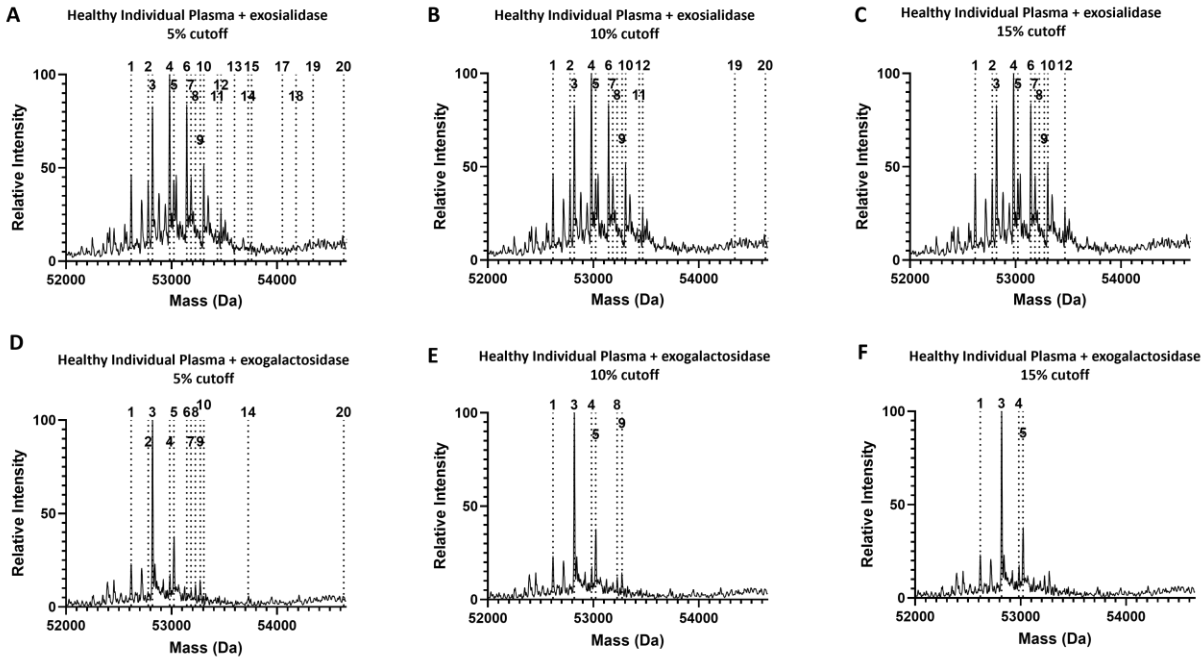
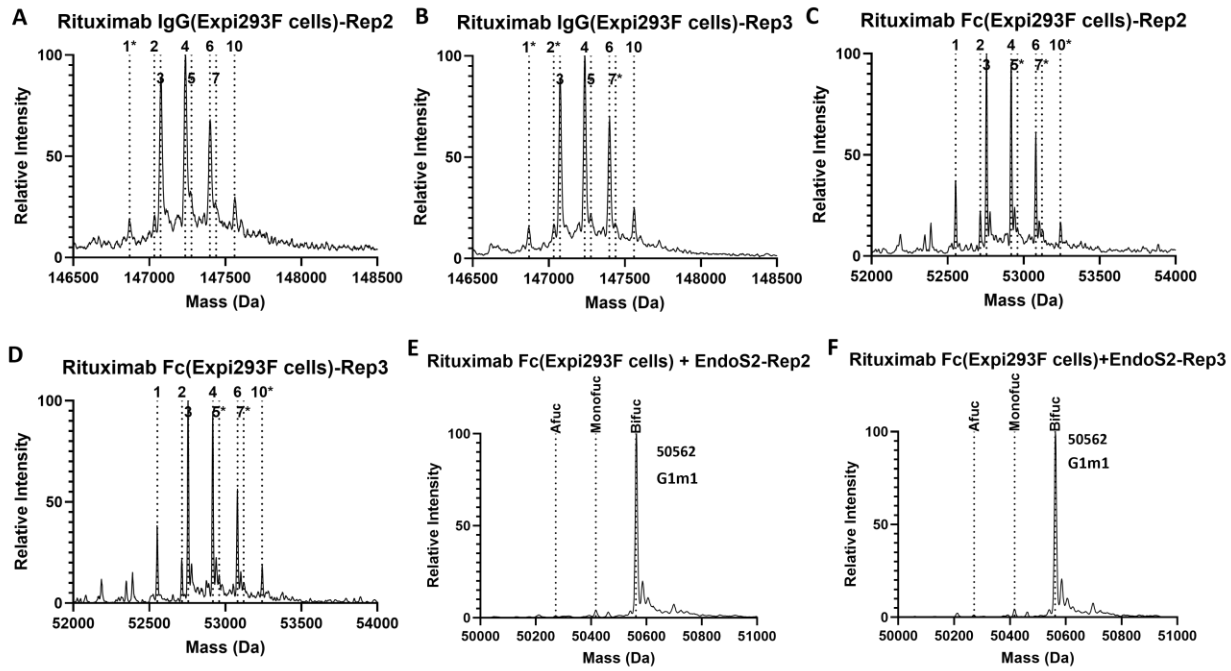
Asymmetrically glycosylated IgG1 antibodies are universal and drive human disease

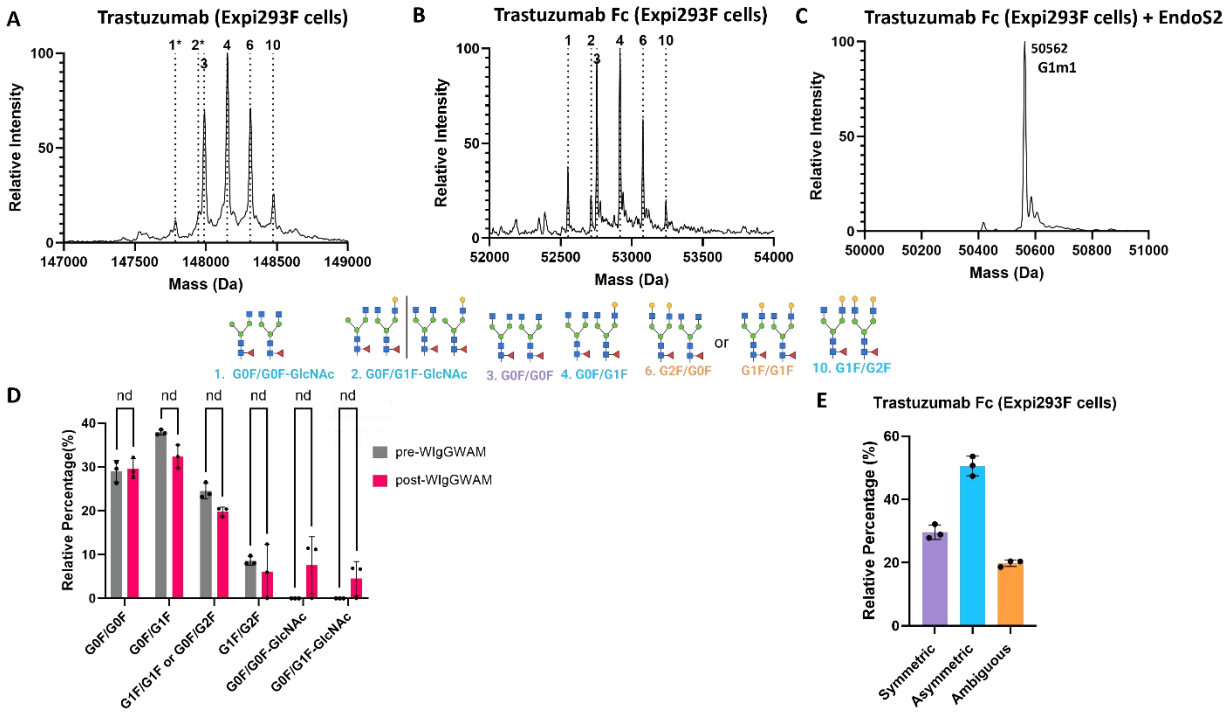
Supplementary Information



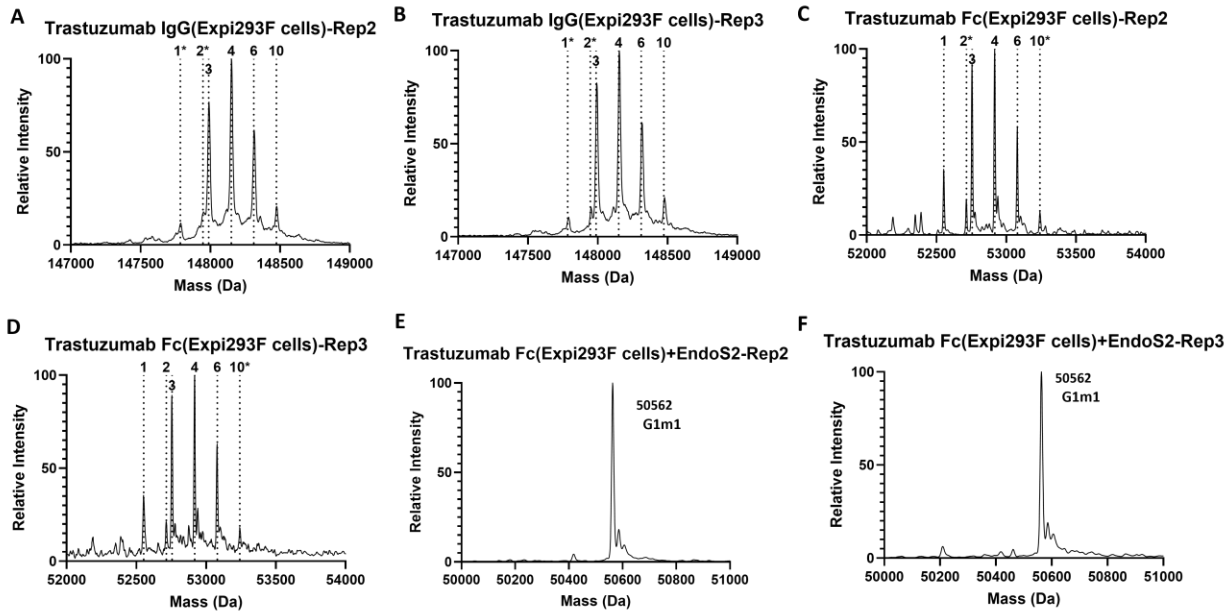
Supplementary Figure 1: The cutoff percentage based on maximum peak height impacts which glycan peaks are identified during WIgGWAM analysis. (A-F) Deconvoluted intact LC/MS spectrum of Fcs purified with WIgGWAM from a healthy individual human plasma sample **(A-C)** treated with an exosialidase. The dotted lines annotate Fc-linked glycoforms whose peak heights were at least greater than **(A)** 5%, **(B)** 10%, or **(C)** 15% of maximum peak height. **(D-F)** treated with an exogalactosidase. The dotted lines annotate Fc-linked glycoforms whose peak heights were at least greater than **(D)** 5%, **(E)** 10%, or **(F)** 15% of maximum peak height. For all intact LC/MS deconvoluted spectra, the highest peak was normalized to 100.



