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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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Transaction Report:

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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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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 Lactoplantibacillus 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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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.
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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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• 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

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Thank you for submitting your paper to Microbiology Spectrum.

Article summary and impression:
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Minor points:

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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.

Manuscript No.: Spectrum 01651-22

Title: 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 Dr. Xiaoyu Tang,

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

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

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

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

Sincerely,
Jiachao Zhang

Yours sincerely,

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

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

Reviewer #1:

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

Metagenome and transcriptome raw data

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

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

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

Reviewer #1 (Comments for the Author):

Article summary and impression:

In the article Spectrum 01651-22, the authors seek to describe the impact of supplementation of the food-derived bacterial strain *lactobacillus 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) SCFAs 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 Lactoplantibacillus 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.

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

Preface to the following comments:

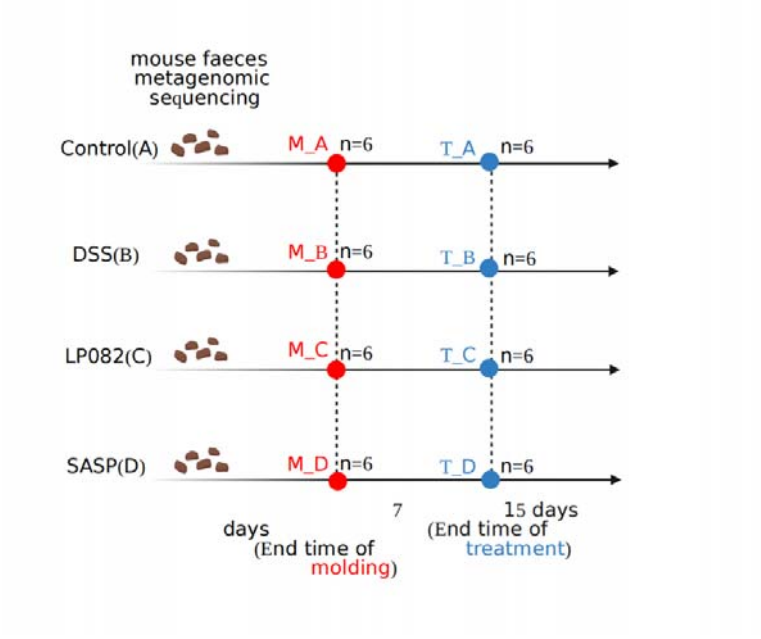
The manuscript does not use page numbers and line numbers. To review this document, I exported the pdf to word and refer to the title page as page 1, with the first line of the title being line 1.

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

Major points:

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

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



SUPPLEMENTARY FIGURE LEGENDS

Fig. S3

(a) Timepoints and grouping of mouse metagenomic sequencing

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

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

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

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

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

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

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

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

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

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

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

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

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

Reference

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

Microbiome. 2021;9(1); doi: 10.1186/s40168-021-01102-0.

