Supplementary Information

Potential Immunomodulatory Effects of CAS+IMD Monoclonal Antibody Cocktail in Hospitalized Patients with COVID-19

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Supplementary Figures

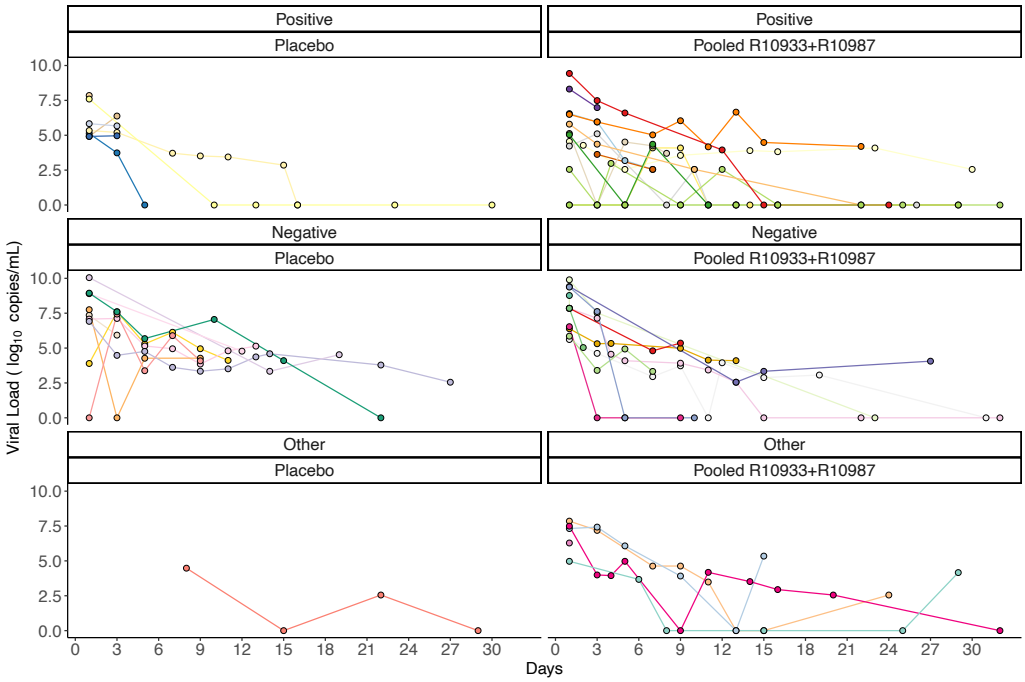

fig S1: Longitudinal viral load changes in patients with different baseline serostatus and treatment. Viral load from patients with different baseline serostatus and treatment group. Lines connecting samples collected from the same individual. All patients were assessed prior to dosing for baseline viral load in nasopharyngeal swabs and anti-SARS-CoV-2 antibodies. Briefly, Anti-spike (S1) immunoglobulin (Ig) A (EUROIMMUNE), anti-S1 IgG (EUROIMMUN), and anti-nucleocapsid IgG (Abbott) were accessed using the cut-offs for negative, positive, or borderline as defined per manufacturer's instructions for use. For patients with board line or missing data, they are categorized as "other" in this study.

