SUPPLEMENTARY FIGURES

Figure S1

A

B

	NVX-CoV2373			Placebo			
Samples	32	32	27	4	4	4
Geo. Mean	0.03	0.17	0.25	0.02	0.02	0.02
% responders	10	53	78	0	0	0

C

	NVX-CoV2373		
Samples	29	31	29
Geo. Mean	0.03	0.03	0.03
% responders	14	13	14

D

	NVX-CoV2373		
Samples	17	17	16
Geo. Mean	0.07	0.07	0.06
% responders	47	47	38

E

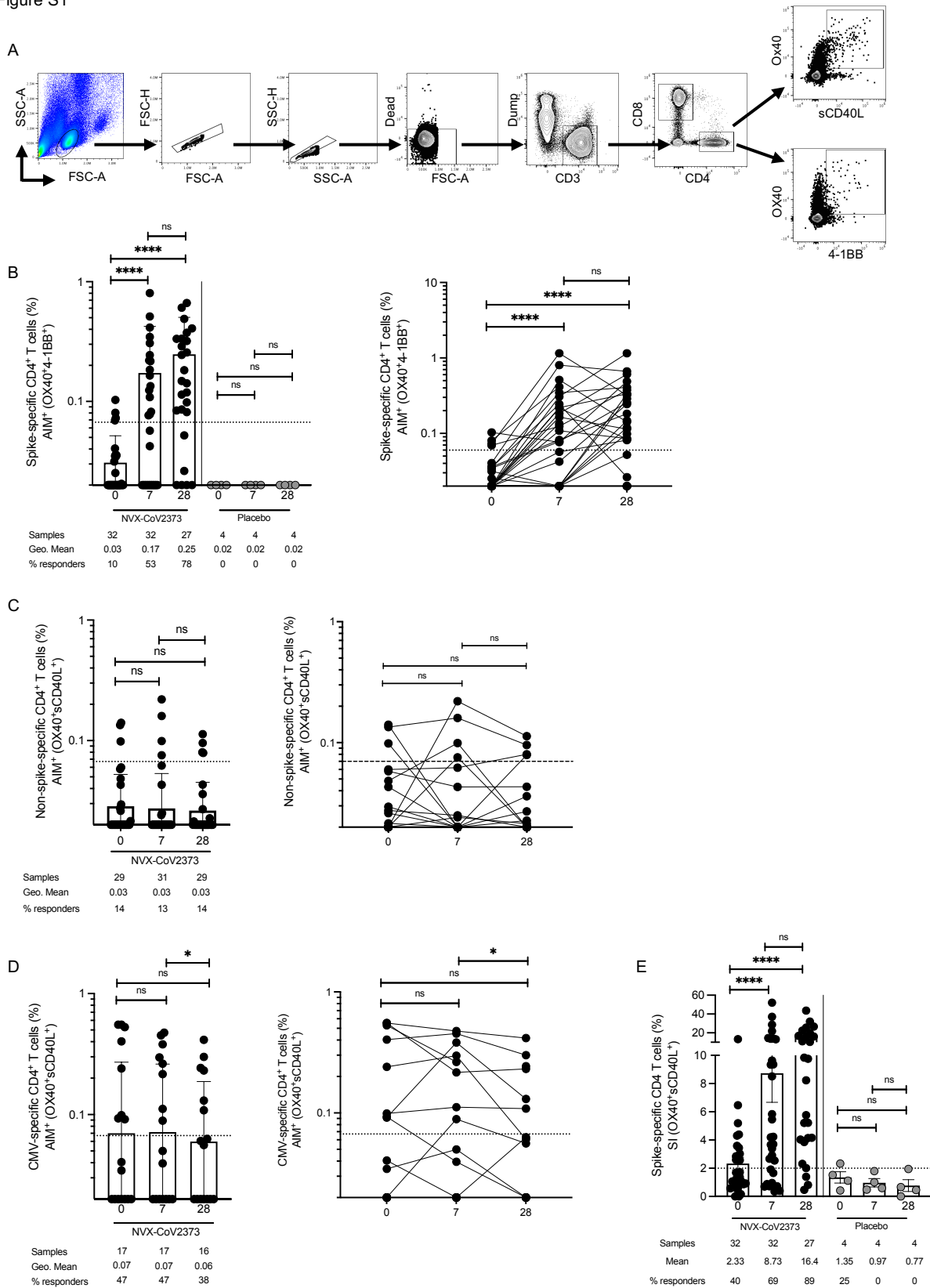|	NVX-CoV2373			Placebo		
Samples	32	32	27	4	4	4
Mean	2.33	8.73	16.4	1.35	0.97	0.77
% responders	40	69	89	25	0	0

