Supplementary material to:

SARS-CoV-2 spike-specific memory B cells express markers of durable immunity after non-severe COVID-19 but not after severe disease

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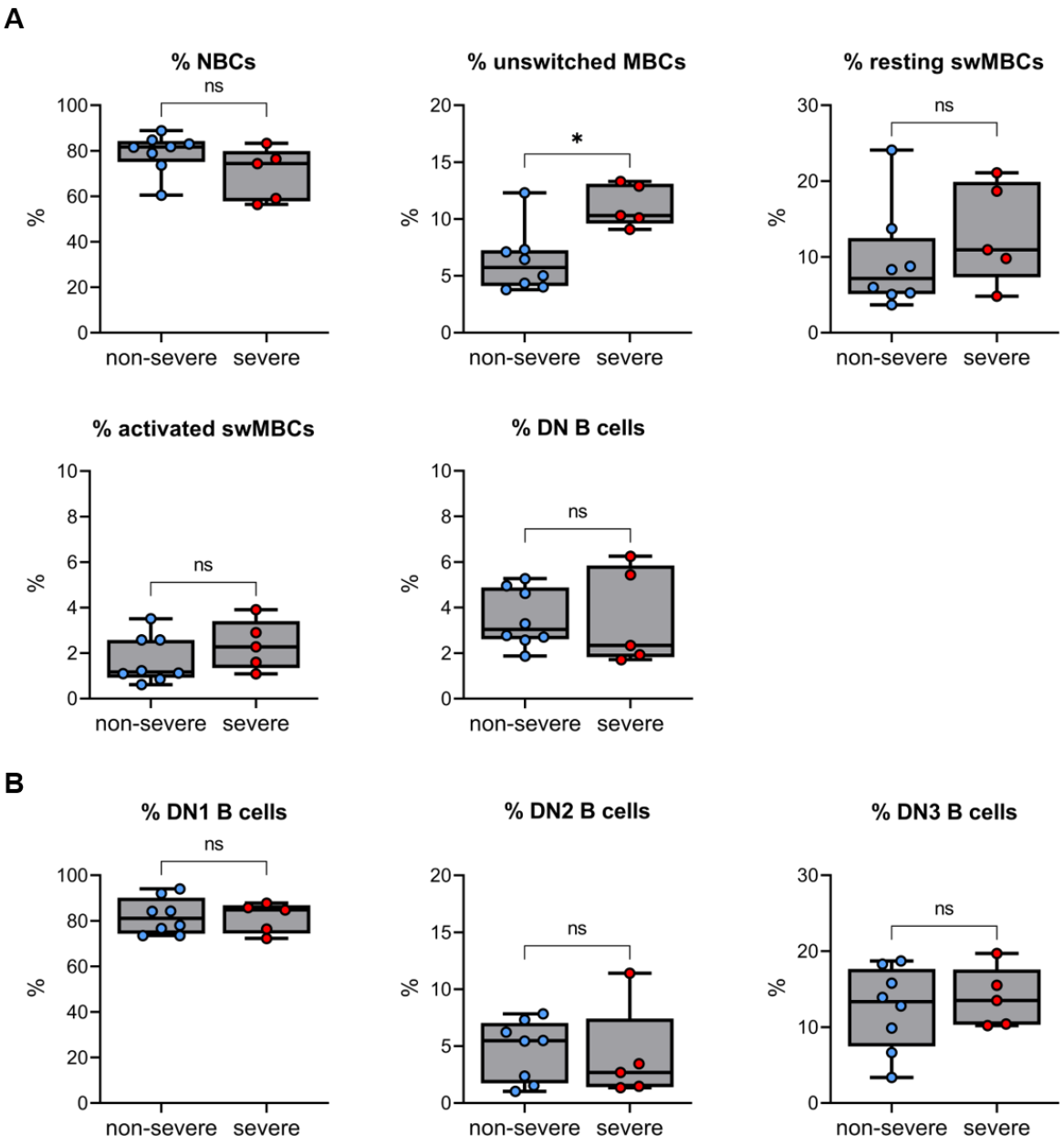

