Electronic Supplementary Information

High-resolution probing heparan sulfate-antithrombin interaction on single endothelial cell surface: Single-molecule AFM studies†

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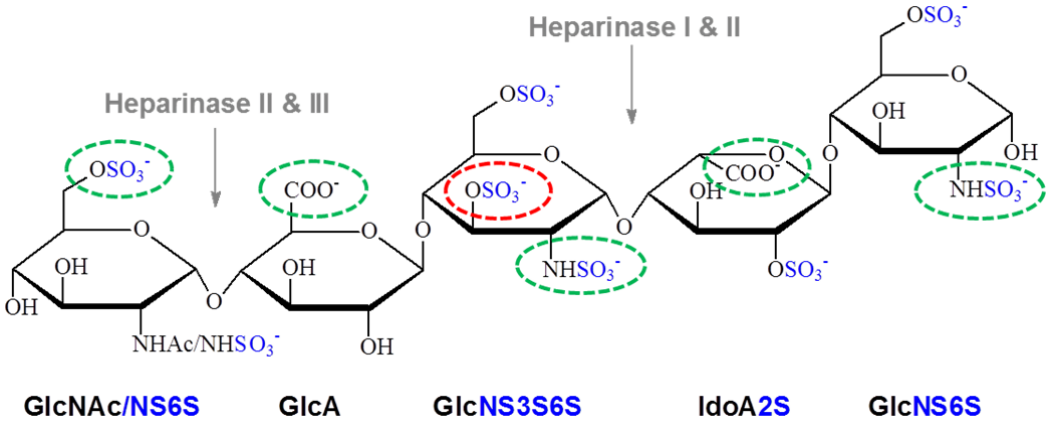

Figure S1. Typical structure of a heparin pentasaccharide required for antithrombin binding (Note: all the possible sulfation sites are shown). Dashed circles show the previously identified groups that are critical in heparin's binding to antithrombin.[1] Dashed red circle shows the central 3-*O*-sulfation (3S) modification. The heparinase cleavage sites are also shown.

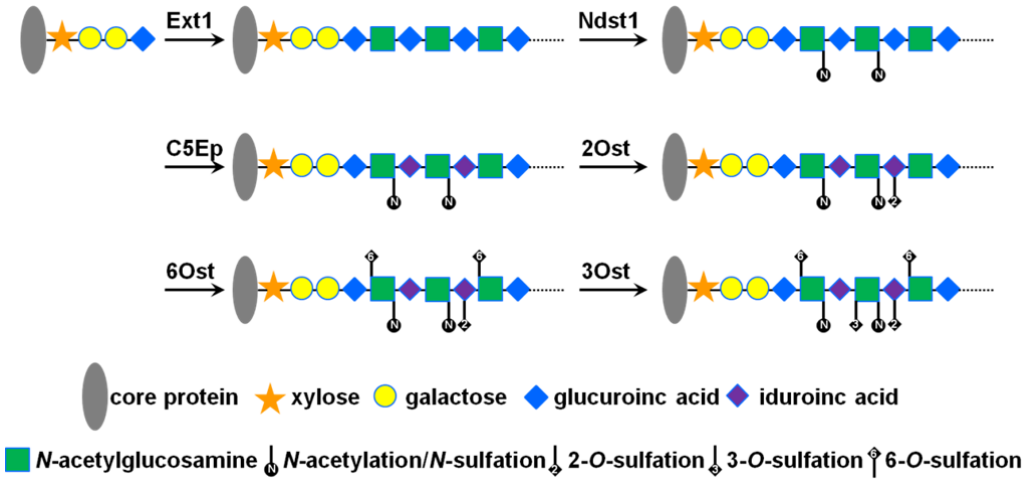

Figure S2. Schematic for biosynthesis of HS.[1]

HS is biosynthesized as proteoglycans by alternatively adding uronic acids (iduronic acid, IdoA, or glucuronic acid, GlcA) and *N*-acetylglucosamine (GlcNAc) repeating units to core proteins with co-polymerase exostosin (Ext 1 shown here as an example).[1] The formed HS backbone is sequentially modified by *N*-deacetylation and *N*-sulfation, epimerization and *O*-sulfation with Ndst (such as Ndst1), C5Ep, and Ost (2Ost, 6Ost, 3Ost), respectively. During this process, Ndst catalyzes the *N*-sulfation of GlcNAc units (NS).[2]