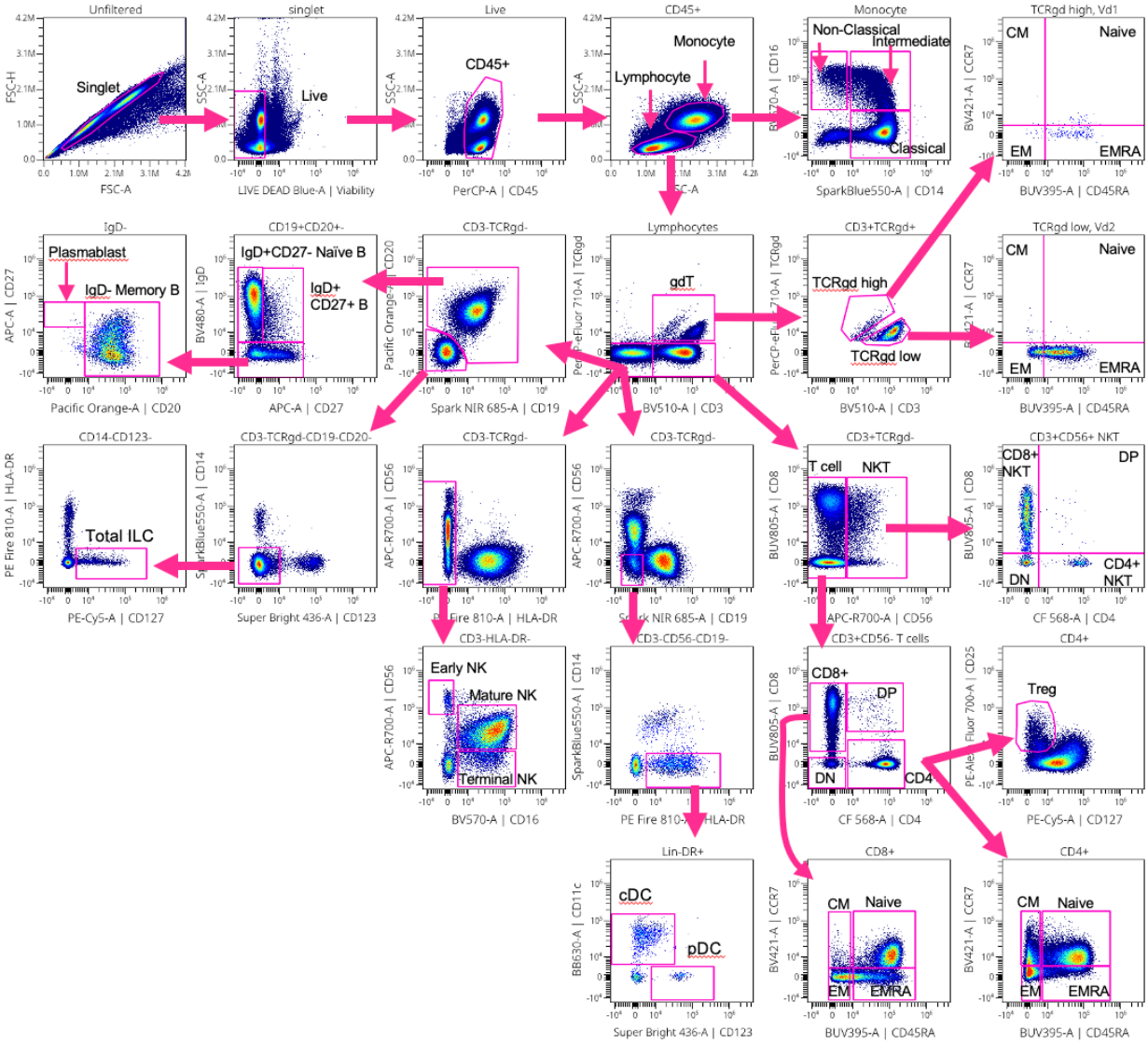

fig S2: Manual flow cytometry gating strategy to identify major immune cell subsets. Flow cytometry data from one patient post Ab treatment with well-representing cell populations was selected and shown here for gating strategy. Arrows connecting parental gate and child gates. Abbreviations: gdT, gamma delta T cells; CM, central memory; EM, effector memory; EMRA, effector memory cells that re-express CD45RA. ILC, innate lymphoid cells; NK, natural killer cells; NKT, natural killer T cells; DP, double positive cells; DN, double negative cells; Treg, regulatory T cells; cDC, classical dendritic cells; pDC, plasmacytoid dendritic cells.