Figure S1. Spike-specific CD4$^+$ T cells following NVX-CoV2373 vaccination. **(A)** FACS gating strategy used to define AIM$^+$CD4$^+$ T cells at D0, D7 and D28 post-vaccination. **(B)** Spike-specific OX40$^+$4-1BB$^+$ AIM$^+$ CD4$^+$ T cell responses in vaccinees (black dots) and placebo controls (grey dots), graphed as both grouped and paired comparisons for the vaccine cohort (**C)** SARS-CoV-2 non-spike-specific OX40$^+$CD40L$^+$ AIM$^+$ CD4$^+$ T cell responses in vaccinees at D0, 7 and 28 post-vaccination. **(D)** CMV class I/II MP-specific OX40$^+$CD40L$^+$ AIM$^+$ CD4$^+$ T cell responses in vaccinees at D0, 7 and 28 post-vaccination. **(E)** Stimulation indices for OX40$^+$CD40L$^+$ CD4$^+$ AIM$^+$ T cells in vaccinees and controls at D0, D7 and D28 post-vaccination. Dotted line indicates LOQ, and is calculated as the geometric mean of all sample DMSO wells multiplied by the geometric SD factor. % responders is calculated as responses $\geq$ LOQ divided by the total samples in the group. Paired data were analyzed by Wilcoxon signed rank test. Log data shown with geometric mean $\pm$ geometric SD. Linear data shown with mean $\pm$ SEM. * p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001.
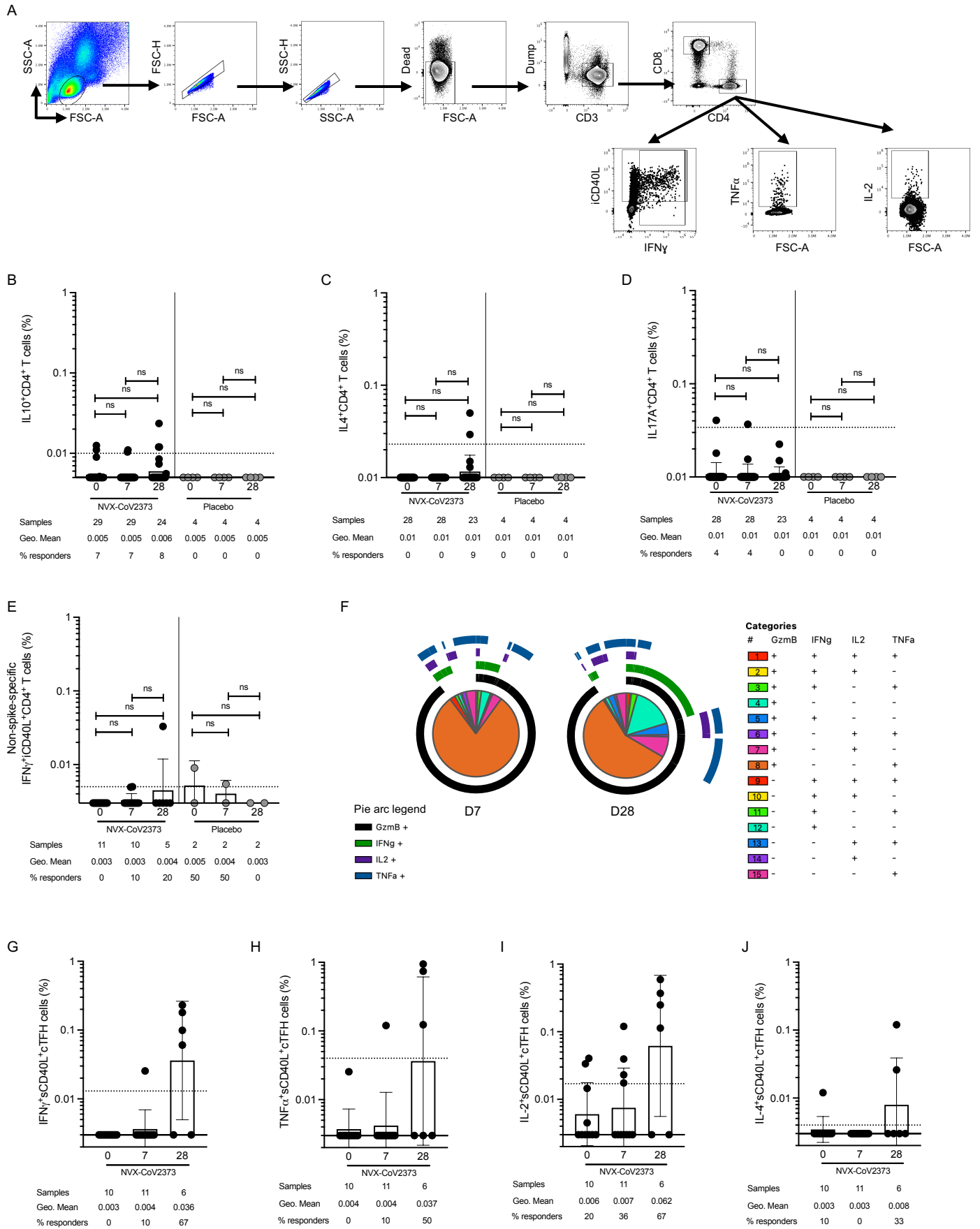
Figure S2

A

B

C

D

		NVX-CoV2373		Placebo		
	0	7	28	0	7	28
Samples	29	29	24	4	4	4
Geo. Mean	0.005	0.005	0.006	0.005	0.005	0.005
% responders	7	7	8	0	0	0

		NVX-CoV2373		Placebo		
	0	7	28	0	7	28
Samples	28	28	23	4	4	4
Geo. Mean	0.01	0.01	0.01	0.01	0.01	0.01
% responders	0	0	9	0	0	0

