Supporting information

Mechanism of Antigen Presentation and Specificity of Antibody Cross-reactivity Elicited by an Oligosaccharide-conjugate Cancer Vaccine

Authors: Szu-Wen Wang[†,‡], Yi-An Ko[†], Chiang-Yun Chen[†], Kuo-Shiang Liao[†], Yi-Hsuan Chang[†,‡], Hsin-Yu Lee[†], Yueh-Hsiang Yu[†], Yu-Hsuan Lih[†], Yang-Yu Cheng[†], Heng-Hsin Lin[†], Tsui-Ling Hsu[†], Chung-Yi Wu[†], Kuo-I Lin[†,*], Chi-Huey Wong[†,§,*]

Affiliations:

[†]Genomics Research Center, Academia Sinica, Taipei, 11529, Taiwan

[‡]Institute of Biochemical Sciences, National Taiwan University, Taipei 10617, Taiwan

[§]Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, USA

*Correspondence to: Kuo-I Lin (kuoilin@gate.sinica.edu.tw) or Chi-Huey Wong (wong@scripps.edu).

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Materials and Methods

Mice and vaccination. Balb/c mice (8-10 weeks) were purchased from National Laboratory Animal Center, Taiwan. FUCA1 knockout (KO) mice (C57BL/6N-*Fuca1*$^{em1(IMPC)J}$/Mmucd) were purchased from Mutant Mouse Resource & Research Centers at University of California, Davis, California, USA. All the mice were maintained in a specific pathogen-free environment. Eight-week-old Balb/c mice were immunized i.m. three times with 2-week intervals. Each vaccination contains 2 μg GH-DT and 2 μg C34 in PBS (100 μL). Sera collected from immunized mice were subjected to glycan array analysis 10 days after the last immunization. The experimental protocol was approved by Academia Sinica's Institutional Animal Care and Utilization Committee.

Preparation of glycan array slides. To prepare the glycan array slides, 15 different globo-series glycans with aminopentyl linker (Supplementary Table1) were dissolved in the printing buffer (100 μM glycan in 300 mM phosphate buffer with 0.005% triton X-100, pH 8.5). The prepared glycans were loaded into 96-well-plates, and 0.7 μL was printed on the NHS ester-coated glass slides (Nexterion H slide, SCHOTT, Germany). The slide was separated into 16 grids and each contained 16 column × 10 rows of glycan spots as described previously.[1] The prepared slides were reacted in an atmosphere of 80% humidity for 2 h, dried overnight, and stored at room temperature in a drier before use.

Serum and antibody analysis on glycan array. Glycan array analysis was performed as described.[2] Briefly, the glycan array slides were blocked by Pierce™ blocking buffer (Thermo Fisher Scientific) at room temperature for 1 h, followed by washing with PBST (0.5% Tween-20 in PBS) 3 times. The slides were then incubated with sera or ch64B7 diluted with 3% BSA in PBST at room temperature for 2 h and washed with PBST for three times and then incubated with Alexa Flour 647 conjugated goat anti-mouse IgG antibody (Jackson ImmunoResearch, 2.5 μg/mL in PBST) at room temperature for 30 min. The slides were rinsed in PBST and ddH_2O, centrifuged and scanned at 635 nm wavelength by microarray fluorescence chip reader (GenePix 4300A; Molecular Devices Corporation). The result was quantified and analyzed by GenePix Pro-6.0 analysis software (Axon Instruments).

Splenic B cells, BMDCs culture, human monocyte-derived dendritic cells, and antigen treatment. To prepare splenic B cells, spleens were homogenized with the frosted end of glass slide followed by passing through the cell strainer (BD Biosciences). RBCs were depleted by treating with RBC lysis buffer (Sigma). Splenic B cells were isolated by positive selection from splenocytes using B220 microbeads by MACS (Miltenyi Biotec, 130-049-501). Bone-marrow derived dendritic cells (BMDCs) were prepared as described.[3] Briefly, bone marrow was isolated from mouse femurs and tibiae and treated with RBC lysis buffer (Sigma-Aldrich) to deplete RBCs. Cells were then cultured in RPMI-1640 containing 10% heat inactivated FBS (Thermo Fisher Scientific), 1% Penicillin/Streptomycin

(Thermo Fisher Scientific), 50 μM 2-mercaptoethanol (Thermo Fisher Scientific), and 20 ng/mL recombinant mouse GM-CSF (eBioscience) at a density of 2×10^5 cells/mL. The cells were supplemented with an equal volume of the complete culture medium described above at day 3 and refreshed with one-half the volume of medium at day 6. At day 8, the suspended cells were then harvested and treated with 1 mg/mL GH-DT or SSEA4-DT.

Human monocyte-derived dendritic cells (MoDCs) were differentiated from periphery blood mononuclear cells (PBMCs) as described.[4] Briefly, PBMCs were isolated from blood of healthy donor by using ficoll and centrifugation at 700×g for 30 min. After isolation with CD14 microbeads by MACS (Miltenyi Biotec, 130-050-201), $CD14^+$ PBMCs were cultured in RPMI 1640 containing 10% heat inactivated FBS, 1% Penicillin/Streptomycin, 50 μM 2-mercaptoethanol, 25 ng/mL human GM-CSF (R&D) and 20 ng/mL human IL-4 (R&D). The cells were supplemented with an equal volume of the complete culture medium at day 3 and incubated for 6 days. At day 8, the suspended MoDCs were then harvested. The Research Ethics Committee of Academia Sinica approved the research project to acquire peripheral blood of healthy donors from Taipei Blood Center.

Flow cytometry staining. For staining SSEA3 specific B cells induced by GH-DT immunization, after blocking with Fc receptor binding inhibitor (clone: 93, eBioscience) for 20 min, splenocytes were stained with antibodies against CD3 (clone: 17A2, BV421-conjugated, Biolegend), CD19 (clone: 1D3, PECy7-conjugated, BD Biosciences), GH-BSA-CF633, SSEA3-BSA-FITC, and SSEA3-BSA-Cy3 for one h. To analyze the antigen presentation by BMDCs, splenic B cells, and human MoDCs, the cells treated with GH-DT or SSEA4-DT were labeled with FITC or Alexa Fluor 488 conjugated antibodies against GH (clone VK9), SSEA3 (clone MC631, Biolegend), or SSEA4 (clone MC813-70, Biolegend). BMDCs were gated by using anti-mouse CD11c-APC antibody (clone N418, Biolegend). To analyze GH presentation by MHC II, BMDCs were incubated with indicated concentration of antibody against IA/IE (clone: M5/114, Biolegend) one h before the treatment of GH-DT. Labeled cells were analyzed using FACSCanto Flow Cytometer (BD Biosciences).

Single B cell isolation. Splenocytes isolated from GH-DT immunized mice were incubated with 30 μg/mL Gb3-BSA-biotin at 4 ^oC for 1 h, followed by washing and incubation with anti-biotin microbeads (Miltenyi Biotec) on ice for 15 min. After washing, Gb3-BSA positive cells were negatively depleted by LS column (Miltenyi Biotec). The flow-through cells were incubated with 10 μg/mL SSEA3-BSA-biotin at 4 ^oC for 1 h, followed by washing and incubation with anti-biotin microbeads (Miltenyi Biotec) on ice for 15 min. SSEA3 positive cells were collected with LD column (Miltenyi Biotec) and stained with antibodies CD19 (clone: 6D5, PE-Cy7-conjugated, BioLegend), CD3 (clone: 17A2, BV421-conjugated, BioLegend) at 1:125 dilution on ice for 15 min. Propidium Iodide (BioLegend) was used at 1:1000 to exclude dead cells. Live single SSEA3-specific B cells ($CD3^-CD19^+$) were sorted into 96-well PCR plates (Applied Biosystems) containing 10 μL/well catch buffer (10 mM Tris-HCl, pH 8, and 5 U/μl RNasin (Promega)) by BD FACSAria II.

Monoclonal antibodies construction and expression. Monoclonal antibodies construction and expression was performed according to the method described previously.[5] Briefly, reverse transcription-polymerase chain reaction (PT-PCR) was performed by using primers designed based on a previous publication.[6] PCR products were then analyzed by electrophoresis and sequencing. The Ig V and L genes were identified on IMGT (the international ImMunoGeneTics information system, http://imgt.org/IMGT_vquest/input). Genes were then amplified from the second round PCR product with single gene-specific V and L gene primers containing restriction sites for cloning into the vectors containing human IgH or IgL expression backbone. The chimeric IgH and IgL expression constructs were co-transfected into Expi293 for antibody production.

