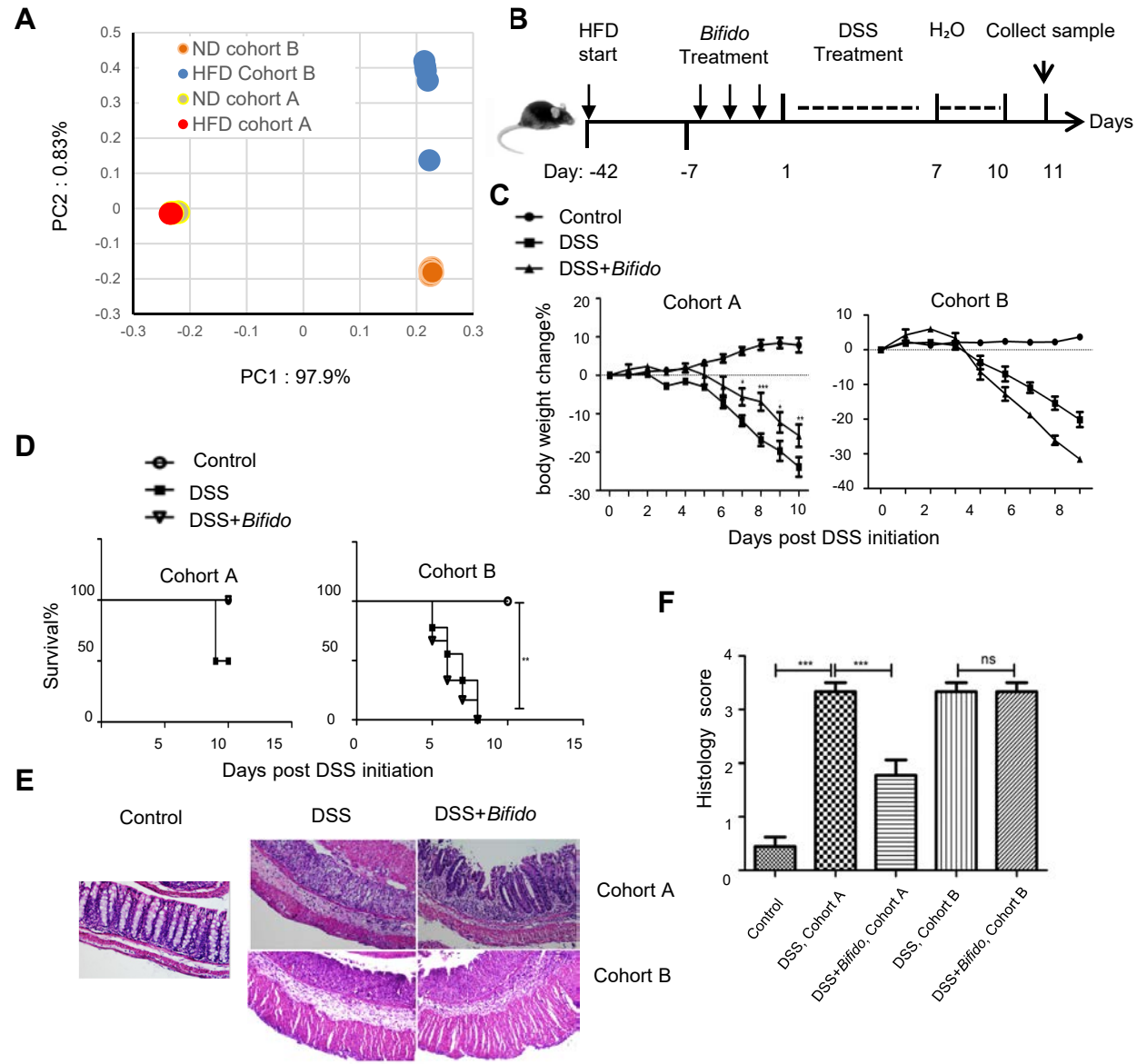


Fig 2

