Supplemental information for:

A single amino acid distorts the Fc γ receptor IIIb / CD16b structure upon binding immunoglobulin G1 and reduces affinity relative to CD16a

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Including:

Table S1

Figures S1-3

Table S1. Data collection and refinement statistics for the IgG1 Fc : CD16b (N38Q/N64Q/N74Q/N169Q) complex (PDB-ID 6EAQ).

Space group	C 2
Protein chains per asymmetric unit	3
Unit cell parameters	
a, b, c (Å)	123.629, 49.388, 139.415
α, β, γ (°)	90, 103.037, 90
Resolution (Å)	45.27 - 2.22 (2.299 - 2.22)*
Total reflections	141822 (10166)
Unique reflections	40439 (3536)
Multiplicity	3.5 (2.9)
Completeness (%)	0.97 (0.88)
Mean I/σ(I)	7.01 (1.41)
Wilson B-factor	38.57
R_{merge}	0.1769 (2.41)
R_{meas}	0.2099 (2.973)
CC1/2	0.974 (0.755)
CC*	0.993 (0.928)
Reflections used in refinement	39714 (3018)
Reflections used for R_{free}	2750 (205)
R_{work}	0.2218 (0.5613)
R_{free}	0.2477 (0.5929)
Number of non-hydrogen atoms	5202
macromolecules	4581
ligands	262
Protein residues	595
RMS bond lengths (Å)	0.010
RMS bond angles (°)	1.08
Ramachandran statistics	
favored (%)	98.3
allowed (%)	1.7
outliers (%)	0
Rotamer outliers (%)	1.2
Clashscore	6.27
B-factors	
average (Å^2)	42.89
macromolecules (Å^2)	41.90
ligands (Å^2)	56.42
solvent (Å^2)	45.63

*values in parentheses refer to the highest resolution shell

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CD16b-NA2     MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYSVLEKDSVTLKCQGAYSPEDNSTQW
CD16b-NA1     MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQW
CD16a-V158    MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQW
CD16a-F158    MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQW
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CD16b-NA2     FHNESLISSQASSYFIDAATVNDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKE
CD16b-NA1     FHNENLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHVGWLLLQAPRWVFKE
CD16a-V158    FHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKE
CD16a-F158    FHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKE
              ****.***************.************************:**************

CD16b-NA2     EDPIHLRCHSWKNTALHKVTYLQNGKDRKYFHHNSDFHIPKATLKDSGSYFCRGLVGSKN
CD16b-NA1     EDPIHLRCHSWKNTALHKVTYLQNGKDRKYFHHNSDFHIPKATLKDSGSYFCRGLVGSKN
CD16a-V158    EDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLVGSKN
CD16a-F158    EDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLFGSKN
              **************************.**********:*****************.****

CD16b-NA2     VSSETVNITITQGLAVSTISSFSPPGYQVSFCLVMVLLFAVDTGLYFSVKTNI-------
CD16b-NA1     VSSETVNITITQGLAVSTISSFSPPGYQVSFCLVMVLLFAVDTGLYFSVKTNI-------
CD16a-V158    VSSETVNITITQGLAVSTISSFFPPGYQVSFCLVMVLLFAVDTGLYFSVKTNIRSSTRDW
CD16a-F158    VSSETVNITITQGLAVSTISSFFPPGYQVSFCLVMVLLFAVDTGLYFSVKTNIRSSTRDW
              ********************** ******************************

CD16b-NA2     ---------------
CD16b-NA1     ---------------
CD16a-V158    KDHKFKWRKDPQDK
CD16a-F158    KDHKFKWRKDPQDK
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Figure S1. The two predominant CD16b alleles (NA2 and NA1) differ at four residues (S/R18, S/N47, N/D64 and I/V88) resulting in four potential N-glycans on NA1 and six for NA2. The text is labeled as follows: *red* type denotes the N-terminal signal peptide; *yellow* type indicates cysteine residues that form disulfides; *green* asparagines (N) indicate a potential N-linked glycosylation site; "*" indicates sequence conservation, "." indicates a difference in one or more sequences; *orange* indicates C-terminal peptide cleaved for a glycosyl-phosphatidyl inositol (GPI) anchor in the mature CD16b protein.

