Dear Editors and **Editorial Committee**:

Thank you for your correspondence regarding our manuscript entitled " Urolithin A Improves Mitochondrial Function and Alleviates Oxidative Stress-Induced Senescence in Nucleus Pulposus-derived Mesenchymal Stem Cells through SIRT1/PGC-1α Pathway (Manuscript NO: 69417)". This comment is valuable and very helpful for improving our study and revising our paper. We have studied and responded the comment carefully. We provided two manuscripts, one with traces of revision and the other without traces of revision. All the revised place in the manuscript is marked in red.

**Reviewer #1**

**Comment 1**: Acceptable in present form.

Response: Thank you very much for approving our research. We have further improved the manuscript and hope that this research could provide reference value for exploring intervertebral disc degeneration (IDD) at the basic level.

**Reviewer #2**

This manuscript investigated the effect of Urolithin A on Nucleus Pulposus-derived Mesenchymal Stem Cell. Authors found that UA could activate SIRT1/PGC-1α signal pathway to protect mitochondrial function, alleviate NPMSCs senescence and IDD.

**Comment 1**: 1. Please reconsider about the group name.

Response: Thank you very much for this valuable feedback. We have re-checked the relevant literature and found that the group name setting is indeed unreasonable, then, we have re-modified the group name: (a) Control group; (b) H2O2 group (80 μM H2O2); (c) H2O2 + UA group (80 μM H2O2 + 20 μM UA); (d) H2O2 + UA + SR-18292 group (80 μM H2O2 + 20 μM UA + 20 μM SR-18292). And the relevant contents had been marked in red in the manuscript.

Thanks again for your feedback to make the manuscript more readable.

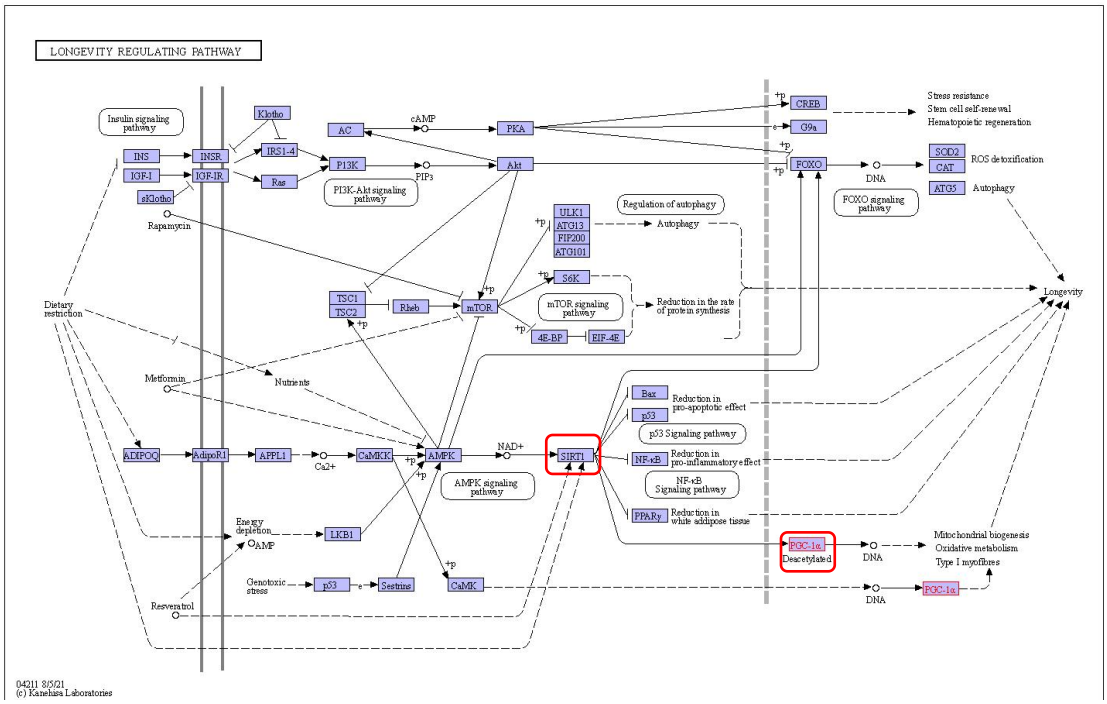
**Comment 2**: Please explain the reason why authors pick SR-18292 SIRT1/PGC-1α

signal pathway as your target.

