Supplementary Data

LARGE2-dependent glycosylation confers laminin-binding ability on proteoglycans

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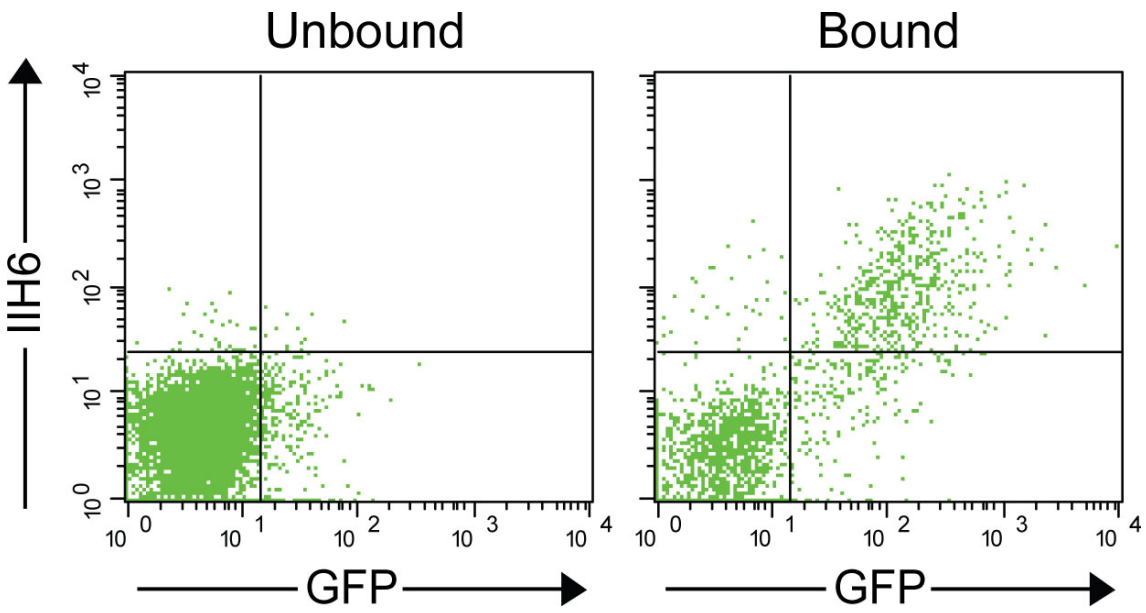

fig. S1. Flow cytometry of separated DG-/- ES cells infected with adenovirus for LARGE2-IRES-eGFP. IIH6+ cells were separated by magnetic cell separator (Unbound/Bound to IIH6) and stained with IIH6 followed by Alexa 647-anti-mouse IgM.