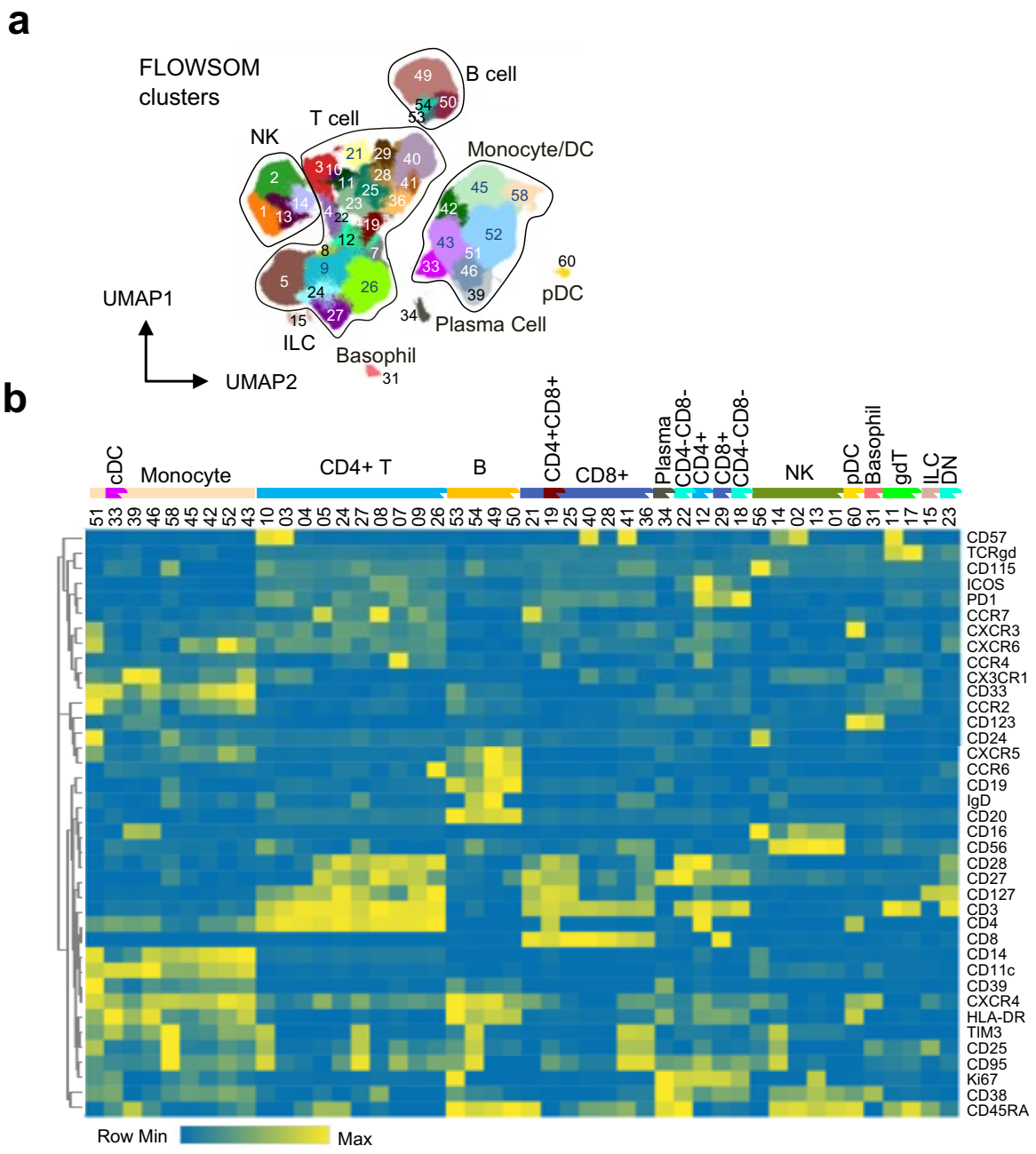

fig S3: Characterization of blood immune cell subsets by flow cytometry analysis. (A) UMAP and unsupervised cluster analysis of PBMC from combined all patient samples from all time points and treatment groups. Major immune cell subsets are marked on the graph. Number of individual clusters is labeled on individual cluster that identified by FLOWSOM. (**B**) Heatmap of selected 38 markers used to phenotyping the immune cell subsets. Expression level was scaled by MFI of individual marker and color coded based on row minimum (Min, blue) and maximum (Max, yellow). Numbers indicate cluster ID and major immune subsets are annotated.

