Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

One step carboxyl group isotopic labeling for quantitative analysis of intact N-glycopeptide by Mass Spectrometry

Zhenyu Sun[a], Guanghui Ji[b], Guoli Wang[a], Lei Wei[b], Ying Zhang[a,*], Haojie Lu[a,b*]

[a] Institutes of Biomedical Sciences and Shanghai Cancer Center, Fudan University. Shanghai 200032 (China).

[b] Department of Chemistry and NHC Key Laboratory of Glycoconjugates Research, Fudan University. Shanghai 200032 (China).

Corresponding Authors:

* Ying Zhang, Phone: (+) 86-21-54237618. Fax: (+) 86-21-54237961. E- mail: ying@fudan.edu.cn

* Haojie Lu, Phone: (+) 86-21-54237618. Fax: (+) 86-21-54237961. E- mail: luhaojie@fudan.edu.cn

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MATERIALS AND METHODS

Materials and Chemicals

Immunoglobulin G from human serum (IgG), (7-azabenzotriazol-1-yloxy) trispyrrolidinophosphonium hexafluorophosphate (PyAOP), 4-methylmorpholine, trifluoroacetic acid (TFA), methylamine hydrochloride (CH3NH2·HCl), methyl-d3-amine hydrochloride (CD3NH2·HCl), triethylammonium bicarbonate (TEAB), urea, thiourea, dithiothreitol (DTT) and iodoacetamide (IAA) were purchased from Sigma-Aldrich (St. Louis, MO). Phosphate-buffered saline (PBS, pH 7.2) was bought from Shanghai BasalMedia Technologies Co., Ltd. (Shanghai, China). Quick start Bradford 1× dye reagent was obtained from Bio-Rad Laboratories, Inc. HPLC-grade acetonitrile (ACN), methanol (MeOH) and hydrophilic interaction chromatography (HILIC) SPE column were purchased from Merck (Darmstadt, Germany). Sep-Pak C18 Vac cartridges were purchased from Waters (Massachusetts, USA). Analytical grade acetic acid (HAc) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Distilled water was purified by Milli-Q system (Milford, MA, USA). MS-grade Trypsin was purchased from Beijing Shengxia Proteins Scientific Ltd (Beijing, China). HepG2 cells and huh7 cells were provided by the Cell Resource Center of the Shanghai Institute for Biology Science, Chinese Academy of Science.

Cell Lysis and Trypsin Digestion

HepG2 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (Gibco, Invitrogen), 10^5 U/L penicillin, 100 mg/L streptomycin, 2 mM glutamine, 25 mM HEPES solution, and 1 mM sodium pyruvate. Cells were cultured in 10 cm culture dishes placed in a humidified incubator maintained at 37 °C and 5% CO_2. Cells were grown to approximately 80% confluence and collected. Collected cells were washed twice with PBS to remove residual cell culture medium and then lysed in a buffer containing 7 M urea, 2 M thiourea, and protease inhibitors. Lysates were sonicated on ice for 5 min and centrifuged at 20,000 × g for 30 min at 4 °C to collect the supernatant. Protein concentration was measured using the Bradford assay. Proteins were reduced with 10 mM dithiothreitol (DTT) for 30 min at 37 °C and subsequently alkylated by 20 mM iodoacetamide for 30 min at 25 °C in the dark. After adding with 5-fold volume cold acetone, proteins were incubated at -20 °C overnight, and then centrifuged at 17,000 × g for 30 min to collect the precipitate. The collected lysates were then dissolved with 100 mM TEAB, and digested overnight at 37 °C with trypsin at an enzyme to substrate ratio of 1:50. Protease digestion was quenched by adding TFA to a final concentration of 0.5%. And the digests were subsequently desalted using a Sep-Pak C18 SPE cartridge. Desalted peptides were lyophilized in vacuum and stored at -80 °C for further use. Huh7 and ZIKV infected cells were provided as a generous gift from Dr. Jian Chen at the Shanghai Public Health Clinical Center. Huh7 were cultured in DMEM (Gibco) supplemented with 10% FBS (Gibco), 100 IU mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin and maintained at 37 °C in a fully humidified atmosphere containing 5% CO_2. Prior to ZIKV infection, huh7 cells were seeded in 150 mm plates (1.5×10^7 cells per well). Twenty-four

hours after seeding, the cells were rinsed once with phosphate-buffered saline (PBS) and then incubated with ZIKV at the indicated multiplicity of infection (MOI=2) in serum-free medium for 1 h at 37°C. The ZIKV-containing medium was then replaced with fresh DMEM supplemented with 2% FBS. Cells were collected 24 hours after infection or mock treated and proceeded with the same protocol described above.

Isotopic Labeling IgG and Cell samples with Methylamine

Our previous experience of glycan methylamidation was extendedly applied to label the intact N-glycopeptides in this work. [1] The dried peptides were dissolved in 5 μL of DMSO containing 5 M methylamine hydrochloride or methyl-d3-amine hydrochloride. Then 5μL of PyAOP (250 mM in 30% 4-methylmorpholine/DMSO) was added. The reaction proceeded at room temperature with constant shaking for 1 hour. The derivatized glycopeptides were enriched using HILIC and lyophilized for further analysis.

Intact N-glycopeptides purification using ZIC-HILIC micro-column

The purification was performed according to previous literature with minor modification. [2] The reaction solution was diluted with 1 mL 80% ACN containing 1% TFA. then the N-glycopeptides were enriched using in-house ZIC-HILIC micro-column. Briefly, 30 mg of cellulose microcrystalline particles was applied to a 200 μL pipet tip. Then, the tip was set on a 2.0 mL Eppendorf tube for centrifugation. Before loading samples, the HILIC stationary phases were prewashed using 200 μL of 0.1% TFA for three times, followed by activation using 200 μL of 80% ACN 1% TFA for three times. The samples were loaded to the tips, subsequently washed using 200 μL 80% ACN 1% TFA for six times. Finally, N-glycopeptides were eluted using 100 μL of 0.1%TFA for three times, followed by lyophilization step for further analysis.

MALDI-TOF MS and Analysis

One microliter of sample and the same volume of CHCA matrix solution (5 mg/mL in 50% ACN containing 0.1% TFA) were spotted on the MALDI plate for MS analysis. The MALDI-MS spectra were acquired using 5800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) equipped with a Nd: YAG laser (355 nm), an acceleration voltage of 20 kV and a repetition rate of 400 Hz. The spectrometer was operated in positive reflection mode accumulated by 1000 laser shots. For the analysis of intact N-glycopeptides from IgG and cells, the assays were performed on an EASYnLC 1000 system (Thermo Fisher Scientific, Waltham, MA) connected to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an online nano electrospray ion source. The parameters of LC-MS in each section were listed in the Supporting Information.

LC-MS/MS Analysis

To test the compatibility of labeling method with ESI-based instruments, IgG digest was labeled by light and heavy methylamine, and then mixed equally prior to MS analysis. The samples were resuspended with solvent A (water with 0.1% formic acid), separated by nano LC, and analyzed by online electrospray tandem mass spectrometry. The experiments were performed on an EASYnLC 1000 system (Thermo Fisher Scientific, Waltham, MA) connected to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an online nano electrospray ion source. Glycopeptides were loaded onto the analytical column (Acclaim PepMap C18, 75 μm×50 cm) and subsequently separated with a linear gradient, Solvent A was 0.1% formic acid in water. Solvent B was 80% acetonitrile with 0.1% formic acid. The gradient was 1 h in total for the glycopeptide from IgG samples: 2-40% from 0 to 45 min, 40-60% from 46 min to 54 min, followed by an increase to 100% in 1 minute, and held for 100% B for the last 5 min. The column flow rate was maintained at 200 nL/min. The parameters for glycopeptides analysis was:

(1) MS: scan range (m/z) = 800-2000; resolution = 120,000; AGC target = 200,000; maximum injection time = 100 ms; included charge state = 2-8; dynamic exclusion after n times, n = 1; dynamic exclusion duration = 15 s; each selected precursor was subject to one HCD-MS/MS; (2) HCD-MS/MS: isolation window = 2; detector type = Orbitrap; resolution = 15,000; AGC target = 500,000; maximum injection time = 250 ms; collision energy = 30%; stepped collision mode on, energy difference of ± 10% (10% as absolute value in the Orbitrap Fusion). To test the feasibility of our strategy, we analyzed the glycopeptide from cells. The gradient was 4 h in total for samples: 2-4% from 0 to 20 min, 4-25% from 21 to 190 min, 25-45% from 191 to 220 min followed by an increase to 90% B from 221 to 226 min, held for another 2 min and held for 2% B for the last 10 min. other parameters followed above parameters.