Figure S1: Distribution of major B cell subsets in patients who recovered from non-severe or severe COVID-19. A) The percentage of naïve B cells (NBC; IgD^+ $CD27^-$), unswitched memory B cells (MBCs; IgD^+ $CD27^+$), resting switched MBCs (swMBC; IgD^- $CD27^+CD21^+$), activated swMBC (IgD^+ $CD27^+$ $CD21^+$), and double negative B cells (DN; IgD^- $CD27^-$). **B)** The percentage of type 1, 2, and 3 DN cells among all DN cells. Results are shown for patients who recovered from non-severe (n = 8) and severe (n = 5) COVID-19. * $P < 0.05$

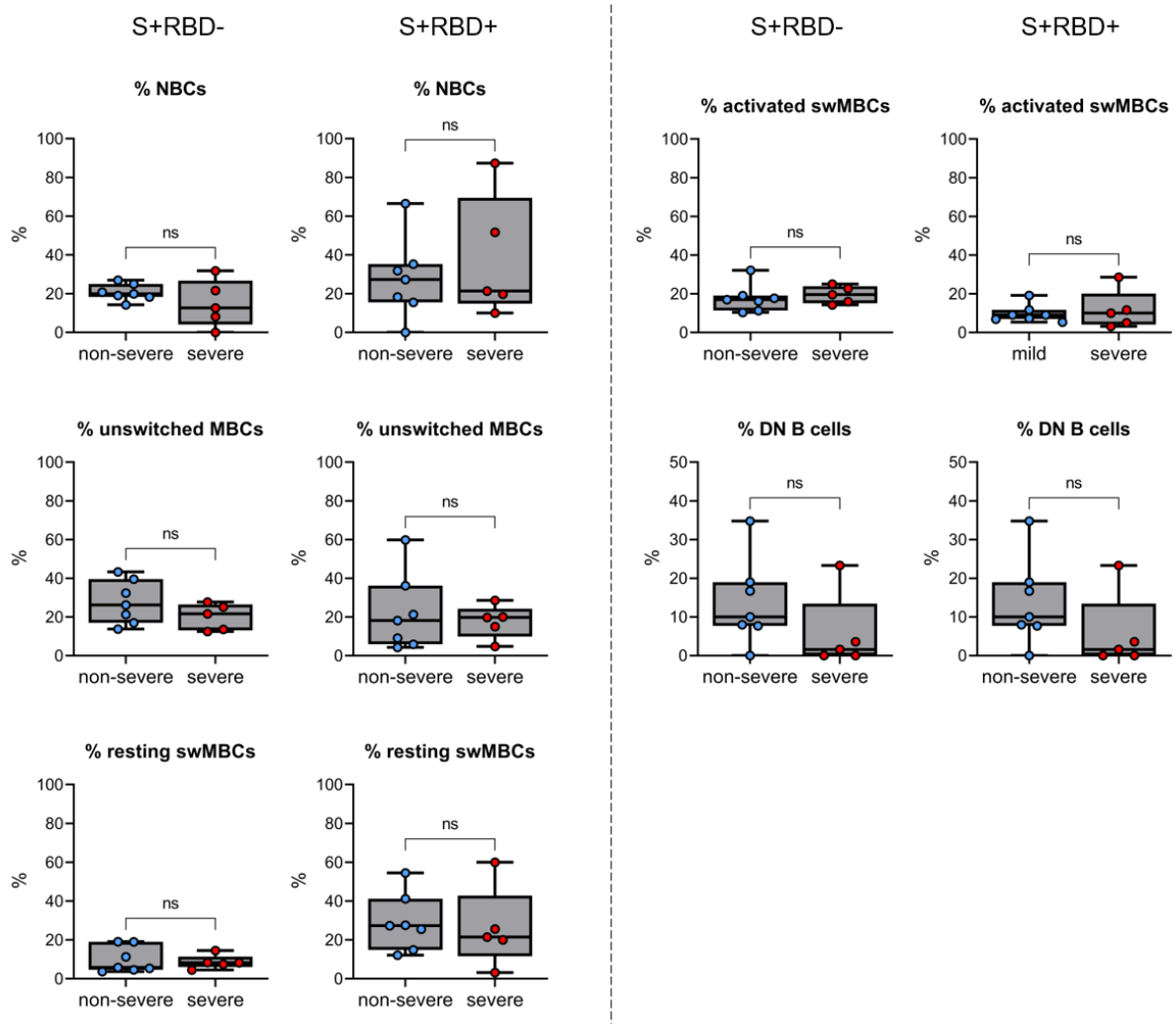

Figure S2: Percentage of spike-specific B cells among the major B cell subsets in patients who recovered from non-severe and severe COVID-19. The percentage of naïve B cells (NBC; IgD^+ $CD27^-$), unswitched memory B cells (MBCs; IgD^+ $CD27^+$), resting switched MBCs (swMBC; IgD^- $CD27^+$ $CD21^+$), activated swMBC (IgD^+ $CD27^+$ $CD21^-$), and double negative B cells (DN; IgD^- $CD27^-$) is shown side-by-side for non-RBD-specific (S+RBD-) B cells (left) and RBD-specific (S+RBD+) B cells (right). Results are shown for patients who recovered from non-severe (n = 7) and severe (n = 5) COVID-19.

