SUPPLEMENTAL DATA

Table S1. Characteristics of K5 polysaccharide derivatives and modified heparin

Test molecule	Characteristics	Source
K5	$(GlcNAc\text{-}GlcA)_n$ (47)	Glycores (Milano, Italy)
NdAcNS K5	*N*-deacetylated/*N*-sulfated K5 (47)	Glycores (Milano, Italy)
epiNdAcNS K5	epimerized *N*-deactylated/*N*-sulfated K5	Glycores (Milano, Italy)
OS K5 (low)	low *O*-sulfated K5: 90% GlcNAc6OS, 10% GlcNAc (47)	Glycores (Milano, Italy)
NdAcNS/OS K5 (low)	*N*-deacetylated/*N*-sulfated/low *O*-sulfated K5: 90% GlcNS6OS, 10% GlcNS (47)	Glycores (Milano, Italy)
NdAcNS/OS K5 (high)	*N*-deacetylated/*N*-sulfated/high *O*-sulfated K5: 100% GlcNS6OS; 70% GlcA2,3OS, 30% GlcA2OS or GlcA3OS (47)	Glycores (Milano, Italy)
heparin	11.3% NAc, 88.7% NS, 69% 2OS, 79% 6OS	G. Ronzoni Institute (Milan, Italy)
CdSNAc heparin	completely desulfated/*N*-acetylated heparin	Seikagaku (Tokyo, Japan)
CdSNS heparin	completely desulfated/*N*-sulfated heparin	Seikagaku (Tokyo, Japan)
NdSNAc heparin	*N*-desulfated/*N*-acetylated heparin: 100% NAc, 0% NS, 69% 2OS, 79% 6OS	G. Ronzoni Institute (Milan, Italy)
2OdS heparin	2-*O*-desulfated heparin: 13% NAc, 87% NS, 0% 2OS, 79% 6OS	G. Ronzoni Institute (Milan, Italy)
partially 6OdS heparin	6-*O*-desulfated heparin: 13% NAc, 87% NS, 67% 2OS, 23% 6OS	G. Ronzoni Institute (Milan, Italy)
OdS heparin	*O*-desulfated heparin	Neoparin (Alameda, CA)
carboxyl-reduced heparin	uronic acid COOH-reduced heparin	Neoparin (Alameda, CA)
HS		Celsus (Cincinnati, OH)

K5 is the non-sulfated precursor polysaccharide of HS/heparin derived from *E. coli*; the modified heparin preparations and HS are from porcine intestinal mucosa.

HS, heparan sulfate; (Glc)NAc, *N*-acetyl(glucosamine); (Glc)NS, *N*-sulfated(glucosamine); GlcA, D-glucuronic acid; IdoA, L-iduronic acid; OS, *O*-sulfate.

