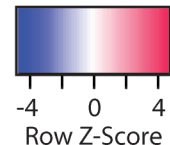
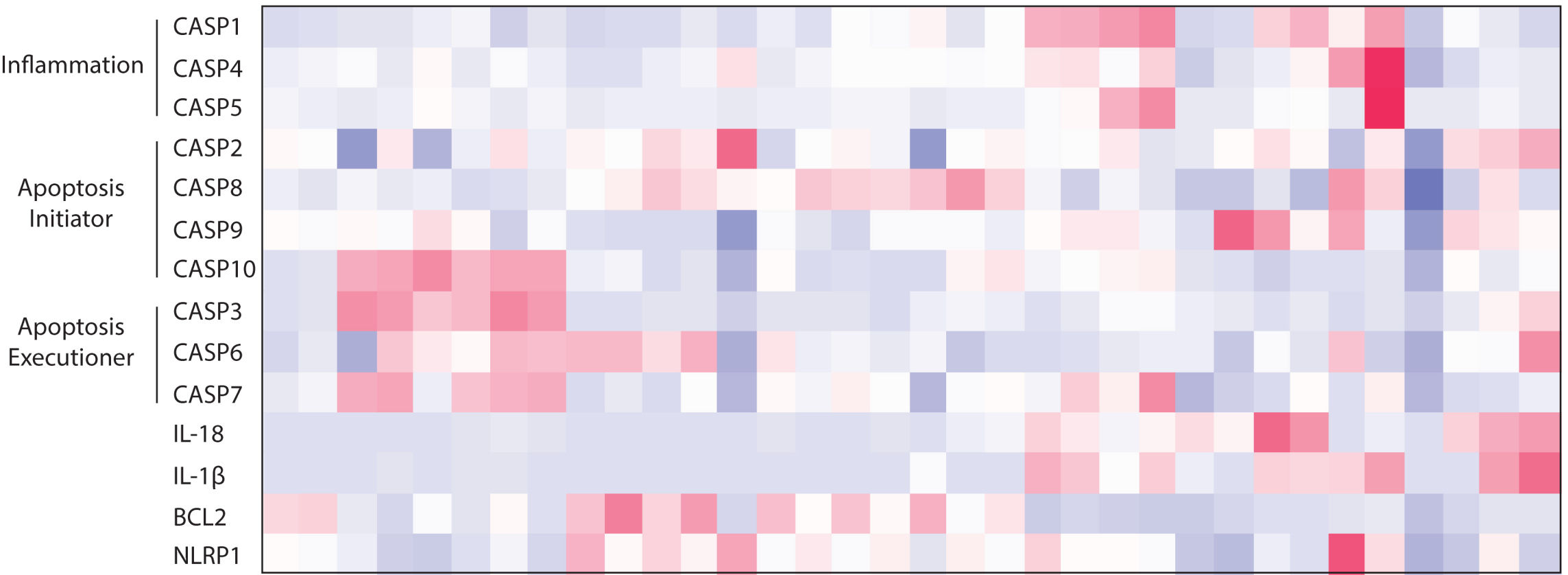
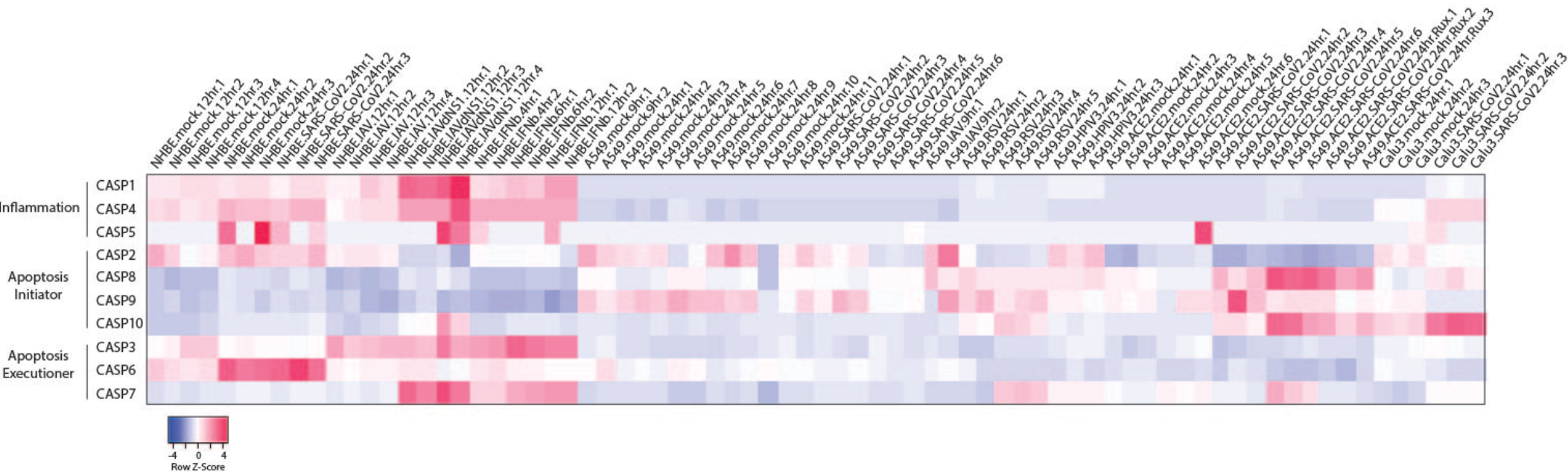


