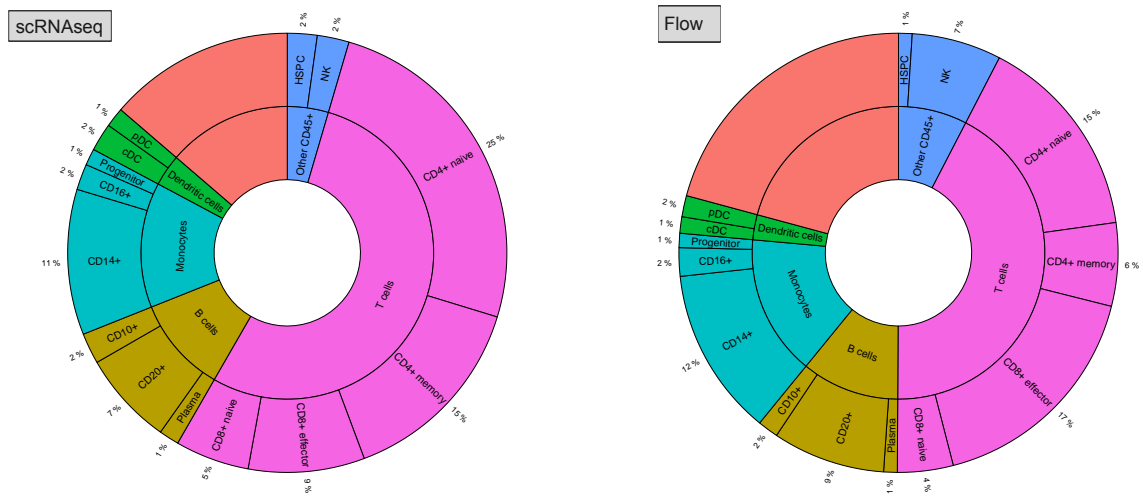
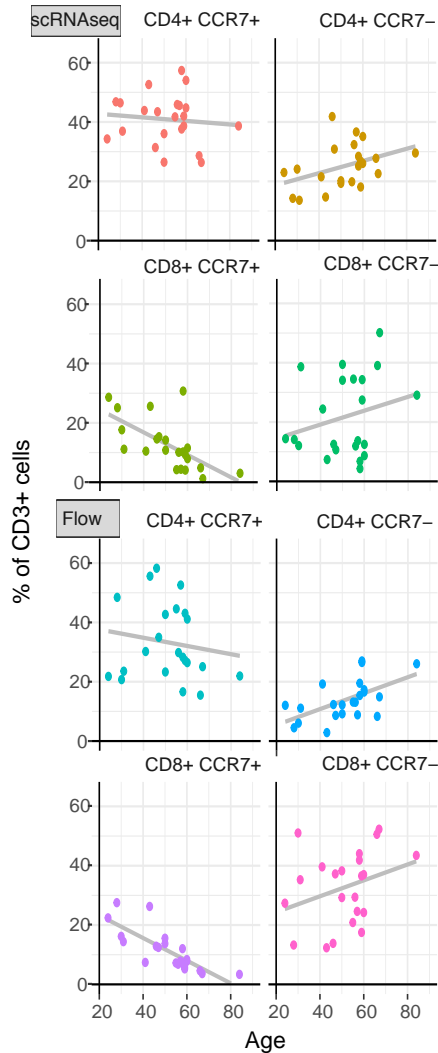


(A)



(B)



(C)