Data Analysis

All mass spectrometric data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX partner repository with the dataset identifier PXD021104. [3] (https://www.iprox.org/page/PSV023.html;?url=1599120575578gasR, password: sf5W) XCalibur 3.0 was used for data processing. Raw data was used directly without any further processing for N-glycopeptide identification using Byonic (Protein Metrics, San Carlos, CA). The following parameters were used for IgG search: Trypsin was selected as enzyme. The search was performed using the following parameters: (1) fixed modification, carbamidomethylamidation of C; (2) dynamic modifications, oxidation of M, and methylamine modifications of D, E and C-terminal of peptides; pyro-Glu conversion of N-terminal E (3) maximum missed cleavages, 0; (4) precursor ion mass tolerance 10 ppm; (5) fragment ion mass tolerance 20 ppm. The IgG glycan database was summarized from previous reports. [2] (Detailed information is in Table S2) The protein databases contain all four IgG subclasses: IgG1 (UniProt ID, P01857), IgG2 (P01859), IgG3 (P01860) and IgG4 (P01861). For complex sample, the parameters were the same except maximum missed cleavage was set to 2. A human N-glycan database (from Byonic database) containing 182 human N-glycans was employed. The Homo sapiens proteome database was downloaded from UniProtKB on 15 Jul. 2019, containing 20431 reviewed protein sequences. In addition, The N-glycopeptides identified were filtered to 1% FDR and Byonic score $\geqslant$100. [4] Further validation was performed manually. The manual check criteria include the retention time, the presence of oxonium ions, e.g., m/z 204.09 for HexNAc, 512.20 for HexHexNAcFuc, and 657.23 for HexHexNAcNeuAc. [5] K-nearest neighbor (k-NN) imputation was applied to impute the missing values. Before missing value imputation, peptides having more than 50% missing data were excluded to ensure that each sample hand enough data for imputation. The imputation method was implemented in the DMwR (version 0.4.1) package in R. [6] *p*-value was calculated using t-test. [7] The fold-change cutoff of >2 was chosen according to the reported isotopic labeling approach for intact N-glycopeptide quantitation. [8]

RESULTS AND DISCUSSION

Methylamidation converted the N-terminal Glu residue to pyro-Glu

Before labeling, native intact N-glycopeptide IgG1-H4N4F1 (EEQFNSTFR with the glycan composition of Hex4HexNAc4Fuc1) was detected as [M + H]+ at m/z 2764.11. (Fig 1a) This glycopeptide possesses three carboxyl groups, with two carboxyl groups on the side chain carboxyl groups of peptides (Glu), and one at the C-terminal. After the amidation reaction, the mass of this glycopeptide increased 8.051 Da (13.031 × 2 – 18.011 Da), which was detected as 7.87 Da. (Fig 1b) It was noted that the methylamidation converted the N-terminal Glu residue to pyro-Glu by losing H_2O, resulting a mass decreases of 18 Da. According to previous report, the pyro-Glu occurred for glycopepides were complete. [9] After labeling, the glycopeptide was detected as [M + H]+ at m/z 2771.98 while unlabeled glycopeptides were not detected, indicating 100% efficiency of methylamidation reaction. In addition, the mass of glutamine and the methylamination product of aspartic acid are very close, but they can be easily distinguished in this study because the introduction of heavy methylamine generated paired peaks for aspartic acid after being labeled with light and heavy methylamine.

Function of the differentially expressed glycoproteins after ZIKV infection

αvβ3 integrin is a cellular receptor common to neuraminidase-sensitive and neuraminidase-resistant rotaviruses and it could determine the cellular susceptibility to rotaviruses. [10] We quantified that integrin subunit alpha V (ITGAV) was down-regulated after ZIKV infected. Lysosomal associated membrane protein 1 (LAMP1) and lysosomal associated membrane protein 2 (LAMP2) were mainly distributed in the lysosomes and late endosomes. The role of these were known to maintain the lysosomal acidification and lysosomal membrane integrity. [11] [12] We found that LAMP1 and LAMP2 were up-regulated in ZIKV infected cells. In addition, LAMP1 and LAMP2 were also reported to be associated with autopha-gosome accumulation andbiogenesis. [13] Dengue virus-2 (DV2) can trigger autophagic process of huh7 cells and facilitate the viral replication by activating the autophagic machinery. [14] Down-regulation was also observed form other glycoproteins including CD63 molecule (CD63) and palmitoyl-protein thioesterase 1 (PPT1). CD63, a late endosomal marker protein, recently was described as a key factor in extracellular vesicle (EV) production and endo-somal cargo sorting. HSV-1 infection causes a decrease in the amount of intracellular CD63 protein with a concomi-tant increase in extracellular CD63. CD63 negatively impacts HSV-1 infection, and that the CD63-positive EVs could control the dissemination of the virus in the host. [15] The N-glycosylation on N197 and N232 of PPT1 is essential for its activity and intracellular transport, and both sites are needed for correct lysosomal targeting of PPT1. [16]

