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Multiplexed single-cell morphometry for hematopathology diagnostics

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Supplementary Table 1

Antibody	Clone	Element	Mass Channel	Stock (µg/mL)	Stain (µg/mL)	Manufacturer	Catalog	Titration Positive Control Example	Titration Negative Control Example
SURFACE									
CD235ab	HIR2	In	113	200	1	BioLegend	306602	erythroids	T-cells
CD71	CY1G4	In	115	200	3	BioLegend	334102	erythroids	T-cells
CD61	VI-PL2	La	139	200	0.5	BioLegend	336402	platelets	T-cells
CD71	CY1G4	Ce	140	300	0.5	BioLegend	334102	erythroids	T-cells
CD3	UCHT1	Pr	141	100	0.5	BD	561416	T-cells	B-cells
CD8	SK1	Nd	142	300	1	BioLegend	344702	T-cells	B-cells
CD2	RPA-2.10	Nd	143	50	0.15	BioLegend	300202	T-cells	B-cells
CD5	UCHT2	Nd	144	100	2	BioLegend	300602	T-cells	B-cells
CD4	RPA-T4	Nd	145	300	1	BioLegend	300502	T-cells	B-cells
CD7	124-1D1	Nd	146	300	3	eBioscience/Invitrogen	14007982	T-cells	B-cells
CD11c	Bu15	Sm	147	300	0.25	BioLegend	337202	monocytes	nRBC
CD23	M-L233	Nd	148	300	0.5	BD	555708	B-cells	nRBC
CD123	6H6	Sm	149	400	0.5	BioLegend	306002	basophils	T-cells
CD56	NCAM16.2	Nd	150	300	0.25	BD	559043	NK-cells	B-cells
IgL	MHL-38	Eu	151	300	6	BioLegend	316602	B-cells	T-cells
IgK	MHK-49	Eu	153	500	6	BioLegend	316502	B-cells	T-cells
CD45	HI30	Sm	154	300	0.5	BioLegend	304002	T-cells	nRBC
CD10	HI10a	Gd	158	300	1.5	BD	555373	B-cell precursors	monocytes
CD13	WM15	Gd	160	300	4	BioLegend	301702	neutrophils	T-cells
CD117	104D2	Dy	161	300	0.75	BioLegend	313202	myeloblasts	T-cells
CD34	581	Ho	165	300	1	BioLegend	343502	myeloblasts	T-cells
CD20	2H7	Er	166	300	0.3	BioLegend	302302	B-cells	T-cells
CD19	HIB19	Er	167	300	0.5	BioLegend	302202	B-cells	T-cells
CD22	HIB22	Er	168	200	2	BioLegend	302502	B-cells	T-cells
CD15	MC-480	Yb	171	300	0.125	BioLegend	125602	neutrophils	T-cells
CD33	P67.6	Yb	172	300	2	BioLegend	366602	monocytes	T-cells
CD14	M5E2	Yb	173	300	3	BioLegend	301802	monocytes	T-cells
CD64	10.1	Yb	174	200	2	BioLegend	305002	monocytes	T-cells
CD16	3G8	Lu	175	300	0.125	BioLegend	302002	neutrophils	B-cells
CD38	HIT2	Yb	176	100	0.25	BioLegend	303502	plasma cells	erythroids
HLA-DR	L243	Bi	209	200	1	BioLegend	307602	monocytes	T-cells

INTRACELLULAR

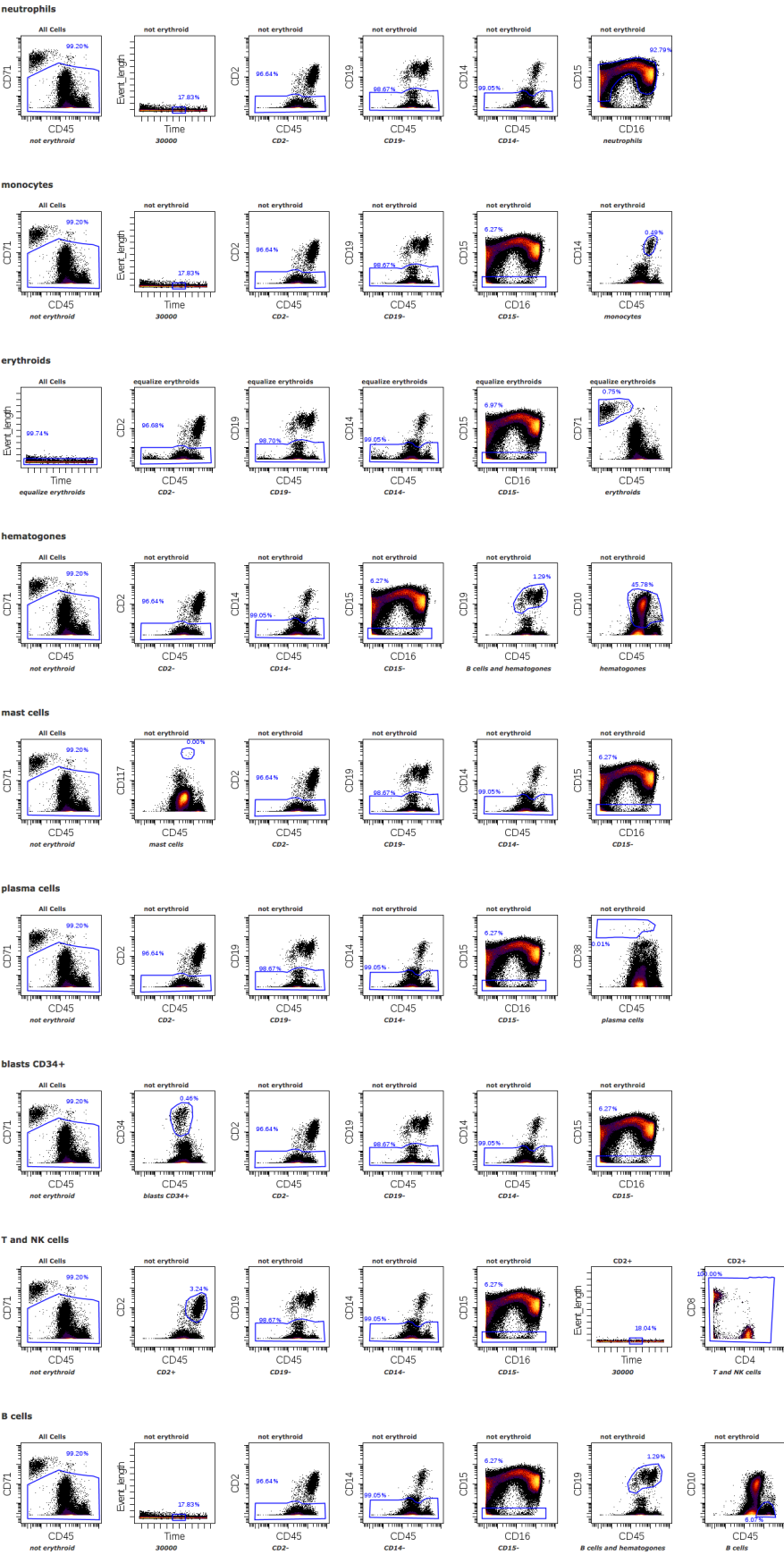
MPO	1B10	Y	89	100	1.5	BD	556035	neutrophils	T-cells
lactoferrin	1C6	Sm	152	200	4	Novus	NB120-10109	Tsai et al. Fig. 1d, 2	
rRNA	Y10b	Gd	155	300	2	Novus	NB100-662	Tsai et al. Fig. 1d, 2	
HP1β	4D7B8	Gd	156	300	8	Santa Cruz	sc-293177	Tsai et al. Fig. 1d, 2	
lamin B1	G-1	Tb	159	300	0.8	Santa Cruz	sc-373918	Tsai et al. Fig. 1d, 2	
VAMP-7	549115	Dy	162	300	8	R&D	MAB6117	Tsai et al. Fig. 1d, 2	
lysozyme	BGN/0696/5B1	Dy	163	300	0.3	Novus	NB100-63062	Tsai et al. Fig. 1d, 2	
serpin B1	OTI2B10	Dy	164	300	0.5	OriGene	CF800117	Tsai et al. Fig. 1d, 2	
lamin A/C	E-1	Tm	169	200	0.5	Santa Cruz	sc-376248	Tsai et al. Fig. 1d, 2	
CD79a	HM47	Er	170	300	2	BioLegend	333502	B-cells	T-cells

β actin	2F1-1	Pt	198	300	0.25	BioLegend	643802	Tsai et al. Fig. 1d, 2
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BARCODING								
β2 microglobulin	2M2	Pt	195	200	1	BioLegend	316302	Hartmann et al. Fig. 1c-d
CD298	LNH-94	Pt	195	200	2	BioLegend	custom	Hartmann et al. Fig. 1c-d
β2 microglobulin	2M2	Pt	196	200	1	BioLegend	316302	Hartmann et al. Fig. 1c-d
CD298	LNH-94	Pt	196	200	2	BioLegend	custom	Hartmann et al. Fig. 1c-d

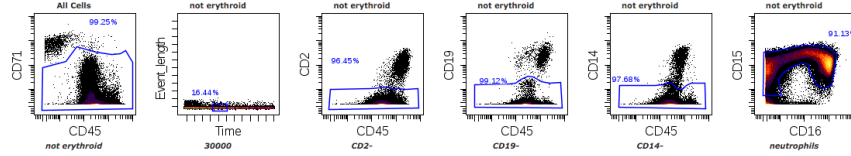
Supplementary Table 1: Summary of mass cytometry antibodies/reagents and example positive and negative controls
List of antibodies, clones, reporter isotopes, stock and staining concentrations, manufacturer, and catalog numbers.

