

Role of *N*-glycosylation in FcγRIIIa interaction with IgG

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Supplementary Material

Figure S1. *N*-glycan profiling of IgG1.

Figure S2. FcγRIIIa construct sequence.

Figure S3. *N*-glycan profiling of FcγRIIIa-158F.

Figure S4. *N*-glycan profiling of FcγRIIIa-158V

Figure S5. Sensorgrams of glycoengineered IgG1 and FcγRIIIa-158F/V binding by SPR.

Table S1. Cell line engineering background

Table S2. Binding affinity of glycoengineered IgG1 to FcγRIIIa-158F/V.

Supplementary Figure 1

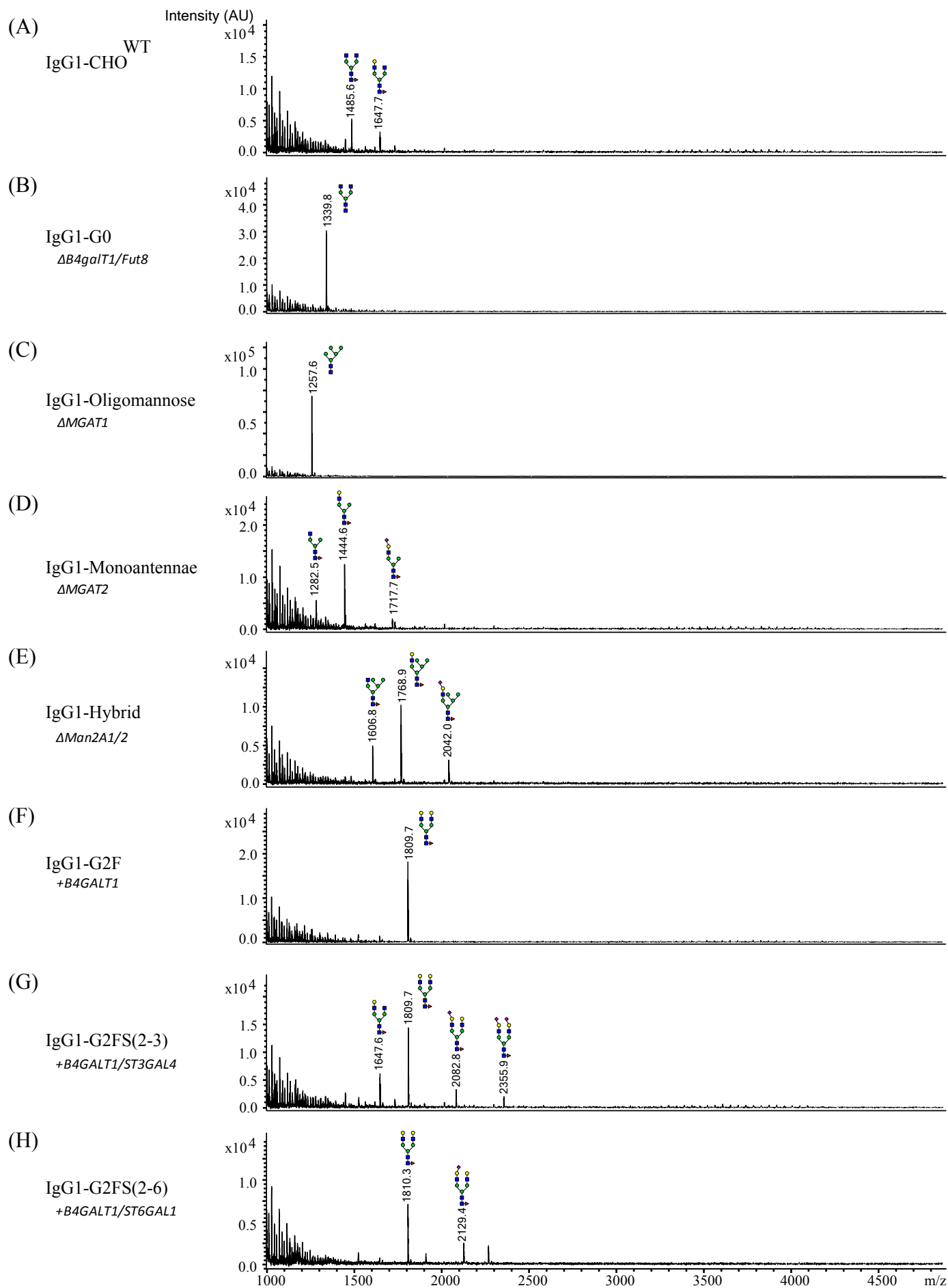


Figure S1. *N*-glycan profiling of IgG1. MALDI-TOF-MS spectrum of esterified released *N*-glycans with *m/z* range of 1000-5000 according to Reiding *et al.* (52). (A) IgG-CHO^{WT}, (B) IgG1-G0 (KO of *B4galT1/Fut8*), (C) IgG1-oligomannose (KO of *Mgat1*), (D) IgG1-Monoantennary (KO of *Mgat2*), (E) IgG1-Hybrid (KO of *Man2A1/2*), (F) IgG1-G2F (KI of *B4GALT1*), (G) IgG1-G2FS(2-3) (KI of *B4GALT1/ST3GAL4*), and (H) IgG1-G2FS(2-6) (KI of *B4GALT1/ST6GAL1*). Annotation represents one possible glycan structure, which is based on gene editing signature and literature. Structures are depicted following the CFG notation (51) and the linkage position of sialic acid is depicted to the left or right for 2-3- or 2-6-linked sialic acid, respectively.

