

Supplementary Materials for

Surface Ig variable domain glycosylation affects autoantigen binding and acts as threshold for human autoreactive B cell activation

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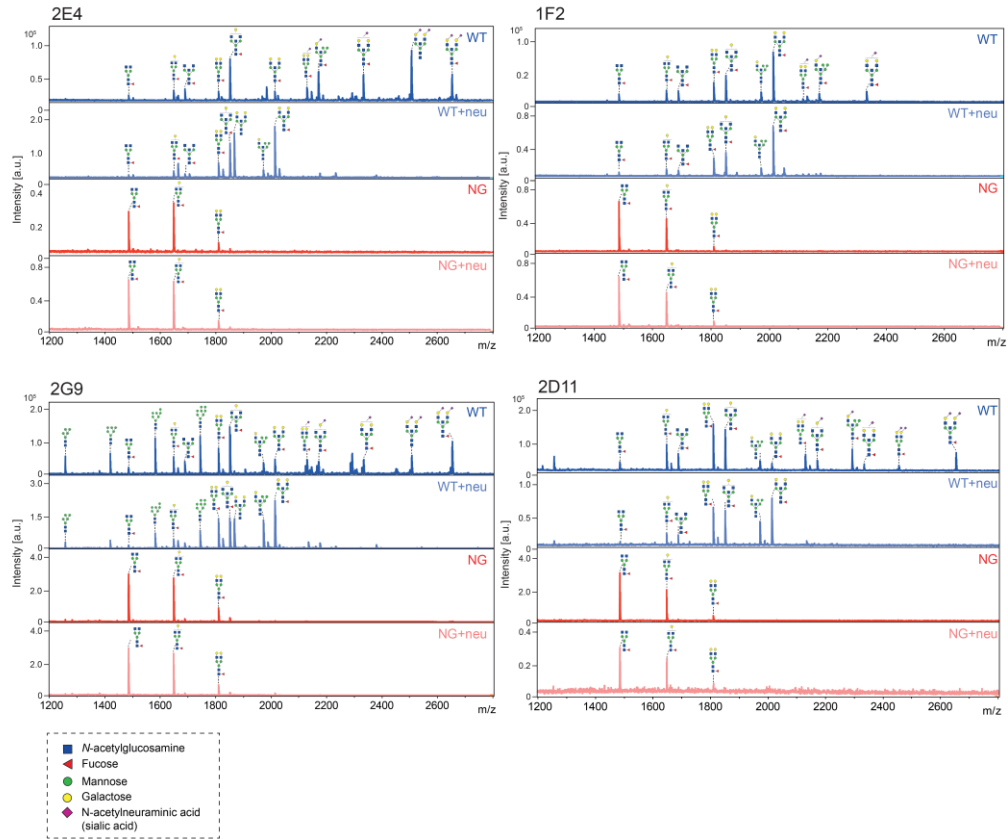


Fig. S1. MALDI-TOF analysis of released and stabilized *N*-linked variable domain and Fc-glycans from WT (blue), WT+neu (light blue), NG (red) and NG+neu (light red) ACPA-IgG 2E4, 1F2, 2G9 and 2D11. Glycan structures of the most abundant *N*-linked glycan peaks are depicted. Blue square: *N*-acetylglucosamine (GlcNAc), green circle: mannose, yellow circle: galactose, red triangle: fucose, purple diamond: α 2,6-linked *N*-acetylneuraminic acid.