Oxidative release of glycans from carbohydrate-conjugated vaccine. Oxidative release of conjugated glycans was performed according to the method described previously with some modification.[7] Briefly, NaClO was added to the membrane fractions of BMDCs to give a final concentration at 1%. The mixture was shaken at room temperature for 1 min. Formic acid at 1% concentration was used to quench the reaction. The mixture was then centrifugated at 16,000×g at 4 ℃ for 5 min. The supernatant was passed through a Sep-Pak C18 cartridge (Waters) and washed with 3 × 3 mL 0.1% formic acid. Glycans were eluted with 1 mL 20% acetonitrile and 0.1% formic acid and dried by SpeedVac evaporator. The glycans were then analyzed by LC-MS/MS (LTQ-FT or Orbitrap Fusion, Thermo Fisher Scientific).

Membrane protein extraction. DC membrane proteins were extracted using ProteoExtract® Native Membrane Protein Extraction Kit (Millipore) according to the manufacturer's instructions. Briefly, GHN_3-DT or GH-DT treated BMDCs were scraped and washed three times with 2 mL ice cold wash buffer. The cell pellet was then suspended with 2 mL extraction buffer I, supplemented with a cocktail of protease inhibitors (Roche) and then incubated at 4 ℃ for 10 min. The cells were pelleted by centrifugation at 16,000×g at 4℃ for 15 min. The pellet was then suspended with 500 μl extraction buffer II supplemented with the protease inhibitor cocktail and then incubated at 4 ℃ for 30 min. After centrifugation at 16,000×g at 4 ℃ for 15 min, the supernatant was transferred to a new tube for the following experiments.

Click chemistry. Lysates of membrane proteins isolated from GHN_3-DT treated BMDCs were conjugated with an alkynyl-biotin linker by mixing with 1 mM of CuSO4, 2 mM of sodium ascorbate, 0.1 mM of alkynyl-biotin and 0.1 mM tris-triazoleamine catalyst, and then incubated at room temperature overnight.

The extra unreacted alkynyl-biotin linker was removed by protein precipitation. 480 μL methanol and 120 μL chloroform were added to a 420 μL reaction sample, followed by addition of 320 μL ddH_2O, mixed well, and then centrifuged for 5 min at 16,000×g. The upper layer was removed. The

sample was then added with 360 μL methanol, mixed well, and then subjected to centrifugation for 5 min at 16,000×g. The supernatant was removed. The pellet was air dried and then resuspend with 400 μL PBST by sonication at 4 ℃. After centrifugation for 5 min at 16,000×g, the supernatant was collected for the following use. GHN_3 associated protein complex was then isolated using SoftLink™ Soft Release Avidin Resin (Promega) according to the manufacturer's instructions. Briefly, the solution containing GHN_3 associated protein complex was passed through the resin packed in a mini column for three times. The resin was then washed with 5 column volumes of PBS. The protein complex was then eluted with 10% acetic acid and the sample was then dried by SpeedVac evaporator.

Filter-aided sample preparation. The purified GHN_3 associated protein complex was applied to a 30 kDa cut off filter unit (Microcon-30 kDa, Millipore), centrifuged at 14,000×g for 10 min and then the flow-through from the collection tube was discarded. 200 μL of urea buffer (8 M urea in 0.1 M Tris-HCl, pH 8.5) were added to the filter unit and centrifuged at 14,000×g for 15 min. The filter unit was added with 100 μL DTT solution (25 mM DTT in urea buffer) and then was incubated at room temperature for 10 min, followed by centrifugation at 14,000×g for 10 min. The filter unit was then added with 100 μL of IAA solution (50 mM iodoacetamide in urea buffer), incubated at room temperature for 10 min and centrifuged at 14,000×g for 10 min, followed by adding 100 μL of urea buffer. The filter unit was centrifuged at 14,000×g for 15 min and then added with 100 μL of 50 mM ammonium bicarbonate in water (ABC buffer), followed by centrifugation at 14,000×g for 10 min. 40 μl of ABC buffer with trypsin (Thermo Fisher Scientific) in an enzyme/protein ratio of 1:20 were added to the filter unit and the solution was mixed well. Subsequently, the filter unit was incubated in a wet chamber at 37 °C for overnight, followed by addition of 40 μl ABC buffer and centrifugation at 14,000×g for 10 min. The trypsin digested peptides in the flow through were collected and dried in a SpeedVac evaporator.

ELISA. For IL-2 detection, $CD4^+$ cells from GH-DT or PBS immunized mice were purified by using MACS (Miltenyi Biotec, 130-104-454) and co-incubated with BMDCs at the ratio of 5:1. Forty-eight hours after stimulation with 1 mg/mL DT, GH-DT, or PBS, the Il-2 level in the supernatant was determined with BD OptEIA Mouse IL-2 ELISA Set (BD Biosciences) according to the manufacturer provided procedures.

To determine the binding affinity of ch64B7, GH glycan-BSA, SSEA3 glycan-BSA, or SSEA4 glycan-BSA was coated on ELISA plate at the concentration of 0.2 μg per well in 100 μL coating buffer (0.05 M Carbonate-Bicarbonate, pH 9.6) at 4 ℃ for overnight. After washing 3 times with PBST, the plates were incubated with 1% BSA/PBST blocking solution at room temperature for 1 hour. After 3 times washing with PBST, the plates were incubated with serially diluted ch64B7 at room temperature for 2 hours. After 5 times washing with PBST, the plates were incubated with goat-anti-human IgG-HRP (1:5000, Jackson Immuno- Research), and incubated at room temperature for 2 hours. After 7 times washing with PBST, the plates were developed with 100 μl of the TMB substrate

reagent set (BD) for 10 minutes at 25°C and stopped with 50 μL 2N H_2SO_4. The OD_{450} was measured by SpectraMax Paradigm, Molecular Devices.

Immunofluorescence staining. BMDCs were cultured on chamber slide (ibidi) and treated with 1 mg/mL GH-DT for various timepoints. The cells were then rinsed with PBS and fixed in 4% paraformaldehyde, followed by permeabilization with 0.1% Triton X-100 and blocked by 1% BSA in PBST. Anti-GH antibody (clone VK9) in blocking buffer was added and incubated with a secondary antibody (goat anti-mouse antibody conjugated with Alexa Fluor 633, Thermo Fisher Scientific). The cells were then stained with rat anti-mouse I-A/I-E antibody (clone M5/114; Alexa Fluor 594 conjugated, 1:100 dilution, Biolegend) and Alexa Fluor 488 conjugated anti-SSEA3 antibody (clone MC631; 1:100 dilution, Biolegend). Fluorescent images were examined by confocal microscope (Leica TCS SP8X WLL Confocal Super resolution Microscope). To investigate the colocalization rate between GH and FUCA1 or FUCA2 in BMDCs, cells were stained with anti-GH antibody (clone VK9) and then with goat anti-mouse antibody conjugated with Alexa Fluor 633 (A21050, 1:200 dilution Thermo Fisher Scientific), and then stained with anti-FUCA1 (PA544145, 1:100 dilution, Thermo Fisher Scientific) or FUCA2 (PA570565, 1:100 dilution, Thermo Fisher Scientific) antibodies, and then with goat anti-rabbit antibody conjugated with Alexa Fluor 555 (A21428, 1:200 dilution, Thermo Fisher Scientific). In some experiments, cells were stained with Alexa Fluor 488 conjugated anti-LAMP-1 antibody (clone 1D4B; 1:100 dilution, BD) and Alexa Fluor 488-conjugated anti-transferrin receptor antibody (clone C2F2; 1:100 dilution, BD).

