# Supplemental Materials

***Molecular Biology of the Cell***

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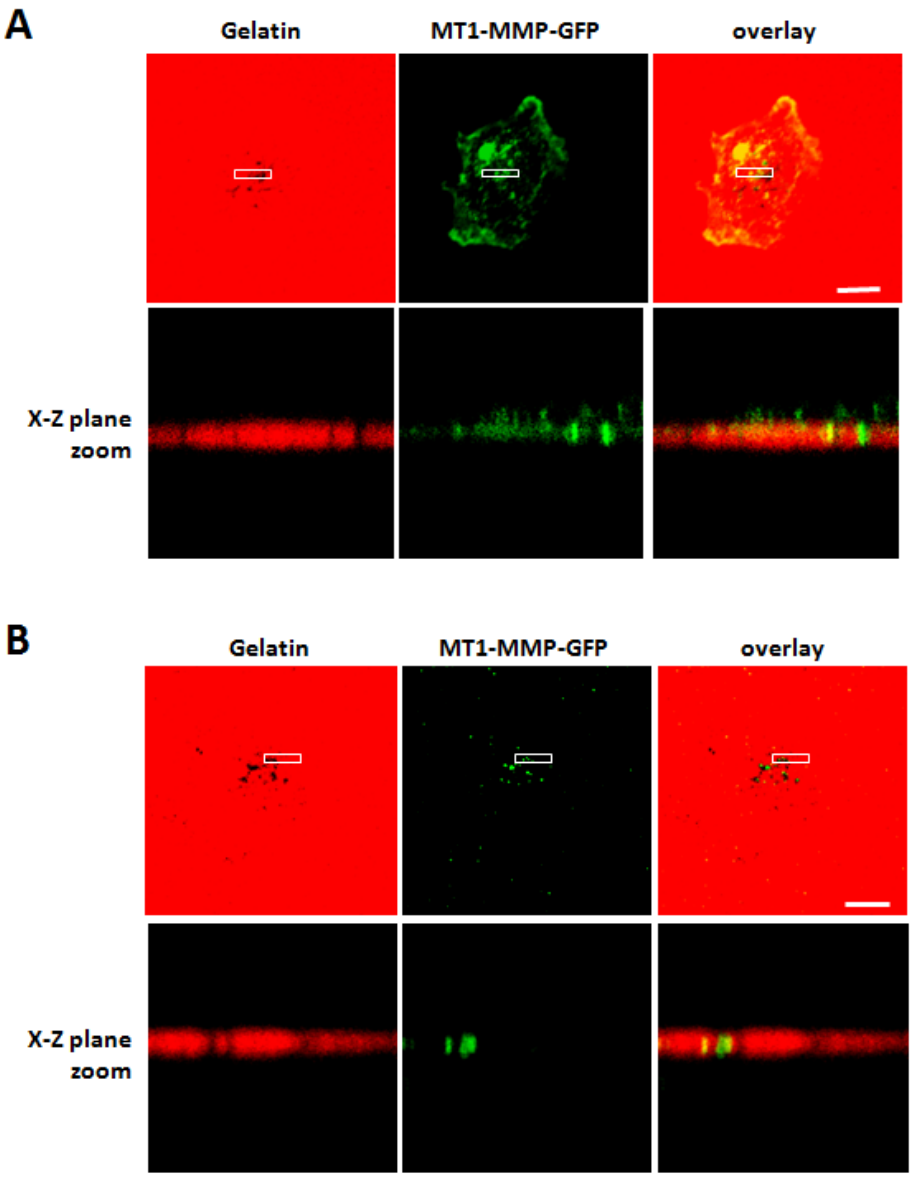
**SUPPLEMENTAL FIGURE 1.** Confocal microscopy showing isolation of invadopodia. Cells were plated on coverslips coated with 594-labeled gelatin and subsequently fixed (A) or sheared to remove cell bodies (as described under Experimental Procedures), followed by fixation of invadopodia embedded in gelatin (B). Confocal microscopy shows MT1-MMP-GFP overlaying areas of invadopodial degradation (dark area in gelatin image). Images in x-y plane, of boxed region in top panels, reveal invadopodial protrusions embedded in the gelatin matrix prior to (A) and after (B) shearing. Scale bar = 10 μm.

