Supplement for:

Restricted motion of the conserved immunoglobulin G1 N-glycan is essential for efficient FcγRIIIa binding

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		Relative Abundance (%)							
Glycoform	Monoisotopic Mass + Na⁺(m/z)	WT	K246F	F241I	F241S	F243I	F243S	F241I F243I	F241S F243S
M5N2	1345							2.01	2.60
NM3N2F	1591		5.68				7.93	2.85	2.97
G0F	1836	73.18	73.58	35.31	20.2	18.41	27.39	8.63	10.42
M7N2	1988							0.83	1.68
G1F	2040	23.85	15.94	21.16	8.67	13.39	16.42	5.31	3.37
G2	2070				2.87	2.92			
N3M3N2F	2081			8.41	7.14	6.26	9.62	2.93	4.04
M8N2	2192							1.49	
Unidentified	2232							1.52	3.22
G2F	2244	2.98	2.56	20.71	11.76	10.16	12.7	6.47	4.14
G1N3M3N2F	2285		2.24	7.99	10.85	12.15	7.53	5.18	2.97
A1G1F	2404							3.57	3.22
G2F2	2418				5.28	5.21	3.9	2.46	2.89
A1G2N3M3N	2432							1.04	1.74
G2N3M3N2F	2489					4.03	5.35	3.87	2.89
A1G1N1M5N2F	2564							1.00	3.64
A1G2F	2605			6.42	9.22	9.96	9.16	11.35	5.76
A1G3N3M3N	2636							2.50	2.52
A1G1N3M3N2F	2646							2.80	2.85
G2N3M3N2F2	2663				4.58	2.6		1.43	1.67
G3N3M3N2F	2693							2.77	2.11
A1G2F2	2780				5.49	4.21		3.45	4.83
A2G2	2793							1.44	3.01
A1G2N3M3N2F	2851				4.91	3.21		2.63	3.38
A2G2F	2967				9.04	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

Figure S1, related to Figure 2. Relative abundance of Fc N-glycoforms as measured from the spectra presented in **Figure 2.**

