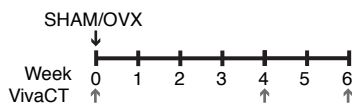
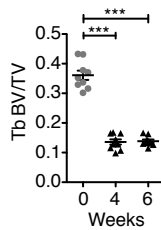
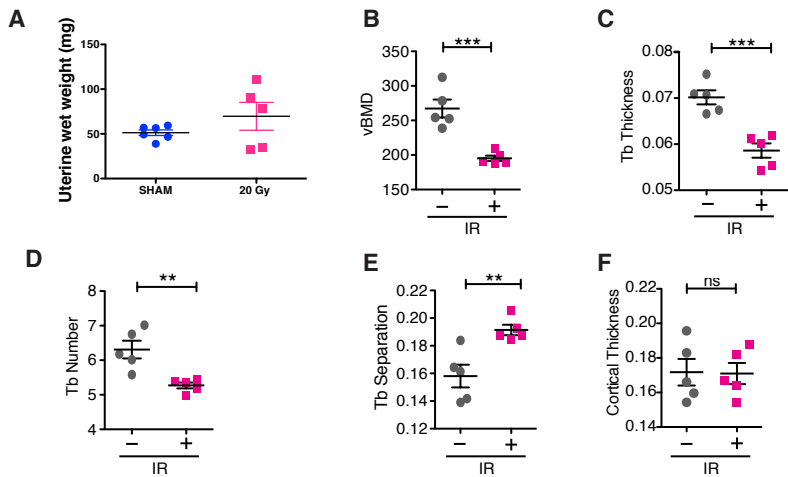
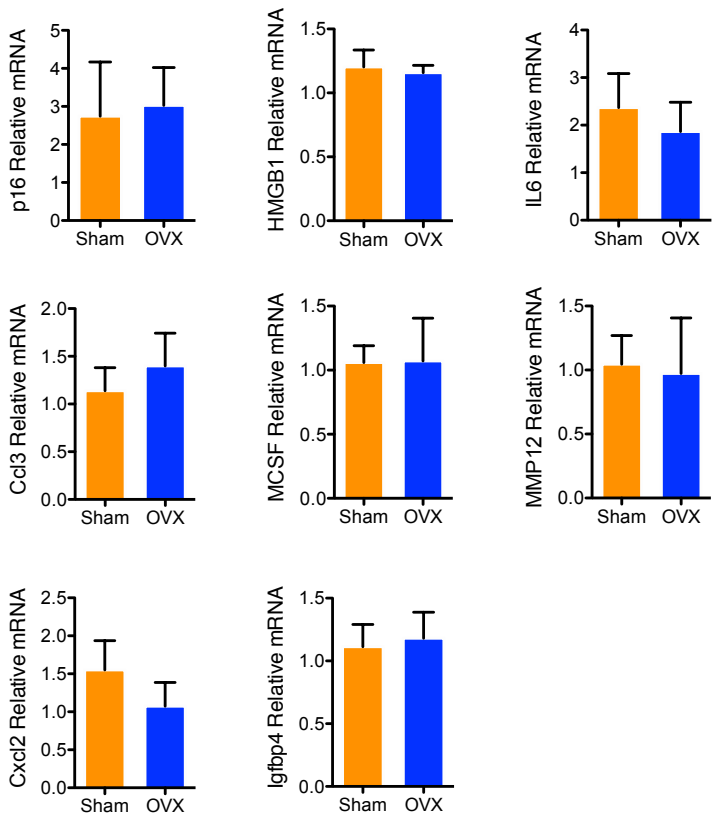
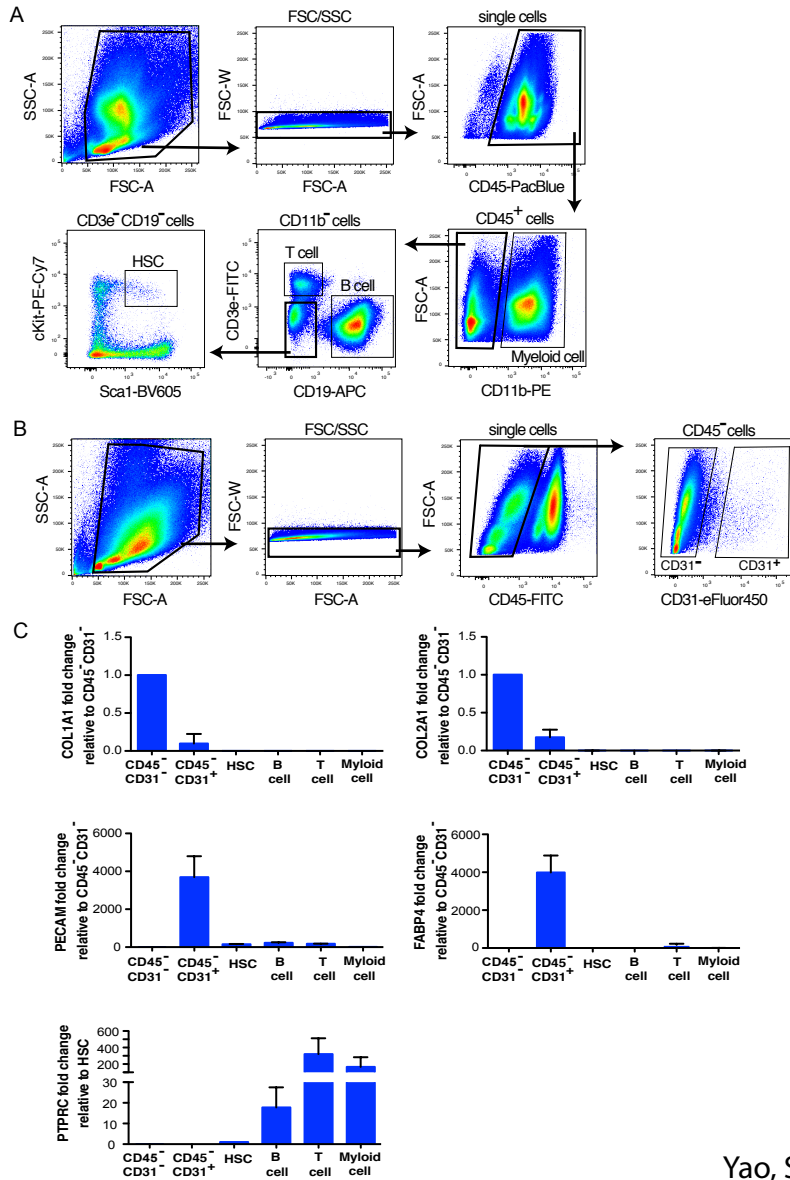
