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Supplemental Methods

Flow cytometry analysis

Immunophenotype analysis on whole peripheral blood was performed according to a standard no-wash procedure, using a PrepPlus™ 2 workstation (Beckman Coulter Inc., Brea, CA, USA). Blood samples were incubated with antibodies (Beckman Coulter Inc., Brea, CA, USA) for 15 min at 20°C. Following is the list of antibodies used:

- FITC-conjugated CD57 (clone NC1), PE-conjugated CD45RA (ALB11), ECD-conjugated CD8 (SFCI21Thy2D3), PC5.5-conjugated CD56 (N901), PC7-conjugated CD4 (SFCI12T4D11), APC-conjugated CD27 (1A4CD27), APC A700-conjugated CD45 (J33), APC A750-conjugated CD3 (UCHT1), PB-conjugated CD16 (3G8), KR OR-conjugated CD19 (J3-119)
- FITC-conjugated CD57 (clone NC1), PE-conjugated HLA-DR (Immu-357), ECD-conjugated CD8 (SFCI21Thy2D3), PC5.5-conjugated CD38 (LS198-4-3), PC7-conjugated CD4 (clone SFCI12T4D11), APC-conjugated CD14 (RMO52), APC A700-conjugated CD45 (J33), APC A750-conjugated CD3 (UCHT1), PB-conjugated CD16 (3G8), KR OR-conjugated CD19 (J3-119)
- PE-conjugated IgD (IA6-2), ECD-conjugated CD3 (UCHT1), PC5.5-conjugated CD27 (1A4CD27), PC7-conjugated CD20 (clone B9E9), APC-conjugated IgM (SA-DA4), APC A700-conjugated CD45 (J33), APC A750-conjugated CD38 (LS198-4-3), PB-conjugated CD21 (BL13), KR OR-conjugated CD19 (J3-119)

Red blood cell lysis and cell fixation were performed using TQ-Prep workstation and ImmunoPrep reagent system (Beckman Coulter Inc., Brea, CA, USA). Samples were acquired with Navios and analyzed with Navios software (Beckman Coulter Inc., Brea, CA, USA).

To determine the intracellular levels of p-STAT1 and p-STAT3, whole peripheral blood was incubated with FcR Blocking reagent (Miltenyi Biotec, Paris, FR) followed by the addition of anti-human FITC-conjugated CD56 (BD Bioscience, San Jose, CA, USA; clone NCAM16.2), PerCP-Cy5.5-conjugated CD3 (BD Bioscience, San Jose, CA, USA; clone UCHT1), PE.Cy7-conjugated CD19 (eBiosciences, ThermoFisher Scientific, Waltham, MA, USA; clone HIB19), APC.H7-conjugated CD14 (BD Bioscience, San Jose, CA, USA; clone MφP9), Brilliant Violet 421™-conjugated CD15 (BD Bioscience, San Jose, CA, USA; clone W6D3) antibodies and Aqua LIVE/DEAD dye (ThermoFisher Scientific, Waltham, MA, USA). Red blood cells were lysed using Cal-Lyse™ Lysing Solution (ThermoFisher Scientific, Waltham, MA, USA) in accordance with the manufacturer's instructions. Cells were then fixed with 2% paraformaldehyde and permeabilized with 90% cold methanol. For the intracellular staining, PE-conjugated p-STA3 (pTyr705) (Cell Signaling Technologies, Danvers, MA, USA, clone D3A7), APC-conjugated p-STA3 (pTyr705) (eBioscience, ThermoFisher Scientific, Waltham, MA, USA; clone LUVNKLA) and Alexa Fluor® 647-conjugated pSTAT1 (pY701) (BD

Bioscience, San Jose, CA, USA; clone 4a) antibodies were used. All steps were performed in ice. Samples were acquired with FACS Canto II (BD, Franklin Lakes, NJ, USA) using the gate strategy exemplified in Supplemental Figure 4. Data were analyzed with FlowJo software (Tree Star, Inc., Ashland, OR, USA).

