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Probiotics (*Lactobacillus plantarum* HNU082) supplementation relieves ulcerative colitis by affecting intestinal barrier functions, immunity-related genes expression, gut microbiota, and metabolic pathways in mice

yuqing Wu, Rajesh Jha, Ao Li, Huanwei Liu, Zeng Zhang, Chengcheng Zhang, Qixiao Zhai, and Jiachao Zhang

Corresponding Author(s): Jiachao Zhang, Hainan University

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June 9, 2022

Prof. Jiachao Zhang
Hainan University
Food Science
58 renmin road
Haikou, Hainan 570228
China

Re: Spectrum01651-22 (**Probiotics (*Lactiplantibacillus plantarum* HNU082) supplementation relieves ulcerative colitis by affecting intestinal barrier functions, immunity-related genes expression, gut microbiota, and metabolic pathways in mice.**)

Dear Prof. Jiachao Zhang:

Thank you for submitting your manuscript to Microbiology Spectrum. When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed instructions on submitting your revised paper are below.

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Sincerely,

Xiaoyu Tang

Editor, Microbiology Spectrum

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American Society for Microbiology
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Washington, DC 20036
E-mail: spectrum@asmusa.org

Reviewer comments:

Reviewer #1 (Public repository details (Required)):

Metagenome and transcriptome raw data

Reviewer #1 (Comments for the Author):

Article summary and impression:

In the article Spectrum01651-22, the authors seek to describe the impact of supplementation of the food-derived bacterial strain *Lactiplantibacillus plantarum* HNU082 (Lp082) on the commonly used DSS-induced IBD model in C57BL/6J adult male mice with otherwise normal microbiota and diet. The authors induce inflammation with DSS supplementation in animal water, stop DSS supplementation, and then add either Lp082 or the compound SASP (although the rationale for using SASP is not provided, I assume this is a positive control for alleviation of DSS induced inflammation) to evaluate Lp082 impacts on DSS treated mice. The authors perform a number of analyses in an attempt to provide a comprehensive assessment of the impact of Lp082 treatment on DSS treated mice including the following: assessment of 1) animal behavior, 2) immune organ weight, 3) serum inflammatory cytokines, 4) colon structure and histopathology and stool formation, 5) colonic mucin and tight junction integrity, 6) microbial taxa changes and abundance, 7) SCFA acid content, 8) host epithelial transcriptional responses, as well as an attempt to connect microbiome changes to host physiology through correlation modeling. If presented accurately and completely, such a compilation is a useful addition to the scientific community and would provide a greater understanding of the impact of *Lactiplantibacillus* on colitis in healthy mouse models. However, the current version of the manuscript has a number of shortcomings, many of which are summarized below. Overall, the text and figures are confusing to follow as key information required to accurately assess the data and author conclusions has been left out. Information omission begins at the beginning of the paper and builds to where it's difficult to assess the content and accuracy of subsequent data.

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Fig. 4C-D - A description of the tree components is missing. Describe the correlation analysis more in the text and figure legend.

V. Figure 5: I think this entire figure would be best placed in the supplement as it's really just a sub-point of the contents of figure 6 (but it won't fit in figure 6). You might also remove "distribution" from the title and legend as this suggests tissue spatial information but is not needed.

VI. Figure 6: Overall, the less color you use, the clearer this figure will be.

Fig 6A-C: I recommend condensing as Fig 6A. Describe what gene ratio is in the figure legend.

Fig 6D-F: I recommend condensing as Fig 6B.

Fig 6G-J: I recommend condensing as Fig 6C. I and j legends are swapped. Describe ifcSE in the legend.

2. The authors confuse whether they are studying Lp082 prevention or treatment of colitis by using verbiage referring to "prevention" and "treatment" interchangeably. This makes it difficult to track what the authors are trying to accomplish (for example, line 60 says "relieving", lines 76 and 87-88 say "prevention"). Because the authors state that the colitis inducer (DSS) is administered at the time of treatment (Lp082) in the beginning of the Results to evaluate prevention (line 87), but Figure 1A shows that Lp082 is being added at day 8 (so not at the time of induction), I cannot assess which is being studied: Lp082 1) treatment or 2) prevention of UC. My best assumption is that the methods section is correct, and the methods says that DSS is used prior to addition of Lp082, and thus the authors are studying Lp082 relief of colitis. Thus, the language in the paper should be altered to indicate that Lp082 was administered after DSS induced colitis and observed effects are Lp082 alleviation of symptoms, not prevention of symptoms.
3. The abstract, discussion section, and figure 7B describe the effect of Lp082 on the animal model through the groups: biological barrier, chemical barrier, mechanical barrier, and immune barrier. I don't recommend subdividing "biological, chemical, and mechanical barrier", as everything you are referring to is biological, chemical, and mechanical in nature. Rather, use categories akin to "microbiota/microbiome alterations, barrier function improvements, and inflammation reduction."
4. In general, the abstract could be re-written to describe the results from a higher level, rather than just listing the altered genes. Close the abstract with a statement connecting the paper results to the broader scientific field.
5. As written, lines 72-73 suggest Yucha has resistance to acid and bile salts, but I assume that the authors mean Lp082 is resistant. Re-wording the sentence and adding a clarification on what point the authors are trying to make about acid and bile salt resistance would help alleviate the confusion here.
6. Referring to lines 77-92: The authors interchange physiological results with techniques as if they are the same things. Before describing the specific things you were evaluating, describe what you were looking for at a high level. Then separate physiological indicators from methods (e.g., rather than say, "evaluated physiological indexes and shotgun metagenomic sequencing," use language like "evaluated inflammation, microbial community composition and activity...using ELISA, immunohistochemistry, metagenomic sequencing, and RNA-seq."
7. Potentially incorrect information: Lines 97-98 days and scores do not line up with the data reported in figure 1B.
8. Abbreviations should be described in the text as they arise, not in an additional section at the end of the paper (page 20).
9. After revising the manuscript, a thorough and detailed assessment and correction of sentence structure would improve the readability of the paper dramatically.
10. Abbreviations, capitalization, italics, and spacing are inconsistent throughout and should be fixed for a final draft. E.g. Lp082(most commonly used in the draft)/LP082 (lines 78-79) or HNU082 (correct)/HNU082 (line 23).
11. Review your usage of "prove" in your manuscript (notably in the discussion section) as the experiments presented provide largely correlative data.

Reviewer #2 (Public repository details (Required)):

metagenomics sequencing and metabolome data are needed to deposit at a repository.

Reviewer #2 (Comments for the Author):

The manuscript aimed to demonstrate the beneficial roles and elucidate the mechanisms of Lp082 on treatment of UC. Study on specific probiotic strain is demanding, and this manuscript is timely and the knowledge obtained from this study would enrich and broaden our understanding on probiotics. However, this manuscript does need MAJOR revision before consideration for acceptance.

Major comments:

1. Authors claim that "we chose LP082 to study the mechanism of probiotics in preventing UC", however, the animal was treated with various reagents followed by DSS challenge. Please explain how this setting could serve well for assessing the effects of probiotics on prevention UC? Authors should discriminate the difference between "prevention" and "treatment", and pay more attention for accuracy of wording.
2. Basically only one biological repeat was conducted in this study. At least two biological repeats are acceptable for this purpose. Please repeat one more animal assay during next round of revision.
3. Please improve layouts of figures, and pay attention to size, location of symbols.
4. Please improve the language and grammar.
5. Please provide the H&E staining results for entire swiss roll in figure 2.
6. Authors claim that "that LP082 could improve UC by regulating gut microbiota, intestinal mucosal barrier, inflammatory pathways and neutrophil infiltration", please provide direct evidence to support Lp082 effects on "mucosal barrier". Manuscript shows the transcriptome data, however, transcriptome analysis on host genes are far away from real expression and function.

Minor comments:

1. Please provide line numbering.
2. Figure 1a depicted the study design and methodology, which might be better to merge into M&M part.

3. Information of study design and methodology are not appropriate present in Results section. The tables or figures should be displayed at a consecutive and sequential order. In current version figure S1b appeared ahead of S1a.

Staff Comments:

Preparing Revision Guidelines

To submit your modified manuscript, log onto the eJP submission site at <https://spectrum.msubmit.net/cgi-bin/main.plex>. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:

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- Upload a compare copy of the manuscript (without figures) as a "Marked-Up Manuscript" file.
- Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file.
- Manuscript: A .DOC version of the revised manuscript
- Figures: Editable, high-resolution, individual figure files are required at revision, TIFF or EPS files are preferred

For complete guidelines on revision requirements, please see the journal Submission and Review Process requirements at <https://journals.asm.org/journal/Spectrum/submission-review-process>. **Submissions of a paper that does not conform to Microbiology Spectrum guidelines will delay acceptance of your manuscript. "**

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Corresponding authors may [join or renew ASM membership](#) to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at Service@asmusa.org.

Thank you for submitting your paper to Microbiology Spectrum.

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Fig 6G-J: I recommend condensing as Fig 6C. I and j legends are swapped. Describe ifcSE in the legend.
2. The authors confuse whether they are studying Lp082 prevention or treatment of colitis by using verbiage referring to “prevention” and “treatment” interchangeably. This makes it difficult to track what the authors are trying to accomplish (for example, line 60 says “relieving”, lines 76 and 87-88 say “prevention”). Because the authors state that the colitis inducer (DSS) is administered at the time of treatment (Lp082) in the beginning of the Results to evaluate prevention (line 87), but Figure 1A shows that Lp082 is being added at day 8 (so not at the time of induction), I cannot assess which is being studied: Lp082 1) treatment or 2) prevention of UC. My best assumption is that the methods section is correct, and the methods says that DSS is used prior to addition of Lp082, and thus the authors are studying Lp082 relief of colitis. Thus, the language in the paper should be altered to indicate that Lp082 was administered after DSS induced colitis and observed effects are Lp082 alleviation of symptoms, not prevention of symptoms.
 3. The abstract, discussion section, and figure 7B describe the effect of Lp082 on the animal model through the groups: biological barrier, chemical barrier, mechanical barrier, and immune barrier. I don’t recommend subdividing “biological, chemical, and mechanical barrier”, as everything you are referring to is biological, chemical, and mechanical in nature. Rather, use categories akin to “microbiota/microbiome alterations, barrier function improvements, and inflammation reduction.”
 4. In general, the abstract could be re-written to describe the results from a higher level, rather than just listing the altered genes. Close the abstract with a statement connecting the paper results to the broader scientific field.
 5. As written, lines 72-73 suggest Yucha has resistance to acid and bile salts, but I assume that the authors mean Lp082 is resistant. Re-wording the sentence and adding a clarification on what point the authors are trying to make about acid and bile salt resistance would help alleviate the confusion here.
 6. Referring to lines 77-92: The authors interchange physiological results with techniques as if they are the same things. Before describing the specific things you were evaluating, describe what you were looking for at a high level. Then separate physiological indicators from methods (e.g., rather than say, “evaluated physiological indexes and shotgun metagenomic sequencing,” use language like “evaluated inflammation, microbial community composition and activity...using ELISA, immunohistochemistry, metagenomic sequencing, and RNA-seq.”
 7. Potentially incorrect information: Lines 97-98 days and scores do not line up with the data reported in figure 1B.
 8. Abbreviations should be described in the text as they arise, not in an additional section at the end of the paper (page 20).

9. After revising the manuscript, a thorough and detailed assessment and correction of sentence structure would improve the readability of the paper dramatically.
10. Abbreviations, capitalization, italics, and spacing are inconsistent throughout and should be fixed for a final draft. E.g. Lp082 (most commonly used in the draft)/LP082 (lines 78-79) or HNU082 (correct)/*HNU082* (line 23).
11. Review your usage of “prove” in your manuscript (notably in the discussion section) as the experiments presented provide largely correlative data.

1 **Manuscript No.: Spectrum 01651-22**
2 **Title: Probiotics (*Lactobacillus plantarum* HNU082) supplementation relieves**
3 **ulcerative colitis by affecting intestinal barrier functions, immunity-related genes**
4 **expression, gut microbiota, and metabolic pathways in mice.**

5 **Dear Dr. Xiaoyu Tang,**

6 On behalf of my co-authors, I thank you very much for allowing us to revise our
7 manuscript. We appreciate the time and effort that you and the reviewers dedicated to
8 providing feedback on our manuscript and are grateful for the insightful comments on
9 and valuable improvements to our manuscript. We have discussed reviewer's
10 comments carefully and revised the manuscript taking all the comments positively.
11 All revisions in the manuscript have been highlighted in yellow. Please find the
12 point-to-point responses to reviewers' comments in the following text. We thoroughly
13 double-checked the manuscript. In addition, the revised manuscript with tracked
14 changes is also uploaded as "Marked Up Manuscript" files.

15 The sequence data reported in this paper have been deposited in the NCBI
16 database (metagenomic sequencing data and transcriptome sequencing
17 data:PRJNA812272). As is customary, our data will be made public after the article is
18 received.

19
20 We would like to have this revised manuscript considered for publication in
21 "*Microbiology Spectrum*." We deeply appreciate your consideration of our manuscript.
22 If you have any queries, please don't hesitate to contact us at the following e-mail
23 address.

24
25 We would like to express our great appreciation again to you and the reviewers for
26 their comments on our paper. We are looking forward to hearing from you.

27
28 Sincerely,
29 Jiachao Zhang

30 Yours sincerely,
31 E-mail: Jiachao Zhang^{1*}, zhjch321123@163.com
32 College of Food Science and Engineering, Hainan University, Haikou 570228, China

33

34 **Reviewer #1:**

35 Reviewer #1 (Public repository details (Required)):

36 Metagenome and transcriptome raw data

37 **Response:** We are very sorry for our negligence of metagenome and transcriptome
38 raw data. We have uploaded the metagenomic and transcriptome raw data, and the
39 modifications in the manuscript have been highlighted. (Page 27, Line: 790-792)

40 The sequence data reported in this paper have been deposited in the NCBI
41 database (metagenomic sequencing data and transcriptome sequencing
42 data:PRJNA812272).

43 As is customary, our data will be made public after the article is received.

44

45 Reviewer #1 (Comments for the Author):

46 Article summary and impression:

47 In the article Spectrum 01651-22, the authors seek to describe the impact of
48 supplementation of the food-derived bacterial strain *lactobacillus plantarum* HNU082
49 (Lp082) on the commonly used DSS-induced IBD model in C57BL/6J adult male
50 mice with otherwise normal microbiota and diet. The authors induce inflammation
51 with DSS supplementation in animal water, stop DSS supplementation, and then add
52 either Lp082 or the compound SASP (although the rationale for using SASP is not
53 provided, I assume this is a positive control for alleviation of DSS induced
54 inflammation) to evaluate Lp082 impacts on DSS treated mice. The authors perform a
55 number of analyses in an attempt to provide a comprehensive assessment of the
56 impact of Lp082 treatment on DSS treated mice including the following: assessment
57 of 1) animal behavior, 2) immune organ weight, 3) serum inflammatory cytokines, 4)

58 colon structure and histopathology and stool formation, 5) colonic mucin and tight
59 junction integrity, 6) microbial taxa changes and abundance, 7) SCFAs acid content, 8)
60 host epithelial transcriptional responses, as well as an attempt to connect microbiome
61 changes to host physiology through correlation modeling. If presented accurately and
62 completely, such a compilation is a useful addition to the scientific community and
63 would provide a greater understanding of the impact of *Lactoplantibacillus* on colitis
64 in healthy mouse models. However, the current version of the manuscript has a
65 number of shortcomings, many of which are summarized below. Overall, the text and
66 figures are confusing to follow as key information required to accurately assess the
67 data and author conclusions has been left out. Information omission begins at the
68 beginning of the paper and builds to where it's difficult to assess the content and
69 accuracy of subsequent data.

70 **Response:** We appreciate the time and effort you dedicated to providing feedback on
71 our manuscript and are grateful for the insightful comments and valuable
72 improvements to our manuscript. We have discussed your comments carefully, and we
73 sincerely accept the suggestions. Your comments provided valuable insights to refine
74 its contents and analysis. In this document, we try to address the issues raised as best
75 as possible. All revisions in the manuscript have been highlighted in yellow. A list of
76 changes to the manuscript has been attached, and you can kindly find the
77 point-to-point responses to your comments in the following text.

78

79 Preface to the following comments:

80 The manuscript does not use page numbers and line numbers. To review this
81 document, I exported the pdf to word and refer to the title page as page 1, with the
82 first line of the title being line 1.

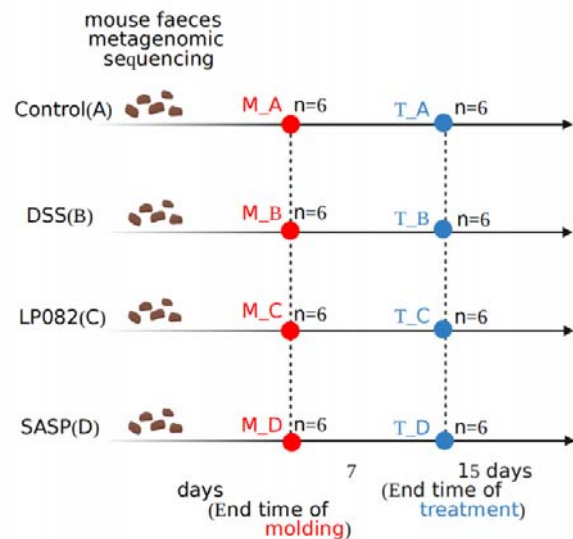
83 **Response:** We appreciate your helpful comments. It was a mistake. We have added
84 the page number and line number to the manuscript now. The title page is also called
85 page 1, and the first line of the title is line 1.

86

87 Major points:

88 1. Conditions used in figure 3A-D are inadequately described, such that I cannot
89 sufficiently assess sample timing, sample size, comparisons made, and biological
90 meaning. A primary contributor to this is a lack of a clear description on what
91 M_A-M_D and T_A-T_D are and how the figures relate to sample timing. This
92 makes it hard to assess other data in the manuscript, including overall conclusions that
93 assess microbiome impact on the host response, which is a primary conclusion that
94 the authors try to address.

95 **Response:** We are extremely grateful to the you for pointing out this problem. We are
96 very sorry for the inadequacy of the condition description. We have added the **Fig. S3**
97 to describe the sampling time and grouping of metagenomics sequencing. In addition,
98 we provide supplementary descriptions of all sample times, sample sizes, and
99 biological significance in the materials and methods and results sections, and
100 modifications in the manuscript are highlighted in yellow. A detailed description of
101 **Fig. S3** has been added to Supplemental materia. (Page 2, Line: 22-33)



102

103 **SUPPLEMENTARY FIGURE LEGENDS**

104 **Fig. S3**

105 (a) Timepoints and grouping of mouse metagenomic sequencing

106 M means the modeling period, T means the treatment period. Respectively, A, B, C

107 and D group mean 7 days normal water (ultrapure water), DSS, Lp082 and SASP
108 treatment after 7 days DSS gavage.

109 M-A means A group represents the control group on the 7th day of DSS
110 modeling, M-B represents the DSS group on the 7th day of DSS modeling, M-C
111 represents the Lp082 group on the 7th day of DSS modeling, M-D represents the
112 SASP on the 7th day of DSS treatment Group.

113 T-A means treating-A group represents the control group at the end of the
114 treatment, T-B represents the DSS group at the end of the treatment, T-C represents
115 the Lp082 group at the end of the treatment, and T-D represents the SASP group at the
116 end of the treatment.

117

118 As shown above, we collected mice fecal samples from group A (Control, $n=6$),
119 group B (DSS, $n=6$), group C (Lp082, $n=6$) and group D (SASP, $n=6$) on days 7 and
120 15 for metagenomic sequencing. On days 1-7, mice in the group B, group C and
121 group D drank DSS-containing water freely, the mice in the group A drank normal
122 water (ultrapure water). On days 8-15, group B, C and D mice stopped drinking DSS
123 water, Mice in groups A and B were gavaged with PBS water, mice in group C were
124 gavaged in PBS water and Lp082, and mice in group D were gavaged in PBS water
125 and SASP. The 7th day was the end of DSS modeling and the 15th day was the end of
126 Lp082 and SASP treatment, so we chose to take samples from the two key time points
127 for sequencing to observe the effect of DSS, Lp082 and SASP on the gut microbiome.
128 We are grateful for the suggestion.

129

130 2. Although Lp082 probiotic introduction is the primary study intervention, the
131 authors do not mention or discuss Lp082 presence in the stool and its own genomic
132 and metabolic contributions to the host response and the SCFAs content. There is a
133 label on Figure 3D that says "*Lactobacillus plantarum*" but it is not discussed. I'd like
134 to see specific Lp082 evaluation and discussion in their metagenome or via another
135 sampling method (like stool qPCR if samples still exist) that indicates the abundance
136 of Lp082 at the times that they sampled in Figure 3 and preferably discussed in light

137 of the experiments and data discussed in Figures 4-6.

138 **Response:** We appreciate your valuable and helpful comment. Previous studies [1],
139 have shown that the abundance of *lactobacillus plantarum* in mice was 0 [2], and it
140 was also found in our experiment (during modeling period, the abundance of
141 *lactobacillus plantarum* in control group (M-A), DSS group (M-B), Lp082 group
142 (M-C) and SASP group (M-D) was 0 , and during the treatment period, the abundance
143 of *lactobacillus plantarum* in the control group (T-A), DSS group (T-B) and SASP
144 group (T-D) was 0.), but we found that the abundance of *lactobacillus plantarum*
145 increased in the Lp082 group (T-C) only after *lactobacillus plantarum* HNU082
146 (Lp082) treatment. This is consistent with Wang et al [3] and Huang et al [4] that
147 probiotic Lp082 can colonize the mouse gut. Therefore, in our experiment, we can
148 infer that the change in *lactobacillus plantarum* was due to the probiotic Lp082
149 intake.

150 Added discussion (Page 10, Line: 287-295)

151 Next, we conducted a correlation analysis between Lp082 (*lactobacillus*
152 *plantarum*) and SCFAs, and found that Lp082 (*lactobacillus plantarum*) was strongly
153 positively correlated with SCFAs (acetic acid, propionic acid, butyric acid) (**Fig. 4c**),
154 the correlation results suggested that Lp082 can increase the content of SCFAs. The
155 above results inspired us to further explore the relationship between Lp082 and
156 SCFAs, and we further analyzed the bacterial species and metabolic pathways
157 associated with SCFAs. Further metagenomic data provided support for our above
158 speculation. Combined with metagenomic data, the species composition of mice gut
159 microbiota was further analyzed. The results showed that the relative abundance of
160 some special bacteria increased in the Lp082 group, such as, *lactobacillus plantarum*,
161 *Bifidobacterium pseudolongum*, *Akkermansia muciniphila*, *Bacteroides ovatus*,
162 *Parabacteroides distasonis*, *Lactobacillus reuteri*, *Anaerotruncus sp G3 2012* (these
163 bacteria are highlighted in red in **Fig. 3d**), all of which can metabolize produces the
164 SCFAs [5].

165 Subsequently, we further analyzed the metabolic pathways of gut microbiota in
166 mice. Results of differential metabolic pathways showed that the abundance of gut

167 microbiota metabolic pathways related SCFAs production decreased in DSS group but
168 increased in Lp082 group (**Fig. 4a**). We infer that Lp082 can promote the content of
169 SCFAs (acetate, propionate and butyrate) by adjust three metabolic pathways,
170 including Pyruvate fermentation to Propanoate I, Pyruvate fermentation to acetate and
171 lactate II, Acetyl CoA fermentation to Butanoate (**Fig. 4a**).

172 To prove the above findings, we further used gas chromatography-mass
173 spectrometry (GC-MS) to detect the content of SCFAs. Compared with control group,
174 the contents of butyric acid, valeric acid, acetic acid, propionic acid and isobutyric
175 acid were significantly decreased after ingestion of DSS ($P < 0.01$). Compared with
176 DSS group, the contents of butyric acid, acetic acid, propionic acid and isobutyric
177 acid were extremely significant increased after ingestion of Lp082 ($P < 0.01$). This
178 confirmed our previous hypothesis based on the correlation that Lp082 intake would
179 increase SCFAs levels (**Fig. 4b**). Based on the above results, we speculate that Lp082
180 increase the content of SCFAs by affecting the abundance of SCFAs-producing
181 microbes, as well as the metabolic pathways of SCFAs-producing microbes.

182 Reference

- 183 1. Pan Y, Ning Y, Hu J, Wang Z, Chen X, Zhao X. The Preventive Effect of
184 *lactobacillus plantarum* ZS62 on DSS-Induced IBD by Regulating Oxidative Stress
185 and the Immune Response. *Oxid Med Cell Longev*. 2021;2021:9416794; doi:
186 10.1155/2021/9416794.
- 187 2. Shao Y, Huo D, Peng Q, Pan Y, Jiang S, Liu B, et al. *lactobacillus plantarum*
188 HNU082-derived improvements in the intestinal microbiome prevent the development
189 of hyperlipidaemia. *Food & Function*. 2017;8(12):4508-16; doi: 10.1039/c7fo00902j.
- 190 3. Wang Y, li J, Ma C, Jiang S, Li C, Zhang L, et al. *lactobacillus plantarum*
191 HNU082 inhibited the growth of *Fusobacterium nucleatum* and alleviated the
192 inflammatory response introduced by *F. nucleatum* invasion. *Food & Function*.
193 2021;12(21):10728-40; doi: 10.1039/d1fo01388b.
- 194 4. Huang S, Jiang S, Huo D, Allaband C, Estaki M, Cantu V, et al. Candidate
195 probiotic *lactobacillus plantarum* HNU082 rapidly and convergently evolves within
196 human, mice, and zebrafish gut but differentially influences the resident microbiome.

197 [Microbiome. 2021;9\(1\); doi: 10.1186/s40168-021-01102-0.](#)

198 5. Y. W. Cheng, J. M. Liu and Z. X. Ling, Short-chain fatty acids-producing
199 probiotics: A novel source of psychobiotics, *Critical Reviews in Food Science and*
200 *Nutrition*, DOI: 10.1080/10408398.2021.1920884.

