*Supplementary Material*

# Potent NTD-targeting neutralizing antibodies against SARS-CoV-2 selected from a synthetic immune system

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## Figure S1

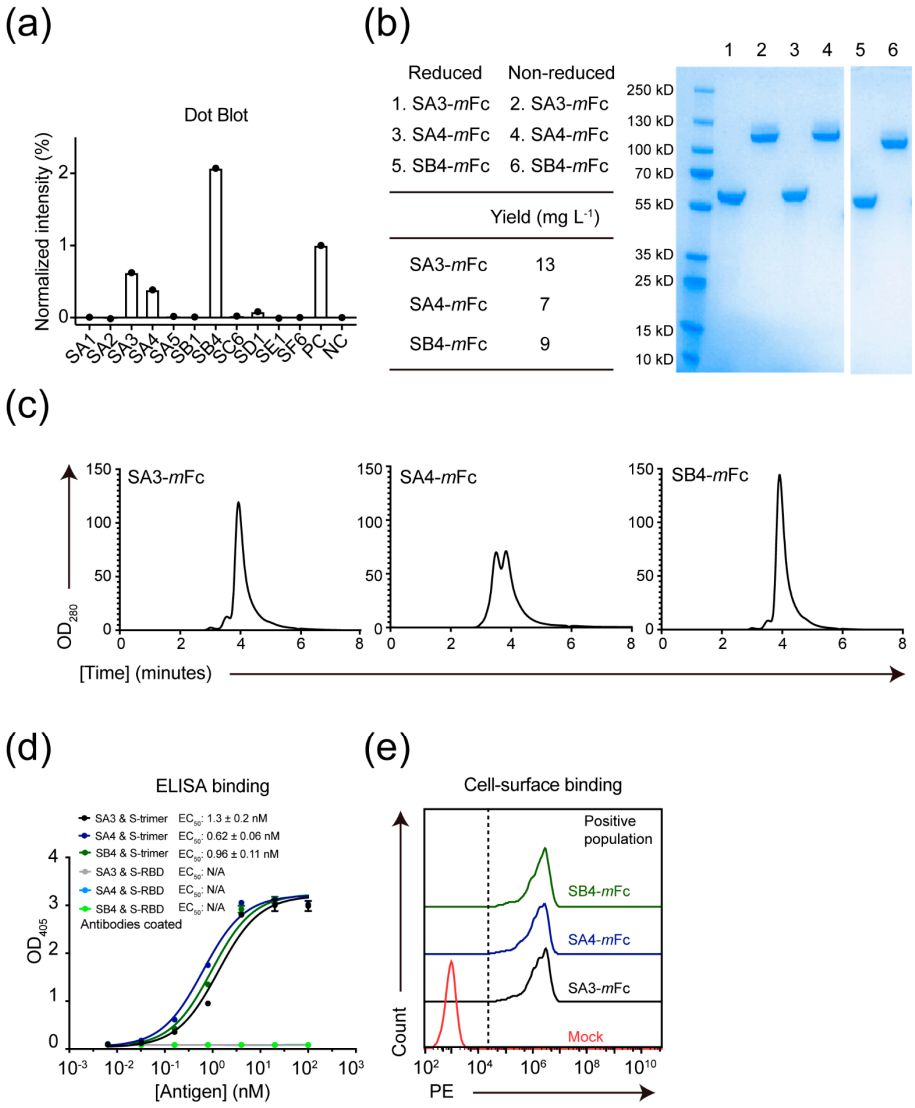



**Figure S1.** Candidate antibodies bind to spike of SARS-CoV-2 in non-RBD regions. **(a)** Dot Blot of these 11 unique antibodies expressing-supernatant were evaluated. Intensity was normalized by the signal of a purified *m*Fc-tag antibody used as a positive control. **(b)** Expression yield of purified SA3, SA4, and SB4 in scFv-*m*Fc format are shown. Three candidate antibodies in scFv-mouse Fc format were analyzed by SDS-PAGE, in non-reduced and reduced forms. **(c)** HPLC-SEC assessment of these three candidate antibodies stability. Aliquots of antibodies protein dissolved in PBS buffer (150 mM NaCl, 20 mM sodium phosphate, pH 7.4) were incubated at 4 ºC for 21 days and subjected to analysis in an Agilent Bio SEC-5, 500A HPLC system running with the same PBS buffer at a flow rate of 0.5 mL $min^{-1}$. **(d)** Binding ELISA for the three candidate antibodies against recombinant, biotinylated SARS-CoV-2 spike protein (S-trimer-His) and SARS-CoV-2 spike RBD protein (S-RBD-His) ($n$=3). $EC_{50}$ of different antibodies are shown. **(e)** HEK293T cells transfected with expression plasmid encoding the full-length spike of SARS-CoV-2 were incubated with purified three antibodies and stained with PE labelled anti-human Fc secondary antibody, then analyzed by FACS.

