



# One tube to find them all

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OMIP



## OMIP 077: Definition of all principal human leukocyte populations using a broadly applicable 14-color panel

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# Aims

Describe the complete immune landscape in blood

What do we want to know?

What type of immune cell?

How many?

Situation *in vivo*?

What do we need to know?

# Leucocyte populations

- Neutrophils
  - 60%, not in PBMC
- Eosinophils
  - < 5%
- Basophils
  - < 1%
- Monocytes
  - 5%, subsets
- T cells
  - 30%
- B cells
  - 5%, incl. Plasma cells
- NK cells
  - 5%, subsets
- Dendritic cells
  - 1%, Various populations
- Progenitors cells
  - 0.02%



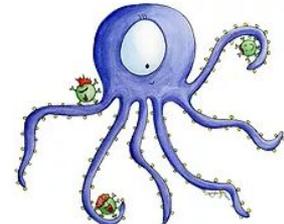
NK Cell



Cytotoxic T Cell



Helper T Cell



Follicular Dendritic Cell



Macrophage



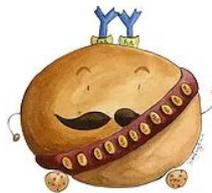
Treg



B Cell



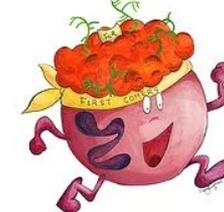
Plasma Cell



Mast Cell



Basophil



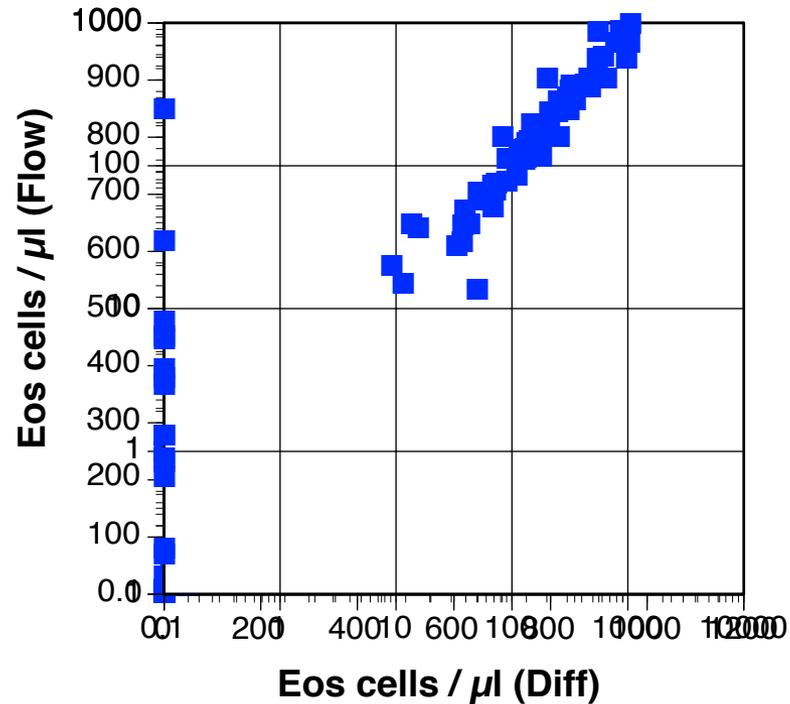
Neutrophil



Eosinophil

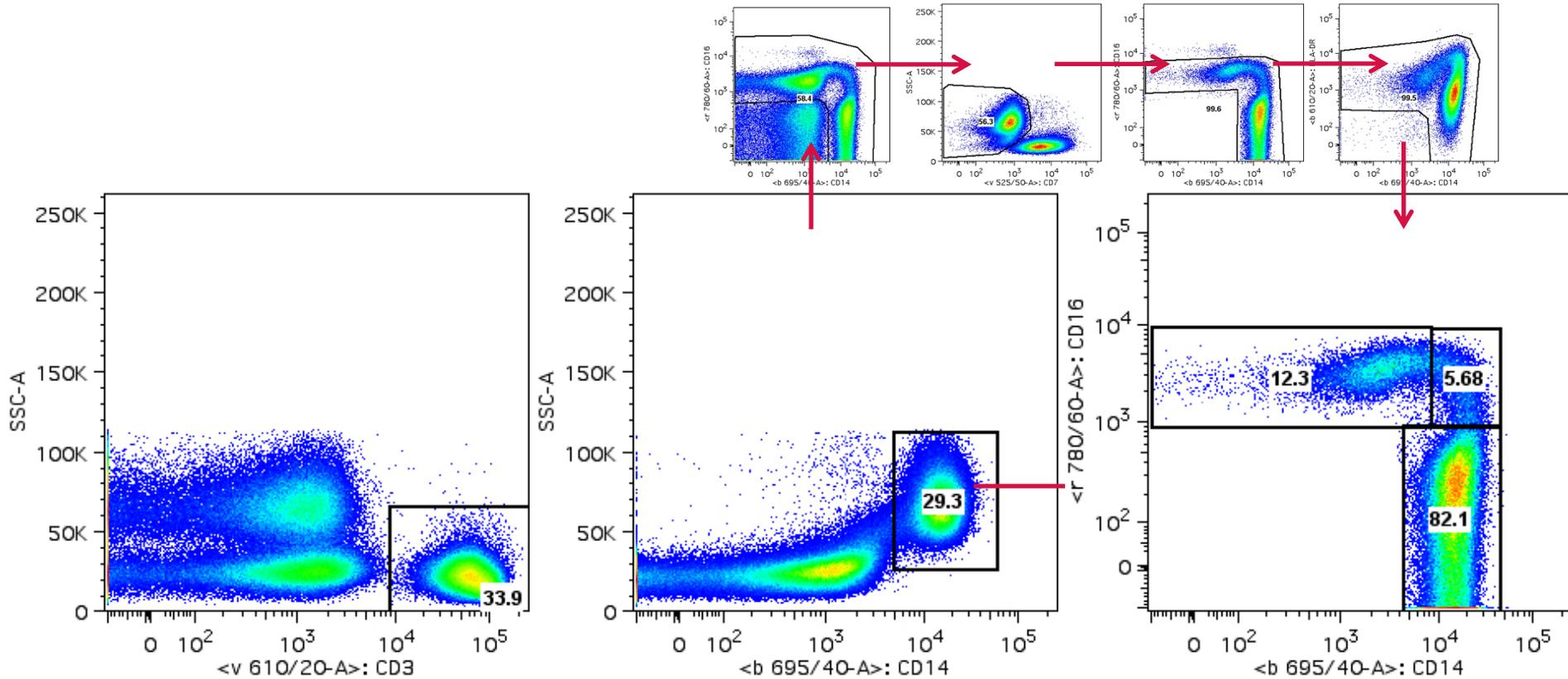
# Leucocyte populations

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# What do we need to know?