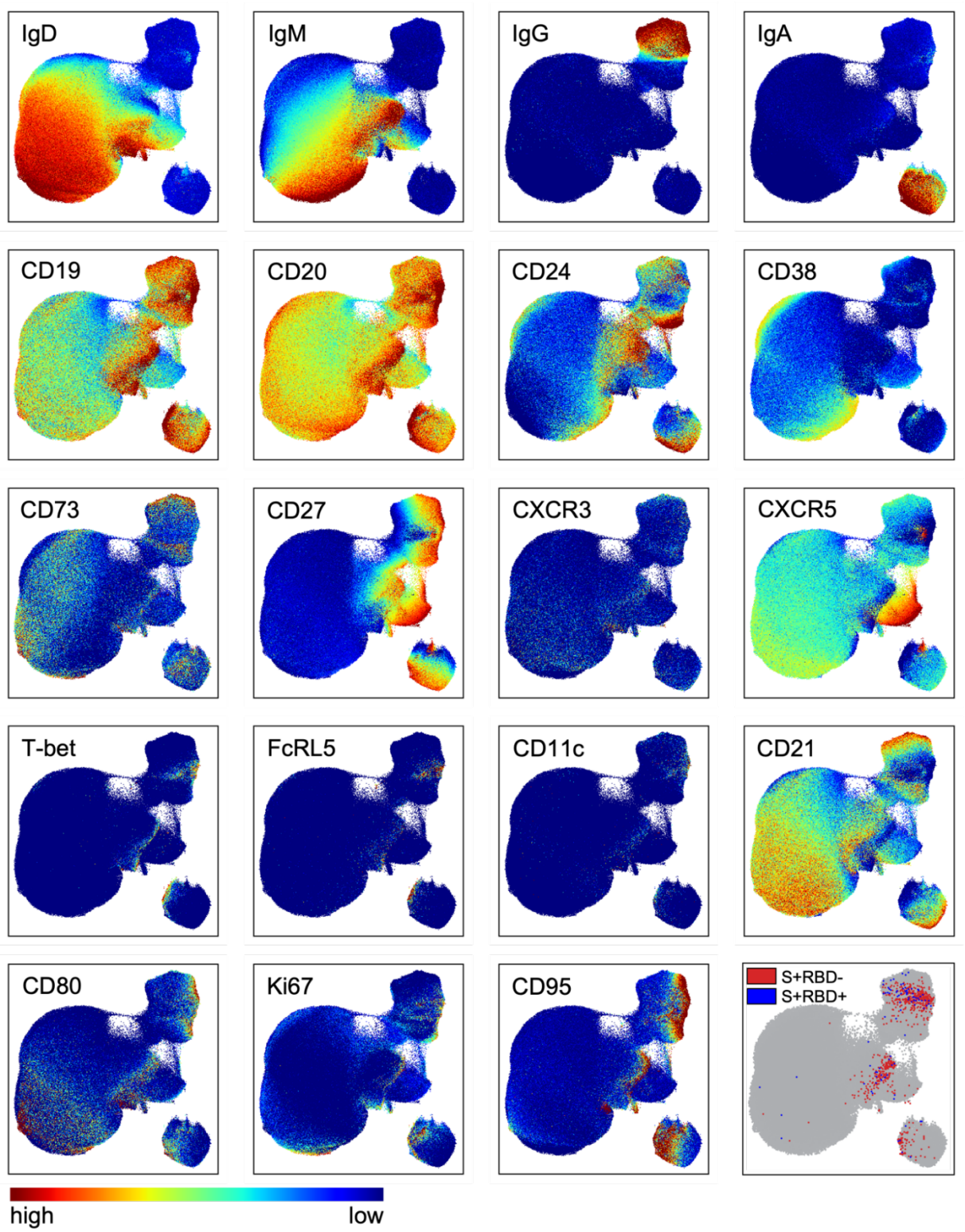

Figure S3: Composite UMAPs for all intracellular and surface markers included in this study. The plot in the bottom right shows the overlay of all non-RBD-specific (S+RBD-) and RBD-specific (S+RBD+) B cells onto the UMAP.

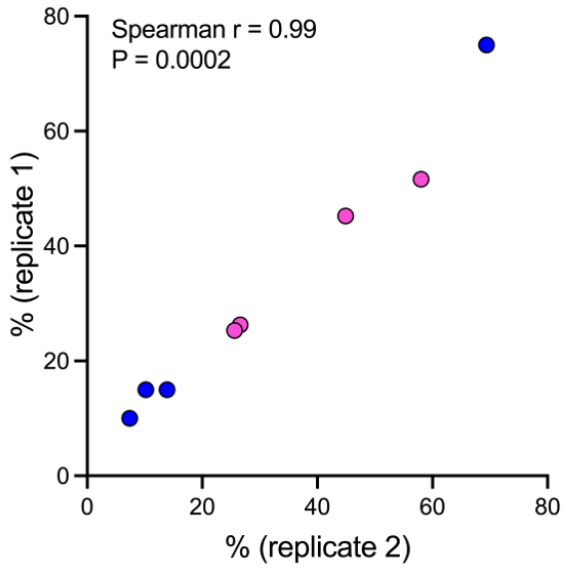

Figure S4: Correlation between technical replicates. Shown are the percentages of spike-specific B cells that express T-bet, FcRL5, CD11c, and CD21 in two technical replicates, one from a non-severe case (pink) and one from a severe case (blue), that were processed and analyzed independently and blinded on separate days. Two data points (pink, ~ 25%) were overlapping and were changed slightly for visualization purposes.

