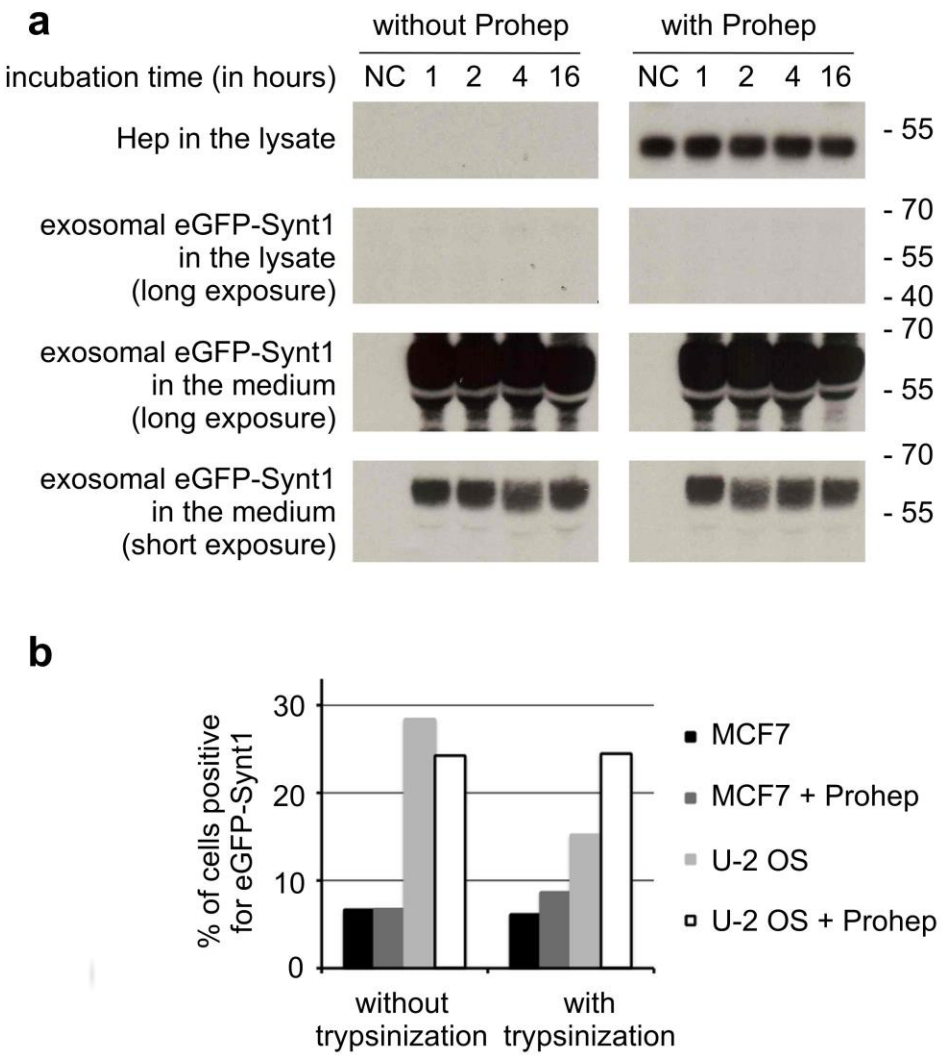


**Supplementary information, Figure S4** Heparanase effects on syntenin-1-exosome binding and uptake.

Recently, heparan sulfate proteoglycans in recipient cells were shown to be involved in exosome uptake or sequestration. Thus, trimming of the heparan sulfate on cells by heparanase might reduce exosomal uptake (re-internalization), leading to a net increase of exosomes in conditioned media. To test for exosomal binding and uptake, MCF-7 cells were transiently transfected with eGFP-syntenin-1. Media conditioned by these cells were spun for 10 minutes at 150xg, 10 min at 800xg and 30 minutes at 10,000xg (to remove cells and debris) and were then administered to wild type MCF-7 cells, left untreated or pre-treated with pro-heparanase (10nM). Cells treated for

16h with conditioned media derived from cells not transfected with eGFP-syntenin-1 served as negative controls (NC). **(a)** When analyzed by western blotting, lysates of MCF-7 cells that had been pre-exposed to pro-heparanase contained large amounts of mature heparanase (Hep). Whether cells were exposed to heparanase or not, lysates of cells incubated for 1, 2, 4 or 16 hours in media conditioned by cells transfected with eGFP-syntenin-1 showed no detectable accumulation of eGFP-syntenin-1. At the same time, exosomal fractions, prepared at the end of the incubations by step-wise ultracentrifugation of the culture media, showed no marked decrease of eGFP-syntenin-1 with time, even in the absence of heparanase. These data illustrate that MCF-7 cells, at best, bind and internalize only small amounts of syntenin-exosomes. **(b)** The uptake of eGFP-syntenin-1-exosomes was also investigated by confocal microscopy. Confocal images are shown in Supplementary figure 5. Upon incubation for 2 hours with media that contained exosomes labeled with eGFP-syntenin-1, a small fraction (+- 6%) of the MCF-7 cells bound or internalized eGFP (i.e. syntenin-1-exosomes). Trypsin treatment, to liberate exosomes bound to cell-surface proteins, did not substantially reduce the number of cells labeled with eGFP, suggesting most of the eGFP-syntenin-1-exosomes associating with MCF-7 cells had been internalized. Exposure to heparanase did not modify (reduce or stimulate) exosomal binding or uptake by MCF-7 cells. Compared to MCF-7 cells, U-2 OS (osteosarcoma) cells showed higher binding/uptake of syntenin-exosomes (+-25% of the cells bound/internalized eGFP), but again heparanase did not reduce this. For each condition, at total of at least 150 cells was scored.