Dear Editor,

Thank you very much for your attention and the referees' valuable evaluation and comments on our manuscript 78512. We have revised the manuscript according to your kind advice and the referee's detailed suggestions. Please find enclosed the responses to the referees.

In addition, as mentioned in the previous email sent to BPG Editorial Office. During the implementation of this study, Dr. Xiang Ao and Dr. Xiang Xu made the same contribution and assumed the same responsibility, so we listed Dr. Xiang Ao and Dr. Xiang Xu as the co-corresponding authors in the first submission. However, during this revision process, we accidentally found that the journal does not allow the designation of co-corresponding authors. Therefore, based on Dr. Xiang Ao's more contributions in the manuscript revision, after discussion and agreement by all authors, we apply to list Dr. Xiang Ao (ax_syd@163.com) as the corresponding author.

We sincerely hope this manuscript will be finally acceptable to be published in the *World Journal of Stem Cells*.

Thank you very much for all your help and looking forward to hearing from you soon.

Sincerely,

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Please find the following responses to the comments of reviewers and Company editor-in-chief:

- **Response to Reviewer #1:**

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: The study developed in this work is very interesting. It is an excellent starting point for future studies. I would prolong the time associated with the expression of TNF-α to better validate the results and repeat them in more biological replicates to be sure of reproducibility. Congratulations to the authors.

Response: Thank you very much for your positive comments on this research. With your encouragement, we will further explore the translational application potential of IFN-γ and TNF-α pretreated MSCs in future studies, as well as other strategies that may improve the immunosuppressive function of MSCs.

- **Response to Reviewer #2:**

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The expression of immunosuppressive molecules, such as PD-L1, in MSCs, poses limitations on the effectiveness of MSCT therapy. Nonetheless, the precise mechanism through which IFN-γ alone or in combination with TNF-α induces a significant increase in PD-L1 expression in hUC-MSCs has not been fully elucidated. In the current research authors have answered tis research question. They demonstrated that the expression of PD-L1 in hUC-MSCs was increased by IFN-γ, leading to an enhancement of their immunosuppressive abilities through the JAK/STAT1/IRF1 pathway. Moreover, TNF-α acted in synergy with IFN-γ to further elevate PD-L1 expression in hUC-MSCs by upregulating the Nuclear Factor kappa-B (NF-κB)-mediated expression of IFN-γ receptor 1 (IFNGR1), which subsequently activated the JAK/STAT1/IRF1 signaling pathway. In my opinion manuscript is well planned and all the experiments are executed nicely. So it suitable for publication after

addressing following queries; 1. Discuss about the future transnational potential of the current study. 2. Include some of the limitations of the current study before translating it.

Response: Thank you very much for your comments. In response to your queries, we have added the following content to the "Discussion" section of this manuscript.

In recent years, MSCT therapy is becoming a popular cell therapy for the treatment of various inflammatory diseases due to the immunosuppressive ability of MSCs. However, due to the differences in the source of MSCs and the techniques of cells extraction and culture, the efficacy of MSCT in patients is not stable. Based on the present study, it is likely that the abundance of immunosuppressive molecules expressed by MSCs plays an important role. Therefore, this study provides two possible solutions to improve the efficacy of MSCT in diseases. On the one hand, MSCs were induced to express higher levels of immunosuppressive molecules (PD-L1) by lower concentrations of inflammatory cytokines (IFN-γ and TNF-α) in vitro. On the one hand, MSCs can express more immunosuppressive molecules (PD-L1) after co-induction with lower concentrations of inflammatory factors (IFN-γ and TNF-α) without impairing cell viability. On the other hand, the efficacy of MSCT can be improved by detecting and screening MSCs with high expression of immunosuppressive molecules before transplantation. In future studies, to compensate for some of the limitations of this study in translational application, it is necessary to investigate the effect of more combinations of inflammatory factors on PD-L1 expression in MSCs and to investigate the immunosuppressive molecules induced by other inflammatory factors. More research in this area will contribute to the translational application of MSCT in clinical practice.

- **Response to Company editor-in-chief:**

Company editor-in-chief's comments: I have reviewed the Peer-Review Report and the full text of the manuscript, all of which have met the basic publishing requirements of the World Journal of Stem Cells, and the manuscript is conditionally accepted. I have

sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/. Uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2023.

Response: Thank you very much for your suggestions. According to your comments, firstly, in order to supplement and improve the highlights of the latest cutting-edge research results, we used RCA tools to find the latest highlight articles and updated references based on RCA search results. Secondly, we double-checked whether the uniform presentation was used for each figure. In addition, we provided decomposable figures and organized them into a single PowerPoint file. We also added the copyright information to the bottom right-hand side of the picture in the PowerPoint file.