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Supplemental information

Immunodeficiency syndromes differentially impact the functional profile of SARS-CoV-2-specific T cells elicited by mRNA vaccination

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Figure S1

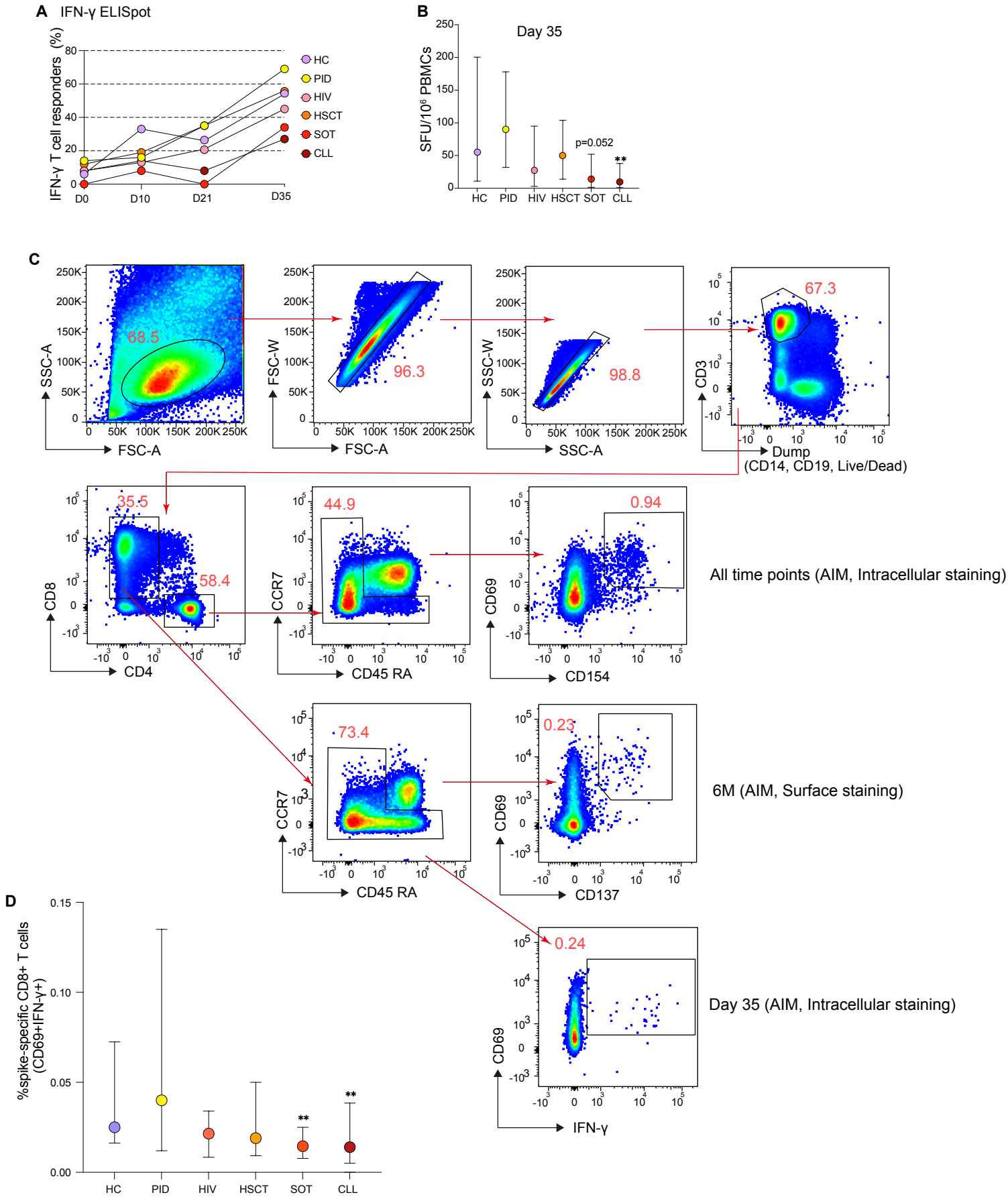
A
IFN-γ ELISpot
IFN-γ T cell responders (%)
D0
D10
D21
D35
HC
PID
HIV
HSCT
SOT
CLL
B
Day 35
SFU/10⁶ PBMCs
p=0.052
**
C
SSC-A
FSC-A
68.5
FSC-W
96.3
SSC-W
98.8
CD3
67.3
Dump
(CD14, CD19, Live/Dead)
CD8
CD4
35.5
58.4
CCR7
CD45 RA
44.9
CD69
CD154
0.94
All time points (AIM, Intracellular staining)
73.4
CD137
0.23
6M (AIM, Surface staining)
0.24
IFN-γ
Day 35 (AIM, Intracellular staining)
D
%spike-specific CD8+ T cells
(CD69+IFN-γ+)

