Supplementary Methods

RNA-sequencing Analysis

Sample and library preparation: samples were divided into batches for RNA isolation and library preparation to avoid batch effects which could affect group comparisons. Whole blood was collected from study participants in PAXgene Blood RNA tubes (Qiagen, Hilden, Germany) and shipped to CeGaT for mRNA isolation, library preparation (KAPA RNA HyperPrep Kit with RiboErase y(HMR) Globin) and sequencing. Globin and rRNA were depleted in library construction. NGS library sequencing was on a NovaSeq 6000 (Illumina) with read length of 100 bp for read 1 and read 2 (in total 2x 100 bp). RNA sample quality was assessed by the RIN value and DV200 value using Qubit RNA (Thermo Fisher) and Fragment Analyzer (Agilent).

Transcriptome sequencing data analysis: demultiplexing of the sequencing reads was performed with Illumina bcl2fastq 2.20 and adapters were trimmed with Skewer 0.2.2. Reads with a Phred score of < 30 were removed using Trimmomatic 0.39. Trimmed raw reads were aligned to the human reference genome (hg19) with transcript annotation from Ensembl (GRCh37, v75)) using STAR 2.5.2b and multimappers removed. The quality of the FASTQ files were analyzed with FastQC. The read length after adapter trimming, the average base quality per read (as Phred score) and average GC content of the reads were examined via histograms.

Differential expression analysis: group comparisons for testing significant differentially expressed genes (DEGs) performed by CeGaT with DESeq2 [1] in R (version 3.6.1) [2]. DESeq2 uses a negative binomial generalized linear model to test for differential expression based on gene counts. As recommended by DESeq2, genes with less than two reads over all samples were removed and finally normalized. Sample RNA quality including possible sample outlier and sample characteristics were investigated by a PCA and dendrogram on rlog- transformed read counts. For each type of group comparison counts were modeled by the design formulas:

- A2, A3, A4: subject_ID + Day
- A5, A6, A7: Dose_group

Log2 fold changes were calculated, and the Wald test was applied for statistical testing with a significance level of 0.05 including a multiple testing correction by Benjamini-Hochberg [3] for adjusting the p-values. For each comparison, genes with a read count of zero in all compared samples or a read count of 1 in only one sample were excluded.

DEGs from group comparisons were further applied to gene set enrichment analysis (GSEA, Linux Version 4.1.0) [4,5] by CureVac Bioinformatics to the Blood Transcription Modules (BTM version 8 from October 2013 [6]) and HALLMARK molecular Signatures Database (MSigDB, version 7.4 [7]) gene set databases. The 50 HALL-MARKs show a wide range of biological processes and were summarized from multiple founder gene sets to reduce variation and redundancy. The BTMs were identified from systems signatures from five human vaccines from a large-scale network integration of public human blood transcriptomes, and systems-scale databases in specific biological contexts. The 382 BTM sets (modules) are annotated by their function and focus more on processes related to immunity.

GSEA parameter: genes for GSEA were pre-ranked (according to metric described in Reimand et al. [8]). Firstly, all significant (cut-off adjusted P value <0.05) differentially expressed genes for each group comparison were extracted. A score per gene $s_{gene\ i}$ was calculated and finally all genes per group comparison were sorted in a decreasing order. The scoring metric uses the sign (*sign*) of the log2 fold change of each gene (log2FC$_{gene\ i}$) with negative logarithm (*log10)* of adjusted P value (padj$_{gene\ i}$) based on multiple testing using the Benjamini-Hochberg method:

$$s_{gene\ i} = sign(\text{log2FC}_{gene\ i}) * (-log10(\text{padj}_{gene\ i}))$$

The ranked gene list per group comparison is received where top genes on the list represent up-regulation and genes at the bottom of the list down-regulation.

GSEA default settings were used, number of permutations was set to 10,000 and type of permutation was "genotype" because of pre-ranked gene list. To include HGNC (HUGO Gene Nomenclature Committee) gene annotations in the analysis report the command "collapse" was used and the chip annotation file Human_Gene_Symbol_with_Remapping_MSigDB.v7.4.chip.

Results from GSEA were visualized in R (4.0.4) as heatmaps using the ComplexHeatmap (2.6.2) package [9] and significant DEGs as bar plots with ggplot2 in R (4.0.4) [10].

Supplementary Tables

Table S1. Cytokines and Chemokines Included in Serum Cytokine Analysis

Cytokine Abbreviation	Abbreviation Meaning
APRIL	A-proliferation-inducing ligand
BAFF	B-cell activating factor
CCL4	C-C chemokine ligand 4
CXCL9	C-X-C chemokine ligand 9
CXCL11	C-X-C chemokine ligand 11
CXCL13	C-X-C chemokine ligand 13
CD40L	CD40 Ligand
CD62L	CD62 Ligand
FasL	Fas Ligand
ICAM-1	Intercellular adhesion molecule 1
IFNα	Interferon α
IFNβ	Interferon β
IFNγ	Interferon γ
IL-1α	Interleukin 1a
IL-2	Interleukin 2
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-6	Interleukin 6
IL-10	Interleukin 10
IL-12(p70)	Interleukin 12(p70)
IL-13	Interleukin 13
IL-17A	Interleukin 17A
IP-10	Interferon gamma-induced protein 10
MCP-1	Monocyte chemotactic protein 1
MCP-4	Monocyte chemotactic protein 4
MDC	Macrophage-derived chemokine
MICA	MHC class I polypeptide-related sequence A
MIP-1α	Macrophage inflammatory protein 1α
SCF	Stem cell factor
TNFα	Tumour necrosis factor α

Table S2. Antibodies Used for ICS

Marker	Clone	Supplier	Catalogue Number
Live/dead	-	Invitrogen	L34966
CD8	SK1	BD Biosciences	641400
CD4	SK3	BD Biosciences	345770
CD3	UCHT1	BD Biosciences	557943
CD40L	TRAP1	BD Biosciences	555700
IL-2	MQ1-17H12	BD Biosciences	554567
TNFα	MAb11	BD Biosciences	557647
IFNγ	4S.B3	BD Biosciences	554551

Table S3. Peptides Used for Mass Cytometry Analysis

No.	Peptide name	Peptide sequence	Antigen	HLA
1	A*02:01_1	KLPDDFTGCV	S	A*02:01
2	A*02:01_2	LITGRLQSL	S	
3	A*02:01_3	RLNEVAKNL	S	
4	A*02:01_4	YLQPRTFLL	S	
5	A*02:01_5	RLQSLQTYV	S	
6	A*02:01_6	VLNDILSRL	S	
7	A*02:01_7	KIADYNYKL	S	
8	A*02:01_8	SIIAYTMSL	S	
9	A*02:01_9	LLFNKVTLA	S	
10	A*02:01_10	RLDKVEAEV	S	
11	A*02:01_11	HLMSFPQSA	S	
12	A*02:01_12	FIAGLIAIV	S	
13	A*02:01_13	ALNTLVKQL	S	
14	A*02:01_14	NLNESLIDL	S	
15	A*02:01_15	VVFLHVTYV	S	
16	A*02:01_C1	VLEETSVML	CMV-IE1	
17	A*02:01_C2	NLVPMVATV	CMV-pp65	
18	A*02:01_C3	CLGGLLTMV	EBV-LMP2A	
19	A*02:01_C4	FLYALALLL	EBV-LMP2A	
20	A*02:01_C5	GILGFVFTL	Flu-M1	
21	A*11:01_1	GVYFASTEK	S	A*11:01
22	A*11:01_2	TLKSFTVEK	S	
23	A*11:01_3	RLFRKSNLK	S	
24	A*11:01_4	NSASFSTFK	S	
25	A*11:01_5	VTYVPAQEK	S	
26	A*11:01_6	SSTASALGK	S	
27	A*11:01_7	ASANLAATK	S	
28	A*11:01_8	GTHWFVTQR	S	
29	A*11:01_9	MTSCCSCLK	S	
30	A*11:01_C1	ATVQGQNLK	CMV-pp65	
31	A*11:01_C2	AVFDRKSDAK	EBV(EBNA 3B)	
32	A*11:01_C3	SSCSSCPLSK	EBV(LMP2)	
33	A*11:01_C4	SIIPSGPLK	Flu-MP1	
34	A*24:02_1	NYNYLYRLF	S	A*24:02
35	A*24:02_2	VYSTGSNVF	S	
36	A*24:02_3	EYVSQPFLM	S	
37	A*24:02_4	QYIKWPWYI	S	
38	A*24:02_5	LYNSASFSTF	S	
39	A*24:02_6	PYRVVVLSF	S	
40	A*24:02_7	YYVGYLQPRTF	S	
41	A*24:02_8	VYSSANNCTF	S	
42	A*24:02_9	IYKTPPIKDF	S	
43	A*24:02_10	RFDNPVLPF	S	

