

Supplementary Materials for
Dynamic transcriptome analysis of osteal macrophages identifies distinct
subset with senescence features in experimental osteoporosis

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15 Supplementary Figures

3 Supplementary Tables

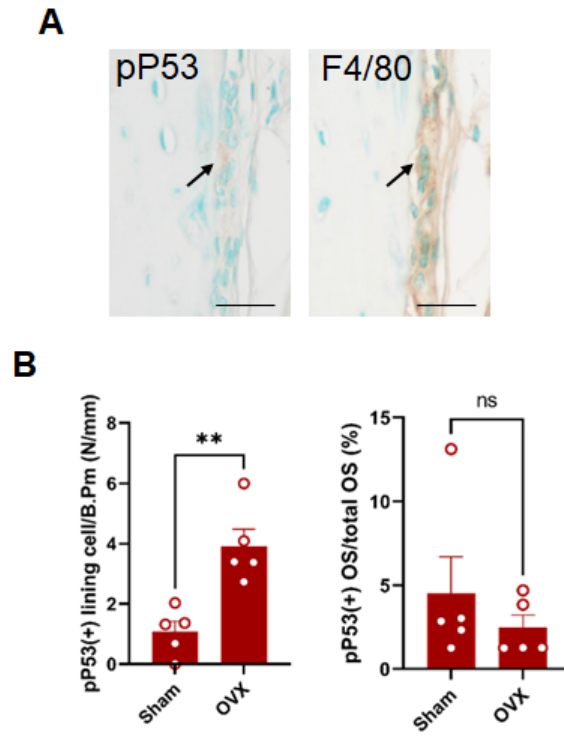


Fig. S2. Detection of pP53 in bone tissue. A) IHC examination for detection of pP53 and F4/80 in femoral bone tissue. Scale bar is 100 μ m. B) Percentage of pP53 stained cells in femoral bone tissue from sham and OVX mice. The positivity was counted in the lining cells and osteocytes of femoral bone tissue. Bars are the mean (n=5) $\pm$ SEM.



Fig. S3. UMAP plots of the scRNA-seq data. Expression of common tissue-resident macrophage markers not bone marrow macrophage or eosinophils in the clusters of Omacs.