Figure S1. Longitudinally evolution of total T cell responses and gating strategy for identifying spike-specific T cells, Related to Figure 1. (A) Plot of the frequency of individuals with detectable IFN-γ T cell responses (ELISpot assay). (B) Magnitude of IFN-γ ELISpot T cell responses at Day 35. (C) Gating strategy for the identification of memory (non $CD45RA^{+}CCR7^{+}$) spike-specific T cells in the $CD4^{+}$ ($CD154^{+}CD69^{+}$) and $CD8^{+}$ compartments ($CD69^{+}CD137^{+}$ for 6M and $CD69^{+}IFN\text{-}\gamma^{+}$ for Day 35) after spike peptide stimulation. Numbers indicate percentages in the drawn gates. (D) Spike-specific ($CD69^{+}IFN\gamma^{+}$) $CD8^{+}$ T cell frequencies at Day 35. Graph shows median ± IQR and Kruskal-Wallis test with Dunn's post test. Mann-Whitney test. * P< 0.05, ** P< 0.01, *** P< 0.001.

Figure S2

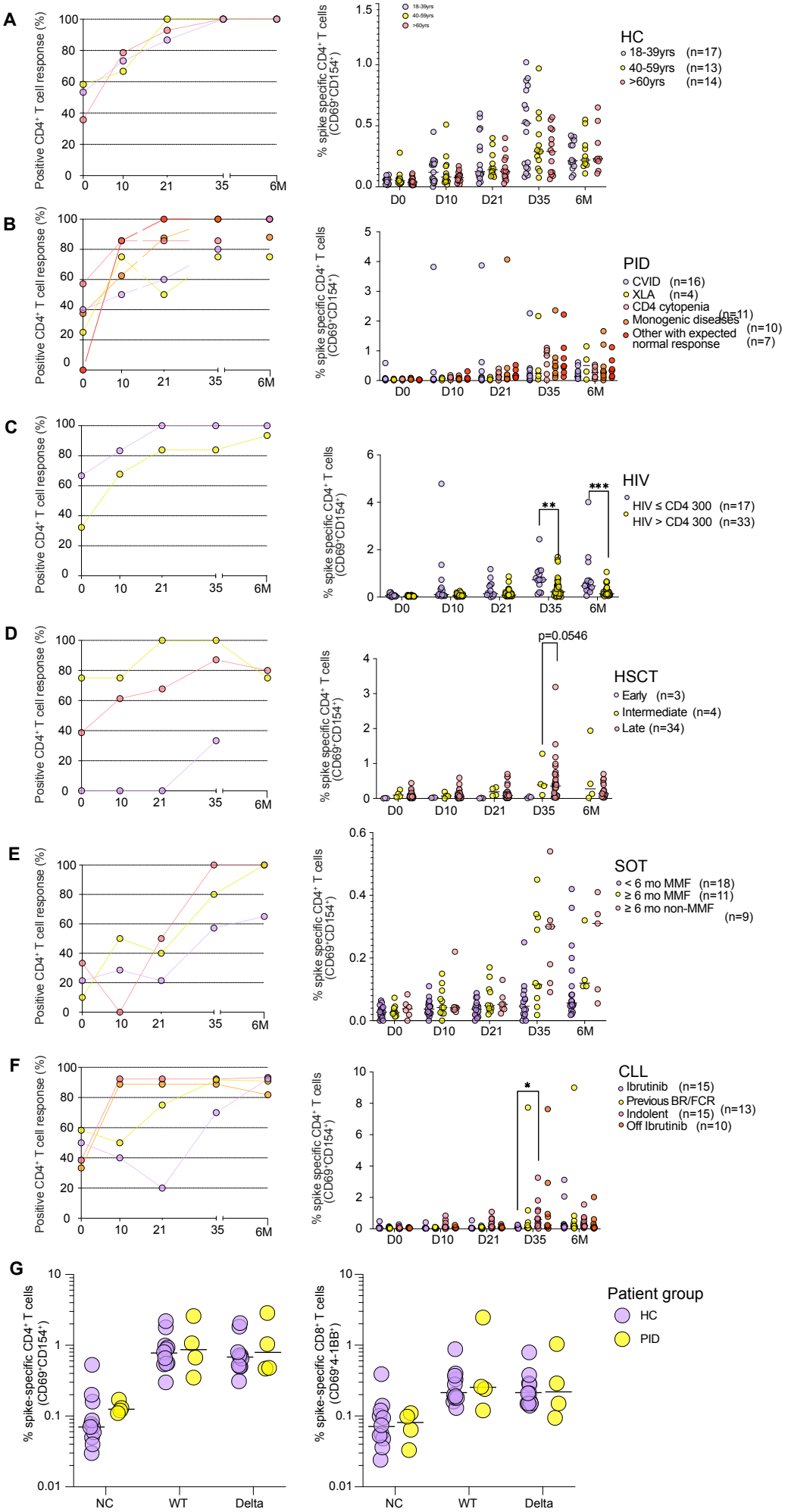
A
B
C
D
E
F
G
Positive CD4+ T cell response (%)
% spike specific CD4+ T cells (CD69+CD154+)
0
10
21
35
6M
D0
D10
D21
D35
6M
18-39yrs
40-59yrs
>60yrs
HC
18-39yrs (n=17)
40-59yrs (n=13)
>60yrs (n=14)
PID
CVID (n=16)
XLA (n=4)
CD4 cytopenia (n=11)
Monogenic diseases (n=10)
Other with expected normal response (n=7)
HIV
HIV ≤ CD4 300 (n=17)
HIV > CD4 300 (n=33)
**
***
HSCT
Early (n=3)
Intermediate (n=4)
Late (n=34)
p=0.0546
SOT
< 6 mo MMF (n=18)
≥ 6 mo MMF (n=11)
≥ 6 mo non-MMF (n=9)
CLL
Ibrutinib (n=15)
Previous BR/FCR (n=13)
Indolent (n=15)
Off Ibrutinib (n=10)
*
% spike-specific CD4+ T cells (CD69+CD154+)
% spike-specific CD8+ T cells (CD69+4-1BB+)
NC
WT
Delta
Patient group
HC
PID

