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Movie S7, related to Figure 4**.** MT1-MMP-null bone marrow myeloid progenitors infected with pRETRO-MT1-MMP E240A and cultured in the presence of M-CSF and RANKL for 4 days.

SUPPLEMENTAL DATA

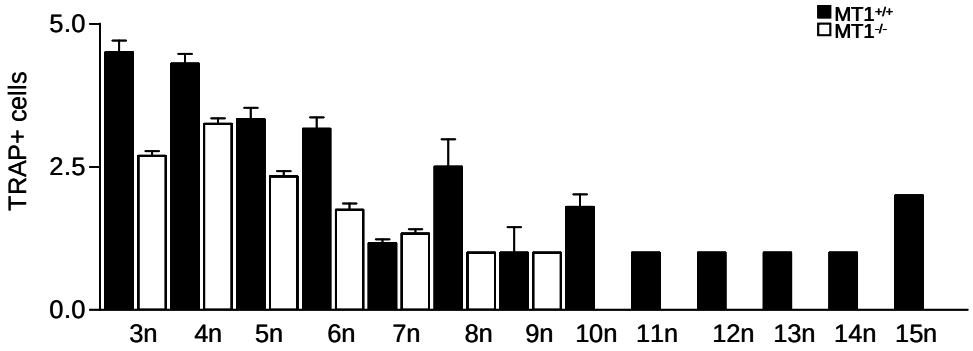

Figure S1, related to Figure 1. Distribution of nuclei in OC obtained from bone marrow cells. Bone marrow myeloid progenitors from wild-type (WT) or MT1-MMP-null mice were cultured in the presence of M-CSF and RANKL for 12 days. The number of TRAP-positive cells containing from 3 to 15 nuclei was counted (n=6 for WT and n=13 for null mice).

