bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

1 # The probiotic effectiveness in experimental colitis is correlated with gut
2 microbiome and host genetic features

3 **Running Title: Probiotics & personalized microbiome**

4 Sharmila Suwal, Qiong Wu, Wenli Liu, Qingya Liu, Hongxiang Sun, Ming Liang, Jing Gao, Bo
5 Zhang, Yanbo Kou, Zhuanzhuan Liu, Yanxia Wei, Yugang Wang*, Kuiyang Zheng*

6 2106906416@qq.com, 582879117@qq.com, 1057341346@qq.com, 991262992@qq.com, 345793901@qq.com,
7 879311431@qq.com, 784974882@qq.com, 1262179198@qq.com, 330624083@qq.com, 109014180@qq.com,
8 470368479@qq.com, wang_yg@hotmail.com, ZKY02@163.com

9

10 Jiangsu Key Laboratory of Immunity and Metabolism, Laboratory of Infection and Immunity,
11 Department of Pathogenic Biology and Immunology, Xuzhou Medical University, Xuzhou,
12 Jiangsu Province, People's Republic of China

13

14 ***Correspondence:** Yugang Wang (wang_yg@hotmail.com), or Kuiyang Zheng
15 (ZKY02@163.com), Xuzhou Medical University, Tongshan Road #209, Xuzhou, Jiangsu
16 Province, P.R. China, 221004. Phone/FAX: 86-516-83262043

17

18

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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.

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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*$^{-/-}$ 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*$^{-/-}$ 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

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 1×10^9 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),

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 ± 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

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 constitutes 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 (1×10^9 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).

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 (α diversity) in both ND- and HFD-fed conditions. *B. longum* JDM

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 301on 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*$^{-/-}$ 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*$^{-/-}$ 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*$^{-/-}$ mice, the mice were pretreated with *B. longum* JDM 301 and challenged with
235 3% DSS. Unlike the WT mice in cohort A, *TLR2*$^{-/-}$ 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*$^{-/-}$ 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

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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.

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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.

277

278

279

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

280 **ACKNOWLEDGEMENTS**

281 Project support was provided in part by the National Natural Science Foundation of China (Grant
282 Nos. 81770853 to Y.W.), the Priority Academic Program Development of Jiangsu Higher
283 Education Institutions (PAPD) in the year of 2014 (Grant No. KYLX14-1448), Natural Science
284 Foundation of the Higher Education Institutions of Jiangsu Province, China (Grant Nos
285 16KJB310016), the Starting Foundation for Talents of Xuzhou Medical University (Grant No.
286 D2016029 to Y.W.).

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

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

296 # REFERENCES

297 1. **Jiang Y, Yang G, Meng F, Yang W, Hu J, Ye L, Shi C, Wang C.** 2016. Immunological
298 mechanisms involved in probiotic-mediated protection against Citrobacter rodentium-
299 induced colitis. Beneficial microbes **7:**397-407.
300 2. **Kim MS, Byun JS, Yoon YS, Yum DY, Chung MJ, Lee JC.** 2017. A probiotic
301 combination attenuates experimental colitis through inhibition of innate cytokine
302 production. Beneficial microbes **8:**231-241.
303 3. **Martin R, Miquel S, Chain F, Natividad JM, Jury J, Lu J, Sokol H, Theodorou V,**
304 **Bercik P, Verdu EF, Langella P, Bermudez-Humaran LG.** 2015. Faecalibacterium
305 prausnitzii prevents physiological damages in a chronic low-grade inflammation murine
306 model. BMC microbiology **15:**67.
307 4. **Leone VA, Cham CM, Chang EB.** 2014. Diet, gut microbes, and genetics in immune
308 function: can we leverage our current knowledge to achieve better outcomes in
309 inflammatory bowel diseases? Current opinion in immunology **31:**16-23.
310 5. **Ruemmele FM.** 2016. Role of Diet in Inflammatory Bowel Disease. Annals of nutrition
311 & metabolism **68 Suppl 1:**33-41.
312 6. **Statovci D, Aguilera M, MacSharry J, Melgar S.** 2017. The Impact of Western Diet
313 and Nutrients on the Microbiota and Immune Response at Mucosal Interfaces. Frontiers
314 in immunology **8:**838.
315 7. **Cheifetz AS, Gianotti R, Luber R, Gibson PR.** 2017. Complementary and Alternative
316 Medicines Used by Patients With Inflammatory Bowel Diseases. Gastroenterology
317 **152:**415-429 e415.
318 8. **Derwa Y, Gracie DJ, Hamlin PJ, Ford AC.** 2017. Systematic review with meta-