Figure S2. Subgroups of immunodeficient cohorts display differential T cell response rates, but maintain cross-variant recognition, Related to Figure 1. Longitudinal plots of the median proportion of individuals with detectable (⩾0.05%) spike-specific $CD4^+$ T cell responses segregated within-cohort subgroups for healthy controls (A), patients with PID (B), HIV (C), HSCT (D), SOT (E), and CLL (F). (G) Frequencies of spike-specific T cell responses to stimulation from wildtype and Delta variant peptide pools for HC and PID cohorts. Graphs show median values. Mann-Whitney test for comparison between two groups and Kruskal-Wallis test with Dunn's post test for comparisons between three or more groups. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. CIVD: Common variable immunodeficiency, XLA: X-linked agammaglobulinemia, MMF: Mycophenolate mofetil, BR/FCR: bendamustine and rituximab/ fludarabine, cyclophosphamide and rituximab

Figure S3

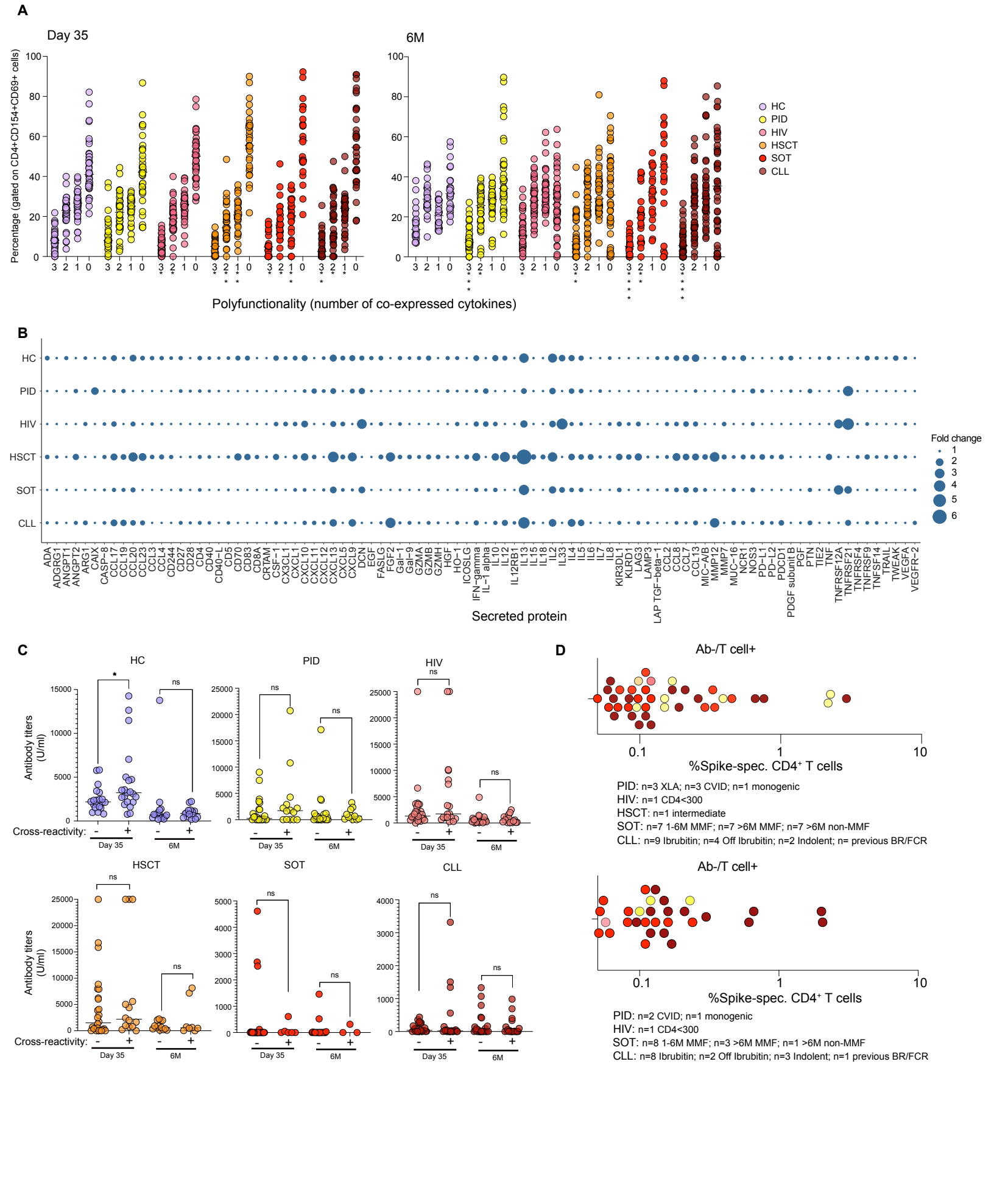
A
Day 35
6M
Percentage (gated on CD4+CD154+CD69+ cells)
Polyfunctionality (number of co-expressed cytokines)
HC
PID
HIV
HSCT
SOT
CLL
B
Fold change
Secreted protein
C
Antibody titers (U/ml)
Cross-reactivity:
Day 35
6M
D
Ab-/T cell+
%Spike-spec. CD4+ T cells
PID: n=3 XLA; n=3 CVID; n=1 monogenic
