

Supplementary Materials for
**Identification and characterization of circulating immune complexes in
IgA nephropathy**

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The PDF file includes:

Figs. S1 to S17
Legend for table S1

Other Supplementary Material for this manuscript includes the following:

Table S1

Supplementary Figure Legends and Figures

fig. S1. Characterization of Tn(+)matrix beads. (a) Simplified representation of Tn(+)matrix beads showing exposed Tn antigens. (b and c) Microscopic analysis of ReBaGs6 or VVA lectin stained for BSM, Asialo-BSM, BSA, and Fetuin beads and beads alone.

fig. S2. Mass spectrometry analysis of the components of the purified anti-Tn antibodies. Tn(+)matrix captured anti-Tn antibodies from human serum were eluted and resolved on SDS-PAGE and stained with Coomassie. Predominant resolved bands were excised from SDS-PAGE gels and digested (in gel) and analyzed by mass spectrometry (See **Supplementary Table S1**).

fig. S3. Purified anti-Tn antibodies bind to Tn glycoform containing IgA1. (a) Similar to **Fig. 1d**, more examples showing Tn(+)matrix-purified anti-Tn antibodies interacting with Tn containing IgA1 glycopeptides on the Tn glycopeptide array. (b and c) Similar to **Fig. 1d**, 4x higher amount of anti-Tn antibodies were used, compared to (a) to probe on the Tn glycopeptide array for IgA and IgG detection. (d) The mammalian glycan array (CFG array) was probed with purified anti-Tn antibody (C1, diluted to 20 $\mu\text{g/ml}$).

fig. S4. Establishment of *Cosmc*KO cell line to generate galactose-deficient IgA1 glycoform, Tn(+)IgA1. (a) Design of the guide RNA for *Cosmc* gene and the gene sequencing in *Cosmc*KO Dakiki subclones. (b) Flow cytometry profile with ReBaGs6 on WT or *Cosmc*KO cell lines. (c) T-synthase enzyme activity of WT and *Cosmc*KO cell lines. Error bars represent SD in three independent experiments with four replicates ($n=3$). (d) Immunoblots showing protein levels of T-synthase or *Cosmc* in WT and *Cosmc*KO cell lines, as indicated on the right. Actin serves as an internal loading control. (e) Cell extracts from WT and *Cosmc*KO cell lines with or without treatment with neuraminidase (Neu) were analyzed by lectin blot with PNA or HPA.

fig. S5. Immune complexes of the total serum, from both healthy and IgAN patients. (a-c) BN-APAGE immunoblots showing serum IgM, IgA, and IgG from healthy donors (C2, C3, and C6) and IgAN patients (P1, P3, and P4).

fig. S6. Characterization data for **3**: ^1H spectrum of compound **3** in DMSO- d_6 .

fig. S7. Characterization data for **3**: ^{13}C NMR spectrum of compound **3** in DMSO- d_6 .

fig. S8. Characterization data for **4**: ^1H spectrum of compound **4** in DMSO- d_6 .

fig. S9. Characterization data for **4**: ^{13}C NMR spectrum of compound **4** in DMSO- d_6 .

fig. S10. Characterization data for **4**: 2D Heteronuclear single quantum coherence NMR spectrum of compound **4** in DMSO- d_6 .

fig. S11. Characterization data for **8**: ^1H spectrum of compound **8** in DMSO- d_6 .

fig. S12. Characterization data for **8**: ^{13}C NMR spectrum of compound **8** in DMSO- d_6 .

fig. S13. Characterization data for **8**: 2D Heteronuclear single quantum coherence NMR spectrum of compound **8** in DMSO- d_6 .

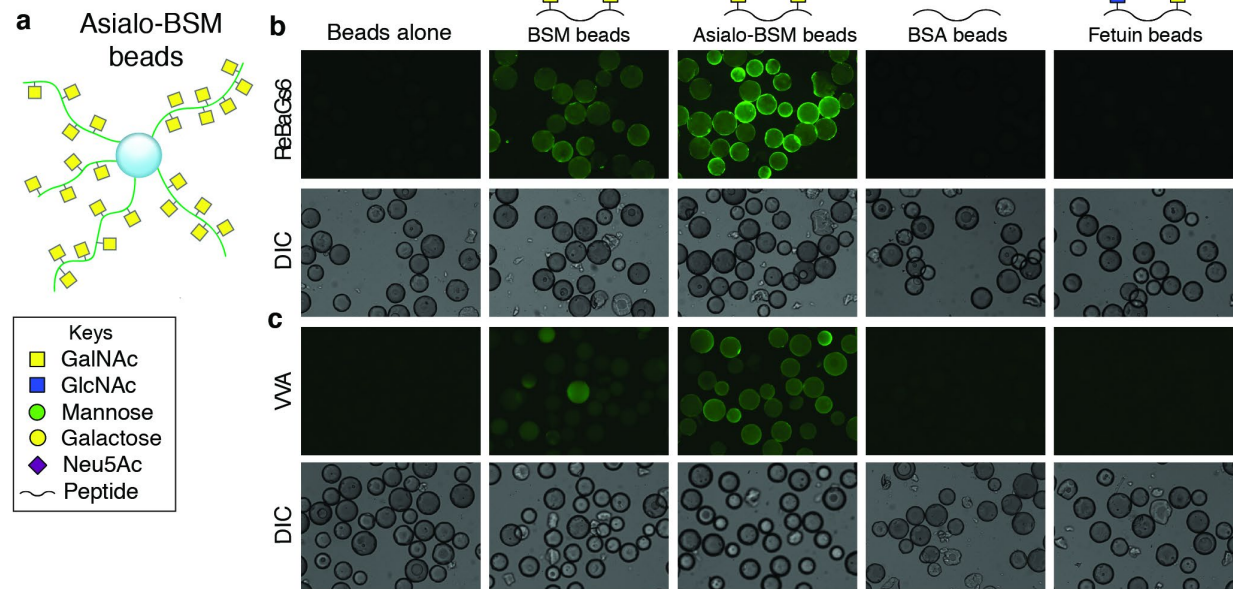
fig. S14. Glycomimetics-Di α GalNAc specifically inhibits the binding of anti-Tn CICs to IgA1 glycopeptide. (a) Similar to **Fig. 3g**, the interaction of Tn(+)matrix-purified anti-Tn antibodies and Tn containing IgA1 glycopeptides was studied on Tn glycopeptide array using Di α GalNAc or control Di α GlcNAc as indicated. (b) The half-maximal inhibitory concentration (IC_{50}) was measured for anti-Tn CICs purified from serum (IgAN Patients- P3, or Pmix-pooled P1-P10, Healthy control- C3 and Cmix-pooled C1-C10) using IgA1 glycopeptide (ID18-Table 1)-coated on ELISA plate. Dash line with circle (GalNAc-pretreated) or solid line with square (Di α GalNAc-pretreated) were plotted. Error bars represent SEM \pm of three replicates. (c) Summary of the inhibition experiments from **b**.

fig. S15. Cell proliferation assay on primary mesangial cells using serum from IgAN patients and healthy controls. (a) Human renal mesangial cells (HRMCs) were stimulated with 1, 2.5, or 5% serum with IgAN (Pmix) or HC (Cmix), and stained with Ki-67 antibody and DAPI. Ki-67 positive cells were counted and plotted on a graph. Error bars represent ± 1 SD of three replicates with two independent experiments ($n=2$). (b) Human renal mesangial cells were stimulated with 2.5% serum with IgAN (P1-P20) or HC (C1-C20), and stained with Ki-67 antibody. All images were taken in three different areas in 96-well plate, and Ki-67 positive cells were counted and plotted on a graph ($n=20$, each IgAN and HC). P, student t-test was performed ($p=1.268\text{E-}06$). (c) Serum with IgAN (P1-P20) or HC (C1-C20) were analyzed on SDS-PAGE gel and stained with Coomassie ($n=1$).