Supporting Figures

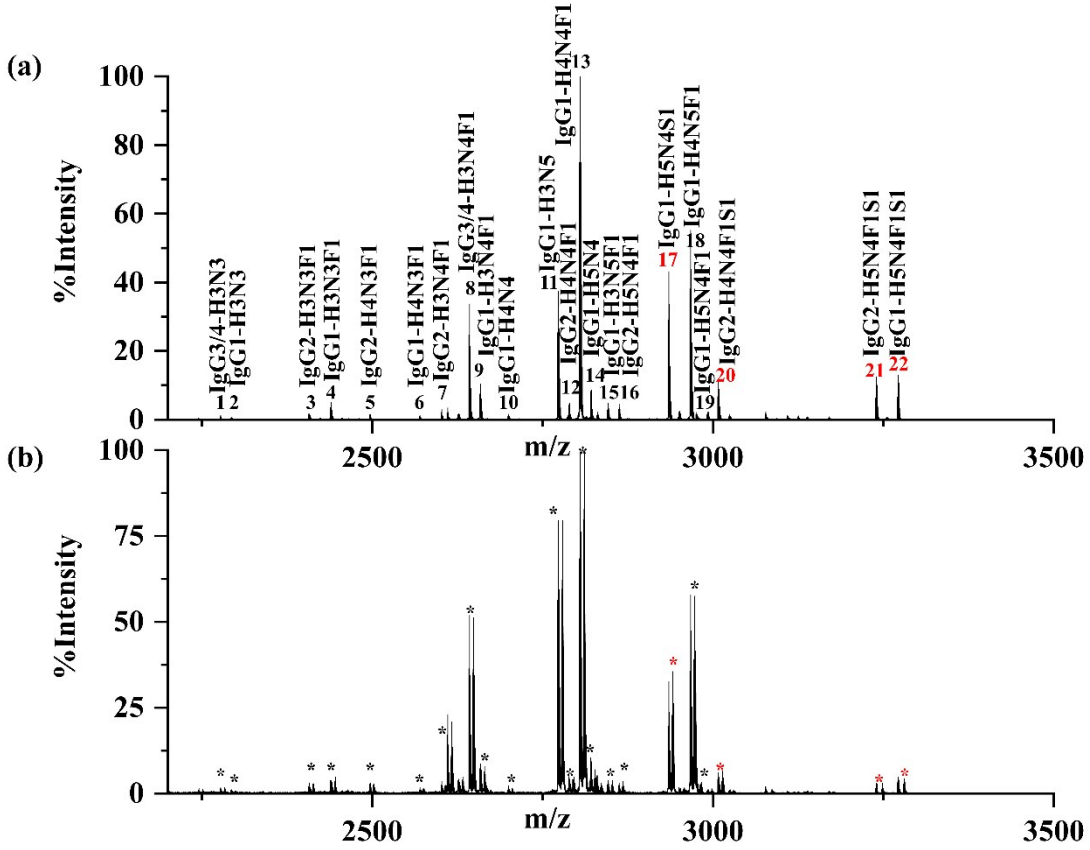

Fig. S1 MALDI-TOF mass spectra of intact N-glycopeptides from human IgG. (a) Methylamidation with light labeling regent. (b) Derivatized with light/heavy methylamine and mixed in a molar ratio of 1:1; Numbers represent intact N-glycopeptides. Their detailed sequences are listed in Table S1. The light and heavy methylamine labeled N-glycopeptides of IgG were marked as black asterisks and the sialylated glycopeptides were marked with red asterisks.

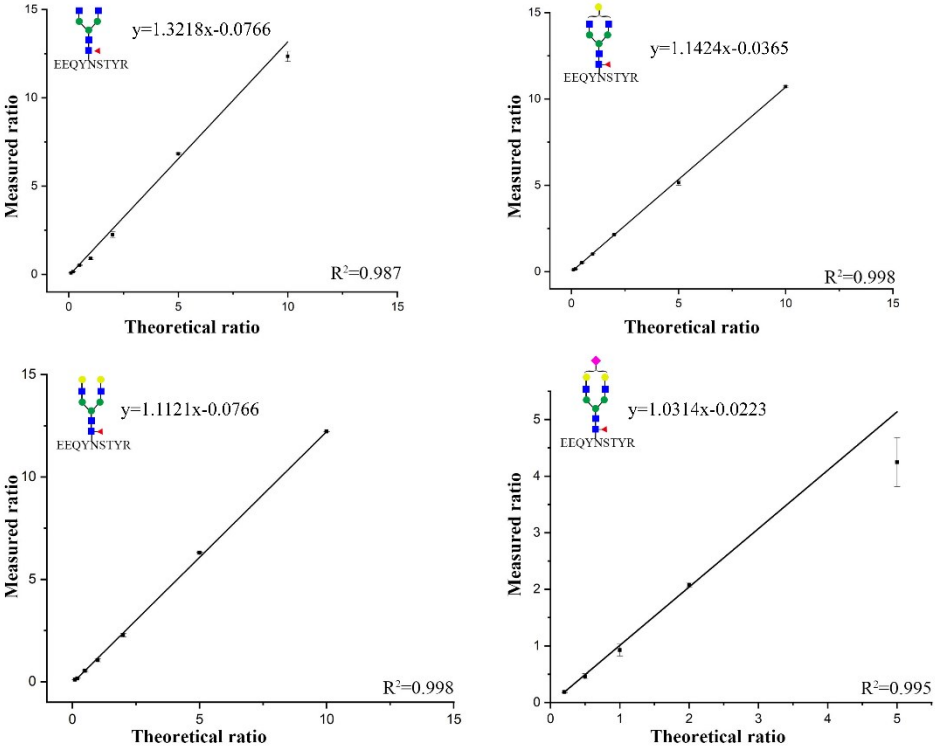

Fig. S2 Plots of the theoretical molar ratios vs the measured ratios of each intact N-glycopeptide from IgG.

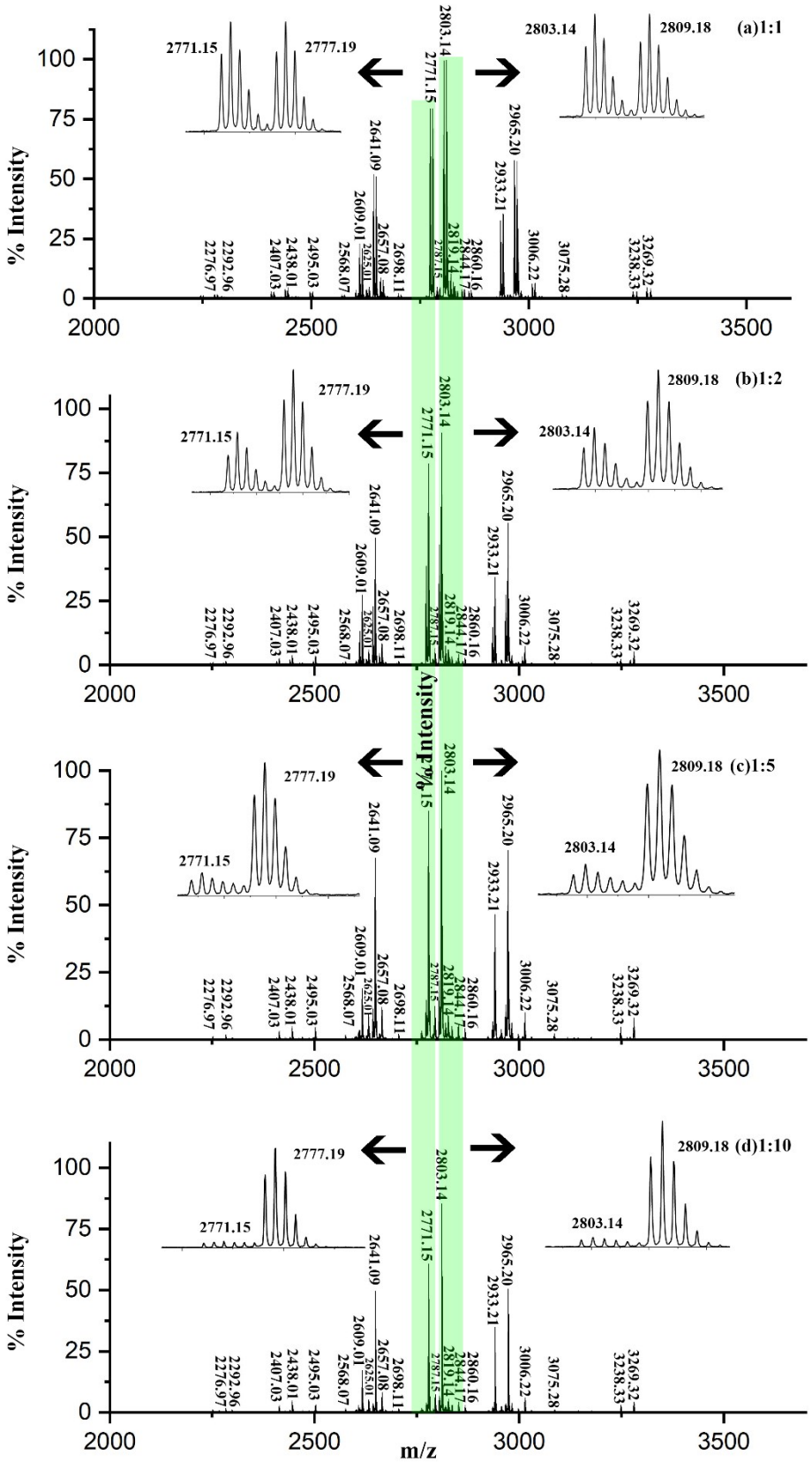

Fig. S3 MALDI-MS spectra of two N-glycopeptides (IgG1- and IgG2- H4N4F1) from human IgG derivatized with light/heavy methylamine, stored at −20 °C for six months and mixed in different molar ratios: (a)1:1, (b) 1:2, (c) 1:5, (d) 1:10.