To quantify the amount of monocytes and monocyte subsets (defined as classical, $CD14^{high}$ $CD16^{low/dim}$; intermediate $CD14^{int}$ $CD16^{+}$; non classical $CD14^{low/dim}$ $CD16^{high}$), peripheral blood was incubated with FcR Blocking reagent (Miltenyi Biotec, Paris, FR) followed by the addition of: anti-human PE-conjugated CD56 (BD Bioscience, San Jose, CA, USA; clone NCAM16.2), FITC-conjugated-CD16 (Biolegend, San Diego, CA, USA; clone 3G8), PerCP-Cy5.5-conjugated CD3 (BD Bioscience, San Jose, CA, USA; clone UCHT1), PE.Cy7-conjugated HLA-DR (eBiosciences, ThermoFisher Scientific, Waltham, MA, USA; clone L243), APC.H7-conjugated CD14 (BD Bioscience, San Jose, CA, USA; clone MφP9), Brilliant Violet 421™-conjugated PD-L1 (BD Bioscience, San Jose, CA, USA; clone MIH1) antibodies and Aqua LIVE/DEAD dye (ThermoFisher Scientific, Waltham, MA, USA). Red blood cells were lysed using Cal-Lyse™ Lysing Solution (ThermoFisher Scientific, Waltham, MA, USA) in accordance with the manufacturer's instructions using the gate strategy exemplified in Supplemental Figure 5.

To perform t-SNE analysis, cells were gated based on CD45 expression and SSC. Cell number was then reduced to $5x10^3$ events and 12 samples per group were concatenated in a single FCS file, using FlowJo software, version 10.6.2. The concatenated file was imported into the R/Bioconductor platform through the flowCore package (https://www.bioconductor.org/packages/release/bioc/html/flowCore.html).

After compensation, data was transformed using the estimated Logicle transformation (1). Subsequently, t-SNE and FlowSOM metaclustering analyses were performed through the Catalyst package (https://github.com/HelenaLC/CATALYST) using the default settings. The following parameters were used for the analysis: FSC-A, SSC-A, CD45, CD3, CD4, CD8, CD19, CD56, CD16, CD57, CD45RA, CD27.

Detection of cytokines and serology

Sera of COVID-19 patients were tested for IL-6, TNFα, IL-1β and IL-8 production by Ella Automated Immunoassay System (RnD system, Minneapolis, MN 55413) according to the manufacturer's instruction. Cytokines released by patients' monocytes and neutrophils were quantified by Human ProcartaPlex™ Panel 1 multiplex (ThermoFisher Scientific, Waltham, MA, USA).

The ELISA assay to detect Immunoglobulins (Ig) used fragment of the SARS-CoV2 spike glycoprotein (S-protein) as antigens based on a recently published protocol (2). The Spike SARS-CoV2 glycoprotein receptor binding domain (RBD) and the soluble full-length trimeric ectodomain used as antigens were expressed in mammalian HEK293 cells at IEO, Milan by Drs. Marina Mapelli and Sebastiano Pasqualato as glycosylated proteins by transient

transfection with pGACCS vectors generated in Dr. Krammer's laboratory. The constructs were synthesized using the genomic sequence of the isolated virus, Wuhan-Hi-1 released in January 2020, and contained codons optimized for expression in mammalian cells. Secreted proteins were purified from the culture medium by affinity chromatography, quantified and stored in liquid nitrogen in aliquots. The ELISA tests to detect IgG and IgA in patients' sera used as antigens both the recombinant fragments of the RBD of the Spike SARS-CoV2 glycoprotein and the soluble ectodomain. After binding of the proteins to a Nunc Maxisorp ELISA plate, patients' sera to be analyzed were applied to the plate to allow antibody binding, then revealed with secondary anti-human-IgG (BD) or IgA (Biolegend) antibodies conjugated to HRP. Reaction was revealed upon addition of TMB (Merck). Optical density at 450 nanometers was measured on a Glomax (Promega) plate reader. All samples were tested with a two-step ELISA assay, as indicated in (3). The first step consisted in the detection of putative positives towards the RBD fragment, that were further validated in a second-step assay on the full-length soluble ectodomain.

