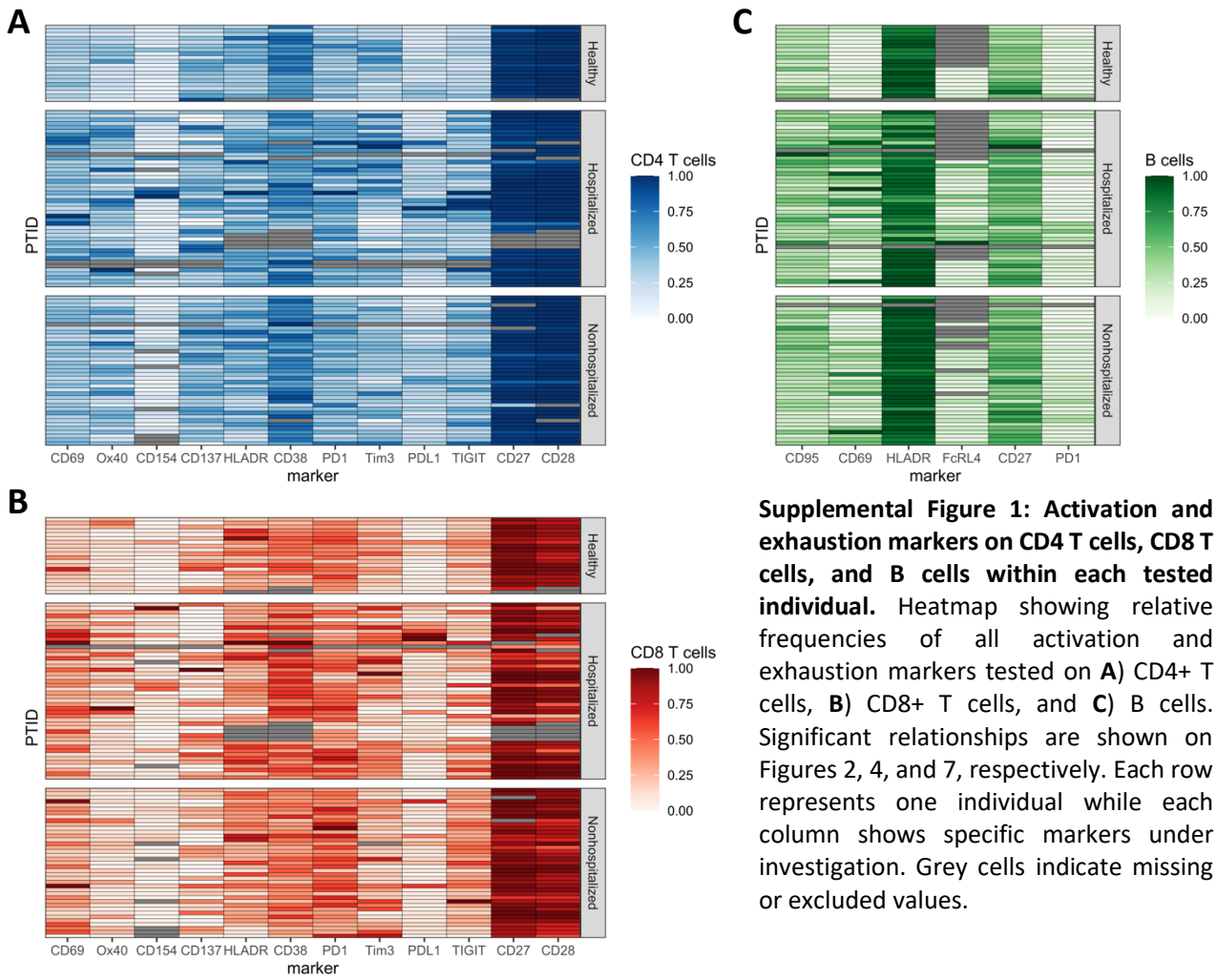
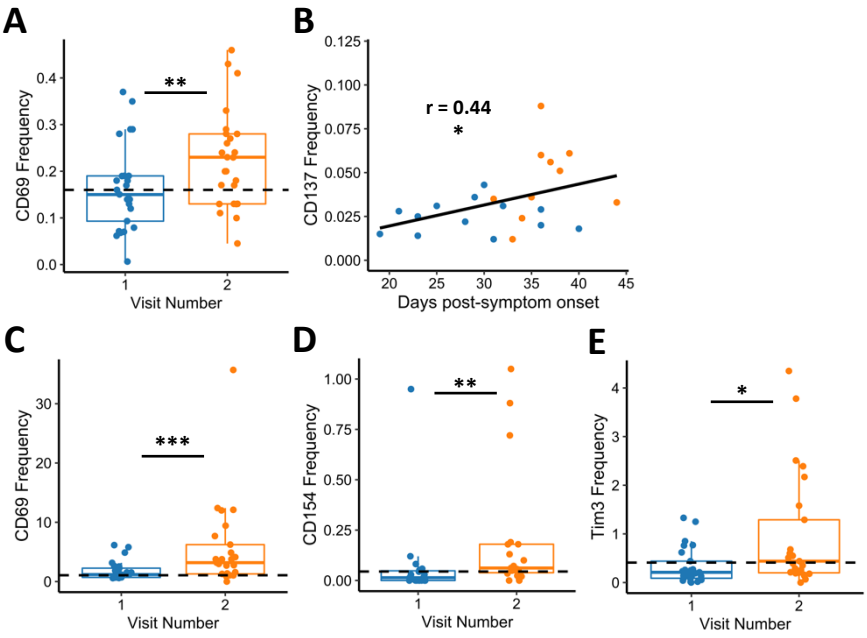
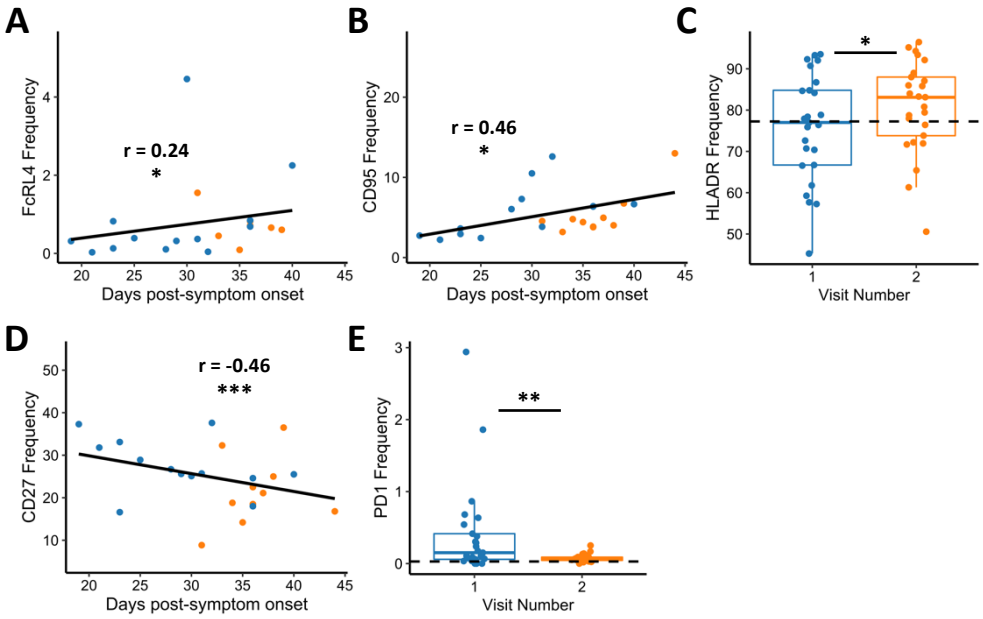
SUPPLEMENTARY MATERIAL



