Table S1. Autopsied Patients with COVID-19 studied by immunofluorescence and multispectral imaging

Duration of hospital stay	1-8 days	16-36 days
	n=5	n=6
Clinical Course		
Hospital stay duration (days) (mean ± s.d.)	3.6 (± 3.2)	25.8 (± 7.9)
Demographics		
Age (years) (mean ± s.d.)	66.2 (± 11.2)	63.8 (± 9.3)
Gender (% male)	60%	80%
BMI (kg/m^2) (mean ± s.d.)	33.0 (± 4.1)	30.2 (± 2.0)
Maximum Laboratory Values		
CRP (mg/L) (mean ± s.d.)	199.2 (± 102.3)	74.5 (± 44.8)
LDH (U/L) (mean ± s.d.)	590.0 (± 149.9)	423.0 (± 128.2)
Ferritin (ug/L) (mean ± s.d.)	703 (± 618.0)	410.8 (± 166.1)
D-dimer (ng/mL) (range)	1374 to >4000	725 to >4000

Body mass index (BMI), C-reactive protein (CRP), lactate dehydrogenase (LDH), standard deviation (s.d.)

Table S2. Summary of information on COVID-19 patients in whom circulating B cells were analyzed by flow cytometry.

	Convalescence	**Moderate**	**Severe (CRP int)** CRP < 200 mg/L	**Severe (CRP hi)** CRP > 200 mg/L
	mean (± SD)	mean (± SD)	mean (± SD)	mean (± SD)
	n=19	n=5	n=4	n=8
Demographics				
Age (years)	44.6 (± 17.1)	46.6 (± 16.3)	59.3 (± 10.8)	59.0 (± 15.3)
Deceased (%)	0	0	0	0
Gender (% male)	42.1	40.0	100.0*	62.5
BMI (kg/m^2)	26.2 (± 4.3)	29.8 (± 9.1)	36.3 (± 7.8)**	35.3 (± 10.5)*
Clinical Course				
Hospitalized due to COVID-19 (%)	0	40	100	100
Admitted to ICU due to COVID-19 (%)	0	0	100	100
Mechanically ventilated due to COVID-19 (%)	0	0	100	100
Total Hospitalization (days)	0	3.0 (± 4.5)	26.5 (± 11.0)	50.6 (± 17.9)$^+$
Total Mechanical Ventilation (days)	0	0	20.5 (± 14.5)	36.3 (± 21.5)
Symptom Duration at Draw (days)	0	13.8 (± 10.8)	9.5 (± 4.7)	32.4 (± 11.2)$^{++}$
Convalescence Period at Draw (days)	32.3 (± 13.9)	0	0	0
Pre-Draw Immunosuppression				
Steroids (≥ prednisone 20 mg daily) (%)	na	20.0	25.0	12.5
Hydroxychloroquine (3-5 day total course) (%)	na	0	25.0	75.0
Tocilizumab (single dose or enrolled in trial) (%)	na	20.0	25.0	12.5
Maximum Laboratory Values				
CRP (mg/L)	na	64.3 (± 20.9)	122.2 (± 36.5)	301.5 (± 33.0)$^{+++}$
ESR (mm/h)	na	77.5 (± 54.4)	46.3 (± 14.9)	116.7 (± 34.3)$^{++}$
LDH (U/L)	na	323.5 (± 19.1)	670.8 (± 240.8)	762.3 (± 491.0)
Ferritin (ug/L)	na	750.0 (± 5.7)	6045.5 (± 8471.8)	2919.1 (± 3452.6)
D-dimer (ng/mL)	na	1306.0 (± 849.0)	5963.8 (± 3242.7)	8333.1 (± 2285.7)

Convalescence was defined as a clinically asymptomatic state on the date of blood draw, either from a baseline asymptomatic state or recuperated from moderate disease. Moderate disease was defined as active clinical symptoms resulting from COVID-19 infection on the date of blood draw that did not require intensive care unit (ICU) admission or mechanical ventilation for supportive care. Severe disease was defined as active clinical symptoms resulting from COVID-19 infection on the date of blood draw that did require ICU admission and mechanical ventilation for supportive care. Severe disease was further subdivided by maximum CRP level during inpatient admission as CRP < 200 mg/L 'CRP intermediate (int)' and CRP > 200 mg/L 'CRP high (hi).' Body mass index (BMI), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), lactate dehydrogenase (LDH), not available (na), standard deviation (SD), *significance by Student's t-test to 'convalescence' group, $^+$significance by Student's t-test to 'severe (CRP int)' group.

