

Supplementary Information for

Integrated Single-Cell Analysis Revealed Immune Dynamics During Ad5-nCoV Immunization

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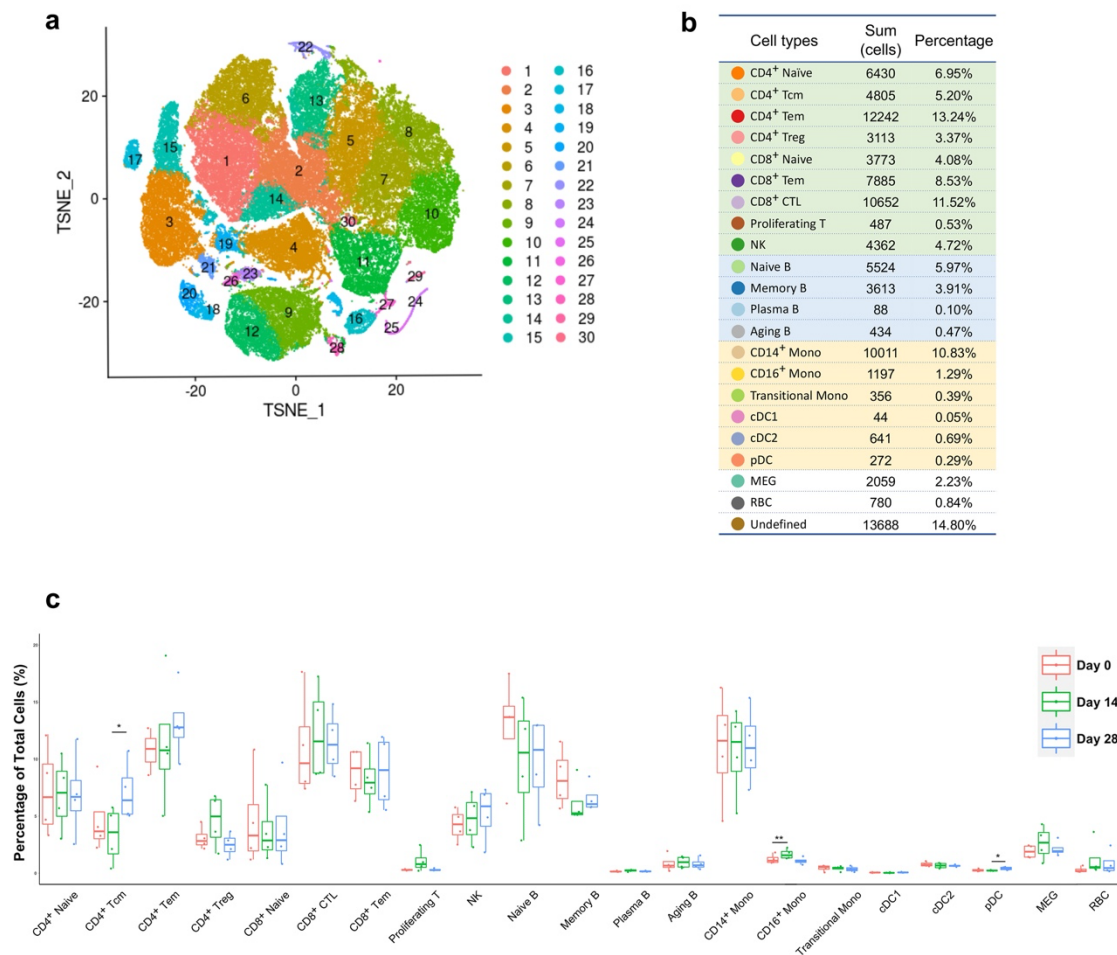
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Supplementary Figures

