Supplementary Table 1: *Ank*$^{+/+}$ and *Ank*$^{KI/KI}$ mCOB cultures in osteogenic media for 7-9, 14 and 21days exhibit no difference in DNA content measured by PicoGreen assays.

Days in osteogenic media	*Ank*$^{+/+}$ (μg DNA)	*Ank*$^{KI/KI}$ (μg DNA)	P value
7-9	7.23±0.73	7.64±0.58	0.49
14	13.64±3.18	12.41±3.51	0.74
21	8.55±1.17	9.11±1.55	0.64

Statistics were performed by *Student's t-test*.

Supplementary Table 2: Gene expression in osteoblast cultures and in bone

Fold-change in calvarial osteoblast cultures (mCOBs)				
Genes	D0	D7	D14	D21
Tnap	0.99 ± 0.09	0.82 ± 0.32	1.09 ± 0.18	0.71 ± 0.2
Ank	1.21 ± 0.21	0.96 ± 0.04	1.3 ± 0.42	1.17 ± 0.32
Bsp	0.74 ± 0.34	1.27 ± 0.13	1.23 ± 0.23	0.86 ± 0.33
ColI	1.01 ± 0.05	1.06 ± 0.18	1.03 ± 0.22	0.77 ± 0.15
Enpp1	1.23 ± 0.5	1.01 ± 0.17	1.3 ± 0.41	1.31 ± 0.5
Mmp13	0.92 ± 0.21	0.95 ± 0.50	0.89 ± 0.10	0.74 ± 0.04**
Ocn	0.92 ± 0.17	0.78 ± 0.49	0.73 ± 0.42	0.30 ± 0.23**
Sp7 (Osx)	0.85 ± 0.22	0.86 ± 0.14	1.16 ± 0.37	0.77 ± 0.07**
Runx2	1.21 ± 0.321	0.91 ± 0.20	1.02 ± 0.03	0.80 ± 0.08**
Phex	0.64 ± 0.067*	0.75 ± 0.004*	0.72 ± 0.284	0.29± 0.203**

Fold-change in whole bone (calvariae/femurs)		
	Calvariae	Femur
ColI	0.78 ± 0.19	0.70 ± 0.08
Bsp	0.86 ± 0.007*	0.71 ± 0.01
Mmp13	0.87 ± 0.26	0.42 ± 0.14
Ocn	0.85 ±0.29	0.57 ± 0.08
Opn	0.95 ± 0.35	0.71 ±0.12
Sp7 (Osx)	0.71 ± 0.24	0.63 ± 0.08
Runx2	0.80 ± 0.21	0.75 ± 0.21
Rankl	0.91 ± 0.37	0.89 ± 0.26
Opg	0.93 ± 0.46	0.77 ± 0.37
Mcsf	1.02 ± 0.27	0.73 ± 0.17
Fgf23	11.44 ± 5.412*	8.39 ± 1.223**
Dmp1	3.10 ± 1.337*	1.44 ± 0.836
Mepe	1.80 ± 0.617*	0.30 ± 0.09*
Phex	1.88 ± 0.88	0.50 ± 0.18

Fold-change of gene expression ($Ank^{KI/KI}$ divided by $Ank^{+/+}$) at each time point determined by qPCR. Gene expression was normalized to 18S RNA. $Ank^{+/+}$ day 0 samples were used as calibrators (*p<0.05, **p<0.01).

Supplementary Table 3: μCT analysis of $Ank^{+/+}$ and $Ank^{KI/KI}$ male mice receiving bone marrow transplantation

