## **Supporting Information**

# Effective Phagocytosis of Low Her2 Tumor Cell Lines with Engineered, Aglycosylated IgG Displaying High FcyRIIa Affinity and Selectivity

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<sup>2</sup>To whom correspondence should be addressed. E-mail: gg@che.utexas.edu; Telephone: 512-471-6975; Fax: 512-471-7963. SI Materials and Methods. Molecular Biology Techniques: All plasmids and primers used in this study are described in supplementary Table S1 and Table S2. A gene encoding the human IgG1 Fc (comprising the hinge, CH2, and CH3 domains (1)), was ligated into pPelBFLAG on Sfil restriction endonuclease sites to generate pPelBFLAG-Fc. To construct pTrc99A-DsbA-Fc2a-FLAG, the Fc2a gene containing mutations S298G and T299A in the CH2 region was PCR amplified using two primers (STJ#422 and STJ#147) with the template pTrc99A-DsbA-Fc-FLAG (1), and ligated into SacII / HindIII restriction enzyme-treated pTrc99A-DsbA-Fc-FLAG. For the construction of pSTJ4-AglycoT-Fc2a, the Fc2a gene was amplified by primers STJ#290 and STJ#291, with pTrc99A-DsbA-Fc2a-FLAG as a template. The amplified PCR fragments were ligated into Sall / EcoRV digested pSTJ4-AglycoT to generate pSTJ4-AglycoT-Fc2a. pSTJ4-AglycoT-Fc5-2a (E382V/M428I/S298G/T299A) was generated by amplifying the Fc5-2a gene using two primers (STJ#490 and STJ#220) and pSTJ4-AglycoT-Fc5 as a template, followed by SacII / EcoRI restriction enzyme digestion, and ligation into digested pSTJ4-AglycoT.

Trastuzumab heavy chains encoding either wild type human Fc or the Fc5, Fc2a, or Fc5-2a mutants were amplified using the primers STJ#474 and STJ#67 with the respective templates pSTJ4-AglycoT, pSTJ4-AglycoT-Fc5, pSTJ4-AglycoT-Fc2a, or pSTJ4-AglycoT-Fc5-2a. Each fragment was ligated into the pPelBFLAG vector using the *Sfi*l restriction enzyme sites to generate pPelB-AglycoT(H)-FLAG, pPelB-AglycoT(H)-Fc5-FLAG, pPelB-AglycoT(H)-Fc2a-FLAG, and pPelB-AglycoT(H)-Fc5-2a-FLAG. pBADNlpAHis-M18 was constructed by ligating the NlpA fused M18 scFv gene amplified from pMoPac1-FLAG-

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M18 (2), and digested with *Xba*I–*Hin*dIII restriction enzymes, into pBAD30-KmR (2) digested with the same restriction endonucleases. Ligation of the trastuzumab VL-Ck gene, amplified using two primers (STJ#475 and STJ#476) and template pSTJ4-AglcoT, into pBADNIpAHis-M18 using *Sfi*I restriction sites generated pBADNIpA-VL-Ck-His. The PelB leader peptide-fused trastuzumab VL-Ck gene was amplified with primers STJ#16 and STJ#340 from pSTJ4-AglycoT as the template, digested with *Xba*I / *Hin*dIII endonucleases, and ligated into pBADNIpA-VL-Ck-His digested with the same endonucleases to generate pBADPelB-VL-Ck. pBAD-AglycoT(L)-His was constructed by ligating *Xba*I digested PCR fragments amplified using the primers STJ#70 and STJ#332 with pBADPelB-VL-Ck as a template into *Xba*I digested pBADNIpA-VL-Ck-His.

The Fc1001, Fc1002, Fc1003, Fc1004, FcG236A, and FcN297D genes were PCR amplified from AglycoT(H)-Fc1001-FLAG, AglycoT(H)-Fc1002-FLAG, AglycoT(H)-Fc1003-FLAG, AglycoT(H)-Fc1004-FLAG, AglycoT(H)-FcG236A-FLAG, and AglycoT(H)-FcN297D-FLAG, respectively by using the primers STJ#290 and STJ#498, then digested with *Sal*I and *Xba*I restriction enzymes, and ligated into the mammalian expression vector, pMAZ-IgH-GlycoT (*1*), to generate pMAZ-IgH-GlycoT-Fc1001, pMAZ-IgH-GlycoT-Fc1002, pMAZ-IgH-GlycoT-Fc1003, pMAZ-IgH-GlycoT-Fc1004, pMAZ-IgH-GlycoT-FcG236A, and pMAZ-IgH-GlycoT-FcN297D, respectively.

For 2B6-N297D gene synthesis (3), 2B6 variable domains from heavy and light chains were gene assembled by PCR with Phusion polymerase (New England Biolabs) from primers WK#158 – WK#169 for the light chain and WK#172 – WK#187 for the heavy

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chain (25 cycles with 98 °C denaturation 1 min, 55 °C denaturation 1 min and 72 °C extension 2 min were performed before a 10 min final extension step). Correctly assembled genes were amplified with the light chain primers WK#158, WK#170 and heavy chain primers WK#171, WK#187 by overlap extension (OLE) PCR (*4*). Briefly, the megaprimer generated in the previous step was added with Phusion polymerase at a 1:250 molar ratio to heavy chain template (pMAZ-IgH-GlycoT-FcN297D) and light chain template (pMAZ-IgL-GlycoT) (25 cycles of amplification were performed with 98 °C denaturation 1 min, 55 °C denaturation 1 min and 72 °C extension 10 min steps). Remaining template plasmid in the PCR reaction was digested with Dpn1 endonuclease for 1 h at 37 °C and the final mixture was transformed into Jude-1 cells (F' [Tn10(Tet<sup>r</sup>) proAB<sup>+</sup> *lacl*<sup>q</sup>  $\Delta$ (*lacZ*)M15] *mcrA*  $\Delta$ (*mrr-hsdRMS-mcrBC*) 80d*lac*Z $\Delta$ M15  $\Delta$ *lacX74 deoR recA1 araD139*  $\Delta$ (*ara leu*)7697 *galU galK rpsL endA1 nupG*) (5).

pMAZ-FcyRIIa<sub>H131</sub>-GST was cloned by the OLE PCR method as described above using a megaprimer generated with primers WK#100 and WK#101 from pDNR-LIB-FcyRIIa (ATCC: MGC-23887). The second PCR step cloned this megaprimer fragment in place of FcyRI in pMAZ-FcyRI-GST, a plasmid derived by OLE PCR from pMAZ-IgH GlycoT by cloning the FcyRI-GST cassette from pcDNA3(oriP)-FcyRI (*6*) with primers WK#56 and WK#57. pMAZ-FcyRIIa<sub>R131</sub>-GST was generated by OLE PCR using pMAZ-FcyRIIa<sub>H131</sub>-GST as a template to generate a megaprimer with primers (WK#100 and WK#116).

For the construction of pPelBHis-Fc $\gamma$ RIIIa<sub>V158</sub>, the Fc $\gamma$ RIIIa<sub>V158</sub> gene was PCR amplified using primers STJ#76 and STJ#82 and the template pCMV-SPORT6-Fc $\gamma$ RIIIa (ATCC: MGC-

45020), and then ligated into pPelBHis (1) using the *Sfi*l sites. pMAZ-FcyRIIIa<sub>V158</sub>-GST was cloned by OLE PCR from pMAZ-FcyRIIIa<sub>V158</sub>, a monomeric mammalian derivative of pPelBHis-FcrRIIIa<sub>V158</sub>, with primers WK#91 and WK#92 used to generate the megaprimer. The recipient vector was pMAZ-FcyRIIIa<sub>H158</sub>-GST. pMAZ-FcyRIII<sub>F158</sub>-GST was likewise cloned by OLE PCR from pMAZ-FcyRIIIa<sub>V158</sub>-GST with primers WK#91 and WK#94 used for megaprimer synthesis and pMAZ-FcyRIIIa<sub>V158</sub>-GST as the recipient vector.

**IgG Display in E. coli for FcyR Binding.** pBAD-AglycoT(L)-His was transformed with either pPelB-AglycoT(H)-FLAG, pPelB-AglycoT(H)-Fc5-FLAG, pPelB-AglycoT(H)-Fc2a-FLAG, or pPelB-AglycoT(H)-Fc5-2a-FLAG for wild type trastuzumab, trastuzumab-Fc5, trastuzumab-Fc2a, or trastuzumab-Fc5-2a, respectively into E. coli JUDE-1. The transformed *E. coli* cells were cultured overnight at 37 °C with 250 rpm shaking in TB (Terrific Broth; Becton Dickinson Diagnostic Systems Difco™) supplemented with 2% (wt/vol) glucose, chloramphenicol (40  $\mu$ g/ml) and kanamycin (50  $\mu$ g/ml). The overnight cultured cells were diluted 1:100 in fresh 7 ml of TB medium with chloramphenicol (40  $\mu$ g/ml) and kanamycin (50  $\mu$ g/ml) in 125 ml Erlenmeyer flask. After incubation at 37 °C for 2 h and cooling at 25 °C for 20 min with 250 rpm shaking, protein expression was induced with 1 mM of isopropyl-1-thio-D-galactopyranoside (IPTG). 20 h after IPTG induction, 6 ml of the culture broth was harvested by centrifugation and washed two times in 1 ml of cold 10 mM Tris-HCl (pH 8.0). After re-suspension in 1 ml cold STE solution (0.5 M Sucrose, 10 mM Tris-HCl, 10 mM EDTA, pH 8.0), the cells were incubated at 37 °C for 30 min on a tube rotator, pelleted by centrifugation at 12,000 x g for 1 min and washed in 1 ml of cold Solution A (0.5 M Sucrose, 20 mM MgCl<sub>2</sub>, 10 mM MOPS, pH 6.8). The washed cells were incubated in 1 ml Solution A with 1 mg/ml of hen egg lysozyme at 37 °C for 15 min. After centrifugation at 12,000 x g for 1 min, the resulting spheroplast pellets were resuspended in 1 ml of cold PBS. 300  $\mu$ l of the spheroplasts were further diluted in 700  $\mu$ l of PBS and labeled with 30 nM FcγRI-FITC to analyze binding. For FACS analysis of FcγRIIa binding, spheroplasts were incubated with 30 nM FcγRIIa C-terminal fused to GST (*6*), washed in 1 ml of PBS, and labeled with polyclonal goat anti-GST-FITC (Abcam) diluted 1:200 in 1 ml of PBS. After incubation for 1 h with vigorous shaking at 25 °C protected from light, the mixture was pelleted by centrifugation at 12,000 x g for 1 min and resuspended in 1 ml of PBS. The fluorescently labeled spheroplasts were diluted in 2.5 ml of PBS and analyzed on BD FACSCalibur (BD Bioscience).

*Library Construction.* An error prone PCR library using the trastuzumab-CH2-CH3 region of Fc5-2a as a template was created using standard techniques (*7*) and the two primers STJ#485 and STJ#67. VH-CH1 was then PCR amplified using the primers STJ#474 and STJ#486 from the template (pSTJ4-AglycoT). The two fragments, hinge-CH2-CH3 regions and VH-CH1 regions, were assembled by gene assembly PCR using the primers STJ#474 and STJ#67 to generate the trastuzumab heavy chain (VH-CH1-Hinge-CH2-CH3) library. The amplified heavy chain library genes were ligated into Sfil-digested pPelBFLAG. The resulting plasmids were transformed into E. coli Jude-1 harboring the light chain plasmid (pBAD-AglycoT(L)-His).

*Culture and Spheroplasting of E. coli for Library Screening.* For screening, *E. coli* Jude-1 cells containing the heavy chain plasmid (pPelB-VH-CH1-Hinge-CH2-CH3) and the light chain plasmid (pBAD-AglycoT(L)-His) were cultured overnight at 37 °C with 250 rpm shaking in TB supplemented with 2% (w/v) glucose and appropriate antibiotics (40  $\mu$ g/ml of chloramphenicol and 50  $\mu$ g/ml of kanamycin). The overnight cultured cells were diluted 1:100 in 110 ml of fresh TB. After incubation at 37 °C for 2 h and cooling at 25 °C with 250 rpm shaking for 20 min, protein expression was induced with 1 mM of isopropyl-1-thio-D-galactopyranoside (IPTG). Following protein expression for 20 h, spheroplasts were prepared from 36 ml of culture broth for library screening.

*Library Screening.* Glycosylated FcyRIIa-R131-GST (*6*) was labeled with Alexa488 using an Alexa488 labeling kit (Invitrogen). A competitive screen was used to isolate clones with high binding affinity for FcyRIIa over FcyRIIb in which spheroplasts were incubated with fluorescent FcyRIIa-R131-GST-Alexa488 with excess amounts of non-fluorescent FcyRIIb-GST present (concentration of FcyRIIa-R131-GST-Alexa488: concentration of nonfluorescent FcyRIIb-GST = 30 nM: 100 nM for the 1<sup>st</sup> round, 10 nM: 100 nM for the 2<sup>nd</sup> round, 10 nM : 100 nM for the 3<sup>rd</sup> round, 5 nM : 100 nM for the 4<sup>th</sup> round, and 5 nM : 200 nM for the 5<sup>th</sup> round of sorting). More than 4 × 10<sup>8</sup> spheroplasts were sorted in the first round of screening on a MoFlo flow cytometer (Dako Cytomation) equipped with a 488 nm argon laser for excitation. In each round, the top 3% of the population showing the highest fluorescence was isolated and resorted immediately after the initial sorting. The heavy chain genes (VH-CH1-CH2-CH3) in the spheroplasts were amplified from the collected spheroplasts by PCR with two specific primers STJ#474 and STJ#67, ligated into *Sfi*I restriction enzyme digested pPelBFLAG-Fc, and transformed in electrocompetent *E. coli* Jude-1 cells. The resulting transformants were grown on chloramphenicol containing LB agar plates and prepared again as spheroplasts for the next round of sorting.

