

Supplementary Materials

Supplementary Figures

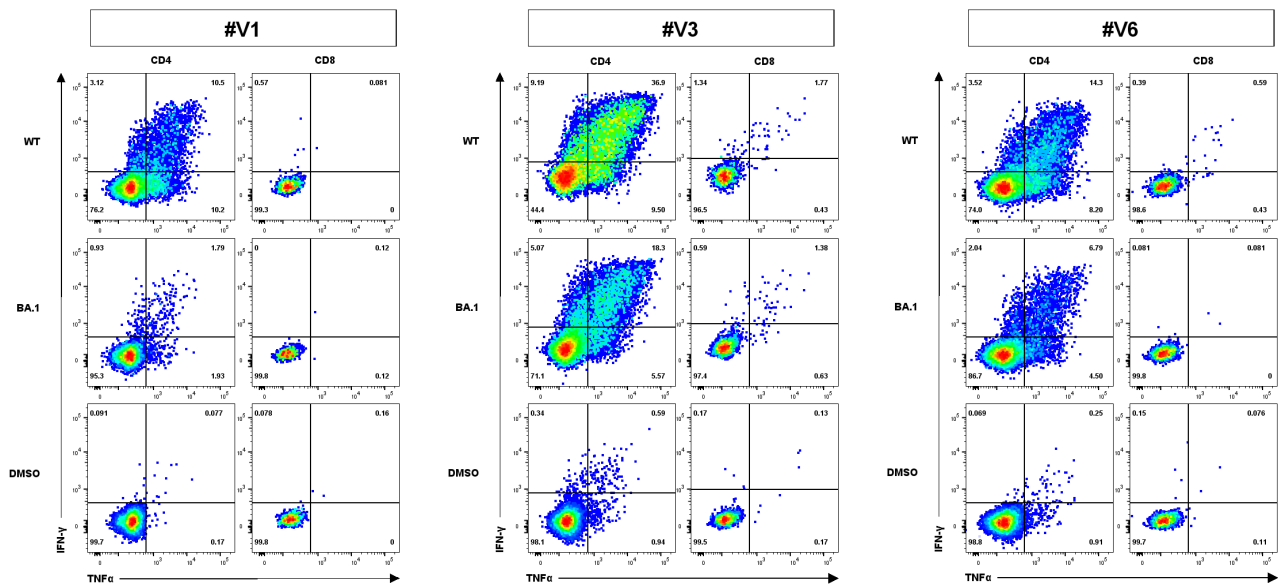


Figure S1. Mainly CD4 $^{+}$ and not CD8 $^{+}$ T cells were activated upon stimulation with selected CD4 $^{+}$ T cell epitope pools

FACS plots showing intracellular cytokine staining of IFN- γ (X-axis) and TNF- α (Y-axis) of stimulated T cell lines of three representative vaccinated subjects (#V1 (left panel), #V3 (middle panel), and #V6 (right panel)). For each of the three subjects, T cells gated on CD4 $^{+}$ expression are shown in the left plots, and T cells gated on CD8 $^{+}$ expression are presented in the right plots. From the top down, T cell lines stimulated with respectively, pool of selected CD4 $^{+}$ T cell epitope candidates from the D614G wild-type (WT) strain (“WT CD4+ pool”), the corresponding Omicron BA.1 variant peptides (“Omicron CD4+ pool”) (BA.1) and DMSO (negative control). Stimulation with the selected promiscuous helper epitopes mainly induced intracellular cytokine expression in CD4 $^{+}$ and not CD8 $^{+}$ T cells. Expression of intracellular cytokines was reduced in response to “Omicron CD4 $^{+}$ pool” compared to “WT CD4 $^{+}$ pool”. Numbers indicate the percentage of cells in each quadrant.