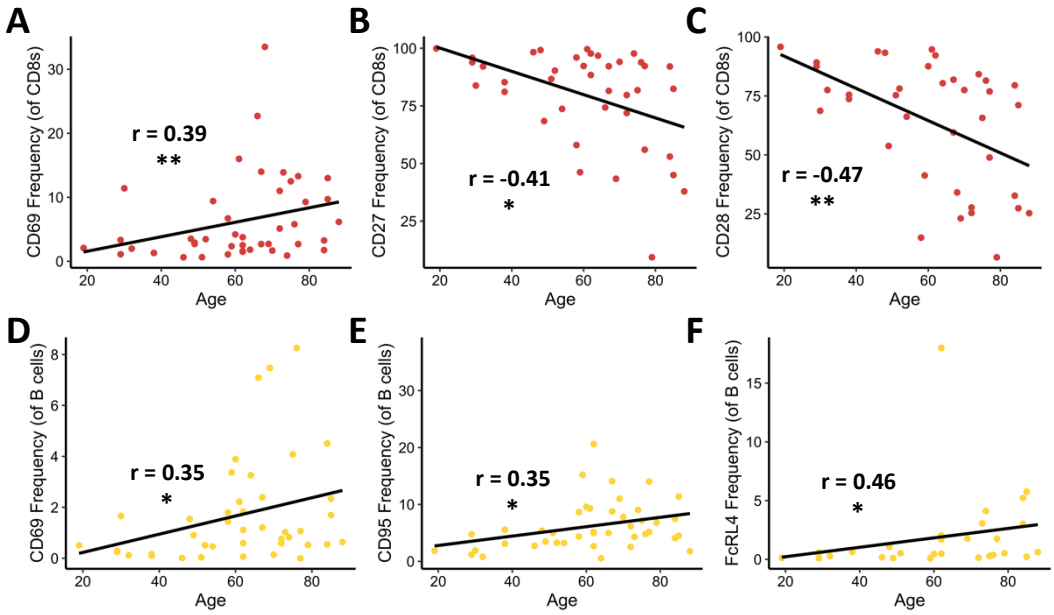
Supplemental Figure 1: Activation and exhaustion markers on CD4 T cells, CD8 T cells, and B cells within each tested individual. Heatmap showing relative frequencies of all activation and exhaustion markers tested on **A**) CD4+ T cells, **B**) CD8+ T cells, and **C**) B cells. Significant relationships are shown on Figures 2, 4, and 7, respectively. Each row represents one individual while each column shows specific markers under investigation. Grey cells indicate missing or excluded values.