Supplementary Figure 2

FcγRIIIA.F MWQLLLPTAL LLLVSAGMRT EDLPKAVVFL EPQWYRVLEK DSVTLKCQGA
 FcγRIIIA.V MWQLLLPTAL LLLVSAGMRT EDLPKAVVFL EPQWYRVLEK DSVTLKCQGA

FcγRIIIA.F YSPED³⁸**N**STQW FH⁴⁵**N**ESLISSQ ASSYFIDAAT VDDSGEYRCQ ⁷⁴**T**NLSTLSDPV
 FcγRIIIA.V YSPED³⁸**N**STQW FH⁴⁵**N**ESLISSQ ASSYFIDAAT VDDSGEYRCQ ⁷⁴**T**NLSTLSDPV

FcγRIIIA.F QLEVHI GWLL LQAPRWFVKE EDPIHLRCHS WKNTALHKVT YLQNGKGRKY
 FcγRIIIA.V QLEVHI GWLL LQAPRWFVKE EDPIHLRCHS WKNTALHKVT YLQNGKGRKY

FcγRIIIA.F FHHNSDFYIP KATLKDSGSY FCRGL¹⁶²**F**GSK¹⁶⁹**N** VSSETV**N**ITI TQEFGGGGSH
 FcγRIIIA.V FHHNSDFYIP KATLKDSGSY FCRGL¹⁶²**V**GSK¹⁶⁹**N** VSSETV**N**ITI TQEFGGGGSH

FcγRIIIA.F HHHHHHHHHG LNDIFEAQ**K**I EWHE
 FcγRIIIA.V HHHHHHHHHG LNDIFEAQ**K**I EWHE








	Signal peptide		Biotinylation site
	Ig-like domain		N-glycan site
	10X HIS-Tag		Allotype
	AviTag™		

Figure S2. FcγRIIIa construct sequence. Construct sequence of soluble human recombinant FcγRIIIa-158F/V with *N*-glycan sites (Asn38, Asn45, Asn74, Asn162, and Asn169) annotated in bold and signal peptide and Ig-like domains highlighted. FcγRIIIa was tagged by a 10X His-tag and AviTag™, of which the latter can be enzymatically biotinylated at the lysine (K).