Protein Expression and Purification. AglycoT-Fc1001, AglycoT-Fc1002, AglycoT-Fc1003, AglycoT-Fc1004, GlycoT-G236A, AglycoT-N297D and the N297D variant of an anti-FcyRIIb 2B6 antibody (3) were produced by transient transfection of HEK293F cells (Invitrogen). pMAZ-IgL and pMAZ-IgH vectors for each of the variants were purified from overnight E. coli cultures by Midiprep (Qiagen). 293Fectin Transfection Reagent (Invitrogen) was used to transfect cells cultured in GIBCO FreeStyle<sup>™</sup> 293 Expression Medium (Invitrogen) following the manufacturer's instructions. After 6 days, the cells were pelleted by centrifugation at 2,000 rpm for 10 min and the supernatant was recovered. 25x PBS was added to the supernatant to make a 1x final concentration and the solution was passed through a 0.22 µm filter. Protein A high capacity agarose resin (Thermo Scientific) was added to a polypropylene column and allowed to settle. The packed slurry was equilibrated with 1x PBS before addition of the buffered supernatant. The flow through was collected and passed twice more through the column. Unbound proteins were washed away with >10 CV (Column Volume) of 1x PBS. IgGs were eluted with 3 ml of 100 mM glycine-HCl (pH 2.7) and immediately neutralized with 1 ml of 1 M Tris (pH 8.0).

Samples were buffer-exchanged into 1x PBS using Amicon Ultra-4 (Millipore) spin columns with a 10 kDa cutoff. Purity of purified samples was assessed by 4-20% gradient SDS-PAGE gel (NuSep).

FcγRIIa-R131-GST, FcγRIIa-H131-GST, FcγRIIb-GST and FcγRIIIa-F158-GST were produced by transient transfection of HEK293F cells (Invitrogen) using the pMAZ-IgH expression vectors described. Receptors with GST fusion partners were purified by Glutathione Sepharose (GE Healthcare) affinity chromatography. 25x PBS was added to filtered supernatants to a 1x concentration and the mixture passed twice over the column. The column was washed with 100 ml of 1x PBS to remove nonspecifically bound protein. 4 ml of 1x PBS containing 10 mM reduced glutathione was used for elution into 10 kDa filter columns.

*SPR Analysis.* Surface plasmon resonance (SPR) was performed using a BIAcore 3000 instrument (GE Healthcare). Herceptin, AglycoT-Fc5-2a, AglycoT-Fc1001, AglycoT-Fc1002, AglycoT-Fc1003, and AglycoT-Fc1004 were individually immobilized on CM5 sensor chips by amine coupling as recommended by the manufacturer. Binding experiments were performed in HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM EDTA, and 0.005% P20 surfactant)(GE Healthcare). Dimeric FcγRIIa-131R-GST, FcγRIIa-H131-GST, and FcγRIIb-GST receptors were injected in duplicate at a flow rate of 30 μl/min for 60 sec with a dissociation time of 5 min. The chip was regenerated after each run by sequential injection of 50 mM glycine, pH 4.0, 50 mM glycine, pH 9.5, and 3

M NaCl for 2 min each. For each run, a bovine serum albumin-coupled (BSA) surface was used to subtract non-specific receptor binding. Equilibrium dissociation constants ( $K_D$ ) for monovalent receptor binding were determined by fitting a 2:1 bivalent analyte model ( $A+2B \rightleftharpoons AB+B \rightleftharpoons AB_2$ ) to the data using BIAevaluation 3.2 software (GE Healthcare) in accordance with earlier analyses (8). To determine binding to FcγRI, purified IgGs were immobilized on activated amine CM5 Biacore chips in 10 mM sodium acetate buffer (pH 5.0) and BSA in 10 mM sodium acetate (pH 5.0) was immobilized to a control lane on each chip for background receptor binding subtraction. 30 µl samples of purified FcγRI (R&D Systems) in HBS-EP running buffer were injected in duplicate and dissociation was monitored over a 5 min period. 10 mM glycine at pH 3.0 was used for chip regeneration between samples. The data were fit to a 1:1 Langmuir binding model as described earlier to obtain kinetic constants (1).

*ELISAs.* ELISA plates (Corning) were coated with 4  $\mu$ g/ml of Her2 protein (Sino Biological) in 0.05M Na<sub>2</sub>CO<sub>3</sub> (pH 9.5) overnight at 4 °C. The next day the plates were blocked at room temperature for 2 h with 2% milk in PBS containing 0.05% Tween (PBST) and washed four times in PBST at pH 7.4 before the addition of 4  $\mu$ g/ml of antibody dissolved in PBS with 2% milk (PBSM). After 1 h of incubation, the plates were washed with PBST 4x and then 66  $\mu$ l of FcγRIIIa-GST at 20  $\mu$ g/ml added to the first well followed by 1:4 serial dilution. The plates were incubated at room temperature for 1 h, washed with PBS 4x and 50  $\mu$ l PBSM was added containing goat anti-GST HRP (GE Healthcare) 1:5000 for 1 h. To develop the plates, the wells were washed 4x with PBST and 50  $\mu$ l TMB substrate was added per well (Thermo Scientific). 50  $\mu$ l of 1 M H<sub>2</sub>SO<sub>4</sub> was used for neutralization and the final Abs<sub>450</sub> was recorded.

*HER2 Cell Surface Density.* To qualitatively evaluate the density of HER2 receptors on the surface of SKBR-3 (ATCC), SKOV-3 (ATCC), and MDA-MB-453 (ATCC) cells, 10  $\mu$ g/ml of Herceptin or IgG1 pooled from human serum (Sigma-Aldrich) as a control were incubated with 10<sup>6</sup> cells for 45 min on ice in 1 ml of Stain buffer (BD Biosciences). Subsequently, the Herceptin or IgG1 bound cells were washed with 1 ml of Stain buffer by centrifugation at 400 x g for 5 min, and labeled with 1:50 diluted donkey anti-human IgG (H+L) FITC-conjugate Fab (Jackson ImmunoResearch Laboratories) on ice for 45 min. The cells were washed twice more with 1 ml of Stain buffer following the centrifugation procedure above and cell fluorescence was analyzed by flow cytometry (BD FACSCalibur).

**Preparation of Human Monocyte-derived Macrophages.** PBMCs were isolated from fresh human pooled blood samples (Gulf Coast Regional Blood Center) by Histopaque (Sigma-Aldrich) gradient centrifugation. Briefly, 20 ml Histopaque was added to 50 ml conical tubes followed by 30 ml of blood gradually. The mixture was centrifuged for 15 min in a swinging bucket rotor without braking at 800 x g. PBMCs were aspirated from the sample and transferred to a fresh tube. The sample was washed twice with 50 ml

PBS containing 2% FBS (Mediatech) and 1 mM EDTA by centrifuging without braking at 120 x g for 10 min to remove platelets from the sample. CD14<sup>+</sup> monocytes were isolated by magnetic bead separation (Stemcell Technologies) according to the manufacturer's instructions. Cells were resuspended in RPMI (Invitrogen) containing 15% FBS and seeded at 1.5 x 10<sup>6</sup> cell per well in 96 well plates containing 3 ml of the same media supplemented with 50 ng/ml GM-CSF (R&D systems). The cells were grown at 37 °C in 5% CO<sub>2</sub> and at days 2 and 5 of culture and an additional 1 ml of media with fresh cytokine was added to each well. After 7 days, non-adherent cells were aspirated and the plate was washed with Dulbecco's PBS (Mediatech). 1 ml HyQTase (Thermo Scientific) solution was added for 15 min at 37 °C for the detachment of macrophages from the plate surface. Recovered cells were washed with 50 ml RPMI media and resuspended in RPMI containing 10% Human AB serum (Mediatech). Macrophage differentiation was confirmed by staining with 10 μg/ml anti-CD14-APC (Clone M5E2, Biolegend) and 10 μg/ml anti-CD11b-APC (Clone ICRF44, Biolegend).

**Quantification of FcyRs on Macrophages.** The anti-FcyRIIb antibody, 2B6-N297D (*3*) was transiently expressed and FITC labeled alongside an aglycosylated human IgG1 isotype control using a FITC conjugation kit (Invitrogen). Monocyte-derived macrophages were cultured as above and labeled separately with 20 µg/ml anti-FcyRI-FITC (Clone 10.1, Genetex), 10 µg/ml anti-FcyRIIa-FITC (Clone IV.3, Stemcell Technologies), 20 µg/ml anti-FcyRIII-FITC (Clone 3G8, ABcam), 1 µg/ml 2B6-N297D-FITC, 1 µg/ml human aglycosylated IgG1-FITC isotype control as well as FITC conjugated murine isotype control antibodies

for IgG1 (20 μg/ml Clone 15H6, Biolegend) and IgG2b (10 μg/ml Clone MG2b-57, Biolegend). Receptor counts were determined in a FACS assay by comparing the fluorescent values of antibody labeled macrophages to standard curves generated by bead standards that capture precisely known numbers of each of the labeling antibodies (Quantum Simply Cellular anti-mouse and anti-human, Bangs Laboratories).

**Construction and Parameterization of Mathematical Model.** A mathematical model was developed to better understand the interaction between Her2-expressing cells (SKOV-3 or MDA-MD-453) and macrophages. Within a "contact" area, Her2-bound IgG can bind to FcγRs, but this interaction is not possible outside of this region because of physical constraints imparted by the curvature of the two cells (Fig. 4A). This contact area was estimated to be 1/3 of the surface area of the smaller SKOV-3/MDA-MD-453 cells based on geometric considerations. A lower bound of 1/10 of the surface area has been calculated for a non-deforming bead (*9*), but the actual contact area is significantly higher because macrophages deform and spread around IgG-bound cells (*10, 11*). Model nomenclature and parameter values are provided in Table S4.

We considered three types of FcyRs on macrophages: FcyRIIa-H131, FcyRIIa-R131, and FcyRIIb. The number of FcyRIIa and FcyRIIb were experimentally quantified (Fig. S5*B*), and the FcyRIIa-H131 and FcyRIIa-R131 variants were assumed to exist in 50/50 proportions (*12*). FcyRs partitioned between the contact area and the free (non-contact) area with equilibrium constant K<sub>diff</sub>:

 $Fc\gamma RIIx_{free} \xrightarrow{K_{diff}} Fc\gamma RIIx_{contact}$ 

where  $K_{diff} = \frac{\left[Fc\gamma RIIx_{free}\right]}{\left[Fc\gamma RIIx_{contact}\right]} = \frac{\left[Macrophage Surface Area - Contact Area\right]}{\left[Contact Area\right]}$ 

#### and FcyRIIx = FcyRII-H131, FcyRII-R131 or FcyRIIb

All Her2 receptors on SKOV-3 and MDA-MD-453 cells were considered to be evenly distributed and receptor numbers were calculated from experimental quantification of relative expression levels (Fig. S5*A*) and total absolute values from literature (*13, 14*). The effective concentration of cell-bound IgG ([L<sub>0</sub>]) was calculated in an "effective contact volume," defined as the product of contact\_area and cell\_gap. These cell-bound IgGs were free to interact with FcyRIIx <sub>contact</sub> with ligand depletion:

 $L + Fc\gamma RIIx_{contact} \leftarrow Fc\gamma RIIx_{cell-bound}$ 

where  $K_{D_x} = K_{D_{1|a-H131}}$ ,  $K_{D_{1|a-R131}}$ , or  $K_{D_{1|b}}$  from SPR data for different Fc variants (Table S3).

To mimic conditions in our *in vitro* experiments, as well as in normal physiology, 10  $\mu$ M serum IgG ([IgG]<sub>s</sub>) was included in the system. Serum IgG was assumed to be in excess and could bind FcyRIIs anywhere on the macrophage surface without ligand depletion:

$$IgG_{s} + Fc\gamma RIIx_{free} \xleftarrow{K_{D \times IgG}} Fc\gamma RIIx_{free | bound}$$

 $IgG_{s} + Fc\gamma RIIx_{contact} \leftarrow Fc\gamma RIIx_{contact | bound}$ 

where  $K_{D_x_{lgG}} = K_{D_{lla_{lgG}}}$  or  $K_{D_{llb_{lgG}}}$ .

Since receptor crosslinking leads to cell activation or inhibition, we assumed that dimers represent the minimal signaling unit (and serve as proxies for any higher-order receptor

clusters). Any IgG-bound FcyRIIs were allowed to dimerize with equilibrium dissociation constant  $K_{cross}$ .  $K_{cross}$  was chosen to be 2500 #/ $\mu$ m<sup>2</sup>, which maximizes the difference in the number of crosslinked receptors with and without MDA-MB-453 cells (chosen for this signal optimization since they express fewer Her2 molecules than SKOV-3 cells and therefore have lower signals). All possible combinations of dimers were allowed between the three FcyRII subunits with the same crosslinking constant, whether occupied by serum IgG or Her2-bound IgG. However, geometric constraints limited receptors in the contact area to only crosslink with those in the contact area, while receptors outside of the contact area could only crosslink with those outside:

$$Fc\gamma RIIx_{cell-bound} + Fc\gamma RIIx_{cell-bound} + Fc\gamma RIIx_{cell-bound}$$

 $\mathsf{Fc} \gamma \mathsf{RIIx}_{\mathsf{contact | bound}} + \mathsf{Fc} \gamma \mathsf{RIIx}_{\mathsf{contact | bound}} \xrightarrow{\mathsf{K}_{\mathsf{cross}}} \mathsf{Fc} \gamma \mathsf{RIIx} - \mathsf{Fc} \gamma \mathsf{RIIx}$ 

 $\mathsf{Fc}\gamma\mathsf{RIIx}_{\mathsf{free}\,\mathsf{I}\,\mathsf{bound}}\,+\,\mathsf{Fc}\gamma\mathsf{RIIx}_{\mathsf{free}\,\mathsf{I}\,\mathsf{bound}} \underbrace{\overset{\mathsf{K}_{\mathsf{cross}}}{\longleftarrow}}_{\mathsf{Fc}\gamma\mathsf{RIIx}-\,\mathsf{Fc}\gamma\mathsf{RIIx}$ 

No discrimination was made between crosslinked receptors in and out of the contact area because all of them could lead to an activating or inhibitory signal. However, local concentration effects made the density of dimers (and potentially higher-order clusters) much higher in the contact area.