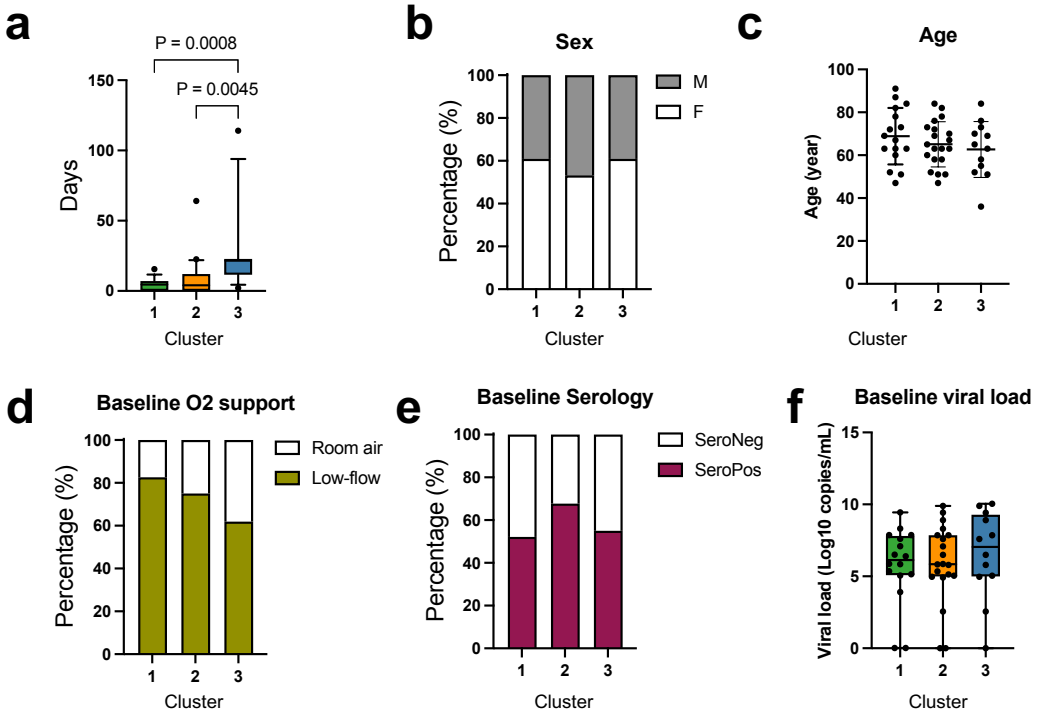

fig S4: Clinical characteristics of samples from three immunophenotypes. (A) Sample collection timepoint (in days, baseline day 0) of each immunophenotypes labeled in X-axis. (B) Sex distribution. (C) Age distribution. (D) Baseline O2 requirement. (E) Baseline serology. (F) Baseline viral load. (Non-parametric Kruskal Wallis test, corrected for multiple comparisons using Dunn's test. ** P<0.01, *** P<0.001.)

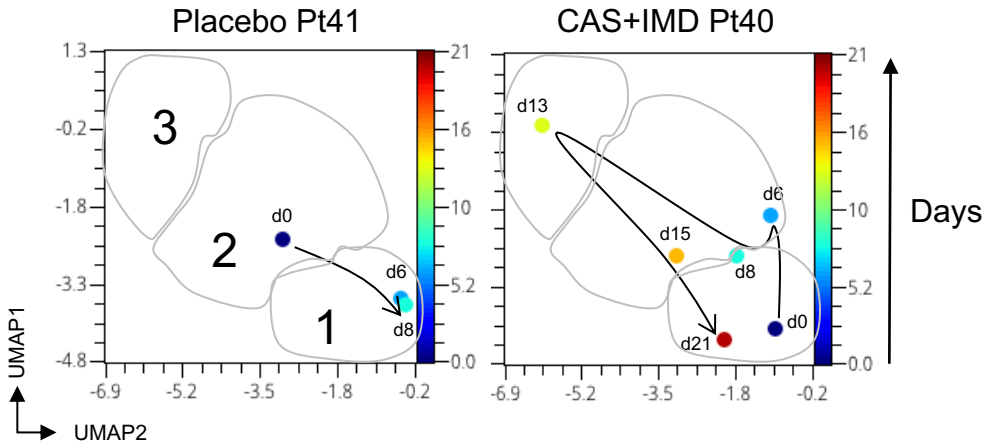

fig S5: Immunophenotype of longitudinal samples from two deceased patients treated with placebo or CAS+IMD. UMAP plots showing the samples from indicated patient. Time points of samples were collected are annotated on the graph. Color matrix is corresponding to the time points. Immunophenotype (1-3) are marked on the UMAP.