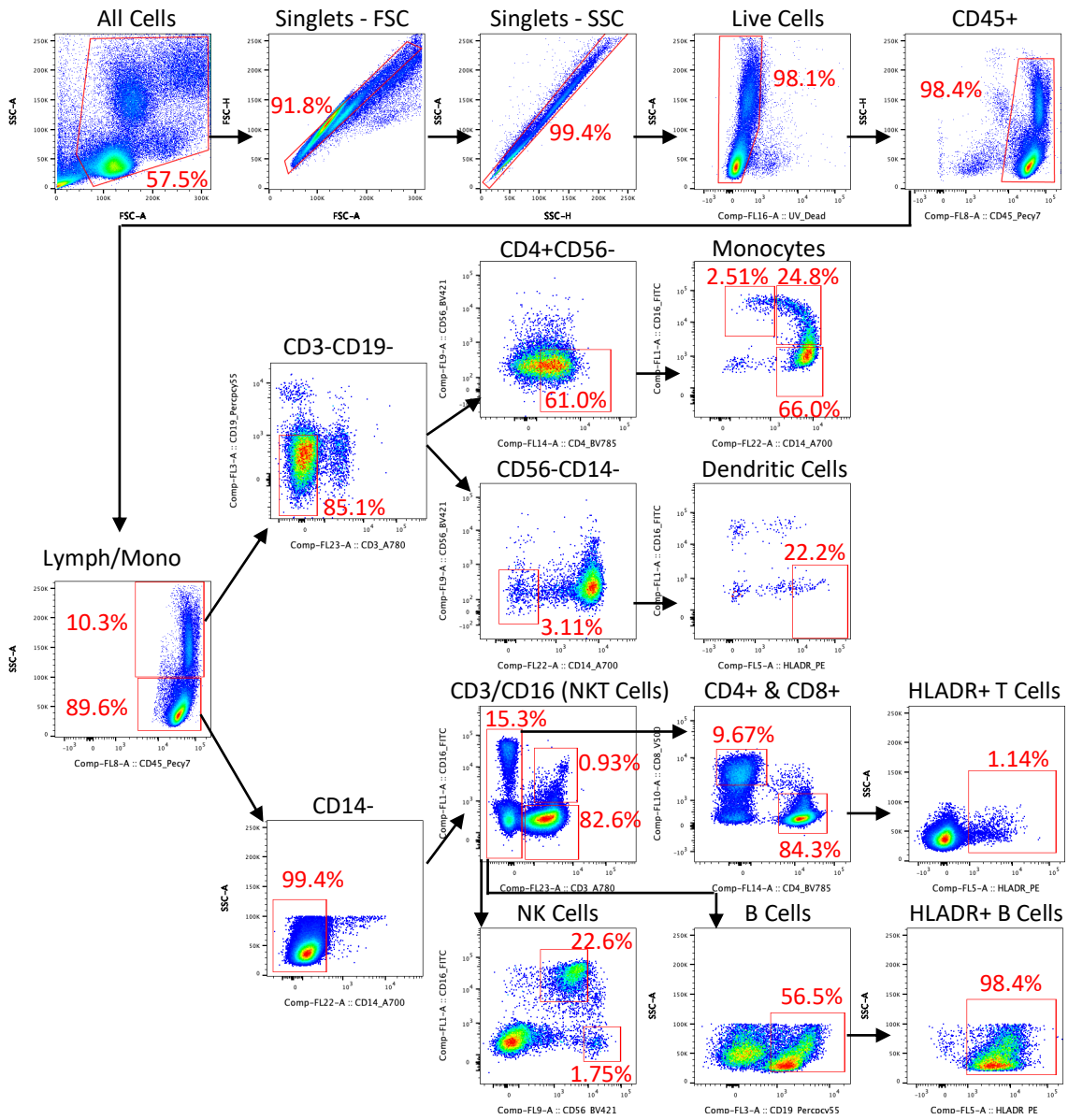
Supplemental Figure 2: A) CD4+ T-cell expression of CD69 is increased in non-hospitalized individuals between visit 1 and visit 2; **B)** CD137 expression increases within CD4+ T cells over time. **C-E)** CD8+ T-cell expression of the activation markers CD69, CD154, and Tim3, are higher at visit 2. Plots **A)** and **C-E)** show paired analysis of first versus second convalescent visits (n=25, Blue: visit 1, Orange: visit 2); p-values were determined by paired Wilcoxon signed-rank test. Boxplots indicate median, IQR, and 95% confidence interval. Dotted line shows median values of Healthy samples as baseline. Plot **B)** shows CD137 expression in CD4+ T cells by days post-symptom onset (n=23); p values determined by mixed effect model and relationship represented by linear regression. All p-values are indicated as follows: *p≤0.05, **p≤0.01, ***p≤0.001.



