
Supplementary information

A dynamic COVID-19 immune signature includes associations with poor prognosis

In the format provided by the
authors and unedited

Supplementary Table 1: Parameters corrected for age and sex-dependency

Parameter	Correction	Control vs. COVID uncorrected	Control vs. COVID corrected	Change significance
CCR4 ⁺ CD4 T cells /mL (panel 2)	age	7.7E-02	4.7E-08	Gained
CD4 ⁺ T cells /mL(panel 3)	age	7.0E-06	3.3E-10	None
CD4 ⁺ T cells /mL (panel 5)	age	1.7E-03	6.9E-07	None
CD45 ⁺ cells /mL	age	1.3E-01	2.8E-01	None
CXCR3 ⁺ CCR6 ⁻ CD45RA ⁻ CD4 ⁺ T cells /mL	age	3.8E-03	8.4E-08	None
PD-1 ⁻ CXCR3 ⁺ CCR6 ⁻ CD45RA ⁻ CD4 ⁺ T cells /mL	age	2.5E-04	9.5E-10	None
PD-1 ⁻ CXCR3 ⁺ CCR6 ⁻ CD45RA ⁻ CD4 ⁺ T cells /mL	sex	1.1E-06	3.7E-06	None
TIM-3 ⁻ PD-1 ⁻ CXCR3 ⁺ CCR6 ⁻ CD45RA ⁻ CD4 ⁺ T cells /mL	sex	9.6E-07	3.5E-06	None
CXCR3 ⁺ CCR6 ⁻ CD45RA ⁺ CD4 ⁺ T cells /mL	age	2.8E-02	3.4E-07	None
Non B/T cells (% of CD45 ⁺) (panel 1)	sex	5.9E-07	3.9E-06	None
CD4 T cells (% T cells) (panel 1)	age	1.0E-02	3.4E-01	Lost
non Treg (% of CD4 T cells) (panel 1)	sex	2.2E-03	2.0E-02	None
Treg (% of CD4 T cells) (panel 1)	sex	1.3E-02	8.1E-02	Lost
CCR4 ⁺ (% of CD4 T cells)	age	7.8E-04	2.9E-01	Lost
Th2 (% of CD4 T cells)	age	2.0E-05	6.8E-01	Lost
Treg (% of CD4 T cells) (panel 2)	sex	5.4E-03	3.9E-02	None
CD4 T cells (% T cells) (panel 3)	age	5.4E-03	1.3E-01	Lost
PD-1 ⁻ (% of CXCR3 ⁺ CCR6 ⁻ CD45RA ⁻ CD4 ⁺ T cells)	age	1.9E-03	8.5E-08	None
PD-1 ⁻ (% of CXCR3 ⁺ CCR6 ⁺ CD45RA ⁻ CD4 ⁺ T cells)	age	2.2E-02	2.2E-04	None
PD-1 ⁻ (% of CD45RA ⁻ CD4 ⁺ T cells)	age	3.2E-03	1.6E-05	None
CXCR3 ⁺ CCR6 ⁻ (% of CD45RA ⁺ CD4 ⁺ T cells)	age	2.1E-02	3.5E-01	Lost
CXCR3 ⁺ CCR6 ⁻ (% of CD45RA ⁺ CD4 ⁺ T cells)	age	4.9E-02	2.8E-01	Lost
HLADR ⁺ (% of non-monocytes)	sex	1.1E-05	7.0E-06	None
CD45RA ⁻ CCR7 ⁺ (% of CD8 T cells) (panel 5)	age	1.7E-01	4.2E-02	Gained
G1 (% of CD45RA ⁺ CCR7 ⁻ CD4 T cells)	age	2.2E-06	6.5E-04	None
CD4 : CD8 T cell ratio (panel 2)	age	2.3E-04	1.3E-01	Lost
CD4 : CD8 T cell ratio (panel 3)	age	2.2E-03	7.5E-01	Lost
Th1 : Th2 CD4 T cell ratio	age	7.6E-04	9.1E-01	Lost

Parameters significantly affected by age and/or sex in control cohort were corrected for statistical analysis as detailed in methods. All corrected parameters listed, with example statistical comparison of total COVID vs. control samples before and after correction. Panel is indicated where same parameter was derived from multiple flow cytometry panels.

Supplementary Table 2: Summary of most different parameters between control and COVID samples

Most increased in COVID vs control			Most decreased in COVID vs control		
Parameter	p-value	Effect size	Parameter	p-value	Effect size
N IgG ELISA	1.7e-24	15.97	CD27 ⁺ IgM ⁺ IgD ⁺ (% of B cells)	1.9e-14	-12.05
N IgG LIPS	2.0e-21	14.15	T cells (% of CD45 ⁺ cells)	3.3e-14	-11.83
Spike IgG ELISA	6.7e-20	13.18	PD-1 ⁺ Vδ2 ⁺ T cells /mL	7.7e-13	-11.49
IP-10	1.1e-18	14.08	NKG2D ⁺ Vδ2 ⁺ T cells /mL	1.0e-11	-10.83
RBD IgG ELISA	4.4e-16	11.34	CXCR3 ⁺ CCR6 ⁺ (% of CD45RA ⁻ CD4 ⁻ T cells)	1.2e-11	-9.67
Spike IgM ELISA	1.8e-15	11.72	CD5 ⁺ (% of B cells)	1.5e-10	-9.53
IL-6	4.2e-15	12.33	NKG2D ⁺ CD8 T cells /mL	1.9e-10	-9.36
RBD IgM ELISA	1.3e-14	11.27	CD45RA ⁺ CD27 ⁺ CD8 T cells /mL	2.0e-10	-9.63
IL-8	4.9e-14	11.66	CD8 ⁻ Vδ2 ⁺ T cells /mL	3.5e-10	-9.83
CD1c ^{neg} DC (% of mDC)	5.1e-12	12.59	T cells /mL	1.0e-09	-8.93
IL-10	1.8e-11	9.92	αβ T cells /mL	1.6e-09	-8.25
TIM-3 ⁺ PD-1 ⁺ (% of CD3 ⁺ CD4 ⁻ T cells)	3.7e-11	10.66	CD8 T cells /mL	2.8e-09	-8.73
CD38 ⁺ HLADR ⁺ activated (% of CD8 T cells)	5.1e-10	9.15	Vδ2 ⁺ T cells /mL	6.9e-09	-8.40
S/G ₂ /M (% of CD45RA ⁻ CCR7 ⁻ CD4 T cells)	1.1e-09	9.02	CXCR3 ⁺ CCR6 ⁺ (% of CD45RA ⁻ CD4 ⁺ T cells)	8.5e-09	-8.82
RBD IgG LIPS	1.8e-09	7.74	CD19 MFI in B cells	1.9e-08	-8.25
G ₁ (% of γδ T cells)	4.0e-09	8.83	CD45RA ⁺ CCR7 ⁺ CD8 T cell number	2.5e-08	-8.24
Plasmablasts (% of B cells)	7.0e-09	8.54	CCR4 ⁺ CD4 T cells /mL	4.7e-08	-7.92
G ₁ (% of CD45RA ⁻ CCR7 ⁻ CD4 T cells)	1.0e-08	7.51	γδ T cells /mL	1.1e-07	-7.59
TIM3 ⁺ PD-1 ⁺ (% of CD45RA ⁻ CD3 ⁺ CD4 ⁻ T cells)	1.2e-08	7.89	pDCs (% of HLADR ⁺ CD16 ⁻ cells)	1.2e-07	-9.25
TIM3 ⁺ PD-1 ⁺ (% of CD45RA ⁺ CD3 ⁺ CD4 ⁻ T cells)	2.2e-08	8.70	Th17.1 CD4 T cells /mL	1.8e-07	-7.59

