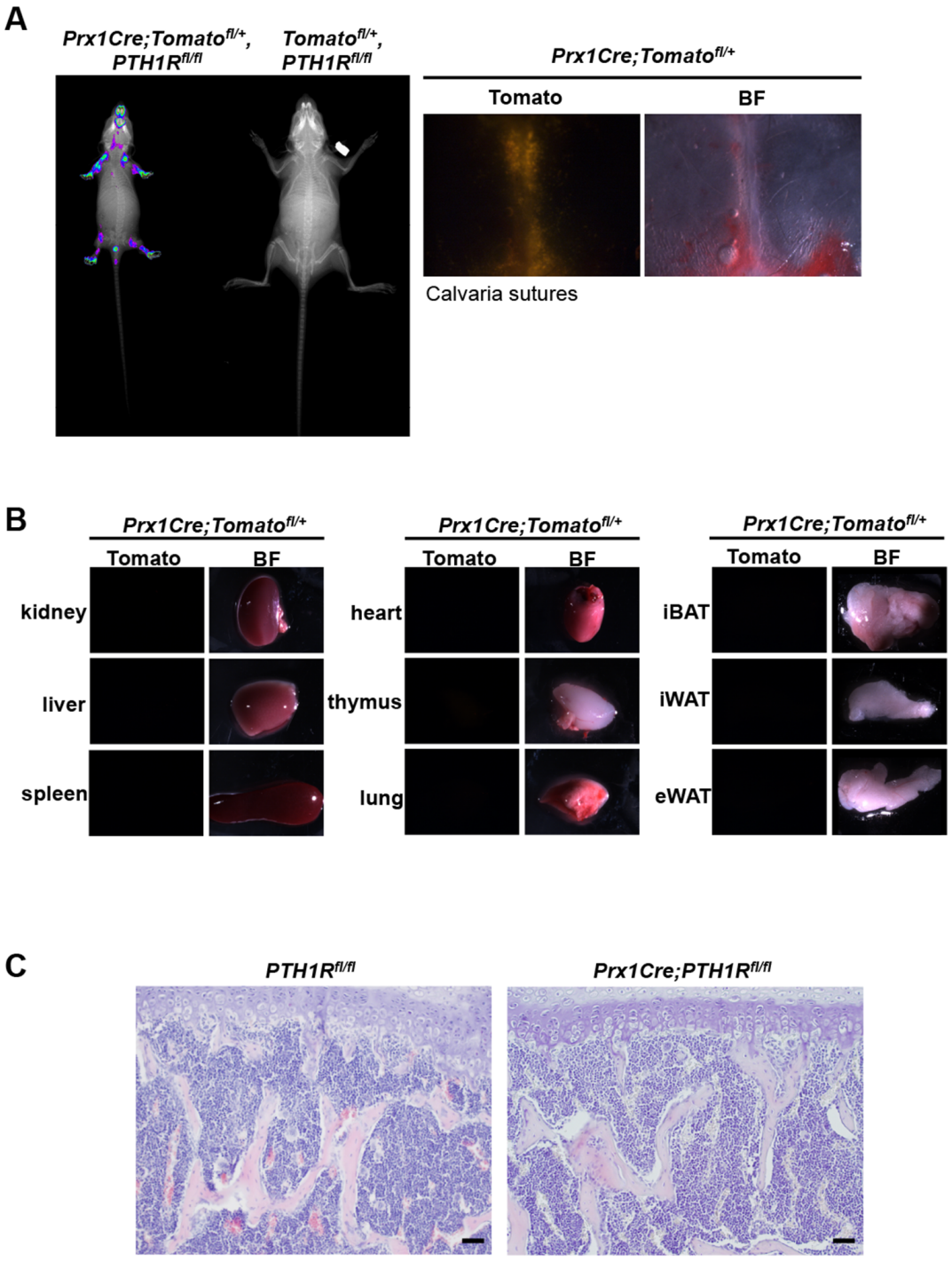

Figure S1. Specificity of *Prx1Cre* expression and PTH1R deletion and no increase in BMAT in mutant spine (Related to Figure 1).

(A) (left) Whole body image of Tomato fluorescence of 3-week-old *Prx1Cre;Tm*$^{fl/+}$,*PTH1R*$^{fl/fl}$ and *Tm*$^{fl/+}$,*PTH1R*$^{fl/fl}$ littermate. (right) Tomato fluorescence and bright field (BF) of calvaria sutures of 3-week-old *Prx1Cre;Tm*$^{fl/+}$ mice.

(B) Microscopic images of Tomato fluorescence and bright field (BF) of isolated single tissues (kidney, liver, spleen, heart, thymus, lung) and peripheral fat depots (iBAT, iWAT, eWAT) of 3-week-old *Prx1Cre;Tm*$^{fl/+}$ mice. n=3.

(C) HE stained paraffin sections of *PTH1R*$^{fl/fl}$ and *Prx1Cre;PTH1R*$^{fl/fl}$ L1 vertebrae of 3-week-old mice showed no increase in BMAT in mutant spine. n=3. Scale bar: 50μm.