		NVX-CoV2373		Placebo		
	0	7	28	0	7	28
Samples	28	28	23	4	4	4
Geo. Mean	0.01	0.01	0.01	0.01	0.01	0.01
% responders	4	4	0	0	0	0

E

		NVX-CoV2373		Placebo		
	0	7	28	0	7	28
Samples	11	10	5	2	2	2
Geo. Mean	0.003	0.003	0.004	0.005	0.004	0.003
% responders	0	10	20	50	50	0

F

Categories

#	GzmB	IFNg	IL2	TNFa
1	+	+	+	+
2	+	+	+	-
3	+	+	-	+
4	+	-	-	-
5	+	+	-	-
6	+	-	+	-
7	+	-	+	-
8	+	-	-	+
9	-	+	+	+
10	-	+	+	-
11	-	+	-	-
12	-	+	-	-
13	-	-	+	+
14	-	-	+	-
15	-	-	-	+

Pie arc legend
GzmB +
IFNg +
IL2 +
TNFa +

D7   D28

G

		NVX-CoV2373	
	0	7	28
Samples	10	11	6
Geo. Mean	0.003	0.004	0.036
% responders	0	10	67

H

		NVX-CoV2373	
	0	7	28
Samples	10	11	6
Geo. Mean	0.004	0.004	0.037
% responders	0	10	50

I

		NVX-CoV2373	
	0	7	28
Samples	10	11	6
Geo. Mean	0.006	0.007	0.062
% responders	20	36	67

J

		NVX-CoV2373	
	0	7	28
Samples	10	11	6
Geo. Mean	0.003	0.003	0.008
% responders	10	0	33

Figure S2. Cytokine-producing spike-specific CD4$^+$ T cell responses following NVX-CoV2373 vaccination. **(A)** FACS gating strategy used to define cytokine$^+$ CD4$^+$ T cells at D0, D7 and D28 post-vaccination. Proportion of **(B)** IL10$^+$, **(C)** IL4$^+$ and **(D)** IL17A$^+$ spike-specific CD4 T cells detected following peptide stimulation. **(E)** IFNɣ$^+$CD40L$^+$ CD4$^+$ T cells measured in response to SARS-CoV-2 Remainder MP stimulation. **(F)** Pie charts depicting the proportion of spike-specific CD4$^+$ T cells exhibiting different combination of cytokines and/or effector molecules at D7 and D28 post-1st immunization. **(G)** sCD40L$^+$IFNɣ$^+$, **(H)** sCD40L$^+$TNFα^+, **(I)** sCD40L$^+$IL-2$^+$, and **(J)** sCD40L$^+$IL-4$^+$ cTFH cells detected following peptide stimulation (cTFH cells defined as CXCR5$^+$CD4$^+$ T cells). Dotted line indicates LOQ, and is calculated as the geometric mean of all sample DMSO wells multiplied by the geometric SD factor. % responders is calculated as responses ≥ LOQ divided by the total samples in the group. Paired data were analyzed by Wilcoxon signed rank test. Data shown with geometric mean $\pm$ geometric SD. * p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001. Boolean data were input into SPICE, an open-source graphing tool for complex data sets (32), to create pie graphs.
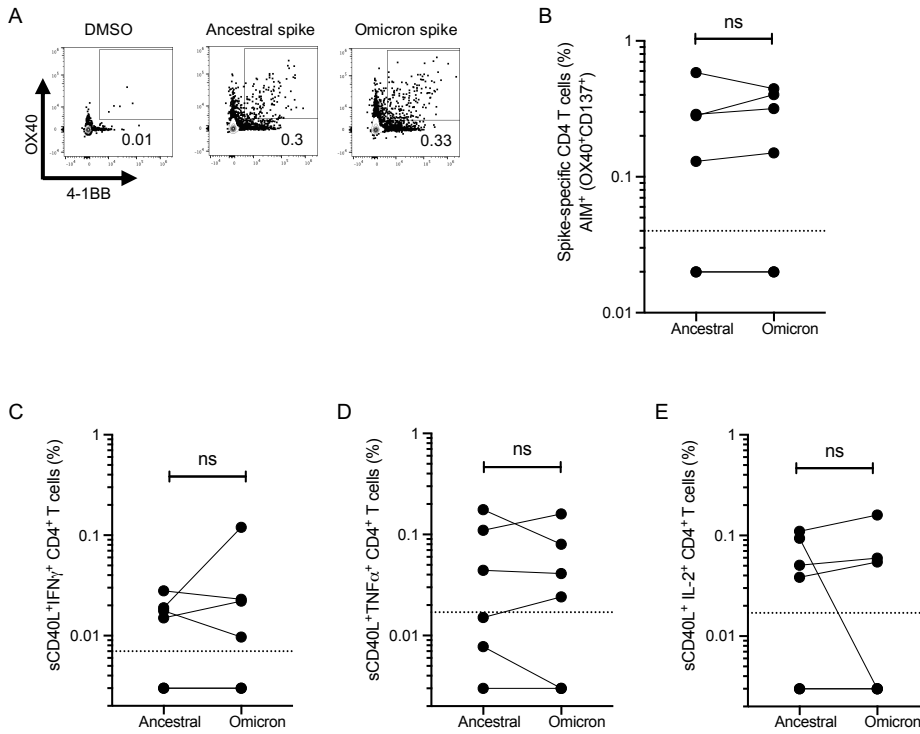
Figure S3. Ancestral and Omicron spike-specific CD4+ T cells responses induced by NVX-CoV2373 vaccination. **(A)** Representative FACS gating of OX40+CD137+ CD4+ T cells following stimulation with Ancestral or Omicron SARS-2 spike MP. **(B)** Paired AIM+ CD4+ **(C)** sCD40L+IFNγ+ **(D)** sCD40L+TNFα+, and **(E)** sCD40L+IL-2+ CD4+ T cells one week post-2nd immunization and stimulated with either Ancestral or Omicron SARS-2 spike MP. Dotted line indicates LOQ, and is calculated as the geometric mean of all sample DMSO wells multiplied by the geometric SD factor. Paired data were analyzed by Wilcoxon signed rank test. Data shown with geometric mean ± geometric SD. * p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001.