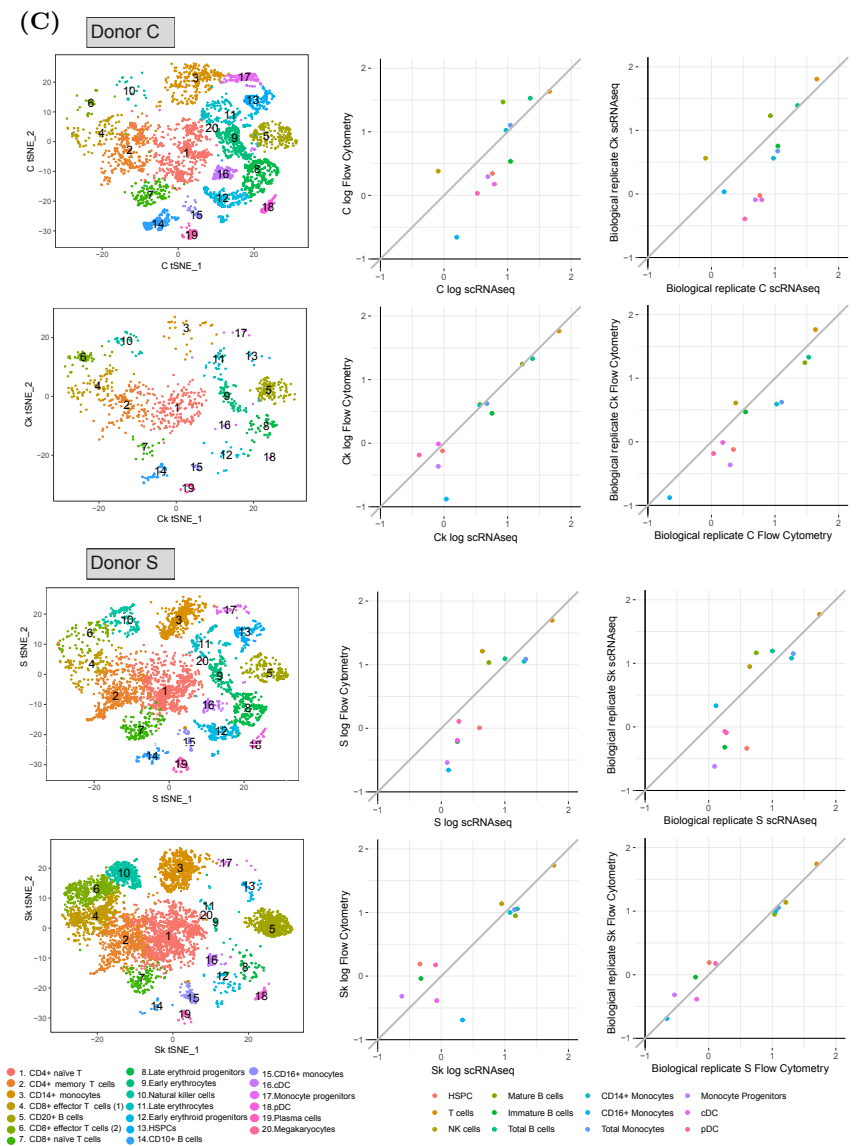


Figure S2: Flow Cytometry compared with Single Cell RNA Sequencing (A) Median frequency of major cell subsets in human bone marrow aspirate assessed by single-cell RNA sequencing (scRNAseq) (left) or flow cytometry (right). Percentages are of CD45 positive cells. Red zone reflects medians do not add to 100%. Aspirate underwent ficoll isolation of mononuclear cells prior to assessment. Cell yields per marrow aspirate varied by subject. (B) Age associated differences in T cell population frequencies (C) Analysis of biological replicates. Two subjects had repeat marrow aspirations analyzed. Shown on left are tSNE plots of scRNAseq data. Cell population frequencies as determined by flow cytometry and scRNAseq at the same timepoint (center) or between two timepoints using the same modality (right).

