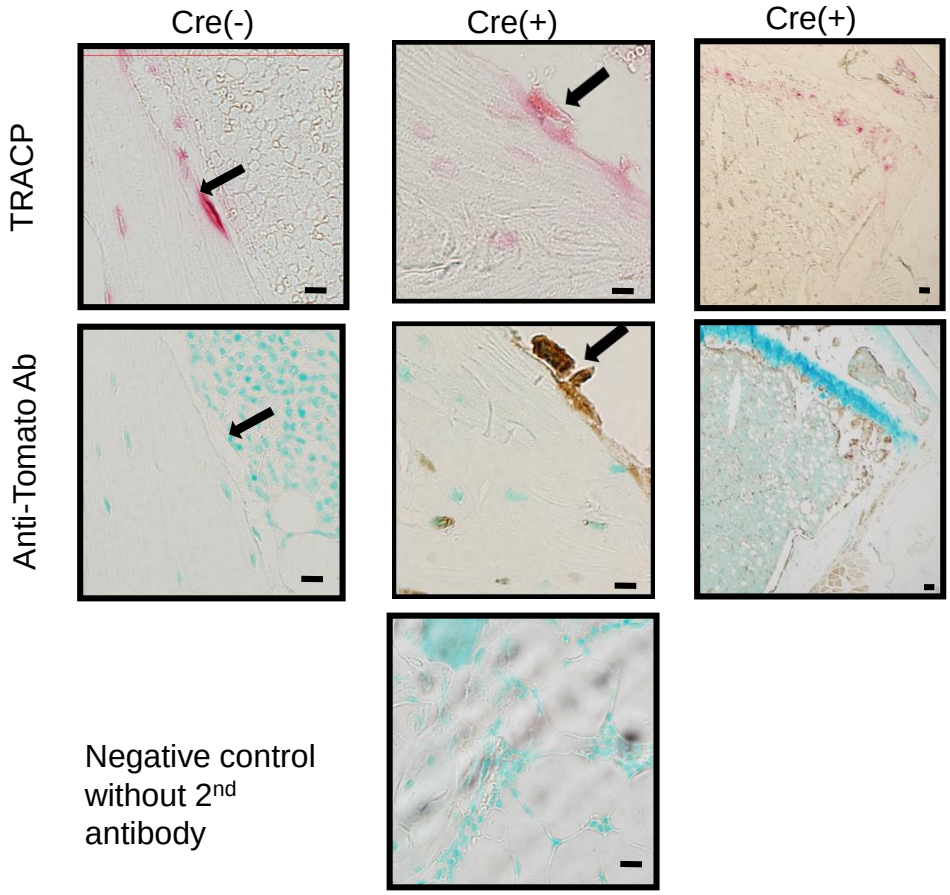
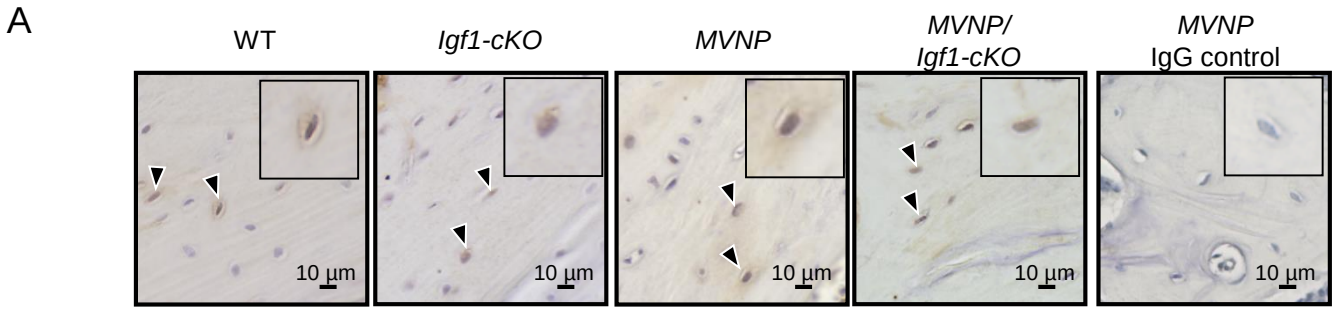
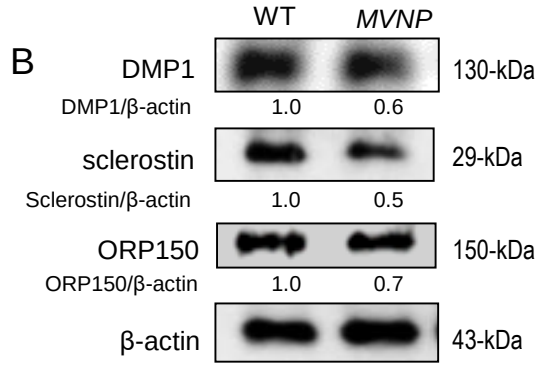
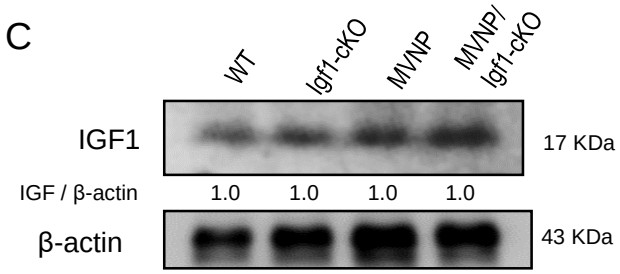