## Figure S2

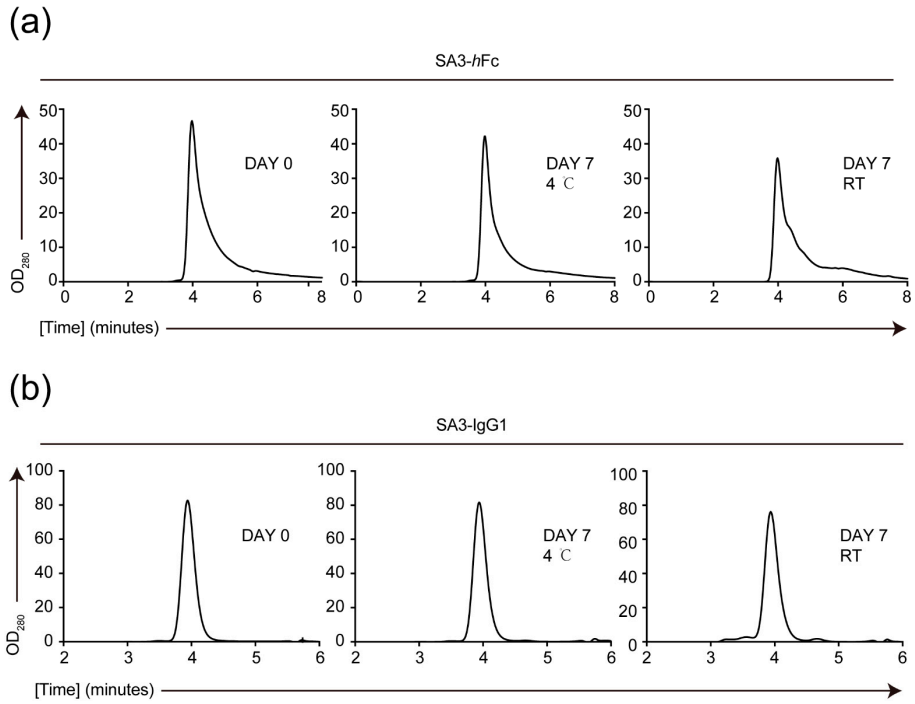
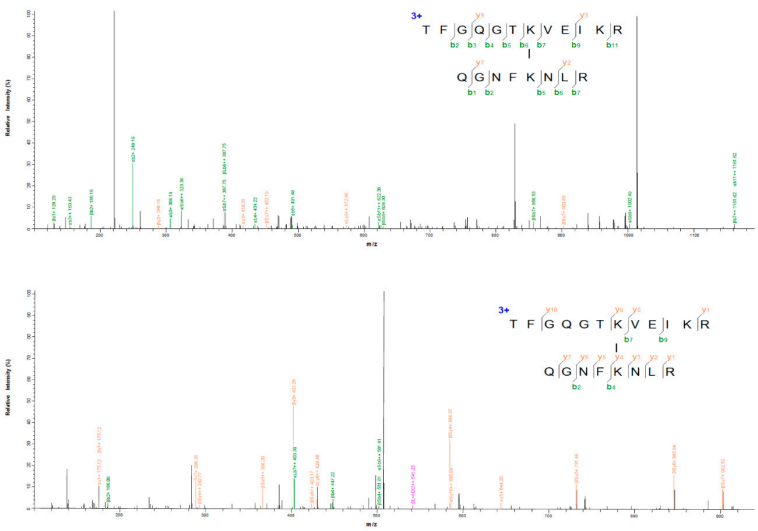



**Figure S2.** HPLC-SEC thermostability assay of SA3-*h*Fc and SA3-IgG1. HPLC-SEC assessment of **(a)** SA3-*h*Fc and **(b)** SA3-IgG1 thermal stability. Aliquots of SA3 protein dissolved in PBS buffer (150 mM NaCl, 20 mM sodium phosphate, pH 7.4) were incubated at 4 ºC or room temperature (RT) for 7 days and subjected to analysis in an Agilent Bio SEC-5, 500A HPLC system running with the same PBS buffer at a flow rate of 0.5 mL $min^{-1}$.

