



BACKGROUND

A 28-color high-dimensional flow cytometry (HDFCM) panel was designed to characterize human B cell subsets in whole blood samples and peripheral blood mononuclear cells (PBMCs) from healthy donors. To ensure data quality, a fixable viability dye was included in the panel to remove dead cells from the samples before downstream data analysis. Then, to investigate a pure B cell subset, CD45, CD15, CD14, CD117, CD56, CD16, CD3, CD123, and CD127 markers were included to ensure the assessment of B cells within CD45+ cells without contamination from other immune cell types, such as granulocytes, monocytes, stem cells, NK cells, T cells, DCs, basophils, and ILC cells. After that, B cells were identified by expression of the canonical marker CD19. The combination of CD27 and CD38 was employed to investigate antibody-secreting cells (ASCs; CD38hiCD27hi), memory B cells (CD27+CD38low/-), and CD27- cells. Under CD27- cells, transitional B cells (T1, T2, and T3), naïve B cells, and double-negative (DN) B cells were identified by the combination of CD24, CD10, CD38, and IgD. For memory B cells, combinations of IgM, IgD, IgG, IgA, and IgE were separately used to identify non-switched memory B cells (IgM+IgD+) and switched memory B cells (IgM-IgD-IgA+/IgG+/IgE+). In addition to B cell subsets, this panel can also assess granulocytes, monocytes, NK cells, T cells, DCs (cDCs and pDCs), and basophils.

SAMPLES

Fresh whole-blood samples were obtained in-house with informed consent and used exclusively for research purposes.

METHODS

Fresh whole-blood samples were treated with BioLegend RBC Lysis Buffer to remove red blood cells (RBCs). After RBC lysis, matched whole-blood and freshly isolated PBMC samples underwent live/dead staining followed by cell-surface staining. Samples were acquired on a Cytex Aurora and analyzed using OMIQ.

PANEL INFORMATION

Specificity	Clone	Purpose
Viability	NA	Viability
CD45	HI30	Pan leukocyte marker
CD15	W6D3	Neutrophil lineage marker
CD14	63D3.rMAb	Monocyte lineage marker
CD56	NCAM16.2	NK cell lineage marker
CD3	Sk7	Pan T cell lineage marker
CD33	WM53	Myeloid lineage marker
CD123	6H6	pDC lineage marker
CD127	eBioRDR5	T cell and ILC lineage marker
CD16	3G8	NK cells and Monocytes
CD11c	B-LY6	cDC lineage marker
CD117	104D2	Hematopoietic stem/progenitor cells, Mast cells
HLA-DR	L243	Myeloid lineage, T cell activation
CD19	SJ25C1	B cell lineage marker
CD20	2H7	B cell differentiation marker
CD38	HIT2	B cell differentiation marker
CD27	O323	B cell differentiation marker
CD10	HI10a	B cell differentiation marker
CD24	ML5	B cell differentiation marker
CD21	B-ly4	B cell differentiation marker
CD185 (CXCR5)	RF8B2	B cell differentiation marker
CD23	M-L233	B cell differentiation marker
IgM	MHM-88	Unswitched B cells
IgD	IA6-2	Unswitched B cells
IgG	G18-145	IgG class-switched B cells
IgA	IS11-8E10	IgA class-switched B cells
IgE	MHE-18	IgE class-switched B cells
CD69	FN50	Activation marker

CONCLUSIONS

This HDFCM panel was developed to detect every marker with optimal resolution and can be used as a foundation for investigating B cell subsets in both frozen PBMC and whole blood samples.