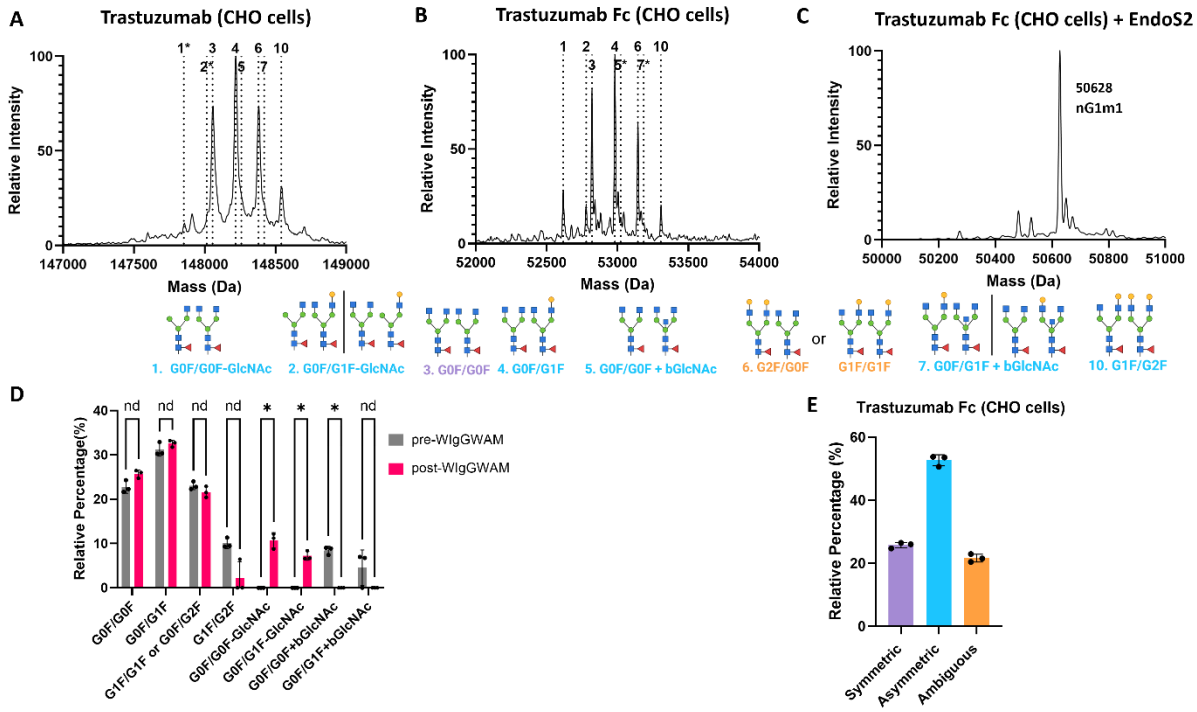
Supplementary Figure 2: Replicate WIgGWAM analysis of recombinant Rituximab IgG1. (A-B) Deconvoluted intact LC/MS spectrum of recombinant Rituximab IgG1 expressed in Expi293F cells with no enzyme added. The dotted lines without an asterisk (*) annotate IgG-linked glycoforms whose peak heights were at least 20% of the maximum peak height. **(C-F)** Deconvoluted intact LC/MS spectrum of recombinant Rituximab Fcs after WIgGWAM **(C-D)** with no enzymes added. The dotted lines without an asterisk (*) annotate Fc-linked glycoforms whose peak heights were at least 20% of the maximum peak height. Or (**E-F)** with EndoS2 added. The dotted lines annotate the peaks corresponding to the bifucosylated, monofucosylated, and afucosylated mass of the G1m1 Fc allele. Lines marked with an asterisk (*) are peaks that did not pass the 20% cutoff for one spectrum but did in another. For all intact LC/MS deconvoluted spectra, the highest peak was normalized to 100.



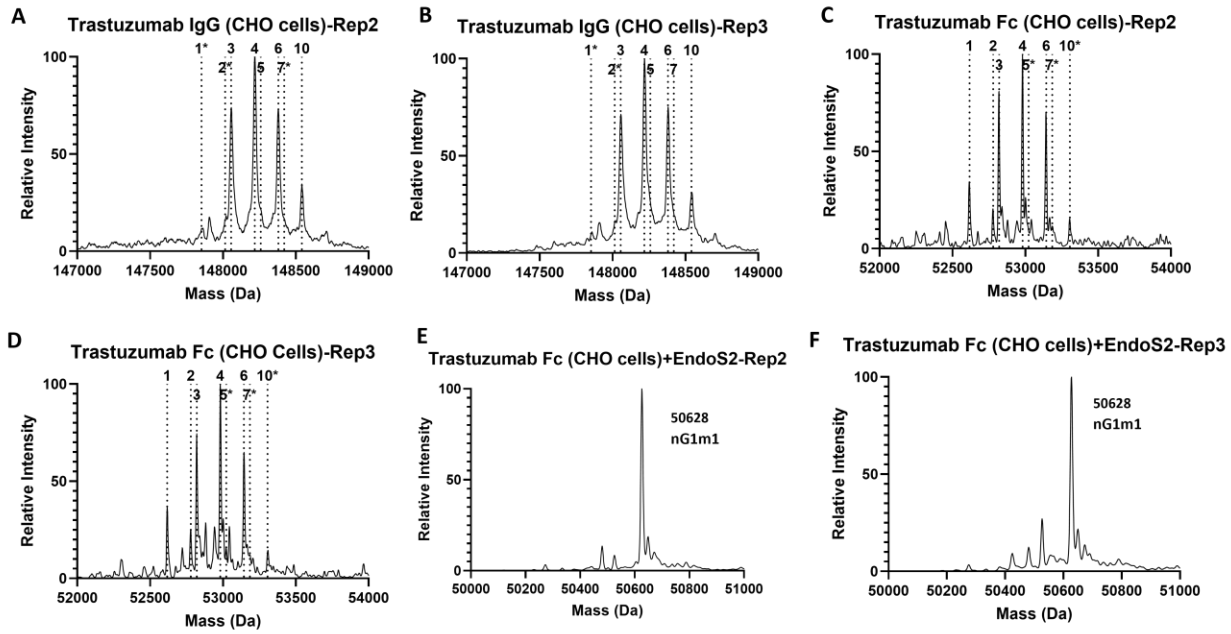
Supplementary Figure 3: WIgGWAM identifies both symmetric and asymmetric glycoforms of recombinant Trastuzumab IgG1 from Expi293F cells. (A) Deconvoluted intact LC/MS spectrum of recombinant Trastuzumab IgG1 expressed in Expi293F cells. The dotted lines without an asterisk (*) annotate IgG-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. **(B-C)** Deconvoluted intact LC/MS spectrum of recombinant Trastuzumab Fcs after WIgGWAM **(B)** with no enzymes added. The dotted lines without an asterisk (*) annotate IgG-linked glycoforms whose peak heights were at least 20% of the maximum peak height. **(C)** with EndoS2 added. The dotted lines annotate the peak corresponding to the bifucosylated mass of the G1m1 Fc allele. Lines marked with an asterisk (*) are peaks that did not pass the 20% cutoff for one spectrum but did in another. For all intact LC/MS deconvoluted spectra, the highest peak was normalized to 100. Legend created in BioRender. D, J. (2025) https://BioRender.com/dvbra9l. **(D)** Comparison of the relative percentages of the glycoforms identified in recombinant Trastuzumab as an IgG1 before WIgGWAM (black) and as an Fc after WIgGWAM (pink). Data are presented as mean values +/- SD. Multiple unpaired t-tests for 3 technical triplicates were used. (nd) or (*) indicate whether there was a significant difference using the two-stage step-up method for false discovery rate (FDR). **(E)** Bar graph showing the relative percentages of symmetric (purple), asymmetric (blue), and ambiguous (orange) glycoforms in recombinant Trastuzumab Fc after WIgGWAM for three technical triplicates. Data are presented as mean values +/- SD.



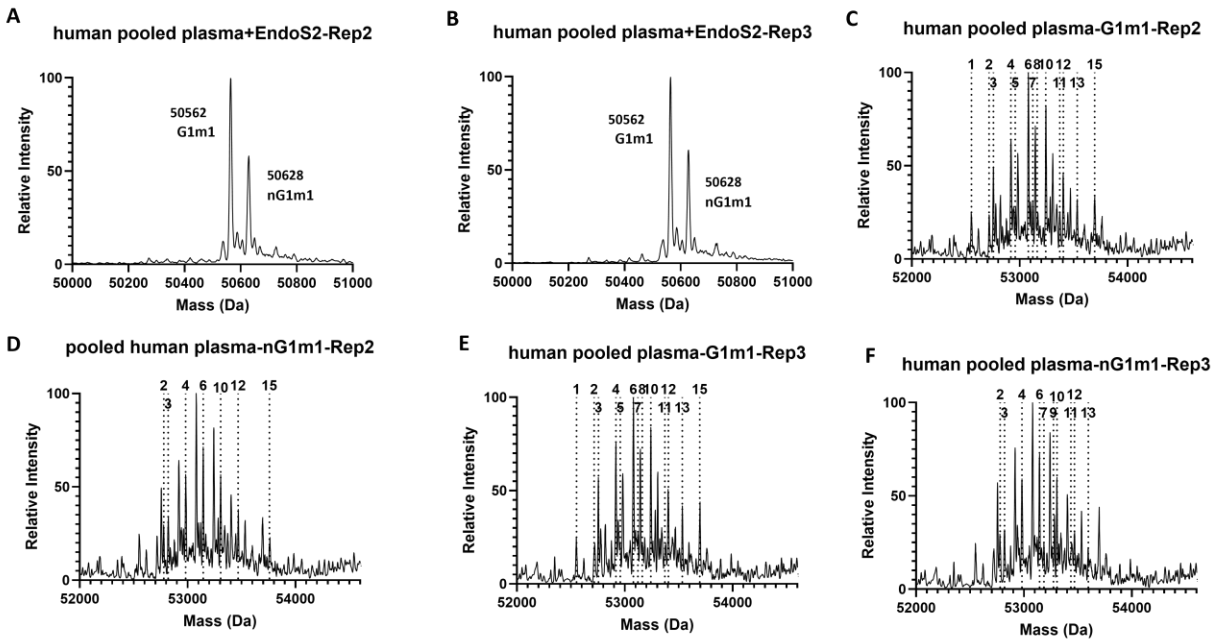
Supplementary Figure 4: Replicate WIgGWAM analysis of recombinant Trastuzumab IgG1 from Expi293F cells. (A-B) Deconvoluted intact LC/MS spectrum of recombinant Trastuzumab IgG1 expressed in Expi293F cells with no enzyme added. The dotted lines without an asterisk (*) annotate IgG-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. **(C-F)** Deconvoluted intact LC/MS spectrum of recombinant Trastuzumab Fcs after WIgGWAM **(C-D)** with no enzymes added. The dotted lines without an asterisk (*) annotate Fc-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. Or (**E-F)** with EndoS2 added. The peak corresponding to the bifucosylated mass of the G1m1 Fc allele is labeled. Lines marked with an asterisk (*) are peaks that did not pass the 20% cutoff for one spectrum but did in another. For all intact LC/MS deconvoluted spectra, the highest peak was normalized to 100.



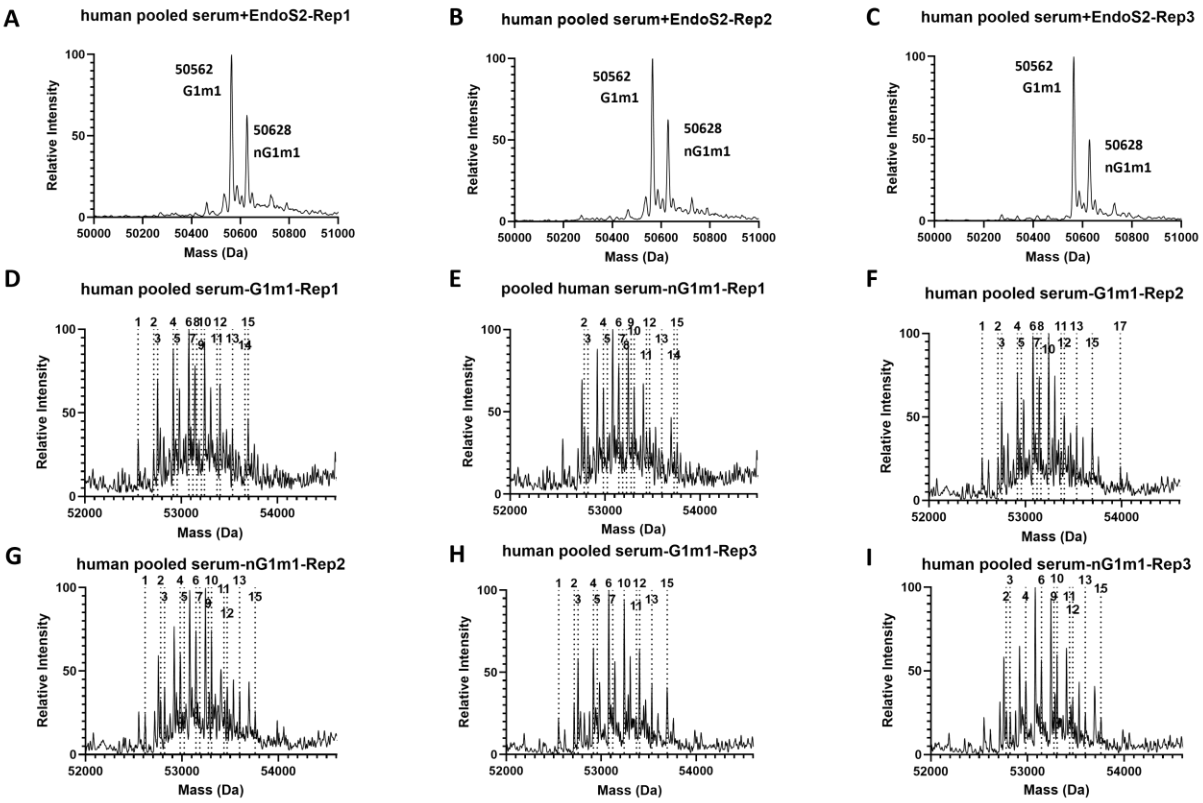
Supplementary Figure 5: WIgGWAM identifies both symmetric and asymmetric glycoforms of recombinant Trastuzumab IgG1 from CHO cells. (A) Deconvoluted intact LC/MS spectrum of recombinant Trastuzumab IgG1 expressed in CHO cells. The dotted lines without an asterisk (*) annotate IgG-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. **(B-C)** Deconvoluted intact LC/MS spectrum of recombinant Trastuzumab Fcs after WIgGWAM **(B)** with no enzymes added. The dotted lines without an asterisk (*) annotate IgG-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. **(C)** with EndoS2 added. The dotted lines annotate the peak corresponding to the bifucosylated mass of the nG1m1 Fc allele. Lines marked with an asterisk (*) are peaks that did not pass the 20% cutoff for one spectrum but did in another. For all intact LC/MS deconvoluted spectra, the highest peak was normalized to 100. Legend created in BioRender. D, J. (2025) https://BioRender.com/dvbra9l. **(D)** Comparison of the relative percentages of the glycoforms identified in recombinant Trastuzumab as an IgG1 before WIgGWAM (black) and as an Fc after WIgGWAM (pink). Data are presented as mean values +/- SD. Multiple unpaired t-tests for 3 technical triplicates were used. (nd) or (*) indicate whether there was a significant difference using the two-stage step-up method for false discovery rate (FDR). **(E)** Bar graph showing the relative percentages of symmetric (purple), asymmetric (blue), and ambiguous (orange) glycoforms in recombinant Trastuzumab Fc after WIgGWAM for three technical triplicates. Data are presented as mean values +/- SD.