HIV: n=1 CD4<300
HSCT: n=1 intermediate
SOT: n=7 1-6M MMF; n=7 >6M MMF; n=7 >6M non-MMF
CLL: n=9 Ibrubitin; n=4 Off Ibrubitin; n=2 Indolent; n= previous BR/FCR
PID: n=2 CVID; n=1 monogenic
HIV: n=1 CD4<300
SOT: n=8 1-6M MMF; n=3 >6M MMF; n=1 >6M non-MMF
CLL: n=8 Ibrubitin; n=2 Off Ibrubitin; n=3 Indolent; n=1 previous BR/FCR

Figure S3. Spike-specific T cells' polyfunctional profile and relationship with antibodies titers after mRNA, Related to Figures 2 and 3. (A) Polyfunctional cytokine profile of AIM+ CD4 T cells after spike peptide pool stimulation based on number of co-expressed cytokines at Day 35 and 6M. Graph shows median values. Pairwise Mann-Whitney comparisons were performed between the same timepoints for each group against HC only. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. (B) Bubble plot of secretome fold change at Day 35 when compared to unstimulated background. Proteins with lower expression after stimulation (fold change < 1) were treated as unchanged (fold change = 1) for visualization. (C) Spike-specific antibody titers at Day 35 and 6M based on the presence or absence of pre-existing Day 0 $CD4^+$ T cell responses. (D) Frequency of spike-specific $CD4^+$ T cell responses in individuals without detectable spike-specific antibodies at Day 35 and 6M.