Supplementary Figure S1

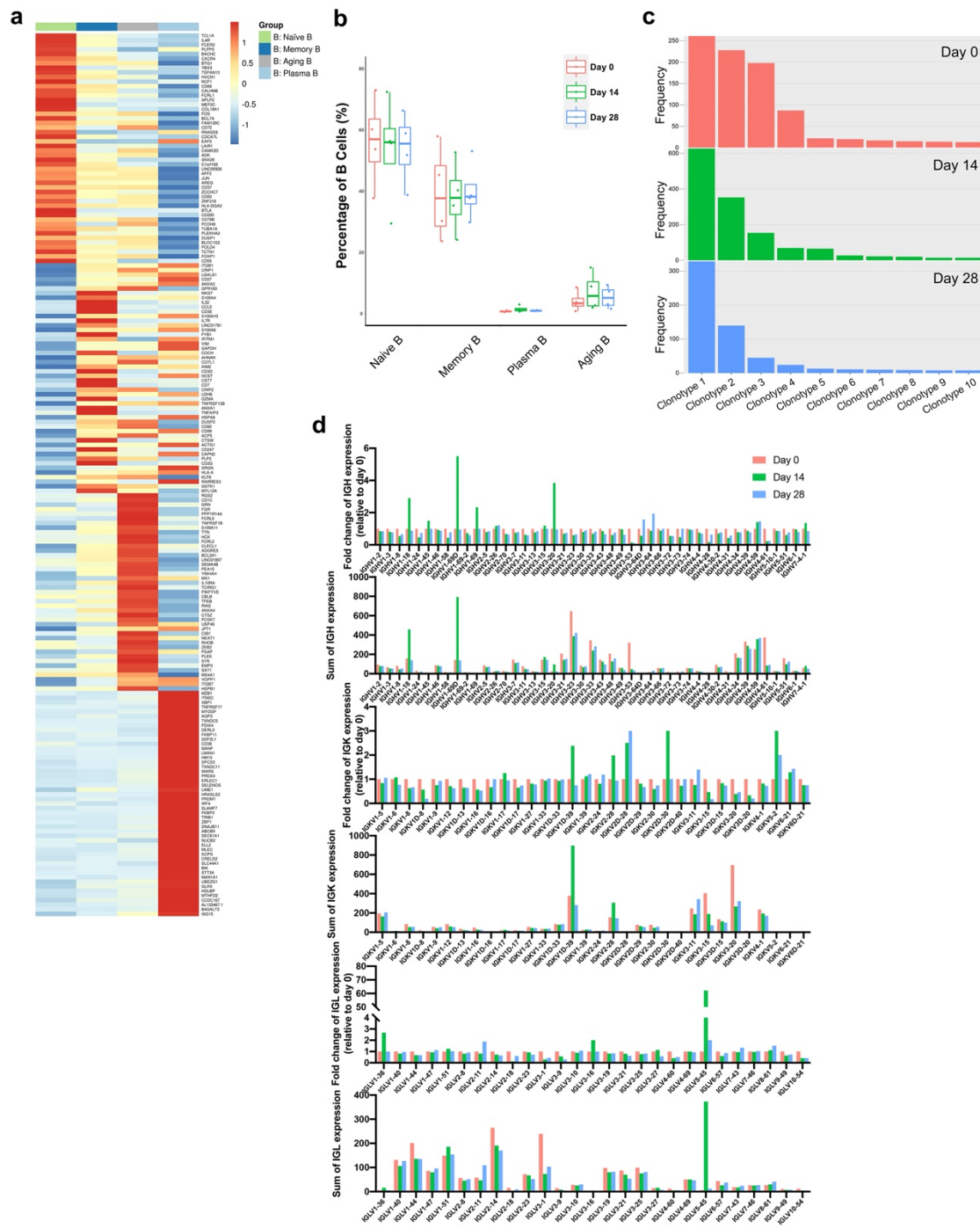


a t-SNE analysis of PBMCs from all participants and timepoints. Cells are primarily unsupervised grouped into 30 clusters.

b Cell counts and percentages of each cell types.

c Proportions of all the cell types in PBMCs from participants in different timepoints by scRNA-seq. Statistical analysis used paired samples t-test. Values are mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$.

Supplementary Figure S2



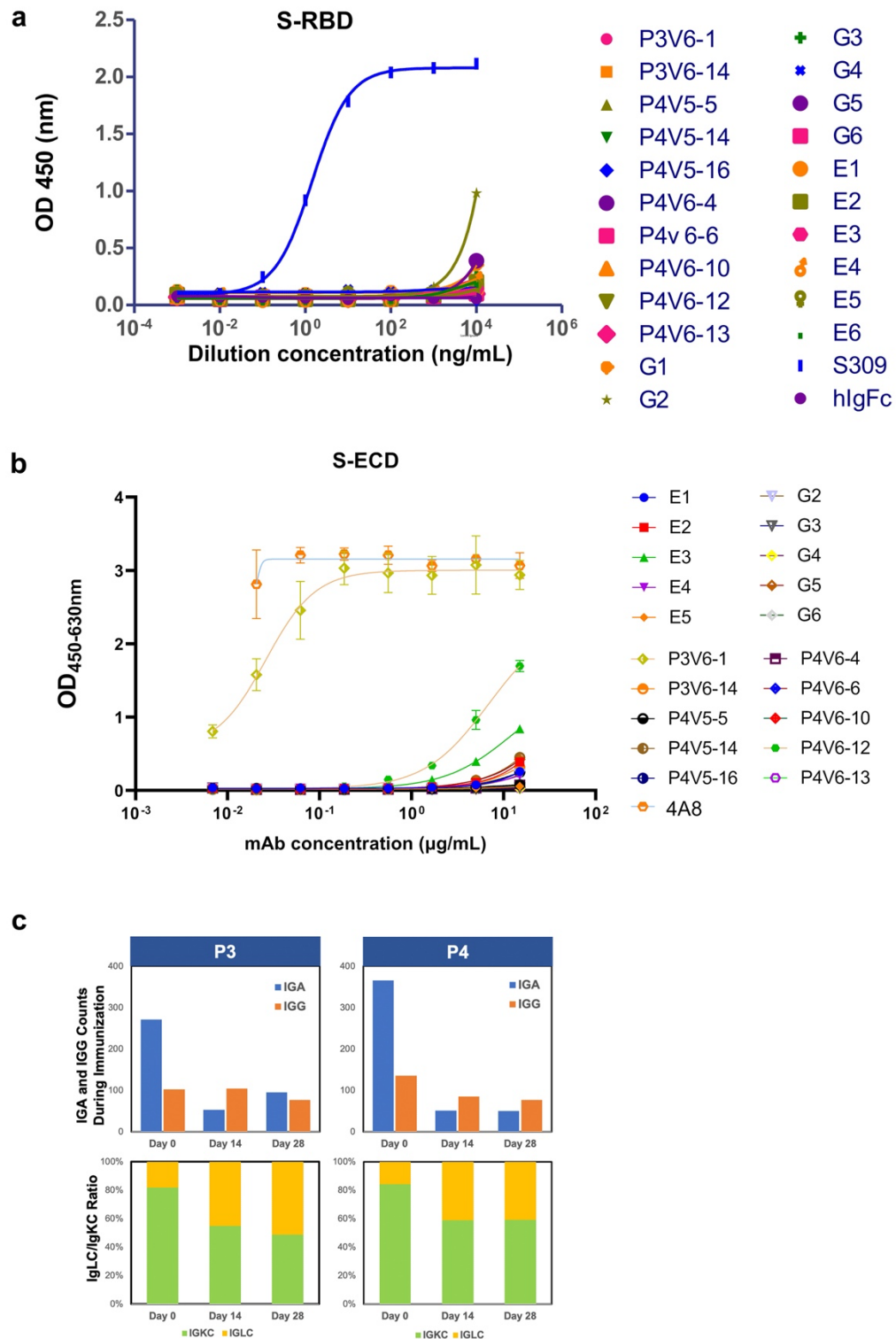
a The heatmaps showing differentially expressed genes (DEGs) upregulated in B cell subtypes.

