Supplementary Methods

Flow cytometry analysis. The MACS-sorted OSCAR+CTR- and OSCAR+CTR+ cells were first washed with PBS, and then incubated with FITC-conjugated goat anti-rat secondary antibody (1:200 dilution, Thermo Scientific, A11006) and PE-conjugated goat anti-rabbit secondary antibody (1:200 dilution, Thermo Scientific, P-2771MP) for 30 min at 4 °C. The cells were then stained with 7-aminoactinomycin D for labeling dead cells. Thereafter, all the stained cell populations were washed with PBS for three times and proceeded to flow cytometry analysis using a FACS Aria III cell sorter (BD Biosciences). The flow cytometric data were further processed and analyzed in FlowJo (BD Biosciences).

Total RNA extraction, reverse transcription, and quantitative real-time PCR. Total RNA was isolated using TRIzol reagent (Thermo Fisher Scientific, 15596018) following the standard protocol provided by the manufacturer. For mRNA qPCR, the first-strand cDNA was synthesized from the total RNA with the High-Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific, 4387406). The expression of TRAP and CTSK mRNA was determined by TaqMan real-time PCR Assay (TRAP, Mm00475698_m1; CTSK, Mm00484039_m1). The real-time PCR reactions contain 10 μl 2× Universal MasterMix (Thermo Fisher Scientific, 4304437), 3μl of the RT product, 1 μl 20× TaqMan assay and 6 μl of nuclease-free water. The real-time PCR program started with 95 °C for 10min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1min on the ABI Viia 7 real-time PCR system (Applied Biosystems). The relative expression of mRNA calculated by the $2^{-\Delta\Delta Ct}$ method with Gapdh (mRNA, Mm99999915_g1).

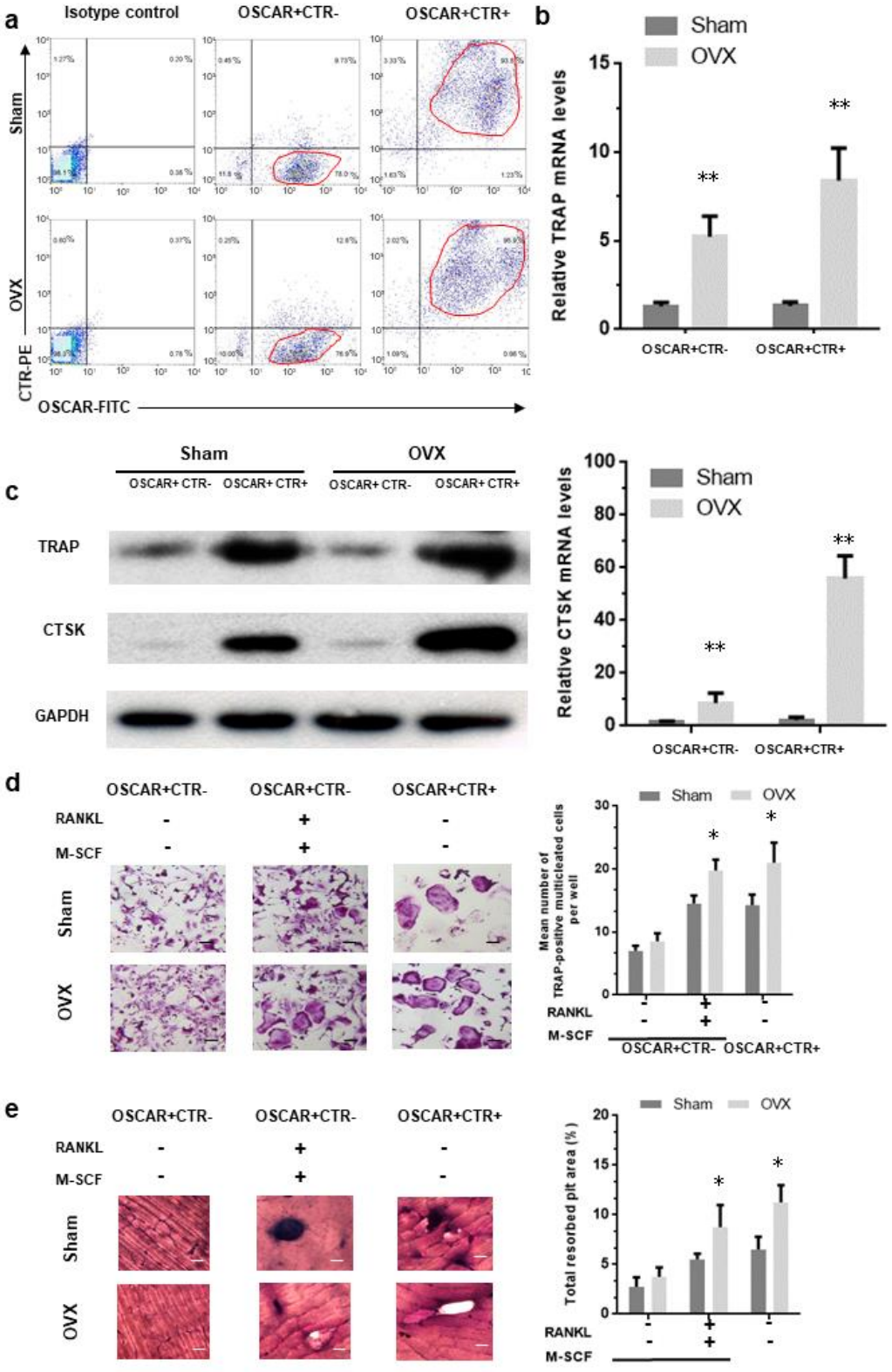
Western Blot. The total protein was extracted from the collected cells with RIPA lysis buffer (Thermo Fisher Scientific, 89900) supplemented with protease/phosphatase inhibitor (Cell Signaling, 5872S) following the manufacturer's instruction and subjected to SDS–PAGE (10% polyacrylamide gel). Proteins were then transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% milk in TBST followed by incubation with primary antibodies against TRAP (1: 1,000 dilution, Abcam ab191406), CTSK (1:1,000 dilution, Abcam ab19027), and GAPDH (1:2,000 dilution, Cell Signaling, 5174) overnight, respectively. All the primary antibodies have been validated by either the manufacturer or other users in the published studies. After washing with TBST three times, the membranes were incubated with a horseradish-peroxidase-conjugated secondary antibody (1:10,000 dilution, Bio-Rad 1662408) at room temperature for 1h. The immunoreactive bands were visualized by using SuperSignal West Dura Extended Duration Substrate (Thermo Fisher Scientific 34075). The band images were analyzed using ImageJ.

