SUPPLEMENTAL MATERIALS:

Supplemental Figure 1. Representative photographs showing the quantification of matrix degradation in the cell. **a)** The original image of the labeling of Alexa 568-gelatin. **b)** The area of degraded matrix in the field was measured using ImageJ 1.73v software. The image was first converted to 8 bit grayscale files. Then automatic thresholding function of ImageJ was applied to the image, followed by manually adjusting the maximum threshold value by moving the bottom horizontal bar in the "Threshold" window from right to left until the upper threshold line just touches the edge of the histogram while keeping the minimum threshold value zero. This adjustment allows optimal matching to the original fluorescence images taken from the microscope. The AlexaFluor568-gelatin image was thresholded at 0-127 after the above adjustments. **c)** The boundary of the black dots and the total degradation area were obtained by automatic outlining. There are 41 degrading spots and the total degradation area is 3909.000 pixel. **d)** The area of the whole cell (37274.7 pixel) was obtained by manually outlining the boundary of the cell surface. Degradation level (10.487%) was then calculated as the total degradation area divided by the total area of the cell.

Supplemental Figure 2. (A) MDA-MB-231 (Y527F c-Src) cells were transfected with GFP-tagged Exo70 (GFP-Exo70), plated on fluorescent Alexa 568 labeled-gelatin film for 4 hrs, and then processed for microscopy. Individual and merged images of GFP-Exo70 fluorescence (green), F-actin (blue) and gelatin (red) were shown. Expression of

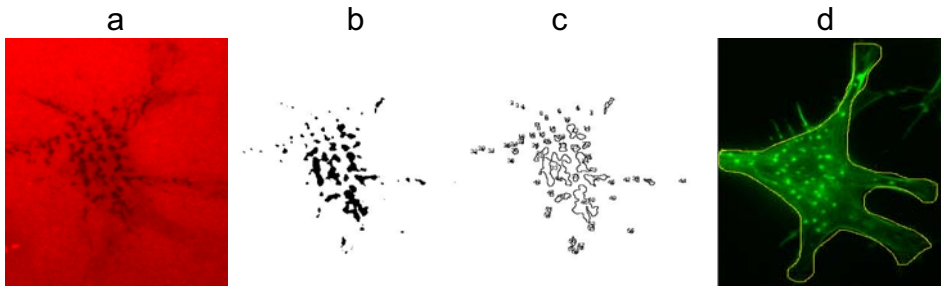
GFP alone did not change the ability of c-Src-expressing cells to form invadopodia (bottom panel). Overexpression of GFP-Exo70 stimulated focal degradation in MDA-MB-231 (Y527F c-Src) cells (upper panel) and more invadopodia were formed compared with GFP-transfected cells. **(B)** Quantification of degradation areas in cells overexpressing Exo70. Three independent measurements (~50 cells each) for each treatment were carried out. Error bars, SD. "*" indicates $p < 0.01$. Scale bar = 5 μm.

Supplemental Figure 3. Exo70 does not directly interact with CA or VCA. Glutathione Sepharose conjugated with 20 μg of GST or GST-Exo70 (labeled on the top) was incubated with either 15 μg of Hisx6-tagged CA or VCA (labeled at the bottom). The input (1/20 of the total) and bound Hisx6-CA or Hisx6-VCA proteins were analyzed by western blot using an anti-Hisx6 monoclonal antibody. The molecular weights ("MW") are marked to the left (in kDa).

Supplemental Figure 4. (A) The final level of actin polymerization in exocyst knockdown and GFP-Exo70 overexpression samples. Cell lysates from all the treatments were ultracentrifuged and mixed with pyrenyl-actin for the actin polymerization reaction as described in *MATERIALS AND METHODS*. After 24 hours of incubation at room temperature, the samples were transferred into a cuvette and the fluorescence intensity was read in the fluorometer. The final extent of actin polymerization was shown to be identical for all the samples tested. **(B)** The amounts of available actin in the reactions of