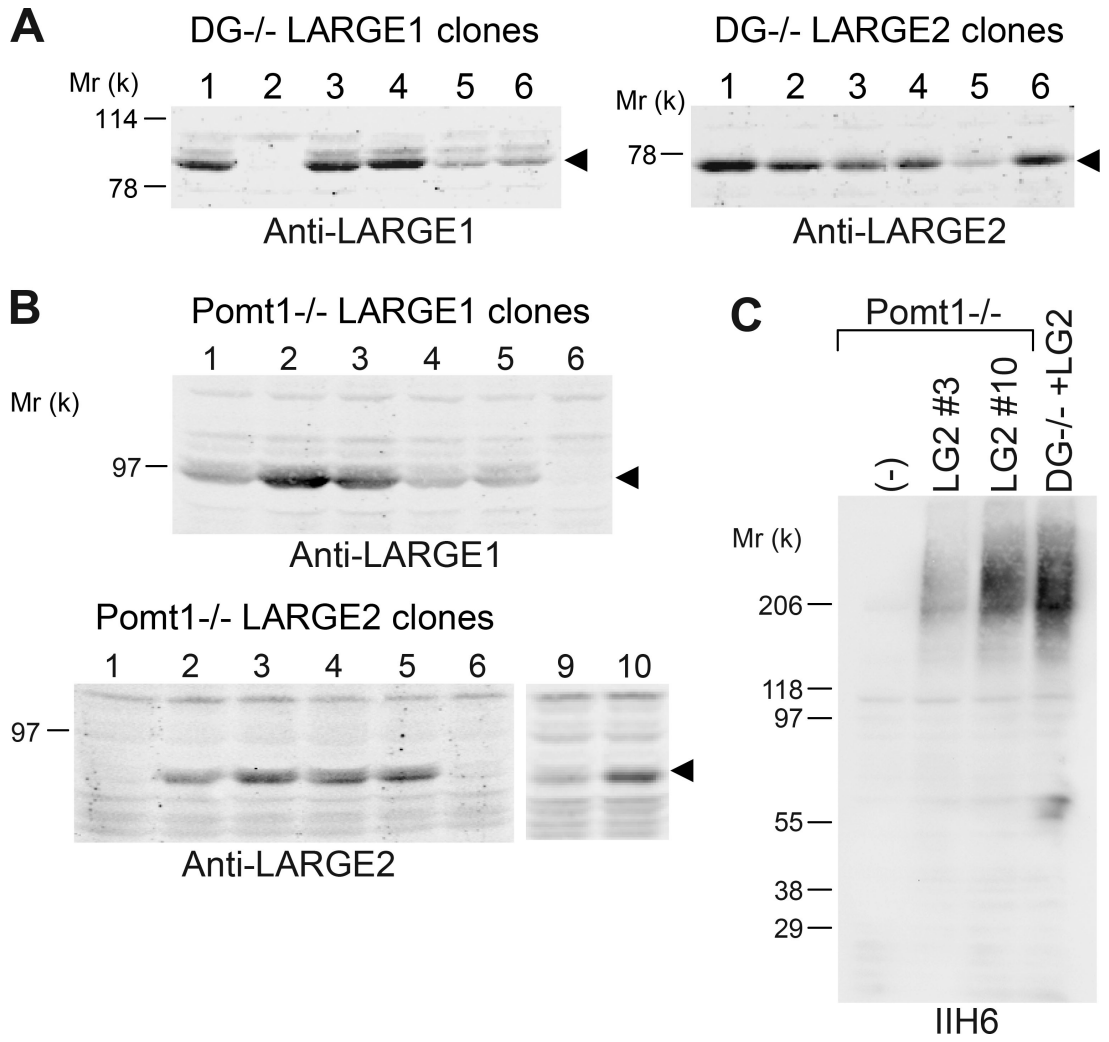

fig. S2. Immunoblotting of cell lysate from ES cell clones stably expressing LARGE1 or LARGE2. (A) Cell lysate extracted from DG-/- cell clones were analyzed by immunoblotting with anti-LARGE1 or anti-LARGE2 antibody. (B) Cell lysate extracted from Pomt1-/- cell clones were analyzed as in A. (C) DEAE-enriched proteins from cell lysate of the Pomt1-/- cell clones with or without stable expression of LARGE2 and the DG-/- cells stably expressing LARGE2 (DG-/-LG2) were analyzed with IIH6.

