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Supplemental information

Directed evolution of potent neutralizing nanobodies against SARS-CoV-2 using CDR-swapping mutagenesis

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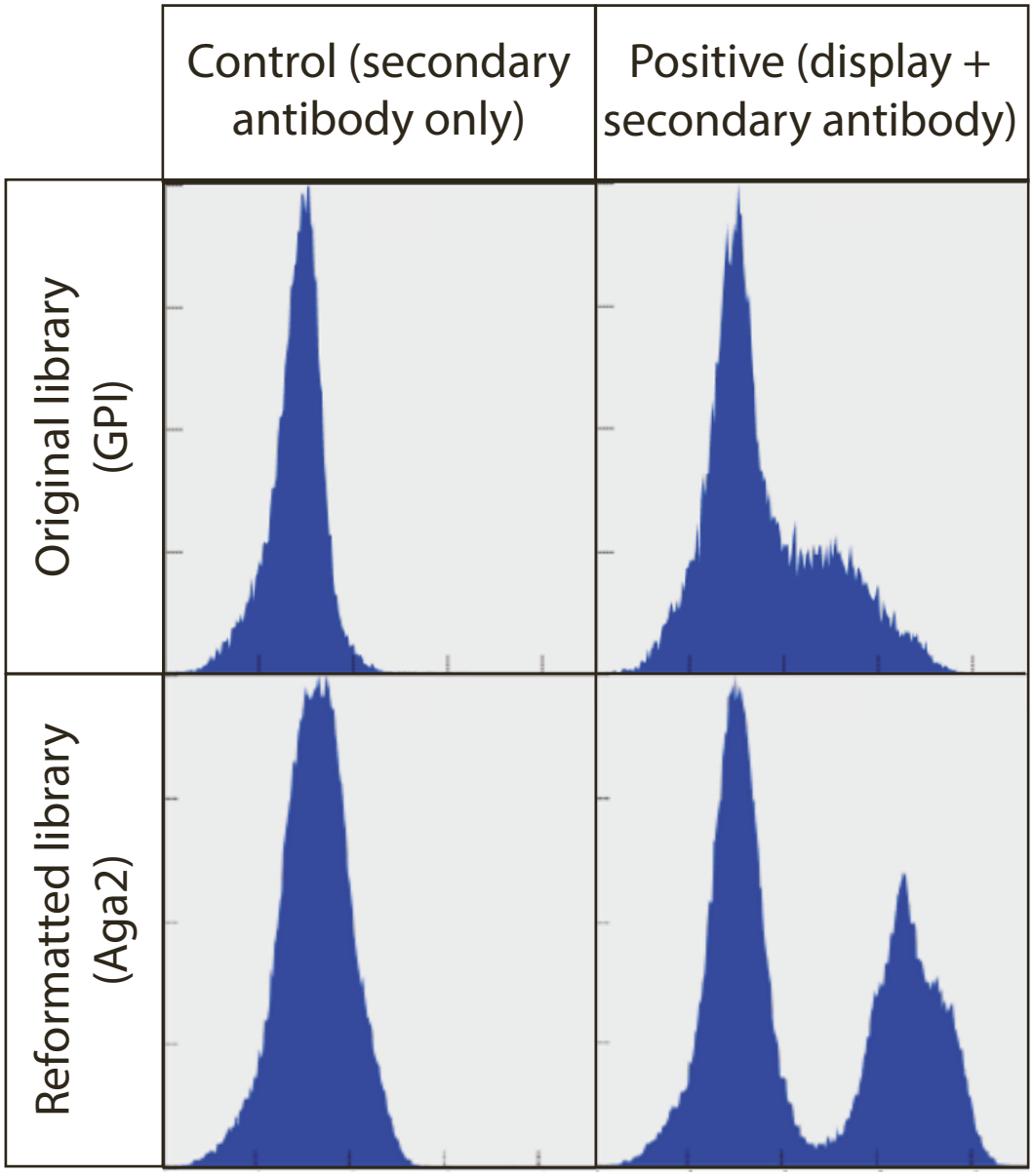

Figure S1. Reformatting the nanobody library from a GPI-anchored format to an Aga2 display format improves nanobody display levels on the yeast surface. The expression levels of the nanobody libraries were compared between two display formats, namely a GPI (glycosylphosphatidylinositol)-anchored format that displays nanobodies as GPI-linker-nanobody fusions and an Aga2 format that displays nanobodies as Aga2-linker-nanobody fusions. The yeast cells were induced to promote nanobody expression for two days, and nanobody expression levels were detected via anti-HA tag (GPI) and anti-Myc tag (Aga2) antibodies. See STAR Methods.