FUCA1 and FUCA2 construction and expression. Mature mouse FUCA1 and FUCA2 genes were amplified from cDNA of mouse BMDCs using primers with restriction enzyme sites (Fuca1: forward 5'-ATATAT<u>AAGCTT</u>CTGGCTCCGCGCCGCTTCAC and reverse 5'-ATATAT<u>TCTAGAT</u>CAGTTCACCTTTGTCAGCT; Fuca2: forward 5'-ATATAT<u>AAGCTT</u>CTTAGCTATGACCCCACTTG and reverse 5'-ATATAT<u>TCTAGA</u>TTAAATCACATTACTTAGCA). The PCR products were then cloned into the expression vector p3xFLAG-CMV-9 (Sigma-Aldrich) with FLAG tag. The expression constructs were then transfected into Expi293 cells using ExpiFectamine™ 293 Transfection Kit (Thermo Fisher Scientific). After 5~7 days, the supernatants were harvested. FUCA1 and FUCA2 were purified by passing the supernatant through anti-FLAG M2 agarose beads (Sigma-Aldrich). The beads were then washed 3 times with PBS, and proteins were eluted with 0.1 M glycine-HCl (pH = 3.4).

FUCA1 and FUCA2 activity test. To investigate the effect of pH on the activity of FUCA1, 1.8 nmole GH glycan was digested with 2 μg FUCA1 in 20 μl acetate buffer at the pH range from 4 to 7 or in PBS. The reaction was incubated at 37 ℃ for 5 h. Fucose in the reactions was detected using L-Fucose Assay Kit (Megazyme). To test the ability of FUCA1 to digest GH glycan on intact GH-DT or GH-DT peptides, GH-DT was digested by FUCA1 first and then by chymotrypsin or by chymotrypsin first and then by FUCA1 in the following conditions by using filter-aided sample preparation: for

chymotrypsin digestion, 20 μg GH-DT or 2 μg FUCA1 digested GH-DT was incubated with 1 μg or 0.1 μg chymotrypsin (Promega) at RT for 16 h; for FUCA1 digestion, 2 μg chymotrypsin digested GH-DT peptide or intact GH-DT was incubated with 4 μg FUCA1 in acetate buffer (50 mM acetic acid, 5 mM $MgCl_2$, pH=5.5) at 37 ℃ for 24 h. The digested peptides were then analyzed by LC-MS/MS.

Fuca1 and Fuca2 knockdown by siRNA. siRNA targeting Fuca1-1 (mm.Ri. fuca1.13.1), Fuca1-2 (mm.Ri. fuca1.13.2), Fuca1-3 (mm.Ri. fuca1.13.3), or Fuca2 (mm.Ri. fuca2.13.1) was purchased from Integrated DNA Technologies. 300 nM of siRNA targeting FUCA1, FUCA2, or control siRNA was used for each transfection which was performed by mixing siRNA with day 8 differentiated BMDCs by using Mouse Dendritic Cell Nucleofector Kit by Amaxa electroporation (program Y-01, Lonza). Transfected DCs were further cultured for 24 h in RPMI 1640 supplemented with 20 ng/mL recombinant mouse GM-CSF (eBiosceince) before treatment with GH-DT.

Statistical analysis. GraphPad Prism version 8 was used to conduct all statistical analysis in this study. All the data are expressed as the means ± standard errors. For all the analyses, P values were obtained from Student's t-test (unpaired, two tailed or paired t-test). Two-way ANOVA was used for multiple comparisons. $p < 0.05$ was statistically significant. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; and ****$p < 0.0001$.

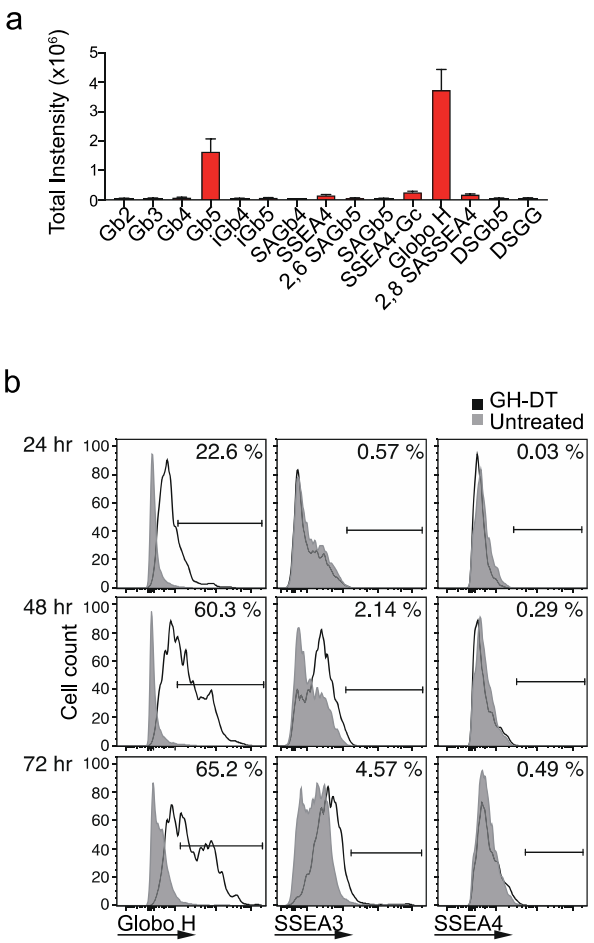


Figure S1. GH-DT vaccine processing in mouse splenic B cells (**a**) Immunization with GH-DT/C34 in Balb/c mice induced anti-serum against GH, SSEA3, and SSEA4 glycans, as determined by glycan array analysis (n=10). (**b**) Mouse splenic B cells presented GH glycan or a very low level of SSEA3 glycan, but not SSEA4 glycan, on the cell surface 24 h, 48 h, and 72 h after GH-DT treatment.

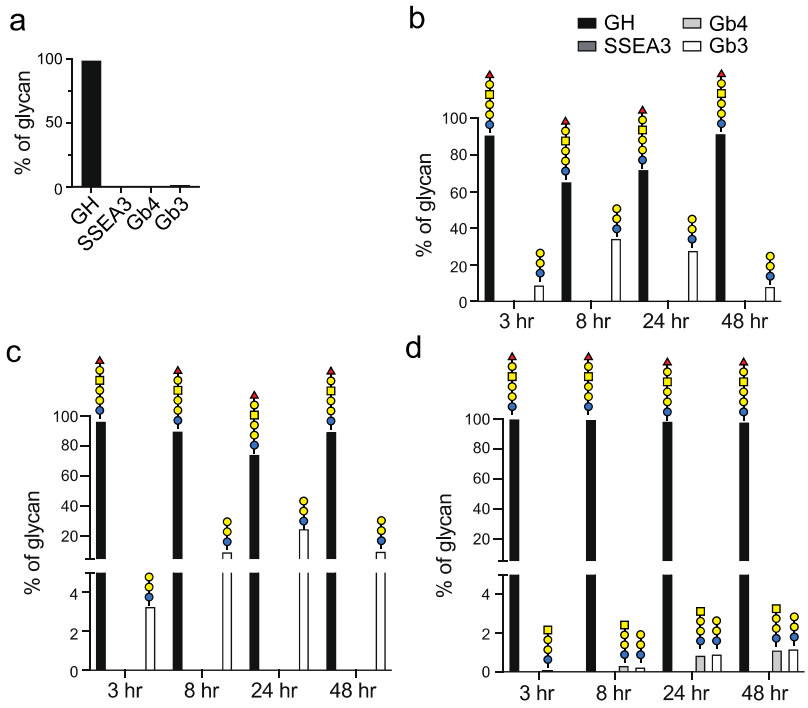
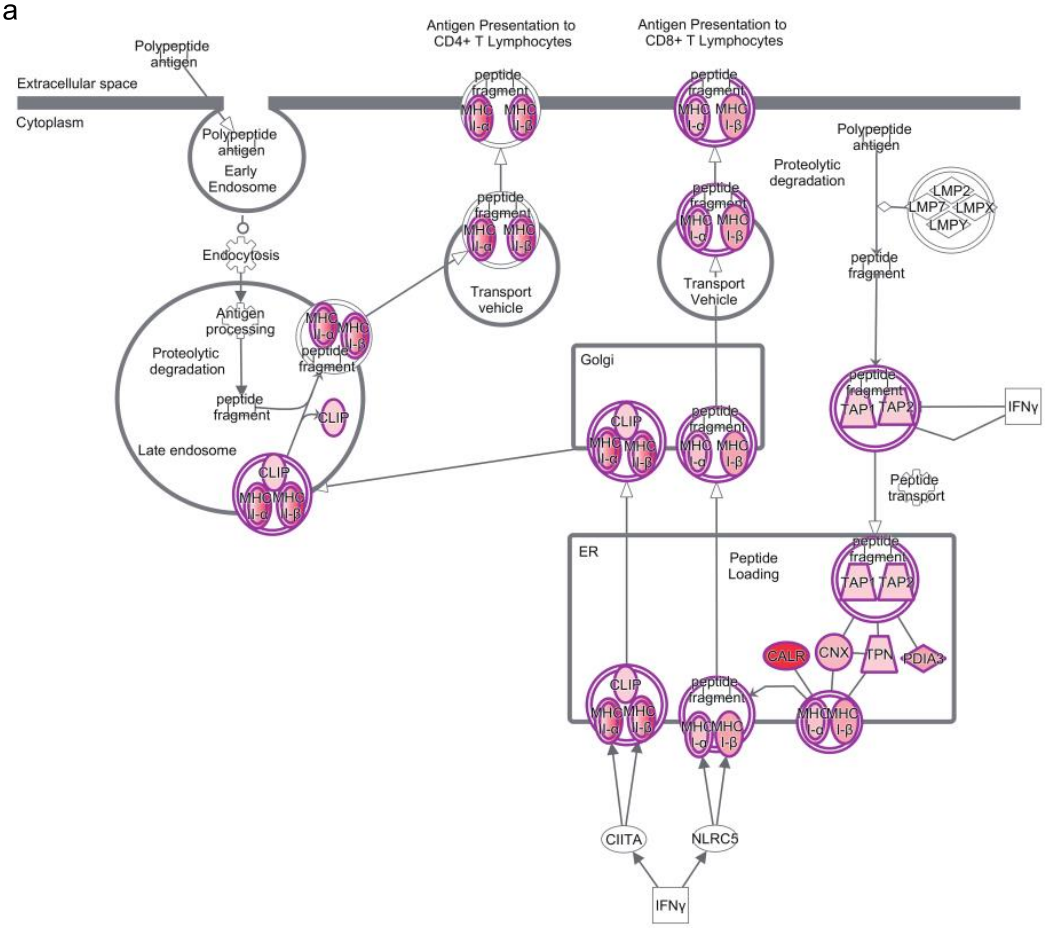


