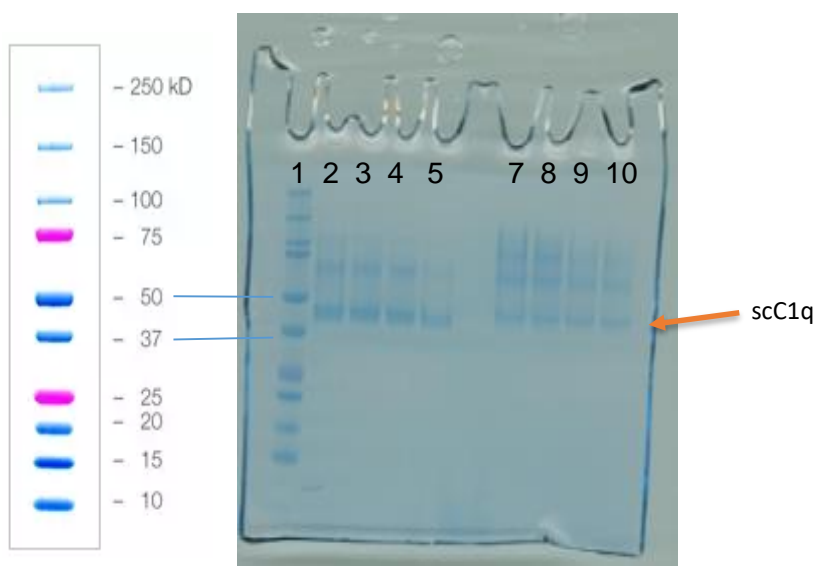
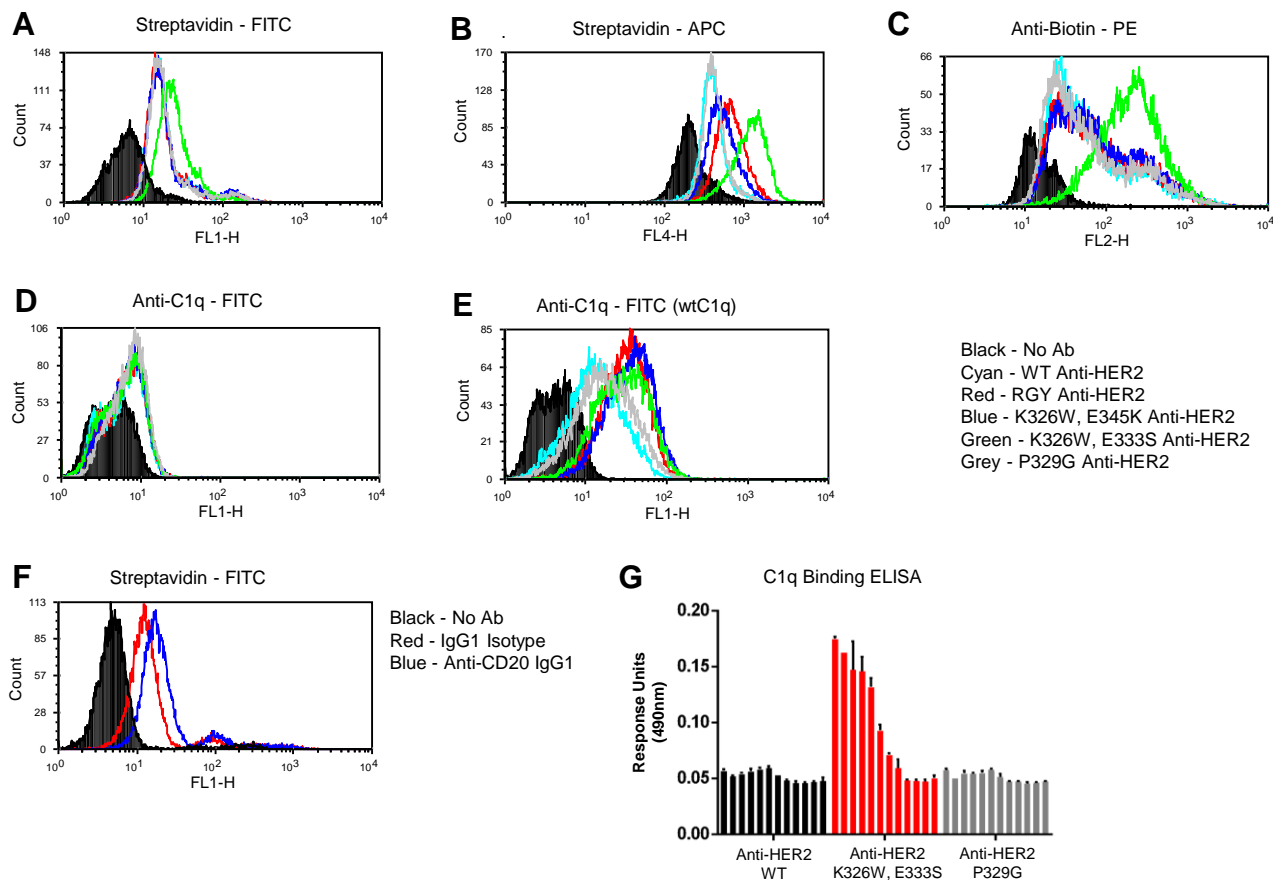