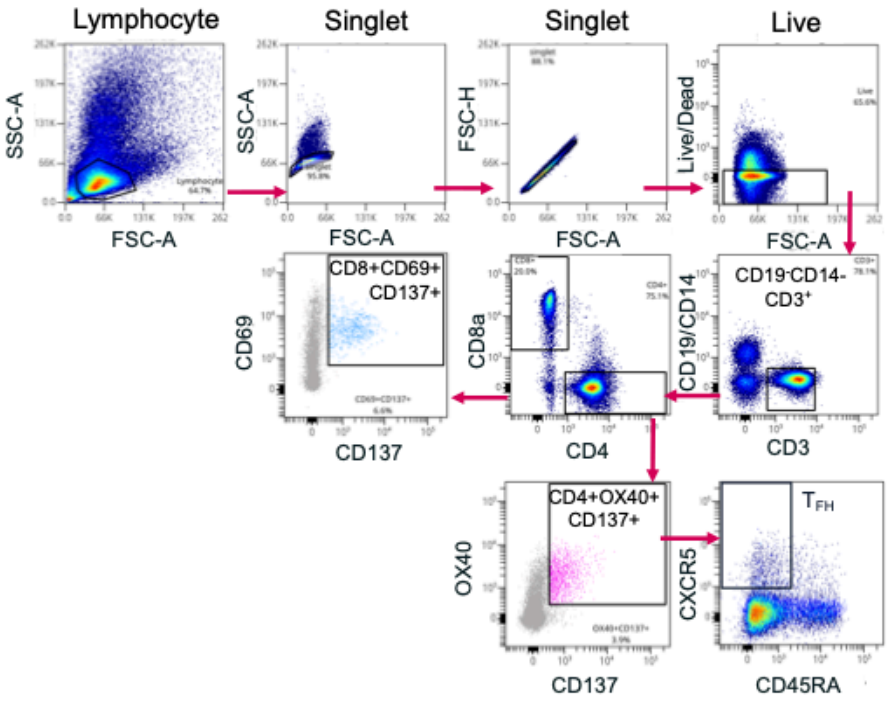

fig S6: Flow cytometry gating for AIM assay. Representative gating of viral-specific $CD4^+$ and $CD8^+$ T cells. Briefly, mononuclear cells were gated out of total events by subsequent singlet gating. Live cells were gated as Fixable Blue (DAPI channel) negative. T cells were then gated as CD19/CD14 negative and $CD3^+$ cells. T cells were further gated into either $CD8^+$ or $CD4^+$ populations. Antigen-specific $CD4^+$ T cells were gated on $OX40^+CD137^+$ subset (overlayed pink color); antigen-specific $CD8^+$ T cells were gated on $CD69^+CD137^+$ subset (overlayed in blue color).

