Supplementary Figure Legend

Figure S1

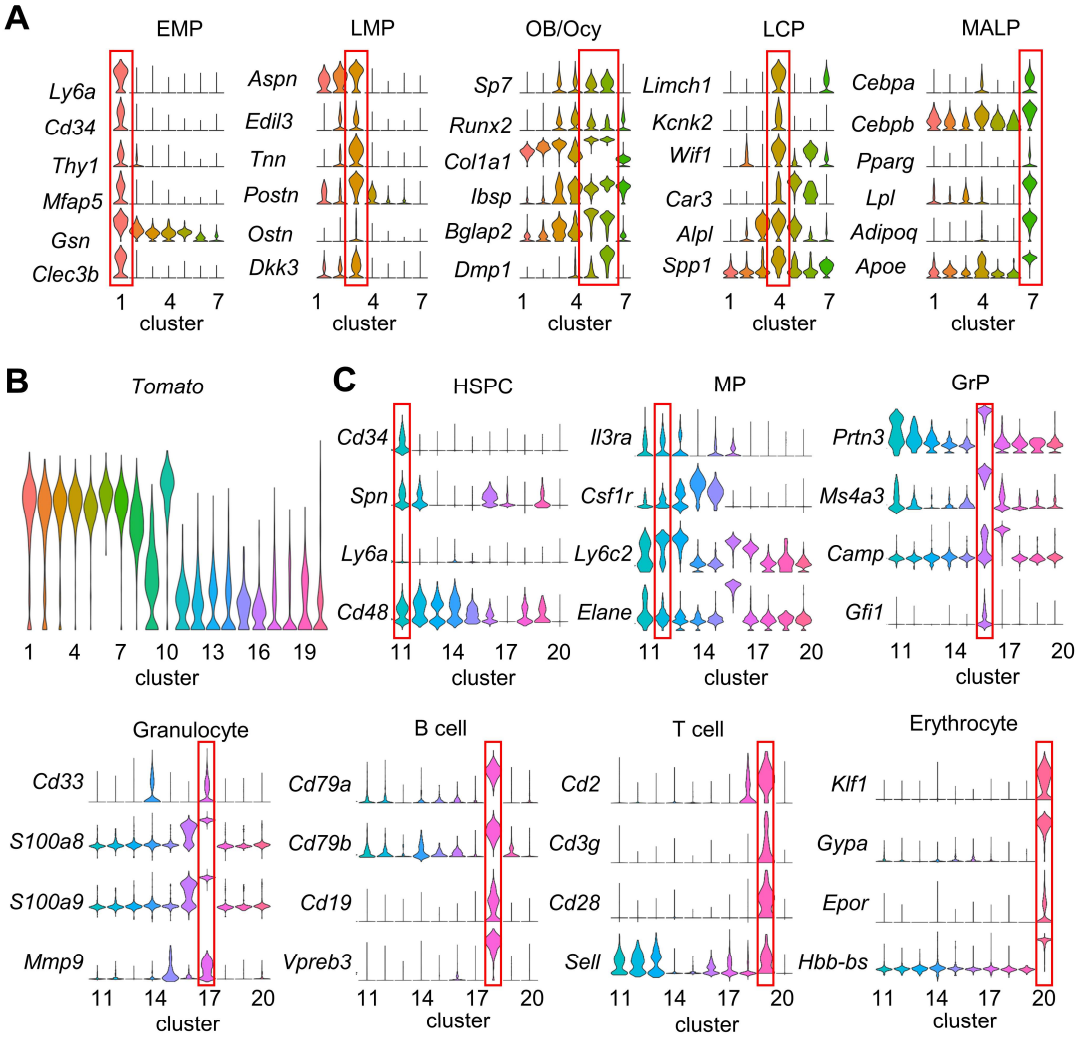

Figure S1. ScRNA-seq analysis of mesenchymal lineage and hematopoietic lineage cell clusters.

(A) Violin plots of marker gene expression in mesenchymal lineage subpopulations. EMP: early mesenchymal progenitor; LMP: late mesenchymal progenitor; OB: osteoblast; Ocy: osteocyte; LCP: lineage committed progenitor.

(B) Violin plot of Tomato expression in all cell clusters.

(C) Violin plots of marker gene expression in hematopoietic lineage subpopulations. HSPC: hematopoietic stem and progenitor cells; MP: monocyte progenitor; GrP: granulocyte progenitor. In A-C, cluster numbers correspond to the numbers in Fig. 1A.

(D) Hierarchy heatmap of hematopoietic lineage cell clusters.