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

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

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

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

The regulatory role of SCFAs

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

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

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

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

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

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

Reference

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

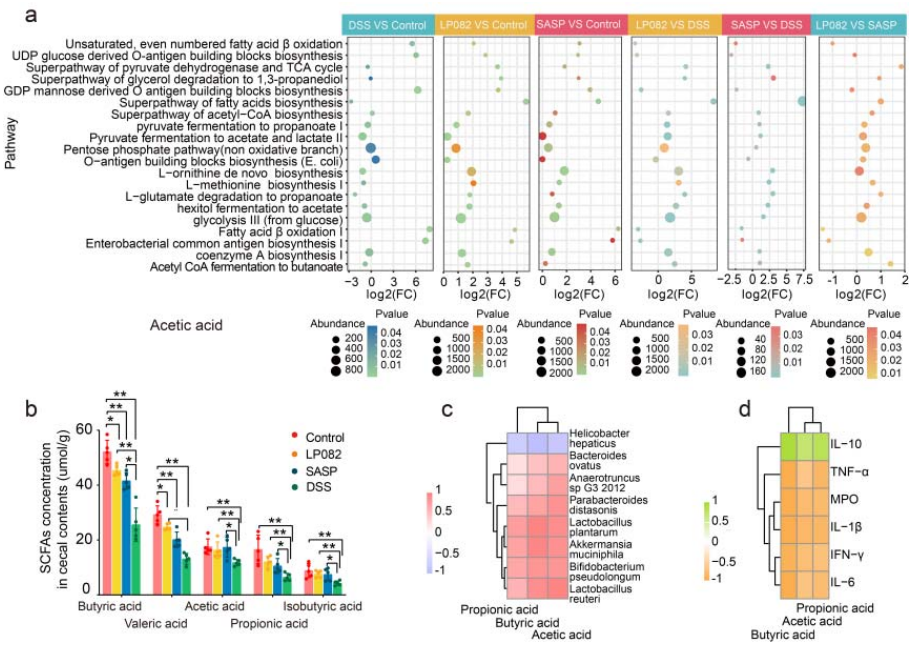


Fig. 4

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

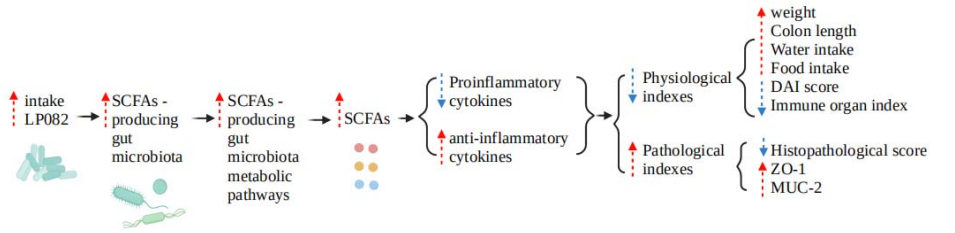


Fig. S4

The underlying mechanism by which Lp082 regulates SCFAs to alleviate UC

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

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

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

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

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

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

To prove the above findings, we further used gas chromatography-mass spectrometry (GC-MS) to detect the content of SCFAS. Compared with control group, the contents of butyric acid, valeric acid, acetic acid, propionic acid and isobutyric acid were significantly decreased after ingestion of DSS ($P < 0.01$). Compared with DSS group, the contents of butyric acid, acetic acid, propionic acid and isobutyric acid were extremely significant increased after ingestion of Lp082 ($P < 0.01$). This confirmed our previous hypothesis based on the correlation that Lp082 intake would 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-1β, 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

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

Reference

1. Cordeiro BF, Alves JL, Belo GA, Oliveira ER, Braga MP, da Silva SH, et al. Therapeutic Effects of Probiotic Minas Frescal Cheese on the Attenuation of UC in a Murine Model. Frontiers in Microbiology. 2021;12; doi: 10.3389/fmicb.2021.623920.
2. Wang J, Ji HF, Wang SX, Liu H, Zhang W, Zhang DY, et al. Probiotic *lactobacillus plantarum* Promotes Intestinal Barrier Function by Strengthening the Epithelium and Modulating Gut Microbiota. Frontiers in Microbiology. 2018;9; doi: 10.3389/fmicb.2018.01953.
3. Pan Y, Ning Y, Hu J, Wang Z, Chen X, Zhao X. The Preventive Effect of *lactobacillus plantarum* ZS62 on DSS-Induced IBD by Regulating Oxidative Stress and the Immune Response. Oxid Med Cell Longev. 2021;2021:9416794; doi: 10.1155/2021/9416794.
4. Shao Y, Huo D, Peng Q, Pan Y, Jiang S, Liu B, et al. *lactobacillus plantarum* HNU082-derived improvements in the intestinal microbiome prevent the development of hyperlipidaemia. Food & Function. 2017;8(12):4508-16; doi: 10.1039/c7fo00902j.
5. Wang Y, li J, Ma C, Jiang S, Li C, Zhang L, et al. *lactobacillus plantarum* HNU082 inhibited the growth of Fusobacterium nucleatum and alleviated the inflammatory response introduced by F. nucleatum invasion. Food & Function. 2021;12(21):10728-40; doi: 10.1039/d1fo01388b.
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probiotic *lactobacillus plantarum* HNU082 rapidly and convergently evolves within human, mice, and zebrafish gut but differentially influences the resident microbiome. Microbiome. 2021;9(1); doi: 10.1186/s40168-021-01102-0.