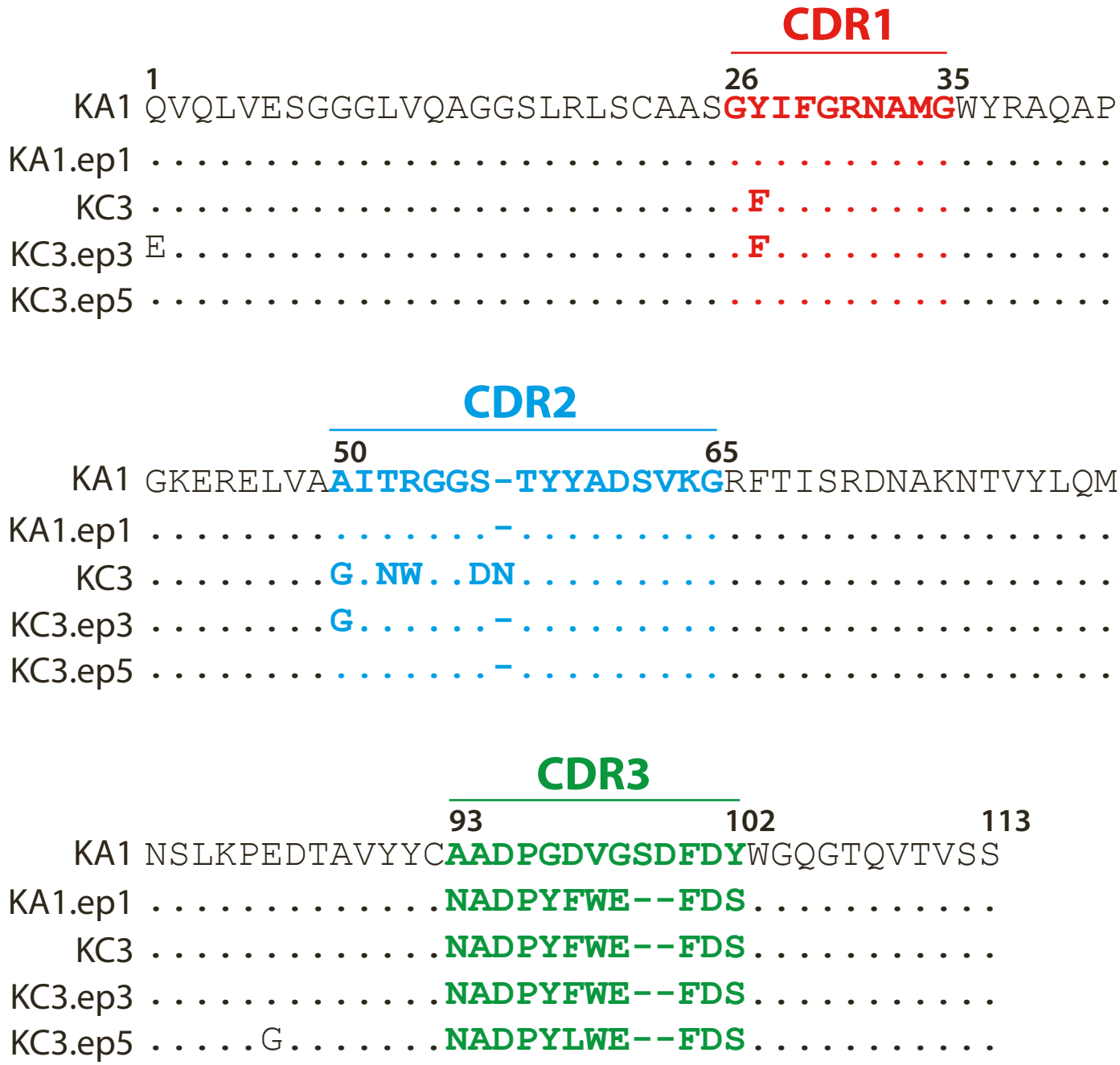

Figure S2. Amino acid sequences of the affinity-matured nanobodies in this work. The sequences are shown for Kabat numbering except CDR1 (combined Chothia and Kabat definition) and CDR3 (additional two N-terminal residues included in the CDR). See also Figure 2.