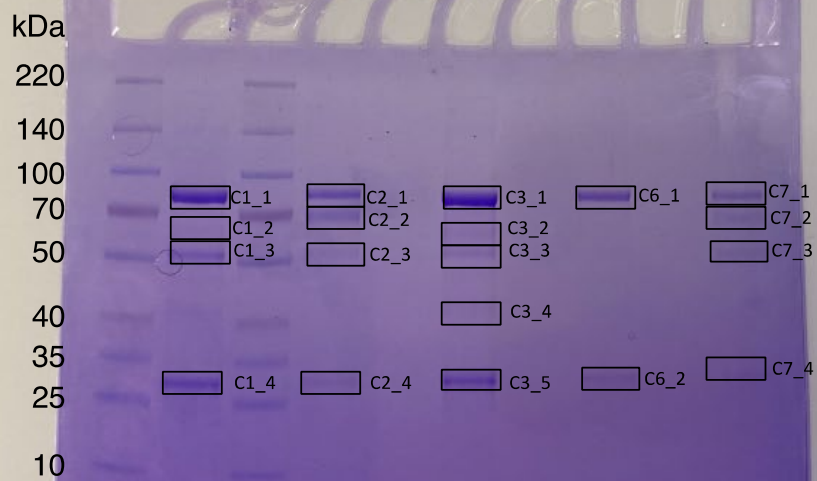
fig. S16. Purified Anti-Tn CICs from both IgAN patient and healthy control serum do not stimulate HEK293T cells. (a) Human embryonic kidney (HEK293T) and in parallel HRMCs cells were stimulated with sera with IgAN (mock), CICs-immunodepleted sera (ID), or exogenously added CICs in ID (ID+anti-Tn CICs), and stained with Ki-67 and DAPI (Green, Ki-67; blue, DAPI, merged images). At right, parallel experiment associated with HC control. All images were taken in three different areas in 96-well plate using IgAN sera and anti-Tn CICs (P10 and Pmix). (b) Quantification of Ki-67 positive cells were counted and plotted on respective graphs as shown. Error bars represent triplicates measure obtained from 3 different areas of each well. Representative example of two independent experiments.

fig. S17. Synthesis of GalNAc dimer (Di α GalNAc) and GlcNAc dimer (Di α GlcNAc). Steps described in the Methods section with compound numbers and steps noted.

fig. S1



down with asialo BSM (gel on 29.06.2020)
fig. S2



Identification of proteins bands on
Supplemental Table 1 XL Sheet

fig. S3

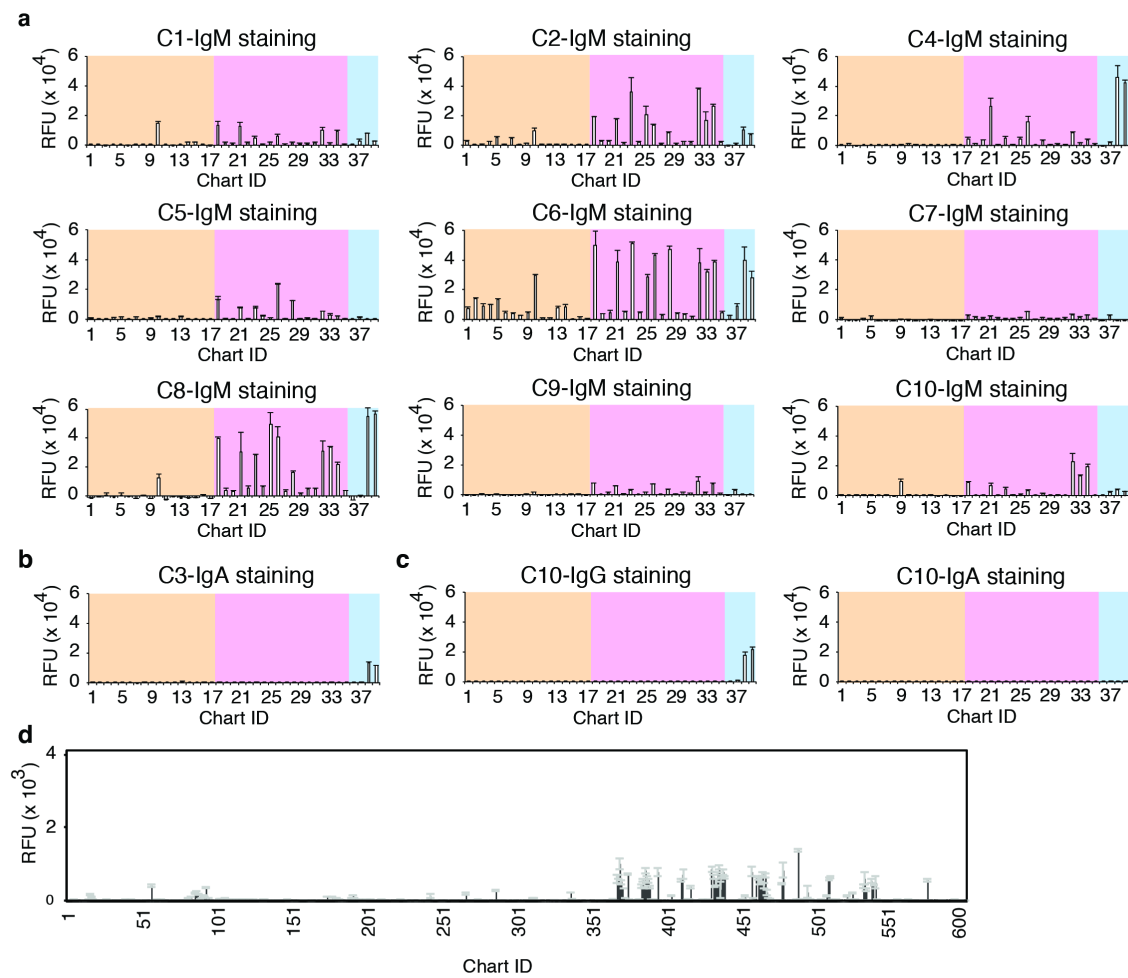


fig. S4

a

WT *cosmc* genomic sequence

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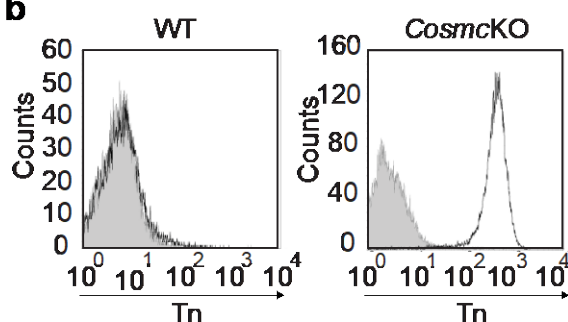
RNA guide target sequence

