INTRODUCTION
Dickkopf 1 (DKK1) is an inhibitor of the Wnt signaling pathway that has been implicated in several bone pathologies including multiple myeloma-induced bone loss (1-2) and rheumatoid arthritis (3). DKK1 expression was demonstrated to be increased in the pathological state of these diseases. Recently, it has been shown that altering Wnt signaling can modulate fracture healing (4). Treatment with lithium, an agonist of Wnt signaling, improved fracture healing, whereas treatment with DKK1 inhibited fracture healing (4). In this study, we sought to determine the temporal pattern of DKK1 expression during fracture healing and the effect of DKK1 inhibition by a neutralizing antibody (DKK1-Ab) on the healing process.

METHODS

Closed Femoral Fracture Model: Male SD young (10 week-old) and adult (6-6.5 month-old) rats underwent a unilateral closed femoral fracture (5).

Temporal DKK1 mRNA Expression in the Fractured Callus: DKK1 expression level within the fractured callus was determined in both young and adult rats at 6 hrs (day 0), 3, 7, 10, 14 and 21 days post fracture (n=3-5each time point). The central 30% of the fractured femur was pulverized in liquid nitrogen, homogenized in RNA Stat60 buffer, extracted, and purified to perform the PCR reaction in an ABI Prism 7700HT.

DKK1-Ab Treatment in Adult Rat Fracture Model: One day post fracture, adult rats were subcutaneously injected with either saline vehicle (Veh, n=12) or DKK1-Ab at 25 mg/kg twice per week (n=14) for 7 weeks. At 7 weeks post fracture, animals were euthanized; the fractured and non-fractured contralateral (CL) femurs were collected for densitometry and biomechanics.

Densitometry: Femurs were scanned ex vivo by DXA (GE Lunar PIXIImus II) at the fracture region (30% mid-shaft) or the corresponding region in the CL femur to determine areal bone mineral density (BMD), areal bone mineral content (BMC), and total area. The mid-plane of these regions was also scanned by pQCT (Stratec XCT research SA+) to determine volumetric BMD, volumetric BMC, and total area.

Biomechanics: After the densitometric evaluations, femurs were tested in 3-point bending to failure at the center of the fractured callus or at the mid-diaphysis of the contralateral femur, and maximum load, stiffness, and energy were assessed (MTS 858 Mini Bionix II; span length = 20 mm; displacement rate = 0.1 mm/sec).

These studies were approved by Amgen’s Institution Animal Care and Use Committee. An unpaired student t-test was used for the statistical comparison between the Veh and DKK1-Ab treated groups. Data are presented as Mean±SE; p<0.05 was reported as statistical significance.

RESULTS
Temporal DKK1 mRNA Expression in the Fractured Callus (Figure 1): In both young and adult animals, DKK1 expression increased during the fracture healing process, peaking between days 7 and 14, and remaining elevated after 21 days.

Fractured Femur Densitometry (Figure 2): aBMD (DXA) was increased by 15% in fractured femurs from DKK1-Ab treated animals. Similarly, there were increases in vBMD (+24%) and in vBMC (+40%) at the fracture line as assessed by pQCT.

Fractured Femur Biomechanics (Figure 3): Fractured femoral maximum load and stiffness in the DKK1-Ab group were increased by 65% (P < 0.01) and 73% (P < 0.06) respectively, compared to the Veh group. Fractures in the DKK1-Ab group achieved a significantly greater functional recovery in maximum load, expressed as a percentage of their CL intact femoral strength (44.7±21.9% vs. 26.2±9.6%, P < 0.01).

Correlations between pQCT BMD and maximum load in fractured femurs were positive and similar across both Veh and DKK1-Ab treated groups, with an overall r value of 0.73.

Non-Fractured CL Femurs: DKK1-Ab treatment had no effect on BMD and BMC (by DXA or pQCT) or on the bending strength of CL femurs.

DISCUSSION
DKK1 mRNA expression level was significantly elevated after closed femur fracture in rats, and DKK1 neutralization resulted in increased callus BMD and bending strength. The efficacy of DKK1-Ab treatment in adult rats appeared to be specific to the fracture site, without an effect on the non-fractured contralateral femur.

SIGNIFICANCE
This study demonstrated that Dkk1 inhibition improved fracture healing and may provide a therapeutic approach to fracture treatment.

REFERENCES