Liposome co-incubation with cancer cells secreted exosomes (extracellular vesicles) with different proteins expressions and different uptake pathways.

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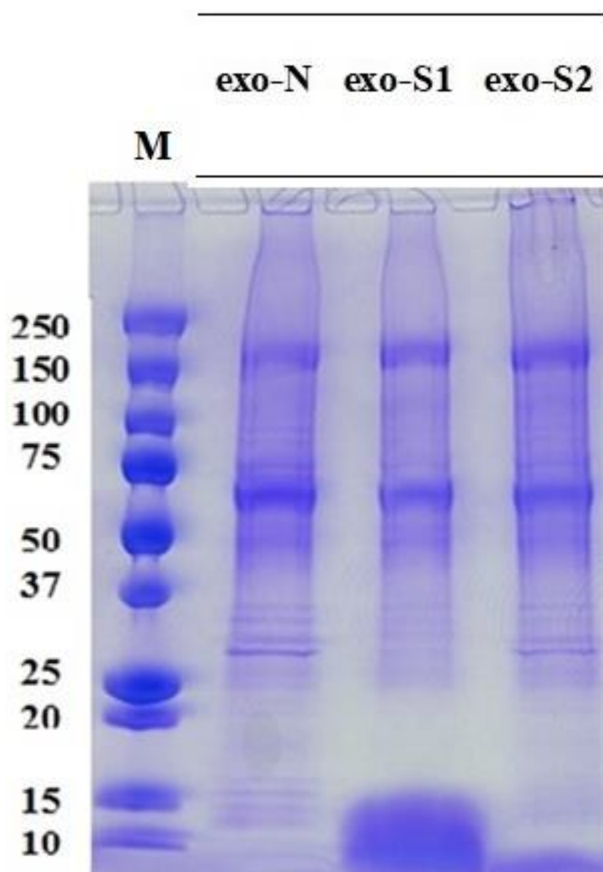
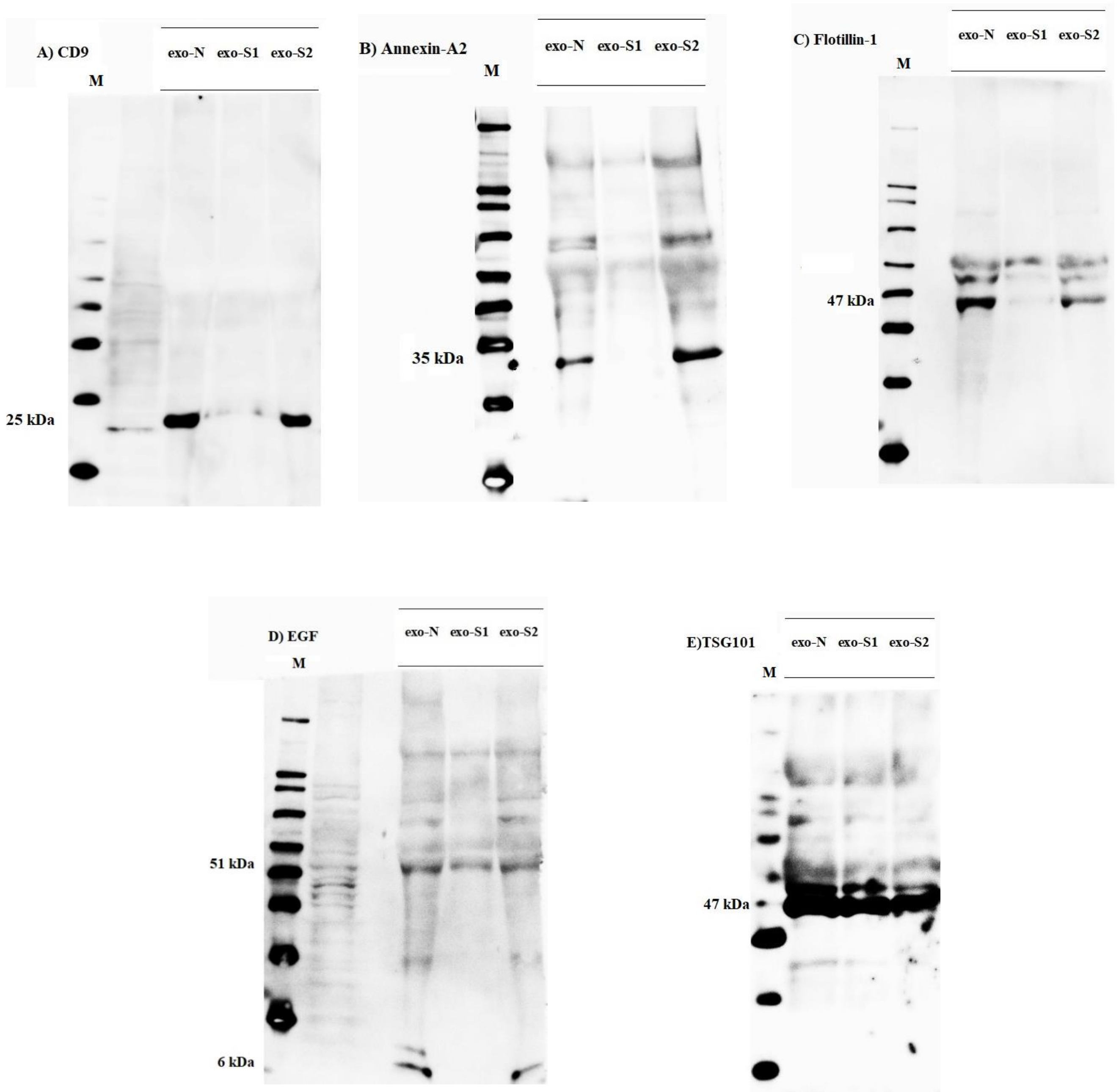
Emam et al., Supplementary Fig. 1



