

Supplementary Appendix

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Supplemental Table 1. List of all fluorochrome-conjugated antibodies used for flow cytometric analysis of immunophenotyping of lymphoid cell subsets.

Antigen	Fluorochrome	Clone	Company
HLA-DR	FITC	L243	BDBioscience
CD56	PE	NCAM16.2	BDBioscience
CD16	PerCP 5.5	3G8	BDBioscience
CD4	PE-Cy7	SK3	BDBioscience
CD19	APC	SJ25C1	BDBioscience
CD8	APC-H7	SK1	BDBioscience
CD3	V450	ICHT1	BDBioscience
CD45	V500	2D1	BDBioscience

Supplemental Table 2. List of all fluorochrome-conjugated antibodies used for flow cytometric analysis of antigen-specific and polyclonal T cells.

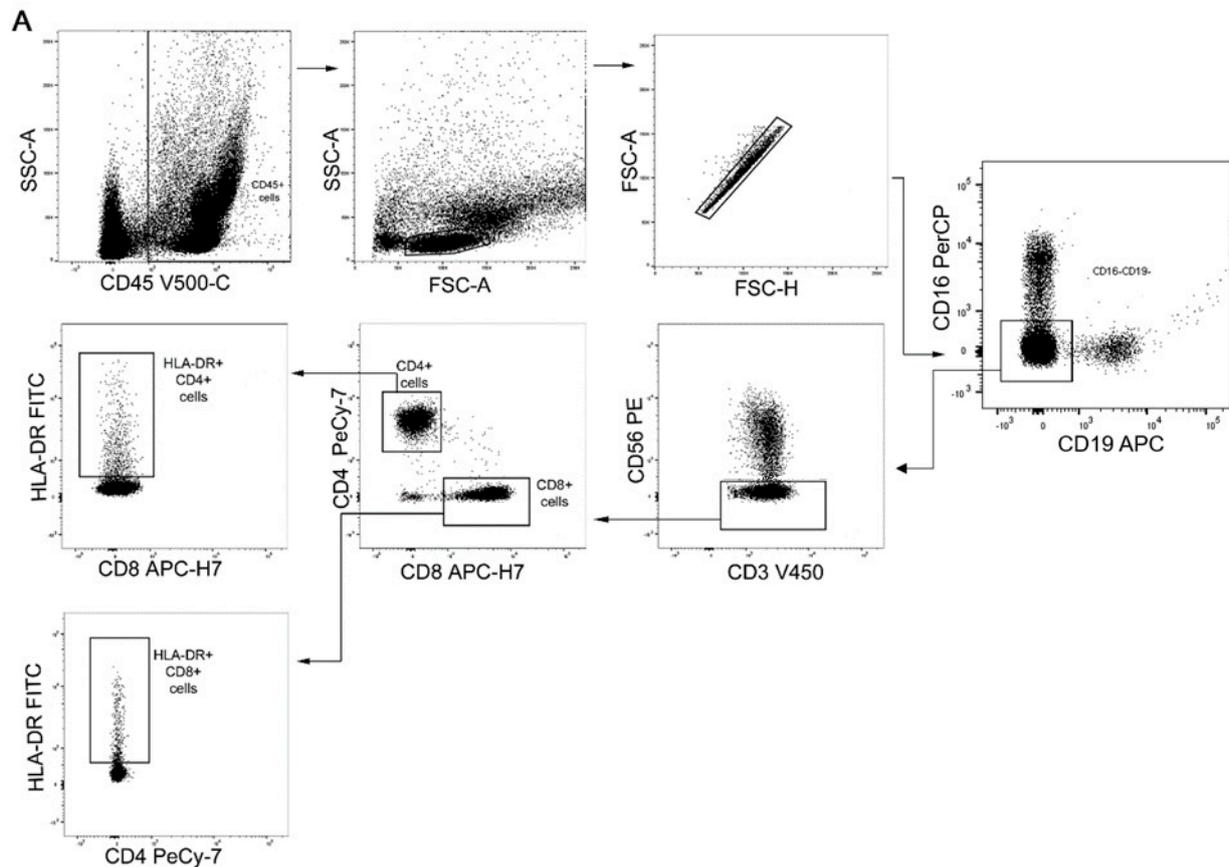
Antigen	Fluorochrome	Clone	Company
TNF- α	FITC	6401.1111 B	BDBioscience
CD154	PE	TRAP1	BDBioscience
CD3	PerCP	SK7	BDBioscience
CD4	PECy-7	SK3	Invitrogen
CD8	SB600	SK1	eBioscience™
IL-2	APC	MQ1-17H12	BDBioscience
IFN- γ	Pacific Blue	B27	BioLegend
L/D	Fixable Viability Stain 780		BDBioscience

Supplemental Table 3. List of all fluorochrome-conjugated antibodies used for flow cytometric analysis of antigen-specific B cells.