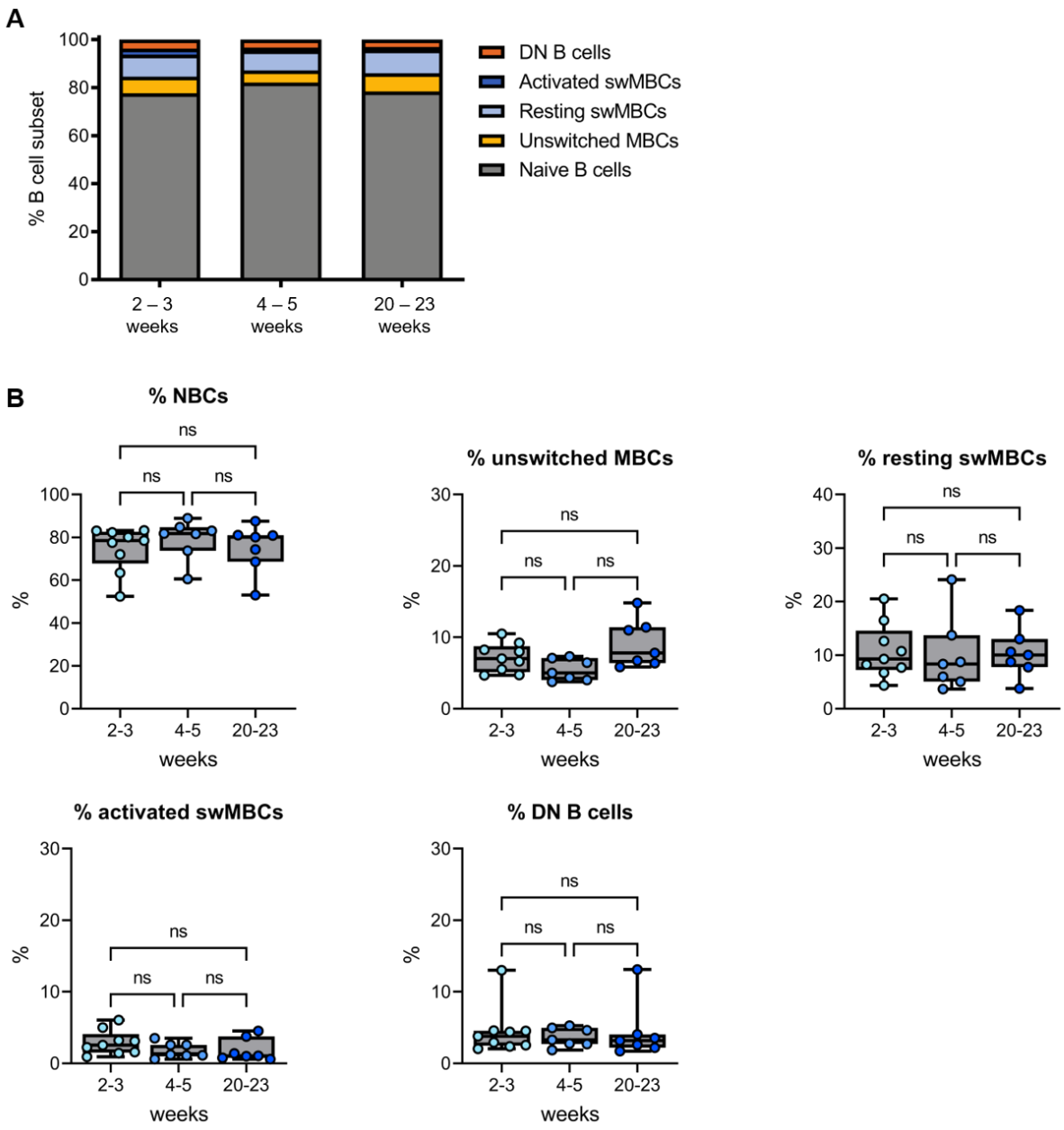

Figure S5: Distribution of major B cell subsets in recovered COVID-19 patients at 2 – 3, 4 – 5, and 20 – 23 weeks post-symptom onset. A) The median distribution of B cell subsets in recovered COVID-19 patients. **B)** The percentage of naïve B cells (NBC; IgD^+ $CD27^-$), unswitched memory B cells (MBCs; IgD^+ $CD27^+$), resting switched MBC (swMBCs; IgD^+ $CD27^+$ $CD21^+$), activated swMBCs (IgD^- $CD27^+$ $CD21^-$), and double negative B cells (DN; IgD^- $CD27^-$). In all graphs, results are shown for samples collected 2 – 3 (n = 9), 4 – 5 (n = 7), and 20 – 23 (n = 7) weeks post-symptom onset.