Donor → Recipient	$Ank^{+/+}$→ $Ank^{+/+}$(n=8)	$Ank^{KI/KI}$→ $Ank^{KI/KI}$(n=6)	$Ank^{+/+}$→ $Ank^{KI/KI}$(n=8)	$Ank^{KI/KI}$→ $Ank^{+/+}$(n=6)
Calvariae				
Cortical width (mm)	0.10±0.01	0.15±0.01*	0.14±0.18	0.12±0.06
BM/TA (%)	5.4±1.76	0.7±0.6**	0.7±0.6**	1.8±1.39**
BA/TA (%)	95.2±2.39	99.4±0.6**	99.3±0.6**	98.2±1.3**
Mandible				
BVF (%)	79.3±1.43	87.3±2.23**	79.5±5.09[a]	81.8±4.13[a]
Femur trabecular bone (metaphyses)				
BVF (%)	7.46±5.16	5.97±1.25	5.69±1.53	7.67±4.61
Trabecular number(N/mm)	3.77±0.43	4.23±0.35	4.09±0.47	4.03±0.56
Trabecular thickness (μm)	39.7±7.10	38.3±1.91	38.3±2.54	44.37±8.19
Trabecular Spacing (μm)	268±39.4	237±21.7	246±29.5	252±33.7
Femur trabecular bone (diaphyses)				
Bone volume (mm^3)	0.003±0.003	0.204±0.082**	0.086±0.085[a]	0.01±0.017[a]
BV/TV (BVF%)	0.14±0.142	4.172±1.78 **	1.99±1.961[a]	0.49±0.907[a]
Femur cortical bone (diaphyses)				
Subperiosteal area (mm^2)	1.48±0.19	2.24±0.37**	2.29±0.20**	1.57±0.30[ab]
Subendosteal area (mm^2)	0.71±0.14	1.37±0.27**	1.36±0.18**	0.84±0.24 [ab]
Cortical porosity (%)	0.32±0.27	2.98±1.60**	3.07±1.24 **	0.85±1.59[ab]
Tissue density (mg/cm^3 HA)	1221±13.94	1166±24.06**	1168±18.92**	1217±34.15[ab]

BM: calvarial total bone marrow space; BA: calvarial bone area; TA: calvarial total area; BV: bone volume; TV: total volume; BVF: BV/TV.

Data are mean±SD. * $p<0.05$, ** $p<0.01$ *: Significant difference compared to $Ank^{+/+} \rightarrow Ank^{+/+}$ group a: significant difference compared to $Ank^{KI/KI} \rightarrow Ank^{KI/KI}$ group; b: significant difference compared to $Ank^{+/+} \rightarrow Ank^{KI/KI}$ group

FIGURE LEGENDS

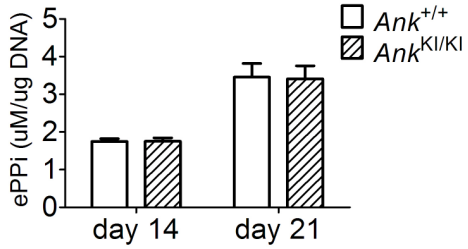
Supplementary Figure 1. Extracellular pyrophosphate (ePPi) assays in $Ank^{+/+}$ and $Ank^{KI/KI}$ day-14 and day-21 mCOB cultures. *Student's t-test* shows no significant differences between two groups at either time points.

Supplementary Figure 2. (A) Expression analysis of *Ank* in RAW 264.7 cells, sorted osteoclasts and bone marrow-derived macrophages (BMM) by RT-PCR. RANKL (30ng/ml; R&D system) was used to stimulate osteoclast differentiation. Cells grown in medium without RANKL treatment remain osteoclast precursors. GAPDH served as loading control. (B) Representative plots of sorted $CD11b^{-/low}CD45R^{-}CD3^{-}$ (triple negative population, TN) in the context of C-fms (CD115) and C-kit (CD117) from freshly isolated $Ank^{+/+}$ and $Ank^{KI/KI}$ bone marrow cells. The P6 population ($CD11b^{-/low}CD45R^{-}CD3^{-}$ C-fms/$CD115^{high}$C-kit/$CD117^{high}$) contains mostly cells with osteoclastogenic activity (Jacquin et al., 2006). No significant difference in the ratio of P6 cells to TN population was found between $Ank^{+/+}$ and $Ank^{KI/KI}$ bone marrow ($p=0.611$). Data shown are mean ± SD from three independent experiments.

Supplementary Figure 3. (A) qPCR gene expression analysis of *Mcsf, Rankl* and *Opg* in $Ank^{+/+}$ and $Ank^{KI/KI}$ mCOBs in differentiation media for 7 and 14 days. Expression levels of genes of interest were normalized to 18S RNA. $Ank^{+/+}$ day 7 samples were used as calibrators. No significant difference in the levels of *Mcsf*, *Rankl* and *Opg* was found between $Ank^{+/+}$ and $Ank^{KI/KI}$ cultures. (B) RANKL concentration in conditioned media from mouse calvarial osteoblast (mCOB) and bone marrow-derived macrophage (BMM) cocultures was measured by ELISA (Mouse TRANCE/RANK Ligand/TNFSF 11 Immunoassay, Quantikine, R&D System). Media were changed every three days and conditioned media were collected from mCOB- BMM cocultures at day 9. No significant difference was found among four groups. Statistics performed