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

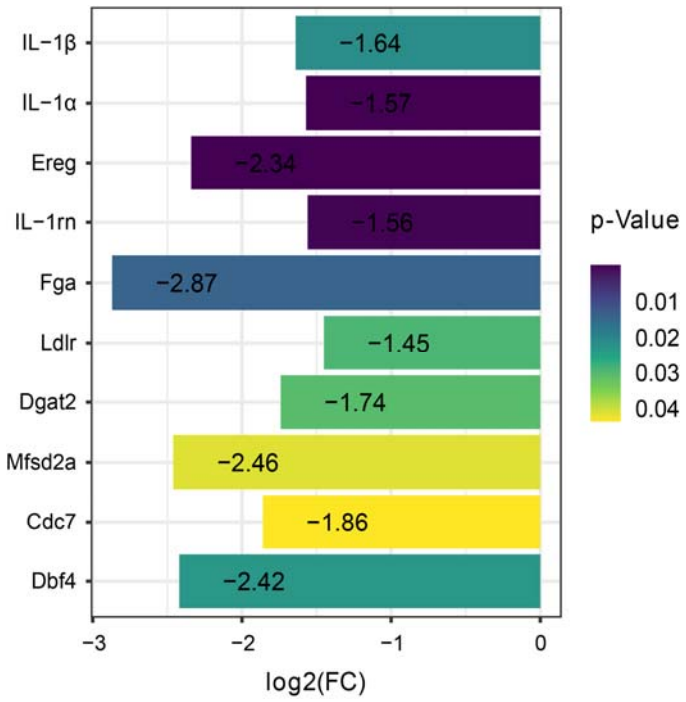
Response: We are grateful for the suggestion. We have added a more detailed interpretation regarding analysis and discussion of Lp082 gene. More detailed statistical analysis was added in the paper. Supplementary Figure **Fig. S6** illustrates the effect of Lp082 treatment on up-regulated inflammatory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

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

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

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

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



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

SUPPLEMENTARY FIGURE LEGENDS

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

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

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

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

a. Rationale:

I. Why was SASP used?

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

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

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

Reference

1. Steinhart AH, Hemphill D, Greenberg GR. Sulfasalazine and mesalazine for the maintenance therapy of Crohn's disease: a meta-analysis. The American journal of gastroenterology. 1994;89(12):2116-24.

2. Klotz U, Maier K, Fischer C, Heinkel K. Therapeutic efficacy of sulfasalazine

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

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II. Why was Lp082 used specifically?

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

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

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

Reference

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

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b. Experimental design, conditions, methods:

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

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

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

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

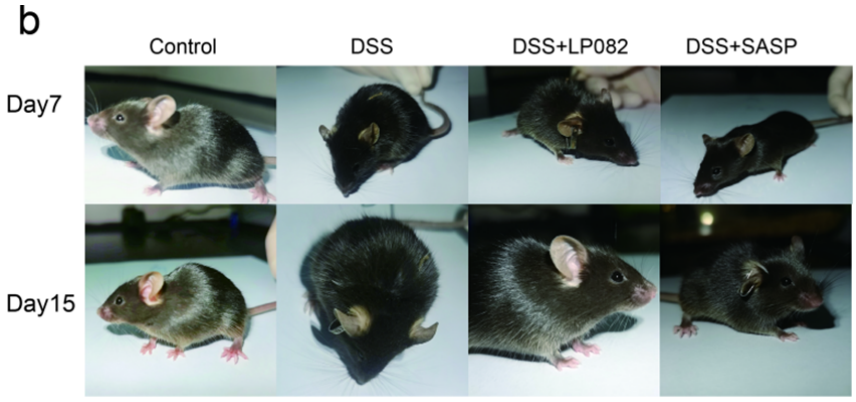

Fig. S1

(b) Mental state of experimental mice.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

e. Statistics:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Minor points:

1. Missing information that should be addressed:

a. Rationale:

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

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

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

There is no consensus on the specific pathogenesis of UC, and many evidences suggest that the pathogenesis of UC is multifactorial, involving genetic susceptibility, epithelial barrier defects, immune response disorders and environmental factors [6]. Differences in gut microbiota (type and amount) between colitis patient and healthy people are thought to be one of the key factors in disease progression [7]. In UC patients, the immune response is activated, the intestinal permeability is increased, the intestinal mucosal barrier structure is destroyed, the homeostasis of gut microbiota is

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

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

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

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

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

Reference

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

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

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

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

Reference

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supernatant increase SCFAs-producing bacteria to restore gut dysbiosis in TNBS-induced colitis. Amb Express. 2021;11(1); doi: 10.1186/s13568-021-01197-6.