Cell isolation and functional assay

Cells were isolated from EDTA-treated tubes (BD Biosciences, NJ, USA) and freshly separated by Ficoll-Hypaque (GE Healthcare, Uppsala, Sweden) gradient centrifugation. PBMCs were counted and the monocyte fraction ($CD14^+$) was further isolated by CD14-microbeads (Miltenyi), following manufacturer's instructions. From the $CD14^-$ fraction the $CD66^+$low density gradient neutrophils (LDNs) were isolated by the sequential addition of CD66b-FITC antibody (BD Biosciences, NJ, USA) and microbeads anti-FITC (Miltenyi), following manufacturers' instructions. The normal density neutrophils (NDNs) $CD66b^+$ were isolated from the red blood cell (RBC) layer by dextran density gradient followed by CD66b-antibody as described for LDNs. The purity of each fraction was evaluated by flow cytometry analysis. Samples with a purity greater than 95% were assessed for their suppressive capacity. $2x10^6$ cells of each cell type were plated in 24-well plates for 12 hours. At the end of the incubation, viability was evaluated by flow cytometry and Trypan blue assay, and both the supernatants and the cells were collected and cultured with CellTrace (Thermo Fisher Scientific) labeled PBMCs, stimulated with coated anti-CD3 (clone OKT-3, eBioscience, Thermo Fisher Scientific) and soluble anti-CD28 (clone28.2, eBioscience, Thermo Fisher Scientific) for 4 days in 37°C and 8% CO_2 incubator. For the cells a ratio of 3:1 (target:effector) was used. At the end of the culture, cells were stained with anti-CD3-PE/Cy7 (UCHT1, eBioscience, Thermo Fisher Scientific) and CellTrace signal of lymphocytes was analyzed with FlowJo software (Tree Star, Inc. Ashland).