No.	Peptide name	Peptide sequence	Antigen	HLA
44	A*24:02_11	PFFSNVTWF	S	
45	A*24:02_12	RFPNITNLCPF	S	
46	A*24:02_13	TYVPAQEKNF	S	
47	A*24:02_C1	QYDPVAALF	CMV-2 pp65 341-349	
48	A*24:02_C2	AYAQKIFKIL	CMV-5-IE1	
49	A*24:02_C3	RYSIFFDYM	EBV-10-EBNA3A NP	
50	A*24:02_C4	RYGFVANF	Flu-8-Pol	
51	A*24:02_C5	TYQWIIRNW	Flu-PB2	
52	A*03:01_1	GVYFASTEK	S	A*03:01
53	A*03:01_2	TLKSFTVEK	S	
54	A*03:01_3	RLFRKSNLK	S	
55	A*03:01_4	VTYVPAQEK	S	
56	A*03:01_5	QIYKTPPIK	S	
57	A*03:01_6	ASANLAATK	S	
58	A*03:01_7	MTSCCSCLK	S	
59	A*03:01_8	KVFRSSVLH	S	
60	A*03:01_9	RQIAPGQTGK	S	
61	A*03:01_10	KCYGVSPTK	S	
62	A*03:01_C1	KLGGALQAK	CMV (IE-1)	
63	A*03:01_C2	RLRAEAQVK	EBV (EMNA 3A)	
64	A*03:01_C3	ILRGSVAHK	Influenza (PR8)	
65	A*01:01_1	WTAGAAAYY	S	A*01:01
66	A*01:01_2	LADAGFIKQY	S	
67	A*01:01_3	LTDEMIAQY	S	
68	A*01:01_4	TSNQVAVLY	S	
69	A*01:01_C1	VTEHDTLLY	CMV (UL44)	
70	A*01:01_C2	YSEHPTFTSQY	CMV (pp65)	
71	A*01:01_C3	CTELKLSDY	Influenza (NP)-A01	
72	A*01:01_C4	VSDGGPNLY	Influenza (PB1)-A01	
73	B*07:02_1	SEPVLKGVKL	S	B*07:02
74	B*07:02_2	LPQGFSAL	S	
75	B*07:02_3	KPFERDISTEI	S	
76	B*07:02_4	QPYRVVVL	S	
77	B*07:02_5	LPFNDGVYF	S	
78	B*07:02_6	SPRRARSVA	S	
79	B*07:02_7	IPTNFTISV	S	
80	B*07:02_8	APHGVVFL	S	
81	B*07:02_C1	RPHERNGFTVL	CMV (pp65 #1)	
82	B*07:02_C2	RPPIFIRRL	EBV (EBNA 3A)	
83	B*07:02_C3	SPIVPSFDM	Influenza (NP)	
84	B*07:02_C4	QPEWFRNVL	Influenza (PB1)	

Table S4. Antibodies Used for Mass Cytometry

Marker	Clone	Catalogue number	Company
CD45	Y-89	HI30 3089003B	Standard BioTools Inc
CD19	HIB19	bl302202	BioLegend
CD8	SK1	bl344727	BioLegend
CD14	TUK4	MCA1568GA	BioRAD
CD4	SK3	bl344602	BioLegend
CD57	HNK-1	bl359602	BioLegend
CD3	UCHT1	bl300414	BioLegend
TCRγ/δ1	B1	331210	BioLegend
PE2	PE001	bl408102	BioLegend
CD56	NCAM16.2	bd559043	BD
HLA-DR	L243	bl307602	BioLegend
CD27	LG.7F9	14-0271-85	Invitrogen
CD24	ML5	311127	Biolegend
CXCR5	RF8B2	bd552032	BD
CRTH2	BM16	13294982	eBioscience
CD95	DX2	305602	BioLegend
CD45RO	UCHL1	304202	BioLegend
CCR6	G034E3	bl353402	BioLegend
CD103	B-Ly7	14-1038-82	eBioscience
CD69	FN50	bl310902	BioLegend
KLRG1	13F12F2	ebio16-9488-85	eBioscience
CLA	HECA-452	bl321302	BioLegend
CXCR3	49801	MAB160-100	R&D
CD45RA	H100	bd555486	BD
PD-1	J105	14-2799-80	invitrogen
CD161	HP-3G10	bl339902	BioLegend
CD127	A019D5	bl351302	BioLegend
CD39	A1	bl328202	BioLegend
CCR5	RF8B2	566191	Genetex
CCR7	150503	MAB197	R&D
CD25	M-A251	bl356102	BioLegend
CD28	CD28.2	bl302914	BioLegend
CD71	CY1G4	bl334102	BioLegend
CD38	HIT2	bl303502	BioLegend
CCR4	L291H4	bl359402	BioLegend
Perforin	B-D48	ab47225	Abcam
CD16	T24/31	BE0212	Standard BioTools Inc
DNA	Ir-191/193	DNA	Standard BioTools Inc
CisPlatin live/dead	Pt-195	CisPlatin live/dead	Sigma

[1]anti-TCRγδ is the PE-labelled primary antibody. [2] secondary antibody: anti-PE.

Table S5. R Packages Used for Analysis

Analysis	R Packages used
CMI	R 4.3.0
	renv 1.0.0
	tidyverse 2.0.0
	DT 0.28
	plyr 1.8.8
	dplyr 1.1.2
	plotly 4.10.2
	openxlsx 4.2.5.2
	readxl 1.4.3
	viridis 0.6.4
	scales 1.2.1
	data.table 1.14.8
	patchwork 1.1.2
	formattable 0.2.1
	EnvStats 2.8.0
	ggforce 0.4.1
	ggh4x 0.2.5
	gghighlight 0.4.0
	ggridges 0.5.4
	ggbeeswarm 0.7.2
	ggthemes 4.2.4
	gridExtra 2.3
	testthat 3.1.10
	shiny 1.7.4.1
	shinythemes 1.2.0
	shinyWidgets 0.7.6
	plotlywrappers 0.1.0
Correlation analysis	R 4.0.4
	shiny 1.7.1
	tidyverse 1.3.1
	DT 0.19
	plotly 4.9.4.1
	scales 1.1.1
	EnvStats 2.4.0
	heatmaply 1.3.0
	reshape2 1.4.4
	readxl 1.3.1
	ggridges 0.5.3
	viridis 0.6.1
	ggthemes 4.2.4
	corrplot 0.92
	xlsx 0.6.5
	dplyr 1.0.7
Cytokine Volcano Plots	R version 4.2.2
	stringr 1.5.0
	tidyverse 1.3.2
	EnhancedVolcano 1.16.0
	yaml 2.3.7

Analysis	R Packages used
	readxl 1.4.1
	dplyr 1.1.0
	rstatix 0.7.2
	openxlsx 4.2.5.1
	renv 0.17.0

CMI, cell mediated immunity.

Table S6. Differentially Expressed Genes Between Different Days and CVnCoV Dose Level Groups

Dose group A	Dose group B	Analysis	Day X	Day Y	Numbers of differentially expressed genes
2 µg	2 µg	A2	D29	D1	48
2 µg	2 µg	A2	D2	D1	9586
2 µg	2 µg	A2	D30	D1	8985
2 µg	2 µg	A2	D30	D29	8877
2 µg	2 µg	A2	D36	D1	58
2 µg	2 µg	A2	D36	D29	234
2 µg	2 µg	A2	D8	D1	53
12 µg	12 µg	A3	D29	D1	51
12 µg	12 µg	A3	D2	D1	16250
12 µg	12 µg	A3	D30	D1	16100
12 µg	12 µg	A3	D30	D29	15668
12 µg	12 µg	A3	D36	D1	207
12 µg	12 µg	A3	D36	D29	534
12 µg	12 µg	A3	D8	D1	1879
20 µg	20 µg	A4	D29	D1	58
20 µg	20 µg	A4	D2	D1	14355
20 µg	20 µg	A4	D30	D1	14501
20 µg	20 µg	A4	D30	D29	15442
20 µg	20 µg	A4	D36	D1	868
20 µg	20 µg	A4	D36	D29	833
20 µg	20 µg	A4	D8	D1	559
2 µg	12 µg	A5	D1	D1	6
2 µg	12 µg	A5	D2	D2	3132
2 µg	12 µg	A5	D29	D29	0
2 µg	12 µg	A5	D30	D30	3677
2 µg	12 µg	A5	D36	D36	0
2 µg	12 µg	A5	D8	D8	501
2 µg	20 µg	A6	D1	D1	3
2 µg	20 µg	A6	D2	D2	6433
2 µg	20 µg	A6	D29	D29	5
2 µg	20 µg	A6	D30	D30	6587
2 µg	20 µg	A6	D36	D36	10
2 µg	20 µg	A6	D8	D8	1
12 µg	20 µg	A7	D1	D1	8
12 µg	20 µg	A7	D2	D2	0
12 µg	20 µg	A7	D29	D29	1
12 µg	20 µg	A7	D30	D30	2
12 µg	20 µg	A7	D36	D36	1
12 µg	20 µg	A7	D8	D8	0

Numbers of significantly differentially expressed genes (DEGs) between different Days (D) X and Y or CVnCoV dose groups A and B. Significant with adjusted p-value <0.05. Analysis from at least n=11 SARS-CoV-2 seronegative participants per dose group and timepoint. For participants who did not receive a second CVnCoV dose, Day 30 and 36 timepoint values were excluded from analysis.

n, number of participants

Table S7. Frequencies of SARS-CoV-2 Spike-Specific CD8+ T Cells in Longitudinal Samples from CVnCoV Vaccinated Participants from CV-NCOV-001 and CV-NCOV-002 Trials

CV-NCOV-001	Day 1	Day 29	Day 36
Participant samples tested (n)	20	18	20
Participant samples with hits (n)	1	7	15
Number of hits	1	7	18
Participants with different hits on the same day (n)	0	0	3
CV-NCOV-002	**Day 1**	**Day 29**	**Day 43**
Participant samples tested (n)	24	21	21
Participant samples with hits (n)	0	10	9
Number of hits	0	10	9
Participants with different hits on the same day (n)	0	0	0

A hit is defined as the detection of a SARS-CoV-2 spike derived tetramer-binding CD8$^+$ T cell.

For CV-NCOV-001, a total of 22 participants were included in the analysis, but 2 baseline samples were not available. For 1 participant who was tested seronegative for SARS-CoV-2, there was a hit detected on baseline. For CV-NCOV-002, a total of 24 participants were included in the analysis.