Supplementary Table 3: Antibody panels for flow cytometry analysis

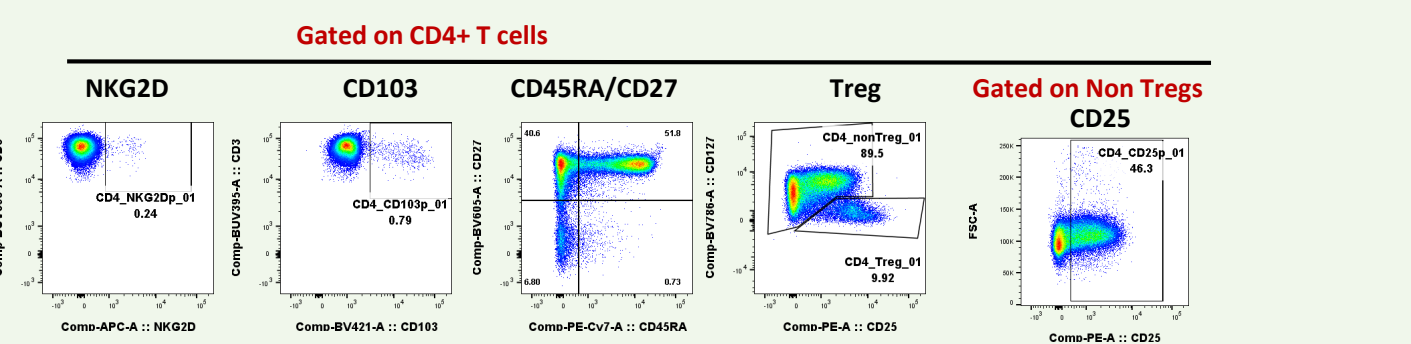
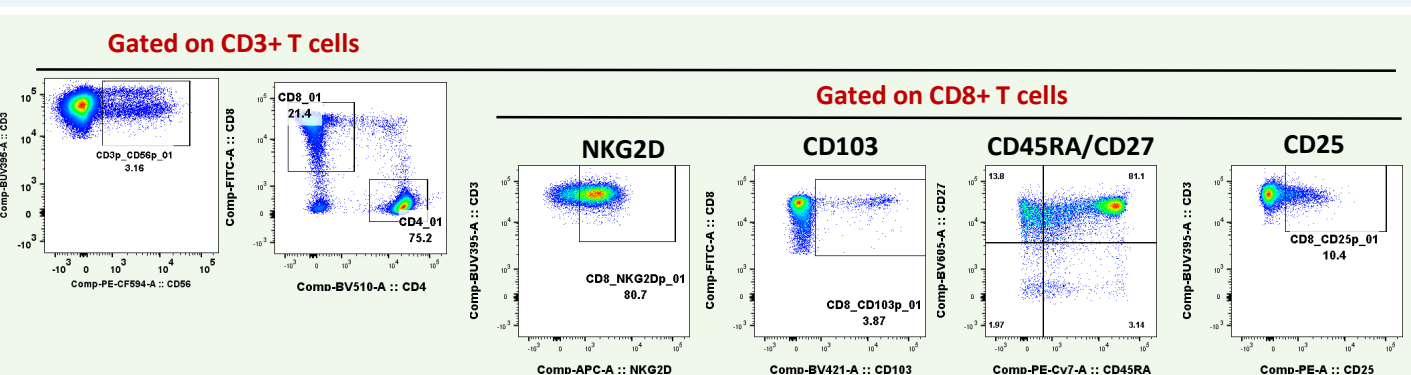
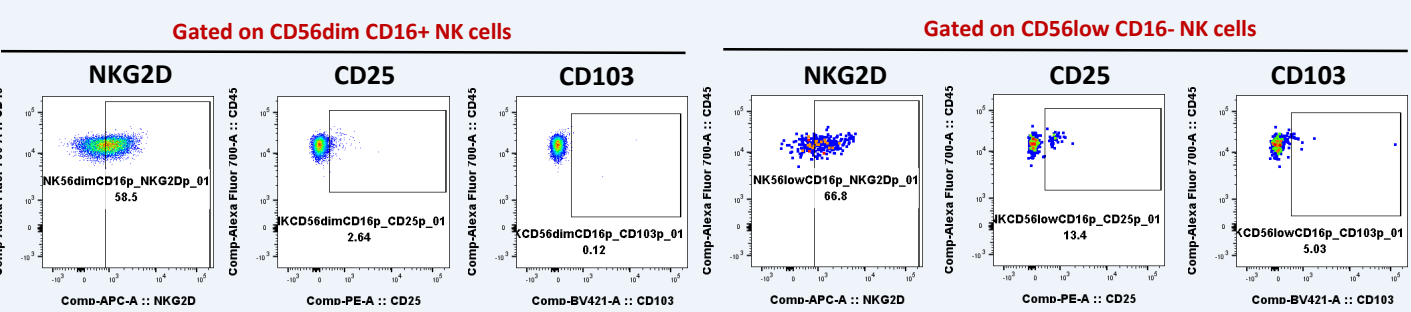
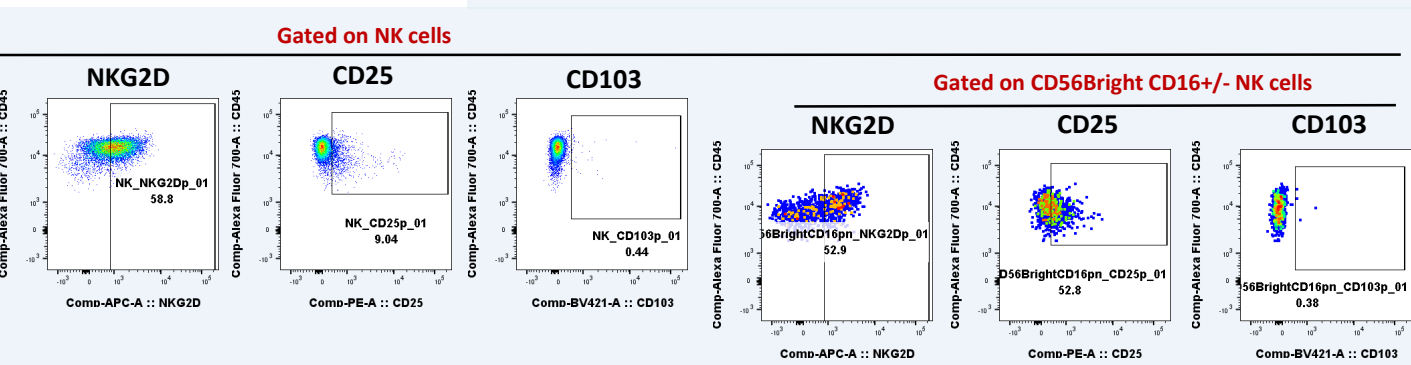
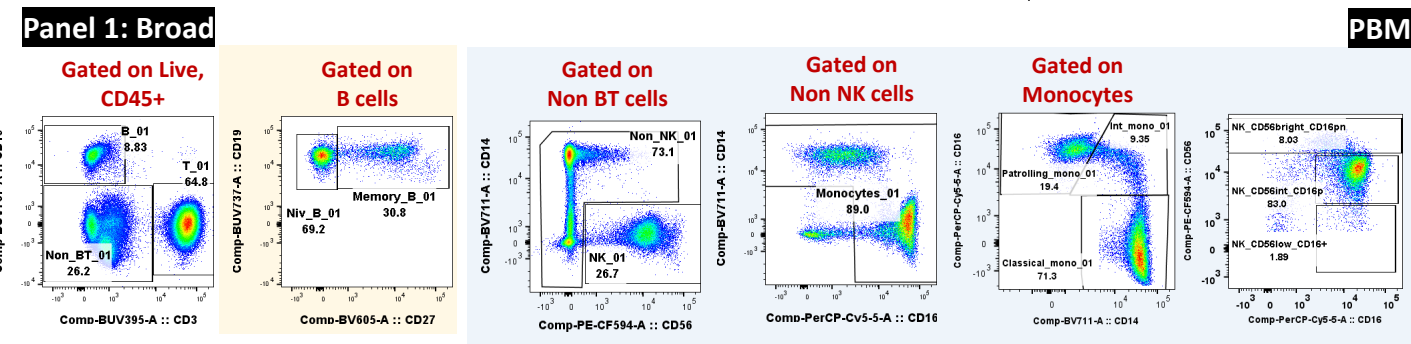
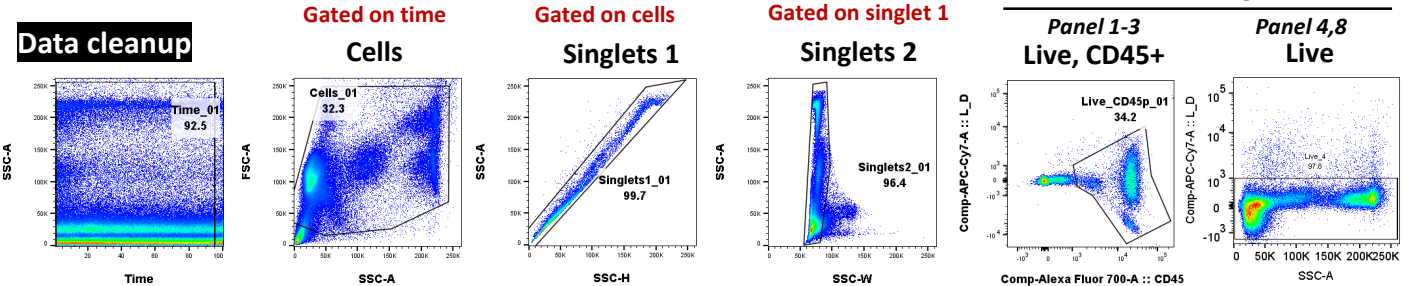
Panel	Target	Fluorophore	Clone	Brand	Volume per 100 microlitres
1	CD103	BV421	BER-ACT08	BD	1.25
1	CD127	BV786	HIL-7R-M21	BD	2.5
1	CD14	BV711	MφP9	BD	0.63
1	CD16	PerCP-Cy5.5	3G8	BD	0.16
1	CD19	BUV737	SJ25C1	BD	1.25
1	CD25	PE	2A3	BD	5
1	CD25	PE	M-A251	BD	5
1	CD27	BV605	L128	BD	1.25
1	CD3	BUV395	UCHT1	BD	2.5
1	CD4	BV510	SK3	BD	1.25
1	CD45	AF700	HI30	BD	1.25
1	CD45RA	PE-Cy7	HI100	BD	1.25
1	CD56	PE-CF594	NCAM16.2	BD	0.625
1	CD8	FITC	SK1	BD	2.5
1	NKG2D	APC	1D11	BD	10
2	CCR4	APC	1G1	BD	2.5
2	CCR6	BV421	11A9	BD	2.5
2	CCR7	PE-CF594	150503	BD	5
2	CD103	BV711	BER-ACT08	BD	2.5
2	CD127	BV786	HIL-7R-M21	BD	2.5
2	CD25	PE	2A3	BD	5
2	CD25	PE	M-A251	BD	5
2	CD27	BV605	L128	BD	1.25
2	CD3	BUV395	UCHT1	BD	2.5
2	CD38	BUV737	HB7	BD	0.63
2	CD4	BV510	SK3	BD	1.25
2	CD45	AF700	HI30	BD	1.25
2	CD45RA	PE-Cy7	HI100	BD	1.25
2	CD8	FITC	SK1	BD	2.5
2	CXCR3	PE-Cy5	1C6	BD	10
2	HLA-DR	PerCP-Cy5.5	L243	BD	5
3	CCR7	PE-CF594	150503	BD	5
3	CD103	BV711	BER-ACT08	BD	2.5
3	CD27	BV605	L128	BD	1.25
3	CD3	BUV395	UCHT1	BD	2.5
3	CD4	BV510	SK3	BD	1.25
3	CD45	AF700	HI30	BD	1.25
3	CD45RA	BV786	HI100	BD	1.25
3	CD8	PerCP-Cy5.5	SK1	BD	1.25
3	NKG2D	APC	1D11	BD	10
3	PD-1	BV421	EH12.1	BD	1.25
3	TCR γδ	PE-Cy7	IMMU510	Beckman	2.5
3	Vδ1	FITC	REA173	Miltenyi	0.5
3	Vδ2	PE	B6	BD	1.25