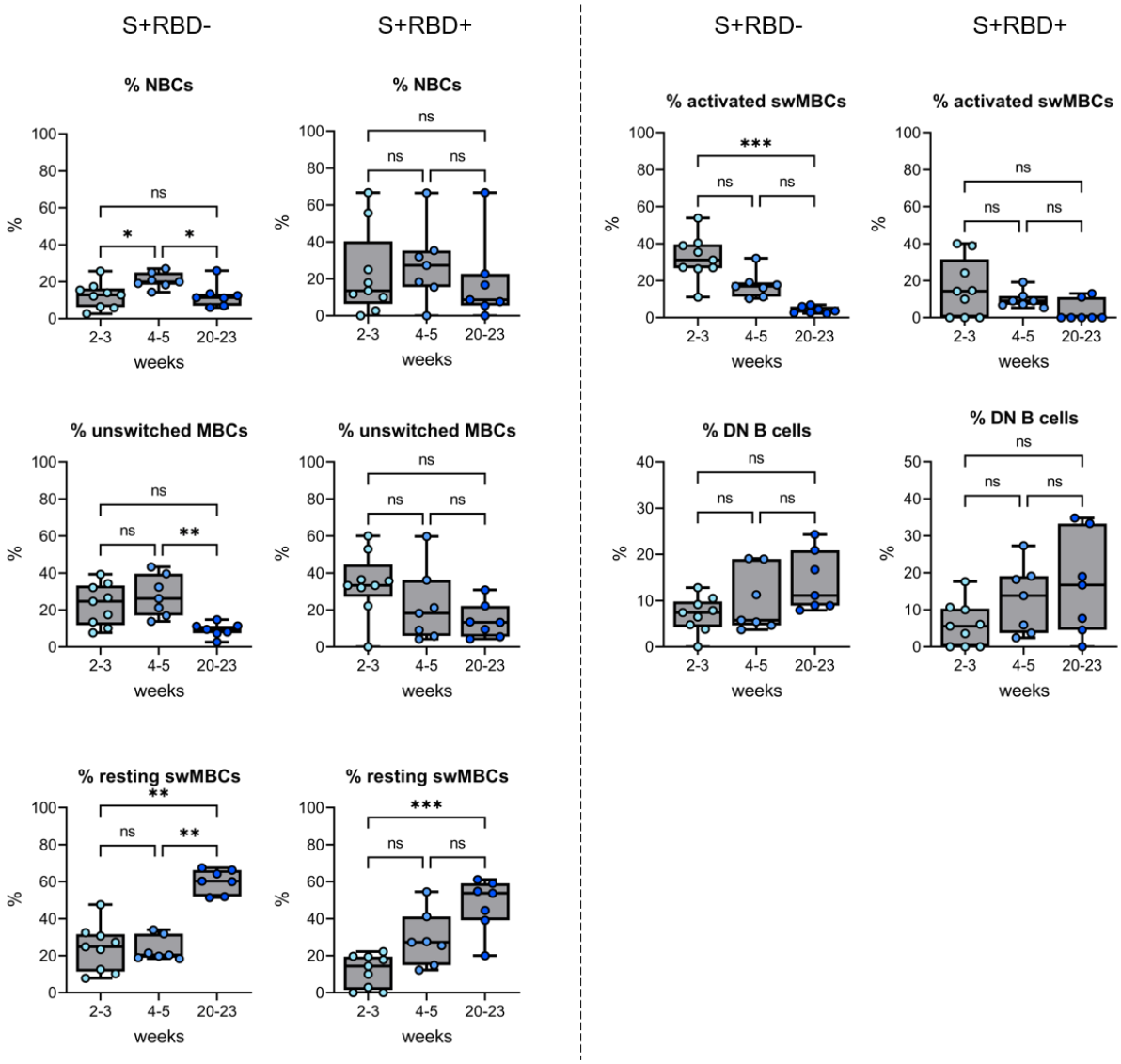

Figure S6: Percentage of spike-specific B cells among the major B cell subsets in recovered COVID-19 patients 2 – 3, 4 – 5, and 20 – 23 weeks post-symptom onset. The percentage of naïve B cells (NBC; IgD+CD27-), unswitched memory B cells (MBCs; IgD^+ $CD27^+$), activated switched MBCs (swMBC; IgD^- $CD27^+$ $CD21^-$), resting swMBC (IgD^+ $CD27^+$ $CD21^+$), and double negative B cells (DN; IgD^- $CD27^-$) is shown side-by-side for non-RBD-specific (S+RBD-) B cells (left) and RBD-specific (S+RBD+) B cells (right). In all graphs, results are shown for samples collected 2 – 3 (n = 9), 4 – 5 (n = 7), and 20 – 23 (n = 7) weeks post-symptom onset. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