Figure S2

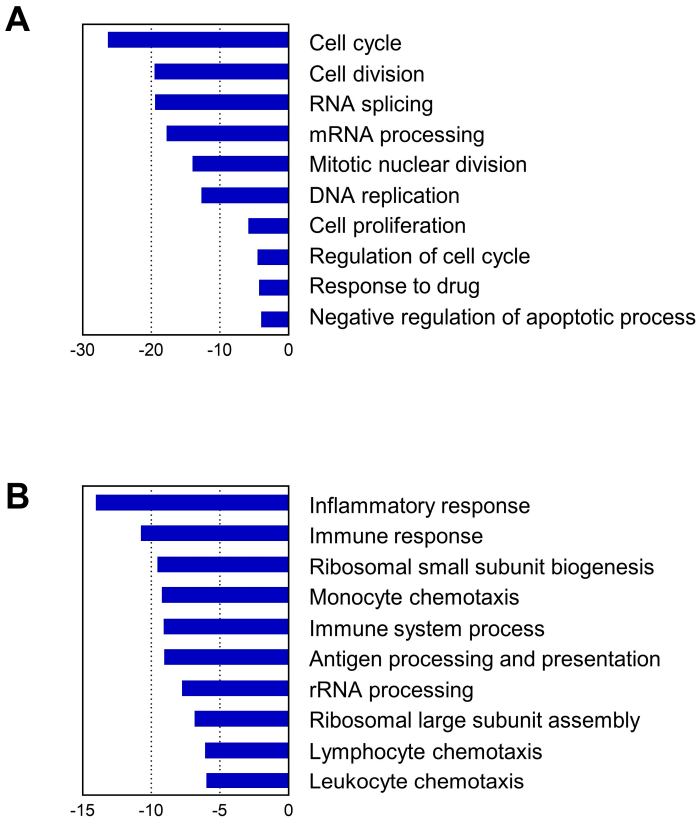

Figure S2. GO term and KEGG pathway analyses of up-regulated genes in early osteoclasts versus late osteoclasts (A) and in Mϕα versus Mϕβ cells (B).

Figure S3

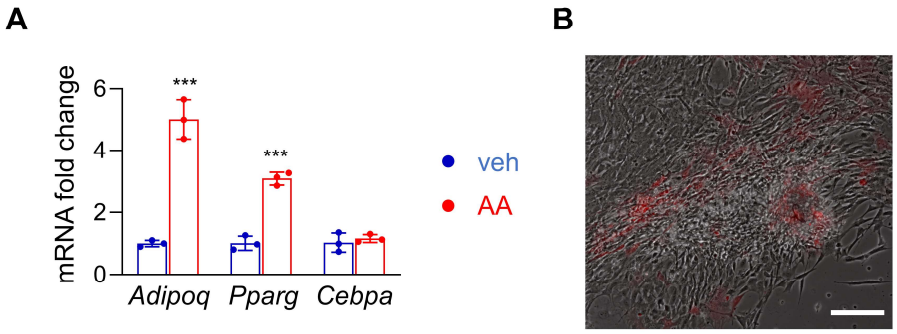

Figure S3. *Adipoq-Cre* does not label most bony nodule-forming osteogenic cells in culture.

(A) qRT-PCR analysis of *Adipoq, Pparg*, and *Cebpa* expression in bone marrow mesenchymal progenitors cultured in the growth medium with or without 50 µg/mL ascorbic acid (AA) for 6 days. ***: $p<0.001$, AA vs vehicle (veh), 2-tailed unpaired Student's *t* test.

(B) A few bony nodules formed after osteogenic differentiation of mesenchymal progenitors from *Adipoq/Td* bone marrow contain Td^+ cells. However, most cells in these nodules are Td^-. Scale bar=500 µm.

Figure S4

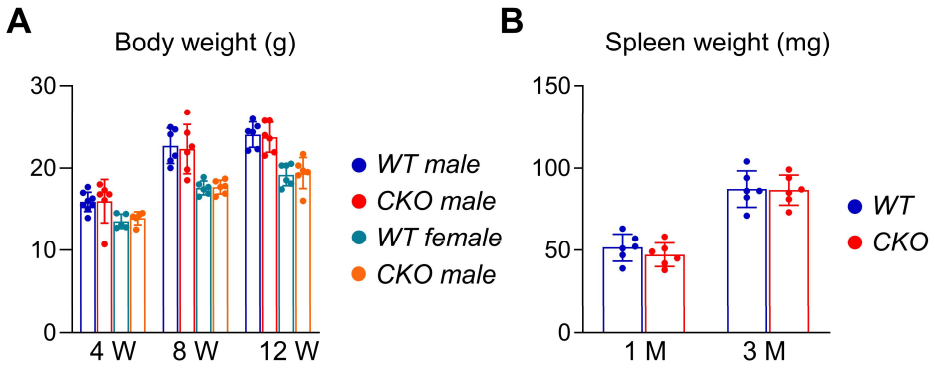

Figure S4. *RANKL CKO*^{Adipoq} mice has normal body and spleen weight.