Panel	Target	Fluorophore	Clone	Brand	Volume per 100 microlitres
4	CD19	BV711	SJ25C1	BD	2
4	CD24	BUV395	ML5	BD	2
4	CD25	APC-R700	2A3	BD	2
4	CD27	BV786	L128	BD	2
4	CD38	PE	HIT-2	BD	10
4	CD43	BV421	1610	BD	2
4	CD5	PE-Cy7	L17F12	BD	2
4	HLA-DR	BV510	G46-6	BD	2
4	IgD	BUV737	IA6-2	BD	2
4	IgG	APC	G18-145	BD	5
4	IgM	BB515	G20-127	BD	2
5	CCR7	PE-CF594	150503	BD	5
5	CD25	PE	2A3	BD	5
5	CD25	PE	M-A251	BD	5
5	CD3	FITC	UCHT1	BD	10
5	CD4	BV711	SK3	BD	1.25
5	CD45RA	BV786	HI100	BD	1.25
5	CD8	PerCP-Cy5.5	SK1	BD	1.25
5	TCR $\gamma\delta$	PE-Cy7	IMMU510	Beckman	2.5
5	Ki-67 (intracellular)	AF700	B56	BD	1
5	FoxP3 (intracellular)	AF647	259D	Biolegend	1
6	CD10	BV711	HI10a	Biolegend	2.5
6	CD14	AF488	HCD14	Biolegend	1
6	CD15	BV605	W6D3	Biolgend	0.25
6	CD16	PE-Cy7	3G8	Biolegend	0.125
6	CD19	PE	HIB19	Biolegend	0.5
6	CD3	APC-Cy7	OKT3	Biolegend	0.5
6	CD45	PerCP	HI30	Biolegend	0.5
6	CD56	AF647	HCD56	Biolegend	2
6	CD62L	BV785	DREG-56	Biolegend	1
6	CD64	BV421	10.1	Biolegend	2.5
7	2B4	APC	(2-69)	BD	2.5
7	CCR6	BB515	11A9	BD	2.5
7	CD3	APC-Cy7	OKT3	Biolegend	1
7	CD4	PE-Cy7	RM4-5	BD	0.5
7	CD45RA	BV786	HI100	BD	1
7	CXCR3	BB700	CXCR3-173	BD	2.5
7	LAG-3	BV510	TA7-530	BD	2.5
7	PD-1	BV421	EH12.1	BD	2.5
7	TIM3	PE-CF594	7D3	BD	2.5
8	CD11c	BV650	3.9	Biolegend	1
8	CD123	PerCP-Cy5.5	6H6	Biolegend	1
8	CD14	BV421	M5E2	Biolegend	1
8	CD16	PE-Cy7	3G8	Biolegend	0.5
8	CD19	BV786	HIB19	Biolegend	1
8	CD1c	APC	L161	Biolegend	1
8	CD3	AF700	OKT3	Biolegend	0.5
8	CD303	FITC	201A	Biolegend	1
8	CD86	PE	IT2.2	Biolegend	1
8	HLA-DR	BV510	G46-6	BD	1
Sort	CD127	BV421	A019D5	Biolegend	1

Supplementary Table 4: Sample selection for NanoString gene expression analysis

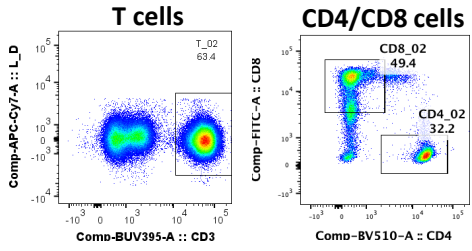
		Age range & sex	Ethnicity	CD8 _{EM} G1 %	Lymph Conc (x10 ⁶ /mL)
Control Seronegative	1	40-53, M	Caucasian	1.8	0.8
	2		Caucasian	2.6	1.486
	3		Caucasian	1.7	0.9
COVID- 19_Severe	1	44-65, M	Chinese	50.4	0.587
	2		Caucasian	55.9	0.4566
	3		Indian	34.4	0.268