## Figure S3

(a)




(b)

MS³ of TFGQGTKVEIKR

| b | | | | y |
|---|---|---|---|---|
| --- | 1 | T | 12 | --- |
| 249.1234 | 2 | F | 11 | 1262.7215 |
| 306.1448 | 3 | G | 10 | 1115.6531 |
| 434.2034 | 4 | Q | 9 | 1058.6317 |
| 491.2249 | 5 | G | 8 | 930.5731 |
| 592.2726 | 6 | T | 7 | 873.5516 |
| 720.3675 | 7 | K | 6 | 772.5039 |
| 819.4359 | 8 | V | 5 | 644.409 |
| 985.67(+DSSO moiety-$NH_3$) | 9 | E | 4 | 545.3406 |
| 1061.5626 | 10 | I | 3 | 416.298 |
| 1189.6575 | 11 | K | 2 | 303.2139 |
| --- | 12 | R | 1 | 175.119 |

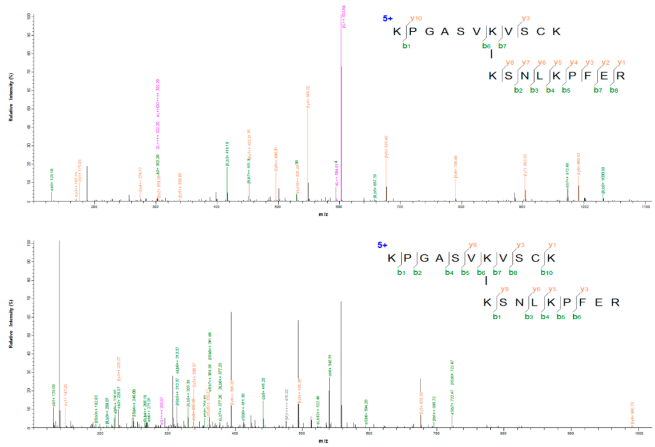
MS³ of QGNFKNLR

| b | | | | y |
|---|---|---|---|---|
| --- | 1 | Q | 8 | --- |
| 186.0873 | 2 | G | 7 | 848.4737 |
| 283.15(-$NH_3$) | 3 | N | 6 | 877.53(+DSSO moiety) |
| 447.1987 | 4 | F | 5 | 763.48(+DSSO moiety) |
| 575.2936 | 5 | K | 4 | 530.3409 |
| 689.3365 | 6 | N | 3 | 402.2459 |
| 802.4206 | 7 | L | 2 | 288.203 |
| --- | 8 | R | 1 | 175.119 |

**Figure S3.** SA3 antibody cross-linked with SARS-CoV-2 spike via TFGQGTKVEIKR and QGNFKNLR peptides. **(a)** Tertiary MS for individual peptides TFGQGTKVEIKR and QGNFKNLR. **(b)** Peptide mass fingerprint for TFGQGTKVEIKR and QGNFKNLR.

## Figure S4

(a)




(b)

MS³ of KPGASVKVSCK

| b | | | | y |
|---|---|---|---|---|
| --- | 1 | K | 11 | --- |
| 226.155 | 2 | P | 10 | 1118.61(+Carbamidomethyl+DSSO moiety) |
| 283.1765 | 3 | G | 9 | 1021.55(+Carbamidomethyl+DSSO moiety) |
| 354.2136 | 4 | A | 8 | 821.455 |
| 441.2456 | 5 | S | 7 | 750.4178 |
| 540.314 | 6 | V | 6 | 663.3858 |
| 722.47(+DSSO moiety) | 7 | K | 5 | 675.46(+Carbamidomethyl+DSSO moiety) |
| 767.4774 | 8 | V | 4 | 493.35(Carbamidomethyl) |
| 854.5094 | 9 | S | 3 | 394.15(+Carbamidomethyl) |
| 957.5186 | 10 | C | 2 | 307.2(+Carbamidomethyl) |
| --- | 11 | K | 1 | 147.1128 |