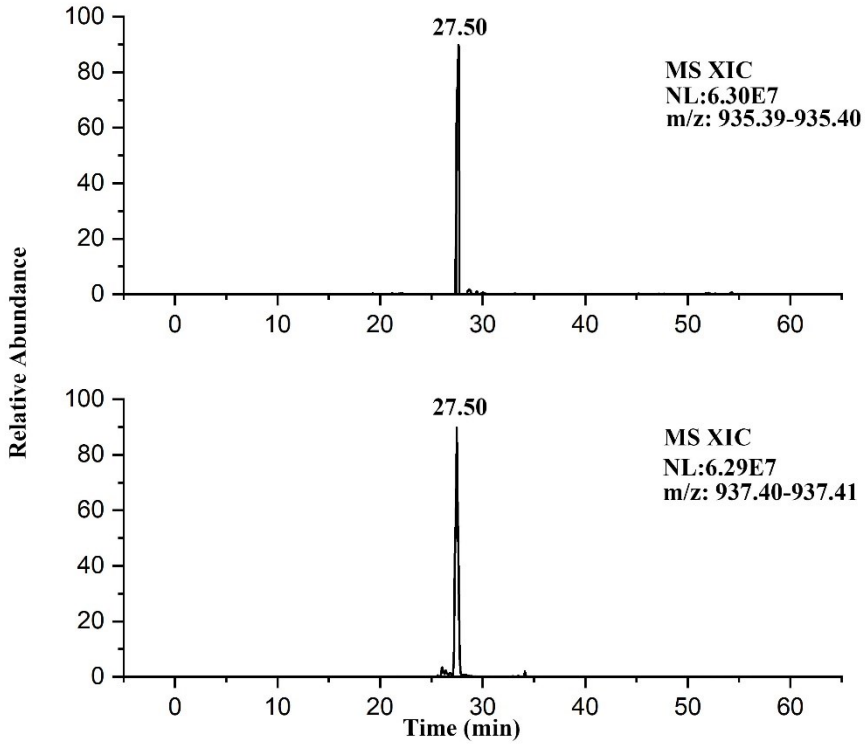

Fig. S4 The chromatographic behavior of glycopeptides IgG1-H4N4F1 (reacted with CH3NH2 or CD3NH2). The light and heavy labeled IgG1-H4N4F1 co-elute at the retention time of 27.5 min.

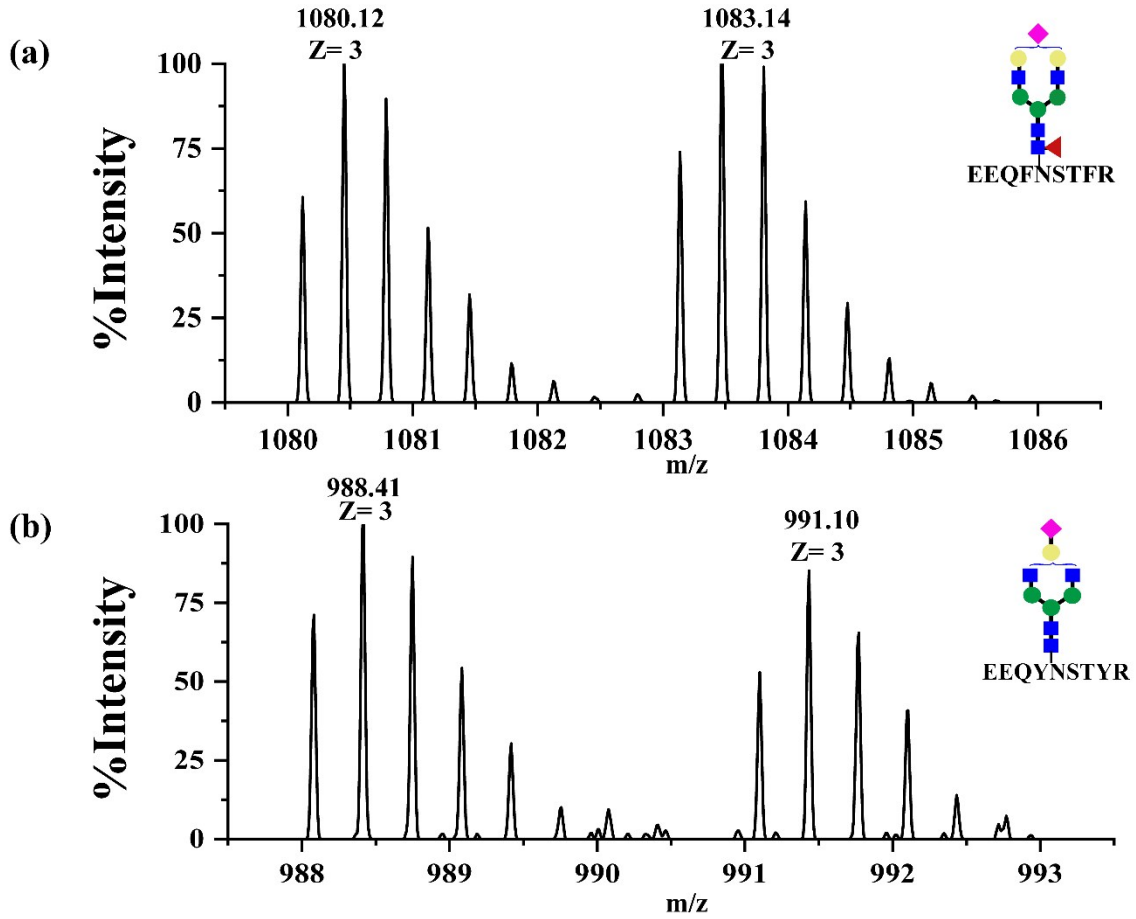

Fig. S5. Dual peaks in mass spectra of intact N-glycopeptides from IgG after processing by the MeSIL method and analyzed by LC-MS/MS. (a) The spectrum was assigned to the EEQFNSTFR with the N-glycan composition of H5N4F1S1. (b) The spectrum was assigned to the EEQFNSTFR with the N-glycan composition of H4N4S1.