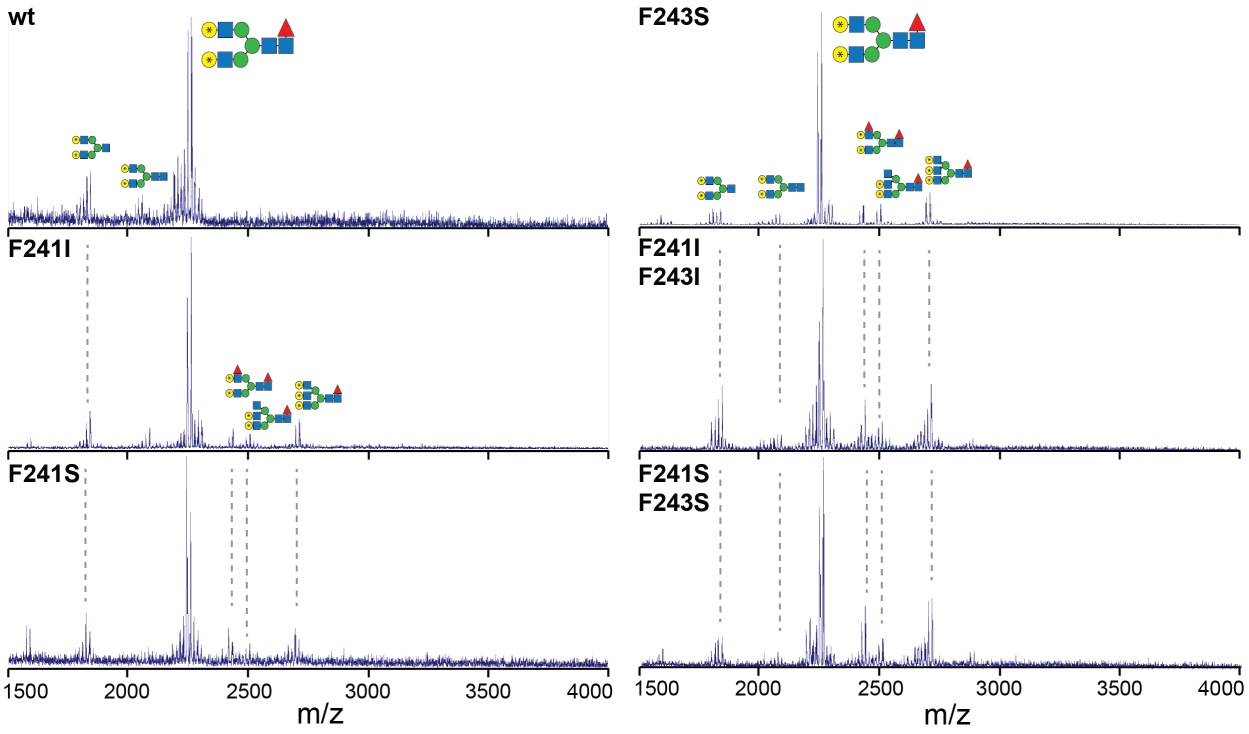

Figure S2, related to Figures 2 and 4**.** MALDI-MS of PNGaseF-released, permethylated N-glycans following in vitro remodeling to incorporate ^{13}C2-Gal (indicated by a "*" within a *yellow* circle). Cartoon diagrams highlight potential configurations that fit the observed masses and known N-glycan structures; isobaric ions were not distinguished. Two peaks corresponding to Na^+ and K^+ adducts are observed for each species.

		Relative Abundance (%)						
Glycoform	Monoisotopic Mass + Na^+(m/z)	WT	F241I	F241S	F243S	F241I F243I	F241S F243S	K246F
i. G*2N2M3N 	1827	20	12	15	5	19	10	5
ii. G*2N2M3N2 	2072	6	2	2	4	2	3	5
iii. G*2N2M3N2F 	2246	74	66	56	69	46	50	90
G*2N2FM3N2F 	2420		5	11	6	10	15	
G*2N3M3N2F 	2491		4	5	5	8	6	
G*3N3M3N2F 	2697		11	11	11	15	16	
i + iii (total G2F)		94	78	71	74	65	60	95
i + ii + iii (total with two Gal-termini)		100	80	73	78	67	63	100

Figure S3, related to Figures 2,4 and S2. Relative abundance of Fc N-glycoforms following in vitro remodeling with [^{13}C2]-Gal as measured from the spectra presented in **Figure S2.**

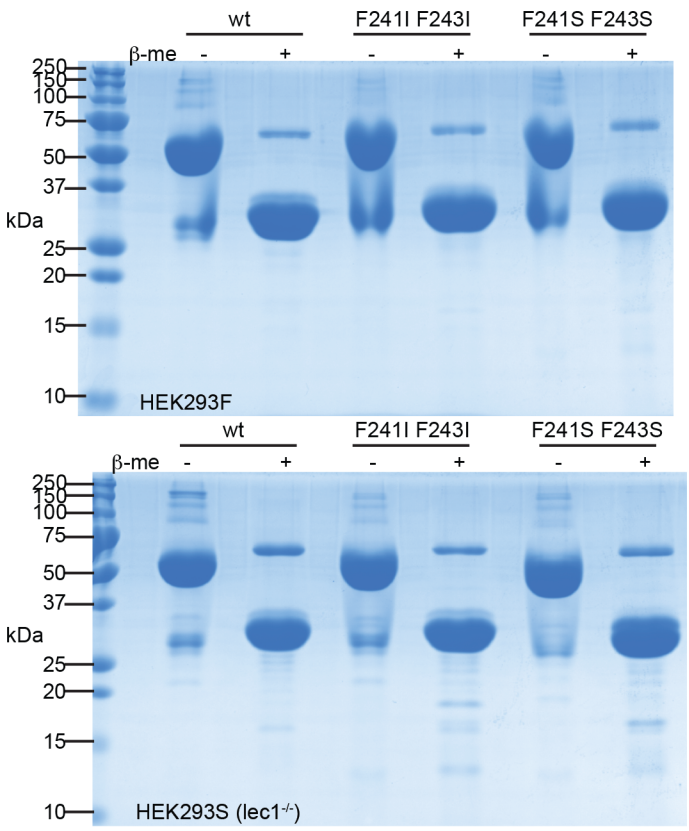

Figure S4, related to Figures 3 and 4. Hinge disulfides formed stabilize Fc dimers (expt ~55 kDa) expressed from both HEK293F and HEK293S(lec1$^{-/-}$) cells. The covalently-linked Cγ2, Cγ3 domains and N-glycan that compose the Fc monomer have a combined mass of ~27.5 kDa and are observed in an SDS-PAGE gel when 1 mM beta-mercaptoethanol (β-me) is included in the sample preparation buffer.