Supplementary Figure 1a

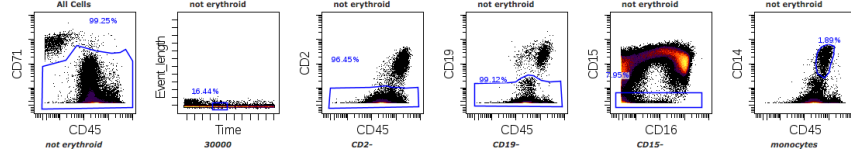


Supplementary Figure 1b

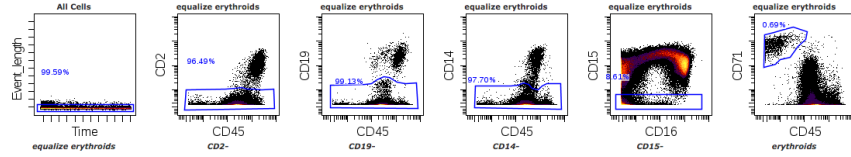
neutrophils



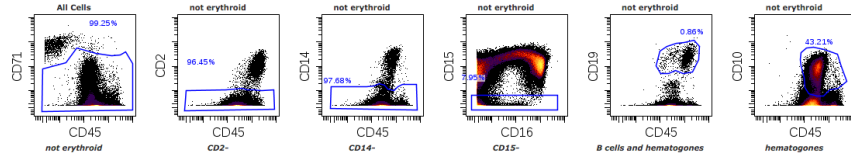
monocytes



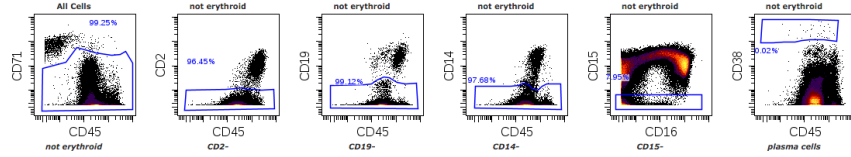
erythroids



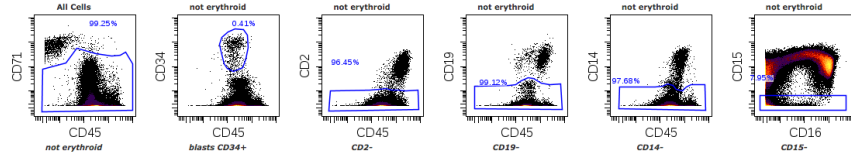
hematogones



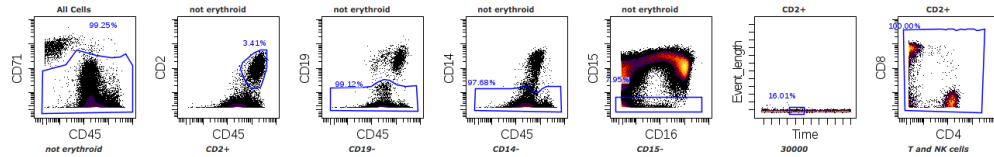
plasma cells



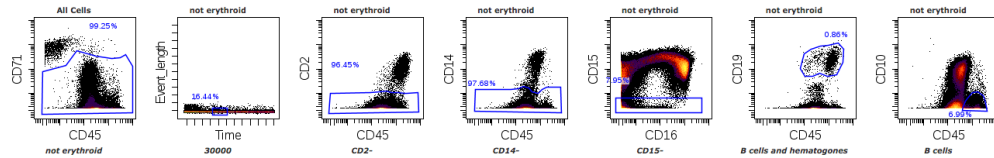
blasts CD34+



T and NK cells

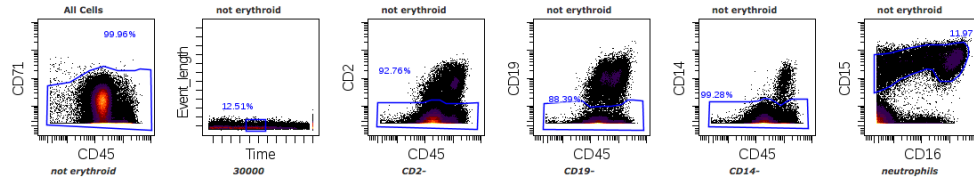


B cells

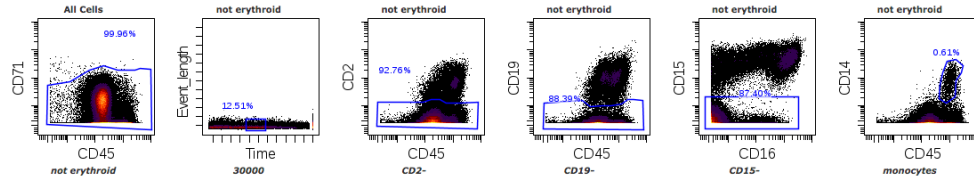


Supplementary Figure 1c

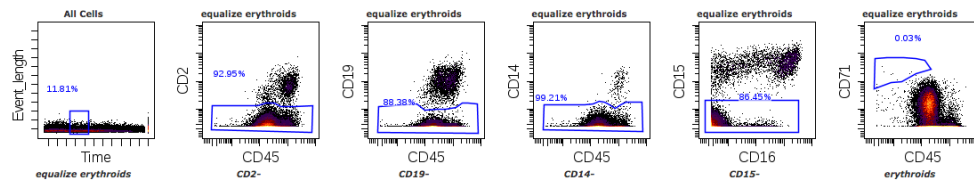
neutrophils



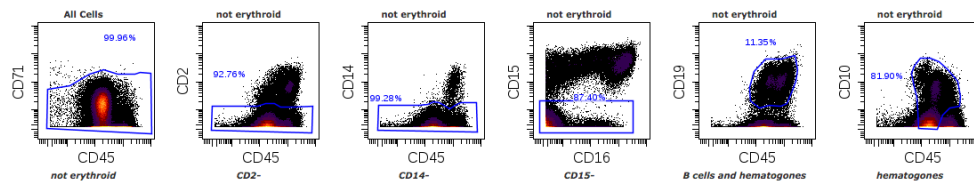
monocytes



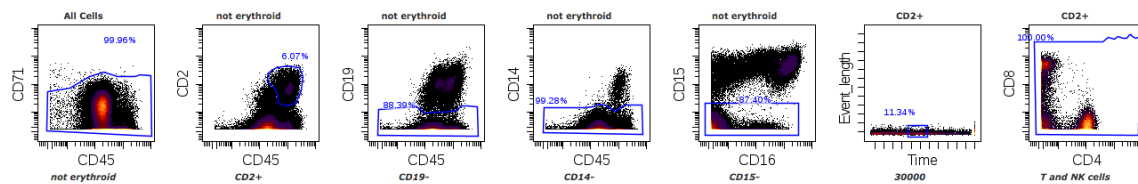
erythroids



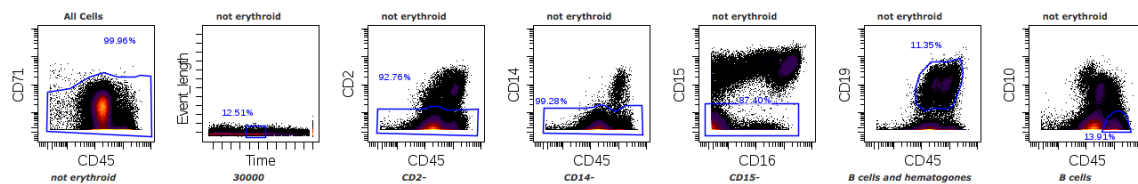
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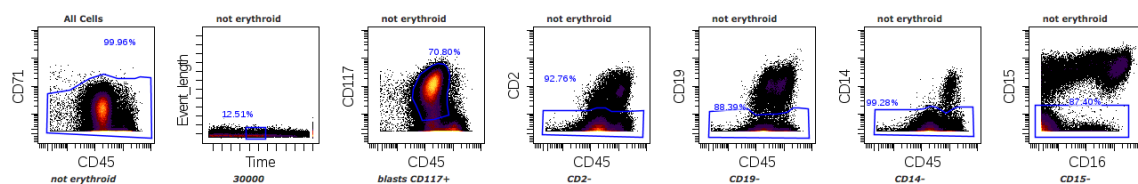
T and NK cells



B cells

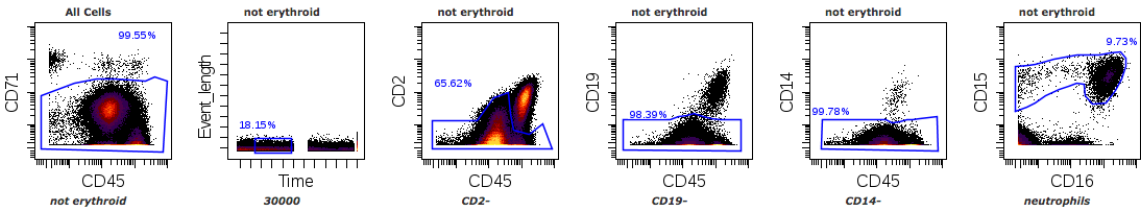


blasts CD117+

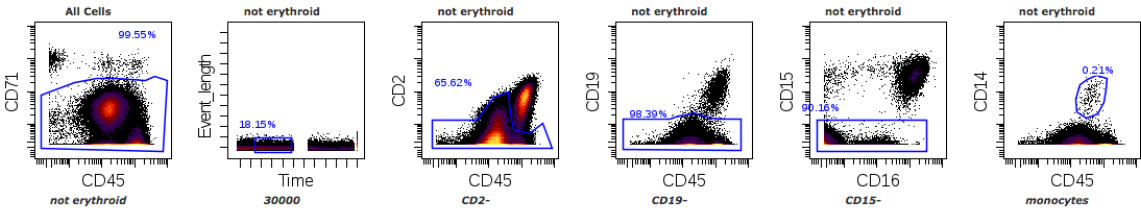


Supplementary Figure 1d

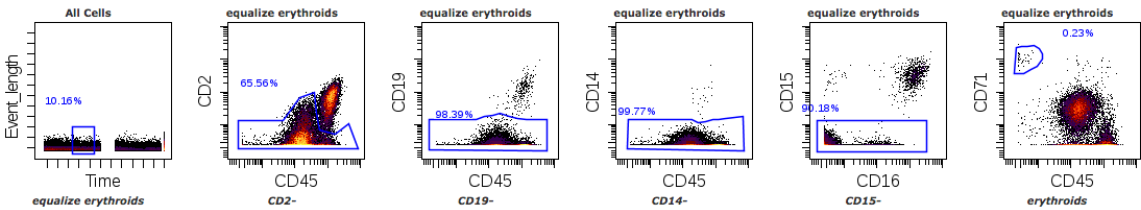
neutrophils



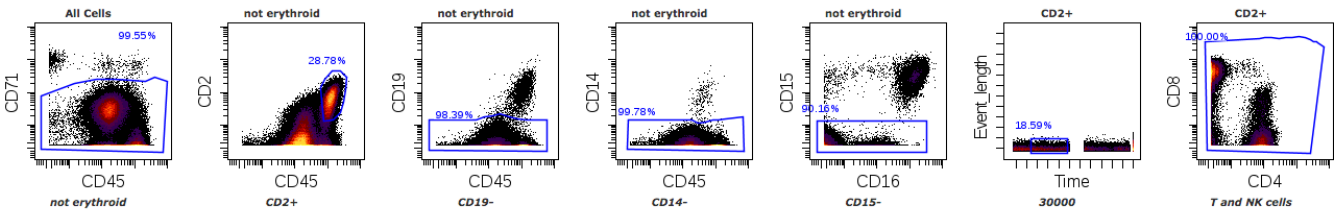
monocytes



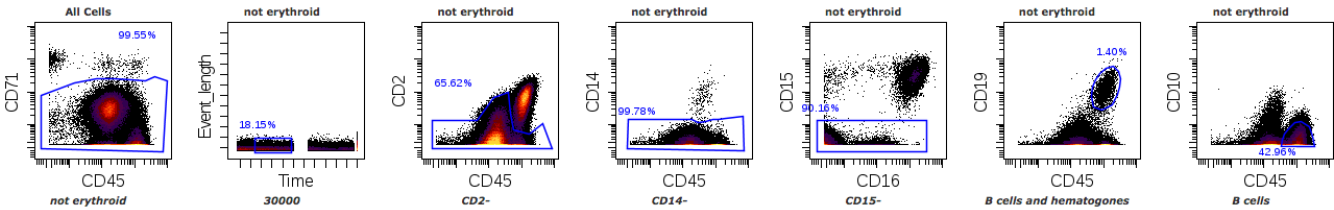
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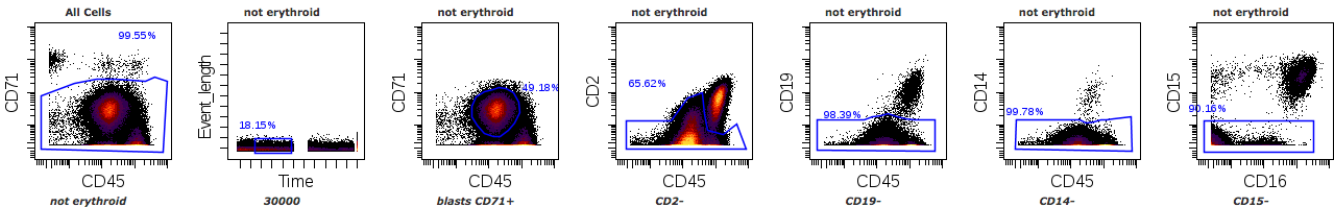
T and NK cells