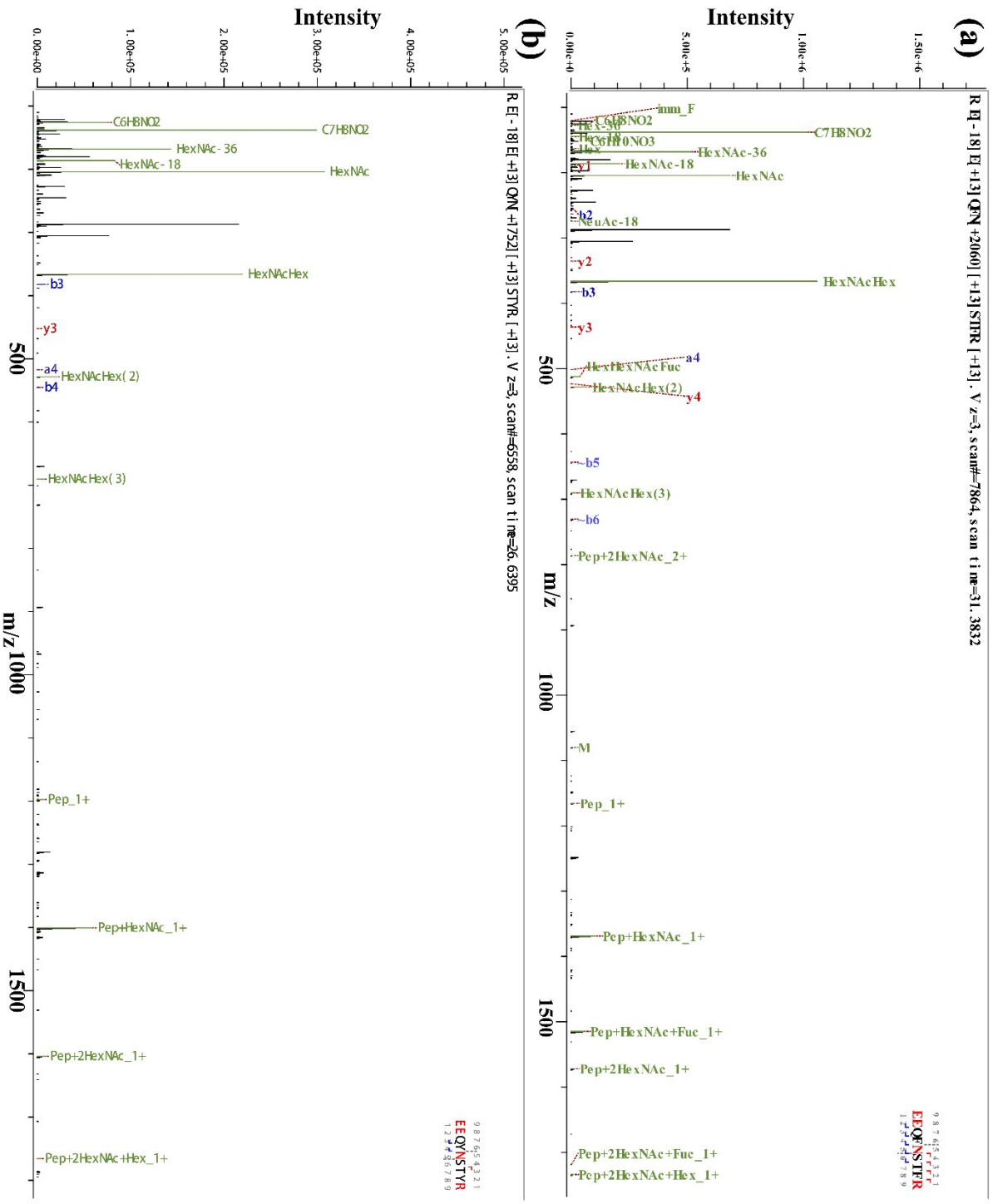

Fig. S6 (a) MS2 spectrum of precursor m/z = 1080.12 at 31.3 min. This spectrum was assigned to the EEQFNSTFR with N-glycan composition of H5N4F1S1. (b) MS2 spectrum of precursor m/z = 988.07 at 16.8 min was assigned to the EEQFNSTFR, which was modified by N-glycan with a composition of H4N4S1.

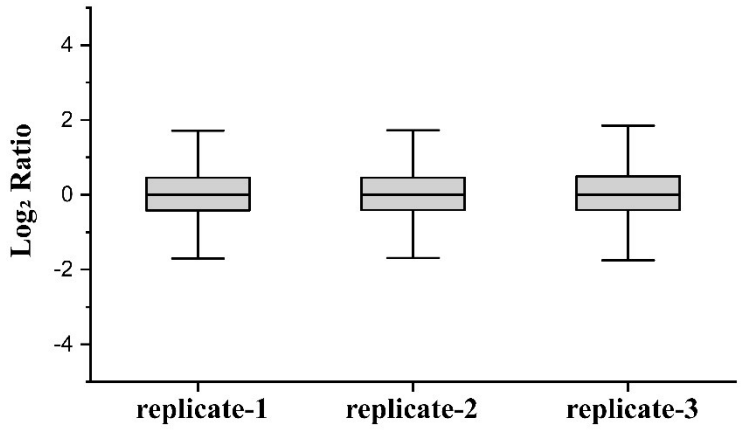

Fig. S7 Box plots displaying the XIC intensity distribution of quantified glycopeptides from HepG2 cells using the MeSIL strategy with proportions of 1:1.

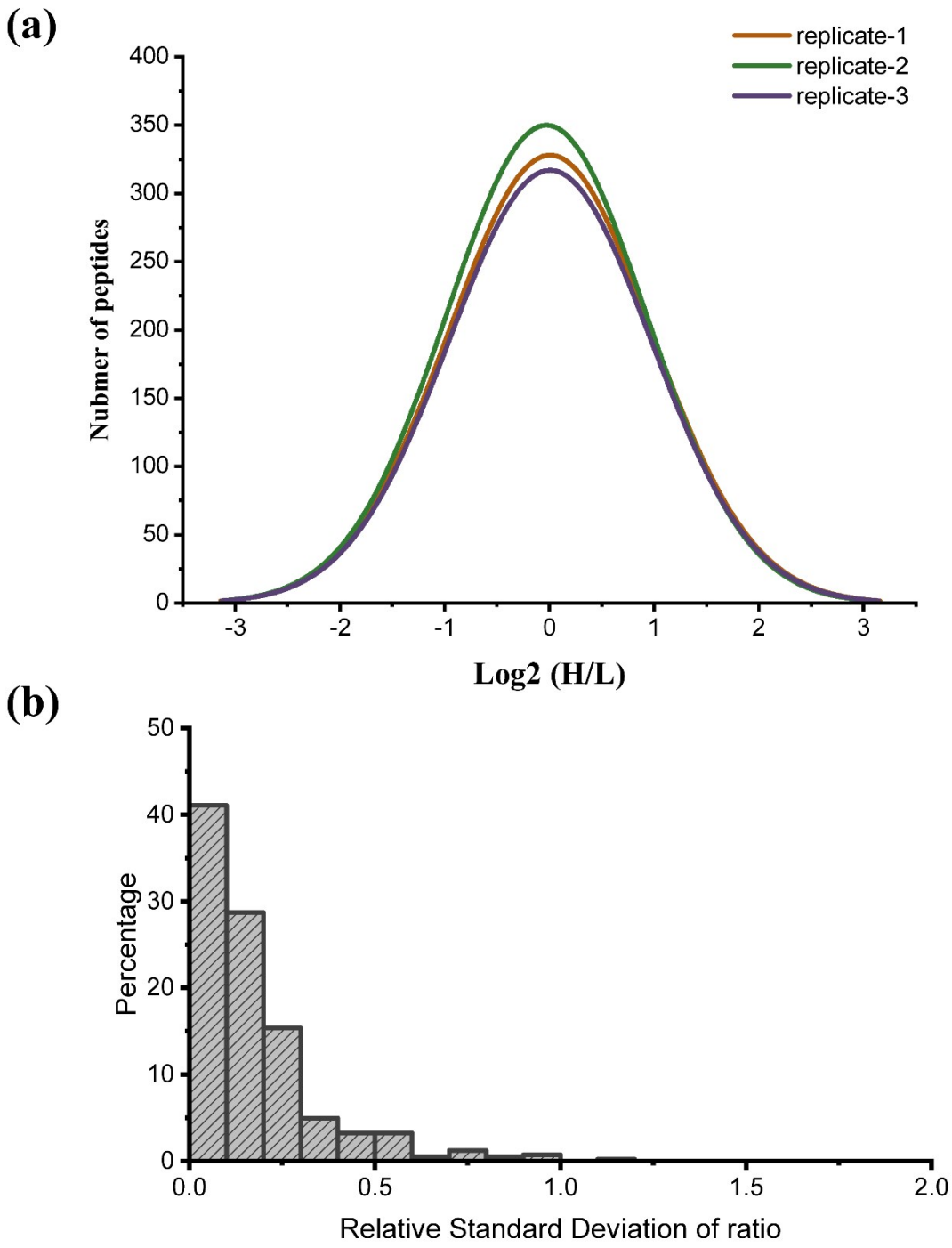

Fig. S8 HepG2 peptides were mixed at 1:1 ratio. (a) Histograms of quantified glycopeptide ratios from three replicates. (b) Histograms demonstrated the distribution of standard deviation from MeSIL strategy.

