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Supplemental information

A third SARS-CoV-2 mRNA vaccine dose in people receiving hemodialysis overcomes B cell defects but elicits a skewed CD4^{+} T cell profile

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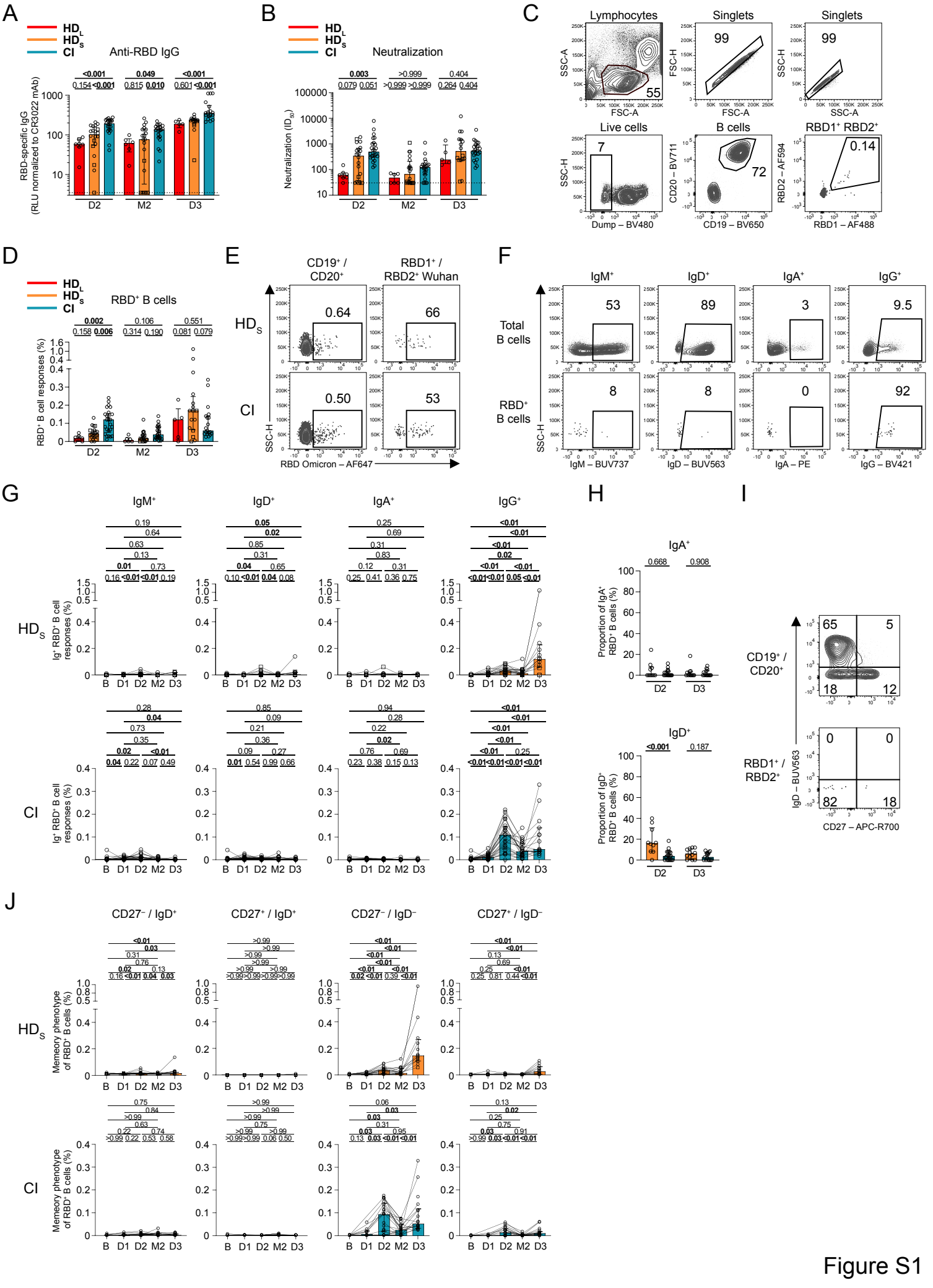
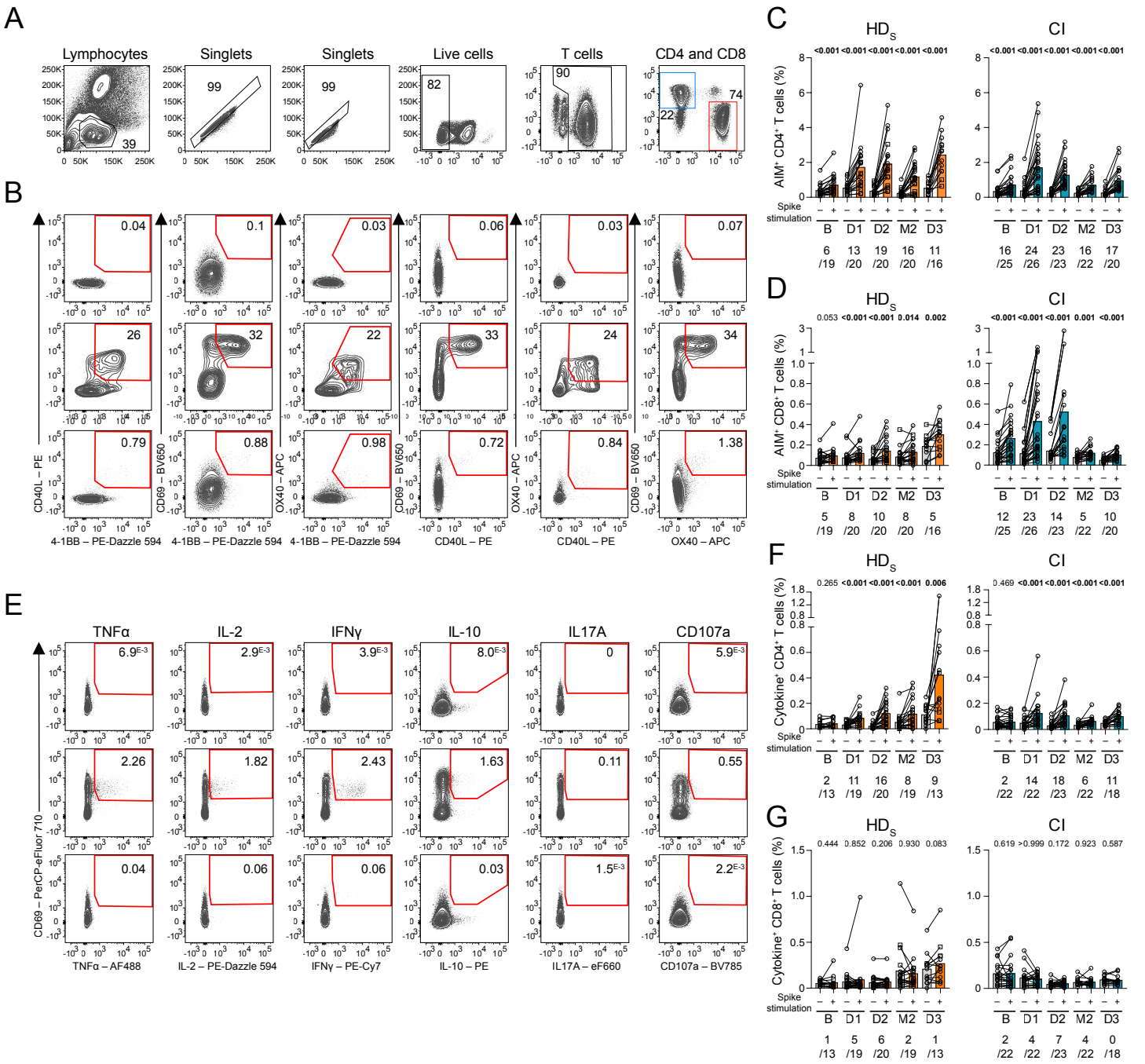