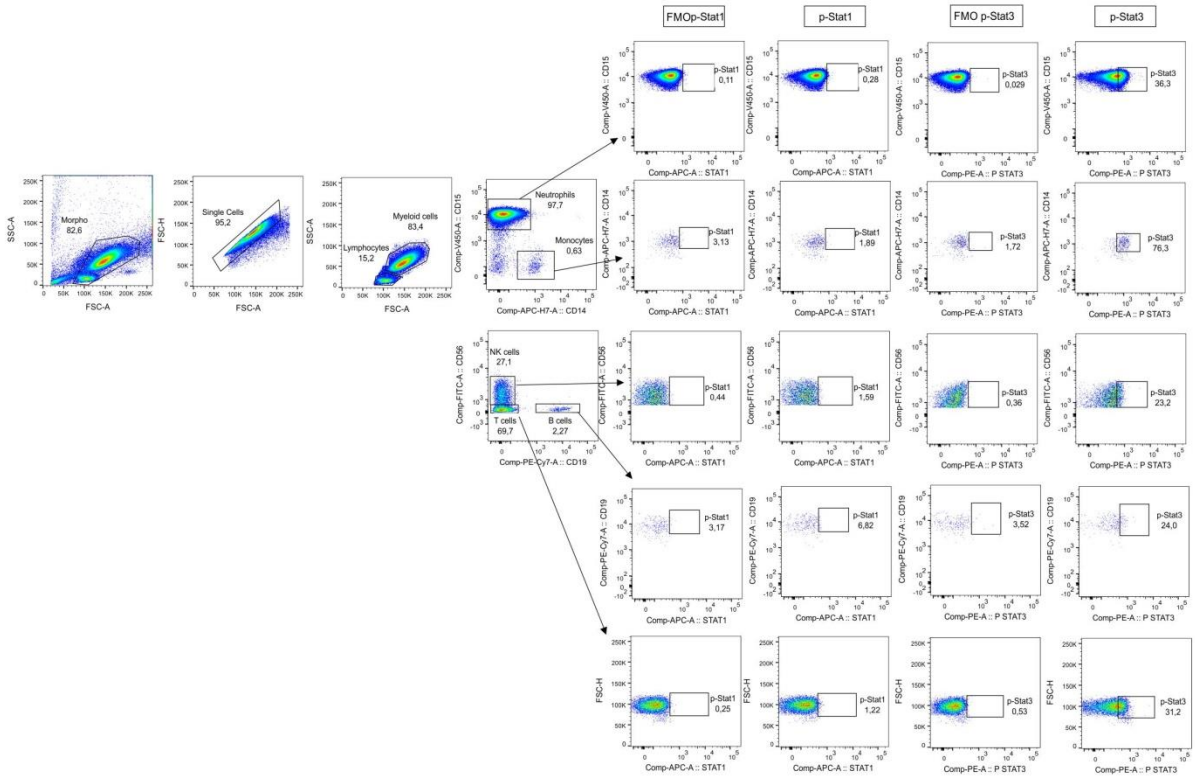
References

1. Parks DR, Roederer M, and Moore WA. A new "Logicle" display method avoids deceptive effects of logarithmic scaling for low signals and compensated data. *Cytometry A.* 2006;69(6):541-51.

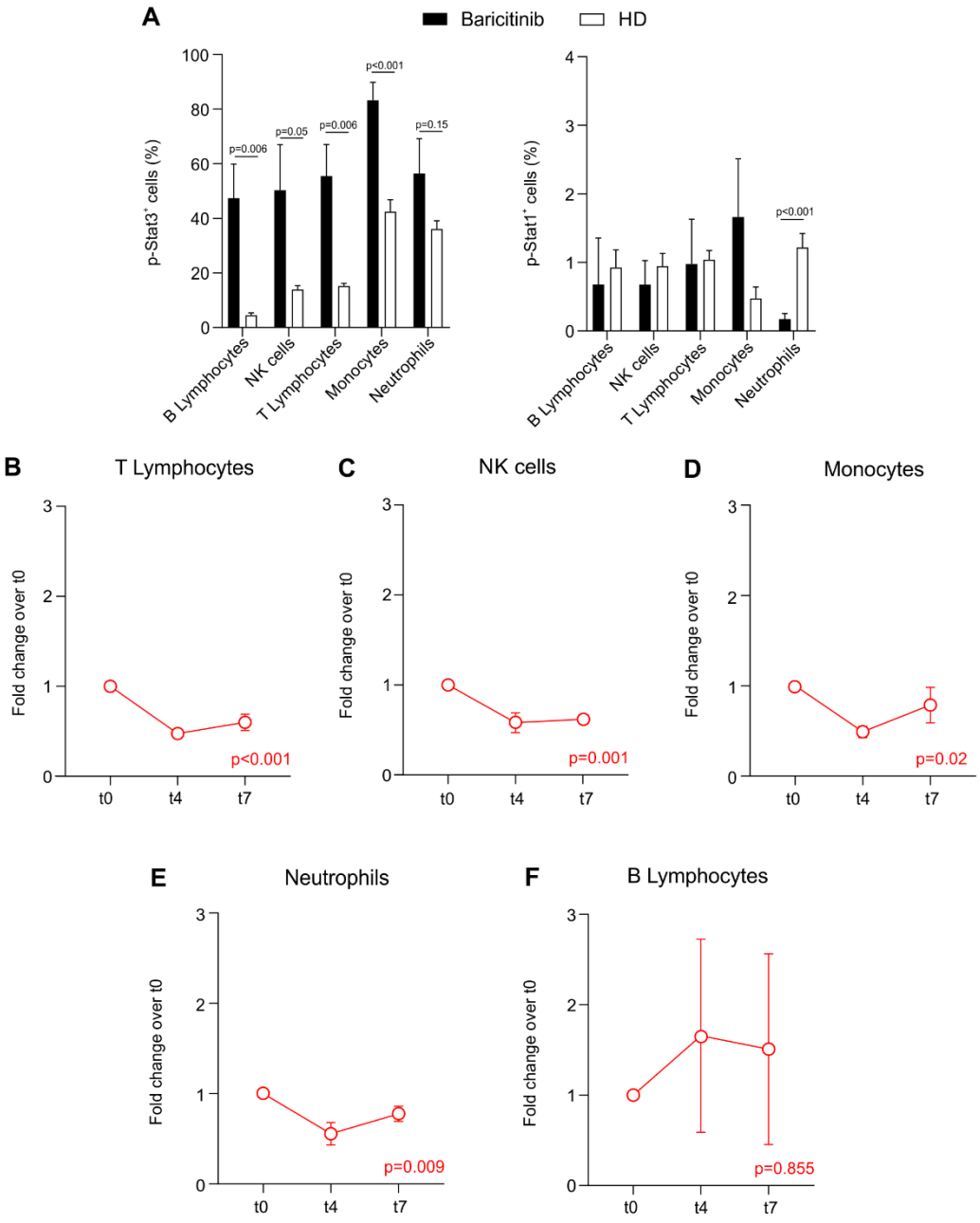
2. Amanat F, Stadlbauer D, Strohmeier S, Nguyen T, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *medRxiv.* 2020:2020.03.17.20037713.
3. Stadlbauer D, Amanat F, Chromikova V, Jiang K, Strohmeier S, Arunkumar GA, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. *Curr Protoc Microbiol.* 2020;57(1):e100.

