Anti-Osteoporotic Effects of combined extract of Lycii Radicis Cortex and Achyranthes japonica in Osteoblast and Osteoclast Cells and Ovariectomized Mice

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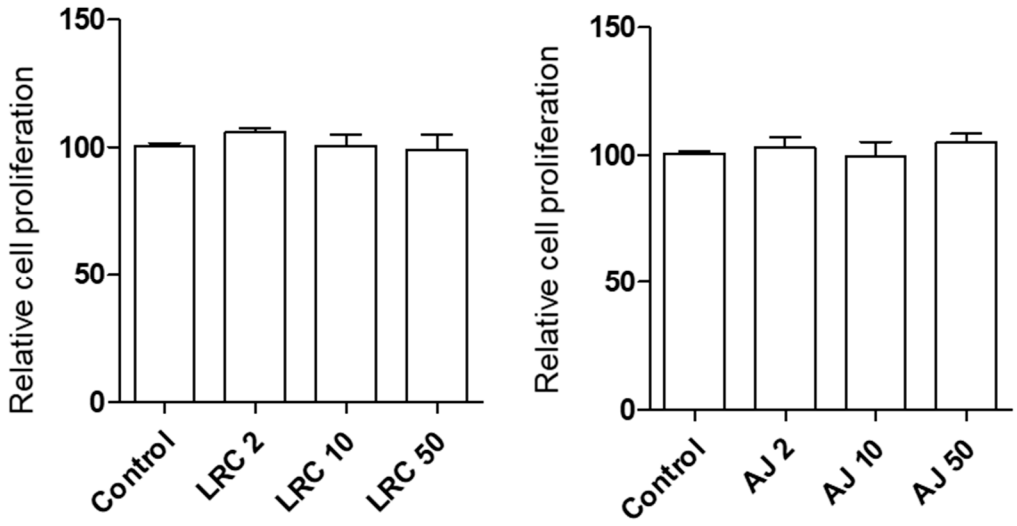

Figure. S1. Effects of LRC and AJ extracts on cell proliferation in preosteoblast MC3T3-E1 cells. Cells were treated with ascorbic acid (50 μg/ml) and β-glycerophosphate (10 mM) and cultured with three different concentrations (2, 10 and 50 μg/ml) and cell proliferation was assessed by WST.

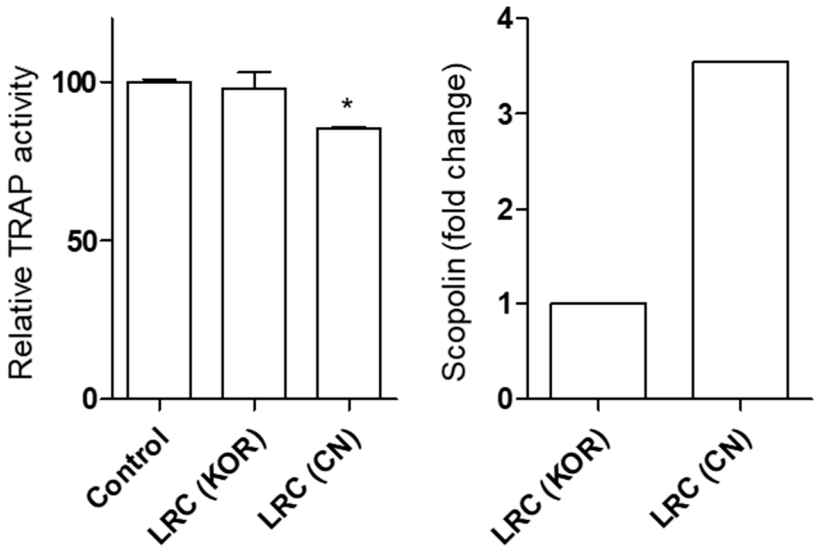

Figure. S2. Effect of LRC extracts from Korean (KOR) or Chinese (CN) plants on osteoclast differentiation of primary-cultured monocytes. Fold change in scopolin concentration.

