SUPPLEMENTAL MATERIAL

Def6 restrains osteoclastogenesis and inflammatory bone resorption

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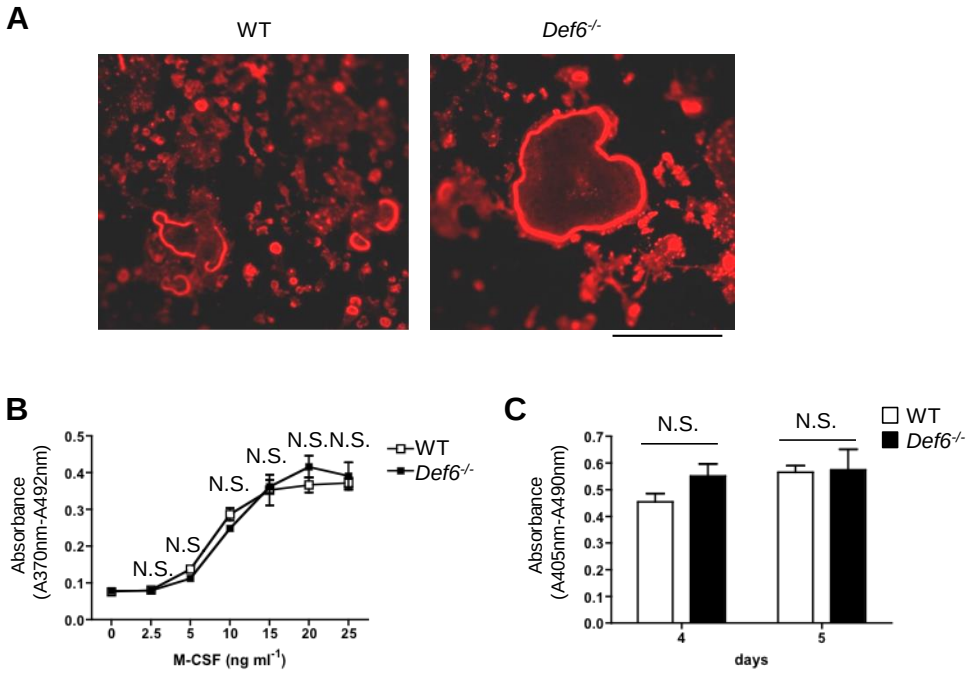
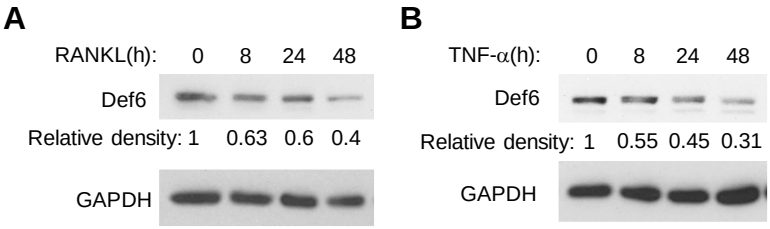
Supplementary Fig. 1



Supplementary Figure 1. (A) Actin ring formation in osteoclasts. The BMMs derived from WT and *Def6*$^{-/-}$ mice were treated with RANKL (40 ng/ml) for 4 days and the F-actin was labeled by rhodamine phalloidin. Scale bar: 100μm. (B) The BMMs derived from WT and *Def6*$^{-/-}$ mice were treated with M-CSF with the indicated doses for two days and the cell proliferation was analyzed using a BrdU proliferation kit as described in the method. (C) The BMMs derived from WT and *Def6*$^{-/-}$ mice were treated with RANKL for 4 or 5 days to induce the formation of mature osteoclasts, which were then starved by culturing in the absence of M-CSF and RANKL for five hours. The cell apoptosis was indicated by the relative amount of the cytoplasmic histone-associated DNA fragments that were measured by the absorbance (A405nm-A490nm) as described in the method. N.S., not statistically significant.

