Supplemental data for:

The structural role of antibody N-glycosylation in receptor interactions

Ganesh P. Subedi and Adam W. Barb*

Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology

Iowa State University, Ames, IA 50011

*corresponding Author

email: abarb@iastate.edu

address: 2214 Molecular Biology Building

Ames, IA, 50011

Supplemental Table 1. Alignment and quality-of-fit (Q) parameters from RDC fits. These data are from the RDC fits shown in Figure 2.

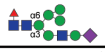
			fit to PDB 1L6X		
		n	Da ($x10^{-4}$)	Dr ($x10^{-4}$)	Q
Fc wt	Cγ2 alone	10	-6.8	-3.4	0.188
	Cγ3 alone	11	6.5	4.0	0.119
	Cγ2 + Cγ3	21	-7.5	-3.5	0.185
T299A	Cγ2 alone	10	-8.8	-3.3	0.245
	Cγ3 alone	10	9.3	5.9	0.091
	Cγ2 + Cγ3	20	-10.0	-4.5	0.177

Supplemental Table 2. Relaxation parameters for IgG1 Fc wt and the T299A variant at 18.8 T. These data are presented in a plot format in Figure 4.

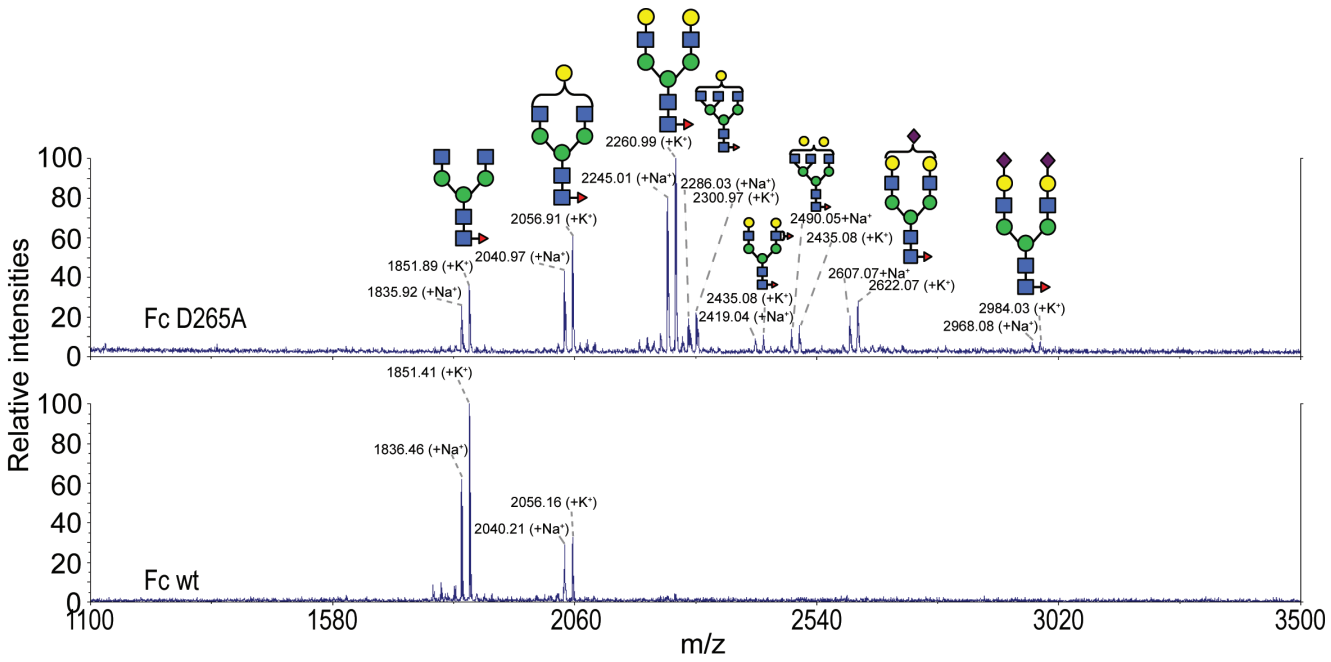
Residue	IgG1 Fc wt R_1 (s^{-1})	error (s^{-1})	IgG1 Fc T299A R_1 (s^{-1})	error (s^{-1})
274	0.60	0.03	0.62	0.02
278	0.56	0.03	0.70	0.09
288	0.54	0.03	0.68	0.04
290	0.58	0.03	0.75	0.04
300	0.59	0.05	0.57	0.02
317	0.59	0.04	0.61	0.01
319	0.48	0.04	0.60	0.02
320	0.59	0.04	0.62	0.02
322	0.59	0.04	0.67	0.03
349	0.47	0.02	0.57	0.03
360	0.68	0.06	0.69	0.04
370	0.57	0.05	0.55	0.03
373	0.53	0.04	0.63	0.02
391	0.66	0.03	0.67	0.02
392	0.55	0.05	0.57	0.02
407	0.55	0.02	0.57	0.02
409	0.55	0.06	0.58	0.03
414	0.53	0.04	0.67	0.03
436	0.63	0.02	0.66	0.02
439	0.55	0.04	0.54	0.03