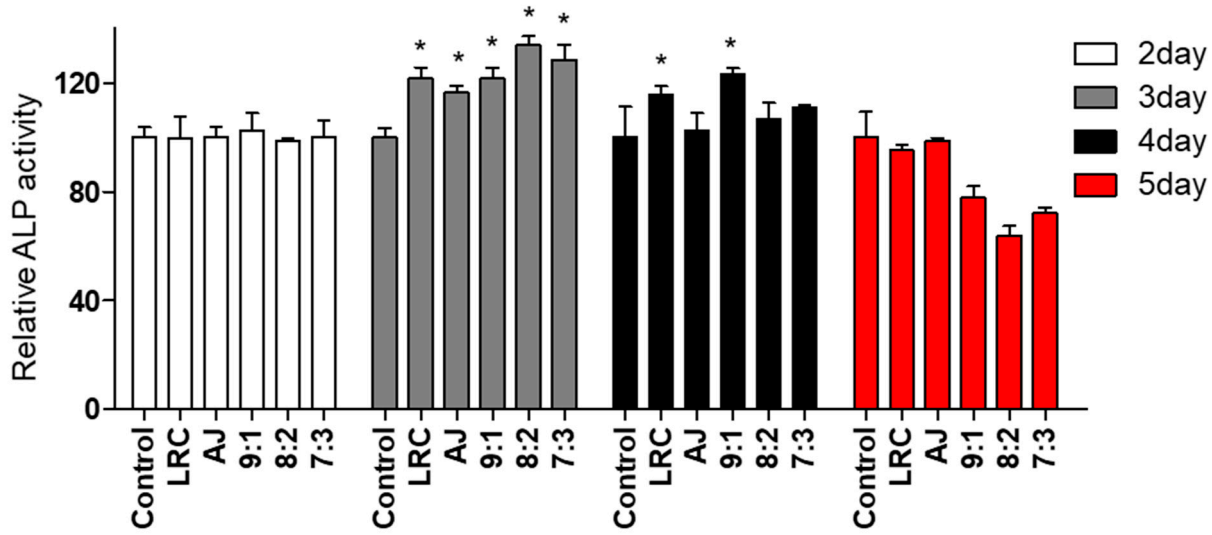

Figure. S3. Effects of single or combined LRC and AJ extract on alkaline phosphatase (ALP) activity in the osteoblast-lineage cell lines. Cells were treated with ascorbic acid (50 μg/ml) and β-glycerophosphate (10 mM) and incubated with 10 μg/ml of single or combined LRC and AJ extracts (9:1, 8:2 or 7:3), and an ALP activity assay was assessed at 2, 3, 4, and 5 days. Control: non-treated cells. *: $p < 0.05$ vs. control.

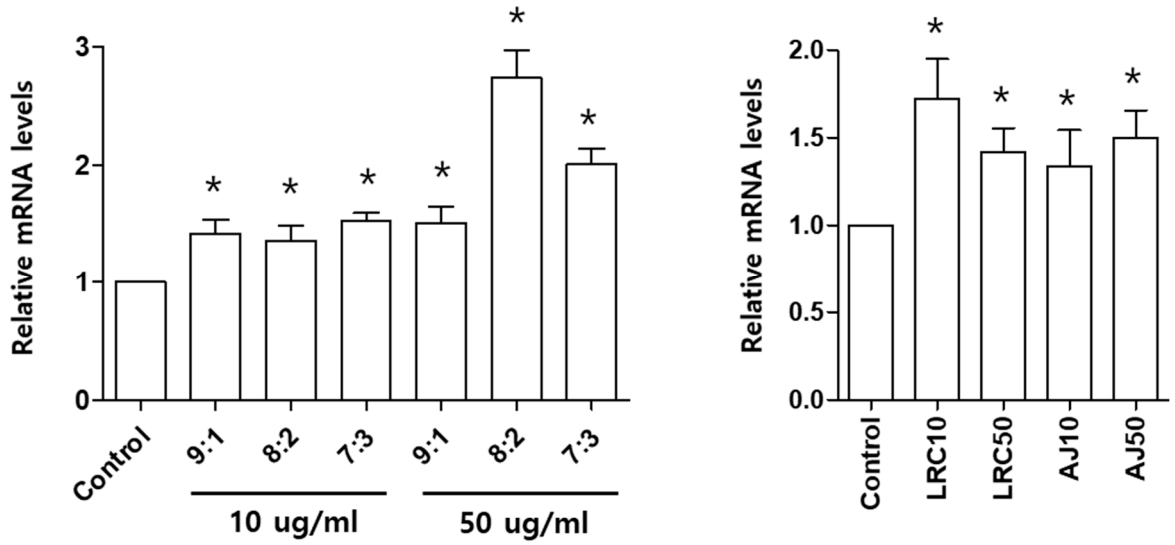

Figure. S4. Effects of single or combined LRC and AJ extract on mRNA expression of *Bglap.* Cells were treated with single extracts of LRC and AJ and combined LRC and AJ. The mRNA expression of *Bglap* was measured by RT-PCR. Control: non-treated cells. *: $p<0.05$ vs. Control.

