**Peer Review File**

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**Reviewer A**

Comment 1: Mention the source of IMR-90 cells.
Reply 1: Thanks, the IMR-90 cells were provided by the Stem Cell Bank, Chinese Academy of Sciences (Beijing, China), and we have revised this in text as be advised (see Page 5, line 97-98).

Comment 2: The methods for treatment of IMR-90 cells with BAMSCs and BMSCs, in vitro, need to be added.
Reply 2: Thanks, we have added it in text (see Page 7, line 138-143).

Comment 3: Line 211-212: It has been reported that transforming growth factor β1 (TGFβ1) and growth differentiation factor 11 (GDF11) are related to aging (1,31). Specify the relation by mentioning overexpression or downregulation is related to aging.
Reply 3: Thanks for the valuable comment. We have modified our text (see Page 10-11, line 220-222)

Comment 4: Line 213-14: we found that the expression of TGFβ1 and GDF11 were decreased in D-gal induced aged cells → we found that the expression of TGFβ1 and GDF11 were decreased in D-gal induced aged cells, in vitro.
Reply 4: Thanks for the valuable comment. We modified the sentence "we found that the expression of TGFβ1 and GDF11 were decreased in D-gal induced aged cells" to "we found that the expression of TGFβ1 and GDF11 were decreased in D-gal induced aged cells, compared to controls" according to the next comment (see Page 11, line 224).

Comment 5: Line 214: GDF11 were decreased in D-gal induced aged cells → mention the controls also, such as GDF11 were decreased in D-gal induced aged cells, compared to controls.
Reply 5: Thanks for the suggestion. We have revised our text as be suggested (see Page 11, line 224).

Comment 6: Line 2015: TGFβ1 and GDF11 was significantly reversed by BAMSCs and BMSCs treatment. Since this is in vitro data, the methods for this experiment (i.e. treatment of IMR-90 cells with BAMSCs and BMSCs, in vitro) are not described. Please update the methods (in methods section).
Reply 6: thanks for your suggestion. We have modified this in text, accordingly (see Page 7, line 138-143).

Comment 7: greatly decreased → statically significantly decreased
Reply 7: Thanks for the valuable advice. We changed the text from "greatly decreased" to "statically significantly decreased" in the text (see Page 12, line 250).

Comment 8: In figure legends, mention how data is presented, especially where statistics is involved. E.g., data is presented as mean +/- SD of n, number of replicates. Also mention the statistical parameter applied, e.g, two-way ANOVA or…. Else.
Reply 8: Thanks for the valuable advice. We have revised in related part in our manuscript (see Page 9, line 186-193 and Page 25, line 533-535, 543-544. Page 26, line 554-555, 561-562. Page 27, line 581-582)

Comment 9: Figure 1B, Figure 3, Figure 4D, and Figure 5E: Figure panels showing bar graphs, especially where statistical analysis is applied, please replace the bar charts by dot plots to show the position/distribution of individual biological replicates. Please see Weissgerber TL, Milic NM, Winham SJ, Garovic VD. (2015) Beyond Bar and Line Graphs: Time for a New Data Presentation Paradigm. PLoS Biol 13(4): e1002128.
Reply 9: Thanks for the valuable advice. We changed the figure panels of the above mentioned statistical chart to the form of bars combinates with the dots, which can reflect both the mean± se and the position/distribution of individual biological replicates. If such a modification is not possible, we would like to negotiate with you for an optimal modification.
Changes in the text: We have modified our figures as advised (see Figure 1B, Figure 2, Figure 3D, and Figure 4E and Figure S2).

