Supplemental Information

CCAAT/enhancer binding protein α (C/EBPα) is important for osteoclast differentiation and activity*

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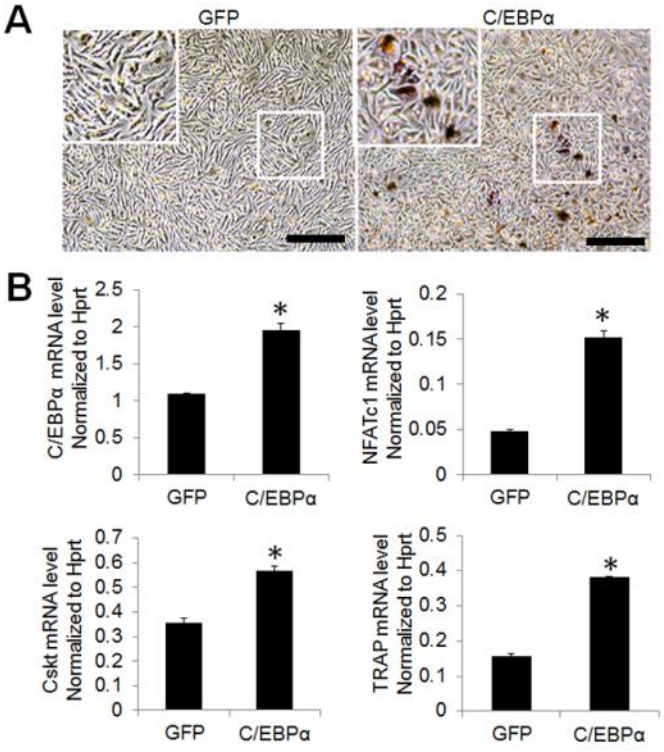
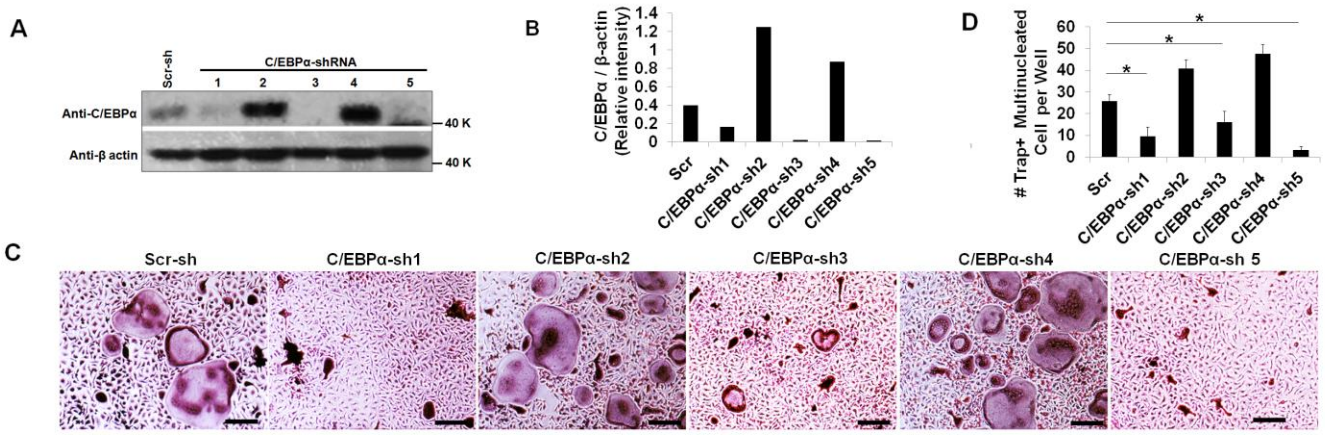
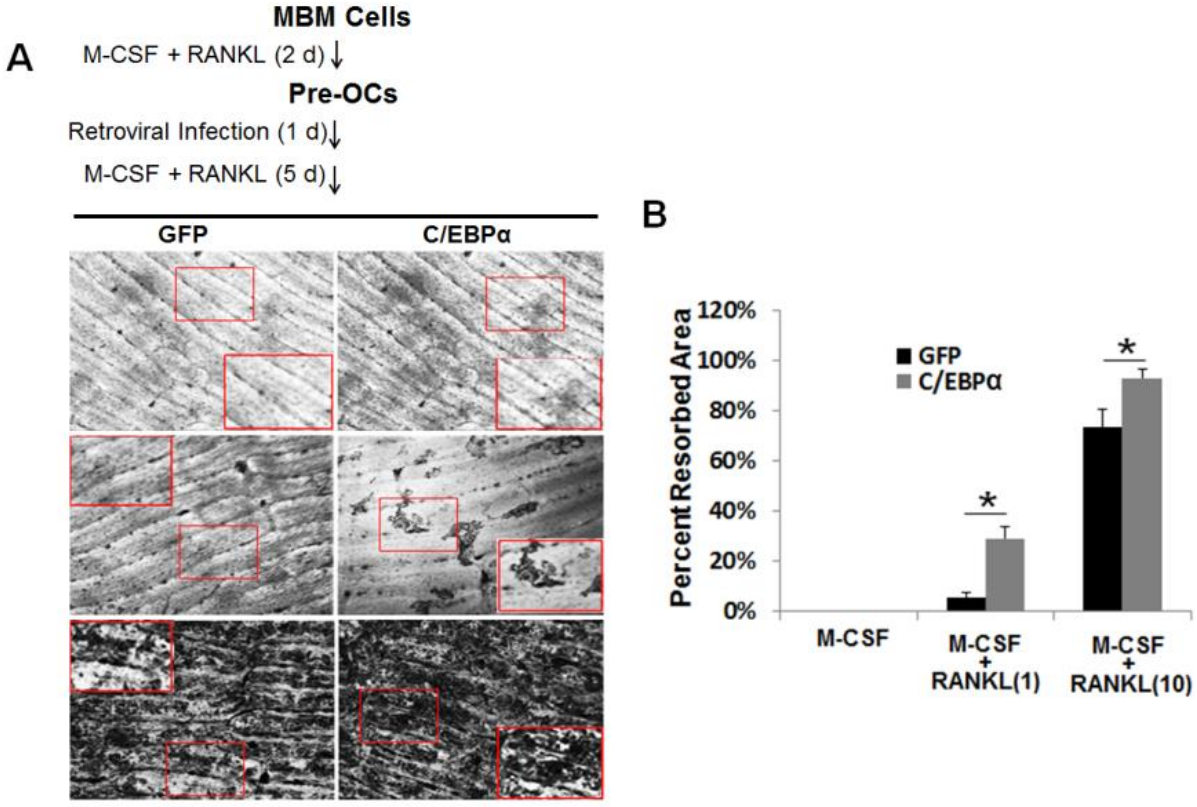