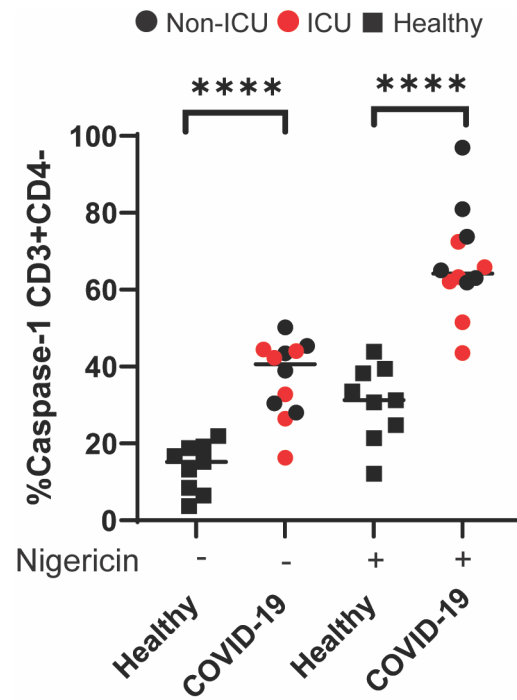
B.Healthy  
 B.COVID  
 PB.IgG.Healthy  
 PB.IgG.COVID  
 PB.IgM.Healthy  
 PB.IgM.COVID  
 PB.IgA.Healthy  
 PB.IgA.COVID  
 T.4Nve.Healthy  
 T.4Nve.COVID  
 T.4Mem.Healthy  
 T.4Mem.COVID  
 T.4.IFN-stim.Healthy  
 T.4.IFN-stim.COVID  
 T.8Mem.Healthy  
 T.8Mem.COVID  
 T.gd.Healthy  
 T.gd.COVID  
 NK.Healthy  
 NK.COVID  
 Mo.CD14.Healthy  
 Mo.CD14.COVID  
 Mo.CD16.Healthy  
 Mo.CD16.COVID  
 pDC.Healthy  
 pDC.COVID  
 DC.Healthy  
 DC.COVID  
 Neu.Healthy  
 Neu.COVID  
 Neu.Dev.Healthy  
 Neu.Dev.COVID  
 Eos.SC.Healthy  
 Eos.SC.COVID



