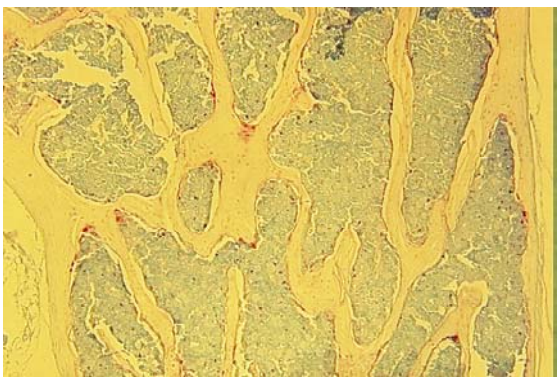
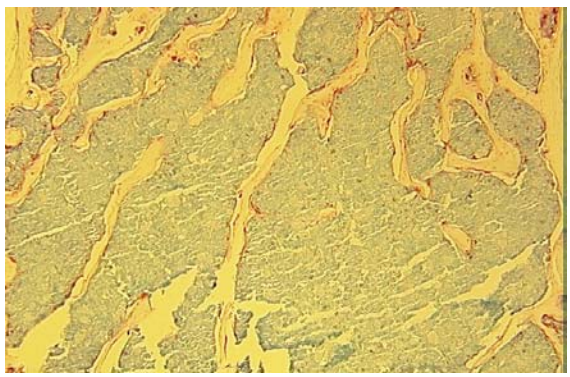
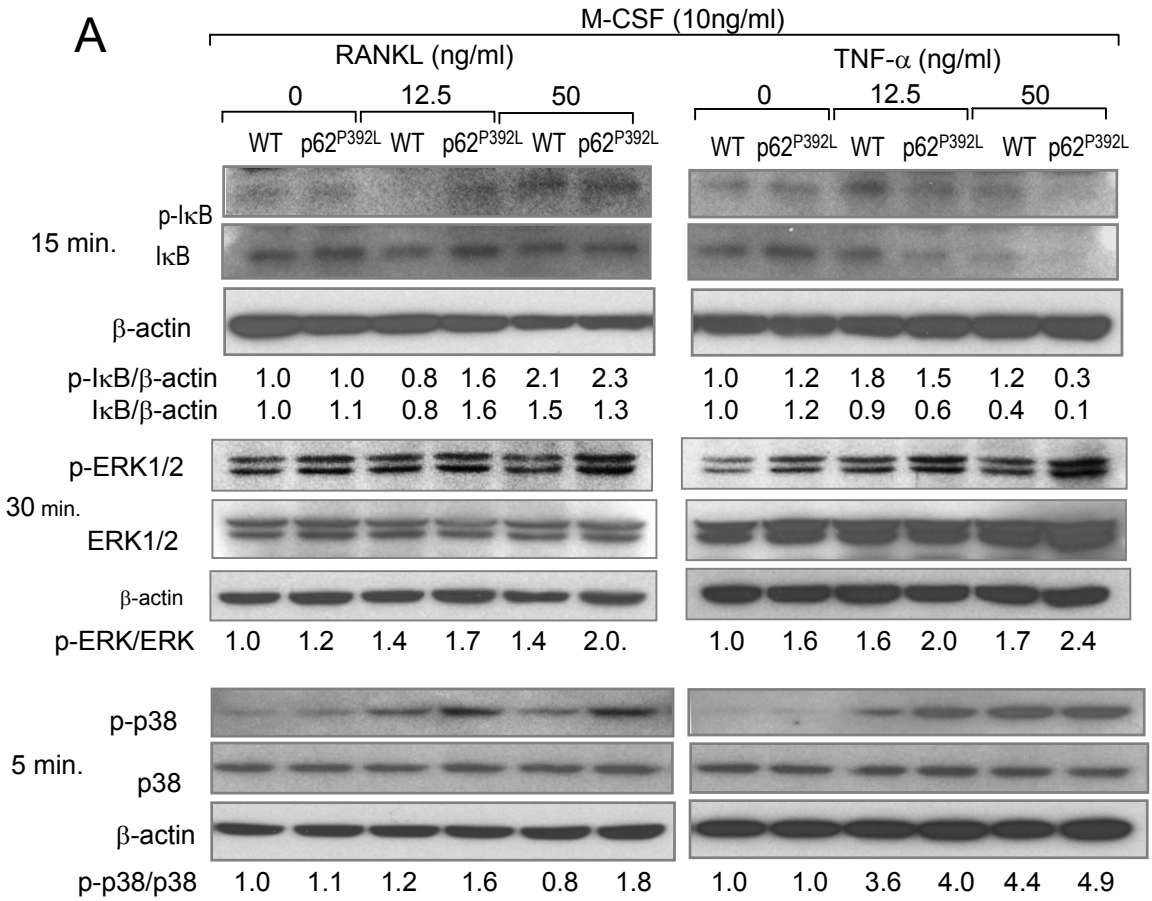
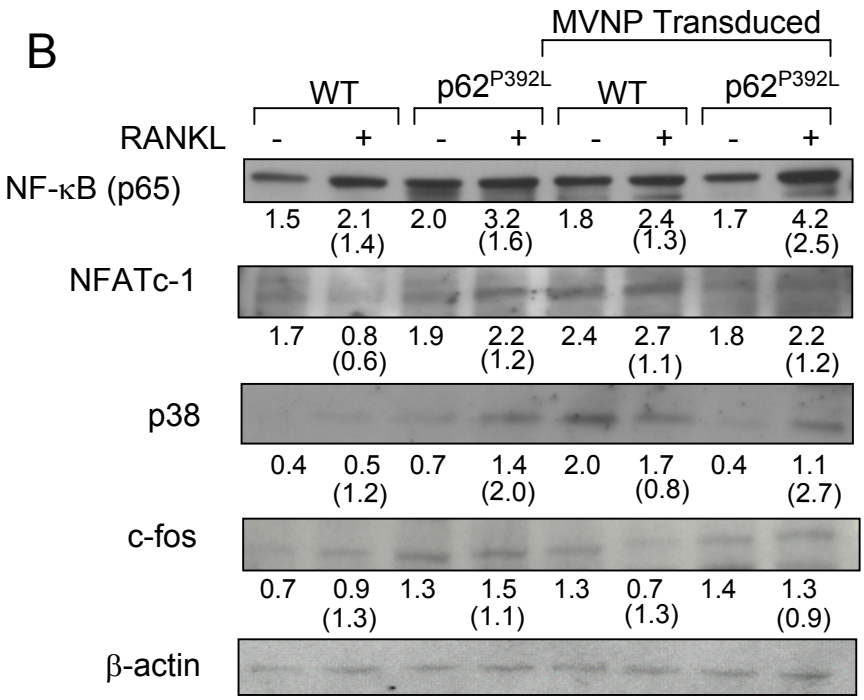
A