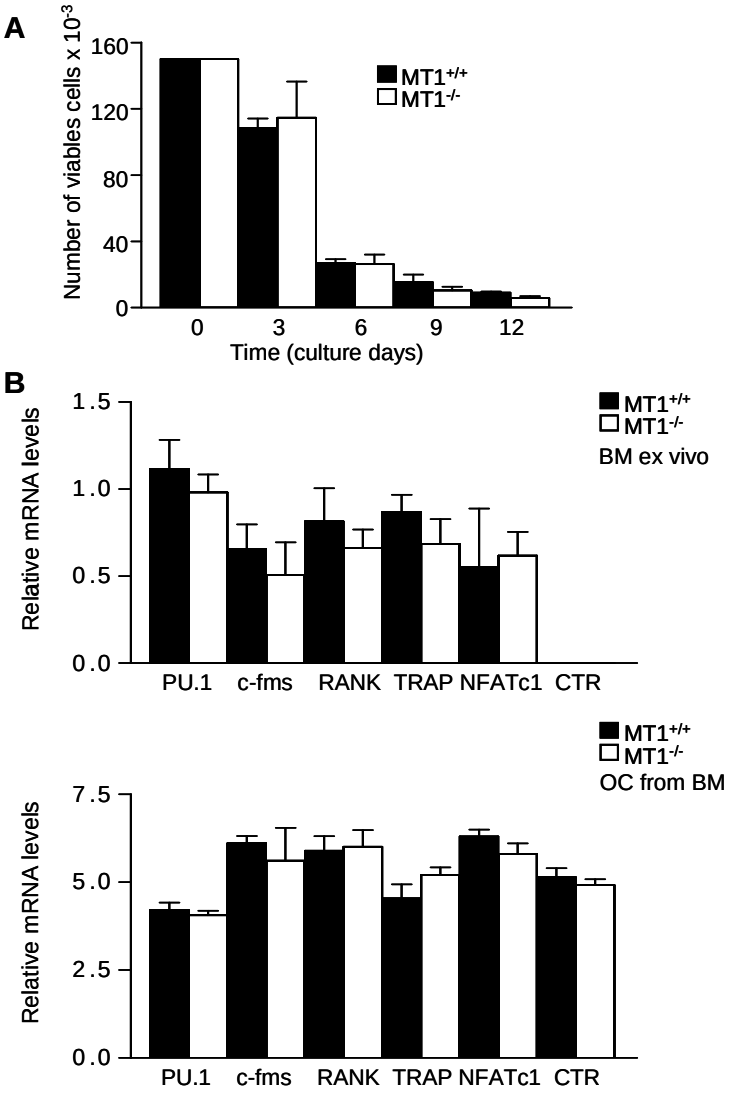

Figure S2, related to Figure 1. Viability and commitment of bone marrow cells during OC differentiation. A) The number of viable cells was counted in cultures established from wild-type and MT1-MMP-null bone marrow cells at different times (n=4). B) Expression of OC markers was detected by RT-PCR in freshly isolated bone marrow cells from wild-type and MT1-MMP-null mice (top) and in cells differentiated to OC in the presence of M-CSF and RANKL (bottom) (n=4).

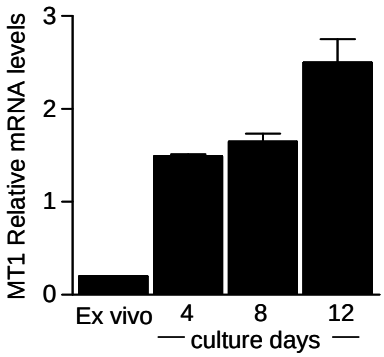

Figure S3, related to Figure1. MT1-MMP expression in bone marrow cells under osteoclastogenic conditions. MT1-MMP mRNA levels were estimated by semi-quantitative RT-PCR in bone marrow cells from wild-type mice differentiated in the presence of M-CSF and RANKL for the times indicated (n=4).