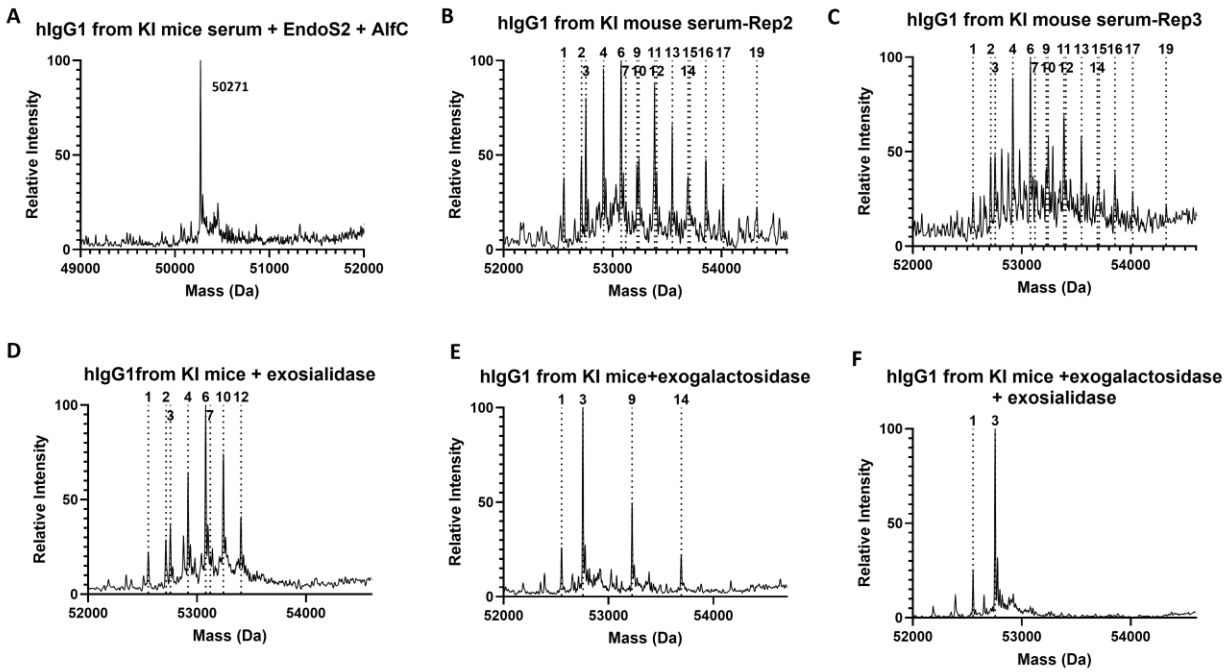
Supplementary Figure 6: Replicate WIgGWAM analysis of recombinant Trastuzumab IgG1 from CHO cells. (A-B) Deconvoluted intact LC/MS spectrum of recombinant Trastuzumab IgG1 expressed in CHO cells with no enzyme added. The dotted lines without an asterisk (*) annotate IgG-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. **(C-F)** Deconvoluted intact LC/MS spectrum of recombinant Trastuzumab Fcs after WIgGWAM **(C-D)** with no enzymes added. The dotted lines without an asterisk (*) annotate Fc-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. Or **(E-F)** with EndoS2 added. The peak corresponding to the bifucosylated mass of the nG1m1 Fc allele is labeled. Lines marked with an asterisk (*) are peaks that did not pass the 20% cutoff for one spectrum but did in another. For all intact LC/MS deconvoluted spectra, the highest peak was normalized to 100.



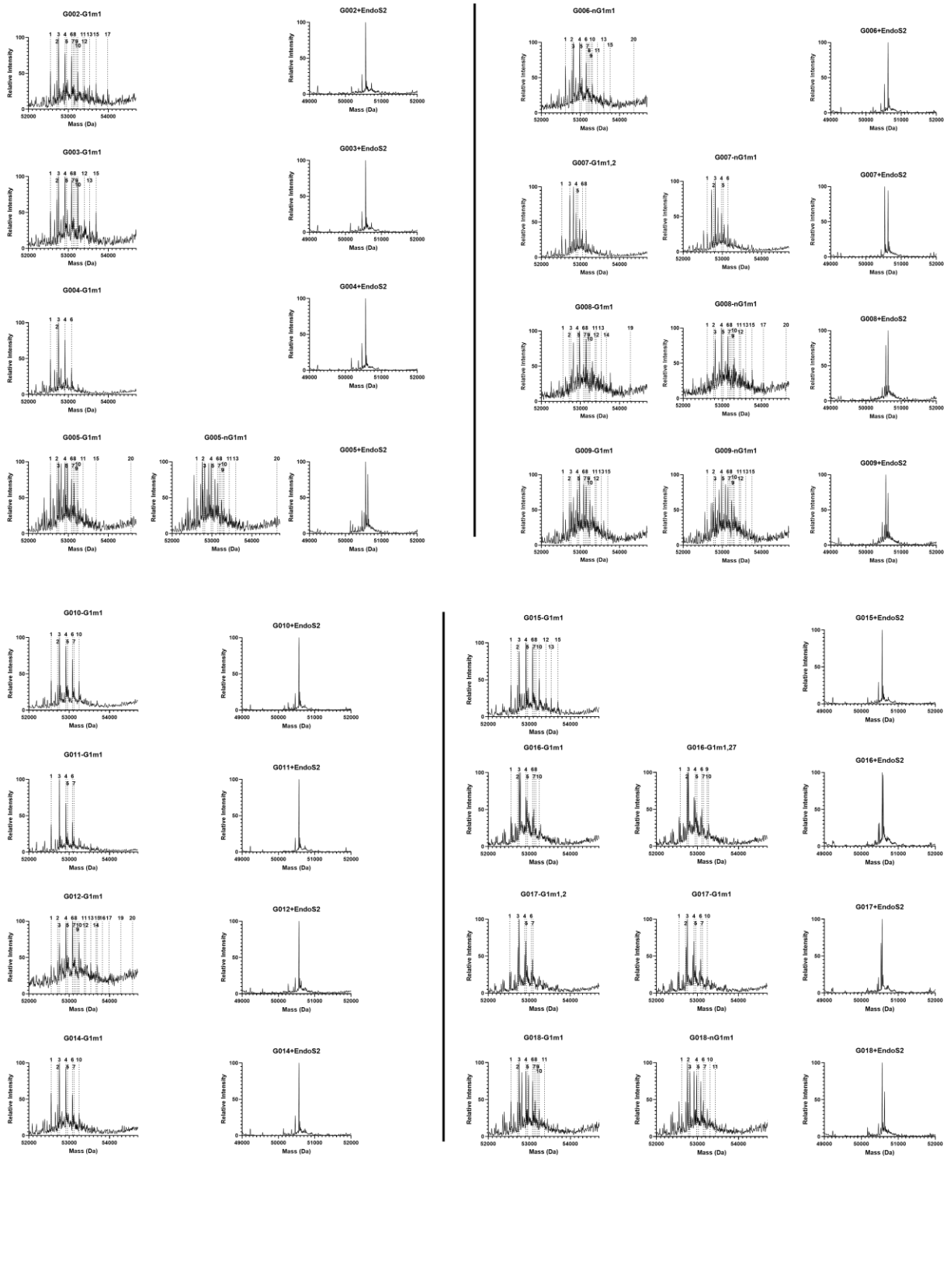
Supplementary Figure 7: Replicate WIgGWAM analysis of pooled human plasma. Deconvoluted intact LC/MS spectrum of a pooled human plasma sample after WIgGWAM **(A-B)** treated with EndoS2. The peaks corresponding to the bifucosylated masses of the G1m1 and nG1m1 Fc alleles are labeled. **(C-F)** with no enzymes added. The dotted lines annotate Fc-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. For all intact LC/MS deconvoluted spectra, the highest peak was normalized to 100.

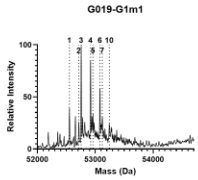


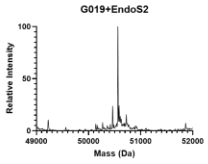
Supplementary Figure 8: WIgGWAM analysis of pooled human serum. Deconvoluted intact LC/MS spectrum of a pooled human serum sample after WIgGWAM **(A-C)** treated with EndoS2. The peaks corresponding to the bifucosylated masses of the G1m1 and nG1m1 Fc alleles are labeled. **(D-I)** with no enzymes added. The dotted lines annotate Fc-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. For all intact LC/MS deconvoluted spectra, the highest peak was normalized to 100.

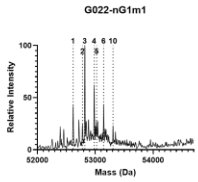


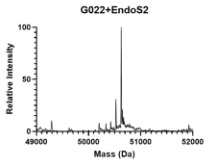
Supplementary Figure 9: WIgGWAM analysis of serum from human IgG1 knock-in mice with replicates and exoglycosidase treatment. Deconvoluted intact LC/MS spectrum of a serum sample from human IgG1 knock-in mice after WIgGWAM **(A)** treated with EndoS2 and the exofucosidase AlfC. The peak corresponding to the afucosylated mass of the G1m1 allele is labeled. **(B-C)** with no enzymes added, **(D)** with exosialidase added, **(E)** with exogalactosidase added, and **(F)** with exogalactosidase and exosialidase added. The dotted lines annotate Fc-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. For all the deconvoluted intact LC/MS spectra, the highest peak was normalized to 100.

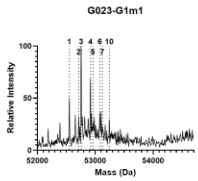

G002-G1m1
G002+EndoS2
G003-G1m1
G003+EndoS2
G004-G1m1
G004+EndoS2
G005-G1m1
G005-nG1m1
G005+EndoS2
G006-nG1m1
G006+EndoS2
G007-G1m1,2
G007-nG1m1
G007+EndoS2
G008-G1m1
G008-nG1m1
G008+EndoS2
G009-G1m1
G009-nG1m1
G009+EndoS2
G010-G1m1
G010+EndoS2
G011-G1m1
G011+EndoS2
G012-G1m1
G012+EndoS2
G014-G1m1
G014+EndoS2
G015-G1m1
G015+EndoS2
G016-G1m1
G016-G1m1,27
G016+EndoS2
G017-G1m1,2
G017-G1m1
G017+EndoS2
G018-G1m1
G018-nG1m1
G018+EndoS2
Relative Intensity
Mass (Da)

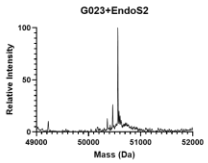

G019-G1m1
Relative Intensity
Mass (Da)

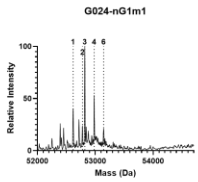

G019+EndoS2
Relative Intensity
Mass (Da)

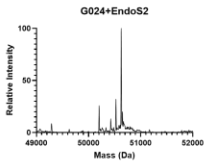

G022-nG1m1
Relative Intensity
Mass (Da)

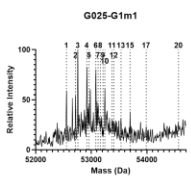

G022+EndoS2
Relative Intensity
Mass (Da)

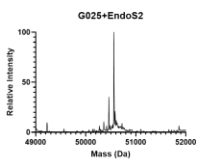

G023-G1m1
Relative Intensity
Mass (Da)

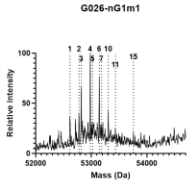

G023+EndoS2
Relative Intensity
Mass (Da)

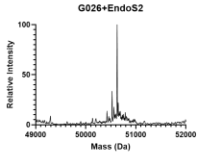

G024-nG1m1
Relative Intensity
Mass (Da)

