

Supplemental Table S1. Primers used for qPCR analysis

HSPG	Primer Sequence
Syndecan 1	5' A G G A T G G A A C T G C C A A T C A G 3' A T C C G G T A C A G C A T G A A A G C
Syndecan 2	5' T C T G A G G C A G A A G A G A A G C T G 3' A G G A T G A G G A A A A T G G C A A A
Syndecan 3	5' A T A C T G G A G C G G A A G G A G G T 3' T T T C T G G T A C G T G A C G C T T G
Syndecan 4	5' A A C C A C A T C C C T G A G A A T G C 3' A G G A A A A C G G C A A A G A G G A T
Glypican 1	5' G G C C A T C A T G A A G T T G G T C T 3' A C A C C G C C A A T G A C A C T C T C
Glypican 2	5' T G A A T G A G C A G C T C C A C A A C 3' T C G T C T G C A T A C T G C T G T C C
Glypican 3	5' T G T G C C C A A G G G T A A A G T T C 3' A G G T G G T G A T C T C G T T G T C C
Glypican 4	5' C G T T T G C A A T G A T G A G A G G A 3' G C C A T G A T C T G A C G A A G G A T
Glypican 5	5' A G A T G G C T G T G G A G G A T C A G 3' G A C T C A G T T C C T G A C G C A C A
Glypican 6	5' G T G C C A C G G A A A C T G A A G A T 3' G G C C G A A G A T T C C T C T T C T C
Betaglycan	5' G C A G A A T A A G A A G A C 3' C T T G C T G C C T T C G T G C
CD44	5' G C C T C A A C T G T G C A C T C A A A 3' C A G C C T G T T G G G T T G G T A T T 5' G A T G A C C A C C C C T G A A A C A C 3' T C A T C A A T G C C T G A T C C A G A

Supplemental Figure Legends

Supplemental Figure S1. Triglyceride Secretion. After a 6 hour fast, triglyceride secretion rate (TGSR) was determined by the method described by Hirano et al. (1). Briefly, Triton WR-1339 (0.5 mg per gm body weight of (Sigma) was injected via tail vein, and triglyceride concentrations were measured in plasma samples taken pre-injection, and 0.5, 1, 1.5, 2, and 24 hours post-injection. TGSR was calculated from the increment in triglyceride concentration per min multiplied by the plasma volume of the mouse (estimated by 0.035% of body weight in g) and expressed in mg/min. The rates did not differ significantly between *Sdc1*^{-/-} (0.51 ± 0.11 mg/min; $n = 3$) and wild-type (0.38 ± 0.07 ; $n = 3$), $P = 0.63$.

Supplemental Figure S2. Syndecan-1 expression and lipolytic activity in adipose tissue.

(A) Lipolytic activity was measured as previously described (4) in extracts of gonadal adipose tissue (5) homogenized in RIPA buffer. Samples were centrifuged at 4°C for 10 min at 1000 x g on a tabletop centrifuge. An aliquot of the supernatant was mixed with 100 µL of [³H]triolein. Samples were then incubated at 37°C for 30 min. The reaction was stopped by the addition of 3.25 mL of methanol/chloroform/heptane (1.4:1.25:1, v/v). Free fatty acids were extracted through addition of 0.1M borate-potassium carbonate buffer (pH 10.5). After centrifugation, the upper phase (1 mL) was removed and counted. Activity was determined as units of activity per gram of wet weight of the tissue (1 U = 1 µmol/min). No difference in activity was observed ($P = 0.37$). **(B)** Proteoglycans were isolated from gonadal fat pads according to a protocol adapted from Reizes et al (2). Proteoglycans in the supernatants were collected by anion-exchange chromatography (DEAE-Sephacel) and digested for 1 hr with a mixture of 2 mU/ml of heparin lyases I and II and 5 mU/ml of heparin lyase III and chondroitinase ABC. Syndecan-1 was detected with mAb 281-2 and goat anti-rat IgG conjugated to HRP. Two bands were detected as indicated by the arrows, a 69 kDa form representing syndecan-1 and a 140 kDa band

representing most likely a dimer (3). The membrane was stripped and reprobed with mAb 3G10, which reacts with the glycan stubs remaining after heparinase digestion. mAb 3G10 was detected with a goat anti-rat IgG conjugated to HRP.

Supplemental Figure S3. Syndecan-1 and LDLR expression. (A) Syndecan-1 expression in control and AdSdc1 infected livers. Hepatocytes were isolated from *Sdc1*^{-/-} mice treated with AdSdc1 or AdGFP three weeks after intravenous virus injection. Equal volumes of hepatocyte lysate were loaded on NuPAGE gradient gels (4-15%). Western blots were performed by transfer of the gel to 0.45 μm nitrocellulose (NuPAGE) using a wet transfer apparatus in NuPAGE transfer buffer. Membranes were blocked in Superblock (Pierce) for 1 hour, then incubated overnight at 4°C with mAb 281-2 or rabbit polyclonal anti-ERK (1:1000, Cell Signaling Technology). Membranes were washed and incubated with HRP-conjugated secondary antibody, washed and developed with ECL West Pico SuperSignal Reagents (Pierce). **(B) LDLR expression.** Equal volumes of hepatocyte lysate from wild-type, *Ndst1*^{fl/fl}*AlbCre*⁺, *Sdc1*^{-/-}, and *Ldlr*^{-/-} mice were separated by SDS-PAGE and Western blotted as described in panel A, using rabbit polyclonal anti-LDLR (1:2500, a kind gift from Dr. Jay Horton, University of Texas, Southwestern) or rabbit polyclonal anti-β-actin (1:1000, Cell Signaling Technology).

Supplemental Figure S4. Syndecan-1 localization in the liver. (A) Immunofluorescence micrographs of wild-type and **(B) *Sdc1*^{-/-}** livers stained with rabbit polyclonal antibody to syndecan-1 and goat anti-rabbit secondary antibody conjugated to Alexa fluor-594 (red). Nuclei were stained with DAPI (blue). Syndecan-1 was localized around the sinusoids (H, hepatocytes, S, sinusoid). The antiserum was specific for syndecan-1 based on the absence of staining in sections from *Sdc1*^{-/-} livers. Images were captured with a DeltaVision Restoration microscope system (AppliedPrecision) using a Photometrics Sony CoolSAP HQ charge-coupled device

(CCD) camera system attached to an inverted, wide-field fluorescence microscope (Nikon TE200). Optical sections were acquired using a 63x Nikon (NA 1.3) oil immersion objective in 0.2- μm steps in the z-axis. **(C)** The pattern of staining was similar in wild-type and *Ndst1^{ff}AlbCre⁺* mice, indicating that alteration of the sulfation pattern on the chains did not affect syndecan-1 expression or localization. **(D)** Electron microscopy of immunogold-labeled sections revealed syndecan-1 on the microvilli extending from the basal surface of the hepatocytes. Bar = 0.5 μ . The area outlined by the box in the center was magnified 1.8X in the inset in the lower right. The images shows syndecan-1 expression restricted to the microvilli of the basal membrane facing the space of Disse, as observed previously (6,7).

References

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