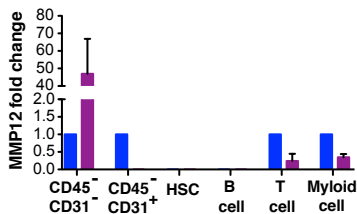
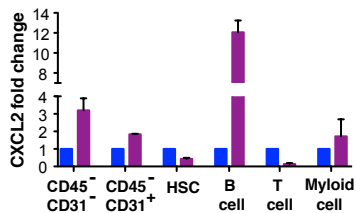
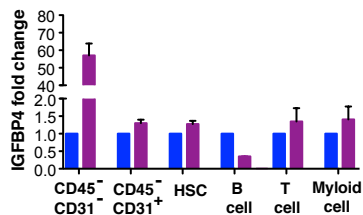
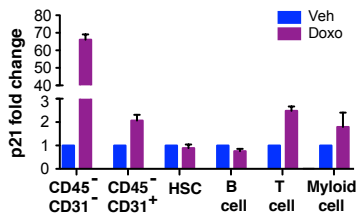
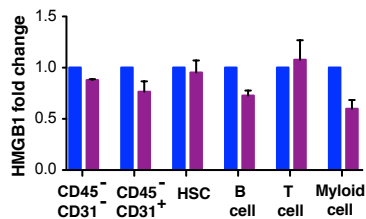


