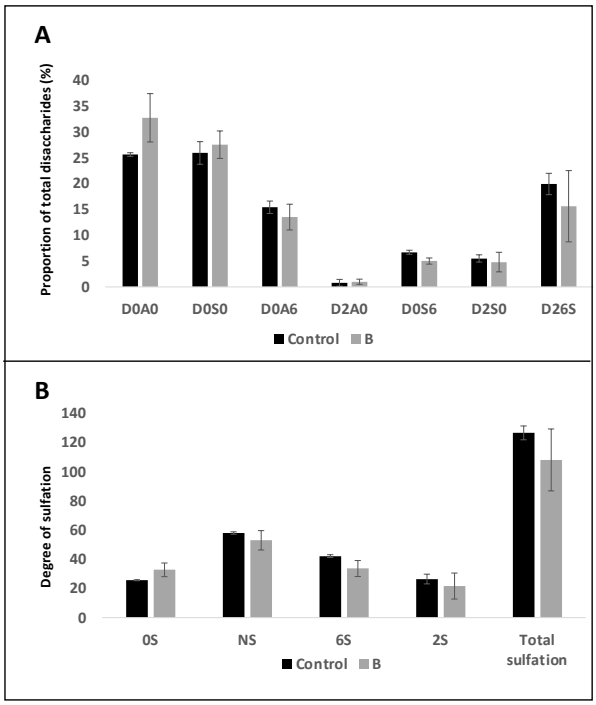
Suppl. Fig 1 Disaccharide composition of HS isolated from control HeLa cells or HeLa cells transiently transfected with NDST1B

Two 10 cm petri dishes containing 60-70% confluent HeLa cells were transfected with the *NDST1B*-pCMV6-vector using Lipofectamine®3000 reagent. In parallel, reagent without the pCMV6-vector was added to HeLa cells which would serve as controls (two 10 cm petri dishes). After 48 h of incubation, the cells which were now confluent, were harvested. Expression of NDST1B was confirmed on a small aliquot of the cells (10%) after SDS-PAGE and Western blotting as described in Materials & Methods, while 80% was used to purify HS for disaccharide analysis. The remaining 10% were allowed to settle in new petri dishes. The control cells were grown in Dulbecco´s Modified Eagle Medium (Gibco® DMEM) containing 10 % of fetal bovine serum and 60 µg/ml penicillin and 50 µg/ml streptomycin, while the NDST1B transfected cells received the same medium supplemented with 0.8 mg/ml G418. When confluent, the two dishes of control cells and the two dishes of transfected cells were harvested for disaccharide analysis.

Disaccharide composition of HS produced by control HeLa cells (black bars) and HeLa cells transiently transfected with NDST1B (grey bars) analyzed by RPIP-HPLC. The values shown ±SD are the mean of the results obtained for cells collected from the four petri dishes of each kind. A. HS disaccharide composition: D0A0, HexAGlcNAc; D0S0, HexAGlcNS; D0A6, HexAGlcNAc(6S); D2A0, HexA(2S) GlcNAc; D0S6, HexAGlcNS(6S); D2S0, HexA(2S) GlcNS; D26S, HexA(2S) GlcNS(6S). B. Percentage of unsulfated disaccharides (OS), *N*-sulfated disaccharides (NS), 6-*O*-sulfated disaccharides (6S) and 2-*O*-sulfated disaccharides (2S). Total sulfation is the sum of *N*-sulfate, 2-*O*-sulfate, and 6-*O*-sulfate groups in 100 disaccharides. Disaccharide abbreviations are according to the published structural code in Lawrence et al., (2008)(Lawrence et al. 2008).


A
Proportion of total disaccharides (%)
40
35
30
25
20
15
10
5
0
D0A0
D0S0
D0A6
D2A0
D0S6
D2S0
D26S
Control
B
B
Degree of sulfation
140
120
100
80
60
40
20
0
0S
NS
6S
2S
Total sulfation
Control
B

Suppl. Fig. 1