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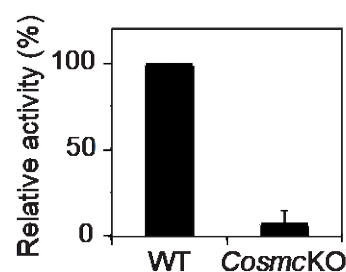
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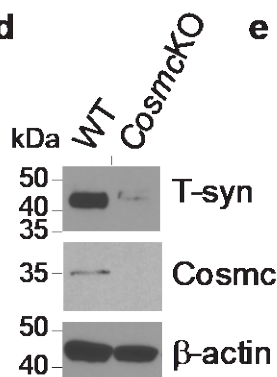
b



c



d



e

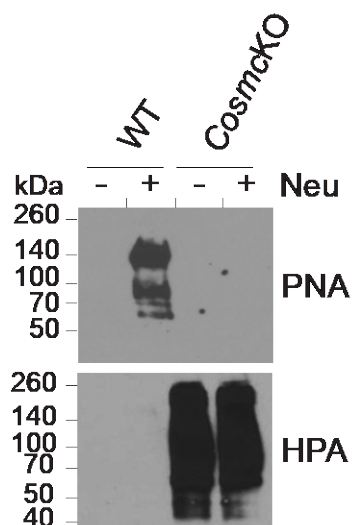


fig. S5

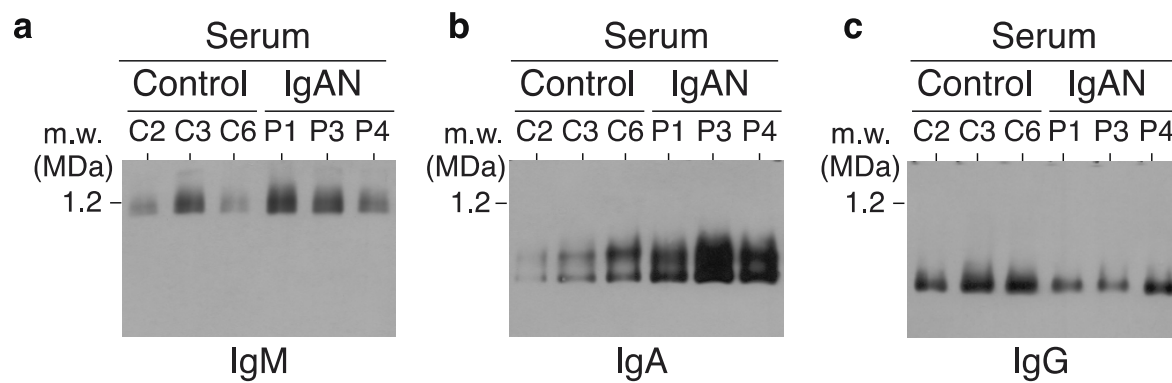


fig. S6

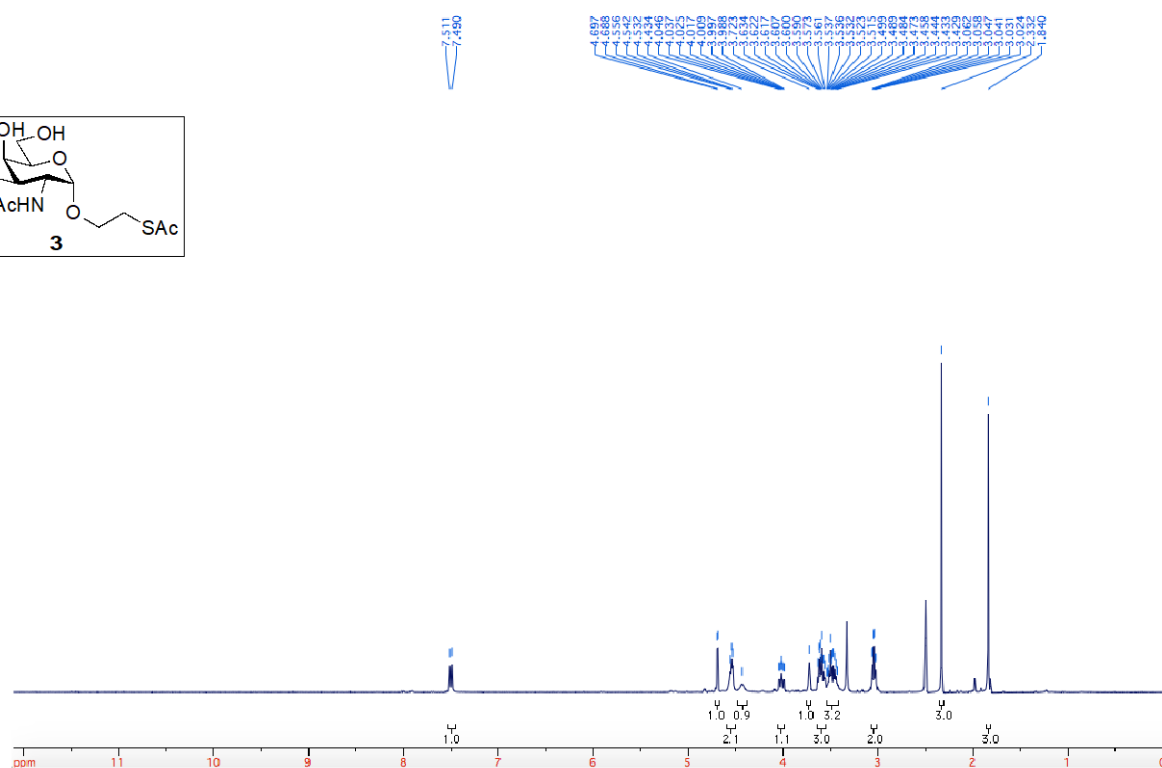
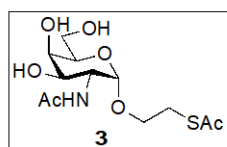
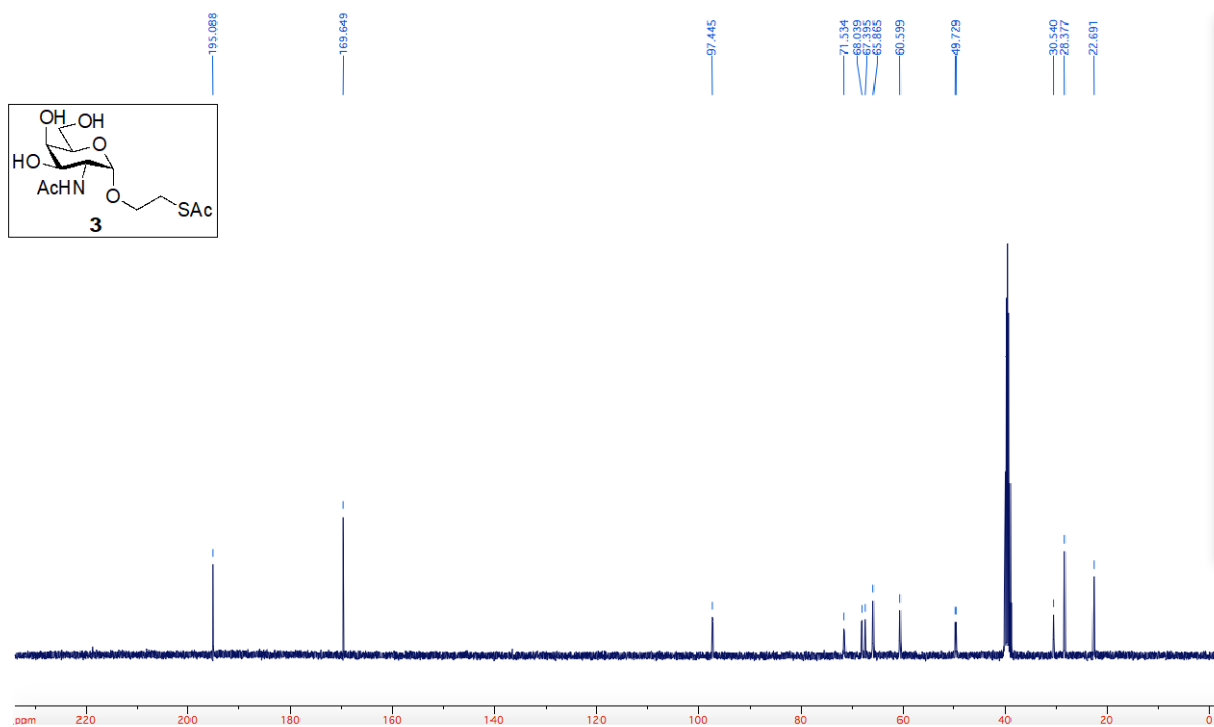