Residue	IgG1 Fc wt R_2 (s^{-1})	error (s^{-1})	IgG1 Fc T299A R_2 (s^{-1})	error (s^{-1})
274	47.1	1.1	48.1	2.2
278	46.6	2.2	51.6	2.1
288	49.9	1.7	45.4	2.2
290	49.7	2.0	40.7	2.1
300	52.2	3.9	75.0	5.0
317	54.9	1.4	52.4	1.6
319	57.8	2.5	50.2	1.7
320	54.6	2.9	52.1	1.5
322	57.3	4.7	51.8	2.3
349	60.6	1.7	55.3	2.1
360	51.0	2.1	50.5	0.8
370	61.0	5.0	57.0	1.4
373	56.7	2.0	47.7	1.2
391	52.4	1.8	52.2	1.0
392	58.5	3.5	56.1	1.7
407	54.7	4.4	53.0	5.0
409	64.7	4.8	63.0	3.0
414	58.0	5.0	57.0	6.0
436	57.2	1.3	54.4	1.6
439	56.0	1.4	51.6	1.5

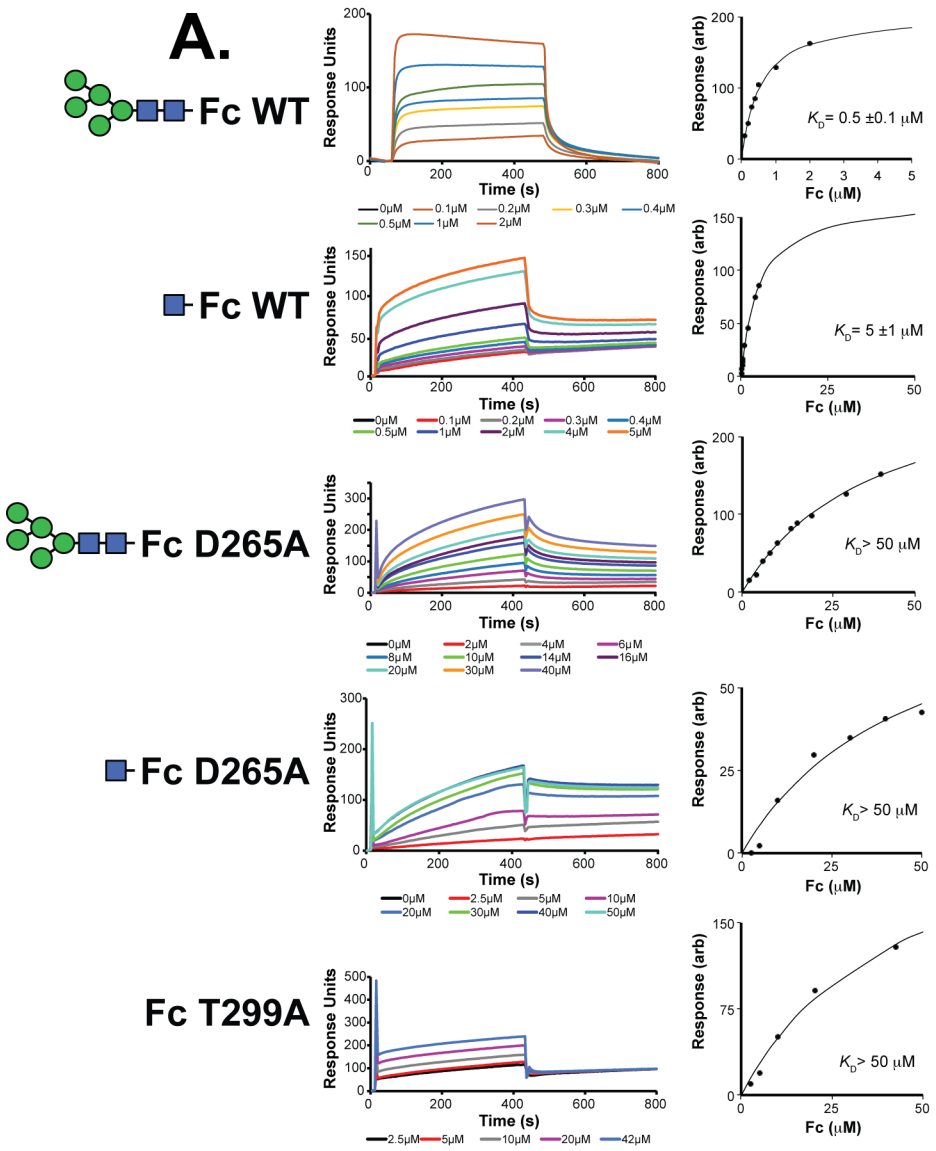
Supplemental Table 3. Relative proportion of N-glycan species following expression of Fc variants in HEK293F cells. Observations deviated from < 1 Da from expected values. Isobaric ions were not distinguished. These data are related to Figure 5.

		Relative Abundance (%)				
Glycoform	Monoisotopic Mass + Na^+(m/z)	WT *	D265A	F243I *	F241I * F243I	F241S * F243S