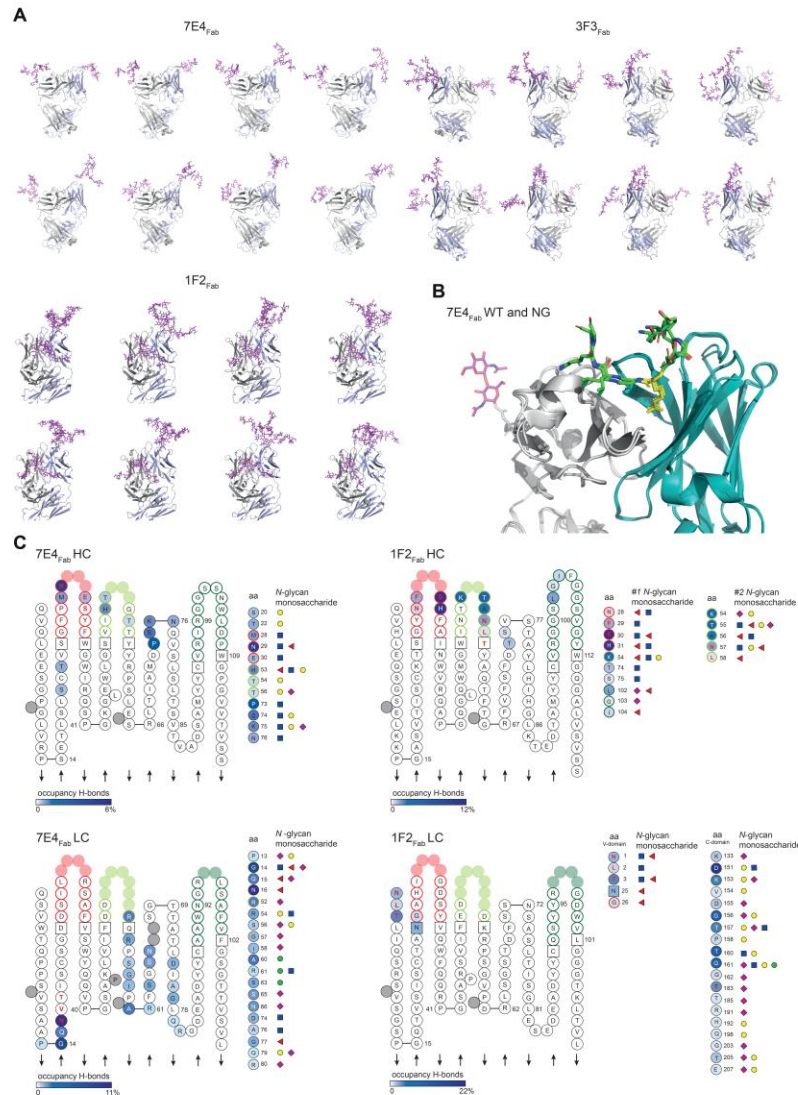


Fig. S2. Molecular dynamics (MD)-simulations to predict VDG-antibody interactions and 7E4_{Fab} crystal structure comparison with and without N-linked glycan sites. (A) MD-simulations, 8 time-points, of 7E4_{Fab}, 3F3_{Fab} and 1F2_{Fab} crystal structures modelled with full length disialylated VDG (sticks in magenta). (B) Superposition of 7E4_{Fab} crystal structures expressed with and without N-linked glycan sites. 7E4_{Fab} WT including N-linked glycan sites was crystallized together with the first two GlcNAcs (sticks in magenta) of the LC VDG. LC is depicted in light grey, HC in steel blue, the peptides bound to the respective Fab as sticks with carbon (green), oxygen (red) and nitrogen (blue) atoms. (C) ACPA 7E4_{Fab} and 1F2_{Fab} HC and LC variable gene aa-sequences, based on IMGT (2D), are depicted. Acceptor/ donor pairs of H-bond interactions between the antibody and HC (#1 and #2)/LC N-glycans are visualized. A high occupancy is depicted in dark blue (HC: 6%; 12%, LC: 11%; 22%) and a low occupancy is depicted in light blue (0.01-0.2%). Amino acids and their respective interaction partners (N-glycan monosaccharides) are shown. Blue square: N-acetylglucosamine, green circle: mannose, yellow circle: galactose, red triangle: fucose, purple diamond: α 2,6-linked N-acetylneuraminic acid (sialic acid).

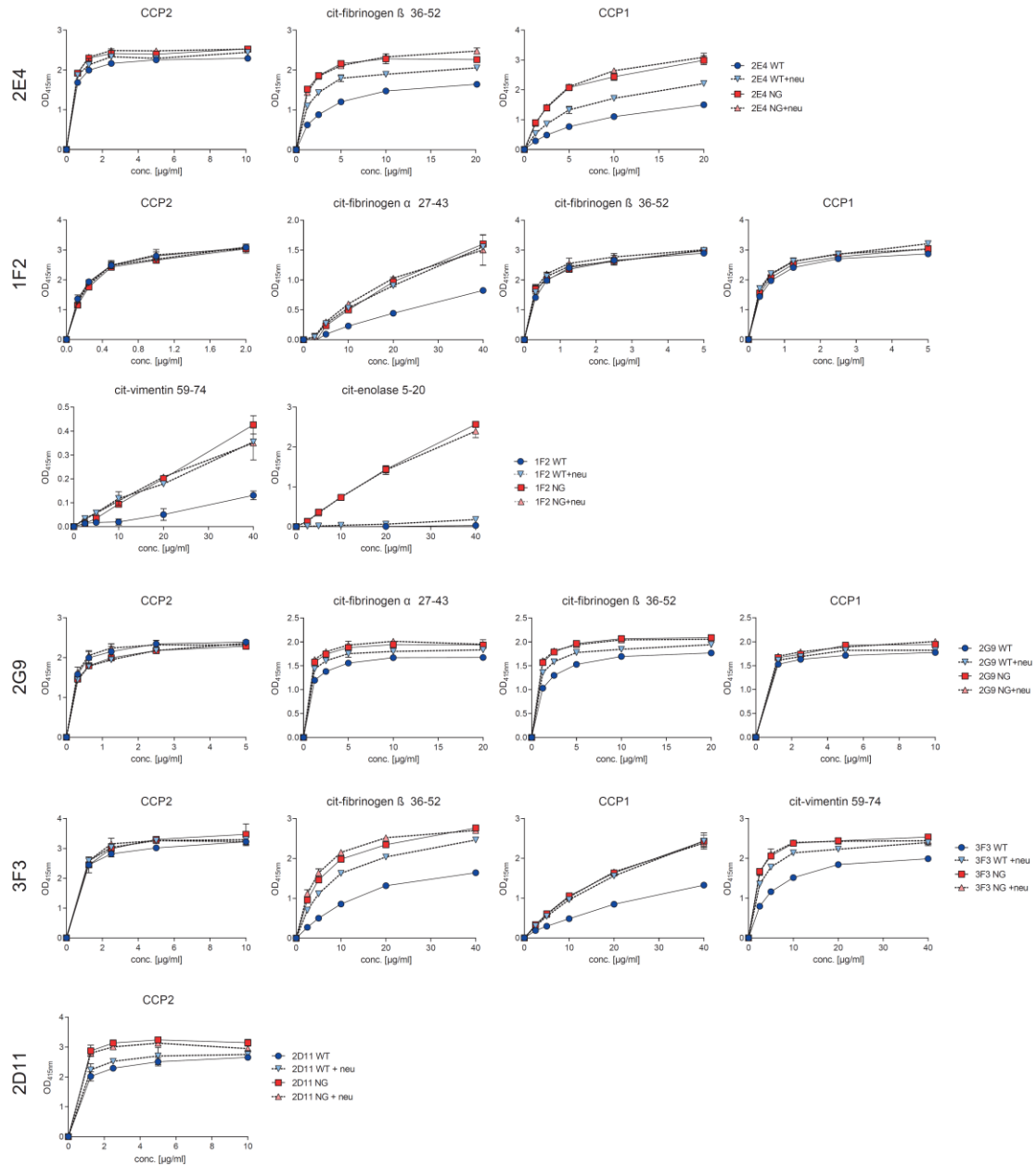


Fig. S3. Impact of disialylated ACPA VDG on citrullinated peptide binding. ELISA titration binding curves of the ACPA 2E4, 1F2, 2D11, 2G9 and 3F3 (0-40µg/ml) variants (WT, WT+neu, NG and NG+neu) towards citrullinated peptides (CCP2, CCP1, cit-fibrinogen α 27-43, cit-fibrinogen β 36-52, cit-vimentin 59-74, cit-enolase 5-20). Binding to the arginine control peptide was subtracted. Reactivity was determined via the OD at 415nm. Each data point represents the mean of two technical replicates. N= 2-3.

