

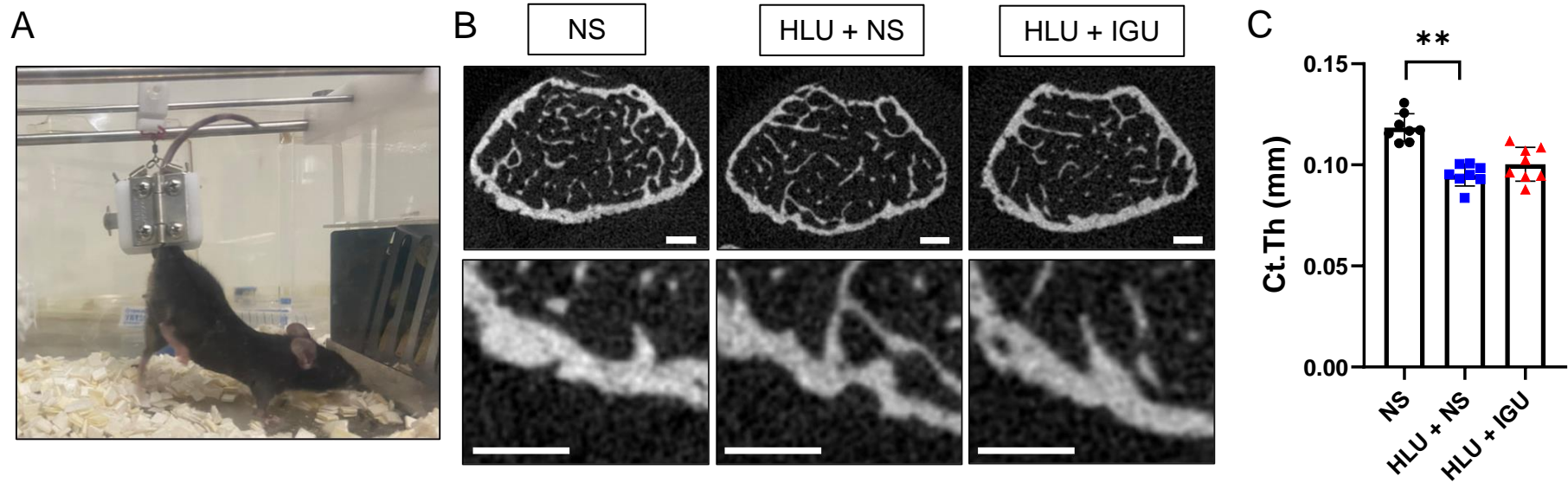


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| Title | Iguratimod suppresses sclerostin and receptor activator of NF- κ B ligand production via the extracellular signal-regulated kinase/early growth response protein 1/tumor necrosis factor alpha pathway in osteocytes and ameliorates disuse osteoporosis in mice |
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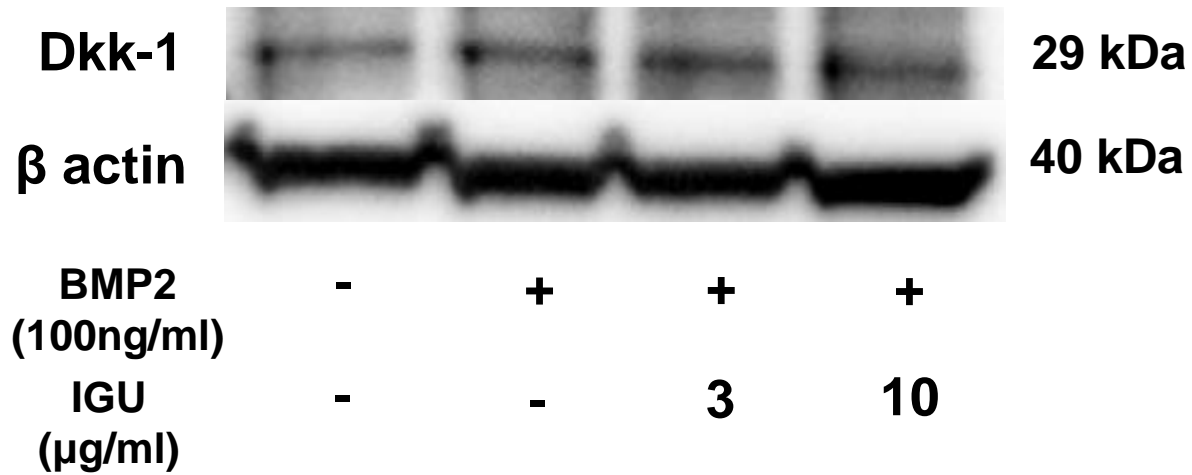
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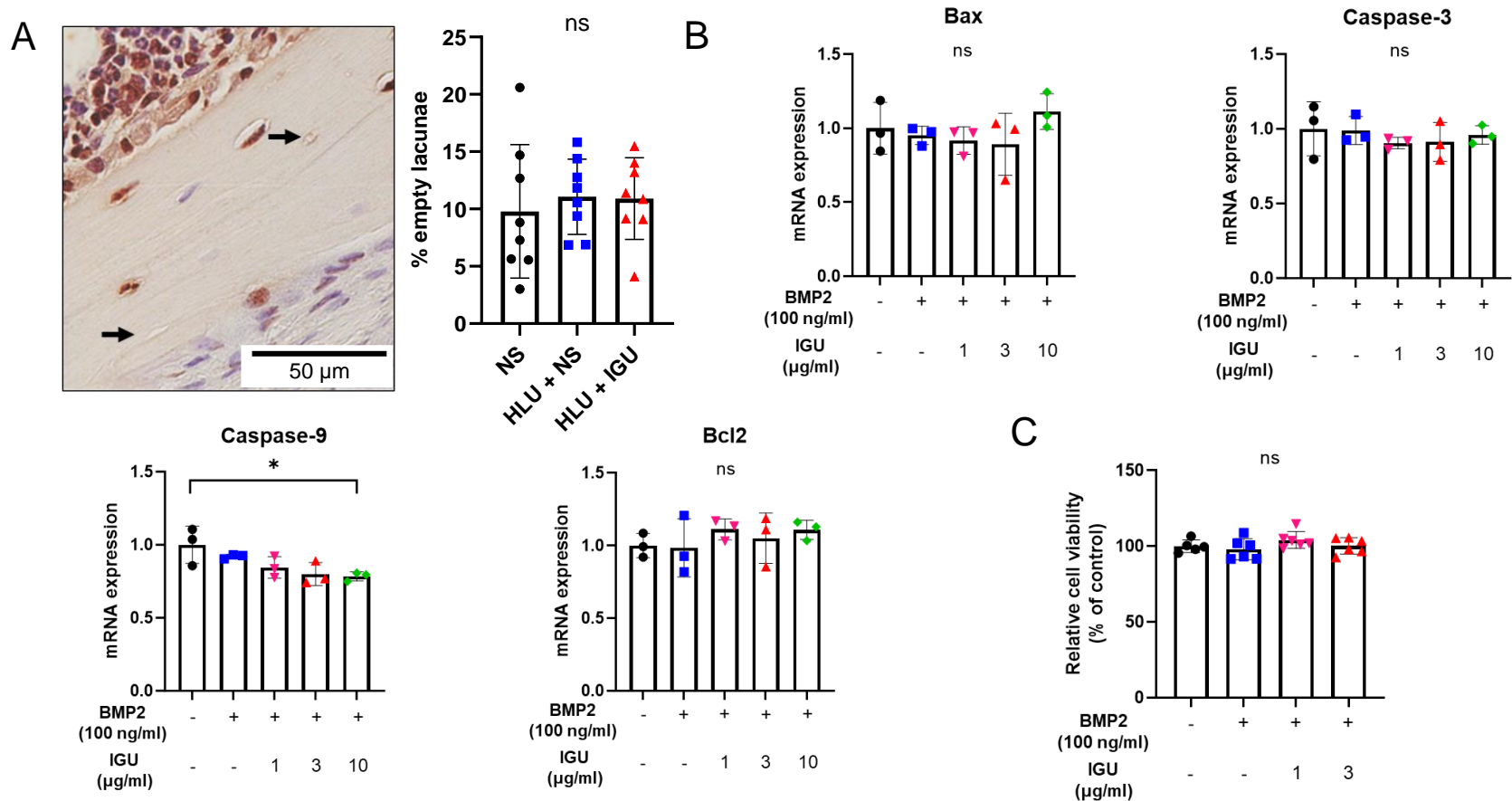
Supplemental Figure 1. Effects of normal saline (NS) or iguratimod (IGU) on cortical bone loss in hindlimb-unloaded (HLU) mice.

(A) Tail-suspended hindlimb-unloaded mice confined in the cage, demonstrating the suspension system. (B) Representative microcomputed tomography images of the distal femur in the three groups after the intervention. Scale bars: 500 μ m. (C) Quantification of the cortical bone parameter: cortical thickness (Ct.Th). One-way ANOVA followed by Tukey's post-hoc analysis, ** $p < 0.01$. All data were expressed as mean \pm standard deviation for eight mice in each group.