b Proportions of the B cell subtypes in the total B cells. Statistical analysis used paired t test. Values are mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$.

c The frequency of top 10 clonotypes in each timepoints.

d The isotype usage (sum and fold change relative to day 0) of IGH, IGK and IGL at different timepoints.

Supplementary Figure S3

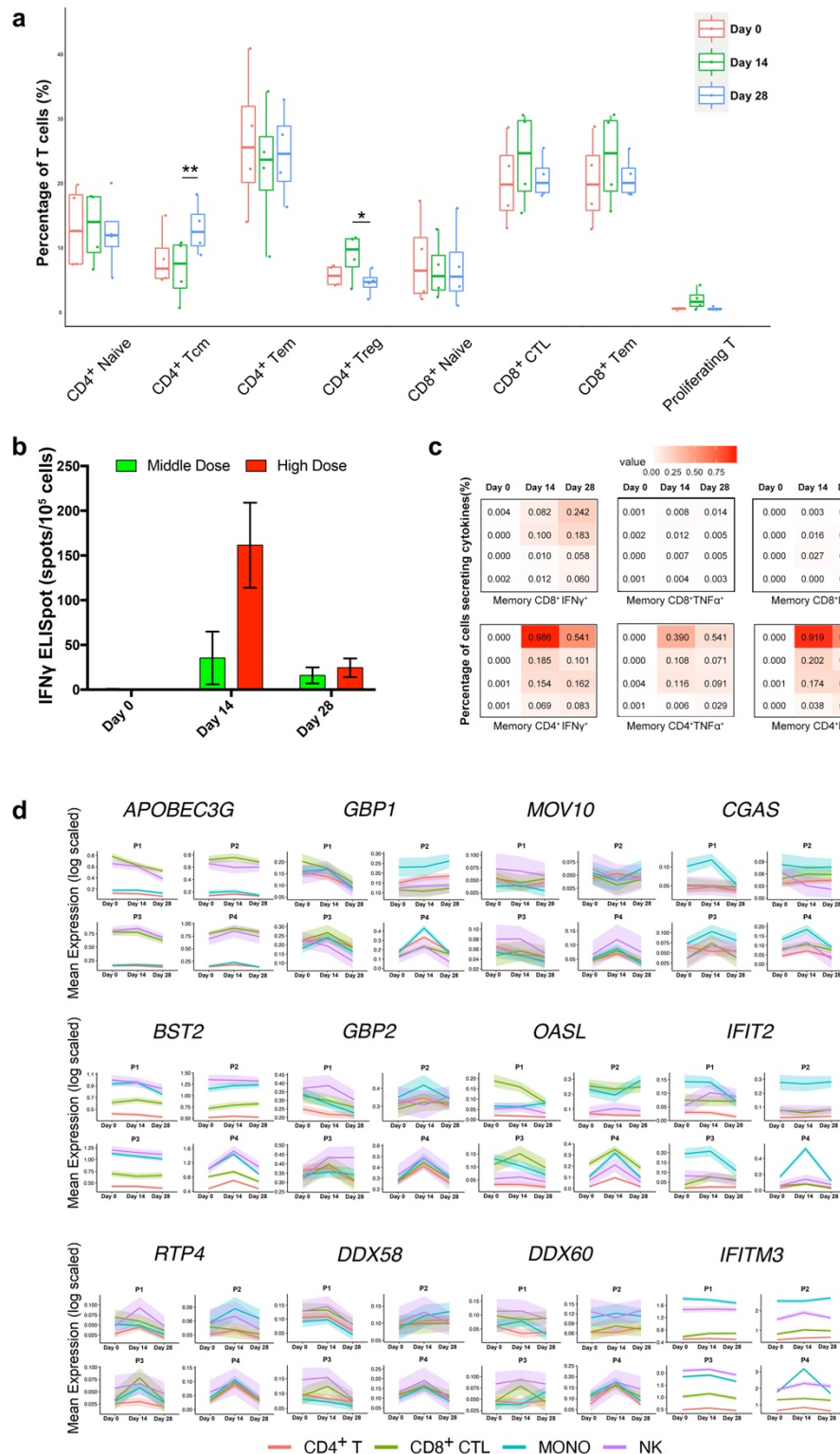


a Binding curves of all synthesized mAbs to S-RBD. S309 is positive control while hIgFc is the negative control.