The diffusion, binding, and crosslinking reactions above yield the following system of equations (x = IIa-H131, IIa-R131, or IIb):

$$\left[Fc\gamma RIIx\right]_{free} = K_{diff} \left[Fc\gamma RIIx\right]_{contact}$$
(E1, E2, E3)

$$\left(\frac{\left[L_{0}\right]_{SK/MD}-\left[Fc\gamma RIIx\right]_{cell-bound}}{\left(N_{AV}\times cell\_gap\right)}\right)\times\left[Fc\gamma RIIx\right]_{contact}=K_{D_{x}}\left[Fc\gamma RIIx\right]_{cell-bound}$$
(E4, E5, E6)

$$\left[IgG\right]_{s}\left[Fc\gamma RIIx\right]_{free} = K_{D_{x}_{l}gG}\left[Fc\gamma RIIx\right]_{free \, l \, bound}$$
(E7, E8, E9)

$$\left[IgG\right]_{s}\left[Fc\gamma RIIx\right]_{contact} = K_{D_{x}_{lgG}}\left[Fc\gamma RIIx\right]_{contact | bound}$$
(E10, E11, E12)

$$\left[Fc\gamma RIIx\right]_{cell-bound} \left[Fc\gamma RIIx\right]_{cell-bound} = K_{cross} \left[Fc\gamma RIIx:Fc\gamma RIIx\right]$$
(E13 to E18)

$$\left[Fc\gamma RIIx\right]_{contact \, I \, bound} \left[Fc\gamma RIIx\right]_{cell-bound} = K_{cross} \left[Fc\gamma RIIx:Fc\gamma RIIx\right]$$
(E19 to E27)

$$\left[Fc\gamma RIIx\right]_{free \mid bound} \left[Fc\gamma RIIx\right]_{free \mid bound} = K_{cross} \left[Fc\gamma RIIx:Fc\gamma RIIx\right]$$
(E28 to E33)

where [FcyRIIx] is in  $\#/\mu m^2$ . Conservation of mass gives:

all[FcyRIIx]in free area×(macrophage total surface area – contact\_area) +all[FcyRIIx]in contact area×contact\_area = Mac\_IIx

#### (E34, E35, E36)

This system of equations was solved in Matlab using *fsolve* to obtain the 36 unknowns. Finally, the resulting distribution of FcyRII dimers was correlated to the experimental output of ADCP. The relative contribution of activating/inhibiting homodimers to this cellular response is not known and, although there is evidence that heterodimers (FcyRIIa crosslinked with FcyRIIb) do form (*15*), it is not known whether they activate or inhibit. Therefore, we did not assign any *a priori* functions or signaling weights to these species, but rather allowed their contributions to be determined by the model by assigning an "intrinsic signaling potency" to each subunit. The signaling potency for FcyRIIb was fixed at -1 (negative for inhibitory) and the signaling potencies for FcyRIIaH131 and FcyRIIa-R131 were allowed to vary freely. We then assumed that the signaling potency of any given dimer was equal to the sum of potency of individual receptors. The overall response was calculated from:

phagocytosis  $\propto \sum (Fc\gamma RIIx : Fc\gamma RIIx \times signaling potency of the respective dimer)$ 

The level of phagocytosis was then compared to experimental data for only two Fc variants to obtain the intrinsic signaling potencies for FcyRIIa-H131 and FcyRIIa-R131. Fitted potency values are presented in Table S5.

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 Table S1.
 Plasmids used in this work.

Plasmids	Relevant characteristics	Reference or Source
pPelBFLAG	Cm <sup>r</sup> , <i>lac</i> promoter, <i>tetA</i> gene, <i>skp</i> gene, C-terminal FL AG tag	(1)
pPelBFLAG-Fc	IgG1-Fc gene in pPelBFLAG	(1)
pTrc99A-DsbA-Fc-FLAG	dsbA fused IgG1-Fc gene, C-terminal FLAG tag in pTrc99A	(1)
pTrc99A-DsbA-Fc2a-FLAG	dsbA fused IgG1-Fc2a gene, C-terminal FLAG tag in pTrc99A	This study
pSTJ4-AglycoT	Trastuzumab IgG1 gene in pMAZ360	(1)
pSTJ4-AglycoT-Fc5	Trastuzumab IgG1-Fc5 gene in pMAZ360	(1)
pSTJ4-AglycoT-Fc2a	Trastuzumab IgG1-Fc2a gene in pMAZ360	This study
pSTJ4-AglycoT-Fc5-2a	Trastuzumab IgG1-Fc5-2a gene in pMAZ360	This study
pPelB-AglycoT(H)-FLAG	Trastuzumab IgG1 heavy chain gene in pPelBFLAG	This study
pPelB-AglycoT(H)-Fc5-FLAG	IgG1-Fc5 heavy chain gene in pPelB-AglycoT(H)-FLAG	This study
pPelB-AglycoT(H)-Fc2a-FLAG	<i>IgG1-Fc2a heavy chain</i> gene in pPelB- AglycoT(H)-FLA G	This study
pPelB-AglycoT(H)-Fc5-2a-FLAG	<i>IgG1-Fc5-2a heavy chain</i> gene in pPelB- AglycoT(H)-FL AG	This study
pPelB-AglycoT(H)-Fc1001-FLAG	<i>IgG1-Fc1001 heavy chain</i> gene in pPelB- AglycoT(H)-FL AG	This study
pPelB-AglycoT(H)-Fc1004-FLAG	<i>IgG1-Fc1004 heavy chain</i> gene in pPelB- AglycoT(H)-FL AG	This study
pPelB-AglycoT(H)-FcG236A-FLA G	<i>IgG1-FcG236A heavy chain</i> gene in pPelB- AglycoT(H)- FLAG	This study
pMoPac1-FLAG-M18	NIpA fused <i>M18 scFv gene,</i> C- terminal FLAG tag in pMoPac1	(2)
pBAD30-KmR	Km <sup>r</sup> , BAD promoter	(2)
pBADNIpAHis-M18	NIpA fused <i>M18 scFv</i> , C- terminal polyhistidine tag in pBAD30	This study
pBADNIpA-VL-Ск-His	NlpA fused <i>trastuzumab VL-Cκ domain</i> , C-terminal polyhistidine tag and c-myc tag in pBAD30-KmR	This study
pBADPelB-VL-Ск-His	PelB fused <i>trastuzumab VL-Cκ domain</i> , C-terminal polyhistidine tag and c-myc tag in pBAD30-KmR	This study
pBAD-AglycoT(L)-His	PelB fused trastuzumab VL-Cκ domain followed by Nlp A fused trastuzumab VL-Cκ-His in pBAD30-KmR for dicistronic expression	This study
pMAZ-IgH-GlycoT	Trastuzumab IgG1 heavy chain gene in pMAZ-IgH-H23	(1)
pMAZ-IgH-GlycoT-Fc1001	IgG1-Fc1001 heavy chain gene in pMAZ-IgH-GlycoT	This study
pMAZ-IgH-GlycoT-Fc1002	IgG1-Fc1002 heavy chain gene in pMAZ-IgH-GlycoT	This study
pMAZ-IgH-GlycoT-Fc1003	IgG1-Fc1003 heavy chain gene in pMAZ-IgH-GlycoT	This study

pMAZ-IgH-GlycoT-Fc1004	IgG1-Fc1004 heavy chain gene in pMAZ-IgH-GlycoT	This study
pMAZ-IgH-GlycoT-FcG236A	lgG1-FcG236A heavy chain gene in pMAZ-IgH-GlycoT	This study
pMAZ-IgH-GlycoT-FcN297D	IgG1-FcN297D heavy chain gene in pMAZ-IgH-GlycoT	This study
pMAZ-IgH-2B6-N297D	2B6-N297D lgG1 heavy chain gene in pMAZ-lgH-GlycoT	This study
pMAZ-IgL-GlycoT	Trastuzumab IgG1 light gene in pMAZ-IgH-H23	(1)
pMAZ-IgL-2B6-N297D	2B6-N297D IgG1 light chain gene in pMAZ-IgH-GlycoT	(1)
pDNR-LIB-FcyRIIa	FcyRIIa <sub>H131</sub> gene in pMAZ-IgH-GlycoT	ATCC
pCMV-SPORT6-FcrRIIIa	<i>FcyRIIIa<sub>v158</sub></i> gene in pMAZ-IgH-GlycoT	ATCC
pMAZ-FcγRI-GST	FcyRI-GST gene in pMAZ-IgH-GlycoT	This study
pMAZ-FcyRIIa <sub>R131</sub> -GST	<i>FcyRIIa<sub>R131</sub>-GST</i> gene in pMAZ-IgH-GlycoT	This study
pcDNA3(oriP)-FcyRl	<i>FcyRI</i> gene with C-Terminal GST fusion for mammalian expression	(6)
рМАZ-FcүRIIa <sub>H131</sub> -GST	<i>FcyRIIa<sub>H131</sub>-GST</i> gene in pMAZ-IgH-GlycoT	This study
pMAZ-FcγRIIIa <sub>v158</sub> -GST	<i>FcyRIIIa<sub>V158</sub>-GST</i> gene in pMAZ-IgH-GlycoT	This study
pMAZ-FcγRIIIa <sub>F158</sub> -GST	<i>FcγRIIIa<sub>F158</sub>-GST</i> gene in pMAZ-IgH-GlycoT	This study

