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Supplemental information

mRNA COVID-19 vaccine elicits potent adaptive immune response without the acute inflammation of SARS-CoV-2 infection

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Table S1

Participant	Cohort	Age	Sex	Race	BMI	Days post onset symptoms or days since first vaccine dose	Fraction inspired O_2	Days since booster	WHO COVID severity scale	COVID Outcome	Co-morbidities
SK-010	Acute COVID-19	36-40	Female	Asian	NA	12	21	NA	5	Recovered	NA
						24	21				
SK-011	Acute COVID-19	56-60	Female	Caucasian	35.7	6	32	NA	7	Recovered	Type 2 Diabetes, Hypertension, Hyperlipidemia, Obesity
						13	24				
SK-012	Acute COVID-19	41-45	Male	Caucasian	24.7	8	38	NA	8	Recovered	Asthma, Anxiety, Depression
						24	38				
SK-013	Acute COVID-19	61-65	Male	NA	NA	6	38	NA	5	Recovered	NA
						11	28				
SK-014	Acute COVID-19	51-55	Female	Asian	NA	9	21	NA	5	Recovered	NA
CV-001	mRNA vaccine	36-40	Male	Asian	28.4	0, 7, 14,21, 28, 35	21	0, 7, 28	NA	NA	NA
CV-003	mRNA vaccine	31-35	Male	Asian	27.9	0, 10, 20, 28, 35	21	0, 7, 28	NA	NA	Hypothyroidism
CV-011	mRNA vaccine	36-40	Male	Caucasian	28.6	0, 7, 14, 21, 29, 36	21	0, 7	NA	NA	NA
CV-012	mRNA vaccine	51-55	Female	Caucasian	28.7	0, 7, 21, 28	21	NA	NA	NA	NA
CV-022	mRNA vaccine	41-45	Male	Caucasian	22.4	0, 7, 21, 28	21	NA	NA	NA	Facial Cellulitis, GERD
CV-053	mRNA vaccine	46-50	Female	African-American	NA	0, 7, 21, 28	21	NA	NA	NA	Fibroids, HSV, GERD
CV-056	mRNA vaccine	51-55	Female	Caucasian	25.4	0, 7, 21, 28	21	NA	NA	NA	NA
SK-007	HC	36-40	Female	Caucasian	20.4	0	21	NA	NA	NA	NA
SK-008	HC	36-40	Female	Caucasian	28.6	0	21	NA	NA	NA	NA

Table S1. Demographic characteristics, clinical features, and outcomes for study participants, Related to Schematic 1.

