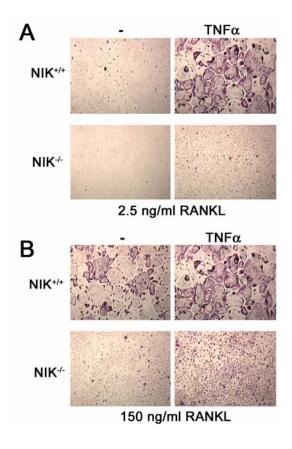


Supplemental Figure 1– RAG transfer flow cytometry. Splenocytes were isolated from $RAG2^{-/-}$ recipients of $NIK^{+/+}$ or $NIK^{-/-}$ splenocytes at the time of sacrifice (day 32), and stained with antibody to CD90. Dotted lines show CD90+ cells, solid lines show staining of splenocytes from an unmanipulated $RAG2^{-/-}$ mouse as negative control. The number of engrafted T cells is similar in recipients of both $NIK^{+/+}$ and $NIK^{-/-}$ cells.



Supplemental Figure 2 – Osteoclast culture in RANKL and TNF α .

Bone marrow macrophages (5 x 10^3 /well in 96-well plate) were differentiated in the presence of RANKL at 2.5 ng/ml (A) or 150 ng/ml (B), with or without added TNF α at 10 ng/ml. Media was changed daily, and cultures were fixed and stained for TRAP on day 5. (A) While TNF α potently induces osteoclastogenesis in $NIK^{+/+}$ cultures with low dose RANKL, there is little effect on $NIK^{-/-}$ cultures at this dose. (B) With high dose RANKL, TNF α has little effect in the presence of NIK, where osteoclastogenesis is already optimal. In these conditions, $NIK^{-/-}$ cultures show a predominance of TRAP+ mononuclear cells, with some small multinucleated cells. Pale stained OCs (ghosts) in $NIK^{+/+}$ cultures are apoptotic, due to cytokine levels above optimal. 100x.