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b. Impact:

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

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

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

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

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

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

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

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

Reference

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

abundance suggest about Lp082 effects?

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

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

Reference

1. Li XX, Wei B, Goodglick L, Wen T, Xia LJ, Braun J. Investigating Therapeutic Approach of IBD Using Recombinant Glycoprotein Mucin2. Faseb Journal. 2009;23.

2. Pan Y, Ning Y, Hu J, Wang Z, Chen X, Zhao X. The Preventive Effect of *lactobacillus plantarum* ZS62 on DSS-Induced IBD by Regulating Oxidative Stress and the Immune Response. Oxid Med Cell Longev. 2021;2021:9416794; doi: 10.1155/2021/9416794.

c. Methods:

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

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

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

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

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

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

The following has been added to the supplementary materials:

FIGURE LEGENDS

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

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

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

SUPPLEMENTARY TABLE LEGENDS

Table S1.

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

colitis.

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

Table S2.

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

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

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

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

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

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

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

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

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

d. Results structure:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

e. Figures:

I. Figure 1:

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

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

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

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

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

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

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

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

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

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

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

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

II. Figure 2:

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

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

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

not labeled in the figure and in the figure legend.

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

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

FIGURE LEGENDS

Fig. 2. Effects of Lp082 on histological parameters and immunofluorescent proteins.

(d) Immunofluorescence staining of MUC-2 (green fluorescence). Scale bar = 100 μm. Blue marker is the color of the negative of the photograph (colon tissue without antigenic markers)

(e) Immunofluorescence staining of ZO-1 (red fluorescence). Scale bar = 100 μm. Blue marker is the color of the negative of the photograph (colon tissue without antigenic markers) (Page 40, line: 1164-1169)

Fig 2C - the y axis is missing a metric

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

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

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

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

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

III. Figure 3:

Fig 3A-C groupings not labeled as indicated above

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

SUPPLEMENTARY FIGURE LEGENDS

Fig.S3

(a) Timing and grouping of mouse metagenomic sequencing

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

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

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

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

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

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

FIGURE LEGENDS

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

(d)The red highlight in the **Fig. 3d** refers to the significantly increased bacteria that can produce SCFAs in the Lp082 group. The tree in the **Fig. 3d** represents the phylogenetic tree, which is obtained by clustering the abundance of each color block based on the unifrac distance after taking log2 (x*100) for the relative abundance at the species level. The clustering does not reflect any evolutionary relationship. It shows the abundance of bacterial species in the sample. 0 has no special meaning in it (it is only used to facilitate the differentiation of overall abundance). The darker the yellow in the color block in the Fig. 3d (the value closer to 2), the higher the relative abundance. Darker blue (values closer to -2) indicate lower relative abundance.

IV. Figure 4:

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

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

The regulatory role of SCFAs

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

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

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

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

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

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

Reference

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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.

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

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

FIGURE LEGENDS

Fig. 4.

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

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

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

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

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

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.

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

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

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

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

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

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

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

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

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

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

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

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

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.

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

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."

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

biological barrier by regulating the intestinal microbiome and increasing the SCFAs. Lp082 improved the chemical barrier by reducing ICAM-1, VCAM, and increasing goblet cells and mucin2. Lp082 ameliorated the mechanical barrier by increasing the ZO-1, ZO-2, and occludin and decreasing claudin-1 and claudin-2. Lp082 optimized the immune barrier by reducing the content of IL-1β, IL-6, TNF-α, MPO, IFN-γ and increasing the IL-10, TGF-β1, and TGF-β2. In conclusion, we believe that it is reasonable to use these four barriers to discuss the effect of Lp082 on DSS induced UC. Maybe we didn't describe it very well, so we rewrote a discussion section that explained the four barriers in more detail, with the following changes. (Page 17, line: 496-637)