Figure S1

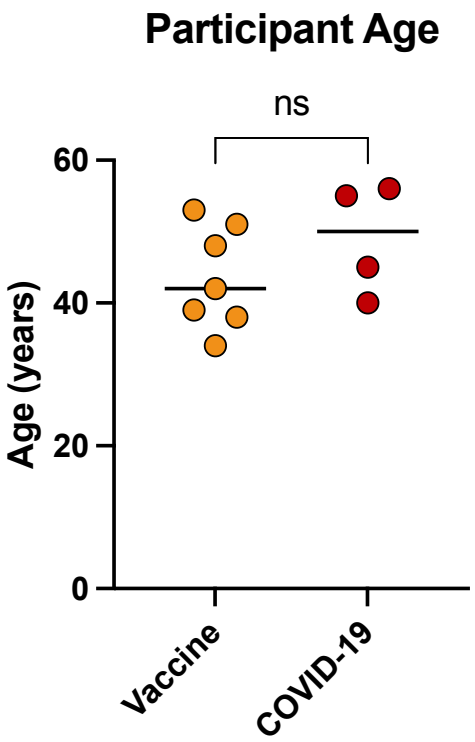
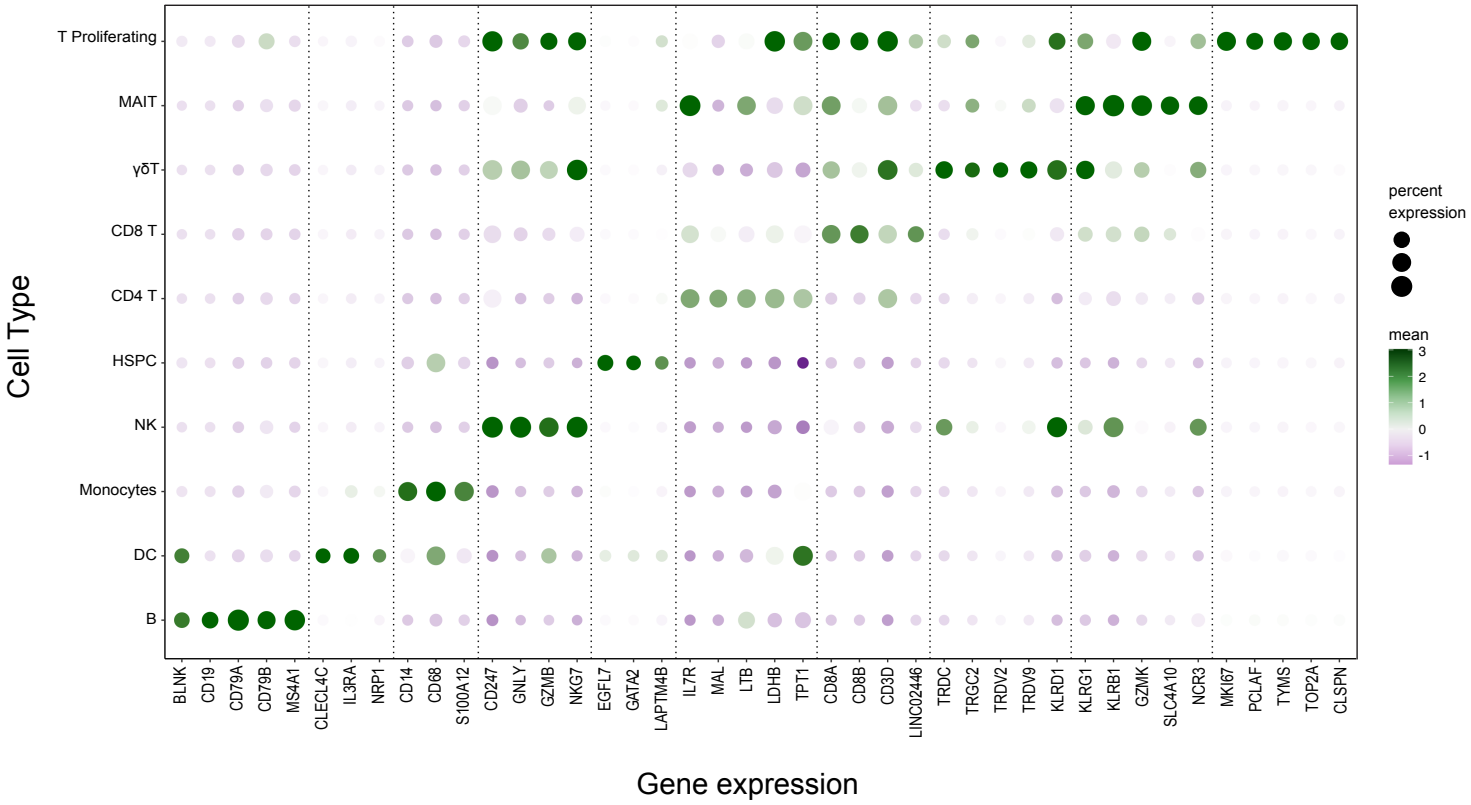


Figure S1. Participant age, Related to Schematic 1. Scatter plot of age for all healthy volunteers who received SARS-CoV2 vaccine and COVID-19 patients in Supplemental Table 1. P-value were determined by Welch's t-test (ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure S2

A


T Proliferating
MAIT
γδT
CD8 T
CD4 T
HSPC
NK
Monocytes
DC
B
Cell Type
BLNK
CD19
CD79A
CD79B
MS4A1
CLECL4C
IL3RA
NRP1
CD14
CD68
S100A12
CD247
GNLY
GZMB
NKG7
EGFL7
GATA2
LAPTM4B
IL7R
MAL
LTB
LDHB
TPT1
CD8A
CD8B
CD3D
LINC02446
TRDC
TRGC2
TRDV2
TRDV9
KLRD1
KLRG1
KLRB1
GZMK
SLC4A10
NCR3
MKI67
PCLAF
TYMS
TOP2A
CLSPN
Gene expression
percent expression
mean
3
2
1
0
-1