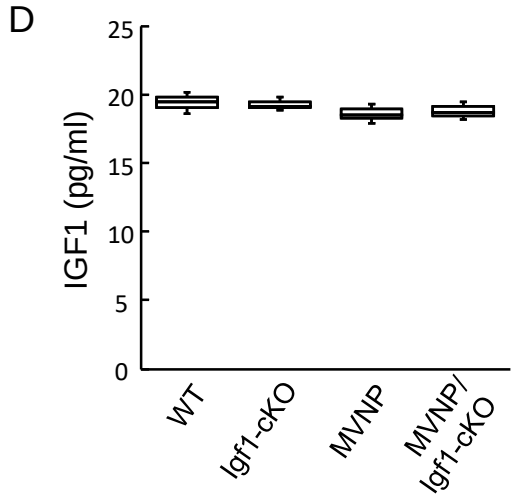
Supplemental Figure 1. Localization of TRACP(+) OCL and staining of anti-Tomato antibody. *TRACP-Cre(+)/tomato* mouse showed the co-localization of OCL with TRACP and anti-tomato antibody in consecutive sections as shown in arrows, but *TRACP-Cre(-) /tomato* mouse did not show positive staining with the Tomato antibody. TRACP is also expressed in chondrocytes and occasional osteoblasts in bone (25), but at very low levels compared with OCLs. Right hand two panels show low power view. Scale bar is 50 µm.



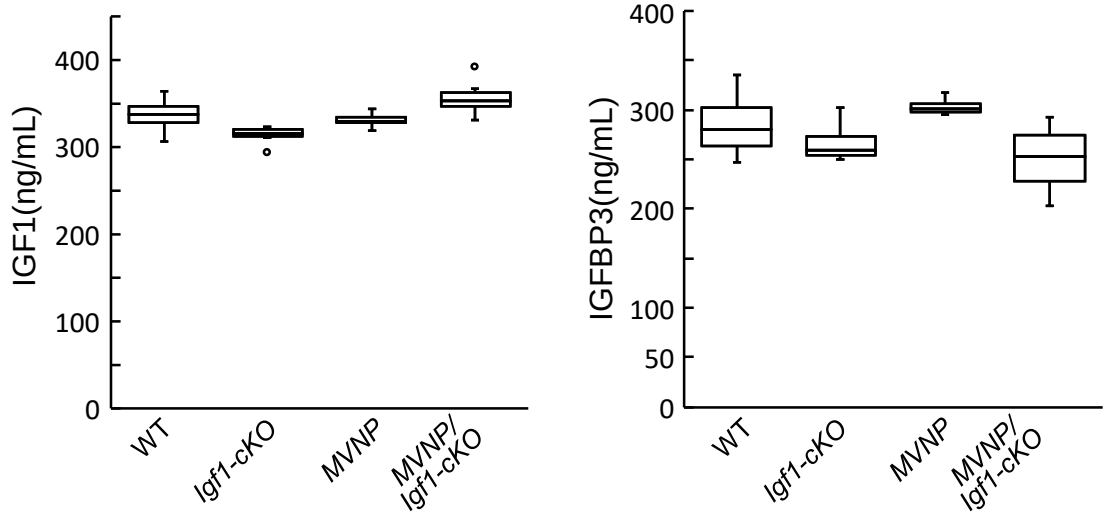
	WT	*Igf1-cKO*	*MVNP*	*MVNP/ Igf1-cKO*
Osteocyte density (#/mm^2)	681.3 ± 25.1	689.1 ± 57.8	693.9 ± 44.9	685.1 ± 25.1
IGF1(+) osteocytes (#/mm^2)	86.3 ± 13.7	88.4 ± 12.5	90.4 ± 23.1	88.8 ± 10.5
IGF1(+) osteocytes per osteocytes(%)	12.7 ± 2.2	12.8 ± 2.5	13.0 ± 2.1	13.2 ± 1.4