Traine         TIGTGAGCGGATAACAATTTC           STIHAT6         TAATTCGGGCCCCGAGGCCCTTTACCCGGGGACAGGGAGAGGCCTTTCTGCGTG           STIH70         CTAACCTGAACGCTTTTATTACCG           STIH710         CTAACCTGAACGCTTTTATTACCG           STIH720         CGCAAGTAGGCCCCGAGGCCGGCATGGCGGCATGCGGAACAGAGATCT           STIH720         CGCAAGTTGGCCCCCGAGGCCGCCTTGGTAACCAGGTGGAAAAAATG           STIH720         CCAATTTGGCCCCCGAGGCCAGCGGCCTTGG           STIH720         CAATTTGTACGCCGCCGAGACAGGAAG           STIH720         CAATTTGTACGCGCCCGAGGACAGGGCACAGGGAAAGGCAAAACTCACACATGCCCACCGG           STIH290         TTTTAGGGGTCGAAAAGATTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCCACGG           STIH291         GGCCAACCGGATATCTATTATTATTACCCGGGGACAGGGGACAGGGAAAGG           STIH4322         CTAGGGAGCCCGCGGGAGGACCTCCCCCTTGGAGCGCTTGGACGGAC	Primer	Primer nucleotide sequence (5' $\rightarrow$ 3')						
31JH30         THISPACESALASATTCE           31JH40         THISPACESALASATTCE           31JH70         CTACCTGACGCITTTATCCGG           STJH70         CTACCTGACGCITTTATCCGG           STJH71         CGCAACTCGGCCCCCCAGGCCCATGGCGGCATGCGGACTGAAGAACTC           STJH42         GGCAATTCGGCCCCCCCCCCTGGTACCCAGGTGGAAAGAATG           STJH42         GGCAATTCGGCCCCCCCCCCCCTGGTACCCAGGTGGAAAGAATG           STJH420         CAATTTTGCAGCCCCCCGAGCAGAAG           STJH420         CAATTTTGCAGCCCCCCGACGAAGC           STJH420         CGCAATCGGCCCGGACAGCAGAAG           STJH420         CGCAACCGGGTTTAGCCGCGACAGGAAAGTTGGACAAAACTCACACATGCCCCCGG           STJH420         TTTTAGGGTTTAGCACTATTACCACGGGCCAAAGCTGGACAAAACTCACACAGCCCCCGG           STJH431         CTGAGGGAGCCCGGGGCAGGACACTTACACGGCGGCGGTACCGTGTGGTCAGCGTCCTC           STJH432         GGCAATCGGCCGGGCCATGCGCGCATGCGCGGCATGCCGGGTACCAGTGTGGTCAGCGTCCTC           STJH434         CGCAGCGGAGGCCCAGCCCGCCGCCCGACTCCCCCTGTGTGGTCAGCGTCCCCGGTGGTCGTGGTCAGTGGTCGC           STJH435         CGCCACCGCGGGAGGAGGAGCAGTACAACGGGGGAGAGG           STJH434         CTAGGGAAGCCGGGCCCCCGACTCCCCAAGGGGACAGGGAAGG           STJH435         CGCACGCGGGCCCCCCCGACTCCCCAAGGGGGACAGGTGAAGCGTGCGCCCGTGGTGGTCAGTGTGGTCAGTGTGGTCCCCGGGCCCCCCCAAGGGGGAAGGGGAAGGTGGAAGCCCGTGGAGCGGCACGGCGCACTCCCCCCCC	STI#16	TTGTGAGCGGATAACAATTTC						
JIH70       FAIT EGUCECCONSTRUCTOR         STH770       CTACCTGACGCTTITTATCCC         STH770       CGCAGCCCCCAGCCOGCCATGGCGGCATGGCGACTGAAGATCT         STH771       GGCAATTCGGCCCCCGAGCCCCTGGGCACTGGGGAAAGAATG         STH720       CAATTTGTTTATCGGCCCTGAGCAGCAGCAGCCCAAATCTTGTGACAAAACTCACACATGCCC         ACCG       ACCG         STH720       TITTAGGGTTCAACCACGCATTAGCCCCCGGGACAGGACGGGACAGGACAGGAAACTCACACATGCCCACCG         STH720       TITTAGGGTTCAACAACAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCG         STH720       TITTAGGGTCAACAAGAAAGTTGAGCCCCAAATCTTGTGACAAAACTCACACATGCCCACCG         STH720       GGCAATTCTATATATTATTACCCGGGGACAGTGACAATCATTGGACAAAACTCACACATGCCCCCCCGC         STH720       GGCCACGCGGCGGCGGCCCCCCCCCCTTGGAGCGCGACGCCCCCCCC	STI#67							
STI#76 CGCACGGAGGCCCAGGCGGCATGGCGGGCATGCGGAAAGAATC STI#22 CGCAATTC <u>GGCCCCCCGGCGCCTTGGTACCCAGGTGGAAAGAATG</u> STI#22 CGCAATTCTGTTTTATCAGAGCGCTTGG STI#220 CAATTCTGTTTTATCAGAGCGCTTGG STI#220 CAATTCTGTTTTATGGG <u>GGTCGAC</u> AAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCC ACCG STI#291 GGCCACC <u>GGATACTTATTATTTACCCGGGGACAGGGAGGGAGGG</u> STI#291 GGCCACC <u>GGATACTTATTATTTACCCGGGGACAGGGAGAGGG</u> STI#291 GGCCACC <u>GGATACTTATTATTACCCGGGGACAGGGAGAGGGAGGG</u> STI#292 CTA <u>GGGACGCACGGAAAGCTTCATTAGTGACCCACAGCCCCCCCG</u> STI#292 CTA <u>GGGACCCGCGGCGGCGCGCGCCCCCCGTTGAAGCTCTTG</u> STI#422 CTA <u>GGGAGCCCGCGGCGAGGAGCAGTACAACGGCGCGTACCGTGGTCAGCGGCCCCC</u> STI#474 CGCAGCG <u>AGCCCCCGCGCCATGCCGGCATGCCAAGCGCGCGCTCCCG</u> STI#475 CGCACGC <u>AGGCCCCCGGCGCCCGCCCGCCGCCGCCGCCGCGCGCG</u>	STI#70							
STIH20       CGCAATTCGGCCCCGAGGGCCCTTGGTACCCAGGTGGAAAGAATG         STIH21       GCCAATTCTGTTTTAGCAGCGCCTTGG         STIH220       CAATTTTGTCAGCCGCCTGAGCAAGAAG         TTTTAGGGTTTTAGGGGCCCCGAGCAAGAAAGTTGAACCCAAATCTTGTGACAAAACTCACACATGCCC         ACCG         STIH290       TTTTAGGGTCGACAAGAAAGTTGAAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCG         STIH291       GGCCACCGAGAGAAGATTATATTTACCCGGGGACAGGGAGAGGGAGG	STJ#70							
STIH22 GGCAATICGGTTTATCAGACCGCTTCG STIH230 ACCG STIH230 TTTTAGGGTTTAGGGGTCGACAAGAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCC ACCG STIH230 TTTTAGGGGTCGACAGAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCG STIH230 GGCCACCGGATTTACTTATATTACCGGGGACGACAGGAAGAGG STIH231 GGCCACCGGGATGTCTTATATTACCGGGGCACGACAGGAGAGGA STH232 GGCAATTCGAGCTTTGAGCTTTTAGCACTCCCCCTGTTGAAGCCCTTTG STIH330 TTTAAGGGAAGCCTGCGGGCCATGCGGGAGGTTCATTAGTGGAACTG STIH422 CTAGGGAAGCCCGCGGGGCCATGCGGGAGGTTCATTAGTGGAAGCTGTTG STIH422 CTAGGGAGCCCCCCGGGCCATGCGGGGAGGTTCATTAGTGGAAGCTGTG STIH475 CGCAGCGA <u>GGCCCCGCCGGCCATGCGGGAGGTTCATTAGTGGAAGCCGAGGCGCGCGGGGGCCCCCGGGGGGGG</u>	51J#70 CT1#00							
31#147       GUGARATTCINACCAGECCTGACCAAGA         STI#220       TTTTAGGGTTTACGAGECCTGACAAGAAGTTGAGECCAAATCTTGTGACAAAACTCACACATGECCCACCG         ACCG       TTTTAGGGTTTAGEGGTCGACAAGAAAGTTGAGECCAAATCTTGTGACAAAACTCACACATGECCCACCG         STI#291       GGCCACCGGATATCTTATATTATTACCCGGGGACAGGGAGAGG         STI#320       GGGAATTCTAGGACTATTAGCACTCTCCCCCTGTTGAAGCTCTTTG         STI#321       GGCAACCGCCGCGGGAGGAGCAGTACAACGGCGCCGCCTTGGGTCAGCGTCACCGTGTGGTCAGCGTCCCC         STI#322       CTAGGGAGGCCCCGGGGAGGAGCAGTACAACGGCGCGCATGCGCGGATACTACGGCGCACCCGCGCGCG	31J#02 STI#147							
STI#220       CHAITTIGEGETTTAGGEGTEGACAAGAAGATGAGCCCAAATCTTGTGACAAAACTCACACATGCCC         ACCG       ACCG         STI#290       TTTTAGGEGTTGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCG         STI#291       GGCACCCGGATATCTTATTATTATTACCCGGGGACAGGCAGAGCG         STI#322       CGGAGCCCGGCGGAGGACACTCCCCCGTTGAAGCTCTTTG         STI#323       GGGAATTCTAGGACTCTCCCCCGTGTGAAGCTGTGGTCAGCGGTGCAGCGGCCCCCCCC	STJ#147							
STI#290       THTAGGGTTGGACAAGAAAGTTGAGCCCAAATCHGTGACAAAACTCACACATGCCCACCG         STJ#290       TTTAGGGGTCGACAAGAAAAGTTGAGCCCAAATCHGTGACAAAACTCACACATGCCCACCG         STJ#291       GGCCACCGGAATACTTATTATTATTACCCGGGGACAGGGACAGGAAGGG         STJ#321       GGGAATTCGACTTTAGCACTCTCCCCTGTTGAAGCTCTTTG         STJ#340       TTTAAGGGACGCGCGGGGAGGAGCAGTACAACGGCGCGTACCGTGTGGTCAGGGGCTCTC         STJ#422       CTAGGGAGCCCGCGGGCGAGGAGCAGTACAACGGCGCGTATCGACGGGCCCTG         STJ#474       CGCAGCGAGCCGCGGGCCATGGCCGGAGGTTCAATTAGTGGAATCTG         STJ#475       CGCAGCGGCGCGGGGCCCGGCCATGGCGGGATTCAAATGAGTGGTCAGTGTCAGTGTCCTG         STJ#476       CGCAATTCGGCCCCGGGAGGAGCAGTACAACGGCGGCTACCGTGTGGTCAGTGTCACTGTGTCAGTGTCAGTGTCAGGGCGCCCCGGAGGAGGAGCAGTACAACGGCGGCACCACAAAAGGCAGTGCAGTGGCGGCACTCCGAAGTGGGACCAGCAGCGGGAGGAGGAGGG         WK#56       TCCACAGGGCGCGCACTCCCGAAGTGGGACCACACAAAGGCAGTG         WK#57       GGCTGATCACGCAGGCTCGAGCTGGGACTGAAGAATCCC         WK#91       CTCCACAGGCGGCACTCCGAAGTGGTGACCCAGCAAAGGCCCC         WK#93       GTTCACAGGGCGCACTCCGAAATTGTGTGGAACAAGTTCCC         WK#100       CTCTCCACAGGCGGCACTCCCAAAGTGGGATCCAACCGGGAAAAAGCTGCCC         WK#116       GCTTGTGGGATGAAGAGGAGGGGGGACCCATCGCAAGGCGGAAGCAGGCCC         WK#116       GCTTGTGGGATGAAGGTGGGATCCAACCGGGAAAGTCAGGCCCC         WK#116       GCTGGTGACACAAAAGTACTGGGGACTGAAAGCCGGGAACGTGGAGACG         WK#116       GCTGTGGGACAAGCTCGGAGAAGGAGGAGCTTGGAAGGCTCCAAGGGGAAGG	313#220							
TITTAGGG <u>GTCGAC</u> AAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCG         STJ#291       GGCCACCG <u>GATATC</u> TTATTATTATTACCCGGGGACAGGGAGAGG         STJ#322       GGGAAT <u>TCTTAGCACTCTCCCCTGTTGAAGCTCTTTG</u> STJ#324       TTTAAGGA <u>AGCTTTAGCACTCTCCCCCTGTTGAAGCTCTTTG</u> STJ#325       GGGAAGCCGCGGGGAGGACCACTCCCCCCTGTGAGCGTCAGCGTCTTG         STJ#440       TTTAAGG <u>AGCCCCGCGGCCATGGCGCATGCCGGCGATACTCAACCGGCGCTCACCGTGTGGTCAGCGCTCTC</u> STJ#474       CGCAGCGA <u>GGCCCCCGCCCCCCCCCCCCCCCCCCCCCC</u>	STJ#290	Arrg						