fig. S7



Disac

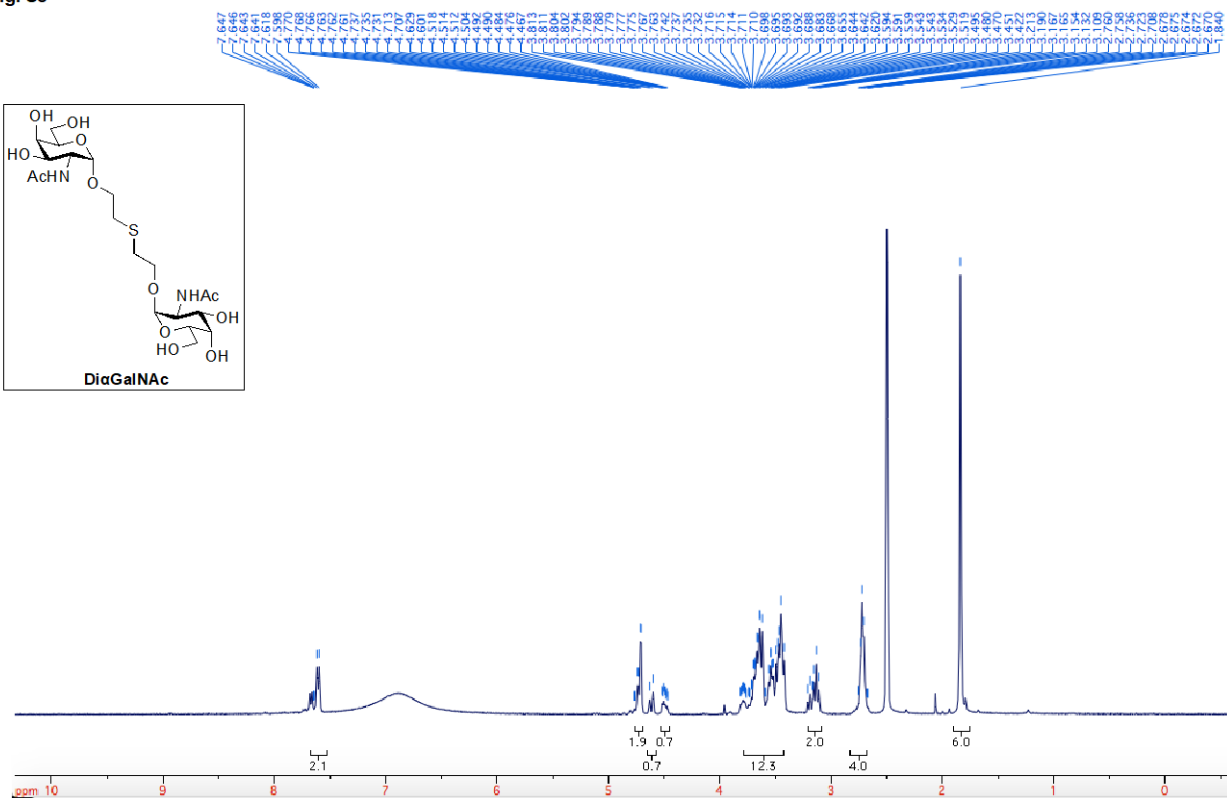


fig. S9

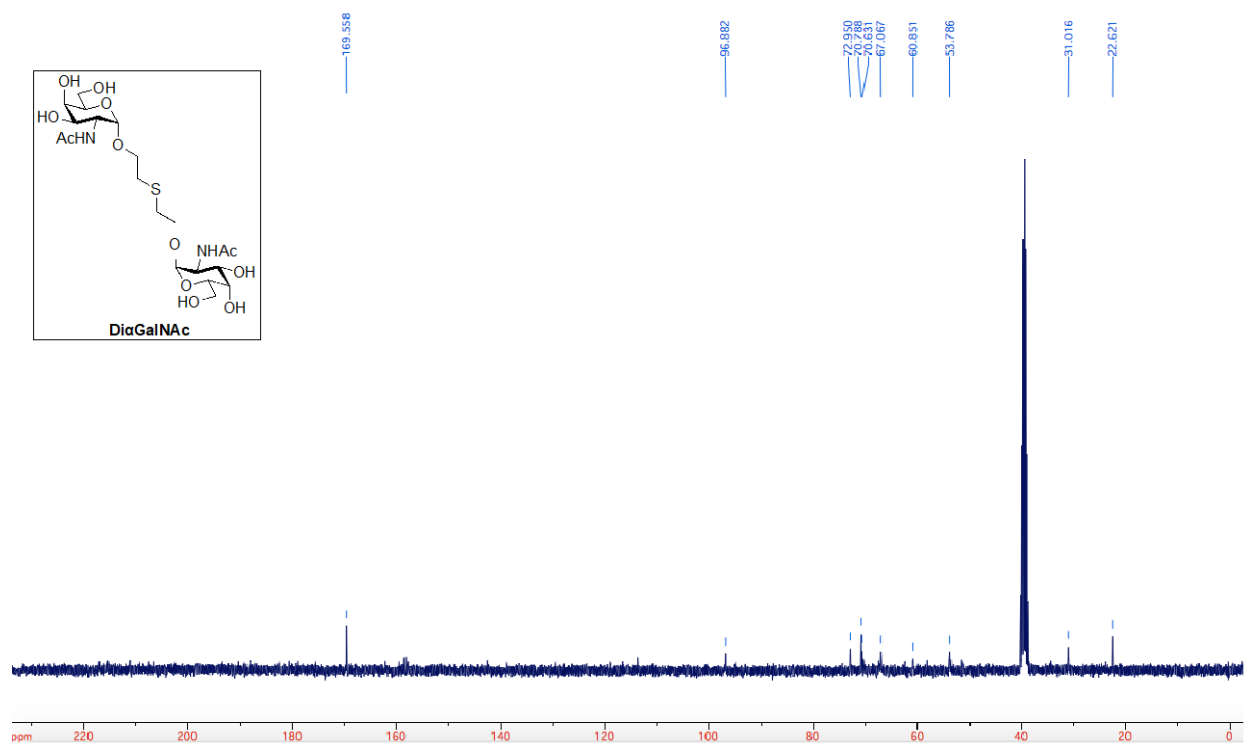


fig. S10

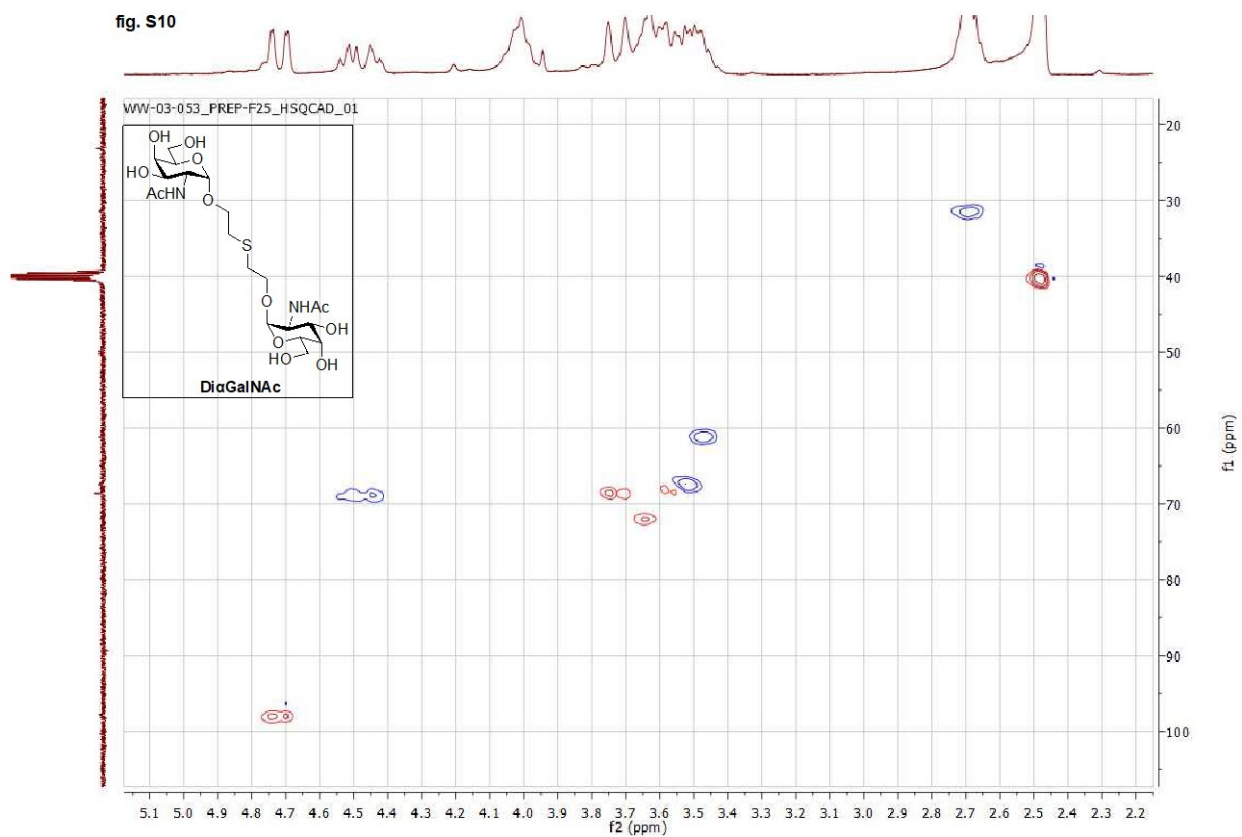