Antigens to define the population of interest



Expression of antigens on other cell populations

# Antigen expression

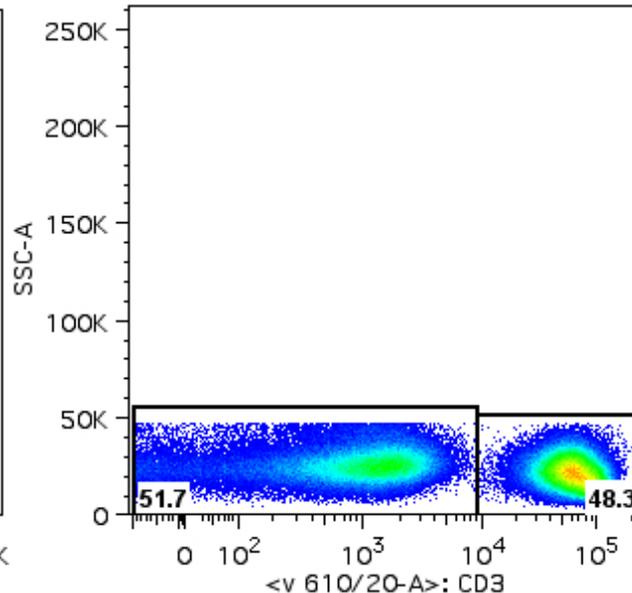
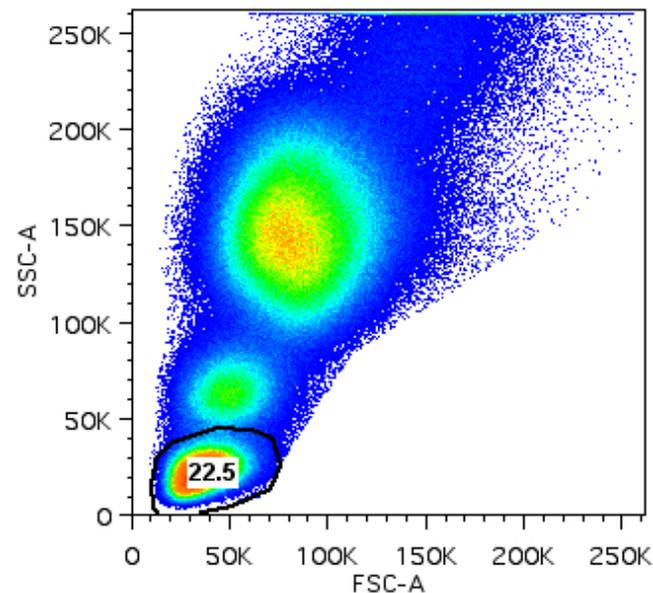
	SSC	Auto-fluor	CD1c	CD3	CD14	CD15	CD16	CD19	CD34	CD38	CD45	CD56	CD123	CD141	CD193	HLA-DR
<b>Eosinophils</b>	++++	+++				+					++				+	
<b>Neutrophils</b>	+++	+				+	+++				++					
<b>Basophils</b>	+									+	++		+		+	
<b>Monocytes (c)</b>	++	+			++						++					+ / ++
<b>Monocytes (i)</b>	++	+			++		+				++					+++
<b>Monocytes (nc)</b>	+	+			±		++				+++					++
<b>Progenitor cells</b>									+		+					+
<b>T cells</b>				+						(+)	+++	(+)			(+)	(+)
<b>B cells</b>			(+)					++		(+)	+++					+++
<b>Plasma cells</b>	+							+		++	++					+
<b>NK cells</b>	+						(+)			+	+++	+				
<b>pDCs</b>											++		+			+++
<b>CD1c<sup>+</sup> mDCs</b>			+								++					+++
<b>CD141<sup>+</sup> mDCs</b>											++			+		+++

# What do we need to know?

Abundance of population of interest

Flow cytometry usually gives only relative numbers

For lymphocytes (T-, B-, NK- cells) FSC/SSC defined population as denominator



T cells	48,3
B cells	7,28
NK cells	37,1
Monocytes	4,2
Basophils	1,49
Progenitors	0,47
Plasma cells	0,41
pDC	0,32
mDC1	0,22
mDC2	0,13
Total	99,92

# What do we need to know?

Absolute numbers

Only possible with whole blood

Two platform method relative values of FC combined with differential blood count

Differences in the denominator, combined variability, influence of abundant populations

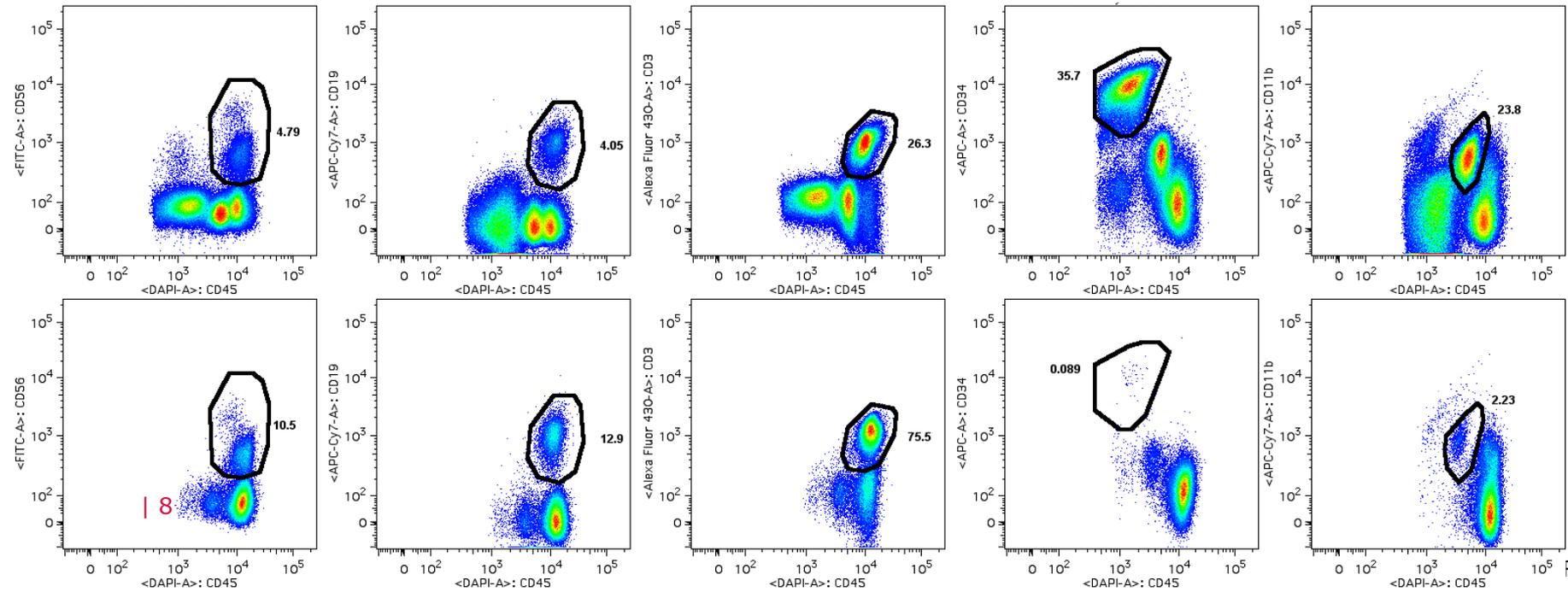
**NK cells**

**B cells**

**T cells**

**progenitors**

**basophils**



# General method

Whole blood

Trucount tubes for absolute enumeration

lyse / no wash (one centrifugation)