Supplementary Figure 1

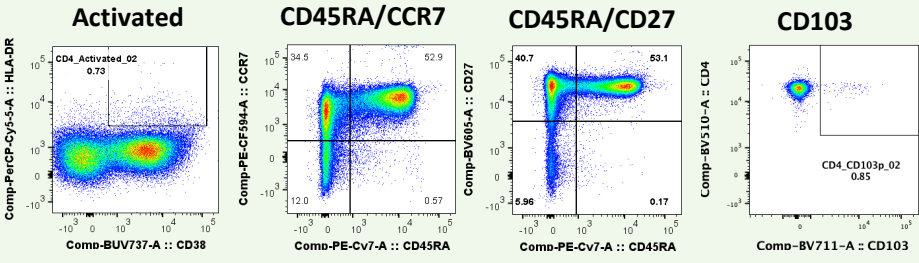


Gated on Live CD45+

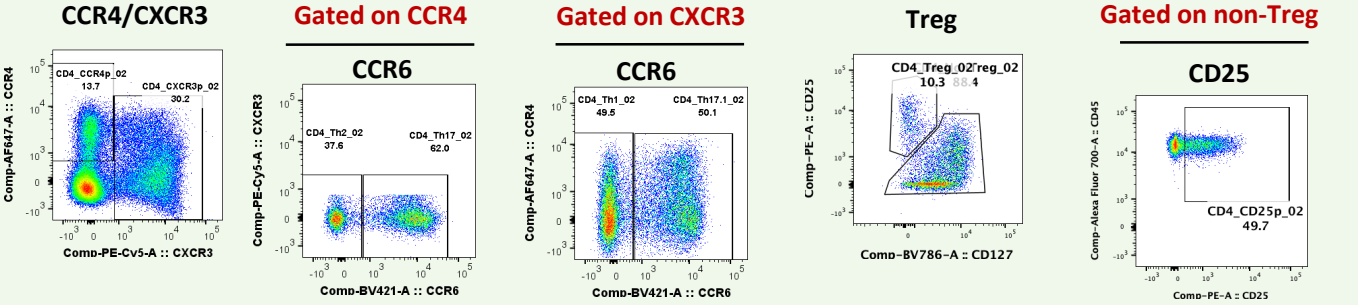
Gated on T cells



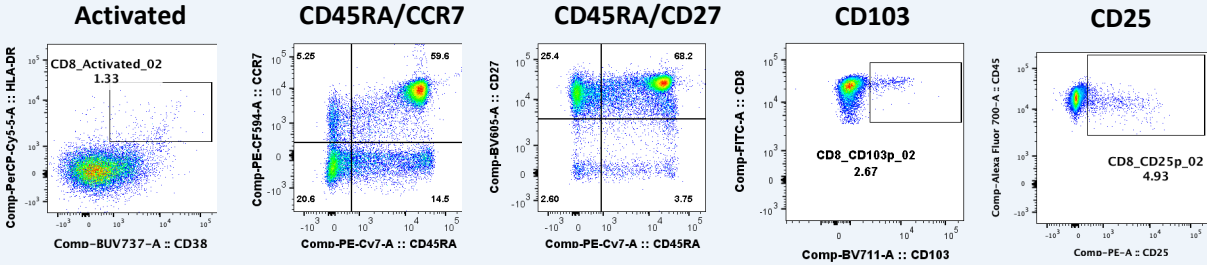
Gated on CD4



Gated on CD4



Gated on CD8

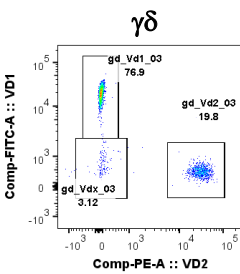
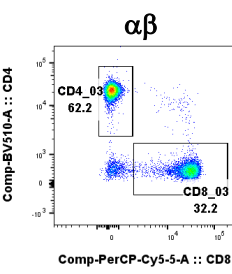
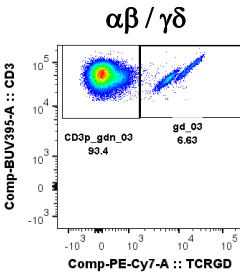
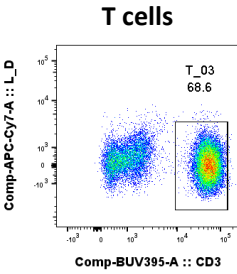


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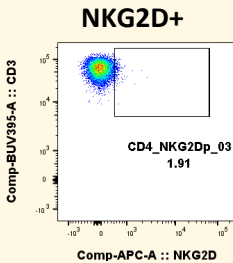
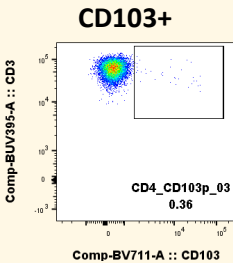
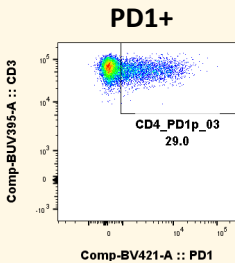
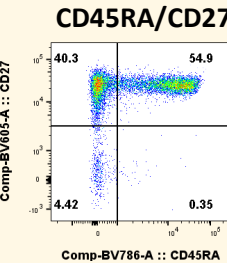
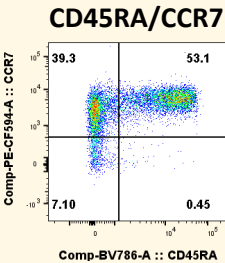
Gated on T cells

Gated on CD3p GDN cells

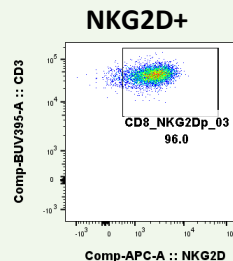
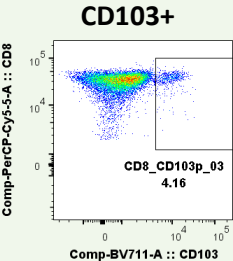
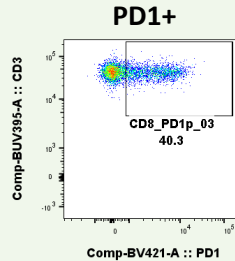
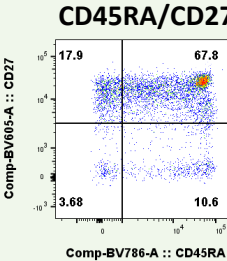
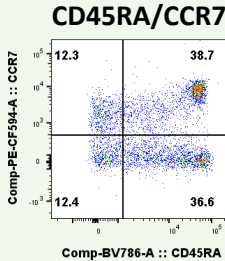
Gated on GD cells



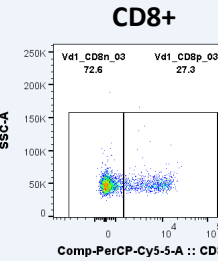
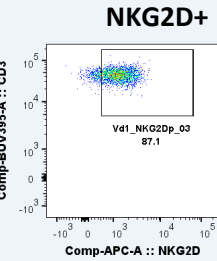
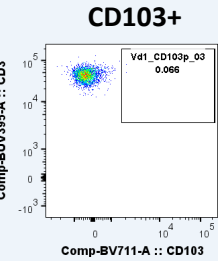
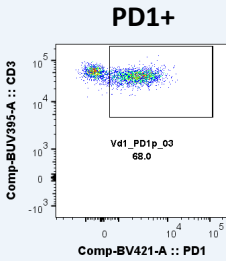
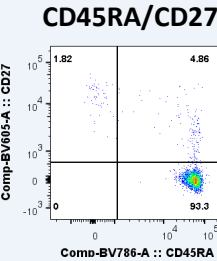
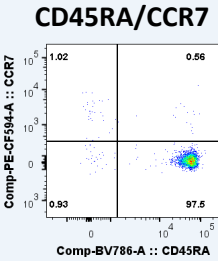
Gated on CD4+ T cells



