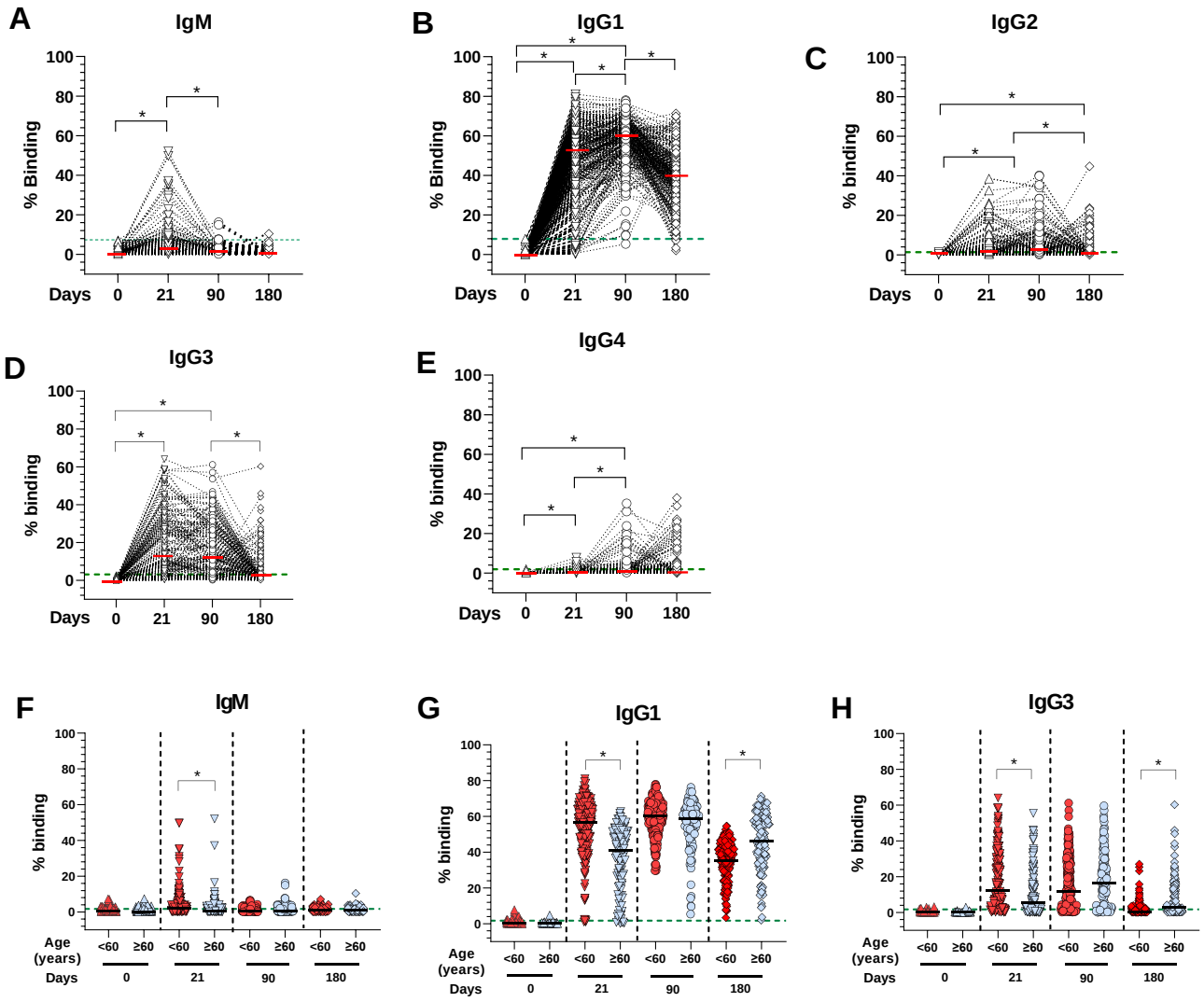
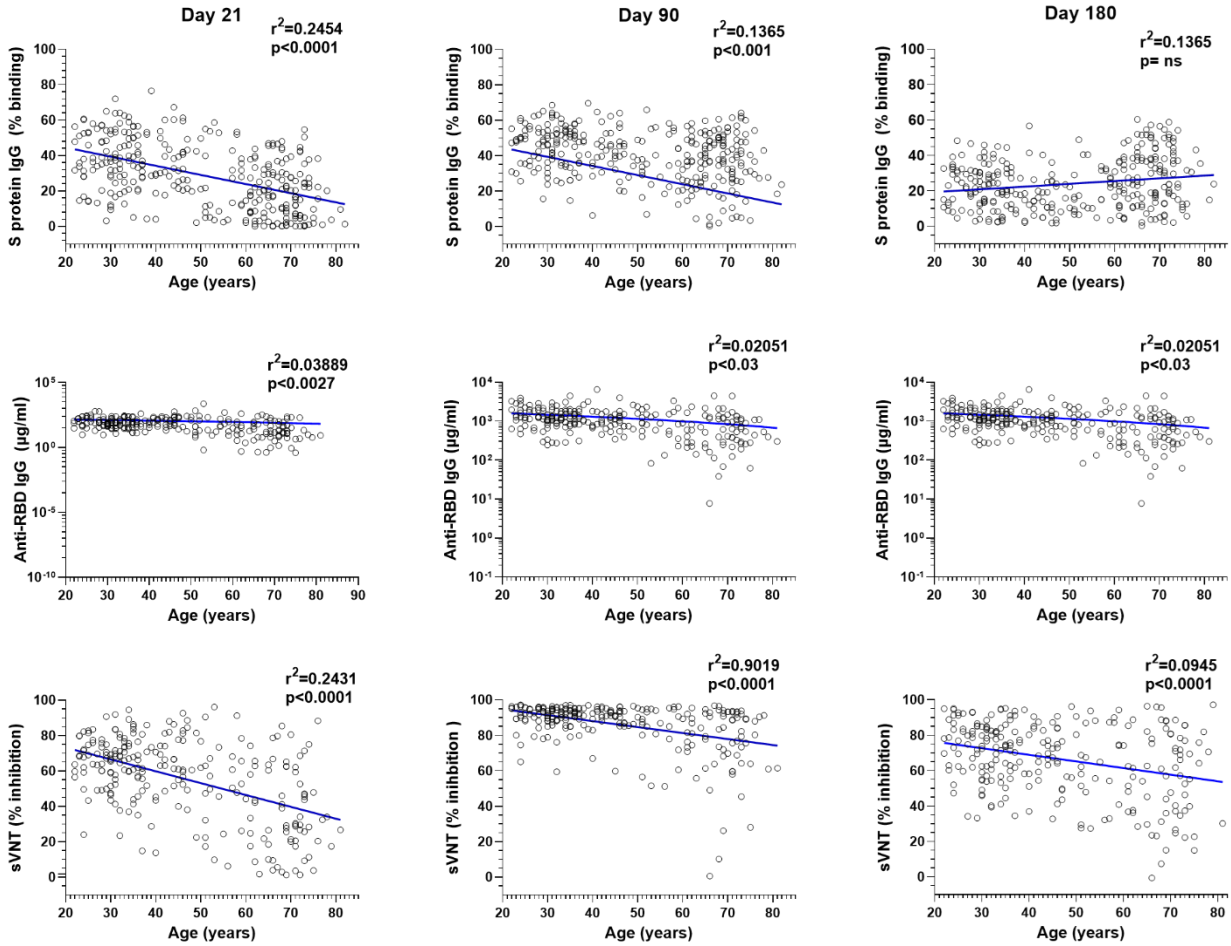
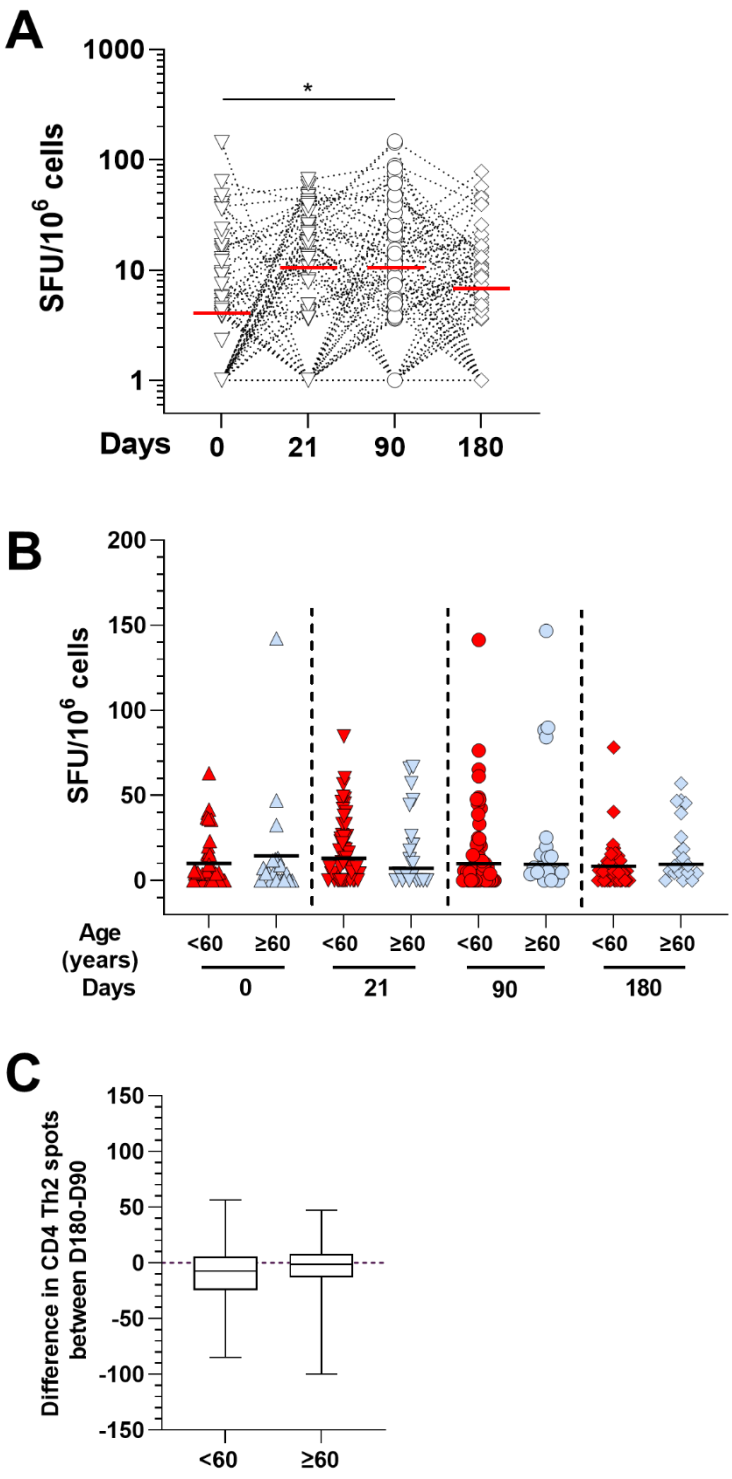
# Lower vaccine-acquired immunity in the elderly population following two-dose BNT162b2 vaccination is alleviated by a third vaccine dose



