



Title	Iguratimod suppresses sclerostin and receptor activator of NF- $\kappa$ B ligand production via the extracellular signal-regulated kinase/early growth response protein 1/tumor necrosis factor alpha pathway in osteocytes and ameliorates disuse osteoporosis in mice
Author(s)	Miura, Taihei; Etani, Yuki; Noguchi, Takaaki et al.
Citation	Bone. 2024, 181, p. 117026
Version Type	AM
URL	<a href="https://hdl.handle.net/11094/95865">https://hdl.handle.net/11094/95865</a>
rights	© 2024. This manuscript version is made available under the CC-BY-NC-ND 4.0 license <a href="https://creativecommons.org/licenses/by-nc-nd/4.0/">https://creativecommons.org/licenses/by-nc-nd/4.0/</a>
Note	

*The University of Osaka Institutional Knowledge Archive : OUKA*

<https://ir.library.osaka-u.ac.jp/>

The University of Osaka

## **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  $5.0 \times 10^3$  cells/well in 96-well plates. After 24 hours of incubation, the cells were treated with or without bone morphogenetic protein-2 (BMP2) and igratimod (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.