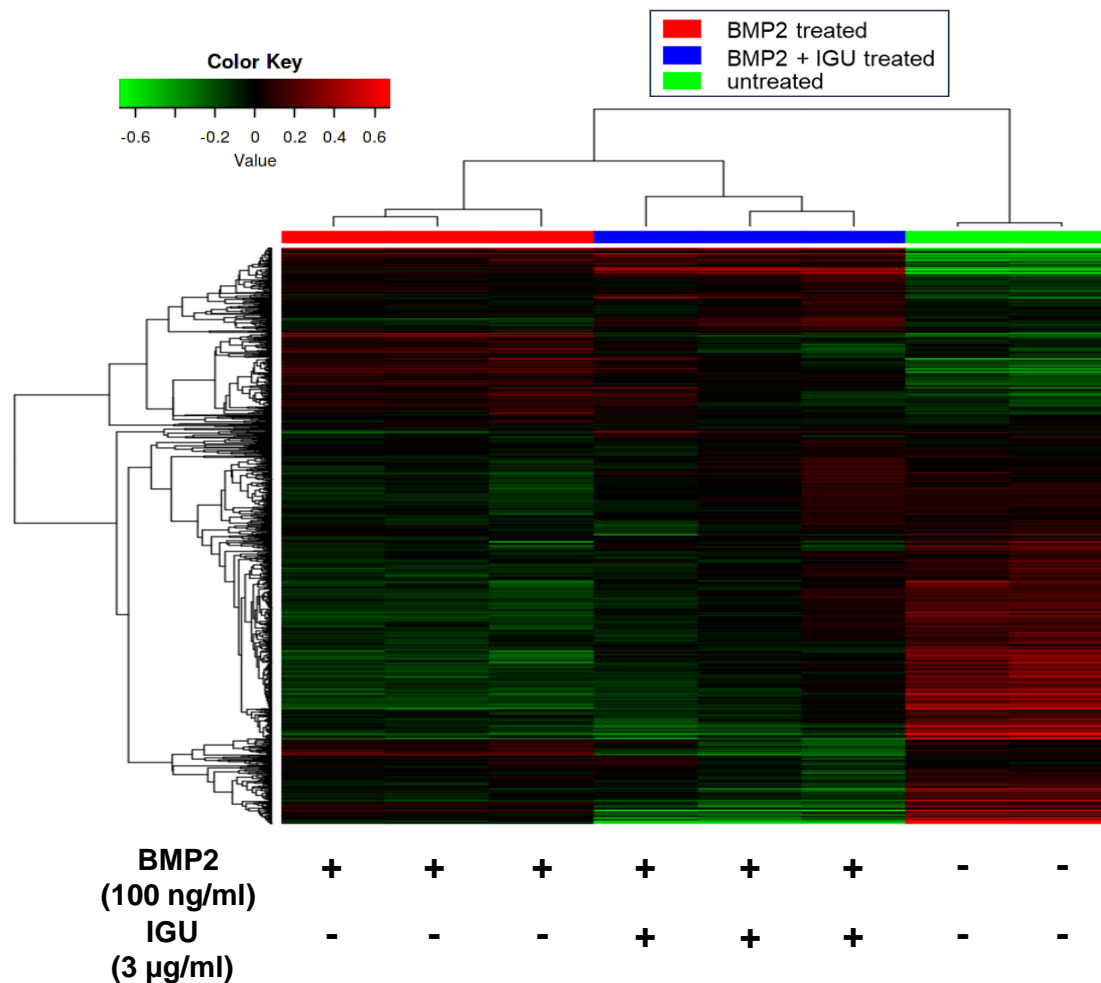
Supplemental Figure 2. Effects of iguratimod (IGU) on osteocyte-related gene, Dkk-1 expression.

Western blotting analysis of Dkk-1 using Saos-2 cells treated with bone morphogenetic protein-2 (BMP2) with or without IGU.