Supplementary File

Supplementary Methods
Supplementary Figures 1-7

Supplementary Methods

Patient Cohorts

Tissue analysis Cohort:

Thoracic lymph nodes and spleens samples from COVID-19 patients were obtained through the Brigham and Women's hospital Department of Pathology. All cases were retrieved from the Anatomic Pathology files of Brigham and Women's Hospital and included 11 patients with laboratory confirmed COVID-19 who underwent autopsy in 2020. All patients had tested positive for SARS-CoV-2 by RT-PCR of nasopharyngeal swabs in a laboratory during hospital admission. All cases were divided to two groups; Early (less than ten days from respiratory symptoms onsets to death, hospitalization of up to 8 days), Late (Hospitalized for 15-36 days prior to death). In addition, 2 mesenteric lymph nodes, ten healthy human tonsils and seven spleens were obtained from the Ragon Institute Tissue Core, which were histologically normal.

Peripheral Blood Cohort:

Peripheral blood samples were drawn from both Outpatients and Inpatients with COVID-19 at Massachusetts General Hospital and fresh blood was analyzed for flow cytometry using two 25 color panels from 180 patients. Data is presented on B cell populations from 36 patients, including moderately ill, severely ill and convalescent patients.

Multi-color immunofluorescence staining

Tissue samples were fixed in formalin, embedded in paraffin, and sectioned. These specimens were incubated with the following antibodies: anti-CD3 (clone: A045229-2; DAKO), anti-CD4 (clone: CM153A; Biocare Medical), anti-CD19 (clone: SKU310; Biocare Medical), anti-Bcl6

(clone: LN22; Biocare Medical), anti-AID (clone: ZA001; Invitrogen), anti-T-bet (clone: ab150440; Abcam), GATA3 (clone: CM405A; Biocare), Rorc (clone: ab212496; Abcam), CXCR5 (clone: MAB190; R&D Systems), Foxp3 (clone: 98377; Cell Signaling Technology), anti-CD8 (clone: ab85792; Abcam), anti-IgD (clone: AA093; DAKO), anti-CD27 (clone: ab131254; Abcam), anti-IgG (clone: ab109489; Abcam), anti-TNF-α (clone: ab6671; Abcam), and anti-CD35 (clone: ab25; Abcam) followed by incubation with a secondary antibody using an Opal™ Multiplex Kit (Perkin Elmer). The samples were mounted with ProLong™ Diamond Antifade mountant containing DAPI (Invitrogen).

Microscopy and Quantitative Image Analysis

Images of the tissue specimens were acquired using the TissueFAXS platform (TissueGnostics). For quantitative analysis, the entire area of the tissue was acquired as a digital grayscale image in five channels with filter settings for FITC, Cy3, Cy5 and AF75 in addition to DAPI. Cells of a given phenotype were identified and quantitated using the TissueQuest software (TissueGnostics), with cut-off values determined relative to the positive controls. This microscopy-based multicolor tissue cytometry software permits multicolor analysis of single cells within tissue sections similar to flow cytometry. In addition, multispectral images (seven-colors staining) were unmixed using spectral libraries built from images of single stained tissues for each reagent using the StrataQuest (TissueGnostics) software. StrataQuest software was also used to quantify cell-to-cell contact. In the Strata Quest cell-to-cell contact application, masks of the nuclei based on DAPI staining establish the inner boundary of the cytoplasm and the software “looks” outwards towards the plasma membrane boundary. Overlap of at least 3 pixels of adjacent cell markers is required to establish a “contact” criterion. Although the software has been developed and validated more recently, the principle of the method and the algorithms used have been described in detail elsewhere (reference Ecker RC, Steiner GE. Microscopy-based multicolor tissue

cytometry at the single-cell level. *Cytometry Part A : the journal of the International Society for Analytical Cytology*. Jun 2004;59(2):182-90. doi:10.1002/cyto.a.20052).