Lp082 improved chemical barrier

The chemical barrier refers to the glue-like mucin layer covering the surface of intestinal epithelial cells, which is mainly composed of MUC-2 secreted by goblet cells, digestive juices and bacteriostatic substances produced by normal parasitic bacteria in the intestinal lumen [1]. The chemical barrier plays an important role in isolating the internal and external environment of the intestinal tract, lubricating the intestinal mucosa, and inhibiting the entry of harmful substances in the intestinal lumen [5]. The intestinal mucosal wall thickness was significantly increased in the DSS group, whereas it was significantly decreased after Lp082 ingestion **(Fig. 2c)**. In DSS-induced UC, the thicker the intestinal mucosal wall, indicating more severe inflammation. In addition,the H&E staining result showed that the number of goblet cells decreased in the DSS group (red arrow), whereas the number of goblet cells increased (yellow arrow) after Lp082 ingestion **(Fig. 2a)**. The immunofluorescent protein content of MUC-2, which is mainly secreted by goblet cells, was significantly decreased in the DSS group **(Fig. 2d)**, and the areal density of MUC-2 **(Fig. 2f)** and the mRNA expression of MUC-2 were also significantly decreased in the DSS group **(Fig. 5c)**, while the immunofluorescence protein content, areal density and mRNA expression of MUC-2 all increased in the Lp082 group,

Sun et al. [6] observed the same phenomenon that *lactobacillus plantarum 12* can repair the intestinal mucosal chemical barrier by increasing the content of MUC-2.

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

Lp082 improved mechanical barrier

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

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

Lp082 improved the immune barrier

Although the exact etiology of UC is complex and uncertain, studies suggest that the NF-κB pathway plays a vital role in the pathogenesis of UC [3]. Our study has proved that Lp082 inhibits the NF-κB pathway by down-regulating the mRNA expression of NF-κB2, NF-κB1, COX-2, Rela, Toll4, iNOS, and that NF-κB can also regulate inflammation by regulating cytokines [16]. Therefore, it can be suggested that Lp082 also has a specific regulatory effect on cytokines. To confirm this, we analyzed the cytokines associated with NF-κB. As expected, we observed that the mRNA expression level content of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) were significantly increased in the DSS group but significantly decreased in the Lp082 group, It is interesting to note that the protein levels of TNF-α, IL-1β, and IL-6 detected by elisa kit were also increased in the DSS group and decreased after Lp082 intake. Among them, TNF-α can promote the proliferation and differentiation of T cells and increase intestinal inflammation [17]. The upregulation of IL-1β is involved in the recruitment and retention of leukocytes in inflamed tissues and can activate

innate immune lymphocytes [18]. IL-6 activates NF-κB to regulate the dextran sulfate sodium-induced colitis in mice [19]. The above results indicate that Lp082 alleviates UC by inhibiting the levels of pro-inflammatory factors (TNF-α, IL-1β, and IL-6). Interestingly, we also found that the mRNA expressions of anti-inflammatory cytokines IL10, TGF-1, and TGF-2 were significantly decreased in the DSS group but increased in the Lp082 group. Il-10 protein levels measured by elisa kit also decreased in the DSS group and increased in the Lp082 group. Surprisingly, IL10, TGF-1, and TGF-2 were shown to activate Treg and anti-inflammatory macrophages to alleviate UC [20]. And Sato et al. [21] also found that the loss of IL-10 spontaneously gave rise to IBD, and Hume et al. [22] found that TGF-β1 and TGF-β2 could dramatically relieve intestinal inflammation in DSS-induced colitis mice. These results suggest that Lp082 alleviates UC by increasing the levels of anti-inflammatory factors IL10, TGF-1, and TGF-2. We further analyzed the specific regulatory effects of Lp082 on intestinal mucosal immunity. In addition to inflammatory factors, we also noticed that a heme protein, MPO, was significantly reduced in the Lp082 group. Trevisin et al. [23] found that MPO caused UC by producing cytokines and hypochlorite and that MPO in the colon of UC patients is mainly produced by neutrophil infiltration [24]. Interestingly, this is consistent with the fact that the DSS group had a severe neutrophil infiltration in this study. However, neutrophil infiltration and MPO content were significantly decreased in Lp082 group. This shows that Lp082 alleviates UC by reducing neutrophil infiltration and its secreted MPO content. In a nutshell, our results suggest that Lp082 may play an anti-UC effect by inhibiting the NF-κB pathway, down-regulating pro-inflammatory cytokines, and up-regulating anti-inflammatory cytokines, reducing MPO content, thereby maintaining immune balance and protecting the immune barrier.