B cells

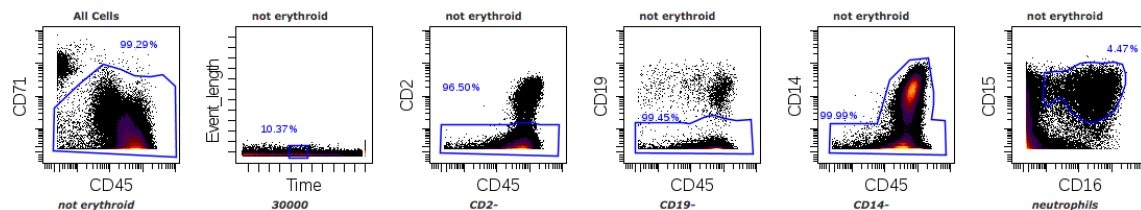


blasts CD71+

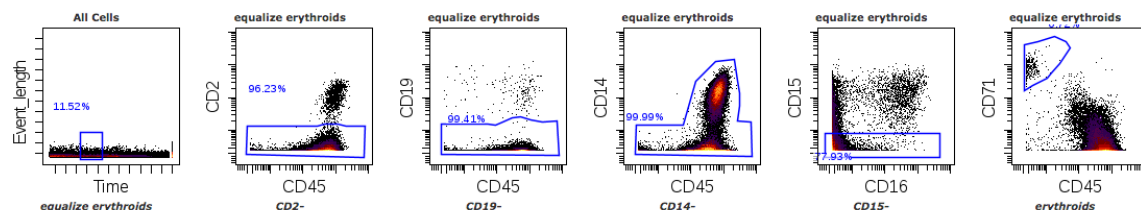


Supplementary Figure 1e

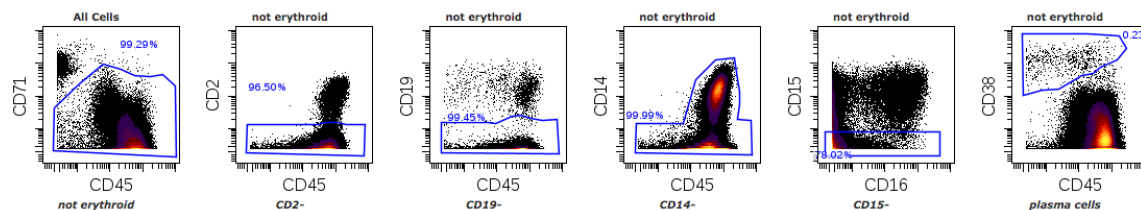
neutrophils



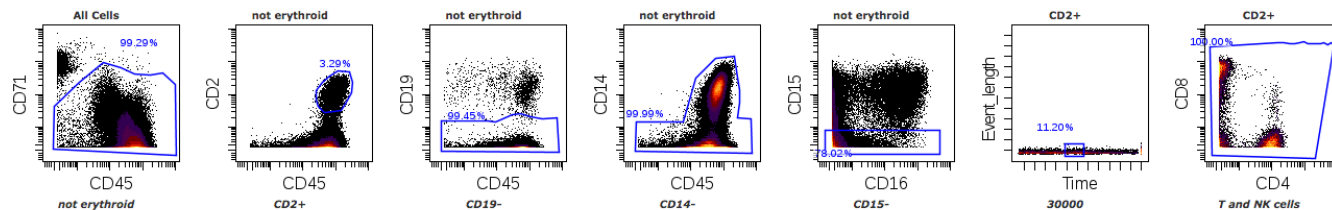
erythroids



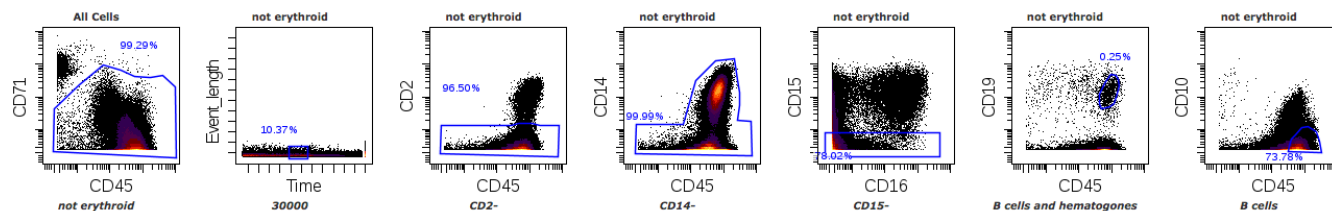
plasma cells



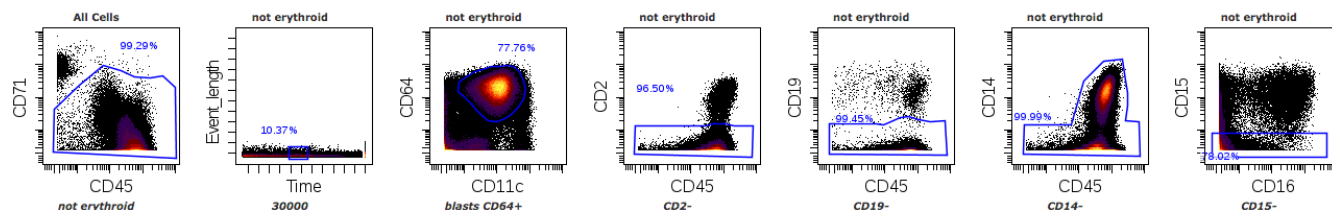
T and NK cells



B cells

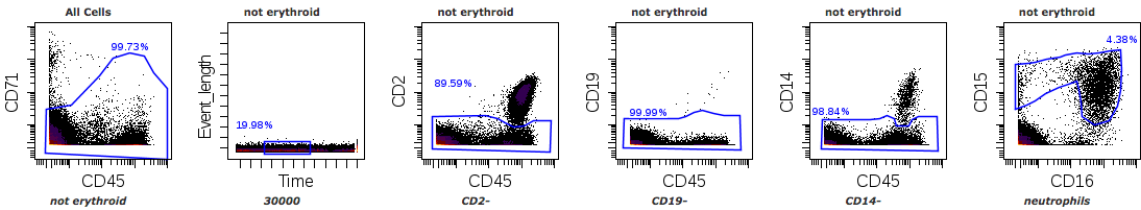


blasts CD64+

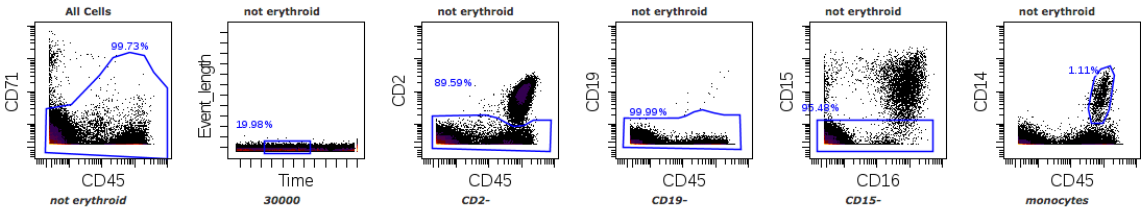


Supplementary Figure 1f

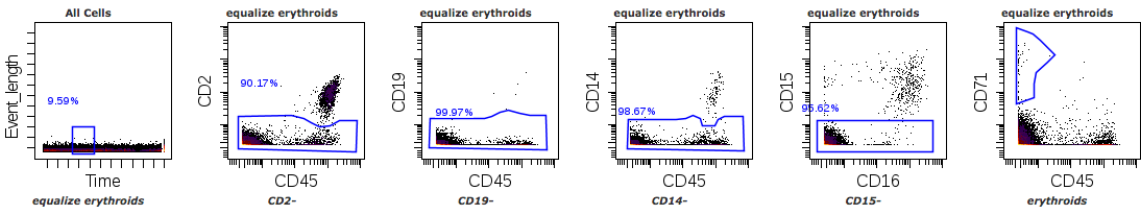
neutrophils



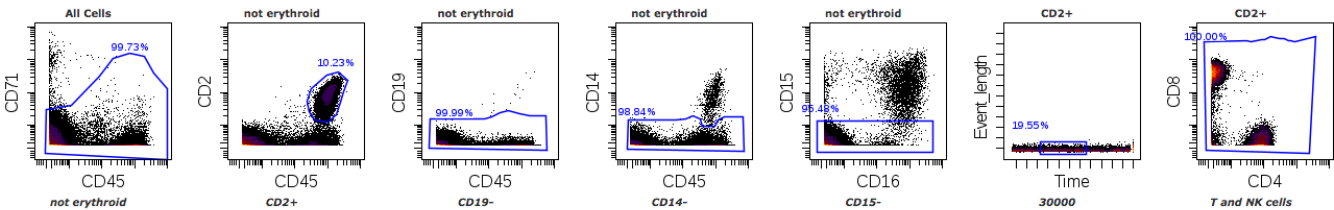
monocytes



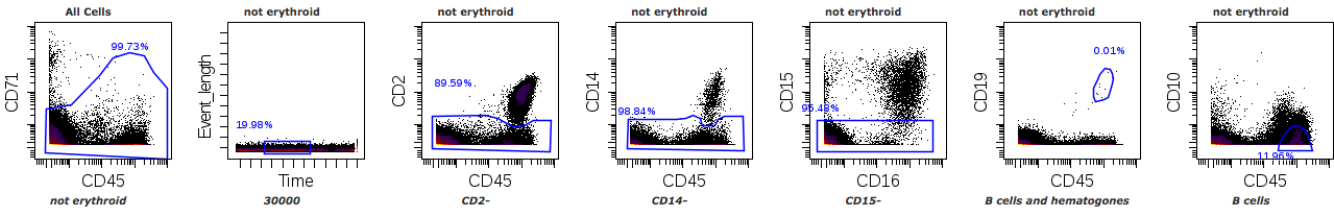
erythroids



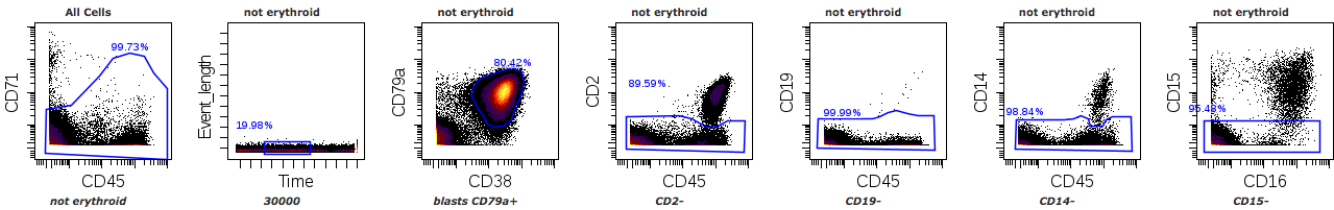
T and NK cells



B cells

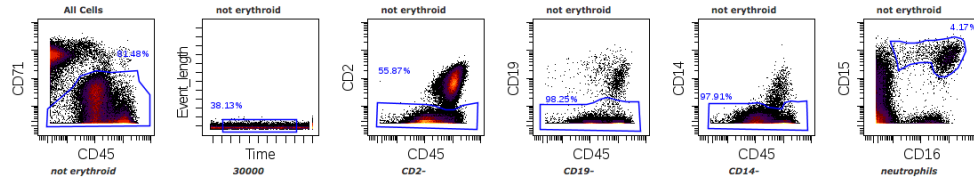


blasts CD79a+

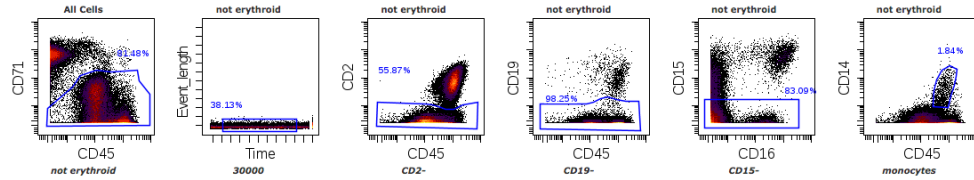


Supplementary Figure 1g

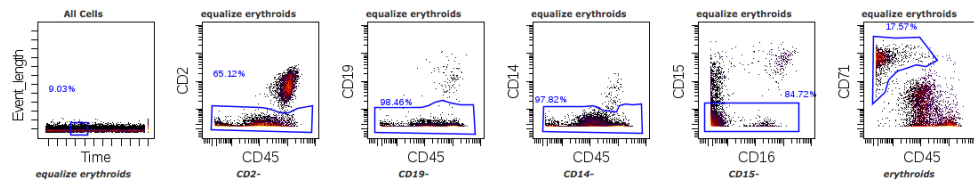
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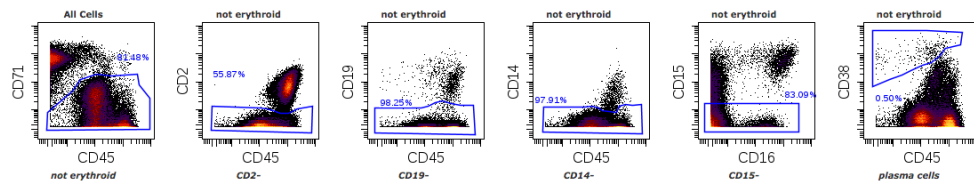
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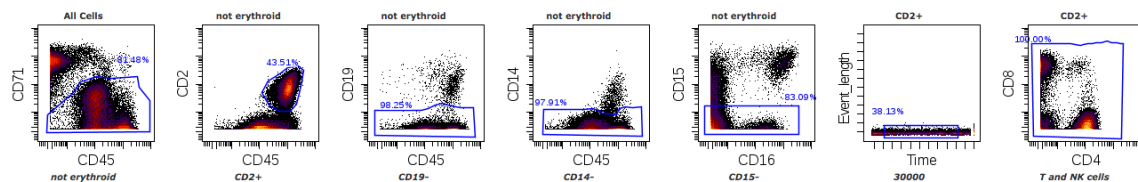
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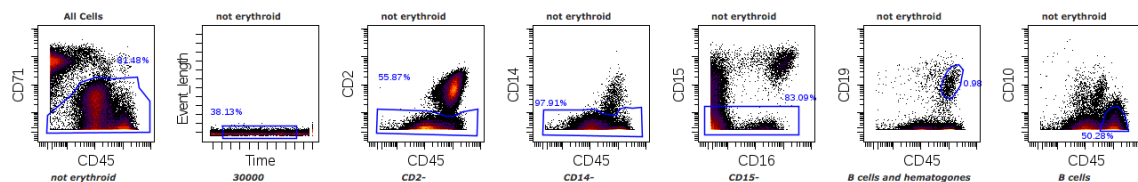
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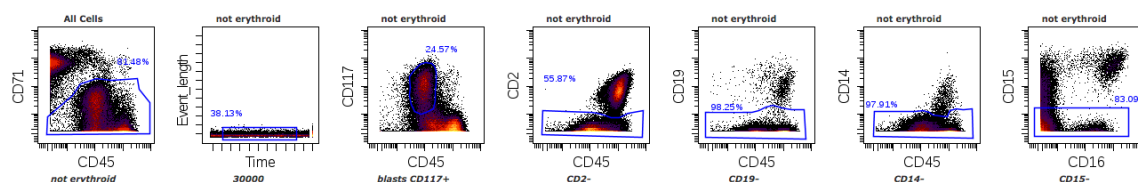
T and NK cells