B

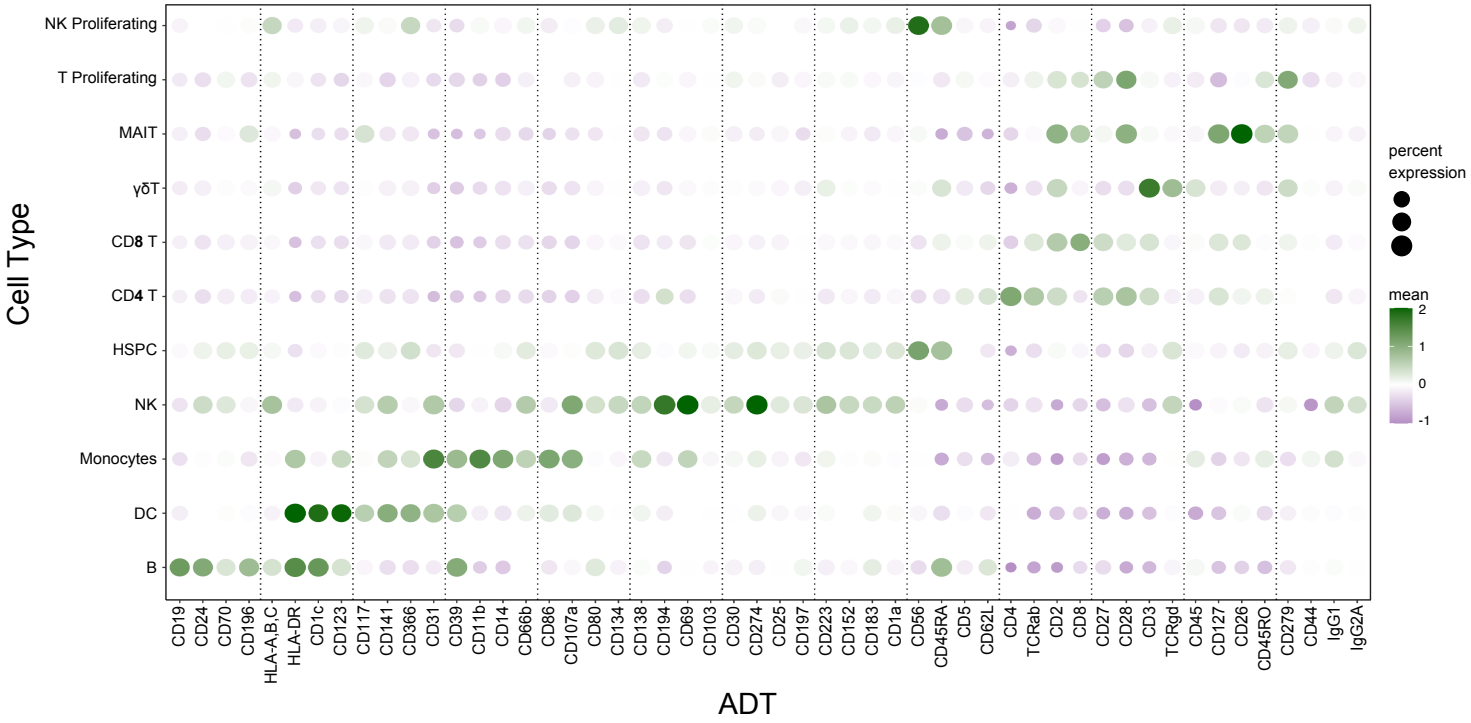

NK Proliferating
T Proliferating
MAIT
γδT
CD8 T
CD4 T
HSPC
NK
Monocytes
DC
B
Cell Type
CD19
CD24
CD70
CD196
HLA-A,B,C
HLA-DR
CD1c
CD123
CD117
CD141
CD366
CD31
CD39
CD11b
CD14
CD66b
CD86
CD107a
CD80
CD134
CD138
CD194
CD69
CD103
CD30
CD274
CD25
CD197
CD223
CD152
CD183
CD1a
CD56
CD45RA
CD5
CD62L
CD4
TCRab
CD2
CD8
CD27
CD28
CD3
TCRgd
CD45
CD127
CD26
CD45RO
CD279
CD44
IgG1
IgG2A
ADT
percent expression
mean
2
1
0
-1

Figure S2. Surface protein and gene expression across identified populations, Related to Figure 1. A. Dot plot illustrating the expression of key markers across the major cell populations identified. The gene names are shown on the x-axis and the identities of the cell populations on the y-axis. Downregulated markers are colored in purple and upregulated markers in green. The size of the dots represents the fraction of cells expressing the specific marker and the color intensity represents the average expression level. **B.** Dot plot illustrating the distribution of antibody-derived tags (ADT) expression across the major cell populations identified. The protein markers are shown on the x-axis and the identities of the cell populations on the y-axis. Downregulated markers are colored in purple and upregulated markers in green. The size of the dots represents the fraction of cells expressing the specific marker and the color intensity represents the average expression level.

Table S2

Cell Population	Subpopulation	Markers
B cells	Memory	MS4A1, COCH, AIM2, BANK1, CD79A
	Resting	IL4R, CXCR4, BTG1, TCL1A, YBX3
	Plasmablasts	MZB1, TNFRSF17, CPNE5, POU2AF1
	Activated	FOSB, CD196 (ADT)
T cells	T Proliferating	MKI67, TOP2A, PCLAF, TYMS, RRM2
	CD4 T CM	CD4, TCF7, IL7R
	CD4 T Regulatory	CD4, CTLA4, FOXP3, IL2RA
	CD4 Activated	CD4, CCL5, FYB1, GZMK, IL32, TRAC, KLRB, GZMA, ZEB2
	CD8 Naïve	CCR7, CD8A, CD8B
	CD8 EM	CD8A, CD28, CD127, CD45RO, CCL5, NKG7
T innate cells	MAIT	TRAV1-2, SLC4A10, KLRB1, GZMK, IL7R, SLC4A10, CXCR6, CD45RO (ADT)
	NK	GNLY, TYROBP, NKG7, FGFBP2, KLRF1
	γδT	TRDC, TRGC2, KLRC1, NKG7, TRDV2, TRGV9, KLRG1
	NK Proliferating	GNLY, NKG7, MKI67, TOP2A, STMN1, TYMS
Myeloid cells	pDC	CLEC4C, IL3RA
	Classical Monocytes	CD14, LYZ, CD36
	NKG7+ Monocytes	LYZ, NKG7, GNLY
	Non-classical monocytes	FCGR3A, MS4A7, CDKN1C
	NEAT1hi Monocytes	CD14, NEAT1

Table S2. Markers used to delineate immune cell subsets, Related to Figure 1.