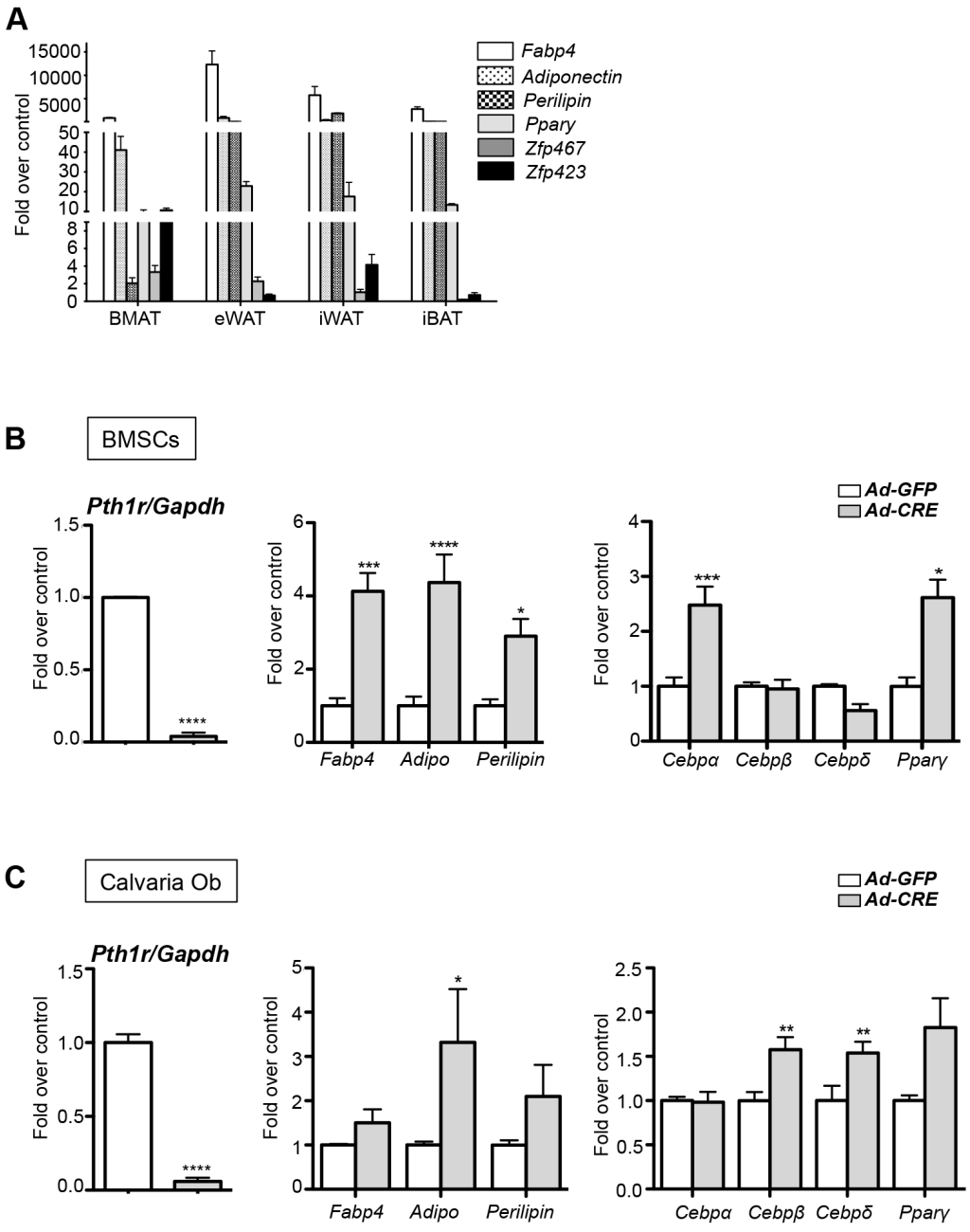

Figure S2. Characterization of population of BMAT and *in vitro* deletion of PTH1R from bone marrow stromal cells (BMSCs) and calvarial osteoblasts (Ob) (Related to Figure 2).

(A) Expression levels of adipogenesis-related genes (*Fabp4, Adiponectin, Perilipin, Pparγ, Zfp467* and *Zfp423*) in BMAT relative to other fat depots (eWAT, iWAT, and iBAT) of *Prx1Cre;PTH1R*$^{fl/fl}$ mice. n=6.

(B) Gene expression of *Pth1r* and adipogenesis-related genes in BMSCs of 3-week-old *PTH1R*$^{fl/fl}$ using Ad-Cre to delete PTH1R *in vitro*. Ad-GFP was used as control. n=3.

(C) Expression of *Pth1r* and adipogenesis-related genes in isolated calvaria osteoblasts of P4 *PTH1R*$^{fl/fl}$ using Ad-Cre to delete PTH1R *in vitro*. n=14.

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus control. All graphs show mean ± SEM.