M5N2 	1579				2.01	2.60
NM3N2F 	1591				2.85	2.97
G0F 	1836	73.18	10.0	18.41	8.63	10.42
M7N2 	1988				0.83	1.68
G1F 	2040	23.85	18.0	13.39	5.31	3.37
G2 	2070		2.6	2.92		
N3M3N2F 	2081			6.26	2.93	4.04
M8N2 	2192				1.49	
Unidentified	2232				1.52	3.22
G2F 	2244	2.98	31.5	10.16	6.47	4.14
G1N3M3N2F 	2285		9.0	12.15	5.18	2.97
A1G1F 	2404				3.57	3.22
G2F2 	2418		4.0	5.21	2.46	2.89
A1G2N3M3N 	2432				1.04	1.74
G2N3M3N2F 	2489		4.4	4.03	3.87	2.89
A1G1N1M5N2F 	2564		2.0		1.00	3.64
A1G2F 	2605		7.0	9.96	11.35	5.76
A1G3N3M3N 	2636				2.50	2.52
A1G1N3M3N2F 	2646		2.0		2.80	2.85
G2N3M3N2F2 	2663			2.6	1.43	1.67
G3N3M3N2F 	2693		2.5		2.77	2.11
A1G2F2 	2780		2.0	4.21	3.45	4.83
A2G2 	2793		2.0		1.44	3.01
A1G2N3M3N2F 	2851			3.21	2.63	3.38
A2G2F 	2967		3.0	7.49	9.23	11.10
A2G3N3M3N 	2997				2.29	3.53
A2G2N3M3N2 	3038				2.55	2.46
A1G3N3M3N2F 	3055				2.99	2.49
A2G2N3M3N2F 	3212				1.46	
A2G3N3M3N2F 	3416				2.50	2.57
A3G3N3M3N2F 	3777				1.45	1.94