Tartrate-Resistant Acid Phosphatase (TRAP) Staining. The OSCAR-, OSCAR+CTR-, and OSCAR+CTR+ cells were planted in 24-well plates at a density of 3×10^4 cells per well. After cell attachment, remove media. Wash with PBS twice and fix the cells with 4%

Paraformaldehyde (PFA) for 10 minutes at room temperature. Prepare TRAP Staining Solution Mix (Sodium Acetate Anhydrous (Sigma, S-2889) 9.2 g, L-(+) Tartaric Acid (Sigma, T-6521) 11.4 g, Distilled water 950 ml, Glacial Acetic Acid 2.8 ml, pH 4.7 - 5.0) and pre-warm to 37°C in waterbath. Take 50ml TRAP Staining Solution Mix and add 0.5 ml Naphthol AS-MX Phosphate Substrate mix (Napthol AS-MX Phosphate (Sigma, N-4875) 20 mg, Ethylene Glycol Monoethyl Ether (Sigma, E-2632)1 ml), add to plates and incubate at 37°C for 50 min protected from light. Prepare solution A (Sodium Nitrite (Sigma, S-2252) 0.25 g, Distilled Water 5 ml) and solution B (Pararosaniline Chloride (Sigma, P-3750) 0.2 g, 2N HCL 5 ml). Mix 1 ml of Solution A and 1 ml of Solution B by gentle inversion for 30 sec and let stand for 2 min. Add this mixed solution to another pre-warmed 50ml TRAP Staining Solution Mix, mix and add the plates without rinsing. Incubate at room temp for 20 min. After staining, observe the osteoclasts (≥3 nuclei) under the microscope, take picture for each well and count the number of osteoclasts.

Bone Resorption Assay. OSCAR-, OSCAR+CTR-, and OSCAR+CTR+ cells were seeded and cultured on the dentin slices in 96-well plate for 5 days. Thereafter, the bone slices were ultra-sonicated in 1 mol/L NH_4OH to remove adherent cells and stained with 0.1% toluidine blue solution. Bone slices were imaged under light microscopy. Pit areas were quantified using Image Pro Plus 6.2 software (Media Cybernetics Inc.)

Supplementary Figure



Supplementary Figure 1. The molecular and functional characterization of the MACS-sorted OSCAR+CTR- and OSCAR+CTR+ cells from the Sham and OVX mice.

(a) The purity assessment of the MACS-sorted OSCAR+CTR- and OSCAR+CTR+ cell fractions from the Sham and OVX mice by flow cytometry analysis. (**b**) Real-time QPCR analysis of TRAP and CTSK mRNA expression in the OSCAR+CTR- and OSCAR+CTR+ cells. **(c)** Western blot analysis of the TRAP and CTSK expression in the OSCAR+CTR- and OSCAR+CTR+ cells. **(d)** TRAP staining of the OSCAR+CTR- cells, OSCAR+CTR- cells in the presence of RANKL (50 ng/ml) and M-CSF (50 ng/ml), and OSCAR+CTR+ cells. The trap-positive cells with more than three nucleic were counted. Scale bars, 100 μm. **(e)** Bone-resorptive assay of the OSCAR+CTR- cells, OSCAR+CTR- cells in the presence of RANKL (50 ng/ml) and M-CSF (50 ng/ml), and OSCAR+CTR+ cells. Scale bars, 100 μm. All data are the mean ± s.d. *P < 0.05 for a comparison of the same cell fractions from the Sham mice.