Figure S4

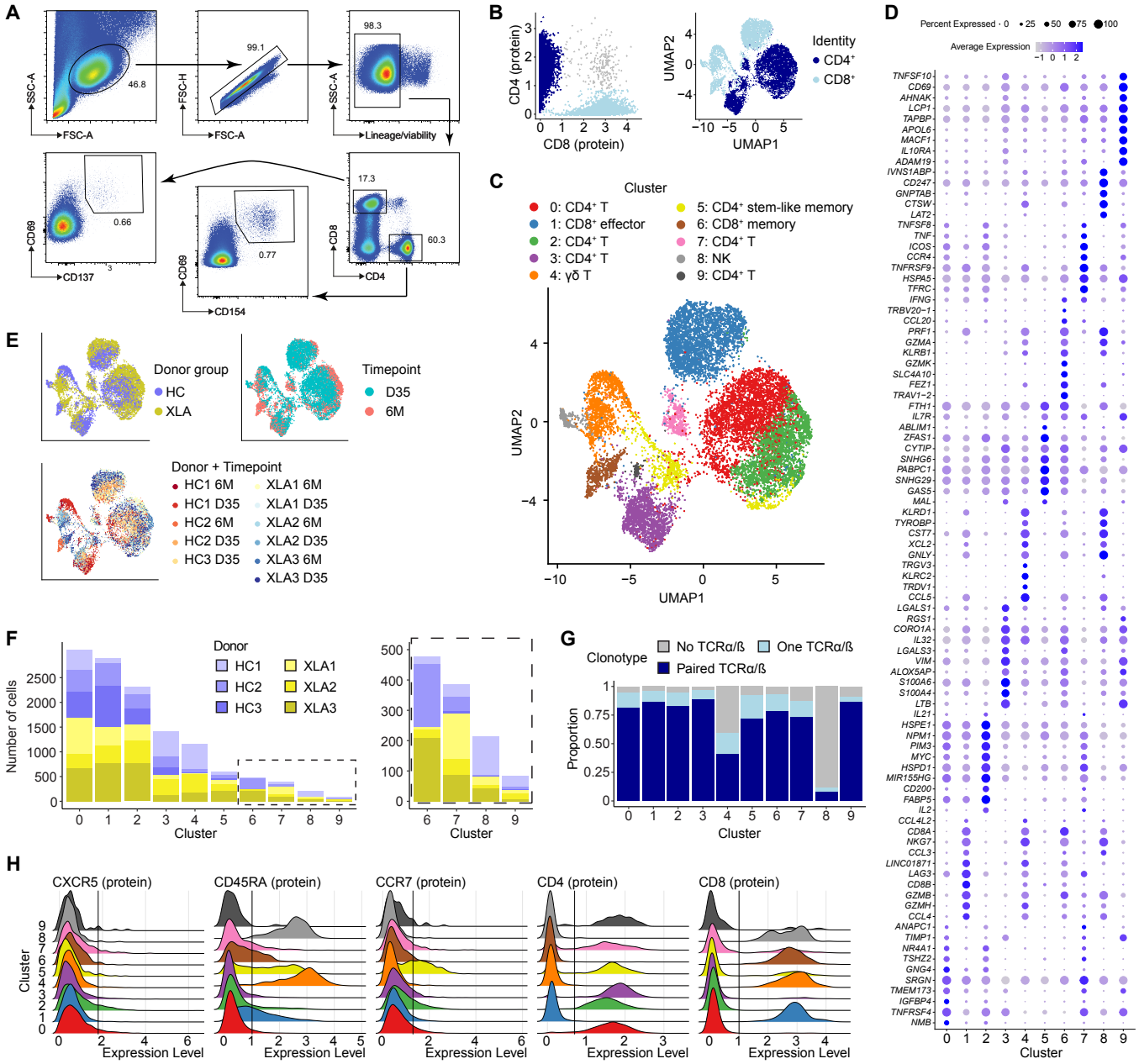

Figure S4. Single-cell analysis of post-vaccination T cell responses, Related to Figures 4 and 5. (A) Sorting strategy for the isolation of spike-specific T cells for single-cell sequencing. (B) Scatter plot and UMAP visualization of cells assigned the CD4 or CD8 expressing subset. Doublets are colored in grey. (C) UMAP visualization of all sequenced cells and clustered by the Louvain method. (D) Dot plot of the top marker genes for each cluster. (E) UMAP visualizations of cells colored according to donor group, timepoint or combined. (F) Stacked barplot of the contribution of each donor to each cluster. (G) Stacked barplot of the proportion of cells with any successfully reconstructed TCR CDR3 sequences. (H) Ridge plots of protein expression measured by antibody-derived tags.