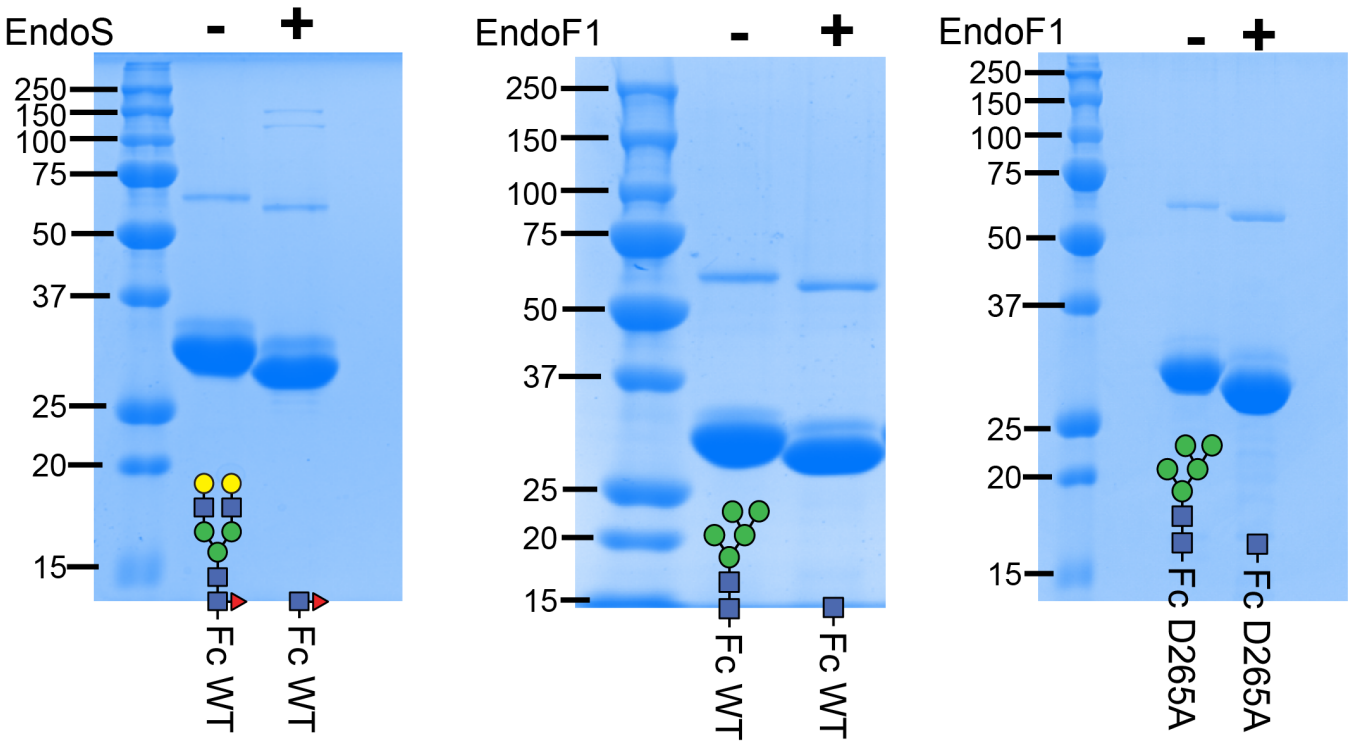
*- values reported in Subedi, Hanson and Barb (2014)



