**Supplementary Table 1.** Summary of the disaccharide analysis of *N*-sulfo heparosan and *N*-sulfo heparosan 6-*O*-sulfate

Disaccharides	N-sulfo heparosan	<i>N</i> -sulfo heparosan 6- <i>O</i> -sulfate
	( <i>mol/mol</i> %)	( <i>mol/mol</i> %)
∆UA-GlcNAc	24.4%	4.6%
ΔUA-GlcNS	75.6%	10.4%
∆UA-GlcNAc6S	N.D.	14.6%
∆UA-GlcNS6S	N.D.	70.4%

## **Supplementary Figure 1**





Supplementary Figure 1. Elution profiles of N-[<sup>35</sup>S]sulfo heparosan oligosaccharides on BioGel P-10. Low specific [<sup>35</sup>S]radioactively labeled N-[<sup>35</sup>S]sulfo heparosan ( $1.5 \times 10^6$  cpm/mg) was prepared by incubating deacetylated heparosan with purified NST and  $[^{35}S]PAPS$ . The polysaccharide (1 mg) was digested with 20 ng of purified heparin lyase III. The products were fractionated on a BioGel P-10 column, which was eluted with a buffer containing 25 mM Tris, 1000 mM NaCl, pH 7.4. The fractions were monitored by both [<sup>35</sup>S]radioactivity and UV 232 nm. The fractions containing the oligosaccharides with the desired size were pooled for further analysis.



**Supplementary Figure 2. Synthetic scheme of recomparin.** The components involved in PAPS regeneration system are boxed. AST-IV represents arylsulfotransferase IV, and PAP represents 3'-phosphoadenosine 5'-phosphate.

**Supplementary Figure 3** 



Supplementary Figure 3. Inhibitory effects of heparin and recomparin on Xa activity. Heparin and recomparin were incubated with AT ( $20 \mu g/ml$ ), factor Xa (10 U/ml) and bovine serum albumin ( $100 \mu g/ml$ ) in 20 mM sodium phosphate and 150 mM NaCl pH 7.2, and 1 mM S-2765 chromogenic Xa substrate. The activity of Xa was determined by the rate of the increase of the absorbance at 405 nm. The activity without polysaccharide was defined as 100%. Each data point represents the average of two determinations. Error bars indicate the range.