ANTIBODY TITRATION

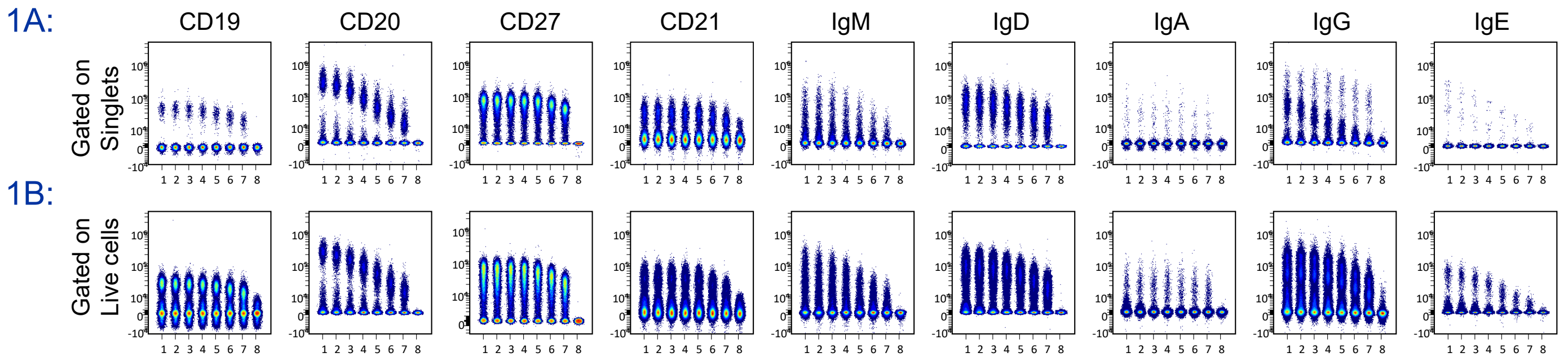
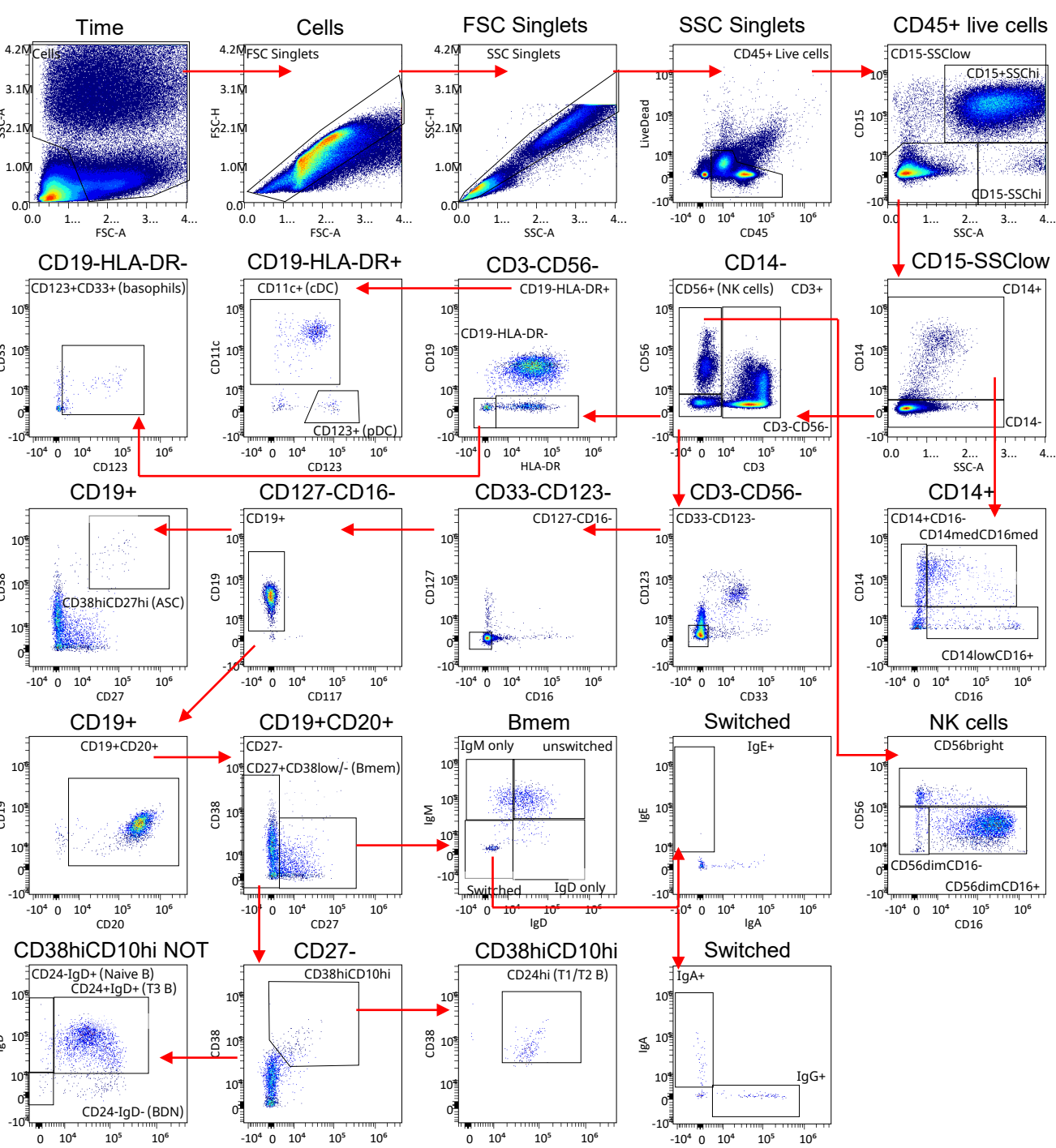


Figure 1. Antibody titrations on whole blood samples (1A) and PBMC samples (1B).