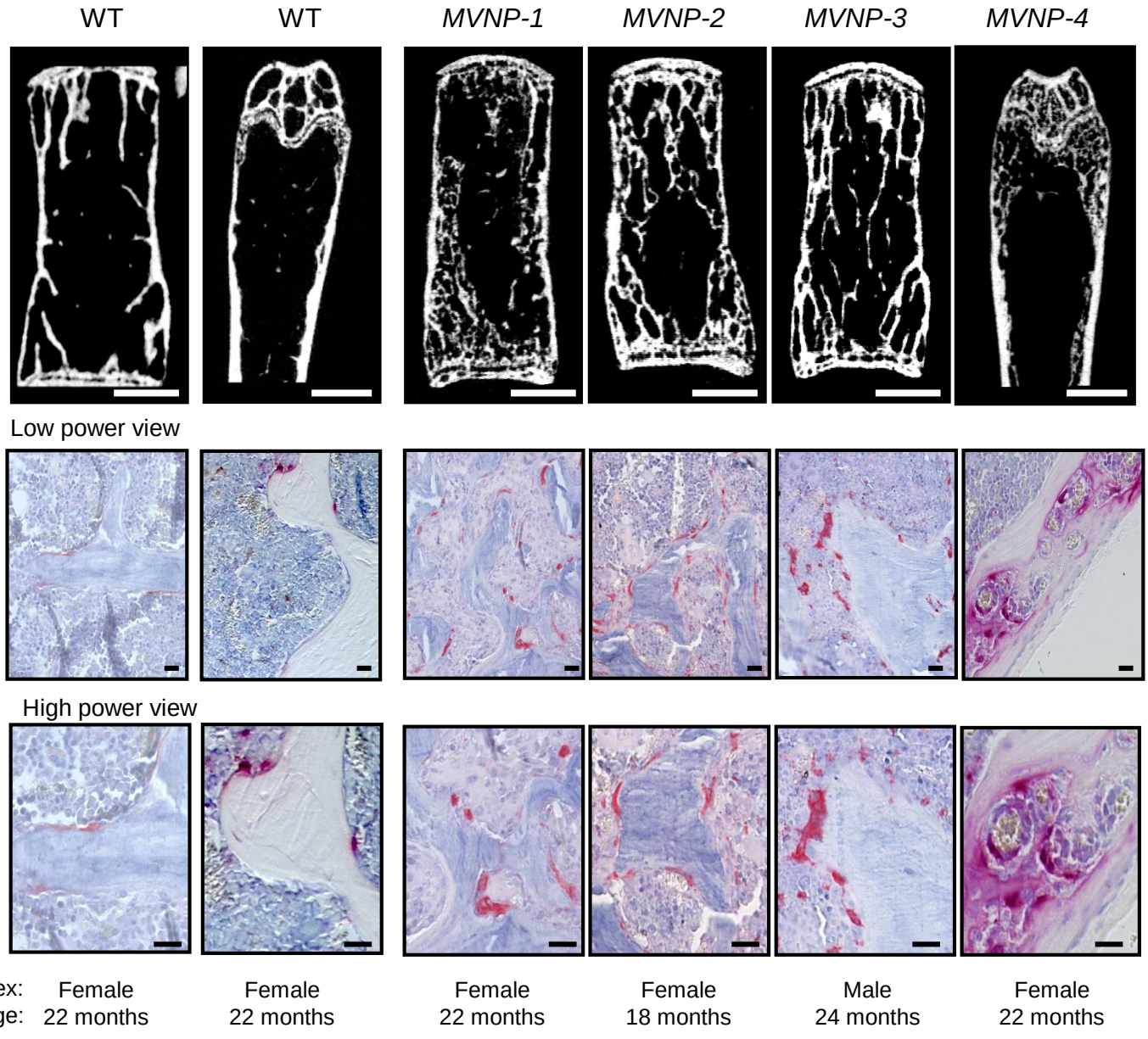




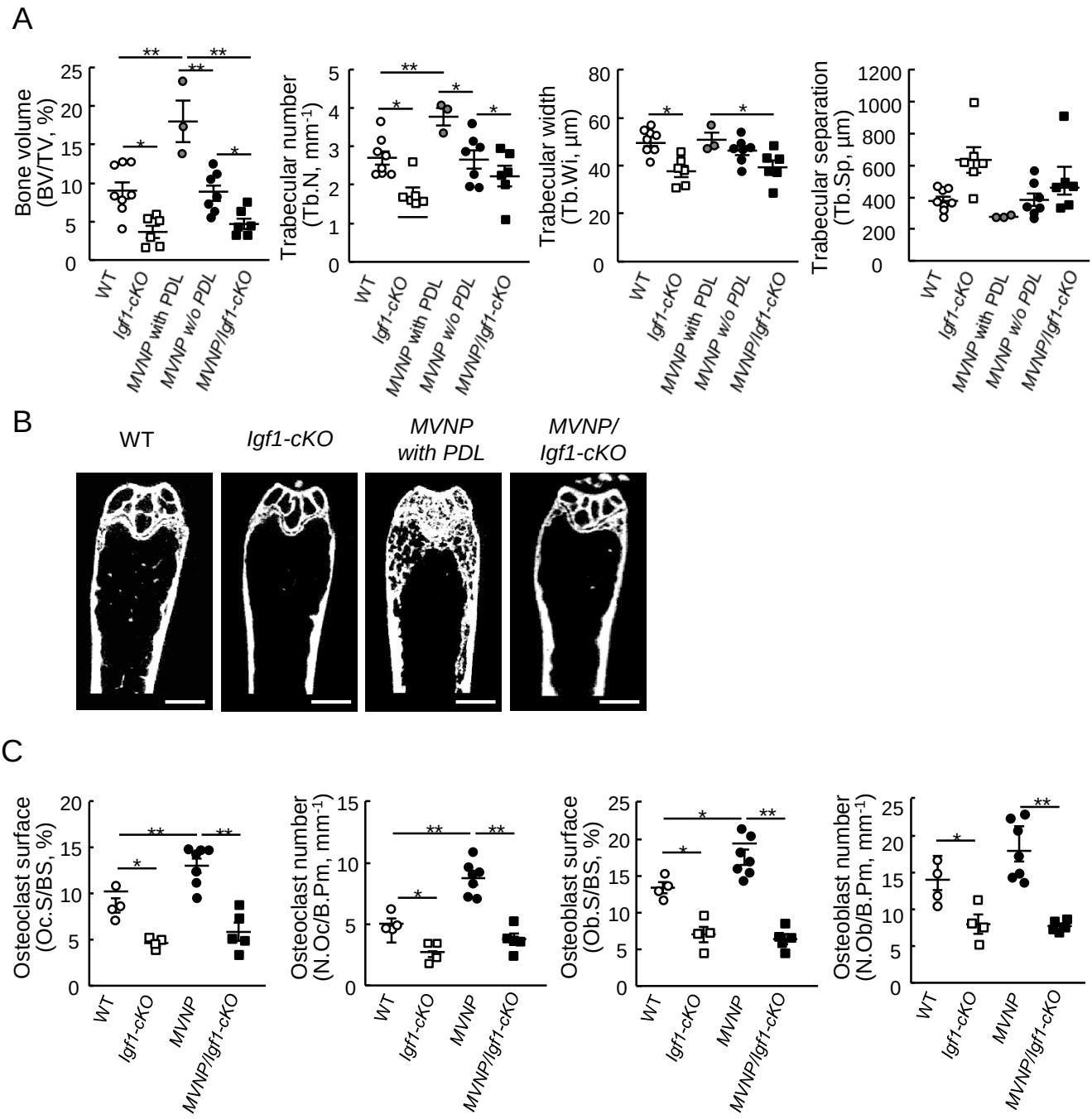
Supplemental Figure 2. IGF1 expression in osteocytes (OCys) in bone and IGF1 secretion by OCy-like cells isolated from bone. (A) Bone sections obtained from femurs of WT, *Igf1-cKO*, *MVNP* and *MVNP/Igf1-cKO* mice were stained with anti-IGF1 antibody. In addition, bone from *MVNP* mice was stained with control IgG. The number of IGF1-expressing OCys (# /mm^2) in the 4 mouse genotypes at 18-24 months of age (mean ± SEM, n=7) as shown in the photomicrographs. (B) Expression of OCy markers in OCy-like cells isolated from bone. OCy-like cells (2x10^5 cells/well) derived from day 30 cultures of bone from 20 month old mice were collected with RIPA buffer. DMP1, sclerostin and ORP150 were measured by Western blotting using these antibody. β-actin was used as loading control. The expression ratios of these proteins were quantitated by ImageJ software (NIH). The basal ratio of these proteins in OCy-like cells from WT mice was set at 1.0. We found that OCy in *MVNP* mice express lower levels of sclerostin and ORP150. (C) IGF1 expression levels in OCy-like cells derived from bones of WT, *Igf1-cKO*, *MVNP* and *MVNP/Igf1-cKO* mice. OCy-like cells (2x10^5 cells/well) derived from day 30 cultures of bone from 20 months old mouse bone were collected with RIPA buffer, and culture media were kept for ELISA assays. IGF1 was measured by Western blot using anti-IGF1 antibody. β-actin was used as loading control. The expression ratios of IGF1 were calculated from densitometric data using by ImageJ software (NIH). The basal ratio of IGF1 in OCy-like cells from WT mice was set at 1.0. (D) IGF1 secretion levels in OCy-like cells(2x10^5 cells/well) derived from bones of WT, *Igf1-cKO*, *MVNP* and *MVNP/Igf1-cKO* mice. Day 30 cultures of OCy-like cells (2x10^5 cells/ml) were cultured with 10% FCS in αMEM for 72 hours, and then IGF1 levels in culture media were measured using the mouse IGF1 ELISA Kit (abcam, ab100695). The box plots depict the minimum and maximum values (whiskers), the upper and lower quartiles, and the mean. The length of box represents the interquartile range. (3 biological replicates from each transgenic mouse line).