Figure S4
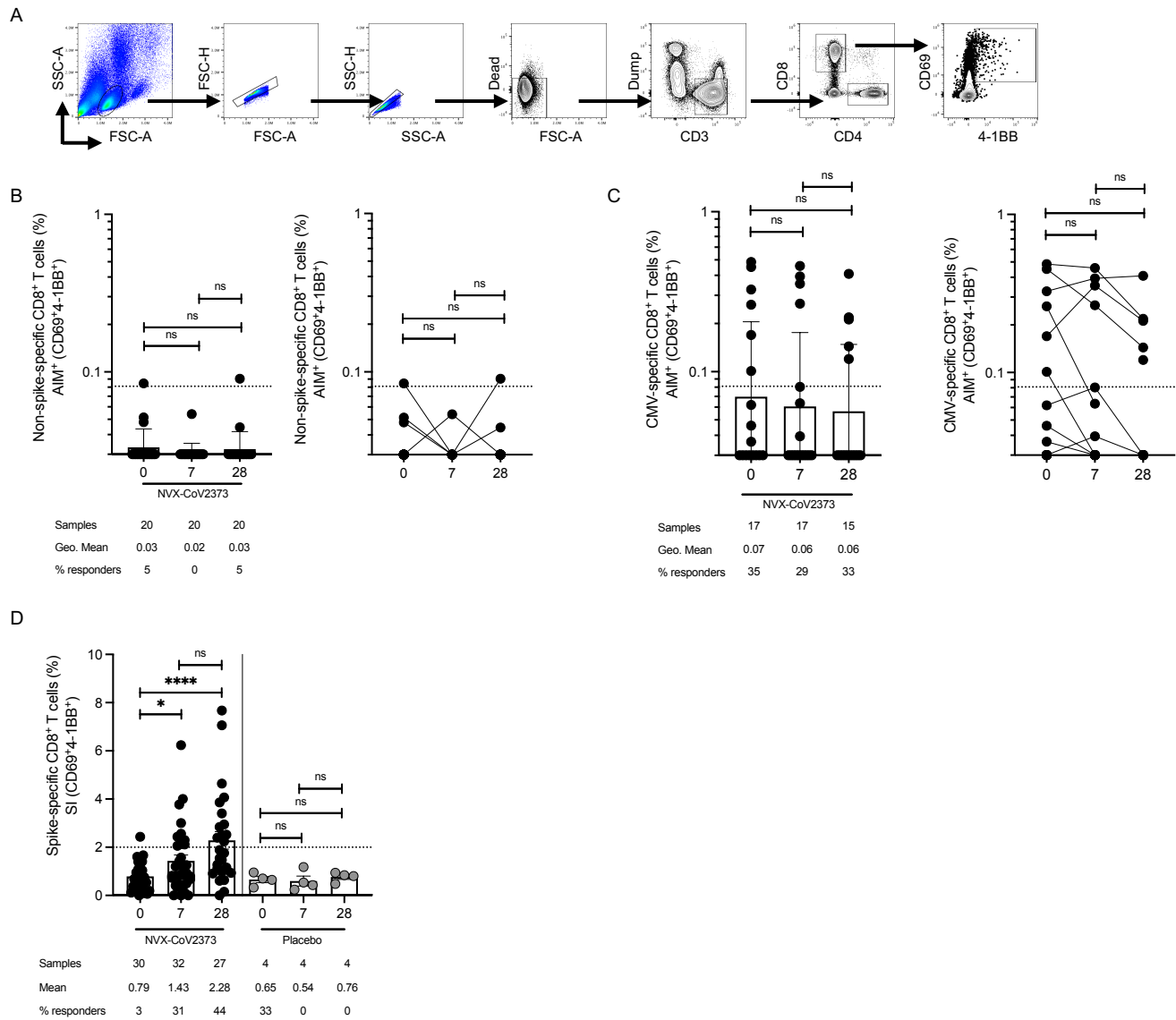
Figure S4. Spike-specific CD8+ T cells are induced following NVX-CoV2373 vaccination. **(A)** FACS gating strategy used to define AIM+ CD8+ T cells at D0, D7 and D28 post-vaccination. **(B)** SARS-CoV-2 Remainder MP-specific CD69+4-1BB+ AIM+ CD8+ T cell responses in vaccinees at D0, 7 and 28 post-vaccination. **(C)** CMV class I/II MP-specific CD69+4-1BB+ AIM+ CD8+ T cell responses in vaccinees at D0, 7 and 28 post-vaccination. **(D)** Stimulation indices for CD69+4-1BB+ CD8+ AIM+ T cells in vaccinees and controls at D0, D7 and D28 post-vaccination. Paired data were analyzed by Wilcoxon signed rank test. Log data shown with geometric mean ± geometric SD. Linear data shown with mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001.