Figure S5

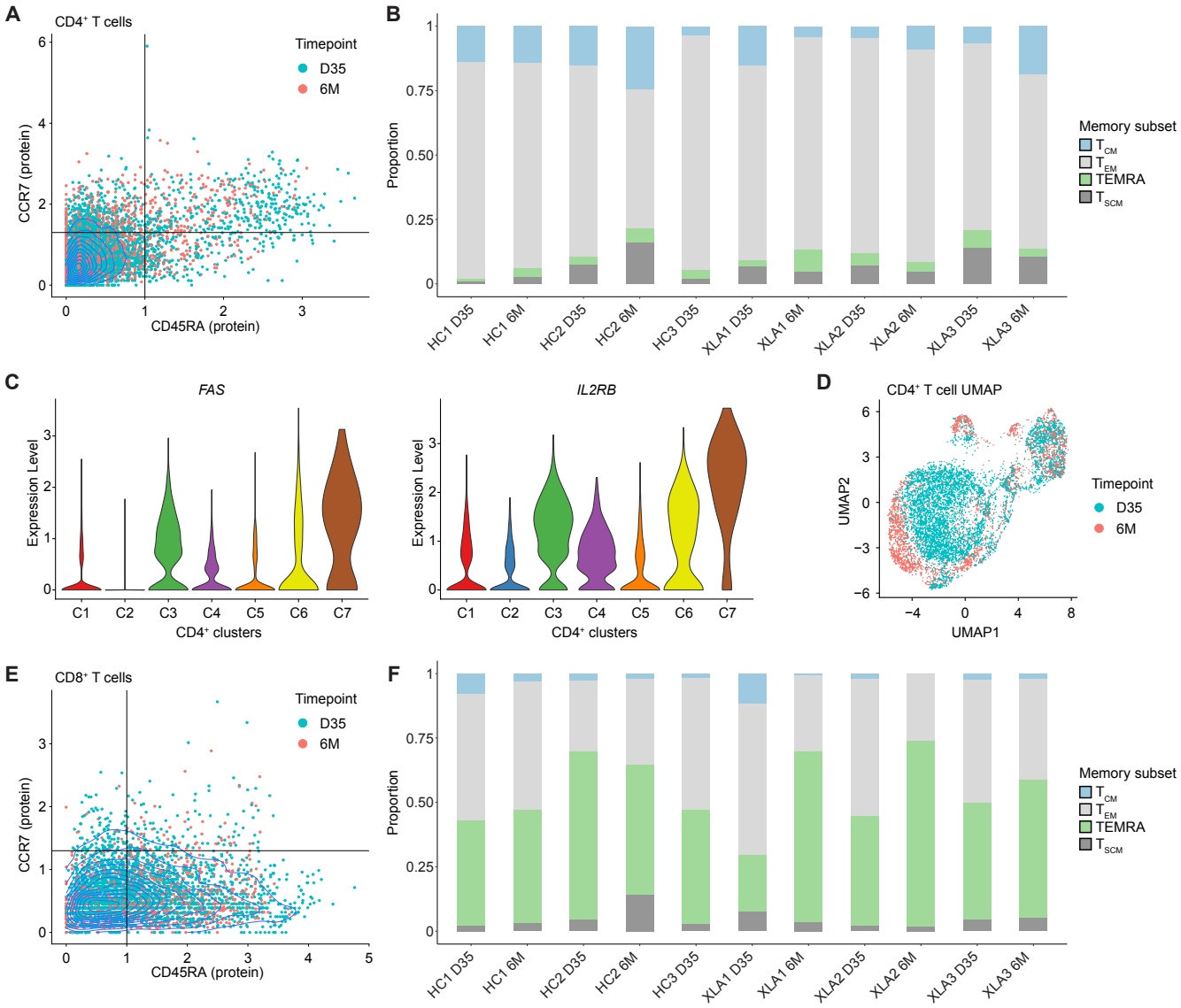

Figure S5. Memory subset composition of post-vaccination T cell responses, Related to Figures 4 and 5. (A) Scatterplot of CD45RA and CCR7 protein expression by CD4$^+$ T cells colored by timepoint. Lines indicate thresholds between negative and positive expression. (B) Stacked barplots of the CD4$^+$ T cell memory composition for each donor and timepoint. (C) Violin plots of gene expression in each CD4 cluster. (D) UMAP visualization for the timepoint of origin of CD4$^+$ T cells. (E) Scatterplot of CD45RA and CCR7 protein expression by CD8$^+$ T cells colored by timepoint. Lines indicate thresholds between negative and positive expression. (F) Stacked barplots of the CD8$^+$ T cell memory composition for each donor and timepoint.