**C**

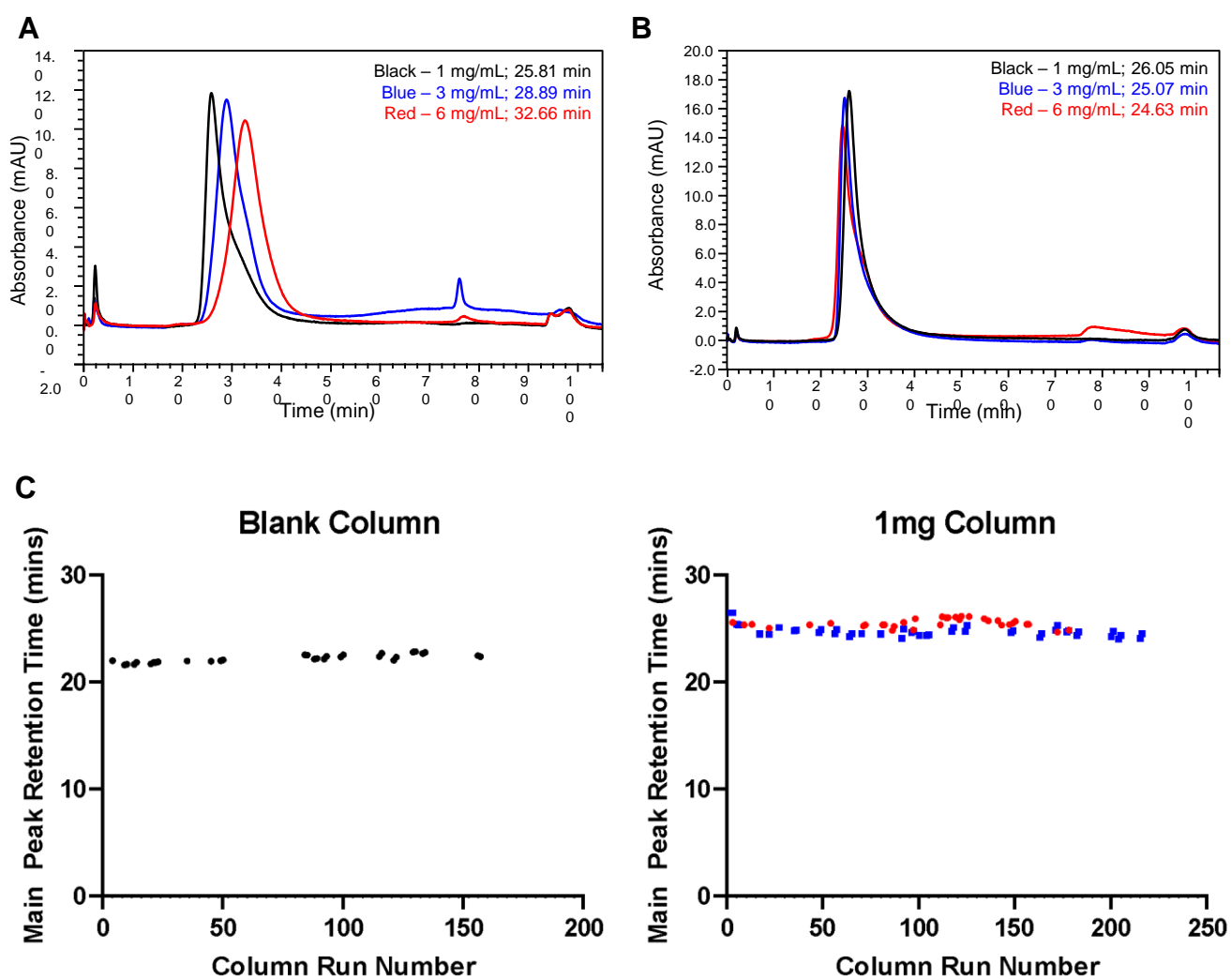


Reduced	Non reduced
Lane 1 = marker	
Lane 2 = C1q control non-biotinylated	Lane 7 = C1q control non-biotinylated
Lane 3 = C1q + Biotin 3 $\mu$ g	Lane 8 = C1q + Biotin 3 $\mu$ g
Lane 4 = C1q + Biotin 2.5 $\mu$ g	Lane 9 = C1q + Biotin 2.5 $\mu$ g
Lane 5 = C1q + Biotin 2 $\mu$ g	Lane 10 = C1q + Biotin 2 $\mu$ g

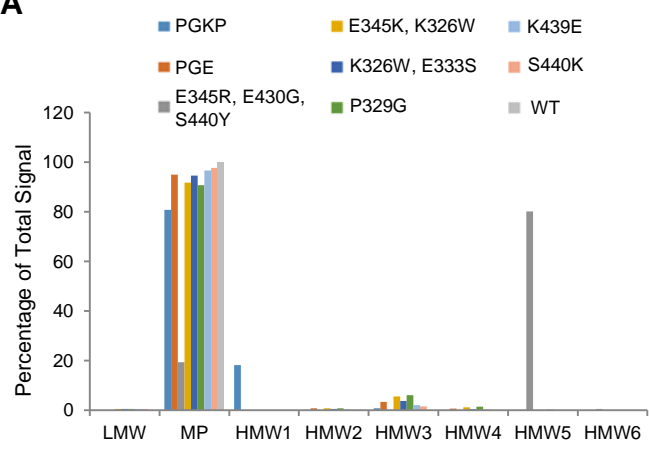
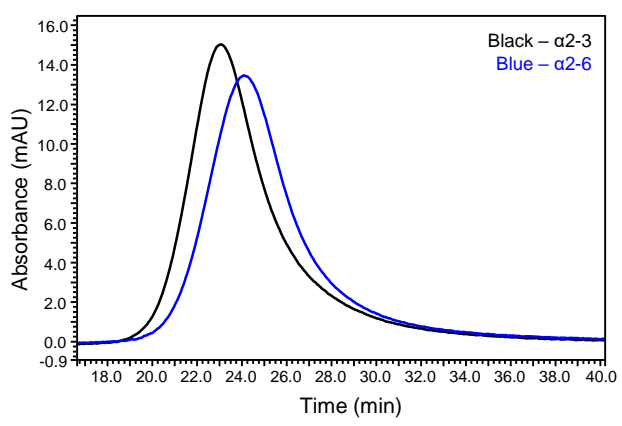
Supplementary Figure 1- Characterisation of scC1q. A- scC1q was biotinylated using a bulk BirA biotinylation kit and the resulting biotinylated product (blue) was compared to non-biotinylated scC1q (black) by size exclusion chromatography. B- Biotinylated scC1q (black) was also compared to non-biotinylated scC1q (blue) by mass spectrometry. C- SDS-PAGE of the non-biotinylated final purified C1q batch in comparison to the biotinylated C1q material.