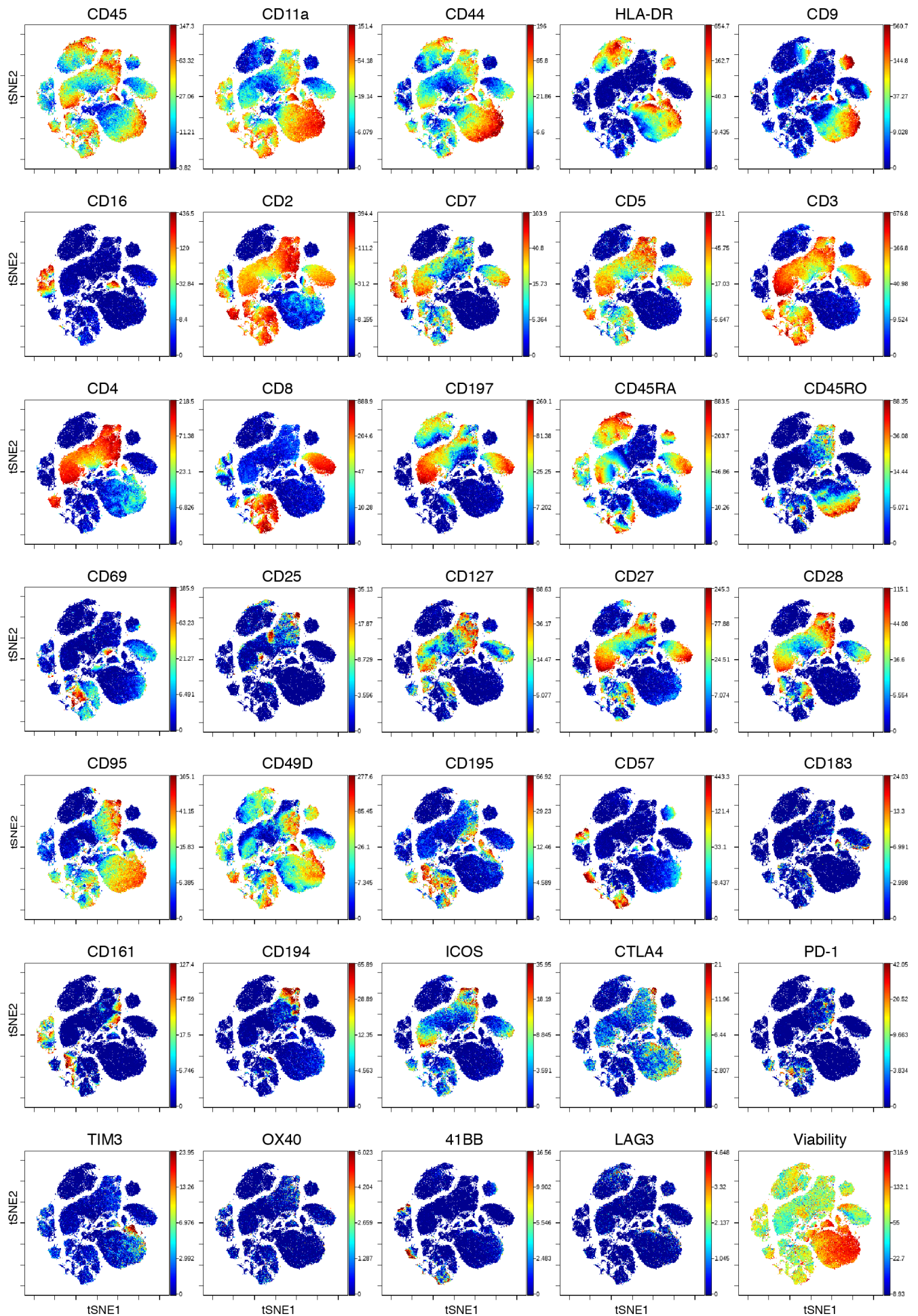


Figure S3: Mass Cytometry The viSNE visualization of CD45+ cells across the panel of markers.

Table S1: 13-Color flow cytometry panel design

Laser	Violet - 405nm					Blue - 488nm		Green - 532nm				Red - 633nm	
Detector	V450	V545	V605	V655	V800	B515	B710	G560	G610	G660	G780	R660	R780
Fluorochrome	Pac Blue/ BV421	Aquablue	BV 605	BV 650	BV 785	FITC	PerCP/Cy5.5	PE	PE Dazzle	PE/Cy5	PE/Cy7	APC	APC/Cy7
T-cells	CCR7	Live/Dead	CD4	CD45RA	CD3	CD27	CXCR4	CD49b	CD14/19	CD69	CD103	CD95	CD8
B-cells	CD80	Live/Dead	CD27	CD19	CD45	CD10	CD138	PD-1	CD20	CD38	CD86	CD21	CD40
NK Cells	CD7	Live/Dead	CD335 (NKp46)	CD314 (NKG2D)	CD45	CD158e1 (KIR3DL1)	CD226 (DNAM-1)	PD-1	CD16	CD69	CD56	NKG2C	CD3
Monocytes	CD15	Live/Dead	CD33	HLA-DR	CD45	Lineage (3,19,56)	CD14	PD-L1	CD16	CD11b	CD124	CD64	CD13
Dendritic Cells	CD123	Live/Dead	CD141 (BDCA-3)	HLA-DR	CD45	Lineage (3,14,16,19)	CD34	PD-L1	CD303 (BDCA-2)	CD11c	CD1c (BDCA-1)	CD117	CD40