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

319 analysis: the efficacy of probiotics in inflammatory bowel disease. Alimentary
320 pharmacology & therapeutics **46:**389-400.
321 9. **Yamamoto T, Shimoyama T, Kuriyama M.** 2017. Dietary and enteral interventions
322 for Crohn's disease. Current opinion in biotechnology **44:**69-73.
323 10. **Kaser A, Zeissig S, Blumberg RS.** 2010. Inflammatory bowel disease. Annual review
324 of immunology **28:**573-621.
325 11. **Hand TW.** 2016. The Role of the Microbiota in Shaping Infectious Immunity. Trends in
326 immunology **37:**647-658.
327 12. **Round JL, Mazmanian SK.** 2009. The gut microbiota shapes intestinal immune
328 responses during health and disease. Nature reviews. Immunology **9:**313-323.
329 13. **Macpherson AJ, de Aguero MG, Ganal-Vonarburg SC.** 2017. How nutrition and the
330 maternal microbiota shape the neonatal immune system. Nature reviews. Immunology
331 **17:**508-517.
332 14. **Saulnier DM, Ringel Y, Heyman MB, Foster JA, Bercik P, Shulman RJ, Versalovic
333 J, Verdu EF, Dinan TG, Hecht G, Guarner F.** 2013. The intestinal microbiome,
334 probiotics and prebiotics in neurogastroenterology. Gut microbes **4:**17-27.
335 15. **Dinan TG, Stanton C, Cryan JF.** 2013. Psychobiotics: a novel class of psychotropic.
336 Biological psychiatry **74:**720-726.
337 16. **Groen RN, de Clercq NC, Nieuwdorp M, Hoenders HJR, Groen AK.** 2018. Gut
338 microbiota, metabolism and psychopathology: A critical review and novel perspectives.
339 Critical reviews in clinical laboratory sciences **55:**283-293.
340 17. **Kashyap PC, Chia N, Nelson H, Segal E, Elinav E.** 2017. Microbiome at the Frontier
341 of Personalized Medicine. Mayo Clinic proceedings **92:**1855-1864.

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

342 18. **Wei YX, Zhang ZY, Liu C, Zhu YZ, Zhu YQ, Zheng H, Zhao GP, Wang S, Guo**
343 **XK.** 2010. Complete genome sequence of Bifidobacterium longum JDM301. Journal of
344 bacteriology **192:**4076-4077.

345 19. **Wei Y, Yang F, Wu Q, Gao J, Liu W, Liu C, Guo X, Suwal S, Kou Y, Zhang B,**
346 **Wang Y, Zheng K, Tang R.** 2018. Protective Effects of Bifidobacterial Strains Against
347 Toxigenic Clostridium difficile. Frontiers in microbiology **9**.

348 20. **Cheng L, Jin H, Qiang Y, Wu S, Yan C, Han M, Xiao T, Yan N, An H, Zhou X,**
349 **Shao Q, Xia S.** 2016. High fat diet exacerbates dextran sulfate sodium induced colitis
350 through disturbing mucosal dendritic cell homeostasis. International
351 immunopharmacology **40:**1-10.

352 21. **Park SY, Kim JS, Seo YR, Sung MK.** 2012. Effects of diet-induced obesity on colitis-
353 associated colon tumor formation in A/J mice. International journal of obesity **36:**273-
354 280.