Figure S3

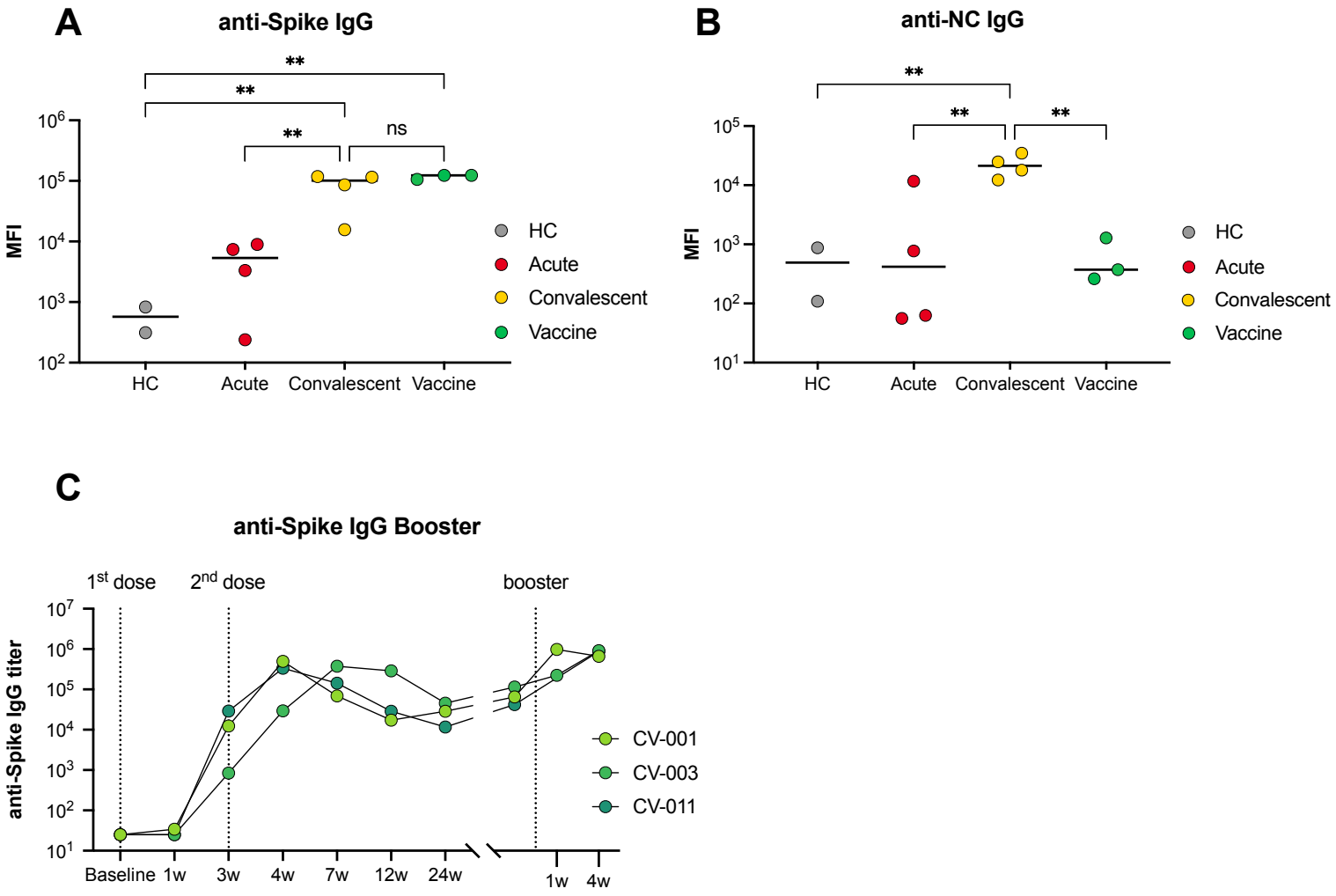


Figure S3. Ab titers and plasmablast responses, Related to Figure 2. A,B. SARS-CoV-2-specific Ab titers were assessed for COVID-19 patients and healthy volunteers using Multiplex Bead Binding Assay (MBBA). IgG anti-Spike responses are shown in (**A**), anti-NC responses in (**B**). COVID-19 patient samples are split by days post-onset (DPO) of symptoms into acute (≤10 DPO) and convalescent (>10 DPO). For the vaccine group, samples collected 4 weeks post-first vaccine dose were used. P-value were determined by Welch's t-test (ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). **C.** Spike-specific Ab titers for 3 healthy volunteers before and after receiving the BNT162b2 mRNA vaccine and booster, assessed by direct ELISA.

