Supplementary data

Impact of IgG1 N-glycosylation on their interaction with Fc gamma receptors

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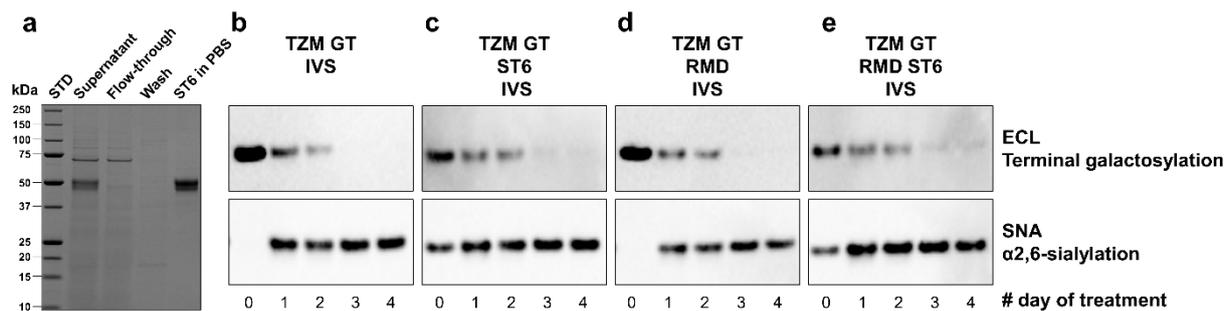
Figure S1: Soluble ST6Gal1 purification and characterization. (a) SDS-PAGE analysis (non-reducing conditions) of soluble ST6Gal1 production and purification (Coomassie Blue staining). (**b-e**) *In vitro* sialylation (IVS) of TZM GT, TZM GTST6, TZM GTRMD and TZM GTRMDST6 was performed with the purified soluble human ST6Gal1. To assess terminal galactosylation and α2,6-sialylation levels during the *in vitro* sialylation process, ECL and SNA blots were performed under reducing conditions, respectively. Only the bands corresponding to the heavy chains of the antibodies are shown.
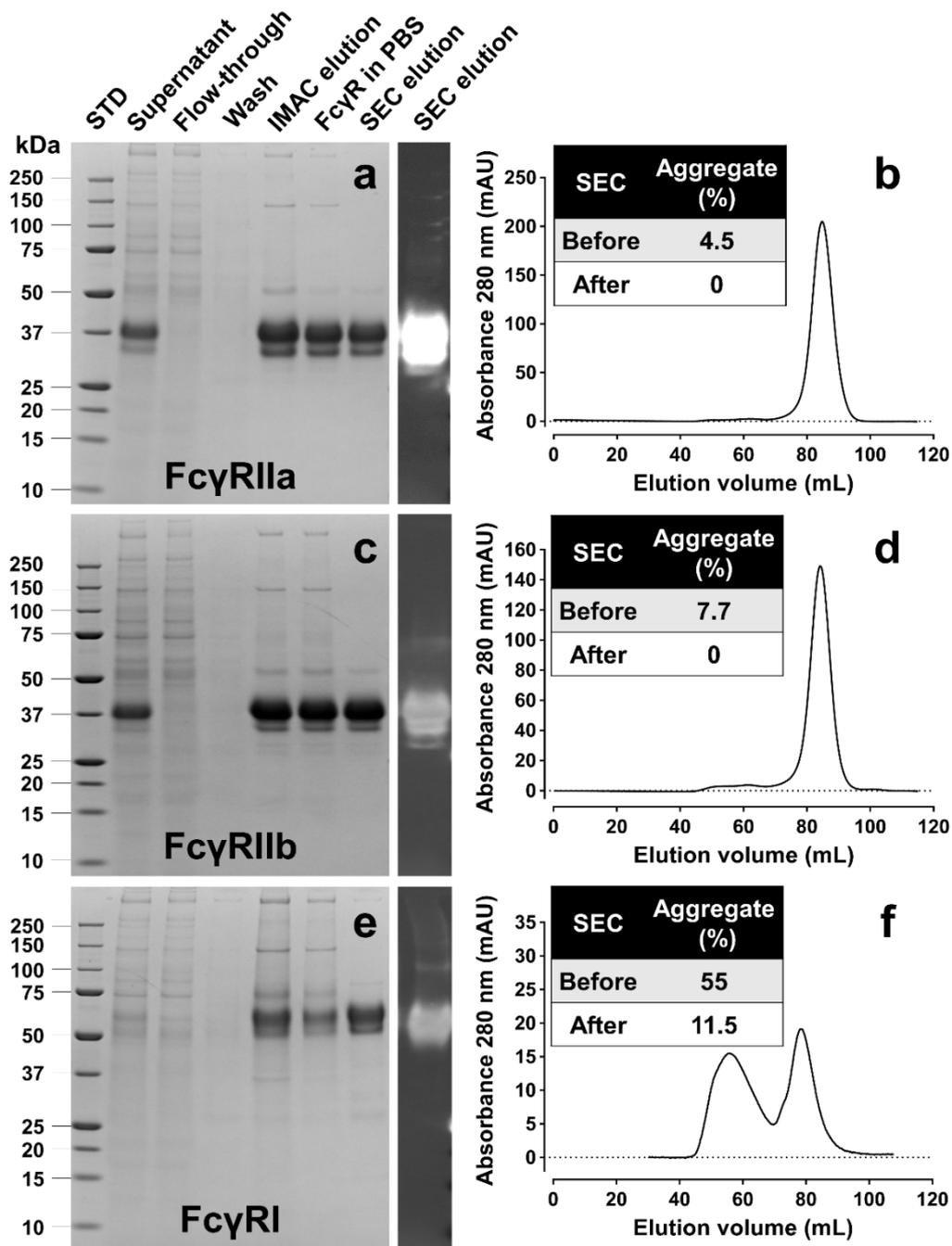
Figure S2: E5-tagged FcγRs production and purification. SDS-PAGE analysis (non-reducing conditions) of E5-tagged FcγRIIa (**a**), E5-tagged FcγRIIb (**c**) and E5-tagged FcγRI (**e**) production and purification (Coomassie Blue staining or transfer to nitrocellulose membrane and probing with Penta-His^TM Alexa Fluor® 488 Conjugate). Chromatograms corresponding to the SEC of E5-tagged FcγRIIa (**b**), E5-tagged FcγRIIb (**d**) and E5-tagged FcγRI (**f**) using a HiLoad 16/600 Superdex 200 Prep grade. Fractions were pooled then aggregate contents were assessed by analytical SEC (see insets).

