Theme: Unswitched Memory B cells in Human Health and Disease

Title: ***Unravelling B cell heterogeneity: Insights into flow cytometry-gated B cells from single-cell multi-omics data***

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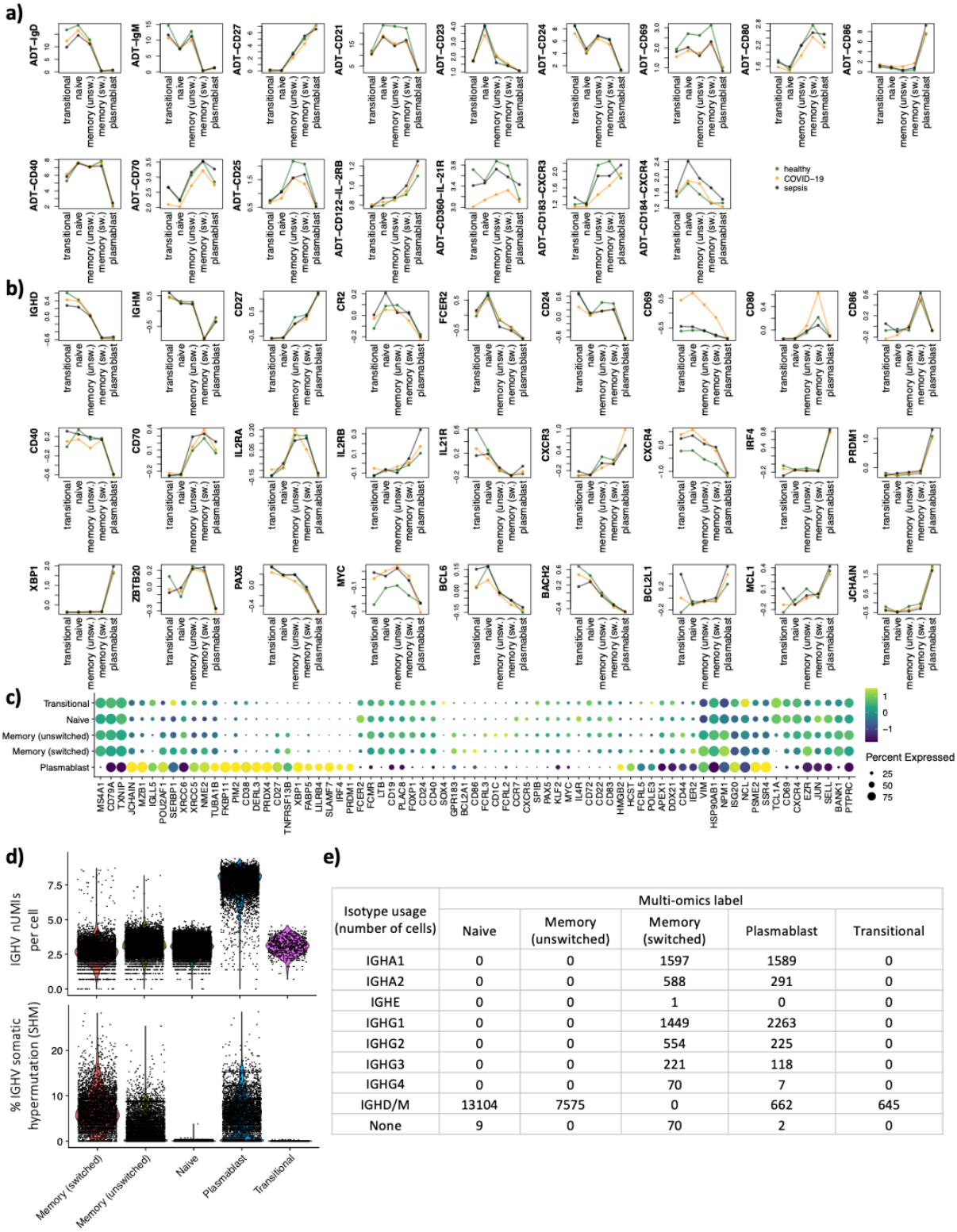
Supplemental Figures



