

**Knockout and Double Knockout of Cathepsin K and Mmp9 reveals a novel  
function of Cathepsin K as a regulator of osteoclast gene expression and bone  
homeostasis**

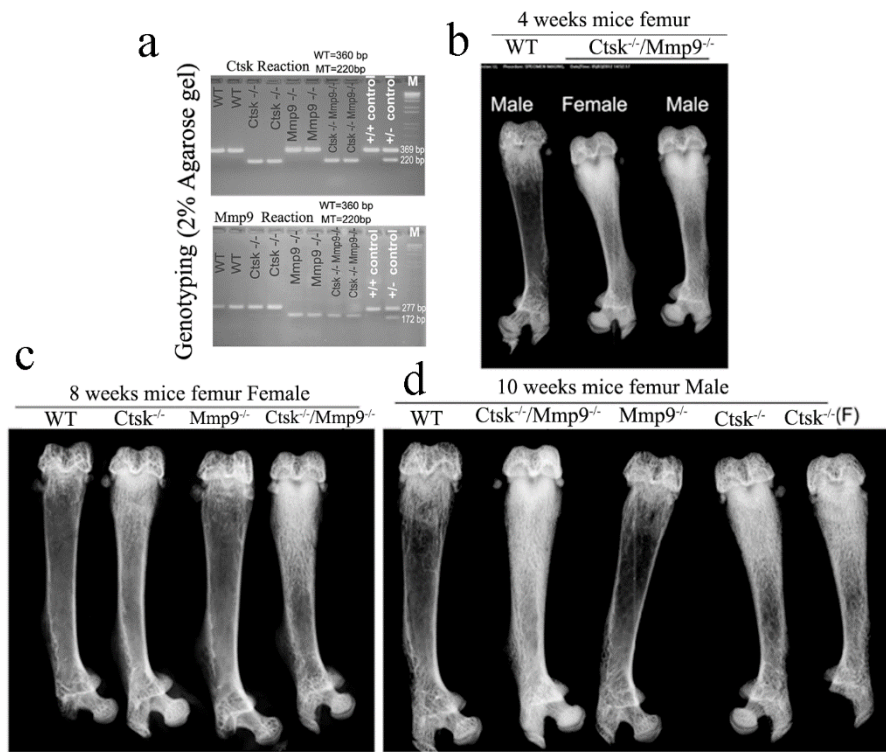
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Jinwen Wang<sup>3</sup>, Matthew McConnell<sup>2</sup>, Shuying Yang<sup>4</sup>, Yang Li<sup>4</sup>, Zhijie Chang<sup>1, \*</sup>, and  
Yi-Ping Li<sup>2, 3, \*</sup>