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

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

Lp082 improved the biological barrier

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

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

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

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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.

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

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

barrier. Specifically, Lp082 rebuilt the biological barrier by regulating the intestinal microbiome and increasing the production of short-chain fatty acids (SCFAs). Lp082 improved the chemical barrier by reducing intercellular cell adhesion molecule-1, vascular cell adhesion molecule and increasing goblet cells and mucin2. Lp082 ameliorated the mechanical barrier by increasing the zonula occludens-1 (ZO-1), zonula occludens-2 (ZO-2), and occludin while decreasing claudin-1 and claudin-2. Lp082 optimized the immune barrier by reducing the content of IL-1β, IL-6, TNF-α, MPO, IFN-γ and increasing the IL-10, TGF-β1, and TGF-β2, inhibiting the NF-kB signalling pathway. Taken together, probiotic Lp082 can play a protective role in a DSS-induced colitis mouse model by protecting the intestinal mucosal barrier, attenuating the inflammatory response, and regulating microbial imbalance. This study provides support for the development of probiotic-based microbial products as an alternative treatment strategy for UC.

Importance

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

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

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.

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

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

Reference

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

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."

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

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

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

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

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

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

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

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

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).

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

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

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

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

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

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

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

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

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

Reviewer #2 (Comments for the Author):

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

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.

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

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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4. Please improve the language and grammar.

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

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

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

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.

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

function of the intestinal mucosal barrier. The intestinal mucosal barrier is damaged, resulting in an increase in the permeability of the intestinal epithelial barrier, and further stimulation of intestinal contents, bacteria, and toxins promotes the immune response to intestinal inflammation. The normal intestinal mucosal barrier consists of mechanical barrier, chemical barrier, immune barrier, and biological barrier. The chemical barrier refers to the glue-like mucin layer covering the surface of intestinal epithelial cells, which is mainly composed of MUC-2 secreted by goblet cells, digestive juices, and bacteriostatic substances produced by normal parasitic bacteria in the intestinal lumen [1]. The mechanical barrier is the most important part of the intestinal mucosal barrier. Its structural basis is the intestinal mucosal epithelial cells and the tight junctions (TJ) between the epithelial cells [2]. The immune barrier is associated with immune cells, and inflammatory factors [3]. The biological barrier is a normal intestinal colony of bacteria that is resistant to colonization by foreign strains [4]. The results of the study found that Lp082 can improve the intestinal mucosal barrier by synergistically optimizing the biological barrier, chemical barrier, mechanical barrier and immune barrier, thereby alleviating UC. Specifically, We found that Lp082 rebuilt the biological barrier by regulating the intestinal microbiome and increasing the SCFAs. Lp082 improved the chemical barrier by reducing ICAM-1, VCAM, and increasing goblet cells and mucin2. Lp082 ameliorated the mechanical barrier by increasing the ZO-1, ZO-2, and occludin and decreasing claudin-1 and claudin-2. Lp082 optimized the immune barrier by reducing the content of IL-1β, IL-6, TNF-α, MPO, IFN-γ and increasing the IL-10, TGF-β1, and TGF-β2. From the above four aspects, we demonstrated that Lp082 can indeed improve the "intestinal mucosal barrier" to treat DSS-induced UC.

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

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

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

16, line: 459-637)