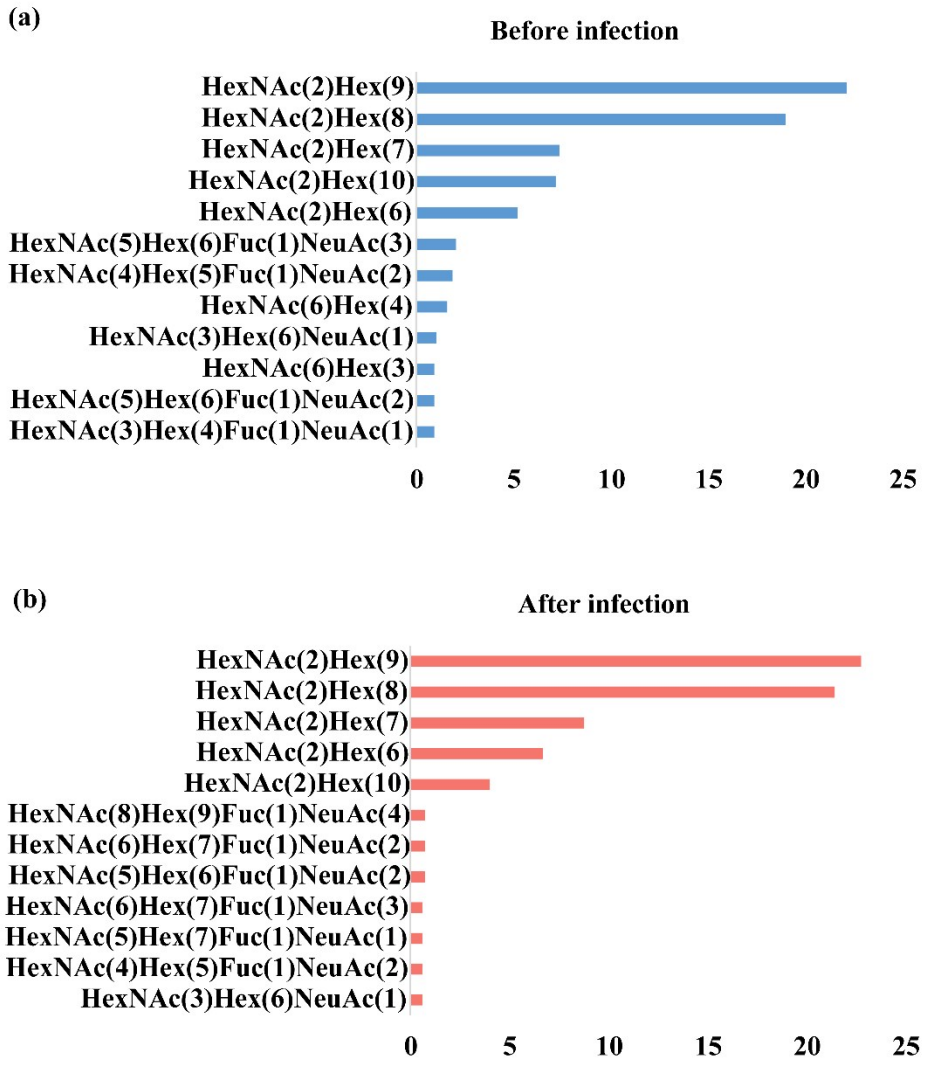

Fig. S9 Frequency distribution of the top 10 abundance N-glycopeptides in cells. (a) N-glycopeptides in huh7 cells (b) N-glycopeptides in ZIKV infected huh7 cells.

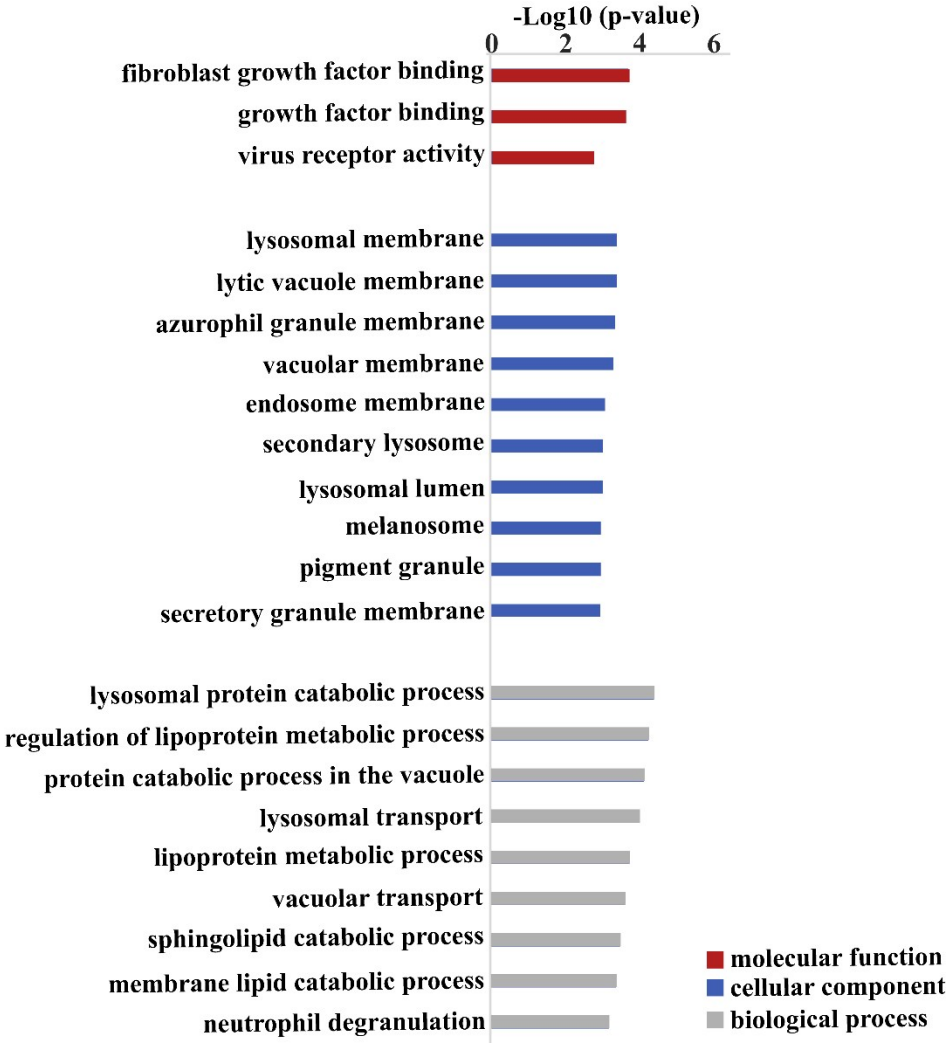

Fig. S10 GO analysis of differentially glycopeptides acquired by MeSIL strategy in terms of (a) molecular function, (b) cellular component, (c) biological process.

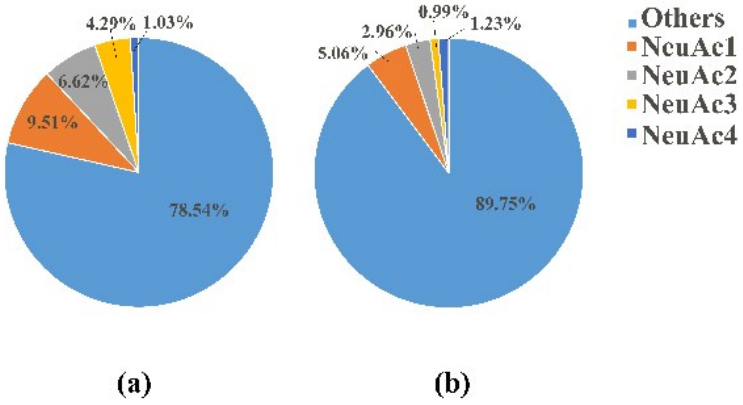

Fig. S11 Distribution of N-glycopeptides within different N-glycan subcategories. (a) N-glycopeptides identified in huh7 cells. (b) N-glycopeptides identified in ZIKV infected huh7 cells.

Supporting Tabels

Table S1 Detailed information of the glycopeptides derivated by MeSIL from an IgG tryptic digest by MALDI-TOF. N# denotes the N-linked glycosylation site.