b Binding curves of all synthesized mAbs to S-ECD. 4A8 is a positive control that was reported to bind the S-ECD of SARS-CoV-2.

c IGA and IGG counts (top) and IgLC/IgKC ratio (bottom) of different timepoints from P3 and P4.

Supplementary Figure S4



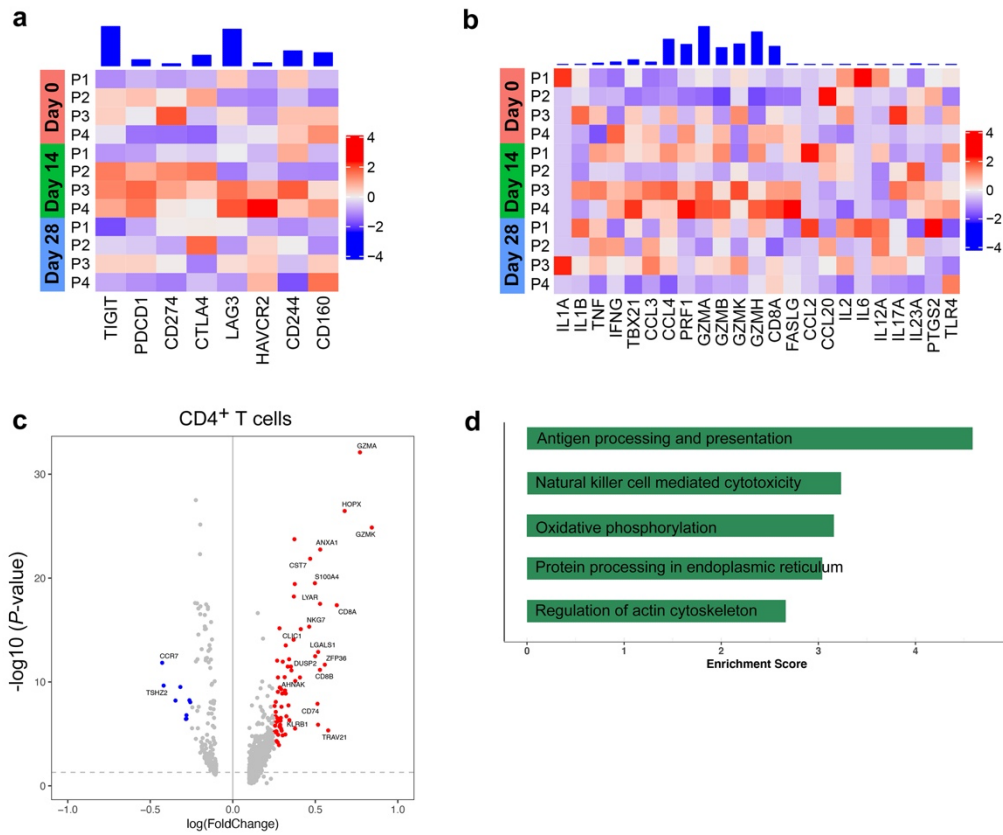
a Proportions of the T and NK cell subtypes in the total T and NK cells. Statistical analysis used paired t test. Values are mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$.

b Specific T-cell response measured by ELISpot. The number of specific T cells with secretion of IFN γ (at days 0, 14 and 28) in each participant, and stratified by pre-existing Ad5 neutralizing antibody titres.

c Percentage of cells secreting IFN γ , TNF α , and IL-2 from CD4⁺ T and CD8⁺ T cells.

d Mean expression of typical ISGs in CD4⁺ T, CD8⁺ T, monocytes and NK cells, which are separated by timepoints and individuals. Shaded area denotes 95% CI of the mean.

