



Supplementary information, Figure S2 Heparanase effects on exosome number and size.

MCF-7 cells were left untreated (no Hep) or 10 nM exogenous proheparanase (Hep) was added to the culture medium of the cells. Prior to NanoSight analysis, exosomes were purified from conditioned media using ultracentrifugation as described elsewhere in this study. At least 3400 particle tracks were recorded with a NanoSight device and analyzed using Nanoparticle tracking analysis software v2.3. Results obtained for two different cultures of each type. **(a)** Particle concentration in function of particle size, for untreated (black curves) and heparanase treated (red curves) MCF-7 cells. **(b)** Total number of particles accumulating in exosomal fractions of untreated cells and cells treated with heparanase, relative to the average of the total particle count values measured in untreated MCF-7 cell cultures. **(c)** Modal sizes of the particles produced by untreated (light grey bar) and heparanase-treated (dark grey bar) MCF-7 cells. Bar heights reflect average values. Individual data points are shown as white dots on top of the corresponding bars.

The particle size distributions observed in media conditioned by MCF-7 cells correspond to a distribution expected for exosomes. On average, media conditioned by heparanase-treated cells contain 30% more particles compared to media conditioned by untreated cells (5.8 versus 4.4 x 10⁸ particles/ml). Note that this (small) difference might correspond to a large increase in syntenin-exosome number if syntenin-exosomes represent only a small fraction of the total pool of exosomes in untreated cells. In other words, a strong increase in syntenin-exosomes might be masked by the presence of a large number of exosomes that are not affected by heparanase. On the other hand, taking into account the large differences between replicate measurements, the observed difference might be the result of normal sample variation. The modal size of the particles does not change upon heparanase treatment.