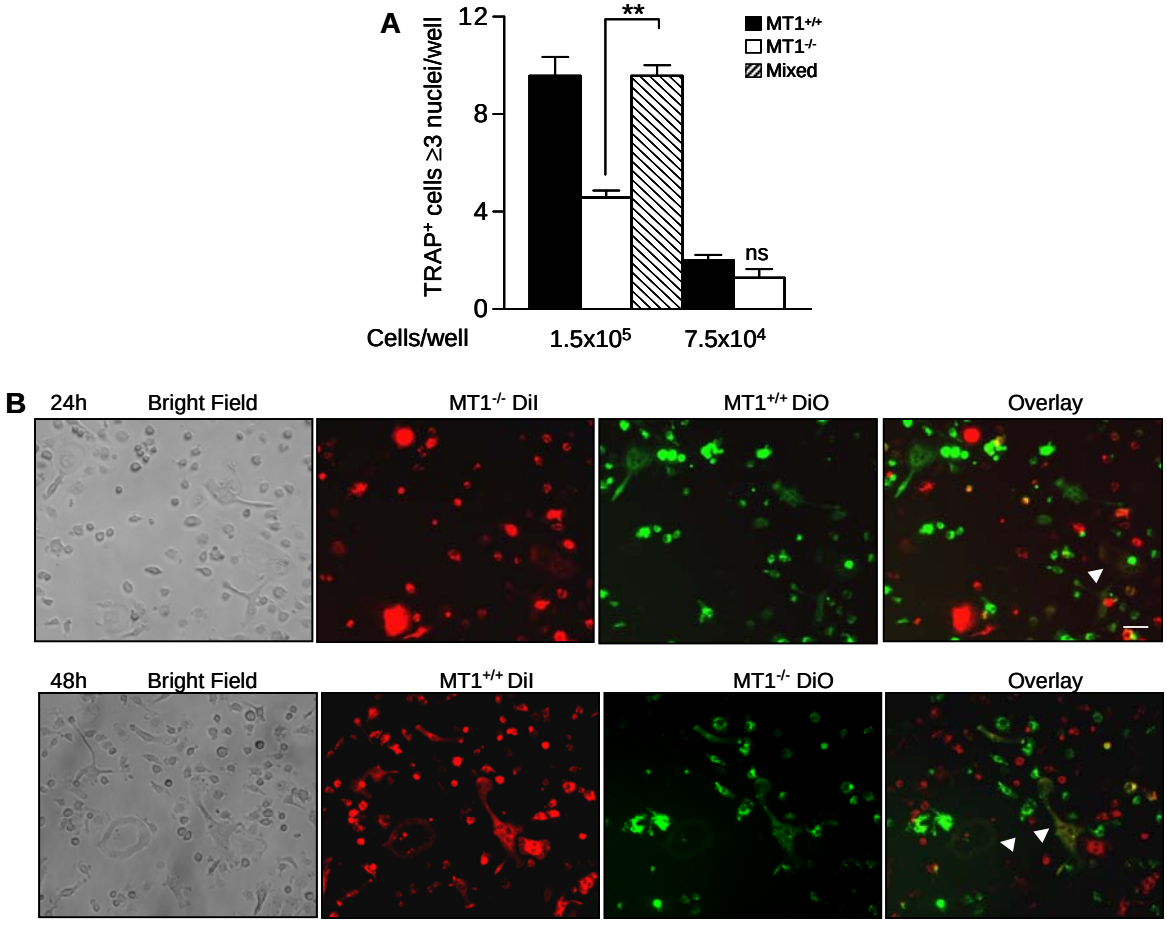

Figure S4, related to Figure 1. Mixed culture of bone marrow cells under osteoclastogenic conditions. Bone marrow cells from wild-type and MT1-MMP-null mice were cultured in the presence of M-CSF and RANKL to induce differentiation. A) Single and mixed cultures were established at the specified cell densities. After 12 days OC were stained for TRAP and the number of cells with more than 3 nuclei were counted. Experiments were run in triplicate (n=5). B) BM progenitors from wild-type and MT1-MMP-null mice were labeled with DiO (green) or DiI (red) probes as indicated and mixed cultured in the presence of M-CSF and RANKL for 24 or 48 h. Representative images are shown. Bar, 20 µm.