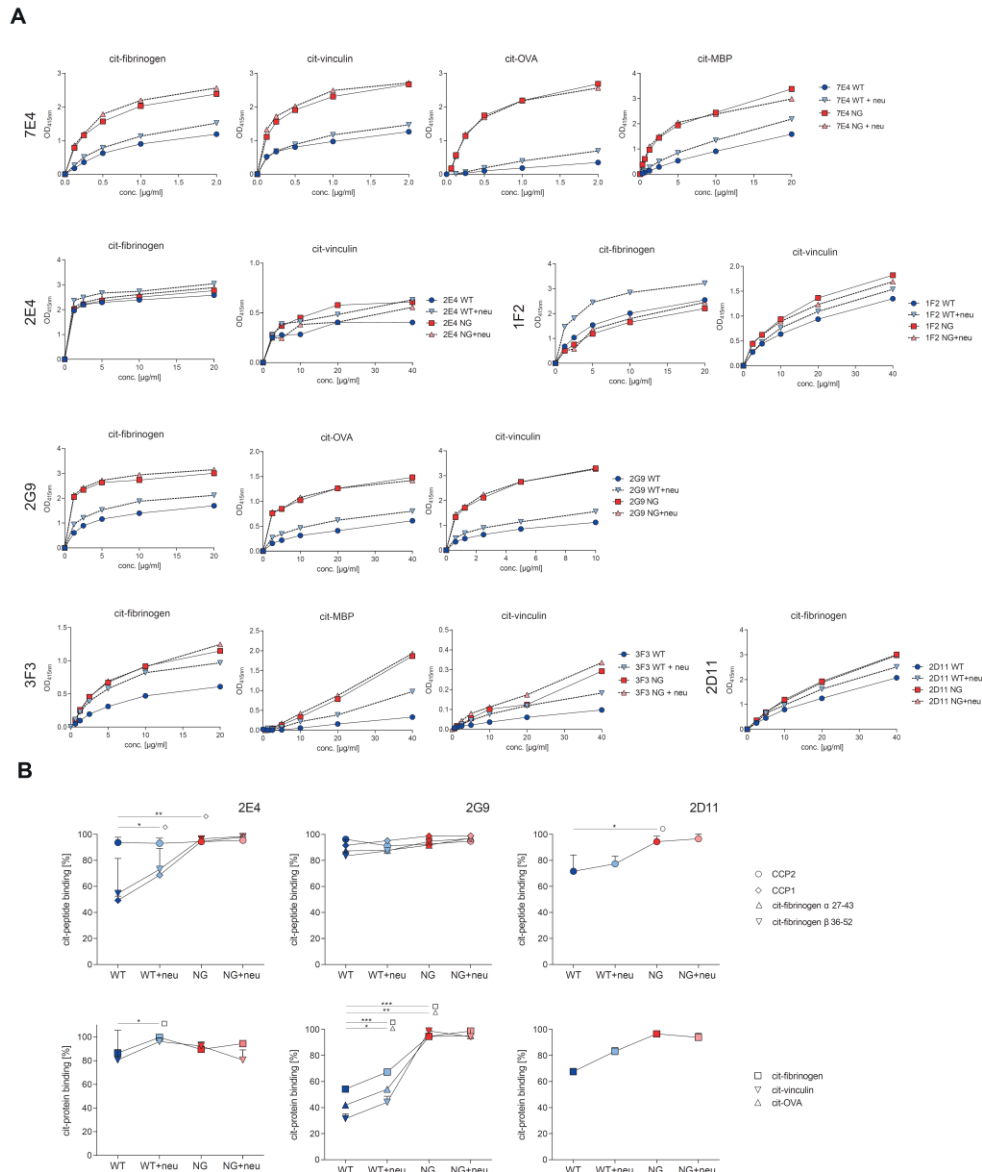


Fig. S4. Impact of disialylated ACPA VDG on citrullinated protein binding. (A) ELISA titration binding curves of the ACPA 2E4, 1F2, 2D11, 2G9 and 3F3 (0-40 μ g/ml) variants (WT, WT+neu, NG and NG+neu) towards citrullinated proteins (cit-fibrinogen, cit-vinculin, cit-MBP and cit-OVA). Binding to the arginine control protein was subtracted. Reactivity was determined via the OD at 415nm. N= 2-3. (B) Relative binding of the ACPA mAb 2E4, 2G9 and 2D11 (10-40 μ g/ml) variants (WT, WT+neu, NG, NG+neu) towards citrullinated peptides and proteins. N=2-3. Unpaired two-tailed t-tests assuming the same SD. 2E4WT-NG: **p(CCP1)=0.0028; 2E4WT-WT+neu: *p(CCP1)=0.0143, *p(cit-fibrinogen)=0.0226; 2G9WT-NG: ***p(cit-fibrinogen)=0.0009, **p(cit-OVA)=0.0047; 2G9WT-WT+neu: ***p(cit-fibrinogen)=0.0004, *p(cit-OVA)=0.0297; 2D11WT-NG: *p(CCP2)=0.0406.

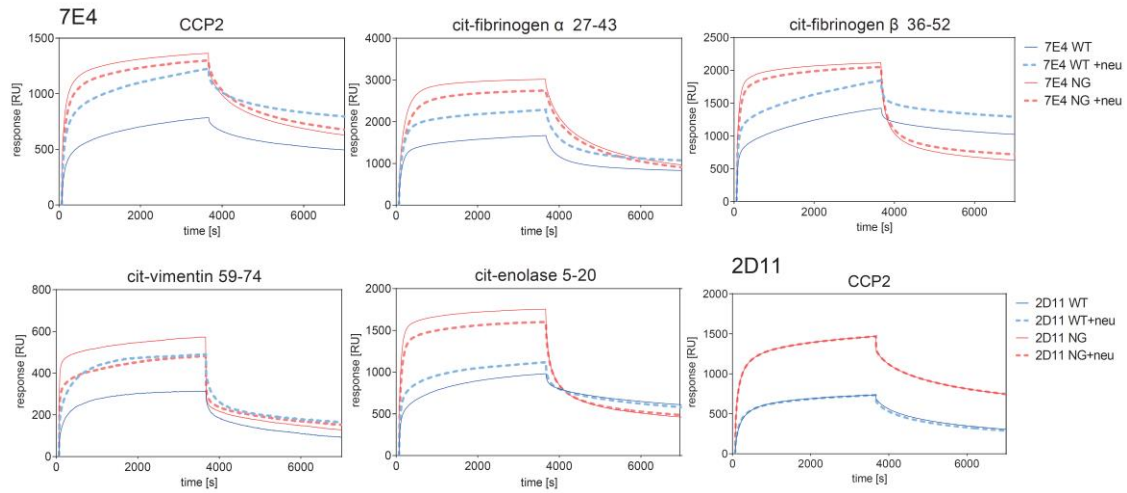


Fig. S5. SPR sensorgrams of ACPA-IgG 7E4 and 2D11 binding to citrullinated peptides. Antigen association and dissociation are represented as response units (RU) over time (s). The experiment represents 2 technical replicates. Four different monoclonal antibody variants were assessed: WT, WT+neu, NG and NG+neu.