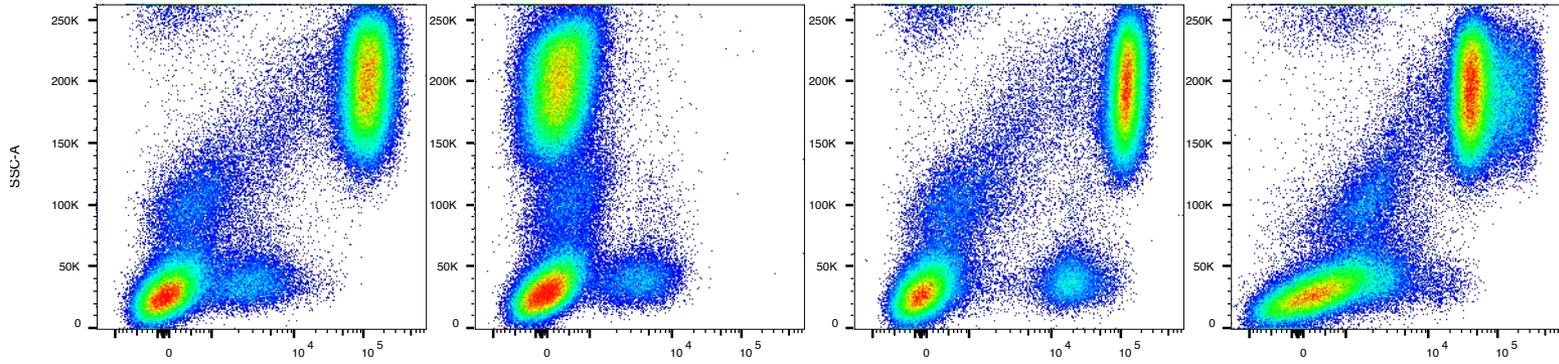
# Whole blood : PBMC

**3G8**

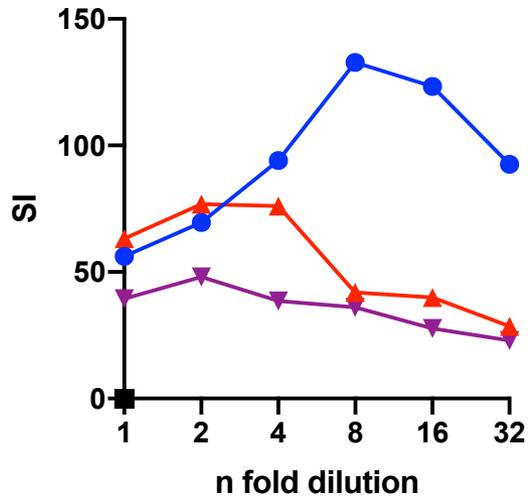
**B73**

**CB16**

**LNK16**



CD16 →



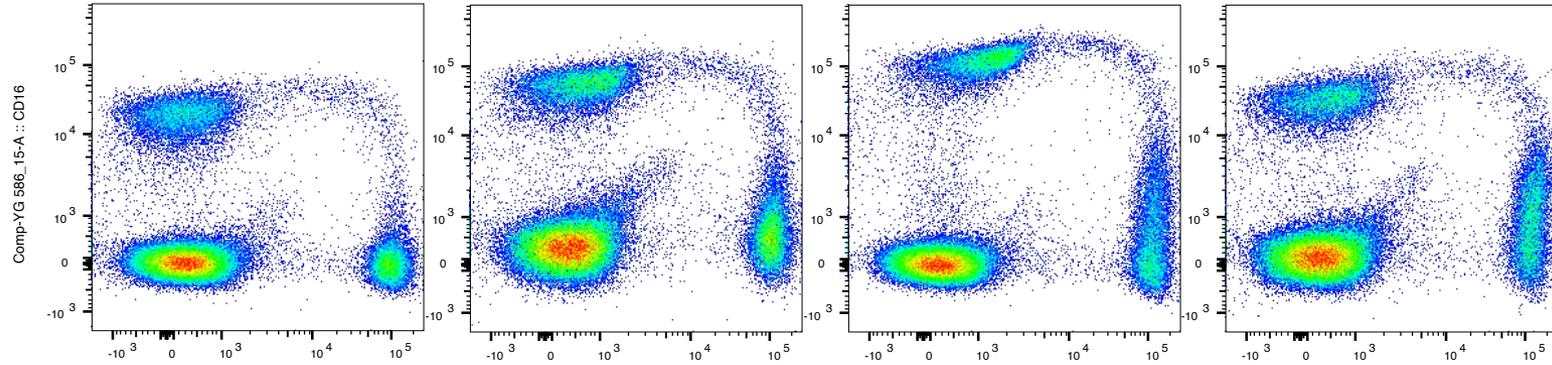
# Whole blood : PBMC

**3G8**

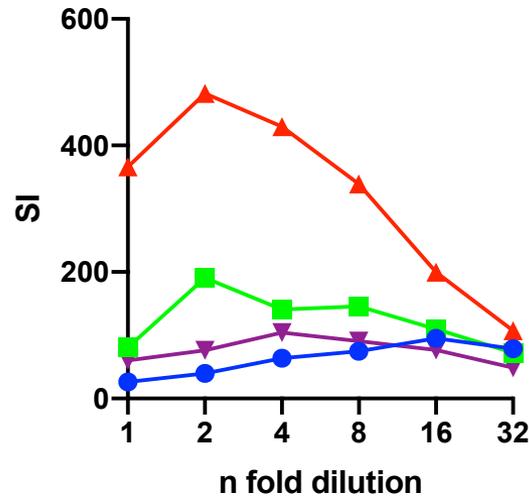
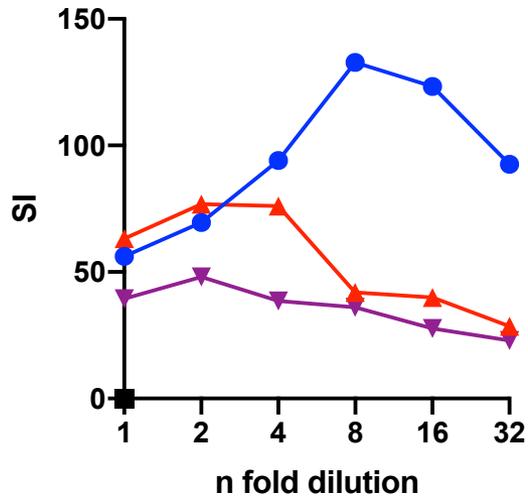
**B73**