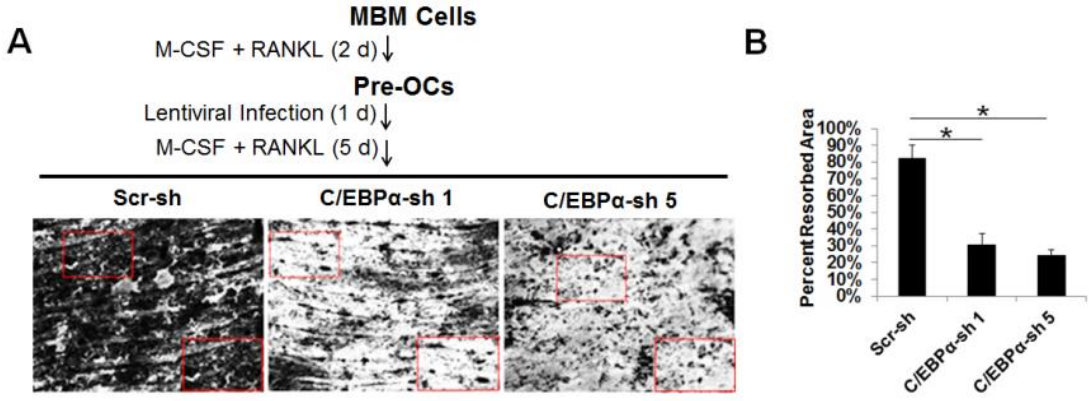
SUPPLEMENTAL FIGURE 1**. C/EBPα overexpression induces OC lineage commitment in the absence of RANKL.** *A*, MBM cells expressing GFP control (GFP) or Flag-C/EBPα (C/EBPα) were treated with M-CSF (10 ng/ml) alone for 4 days. Cultures from three independent experiments were then stained for TRAP activity. Scale bars are 200 μm *B*, MBM cells expressing GFP or C/EBPα were cultured with M-CSF (10 ng/ml) alone for 3 days. Gene expression was assessed by qPCR using Hprt as a loading control in three independent experiments. *Bars* show averages ± S.D. *, $p < 0.05$.



SUPPLEMENTAL FIGURE 2. **C/EBPα silencing inhibits osteoclastogenesis**. *A,* MBM cells expressing Scramble shRNA control (Scr-sh) or various C/EBPα shRNA constructs (C/EBPα-sh 1-5) were treated with M-CSF (10 ng/ml) and RANKL (10 ng/ml) for 48 hours and then submitted to Western blot analysis using β-actin as loading control. *B,* quantification for A is shown as relative intensity of C/EBPα expression. *C,* MBM cells expressing Scr-sh or C/EBPα shRNA 1-5 (C/EBPα-sh1-5) were treated with M-CSF (10 ng/ml) plus RANKL (10 ng/ml) for 4 days. All cultures were then stained for TRAP activity. *D,* quantification for *C* is shown from three independent experiments. Scale bars are 200 μm. Bars show averages ± S.D. *, $p < 0.05$.



SUPPLEMENTAL FIGURE 3. **C/EBPα overexpression can stimulate bone resorption by OCs from hematoxylin staining of bone slices**. *A*, MBM cells seeded on bone slices were first treated with M-CSF (10 ng/ml) and RANKL (10 ng/ml) for 2 days (d) before infection with retrovirus encoding GFP control (GFP) or Flag-C/EBPα (C/EBPα) for 1 day. The treatments were then continued for five additional days. The bone slices were stained by hematoxylin for analysis of the resorption pits. *B*, quantification for the bone resorption shown in A for three independent experiments. Numbers in parenthesis show concentration in ng/ml. *Bars* show mean ± S.D. *, $p < 0.05$.



SUPPLEMENTAL FIGURE 4. **C/EBPα silencing abrogates bone resorption by OCs from hematoxylin staining of bone slices**. *A*, MBM cells seeded on bone slices were first treated with M-CSF (10 ng/ml) and RANKL (10 ng/ml) for 2 days (d) before infection with lentivirus encoding Scramble shRNA control (Scr-sh) or C/EBPα-shRNA constructs 1 or 5 (C/EBPα-sh 1 or 5) for 1 day. The treatments were then continued for five additional days. The bone slices were stained by hematoxylin for analysis of the resorption pits. *B*, quantification for the bone resorption shown in A from three independent experiments. Numbers in parenthesis show concentration in ng/ml. *Bars* show mean ± S.D. *, $p < 0.05$.