Figure S2. Oxidative release of globo-series glycan analyzed by LC-MS/MS. (**a**) Glycan released from GH-DT vaccine. Glycan released from soluble fraction (**b**), membrane fraction (**c**), or condition medium **(d)**, of BMDC treated with GH-DT.

a


Antigen Presentation to CD4+ T Lymphocytes
Antigen Presentation to CD8+ T Lymphocytes
Polypeptide antigen
Extracellular space
Cytoplasm
Polypeptide antigen
Early Endosome
Endocytosis
Antigen processing
Proteolytic degradation
peptide fragment
CLIP
Late endosome
MHC II-α
MHC II-β
peptide fragment
Transport vehicle
Transport Vehicle
Golgi
Proteolytic degradation
Polypeptide antigen
peptide fragment
LMP2
LMP7
LMPX
LMPY
TAP1
TAP2
IFNγ
Peptide transport
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NLRC5
IFNγ

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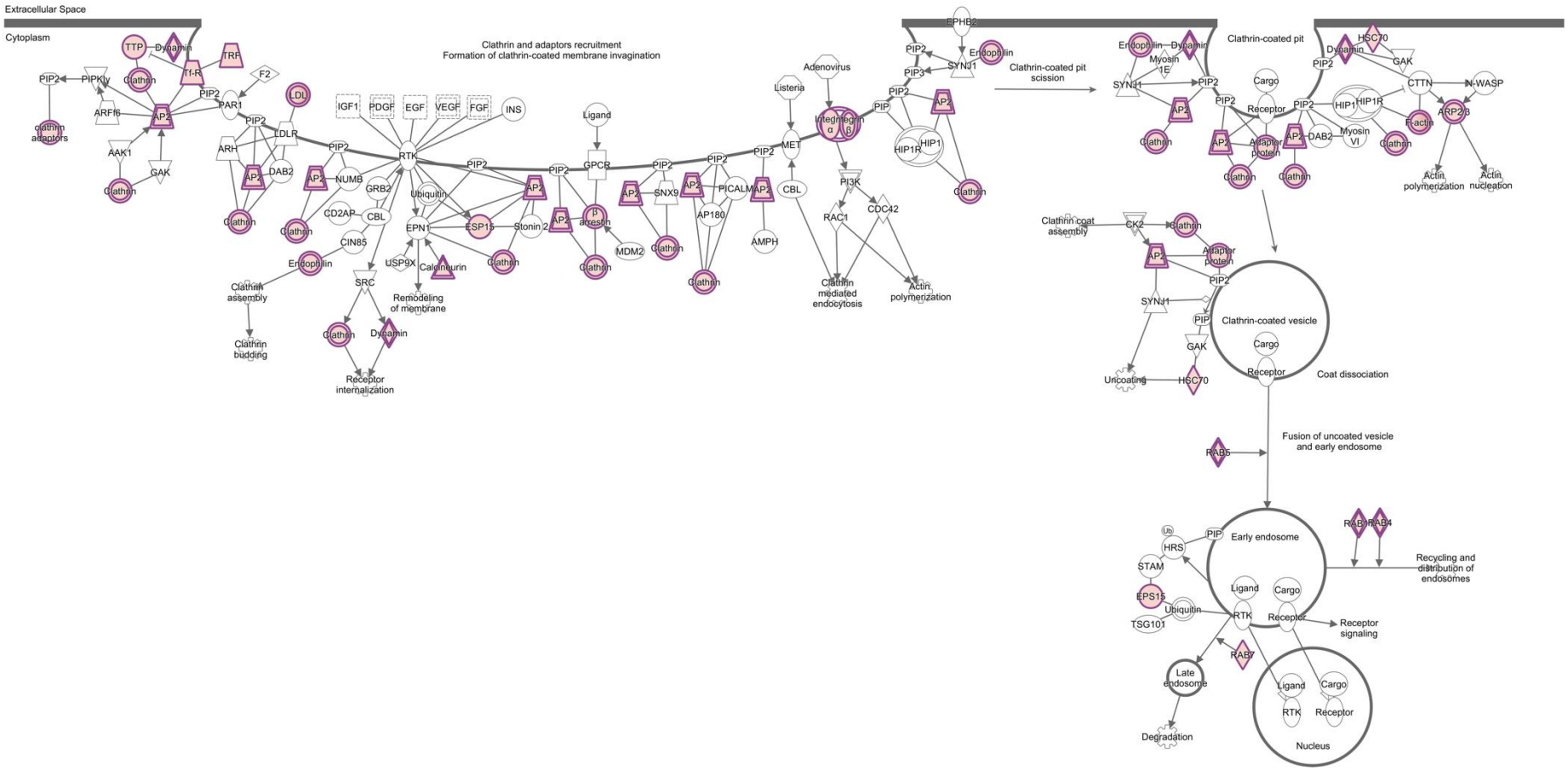
b


Extracellular Space
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Clathrin-coated pit
Clathrin coat assembly
Clathrin-coated vesicle
Coat dissociation
Uncoating
Fusion of uncoated vesicle and early endosome
Early endosome
Recycling and distribution of endosomes
Receptor signaling
Late endosome
Degradation
Nucleus
Clathrin assembly
Clathrin budding
Receptor internalization
Remodeling of membrane
Clathrin mediated endocytosis
Actin polymerization
Actin nucleation
TTP
Dynamin
TRF
Tf-R
F2
PIP2
PIPKIγ
Clathrin
ARFI6
AP2
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Adenovirus
Listeria
Integrin α β
PIP3
PIP
HIP1
HIP1R
EPHB2
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Nucleus