B cells

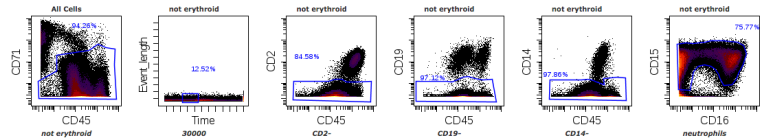


blasts CD117+

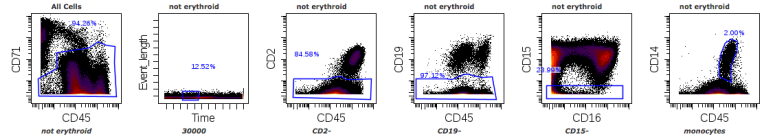


Supplementary Figure 1h

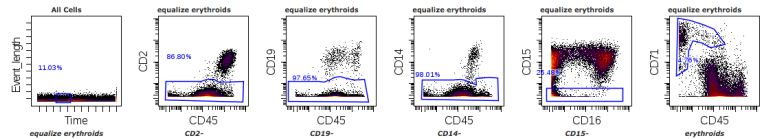
neutrophils



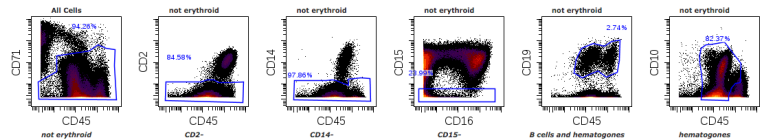
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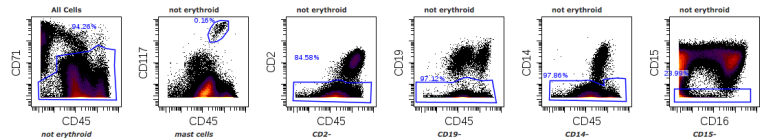
erythroids



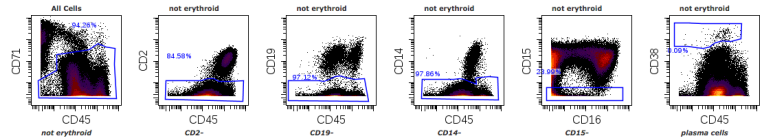
hematogones



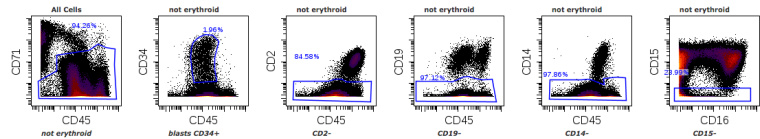
mast cells



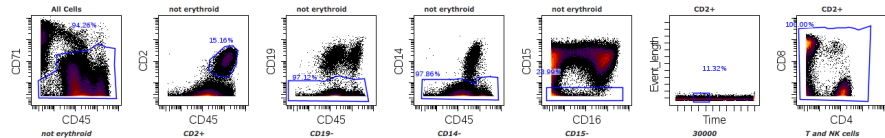
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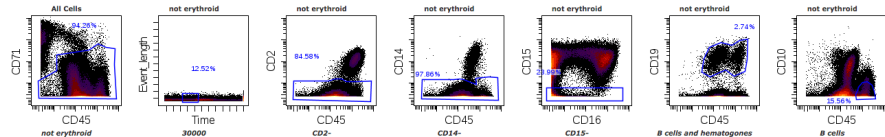
blasts CD34+



T and NK cells

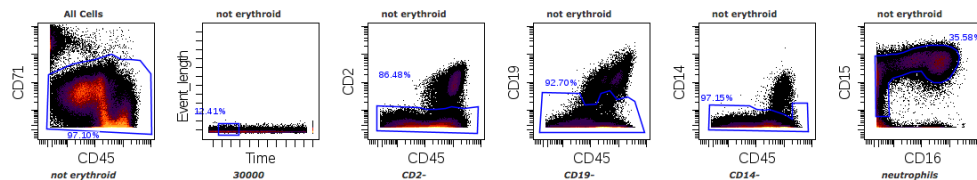


B cells

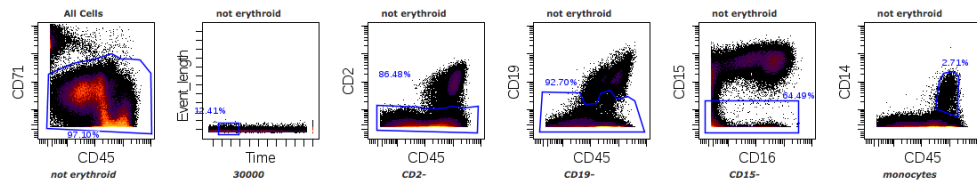


Supplementary Figure 1i

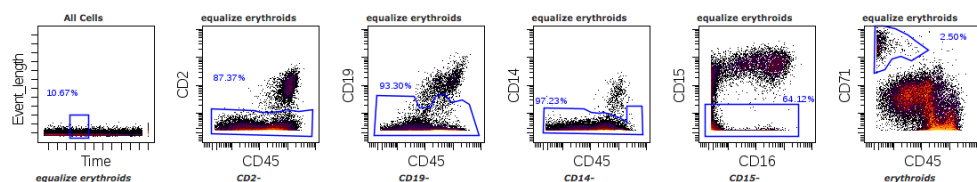
neutrophils



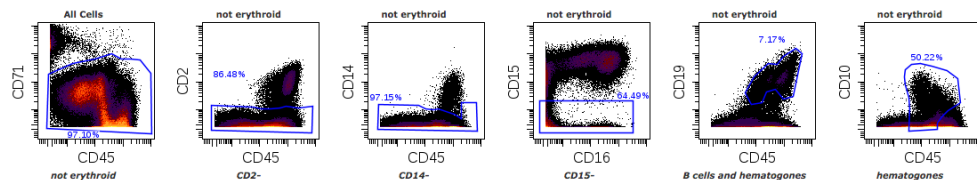
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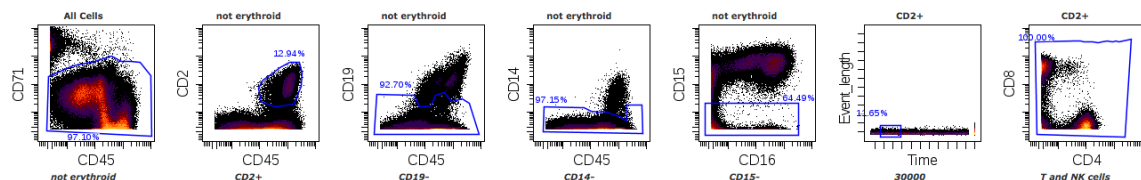
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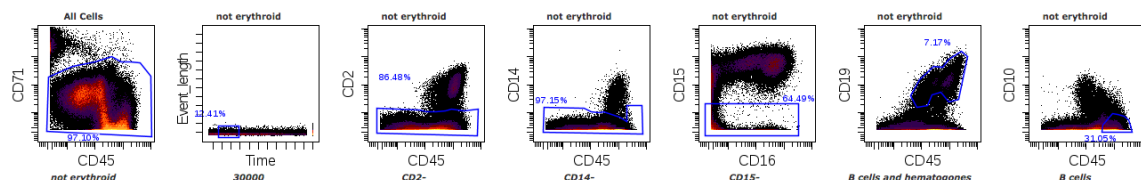
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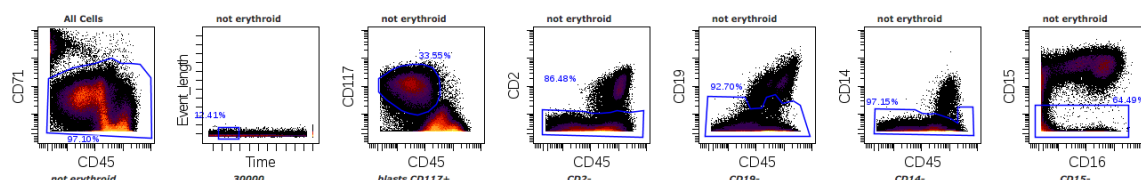
T and NK cells



B cells

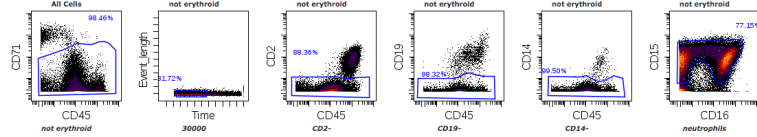


blasts CD117+

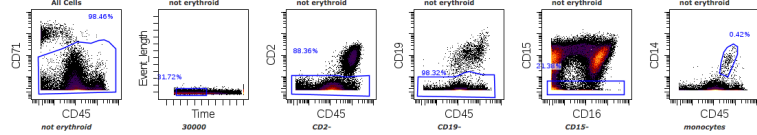


Supplementary Figure 1j

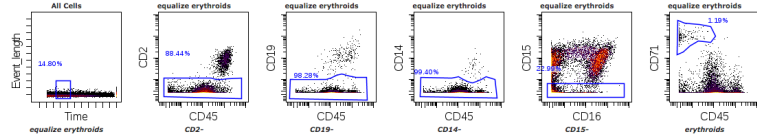
neutrophils



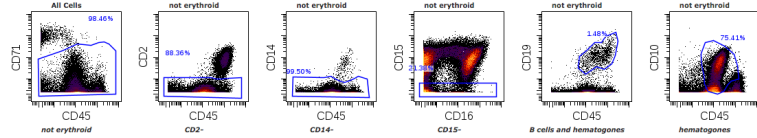
monocytes



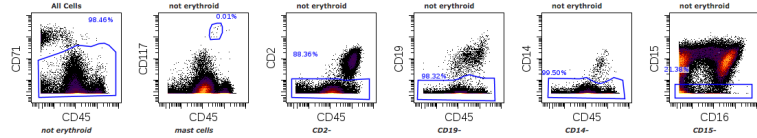
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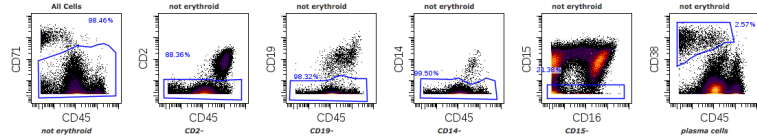
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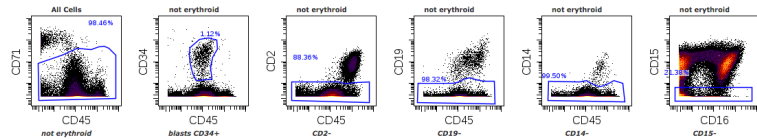
mast cells