**CB16**

**LNK16**



**CD14** →



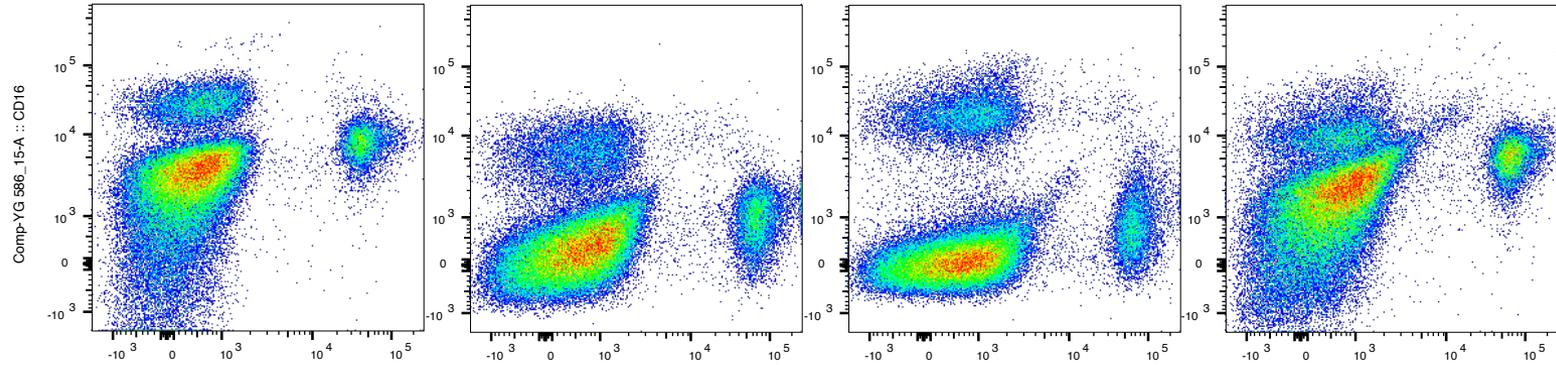
# Whole blood : PBMC

**3G8**

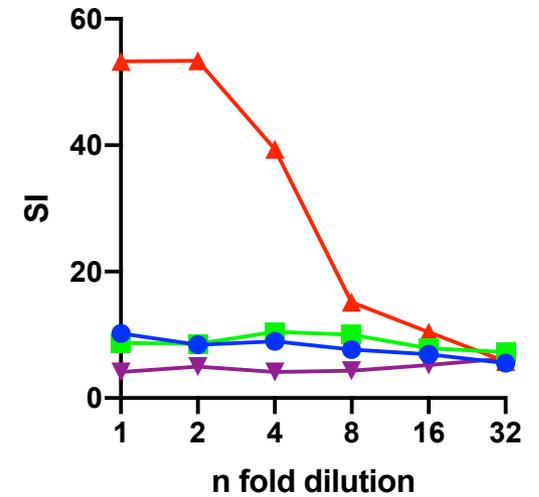
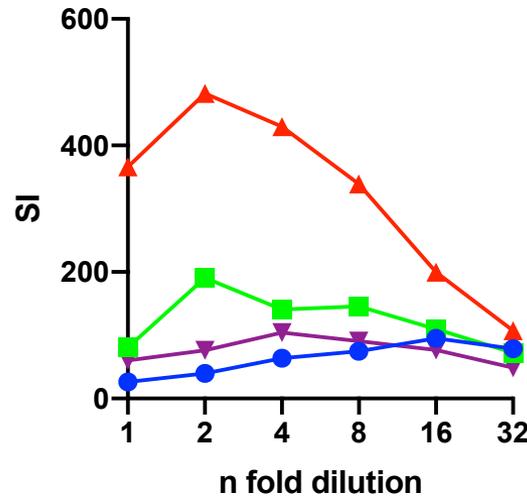
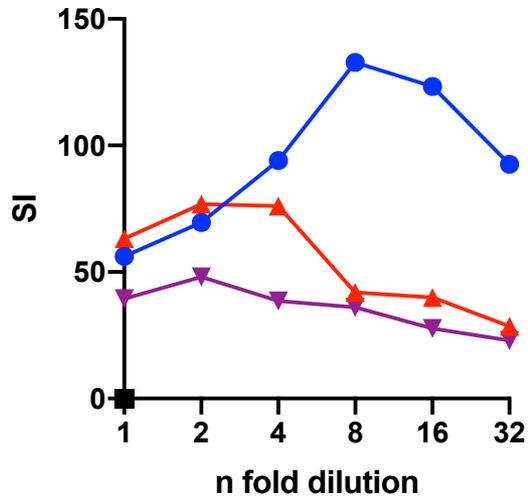
**B73**