Supplementary Figure 1. Analysis of exosomal proteins by SDS PAGE.

Exosomal proteins in each sample (exo-N, exo-S1 and exo-S2) were electrophoretically separated and then stained by Coomassie brilliant blue dye (0.05%). M, molecular weight marker.

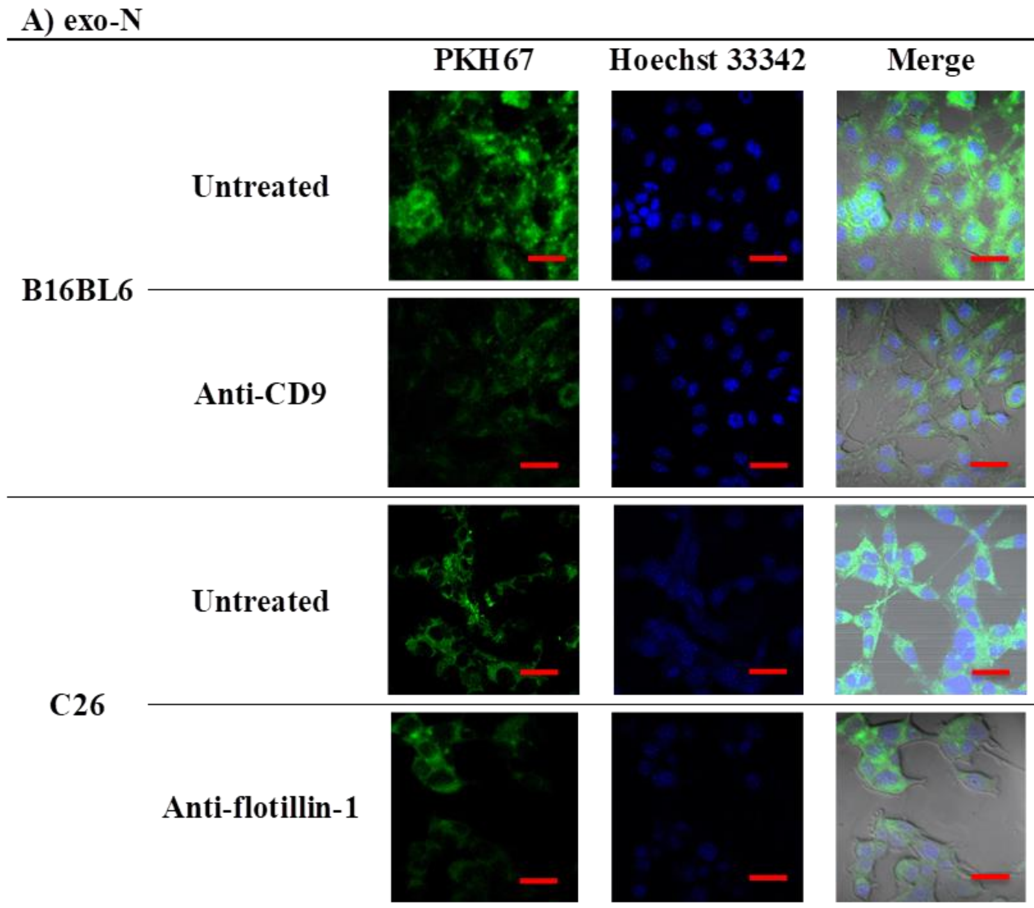
Emam et al., Supplementary Fig. 2



Supplementary Figure 2. Identification of exosomal marker proteins by Western blotting

Exosomal proteins in each sample (exo-N, exo-S1 and exo-S2) were electrophoretically separated and then blotted in presence of different Abs such as anti-CD9, anti-flotillin-1, anti-annexin-A2, anti-EGF and anti-TSG101. TSG101 was used as a reference protein (housekeeping protein). M, molecular weight marker.