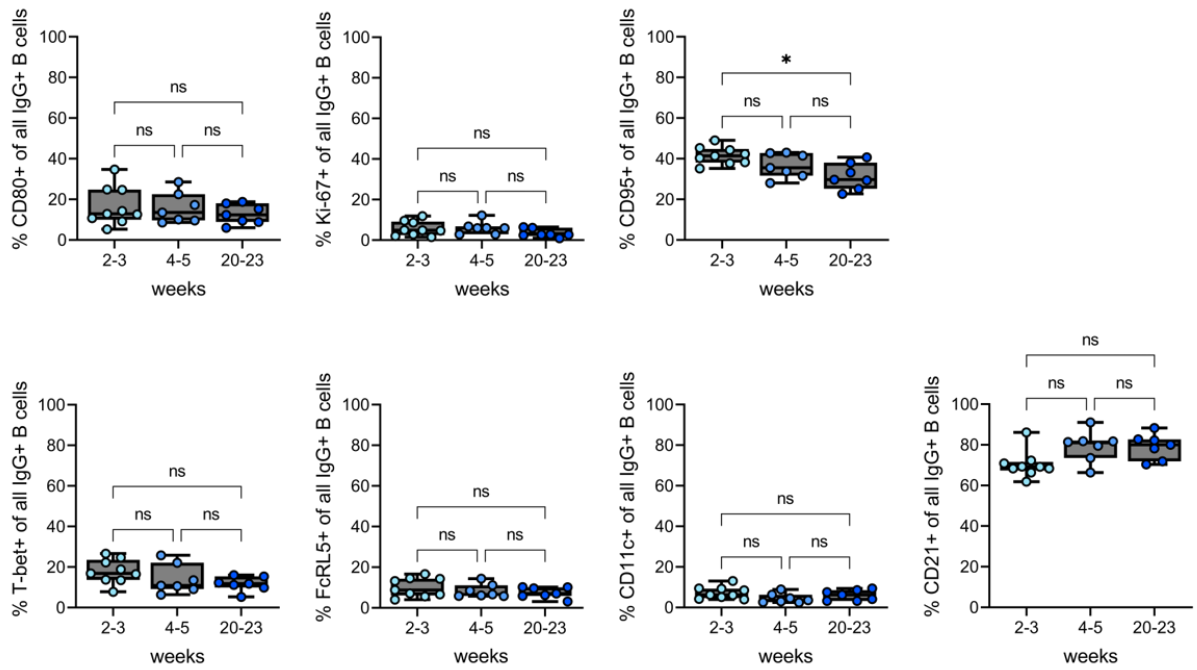

Figure S7: Expression of activation markers in all IgG$^+$ B cells shortly after recovery and five months post-symptom onset. In all graphs, results are shown for samples collected 2 – 3 (n = 9), 4 – 5 (n = 7), and 20 – 23 (n = 7) weeks post-symptom onset. * P < 0.05

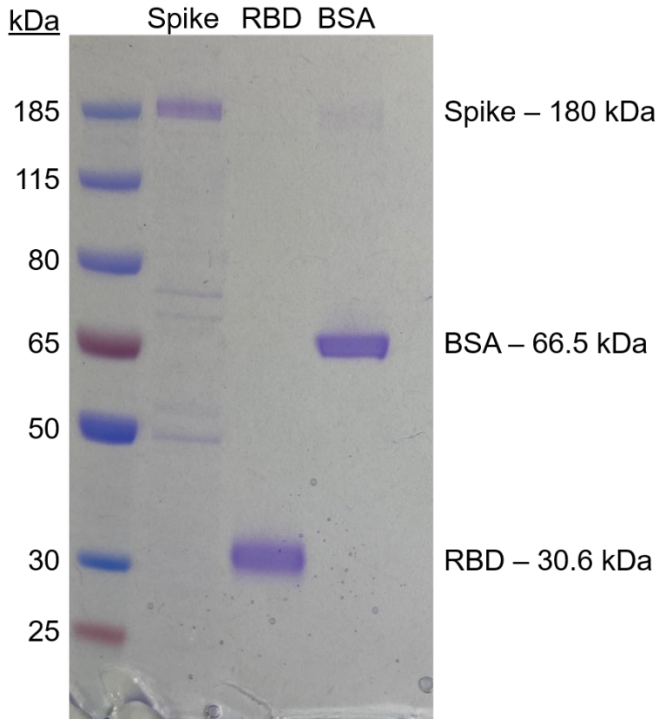

Figure S8: Quality control of purified S and RBD proteins by SDS-PAGE. 800 ng of spike, RBD, and BSA was run on a 4 – 12% Bis-Tris gel and stained using Imperial Protein Stain.