(A) Body weight was measured in male and female mice at 4, 8, and 12 weeks of age. n=5-8 mice/group.

(B) Spleen weight was measured at 1 and 3 months of age. n=6 mice/group.

Figure S5

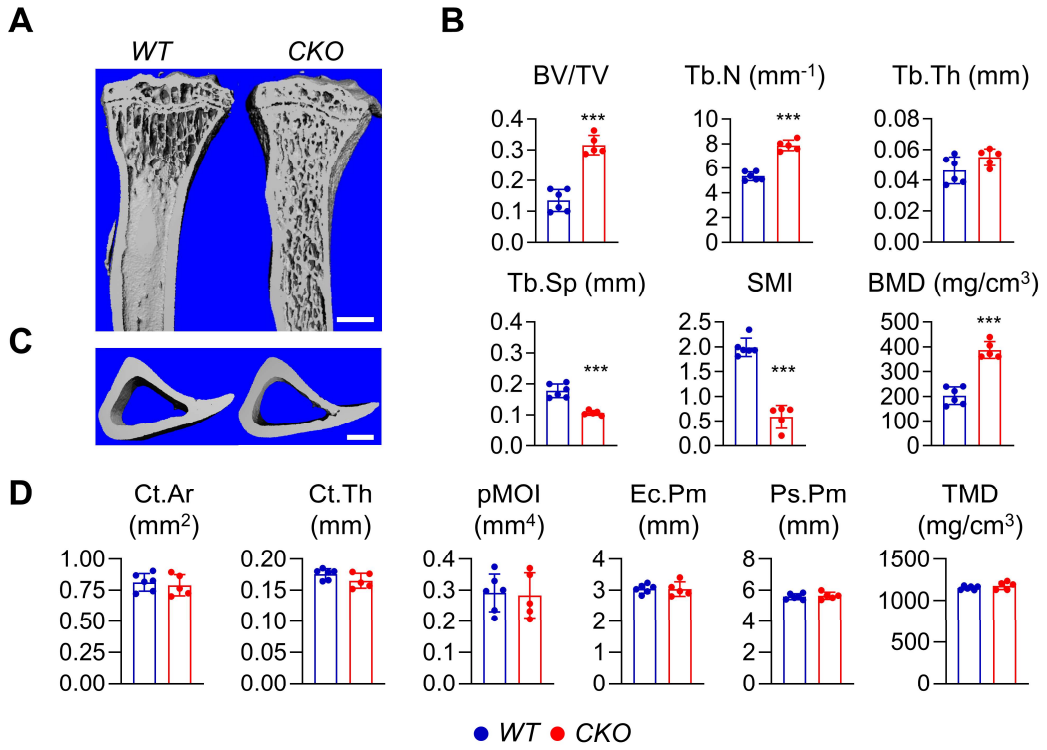

Figure S5. 5-month-old male *RANKL CKO*^{Adipoq} mouse tibiae have high trabecular bone mass.

(A) 3D microCT reconstruction of *WT* and *RANKL CKO*^{Adipoq} mouse tibiae reveals a drastic increase of trabecular bone at 5 months of age. Scale bar=1 mm.

(B) MicroCT measurement of trabecular bone structural parameters from the secondary spongiosa region. BV/TV: bone volume fraction; Tb.N: trabecular number; Tb.Th: trabecular thickness; Tb.Sp: trabecular separation; SMI: structural model index; BMD: bone mineral density. n=5-6 mice/group.

(C) 3D microCT reconstructions of the midshaft region. Scale bar=0.4 mm.

(D) MicroCT measurement of cortical bone structural parameters from the midshaft region. Ct.Ar: cortical area; Ct.Th: cortical thickness; pMOI: Polar moment of inertia; Ec.Pm: endosteal perimeter; Ps.Pm: periosteal perimeter; TMD: tissue mineral density. n=5-6 mice/group.