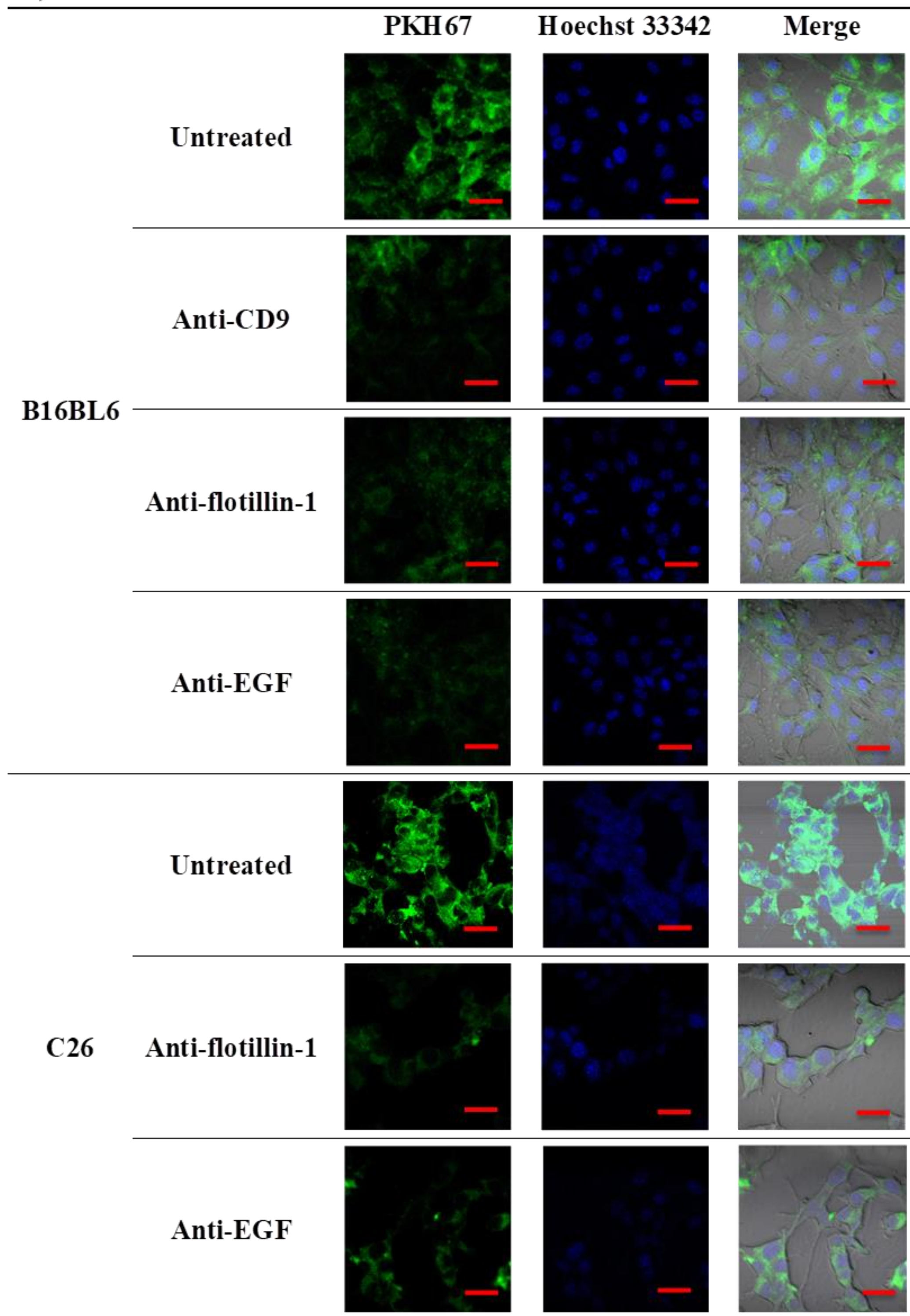
Emam et al., Supplementary Fig. 3A

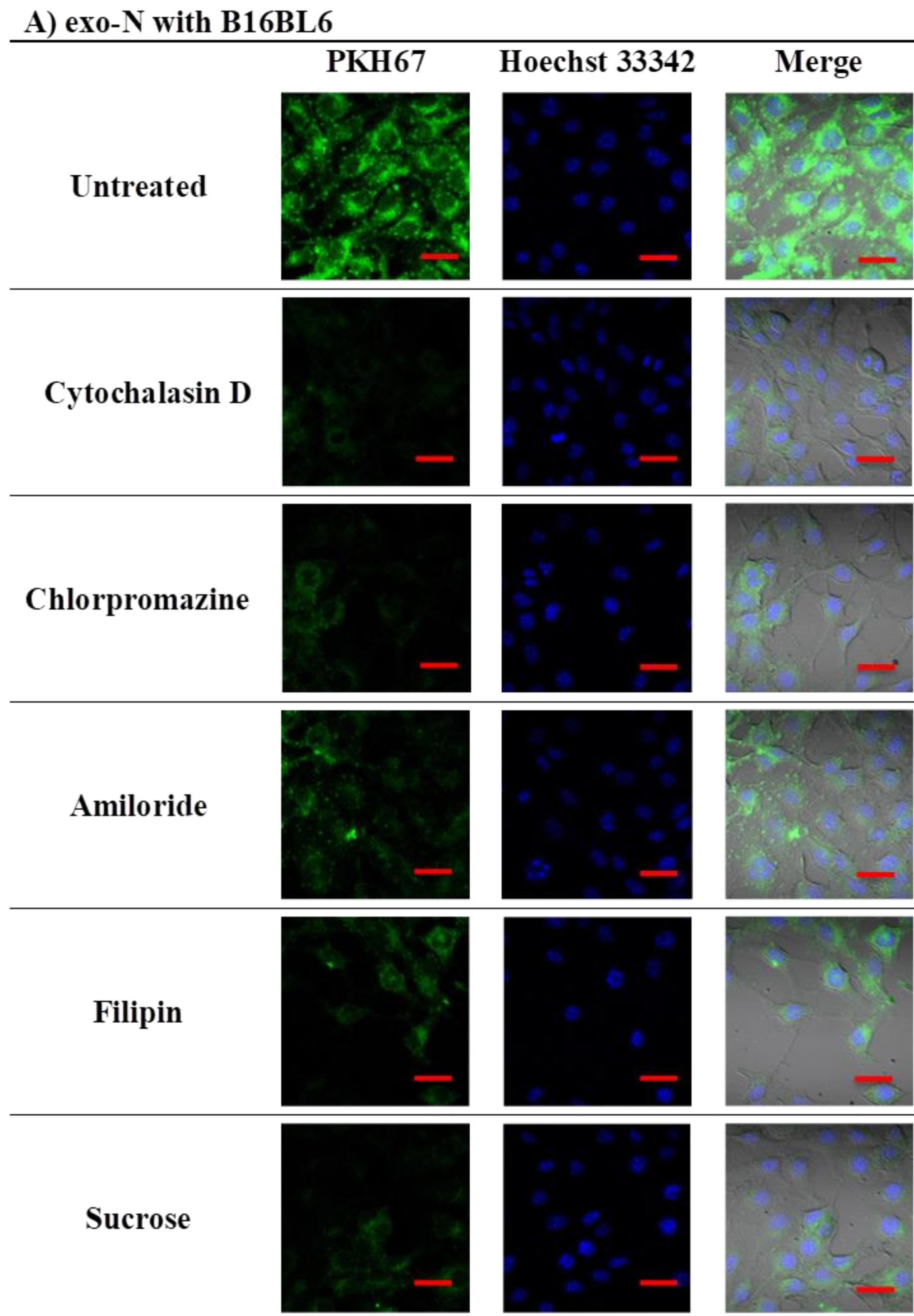


Supplementary Figure 3. Role of certain marker proteins in the uptake of exo-N and exo-S2 by donor cells B16BL6 and other allogeneic cells C26.

Labeled exo-N (A) or exo-S2 (B) were incubated with different Abs in a ratio 1:1 for 2 h at 4 ° C and then added to different cancer cell lines. After 4 h incubation, cancer cells were imaged by laser scanning confocal microscope after staining the DNA core with Hoechst 33342. All data represent one set of triplicates. Exosomes (EVs) were labeled with PHK67 (green) and the DNA core was stained with Hoechst 33342 (blue). Scale bar indicates 20 µm.