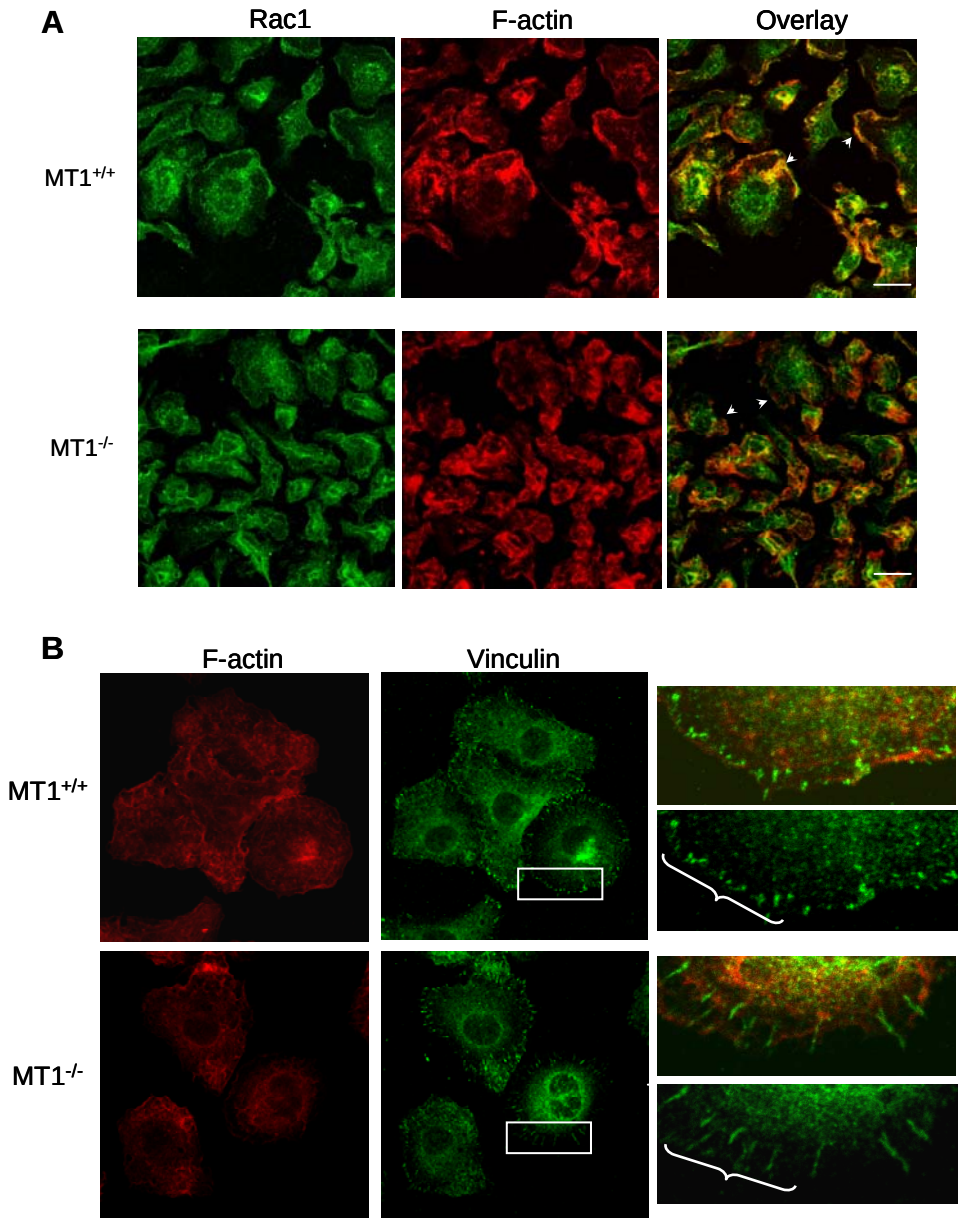

Figure S5, related to Figure 5. A) Rac1 subcellular localization in myeloid progenitors. Bone marrow cells from wild-type or MT1-MMP-null mice were cultured in the presence of M-CSF and RANKL for 4 days. Cells were fixed and double-stained for Rac1 (green) and F-actin (red). Representative maximal projections of confocal sections and the overlay images are shown for each genotype from 4 independent experiments. Bar, 20 µm. B) Focal complexes on the lamellipodia of OC progenitors. Bone marrow cells from wild-type and MT1- MMP-null mice were treated with M-CSF and RANKL for 4 days and then detached and plated on FN-coated coverslips for 1 h. Cells were double-stained for F-actin (red) and vinculin (green). Representative confocal sections at the attached surface are shown for each genotype. Bar, 20 µm. Insets show the distribution of focal complexes at the edge of the lamellipodia (brackets).

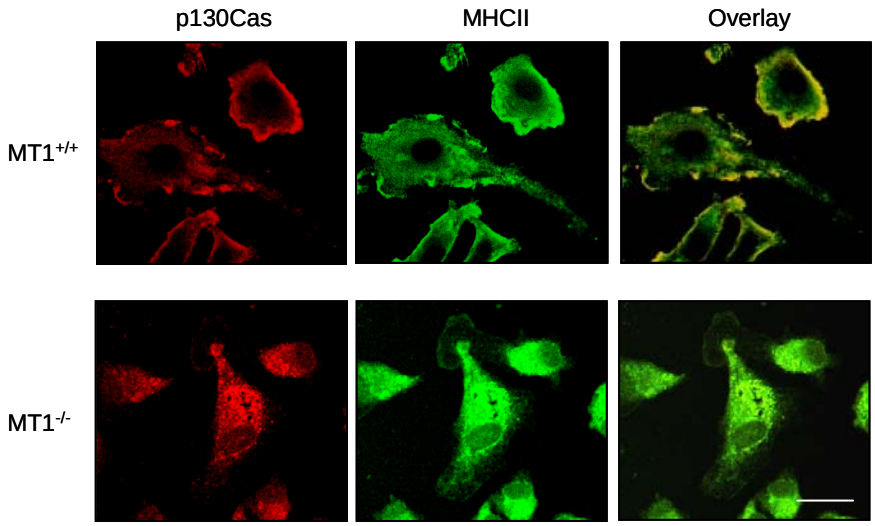

Figure S6, related to Figure 6. p130Cas subcellular localization in myeloid progenitors. Bone marrow cells from wild-type or MT1-MMP-null mice were cultured in the presence of M-CSF and RANKL for 4 days. Cells were fixed and double-stained for p130Cas (red) and MHCII (green). Representative single and overlay images are shown for each genotype. Bar, 5 μm.