***: p<0.001 *CKO* vs *WT,* 2-tailed unpaired Student's *t* test

Figure S6

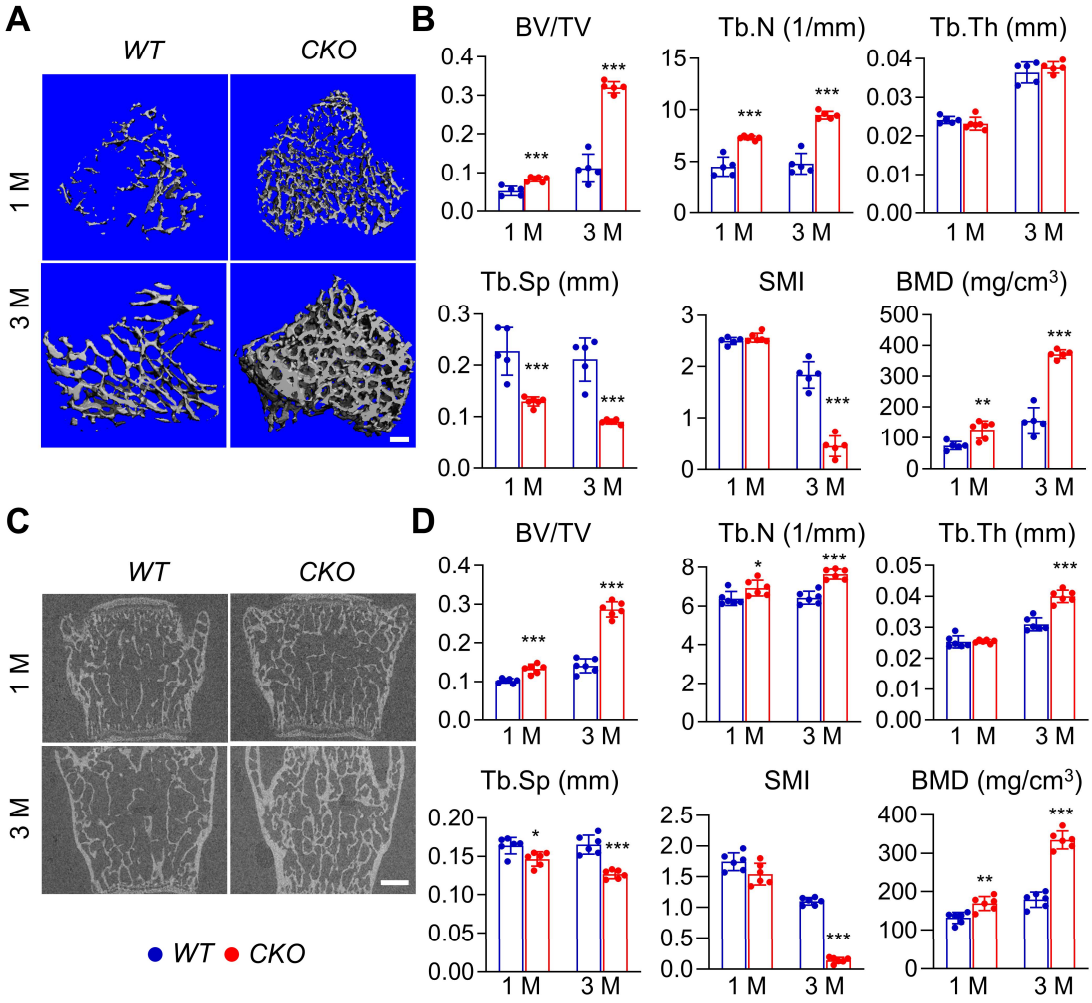

Figure S6. Female *RANKL CKO*^{Adipoq} mice also display osteopetrosis phenotype.

(A) 3D microCT reconstruction of metaphyseal region in *WT* and *CKO* tibiae. Scale bar=200 μm.

(B) MicroCT measurement of trabecular bone structural parameters from the secondary spongiosa region. BV/TV: bone volume fraction; Tb.N: trabecular number; Tb.Th: trabecular thickness; Tb.Sp: trabecular separation; SMI: structural model index; BMD: bone mineral density. n=5-6 mice/group.

(C) 2D microCT images of *WT* and *CKO* vertebrates. Scale bar=500 μm.

(D) MicroCT measurement of trabecular bone structural parameters in vertebrates. n=5-6 mice/group.

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ *CKO* vs *WT*, 2-tailed unpaired Student's *t* test.