Emam et al., Supplementary Fig. 3B

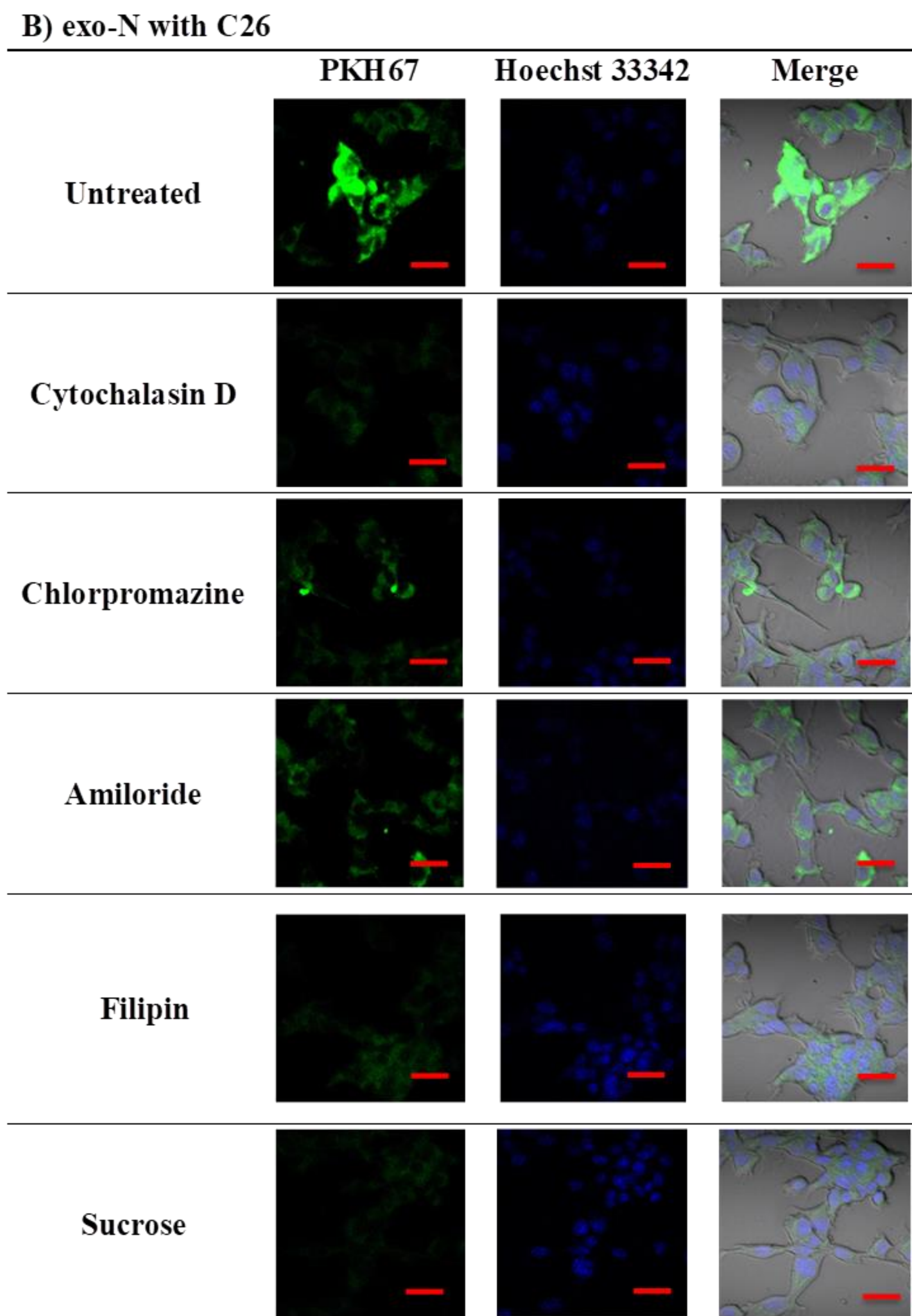




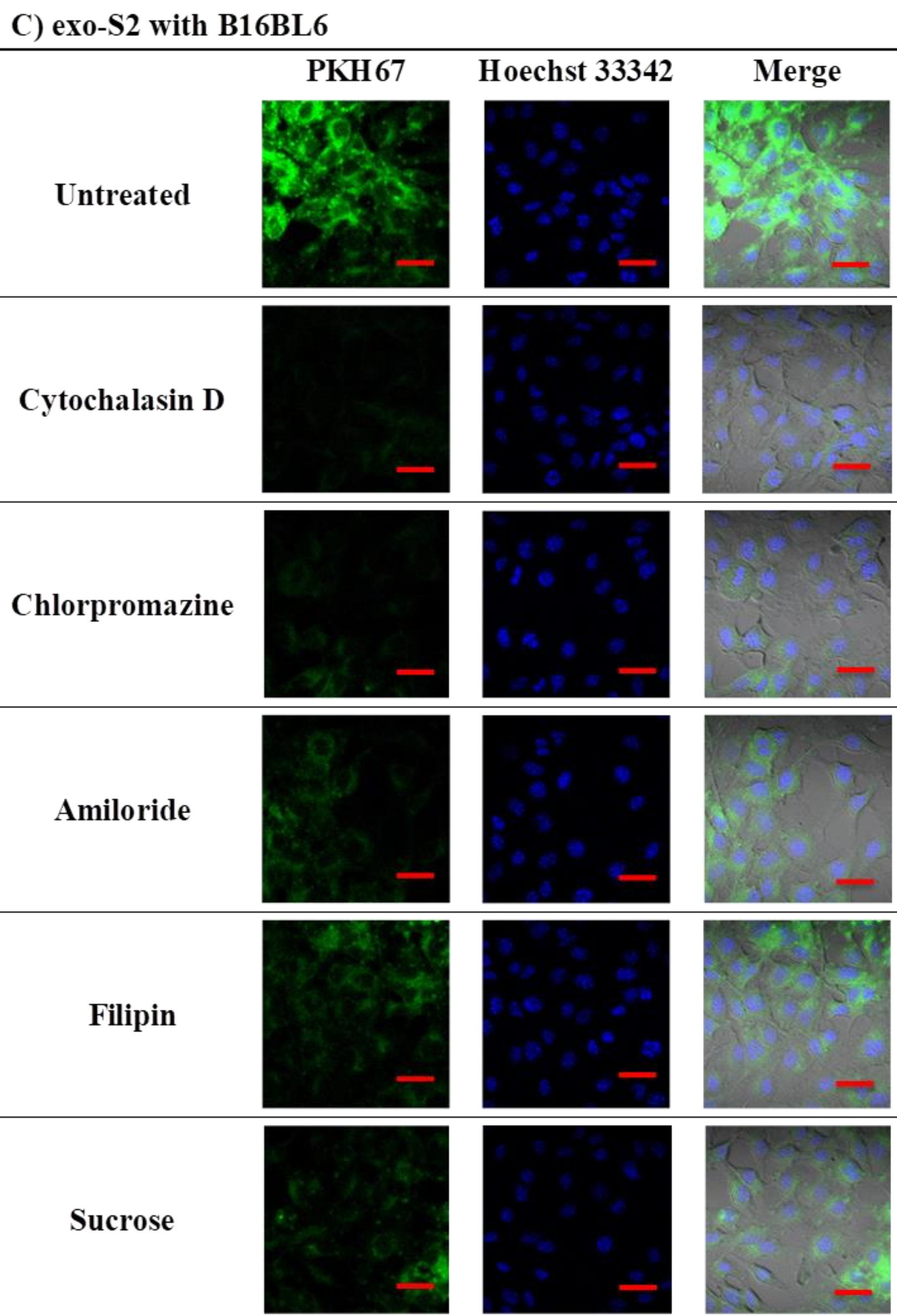
Supplementary Figure 4. Uptake mechanisms for the internalization of exo-N and exo-S2 by donor cells B16BL6 and other allogeneic cells C26.

