Matsumoto et al.

Supplementary Figure Legends

Fig. S1. **Characterization of mouse ascites BaGs6**. (*A*) The binding profiles of BaGs6 ascites (a) and VVA lectin with and without GalNAc inhibition (b) with LS174T and MDA-MB-231 cells by flow cytometry. Green lines = ascites or VVA, red lines = VVA + GalNAc, purple = isotype control or streptavidin-FITC alone. (*B*) Inhibition assay with pre-incubation of LSC cells with GalNAc, lactose, and Asialo-BSM with ascites (a, top) and VVA (b, bottom). Green lines = ascites or VVA, purple = isotype control or streptavidin-FITC alone. (*C*) Cell extracts were probed with ascites (a), VVA (b), and anti-actin antibody (c) by western blot.

Fig. S2. Total cell extracts (TCE) and purified IgA from wild-type Dakiki cells (Tn-) versus *Cosmc* KO Dakiki cells (Tn+) were analyzed by Western blot using ReBaGs6, Remab6, VVA, and goat anti-human IgA antibody.

Fig. S3. **Generation and quality control of STn and T glycopeptide arrays**. (*A*) The STn glycopeptide array was probed with Alexa Fluor[®] 488-streptavidin, which binds to the biotinylated sialic acid (a). MALDI-TOF MS analyses of ID6 (b) and ID7 (c) before and after enzyme reactions are shown. (*B*) The T glycopeptide array was probed with biotinylated PNA and Alexa Fluor[®] 488-streptavidin, which binds to the T antigen (a). Mass spectrometric analyses of ID6 (b) and ID7 (c) before and after enzyme reactions.

Fig. S4. Remab6 binding to the CFG glycan microarray. Remab6 tested at 20 μ g/ml (top) and 2 μ g/ml (bottom). RFU = relative fluorescence units.

Fig. S5. Immunofluorescence studies. Corresponding to **Fig. 3B**, replicate immunofluorescence images showing localization of Tn+ staining with respect to nuclear (DAPI), cis-medial Golgi (Giantin; top), trans Golgi (TGN46; middle) and ER (Calnexin; bottom) of Tn-positive (left) and Tn-negative (right) MDA-MB-231 cell line. Images were collected by confocal microscopy (Zeiss).

Fig. S6. Immunohistochemical staining in IEC-*Cosmc* KO mice with Remab6-HRP and Remab6-Fab-HRP. Small intestine-colon-rectum sections in villi-specific *Cosmc* KO mice were stained with Remab6-HRP (top) and Remab6-Fab-HRP (bottom). Brown indicates Tn staining, and blue indicates nuclear staining. Scale bar represents 100 μm.

Fig. S7. IHC staining in human cancer tissue array, FDA808k-1 and k-2. Human tumor tissue array with normal tissues (FDA808k-1 and k-2) stained with Remab6-Fab-HRP. Squares in red indicate Tn positive staining: in normal tissues, intracellular staining in stomach, small intestine, and colon tissues; in tumor tissues, 12 different tissues demonstrate staining with Remab6, as noted by red boxes and corresponding with **Table 3** Brown indicates Tn staining, blue indicates nuclear staining.

Fig. S8. LC/ESI-MS/MS analysis using Asialo-BSM. (*A*) Asialo-BSM was analyzed on a FusionTM LumosTM at two different higher collision dissociation energies (HCD, 25 and 32 eV) to optimize the peptide spectral matches. The precursor monoisotopic patterns for BSM peptides were observed in the top trace for the two different HCD, and matched between the experimental and the theoretical data as a histogram. (*B*) The bottom trace of the profile shows the product ion data for the identified monoisotopic peak for BSM identification. (*C*) Orange bar corresponds to the experimental precursor isotope match, and the blue bar corresponds to the theoretical data.

Supplementary Figures





Fig. S3



Fig. S4





Fig. S5







Blue; DAPI Green; Remab6 Red; Golgi (cis-medial)

Blue; DAPI Green; Remab6 Red; Golgi (trans)

Blue; DAPI Green; Remab6 Red; ER

Fig. S6





muscle

k2-H9

FDA808k-1 and k-2 tissue array

Fig. S7