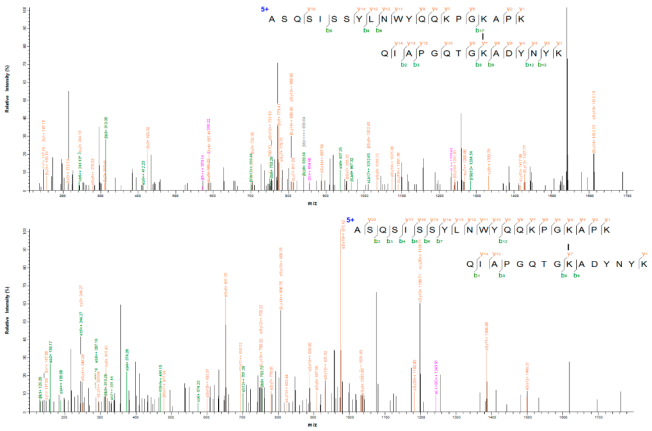
MS³ of KSNLKPFER

| b | | | | y |
|---|---|---|---|---|
| --- | 1 | K | 9 | --- |
| 270.18(+DSSO moiety) | 2 | S | 8 | 990.5367 |
| 330.1772(+DSSO moiety) | 3 | N | 7 | 903.5047 |
| 529.38(+DSSO moiety) | 4 | L | 6 | 789.4618 |
| 571.3562 | 5 | K | 5 | 676.3777 |
| 668.409 | 6 | P | 4 | 548.2827 |
| 815.4774 | 7 | F | 3 | 451.23 |
| 944.52 | 8 | E | 2 | 304.1615 |
| --- | 9 | R | 1 | 175.119 |

**Figure S4.** SA3 antibody cross-linked with SARS-CoV-2 spike via KPGASVKVSCK and KSNLKPFER peptides. **(a)** Tertiary MS for individual peptides KPGASVKVSCK and KSNLKPFER. **(b)** Peptide mass fingerprint for KPGASVKVSCK and KSNLKPFER.

## Figure S5

(a)



(b)

MS³ of ASQSISSYLNWYQQKPGKAPK

| b | | | | y |
|---|---|---|---|---|
| --- | 1 | A | 21 | --- |
| 159.0764 | 2 | S | 20 | 2310.1877(+DSSO moiety) |
| 287.135 | 3 | Q | 19 | 2223.1557 |
| 356.23(-$H_2O$) | 4 | S | 18 | 2095.0971 |
| 487.2511 | 5 | I | 17 | 2008.0651(+DSSO moiety) |
| 574.2831 | 6 | S | 16 | 1894.981(+DSSO moiety) |
| 661.3151 | 7 | S | 15 | 1807.949 |
| 824.3785 | 8 | Y | 14 | 1720.917 |
| 937.4625 | 9 | L | 13 | 1557.8536 |
| 1051.5055 | 10 | N | 12 | 1482.0(+DSSO moiety-$NH_3$) |
| 1237.5848 | 11 | W | 11 | 1330.7266 |
| 1400.6481 | 12 | Y | 10 | 1198.71(+DSSO moiety) |
| 1528.7067 | 13 | Q | 9 | 981.584 |
| 1656.7653 | 14 | Q | 8 | 853.5254 |
| 1784.8602 | 15 | K | 7 | 725.4668 |
| 1881.913 | 16 | P | 6 | 597.3719(+DSSO moiety) |
| 1938.9345 | 17 | G | 5 | 500.3191 |
| 2067.0294 | 18 | K | 4 | 443.2976 |
| 2138.0665 | 19 | A | 3 | 315.2027 |
| 2235.1193 | 20 | P | 2 | 244.1656 |
| --- | 21 | K | 1 | 147.1128 |