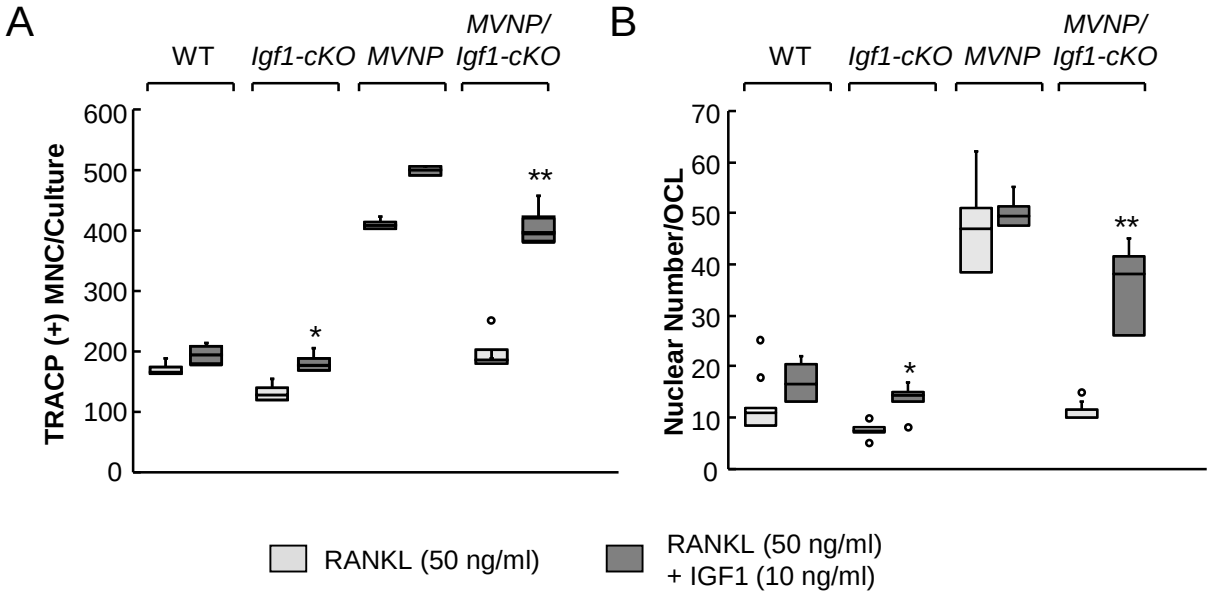
Supplemental Figure 3. Serum IGF1 and IGFBP3 levels in 12 - 20 months of age mice. The box plots depict the minimum and maximum values (whiskers), the upper and lower quartiles, and the mean. The length of box represents the interquartile range from 7 biological replicates. ELISA assay for IGF1 and IGFBP3 were described in Methods. Mice of similar age were used in each experiment.