Figure S7

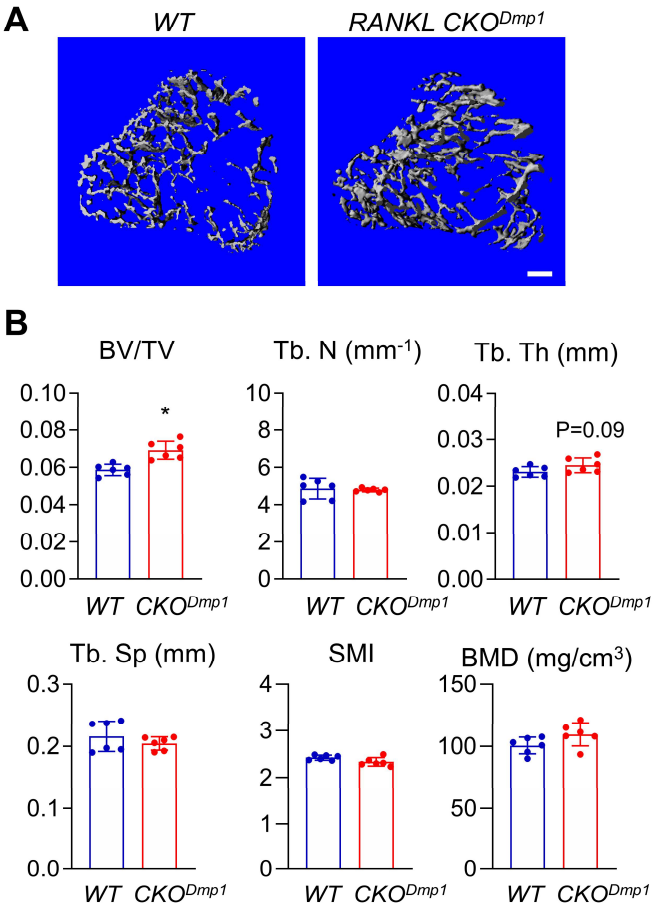

Figure S7. Mice with osteocyte-specific RANKL deficiency have a minor increase in trabecular bone mass at 1 month of age.

(A) 3D microCT reconstruction of metaphyseal region in *WT* and *RANKL CKO^{Dmp1}* mouse tibiae. Scale bar=200 μm.

(B) MicroCT measurement of trabecular bone structural parameters from the secondary spongiosa region. BV/TV: bone volume fraction; Tb.N: trabecular number; Tb.Th: trabecular thickness; Tb.Sp: trabecular separation; SMI: structural model index; BMD: bone mineral density. *: $p<0.05$ *CKO* vs *WT*. n=6 mice/group, 2-tailed unpaired Student's *t* test.

Figure S8

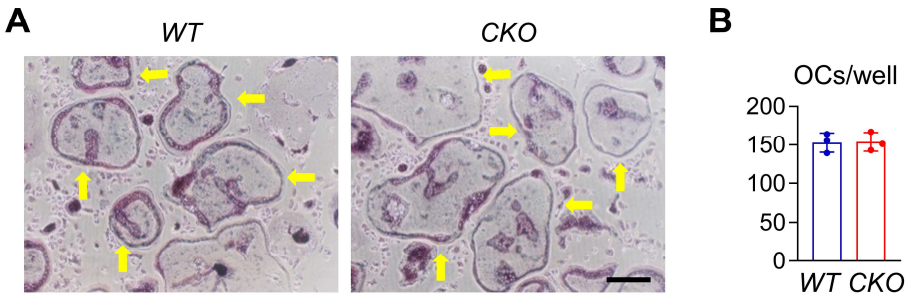

Figure S8. Osteoclast progenitors are normal in *RANKL CKO*^{Adipoq} mice.

(A) Representative TRAP staining images of osteoclast culture derived from *WT* and *RANKL CKO*^{Adipoq} BMCs at 7 days after addition of RANKL and M-Csf. Arrows point to giant multinucleated osteoclasts. Scale bar=200 μm.

(B) Quantification of TRAP^{+} multinucleated cells (>3 nuclei/cell) per well. n=3 mice/group.

