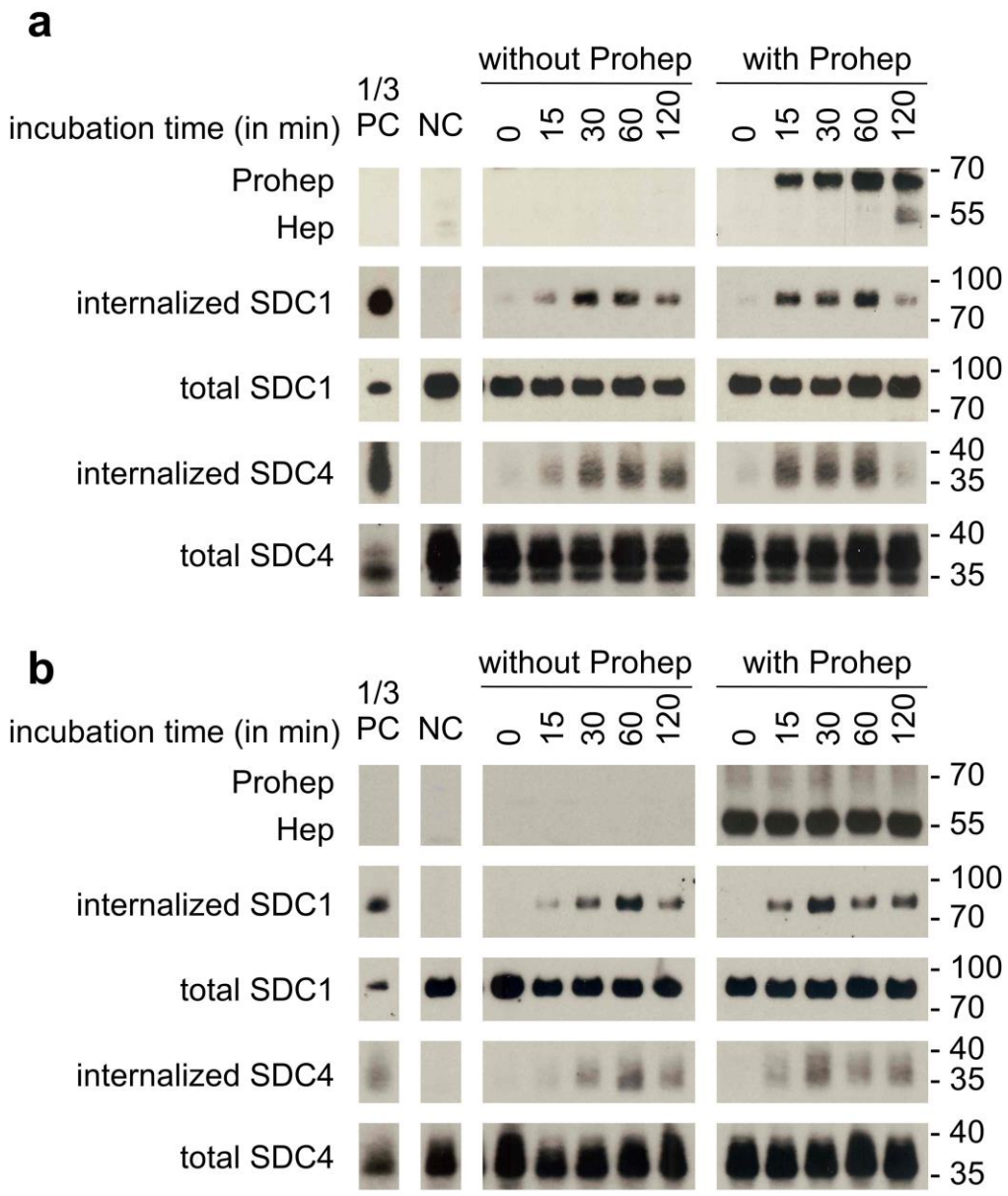

Supplementary information, Figure S6 Heparanase stimulates the endocytosis of syndecan-1 and syndecan-4.

To study the effect of heparanase on syndecan internalization, cell surface proteins including syndecans were reversibly biotinylated using Sulfo-NHS-SS-Biotin. After various times of treatment or incubation of the cells in culture medium supplemented with 0.1% BSA (as indicated in the figure), the remaining cell surface biotin was removed by reducing the disulfide bond. Endocytosed biotin is protected from reduction and removal. Biotinylated proteins were purified

from the lysates using streptavidin-Sepharose and analyzed by western blotting using antibodies against syndecan-1 (mAb 2E9) and syndecan-4 (mAb 8G3), allowing detection of endocytosed syndecans. Two different modes of heparanase-exposure were studied. **(a)** First, the acute effect of proheparanase on syndecan internalization was investigated. To this end, 10 nM proheparanase was added to biotinylated cells for the indicated amounts of time before removing the cell surface biotin. The same time course was applied to cells without proheparanase. Non-biotinylated cells served as negative control (NC). Non-incubated biotinylated cells of which the cell surface biotin was not removed served as positive control (PC, with only 1/3 of the sample loaded compared to the other samples). After 15, 30 and 60 minutes of incubation, the amount of endocytosed syndecan-1 and syndecan-4 increases with the addition of proheparanase. Note that the amount of endocytosed biotinylated syndecan decreases after 120 minutes of incubation, possibly because cell surface levels of biotinylated syndecan remaining available for internalization start decreasing and endocytosed syndecan is degraded. **(b)** Second, the effect of heparanase activity on syndecan endocytosis was examined. To this end the cells were first incubated with 10 nM proheparanase or left untreated (without heparanase). After several hours of heparanase uptake, the remaining extracellular proheparanase was washed away and cell surface proteins were biotinylated. Then, cells were allowed to internalize syndecans for the indicated amount of time before cell surface biotin was removed. While in the first experiment a large amount of proheparanase was present in the lysates, all proheparanase had been converted to active heparanase in the second experiment. Cells having acquired heparanase activity endocytosed more syndecan-1 and syndecan-4 compared to cells without heparanase activity. This suggests heparanase activity and the consequent trimming of heparan sulfate chains on syndecans influences their internalization rate. These experiments indicate both proheparanase and heparanase activity stimulate the endocytosis of syndecans, possibly leading to increased endosomal concentrations of syndecans.