Figure S5

A

B

	0	7	28	0	7	28
		NVX-CoV2373			Placebo	
Samples	28	28	22	4	4	4
Mean	1.12	1.87	5.03	3.78	2.27	0.97
% responders	4	32	41	25	25	0

C

D

E

F

D7   D28

Pie arc legend

■ GzmB +
■ IFNg +
■ IL2 +
■ TNFa +

Categories

#	GzmB	IFNg	IL2	TNFa
1	+	+	+	+
2	+	+	+	-
3	+	+	-	+
4	+	+	-	-
5	+	-	+	+
6	+	-	+	-
7	+	-	-	+
8	-	+	+	+
9	-	+	+	-
10	-	+	-	+
11	-	+	-	-
12	-	-	+	+
13	-	-	+	-
14	-	-	-	+
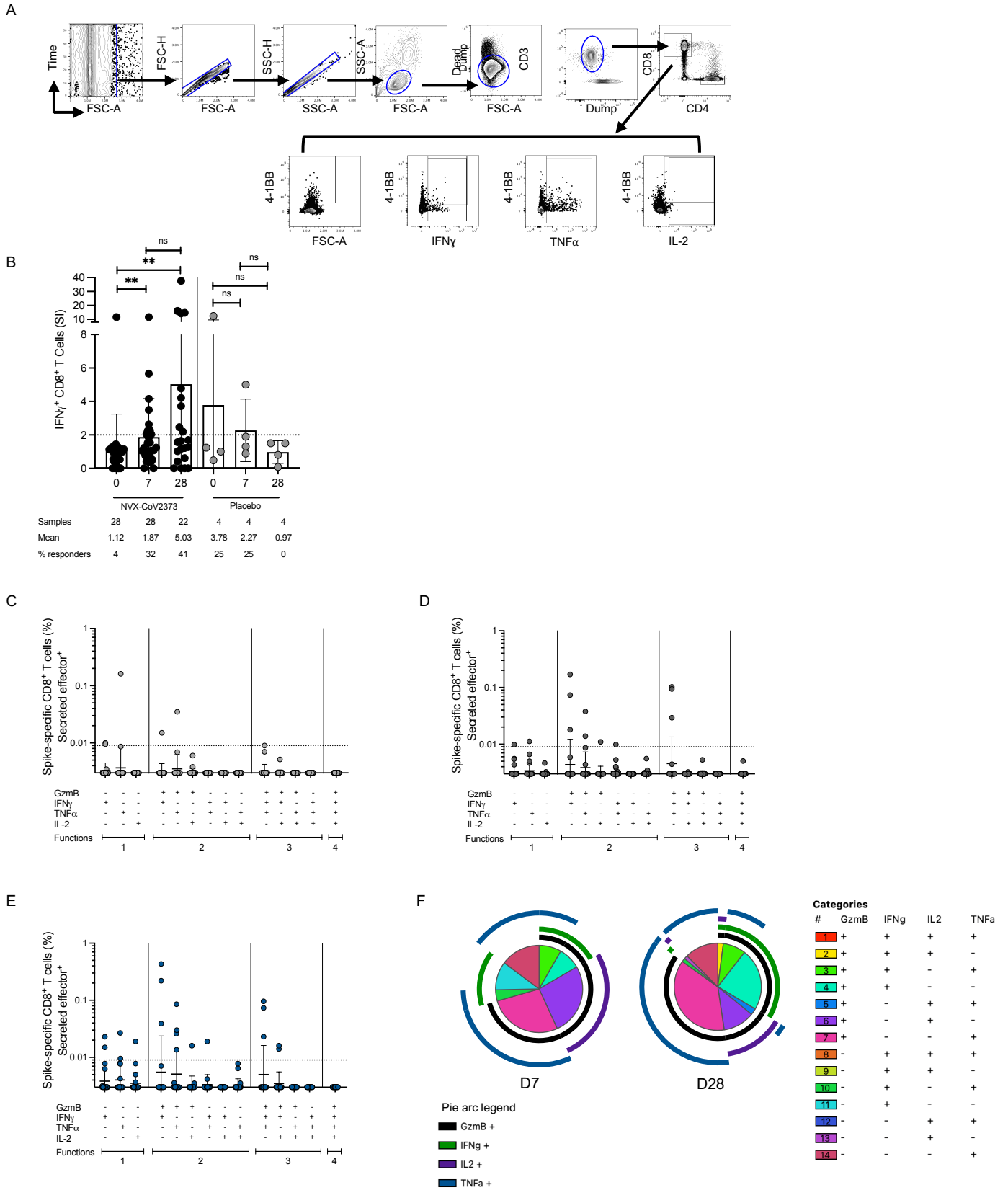
Figure S5. CD8$^+$ T cell cytokine responses induced by NVX-CoV2373 vaccination. **(A)** Gating strategy used to identify cytokine-producing CD8$^+$ T cells. **(B)** IFNɣ$^+$CD8$^+$ T cells measured in response to SARS-CoV-2 non-spike MP stimulation. Predominant multifunctional profiles of spike-specific CD8$^+$ T cells with one, two, three or four functions were analyzed at **(C)** D0, **(D)** D7, and **(E)** D28 post vaccination. Dotted line indicates LOQ for the assay, and is calculated as the geometric mean of all sample DMSO wells multiplied by the geometric SD factor. % responders is calculated as responses ≥ LOQ divided by the total samples in the group. **(F)** Pie charts depicting the proportion of spike-specific CD4$^+$ T cells exhibiting different combination of cytokines and/or effector molecules at D7 and D28 post-1st immunization. Paired data were analyzed by Wilcoxon signed rank test. Data shown with geometric mean $\pm$ geometric SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ****$p<0.0001$. Boolean data were input into SPICE, an open-source graphing tool for complex data sets (32), to create pie graphs.