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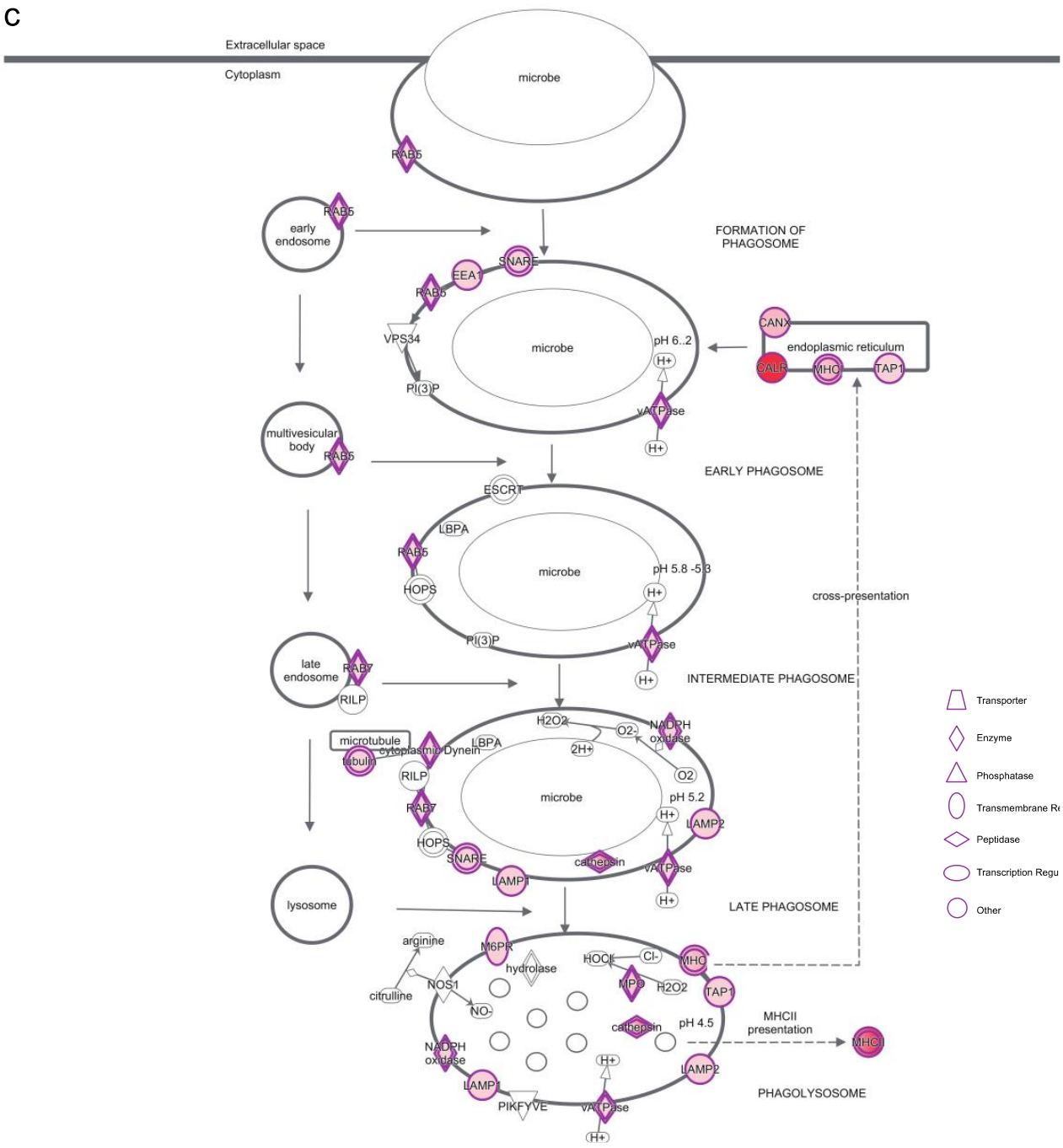


Figure S3. Identification of proteins interacting with GHN3 and intermediates. (**a**) antigen presentation pathway, (**b**) Clathrin-mediated endocytosis signaling, and (**c**) Phagosome maturation. Identified proteins were labeled in pink. The deeper color represents the proteins identified with higher intensity.

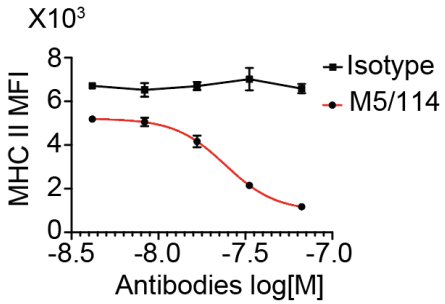


Figure S4. FACS analysis of MHC II on BMDCs (n = 3) 24 h after various doses of anti-MHC II antibody or rat IgG2b isotype control treatment, showing the level of surface MHC II is reduced.

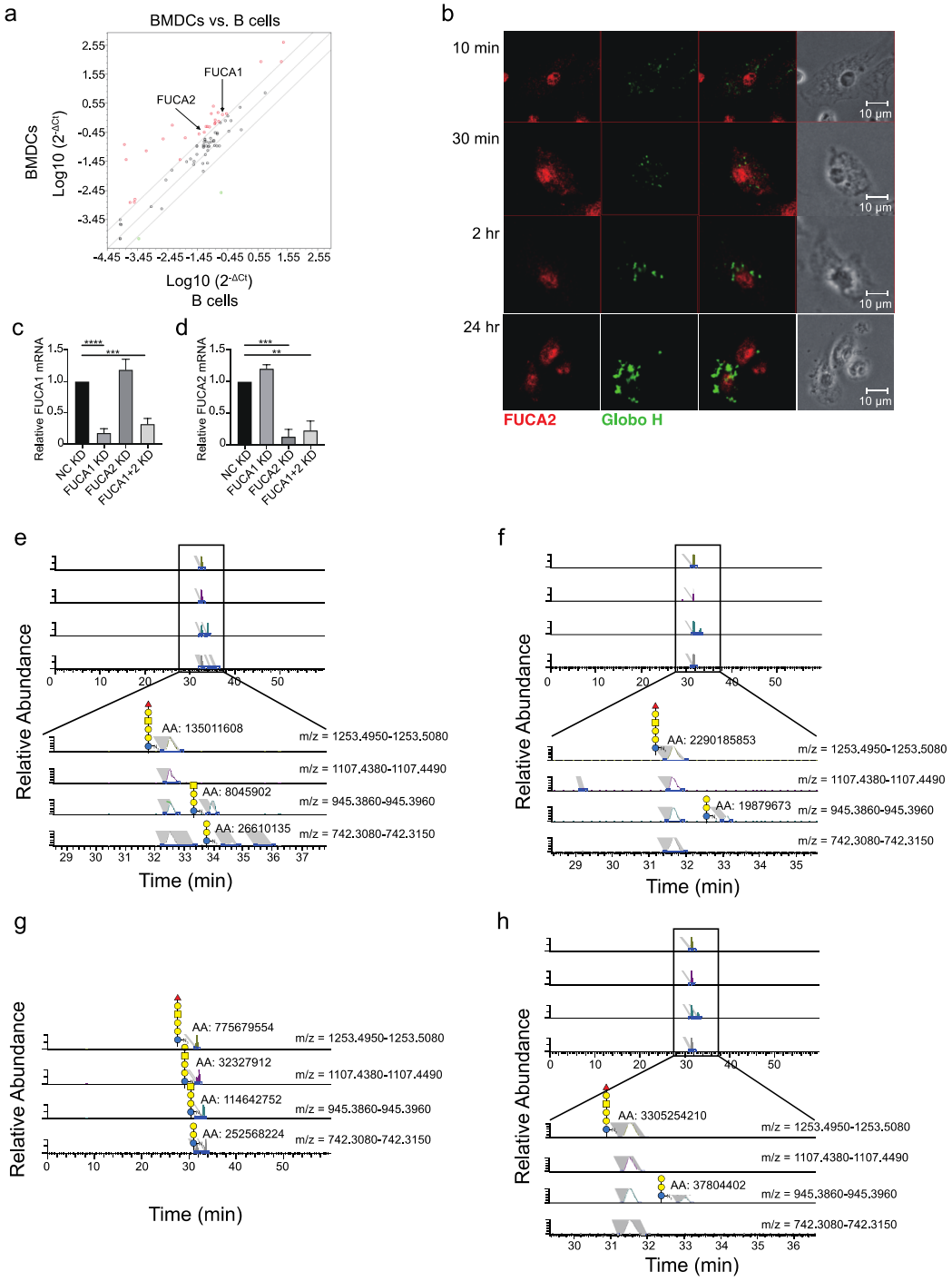


Figure S5. GH processing is initiated by FUCA1 in DCs (**a**) Compared with splenic B cells, BMDCs treated with GH-DT expressed higher levels of FUCA1 and FUCA2 mRNA. The abbreviation deltaCt is the Ct (threshold cycle) of target gene minus the Ct of control gene, HSP90AB1 (Heat shock protein HSP 90-beta). (**b**) Confocal microscope analysis showing that FUCA2 (red) and GH glycan (green) were rarely colocalized. (**c**, **d**) RT-qPCR showing the knockdown efficiency of siRNA against FUCA1 or FUCA2, or both. FUCA1 (**c**) or FUCA2 (**d**) mRNA were analyzed by RT-qPCR. NC represents negative control. (**e**–**h**) LC-MS/MS showing the glycan released from GHN_3-DT treated, control

siRNA (**e**), FUCA1 siRNA (**f**), FUCA2 siRNA (**g**), or both FUCA1 and FUCA2 siRNAs (**h**) transfected BMDCs.

