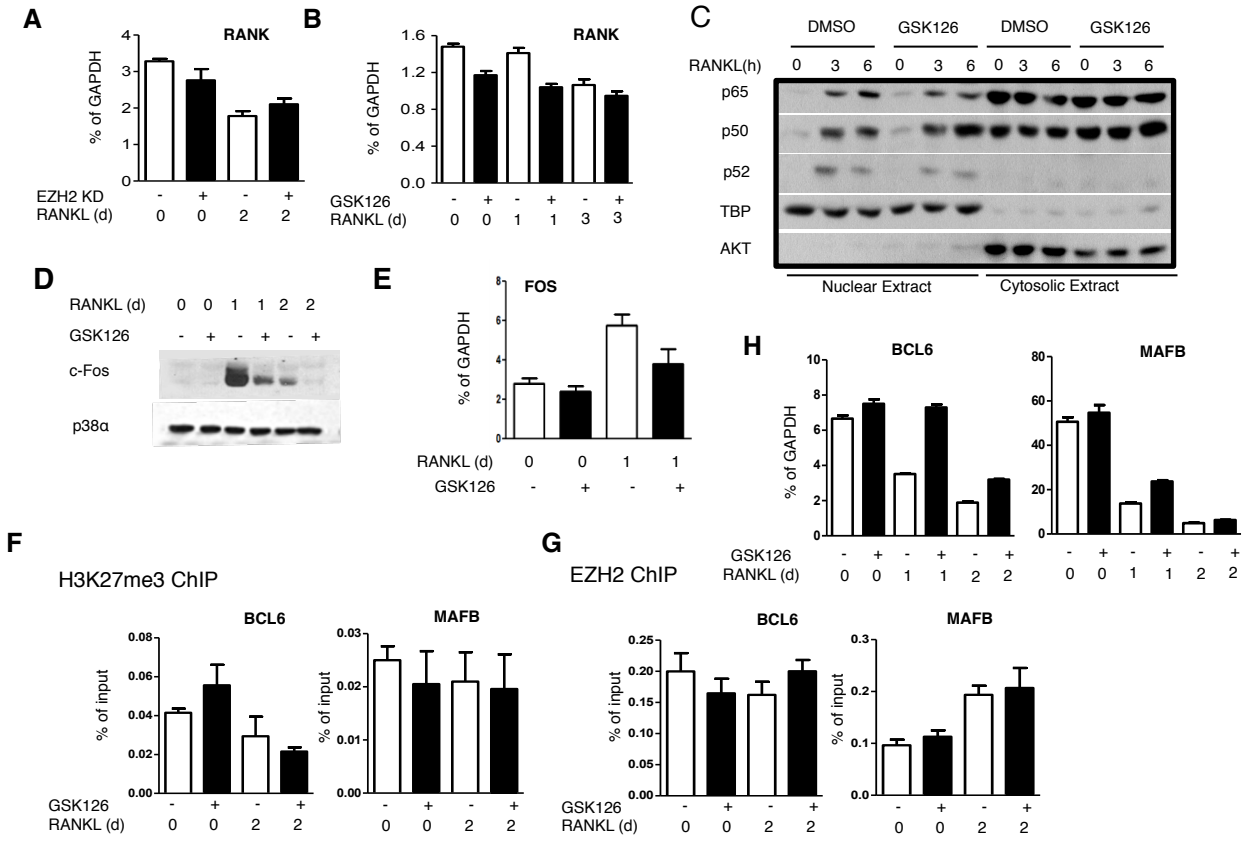
Supplementary Figure 1. EZH2 has a minimal effect on RANKL-induced NF-κB signaling but regulates RANKL-induced Fos expression.



A. RANK expression in EZH2 knockdown and control cells with or without RANKL stimulation. Data are shown as mean ± SEM of triplicates from one experiment and are representative of 4 experiments.

B. RANK expression in monocytes pre-treated with GSK126 or DMSO for 1 day and stimulated with RANKL for 0, 1 or 3 days. Data are shown as mean ± SEM of triplicates from one experiment and are representative of 3 experiments.

C. OCPs were treated with or without GSK126 for one day and then stimulated with RANKL for the indicated times. Nuclear lysates were blotted with anti-p65, p50, p52, TBP and AKT antibodies. TBP was used as a loading control for nulcear lysates.

D. Immunoblot of c-Fos expression in cells treated with GSK126. p38α is shown as a loading control. Blots shown are representative of 3 independent experiments.