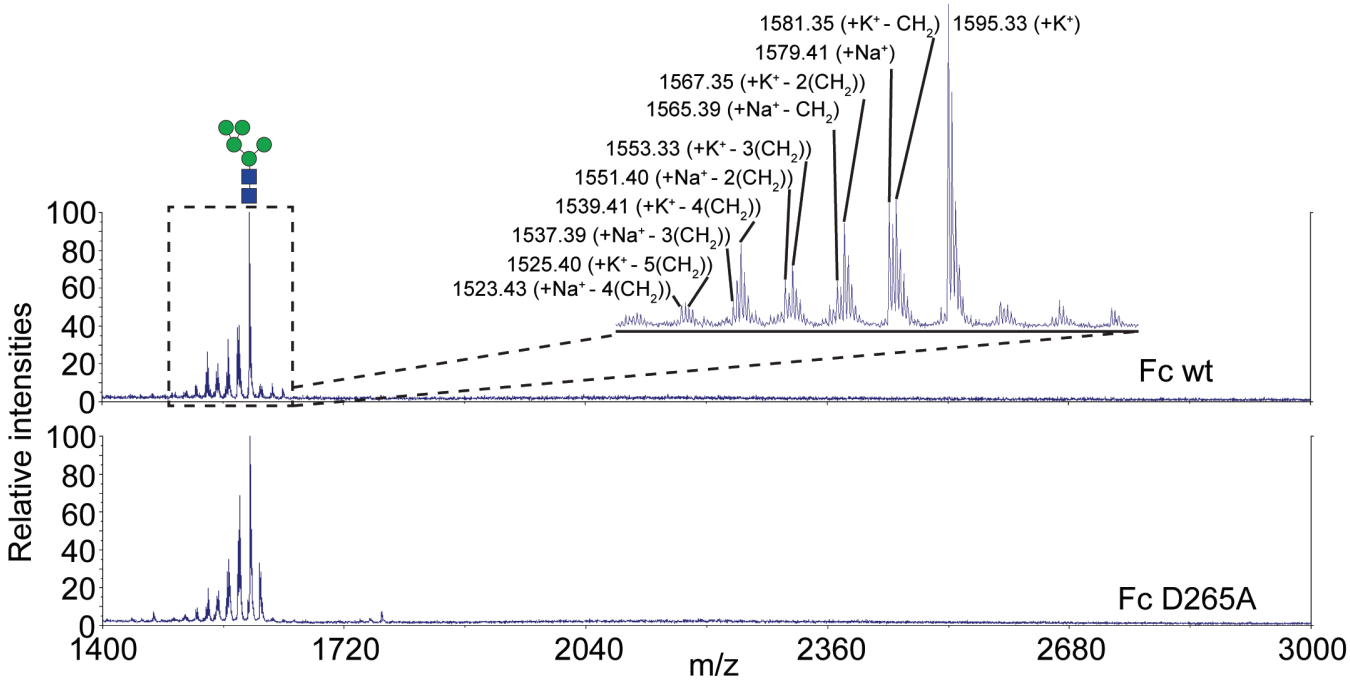
Supplemental Figure 1. N-glycans from IgG1 Fc D265A (*top* panel) experience a greater degree of modification during protein expression and show more glycoforms than those observed on IgG1 Fc WT (*bottom* panel). MALDI-MS analysis is conducted using PNGaseF-released and permethylated N-glycans. Cartoon diagrams represent a potential configuration based upon the observed mass and reflect the CfG convention (Varki, 2009). Isobaric ions were not distinguished and peaks corresponding to Na^+ and K^+ adducts were observed. Observed m/z values are indicated. These data are related to Figure 5.