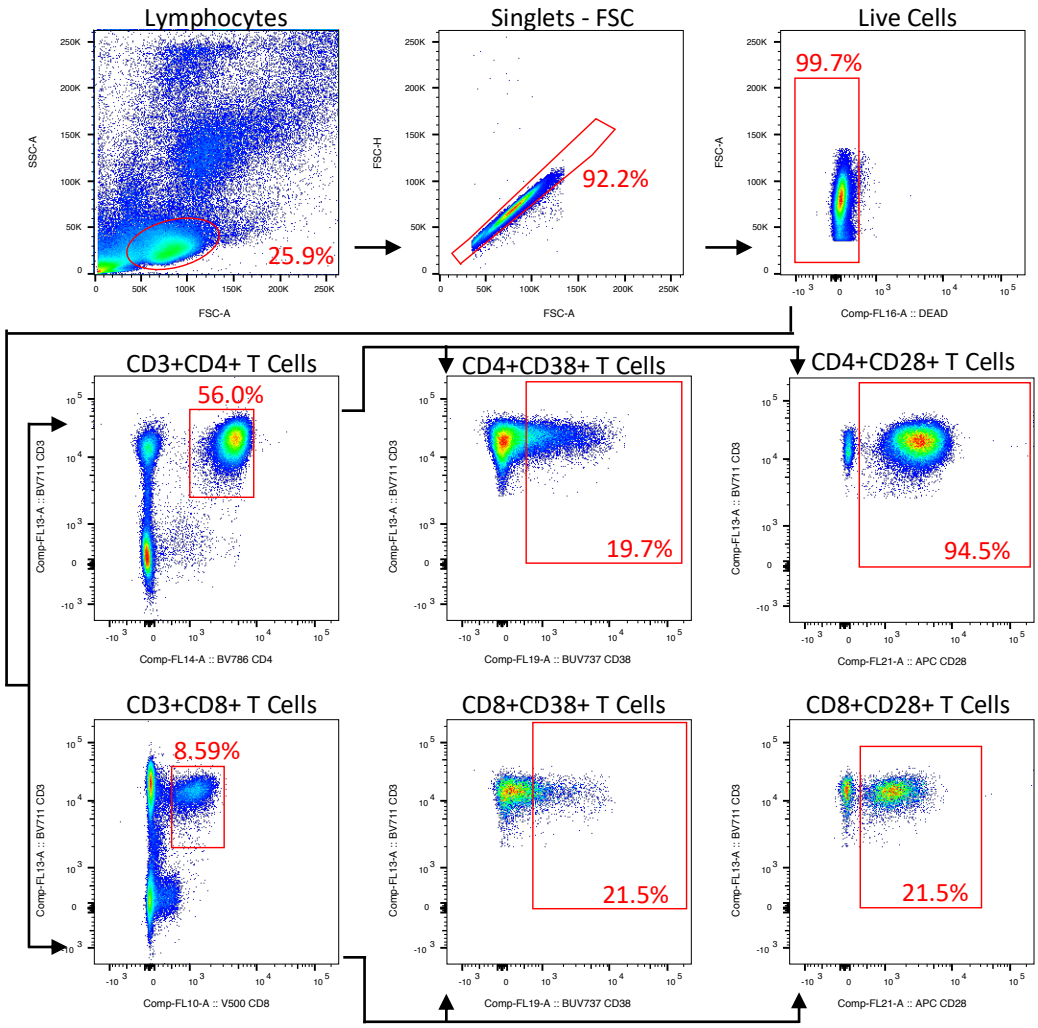
Supplemental Figure 3: B cell activation and exhaustion over time in non-hospitalized individuals. A) FcRL4 and CD95 frequencies increase over time based on days post-symptom onset (n=20; Blue: visit 1, Orange: visit 2), while **C)** HLA-DR frequencies increase between visit 1 and visit 2 (n=29); **D**) CD27 frequency decreases over time (n = 20; Blue: visit 1, Orange: visit 2), and **E**) decreased PD1 frequency decreased from visit 1 to visit 2 (n = 29). P-values for days post-symptom onset analysis determined by mixed effect model with relationship represented by linear regression; p-values for visit 1/visit 2 analyses determined by paired Wilcoxon signed-rank test. Boxplots indicate median, IQR, and 95% confidence interval. Dotted line shows median values of Healthy samples as baseline. All p-values are indicated as follows: *p≤0.05, **p≤0.01, ***p≤0.001.