A
HD_L
HD_S
CI
Anti-RBD IgG
RBD-specific IgG (RLU normalized to CR3022 mAb)
D2
M2
D3
B
Neutralization
Neutralization (ID50)
C
Lymphocytes
Singlets
Live cells
B cells
RBD1+ RBD2+
SSC-A
FSC-A
SSC-H
Dump – BV480
CD20 – BV711
CD19 – BV650
RBD2 – AF594
RBD1 – AF488
D
RBD+ B cells
RBD+ B cell responses (%)
E
CD19+ / CD20+
RBD1+ / RBD2+ Wuhan
HD_S
CI
RBD Omicron – AF647
F
IgM+
IgD+
IgA+
IgG+
Total B cells
RBD+ B cells
IgM – BUV737
IgD – BUV563
IgA – PE
IgG – BV421
G
IgM+
IgD+
IgA+
IgG+
Ig+ RBD+ B cell responses (%)
B D1 D2 M2 D3
H
IgA+
IgD+
Proportion of IgA+ RBD+ B cells (%)
Proportion of IgD+ RBD+ B cells (%)
I
CD19+ / CD20+
RBD1+ / RBD2+
IgD – BUV563
CD27 – APC-R700
J
CD27− / IgD+
CD27+ / IgD+
CD27− / IgD−
CD27+ / IgD−
Memeory phenotype of RBD+ B cells (%)

Figure S1

Figure S1. Antibody and SARS-CoV-2-specific B cell responses in hemodialysis patients. Related to Figure 1. (A) Comparison of RBD-specific IgG responses between HD_L (red), HD_S (orange) and CI (blue) participants at D2, M2 and D3. HD_S on immunosuppressive drugs are represented by square symbols, and HD_S not on immunosuppressants are represented by circles. Bars represent medians ± interquartile ranges. Intercohort statistical comparisons using a linear mixed model are shown. **(B)** Comparison of neutralizing activity between HD_L (red), HD_S (orange) and CI (blue) participants at D2, M2 and D3. HD_S on immunosuppressive drugs are represented by square symbols, and HD_S not on immunosuppressants are represented by circles. Bars represent medians ± interquartile ranges. Intercohort statistical comparisons using a linear mixed model are shown. **(C)** Gating strategy to identify RBD^+ B cells. **(D)** Comparison of RBD^+ B cell responses between HD_L (red), HD_S (orange) and CI (blue) participants at D2, M2 and D3. HD_S on immunosuppressive drugs are represented by square symbols, and HD_S not on immunosuppressants are represented by circles. Bars represent medians ± interquartile ranges. Intercohort statistical comparisons using a linear mixed model are shown. **(E)** Gating strategy of Omicron RBD^+ B cells among total B cells (left) and among Wuhan-1-RBD^+ B cells (right). **(F)** Examples of gatings for IgD, IgM, IgA and IgG expression on total $CD19^+CD20^+$ B cells (left) or RBD^+ B cells (right). **(G)** Histograms reporting the longitudinal frequency of isotype expression in HDs (orange) and CI (blue) participants. Lines connect data points for individual participants. Wilcoxon tests are shown above each panel. **(H)** Comparison of IgD^+ and IgA^+ RBD^+ B cells between HD_S and CI participants at D2 and D3. Mann-Whitney tests are shown. **(I)** Gating strategy of $IgD^{+/-}$ and $CD27^{+/-}$ of total (left) and RBD^+ memory B cells (right). **(J)** Histograms reporting the longitudinal frequency of each IgD and CD27 RBD-B phenotypes in $CD19^+$ $CD20^+$ B cells for HDs (orange) and CI (blue) participants. In support of the pie charts displayed in Figure 1K. Wilcoxon tests are shown above. In ABD) n=7 HD_L, n=20 HD_S, n=26 CI. GJ) n=20 HDs, n=26 CI. H) n=16 HDs, n=23 CI.