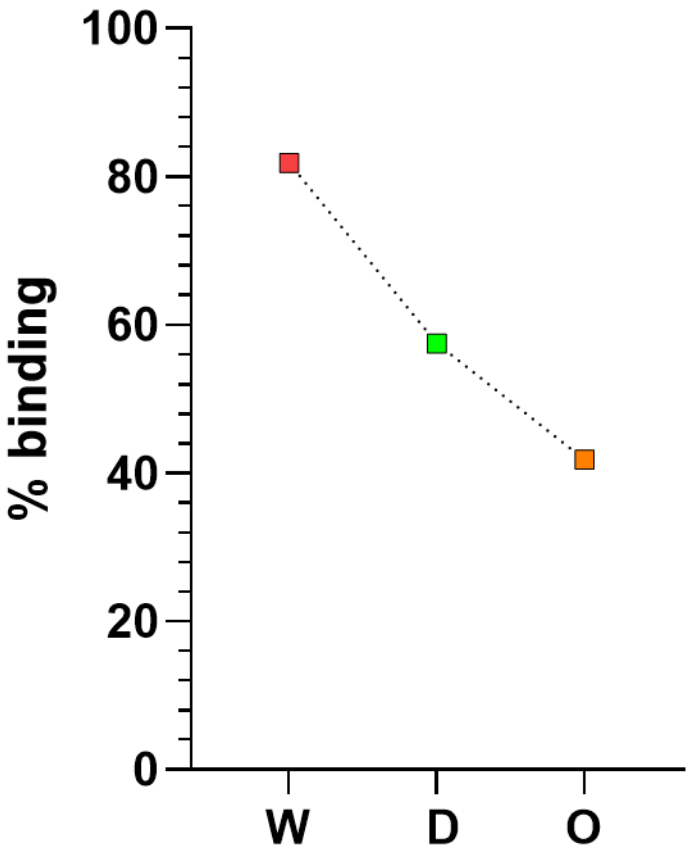

**Supplementary Figure 1. Anti-SARS-CoV-2 spike protein antibody isotype response after vaccination.** A flow cytometry-based assay using the full Spike protein (SFB) assay was used to determine antibody isotype in the same 312 vaccinated individuals described in Figure 1. (A) IgM. Median of group values at day 0, 21, 90 and 180: was 0.4, 1.04, 0.59 and 0.64%, respectively. Antibody levels below the maximum range (7.385) were considered baseline values . *, $p < 0.001$, Friedman test; (B) IgG1. Median of group values at day 0, 21, 90 and 180: was 0.12, 51.8, 60 and 39.7%, respectively. Antibody levels below the maximum range (7.893) were considered baseline values. *, $p < 0.001$, Friedman test; (C) IgG2. Median of values at day 0, 21, 90 and 180: was 0.37, 0.52, 1.3 and 0.3, respectively. Antibody levels below the maximum range (1.44) were considered baseline values. *, $p < 0.001$, Friedman test; (D) IgG3. Median of values at day 0, 21, 90 and 180: was 0.08, 12.53, 11.8 and 1, respectively. Antibody levels below the maximum range (3.19) were considered baseline values. *, $p < 0.001$, Friedman test; (E) IgG4. Median of values at day 0, 90 and 180: was 0.03, 0.06, 0.38 and 0.35, respectively. Antibody levels below the maximum range (1.953) were considered baseline values. *, $p < 0.001$, Friedman test. Median of group values are indicated by a red bar. (F) IgM, (G) IgG1 and (H) IgG3 antibody levels comparison between the analyzed age groups: <60 (n=186) and ≥60 (n=144), at different time points (day 0, 21 and 90). The Median of group values are represented as a black line through the dots. The red lines or boxes represent the maximum range of the samples. *, $p < 0.001$, Mann Whitney test.



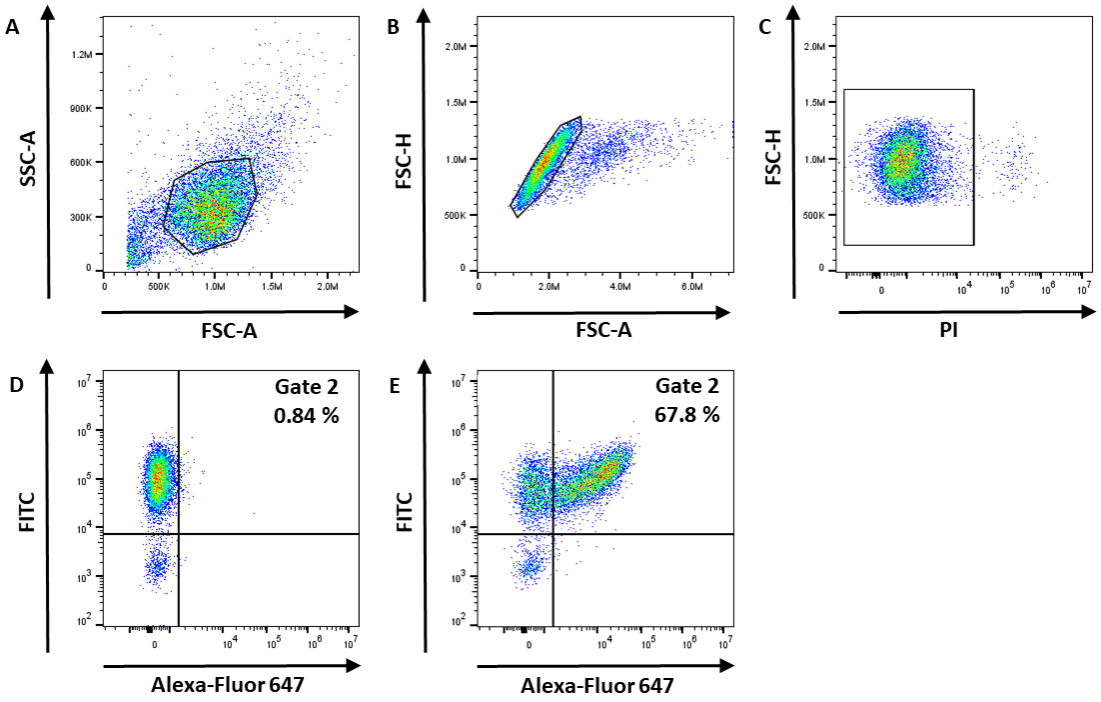

**Supplementary Figure 2. Linear correlations between participant's age and SARS-CoV-2 specific antibody titer measured at different time after vaccination.** Correlation was analyzed by simple linear regression at day 21, 90 and 180 for the Flow cytometry-based assay for the S-pike protein (SFB) (Higher panels); the commercial RBD Roche S assay (mid panels) ; and the sVNT assay (Lower panels).




**Supplementary Figure 3. CD4 Th2 responses.** (A) Kinetics of Spike-protein-specific Th2 cells overtime in vaccinees. CD4 Th2 cells were assayed on a subset of vaccinees \on a subset of vaccinees (n =71) by IL4/IL5/IL13 ELISPOT using 15 mer pool peptides. Data are presented are spot forming units (SFU) per million of PBMC from paired samples from vaccinated individuals at 4 time points. Each data point represents the normalized mean spot count from duplicate wells for one study participant, after subtraction of the medium-only control . The median values of each group are represented by a red line and were at day 0, 21, 90 and 180: 4.1, 10.4, 10 and 7.4 SFU for CD4 Th2 T cells. *, $p < 0.01$, (B) Comparison between the analyzed age groups at day 0, 21 , 90 and 180, (n=83), <60: (n= 51) and ≥60 (n=20). Median values are indicated by a dark line. *, $p < 0.01$Mann Whitney test. *, $p < 0.01$, Mann Whitney test. (C) Box plots (with whiskers) showing difference in CD4 Th2 cell responses measure in the different assays between days 180 and 90 for paired samples for both age groups, <60: (n= 51), and ≥60 (n=20). $p = 0.003$, Mann Whitney test.