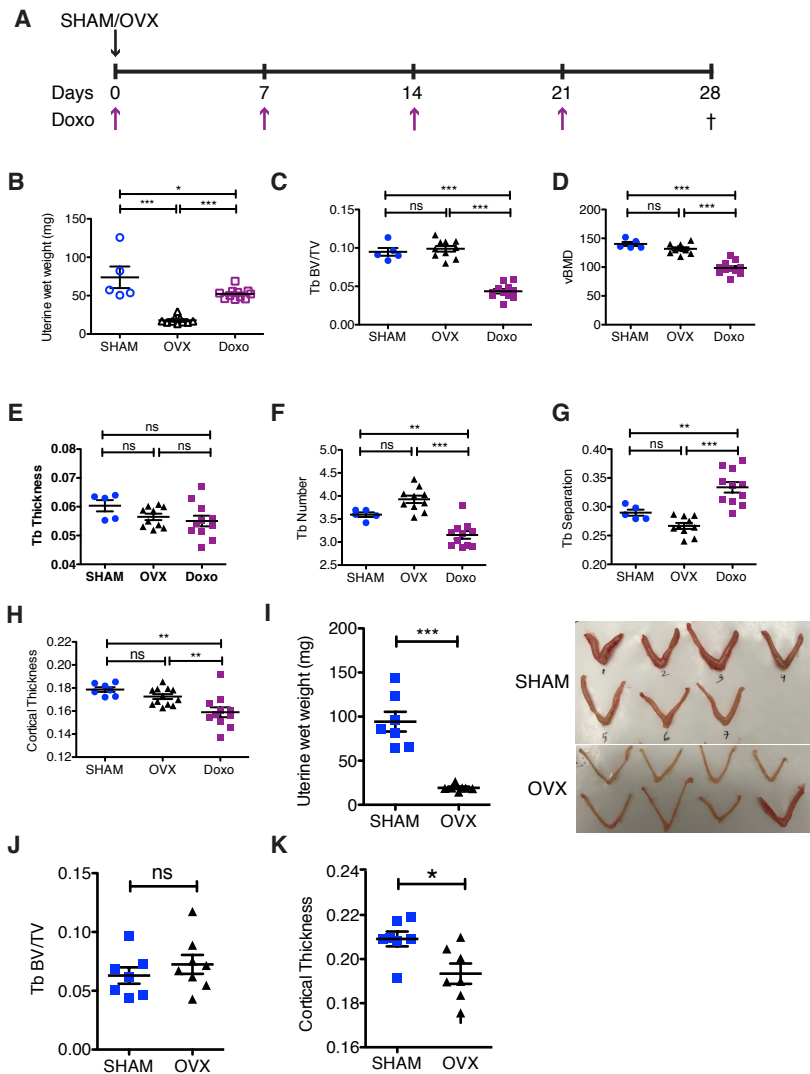
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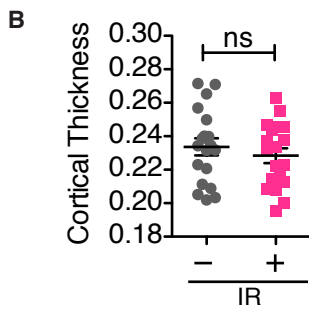
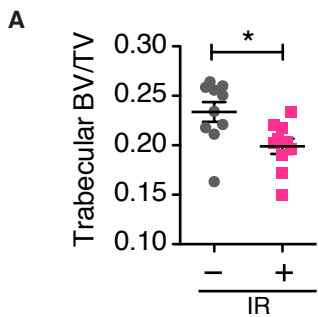