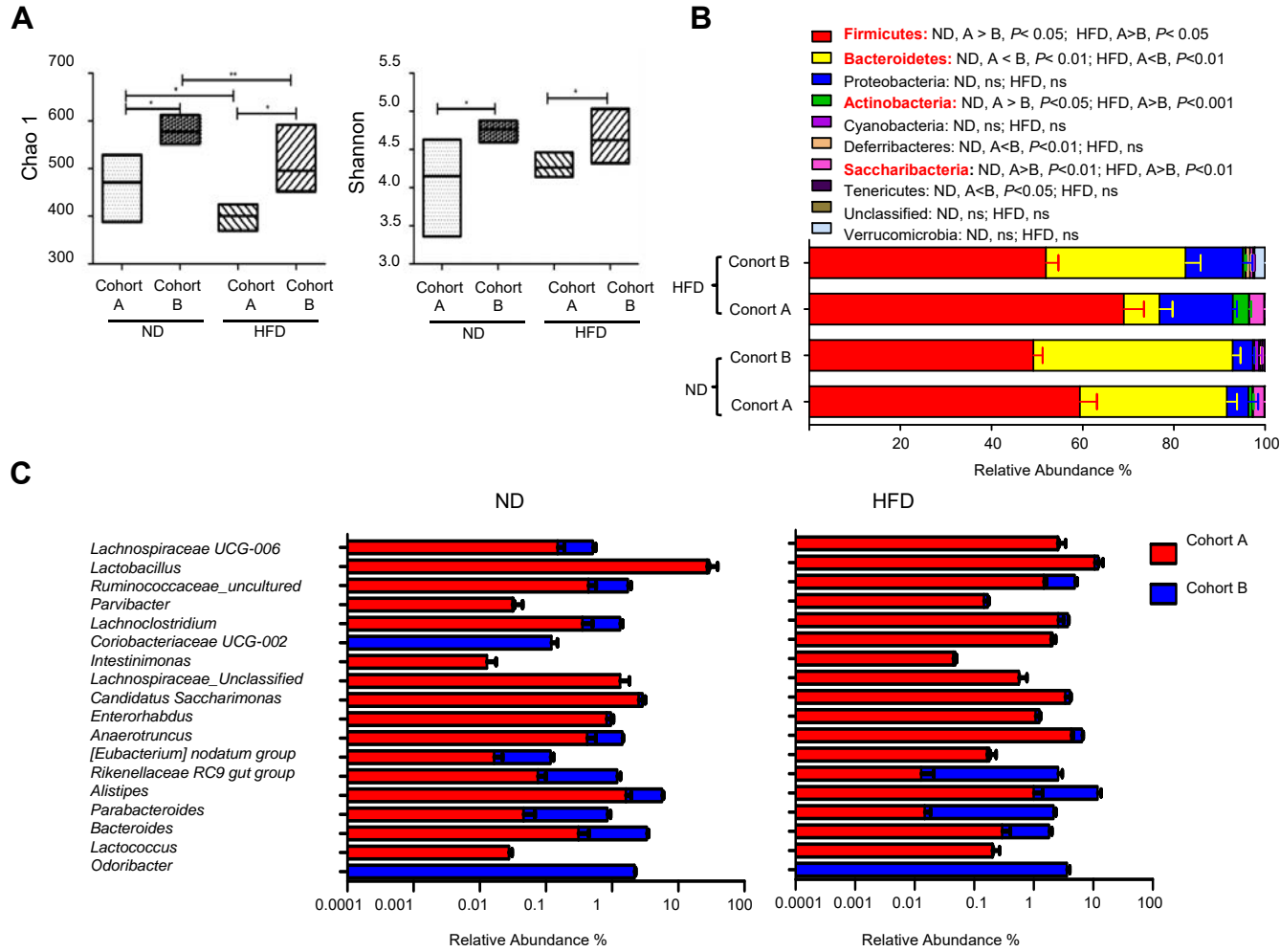


Fig 3