Supplemental Table 1: Baseline clinical characteristics of enrolled patients.

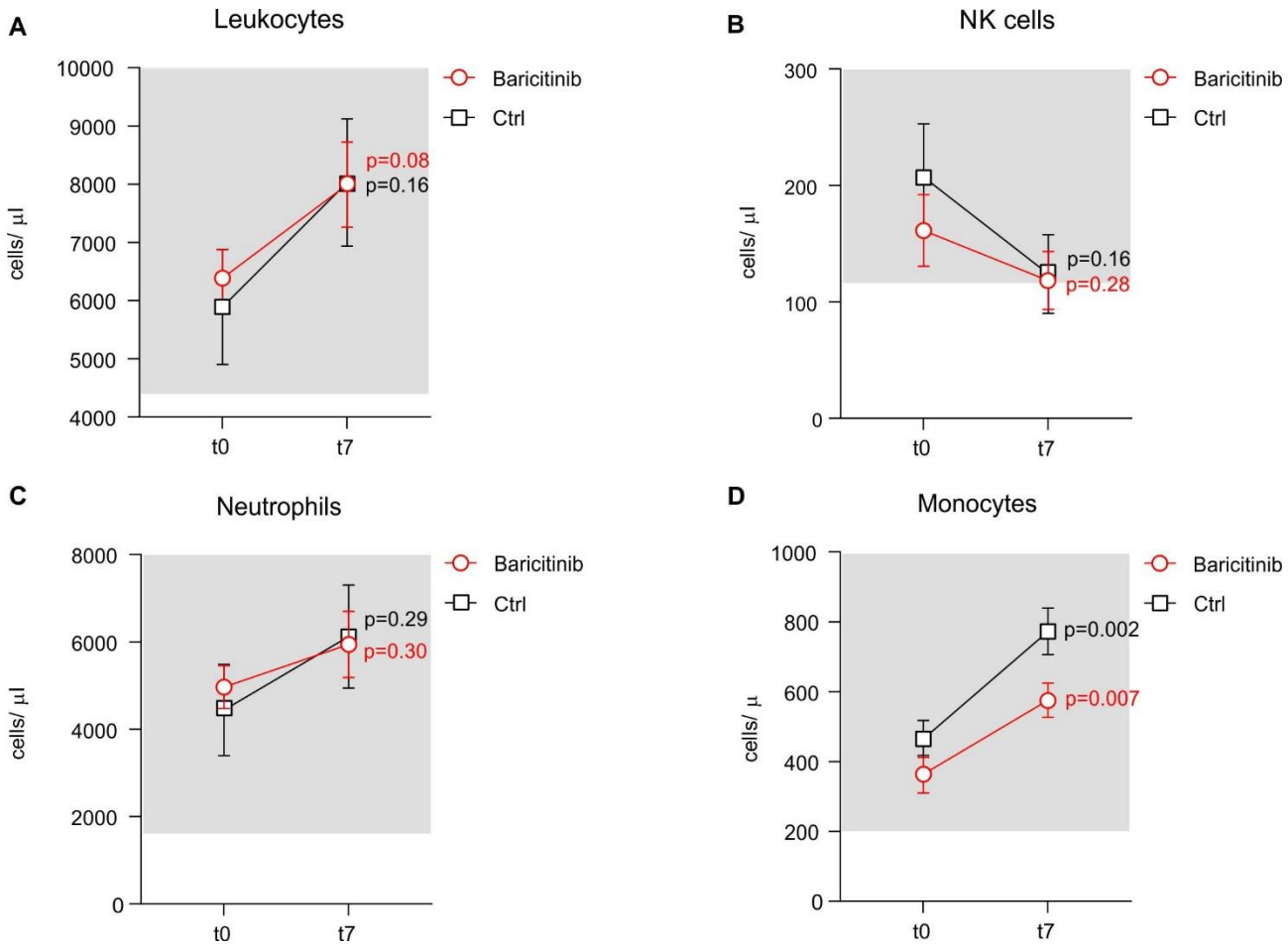
Table S1. Patients Selection Criteria			
Inclusion criteria	**Exclusion criteria**		
Years > 18	Active malignancies		
Symptoms' onset not exceeding 9 days	Personal hystory of primary immunodeficiency and/or thrombophilia		
Presence of interstitial lung involvement less than or equal to 50% (data from HRCT or chest X-ray)	Recent history (< 1 year) of deep vein thrombosis and/or pulmonary thromboembolism		
	Stroke or myocardial infarction in the last 5 years		
	Diagnosis of chirrosis (child pugh c)		
	Chronic kidney disease (eGFR < 30 ml/min)		
	Concomitant bacterial or opportunistic infections		
	Major surgery in the last 3 months		
	Detection of Hb <8 g/dl or reduction of leukocytes' count (neutrophils < 1000/mmc o lymphocytes < 500/mmc)		
Baseline Clinical Characteristics of Enrolled Patients			
Characteristic	**BARICITINIB**	**NO BARICITINIB**	**P VALUE**
Patient number, n (%)	20 (100)	56 (100)	
Male/female, n (%)	7/13 (35/65)	31/25 (55/45)	0.19
Age years, median (IQR)	68 (64.5-78.5)	77.5 (62-87.5)	0.17
Comorbidities			
Hypertension, n (%)	16 (80)	27 (48.2)	0.02
Diabetes, n (%)	4 (20)	9 (16)	0.73
Chronic obstructive pulmonary disease, n (%)	2 (10)	0 (0)	0.07
Cardiovascular disease, n (%)	4 (20)	21 (37.5)	0.18
Chronic kidney disease, n (%)	1 (5)	6 (10.7)	0.67
Active malignancies , n (%)	0 (0)	0 (0)	-
Laboratory findings at baseline			
Ferritin (µg/L), median (IQR)	613 (441-974.2)	590 (322-1175)	0.62
Creatin kynase, (U/L), median (IQR)	94 (56 -134)	107 (61.5-185.5)	0.69
Lactate dehydrogenase, (U/L), median (IQR)	438.5 (377-507.5)	309.5 (268-384.5)	<0.001
D-dimer (µg/L), median (IQR)	819.5 (563-1670)	1744 (975-2331)	0.02
Treatment during hospitalization			
Hydroxycloroquine with antiviral therapy, n (%)	8 (40)	26 (46.4)	0.79
Hydroxycloroquine without antiviral therapy, n (%)	12 (60)	7 (13)	<0.001
Antiviral therapy without hydroxycloroquine, n (%)	0 (0)	1 (2)	-
Anticoagulant prophylaxis, n (%)	20 (100)	54 (96.4)	1.00
Antibiotic prophylaxis, n (%)	13 (65)	23 (41.1)	0.08