Figure S4

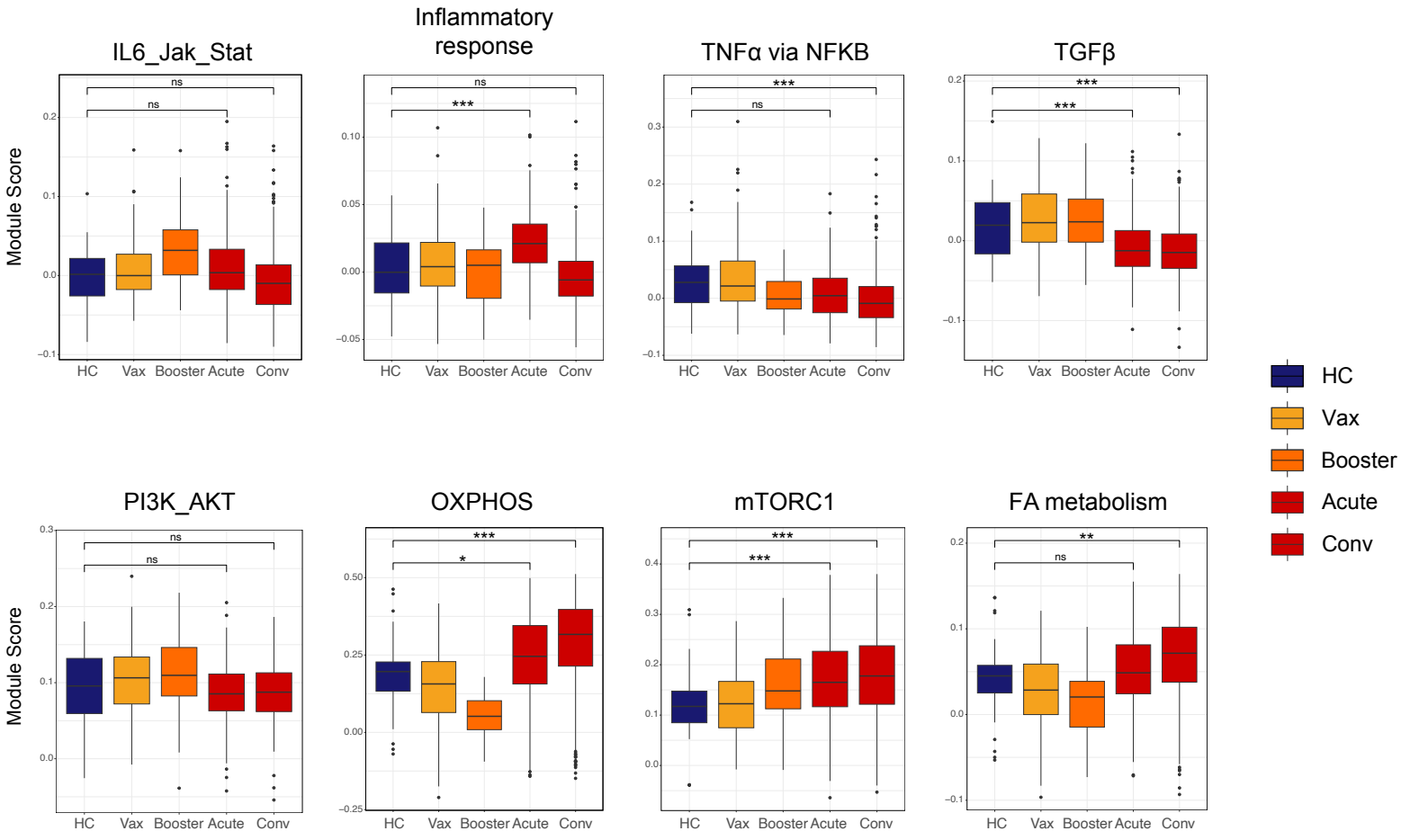


Figure S4. Pathway enrichment in plasmablasts module score, Related to Figure 2. Module scores for the pathways in **Figure 2A** were calculated using the AddModuleScore function from the Seurat package, which calculates the mean expression for a set of genes and adjusts for the collective expression of control features. The p-values were computed using the Wilcoxon test to compare the median module scores across a range of conditions (ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure S5