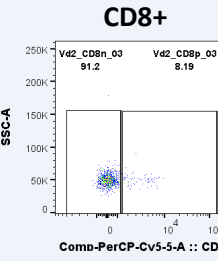
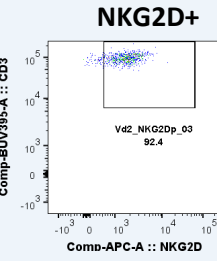
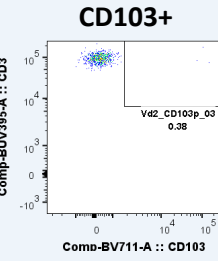
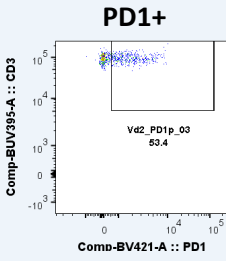
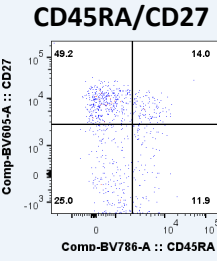
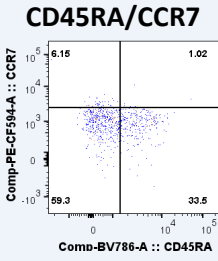
Gated on CD8+ T cells



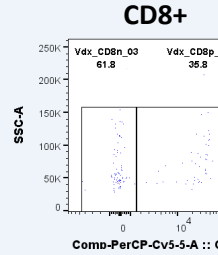
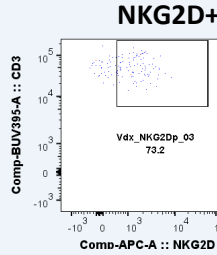
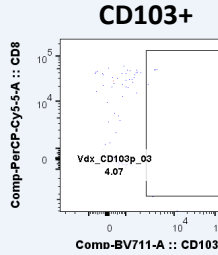
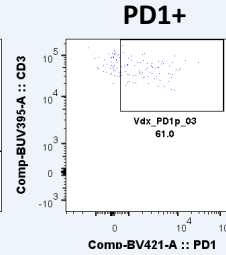
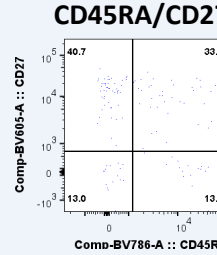
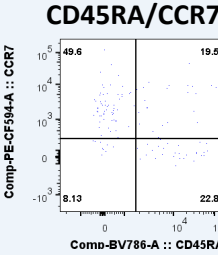
Gated on V01 T cells



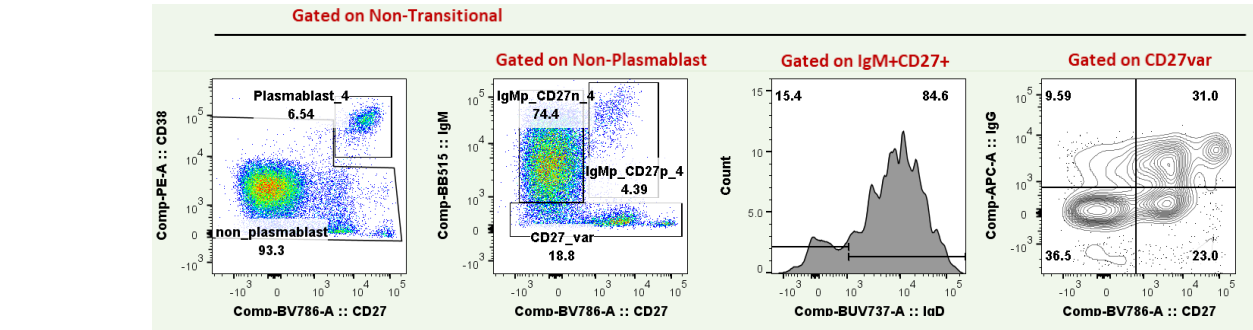
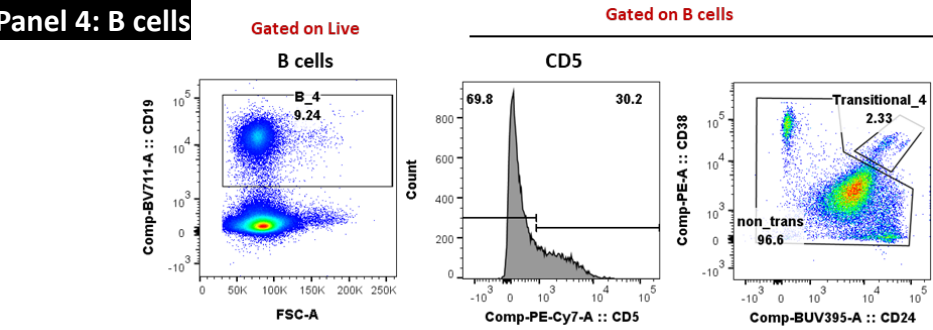
Gated on V02 T cells



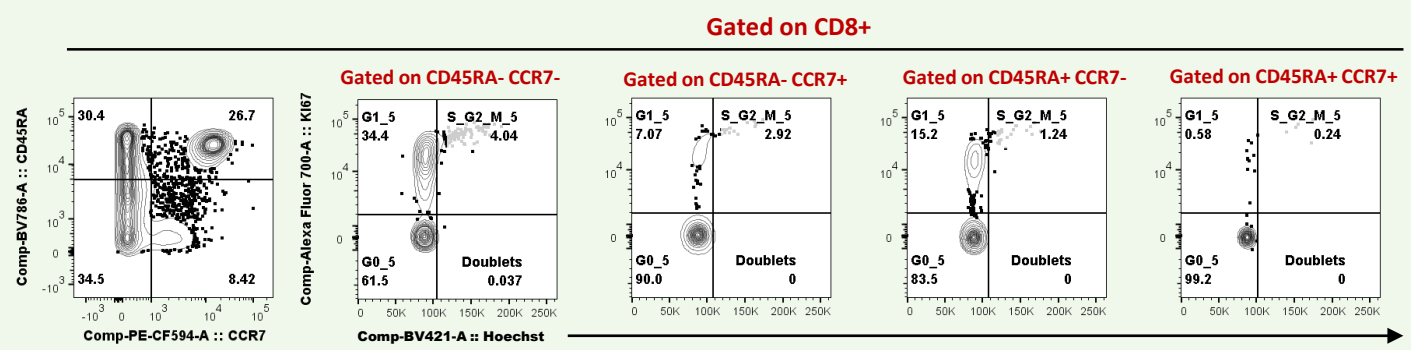
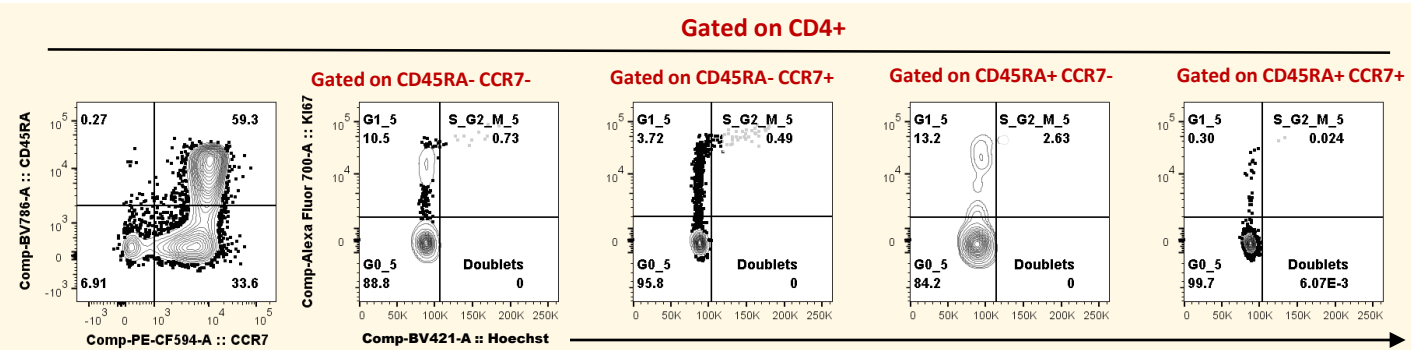
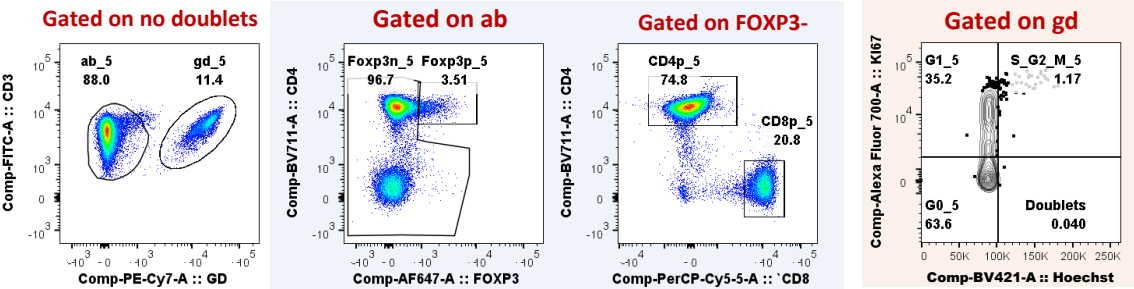
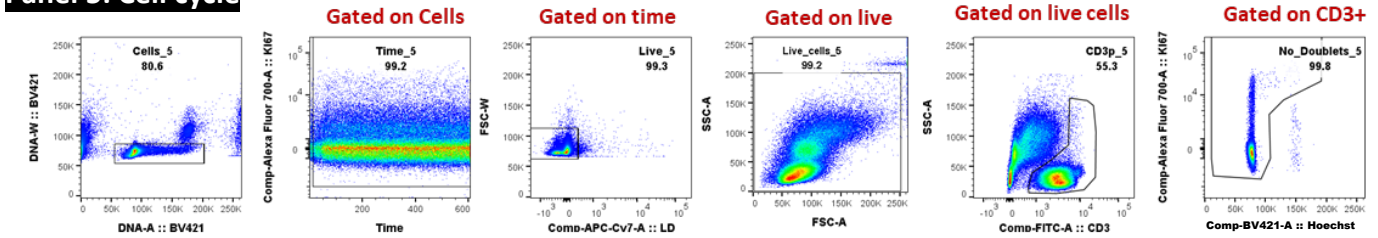
Gated on V0x T cells