Flow cytometry

Fresh PBMCs were stained. Prior to antibody staining, Fc receptors were blocked using Human TruStain FcX (BioLegend, 422302) at a concentration of 1:20 on ice for 15 minutes. Cells were surface stained on ice, protected from light, using optimized concentrations of fluorochrome-conjugated primary antibodies for 30 minutes using the following antibody panel (manufacturer, clone, concentration used): CD3 (HIT3a, BioLegend), CD19 (SJ25C1, BioLegend), CD27 (M-T271, BD Biosciences), IgD (IA6-2, BioLegend), CD38 (HIT2, BD Biosciences), CD24 (ML5, BioLegend), CD10 (HI10a, BioLegend), CD45RB (MEM-55, Thermo Fisher), CD21 (B-ly4, BD Biosciences). CD73 (AD2, BioLegend),

Flow cytometry was performed on a BD Symphony (BD Biosciences, San Jose, CA) and rainbow tracking beads were used to ensure consistent signals between flow cytometry batches. FCS files were analyzed, and B cell subsets were quantified using FlowJo software (version 10). Assembly of quantified CD19+ B cell subsets and statistical analysis was performed in GraphPad Prism (version 7.01).

Statistics

Flow cytometry, clinical correlations and tissue studies. GraphPad Prism version 8 was

used for statistical analysis, curve fitting and linear regression. A two-tailed Mann-Whitney U

test was used to calculate p-values for continuous, non-parametric variables. For comparing more than one population, Kruskal-Wallis testing was used with Dunn's multiple comparison testing.

A p-value of < 0.05 was considered significant.

Study Approval

This study was performed with the approval of the Institutional Review Boards at the Massachusetts General Hospital and the Brigham and Women's Hospital.

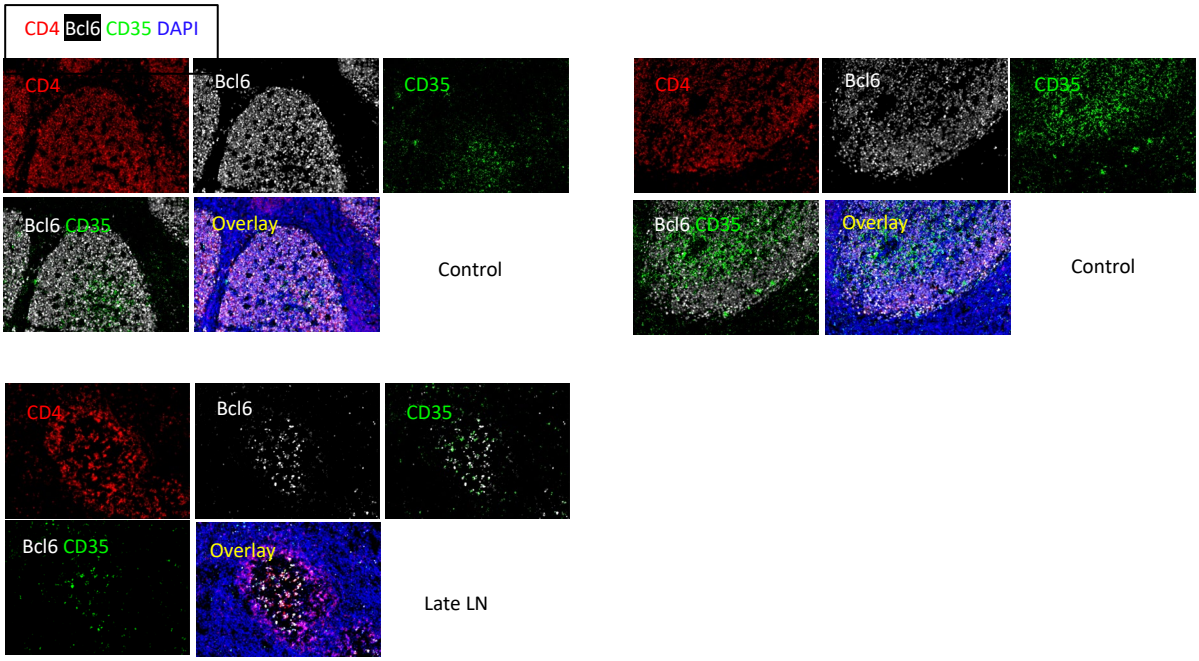

Fig. S1. No loss of FDCs in COVID-19
Representative multi-color immunofluorescence images of CD4 (red), Bcl6 (white), CD35 (green) and DAPI (blue) staining in a lymph node from late COVID-19 patients and controls.