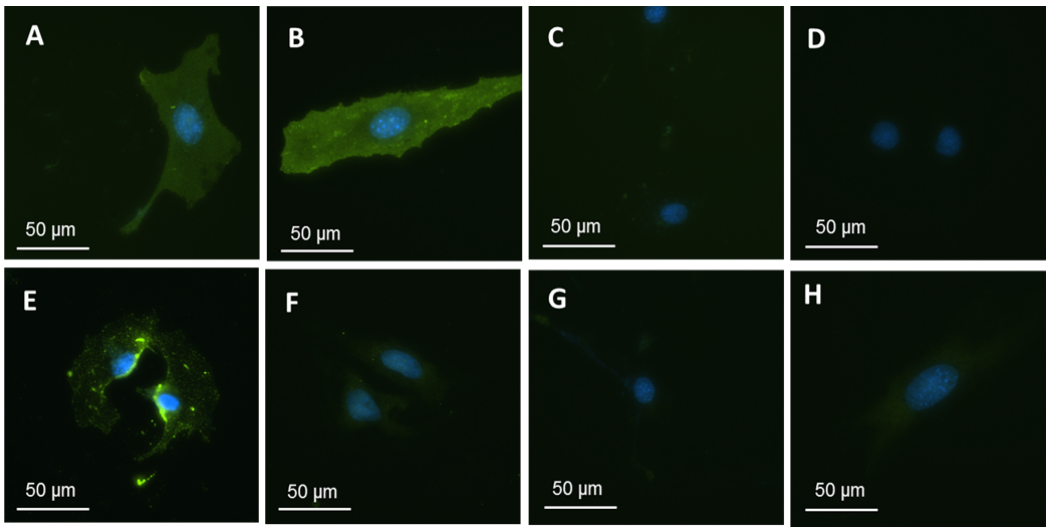

Figure S3. Immunofluorescence staining of PECAM-1 and HS expression on endothelial cell surface. The *Ndst1*$^{f/f}$ and *Ndst1*$^{-/-}$ endothelial cells were first stained with the primary anti-PECAM-1 antibody (A, B) or anti-HS antibody 10E4 (E, F), and then stained with corresponding FITC-conjugated secondary antibody (green color). The cells that were not stained with the primary anti-PECAM-1 antibody (C, D) or anti-HS antibody 10E4 (G, H), but only the FITC-conjugated secondary antibody were served as background controls. The cell nuclei were stained by DAPI (blue color).

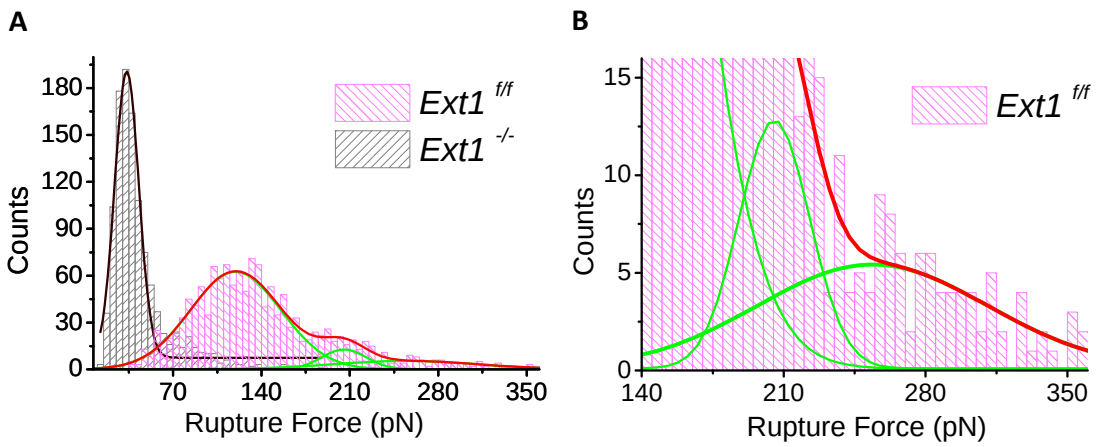

Figure S4. Comparison of rupture forces between *Ext1*$^{f/f}$ and *Ext1*$^{-/-}$ cells. (A) Distributions of rupture force for *Ext1*$^{f/f}$ and *Ext1*$^{-/-}$ cells. (B) Partially zoom in of histogram for *Ext1*$^{f/f}$ cell to clearly show the third peak.

References

1. M. Petitou, B. Casu and U. Lindahl, *Biochimie*, 2003, **85**, 83-89.
2. R. Sasisekharan and G. Venkataraman, *Curr. Opin. Chem. Biol.*, 2000, **4**, 626-631.