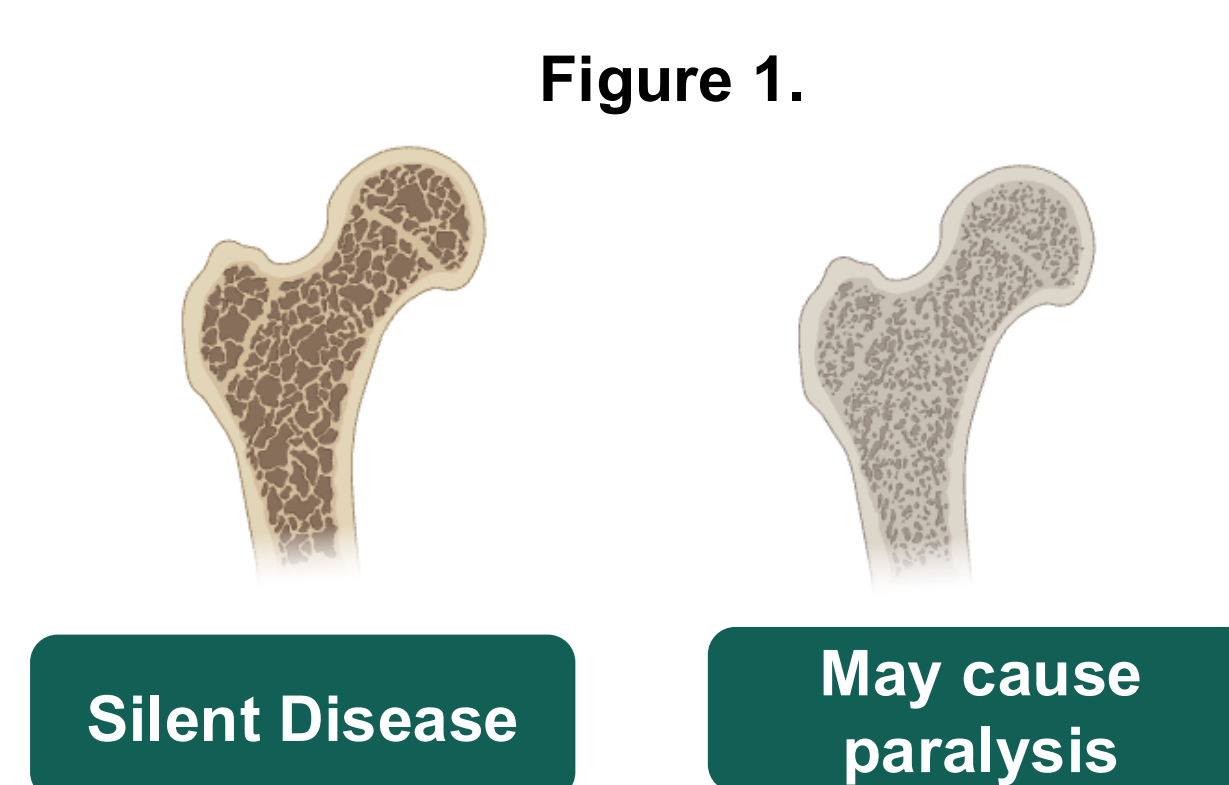


Engineering Trb3-Targeted Exosome Mimetics to Promote Bone Regeneration in Osteoporosis