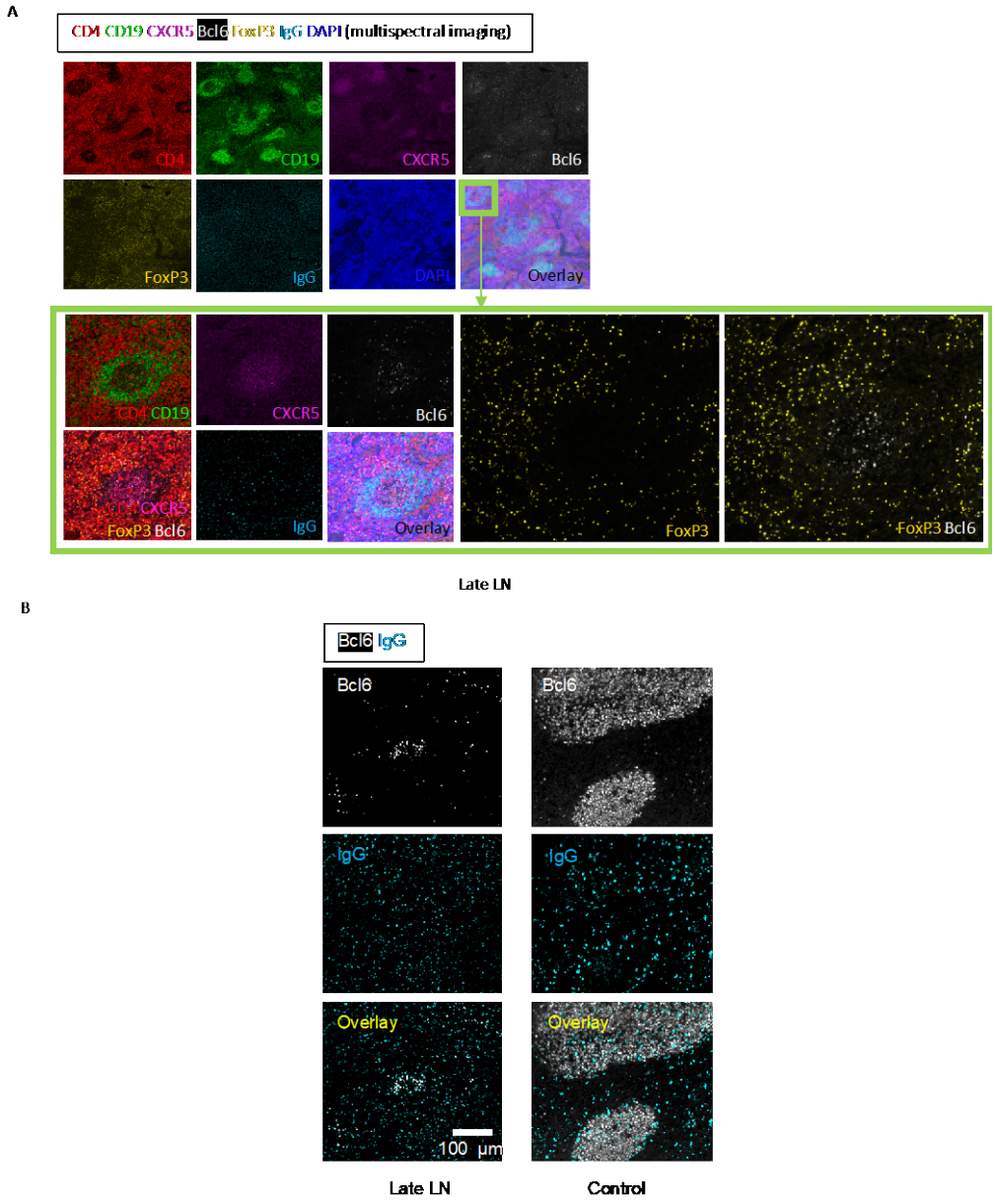

Fig. S2. A. Increased T regs but no differentiation into TFR cells late in COVID-19. Representative multi-spectral 7 colors immunofluorescence images showing CD4 (red), CD19 (green), CXCR5 (purple), Bcl6 (white) FoxP3 (yellow), IgG (light blue) and DAPI (blue) staining in lymph nodes from late COVID-19 patients. Images circled green rectangular showing high-power images of a follicle. No FoxP3+/Bcl6+ cells are seen (red staining with no green overlap) in a follicle.

B. IgG+ plasmablasts are found in follicular and extrafollicular areas in COVID-19 lymph nodes. IgG+ cells were abundant in follicular and extrafollicular areas in both COVID-19 lymph nodes and controls.