JI#230       TTTIAGGG_DISCARGAMACTICATCCCGGGAACGGGAGAGG         STI#231       GGCAACCCGGATATCTTATTATTTATCCCCGGGGACAGGGAGAGG         STI#332       GGGAATCTTATTATTATTTACCCGGGGACAGGGAGAGG         STI#340       TTTAAAGGAAGCTTATTAGCACTCTCCCCTGTTGAAGCTCTTTG         STI#341       TTTAAAGGAAGCTTCATTAGCACTCTCCCCTGTTGAAGCTCTTTG         STI#442       CGCAGCGAGGCCCCGGCCATGGCGGAGGTCAATTAGTGGAATCTG         STI#474       CGCAGCGAGGCCCCCGCAGCGGCCATGGCGGAGAGTCAATTAGTGGAATCTG         STI#475       CGCAGCGAGGCCCCCCGCACTCCCCCGGAGGAGAGGG         STI#476       CGCAATTCGGGCCCCCGGAGGAGAGCAGTCCAACGGGCGCGTACCGTGTGGTCAGTGTCCTC         STI#498       TTTTAGGGTCTAGATCAGGGAGAGCAGTACAACGGCGCGCGTACCGTGTGGTCAGTGTCCTC         STI#498       TTTTAGGGCTCTAGATCAGGGAGACAGCAGGAAGAGGGAGAGG         WK#56       TCCACAGGCGGCGCACTCCCAAGGCTGGCGCCCCCCCAAGGCGGGACGAAGAGTGGCC         WK#91       CTCCACAGGCGGCGCACTCCCAAGGCTGGCTCCCCCCAAGGCTGGT         WK#92       CCACGCGGAACCAGCTCGAAGCTGGGATCCAACGGGAGAAGATGATG         WK#110       CCACGCGGAACCAGCTCGAAGCTGGGATCCAACGGGAGACAGTTCCAGGGCCC         WK#111       CCACGCGGCACCCCAAGGCGCGCCCATTGGCAGAAGCTGGAGACCAGTCCAGGACCAGTCAGAGC         WK#116       CTTGTGGCAACCAAAGATACTTGGCGAGAAGCTCGAGAGCCCCCCAAGGCCCCCCCAAGGCGGAGCCCCCAAGGCGGAGACCTGCAAGAGCCCCAGGCAGCCCCAAGGCGCCCCCAAGGCAGGACCTTGCAAGGACCTGGAAGCTGGAAACCAAGATTCTGGCAGAAACCAAGCTTGCAAGGAACCAGGTCCAAGGACCAGGTCAGGGG         WK#113       CTCCACAGGCGGCACTCCGAGAGA	ST1#200							
STI#321       GGCACCGQMINIC ITATTAGCACTCTCCCCTGTTGAAGCTCTTTG         STI#320       TTTAAGGGAAGCTTATTAGCACTCTCCCCTGTTGAAGCTCTTTG         STI#340       TTTAAGGGAAGCCGCGGGAGGAGCAGCATCCCCCTGTTGAAGCTCTTTG         STI#422       CTAAGGAAGCCGCGGGCAGGAGCAGCATGCCCGGAGGTTCCATTAGTGGAATCTG         STI#474       CGCAGCGAGGCCCAGCCGGCCATGGCGGAGGATCAATGAACGGCACAAAGCCCG         STI#476       CGCAAGCGCGCGCAGCCGGGCCATGGCGGAGAACAACGGCGGTACCGTGTGGACAGTGTCCTC         STI#476       CGCAATTCGGCCCCGAGGCGCCCGGCACTCCCCCTGCTGAAGAGCTGTGGCAGGAGGAGG         WK#57       GGCTGATCAGCGGAGCACTCCCAAGTGGGACAACGACGAGGAGAGG         WK#91       CTCCACAGGCGCGCACTCCCGAGTGGGACAACGACGAAGAAGAATGATG         WK#92       CCACGCGGAACCAGCTGGAGTGGCACCACGAGGGAAGAAGAATGATG         WK#910       CTCTCCACAGGCGCGCACTCCCGAAGTGGTACCGAGGAGAGATTTCTGGG         WK#100       CTCTCCACAGGCGCGCACTCCCGAAGTGGTCCCCCAAAGGCTGCC         WK#116       GCTTGTGGGAACCAAGCTGGAGGGGATCCAACCGGCAGAGATTTCTGGG         WK#116       GCTGGTACAACCAAGCAGGGGAGACCAATCGAGCAGAGACTGAGTCAGAGCC         WK#116       GCTGGTACAACCAAACAAACATACTTGGTGAAACCAGCTGCAGAGCACAGTTCC         WK#117       GGGGAGTAAAACTAACTTGGTACCACGCAGGAGAAGCAGAGTGCAGAGCTGAGGACAGATTCC         WK#118       GAGCGTGAAAACAACAACATACTTGGTACCACGAGAAACCAGACTGCAGAGCACAAGTTC         WK#119       GGTGACTTTCTCCTTGGTGTCACCACGCCACGCAGGAGAACCAGACTTCAGAGGCACGAGTGG         WK#113       GACTCCAAGGGGGA	STJ#290							
STI#332       GOGAATI <u>CLIADUCATI</u> TAGCACCTCTCCCCTGTTGAAGCTCTTTG         STI#342       CTAGGGAAG <u>CTTCTATTAGCACCTCTCCCCTGTTGAAGCTCTTTG</u> STI#422       CTAGGGAAG <u>CTTCTATTAGCACCTCTCCCCTGTTGAAGCTCTTTG</u> STI#442       CGCAGCGA <u>GGCCCAGCCGGCCATGCGCGAGGTTCAATTAGTGGAATCTG</u> STI#475       CGCAGCGA <u>GGCCCCGGAGGCCCGCCGCACTCTCCCCTGTTGAAGCTCTTTG</u> STI#476       CGCAGTT <u>GGCCCCCGAGGGCCCGCGCACTCCCCCGTGTGAAGCGTCTTTG</u> STI#479       CTAGGGA <u>GCCCCGG</u> AGGAGCAGTACAACGGCGGTGGTGGTCAGTGTCCTC         STI#498       TTTTAGGGC <u>CCCGG</u> AGCATCCCAAGTGGACACCACAAAGGGCAGTG         WK#56       TCCCACAGGCGCCACTCCCGAGTGGGACACCCAAAGGGCAGTG         WK#51       CGCCGGCAACCCGGCACTCCCGAGTTGGTAACCAAGGGCGGCG         WK#92       CCACGCGGAACCAGCTCGAGTTGGTACCCAGGTGGAAAGAATGATG         WK#91       CTTCCACAGGCGCGCACTCCCGACTGCCGAAAGGAGGCCCC         WK#100       CTCTCCACAGGCGCGCACTCCCAAGCTGGTGAACAGCTCTCCCCAAGGCGCCC         WK#116       GCTTGTGGGATGAGAAGAGGGGGATCCAACCGGGAGAATTTCTGGG         WK#116       GCTTGTGGGATGGAGAAGGTGGGATCCAACCGGGAGAATTTCTGGG         WK#118       CTCCCACAGGCGCCCCCCCAAGGCGCACTCCCCAAAGGCGCCCGGAGCCCCGGAGCCCCCCCC	STI#231							
S1#340       THAAGGGAGGCCGGGAGAGGAGACAGTACAACGGGGGGGGGG	STI#270							
31J#472       CGCAGCGAGCCCAGCCGACCATGCCGAGGTTCATTGTGGCAAGCTCTC         STJ#474       CGCAGCGAGGCCCAGCCGGCCATGCGCGAGGTTCAATTGTGGAAATCTG         STJ#475       CGCAGCGAGGCCCAGCCGGCCATGCGCGAGGTTCAATTGAAGCTCTTG         STJ#476       CGCAATTC <u>GGCCCCCGAGCCCGCGCCCGCACTCCCCCCGTTGAAAGCTCTTTG</u> STJ#490       CTAGGGAG <u>CCCGGCGGGGCCGGGGCCGCGCGCGCGCGCGCG</u>	STJ#340 STI#422							
31J#741       CGCAGCGA <u>GGCCCAGCCGGCCA</u> TGGCGGATATTCAATGACCCAAAGCCCG         STJ#475       CGCAGCGA <u>GGCCCCGGCCGCGCCCGCCCCCCCCTGTGAAGCCCCAAAGCCCGG</u> STJ#490       CTAGGGA <u>GCCCCGGGG</u> GAGGAGCAGTACAACGGCGGTACTCGTGGTCAGTGTCCTC         STJ#490       CTAGGGA <u>GCCCCCCCAAGTGG</u> GACAAGGACAAGGGAGAGG         WK#56       TCCACAGGCGCCCCCCAAGTGGACACCACAAAGGCAGTG         WK#57       GGCTGATCAGCGAGCTTCTAGATCAGGACACCACAAAGGCAGTG         WK#94       CTCCACAGGCGCCCCCCCAAGTGGACACCACAAAGCCCCCC         WK#92       CCACGCGGAACCAGCTCGAGCTGGTACCCAAGGTGGAAAGAATGATG         WK#94       GTTCACACGCGGCACCTCCGAAGCTGCTCCCCCAAAGGCTGT         WK#910       CCTCCCACAGGCGCGCACTCCCAAGCTGGTCCCCCAAAGGCTGT         WK#100       CCCACGGGAACCAGCTCCGAAGCTGGTGCCCCCAAAGGCTGG         WK#116       GCTGGACCAAGCTCGAAGCTCCAACCGGGAGACATTTCGGG         WK#158       CTCCACAGGCGCGCACTCCGAAAGTGGGATCCAACCGGGAAGCTGAGACCAGACTAGCACAATTCC         WK#159       GGTGACCTTTGCTTTGGTGCACGCTCGAAAGCTCGAGCCCACTTCC         WK#151       GCTGGTACCAATGATGTTTGTGCCCAATGCTTGGAGAACTGAGCTCAGAGCACAACATTC         WK#151       GCTGGTGACCAATACATTGTTGTGCCAAGCCCACAGGCCCACTCCGGAAGCCTGCAGGCGCACTCC         WK#161       AGCTGGTGACACCAAAGAATACATTGTCTCGAGAACCAGGATCAGCTCCAGGGAACCTGAGGTGCAGTGTGG         WK#163       GACTCCAAGAGATCAACATTGTGTGCCCCCCCCCGCAGGGAGCTTGGAGACTGAGGTGCAGTGGGAGCTTCCCAAGGGTGAAAACTCTGTCCCCACACAGCAGGCCCCATGGGAGGGCCCCAGGGCCCCCCCC	51J#422 STI#474							
STIH475       CGCAATTCGGCCCCGGAGGCCCGGCACTCTCCCCTGTTGAAGCCTCG         STIH476       CGCAATTCGGCCCCGGAGGCACGCACCTCTCCCCTGTTGAAGCTCATTG         STIH476       CGCAATTCGGCCCCCGAGGCCCGCACTCTCCCCTGTTGAAGCTCAGTGTCCTC         STIH479       TTTTAGGGTCTAGATCATTTACCCGGGACCACGCAGGGGAGAGG         WK#56       TCCCACAGGCGCGCACTCCCAAGTGGACACCACAAAGGCAGTG         WK#57       GGCTGATCAGCGGCACTCCGGCATGCGGACTGAAAGCCCC         WK#92       CCACGCGGAACCAGCTCGAGTTGGTACCCAGGTGAAAGAATGATG         WK#94       GTTCACAGGCGCGCACTCCCAAGCTGGTCCCCCAAAAGCCCCCTGCAGAAG         WK#910       CTCCCACAGGCGCGCACTCCCAAGCTGGTGAAGAGCTGCCC         WK#101       CCACGCGGAACCAGCTCGAGGCCCCCATTGGTGAAAGACCCGGGAGAATTTCTGGG         WK#110       CCACGCGGAACCAGCTGGAGCCCCCATTGGTGAAAGCCCGGAGACTGAGTCAGCACAATTTC         WK#158       CTCCACAGGCGCGCACTCCCAAATTGTGTGACACCAGGAGAGACTGAGTCAGGCACGACAAATTC         WK#159       GGTGACTTTCTCCTTTGGTGTCACGCTCTGAAAGCTGGAGCCCAGTCAGAGC         WK#151       GCTGGTACCAATGATGTTTTGGTCCCAATGCTCTGACAGCAGCAGTCAGGCAGTCAGAGC         WK#161       GCTGGTACACAAAACATACTAGTTCTGGAGACCTGAGAGCTGCAGGGGAGCTTTCC         WK#161       GCTGGTGACACCAAAGACTCAGTAGCCTGGAAGCTGGAGGGGGGCCCCCACGGGAGGG         WK#163       GACTCCAAGGAGTGAACCCAGAAAGACAGACTGGGAGCTTGCAGGGGGAGGGGGGGG	STJ#474							
STIR490 CTAGGAATCGUCCCCGGAGGAGCAGTACAACGGCGGTACGGTGTGGTCAGTGTCCTC STIR490 TTTTAGGGTCTAGATCATTTACCCGGGGACAGGGCAGGG	51J#475 STI#476							
STJ#490       CTAGGGGCCCCGGCACTGCCGAGGACAGGGGGACAGGGGGACAGGGG         WK#56       TCCACAGGCGCGCACTCCCAAGTGGACAGGACAGGAGG         WK#57       GGCTGATCAGCGAGCTTCTAGATCAGGACAGGAAGGAGG         WK#91       CTCCACAGGCGCGCACTCCCGACTGCGGACTGAAGATCTCCC         WK#92       CCACGCGGAACCAGCTCCGAGACTGAGGACAGGAAGAAGATGATG         WK#94       GTTCACAAGTCCTGAAGACACATTTTTACTCCCCAAAGGCTGCAGAGAG         WK#100       CTCTCCACAGGCGCGCACTCCCAAGCTGGTGCAAAGAAGTCTCCC         WK#101       CCACGCGGAACCAGCTCGAAGCTGGTGCTCCCCAAAGGCTGCT         WK#116       GCTTGTGGGATGGAGAAGGTGGGATCCAACCGGGAGAATTTCTGGG         WK#116       GCTTGTGGGATGGAGAAGGTGGGATCCAACCGGGAGACTGAGCCAGACTTCC         WK#118       CTCCACAGGCGCGCACTCCGAAATTGTGCTGACACTCGGCAGCAGGCAG	STJ#470							
S1J#456       TTTAGGG_LIAQATCATTACCCGGGGACACCACAAAGGCAGTG         WK#56       TCCACAGGCGCGCACTCCCACTAGGTGGACACCACAAAGGCAGTG         WK#57       GGCTGATCAGCGGGCACTCCGGCATGCGGACAGAAGGATGGTCGC         WK#91       CTCCACAGGCGGCACTCCGGGATGGGGACAGAAGAATGATG         WK#92       CCACGCGGAACCAGCTCGAGGTGGTACCCAGGTGGAAAGAATGATG         WK#00       CTCTCCACAGGCGCGCACTCCCAAGCTGCTCCCCCAAAGGCCCGTGT         WK#101       CCACCGGGAACCAGCTCGAGCCCAATGGTGAAAAGCCCCCGCGCACCCC         WK#110       CCACGCGGAACCAGCTGGAGGGGATCCAACCGGGAGAATTTTCGGG         WK#1116       GCTTGTGGGATGGAGAAGGTGGGATCCAACCGGGAGAATTTCTGGG         WK#1158       CTCCCACAGGCGCCACTCCCGAAATTGGTGACTCAGCTGAGACCAGTCAGGCACAATTTC         WK#150       AGAGCGTGACACCAAAGGAGGAAAGTCACCATCACCTGCAGGACCAGTCAGGACCAATTTC         WK#161       GCTGGTACCAATGATTGTTGTGCCAATGCTGAACACTGGACCAGTCAGGCACAATTTC         WK#162       ATTGGCACAAACATACATTGGTACCAGCAGAAACATCATGGTCTGACTGGCAGTGA         WK#163       GACTCCAGAGATAGACTACATTGATGAGAGAGCTTTGAAGGAGCTTCCAAGGCTCC         WK#164       TCATCAAGGAGAAGATCTGTCCCGAACACAAACATACTTGATGAGAGAGCTTCAGGGGGGGG	STJ#490							