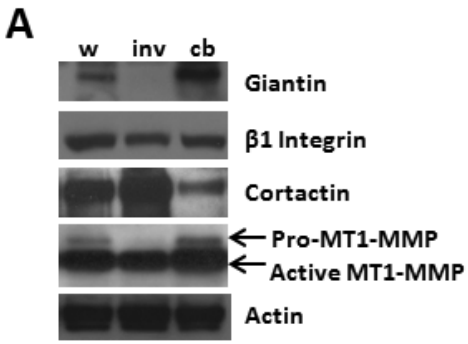
**SUPPLEMENTAL FIGURE 2.** Western blot analysis of cell body and isolated invadopodium fractions. (A) Representative blots of giantin (Golgi marker), β1 integrin, cortactin, MT1-MMP and actin are shown. Amounts of proteins in cell body fractions (cb) and invadopodial fractions (inv), compared to whole cell lysate (w), can be seen. The Golgi resident protein giantin and pro-MT1-MMP are only present in the cell body fraction, while cortactin is enriched in the invadopodial fraction. β1 integrin and active MT1-MMP are found in both invadopodial and cell body fractions. Actin is present in all fractions and was used as a loading control. (B) Quantification of protein levels from replicate (3 or more) Western blots as shown in A. 20 μg of whole cell lysate was used on all blots.

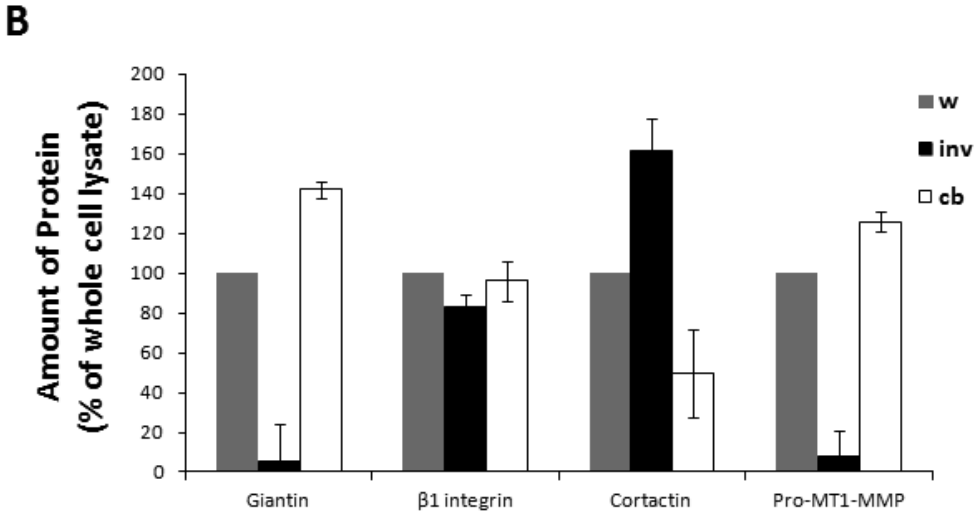
**SUPPLEMENTAL FIGURE 3.** Analysis of SNARE localization in cell body (cb) and invadopodial (inv) fractions. Integrin and actin are both used as loading controls. (A) Western blot analysis of cell fractionation. Representative blots of SNAP23, SNAP29, Syntaxin4, Syntaxin3, VAMP7 and VAMP3 are shown. (B) Quantification of blots, as in A, from triplicate experiments. Whole cell lysate was used as an internal standard to compare directly to each fraction.

**SUPPLEMENTAL FIGURE 4.** Analysis of antibody and GFP-SNARE specificity. (A) Cells co-transfected with GFP and siRNA to Syntaxin4, or GFP and shRNA to SNAP23 were probed with anti-Syntaxin4 or anti-SNAP23 antibodies. (B) Cells transfected with GFP-SNAP23, GFP-VAMP7 or GFP-Syntaxin4 were probed with anti-Syntaxin4, anti-SNAP23, or anti-VAMP7 antibodies.