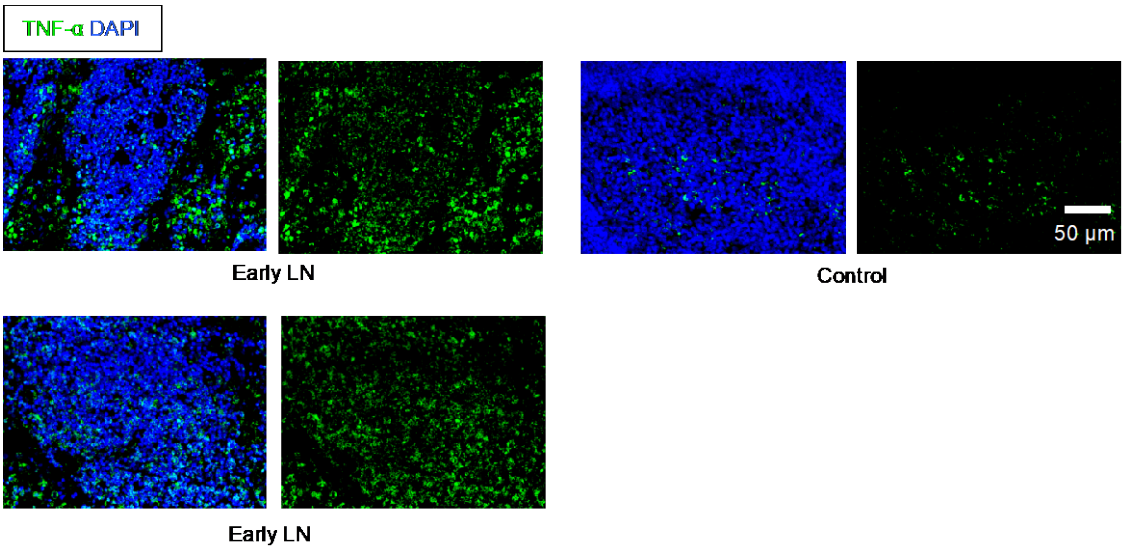

Fig. S3. Large increase in TNF-α production at both follicular and extra-follicular sites in COVID-19 lymph nodes. Controls have low levels but only in follicle
Representative multi-color immunofluorescence images of TNF-α(green) and DAPI (blue) staining in lymph nodes from early COVID-19 patients and controls.