B




A
M-CSF (10ng/ml)
RANKL (ng/ml)
TNF-α (ng/ml)
0
12.5
50
WT
p62P392L
15 min.
p-IκB
IκB
β-actin
p-IκB/β-actin 1.0 1.0 0.8 1.6 2.1 2.3 1.0 1.2 1.8 1.5 1.2 0.3
IκB/β-actin 1.0 1.1 0.8 1.6 1.5 1.3 1.0 1.2 0.9 0.6 0.4 0.1
p-ERK1/2
30 min.
ERK1/2
β-actin
p-ERK/ERK 1.0 1.2 1.4 1.7 1.4 2.0. 1.0 1.6 1.6 2.0 1.7 2.4
p-p38
5 min.
p38
β-actin
p-p38/p38 1.0 1.1 1.2 1.6 0.8 1.8 1.0 1.0 3.6 4.0 4.4 4.9


B
MVNP Transduced
WT
p62P392L
WT
p62P392L
RANKL
- + - + - + - +
NF-κB (p65)
1.5 2.1 (1.4) 2.0 3.2 (1.6) 1.8 2.4 (1.3) 1.7 4.2 (2.5)
NFATc-1
1.7 0.8 (0.6) 1.9 2.2 (1.2) 2.4 2.7 (1.1) 1.8 2.2 (1.2)
p38
0.4 0.5 (1.2) 0.7 1.4 (2.0) 2.0 1.7 (0.8) 0.4 1.1 (2.7)
c-fos
0.7 0.9 (1.3) 1.3 1.5 (1.1) 1.3 0.7 (1.3) 1.4 1.3 (0.9)
β-actin

Supplemental Figure 1. Histologic studies of bones from TRAP-p62^{P392L} mice. Vertebral cancellous bone from wild type littermates (**A**) or TRAP-p62^{P392L} mice (**B**). Note increased osteoclast perimeter (red stain), reduced cancellous bone volume, fewer and thinner trabeculae, and loss of trabecular connectivity in TRAP-p62^{P392L} bone.

Supplemental Figure 2.

(A) RANKL and TNF-α increase phospho-IκBα, ERK and p38MAPK in OCL precursors from TRAP-p62^{P392L} and wild type mice. OCL precursors (5×10^5 cells/well) from TRAP-p62^{P392L} and wild type mice were pretreated with M-CSF (10 ng/ml) for 4 days. Cells were then exposed to RANKL or TNF-α for the indicated periods. Cells were lysed, fractionated by SDS-PAGE, and analyzed by immunoblot using antibodies recognizing phosphorylated and total signaling molecules, and the ratios were shown. β-actin served as the loading control.

(B) Expression of signaling molecules by OCL precursors from MVNP or EV transduced TRAP-p62^{P392L} and WT mice. Cell lysates were obtained from day 4 CFU-GM-derived cells and were immunoblotted using anti- NF-κB (p65), NFATc-1, p38MAPK or c-fos rabbit polyclonal antibody. The ratios with β-actin are shown.