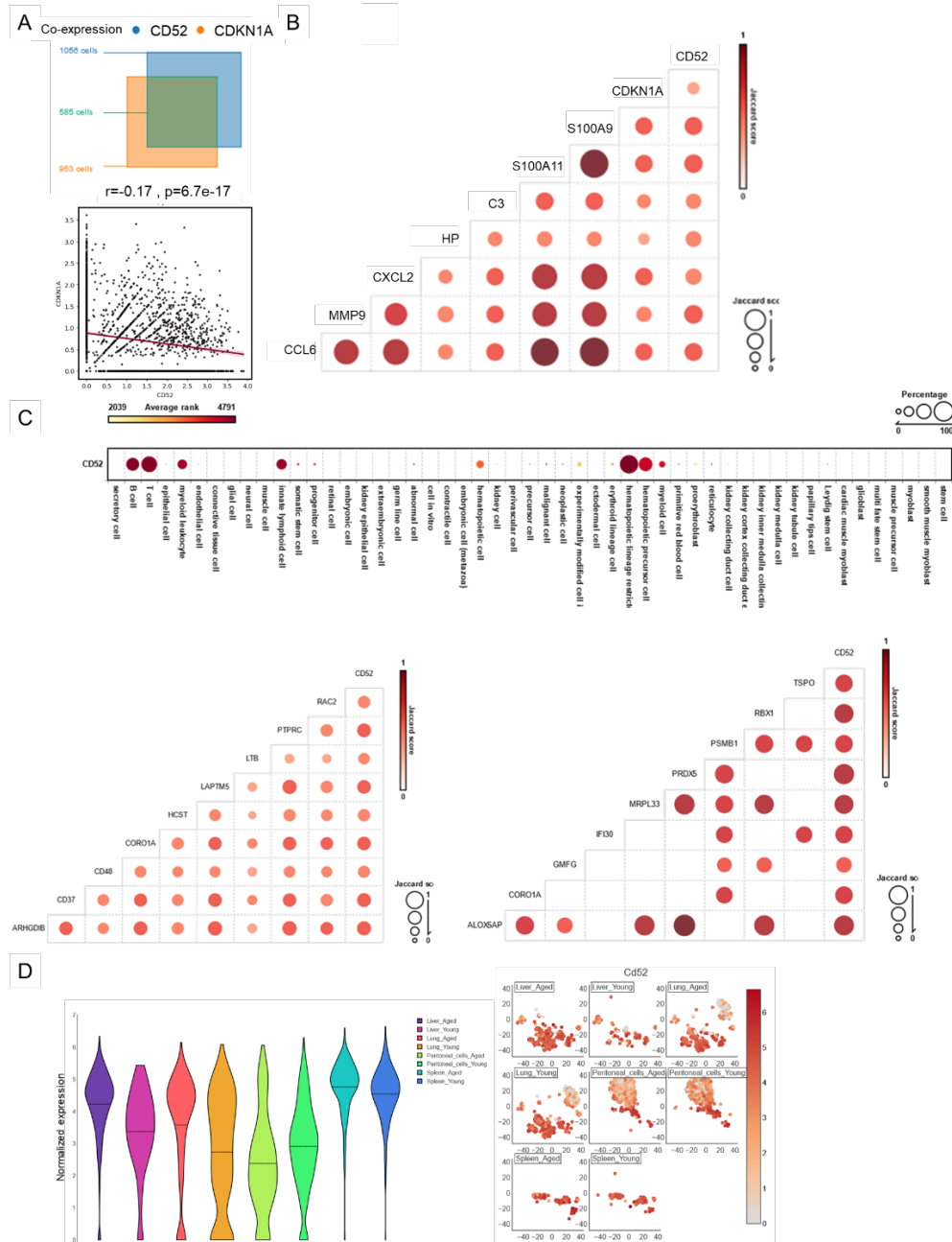


Fig. S6. Molecular characterizations of cells expressing CD52. A) Co-expression of Cd52 with Cdkn1A and analyzed by correlation coefficient test. Jaccard coefficient analysis for the genes set co-expressed in Cdkn1A expressing cells. B) Co-expression analysis of Cd52 and senescence markers in Omics. C). Expression of Cd52 in different murine cells (upper panel) and co-expression analysis (lower panel) from public databases, as right panel for all Cd52 expressing cells and left panel for only myeloid cells. D) Expression of Cd52 in aged myeloid cells from 4 organs shown as violin plot (left panel) and UMAP plot of color-coded cell cluster (right panel).

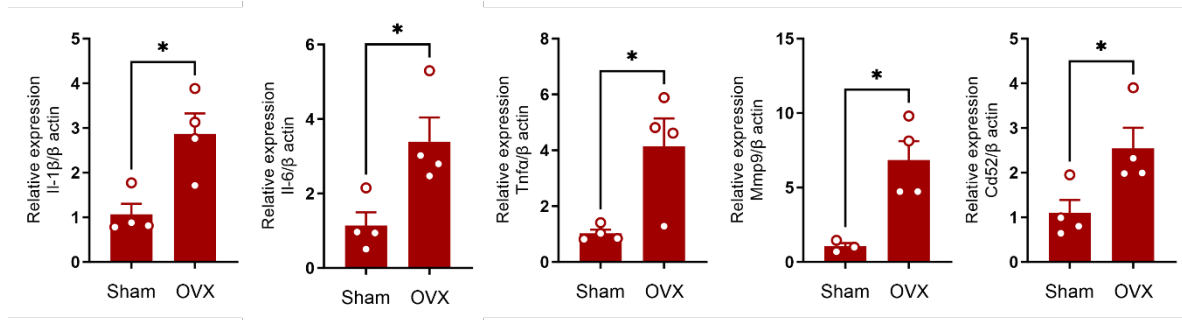


Fig. S7. Gene expression analysis of senescence markers in peritoneal macrophages of sham and OVX mice by qRT-PCR. Results represent relative expression values of each target gene to the expression of β -actin. Results represent means of 4 samples $\pm$ SEM and significant difference was determined by the two-tailed Student's *t* test.