**Supplementary Figure 4**. Detection of expression the Spike protein from different strains of SARS-CoV-2 on the surface of HEK203 transduced cells. The flow cytometry-based assay using the full Spike protein (SFB) assay was used as described in Figure 1. The serum used to detect S protein expression of different SARS-CoV-2 (W, Wuhan; D, Delta; and O, Omicron (BA.1) strains) was from an individual who recovered from a previous infection and then was vaccinated with two doses of the BNT162b2 vaccine and taken at 90 days postsecond doses.




**Supplementary Figure 5. Representative gating strategy for SFB assay to determine spike-specific antibody response.** Cells were gated on: (A) FSC-A/SSC-A to exclude cell debris, (B) FSC-A/FSC-H to select for single cells, (C) FSC-H/PI to select for live cells (PI-negative population), (D, E) FITC/Alexa Fluor 647. Binding is determined by the percentage of GFP-positive S protein-expressing cells that are bound by specific antibody, indicated by the events that are Alexa Fluor 647- and FITC-positive (Gate 2). (D) Healthy control individual plasma, 1:100 diluted; (E) COVID-19 patient plasma, 1:100 diluted

**Supplementary Table 1. Characteristics of the study population**

| Characteristics | <60 years | ≥60 years | Total cohort number (%) |
|---|---|---|---|
| **Total** | 178 (57.1) | 134 (42.9) | 312 |
| **Gender** | | | |
| Male (%) | 51 (28.3) | 79 (61.7) | 130 (41.7) |
| Female (%) | 127 (69.7) | 55 (30.3) | 182 (58.3) |
| **Ethnicity** | | | |
| Chinese (%) | 106 (46.9) | 120 (53.1) | 226 (72.4) |
| Indians (%) | 14 (60.9) | 9 (39.1) | 23 (7.6) |
| Malays (%) | 26 (89.7) | 3 (10.3) | 29 (8.8) |
| Others (%) | 32 (94.1) | 2 (5.9) | 34 (11.2) |
| Median years (minimum-maximum) | 35 (22-59) | 69 (60-82) | 50.85 (22-82) |

**Supplementary Table 2.** Immune reactivity across timepoints

| Assays | Proportion of volunteers with response above individual baseline * (%) | | |
|---|---|---|---|
| | At day 21 | At day 90 | At day 180 |
| Anti-spike protein IgG (SFB) | 306/312 (98) | 311/312 (99) | 311/312 (99) |
| Anti-RBD IgG (Roche S) | 310/312 (99.3) | 312/312 (100) | 312/312 (100) |
| sVNT | 247/312 (79.1) | 309/312 (99) | 293/312 (93.9) |
| B cell Elispot | 17/35 (48.6) | 28/35 (80) | 30/35 (85.7) |
| IL-2 T cells | 153/155 (98.7) | 155/155 (100) | 154/155 (99.3) |
| IFNγ T cells | 144/155 (92.9) | 149/155 (96.1) | 153/155 (98.7) |
| CD8 T cell Elispot | 39/72 (54.2) | 54/72 (75) | 29/72 (40.3) |
| CD4 Th1 Elispot | 54/78 (69.2) | 66/78 (84.6) | 65/78 (83.3) |
| CD4 Th2 Elispot | 42/71 (59) | 42/71 (59) | 35/71 (49) |

* Paired samples

**Supplementary Table 3.** Long-term immune responses in vaccinated individuals

| Assays | Proportion of individuals with a lower response at day 180 compared with day 90 (%)* | | | |
|---|---|---|---|---|
| | Total population | < 60 years | ≥60 years | *P value*** |
| Anti-spike protein IgG (SFB) | 243/312 (78) | 176/178 (98.9) | 66/134 (49.2) | 0.0001 |
| Anti-RBD IgG (Roche S) | 243/312 (77.8) | 153/178 (85.9) | 89/134 (66.4) | 0.0001 |
| sVNT | 243/312 (77.5) | 119/178 (66.8) | 123/134 (91.8) | NS |
| B cell Elispot | 10/34 (29.4) | 3/16 (18.75) | 7/18 (52.9) | NS |
| IL-2 T cells | 97/155 (62.5) | 56/81 (69.1) | 40/74 (54) | NS |
| IFNγ T cells | 72/155 (46.5) | 49/81 (60.4) | 24/74 (32) | 0.0003 |
| CD8 T cell Elispot | 45/72 (67.9) | 31/45 (68.9) | 13/27 (48.1) | NS |
| CD4 Th1 Elispot | 37/79 (46.8) | 21/51 (41.2) | 16/28 (57.1) | NS |
| CD4 Th2 Elispot | 35/71 (50.7) | 30/51 (58.2) | 11/20 (55) | NS |

* Paired samples

** Difference between age groups

**Supplementary Table 4**. Low responders as determined by the SFB assay

| Days | 21 | | 90 | | 180 | |
|---|---|---|---|---|---|---|
| Median antibody response (% binding) | 28.3% | | 41.98% | | 23.35% | |
| Proportion of low responders* (n / tested number) | - | | 37.2 (116/312) | | 22.2 (88/312) | |
| | | | | | | |
| Age (Years) | N | % of total cohort | N | % of total cohort | N | % of total cohort |
| <60 | 57 | 18.2 | 42 | 13.5 | 40 | 12.8 |
| >60 | 99 | 31.7 | 74 | 23.7 | 48 | 13.5 |

* Low responders are defined as fully vaccinated individuals with antibody response below cohort's median response at consecutive timepoints (%). For example, the low responders, at day 90, have antibody response below cohort's median response at both day 21, 90 and 180.

**Supplementary Table 5**. Low responders as determined by Roche S

| Days | 21 | | 90 | | 180 | |
|---|---|---|---|---|---|---|
| Median antibody response (U/ml) | 50.28 | | 927 | | 677 | |
| Proportion of low responders* (n/tested number) | - | | 36.5 (114/312) | | 31.4 (98/312) | |
| Age (Years) | N | % of total cohort | N | % of total cohort | N | % of total cohort |
| <60 | 60 | 19.2 | 32 | 10.25 | 26 | 8.3 |
| >60 | 99 | 31.7 | 82 | 26.2 | 69 | 22 |

* These values were determined as in Supplementary table 4.

**Supplementary Table 6.** Low responders as determined by sVNT

| Days | 21 | | 90 | | 180 | |
|---|---|---|---|---|---|---|
| Median inhibition (%l) | 56.39 | | 89.87 | | 67.4 | |
| Proportion of low responders* (n/tested number) | - | | 39.1 (122/312) | | 34.9 (109/312) | |
| Age (Years) | N | % of total cohort | N | % of total cohort3 | N | % of total cohort |
| <60 | 53 | 16.9 | 38 | 12.18 | 35 | 11.2 |
| >60 | 104 | 33 | 84 | 26.9 | 74 | 23.7 |

* These values were determined as in Supplementary table 4.