355 22. **Thaiss CA, Levy M, Grosheva I, Zheng D, Soffer E, Blacher E, Braverman S,**
356 **Tengeler AC, Barak O, Elazar M, Ben-Zeev R, Lehavi-Regev D, Katz MN,**
357 **Pevsner-Fischer M, Gertler A, Halpern Z, Harmelin A, Aamar S, Serradas P,**
358 **Grosfeld A, Shapiro H, Geiger B, Elinav E.** 2018. Hyperglycemia drives intestinal
359 barrier dysfunction and risk for enteric infection. Science **359:**1376-1383.

360 23. **Litvak Y, Byndloss MX, Tsolis RM, Baumler AJ.** 2017. Dysbiotic Proteobacteria
361 expansion: a microbial signature of epithelial dysfunction. Current opinion in
362 microbiology **39:**1-6.

363 24. **Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, Zhang D, Xia H, Xu X, Jie Z,**
364 **Su L, Li X, Li J, Xiao L, Huber-Schonauer U, Niederseer D, Al-Aama JY, Yang H,**

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

365 **Wang J, Kristiansen K, Arumugam M, Tilg H, Datz C.** 2015. Gut microbiome
366 development along the colorectal adenoma-carcinoma sequence. Nature
367 communications **6:**6528.
368 25. **Khailova L, Mount Patrick SK, Arganbright KM, Halpern MD, Kinouchi T,**
369 **Dvorak B.** 2010. Bifidobacterium bifidum reduces apoptosis in the intestinal epithelium
370 in necrotizing enterocolitis. American journal of physiology. Gastrointestinal and liver
371 physiology **299:**G1118-1127.
372 26. **Lowe EL, Crother TR, Rabizadeh S, Hu B, Wang H, Chen S, Shimada K, Wong**
373 **MH, Michelsen KS, Arditi M.** 2010. Toll-like receptor 2 signaling protects mice from
374 tumor development in a mouse model of colitis-induced cancer. PloS one **5:**e13027.
375 27. **Brun P, Giron MC, Qesari M, Porzionato A, Caputi V, Zoppellaro C, Banzato S,**
376 **Grillo AR, Spagnol L, De Caro R, Pizzuti D, Barbieri V, Rosato A, Sturniolo GC,**
377 **Martines D, Zaninotto G, Palu G, Castagliuolo I.** 2013. Toll-like receptor 2 regulates
378 intestinal inflammation by controlling integrity of the enteric nervous system.
379 Gastroenterology **145:**1323-1333.
380 28. **El Hage R, Hernandez-Sanabria E, Van de Wiele T.** 2017. Emerging Trends in
381 "Smart Probiotics": Functional Consideration for the Development of Novel Health and
382 Industrial Applications. Frontiers in microbiology **8:**1889.
383 29. **Abraham BP, Quigley EMM.** 2017. Probiotics in Inflammatory Bowel Disease.
384 Gastroenterology clinics of North America **46:**769-782.
385 30. **Maldonado-Gomez MX, Martinez I, Bottacini F, O'Callaghan A, Ventura M, van**
386 **Sinderen D, Hillmann B, Vangay P, Knights D, Hutkins RW, Walter J.** 2016. Stable
387 Engraftment of Bifidobacterium longum AH1206 in the Human Gut Depends on

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

388 Individualized Features of the Resident Microbiome. Cell host & microbe **20:**515-526.

389 31. **Lee SM, Donaldson GP, Mikulski Z, Boyajian S, Ley K, Mazmanian SK.** 2013.
390 Bacterial colonization factors control specificity and stability of the gut microbiota.
391 Nature **501:**426-429.

392 32. **Stecher B, Chaffron S, Kappeli R, Hapfelmeier S, Freedrich S, Weber TC, Kirundi**
393 **J, Suar M, McCoy KD, von Mering C, Macpherson AJ, Hardt WD.** 2010. Like will
394 to like: abundances of closely related species can predict susceptibility to intestinal
395 colonization by pathogenic and commensal bacteria. PLoS pathogens **6:**e1000711.

396 33. **Schieber AM, Lee YM, Chang MW, Leblanc M, Collins B, Downes M, Evans RM,**
397 **Ayres JS.** 2015. Disease tolerance mediated by microbiome E. coli involves
398 inflammasome and IGF-1 signaling. Science **350:**558-563.