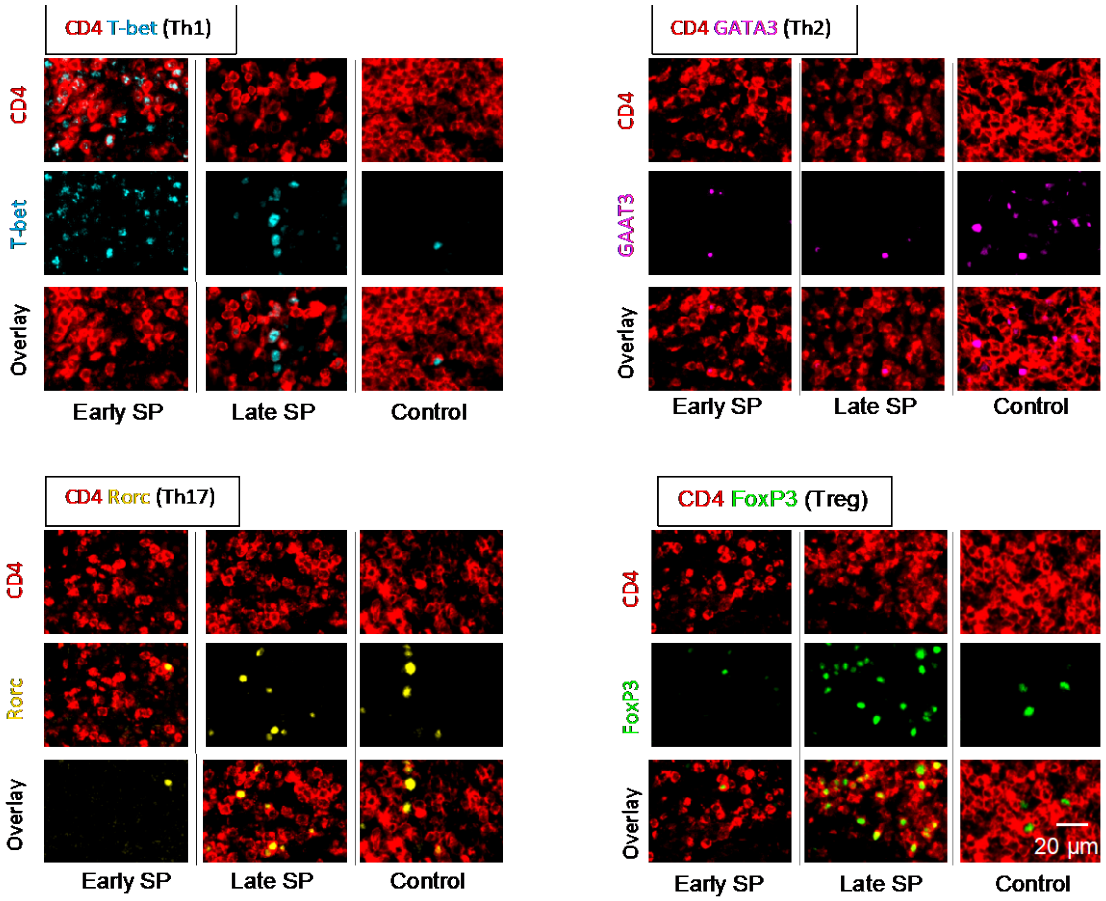

Fig. S4. Increased Th1 cells in Spleen in COVID-19
Representative multi-color staining showing T_{H1}, T_H2, T_{H17} and Treg cells in spleens from early and late COVID-19 patients and control.