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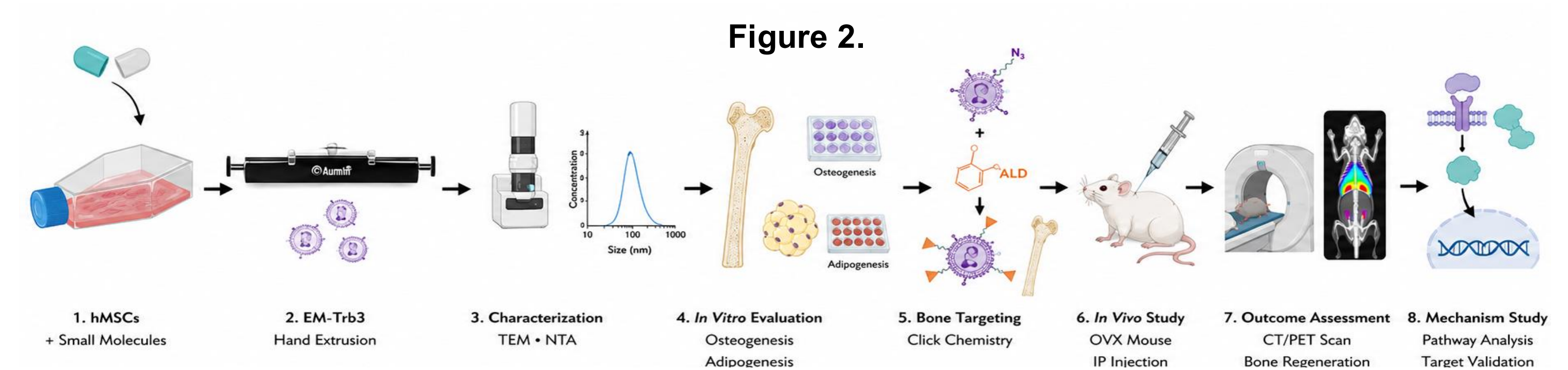
INTRODUCTION & AIM

- Age-related osteoporosis is characterized by reduced bone formation, increased marrow adiposity, and impaired skeletal regeneration.
- Dysregulated mesenchymal stem cell (MSC) differentiation contributes to bone loss by shifting lineage commitment from osteogenesis toward adipogenesis. Targeting key regulators of MSC fate may provide an effective strategy for restoring bone homeostasis.
- This study investigates the role of Tribbles homolog 3 (Trb3) in regulating MSC differentiation and promoting bone regeneration in osteoporosis.



METHOD

- A novel exosome mimetics engineering approach was developed to generate Trb3-enriched exosome mimetics (EM-Trb3) from small molecule-treated MSCs via hand extruder.
- The osteogenic and adipogenic effects of EM-Trb3 were evaluated using molecular and cellular assays.
- To enhance skeletal targeting, EM-Trb3 were functionalized with alendronate through a metabolic engineering and click chemistry strategy, generating bone-targeting EM-Trb3-ALD.
- An ovariectomized (OVX) C57BL/6 mouse model was established to mimic postmenopausal osteoporosis. EM-Trb3-ALD was administered via intraperitoneal injection for 12 weeks, and therapeutic efficacy was assessed through imaging and mechanistic analysis.



