Figure S1

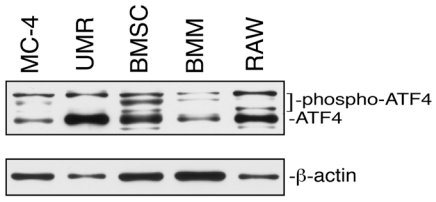

Figure S2

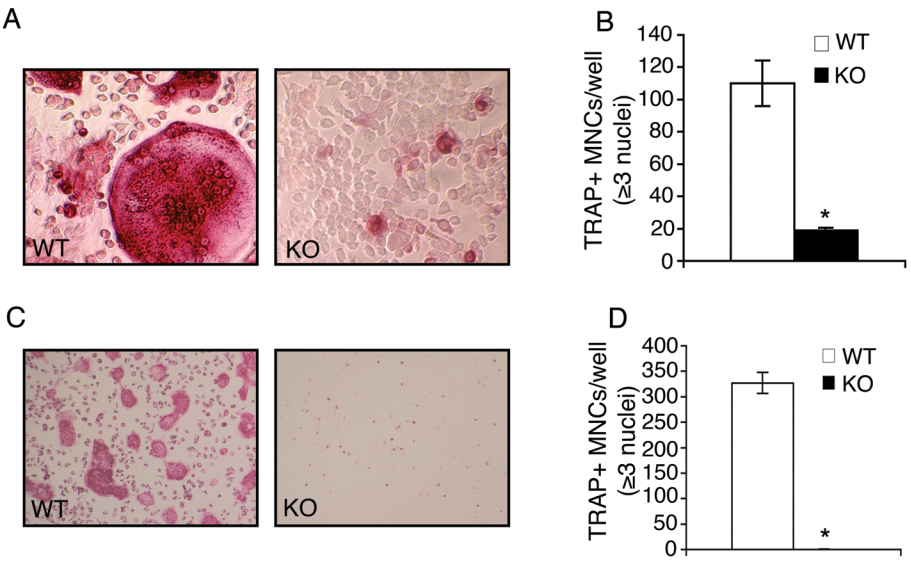
A
WT
KO
B
TRAP+ MNCs/well (≥3 nuclei)
140
120
100
80
60
40
20
0
WT
KO
*
C
WT
KO
D
TRAP+ MNCs/well (≥3 nuclei)
400
350
300
250
200
150
100
50
0
WT
KO
*

Figure S3

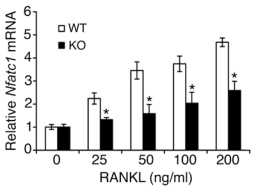

Figure S4

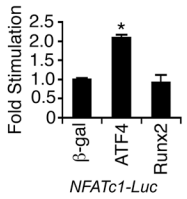

Figure S5

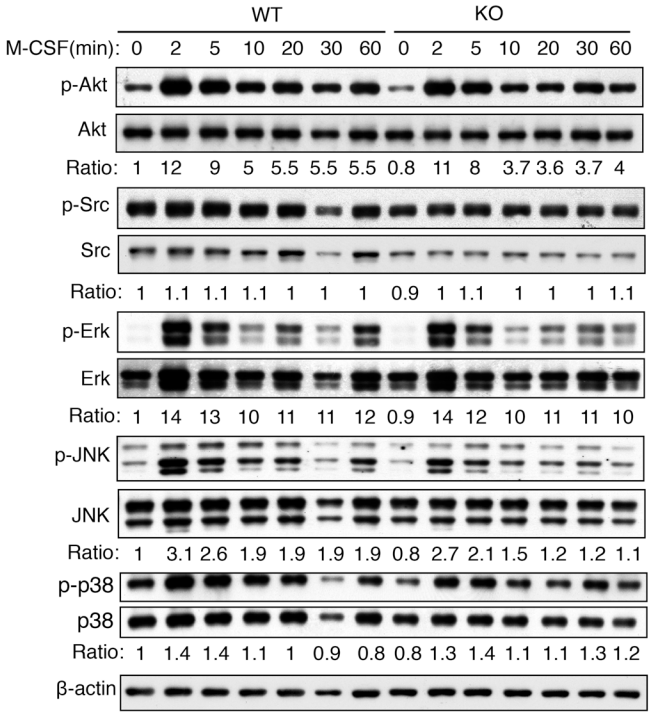
WT
KO
M-CSF(min): 0 2 5 10 20 30 60 0 2 5 10 20 30 60
p-Akt
Akt
Ratio: 1 12 9 5 5.5 5.5 5.5 0.8 11 8 3.7 3.6 3.7 4
p-Src
Src
Ratio: 1 1.1 1.1 1.1 1 1 1 0.9 1 1.1 1 1 1 1.1
p-Erk
Erk
Ratio: 1 14 13 10 11 11 12 0.9 14 12 10 11 11 10
p-JNK
JNK
Ratio: 1 3.1 2.6 1.9 1.9 1.9 1.9 0.8 2.7 2.1 1.5 1.2 1.2 1.1
p-p38
p38
Ratio: 1 1.4 1.4 1.1 1 0.9 0.8 0.8 1.3 1.4 1.1 1.1 1.3 1.2
β-actin