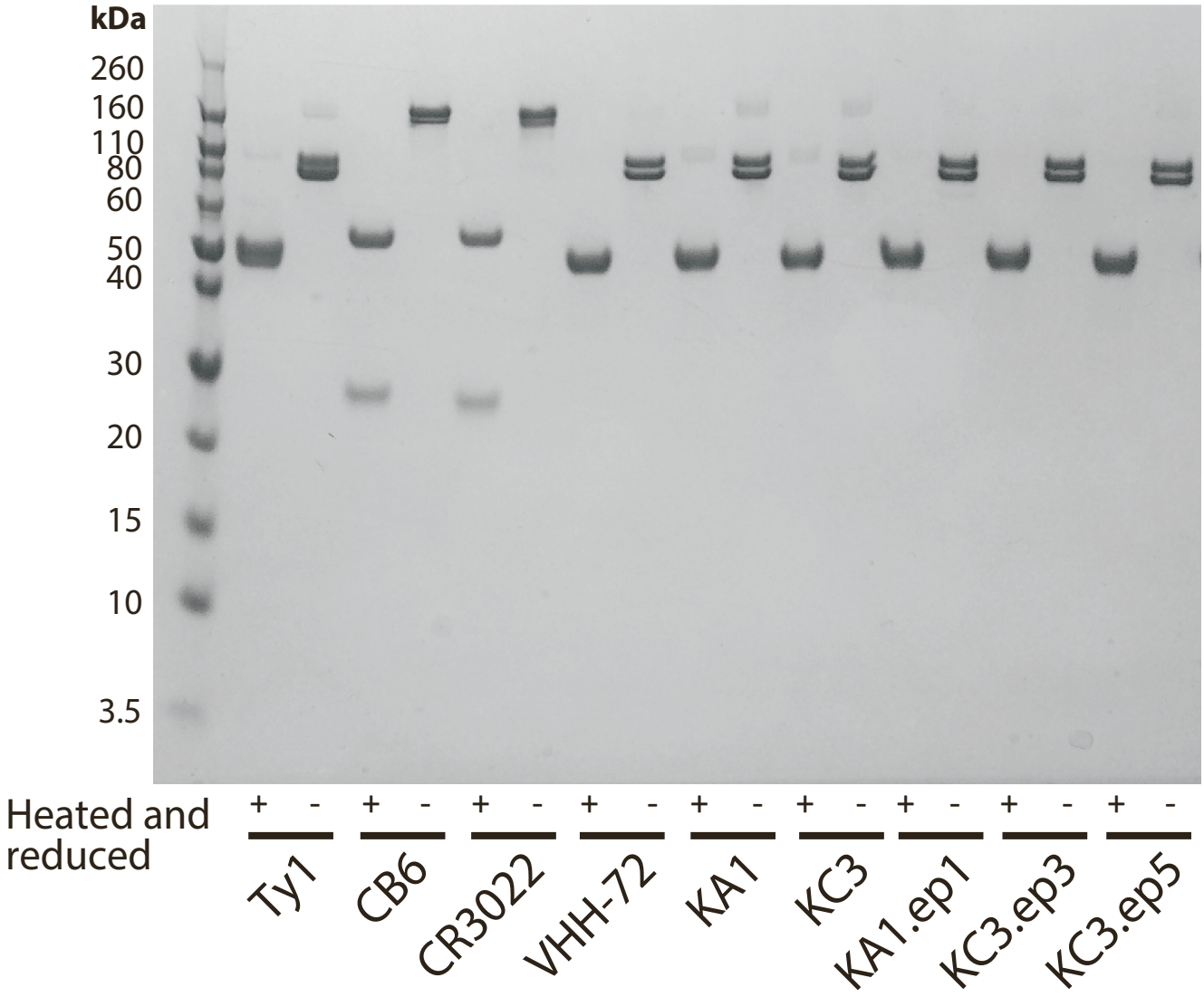

Figure S3. SDS-PAGE analysis of the nanobodies and antibodies used in this work. The nanobodies and antibodies were evaluated for samples without both heating and reducing (-) or with both heating and reducing (+). See STAR Methods.