**CB16**

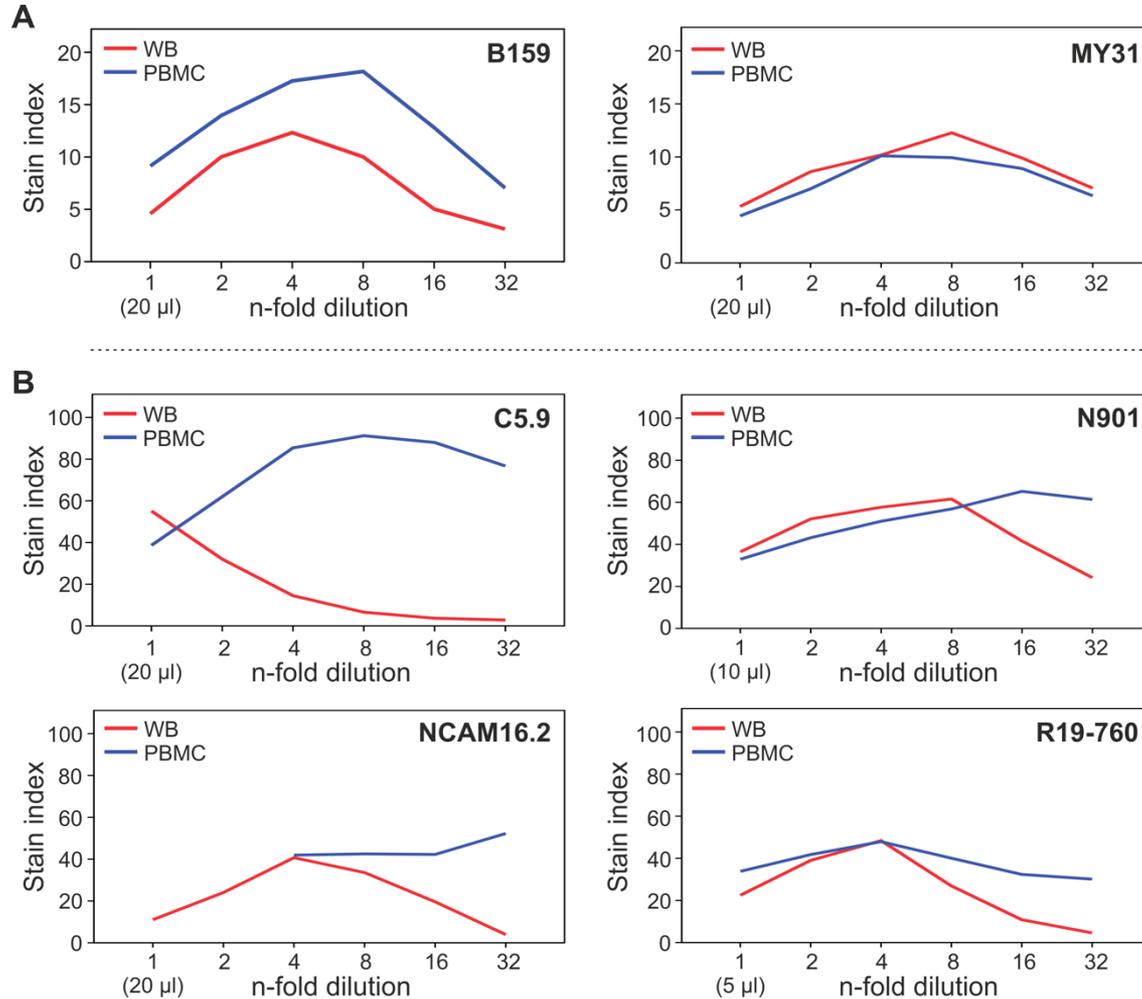
**LNK16**



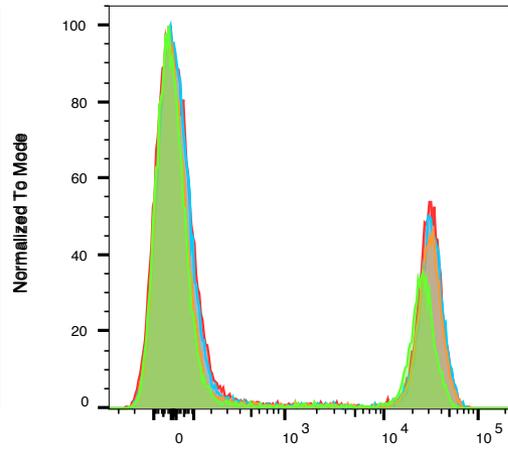
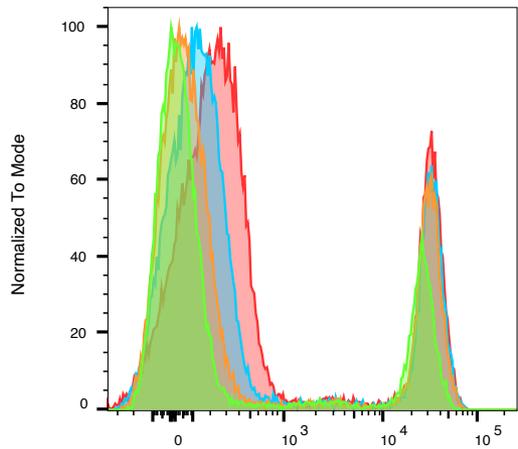
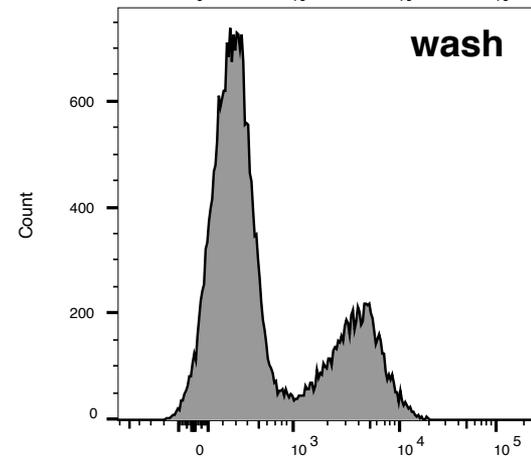
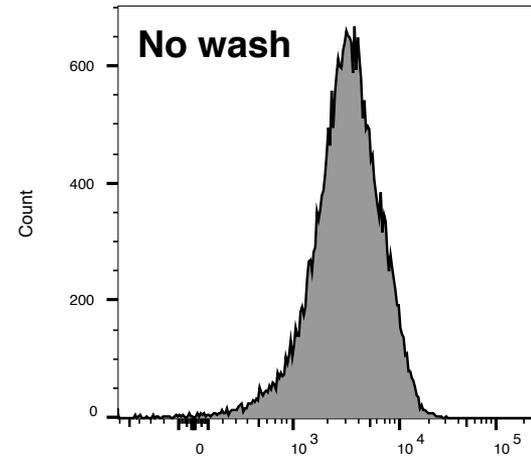
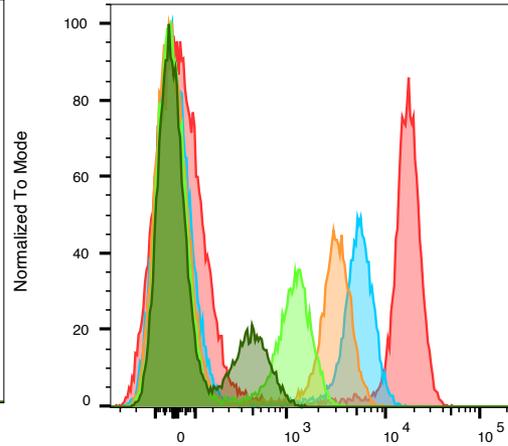
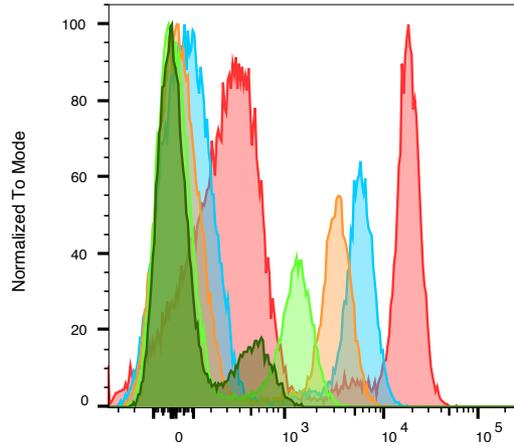
**CD14** →