DISCUSSION

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

Lp082 improved chemical barrier

The chemical barrier refers to the glue-like mucin layer covering the surface of intestinal epithelial cells, which is mainly composed of MUC-2 secreted by goblet cells, digestive juices and bacteriostatic substances produced by normal parasitic bacteria in the intestinal lumen [1]. The chemical barrier plays an important role in isolating the internal and external environment of the intestinal tract, lubricating the intestinal mucosa, and inhibiting the entry of harmful substances in the intestinal lumen [5]. The intestinal mucosal wall thickness was significantly increased in the DSS group, whereas it was significantly decreased after Lp082 ingestion **(Fig. 2c)**. In DSS-induced UC, the thicker the intestinal mucosal wall, indicating more severe inflammation. In addition,the H&E staining result showed that the number of goblet cells decreased in the DSS group (red arrow), whereas the number of goblet cells increased (yellow arrow) after Lp082 ingestion **(Fig. 2a)**. The immunofluorescent protein content of MUC-2, which is mainly secreted by goblet cells, was significantly decreased in the DSS group **(Fig. 2d)**, and the areal density of MUC-2 **(Fig. 2f)** and the mRNA expression of MUC-2 were also significantly decreased in the DSS group **(Fig. 5c)**, while the immunofluorescence protein content, areal density and mRNA expression of MUC-2 all increased in the Lp082 group,

Sun et al. [6] observed the same phenomenon that *lactobacillus plantarum 12* can repair the intestinal mucosal chemical barrier by increasing the content of MUC-2.

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

Lp082 improved mechanical barrier

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

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

Lp082 improved the immune barrier

Although the exact etiology of UC is complex and uncertain, studies suggest that the NF-κB pathway plays a vital role in the pathogenesis of UC [3]. Our study has proved that Lp082 inhibits the NF-κB pathway by down-regulating the mRNA expression of NF-κB2, NF-κB1, COX-2, Rela, Toll4, iNOS, and that NF-κB can also regulate inflammation by regulating cytokines [16]. Therefore, it can be suggested that Lp082 also has a specific regulatory effect on cytokines. To confirm this, we analyzed the cytokines associated with NF-κB. As expected, we observed that the mRNA expression level content of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) were significantly increased in the DSS group but significantly decreased in the Lp082 group, It is interesting to note that the protein levels of TNF-α, IL-1β, and IL-6 detected by elisa kit were also increased in the DSS group and decreased after Lp082 intake. Among them, TNF-α can promote the proliferation and differentiation of T cells and increase intestinal inflammation [17]. The upregulation of IL-1β is involved in the recruitment and retention of leukocytes in inflamed tissues and can activate

innate immune lymphocytes [18]. IL-6 activates NF-κB to regulate the dextran sulfate sodium-induced colitis in mice [19]. The above results indicate that Lp082 alleviates UC by inhibiting the levels of pro-inflammatory factors (TNF-α, IL-1β, and IL-6). Interestingly, we also found that the mRNA expressions of anti-inflammatory cytokines IL10, TGF-1, and TGF-2 were significantly decreased in the DSS group but increased in the Lp082 group. Il-10 protein levels measured by elisa kit also decreased in the DSS group and increased in the Lp082 group. Surprisingly, IL10, TGF-1, and TGF-2 were shown to activate Treg and anti-inflammatory macrophages to alleviate UC [20]. And Sato et al. [21] also found that the loss of IL-10 spontaneously gave rise to IBD, and Hume et al. [22] found that TGF-β1 and TGF-β2 could dramatically relieve intestinal inflammation in DSS-induced colitis mice. These results suggest that Lp082 alleviates UC by increasing the levels of anti-inflammatory factors IL10, TGF-1, and TGF-2. We further analyzed the specific regulatory effects of Lp082 on intestinal mucosal immunity. In addition to inflammatory factors, we also noticed that a heme protein, MPO, was significantly reduced in the Lp082 group. Trevisin et al. [23] found that MPO caused UC by producing cytokines and hypochlorite and that MPO in the colon of UC patients is mainly produced by neutrophil infiltration [24]. Interestingly, this is consistent with the fact that the DSS group had a severe neutrophil infiltration in this study. However, neutrophil infiltration and MPO content were significantly decreased in Lp082 group. This shows that Lp082 alleviates UC by reducing neutrophil infiltration and its secreted MPO content. In a nutshell, our results suggest that Lp082 may play an anti-UC effect by inhibiting the NF-κB pathway, down-regulating pro-inflammatory cytokines, and up-regulating anti-inflammatory cytokines, reducing MPO content, thereby maintaining immune balance and protecting the immune barrier.

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

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

Lp082 improved the biological barrier

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

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

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

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Minor comments:

1. Please provide line numbering.

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

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

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

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.

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

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

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

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

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

Reference

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

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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:

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• Upload a compare copy of the manuscript (without figures) as a "Marked-Up Manuscript" file.
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• 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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Thank you for submitting your paper to Microbiology Spectrum.

