Supplementary figure legends

Suppl. Figure 1. Heparanase enhances signaling cascade: densitometry analysis. Over expression. FaDu pharynx carcinoma (**A-D**) and LNCaP prostate carcinoma (**E-G**) cells were stably transfected with control empty vector (Vo), wild type (Hepa), or mutated inactive (DM) heparanase gene constructs and cell lysates were subjected to immunoblotting applying the indicated antibody. Shown are bar graphs of phosphorylation index calculated by densitometry analysis (fold increase; average ± SE, Vo cells arbitrary set to a value of 1) of phospho-EGFR (A, E), phospho-Src (B, F), phospho-Akt (C, G) and phospho-ERK (D). Gene silencing. LNCaP prostate carcinoma cells were transfected with anti-GFP (siGFP) or anti-heparanase (siHepa) siRNA oligonucleotides. Cell lysates were prepared and subjected to immunoblotting applying the antibodies described above. Shown are bar graphs of phosphorylation index calculated by densitometry analysis (siGFP cells arbitrary set to a value of 1) of phospho-EGFR (H), phospho-Src (I), and phospho-Akt (J). The actual Western blots are shown in Fig. 1.

Suppl. Figure 2. Heparanase stimulates STAT5 phosphorylation: densitometry analysis. **A**. Lysates of FaDu cell transfected with wild type (Hepa) or inactive, double-mutated (Glu^{225} and Glu^{343}; DM) heparanase gene constructs or an empty vector (Vo) were subjected to immunoprecipitation (IP) with anti-STAT5b antibody, followed by immunoblotting with anti-phospho-tyrosine (PY) antibody. Shown are bar graphs of pSTAT5b phosphorylation index calculated by densitometry analysis (Vo cells arbitrary set to a value of 1) of at least 5 independent experiments. **B, C**. Shown is densitometry analysis (fold increase; average ± SE) of phospho-STAT5b levels in LNCaP cells following heparanase over expression (B) or gene silencing (C). **D, E**. Shown is densitometry analysis (fold increase; average ± SE) of phospho-STAT5b (D) and phospho-STAT5a (E) levels in T47D cells following heparanase over expression. **F, G**. Association (fold-increase; average ± SE) of STAT5b with the β-casein promoter following heparanase over expression in T47D (**F**) and FaDu cells (**G**). The actual Western blots are shown in Fig. 1.

Suppl. Figure 3. **A**. Immunofluorescent staining. Control (Vo) and heparanase transfected (Hepa) LNCaP (left panels) and CAG (right panels) cells were stained with anti-STAT5b antibody. **B.** Immunohistochemistry analysis of pSTAT3. Formalin-fixed, paraffin-embedded 5 micron sections of xenografts produced by control (Vo) and heparanase transfected (Hepa) CAG myeloma cells were stained with anti-pSTAT3 antibody. Note increased reactivity in endothelial cells in xenograft produced by heparanase transfected CAG cells. Arrows point to unstained blood vessels in control xenograft. **C, D**. Clinical association. **C**. Lymph nodes metastasis. The number of metastatic lymph nodes found in neck dissection was compared in

head and neck cancer patients exhibiting cytoplasmic (Cyto) vs. nuclear (Nuc) pSTAT3 localization. Note increased number of lymph nodes metastasis in patients exhibiting cytoplasmic vs. nuclear pSTAT3 (3 vs. 1.5 in average, respectively; p=0.05). **D**. Association with pEGFR. The extent (i.e., percent of positively-stained cells) of pEGFR staining was compared in head and neck cancer patients exhibiting cytoplasmic (Cyto) vs. nuclear (Nuc) localization of pSTAT3. Note higher pEGFR staining extent in patients exhibiting cytoplasmic vs. nuclear pSTAT3 (59 vs. 27%, respectively; p=0.0007).

Suppl. Figure 4. Cells over expressing heparanase are less sensitive to EGFR inhibitor: densitometry analysis. Control (Vo, grey bars) and heparanase transfected (Hepa, black bars) FaDu cells were incubated with the indicated concentration (μM) of CL-387,785 for 2h. Vehicle (DMSO) was used as control (0). Shown are bar graphs of phosphorylation index calculated by densitometry analysis of EGFR phosphorylated at tyrosine residue 1173 or 845 (upper and second panels, respectively), phospho-Akt (third panel), and phospho-STAT3 (lower panel). Note sustained EGFR signaling in heparanase over expressing cells even in the presence of high doses (1μM) of CL-387,785. The actual Western blots are shown in Fig. 5.