Supplementary Figure S5



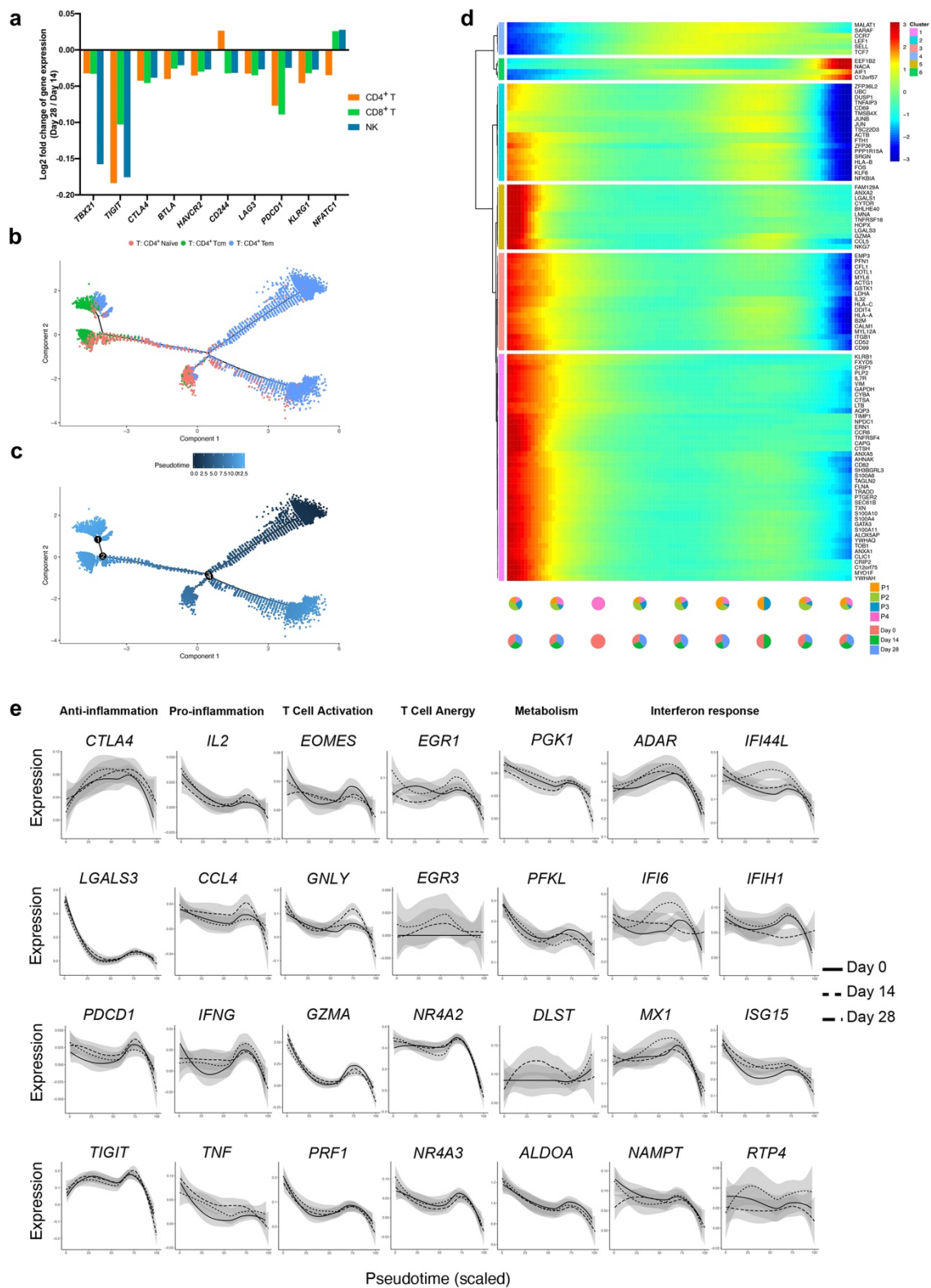
a Heatmap of z-scored mean expression of T cell terminal differentiation signature (defined as the normalized mean expression of genes in the activation signature in Supplementary Table S5) across T cells from each participants and timepoints. Top: Bar plot of total expression of each gene, across all patients. Expression of T cell terminal differentiation signature shows variability across individuals.

b Heatmap of z-scored mean expression of pro-inflammatory signature (defined as the normalized mean expression of genes in the activation signature in Supplementary Table S6) across T cells from each participants and timepoints. Top: Bar plot of total expression of each gene, across all patients. Expression of pro-inflammatory signature shows variability across individuals.

c The volcano plot showing the DEGs between CD4⁺ T cells with expanded and unexpanded TCR clones at day 14 post-vaccination.

d KEGG analysis of the DEGs between T cells with expanded and unexpanded TCR clones at day 14 post-vaccination.

Supplementary Figure S6



a Pseudotime trajectories for CD4⁺ T cells based on Monocle2, color-coded for the CD4⁺ T cell phenotypes.

- b** Pseudotime trajectories for CD4⁺ T cells, color-coded for the pseudotime.
- c** Gene expression dynamics along the CD4⁺ T cells lineage. Genes cluster into 6 gene sets, each of them characterized by specific expression profiles.
- d** Log2 fold change of inhibitory markers between day 14 and day 28 in NK and T cells.
- e** Genes involved in the function and response of T cells modelled along the CD4⁺ T cell lineages in different timepoints. See also the Method for constructing single-cell trajectories.