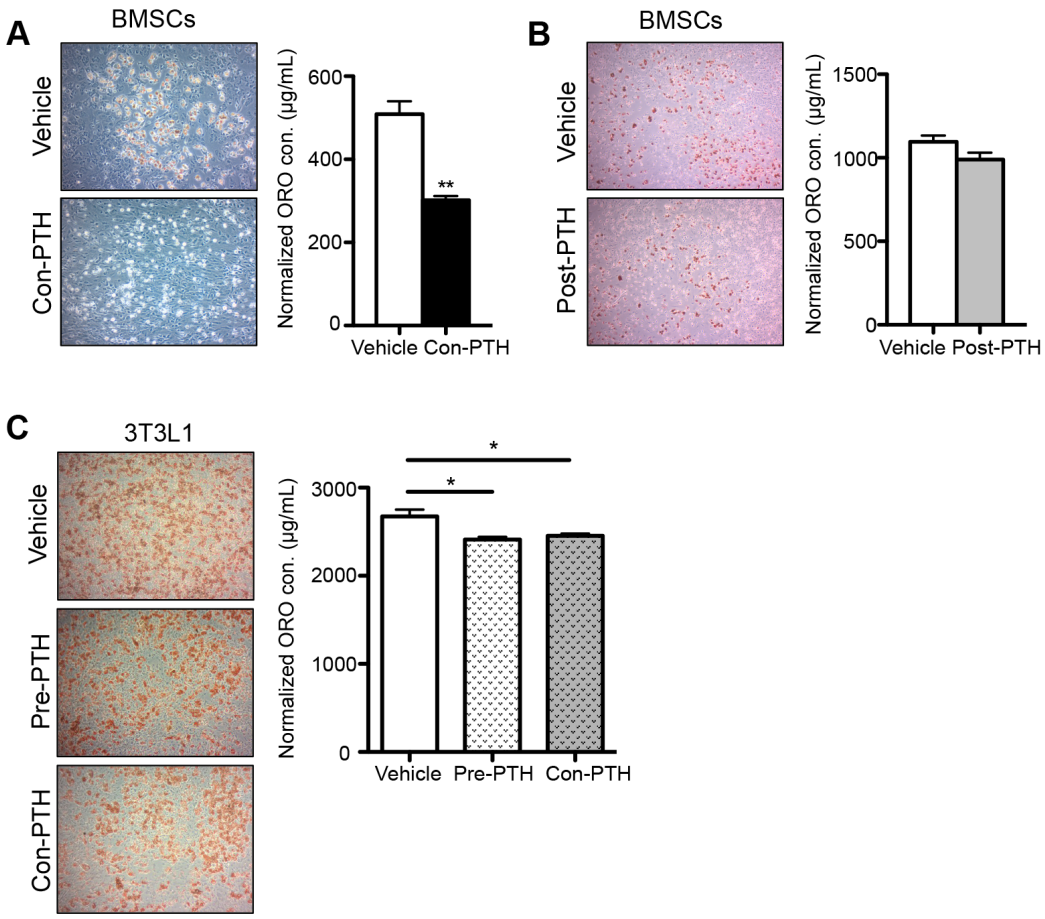

Figure S3. Different stage of PTH treatment on adipogenesis of BMSCs and 3T3-L1 cells (Related to Figure 3).

(A) Oil red O staining and quantification of BMSCs under vehicle or continuous PTH (Con-PTH) treatment. Con-PTH: cells were treated with 100 nM PTH(1-34) continuously for 6 days under adipogenic condition. The medium was changed every other day.

(B) Oil red O staining and quantification of BMSCs under vehicle or post 100 nM PTH(1-34) treatment. Post-PTH: cells were treated with 100 nM PTH(1-34) only for the last 24 hours before collection.

(C) Oil red O staining and quantification of 3T3-L1 cells under vehicle, Pre-PTH and Con-PTH treatment. Pre-PTH: cells were treated with 100 nM PTH(1-34) for 24 hours before the adipogenic induction. n=3.

*$p<0.05$, **$p<0.01$ versus vehicle control. All graphs show mean ± SEM.