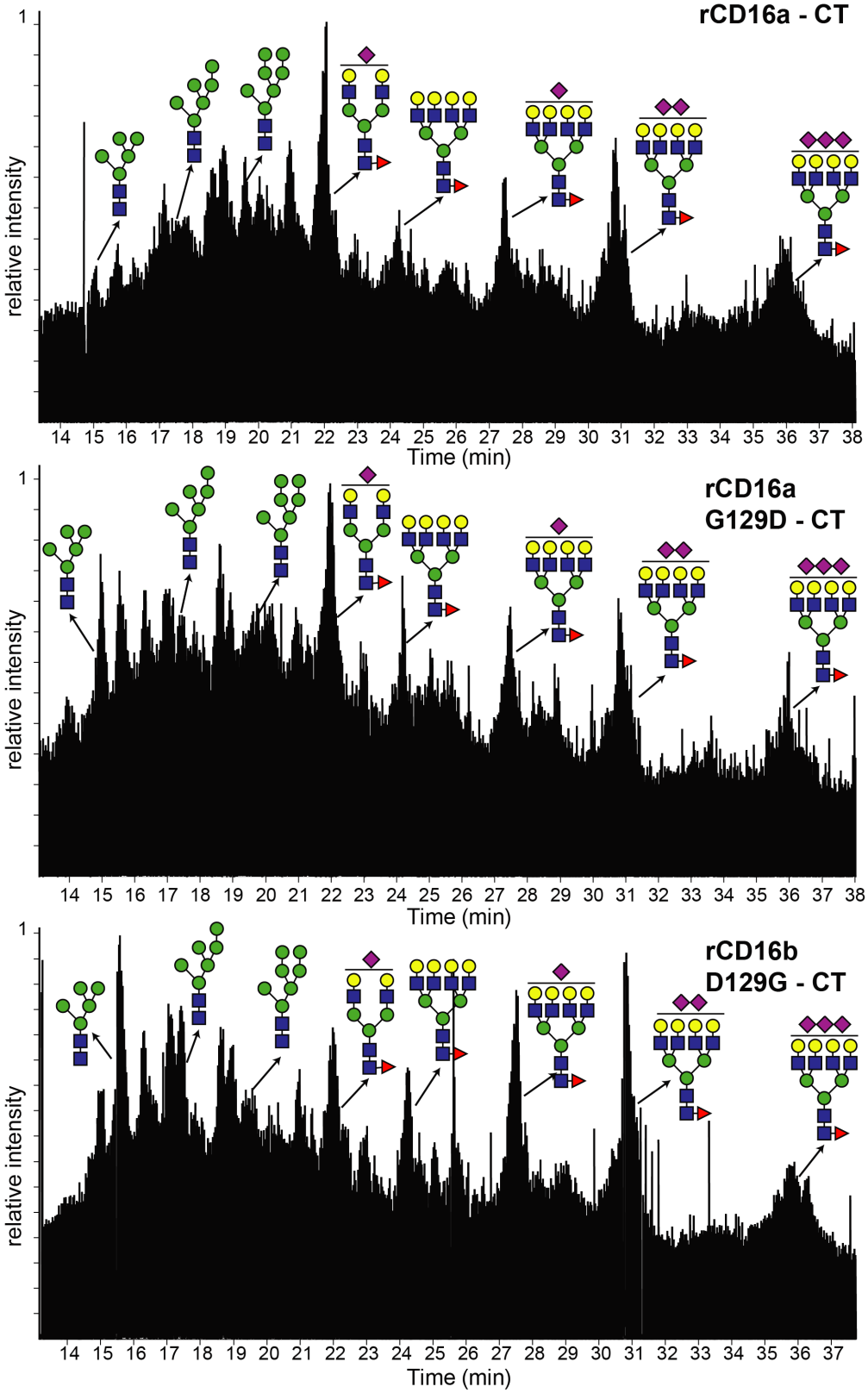

Figure S2. Total ion current of procainamide-derivatized CD16 N-glycans expressed using HEK293F cells and analyzed by HILIC-MS.

rCD16b:IgG1-Fc complex (reported here, pdb 6eaq)		rCD16a:IgG1-Fc complex (pdb 5vu0)	
rCD16b	**IgG1-Fc-Chain B**	**rCD16a**	**IgG1-Fc-Chain B**
GLU 21	PRO 329	GLU 21	PRO 329
ILE 88	ALA 330	ILE 88	PRO 329, ALA 330
GLY 89	PRO 329	GLY 89	PRO 329
TRP 90	LEU 235, GLY 236, GLY 237, PRO 238, ALA 327, LEU 328, PRO 329	TRP 90	LEU 235, GLY 236, ALA 327, LEU 328, PRO 329
TRP 113	ALA 327, LEU 328, PRO 329	TRP 113	LYS 326, ALA 327, LEU 328, PRO 329
THR 116	LEU 235	THR 116	LEU 235
ALA 117	LEU 235	ALA 117	LEU 235
VAL 158	LEU 235, GLY 236	VAL 158	LEU 235, GLY 236
GLY 159	LEU 235, GLY 237	GLY 159	LEU 235
LYS 161	GLY 236, GLY 237, PRO 238, SER 239	LYS 161	GLY 236, GLY 237, PRO 238, SER 239, LEU 328
	IgG1-Fc-Chain A		**IgG1-Fc-Chain A**
HIS 119	LEU 235, GLY 236	HIS 119	LEU 235, GLY 236
LYS 120	GLY 236, GLY 237, PRO 238, SER 239, **ASP 265**	LYS 120	GLY 236, GLY 237, PRO 238, SER 239, **ASP 265**
THR 122	(1)GlcNAc, ASN 297	THR 122	(1)GlcNAc, ASN 297
		ASN 126	TYR 296
GLY 127	TYR 296	GLY 127	TYR 296
LYS 128	TYR 296, ASN 297, SER 298	LYS 128	TYR 296
ASP 129	GlcNac1, ASN 297, SER 298	GLY 129	TYR 296, ASN 297, SER 298
ARG 130	SER 298	ARG 130	SER 298
LYS 131	HIS 268, **Glu 269**, SER 298	LYS 131	HIS 268, **GLU 269**
TYR 132	(1)GlcNAc , GLY 237, ASP 265, SER 267, SER298,	TYR 132	(1)GlcNAc , GLY 237, ASP 265, SER 267, ASN 297, SER 298
PHE 133	GLU 269	PHE 133	GLU 269
HIS 134	GLY 236, GLY 237, PRO 238, ASP 265, SER 267, ALA 327	HIS 134	GLY 236, GLY 237, PRO 238, ASP 265, VAL 266, SER 267, ALA 327
HIS 135	LEU 235, GLY 236, GLY 237	HIS 135	LEU 235, GLY 236
		ARG 155	(1)GlcNAc , ASN 297
		GLY 159	LEU 235

Figure S3. A catalog of residues found at the CD16 – IgG1 Fc interface. All residue contacts are < 5 Å and were identified using the PDBe Pisa online utility tool (49). Residues that form hydrogen bonds are *underlined*. Residues that form ionic interactions are *underlined* and *bold*. *Red* type denotes a differences between CD16a and CD16b.