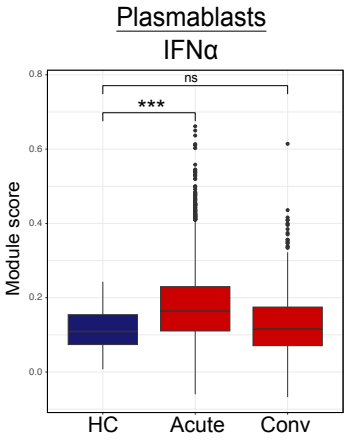
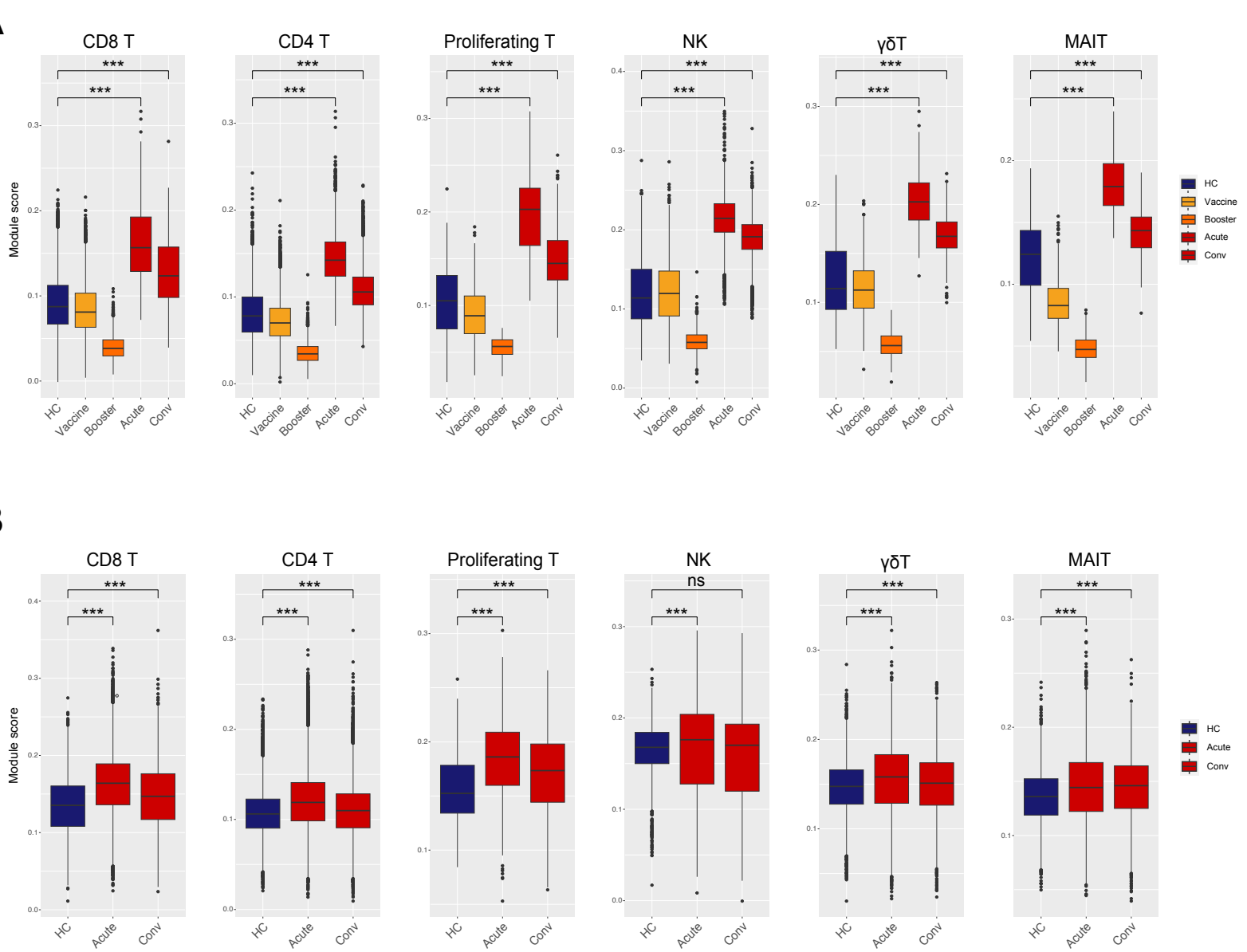


Figure S5. IFN-I gene pathway enrichment in plasmablasts module score in Haniffa data set, Related to Figure 2A,B. Module scores were calculated for plasmablasts in the Haniffa acute and convalescent COVID-19 samples [S1] using the AddModuleScore function from the Seurat package, which calculates the mean expression for a set of genes and adjusts for the collective expression of control features. The p-values were computed using the Wilcoxon test to compare the median module scores across a range of conditions (ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure S6


A
CD8 T
CD4 T
Proliferating T
NK
γδT
MAIT
Module score
HC
Vaccine
Booster
Acute
Conv
***
B
ns
C
CD4 T Activated
CD4 T CM
Scaled Expression
IL7R
CXCR4
TNFAIP3
JUN
FOS
NFKBIA
DUSP1
CCL4
KLRD1
NKG7
GZMA
IFITM2
IFITM1
PRF1
GZMB
GNLY
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FCER1G
CCL5
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PTPRC
ANXA1
HLA-B
HLA-E
HLA-C
HLA-A
B2M
FLNA
CD74
HMGB2
RPS27A
UBA52
RPS19
IFI6
LY6E
RAC2
PSMB9
PSMB8
CORO1A
HMGB1
HSP90AA1
PPIB
MIF
CALR
HSP90AB1
MT2A
UBC
UBB
PCBP2

