Supplementary materials and methods

Immunohistochemistry

FFPE tissue sections of 3 μm thickness were deparaffinized in xylene and rehydrated in ethanol, and endogenous peroxidase activity was quenched by soaking in absolute methanol containing 0.3% H_2O_2 for 30 min. In the case of CD34 staining, antigens were retrieved by boiling sections in 10 mM Tris/HCl buffer (pH 8.0) containing 1 mM EDTA for 25 min in a microwave oven. After blocking with 1% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA) in Tris-buffered saline (TBS) (pH 7.6) for 15 min, sections were incubated with primary antibodies at 4°C overnight. After washing with TBS, sections were incubated with horseradish peroxidase (HRP)-conjugated species- and class-matched secondary antibodies (Dako, Kyoto, Japan) for 60 min for the staining of QBEND10, MECA-79, and HECA-452. For NCC-ST-439 staining, sections were incubated with biotin-conjugated anti-mouse immunoglobulins (Dako) for 60 min, followed by HRP-conjugated streptavidin for 60 min. The color reaction was developed with 3,3'-diaminobenzidine (DAB) (Dojindo, Kumamoto, Japan) containing 0.02% H_2O_2. Sections were briefly counterstained with hematoxylin. Negative controls were obtained by replacing primary antibodies with isotype-matched immunoglobulins, which showed no specific staining.

Stable expression of a set of sLeX-capped glycans on CHO cells

CHO cells were first cotransfected with pCDM8-CD34, as a scaffold protein [13], and pcDNA3.1/Hyg (Invitrogen, Carlsbad, CA, USA) and selected in 400 μg/ml of Hygromycin B (Invitrogen). QBEND10-positive cells were cloned by limited dilution, resulting in CHO/CD34. Cells were then cotransfected with pCDM8-α1,3-fucosyltransferase 7 (FucT-7) [27] and pcDNA3.1/Zeo (Invitrogen) and selected in 100 μg/ml of Zeocin (Invitrogen) and Hygromycin B. Cells positive for HECA-452 were cloned, resulting in CHO/CD34/F7. Cells were further

transfected with pcDNA3.1-core 1 extending β1,3-*N*-acetylglucosaminyltransferase (Core1-β3GlcNAcT) [12], and selected in 1,200 μg/ml of Geneticin (Sigma-Aldrich), Zeocin, and Hygromycin B. After transient transfection with pcDNA1.1-*N*-acetylglucosamine-6-*O*-sulfotransferase 2 (GlcNAc6ST-2), otherwise known as L-selectin ligand sulfotransferase (LSST) [13,28], cells positive for MECA-79 were cloned, resulting in CHO/CD34/F7/C1. Cells were then cotransfected with pcDNA1.1-GlcNAc6ST-2 and pCMV/Bsd (Invitrogen) and selected in 10 μg/ml of Blasticidin S (Invitrogen) plus Geneticin, Zeocin, and Hygromycin B. Cells positive for MECA-79 were cloned, resulting in CHO/CD34/F7/C1/LSST.

In parallel, CHO/CD34/F7 cells were cotransfected with pcDNA1.1-core 2 branching β1,6-*N*-acetylglucosaminyltransferase 1 (Core2GlcNAcT-1) [26] and pcDNA3.1, and selected in Geneticin, Zeocin, and Hygromycin B. Cells positive for NCC-ST-439 were cloned, resulting in CHO/CD34/F7/C2. Cells were further cotransfected with either pcDNA1.1-GlcNAc6ST-2 or pcDNA3.1-Core1-β3GlcNAcT together with pCMV/Bsd, and selected in Blasticidin S, Geneticin, Zeocin, and Hygromycin B. After transient transfection with either pcDNA3.1-Core1-β3GlcNAcT or pcDNA1.1-GlcNAc6ST-2, cells positive for MECA-79 were cloned, resulting in CHO/CD34/F7/C2/LSST and CHO/CD34/F7/C2/C1, respectively. CHO/CD34/F7/C2/C1 cells were further cotransfected with pcDNA1.1-GlcNAc6ST-2 and pBApo-CMV Pur (Takara, Otsu, Japan), and selected in 4 μg/ml Puromycin (Sigma-Aldrich) plus Blasticidin S, Geneticin, Zeocin, and Hygromycin B. MECA-79-positive cells were cloned, resulting in CHO/CD34/F7/C2/C1/LSST.

Additionally, CHO/CD34/F7 cells were cotransfected with pcDNA1.1-GlcNAc6ST-2 and pBApo-CMV Pur and selected in Puromycin, Zeocin, and Hygromycin B. After transient transfection with pcDNA3.1-Core1-β3GlcNAcT, cells positive for MECA-79 were cloned, resulting

in CHO/CD34/F7/LSST.