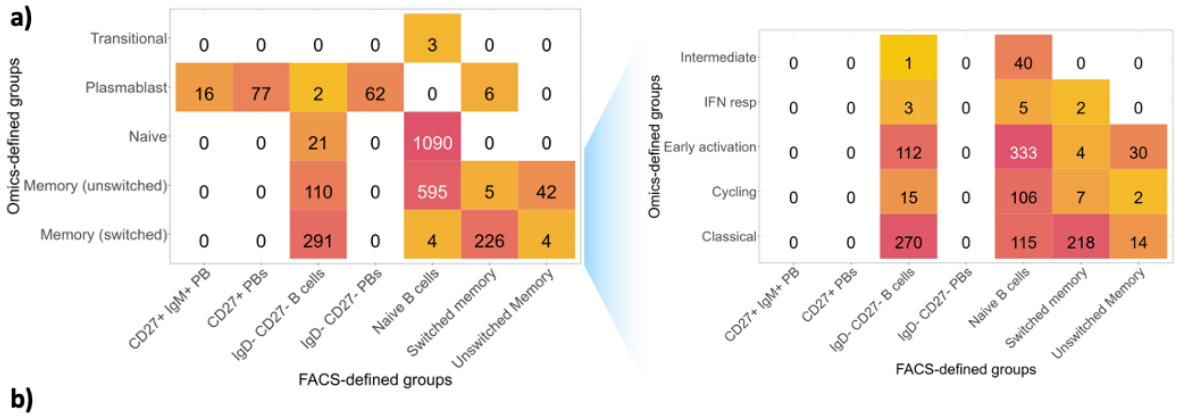
Isotype usage (number of cells)	Multi-omics label				
	Naive	Memory (unswitched)	Memory (switched)	Plasmablast	Transitional
IGHA1	0	0	1597	1589	0
IGHA2	0	0	588	291	0
IGHE	0	0	1	0	0
IGHG1	0	0	1449	2263	0
IGHG2	0	0	554	225	0
IGHG3	0	0	221	118	0
IGHG4	0	0	70	7	0
IGHD/M	13104	7575	0	662	645
None	9	0	70	2	0

Figure S1. Gene expression and VDJ signatures for the single cell multi-omics B cell annotations.

a) Gene expression profiles of B cell subpopulations of the top differentially expressed and marker genes.

b) The per cell subpopulation (left) somatic hypermutation levels (SHM) and (right) IGHV expression level (nUMIs). Naïve and transitional B cells are marked by low/zero SHM and plasmablasts have the highest IGHV expression.

c) The isotype usage percentages across cell types within each cell population. Naïve, transitional B cells amd unswithced activated and memory B cells are marked by IGHD/IGHM expression, with only class-switched B cells within the other populations.



b)

FACS-defined group	True multi-omics label population	% Accuracy	% Sensitivity	% Specificity	% Purity
CD27+ IgM+ PBs, CD27+ PBs, IgD- CD27- PBs	Plasmablast	99.69	95.09	100	100
Naive B cells	Naive	75.61	98.11	58.28	64.42
Switched memory	Memory (switched)	87.86	43.05	99.46	95.36
Unswitched Memory	Memory (unswitched)	72.04	5.59	99.78	91.3

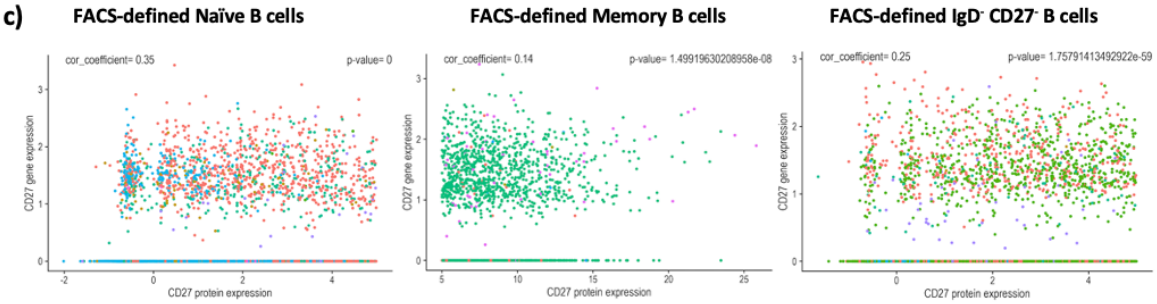


Figure S2.

a) Heatmap of the heterogeneity of B cells captured within each standard B cell flow cytometric-style gating for only healthy individuals. The number represents the number of cells captured with in the corresponding flow-cytometric gate with the corresponding multi-omics label.

b) Table of the accuracy, sensitivity and specificity of the flow cytometric-style gating to capture target B cell populations for only healthy individuals, where the true annotations were defined using the multi-omics labelling.

c) The correlation between CD27 protein and gene expression within cell subsets. Correlations were performed using Spearman Rank with the corresponding p-values.