Figure S6. Cytotoxic responses and clonality of conventional and innate-like T cells in COVID-19 and SARS-CoV-2 vaccine recipients, Related to Figure 3C. A,B. Module scores were calculated based on expression of genes associated with cytotoxic effector function from the gene set T cell mediated cytotoxicity (GO:0001913) in major conventional and innate-like T cell populations in our data set (**A**) andvalidated in Haniffa data set (**B**) [S1]. The p-values were computed using the Wilcoxon test to compare the median module scores across a range of conditions (ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). **C.** Average per-sample scaled expression of genes associated with cytotoxic effector function from the gene set T cell mediated cytotoxicity (GO:0001913) in activated CD4 and CD4 CM T cells.

Figure S7

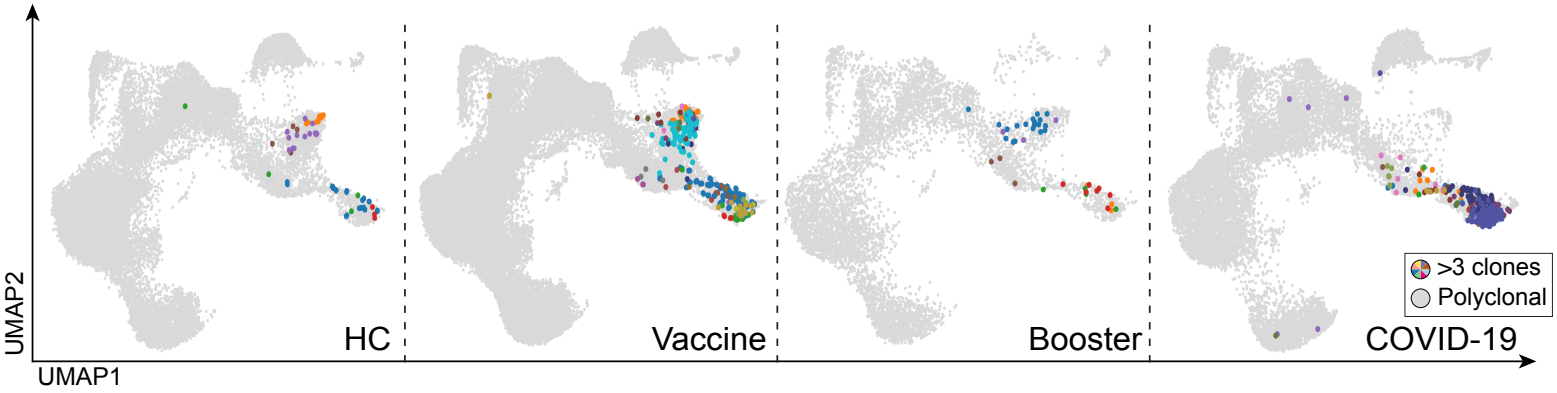

Figure S7. SARS-CoV-2 reactive CD8 T cell clones, Related to Figure 3D,E. UMAP visualization of CD8 T cell clones that match reported TCRβ sequences from natural and synthetic exposure to SARS-CoV-2. Since clusters CD8 T Effector Memory and CD4 T Activated overlap in UMAP space, we confirmed that all clonal cells were from the CD8 T Effector Memory population. At a 100% sequence identity threshold, clonal TCRαβ CDR3 amino acid sequences were compared to CDR3 sequences from spike-specific T cell clones using the cd-hit-2d command from the CD-HIT package. Identical CDR3 sequences in at least 3 cells that had matches to CDR3s in the AIM assay are colored uniquely.