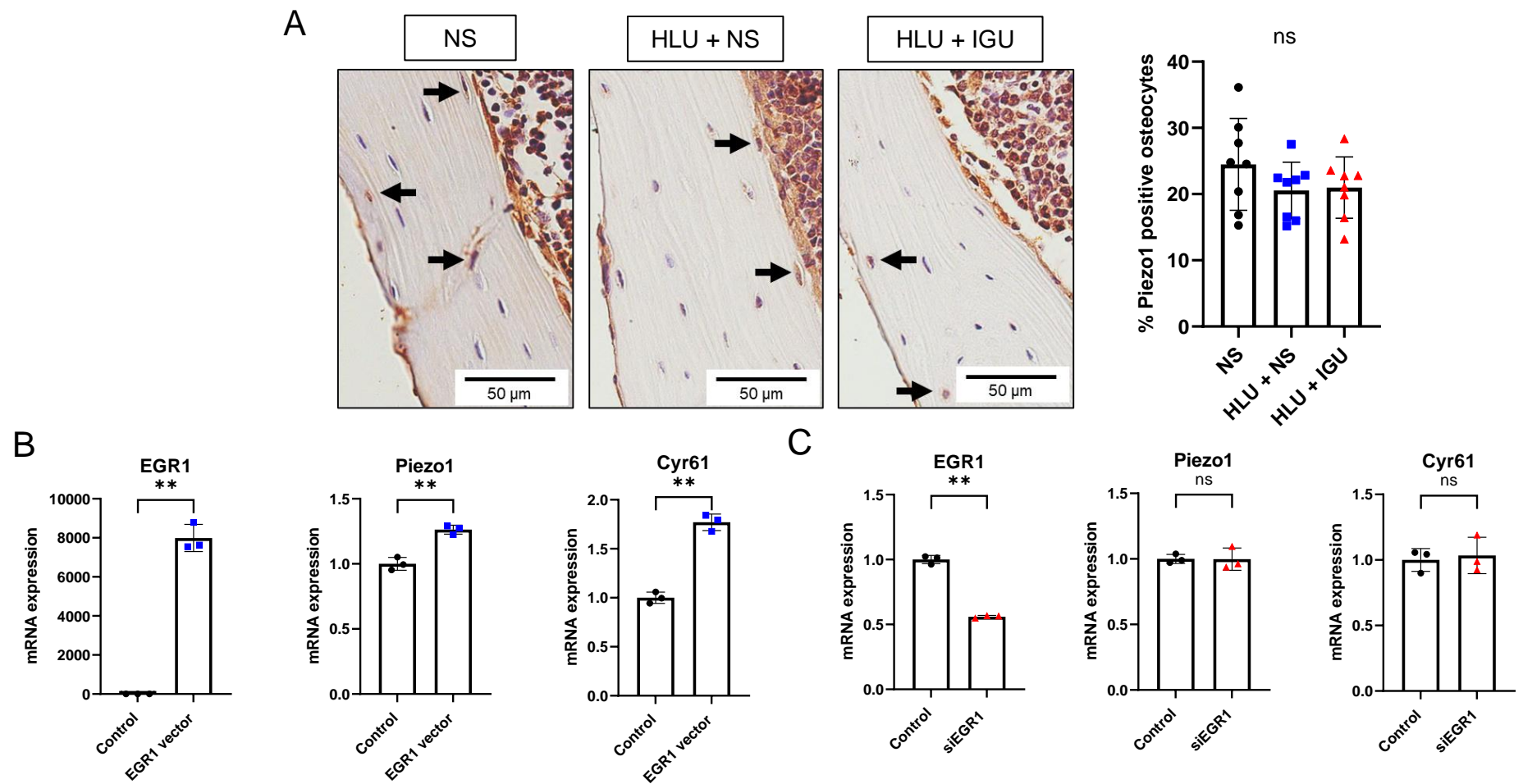


Supplemental Figure 3. Effects of iguratimod (IGU) on osteocyte apoptosis.

(A) Quantification of the relative number of empty lacunae in the cortical bone of the distal femur obtained from each group (normal saline (NS), hindlimb-unloaded (HLU) + NS, and HLU + IGU). The black arrows indicate the empty lacunae. (B) Reverse transcription quantitative polymerase chain reaction analysis of apoptosis-related gene expression in Saos-2 cells treated with bone morphogenetic protein-2 (BMP2) with or without IGU (data from three independent experiment data for each group). (C) Evaluation of cell proliferation in Saos-2 cells treated with different concentrations of IGU in the presence of BMP2 (data from five to six independent experiments for each group). Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test (* $p < 0.05$). All data are presented as mean \pm standard deviation. ns = not significant.



Supplemental Figure 4. The heatmap of differentially expressed genes, which were detected by RNA sequencing analysis on Saos-2 cells from untreated, bone morphogenetic protein-2 (BMP2)-treated, and BMP2 + iguratimod (IGU)-treated groups. Hierarchical clustering analysis was performed to generate a gene expression profile map of Saos-2 cells under untreated conditions, as well as conditions treated with BMP2 or BMP2 + IGU. The gene expression levels are represented by different colors: red points indicate up-regulated genes, green points indicate down-regulated genes and black points represent genes with no change in expression.



Supplemental Figure 5. Effects of iguratimod (IGU) on Piezo1-related genes.

(A) Immunohistochemical staining of Piezo1 in the distal femur cortical bone of each group (normal saline (NS), hindlimb-unloaded (HLU) + NS, and HLU + IGU) (data eight independent experiments for each group). The black arrows indicate Piezo1-positive osteocytes. (B and C) Saos-2 cells were transiently transfected with the early growth response protein 1 (EGR1) overexpression vector (B) or the EGR1-specific siRNA (C). Reverse transcription quantitative polymerase chain reaction analysis was conducted to examine the expression of EGR1 and Piezo1-related genes (data from three independent experiment data for each group). Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test (** $p < 0.01$). All data are expressed as the mean \pm standard deviation. ns = not significant.