WK#30TCCACAGGCGCGCACTCCCCAAGTGGACACCACACAGAGGCGGCGCWK#57GGCTGATCAGCGAGGCTTCTGAGATCAGGATCTTTTTGGAGGATGGCCCWK#92CCACGCGGAACCAGCTCCGAGTTGGTACCCAGGTGGAAAGAATGATGWK#94GTTCACAGTCTCTGAAGACACATTTTTACTCCCGAAAAGCCCCCTGCAGAAGWK#100CTCTCCACAGGCGCGCACTCCCAAGCTGCTCCCCCAAAGGCTGCCWK#110CCACGCGGAACCAGCTCGAAGCTGCCCCCATGGTGAAGAAGCTGCCCWK#111CCACGCGGAACCAGCTCGAAGCTGCCCACCCGGAGAATTTCTGGGWK#112CCACGGGACCAGGCGCACTCCGAAATGTGCTGACACAGTCCAGCCCGAGACTGAGTCAGGCWK#115GCTCACAGGCGCGCACTCCGAAATTGTGCTGACACTCGGAGACTGAGTCAGCACAATTTCWK#158CTCCACAGGCGCGCACTCCGAAATGTGCTGACACCAGCGAGGAGCAGTGAGTCAGCACAATTTCWK#159GGTGACTTTCTCTTTGGTGTCACGCTCTGAAAGTCAGGACCAGTCAGGACCAGAGCWK#159GGTGACTTTCTCCTTTGGTGTCACGCTCTGAAAGTCCTGCAGGACCAGTCAGAGCWK#161GCTGGTACCAATGTATTTTGTGCCAATGCTCGACTGGCCCTGCAAGGCGCWK#162ATTGGCACAAAACATACATTGGTACCAGCAGAAACCAGATCAGCTCCAAAGCTCCWK#163GACTCCAGAGATAGACTCAGAACATACTTGATGAGGAGCTTGGAGACTGATCTGGTTTCTWK#164TCATCAAGGAGTGAAATCTGTCCCTGCACTGCACCATGGAGGCGGCAGTGGWK#165GGTGAGGGTGAAATCTGTCCCTGACCATGCACCTGCAAGCTGAGGGGGAGAGGWK#167ATCAGGGACAGATTTCACCTCACCTTGGACCCCTGCAAGCTGAGGGAGG	31J#490							
WK#37GGGCGATCAGGGAGGCACTCCGGCATGGAAGATCTCCCWK#91CTCCACAGGCGCGCACTCCGAGTTGGTACCCAGGTGGAAAGACTGATGWK#92CCACGCGGAACCAGCTCGAAGATGGTACCCAGGTGGAAAAGAATGATGWK#94GTTCACAGTCTCTGAAGACCACATTTTACTCCCGAAAAGCCCCCTGCAGAAGWK#100CTCTCCACAGGCGCGCACTCCCAAGCTGCTCCCCCAAAGGCTGCCCWK#101CCACGCGGAACCAGCTCGAAGCTGGGATCCAACCGGGAGAATTCTGGGWK#116GCTTGTGGGATGGAGAAGGTGGGATCCAACCGGGAGAATTCTGGGWK#116GCTCACAGGCGCGCACTCCGAAATTGTGCTGACTCAGTCCCAGACTGAGTCAGCACAATTTCWK#158CTCCACAGGCGCGCACTCCGAAATGTGCTGACAAGCTGGAGACTGAGTCAGCACAAATTCWK#159GGTGACTTTCTCCTTTGGTGTCACGCTCTGAAAGTCTGCGAGGACCAGTCAGCACAATTTCWK#160AGAGCGTGACCAAAGAAGATAGCCCCATGACCCAGCAGAGCCAGGCWK#161GCTGGTACCAATGATGTTGTGCCAATGCTCTGACTGACTG								
WK#91CICCACAGGCGCGGACCCGGGTTGGCACCGGGGAACGAAGAAGAATGATGWK#92CCACGCGGAACCAGCTCGAGCTCGGGTGCACCCCGGGGAAAGGAATGATGWK#94GTTCACAGTCTCTGAAGACACATTTTTACTCCCGAAAAGCCCCCGGAGAGGWK#100CTCTCCACAGGCGCGCACTCCCAAGCTGGCCCCAAAGCGCGCCWK#111CCACGCGGAACCAGCTCGAGCCCCATTGGTGAAGAGCTGCCCWK#116GCTTGTGGGATGGAGAAGGTGGGATCCAACCGGGAGAATTTCTGGGWK#158CTCCACAGGCGCGCACTCCGAAATTGTGCTGACTCAGTCTCCAGACTCAGCACAATTTCWK#159GGTGACTTTCTCCTTTGGTGTCACGCTCTGAAAGTCTGGAGACCAGGCAGG	WK#37							
WK#92CCCACGCGGACCCGAG TIGGTACCCACGTGACAGAGAGAGAGAGAGAGGWK#94GTTCACAGTCTTGAAGACCTCGAGACACATTTTTACTCCCCGAAAAGACGCCCCTGCAGAAGWK#100CTCTCCACAGGCGCGCACTCCCAAGCTGCTCCCCAAAGGGCTGCWK#101CCACGCGGAACCAGCTCGAAGCCCCCATTGGTGAAGAGCCGCCCWK#116GCTTGTGGGATGGAGAAGGTGGGATCCAACCGGGAGACATTTCGGGWK#158CTCCACAGGCGCGCACTCCGAAATTGTGCTGACTCAGTCTCCAGACTCAGCACAATTTCWK#159GGTGACTTTCTCCTTTGGTGTCACGCTCTGAAAGTCTGGAGACCAGACCAGACACAATTTCWK#160AGAGCGTGACACCAAAGGAGAAAGTCACCATCACCTGCAGGACCAGTCAGAGCWK#161GCTGGTACCAATGTATGTTTGTGCCAATGCTCTGACTGGTCCTGCAGGGAGTWK#162ATTGGCACAAACATACATTGGTACCAGCAGAAACACAGATCAGTCTCCAAAGCTCCWK#163GACTCCCAGAGATAGACTCAGCAGCAGAAACATACTTGATGAGGAGACTTGATCTGGTTTCTWK#164TCATCAAGTATGTTTCTGAGTCTATCTCTGGAGTCCACTGCAACGAGAGCTGATCTGGTTTCTWK#165GGTGAGGGTGAAATCTGTCCCTGATCCACTGCACTGCAACGAGGAGCTGAGGGWK#167ATCAGGGACAGATTTCACCCTCACCATCAATAGCCTGGAAGCTGAGGAGGGGGGAGAGATTACTTTGTGAACAAAGTAATACCTGGCCGTTCAGGTTCAGGTGCAACTGGTWK#168GCCAGGTATTACTTTGTGAACAAAGTAATACCTGGCCGTTCACGTTCGAGCGAG	WK#91							
WK#100CTCACAGGCGCGCACTCCCAAGCTGCAAGGCTGTWK#101CCACGCGGAACCAGGCTCGAGCCCCATGGTGAAGAGGCTGCCWK#101CCACGCGGAACCAGCTCCGAAGCTCCCAAGCGGGAAGAAGGCGCCWK#116GCTTGTGGGATGAAGAGGGGGATCCAACCGGGAACACGGGAGACTTTCWK#158CTCCACAGGCGCGCACTCCGAAATTGTGCTGACTCAGTCTCCAGACTTTCWK#159GGTGACTTTCTCCTTTGGTGTCACGCTCTGAAAGTCTGGAGACTGAGCACAATTTCWK#160AGAGCGTGACACCAAAGGAGAAAGTCACCATCACCTGCAGGGACCAGTCAGAGCWK#161GCTGGTACCAATGTTTGTGCCAATGCTCTGAACGTCCTGCAGGGCAGTWK#162ATTGGCACAACATACATTGGTACCAGCAGCAGAAACCAGATCAGTCTCCAAAGCTCCWK#163GACTCCCAGAGATAGACTCAGAAACATACTTGATGAGAGAGCTTTGGAGACTGATCTGGTTTCTWK#164TCATCAAGTATGTTTCTGAGTCATCTCTGGAGTCCACTGCAGGGCAGTGGWK#165GGTGAGGGTGAAATCTGTCCCTGATCCACTGCCACTGAAGCTGAAGATGCTGWK#166ATCAGGGACAGATTTCACCCTCACCATCACTAGCCTGGAAGCTGAAGATGCTGWK#167ATCAGGGACAGATTTCACCCTCACCATCACATAGCCTGGAAGCTGAAGATGCTGWK#168GCCAGGTATTACTTTGTGAACAAAGTAATACCTGGCCGTTCACGTTCGGCGAAGGWK#170CGATGGGCCCTTGGTGCTAGCTTTGATCTCCACCTTGGTCWK#171GCGGCCGCGGCGCACTCCWK#172CTCCACAGGGCGCGCACTCCWK#173CTCCACAGGCGCGCACTCCWK#174CCCCAGGCTGCACTCCAGGTTCAGGTGCAGCCTGGAACCTGWK#175AGCTGAGGTGAAAGCCTGCAGCCTCAGGACCTTGCAGGAGACCTTCACGTGAAGGWK#175GACTCAGTAGTAGTAGTAGTAGCAGCACCAGCCAGCCTGCAAAGGWK#175GTATCCAGTAGTAGTAGTAGGTGAAACCAGACCTGCAAGACCTTCACTGAAGGWK#175GTATCCAGTAGTAGTAGTAGTACCAGAAGCCTTGCAGAAGCCTTCCATGAAGGWK#176GTATCCAGTAGTAGTAGTAGTACCAGAAGCCTTGCAGAAGCCTTCACTGAAGGWK#175GTCCAGTAGTAGTAGTAGTACCAGAACCCTG	WK#92							
WK#100CTCTCCACAGGCGCGCACTCCCAAGCTGCTCCCCAAGGCTCCCCCAAGGCGCCCWK#101CCACGCGGAACCAGCCGCGCACTCCGAGCCCCATTGGTGAAGAGCTGCCCWK#116GCTTGTGGGATGGAGAAGGTGGGATCCAACCGGGAGAATTTCTGGGWK#158CTCCACAGGCGCGCACTCCGAAATTGTGCTGACCAGCAGACTGAGTCAGCACAATTTCWK#159GGTGACTTTCTCCTTTGGTGTCACGCTCTGAAAGTCTGGAGACTGAGTCAGCACAATTTCWK#160AGAGCGTGACACCAAAGGAGAAAGTCACCATCACCTGCAGGAACTGAGTCAGCAGCAWK#161GCTGGTACCAATGTATGTTTGTGCCAATGCTCTGAACGTCTGCAGGACCAGTCAGAGCWK#162ATTGGCACAAACATACATTGGTACCAGCAGAAACCAGATCAGTCTCCAAAGCTCCWK#163GACTCCAGAGATAGACTCAGAAACATACTTGATGAGGAGACCTTGGAGACTGATCTGGTTTCTWK#164TCATCAAGTATGTTTCTGAGTCTATCTCTGGAGGCCCACTGAAGCTGAGGGGGGAGACTTCAGGTGAGGGGGAAATCTGTCCCTCACCATCAATAGCCTGGAAGATGGCTGWK#165GGTGAGGGTGAAATCTGTCCCTCACCATCAATAGCCTGGAAGCTGAAGATGCTGWK#167ATCAGGGACAGATTTCACCCTCACCATCAATAGCCTGGAAGCTGAAGATGCTGWK#168GCCAGGTATTACTTTGTTGACAGTAATACCTGGCCGTTCACGTTCGGCGGAGGWK#170CGATGGGCCCTTGGTGCTAGCTTTGATCTCCACCTTGGTCCCTCCGCCGAACGTGAACGWK#171GCGGCCGCGGCGCACTCCWK#173CTCCACAGGCGCGCACTCCWK#174CCCCAGGCGCGCACTCCWK#175AGCTGAGGTGAAAGCCTGGGGCCTCAGTGAAAGGTCTACGGTGCAGCCTGCAAGGWK#174CCCCAGGCGCACTCCWK#175AGCTGAGGTAAAGCCTGGGGCCTCAGTGAAAGCCTGGAGACCTTGCAGGAGGCCTCCAGGAGGCCTCCGAAGGGGGACCTTCACCAGGAGGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCCCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCTGCAGGAGGCCTCCCCCAGGAGGGCCCTCAGTGAAGGCCTGCAAGGGGCGCCCTCAGTGAAGGCCTTGCAGCAGGGGCCC	WK#94							
WK#101CCACGGGGAACCAGCTCCAAGCCCCCATTGGTGAAGCTGCCCCWK#116GCTTGTGGGATGGAGAAGGTGGGATCCAACCGGGAGCACTTCTGGGWK#158CTCCACAGGCGCGCACTCCGAAATTGTGCTGACTCAGTCTCCAGACTTTCWK#159GGTGACTTTCTCCTTTGGTGTCACGCTCTGAAAGTCTGGAGACTGAGTCAGCACAATTTCWK#160AGAGCGTGACACCAAAGGAGAAAGTCACCATCACCTGGAGGACCAGTCAGAGCWK#161GCTGGTACCAATGTATGTTTGTGCCAATGCTCTGAACTGGTCCTGCAGGAGCAGTCWK#162ATTGGCACAAACATACATTGGTACCAGCAGAAACCAGATCAGTCCGCAGGGGATWK#163GACTCCAGAGATAGACTCAGAAACATACTTGGAGAGCCTGGAGAGCTGACTGGGTCTCWK#164TCATCAAGTATGTTTCTGAGTCTACTCTGGAGTCCCATCGAGGGTCAGTGGCAGTGGWK#165GGTGAGGGTGAAATCTGTCCCTGATCCACTGCCACTGGAAGCTGAAGGTGGGWK#167ATCAGGGACAGATTTCACCCTCACACTGCACTGCACCTGGAAGCTGAAGAGTGCTGWK#168GCCAGGTATTACTTTGTTGACGTAATACCTGGCCGTTCAGGTGGGAGGCGAGGGWK#170CGATGGGCCCTTGGTGCTAGCTTGATCTCCACCTTGGTCCCTCCGCGAAGGGGAGGWK#171GCGGCCGCGGTGCGACTCCWK#172CTCCACAGGCGCGCACTCCWK#173CTCCACAGGCGCGCACTCCCAGGTTCAGCTGGTGCAGCTGGGWK#174CCCCAGGCTTCTTTGATCCCAGGTTCAGCTGGAAGCTGGAACCTGWK#175AGCTGAGGTGAAAGCCTGGGGCCTCAGGTGGAACCTGGAACCTGWK#176GTATCCAGTGAGAAGACCTGGGGCCTCAGGTGAAGCCTTGCAGGAGACCTTGCAAGGGGWK#174CCCCAGGCTGAAGAAGCCTGGGGGCCTCAGTGAAAGGTTGCAGCAGGAGACCTGCAACGGGGWK#175AGCTGAGGTGAAAGGCTGGGGGCCTCAGTGAAAGGCTTGCAGGAGACCTGCACCGGGGWK#176GTATCCAGTAGTGAAAGGTGTAACCAGAAGCCTTGCAAGGAGACCTGCACCGGGGWK#177CTCCACAGGGTGAAAGGCTGGGGGCCTCAGTGAAAGGCTTGCAGGAGACCTGCACCGGGGWK#175AGCTGAGGTGAAAGGCTGGGGGCCTCAGTGAAGGCCTGCAGGAGACCTGCACGGGGWK#174CCCCAGGCT	WK#100							