**Supplementary Table 7.** Low responders as determined by measurement of IL-2 production by T cells

| Days | 21 | | 90 | | 180 | |
|---|---|---|---|---|---|---|
| Median antibody response (pg/ml) | 54.48 | | 89.39 | | 78.06 | |
| Proportion of low responders* (n / tested number) | - | | 29.7 (46/155) | | 25.2 (39/155) | |
| Age (Years) | N | % of total cohort | N | % of total cohort | N | % of total cohort |
| <60 | 40 | 25.8 | 25 | 16.1 | 21 | 13.5 |
| >60 | 37 | 23.9 | 21 | 13.5 | 18 | 11.6 |

* These values were determined as in Supplementary table 4.

**Supplementary Table 8.** Low responders as determined by measurement of IFN-γ production by T cells

| Days | 21 | | 90 | | 180 | |
|---|---|---|---|---|---|---|
| Median antibody response (U/ml) | 11.69 | | 33.81 | | 32.43 | |
| Proportion of low responders* (n / tested number) | - | | 32.2 (50/155) | | 26.5 (41/155) | |
| Age (Years) | N | % of total cohort | N | % of total cohort | N | % of total cohort |
| <60 | 33 | 21.2 | 22 | 14.2 | 20 | 12.9 |
| >60 | 45 | 29 | 28 | 18 | 21 | 13.5 |

* These values were determined as in Supplementary table 4. The median values were at day 21: 11.69 pg/ml, at day 90: 33.81 pg/ml and day 180: 32.43 pg/ml).

**Supplementary Table 9.** Low responders as determined by CD4 Th1 ELISPOT

| Days | 21 | | 90 | | 180 | |
|---|---|---|---|---|---|---|
| Median antibody response (Spot forming units) | 53.48 | | 89.39 | | 78.06 | |
| Proportion of low responders* (n / tested number) | - | | 19.2 (15/78) | | 6.4 (5/78) | |
| Age (Years) | N | % of total cohort | N | % of total cohort | N | % of total cohort |
| <60 | 22 | 28.2 | 8 | 10.2 | 1 | 1.2 |
| >60 | 13 | 16.7 | 7 | 9 | 4 | 6.4 |

* These values were determined as in Supplementary table 4.

**Supplementary Table 10.** Low responders as determined by CD8 ELISPOT

| Days | 21 | | 90 | | 180 | |
|---|---|---|---|---|---|---|
| Median antibody response (Spot forming units) | 44.85 | | 71.65 | | 180.42 | |
| Proportion of low responders* (n / tested number) | - | | 36 (26/72) | | 23.6 (17/72) | |
| Age (Years) | N | % of total cohort | N | % of total cohort | N | % of total cohort |
| <60 | 25 | 42.3 | 18 | 11.5 | 13 | 1.2 |
| >60 | 11 | 57.7 | 8 | 7.7 | 4 | 6.4 |

* These values were determined as in Table S4. The median values were at day 21: 44.85 SFU, at day 90: 71.65 SFU and day 180: 42.1 SFU).

**Supplementary Table 11**: Spike protein peptide pool utilized in whole blood cytokine release assay

| Spike peptide pool | | | |
|---|---|---|---|
| Amino Acid Sequence | Amino Acid Position | Amino Acid Sequence | Amino Acid Position |
| IRGWIFGTTLDSKTQ | 101-115 | SIIAYTMSLGAENSV | 691-705 |
| FGTTLDSKTQSLLIV | 106-120 | TMSLGAENSVAYSNN | 696-710 |
| CTFEYVSQPFLMDLE | 166-180 | STECSNLLLQYGSFC | 746-760 |
| VSQPFLMDLEGKQGN | 171-185 | NLLLQYGSFCTQLNR | 751-765 |
| TRFQTLLALHRSYLT | 236-250 | KNTQEVFAQVKQIYK | 776-790 |
| LLALHRSYLTPGDSS | 241-255 | VFAQVKQIYKTPPIK | 781-795 |
| RSYLTPGDSSSGWTA | 246-260 | KQIYKTPPIKDFGGF | 786-800 |
| CALDPLSETKCTLKS | 291-305 | TPPIKDFGGFNFSQI | 791-805 |
| LSETKCTLKSFTVEK | 296-310 | NFSQILPDPSKPSKR | 801-815 |
| CTLKSFTVEKGIYQT | 301-315 | AGFIKQYGDCLGDIA | 831-845 |
| FTVEKGIYQTSNFRV | 306-320 | QYGDCLGDIAARDLI | 836-850 |
| GIYQTSNFRVQPTES | 311-325 | GAALQIPFAMQMAYR | 891-905 |
| SASFSTFKCYGVSPT | 371-385 | QMAYRFNGIGVTQNV | 901-915 |
| TFKCYGVSPTKLNDL | 376-390 | FNGIGVTQNVLYENQ | 906-920 |
| YNYKLPDDFTGCVIA | 421-435 | DSLSSTASALGKLQD | 936-950 |
| WNSNNLDSKVGGNYN | 436-450 | TASALGKLQDVVNQN | 941-955 |
| LDSKVGGNYNYLYRL | 441-455 | AQALNTLVKQLSSNF | 956-970 |
| GGNYNYLYRLFRKSN | 446-460 | VLNDILSRLDKVEAE | 976-990 |
| YLYRLFRKSNLKPFE | 451-465 | LITGRLQSLQTYVTQ | 996-1010 |
| FRKSNLKPFERDIST | 456-470 | QLIRAAEIRASANLA | 1011-1025 |
| LKPFERDISTEIYQA | 461-475 | AEIRASANLAATKMS | 1016-1030 |
| GPKKSTNLVKNKCVN | 526-540 | APHGVVFLHVTYVPA | 1056-1070 |
| TNLVKNKCVNFNFNG | 531-545 | HWFVTQRNFYEPQII | 1101-1115 |
| FNFNGLTGTGVLTES | 541-555 | KEIDRLNEVAKNLNE | 1181-1195 |
| LTGTGVLTESNKKFL | 546-560 | LNEVAKNLNESLIDL | 1186-1200 |
| RAGCLIGAEHVNNSY | 646-660 | KNLNESLIDLQELGK | 1191-1205 |
| IGAEHVNNSYECDIP | 651-665 | IWLGFIAGLIAIVMV | 1216-1230 |
| SVASQSIIAYTMSLG | 686-700 | | |