Figure S9

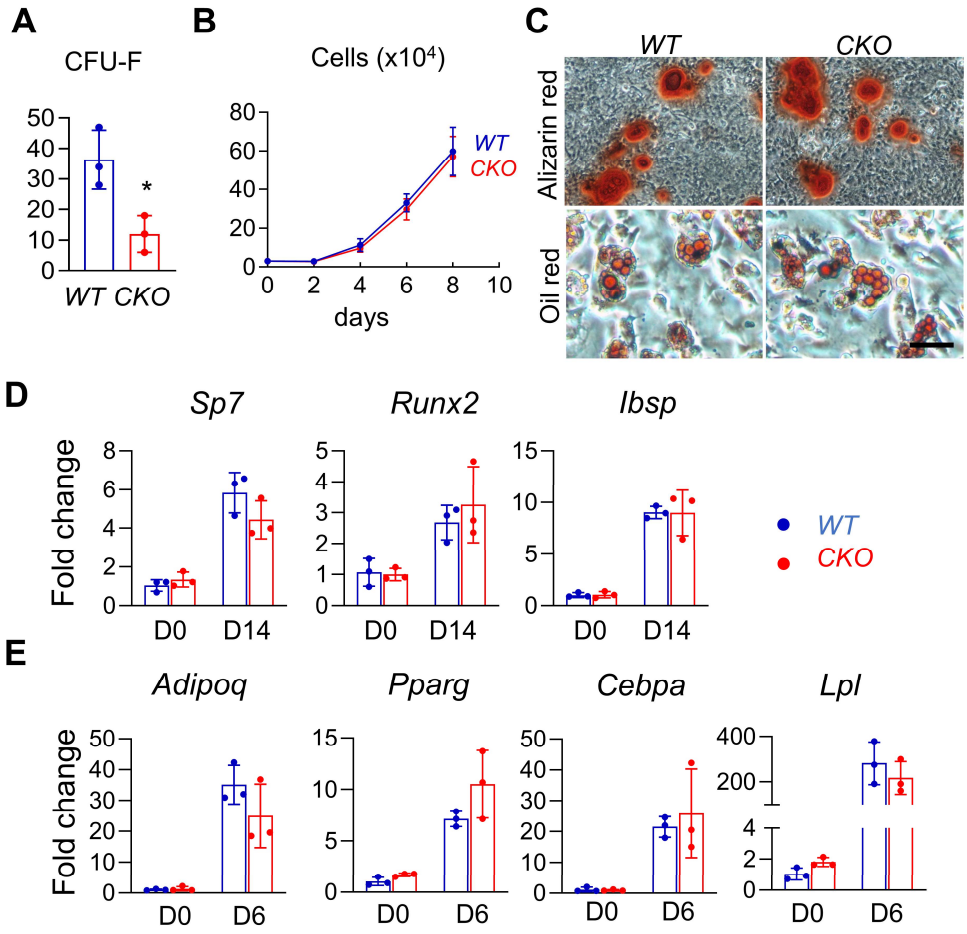

Figure S9. *RANKL CKO*^{Adipoq} mice have a reduced pool of bone marrow mesenchymal progenitors.

(A) CFU-F assay of mesenchymal progenitors from femoral bone marrow of *WT* and *CKO* mice. *: $p<0.05$, *CKO* vs *WT,* 2-tailed unpaired Student's *t* test. n=3 mice/group.

(B) Growth curves of bone marrow mesenchymal progenitors in culture.

(C) Representative Alizarin red and Oil red staining of cells after cultured in osteogenic and adipogenic medium, respectively. Scale bar= 100 μm.

(D) qRT-PCR analysis of osteogenic marker gene expression in cells after 2 weeks of osteogenic differentiation.

(E) qRT-PCR analysis of adipogenic marker gene expression in cells after 1 weeks of adipogenic differentiation.

Figure S10

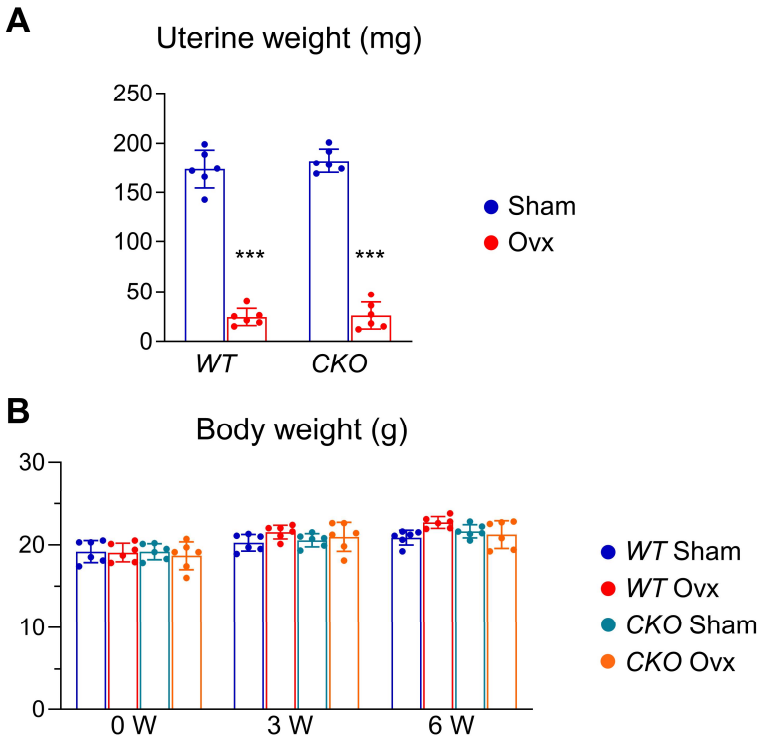

Figure S10. Examination of mouse uterine and body weight after ovx surgery.

(A) Uterine weight of *WT* and *CKO* mice after ovx. ***: p<0.001 *CKO* vs *WT,* 2-tailed unpaired Student's *t* test, n=6 mice/group. n=6 mice/group.

(B) Body weight of *WT* and *CKO* mice is recorded at 0, 3, and 6 weeks after ovx surgery.