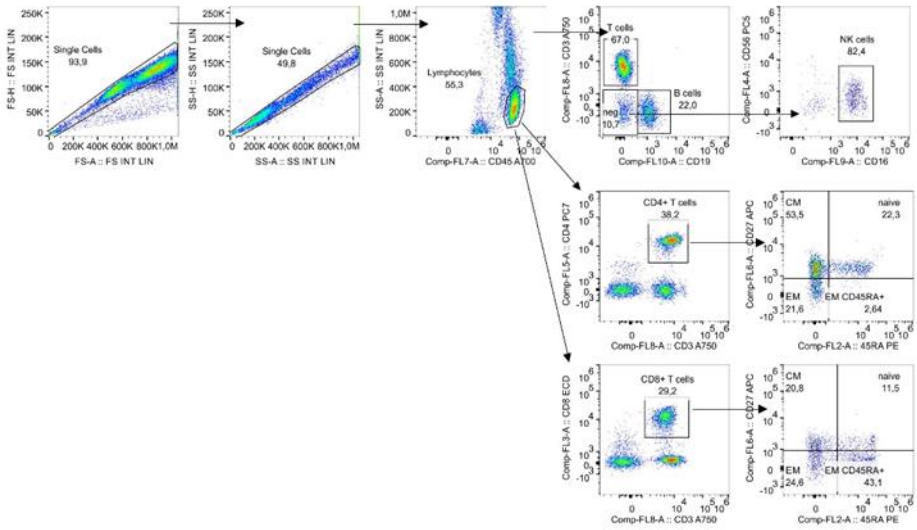
Supplemental Figure S1: Gating strategy for p-STAT1 and p-STAT3 evaluation.