**Supplementary Table 12.** Spike peptides (n=211) for CD8 ELISPOT

| | | | |
|---|---|---|---|
| LTDEMIAQY | GQTGKIADY | NIDGYFKIY | SVYAWNRKR |
| VRFPNITNL | YRLFRKSNL | HVTYVPAQEK | GLTVLPPLL |
| VRFPNITNL | VRDPQTLEI | YYVGYLQPRTF | IYQTSNFRV |
| RLFRKSNLK | VYSTGSNVF | RFDNPVLPF | KFLPFQQF |
| VRFPNITNL | WTAGAAAYY | YLQPRTFLL | SLIDLQELGK |
| VYDPLQPEL | GVYYHKNNK | TRFASVYAW | KWPWYIWLGF |
| YLQPRTFLL | VRDPQTLEI | LVRDLPQGF | TRFASVYAW |
| LLTDEMIAQY | HLMSFPQSA | IVRFPNITNL | SGWTAGAAAYY |
| VLKGVKLHY | YYHKNNKSW | YLQPRTFLL | VFKNIDGYF |
| VLNDILSRL | RLQSLQTYV | NSFTRGVYY | ILPDPSKPSK |
| VYSTGSNVF | FRVQPTESI | FRKSNLKPF | QRNFYEPQI |
| IRGDEVRQI | RFPNITNLCPF | YKTPPIKDF | ALNTLVKQL |
| TRFQTLLAL | LVKQLSSNF | CVADYSVLY | VFVSNGTHWF |
| QIYKTPPIK | INITRFQTL | YKTPPIKDF | LPQGFSAL |
| TRFQTLLAL | YQPYRVVVL | VYYHKNNKSW | QPYRVVVL |
| RQIAPGQTGK | TDEMIAQY | NLTTRTQL | SIIAYTMSL |
| TYVPAQEKNF | TQLPPAYTNSF | SIVRFPNITNL | YRVVVLSF |
| GVYFASTEK | LQIPFAMQMAY | YQPYRVVVL | LLALHRSYL |
| TLKSFTVEK | NTSNQVAVLY | KRFDNPVL | GPKKSTNL |
| QYIKWPWYI | PLLTDEMIAQY | QIAPGQTGK | VYYPDKVF |
| YLQPRTFLL | LITGRLQSL | SKHTPINLV | IYSKHTPINL |
| TRTQLPPAY | IRGDEVRQI | LTDEMIAQY | SAPHGVVFL |
| TRFQTLLAL | KPFERDISTEI | SPRRARSV | CYFPLQSYGF |
| KQIYKTPPIK | LQIPFAMQM | NYNYLYRLF | KVTLADAGFIK |
| EPVLKGVKL | EYVSQPFLM | KCYGVSPTK | RVYSTGSNVF |
| TLDSKTQSL | YTNSFTRGVYY | HWFVTQRNF | VYDPLQPEL |
| VYSSANNCTF | YQPYRVVVL | YSSANNCTFEY | NLNESLIDL |
| LYNSASFSTF | FRKSNLKPF | FIAGLIAIV | GYLQPRTFL |
| KRFDNPVLPF | KRFDNPVLPF | VYYPDKVF | GVYYPDKVF |
| VYDPLQPEL | YTNSFTRGVY | QSAPHGVVF | YYHKNNKSW |
| IYKTPPIKDF | NQKLIANQF | YSKHTPINL | ALDPLSETK |
| YYVGYLQPRTF | YRLFRKSNL | VVFLHVTYV | YFPLQSYGF |
| TRTQLPPAY | QLTPTWRVY | VYDPLQPEL | EPVLKGVKL |
| STECSNLLLQY | TRTQLPPAY | DLLFNKVTL | FPQSAPHGVVF |
| VRDPQTLEI | LLFNKVTL | KRFDNPVL | SIVRFPNITNL |
| TLDSKTQSL | VASQSIIAY | YPDKVFRSSVL | TTEILPVSMTK |
| FRVQPTESI | FRVQPTESI | RFDNPVLPF | VRDPQTLEIL |
| VYDPLQPEL | VFVSNGTHW | YKTPPIKDF | IGAEHVNNSY |
| TSNQVAVLY | NLDSKVGGNY | PYRVVVLSF | FRSSVLHST |
| KIADYNYKL | TEILPVSMTK | YRLFRKSNL | GINASVVNIQK |
| GRLQSLQTY | KCTLKSFTVEK | KTSVDCTMY | KSTNLVKNK |
| GRLQSLQTY | YLQPRTFLLK | ASANLAATK | KMSECVLGQSK |
| VTYVPAQEK | NITRFQTL | GRLQSLQTY | FQPTNGVGY |
| NYNYLYRLF | TLLALHRSY | YPDKVFRSSV | QYIKWPWYIW |
| SPRRARSVA | IVRFPNITNL | VADYSVLY | GVVFLHVTY |
| QPYRVVVL | ARSVASQSI | RLNEVAKNL | GPKKSTNLV |
| KVFRSSVLH | LLFNKVTLA | SLGAENSVAY | RVDFCGKGY |
| YFPLQSYGF | GAEHVNNSY | KLNDLCFTNVY | TPINLVRDL |
| LIDLQELGKY | SSANNCTFEY | YLQPRTFLL | AHFPREGVF |
| RLDKVEAEV | QYGSFCTQL | YTNSFTRGV | TLKSFTVEK |
| LADAGFIKQY | KRFDNPVLPF | GPKKSTNL | ASVYAWNRK |
| RVYSTGSNVF | FRKSNLKPF | SVASQSIIAY | FQFCNDPFL |
| YLQPRTFL | SEPVLKGVKL | NSFTRGVYY | |

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Peptide pool adapted from Zhang et al,(2021) and from Kared .(2021).