B16BL6 (A and C) and C26 (B and D) cancer cell lines were incubated in the presence of different uptake inhibitors for 30 min and then labeled exo-N (A and B) or exo-S2 (C and D) were added. After 4 h incubation, cancer cells were imaged by laser scanning confocal microscopy after staining the DNA core with Hoechst 33342 (C, D). All data represent one set of triplicates. Exosomes (EVs) were labeled with PHK67 (green) and the DNA. Scale bar indicates 20 µm.

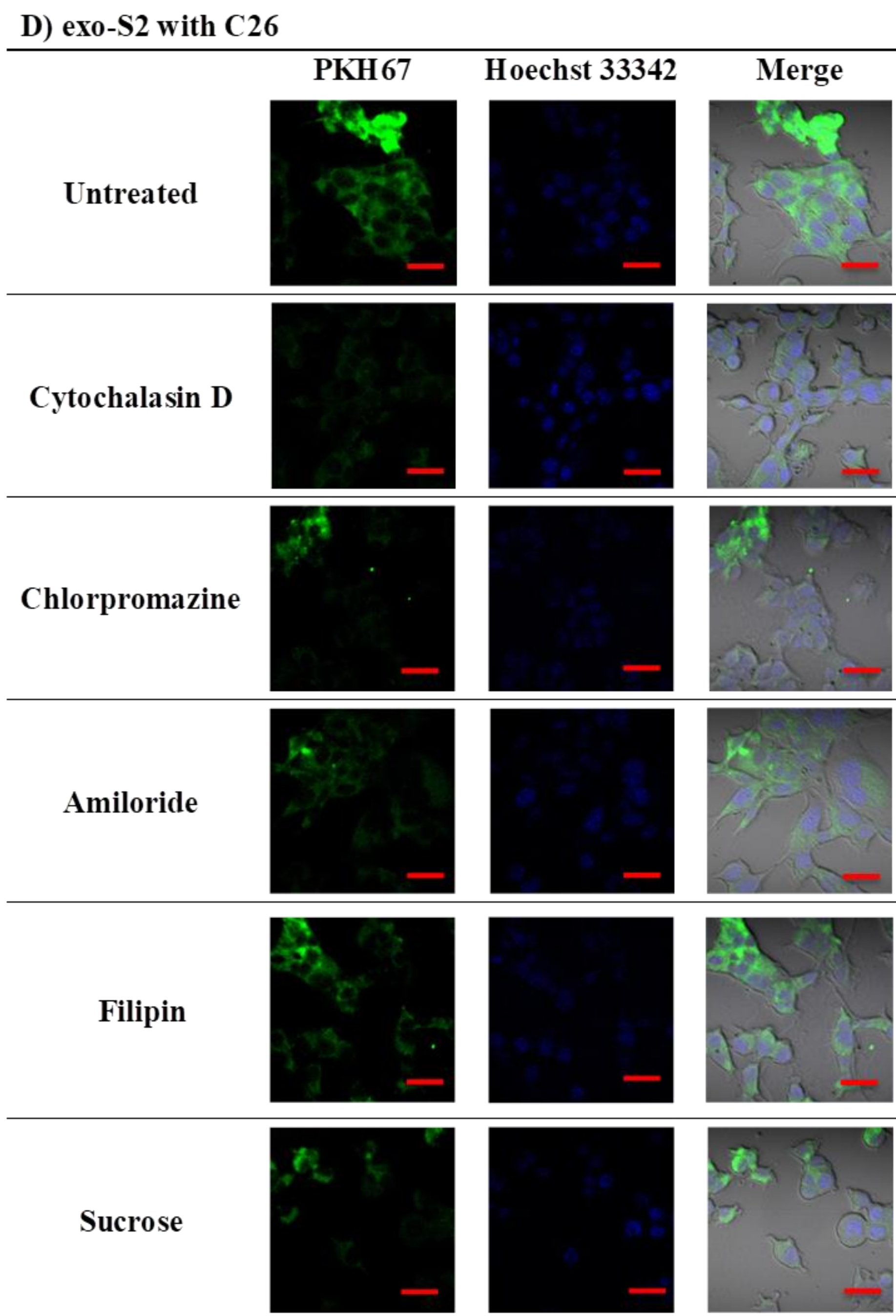
Emam et al., Supplementary Fig. 4B