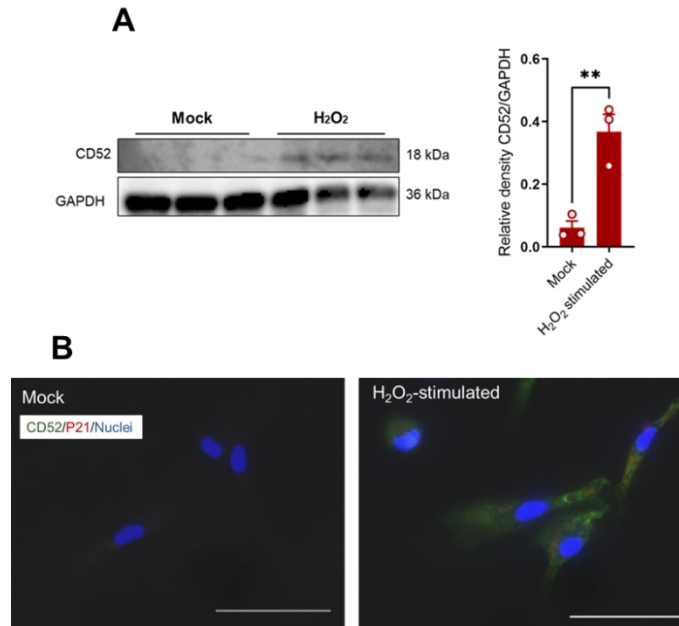


Fig. S8. Expression of CD52 in peritoneal macrophages exposed to H₂O₂-induced oxidative stress. A) Western blotting results of stimulated macrophages. The left panel represents blots analysis with antibodies and right panel represents quantification of band density for each target. Results represent means of 3 samples $\pm$ SEM and significant difference was determined by the two-tailed Student's *t* test. B) Immunofluorescence test of stimulated macrophages as stained by antibodies to CD52 and P21. Scale bars are 50 μ m.