Movie S1, related to Figure 3**.** Bone marrow cells from wild-type mice were cultured in the presence of M-CSF and RANKL for 4 days, and images were recorded every 15 min for 16 h.

Movie S2, related to Figure 3**.** Bone marrow cells from MT1-MMP-null mice were cultured in the presence of M-CSF and RANKL for 4 days, and images were recorded every 15 min for 16 h.

Movie S3, related to Figure 3**.** Mac-1+ bone marrow cells from wild-type mice were cultured in the presence of M- CSF and RANKL for 4 days, and images were recorded every 15 min for 16 h.

Movie S4, related to Figure 3**.** Mac-1+ bone marrow cells from MT1-MMP-null mice were cultured in the presence of M-CSF and RANKL for 4 days, and images were recorded every 15 min for 16 h.

Movie S5, related to Figure 4**.** Bone marrow cells from MT1-MMP-null mice were infected with pRETRO (mock) and cultured in the presence of M-CSF and RANKL for 4 days. Images were recorded every 15 min for 16 h.

Movie S6, related to Figure 4**.** Bone marrow cells from MT1-MMP-null mice were infected with pRETRO-MT1-MMP and cultured in the presence of M-CSF and RANKL for 4 days. Images were recorded every 15 min for 16 h.

Movie S7, related to Figure 4**.** Bone marrow cells from MT1-MMP-null mice were infected with pRETRO-MT1-MMP E240A mutant and cultured in the presence of M-CSF and RANKL for 4 days. Images were recorded every 15 min for 16 h.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Flow cytometry. Cell suspensions of individual or pooled cultures were used for flow cytometry and cell sorting, respectively. Cells were incubated with the Fc-blocking anti-CD16/CD32 mAb (Pharmingen), and then with appropriate antibody conjugated to biotin, FITC or Cy5. Biotinylated mAb were revealed with streptavidin conjugated to phycoerythrin, fluorescein, or Cy5. Cells were analyzed in a FACScanto (Becton Dickinson) cytometer equipped with the CellQuest System (Immunocytometry Systems, BD Biosciences). Cells were purified in MoFlo (Cytomation) or FACSaria (Becton Dickinson) cell sorters under sterile conditions. Anti-Mac-1/CD11b (M1/70), anti-c-fms/CD115 mAb, and the isotype controls IgG2b and IgG2a were from Pharmingen.

Retroviral gene constructs and cell infection. The MT1-MMP retroviral construct has been described (Bartolome et al., 2009). The E240A and Y573F mutants were generated from the pRETRO-MT1-MMP plasmid with the QuickChange site-directed Mutagenesis kit (Stratagene). Retroviral constructs for p130Cas and constitutively active Rac1 were generated as described (Ambrogio et al., 2005; Guo and Zheng, 2004). Retroviral particles were collected from conditioned medium after transfection of plasmids into the proper packaging cell lines. As a control, BM cells were infected with retroviral particles obtained with the empty plasmids (pMSCV/pRETRO, pMIEG and Pallino). BM cells were infected on day 0 and washed 48 h after infection.

RT-PCR. Total RNA was extracted with Trizol (Invitrogen), and cDNA prepared with Omniscript (Qiagen). RT-PCR was conducted with primers for mouse GAPDH, PU.1, c-fms, RANK, TRAP, NFATc1, calcitonin R and MT1-MMP, and human MT1-MMP (Galvez et al., 2005; Hikita et al., 2006; Hotokezaka et al., 2007; Kim et al., 2006; Kobayashi et al., 2005; Kwon et al., 2005). Products of nonsaturated PCR reactions were separated on 2% agarose gels, and semiquantitative analysis of transcript levels relative to GAPDH was performed by densitometry using Quantity-one software (Bio-Rad).