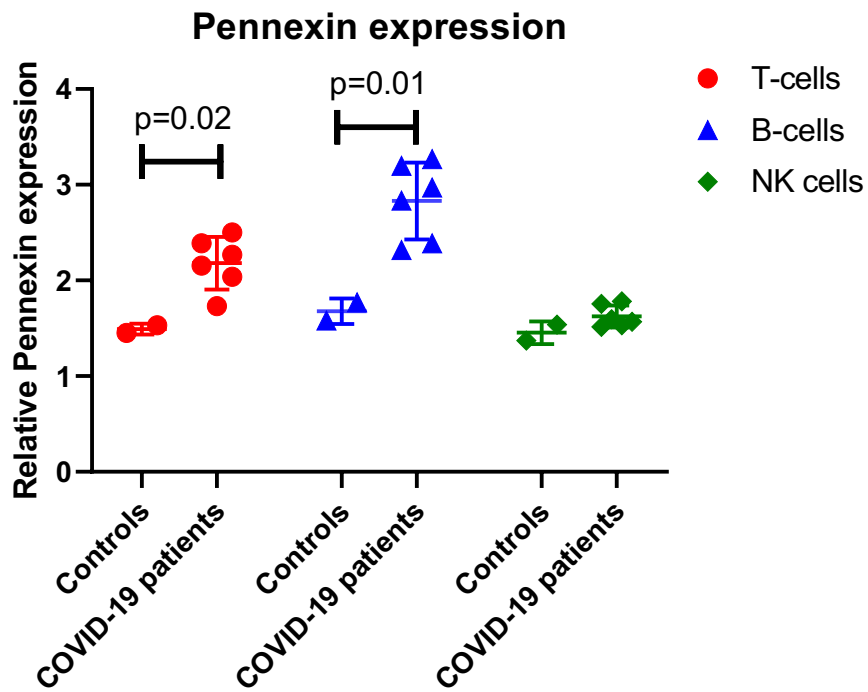
Supplementary Figure 1. Single-cell caspase gene expression of immune cells. Data was compiled from three COVID-19 participants and from 6 healthy controls. Expression values for the caspase genes were normalized by DESeq2.



Supplementary Figure 2. Transcriptome profiling caspase datasets of in vitro SARS-CoV-2 infection models. Duration of infection/stimulation is as indicated. NHBE=primary human bronchial epithelial cells; A549=lung adenocarcinoma; Calu-3=human lung epithelial cells; IAV=Influenza A virus; IAVdNS1=IAV lacking antiviral NS1 gene; IFN-  $\beta$ =interferon beta; RSV=Respiratory Syncytial virus; HPIV3=human parainfluenza virus type 3.

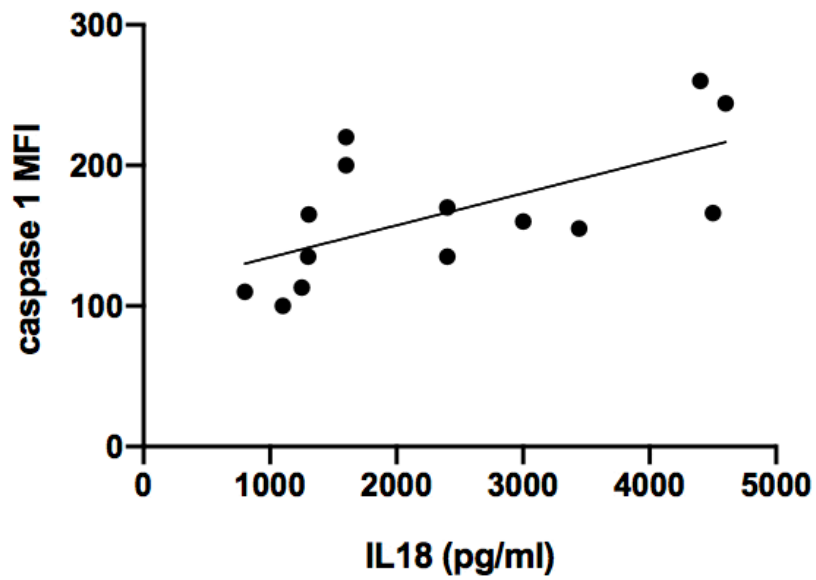


**Figure 3. Caspase-1 expression in immune cells.** Caspase-1 expression is shown in CD3+CD4- T cell lymphocytes from healthy and COVID-19 patients as indicated.