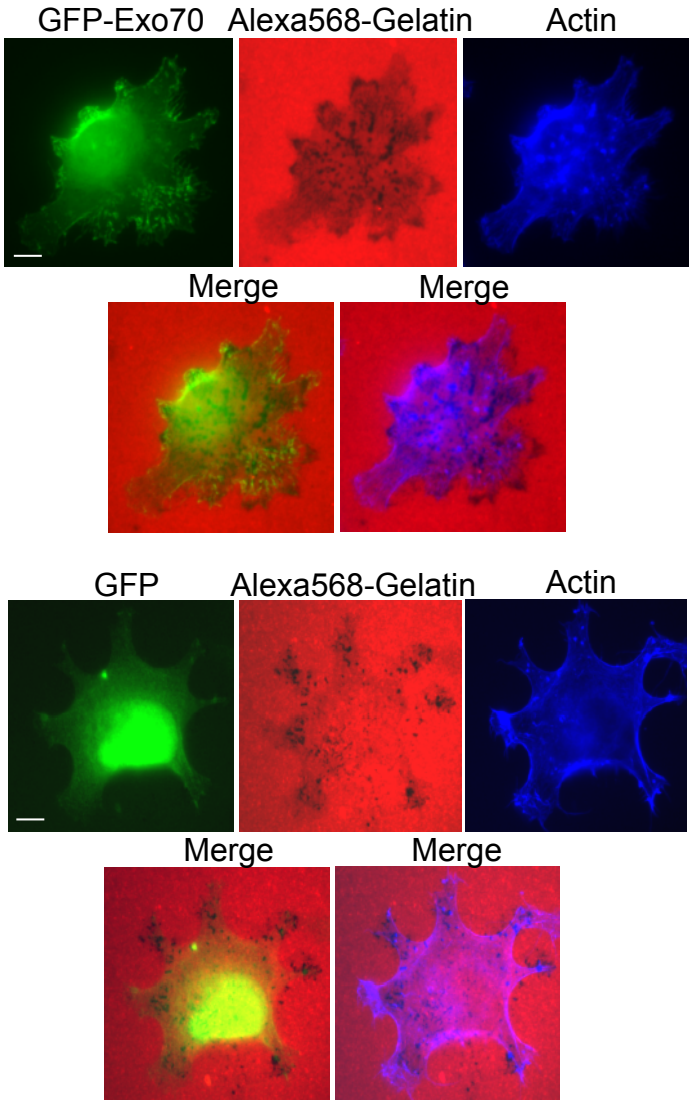
all the samples. Actin polymerization reactions were set up as described above. After 24 hours of incubation for the reactions to reach the steady state, the samples were ultracentrifuged and the pellet / supernatant fractions of each sample were collected. The fractions were then subjected to SDS-PAGE and stained with SYPRO-Red. The amounts of actin in pellet and supernatant fractions were at the same level in each treatment.

Supplementary Figure 1.



Supplementary Figure 2.

A.