Supplementary Data
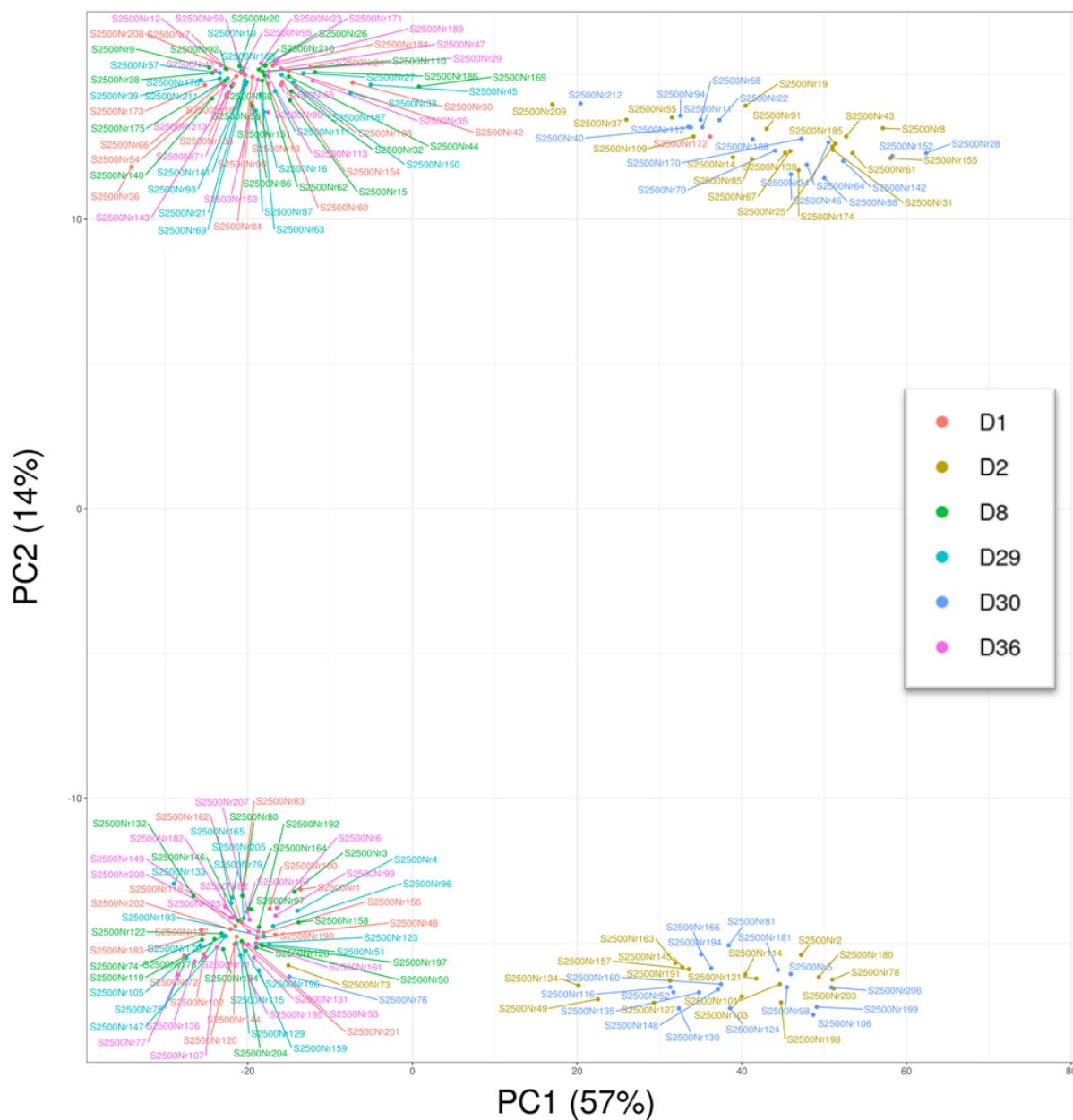
Figure S1. Principal Component Analysis of Gene Expression Data. Principal component analysis (PCA) from expression data of genes with at least two sequencing reads. The percentage values on the axes describe how much of the variance between samples is captured in this principal component (PC). Samples (S) (n=210) are colored according to Days (D) group and are from SARS-CoV-2 seronegative participants vaccinated with 2, 12 or 20 μg doses of CVnCoV. For participants who did not receive a second CVnCoV dose, Day 30 and 36 timepoint values were excluded from analysis.
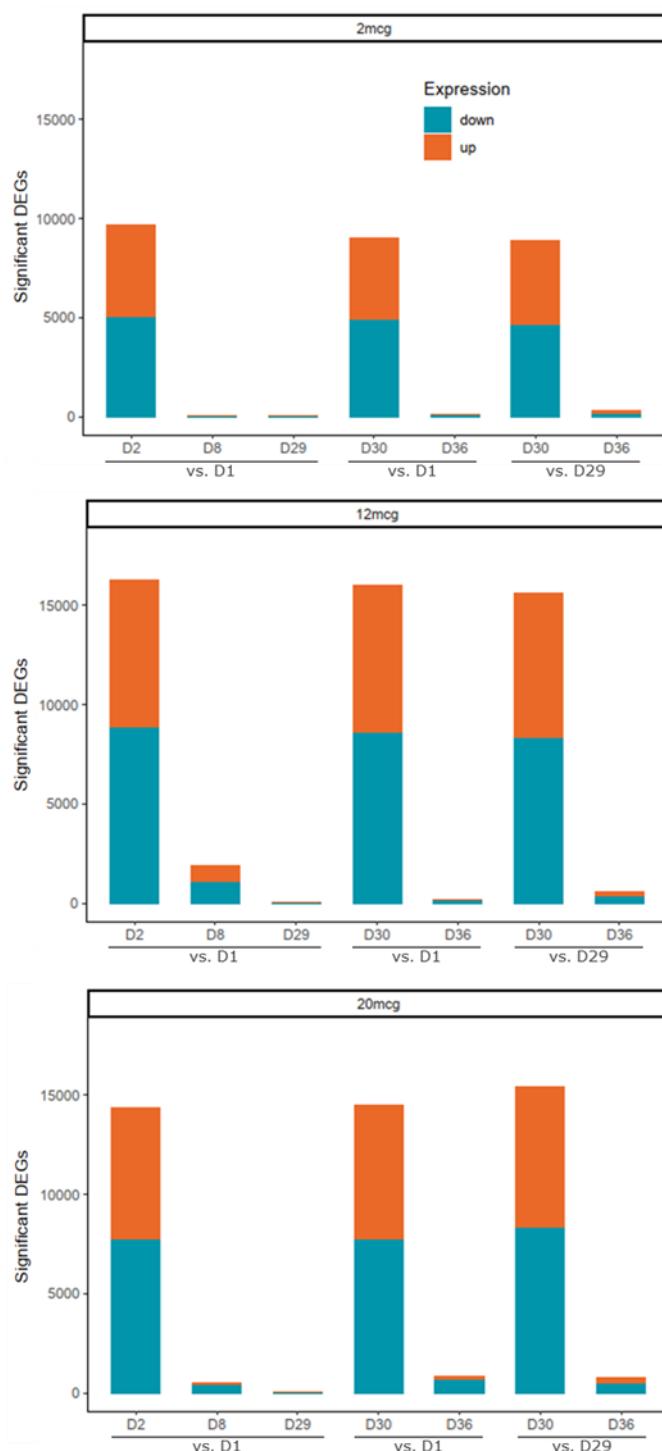
Figure S2. Significantly Differentially Expressed Genes Between Different Time Points per CVnCoV Dose Group. Numbers of significantly differentially expressed genes (DEGs) between different Days (D) per dose group (from top to bottom 2, 12 and 20 µg of CVnCoV). Significance level for adjusted p-value <0.05; downregulated DEGs in turquoise and upregulated in orange. Analysis from at least n=11 per dose group who were SARS-CoV-2 seronegative at shown timepoints. For participants who did not receive a second CVnCoV dose, Day 30 and 36 timepoint values were excluded from analysis.
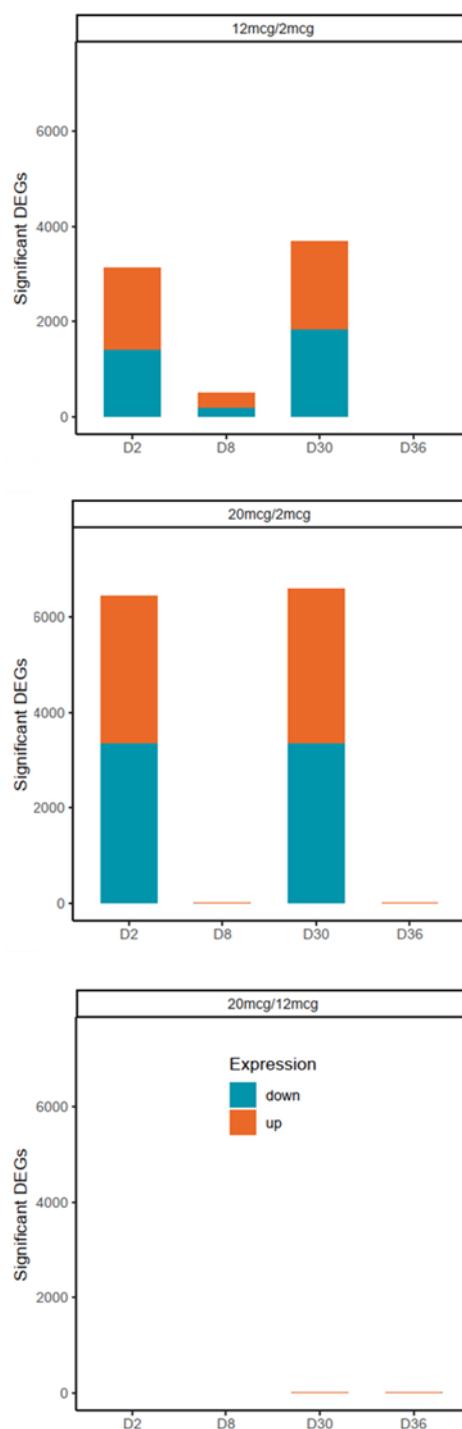
Figure S3. Significantly Differentially Expressed Genes on Different Days Between CVnCoV Dose Group. Numbers of significantly differentially expressed genes (DEGs) between different CVnCoV dose groups (from top to bottom: 12 vs. 2 µg, 20 vs. 2 µg and 20 vs. 12 µg) on one Day (D). Significance level for adjusted p-value <0.05; downregulated DEGs in turquoise and upregulated in orange. Analysis from at least n=11 per dose group who were SARS-CoV-2 seronegative at shown timepoints. For participants who did not receive a second CVnCoV dose, Day 30 and 36 timepoint values were excluded from analysis.
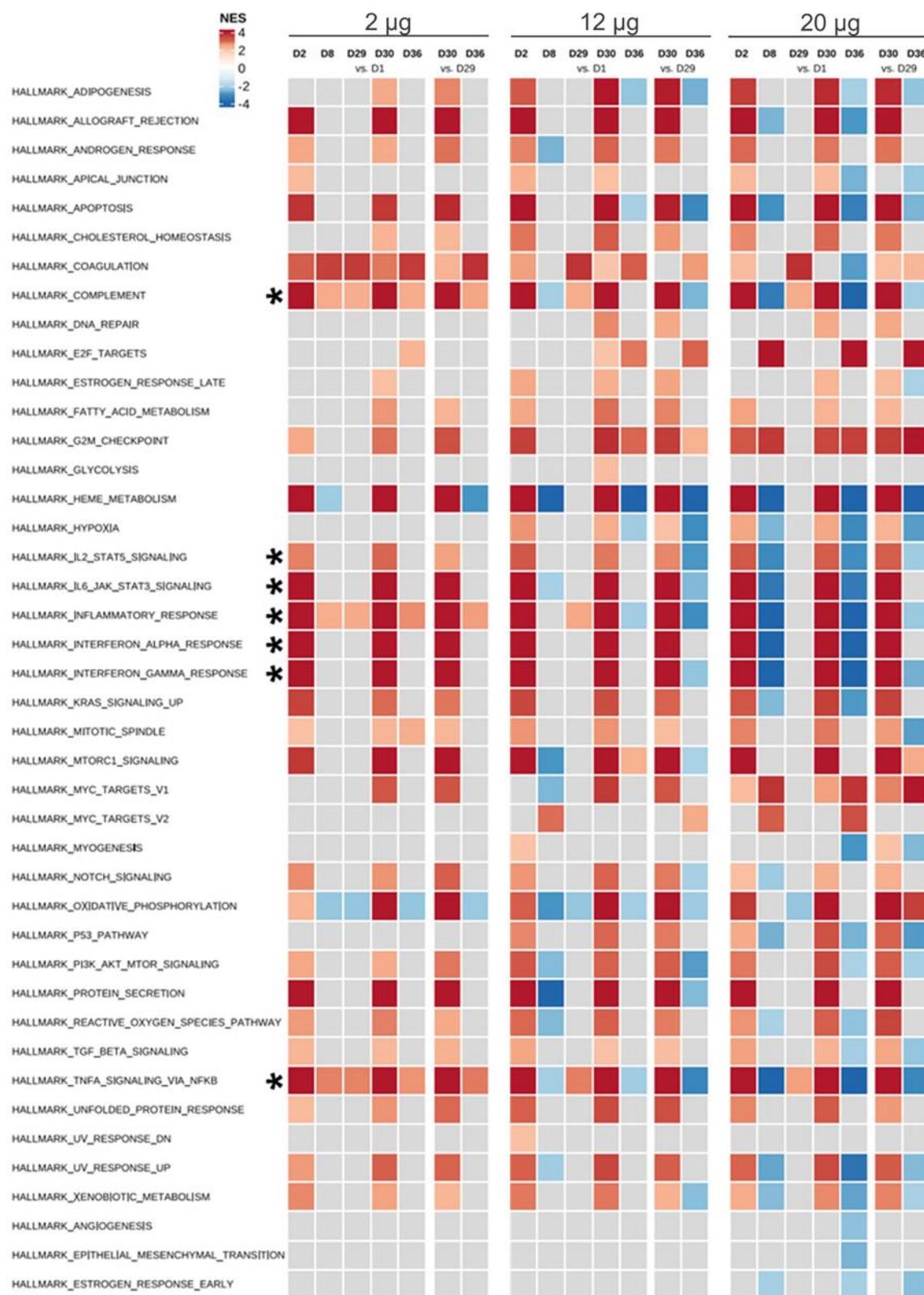
Figure S4. GSEA with HALLMARK Gene Sets After CVnCoV Vaccination. Gene set enrichment analysis (GSEA) from significantly differentially expressed genes (DEGs) summarized by HALLMARK gene sets. Analysis from at least n=11 participants per 2, 12 or 20 µg CVnCoV dose group who were SARS-CoV-2 seronegative at shown timepoints (CV-NCOV-001). For participants who did not receive a second CVnCoV dose, Day 30 and 36 timepoint values were excluded from analysis. Normalized enrichment score (NES) in grey when not enriched in GSEA or with non-significant false discovery rate (FDR > 0.01); colored NES indicates significance with FDR <0.01, red with positive score indicates upregulation and blue (negative score) downregulation of listed gene sets between compared groups. * indicates gene sets related to inflammatory processes.