Supplemental Figure 4. Whole blood was stained with anti-CD3, anti-CD20, anti-CD16 and anti-CD56 to identify T-cells, B-cells, and NK-cells. The Pannexin level was calculated as MFI fold change compared to a FMO control. P-value calculated by a students t-test.

### Plasma IL-18 to CD4T cell caspase-1 correlation

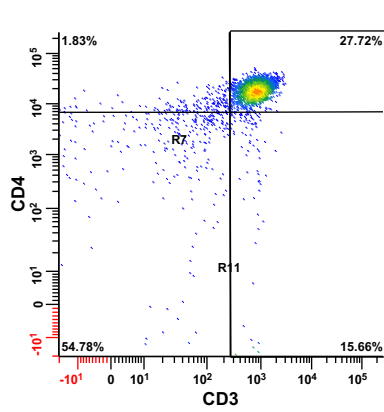
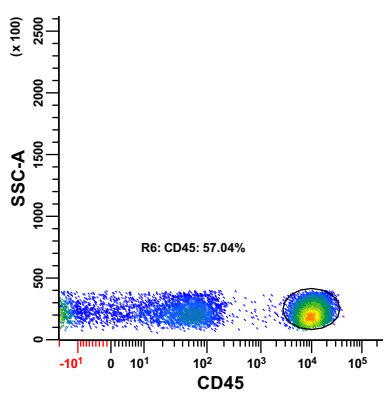
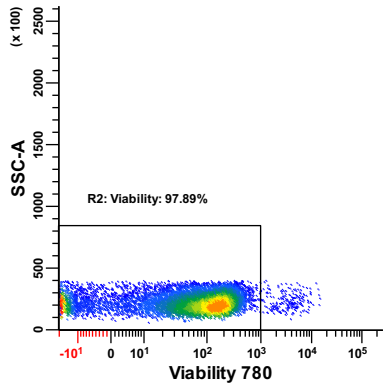
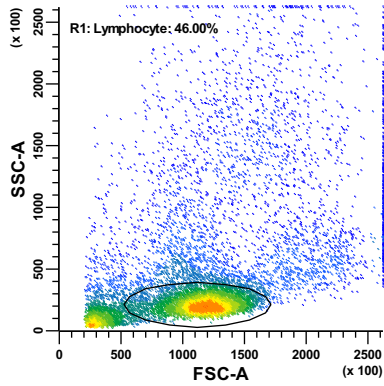


Supplemental Figure 5. Correlation of active Caspase-1 mean fluorescence intensity (MFI) in T-helper cells to serum IL-18 levels in hospitalized COVID-19.  $r = 0.023$ ,  $p = 0.016$