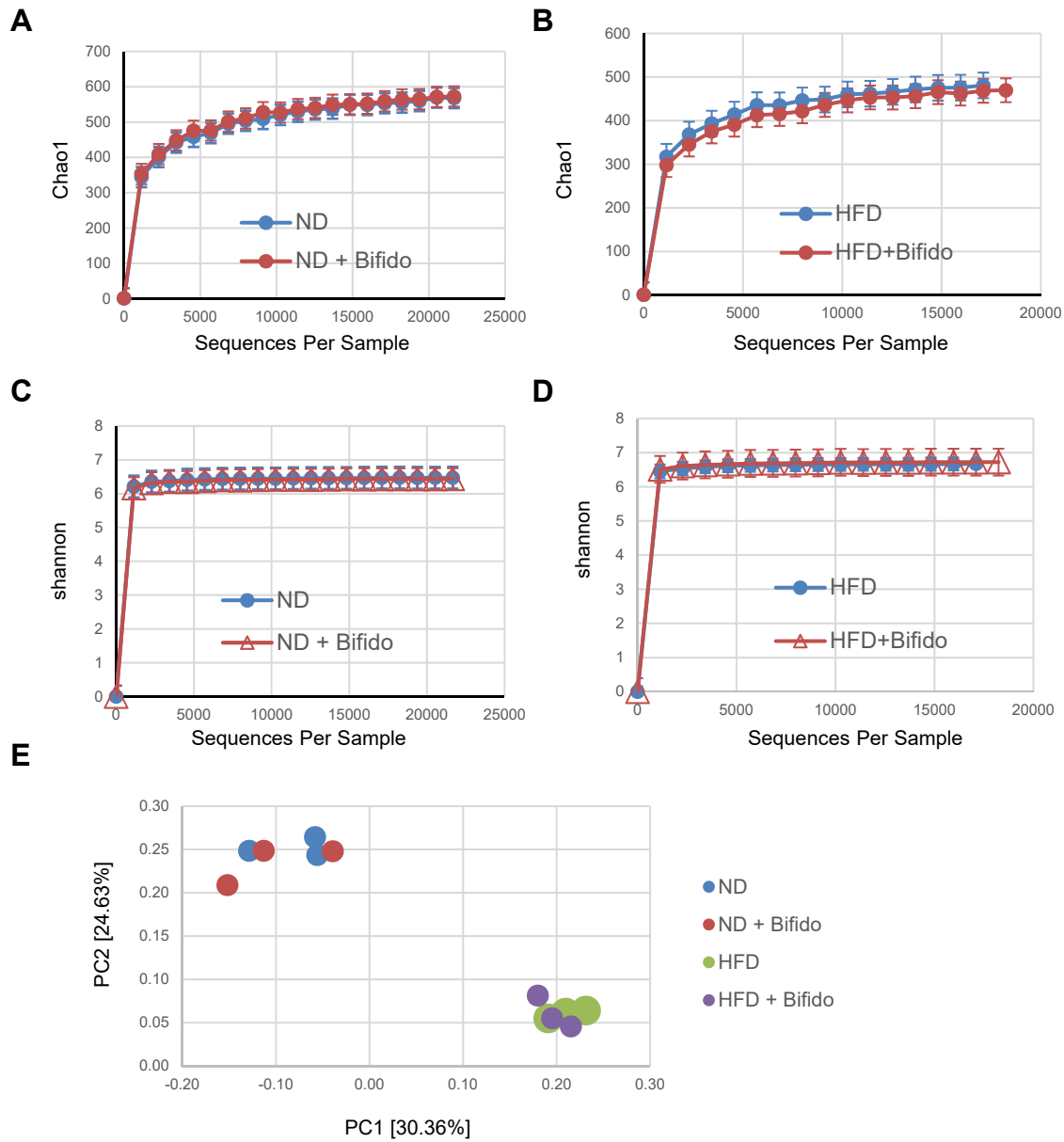


Fig 4