Panel 4: B cells

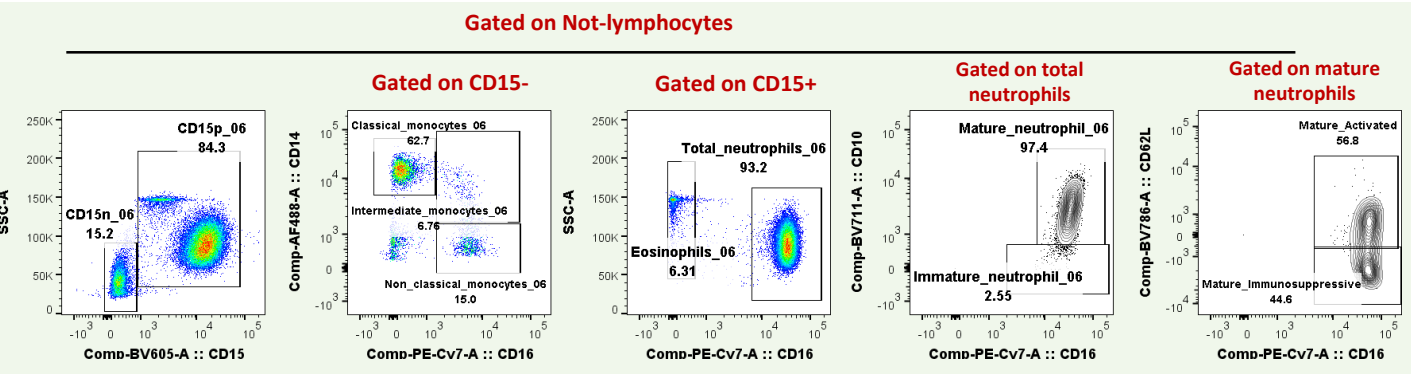
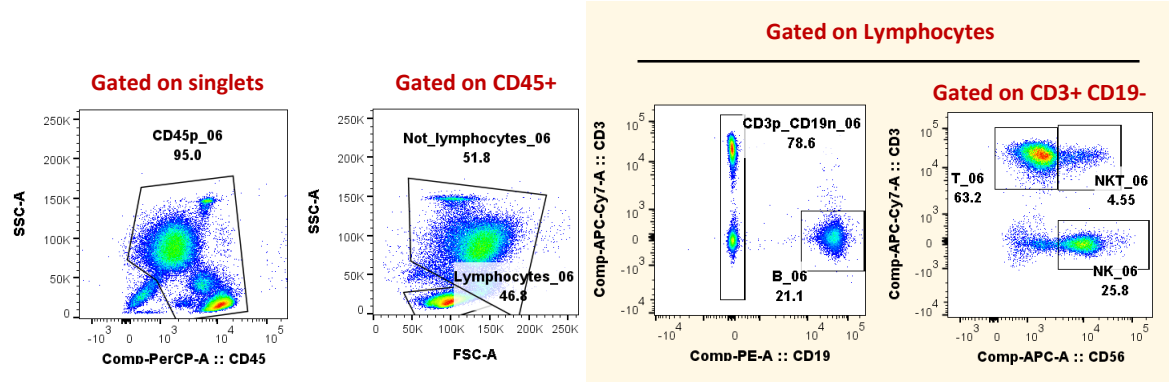


Panel 5: Cell cycle

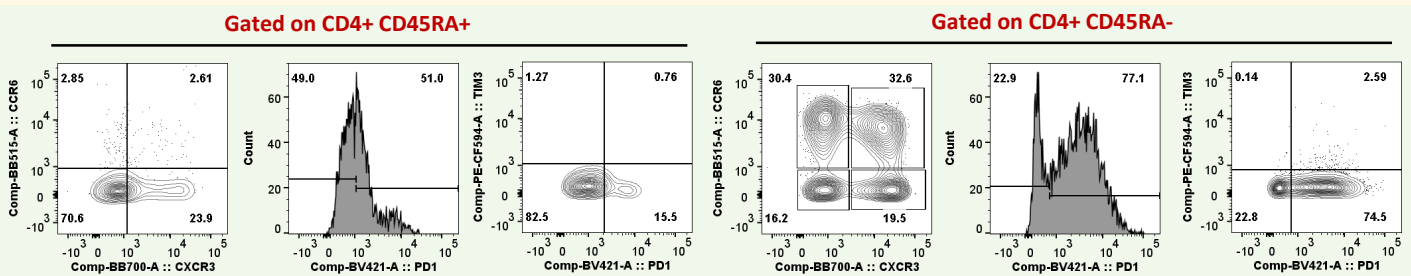
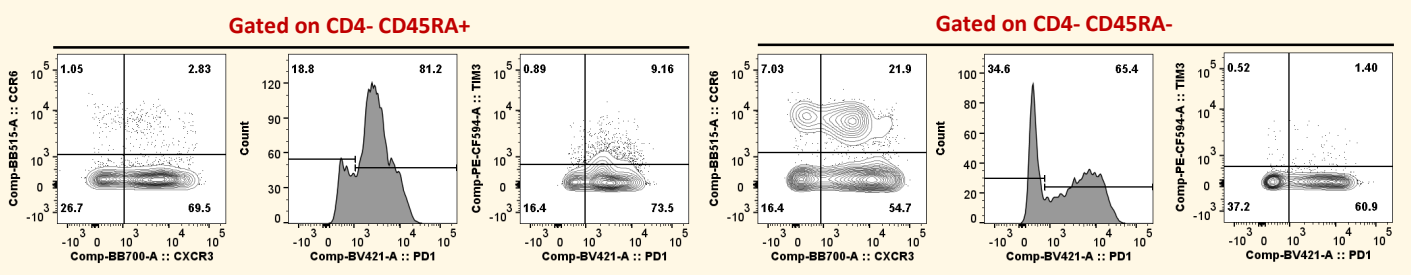
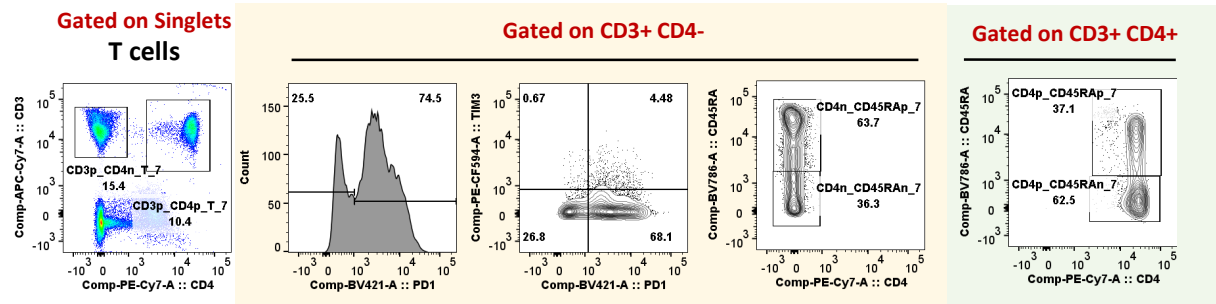