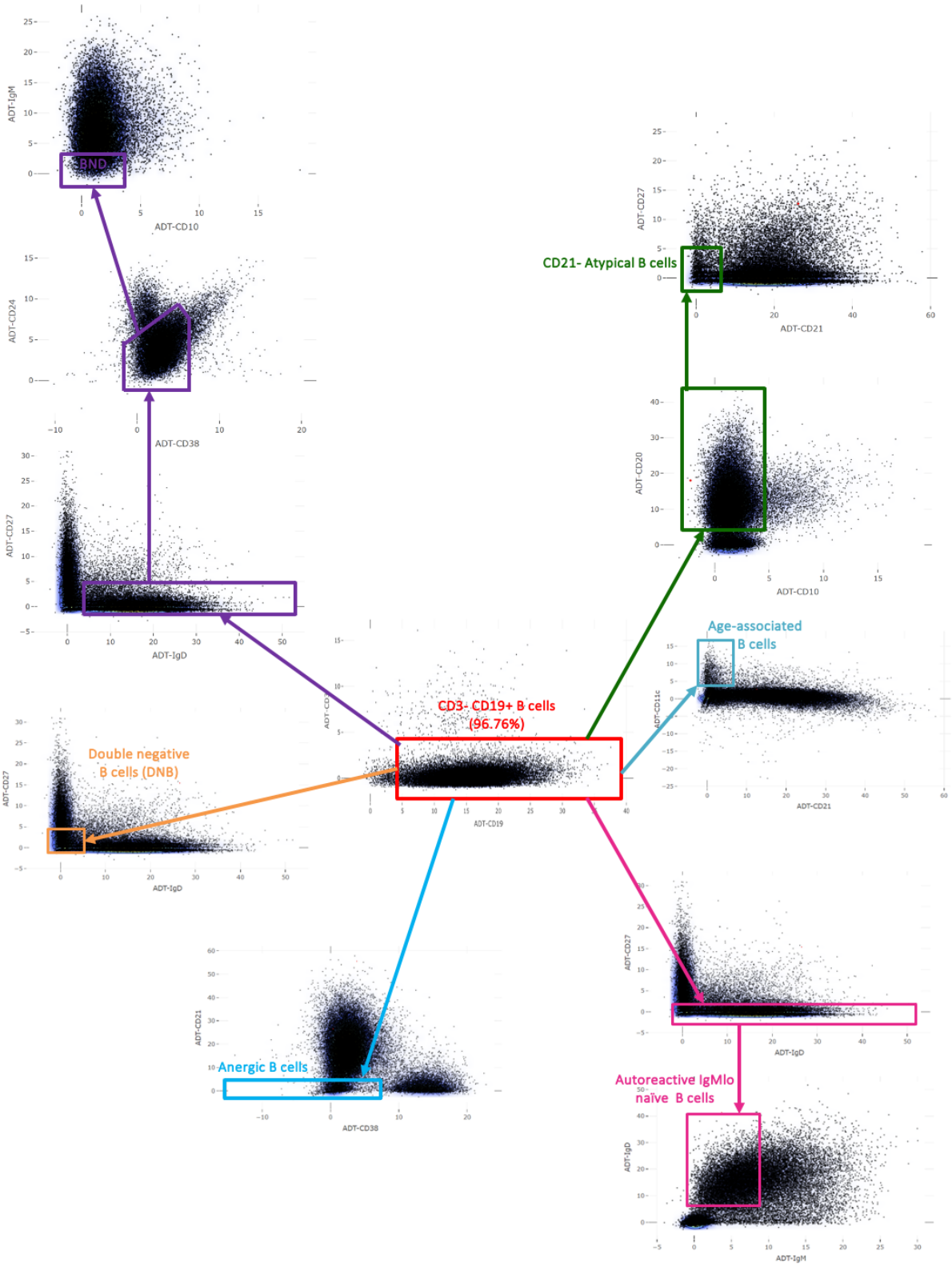


Figure S3. Gating for the anergic, age-associated, B_{ND}, CD21- atypical, and DNB B cells, and autoreactive IgMlo naïve B cells based on flow cytometric gating strategies used in the literature from (**Figure 4a**).