201

202 3. The Results section "The regulatory roles of SCFAs" and Figure 4 appear to be
203 among the weaker sections in the paper. The figures are not well described, making it
204 difficult to understand the graphs and interpret the data (specific points made below in
205 "minor points"). Lines 172-175 claim "the contents of acetic acid, propionic acid,
206 butyric acid were significantly decreased in the DSS group but significantly increased
207 in the Lp082 group ($p < 0.05$) (Fig. 4b)," but this information does not match the data
208 in Fig. 4b. Fig. 4b shows that the cecal levels of all five evaluated SCFAs are lower
209 than the control in DSS, Lp082, and SASP. Additionally, none of the five SCFAs are
210 higher in Lp082 cecal contents than DSS, and in most cases, the five SCFAs appear
211 lower in Lp082 than DSS. Thus, Fig 4b contradicts their claim that SCFAs improve
212 host outcomes in response to Lp082 treatment after DSS. This is further reiterated by
213 the rather small fold-change increase in the two pathways they indicate promote
214 SCFAs production in Lp082 "the fermentation of pyruvate to propionate I and the
215 fermentation of pyruvate to acetate and lactate II" in figure 4A. The authors'
216 conclusion that Lp082 promotes SCFAs production is heavily leveraged in the
217 discussion section, but is not well supported in their data.

218 **Response:** We apologize for any confusion caused and appreciate the valuable
219 suggestions. We sincerely thank you for pointing out the inconsistency between the
220 figure information and the manuscript information. After carefully examining and
221 comparing of the original drawing data, we found that the grouping in **Fig. 4b** was
222 wrong. We sincerely apologize for this, and the correct grouping is as follows. In **Fig.**
223 **4b**, red represents the control group, yellow represents the Lp082 group, blue
224 represents the SASP group, and green represents the DSS group. The content of
225 SCFAs described in the original manuscript is based on the correct grouping
226 mentioned above. We have revised the grouping of **Fig. 4b** and carefully checked all

227 the figures and full text to ensure the consistency of the manuscript and figures. In
228 addition, we have rewritten the results section "The regulatory roles of SCFAs" and
229 we have redescribed all panels in **Figure 4** including **Fig. 4a-Fig. 4d**. (Page 10, Line:
230 286-346). All revisions in the manuscript have been highlighted.

231 **The regulatory role of SCFAs**

232 Next, we conducted a correlation analysis between Lp082 (*Lactobacillus*
233 *plantarum*) and SCFAs, and found that Lp082 (*Lactobacillus plantarum*) was strongly
234 positively correlated with SCFAs (acetic acid, propionic acid, butyric acid) (**Fig. 4c**),
235 the correlation results suggested that Lp082 can increase the content of SCFAs. The
236 above results inspired us to further explore the relationship between Lp082 and
237 SCFAs, and we further analyzed the bacterial species and metabolic pathways
238 associated with SCFAs. Further metagenomic data provided support for our above
239 speculation. Combined with metagenomic data, the species composition of mice gut
240 microbiota was further analyzed. The results showed that the relative abundance of
241 some special bacteria increased in the Lp082 group, such as, *Lactobacillus plantarum*,
242 *Bifidobacterium pseudolongum*, *Akkermansia muciniphila*, *Bacteroides ovatus*,
243 *Parabacteroides distasonis*, *Lactobacillus reuteri*, *Anaerotruncus sp G3 2012* (these
244 bacteria are highlighted in red in **Fig. 3d**), all of which can metabolize produces the
245 SCFAs [1].

246 Subsequently, we further analyzed the metabolic pathways of gut microbiota in
247 mice. Results of differential metabolic pathways showed that the abundance of gut
248 microbiota metabolic pathways related SCFAs production decreased in DSS group but
249 increased in Lp082 group (**Fig. 4a**). We infer that Lp082 can promote the content of
250 SCFAs (acetate, propionate and butyrate) by adjust three metabolic pathways,
251 including Pyruvate fermentation to Propanoate I, Pyruvate fermentation to acetate and
252 lactate II, Acetyl CoA fermentation to Butanoate (**Fig. 4a**).

253 To prove the above findings, we further used gas chromatography-mass
254 spectrometry (GC-MS) to detect the content of SCFAs. Compared with control group,
255 the contents of butyric acid, valeric acid, acetic acid, propionic acid and isobutyric
256 acid were significantly decreased after ingestion of DSS ($P < 0.01$). Compared with

257 DSS group, the contents of butyric acid, acetic acid, propionic acid and isobutyric
258 acid were extremely significant increased after ingestion of Lp082 ($P < 0.01$). This
259 confirmed our previous hypothesis based on the correlation that Lp082 intake would
260 increase SCFAs levels (**Fig. 4b**). Based on the above results, we speculate that Lp082
261 increase the content of SCFAs by affecting the abundance of SCFAs-producing
262 microbes, as well as the metabolic pathways of SCFAs-producing microbes.

263 To further understand the role of SCFAs, we performed a Pearson correlation
264 analysis. The results showed that *helicobacter hepatica*, which was significantly
265 increased in the DSS group, was strongly negatively correlated with acetic acid,
266 propionic acid, and butyric acid (**Fig. 4c**). *lactobacillus plantarum*, *Bifidobacterium*
267 *pseudolongum*, *Akkermansia muciniphila*, *Parabacteroides distasonis*, *Lactobacillus*
268 *reuteri*, which were significantly increased in Lp082 group showed strong positive
269 correlation with acetic acid, propionic acid, and butyric acid. *Anaerotruncus sp G3*
270 *2012* and *Bacteroides ovatus* showed a strong positive correlation with butyric acid
271 and acetic acid, and a weak positive correlation with propionic acid (**Fig. 4c**). These
272 SCFAs including acetic acid, propionic acid, and butyric acid were all strong
273 negatively correlation with the pro-inflammatory factors TNF- α , IL-1 β , IFN- γ , IL-6,
274 MPO but strongly positively correlated with the inflammatory suppressor IL-10 (**Fig.**
275 **4d**). As important products of gut microbiota metabolism, SCFAs have certain
276 anti-inflammatory effects and play an important role in maintaining normal intestinal
277 morphology and function. Combined with the results of **Fig. 3d**, **Fig. 4a-4d**, as well
278 as the improvement of physiological indicators (**Fig. 1b-1d**), pathological indicators
279 (**Fig. 2a-2g**) and inflammatory factors (**Fig. 1e**) after ingestion of Lp082, we
280 speculated that Lp082 may alleviate DSS-induced UC by regulating SCFAs through
281 the following mechanisms (**Fig. S4**). That is, after the ingestion of Lp082, the
282 abundance of the intestinal microbes of SCFAs-producing increased, which promoted
283 the content of SCFAs. The SCFAs has the function of promoting the secretion of
284 inflammatory cytokine and suppressing the secretion of inflammatory factors. The
285 changes in inflammatory cytokines affect the physiological indicators of mice, which
286 increases the weight, colon length, drinking water and eating volume of mice, and

287 reduces the DAI score and immune organs index. The changes in inflammatory
 288 cytokines also affected the pathological indexes of mice, resulting in a decrease in
 289 histopathological score and an increase in immunofluorescence protein content of
 290 ZO-1 and MUC-2.

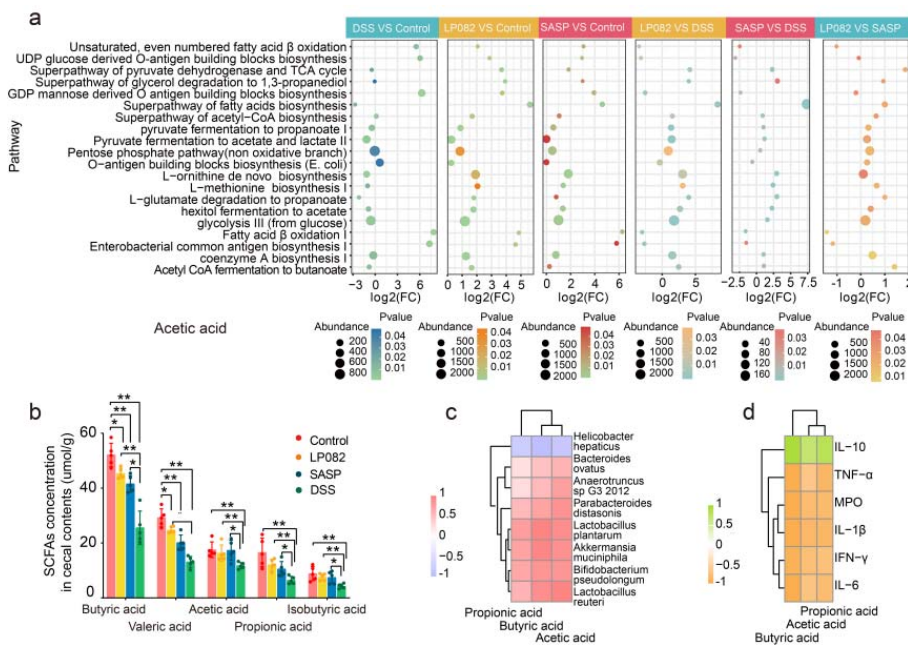
291

292 **Reference**

293 1. Y. W. Cheng, J. M. Liu and Z. X. Ling, Short-chain fatty acids-producing
 294 probiotics: A novel source of psychobiotics, Critical Reviews in Food Science and
 295 Nutrition, DOI: 10.1080/10408398.2021.1920884.

296

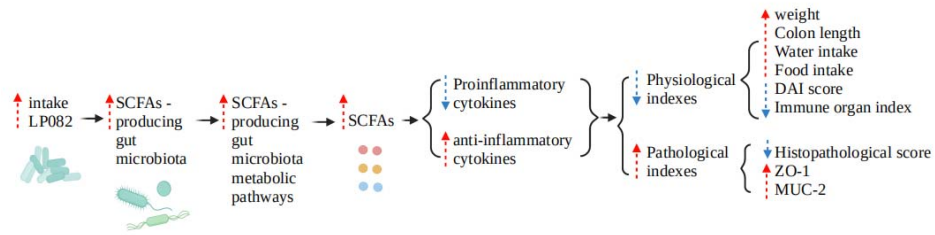
297



298

299 **Fig. 4**

300 The important role of SCFAs in alleviation of DSS-induced UC.



301

302 **Fig. S4**

303 **The underlying mechanism by which Lp082 regulates SCFAs to alleviate UC**

304 4. The authors attempt to model microbial impact on the host using the bacterial
 305 metagenome and a host transcriptional analysis. This comparison would be better
 306 made if there was a microbial metatranscriptome/proteome included in this paper to
 307 support the microbial genomic data. In the absence of this, an evaluation of Lp082
 308 itself in the host, and a weak finding on SCFAs changes in response to Lp082, I find
 309 the correlations reported in figure 7 to be more speculative rather than well supported
 310 by the manuscript.

311 **Response:** We appreciate your valuable and helpful comment. Indeed, it is a pity that
 312 the microbiome lacks transcriptome, but the absence of a microbial transcriptome in
 313 the Cordeiro et al. [1] and Wang et al. [2] articles did not affect the demonstration of
 314 the impact of microorganisms on the host.

315 Previous studies [3], have shown that the abundance of *lactobacillus plantarum*
 316 in mice was 0 [4], and it was also found in our experiment (during modeling period,
 317 the abundance of *lactobacillus plantarum* in control group (M-A), DSS group (M-B),
 318 Lp082 group (M-C) and SASP group (M-D) was 0 , and during the treatment period,
 319 the abundance of *lactobacillus plantarum* in the control group (T-A), DSS group (T-B)
 320 and SASP group (T-D) was 0.), but we found that the abundance of *lactobacillus*
 321 *plantarum* increased in the Lp082 group (T-C) only after *lactobacillus plantarum*
 322 Lp082 treatment. This is consistent with Wang et al [5] and Huang et al [6] that
 323 probiotic Lp082 can colonize the mouse gut. Therefore, in our experiment, we can
 324 infer that the change in *lactobacillus plantarum* was due to the probiotic Lp082
 325 intake.

326 Added discussion (Page 10, Line: 287-318)

327 Next, we conducted a correlation analysis between Lp082 (*lactobacillus*
328 *plantarum*) and SCFAs, and found that Lp082 (*lactobacillus plantarum*) was strongly
329 positively correlated with SCFAs (acetic acid, propionic acid, butyric acid) (**Fig. 4c**),
330 the correlation results suggested that Lp082 can increase the content of SCFAs. The
331 above results inspired us to further explore the relationship between Lp082 and
332 SCFAs, and we further analyzed the bacterial species and metabolic pathways
333 associated with SCFAs. Further metagenomic data provided support for our above
334 speculation. Combined with metagenomic data, the species composition of mice gut
335 microbiota was further analyzed. The results showed that the relative abundance of
336 some special bacteria increased in the Lp082 group, such as, *lactobacillus plantarum*,
337 *Bifidobacterium pseudolongum*, *Akkermansia muciniphila*, *Bacteroides ovatus*,
338 *Parabacteroides distasonis*, *Lactobacillus reuteri*, *Anaerotruncus sp G3 2012* (these
339 bacteria are highlighted in red in **Fig. 3d**), all of which can metabolize produces the
340 SCFAs [7].

341 Subsequently, we further analyzed the metabolic pathways of gut microbiota in
342 mice. Results of differential metabolic pathways showed that the abundance of gut
343 microbiota metabolic pathways related SCFAs production decreased in DSS group but
344 increased in Lp082 group (**Fig. 4a**). We infer that Lp082 can promote the content of
345 SCFAs (acetate, propionate and butyrate) by adjust three metabolic pathways,
346 including Pyruvate fermentation to Propanoate I, Pyruvate fermentation to acetate and
347 lactate II, Acetyl CoA fermentation to Butanoate (**Fig. 4a**).

348 To prove the above findings, we further used gas chromatography-mass
349 spectrometry (GC-MS) to detect the content of SCFAS. Compared with control group,
350 the contents of butyric acid, valeric acid, acetic acid, propionic acid and isobutyric
351 acid were significantly decreased after ingestion of DSS ($P < 0.01$). Compared with
352 DSS group, the contents of butyric acid, acetic acid, propionic acid and isobutyric
353 acid were extremely significant increased after ingestion of Lp082 ($P < 0.01$). This
354 confirmed our previous hypothesis based on the correlation that Lp082 intake would
355 increase SCFAs levels (**Fig. 4b**). Based on the above results, we speculate that Lp082

356 increase the content of SCFAs by affecting the abundance of SCFAs-producing
357 microbes, as well as the metabolic pathways of SCFAs-producing microbes.

358 The above evidence is obtained from actual measurements, and the data is
359 objective and true, which is enough to prove that the increase in SCFAs is indeed
360 caused by the introduction of Lp082.

361 **Fig. 6a** (original named **Fig. 7a**) is a comprehensive network diagram. We have
362 performed pearson correlation analysis based on the actual measured data and
363 simulated possible mechanisms. In **Fig. 6a**, red lines indicate positive correlation,
364 blue lines indicate negative correlation, and thicker lines indicate stronger correlation.
365 The purpose of this picture is to combine the possible mechanism diagrams to better
366 understand the theme of the article, which is the usual method of many [8] articles [9].
367 **Fig. 6a** does not only analyze the correlation, we have really done a lot of
368 experiments and verifications in it. First, we studied some basic indicators and found
369 that Lp082 could not only significantly inhibit the decrease of body weight, water
370 intake and food intake induced by DSSS in mice, but also significantly inhibit the
371 increase of DAI and immune organ index induced by DSSS, as well as the decrease of
372 colon length caused by DSS (**Fig. 1a-1d**). Second, we measured the protein content of
373 six inflammatory cytokines in mouse serum, and found that Lp082 could significantly
374 reduce the increase of IL-1 β , IL-6, TNF- α , MPO, IFN- γ induced by DSS, and increase
375 the protein content of IL-10 in mice (**Fig. 1e**). Third, we performed HE staining
376 section experiment and immunofluorescence protein experiment. The results showed
377 that Lp082 could not only improve the crypt infiltration, goblet cell loss and intestinal
378 mucosal ulcer induced by DSS, but also could reduce the increase of histopathology
379 score caused by DSS and reduce the loss of ZO-1 and MUC-2 proteins caused by
380 DSS (**Fig. 2a-2g**). Fourth, we collected fecal samples on day 7 for metagenomic
381 sequencing. The results of Shotgun metagenomic data analysis showed that Lp082
382 could increase α -diversity and β -diversity, reduce the differences in species
383 composition, increase the content of beneficial bacteria and inhibit the abundance of
384 harmful bacteria in mice (**Fig. 3a-3d**). Fifth, we used gas chromatography-mass
385 spectrometry to determine the content of SCFAs in the intestinal contents of mice, and

386 found that Lp082 could significantly inhibit the reduction of acetic acid, propionic
387 acid, butyric acid, isobutyric acid and valeric acid induced by DSS, and restore the
388 content of SCFAs in mice (**Fig. 4b**). Sixth, we sequenced the transcriptome of colon
389 tissue, and the results showed that Lp082 not only affected gene expression
390 distribution, but also affected inflammation and cancer-related and KEGG,GO-BP
391 pathways (**Fig. 5a-5g**). From the above, it can be seen that our correlations are not
392 unreasonable speculation, but are based on experimental data from a large number of
393 real measurements. Our data were not less than 6 replicates in each group, and our
394 data were absolutely reliable . Collectively, our current data are objective and accurate
395 enough to support our conclusions.

396 **Reference**

- 397 1. Cordeiro BF, Alves JL, Belo GA, Oliveira ER, Braga MP, da Silva SH, et al.
398 Therapeutic Effects of Probiotic Minas Frescal Cheese on the Attenuation of UC in a
399 Murine Model. *Frontiers in Microbiology*. 2021;12; doi: 10.3389/fmicb.2021.623920.
- 400 2. Wang J, Ji HF, Wang SX, Liu H, Zhang W, Zhang DY, et al. Probiotic
401 *lactobacillus plantarum* Promotes Intestinal Barrier Function by Strengthening the
402 Epithelium and Modulating Gut Microbiota. *Frontiers in Microbiology*. 2018;9; doi:
403 10.3389/fmicb.2018.01953.
- 404 3. Pan Y, Ning Y, Hu J, Wang Z, Chen X, Zhao X. The Preventive Effect of
405 *lactobacillus plantarum* ZS62 on DSS-Induced IBD by Regulating Oxidative Stress
406 and the Immune Response. *Oxid Med Cell Longev*. 2021;2021:9416794; doi:
407 10.1155/2021/9416794.
- 408 4. Shao Y, Huo D, Peng Q, Pan Y, Jiang S, Liu B, et al. *lactobacillus plantarum*
409 HNU082-derived improvements in the intestinal microbiome prevent the development
410 of hyperlipidaemia. *Food & Function*. 2017;8(12):4508-16; doi: 10.1039/c7fo00902j.
- 411 5. Wang Y, li J, Ma C, Jiang S, Li C, Zhang L, et al. *lactobacillus plantarum*
412 HNU082 inhibited the growth of *Fusobacterium nucleatum* and alleviated the
413 inflammatory response introduced by *F. nucleatum* invasion. *Food & Function*.
414 2021;12(21):10728-40; doi: 10.1039/d1fo01388b.
- 415 6. Huang S, Jiang S, Huo D, Allaband C, Estaki M, Cantu V, et al. Candidate

416 probiotic *Lactobacillus plantarum* HNU082 rapidly and convergently evolves within
417 human, mice, and zebrafish gut but differentially influences the resident microbiome.
418 *Microbiome*. 2021;9(1); doi: 10.1186/s40168-021-01102-0.

419 7. Y. W. Cheng, J. M. Liu and Z. X. Ling, Short-chain fatty acids-producing
420 probiotics: A novel source of psychobiotics, *Critical Reviews in Food Science and*
421 *Nutrition*, DOI: 10.1080/10408398.2021.1920884.

422 8. Ma C, Wasti S, Huang S, Zhang Z, Mishra R, Jiang S, et al. The gut microbiome
423 stability is altered by probiotic ingestion and improved by the continuous
424 supplementation of galactooligosaccharide. *Gut Microbes*. 2020;12(1); doi:
425 10.1080/19490976.2020.1785252.

426 9. Z. P. Gu, Y. J. Zhu, S. M. Jiang, G. H. Xia, C. Li, X. Y. Zhang, J. C. Zhang and X.
427 R. Shen, Tilapia head glycolipids reduce inflammation by regulating the gut
428 microbiota in dextran sulphate sodium-induced colitis mice, *Food & Function*, 2020,
429 11, 3245-3255.

430

431 5. I'd like to see an analysis or discussion of the genes in Figure 6D for Lp082. The
432 authors indicate that these genes in 6D are upregulated in DSS and some are
433 pro-inflammatory. I'd like to know if Lp082 treatment suppresses these genes when
434 compared to DSS alone.

435 **Response:** We are grateful for the suggestion. We have added a more detailed
436 interpretation regarding analysis and discussion of Lp082 gene. More detailed
437 statistical analysis was added in the paper. Supplementary Figure **Fig. S6** illustrates
438 the effect of Lp082 treatment on up-regulated inflammatory genes in the DSS group
439 in **Fig. 6d**.

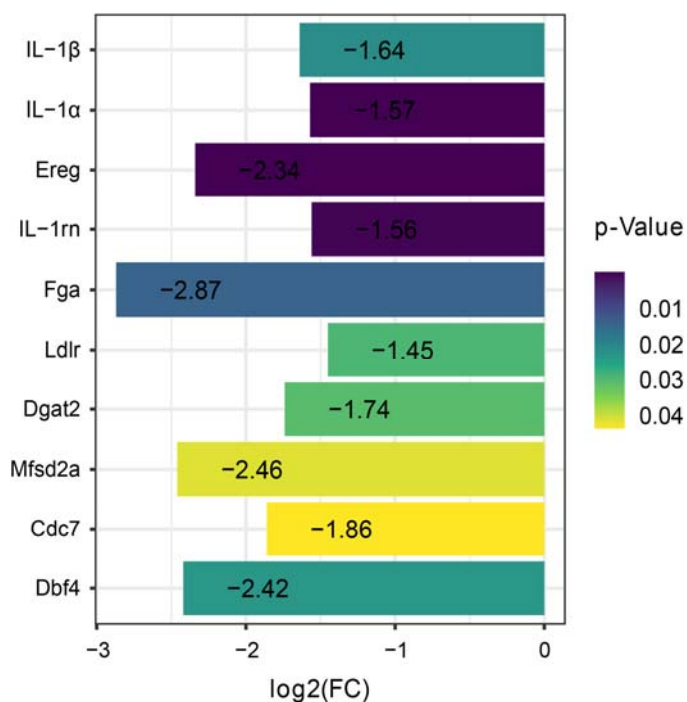
440 Our previous analysis idea was as follows: Since the preliminary analysis of
441 transcriptome data showed that the intake of Lp082 affects the gene expression
442 distribution (**Fig. 5**), in order to explore whether Lp082 also affects gene enrichment
443 pathways, we analyzed the GO pathway and KEGG pathway.

444 Since the differentially expressed genes (DEGs) were more enriched in the
445 biological process (BP) pathway among the three major GO pathway categories (**Fig.**

446 **5a-5c**), and the number of significantly up-regulated genes in Lp082 group is more
447 than the down-regulated genes compared with the DSS group (**Fig. 5d**), so we
448 performed further GO-BP analysis on the significantly up-regulated differentially
449 expressed genes (**Fig. 6d-6f**). Therefore, in **Fig. 6d**, more attention was paid to
450 inflammatory pathways enriched by up-regulated genes in the DSS group. We added
451 **Fig. S6** to see the changes of genes enriched in inflammatory pathways in the DSS
452 group, and their changes in the Lp082 group. We have supplemented **Fig. S6** content
453 in the article and highlighted it, the supplementary content is as follows (Page 14, line:
454 385-391):

455 To further observe whether Lp082 treatment would suppress these inflammatory
456 and cancer genes enriched on inflammatory pathways in the DSS group, we
457 supplemented Fig. S6. As can be seen from Fig. S6, among the 13 inflammatory genes
458 or oncogenes that were up-regulated and enriched in the inflammatory pathway in the
459 DSS group, the following 10 genes were significantly down-regulated in the Lp082
460 group: IL-1 β , IL-1 α , Ereg, IL-1rn, Fga, Ldlr, Dgat2, Mfsd2a, Cdc7, Dbf4 (Fig. S6).

461



462

463 A supplementary legend to Figure S6 has been added to the supplementary material
464 (Page 6, line: 51-58)

465 **SUPPLEMENTARY FIGURE LEGENDS**

466 **Fig. S6.** The effect of Lp082 treatment on up-regulated inflammatory genes in the
467 DSS group in **Fig. 6d**.

468 The 13 inflammatory genes or oncogenes that were up-regulated and enriched in the
469 inflammatory pathway in the DSS group, the following 10 genes were significantly
470 down-regulated in the Lp082 group: IL-1 β , IL-1 α , Ereg, IL -1rn, Fga, Ldlr, Dgat2,
471 Mfsd2a, Cdc7, Dbf4.

472 Wilcoxon signed-rank test is used here. Each group had at least 6 biological
473 replicates.

474

475 2. Other missing information that should be addressed in the manuscript:

476 a. Rationale:

477 I. Why was SASP used?

478 **Response:** Thank you for pointing this out. We have supplemented the description of
479 SASP, and relevant content has been added to the manuscript now (Page 5, line:
480 126-132). The details are as follows:

481 Sulfasalazine (SASP) is a commonly used medicine to treat UC at present [1].
482 Sulfasalazine is hydrolyzed into 5'-aminosalicylic acid and sulfamerydine by
483 intestinal bacteria when it enters the human intestine. The decomposed 5'
484 -aminosalicylic acid not only has good anti-inflammatory and antibacterial effects but
485 also can effectively suppress the outbreak of UC through immunosuppression [2].
486 Zhipeng Gu [3] used SASP as the positive control group of tilapia head sugar lipids in
487 the treatment of colitis.

