Supplementary information:

Supplemental Materials and Methods

Western Blotting

Western blotting was conducted as previously described [22].

The primary antibodies were as follows: anti-Dkk1 antibody (1:1000) was acquired from Abcam.

Cell proliferation assay

Saos-2 cells were cultured at a concentration of 5.0×10^3 cells/well in 96-well plates. After 24 hours of incubation, the cells were treated with or without bone morphogenetic protein-2 (BMP2) and iguratimod (IGU). The following day, cell proliferation was assessed using the Cell Count Reagent SF cell proliferation assay system (Nacalai Tesque) according to the manufacturer's instructions.

Immunohistochemical analysis

Samples were incubated with anti-Piezo1 antibody (Proteintech Group, Chicago, IL, USA). The sections were then incubated with a secondary antibody (Histofine Simple Stain Mouse MAX PO; Nichirei Bioscience Inc.) and stained with 3, 3′-Diaminobenzidine tetrahydrochloride (Dako). The number of empty lacunae and Piezo1-positive osteocytes was measured in the cortical bone.