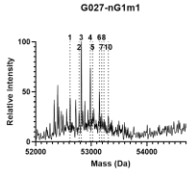

G024+EndoS2
Relative Intensity
Mass (Da)

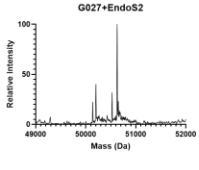

G025-G1m1
Relative Intensity
Mass (Da)

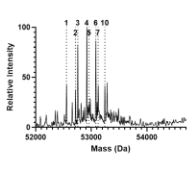

G025+EndoS2
Relative Intensity
Mass (Da)

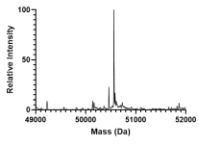

G026-nG1m1
Relative Intensity
Mass (Da)

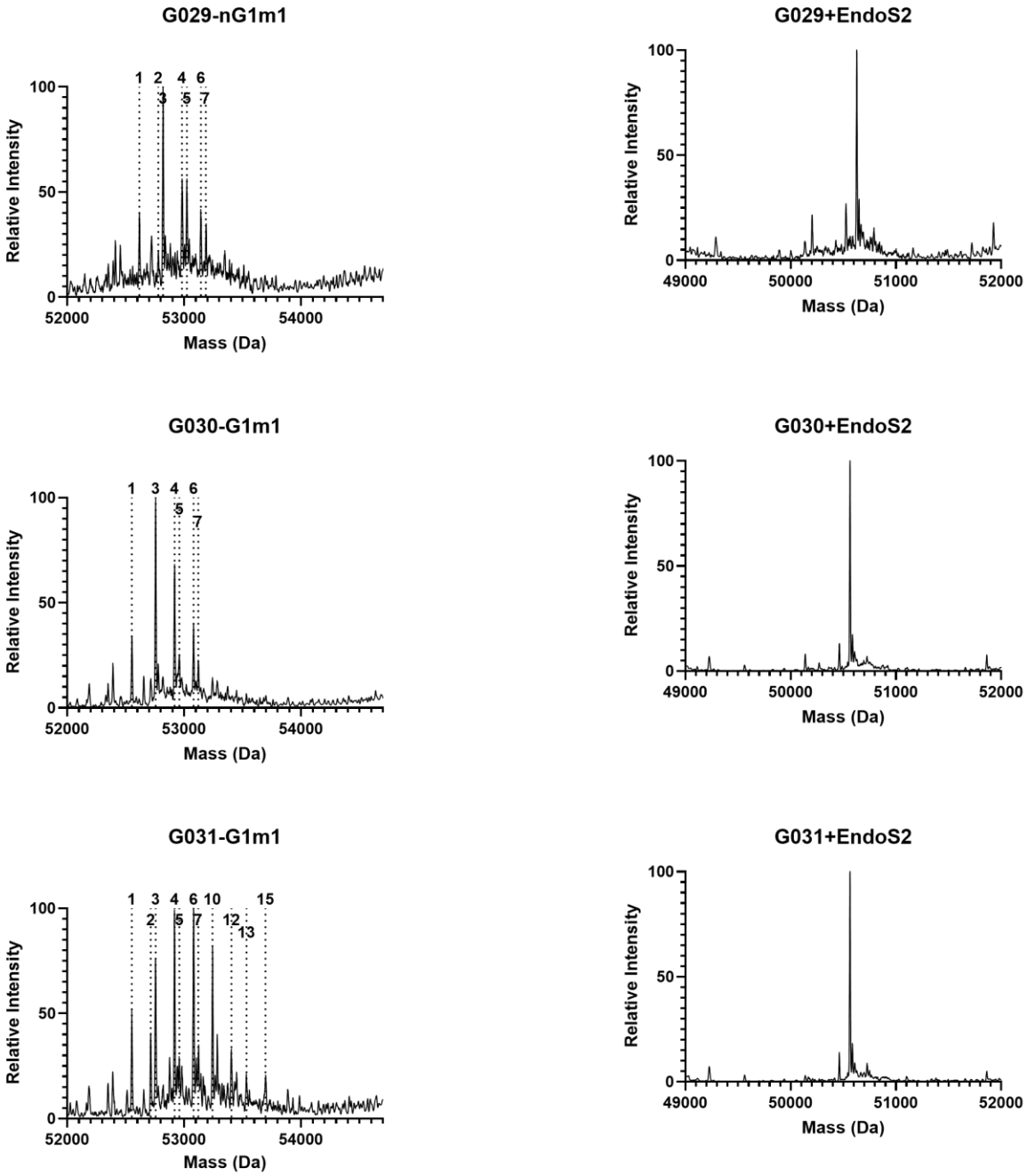

G026+EndoS2
Relative Intensity
Mass (Da)


G027-nG1m1
Relative Intensity
Mass (Da)

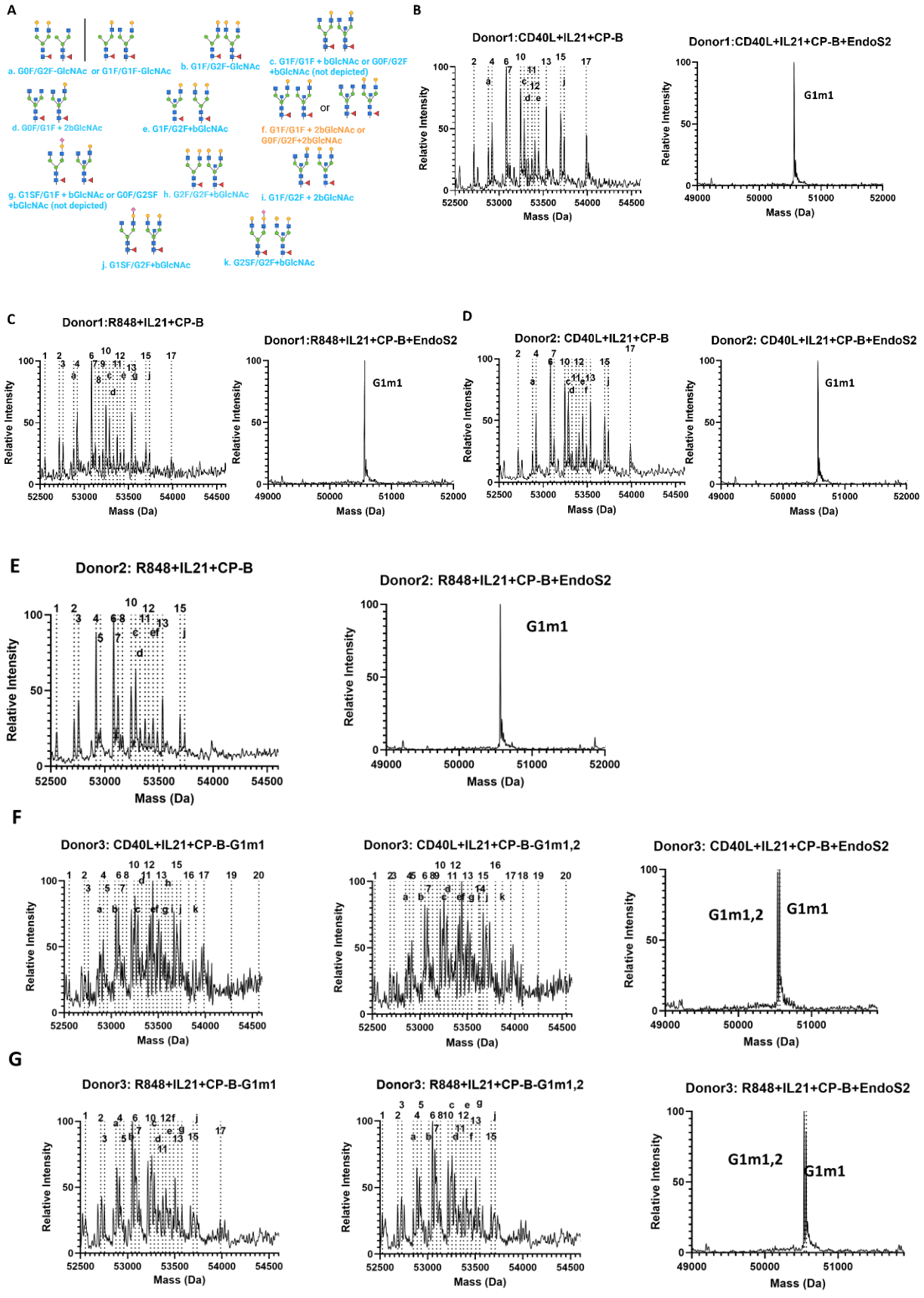

G027+EndoS2
Relative Intensity
Mass (Da)

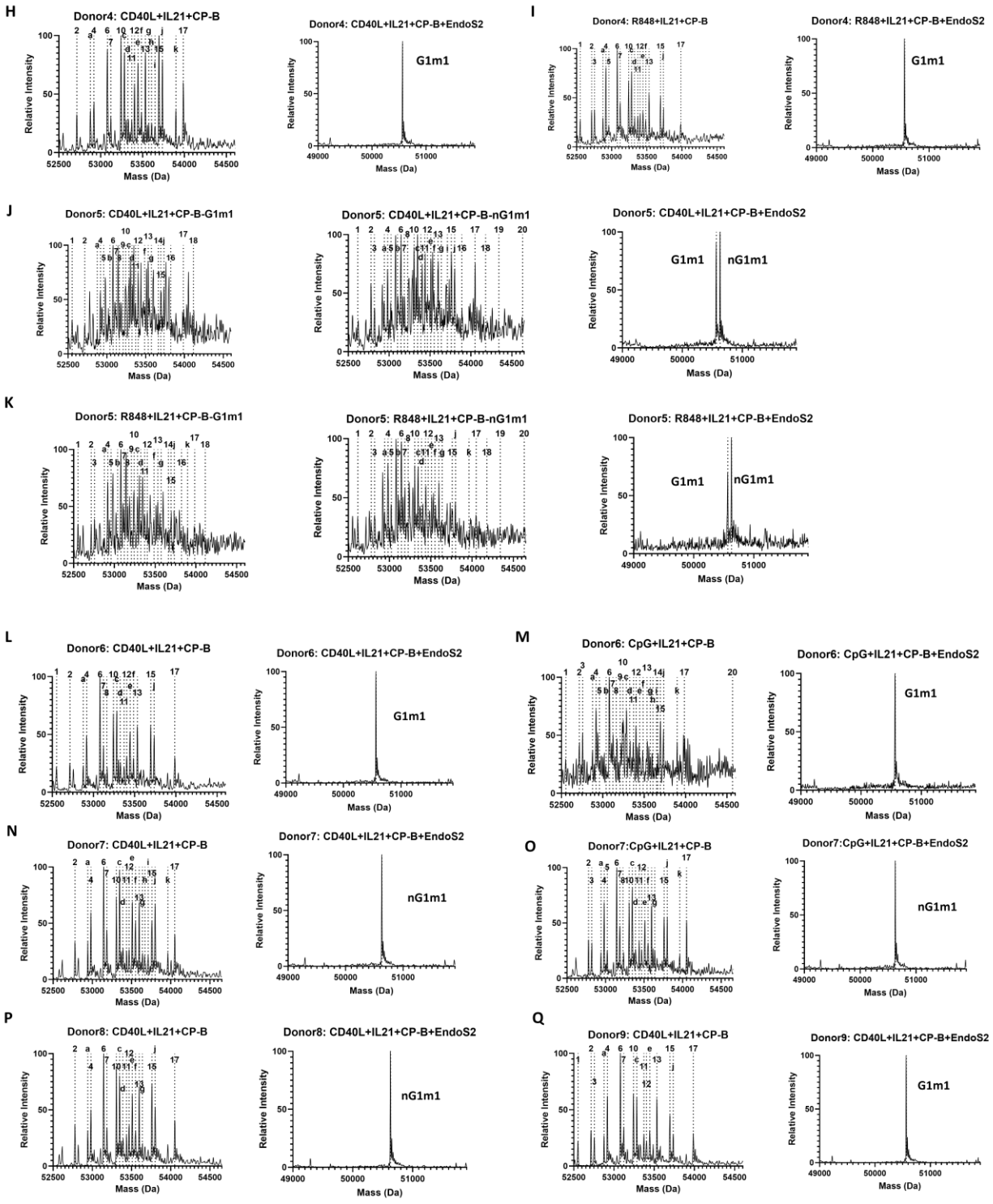

G028-G1m1
Relative Intensity
Mass (Da)