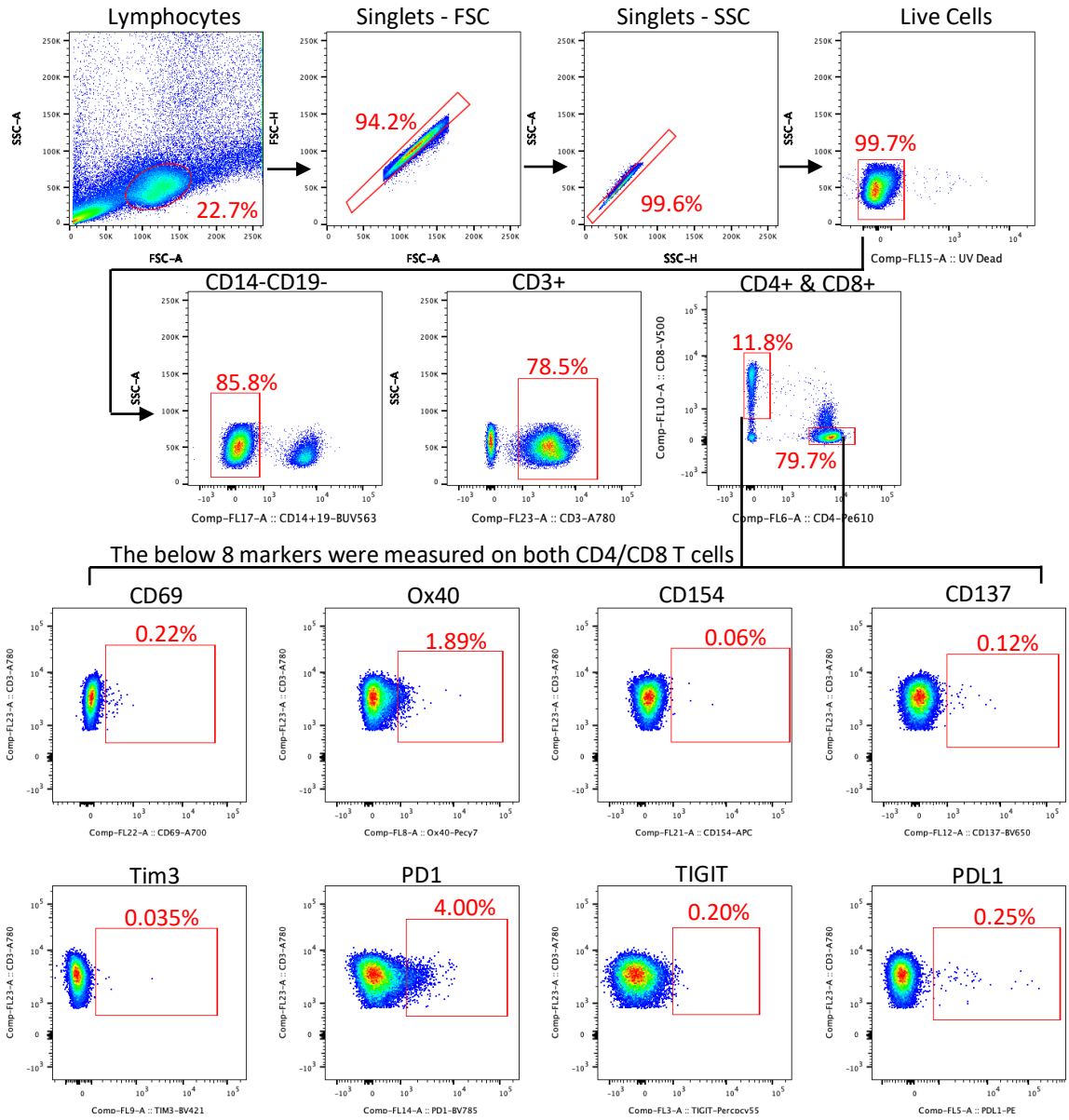
Supplemental Figure 4: Correlations between CD8 T-cell and B-cell marker frequencies and age in hospitalized individuals. Analysis of CD8+ T cells (red dots) shows **A)** positive correlation between CD69 expression and age, and **B-C)** negative correlations between CD27 and CD28 with age. Analysis of B cells (yellow dots) shows **D-F)** positive correlations between CD69, CD95 and FcRL4. R and p-values determined by Spearman rank correlation tests and are indicated as follows: *p≤0.05, **p≤0.01, ***p≤0.001; n = 45.