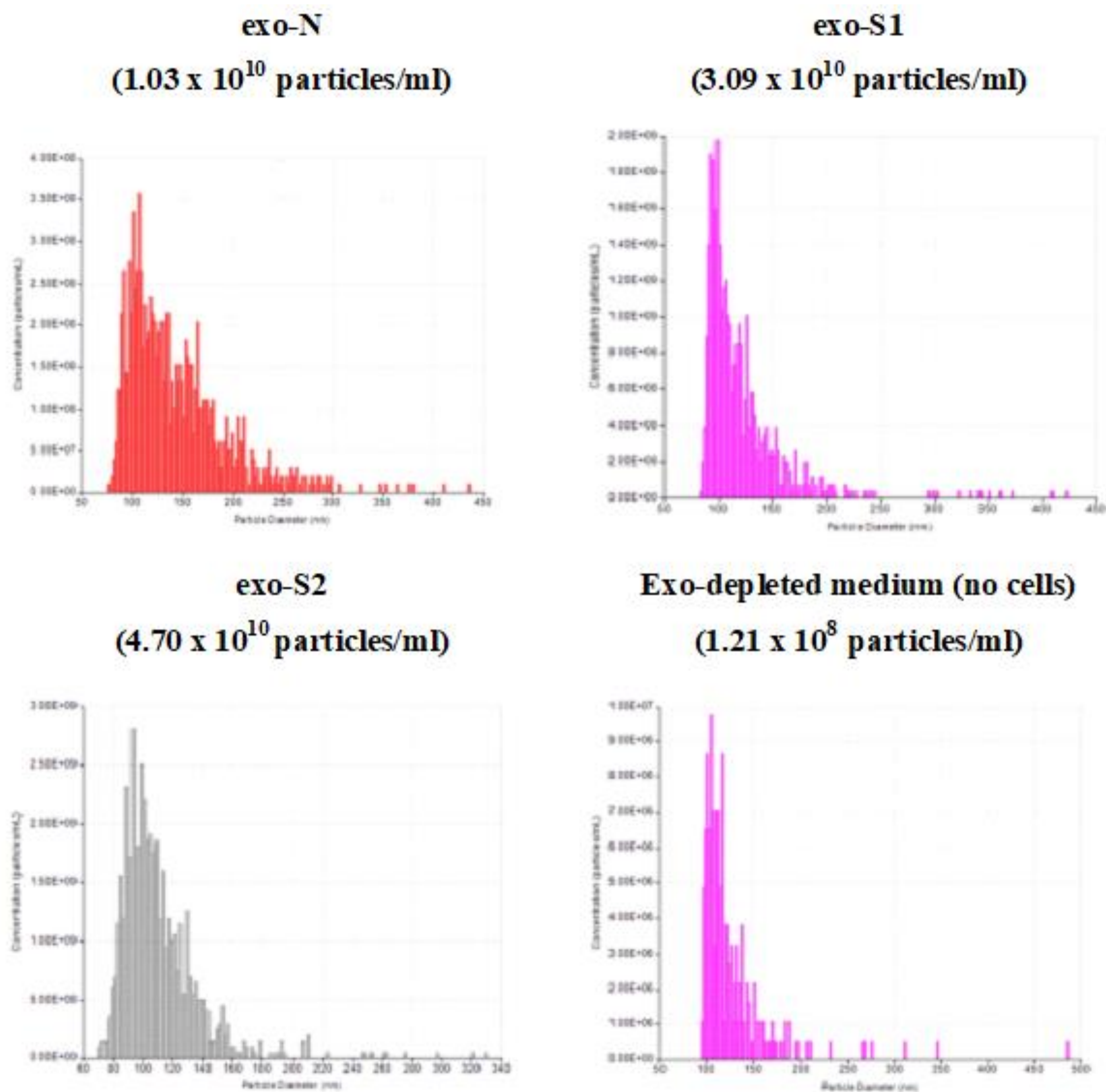
Emam et al., Supplementary Fig. 4C



Emam et al., Supplementary Fig. 4D

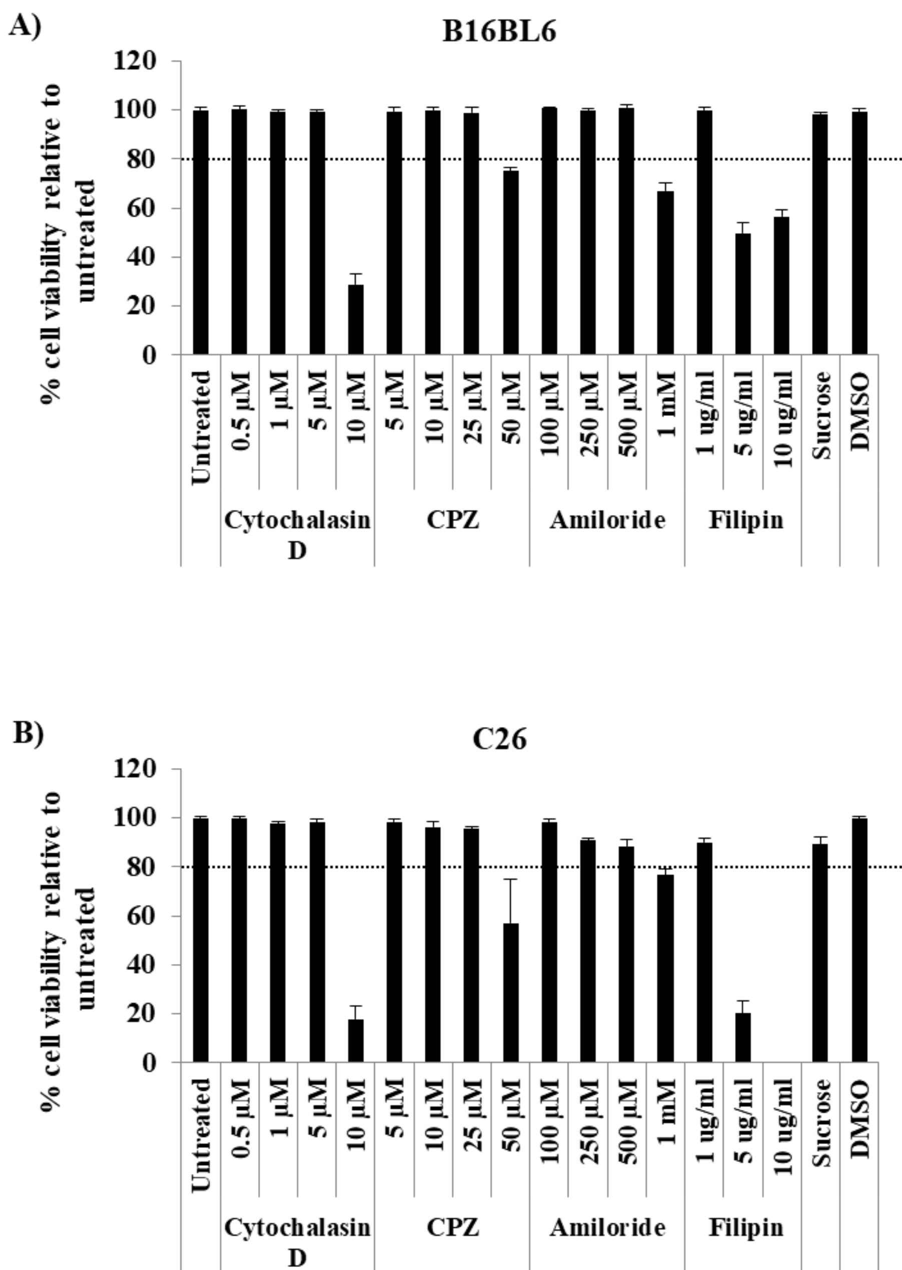


Emam et al., Supplementary Fig. 5



Supplementary Figure 5. Concentration and size distribution of different exosomes (EVs). The concentration and size distribution of exo-N, exo-S1 and exo-S2 in addition to the control medium (fresh conditioned medium treated in a similar manner as the isolation of exosomes, EVs) via nanoparticle tracking analysis using qNano.

Emam et al., Supplementary Fig. 6



Supplementary Figure 6. Effect of the uptake inhibitor concentration on cell viability of B16BL6 and C26.

B16BL6 (A) and C26 (B) cancer cell lines were incubated in presence of different concentrations of each uptake inhibitor and then cell viability was evaluated via a Countess II automated cell counter after staining cells with trypan blue dye. The cell viability was calculated as a relative percentage compared to untreated cells. All data represent the mean ± SD.