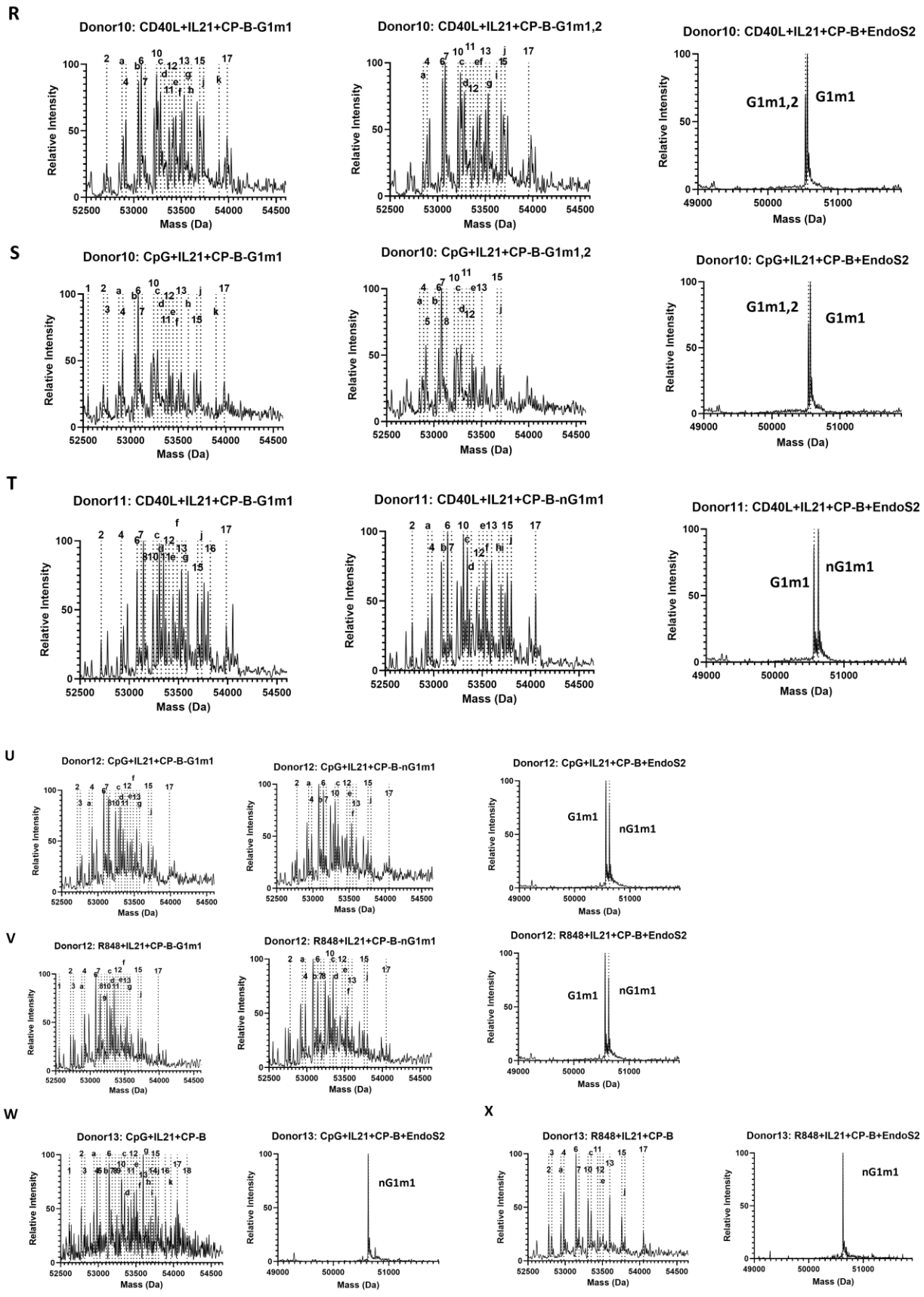

G028+EndoS2
Relative Intensity
Mass (Da)

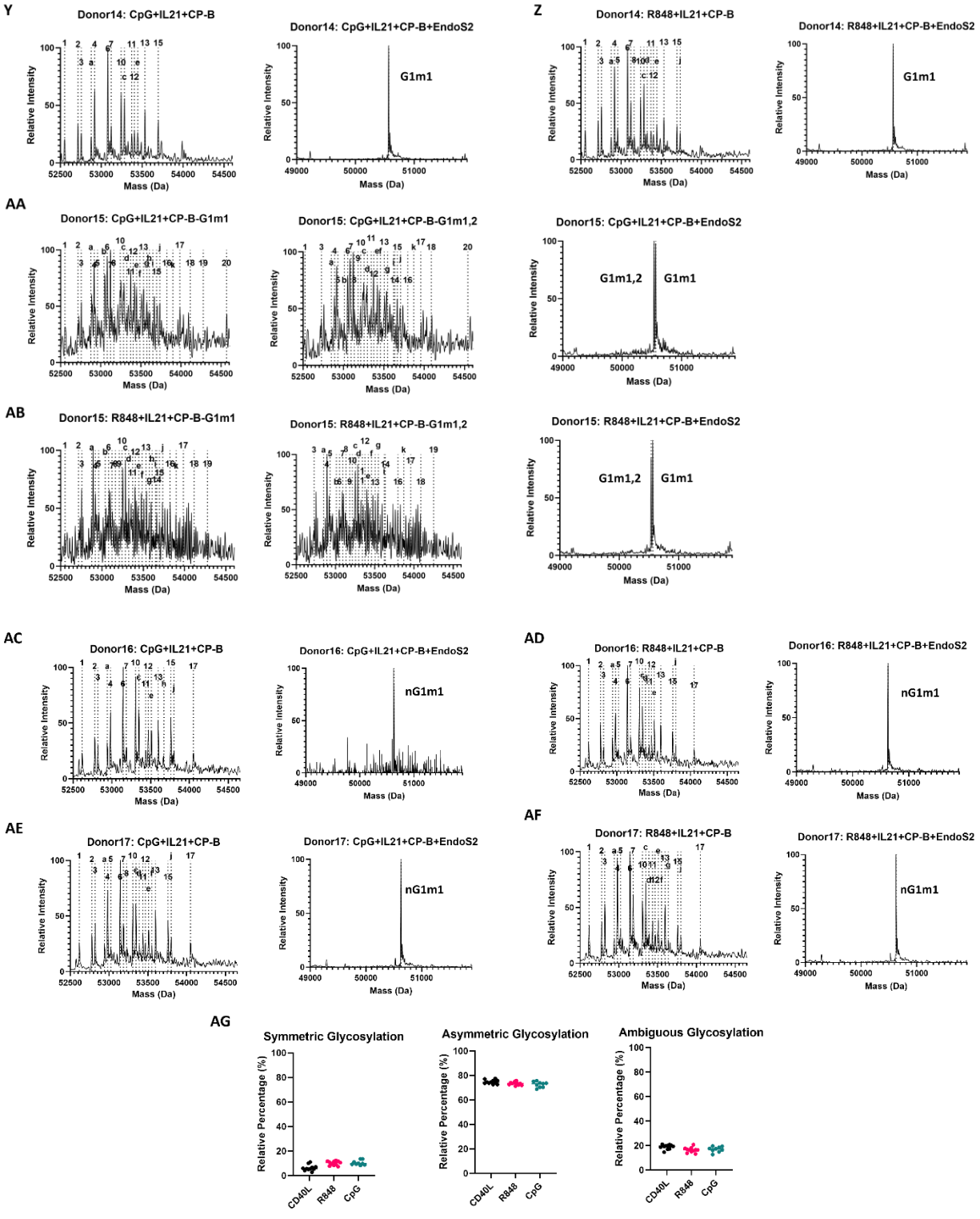


Supplementary Figure 10: WIgGWAM analysis of COVID and healthy patient plasma samples. Deconvoluted spectra of COVID and healthy patient plasma samples with our without EndoS2. The dotted lines annotate Fc-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. For all the deconvoluted intact LC/MS spectra, the highest peak was normalized to 100.

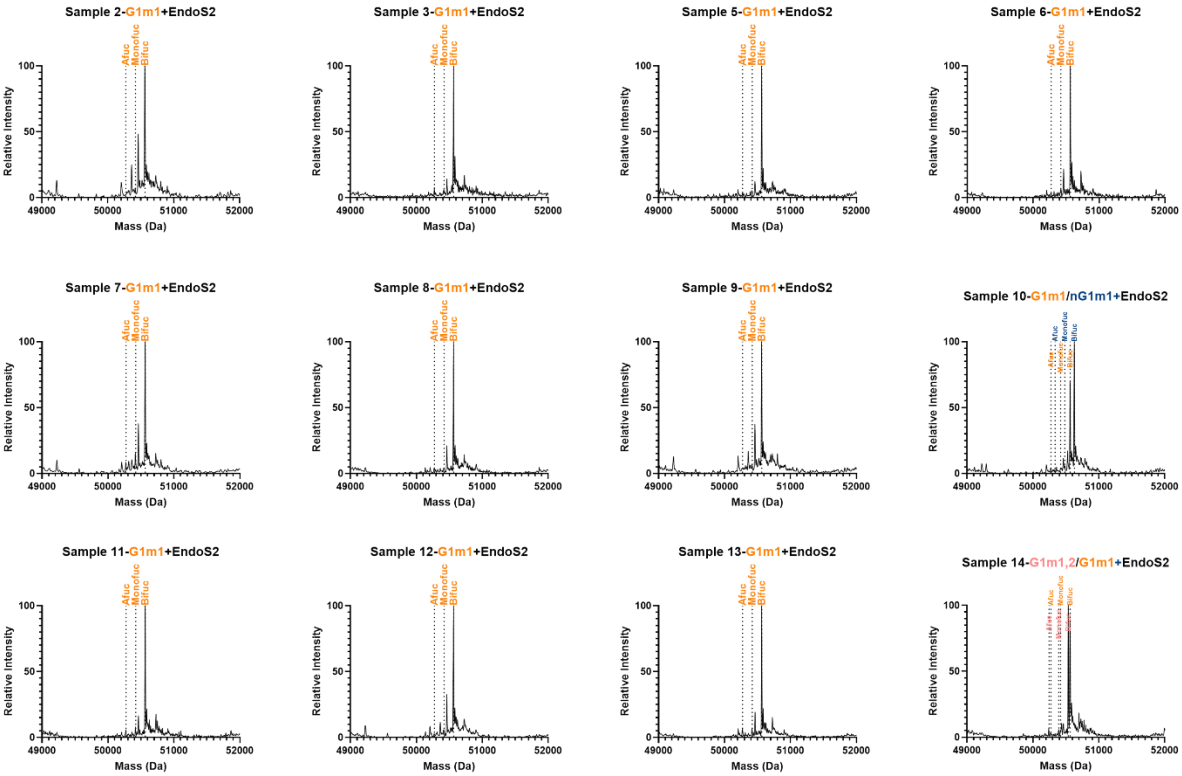

A
a. G0F/G2F-GlcNAc or G1F/G1F-GlcNAc
b. G1F/G2F-GlcNAc
c. G1F/G1F + bGlcNAc or G0F/G2F +bGlcNAc (not depicted)
d. G0F/G1F + 2bGlcNAc
e. G1F/G2F+bGlcNAc
or
f. G1F/G1F + 2bGlcNAc or G0F/G2F+2bGlcNAc
g. G1SF/G1F + bGlcNAc or G0F/G2SF +bGlcNAc (not depicted)
h. G2F/G2F+bGlcNAc
i. G1F/G2F + 2bGlcNAc
j. G1SF/G2F+bGlcNAc
k. G2SF/G2F+bGlcNAc
B
Donor1:CD40L+IL21+CP-B
Donor1:CD40L+IL21+CP-B+EndoS2
G1m1
C
Donor1:R848+IL21+CP-B
Donor1:R848+IL21+CP-B+EndoS2
G1m1
D
Donor2: CD40L+IL21+CP-B
Donor2: CD40L+IL21+CP-B+EndoS2
G1m1
E
Donor2: R848+IL21+CP-B
Donor2: R848+IL21+CP-B+EndoS2
G1m1
F
Donor3: CD40L+IL21+CP-B-G1m1
Donor3: CD40L+IL21+CP-B-G1m1,2
Donor3: CD40L+IL21+CP-B+EndoS2
G1m1,2
G1m1
G
Donor3: R848+IL21+CP-B-G1m1
Donor3: R848+IL21+CP-B-G1m1,2
Donor3: R848+IL21+CP-B+EndoS2
G1m1,2
G1m1
Relative Intensity
Mass (Da)

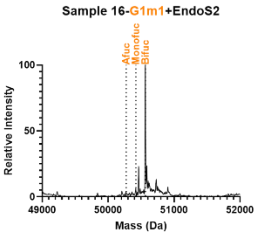

H
Donor4: CD40L+IL21+CP-B
Donor4: CD40L+IL21+CP-B+EndoS2
G1m1
I
Donor4: R848+IL21+CP-B
Donor4: R848+IL21+CP-B+EndoS2
G1m1
J
Donor5: CD40L+IL21+CP-B-G1m1
Donor5: CD40L+IL21+CP-B-nG1m1
Donor5: CD40L+IL21+CP-B+EndoS2
G1m1
nG1m1
K
Donor5: R848+IL21+CP-B-G1m1
Donor5: R848+IL21+CP-B-nG1m1
Donor5: R848+IL21+CP-B+EndoS2
G1m1
nG1m1
L
Donor6: CD40L+IL21+CP-B
Donor6: CD40L+IL21+CP-B+EndoS2
G1m1
M
Donor6: CpG+IL21+CP-B
Donor6: CpG+IL21+CP-B+EndoS2
G1m1
N
Donor7: CD40L+IL21+CP-B
Donor7: CD40L+IL21+CP-B+EndoS2
nG1m1
O
Donor7:CpG+IL21+CP-B
Donor7:CpG+IL21+CP-B+EndoS2
nG1m1
P
Donor8: CD40L+IL21+CP-B
Donor8: CD40L+IL21+CP-B+EndoS2
nG1m1
Q
Donor9: CD40L+IL21+CP-B
Donor9: CD40L+IL21+CP-B+EndoS2
G1m1
Relative Intensity
Mass (Da)