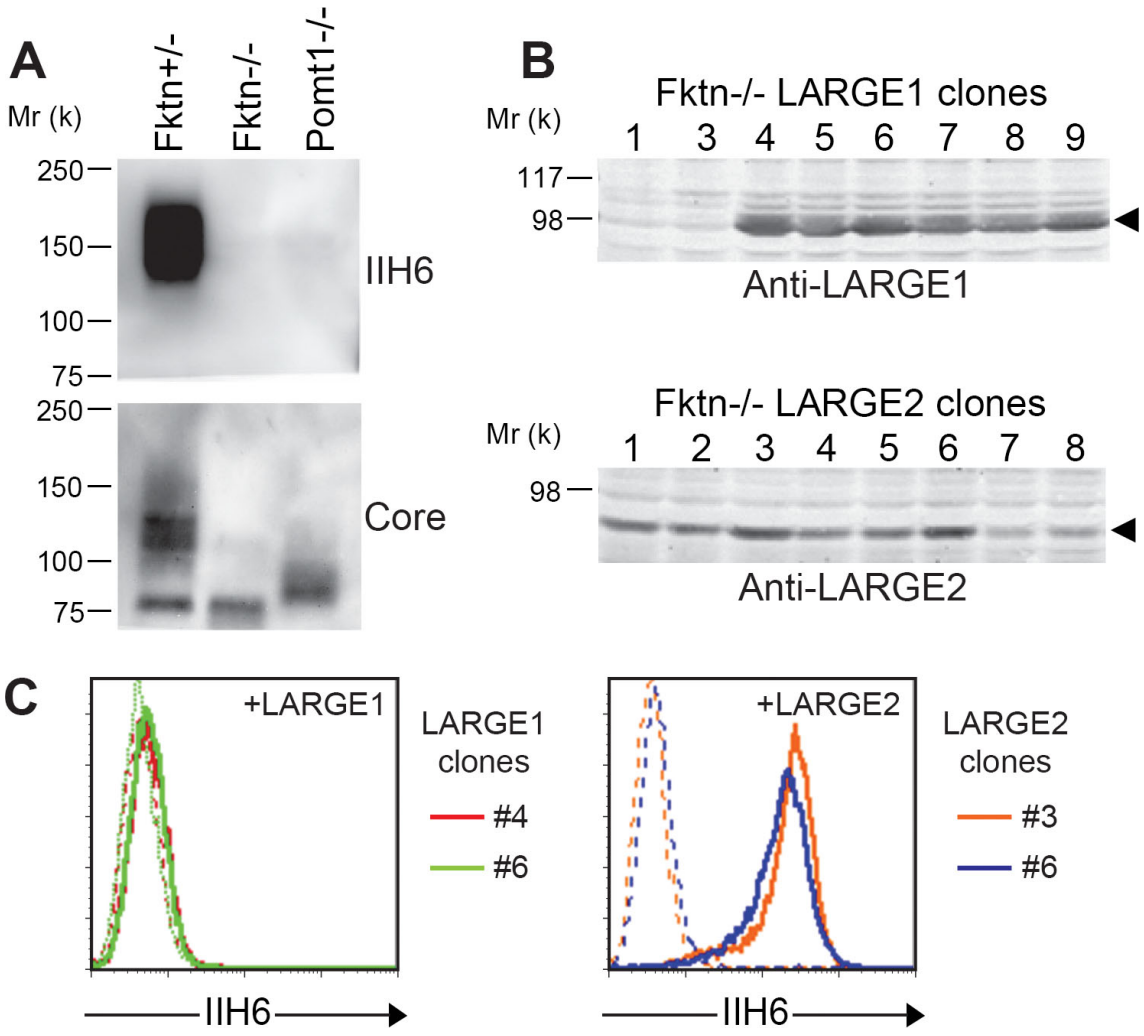

fig. S3. Overexpression of LARGE2 but not LARGE1 overcome the glycosylation defect in Fktn-/- ES cells. (A) Immunoblotting of WGA-enriched glycoproteins extracted from Fktn-/- and Fktn+/- ES cells for functionally modified α-DG (IIH6) and the α-DG core protein (Core). Pomt1-/- ES cells were served as a control for the defective glycosylation of α-DG. (B) Immunoblotting of cell lysates extracted from Fktn-/- ES cell clones stably expressing LARGE1 or LARGE2, using anti-LARGE1 or anti-LARGE2 antibody. (C) Flow cytometry of the Fktn-/- cell clones expressing either LARGE1 or LARGE2 for surface staining with IIH6. Two independent clones of each (#4 and #6 of LARGE1-, and #3 and #6 of LARGE2-expressing clones) were analyzed. Dotted line, secondary antibody alone.

MARLGLLALLCTLAALSASLLAAELKSKSCSEVRRLYVSKGFNKNDAPLY 50
EINGDHLKICPQDYTCCSQEMEEKYSLQSKDDFKTVVSEQCNHLQAIFAS 100
RYKKFDEFFKELLENAEKSLNDMFVKTYGHLYMQNSELFKDLFVELKRYY 150
VAGNVNLEEMLNDFWARLLERMFRLVNSQYHFTDEYLECVSKYTEQLKPF 200
GDVPRKLKLQVTRAFVAARTFAQGLAVARDVVSKVSVVNPTAQCTHALLK 250
MIYCSHCRGLVTVKPCYNYCSNIMRGCLANQGDLDFEWNNFIDAMLMVAE 300
RLEGPFNIESVMDPIDVKISDAIMNMQDNSVQVSQKVFQGCGPPKPLPAG 350
RISRSISESAFSARFRPYHPEQRPTTAAGTSLDRLVTDVKEKLKQAKKFW 400
SSLPSTVCNDERMAAGNENEDDCWNGKGKSRYLFAVTGNGLANQGNNPEV 450
QVDTSKPDILILRQIMALRVMTSKMKNAYNGNDVDFFDISDESSGEGSGS 500
GCEYQQCPSEFEYNATDHSGKSANEKADSAGGAHAEAKPYLLAALCILFL 550
AVQGEWR 557

fig. S4. Amino acid sequence of mouse GPC4. Signal sequences for secretion and GPI-anchoring (dotted lines), potential GAG attachment sites (Ser-Gly, bold letters), minimal furin cleavage sites (boxed), peptide sequences identified by mass spectrometric analyses (solid lines, from rabbit kidney Triton extract; bold line, from DG-/- LARGE2 cells) are indicated.