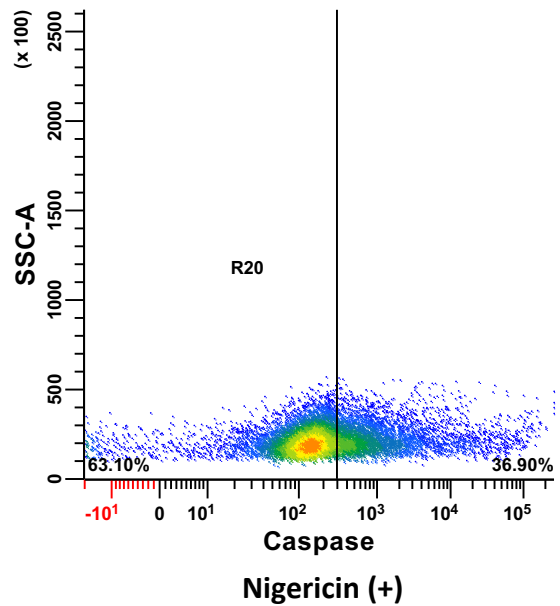
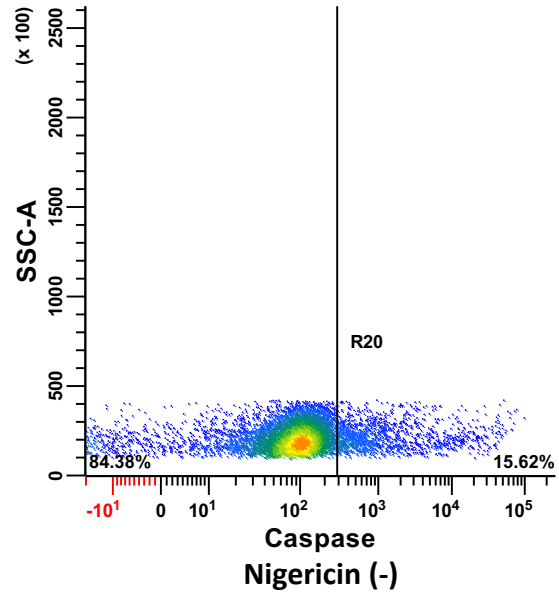
Supplemental Table 1 and Caspase-1 gating strategy