Manuscript No.: Spectrum 01651-22

Title: 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 Dr. Xiaoyu Tang,

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

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

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

Sincerely,

Jiachao Zhang
Yours sincerely,
E-mail: Jiachao Zhang1*, zhjch321123@163.com
College of Food Science and Engineering, Hainan University, Haikou 570228, China

Responds to the reviewer's comments

Reviewer #2 (Comments for the Author):

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

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

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

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

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

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

The intake of Lp082 alleviated pathological lesions in DSS-induced colitis mice

(Page 8, line: 203)

The intake of Lp082 regulated the gut microbiota in DSS-induced colitis mice

(Page 9, line: 238)

The intake of Lp082 regulated the short chain fatty acid in DSS-induced colitis mice

(Page 10, line: 265-266)

The intake of Lp082 regulated the transcriptome of intestinal epithelial cells in DSS-induced colitis mice

(Page 12, line: 328-329)

The potential mechanism of Lp082 alleviated the DSS-induced colitis

(Page 14, line: 398)

The intake of Lp082 improved the chemical barrier

(Page 16, line: 449)

The intake of Lp082 improved the mechanical barrier

(Page 17, line: 482)

The intake of Lp082 improved the immune barrier

(Page 18, line: 513)

The intake of Lp082 improved the biological barrier

(Page 20, line: 576)

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

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

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

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

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

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

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

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

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

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

Reference

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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.

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

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

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

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

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

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

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

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

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

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

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

Reference

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

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

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

processes, Cellular Component and Molecular Function. In the initial analysis, I tried to show the specific gene results and the up-regulation and down-regulation of specific genes in the Gene Ontology pathway, but we did not do so in the end.

The reason we focus on the pathways in which genes are enriched, rather than the genes in the pathways are as follows: By annotating the transcriptome data, we have a volcanic map that reveals the distribution of gene expression and shows that the total number of annotated genes is close to 20,000 (Fig. S5). There are so many genes that it's too difficult for us to find rules among them. Through the investigation of references [1], we found that a large number of disordered genes could be enriched into a small number of pathways by gene enrichment analysis, so as to facilitate us to explore the characteristics and rules between pathways. Gene enrichment analysis is a common way to process a large amount of gene data, which can facilitate us to find the rules among genes and GO enrichmen analysis is one of the enrichment methods [2]. The minimum value of GeneRatio of the GO term in Fig. 5a is 0.1, if the input data used for enrichment analysis is assumed to be 1000 genes, then according to the formula [3]: GeneRatio= the number of genes enriched to this GO term / the number of all input genes used for enrichment analysis, it can be concluded that the number of genes enriched to the GO entry is 100 genes. There were 100 genes in one GO term, 1,000 genes in 10 GO terms. In fact, we calculated that the number of genes enriched in a certain GO pathway was much greater than 100, because the number of differentially expressed genes we input was much greater than 1000. That's why we chose to analyze and present the pathway results, rather than listing every single gene up-regulation and down-regulation in the pathway, because the amount of genetic data is too large to find regular. Maza et al.[4] and Wang et al. [5]process a large number of gene data through enrichment analysis, and finally find rules in pathway.

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

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

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

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

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

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

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

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

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

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

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

Results of gene analysis related to NF-κB pathway showed that Lp082 also inhibited the mRNA expression of NF-κB1, NF-κB2, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), Toll-4, and RelA. These genes are signaling molecules in the NF-κB signaling pathway (Fig.5g-5j).

Reference

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

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

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

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

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

Although the exact etiology of UC is complex and uncertain, studies suggest that the NF-κB pathway plays a vital role in the pathogenesis of UC [10]. Our study has proved that Lp082 inhibits the NF-κB pathway by down-regulating the mRNA expression of NF-κB2, NF-κB1, COX-2, Rela, Toll4, iNOS, and that NF-κB 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-κB. As expected, we observed that the
449 mRNA expression level of pro-inflammatory cytokines (TNF-α, IL-1β, 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-1β, 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-1β 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-κB 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-1β, 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-β1 and TGF-β2 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

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

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

October 14, 2022

Prof. Jiachao Zhang
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