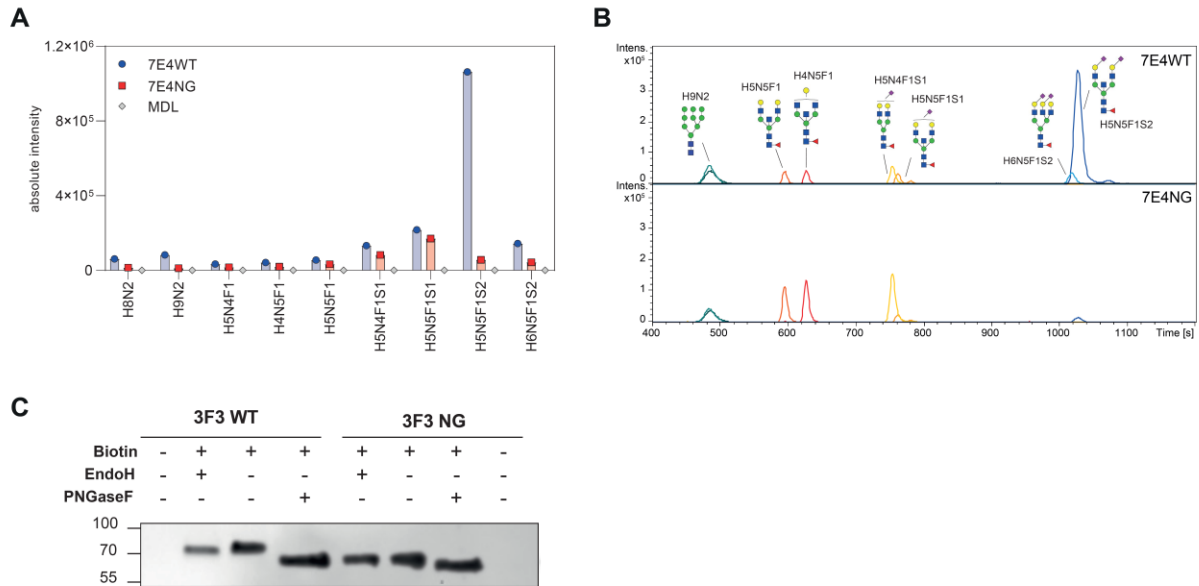


Fig. S6. Human Ramos IgG B cell receptor glycan analysis. (A) Absolute intensity of glycan traits expressed on the 7E4 WT, NG and MDL IgG BCR after passing QC settings. (B) LC-chromatogram of glycan traits expressed on the 7E4 WT and NG (IgG BCR after passing QC settings). The respective glycan traits are schematically illustrated. (C) Western blot analyses of PBS-treated (- Biotin) or biotinylated (+ Biotin) surface IgG 3F3 WT and NG BCRs after NeutrAvidin capturing. Biotinylated mIgG with and without VDG were treated with 2U EndoH (cleavage of high-mannose structures) or 2U PNGaseF (cleavage of all *N*-glycans).

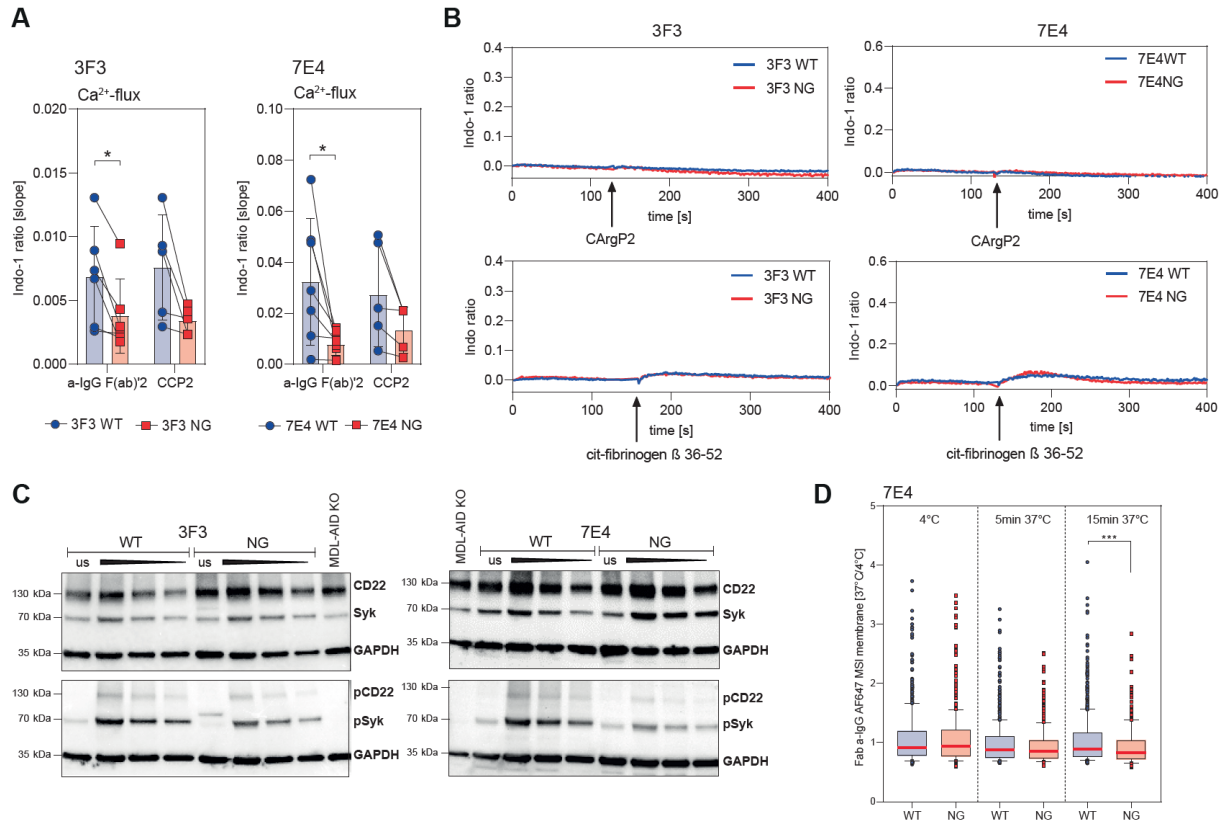


Fig. S7. Impact of mIgG VDG on human Ramos B cell activation and BCR downmodulation. (A) Paired analysis of Ca²⁺-flux speed (slope) for mIgG 3F3/ 7E4 WT and NG Ramos B cells after a-IgG F(ab)₂ and antigen (CCP2-SA) stimulation. Paired two-tailed t-test. N=5-7. 3F3: *p=0.0377; 7E4: *p=0.0206. (B) Overlays of WT and NG Ca²⁺-flux (Ca²⁺ bound Indo-1/ unbound Indo-1) of mIgG 3F3/ 7E4 WT and NG Ramos B cells after stimulation with CArgP2-SA or cit-fibrinogen β 36-52. (C) Western blot analyses of mIgG 3F3/ 7E4 WT and NG Ramos B cells after 5 minutes of CCP2 stimulation or unstimulated (us). CD22, Syk, pCD22 (Y822) and pSyk(Y352) expression are shown. Cell lysis of 1 million (unstimulated and stimulated 1st slot), 0.5 million (stimulated 2nd slot) and 0.25 million (stimulated 3rd slot) were blotted. GAPDH was used as loading control. Cell lysates of 1 million MDL-AID KO cells were added as additional control. (D) Remaining mIgG 7E4 WT and NG expression after CCP2-SA stimulation and incubation at 4°C or 5, 15 minutes at 37°C. N = 619, 459, 645, 433, 738 and 302 cell slices respectively. Ordinary one-way ANOVA, ***p=0.0002.