fig. S11

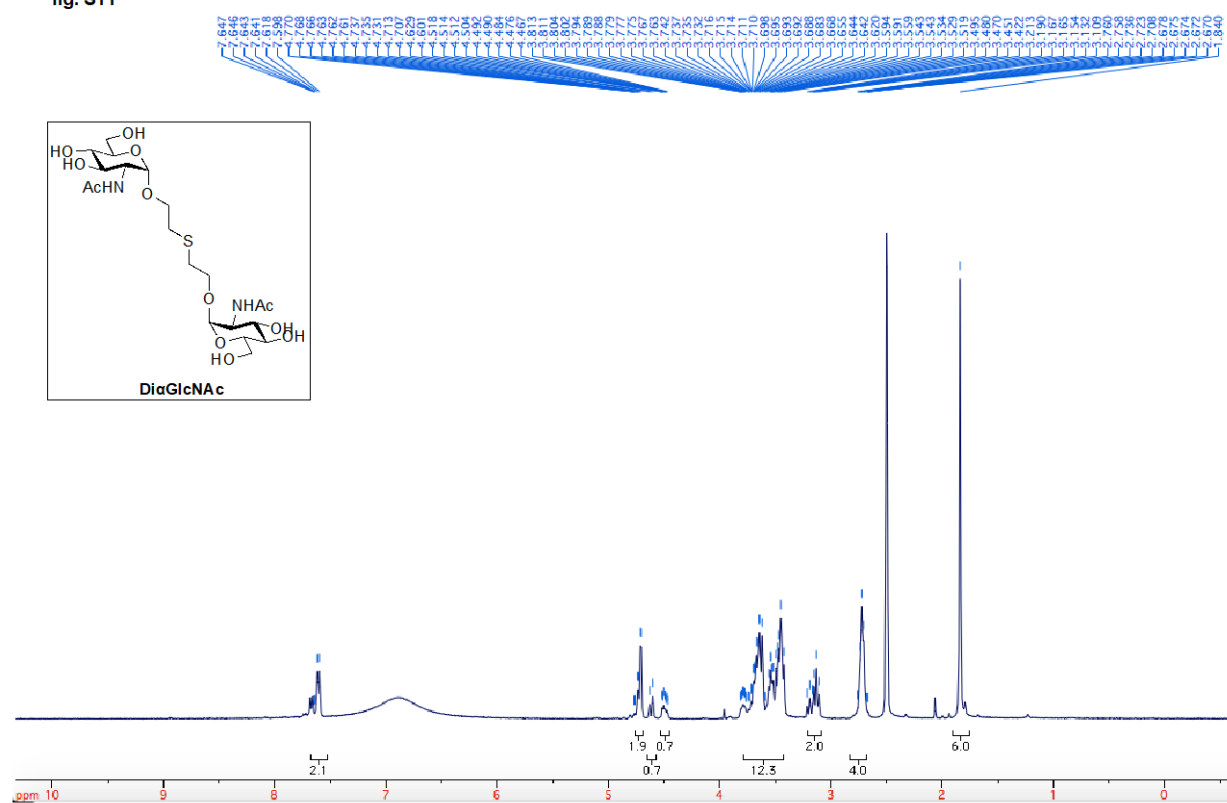


fig. S12

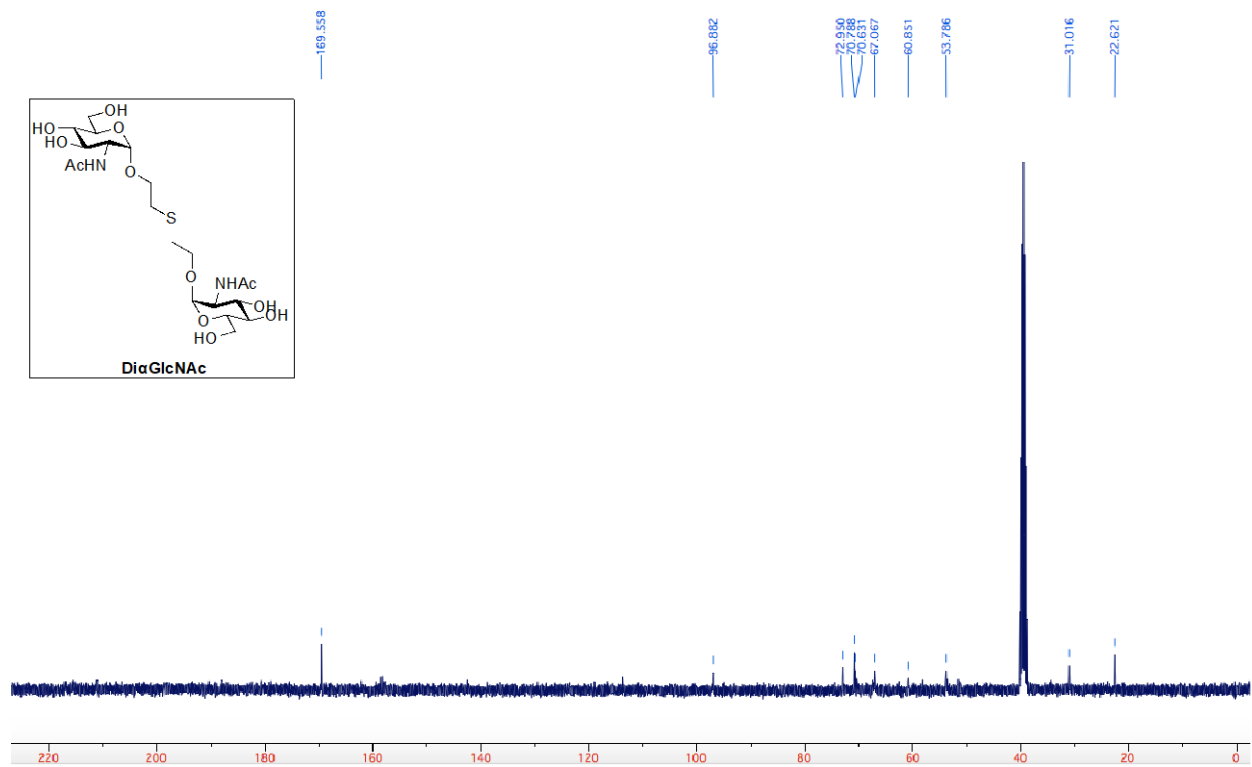


fig. S13

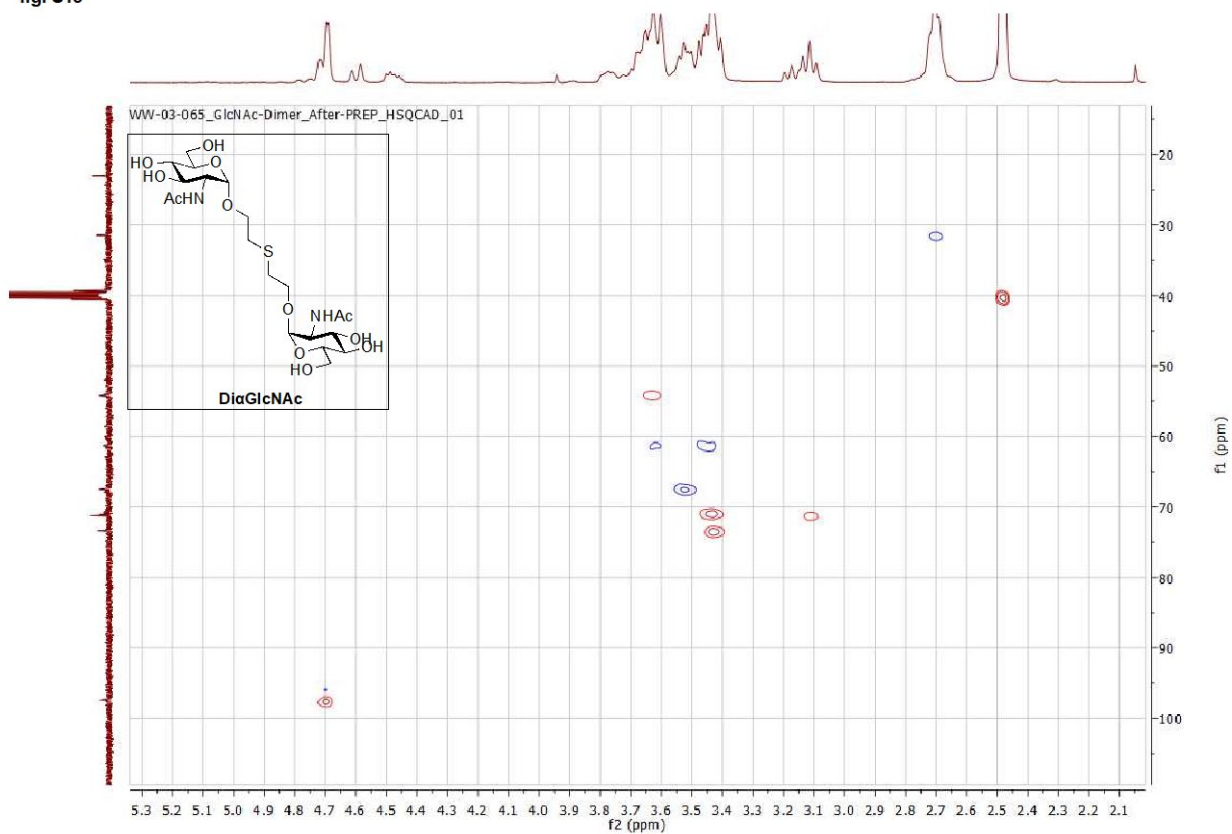