**Supplemental Material**

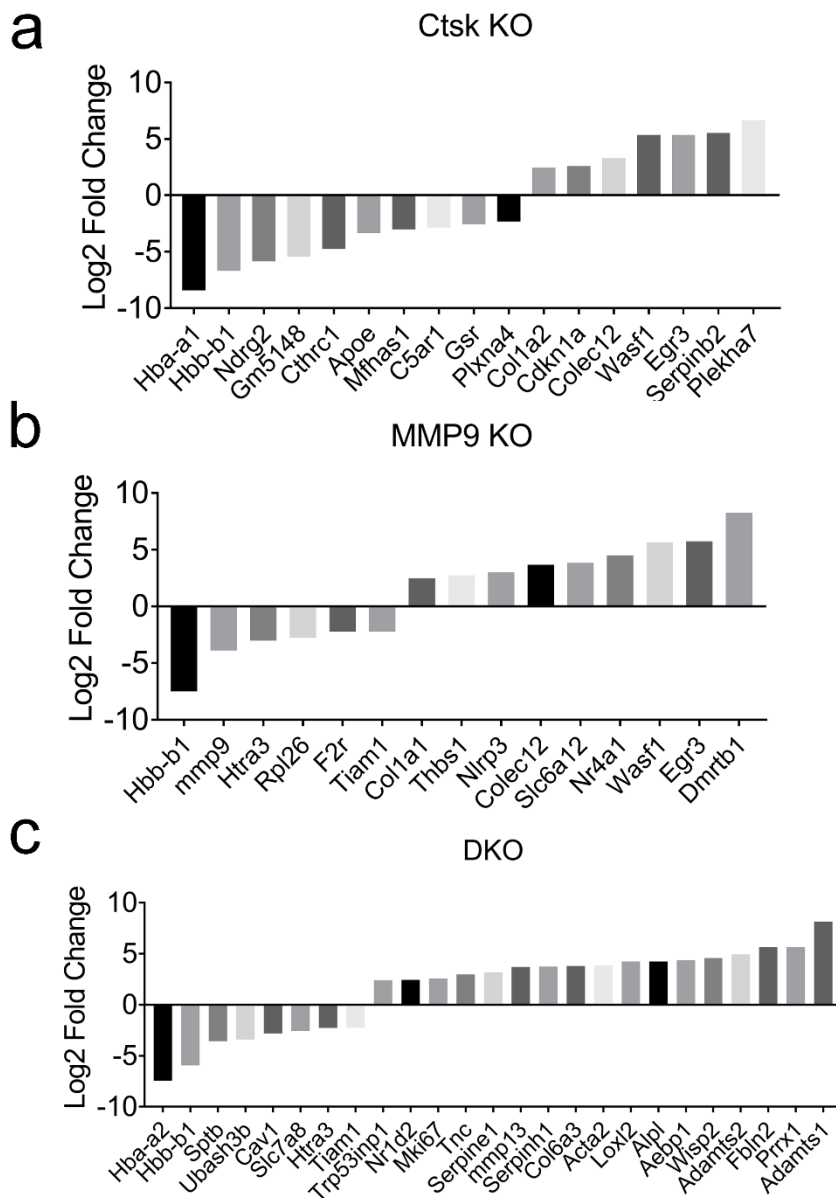
**Primers list for qPCR**

gene symbol	Forward primers (5'-3')	Reverse primers (5'-3')
c-Fos	CGGGTTTCAACGCCGACTA	TTGGCACTAGAGACGGACAGA
Hprt	GGTGGAGATGATCTCTCAACTTTAA	AGGAAAGCAAAGTCTGCATTGTT
NFATC1	TGCCTTTTGCGAGCAGTATCT	CAGGCAAGGATGGGCTCATAT
Pu.1	CCCCACACCGGCCTCAGTCACCAG	CCCCACGGCCCAGCACCTC
Cebp $\alpha$	CAAGAACAGCAACGAGTACCG	GTCACTGGTCAACTCCAGCAC
ATP6i	CACAGGGTCTGCTTACAACCTG	CGTCTACCACGAAGCGTCTC

## Supplemental Figures and Figure Legends

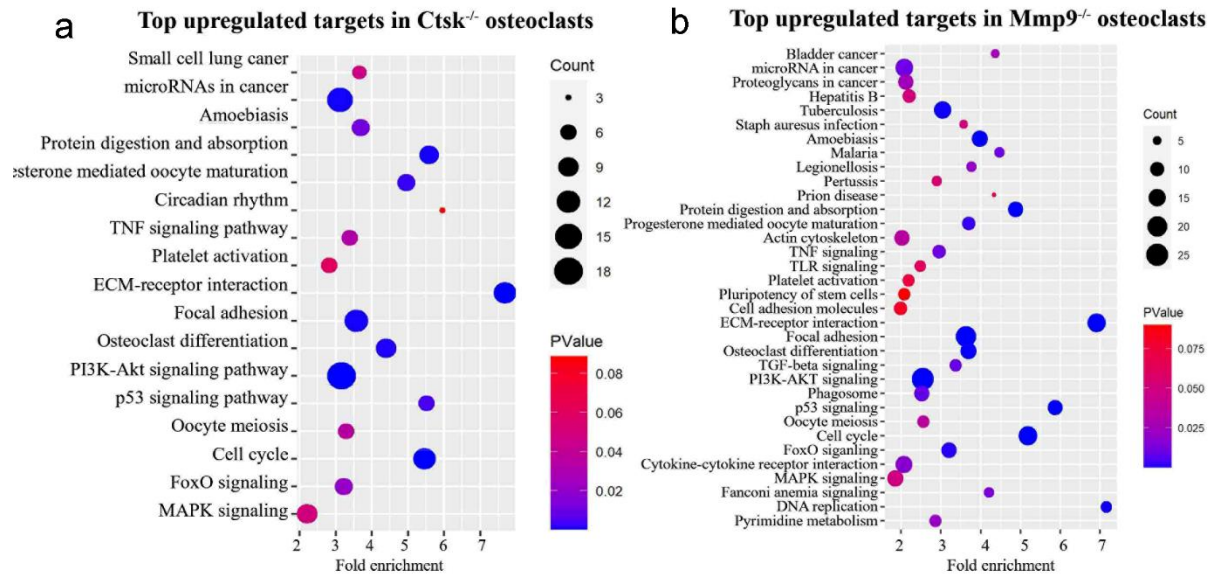


**Supplemental Figure 1. Double deletion of *Mmp9* and *Ctsk* causes increased bone mass and severe osteopetrosis in mice.** (a) PCR was used to determine the genotypes of the mice. (b-d) X-ray analysis of (b) 4-week old (c) 8-week-old, and (d) 10-week-old mouse femurs. Data showed representative images, n=10.



