

Supporting Information

**CD16a with oligomannose-type N-glycans is the only “low affinity” Fc  $\gamma$  receptor that binds the IgG crystallizable fragment with high affinity in vitro**

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Including:

Table S1

Figure S1

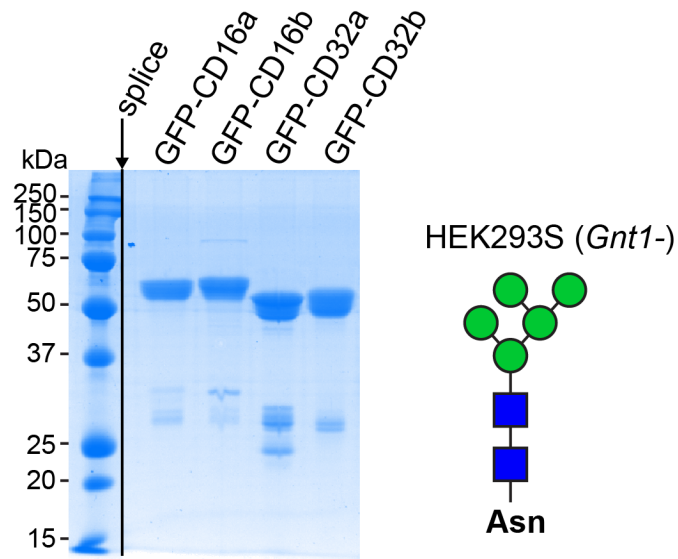
Figure S2

Figure S3

**Table S1.** Binding affinity measurements for IgG1 Fc and receptor glycovariants and amino acid variants.

	Fucosylated IgG1-Fc				Afucosylated IgG1-Fc			
	G0F K <sub>D</sub> (nM) ± err	G2F K <sub>D</sub> (nM) ± err	A2G2F K <sub>D</sub> (nM) ± err		G0 K <sub>D</sub> (nM) ± err	G2 K <sub>D</sub> (nM) ± err	A2G2 K <sub>D</sub> (nM) ± err	
<b>Complex-Type Receptors</b>	<b>N-glycan Composition</b>							
<b>Oligomannose-Type Receptors</b>								
rCD16a-Man5	25	17	13	9	21	1	1	1
rCD16b-Man5	3300	120	1500	100	1300	80	190	20
rCD32a-Man5	720	60	480	40	450	40	440	30
rCD32b-Man5	3000	200	1900	200	1700	120	1700	200
<b>CD16a Variants</b>								
rCD16a-CT N38Q/N74Q/N169Q	56	4	26	1	7.8	0.5	1.0	0.1
rCD16a-CT N162Q	150	80	80	20	88	20	130	10
rCD16a-CT N45Q	1200	130	590	50	130	10	870	30
rCD16a-CT N45Q/N162Q	340	30	160	20	160	20	500	200
rCD16a-Man5 N38Q/N74Q/N169Q	4.0	0.2	1.9	0.1	1.5	1.0	1.6	0.3
rCD16a-Man5 N162Q	63	3	34	2	38	3	329	26
rCD16a-Man5 N45Q	32	4	17	1	12	1	765	31
rCD16a-Man5 N45Q/N162Q	285	6	105	8	83	14	1350	131
<b>Afucosylated CD16a</b>								
rCD16a-CT	260	20	140	10	23	2	1.0	0.3
rCD16a-CT N38Q/N74Q/N169Q	120	20	70	7	6	3	440	30
rCD16a-CT N162Q	100	10	55	4	60	5	1700	200

\* - Values reported in Subedi and Barb (2016) MAbs 8(8):1512-1524 doi: 10.1080/19420862.2016.1218566



**Figure S1.** SDS-PAGE analysis of low affinity Fc  $\gamma$  receptors extracellular fragments expressed with HEK293S (*Gnt1*-) cells. Receptors were expressed with an N-terminal green fluorescent protein (GFP) fusion containing a His8 purification tag. These proteins contain primarily Man5 N-glycans. This gel image was spliced to remove lanes not pertinent to the expression of Fc  $\gamma$  receptors HEK293S (*Gnt1*-) cells.