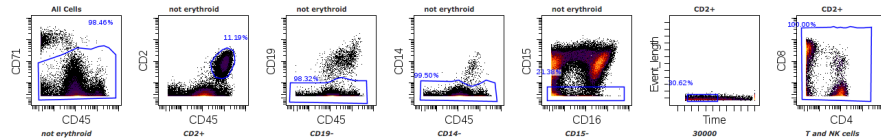
plasma cells



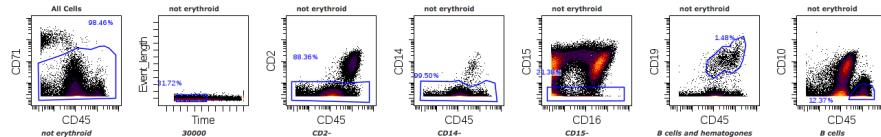
blasts CD34+



T and NK cells

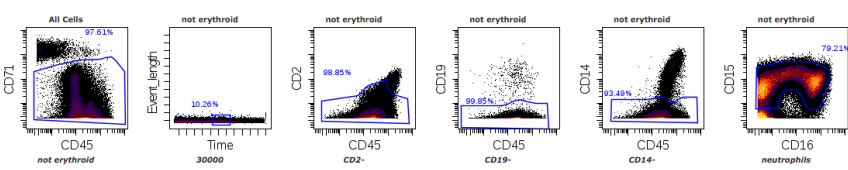


B cells

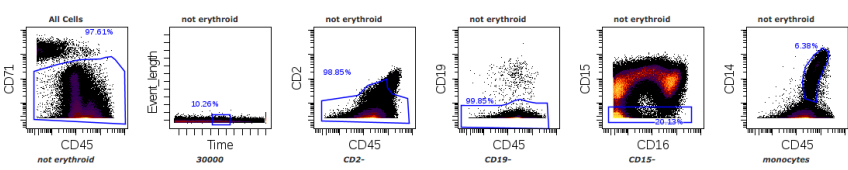


Supplementary Figure 1k

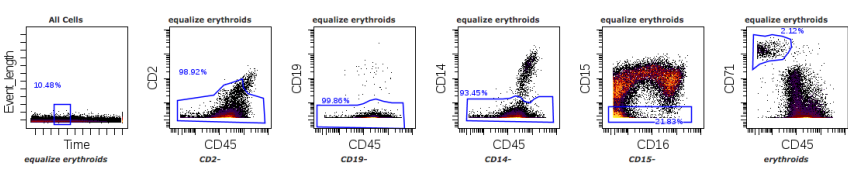
neutrophils



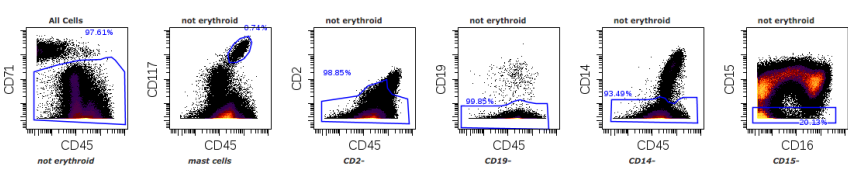
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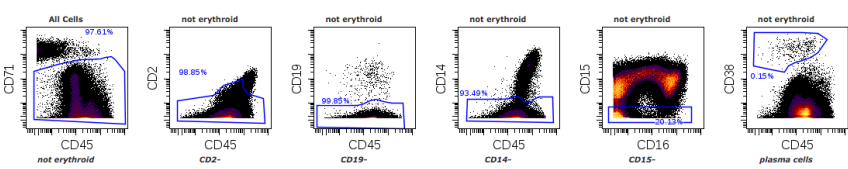
erythroids



