

## Supplementary material

### Supplementary Table I. Disaccharide composition of CS/DS chains in WT and *Hsepi* mutant MEFs

<sup>35</sup>S-labeled CS/DS were isolated from cellular lysates as outlined in “Experimental Procedures”. Disaccharides obtained after complete digestion of chains by chondroitinase ABC were separated on a CarboPac PA-1 HPCL column and quantified by liquid scintillation counting. Results are expressed as proportion of each disaccharide species.

Disaccharide unit \ Hsepi genotype	WT	<i>Hsepi</i> <sup>-/-</sup>
	%	%
ΔHexU-GalNAc4S	95.5	96.3
ΔHexU-GalNAc6S	2.7	2.3
ΔHexU-GalNAc4,6diS	0.0	0.0
ΔHexU2S-GalNAc4S	1.5	1.1
ΔHexU2S-GalNAc6S	0.3	0.3

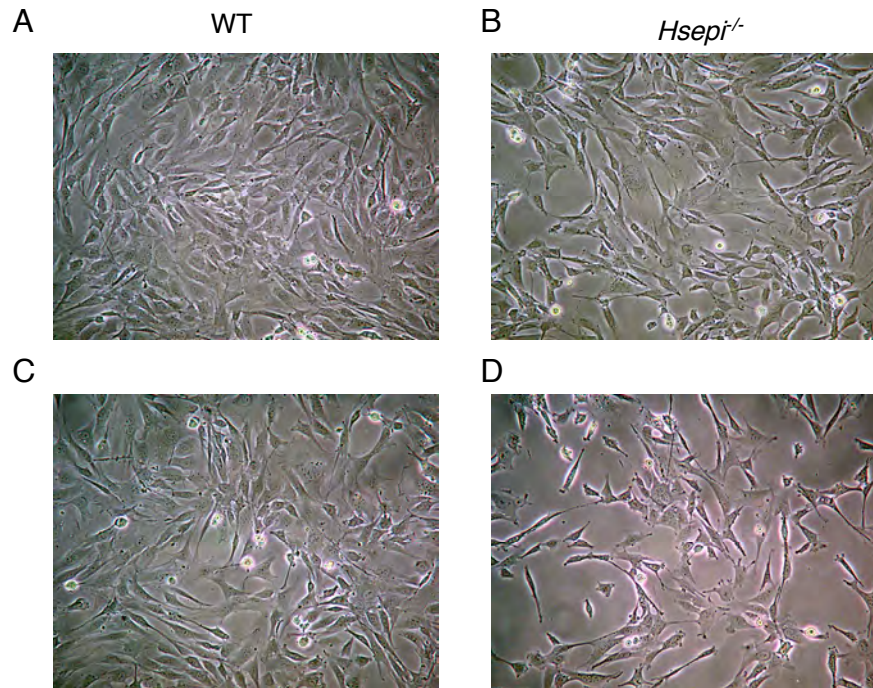


Fig. S1. Morphology of MEF cells.

MEF cells were isolated from WT and *Hsepi*<sup>-/-</sup> embryos. (A, B) Cells seeded at a density of 4 x 10<sup>5</sup> in 6-well plate were cultured for 24 hours; (C, D) Cells seeded at a density of 1 x 10<sup>5</sup> in 6-well plate were cultured for 72 hours.

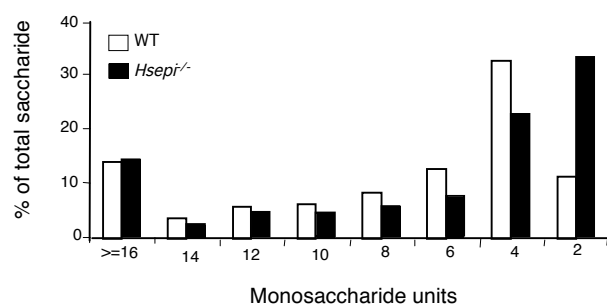


Fig. S2. Proportions of oligosaccharides generated by deaminative cleavage of WT and *Hsepi*<sup>-/-</sup> HS samples  
 Data are based on areas of the peaks shown in Fig. 2C.