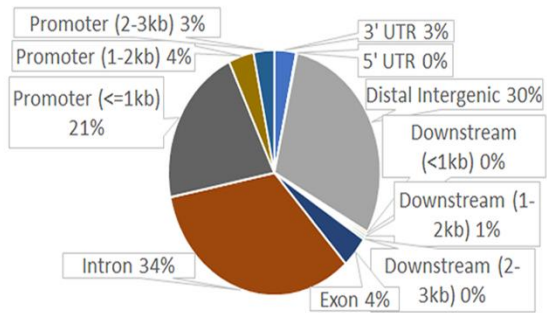
**Supplemental Figure 2. RNA-seq analysis of *Ctsk* and *Mmp9* modulated genes.**

Quantification data from volcano plot illustrating differentially regulated gene expression from RNA-seq analysis between the control (c) *Ctsk*<sup>-/-</sup>, (b) *Mmp9*<sup>-/-</sup> and (c) *Ctsk*<sup>-/-</sup>/*Mmp9*<sup>-/-</sup> osteoclasts from Figure 4a. Values are presented as the log<sub>2</sub> of tag counts.

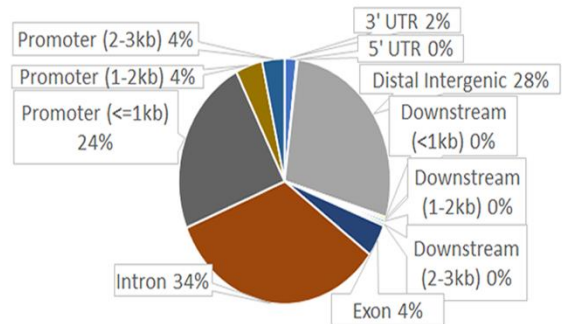


**Supplemental Figure 3. RNA-seq analysis of *Ctsk* and *Mmp9* modulated genes in osteoclast signaling.** Top upregulated signaling pathways in (a) *Ctsk*<sup>-/-</sup> and (b) *Mmp9*<sup>-/-</sup> osteoclasts, respectively, as analyzed by KEGG database.

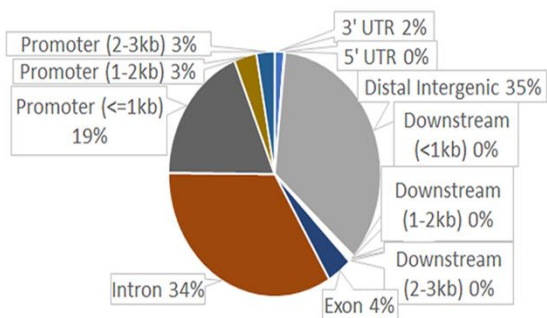
**a** Wild Type Osteoclasts (WT OC)



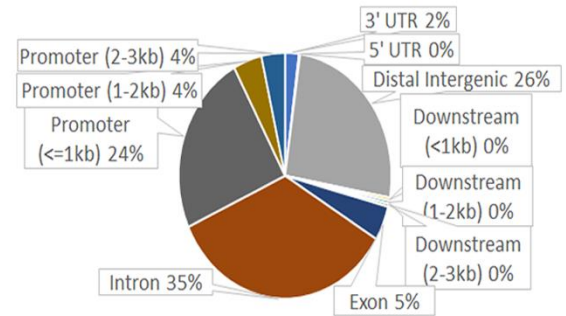
**b** *Ctsk*<sup>-/-</sup> Osteoclasts (*Ctsk* OC)



**c** *Mmp9*<sup>-/-</sup> Osteoclasts (*Mmp9* OC)



**d** *Ctsk*<sup>-/-</sup>; *Mmp9*<sup>-/-</sup> Osteoclasts (DKO OC)



**Supplemental Figure 4. ATAC-Seq Profiling of WT, *Ctsk*<sup>-/-</sup>, *Mmp9*<sup>-/-</sup>, and DKO**

**osteoclasts. (a-d)** Distribution of ATAC-seq THSs relative to genomic features in (a)

WT, (b) *Ctsk*<sup>-/-</sup>, (c) *Mmp9*<sup>-/-</sup>, and (d) *Ctsk*<sup>-/-</sup>; *Mmp9*<sup>-/-</sup> osteoclasts.