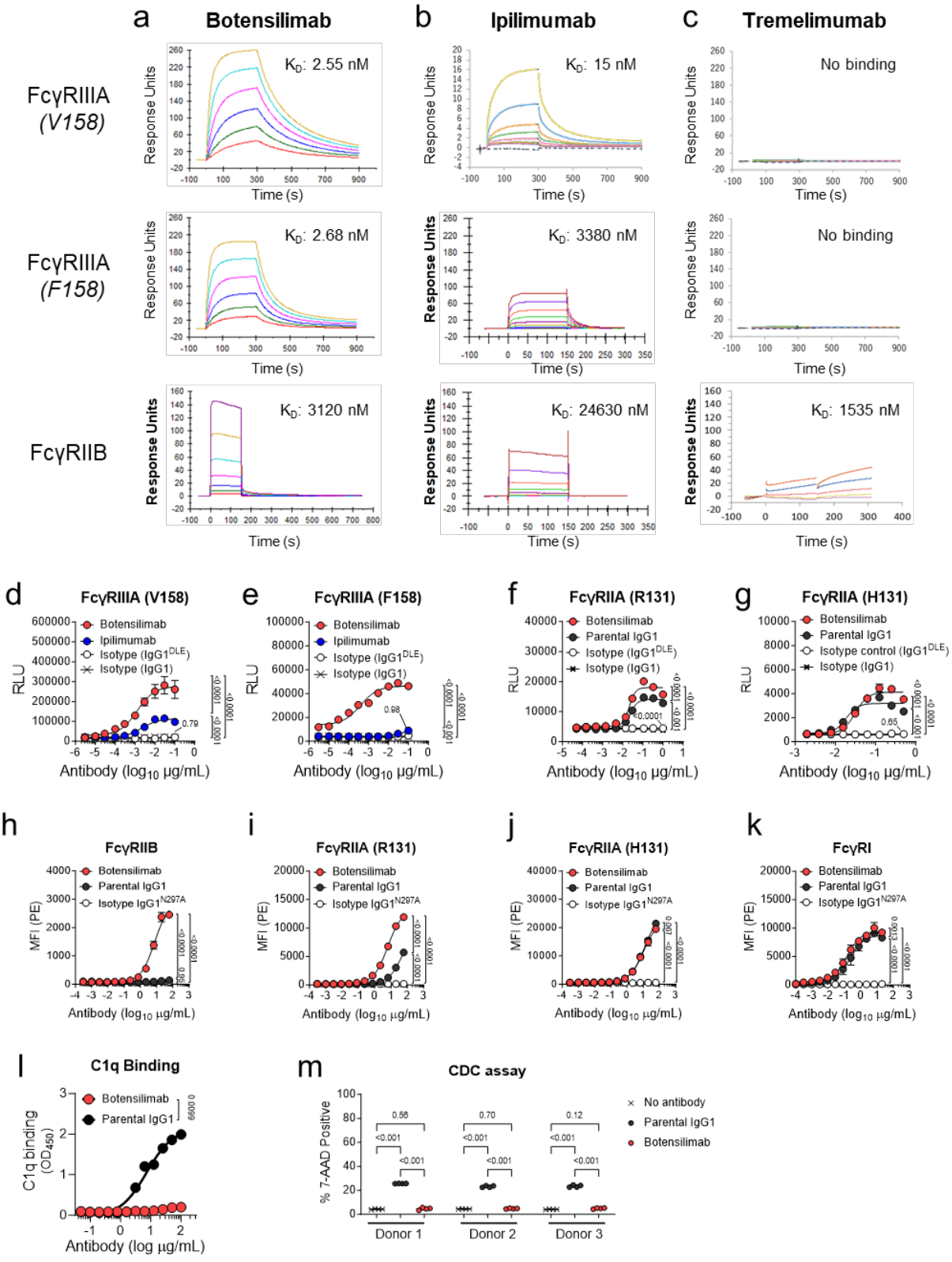
Supplementary Figure S8



Supplementary Figure S8. Botensilimab demonstrates enhanced binding to Fcγ receptors and superior induction of FcγRIIIA signaling as compared to ipilimumab but does not mediate complement-dependent cytotoxicity (CDC). Binding kinetics of **(a)** botensilimab, **(b)** ipilimumab or **(c)** tremelimumab to human FcγRIIIA V158 (valine at position 158; top row), FcγRIIIA F158 (phenylalanine at position 158; middle row) and FcγRIIB (bottom row) proteins as determined by surface plasmon resonance. FcγR proteins were captured on a sensor chip and a range of antibody concentrations were injected. Representative sensorgrams are shown, and the binding affinity (K_D) for each interaction is indicated in the respective panels. Activation of Jurkat cells genetically engineered to express **(d)** FcγRIIIA V158, **(e)** FcγRIIIA F158, **(f)** FcγRIIA R131 (arginine at position 131), or (**g**) FcγRIIA H131 (histidine at position 131) upstream of a NFAT-dependent luciferase reporter. Cells were co-cultured with CTLA-4-expressing cells and treated with a dose range of the indicated antibodies. Receptor activation was assessed by luciferase expression, shown as relative light units (RLU). Results representative of two independent experiments. Botensilimab binding to Chinese hamster ovarian cells genetically engineered to express **(h)** Fcγ receptor (FcγR) IIB, **(i)** FcγRIIA R131 (arginine at position 131), **(j)** FcγRIIA H131 (histidine at position 131), or **(k)** FcγRI. Cells were incubated with botensilimab, parental IgG1, or isotype $IgG1^{N297A}$ aglycosylated control antibody (negative control) and binding analyzed by flow cytometry using a fluorochrome conjugated anti-human F(ab')2 secondary antibody. **(l)** Binding to C1q was evaluated by enzyme-linked immunosorbent assay. A dose titration of plate bound botensilimab or parental IgG1 was exposed to C1q. To detected C1q binding, antibodies were incubated with a biotinylated anti-C1q followed by streptavidin-horse radish peroxidase and absorbance measured at 450 nm. **(m)** CTLA-4^+ Jurkat cells were incubated with 20% normal human serum from three healthy donors and treated with 10 μg/mL of the indicated antibodies. Cells were incubated for 4 hours followed by staining with a viability dye. Percent cell death was determined by flow cytometric analysis. All samples were tested in quadruplicate. Data were

analyzed using a two-way ANOVA followed by a Tukey's multiple comparisons test **(d-k, m)** or unpaired t-test **(l)**.