Response: Thank you very much for this comment. Our research team is always focusing on exploring the pathological mechanism of IDD. SIRT1, a member of the family of NAD+-dependent Sir2 histone deacetylases, is an essential role in the regulation of oxidative stress, energy metabolism and senescence [1, 2]. Our previous study has already found that the expression of SIRT1 in the senescent NPMSCs induced by high glucose is significantly lower than that of the control group [3]. Previous study found that overexpression of SIRT1 can prevent MSC from entering senescence and restore its differentiation ability [4]. To further evaluate the mechanism of SIRT1 in regulating the senescence of NPMSCs, we choose SIRT1 as the target of further research in this study.

To clarify the mechanism that SIRT1 regulates senescence, we found that there are many studies focus on the PGC-1α pathway, but few studies elucidate whether the PGC-1α pathway plays a role in NPMSCs senescence. The PGC-1α pathway mainly involved in longevity regulating and energy metabolism (Figure 1). By simulating the unfavorable environment of intervertebral disc degeneration induced by oxidative stress, we have confirmed that SIRT1/ PGC-1α plays an important role in the regulation of senescence. We can also infer that: energy metabolism abnormalities may also affect NPMSCs senescence process in the unfavorable intervertebral disc environment. Therefore, we pick SIRT1/PGC-1α signal pathway as our target from comprehensive consideration.

Figure 1. Details of Longevity regulating pathway.




**Comment 3**: There needs to be more statistics to prove the conclusion in this article. For example, EdU alone is not enough to prove the change of cell proliferation.

Response: Thank you very much for this valuable feedback. Based on your suggestions, we have carefully reviewed the research content. The purpose of this study is to evaluate the effect of UA on senescent NPMSCs and explore its mechanism. Therefore, the focus of this research may be more biased towards the evaluation of NPMSC senescence indicators, while ignoring the evaluation of cell proliferation and cytotoxicity caused by different interventions. Therefore, to further confirm the protective effect of UA on NPMSCs, we have supplemented the cytotoxicity assay, which is to detect the cytotoxic effects of NPMSCs caused by different interventions by detecting Lactate Dehydrogenase (LDH) activity in the supernatant. Cytotoxicity-related content has been added to the manuscript, and the results confirmed that $H_2O_2$ is cytotoxic to NPMSCs and the cytotoxicity induced by $H_2O_2$ was alleviated after pretreating with UA. Combined with other research result, we conclude that UA could alleviate NPMSCs senescence and IDD.

**Comment 4**: Figure6 A-E is not right way to present the WB result. Besides, the fold

change is not consistent among the four bar graphs.

Response: Thank you very much for your comment. Due to our carelessness, the annotation of the vertical coordinate of the histogram was incorrectly marked and then the wrong WB quantitative result appeared when we performed quantitative analysis of the WB results. We have adjusted the vertical coordinate of the histogram ("the ratio of P16/β-actin (of control)" change to "relative protein expression of P16"). On the other hand, Figure 6 D-E is the result of qRT-PCR. We did not mark the annotation of the vertical coordinate clearly in the previous figure due to carelessness. The reason why the fold change may be inconsistent is that we conducted a comprehensive analysis of the results of several experiments, and the overall trend presented by the results is roughly consistent with the WB bands.

Thanks again for your valuable comment.

**Comment 5**: Please write clearly about the brand and product code of antibodies you used in the method part.

Response: Thank you for your comments. Due to our negligence and carelessness, we failed to provide complete information about the antibody. We have checked the manuscript again and supplemented the brand and production code of all the antibody, which has been marked in red in the manuscript.

We also read the Guidelines and Requirements for Manuscript Revision and the Format for Manuscript Revision to make sure the manuscript meets the submission requirements of the magazine.

Thank you again for your feedback, so that we can further realize the limitations the manuscript, which can further improve the manuscript contents.