Supplementary Figure 3

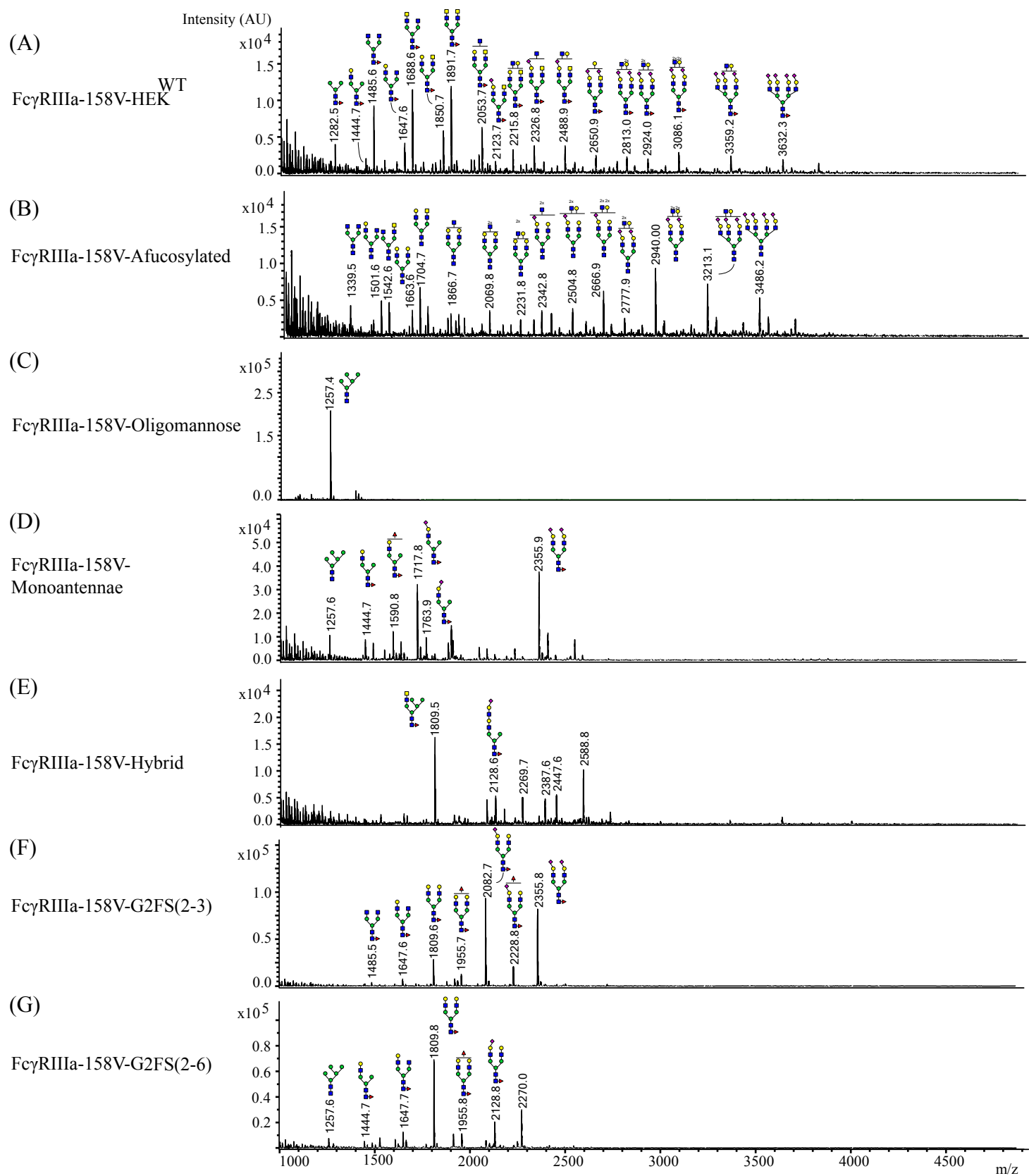


Figure S3. *N*-glycan profiling of FcγRIIIa-158F. MALDI-TOF-MS spectrum of esterified released *N*-glycans of FcγRIIIa-158V with m/z range of 1000-5000 according to Reiding *et al.* (52). (A) FcγRIIIa-HEK^{WT}, (B) FcγRIIIa-Afucosylated by KO of *FUT8*, (C) FcγRIIIa-Oligomannose with (KO of *MGAT1*), (D) FcγRIIIa-Monoantennary (KO of *MGAT2/3*, *KIB4GALT1*), (E) FcγRIIIa-Hybrid (KO of *MAN2A1/2*), (F) FcγRIIIa-G2FS(2-3) (KO of *B4GALNT3/4*, Δ *MGAT3/4A/4B/5*, *ST6GAL1*, *FUT4*) and (G) FcγRIIIa-G2FS(2-6) (KO of *B4GALNT3/4*, *MGAT3/4A/4B/5*, *ST3GAL3/4/6*, *FUT4*). Annotation represents one possible glycan structure, which is based on gene editing signature and literature. Structures are depicted following the CFG notation (52) and the sialic acid linkage is depicted to the left or right for 2-3- or 2-6-linked sialic acid, respectively.

