INTRODUCTION
Aging decreases chondrogenesis, bone formation, callus vascularization and remodeling during fracture healing in mice, which is associated with reduced cyclooxygenase-2 (COX-2) expression, and is restored by administration of an EP4 agonist. Activation of the EP4 receptor stimulates cAMP and activation of the protein kinase A (PKA) signaling pathway. These findings suggest that enhanced PKA signaling compensates for deficient fracture healing associated with aging.

The parathyroid hormone receptor (PTHR1), similar to the EP receptors, is a G-coupled 7-transmembrane receptor that activates PKA signaling. PTH mediates the anabolic effect of Forteo (PTH 1-34) in osteoporosis patients via PKA signaling. Because of the similar signaling effects of PGE2/EP4 and PTH/PTH1R, we hypothesized that PTH 1-34 would rescue the reduced fracture healing that occurred in aging. Here we tested this using stabilized tibia fractures in mice, and a complementary uni-cortical defect-bone-graft model.

MATERIALS AND METHODS
Experimental Animals: C57/B6, 7- to 8-week-old (young) and 52- to 53-week-old (aged) female mice were subjected to tibia fracture and uni-cortical tibia bone grafting. After surgery, mice from both models were divided into two treatment groups: (1) PTH 1-34 (Forteo; 40ug/kg); and (2) normal saline. Daily subcutaneous drug administration was delivered for either 2 weeks (grafting) or 3 weeks (tibia fracture).

Animal Models: (1) Tibia fracture: An intramedullary pin was inserted into the tibia at the knee and an open fracture in the proximal tibia diaphysis was performed. (2) Tibia uni-cortical defect-graft model: A 2mm x 1mm segment of the anterior cortex was removed and was used to graft a similarly sized tibial defect. Young/aged grafts were transplanted into defects in young or aged mice, and harvested at days 5, 11, 14, and 21 for micro-CT, histology, and RNA extraction.

Radiographs and μCT: Weekly radiographs (Faxitron X-ray, Wheeling, IL) were obtained to monitor bone healing. Specimens were scanned at 10.5-micron isotropic resolution using a Scanco VivaCT 40 (Scanco Medical AG, Switzerland) at indicated time points. Mineralized callus volume, callus bone mineral density (BMD) and total mineral content (BMC) were determined.

Biomechanical Torsion Testing: Fracture specimens were mounted on an EnduraTec TestBench® system with a 200 N.mm torque cell (Bose Corp., Minnetonka, MN) and was tested in torsion at a rate of 1/2/sec until failure to determine the torsional stiffness, ultimate torque, ultimate rotation, and strain energy to failure.

Quantitative Real-time RT-PCR: The fracture callus and 1mm of adjacent bone was excised and total RNA extracted using the QIAGEN RNasey kit. cDNA was synthesized from 1ug of RNA per callus using a first-strand cDNA synthesis kit (Invitrogen). Real-time RT-PCR analyses were performed using murine specific primers for chondrogenesis and osteogenesis related genes (col2a1, colX, sox9, ihh, oster1, runx2 and osteocalcin).

Histology & Histomorphometry: Specimens were harvested at 5, 7, 10, 14, and 21 days and paraffin embedded after 3 days of fixation in 10% NB-Formalin. 4-μm sections underwent H&E staining and histomorphometric analyses using Osteometrics software (Decatur, GA).

Statistics: Results are shown as the mean +/- standard deviation. Statistical tests included Student’s t-tests and two-way ANOVA followed by Tukey-Kramer test. P <0.05 was considered significant.

RESULTS
Radiographs and μCT showed that administration of PTH 1-34 treatment enhanced bone callus formation in both young and aged mice. Micro-CT showed a significant increase in bone volume of the external fracture callus in both young and aged mice treated with PTH for 10, 14, and 21 days compared to their age-matched controls. Moreover, aged mice treated with PTH 1-34 for 14 days had elevated bone callus volume compared to untreated young control mice (Fig. 1A and 1B).

Histology and quantitative histomorphometry showed that PTH 1-34 enhanced bone formation in both young and aged control mice (Fig. 2). Detailed histomorphometry data is shown for aged mice (Fig. 2B) and demonstrates that PTH 1-34 significantly increased bone area at days 10, 14, and 21. No measurable effect was observed on the area of mesenchymal tissue. While cartilage area was similar at early time points, PTH 1-34 treatment resulted in persistence of cartilage at 14 days consistent with a delay in endochondral bone formation (Fig. 2).

Gene expression showed persistence of cartilage genes (colII) and increased bone marker expression (osteix) in PTH 1-34 treated cultures. Biomechanical testing showed that maximum torque of tibiae in treated aged mice was increased versus matched controls (157%, day 14). Similar effects were noted in young mice (Data not shown).

DISCUSSION
The current study shows that PTH 1-34 treatment increases bone callus formation and mechanical strength during fracture healing in both young and aged mice. The bone graft model further shows that PTH 1-34 increases the proliferation of cartilage formation of periosteal cells in the bone graft model. Interestingly, completion of endochondral ossification was delayed. These results suggest that PTH acts by increasing stem cell proliferation, and osteoblast differentiation. While similar amounts of cartilage tissue forms, completion of endochondral ossification is delayed. A more complete understanding of the effects of PTH 1-34 and its target cell populations will enhance the translation of this important anabolic agent into new treatments for bone repair.

REFERENCE
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