Enzyme-linked immunosorbent assay (ELISA). Serum TRAP was measured by ELISA (R&D Systems).

Histological analysis. Bone histology was performed on mouse long bones (femur and tibia). Paraffin-embedded bone sections (5μm) were stained for TRAP (Sigma). Nuclei within TRAP+ cells (OC) were identified by hematoxylin (blue) staining. Nuclei were

counted in OC at the cartilage/bone interface in two independent sections from each bone. Two blinded observers examined at least 10 OC per section.

TRAP image acquisition and analysis in histological cross-sections. Images of histological cross-sections of mice hind limbs stained for TRAP were acquired using a Leica DMR microscope and a Retiga 2000R CCD digital camera (Q-Imaging). For quantitative analysis of OC morphometrics, large field-of-view FOV images were batch processed using customized macros and algorithms generated for Image-Pro Plus 6.1 (Media Cybernetics). In each large FOV image, the region of interest was focused in zone 1 closest to the cartilage bone interface and extending 200 μm into the metaphysis. Using predetermined size and shape thresholds that limited segmentation of TRAP to objects consistent with OC morphology (eliminating large TRAP positive areas around trabecular bone), a mask of TRAP+ OC was generated for each image. Applying the previously created zone masks, parameters including TRAP+ OC-like cells and total TRAP area were exported to Prism.

ICTP RIA. Sera were frozen at -20ºC until analysis. Serum ICTP concentrations were measured by competitive RIA using 15 μl samples.

Transmigration assays. Transmigration assays were performed in 3 μm–pore Transwell chambers (Costar Corp.). A total of 10^5 HUVEC were plated on 1% gelatin-coated filters for 24 h and stimulated for 2 h before assay with 20 ng/mL TNFα (R&D systems). 2.5 x 10^5 BM cells or 2.5 x 10^4 cells from the sorted Mac-1^{dull} population were resuspended in 150 μL RPMI 1640 medium containing 0.5% BSA, and added to the upper chamber of transwells. RANKL (25 ng/mL) was added or not to the lower chamber. Transmigrated cells were collected from the lower chamber after 4 h and counted.

Fluorescence microscopy and confocal analysis. BM cells were cultured with M-CSF and RANKL for 4 days and then either fixed and stained or detached and replated on fibronectin-coated glass coverslips for 1 h before fixation. Cells were fixed with 4% PFA for 15 min and permeabilized in 0.2% Tx100 in PBS 3% BSA for 10 min before staining. Anti-vinculin mAb was from Sigma-Aldrich. Anti-MHCII-FITC antibody was from Pharmingen. Primary antibodies were labelled with the appropriate secondary Ab, and F-actin was stained with rhodamine-phalloidin (Molecular Probes). Samples were mounted in ProLong Gold (Invitrogen). Samples were examined with a confocal microscope (Leica SP5) fitted with a 63x/1.4 NA oil-immersion objective, and images were acquired with a digital camera and LAS AF software (Leica).

GST-protein pull-down assay. The MT1-MMP cytosolic domain-GST fusion protein was obtained by direct cloning of the MT1-MMP cytosolic nucleotide sequence into pGEX plasmid. The GST-MT1-MMP cytosolic tail Y573F mutant (GST-MT1Y-F) was generated with the QuickChange site-directed Mutagenesis kit (Stratagene). Pull-down assays were performed by incubating cell lysate from p130Cas-infected or non-infected BM progenitors with 40 μg GST, GST-MT1 or GST-MT1Y-F fusion proteins. After washing, bound proteins were eluted by boiling in Laemmli buffer. Samples were run in SDS-PAGE and western blotted for p130Cas. Bound antibody was detected by enhanced chemiluminescence. Densitometric analysis of band intensity was done using Image J software, and values were normalized to GST fusion proteins.

SUPPLEMENTAL REFERENCES

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