Supplementary Figure 4

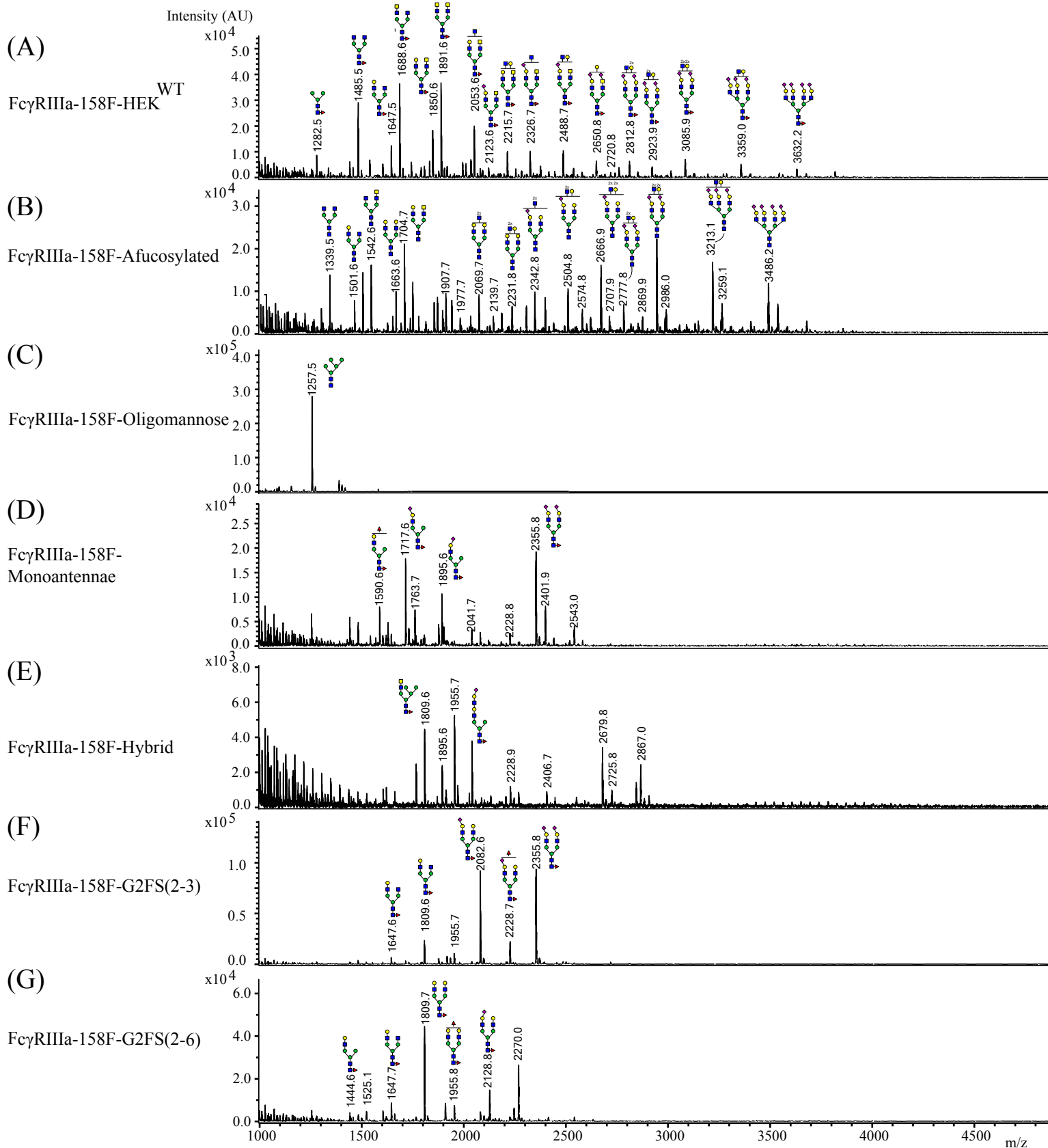