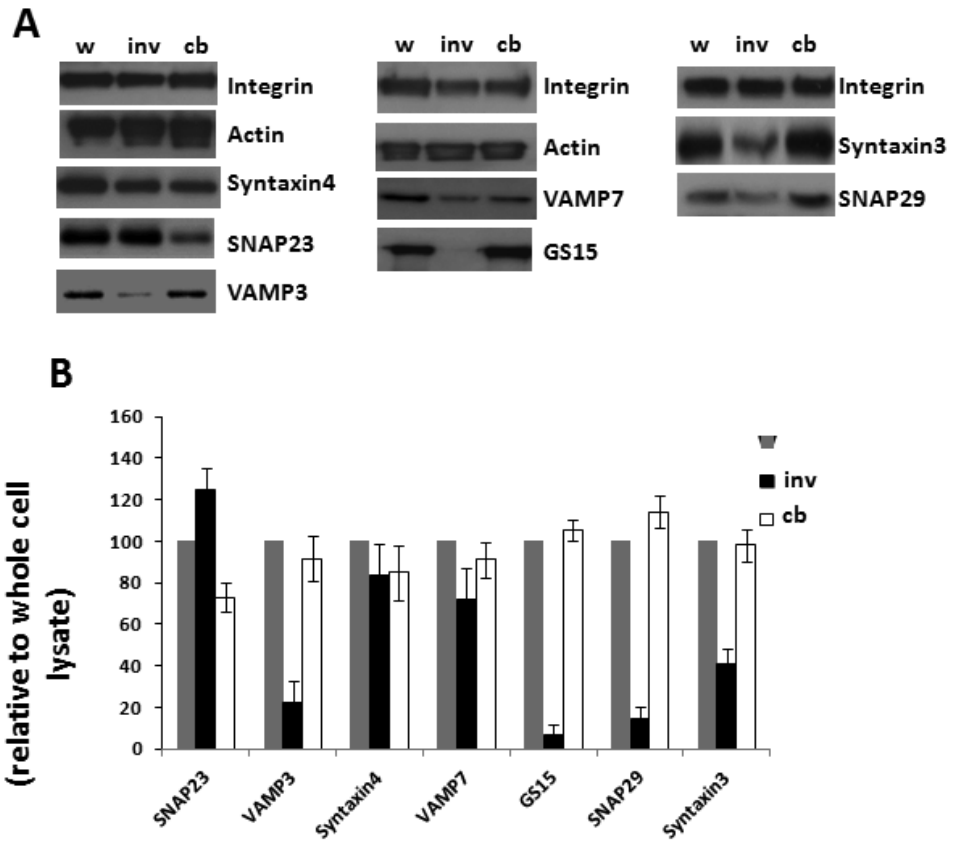



Williams et al. Supplemental Fig. 1

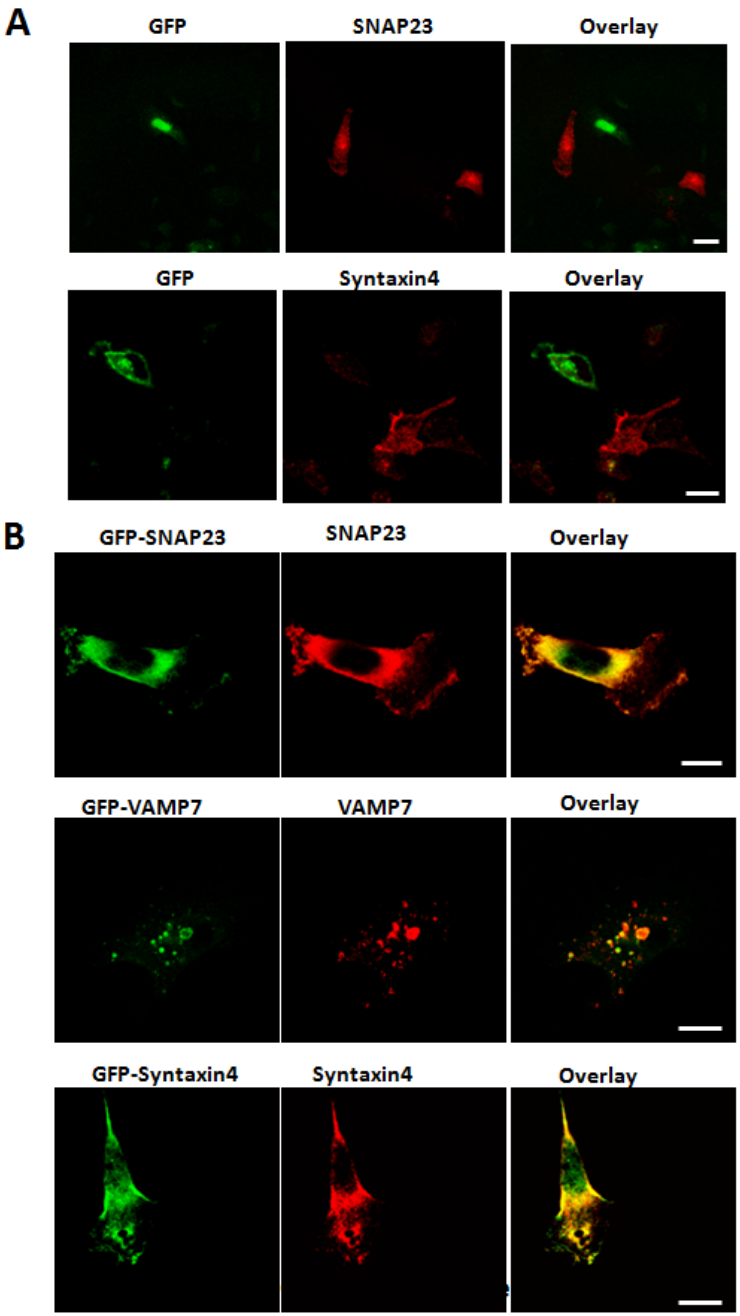



**Williams et al. Supplemental Fig. 2**




Williams et al. Supplemental Fig. 3




Williams et al. Supplemental Fig. 4