Figure S11

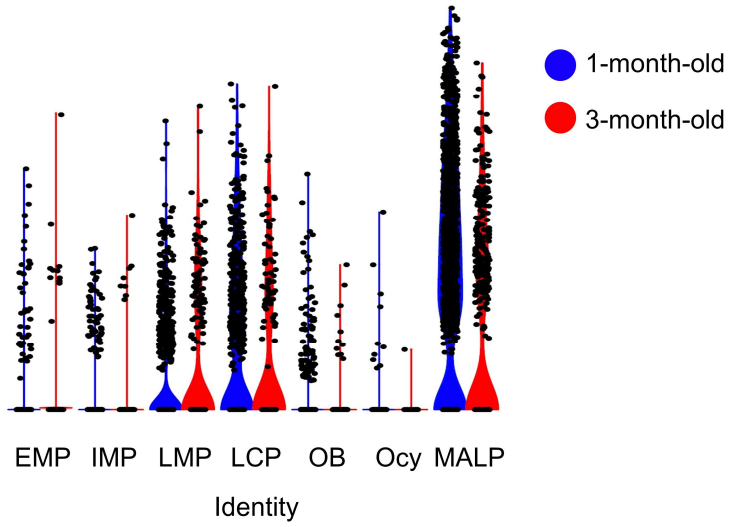

Figure S11. Violin plot of *Tnfsf11* expression in bone marrow mesenchymal lineage cells at 1 and 3 months of age.

EMP: early mesenchymal progenitor; IMP: intermediate mesenchymal progenitor; LMP: late mesenchymal progenitor; OB: osteoblast; Ocy: osteocyte; LCP: lineage committed progenitor.