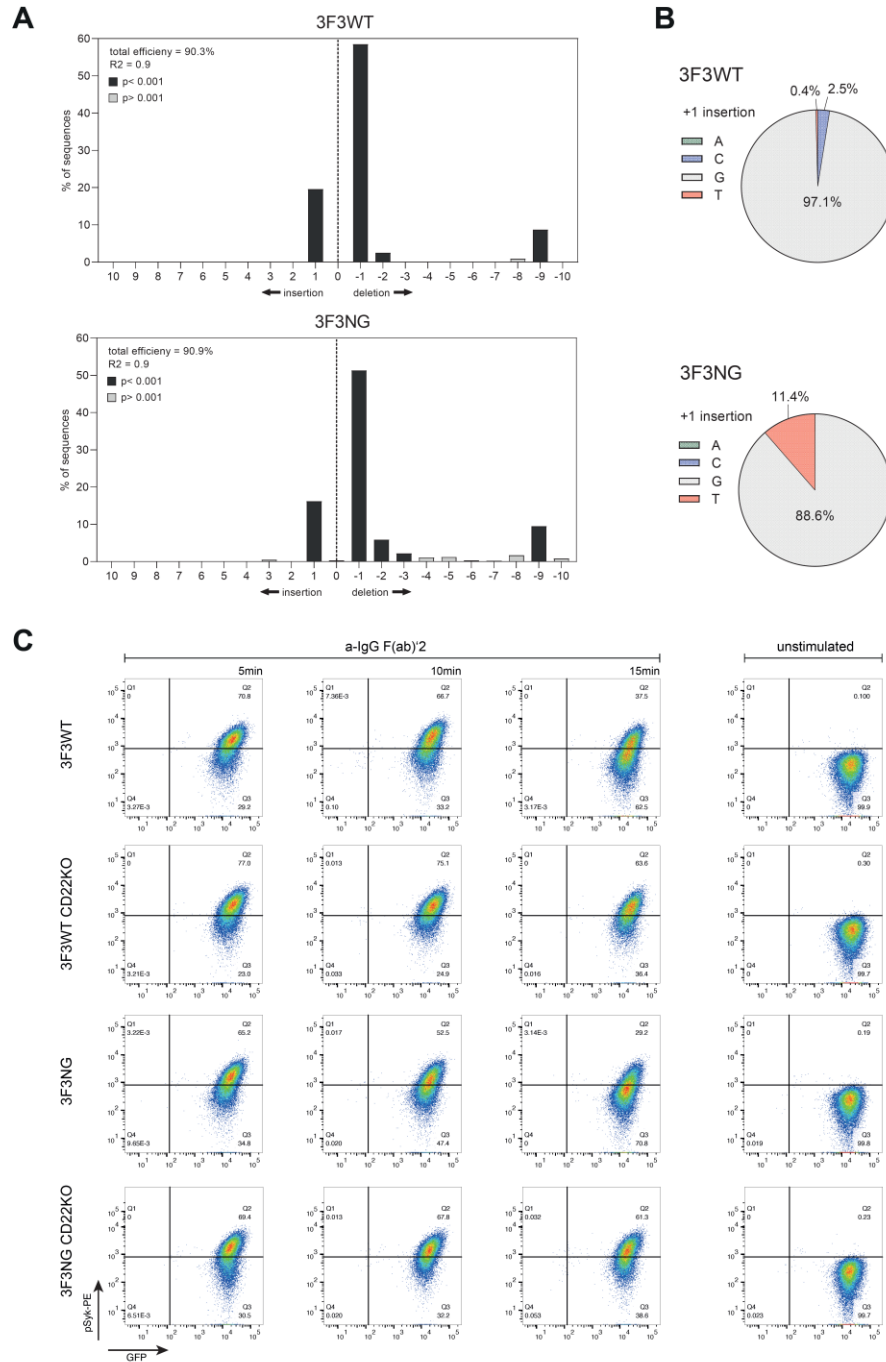


Fig. S8. TIDE analysis 3F3 CD22KO Ramos B cells. (A) Comprehensive profile of all insertions and deletions (indels) in the CD22 KO edited sample according to the TIDE analysis. The total KO efficiency is depicted. (B) An estimate of the +1 insertion base composition according to the TIDE analysis. (C) pSyk(Y348) expression of mIgG 3F3 WT, WT CD22KO, NG and NG CD22KO BCRs after 5, 10 and 15 minutes of a-IgG F(ab)'2 stimulation. Gating is based on the unstimulated cells. The pSyk expression is depicted on the y-axis and the GFP expression on the x-axis. One experiment is exemplarily shown.

Table S1. SPR Kd1 and Kd2 values of monoclonal ACPA-IgG with VDG (WT) and w/o VDG (NG) calculated based on biphasic curve fits.

Kd1	CCP2	cit-fibrinogen α 27-43	cit-fibrinogen β 36-52	cit-vimentin 59-74	cit-enolase 5-20	Kd1	CCP2
7E4 WT	8.80E-05	nd*	7.14E-05	1.78E-05	3.72E-05	2D11 WT	1.21E-04
7E4 WT+neu	2.89E-04	6.10E-04	1.19E-04	2.90E-05	5.18E-05	2D11 WT+neu	7.82E-05
7E4 NG	9.36E-05	7.33E-05	nd*	4.83E-04	3.99E-03	2D11 NG	5.94E-05
7E4 NG+neu	nd*	7.93E-04	nd*	3.96E-05	nd*	2D11 NG+neu	7.53E-05
Kd2	CCP2	cit-fibrinogen α 27-43	cit-fibrinogen β 36-52	cit-vimentin 59-74	cit-enolase 5-20	Kd2	CCP2
7E4 WT	3.74E-05	2.22E-04	nd*	1.41E-04	3.20E-04	2D11 WT	nd*
7E4 WT+neu	nd*	1.39E-04	nd*	nd*	4.60E-03	2D11 WT+neu	5.75E-05
7E4 NG	1.94E-04	4.36E-05	nd*	nd*	nd*	2D11 NG	7.96E-05
7E4 NG+neu	5.39E-05	6.06E-05	nd*	nd*	nd*	2D11 NG+neu	nd*

*nd= not-determined, negative Kon values.

