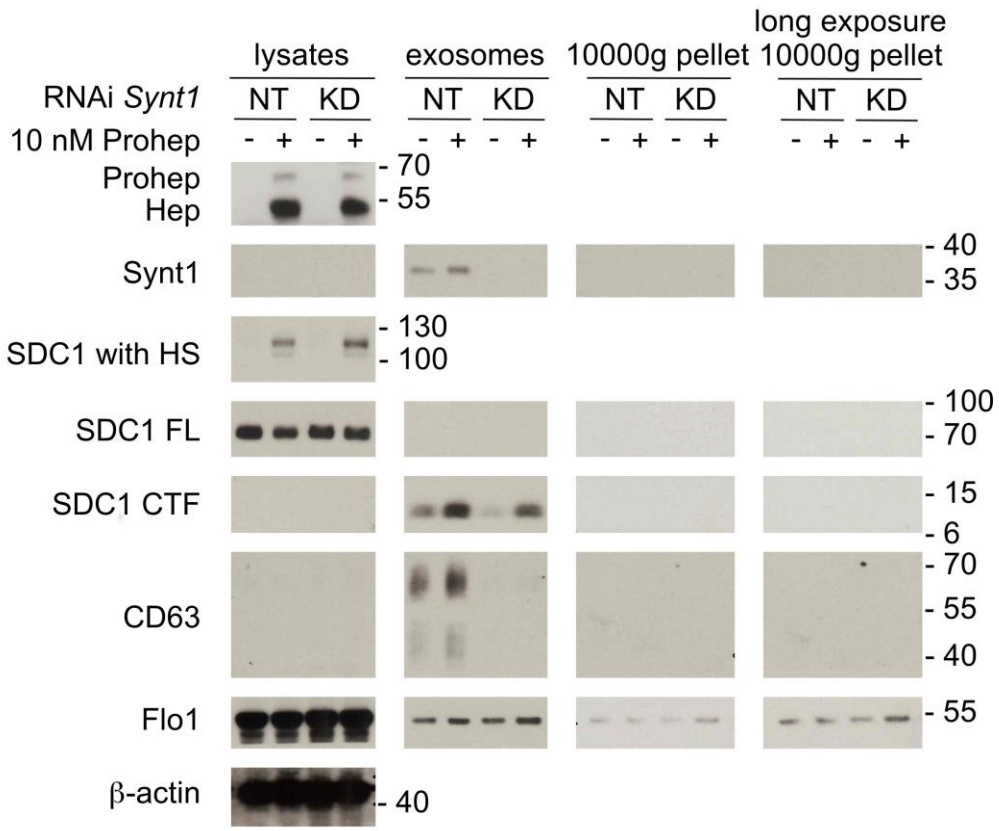

Supplementary information, Figure S3 Heparanase effects on exosome sedimentation.

To prepare exosome fractions, materials present in conditioned culture media are first pelleted at 10,000xg, to remove cell fragments and cell debris, and then at 140,000xg, to recover exosomes (see also 'Materials and methods' section). Heparanase causes an increase in exosomal markers in the 140,000xg pellet, stimulating the accumulations of syntenin-1, syndecan-1 CTFs and CD63, but leaving flotillin-1 unaffected. This stimulation is attenuated by syntenin knock-down. In theory, heparanase might reduce the aggregation of syntenin-exosomes. As a consequence, exosomal markers might shift from the 10,000xg pellet (containing exosomal aggregates) to the 140,000xg pellet (containing non-aggregated exosomes), explaining the observed effect of heparanase on exosomal markers. To investigate such possible effect of heparanase, MCF-7 cells were incubated with or without 10 nM proheparanase. In addition, the cells were treated with non-targeting siRNA (NT) or siRNA targeting syntenin-1 (KD). The 10,000xg pellets and exosomes (140,000xg pellets) prepared from the media conditioned by these cells were analyzed

by Western blotting. Unlike flotillin-1, present in both low- and high-speed pellets, syntenin-1, syndecan-CTFs and CD63 were exclusively detected in the 140,000xg pellets. Thus, possible trivial effects of heparanase on the partitioning of exosomes between low- and high-speed pellets during centrifugation seem excluded.