Supplemental Information

Materials and Methods

Topoisomerase I Assay

Topoisomerase I activity was measured using a commercially available topoisomerase assay kit (TopoGEN, FL). The kit is based on the detection of topoisomerase I activity using relaxation of plasmid DNA as the assay. Nuclear extracts from CAG HPSE-high and HPSE-low cells were incubated with assay buffer (10 mM tris-HCl, pH 7.9, 1 mM EDTA, 0.15 M NaCl, 0.1% BSA, 0.1 mM spermidine, 5% glycerol) and supercoiled DNA for 30 minutes at 37ºC . The reactions were then terminated by adding 5 µl stop loading dye (5% sarkosyl, 0.125% bromophenol blue, 25% glycerol). Relaxation of supercoiled DNA plasmid was determined by running the samples on 1% agarose. Gel was post-stained with 0.5 µg/ml ethidium bromide and visualized under UV light.

Cell Cycle Analysis

Following 24 h in culture, cells (10^6) were harvested, washed with PBS solution, fixed and permeabilized with cold ethanol. Cells were then resuspended in 0.1 % Triton X100 in PBS containing 20 µg/ml propidium iodide and 2 mg DNase free RNaseA. Samples were analyzed using a Becton Dickinson FACSCalibur flow cytometer. Data were analyzed using FlowJo software.

HAT Assay

SST001 (50 µg/ml) or heparin (50 µg/ml) was added directly to the nuclear extracts from heparanase-high cells and HAT activity measured as described.

Western Blot

Nuclear extracts from heparanase low and high cells were subjected to 10% SDS-PAGE under reducing conditions, transferred to nitrocellulose membrane (Schleicher and Schuell), and probed with pCAF (Millipore, Temecula, CA), CBP (Millipore, Temecula, CA) or p300 (Santa Cruz Biotechnology Inc., CA). Equal loading of protein was confirmed by staining membranes for lamin (abcam).

Supplemental Table 1. Disaccharide composition of the heparan sulfate (HS) and heparin preparations.

HS from bovine kidney (Seikagaku Co, Tokyo, Japan) and porcine intestine (Sigma, Saint Louis, MO), and heparin from porcine intestine (Nacalai tesque, Kyoto, Japan) were individually digested with a mixture of heparinases-I and –III (IBEX Technologies, Montreal, Canada), and each digest was labeled with 2-aminobenzamide and analyzed by anion-exchange HPLC using a PA-03 column (YMC Co., Kyoto, Japan) (Kinoshita, A., and Sugahara, K. (1999) *Anal Biochem* **269**, 367-378). The amount of specific disaccharide units in each sample was calculated on the basis of the peak area of the chromatograms.

	HS (Bovine kidney)	HS (Porcine intestine)	heparin (Porcine intestine)
	mol%		
ΔHexUA-GlcNAc	54.3	46.4	5.3
ΔHexUA-GlcNAc(6S)	11.7	11.7	4.2
ΔHexUA-GlcN(NS)	20.7	32.0	4.7
ΔHexUA-GlcN(NS,6S)	5.0	3.3	14.5
ΔHexUA(2S)-GlcN(NS)	4.3	2.4	4.3
ΔHexUA(2S)-GlcN(NS,6S)	4.1	4.2	67.0
Total	100.0	100.0	100.0

Abbreviations:
ΔHexUA, 4,5-unsaturated hexuronic acid; GlcNAc, *N*-acetyl-D-glucosamine; GlcN, D-glucosamine; NS, 2-*N*-sulfate; 2S, 2-*O*-sulfate; 6S, 6-*O*-sulfate.

Figure Legend

Supplemental Fig 1. (A) Nuclear extracts from CAG cells expressing high and low levels of heparanase were assessed for their level of HAT enzymes CBP, pCAF, and p300. (B) SST0001 (50 μg/ml) or heparin (50 μg/ml) were added to the nuclear extracts from HPSE-high cells and HAT activity was assessed by ELISA using histone H3 and histone H4 peptides as substrates. Data are expressed as absorbance ± SD. * $P < 0.05$ vs. untreated cells. (C) Cell cycle distribution of CAG HPSE-high or low cells stained with propidium iodide. (D) Nuclear extracts isolated from CAG human myeloma cells expressing either high or low levels of HPSE were assayed for their topoisomerase I activity.

Supplemental Fig. 1