488 Therefore, SASP was selected as the positive control group for Lp082 in the
489 treatment of UC.

490 **Reference**

- 491 1. Steinhart AH, Hemphill D, Greenberg GR. Sulfasalazine and mesalazine for the
492 maintenance therapy of Crohn's disease: a meta-analysis. The American journal of
493 gastroenterology. 1994;89(12):2116-24.
- 494 2. Klotz U, Maier K, Fischer C, Heinkel K. Therapeutic efficacy of sulfasalazine

495 and its metabolites in patients with UC and Crohn's disease. The New England journal
496 of medicine. 1980;303(26):1499-502; doi: 10.1056/nejm198012253032602.

497 3. Gu ZP, Zhu YJ, Jiang SM, Xia GH, Li C, Zhang XY, et al. Tilapia head
498 glycolipids reduce inflammation by regulating the gut microbiota in dextran sulphate
499 sodium-induced colitis mice. Food & Function. 2020;11(4):3245-55; doi:
500 10.1039/d0fo00116c.

501

502 II. Why was Lp082 used specifically?

503 **Response:** We are grateful for the suggestion. We have added a more detailed
504 interpretation regarding Lp082. Relevant content has been added to the text (Page 4,
505 line: 98-111). The revised content is as follows:

506 The strain of *Lactobacillus plantarum* HNU082 (Lp082) was originally isolated
507 from a traditional fermented food-fish tea of the Li people in Hainan Province,
508 China ,which has a good safety profile and tolerance to acids and bile salts [1]. The
509 results of Lp082 whole genome sequencing showed showed that this bacterium has
510 great potential to develop as a probiotic in terms of physiology and function [2]. In
511 our previous study, Lp082 not only can enhance the ecological and genetic stability of
512 the intestinal microbiota [3]. But also can inhibit the growth of *Fusobacterium*
513 *nucleatum* and reduce the inflammatory response [4]. Previous studies have also
514 shown that Lp082 exerts a preventive effect on hyperlipidemia through the
515 modulation of metabolism [5]. In addition, ingestion of Lp082 and supplementation
516 with prebiotics improved the stability of the intestinal microbiota and reduced the
517 occurrence of disorders associated with disease. These results invariably demonstrate
518 the probiotic potential of Lp082. However, the treatment effect of Lp082 on UC has
519 not been studied.

520 Therefore, we chose Lp082 to study the mechanism of probiotics in treating UC.

521 **Reference**

522 1. Zhang J, Wang X, Huo D, Li W, Hu Q, Xu C, et al. Metagenomic approach
523 reveals microbial diversity and predictive microbial metabolic pathways in Yucha, a
524 traditional Li fermented food. Scientific Reports. 2016;6; doi: 10.1038/srep32524.

525 2. Ma C, Wasti S, Huang S, Zhang Z, Mishra R, Jiang S, et al. The gut microbiome
526 stability is altered by probiotic ingestion and improved by the continuous
527 supplementation of galactooligosaccharide. *Gut Microbes*. 2020;12(1); doi:
528 10.1080/19490976.2020.1785252.

529 3. Huang S, Jiang S, Huo D, Allaband C, Estaki M, Cantu V, et al. Candidate
530 probiotic *Lactiplantibacillus plantarum* HNU082 rapidly and convergently evolves
531 within human, mice, and zebrafish gut but differentially influences the resident
532 microbiome. *Microbiome*. 2021;9(1); doi: 10.1186/s40168-021-01102-0.

533 4. Wang Y, li J, Ma C, Jiang S, Li C, Zhang L, et al. *Lactiplantibacillus plantarum*
534 HNU082 inhibited the growth of *Fusobacterium nucleatum* and alleviated the
535 inflammatory response introduced by *F. nucleatum* invasion. *Food & Function*.
536 2021;12(21):10728-40; doi: 10.1039/d1fo01388b.

537 5. Shao Y, Huo D, Peng Q, Pan Y, Jiang S, Liu B, et al. *Lactobacillus plantarum*
538 HNU082-derived improvements in the intestinal microbiome prevent the development
539 of hyperlipidaemia. *Food & Function*. 2017;8(12):4508-16; doi: 10.1039/c7fo00902j.

540

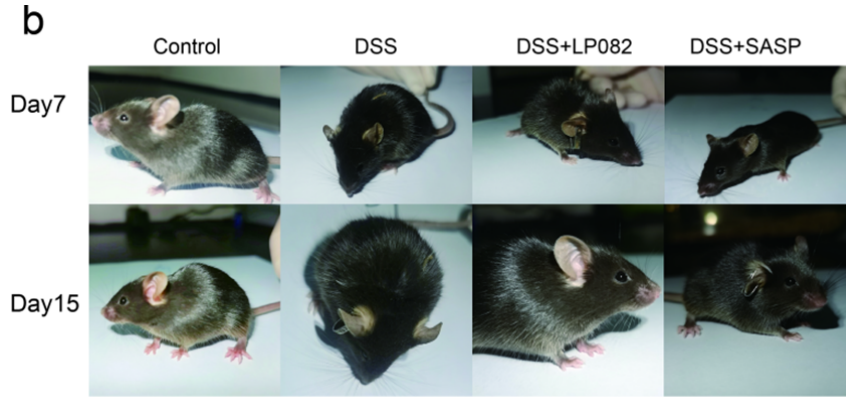
541 b. Experimental design, conditions, methods:

542 I. Fig S1B does not adequately describe mouse behavior as it's a single
543 non-descriptive image of each mouse.

544 **Response:** We are grateful for the suggestion. As suggested by the reviewer, we have
545 added more details of mouse behavior. Relevant content has been added to the text
546 (Page 7, line: 185-197). The details are as follows:

547 The mental state of the mice was observed daily, and the results are shown in **Fig.**
548 **S1 b**. On the 7th day of modeling, mice in the control group were in a normal state,
549 with normal urine and feces, shiny hair, active spirit, sensitive reaction and increased
550 body size. However, mice in the B,C and D group had yellow and smelly urine,
551 difficult defecation, bloody stool, dark and fried hair, slow reaction and easy panic,
552 arched back, and reduced body size (**Fig. S1 b**). On the last day of treatment (Day 15),
553 compared with the arched back, retarded response, hematochezia and lethargic in the
554 DSS group, the mental state of mice in the Lp082 and SASP groups gradually

555 returned to normal, with active spirit, no arched back, no hematochezia and shiny hair
556 (Fig. S1 b). These results indicated that Lp082 intake could alleviate the symptoms of
557 depression, crouching and untidy hair of mice in the DSS group in the middle and late
558 stage of the experiment (Fig. S1 b).



559

Fig. S1

560

(b) Mental state of experimental mice.

561

562

563 c. Timing of experiments: After line 101, the sampling times of most experiments are
564 omitted or inadequately described.

565 **Response:** We appreciate your valuable and helpful comment. It is true that the
566 sampling times of most experiments are inadequately described. We have rewritten
567 this section. The rewritten content is more detailed, and the details are as follows:

568

569 After the experiment, the spleen, liver, kidney and colon of 8 mice were selected from
570 each group for observation and measurement. (Page 6, line: 170-172)

571

572 To further evaluate the effects of Lp082 on inflammatory cytokines in mice with
573 colitis, serum of 6 mice in each group was randomly collected after the experiment,
574 and the levels of pro-inflammatory cytokines TNF- α , IL-1 β , IFN- α , IL-6, MPO and
575 anti-inflammatory cytokines IL-10 were detected by ELISA kit. (Page 8, line:
576 208-213)

577

578 At the end of the experiment, the 1cm portion of the distal colon of 6 mice in each
579 group was selected randomly for HE staining, and histopathological score and
580 intestinal wall thickness were further measured ($n=6$). (Page 8, line: 220-224)

581

582 At the end of modeling (day 7 of the experiment), feces of 6 mice in each group were
583 randomly selected for metagenomic sequencing, and at the end of treatment (day 15
584 of the experiment), feces of 6 mice in each group were selected for metagenomic
585 sequencing, to observe the effects of DSS and Lp082 on the intestinal microecology
586 of mice. (Page 9, line: 258-262)

587

588 To prove the above findings, we further used gas chromatography-mass spectrometry
589 (GC-MS) to detect the content of SCFAs in cecal contents of 6 mice in each group.
590 (Page 11, line: 308-309)

591

592 At the end of the experiment, 6 mice from each group were randomly selected for
593 colon transcriptome sequencing, and the volcanic map was drawn based on the
594 preliminary gene distribution analysis results. (Page 13, line: 350-352)

595

596 C57BL/6J mice aged 7 weeks were randomly divided into 4 groups: control group
597 ($n=8$), dextran sulfate sodium (DSS) group ($n=8$), lactobacillus plantarum HNU082
598 (Lp082) group ($n=8$), and salazosulfapyridine (SASP) group ($n=8$). (Page 23, line:
599 659-661)

600

601 After the mice were euthanized, the colon length of 8 mice in each group was
602 measured, the weight of spleen, liver, and kidney of 8 mice in each group was
603 measured. (Page 23, line: 677-679)

604

605 Before euthanasia, 6 mice were randomly selected from each group, and blood was
606 collected from the orbital venous plexus by a capillary tube. (Page 24, line: 696-697)

607

608 Finally, the levels of interleukin-1beta (IL-1 β), interleukin-6 (IL-6), interleukin-10
609 (IL-10), interleukin-17A (IL-17A), interferon-gamma (IFN- γ), Tumor necrosis
610 factor-alpha (TNF- α), and Myeloperoxidase (MPO) in the serum of 6 randomly
611 selected mice from each group were measured using the corresponding ELISA kits
612 (X-Y Biotechnology, Shanghai, China), as previously described. (Page 24, line:
613 686-687)

614

615 After euthanasia, the distal 1cm colons of 6 mice in each group were randomly
616 selected for HE staining section, histopathological score, and intestinal wall thickness
617 measurement. (Page 24, line: 697-688)

618

619 On the other hand, 8 mice were selected from each group, and their colonic tissues
620 were labeled with mucin 2 and ZO-1 antibodies, respectively [75], for further
621 immunofluorescence staining (Servicebio, Wuhan, China). (Page 25, line: 710-712)

622

623 Six mice were randomly selected at two time points (day 7 and day 15 of the
624 experiment) for metagenomic sequencing of feces. (Page 25, line: 728-7429)

625

626 At the end of the experiment, the cecal contents of 6 mice from each group were
627 randomly selected for SCFAs determination, and the specific steps were as follows:
628 (Page 26, line: 742-743)

629

630 At the end of the experiment, colon tissues of 6 mice from each group were randomly
631 selected for RNA sequencing. (Page 26, line: 757-758)

632

633

634 d. Sample sizes: Sample sizes and number of repeats are omitted. In most cases, the
635 specific datapoints in figures are not well described as to what they are measuring.

636 **Response:** We appreciate your valuable and helpful comment and we deeply agree
637 with the opinions of reviewer. According to your helpful suggestions, we have

638 carefully checked the whole paper, and added descriptions of sample size and number
639 of repeats in material and methods, legends and corresponding places in the article.
640 The changes have been highlighted in the text in yellow. The rewritten content is
641 more detailed, and the details are as follows:

642 After the experiment, the spleen, liver, kidney and colon of 8 mice were selected from
643 each group for observation and measurement. (Page 6, line: 170-172)

644
645 To further evaluate the effects of Lp082 on inflammatory cytokines in mice with
646 colitis, serum of 6 mice in each group was randomly collected after the experiment,
647 and the levels of pro-inflammatory cytokines TNF- α , IL-1 β , IFN- α , IL-6, MPO and
648 anti-inflammatory cytokines IL-10 were detected by ELISA kit. (Page 8, line:
649 208-213)

650
651 At the end of the experiment, the 1cm portion of the distal colon of 6 mice in each
652 group was selected randomly for HE staining, and histopathological score and
653 intestinal wall thickness were further measured ($n=6$). (Page 8, line: 220-224)

654
655 At the end of modeling (day 7 of the experiment), feces of 6 mice in each group were
656 randomly selected for metagenomic sequencing, and at the end of treatment (day 15
657 of the experiment), feces of 6 mice in each group were selected for metagenomic
658 sequencing, to observe the effects of DSS and Lp082 on the intestinal microecology
659 of mice. (Page 9, line: 258-262)

660
661 To prove the above findings, we further used gas chromatography-mass spectrometry
662 (GC-MS) to detect the content of SCFAs in cecal contents of 6 mice in each group.
663 (Page 11, line: 308-309)

664
665 At the end of the experiment, 6 mice from each group were randomly selected for
666 colon transcriptome sequencing, and the volcanic map was drawn based on the
667 preliminary gene distribution analysis results. (Page 13, line: 350-352)

668

669 C57BL/6J mice aged 7 weeks were randomly divided into 4 groups: control group
670 ($n=8$), dextran sulfate sodium (DSS) group ($n=8$), lactobacillus plantarum HNU082
671 (Lp082) group ($n=8$), and salazosulfapyridine (SASP) group ($n=8$). (Page 23, line:
672 659-661)

673

674 After the mice were euthanized, the colon length of 8 mice in each group was
675 measured, the weight of spleen, liver, and kidney of 8 mice in each group was
676 measured. (Page 23, line: 677-679)

677

678 Before euthanasia, 6 mice were randomly selected from each group, and blood was
679 collected from the orbital venous plexus by a capillary tube. (Page 24, line: 696-697)

680

681 Finally, the levels of interleukin-1beta (IL-1 β), interleukin-6 (IL-6), interleukin-10
682 (IL-10), interleukin-17A (IL-17A), interferon-gamma (IFN- γ), Tumor necrosis
683 factor-alpha (TNF- α), and Myeloperoxidase (MPO) in the serum of 6 randomly
684 selected mice from each group were measured using the corresponding ELISA kits
685 (X-Y Biotechnology, Shanghai, China), as previously described. (Page 24, line:
686 686-687)

687

688 After euthanasia, the distal 1cm colons of 6 mice in each group were randomly
689 selected for HE staining section, histopathological score, and intestinal wall thickness
690 measurement. (Page 24, line: 697-688)

691

692 On the other hand, 8 mice were selected from each group, and their colonic tissues
693 were labeled with mucin 2 and ZO-1 antibodies, respectively [75], for further
694 immunofluorescence staining (Servicebio, Wuhan, China). (Page 25, line: 710-712)

695

696 Six mice were randomly selected at two time points (day 7 and day 15 of the
697 experiment) for metagenomic sequencing of feces. (Page 25, line: 728-7429)

698

699 At the end of the experiment, the cecal contents of 6 mice from each group were
700 randomly selected for SCFAs determination, and the specific steps were as follows:
701 (Page 26, line: 742-743)

702

703 At the end of the experiment, colon tissues of 6 mice from each group were randomly
704 selected for RNA sequencing. (Page 26, line: 757-758)

705

706 e. Statistics:

707 a. Only one statistical test is indicated in the paper, Wilcoxin signed rank test, line 546
708 in the methods. Adding the test run to each figure legend would be appropriate and
709 helpful.

710 **Response:** We appreciate your valuable and helpful comment. We have added
711 statistical test methods to each of the graphical legends. The revised content is as
712 follows:

713 Wilcoxon signed-rank test is used here. The significant difference was
714 considered at $*p<0.05$, $** p<0.01$ and $***p<0.001$. Each group had at least 6
715 biological replicates.

716

717 b. Conditions statistical tests being used on are not obvious, in part due to the lack of
718 descriptions on sample sizes and replicates.

719 **Response:** Thank you for your comments. We deeply agree with the opinions of
720 reviewer and we have carefully checked the whole paper, and added descriptions of
721 sample size and replicates in material and methods, legends and corresponding places
722 in the article. The changes have been highlighted in the text in yellow. The details are
723 as follows:

724

725 After the experiment, the spleen, liver, kidney and colon of 8 mice were selected from
726 each group for observation and measurement. (Page 6, line: 170-172)

727

728 To further evaluate the effects of Lp082 on inflammatory cytokines in mice with
729 colitis, serum of 6 mice in each group was randomly collected after the experiment,
730 and the levels of pro-inflammatory cytokines TNF- α , IL-1 β , IFN- α , IL-6, MPO and
731 anti-inflammatory cytokines IL-10 were detected by ELISA kit. (Page 8, line:
732 208-213)

733

734 At the end of the experiment, the 1cm portion of the distal colon of 6 mice in each
735 group was selected randomly for HE staining, and histopathological score and
736 intestinal wall thickness were further measured ($n=6$). (Page 8, line: 220-224)

737

738 At the end of modeling (day 7 of the experiment), feces of 6 mice in each group were
739 randomly selected for metagenomic sequencing, and at the end of treatment (day 15
740 of the experiment), feces of 6 mice in each group were selected for metagenomic
741 sequencing, to observe the effects of DSS and Lp082 on the intestinal microecology
742 of mice. (Page 9, line: 258-262)

743

744 To prove the above findings, we further used gas chromatography-mass spectrometry
745 (GC-MS) to detect the content of SCFAs in cecal contents of 6 mice in each group.
746 (Page 11, line: 308-309)

747

748 At the end of the experiment, 6 mice from each group were randomly selected for
749 colon transcriptome sequencing, and the volcanic map was drawn based on the
750 preliminary gene distribution analysis results. (Page 13, line: 350-352)

751

752 C57BL/6J mice aged 7 weeks were randomly divided into 4 groups: control group
753 ($n=8$), dextran sulfate sodium (DSS) group ($n=8$), lactobacillus plantarum HNU082
754 (Lp082) group ($n=8$), and salazosulfapyridine (SASP) group ($n=8$). (Page 23, line:
755 659-661)

756

757 After the mice were euthanized, the colon length of 8 mice in each group was

758 measured, the weight of spleen, liver, and kidney of 8 mice in each group was
759 measured. (Page 23, line: 677-679)

760

761 Before euthanasia, 6 mice were randomly selected from each group, and blood was
762 collected from the orbital venous plexus by a capillary tube. (Page 24, line: 696-697)

763

764 Finally, the levels of interleukin-1beta (IL-1 β), interleukin-6 (IL-6), interleukin-10
765 (IL-10), interleukin-17A (IL-17A), interferon-gamma (IFN- γ), Tumor necrosis
766 factor-alpha (TNF- α), and Myeloperoxidase (MPO) in the serum of 6 randomly
767 selected mice from each group were measured using the corresponding ELISA kits
768 (X-Y Biotechnology, Shanghai, China), as previously described. (Page 24, line:
769 686-687)

770

771 After euthanasia, the distal 1cm colons of 6 mice in each group were randomly
772 selected for HE staining section, histopathological score, and intestinal wall thickness
773 measurement. (Page 24, line: 697-688)

774

775 On the other hand, 8 mice were selected from each group, and their colonic tissues
776 were labeled with mucin 2 and ZO-1 antibodies, respectively [75], for further
777 immunofluorescence staining (Servicebio, Wuhan, China). (Page 25, line: 710-712)

778

779 Six mice were randomly selected at two time points (day 7 and day 15 of the
780 experiment) for metagenomic sequencing of feces. (Page 25, line: 728-7429)

781

782 At the end of the experiment, the cecal contents of 6 mice from each group were
783 randomly selected for SCFAs determination, and the specific steps were as follows:
784 (Page 26, line: 742-743)

785

786 At the end of the experiment, colon tissues of 6 mice from each group were randomly
787 selected for RNA sequencing. (Page 26, line: 757-758)

788

789 Minor points:

790 1. Missing information that should be addressed:

791 a. Rationale:

792 I. The introduction provides weak descriptions and evidence for use of a probiotic in
793 general to treat UC and Lp082 specifically. The introduction would benefit from
794 further elaboration on what is known about probiotic treatment of UC and indicate
795 what is or isn't known about Lp082 usage in UC specifically rather than using general
796 "probiotics" references. Along with this, lines 55-59 are confusing as written, but this
797 may be addressed when more information is added about those two points.

798 **Response:** We appreciate your valuable comment. According to your helpful
799 suggestions, we have rewritten this section to include more references detailing the
800 etiology of UC, current status of *lactobacillus plantarum* in the treatment of UC and
801 the reasons for using Lp082. The revised content is as follows: (Page 3, line: 62-111)

802 Inflammatory bowel disease (IBD) is a chronic non-specific inflammatory
803 disease occurring in the gastrointestinal tract, mainly including ulcerative colitis (UC)
804 and crohn's disease (CD) [1]. The clinical manifestations of UC patients are diarrhea,
805 blood in the stool, weight loss, and diffuse inflammation of the colonic mucosa [2].
806 UC has become a major health problem worldwide due to its chronicity, recurrence,
807 and high morbidity [3], high risk of developing into Colorectal cancer (CRC) [4]. Due
808 to the disadvantages of traditional surgery and drug therapy of UC, such as
809 postoperative complications, side effects, and high cost [5], there is an urgent need to
810 develop a new UC treatment method.

811 There is no consensus on the specific pathogenesis of UC, and many evidences
812 suggest that the pathogenesis of UC is multifactorial, involving genetic susceptibility,
813 epithelial barrier defects, immune response disorders and environmental factors [6].

814 Differences in gut microbiota (type and amount) between colitis patient and healthy
815 people are thought to be one of the key factors in disease progression [7]. In UC
816 patients, the immune response is activated, the intestinal permeability is increased, the
817 intestinal mucosal barrier structure is destroyed, the homeostasis of gut microbiota is

818 disturbed, and the intestinal symbiotic bacteria are destroyed, thus activating a more
819 serious immune response, leading to the recurrence of the disease [8].

820 Due to the shortcomings of traditional treatments, it is urgent to develop new
821 treatments for UC, among which probiotics, as a substitute for antibiotics, have
822 attracted much attention for regulating gut microbiota to effectively alleviate UC
823 [9].As one of the main probiotics, *lactobacillus plantarum* has the characteristics of
824 regulating the balance of gut microbiota, increasing the adhesion of beneficial bacteria
825 to intestinal mucosa, inhibiting the adhesion of pathogenic bacteria and inhibiting the
826 inflammatory reaction [10]. Both animal [11] and clinical trials [12] have reported
827 that *lactobacillus plantarum* can reduce chronic mucosal inflammation in patients
828 with UC and prevent the occurrence of experimental colitis induced by DSS. In
829 addition, Bibiloni et al. evaluated the efficacy of *lactobacillus VSL#3* in 20 patients
830 with IBD and VSL#3 in newly diagnosed children with IBD and found that the
831 *lactobacillus strain* was effective in mild to moderate adult patients with IBD [13].
832 Yin et al. [14] believe that *lactobacillus plantarum* can restore the damaged mucosal
833 barrier function, regulate the imbalance of intestinal microbiota, inhibit pathogenic
834 bacteria, enhance intestinal system immunity, and have a good effect on relieving IBD
835 symptoms and maintaining remission. However, there are few studies on the specific
836 mechanism of action of *lactobacillus plantarum* in UC treatment, and there is no
837 unified argument [15].

838 The strain of *lactobacillus plantarum* HNU082 (Lp082) was originally isolated
839 from a traditional fermented food-fish tea of the Li people in Hainan Province, China
840 [16],which has a good safety profile and tolerance to acids and bile salts [17]. The
841 results of Lp082 whole genome sequencing showed showed that this bacterium has
842 great potential to develop as a probiotic in terms of physiology and function [5]In our
843 previous study, Lp082 not only can enhance the ecological and genetic stability of the
844 intestinal microbiota [18]. But also can inhibit the growth of *Fusobacterium*
845 *nucleatum* and reduce the inflammatory response [19]. Previous studies have also
846 shown that Lp082 exerts a preventive effect on hyperlipidemia through the
847 modulation of metabolism [20]. In addition, ingestion of Lp082 and supplementation

848 with prebiotics improved the stability of the intestinal microbiota and reduced the
849 occurrence of disorders associated with disease. These results invariably demonstrate
850 the probiotic potential of Lp082. However, the treatment effect of Lp082 on UC has
851 not been studied.

852 Therefore, we chose Lp082 to study the mechanism of probiotics in treating UC.

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927

928 II. The intro (starting at line 62) provides weak background on data for SCFAs
929 alleviation of IBD. Citing work and adding text of SCFAs impact of IBD (preferably
930 UC and action through immune cells) would be helpful.

931 **Response:** We appreciate your valuable comment. According to your helpful
932 suggestions, we have rewritten this part and added more literature describing the
933 effects of SCFAs on UC and its effects on immune cells. The revised content is as
934 follows: (Page 4, line: 112-125)

935 *Lactobacillus* has been reported to have potential benefits for inflammatory
936 Bowel Disease (IBD) and colorectal cancer (CRC) symptoms due to its ability to
937 promote the formation of short-chain fatty acids (SCFAs) [1]. SCFAs are one of the

938 important metabolites of gut microbiota, and the main components in intestinal tract
939 are butyrate, acetate and propionate. Many studies have shown that SCFAs has
940 immunomodulatory effects [2], can reduce the expression of pro-inflammatory factors,
941 reduce inflammatory response, and play an important role in the treatment of UC [3].
942 Studies have shown that SCFAs can act on immune cells such as monocyte
943 macrophages and lymphocytes, change their gene expression, affect differentiation,
944 chemotaxis, proliferation and apoptosis, and thus participate in immune regulation [4].
945 In inflammatory response, SCFAs can reduce the expression of C5aR, thus regulating
946 the aggregation of macrophages and neutrophils [5], In addition, SCFAs can maintain
947 the integrity and permeability of intestinal epithelial cells, promote the secretion of
948 mucin in goblet cells, and protect the intestinal epithelial barrier so as to alleviate UC
949 [6].

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974

975 **b. Impact:**

976 I. Referencing lines 94-115: No text is provided to indicate what the alterations in
977 water intake, food intake, body weight, DAI, neurological responses, immune organ
978 index, spleen and colon color and structure, hyperemia, and feces structure mean in
979 the context of disease in DSS or in the Lp082 treated animals. This is not addressed
980 elsewhere in the paper and would help the reader understand the impact of your
981 results.