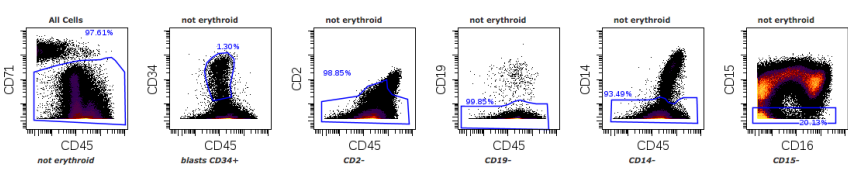
mast cells



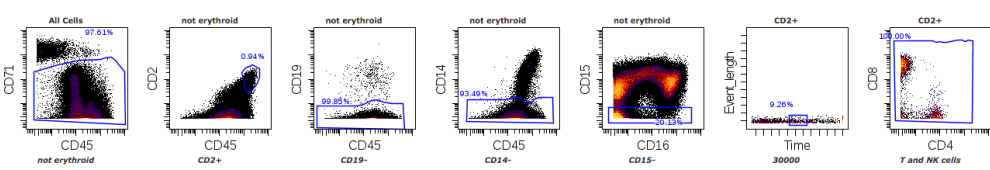
plasma cells



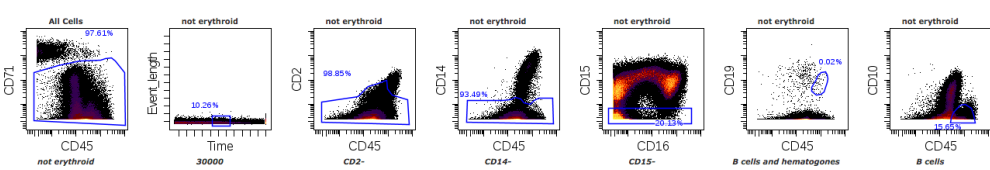
blasts CD34+



T and NK cells

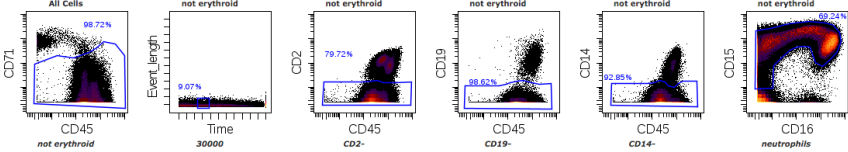


B cells

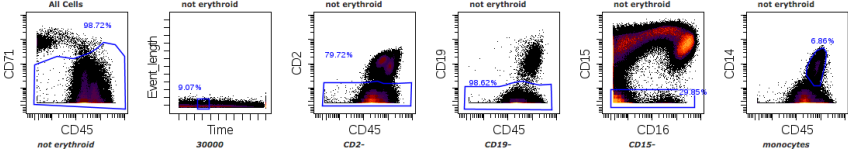


Supplementary Figure 11

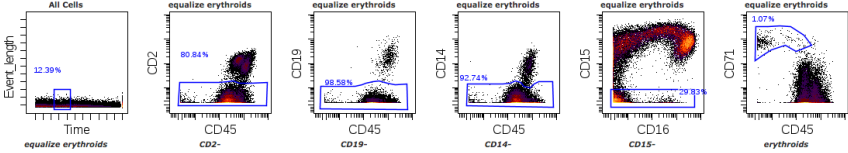
neutrophils



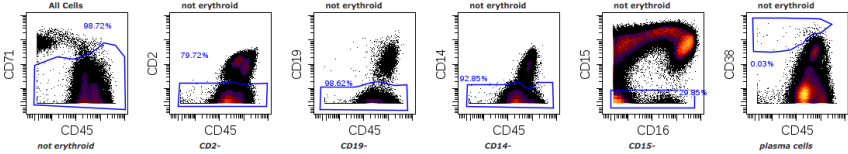
monocytes



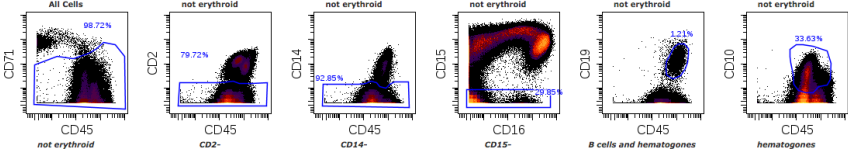
erythroids



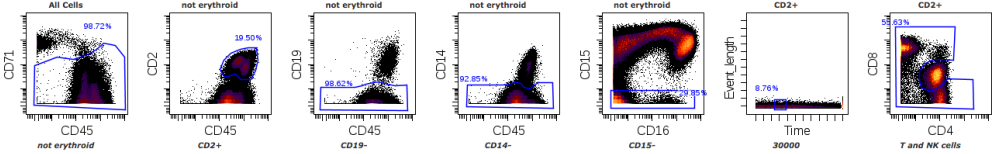
plasma cells



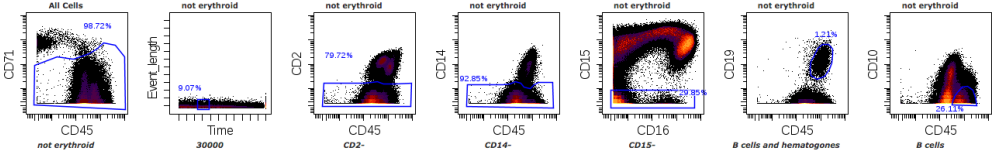
hematogones



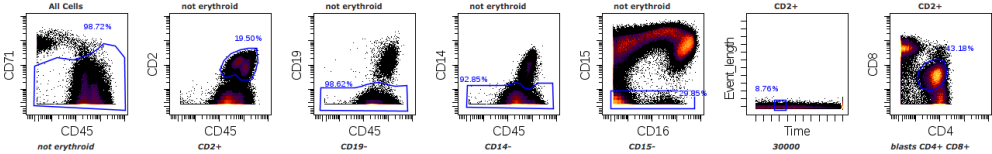
T and NK cells



B cells

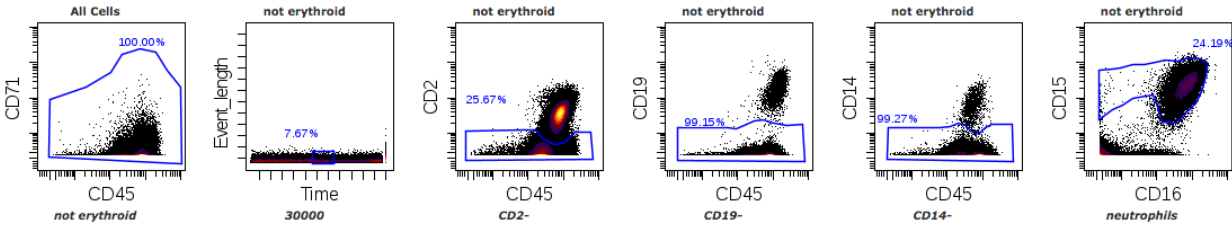


blasts CD4+ CD8+

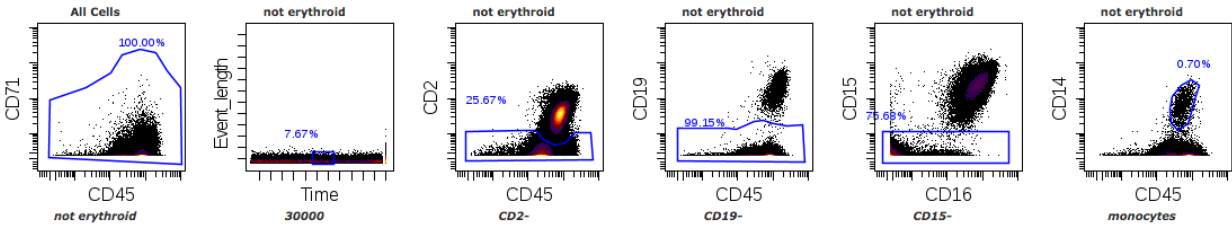


Supplementary Figure 1m

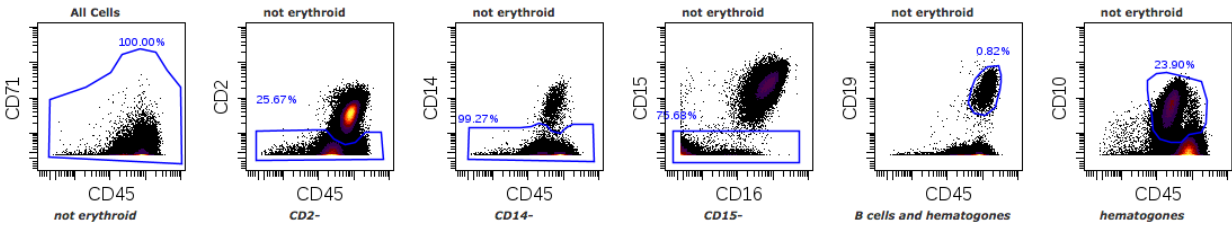
neutrophils



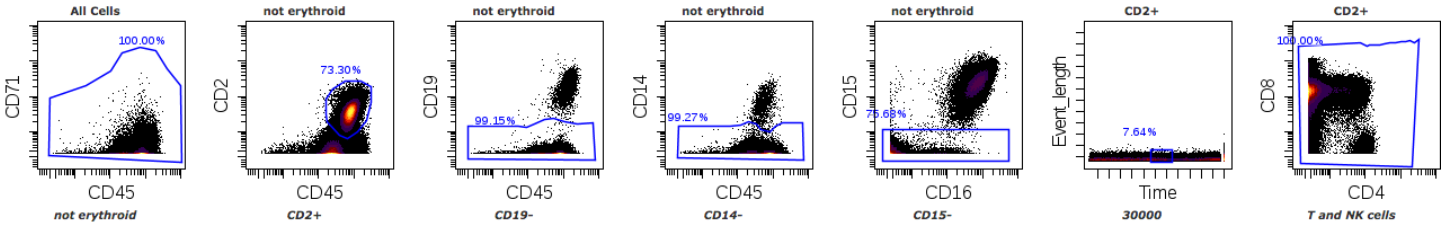
monocytes



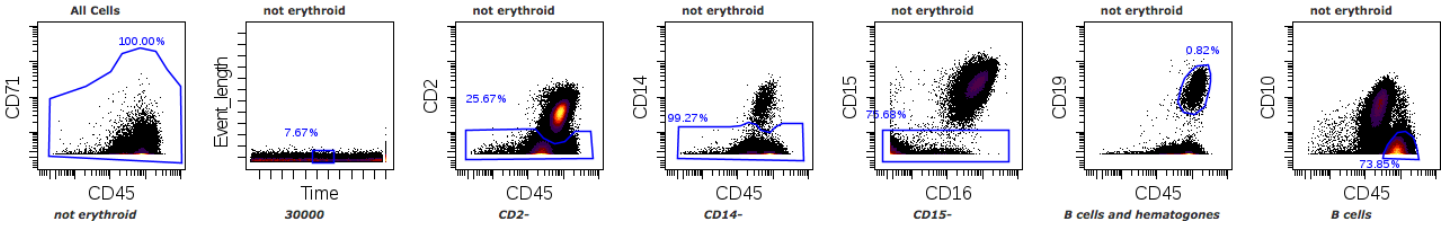
hematogones



T and NK cells



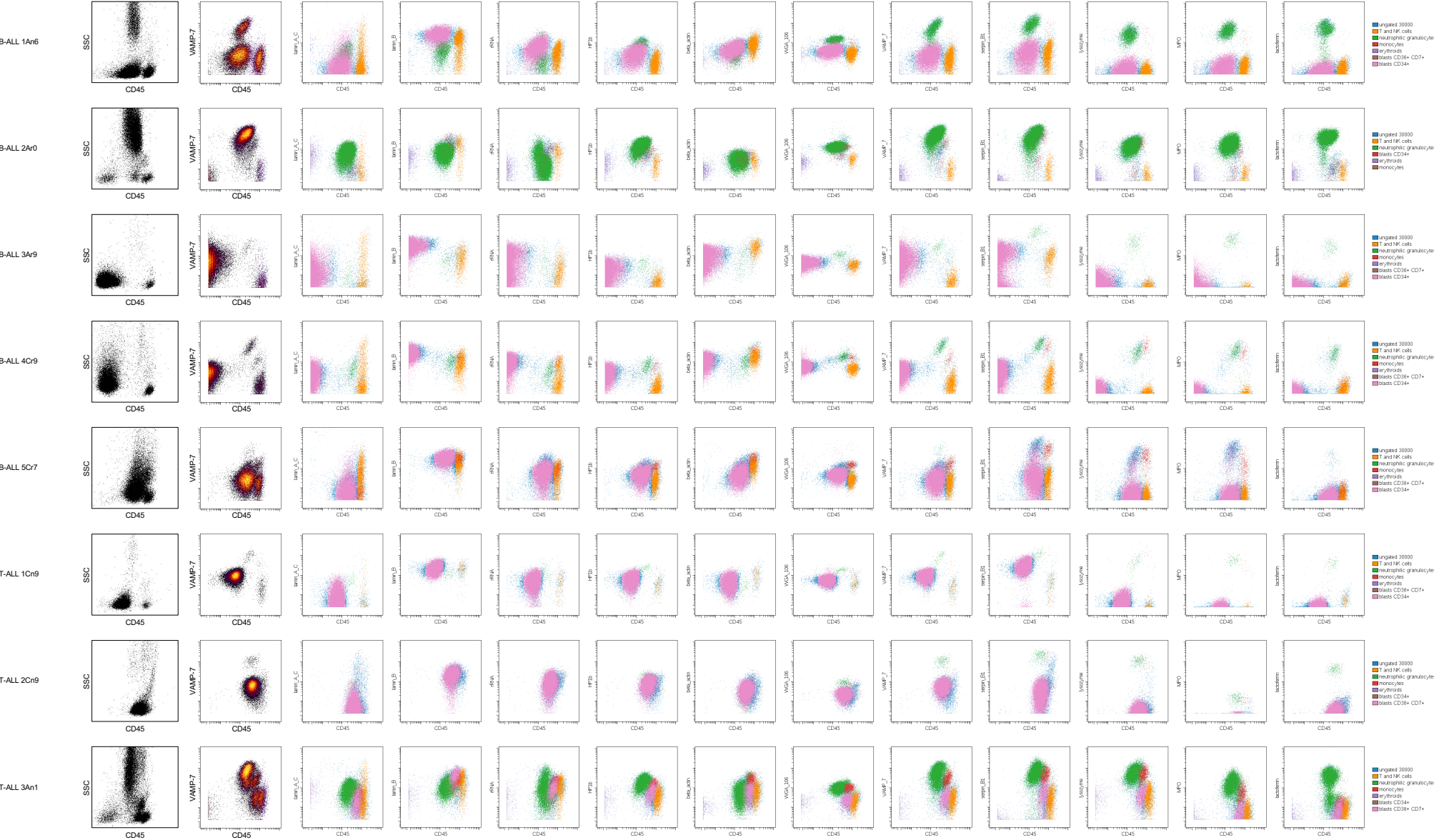
B cells



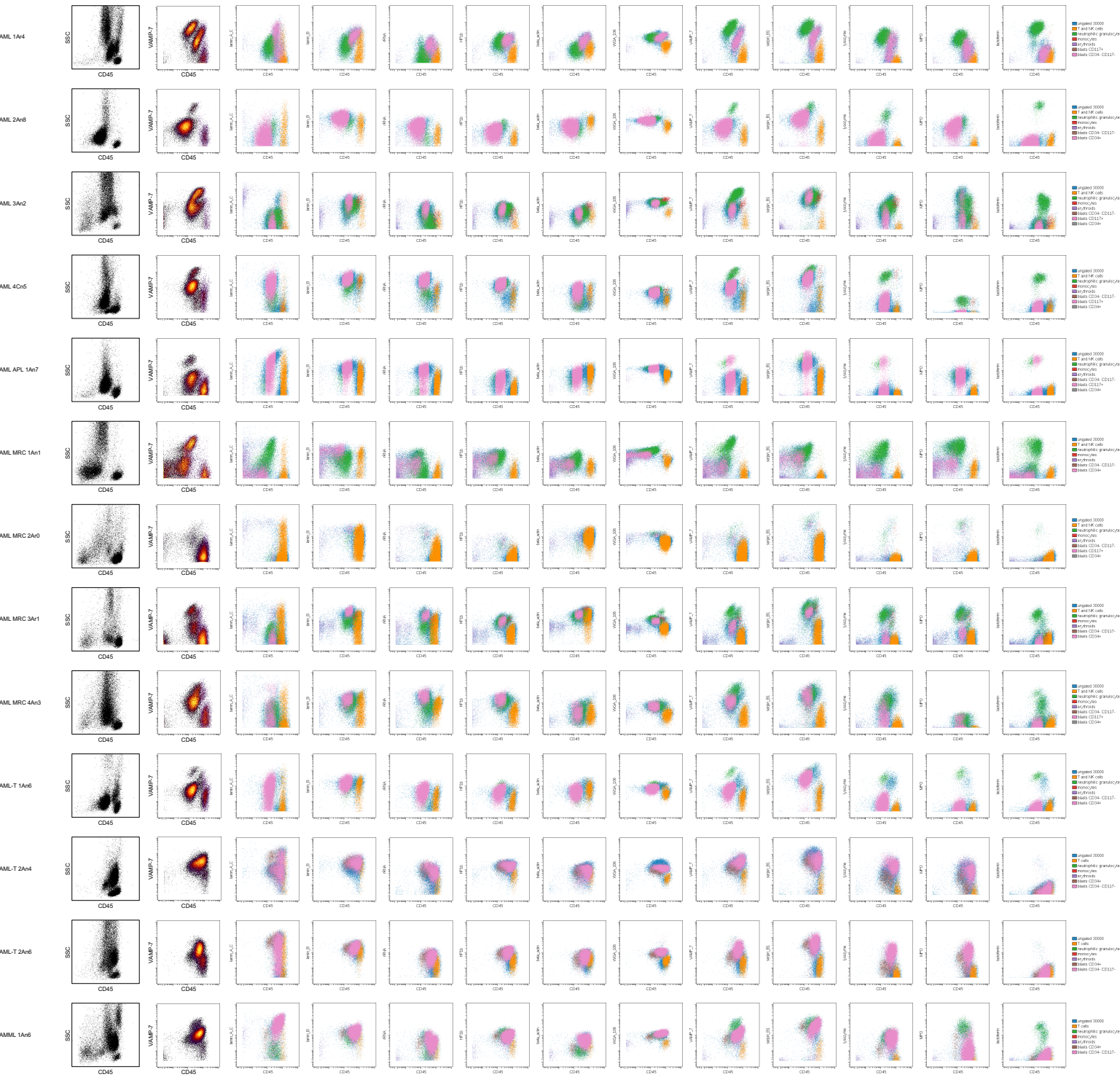
Supplementary Figure 1: Surface marker gating scheme for eleven exemplary samples

Gating strategy for eleven exemplary samples chosen for further processing and analysis. Major cell populations were gated *ad hoc* by surface marker expression using *a priori* knowledge of sample composition and population immunophenotypes. A) Healthy bone marrow 1. B) Healthy bone marrow 2. C) AML. D) APL. E) AMML. F) B-ALL. G) MDS-EB2. H) MDS-RS. I) MPAL. J) PCM. K) SM-CMML. L) T-ALL. M) TCL