Antigen	Fluorochrome	Clone	Company
CD19	APC-Vio770	LT19	Miltenyi
CD27	VioBright FITC	M-T271	Miltenyi
IgA	VioGreen	IS11-8E10	Miltenyi
IgG	VioBlue	IS11-3B2.2.3	Miltenyi
CD14	PerCP	TÜK4	Miltenyi
IgM	APC	PJ2-22H3	Miltenyi
CD3	PerCP	BW264/56	Miltenyi
7AAD			Miltenyi
Spike	PE		Miltenyi
Spike	PE-Vio770		Miltenyi

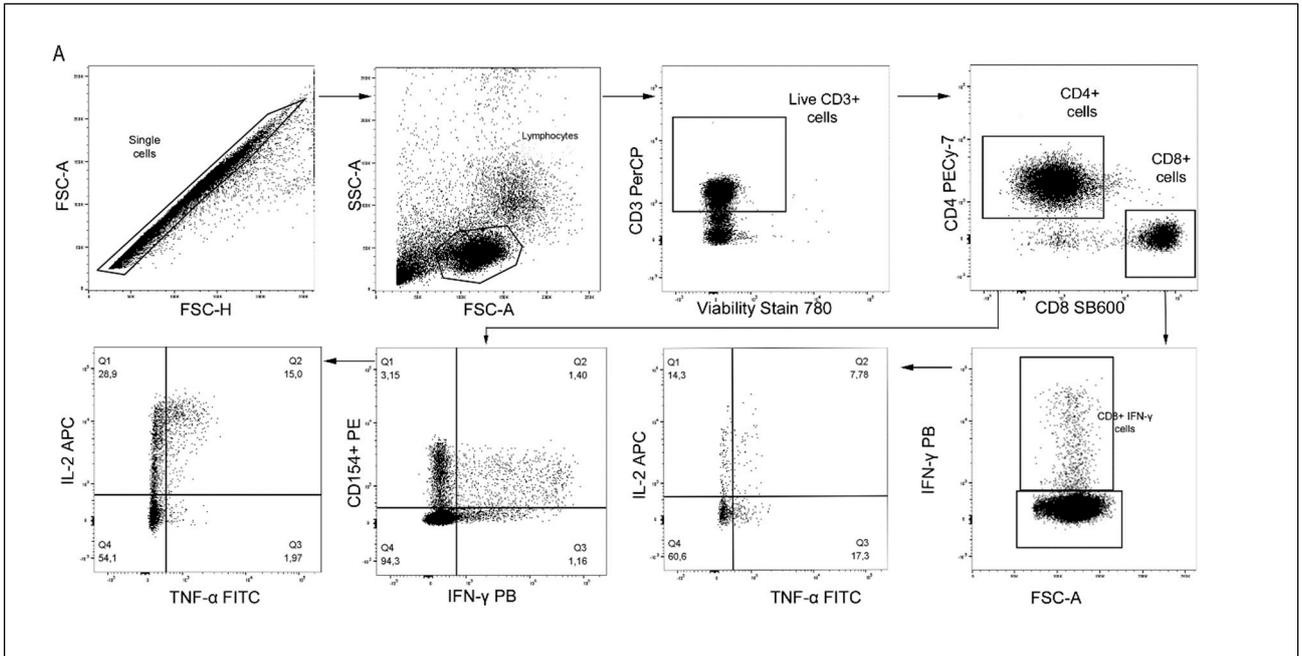
Supplemental Table 4. List of all fluorochrome-conjugated antibodies used for flow cytometric analysis of antigen-specific B cells.