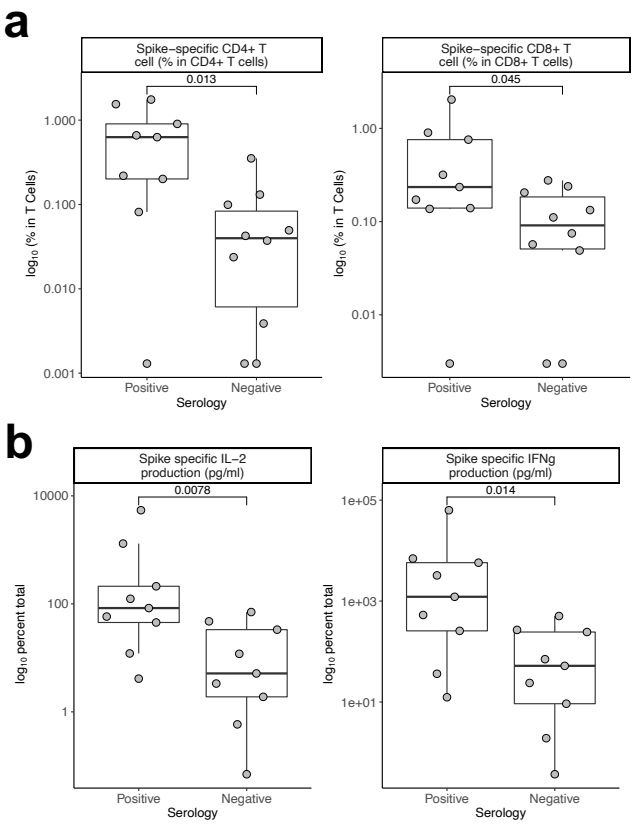

fig S7: Baseline spike-specific T cell response in seropositive and seronegative patients. (**A**). Magnitude of spike-specific $CD4^+$ and $CD8^+$ T cell responses from seropositive versus seronegative patients. (**B**). Spike-specific IL-2 and IFN-g production from AIM assay. Nominal P values are annotated on individual graph.