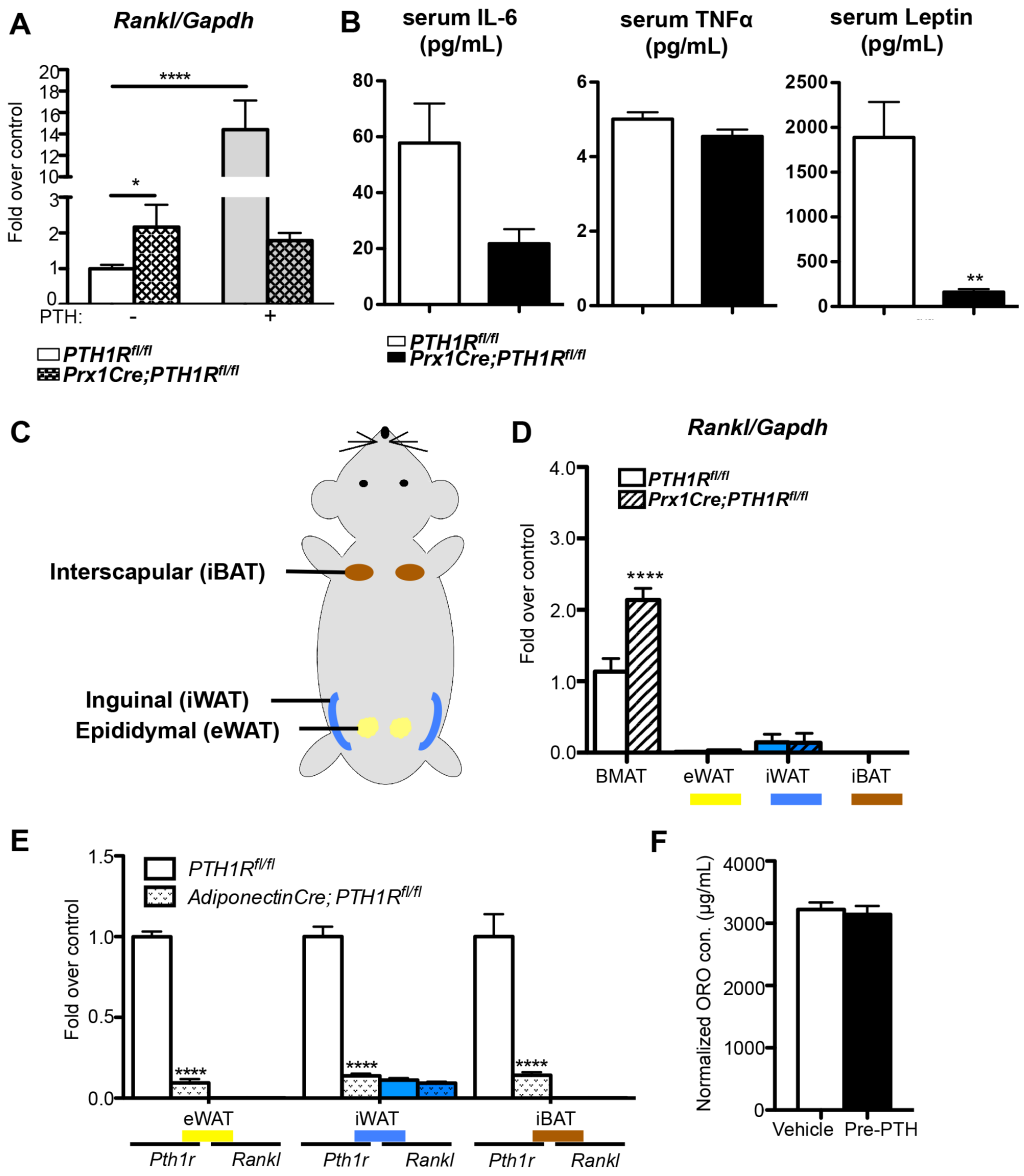

Figure S4. RANKL production is limited to bone marrow adipose tissue (BMAT) (Related to Figure 5).

(A) Cortical bone *Rankl* mRNA expression was significantly elevated after 2-week PTH administration in control mice, whereas the already elevated basal *Rankl* levels in mutant bones did not further increase. n=4/control, 5/mutant.

(B) Serum IL-6, TNFα and Leptin levels in 3-week-old *PTH1R*$^{fl/fl}$ and *Prx1Cre;PTH1R*$^{fl/fl}$ mice. n=17/control, 11/mutant.

(C) Diagram depicting the areas of peripheral fat collection.

(D) Comparison of *Rankl* expression in these compartments (eWAT, iWAT, iBAT) to BMAT of *PTH1R*$^{fl/fl}$ and *Prx1Cre;PTH1R*$^{fl/fl}$ mice at 3wks. n=5/control, 6/mutant.

(E) *Pth1r* and *Rankl* gene expression in eWAT, iWAT and iBAT of control and *AdiponectinCre;PTH1R*$^{fl/fl}$ mice. n=6/control, 4/mutant.

(F) Quantification of oil red O staining of *in vitro* cultured primary stromal vascular fraction pre-adipocytes under adipogenic condition with vehicle or Pre-PTH treatment. Pre-PTH: cells were treated with 100nM PTH(1-34) for 24 hours before the adipogenic induction. n=3.

*p<0.05, **p<0.01, ****p<0.0001 versus control. All graphs show mean ± SEM.