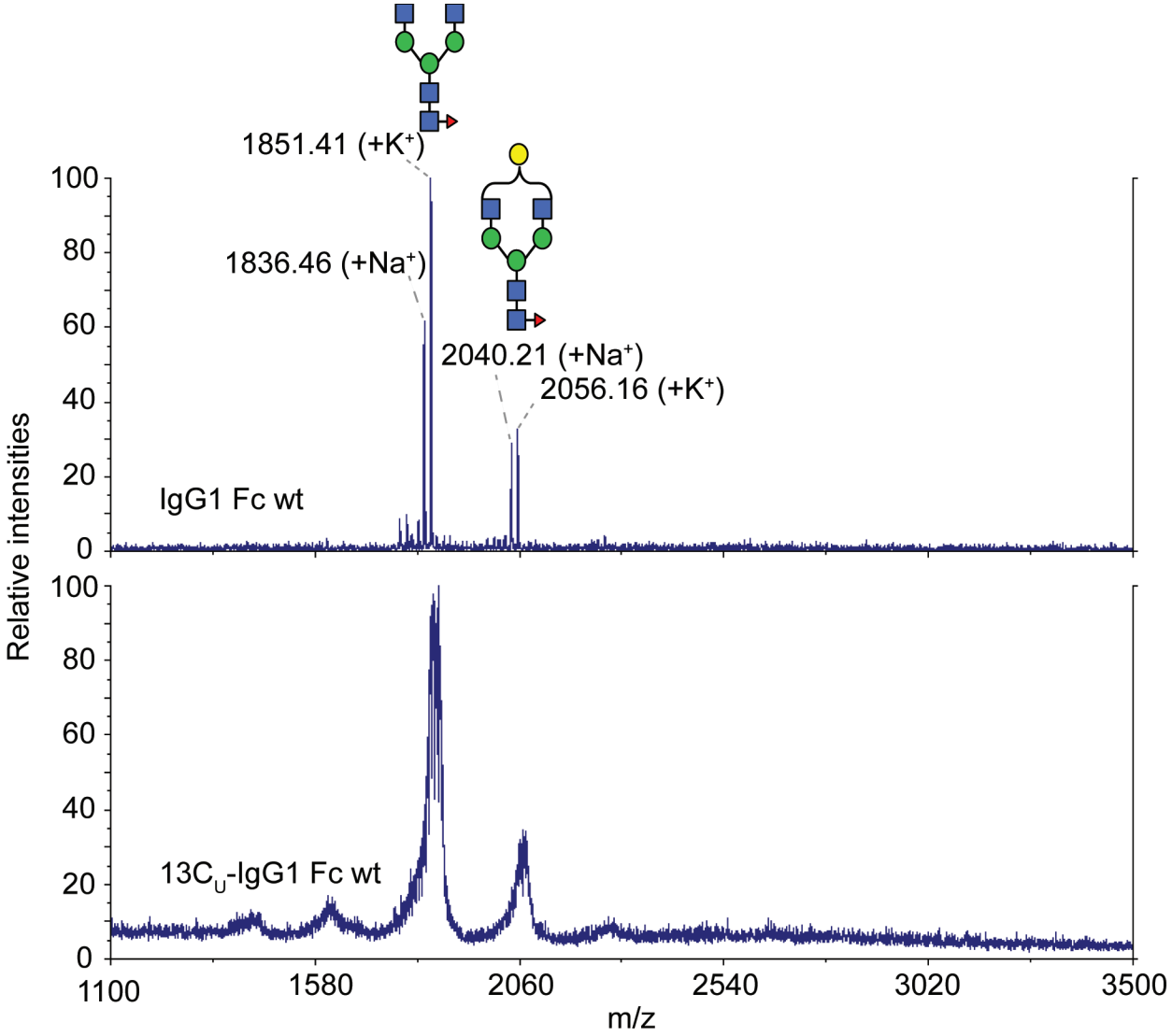
Supplemental Figure 2. SPR analysis of IgG1 Fc binding to FcγRIIIa. (**A**) Interferograms from the SPR experiment are shown in the left column. In the right column, extracted changes in intensities are plotted and fitted K_D values are indicated. To prepare the plots in the right column, the effect of instrument drift was removed using baseline subtraction (most notable in the second, fourth and fifth interferograms from the top). Values for the top two K_Ds are similar to our previously published results (Subedi et al., 2014). All response curves fit to maximum values of ~200 units. Clear evidence of saturation was not observed for the third, fourth and fifth samples (from the top) due to limiting concentrations, therefore K_D values are given as a lower bound for these weak-binding species. The K_D values are reported in Figure 5A.