Figure S2


A
Lymphocytes
Singlets
Singlets
Live cells
T cells
CD4 and CD8
B
4-1BB – PE-Dazzle 594
CD40L – PE
CD69 – BV650
OX40 – APC
C
HDS
CI
AIM+ CD4+ T cells (%)
Spike stimulation
B D1 D2 M2 D3
D
AIM+ CD8+ T cells (%)
E
TNFα
IL-2
IFNγ
IL-10
IL17A
CD107a
CD69 – PerCP-eFluor 710
TNFα – AF488
IL-2 – PE-Dazzle 594
IFNγ – PE-Cy7
IL-10 – PE
IL17A – eF660
CD107a – BV785
F
Cytokine+ CD4+ T cells (%)
G
Cytokine+ CD8+ T cells (%)

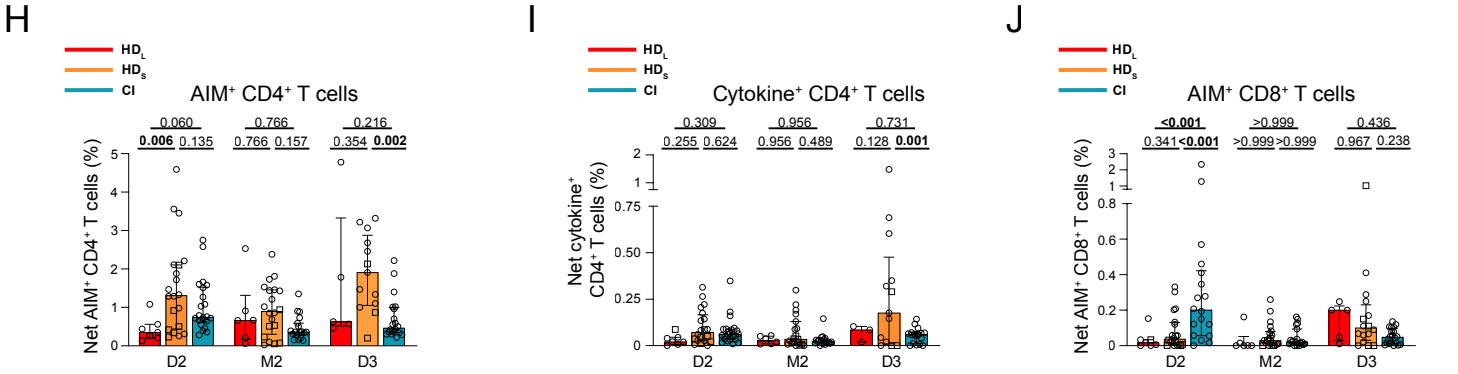

H
HDL
HDS
CI
AIM+ CD4+ T cells
Net AIM+ CD4+ T cells (%)
D2 M2 D3
I
Cytokine+ CD4+ T cells
Net cytokine+ CD4+ T cells (%)
J
AIM+ CD8+ T cells
Net AIM+ CD8+ T cells (%)

Figure S2. SARS-CoV-2-specific T cell responses in hemodialysis patients. Related to Figure 2. (A) Representative upstream generic gating and **(B)** ORgate strategy to identify SARS-CoV-2-specific AIM^+ T cells. For simplicity, the example focuses on $CD4^+$ T cells. **(CD)** Raw frequencies of **(C)** AIM^+ CD4+ and **(D)** $CD8^+$ T cells following *ex vivo* stimulation of PBMCs with a pool of SARS-CoV-2 Spike peptides (colored). HD_S are represented on the left and CI on the right for each panel. As a control, PBMCs cells were left unstimulated (grey bars). HD_S on immunosuppressive drugs are represented by square symbols, and HD_S not on immunosuppressants are represented by circles. The bars represent median values. Wilcoxon tests are shown. The numbers of responders at least two times over unstimulated conditions are written below the histograms for each timepoint. **(E)** Representative ORgate strategy to identify SARS-CoV-2-specific cytokine-expressing T cells. For simplicity, the example focuses on CD4 T cells. **(FG)** Raw frequencies of **(F)** cytokine-expressing $CD4^+$ T cells and **(G)** $CD8^+$ T cells following *ex vivo* stimulation of PBMCs with a pool of SARS-CoV-2 Spike peptides (colored). HD_S are represented on the left and CI on the right for each panel. As a control, PBMCs cells were left unstimulated (grey bars). HD_S on immunosuppressive drugs are represented by square symbols, and HD_S not on immunosuppressants are represented by circles. The bars represent median values. Wilcoxon tests are shown. The numbers of responders at least two times over unstimulated conditions are written below the histograms for each timepoint. **(HIJ)** Comparison at D2, M2 and D3 of **(H)** net AIM^+ $CD4^+$ T cell responses, **(I)** net $cytokines^+$ $CD4^+$ responses and **(J)** net AIM^+ $CD8^+$ responses between HD_L (red), HD_S (orange) and CI (blue) participants. HD_S on immunosuppressive drugs are represented by square symbols, and HD_S not on immunosuppressants are represented by circles. The bars represent median and interquartile ranges. Intercohort statistical comparisons using a linear mixed model. In A-G) n=20 HD_S, n=26 CI participants, in H-J) n=20 HD_S, n=26 CI, n=7 HD_L participants.