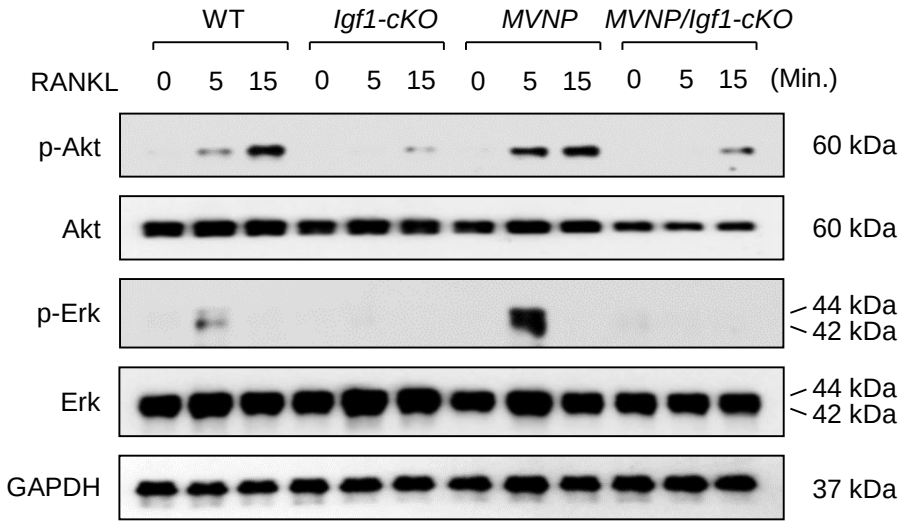
Supplemental Figure 4. µCT and histologic analysis of PDLs in *MVNP and* WT mice. Vertebrae and femurs from mice were examined as described in Methods. Markedly abnormal bone structure was seen in 4 of the 10 *MVNP* mice (40%) at 18 -24 months of age (Table 1). These lesions were histologically similar to those seen in PD and were characterized by focally thickened and irregular trabeculae composed mainly of woven bone. The scale bars in CT are 1 mm, scale bars in TRACP-stained sections are 50 µm.



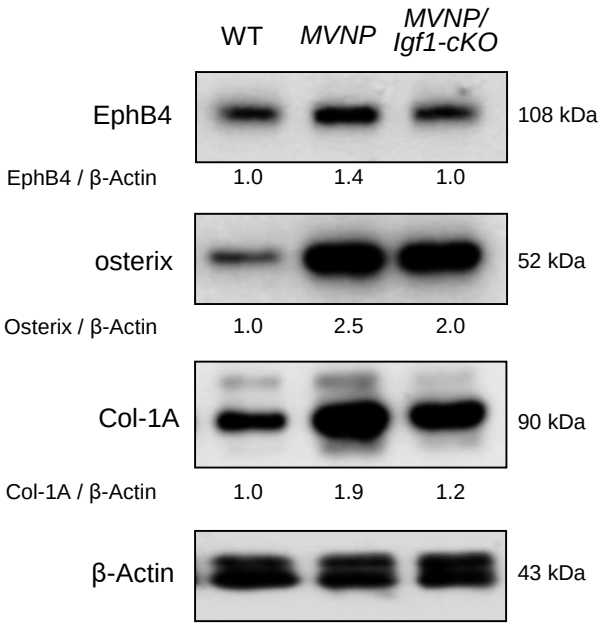
Supplemental Figure 5. Bone structure and remodeling in WT, *Igf1-cKO*, *MVNP* and *MVNP/Igf1-cKO* mice. (A) Bone volume and parameters of trabecular bone in femur. Results are expressed as the mean ± SEM for WT (4 male, 4 female, 17 ± 2 months old), *Igf1-cKO* (4 male, 2 female, 14 ± 2 months old), *MVNP* with PDL (1 male, 2 female, 21 ± 2 months old), *MVNP* without PDL (6 male, 1 female, 17 ± 3 months old), and *MVNP/Igf1-cKO* (5 male, 1 female, 20 ± 3 months old) mice. The data were analyzed using 1-way ANOVA with Tukey test. Asterisk indicates significantly different from *, *p<0.05*. **, *p<0.01* as compared each indicated group. (B) Representative μCT images of femur. Scale bars are 1 mm. (C) Bone morphometric analysis in femur. Results are expressed as the mean ± SEM for WT (4 male, 14 ± 2 months old), *Igf1-cKO* (4 male, 14 ± 3 months old), *MVNP* (7 male, 17 ± 3 months old) and *MVNP/Igf1-cKO* (5 male, 18 ± 3 months old) mice. The data were analyzed using 1-way ANOVA with Tukey test. Asterisk indicates significantly different ; *, *p<0.05.* **, *p<0.01* as compared with each indicated group. Results in panels A and C are representative of 3 - 8 biological replicates. There were no significant differences within each genotype as a function of age.