No.	m/z	Glycan composition	Peptides sequence
1	2276.97	HexNAc(3)Hex(3)	EEQFN#STYR, or EEQFN#STFR
2	2292.96	HexNAc(3)Hex(3)	EEQYN#STYR
3	2407.03	HexNAc(3)Hex(3)Fuc(1)	EEQFN#STFR
4	2439.01	HexNAc(3)Hex(3)Fuc(1)	EEQYN#STYR
5	2495.03	HexNAc(3)Hex(4)Fuc(1)	EEQFN#STFR
6	2569.08	HexNAc(3)Hex(4)Fuc(1)	EEQYN#STYR
7	2610.11	HexNAc(4)Hex(3)Fuc(1)	EEQFN#STFR
8	2626.10	HexNAc(4)Hex(3)Fuc(1)	EEQFN#STYR, or EEQFN#STFR
9	2642.09	HexNAc(4)Hex(3)Fuc(1)	EEQYN#STYR
10	2658.09	HexNAc(4)Hex(4)	EEQYN#STYR
11	2699.11	HexNAc(5)Hex(3)	EEQYN#STYR
12	2772.16	HexNAc(4)Hex(4)Fuc(1)	EEQFN#STFR
13	2804.14	HexNAc(4)Hex(4)Fuc(1)	EEQYN#STYR
14	2820.15	HexNAc(4)Hex(5)	EEQYN#STYR
15	2845.18	HexNAc(5)Hex(3)Fuc(1)	EEQYN#STYR
16	2861.17	HexNAc(4)Hex(5)Fuc(1)	EEQFN#STFR
17	2934.21	HexNAc(4)Hex(5)NeuAc(1)	EEQYN#STYR
18	2966.20	HexNAc(4)Hex(5)Fuc(1)	EEQYN#STYR
19	3007.23	HexNAc(5)Hex(4)Fuc(1)	EEQYN#STYR
20	3075.28	HexNAc(4)Hex(4)Fuc(1)NeuAc(1)	EEQFN#STFR
21	3238.33	HexNAc(4)Hex(5)Fuc(1)NeuAc(1)	EEQFN#STFR
22	3270.33	HexNAc(4)Hex(5)Fuc(1)NeuAc(1)	EEQYN#STYR

Table S2 The N-glycan database of IgG.

No.	Glycan composition
1	HexNAc(10)Hex(11) @ NGlycan | common1
2	HexNAc(2)Hex(11) @ NGlycan | common1
3	HexNAc(2)Hex(12) @ NGlycan | common1
4	HexNAc(2)Hex(3) @ NGlycan | common1
5	HexNAc(2)Hex(3)Fuc(1) @ NGlycan | common1
6	HexNAc(2)Hex(4) @ NGlycan | common1
7	HexNAc(2)Hex(5) @ NGlycan | common1
8	HexNAc(2)Hex(6) @ NGlycan | common1
9	HexNAc(2)Hex(7) @ NGlycan | common1
10	HexNAc(2)Hex(8) @ NGlycan | common1
11	HexNAc(2)Hex(9) @ NGlycan | common1
12	HexNAc(2)Hex(10) @ NGlycan | common1
13	HexNAc(3)Hex(3)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
14	HexNAc(3)Hex(3) @ NGlycan | common1
15	HexNAc(3)Hex(3)Fuc(1) @ NGlycan | common1
16	HexNAc(3)Hex(3)Fuc(1)NeuAc(2) (26.062) @ NGlycan | common1
17	HexNAc(3)Hex(3)NeuAc(1) @ NGlycan | common1
18	HexNAc(3)Hex(4) @ NGlycan | common1
19	HexNAc(3)Hex(4)Fuc(1) @ NGlycan | common1
20	HexNAc(3)Hex(4)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
21	HexNAc(3)Hex(4)Fuc(2) @ NGlycan | common1
22	HexNAc(3)Hex(4)NeuAc(1) (13.031) @ NGlycan | common1
23	HexNAc(3)Hex(4)NeuAc(2) (26.062) @ NGlycan | common1
24	HexNAc(3)Hex(5) @ NGlycan | common1
25	HexNAc(3)Hex(5)Fuc(1) @ NGlycan | common1
26	HexNAc(3)Hex(5)Fuc(1)NeuAc(1) (13.031)@ NGlycan | common1
27	HexNAc(3)Hex(5)NeuAc(1) (13.031) @ NGlycan | common1
28	HexNAc(3)Hex(5)NeuAc(2) (26.062) @ NGlycan | common1
29	HexNAc(3)Hex(6) @ NGlycan | common1
30	HexNAc(3)Hex(6)Fuc(1) @ NGlycan | common1
31	HexNAc(3)Hex(6)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
32	HexNAc(3)Hex(6)NeuAc(1) (13.031) @ NGlycan | common1
33	HexNAc(3)Hex(6)NeuAc(2) (26.062)@ NGlycan | common1
34	HexNAc(3)Hex(7)Fuc(1) @ NGlycan | common1
35	HexNAc(3)Hex(7)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
36	HexNAc(3)Hex(7)NeuAc(1) (13.031) @ NGlycan | common1
37	HexNAc(3)Hex(7)NeuAc(2) (26.062) @ NGlycan | common1
38	HexNAc(3)Hex(8)NeuAc(1) (13.031) @ NGlycan | common1
39	HexNAc(3)Hex(8)NeuAc(2) (26.062)@ NGlycan | common1
40	HexNAc(3)Hex(9)Fuc(2) @ NGlycan | common1
41	HexNAc(4)Hex(11)Fuc(1)NeuAc(4) (52.124) @ NGlycan | common1

HexNAc(4)Hex(11)NeuAc(1) @ NGlycan | common1
HexNAc(4)Hex(14)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(4)Hex(14)Fuc(3)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(4)Hex(14)NeuAc(1) (13.031) @ NGlycan | common1
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HexNAc(4)Hex(3)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(4)Hex(3)Fuc(3) @ NGlycan | common1
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HexNAc(4)Hex(4) @ NGlycan | common1
HexNAc(4)Hex(4)Fuc(1) @ NGlycan | common1
HexNAc(4)Hex(4)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(4)Hex(4)Fuc(3) @ NGlycan | common1
HexNAc(4)Hex(4)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(4)Hex(4)NeuAc(2) (26.062)@ NGlycan | common1
HexNAc(4)Hex(5) @ NGlycan | common1
HexNAc(4)Hex(5)Fuc(1) @ NGlycan | common1
HexNAc(4)Hex(5)Fuc(2) @ NGlycan | common1
HexNAc(4)Hex(5)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(4)Hex(5)Fuc(1)NeuAc(2) (26.062) @ NGlycan | common1
HexNAc(4)Hex(5)Fuc(2)NeuAc(2) (26.062) @ NGlycan | common1
HexNAc(4)Hex(5)Fuc(3) @ NGlycan | common1
HexNAc(4)Hex(5)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(4)Hex(5)NeuAc(2) (26.062) @ NGlycan | common1
HexNAc(4)Hex(6) @ NGlycan | common1
HexNAc(4)Hex(6)Fuc(1) @ NGlycan | common1
HexNAc(4)Hex(6)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(4)Hex(6)Fuc(1)NeuAc(2) (26.062) @ NGlycan | common1
HexNAc(4)Hex(6)Fuc(3)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(4)Hex(6)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(4)Hex(6)NeuAc(2) (26.062) @ NGlycan | common1
HexNAc(4)Hex(7) @ NGlycan | common1
HexNAc(4)Hex(7)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(5)Hex(10)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(5)Hex(10)NeuAc(2) (26.062) @ NGlycan | common1
HexNAc(5)Hex(11)Fuc(1)NeuAc(4) (52.124) @ NGlycan | common1
HexNAc(5)Hex(13)Fuc(3)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(5)Hex(3) @ NGlycan | common1
HexNAc(5)Hex(3)Fuc(1) @ NGlycan | common1
HexNAc(5)Hex(4) @ NGlycan | common1
HexNAc(5)Hex(4)Fuc(1) @ NGlycan | common1
HexNAc(5)Hex(4)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
HexNAc(5)Hex(4)Fuc(2) @ NGlycan | common1