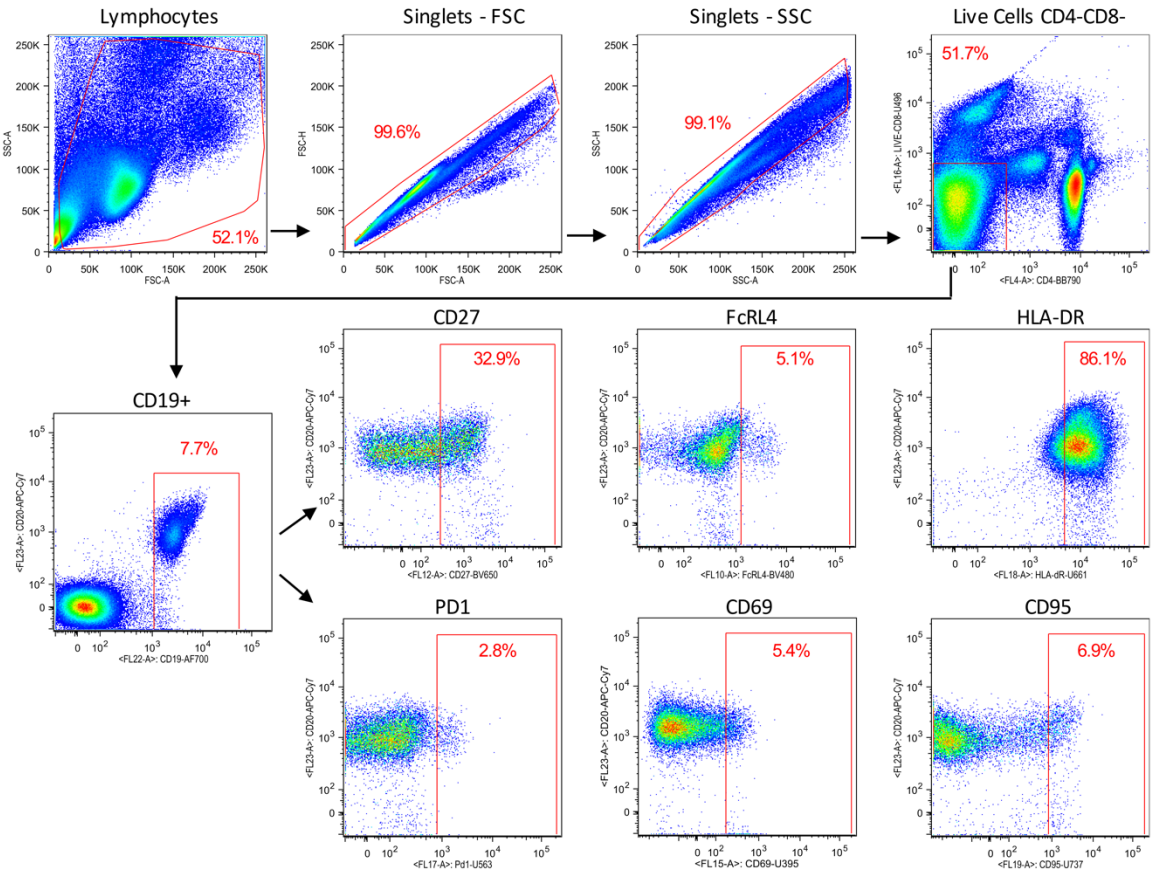
Supplemental Figure 5: Immunophenotyping Gating Strategy