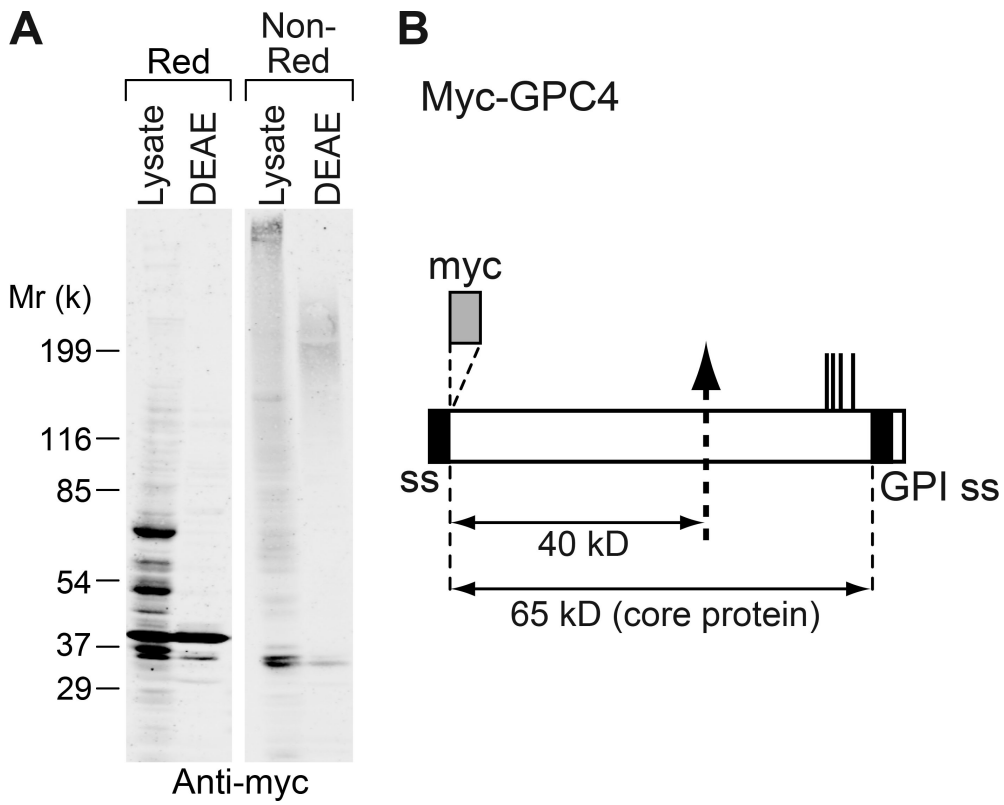

fig. S5. Myc-GPC4 stably expressed in HEK293 cells. (A) Lysate and DEAE-enriched lysate (DEAE) was separated by SDS-PAGE under reducing (Red) or non-reducing (Non-Red) conditions followed by immunoblotting with anti-myc antibody. (B) Schematic representation of Myc-GPC4 construct. Solid bars, potential GAG attachment sites. ss, signal sequence.

