Editor's comments:

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

Response: Thank you for your suggestions. We revised the title on Page 1 "Low intensity pulsed ultrasound reduces alveolar bone resorption during orthodontic treatment via Lamin A/C-YAP axis"

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

Response: We appreciate the reviewer's concerns and modify the objective of this study on Page 3 to be "To investigate the effects of LIPUS on bone remodeling in orthodontic tooth movement (OTM) model and explore the underlying mechanisms.".

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

Response: Thank you for your suggestions. We revised the abstract to highlight the gaps in the current research field in the background section on Page 3: "The bone remodeling of orthodontic treatment for malocclusion often requires a long duration around two to three years, which also may lead to some complications such as alveolar bone resorption or tooth root resorption. Low-intensity pulsed ultrasound (LIPUS), a noninvasive physical therapy, has been shown to promote bone fracture healing. It's also reported that LIPUS could reduce the duration of orthodontic treatment, however, how LIPUS regulates the bone metabolism during orthodontic treatment process is still unclear."

And we revise the aim section in a more scientific language on Page 3: "AIM: To investigate the effects of LIPUS on bone remodeling in orthodontic tooth movement (OTM) model and explore the underlying mechanisms.".

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?

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.

Response: We appreciate the reviewer's comments. It's reported that LIPUS may decrease the duration of orthodontic treatment by promote osteoclast differentiation, which may lead to alveolar bone resorption. However, it's unclear whether LIPUS affect the osteogenic capacity of mesenchymal stem cells and its effects on alveolar bone resorption especially during the orthodontic tooth movement process. We revised this part to interpret the significance and novelty of this study in the introduction section on page 6: "LIPUS has also gained attention in the field of orthodontics. *In vivo*, LIPUS can increase the distance of the teeth movement[1]; a retrospective clinical study also showed that LIPUS reduced the duration of invisible treatment by an average of 49%[2]. In a clinical study, buccal alveolar bone thickness and height did not respond to LIPUS during maxillary arch expansion[3].In a tooth movement model in rats, LIPUS application increased the number of osteoclasts on the compression side [4]. The increased osteoclasts may lead to alveolar bone resorption and periodontal supporting tissue destruction. It's unclear whether LIPUS regulate the alveolar bone remodeling.

Bone marrow mesenchymal stem cells (BMSCs), as bone marrow-derived stem cells, exhibit self-renewal capacity and multiple differentiation potential and can differentiate into multiple types of cells, tissues and organs under certain conditions[5]. LIPUS could regulate MSC growth[6], and can promote chondrogenesis of MSCs seeded on 3D printed scaffolds[7]. Besides, MSCs encapsulated in hydrogels of certain stiffness shows enhanced osteogenesis ability under LIPUS[8]. Few researches reported the effect of LIPUS on cells loaded with compression. Whether LIPUS could regulate the property of MSCs under compression force to control the alveolar bone remodeling during orthodontic treatment and the underlying mechanism need to be investigated.

Here, we show that LIPUS could accelerate the orthodontic tooth movement and increase alveolar bone density and decreased vertical bone absorption via Lamin A/C-YAP axis. This study shed light LIPUS is promising strategy to accelerate the orthodontic treatment with little side effects.

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

Response: Thank you for your concerns. We carefully checked the methods part and provided more details of the experiment procedures to ensure reproducibility. The experimental procedures for the isolation and osteogenic differentiation induction of stem cells were following the previously study[9, 10] The procedures for transfections of siRNA, alkaline phosphatase (ALP) activity and alizarin red staining, qRT-PCR, western blot, immunofluorescence staining and sections staining all followed the manufacturer's instructions. Moreover, the details for method were added to ensure that the results matched the experimental methods described on page 7-9: "BMSCs (1×10^5/ml) were seeded onto 6-well plates and cultured until the cell confluence reached 70-80% before the medium was changed to osteogenic differentiation medium……. During osteogenic induction of stem cells, the induction differentiation medium was changed every 3 days. After induction for 14 days, ALP staining (Beyotime, Shanghai, China) were conducted in accordance with specific protocols, and so as Alizarin Red staining (Sigma, St. Louis, Missouri, USA) after induction for 21 days. For the quantification of mineralization, we dissolved red matrix sediment in 10% cetyl-pyridinium chloride (CPC; Macklin, Shanghai, China), and the absorbance of the solution was measured to determine the degree of mineralization quantitatively[11]."

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

Response: We appreciate the opportunity to clarify the novelty of our results.