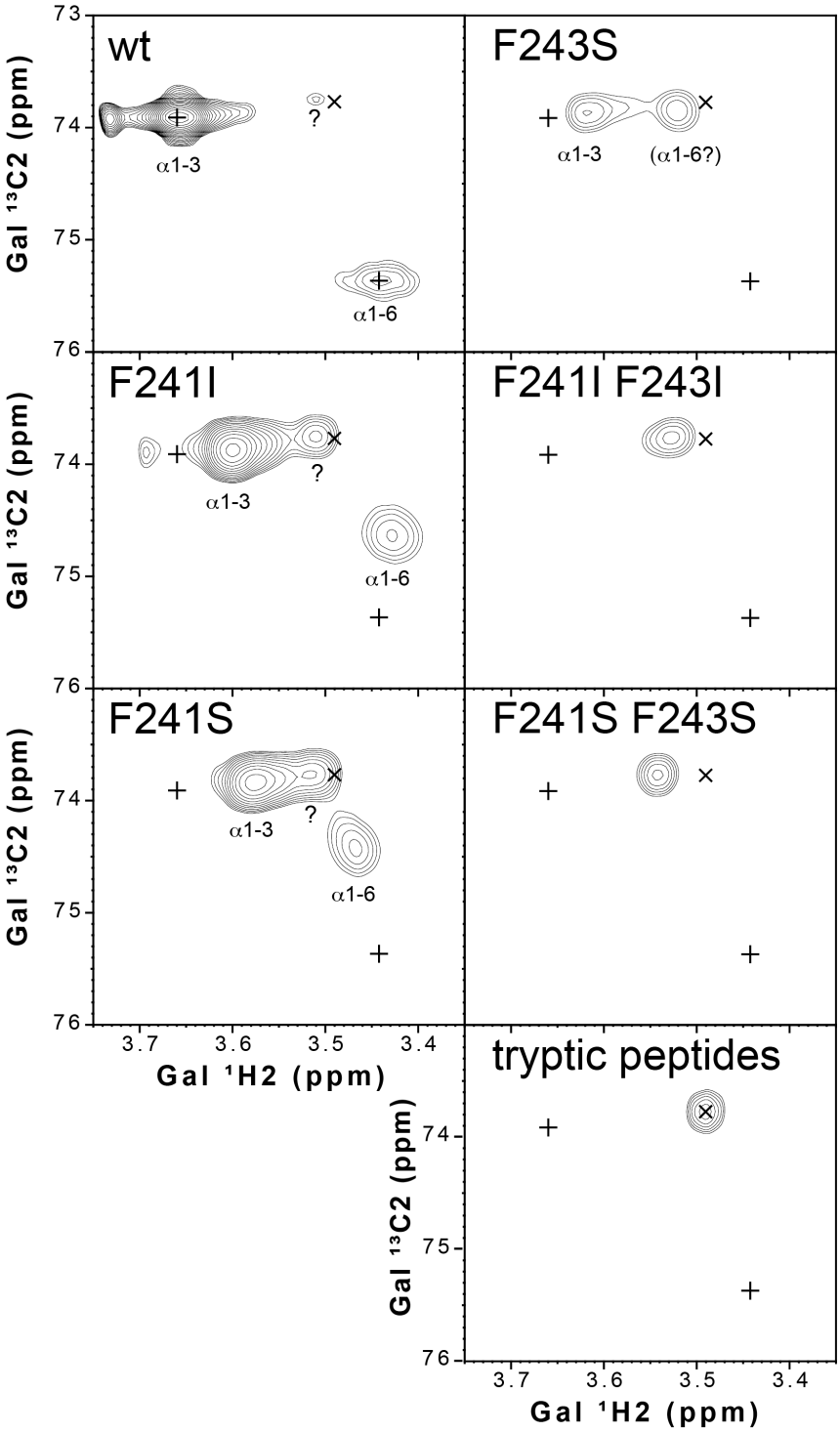

Figure S5, related to Figure 4. ^{1}H-^{13}C HSQC spectra of Fc mutants collected at 50°C following enzymatic remodeling to incorporate $^{13}C2$-Gal. Positions of wild type coherences are highlighted in each spectrum with "+" signs, and the position of coherences following trypsin proteolysis are marked with "x". Peaks labeled by "?" have not been identified, but are present in wild type. These peaks are very narrow and intense, but according to 1d ^{13}C NMR spectroscopy only represent a small fraction of the total ^{13}C signal.