Figure S6

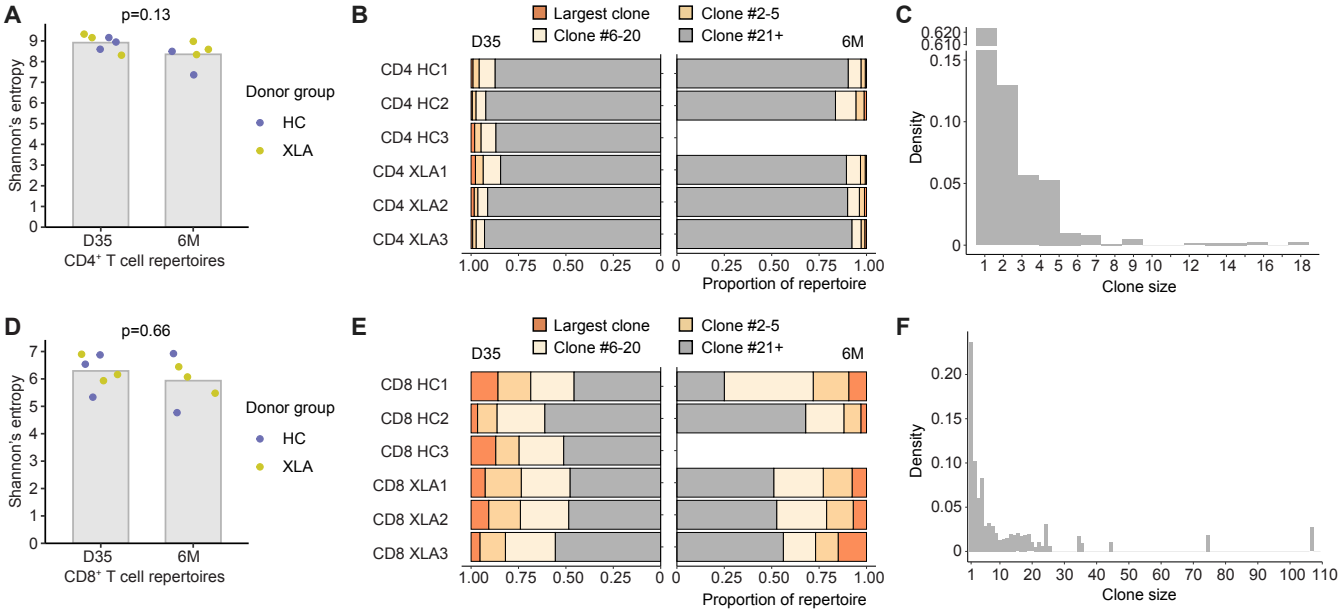

Figure S6. Repertoire characteristics of spike-specific CD4⁺ and CD8⁺ T cells after mRNA vaccination, Related to Figures 4 and 5. (A) Barplot of the diversity of each donor/timepoint CD4⁺ T-cell repertoire measured by Shannon's entropy. (B) Stacked barplot of the repertoire occupied by CD4⁺ T cell clones ordered by size. (C) Density plot of the size of each CD4⁺ T cell clone from all donors and timepoints. (D) Barplot of the diversity of each donor/timepoint CD8⁺ T-cell repertoire measured by Shannon's entropy. (E) Stacked barplot of the repertoire occupied by CD4⁺ T cell clones ordered by size. (F) Density plot of the size of each CD8⁺ T cell clone from all donors and timepoints.

Table S1. Clinical information about each study subgroup. Related to Figures 1 and 2.