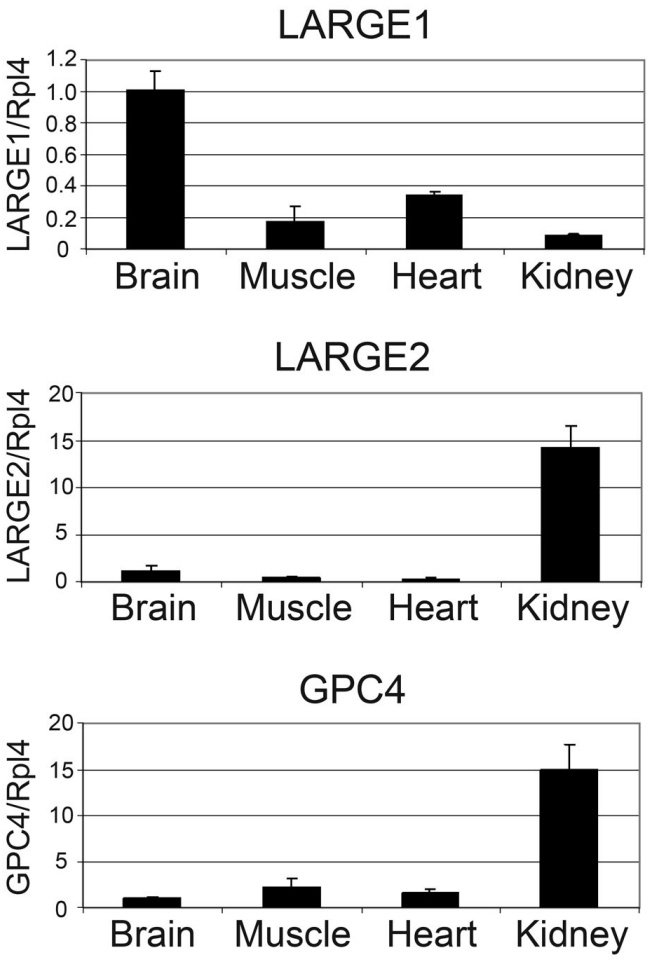

fig. S6. Analysis of mRNA for LARGE1, LARGE2, and GPC4 by quantitative RT-PCR. Complementary DNA was synthesized from total RNA of each mouse tissue. For each tissue, the expression of LARGE1, LARGE2 and GPC4 are shown as relative expression to Rpl4 (normalization control). The relative expression of each gene with respect to the brain is shown.

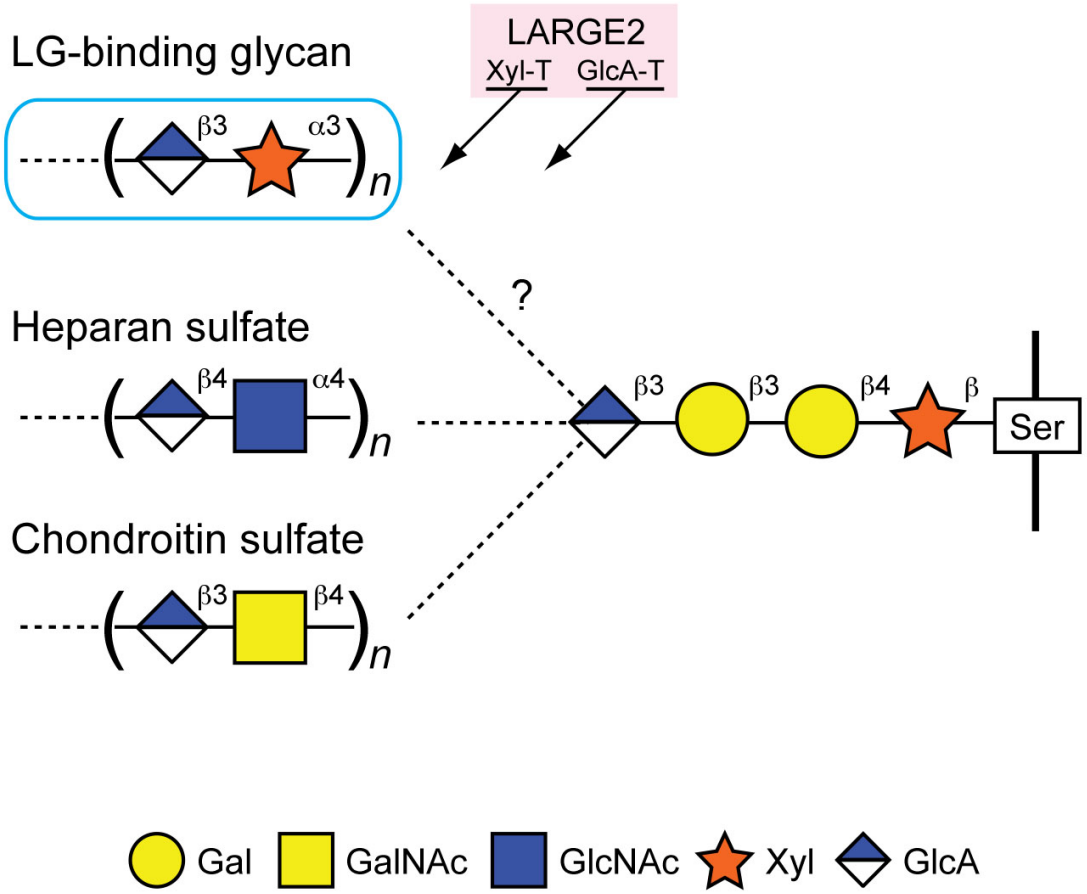

fig. S7. Proposed model for synthesis of LARGE2-mediated LG-binding glycan on proteoglycan core protein. The β1,3-linked GlcA in the GAG-protein linkage region is known to be extended by heparan sulfate (HS)- and chondroitin sulfate (CS)-GAG synthesis. HS- and CS-GAGs undergo further modifications such as sulfation and epimerization. Although the glycan structure of the LARGE2-dependent proteoglycan has not yet been solved, its known Xyl-T and GlcA-T activities suggest that LARGE2 is most likely to compete with HS- and CS-GAGs to introduce a xylose to the same β1,3-linked GlcA in seeding polymerization of the LG-binding glycan.