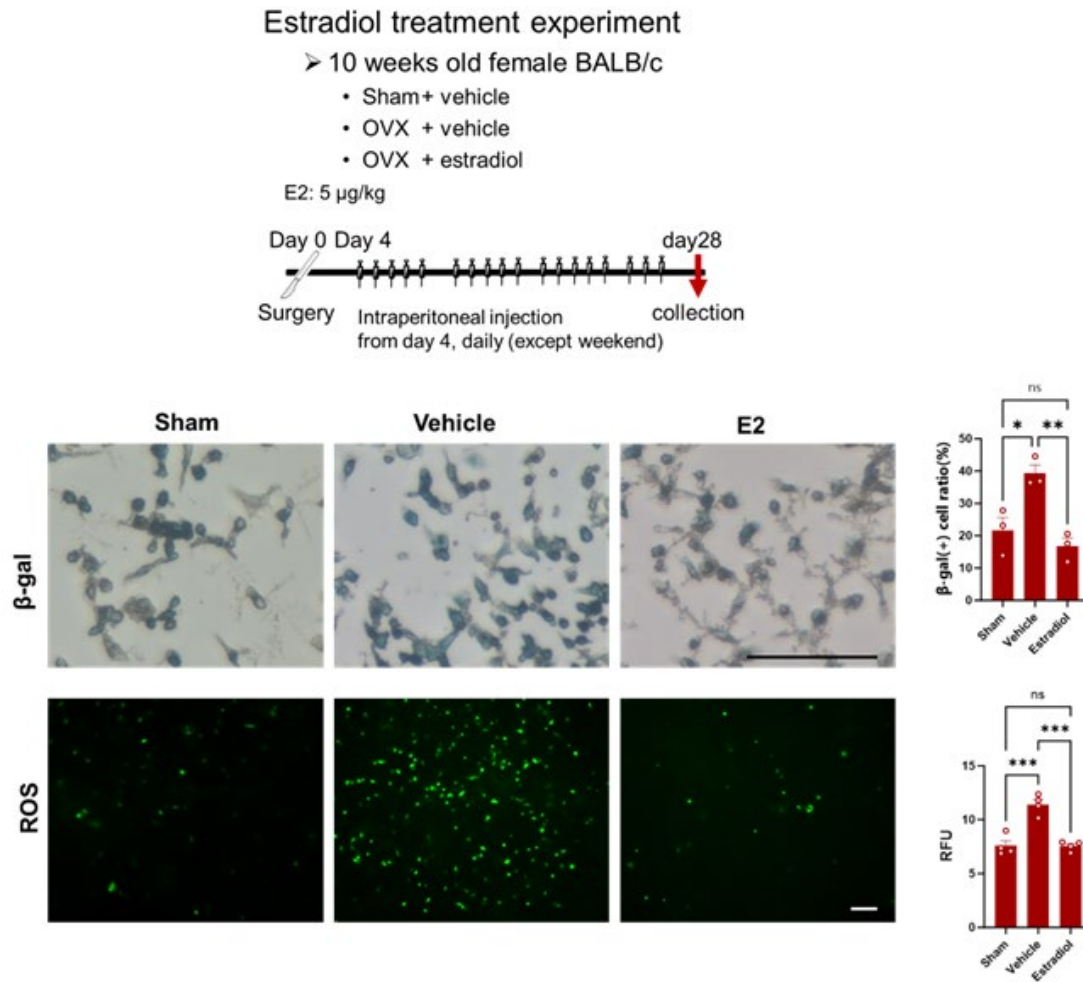


Fig. S9. Effects of estradiol treatment on development of senescent macrophages. Upper panel shows the treatment procedure. Lower panels represent number of positive peritoneal macrophages to β -gal and ROS in OVX mice. The left panels are representative images of stained cells. The right panels show quantification analysis. Scale bars are 100µm. Results represent means of 3 and 4 samples $\pm$ SEM and significant difference was determined by the one-way ANOVA, followed by Tukey's multiple-comparison procedure. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; ns: not significantly different.

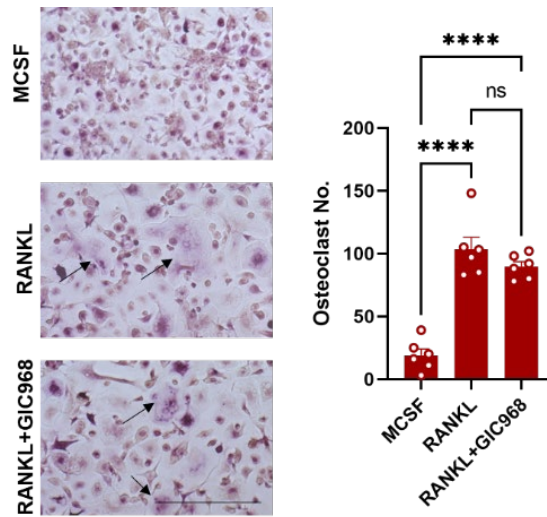


Fig. S10. Effects of GIC968 on the number of differentiated osteoclasts in vitro. Left panels show representative images for each treatment. Right panels show the quantification of TRAP⁺ cells. Scale bar is 100 μ m. Results represent the count of positive cell $\pm$ SEM (n=6). The significant difference was determined by the one-way ANOVA, followed by Tukey's multiple-comparison procedure.