Supplemental Figure 6: T-Cell Panel #1 Gating Strategy



Supplemental Figure 7: T-Cell Panel #2 Gating Strategy



Supplemental Figure 8: B-Cell Panel Gating Strategy

Supplemental Table 1: Linear Model Analyses for immune subsets analysis

Cell Type	Group 1	Group 2	Wilcoxon	LM- Hosp	LM- Age	LM- Sex	LM- Race
CD8 T cells	H	NH	***0.0027***	***0.0003***	***7.94E-06***	0.6978	0.1865
B cells	CoV-	NH	***0.0024***	***0.0036***	0.4940	0.4893	0.1070
	H	NH	***1.56E-05***	***1.22E-06***	0.5228	0.0965	0.1021
DP NK cells	CoV-	H	***0.0230***	0.3208	0.1493	0.1012	0.0627
	H	NH	***0.0107***	***0.0351***	***0.0102***	***0.0067***	0.2777
CD56+ NK cells	H	NH	***0.0088***	0.0563	0.6037	0.5296	0.4241
CD16+ monocytes	H	NH	***0.0125***	0.7951	0.4154	***0.0097***	0.7160
DP monocytes	CoV-	NH	***0.0325***	0.7611	0.2103	0.5789	0.8263
CD14+ monocytes	CoV-	H	***0.0007***	0.1312	0.4969	0.7939	0.7411
	H	NH	***0.0042***	0.6029	0.9797	0.4185	0.7380
Dendritic cells	CoV-	H	***0.0029***	0.1139	0.7283	0.9036	0.9535
	CoV-	NH	***0.0071***	0.1071	0.1237	0.1013	0.4518

Note: Wilcoxon: p-values as determined by Wilcoxon rank sum test; LM: p-values determined by ANOVA after linear modeling. Hosp = Hospital Status (Hospitalized/Non-hospitalized/Healthy). Significance determined by $p \leq 0.05$ and indicated by bold and italics.

Supplemental Table 2: Linear Model Analyses for T-Cell/B-Cell Activation and Exhaustion Markers

Subset	Marker	Group 1	Group 2	Wilcoxon	LM- Hosp	LM- Age	LM- Sex	LM- Race
CD4	CD69	CoV-	H	***8.46E-05***	***0.0045***	0.1915	0.7189	0.3266
		H	NH	***0.0002***	***0.0003***	0.1330	0.8110	0.1785
	Ox40	CoV-	H	***0.0002***	***0.0104***	0.3329	0.2542	0.8825
		H	NH	***0.0002***	***0.0007***	0.6612	***0.0337***	0.9735
	PD1	CoV-	H	***0.0003***	***0.0074***	0.2850	0.9485	***0.0384***
		H	NH	***0.0049***	***0.0036***	***0.0193***	0.9096	***0.0288***
	HLADR	H	NH	***0.0090***	***0.0065***	***0.0194***	0.7144	0.0928
	CD154	H	NH	***0.0278***	***0.0171***	0.0705	0.0583	0.0780
	Tim3	H	NH	***0.0473***	***0.0107***	0.6969	0.0792	0.7506
	TIGIT	CoV-	H	***2.56E-05***	***0.0026***	0.2814	0.9019	0.0849
		CoV-	NH	***2.70E-05***	***3.99E-05***	***0.0437***	0.0731	0.7789
	PDL1	CoV-	H	***0.0072***	***0.0337***	0.2979	0.9511	0.5307
		CoV-	NH	***0.0031***	***0.0035***	0.7585	0.9496	0.3551
	CD38	H	NH	***0.0305***	***0.0407***	***0.0071***	0.6066	0.3370
CD8	CD69	CoV-	H	***8.24E-05***	***0.0376***	***0.0112***	0.1524	0.2504
		H	NH	***0.0001***	***0.0166***	0.1567	0.1669	0.4386
	CD38	CoV-	H	***0.0019428***	***0.0102***	0.8129	0.3883	0.6824
		H	NH	***7.93E-05***	***8.04E-05***	0.6010	0.2347	0.4981
	Ox40	H	NH	***0.0331***	***0.0185***	***0.0215***	0.1226	0.0763
	CD154	H	NH	***0.0128***	0.0674	0.5114	0.7558	0.8356
	HLADR	H	NH	***0.0047***	***0.0141***	0.1424	0.3045	0.6171
	Tim3	H	NH	***0.0043***	***0.0018***	0.4180	***0.0098***	0.1833
	PDL1	CoV-	H	***0.0012***	0.1758	0.3393	0.1779	0.7140
		H	NH	***0.0003***	0.0617	0.6121	0.0885	0.7529
B	CD69	CoV-	H	***7.03E-06***	***0.0025***	***0.0133***	0.1358	0.1530
		CoV-	NH	***0.0004***	***0.0074***	0.0676	0.5787	0.1595
		H	NH	***0.0390***	***0.0254***	***0.0063***	0.1872	0.1428
	CD95	CoV-	H	***0.0207***	***0.0089***	***0.0225***	0.6015	0.1436
		H	NH	***0.0339***	0.1545	***0.0108***	0.2239	0.1940
	HLADR	H	NH	***0.0508***	***0.0317***	0.6440	0.6904	0.6224
	CD27	CoV-	NH	***0.0042***	***0.0020***	***0.0321***	0.5078	0.9163
	PD1	CoV-	NH	***0.0162***	0.0797	0.8880	0.9663	0.6753
		H	NH	***0.0264***	***0.0301***	0.8937	0.9812	0.7671