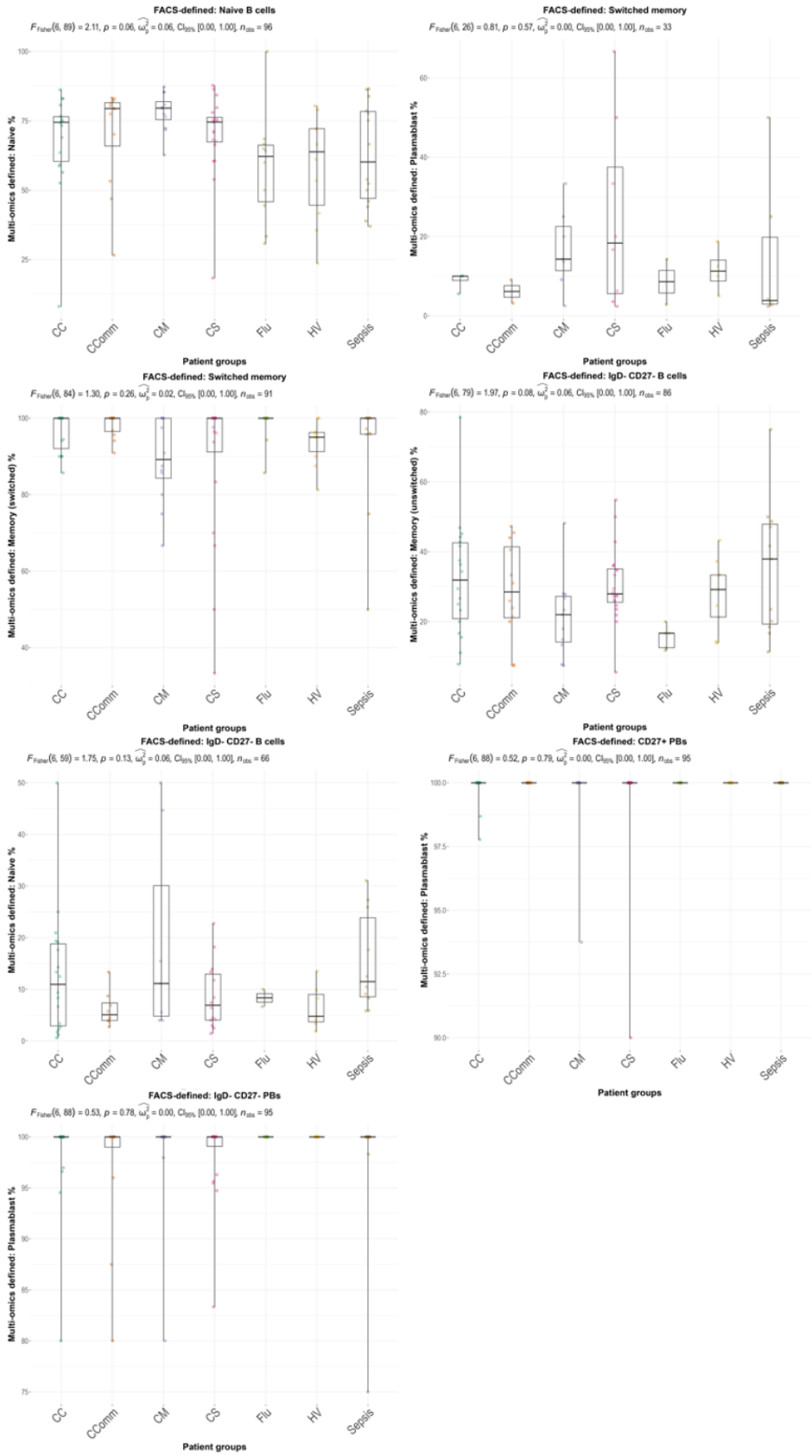
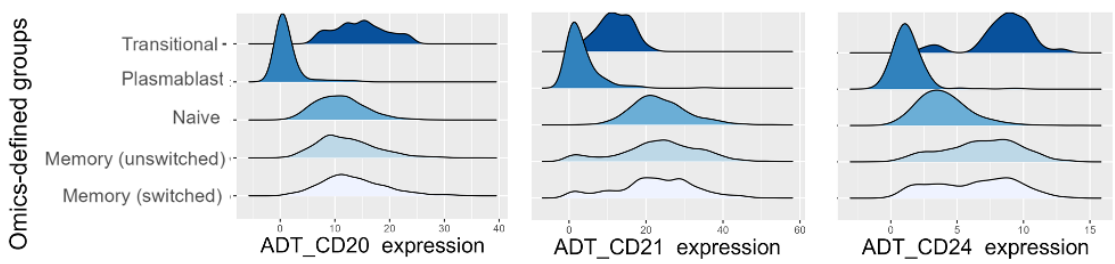