Figure S6
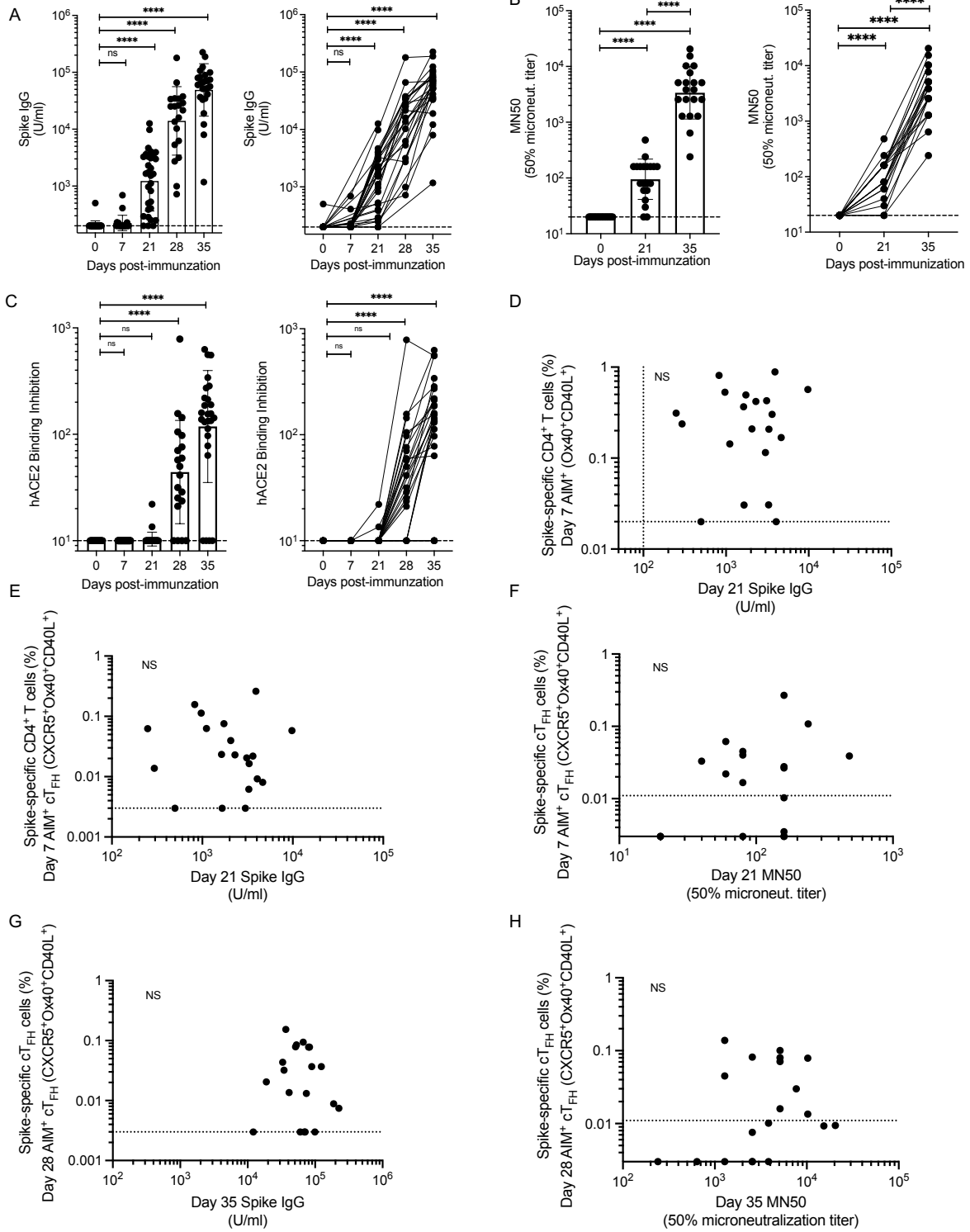
Figure S6. Association between T cells and antibody responses following NVX-CoV2373 vaccination. **(A)** Spike IgG titers, **(B)** SARS-CoV-2 neutralization titers, and **(C)** hACE2 binding inhibition measured in vaccinees at D0, 7, and 28 post-immunization. **(D)** Correlation of D7 SARS-CoV-2 Spike-specific AIM$^+$ CD4$^+$ T cells and D21 spike-specific IgG. Spike IgG and neutralizing antibody correlations with AIM$^+$ cT$_{FH}$ cells at **(E, F)** D7 post-1st, or **(G, H)** D7 post-2nd immunization. Paired data were analyzed by Wilcoxon signed rank test **(A-C)** and Spearman correlation **(D-H)**. Data shown with geometric mean $\pm$ geometric SD. * p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001.
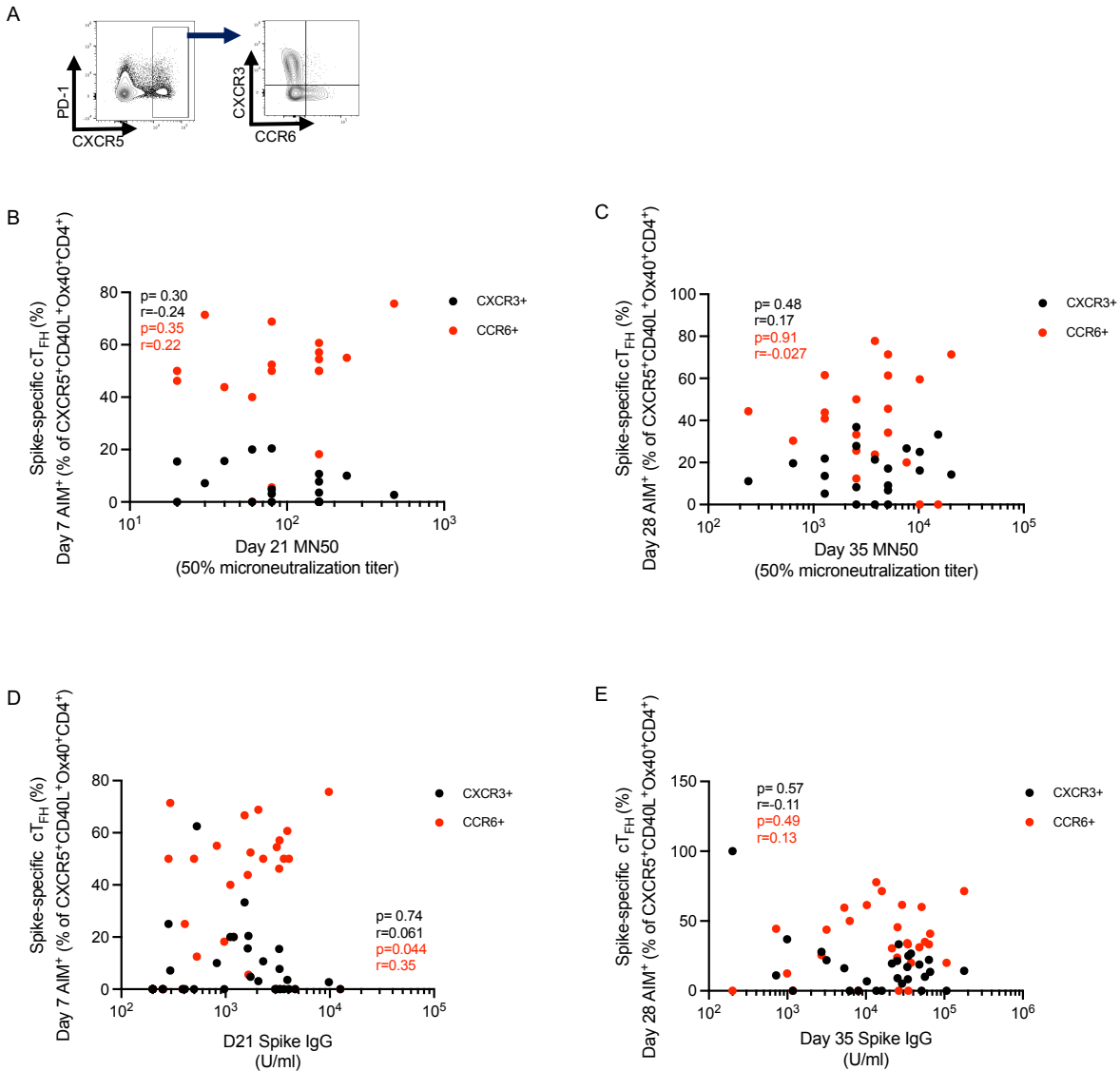