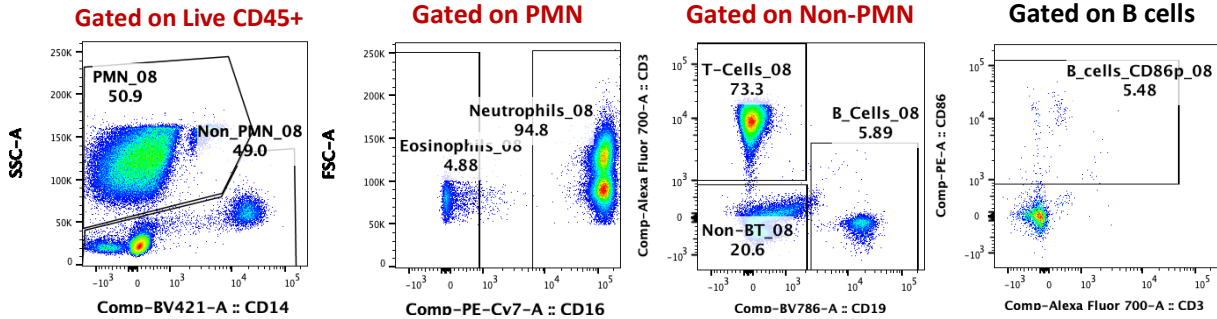
Panel 6: Cell counts

Whole blood

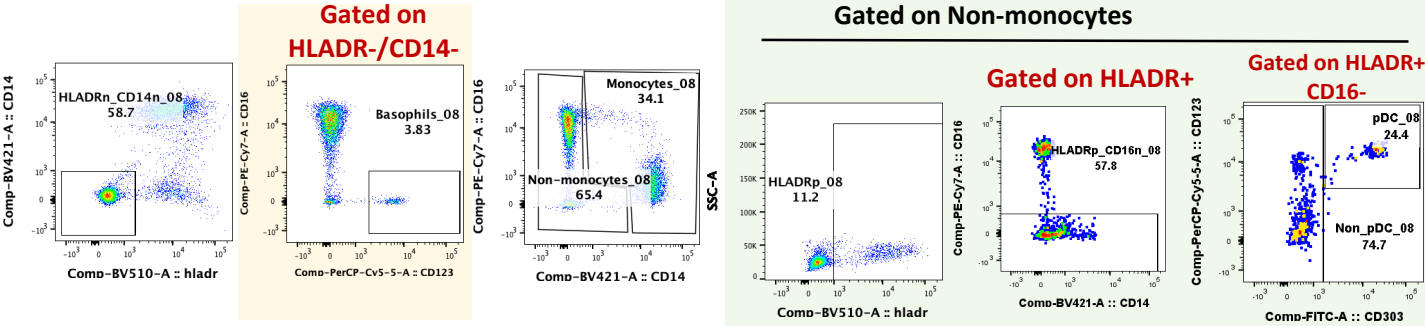


Panel 7: T cell exhaustion

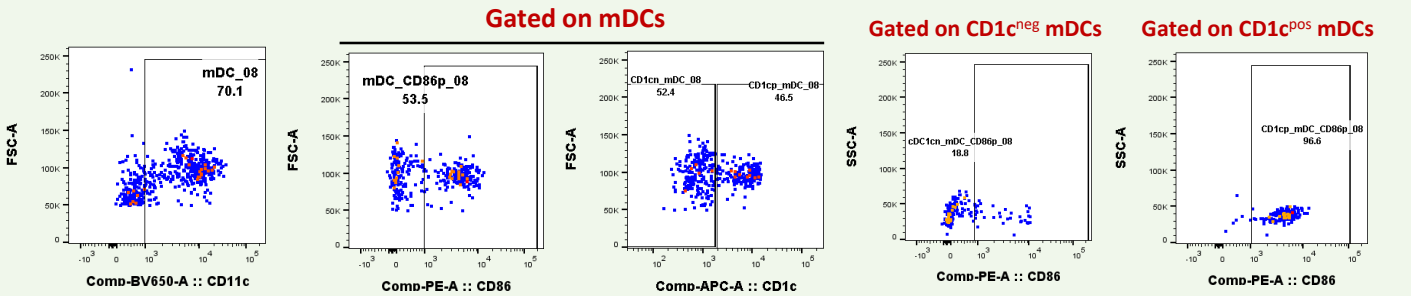




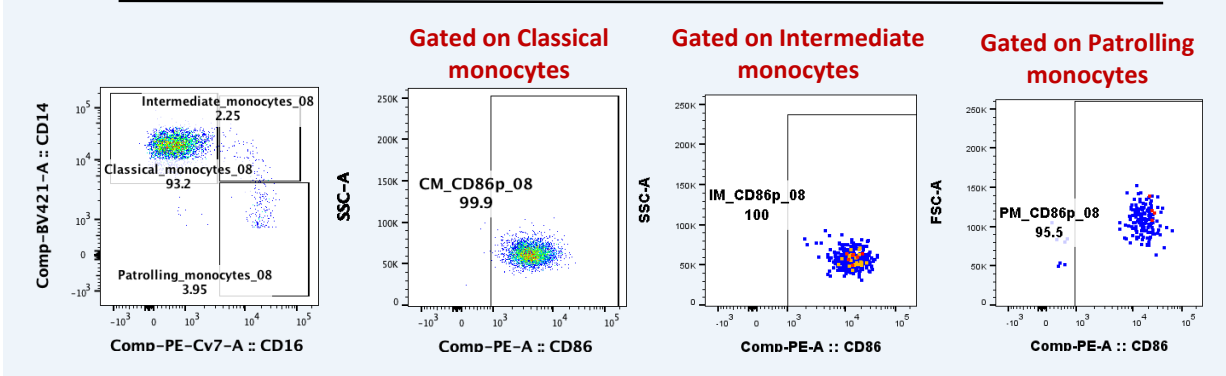
Gated on Non-BT cells

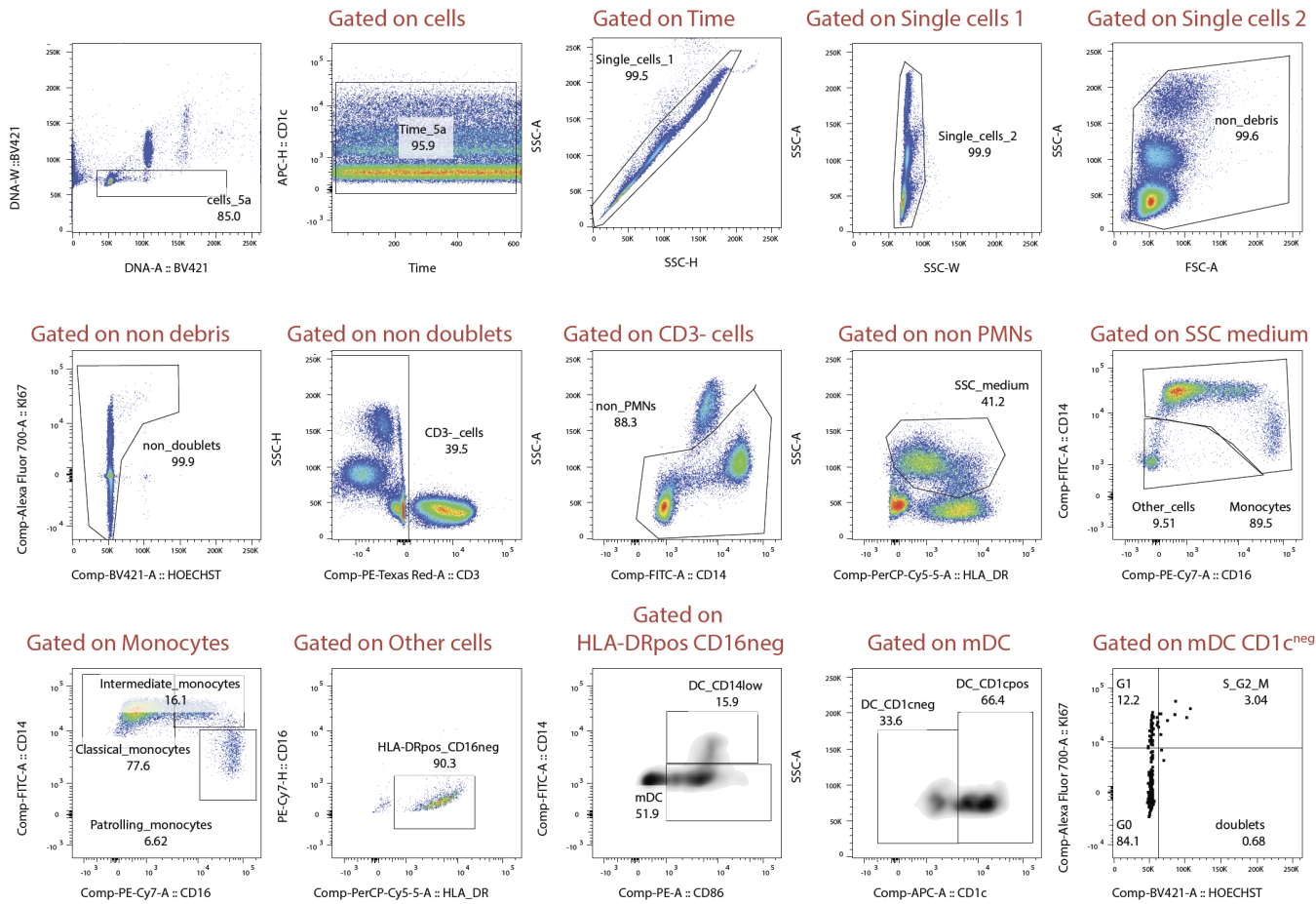


Gated on Non-pDCs

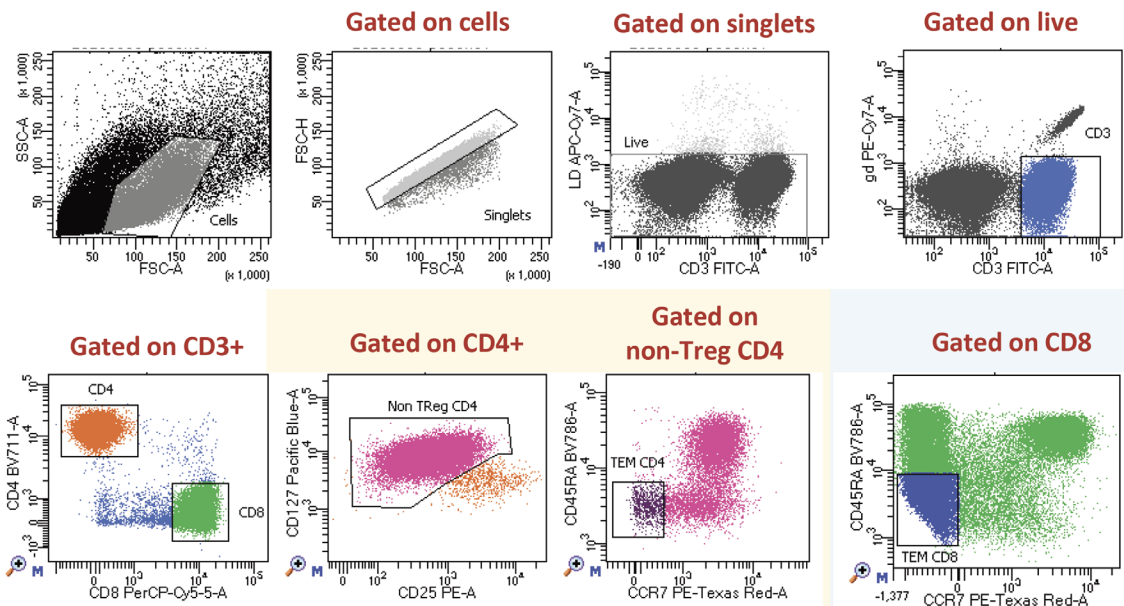


Gated on Monocytes





Sorting gating strategy



Supplementary Material: Flow cytometry gating strategies.

Data cleanup: Pre-gating for all panels as indicated.

Panel 1: Gating strategy used to examine CD4 T cells, CD8 T cells, Tregs, B cells, NK cells and monocytes in human PBMCs by flow cytometry. In addition, naïve, effector and memory markers on T cells as well as NK cell subsets and CD25, CD103 and NKG2D expression were analysed. This gating strategy was used to examine relevant parameters in figure 1 and Extended Data 4 and 5.

Panel 2: Gating strategy used to examine naïve, effector and memory markers, CD25, CD103, CD38 and HLADR in CD4 and CD8 T cells and Tregs, Th1, Th17.1 and Th17 cells in CD4 T cells in human PBMCs by flow cytometry. This gating strategy was used to examine relevant parameters shown in figures 1, 2 and 5 and Extended Data 4, 5 and 6.

Panel 3: Gating strategy used to examine V δ 1, V δ 2 and V δ x $\gamma\delta$ T cells subsets and naïve and memory populations, PD-1, CD103 and NKG2D expression within CD4, CD8 and $\gamma\delta$ T cells in human PBMCs by flow cytometry. This gating strategy was used to examine relevant parameters shown in figures 1 and 5 and Extended Data 4 and 5.

Panel 4: Gating strategy used to examine different B cell subpopulations: CD5⁺ B cells, plasmablasts, transitional B cells and naïve and memory B cells in human PBMCs by flow cytometry. This gating strategy was used to examine relevant parameters in figures 1 and 2 and Extended Data 1.