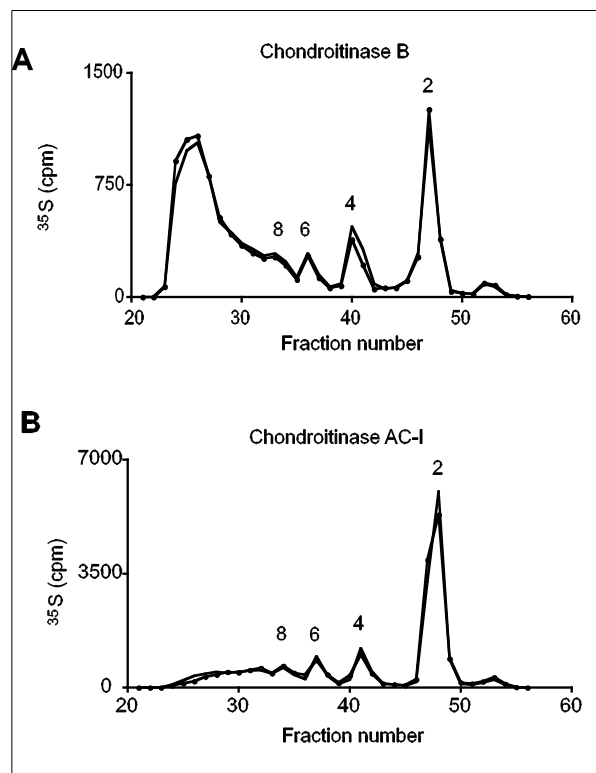
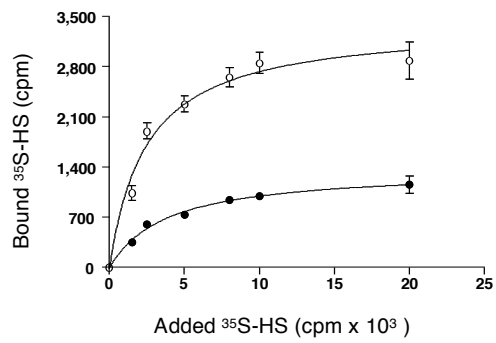


Fig. S3. Amount and distribution of IdoA in CS/DS derived from MEF lysates.  
 MEF were metabolically labelled, and cellular CS/DS chains were isolated. The products after chondroitinase B (A) or chondroitinase AC-I (B) digestion were size fractionated on Superdex Peptide column. WT (*line*), *Hsepi*<sup>-/-</sup> (*filled circle*).

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**Fig. S4. Interactions of HS from MEF with FGF2**

Metabolically  $^{35}\text{S}$ -labeled HS from WT (*empty circle*) and  $Hsepi^{-/-}$  (*filled circle*) MEF were incubated with 50 ng FGF2 for 2 hours. Bound HS was trapped on a nitrocellulose filter and recovered for radioisotope counting (see “Experimental Procedures”). Data shown represent 4 independent experiments (means  $\pm$  s.d.).

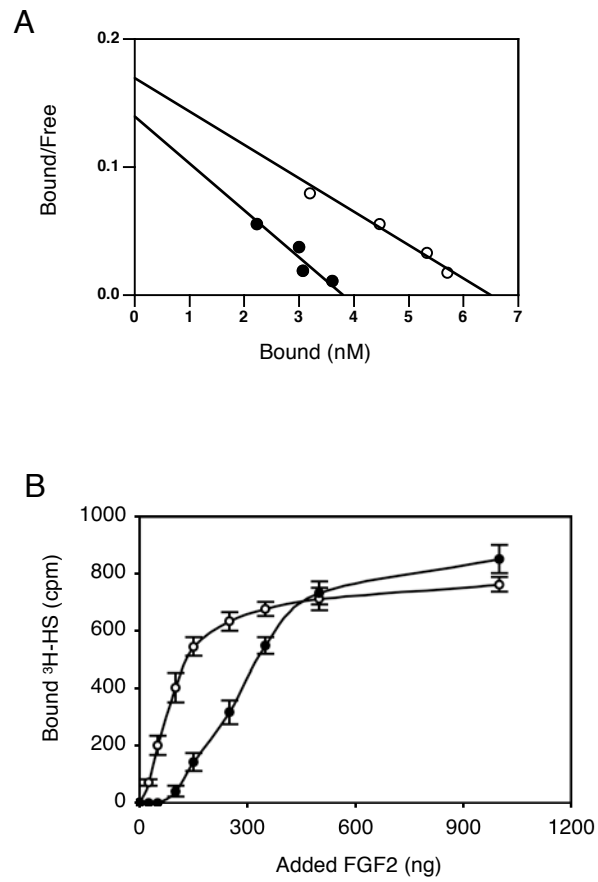


Fig. S5. Interactions of HS with FGF2

(A) Scatchard-plot of Fig. 6A by GraphPad Prism Software. (B) Saturation binding curve (*empty circle*) and *Hsep1<sup>-/-</sup>* (*filled circle*).