# Comparison of CD56 antibodies



# No wash / wash



Comp-YG 586\_15-A :: CD4

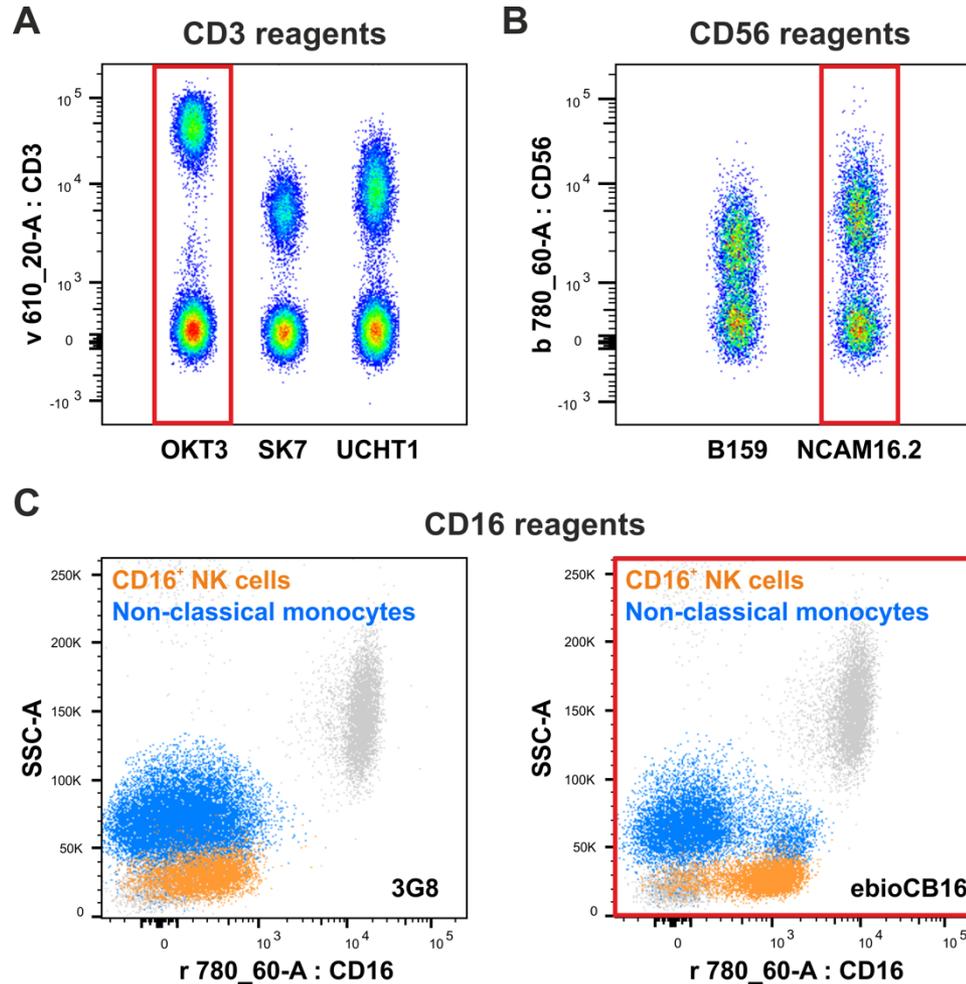
Comp-YG 586\_15-A :: CD4

Comp-B 710\_50-A :: CD314

**No wash**

**wash**

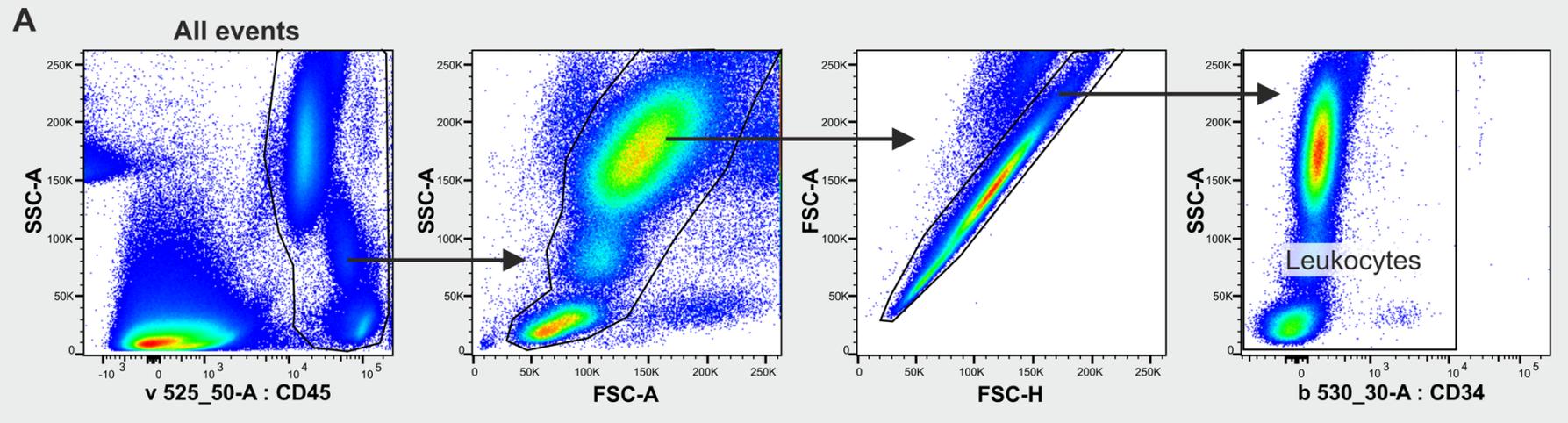
# Comparison of Abs with final fluorochrome