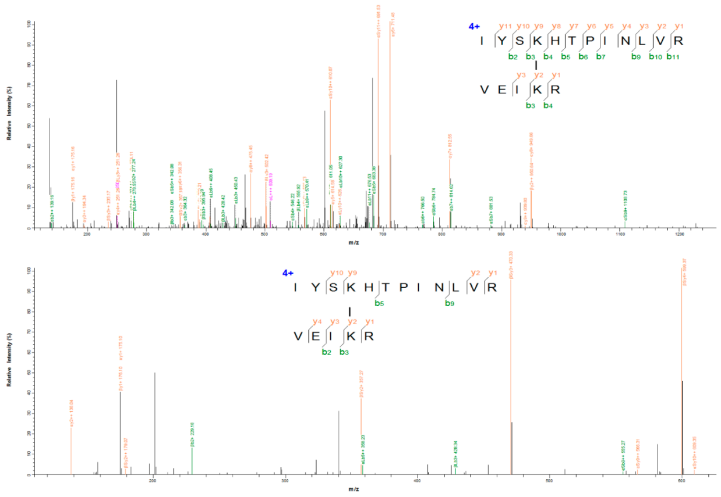
MS³ of QIAPGQTGKIADYNYK

| b | | | | y |
|---|---|---|---|---|
| --- | 1 | Q | 16 | --- |
| 242.1499 | 2 | I | 15 | 1638.8486 |
| 313.187 | 3 | A | 14 | 1612.13(+DSSO moiety) |
| 410.2398 | 4 | P | 13 | 1540.88(+DSSO moiety) |
| 467.2613 | 5 | G | 12 | 1443.79(+DSSO moiety) |
| 595.3198 | 6 | Q | 11 | 1386.66(+DSSO moiety) |
| 696.3675 | 7 | T | 10 | 1258.82(+DSSO moiety) |
| 753.389 | 8 | G | 9 | 1071.5469(+DSSO moiety) |
| 881.4839 | 9 | K | 8 | 1014.5255 |
| 994.568 | 10 | I | 7 | 886.4305 |
| 1065.6051 | 11 | A | 6 | 773.3464 |
| 1180.6321 | 12 | D | 5 | 702.3093 |
| 1343.6954 | 13 | Y | 4 | 587.2824 |
| 1543.51(+DSSO moiety) | 14 | N | 3 | 424.2191 |
| 1689.19(+DSSO moiety-$H_2O$) | 15 | Y | 2 | 310.1761 |
| --- | 16 | K | 1 | 147.1128 |

**Figure S5.** SA3 antibody cross-linked with SARS-CoV-2 spike via ASQSISSYLNWYQQKPGKAPK and QIAPGQTGKIADYNYK peptides. **(a)** Tertiary MS for individual peptides ASQSISSYLNWYQQKPGKAPK and QIAPGQTGKIADYNYK. **(b)** Peptide mass fingerprint for ASQSISSYLNWYQQKPGKAPK and QIAPGQTGKIADYNYK.

## Figure S6

(a)




(b)

MS³ of IYSKHTPINLVR

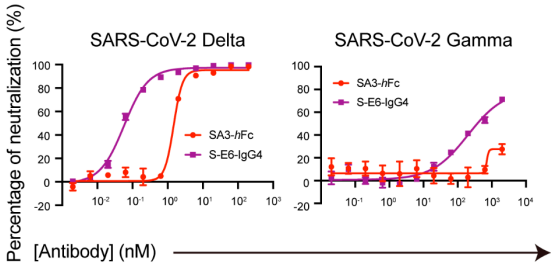
| b | | | | y |
|---|---|---|---|---|
| --- | 1 | I | 12 | --- |
| 277.1547 | 2 | Y | 11 | 682.55(charge2,+DSSO moiety-$H_2O$) |
| 364.1867 | 3 | S | 10 | 1250.83(+DSSO moiety) |
| 492.2817 | 4 | K | 9 | 1077.6527 |
| 629.3406 | 5 | H | 8 | 949.5578 |
| 730.3883 | 6 | T | 7 | 812.4989 |
| 827.441 | 7 | P | 6 | 711.4512 |
| 940.5251 | 8 | I | 5 | 614.3984 |
| 1054.568 | 9 | N | 4 | 501.3144 |
| 1167.6521 | 10 | L | 3 | 387.2714 |
| 1266.7205 | 11 | V | 2 | 274.1874 |
| --- | 12 | R | 1 | 175.119 |

MS³ of VEIKR

| b | | | | y |
|---|---|---|---|---|
| --- | 1 | V | 5 | --- |
| 229.1183 | 2 | E | 4 | 599.37(+DSSO moiety) |
| 342.2023 | 3 | I | 3 | 470.33(+DSSO moiety) |
| 470.2973 | 4 | K | 2 | 357.27(+DSSO moiety) |
| --- | 5 | R | 1 | 175.119 |

**Figure S6.** SA3 antibody cross-linked with SARS-CoV-2 spike via IYSKHTPINLVR and VEIKR peptides. **(a)** Tertiary MS for individual peptides IYSKHTPINLVR and VEIKR. **(b)** Peptide mass fingerprint for IYSKHTPINLVR and VEIKR.