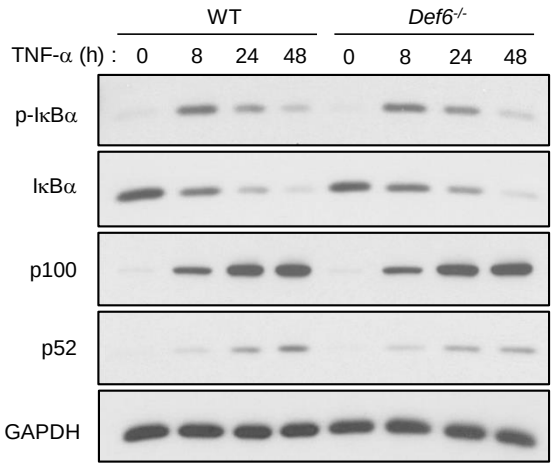
Supplementary Fig. 2



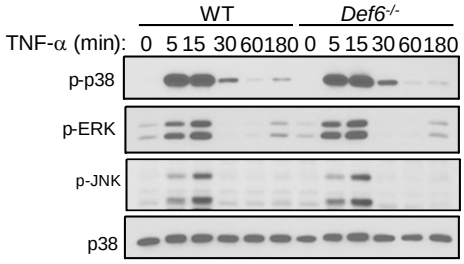
Supplementary Figure 2. Downregulation of Def6 expression in BMMs by TNF-α or RANKL. The WT BMMs was treated with TNF-α (40 ng/ml) or RANKL (40 ng/ml) for the indicated times and immunoblot analysis was performed to detect Def6 expression. GAPDH was used as a loading control. Quantification of the immunoblot bands of Def6 indicated by relative density determined by densitometry was shown below each Def6 band.

Supplementary Fig. 3

A

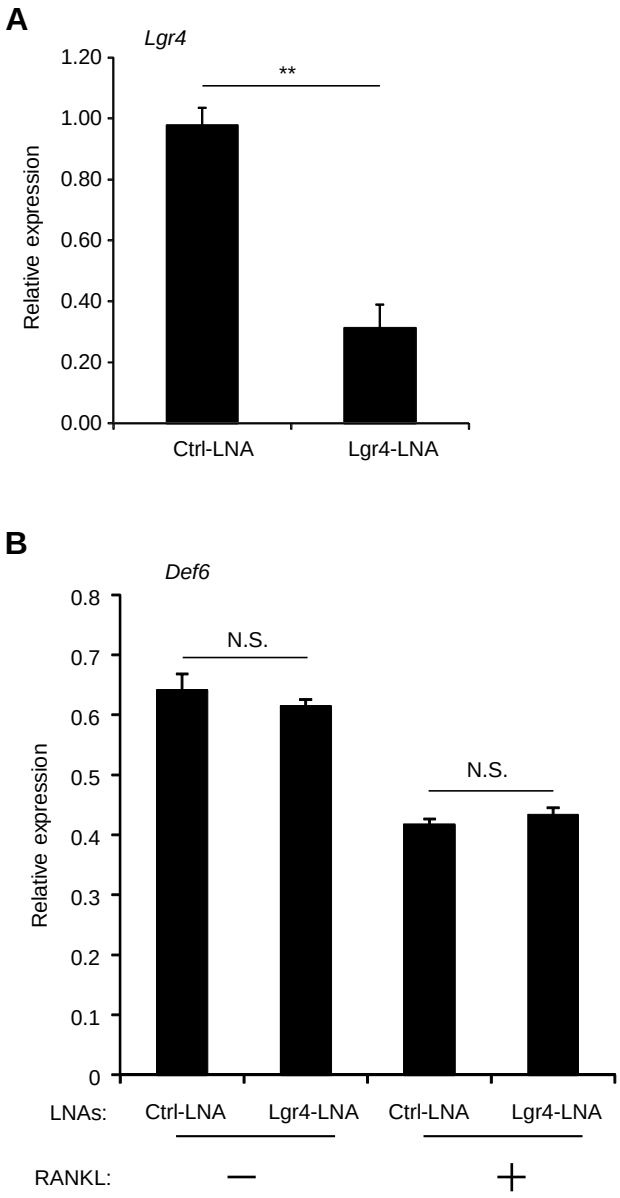


B



Supplementary Figure 3. Def6 deficiency does not affect NF-κB or MAPK signaling pathways during osteoclast differentiation induced by TNF-α. (A) Immunoblot analysis of canonical NF-κB signaling components p-IκB and IκB as well as non-canonical NF-κB signaling components P100 and p52. GAPDH was used as a loading control. (B) Immunoblot analysis of p-p38, p-ERK and p-JNK. Total p38 and ERK were used as loading controls. The concentration of TNF was 40 ng/ml.

Supplementary Fig. 4



Supplementary Figure 4. Knockdown of Lgr4 does not affect Def6 expression. The WT BMMs were transfected with the Lgr4-locked nucleic acid (LNA) or non-targeting control LNA for 24 hours to knockdown Lgr4 expression. The knockdown efficiency of Lgr4 was analyzed by RT-PCR (A). After the knockdown of Lgr4, the cells were treated without or with RANKL (40 ng/ml) for 48 hours and Def6 expression was analyzed by RT-PCR (B). Data are representative of 3 independent experiments. *p<0.01; N.S., not statistically significant.