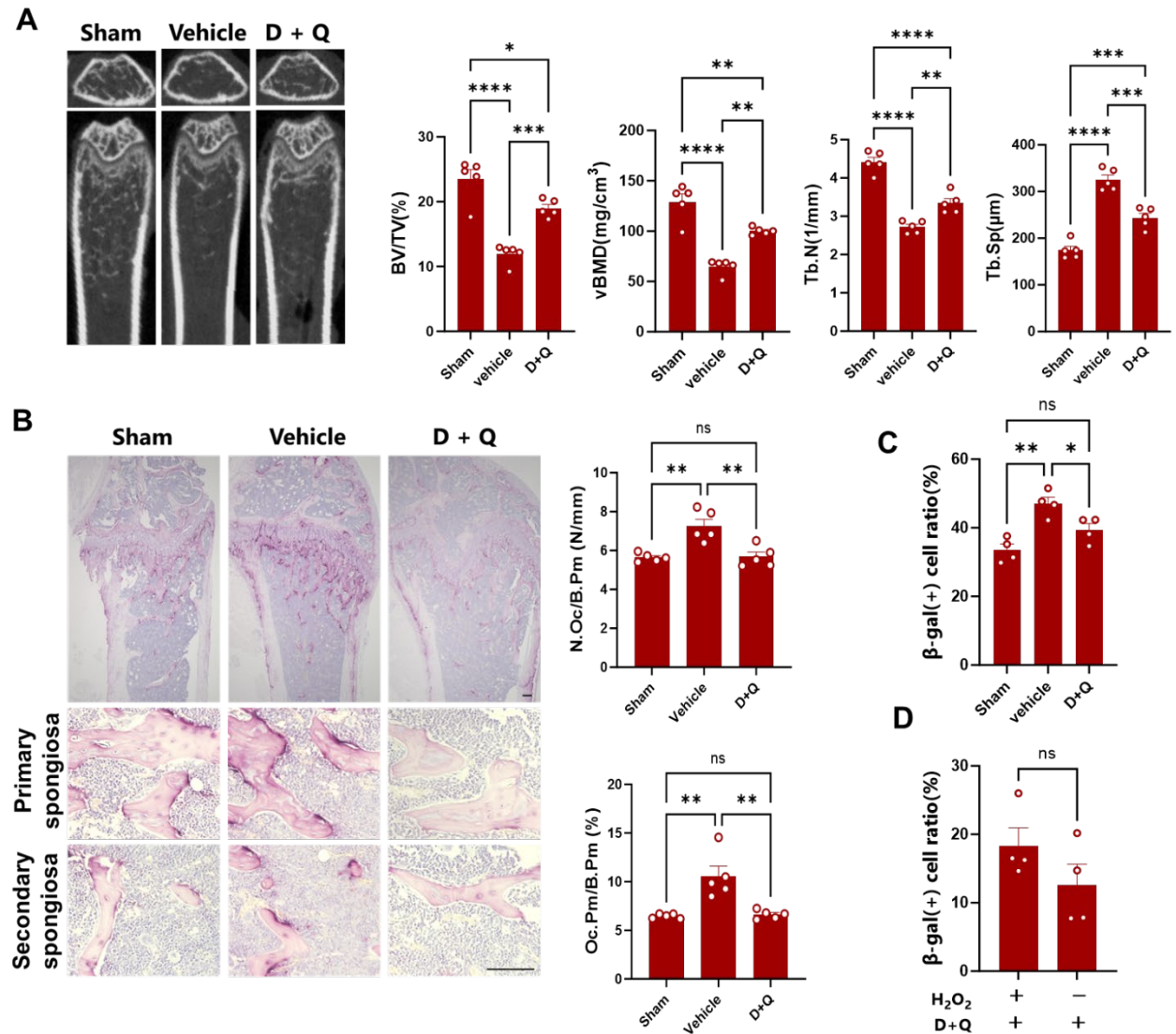


Figure S11. Therapeutic effects of dasatinib and quercetin in experimental osteoporosis.

A) Representative micro-CT images of femoral bones in OVX mice. The right panels show bone parameters including BV/TV, vBMD, Tb.N, and Tb.Sp of femoral bones. B) TRAP-stained sections of femoral bone, including primary and secondary spongiosa. Scale bars are 100 µm. The right panels show quantification of osteoclasts number and sizes on the surface of femoral bone. Results represent means of 5 samples $\pm$ SEM and significant difference was determined by the one-way ANOVA, followed by Tukey's multiple-comparison procedure. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$; ns: not significantly different. C) Percentage of β -gal-positive macrophages collected from peritoneal cavity. D) Percentage of β -gal-positive macrophages stimulated by H₂O₂ with or without D and Q. Results represent means of 4 samples $\pm$ SEM and significant difference was determined by the two-tailed Student's *t* test.

