SUPPLEMENTAL FIGURE LEGENDS

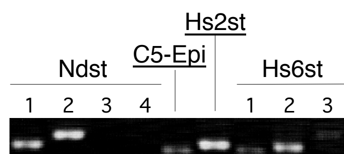
Supplemental Figure S1. Major heparan sulfate biosynthetic enzymes expressed by mouse lymphatic endothelial cells: RNA was isolated from murine lymphatic endothelial cells purified from oil-granuloma/ lymphangioma lesions, reverse transcribed, and amplified (40 cycles) by RT-PCR using murine gene specific primers to each of the enzymes shown. The cells expressed enzymes involved in both the biosynthesis of the heparan sulfate linkage tetrasaccharide and chain polymerization (XylT1,2; GalT1,2; GlcAT1; Ext1,2) as well as several sulfate-modifying enzymes typically involved in the generation of growth factor binding/ signaling motifs, including N-deacetylase/ N-sulfotransferases-1 and -2 (Ndst1, Ndst2), HS 2-O sulfotransferase (Hs2st), and HS 6-O sulfotranferases (Hs6st). Otherwise, only Hs3st3a was expressed along with endosulfatases-1 and –2 (Sulf1, Sulf2). Legend for enzymes: XylT1,2 (Xylosyltransferase-1, -2); GalT1,2 (Galactosyltransferase-1, -2); GlcAT1 (Glucuronosyl-transferase-1); Ext1,2 (Exostosin-1); Ndst1-4 (N-deacetylase/ N-sulfotransferase 1-4); C5-Epi (C5-Epimerase); Hs2st (HS 2-O-sulfotransferase); Hs6st1-3 (HS 6-O-sulfotransferases 1-3); Hs3st1-6 (HS 3-O-sulfotransferases 1-6); Sulf1,2 (HS endosulfatase-1, -2).

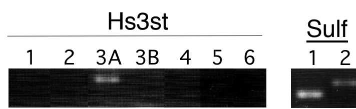
Supplemental Figure S2: VEGFR-3 receptor activation in response to VEGF-C is altered in the presence of heparin. VEGFR-3 phosphorylation in response to treatment with 100 ng/ml VEGF-C was examined in the absence/ presence of 10 μg/ml heparin (a level sufficient to abrogate Erk1/2 phosphorylation in response to VEGF-C; see Fig.1B). Receptor was immunoprecipitated from lysates of either serum-starved hLEC (baseline) or serum-starved hLEC incubated in the absence/ presence of heparin during stimulation with recombinant human VEGF-C. Immunoblotting with anti-phospho-tyrosine was carried out, and revealed a phosphorylated product in response to stimulation with VEGF-C (WB:P-Tyr; middle lane), consistent with phosphorylated VEGFR-3. The response of heparin-treated cells is shown in the right lane. Total receptor in the original lysates (TL) is shown below (WB:VEGFR-3).

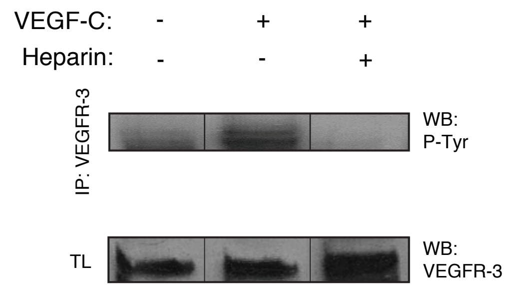
Supplemental Figure S3: Effect of targeting lymphatic Ndst1 on cell cycle progression in response to VEGF-C**.** Serum-starved early-passage primary human lung LEC transfected with either control RNA (scrambled-duplex; siDS) or with siRNA targeting Ndst1 (siNdst1) were incubated in the absence vs presence of VEGF-C (300 ng/ml) for 24hr in minimally supplemented medium. Cell cycle analysis was then carried out using flow cytometry to measure cell DNA content, with G1, S, and G2/M cytometry data fit to a Watson Pragmatic mathematical model. The ratio of (S+G2/M) to G1 cells is presented for each condition.

Supplemental Figure S4: Summary of sulfate modifications on heparan sulfate disaccharides purified from wildtype versus *Ndst1*-mutant primary lymphatic endothelia. Heparan sulfate was isolated from primary mouse lymphatic endothelial cells bearing a mutation in *Ndst1*, and the sulfation of heparinase-liberated disaccharides was determined by liquid chromatography/ mass spectrometry. (See Fig.6D for graph showing relative abundances of each disccharide species.) The degree to which the disaccharides from mutant versus wildtype cells were unsulfated, N-sulfated, 2-O-sulfated, or 6-O-sulfated is shown as a percentage of total disaccharides. (Sum of percentages for any given genotype may exceed 100% since disaccharides may be multi-sulfated with combinations of N-, 2-O-, and/or 6-O- sulfate modifications.)