Supplementary Figure 2- A - G In order to show scC1q could be recruited to opsonised cells, MDA-MB-453 cells were opsonised with 50  $\mu\text{g}/\text{ml}$  anti-HER2 antibody variants and then incubated with 0.1 mg/ml biotinylated scC1q. Bound scC1q was then detected with 10  $\mu\text{l}$  of neat streptavidin-FITC (A) or streptavidin-APC (B) conjugates, an anti-biotin monoclonal antibody (clone 1D4-C5) conjugated to PE (C) or 1  $\mu\text{l}$  per sample of anti-C1q polyclonal antibody conjugated to FITC (E). All samples were analysed by flow cytometry and data processed using FCS Express V3 and GraphPad Prism version 6.05. F- To compare scC1q recruitment with WT C1q, MDA-MB-453 cells were opsonised with 50  $\mu\text{g}/\text{ml}$  anti-HER2 antibody variants and then incubated with 8  $\mu\text{g}/\text{ml}$  purified human WT C1q. Bound scC1q was then detected with 1  $\mu\text{l}$  per sample of anti-C1q polyclonal antibody conjugated to FITC. All samples were analysed by flow cytometry and data processed using FCS Express V3 and GraphPad Prism version 6.05. G- a second cell type, SU-DHL-4 cells, were opsonised with 20  $\mu\text{g}/\text{ml}$  anti-CD20 IgG1 (blue) or an irrelevant isotype control (red) and then incubated with 0.1 mg/ml scC1q. Bound scC1q was detected with 10  $\mu\text{l}$  streptavidin-FITC conjugate and analysed by flow cytometry. Data was processed using FCS Express V3 and GraphPad Prism version 6.05. I- scC1q was immobilised onto an ELISA plate and incubated with 1  $\mu\text{g}/\text{ml}$  anti-HER2 antibody variants. Bound antibody was detected using a polyclonal HRP-conjugated anti-human IgG Fc region specific antibody.



Supplementary Figure 3- Stability of scC1q columns. Three different scC1q column densities (1, 3 and 6 mg scC1q per ml of sepharose) were prepared and assessed for their binding to a human IgG1 antibody. A- 25  $\mu$ g samples of IgG1 antibody were applied to either a 1 mg/mL (black), 3 mg/mL (blue) or 6 mg/mL (red) column under the same conditions immediately after their production and the resulting elution profiles overlaid. B- 25  $\mu$ g samples of IgG1 antibody were again applied to the same columns after a period of storage at 4  $^{\circ}$ C, and the resulting elution profiles overlaid. C- Column run stability of the blank column and 1 mg scC1q columns was monitored by including a standard IgG1 antibody in each run. The elution times of this 25  $\mu$ g application were collated and plotted against the cumulative number of samples run for the blank column (left- black) and two separate 1mg scC1q columns (right- red and blue).

**A****B**

Supplementary Figure 4 A - A series of anti-HER2 antibody mutants were applied to a size exclusion column, and the relative percentage of the antibody mutants identified as a low molecular weight peak (LMW), main peak (MP) or high molecular weight peak (HMW- HMW1 applies to a product approximately twice the size of the main peak). B -Anti-HER2 IgG1 were glycoengineered to contain a high level of sialic acid using alpha-2,3-sialyltransferase or alpha-2,6-sialyltransferase enzymes. 25  $\mu$ g samples were applied to the scC1q column and the elution profiles overlaid. Alpha-2,3-sialyltransferase (black) or alpha-2,6-sialyltransferase (blue).