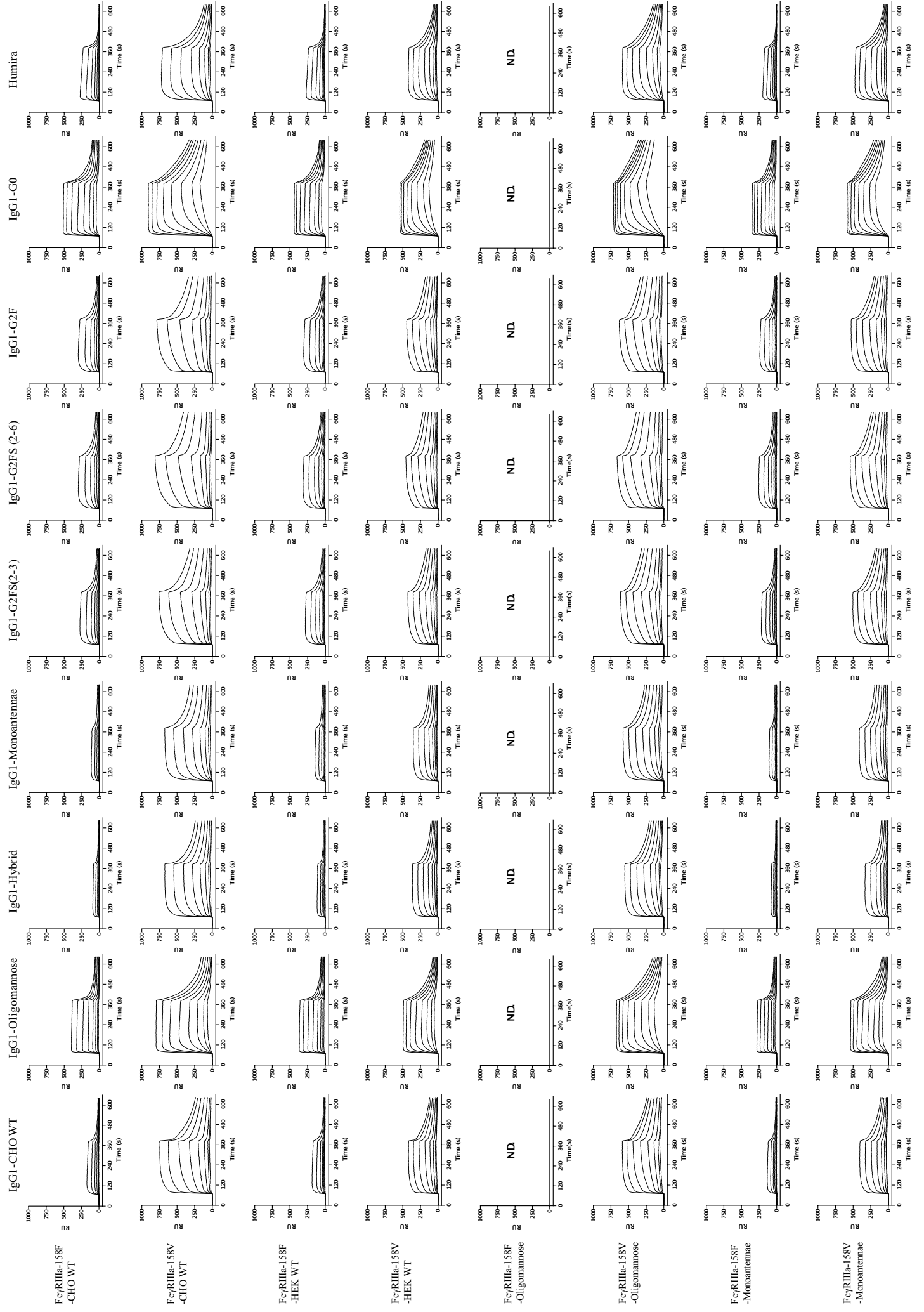