**EDITORIAL OFFICE'S COMMENTS**

**Science editor:**

The manuscript is a basic study with two sections, one of them is an in-vitro and the other is in-vivo part. The authors proved that Urolithin A can protect the mitochondrial function and delay the nucleus pulposus-derived mesenchymal stem

cells senescence by activating the SIRT1/PGC-1α pathway in-vitro and alleviate animal model of intervertebral disc degeneration in-vivo. The topic is not within the scope of the World Journal of Stem Cells. 1. Scientific quality: 1) Classification Grades C, B; 2) Summary of the Peer-Review Report: Overall, the authors drafted a well-written manuscript with an interesting topic. The questions raised by the reviewer should be answered; 3) Format: 9 figures are found and there is only one table; 4) References: A total of 40 references are cited, including about 18 references published in the last 3 years and there is no any self-cited references; 5) Self-Cited references: Self-cited references not found. 2. Language evaluation: Classification: Grades B, B. A language editing certificate was provided (American Journal Expert). 3. Academic norms and rules: The authors didn't provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement. No academic misconduct was found by the Bing search. 4. Supplementary comments: This is unsolicited manuscript, supported by grants from the National Natural Science Foundation of China (81972136); Young Medical Scholars Major Program of Jiangsu Province (QNRC2016342); Key Funding Project of Maternal and Child Health Research of Jiangsu Province (F201801); High-level Health Professionals "Six projects" Top-notch Talent Research Program of Jiangsu Province (LGY2019035). The topic has not previously been published. 5. Issues raised: 1) The abbreviations should be properly handled. 6. Re-Review: Required; 7. Recommendation: Conditional acceptance.

**Comment 1**: Academic norms and rules: The authors didn't provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement.

Response: Thank you very much for this valuable feedback. We have supplemented the Conflict-of-Interest Disclosure Form and Copyright License Agreement, which has been signed and will be uploaded when the revised manuscript is returned.

**Comment 2**: Issues raised: 1) The abbreviations should be properly handled.

Response: Thank you for your comments. Based on your suggestion, we reviewed the manuscript according to the abbreviation requirement. All the abbreviations in the

manuscript are presented in a unified format. At the same time, we have polished the manuscript by the native English speaker to further enhance the readability of the manuscript. We sincerely hope that the revised manuscript could meet the publication requirement.

Thanks again for your valuable feedback.

**Company editor-in-chief:**

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, 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.

**Comment 1**: Before final acceptance, 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: ...".

Response: Thank you very much for your comments. According to your suggestions, we have checked the figure legend and adjusted its format and content. At the same time, we have further beautified and improved the figure to make it more beautiful. Thanks again for your valuable comment.

We would like to express our great appreciation to you and reviewers for comments on our paper. If you have any queries, please don't hesitate to contact me at the address below (zhangliang6320@sina.com).

Once again, thank you very much for your comments and suggestions.

Yours sincerely,

Liang Zhang

## Reference:


1 Kitada M, Kume S, Takeda-Watanabe A, Kanasaki K, Koya D. Sirtuins and renal diseases: Relationship with aging and diabetic nephropathy. Clin Sci (Lond) 2013; 124: 153-164 [PMID: PMC3466784 DOI: 10.1042/CS20120190

2 Ou X, Lee MR, Huang X, Messina-Graham S, Broxmeyer HE. Sirt1 positively regulates autophagy and mitochondria function in embryonic stem cells under oxidative stress. Stem Cells 2014; 32: 1183-1194 [PMID: PMC3991763 DOI: 10.1002/stem.1641

3 Liu Y, Li Y, Nan LP, Wang F, Zhou SF, Wang JC, Feng XM, Zhang L. The effect of high glucose on the biological characteristics of nucleus pulposus-derived mesenchymal stem cells. Cell Biochem Funct 2020; 38: 130-140 [PMID: DOI: 10.1002/cbf.3441

4 Khanh VC, Zulkifli AF, Tokunaga C, Yamashita T, Hiramatsu Y, Ohneda O. Aging impairs beige adipocyte differentiation of mesenchymal stem cells via the reduced expression of sirtuin 1. Biochem Biophys Res Commun 2018; 500: 682-690 [PMID: DOI: 10.1016/j.bbrc.2018.04.136