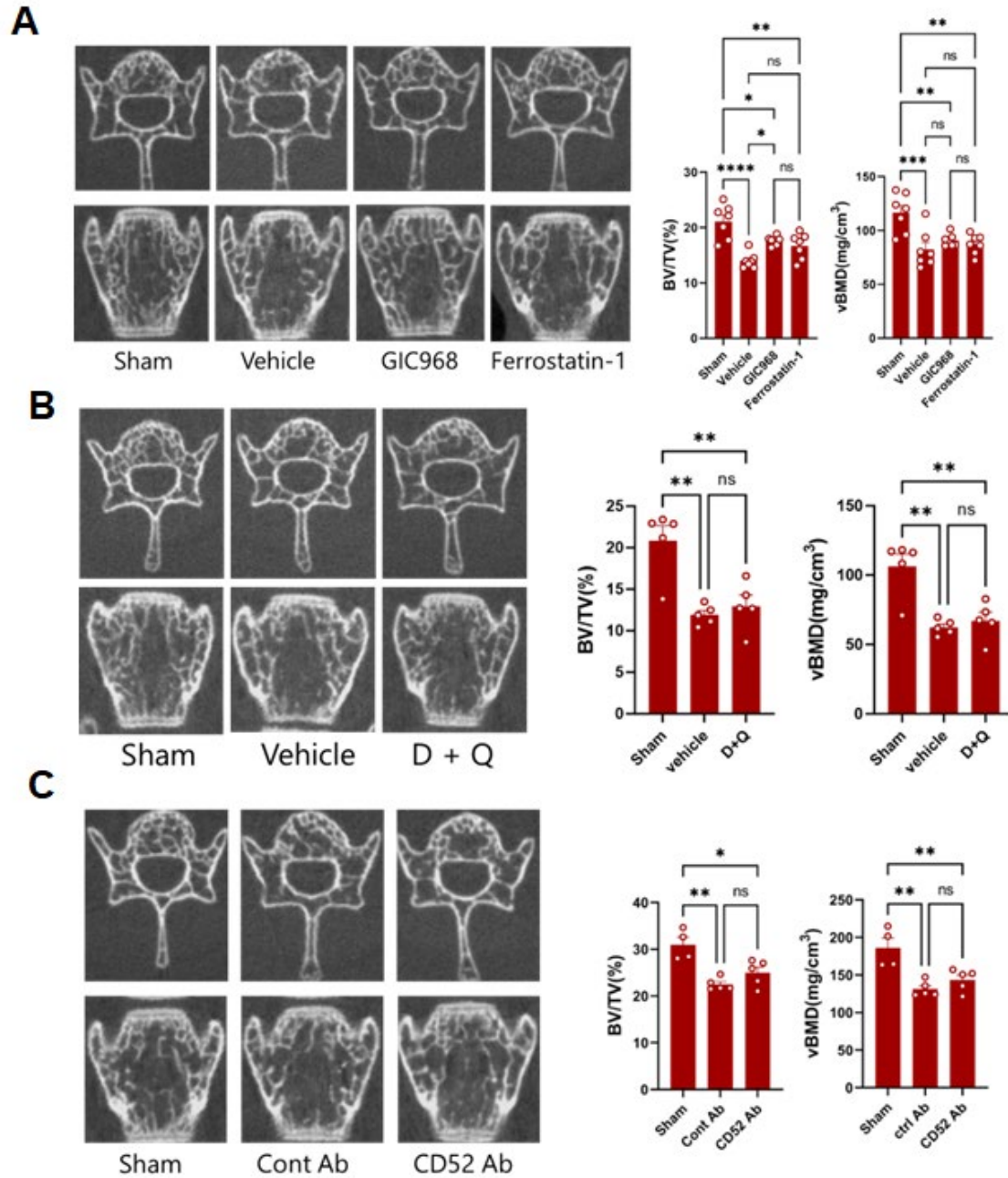
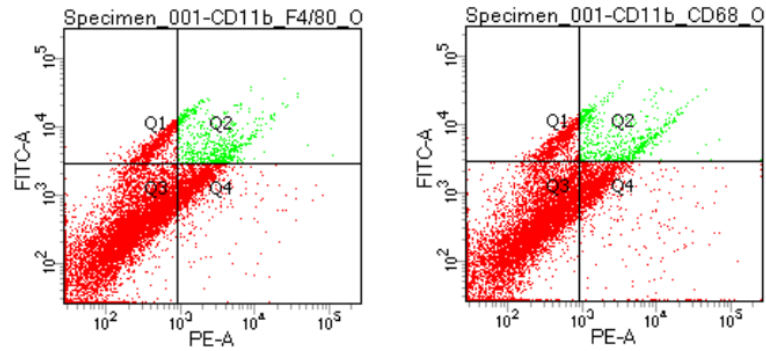


Fig. S12. Beneficial effects of elimination of senescent macrophages in postmenopausal osteoporosis model. A) For treatment with GIC968 and Ferrostatin-1. B) For treatment with dasatinib and quercetin. C) For treatment with CD52 antibody. Left panels are representative micro-CT images of vertebral bones of the mice. Right panels show major bone parameters including BV/TV, and vBMD. Bars are the mean 7 and $5 \pm$ SEM. The significant difference was determined by the one-way ANOVA, followed by Tukey's multiple-comparison procedure.

Sham



OVX

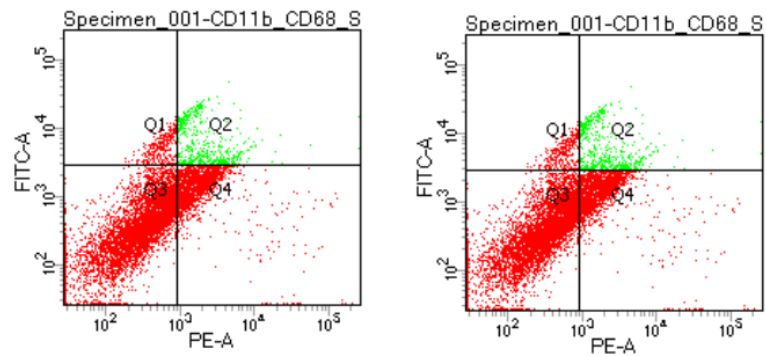


Fig. S13. Gating strategy for separation of osteal macrophages using fluorescence-activated cell sorting targeting CD11b+CD68 or F4/80 positive cells. Cells were isolated and further subjected to RNA-seq analyses.