Marker	Fluorochrome	Clone	Cat #	Company	Marker	Fluorochrome	Clone	Cat #	Company
BDCA-1	PE/Cy7	L161	331516	Biologend	CD27	FITC	O323	302806	Biologend
BDCA-2	PE Dazzle	201A	354226	Biologend	CD3	BV785	OKT3	317330	Biologend
CCR7	BV421	G043H7	353208	Biologend	CD3	FITC	OKT3	317306	Biologend
CD10	FITC	H110a	312208	Biologend	CD3	APC/Cy7	OKT3	317342	Biologend
CD103	PE/Cy7	Ber-ACT8	350212	Biologend	CD314	BV650	1D11	563408	BD Biosciences
CD117	APC	104D2	313206	Biologend	CD33	BV605	P67.6	366612	Biologend
CD11b	PE/Cy5	ICRF44	301308	Biologend	CD335	BV605	9E+2	331926	Biologend
CD11c	PE/Cy5	3.9	301610	Biologend	CD34	PerCP/Cy5.5	581	343522	Biologend
CD123	BV421	6H6	306018	Biologend	CD38	PE/Cy5	HIT2	303508	Biologend
CD124	PE/Cy7	G077F6	355008	Biologend	CD4	BV605	OKT4	317438	Biologend
CD13	APC/Cy7	WM15	301710	Biologend	CD40	APC/Cy7	5C3	334324	Biologend
CD138	PerCP/Cy5.5	M15	356510	Biologend	CD45	BV785	H130	304048	Biologend
CD14	FITC	M5E2	301804	Biologend	CD45RA	BV650	H1100	304136	Biologend
CD14	PerCP/Cy5.5	HCD14	325622	Biologend	CD49b	PE	P1E6-C5	359308	Biologend
CD14	PE Dazzle	M5E2	301852	Biologend	CD56	FITC	5.1H11	362546	Biologend
CD141	BV605	M80	344118	Biologend	CD56	PE/Cy7	5.1H11	362510	Biologend
CD15	BV421	W6D3	323040	Biologend	CD64	APC	10.1	305014	Biologend
CD158	FITC	DX9	312706	Biologend	CD69	PE/Cy5	FN50	310908	Biologend
CD16	FITC	3G8	302006	Biologend	CD7	eFluor® 450	eBio124-1D1	48-0079-42	eBioscience
CD16	PE Dazzle	3G8	302054	Biologend	CD8	APC/Fire	SK1	344746	Biologend
CD19	BV650	SJ25C1	563226	BD Biosciences	CD80	BV421	2D10	305222	Biologend
CD19	BV650	SJ25C1	363026	Biologend	CD86	PE/Cy7	IT2.2	305422	Biologend
CD19	FITC	HIB19	302206	Biologend	CD95 (Fas)	APC	DX2	305612	Biologend
CD19	PE Dazzle	HIB19	302252	Biologend	CXCR4	PerCP/Cy5.5	12G5	306516	Biologend
CD20	PE Dazzle	2H7	302348	Biologend	HLA-DR	BV650	L243	307650	Biologend
CD21	APC	Bu32	354906	Biologend	NKG2C	APC	REA205	130-103-636	Miltenyi
CD226	PerCP/Cy5.5	11A8	338314	Biologend	PD-1	PE	MIH4	12-9969-42	eBioscience
CD27	BV605	O323	302830	Biologend	PD-L1	PE	29E.2A3	329706	Biologend