D, Day; IL, interleukin; KRAS, 15kirsten rat sarcoma viral oncogene homolog; MYC, myelocytomatosis oncogene; n, number of participants; NFKB, nuclear factor kappa B; TGF, tumor growth factor; TNFA, tumor necrosis factor alpha.
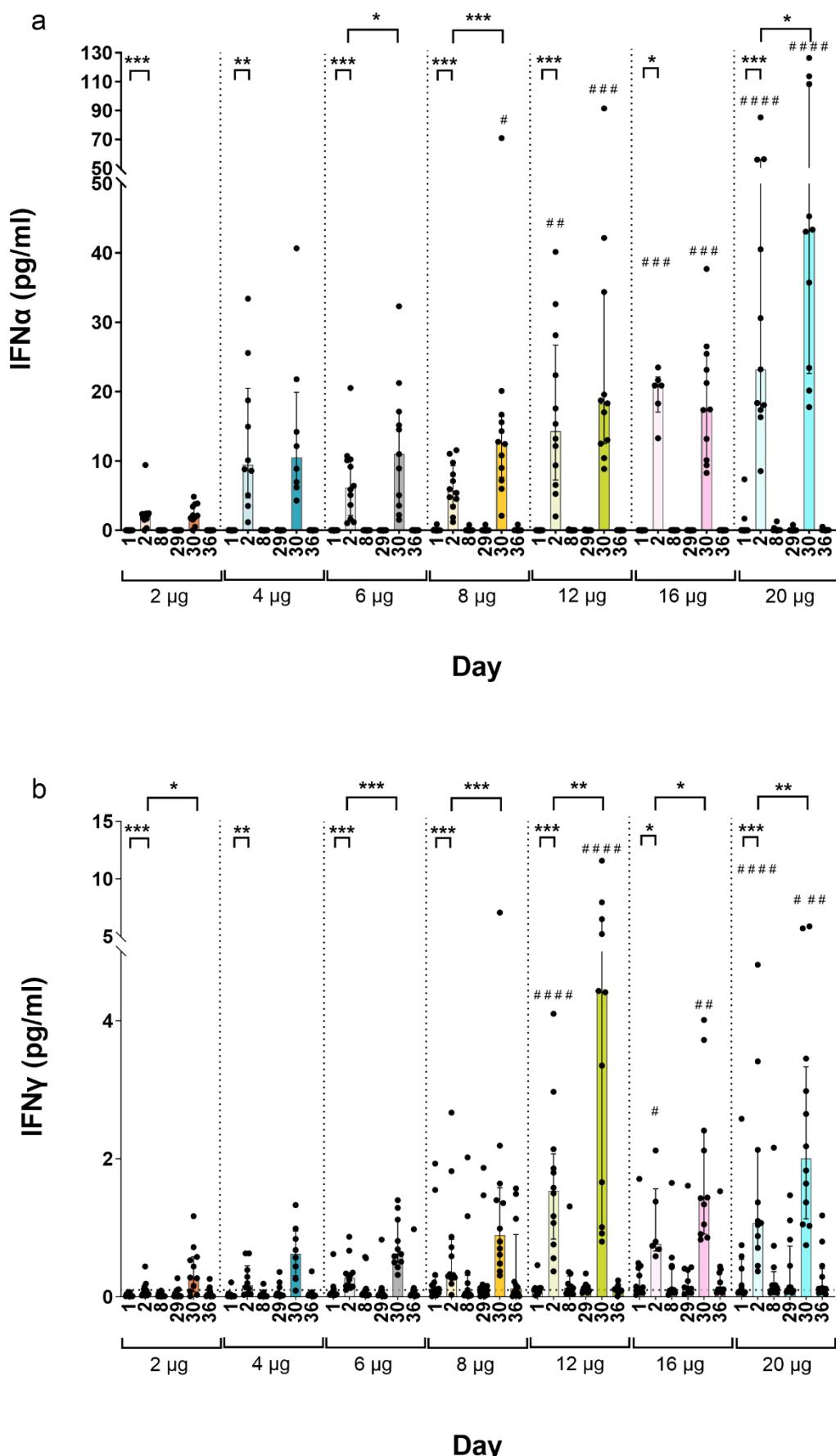
Figure S5. Serum Interferon Concentration by Dose and Trial Visit. IFNα (a) and IFNγ (b) serum concentrations on Days 1, 2, 8, 29, 30 and 36 in SARS-CoV-2 seronegative participants vaccinated with two doses of 2, 4, 6, 8, 12, 16 or 20 μg of CVnCoV (CV-NCOV-001). If participants did not receive a second CVnCoV dose, Day 30 and 36 values were excluded from analysis. Bars show median values with interquartile range. For IFNγ (b), dotted line indicates the lower level of quantification (LLOQ). Values below LLOQ were reported as extrapolated value. Values below detection limit were set to the lowest extrapolated value reported for respective cytokine. Statistical comparisons in one dose group (indicated as *) were analyzed using a two-tailed Wilcoxon signed-rank test and for Day 2 and Day 30 timepoints between dose groups using Kruskal-Wallis ANOVA with Dunn's multiple comparisons testing with 2 μg as the reference (indicated as #). Significance is defined as: */# $p \leq 0.05$, **/# # $p \leq 0.01$, ***/# # # $p \leq 0.001$ or # # # # $p \leq 0.0001$.

IFNα, interferon alpha; IFNγ, interferon gamma.