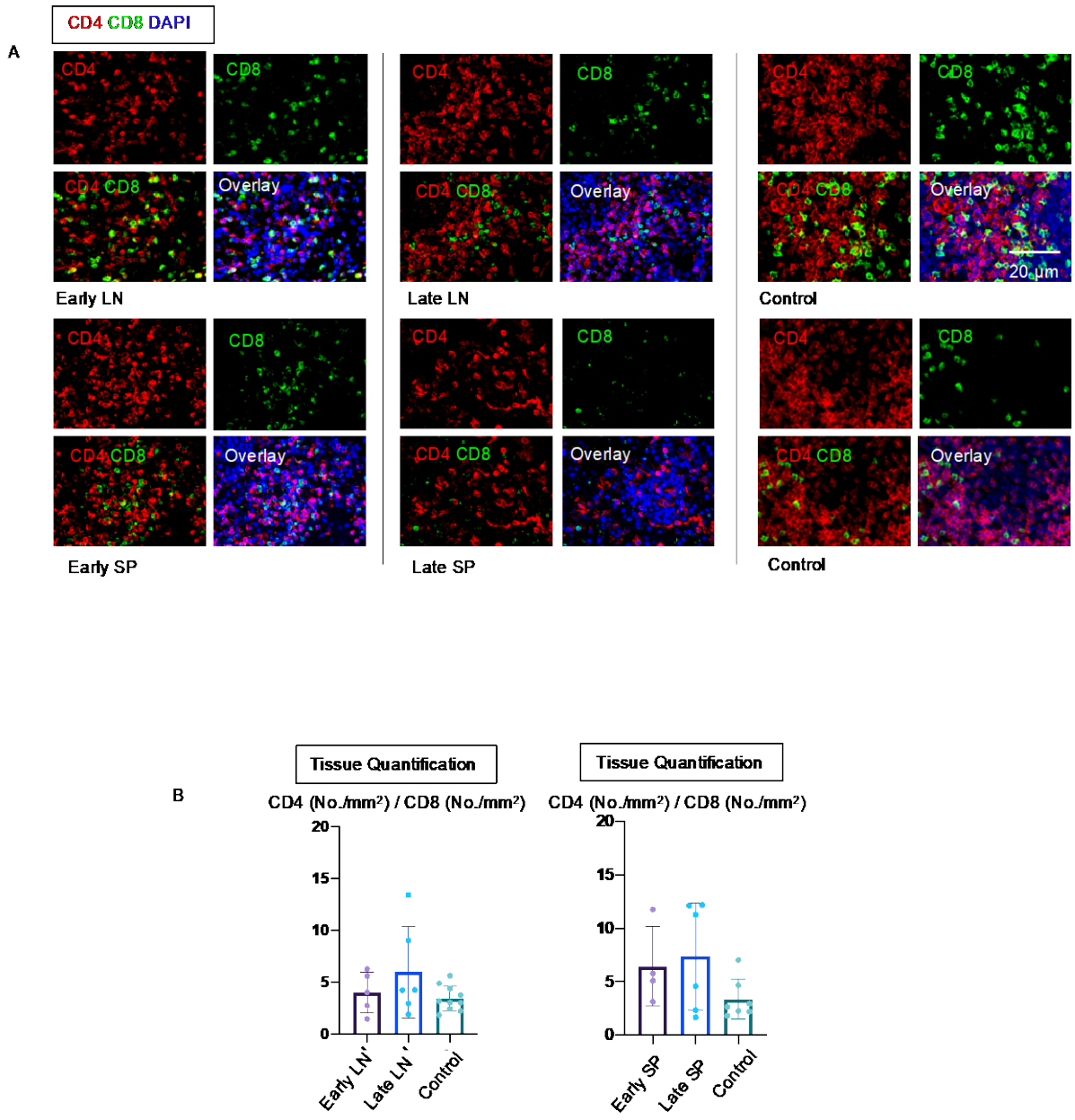

Fig. S5. Increase in CD4+/CD8+ T cell ratio in lymph nodes and spleens in COVID-19
(A) Representative multi-color immunofluorescence images of CD4 (red), CD8 (green) and DAPI (blue) staining in lymph nodes and spleens from early (left) and late (middle) COVID-19 patients and controls (right). (B) Relative ratios of CD4 and CD8 T cells (No./mm^2) in lymph nodes and spleens from early and late COVID-19 patients and controls.

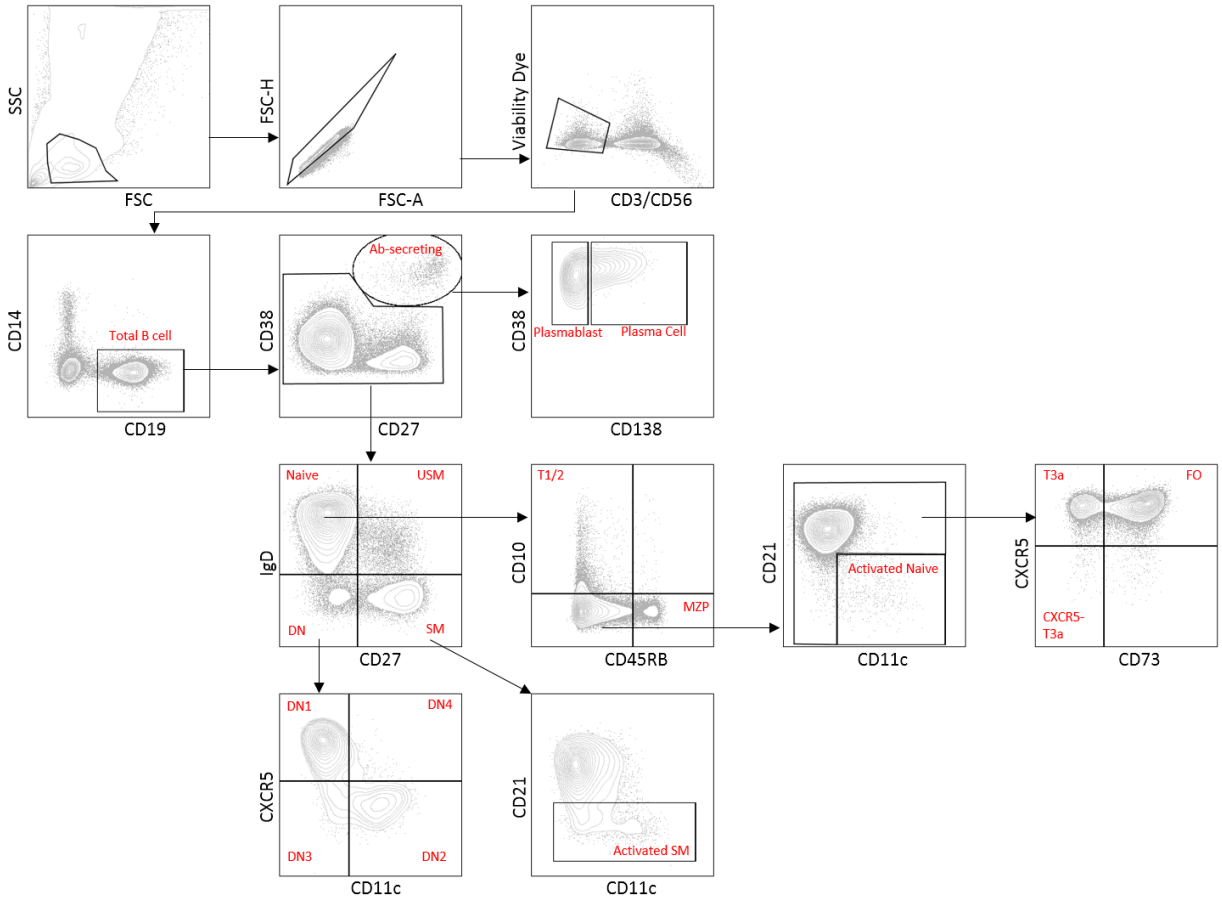

Fig. S6. B cell gating strategy for COVID-19 PBMCs. Antibody-secreting (Ab-secreting), double negative (DN), follicular (FO), marginal zone precursor (MZP), switched memory (SM), transitional (T), unswitched memory (USM).