Table S2: Flow cytometry gating strategy

<u>Cell population</u>	<u>Flow cytometry gating</u>
B cells	CD45+ CD19+
Immature B cells	CD45+ CD19+ CD10+
Pre-B cell (CD20-)	CD45+ CD19+ CD20- CD10+
Immature B cells (CD20+)	CD45+ CD19+ CD20+ CD10+
Translational B cells	CD45+ CD19+ CD20+ CD10+ CD27-
Mature B cells	CD45+ CD19+ CD20+ CD10-
Naïve Mature B cells	CD45+ CD19+ CD20+ CD10- CD27- CD21hi
Exhausted/Tissue-like Memory	CD45+ CD19+ CD20+ CD10- CD27- CD21lo
Memory B cells	CD45+ CD19+ CD20+ CD10- CD27+
Activated Mature	CD45+ CD19+ CD20+ CD10- CD27+ CD21lo
Resting Memory	CD45+ CD19+ CD20+ CD10- CD27+ CD21hi
CD20- CD10-	CD45+ CD19+ CD20- CD10-
Plasmablast	CD45+ CD19+ CD20- CD10- CD27+ CD38+
Plasma Cell	CD45+ CD19+ CD20- CD10- CD27+ CD38+ CD138+
pDC	CD45+CD11c-CD123+
cDC	CD45+CD3-CD14-CD16-CD19-HLA-DR+CD11c+
cDC1	CD45+CD3-CD14-CD16-CD19-HLA-DR+CD11c+CD141+
CD1c+ DC	CD45+CD3-CD14-CD16-CD19-HLA-DR+CD11c+CD141-CD1c+
cDC2	CD45+CD3-CD14-CD16-CD19-HLA-DR+CD11c+CD141-CD1c-
HSPC	CD45+CD34+
Monoblast/myeloblast/promyelocyte	CD45+CD3-CD14-CD16-CD19-CD34+HLA-DR+CD117+
Monocytes	CD45+CD3-CD19-CD56-CD33+CD13+CD11B+HLA-DR+CD64+
CD14+ Monocytes	CD45+CD3-CD19-CD56-CD33+CD13+CD11B+HLA-DR+CD64+CD14+
Classical Monocytes	CD45+CD3-CD19-CD56-CD33+CD13+CD11B+HLA-DR+CD64+CD14+CD16-
Intermediate Monocytes	CD45+CD3-CD19-CD56-CD33+CD13+CD11B+HLA-DR+CD64+CD14+CD16int
Non-classical Monocytes	CD45+CD3-CD19-CD56-CD33+CD13+CD11B+HLA-DR+CD64+CD14intCD16+
NK cells	CD45+ CD3- CD7+ CD56+
Immunoregulatory NK	CD45+ CD3- CD7+ CD56bright CD16 dim
Cytotoxic NK	CD45+ CD3- CD7+ CD56dim CD16 bright
CD3+	CD45+ CD3+
CD8+	CD3+CD14-CD19-CD8+
CD8+ CCR7+	CD3+CD14-CD19-CD8+CCR7+
CD8+ Naïve	CD3+CD14-CD19-CD8+CCR7+CD45RA+
CD8+ CM	CD3+CD14-CD19-CD8+CCR7+CD45RA-
CD8+ CCR7-	CD3+CD14-CD19-CD8+CCR7-
CD8+ TEMRA	CD3+CD14-CD19-CD8+CCR7-CD45RA+
CD8+ EM	CD3+CD14-CD19-CD8+CCR7-CD45RA-
CD8+ EM CD69+	CD3+CD14-CD19-CD8+CCR7-CD45RA-CD69+
CD8+ EM CD69+ CD103+	CD3+CD14-CD19-CD8+CCR7-CD45RA-CD69+CD103+
CD4+	CD3+CD14-CD19-CD4+
CD4+ CCR7+	CD3+CD14-CD19-CD4+CCR7+
CD4+ Naïve	CD3+CD14-CD19-CD4+CCR7+CD45RA+
CD4+ CM	CD3+CD14-CD19-CD4+CCR7+CD45RA-
CD4+ CCR7-	CD3+CD14-CD19-CD4+CCR7-
CD4+ TEMRA	CD3+CD14-CD19-CD4+CCR7-CD45RA+
CD4+ EM	CD3+CD14-CD19-CD4+CCR7-CD45RA-
CD4+ EM CD69+	CD3+CD14-CD19-CD4+CCR7-CD45RA-CD69+
CD4+ EM CD69+ CD103+	CD3+CD14-CD19-CD4+CCR7-CD45RA-CD69+CD103+
DPT	CD3+CD14-CD19-CD4+CD8+
DNT	CD3+CD14-CD19-CD4-CD8-