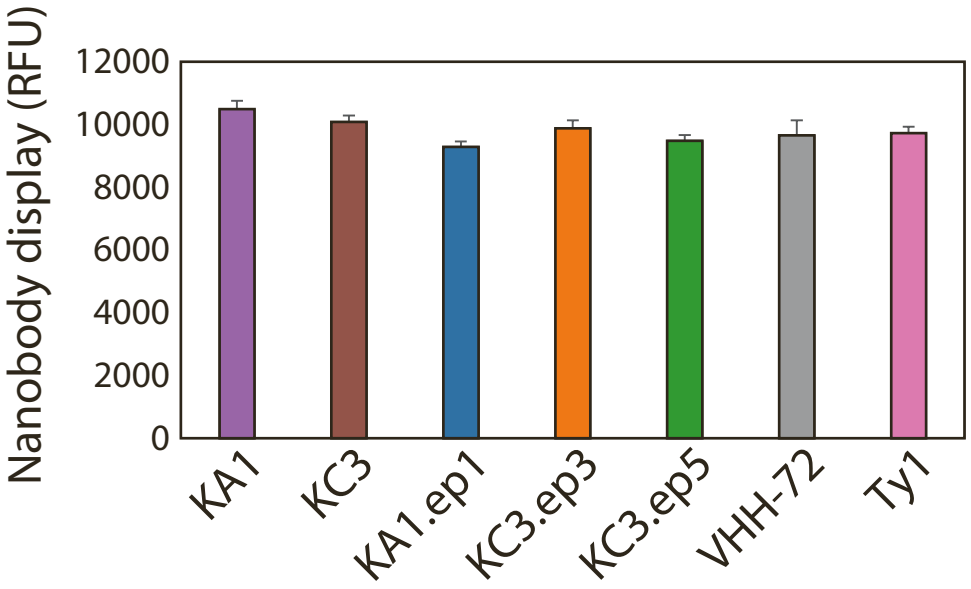

Figure S4. Display levels of nanobodies as Aga2 fusion proteins on the yeast surface. Nanobody-Aga2 expression levels on the yeast surface were detected using mouse anti-Myc antibody (1000x dilution) and goat anti-mouse IgG AF488 (200x dilution) using a Bio-Rad ZE5 analyzer. The results are averages from two independent repeats and the error bars are standard deviations. See STAR Methods.