Figure S3

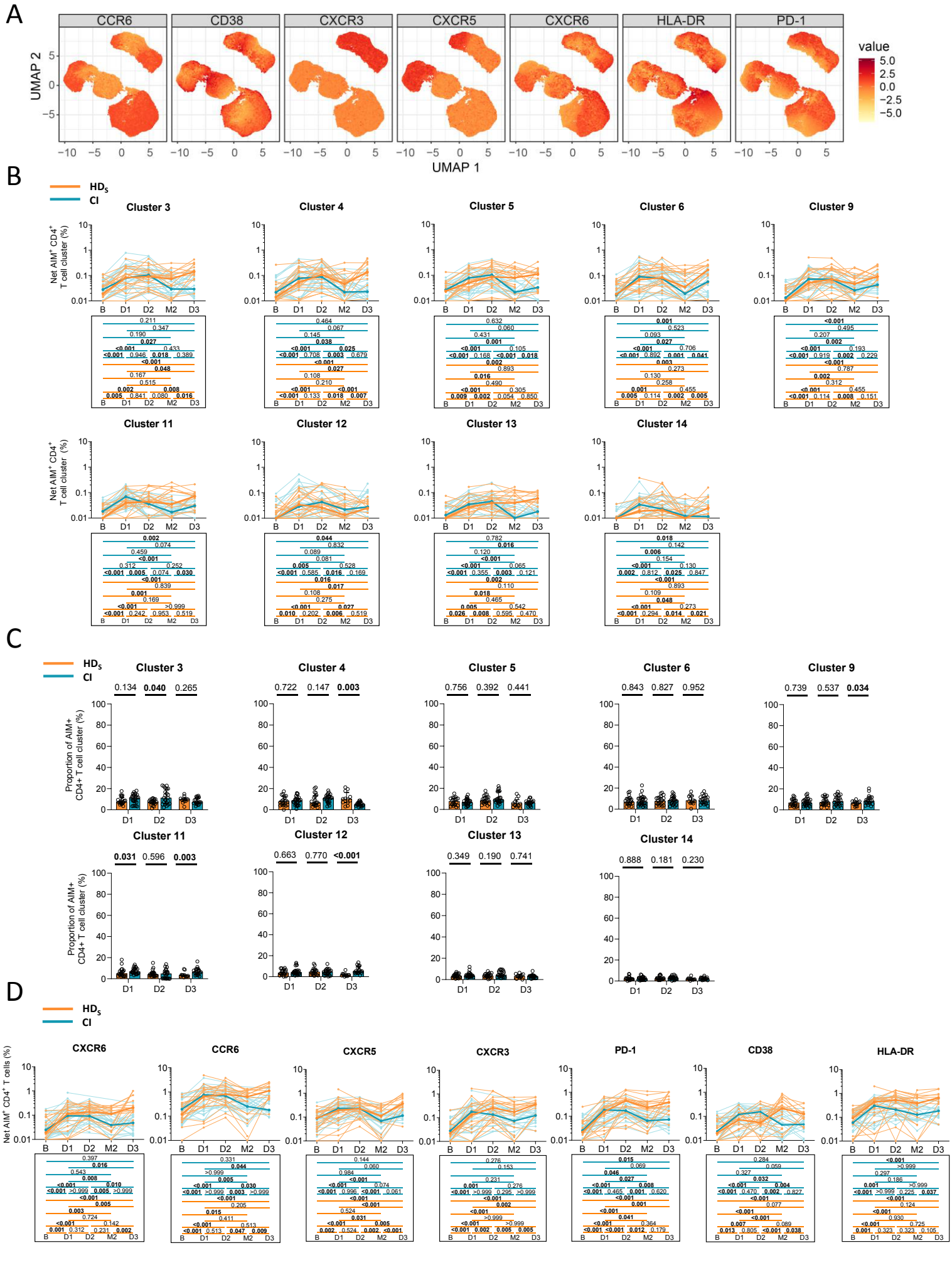

A
CCR6
CD38
CXCR3
CXCR5
CXCR6
HLA-DR
PD-1
UMAP 2
UMAP 1
value
5.0
2.5
0.0
−2.5
−5.0
B
HDs
CI
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 9
Cluster 11
Cluster 12
Cluster 13
Cluster 14
Net AIM+ CD4+ T cell cluster (%)
B D1 D2 M2 D3
C
HDs
CI
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 9
Cluster 11
Cluster 12
Cluster 13
Cluster 14
Proportion of AIM+ CD4+ T cell cluster (%)
D1 D2 D3
D
HDs
CI
CXCR6
CCR6
CXCR5
CXCR3
PD-1
CD38
HLA-DR
Net AIM+ CD4+ T cells (%)
B D1 D2 M2 D3

Figure S3. Phenotypic characterization of SARS-CoV-2-specific CD4$^+$ T cell responses in hemodialysis patients. Related to Figure 3. (A) Heat map overlaid on the AIM$^+$ UMAP showing the gradient of expression for each marker. **(B)** Longitudinal analysis of net AIM$^+$ CD4$^+$ T cell clusters, regarding clusters 3, 4, 5, 6, 9, 11, 12, 13, and 14 for HD_S (orange, n=20) and CI (blue; n=26) participants. Lines connect data from the same donor. Bold lines represent median values. Wilcoxon tests are shown below for each pairwise comparison. Complement Figure 3E. **(C)** Proportions of AIM$^+$ clusters 3, 4, 5, 6, 9, 11, 12, 13 and 14 among AIM$^+$ CD4$^+$ T cells in HD_S and CI at D1, D2 and D3. Bars represent medians ± interquartile ranges. Mann-Whitey tests are shown. Complement Figure 3F. **(D)** Longitudinal frequencies of CCR6$^+$, CXCR5$^+$, CXCR3$^+$, PD-1$^+$, CD38$^+$ and HLA-DR$^+$ AIM$^+$ CD4$^+$ T cells in HD_S (orange) and CI (blue). Lines connect data from the same donor. The bold lines represent the median value of each cohort. Statistical comparisons using a linear mixed model.