Table S2. Equilibrium K_D values of monoclonal ACPA-IgG with VDG (WT) and without VDG (NG). Equilibrium K_D and B_{max} values were calculated based on ELISA curves and shown including their standard errors.

peptide	7E4 WT		7E4 WT + neu		7E4 NG		7E4 NG + neu	
	K_D [M]	B_{max}	K_D [M]	B_{max}	K_D [M]	B_{max}	K_D [M]	B_{max}
CCP2	1.04E-08 ± 6.56E-10	2.54 ± 0.048	6.78E-09 ± 5.02E-10	2.81 ± 0.051	2.66E-09 ± 4.77E-10	3.25 ± 0.082	2.88E-09 ± 2.29E-10	3.16 ± 0.037
Cit-fibrinogen α 27-43	6.64E-09 ± 5.67E-10	3.20 ± 0.066	5.26E-09 ± 6.84E-10	3.48 ± 0.097	2.83E-09 ± 3.47E-10	3.43 ± 0.062	2.32E-09 ± 3.47E-10	3.29 ± 0.063
CCP1	NM*	NM*	NM*	NM*	7.16E-08 ± 6.38E-09	4.83 ± 0.280	5.82E-08 ± 6.91E-09	4.11 ± 0.298
Cit-vimentin 59-74	NM*	NM*	NM*	NM*	4.91E-08 ± 9.56E-09	4.44 ± 0.497	4.76E-08 ± 1.03E-08	4.51 ± 0.553
Cit-enolase 5- 20	NM*	NM*	NM*	NM*	1.73E-07 ± 2.10E-08	5.39 ± 0.453	1.81E-07 ± 1.92E-08	5.54 ± 0.413
Cit-fibrinogen β 36-52	1.30E-08 ± 3.67E-10	2.11 ± 0.022	9.24E-09 ± 3.54E-10	2.31 ± 0.029	5.53E-09 ± 6.03E-11	2.70 ± 0.009	5.12E-09 ± 1.81E-10	2.61 ± 0.028

*NM= not-measurable, ELISA curves not at saturation.

Table S3. Data collection and refinement statistics. Statistics for the highest-resolution shell are shown in parentheses.

	3F3:cit-vimentin 59-74	1F2:cit-CII-C-39	1F2	7E4:cit-CII-C-48
Wavelength [Å]	0.91840	0.97622	0.97622	0.91840
Resolution range [Å]	52.28 - 2.0 (2.071 - 2.0)	36.19 - 2.85 (2.952 - 2.85)	35.01 - 3.05 (3.159 - 3.05)	59.78 - 2.45 (2.538 - 2.45)
Space group	P 21 21 21	P 21 21 21	C 1 2 1	C 2 2 21
Unit cell a, b, c [Å]	53.379, 82.094, 135.613	53.01, 89.65, 118.68	152.43, 88.8, 88.02	77.183, 151.774, 97.066
Unit cell a, b, c [Å]	90, 90, 90	90, 90, 90	90, 113.26, 90	90, 90, 90
Total reflections	325932 (33061)	121917 (11635)	95969 (9446)	195694 (19513)
Unique reflections	40868 (4035)	13784 (1351)	20678 (2032)	21351 (2104)
Multiplicity	8.0 (8.2)	8.8 (8.5)	4.6 (4.6)	9.2 (9.3)
Completeness [%]	99.37 (99.51)	98.10 (98.69)	99.63 (99.61)	99.96 (99.86)
Mean I/sigma [I]	9.93 (2.16)	9.97 (3.66)	9.92 (3.31)	22.47 (4.80)
Wilson B-factor	32.61	44.66	77.47	57.62
R-merge	0.1256 (0.886)	0.1524 (0.5557)	0.09467 (0.3793)	0.05246 (0.4131)
R-meas	0.1344 (0.9457)	0.1623 (0.5933)	0.1073 (0.4305)	0.05567 (0.438)
R-pim	0.04728 (0.3275)	0.05479 (0.2044)	0.04964 (0.2002)	0.01827 (0.1431)
CC1/2	0.996 (0.682)	0.992 (0.901)	0.993 (0.938)	0.999 (0.951)
CC*	0.999 (0.901)	0.998 (0.974)	0.998 (0.984)	1 (0.987)
Reflections used in refinement	40845 (4025)	13532 (1351)	20638 (2032)	21348 (2104)
Reflections used for R-free	2023 (193)	638 (74)	1054 (102)	1129 (106)
R-work	0.2011	0.2117	0.2349	0.2384
R-free	0.2414	0.2610	0.2663	0.2746
Number of non-hydrogen atoms	3762	3372	6444	3363
macromolecules	3395	3275	6392	3276
ligands	64	64	52	39
solvent	303	33		48
Protein residues	447	434	848	440
RMS [bonds]	0.019	0.013	0.012	0.013
RMS [angles]	2.39	1.77	1.84	1.89
Ramachandran favored [%]	97.00	95.01	96.12	96.69
Ramachandran allowed [%]	3.00	4.75	3.03	3.31
Ramachandran outliers [%]	0.00	0.24	0.85	0.00
Number of TLS groups	3	3	4	3

Table S4. H-bond interaction details between the ACPA 7E4 HC and LC Fab fragments and the respective *N*-linked glycan. Shown are the H-bond donor and acceptor pairs (three letter code of the respective amino acids and monosaccharides) and the occupancy of the interaction in percentage. GlcNAc: *N*-acetyl-glucosamine, Neu5Ac: *N*-acetylneuraminic acid, Fuc: fucose, Gal: galactose, Man: mannose.