Supplemental Figure 6. Addition of IGF1 restores OCL differentiation in *Igf1-cKO* and *MVNP-Igf1cKO* mouse cultures. All experiments used bone marrow mononuclear cells from 15 month old mice. (A) OCLs formation in cultures of the 4 genotypes. OCLs formation was performed as described in Methods. The box plots depict the minimum and maximum values (whiskers), the upper and lower quartiles, and the mean. The length of box represents the interquartile range of 4 technical replicates from the 4 genotypes. The data were analyzed using a 1-way ANOVA with Tukey test. *, *p<0.01* compared with OCLs numbers from *Igf1-cKO* mice treatment with RANKL (50 ng/ml), **, *p<0.01* compared with OCLs numbers from *MVNP/Igf1-cKO* mice treatment with RANKL (50 ng/ml). Similarly results were found in cultures derived from the 3 biological replicates. (B) Nuclear numbers/OCL. Results are expressed as the box plots depict the minimum and maximum values (whiskers), the upper and lower quartiles, and the mean. The length of box represents the interquartile range of nuclei/OCL in 10 randomly counted OCLs from the 4 genotypes. The data were analyzed using 1-way ANOVA with Tukey test. *, *p<0.01* compared with OCLs numbers from *Igf1-cKO* mice treatment with RANKL (50 ng/ml), **, *p<0.01* compared with OCLs numbers from *MVNP/ Igf1-cKO* mice treatment with RANKL (50 ng/ml). Similar results were found in 3 biological replicates.



Supplemental Figure 7. Analysis of Akt and Erk activation in WT, *Igf1-cKO*, *MVNP* and *MVNP/Igf1-cKO* OCLs. OCLs (5×10^4 cells/well) derived from WT, *Igf1-cKO*, *MVNP* or *MVNP/Igf1-cKO* mice were isolated with a rubber policeman as described in Methods, then re-plated and cultures were starved in 2% FCS in αMEM overnight. Results are from lysates from cultures of purified OCLs of transgenic or WT mice induced with RANKL (50 ng/ml) for the times indicated. Akt and Erk activation was determined by Western blotting using p-Akt (Cell Signaling, #4060), Akt (Cell Signaling, #9272), p-Erk (Cell Signaling, #4370), Erk (Cell Signaling, #9102) and GAPDH (Cell Signaling, #3683) as described previously(9). The figure represents a typical experiment. Similar results were seen in three biological replicates.



Supplemental Figure 8. Osteoblast (OB) differentiation of WT OBs co-cultured with OCLs from WT, *MVNP* and *MVNP/Igf1-cKO* OCLs. OCLs (5×10^4 cells/well) derived from WT, *MVNP or MVNP/Igf1-cKO* mice were isolated with a rubber policeman as described in Methods, then re-plated and cultured with 50 ng/ml RANKL overnight. WT OBs (1×10^5 cells/well) were plated on top of the OCLs the next day, and the cells co-cultured for 72 hours. The expression levels of EphB4, osterix and Col-1A were determined by Western blotting using the appropriate antibodies as described in Methods. β-actin was used as the loading control. The expression levels of EphB4, osterix and Col-1A were quantitated by ImageJ software. The basal ratio for each protein/loading control for OBs from WT mice co-cultured with WT-OCLs was set at 1.0.

Supplemental Table 1. Location of PDLs in *MVNP* mice

Mouse ID	*MVNP-1*	*MVNP-2*	*MVNP-3*	*MVNP-4*
	Female	Female	Male	Male
Lumbar	7	7	1	0
Femur	2	5	3	2
Tibia	1	1	0	1

PDL ware examined by µCT as described in Methods.