Supplementary material

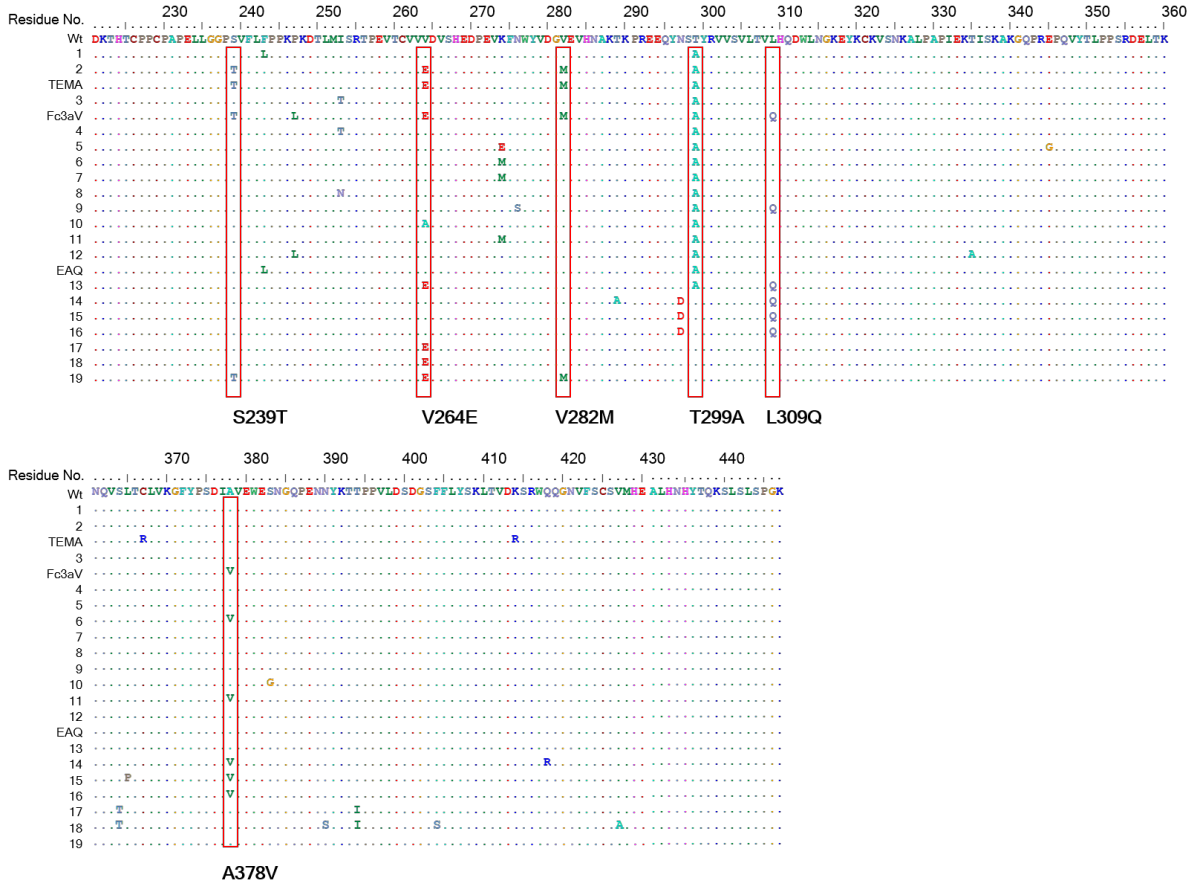

Figure S1. Amino acids sequence alignment of isolated Fc variants. Twenty-two Fc variants were identified from the screened library after 4 rounds of sorting. Highly enriched mutations in several Fc variants are highlighted by red box.