WK#110GetTististicationadataset is district accord accord action in the formWK#158CTCCACAGGCGCGCACTCCGAAATTGTGCTGAACTCAGTCTCCAGACTTTCWK#159GGTGACTTTCTCCTTTGGTGTCACGCTCTGAAAGTCTGGAGACTGAGCACAATTTCWK#160AGAGCGTGACACCAAAGGAGAAAGTCACCATCACCATCACCTGCAGGACCAGATCAGAGCWK#161GCTGGTACCAATGTATGTTTGTGCCAATGCTCTGACAGGTCCGGAGGCAGAGACWK#162ATTGGCACAAACATACATTGGTACCAGCAGAAACCAGATCAGGTCCCAAAGCTCCWK#163GACTCCAGAGATAGACTCAGAAACATACTTGATGAGGAGACTGATCTGGAGACTGATCTGGTTTCTWK#164TCATCAAGTATGTTTCTGAGTCTATCTCTGGAGTCCACTGCAGGGGCAGTGGWK#165GGTGAGGGTGAAATCTGTCCCTGATCCACTGCCACTGAAGCTCAGTGGCAGTGGWK#167ATCAGGGACAGATTTCACCCTCACCATCAATAGCCTGGAAGCTGAAGATGCTGWK#168GCCAGGTATTACTTTGTTGACAGTAATACGTTGCAGCATCTTCAGCTTCCAGGCTATTGATWK#169CAACGTATTACTGTCAACAAAGTAATACCTGGCCGTTCAGCTTCGGCGGAGGWK#170CGATGGGCCCTTGGTGCTAGCTTTGATCTCCACCTTGGTCWK#172CTCCACAGGCGCGCACTCCWK#173CTCCACAGGCGCGCACTCCWK#174CCCCAGGCTTCTCCACGTGGAGCAGCTGGAACCTGWK#175AGCTGAGGTGAAAGACTGGAGCCCTAGGGAAGCCTGCAGGGAGAACCTGGAAGCTGAACCTGWK#176GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGAGCCTTGCAGGAGACCTTCACTGAGGGWK#1776GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGCAGACCTGCAAGCGWK#174CCCCAGGCTTCTTCACCTCAGCTGAGACCTGGAACCTGWK#175AGCTGAGGTGAAAGAAGCCTGGAACCTGGAAGCCTTGCAGGAGACCTTCACTGAGGGWK#176GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAAGCCTTGCAGCAGACCTTCACTGAGGGWK#177CTCCCATAGTGATAAAGGTGTAACCAGAAGCCTTGCAGCAGCCTTCACTGAGGGWK#176GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGCGGAACCTTCACTGAGGG	WK#101							
WK#133CTCCACAGGCGCGCACTCCCGAAAATGTGCTCAGGCTCAGGACTGAGGCAGACAAATTTCWK#159GGTGACTTTCTCCTTTGGTGTCACGCTCTGAAAGTCAGCTCGAGAGCCAGCAGCACAATTTCWK#160AGAGCGTGACACCAAAGGAGAAAGTCACCATCACCTGCACGGACCAGTCAGAGCWK#161GCTGGTACCAATGTATGTTTGTGCCAATGCTCTGACTGGTCCTGCAGGGTGATWK#162ATTGGCACAAACATACATTGGTACCAGCAGCAGAAACCAGATCAGTCCCAAGGCTCCWK#163GACTCCAGAGATAGACTCAGAAACATACTTGATGAGGAGAGCTTTGGAGACTGATCTGGTTTCTWK#164TCATCAAGTATGTTTCTGAGTCTATCTCTGGAGTCCCATCGAGGGTCAGTGGCAGTGGWK#165GGTGAGGGTGAAATCTGTCCCTGATCCACTGCACTGCAC	WK#110							
WK#133GGTGACTTTCCCTTTGATGCCAAAGGCCTGGAAGGCCGAGGCGAGGCCAAGGGGAGCCAAGGGGGGCGCCAAAGGGGGG	WK#150							
WK#100AGAGGGTGACACCAAAGGAGAGAGAGAGAGACCACCTGCACGGAGGAGCCAGTCAGAGCWK#161GCTGGTACCAATGTATGTTTGTGCCAATGCTCTGACTGGTCCTGCAGGGTGATWK#162ATTGGCACAAACATACATTGGTACCAGCAGAAACCAGATCAGTCTCGAAGCTCCWK#163GACTCCAGAGATAGACTCAGAAACATACTTGATGAGGAGGAGCTGAAGCTGGATCTGGTTTCTWK#164TCATCAAGTATGTTTCTGAGTCTATCTCTGGAGTCCCATCGAGGGTTCAGTGGCAGTGGWK#165GGTGAGGGTGAAATCTGTCCCTGATCCACTGCCACTGAACCTCGATGGWK#167ATCAGGGACAGATTTCACCCTCACCATCAATAGCCTGGAAGCTGAAGATGCTGWK#168GCCAGGTATTACTTTGTTGACAGTAATACGTTGCAGCATCTTCAGGTTCCAGGCTATTGATWK#169CAACGTATTACTGTCAACAAAGTAATACCTGGCCGTTCACGTTCGGCGGAGGWK#170CGATGGGCCCTTGGTGCTAGCTTTGATCTCCACCTTGGTCWK#171GCGGCCGCGGTGCGTTTGATCTCCACCTTGGTCWK#172CTCCACAGGCGCGCACTCCWK#173CTCCACAGGCGCGCACTCCCAGGTTCAGCTGGAACCTGGWK#174CCCCAGGCTTCTTCACCTCAGGCTCCAGGACGCACCCAGCTGAACCTGWK#175AGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGWK#176GTATCCAGTAGTTGGTAAACGAGTGTAACCAGAAGCCTTGCAGGAGACCTTCACTGAGGGWK#176GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGGAGACCTTCACTGAGG	WK#159							
WK#101GCTGGTACCAAIGTTTGTGCCAAIGCTCGACGGCAGGAGCCGGCAGGGAGGTCCGGCGGGGGGGG	WK#161							
WK#102ATTGGCACAAACATACATTGGTACCAGCAGAAACCAGATCAGTCTCGATTCCCWK#163GACTCCAGAGATAGACTCAGAAACATACTTGATGAGGAGGAGCTTTGGAGACTGATCTGGTTTCTWK#164TCATCAAGTATGTTTCTGAGTCTATCTCTGGAGTCCCATCGAGGGTTCAGTGGCAGTGGWK#165GGTGAGGGTGAAATCTGTCCCTGATCCACTGCACTGCAC	WK#101							
WK#105GACTCCAGAGATAGACTCAGAAACATACTTGATGAGGAGCTTTGGAGACTGATCTGGTTCTGGTGCTGGTTCTGAGTGTGAGGGTGAAGCTCAGTGGCAGTGGWK#164TCATCAAGTATGTTTCTGAGTCTATCTCTGGAGTCCCACTGCAGTGGAGGCTCAGTGGCAGTGGWK#165GGTGAGGGTGAAATCTGTCCCCTGATCCACTGCCACTGAACCTCGATGGWK#167ATCAGGGACAGATTTCACCCTCACCATCAATAGCCTGGAAGCTGAAGATGCTGWK#168GCCAGGTATTACTTTGTTGACAGTAATACGTTGCAGCATCTTCAGCTTCCAGGCTATTGATWK#169CAACGTATTACTGTCAACAAAGTAATACCTGGCCGTTCACGTTCGGCGGAGGWK#170CGATGGGCCCTTGGTGCTAGCTTTGATCTCCACCTTGGTCCCTCCGCCGAACGTGAACGWK#171GCGGCCGCCGTGCGTTTGATCTCCACCTTGGTCWK#172CTCCACAGGCGCGCACTCCWK#173CTCCACAGGCGCGCACTCCCAGGTTCAGCTGGTGCAGTCTGGWK#174CCCCAGGCTTCTTCACCTCAGCTCCAGACTGCACCAGCTGAACCTGWK#175AGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGGWK#176GTATCCAGTAGTTGGTAAAAGTGTAACCAGAAGCCTTGCACCAGGAGACCTTCACTGAGGWK#174CCCCAGTGTTAGCTAACGACGTGTAACCAGAAGCCTTGCAGGAGACCTTCACTGAGG	WK#162							
WK#164ICATCAAGTAIGTTTCTGAGTCTATCTCTGGAGGTCCAATGGAGGTTCAGTGGCAGTGGWK#165GGTGAGGGTGAAATCTGTCCCCTGATCCACTGCCACTGAAGCTCGAAGCTGGAWK#167ATCAGGGACAGATTTCACCCTCACCATCAATAGCCTGGAAGCTGAAGATGCTGWK#168GCCAGGTATTACTTTGTTGACAGTAATACGTTGCAGCATCTTCAGCTTCCAGGCTATTGATWK#169CAACGTATTACTGTCAACAAAGTAATACCTGGCCGTTCACGTTCGGCGGAGGWK#170CGATGGGCCCTTGGTGCTAGCTTTGATCTCCACCTTGGTCCCTCCGCCGAACGTGAACGWK#171GCGGCCGCCGTGCGTTTGATCTCCACCTTGGTCWK#172CTCCACAGGCGCGCACTCCWK#173CTCCACAGGCGCGCACTCCCAGGTTCAGCTGGTGCAGTCTGGWK#174CCCCAGGCTTCTTCACCTCAGCTCCAGACTGCACCAGCTGAACCTGWK#175AGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGWK#176GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGGAGACCTTCACTGAGGWK#174CCCCAGTGAAGAAGCCTGGGGCCTCAGTGAAGGCCTTGCAGGAGACCTTCACTGAGGWK#176GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGGAGACCTTCACTGAGG	WK#103							
WK#103GGTGAGGGTGAAAACTGTCCCCTGAACCACTGCCACTGAACCTCGATGGWK#167ATCAGGGACAGATTTCACCCTCACCATCAATAGCCTGGAAGCTGAAGATGCTGWK#168GCCAGGTATTACTTTGTTGACAGTAATACGTTGCAGCATCTTCAGCTTCCAGGCTATTGATWK#169CAACGTATTACTGTCAACAAAGTAATACCTGGCCGTTCACGTTCGGCGGAGGWK#170CGATGGGCCCTTGGTGCTAGCTTTGATCTCCACCTTGGTCCCTCCGCCGAACGTGAACGWK#171GCGGCCGCCGTGCGTTTGATCTCCACCTTGGTCWK#172CTCCACAGGCGCGCACTCCWK#173CTCCACAGGCGCGCACTCCCAGGTTCAGCTGGTGCAGTCTGGWK#174CCCCAGGCTTCTTCACCTCAGCTCCAGACTGCACCAGCTGAACGGWK#175AGCTGAGGTGAAGAAGCCTGGGGGCCTCAGTGAAGGTCTCCTGCAAGGGWK#176GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGCAGGAGACCTTCACTGAGGWK#177CTTCCCTTACCACCTCAGACTGCACCAGAAGCCTTGCAGGAGACCTTCACTGAGG	WK#104							
WK#107ATCAGGGACAGATTTCACCCTCACCATCAATAGCCTGGAAGCTGAAGATGCTGWK#168GCCAGGTATTACTTTGTTGACAGTAATACGTTGCAGCATCTTCAGCTTCCAGGCTATTGATWK#169CAACGTATTACTGTCAACAAAGTAATACCTGGCCGTTCACGTTCGGCGGAGGWK#170CGATGGGCCCTTGGTGCTAGCTTTGATCTCCACCTTGGTCCCCCCGCGAACGTGAACGWK#171GCGGCCGCCGTGCGTTTGATCTCCACCTTGGTCWK#172CTCCACAGGCGCGCACTCCWK#173CTCCACAGGCGCGCACTCCCAGGTTCAGCTGGTGCAGTCTGGWK#174CCCCAGGCTTCTTCACCTCAGCTCCAGACTGCACCAGCTGAACCTGWK#175AGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGWK#176GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGGAGACCTTCACTGAGGWK#176GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTGCACCAGCAGGAGACCTTCACTGAGG	WK#103							
WK#108GCCAGGTATTACTTTGTTGACAGTAATACGTTGCAGGAGCATCTTCAGCTTCCAGGTTCCAGGTAATACATTGATWK#169CAACGTATTACTGTCAACAAAGTAATACCTGGCCGTTCACGTTCGGCGGAGGWK#170CGATGGGCCCTTGGTGCTAGCTTTGATCTCCACCTTGGTCCCCCCGCGAACGTGAACGWK#171GCGGCCGCCGTGCGTTTGATCTCCACCTTGGTCWK#172CTCCACAGGCGCGCACTCCWK#173CTCCACAGGCGCGCACTCCCAGGTTCAGCTGGTGCAGTCTGGWK#174CCCCAGGCTTCTTCACCTCAGCTCCAGACTGCACCAGCTGAACCTGWK#175AGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGWK#176GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGGAGAACCTTCACTGAGGWK#177CTTCCCCTTACACGTTTACCAACGACTGCACTGCACCAGCAGGAGACCTTCACTGAGG	WK#107							
WK#109       CAACGTATTACTGTCAACAAAGTAATACCTGGCCGTTCACGTTCGGCGGGGGGGG	WK#160							
WK#170       CCGACGCCGTGCGTTTGATCTCCACCTTGATCTCCACCTTGGTC         WK#171       GCGGCCGCCGTGCGTTTGATCTCCACCTTGGTC         WK#172       CTCCACAGGCGCGCACTCC         WK#173       CTCCACAGGCGCGCACTCCCAGGTTCAGCTGGTGCAGTCTGG         WK#174       CCCCAGGCTTCTTCACCTCAGCTCCAGACTGCACCAGCTGAACCTG         WK#175       AGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGG         WK#176       GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGGAGAACCTTCACTGAGG         WK#177       CTTCCCTTACACGTTAGCAACTACTACTACCAGAAGCCTTGCAGGAGACCTTCACTGAGG	WK#109							
WK#171       GCCGCCCCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC	WK#170	CCGCCCCCCCGTCCGTTCGTCCCCCCCCCCCCCCCCCCC						
WK#172       CTCCACAGGCGCGCACTCCCAGGTTCAGCTGGTGCAGTCTGG         WK#173       CTCCACAGGCGCGCACTCCCAGGTTCAGCTGGTGCAGTCTGG         WK#174       CCCCAGGCTTCTTCACCTCAGCTCCAGACTGCACCAGCTGAACCTG         WK#175       AGCTGAGGTGAAGAAGCCTGGGGGCCTCAGTGAAGGTCTCCTGCAAGG         WK#176       GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGGAGACCTTCACTGAGG         WK#177       CTTCTCCTTACACGTTAGCAACTACTCCATACAGCACGCCTCCCAGGAGACCTTCACTGAGG	WK#171							
WK#175       CCCCAGGCTTCTTCACCTCAGCTCCAGACTGCACCAGCTGAACCTG         WK#175       AGCTGAGGTGAAGAAGCCTGGGGGCCTCAGTGAAGGTCTCCTGCAAGG         WK#176       GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGCCTTGCAGGAGACCTTCACTGAGG         WK#177       CTTCTCCTTAGAGCTTTAGCAAGTGTAACCAGAAGCCTTGCAGGAGACCTTCACTGAGG	W/K#172							
WK#174       CCCCAGGCTCAGCTCAGCTGCAGCAGCAGCTGCAGCTGCAGCTGCAGCTGGCAGCTGGCAGCTGGCAGGGGGGGG	WIX#173							
WK#175 GTATCCAGTAGTTGGTAAAGGTGTAACCAGAAGGTCTCCCGCAAGG	VVIC#174							
	WK#175							
	W/K#177	CTTCTGGTTACACCTCTACTACTACTACTACTACTACTACTACTACT						