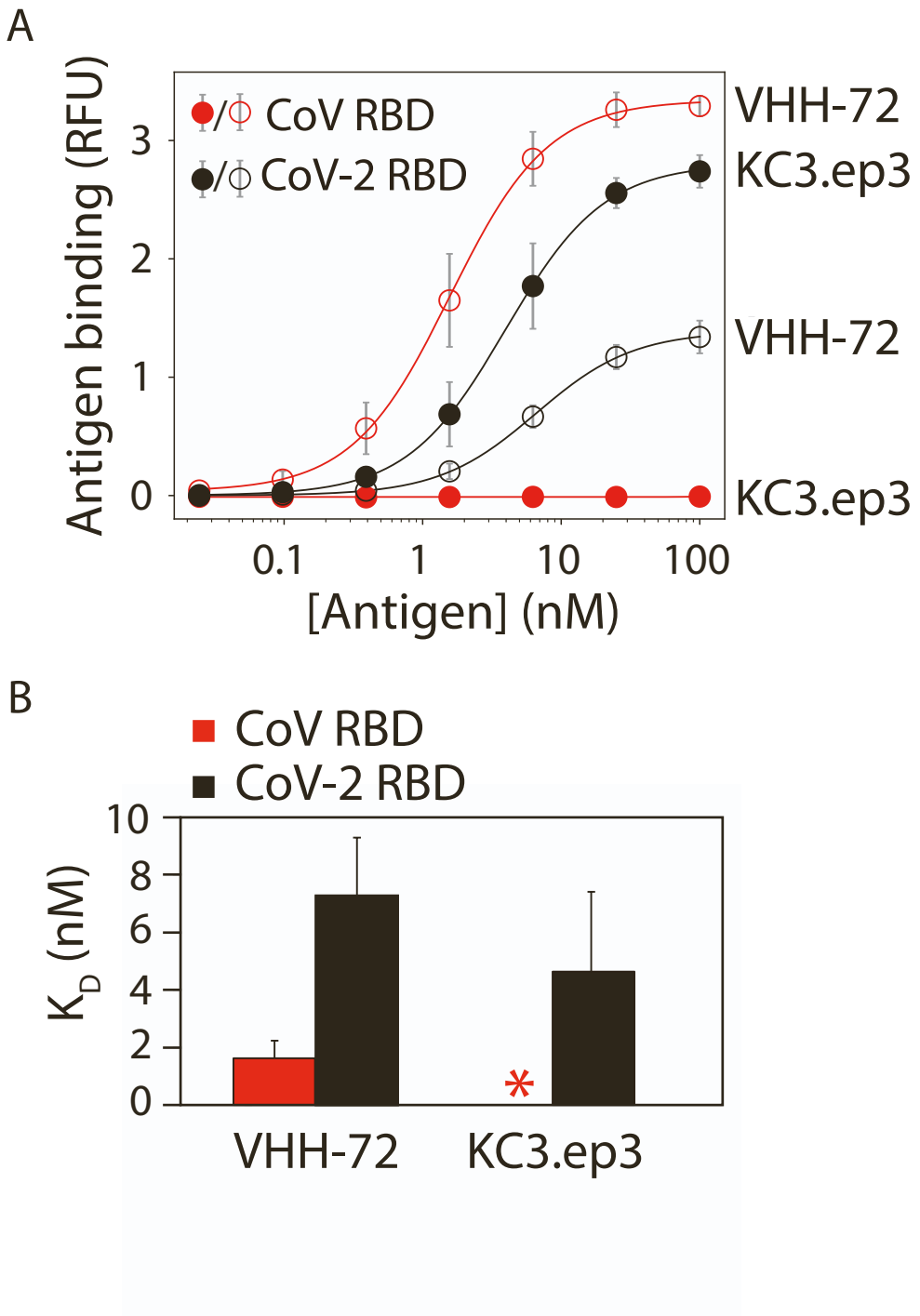

Figure S5. Affinity-matured nanobody selectively recognizes SARS-CoV-2 receptor-binding domain. (A) Concentration-dependent, monovalent nanobody binding to the receptor-binding domains of SARS-CoV and SARS-CoV-2. The nanobodies were displayed on the yeast surface and binding to the biotinylated receptor-binding domains was detected via flow cytometry. VHH-72 was originally identified against SARS-CoV and cross reacts with SARS-CoV-2. (B) Equilibrium binding constants (K_D) for nanobodies binding to the receptor-binding domains of SARS-CoV and SARS-CoV-2. In (A) and (B), the results are averages from three independent repeats and error bars are standard deviations. See STAR Methods.

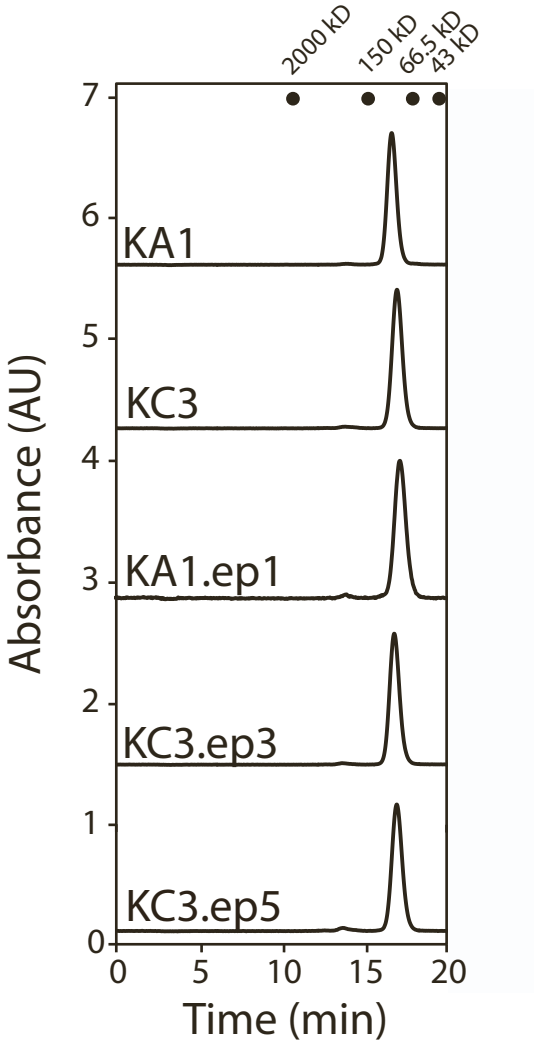

Figure S6. Size-exclusion chromatography analysis of the lead and affinity-matured nanobodies. The nanobodies were purified using Protein A and size-exclusion chromatography. The running buffer was PBS (pH 7.4) with 200 mM arginine. See STAR Methods.