Supplementary Table

Supplementary Table S1: Demographical information and blood results of vaccine participants

ID		P1	P2	P3	P4
Dose		Middle	Middle	High	High
Gender		Female	Male	Female	Male
Age		45	46	41	42
SARS-CoV-2 NAb Titer	Day 0	< 8	< 8	< 8	< 8
	Day 14	9	9	33	13
	Day 28	< 8	37	45	72
RBD ELISA Titer	Day 0	< 40	< 40	< 40	64
	Day 14	93	< 40	1112	199
	Day 28	571	138	2579	10694
S ELISA Titer	Day 0	20	20	20	54
	Day 14	104	242	689	1354
	Day 28	246	491	1303	1983
Pseudovirus NAb Titer	Day 0	5	5	5	5
	Day 14	70	5	148	20
	Day 28	16	143	46	284
Ad5 NAb Titer	Day 0	254	232	6	6
	Day 14	1380	8748	100	145
	Day 28	924	8748	107	106
IFN γ ELISpot (spots/1e5 cells)	Day 0	1	0	0	0
	Day 14	6	65	114	209
	Day 28	7	25	14	35
Memory CD8 ⁺ IFN γ ⁺ (%)	Day 0	0.002	0.000	0.000	0.004
	Day 14	0.012	0.010	0.100	0.082
	Day 28	0.060	0.058	0.183	0.242
Memory CD8 ⁺ TNF α ⁺ (%)	Day 0	0.001	0.000	0.002	0.001
	Day 14	0.004	0.007	0.012	0.008
	Day 28	0.003	0.005	0.005	0.014
Day 0		0.000	0.000	0.000	0.000

Memory CD8 ⁺ IL2 ⁺ (%)	Day 14	0.000	0.027	0.016	0.003
	Day 28	0.011	0.002	0.013	0.002
Memory CD4 ⁺ IFN γ ⁺ (%)	Day 0	0.001	0.001	0.000	0.000
	Day 14	0.069	0.154	0.185	0.986
	Day 28	0.083	0.162	0.101	0.541
Memory CD4 ⁺ TNF α ⁺ (%)	Day 0	0.001	0.004	0.000	0.000
	Day 14	0.006	0.116	0.108	0.390
	Day 28	0.029	0.091	0.071	0.158
Memory CD4 ⁺ IL2 ⁺ (%)	Day 0	0.000	0.001	0.000	0.000
	Day 14	0.038	0.174	0.202	0.919
	Day 28	0.083	0.181	0.100	0.582

Abbreviations: IFN, interferon; TNF, tumour necrosis factor; IL, interleukin.

< under detectable baseline

Supplementary Table S2: 10X genomics sequencing parameters of each samples

Patient ID	Timepoint	Estimated Number of Cells	Mean Reads per Cell	Median Genes per Cell
P1	Day 0	6867	68110	1324
	Day 14	7682	55527	1276
	Day 28	8082	54674	1208
P2	Day 0	8384	57276	1202
	Day 14	7229	63621	1285
	Day 28	9603	45795	1190
P3	Day 0	8212	60029	1392
	Day 14	7703	54200	1323
	Day 28	6483	67925	1337
P4	Day 0	7572	54039	1415
	Day 14	6984	60501	1473
	Day 28	7655	54121	1307

Supplementary Table S3: Expression of Interferon response signature (defined as the normalized mean expression of genes)

Patient ID	P1			P2			P3			P4		
Timepoint	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
IRF1	1.00820	0.84289	1.17245	0.76073	0.88340	0.84392	1.23655	1.02655	1.09436	0.83280	1.09289	0.77246
IFIH1	0.13414	0.13349	0.12881	0.13233	0.12137	0.14243	0.12458	0.21887	0.06679	0.13650	0.10771	0.13465
IFITM3	0.84560	0.93071	1.05091	1.43401	0.94434	1.07080	1.18082	1.58309	0.90583	1.13119	0.98612	1.34321
DDX58	0.23472	0.18016	0.19321	0.24178	0.23936	0.21184	0.20048	0.29219	0.13839	0.18943	0.14434	0.18665
IFI44L	0.10579	0.42143	0.13807	0.09634	0.06722	0.34350	0.13722	0.51202	0.07884	0.51051	0.11867	0.13270
IFI6	0.55780	0.83359	0.59020	0.58338	0.52263	0.75417	0.58256	1.07752	0.49880	0.91883	0.50375	0.54318
IFITM2	1.96235	1.99979	1.99874	2.45847	1.99246	2.07260	2.07190	2.59892	1.86129	2.06499	1.95531	2.39346
NAMPT	0.15534	0.15937	0.17591	0.13709	0.16978	0.14610	0.22585	0.16616	0.16374	0.13994	0.16681	0.16055
OASL	0.17101	0.21976	0.11160	0.26112	0.17544	0.21555	0.18350	0.50907	0.08889	0.21737	0.09693	0.17827
RTP4	0.10555	0.05914	0.05991	0.06917	0.10754	0.07783	0.10008	0.20618	0.05651	0.04662	0.04415	0.06532
TREX1	0.02309	0.04798	0.04015	0.05134	0.03355	0.03852	0.04146	0.06204	0.01788	0.03752	0.02201	0.03157
ADAR	0.67533	0.62218	0.65196	0.69334	0.66942	0.63538	0.70141	0.83252	0.66440	0.64330	0.67112	0.65945
TENT5C	0.54075	0.58769	0.60846	0.28390	0.35811	0.51926	0.60498	0.33767	0.66170	0.43421	0.55706	0.34691
LY6E	1.52678	1.63529	1.57032	1.72298	1.52345	1.57072	1.63079	2.18910	1.54468	1.71005	1.57045	1.76020
MCOLN2	0.12809	0.05752	0.12066	0.13745	0.14460	0.08492	0.14575	0.21924	0.12876	0.07504	0.13067	0.13297
APOBEC3G	0.75360	0.54755	0.71980	0.88558	0.68215	0.62639	0.81718	1.13405	0.49378	0.57596	0.63330	0.81825
IL15	0.05152	0.02740	0.05540	0.03101	0.03997	0.03833	0.06600	0.05273	0.03374	0.02454	0.04126	0.02985
ISG15	0.48546	0.64084	0.51472	0.58527	0.48190	0.64248	0.62276	1.03857	0.44385	0.72723	0.53883	0.59224
MX1	0.27429	0.45481	0.26401	0.28800	0.21293	0.40180	0.25116	0.81249	0.21865	0.47562	0.22121	0.32984
TLR3	0.02148	0.01399	0.01522	0.02798	0.01999	0.01901	0.02752	0.04135	0.01011	0.01011	0.01857	0.02551

