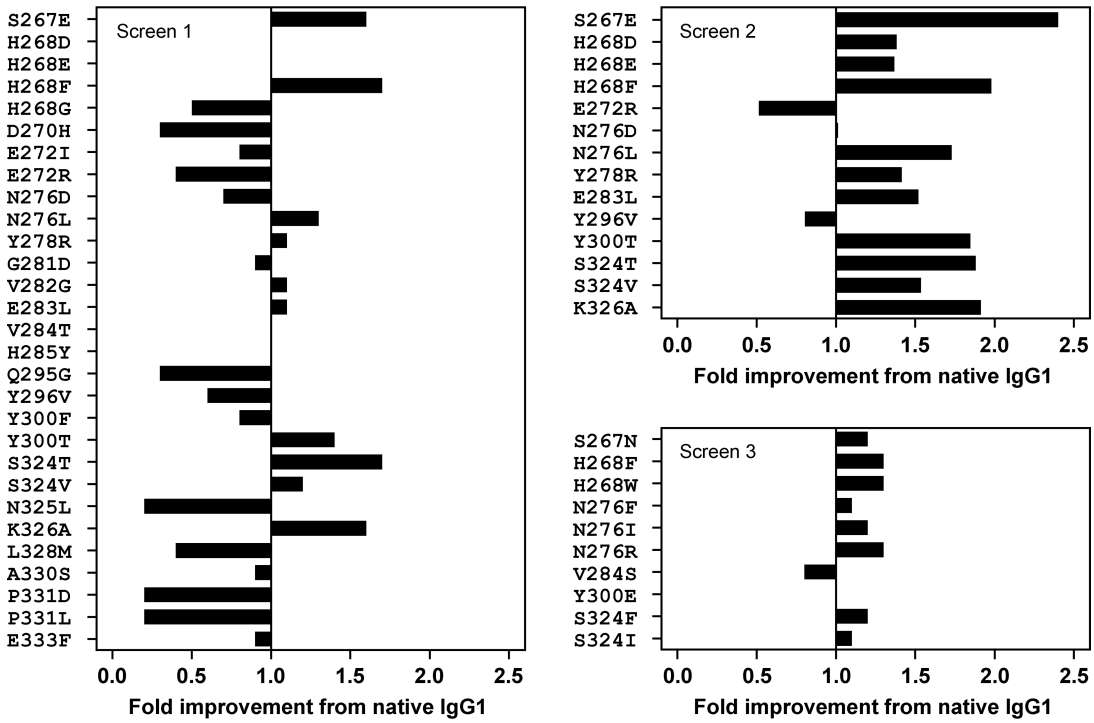
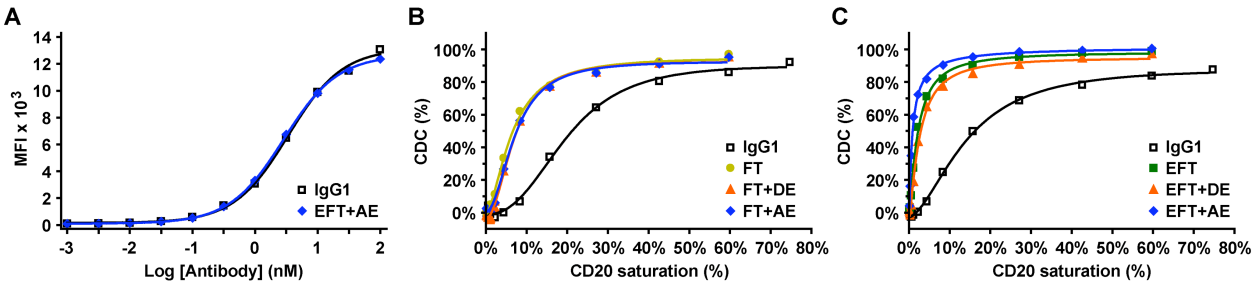
Supplemental Figure 1



Primary screens for CDC activity of Fc variant anti-CD20 mAbs against opsonized Raji cells using human complement. Screens 1 and 2 used 50,000 Raji cells as targets; Screen 3 used 40,000 Raji cells. Cell viability was measured by Alamar Blue-based detection. Data were fit to a four-parameter sigmoidal dose-response curve using GraphPad Prism (La Jolla, CA). Fold improvements were calculated as Fold = EC_{50} (Native IgG1) / EC_{50} (variant).

Supplemental Figure 2



Estimation of antigen saturation required for a given level of CDC activity based on cell-surface binding data. (A) A Ramos cell-surface binding assay was used to measure CD20 binding affinity of the indicted anti-CD20 antibodies. Antibodies were detected using fluorescently labeled secondary antibodies on a FACSCanto II flow cytometer. The fitted EC_{50} of native IgG1 was 3.4 nM; the fitted EC_{50} of variant EFT+AE was 2.9 nM. (B-C) Replotting of CDC activities from antibody concentration to % CD20 saturation. The CDC data from Fig. 3A-B were transformed using the native IgG1 binding data from panel A according to the following equation:

$$\% \text{ CD20 saturation} = 100\% \cdot (1 / (1 + [\text{native IgG1 } EC_{50}] / [\text{Antibody}]))$$

Materials and Methods: Cell-surface binding

Ramos cells were washed 2x in RHB Buffer (RPMI Medium 1640 containing 20 mM HEPES, 2 mM glutamine, 0.1% BSA, pH 7.2) by centrifugation and resuspension and seeded at 40,000 cells per well. Native IgG1 or variant antibody was added at the indicated final concentrations. Plates were incubated for 30 min at room temperature, and then washed 4x in PBS. Then, PE-labeled goat secondary anti-human IgG Fc antibodies (Jackson ImmunoResearch, West Grove, PA) were added and the mixture incubated for an additional 30 minutes on ice. Cells were washed in PBS twice, fixed with 1% paraformaldehyde, then analyzed on a FACSCanto II (BD Biosciences, San Jose, CA). Resulting mean fluorescent intensity (MFI) values were fit to a three-parameter sigmoidal dose-response curve using GraphPad Prism (La Jolla, CA).