**Supplementary Table 13.** Spike peptides (n=315) for CD4 ELISPOT

| | | | |
|---|---|---|---|
| MFVFLVLLPLVSSQC | DSSSGWTAGAAAYYV | YQPYRVVVLSFELLH | GSFCTQLNRALTGIA |
| LVLLPLVSSQCVNLT | GWTAGAAAYYVGYLQ | RVVVLSFELLHAPAT | TQLNRALTGIAVEQD |
| PLVSSQCVNLTTRTQ | GAAAYYVGYLQPRTF | LSFELLHAPATVCGP | RALTGIAVEQDKNTQ |
| SQCVNLTTRTQLPPA | YYVGYLQPRTFLLKY | LLHAPATVCGPKKST | GIAVEQDKNTQEVFA |
| NLTTRTQLPPAYTNS | YLQPRTFLLKYNENG | PATVCGPKKSTNLVK | EQDKNTQEVFAQVKQ |
| RTQLPPAYTNSFTRG | RTFLLKYNENGTITD | CGPKKSTNLVKNKCV | NTQEVFAQVKQIYKT |
| PPAYTNSFTRGVYYP | LKYNENGTITDAVDC | KSTNLVKNKCVNFNF | VFAQVKQIYKTPPIK |
| TNSFTRGVYYPDKVF | ENGTITDAVDCALDP | LVKNKCVNFNFNGLT | VKQIYKTPPIKDFGG |
| TRGVYYPDKVFRSSV | ITDAVDCALDPLSET | KCVNFNFNGLTGTGV | YKTPPIKDFGGFNFS |
| YYPDKVFRSSVLHST | VDCALDPLSETKCTL | FNFNGLTGTGVLTES | PIKDFGGFNFSQILP |
| KVFRSSVLHSTQDLF | LDPLSETKCTLKSFT | GLTGTGVLTESNKKF | FGGFNFSQILPDPSK |
| SSVLHSTQDLFLPFF | SETKCTLKSFTVEKG | TGVLTESNKKFLPFQ | NFSQILPDPSKPSKR |
| HSTQDLFLPFFSNVT | CTLKSFTVEKGIYQT | TESNKKFLPFQQFGR | ILPDPSKPSKRSFIE |
| DLFLPFFSNVTWFHA | SFTVEKGIYQTSNFR | KKFLPFQQFGRDIAD | PSKPSKRSFIEDLLF |
| PFFSNVTWFHAIHVS | EKGIYQTSNFRVQPT | PFQQFGRDIADTTDA | SKRSFIEDLLFNKVT |
| NVTWFHAIHVSGTNG | YQTSNFRVQPTESIV | FGRDIADTTDAVRDP | FIEDLLFNKVTLADA |
| FHAIHVSGTNGTKRF | NFRVQPTESIVRFPN | IADTTDAVRDPQTLE | LLFNKVTLADAGFIK |
| HVSGTNGTKRFDNPV | QPTESIVRFPNITNL | TDAVRDPQTLEILDI | KVTLADAGFIKQYGD |
| TNGTKRFDNPVLPFN | SIVRFPNITNLCPFG | RDPQTLEILDITPCS | ADAGFIKQYGDCLGD |
| KRFDNPVLPFNDGVY | FPNITNLCPFGEVFN | TLEILDITPCSFGGV | FIKQYGDCLGDIAAR |
| NPVLPFNDGVYFAST | TNLCPFGEVFNATRF | LDITPCSFGGVSVIT | YGDCLGDIAARDLIC |
| PFNDGVYFASTEKSN | PFGEVFNATRFASVY | PCSFGGVSVITPGTN | LGDIAARDLICAQKF |
| GVYFASTEKSNIIRG | VFNATRFASVYAWNR | GGVSVITPGTNTSNQ | AARDLICAQKFNGLT |
| ASTEKSNIIRGWIFG | TRFASVYAWNRKRIS | VITPGTNTSNQVAVL | LICAQKFNGLTVLPP |
| KSNIIRGWIFGTTLD | SVYAWNRKRISNCVA | GTNTSNQVAVLYQDV | QKFNGLTVLPPLLTD |
| IRGWIFGTTLDSKTQ | WNRKRISNCVADYSV | SNQVAVLYQDVNCTE | GLTVLPPLLTDEMIA |
| IFGTTLDSKTQSLLI | RISNCVADYSVLYNS | AVLYQDVNCTEVPVA | LPPLLTDEMIAQYTS |
| TLDSKTQSLLIVNNA | CVADYSVLYNSASFS | QDVNCTEVPVAIHAD | LTDEMIAQYTSALLA |
| KTQSLLIVNNATNVV | YSVLYNSASFSTFKC | CTEVPVAIHADQLTP | MIAQYTSALLAGTIT |
| LLIVNNATNVVIKVC | YNSASFSTFKCYGVS | PVAIHADQLTPTWRV | YTSALLAGTITSGWT |

| | | | |
|---|---|---|---|
| LLALHRSYLTPGDSS | QSYGFQPTNGVGYQP | DSTECSNLLLQYGSF | ITGRLQSLQTYVTQQ |
| HRSYLTPGDSSSGWT | FQPTNGVGYQPYRVV | CSNLLLQYGSFCTQL | LQSLQTYVTQQLIRA |
| LTPGDSSSGWTAGAA | NGVGYQPYRVVVLSF | LLQYGSFCTQLNRAL | QTYVTQQLIRAAEIR |
| TQQLIRAAEIRASAN | KNFTTAPAICHDGKA | VYDPLQPELDSFKEE | QELGKYEQYIKWPWY |
| IRAAEIRASANLAAT | TAPAICHDGKAHFPR | LQPELDSFKEELDKY | KYEQYIKWPWYIWLG |
| EIRASANLAATKMSE | ICHDGKAHFPREGVF | LDSFKEELDKYFKNH | YIKWPWYIWLGFIAG |
| SANLAATKMSECVLG | GKAHFPREGVFVSNG | KEELDKYFKNHTSPD | PWYIWLGFIAGLIAI |
| AATKMSECVLGQSKR | FPREGVFVSNGTHWF | DKYFKNHTSPDVDLG | WLGFIAGLIAIVMVT |
| MSECVLGQSKRVDFC | GVFVSNGTHWFVTQR | KNHTSPDVDLGDISG | IAGLIAIVMVTIMLC |
| VLGQSKRVDFCGKGY | SNGTHWFVTQRNFYE | SPDVDLGDISGINAS | IAIVMVTIMLCCMTS |
| SKRVDFCGKGYHLMS | HWFVTQRNFYEPQII | DLGDISGINASVVNI | MVTIMLCCMTSCCSC |
| DFCGKGYHLMSFPQS | TQRNFYEPQIITTDN | ISGINASVVNIQKEI | MLCCMTSCCSCLKGC |
| KGYHLMSFPQSAPHG | FYEPQIITTDNTFVS | NASVVNIQKEIDRLN | MTSCCSCLKGCCSCG |
| LMSFPQSAPHGVVFL | QIITTDNTFVSGNCD | VNIQKEIDRLNEVAK | CSCLKGCCSCGSCCK |
| PQSAPHGVVFLHVTY | TDNTFVSGNCDVVIG | KEIDRLNEVAKNLNE | KGCCSCGSCCKFDED |
| PHGVVFLHVTYVPAQ | FVSGNCDVVIGIVNN | RLNEVAKNLNESLID | SCGSCCKFDEDDSEP |
| VFLHVTYVPAQEKNF | NCDVVIGIVNNTVYD | VAKNLNESLIDLQEL | CCKFDEDDSEPVLKG |
| VTYVPAQEKNFTTAP | VIGIVNNTVYDPLQP | LNESLIDLQELGKYE | DEDDSEPVLKGVKLHYT |
| PAQEKNFTTAPAICH | VNNTVYDPLQPELDS | LIDLQELGKYEQYIK | |