399 34. **Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O,**
400 **Lador D, Avnit-Sagi T, Lotan-Pompan M, Suez J, Mahdi JA, Matot E, Malka G,**
401 **Kosower N, Rein M, Zilberman-Schapira G, Dohnalova L, Pevsner-Fischer M,**
402 **Bikovsky R, Halpern Z, Elinav E, Segal E.** 2015. Personalized Nutrition by
403 Prediction of Glycemic Responses. Cell **163:**1079-1094.

404

405

406

407

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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)** α-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

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 $TLR2^{-/-}$ 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.

446
447
448
449
450
451
452
453

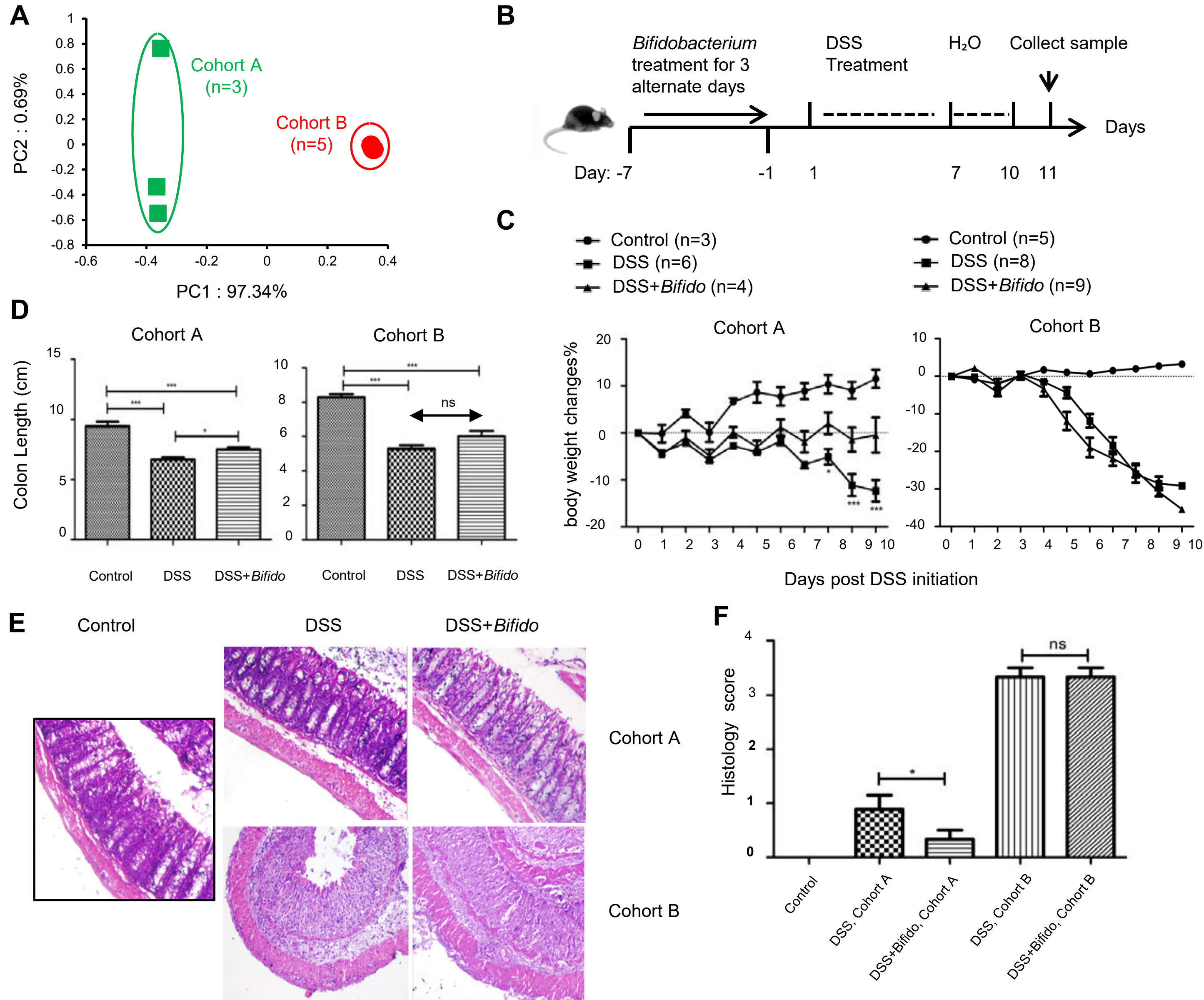

Fig 1