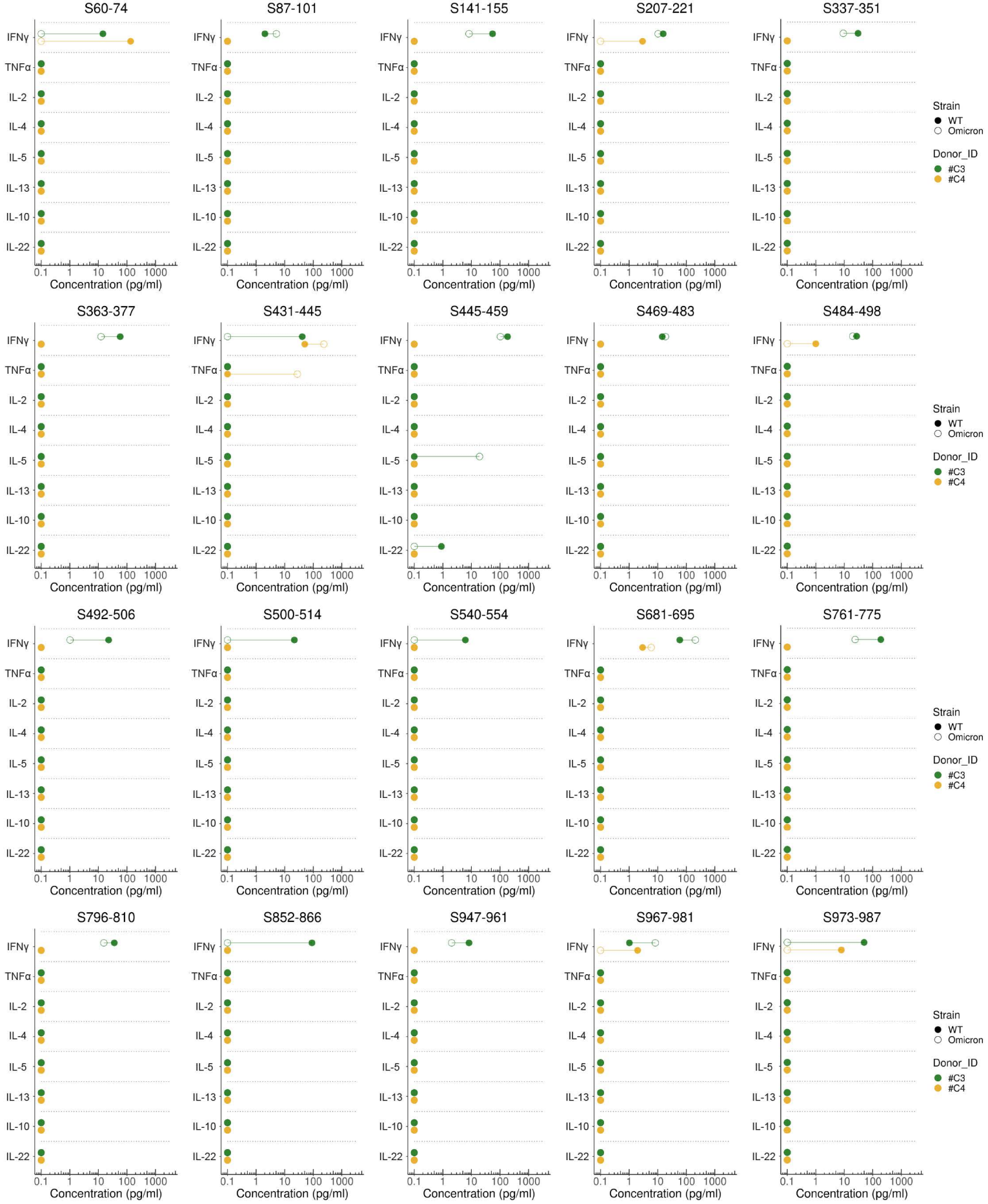


Figure S2. Cytokine production of T cell lines from two convalescent subjects in response to individual spike epitopes harboring Omicron BA.1 mutations

T cell lines were generated by 14-days in vitro stimulation of PBMCs with a pool of selected CD4+ T cell epitope candidates from the D614G wild-type (WT) strain ("WT CD4+ pool") in the presence of IL-2. T cell lines from two convalescent subjects, i.e., donor #C3 (orange) and donor #C4 (dark green), were analyzed for secretion of various cytokines detected using a flowcytometric assay.

