

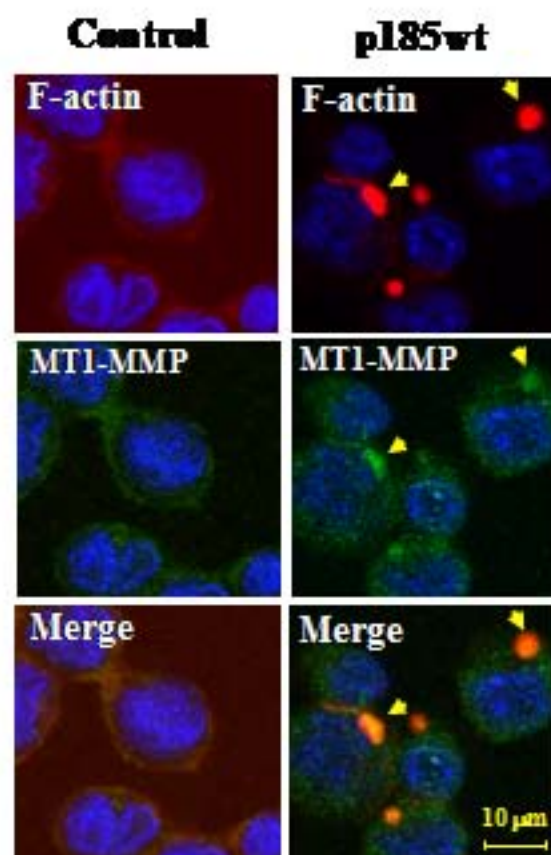
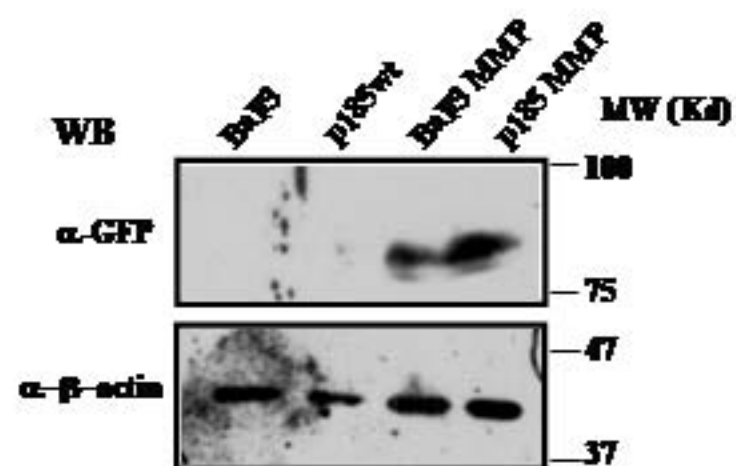
were visualized by fluorescence microscopy (upper panel) and were counted (lower panel, represented as average percentage of three randomly picked areas). * P<0.01.

Figure 3. Knockdown of MT1-MMP expression impaired Bcr-Abl-stimulated cell migration. (A) Expression of MT1-MMP shRNA resulted in a decrease in mRNA level (lower panel) and proteolytic activity (upper panel) of MT1-MMP in p185wt cells. Ba/F3 cells transformed by p185wt were transduced with control retrovirus (p185wt) or the retrovirus expressing the sequence specific MT1-MMP shRNA (p185wt MMP kd). The proteolytic activity in conditioned medium was analyzed by gelatin zymography and the relative level of MT1-MMP mRNA was analyzed by real time quantitative PCR and the data represents the mean +/- s.d. of triplicate experiments. * P<0.001. (B) Knockdown of MT1-MMP expression impaired the ability of p185wt cells to transmigrate through fibronectin coated membrane. Data represents the mean +/- s.d. of triplicate experiments. * P<0.001.

Supplementary Figure 1. Bcr-Abl induces polarized distribution of MT1-MMP around F-actin rich structures. (A) Ba/F3 cells transduced with control retrovirus (control) and the retrovirus expressing p185^{Bcr-Abl} were probed with anti-MT1-MMP antibody. This was followed by staining with FITC-conjugated secondary antibody. Cells were then counterstained with TRITC-conjugated phalloidin and DAPI to visualize F-actin and nuclei, respectively. The pictures were captured by two-photon confocal microscopy. Arrows indicate the polarized distribution of F-actin and Mt1-MMP. (B) Control Ba/F3 cells (control) or p185wt cells (p185wt) were transfected with a plasmid expressing GFP-MT1-MMP. The expression of GFP-MT1-MMP was examined by Western blot analysis using anti-GFP antibody, as indicated and subcellular localization of GFP-MT1-MMP was analyzed by fluorescence microscopy (C).

Arrowheads indicate the polarized distribution of GFP-MT1-MMP around F-actin rich structures.

Supplementary Figure 2. Expression of p185wt and p185 Δ SH3 Δ C in Ba/F3 cells expressing GFP-MT1-MMP (A) and, (B) knockdown of Abi1 expression in p185wt cells expressing GFP-MT1-MMP.

A**B****C**