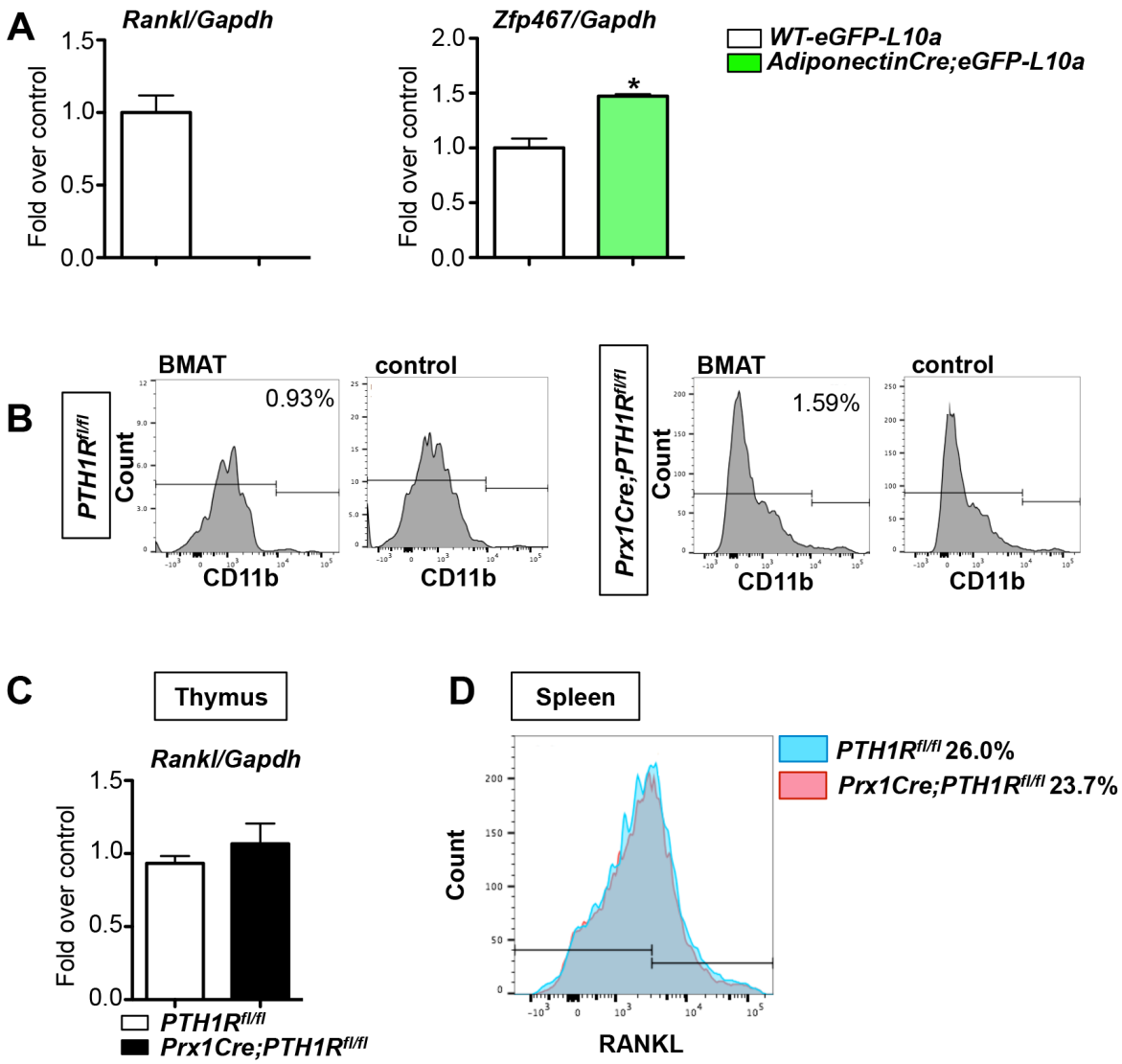

Figure S5. No change of RANKL expression in other tissues (Related to Figure 5).

(A) GFP^{+} cells from inguinal depot of *AdiponectinCre;eGFP-L10a* mice were isolated by FACS. Whole tissue RNA of iWAT was isolated from WT-eGFP-L10a mice and served as control. Gene expression analysis showed absolutely no *Rankl* expression in the adipose depot of *AdiponectinCre;eGFP-L10a* mice. *Zfp467* mRNA, a marker of adipocytes that is regulated by PTH, was 60% enriched in the eGFP^{+} cells from the *AdiponectinCre;eGFP-L10a* mice *versus* controls. n=3, *p<0.05.

(B) Flow cytometric analysis of CD11b showed less than 2% of macrophage cells in isolated BMAT of *PTH1R*$^{fl/fl}$ and *Prx1Cre;PTH1R*$^{fl/fl}$ mice. n=4. Histograms show CD11b staining with percent positive cells in each experiment panel. No antibody groups served as controls.

(C) No change of *Rankl* mRNA was detected in thymus of *Prx1Cre;PTH1R*$^{fl/fl}$ when compared to *PTH1R*$^{fl/fl}$ at 3 weeks of age. n=3.

(D) Flow cytometric analysis of RANKL expression from whole spleen of control and mutant mice. Overlay of histograms for spleen cells of *PTH1R*$^{fl/fl}$ and *Prx1Cre;PTH1R*$^{fl/fl}$, showing comparable RANKL positive cells. 26.0% and 23.7% of RANKL^{+} cells in spleen of *PTH1R*$^{fl/fl}$ (blue) and *Prx1Cre;PTH1R*$^{fl/fl}$ (red), respectively. n=3.

All graphs show mean ± SEM.