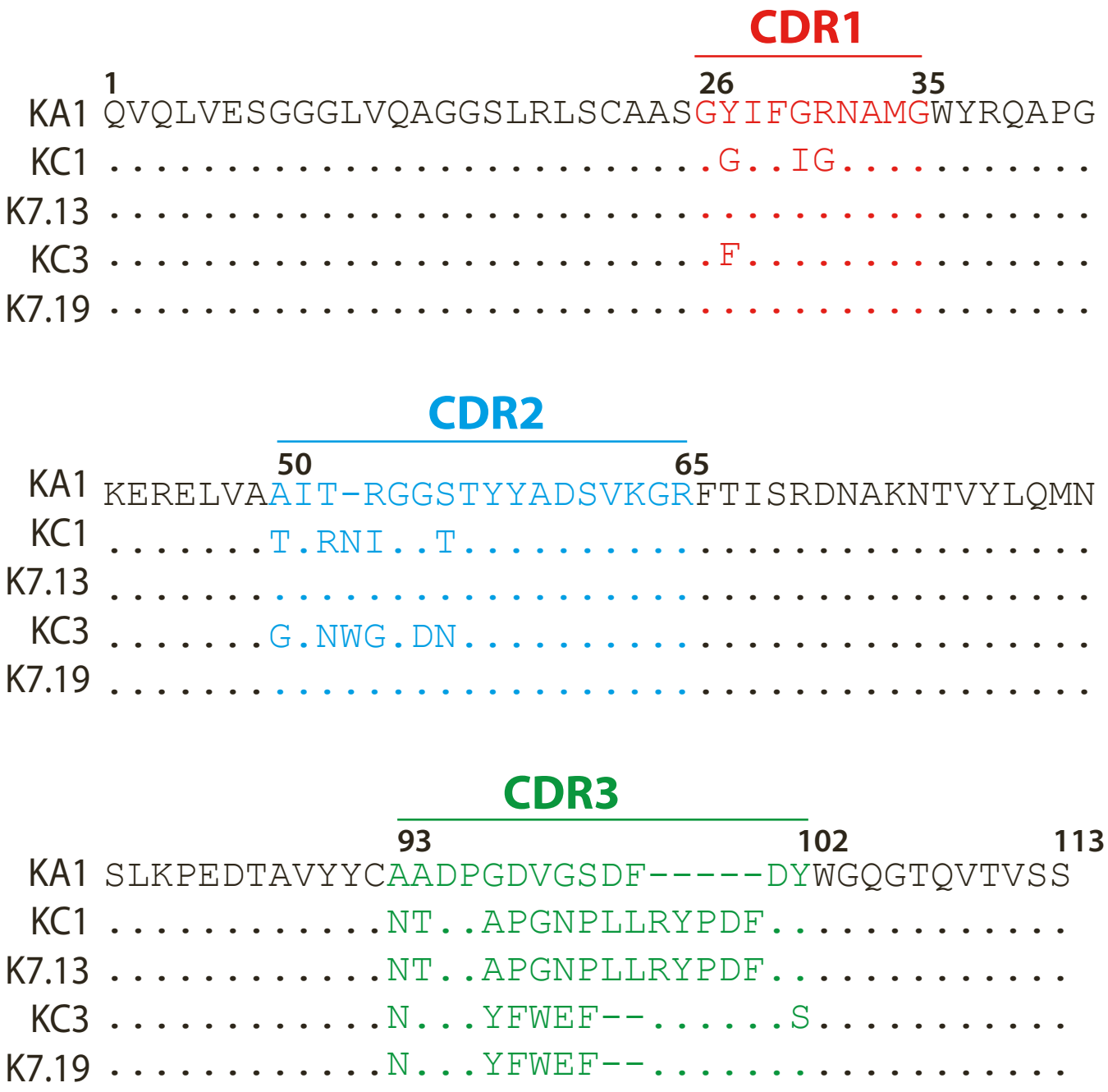

Figure S7. Amino acid sequences of the nanobodies generated via CDR-swapping mutagenesis relative to the initial non-mutagenized lead clones. The clones identified by CDR-swapping mutagenesis are K7.13, and K7.19 in addition to KA1.ep1 (not shown). The sequences are shown for Kabat numbering except CDR1 (combined Chothia and Kabat definition) and CDR3 (additional two N-terminal residues included in the CDR). See also Figure 7.