Figure S8

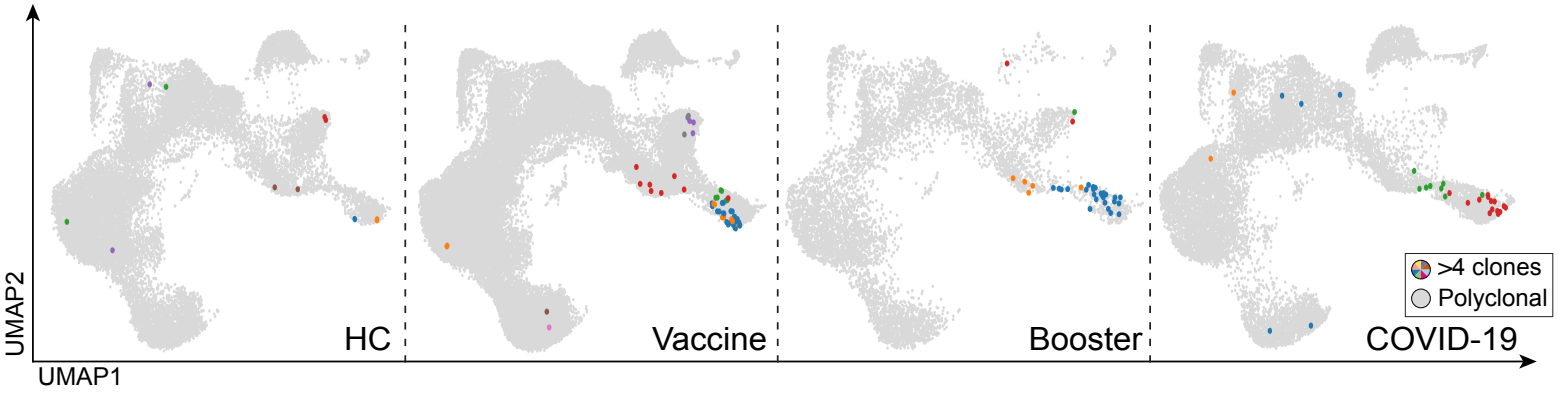

Figure S8. SARS-CoV-2 reactive CD4 T cell clones, Related to Figure 3D,E. UMAP visualization of T cells expressing TCRs with CDR3 sequences found in SARS-CoV-2 reactive T cells in antigen-induced activation (AIM) assay using Spike peptides [S2]. At a 95% sequence identity threshold, clonal TCRαβ CDR3 amino acid sequences were compared to CDR3 sequences from spike-specific T cell clones using the cd-hit-2d command from the CD-HIT package. Identical CDR3 sequences in at least 4 cells that had matches to CDR3s in the AIM assay are colored uniquely.

Figure S9

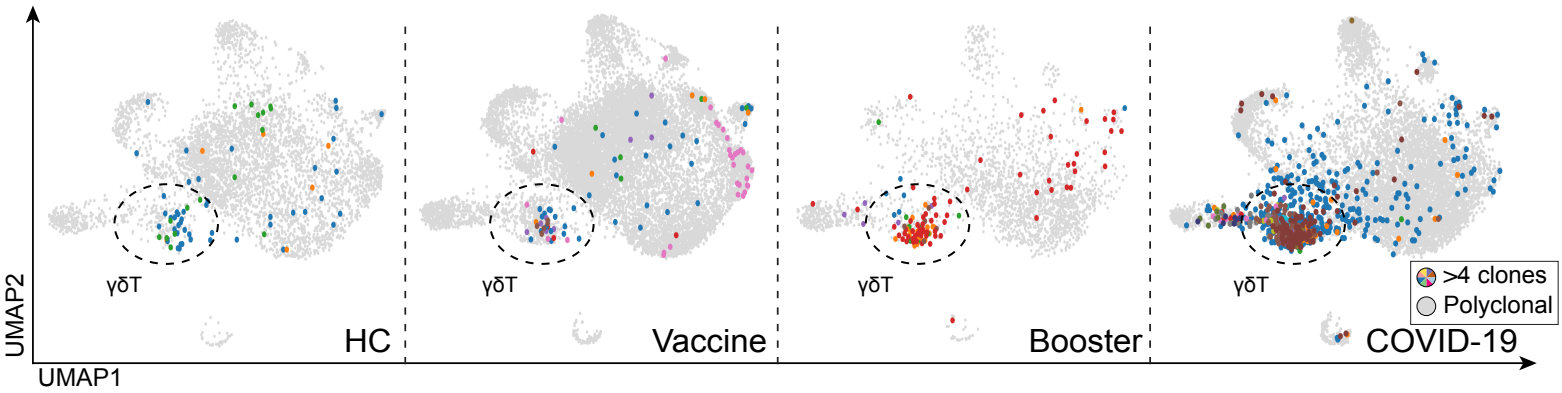

Figure S9. Clonality of γδ T cell, Related to Figure 3F. UMAP visualization of clonal γδ T cells from healthy volunteers before (first panel) and after (second panel) receiving the BNT162b2 mRNA vaccine and booster (third panel), and COVID-19 patients (fourth panel). Clonality is determined by the CDR3 sequence in TCRδ chain. Identical CDR3 sequences in at least 5 cells are colored uniquely.

References:

S1. Stephenson, E., Reynolds, G., Botting, R.A., Calero-Nieto, F.J., Morgan, M.D., Tuong, Z.K., Bach, K., Sungnak, W., Worlock, K.B., Yoshida, M., et al. (2021). Single-cell multi-omics analysis of the immune response in COVID-19. Nat Med *27*, 904-916. 10.1038/s41591-021-01329-2.

S2. Gray-Gaillard, S.L., Solis, S., Monteiro, C., Chen, H.M., Ciabattoni, G., Samanovic, M.I., Cornelius, A.R., Williams, T., Geesey, E., Rodriguez, M., et al. (2022). Molecularly distinct memory CD4+ T cells are induced by SARS-CoV-2 infection and mRNA vaccination. bioRxiv. 10.1101/2022.11.15.516351.