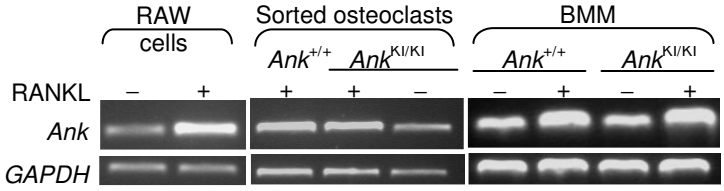
by one-way ANOVA. (C) Resorption assay evaluated after 12 days of plating mCOB-BMM cocultures on calcium-phosphate coated slides. *Ank*$^{KI/KI}$ mCOB- *Ank*$^{KI/KI}$ BMM cocultures with or without RANKL treatment (0, 10 or 30 ng/ml) resorbed significantly less mineral than *Ank*$^{+/+}$ mCOB-*Ank*$^{+/+}$BMM cocultures (* $p<0.01$ shown by one-way ANOVA). No significant difference was seen in the *Ank*$^{KI/KI}$ mCOB- *Ank*$^{KI/KI}$ BMM cocultures with or without RANKL treatment.

Supplementary Fig.1



Supplementary Fig. 2

A



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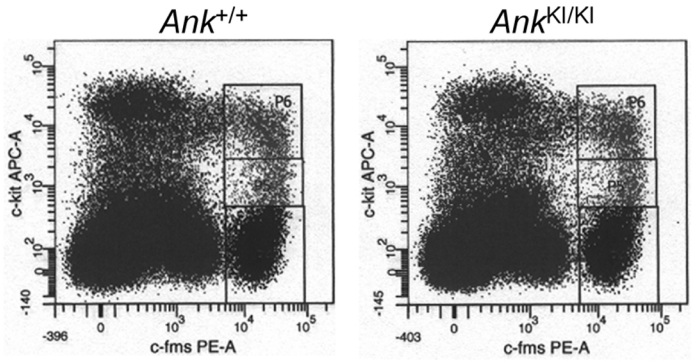
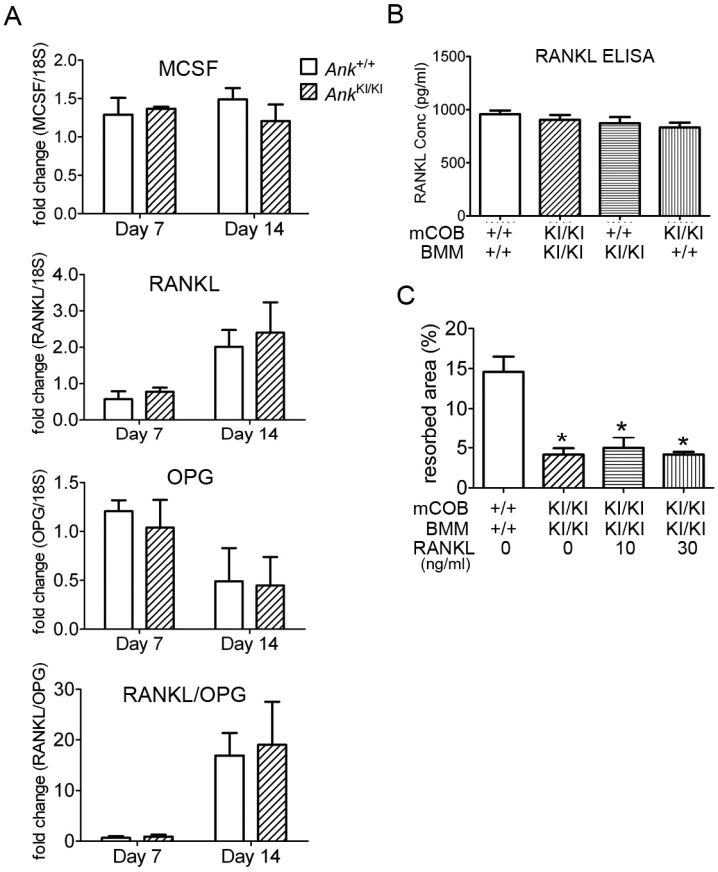


	Ank$^{+/+}$	*Ank*$^{KI/KI}$
% of P6 in TN	1.43 ± 0.40	1.56 ± 0.11

Supplementary Fig. 3



References:

Jacquin, C., Gran, D.E., Lee, S.K., Lorenzo, J.A., and Aguila, H.L. (2006). Identification of multiple osteoclast precursor populations in murine bone marrow. *J. Bone Miner. Res.* ***21***, 67-77.