GlcAT1
XylT
GalT
Ext
1 2 1 2 1 2


Hs2st
Ndst
C5-Epi
Hs6st
1 2 3 4 1 2 3


Hs3st
1 2 3A 3B 4 5 6
Sulf
1 2


VEGF-C: - + +
Heparin: - - +
IP: VEGFR-3
WB:
P-Tyr
TL
WB:
VEGFR-3

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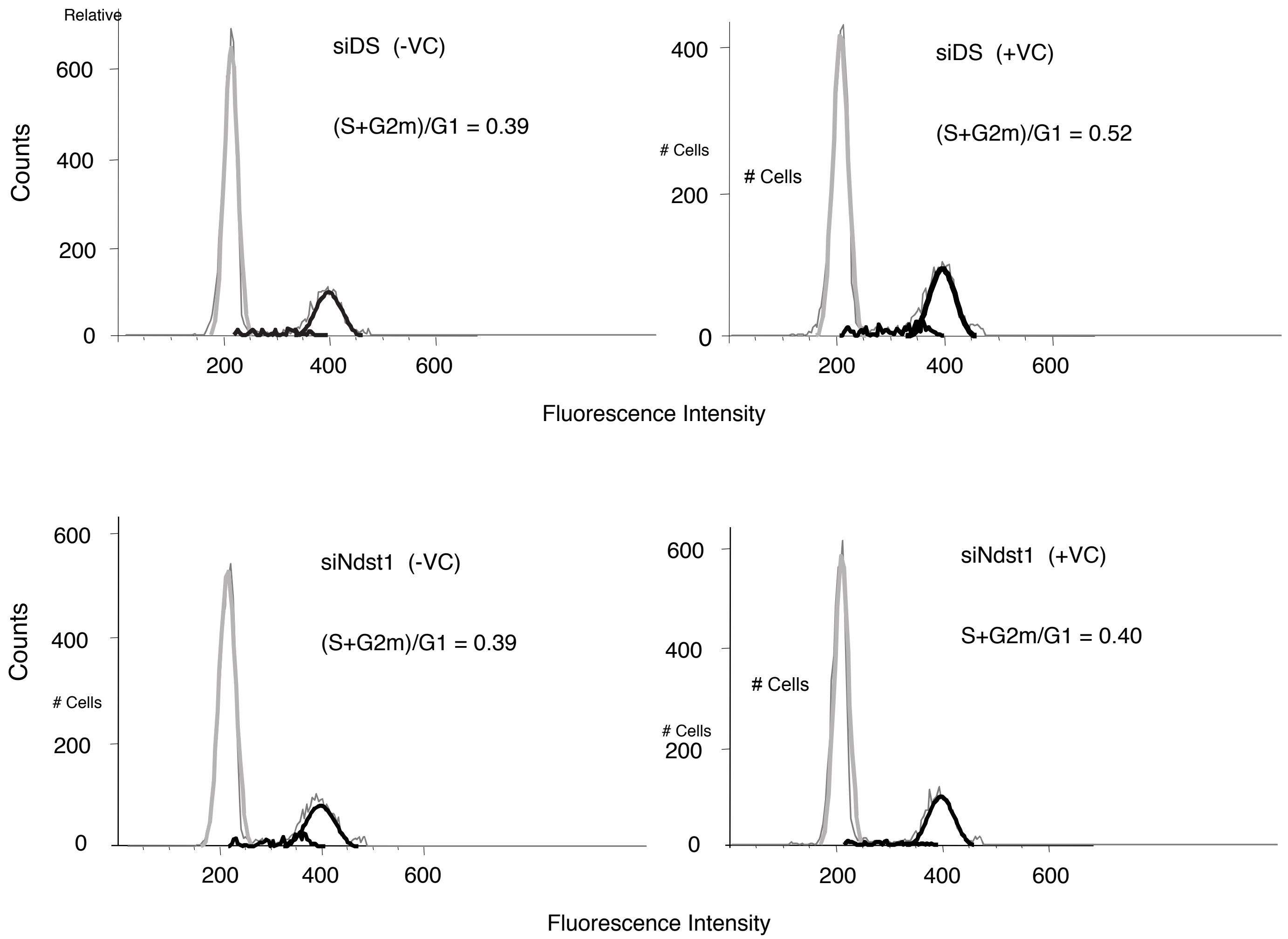
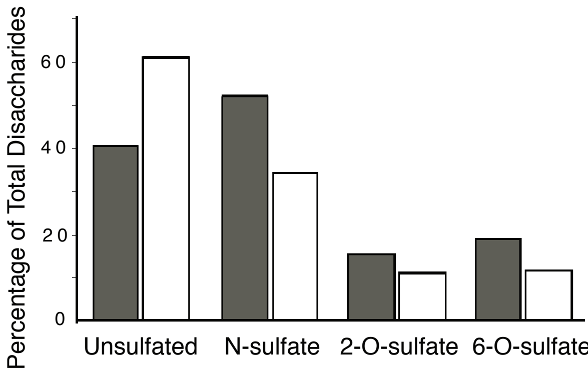
Cells



Cells


Percentage of Total Disaccharides
60
40
20
0
Unsulfated
N-sulfate
2-O-sulfate
6-O-sulfate