Supplementary Figure 2a



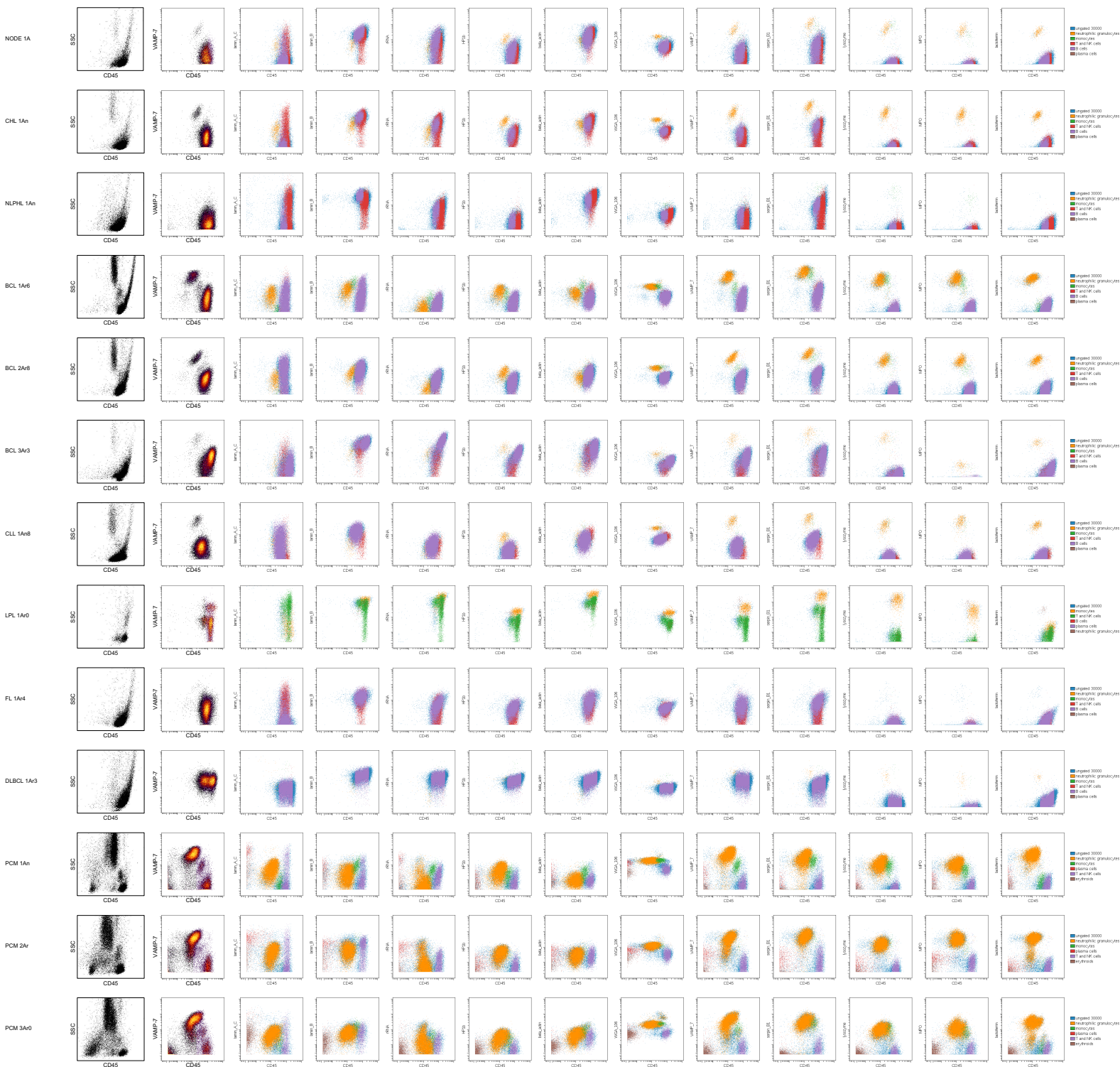
Supplementary Figure 2b



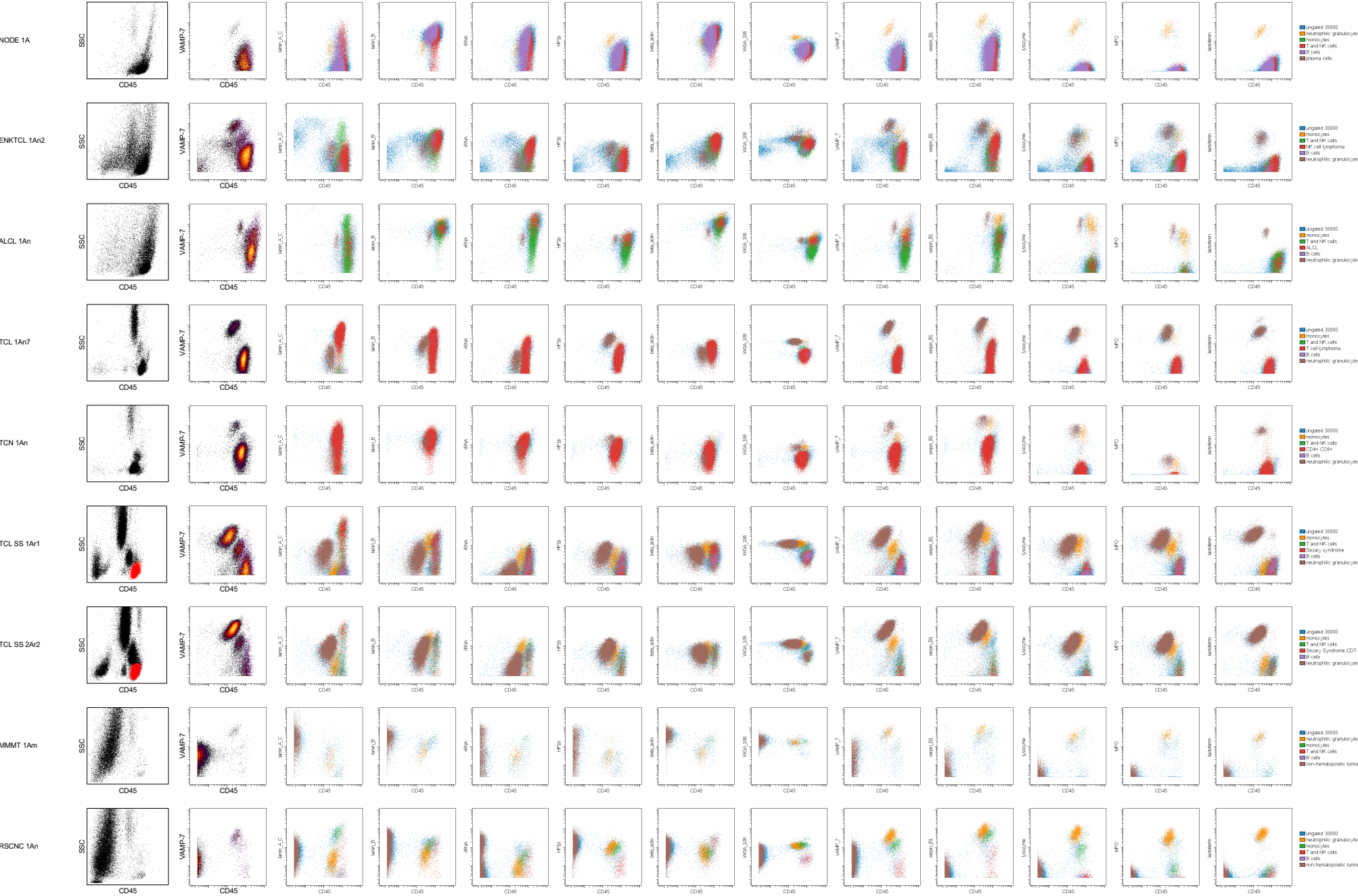
Supplementary Figure 2c



Supplementary Figure 2d



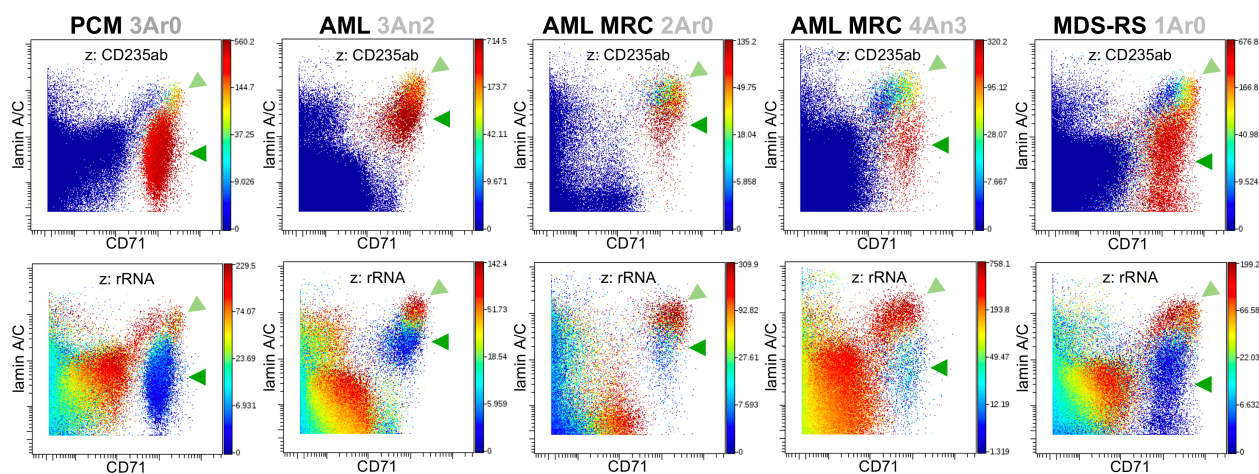
Supplementary Figure 2e



Supplementary Figure 2: Scatterbody vs. CD45 plots for the initial set of 54 clinical samples

Major cell populations for the initial set of 54 clinical samples were gated *ad hoc* by surface marker expression using *a priori* knowledge of sample composition and population immunophenotypes. For reference, the ungated clinical side scatter vs. CD45 plot is provided, followed by an ungated density plot of VAMP-7 vs. CD45. Then, the gated populations were overlaid on scatterbody vs. CD45 plots for each scatterbody, colored by the population keys to the far right. Each sample was only run once and no samples were discarded. AML-T 2An6 and AML-T 2An4 are peripheral blood and bone marrow, respectively, from one patient. A) Acute lymphoid leukemias. B) Acute myeloid leukemias. C) Acute leukemias of ambiguous lineage and chronic myeloid neoplasms. D) Mature B cell and plasma cell neoplasms. E) Mature T and NK cell neoplasms and non-hematopoietic tumors.

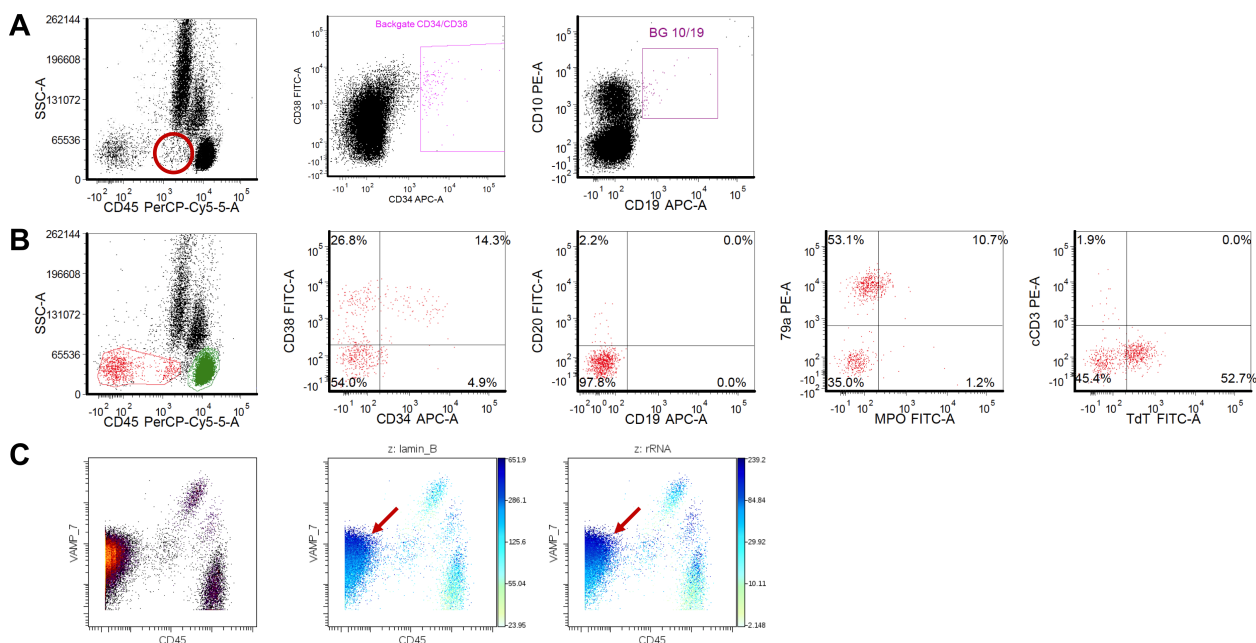
Supplementary Figure 3



Supplementary Figure 3: Lamin A/C in evaluating erythroid dysplasia

Lamin A/C vs. CD71 is plotted for five samples, the top row z-colored by CD235ab and the bottom by rRNA. In these plots, CD71 separates the erythroids from the other marrow elements, with the light green arrow representing the more immature, dim CD45⁺ variably CD235⁺ brightly Lamin A/C⁺ population and the dark green the more mature, CD45⁻ CD235⁺ moderately Lamin A/C⁺ population. The populations in the PCM 3Ar0 sample are normal. Each sample was only run once.