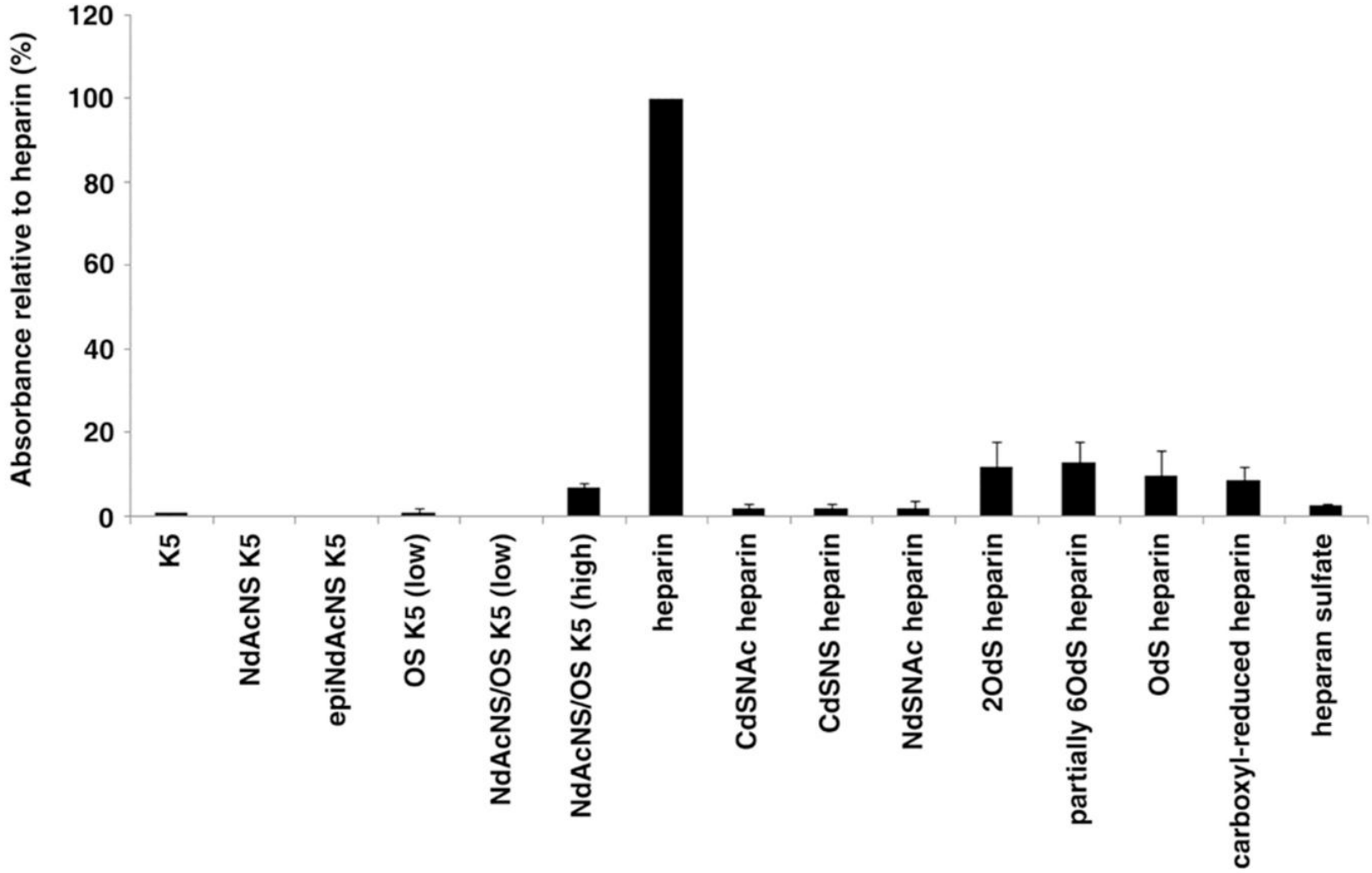
Absorbance relative to heparin (%)
120
100
80
60
40
20
0
K5
NdAcNS K5
epiNdAcNS K5
OS K5 (low)
NdAcNS/OS K5 (low)
NdAcNS/OS K5 (high)
heparin
CdSNAc heparin
CdSNS heparin
NdSNAc heparin
2OdS heparin
partially 6OdS heparin
OdS heparin
carboxyl-reduced heparin
heparan sulfate

Fig. S1. Characterization of the NS4F5 epitope by ELISA. Antibody NS4F5 was applied to various immobilized GAG preparations. Bound antibodies were detected using mouse anti-VSV-tag IgG antibody P5D4, followed by alkaline phosphatase–conjugated rabbit anti-mouse IgG. Enzymatic activity was measured using *p*-nitrophenyl phosphate as a substrate. Bars represent the mean reactivity ± SD (n = 4) of the antibodies in percent relative to the reactivity with heparin.