FULL PANEL ASSESSMENT

2A: Fresh Whole Blood



2B: Fresh Isolated PBMC

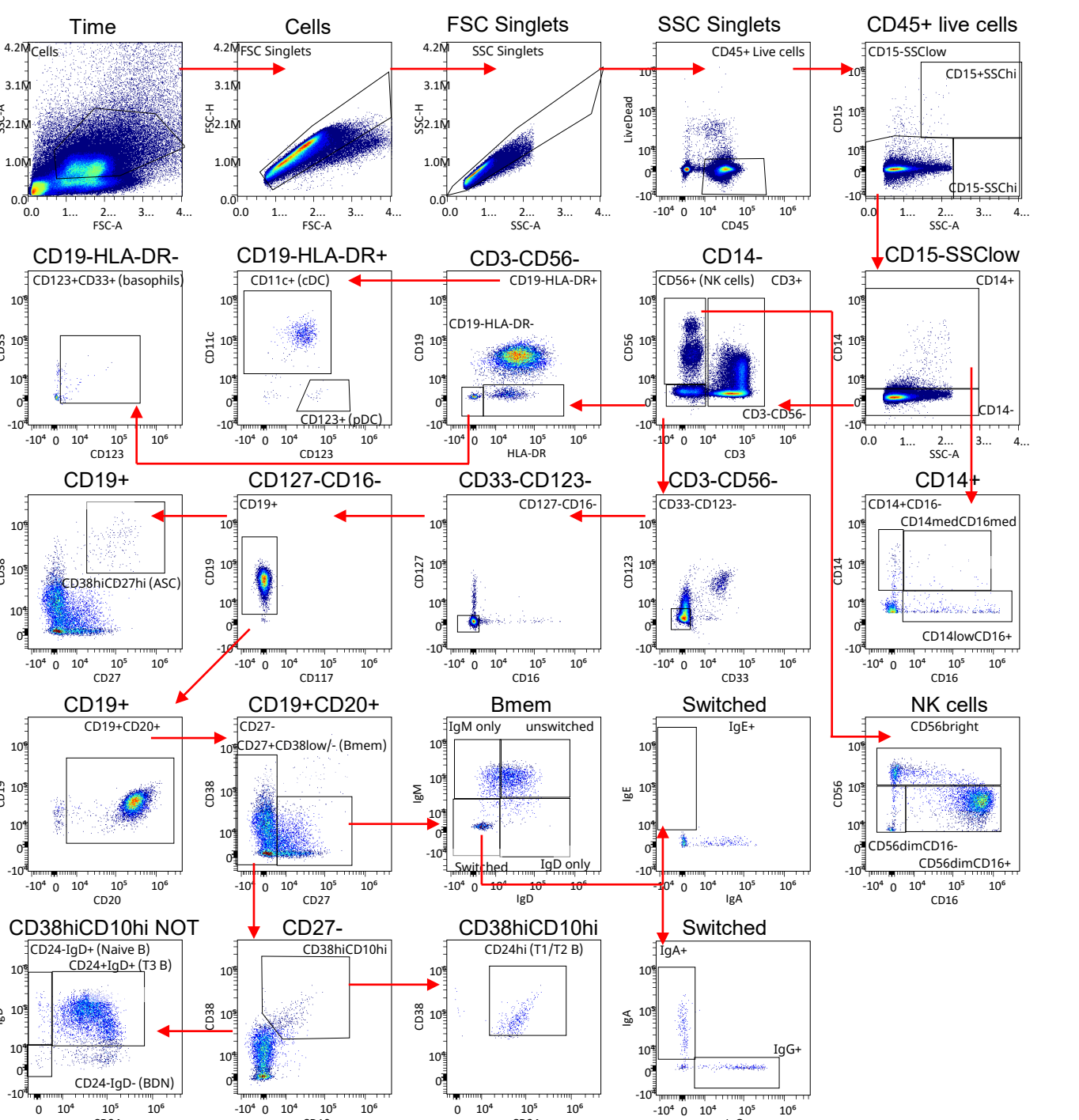


Figure 2. A representative gating hierarchy in fresh whole blood sample (2A) and in fresh isolated PBMC sample (2B) were showed.