

Answering Reviewers

Review 1:

The following points need to be addressed before considering this work to be published.

Major Concerns 1. Rationale and Novelty: The introduction implies a lack of studies on the association between HIF-2 α and osteoporosis, but this relationship is well-documented. The study's novelty requires clarification to highlight its unique contributions amidst existing research.

Answer: We changed as: Recently a few studies have demonstrated that HIF-2 α was involved in BMSCs osteogenesis, but the molecular mechanism remains unclear.

2. In Vitro Experiments and Mice Group Clarification: It remains unclear from which group of mice the MSCs were extracted for the in vitro experiments in Figure 6D-K and Fig. 7. Were these BMSCs from naïve mice? If so, the rationale for not exploring similar parameters in other mouse genotypes needs clarification instead of solely focusing on naïve mice in these particular experiments.

Answer: In Figure 6D-K and Fig. 7, BMSCs were extracted from naïve mice. In vitro experiments, because the longtime of cell differentiation, there was no differences in BMSCs extracted from Prx1-Cre; Hif-2 $\alpha^{fl/fl}$ and Hif-2 $\alpha^{fl/fl}$ mice with interventions (bilateral ovariectomy, semi-lethal irradiation, and dexamethasone treatment), so in the rest researches, we all used BMSCs from naïve mice(Hif-2 $\alpha^{fl/fl}$ mice).

3. Supplementary Data for WB Experiments: The uncropped whole blots of the Western blot experiments should ideally be provided as supplementary figures, including replicates for comprehensive evaluation.

Answer: The uncropped whole blots of the Western blot experiments will be provided as Supplementary Material.

Minor Concerns 1. Abstract Revision: The abstract requires substantial improvement, especially in the background and methodology sections. The claim that few studies exist on HIF-2 α 's role in osteogenesis needs removal, and the study's rationale must be articulated clearly. Detailed methodology is also necessary.

Answer: Abstract is modified according to reviewer's comments.

2. Detailed Quantitative Assessments: The histology experiment's quantitative assessments need elaboration for better comprehension.

Answer: We added literature to explain the histology experiment's quantitative assessments.

3. Language Editing: The manuscript requires language editing for improved clarity and readability.

Answer: Because the limitation of revision time, we didn't perform further language polishing. If the revision time enough, we will use language editing services provided by your recognized high-quality biomedical editing companies.

Review 2:

Thank you for asking my opinion about the manuscript entitled "HIF-2 α increases bone mass by promoting BMSCs osteogenesis in bone marrow microenvironment via inhibiting mTOR signaling". I believe that this manuscript should be MAJOR revision:

Q1. It is very important to change and modify the title. the title is not appropriate.

Answer: We changed our title as "Unveiling the Role of HIF-2a in Osteoporosis: Implications for Bone Health".

Q2. Are the objectives and the rationale of the study clearly stated?

Answer: We rewrite the rationale of the study as: Recently a few studies have demonstrated that HIF-2 α was involved in BMSCs osteogenesis, but the molecular mechanism remains unclear.

Q3. In the abstract, the research gap was not clearly stated. In addition, the authors need to rewrite the study objectives to be more academic writing

Answer: We rewrite the study objectives as: To investigate the effect of HIF-2 α in BMSCs osteogenesis and the hematopoietic function of hematopoietic stem cells (HSCs) in bone marrow niche on the progression of OP.

Q4. In the introduction, include the study's significance and novelty. What makes the study different from the rest and what does it add to the current knowledge?.

Answer: We revised as :Recently a few studies have demonstrated that HIF-2 α was involved in BMSCs osteogenesis, but the molecular mechanism and role of HIF-2 α in the hematopoietic function in bone marrow niche remains unclear.

Q5. In the introduction, the authors should have explained the purpose of this study and the existing gaps in this field and explained why this study was conducted.

Answer: We explained as: In this study, we constructed BMSCs-specific HIF-2 α knockout mice (Prx1-Cre; Hif-2 α fl/fl) to study BMSCs osteogenic/adipogenic differentiation capacity and hematopoietic microenvironment of HSCs, to reveal the influence and mechanism of HIF-2 α regulation in bone marrow niche on osteoporosis and provide drug target genes that promote bone formation for the clinical treatment of osteoporosis.

Q6. Are the methods clear and replicable? Do all the results presented to match the methods described?

Answer: Yes, all the methods clear and replicable. All the results presented to match the methods described.

Q7. If relevant are the results novel? Does the study provide an advance in the field? Is the data plausible?

Answer: Yes, the results are novel. The study provides an advance in the field. And the data is plausible.

Q8. References are relevant, correct, and not recent. The number of references should be increased. please add some references. since this is a scientific review, all the sentences need to be supported with references. This study is very beautiful. I liked the sequence and enjoyed reading. Please add more references on similar studies.

Answer: We added some recent references.

Q9. There are a lot of grammatical errors. This must be taken care of and addressed.

Answer: Because the limitation of revision time, we didn't perform further language polishing. If the revision time enough, we will use language editing services provided by your recognized high-quality biomedical editing companies.

Q10. What are the limitations of the study? A description of limitations is missing at the end of the discussion section.

Answer: We revised as: this study still has limitations. We have not verified the effect of HIF-2 α on osteoclasts. Since bone is a balance process between

osteoblasts and osteoclasts, the relationship between osteogenesis and osteoclasia should be observed while observing the phenotype of bone, which is also the focus of our next study.

Review 3:

Although biological analyses are well done, analyses on bones are rather insufficient. In addition of simple mass, some features such as density, chemical compositions, and inside morphologies may be affected. Without such multi-angle analyses, true phenomena cannot be discussed. More detailed analyses on bones are necessary.

Answer: Thank you for your valuable suggestions. We add features such as bone mineral density in the analyses on bones, see Figure 3E, 3K, 3Q.