Figure S7. Association between antibody responses and cT$_{FH}$ cell subsets following NVX-CoV2373 vaccination. **(A)** Representative FACS gating of CXCR3- and CCR6- expressing cTFH cells. **(B)** Correlation of CXCR3$^+$ CCR6$^+$ cTFH 7 days post-1st immunization and d21 post-1st immunization microneutralization titers. **(C)** Correlation of CXCR3$^+$ CCR6$^+$ cTFH 7 days post-2nd immunization and d14 post-2nd immunization microneutralization titers. **(D)** Correlation of CXCR3$^+$ CCR6$^+$ cTFH 7 days post-1st immunization and d21 post-1st immunization SARS-2 spike IgG titers. **(E)** Correlation of CXCR3$^+$ CCR6$^+$ cTFH 7 days post-2nd immunization and d14 post-2nd immunization SARS-2 spike IgG titers. CXCR3$^+$CXCR5$^+$CD4$^+$ T cells are shown in black and CCR6$^+$CXCR5$^+$CD4$^+$ T cells are shown in red. Data were analyzed by Spearman correlation.
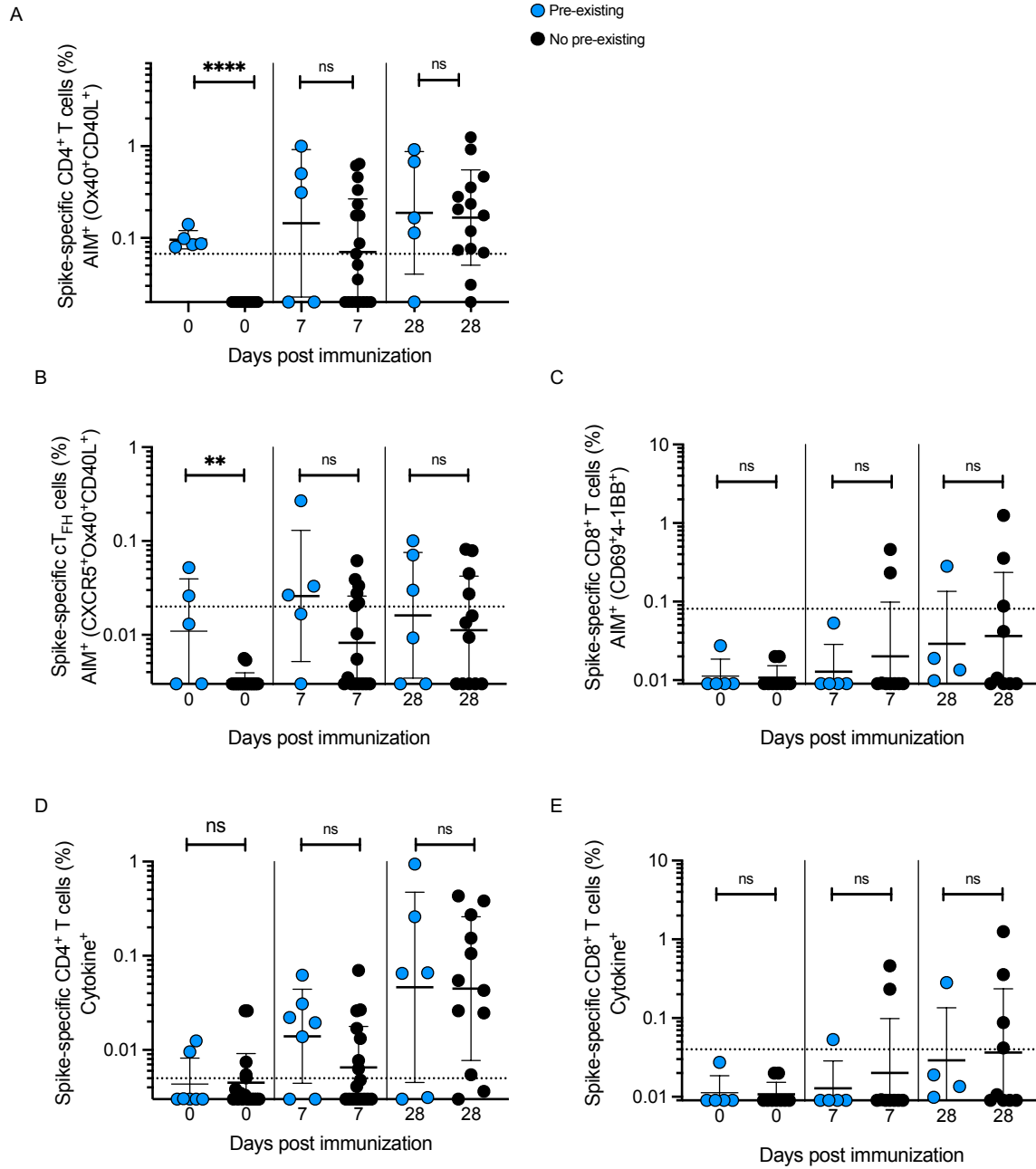
Figure S8

Figure S8. Pre-existing T cell immunity does not impact NVX-CoV2373 vaccine responses. **A)** Spike-specific CD4$^+$ AIM$^+$ T cells at baseline and post-vaccination grouped according to donors with CD4$^+$AIM$^+$ T cell responses at D0 above the LOS for the assay (blue dots) or no detectable CD4$^+$ AIM$^+$ T cell responses at D0 (black dots). Effect of high pre-existing CD4$^+$ AIM$^+$ T cells on **(B)** spike specific AIM$^+$ cT$_{FH}$ cells, **(C)** spike-specific AIM$^+$ CD8$^+$ T cells, **(D)** total Spike-specific CD4$^+$ T cell and **(E)** spike-specific CD8$^+$ T cell cytokine production. Dotted line indicates LOQ for the assay, and is calculated as the geometric mean of all sample DMSO wells multiplied by the geometric SD factor. Data were analyzed by Mann-Whitney test. Data shown with geometric mean $\pm$ geometric SD. * p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001.