Figure S4. Boxplots of the variation of the proportion of multi-omics-defined B cell populations within flow-cytometric style gating between diseases for naïve B cells, unswitched and switched memory B cells, IgD- CD27- B cells, CD27+ IgM+ plasmablasts, CD27+ plasmablasts and IgD- CD27- plasmablasts.

Overall p-values of frequencies associating with disease status is provided at the top of each figure (by ANOVA) and p-values between disease states are provided (by Wilcoxon test using Holm multiple testing correction).

a)



b) ***Relative change in percentage of cells with additional gates 1-3 compared to the gating in Figure 1 (without CD20, CD21 and CD24 gates):***

	FACS-defined groups		
Multi-omics defined labels	**Naive B cells (CD20lo/mid CD24lo/mid CD21mid)**	**Switched memory (CD24+ CD20lo/mid CD21hi)**	**Unswitched Memory (CD24-)**
Transitional	-0.127	NA	NA
Plasmablast	-0.373	-1.000	-1.000
Naive	-0.052	NA	NA
Memory (unswitched)	-0.246	-0.214	-0.635
Memory (switched)	-0.184	-0.290	-0.840

c)

FACS-defined population	Multi-omics defined population	p-value (significance of % FACS impurity associated with disease status) using gating from Figure 6
IgD-CD27- B cells (CD24-CD20hiCD21-)	Memory (switched)	4.21E-03
IgD-CD27- B cells (CD24-CD20lo)	Memory (switched)	0.83
IgD-CD27- B cells (CD24CD20lomidCD21hi)	Memory (switched)	1.06E-03
Nave B cells (CD20lomidCD24lomidCD21mid)	Memory (switched)	0.02
Switched memory(CD24-)	Memory (switched)	0.24
Switched memory(CD24CD20lomidCD21hi)	Memory (switched)	0.71
Unswitched Memory(CD24CD20lo)	Memory (switched)	NA
IgD-CD27- B cells (CD24-CD20hiCD21-)	Memory (unswitched)	0.91
IgD-CD27- B cells (CD24CD20lomidCD21hi)	Memory (unswitched)	0.11
Nave B cells (CD20lomidCD24lomidCD21mid)	Memory (unswitched)	8.94E-04
Unswitched Memory(CD24CD20lo)	Memory (unswitched)	0.34
IgD-CD27- B cells (CD24-CD20hiCD21-)	Naïve	0.04
Nave B cells (CD20lomidCD24lomidCD21mid)	Naïve	0.07
Nave B cells (CD20lomidCD24lomidCD21mid)	Plasmablast	0.28
Switched memory(CD24-)	Plasmablast	0.57
Unswitched Memory(CD24-)	Plasmablast	NA

Figure S5. a) The distribution of CD20, CD21 and CD24 expression across cells defined by multi-omics. b) Relative change in frequency with additional gates from Figure 6 compared to the gating in Figure 1 (without the additional CD20, CD21 and CD24 gates). The relative change value is between -1 to 0 where -1 represents a complete reduction of a population after the additional gating, and zero represents identical frequencies after the additional gating.

c) Table of the variation of the significance (p-value) of association of the percentage of cells labelled via multi-omics-definitions within flow-cytometric style gating between diseases. Overall p-values (calculated by ANOVA) of frequencies associating with disease status is provided only for combinations with >3 individuals with non-zero frequencies across each disease state. Significant values (p-values<0.05) are highlighted in red.