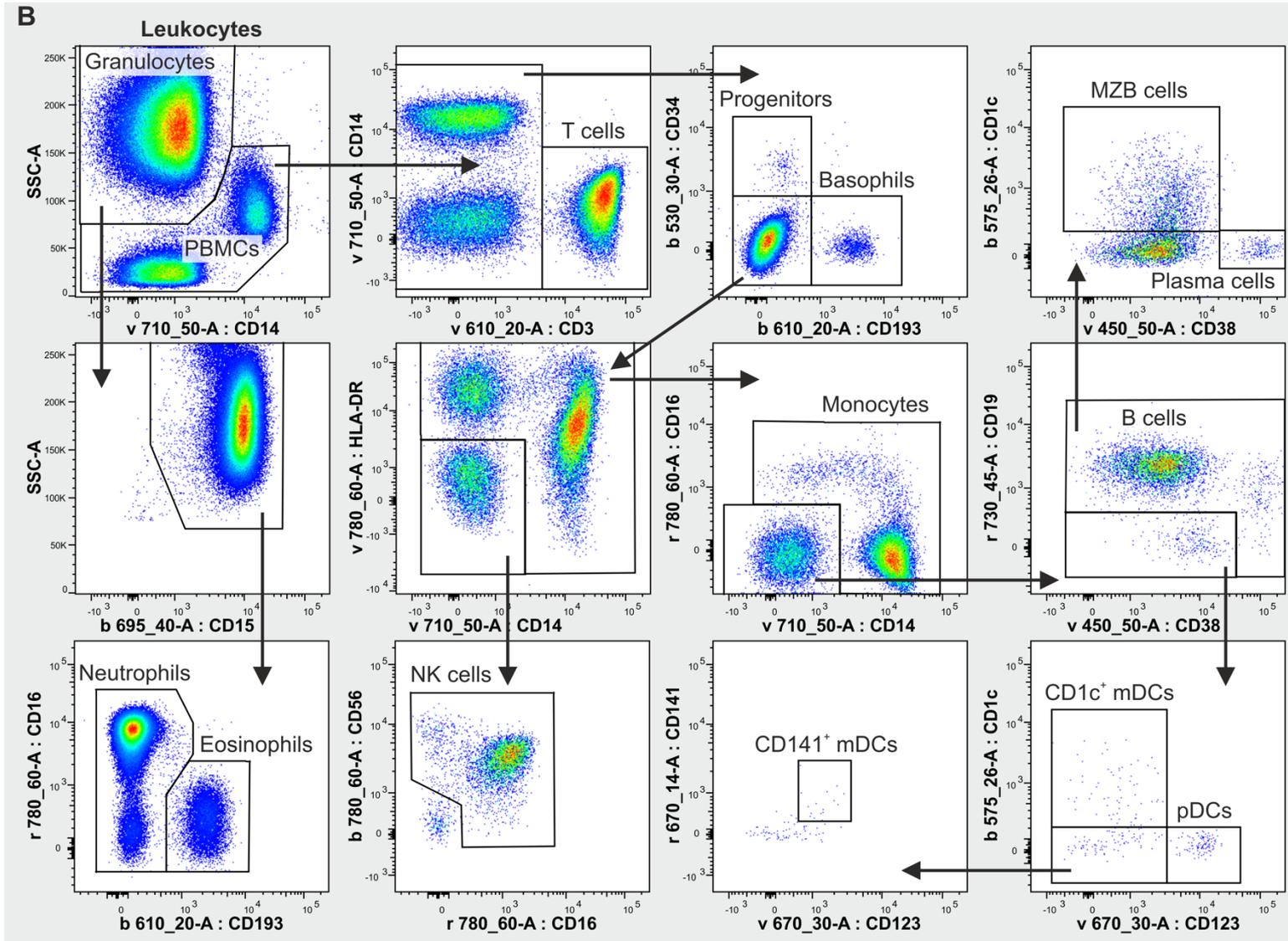
# final panel

Specificity	Fluorochrome	Clone	Vendor	Cat.No.	$\mu\text{l}/100 \mu\text{l}$
<b>CD1c</b>	PE	AD5-8E7	Miltenyi Biotec	130-090-508	1
<b>CD3</b>	BV605	OKT3	BioLegend	317322	1
<b>CD14</b>	BV711	MFP9	BD Horizon	563372	0.5
<b>CD15</b>	PerCP-Cy5.5	HI98	BD Pharmingen	560828	2
<b>CD16</b>	APC-efluor780	ebioCB16	Thermo Fisher	47-0168-42	4
<b>CD19</b>	APC-R700	HIB19	BD Horizon	564977	0.25
<b>CD34</b>	FITC	HPCA-2	BD Biosciences	345801	5
<b>CD38</b>	BV421	HIT2	BD Horizon	562444	0.125
<b>CD45</b>	BV480	HI30	BD Horizon	566115	0.5
<b>CD56</b>	PE-Cy7	NCAM16.2	BD Biosciences	335826	1
<b>CD123</b>	BV650	7G3	BD Horizon	563405	0.5
<b>CD141</b>	APC	AD5-14H12	Miltenyi Biotec	130-090-907	1
<b>CD193</b>	PE-CF594	5E8	BD Horizon	562571	0.3
<b>HLA-DR</b>	BV786	G46-6	BD Horizon	564041	1

# Gating I cleaning



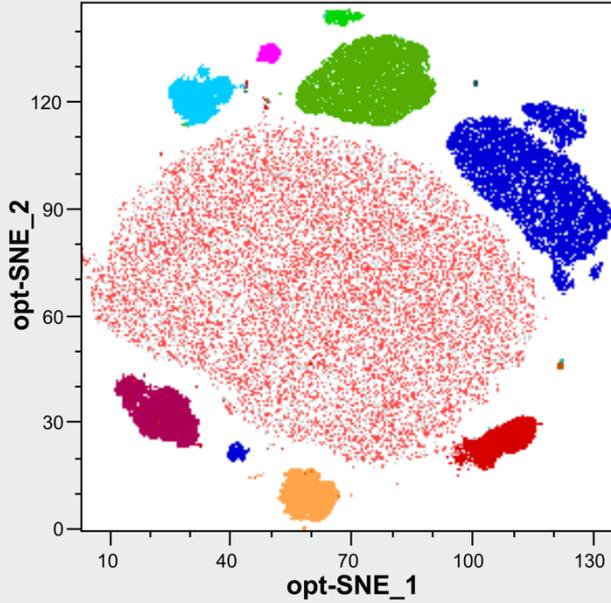
# Gating II populations



# Dimension reduction

C

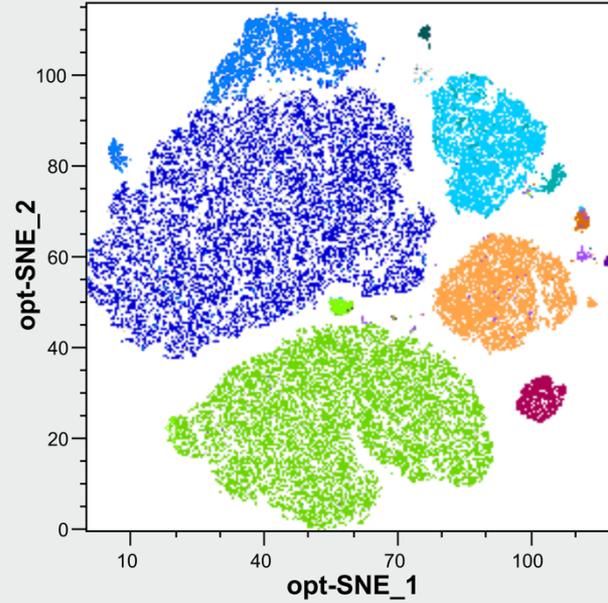
Leukocytes



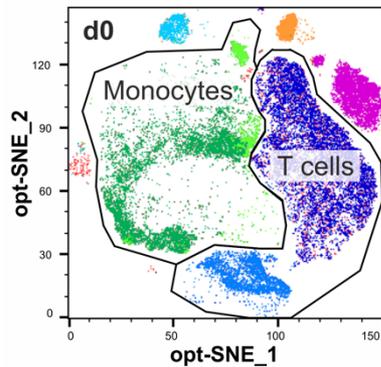
Subset	Count
Monocytes (c)	18124
Monocytes	19176
NK cells	5478
T cells	30359
Plasma cells	208
B cells	5858
Progenitors	168
Basophils	1057
CD16 <sup>neg</sup>	4876
Neutrophils	201873
Eosinophils	8334
Leukocytes	273080