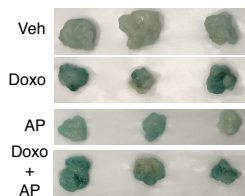
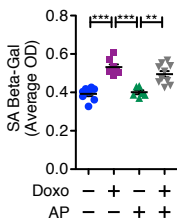


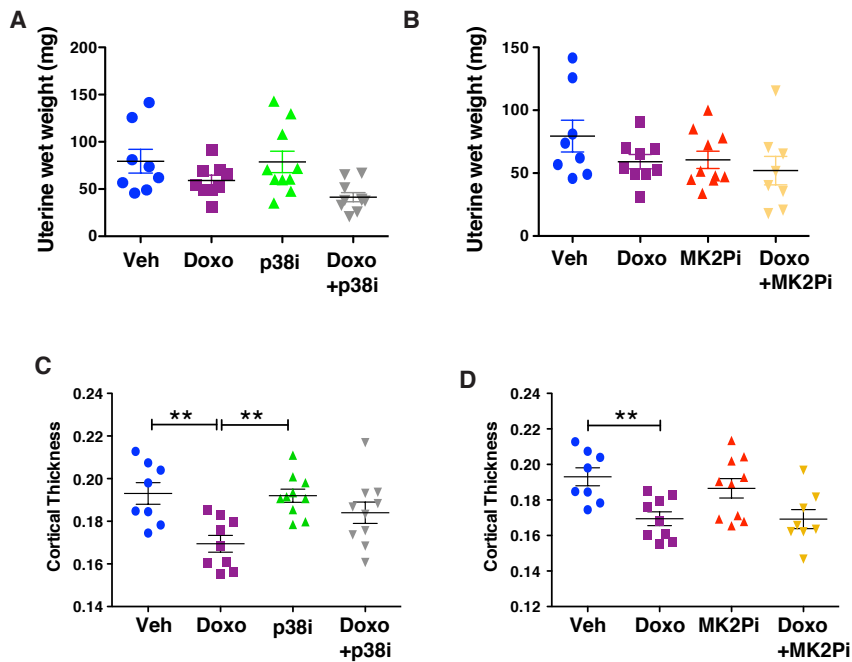








A**B**



Supplementary Figure Legends S1-S11

Supplementary Figure 1: Doxorubicin induces bone loss in wild-type, female 6-week old FVB/NJ or C57BL/6 mice. (A-D) FVB/NJ Mice were treated with 4 once weekly doses of doxorubicin (Doxo; 5mg /kg) or vehicle (Veh) and subject to SHAM (-) surgery or ovariectomy (OVX) (+) and bone measurements carried out by μ CT including: (A) trabecular (Tb) thickness, (B) volumetric bone mineral density (vBMD), (C) Tb separation, and (D) Tb number. $N \geq 4$ per group. (E) 6-week old C57BL/6 mice were treated with 4 cycles of weekly doses of Veh or Doxo (5mg /kg). Trabecular bone parameter presented as trabecular (Tb) bone volume (BV) to trabecular volume (Tb BV/TV). $N=5$ per group. Data are presented as mean \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns=not significant. Comparisons are indicated by capped lines.

Supplementary Figure 2: Paclitaxel induces bone loss in wild-type, female 6-week old C57BL/6 mice. C57BL/6 female mice were treated with Paclitaxel (PTX; 10 mg/kg) and bone measurements were performed by μ CT. (A) Tb number (TB. N), (B) Tb spacing (Sp), (C) Tb thickness (Th), (D) volumetric bone mineral density (vBMD), and (E) Uterine wet weights. $N=8$ per group. Data are presented as mean \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Supplementary Figure 3: Bone loss stabilizes in OVX mice after 4 weeks. (A) Schematic of experimental timeline for VivaCT scanning. (B) Trabecular (Tb) bone volume to trabecular volume (BV/TV) of femurs over the course of the experiment. Dagger indicates time of sacrifice. Baseline measurement (Week 0) was taken before SHAM/OVX

surgery and then 4 or 6 weeks after surgery in the same mice. N=10 per group. Data are presented as mean±SEM. ***p≤0.001.

Supplementary Figure 4: Single-limb irradiation of 6-week old mice induces bone loss in irradiated limb only. 6-week old FVB/NJ mice were shielded and the right hind limb was exposed to 20 Gy radiation. Bone parameters were measured by μ CT. **(A)** Uterine wet weights at endpoint for irradiated mice compared to mice of identical age that underwent SHAM surgery. **(B)** Volumetric bone mineral density (vBMD); **(C)** trabecular (Tb) thickness, **(D)** number, **(E)** separation; and **(F)** cortical thickness was measured. N=5 per group. All data are presented as mean±SEM. **p≤0.01, ***p≤0.001, ns=not significant.

Supplementary Figure 5: SASP factors are not increased in the bone of mice following OVX. 16-week old C57BL/6 mice were subject to SHAM surgery or ovariectomy (OVX). Five weeks post-surgery, legs were isolated, marrow was removed, and bones were crushed and used to make mRNA from bone-resident cells. Expression of p16, HGMB1, IL6, CCL3, MCSF, MMP12, CXCL2, and Igfbp4 mRNA levels in crushed tibias was measured by qRT-PCR. N=5 per group. All RT-PCR data are presented as mean±SD.