Supplementary Table S4: Expression of T cell activation signature (defined as the normalized mean expression of genes)

Patient ID	P1			P2			P3			P4		
Timepoint	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
CD69	1.71648	2.03847	1.94077	1.41654	1.50139	1.93701	1.90391	1.24944	1.88411	1.90219	1.94035	1.47749
CCR7	0.20144	0.40009	0.53638	0.77227	0.24860	0.48596	0.45868	0.82787	0.15154	0.42967	0.41636	0.85267
CD27	0.74480	0.84141	1.02396	0.82803	0.67315	0.92145	1.11484	1.00234	0.55941	0.70273	0.96347	0.95740
BTLA	0.05821	0.06306	0.08292	0.05085	0.06106	0.05920	0.08208	0.08385	0.02469	0.03568	0.05122	0.06434
CD40LG	0.24788	0.24425	0.16967	0.15270	0.13574	0.28594	0.12836	0.17091	0.20503	0.22674	0.10889	0.12598
IL2RA	0.00802	0.01499	0.00974	0.00312	0.01082	0.01119	0.00918	0.00572	0.00996	0.00583	0.01091	0.00396
CD3E	2.63429	2.70226	2.73630	2.72577	2.68704	2.74797	2.70479	2.71095	2.63201	2.70563	2.71496	2.70886
CD47	0.90091	0.94181	0.86841	0.83986	0.92407	0.90536	0.87975	0.98825	0.96417	0.95799	0.76705	0.86345
EOMES	0.54105	0.51162	0.54163	0.47563	0.46403	0.55395	0.55907	0.55567	0.42743	0.43512	0.49845	0.42353
GNLY	3.15915	3.45795	3.15755	3.27290	3.53107	3.51892	3.32752	3.29697	3.40267	3.74371	3.30059	3.10061
GZMA	2.49496	2.54909	2.44710	2.30355	2.60125	2.64510	2.61637	2.55225	2.41290	2.53808	2.42990	2.28270
GZMB	1.71362	1.71165	1.49039	1.82224	2.15004	1.89297	1.71497	2.02915	1.93595	2.07263	1.82312	1.59337
PRF1	1.87849	2.03592	1.72732	2.02710	2.12452	2.17325	1.94236	2.19282	1.77731	2.10111	1.76290	1.79707
IFNG	0.35735	0.60294	0.34807	0.68165	0.66204	0.60731	0.51949	0.53564	0.35628	0.82059	0.51851	0.46813
CD8A	1.68600	1.74903	1.76253	1.84830	1.77796	1.86460	1.90571	2.06191	1.76027	1.65562	1.76894	1.84566
CD8B	1.33602	1.64937	1.73461	1.89964	1.49134	1.75232	1.78427	1.97791	1.35203	1.56104	1.70275	1.98326
FASLG	0.10174	0.06547	0.09226	0.11543	0.14299	0.11975	0.14810	0.19396	0.05409	0.10607	0.09703	0.10647
LAMP1	0.99245	1.06135	0.87974	0.86182	1.01207	0.96763	0.83136	0.86086	0.99644	1.04186	0.89152	0.83298
LAG3	0.68794	0.64003	0.59110	0.51902	0.65576	0.57209	0.70824	0.71928	0.64396	0.60320	0.61873	0.49421
CTLA4	0.02726	0.04922	0.03181	0.01704	0.02289	0.07753	0.08211	0.07806	0.03941	0.06489	0.04083	0.04680
HLA.DRA	0.50783	0.78848	0.66032	0.70657	0.56372	0.81490	1.02737	1.18742	0.60936	0.66835	0.91706	0.76646
TNFRSF4	0.02704	0.14962	0.03096	0.01671	0.01828	0.15130	0.03535	0.02946	0.02262	0.14751	0.01819	0.02825
ICOS	0.05900	0.10550	0.09530	0.07118	0.06892	0.13357	0.12960	0.10808	0.07429	0.10214	0.07050	0.07685
TNFRSF9	0.02596	0.11279	0.01738	0.05127	0.01800	0.08639	0.00512	0.05769	0.01391	0.07270	0.01701	0.01997
TNFRSF18	0.01208	0.33197	0.02355	0.03353	0.00573	0.36090	0.02445	0.02282	0.02340	0.46554	0.01180	0.01987

Supplementary Table S5: Expression of T cell terminal differentiation signature
(defined as the normalized mean expression of genes)