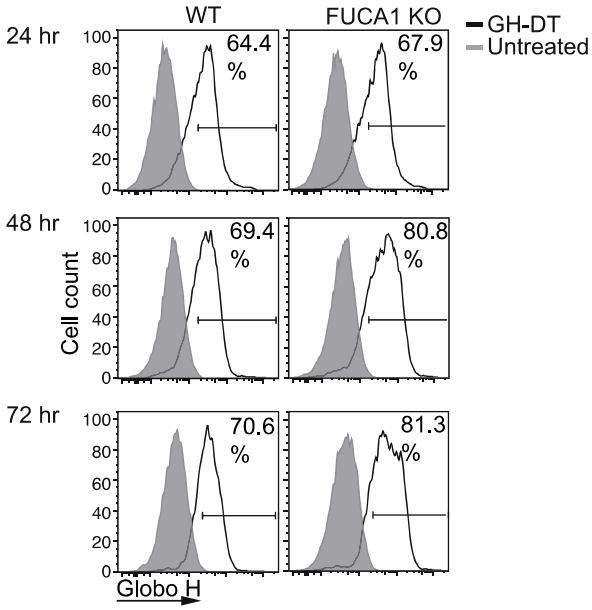


Figure S6. Knockout of FUCA1 in BMDCs presented more GH on cell surface as compared to WT BMDCs. BMDCs with FUCA1-KO were treated with GH-DT and analyzed using FACSCanto II and the acquired data were treated with FlowJo (ver.10.5.0).

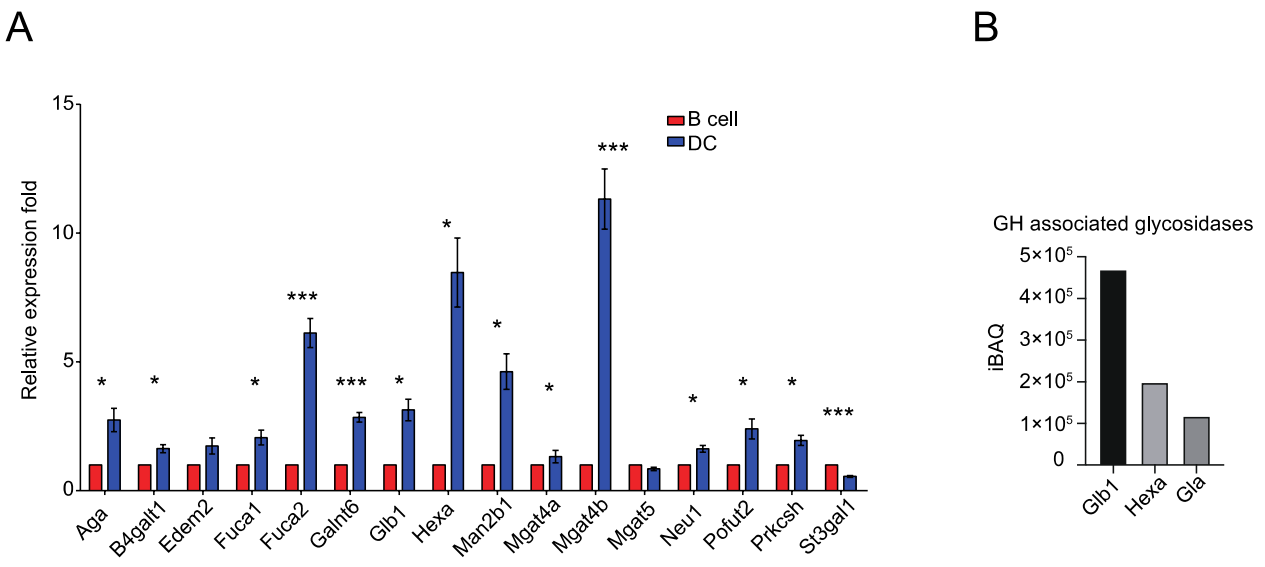
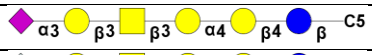
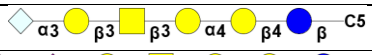
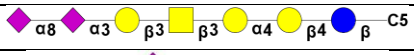
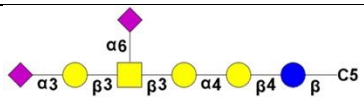
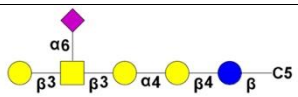
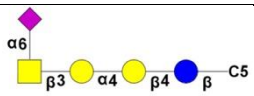
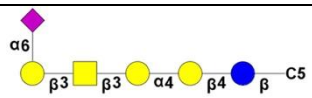
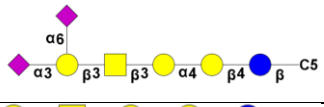
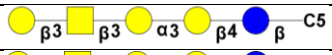
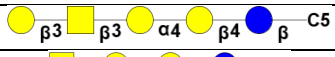


Figure S7. Glycosidase gene expression or association with GH glycan. (**a**) Glycosidase or glycosyl transferase gene expression in mouse splenic B cells or BMDCs. (**b**) Level of GH-associated glycosidases that may involve in GH glycan degradation. Aga, aspartylglucosaminidase; B4galt1, beta-1,4-galactosyltransferase; Edem2, ER degradation-enhancing alpha-mannosidase-like protein 2; Fuca1, Alpha-L-Fucosidase 1; Fuca2, Alpha-L-fucosidase 2; Galnt6, Polypeptide N-Acetylgalactosaminyltransferase 6; Glb1, beta-galactosidase; Hexa, beta-hexosaminidase A; Man2b1, Lysosomal alpha-mannosidase; Mgat4a, Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase A; Mgat4b, Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase B; Mgat5, Alpha-1,6-mannosylglycoprotein 6-beta-N-acetylglucosaminyltransferase A; Neu1, Neuraminidase-1; Pofut2, GDP-fucose protein O-fucosyltransferase 2; Prkcsh, Glucosidase 2 subunit beta; St3gal1, CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1.

Table S1. Glycan structure on the glycan array slide.