D

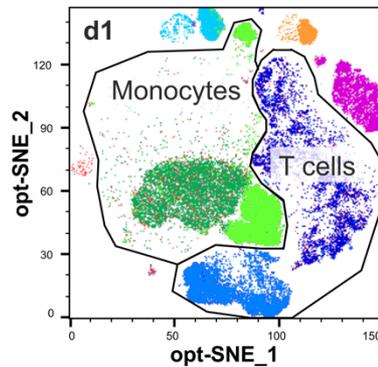
PBMCs



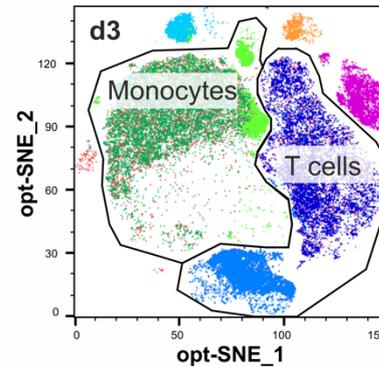
Subset	Count
mDCs	177
pDCs	116
Monocytes (nc)	301
Monocytes	19176
NKT cells	3461
T cells	30359
CD56 <sup>hi</sup> cells	400
NK cells	5478
Plasma cells	208
B cells	5858
Progenitors	168
Basophils	1057
PBMCs	62788



<u>Monocytes</u>	<u>T cells</u>
33.7%	50.3%
1150 cells/ $\mu$ l	1730 cells/ $\mu$ l

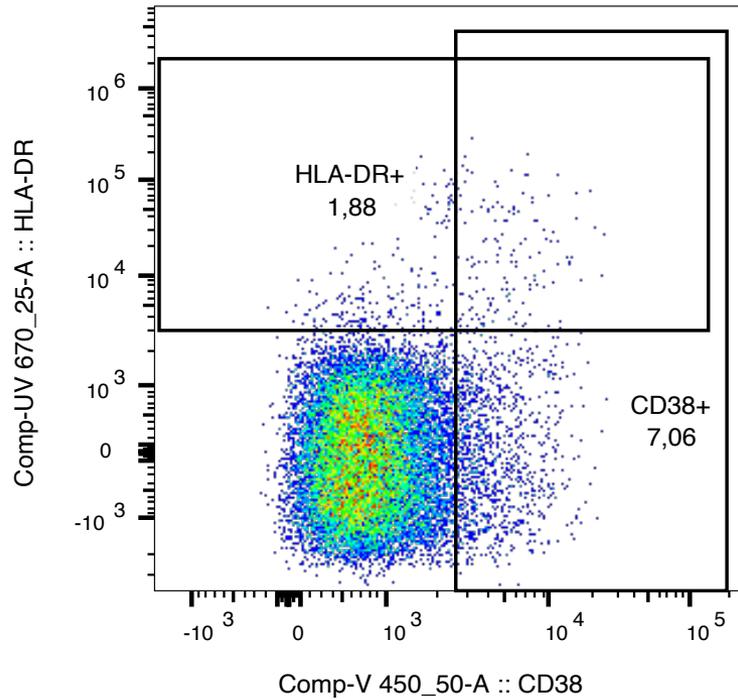


<u>Monocytes</u>	<u>T cells</u>
55.5%	30.7%
3100 cells/ $\mu$ l	1720 cells/ $\mu$ l

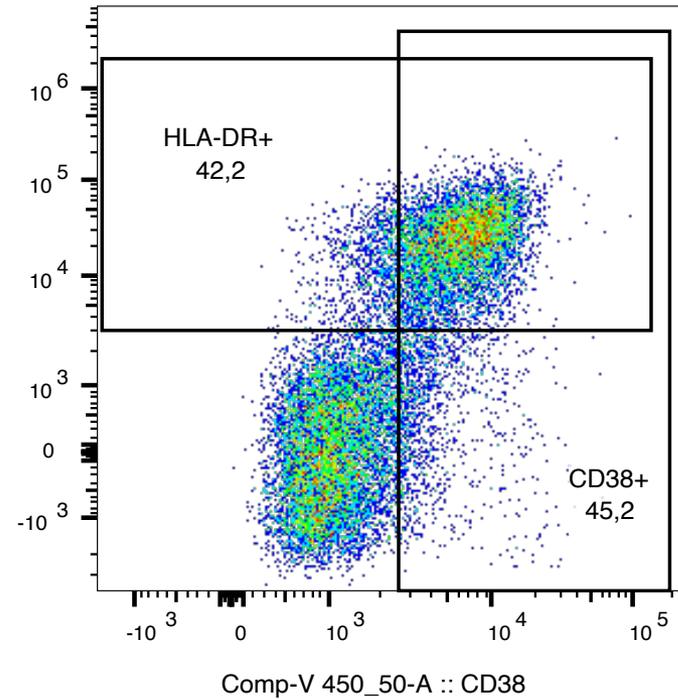


<u>Monocytes</u>	<u>T cells</u>
57.8%	34.3%
3770 cells/ $\mu$ l	2230 cells/ $\mu$ l

# Cell status



**healthy**



**Hospitalized COVID-19 patient**

# Conclusion

Panel established that defines >99% of all CD45+ cells

Absolute enumeration even for very small subsets

Panel suitable for a variety of mid class instruments

Slight adaptation for new 13-color diagnostic instruments

# Outlook



530_30	FITC		CD34
610_20	BB630P		
670_30	BB660		
710_50	PerCp-Cy5.5		CD11c
780_60	BB790P		
670_30	APC		CD141
730_45	APC-R700		CD19
780_60	APC-eF780		CD16
379_28	BUV395		CD56
515_30	BUV496		CD3
580_20	BUV563		CD8
605_20	BUV615P		
670_25	BUV661		HLA-DR
735_30	BUV737		CD4
810_40	BUV805		CD45
450_50	BV421		CD38
525_50	BV510		CD7
586_15	BV570		
605_40	BV605		CD27
677_20	BV650		CD123
710_20	BV711		CD14
750_30	BV750		
780_60	BV786		CD15
586_15	PE		CD1c
610_20	PE-CF594		CD193
670_30	PE-Cy5		TCRgd
780_60	PE-Cy7		CD20

Transferable and extendable for high end instruments