


Supplementary figure legends

Suppl. Figure 1. A. Alignment of Hpa2c (blue) and heparanase (black) protein sequences. Identical amino acids are lined in red; similar amino acids are marked as (+). B. Schematic

presentation of Hpa2 splice variants. Also shown are putative N-glycosylation sites (), and the region utilized to immunize rabbits for the production of Ab58 (arrows).

Suppl. Fig. 2. Hpa2 is localized in the ER. HEK293 cells stably expressing the indicated Myc-tagged Hpa2 variant were subjected to immunofluorescent staining applying anti-Myc (left panels, red) and anti-calnexin (an ER marker; middle panels, green) antibodies. Merge images are shown in the right panels together with nuclear staining (blue).

Suppl. Figure 3. A. Syndecan clustering. U87 glioma cells were left untreated (Con, upper panels) or incubated with Hpa2c (1 $\mu\text{g}/\text{ml}$; second panels) for 30 min. Cells were then fixed and subjected to immunofluorescent staining applying anti-syndecan-4 (left panels, red) and anti-Hpa2 (Ab58, green) antibodies. Merge images are shown in the right panel. B. Hpa2c inhibits heparanase uptake. JSQ-3 nasal vestibule carcinoma (left panels), U87 glioma (middle panels) and 293 (right panels) cells were left untreated as control (Con) or incubated with Myc-tagged heparanase (1 $\mu\text{g}/\text{ml}$; Hepa) and the indicated concentration of Hpa2c (2c) for 30 min. Cell lysates were prepared and subjected to immunoblotting applying anti-Myc (upper panel) or anti actin (lower panel) antibodies. C. Ab58 specificity. 5 micron sections of tumor xenograft produced by control (Mock), heparanase (Hepa-1)-, and Hpa2c (Hpa2)-transfected U87 glioma cells were subjected to immunostaining applying Ab58 without or with the peptide used for immunization (Hpa2+peptide). Note reactivity of antibody 58 in tumor xenograft generated by Hpa2c transfected cells, which practically diminishes by the inclusion of the immunizing peptide. No reactivity was observed in specimen derived from xenograft produced by cells over expressing heparanase.