Supplementary Material

Supplemental Results:

Analysis of apoptosis in *cathepsin* $K^{+/+}$ wild type mice and *cathepsin* $K^{-/-}$ mutant mice in the presence of various survival factors

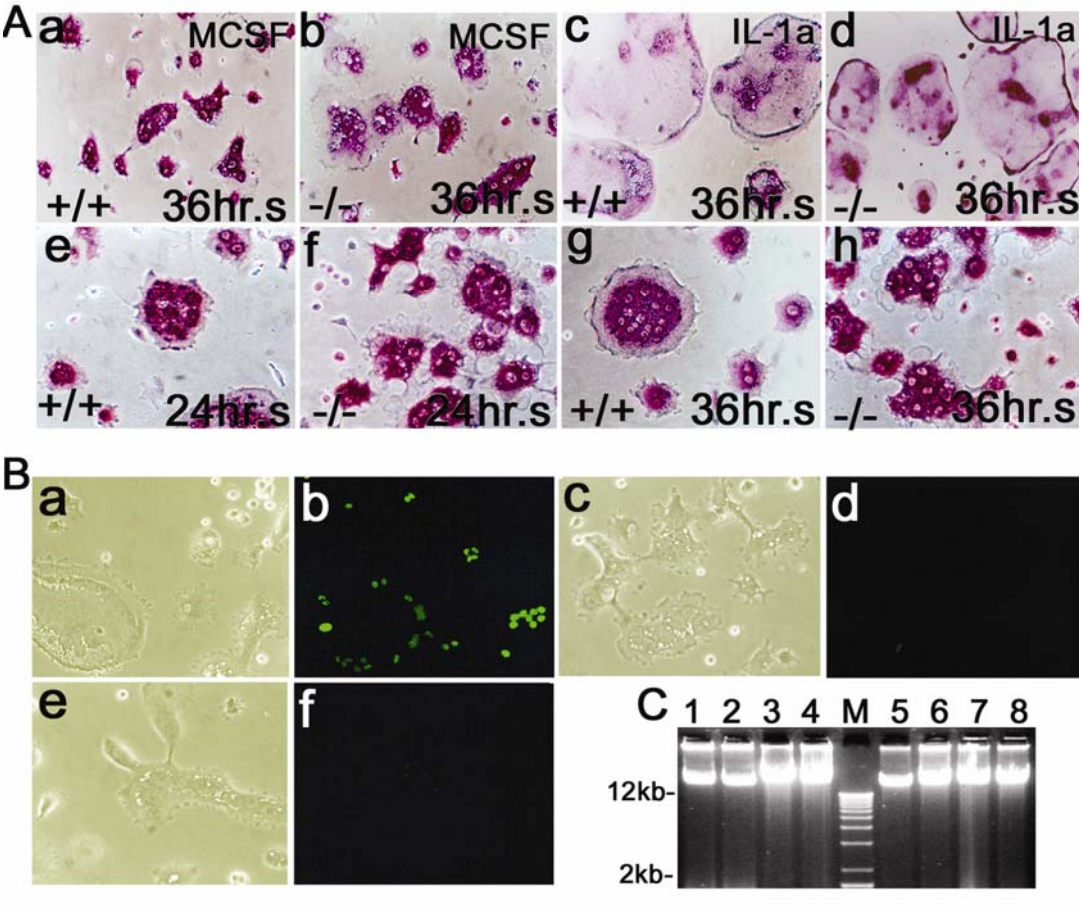
To mimic *in vivo* conditions, we re-examined the apoptosis status of *cathepsin* $K^{-/-}$ osteoclasts and wild type osteoclasts in the presence of M-CSF or IL-1α. The cytokines IL-1α and M-CSF were shown to prolong the survival of purified OCLs. No apoptosis bodies were found in either the *cathepsin* $K^{+/+}$ OCL cultures (Fig. S1Aa,c) or in the *cathepsin* $K^{-/-}$ OCL cultures (Fig. S1Ab,d). Subsequently, the conditioned medium obtained from the MS12 stromal cell culture was introduced into the OCL culture (Fig. S1Ae-h). TRAP staining showed no significant difference between cathepsin $K^{+/+}$ OCLs (Fig. S1Ae,g) and *cathepsin* $K^{-/-}$ OCLs (Fig. S1Af,h) after 24- and 36-hour cultures.

TUNEL experiments were performed to detect the apoptosis of OCLs. Cells strongly displayed blue fluorescence-labeled nuclei in the positive control provided in the kit (Fig. S1Ba,b). There was no detectable fluorescence in either the *cathepsin* $K^{+/+}$ OCLs (Fig. S1Bc,d) or *cathepsin* $K^{-/-}$ OCLs (Fig. S1Be,f). To further confirm that there was no apoptosis in the OCL conditioned medium culture, we analyzed DNA degradation in both *cathepsin* $K^{+/+}$ and *cathepsin* $K^{-/-}$ OCLs. No nuclear DNA degradation was found in DNA obtained from *cathepsin* $K^{+/+}$ OCLs (Fig. S1C lanes 1,3,5,7) or *cathepsin* $K^{-/-}$ OCLs (Fig. S1C lanes 2,4,6,8), which were cultured for 6, 12, 24, and 36 hrs, respectively. These results provide strong evidence that apoptosis may not be the major osteoclast mortality mechanism *in vivo*. There must be other mechanisms that control osteoclast homeostasis.

Supplemental Figure Legend:

Figure S1. (A) Apoptosis analysis of OCLs derived from *cathepsin* $K^{+/+}$ wild type mice and *cathepsin* $K^{-/-}$ mice in the presence of various survival factors. (Aa,b) Analysis of OCL apoptosis in the presence of M-CSF. OCLs derived from either the *cathepsin* $K^{+/+}$ or *cathepsin* $K^{-/-}$ mice did not show apoptosis bodies in the presence of M-CSF. (Ac,d) Analysis of OCL apoptosis in the presence of IL-1α. Neither OCLs derived from the *cathepsin* $K^{+/+}$ or from *cathepsin* $K^{-/-}$ mice showed apoptosis bodies in the presence of IL-1α. (Ae-h) TRAP staining of the *cathepsin* $K^{+/+}$ OCLs (Ae,g) and *cathepsin* $K^{-/-}$ OCLs (Af,h) in stromal culture suggests no apoptosis occurring in either. (B) DNA fragmentation analyses. (Ba,b) The fluorescence detected in osteoclast cells stimulated by RANKL indicates that apoptosis occurred. (Bc-f) Fluorescence cannot be detected in *cathepsin* $K^{-/-}$ and *cathepsin* $K^{+/+}$ OCLs in stromal culture. (C) DNA from *cathepsin* $K^{+/+}$ OCLs (lanes 1,3,5,7) and *cathepsin* $K^{-/-}$ OCLs (lanes 2,4,6,8) cultured for 6, 12, 24, and 36 hrs, respectively, was electrophorated for DNA ladder analysis, indicating intact DNA in each OCL. N=6.

Figure S1


A
a MCSF +/+ 36hr.s
b MCSF -/- 36hr.s
c IL-1a +/+ 36hr.s
d IL-1a -/- 36hr.s
e +/+ 24hr.s
f -/- 24hr.s
g +/+ 36hr.s
h -/- 36hr.s
B
a
b
c
d
e
f
C
1 2 3 4 M 5 6 7 8
12kb-
2kb-