Figure S4

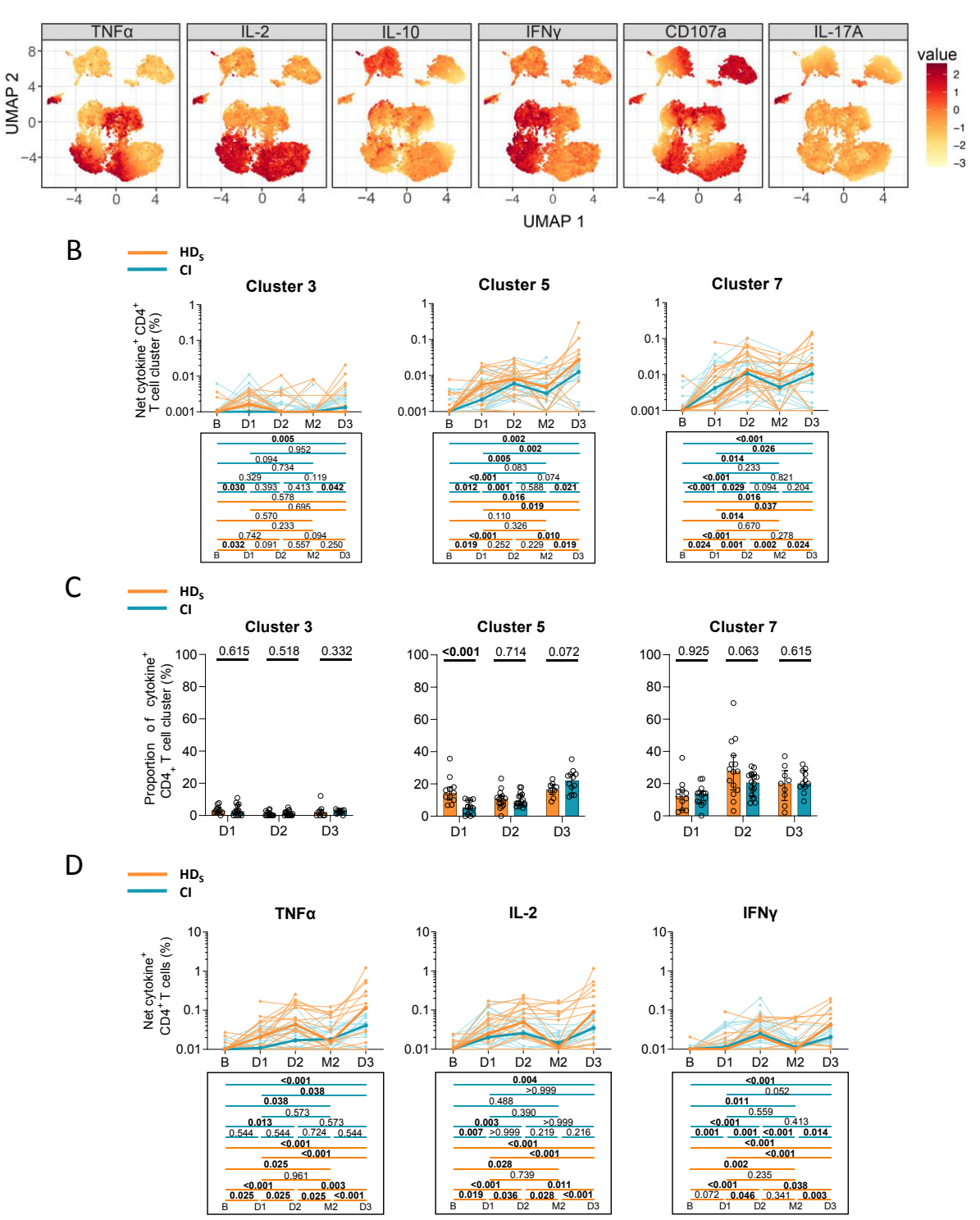

A
TNFα
IL-2
IL-10
IFNγ
CD107a
IL-17A
value
UMAP 2
UMAP 1
B
HDs
CI
Cluster 3
Cluster 5
Cluster 7
Net cytokine+ CD4+ T cell cluster (%)
C
Proportion of cytokine+ CD4+ T cell cluster (%)
D
Net cytokine+ CD4+ T cells (%)
TNFα
IL-2
IFNγ

Figure S4. Effector functions profiling of SARS-CoV-2-specific CD4$^+$ T cells responses in hemodialysis patients. Related to Figure 4. (A) Heat map overlaid on the cytokine$^+$ UMAP showing the gradient of expression for each marker. **(B)** Longitudinal analysis of net cytokine$^+$ CD4$^+$ T cell clusters, regarding clusters 3, 5 and 7 for HD_S (orange, n=20) and CI (blue; n=26) participants. Lines connect data from the same donor. Bold lines represent median values. Wilcoxon tests are shown below for each pairwise comparison. Complement Figure 4E. **(C)** Proportions of cytokine$^+$ clusters 3, 5 and 7 among cytokine$^+$ CD4$^+$ T cells in HD_S and CI at D1, D2 and D3. Bars represent medians ± interquartile ranges. Mann-Whitey tests are shown. Complement Figure 4F. **(D)** Longitudinal frequencies of TNFα$^+$, IL-2$^+$ and IFNγ$^+$ CD4$^+$ T cells in HD_S (orange) and CI (blue). Lines connect data from the same donor. The bold line represents the median value of each cohort. Statistical comparisons using a linear mixed model.