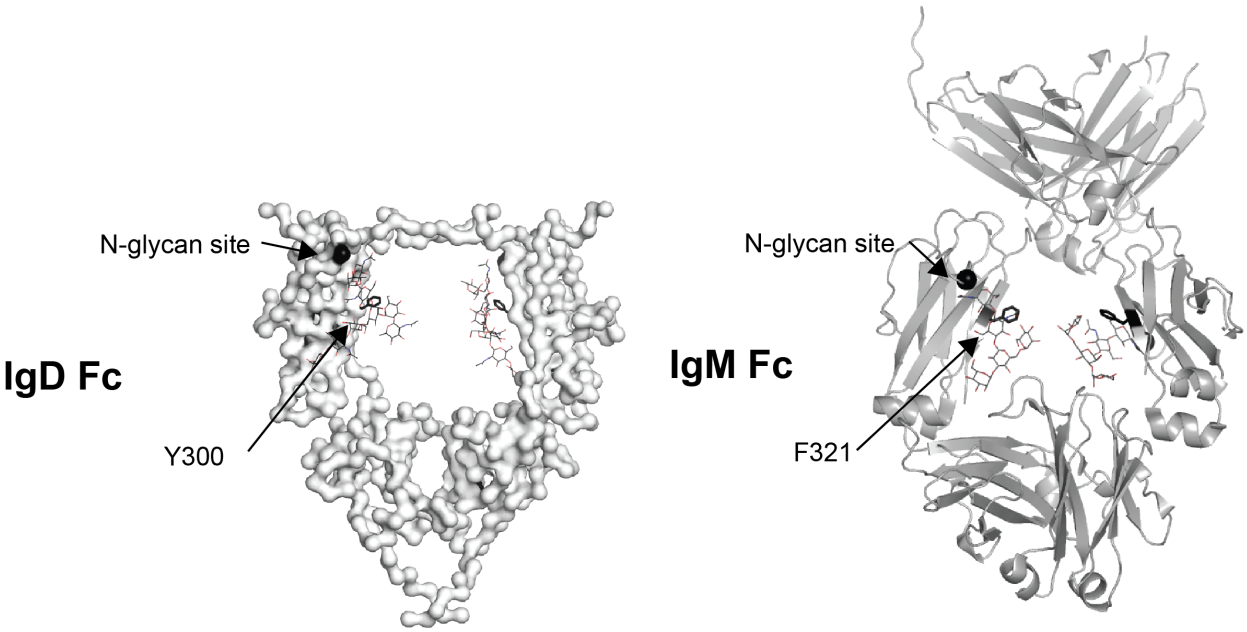

Figure S6, related to Figure 7. Models of IgD and IgM Fc show the position of conserved N-glycosylation sites and aromatic residues. The IgD figure was generated from solution scattering data (pdb 1zvo (Sun et al., 2005)) and IgM upon x-ray scattering, crystallography and NMR data (Muller et al., 2013; Perkins et al., 1991).

Supplement References

Muller, R., Grawert, M.A., Kern, T., Madl, T., Peschek, J., Sattler, M., Groll, M., and Buchner, J. (2013). High-resolution structures of the IgM Fc domains reveal principles of its hexamer formation. Proc Natl Acad Sci U S A *110*, 10183-10188.

Perkins, S.J., Nealis, A.S., Sutton, B.J., and Feinstein, A. (1991). Solution structure of human and mouse immunoglobulin M by synchrotron X-ray scattering and molecular graphics modelling. A possible mechanism for complement activation. J Mol Biol *221*, 1345-1366.

Sun, Z., Almogren, A., Furtado, P.B., Chowdhury, B., Kerr, M.A., and Perkins, S.J. (2005). Semi-extended solution structure of human myeloma immunoglobulin D determined by constrained X-ray scattering. J Mol Biol *353*, 155-173.