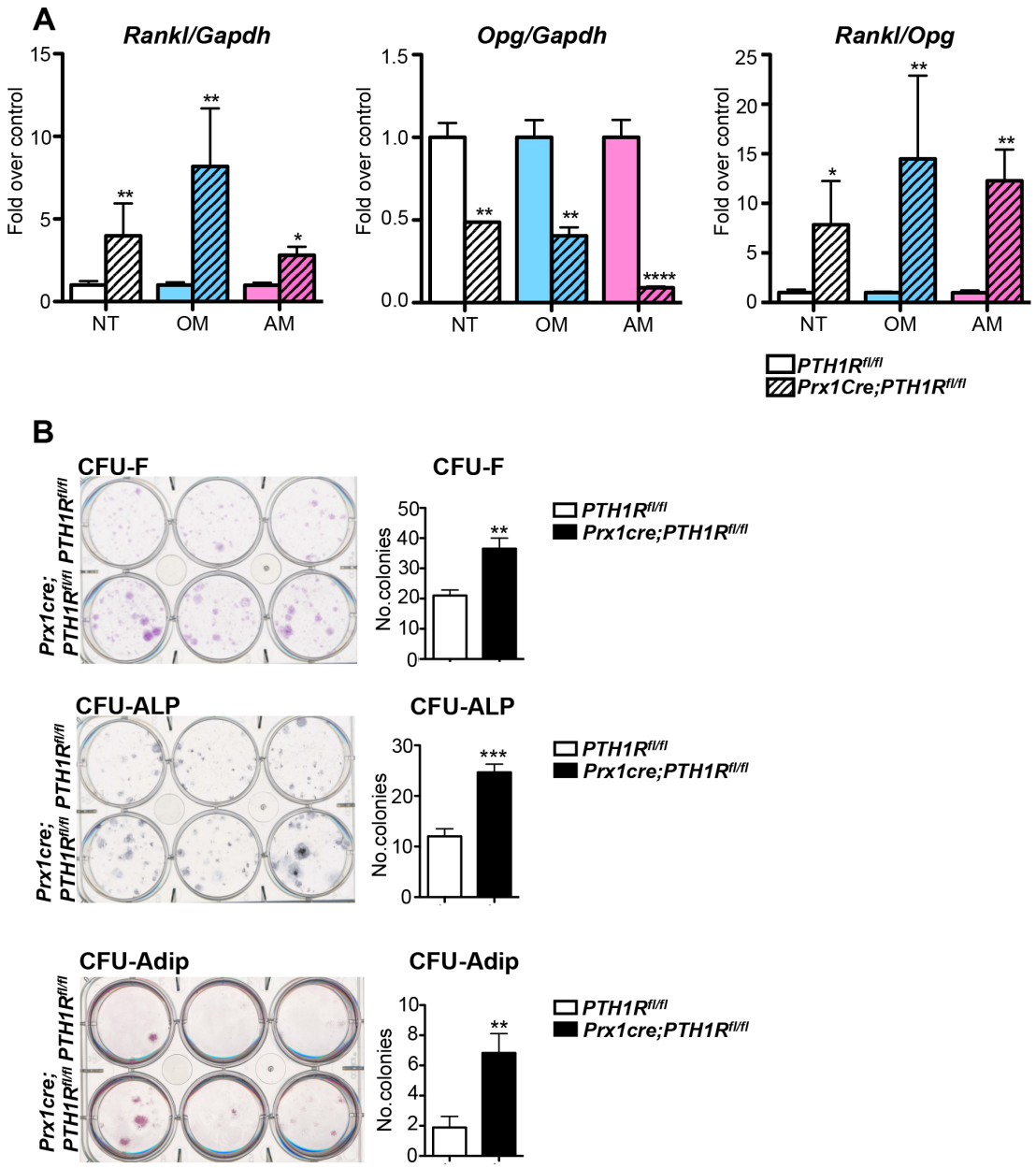

Figure S6. Increased *Rankl* expression in bone marrow of *Prx1Cre;PTH1R$^{fl/fl}$* mice is independent of culture conditions (Related to Figure 6).

(A) *Rankl*, *Opg* mRNA levels and *Rankl/Opg* ratio of *PTH1R$^{fl/fl}$* and *Prx1Cre;PTH1R$^{fl/fl}$* BMSCs under no treatment (NT), osteogenic medium (OM), adipogenic medium (AM). n=6.

(B) The frequency of CFU-F mesenchymal progenitors, CFU-ALP osteoblast precursors as well as CFU-Adipo adipocyte precursors are significantly increased in *Prx1Cre;PTH1R$^{fl/fl}$* mice, with a higher increase in CFU-Adipo adipocyte precursors. n=6.

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. All graphs show mean ± SEM.

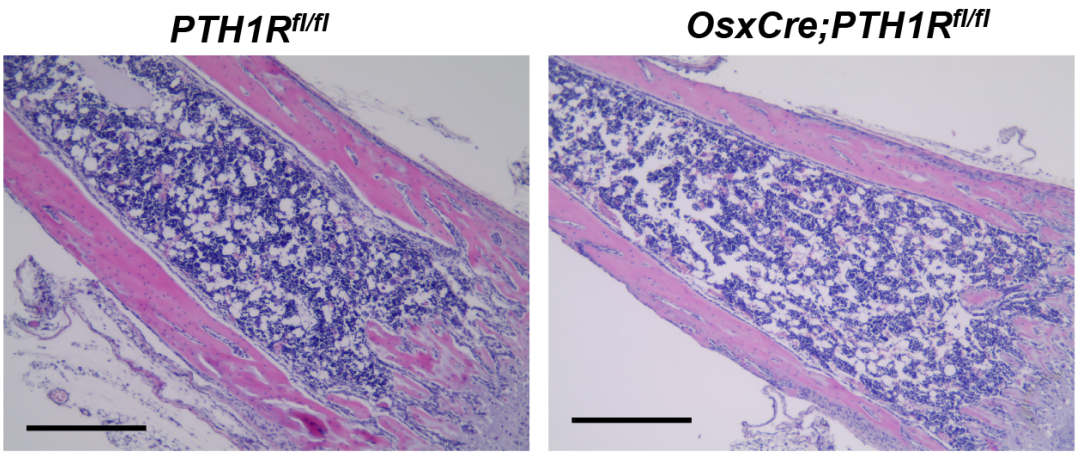

Figure S7. No change of BMAT in *OsxCre;PTH1R$^{fl/fl}$* mice (Related to Figure 2).

HE stained paraffin sections of *PTH1R$^{fl/fl}$* and *OsxCre;PTH1R$^{fl/fl}$* distal tibia at 3-week-old showed no difference in BMAT when PTH1R is ablated. n=3. Scale bar: 400μm.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Marrow adipose tissue quantification by osmium tetroxide staining and μCT

At the time of sacrifice, tibiae were isolated and placed into 10% neutral buffered formalin overnight at room temperature. Soft tissue was then carefully removed to ensure that the fibula remained intact and the bones were washed under continuous cold water for one hour, then stored in PBS at 4°C. Quantification and visualization of marrow adipose tissue was performed as described previously (Scheller et al., 2014). Briefly, bones were decalcified in 14% EDTA (pH 7.4) for 14 days, with EDTA changes every 3-4 days. Bones were then washed for 10 minutes in PBS (3 times) and stained with a 1:1 mixture of 2% aqueous osmium tetroxide (cat# 23310-10, Polysciences, Inc., Warrington, PA, USA) and 5% potassium dichromate for 48 hours. Stained bones were then washed with PBS (pH 7.4) for 5 hours (3 times), and subsequently scanned by μCT. Bone marrow adipose tissue (BMAT) content was calculated by determining the BMAT volume and dividing by the total marrow volume for each region of interest (ROI) which includes ROI1: proximal tibial epiphysis, ROI2: tibial growth plate to tibia-fibula junction, ROI3: tibia-fibula junction to distal tibia.