Supplementary Figure 4

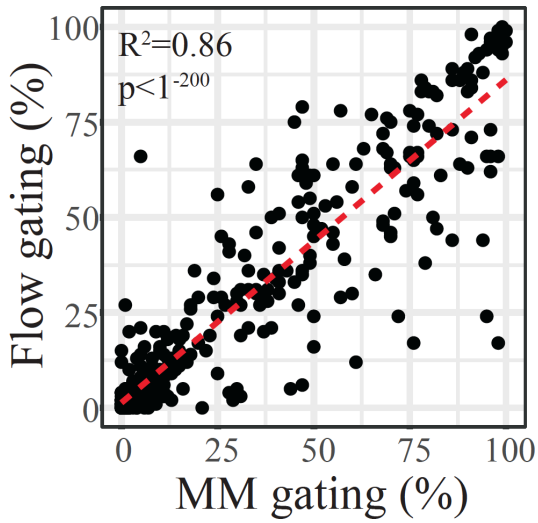


Supplementary Figure 4: Lamin B1 and rRNA in identifying blasts

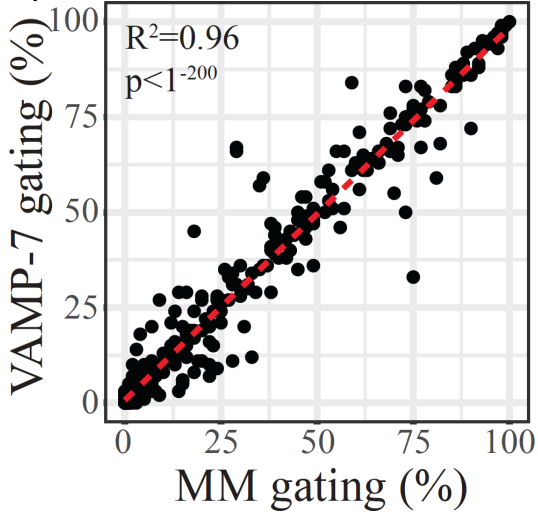
A) Diagnostic flow cytometry was performed on peripheral blood from a patient with a history of B-ALL, status post anti-CD19 CAR-T therapy. The initial blast gate (red circle) and backgating strategy did not identify the tumor. B) Regating and additional tubes revealed the CD79a⁺ Tdt⁺ tumor. C) As very little viable peripheral blood sample remained for testing, CyTOF was performed on the subsequent bone marrow. Lamin B1 and rRNA clearly identify the blast population (red arrows), independent of surface antigens. Lamin B1 quantitation on blasts was performed for the 39 initial samples with blast populations >20 events, with statistical analysis shown in **Fig. 3E**. Lamin B1 and rRNA z-coloring are also demonstrated for the other 10 clinical samples and two normal marrows analyzed in-depth in the source data plots.

Supplementary Figure 5

A)



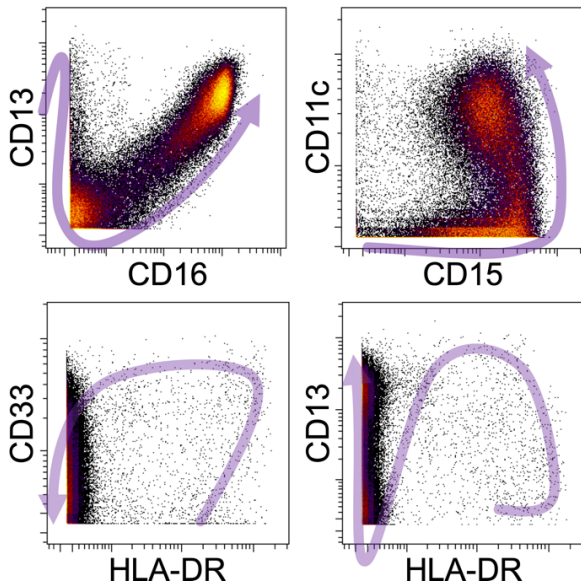
B)



Supplementary Figure 5: SSC, VAMP-7, and MM gating are highly comparable

Percent of events positive for each marker in every daughter plot across eleven samples generated by parent gating using A) MM gating (x-axis) or flow (y-axis) cytometry and B) MM gating (x-axis) or VAMP-7 (y-axis). Correlation was evaluated by the Pearson method. $n=11$ biologically independent samples, 22 independent cell populations, 483 independent axes. A simple linear regression model was generated to determine R^2 and p-values.

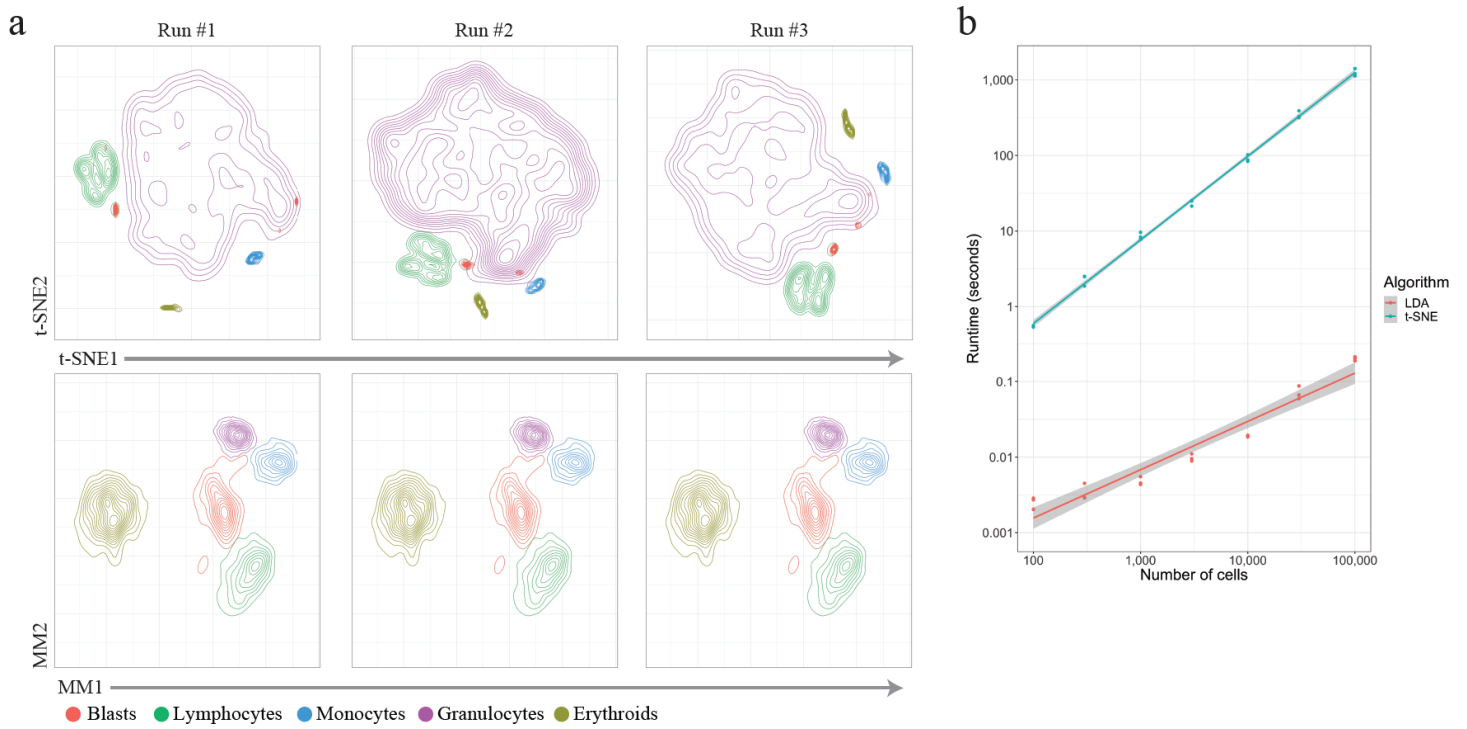
Supplementary Figure 6



Supplementary Figure 6: Trajectory of normal neutrophil maturation on biaxial plots of standard surface markers

Normal bone marrow run on CyTOF was gated on neutrophilic granulocytes based on VAMP-7 vs. CD45, and plotted with the purple trajectory based on Kussick *et al.*¹⁴ Early time points (HLA-DR+, bottom row) are sparsely cellular. Patterns were similar for both healthy marrows from the initial set 1 and the three reactive marrows from set 2 (n=5 biologically independent samples) and have been demonstrated previously.¹⁴

Supplementary Figure 7



Supplementary Figure 7: Morphometric axes are reproducible, interpretable, and computationally inexpensive

A) t-SNE plots and MM axes generated from three separate runs using the same 10,000 cells randomly sampled from two healthy controls. B) t-SNE (green) and LDA (red) was run on varying cell numbers with six scatterbodies as inputs (three replicates per cell count). Reported runtimes are based on performance on an Apple laptop. Best fit line and 95% CI are plotted over raw data (dots).

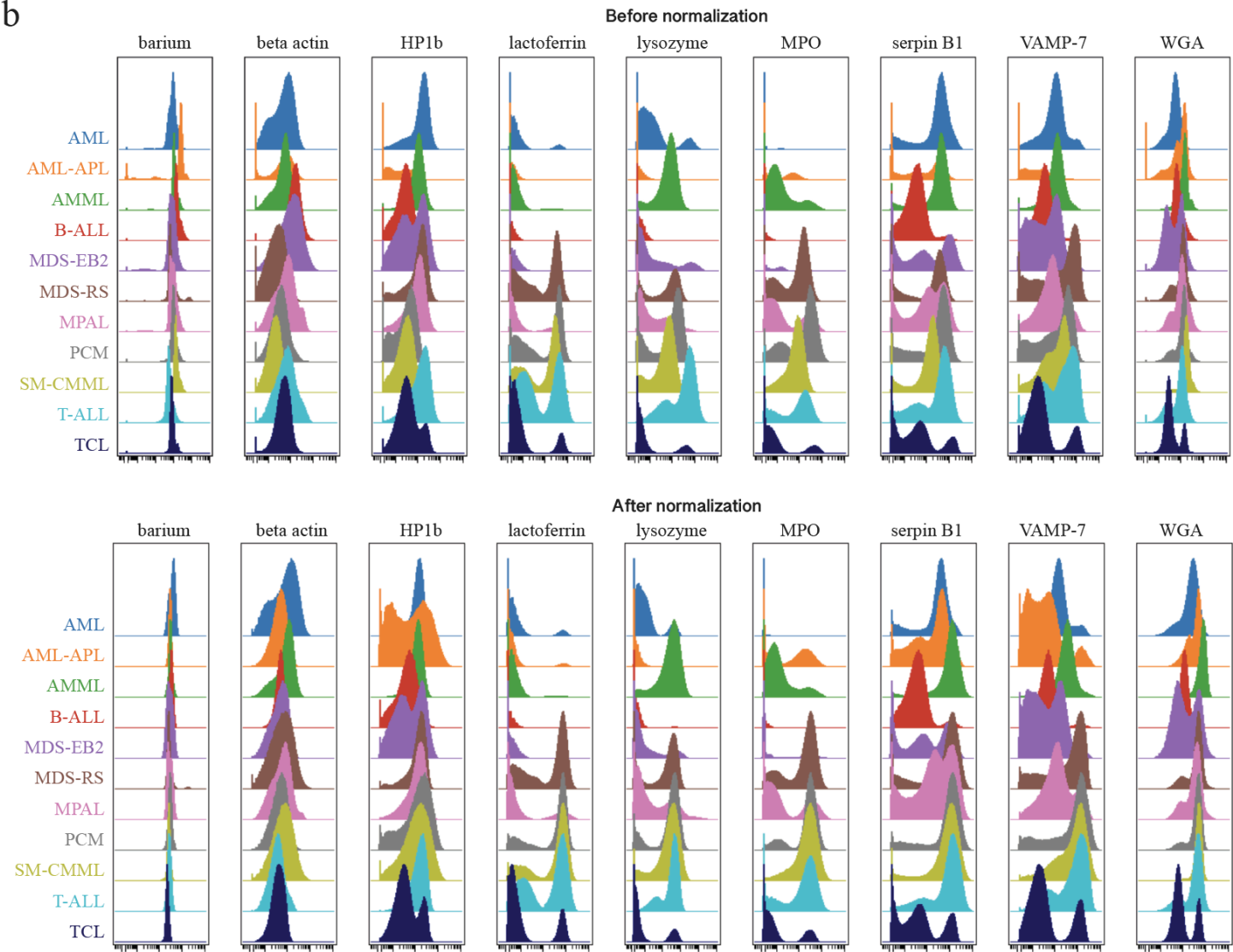
Supplementary Figure 8

a

Population aligned for each scatterbody

Scatterbody	barium	beta actin	HP1b	lactoferrin	lysozyme	MPO	serpin B1	VAMP-7	WGA	lamin A/C	lamin B	rRNA
Population	monocytes	monocytes	monocytes	neutrophils	neutrophils	neutrophils	neutrophils	neutrophils	neutrophils	none	none	none

b



Supplementary Figure 8: Scatterbody normalization

A) Table of hematopoietic population used for normalization of each scatterbody. B) Histograms of ungated singlets before (top) and after (bottom) normalization from eleven representative samples. Each sample was only run once.