Figure S12

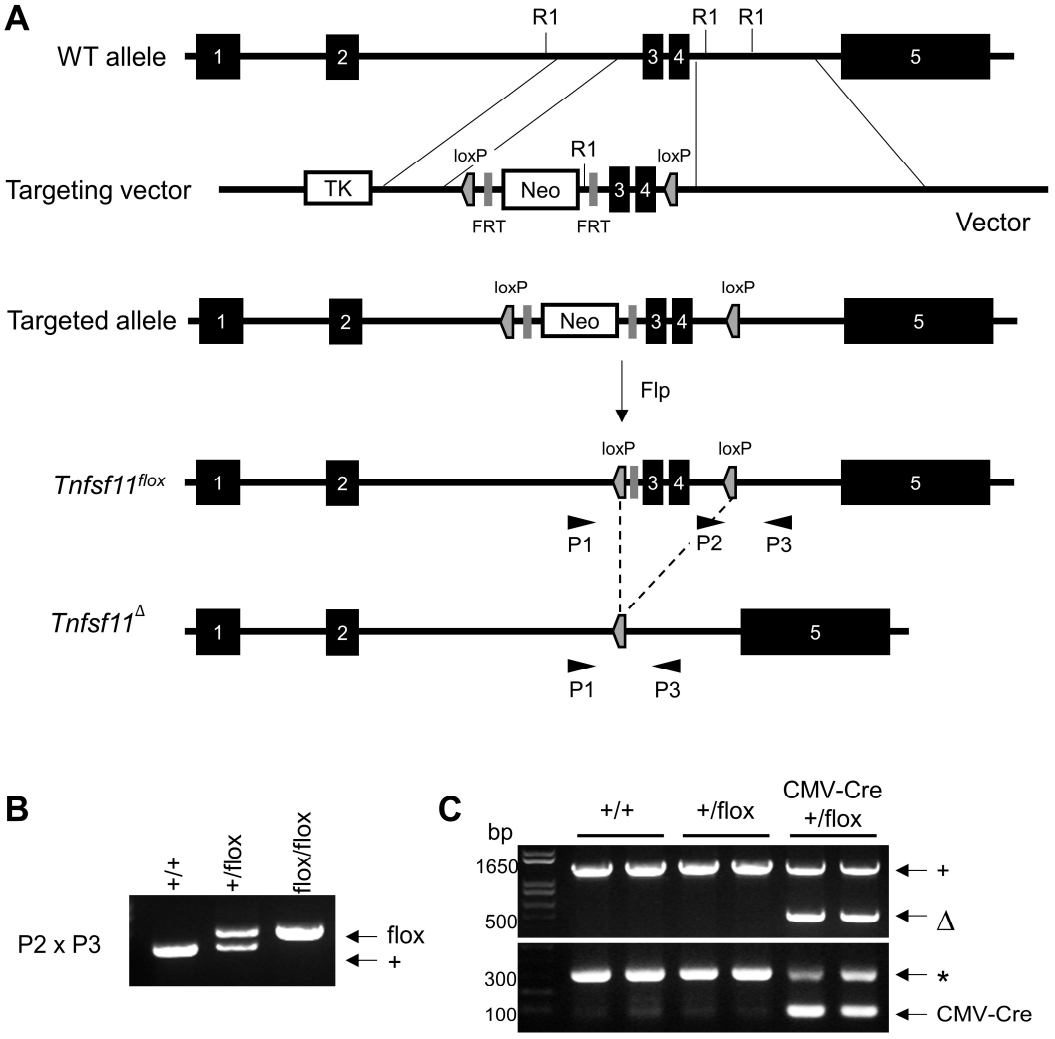

Figure S12. Targeting strategy and conditional deletion of the *Tnfsf1*^{flox} allele.

(A) Schematic diagram of the *loxP*-flanked gene segment generated by homologous recombination. Depicted are the Tyrosine Kinase cassette (TK) and neo gene (Neo).

(B) PCR confirmation of the *Tnfsf1*^{flox} allele using mouse tail DNA. The P2 and P3 primers separate *WT* and *floxed* alleles.

(C) PCR confirmation of Cre-mediated deletion of exons 3 and 4 in *Tnfsf11*. Genotyping of the offsprings of *Tnfsf11*$^{+/flox}$ (CMV-Cre+) mouse crosses were performed using P1 and P3 primers (upper panel) and Cre primers (bottom panel). +, wild type. *, internal positive control.

Table S1. Flow analysis of Td^+ cells in bone marrow hematopoietic cells of *Col2/Td* mice.

Cell type	BM frequency (%)	Td^+ cells (%)	Td^{high} cells (%)
SLAM LSK	0.03±0.01	17.67±3.38	0.31±0.27
MPP	0.19±0.03	13.27±2.01	0.18±0.12
LSK	0.28±0.04	18.30±1.45	0.34±0.13
LKS-	2.83±0.17	10.07±1.16	0.42±0.20
LK	3.15±0.20	11.10±1.11	0.55±0.22
Myeloid cells	37.27±1.37	14.40±1.14	1.17±0.03
B cells	35.60±1.47	5.56±0.16	0.19±0.04
T cells	0.99±0.09	9.28±0.70	0.59±0.02
Megakaryocytes	1.38±0.14	15.67±0.47	2.76±0.61
Erythroid	25.57±1.59	13.97±1.33	1.09±0.05

Bone marrow (BM) frequency of various hematopoietic stem and progenitor cell compartments were assessed by flow cytometry using SLAM marker scheme. Hematopoietic stem cell (SLAM LSK): $Lin^-Sca1^+cKit^+CD48^-CD150^+$; multipotent progenitor (MPP): $Lin^-Sca1^+cKit^+CD48^+CD150^-$. LSK: $Lin^-Sca1^+cKit^+$; LKS^-: $Lin^-Sca1^-cKit^+$; LK: Lin^-cKit^+. Myeloid cells ($Gr1^+/Mac1^+$); B Cells ($B220^+$); T cells ($CD3^+$), Megakaryocytes ($CD41^+$); Erythroid Cells ($Ter119^+$). Td^+ cells % were gated that the controls show less than 0.1% positivity. Td^{high} cells % were gated according to the sorting strategy used in the scRNA-seq experiments (12).

Table S4. Mouse real-time PCR primer sequences used in this study.

Gene	Forward primer	Reverse primer
β-actin	5’-TCCTCCTGAGCGCAAGTACTCT-3’	5’-CGGACTCATCGTACTCCTGCTT-3’
Tnfsf11	5’-GGAAGCGTACCTACAGACTA-3’	5’-TGCTCCCTCCTTTCATCA-3’
Sp7	5’-AGAGGTTCACTCGCTCTGACGA-3’	5’-TTGCTCAAGTGGTCGCTTCTG-3’
Runx2	5’-TAAAGTGACGGACGGTCCC-3’	5’-TGCGCCCTAAATCACTGAGG-3’
Ibsp	5’-ACCAGTTATGGCACCACGACA-3’	5’-TCAACCGTGCTGCTCTTTCTG-3’
Adipoq	5’-AAAGGAGAGCCTGGAGAA-3’	5’-GAATGGGTACATTGGGAACA-3’
Pparg	5’-CCAGCGTGAAGCCAGAGTAG-3’	5’-ACCGTGGCTGTGCTCATCCT-3’
Cebpa	5’-CAAGAACAGCAACGAGTACCG-3’	5’-GTCACTGGTCAACTCCAGCAC
Lpl	5’-GGGAGTTTGGCTCCAGAGTTT-3’	5’-TGTGTCTTCAGGGGTCCTTAG