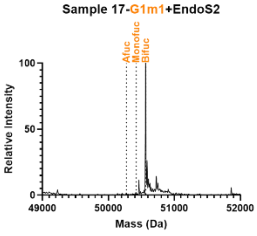

R
Donor10: CD40L+IL21+CP-B-G1m1
Donor10: CD40L+IL21+CP-B-G1m1,2
Donor10: CD40L+IL21+CP-B+EndoS2
G1m1,2
G1m1
S
Donor10: CpG+IL21+CP-B-G1m1
Donor10: CpG+IL21+CP-B-G1m1,2
Donor10: CpG+IL21+CP-B+EndoS2
G1m1,2
G1m1
T
Donor11: CD40L+IL21+CP-B-G1m1
Donor11: CD40L+IL21+CP-B-nG1m1
Donor11: CD40L+IL21+CP-B+EndoS2
G1m1
nG1m1
U
Donor12: CpG+IL21+CP-B-G1m1
Donor12: CpG+IL21+CP-B-nG1m1
Donor12: CpG+IL21+CP-B+EndoS2
G1m1
nG1m1
V
Donor12: R848+IL21+CP-B-G1m1
Donor12: R848+IL21+CP-B-nG1m1
Donor12: R848+IL21+CP-B+EndoS2
G1m1
nG1m1
W
Donor13: CpG+IL21+CP-B
Donor13: CpG+IL21+CP-B+EndoS2
nG1m1
X
Donor13: R848+IL21+CP-B
Donor13: R848+IL21+CP-B+EndoS2
nG1m1
Relative Intensity
Mass (Da)

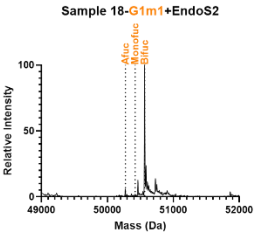

Y
Donor14: CpG+IL21+CP-B
Donor14: CpG+IL21+CP-B+EndoS2
G1m1
Z
Donor14: R848+IL21+CP-B
Donor14: R848+IL21+CP-B+EndoS2
G1m1
AA
Donor15: CpG+IL21+CP-B-G1m1
Donor15: CpG+IL21+CP-B-G1m1,2
Donor15: CpG+IL21+CP-B+EndoS2
G1m1,2
G1m1
AB
Donor15: R848+IL21+CP-B-G1m1
Donor15: R848+IL21+CP-B-G1m1,2
Donor15: R848+IL21+CP-B+EndoS2
G1m1,2
G1m1
AC
Donor16: CpG+IL21+CP-B
Donor16: CpG+IL21+CP-B+EndoS2
nG1m1
AD
Donor16: R848+IL21+CP-B
Donor16: R848+IL21+CP-B+EndoS2
nG1m1
AE
Donor17: CpG+IL21+CP-B
Donor17: CpG+IL21+CP-B+EndoS2
nG1m1
AF
Donor17: R848+IL21+CP-B
Donor17: R848+IL21+CP-B+EndoS2
nG1m1
Relative Intensity
Mass (Da)
AG
Symmetric Glycosylation
Asymmetric Glycosylation
Ambiguous Glycosylation
Relative Percentage (%)
CD40L
R848
CpG

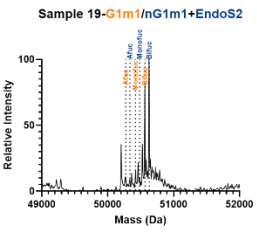
Supplementary Figure 11: In vitro memory B-cell stimulation results in asymmetrically glycosylated IgGs whose glycosylation is regulated by cytokines. **(A)** Depictions of the 11 glycan pairs added to the WIgGWAM analysis of IgGs from memory B-cell culture supernatants. Glycoforms in blue text are asymmetric and in orange are ambiguous. Created in BioRender. D, J. (2025) https://BioRender.com/dvbra9l. **(B-AF)** Deconvoluted spectra resulting from WIgGWAM of IgGs secreted from healthy donor-derived memory B-cells which were stimulated with IL21 in combination with CD40L, R848, or CpG. The Fc samples were treated with rat carboxypeptidase-B (CP-B) alone or with CP-B and EndoS2. The dotted lines annotate Fc-linked glycoforms whose peak heights were at least greater than 20% of the maximum peak height. For all the deconvoluted intact LC/MS spectra, the highest peak was normalized to 100. **(AG)** The relative percentage of symmetric **(left)**, asymmetric **(middle)**, and **(right)** ambiguous Fc-linked glycoforms after WIgGWAM of IgGs from memory B-cells stimulated with IL21 in combination with CD40L (black), R848 (pink), or CpG (green) (n=11 for CD40L, n=11 for R848, n=9 for CpG, with 17 unique donors).

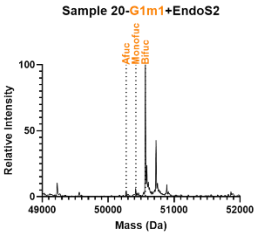

Sample 2-G1m1+EndoS2
Sample 3-G1m1+EndoS2
Sample 5-G1m1+EndoS2
Sample 6-G1m1+EndoS2
Sample 7-G1m1+EndoS2
Sample 8-G1m1+EndoS2
Sample 9-G1m1+EndoS2
Sample 10-G1m1/nG1m1+EndoS2
Sample 11-G1m1+EndoS2
Sample 12-G1m1+EndoS2
Sample 13-G1m1+EndoS2
Sample 14-G1m1,2/G1m1+EndoS2
Afuc
Monofuc
Bifuc
Relative Intensity
Mass (Da)
0
50
100
49000
50000
51000
52000

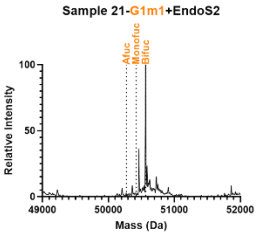

Sample 16-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

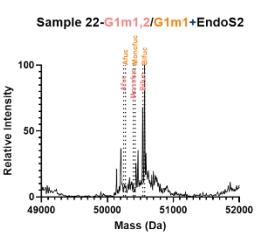

Sample 17-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

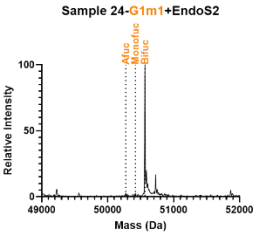

Sample 18-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

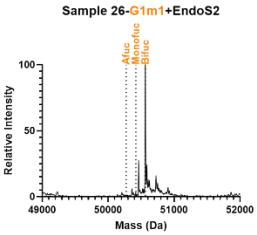

Sample 19-G1m1/nG1m1+EndoS2
Relative Intensity
Mass (Da)

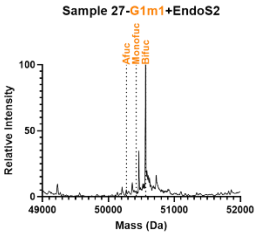

Sample 20-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

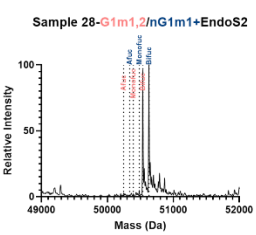

Sample 21-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

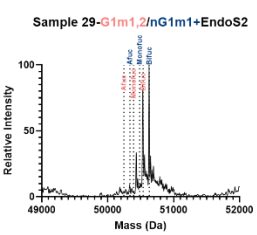

Sample 22-G1m1,2/G1m1+EndoS2
Relative Intensity
Mass (Da)

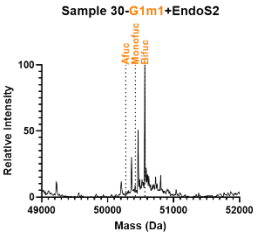

Sample 24-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

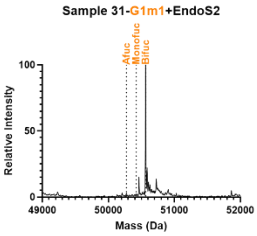

Sample 26-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

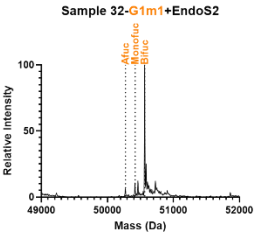

Sample 27-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

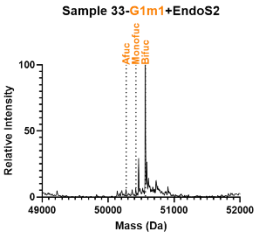

Sample 28-G1m1,2/nG1m1+EndoS2
Relative Intensity
Mass (Da)

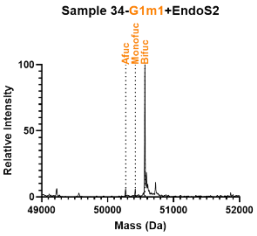

Sample 29-G1m1,2/nG1m1+EndoS2
Relative Intensity
Mass (Da)

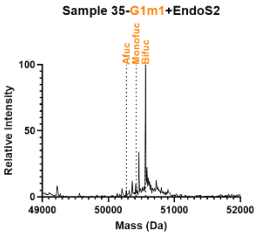

Sample 30-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

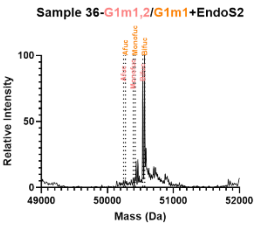

Sample 31-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

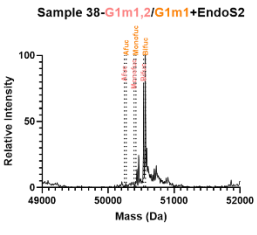

Sample 32-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

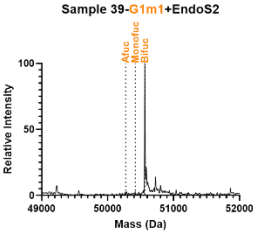

Sample 33-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

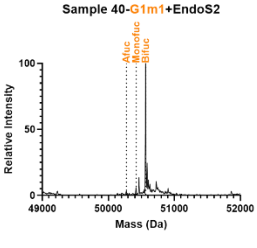

Sample 34-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

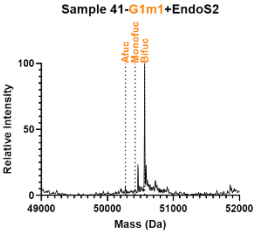

Sample 35-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

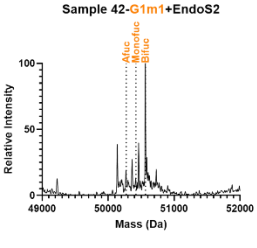

Sample 36-G1m1,2/G1m1+EndoS2
Relative Intensity
Mass (Da)