Antigen	Fluorochrome	Clone	Company
CD19	APC-Vio770	LT19	Miltenyi
CD27	VioBright FITC	M-T271	Miltenyi
Wuhan WT Spike	APC		Miltenyi
Wuhan WT Spike	VioBlue		Miltenyi
CD14	PerCP	TÜK4	Miltenyi
CD3	PerCP	BW264/56	Miltenyi
7AAD			Miltenyi
Spike Omicron B1.1.529	PE		Miltenyi
Spike Omicron B1.1.529	PE-Vio770		Miltenyi



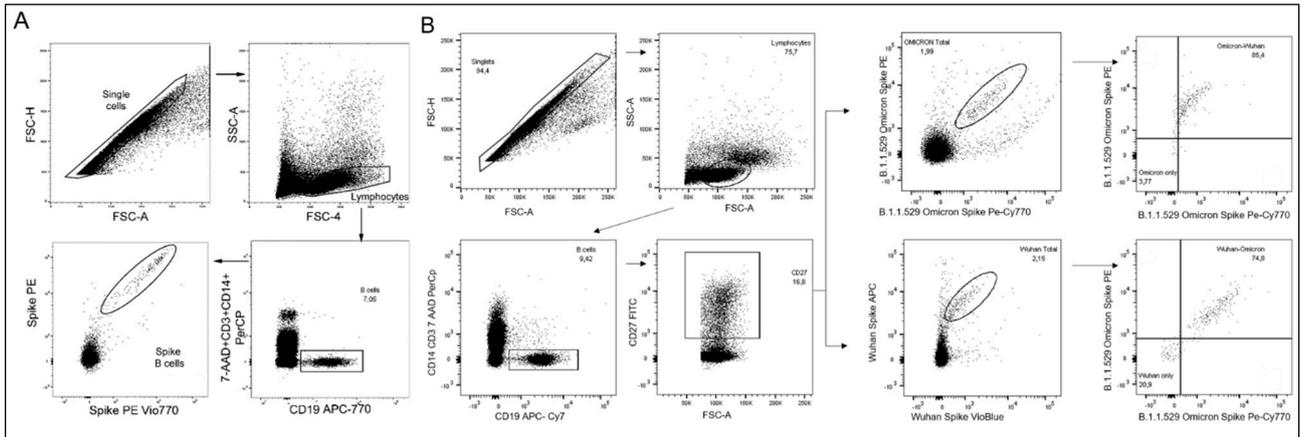
Supplemental Figure 1. Gating strategy for immunophenotyping of lymphoid cell subsets.

(A) PBMCs were gated based on physical parameter SSC and CD45 surface expression, lymphocytes were then gated basing on physical parameters (SSC, FSC). Doublets were removed using FSC-A and FSC-H parameters. CD16-CD19- and CD56-CD3+ cells were identified. In the resulting CD3+ population, CD4+ and CD8+ T lymphocytes were identified. Among these, we identified HLA-DR expressing cells.



Supplemental Figure 2. Gating strategy for the identification of SARS-CoV-2-specific T cells.

(A) Doublets were removed using FSC-A and FSC-A parameters and lymphocytes were gated based on physical parameters (FSC-SSC). Dead cells were excluded using viability stain 780. T cells were identified as CD3+. We then identified CD4+ and CD8+ T cells. CD4+ T cells were evaluated for CD154, IFN- γ , TNF- α and IL-2 expression. CD8+ T cells were evaluated for IFN- γ , TNF- α and IL-2 expression.



Supplemental Figure 3. Gating strategy for the identification of Spike-specific B cells.

(A) Doublets were removed using FSC-A and FSC-A parameters and lymphocytes were gated based on physical parameters (FSC-SSC). PerCP was used as dump channel for the exclusion of dead cells (7AAD), CD3+ T cells and CD14 monocytes. B cells were identified as CD19+. B cells binding PE- and PE Vio770-conjugated spike protein were then identified as Wuhan (WT) Spike-specific. (B) Doublets were removed using FSC-A and FSC-A parameters and lymphocytes were gated based on physical parameters (FSC-SSC). PerCP was used as dump channel for the exclusion of dead cells (7AAD), CD3+ T cells and CD14 monocytes. Memory B cells were identified as CD19+CD27+. B cells binding PE- and PE Vio770-conjugated spike protein were then identified as B.1.1.529 (Omicron) Spike-specific and B cells binding APC- and Pacific Blue-conjugated Spike protein were then identified as Wuhan (WT) Spike-specific.