Figure S6

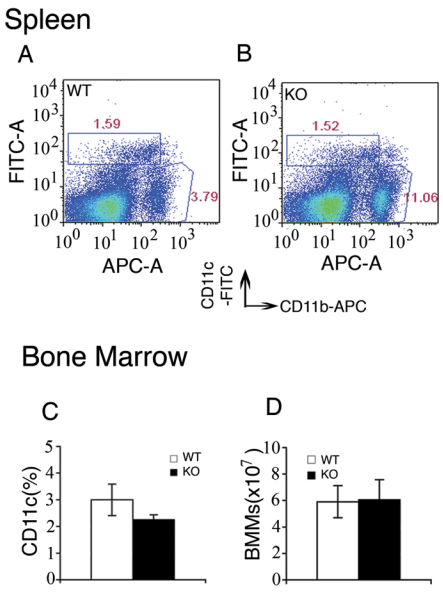
Spleen
A
B
WT
KO
1.59
3.79
1.52
11.06
FITC-A
APC-A
CD11c -FITC
CD11b-APC
Bone Marrow
C
D
CD11c(%)
BMMs(x10^7)
WT
KO

Table S1: real-time PCR primers

Name	5’ primer	3’ primer
Atf4	GAGCTTCCTGAACAGCGAAGTG	TGGCCACCTCCAGATAGTCATC
Cat K	AATACGTGCAGCAGAACGGAGGC	CTCGTTCCCCACAGGAATCTCTCTGTAC
c-Fms	CCTCCTCTGGTCCTGCTGCTGG	GCTCACACATCGCAGGGTCACC
Gapdh	CAGTGCCAGCCTCGTCCCGTAGA	CTGCAAATGGCAGCCCTGGTGAC
Mmp9	TGCCCTGGAACTCACACGACATCTTC	TGCCCTGGAACTCACACGACATCTTC
Nfatc1	CCCCATCCGCCAGGCTACA	GGTTGTCTGCACTGAGCCAACTCC
PU.1	CTCCAGGTGTACCCCCCAGAGG	CTCCAGGGCGGGGCTGTC
Rank	AGAGGGGAGCCTCAGGGTCC	AAGTTCATCACCTGCCCGCTAGA
Trap	CACTCCCACCCTGAGATTTGTG	ACGGTTCTGGCGATCTCTTTG

Legends for Supplementary Figures:

Fig. S1. ATF4 is expressed in OCL-like cells. Mouse MC-4 preosteoblastic cells, rat UMR106-01 osteoblastic cells, primary mouse bone marrow stromal cells (BMSCs), RAW264.7 cells (a mouse monocyte/macrophage cell line), and primary mouse bone marrow-derived monocytes (BMMs) were cultured in 10%FBS media for 24 h. Whole cell extracts were used for Western blot analysis for ATF4.

Fig. S2. TRAP staining in CD11b$^+$ BMMs and BMMs from aged mice. (A) TRAP staining. Purified CD11b$^+$ BMMs from 4-week-old wt and *Atf4*$^{-/-}$ mice were differentiated for 5d followed by TRAP staining and TRAP-positive multinucleated cells (MNCs) were scored. (**B**) Statistical analysis of TRAP staining in (A). Magnification: 100X. **(C**) TRAP staining. Primary BMMs from 15-month-old wt and *Atf4*$^{-/-}$ mice were differentiated for 5d followed by TRAP staining. (**D**) Statistical analysis of TRAP staining in (C). Magnification: 40X.

Fig. S3. RANKL induction of *Nfatc1* mRNA expression in wt and *Atf4*$^{-/-}$ BMMs (dose-response). Wt and *Atf4*$^{-/-}$ BMMs were treated with indicated concentrations of RANKL (25-200 ng/ml) for 7d followed by quantitative real-time RT/PCR for *Nfatc1* mRNA. *Gapdh* mRNA was used for loading. $^*P<0.01$ wt vs. *Atf4*$^{-/-}$).

Fig. S4. ATF4 but not Runx2 activates the *Nfatc1 P1* promoter. COS-7 cells were transfected with 0.125 μg 0.8-kb *Nfatc1*-luc and 1.0 ng pRL-SV40 with 1.0 μg expression plasmid for ATF4 or Runx2. *P<0.01 (β-gal vs. ATF4).

Figure S5. Effects of M-CSF on activation of the AKT, Src, and MAPKs pathways in wt and *Atf4*$^{-/-}$ BMMs. Wt and *Atf4*$^{-/-}$ BMMs were cultured in proliferation medium for 3 d and switched to 2% FBS α-MEM without M-CSF overnight. Cells were then exposed to 30 ng/ml M-CSF for the indicated times. Cells were then lysed, fractionated by SDS–PAGE, and analyzed by Western blot analysis using antibodies recognizing phosphorylated and total ERK1/2, p38, JNK, AKT, and Src. β-actin served as the loading control. Similar results were obtained from three independent experiments.

Fig. S6. *Atf4*$^{-/-}$ mice display an increase in macrophage. (**A-B**) Flow cytometry. Splenocytes from wt and *Atf4*$^{-/-}$ mice were stained with CD11b-APC and CD11c-FITC antibodies and analyzed with flow cytometry as described in Experimental Procedures. (**C**) CD11c^{+} cells were purified and counted from wt and *Atf4*$^{-/-}$ BMMs as described in Experimental Procedures. (**D**) Total nucleated bone marrow cells were counted from long bones (two femurs and two tibias) from 6-week-old wt and *Atf4*$^{-/-}$ mice (6 mice per group).