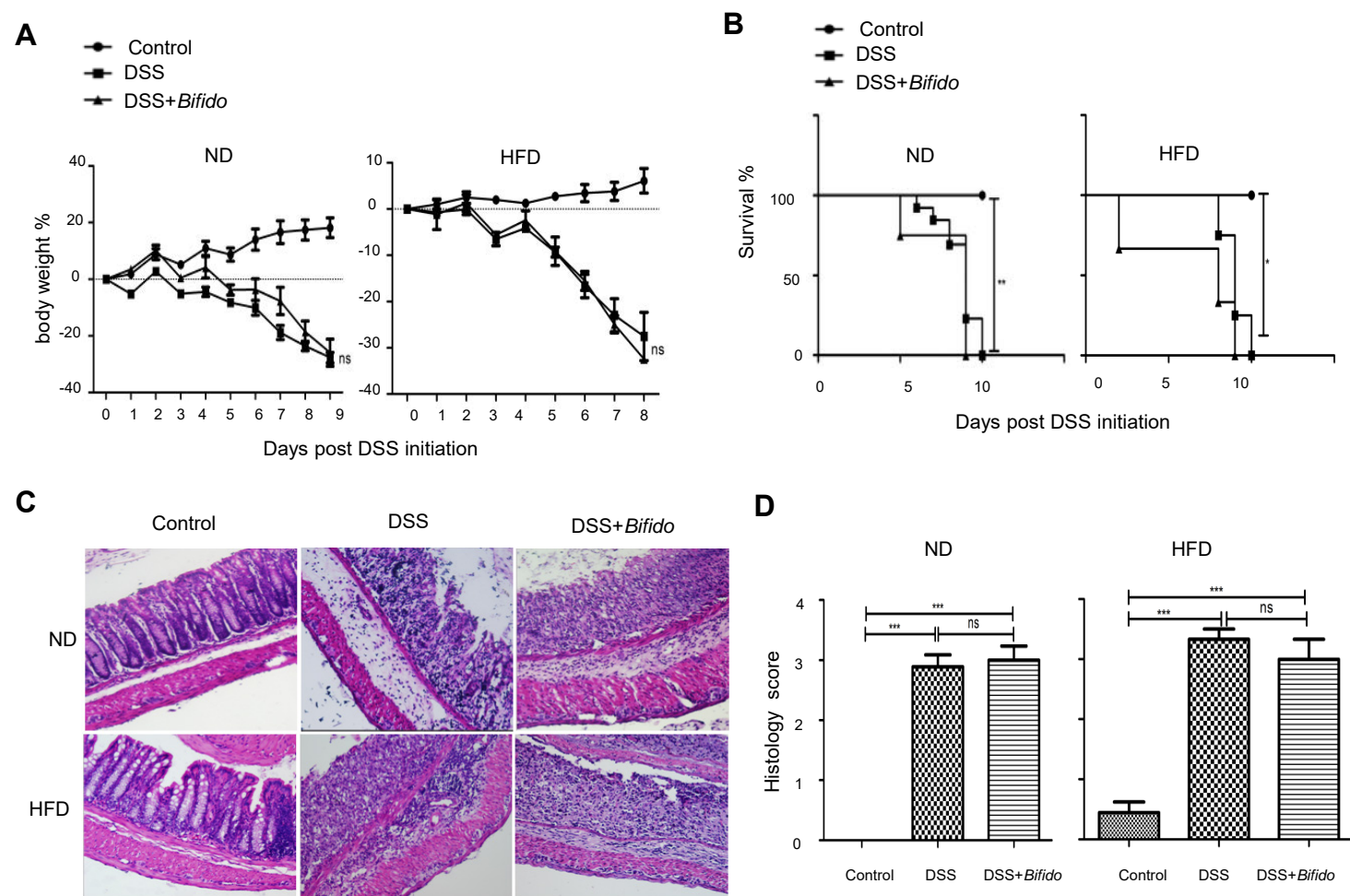


Fig 5