RESULTS & DISCUSSION

EM-Trb3 with enhanced Trb3 Levels

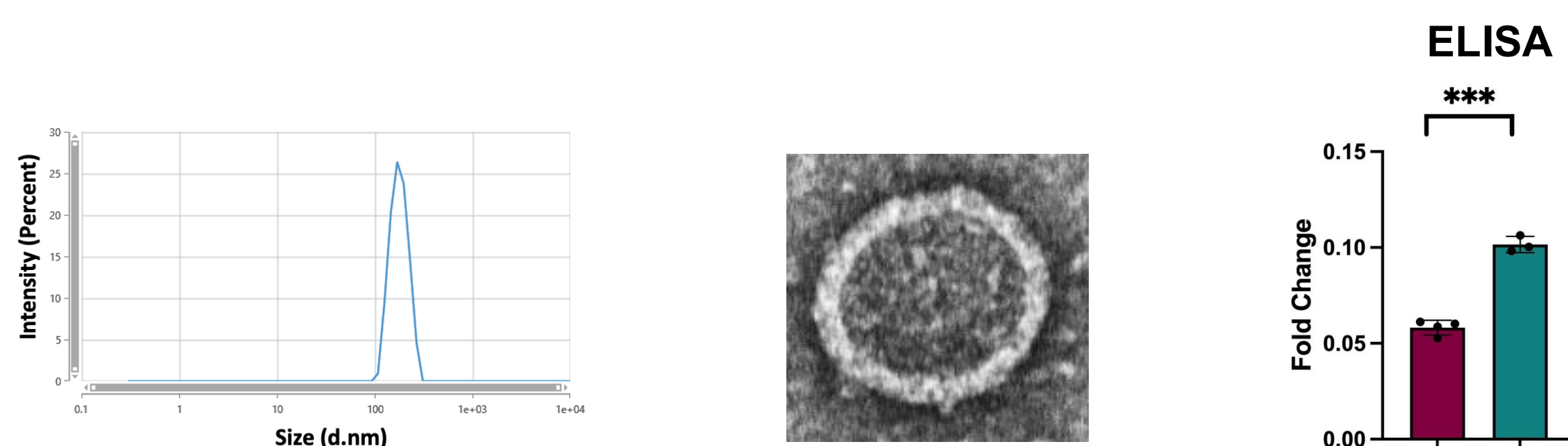


Figure 3. By transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA), the modified EM-Trb3 displayed spherical nanostructures with appropriate diameters from 100 to 200 nm. Enhanced Trb3 protein in EM-Trb3 was confirmed via ELISA.

EM-Trb3 induced osteogenesis via the elevation of key osteogenic genes and increased ALP Activity

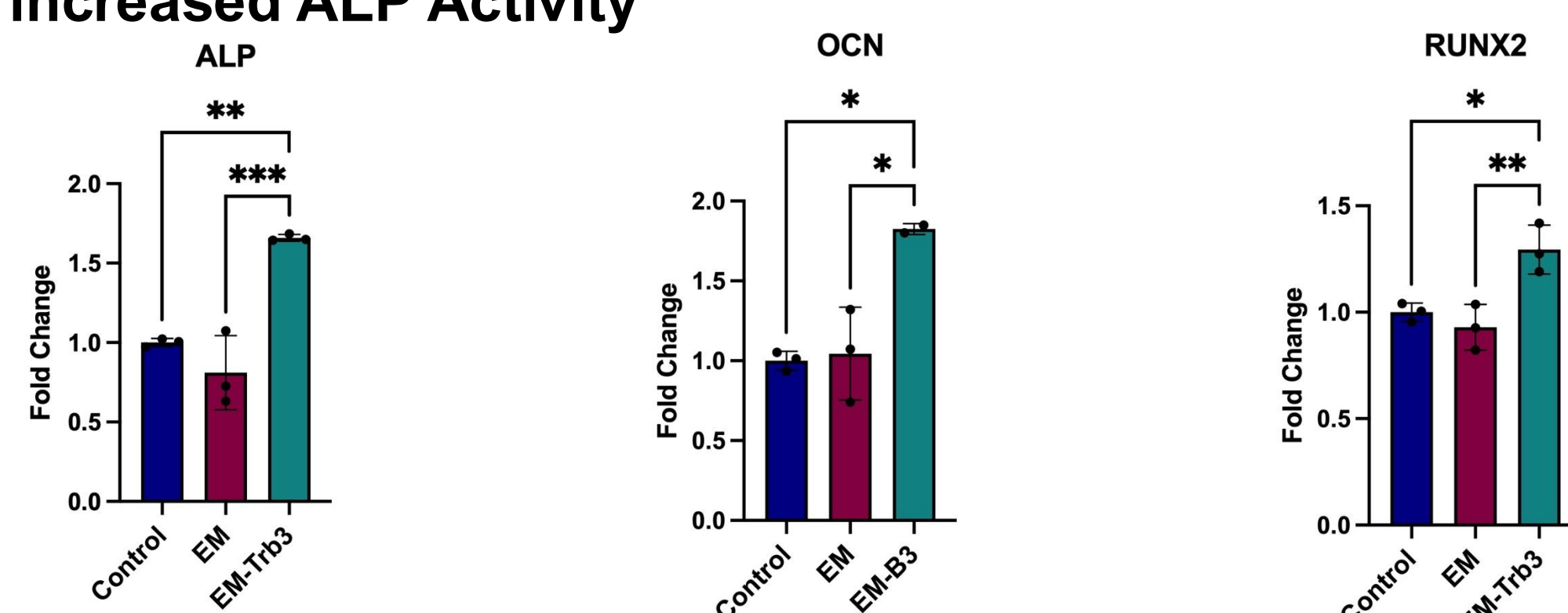


Figure 4. Expression of osteogenic genes (*ALP*, *OCN*, and *RUNX2*) was measured by a real-time PCR assay at day 5.