Table S14. Reagents used for performing scRNA-seq data and analysis.

Hashtag / CITE-seq	Hashtag (Yes/No)	Yes
	CITE-seq (Yes/No)	No
	Antibodies Used:	TotalSeqB 0301, 0302
Single-cell Library Preparation	GEM Kit/PN#	Next GEM Single Cell 3' GEM Kit v3.1/PN-1000123
	Library Kit/PN #	Next GEM Single Cell 3' Library Kit v3.1/PN-1000158
	Gel Beads Kit/PN #	Next GEM Single Cell 3' Gel Bead Kit v3.1/PN-1000129
	Next GEM Chip/PN #	Next GEM Chip G Single Cell Kit/PN-1000120
	Index Kit/PN #	Single Index Kit T Set A/PN-1000213
	Thermal Cycler Instrument	ThermoFisher ProFlex PCR Sytem
	cDNA QC Instrument	Perkin Elmer LabChip
	Library QC Instrument	Perkin Elmer LabChip
	PCR Cycles for cDNA	12
	cDNA Concentration (ng/uL)	1.35
	PCR Cycles for Library	14
	library concentration (ng/uL)	18.3
Sequencing	Sequencer	MGI DNBSEQ-G400
	Flowcell Type	DNBSEQ-G400RS High-throughput Sequencing Set (App-A FCL PE100)
	R1 Sequencing Cycles (cycle)	28
	i7 Index (cycle)	8
	i5 Index (cycle)	-
	R2 Sequencing Cycles (cycle)	100
	Number of reads	435,107,683
Cell Ranger	Cellranger Version	cellranger-6.0.0
	Reference Genome	mm10-2020-A
	Total Number of Cells Recovered	2349
	Mean Reads/Cell	185231
	Median Genes/Cell	1203
	Median UMIs/Cell	4387

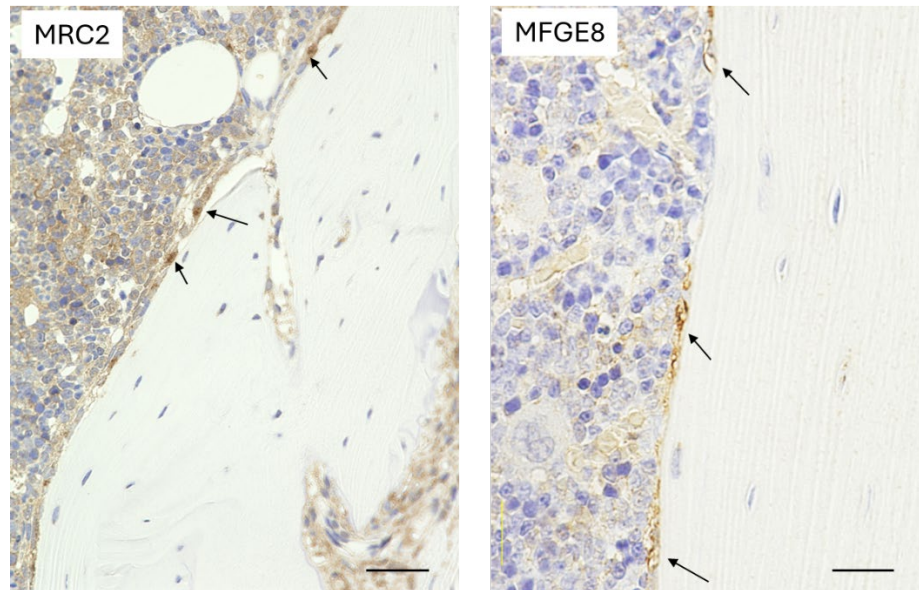


Fig. S15. Detection of Omacs 1 and 2 in bone lining cells using specific antibodies. Bone tissues were stained by MRC2 and MFGE8 antibodies. Arrows indicate positive-stained cells. Scale bars are 50 μm .