**Table S2.** Primers used in this study (Underlining indicates the restriction enzyme sites).

WK#178	CCATCCACTCAAGCCCTTGTCCAGGCGCCTGTCGCACCCAGT
WK#179	TGGACAAGGGCTTGAGTGGATGGGAGTGATTGATCCTTCTGATACTTATCCAAATTAC
WK#180	CATGGTGACTCTGCCCTTGAACTTTTATTGTAATTTGGATAAGTATCAGAAGGATCAATCA
WK#181	AATAAAAAGTTCAAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAG
WK#182	CTCAGGCTCCTCAGCTCCATGTAGGCTGTGCTCGTGGATGTGTCTGTGGT
WK#183	CCTACATGGAGCTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTAC
WK#184	CGGAATCACCGTTTCTCGCACAGTAATACACGGCCGTGTCGTCAGAT
WK#185	TGTGCGAGAAACGGTGATTCCGATTATTACTCTGGTATGGACTACTGGGGGC
WK#186	GAGACGGTGACCGTGGTCCCTTGCCCCCAGTAGTCCATACCAGAGTAATAAT
WK#187	AAGGGACCACGGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCG

		FcγRI		FcyRlla-H131			
Variant	K <sub>on</sub> (M <sup>-1</sup> s <sup>-1</sup> )	k <sub>off</sub> (s⁻¹)	К <sub>D</sub> (nM)	k <sub>on</sub> (M <sup>-1</sup> s <sup>-1</sup> )	k <sub>off</sub> (s <sup>-1</sup> )	κ <sub>⊳</sub> (μΜ)	
Herceptin	1.8 x 10 <sup>5</sup>	2.7 x 10 <sup>-4</sup>	1.5 <sup>#</sup>	7.2 x 10 <sup>5</sup>	8.5 x 10 <sup>-2</sup>	0.12	
AglycoT-Fc5-2a	1.5 x 10 <sup>5</sup>	2.3 x 10 <sup>-3</sup>	16	3.1 x 10 <sup>5</sup>	1.2 x 10 <sup>-1</sup>	0.37	
AglycoT-Fc1001	4.3 x 10 <sup>5</sup>	3.3 x 10 <sup>-2</sup>	77	2.6 x 10 <sup>5</sup>	5.4 x 10 <sup>-2</sup>	0.20	
AglycoT-Fc1002	N/A	N/A	N/A	1.5 x 10 <sup>5</sup>	2.8 x 10 <sup>-2</sup>	0.19	
AglycoT-Fc1003	N/A	N/A	N/A	3.3 x 10 <sup>5</sup>	5.9 x 10 <sup>-2</sup>	0.18	
AglycoT-Fc1004	4.0 x 10 <sup>5</sup>	2.6 x 10 <sup>-2</sup>	64	5.3 x 10 <sup>5</sup>	1.1 x 10 <sup>-2</sup>	0.021	

**Table S3.** Kinetic on and off rates for trastuzumab Fc variant binding to FcγRs as determined by SPR analysis.

		FcyRlla-R131		FcγRIIb			
Variant	k <sub>on</sub> (M⁻¹ s⁻¹)	k <sub>off</sub> (s <sup>-1</sup> )	κ <sub>ο</sub> (μΜ)	k <sub>off</sub> (s⁻¹)	k <sub>on</sub> (M⁻¹ s⁻¹)	Κ <sub>D</sub> (μΜ)	
Herceptin	1.2 x 10 <sup>5</sup>	3.7 x 10 <sup>-2</sup>	0.31	3.7 x 10 <sup>-2</sup>	1.2 x 10 <sup>5</sup>	1.3	
AglycoT-Fc5-2a	1.3 x 10 <sup>5</sup>	3.6 x 10 <sup>-2</sup>	0.27	3.6 x 10 <sup>-2</sup>	1.3 x 10 <sup>5</sup>	1.6	
AglycoT-Fc1001	2.6 x 10 <sup>5</sup>	3.9 x 10 <sup>-3</sup>	0.015	3.9 x 10 <sup>-3</sup>	2.6 x 10 <sup>5</sup>	0.47	
AglycoT-Fc1002	1.8 x 10 <sup>5</sup>	4.0 x 10 <sup>-2</sup>	0.22	$4.0 \times 10^{-2}$	1.8 x 10 <sup>5</sup>	1.9	
AglycoT-Fc1003	2.6 x 10 <sup>5</sup>	3.2 x 10 <sup>-2</sup>	0.12	3.2 x 10 <sup>-2</sup>	2.6 x 10 <sup>5</sup>	1	
AglycoT-Fc1004	3.2 x 10 <sup>5</sup>	6.2 x 10 <sup>-4</sup>	0.0019	6.2 x 10 <sup>-4</sup>	3.2 x 10 <sup>5</sup>	0.2	

<sup>#</sup>Affinity reported in previous study (1), using the same method.

Parameter	Description	Value	Reference or Source					
Physical parameters								
SK_dia	Diameter of SKOV-3 cell	10 µm	(16)					
MD_dia	Diameter of MDA-MB-453 cell	10 µm	(16, 17)					
Mac_dia	Diameter of macrophage	21 µm	(18)					
Cell_gap	Gap distance between SKOV-3/MDA-MB-453 cell and macrophage	12 nm	(19, 20)					
Contact_area	Contact area of FcyRII receptors on macrophage	$104.7  \mu m^2$	(9-11)					
Expression level	parameters							
SK_HER2	HER2 expression level on SKOV-3	7.36 x 10 <sup>5</sup>	(13, 14)					
MD_HER2	HER2 expression level on MDA-MB-453	4.00 x 10 <sup>5</sup>	(13, 14)					
Mac_llaH	Number of FcyRIIa-H131 on macrophage	171271	This work					
Mac_IIaR	Number of FcyRIIa-R131 on macrophage	171271	This work					
Mac_lib	Number of FcyRIIb on macrophage	291150	This work					
[lgG] <sub>s</sub>	Free serum IgG concentration	10 µM	This work					
[L <sub>0</sub> ] <sub>SK</sub>	SKOV-3 Her2-bound IgG effective concentration	324.2 μM	This work					
[L <sub>0</sub> ] <sub>MD</sub>	MDA-MB-453 Her2-bound IgG effective concentration	176.2 μM	This work					
Affinity paramet	ers							
K <sub>diff</sub>	Equilibrium constant for partitioning of FcyRII receptors in/out of the contact area on macrophage	16.64	This work					
K <sub>cross</sub>	Equilibrium dissociation constant for the crosslinking of FcyRII receptors	2500 /µm²	This work					
$K_{D_{lla_{lgG}}}$	Equilibrium dissociation constant between serum IgG and FcyRIIa-H131/FcyRIIa-R131	0.72 μΜ	(21)					
$K_{D_1Ib_1gG}$	Equilibrium dissociation constant between serum IgG a nd FcγRIIb	2.4 μM	(21)					
K <sub>D_IIa-H131</sub>	Equilibrium dissociation constant between Fc variants and FcγRIIa-H131	(Table S3)	This work					
K <sub>D_IIa-R131</sub>	Equilibrium dissociation constant between Fc variants and FcγRIIa-R131	(Table S3)	This work					
K <sub>D_IIb</sub>	Equilibrium dissociation constant between Fc variants and FcγRIIb	(Table S3)	This work					

## **Table S4.** Parameters used to generate FcγRIIa/b activation model

ADCD data associational	Intrinsic signaling potency					
ADCP data considered	FcyRlla-H131	FcyRlla-R131	FcγRIIb <sup>*</sup>			
Fitting Herceptin and AglycoT-Fc1001 only	2.8	0.4	-1			
Fitting Herceptin and AglycoT-Fc1004 only	2.0	0.3	-1			
Fitting AglycoT-Fc1001 and AglycoT-Fc1004 only	11.3	0.1	-1			

### **Table S5.** Intrinsic signaling potencies of FcyRIIa-H131, FcyRIIa-R131, and FcyRIIb.

\* The signaling potency of FcyRIIb was always held fixed at -1, since the goal of each fit was to determine the *relative* potencies among the three receptor subunits.

**Figure S1.** *E. coli* bacterial expression system for the display of full length IgGs. (*A*) Expression cassettes for the display of covalently anchored full length IgGs. (*B* and *C*) Fluorescence histograms of spheroplasts expressing full length AgylcoT-Fc5 (E382V/M428I) binding to 30 nM FcyRI-FITC (*B*) or AgylcoT-2a (S298G/T299A) binding to 30 nM FcyRIIa-GST followed by 1:200 diluted goat anti-GST-FITC (*C*). (*D*) AglycoT-Fc5-2a shows high affinity binding for 30 nM FcyRI, FcyRIIa, and FcyRIIb.



### Figure S2. Sequences and corresponding FACS signals for isolated Fc variants.

		Hinge				C	H2		
		220	230	240	250	260	270	280	290
FcWT Fc5-2a Fc1001 Fc1002 Fc1003 Fc1004	$\begin{array}{r} (75.76) \\ (135.76) \\ (408.55) \\ (303.82) \\ (276.75) \\ (256.43) \end{array}$	[ [ <u>D</u> KTHTCPI [ [		GGPS <u>V</u> FLFPP	KPKD <u>T</u> LMISF	RTPEV <u>T</u> CVVVE	VSHEDPEVKF	NWYV <u>D</u> GVEVHI	IAKT <u>K</u> PREEQYNGA K K
								СНЗ	
		300	310	320	330	340	350	360	370
FcWT Fc5-2a Fc1001 Fc1002 Fc1003 Fc1004	$\begin{array}{r} \underline{(75.76)}\\ \underline{(135.76)}\\ \underline{(408.55)}\\ \underline{(303.82)}\\ \underline{(276.75)}\\ \underline{(256.43)} \end{array}$	<u>Y</u> RVVSVLTV	/L <u>H</u> QDWLNG	KEY <u>K</u> CKVSNK.	ALP <u>A</u> PIEKTI	[ SKA <u>K</u> [GQPRE [ [	:PQVY <u>T</u> LPPSRI	DELT <u>K</u> NQVSL1	CLV <u>K</u> GFYPSDIAV
					CH3				
		380	390	400	410	420	430	440	
FcWT Fc5-2a Fc1001 Fc1002 Fc1003 Fc1004	$\begin{array}{r} (75.76) \\ (135.76) \\ (408.55) \\ (303.82) \\ (276.75) \\ (256.43) \end{array}$	E EWVSNGQPI	EN <u>N</u> YKTTPP EI	VLD <u>S</u> DGSFFL	YSK <u>L</u> TVDKSF	RWQQ <u>G</u> NVFSCS	-M VIH <u>E</u> ALHNHY -L	rok <u>s</u> lslspgf	•] <] -] -] -]

1) FACS mean values are indicated in the parenthesis

**Figure S3.** Characterization of isolated aglycosylated Fc variants. (A) Fluorescent histogram of variant binding to 30 nM FcγRIIa as detected by secondary goat anti-GST-FITC diluted at 1:200 from a 1 mg/ml stock. (B) SDS-PAGE showing full length trastuzumab Fc variants purified from HEK293F cells; M: molecular weight ladder, 1: AglycoT-Fc1001, 2: AglycoT-Fc1002, 3: AglycoT-Fc1003, and 4: AglycoT-Fc1004. (C and D) ELISA analysis of isolated aglycosylated Fc variants and Herceptin for (C) binding to FcRn at pH 6.0 and 7.4.



**Figure S4.** Biacore sensorgrams for FcyRIIa-R131-GST, FcyRIIa-H131-GST and FcyRIIb-GST binding to aglycosylated mutants. Antibody variants were immobilized on CM5 chips and soluble dimeric FcyRs used as analytes. A bivalent kinetic model was used to fit a minimum of four concentrations in duplicate for each variant.



**Figure S5.** Expression level of Her2 and FcyR on tumor cell lines and macrophages for ADCP assay. (*A*) Her2 expression level on tumor cell lines used for ADCP was confirmed by labeling with 10  $\mu$ g/ml Herceptin or IgG1 pooled from human serum followed by fluorescent donkey anti-human IgG (H+L) FITC Fab at a 1:50 dilution. Bars are labeled with the immunohistochemical staining category assigned to each tumor cell line (*17*) (*B*) FcyR counts on macrophages were determined using a fluorescent Quantum Simply Cellular bead assay. Macrophages were labeled with 20  $\mu$ g/ml anti-FcyRII-FITC, 10  $\mu$ g/ml anti-FcyRII-FITC, 1  $\mu$ g/ml 2B6-N297D-FITC and 20  $\mu$ g/ml anti-FcyRIII-FITC. (C) Schematic diagram showing the experimental workflow of the ADCP analysis.



**Figure S6.** ADCP analysis for ovarian cancer cells expressing medium HER2 density (SKOV-3) and low HER2 density (MDA-MB-453). Tumor cells were labeled with PKH67 membrane dye, opsonized with 0.5  $\mu$ g/ml antibody and mixed with macrophages at a 1:5 ratio for MDA-MB-453 and 1:10 for SKOV-3 tumor cells. Macrophages were labeled with 10  $\mu$ g/ml anti-CD11b-APC and 10  $\mu$ g/ml anti-CD14-APC before FACS interrogation. (*A* - *F*) FACS dot plots for SKOV-3 with No Ab (*A*), AglycoT-N297D (*B*), Herceptin (*C*), GlycoT-G236A (*D*), AglycoT-Fc1001 (*E*), and AglycoT-Fc1004 (*F*). Blue population = macrophages, Red population = SKOV-3 cells, green = double positive phagocytosed SKOV-3 cells. (*G* - *L*) FACS dot plots for MDA-MB-453 with No Ab (*G*), AglycoT-N297D (*H*), Herceptin (*I*), GlycoT-G236A (*J*), AglycoT-Fc1001 (*K*), and AglycoT-Fc1004 (*L*). Blue population = macrophages, Red population = MDA-MB-453 cells, green = double positive phagocytosed MDA-MB-453 cells.



Figure S7. The model returns similar predictions for receptor signaling potency independent of experimental data used for parameterization. (A and B) ADCP data for Herceptin and AglycoT-Fc1004 were used to obtain intrinsic signaling potency values in the model, which was then used to predict phagocytic response of AglycoT-Fc1001 with both SKOV-3 cells (A) and MDA-MB-453 cells (B). Blue bars represent experimental values and green bars represent model predictions. (C and D) ADCP data for AglycoT-Fc1001 and AglycoT-Fc1004 were used to obtain intrinsic signaling potency values in the model, which was then used to predict phagocytic potency of AglycoT-Fc1001 with both SKOV-3 cells (C) and MDA-MB-453 cells (D). Blue bars represent experimental values and orange bars represent model predictions. (E) Predicted phagocytic responses of other Fc variants. Based on  $K_{D ||a-H131}$ ,  $K_{D ||a-R131}$ , and  $K_{D ||b}$  values from SPR analysis (Table S3), phagocytic response was predicted by the mathematical model for both SKOV-3 cells (dark red bar) and MDA-MB-453 cells (light red bar). Intrinsic signaling potencies for FcyRIIa-H131, FcyRIIa-R131 and FcyRIIb were the same as in Fig. 4 (2.8, 0.4, and -1 respectively).