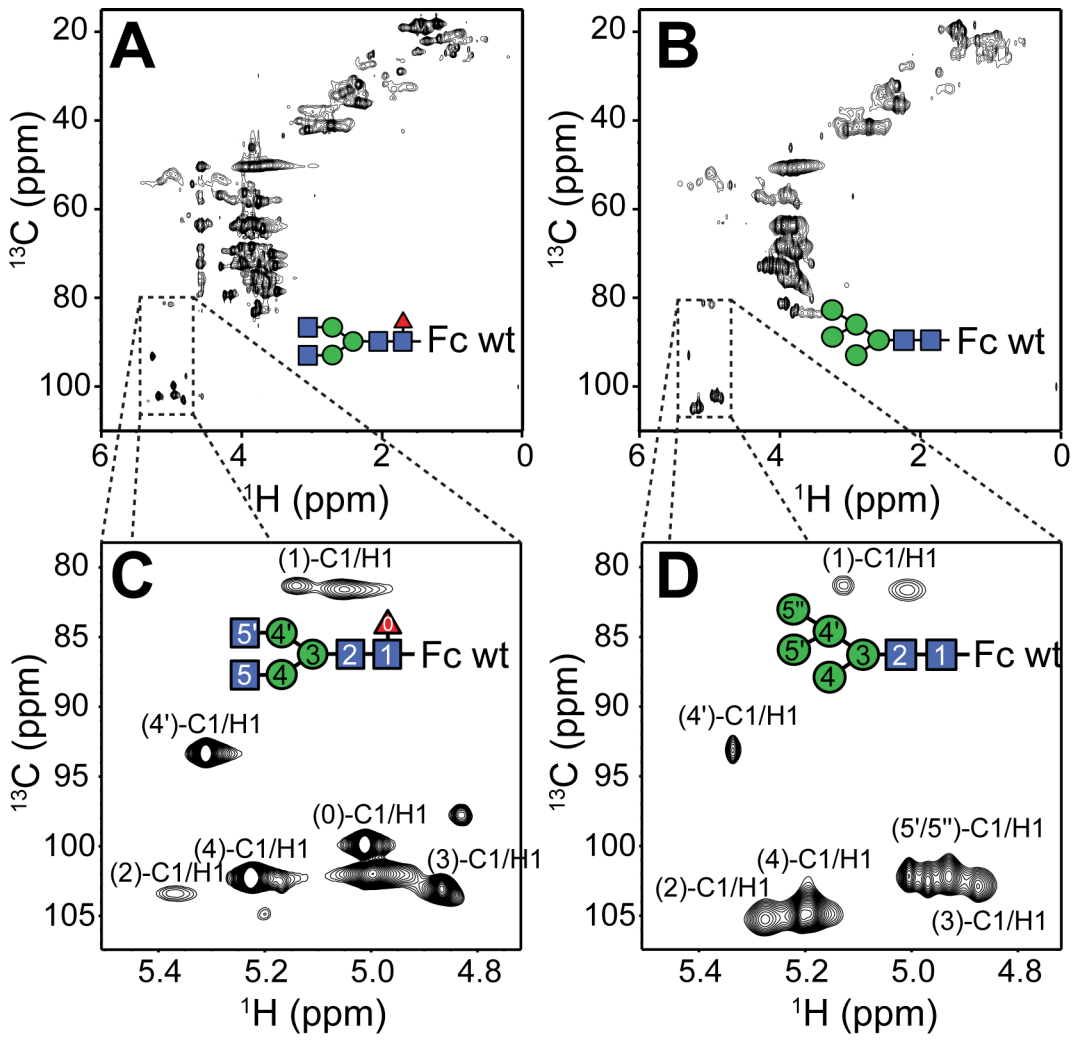
Supplemental Figure 3. Endoglycosidase S and F1 reactions resulted in complete conversion based on an SDS-PAGE analysis of reaction material before (-) and after (+) treatment. These data are related to Fc glycoforms presented in Figure 5 and 6.



