SUPPLEMENTARY FIGURES:

SUPPLEMENTARY FIGURE 1. Representative sections of bone tumors from WT (A-C) and CTSK KO (D-F) mice at 2, 4, and 6 weeks (n = 6 mice/group/time point). H&E sections showing section of the area of the tibia occupied by the tumor; tumor cells, purple; bone tissue, pink; magnification 40x.

SUPPLEMENTARY FIGURE 2. CTSK deficiency has no effect on subcutaneous tumor growth. Vertical scatter plot of tumor-weight values from subcutaneous tumors grown in WT and CTSK KO hosts. Data were analyzed by Mann-Whitney test. No significant differences (n.s) in tumor size were observed between the two hosts.

SUPPLEMENTARY FIGURE 3. CTSK expression in the bone tumor microenvironment is host derived. A: TaqMan RT-PCR analysis of host CTSK levels in control and tumor-bearing tibiae of WT and CTSK KO mice. CTSK transcripts are detected in control (blue bars) and tumor bearing (green bars) tibiae from WT mice. Only background levels of CTSK transcripts are detected in control (red bars) and tumor bearing (orange bars) tibiae from CTSK KO mice. Data were normalized to GUSB and HPRT1 and are the mean of 3 biological replicates (i.e., RNA from 3 mice) +/- SD. **B:** Comparison of CTSK transcript levels in murine bone marrow macrophages (BMMs) from WT mice and in human PC3, DU145, and C4-2B prostate cancer cells. Only background levels of murine CTSK transcripts are detected in human cell lines. Samples were run in triplicate, data were calculated based on C_T values and results are expressed as relative CTSK gene expression ± SD.

SUPPLEMENTARY FIGURE 4. CTSK KO cells differentiate more efficiently into osteoclasts but form fewer macrophages than WT cells. Non-adherent bone marrow cells were stimulated with

M-CSF (20 ng/ml) or M-CSF/RANKL (20 ng/ml each) for up to 8 days and TRAcP staining was performed. M-CSF treatment for 5 days resulted in fewer numbers of adherent macrophages from CTSK KO cells (**B, F)**, than WT cells **(A, E).** In the presence of RANKL, CTSK KO cells formed large osteoclasts (**D, H)** and their numbers were higher than those found for WT cells **(C, G)**. The size and osteoclast numbers for WT and CTSK KO cells further increased with 8-day treatment (**I, J**).

SUPPLEMENTARY FIGURE 5. Taqman Mouse Inflammation Array analysis of host (mouse) cytokines in response to PC3 tumor challenge. A: Heat map illustrating results for most highly differentially expressed genes. Samples were a pool of three biological replicates run in duplicate. Data were normalized to 18S and GUSB housekeeping genes using Data Assist software and shown as fold changes in tumor bone relative to control bone in the same host. **Red,** upregulated genes; **green,** downregulated genes. **B:** Table of fold changes in gene expression relative to control bone in the same host. COX-2 and CCL2 are among the top upregulated genes in the WT host.

SUPPLEMENTARY FIGURE 6. CTSK contributes to vascularization in the bone tumor microenvironment. A: Representative CD34-positive (left panels) and CD31-positive blood vessel staining of WT and CTSK KO prostate bone tumors 6 weeks post tumor implantation; magnification, 20x; arrows indicate blood vessels. **B:** Vertical scatter plot of CD-34 positive blood vessels/field in 10 tumor sections derived from WT and CTSK KO mice**.** Data were analyzed by Mann-Whitney test; $p < 0.001$ indicates high significance.

SUPPLEMENTARY FIGURE 7. Secretion of CCL2 and CTSB is enhanced upon interaction of ARCaP(M) and RM-1 prostate carcinoma cells with bone marrow macrophages. WT and CTSK KO BMMs were cultured alone (A) or Transwell system (T) with ARCaP(M) (**A, C**; left panels) or RM-1 cells (**A, C**; right panels). Lysates and media were collected for immunoblot analyses of

secreted CCL2 and CTSB**.** Samples were loaded based on DNA in cell lysates. ß-actin was used as a loading control. Data are representative of three experiments. Densitometric analysis of secreted BMM-derived **(B)** and tumor cell-derived **(D)** CCL2 (**top**) and CTSB (**bottom**) normalized to ß -actin in corresponding cell lysates. **B:** Black bars, BMM alone; off white bars, BMM in transwell co-culture with ARCaP(M) cells; and grey bars, BMM in transwell co-culture with RM-1 cells. **D:** Black bars, Tumor cells alone; off white bars, tumor cells in transwell co-culture with WT BMMs; and grey bars, tumor cells in transwell co-culture with CTSK KO BMMs. Data are expressed as % control (tumor cells alone) of AU/mm^2 (arbitrary units per square millimeter), and are representative of at least three replicate samples/condition.