Figure S4. *N*-glycan profiling of Fc γ RIIIa-158V. MALDI-TOF-MS spectrum of esterified released *N*-glycans of Fc γ RIIIa-158V with *m/z* range of 1000-5000 according to Reiding *et al.* (52). **(A)** Fc γ RIIIa-HEK^{WT}, **(B)** Fc γ RIIIa-Afucosylated by KO of *FUT8*, **(C)** Fc γ RIIIa-Oligomannose with (KO of *MGAT1*), **(D)** Fc γ RIIIa-Monoantennary (KO of *MGAT2/3*, *KI B4GALT1*), **(E)** Fc γ RIIIa-Hybrid (KO of *MAN2A1/2*), **(F)** Fc γ RIIIa-G2FS(2-3) (KO of *B4GALNT3/4*, Δ *MGAT3/4A/4B/5*, *ST6GAL1*, *FUT4*) and **(G)** Fc γ RIIIa-G2FS(2-6) (KO of *B4GALNT3/4*, *MGAT3/4A/4B/5*, *ST3GAL3/4/6*, *FUT4*). Annotation represents one possible glycan structure, which is based on gene editing signature and literature. Structures are depicted following the CFG notation (51) and the sialic acid linkage is depicted to the left or right for 2-3- or 2-6-linked sialic acid, respectively.

Supplementary Figure 5



IgG1-CHOWT

IgG1-Oligomannose

IgG1-Hybrid

IgG1-Monoantennae

IgG1-G2FS(2-3)

IgG1-G2FS(2-6)

IgG1-G2F

IgG1-G0

Humira

FcγRIIIa-158F-Hybrid

FcγRIIIa-158V-Hybrid

FcγRIIIa-158F-G2FS(2-3)