Table S1: COVID-19 patient characteristics

Donor ID	Sex	Age	Race / ethnicity	Severity score	Medical history[1]	Treatment regimen[2]	Hospital stay (days)	Days PSO[3] T1	Days PSO T2	Days PSO T3
Non-severe										
25	M	78	White, non-Hispanic	4	DM2, OB, CKD, HTN	R + P	4	18	n.a.	156
27	F	48	White, Hispanic	4	OB, HTN	R + P	2	20	34	147
29	M	39	White, Hispanic	4	DM2, OB	R + B	2	15	29	145
32	F	59	White, non-Hispanic	4	None	R + B	2	14	31	139
34	M	59	White, Hispanic	4	OB, CRD, HTN	R + P	2	20	31	n.a.
35	M	40	Black	4	DM1, HTN	R + B	3	16	28	n.a.
38	F	68	White, Hispanic	4	DM2, OB, HTN	R + P	4	19	33	n.a.
40	F	25	White, non-Hispanic	4	Asthma	R + B	2	14	29	n.a.
33	F	20	White, Hispanic	5	OB	R + P	2	n.a.	28	138
22	M	75	White, Hispanic	5	DM2, HTN	R + B	3	17	n.a.	155
21	M	68	White, Hispanic	6	DM2, OB, HTN	R + B	9	21	n.a.	157
Severe										
16	M	64	White, Hispanic	7	OB	P	12	n.a.	35	n.a.
28	M	50	White, Hispanic	7	OB	R + P	22	n.a.	31	n.a.
57	F	47	White, Hispanic	7	HTN	R + B	12	n.a.	32	n.a.
66	M	36	White, Hispanic	7	OB	R + B	24	n.a.	31	n.a.
73	M	50	White, Hispanic	7	OB, HTN	R + P	11	n.a.	37	n.a.

[1] DM2, diabetes mellitus type 2; HTN, hypertension; OB, obesity; CKD, chronic kidney disease; CRD, chronic respiratory disease

[2] R, remdesivir; B, baricitinib; P, placebo

[3] PSO, post-symptom onset; n.a., not available

Table S2: Reagents and antibodies used for spectral flow cytometry

Reagent / antibody	Fluorophore	Clone	Company / catalog number
Live/dead stain	Zombie UV		BioLegend / 423107
Streptavidin (RBD)	BV421		BioLegend / 405226
Streptavidin (spike1)	PE		Tonbo / 504317U100
Streptavidin (spike2)	APC		Tonbo / 204317U100
CD11c	AF532	N418	Thermo / 58011482
CD19	SB645	SJ25C1	Thermo / 64019842
CD20	BV785	2H7	BioLegend / 302355
CD21	PerCP-eF710	HB5	Thermo / 46021942
CD24	BV605	ML5	BioLegend / 311123
CD27	PE-Cy7	LG.3A10	BioLegend / 124215
CD38	APC-Fire/810	HIT2	BioLegend / 303549
CD73	PerCP-Cy5.5	AD2	BioLegend / 344013
CD80	PE-Cy5	2D10	BioLegend / 305210
CD95	BUV737	DX2	BD / 612790
CXCR3	PerCP	G025H7	BioLegend / 353740
CXCR5	APC-Cy7	J252D4	BioLegend / 356925
FcRL5	BUV805	509F6	BD / 749599
IgA	FITC	IS11-8E10	Miltenyi / 130093073
IgD	PE/Dazzle	IA6-2	BioLegend / 348239
IgG	BV510	M1310G05	BioLegend / 410715
IgM	BV711	MHM-88	BioLegend / 314539
Ki-67 (intracellular)	AF700	Ki-67	BioLegend / 350529
T-bet (intracellular)	Pacific Blue	4B10	BioLegend / 644807