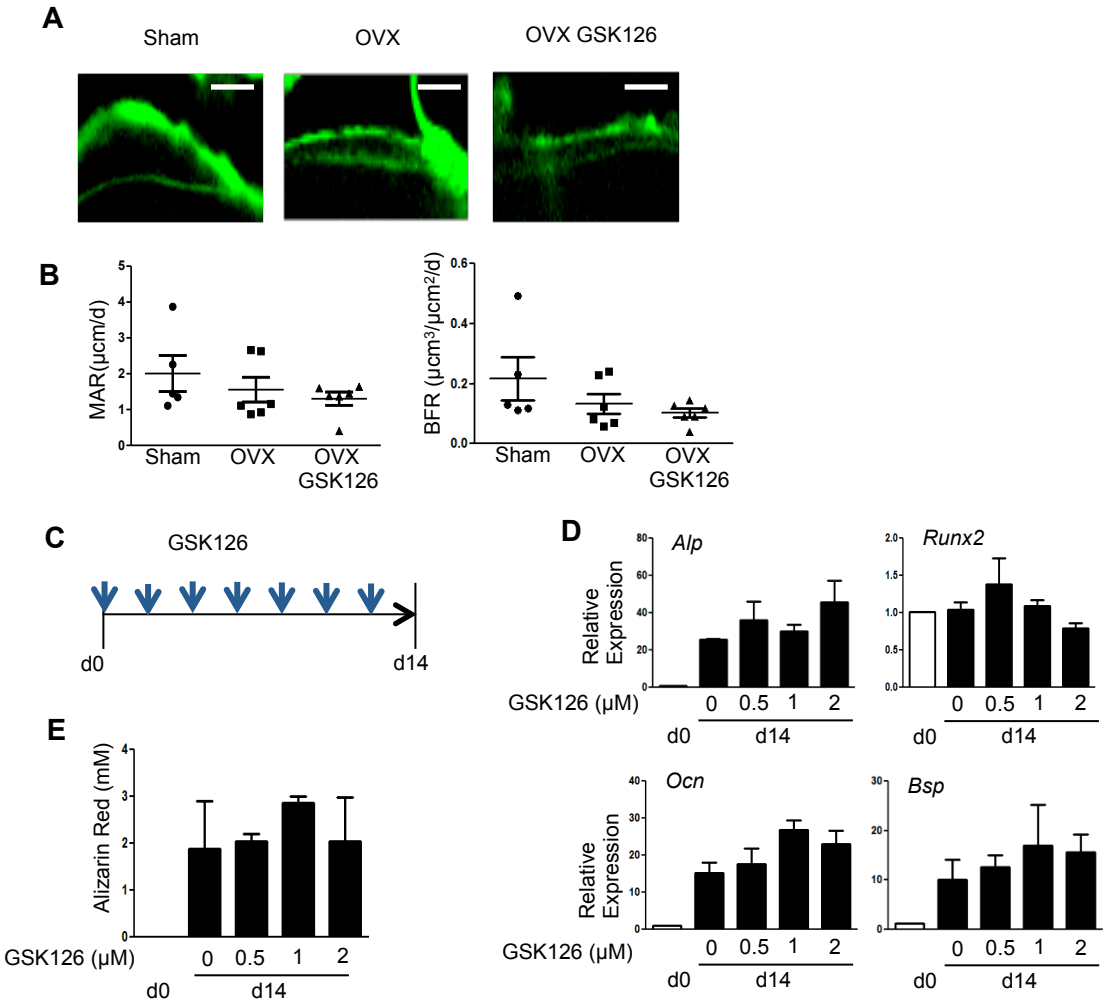
E. RT-PCR analysis of mRNA from GSK126 treated cells. mRNA levels were normalized relative to the expression of GAPDH and results are shown as mean ± SEM from two independent experiments. The kinetics of Fos mRNA induction varied among donors and the suppression of Fos mRNA was not consistently observed at different time points.

F. ChIP assays for H3K27me3 at the BCL6 or MAFB promoter in cells pretreated with GSK126 or DMSO and stimulated with RANKL. Data are shown as mean ± SEM of triplicates from one representative experiment out of 4.

G. ChIP assays for EZH2 at the *BCL6* or *MAFB* promoter in cells pretreated with GSK126 or DMSO and stimulated with RANKL. Data shown as mean ± SEM of triplicates from one representative experiment out of 4.

H. RT-PCR analysis of mRNA from GSK126 treated cells. PCR data is shown as mean ± SEM of triplicates; $p < 0.05$ for percent suppression in pooled data where N = 4 (one-tailed t-test).

Supplementary Figure 2. GSK-126 did not significantly suppress bone formation in the post-ovariectomy model. The dose-dependent effect of GSK-126 on osteoblast cultures.



A. Representative images showing casein double labeling in trabecular bone. Scale bars 10 μm.

B. Bone formation parameters including mineral apposition rate (MAR) and bone formation rate (BFR) were measured in sham, OVX and GSK-126 treated OVX group (n>5). All data are shown as mean ± SEM and the statistical analysis (One-way ANOVA) showed no significant differences among groups.

C-E. Primary osteoblasts were isolated from calvariae and cultured with α-MEM containing 10% FBS, 50 μg/ml of ascorbic acid, and 8 mM beta-glycerophosphate.

C. Experimental scheme. Cells were treated with either DMSO or GSK-126 at the indicated doses every other day from the beginning of the culture.

D. mRNA was measured using real-time PCR at day 14. mRNA levels were normalized relative to the expression of GAPDH and results are shown as mean ± SEM from two independent experiments.

E. Alizarin Red Staining of mineralizing osteoblast cells.