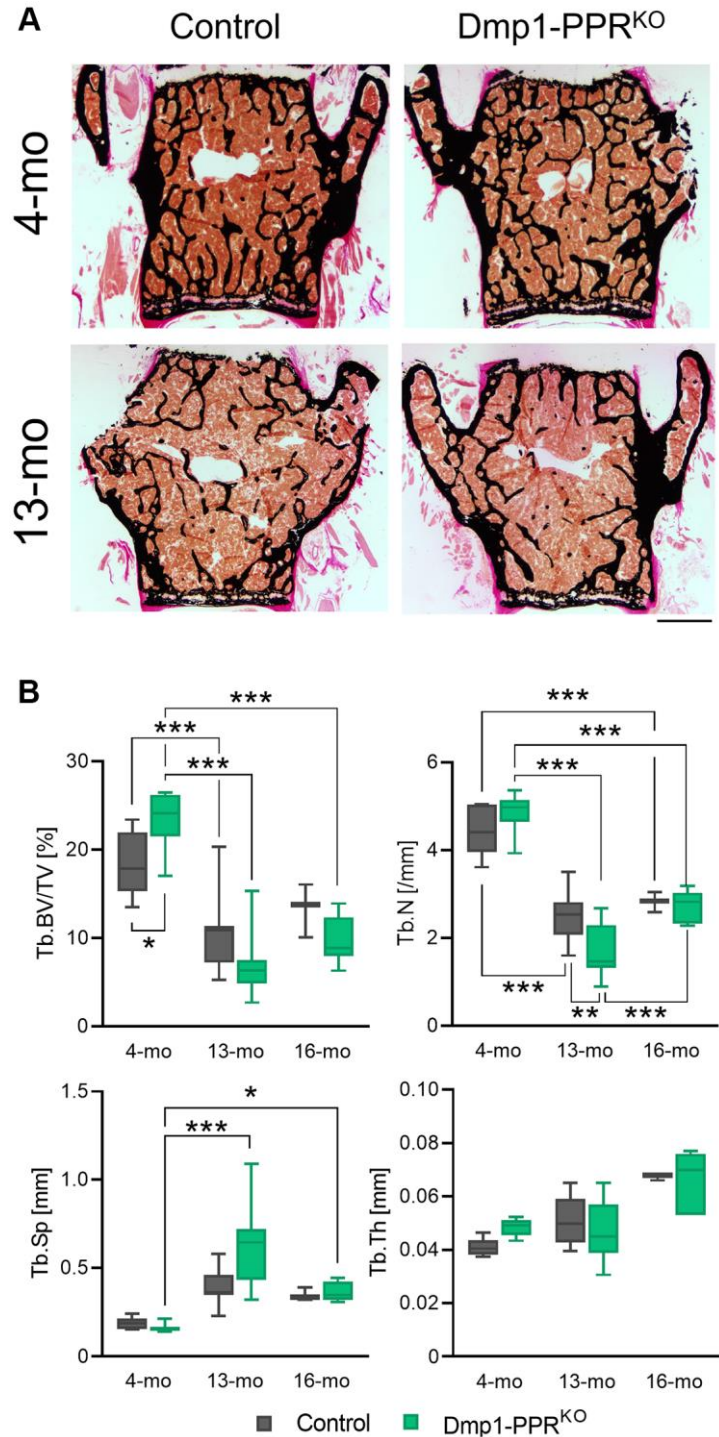
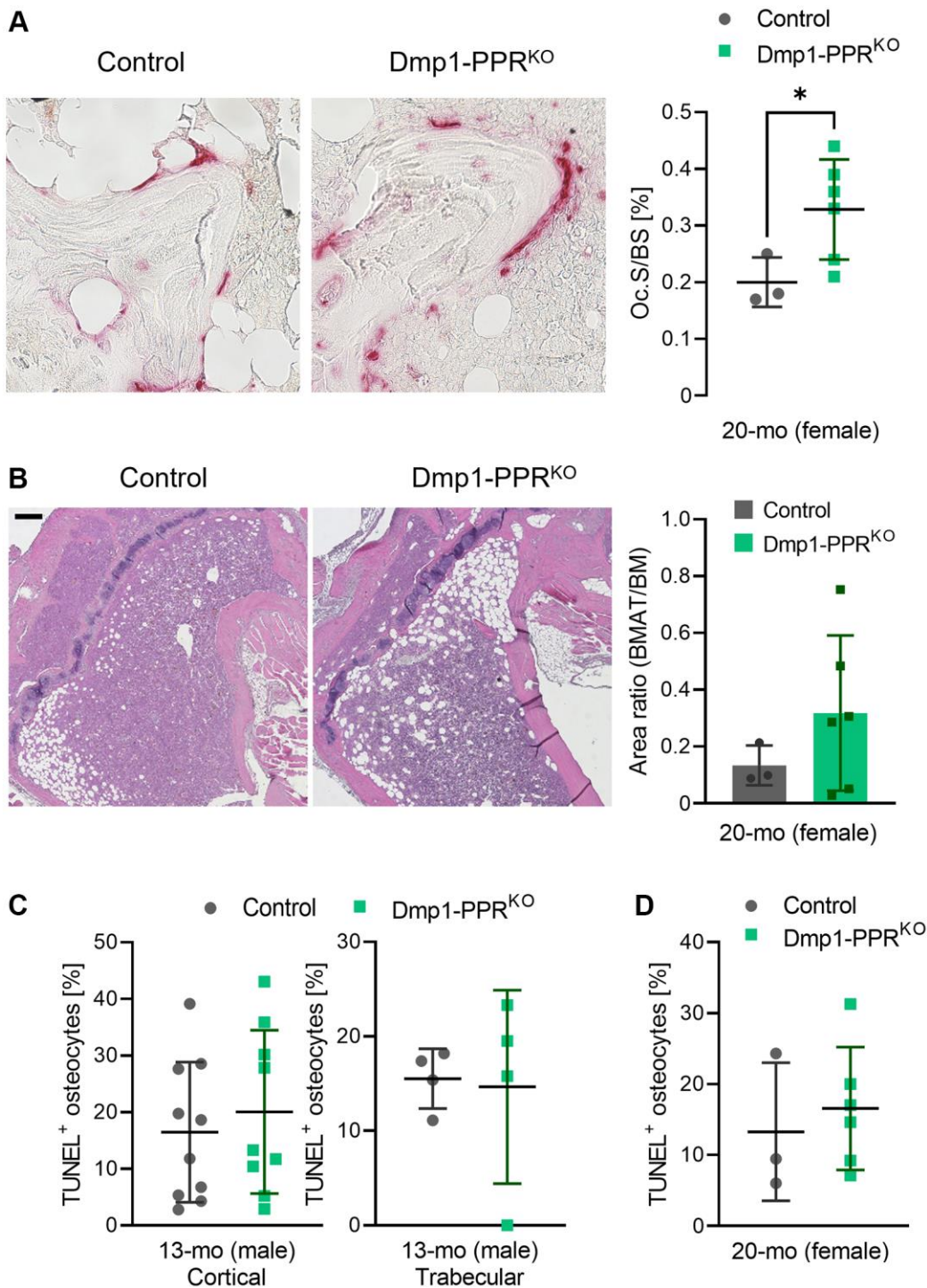


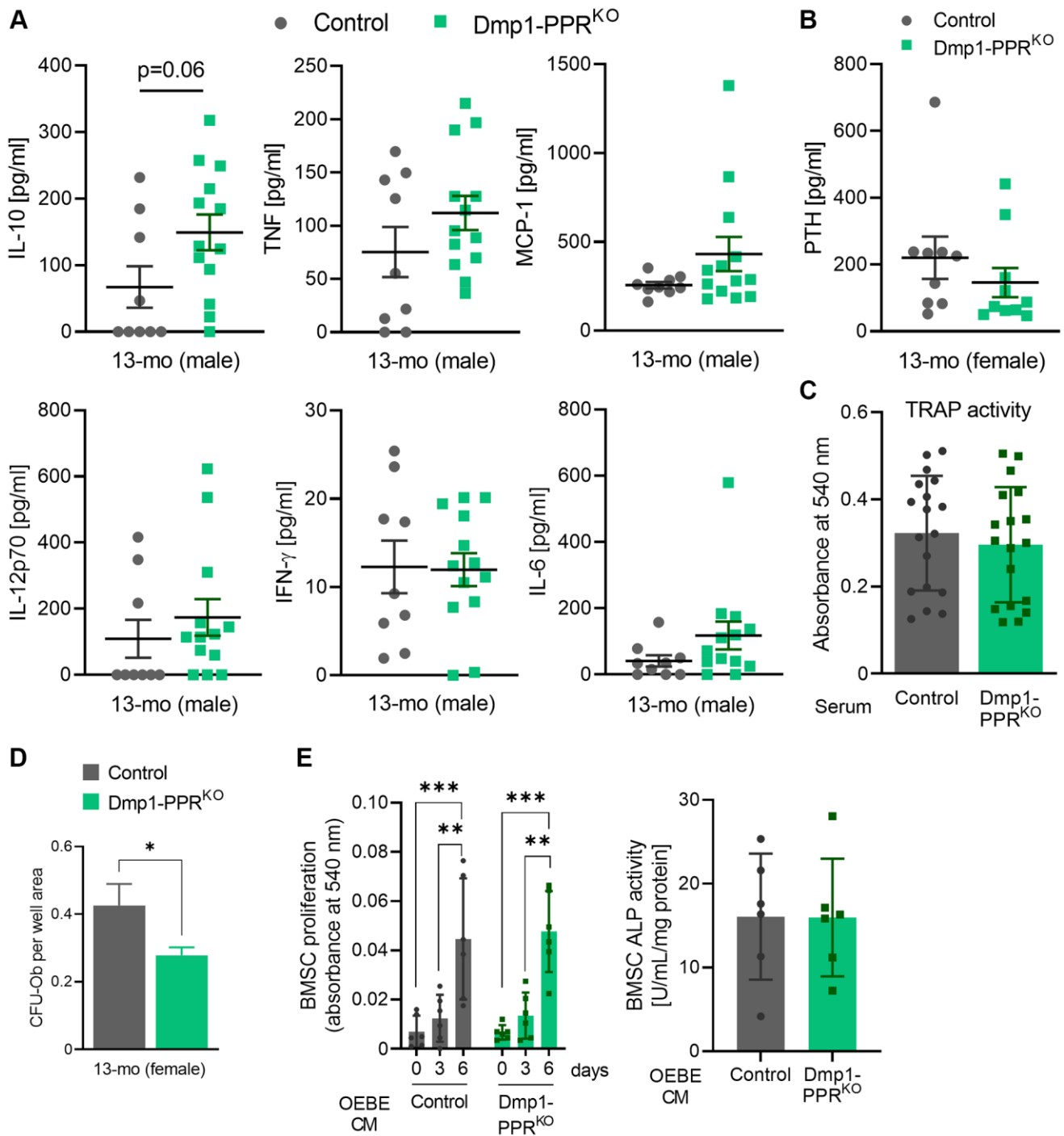
SUPPLEMENTARY FIGURES



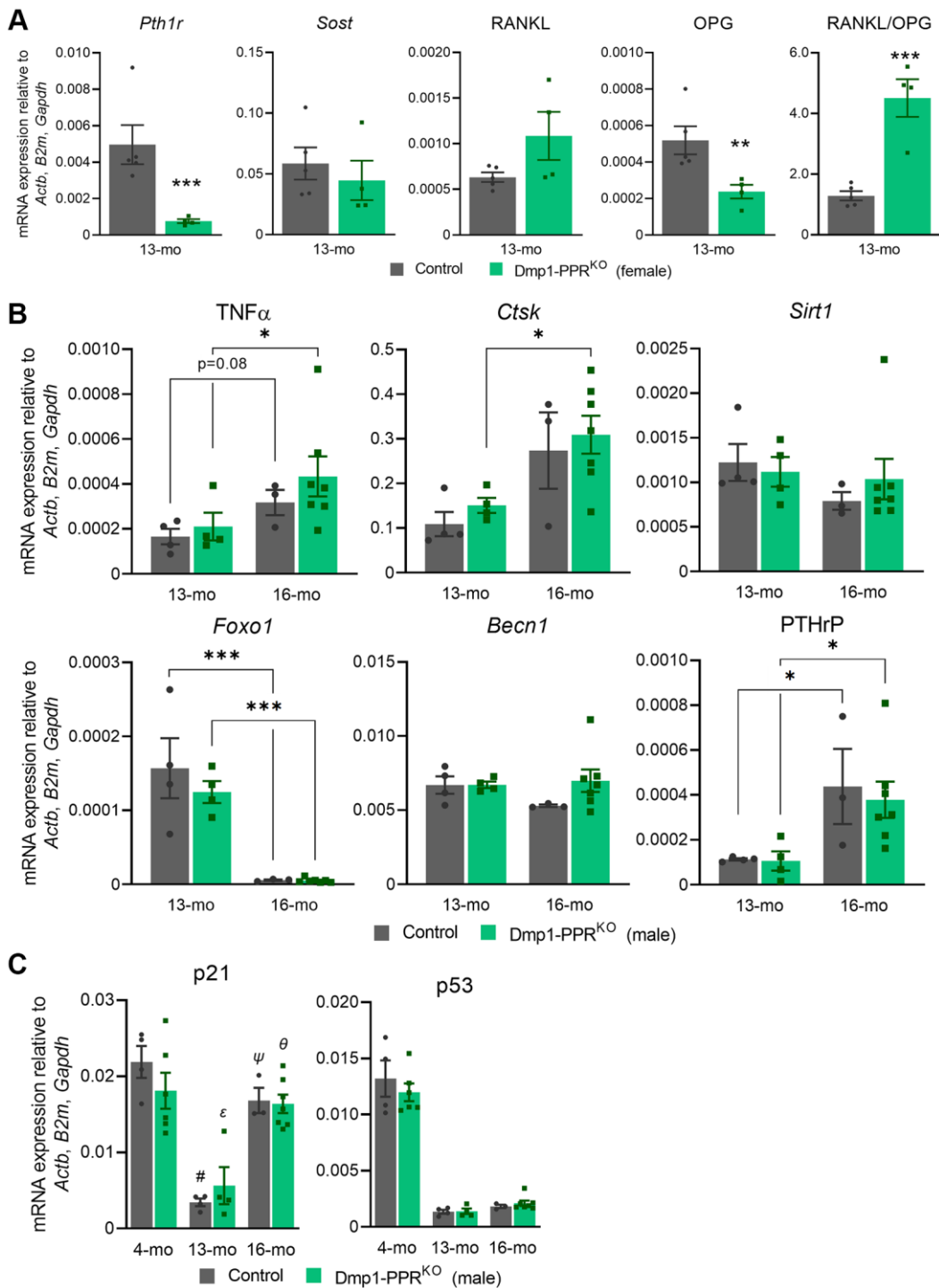
Supplementary Figure 1. Analysis of the skeletal phenotype of Dmp1-PPR^{KO} mice. (A) Representative images of Von Kossa staining on the L5 vertebrae of 4- and 13-month-old male control and Dmp1-PPR^{KO} mice. (B) Skeletal parameters of the distal femora analyzed by μ CT were compared among male control and Dmp1-PPR^{KO} animals at different ages (4, 13, or 16 months of age). Data is shown as box and whisker plot. $N = 7-15$ per group. Two-way ANOVA with Tukey's *post hoc* test was performed. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