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

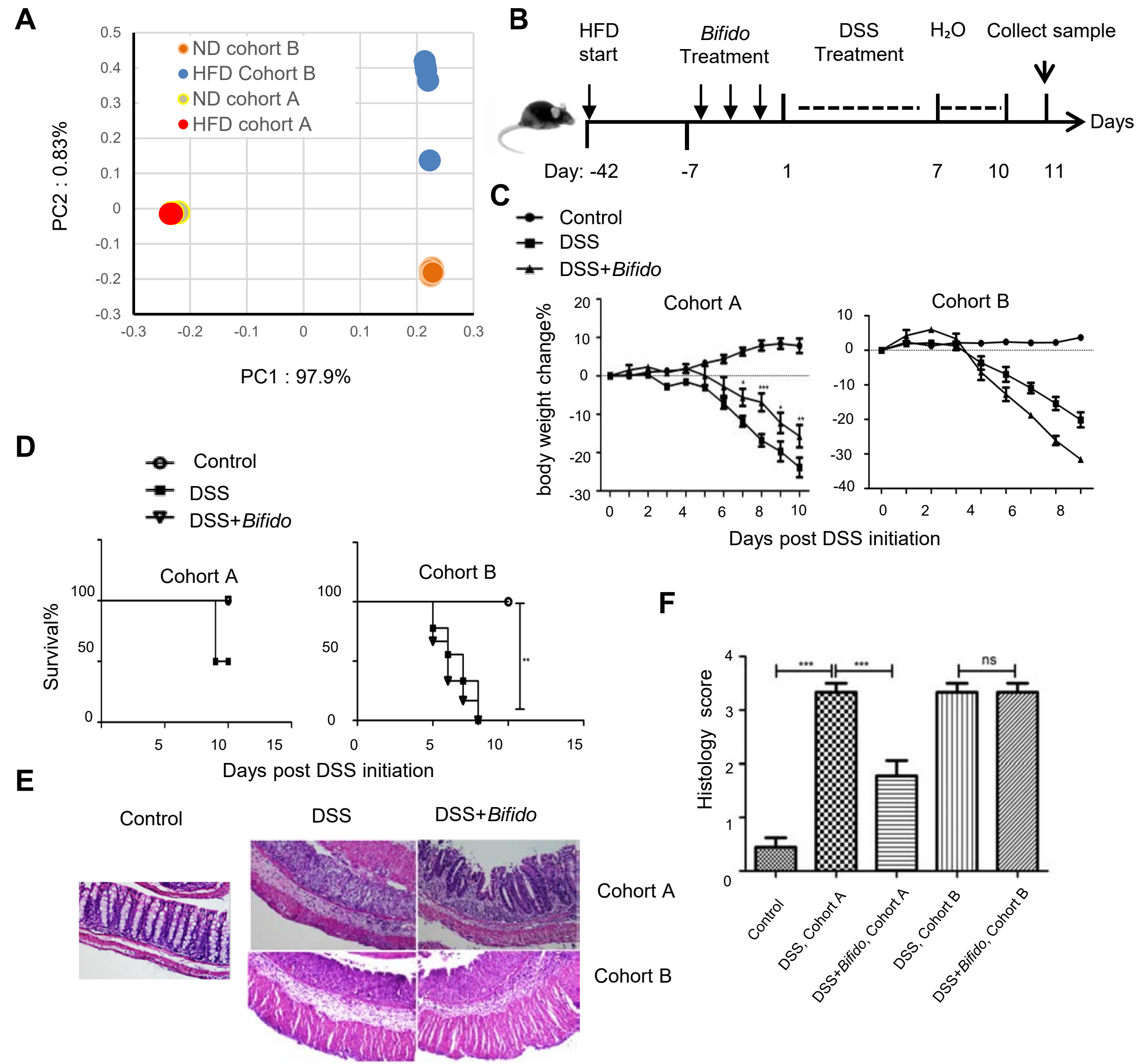

Fig 2

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

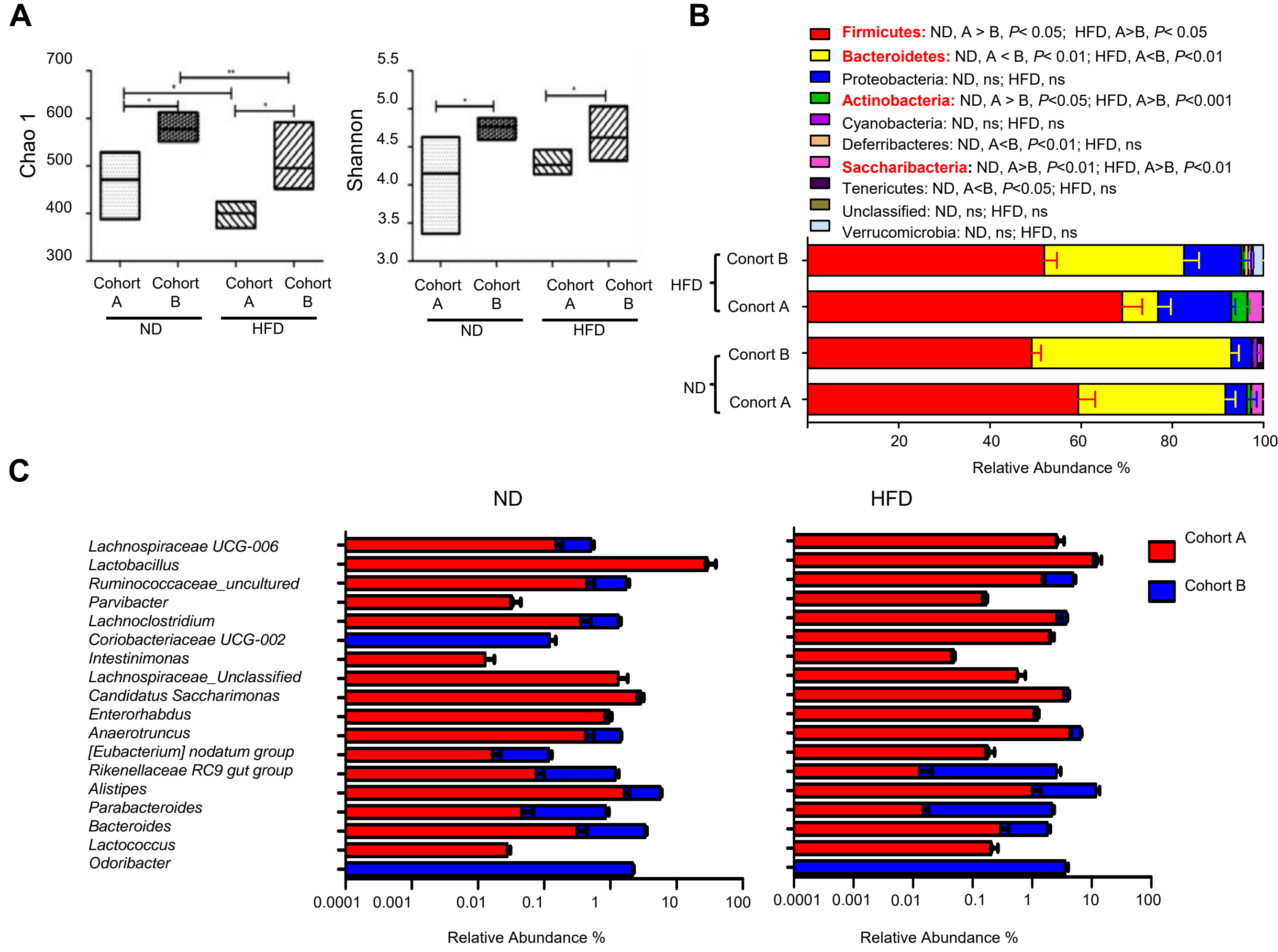

Fig 3

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