Lymphocyte Monitoring	18+ Normal Range		Healthy		Covid-19 +		Covid-19+ ICU		Covid-19+ Non ICU	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Monocytes	3.1 - 11.3	5.86	0.30	5.78	0.45	4.06	0.37	7.82	0.62	
Lymphocytes	8.6 - 47.2	36.99	2.76	17.44	1.65	11.91	1.48	25.46	2.52	
CD3	60.3 - 90.6	73.30	3.01	70.94	1.54	68.33	1.98	74.61	2.50	
CD20	2.4 - 21.4	10.39	1.06	15.31	1.53	17.08	2.28	12.03	1.93	
CD3-CD16+ or CD56+	2.3 - 24.7	14.13	2.62	10.22	1.09	10.03	1.38	11.00	1.95	
%TBNK		97.82	0.38	96.47	0.44	95.43	0.69	97.64	0.34	
CD3+CD16+ or CD56+	0.3 - 7.9	5.73	0.88	5.75	0.59	5.20	0.89	6.84	0.75	
<b>T Cell Monitoring and Activation</b>										
CD3+/CD4+	28.3 - 69.4	46.52	2.65	46.08	1.61	47.63	2.35	42.89	2.10	
CD3+/CD8+	8.6 - 39.5	21.47	2.14	21.43	1.72	17.60	1.42	27.60	3.33	
CD3+/CD4-/CD8-	0.4 - 5	4.49	0.81	2.77	0.26	2.58	0.30	3.26	0.49	
CD3/alpha-beta	56 - 87.6	67.37	3.17	66.60	1.52	64.33	1.89	69.67	2.65	
CC3/gamma-delta	0.2 - 5.8	3.36	0.81	1.93	0.31	1.50	0.35	2.67	0.58	
DNT/alpha-beta	0.1 - 1.7	1.06	0.12	0.92	0.08	1.02	0.13	0.84	0.09	
DNT/gamma-delta	0.2 - 4.3	3.24	0.79	1.57	0.26	1.21	0.29	2.20	0.47	
CD3+/CD8+/CD57+	1.2 - 17.2	7.31	1.73	8.72	1.30	5.60	0.98	13.39	2.58	
CD3+/HLA-DR	1.0 - 9.6	1.35	0.25	3.31	0.37	3.21	0.31	3.35	0.84	
CD3+/CD25	11.2 - 53.9	12.20	1.20	13.67	0.54	14.05	0.82	12.76	0.60	
T4/T8 Ratio	0.71 - 4.3	2.50	0.25	2.80	0.29	3.25	0.44	2.04	0.30	
<b>T Cell Memory</b>										
CD3/CD4/CD45RO	9.9 - 37.7	26.70	1.77	32.43	1.98	29.27	2.92	35.82	2.61	
CD3/CD4/CD45RA	3.4 - 37.9	23.68	2.19	19.79	2.37	26.12	3.38	10.87	2.30	
CD3/CD8/CD45RO	1.0 - 8.3	6.71	1.00	5.02	0.67	3.41	0.58	7.39	1.28	
CD3/CD8/CD45RA	2.4 - 23	15.09	1.77	16.22	1.54	14.88	1.79	18.73	2.90	
CD4/CRTH2	0.2 - 3.29	1.31	0.14	1.46	0.12	1.41	0.15	1.53	0.21	
CD4/CD45RO/CRTH2	0.07 - 2.97	1.06	0.11	1.13	0.11	1.07	0.15	1.22	0.18	
CD8/CRTH2	<2.43	3.67	1.19	1.72	0.35	1.45	0.40	2.07	0.67	
CD8/CD45RO/CRTH2	<1.45	1.76	0.58	0.72	0.20	0.47	0.12	1.09	0.46	
<b>B Cell Maturation and Subtypes</b>										
CD20/CD5	0.1 - 4.5	0.97	0.16	0.66	0.11	0.65	0.15	0.67	0.18	
CD20/CD27	0.4 - 5.4	1.91	0.26	2.19	0.31	2.23	0.40	1.95	0.54	
CD21 B-cells	73.2 - 95	89.17	0.92	57.27	3.43	53.80	4.66	63.54	5.40	
CD21 dim B-cells	3.7 - 21.1	8.35	0.56	34.03	2.95	35.48	3.85	29.90	4.87	
IgG B-cells	2.5 - 17.4	4.80	0.40	8.21	0.71	7.67	0.78	9.17	1.42	
IgA B-cells	1.5 - 7.3	6.61	0.62	5.55	0.55	5.83	0.86	4.82	0.63	
IgM B-cells	69.1 - 97.6	85.39	0.79	84.49	0.98	84.29	1.24	84.73	1.81	
IgD B-cells	69.0 - 97.6	85.17	1.08	77.97	1.39	77.42	1.54	78.48	2.81	
IgMCD27 B-cells	5.6 - 27.2	13.60	1.43	8.76	0.84	9.02	1.17	8.79	1.36	
<b>Dendritic Cell Monitoring</b>										
DC	0.04 - 0.5	0.17	0.02	0.13	0.03	0.09	0.02	0.19	0.06	
CD11d	32.6 - 86	60.17	3.03	60.89	4.22	50.91	5.82	72.42	5.44	
BDCA2	8.9 - 59.9	31.10	3.35	9.80	1.77	8.02	2.02	13.38	3.27	