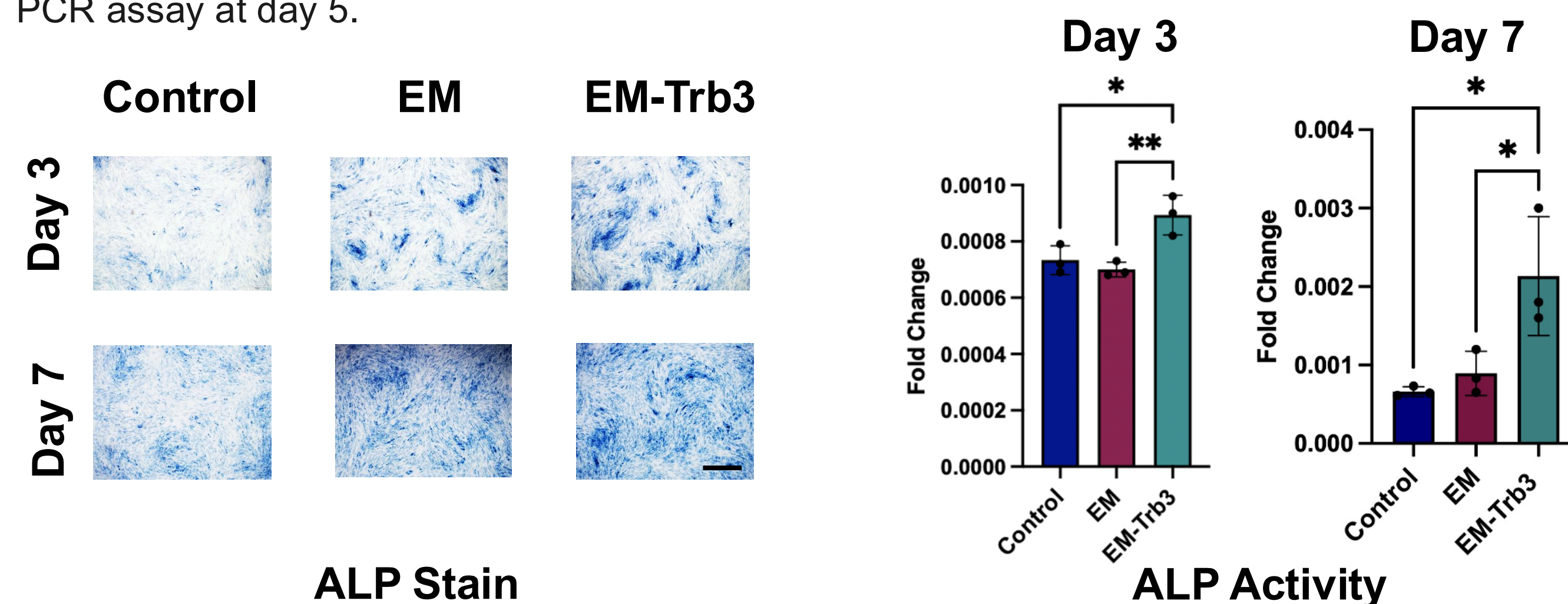


Figure 5. ALP expression was detected by ALP stain and activity at day 3 and day 7. Scale bar: 100 μ m.

CONCLUSIONS

This study presents a novel bone-targeted exosome mimetics platform that combines Trb3 enrichment with alendronate-mediated targeting via copper-free click chemistry. The engineered EM-Trb3-ALD enhanced osteogenic activity and showed encouraging therapeutic potential for osteoporosis. Ongoing μ CT and histological analyses will further define its effects on bone regeneration and skeletal repair.