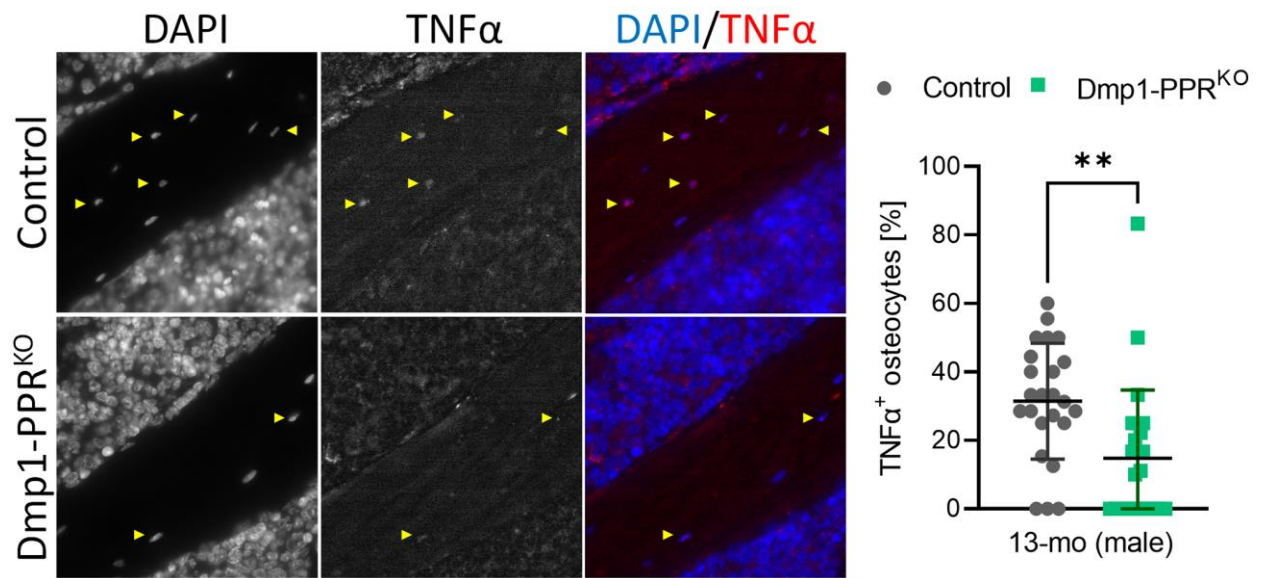
Supplementary Figure 2. (A) Representative images of TRAP staining and quantification of Oc.S/BS on the proximal tibiae (trabecular compartment) of female control and Dmp1-PPR^{KO} mice at 20 months of age are shown. *N* = 3–6 per group. (B) Representative images of H&E staining and quantification of BMAT/BM ratio on the proximal tibiae of female control and Dmp1-PPR^{KO} mice at 20 months old are shown. *N* = 3–6 per group. (C, D) TUNEL analysis on the tibiae from aging control and Dmp1-PPR^{KO} mice. (C) Quantification of TUNEL+ osteocytes in the tibiae of 13-month-old male control and Dmp1-PPR^{KO} mice is shown. Analysis was performed on the cortical region (left, midshaft) and the trabecular region (right, proximal) of the tibiae. *N* = 4–10 per group. (D) Quantification of TUNEL+ osteocytes in the tibiae (cortical region) of 20-month-old female control and Dmp1-PPR^{KO} mice is shown. *N* = 3–6 per group. Data are presented as mean ± SD. Unpaired student's or Welch's *t* test was performed. **p* < 0.05.



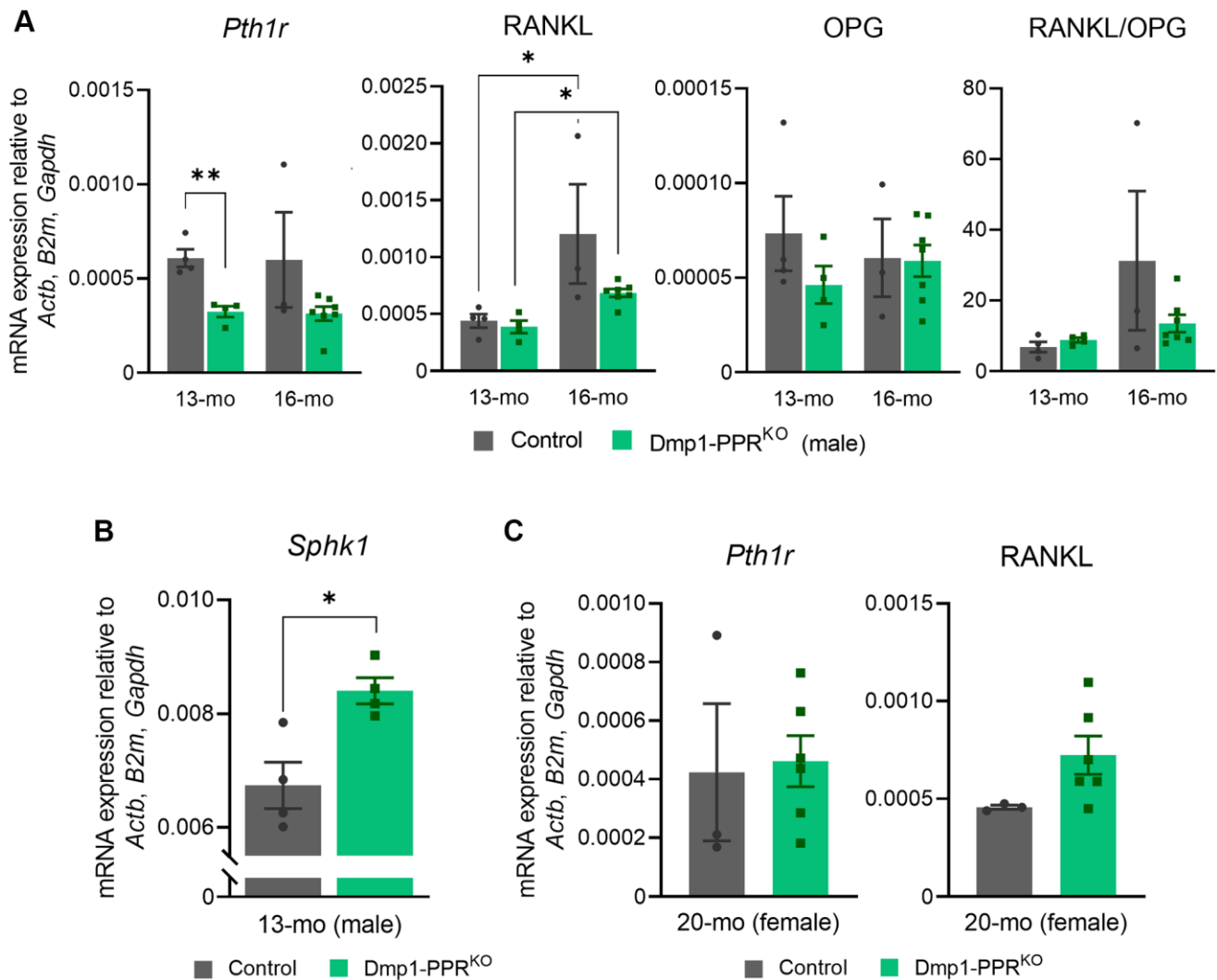
Supplementary Figure 3. (A) Serum cytokines in 13-month-old Dmp1-PPR^{KO} mice were analyzed. Quantification of serum cytokines of 13-month-old male control and Dmp1-PPR^{KO} mice is shown. Data are expressed as mean \pm SEM. $N = 7$ –13 per group. (B) Serum PTH levels in 13-month-old female mice were analyzed by ELISA. Means \pm SEM are shown. (C) TRAP activity was measured in the conditioned medium