Cell culture

Bone marrow stromal cells (BMSCs) from tibia and femur of 3-week-old mice were flushed out and cultured in α-MEM (Invitrogen) containing 1% penicillin/streptomycin (PS, Gibco) and 20% fetal bovine serum (FBS) (Sigma-Aldrich) at 37°C with 5% CO2. Non-adherent cells were removed and the attached cells were passaged at day 6 and maintained in complete medium. Cells were subjected to one passage using 0.25% trypsin. Cells in passage 1 of these BMSCs were used for differentiation assays and plated at 5×10^4 cells/cm2. Osteogenic differentiation was induced with osteoblast differentiation medium (α-MEM supplemented with 1% PS, 10% FBS, 50 μg/ml ascorbic acid (Sigma-Aldrich), 10 mM β-glycerophosphate (Sigma-Aldrich)). The medium was changed every other day for 14 days. For adipogenic differentiation, the cells were induced with adipocyte differentiation medium (α-MEM supplemented with 1% PS, 20% FBS, 500 μM IBMX, 1 μM Dexamethasone, 10 μg/ml Insulin and 1 μM Rosiglitazone) for the first 2 days. The medium was then changed to adipocyte differentiation base medium (α-MEM supplemented with 1% PS, 20% FBS, 10 μg/ml Insulin and 1 μM Rosiglitazone) for the next 4 days with medium changes every other day. For PTH treatment, 100 nM hPTH(1-34) (Bachem) was added into conditioned medium and changed every other day. Calvarial osteoblasts were obtained by serial collagenase digestion from P4 *PTH1R*$^{fl/fl}$ mice as described previously (Yang et al., 2008). Prior to reaching confluence, cells were infected with either Ad-Cre or Ad-GFP (2.5%) overnight (Vector Biolabs) (Duan et al., 2016) and then induced to adipogenic differentiation for 6 days. After 6 days, cells were washed in PBS and collected for qPCR analysis or fixed with 10% formalin for staining. Primary stromal vascular fraction pre-adipocytes were isolated from inguinal white adipose tissue from 3-week-old mice. Inguinal fat pads were incubated for 1hr at 37°C in an isolation buffer composed of 123 mM of NaCl, 1.3 mM $CaCl_2$, 5 mM glucose, 4% Bovine Serum Albumin, 0.1% Collagenase P and 100 mM HEPES in distilled sterile water. The digested solution was passed through a 70μm sterile filter and centrifuged at 500g for 5min at 4°C. The pellets were washed with PBS before being centrifuged a second time at 500g for 5min at 4°C. The pellets were resuspended in culture media made of DMEM with 20% FBS and 1% PS. Cultures were allowed to attach for 72hrs before media change and were subjected to one passage using 0.25% trypsin before being plated for adipogenesis. 3T3-L1 cells were obtained from ATCC and cultured in DMEM with 10% FBS and 1% PS. Cells were passaged using 0.25% trypsin no more than 30 times. Rosiglitazone was not necessary for 3T3-L1 pre-adipocytes cultures.

RNA extraction and qRT-PCR

Total RNA was extracted from the cortical region of long bones of the limbs after flushing out the bone marrow, lumbar vertebrae (L1-L5), whole bone marrow and bone marrow adipocytes, interscapular BAT, inguinal WAT, epididmyal WAT and *in vitro* cultured cells using Trizol (Invitrogen) according to the manufacturer's protocol. The RNA concentration was determined with a NanoDrop ND-1000 (Thermo Fisher Scientific). cDNA was generated using SuperscriptRT II (Invitrogen). Taqman primers were used to perform quantitative real-time PCR (qRT-PCR). Relative gene expression levels were normalized by GAPDH (glyceraldehyde-3-phosphate dehydrogenase) using the ΔΔCT method.

Intermittent PTH(1-34) treatment

25 nmol/kg of human recombinant PTH(1-34) (Bachem, Torrance, CA, USA) was subcutaneous injected daily for a 2-week period starting at P8. Animals of the vehicle group were injected with an equal volume of sterile saline.

Whole animal image and Tomato fluorescence detection

A whole body image was obtained by "Bruker MS FX Pro in-vivo animal imager" to visualize Tomato fluorescence tissues and simultaneously obtain X-ray images of *Prx1Cre;Tm*$^{fl/+}$*,PTH1R*$^{fl/fl}$ and *Tm*$^{fl/+}$*,PTH1R*$^{fl/fl}$ littermates at 3-weeks of age. Tomato fluorescence and bright field images were obtained using a fluorescence stereomicroscope (Leica MZ FLIII).

Histology
Mouse hind limbs and L1 vertebrae from 3-week-old *PTH1R*$^{fl/fl}$ and *Prx1Cre;PTH1R*$^{fl/fl}$ animals and tibiae from 3-week-old *PTH1R*$^{fl/fl}$ and *OsxCre;PTH1R*$^{fl/fl}$ animals were fixed in 10% buffered formalin at 4°C overnight and embedded in paraffin. 5μm sections were cut using an HM360 microtome (Microm). The sections were stained with hematoxylin (VWR) and eosin (Sigma-Aldrich). TRAP staining was performed according to the manufacturer's protocol (Sigma-Aldrich). 3-week-old mice were prepared and stained with alizarin red S and alcian blue as described previously (McLeod, 1980).