## Figure S7




| $NT_{50}$ (nM) | Delta | Gamma |
|---|---|---|
| SA3-*h*Fc | 1.5 ± 0.2 | > 500 |
| S-E6-IgG4 | 0.058 ± 0.003 | > 100 |

**Figure S7.** Neutralization of SA3-hFc against Delta and Gamma pseudovirus (PSV) strains. Dose-dependent neutralization of SA3-*h*Fc against SARS-CoV-2 Delta and Gamma PSVs were performed in triplicates (*n*=3). The apparent $NT_{50}$ values are determined by Hill analyses using one-way ANOVA of GraphPad Prism.

## Figure S8

(a)

```
IGHV1-18   CDR-H1   CDR-H2                    CDR-H3
SA3        DYGIS    WVSTYNVDTNYAQKFQG   --------DEVGSDWLGR-----Y 11
COV2-2034  TYGIS    WISAYNGNSNYGKKFQG   DLPIKVVVPAADYNWFDP------ 18
CV42       SYGIS    WISAYNGNTNYAQKVQG   DRGYAATFGVFDY----------- 13
C1636      SYGIS    WISAHNGNTNYAQKFQG   -------FLALTYYYDSSGYPFDY 17
C1736      SYGIS    WISGYDGNTNYAQKVQG   DHRTVTTFGTFDY----------- 13
C1771      NYGIS    WSSVDNGHTNYAQKFQA   DHRTVVTFGTFDY----------- 13
R616-1D2   NYGLS    WISGNNGNTNYAQKFQG   DQSTVVTFGTFDY----------- 13
            **:*    * *  : .:**.:*.*.
```

(b)

```
IGKV1-39     CDR-L1       CDR-L2      CDR-L3
SA3          RASQSISSYLN  AASSLQS     QQSYSTPRT--   9
COV2-2034    RASQSISSYLN  AASSLQS     QQSYSTPPT--   9
CV42         RASQSISSYLN  AASSLQS     QQTYITAFT--   9
C1636        RASQSISSYLN  AASSLQS     QQSYSTPRMYT   11
C1736        RASQSIGYYLN  AAASLQS     QQSYSTPFT--   9
C1771        RASHNINTFLN  APSILQS     QQTYRTPFT--   9
R616-1D2     RASQNINRYLN  AASGVQS     QQSYITPFT--   9
             ***:.*. :**  * : :**     **:* *
```

**Figure S8.** Amino acid sequence analysis of SA3 antibody. **(a)** Multiple sequence alignment of heavy chain between SA3 and IGHV1-18/IGKV1-39 antibodies against the non-RBD spike of SARS-CoV-2. Antibody sequences were obtained from the CoV-AbDab database of the University of Oxford and aligned using CLUSTAL O (1.2.4) multiple sequence alignment. **(b)** Multiple sequence alignment of light chain between SA3 and IGHV1-18/IGKV1-39 antibodies against the non-RBD spike of SARS-CoV-2. Antibody sequences were obtained from the CoV-AbDab database of the University of Oxford and aligned using CLUSTAL O (1.2.4) multiple sequence alignment. Antibody complementarity determining regions (CDRs) were analyzed by abYsis.

## Table S1

**Table S1.** The germline information and somatic hypermutations numbers (mutations) of heavy chain or light chain of the selected clones.

| Clone | HC Germline | HC Mutations | LC Chain | LC Germline | LC Mutations |
|---|---|---|---|---|---|
| SA1 | 5-51 | 13 | Kappa | 3-20 | 5 |
| SA2 | 3-30 | 7 | Lambda | 6-57 | 8 |
| SA3 | 1-18 | 11 | Kappa | 1-39 | 0 |
| SA4 | 1-18 | 11 | Kappa | 1-39 | 4 |
| SA5 | 1-69 | 12 | Lambda | 5-45 | 5 |
| SB1 | 3-30 | 7 | Lambda | 6-57 | 8 |
| SB4 | 1-18 | 11 | Kappa | 1-39 | 6 |
| SC6 | 1-18 | 11 | Kappa | 3-20 | 2 |
| SD1 | 3-30 | 6 | Lambda | 5-45 | 6 |
| SE1 | 3-30 | 11 | Lambda | 1-47 | 14 |
| SF6 | 3-30 | 7 | Lambda | 6-57 | 8 |

Antibody sequences were analyzed by IMGT/V-QUEST database.