SUPPLEMENTARY DATA

**Supplementary Figure 1. Expression of CSHY in COS-7 cells.** The culture medium of COS-7 cells transfected with HYAL4 (lanes 2, 4, and 6) or mock-transfected cells (lanes 3, 5, and 7) was purified with ANTI-FLAG M2 affinity gel. The resin was subjected to SDS-PAGE under reducing (panel A and lanes 4 and 5 in panel B) and nonreducing (lanes 6 and 7 in panel B) conditions and analyzed by silver staining (panel A) as well as western blotting (panel B) with the anti-FLAG antibody. The apparent molecular weights of the protein standards (lane 1) are indicated. Bands indicated by asterisks were not observed in the mock-transfected samples, suggesting that they are the protein bands of CHSY. Bands observed at around 60 and 30 kDa correspond to those of immunoglobulin heavy and light chains, respectively.

**Supplementary Figure 2. Substrate specificity of bacterial CSase ABC and AC-II.** CSase ABC cleaves all the N-acetylgalactosaminic linkages in CS chains to yield disaccharides (A). However, when the reducing terminal GalNAc residue is derivatized with 2AB, it does not act on the linkage adjacent to the 2AB-labeled disaccharide unit, giving rise to 2AB-tetrasaccharide (B). In contrast, CSase AC-II can act on the reducing terminal N-acetylgalactosaminic linkages in CS chains even if they are 2AB-derivatized (B). However, 2-O-sulfation (shown in larger font size) renders the GalNAc-GlcUA(2S) linkage resistant to the enzyme (A, B).
Supplementary Figure 1
Supplementary Figure 2