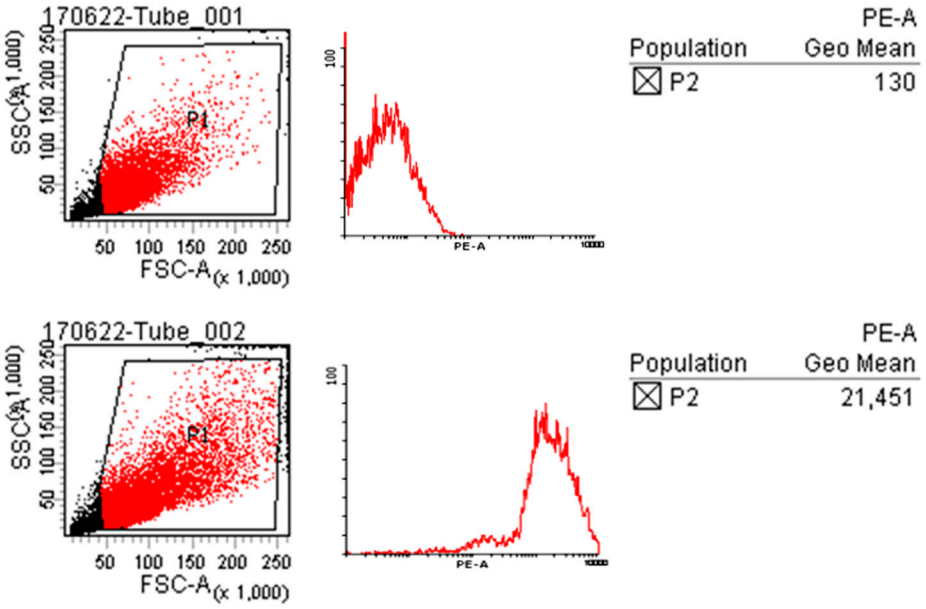

Figure. S5. The validation of the successful isolation of monocytes from mouse bone marrow. Primary-cultured monocytes were identified by an immunophenotypic analysis with a monocyte-specific surface positive marker (PE-conjugated CD11b antibody) using a fluorescence-activated cell sorting analysis.

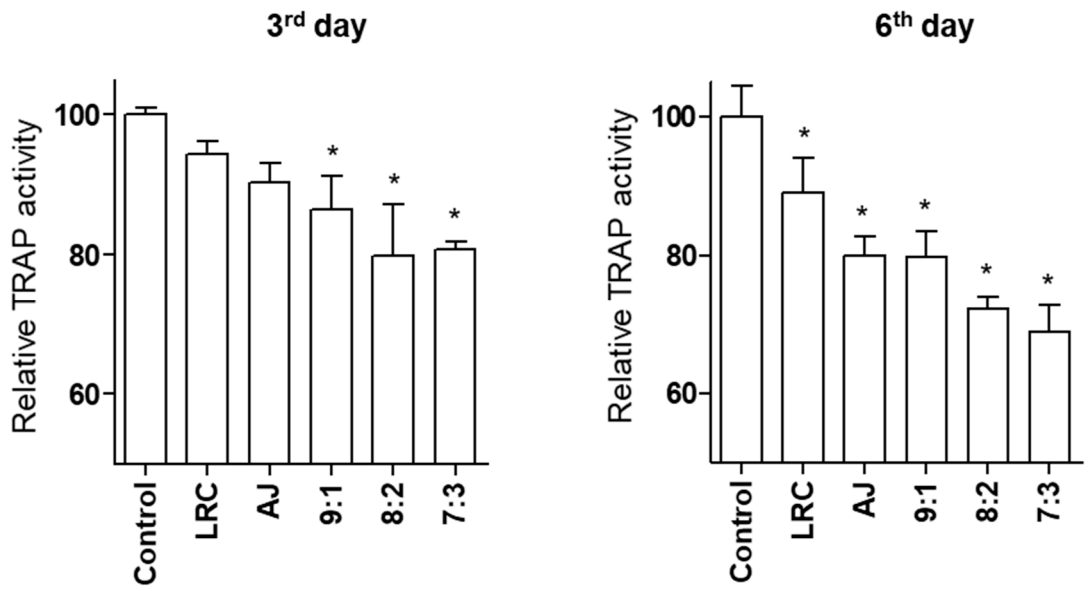

Figure. S6. Effects of single or combined LRC and AJ extract on osteoclast differentiation of primary-cultured monocytes. After induction of osteoclast differentiation by treatment of 30 ng/ml of M-CSF and 50 ng/ml of RANKL (Induction), cells were co-treated with single extracts of LRC and AJ and combined LRC and AJ for 3 and 6 days, and tartrate-resistant acid phosphatase (TRAP) activity was assessed. *: $p < 0.05$ vs. Induction.

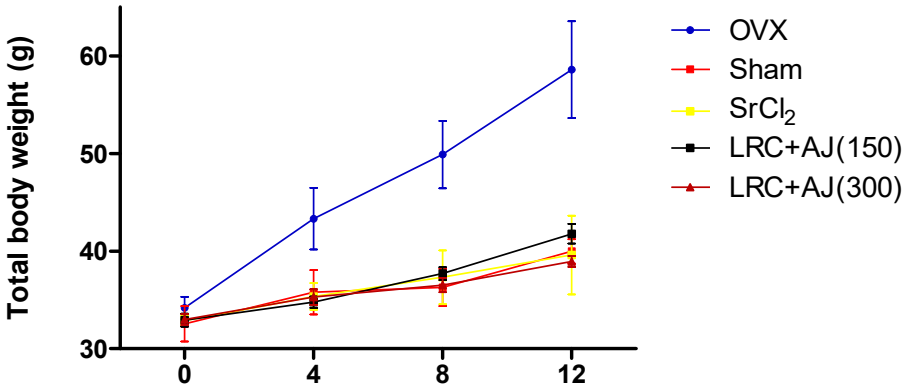

Figure. S7. Changes of total body weight for 12 weeks in non-surgery mice