Figure S5

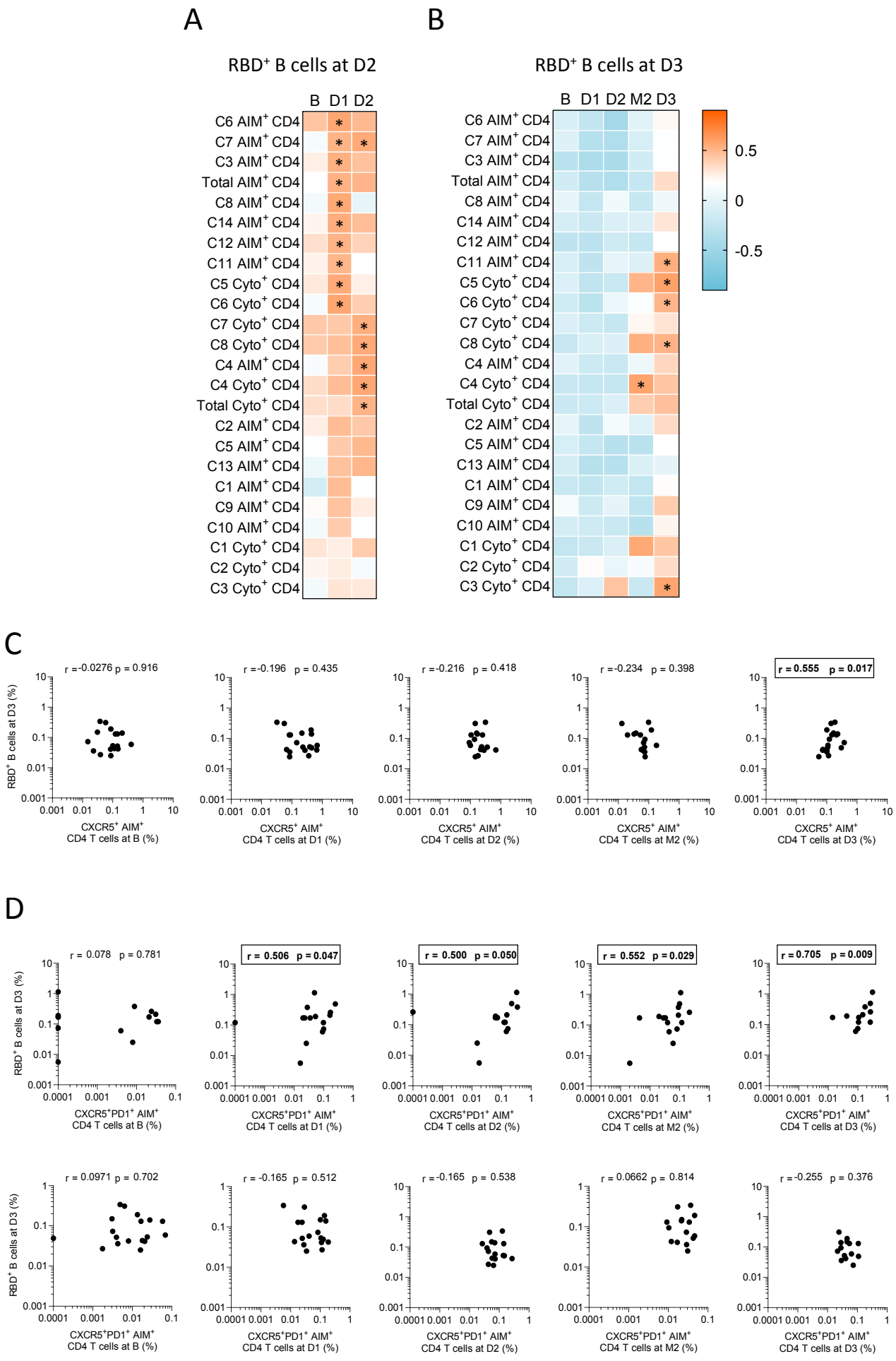

A
RBD+ B cells at D2
B D1 D2
C6 AIM+ CD4
C7 AIM+ CD4
C3 AIM+ CD4
Total AIM+ CD4
C8 AIM+ CD4
C14 AIM+ CD4
C12 AIM+ CD4
C11 AIM+ CD4
C5 Cyto+ CD4
C6 Cyto+ CD4
C7 Cyto+ CD4
C8 Cyto+ CD4
C4 AIM+ CD4
C4 Cyto+ CD4
Total Cyto+ CD4
C2 AIM+ CD4
C5 AIM+ CD4
C13 AIM+ CD4
C1 AIM+ CD4
C9 AIM+ CD4
C10 AIM+ CD4
C1 Cyto+ CD4
C2 Cyto+ CD4
C3 Cyto+ CD4
B
RBD+ B cells at D3
B D1 D2 M2 D3
0.5
0
-0.5
C
r =-0.0276 p = 0.916
r =-0.196 p = 0.435
r =-0.216 p = 0.418
r =-0.234 p = 0.398
r = 0.555 p = 0.017
RBD+ B cells at D3 (%)
CXCR5+ AIM+ CD4 T cells at B (%)
CXCR5+ AIM+ CD4 T cells at D1 (%)
CXCR5+ AIM+ CD4 T cells at D2 (%)
CXCR5+ AIM+ CD4 T cells at M2 (%)
CXCR5+ AIM+ CD4 T cells at D3 (%)
D
HDS
r = 0.078 p = 0.781
r = 0.506 p = 0.047
r = 0.500 p = 0.050
r = 0.552 p = 0.029
r = 0.705 p = 0.009
CXCR5+PD1+ AIM+ CD4 T cells at B (%)
CXCR5+PD1+ AIM+ CD4 T cells at D1 (%)
CXCR5+PD1+ AIM+ CD4 T cells at D2 (%)
CXCR5+PD1+ AIM+ CD4 T cells at M2 (%)
CXCR5+PD1+ AIM+ CD4 T cells at D3 (%)
CI
r = 0.0971 p = 0.702
r = -0.165 p = 0.512
r = -0.165 p = 0.538
r = 0.0662 p = 0.814
r = -0.255 p = 0.376

Figure S5: Associations between RBD$^+$ B cell and SARS-CoV-2-specific CD4$^+$ T cell responses in hemodialysis patients. Related to Figure 5. Temporal relationships between S-specific CD4$^+$ T cells and RBD$^+$ B cells. **(A)** Correlation between total CD4$^+$ T cell frequencies at B-D2 and RBD$^+$ B cell frequencies at D2 in CI (n=26). **(B)** Correlation between total CD4$^+$ T cell frequencies at B-D3 and RBD$^+$ B cell frequencies at D3 in CI (n = 26). Asterisks indicate statistically significant p value from a Spearman test ($p<0.05$). Colors indicate Spearman r. **(C)** Correlations between frequencies of AIM$^+$ CXCR5$^+$ CD4$^+$ T cells (for cTfh) at the B–D3 visits and RBD$^+$ B cell frequencies at D3 in CI. The r and p values from a Spearman test are indicated in each graph. **(D)** Correlations between frequencies of AIM$^+$ CXCR5$^+$ PD-1$^+$ CD4$^+$ T cells (for PD-1$^+$ cTfh) at the B–D3 visits and RBD$^+$ B cell frequencies at D3 in HD_S (top) and CI (bottom). The r and p values from a Spearman test are indicated in each graph.