Supplementary Figure 6: Flow cytometric gating strategy for isolating bone resident cells/hematopoietic cells and validation of cell populations. 6-week old C57BL/6 mice were received vehicle (Veh) or 25 mg/kg doxorubicin (Doxo). 48 h later,

legs were digested and sorted into different populations of myeloid cells, T cells, B cells, HSCs, (A) and CD45⁻CD31⁻, CD45⁻CD31⁺ (B) as indicated in the gating strategy. (C) qRT-PCR analysis of Col1A, Col2A, PECAM, FABP4, and PTPRC (CD45) in the sorted cells from the bones of mice treated with vehicle showing that expected populations were successful isolated. N=2. All RT-PCR data are presented as mean±SD.

Supplementary Figure 7: SASP factors are increased in the isolated CD45⁻CD31⁻ cells following chemotherapy. 6-week old C57BL/6 mice were received vehicle (Veh) or 25 mg/kg doxorubicin (Doxo). Expression of HMGB1, p21 and SASPs (IGFBP4, CXCL2, and MMP12) mRNA levels in each of the indicated sorted cell population were measured by qRT-PCR. N=2. All RT-PCR data are presented as mean±SD.

Supplementary Figure 8: Bone loss in C57BL/6, 16-week old mice is induced by chemotherapy but not OVX. (A) Schematic of experimental timeline showing dosing regimen for doxorubicin (Doxo; 5 mg/kg) or after subjecting mice to sham or OVX surgery. Dagger indicates time of sacrifice. (B) Uterine wet weights at endpoint. Femurs were isolated from the indicated groups and subjected to μ CT analysis. Shown are data for (C) trabecular (Tb) bone volume (BV) compared to trabecular volume (TV) (Tb BV/TV); (D) volumetric bone mineral density (vBMD); (E) Tb thickness, (F) number, and (G) separation; and (H) cortical thickness at endpoint. N \geq 5 per group. A second set of 16-week C57BL/6 mice was subjected to sham or OVX surgery and allowed to recover for 12 additional weeks before assessing uterine weight and bone parameters. (I) Uterine wet weights at endpoint, 12 weeks post-surgery. Left: quantification. Right:

Representative images. Femurs were isolated from the indicated groups and subjected to μ CT analysis. Shown are data for **(J)** Tb BV/TV and **(K)** cortical thickness of femurs at endpoint, 12 weeks post-surgery. $N \geq 7$ per group. All data are presented as mean \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns=not significant.

Supplementary Figure 9: Bone loss in 16-week old C57BL/6 mice following single-limb irradiation. The right hind leg of 16-week C57BL/6 mice was subject to 20 Gy irradiation (IR, +) and compared to the contralateral leg (-) by μ CT. Shown are data for **(A)** trabecular (Tb) bone volume (BV) compared to trabecular volume (TV) (Tb BV/TV) and **(B)** cortical thickness. $N=10$ per group. All data are presented as mean \pm SEM. * $p \leq 0.05$, ns=not significant.

Supplementary Figure 10: SA- β -Gal staining of visceral fat in INKATTAC mice treated with chemotherapy. 16-week-old C57BL/6 mice were treated with 4 weekly doses of doxorubicin (Doxo; 5 mg/kg) and 2 weekly doses of AP20187 (AP; 2 mg/kg) vehicle. **(A)** Visceral fat was removed at endpoint and stained with SA- β -Gal. **(B)** Quantification of SA- β -Gal staining shown in A. $N \geq 6$ per group. Data are presented as mean \pm SEM. ** $p \leq 0.001$, *** $p \leq 0.0001$.

Supplementary Figure 11: MK2 or p38MAPK inhibition (MK2Pi and p38i, respectively) protects against senescence-induced bone loss. Sixteen-week old C57BL/6 mice were treated with 4 once weekly doses of vehicle (Veh) or doxorubicin (Doxo; 5 mg/kg) and provided *ad libitum* chow compounded with either CDD-111 (p38i)

or CDD-450 (MK2Pi). **(A)** Uterine wet weight for Veh and Doxo groups +/- p38i. **(B)** Uterine wet weight for Veh and Doxo groups +/- MK2Pi. **(C)** Cortical thickness as measured by μ CT for Veh and Doxo groups +/- p38i. **(D)** Cortical thickness as measured by μ CT for Veh and Doxo groups +/- MK2Pi. * $p \leq 0.05$, ** $p \leq 0.01$.