Immunostaining
BMSCs under adipogenesis culture from *Prx1Cre;Tm*$^{fl/+}$ and *Prx1Cre;PTH1R*$^{fl/fl}$*,Tm*$^{fl/+}$ animals were fixed in 10% formalin and blocked with 2% BSA. Cells were incubated with primary antibodies: Adiponectin (Abcam, 1:400), Red Fluorescent Protein (RFP) (Rockland, 1:200) at 4°C overnight and then stained with Alexa Fluor 488 (Invitrogen, 1:200) and Alexa Fluor 568 (Invitrogen, 1:200), respectively. DAPI (Vector) was used as counterstaining.

Flow cytometry
For preparation of flow cytometry analysis, bone marrow cells were isolated from femur and tibia of 3-week-old mice (3 controls and 3 mutants) and then suspended in αMEM and washed with PBS with 5% BSA. Cells were incubated with FITC rat anti-mouse CD45R/B220 (BD Bioscience), PE anti-Pref-1 (MBL), biotin anti-mouse CD245 (RANKL) (BioLegend) and APC/Cy7 Streptavidin (BioLegend). Flow cytometry was carried out using the FACSCalibur (BD Biosciences) and Cell Quest software (BD Bioscience) after labeling with each primary antibody and second antibody. For CD11b flow cytometry, bone marrow adipose tissue (BMAT) was isolated as described previously. For preparation of spleen cells, mouse spleens from 3-week-old *PTH1R*$^{fl/fl}$ and *Prx1Cre;PTH1R*$^{fl/fl}$ mice were collected. Whole spleens were dissected and cut into small pieces using scissors and blades, and then digested with collagenase type 2 (1 mg/ml) for 1 hour at 37 °C, with shaking (200 rpm). Digested tissue was filtered, spun down with αMEM and 10% fetal bovine serum (FBS) (1000 rpm, 5 minutes), and then washed twice with PBS with 5% FBS. Isolated BMAT cells or whole splenic cells were incubated with PE conjugated anti-mouse CD11b (eBioscience) at 0.124 μg per million cells in 100 μl total volume at room temperature for 30 minutes. Flow cytometry was carried out using the FACSCalibur (BD Biosciences) and Cell Quest software (BD Bioscience) after labeling with each antibody. Each data set was analyzed by FlowJo software (FLOWJO, LLC, OR, USA).

CFU assays
Bone marrow cells from femur and tibia of 3-week-old *PTH1R*$^{fl/fl}$ and *Prx1Cre;PTH1R*$^{fl/fl}$ mice were flushed out using ice-cold α-MEM and plated in 6-well plates at 1×10^6 cells per well. Cells were cultured in α-MEM with 20% FBS for 10 days without changing medium to establish colonies. For colony-forming unit fibroblastic (CFU-F) assays, cells were fixed with 10% formalin at day 10 and then stained with crystal violet. At day 10, a batch of cells was continued cultured in osteogenic medium or adipogenic medium for additional 6 days. Osteoblast differentiation medium was added for CFU-alkaline phosphatase (CFU-ALP) assay for 6 days and colonies were fixed in formalin and stained for alkaline phosphatase (ALP) activity (Aubin, 1999). For CFU-Adipocyte (CFU-Adip) assays, colonies were cultured under adipocyte differentiation medium at day 10 and then changed to adipocyte differentiation base medium at day 12 for 4 days and stained with oil red O at day 16. Note that the mutants had initially markedly higher number of CFU-F before differentiation assay in both circumstances. After staining, colonies containing >20 positive cells were observed and counted under a phase contrast microscope. Bone marrow cells from 10 mice for each genotype were plated in triplicate dishes and repeated at least 3 times for each experiment.

Elisa assays
Blood was obtained by cheek pouch puncture. Serum IL-6, TNFα and Leptin were measured using Milliplex Multiplex Assays (EMD Millipore). The ELISA kits for 1,25-dihydroxy Vitamin D was purchased from IDS (Fountain Hills, AZ). Serum and bone marrow supernatant RANKL levels were measured using commercially available kit from R&D Systems (Minneapolis, MN) according to the manufacturer's instructions. The assay sensitivity was less than 5 pg/mL. The intra- and inter-assay variations were 4.3 and 6.9 pg/mL respectively. All measurements were performed in duplicate.

Translating ribosome affinity purification (TRAP)
TRAP method was utilized to identify cell type-specific changes in mRNA populations at the whole-organ level (Doyle et al., 2008; Heiman et al., 2008). By crossing *AdiponectinCre* mice to *eGFP-L10a* mice that carry a ribosomal GFP, a pure population of ribosomal RNA was isolated using TRAP technology as described previously (Liu et al., 2014). FACS was used to obtain GFP$^+$ cells of inguinal depot. Females *WT-eGFP-L10a* and *AdiponectinCre;eGFP-10a* mice were sacrificed at 8 weeks of age. TRAP was performed in inguinal white adipose tissue (iWAT) to isolate and purify actively translating

mRNA from mature white adipocytes of *AdiponectinCre;eGFP-10a* mice. Whole tissue RNA of iWAT was isolated from *WT-eGFP-L10a* mice.

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