982 **Response:** Thank you for pointing this out. We have added the description according
983 to your suggestion. The revised content is as follows. (Page 6, line: 146-203)

984 People with UC have a disorder of colon function, poor absorption, loss of
985 appetite, weight loss, diarrhea, and bloody stools [1]. Therefore, the lower the body
986 weight, the lower the amount of water and food intake, and the higher the DAI score
987 (The scoring criteria is shown in **TABLE S1**), indicating the more severe enteritis.
988 Therefore, water intake, food intake, body weight, and DAI were monitored daily to
989 assess the severity of ulcerative enteritis modeling. "Molding ending" in **Fig. 1b** refers
990 to the end date of modeling UC with DSS on days 1-7, and no DSS water was
991 administered to mice beginning with day 8. The results showed that from 1 to 7 days,
992 the water intake, food intake, and body weight of the DSS group, the Lp082 group,
993 and the SASP group all showed a similar degree of gradual decrease, and these three
994 groups were all significantly different from the Control group on day 7 ($p < 0.05$),
995 which may be because these three groups were all under the same DSS modeling
996 conditions on days 0-7. Then on the 8th to 15th day, the water intake, food intake, and
997 body weight of the DSS group were still decreasing, but the water intake, food intake,

998 and body weight of Lp082 and SASP group gradually increased. Specifically, the
999 water and food intake of the Lp082 combined SASP group increased significantly
1000 from day 9 ($p < 0.05$), and body weight increased significantly from day 12 ($p < 0.05$).
1001 The DAI index of the DSS group, Lp082 group, and SASP group increased
1002 significantly ($p < 0.05$) from the third day compared with the Control group. After
1003 stopping DSS gavage on the 8th day, the DAI index of the DSS self-healing group
1004 still increased, while that of the Lp082 group and SASP group gradually decreased
1005 from the 10th day, and the degree of decrease in the Lp082 group was greater than
1006 that in the SASP group (**Fig. 1b**).

1007 In DSS-induced UC mice, the immune organ index gradually increased and the
1008 colon length gradually shortened with increasing disease severity [2]. Therefore, we
1009 measured the spleen, liver, kidney, and colon of the mice. The results showed that the
1010 immune organ index of the DSS group was significantly increased ($p < 0.05$), and the
1011 immune organ index was significantly decreased after Lp082 intake ($p < 0.05$) (**Fig.**
1012 **1c**). The colon length of the mice in the DSS group was significantly decreased ($p <$
1013 0.05), and the colon length in Lp082 group was significantly increased ($p < 0.05$) (**Fig.**
1014 **1d**). In addition, we also observed that the intestinal contents of the colitis mice in the
1015 DSS group were loose, unformed and there was blood in the intestinal lumen, while
1016 the intestinal contents in the Lp082 and Control groups were clear particles, hard stool,
1017 and no blood (**Fig. 1d**). The fecal morphology of the intestinal contents was similar to
1018 the results observed in mouse feces on the buttocks of mice. The feces of the mice in
1019 the DSS group were blood-red, and the feces were loose and unformed, while there
1020 was no blood in the feces after Lp082 ingestion (**Fig. S1 a**).

1021 With the increase of disease degree, DSS-induced UC mice will have a worse
1022 mental state, even abdominal pain, arch back, panic and other symptoms [3]. The
1023 mental state of the mice was observed daily, and the results are shown in **Fig. S1 b**.
1024 On the 7th day of modeling, mice in the control group were in a normal state, with
1025 normal urine and feces, shiny hair, active spirit, sensitive reaction, and increased body
1026 size. However, mice in the BCD group had yellow and smelly urine, difficult
1027 defecation, bloody stool, dark and fried hair, slow reaction and easy panic, arched

1028 back, and reduced body size (**Fig. S1 b**). On the last day of treatment (Day 15),
1029 compared with the arched back, retarded response, hematochezia, and lethargic in the
1030 DSS group, the mental state of mice in the Lp082 and SASP groups gradually
1031 returned to normal, with an active spirit, no arched back, no hematochezia and shiny
1032 hair (**Fig. S1 b**). These results indicated that Lp082 intake could alleviate the
1033 symptoms of depression, crouching, and untidy hair of mice in the DSS group in the
1034 middle and late stage of the experiment (**Fig. S1 b**).

1035 Studies have shown that under the condition of inflammation, the spleen of mice
1036 induced by DSS will increase hyperemia and even appear infection blackening.
1037 Therefore, we looked at the spleens of mice and found that the spleens of mice in the
1038 DSS group were significantly larger and darker than those of mice in the normal
1039 group. The spleens of mice in the Lp082 and SASP groups were smaller and redder
1040 rather than black than those in the DSS group (**Fig. S1 c**).

1041 **Reference**

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1054

1055

1056 II. Lines 125-145: Text here would benefit from at least a little description on what
1057 this data means at this point in the writing. E.g. what does MUC2 loss and ZOI

1058 abundance suggest about Lp082 effects?

1059 **Response:** Thank you for pointing this out; we have added the description according
1060 to your suggestion, and the revised content is as follows. (Page 9, line: 239-254)

1061 MUC-2 is the mucin secreted by goblet cells, which can form the protective layer
1062 of intestinal mucosa epithelium [1]. Tight junction protein ZO-1 is an important
1063 physical barrier located in the gap between intestinal epithelial cells [2]. Studies have
1064 shown that the content of ZO-1 and MUC-2 is reduced in UC, and its structure and
1065 function are destroyed, resulting in increased intestinal permeability and harmful
1066 substances entering the body, aggravating inflammation. Therefore, the levels of
1067 MUC-2 and ZO-1 in colon were determined by immunofluorescence protein assay.
1068 The results showed that the MUC-2 protein (green fluorescence) and ZO-1 protein
1069 (red fluorescence) contents were higher in the control group, almost disappeared in
1070 the DSS group, and significantly recovered in the Lp082 and SASP groups ($p < 0.05$),
1071 and even increased more than SASP in Lp082 group (**Fig. 2d-e**). These results were
1072 consistent with the surface density results of the two proteins (**Fig. 2f-g**). This
1073 suggests that Lp082 can reduce the decrease in the number of ZO-1 and MUC-2
1074 caused by DSS, and maintain the normal structure and function of the intestinal
1075 mucus protein layer and intestinal epithelial cells.

1076 **Reference**

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1078 Approach of IBD Using Recombinant Glycoprotein Mucin2. *Faseb Journal*. 2009;23.
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1081 and the Immune Response. *Oxid Med Cell Longev*. 2021;2021:9416794; doi:
1082 10.1155/2021/9416794.

1083

1084 c. Methods:

1085 I. Scoring: Since understanding the scoring system used is important to understanding
1086 the data, further describing the numbering and what that means would help the reader
1087 understand the severity of the DSS model and the subsequent relief without looking

1088 up the methods reference (either in the figure legends (Figure 1B) or in the methods
1089 (see lines 480-481 where the modifications to DAI are not indicated). DAI and
1090 immune organ index should be described at some level in the results and figure
1091 legends as well so the reader knows what the data is describing without the methods.)

1092 **Response:** Thank you for pointing this out. We deeply agree with the opinions of
1093 reviewer. According to your helpful suggestions, we have added the description, and
1094 the revised content is as follows.

1095 the higher the DAI score (The scoring criteria is shown in **TABLE S1**), indicating
1096 the more severe enteritis. (Page 6, line: 148-149)

1097 The immune organ index (mg/g) of mouse spleen, liver, and kidney. The immune
1098 organ index = immune organ weight (mg)/body weight (g). Increased coefficient of
1099 immune organs indicates congestion and edema of organs and increased inflammation.
1100 (Page 40, line: 1147-1150)

1101 In DSS-induced UC, the higher the histopathological scores, the thicker the
1102 intestinal mucosal wall, indicating more severe disease and more severe inflammation.
1103 (Page 8, line: 222-224)

1104

1105 The following has been added to the supplementary materials:

1106 **FIGURE LEGENDS**

1107 **Fig. 1.** Effects of Lp082 on DSS-induced UC mice.

1108 (b) Water intake, food intake, body weight, and disease activity index (DAI scoring
1109 system modified from previous studies (Table. S1)) in mice.

1110 (c) The immune organ index (mg/g) of mouse spleen, liver, and kidney. The immune
1111 organ index = immune organ weight (mg)/body weight (g). Increased coefficient of
1112 immune organs indicates congestion and edema of organs and increased inflammation.
1113 (Page 40, line: 1143-1150)

1114

1115 **SUPPLEMENTARY TABLE LEGENDS**

1116 **Table S1.**

1117 Disease activity index (DAI) scoring system of dextran sodium sulfate-induced

1118 colitis.

1119 The DAI scoring system consists of three parts: weight loss, stool consistency and
1120 visible blood in feces. Each part has 5 grades from 0 to 4. A score of 0 means that the
1121 three indicators are normal, and the closer the score is to 4, the more serious
1122 inflammation it is. (Page 7, line: 65-70)

1123

1124 **Table S2.**

1125 Histopathology scoring system of dextran sodium sulfate-induced colitis. The
1126 Histopathology scoring system scoring system was modified from previous studies [2].
1127 The modified scoring system consists of six parts, namely, depth of Inflammation,
1128 range of inflammation (%), crypt damage, goblet cell loss and the degree of
1129 neutrophil Infiltration. Each component was rated on a scale of 0 to 4, a score of 0
1130 means that the three indicators are normal, and the closer the score is to 4, the more
1131 serious inflammation it is. (Page 9, line: 75-81)

1132

1133 II. How was "surface density" quantified? Line 144, figure 2F-G.

1134 **Response:** Thank you for pointing this out; we have added the surface density
1135 description according to your suggestion, and the revised content is as follows. (Page
1136 25, line: 716-724)

1137 The surface density of immunofluorescence ZO-1 and MUC-2 was measured and
1138 calculated as follows: Eclipse CI-L fluorescence photography microscope was used to
1139 select the target area of tissues for 200-fold imaging. After the imaging was completed,
1140 image-Pro Plus 6.0 analysis software was used to convert green/red fluorescent
1141 monochrome photos into black and white pictures, and then the same black was
1142 selected as the unified standard to judge the positivity of all photos. The pixel area
1143 was used as the standard unit. The positive cumulative optical DENSITY (IOD) and
1144 the corresponding tissue pixel area in each section were measured, respectively, and
1145 areal density =IOD/area was calculated.

1146

1147 III. Indicate the specific diet provided to the mice (line 459).

1148 **Response:** Thank you for your comment. We added the description of the specific
1149 diet of mice according to your suggestion, and the revised content is as follows. (Page
1150 22, line: 645-650)

1151 Mice in all groups were fed standard normal commercial mouse chow (It is
1152 mainly composed of crude protein, crude fiber, crude fat and trace elements). Mice in
1153 the Control group were free to drink normal water within 15 days, and the other three
1154 groups were free to drink DSS water for the first 7 days, and were changed to normal
1155 water from the 8th day.

1156

1157 IV. Elaborate on what you mean by "mouse colon samples" on line 537 for RNA-seq.

1158 **Response:** Thank you so much for pointing this out, and so sorry we didn't make it
1159 clear here. The mouse colon sample here refers to the middle 1 cm of the mouse colon
1160 for transcriptome sequencing. Requires RNA extraction mini-kit (Qiagen, Hilden,
1161 Germany) to extract total RNA from mouse colon samples for transcriptome
1162 sequencing.

1163

1164 d. Results structure:

1165 I. The experiments, including the rationale, the samples, and the conditions, should be
1166 described at some level prior to discussing the results in the Results text so the readers
1167 know what the results are referencing.

1168 **Response:** Thank you for your comment. We deeply agree with the opinions of
1169 reviewer. At your wise suggestion, We have carefully reviewed the entire article and
1170 added explanations of experimental principles, samples, and conditions at the
1171 beginning of all Discussion and Results sections.

1172 People with UC have a disorder of colon function, poor absorption, loss of
1173 appetite, weight loss, diarrhea, and bloody stools [8]. Therefore, the lower the body
1174 weight, the lower the amount of water and food intake, and the higher the DAI score
1175 (The scoring criteria is shown in **TABLE S1**), indicating the more severe enteritis.
1176 Therefore, water intake, food intake, body weight, and DAI were monitored daily to
1177 assess the severity of ulcerative enteritis modeling. (Page 6, line: 146-151)

1178 With the increase of disease degree, DSS-induced UC mice will have a worse
1179 mental state, even abdominal pain, arch back, panic and other symptoms [30]. The
1180 mental state of the mice was observed daily, and the results are shown in **Fig. S1 b**.
1181 (Page 7, line: 184-186)

1182 In DSS-induced UC mice, the immune organ index gradually increased and the
1183 colon length gradually shortened with increasing disease severity [23]. Therefore,
1184 after the experiment, the spleen, liver, kidney and colon of 8 mice were selected from
1185 each group for observation and measurement. (Page 6, line: 169-172)

1186 To further evaluate the effects of Lp082 on inflammatory cytokines in mice with
1187 colitis, serum of 6 mice in each group was randomly collected after the experiment,
1188 and the levels of pro-inflammatory cytokines TNF-, IL-1 β , IFN- α , IL-6, MPO, and
1189 anti-inflammatory cytokines IL-10 were detected by ELISA kit. (Page 8, line:
1190 208-213)

1191 At the end of the experiment, the 1cm portion of the distal colon of 6 mice in
1192 each group was randomly selected for HE staining, and histopathological score and
1193 intestinal wall thickness were further measured ($n=6$). In DSS-induced UC, the higher
1194 the histopathological scores, the thicker the intestinal mucosal wall, indicating more
1195 severe disease and more severe inflammation. (Page 8, line: 220-224)

1196 MUC-2 is the mucin secreted by goblet cells, which can form the protective layer
1197 of intestinal mucosa epithelium [30]. Tight junction protein ZO-1 is an important
1198 physical barrier located in the gap between intestinal epithelial cells [10]. Studies
1199 have shown that the content of ZO-1 and MUC-2 is reduced in UC, and its structure
1200 and function are destroyed, resulting in increased intestinal permeability and harmful
1201 substances entering the body, aggravating inflammation. Therefore, the levels of
1202 MUC-2 and ZO-1 in the colon were determined by immunofluorescence protein assay.
1203 (Page 9, line: 239-246)

1204 To further observe the effects of Lp082 on the gut microbiota of mice, we
1205 sequenced the metagenome of feces of mice. At the end of modeling (day 7 of the
1206 experiment), feces of 6 mice in each group were randomly selected for metagenomic
1207 sequencing. At the end of treatment (day 15 of the experiment), feces of 6 mice in

1208 each group were randomly selected for metagenomic sequencing, to observe the
1209 effects of DSS and Lp082 on the intestinal microecology of mice. (Page 9, line:
1210 257-262)

1211 To prove the above findings, we further used gas chromatography-mass
1212 spectrometry (GC-MS) to detect the content of SCFAs in cecal contents of 6 mice in
1213 each group. (Page 11, line: 308-318)

1214 At the end of the experiment, 6 mice from each group were randomly selected
1215 for colon transcriptome sequencing, and the volcanic map was drawn based on the
1216 preliminary gene distribution analysis results. (Page 13, line: 350-352)

1217

1218 II. Brief overall conclusions should be provided in the Results text to continue
1219 engaging the reader and leading them along your thought process. This can be
1220 partially addressed by moving text from the Discussion section to the Results. E.g.
1221 lines 302-306 can be moved to the results section where diversity is discussed.

1222 **Response:** We agree with the comment. According to your excellent suggestion, we
1223 moved the Discussion lines 302-306 to the Results section, where we discuss diversity,
1224 with a slight modification. The revised content is as follows: (Page 10, line: 282-284)

1225 The above results show that Lp082 treatment remarkably increased the gut
1226 microbiota diversity and reduced gut microbiota structural differences in gut
1227 microbiota, as shown by the cluster analysis and PCoA analysis, also optimized
1228 species composition.

1229

1230 e. Figures:

1231 I. Figure 1:

1232 Fig 1A - the arrows make it look like PBS only led to weight and colon assessment,
1233 probiotics to immune indices, SASP to sequencing. Collapsing the arrows would
1234 address this.

1235 **Response:** Thanks for your nice comments. In the revised manuscript, we have
1236 corrected the figure. The folded arrow has been added to **Fig. 1a**. Here, PBS refers to
1237 phosphate buffered solution, which can provide a relatively stable ionic environment

1238 and pH buffering capacity, and is a buffer salt solution commonly used in biology. **Fig.**
1239 **1a** shows that on days 8-15, mice in Control group and DSS group were intragastric
1240 with PBS solution, mice in the Lp082 group were intragastric with probiotics solution,
1241 and mice in the SASP group were intragastric with SASP solution. The purpose of
1242 such different gavage is to observe the effect of Lp082 on UC by comparing with DSS
1243 self-healing and SASP positive drugs.

1244

1245 Fig 1B - what's being compared for the stats is not well described

1246 **Response:** We really appreciate your efforts and comments on our manuscript. We
1247 have revised our manuscript according to your comments and suggestions. The
1248 statistical data in **Fig. 1b** are re-described, and the revised content is as follows: (Page
1249 6, line: 153-168)

1250 The results showed that from 1 to 7 days, the water intake, food intake, and body
1251 weight of the DSS group, the Lp082 group and the SASP group all showed a similar
1252 degree of gradual decrease, and these three groups were all significantly different
1253 from the Control group on day 7 ($p < 0.05$), which because these three groups were all
1254 under the same DSS modeling conditions on days 0-7. Then on the 8th to 15th day,
1255 the water intake, food intake, and body weight of the DSS group were still decreasing,
1256 but the water intake, food intake, and body weight of Lp082 and SASP group
1257 gradually increased. Specifically, the water and food intake of the Lp082 in SASP
1258 group increased significantly from day 8 ($p < 0.05$), and body weight increased
1259 significantly from day 11 ($p < 0.05$). The DAI index of the DSS group, Lp082 group,
1260 and SASP group increased significantly ($p < 0.05$) from the second day compared
1261 with the Control group. After stopping DSS gavage on the seventh day, the DAI index
1262 of the DSS self-healing group still increased, while that of the Lp082 group and SASP
1263 group gradually decreased from the 9th day, and the degree of decrease in the Lp082
1264 group was greater than that in the SASP group. (**Fig. 1b**)

1265

1266 Fig 1C - the bars for stats are shifted (also make sure the lines are the same point
1267 thickness for stats in each figure)

1268 **Response:** Thanks for your helpful comments. We are very sorry for our negligence
1269 and we have corrected **Fig. 1c** according to your helpful suggestion. We have checked
1270 all the pictures carefully to make sure we don't have the same problem again.

1271

1272 Fig 1B - "molding ending" is not described in the text. Rephrase or define. Also
1273 decrease the numbers in the X axis as they are too condensed. The title "duration of
1274 probiotic intervention (day)" is an incorrect title as this figure shows duration of the
1275 entire experiment, including pre-treatment with DSS before probiotics.

1276 **Response:** Thank you for your helpful comment. We deeply agree with your
1277 suggestion and we have made correction according to your nice suggestions.
1278 "Molding ending" in **Fig. 1b** refers to the end date of modeling UC with DSS on days
1279 1-7, no DSS water was administered to mice beginning with day 8. We have added the
1280 description of "molding ending" in both the figure legend and the results section,
1281 reduced the number on the X axis, and changed the "duration of probiotic intervention
1282 (day)" to the duration of the entire experiment "Days" based on your good idea.

1283

1284 Fig 1E - there's no Y-axis label and the datapoints are not described

1285 **Response:** Thank you for your helpful comment. We are very sorry for our
1286 negligence and we have modified the figure according to your suggestion. The
1287 changes have been highlighted in yellow in the text.

1288

1289 II. Figure 2:

1290 Fig 2A - you might try to line up your red boxes better so they better represent the
1291 blow ups (and make straighter red lines).

1292 **Response:** Thank you for your helpful comment. We deeply agree with your
1293 suggestion and we have made correction according to your nice suggestions.

1294

1295 Fig 2B - add microscopy information for the antibody stains in the legend and/or the
1296 methods section. Although the staining method cites another paper, it's best to include
1297 antibody information in the methods section. MUC2, ZO-1, and the blue marker are

1298 not labeled in the figure and in the figure legend.

1299 **Response:** Thank you for your helpful comment. We agree with your suggestion, and
1300 we have added the description in the legend and method section according to your
1301 suggestion. The details of the modification are as follows:

1302 On the other hand, 8 mice were selected from each group, and their colonic tissues
1303 were labeled with mucin 2 and ZO-1 antibodies, respectively [75], for further
1304 immunofluorescence staining (Servicebio, Wuhan, China). Fluorescein is linked to the
1305 antibodies ZO-1 and MUC-2 to form fluorescent antibodies. By specifically binding
1306 to the antigen to form a multi-component complex, ZO-1 and MUC-2 can be
1307 characterized and localized in the intestinal tissue by means of a fluorescence
1308 microscope research. (Page 25, line: 710-716)

1309 **FIGURE LEGENDS**

1310 Fig. 2. Effects of Lp082 on histological parameters and immunofluorescent proteins.
1311 (d) Immunofluorescence staining of MUC-2 (green fluorescence). Scale bar = 100 μ m.
1312 Blue marker is the color of the negative of the photograph (colon tissue without
1313 antigenic markers)
1314 (e) Immunofluorescence staining of ZO-1 (red fluorescence). Scale bar = 100 μ m.
1315 Blue marker is the color of the negative of the photograph (colon tissue without
1316 antigenic markers) (Page 40, line: 1164-1169)

1317

1318 Fig 2C - the y axis is missing a metric

1319 **Response:** Thank you very much for your reminder. We are very sorry for our
1320 negligence of metric. **Fig. 2c**-Y axis refers to the thickness of the intestinal mucosal
1321 wall, and its measurement method has been added to the material method section. We
1322 have carefully checked the full text and have highlighted the changes in yellow. The
1323 details are as follows. (Page 24, line: 706-709)

1324 The thickness of the intestinal mucosal wall was measured in the following ways:
1325 Image-Pro Plus 6.0 analysis software was used to measure the thickness of the
1326 mucosal layer at 5 positions of each layer (first from the right) in a unified mm
1327 standard unit, and the average value was calculated.

1328

1329 Fig 2f-g - the y axes are missing metrics (as noted above, the method to define these
1330 numbers is not stated).

1331 **Response:** Thank you for your helpful comment. We are very sorry for our
1332 negligence of metric. **Fig. 2c**-Y axis refers to the areal density of MUC-2 and ZO-1,
1333 and its measurement method has been added to the material method section. We have
1334 carefully checked the full text and have highlighted the changes in yellow. The details
1335 are as follows. (Page 25, line: 716-724)

1336 The surface density of immunofluorescence ZO-1 and MUC-2 was measured and
1337 calculated as follows: Eclipse CI-L fluorescence photography microscope was used to
1338 select the target area of tissues for 200-fold imaging. After the imaging was completed,
1339 image-Pro Plus 6.0 analysis software was used to convert green/red fluorescent
1340 monochrome photos into black and white pictures, and then the same black was
1341 selected as the unified standard to judge the positivity of all photos. The pixel area
1342 was used as the standard unit. The positive cumulative optical DENSITY (IOD) and
1343 the corresponding tissue pixel area in each section were measured, respectively, and
1344 areal density =IOD/area was calculated.

1345

1346 III. Figure 3:

1347 Fig 3A-C groupings not labeled as indicated above

1348 **Response:** Thank you for your comment. We are grateful for your reminder. To be
1349 more clear and in accordance with the reviewer's concerns,, we have added **Fig. S3**
1350 to explain the groupings in **Fig 3a-3c**. We also supplemented the description of this part
1351 in the supplementary material. The revised content is highlighted in yellow. The
1352 specific content is as follows. (Page 3, line: 22-33)

1353 **SUPPLEMENTARY FIGURE LEGENDS**

1354 **Fig.S3**

1355 (a) Timing and grouping of mouse metagenomic sequencing

1356 M means the modeling period, T means the treatment period. Respectively, A, B, C

1357 and D group mean 7 days normal water (ultrapure water), DSS, Lp082 and SASP
1358 treatment after 7 days DSS gavage.

1359 M-A means A group represents the control group on the 7th day of DSS modeling,
1360 M-B represents the DSS group on the 7th day of DSS modeling, M-C represents the
1361 Lp082 group on the 7th day of DSS modeling, M-D represents the SASP on the 7th
1362 day of DSS treatment Group.

1363 T-A means treating-A group represents the control group at the end of the treatment,
1364 T-B represents the DSS group at the end of the treatment, T-C represents the Lp082
1365 group at the end of the treatment, and T-D represents the SASP group at the end of the
1366 treatment.

1367

1368 Fig 3D - The meaning of the red highlighting is not indicated in the figure legend. No
1369 information is provided about the tree, including what it represents and what the
1370 colors indicate. The heat map values are not described - what is being compared and
1371 what does a value of zero mean?

1372 **Response:** Thank you for your helpful comment and your remind, we have
1373 supplemented the description of the figure in the legend and all revisions have been
1374 highlighted, and the revised content is as follows. (Page 41, line: 1181-1193)

1375 FIGURE LEGENDS

1376 **Fig. 3.** Effects of Lp082 strains on the gut microbiota in mice.

1377 (d)The red highlight in the **Fig. 3d** refers to the significantly increased bacteria that
1378 can produce SCFAs in the Lp082 group. The tree in the **Fig. 3d** represents the
1379 phylogenetic tree, which is obtained by clustering the abundance of each color block
1380 based on the unifracs distance after taking $\log_2(x*100)$ for the relative abundance at
1381 the species level. The clustering does not reflect any evolutionary relationship. It
1382 shows the abundance of bacterial species in the sample. 0 has no special meaning in it
1383 (it is only used to facilitate the differentiation of overall abundance). The darker the
1384 yellow in the color block in the Fig. 3d (the value closer to 2), the higher the relative
1385 abundance. Darker blue (values closer to -2) indicate lower relative abundance.