from BMSCs under osteoclastic differentiation in the presence of control or Dmp1-PPR^{KO} serum. Means \pm SD are shown. (D) CFU-Ob assay was performed on BMSCs isolated from female control and Dmp1-PPR^{KO} mice at 13 months of old is shown. Means \pm SEM are shown. (E) Proliferation (left) and ALP activity measured on BMSCs under osteogenic differentiation in the presence of conditioned medium from control and Dmp1-PPR^{KO} OEBEs culture. Means \pm SD are shown. Unpaired student's t test was performed. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



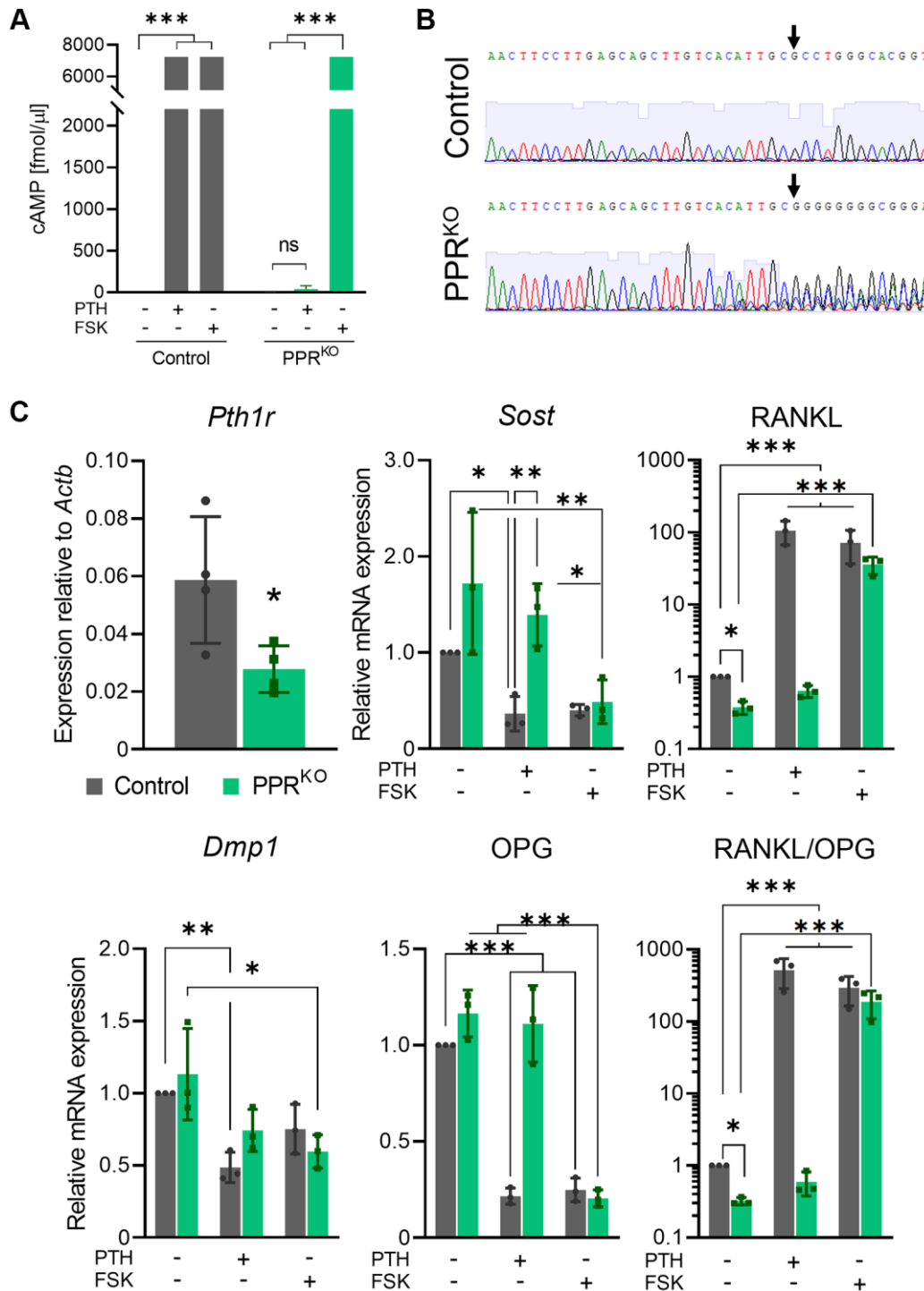
Supplementary Figure 4. (A) Gene expression in marrow-removed femora from 13-month-old female mice is shown. $N = 4-5$ per group. (B, C) Gene