According to our experimental results, LIPUS can effectively accelerate orthodontic tooth movement while reducing the loss of alveolar bone height, which has been rarely reported before. Compared to previously study, we innovatively applied LIPUS to the stem cells loaded with pressure to better simulate the force on the cells during orthodontic tooth movement, thus making our findings more reliable and more valuable. In addition, we focused on changes in the cytoskeleton following LIPUS application, which haven't been reported before. We make revisions according to your suggestions on page 15 and page 18: "Our experiments showed that LIPUS could effectively ameliorate the aesthetic and health problems caused by alveolar bone height loss by reducing vertical alveolar bone resorption and improving the morphology of alveolar bone remodeling, and filled the research gap in the relevant field." and "In summary, LIPUS can promote local alveolar bone remodeling, increase bone mineral density, reduce vertical alveolar bone resorption and consequent gingival recession by regulating the osteogenic ability of BMSCs. In terms of mechanism, LIPUS upregulated the expression and nuclear translocation of YAP, which was decreased by mechanical stress through effects on the cytoskeleton and nuclear skeleton, thereby affecting the osteogenic differentiation of BMSCs."

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.

Response: Thank you for your appreciation and advice. Accordingly, we add the references of recent studies on the role and mechanism of LIPUS on page 15-16: "Bone mineral density is positively correlated with OCN protein level[12], In our study, static pressure on BMSCs inhibited the mRNA and protein expression of osteogenic differentiation markers such as COL1 and OCN,

which partly explained the reduction in alveolar bone mineral density around moving teeth under orthodontic force." and "The process of orthodontics sometimes is accompanied by some adverse reactions, such as root resorption and bone mineral density decline. LIPUS has been reported alleviating chondrocyte damage in temporomandibular disorders[13], reducing root resorption[14] and enhancing bone remodeling[4]. However, there is a lack of research on the effect of LIPUS on the aesthetic problem of gingival recession due to the loss of alveolar bone height during orthodontic treatment. ……LIPUS is effective in various cell processes, such as growth, differentiation[15, 16], extracellular matrix formation, and mineralization of osteoblasts[17], and involves multiple signaling pathways, such as the hedgehog and TRPM7 pathways[18, 19]."

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

Response: Thank you for your careful review of our manuscript. The grammar through the manuscript were revised by native English speaker.

Q10. What are the limitations of the study? A description of limitations is missing at the end of the discussion section. If your manuscript is related to mine, you can cite it (ORCID: https://orcid.org/0000-0002-5107-5550).

Response: Thank you for your suggestions. We add the possible limitations of the study at the end of the discussion section on page 18: "This study still has some limitations. Osteoblasts and osteoclasts jointly participate in the process of bone metabolism. This study mainly focused on the effect of LIPUS on the osteogenic function of stem cells, whether LIPUS could regulate the crosstalk of osteoclast and osteoblast is unclear, which need further investigation. In addition, the underlying mechanism of how LIPUS controls cytoskeleton changes remain unclear." Your study " Evaluation of Bone Mineral Density, Serum Osteocalcin, and Osteopontin Levels in Postmenopausal Women with

Type 2 Diabetes Mellitus, with/without Osteoporosis" has some relevance to ours, and we cite it on page 16: "Bone mineral density is positively correlated with OCN protein level[12], In our study, static pressure on BMSCs inhibited the mRNA and protein expression of osteogenic differentiation markers such as COL1 and OCN, which partly explained the reduction in alveolar bone mineral density around moving teeth under orthodontic force."

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EIC Specific comments:

Q1. The current title did not reflect on the content: "Low-intensity pulsed ultrasound reduces alveolar bone resorption during orthodontic treatment via Lamin A/C-yes-associated protein axis" – neither specific to the cells applied nor captured.

Response: Thank you for your suggestions. We revised the title on Page 1 "Low-intensity pulsed ultrasound reduces alveolar bone resorption during orthodontic treatment via Lamin A/C-yes-associated protein axis in stem cells"

Q2. Page 3, paragraph 3: "It's also reported that LIPUS could reduce the duration of orthodontic treatment, however, how LIPUS regulates the bone metabolism during orthodontic treatment process is still unclear."

Comment: The grammar error surfaced as a comma splice error.

Response: We appreciate you for pointing out the grammar error, and corrected it on Page 3: "It's also reported that LIPUS could reduce the duration of orthodontic treatment. However, how LIPUS regulates the bone metabolism during orthodontic treatment process is still unclear.".

Q3. Page 5, the second to the last paragraph: "It's unclear whether LIPUS regulate the alveolar bone remodeling." [use regultes].

Response: Thanks for your correcting. We corrected it on Page 5: "It's unclear whether LIPUS regulates the alveolar bone remodeling."