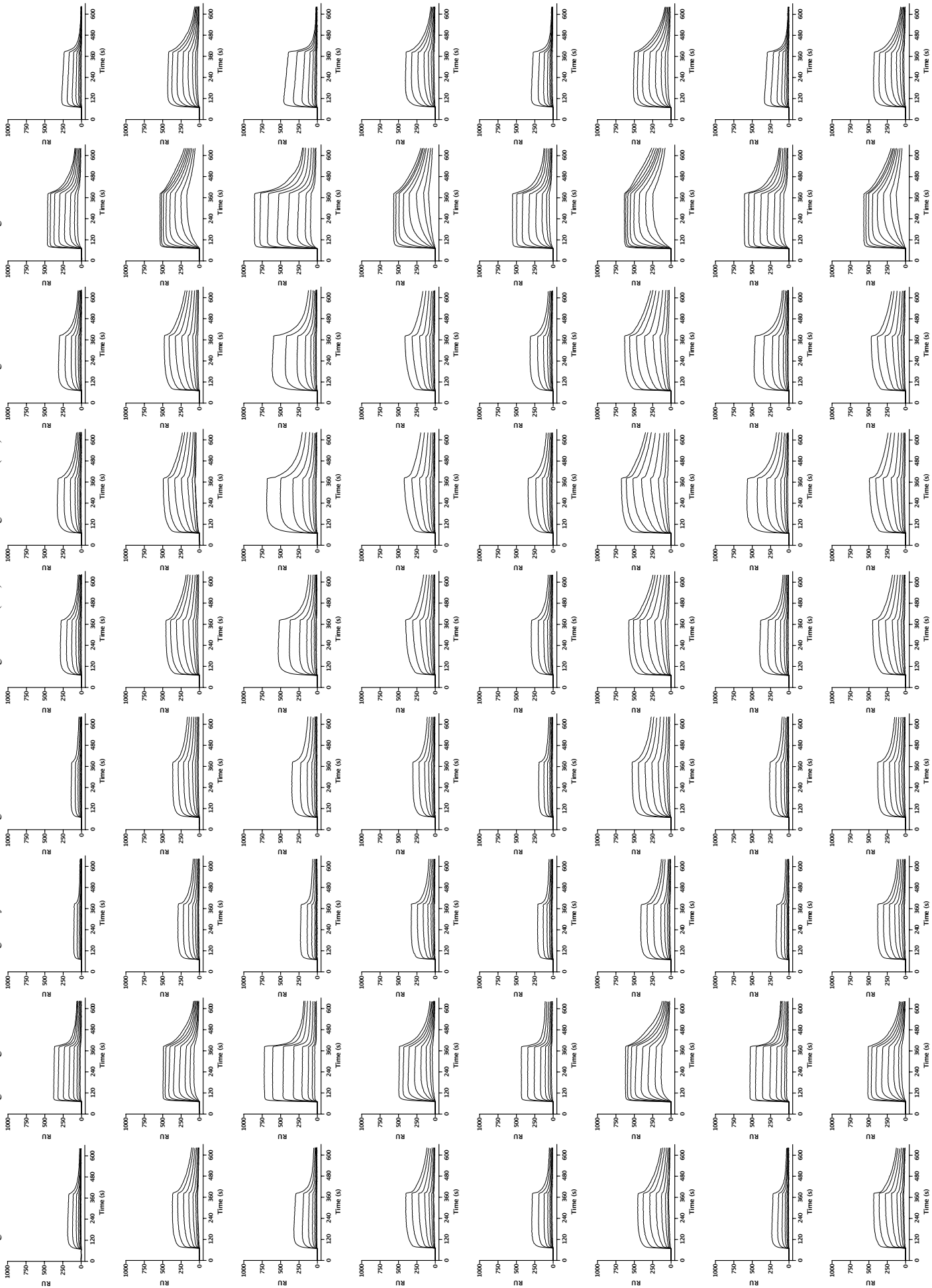
FcγRIIIa-158V-G2FS(2-3)

FcγRIIIa-158F-G2FS(2-6)

FcγRIIIa-158V-G2FS(2-6)

FcγRIIIa-158F-A-fucosylated

FcγRIIIa-158V-A-fucosylated



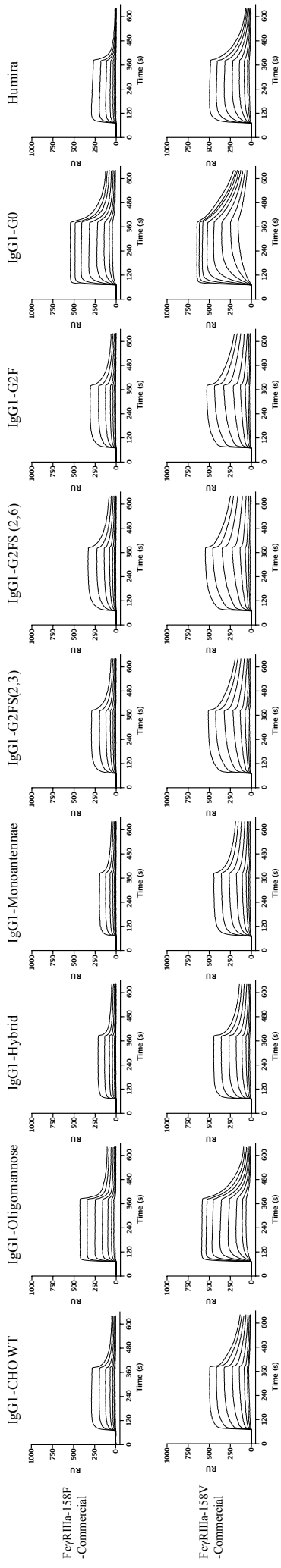


Figure S5. Sensorgrams of glycoengineered IgG1 and FcγRIIIa-158F/V binding by SPR. SPR sensorgrams are shown after biotinylated glycoengineered FcγRIIIa-158F/V was spotted in 4-fold dilutions and overflown with glycoengineered IgG1 for 8 dilution series.