fig. S14

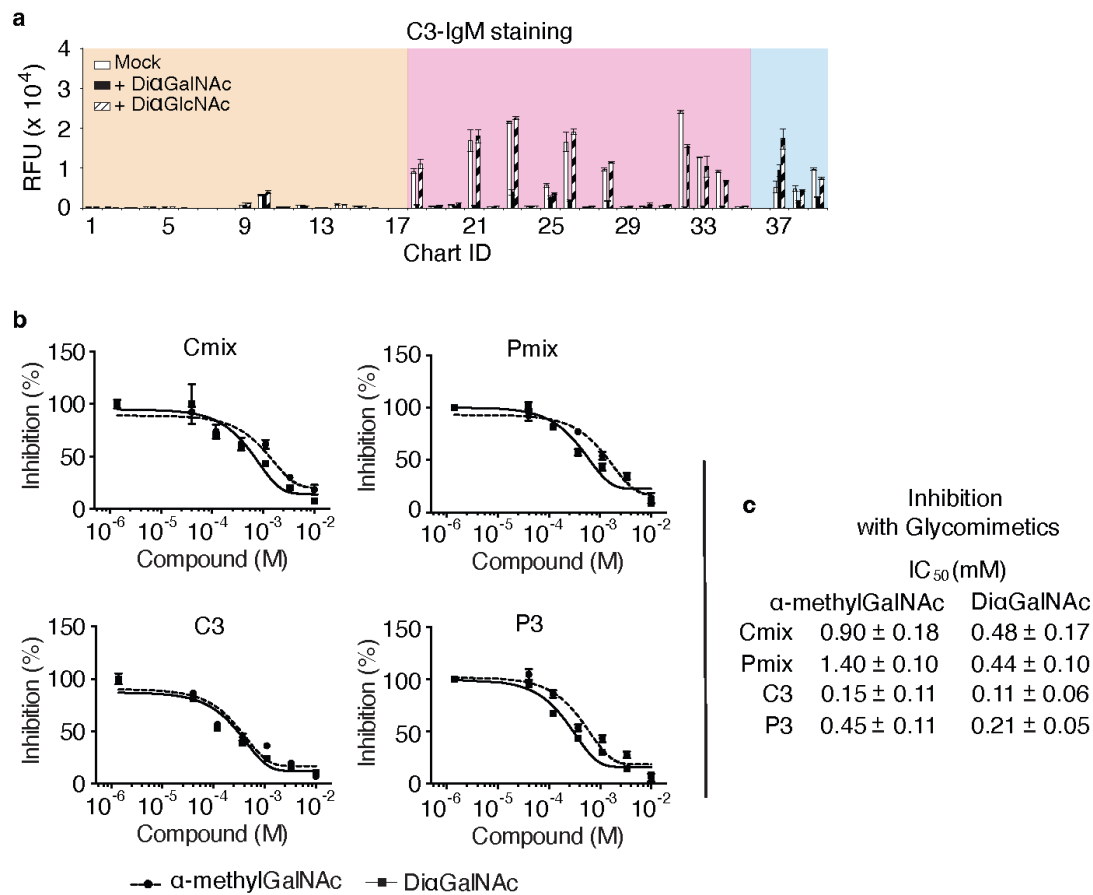
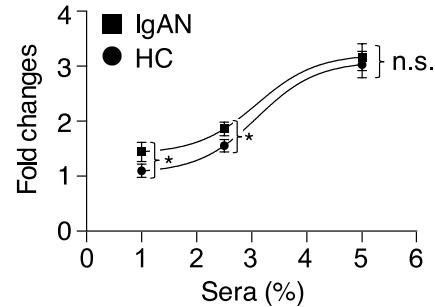
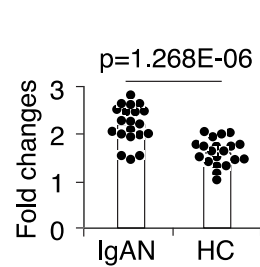