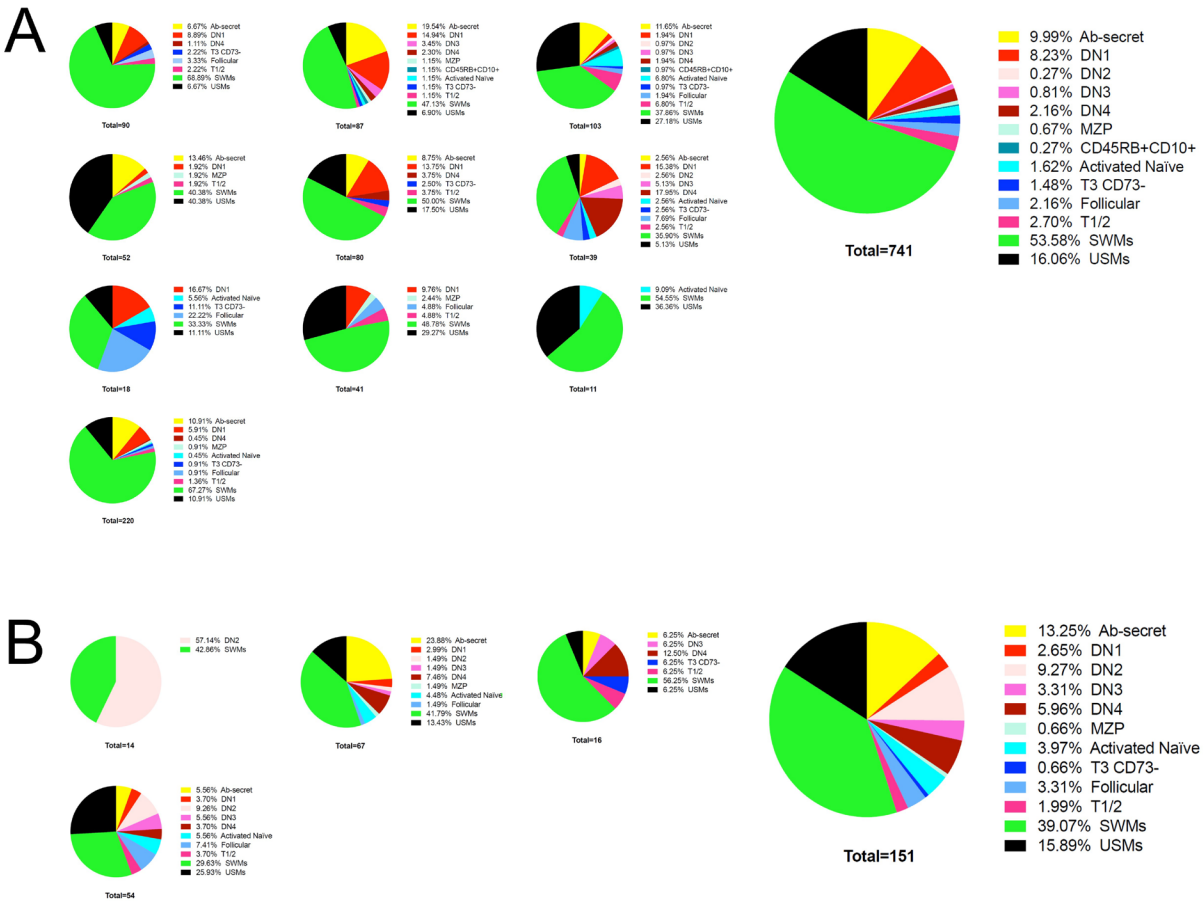

Fig. S7. CoV-2-RBD binding B cell subsets. All CD19+ B cells binding to CoV-2-RBD shown for (**A**) convalesced COVID-19 patients (n=10) and (**B**) hospitalized COVID-19 patients (n=4). Total reactive CD19+ B cells per patient (small plots) and summation of all CoV-2-RBD binding B cells per clinical criteria (large plots) shown. Antibody-secreting (Ab-secret), double negative (DN), marginal zone precursor (MZP), switched memory (SWM), transitional (T), unswitched memory (USM).