Western blot analysis

To express soluble CD34, CHO cell lines listed in Table 1 were transiently transfected with pcDNA1-CD34•IgG [21] using Lipofectamine Plus (Invitrogen). Forty-eight hours later, CD34•IgG in the culture medium of each CHO cell line was purified by Protein A-Sepharose (Sigma-Aldrich), lysed in sample buffer and incubated at 65ºC for 15 min. Each sample was separated by 7% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blocking in Tris-buffered saline (TBS) (pH 7.6) containing 5% nonfat dry milk for 60 min, the membrane was incubated with QBEND10, HECA-452, MECA-79, or NCC-ST-439, followed by HRP-conjugated goat anti-mouse IgG, anti-rat IgM (Jackson Immunoresearch, West Grove, PA, USA), or anti-mouse Immunoglobulins (Dako). The membrane was developed using ECL system (Amersham Pharmacia Biotech, Piscataway, NJ, USA). For *N*-glycanase digestion experiment, the membrane was treated with 0.05% sodium azide overnight, then digested with 5,000 U/ml *N*-glycanase (PNGase F) (New England BioLabs, Pickering, ON, Canada) at 37ºC for 4 hours, and reprobed with HECA-452.

Cell-enzyme-linked immunosorbent assay (ELISA)

CHO lines were seeded into 96-well culture plates at 1 x 10^4 cells/well 24 hours prior to assay. Cells were fixed with phosphate-buffered neutralized 20% formalin for 15 min. To quench endogenous peroxidase activity, cells were treated with 0.3% H_2O_2 in methanol for 30 min and washed with TBS. After blocking with 1% BSA-TBS, cells were incubated with HECA-452 antibody for 60 min. After washing with TBS, cells were incubated with HRP-conjugated goat

anti-rat IgM (Jackson ImmunoResearch) diluted 1:5,000 for 60 min. After washing, 100 μl of 1-Step ABTS (Pierce) was applied to each well, and absorbance at 405 nm was read using a microplate reader (DS Pharma Biomedical, Osaka, Japan).

Mass spectrometry analysis

Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) and MS/MS analyses were carried out as described previously [32,33]. CHO cell lines were suspended in 0.1 M NH_4HCO_3, boiled for 5 min, and lyophilized. Dried samples were delipidated by chloroform-methanol (2:1 by volume) and then extracted by a standard 6 M guanidine chloride protocol, followed by reduction and alkylation with dithiothreitol/iodoacetic acid. After dialysis, samples were digested with trypsin/chymotrypsin (Sigma-Aldrich) and then with *N*-glycanase F (Roche, Basel, Switzerland) and passed through a C_{18} Sep-Pak cartridge (Waters, Milford, MA, USA). De-*N*-glycosylated glycopeptides were eluted stepwise from the C_{18} cartridge by 20-40% 1-propanol in 5% acetic acid and then treated with 0.05 M NaOH, 1M $NaBH_4$ at 37ºC for 3 days to release *O*-glycans. Samples were neutralized using acetic acid on ice until bubbling stopped and then passed through a Dowex 50-X8 column in 5% acetic acid and dried. Borates were removed by repeated co-evaporation with 10% acetic acid in methanol under a nitrogen stream. An aliquot of released and desalted *O*-glycans was permethylated and analyzed by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) and MS/MS on a 4700 Proteomics Analyzer (Applied Biosystems, Farmington, MA, USA).

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from FFPE tissue sections, and single-stranded cDNA was synthesized as described [20]. PCR was then carried out as described previously [20] with primers for

Core1-β3GlcNAcT, 5’-GGACTTCCACGACTCCTTCTTCAA-3’ (5’ primer) and 5’-TCCTGCAGGTAGAAGACCATGTTG (3’-primer); Core2GlcNAcT-1, 5’-AGCTTGCTGGGGAGAATCCTAGTA-3’ (5’-primer) and 5’-TTATATAGTCGTCAGGTGTCCACC-3’ (3’-primer); and glyceraldehyde-6-phosphate dehydrogenase (GAPDH), 5’-TGAGTACGTCGTGGAGTCCACT-3’ (5’-primer) and 5’-CAGAGATGATGACCCTTTTGGCTC-3’ (3’-primer). After initial denaturation at 94ºC for 2 min, PCR conditions were: denaturation at 96ºC for 20 sec, annealing at 60ºC for 30 sec, extension at 72ºC for 30 sec, and final extension at 72ºC for 2 min. The number of amplification was 42 for Core1-β3GlcNAcT and Core2GlcNAcT-1, and 32 for GAPDH. Negative (minus template) and positive (1 pg of plasmid DNA harboring target cDNA) control amplifications were performed in every experiment. PCR product were electrophoresed on 3% agarose gels containing 0.1 μg/ml of ethidium bromide and visualized under ultraviolet light.