Figure S6. Induction of Serum Cytokines Post CVnCoV Vaccination. IP-10 (a), CXCL11 (b), MCP-1 (c) and IL-10 (d) serum concentrations on Days 1, 2, 8, 29, 30 and 36 in SARS-CoV-2 seronegative participants vaccinated with two CVnCoV doses of 2, 4, 6, 8, 12, 16 or 20 µg (CV-NCOV-001). If participants did not receive a second CVnCoV dose, Day 30 and 36 values were excluded from analysis. Bars show median values with interquartile range, dotted line indicates the lower level of quantification (LLOQ). Values below LLOQ were reported as extrapolated value. Values below detection limit were set to the lowest extrapolated value reported for respective cytokine. Statistical comparisons in one dose group (indicated as *) were analyzed using a two-tailed Wilcoxon signed-rank test and for Day 2 and Day 30 timepoints between dose groups using Kruskal-Wallis ANOVA with Dunn's multiple comparisons testing with 2 µg as the reference (indicated as #). Significance is defined as: */# $p \leq 0.05$, **/## $p \leq 0.01$, ***/### $p \leq 0.001$ or #### $p \leq 0.0001$.

CXCL, chemokine (C-X-C motif) ligand 1; IL-10, interleukin 10; IP-10, interferon-gamma inducible protein 10kDa; MCP-1, membrane cofactor protein.
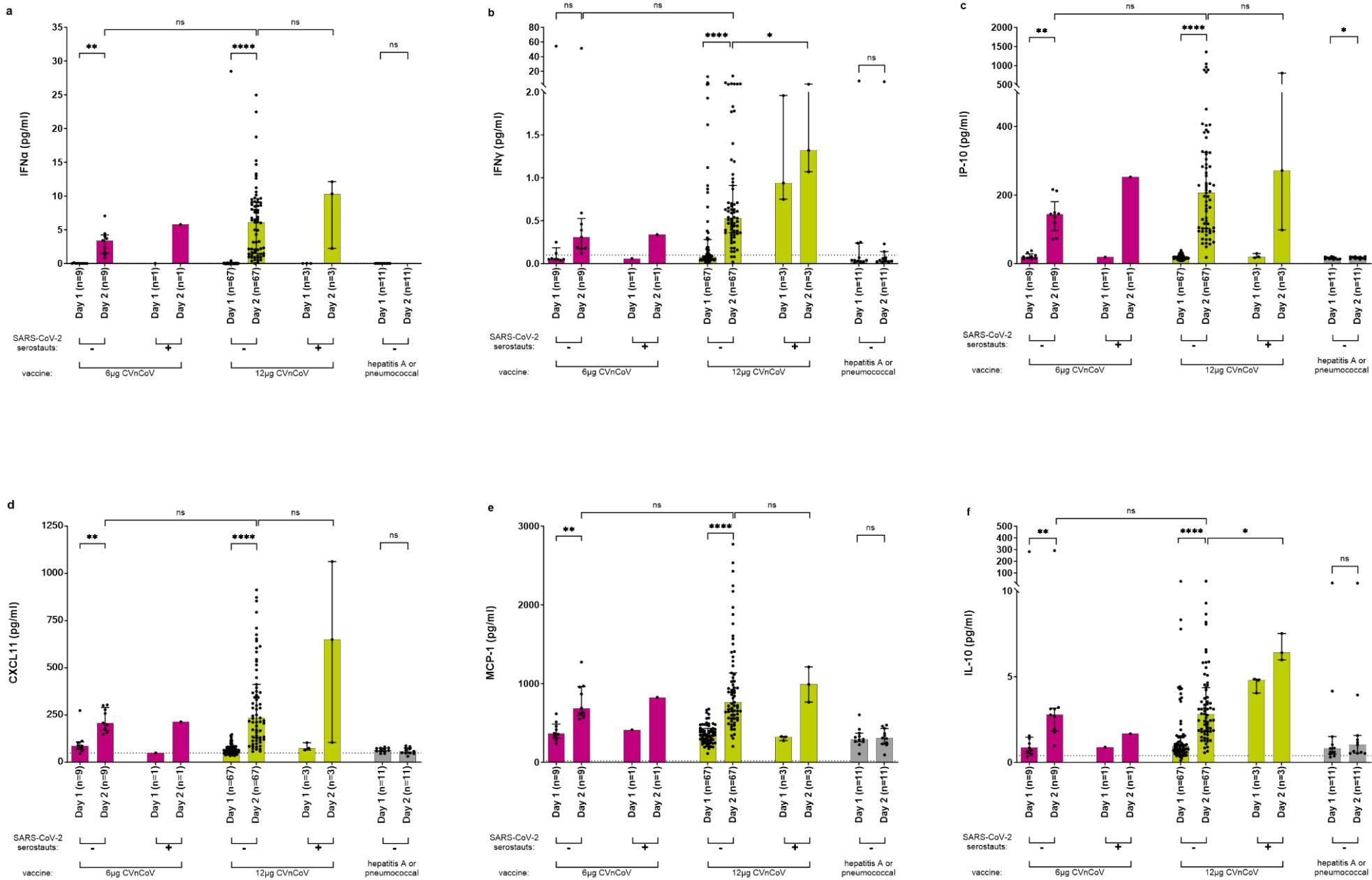
Figure S7. Serum Cytokine Concentrations Post CVnCoV Vaccination By SARS-CoV-2 Serostatus. CV-NCOV-002 cytokine serum concentrations on Day 1 and Day 2 in participants vaccinated with 6 or 12 μg of CVnCoV stratified by SARS-CoV-2 seronegative or seropositive serostatus and Active Control participants who received licensed hepatitis A or pneumococcal vaccine. Bars show median with interquartile range, dotted line indicates the lower level of quantification (LLOQ). Values below LLOQ were reported as extrapolated value. If no value was detected (below limit), the lowest extrapolated value for respective cytokine was used. Groups were compared using either a two-tailed Wilcoxon signed-rank test (pairwise comparison between Day 1 and 2 values in the same group) or by Mann-Whitney test (unpaired comparison between Day 2 values of distinct groups). Statistical significance was defined as: * p≤0.05, ** p≤0.01 or **** p≤0.0001; not significant (ns) p>0.05.

CXCL, chemokine (C-X-C motif) ligand 1; IFNα, interferon alpha; IFNγ, interferon gamma; IL, interleukin; IP-10, interferon-gamma inducible protein 10kDa; MCP-1, membrane cofactor protein 1.
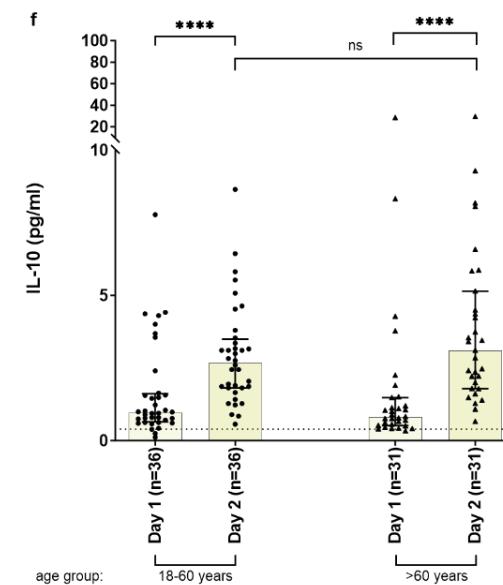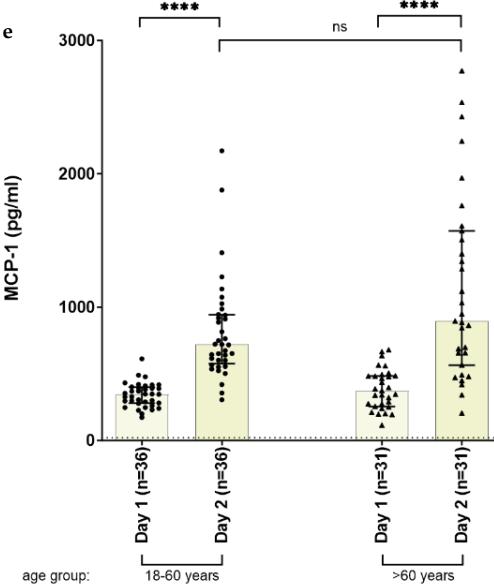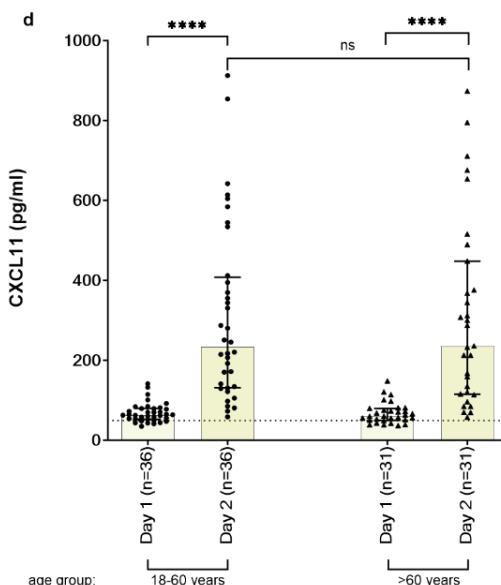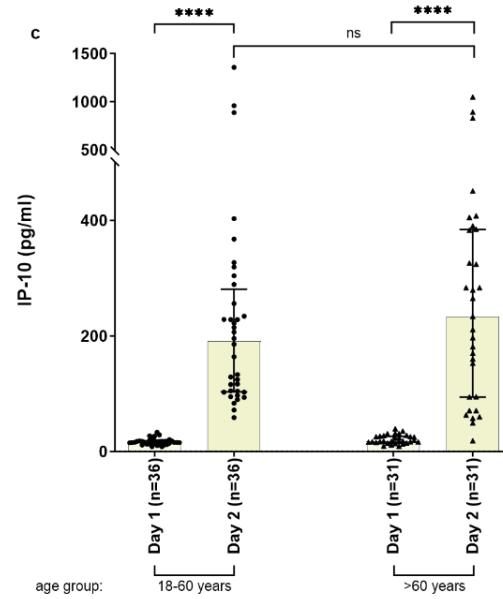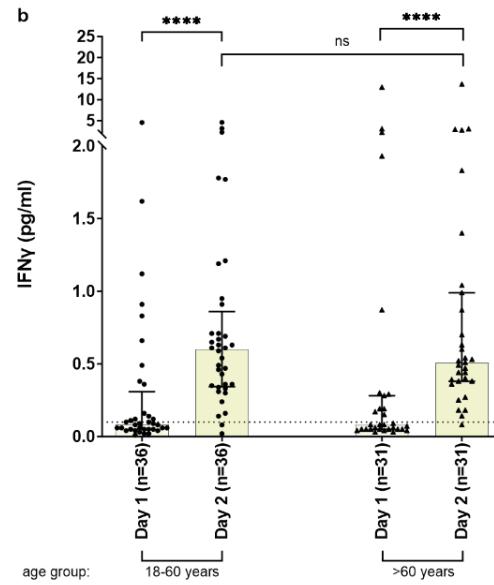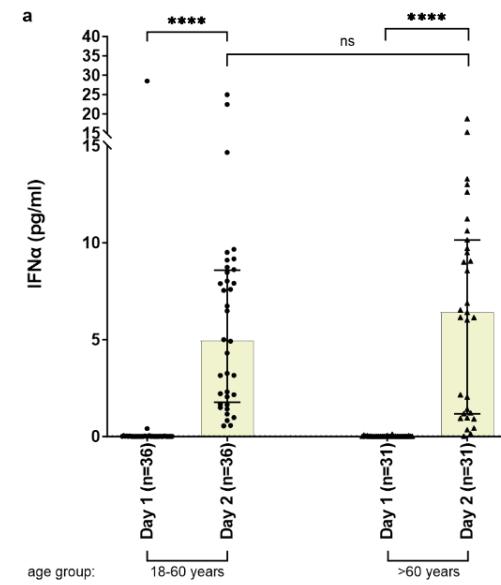
Figure S8. Serum Cytokine Concentrations by Trial Visit and Age Group. IFNα (a), IFNγ (b), IP-10 (c), CXCL11 (d), MCP-1 (e) and IL-10 (f) serum concentrations on Day 1 and Day 2 in SARS-CoV-2 seronegative participants from CV-NCOV-002 trial. Participants administered with a 12 μg CVnCoV dose were stratified by younger (18–60 years of age) and older (over 60 years of age) groups. Bars show median values with interquartile range. Dotted lines indicate lower level of quantification (LLOQ). Values below LLOQ were reported as extrapolated value. If no value was detected (below limit), the lowest extrapolated value for respective cytokine was used. Groups were statistically compared using either a two-tailed Wilcoxon signed-rank test (pairwise comparison between Day 1 and 2 values in the same age group) or by Mann-Whitney test (unpaired comparison between Day 2 values of distinct age groups). Statistical significance was defined as: **** $p \leq 0.0001$; not significant (ns) if $p > 0.05$.