Table S1: Apparent K_D values measured by our surface plasmon resonance assay.

	FcγRIIIa$_{F158}$*		FcγRIIIa$_{V158}$*		FcγRIIa*		FcγRIIb*		FcγRI**	
	CHO	HEK293	CHO	HEK293	CHO	HEK293	CHO	HEK293	CHO	
	K_D (nM) SD	K_D (nM) SD	K_D (nM) SD	K_D (nM) SD	K_D (nM) SD	K_D (nM) SD	K_D (nM) SD	K_D (nM) SD	K_D1 (nM) SD	K_D2 (nM) SD
TZM	3852 ± 116	3285 ± 198	879 ± 85	852 ± 144	1916 ± 257	1988 ± 322	4670 ± 407	5245 ± 315	0.19 ± 0.03	1.84 ± 0.24
TZM GT	1878 ± 368	1608 ± 395	408 ± 74	373 ± 71	1573 ± 153	1639 ± 225	3971 ± 544	3671 ± 1630	0.13 ± 0.01	1.32 ± 0.11
TZM GTST6	1747 ± 43	1466 ± 44	396 ± 44	358 ± 45	1545 ± 209	1508 ± 166	3651 ± 605	3947 ± 631	0.11 ± 0.01	1.15 ± 0.15
TZM GTIVS	2055 ± 215	1664 ± 194	473 ± 62	422 ± 51	1816 ± 214	1794 ± 153	4174 ± 933	3853 ± 1733	0.14 ± 0.03	1.26 ± 0.07
TZM GTST6IVS	2255 ± 37	1916 ± 19	473 ± 21	414 ± 14	1860 ± 161	1811 ± 91	4224 ± 854	3758 ± 1782	0.13 ± 0.01	1.15 ± 0.16
TZM RMD	120 ± 17	100 ± 15	32 ± 6	27 ± 4	1952 ± 403	1915 ± 374	3435 ± 573	3741 ± 455	0.15 ± 0.04	1.42 ± 0.23
TZM GTRMD	89 ± 8	76 ± 3	23 ± 4	19 ± 3	1610 ± 205	1598 ± 191	3097 ± 512	2774 ± 1289	0.14 ± 0.05	1.29 ± 0.31
TZM GTRMDST6	85 ± 3	66 ± 6	22 ± 3	19 ± 2	1523 ± 163	1516 ± 103	2982 ± 302	2982 ± 648	0.14 ± 0.06	1.35 ± 0.41
TZM GTRMDIVS	107 ± 14	85 ± 13	32 ± 6	26 ± 4	1835 ± 264	1811 ± 217	3378 ± 752	2975 ± 1621	0.14 ± 0.05	1.26 ± 0.49
TZM GTRMDST6IVS	110 ± 13	87 ± 11	28 ± 1	22 ± 1	1857 ± 119	1833 ± 65	3405 ± 628	2898 ± 1616	0.14 ± 0.03	1.26 ± 0.31

The K_D values provided here should be used to compare antibodies with one another but not as absolute values.
SD- Standard deviation
*- K_D values were obtained by steady-state affinity with a one-to-one binding model.
**- K_D values were obtained by globally fitting the data with a kinetic model that assumes that monomeric and agregated FcγRI bind to TZM glycoforms with distinct kinetics.

Table S2: EC$_{50}$ of the TZM glycoforms.

	EC$_{50}$ (ng/mL)	
	FcγRIIIa$_{F158}$	FcγRIIIa$_{V158}$
Synagis	n.d.	n.d.
Herceptin	24.29	16.11
TZM ng	n.d.	n.d.
TZM	71.04	36.34
TZM GT	46.31	25.02
TZM GT ST6	35.01	20.21
TZM GT IVS	n.d.	22.67
TZM GT ST6 IVS	57.24	26.65
TZM RMD	22.22	9.215
TZM GT RMD	15.51	6.79
TZM GT RMD ST6	14.71	7.251
TZM GT RMD IVS	20.90	8.944
TZM GT RMD ST6 IVS	17.24	7.977

n.d.- not determined