1386

1387 IV. Figure 4:

1388 Fig. 4A - It is not entirely clear where this data comes from. My assumption was the
1389 metagenome, but the Acetic acid sub section has me unsure. Describe this figure more,
1390 taking care to describe what the acetic acid subsection is evaluating.

1391 **Response:** Thank you for your helpful comment. We are sorry to have failed to make
1392 it clear and are very sorry about the inconvenience caused. According to your helpful
1393 suggestions, we re-describe **Fig. 4** and the rewritten content is as follows: (Page 10,
1394 line: 286-346)

1395 **The regulatory role of SCFAs**

1396 Next, we conducted a correlation analysis between Lp082 (*lactobacillus*
1397 *plantarum*) and SCFAs, and found that Lp082 (*lactobacillus plantarum*) was strongly
1398 positively correlated with SCFAs (acetic acid, propionic acid, butyric acid) (**Fig. 4c**),
1399 the correlation results suggested that Lp082 can increase the content of SCFAs. The
1400 above results inspired us to further explore the relationship between Lp082 and
1401 SCFAs, and we further analyzed the bacterial species and metabolic pathways
1402 associated with SCFAs. Further metagenomic data provided support for our above
1403 speculation. Combined with metagenomic data, the species composition of mice gut
1404 microbiota was further analyzed. The results showed that the relative abundance of
1405 some special bacteria increased in the Lp082 group, such as, *lactobacillus plantarum*,
1406 *Bifidobacterium pseudolongum*, *Akkermansia muciniphila*, *Bacteroides ovatus*,
1407 *Parabacteroides distasonis*, *Lactobacillus reuteri*, *Anaerotruncus sp G3 2012* (these
1408 bacteria are highlighted in red in **Fig. 3d**), all of which can metabolize produces the
1409 SCFAs [1].

1410 Subsequently, we further analyzed the metabolic pathways of gut microbiota in
1411 mice. Results of differential metabolic pathways showed that the abundance of gut
1412 microbiota metabolic pathways related SCFAs production decreased in DSS group but
1413 increased in Lp082 group (**Fig. 4a**). We infer that Lp082 can promote the content of
1414 SCFAs (acetate, propionate and butyrate) by adjust three metabolic pathways,
1415 including Pyruvate fermentation to Propanoate I, Pyruvate fermentation to acetate and

1416 lactate II, Acetyl CoA fermentation to Butanoate (**Fig. 4a**).

1417 To prove the above findings, we further used gas chromatography-mass
1418 spectrometry (GC-MS) to detect the content of SCFAs. Compared with control group,
1419 the contents of butyric acid, valeric acid, acetic acid, propionic acid and isobutyric
1420 acid were significantly decreased after ingestion of DSS ($P < 0.01$). Compared with
1421 DSS group, the contents of butyric acid, acetic acid, propionic acid and isobutyric
1422 acid were extremely significant increased after ingestion of Lp082 ($P < 0.01$). This
1423 confirmed our previous hypothesis based on the correlation that Lp082 intake would
1424 increase SCFAs levels (**Fig. 4b**). Based on the above results, we speculate that Lp082
1425 increase the content of SCFAs by affecting the abundance of SCFAs-producing
1426 microbes, as well as the metabolic pathways of SCFAs-producing microbes.

1427 To further understand the role of SCFAs, we performed a Pearson correlation
1428 analysis. The results showed that *helicobacter hepatica*, which was significantly
1429 increased in the DSS group, was strongly negatively correlated with acetic acid,
1430 propionic acid, and butyric acid (**Fig. 4c**). *lactobacillus plantarum*, *Bifidobacterium*
1431 *pseudolongum*, *Akkermansia muciniphila*, *Parabacteroides distasonis*, *Lactobacillus*
1432 *reuteri*, which were significantly increased in Lp082 group showed strong positive
1433 correlation with acetic acid, propionic acid, and butyric acid. *Anaerotruncus sp G3*
1434 *2012* and *Bacteroides ovatus* showed a strong positive correlation with butyric acid
1435 and acetic acid, and a weak positive correlation with propionic acid (**Fig. 4c**). These
1436 SCFAs including acetic acid, propionic acid, and butyric acid were all strong
1437 negatively correlation with the pro-inflammatory factors TNF- α , IL-1 β , IFN- γ , IL-6,
1438 MPO but strongly positively correlated with the inflammatory suppressor IL-10 (**Fig.**
1439 **4d**). As important products of gut microbiota metabolism, SCFAs have certain
1440 anti-inflammatory effects and play an important role in maintaining normal intestinal
1441 morphology and function. Combined with the results of **Fig. 3d**, **Fig. 4a-4d**, as well
1442 as the improvement of physiological indicators (**Fig. 1b-1d**), pathological indicators
1443 (**Fig. 2a-2g**) and inflammatory factors (**Fig. 1e**) after ingestion of Lp082, we
1444 speculated that Lp082 may alleviate DSS-induced UC by regulating SCFAs through
1445 the following mechanisms (**Fig. S4**). That is, after the ingestion of Lp082, the

1446 abundance of the intestinal microbes of SCFAs-producing increased, which promoted
1447 the content of SCFAs. The SCFAs has the function of promoting the secretion of
1448 inflammatory cytokine and suppressing the secretion of inflammatory factors. The
1449 changes in inflammatory cytokines affect the physiological indicators of mice, which
1450 increases the weight, colon length, drinking water and eating volume of mice, and
1451 reduces the DAI score and immune organs index. The changes in inflammatory
1452 cytokines also affected the pathological indexes of mice, resulting in a decrease in
1453 histopathological score and an increase in immunofluorescence protein content of
1454 ZO-1 and MUC-2.

1455 **Reference**

1456 1. Y. W. Cheng, J. M. Liu and Z. X. Ling, Short-chain fatty acids-producing
1457 probiotics: A novel source of psychobiotics, Critical Reviews in Food Science and
1458 Nutrition, DOI: 10.1080/10408398.2021.1920884.

1459

1460 Fig. 4C-D - A description of the tree components is missing. Describe the correlation
1461 analysis more in the text and figure legend.

1462 **Response:** Thank you for your helpful comment and your reminder. We are sorry to
1463 have failed to describe it clearly and are very sorry about the inconvenience caused.
1464 According to your helpful suggestions, we have supplemented the description of the
1465 figure in the legend, and all revisions have been highlighted, and the revised content is
1466 as follows:

1467 The following sections have been added to the legend: (Page 41, line: 1198-1241)

1468 **FIGURE LEGENDS**

1469 **Fig. 4.**

1470 (c)Relationship between SCFAs and gut microbiota. The tree in the **Fig. 4c** represents
1471 the phylogenetic tree, which is obtained by clustering the data. This clustering does
1472 not reflect any evolutionary relationships but rather shows the abundance of the
1473 samples. **Fig. 4c** is a correlation heat map drawn by Pearson correlation analysis
1474 based on bacterial abundance and SCFAs abundance. The correlation range is from -1
1475 to +1. The closer to -1 or +1, the stronger the correlation between bacterial species

1476 and SCFAs. 0 means no correlation, a negative value means negative correlation, and
1477 a positive value means positive correlation.

1478 (d) Relationship between SCFAs and inflammatory cytokines. The tree in the **Fig. 4d**
1479 represents the phylogenetic tree, which is obtained by clustering the data. This
1480 clustering does not reflect any evolutionary relationships but rather shows the
1481 abundance of the samples. **Fig. 4d** is a correlation heat map drawn by Pearson
1482 correlation analysis based on the content of inflammatory cytokines and the
1483 abundance of SCFAs. The horizontal axis in the **Fig. 4d** is the clustering based on the
1484 abundance of SCFAs, and the vertical axis is based on the abundance of inflammatory
1485 cytokines. 0 means no correlation, a negative value means negative correlation, and a
1486 positive value means positive correlation.

1487 The following sections have been added to the manuscript: (Page 12, line: 319-330)

1488 To further understand the role of SCFAs, we performed a Pearson correlation
1489 analysis. The results showed that helicobacter hepatica, which was significantly
1490 increased in the DSS group, was strongly negatively correlated with acetic acid,
1491 propionic acid, and butyric acid (**Fig. 4c**). lactobacillus plantarum, Bifidobacterium
1492 pseudolongum, Akkermansia muciniphila, Parabacteroides distasonis, Lactobacillus
1493 reuteri ,which were significantly increased in Lp082 group showed strong positive
1494 correlation with acetic acid, propionic acid, and butyric acid. Anaerotruncus sp G3
1495 2012 and Bacteroides ovatus showed a strong positive correlation with butyric acid
1496 and acetic acid, and a weak positive correlation with propionic acid (**Fig. 4c**). These
1497 SCFAs including acetic acid, propionic acid, and butyric acid were all strong
1498 negatively correlation with the pro-inflammatory factors TNF- α , IL-1 β , IFN- γ , IL-6,
1499 MPO but strongly positively correlated with the inflammatory suppressor IL-10 (**Fig.**
1500 **4d**).

1501

1502 V. Figure 5: I think this entire figure would be best placed in the supplement as it's
1503 really just a sub-point of the contents of figure 6 (but it won't fit in figure 6). You
1504 might also remove "distribution" from the title and legend as this suggests tissue
1505 spatial information but is not needed.

1506 **Response:** Thank you for your helpful comment. We agree with the suggestions of the
1507 reviewer. To be more clear and in accordance with the reviewer's concerns, we
1508 re-described **Fig. 4a** and **Fig. 4b** and have put the entire figure of **Fig. 5** in the
1509 supplement according to your suggestion and named it **Fig. S5**. The revised content
1510 has been highlighted in yellow.

1511

1512 VI. Figure 6: Overall, the less color you use, the clearer this figure will be.

1513 **Response:** Thank you for your comment. We will take this into account in future
1514 drawings. We are grateful for the suggestion. As suggested by the reviewer, we have
1515 made some adjustments to the graphics. We have been deeply aware of this problem,
1516 and we will also pay attention to reducing the use of colors in future drawings. Thank
1517 you again for your help.

1518

1519 Fig 6A-C: I recommend condensing as Fig 6A. Describe what gene ratio is in the
1520 figure legend.

1521 **Response:** Thank you for your comment. We agree with your suggestion. According
1522 to your helpful suggestions, we have renamed **Fig. 6a-6c** to **Fig. 6a** and have added a
1523 description of the gene ratio in the legend. All revisions have been highlighted, and
1524 the revised content is as follows: (Page 42, line: 1223-1224)

1525 Gene Ratio: Ratio of the number of genes related to this Term to the total number of
1526 genes

1527

1528 Fig 6D-F: I recommend condensing as Fig 6B.

1529 **Response:** Thank you for your comment. We agree with your suggestion. According
1530 to your helpful suggestions, we have renamed **Fig. 6d-6f** to **Fig. 6b**.

1531

1532 Fig 6G-J: I recommend condensing as Fig 6C. I and j legends are swapped. Describe
1533 ifcSE in the legend.

1534 **Response:** Thank you for your comment. We agree with your suggestion. According
1535 to your helpful suggestions, we have renamed **Fig. 6g-6j** to **Fig. 6c**. and have

1536 supplemented the description of the figure in the legend, all revisions have been
1537 highlighted, and the revised content is as follows: (Page 43, line: 1233-1236)

1538 The IfcSE is the standard error, which is the value obtained from the standard
1539 deviation (SD) of the sample divided by the square root of the previous sample size.
1540 The smaller the standard error is, the smaller the difference between sample mean and
1541 population mean is.

1542

1543 2. The authors confuse whether they are studying Lp082 prevention or treatment of
1544 colitis by using verbiage referring to "prevention" and "treatment" interchangeably.
1545 This makes it difficult to track what the authors are trying to accomplish (for example,
1546 line 60 says "relieving", lines 76 and 87-88 say "prevention"). Because the authors
1547 state that the colitis inducer (DSS) is administered at the time of treatment (Lp082) in
1548 the beginning of the Results to evaluate prevention (line 87), but Figure 1A shows that
1549 Lp082 is being added at day 8 (so not at the time of induction), I cannot assess which
1550 is being studied: Lp082 1) treatment or 2) prevention of UC. My best assumption is
1551 that the methods section is correct, and the methods says that DSS is used prior to
1552 addition of Lp082, and thus the authors are studying Lp082 relief of colitis. Thus, the
1553 language in the paper should be altered to indicate that Lp082 was administered after
1554 DSS induced colitis and observed effects are Lp082 alleviation of symptoms, not
1555 prevention of symptoms.

1556 **Response:** We appreciate your valuable and helpful comment. We apologize for the
1557 language problems in the original manuscript. We sincerely apologize for the
1558 confusion caused to you. We used DSS to establish a model of UC and then treated it
1559 with Lp082. We have carefully checked the wording of the full text and corrected the
1560 preventive effect to the therapeutic effect. Thank you very much for pointing this out.
1561 It was very helpful. The changes have been highlighted in yellow in the article. And
1562 the language presentation was improved with assistance from a native English speaker
1563 with appropriate research background.

1564

1565

1566

1567 3. The abstract, discussion section, and figure 7B describe the effect of Lp082 on the
1568 animal model through the groups: biological barrier, chemical barrier, mechanical
1569 barrier, and immune barrier. I don't recommend subdividing "biological, chemical,
1570 and mechanical barrier", as everything you are referring to is biological, chemical,
1571 and mechanical in nature. Rather, use categories akin to "microbiota/microbiome
1572 alterations, barrier function improvements, and inflammation reduction."

1573 **Response:** We appreciate your valuable and helpful comment. You have provided an
1574 excellent suggestion. Thank you for pointing out this problem. We agree with your
1575 views on this issue. Following your suggestion, the discussion of these four intestinal
1576 barriers has been rewritten in the discussion section, but we think it is reasonable to
1577 describe it in terms of these four barriers. The pathogenesis of UC is the result of the
1578 combined effect of genetically susceptible hosts and the environment, and its common
1579 pathological outcome is the damage of the structure and function of the intestinal
1580 mucosal barrier. The intestinal mucosal barrier is damaged, resulting in an increase in
1581 the permeability of the intestinal epithelial barrier, and further stimulation of intestinal
1582 contents, bacteria, and toxins promotes the immune response to intestinal
1583 inflammation. The normal intestinal mucosal barrier consists of mechanical barrier,
1584 chemical barrier, immune barrier, and biological barrier. The chemical barrier refers to
1585 the glue-like mucin layer covering the surface of intestinal epithelial cells, which is
1586 mainly composed of MUC-2 secreted by goblet cells, digestive juices, and
1587 bacteriostatic substances produced by normal parasitic bacteria in the intestinal lumen
1588 [1]. The mechanical barrier is the most important part of the intestinal mucosal barrier.
1589 Its structural basis is the intestinal mucosal epithelial cells and the tight junctions (TJ)
1590 between the epithelial cells [2]. The immune barrier is associated with immune cells,
1591 and inflammatory factors [3]. The biological barrier is a normal intestinal colony of
1592 bacteria that is resistant to colonization by foreign strains [4]. The results of the study
1593 found that Lp082 can improve the intestinal mucosal barrier by synergistically
1594 optimizing the biological barrier, chemical barrier, mechanical barrier and immune
1595 barrier, thereby alleviating UC. Specifically, We found that Lp082 rebuilt the

1596 biological barrier by regulating the intestinal microbiome and increasing the SCFAs.
1597 Lp082 improved the chemical barrier by reducing ICAM-1, VCAM, and increasing
1598 goblet cells and mucin2. Lp082 ameliorated the mechanical barrier by increasing the
1599 ZO-1, ZO-2, and occludin and decreasing claudin-1 and claudin-2. Lp082 optimized
1600 the immune barrier by reducing the content of IL-1 β , IL-6, TNF- α , MPO, IFN- γ and
1601 increasing the IL-10, TGF- β 1, and TGF- β 2. In conclusion, we believe that it is
1602 reasonable to use these four barriers to discuss the effect of Lp082 on DSS induced
1603 UC. Maybe we didn't describe it very well, so we rewrote a discussion section that
1604 explained the four barriers in more detail, with the following changes. (Page 17, line:
1605 496-637)

1606 Lp082 improved chemical barrier

1607 The chemical barrier refers to the glue-like mucin layer covering the surface of
1608 intestinal epithelial cells, which is mainly composed of MUC-2 secreted by goblet
1609 cells, digestive juices and bacteriostatic substances produced by normal parasitic
1610 bacteria in the intestinal lumen [1]. The chemical barrier plays an important role in
1611 isolating the internal and external environment of the intestinal tract, lubricating the
1612 intestinal mucosa, and inhibiting the entry of harmful substances in the intestinal
1613 lumen [5]. The intestinal mucosal wall thickness was significantly increased in the
1614 DSS group, whereas it was significantly decreased after Lp082 ingestion (**Fig. 2c**). In
1615 DSS-induced UC, the thicker the intestinal mucosal wall, indicating more severe
1616 inflammation. In addition, the H&E staining result showed that the number of goblet
1617 cells decreased in the DSS group (red arrow), whereas the number of goblet cells
1618 increased (yellow arrow) after Lp082 ingestion (**Fig. 2a**). The immunofluorescent
1619 protein content of MUC-2, which is mainly secreted by goblet cells, was significantly
1620 decreased in the DSS group (**Fig. 2d**), and the areal density of MUC-2 (**Fig. 2f**) and
1621 the mRNA expression of MUC-2 were also significantly decreased in the DSS group
1622 (**Fig. 5c**), while the immunofluorescence protein content, areal density and mRNA
1623 expression of MUC-2 all increased in the Lp082 group,
1624 Sun et al. [6] observed the same phenomenon that *lactobacillus plantarum 12* can
1625 repair the intestinal mucosal chemical barrier by increasing the content of MUC-2.

1626 Burger-van Paassen et al. [7] found that intake of SCFAS could increase the
1627 expression abundance of MUC-2 mRNA in cells. The mRNA expressions of ICAM-1
1628 and VCAM-1 were significantly decreased after Lp082 intake. Taniguchi et al. [8]
1629 found that anti-ICAM-1 treatment significantly attenuated colonic mucosal damage,
1630 while Philpott et al. [9] found that adhesion molecules ICAM-1 & VCAM-1 induced
1631 intestinal mucosal lesions. Lp082 has been shown to be effective in relieving
1632 intestinal mucosal lesions (i.e., reduced ulceration and inflammatory cell infiltration
1633 caused by DSS). So, we speculate that Lp082 reduces mucosal lesions by reducing
1634 ICAM-1 and VCAM. The above results showed that probiotic Lp082 increased the
1635 MUC-2 content in the mucus layer by restoring the number of goblet cells, relieved
1636 the intestinal mucosal damage caused by ICAM-1 and VCAM-1, so as to repaired the
1637 chemical barrier.

1638 Lp082 improved mechanical barrier

1639 The mechanical barrier is the most important part of the intestinal mucosal
1640 barrier. Its structural basis is the intestinal mucosal epithelial cells and the tight
1641 junctions (TJ) between the epithelial cells [2]. The mechanical barrier can effectively
1642 prevent harmful substances such as bacteria and endotoxins from entering the blood
1643 through the intestinal mucosa.iers The aberrant structure of tight junction (TJ)
1644 proteins between intestinal epithelial cells, such as the reduction of ZO-1, ZO-2, and
1645 occludin, is one of the critical factors leading to the disruption of the gut mechanical
1646 barrier in UC patients [10]. Several studies have identified TJ protein as a new target
1647 for the current treatment of UC [11]. Because Lp082 excellently improved
1648 histopathology, we speculated that Lp082 also has a regulatory effect on TJ molecules.
1649 To this end, we analyzed major TJ proteins, including ZO-1, ZO-2, occludin. As
1650 expected, the mRNA expression and immunofluorescence protein content of ZO-1
1651 and the mRNA expression of ZO-2 and occludin were significantly decreased in
1652 DSS-induced UC mice but improved in the Lp082 treatment group. These are
1653 consistent with the findings of Cordeiro et al. [12] that ZO-1 and ZO-2 were
1654 significantly decreased in UC but increased after probiotic Minas Frescal cheese
1655 intake, indicating that the improvement of the mechanical barrier by regulating TJ

1656 may be one of the mechanisms by which probiotic Lp082 exerts anti-UC. In addition,
1657 the mRNA expression of another particular tight junction protein, ICAM-1 and
1658 VCAM-1, was increased in the DSS group. It is consistent with the findings of
1659 elevated ICAM-1 and VCAM-1 in IBD patients in clinical studies [13]. Mitselou et al.
1660 [14] found that the adhesion molecules ICAM-1 and VCAM-1 induced intestinal
1661 mucosal injury. Taniguchi et al. [8] found that anti-ICAM-1 treatment attenuated
1662 colonic mucosal injury. It has been reported that ICAM-1 and VCAM-1 can increase
1663 the permeability of intestinal mucosa [15]. Interestingly, the mRNA expression of
1664 ICAM-1 and VCAM-1 was found to decrease after Lp082 ingestion. Therefore, it can
1665 be thought that the alleviation of UC by Lp082 may be due to down-regulation of
1666 ICAM-1, VCAM-1 and increase protein quantity and mRNA expression of
1667 ZO-1, ZO-2, so as to reduce intestinal mucosal permeability, thereby inhibiting the
1668 entry of harmful bacteria and undigested food and toxins into the body and reducing
1669 inflammation. These results suggest that Lp082 repairs the intestinal mechanical
1670 barrier by regulating TJ.

1671 Lp082 improved the immune barrier

1672 Although the exact etiology of UC is complex and uncertain, studies suggest that
1673 the NF- κ B pathway plays a vital role in the pathogenesis of UC [3]. Our study has
1674 proved that Lp082 inhibits the NF- κ B pathway by down-regulating the mRNA
1675 expression of NF- κ B2, NF- κ B1, COX-2, Rel α , Toll4, iNOS, and that NF- κ B can also
1676 regulate inflammation by regulating cytokines [16]. Therefore, it can be suggested
1677 that Lp082 also has a specific regulatory effect on cytokines. To confirm this, we
1678 analyzed the cytokines associated with NF- κ B. As expected, we observed that the
1679 mRNA expression level content of pro-inflammatory cytokines (TNF- α , IL-1 β , and
1680 IL-6) were significantly increased in the DSS group but significantly decreased in the
1681 Lp082 group, It is interesting to note that the protein levels of TNF- α , IL-1 β , and IL-6
1682 detected by elisa kit were also increased in the DSS group and decreased after Lp082
1683 intake. Among them, TNF- α can promote the proliferation and differentiation of T
1684 cells and increase intestinal inflammation [17]. The upregulation of IL-1 β is involved
1685 in the recruitment and retention of leukocytes in inflamed tissues and can activate

1686 innate immune lymphocytes [18]. IL-6 activates NF- κ B to regulate the dextran sulfate
1687 sodium-induced colitis in mice [19]. The above results indicate that Lp082 alleviates
1688 UC by inhibiting the levels of pro-inflammatory factors (TNF- α , IL-1 β , and IL-6).
1689 Interestingly, we also found that the mRNA expressions of anti-inflammatory
1690 cytokines IL10, TGF-1, and TGF-2 were significantly decreased in the DSS group but
1691 increased in the Lp082 group. Il-10 protein levels measured by elisa kit also decreased
1692 in the DSS group and increased in the Lp082 group. Surprisingly, IL10, TGF-1, and
1693 TGF-2 were shown to activate Treg and anti-inflammatory macrophages to alleviate
1694 UC [20]. And Sato et al. [21] also found that the loss of IL-10 spontaneously gave rise
1695 to IBD, and Hume et al. [22] found that TGF- β 1 and TGF- β 2 could dramatically
1696 relieve intestinal inflammation in DSS-induced colitis mice. These results suggest that
1697 Lp082 alleviates UC by increasing the levels of anti-inflammatory factors IL10,
1698 TGF-1, and TGF-2. We further analyzed the specific regulatory effects of Lp082 on
1699 intestinal mucosal immunity. In addition to inflammatory factors, we also noticed that
1700 a heme protein, MPO, was significantly reduced in the Lp082 group. Trevisin et al.
1701 [23] found that MPO caused UC by producing cytokines and hypochlorite and that
1702 MPO in the colon of UC patients is mainly produced by neutrophil infiltration [24].
1703 Interestingly, this is consistent with the fact that the DSS group had a severe
1704 neutrophil infiltration in this study. However, neutrophil infiltration and MPO content
1705 were significantly decreased in Lp082 group. This shows that Lp082 alleviates UC by
1706 reducing neutrophil infiltration and its secreted MPO content. In a nutshell, our results
1707 suggest that Lp082 may play an anti-UC effect by inhibiting the NF- κ B pathway,
1708 down-regulating pro-inflammatory cytokines, and up-regulating anti-inflammatory
1709 cytokines, reducing MPO content, thereby maintaining immune balance and
1710 protecting the immune barrier.

1711 The mucosal immune system of the intestine mainly consists of Peyer's patch
1712 and lamina propria under enterocyte [25]. The Peyer's patch can deliver captured
1713 antigens to dendritic cells [26]. Then dendritic cells can not only trigger T
1714 cell-mediated cellular immunity and B cell-mediated humoral immunity by presenting
1715 antigens but also affect lamina propria immunity [27]. Combining previous studies,

1716 we found that DSS causes inflammation through the following six ways. First, gut
1717 permeability increases, and harmful substances enter to activate innate immunity, such
1718 as stimulating innate immune cells to produce TNF- α , IL-1 β , and IL-6 [28]. Second,
1719 regulatory T cells produce less IL-10 and have a less inhibitory effect on effector T
1720 cells, resulting in the phenomenon of effector T and regulatory T cell dysregulation in
1721 UC patients [29]. Third, effector T cells promote B cell-mediated humoral immunity
1722 by promoting the secretion of IFN- γ and L-17A [30]. Fourth, effector T cells carried
1723 out immune cell recruitment and formed a vicious immune cycle with chemokines
1724 and cytokines [31]. Fifth, Peyer's patch recognizes antigens and presents them to other
1725 immune cells through dendritic cells [26]. Sixth, antigen-activated neutrophils can
1726 both secrete MPO and recruit more immune cells from the bloodstream to the site of
1727 inflammation, further exacerbating inflammation [32] (**Fig. 6b**). Based on the above 6
1728 reasons, we suggest that in addition to relieving inflammation by inhibiting the NF- κ B
1729 pathway, Lp082 can also regulate inflammatory factors to maintain the balance
1730 between regulatory T cells and effector T cells to regulate intestinal mucosal
1731 immunity, thus maintaining the intestinal mucosal barrier.