EM-Trb3 suppressed adipogenesis via suppression of key adipogenic genes and decreased fat deposition

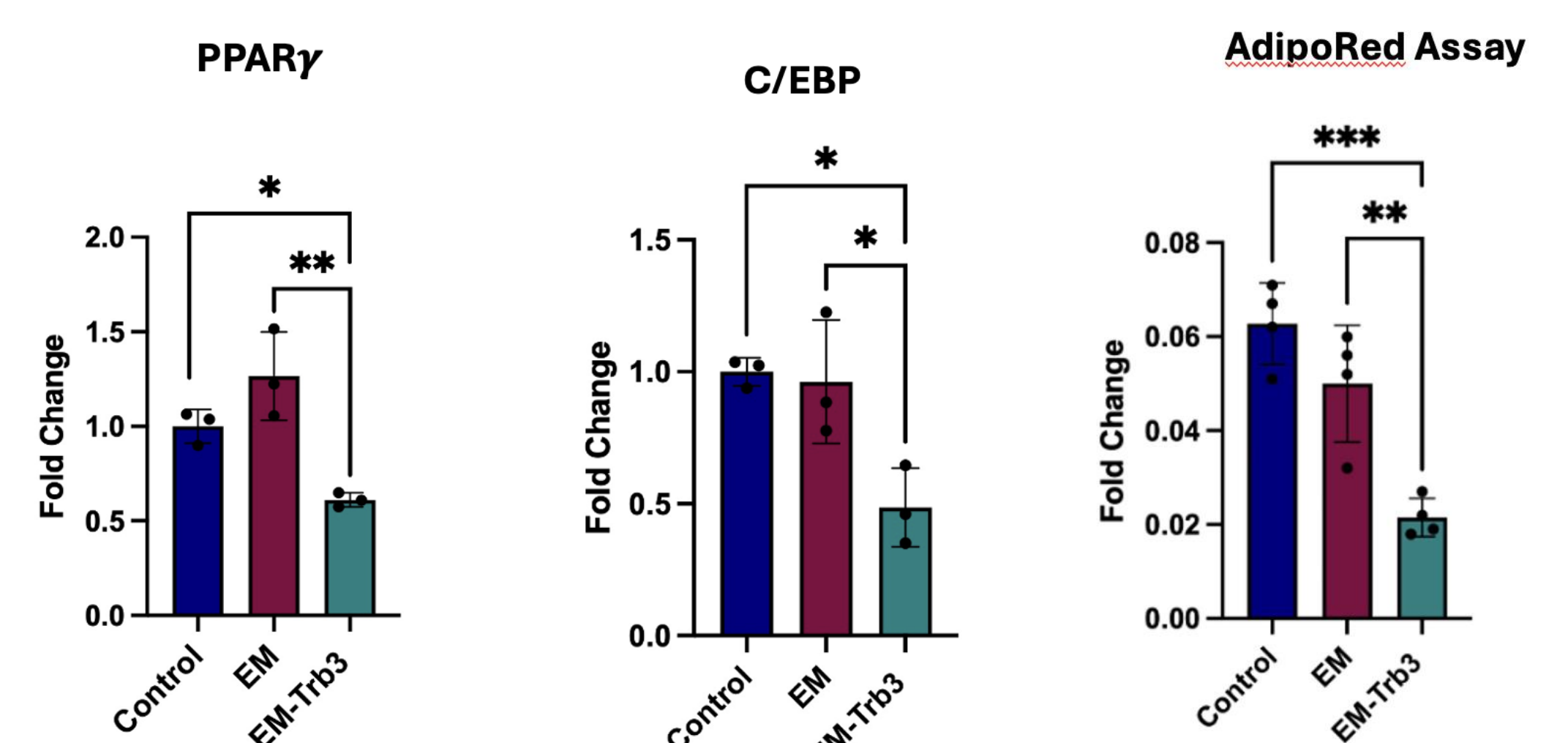


Figure 6. Expression of adipogenic genes (*PPAR γ* and *C/EBP*) was measured by a real-time PCR assay at day 5. Fat deposition detected by AdipoRed Assay at 21 days.