Lymphocyte Monitoring	% CD3+CD4+ Caspase 1 Nigericin -
Monocytes	0.1902
Lymphocytes	0.5053
CD3	0.4789
CD20	0.2798
CD3-CD16+ or CD56+	<b>0.0014</b>
%TBNK	0.2105
CD3+CD16+ or CD56+	0.2402
<b>T Cell Monitoring and Activation</b>	
CD3+/CD4+	0.4624
CD3+/CD8+	0.7696
CD3+/CD4-/CD8-	<b>0.019</b>
CD3/alpha-beta	0.2596
CC3/gamma-delta	<b>0.0169</b>
DNT/alpha-beta	0.6647
DNT/gamma-delta	<b>0.0043</b>
CD3+/CD8+/CD57+	0.6792
CD3+/HLA-DR	0.4755
CD3+/CD25	0.5518
T4/T8 Ratio	0.7592
<b>T Cell Memory</b>	
CD3/CD4/CD45RO	0.1758
CD3/CD4/CD45RA	0.1213
CD3/CD8/CD45RO	0.5101
CD3/CD8/CD45RA	0.4088
CD4/CRTH2	<b>0.0081</b>
CD4/CD45RO/CRTH2	<b>0.007</b>
CD8/CRTH2	0.963
CD8/CD45RO/CRTH2	0.2389
<b>B Cell Maturation and Subtypes</b>	
CD20/CD5	0.3127
CD20/CD27	0.2098
CD21 B-cells	0.6533
CD21 dim B-cells	0.9084
IgG B-cells	0.107
IgA B-cells	0.3013
IgM B-cells	0.1667
IgD B-cells	0.1857
IgMCD27 B-cells	0.1732
<b>Dendritic Cell Monitoring</b>	
DC	0.1093
CD11d	0.8607
BDCA2	<b>0.0187</b>



## CD4 T Cells



PBMC Gating Scheme: Lymphocytes are gated on the FSC vs SSC Plot. Viable CD45+ cells gated on the CD45 vs SSC plot. The CD45+CD4+ cells are identified on the CD3 vs CD4 plot. The CD4+ Caspase cells are shown.

### Supplementary information on antibodies:

The antibodies utilized from Thermo Fisher Scientific were CD56 SB436 [TULY56], CD45 eF506 [HI130], CD3 FITC [SK7], CD16 PE [B73.1], CD8 PerCP-eF710 [SK1] CD14 PE-CY7 [61D3], CD4 APC [SK-3], CD20 APC-eF780 [2H7] CD25 EF450 [CD25-4E3], CD57 FITC [TBo1], TCR $\gamma$ - $\delta$  PE [B1.1], CD4 PerCP-eF710 [SK-3], CD3 PE-CY7 [SK7], TCR  $\alpha\beta$  APC [IP26], HLA-DR AF700 [LN3], CD8 APC-eF780 [SL1], IgD SB436 [IA6-2], IgA FC Secondary Antibody FITC, IgG FC Secondary Antibody PE, IgM PerCP-eF710 [SA-DA4], CD19 PE-CY7 [SJ25C1], CD27 APC [O323], CD5 FITC [UCHT2], CD21 PE [HB5], CD27 PerCP-eF710 [O323], CD45RA FITC [HI100], CD45RO PerCP-eF710 [UCHL1], CD294 APC [BM16], CD4 AF700 [RPA-T4], CD3 FITC [SK7], CD14 FITC [61D3], CD16 FITC [3G8], CD19 FITC [SJ25-C1], CD20 FITC [2H7], CD56 FITC [TULY56], CD34 FITC [4H11], CD11c PE [3.9], HLA-DR PerCP-EF710 [L243], CD303a APC [201A], CD4 SB600 [SK-3], CD45RA FITC [HI100], CD3 PE-CY7 [SK7], CD8 AF700 [SK1], CCR5 APC [NP-6G4], CD25 APC [BC96], CD317 PE [26F8], IL-6 PE-CY7 [MQ2-13A5], MIP1- $\beta$  APC [FL34Z3L].

The antibodies utilized from BD Bioscience were HLADR BV480 [G46-6], CD38 PerCP-CY5.5 [HIT2], CD28 APC [CD28.2], CD45 APC H7 [2D1], CD278 BV421 [DX29], CXCR5 PerCP-CY5.5 [RF8B2], CD127 BV480 [HIL-7R-M21], CD45RO PerCP-CY5.5 [UCHL1], CD20 APC-H7 [2H7], CD11b BV421 [ICRF44], CD16 FITC [NKP15], MIP1- $\alpha$  PE [11A3], HLA-DR PerPC-Cy5.5 [L243]. TNF- $\alpha$  BV421 [Mab11] was from Biolegend (San Diego, CA).