No.	Glycan	Molecular weight	Structure
1	SSEA-4	1245.5566	
2	SSEA4-Gc	1261.5515	
3	SASSEA4	1536.6520	
4	DSGb5	1536.6520	
5	SAGb5	1245.5566	
6	SAGb4	1084.0400	
7	2,6 SAGb5	1245.5566	
8	DSGG	1536.6520	
9	i-Gb5	954.4612	
10	Gb5	954.4612	
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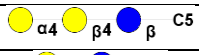
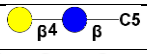
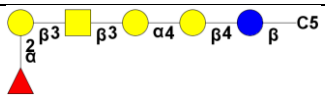
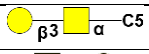
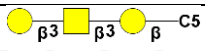
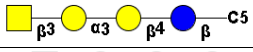
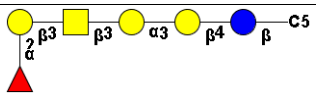
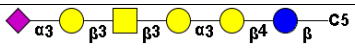
12	Gb3	589.3290	
13	Gb2	427.2762	
14	GH	1100.5191	
15	TF	468.3028	
16		630.3556	
17	i-Gb4	792.4084	
18	i-GH	1100.5191	
19	i-SSEA4	1245.5566	

297 **Table S2.** Plasma membrane proteins associated with GH

Rank	iBAQ*	Symbol	Entrez Gene Name	Type(s)
1	166930000	HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1	transmembrane receptor
2	30856000	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	other
3	29156000	HLA-DRB5	major histocompatibility complex, class II, DR beta 5	transmembrane receptor
4	15148000	HLA-DRA	major histocompatibility complex, class II, DR alpha	transmembrane receptor
5	8920600	B2M	beta-2-microglobulin	transmembrane receptor
6	5508100	HLA-A	major histocompatibility complex, class I, A	other
7	5170700	LGALS3BP	galectin 3 binding protein	transmembrane receptor
8	4389000	CD14	CD14 molecule	transmembrane receptor
9	4166800	Cdc42	cell division cycle 42	enzyme
10	3875500	CR1L	complement C3b/C4b receptor 1 like	other
11	3669400	HLA-DMA	major histocompatibility complex, class II, DM alpha	transmembrane receptor
12	3343000	LAMP1	lysosomal associated membrane protein 1	other
13	3164600	HLA-DMB	major histocompatibility complex, class II, DM beta	transmembrane receptor
14	3049900	IFITM3	interferon induced transmembrane protein 3	other
15	3041200	CD74	CD74 molecule	transmembrane receptor
16	2983100	CD63	CD63 molecule	other
17	2805100	ITGB2	integrin subunit beta 2	transmembrane receptor
18	2455700	CD47	CD47 molecule	transmembrane receptor
19	2432000	HLA-A	major histocompatibility complex, class I, A	other
20	2258800	GNAI2	G protein subunit alpha i2	enzyme
21	2222300	CD9	CD9 molecule	other
22	1988800	GPNMB	glycoprotein nmb	enzyme
23	1965800	ANPEP	alanyl aminopeptidase, membrane	peptidase
24	1896600	ICAM1	intercellular adhesion molecule 1	transmembrane receptor
25	1856200	ITGAM	integrin subunit alpha M	transmembrane receptor

26	1739100	LAMP2	lysosomal associated membrane protein 2	enzyme
27	1588200	RAP2B	RAP2B, member of RAS oncogene family	enzyme
28	1586400	PGRMC1	progesterone receptor membrane component 1	transmembrane receptor
29	1575900	ANXA2	annexin A2	other
30	1574200	STX7	syntaxin 7	transporter
31	1542800	CD36	CD36 molecule	transmembrane receptor
32	1523600	ALOX5AP	arachidonate 5-lipoxygenase activating protein	other
33	1419400	CD44	CD44 molecule (Indian blood group)	other
34	1404500	GNB2	G protein subunit beta 2	enzyme
35	1371500	CD68	CD68 molecule	other
36	1285100	TMEM14C	transmembrane protein 14C	other
37	1214000	LRPAP1	LDL receptor related protein associated protein 1	other
38	1209600	CCR7	C-C motif chemokine receptor 7	G-protein coupled receptor
39	1126400	LGALS3BP	galectin 3 binding protein	transmembrane receptor
40	1116200	MSN	moesin	other
41	1101300	CLTA	clathrin light chain A	other
42	1022900	PDCD1LG2	programmed cell death 1 ligand 2	enzyme
43	1000000	ACTR3	actin related protein 3	other
44	994390	TCIRG1	T cell immune regulator 1, ATPase H+ transporting V0 subunit a3	enzyme
45	989640	ACTR2	actin related protein 2	other
46	979890	GNG12	G protein subunit gamma 12	enzyme
47	929740	MLEC	malectin	other
48	890220	HLA-A	major histocompatibility complex, class I, A	other
49	882160	SLC9A3R1	SLC9A3 regulator 1	other
50	881210	SNAP23	synaptosome associated protein 23	transporter
51	819630	CD40	CD40 molecule	transmembrane receptor
52	806690	CD48	CD48 molecule	other

53	769270	ITGB1	integrin subunit beta 1	transmembrane receptor
54	727180	VAMP3	vesicle associated membrane protein 3	other
55	683350	CLEC10A	C-type lectin domain containing 10A	other
56	679460	Olfr1216	olfactory receptor 1216	G-protein coupled receptor
57	573080	ABHD12	abhydrolase domain containing 12	enzyme
58	567090	GNG2	G protein subunit gamma 2	enzyme
59	559340	SEC61G	SEC61 translocon gamma subunit	transporter
60	548020	GNG5	G protein subunit gamma 5	other
61	510680	VAMP8	vesicle associated membrane protein 8	transporter
62	486240	NCEH1	neutral cholesterol ester hydrolase 1	enzyme
63	481450	STX8	syntaxin 8	other
64	479280	VAT1	vesicle amine transport 1	transporter
65	469820	ANXA5	annexin A5	transporter
66	450530	SLC2A6	solute carrier family 2 member 6	transporter
67	432340	SCARB2	scavenger receptor class B member 2	transmembrane receptor
68	422580	Marcks	myristoylated alanine rich protein kinase C substrate	other
69	403780	LEPROT	leptin receptor overlapping transcript	other
70	397840	PTPRC	protein tyrosine phosphatase receptor type C	phosphatase
71	390820	NCSTN	nicastrin	peptidase
72	381760	CNPY2	canopy FGF signaling regulator 2	other
73	374340	PILRA	paired immunoglobin like type 2 receptor alpha	other
74	371510	PLSCR1	phospholipid scramblase 1	enzyme
75	350990	ATP1B3	ATPase Na+/K+ transporting subunit beta 3	transporter
76	349510	VAPA	VAMP associated protein A	other
77	331510	ITGA5	integrin subunit alpha 5	transmembrane receptor
78	327610	SIRPA	signal regulatory protein alpha	phosphatase
79	317950	NTRK2	neurotrophic receptor tyrosine kinase 2	kinase

80	316060	CD81	CD81 molecule	other
81	296840	STOM	stomatin	other
82	275340	DNAJC5	DnaJ heat shock protein family (Hsp40) member C5	other
83	272430	ATP1A1	ATPase Na+/K+ transporting subunit alpha 1	transporter
84	261980	SLC3A2	solute carrier family 3 member 2	transporter
85	258750	KRTCAP2	keratinocyte associated protein 2	enzyme
86	255590	ARL8B	ADP ribosylation factor like GTPase 8B	enzyme
87	244080	STOML2	stomatin like 2	other
88	238470	ATP6V1A	ATPase H+ transporting V1 subunit A	transporter
89	234200	DPP4	dipeptidyl peptidase 4	peptidase
90	232480	LY6E	lymphocyte antigen 6 family member E	other
91	229670	SIGMAR1	sigma non-opioid intracellular receptor 1	transmembrane receptor
92	226520	LAMTOR1	late endosomal/lysosomal adaptor, MAPK and MTOR activator 1	other
93	219350	Bst2	bone marrow stromal cell antigen 2	other
94	219130	EMB	embigin	other
95	214400	KCTD12	potassium channel tetramerization domain containing 12	ion channel
96	211430	L1CAM	L1 cell adhesion molecule	other
97	199860	HOMER1	homer scaffold protein 1	other
98	195390	RRAS2	RAS related 2	enzyme
99	193870	STT3A	STT3 oligosaccharyltransferase complex catalytic subunit A	enzyme
100	192430	SYPL1	synaptophysin like 1	transporter
101	186520	P2RX4	purinergic receptor P2X 4	ion channel
102	185640	SIRPA	signal regulatory protein alpha	phosphatase
103	172070	CD180	CD180 molecule	other
104	170800	TMEM165	transmembrane protein 165	other
105	170360	CLTC	clathrin heavy chain	other
106	162370	GNB1	G protein subunit beta 1	enzyme