7E4 HC			7E4 LC		
donor	acceptor	oc. [%]	donor	acceptor	oc. [%]
Asn 29	GlcNAc 221	5.85	Asn 16	GlcNAc 214	10.76
GlcNAc 221	Pro 73	1.15	Fuc 226	Gly 14	8.84
Ser 74	GlcNAc 222	0.94	Fuc 226	Asn 16	2.44
GlcNAc 222	Ser 74	0.84	Fuc 226	Gln 15	1.56
Ser 74	GlcNAc 222	0.49	Gln 15	Fuc 226	1.36
Fuc 233	Asn 29	0.49	Arg 52	Neu5Ac 220	1.04
His 53	Fuc 233	0.36	Man 217	Ala 60	0.9
Fuc 233	His 53	0.35	Arg 52	Neu5Ac 220	0.88
Lys 75	GlcNAc 225	0.35	Neu5Ac 220	Asn 66	0.73
GlcNAc 222	Ser 74	0.35	Neu5Ac 220	Ser 65	0.55
Lys 75	GlcNAc 232	0.32	GlcNAc 214	Asp 74	0.42
Met 28	GlcNAc 221	0.29	Arg 52	GlcNAc 218	0.37
Lys 75	GlcNAc 229	0.22	Arg 54	Man 217	0.35
Asn 76	GlcNAc 221	0.22	Gly 77	Fuc 226	0.31
Asn 76	GlcNAc 222	0.19	Man 217	Ser 63	0.28
GlcNAc 222	Asn 76	0.14	Ser 63	Man 217	0.28
Gal 226	Thr 22	0.14	Arg 54	Neu5Ac 220	0.26
Gln 77	Gal 226	0.11	Neu5Ac 220	Ser 63	0.25
Thr 22	Gal 226	0.1	Neu5Ac 220	Ser 63	0.21
Ser 20	Gal 226	0.04	Neu5Ac 220	Ile 58	0.21
GlcNAc 232	Lys 75	0.04	Neu5Ac 220	Gly 57	0.17
GlcNAc 232	Ser 74	0.04	Arg 54	Gal 219	0.16
Gal 226	Ser 74	0.02	Ala 60	GlcNAc 218	0.11
Gal 226	Lys 75	0.02	Ser 56	Neu5Ac 220	0.08
Ser 74	GlcNAc 221	0.01	GlcNAc 215	Ala 60	0.08
Fuc 233	His 53	0.01	Ile 58	Neu5Ac 220	0.06
Lys 75	Neu5Ac 231	0.01	Neu5Ac 220	Ser 56	0.05
His 53	GlcNAc 221	0.01	Ala 60	Man 217	0.05
GlcNAc 221	Ser 74	0.01	Neu5Ac 220	Ser 56	0.05
Neu5Ac 231	Thr 56	0.01	Arg 61	Man 217	0.04
Gal 230	Thr 54	0.01	Gal 223	Gln 79	0.04
Glu 30	GlcNAc 221	0.01	Arg 54	GlcNAc 218	0.04
His 53	Gal 230	0.01	GlcNAc 215	Ala 76	0.03
			Neu5Ac 224	Gly 14	0.03
			Neu5Ac 224	Gln 15	0.03
			Ser 56	Gal 219	0.02
			Gal 219	Ser 56	0.01
			Gln 79	Neu5Ac 224	0.01
			Neu5Ac 224	Gln 79	0.01
			Neu5Ac 224	Pro 13	0.01
			Arg 61	Man 217	0.01
			Arg 80	Neu5Ac 224	0.01
			Neu5Ac 224	Pro 13	0.01
			Gln 15	Neu5Ac 224	0.01
			Neu5Ac 220	Gly 57	0.01

		Gly 57	Neu5Ac 220	0.01
		GlcNAc 214	Ala 76	0.01
		Gal 223	Pro 13	0.01

Table S5. H-bond interaction details between the ACPA 3F3 HC and LC Fab fragments and the respective *N*-linked glycan. Shown are the H-bond donor and acceptor pairs (three letter code of the respective amino acids and monosaccharides) and the occupancy of the interaction in percentage. GlcNAc: *N*-acetyl-glucosamine, Neu5Ac: *N*-acetylneuraminic acid, Fuc: fucose, Gal: galactose, Man: mannose.

3F3 HC #1 N-glycan			3F3 HC #2 N-glycan			3F3 LC		
donor	acceptor	oc. [%]	donor	acceptor	oc. [%]	donor	acceptor	oc. [%]
Fuc 236	Glu 81	13.02	Fuc 249	Glu 22	10.53	Ser 71	GlcNAc 219	15.43
Lys 18	Fuc 236	1.68	Fuc 249	Ser 74	4.57	Asn 78	GlcNAc 219	8.69
Fuc 236	Thr 70	1.21	Gal 246	Gly 25	2.00	GlcNAc 220	Asp 76	5.96
Fuc 236	Gln 64	0.68	Phe 28	GlcNAc 237	0.47	GlcNAc 219	Asp 76	4.23
GlcNAc 224	Glu 81	0.65	GlcNAc 237	Ser 24	0.41	Lys 24	GlcNAc 227	1.18
Thr 70	Fuc 236	0.26	Gal 246	Ser 24	0.21	Gal 228	Ser 26	0.96
Neu5Ac 230	Gly 65	0.24	Neu5Ac 243	Tyr 100	0.08	Lys 24	GlcNAc 220	0.54
Asn 68	GlcNAc 224	0.21	Lys 53	Man 240	0.03	Lys 24	Man 226	0.50
Fuc 236	Asn 68	0.14	Asn 29	GlcNAc 237	0.03	Thr 20	Fuc 231	0.34
Neu5Ac 230	Gln 64	0.12	Lys 53	GlcNAc 238	0.03	GlcNAc 227	Thr 75	0.31
Gln 64	Fuc 236	0.06	Neu5Ac 247	Gly 25	0.03	Neu5Ac 229	Asp 9	0.28
Met 69	GlcNAc 224	0.04	Tyr 100	Neu5Ac 243	0.03	Fuc 231	Thr 20	0.26
Lys 53	Neu5Ac 234	0.03	Lys 53	Neu5Ac 243	0.02	Neu5Ac 229	Asp 9	0.21
Lys 53	Gal 229	0.02	Ile 27	Gal 246	0.01	GlcNAc 219	Asp 76	0.19
Neu5Ac 230	Gly 14	0.02	Tyr 100	Neu5Ac 243	0.01	Ser 28	Gal 228	0.17
Fuc 236	Gly 65	0.02				Fuc 231	Thr 20	0.14
Lys 53	Neu5Ac 230	0.01				Neu5Ac 225	Ser 26	0.14
Thr 83	Neu5Ac 230	0.01				GlcNAc 227	Ser 25	0.14
GlcNAc 224	Thr 83	0.01				Thr 75	GlcNAc 227	0.13
Thr 70	Neu5Ac 234	0.01				GlcNAc 227	Asp 76	0.08
						Neu5Ac 225	Ser 73	0.06
						Arg 18	Fuc 231	0.05
						Gal 228	Tyr 7	0.05
						Gal 228	Gln 27	0.05
						Asp 9	Gal 228	0.04
						Neu5Ac 229	Tyr 7	0.03
						Ser 73	Neu5Ac 225	0.03
						Tyr 7	Neu5Ac 229	0.02
						Neu5Ac 225	Gln 27	0.02
						Tyr 7	Gal 228	0.02
						Fuc 231	Asn 22	0.02
						Tyr 7	GlcNAc 227	0.02
						Gal 228	Thr 75	0.02
						Thr 5	Neu5Ac 229	0.01
						Neu5Ac 225	Thr 75	0.01
						Ser 28	Neu5Ac 225	0.01
						Ser 73	Neu5Ac 225	0.01
						Gal 228	Asp 9	0.01
						Gln 27	Gal 228	0.01
						Thr 75	Gal 228	0.01
						Neu5Ac 225	Ser 71	0.01