Lollipop plots show concentrations of different cytokines measured in T cell line supernatants after stimulation of T cells with individual peptides of spike protein of D614G wild-type (WT) strain (closed dots) and corresponding Omicron BA.1 variant peptides (open dots). Differences between the response to an individual WT and a corresponding Omicron peptide are presented by a connecting line. Above each plot, the location of first and last amino acid position within WT spike protein (S) of the peptide used for stimulation are presented. In general, lower IFN- γ concentrations were observed after stimulation with the Omicron BA.1 peptides, although this was not the case with all epitopes.

Table S1. Selected CD4⁺ T cell epitope candidates of the spike protein of the D614G wild-type reference strain with best matching epitope sequence available from IEDB

Location peptide	WT CD4 ⁺ spike epitopes	IEDB	IEDB epitope sequences ^a
S ₆₀₋₇₄	SNVTWFHAIHVS GTN	1310701	NVTWFHAIHVS GTNG
S ₈₇₋₁₀₁	NDGVYFASTEKSNII	1397173	NDGVYFASTEK SNIIIR
S ₁₄₁₋₁₅₅	LG VYYHKNNKSWMES	1310575	LG VYYHKNNK SWMES
S ₂₀₇₋₂₂₁	HTPINLV RDLPQGFS	1309123	KHTPINLV RDLP QGF
S ₃₃₇₋₃₅₁	PFGEVFNATRFASVY	1310312	CPFGEVFNATRF ASV
S ₃₆₃₋₃₇₇	ADYSVL YNSASFSTF	1069291	CVADYSVL YNS ASF
S ₄₃₁₋₄₄₅	GC V IAWNSNNLDSKV	1310437	GC V IAWNSNN LDSKV
S ₄₄₅₋₄₅₉	VGGNYNYL R LFRKS	1073698	VGGNYNYL R L FRKS
S ₄₆₉₋₄₈₃	STEIYQAGSTPCNGV	1313689	STEIYQAGSTPC NGV
S ₄₈₄₋₄₉₈	EGFNCYFPLQSYGFQ	1397221	VEGFNCYFPLQ SYGFQPT
S ₄₉₂₋₅₀₆	LQSYGFQPTNGVGYQ	1397166	LQSYGFQPTNG VGYPY
S ₅₀₀₋₅₁₄	TNGVG YQPYRVVLS	1540449	GFQPTNGVG Y QPYRVVLSF
S ₅₄₀₋₅₅₄	NFNFNGLTGTGVLTE	1069550	FNFNGLTGTG VLTES
S ₆₈₁₋₆₉₅	PRRARSVASQSIIAY	1394068	SPRRARSVASQ SIIAYT
S ₇₆₁₋₇₇₅	TQLNRALTGI AVEQD	1310863	TQLNRALTGI A VEQD
S ₇₉₆₋₈₁₀	DFGGFNFSQILPDPS	1312421	FGGFNFSQILP DPSK
S ₈₅₂₋₈₆₆	AQKFNGLT VLPPLL	1310303	CAQKFNGLT V LPLL
S ₉₄₇₋₉₆₁	KLQDVVNQNAQALNT	1310448	GKLQDVVNQNA QALN
S ₉₆₇₋₉₈₁	SSNFGAISSVLNDIL	1313359	QLSSNFGAISS VLND
S ₉₇₃₋₉₈₇	ISSVLNDILSR LDKV	1312775	ISSVLNDILSR LDKV

^aIdentified IEDB epitope sequences of SARS-CoV-2 spike protein (UniProtKB: P0DTC2, host: human) that were tested positive in a T cell assay according to information in IEDB

The location of the peptide is shown as the position of the first and last amino acid of the peptide within the spike protein. Each matching amino acid sequence of the selected WT CD4⁺ spike epitope candidates with the closest matching SARS-CoV-2 spike epitope sequence from IEDB is shown as bold character.

Abbreviations: IEDB, The Immune Epitope Database (www.iedb.org)