

Dear editors and reviewers,

We appreciate the reviewer's comments. The followings are our point-by-point responses:

- 1) The authors should seek help from a co-author or reviewer to help with the English language. For example, the title should say "osteogenic bone marrow stromal cells" (s), plural not single cell.

Response:

A native speaker helped us carefully walk through the manuscript with grammar and we corrected all the typos.

- 2) The results are interesting, and the data is quite comprehensive, but there are additional controls needed. Some of the data is quite unique and novel. For example, the transcriptomic signatures that are in Figure 3. However, there are other sections of the manuscript, which are required. For example, the histopathology section and Figure 2 and the colony assays, which are largely out of focus or overstained in some of the panels in Figure B at the bottom. The authors should include a number of additional datasets in the revised manuscript if this is appropriate.

Response:

We thank for Reviewer's comments. We re-checked our original pictures and these were the most representative pictures. To reduce the bias of staining, we added quantitative data. We were unable to re-take the picture of sections and dishes due to the limit access to the facility. We thank for Reviewer's understanding.

- 3) Abstract: This is not grammatically correct English. "Coordinates peripheral adjuncts to exercise movement. The bone marrow stromal cells are being discussed, and this should not be a sentence that has skeletal system and peripheral adjuncts. Adjunct is not an appropriate word for this sentence. The abstract rambles and does not address the facts of the manuscript. There are imaging and histologic specimens from mice studied at the age of 1, 3, 7, 12, 15, and 18 months, and the manuscript lacks studies of bone marrow stromal cells, not imaging histologic specimens. The grammar and the writing is detracting from the potential richness of the data. The last sentence in the abstract says "The enrichment of the osteogenic features and osteogenic signaling pathways as WNT and MAPK..." is not a sentence, and does not appropriately discuss what is in the manuscript. Quantitative information should be put in the abstract.

Response:

We prepare a new abstract that addressed both the histological and transcriptomic findings. We also walked through the content to make sure all the grammar errors were corrected.

- 4) Introduction: This rambles and should be focused on what is actually being studied. Bone marrow stromal cells are not described to any degree. The choice of describing PDGF-AA and BMP2 is a choice of two out of hundreds of genes that have been associated with bone marrow stromal cells.

Response:

We prepared a new introduction that focused on the BMSCs and the transcriptomic findings.

- 5) **Materials and Methods Sections:** The materials and methods sections rambles, and is not specific for what is in the paper. The section on bone marrow stromal cells and CFU-F assay is 40 years behind the standards of how these studies should be done. Clonal cell lines of bone marrow stromal cells should be studied, in addition, to purified bone marrow stromal cells either by a flow sorting technique or by another adherence technique.

Response:

The CFU assay is still the gold standard in assessing the self-renewal potential of the BMSCs. Flow sorting with specific surface marker is helpful to isolate some populations of MSCs but not all of them. Using previously established markers and focus on each distinct stromal cell type is a novel idea to pursue. However, in this study, we tried to focus more on the global features of total BMSCs.

- 6) **Results:** The Results section is confined to several figures. It is not clear what is being shown in Figure 1, which is quantitative measurement of skeletal features. What features? There have to be specific parameters and metrics studied. Panel B does not have a label for the ordinate/vertical axis.

Response:

We measured bone volume, tissue volume, trabecular number, trabecular thickness, trabecular spaces from the MicroCT imaging. We added the label of the vertical axis, which is the normalized fold-change computed by dividing the measurement of target age to the one-month baseline.

- 7) **Figure 2** has histologic sections, which show bone and marrow, however, the staining is different, the focus is different, several are overstained, and several are understained. The panel A bar graph does not have a label on the vertical axis. Panel B the photographs of the tissue culture plates are overstained as in 1 month, 15 months, 18 months, or understained as in 3 months and 7 months. They are not uniform.

Response:

The HE staining and TRAP staining were distinct to identify the histological features, including the number of osteoblast and osteoclast. We are happy to collect more samples, but due to the limited access to the facility, we are not able to do so. We thank for Reviewer's understanding. We added the label to the vertical axis.

- 8) **Figure 4**, which is the signature genes and transcriptional regulators needs to be quantitated and analyzed.

Response:

The gene expression level was first normalized and log transformed, and then scaled across three different time points. The value of expression on the heatmap reflects the relative quantitative measurement.

- 9) The language classification is Grade C. Please visit the following website for the professional English language editing companies we recommend: <https://www.wjgnet.com/bpg/gerinfo/240>

Response:

A native speaker helped us carefully walk through the manuscript with grammar and we corrected all the typos.

- 10) **The "Author Contributions" section is missing. Please provide the author contributions**

Response:

The author contributions is listed on the first page of the manuscript. Liu, SF and Bian, Q performed in vivo and ex vivo experiments, respectively. Cheng, YH wrote the manuscript and analyzed the data. Dong, J and Bian, Q edited the manuscript and provided feedback. Bian, Q designed the project.

- 11) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

Response:

A copy of the grant approval form will be uploaded.

- 12) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

Response:

The original pictures will be uploaded along with the revision submission.

- 13) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.

Response: The article highlights were added at the end of the first page of manuscript.