Table S1. Clinical characteristics of the hemodialysis cohorts†. Related to Table 1.

Cohorts	Patients ID	Cause of ESRD	Years on HD	Comorbidities										Immunomodulating characteristics		
				DM	Cancer	CAD	Heart failure	Stroke / TIA	PVD	COPD	HBV	HCV	Cirrhosis	Previous kidney transplant	Immuno-suppressant medication	HIV
HD_S	1072-36	PCKD	15	N	N	N	N	N	N	N	N	N	N	Y	Prednisone, Cyclosporine	N
	1072-37	GN	4.3	Y	Y	N	N	N	N	N	N	N	N	N	N	N
	1072-38	GN	2.6	N	N	N	N	N	N	N	N	N	N	N	N	N
	1072-46	Atypical HUS	14.4	N	N	N	N	N	N	N	N	N	N	Y	Prednisone	N
	1072-56	HTN	28.4	N	N	N	N	Y	N	N	N	N	N	Y	N	N
	1072-61	GN	13.2	N	N	N	N	N	N	N	N	N	N	Y	Prednisone, Tacrolimus	N
	1072-64	DM vs GN	2.7	Y	N	Y	N	N	N	N	N	N	N	N	N	N
	1072-66	GN	10.4	N	N	N	N	N	N	N	N	N	N	N	N	N
	1072-68	GN	8.2	N	N	N	N	N	N	N	N	Y	N	N	N	N
	1072-72	DM	3.7	Y	N	N	N	N	N	N	N	N	N	N	N	N
	1072-84	HTN	8.5	N	N	N	N	N	N	N	Y	N	N	N	N	N
	1072-86	Alport syndrome	23.4	N	N	N	N	N	N	N	N	N	N	Y	Tacrolimus	N
	1072-94	GN	13.4	Y	N	N	N	N	N	N	N	N	N	Y	Prednisone, Tacrolimus	N
	1072-103	DM	4.2	Y	N	N	N	N	N	N	Y	Y	N	N	N	N
	1072-104	DM	0.9	Y	Y	N	N	N	N	N	N	N	N	N	N	N
	1072-105	GN	5.5	Y	Y	N	N	N	N	N	N	N	N	Y	Tacrolimus	N
	1072-106	DM	0.4	Y	N	N	N	N	N	N	N	N	N	N	N	N
	1072-116	DM & HTN	2.6	Y	N	N	N	N	N	N	N	N	N	N	N	N
	1072-117	GN	0.2	N	N	N	N	N	N	N	N	N	N	N	N	N
	1073-111	DM	0.2	Y	N	Y	Y	N	N	N	N	N	N	N	N	N
Median (Interquartile range)			***4.9 (2.6-13.3)***	***10***	***2***	***2***	***1***	***1***	***0***	***0***	***2***	***2***	***2***	***7***	***6***	***0***
HD_L	1071-03	DM	3.9	Y	N	Y	N	Y	N	N	N	N	N	N	N	N
	1071-07	DM	6.8	Y	N	N	N	N	N	N	N	N	N	N	N	N
	1071-08	DM	6.3	Y	N	N	N	N	N	N	N	N	N	N	Prednisone	N
	1071-38	DM	0.6	Y	N	N	N	N	N	N	Y	N	N	N	N	N
	1071-42	DM	5.9	Y	N	N	N	N	N	Y	Y	N	N	N	N	N
	1071-43	GN	2.0	N	N	N	N	N	N	Y	N	N	N	N	N	N
	1071-51	HTN	3.5	N	N	N	N	N	N	N	N	N	N	N	N	N
Median (Interquartile range)			***3.9 (2.8-6.1)***	***5***	***0***	***1***	***0***	***1***	***0***	***2***	***2***	***0***	***0***	***0***	***1***	***0***

† Values displayed are absolute numbers and percentages for categorical variables.