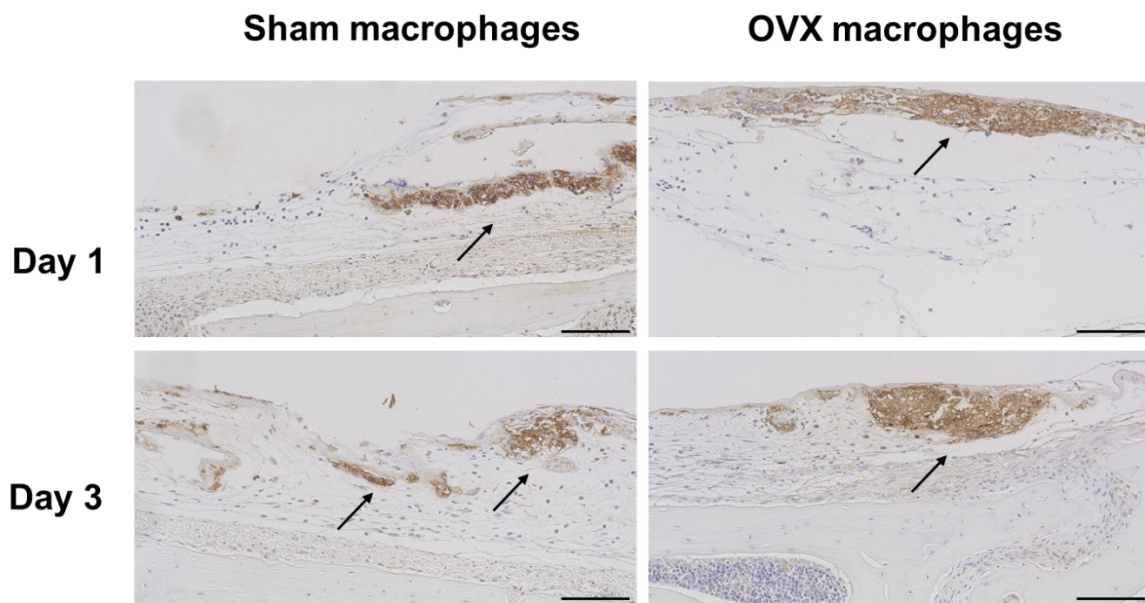


Fig. S16. Detection of macrophages grafted on calvarial bone. GFP-positive cells were stained by immunohistochemistry using specific antibody. Grafted cells were from sham and OVX of GFP-transgenic mice. Sampling was performed on day 1 and day 3 post-grafting. Arrows indicate the positive-stained cells. Scale bars are 100 μm .

Table S17. Antibodies list used in this study.

Antibody	Dilution	Manufacturer	Research Resource Identifier (RRID)
CD11b FITC	1:100	BioLegend	AB_312788
F4/80 PE	1:100	BioLegend	AB_2832546
CD68 PE	1:100	BioLegend	AB_10613469
CD169 FITC	1:100	BioLegend	AB_2563106
MFGE8	1:100	Invitrogen	AB_2855366
MRC2	1:100	EnoGene Biotech	—
GFP	1:1000	Invitrogen	AB_221570
P21	1:1000	GeneTex	AB_2888121
pP21	1:1000	Bioss	AB_11097560
P53	1:1000	GeneTex	AB_1952339
pP53	1:1000	GeneTex	AB_2886801
IL-1β	1:1000	Cell signaling	AB_2715503
β-Galactosidase	1:1000	Invitrogen	AB_2851905
CD52	1:1000	Invitrogen	AB_2901327
GAPDH	1:1000	Bioss	AB_10856675
β actin	1:1000	Cell Signaling	AB_330288
Anti-rabbit HRP	1:3000	BioLegend	AB_2099368
Anti-mouse HRP	1:3000	BioLegend	AB_315009

Table S18. Primer list used in this study.

Target	Forward	Reverse
<i>β-actin</i>	GGCTGTATTCCCCTCCATCG	CCTTCTGACCCATTCCCACC
<i>Il-6</i>	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
<i>Il-1β</i>	TGAAATGCCACCTTTTGACAGTG	AGCCCTTCATCTTTTGGGGTC
<i>Tnf-α</i>	AGCCGATGGGTTGTACCTT	ATAGCAAATCGGCTGACG
<i>Mmp-9</i>	GCCAGCCGACTTTTGTGGTC	GTGTCCGTGAGGTTGGAGGT
<i>Cd52</i>	CTTGGGACAAGCCACTACGG	TGGATGAGGCCCCCACTCTTT