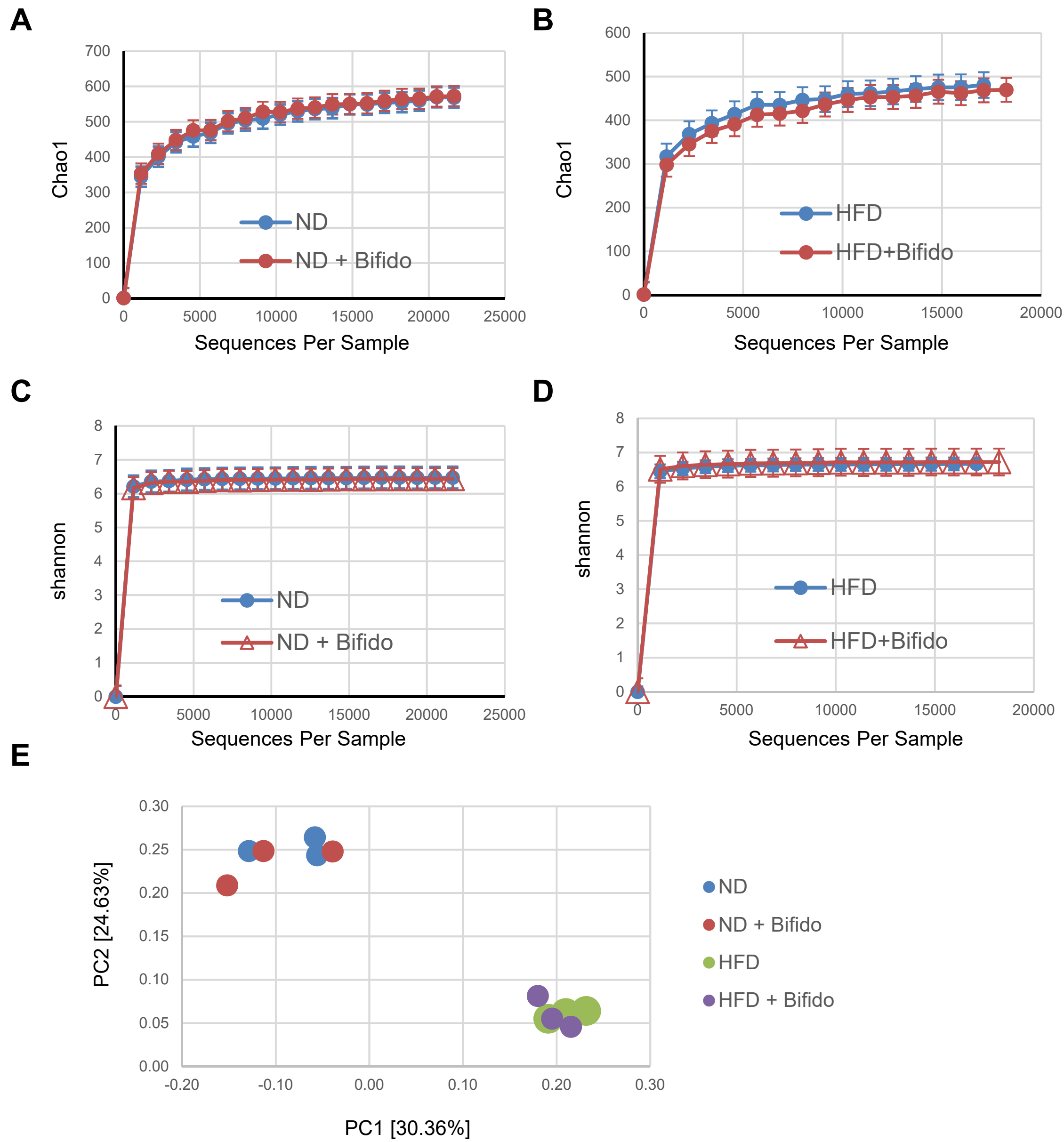

Fig 4

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

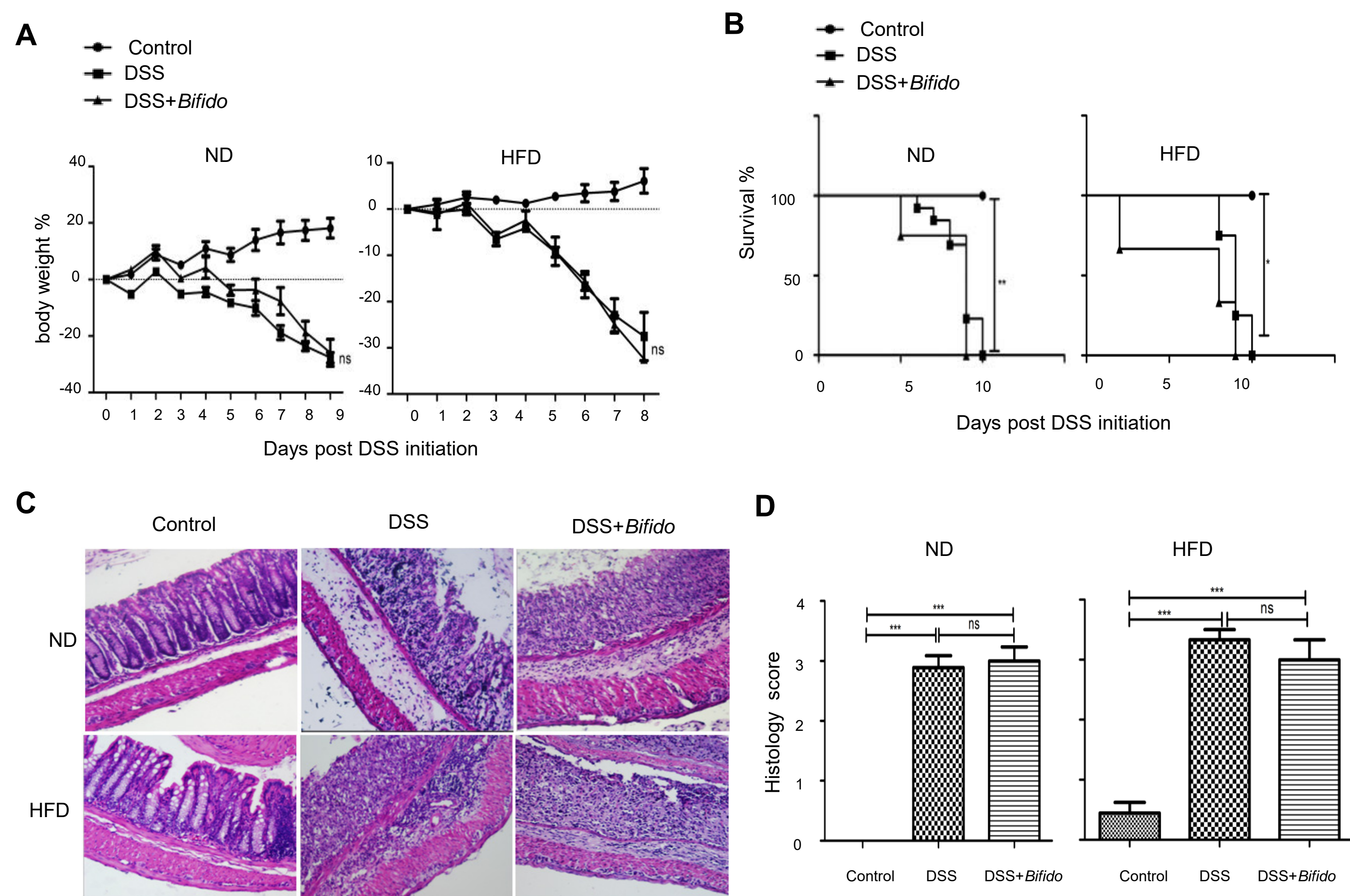

Fig 5

bioRxiv preprint doi: https://doi.org/10.1101/340331; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.