Panel 5: Gating strategy used to examine proliferation status of Tregs, $\gamma\delta$ T cells, naïve, effector, memory and EMRA CD4 and CD8 T cell subsets in human PBMCs by flow cytometry. This gating strategy was used to examine relevant parameters in figures 1 and 5 and Extended Data 5 and 6.

Panel 6: Gating strategy used to examine T cells, B cells, NK cells, NKT cells, monocytes, neutrophils (and subtypes), basophils, and eosinophils in human whole blood by flow cytometry. Data from this panel was used to determine cell counts and is relevant to all figures depicting cell count parameters.

Panel 7: Gating strategy used to examine PD-1, LAG-3, TIM-3 and 2B4 expression by CD4 and CD8 T cells as well as by Th1, Th2, Th17 and Th17.1 and CD4 and CD8 memory populations in human whole blood by flow cytometry. This gating strategy was used to examine relevant parameters in figures 1 and 5 and Extended Data 5.

Panel 8: Gating strategy used to examine eosinophils, neutrophils, basophils, monocytes (and subtypes), plasmacytoid DCs, myeloid DCs, CD1c^{pos} and CD1c^{neg} mDCs in human whole blood by flow cytometry. HLADR MFI and CD86 expression by monocyte and DC subsets

were also analysed. This gating strategy was used to examine relevant parameters in figures 1 and 8 and Extended data 3.

Panel 9: Gating strategy used to examine cell cycle status of classical, intermediate and patrolling monocytes, CD14^{low} DCs and CD1c^{pos} and CD1c^{neg} mDCs. This gating strategy was used to examine relevant parameters in supplementary figure 3.

Sorting gating strategy: Gating strategy used to sort CD8 and CD4 T_{EM} for NanoString analysis.