1732 Lp082 improved the biological barrier

1733 Numerous studies [23] have shown that probiotics improve the clinical outcome
1734 of IBD patients by influencing host gut microbiota [4]. Herein, we performed a
1735 shotgun metagenomic analysis to investigate whether Lp082 can improve gut
1736 dysbiosis in the UC mice model. As expected, we observed that the intake of DSS
1737 significantly reduced the shannon value but increased PCoA distance, a finding that is
1738 consistent with Wang et al. [33]. The Shannon index reflects gut microbiota richness
1739 and uniformity and is positively correlated with gut microbiota diversity, while the
1740 PCoA distance reflects the difference in the structure of the gut microbiota between
1741 different groups; the higher the PCoA value, the greater the difference in the gut
1742 microbiota structure [34]. In particular, Lp082 treatment remarkably increased the gut
1743 microbiota diversity and reduced gut microbiota structural differences in gut
1744 microbiota, as shown by the cluster analysis and PCoA analysis. On the other hand,
1745 Lp082 also optimized species composition; that is, the abundance of

1746 pro-inflammatory microbiota decreased in the Lp082 group, such as *Helicobacter*
1747 *hepaticus*, a potential pathogen of colitis. Likewise, we observed an increasing trend
1748 in the abundance of potential probiotics in the Lp082 group, such as *Bifidobacterium*
1749 *pseudolongum* and *Bacteroides ovatus*, which reduces colonic inflammation [35],
1750 *Parabacteroides distasonis*, which is negatively associated with obesity and diabetes
1751 [36], *Akkermansia muciniphila* and *Lactobacillus reuteri*, a widely studied probiotic,
1752 *Anaerotruncus sp G3 2012* and *lactobacillus plantarum*, potential SCFAs-producing
1753 bacteria [37]. The above results indicate that Lp082 is beneficial to optimizing the
1754 diversity, structure, and composition of gut microbiota. After demonstrating that
1755 Lp082 can increase the abundance of potential SCFAs-producing bacteria, further
1756 analysis found that Lp082 can activate two SCFAs-producing microbial metabolic
1757 pathways and the content of SCFAs. Subsequently, correlation analysis proved that
1758 Lp082 may increase SCFAs by activating the SCFAs-producing metabolic pathway of
1759 SCFAs-producing bacteria, so as to inhibit inflammation [38] and regulate host
1760 physiological activity through SCFAs [39]. All of these suggest that Lp082 repaired
1761 the microbial barrier by regulating the gut microbiome.

1762 In conclusions, the Lp082 has an exciting therapeutic effect on UC than SASP.
1763 Also, shotgun metagenome and transcriptome analysis confirmed that Lp082 could
1764 improve gut microbiota dysbiosis, protect intestinal mucosal barrier, regulate
1765 inflammatory pathways, and affect neutrophil infiltration. These findings firmly
1766 support and advocate the clinical translation of Lp082 in the treatment of UC. It can
1767 be suggested that the application of gut microbiota and probiotics in the treatment of
1768 UC should receive more attention. The findings of this study not only provide new
1769 clues for revealing the complex mechanism of gut microbiota in relieving UC, but
1770 also provide evidence for Lp082 as a potential gut microbiota regulator to treat UC.

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1908

1909 4. In general, the abstract could be re-written to describe the results from a higher
1910 level, rather than just listing the altered genes. Close the abstract with a statement
1911 connecting the paper results to the broader scientific field.

1912 **Response:** We appreciate your valuable and helpful comment. According to your
1913 suggestion, we have rewritten the abstract. The rewritten content links the results of
1914 the paper with the broader scientific field. The revised content is as follows. (Page 2,
1915 line: 25-59)

1916 Probiotics can effectively improve ulcerative colitis (UC), but the mechanism is still
1917 unclear. Here, shotgun metagenomic and transcriptome analyses were performed to
1918 explore the therapeutic effect and the mechanism of the probiotic *lactobacillus*
1919 *plantarum* HNU082 (Lp082) on UC. The results showed that Lp082 treatment
1920 significantly ameliorated dextran sulfate sodium (DSS) -induced UC in mice, which
1921 was manifested as increases in body weight, water intake, food intake, colon length,
1922 and decreases in disease activity index (DAI), immune organ index, inflammatory
1923 factors, and histopathological scores after Lp082 intake. An in-depth study discovered
1924 that Lp082 could improve the intestinal mucosal barrier and relieve inflammation by
1925 co-optimizing the biological barrier, chemical barrier, mechanical barrier and immune

1926 barrier. Specifically, Lp082 rebuilt the biological barrier by regulating the intestinal
1927 microbiome and increasing the production of short-chain fatty acids (SCFAs). Lp082
1928 improved the chemical barrier by reducing intercellular cell adhesion molecule-1,
1929 vascular cell adhesion molecule and increasing goblet cells and mucin2. Lp082
1930 ameliorated the mechanical barrier by increasing the zonula occludens-1 (ZO-1),
1931 zonula occludens-2 (ZO-2), and occludin while decreasing claudin-1 and claudin-2.
1932 Lp082 optimized the immune barrier by reducing the content of IL-1 β , IL-6, TNF- α ,
1933 MPO, IFN- γ and increasing the IL-10, TGF- β 1, and TGF- β 2, inhibiting the NF-kB
1934 signalling pathway. Taken together, probiotic Lp082 can play a protective role in a
1935 DSS-induced colitis mouse model by protecting the intestinal mucosal barrier,
1936 attenuating the inflammatory response, and regulating microbial imbalance. This
1937 study provides support for the development of probiotic-based microbial products as
1938 an alternative treatment strategy for UC.

1939

1940 Importance

1941 Many studies have focused on the therapeutic effect of probiotics on UC, but few
1942 studies have paid attention to the mechanism of probiotics, especially the therapeutic
1943 effect. This study suggests that Lp082 has a therapeutic effect on colitis in mice. Its
1944 mechanisms of action include protect the mucosal barrier and actively modulate the
1945 gut microbiome, modulate inflammatory pathways and reduce neutrophil infiltration.
1946 Our study enriches the mechanism and provides a new prospect for probiotics in the
1947 treatment of colitis, helps to deepen the understanding of the intestinal mucosal barrier,
1948 and provides guidance for the future probiotic treatment of human colitis.

1949 Keywords: Lactobacillus plantarum HNU082, ulcerative colitis, intestinal mucosal
1950 barrier, short chain fatty acid, transcriptome, shotgun metagenome, cytokine

1951

1952 5. As written, lines 72-73 suggest Yucha has resistance to acid and bile salts, but I
1953 assume that the authors mean Lp082 is resistant. Re-wording the sentence and adding
1954 a clarification on what point the authors are trying to make about acid and bile salt
1955 resistance would help alleviate the confusion here.

1956 **Response:** Thank you for your comment. We deeply agree with your suggestion. It is
1957 true that we did not express it clearly. We apologize for the confusion caused to you.
1958 According to your helpful advice, we have revised this sentence and the revised
1959 content is as follows. (Page 4, line: 98-100)

1960 The strain of *Lactobacillus plantarum* HNU082 (Lp082) was originally isolated
1961 from a traditional fermented food-fish tea of the Li people in Hainan Province,
1962 China, which has a good safety profile and tolerance to acids and bile salts [1].

1963

1964 **Reference**

1965 1. Zhang J, Wang X, Huo D, Li W, Hu Q, Xu C, et al. Metagenomic approach
1966 reveals microbial diversity and predictive microbial metabolic pathways in Yucha, a
1967 traditional Li fermented food. *Scientific Reports*. 2016;6; doi: 10.1038/srep32524.

1968

1969 6. Referring to lines 77-92: The authors interchange physiological results with
1970 techniques as if they are the same things. Before describing the specific things you
1971 were evaluating, describe what you were looking for at a high level. Then separate
1972 physiological indicators from methods (e.g., rather than say, "evaluated physiological
1973 indexes and shotgun metagenomic sequencing," use language like "evaluated
1974 inflammation, microbial community composition and activity...using ELISA,
1975 immunohistochemistry, metagenomic sequencing, and RNA-seq."

1976 **Response:** We appreciate your valuable and helpful comment. We deeply agree with
1977 your suggestion. We do indeed have a language problem on this issue which created
1978 confusion. According to your helpful advice, we have changed this sentence and other
1979 places in the article. The revised content is as follows. (Page 23, line: 666-671)

1980 After the UC model was established by DSS, mice were given Lp082 by gavage to
1981 observe the therapeutic effect of the bacteria on DSS-induced UC.. Various tissue
1982 samples, including immune organs, serum, proximal colon, fecal, cecal contents,
1983 distal colon, and other tissues, were collected. Techniques such as ELISA,
1984 immunohistochemistry, metagenomic sequencing, and RNA-seq were used to assess
1985 inflammation, microbial community composition, and gene expression. (Fig. 1a).

1986

1987 7. Potentially incorrect information: Lines 97-98 days and scores do not line up with
1988 the data reported in figure 1B.

1989 **Response:** Thank you for your comment. We are very sorry for our incorrect writing.
1990 We apologize for the confusion caused to you. We have redescribed **Fig. 1b**, and the
1991 modified contents are as follows. (Page 6, line: 153-168)

1992 The results showed that from 1 to 7 days, the water intake, food intake, and body
1993 weight of the DSS group, the Lp082 group, and the SASP group all showed a similar
1994 degree of gradual decrease, and these three groups were all significantly different
1995 from the Control group on day 7 ($p < 0.05$), which may be because these three groups
1996 were all under the same DSS modeling conditions on days 0-7. Then on the 8th to
1997 15th day, the water intake, food intake, and body weight of the DSS group were still
1998 decreasing, but the water intake, food intake, and body weight of Lp082 and SASP
1999 group gradually increased. Specifically, the water and food intake of the Lp082
2000 combined SASP group increased significantly from day 9 ($p < 0.05$), and body weight
2001 increased significantly from day 12 ($p < 0.05$). The DAI index of the DSS group,
2002 Lp082 group, and SASP group increased significantly ($p < 0.05$) from the third day
2003 compared with the Control group. After stopping DSS gavage on the 8th day, the DAI
2004 index of the DSS self-healing group still increased, while that of the Lp082 group and
2005 SASP group gradually decreased from the 10th day, and the degree of decrease in the
2006 Lp082 group was greater than that in the SASP group (**Fig. 1b**).

2007

2008 8. Abbreviations should be described in the text as they arise, not in an additional
2009 section at the end of the paper (page 20).

2010 **Response:** We are grateful for the suggestion. Thank you very much for pointing out
2011 our problem, we deeply agree with your suggestion. According to your helpful advice,
2012 we have corrected this by adding a description of abbreviations to the article.

2013

2014 9. After revising the manuscript, a thorough and detailed assessment and correction of
2015 sentence structure would improve the readability of the paper dramatically.

2016 **Response:** We appreciate the reviewer's attention to the flaws of our text. After
2017 revising the manuscript, we have made a comprehensive and careful assessment and
2018 correction of the sentence structure and carefully checked the full text. The language
2019 presentation was improved with assistance from a native English speaker with an
2020 appropriate research background.

2021

2022 10. Abbreviations, capitalization, italics, and spacing are inconsistent throughout and
2023 should be fixed for a final draft. E.g. Lp082(most commonly used in the draft)/Lp082
2024 (lines 78-79) or HNU082 (correct)/HNU082 (line 23).

2025 **Response:** Thank you for your comment. We have carefully checked abbreviations,
2026 capitals, italics and spaces. We tried our best to improve the manuscript and made
2027 some changes in the manuscript. These changes will not influence the content and
2028 framework of the paper. And here we did not list the changes but marked in yellow in
2029 revised paper.

2030

2031 11. Review your usage of "prove" in your manuscript (notably in the discussion
2032 section) as the experiments presented provide largely correlative data.

2033 **Response:** Thank you for your comment and we have corrected this error and used
2034 the word "prove" more carefully. We also carefully checked the text to ensure the
2035 accuracy of our other words.

2036

2037 Once again, we thank you for the time you put into reviewing our paper. We have
2038 worked hard to answer your questions and look forward to meeting your expectations.
2039 If you have any dissatisfaction, please communicate with us, and we will make
2040 changes and improvements as quickly as possible. We are very grateful for your effort
2041 in reviewing our paper and your positive feedback. Your evaluation of our work is
2042 precise, and your dedication is commendable. Since your input is invaluable for future
2043 publications, we would like to expressly thank you for your contribution.

2044

2045

2046

2047

2048

2049

2050

2051 Reviewer #2 (Public repository details (Required)):

2052 metagenomics sequencing and metabolome data are needed to deposit at a repository.

2053 **Response:** We really appreciate your reminder from the bottom of our hearts. We are
2054 very sorry for our negligence of metagenome and transcriptome raw data. We have
2055 uploaded the metagenomic and transcriptome raw data, and the modifications in the
2056 manuscript have been highlighted. (Page 27, Line: 791-792)

2057 The sequence data reported in this paper have been deposited in the NCBI
2058 database (metagenomic sequencing data and transcriptome sequencing
2059 data:PRJNA812272).

2060 As is customary, our data will be made public after the article is received.

2061

2062 Reviewer #2 (Comments for the Author):

2063 **Response:** We appreciate the time and effort you dedicated to providing feedback on
2064 our manuscript and are grateful for the insightful comments and valuable
2065 improvements to our manuscript. We have discussed your comments carefully and we
2066 sincerely accept the suggestions. Your comments provided valuable insights to refine
2067 its contents and analysis. In this document, we try to address the issues raised as best
2068 as possible. All revisions in the manuscript have been highlighted in yellow. You can
2069 kindly find the point-to-point responses to reviewers' comments in the following text.
2070 We thoroughly double-checked the manuscript. For detail, please see the following
2071 answers.

2072

2073 Major comments:

2074 1. Authors claim that "we chose Lp082 to study the mechanism of probiotics in
2075 preventing UC", however, the animal was treated with various reagents followed by

2076 DSS challenge. Please explain how this setting could serve well for assessing the
2077 effects of probiotics on prevention UC? Authors should discriminate the difference
2078 between "prevention" and "treatment", and pay more attention for accuracy of
2079 wording.

2080 **Response:** We appreciate your valuable and helpful comment. We apologize for the
2081 language problems in the original manuscript. The language presentation was
2082 improved with assistance from a native English speaker with appropriate research
2083 background. We apologize for the confusion and inconvenience caused to you. In fact,
2084 we are studying the effect of Lp082 in the treatment of UC. We used DSS to establish
2085 a model of UC and then treated it with Lp082. We have changed the sentence you
2086 mentioned above to: So the Lp082 strain becomes a good choice for the study of
2087 *lactobacillus plantarum* in the treatment of UC. The changes have been highlighted in
2088 the article. We have carefully checked the wording of the full text and corrected the
2089 preventive effect to the therapeutic effect. Thank you very much for pointing this out.
2090 It was very helpful.

2091

2092 2. Basically only one biological repeat was conducted in this study. At least two
2093 biological repeats are acceptable for this purpose. Please repeat one more animal
2094 assay during next round of revision.

2095 **Response:** We appreciate your valuable and helpful comment. Thank you very much
2096 for pointing out this issue. It is true that we did not express clearly. In fact, we set up 6
2097 biological replicates for each group. According to your helpful suggestions, we have
2098 carefully checked the whole paper, and added descriptions of sample size and number
2099 of repeats in material and methods, legends and corresponding places in the article.
2100 The changes have been highlighted in the text in yellow. The rewritten content is
2101 more detailed, and the details are as follows:

2102 After the experiment, the spleen, liver, kidney and colon of 8 mice were selected from
2103 each group for observation and measurement. (Page 6, line: 170-172)

2104

2105 To further evaluate the effects of Lp082 on inflammatory cytokines in mice with

2106 colitis, serum of 6 mice in each group was randomly collected after the experiment,
2107 and the levels of pro-inflammatory cytokines TNF- α , IL-1 β , IFN- α , IL-6, MPO and
2108 anti-inflammatory cytokines IL-10 were detected by ELISA kit. (Page 8, line:
2109 208-213)

2110

2111 At the end of the experiment, the 1cm portion of the distal colon of 6 mice in each
2112 group was selected randomly for HE staining, and histopathological score and
2113 intestinal wall thickness were further measured ($n=6$). (Page 8, line: 220-224)

2114

2115 At the end of modeling (day 7 of the experiment), feces of 6 mice in each group were
2116 randomly selected for metagenomic sequencing, and at the end of treatment (day 15
2117 of the experiment), feces of 6 mice in each group were selected for metagenomic
2118 sequencing, to observe the effects of DSS and Lp082 on the intestinal microecology
2119 of mice. (Page 9, line: 258-262)

2120

2121 To prove the above findings, we further used gas chromatography-mass spectrometry
2122 (GC-MS) to detect the content of SCFAs in cecal contents of 6 mice in each group.
2123 (Page 11, line: 308-309)

2124

2125 At the end of the experiment, 6 mice from each group were randomly selected for
2126 colon transcriptome sequencing, and the volcanic map was drawn based on the
2127 preliminary gene distribution analysis results. (Page 13, line: 350-352)

2128

2129 C57BL/6J mice aged 7 weeks were randomly divided into 4 groups: control group
2130 ($n=8$), dextran sulfate sodium (DSS) group ($n=8$), lactobacillus plantarum HNU082
2131 (Lp082) group ($n=8$), and salazosulfapyridine (SASP) group ($n=8$). (Page 23, line:
2132 659-661)

2133

2134 After the mice were euthanized, the colon length of 8 mice in each group was
2135 measured, the weight of spleen, liver, and kidney of 8 mice in each group was

2136 measured. (Page 23, line: 677-679)

2137

2138 Before euthanasia, 6 mice were randomly selected from each group, and blood was
2139 collected from the orbital venous plexus by a capillary tube. (Page 24, line: 686-687)

2140

2141 Finally, the levels of interleukin-1beta (IL-1 β), interleukin-6 (IL-6), interleukin-10
2142 (IL-10), interleukin-17A (IL-17A), interferon-gamma (IFN- γ), Tumor necrosis
2143 factor-alpha (TNF- α), and Myeloperoxidase (MPO) in the serum of 6 randomly
2144 selected mice from each group were measured using the corresponding ELISA kits
2145 (X-Y Biotechnology, Shanghai, China), as previously described. (Page 24, line:
2146 690-694)

2147

2148 After euthanasia, the distal 1cm colons of 6 mice in each group were randomly
2149 selected for HE staining section, histopathological score, and intestinal wall thickness
2150 measurement. (Page 24, line: 697-699)

2151

2152 On the other hand, 8 mice were selected from each group, and their colonic tissues
2153 were labeled with mucin 2 and ZO-1 antibodies, respectively [75], for further
2154 immunofluorescence staining (Servicebio, Wuhan, China). (Page 25, line: 710-712)

2155

2156 Six mice were randomly selected at two time points (day 7 and day 15 of the
2157 experiment) for metagenomic sequencing of feces. (Page 25, line: 728-729)

2158

2159 At the end of the experiment, the cecal contents of 6 mice from each group were
2160 randomly selected for SCFAs determination, and the specific steps were as follows:
2161 (Page 26, line: 742-743)

2162

2163 At the end of the experiment, colon tissues of 6 mice from each group were randomly
2164 selected for RNA sequencing. (Page 26, line: 757-758)

2165

2166 We consider our results to be credible on the premise of 6 biological replicates per
2167 group. We have carefully reviewed the full text and supplemented descriptions of data
2168 volumes and biological replicates where measurement data appeared. Modifications in
2169 the article are highlighted in yellow.

2170

2171 3. Please improve layouts of figures, and pay attention to size, location of symbols.

2172 **Response:** We appreciate your valuable and helpful suggestion. According to the your
2173 comment, we have gone through all the images carefully and refined the layout, size
2174 and placement of symbols.

2175

2176 4. Please improve the language and grammar.

2177 **Response:** We apologize for the language problems in the original manuscript. The
2178 language presentation was improved with assistance from a native English speaker
2179 with an appropriate research background. We deeply appreciate your valuable and
2180 helpful comments.

2181

2182 5. Please provide the H&E staining results for entire swiss roll in figure 2.

2183 **Response::** We appreciate your valuable and helpful comment. Indeed, our slicing
2184 pictures that are not in line with the rules. We supplement the full slicing results of
2185 40X and use this to zoom in at 100X and 200X. Thank you very much for your
2186 suggestion; we will pay more attention in the following writing.

2187

2188 6. Authors claim that "that Lp082 could improve UC by regulating gut microbiota,
2189 intestinal mucosal barrier, inflammatory pathways and neutrophil infiltration", please
2190 provide direct evidence to support Lp082 effects on "mucosal barrier". Manuscript
2191 shows the transcriptome data, however, transcriptome analysis on host genes are far
2192 away from real expression and function.

2193 **Response:** We appreciate your valuable and helpful comment. The pathogenesis of
2194 UC is the result of the combined effect of genetically susceptible hosts and the
2195 environment, and its common pathological outcome is the damage of the structure and

2196 function of the intestinal mucosal barrier. The intestinal mucosal barrier is damaged,
2197 resulting in an increase in the permeability of the intestinal epithelial barrier, and
2198 further stimulation of intestinal contents, bacteria, and toxins promotes the immune
2199 response to intestinal inflammation. The normal intestinal mucosal barrier consists of
2200 mechanical barrier, chemical barrier, immune barrier, and biological barrier. The
2201 chemical barrier refers to the glue-like mucin layer covering the surface of intestinal
2202 epithelial cells, which is mainly composed of MUC-2 secreted by goblet cells,
2203 digestive juices, and bacteriostatic substances produced by normal parasitic bacteria
2204 in the intestinal lumen [1]. The mechanical barrier is the most important part of the
2205 intestinal mucosal barrier. Its structural basis is the intestinal mucosal epithelial cells
2206 and the tight junctions (TJ) between the epithelial cells [2]. The immune barrier is
2207 associated with immune cells, and inflammatory factors [3]. The biological barrier is a
2208 normal intestinal colony of bacteria that is resistant to colonization by foreign strains
2209 [4]. The results of the study found that Lp082 can improve the intestinal mucosal
2210 barrier by synergistically optimizing the biological barrier, chemical barrier,
2211 mechanical barrier and immune barrier, thereby alleviating UC. Specifically, We
2212 found that Lp082 rebuilt the biological barrier by regulating the intestinal microbiome
2213 and increasing the SCFAs. Lp082 improved the chemical barrier by reducing ICAM-1,
2214 VCAM, and increasing goblet cells and mucin2. Lp082 ameliorated the mechanical
2215 barrier by increasing the ZO-1, ZO-2, and occludin and decreasing claudin-1 and
2216 claudin-2. Lp082 optimized the immune barrier by reducing the content of IL-1 β ,
2217 IL-6, TNF- α , MPO, IFN- γ and increasing the IL-10, TGF- β 1, and TGF- β 2. From the
2218 above four aspects, we demonstrated that Lp082 can indeed improve the "intestinal
2219 mucosal barrier" to treat DSS-induced UC.

2220 This result is not only supported by transcriptomic data, we have indeed done a
2221 lot of experiments and validation. First, we studied some basic indicators and found
2222 that Lp082 could not only significantly inhibit the decrease of body weight, water
2223 intake and food intake induced by DSSS in mice, but also significantly inhibit the
2224 increase of DAI and immune organ index induced by DSSS, as well as the decrease of

2225 colon length caused by DSS (**Fig. 1a-1d**). Second, we measured the protein content of
2226 six inflammatory cytokines in mouse serum, and found that Lp082 could significantly
2227 reduce the increase of IL-1 β , IL-6, TNF- α , MPO, IFN- γ induced by DSS, and increase
2228 the protein content of IL-10 in mice (**Fig. 1e**). Third, we performed HE staining
2229 section experiment and immunofluorescence protein experiment. The results showed
2230 that Lp082 could not only improve the crypt infiltration, goblet cell loss and intestinal
2231 mucosal ulcer induced by DSS, but also could reduce the increase of histopathology
2232 score caused by DSS and reduce the loss of ZO-1 and MUC-2 proteins caused by
2233 DSS (**Fig. 2a-2g**). Fourth, we collected fecal samples on day 7 for metagenomic
2234 sequencing. The results of Shotgun metagenomic data analysis showed that Lp082
2235 could increase α -diversity and β -diversity, reduce the differences in species
2236 composition, increase the content of beneficial bacteria and inhibit the abundance of
2237 harmful bacteria in mice (**Fig. 3a-3d**). Fifth, we used gas chromatography-mass
2238 spectrometry to determine the content of SCFAs in the intestinal contents of mice, and
2239 found that Lp082 could significantly inhibit the reduction of acetic acid, propionic
2240 acid, butyric acid, isobutyric acid and valeric acid induced by DSS, and restore the
2241 content of SCFAs in mice (**Fig. 4b**). Sixth, we sequenced the transcriptome of colon
2242 tissue, and the results showed that Lp082 not only affected gene expression
2243 distribution, but also affected inflammation and cancer-related and KEGG,GO-BP
2244 pathways (**Fig. 5a-5g**). These experiments provide data support for our derivation,
2245 because the study did integrate metagenomics, transcriptomics, proteomics, HE
2246 stained sections, immunofluorescent proteins and other experimental data, and found
2247 that Lp082 can modulate the immune, chemical, mechanical and biological barriers,
2248 which means that Lp082 can improve the intestinal mucosal barrier. Our data were
2249 not less than 6 replicates in each group, and our data were absolutely reliable and
2250 sufficient to support the results of our paper.