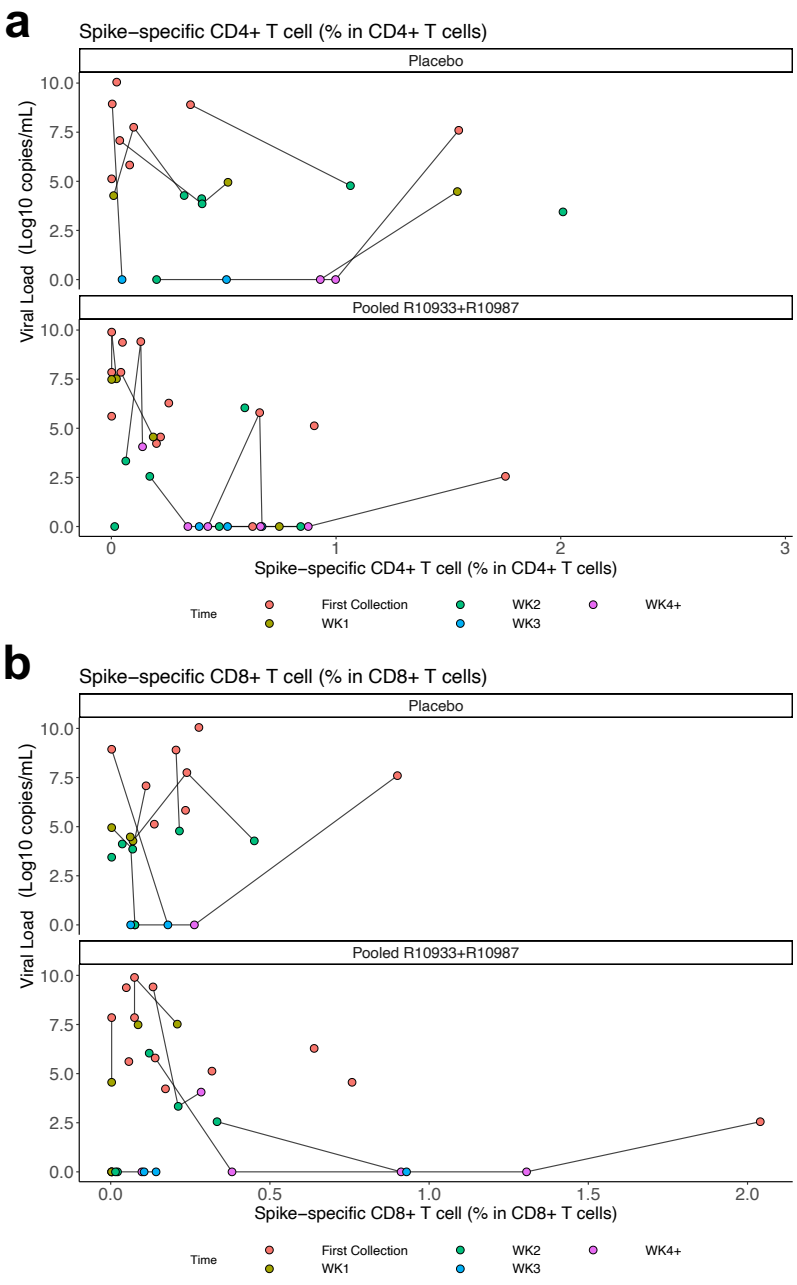

fig. S8. Correlation of viral load and spike-specific T cell response longitudinally. Correlation between viral load and spike-specific $CD4^+$ (A) or $CD8^+$ T cell responses from different time point (binned in weeks). Lines connecting the samples from the same patient.

Supplementary Table 1. Flow cytometry antibodies

Antibody	Vendor	Clone	Cat. #	Conjugate	Dilution
4-1BB	BioLegend	4B4-1	309804	PE	1:20
CCR2	BD Bioscience	48607	558406	AF647	1:20
CCR4	BD Bioscience	1G1	624296	BB790	1:100
CCR6	BD Bioscience	11A9	751515	BUV615	1:25
CCR7	BD Bioscience	3D12	740052	BV421	1:25
CD115	BD Bioscience	9-4D2-1E4	565346	BB515	1:20
CD11c	BD Bioscience	B-ly6	624294	BB630	1:200
CD123	eBioscience	6H6	62-1239-42	Super Bright 436	1:50
CD127	Invitrogen	PCH101	15-4776-42	PE-CY5	1:50
CD138	BD Bioscience	MI15	749874	BUV496	1:20
CD14	BioLegend	63D3	367148	Spark Blue 550	1:400
CD14	BioLegend	63D3	367110	PerCP/Cyanine5.5	1:50
CD16	BioLegend	3G8	302036	BV570	1:100
CD19	BioLegend	HIB19	302270	Spark NIR 685	1:100
CD19	BioLegend	HIB19	302230	PerCP/Cyanine5.5	1:50
CD20	Invitrogen	HI47	MHCD2030	Pacific Orange	1:25
CD24	BD Bioscience	ML5	751874	R718	1:50
CD25	Invitrogen	CD25-3G10	MHCD2524	PE-AlexaFluor700	1:50
CD27	BioLegend	O323	302810	APC	1:50
CD28	BD Bioscience	CD28.2	562976	BV605	1:20
CD3	BioLegend	UCHT-1	300448	BV510	1:50
CD3	BioLegend	UCHT-1	300436	BV570	1:50
CD33	BioLegend	P67.6	366616	PerCP/Cyanine5.5	1:200
CD38	BD Bioscience	HIT2	741837	BUV737	1:20
CD38	BioLegend	HIT2	303550	APC/Fire810	1:100
CD39	BD Bioscience	TU66	749967	BUV661	1:20
CD4	Biotium	C4/206	BNC680206-500	CF568	1:200
CD4	BioLegend	T4, Leu3a	344656	Spark Blue™ 550	1:25
CD45	BioLegend	2D1	368506	PerCP	1:50
CD45RA	BD Bioscience	HI100	740298	BUV395	1:100
CD56	BD Bioscience	NCAM16.2	565139	APC-R700	1:100
CD57	BioLegend	HNK-1	359608	Pacific Blue	1:100
CD66b	BioLegend	G10F5	984102	FITC	1:50
CD69	BD Bioscience	FN50	563834	BV786	1:20
CD8	BD Bioscience	RPA-T8	749366	BUV805	1:100

