



Supplemental Fig. 1

Supplemental Fig. 1. MT1-MMP stimulates cell invasion, metastasis and tumor growth. (A) cell surface expression of MT1-MMP. HT, HT-neo, HT-siRNAsc, HT-siRNA and HT-MT cells (*left*), U-zeo, U-MT and U-MT-E240A cells (*middle*) and MCF7-zeo, MCF7-MT and MCF7-MT-E240A cells (*right*) were each surface labeled with membrane-impermeable biotin and then lysed. The lysates (1 mg total protein each) were immunoprecipitated with an MT1-MMP antibody and protein G-agarose beads. The precipitated samples were analyzed by Western blotting. Where indicated, GM6001 (50 μ M) was added to HT-MT cells. (B) gelatin zymography. HT, HT-neo, HT-MT, HT-siRNAsc and HT-siRNA cells (*left*), U-zeo, U-MT and U-MT-E240A cells (*middle*), and MCF7-zeo, MCF7-MT and MCF7-MT-E240A cells (*right*) were incubated 8 h in serum-free DMEM. Aliquots of the medium were analyzed by gelatin zymography to demonstrate the conversion of the 68 kDa pro-MMP-2 into the 64 kDa activation intermediate and the 62 kDa mature enzyme. In contrast to HT1080 and U251 cells which produce MMP-2 naturally, MCF7 cells do not produce MMP-2 (“cells alone” lane) and, therefore, the purified MMP-2 proenzyme (“no cells” lane) was added to the MCF7 cell samples. (C) Q-RT-PCR of endogenous, chromosomal MT1-MMP. RNA samples isolated from the HT, HT-MT, and HT-siRNA cells were used as templates in the PCR reactions. The expression values were normalized relative to GAPDH. The levels of the MT1-MMP mRNA in HT-MT and HT-siRNA cells are shown in percent relative to HT cells (100%). (D) invasion through the Matrigel. Cells were allowed to invade through the reconstituted basement membrane, Matrigel. The invading cells are shown in percent relative to the total number of cells. The data between HT-MT and HT-siRNA are statistically significant ($p < 0.05$). (E) tumorigenicity of MT1-MMP. HT-neo, HT-MT and HT-siRNA fibrosarcoma, and U-zeo, U-MT and U-MT-E240A glioma cells were injected subcutaneously while MCF7-zeo, MCF7-MT and MCF7-MT-E240A breast carcinoma cells were injected in the mammary fat pads of immunodeficient mice. Tumor size is expressed as mean tumor volume \pm SE (mm^3). (F) metastases-promoting activity of MT1-MMP. Cells were injected i.v. in immunodeficient mice. Mice were sacrificed 25 days after the injection. Metastases were counted in the lungs. The data were analyzed using Fisher’s LSD test ($p < 0.05$).