expression in the tibiae and/or femora of control and Dmp1-PPR^{KO} mice in male (4, 13, and 16 months of age). Expression of genes involved in osteoclasts, autophagy and/or senescence was analyzed by qPCR. $N = 4-11$ per group. Data are presented as mean \pm SEM. One-way ANOVA with Sidak's *post hoc* test, Two-way ANOVA with Tukey's *post hoc* test or unpaired student's *t* test was performed. * $p < 0.05$, *** $p < 0.001$, # $p < 0.001$ (vs. 4-mo Control), $\epsilon p < 0.001$ (vs. 4-mo Dmp1-PPR^{KO}), $\psi p < 0.001$ (vs. 13-mo Control), $\theta p < 0.001$ (vs. 13-mo Dmp1-PPR^{KO}).



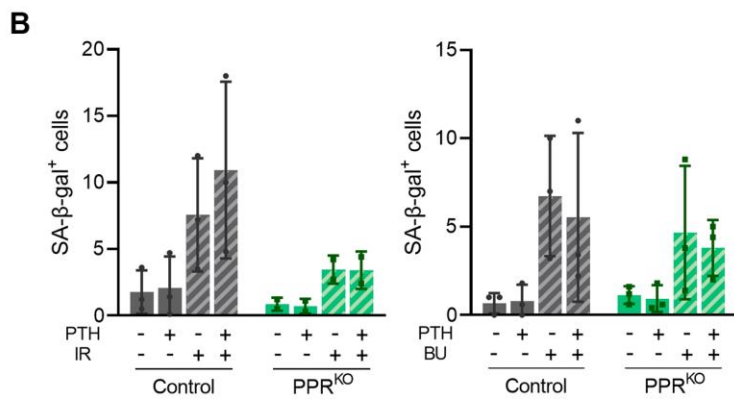
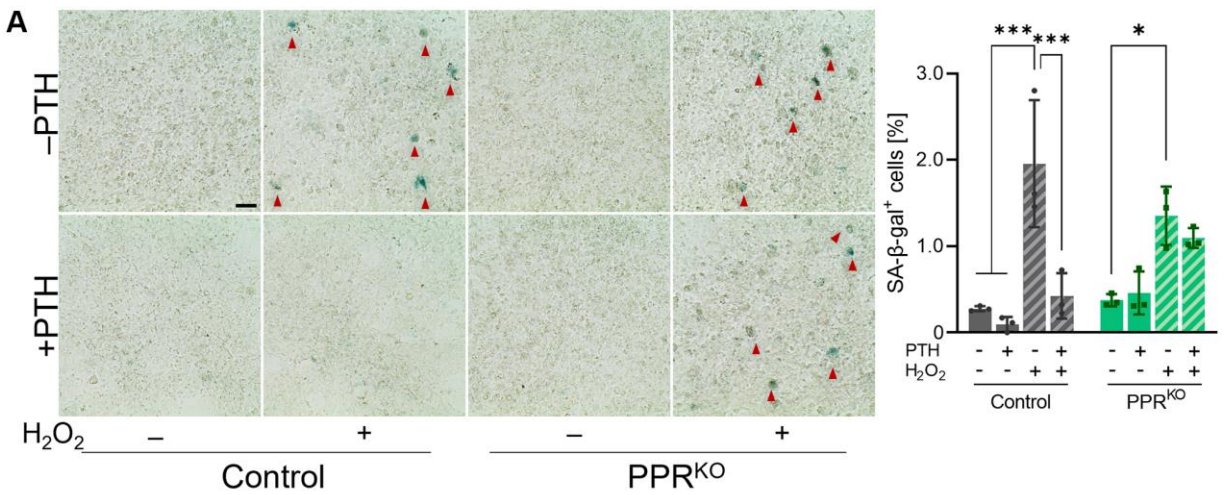