| | | | |
|---|---|---|---|
| NNATNVVIKVCEFQF | SFSTFKCYGVSPTKL | HADQLTPTWRVYSTG | LLAGTITSGWTFGAG |
| NVVIKVCEFQFCNDP | FKCYGVSPTKLNDLC | LTPTWRVYSTGSNVF | TITSGWTFGAGAALQ |
| KVCEFQFCNDPFLGV | GVSPTKLNDLCFTNV | WRVYSTGSNVFQTRA | GWTFGAGAALQIPFA |
| FQFCNDPFLGVYYHK | TKLNDLCFTNVYADS | STGSNVFQTRAGCLI | GAGAALQIPFAMQMA |
| NDPFLGVYYHKNNKS | DLCFTNVYADSFVIR | NVFQTRAGCLIGAEH | ALQIPFAMQMAYRFN |
| LGVYYHKNNKSWMES | TNVYADSFVIRGDEV | TRAGCLIGAEHVNNS | PFAMQMAYRFNGIGV |
| YHKNNKSWMESEFRV | ADSFVIRGDEVRQIA | CLIGAEHVNNSYECD | QMAYRFNGIGVTQNV |
| NKSWMESEFRVYSSA | VIRGDEVRQIAPGQT | AEHVNNSYECDIPIG | RFNGIGVTQNVLYEN |
| MESEFRVYSSANNCT | DEVRQIAPGQTGKIA | NNSYECDIPIGAGIC | IGVTQNVLYENQKLI |
| FRVYSSANNCTFEYV | QIAPGQTGKIADYNY | ECDIPIGAGICASYQ | QNVLYENQKLIANQF |
| SSANNCTFEYVSQPF | GQTGKIADYNYKLPD | PIGAGICASYQTQTN | YENQKLIANQFNSAI |
| NCTFEYVSQPFLMDL | KIADYNYKLPDDFTG | GICASYQTQTNSPRR | KLIANQFNSAIGKIQ |
| EYVSQPFLMDLEGKQ | YNYKLPDDFTGCVIA | SYQTQTNSPRRARSV | NQFNSAIGKIQDSLS |
| QPFLMDLEGKQGNFK | LPDDFTGCVIAWNSN | QTNSPRRARSVASQS | SAIGKIQDSLSSTAS |
| MDLEGKQGNFKNLRE | FTGCVIAWNSNNLDS | PRRARSVASQSIIAY | KIQDSLSSTASALGK |
| GKQGNFKNLREFVFK | VIAWNSNNLDSKVGG | RSVASQSIIAYTMSL | SLSSTASALGKLQDV |
| NFKNLREFVFKNIDG | NSNNLDSKVGGNYNY | SQSIIAYTMSLGAEN | TASALGKLQDVVNQN |
| LREFVFKNIDGYFKI | LDSKVGGNYNYLYRL | IAYTMSLGAENSVAY | LGKLQDVVNQNAQAL |
| VFKNIDGYFKIYSKH | VGGNYNYLYRLFRKS | MSLGAENSVAYSNNS | QDVVNQNAQALNTLV |
| IDGYFKIYSKHTPIN | YNYLYRLFRKSNLKP | AENSVAYSNNSIAIP | NQNAQALNTLVKQLS |
| FKIYSKHTPINLVRD | YRLFRKSNLKPFERD | VAYSNNSIAIPTNFT | QALNTLVKQLSSNFG |
| SKHTPINLVRDLPQG | RKSNLKPFERDISTE | NNSIAIPTNFTISVT | TLVKQLSSNFGAISS |
| PINLVRDLPQGFSAL | LKPFERDISTEIYQA | AIPTNFTISVTTEIL | QLSSNFGAISSVLND |
| VRDLPQGFSALEPLV | ERDISTEIYQAGSTP | NFTISVTTEILPVSM | NFGAISSVLNDILSR |
| PQGFSALEPLVDLPI | STEIYQAGSTPCNGV | SVTTEILPVSMTKTS | ISSVLNDILSRLDKV |
| SALEPLVDLPIGINI | YQAGSTPCNGVEGFN | EILPVSMTKTSVDCT | LNDILSRLDKVEAEV |
| PLVDLPIGINITRFQ | STPCNGVEGFNCYFP | VSMTKTSVDCTMYIC | LSRLDKVEAEVQIDR |
| LPIGINITRFQTLLA | NGVEGFNCYFPLQSY | KTSVDCTMYICGDST | DKVEAEVQIDRLITG |
| INITRFQTLLALHRS | GFNCYFPLQSYGFQP | DCTMYICGDSTECSN | AEVQIDRLITGRLQS |
| RFQTLLALHRSYLTP | YFPLQSYGFQPTNGV | YICGDSTECSNLLLQ | IDRLITGRLQSLQTY |

Peptide pool adapted from Cassaniti et al (2021).

## Supplementary References


1. Zhang et al. Profiling CD8 $^{+}$ T cell epitopes of COVID-19 convalescents reveals reduced cellular immune responses to SARS-CoV-2 variants. *Cell Rep*. **36**, 109708 (2021).

2. Kared et al, SARS-CoV-2-specific CD8+ T cell responses in convalescent COVID-19 individuals. *J. Clin. Invest*. **131**, e145476 (2021).

3. Cassaniti *et al.* SARS-CoV-2 specific T-cell immunity in COVID-19 convalescent patients and unexposed controls measured by ex vivo ELISpot assay. *Clin. Microbiol. Infect.* **27**,1029-1034 (2021).

§ **SCOPE cohort study group**

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