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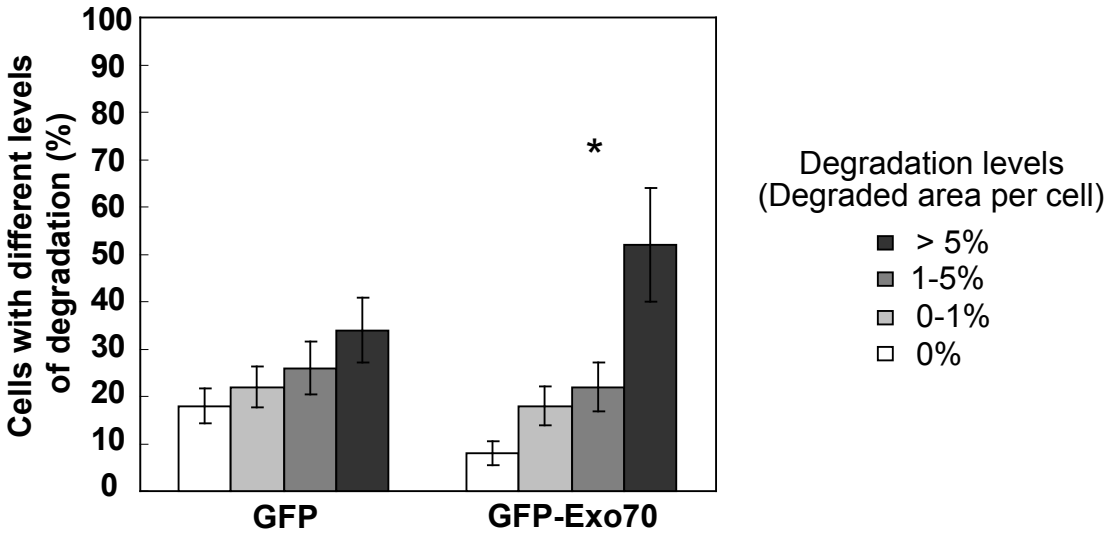
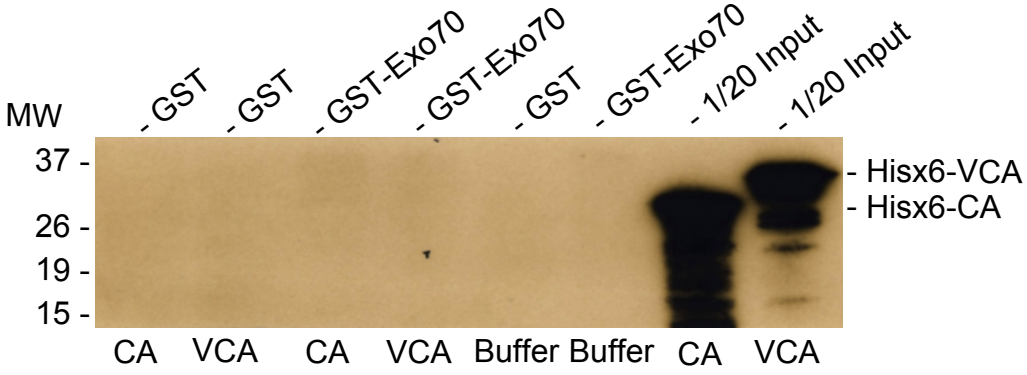
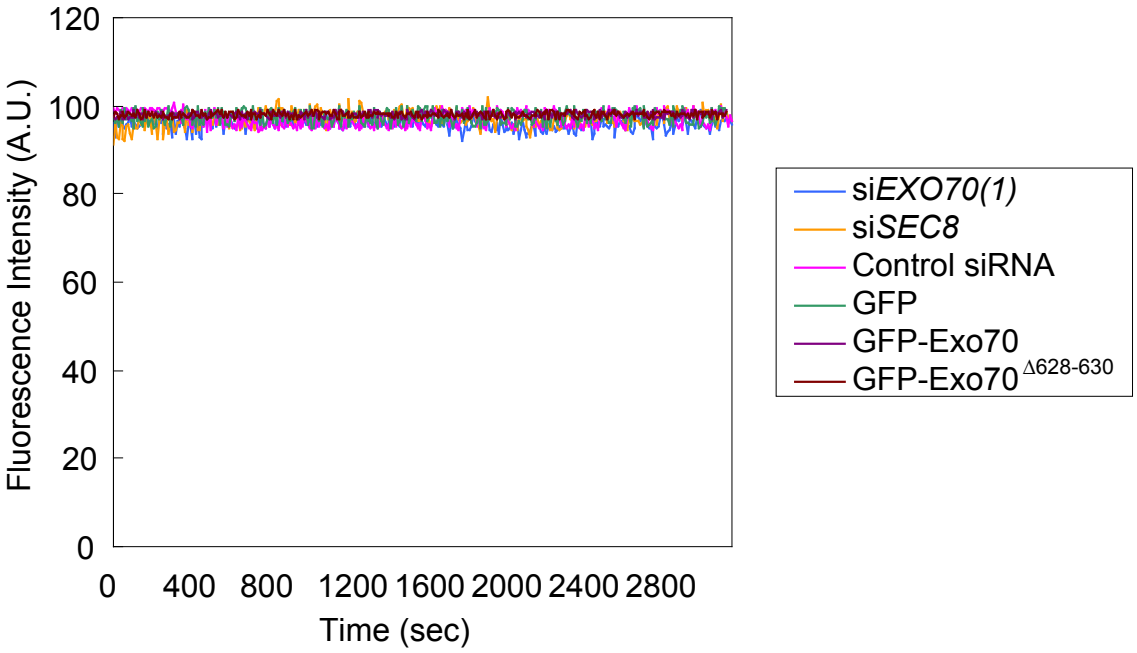


Supplemental Figure 3.



Supplemental Figure 4.

A.



B.