CXCL, chemokine (C-X-C motif) ligand 1; IFNα, interferon alpha; IFNγ, interferon gamma; IL-10, interleukin 10; IP-10, interferon-gamma inducible protein 10kDa; MCP-1, membrane cofactor protein 1; n, number of participants.

	CXCL11 Day 2	CXCL11 Day 30	IFNα Day 2	IFNα Day 30	IFNγ Day 2	IFNγ Day 30	IL-10 Day 2	IL-10 Day 30	IP-10 Day 2	IP-10 Day 30	MCP-1 Day 2	MCP-1 Day 30
Any symptom_Vaccination 2	0.52	0.46	0.56	0.64	0.58	0.55	0.62	0.55	0.42	0.47		0.26
Cytokine CXCL11_Day 2		0.78	0.88	0.74	0.61	0.58	0.65	0.53	0.82	0.71	0.65	0.58
Cytokine CXCL11_Day 30			0.65	0.81	0.58	0.59	0.52	0.65	0.58	0.86	0.41	0.68
Cytokine IFNα_Day 2				0.83	0.67	0.54	0.71	0.55	0.86	0.71	0.62	0.5
Cytokine IFNα_Day 30					0.61	0.55	0.69	0.71	0.68	0.86	0.44	0.58
Cytokine IFNγ_Day 2						0.85	0.72	0.7	0.58	0.56	0.39	0.4
Cytokine IFNγ_Day 30							0.58	0.65	0.44	0.56	0.35	0.41
Cytokine IL-10_Day 2								0.77	0.64	0.56	0.41	0.39
Cytokine IL-10_Day 30									0.46	0.65	0.33	0.56
Cytokine IP-10_Day 2										0.71	0.54	0.42
Cytokine IP-10_Day 30											0.42	0.63
Cytokine MCP-1_Day 2												0.76

Figure S9. Correlation of Serum Cytokine Levels After First and Second Doses of CVnCoV Vaccine. Spearman correlation analysis from n=65 from a pool of CV-NCOV-001 participants who received both two doses of CVnCoV (2,4,6,8,12,16 or 20 µg) and who were SARS-CoV-2 seronegative for shown time points. Fields with significant p≤0.05 are colored according to Spearman correlation coefficient. Reactogenicity was calculated per participant as maximum severity grade (0=absent, 1=mild, 2=moderate, 3=severe) of vaccine-related solicited adverse events (any local or systemic sign/symptom) after both doses. Analysis done with serum cytokine concentrations on indicated days.

CXCL, chemokine (C-X-C motif) ligand 1; IFNα, interferon alpha; IFNg, interferon gamma; IL-10, interleukin 10; IP-10, interferon-gamma inducible protein 10kDa; MCP-1, membrane cofactor protein 1; n, number of participants.

a 6 µg dose

b 12 µg dose
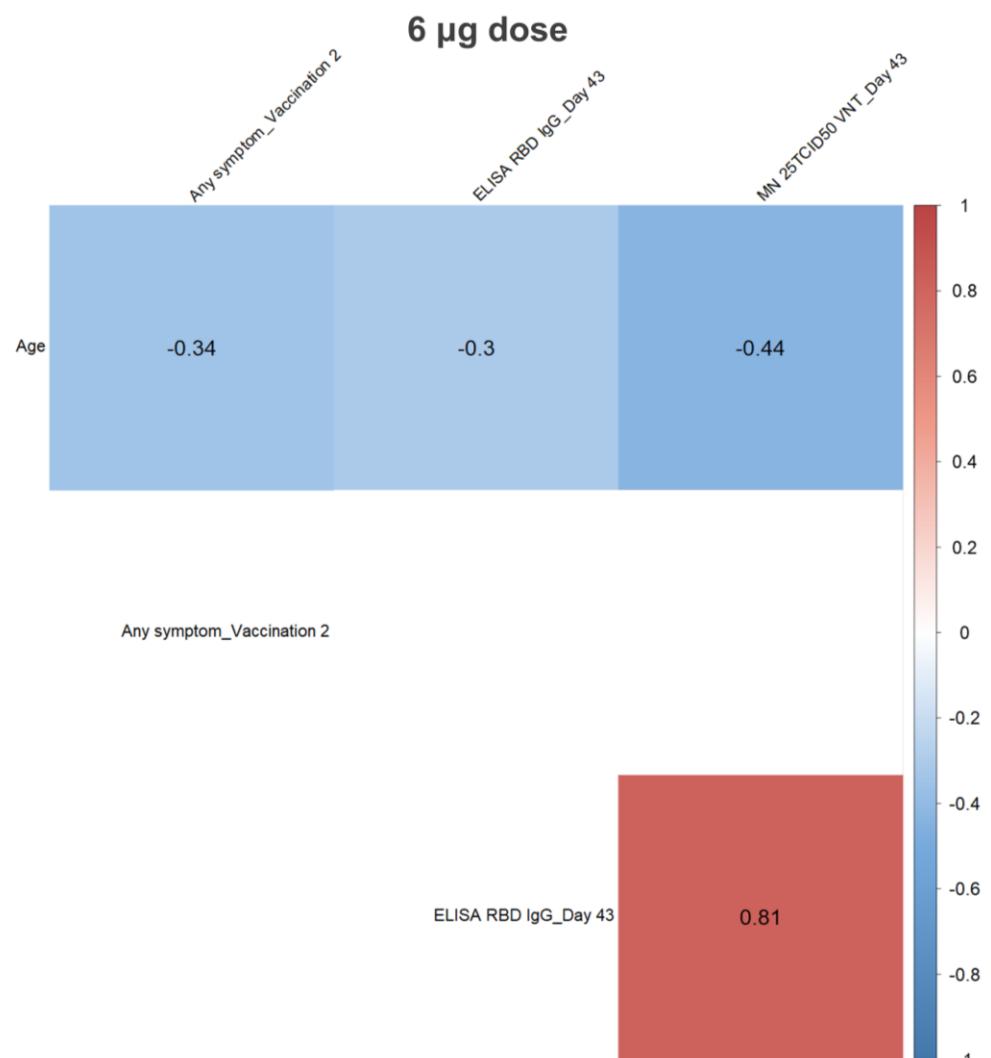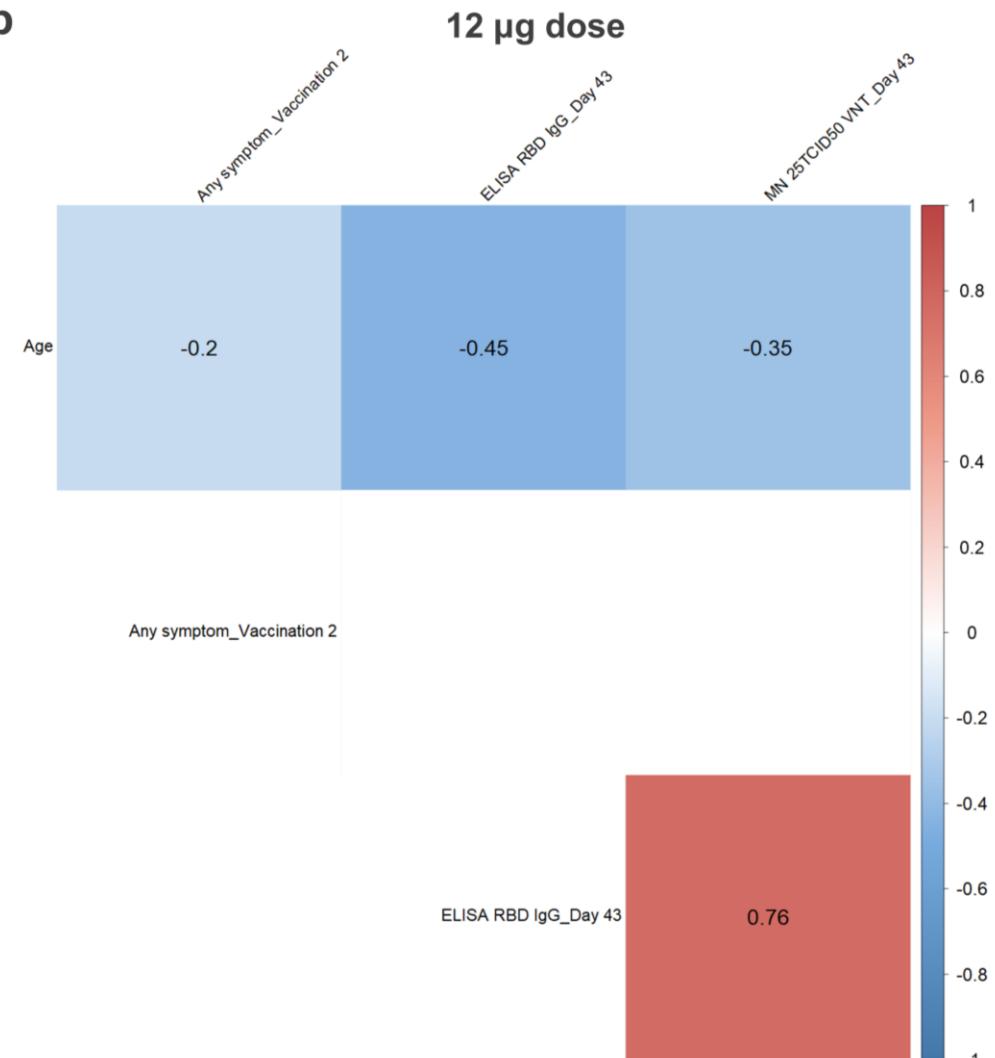
Figure S10. Correlation of CVnCoV Reactogenicity and Humoral Immunity with Participant's Age. Spearman correlation analysis from n=58 (a, 6 μg) and n=344 (b, 12 μg) pairs from a pool of CV-NCOV-001 and -002 participants who received both CVnCoV doses and who were SARS-CoV-2 seronegative for shown time points. Fields with significant p≤0.05 are colored according to Spearman correlation coefficient. Reactogenicity was calculated per participant as maximum severity grade (0=absent, 1=mild, 2=moderate, 3=severe) of vaccine-related solicited adverse events (any local or systemic sign/symptom) after both doses. Analysis performed using VNT and RBD IgG assay titers on indicated days and participants' age in years.

ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; n, number of participants; RBD, receptor binding domain; VNT, virus neutralizing antibody.

a

CV-NCOV-002 CD4/Poly-functional T cells (Sp1+Sp2)

b

CV-NCOV-002 CD8/Poly-functional T cells (Sp1+Sp2)
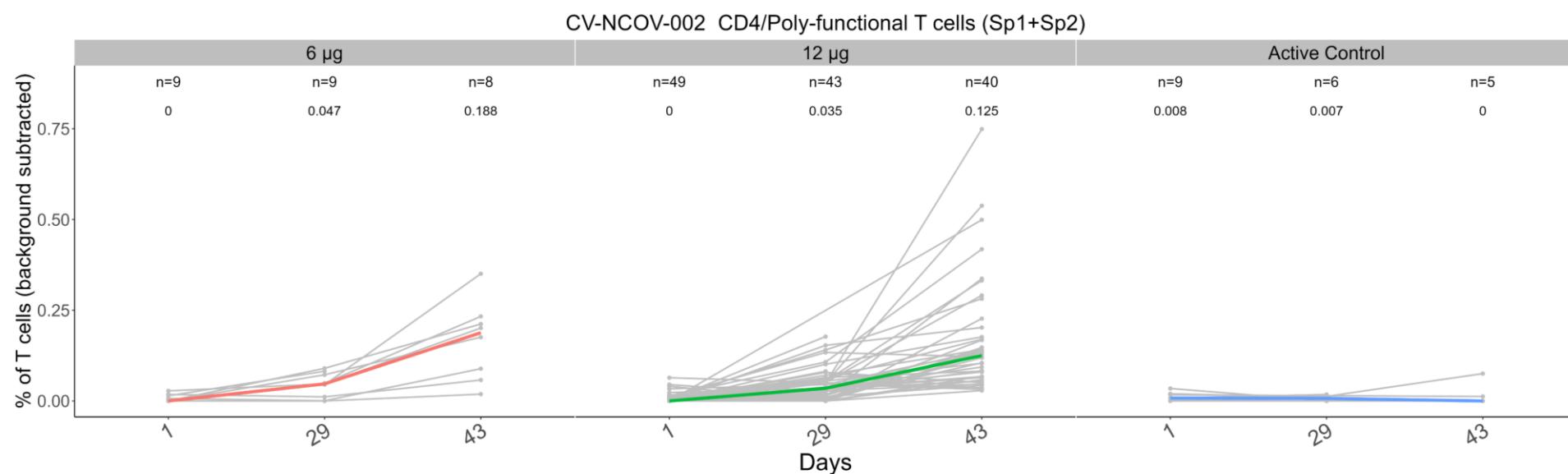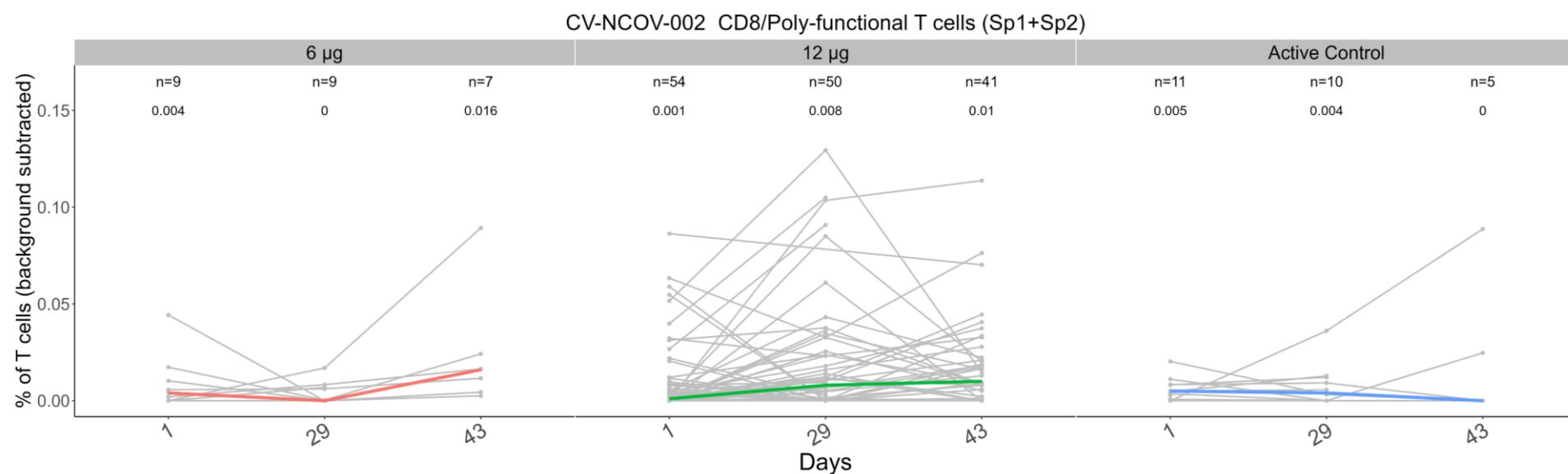
Figure S11. Ex Vivo ICS Analysis of Spike-Specific CD4^{+} and CD8^{+} T Cell Responses Post Vaccination in CV-NCOV-002 clinical trial. SARS-CoV-2-spike-specific CD4^{+} and CD8^{+} T cells were measured by intracellular cytokine staining after stimulation with individual spike pool 1 (Sp1) and spike pool 2 (Sp2). The total T cell frequencies were plotted for Day 1, Day 29 and Day 43 after participants received CVnCoV (6 or 12 μg) or an active control vaccine. Data from participants who were seronegative at baseline are shown. Values from individual participants are represented by grey symbols connected by grey lines, whilst colored lines display group medians. Values above data points indicate median of CD4^{+} (a) and CD8^{+} (b) T cells frequencies per time point.

ICS, intracellular cytokine staining; n, number of participants.
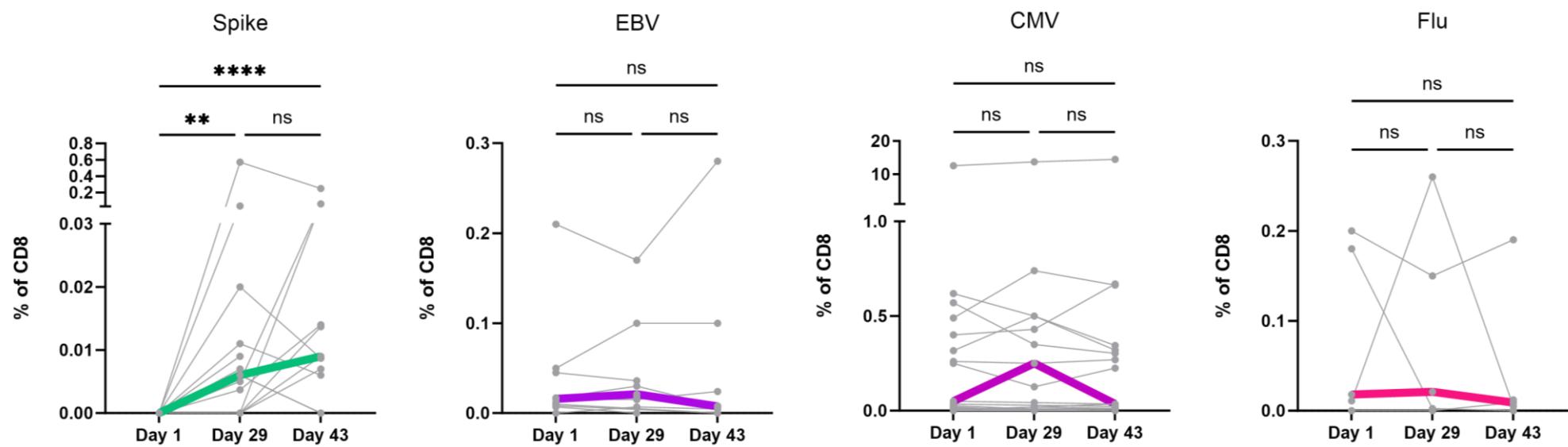
Figure S12. Frequencies of Antigen-Specific CD8+ T cells in Longitudinal Samples from CVnCoV Vaccinated Trial Participants. Virus specific CD8+ T cell responses were assessed using pooled data from CV-NCOV-002 participants vaccinated with different dose levels of CVnCoV (6 and 12 µg). HLA class I tetramer peptide antigens derived from CMV, EBV, influenza virus (Flu) or SARS-CoV-2 spike protein were used to stimulate antigen-specific CD8+ T cell responses. The percentage of CD8+ T cells for individual participants are represented by grey symbols connected by grey lines, whilst colored lines display group medians. Statistical analysis was performed using Kruskal Wallis test followed by Dunn's test for multiple comparisons. Statistical significance defined as ** $p \leq 0.01$, *** $p \leq 0.001$ or not significant (ns) if $p > 0.05$.