Supplementary Figure 5. Representative TNF α immunofluorescent images of the tibia of 13-month-old male mice. The frequency of TNF α ⁺ (red) osteocytes (yellow arrowheads) per field was quantified over the total number of osteocytes (stained with DAPI, blue). $N = 4$ per group. Data are presented as mean \pm SD. Unpaired Welch's t test was performed. ** $p < 0.01$.



Supplementary Figure 6. Gene expression in bone marrow cells of control and Dmp1-PPR^{KO} mice. (A) Expression of PPR, RANKL, OPG and RANKL/OPG ratio in bone marrow from middle-aged male control and Dmp1-PPR^{KO} mice (13 and 16 months old) was analyzed by qPCR. $N = 3-11$ per group. (B) Expression of *Sphk1* in the bone marrow isolated from the femora of middle-aged male animals (13-month-old) was analyzed by qPCR. $N = 4$ per group. (C) Expression of PPR and RANKL in bone marrow from female control and Dmp1-PPR^{KO} mice (20 months old) was analyzed by qPCR. $N = 3-6$ per group. Data are presented as mean \pm SEM. Unpaired Student's t test was performed. * $p < 0.05$, ** $p < 0.01$.



Supplementary Figure 7. Characterization of Ocy454-12H PPR^{KO} cells. (A) cAMP accumulation assay in 10 nM PTH- or 10 μ M forskolin-treated control and PPR^{KO} cells. PPR^{KO} cells showed no cAMP response to PTH but forskolin. (B) Sanger DNA sequencing results of the targeted *Pth1r* exon 3 sequence in control and PPR^{KO} cells are shown. Black arrows indicate the cleavage site by Cas9 protein. Unlike control cells, the sequence of PPR^{KO} after the cleavage site was unreadable due to random repair via nonhomologous end joining (NHEJ). (C) Expression of PPR and osteocytic genes in control and PPR^{KO} cells treated with 10 nM PTH or 10 μ M forskolin was analyzed by qPCR. $N = 3$ per group. Data are presented as mean \pm SD. Two-way ANOVA with Tukey's *post hoc* test was performed. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 8. (A) SA β-gal staining on Ocy454-12H PPR^{KO} under oxidative stress. Cells were pretreated with 10 nM hPTH(1–34) for 18–22 hrs and then exposed to H₂O₂ (150 μM, 7 days). (B) SA β-gal staining on irradiated (5 Gy) or busulfan (50 μM)-treated control and PPR^{KO} cells. *N* = 3 per group. Data are presented as mean ± SD. Two-way ANOVA with Tukey's *post hoc* test was performed. **p* < 0.05, ****p* < 0.001.