

Supplementary Figure 1. The amino acid sequences of m610.27 (A) and m630.3 (B) in alignment with their corresponding germlines of human antibody V genes and/or wild types. The complementarity-determining regions (CDRs) and framework regions (FRs) are indicated according to the ImMunoGeneTics annotation (<http://imgt.cines.fr/>). The somatic mutations in the V regions of the antibodies and the mutations induced by random mutagenesis are highlighted with gray background.

Supplementary Figure 2. Schematic representation of antibody structures and SDS-PAGE analysis. (A) Fab m610.27 was converted to a standard IgG1 format. (B) m630.3Fc was constructed by joining eAd m630.3 to the N terminus of human IgG1 Fc through a hinge linker. (C) The bispecific antibody, m660, was generated by fusing scFv m610.27 and eAd m630.3 to the N termini of the heavy and light chain constant regions of a human IgG1, respectively, via a linker composed of three repeats of the G₄S motif. (D) Reducing and nonreducing SDS-PAGE of the antibodies purified from 293 free style cell cultures.