CMV, cytomegalovirus; EBV, Epstein-Barr virus; Flu, influenza; ns, not significant.
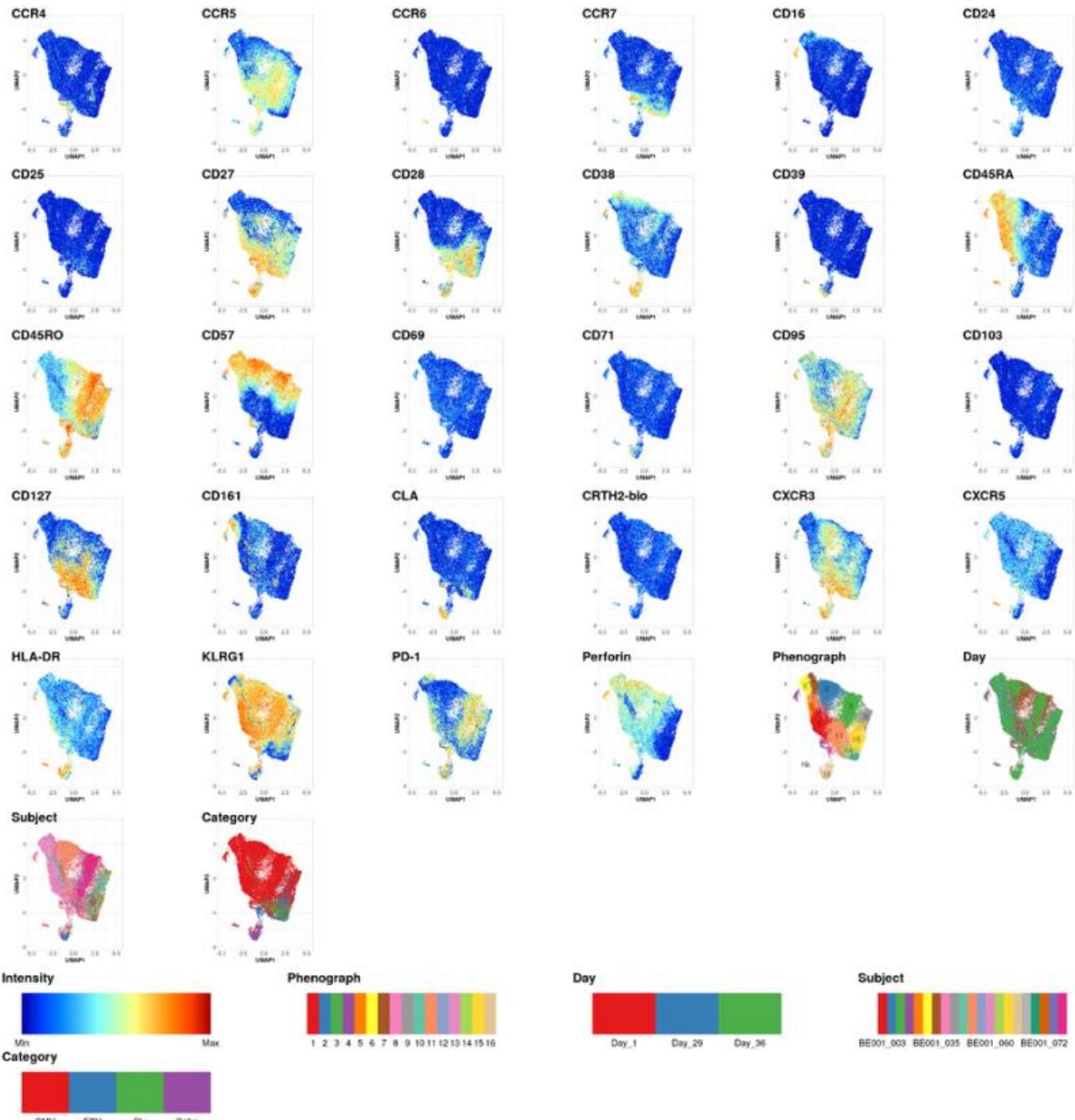
Figure S13. Phenotypes of Antigen-Specific CD8⁺ T cells From CVnCoV Vaccinated Individuals. UMAP plots display the expression intensity of phenotypical markers in CD8⁺ T cells specific to HLA class I tetramer peptide antigens from subjects analyzed in CV-NCOV-001. Category shows the HLA class I tetramer peptide antigens derived from CMV, EBV, or Influenza virus (Flu) controls or from SARS-CoV-2 Spike protein. Phenograph overlay created by unsupervised clustering algorithm.

CMV, cytomegalovirus; EBV, Epstein-Barr virus; Flu, influenza.
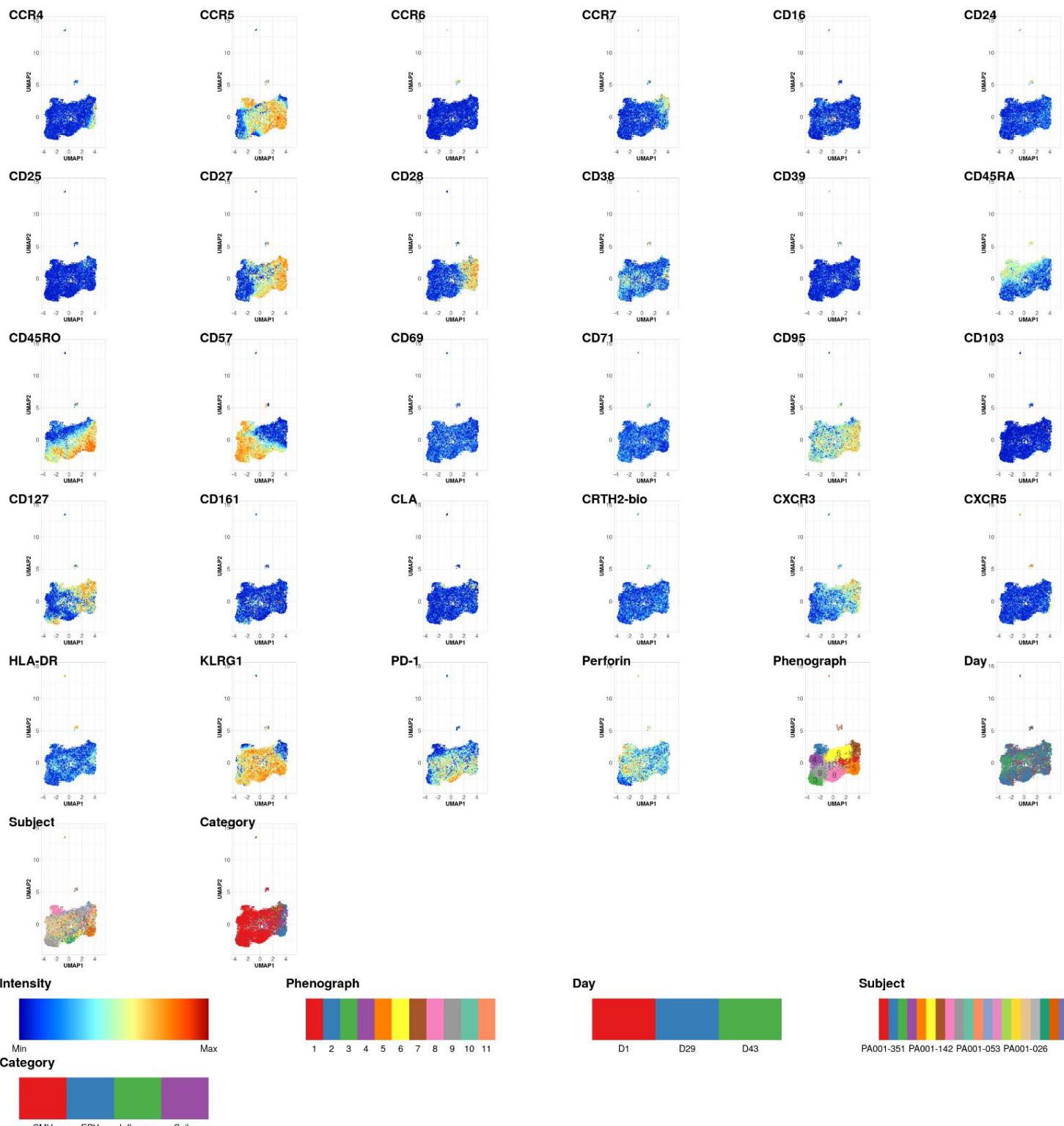
Figure S14. Phenotypes of Antigen-Specific CD8⁺ T cells From CVnCoV Vaccinated Individuals. UMAP plots display the expression intensity of phenotypical markers in CD8⁺ T cells specific to HLA class I tetramer peptide antigens from subjects analyzed in CV-NCOV-002. Category shows the HLA class I tetramer peptide antigens derived from CMV, EBV, or Influenza virus controls or from SARS-CoV-2 Spike protein. Phenograph overlay created by unsupervised clustering algorithm.

CMV, cytomegalovirus; EBV, Epstein-Barr virus.

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