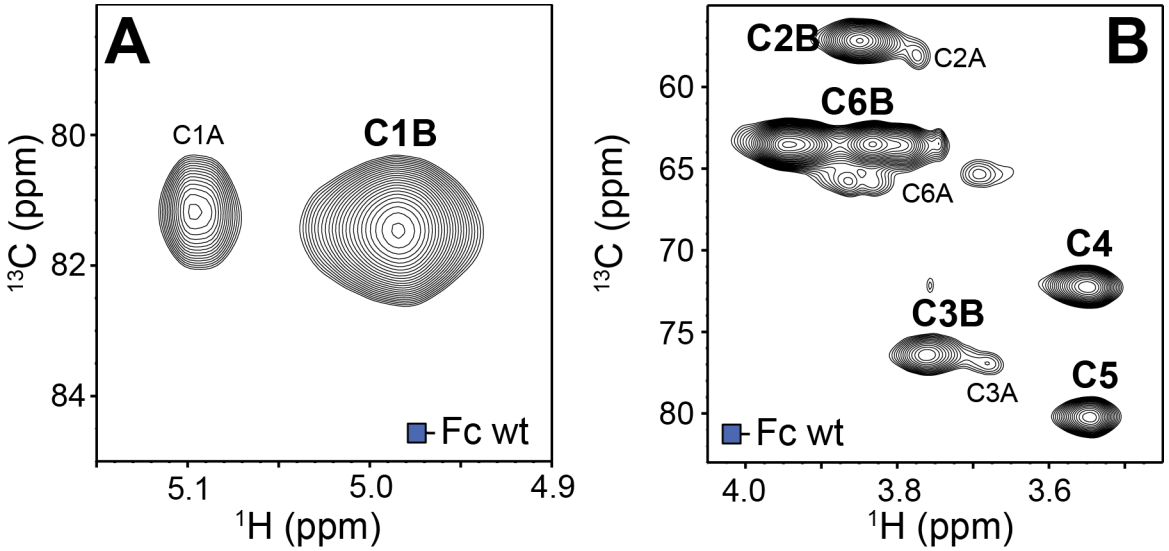
Supplemental Figure 4. N-glycans from IgG1 Fc WT (*top* panel) and IgG1 Fc D265A (*bottom* panel) were predominantly of a Man5 type following protein expression using the HEK293S *lec1*$^{-/-}$ cell line (Reeves et al., 2002) as judged by MALDI-MS analysis of PNGaseF-released and permethylated N-glycans. Cartoon diagrams reflect the CfG convention (Varki, 2009). Isobaric ions were not distinguished and peaks corresponding to Na^+ and K^+ adducts were observed. Observed m/z values are indicated. These data are related to Fc glycoforms presented in Figure 5.



Supplement Figure 5. N-glycans from IgG1 Fc expressed in normal medium (*top* panel) or medium enriched with ^{13}C(1,2,3,4,5,6)-glucose (*bottom* panel) show similar distributions following MALDI-MS analysis of PNGaseF-released and permethylated N-glycans. Cartoon diagrams represent a potential configuration based upon the observed mass and reflect the CfG convention (Varki, 2009). Isobaric ions were not distinguished and peaks corresponding to Na^+ and K^+ adducts were observed. Observed m/z values are indicated. These data are related to Figure 6.



Supplement Figure 6. ^{1}H-^{13}C HSQC spectra of purified Fc, expressed in the presence of ^{13}C(1,2,3,4,5,6)-glucose, indicate that a significant proportion of the ^{13}C nuclei are found in the N-glycan. (**A**) Fc wt expressed in the HEK293F cell line. (B) Fc wt expressed in the HEK293S *lec1*$^{-/-}$ cell line. (**C**) and (**D**) show the spectral region corresponding to anomeric resonances of (**A**) and (**B**), respectively. Partial assignments are available from previous reports (Vliegenthart et al., 1983; Yamaguchi, 2008; Yamaguchi and Kato, 2006). These data are related to Figure 6.



Supplemental Figure 7. ^{1}H-^{13}C HSQC spectra of Fc, grown in the presence of ^{13}C(1,2,3,4,5,6)-glucose using the HEK293S *lec1*$^{-/-}$ cell line and treated with Endo F1 to remove all glycan residues except (1)GlcNAc, reveal a weaker, duplicate set of peaks for the (1)GlcNAc residue in the anomeric region (**A**) or for resonances at the 2-6 positions (**B**). The strongest signals are labeled "C#B" and the weaker signals "C#A." These data are related to Figure 6.

Supplemental References

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