107	159730	TSPAN31	tetraspanin 31	other
108	158110	ALCAM	activated leukocyte cell adhesion molecule	other
109	150340	ITM2B	integral membrane protein 2B	other
110	142010	SYPL1	synaptophysin like 1	transporter
111	141470	CD84	CD84 molecule	other
112	140680	TBXAS1	thromboxane A synthase 1	enzyme
113	130290	TNFRSF9	TNF receptor superfamily member 9	transmembrane receptor
114	128620	ANXA4	annexin A4	other
115	127540	TSPAN13	tetraspanin 13	other
116	126580	BST1	bone marrow stromal cell antigen 1	enzyme
117	122200	DEGS1	delta 4-desaturase, sphingolipid 1	enzyme
118	121090	Ap2b1	adaptor-related protein complex 2, beta 1 subunit	other
119	121050	EPS15L1	epidermal growth factor receptor pathway substrate 15 like 1	other
120	120450	PDGFRB	platelet derived growth factor receptor beta	kinase
121	109460	CD80	CD80 molecule	transmembrane receptor
122	106680	TM9SF2	transmembrane 9 superfamily member 2	transporter
123	105200	Nptn	neuroplastin	other
124	99945	LY75	lymphocyte antigen 75	transmembrane receptor
125	88045	PPIL1	peptidylprolyl isomerase like 1	enzyme
126	86164	Clec2d	C-type lectin domain family 2, member d	transmembrane receptor
127	84999	FCGRT	Fc fragment of IgG receptor and transporter	transmembrane receptor
128	81611	SLC18A2	solute carrier family 18 member A2	transporter
129	79915	LRP1	LDL receptor related protein 1	transmembrane receptor
130	76473	SLC2A1	solute carrier family 2 member 1	transporter
131	76140	RAB8A	RAB8A, member RAS oncogene family	enzyme
132	75529	ITGAL	integrin subunit alpha L	transmembrane receptor
133	74806	FXYD5	FXYD domain containing ion transport regulator 5	ion channel

134	67733	CD83	CD83 molecule	transmembrane receptor
135	67191	LPCAT3	lysophosphatidylcholine acyltransferase 3	enzyme
136	67171	FPR2	formyl peptide receptor 2	G-protein coupled receptor
137	66583	CLEC6A	C-type lectin domain containing 6A	transmembrane receptor
138	66228	TRPV2	transient receptor potential cation channel subfamily V member 2	ion channel
139	61605	TM6SF1	transmembrane 6 superfamily member 1	other
140	59298	ADPGK	ADP dependent glucokinase	kinase
141	57972	ITGA1	integrin subunit alpha 1	other
142	55463	OSBPL8	oxysterol binding protein like 8	transporter
143	52147	FCGR1A	Fc fragment of IgG receptor Ia	transmembrane receptor
144	48667	ANXA1	annexin A1	enzyme
145	48326	SLC7A2	solute carrier family 7 member 2	transporter
146	47464	MPC2	mitochondrial pyruvate carrier 2	other
147	45540	SLC29A3	solute carrier family 29-member 3	transporter
148	41915	VTI1B	vesicle transport through interaction with t-SNAREs 1B	transporter
149	41871	PCDHA3	protocadherin alpha 3	other
150	40611	ATP2B1	ATPase plasma membrane Ca2+ transporting 1	transporter
151	40164	FCGR2A	Fc fragment of IgG receptor IIa	transmembrane receptor
152	36524	CD274	CD274 molecule	enzyme
153	36395	SLAMF7	SLAM family member 7	other
154	36027	SLC39A4	solute carrier family 39-member 4	transporter
155	35145	RAB4B	RAB4B, member RAS oncogene family	enzyme
156	34836	VTI1A	vesicle transport through interaction with t-SNAREs 1A	transporter
157	34523	STX4	syntaxin 4	transporter
158	33658	EMC1	ER membrane protein complex subunit 1	other
159	33604	CAP1	cyclase associated actin cytoskeleton regulatory protein 1	other
160	32886	ITGAX	integrin subunit alpha X	transmembrane receptor

161	32323	CSF1R	colony stimulating factor 1 receptor	kinase
162	31810	RFTN1	raftlin, lipid raft linker 1	other
163	31340	VASP	vasodilator stimulated phosphoprotein	other
164	30821	FKBP15	FKBP prolyl isomerase 15	enzyme
165	29756	Olfr1118	olfactory receptor 1118	G-protein coupled receptor
166	29496	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1	enzyme
167	29088	FAS	Fas cell surface death receptor	transmembrane receptor
168	28066	CD200R1	CD200 receptor 1	transmembrane receptor
169	26624	LILRB3	leukocyte immunoglobulin like receptor B3	transmembrane receptor
170	26380	SLC12A9	solute carrier family 12-member 9	transporter
171	24260	ADGRE5	adhesion G protein-coupled receptor E5	G-protein coupled receptor
172	23188	TNFRSF1B	TNF receptor superfamily member 1B	transmembrane receptor
173	22186	DNM2	dynamin 2	enzyme
174	21855	ADAM17	ADAM metallopeptidase domain 17	peptidase
175	21526	MRC1	mannose receptor C-type 1	transmembrane receptor
176	19285	EHD4	EH domain containing 4	enzyme
177	18485	CLPTM1	CLPTM1 regulator of GABA type A receptor forward trafficking	other
178	17864	MPP1	membrane palmitoylated protein 1	kinase
179	17038	LOC102723996	inducible T cell costimulator ligand	other
180	16670	SEMA4A	semaphorin 4A	other
181	15756	ECE1	endothelin converting enzyme 1	peptidase
182	15581	SPTAN1	spectrin alpha, non-erythrocytic 1	other
183	15252	ABCD1	ATP binding cassette subfamily D member 1	transporter
184	15049	PNN	pinin, desmosome associated protein	other
185	14270	STIM1	stromal interaction molecule 1	ion channel
186	14225	PEAR1	platelet endothelial aggregation receptor 1	other
187	14165	CCR1	C-C motif chemokine receptor 1	G-protein coupled receptor

188	9575.4	TLR7	toll like receptor 7	transmembrane receptor
189	8507.9	SLC1A5	solute carrier family 1 member 5	transporter
190	7849.4	EPB41L2	erythrocyte membrane protein band 4.1 like 2	other
191	5567.4	PKD1	polycystin 1, transient receptor potential channel interacting	ion channel
192	5484.8	IGF2R	insulin like growth factor 2 receptor	transmembrane receptor
193	5237.4	CELSR3	cadherin EGF LAG seven-pass G-type receptor 3	G-protein coupled receptor
194	2060.2	UTRN	utrophin	transmembrane receptor

* iBAQ: intensity-based absolute quantification

Table S3. Fold increase of glycosidase related gene expression in BMDCs as compared to B cells.

Gene Symbol	Enzyme	Fold Increase
Aga	Aspartylglucosaminidase	7.3711
B3gnt3	Beta-1,3-N-Acetylglucosaminyltransferase 3	273.8825
B4galt1	Beta-1,4-galactosyltransferase 1	4.8574
B4galt2	Beta-1,4-Galactosyltransferase 2	6.8326
B4galt5	Beta-1,4-galactosyltransferase 5	5.2379
Edem2	ER degradation-enhancing alpha-mannosidase-like 2	7.0189
Fuca1	Alpha-L-Fucosidase 1	5.9906
Fuca2	Alpha-L-Fucosidase 2	8.1859
Galnt3	Polypeptide N-acetylgalactosaminyltransferase 3	119.1723
Galnt6	Polypeptide N-acetylgalactosaminyltransferase 6	16.5343
Galnt9	Polypeptide N-acetylgalactosaminyltransferase 9	1052.1387
Glb1	Beta-galactosidase	7.952
Hexa	Beta-hexosaminidase subunit alpha	23.5368
Man1c1	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IC	82.6274
Man2b1	Lysosomal alpha-mannosidase	6.1326
Mgat3	Beta-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase	5.9512
Mgat4a	Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase A	5.5296
Mgat4b	Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase B	123.8913
Mgat5	Alpha-1,6-mannosylglycoprotein 6-beta-N-acetylglucosaminyltransferase A	4.4901
Neu1	Neuraminidase 1	15.1744
Pofut2	GDP-fucose protein O-fucosyltransferase 2	6.0998
Prkcsh	Glucosidase 2 subunit beta	4.6331
St3gal1	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1	10.0218
St8sia2	Alpha-2,8-sialyltransferase 8B	5.088
Galntl5	N-acetylgalactosaminyltransferase-like protein 5	-5.0486
St8sia6	Alpha-2,8-sialyltransferase 8F	-73.7251

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