CAD = Coronary Artery Disease; COPD = Chronic Obstructive Pulmonary Disease; DM = Diabetes Mellitus; GN = Glomerulonephritis; HBV = Hepatitis B Virus; HCV = Hepatitis C Virus; HIV = Human Immunodeficiency Virus; HTN = Hypertension; HUS = Hemolytic Uremic Syndrome; PCKD = Polycystic Kidney Disease; PVD = Perivascular Disease; TIA = Transient Ischemic Attack.

N = No; Y = Yes.

Table S2. Flow cytometry antibody staining panel for B cells characterization. Related to STAR Methods, Main Figure 1, Supplementary Figure 1.

Marker – Fluorophore	Clone	Source	Catalog #
CD3 – BV480	UCHT1	BD Biosciences	566105
CD14 – BV480	M5E2	BD Biosciences	746304
CD16 – BV480	3G8	BD Biosciences	566108
CD19 – BV650	SJ25C1	Biolegend	363026
CD20 – BV711	2H7	Biolegend	563126
CD21 – BV786	B-LY4	BD Biosciences	740969
CD24 – BUV805	ML5	BD Biosciences	742010
CD27 – APC-R700	M-T271	BD Biosciences	565116
CD38 – BB790	HIT2	BD Biosciences	CUSTOM
CD56 – BV480	NCAM16.2	BD Biosciences	566124
CD138 – BUV661	MI15	BD Biosciences	5 749873
CCR10 – BUV395	1B5	BD Biosciences	565322
HLA-DR – BB700	G46-6	BD Biosciences	566480
IgA – PE	IS11-8E10	Miltenyi	130-113-476
IgD – BUV563	IA6-2	BD Biosciences	741394
IgG – BV421	G18-147	BD Biosciences	562581
IgM – BUV737	UCH-B1	Thermo Fisher Scientific	748928
LIVE/DEAD Fixable dead cell	N/A	Thermo Fisher Scientific	L34960

Table S3. Flow cytometry antibody staining panel for activation-induced marker assay. Related to STAR Methods, Main Figure 2 and 3, Supplementary Figure 2 and 3.

Marker – Fluorophore	Clone	Source	Catalog #
CD3 – BUV496	UCHT1	BD Biosciences	612941
CD4 – BB630	SK3	BD Biosciences	624294
CD8 – BV570	RPA-T8	Biolegend	301037
CD14 – BV480	M5E2	BD Biosciences	746304
CD19 – BV480	HIB19	BD Biosciences	746457
CD38 – BB790	HIT2	BD Biosciences	CUSTOM
CD45RA – PerCP Cy5.5	HI100	BD Biosciences	563429
CD69 – BV650	FN50	Biolegend	310934
CD134 (OX40) - APC	ACT35	BD Biosciences	563473
CD137 (4-1BB) – PE-Dazzle 594	4B4-1	Biolegend	309826
CD154 (CD40L) - PE	TRAP1	BD Biosciences	555700
CD183 (CXCR3) – BV605	G025H7	Biolegend	353728
CD185 (CXCR5) – BV421	J25D4	Biolegend	356920
CD186 (CXCR6) – BUV805	13B 1E5	BD Biosciences	748448
CD196 (CCR6) – BUV737	11A9	BD Biosciences	564377
CD279 (PD1) – BV711	EH122H	Biolegend	329928
HLA-DR – FITC	LN3	Biolegend	327005
LIVE/DEAD Fixable dead cell	N/A	Thermo Fisher Scientific	L34960

Table S4. Flow cytometry antibody staining panel for intracellular cytokine staining assay. Related to STAR Methods, Main Figure 2 and 4, Supplementary Figure 2 and 4.

Marker – Fluorophore	Clone	Source	Catalog #
CD3 – BUV395	UCHT1	BD Biosciences	563546
CD4 – BV711	L200	BD Biosciences	563913
CD8 – BV570	RPA-T8	Biolegend	301037
CD14 – BUV805	M5E2	BD Biosciences	612902
CD16 – BV650	3G8	Biolegend	302042
CD19 – APC-eFluor780	HIB19	Thermo Fisher Scientific	47-0199
CD56 – BUV737	NCAM16.2	BD Biosciences	564448
CD69 – PerCP-eFluor710	FN50	Thermo Fisher Scientific	46-0699-42
CD107A – BV786	H4A3	BD Biosciences	563869
IFN-γ – PECy7	B27	BD Biosciences	557643
CD154 (CD40L) – BV421	TRQP1	BD Biosciences	563886
IL-2 – PE-Dazzle 594	MQ1-17H12	Biolegend	500344
IL-10 – PE	JES3-9D7	BD Biosciences	554498
IL-17A – eFluor660	eBio64CAP17	Thermo Fisher Scientific	50-7179-42
TNF-α – Alexa Fluor 488	Mab11	Thermo Fisher Scientific	502915
Granzym B – Alexa Fluor 700	GB11	BD Biosciences	561016
LIVE/DEAD Fixable dead cell	N/A	Thermo Fisher Scientific	L34960