Table S3: Bulk RNA-seq deconvolution

See attached Excel spreadsheet

Table S4: Flow cytometry instrument configuration

BD LSR Fortessa SORP Instrument configuration with HTS

Laser	Laser Wave Length	LASER Type and Source	Laser Power	Detector name	Fluorochrome detected	Dichroic LP Filter	BP filter	Signal Collected	Signal Amplification	PMT
BLUE LASER LINE	488nm	DPSS; Coherent	100 mW	FSC	Forward Scatter	NA	NA	Hight, Area and Width	Linear	PhotoDiode
				B710	PerCP CY5.5	685	710/50	Area	Log	A
				B515	FITC	505	515/20	Area	Log	B
				SSC	Side Scatter	488		Hight, Area and Width	Linear	C
GREEN LASER LINE	532nm	DPSS; Coherent	150 mW	G780	PECy7	740	780/40	Area	Log	A
				G710		690	710/50	Area	Log	B
				G660	PECy5	640	660/40Δ	Area	Log	C
				G610	PECF594	600	610/20	Area	Log	D
				G560	PE	empty	575/25	Area	Log	E
RED LASER LINE	637nm	DPSS; OBIS	140 mW	R780	APC-Cy7	740	780/60	Area	Log	A
				R710	APC-700	685	710/50	Area	Log	B
				R660	APC or AF-647	empty	660/20Δ	Area	Log	C
VIOLET LASER LINE	405nm	DPSS; OBIS	100 mW	V800	BV786	740	780/60	Area	Log	A
				<i>V705^v</i>		670	705/70	Area	Log	B
				V655	BV650	630	660/40	Area	Log	C
				V605	BV605	595	605/40	Area	Log	D
				<i>V585^v</i>		570	585/42	Area	Log	E
				V565		557	560/40	Area	Log	F
				V545	Fixable Live Dead Yellow viability stain (Invitrogen)	535	560/40	Area	Log	G
				V450	BV421 or Pacific Blue	empty	450/50	Area	Log	H
ULTRA VIOLET LASER LINE	355nm	DPSS; Genesis 355-100	45mW	UV525	BUV 496	505	525/50Δ	Area	Log	A
				UV450	BUV 395	empty	450/50	Area	Log	B

Notch Filter: *A*

v: Not used in configuration.

Equipped with High Throughput Sampler System (HTS) (BD)

DPSS = Diode Pumped Solid State

Table S5: Mass cytometry panel design

Marker	Metal	Clone
CD11a	142Nd	HI111
CD4	145Nd	RPA-T4
CD8a	146Nd	RPA-T8
CD16	148Nd	3G8
CD25	149Sm	2A3
CD45	154Sm	HI30
CCR7	159Tb	G043H7
CD69	162Dy	FN50
CD45RO	165Ho	UCHL1
CD44	166Er	BJ18
CD27	167Er	O323
CD45RA	169Tm	HI100
CD3	170Er	UCHT1
CD57	172Yb	HCD57
HLA-DR	174Yb	L243
CD127	176Yb	A019D5
CD134 [OX40]	150Nd	ACT35
CD95 [Fas]	152Sm	DX2
CD366 [Tim-3]	153Eu	F38-2E2
CD279 [PD-1]	155Gd	EH12.2H7
CD152 [CTLA-4]	161Dy	14D3
CD278 [ICOS]	168Er	C398.4A
CD137 [4-1BB]	173Yb	4B4-1
CD223 [LAG3]	175Lu	11C3C65
CD2	151Eu	TS1/8
CD5	143Nd	UCHT2
CD7	147Sm	CD7-6B7
CD9	171Yb	SN4 C3-3A2
CD28	160Gd	CD28.2
CD49d	141Pr	9F10
CD161	164Dy	HP-3G10
CCR4	158Gd	L291H4
CCR5	144Nd	NP-6G4
CXCR3	156Gd	G025H7

Fluidigm Maxpar Complete Human T Cell Immuno-Oncology Panel Set (Product # 201322)

From: <https://www.fluidigm.com/reagents/proteomics/201322-maxpar-complete-human-t-cell-immuno-oncology-panel-set> (accessed October 18th 2018)