Patient ID	P1			P2			P3			P4		
Timepoint	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
TIGIT	0.28457	0.43261	0.43525	0.37954	0.29340	0.54285	0.53348	0.49085	0.21284	0.36146	0.43312	0.31640
PDCD1	0.05924	0.08287	0.06996	0.04469	0.05406	0.08853	0.10508	0.10106	0.05609	0.06314	0.07883	0.05082
CD274	0.01867	0.02347	0.03254	0.01613	0.02183	0.02954	0.02812	0.02361	0.02331	0.01916	0.02330	0.01692
CTLA4	0.09866	0.15701	0.10458	0.06675	0.10756	0.17826	0.13987	0.12175	0.12467	0.18451	0.07766	0.10296
LAG3	0.41419	0.28302	0.39298	0.34738	0.37041	0.26883	0.50188	0.52782	0.37496	0.29459	0.39208	0.29506
HAVCR2	0.02193	0.01704	0.02149	0.03343	0.02609	0.02872	0.04892	0.07058	0.02381	0.04067	0.04234	0.04641
CD244	0.18075	0.15884	0.18190	0.18007	0.18768	0.14614	0.20745	0.18119	0.14678	0.16954	0.15873	0.13365
CD160	0.10817	0.05635	0.19030	0.23586	0.10616	0.06620	0.16794	0.22731	0.10058	0.07088	0.17947	0.26011

Supplementary Table S6: Expression of pro-inflammatory signature (defined as the normalized mean expression of genes)

Patient ID	P1			P2			P3			P4		
Timepoint	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
IL1B	0.02264	0.01849	0.04414	0.01061	0.00773	0.00603	0.03899	0.00883	0.04448	0.01485	0.02307	0.01420
TNF	0.11995	0.13610	0.20020	0.08682	0.22177	0.15649	0.24093	0.15446	0.19686	0.22624	0.19902	0.10508
IFNG	0.19177	0.25330	0.20004	0.44752	0.36894	0.27765	0.35926	0.37867	0.19364	0.38907	0.30944	0.27932
TBX21	0.51928	0.42439	0.54715	0.53230	0.52907	0.45901	0.58773	0.66198	0.53052	0.45335	0.53140	0.41496
CCL3	0.19144	0.20548	0.26703	0.34302	0.32598	0.23816	0.41692	0.32895	0.21843	0.36798	0.40535	0.22619
CCL4	1.27653	1.30311	1.70566	1.36431	1.80828	1.35560	1.94258	1.64382	1.62425	1.55272	1.63129	1.16050
PRF1	1.31417	1.13227	1.26173	1.56790	1.48183	1.32217	1.49575	1.76447	1.20424	1.26390	1.28835	1.27614
GZMA	1.88693	1.64152	1.93587	1.84416	1.93245	1.80660	2.16211	2.17753	1.78671	1.69206	1.91532	1.72525
GZMB	1.15690	0.85483	1.03555	1.37049	1.48749	1.05899	1.27840	1.59487	1.32380	1.21233	1.30940	1.08508
GZMK	1.42051	1.31447	1.66820	1.08601	1.06699	1.39835	1.83064	1.39927	1.28262	1.13509	1.63180	1.18604
GZMH	1.70387	1.41996	1.50485	1.88676	1.97238	1.62691	1.78190	2.08093	1.88518	1.71566	1.66951	1.71593
CD8A	1.17431	1.01633	1.31912	1.43807	1.21223	1.14938	1.49336	1.67813	1.21820	0.99396	1.34552	1.35892
FASLG	0.05052	0.02105	0.05169	0.06887	0.06883	0.04645	0.08838	0.12475	0.02553	0.04219	0.05287	0.05524
CCL2	0.00000	0.00000	0.00000	0.00000	0.00057	0.00000	0.00000	0.00000	0.00053	0.00000	0.00000	0.00000
CCL20	0.00292	0.00739	0.00289	0.00165	0.00496	0.00442	0.00183	0.00219	0.00149	0.00507	0.00336	0.00184
IL2	0.01575	0.01168	0.01569	0.00508	0.01161	0.01190	0.00914	0.00369	0.01618	0.00769	0.01467	0.00402
IL6	0.00076	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00060	0.00000	0.00000	0.00000
IL12A	0.01038	0.00693	0.00621	0.00673	0.00895	0.00494	0.00505	0.00569	0.01121	0.01094	0.00910	0.00538
IL17A	0.00088	0.00041	0.00273	0.00036	0.00011	0.00105	0.00222	0.00000	0.00084	0.00084	0.00149	0.00000
IL23A	0.05363	0.07979	0.08547	0.06933	0.06590	0.10864	0.09303	0.08104	0.04561	0.08988	0.07378	0.05159
PTGS2	0.00091	0.00197	0.00000	0.00046	0.00000	0.00000	0.00157	0.00192	0.00400	0.00031	0.00000	0.00000
TLR4	0.00218	0.00052	0.00302	0.00172	0.00352	0.00177	0.00218	0.00340	0.00000	0.00159	0.00050	0.00380

Supplementary Dataset

Supplementary Dataset S1: Gene and antibody sequences of 21 mAb

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Supplementary Dataset S2: Sequences of Spike-RBD(aa319-591)-mFC

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