2251 Maybe we didn't describe it very well, so based on your suggestion, we have
2252 rewritten the discussion section to more clearly describe the improvement effect of
2253 Lp082 on the intestinal mucosal barrier, and the rewritten content is as follows: (Page

2254 16, line: 459-637)

2255 DISCUSSION

2256 The normal intestinal mucosal barrier is composed of mechanical, chemical immune
2257 and biological barriers. The Lp082 has good efficacy in treating UC, which motivates
2258 us to explore further its mechanism of action in the treatment of UC. The results of
2259 the study found that Lp082 can improve the intestinal mucosal barrier by
2260 synergistically optimizing the biological, chemical, mechanical and immune barriers,
2261 thereby alleviating UC. In addition to optimizing the intestinal mucosal barrier,
2262 regulating inflammatory pathways and influencing neutrophil infiltration are potential
2263 mechanisms of Lp082 in treating UC.

2264 Lp082 improved chemical barrier

2265 The chemical barrier refers to the glue-like mucin layer covering the surface of
2266 intestinal epithelial cells, which is mainly composed of MUC-2 secreted by goblet
2267 cells, digestive juices and bacteriostatic substances produced by normal parasitic
2268 bacteria in the intestinal lumen [1]. The chemical barrier plays an important role in
2269 isolating the internal and external environment of the intestinal tract, lubricating the
2270 intestinal mucosa, and inhibiting the entry of harmful substances in the intestinal
2271 lumen [5]. The intestinal mucosal wall thickness was significantly increased in the
2272 DSS group, whereas it was significantly decreased after Lp082 ingestion (**Fig. 2c**). In
2273 DSS-induced UC, the thicker the intestinal mucosal wall, indicating more severe
2274 inflammation. In addition, the H&E staining result showed that the number of goblet
2275 cells decreased in the DSS group (red arrow), whereas the number of goblet cells
2276 increased (yellow arrow) after Lp082 ingestion (**Fig. 2a**). The immunofluorescent
2277 protein content of MUC-2, which is mainly secreted by goblet cells, was significantly
2278 decreased in the DSS group (**Fig. 2d**), and the areal density of MUC-2 (**Fig. 2f**) and
2279 the mRNA expression of MUC-2 were also significantly decreased in the DSS group
2280 (**Fig. 5c**), while the immunofluorescence protein content, areal density and mRNA
2281 expression of MUC-2 all increased in the Lp082 group,
2282 Sun et al. [6] observed the same phenomenon that *lactobacillus plantarum 12* can
2283 repair the intestinal mucosal chemical barrier by increasing the content of MUC-2.

2284 Burger-van Paassen et al. [7] found that intake of SCFAS could increase the
2285 expression abundance of MUC-2 mRNA in cells. The mRNA expressions of ICAM-1
2286 and VCAM-1 were significantly decreased after Lp082 intake. Taniguchi et al. [8]
2287 found that anti-ICAM-1 treatment significantly attenuated colonic mucosal damage,
2288 while Philpott et al. [9] found that adhesion molecules ICAM-1 & VCAM-1 induced
2289 intestinal mucosal lesions. Lp082 has been shown to be effective in relieving
2290 intestinal mucosal lesions (i.e., reduced ulceration and inflammatory cell infiltration
2291 caused by DSS). So, we speculate that Lp082 reduces mucosal lesions by reducing
2292 ICAM-1 and VCAM. The above results showed that probiotic Lp082 increased the
2293 MUC-2 content in the mucus layer by restoring the number of goblet cells, relieved
2294 the intestinal mucosal damage caused by ICAM-1 and VCAM-1, so as to repaired the
2295 chemical barrier.

2296 Lp082 improved mechanical barrier

2297 The mechanical barrier is the most important part of the intestinal mucosal
2298 barrier. Its structural basis is the intestinal mucosal epithelial cells and the tight
2299 junctions (TJ) between the epithelial cells [2]. The mechanical barrier can effectively
2300 prevent harmful substances such as bacteria and endotoxins from entering the blood
2301 through the intestinal mucosa.iers The aberrant structure of tight junction (TJ)
2302 proteins between intestinal epithelial cells, such as the reduction of ZO-1, ZO-2, and
2303 occludin, is one of the critical factors leading to the disruption of the gut mechanical
2304 barrier in UC patients [10]. Several studies have identified TJ protein as a new target
2305 for the current treatment of UC [11]. Because Lp082 excellently improved
2306 histopathology, we speculated that Lp082 also has a regulatory effect on TJ molecules.
2307 To this end, we analyzed major TJ proteins, including ZO-1, ZO-2, occludin. As
2308 expected, the mRNA expression and immunofluorescence protein content of ZO-1
2309 and the mRNA expression of ZO-2 and occludin were significantly decreased in
2310 DSS-induced UC mice but improved in the Lp082 treatment group. These are
2311 consistent with the findings of Cordeiro et al. [12] that ZO-1 and ZO-2 were
2312 significantly decreased in UC but increased after probiotic Minas Frescal cheese
2313 intake, indicating that the improvement of the mechanical barrier by regulating TJ

2314 may be one of the mechanisms by which probiotic Lp082 exerts anti-UC. In addition,
2315 the mRNA expression of another particular tight junction protein, ICAM-1 and
2316 VCAM-1, was increased in the DSS group. It is consistent with the findings of
2317 elevated ICAM-1 and VCAM-1 in IBD patients in clinical studies [13]. Mitselou et al.
2318 [14] found that the adhesion molecules ICAM-1 and VCAM-1 induced intestinal
2319 mucosal injury. Taniguchi et al. [8] found that anti-ICAM-1 treatment attenuated
2320 colonic mucosal injury. It has been reported that ICAM-1 and VCAM-1 can increase
2321 the permeability of intestinal mucosa [15]. Interestingly, the mRNA expression of
2322 ICAM-1 and VCAM-1 was found to decrease after Lp082 ingestion. Therefore, it can
2323 be thought that the alleviation of UC by Lp082 may be due to down-regulation of
2324 ICAM-1, VCAM-1 and increase protein quantity and mRNA expression of
2325 ZO-1, ZO-2, so as to reduce intestinal mucosal permeability, thereby inhibiting the
2326 entry of harmful bacteria and undigested food and toxins into the body and reducing
2327 inflammation. These results suggest that Lp082 repairs the intestinal mechanical
2328 barrier by regulating TJ.

2329 Lp082 improved the immune barrier

2330 Although the exact etiology of UC is complex and uncertain, studies suggest that
2331 the NF- κ B pathway plays a vital role in the pathogenesis of UC [3]. Our study has
2332 proved that Lp082 inhibits the NF- κ B pathway by down-regulating the mRNA
2333 expression of NF- κ B2, NF- κ B1, COX-2, Rel α , Toll4, iNOS, and that NF- κ B can also
2334 regulate inflammation by regulating cytokines [16]. Therefore, it can be suggested
2335 that Lp082 also has a specific regulatory effect on cytokines. To confirm this, we
2336 analyzed the cytokines associated with NF- κ B. As expected, we observed that the
2337 mRNA expression level content of pro-inflammatory cytokines (TNF- α , IL-1 β , and
2338 IL-6) were significantly increased in the DSS group but significantly decreased in the
2339 Lp082 group, It is interesting to note that the protein levels of TNF- α , IL-1 β , and IL-6
2340 detected by elisa kit were also increased in the DSS group and decreased after Lp082
2341 intake. Among them, TNF- α can promote the proliferation and differentiation of T
2342 cells and increase intestinal inflammation [17]. The upregulation of IL-1 β is involved
2343 in the recruitment and retention of leukocytes in inflamed tissues and can activate

2344 innate immune lymphocytes [18]. IL-6 activates NF- κ B to regulate the dextran sulfate
2345 sodium-induced colitis in mice [19]. The above results indicate that Lp082 alleviates
2346 UC by inhibiting the levels of pro-inflammatory factors (TNF- α , IL-1 β , and IL-6).
2347 Interestingly, we also found that the mRNA expressions of anti-inflammatory
2348 cytokines IL10, TGF-1, and TGF-2 were significantly decreased in the DSS group but
2349 increased in the Lp082 group. IL-10 protein levels measured by elisa kit also decreased
2350 in the DSS group and increased in the Lp082 group. Surprisingly, IL10, TGF-1, and
2351 TGF-2 were shown to activate Treg and anti-inflammatory macrophages to alleviate
2352 UC [20]. And Sato et al. [21] also found that the loss of IL-10 spontaneously gave rise
2353 to IBD, and Hume et al. [22] found that TGF- β 1 and TGF- β 2 could dramatically
2354 relieve intestinal inflammation in DSS-induced colitis mice. These results suggest that
2355 Lp082 alleviates UC by increasing the levels of anti-inflammatory factors IL10,
2356 TGF-1, and TGF-2. We further analyzed the specific regulatory effects of Lp082 on
2357 intestinal mucosal immunity. In addition to inflammatory factors, we also noticed that
2358 a heme protein, MPO, was significantly reduced in the Lp082 group. Trevisin et al.
2359 [23] found that MPO caused UC by producing cytokines and hypochlorite and that
2360 MPO in the colon of UC patients is mainly produced by neutrophil infiltration [24].
2361 Interestingly, this is consistent with the fact that the DSS group had a severe
2362 neutrophil infiltration in this study. However, neutrophil infiltration and MPO content
2363 were significantly decreased in Lp082 group. This shows that Lp082 alleviates UC by
2364 reducing neutrophil infiltration and its secreted MPO content. In a nutshell, our results
2365 suggest that Lp082 may play an anti-UC effect by inhibiting the NF- κ B pathway,
2366 down-regulating pro-inflammatory cytokines, and up-regulating anti-inflammatory
2367 cytokines, reducing MPO content, thereby maintaining immune balance and
2368 protecting the immune barrier.

2369 The mucosal immune system of the intestine mainly consists of Peyer's patch
2370 and lamina propria under enterocyte [25]. The Peyer's patch can deliver captured
2371 antigens to dendritic cells [26]. Then dendritic cells can not only trigger T
2372 cell-mediated cellular immunity and B cell-mediated humoral immunity by presenting
2373 antigens but also affect lamina propria immunity [27]. Combining previous studies,

2374 we found that DSS causes inflammation through the following six ways. First, gut
2375 permeability increases, and harmful substances enter to activate innate immunity, such
2376 as stimulating innate immune cells to produce TNF- α , IL-1 β , and IL-6 [28]. Second,
2377 regulatory T cells produce less IL-10 and have a less inhibitory effect on effector T
2378 cells, resulting in the phenomenon of effector T and regulatory T cell dysregulation in
2379 UC patients [29]. Third, effector T cells promote B cell-mediated humoral immunity
2380 by promoting the secretion of IFN- γ and L-17A [30]. Fourth, effector T cells carried
2381 out immune cell recruitment and formed a vicious immune cycle with chemokines
2382 and cytokines [31]. Fifth, Peyer's patch recognizes antigens and presents them to other
2383 immune cells through dendritic cells [26]. Sixth, antigen-activated neutrophils can
2384 both secrete MPO and recruit more immune cells from the bloodstream to the site of
2385 inflammation, further exacerbating inflammation [32] (**Fig. 6b**). Based on the above 6
2386 reasons, we suggest that in addition to relieving inflammation by inhibiting the NF- κ B
2387 pathway, Lp082 can also regulate inflammatory factors to maintain the balance
2388 between regulatory T cells and effector T cells to regulate intestinal mucosal
2389 immunity, thus maintaining the intestinal mucosal barrier.

2390 Lp082 improved the biological barrier

2391 Numerous studies [23] have shown that probiotics improve the clinical outcome
2392 of IBD patients by influencing host gut microbiota [4]. Herein, we performed a
2393 shotgun metagenomic analysis to investigate whether Lp082 can improve gut
2394 dysbiosis in the UC mice model. As expected, we observed that the intake of DSS
2395 significantly reduced the shannon value but increased PCoA distance, a finding that is
2396 consistent with Wang et al. [33]. The Shannon index reflects gut microbiota richness
2397 and uniformity and is positively correlated with gut microbiota diversity, while the
2398 PCoA distance reflects the difference in the structure of the gut microbiota between
2399 different groups; the higher the PCoA value, the greater the difference in the gut
2400 microbiota structure [34]. In particular, Lp082 treatment remarkably increased the gut
2401 microbiota diversity and reduced gut microbiota structural differences in gut
2402 microbiota, as shown by the cluster analysis and PCoA analysis. On the other hand,
2403 Lp082 also optimized species composition; that is, the abundance of

2404 pro-inflammatory microbiota decreased in the Lp082 group, such as *Helicobacter*
2405 *hepaticus*, a potential pathogen of colitis. Likewise, we observed an increasing trend
2406 in the abundance of potential probiotics in the Lp082 group, such as *Bifidobacterium*
2407 *pseudolongum* and *Bacteroides ovatus*, which reduces colonic inflammation [35],
2408 *Parabacteroides distasonis*, which is negatively associated with obesity and diabetes
2409 [36], *Akkermansia muciniphila* and *Lactobacillus reuteri*, a widely studied probiotic,
2410 *Anaerotruncus sp G3 2012* and *Lactobacillus plantarum*, potential SCFAs-producing
2411 bacteria [37]. The above results indicate that Lp082 is beneficial to optimizing the
2412 diversity, structure, and composition of gut microbiota. After demonstrating that
2413 Lp082 can increase the abundance of potential SCFAs-producing bacteria, further
2414 analysis found that Lp082 can activate two SCFAs-producing microbial metabolic
2415 pathways and the content of SCFAs. Subsequently, correlation analysis proved that
2416 Lp082 may increase SCFAs by activating the SCFAs-producing metabolic pathway of
2417 SCFAs-producing bacteria, so as to inhibit inflammation [38] and regulate host
2418 physiological activity through SCFAs [39]. All of these suggest that Lp082 repaired
2419 the microbial barrier by regulating the gut microbiome.

2420 In conclusions, the Lp082 has an exciting therapeutic effect on UC than SASP.
2421 Also, shotgun metagenome and transcriptome analysis confirmed that Lp082 could
2422 improve gut microbiota dysbiosis, protect intestinal mucosal barrier, regulate
2423 inflammatory pathways, and affect neutrophil infiltration. These findings firmly
2424 support and advocate the clinical translation of Lp082 in the treatment of UC. It can
2425 be suggested that the application of gut microbiota and probiotics in the treatment of
2426 UC should receive more attention. The findings of this study not only provide new
2427 clues for revealing the complex mechanism of gut microbiota in relieving UC, but
2428 also provide evidence for Lp082 as a potential gut microbiota regulator to treat UC.

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2565 10.1371/journal.pone.0220642.

2566

2567 **Minor comments:**

2568 1. Please provide line numbering.

2569 **Response:** We are grateful to the reviewer for pointing out this problem. We are very
2570 sorry for our negligence with page numbers and line numbers. We have added the
2571 page number and line number to the article. The title page is also called page 1, and
2572 the first line of the title is line 1.

2573

2574 2. Figure 1a depicted the study design and methodology, which might be better to
2575 merge into M&M part.

2576 **Response:** We appreciate your valuable and helpful comment. Thank you for pointing
2577 out this problem. We deeply agree with the reviewer's opinion on this problem, and
2578 we have moved the content of this part to M&M. The changes in the text are
2579 highlighted in yellow. (Page 24, line: 676-681)

2580

2581 3. Information of study design and methodology are not appropriate present in Results
2582 section. The tables or figures should be displayed at a consecutive and sequential
2583 order. In current version figure S1b appeared ahead of S1a.

2584 **Response:** We appreciate your valuable and helpful comment. We have corrected this
2585 problem and redescribed this part to make the article more coherent, and the rewritten
2586 content is as follows: (Page 7, line: 166-200)

2587 In DSS-induced UC mice, the immune organ index gradually increased and the colon
2588 length gradually shortened with increasing disease severity [1]. Therefore, we
2589 measured the spleen, liver, kidney, and colon of the mice. The results showed that the
2590 immune organ index of the DSS group was significantly increased ($p < 0.05$), and the
2591 immune organ index was significantly decreased after Lp082 intake ($p < 0.05$) (**Fig.**
2592 **1c**). The colon length of the mice in the DSS group was significantly decreased ($p <$
2593 0.05), and the colon length in Lp082 group was significantly increased ($p < 0.05$) (**Fig.**
2594 **1d**). In addition, we also observed that the intestinal contents of the colitis mice in the
2595 DSS group were loose, unformed and there was blood in the intestinal lumen, while
2596 the intestinal contents in the Lp082 and Control groups were clear particles, hard stool,
2597 and no blood (**Fig. 1d**). The fecal morphology of the intestinal contents was similar to
2598 the results observed in mouse feces on the buttocks of mice. The feces of the mice in
2599 the DSS group were blood-red, and the feces were loose and unformed, while there
2600 was no blood in the feces after Lp082 ingestion (**Fig. S1 a**).

2601 With the increase of disease degree, DSS-induced UC mice will have a worse mental
2602 state, even abdominal pain, arch back, panic and other symptoms [2]. The mental state
2603 of the mice was observed daily, and the results are shown in **Figure S1 b**. On the 7th
2604 day of modeling, mice in the control group were in a normal state, with normal urine
2605 and feces, shiny hair, active spirit, sensitive reaction, and increased body size.
2606 However, mice in the BCD group had yellow and smelly urine, difficult defecation,
2607 bloody stool, dark and fried hair, slow reaction and easy panic, arched back, and
2608 reduced body size (**Fig. S1 b**). On the last day of treatment(Day 15), compared with
2609 the arched back, retarded response, hematochezia, and lethargic in the DSS group, the
2610 mental state of mice in the Lp082 and SASP groups gradually returned to normal,
2611 with an active spirit, no arched back, no hematochezia and shiny hair (**Fig. S1 b**).
2612 These results indicated that Lp082 intake could alleviate the symptoms of depression,
2613 crouching, and untidy hair of mice in the DSS group in the middle and late stage of

2614 the experiment (**Fig. S1 b**).

2615 Studies have shown that under the condition of inflammation, the spleen of mice
2616 induced by DSS will increase hyperemia and even appear infection blackening.
2617 Therefore, we looked at the spleens of mice and found that the spleens of mice in the
2618 DSS group were significantly larger and darker than those of mice in the normal
2619 group. The spleens of mice in the Lp082 and SASP groups were smaller and redder
2620 rather than black than those in the DSS group (**Fig. S1 c**).

2621 **Reference**

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2623 Chueca N, et al. Differential intestinal anti-inflammatory effects of *Lactobacillus*
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2628 effect of *lactobacillus plantarum*-12 on DSS-induced murine colitis. *Food & Function*.
2629 2020;11(6):5205-22; doi: 10.1039/d0fo00007h.

2630

2631 Once again, we thank you for the time you put into reviewing our paper, and we are
2632 very grateful for your effort in reviewing our paper and your positive feedback. The
2633 summary of our work as written by you is precise. Since your inputs have been
2634 precious, we would like to acknowledge your contribution explicitly in the eventuality
2635 of a publication.

October 7, 2022

Prof. Jiachao Zhang
Hainan University
Food Science
58 renmin road
Haikou, Hainan 570228
China

Re: Spectrum01651-22R1 (Probiotics (*Lactobacillus plantarum* HNU082) supplementation relieves ulcerative colitis by affecting intestinal barrier functions, immunity-related genes expression, gut microbiota, and metabolic pathways in mice)

Dear Prof. Jiachao Zhang:

Thank you for submitting your manuscript to Microbiology Spectrum. As you will see your paper is very close to acceptance. Please modify the manuscript along the lines the reviewer has recommended. As these revisions are quite minor, I expect that you should be able to turn in the revised paper in less than 30 days, if not sooner. If your manuscript was reviewed, you will find the reviewers' comments below.

When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript. Detailed instructions on submitting your revised paper are below.

Link Not Available

Thank you for the privilege of reviewing your work. Below you will find instructions from the Microbiology Spectrum editorial office and comments generated during the review.

The ASM Journals program strives for constant improvement in our submission and publication process. Please tell us how we can improve your experience by taking this quick [Author Survey](#).

Sincerely,

Xiaoyu Tang

Editor, Microbiology Spectrum

Reviewer comments:

Reviewer #2 (Comments for the Author):

The manuscript has been improved a lot, please fix the following.

1. In results, the title of each section should be same as the line 145 that show a specific conclusion.
2. Experiment details should not be appeared in "Result sections".
3. In Results and Discussion, the author should be described the results more concisely, rather than a repetitive description. For example, Fig.S1a should be a part of the Disease Activity Index (DAI) score and so on. Please reorganize the description in both sections.
4. In Fig 5a, the data should be better presented regarding up-regulated genes and down-regulated genes involved in metabolic pathway, respectively.
5. In discussion, the creativity of manuscript should be noted compared with the similarity studies which published before.

Preparing Revision Guidelines

To submit your modified manuscript, log onto the eJP submission site at <https://spectrum.msubmit.net/cgi-bin/main.plex>. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:

- Point-by-point responses to the issues raised by the reviewers in a file named "Response to Reviewers," NOT IN YOUR COVER LETTER.
- Upload a compare copy of the manuscript (without figures) as a "Marked-Up Manuscript" file.
- Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file.
- Manuscript: A .DOC version of the revised manuscript
- Figures: Editable, high-resolution, individual figure files are required at revision, TIFF or EPS files are preferred

For complete guidelines on revision requirements, please see the journal Submission and Review Process requirements at <https://journals.asm.org/journal/Spectrum/submission-review-process>. **Submissions of a paper that does not conform to Microbiology Spectrum guidelines will delay acceptance of your manuscript. "**

Please return the manuscript within 60 days; if you cannot complete the modification within this time period, please contact me. If you do not wish to modify the manuscript and prefer to submit it to another journal, please notify me of your decision immediately so that the manuscript may be formally withdrawn from consideration by Microbiology Spectrum.

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Thank you for submitting your paper to Microbiology Spectrum.

1 **Manuscript No.: Spectrum 01651-22**

2 **Title: Probiotics (*Lactobacillus plantarum* HNU082) supplementation relieves**
3 **ulcerative colitis by affecting intestinal barrier functions, immunity-related genes**
4 **expression, gut microbiota, and metabolic pathways in mice.**

5 **Dear Dr. Xiaoyu Tang,**

6 I am very glad to receive your email again! On behalf of my co-authors, I thank
7 you very much for allowing us to revise our manuscript. We appreciate the time and
8 effort that you and the reviewers dedicated to providing feedback on our manuscript
9 and are grateful for the insightful comments on and valuable improvements to our
10 manuscript. We have discussed reviewer's comments carefully and revised the
11 manuscript taking all the comments positively. All revisions in the manuscript have
12 been highlighted in yellow. Please find the point-to-point responses to reviewers'
13 comments in the following text. We thoroughly double-checked the manuscript. In
14 addition, the revised manuscript with tracked changes is also uploaded as "Marked Up
15 Manuscript" files.

16

17 We sincerely hope that this revised manuscript will be published in "*Microbiology*
18 *Spectrum*." We deeply appreciate your consideration of our manuscript. If you have
19 any queries, please don't hesitate to contact us at the following e-mail address.

20

21 We would like to express our great appreciation again to you and the reviewers for
22 their comments on our paper. We are looking forward to hearing from you.

23

24 Sincerely,

25 Jiachao Zhang

26 Yours sincerely,

27 E-mail: Jiachao Zhang1*, zhjch321123@163.com

28 College of Food Science and Engineering, Hainan University, Haikou 570228, China

29 **Responds to the reviewer's comments**

30 Reviewer #2 (Comments for the Author):

31

32 The manuscript has been improved a lot, please fix the following.

33 **Response:** We appreciate the time and effort you dedicated to providing feedback on
34 our manuscript and are grateful for the insightful comments and valuable
35 improvements to our manuscript. We have discussed your comments carefully, and we
36 sincerely accept the suggestions. Your comments provided valuable insights to refine
37 its contents and analysis. In this document, we try to address the issues raised as best
38 as possible. All revisions in the manuscript have been highlighted in yellow. A list of
39 changes to the manuscript has been attached, and you can kindly find the
40 point-to-point responses to your comments in the following text.

41

42 1. In results, the title of each section should be same as the line 145 that show a
43 specific conclusion.

44 **Response:** We appreciate your valuable and helpful comment and we deeply agree
45 with the opinions of reviewer. According to your helpful suggestions, we have
46 rewritten the title of each section in results, and we have also improved the title of the
47 conclusion. We sincerely thank you again for pointing this out. It was very helpful.
48 The changes have been highlighted in the manuscript in yellow. And the revised
49 content is as follows.

50

51 The intake of Lp082 alleviated physiological lesions in DSS-induced colitis mice
52 (Page 6, line:145)

53

54 The intake of Lp082 up-regulated the anti-inflammatory cytokines and
55 down-regulated the pro-inflammatory cytokines in DSS-induced colitis mice
56 (Page 7, line:192-193)

57 The intake of Lp082 alleviated pathological lesions in DSS-induced colitis mice
58 (Page 8, line: 203)

59

60 The intake of Lp082 regulated the gut microbiota in DSS-induced colitis mice
61 (Page 9, line: 238)

62

63 The intake of Lp082 regulated the short chain fatty acid in DSS-induced colitis mice
64 (Page 10, line: 265-266)

65

66 The intake of Lp082 regulated the transcriptome of intestinal epithelial cells in
67 DSS-induced colitis mice
68 (Page 12, line: 328-329)

69

70 The potential mechanism of Lp082 alleviated the DSS-induced colitis
71 (Page 14, line: 398)

72

73 The intake of Lp082 improved the chemical barrier
74 (Page 16, line: 449)

75

76 The intake of Lp082 improved the mechanical barrier
77 (Page 17, line: 482)

78

79 The intake of Lp082 improved the immune barrier
80 (Page 18, line: 513)

81

82 The intake of Lp082 improved the biological barrier
83 (Page 20, line: 576)

84

85 2. Experiment details should not be appeared in "Result sections".

86 **Response:** We are grateful to the reviewer for pointing out this problem. We deeply
87 agree with the opinions of reviewer. We are very sorry for our negligence and we
88 sincerely apologize for the inconvenience caused to you. According to your helpful
89 suggestions, we have moved the contents of the experimental details appeared in
90 "Result" sections to the "Materials and methods" section, and we have rewritten the
91 relevant content in the results section. We have carefully checked and verified the
92 contents of the "Result" section again. The changes have been highlighted in the
93 manuscript in yellow. And the revised content is as follows. We sincerely thank you
94 again for pointing this out. It was very helpful.