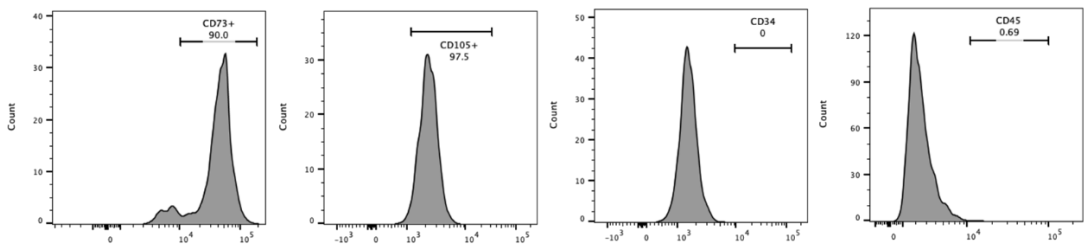
Q4. Page 4, the last paragraph: "Besides, MSCs encapsulated in hydrogels of certain specific stiffness shows enhanced osteogenesis ability under LIPUS[14]." [use show, as MSCs, plural nous is the sentence's subject]

Response: Thanks for your correcting. We corrected it on Page 5: "Besides, MSCs encapsulated in hydrogels of certain stiffness show enhanced osteogenesis ability under LIPUS [14]."

Q5. Page 6, paragraph 4: "Briefly, we obtained nucleated cells from the jaw bone," – indicating that the authors used nucleated cells, not BMSCs, which is specifically for bone marrow-derived MSCs (Citation 16). The authors should provide the biomarker profiles for the jaw bone-derived nucleated cells. It contradicted page 5: "human bone marrow mesenchymal stem cells (hBMSCs) were isolated to detect their osteogenic differentiation." Citation 16 was for BMSC; however, citation 15 is not for BMSC "15 Yamaza T, Ren G, Akiyama K, Chen C, Shi Y, Shi S. Mouse mandible contains distinctive mesenchymal stem cells. J Dent Res 2011; 90: 317-324 [PMID: 21076121 DOI: 10.1177/0022034510387796]." Which cell type did they use?

Response: Thanks for your suggestions. We provided the experimental details for isolation and culture of jaw bone derived MSCs and updated reference on page 6: "BMSCs were isolated and cultured following the previously reported protocol [15]. Briefly, we obtained a small piece of cortical bone located above the tooth crown, which required removal during the extraction of the donor's impacted third molars. The bone was carefully sectioned using a scalpel and subsequently digested using collagenase type I (2 mg/mL; Worthington Biochem, Lakewood, NJ, United States) and dispase II (4 mg/mL; Roche Diagnostic, Indianapolis, IN, United States) for an hour at 37 °C. Single-cell suspensions from the jaw bone were subsequently acquired using 70-μm cell strainers (BD Bioscience, United States).

The expression of stem cell surface markers in BMSC- were characterized by flow cytometry according to the manufacturer's protocol (BD Bioscience)."



Supplementary Figure BMSCs were positive for expression of CD73 and CD105, and negative for expression of CD45 and CD34.

Q6. Page 7: "Compression force was applied to the BMSCs to mimic stress during

orthodontic movement." What was the citation? What was the calibration of the force?

Response: Thank you for your concerns. We have added the reference on Page 7: "Compression force was applied to the BMSCs to mimic stress during orthodontic movement [17]". The magnitude and method of applied compressive force were also referred to the above literature.

Q7. Page 7: "LIPUS treatment" – any citations?

Response: Thanks for your review. We have added the reference on Page 8: "The cells were stimulated with LIPUS following the following specifications: 1.5 MHz frequency, 0.2 pulse duration ratio, 30 mW/cm2 incident intensity, and 1.0 kHz repetition rate. Stimulation was applied for 20 min every day in vivo and in vitro until the rats and cells were harvested, and the control group and force group were treated with pseudo-LIPUS. In vivo, the rats under anesthesia were placed at a constant location, after which the transducer was pressed against the side of the cheek closest to the maxillary first molar. In vitro, we attached the transducer to the bottom of the plate corresponding to the well [18]"

Q8. Page 13, last paragraph: "To explore whether the role of LIPUS in regulating the cytoskeleton in BMSCs" is Not an English expression.

Response: Thank you for your careful review of our manuscript. We have changed this sentence on Page 13: "To explore the role of LIPUS in regulating the cytoskeleton in BMSCs, we performed F-actin and Lamin A/C immunofluorescence co-staining in vitro."

Q9. Page 9, last paragraph: "In total, 27 rats were randomized to the control, force or force + LIPUS group." Male? Female? How did they determine the number of rats? Statistical power calling? Did they access the stem cell status? If not, there was a disjoint between the in vitro and in vivo data sets.

Response: Thank you for your suggestions. 6-week-old male SD rats were purchased

from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), We have added emphasis on the gender on Page 9: “In total, 27 male rats were randomized to the control (n=3), force (day 7 and day 14) (n=6 per group) or force + LIPUS group (day 7 and day 14) (n=6 per group).” No statistical methods were used to predetermine sample sizes, our sample sizes are similar to those reported in previous publications (citation 18).

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