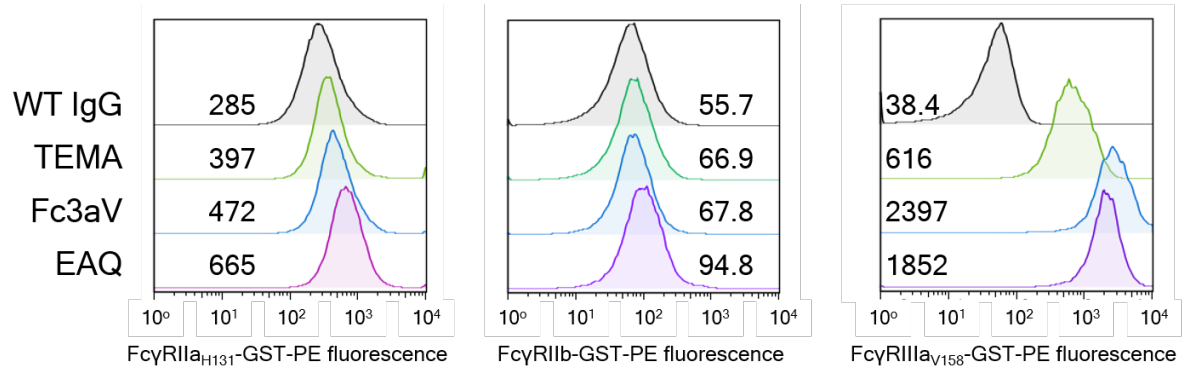

Figure S2. Flow cytometry scanning of Fc variants isolated from library screenings. *E.coli* spheroplasts displaying wild type, TEMA, EAQ, and Fc3aV Fc portions were incubated with 10 nM of PE-labeled GST tagged-FcγRIIa$_{H131}$, -FcγRIIb, or -FcγRIIIa. Mean fluorescence intensity (MFI) values of GST-PE for each Fc variants are presented.

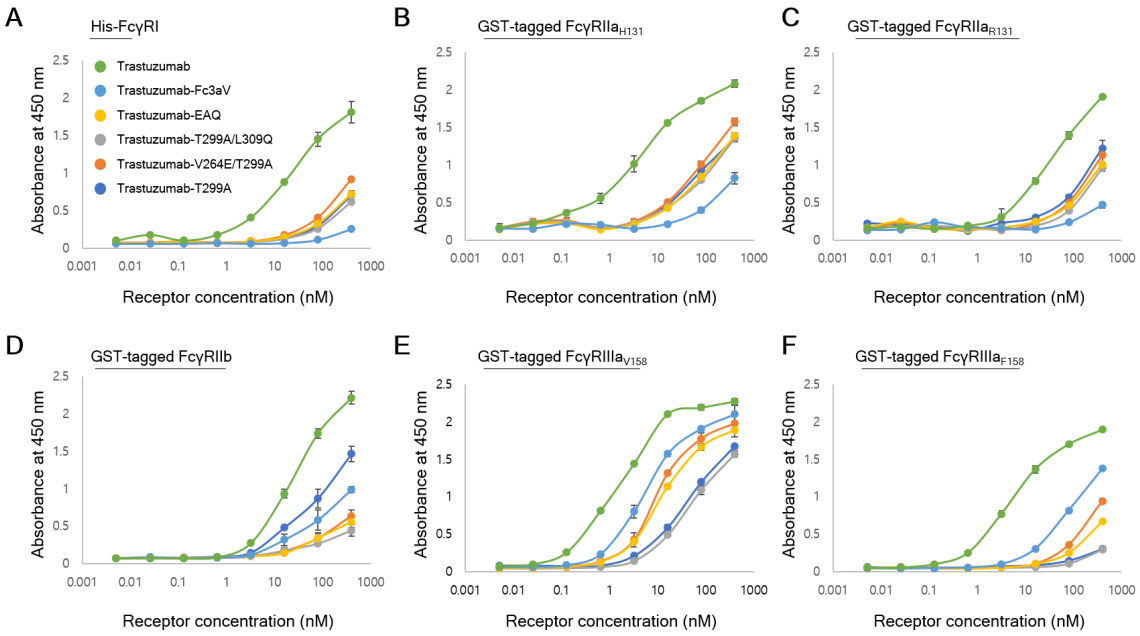

Figure S3. ELISA analysis for the binding of Trastuzumab-Fc variants to ectodomains of human FcγRs. Antibodies were coated to 96 well plates and the binding of high affinity receptor, His-tagged FcγRI (A), and low affinity receptors, GST-tagged FcγRIIa$_{H131}$ (B), FcγRIIa$_{R131}$ (C), FcγRIIb (D), FcγRIIIa$_{F158}$ (E), and FcγRIIIa$_{V158}$ (F) was detected by anti-GST antibodies coupled to HRP. Error bars represent the standard deviation from duplicate experiments.