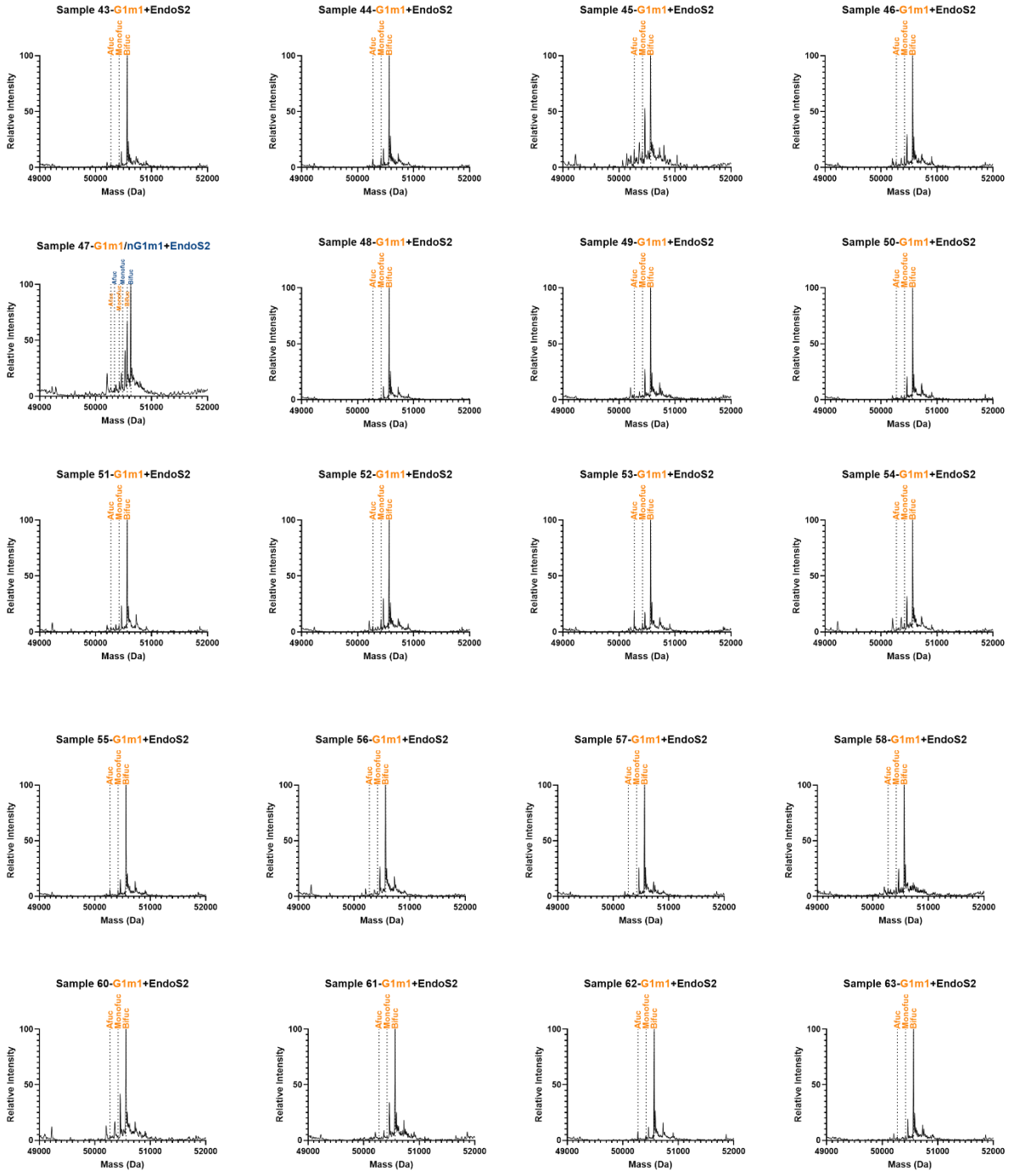

Sample 38-G1m1,2/G1m1+EndoS2
Relative Intensity
Mass (Da)


Sample 39-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

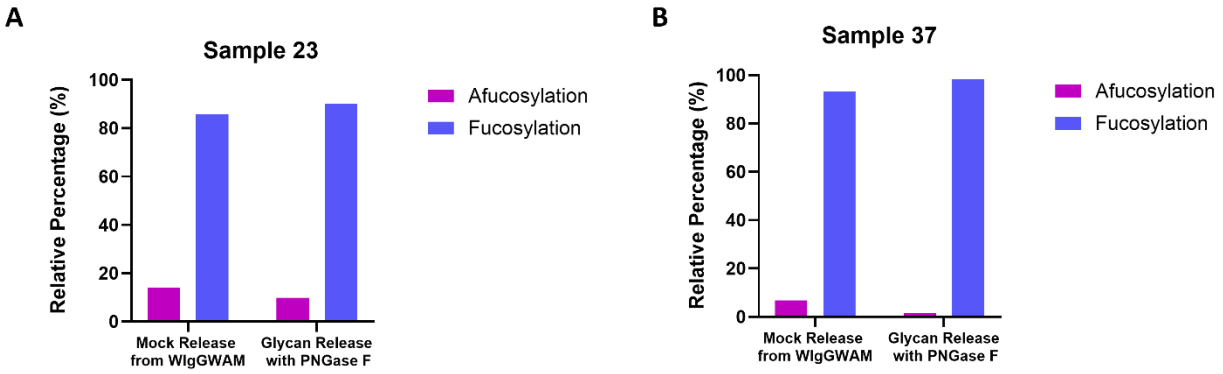

Sample 40-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)


Sample 41-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)

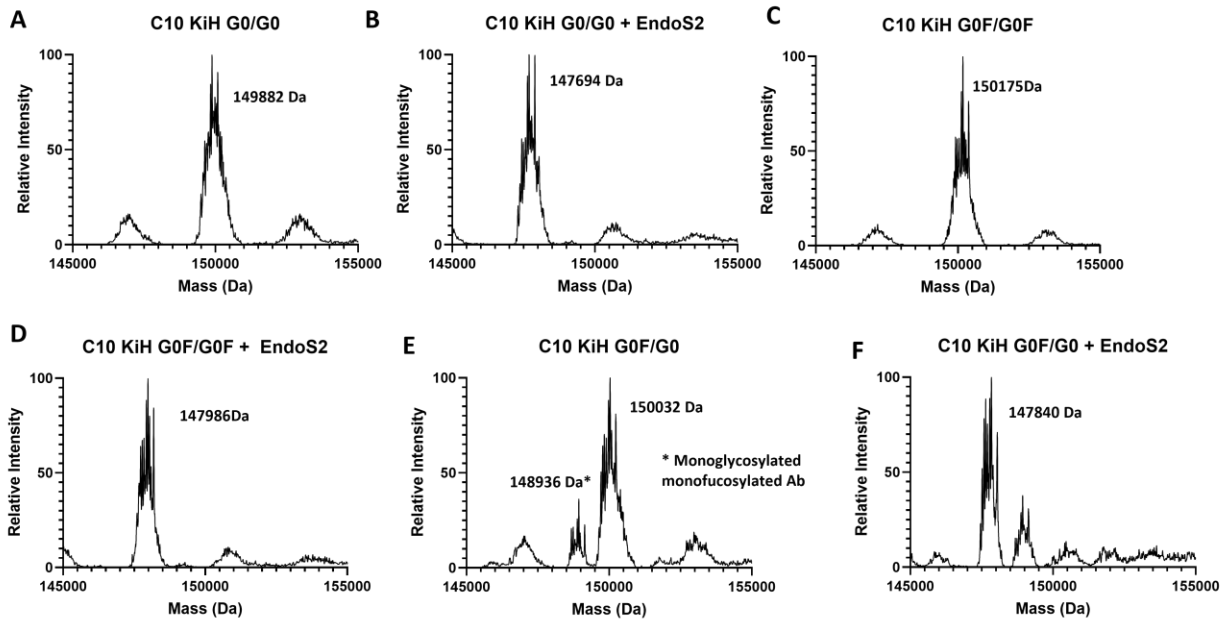

Sample 42-G1m1+EndoS2
Afuc
Monofuc
Difuc
Relative Intensity
Mass (Da)



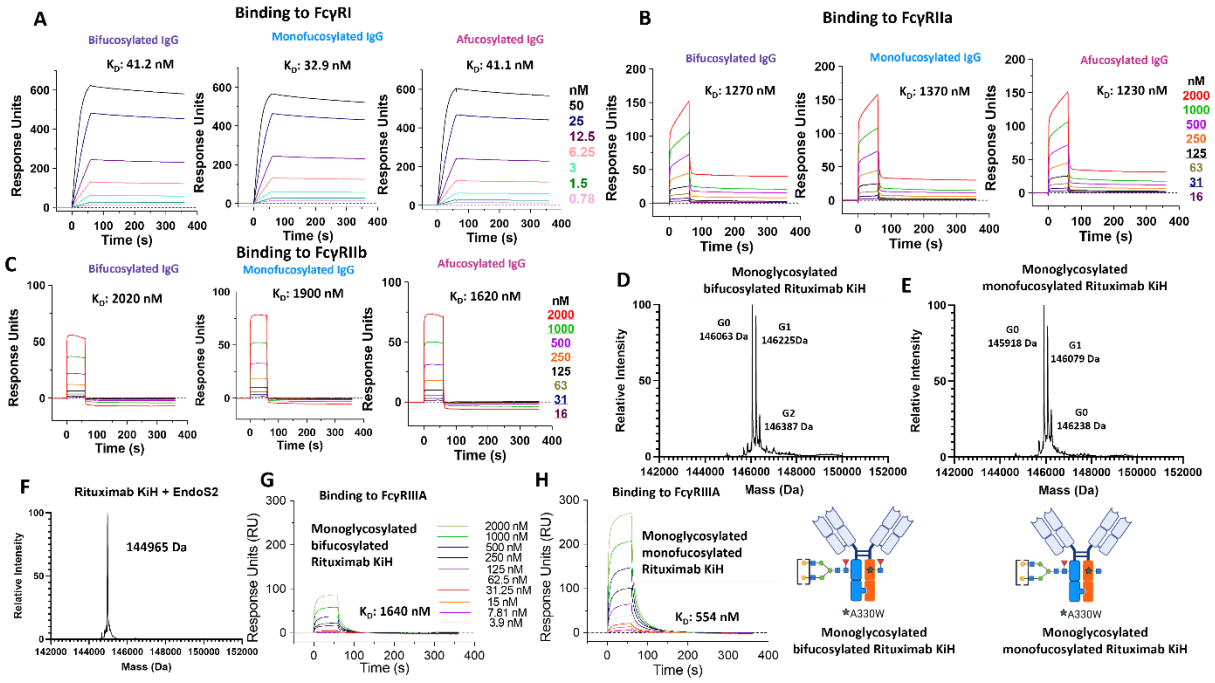
Supplementary Figure 12: WIgGWAM analysis of dengue patient plasma samples. Deconvoluted spectra of dengue patient plasma samples with EndoS2. The dotted lines annotate the peaks corresponding to the bifucosylated, monofucosylated, and afucosylated mass of the Fc alleles present. For all the deconvoluted intact LC/MS spectra, the highest peak was normalized to 100.



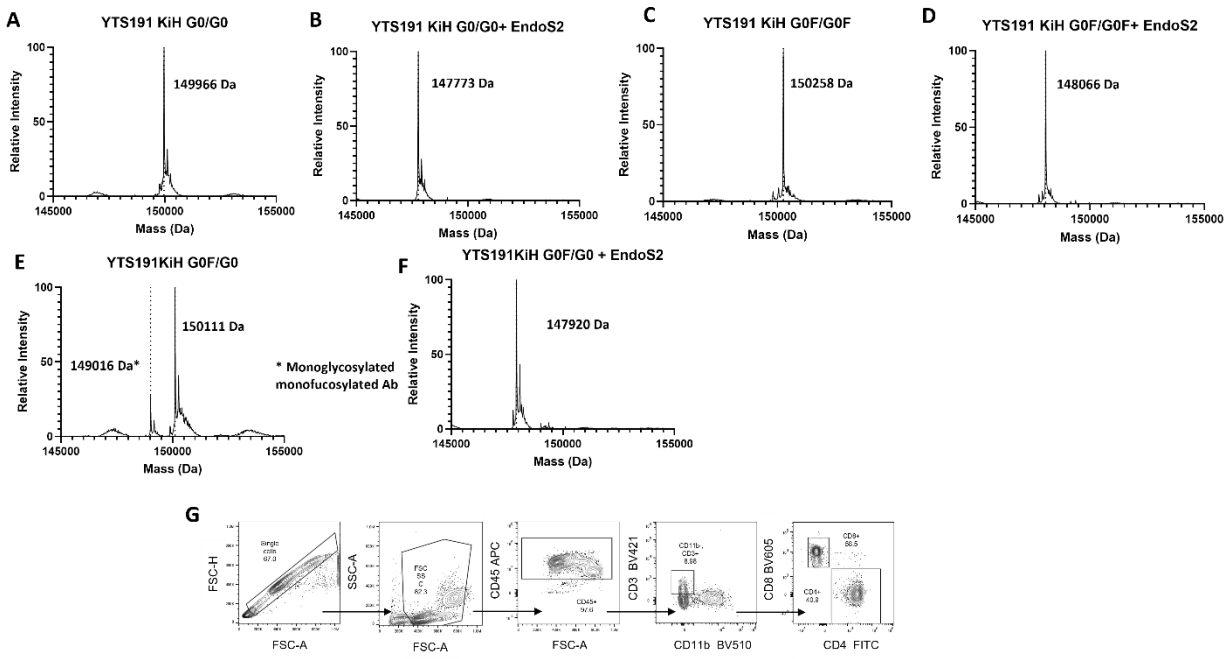
Supplementary Figure 13: WIgGWAM and glycan release glycoprofiling methods have similar results. (A-B) Bar graphs comparing the relative percentages of afucosylation (purple) and fucosylation (blue) when glycans were released with PNGase F from plasma of dengue patients 23 **(A)** and 37 **(B)** or when WIgGWAM was performed on the same plasma samples after a mock release calculation.