Table S6. H-bond interaction details between the ACPA 1F2 HC and LC Fab fragments and the respective *N*-linked glycan. Shown are the H-bond donor and acceptor pairs (three letter code of the respective amino acids and monosaccharides) and the occupancy of the interaction in percentage. GlcNAc: *N*-acetyl-glucosamine, Neu5Ac: *N*-acetylneuraminic acid, Fuc: fucose, Gal: galactose, Man: mannose.

1F2 HC #1 N-glycan			1F2 HC #2 N-glycan			1F2 LC		
donor	acceptor	oc. [%]	donor	acceptor	oc. [%]	donor	acceptor	oc. [%]
Ser 30	GlcNAc 223	11.08	GlcNAc 236	Thr 55	4.27	Gal 222	Asp 151	21.55
His 31	Fuc 235	9.5	Lys 54	Neu5Ac 246	2.7	Neu5Ac 223	Gly 156	10.44
Fuc 235	His 31	2.4	Fuc 248	Ala 56	1.65	GlcNAc 224	Thr 160	10.39
Lys 54	Fuc 235	1.98	Thr 55	GlcNAc 236	0.93	Neu5Ac 219	Gln 161	9.88
Fuc 235	Ser 30	1.32	Fuc 248	Thr 55	0.86	Lys 153	Gal 222	7.29
Neu5Ac 233	Leu 102	1.14	Neu5Ac 246	Lys 54	0.74	Gal 218	Thr 157	5.81
Ser 30	GlcNAc 223	1.13	Asn 57	GlcNAc 236	0.22	Gln 161	GlcNAc 224	4.83
Phe 29	GlcNAc 223	0.89	Gal 245	Asn 57	0.2	Neu5Ac 219	Thr 183	3.81
Thr 74	GlcNAc 224	0.25	GlcNAc 236	Ala 56	0.15	GlcNAc 213	Thr 3	2.83
Ile 104	Fuc 235	0.13	Fuc 248	Asn 57	0.12	Thr 157	Neu5Ac 223	2.75
Fuc 235	Asn 28	0.09	Lys 54	Neu5Ac 246	0.1	Gal 218	Gln 161	2.48
Asn 28	GlcNAc 223	0.05	Thr 55	Fuc 248	0.1	Fuc 225	Asn 25	2.15
GlcNAc 231	Lys 54	0.04	Thr 55	Gal 245	0.07	Neu5Ac 223	Gly 156	1.85
GlcNAc 224	Thr 74	0.03	Neu5Ac 246	Thr 55	0.03	Neu5Ac 223	Thr 157	1.74
Fuc 235	Leu 102	0.02	Gal 245	Lys 54	0.02	Asn 1	GlcNAc 213	1.59
Lys 54	Gal 232	0.02	Leu 58	Fuc 248	0.01	Gln 161	Neu5Ac 219	1.42
His 31	GlcNAc 223	0.01	Thr 55	GlcNAc 236	0.01	Gal 218	Gln 161	1.34
GlcNAc 224	Ser 75	0.01				Gln 161	GlcNAc 217	1.16
Neu5Ac 233	Gly 103	0.01				GlcNAc 224	Gln 161	1.04
						Thr 160	GlcNAc 224	1.02
						GlcNAc 217	Gln 161	0.85
						Gal 222	Gly 156	0.84
						Fuc 225	Asn 25	0.81
						Gln 161	GlcNAc 224	0.62
						Lys 153	Neu5Ac 223	0.55
						Lys 133	Neu5Ac 219	0.53
						Neu5Ac 223	Asp 155	0.49
						Neu5Ac 219	Gln 161	0.46
						Fuc 225	Asn 1	0.42
						Neu5Ac 219	Thr 183	0.39
						Thr 205	Neu5Ac 223	0.39
						GlcNAc 217	Gln 161	0.34
						Fuc 225	Thr 3	0.27
						Thr 160	GlcNAc 224	0.27
						Thr 157	Gal 218	0.26
						Gal 218	Thr 160	0.23
						Gln 161	Gal 218	0.22
						GlcNAc 217	Thr 160	0.22
						Thr 160	Gal 218	0.2
						Thr 3	Fuc 225	0.18
						GlcNAc 213	Leu 2	0.17
						Fuc 225	Asn 1	0.16
						Gly 162	Neu5Ac 219	0.15
						GlcNAc 224	Pro 158	0.15

						Neu5Ac 223	Gly 203	0.15
						Neu5Ac 223	Asp 155	0.14
						Gal 222	Thr 157	0.13
						GlcNAc 221	Asp 151	0.13
						Thr 185	Neu5Ac 219	0.13
						Asn 25	Fuc 225	0.1
						Gal 222	Thr 205	0.08
						Gln 198	Gal 222	0.07
						Neu5Ac 223	Thr 157	0.05
						Arg 191	Neu5Ac 219	0.05
						GlcNAc 221	Thr 160	0.05
						Thr 160	GlcNAc 217	0.04
						Thr 183	Neu5Ac 219	0.03
						GlcNAc 217	Thr 160	0.03
						Neu5Ac 223	Glu 207	0.03
						Gln 161	Man 216	0.02
						Gln 161	GlcNAc 217	0.02
						Thr 205	Neu5Ac 223	0.02
						Thr 185	Neu5Ac 219	0.02
						Gal 222	Glu 207	0.02
						Thr 183	Neu5Ac 219	0.01
						Thr 160	GlcNAc 221	0.01
						Gal 218	Pro 158	0.01
						Gal 218	His 192	0.01
						Neu5Ac 219	Thr 185	0.01
						Gal 218	Val 154	0.01
						Thr 157	GlcNAc 224	0.01
						Fuc 225	Gly 26	0.01
						Thr 157	Neu5Ac 223	0.01