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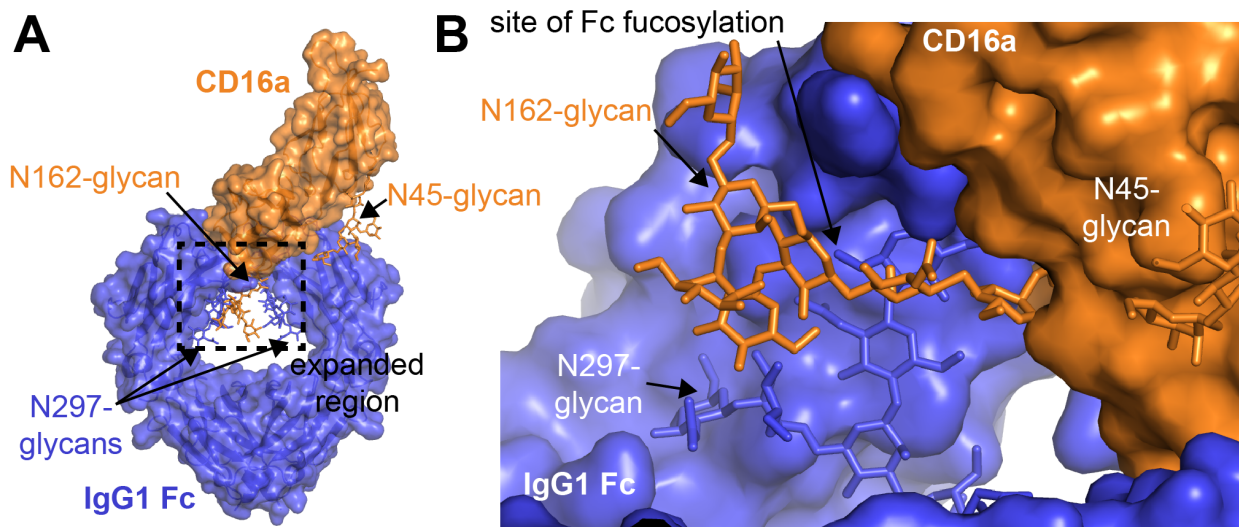
                                     38      45
                                     |      |
CD16a      1  RTEDLPKAVVFLEPQWYRVVLEKDSVTLKCGAYS PEDNSTQWFHNESLISSQASSYFIDA
CD16b      1  RTEDLPKAVVFLEPQWYSVVLEKDSVTLKCGAYS PEDNSTQWFHNESLISSQASSYFIDA
CD32a      1  QAAAPPKAVLKLEPPWINV LQEDSVTLTCQGARS PESDSIQWFHNGNLIPTHTQPSYRFK
CD32b      1  TPAAPPKAVLKLEPQWINV LQEDSVTLTCRGT HS PESDSIQWFHNGNLIPTHTQPSYRFK
CD32c      1  TPAAPPKAVLKLEPQWINV LQEDSVTLTCRGT HS PESDSIQWFHNGNLIPTHTQPSYRFK
          .   ****: *** *   **:*****.**: * **.* ***** .**.:... :

                                     74
                                     |
CD16a      61  ATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALHK
CD16b      61  ATVNDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALHK
CD32a      61  ANNNDSGEYTCQTGQTSLSDPVH LTVLSEWLVLQTPHLEFQEGETIMLRCHSWKDKPLVK
CD32b      61  ANNNDSGEYTCQTGQTSLSDPVH LTVLSEWLVLQTPHLEFQEGETIVLRCHSWKDKPLVK
CD32c      61  ANNNDSGEYTCQTGQTSLSDPVH LTVLSEWLVLQTPHLEFQEGETIVLRCHSWKDKPLVK
          *. :***** ***. :*****:* *   **:***: *:* :.* *****:..* *

                                     162    169
                                     |      |
CD16a      121 VTYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLVGSKNVSSETV NITITQG
CD16b      121 VTYLQNGKDRKYFHHNSDFHIPKATLKDSGSYFCRGLVGSKNVSSETV NITITQG
CD32a      121 VTFFQNGKSKKFSRSLDPTFSIPQANHSHSGDYHCTGNIGYTLFSSKPV TITVQV-
CD32b      121 VTFFQNGKSKKFSRSDPNF SIPQANHSHSGDYHCTGNIGYTLYSSKPV TITVQA-
CD32c      121 VTFFQNGKSKKFSRSDPNF SIPQANHSHSGDYHCTGNIGYTLYSSKPV TITVQA-
          **:*****.:*: : . * **:*. .**.*.* * :* . **:*.*.***:

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**Figure S2.** Sequence alignment of the CD16a (V158), CD16b (NA2), CD32a, CD32b and CD32c extracellular domains studied here. The extracellular antibody-binding domains of CD32b and CD32c are identical. The locations of only four differences in the CD16a and CD16b amino acid sequences are indicated with a red background. Five CD16a N-glycosylation sites are indicated above the sequence.



**Figure S3.** The region of the CD16a N162 glycan in the complex formed with IgG1 Fc. Polypeptide and partial N-glycan coordinates are from the PDB 5vu0 (Falconer et al., 2018). Remaining N-glycan coordinates were generated using GLYCAM ([www.glycam.org](http://www.glycam.org)).