Response to the comments from Reviewer

To the Reviewer 1:

Question: This study is very comprehensive and contributes new insights. Unfortunately, this working group does not adequately integrate its results with the research results of other working groups, which have also shown RUNX2-independent mechanisms during the osteogenic differentiation of DFSCs. The introduction and the discussion would benefit from this. There is also a lack of further investigations, such as what effects the miR-7974/FKBP15 axis has on the TF ZBTB16. A major revision would be necessary.

Answer: We appreciate the reviewer's constructive suggestions. We have now included a more comprehensive discussion and relevant references on the RUNX2-independent mechanisms during the osteogenic differentiation of DFSCs, integrating the research results of other working groups that have also demonstrated similar findings (Line 217-223, Line 483-496).

The transcription factor ZBTB16 is closely associated with the process of osteogenic differentiation in DFSCs [1, 2]. We further investigated the expression pattern of ZBTB16 in FTO-OE DFSCs (Figure R1). Our real-time PCR data revealed that overexpression of FTO, miR-7974 inhibitor or miR7974 mimic did not significantly changed ZBTB16 mRNA level in DFSCs. In addition, the effects of the miR-7974/FKBP15 on ZBTB16 expression or other important regulators during DFSCs osteogenesis will be explored in our future studies.

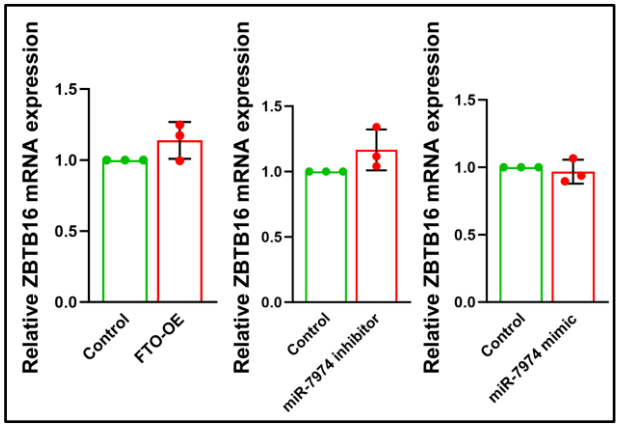

Figure R1. The mRNA level of ZBTB16 in DFSCs under various conditions. Overexpression of FTO, miR-7974 inhibitor or miR7974 mimic did not significantly changed ZBTB16 mRNA level in DFSCs. n = 3.

References

1. Morsczeck C, Schmalz G, Reichert TE, Völlner F, Saugspier M, Viale-Bouroncle S, Driemel O. Gene expression profiles of dental follicle cells before and after osteogenic differentiation in vitro. Clin Oral Investig. 2009 Dec;13(4):383-91. doi: 10.1007/s00784-009-0260-x. Epub 2009 Feb 28. PMID: 19252934.
2. Felthaus O, Gosau M, Morsczeck C. ZBTB16 induces osteogenic differentiation marker genes in dental follicle cells independent from RUNX2. J Periodontol. 2014 May;85(5):e144-51. doi: 10.1902/jop.2013.130445. Epub 2013 Dec 22. PMID: 24359167.