fig. S15

a



b



c

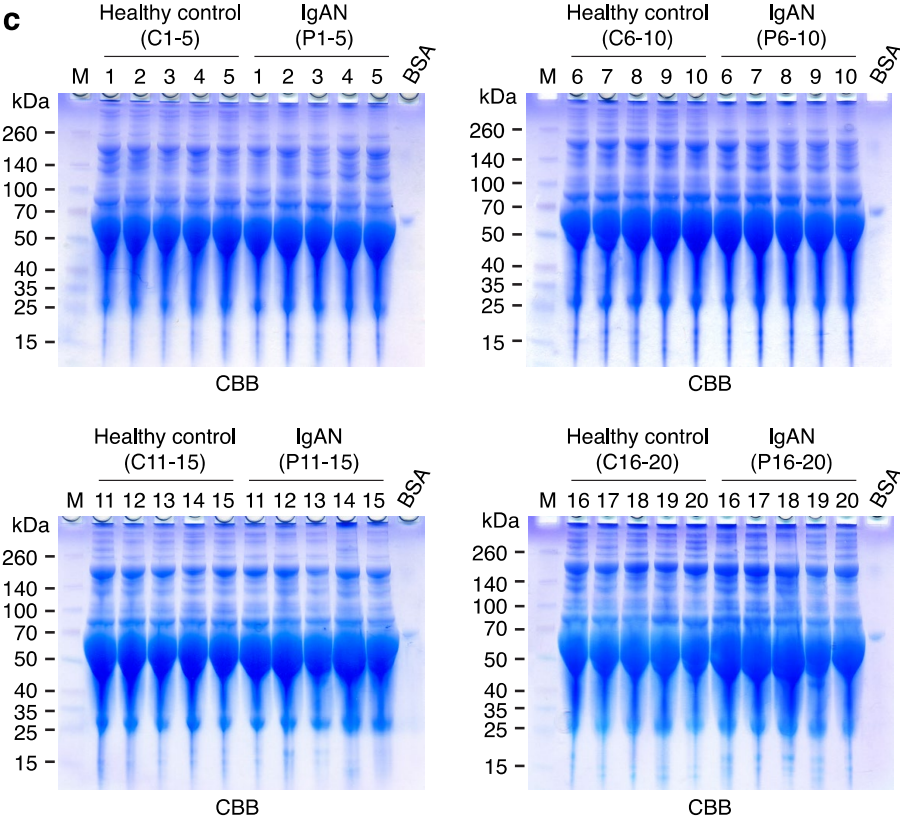


fig. S16

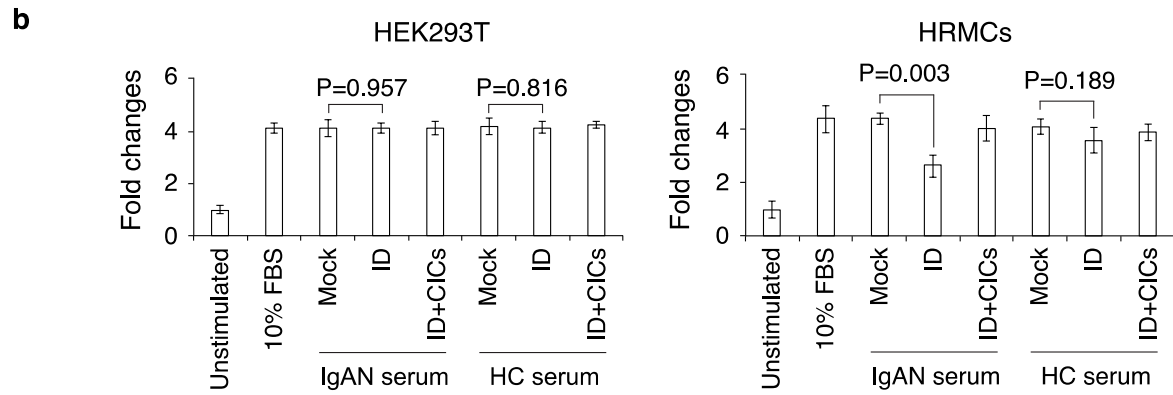
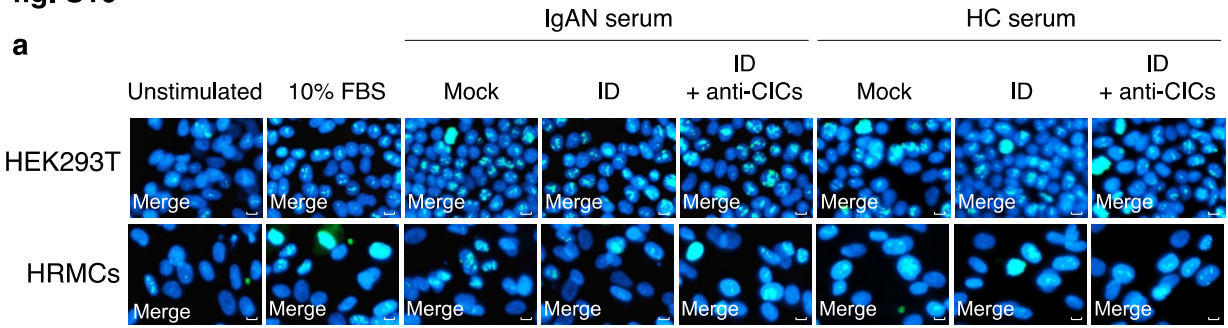
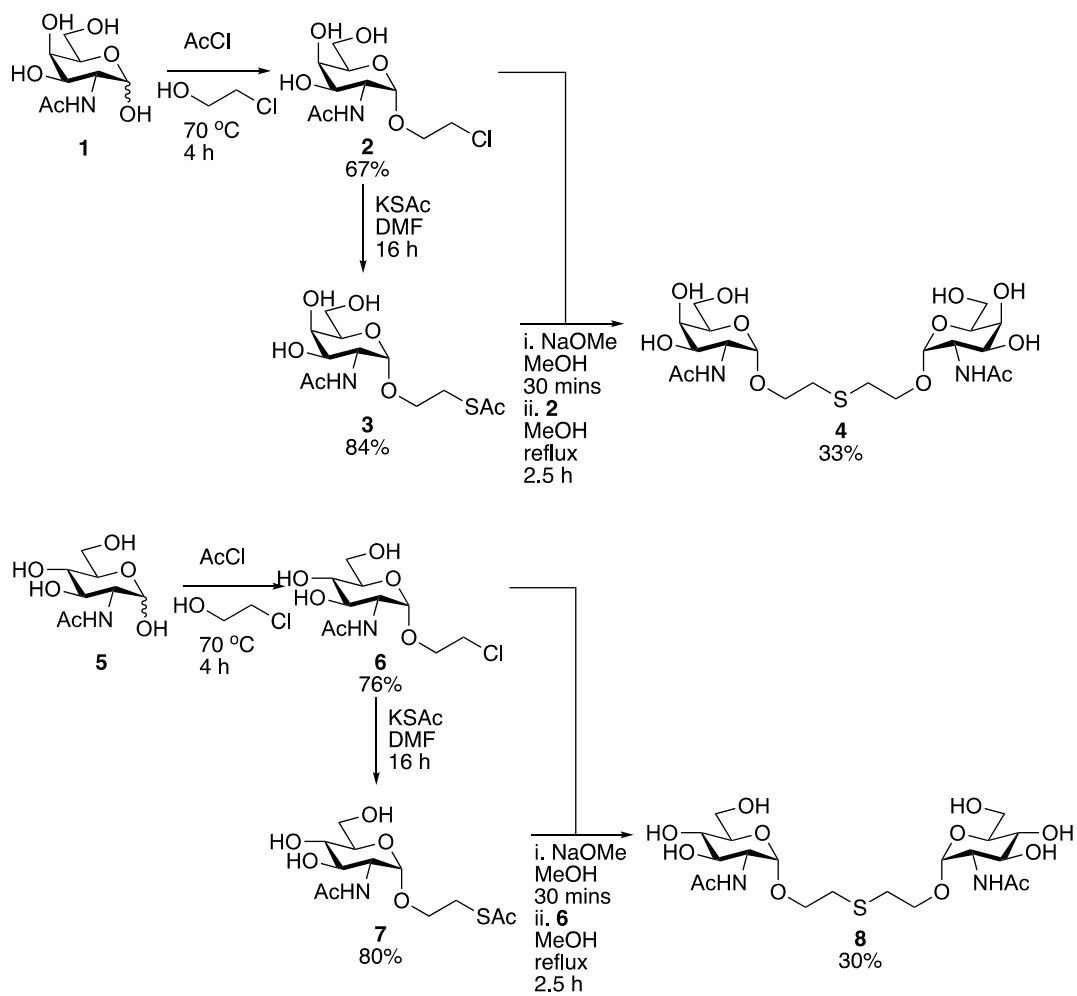


fig. S17



Supplementary Data files

Table S1: Identification of serum protein enriched with asialo-BSM beads. Protein were loaded onto a SDS-PAGE and subjected to in-gel trypsin treatment of selected bands, followed by C18-RP-LC-MS/MS analysis. Main protein hits (bold, grey) were identified based on their number of peptide spectra matches (PSMs). (*Excel file*)