86 HexNAc(5)Hex(4)Fuc(2)NeuAc(1) (13.031) @ NGlycan | common1
87 HexNAc(5)Hex(4)Fuc(2)NeuAc(2) (26.062) @ NGlycan | common1
88 HexNAc(5)Hex(4)NeuAc(1) (13.031) @ NGlycan | common1
89 HexNAc(5)Hex(4)NeuAc(2) (26.062) @ NGlycan | common1
90 HexNAc(5)Hex(5) @ NGlycan | common1
91 HexNAc(5)Hex(5)Fuc(1) @ NGlycan | common1
92 HexNAc(5)Hex(5)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
93 HexNAc(5)Hex(5)Fuc(1)NeuAc(2) (26.062) @ NGlycan | common1
94 HexNAc(5)Hex(5)Fuc(2) @ NGlycan | common1
95 HexNAc(5)Hex(5)Fuc(2)NeuAc(1) (13.031) @ NGlycan | common1
96 HexNAc(5)Hex(5)NeuAc(1) (13.031) @ NGlycan | common1
97 HexNAc(5)Hex(5)NeuAc(2) (26.062) @ NGlycan | common1
98 HexNAc(5)Hex(6) @ NGlycan | common1
99 HexNAc(5)Hex(6)Fuc(1) @ NGlycan | common1
100 HexNAc(5)Hex(6)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
101 HexNAc(5)Hex(6)Fuc(1)NeuAc(2) (26.062) @ NGlycan | common1
102 HexNAc(5)Hex(6)Fuc(1)NeuAc(3) (39.093) @ NGlycan | common1
103 HexNAc(5)Hex(6)Fuc(2)NeuAc(3) (39.093) @ NGlycan | common1
104 HexNAc(5)Hex(6)Fuc(2)NeuAc(5) (65.155) @ NGlycan | common1
105 HexNAc(5)Hex(6)NeuAc(1) (13.031) @ NGlycan | common1
106 HexNAc(5)Hex(6)NeuAc(2) (26.062)@ NGlycan | common1
107 HexNAc(5)Hex(6)NeuAc(3) @ NGlycan | common1
108 HexNAc(5)Hex(6)NeuAc(4) @ NGlycan | common1
109 HexNAc(5)Hex(7)Fuc(1) @ NGlycan | common1
110 HexNAc(5)Hex(7)Fuc(1)NeuAc(1) (13.031)@ NGlycan | common1
111 HexNAc(5)Hex(7)Fuc(1)NeuAc(3) (39.093) @ NGlycan | common1
112 HexNAc(5)Hex(7)Fuc(3)NeuAc(2) (26.062) @ NGlycan | common1
113 HexNAc(5)Hex(7)NeuAc(1) (13.031) @ NGlycan | common1
114 HexNAc(5)Hex(8)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
115 HexNAc(5)Hex(8)Fuc(2)NeuAc(1) (13.031) @ NGlycan | common1
116 HexNAc(5)Hex(8)Fuc(4)NeuAc(1) (13.031) @ NGlycan | common1
117 HexNAc(5)Hex(8)NeuAc(1) (13.031) @ NGlycan | common1
118 HexNAc(6)Hex(10)Fuc(5)NeuAc(3) (39.093) @ NGlycan | common1
119 HexNAc(6)Hex(4)Fuc(1) @ NGlycan | common1
120 HexNAc(6)Hex(6)Fuc(1) @ NGlycan | common1
121 HexNAc(6)Hex(6)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
122 HexNAc(6)Hex(6)Fuc(1)NeuAc(2) (26.032) @ NGlycan | common1
123 HexNAc(6)Hex(6)Fuc(1)NeuAc(3) (39.093) @ NGlycan | common1
124 HexNAc(6)Hex(6)Fuc(3) @ NGlycan | common1
125 HexNAc(6)Hex(6)Fuc(3)NeuAc(1) (13.031) @ NGlycan | common1
126 HexNAc(6)Hex(7) @ NGlycan | common1
127 HexNAc(6)Hex(7)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
128 HexNAc(6)Hex(7)Fuc(1)NeuAc(2) (26.032) @ NGlycan | common1
129 HexNAc(6)Hex(7)Fuc(1)NeuAc(3) (39.093) @ NGlycan | common1

130	HexNAc(6)Hex(7)Fuc(1)NeuAc(4) (52.124) @ NGlycan | common1
131	HexNAc(6)Hex(7)Fuc(2)NeuAc(1) (13.031) @ NGlycan | common1
132	HexNAc(6)Hex(7)Fuc(2)NeuAc(2) (26.032) @ NGlycan | common1
133	HexNAc(6)Hex(7)Fuc(2)NeuAc(3) (39.093) @ NGlycan | common1
134	HexNAc(6)Hex(7)Fuc(2)NeuAc(4) (52.124) @ NGlycan | common1
135	HexNAc(6)Hex(7)Fuc(3)NeuAc(1) (13.031) @ NGlycan | common1
136	HexNAc(6)Hex(7)NeuAc(1) (13.031) @ NGlycan | common1
137	HexNAc(6)Hex(7)NeuAc(2) (26.032) @ NGlycan | common1
138	HexNAc(6)Hex(7)NeuAc(3) (39.093) @ NGlycan | common1
139	HexNAc(6)Hex(7)NeuAc(4) (52.124) @ NGlycan | common1
140	HexNAc(6)Hex(8)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
141	HexNAc(6)Hex(8)Fuc(1)NeuAc(2) (26.032) @ NGlycan | common1
142	HexNAc(6)Hex(8)Fuc(1)NeuAc(3) (39.093) @ NGlycan | common1
143	HexNAc(6)Hex(8)Fuc(2)NeuAc(2) (26.032) @ NGlycan | common1
144	HexNAc(6)Hex(8)Fuc(3)NeuAc(3) (39.093) @ NGlycan | common1
145	HexNAc(7)Hex(10)Fuc(4)NeuAc(1) (13.031) @ NGlycan | common1
146	HexNAc(7)Hex(11)Fuc(4)NeuAc(1) (13.031) @ NGlycan | common1
147	HexNAc(7)Hex(12)Fuc(1)NeuAc(4) (52.124) @ NGlycan | common1
148	HexNAc(7)Hex(12)Fuc(4)NeuAc(1) (13.031) @ NGlycan | common1
149	HexNAc(7)Hex(13)Fuc(2)NeuAc(1) (13.031) @ NGlycan | common1
150	HexNAc(7)Hex(5) @ NGlycan common1
151	HexNAc(7)Hex(5)Fuc(1)NeuAc(1) (13.031) @ NGlycan | common1
152	HexNAc(7)Hex(6)Fuc(2)NeuAc(6) (78.186) @ NGlycan | common1
153	HexNAc(7)Hex(7)NeuAc(3) (39.093) @ NGlycan | common1
154	HexNAc(7)Hex(8) @ NGlycan | common1
155	HexNAc(7)Hex(8)Fuc(1)NeuAc(2) (26.032) @ NGlycan | common1
156	HexNAc(7)Hex(8)Fuc(2)NeuAc(3) (39.093) @ NGlycan | common1
157	HexNAc(7)Hex(8)Fuc(3)NeuAc(3) (39.093) @ NGlycan | common1
158	HexNAc(7)Hex(9)Fuc(1)NeuAc(2) (26.032) @ NGlycan | common1
159	HexNAc(7)Hex(9)Fuc(4)NeuAc(2) (26.032) @ NGlycan | common1
160	HexNAc(7)Hex(9)NeuAc(1) (13.031) @ NGlycan | common1
161	HexNAc(8)Hex(8)Fuc(2) @ NGlycan | common1
162	HexNAc(8)Hex(9)Fuc(1) @ NGlycan | common1
163	HexNAc(9)Hex(9)Fuc(1) @ NGlycan | common1

Note: Table S3, Table S4, S5 and S6 are provided as separate files.

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