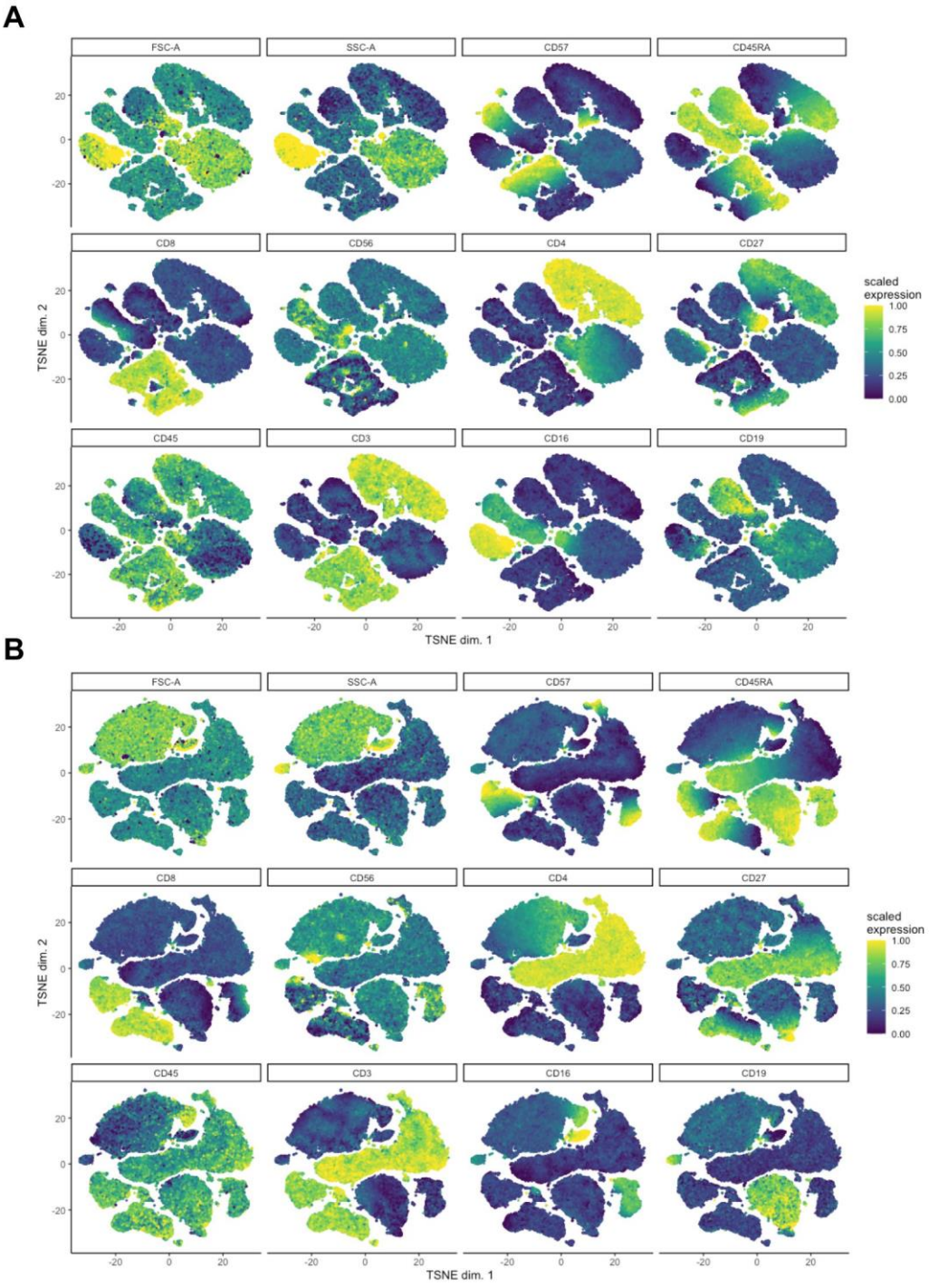
Supplemental Figure S2: Baricitinib alters STAT3 signaling pathway in leukocytes. (A) Levels of p-STAT3 (Tyr705) and p-STAT1 (Tyr701) were analyzed by flow cytometry in the peripheral blood of COVID-19 patients before treatment (n=6) and HDs (n=6). (B-F) Activation of STAT3 pathway was analyzed in peripheral blood of patients (n=6) undergoing baricitinib treatment by flow cytometry. MFI of p-STAT3 was measured at t0 (baseline), t4 (4 days following treatment), t7 (7 days following treatment) on NK cells (B), T lymphocytes (C), monocytes (D), neutrophils (E), B lymphocytes (F) and normalized to t0 for each patient. Data are reported as mean ± SEM. Statistic performed by One-way RM ANOVA.



Supplemental Figure S3: Baricitinib does not alter the number of some leukocyte subsets. Peripheral blood of COVID-19 patients enrolled in either baricitinib (n=12) or basic treatment (n=8, Ctrl) arms was analyzed at t0 (baseline), and t7 (7 days following treatment) by flow cytometry. Number of cells/µl was reported for leukocytes (A), NK cells (B), neutrophils (C), monocytes (D). Normal reference range is shown in light gray boxes. Data are reported as mean ± SEM. Statistic performed by One-way RM ANOVA.



Supplemental Figure S4: Gating strategy used for enumeration of lymphocyte subsets



Supplemental Figure S5: Expression intensity of single markers used for t-SNE analysis of peripheral blood from 12 patients at t0 (A) and t7 (B) of baricitinib treatment.