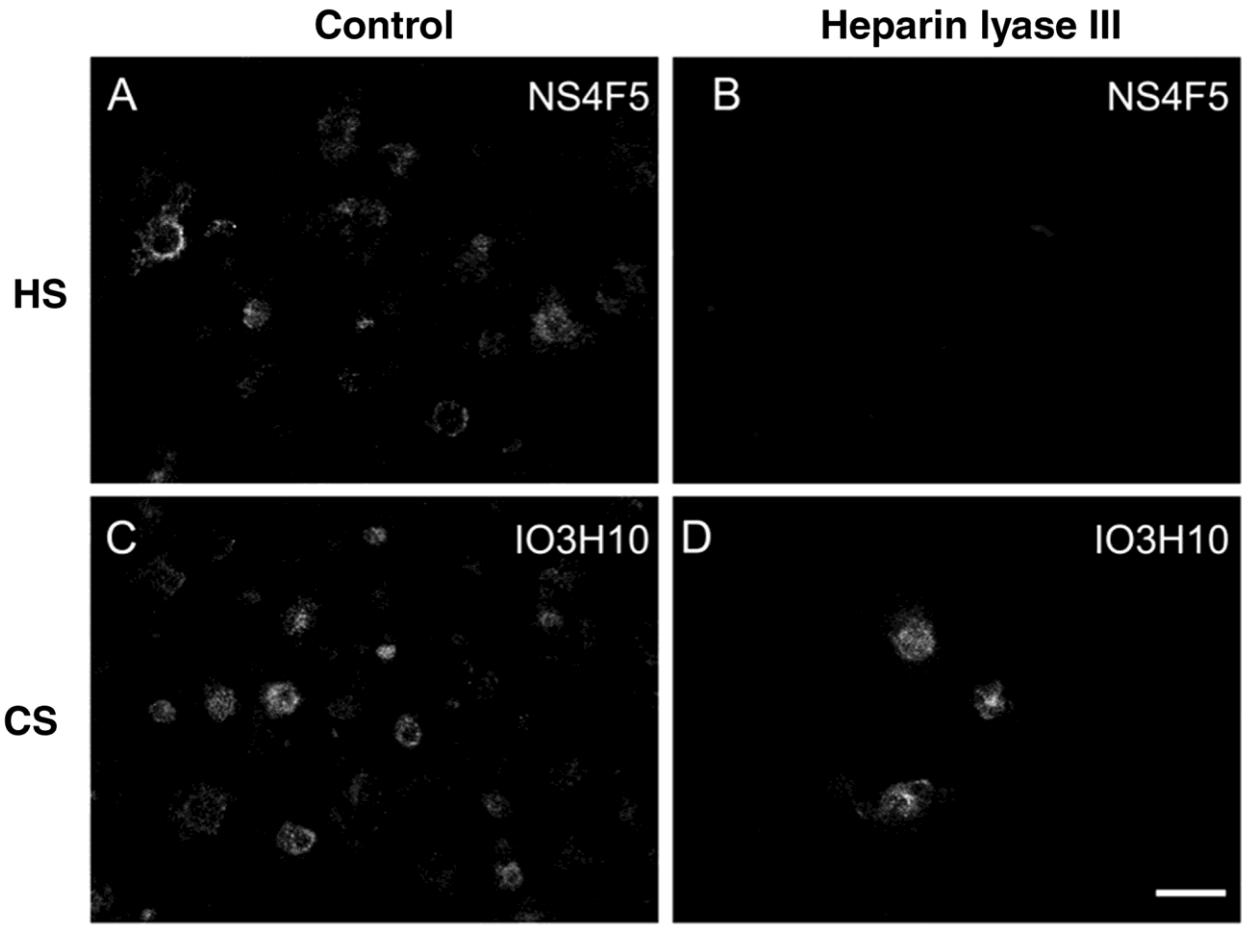

Fig. S2. Expression of the HS^{NS4F5} motif by human lung epithelial cells. Human lung epithelial cells were stained with antibody NS4F5 (A) or anti-CS antibody IO3H10 (C). Bound antibodies were detected using mouse monoclonal antibody P5D4 followed by Alexa 488-conjugated goat anti-rabbit IgG. Human lung epithelial cells produce the HS^{NS4F5} motif (A), which can be removed by heparin lyase III-treatment (B), indicating the epitope to be present in HS. Note that heparin lyase III-treatment had no effect on CS (C, D). n = 5, Bar, 50 µm