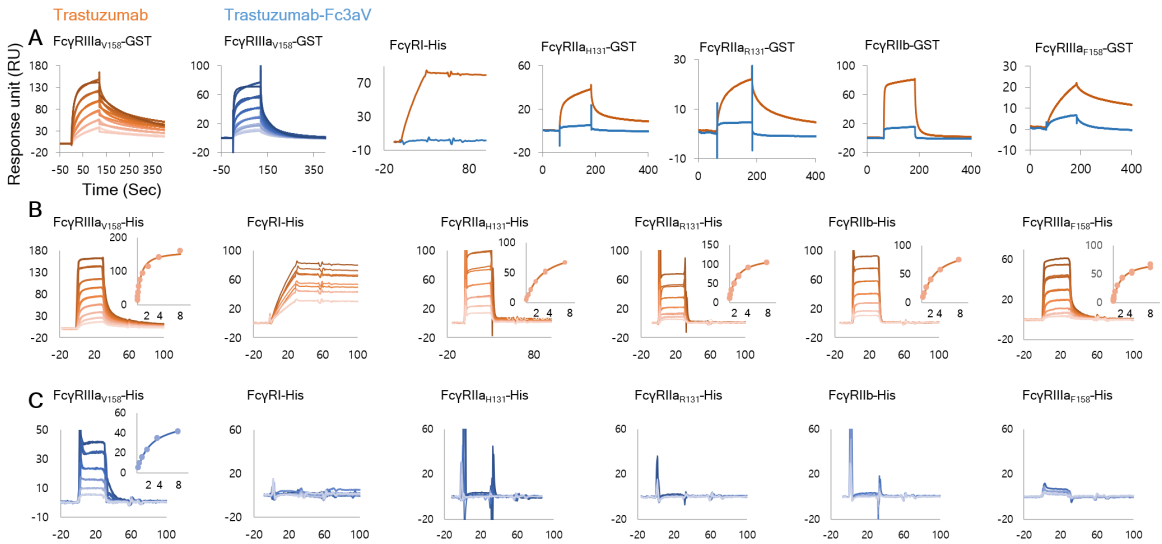

Figure S4. Kinetic binding analysis of Trastuzumab or Trastuzumab-Fc3aV binding to FcγRs. (**A**) SPR sensorgrams of the interaction between wt Trastuzumab (orange) or Trastuzumab-Fc3aV (sky blue) with serially diluted GST-tagged dimeric ectodomains of FcγRIIIa$_{V158}$. Sensorgrams of the interaction between wt Trastuzumab (orange) and Trastuzumab-Fc3aV (sky blue) with 400 nM of his-tagged FcγRI or GST-tagged low-affinity FcγRs are shown using overlays. (**B-C**) SPR sensorgrams and steady state model fit of wt Trastuzumab (orange) and Trastuzumab-Fc3aV (sky blue) with either monomeric his-tagged or dimeric GST-tagged human FcγRs. Antibodies were immobilized on a CM5 chip and the binding of the serial diluted monomeric FcγRI (120 nM-40 nM), FcγRIIa$_{H131}$, FcγRIIa$_{R131}$, and FcγRIIb (8 µM-125 nM) is represented. Inserted plot: Response unit (RU) as a function of receptor concentration (µM). In all sensorgrams, x axis represents time (sec) and y axis response unit (RU).

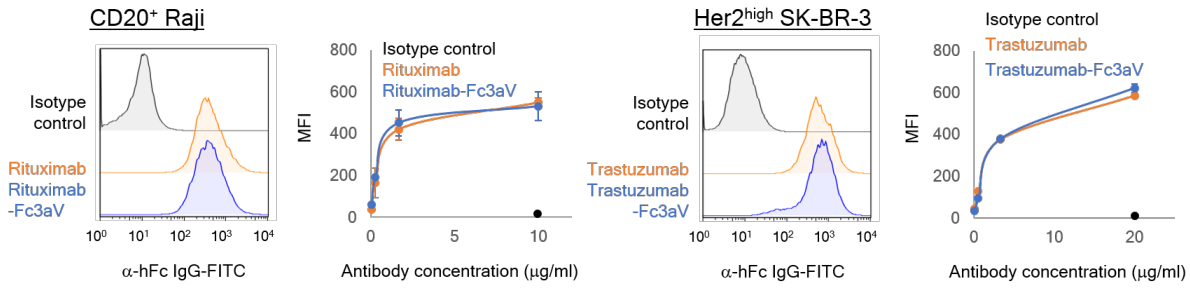

Figure S5. Cell surface binding analysis of antibody variants with engineered Fc variant. Binding activity of the serially diluted Rituximab or Trastuzumab Fc variant to $CD20^{+}$ Raji or $Her2^{high}$ SK-BR-3 cells was detected by a $F(ab')_2$ fragment against hIgG Fc, conjugated with FITC.

Table S1. K_D values for the binding Fc3aV to dimeric, GST-tagged low affinity FcγRs

K_D (μM)	WT	Fc3aV	T299A	EA	EAQ	TEMA
His-FcγRI	0.0057 ± 0.0017	-	11.7	1.13	0.90	N.T.
GST-FcγRIIa$_{R131}$	1.16	-	3.44	2.63	1.71	N.T.
GST-FcγRIIa$_{H131}$	0.22	-	1.81	1.00	0.98	N.T.
GST-FcγRIIb	7.64	-	0.69	0.11	0.96	-
GST-FcγRIIIa$_{F158}$	1.47	-	71.4	0.91	0.93	-
GST-FcγRIIIa$_{V158}$	0.068	0.2 ± 0.01	24.9	0.48	2.60	1.3 ± 0.02

K_D values were calculated by fitting the experimental curves using models provided by the BIAevaluation software: the 1:1 langmuir model for ectodomains of FcγRI; the bivalent analyte model for all other ectodomains of low-affinity FcγRs. "-" = not detectable (lower response than 5 RU at 400 nM of dimeric FcγR), N.T.= not tested.