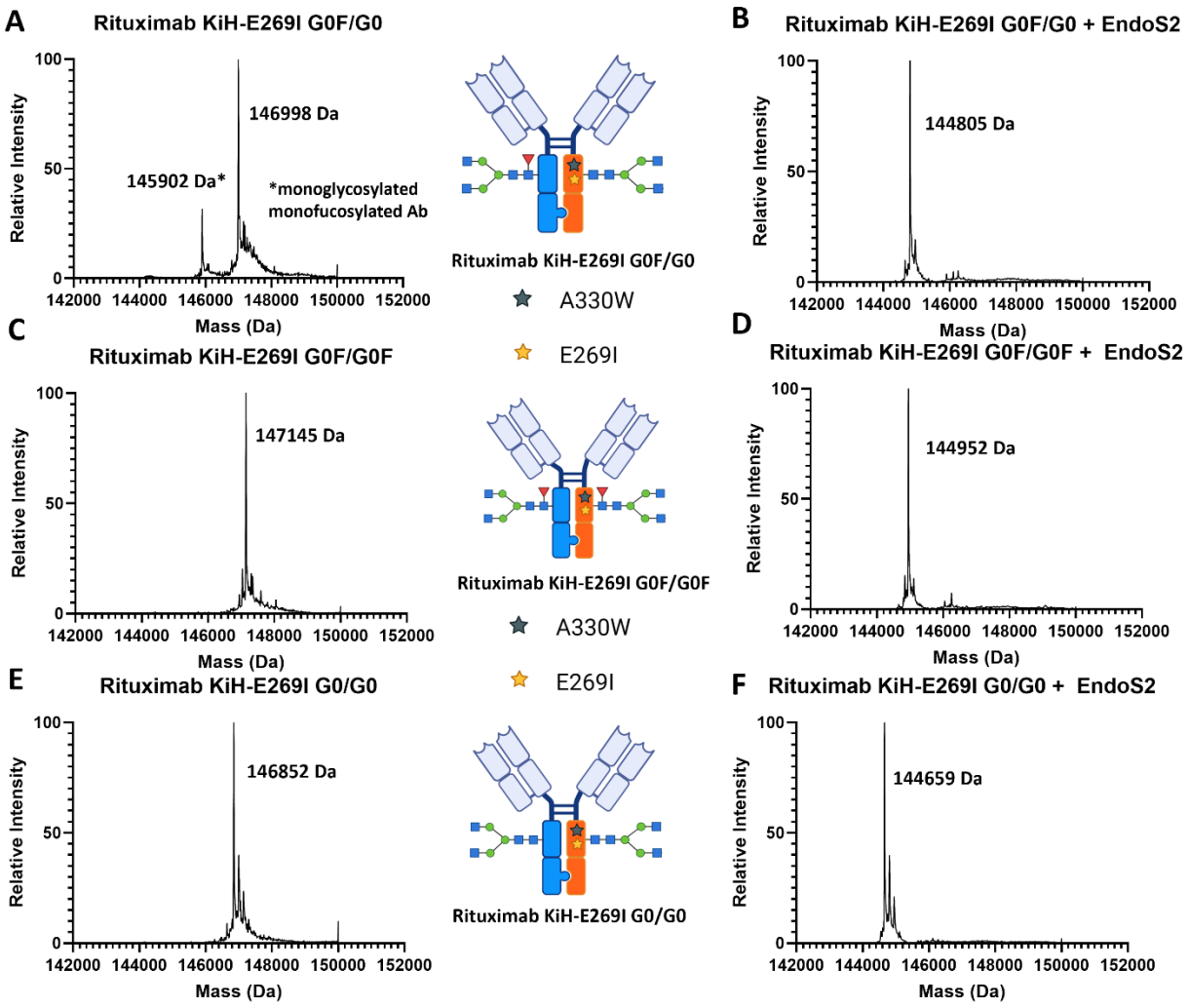
Supplementary Figure 14: Glycoengineering of KiH-A330W anti-DENV2 E protein IgG1 (C10). Deconvoluted intact LC/MS spectra depicting **(A-B)** the afucosylated agalactosylated knob-in-hole A330W C10 IgG1 **(A)** untreated or after addition of **(B)** EndoS2, **(C-D)** the bifucosylated agalactosylated knob-in-hole A330W C10 IgG1 **(C)** untreated or after addition of **(D)** EndoS2, and **(E-F)** the monofucosylated agalactosyalted knob-in-hole A330W C10 IgG1 **(E)** untreated or after addition of **(F)** EndoS2. For all the deconvoluted intact LC/MS spectra, the highest peak was normalized to 100.



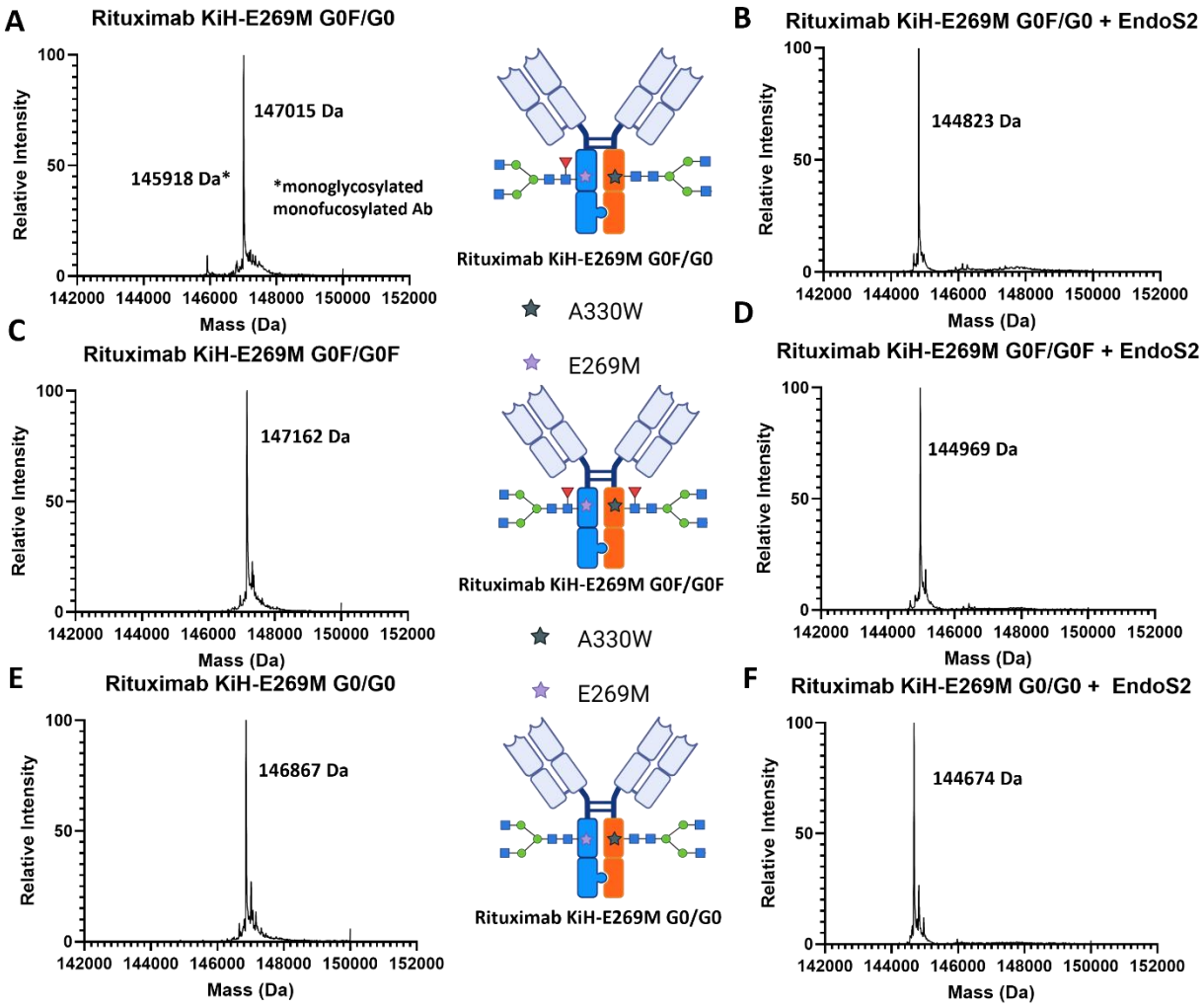
Supplementary Figure 15: Binding of bifucoyslated, monofucosylated, and afucosylated IgG1s to Fcγ receptors. **(A-C)** Surface plasmon resonance (SPR) sensograms of bifucosylated, monofucosylated, and afucosylated anti-DENV2 E protein IgG1 (C10) binding to **(A)** FcγRI, **(B)** FcγRIIa, and **(C)** FcγRIIb. **(D-F)** Deconvoluted intact LC/MS spectra depicting **(D)** monoglycosylated bifucosylated knob-in-hole A330W Rituximab IgG1 **(E)** monoglycosylated monofucosylated knob-in-hole A330W Rituximab IgG1, and **(F)** knob-in-hole A330W Rituximab IgG1 after addition of EndoS2. For all the deconvoluted intact LC/MS spectra, the highest peak was normalized to 100. **(G-H)** Surface plasmon resonance (SPR) sensograms of monoglycosylated bifucosylated knob-in-hole A330W Rituximab IgG1 **(G)** and monoglycosylated monofucosylated knob-in-hole A330W Rituximab IgG1 **(H)** binding to FcγRIIIA-F158. Antibody diagrams created in BioRender. D, J. (2025) https://BioRender.com/yzzhk0x.



Supplementary Figure 16: Glycoengineering of KiH-A330W anti-CD4 IgG1 (YTS191). Deconvoluted intact LC/MS spectra depicting **(A-B)** the afucosylated agalactosylated knob-in-hole A330W YTS191 IgG1 **(A)** untreated or after addition of **(B)** EndoS2, **(C-D)** the bifucosylated agalactosylated knob-in-hole A330W YTS191 IgG1 **(C)** untreated or after addition of **(D)** EndoS2, and **(E-F)** the monofucosylated agalactosyalted knob-in-hole A330W YTS191 IgG1 **(E)** untreated or after addition of **(F)** EndoS2. For all the deconvoluted intact LC/MS spectra, the highest peak was normalized to 100. (G) Gating strategy for the CD4+ T-cell depletion assays in Figure 7G-H.



Supplementary Figure 17: Glycoengineering of KiH-A330W-E269I Rituximab IgG1. Deconvoluted intact LC/MS spectra depicting **(A-B)** the monofucosylated agalactosylated knob-in-hole A330W-E269I Rituximab IgG1 **(A)** untreated or after addition of **(B)** EndoS2, **(C-D)** the bifucosylated agalactosylated knob-in-hole A330W-E269I Rituximab IgG1 **(C)** untreated or after addition of **(D)** EndoS2, and **(E-F)** the afucosylated agalactosyalted knob-in-hole A330W-E269I Rituximab IgG1 **(E)** untreated or after addition of **(F)** EndoS2. For all the deconvoluted intact LC/MS spectra, the highest peak was normalized to 100. Antibody diagrams created in BioRender. D, J. (2025) https://BioRender.com/yzzhk0x.



Supplementary Figure 18: Glycoengineering of KiH-A330W-E269M Rituximab IgG1. Deconvoluted intact LC/MS spectra depicting **(A-B)** the monofucosylated agalactosylated knob-in-hole A330W-E269M Rituximab IgG1 **(A)** untreated or after addition of **(B)** EndoS2, **(C-D)** the bifucosylated agalactosylated knob-in-hole A330W-E269M Rituximab IgG1 **(C)** untreated or after addition of **(D)** EndoS2, and **(E-F)** the afucosylated agalactosyalted knob-in-hole A330W-E269M Rituximab IgG1 **(E)** untreated or after addition of **(F)** EndoS2. For all the deconvoluted intact LC/MS spectra, the highest peak was normalized to 100. Antibody diagrams created in BioRender. D, J. (2025) https://BioRender.com/yzzhk0x.

Diagnosis	Healthy	COVID-19*
Number	12	19
Age		
Mean (SD)	63.6 (15.7)	67.9 (14.5)
Sex n (%),		
Male	10 (83.3)	10 (52.6)
Female	2 (16.7)	8 (42.1)
Race n (%)		
White	5 (41.7)	4 (21.0)
African American	6 (50)	14 (73.7)
Asian/Pacific Islander	1 (8.3)	0 (0)

*missing demographic data for 1 patient

Supplementary Table 1: Demographic data for plasma samples in the COVID study.

Classification according to WHO 1997 criteria	**DF**	**DHF**	**DSS**
Number	31	14	13
Age			
Mean (SD)	8 (3.5)	11 (2.5)	8 (3.7)
Sex n (%),			
Male	19 (61.3)	7 (50)	5 (38.5)
Female	12 (38.7)	7 (50)	8 (61.5)
Immune Status* n (%),			
Primary	14 (45.2)	0 (0)	0 (0)
Secondary	17 (54.8)	14 (100)	13 (100)
Viral Load † (median FFU/ml)	10.7	6.16	10900

*Determined by HI test; † Determined by qRT-PCR

Supplementary Table 2: Demographic and clinical data for the dengue patient plasma samples.