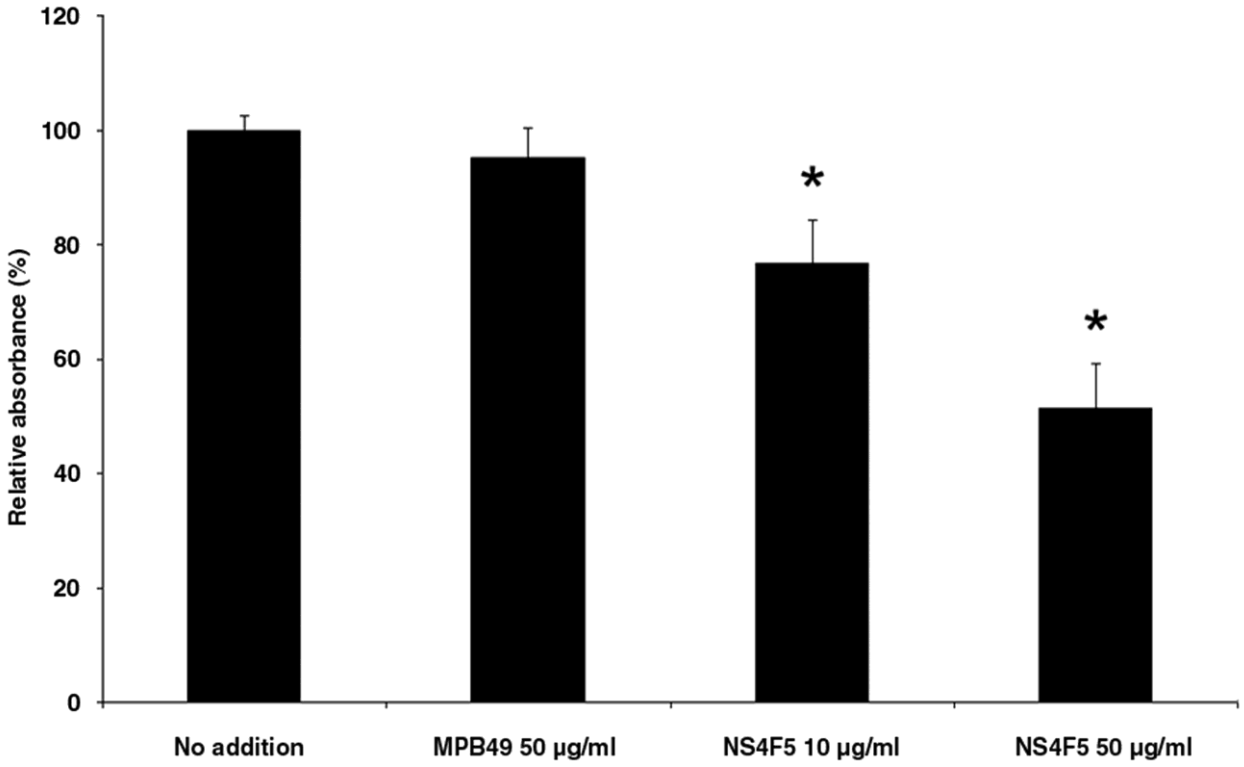

Fig. S3. HS^{NS4F5} immunoblocking reduces cell proliferation. Human lung epithelial cells were incubated with or without the NS4F5 antibody (10 and 50 µg/ml) for 16 h. Proliferation was measured at 450 nm using the WST-1 assay (*see Methods*). Values are expressed as means ± SD, $n = 5$). Note that the effect of the NS4F5 antibody is dose dependent. As a control, cells were incubated with control antibody MPB49. *, $P < 0.05$