EM-Trb3-ALD Promotes Bone Regeneration Through Activation of Wnt/ β -Catenin Signaling

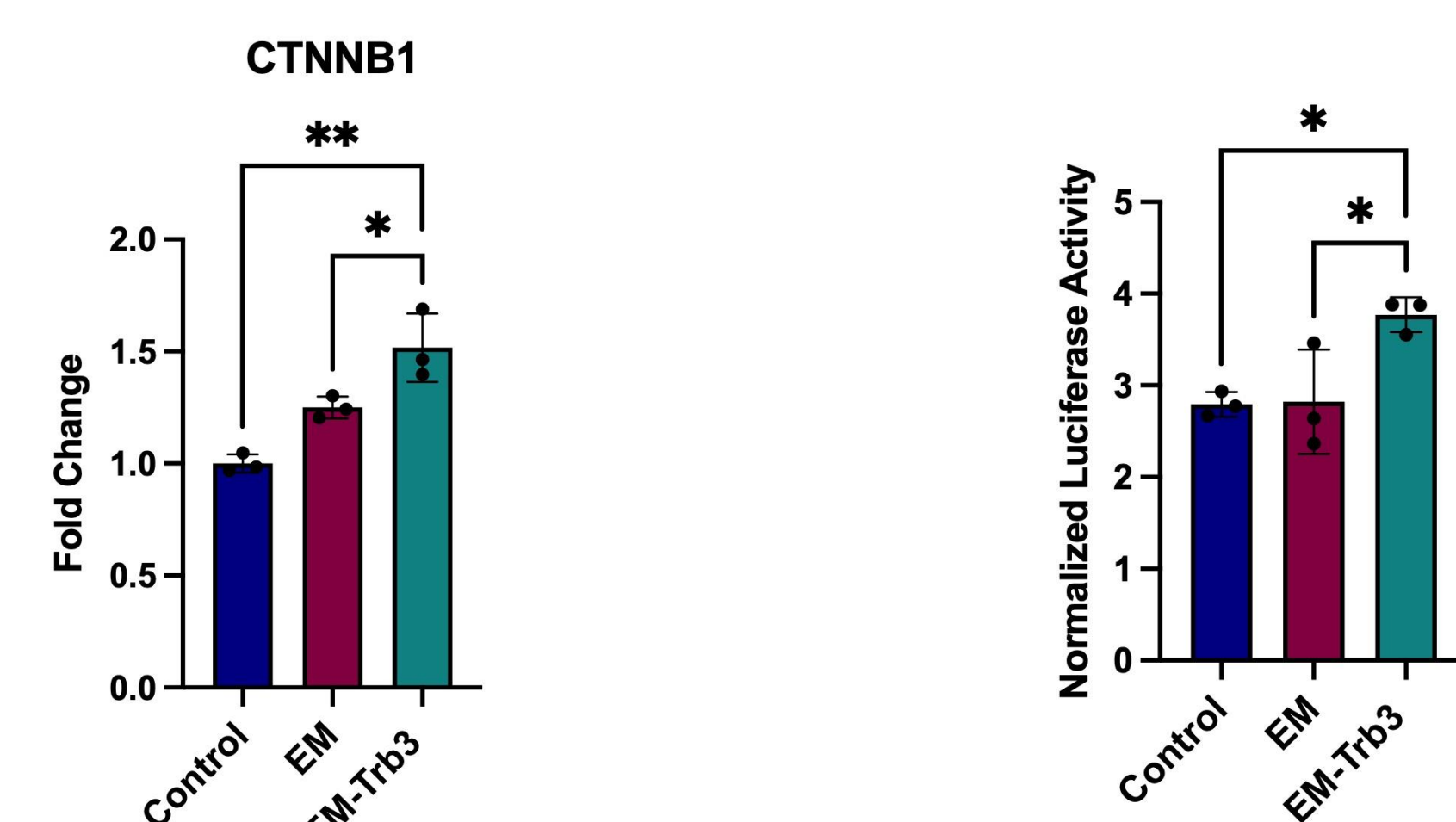


Figure 7. EM-Trb3-ALD enhanced bone regeneration via Wnt/ β -catenin signaling, evidenced by elevated CTNNB1 expression and increased LEF reporter activity.

FUTURE WORK/ REFERENCES/ACKNOWLEDGMENT

Comprehensive μ CT and histological analyses are currently underway to further evaluate the therapeutic efficacy of EM-Trb3-ALD in OVX mice. Early observations are encouraging and suggest promising benefits for the treatment of osteoporotic bone loss.

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