95

96 To further evaluate colon injury, we quantified the pro-inflammatory cytokines
97 interleukin-1beta (IL-1 β), interleukin-6 (IL-6), interferon-gamma (IFN- γ), tumor
98 necrosis factor-alpha (TNF- α), and myeloperoxidase (MPO), and anti-inflammatory
99 cytokines interleukin-10 (IL-10) in serum of 6 mice in each group. The results showed
100 that compared with the control group, the pro-inflammatory cytokines TNF-, IL-1 β ,
101 IFN- α , IL-6, and MPO in DSS group were significantly increased ($p < 0.05$), while
102 the anti-inflammatory cytokines IL-10 were significantly decreased ($p < 0.05$), while
103 the opposite was observed in Lp082 and SASP groups (Fig. 1e). (Page 7, line:
104 194-201)

105

106 The results of Shotgun metagenomic data diversity analysis demonstrated the effect of
107 Lp082 on the diversity of intestinal microbiota in mice. The results of α diversity
108 analysis showed that on days 1 - 7 of the study, the Shannon index in DSS, Lp082,
109 and SASP groups were all significantly decreased (Fig. 3a) , but the Shannon index
110 was significantly increased after the intake of Lp082 ($p < 0.05$) (Fig. 3a). The results
111 of β diversity analysis showed that the DSS group, LP082 group and SASP group
112 (M_B, M_C, M_D) and control group (M_A) were significantly separated on day 7 (p
113 < 0.05) (Fig. 3b). However, on day 15, the DSS group was still significantly separated
114 from the control group (T_B), while the distance between Lp082 group (T_C), SASP
115 group (T_D), and control group (T_A) was significantly reduced (p values < 0.05),

116 and the distance between Lp082 group and control group was closer, the above results
117 were consistent with the principal co-ordinates analysis (PCoA) distance results (Fig.
118 3c). The above diversity analysis results showed that Lp082 increased the α
119 -diversity and optimized the β -diversity of cecal microbiota in mice. (Page 9, line:
120 239-252)

121

122 Gene distribution was analyzed using colonic transcriptome data, the volcano map the
123 results show that Lp082 significantly affected gene expression distribution (Fig. S5
124 a-f). To further explore the impact of these differentially expressed genes (DEGs), we
125 analyzed the pathways involved in DEGs. (Page 12, line: 330-333)

126

127 At the end of the experiment, we euthanized the mice , and the 1cm portion of the
128 distal colon of 6 mice in each group was randomly selected for HE staining, and
129 histopathological score and intestinal wall thickness were further measured (n=6).
130 (Page 23, line: 674-676)

131

132 Six mice were randomly selected at two time points for metagenomic sequencing of
133 feces. At the end of modeling (day 7 of the experiment), feces of 6 mice in each group
134 were randomly selected for metagenomic sequencing. At the end of treatment (day 15
135 of the experiment), feces of 6 mice in each group were randomly selected for
136 metagenomic sequencing, to observe the effects of DSS and Lp082 on the intestinal
137 microecology of mice. (Page 24, line: 706-711)

138

139 At the end of the experiment, 6 mice from each group were randomly selected for
140 colon transcriptome RNA sequencing, and the volcanic map was drawn based on the
141 preliminary gene distribution analysis results. The sequencing was performed by
142 Beijing Novogene Co., Ltd. (Beijing, China). The RNA extraction mini kit (Qiagen,
143 Hilden, Germany) was used for total RNA extraction from the mouse colon samples,
144 and NanoDrop 2000 was used for quantification. Then the library construction and the
145 quality control were carried on, and the raw RNA-seq data was filtered [1]. After

146 constructing the RNA library, Illumina Novaseq 6000 was used for sequencing, and
147 the FeatureCounts were used to estimate the gene expression [2]. (Page 26, line:
148 739-747)

149

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152 ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;29(1):15-21; doi:
153 10.1093/bioinformatics/bts635.

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155 assigning sequence reads to genomic features. *Bioinformatics*. 2014;30(7):923-30;
156 doi: 10.1093/bioinformatics/btt656.

157

158 3. In Results and Discussion, the author should be described the results more
159 concisely, rather than a repetitive description. For example, Fig.S1a should be a part
160 of the Disease Activity Index (DAI) score and so on. Please reorganize the description
161 in both sections.

162 **Response:** We appreciate your valuable and helpful comment. We apologize for the
163 language problems in the original manuscript. We sincerely apologize for the
164 confusion caused to you. The language presentation was improved with assistance
165 from a native English speaker with appropriate research background. We deeply and
166 sincerely agree with you that Fig. S1a should indeed be part of the Disease Activity
167 Index (DAI) score, we have put the two parts of the description together and
168 reorganize the description. In addition, according to your helpful suggestions, We
169 have rewritten the relevant content of the results and discussion section, and have
170 described the results in more concise language, deleted the repeated description, and
171 deepened the discussion. The changes have been highlighted in the manuscript in
172 yellow. And the revised content is as follows.

173

174 People with UC have a disorder of colon function, poor absorption, loss of appetite,
175 weight loss, diarrhea, and bloody stools [8]. Therefore, the lower the body weight, the

176 lower the amount of water and food intake, and the higher the disease activity index
177 (DAI) score (The scoring criteria is shown in TABLE S1), indicating the more severe
178 enteritis. (Page 6, line: 146-150)

179

180 From 1 to 7 days, the water intake, food intake, and body weight of the DSS group,
181 the Lp082 group, and the SASP group all showed a similar degree of gradual decrease,
182 which may be because these three groups were all under the same DSS modeling
183 conditions on days 0-7. Then on the 8th to 15th day, the water intake, food intake, and
184 body weight of the DSS group were still decreasing, but the water intake, food intake,
185 and body weight of Lp082 and SASP group gradually increased. However, the water
186 and food intake of the Lp082 combined SASP group increased significantly from day
187 9 ($p < 0.05$), and body weight increased significantly from day 12 ($p < 0.05$). (Page 6,
188 line: 151-158)

189

190 The DAI index of the DSS group, Lp082 group, and SASP group increased
191 significantly ($p < 0.05$) since the third day compared with the Control group. But after
192 stopping DSS gavage on the 8th day, the DAI index of the DSS self-healing group
193 still increased, while that of the Lp082 group and SASP group gradually decreased
194 from the 10th day. And the degree of decrease in the Lp082 group was greater than
195 that in the SASP group, indicating that Lp082 had a better improvement effect on DAI
196 index (Fig. 1b). In addition, we observe that the feces of the mice in the DSS group
197 were blood-red, but there was no blood in the feces after Lp082 and SASP ingestion
198 (Fig. S1 a). This phenomenon is consistent with the measurement results of DAI
199 index. (Page 6, line: 159-168)

200

201 An increase in immune organ index and a decrease in colon length indicate an
202 increase in inflammation [2]. The results showed that the immune organ index of the
203 DSS group was significantly increased ($p < 0.05$), but was significantly decreased
204 after Lp082 intake ($p < 0.05$) (Fig. 1c). And the colon length of the mice in the DSS
205 group was significantly decreased ($p < 0.05$), but was significantly increased after

206 Lp082 intake ($p < 0.05$) (Fig. 1d). (Page 6, line: 169-174)

207

208 Studies have shown that DSS-induced UC mice will have a worse mental state, even
209 abdominal pain, arch back, panic and other symptoms with the increase of disease
210 degree, and the spleen will also increase hyperemia and infection blackening [30].
211 After successful modeling of UC, we observed that the mice in the control group were
212 in a normal state, with normal urine and feces, shiny hair, active spirit, sensitive
213 reaction, and increased body size. However, mice in the DSS, Lp082 and SASP
214 groups had yellow and smelly urine, difficult defecation, bloody stool, dark and fried
215 hair, slow reaction and easy panic, arched back, and reduced body size (Fig. S1 b). On
216 the last day of treatment (Day 15), the mental state of the DSS mice was still poor, but
217 the mental state of mice in the Lp082 and SASP groups gradually returned to normal,
218 with an active spirit, no arched back, no hematochezia and shiny hair (Fig. S1 b). In
219 addition, we found that the spleens of mice in the DSS group were significantly larger
220 and darker than those of mice in the normal group, but the spleen gradually returned
221 to normal in size and color after the Lp082 and SASP intake. (Fig. S1 c). (Page 7, line:
222 175-188)

223

224 The results of Shotgun metagenomic data diversity analysis demonstrated the effect of
225 Lp082 on the diversity of intestinal microbiota in mice. The results of α diversity
226 analysis showed that on days 1 - 7 of the study, the Shannon index in DSS, Lp082,
227 and SASP groups were all significantly decreased (Fig. 3a) , but the Shannon index
228 was significantly increased after the intake of Lp082 ($p < 0.05$) (Fig. 3a). The results
229 of β diversity analysis showed that the DSS group, LP082 group and SASP group
230 (M_B, M_C, M_D) and control group (M_A) were significantly separated on day 7 (p
231 < 0.05) (Fig. 3b). However, on day 15, the DSS group was still significantly separated
232 from the control group (T_B), while the distance between Lp082 group (T_C), SASP
233 group (T_D), and control group (T_A) was significantly reduced (p values < 0.05),
234 and the distance between Lp082 group and control group was closer, the above results
235 were consistent with the principal co-ordinates analysis (PCoA) distance results (Fig.

236 3c). The above diversity analysis results showed that Lp082 increased the α
237 -diversity and optimized the β -diversity of cecal microbiota in mice. (Page 9, line:
238 239-252)

239

240 Gene distribution was analyzed using colonic transcriptome data, the volcano map the
241 results show that Lp082 significantly affected gene expression distribution (Fig. S5
242 a-f). To further explore the impact of these differentially expressed genes (DEGs), we
243 analyzed the pathways involved in DEGs. (Page 12, line: 330-333)

244

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256 effect of *Lactobacillus plantarum*-12 on DSS-induced murine colitis. *Food &*
257 *Function*. 2020;11(6):5205-22; doi: 10.1039/d0fo00007h.

258

259 We sincerely thank you again for pointing this out. It was very helpful.

260

261 4. In Fig 5a, the data should be better presented regarding up-regulated genes and
262 down-regulated genes involved in metabolic pathway, respectively.

263 **Response:** We appreciate your valuable and helpful comment. We deeply and
264 sincerely understand the reviewer's idea. Fig. 5a is the results of Gene Ontology (GO)
265 enrichment analysis, GO can be divided into three categories, namely Biological

266 processes, Cellular Component and Molecular Function. In the initial analysis, I tried
267 to show the specific gene results and the up-regulation and down-regulation of
268 specific genes in the Gene Ontology pathway, but we did not do so in the end.
269 The reason we focus on the pathways in which genes are enriched, rather than the
270 genes in the pathways are as follows: By annotating the transcriptome data, we have a
271 volcanic map that reveals the distribution of gene expression and shows that the total
272 number of annotated genes is close to 20,000 (Fig. S5). There are so many genes that
273 it's too difficult for us to find rules among them. Through the investigation of
274 references [1], we found that a large number of disordered genes could be enriched
275 into a small number of pathways by gene enrichment analysis, so as to facilitate us to
276 explore the characteristics and rules between pathways. Gene enrichment analysis is a
277 common way to process a large amount of gene data, which can facilitate us to find
278 the rules among genes and GO enrichment analysis is one of the enrichment methods
279 [2]. The minimum value of GeneRatio of the GO term in Fig. 5a is 0.1, if the input
280 data used for enrichment analysis is assumed to be 1000 genes, then according to the
281 formula [3]: $\text{GeneRatio} = \frac{\text{the number of genes enriched to this GO term}}{\text{the number of all input genes used for enrichment analysis}}$, it can be concluded that the number of
282 genes enriched to the GO entry is 100 genes. There were 100 genes in one GO term,
283 1,000 genes in 10 GO terms. In fact, we calculated that the number of genes enriched
284 in a certain GO pathway was much greater than 100, because the number of
285 differentially expressed genes we input was much greater than 1000. That's why we
286 chose to analyze and present the pathway results, rather than listing every single gene
287 up-regulation and down-regulation in the pathway, because the amount of genetic data
288 is too large to find regular. Maza et al.[4] and Wang et al. [5] process a large number of
289 gene data through enrichment analysis, and finally find rules in pathway.
290

291 Our previous analysis idea was as follows: Since the preliminary analysis of
292 transcriptome data showed that the intake of Lp082 affects the gene expression
293 distribution (**Fig. S5**), in order to explore the relationship between a large number of
294 genes, we conducted GO pathway enrichment analysis and KEGG pathway
295 enrichment analysis for the differentially expressed genes (DEGs). Since the

296 differentially expressed genes (DEGs) were more enriched in the biological process
297 (BP) pathway among the three major GO pathway categories (**Fig. 5a-c**). And
298 compared with the DSS group, the number of significantly up-regulated genes in
299 Lp082 group is more than the down-regulated genes (**Fig. 5d**), so we performed
300 further GO-BP pathway enrichment analysis on the significantly up-regulated
301 differentially expressed genes (**Fig. 6d-6f**). Subsequently, we learned about some
302 genes that are abnormally expressed in inflammatory situations through literature,
303 analyzed the up-down regulation of these specific inflammatory genes, and found
304 similar rules in our data (**Fig. 6g-6i**). We have 6 biological replicates in each group,
305 and our data are realistic and objective enough to support our conclusion.

306 We appreciate your valuable and helpful comment again and we deeply agree
307 with the opinions of reviewer. We are deeply sorry for our not clear description.
308 According to your helpful suggestions, we have rewritten this part. The changes have
309 been highlighted in the manuscript in yellow. The rewritten content is more detailed,
310 and the details are as follows. (Page 12, line: 330-396)

311 Gene distribution was analyzed using colonic transcriptome data, the volcano
312 map the results show that Lp082 significantly affected gene expression distribution
313 (Fig. S5 a-f). To further explore the impact of these differentially expressed genes
314 (DEGs), we analyzed the pathways involved in DEGs.

315 Fig. 5a is the results of Gene Ontology (GO) enrichment analysis, GO can be
316 divided into three categories, namely Biological processes, Cellular Component and
317 Molecular Function. The results of gene ontology (GO) analysis (n=6) showed that
318 the DEGs of the DSS group and the control group were mainly involved in biological
319 processes such as the humoral immune response, activation of an immune response,
320 negative regulation of hemostasis; and cellular components such as blood
321 microparticle, membrane attack complex; and molecular functions such as lipid
322 binding, lipopolysaccharide-binding, thrombospondin receptor activity (Fig. 5a). On
323 the other hand, the DEG of the Lp082 and DSS groups was mainly involved in
324 biological processes such as blood coagulation, fibrin clot formation, regulation of
325 humoral immune markers, regulation of inflammatory cytokines; and cellular

326 components such as Golgi lumen, endoplasmic reticulum, and molecular functions
327 such as endopeptidase activity and peptidase activity (Fig. 5b).

328 Considering that in the Lp082, the up-regulated DEGs were far more than
329 down-regulated DEGs (Fig. S5 a-f), and the DEGs have the largest proportion of
330 participation in biological processes (Fig. 5a-5c), we further conducted GO-BP
331 analysis (n=6) on significantly up-regulated DEGs. The results of GO-BP analysis
332 showed that compared to control group, up-regulated DEGs in DSS group were
333 mainly enriched in the 6 inflammation-related GO-BP. Among those, the genes IL-1 β
334 and IL-1 α were both involved in the IL-1 β production and TNF production, the
335 oncogene Ereg were involved in the IL-1 β production, the genes IL-1 β and IL-1rn,
336 oncogene Fga were all involved in positive regulation of nuclear factor kappa-B
337 (NF- κ B) transcription factor activity, the oncogene Ldlr, Dgat2, and Mfsd2a were all
338 involved in the regulation of toll-like receptor 4 signaling pathway, the pro-oncogenes
339 Cdc7, Dbf4 were all involved in the acute inflammatory response, the anti-tumour
340 gene Syk and the inflammatory genes Nlrp3 as well as Syk were all involved in the
341 pro-inflammatory factor IL-6 production (Fig. 5d). Compared to DSS group, the
342 up-regulated genes in Lp082 group were mainly enriched in the 6
343 anti-inflammatory-related GO-BP. Among them, the gene Isg15, which exerted both
344 its antiviral and anti-inflammatory effects in innate immunity, and the gene Prg2,
345 which played an important role in wound healing, were involved in the
346 anti-inflammatory factors IL-10 production (Fig. 5e).

347 To further observe whether Lp082 treatment would suppress these inflammatory
348 and cancer genes enriched on inflammatory pathways in the DSS group, we
349 supplemented Fig. S6. As can be seen from Fig. S6, among the 13 inflammatory genes
350 or oncogenes that were up-regulated and enriched in the inflammatory pathway in the
351 DSS group, the following 10 genes were significantly down-regulated in the Lp082
352 group: IL-1 β , IL-1 α , Ereg, IL -1rn, Fga, Ldlr, Dgat2, Mfsd2a, Cdc7, Dbf4 (Fig. S6)

353 The results of kyoto encyclopedia of genes and genomes (KEGG) analysis (n=6)
354 showed that the DEGs in DSS and control groups were mainly enriched in systemic
355 lupus erythematosus, Staphylococcus aureus infection, Viral carcinogenesis, Pathways

356 in cancer, TNF signaling pathway, Cellular senescence, and mitogen-activated protein
357 kinase (MAPK) signaling pathway (Fig. S2a). However, the DEG in both Lp082 and
358 DSS groups, SASP and DSS groups, and SASP and Lp082 groups were mainly
359 enriched in the following five pathways: Complement and coagulation cascades,
360 Platelet activation, Autophagy - animal, Phagosome and N-Glycan biosynthesis (Fig.
361 S2b-S2d). Besides, the DEGs in Lp082 and DSS groups, as well as SASP and DSS
362 groups were involved in protein processing in the endoplasmic reticulum and
363 metabolic pathways (Fig. S2b-S2c).

364 The results of gut mucosal barrier analysis showed that gene expression of
365 MUC-2, ZO-1, ZO-2, occludin was significantly reduced in the DSS group but
366 significantly increased in the Lp082 and SASP groups (p values < 0.05), and the gene
367 expression of intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion
368 molecule (VCAM,) claudin-1, and claudin-2 increased significantly in the DSS group
369 but decreased significantly in the Lp082 and SASP groups (p values < 0.05)
370 (Fig.5g-5j). It is worth mentioning that MUC-2 is an essential component of gut
371 mucosa; ICAM-1 and VCAM induce gut mucosal lesions; ZO-1, ZO-2, and occludin
372 promote tight junctions of gut epithelial cells; claudin-1 and claudin-2 increase
373 intestinal permeability and aggravate inflammation.

374 Results of gene analysis related to NF- κ B pathway showed that Lp082 also
375 inhibited the mRNA expression of NF- κ B1, NF- κ B2, cyclooxygenase-2 (COX-2),
376 inducible nitric oxide synthase (iNOS), Toll-4, and RelA. These genes are signaling
377 molecules in the NF- κ B signaling pathway (Fig.5g-5j).

378

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381 program for assigning sequence reads to genomic features, *Bioinformatics*, 2014,
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395 HNU082 inhibited the growth of *Fusobacterium nucleatum* and alleviated the
396 inflammatory response introduced by *F. nucleatum* invasion. *Food & Function*.
397 2021;12(21):10728-40; doi: 10.1039/d1fo01388b.

398

399 5. In discussion, the creativity of manuscript should be noted compared with the
400 similarity studies which published before.

401 **Response:** We appreciate your valuable and helpful comment. We are very sorry for
402 our negligence of the creativity of manuscript. We sincerely apologize for the
403 confusion caused to you. According to your helpful suggestions, We have rewritten
404 the relevant content of the discussion section. The rewritten content focuses more on
405 creativity and innovation compared with similar studies published in the past. The
406 changes have been highlighted in the manuscript in yellow. And the revised content is
407 as follows.

408 Taniguchi et al. [1] found that ICAM-1 increases colonic mucosal damage. In our
409 study, we found that the Lp082 can not only decreased the mRNA expressions of
410 ICAM-1 and VCAM-1 but also can be effective in relieving intestinal mucosal lesions
411 (i.e., reduced ulceration and inflammatory cell infiltration caused by DSS). While the
412 adhesion molecules ICAM-1 and VCAM-1 are the key to the induction of intestinal
413 mucosal lesions[2]. This suggests that Lp082 may reduce intestinal mucosal lesions
414 by reducing mRNA expression of ICAM-1 and VCAM, thereby alleviating neutrophil
415 infiltration and ulceration. The above results showed that probiotic Lp082 increased

416 the MUC-2 content in the mucus layer by restoring the number of goblet cells, and
417 relieved the intestinal mucosal damage caused by ICAM-1 and VCAM-1, so as to
418 repaired the chemical barrier. (Page 17, line: 470-480)

419

420 Cordeiro et al. [6] found that the content of ZO-1 and ZO-2 were significantly
421 decreased in UC mice, but were increased after probiotic minas frescal cheese intake.
422 Because Lp082 excellently improved histopathology, we speculated that Lp082 also
423 has a regulatory effect on TJ molecules. To this end, we analyzed major TJ proteins,
424 including ZO-1, ZO-2, and occludin. As expected, the mRNA expression and
425 immunofluorescence protein content of ZO-1, the mRNA expression of ZO-2 and
426 occludin were significantly decreased in DSS-induced UC mice, but were
427 significantly improved in the Lp082 group, indicating that the improvement of the
428 mechanical barrier by regulating TJ may be one of the mechanisms by which
429 probiotic Lp082 exerts anti-UC. In addition, Icam-1 and VCAM-1, which are
430 abnormally expressed in UC patients, were increased in DSS group [7]. Adhesion
431 molecules ICAM-1 and VCAM-1 can not only induce intestinal mucosal injury [8],
432 but also increase the permeability of intestinal mucosa [1] while anti-ICAM-1
433 treatment can alleviate colonic mucosal injury [9]. Interestingly, the mRNA
434 expression of ICAM-1 and VCAM-1 was found to decrease after Lp082 ingestion.
435 Therefore, it can be thought that the alleviation of UC by Lp082 may be due to
436 down-regulation of ICAM-1, VCAM-1 and increase protein quantity and mRNA
437 expression of ZO-1, ZO-2 to reduce intestinal mucosal permeability, thereby
438 inhibiting the entry of harmful bacteria and undigested food and toxins into the body
439 and reducing inflammation. These results suggest that Lp082 repairs the intestinal
440 mechanical barrier by regulating TJ. (Page 17, line: 491-511)

441

442 Although the exact etiology of UC is complex and uncertain, studies suggest that the
443 NF- κ B pathway plays a vital role in the pathogenesis of UC [10]. Our study has
444 proved that Lp082 inhibits the NF- κ B pathway by down-regulating the mRNA
445 expression of NF- κ B2, NF- κ B1, COX-2, Rela, Toll4, iNOS, and that NF- κ B can also

446 regulate inflammation by regulating cytokines [11]. Therefore, it can be suggested
447 that Lp082 also has a specific regulatory effect on cytokines. To confirm this, we
448 analyzed the cytokines associated with NF- κ B. As expected, we observed that the
449 mRNA expression level of pro-inflammatory cytokines (TNF- α , IL-1 β , and IL-6) was
450 significantly increased in the DSS group but significantly decreased in the Lp082
451 group. It is interesting to note that the protein levels of TNF- α , IL-1 β , and IL-6
452 detected by ELISA kit were also increased in the DSS group and decreased after
453 Lp082 intake. Among them, TNF- α can promote the proliferation and differentiation
454 of T cells and increase intestinal inflammation [12]. The upregulation of IL-1 β is
455 involved in the recruitment and retention of leukocytes in inflamed tissues and can
456 activate innate immune lymphocytes [13]. IL-6 activates NF- κ B to regulate the
457 dextran sulfate sodium-induced colitis in mice [14]. The above results indicate that
458 Lp082 alleviates UC by inhibiting the levels of pro-inflammatory factors (TNF- α ,
459 IL-1 β , and IL-6). Interestingly, we also found that the mRNA expressions of
460 anti-inflammatory cytokines IL10, TGF-1, and TGF-2 were significantly decreased in
461 the DSS group but increased in the Lp082 group. IL-10 protein levels measured by
462 ELISA kit also decreased in the DSS group and increased in the Lp082 group.
463 Surprisingly, IL10, TGF-1, and TGF-2 were shown to activate Treg and
464 anti-inflammatory macrophages to alleviate UC [15]. And Sato et al. [16] also found
465 that the loss of IL-10 spontaneously gave rise to IBD, and Hume et al. [17] found that
466 TGF- β 1 and TGF- β 2 could dramatically relieve intestinal inflammation in
467 DSS-induced colitis mice. These results suggest that Lp082 alleviates UC by
468 increasing the levels of anti-inflammatory factors IL10, TGF-1, and TGF-2. We
469 further analyzed the specific regulatory effects of Lp082 on intestinal mucosal
470 immunity. In addition to inflammatory factors, we also noticed that a heme protein,
471 MPO, was significantly reduced in the Lp082 group. Trevisin et al. [18] found that
472 MPO caused UC by producing cytokines and hypochlorite and that MPO in the colon
473 of UC patients is mainly produced by neutrophil infiltration [19]. Interestingly, this is
474 consistent with the fact that the DSS group had a severe neutrophil infiltration in this
475 study. However, neutrophil infiltration and MPO content were significantly decreased

476 in the Lp082 group. This shows that Lp082 alleviates UC by reducing neutrophil
477 infiltration and its secreted MPO content. Thus, our results suggest that Lp082 may
478 play an anti-UC effect by inhibiting the NF- κ B pathway, down-regulating
479 pro-inflammatory cytokines, and up-regulating anti-inflammatory cytokines, reducing
480 MPO content, thereby maintaining immune balance and protecting the immune barrier.
481 (Page 18, line: 514-553)

482

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