Comment 10: Grammar check and language typesetting:
There are several mistakes both in typing and grammar, I recommend authors to carefully re-read the manuscript or get assistance from a native English speaker. I am placing few examples but not limited to;
(i). Apply the past tense (cells were passaged) in the following statement: ‘’Passage the cells with the ratio of when the confluence degree reached 80-90%’’.
(ii) Similarly, in the following sentence: ‘’The cell aging model can be induced. → was induced.
(iii) phenomenon that associated with… → that is associated with….
(iv) ischemic injury etc. → ischemic injury, among others.
(v) Planted → seeded or cultured.
Reply 10: Thank you very much for the valuable advice. We corrected the grammatical errors mentioned above one by one, and perform the grammar check and language typesetting through the full text and made some modifications as follows.
Changes in the text: We have modified our text as advised ((i) see Page 5, line 100. (ii) see Page 5, line 101. (iii) see Page 2, line 24. (iv) see Page 2, line 27. (v) see Page 5, line 105).
We also modified the other grammatical problems and marked them in red in the text

Comment 11: The separate note,
Title says cells and mice. In fact, mice also have cells. Thus, authors may wish to rephrase the title, as in vitro and in vivo.
Reply 11: Thank you very much for the valuable advice. We rephrase the title as “Mesenchymal stem cells alleviate aging in vitro and in vivo” according to the comment (see Page 1, line 2).

**Reviewer B**

Major comments

Comment 1: Figure1: The authors have elucidated cell senescent with beta-Gal expression and population doubling. However, I think other senescent markers should be investigated for more detailed elucidation. Please indicate some other data for cell senescent markers.
Reply 1: Thank you very much for your valuable comment. β-gal staining is convincing and solid to the detection of aging, and we also tested the proliferation of cells using cell doubling assay. We would like to investigate other senescent markers. But we are so sorry and hope you could understand about that we have run out of IMR-90 cells, and we cannot get new cells due to the COVID-19 epidemic.
Changes in the text: There is no change in the text.

Comment 2: Figures 4 and 5: these figures are only the results with BMSC. However, the effects of WAMSC or BAMSC for cell senescent are similar in Figures 2 and 3. Therefore, I recommend that the authors should indicate the in vivo data with WAMSC and BAMSC. Please show these additional data if you have any. If you do not have such data, I recommend transferring BAMSC/WAMSC data in Figures 2 and 3 to supplemental data
Reply 2: Thank you very much for the valuable advice. We do not have the in vivo data with WAMSC and BAMSC, so I transfer the Figure 2 and the BAMSC/WAMSC data in Figures 3 to supplemental data according to the advice. And we modified the order of the figures accordingly. We have modified our text as advised (see Page 10, line 217. Page 11, line 227, 230, 239. Page 12, line 243, 255. Page 26-27, line 565-587 and Figure S1 and S2)

Minor comments

Comment 1: Materials and methods: There is no information on the origin of each MSC. If you isolated them by yourself, please mention the isolation protocol in Materials and methods.
Reply 1: Thank you very much for the valuable advice. We isolated the BMSCs, BAMSCs and WAMSCs by myself from the C57BL/6J male mice. The protocol we mentioned previously in "Materials and methods" (see Page 6, line 118).

Comment 2: Figure 2: Please indicate these N numbers and perform their statistical analysis. Moreover, the text in the FACS histogram figure is too small to read and understand.
Reply 2: Thanks for the valuable advice. We transfer the data in figure 2 to figure S1. And the results of 3 repeated experiments were summarized and made a statistical chart. Also, we changed the text size in the FACS histogram figure to make it easier to read and understand. We have modified our text and figure as advised (see Page 26-27, line 570-571, and Figure S1)

Comment 3: Figures 2 and 3: These MSCs show different phenotypes (surface antigen and cell shapes) and the reaction for senescent genes. However, there is no discussion of these differences. Please mention these differences in aspects of the mechanisms with proper references.
Reply 3: Thanks a lot for the valuable advice. We mentioned the differences and the possible mechanisms of anti-aging effects of different MSCs in the discussion section according to the

comment (see Page 16, line 336-341, 345-352).

Comment 4: Figure 4B: Please indicate each standard deviation.
Reply 4: Thanks a lot for the valuable advice. The survival curve recorded the survival rate of mice after we injected MSCs, and we used the Log-rank (Mantel-Cox) test for statistical analysis, in which Chi square=1.967, p value = 0.167, but there is no value of standard deviation. We have modified our text as advised (see Page 26, line 554-555).