CD95	BioLegend	DX2	305638	APC/Fire750	1:50
CX3CR1	BD Bioscience	2A9-1	749355	BUV737	1:200
CXCR3	BD Bioscience	1C6/CXCR 3	740603	BV650	1:20
CXCR4	BioLegend	12G5	306506	PE	1:100
CXCR5	BD Bioscience	RF8B2	747111	BV750	1:50
CXCR5	eBioscience	MU5UBEE	15-9185-42	PE-Cy5	1:20
CXCR6	BD Bioscience	13B 1E5	748450	BUV563	1:20
HLA-DR	BioLegend	L243	CUSTOM	PE Fire 810	1:100
HLA-DR	BioLegend	Tu36	361612	PE-Cy7	1:200
ICOS	BioLegend	C398.4A	313532	PE/Dazzle594	1:400
IgD	BD Bioscience	IA6-2	566138	BV480	1:50
Ki67	BD Bioscience	B56	563755	BV711	1:200
OX40	BioLegend	Ber-ACT35	350032	APC/Fire™ 750	1:20
PD-1	BioLegend	EH12.2H7	329930	BV785	1:50
PD-1	BioLegend	EH12.2H7	329924	BV605	1:50
TCRgd	Invitrogen	B1.1	46-9959-42	PerCP-eFluor710	1:50
Tim3	BioLegend	F38-2E2	345014	PE-CY7	1:100

Supplementary Table 2. A complete list of significantly expressed markers or immune cell subsets in each cluster. *Provided in a sperate excel file.*

Supplementary Table 3. Proteomics enriched pathway analysis (related to Fig 1C)

Pathways	-LogP	-Log(q-value)	Number of markers	Marker ratio	Markers
cellular response to cytokine stimulus	18.57	14.89	26	3.616	CD40,CRKL,CSF1,GBP2,CXCL1,CXCL3,IL5,IL5RA,IL6,CXCL8,IL15,IL15RA,CXCL10,IRAK1,LGALS9,OSM,PDGFB,CCL3,CCL7,CCL23,SHMT1,TFF2,TNFRSF14,TNFRSF11A,SPRY2,LILRB4
leukocyte activation	17.19	13.76	23	3.938	CD40,CD40LG,CD79B,CSF1,CXADR,FKBP1B,HLA-E,IL6,CXCL8,IL15,IL15RA,JUN,MICB,CCL3,LY6D,SKAP2,LAT,SLAMF7,CLEC7A,CEACAM21,MILR1,CLEC4D,MICA
positive regulation of MAPK cascade	16.33	13.00	21	4.242	CD40,CRKL,F2R,HGF,IL6,IRAK1,JUN,LGALS9,OSM,PDGFB,MAP2K6,CCL3,CCL7,CCL23,TGFA,TIMP3,VEGFA,TNFRSF11A,SPRY2,LILRB4,PROK1,TRIM5,CXCL17
cell chemotaxis	15.26	12.10	15	7.426	CRKL,CXADR,CXCL1,CXCL3,HGF,IL6,CXCL8,CXCL10,PDGFB,CCL3,CCL7,CCL23,VEGFA,TNFRSF11A,CXCL17
adaptive immune response	14.94	11.81	21	3.614	CD40,CD40LG,CD79B,CTSC,HLA-E,IL6,KLRD1,LAIR1,MICB,TNFRSF14,TNFRSF11A,LILRB4,BTN3A2,TNFRSF13B,LAT,LAMP3,DBNL,SLAMF7,SIGLEC10,CLEC4D,MICA,CXADR,CCL3,MILR1,PTX3,TRIM21
regulation of defense response	14.64	11.53	23	2.999	CD40,CTSC,HGF,HLA-E,HSPA1A,IL6,IL15,IRAK1,KLRD1,LGALS9,MICB,OSM,MAP2K6,CCL3,TRIM21,CST7,TNFRSF11A,HEXIM1,CLEC7A,TRIM5,SIGLEC10,CXCL17,MICA
myeloid leukocyte migration	14.54	11.47	13	9.286	CSF1,CXADR,CXCL1,CXCL3,IL6,CXCL8,CXCL10,PDGFB,CCL3,CCL7,CCL23,TNFRSF11A,CXCL17
SARS-CoV-2 innate immunity evasion and cell-specific immune response	8.45085485	6.179	7	10.606	CXCL1,CXCL3,IL6,CXCL8,CXCL10,CCL3,CXCL17
COVID-19 adverse outcome pathway	6.72033674	4.586	4	26.667	IL6,CXCL8,CXCL10,CCL3