**Supplementary Materials: Anti-Osteoporotic Effects of Kukoamine B in Osteoblast and Osteoclast Cells and Ovariectomized Mice**

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**Figure S1.** Fractionation and isolation of the bioactive component enhancing osteoblast differentiation from 70% ethanol extract of *Lycii radicis* cortex.



**Figure S2.** Results of proton nuclear magnetic resonance(1H-NMR). (A) Carbon**-**13 nuclear magnetic resonance (13C-NMR), (B) mass spectrum, and (C) analyses of the B4 fraction of Supplementary Figure S1.

 

**Figure S3.** Effects of Kukoamin B (KB) on alkaline phosphatase (ALP) activity in preosteoblast MC3T3-E1 cells. Cells were treated with ascorbic acid (50 μg/ml) and β-glycerophosphate (10 mM) and cultured with three different concentrations of KB (5, 10, and 20 μM), and an ALP activity assay was done at 2, 3, 4, and 5 days of incubation. Control: KB non-treated cells. \*: *p* < 0.05 vs. control.



**Figure S4.** Effects of Kukoamin B (KB) on cellular proliferation of primary-cultured monocytes. After induction of osteoclast differentiation by treatment of 30 ng/ml of M-CSF and 50 ng/ml of RANKL (Induction), cells were co-treated with three different concentrations of KB (5, 10, and 20 μM) for 6 days, and then cell viability was assessed.



**Figure S5.** Effects of Kukoamin B (KB) on osteoclast differentiation of primary-cultured monocytes. After induction of osteoclast differentiation by treatment of 30 ng/ml of M-CSF and 50 ng/ml of RANKL (Induction), cells were co-treated with three different concentrations of KB (5, 10, and 20 μM) for 3 and 6 days, and tartrate-resistant acid phosphatase (TRAP) activity was assessed. \*: *p* < 0.05 vs. Induction.



**Figure S6.** Effects of Kukoamin B (KB) on osteoblast and osteoclast differentiations in the co-culture of preosteoblasts and primary monocytes. Co-cultured MC3T3-E1 and primary monocyte cells were treated with osteoblast differentiation reagents, 50 μg/mL of ascorbic acid, and 10 mM of β-glycerophosphate and then co-treated with three different concentrations of KB (5, 10, and 20 μM) for 3 and 6 days. Alkaline phosphatase (ALP) activity (A) and tartrate-resistant acid phosphatase (TRAP) activity (B) were assessed in the co-culture cells. Control: KB non-treated cells. \*: *p* < 0.05 vs. Control.