Note: Wilcoxon: p-values as determined by Wilcoxon rank sum test; LM: p-values determined by ANOVA after linear modeling. Hosp = Hospital Status (Hospitalized/Non-hospitalized/Healthy). Significance determined by $p \leq 0.05$ and indicated by bold and italics.

Supplemental Table 3: Linear Model Analyses for T-Cell/B-Cell Activation and Exhaustion Markers in Hospitalized Patients based on ICU vs No ICU

Subset	Marker	Group 1	Group 2	Wilcoxon	LM- Hosp	LM- Age	LM- Sex	LM- Race
B	CD27	ICU	No ICU	***0.0288***	0.0659	0.3587	0.9852	0.4125
	CD95	ICU	No ICU	***0.0547***	0.1801	0.1182	0.0989	0.2775
	HLADR	ICU	No ICU	***0.0451***	***0.0333***	0.9502	0.6012	0.6356
CD4	CD69	ICU	No ICU	***0.0044***	***0.0245***	0.3295	0.7362	0.4688
	CD38	ICU	No ICU	***0.0497***	***0.0372***	0.0679	0.8076	0.1868
CD8	CD137	ICU	No ICU	***0.0018***	***0.0128***	0.3691	0.4160	0.7974
	PDL1	ICU	No ICU	***0.0176***	0.1368	0.7283	0.1886	0.8374
	CD38	ICU	No ICU	***0.0249***	0.0564	0.8979	0.6325	0.4485

Note: Wilcoxon: p-values as determined by Wilcoxon rank sum test; LM: p-values determined by ANOVA after linear modeling. Hosp = Hospital Status (Hospitalized/Non-hospitalized/Healthy). Significance determined by $p \leq 0.05$ and indicated by bold and italics.

Supplemental Table 4: Flow Cytometry Panels

	Fluorophore	Antigen	Clone	Manufacturer
Immunopheno-typing Panel	FITC	CD16	CB16	Invitrogen
	Percpcy5.5	CD19	SJ25C1	BD Biosciences
	PE	HLA-DR	G46-6	BD Biosciences
	PE Alexa 610	CD27	0323	Invitrogen
	PeCy7	CD45	HI30	BD Biosciences
	BV421	CD56	NCAM16.2	BD Biosciences
	V500	CD8	RPA-T8	BD Biosciences
	BV785	CD4	SK3	Biolegend
	A700	CD14	M5E2	BD Biosciences
	A780	CD3	UCHT1	Invitrogen
T-cell Panel #1	V500	CD8	RPA-T8	BD Biosciences
	BV711	CD3	UCHT1	BD Biosciences
	BV786	CD4	SK3	BD Biosciences
	BUV737	CD38	HB7	BD Biosciences
	APC	CD28	CD28.2	BD Biosciences
T-cell Panel #2	PerCpCy5.5	TIGIT	A15153G	Biolegend
	PE	PDL1	MIH1	BD Biosciences
	PE Alexa 610	CD4	S3.5	Life Technologies
	Pecy7	Ox40	Ber-ACT35	Biolegend
	BV421	Tim3	F38-2E2	Biolegend
	V500	CD8	RPA-T8	BD Biosciences
	BV650	CD137	4B4-1	BD Biosciences
	BV785	PD1	EH12.2H7	Biolegend
	BUV563	CD14	MΦP9	BD Biosciences
	BUV563	CD19	SJ25C1	BD Biosciences
	APC	CD154	TRAP1	BD Biosciences
	A780	CD3	UCHT1	Invitrogen
B-cell Panel	BB790	CD4	L200	BD Biosciences
	AF700	CD19	J3-119	Beckman Coulter
	BV 480	FcRL4	A1	BD Biosciences
	BV 650	CD27	O323	Biolegend
	BUV395	CD69	FN-50	BD Biosciences
	BUV496	CD8	RPA-T8	BD Biosciences
	BUV563	PD-1	MIH18	BD Biosciences
	BUV661	HLA-DR	G46-6	BD Biosciences
	BUV737	CD95	DX2	BD Biosciences