Group	Subgroup	Medical treatment
HC	18-39yrs	-
	40-59yrs	-
	> 60yrs	-
PID	CVID	-
	XLA	-
	CD4 cytopenia	-
	Monogenic diseases	-
	Other with expected normal response	-
HIV	nCD4 ≤ 300/mm3	-
	nCD4 > 300/mm3	-
HSCT	Early	<6 months after transplantation
	Intermediate	6-12 months after transplantation
	Late	>12 months after transplantation
SOT	< 6 mo MMF	<6 months after transplantation with mycophenolate mofetil
	≥ 6 mo MMF	≥6 months after transplantation with mycophenolate mofetil
	≥ 6 mo Non-MMF	≥6 months after transplantation no mycophenolate mofetil
CLL	Ibrutinib	ongoing ibrutinib treatment
	Previous BR/FCR	previous treatment with BR/FCR bendamustine and rituximab / fludarabine, cyclophosphamide and rituximab
	Indolent	indolent and not treated
	Off Ibrutinib	off ibrutinib treatment for >2 months

Table S2. List of individuals with detectable T-cell responses in the absence of antibody responses after vaccination. Related to Figure 3.

Group	ID	AIM$^+$ CD4$^+$ T cells at Day35 (% of CD4$^+$ T cells)	Ab titer at Day 35 (U/ml)	Subgroup info
	WP1138	2.26	0.4	CVID
	WP1114	0.15	0.4	XLA
	WP1109	0.39	0.4	XLA
	WP1111	0.095	0.4	Monogenic diseases
	WP1102	0.17	0.4	CVID
	WP1105	0.15	0.4	CVID
PID	WP1103	2.17	0.4	XLA
HIV	WP2104	0.12	0.4	≤CD4 300
HSCT	WP3168	0.098	0.4	Intermediate
	WP4135	0.34	0.4	> 6 mon MMF
	WP4139	0.12	0.4	> 6 mon MMF
	WP4141	0.072	0.4	> 6 mon MMF
	WP4111	0.06	0.4	1-6 mo MMF
	WP4117	0.11	0.4	> 6 mon MMF
	WP4118	0.097	0.4	1-6 mo MMF
	WP4119	0.07	0.44	1-6 mo MMF
	WP4122	0.33	0.4	1-6 mo MMF
	WP4123	0.11	0.4	1-6 mo MMF
	WP4124	0.45	0.4	> 6 mon MMF
	WP4125	0.29	0.4	> 6 mon MMF
	WP4126	0.083	0.4	1-6 mo MMF
	WP4128	0.11	0.4	> 6 mon MMF
	WP4101	0.088	0.4	> 6 mon non-MMF
SOT	WP4102	0.05	0.4	1-6 mo MMF
	WP5129	0.17	0.4	Off Ibrutinib
	WP5132	0.099	0.4	Ibrutinib
	WP5119	0.084	0.4	Ibrutinib
	WP5120	0.088	0.4	Off Ibrutinib
	WP5121	0.059	0.4	Ibrutinib
	WP5122	2.93	0.4	Off Ibrutinib
	WP5123	0.066	0.4	Ibrutinib
	WP5126	0.21	0.4	Ibrutinib
	WP5136	0.19	0.4	Indolent
CLL	WP5137	0.76	0.4	Off Ibrutinibtinib

	WP5145	0.062	0.4	Previous BR/FCR
	WP5104	0.12	0.4	Ibrutinib
	WP5107	0.11	0.4	Ibrutinib
	WP5112	0.065	0.4	Ibrutinib
	WP5152	0.26	0.4	Ibrutinib
	WP5153	0.66	0.4	Ibrutinib

Table S3. Cell metrics for cells selected for single-cell sequencing. Related to Figures 4 and 5.

			Flow cytometry		Sequenced cells passing QC	
ID	**Timepoint**	**Antibody Titre (U/ml)**	**% SARS-CoV-2 specific CD4**	**% SARS-CoV-2 specific CD8**	**$CD4^+$ T cells**	**$CD8^+$ T cells**
WP6127 (HC1)	Day 35	5564	0.59	0.33	726	184
WP6133 (HC2)	Day 35	5775	0.85	1.63	781	468
WP6173 (HC3)	Day 35	973	0.77	1.53	996	839
WP1103 (XLA1)	Day 35	0.4	0.77	0.66	697	283
WP1109 (XLA2)	Day 35	0.4	0.66	0.63	803	413
WP1114 (XLA3)	Day 35	0.4	1.17	2.34	1037	761
WP6127 (HC1)	6 months	966	0.34	0.021	452	34
WP6133 (HC2)	6 months	2146	0.42	0.27	242	155
WP1103 (XLA1)	6 months	154	1.15	1.55	594	202
WP1109 (XLA2)	6 months	473	0.3	0.12	378	114
WP1114 (XLA3)	6 months	8.23	0.71	0.25	695	320