SUPPLEMENTARY APPENDIX

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SUPPLEMENTARY METHODS

Leukocyte preparation and experimental design

Whole blood samples from healthy donors (N=6) were apheresed, and leukocyte-enriched fractions were transferred to the company Primity Bio (Fremont, CA, USA). Immediately following apheresis, approximately 600,000 cells were plated in 100 µL into 96-well plates and incubated with baricitinib using an 8-point dose range from 0.1 nM to 10, 0000 nM for 1 hour prior to stimulation with cytokines for 15 minutes at 37°C. Baricitinib (Eli Lilly and Company) was prepared as 10 mM stocks in dimethyl sulfoxide. Cytokines were used at the following concentrations: IL-2 (4 ng/mL), IL-3 (12 ng/mL), IL-4 (350 pg/mL), IL-6 (1.5 ng/mL), IL-10 (12 ng/mL), IL-15 (850 pg/mL), and IL-21 (200 pg/mL), IFN-γ (100 pg/mL), G-CSF (5 ng/mL), GM-CSF (5 pg/mL). The choice of cytokine concentrations and incubation conditions were optimized in order to ensure consistent signaling in alternate cell types and pSTAT readouts. After stimulation, cells were fixed, permeabilized, fluorescence barcoded, and multicolor flow cytometry was performed as previously described to quantify STAT phosphorylation in gated leukocyte subpopulations[17]. For a given case (stimulation, cell type, and pSTAT combination), the IC_{50} was determined if there was a consistent response to the stimulus as described in the statistical analysis section. The primary pSTAT observed for each stimulus is reported. Leukocyte populations were defined as $CD20^+$ (B cells), $CD3^+CD4^+$ ($CD4^+$ T cells), $CD3^+CD4^-$ ($CD8^+$ T cells), $CD3^-CD56^+$ (natural killer cells), and by forward and side scatter (monocytes).

Human numb-associated kinase (NAK) assays

AAK1 Kinase-tagged T7 phage strains were prepared in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage and incubated with shaking at 32°C until lysis. The lysates were centrifuged and filtered to remove cell debris. The remaining kinases (BIKE, GAK, and STK16) were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection.

606 Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30
607 minutes at room temperature to generate affinity resins for kinase assays. The liganded beads were
608 blocked with excess biotin and washed with blocking buffer (SeaBlock; Thermo Scientific Pierce, 1% BSA,
609 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific binding. Binding
610 reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x
611 binding buffer (20% SeaBlock, 0.17x PBS, 0.05% Tween 20, 6 mM DTT). Test compounds were prepared
612 as 111x stocks in 100% DMSO. Equilibrium affinity constants (Kds) were determined using an 11-point 3-
613 fold compound dilution series with three DMSO control points.
614
615 All compounds for Kd measurements were distributed by acoustic transfer (non-contact dispensing) in
616 100% DMSO. The compounds were then diluted directly into the assays such that the final concentration
617 of DMSO was 0.9%. All reactions performed in polypropylene 384-well plate. Each was a final volume of
618 0.02 mL. The assay plates were incubated at room temperature with shaking for one hour and the
619 affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-
620 suspended in elution buffer (1x PBS, 0.05% Tween 20, 0.5 µM non-biotinylated affinity ligand) and
621 incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates
622 was measured by qPCR.
623
624 **Clinical case study**
625 *Procedures*
626 Clinical samples for SARS-CoV-2 diagnostic testing were obtained according to WHO guidelines[24]. For
627 each patient, a sampling strategy was implemented in which nasopharyngeal and blood samples were
628 obtained regularly from hospital admission, and subsequently once every two or three days until patient
629 discharge. At the time of manuscript submission, these four patients continue to be monitored for

630 COVID-19 disease. Upper respiratory samples were nasopharyngeal swabs and blood samples were
631 EDTA tubes adapted for RT-PCR. All samples were analyzed in the same center as the patients where
632 procedures for RNA extraction, real-time RT-PCR (rRT-PCR), were undertaken.
633
634 Throat-swabs and plasma for each patient were processed using the automated ELITe InGenius® system
635 and the GeneFinderTM COVID-19 Plus RealAmp Kit assay (ELITechGroup, France). The reaction mix was
636 manually prepared (according to manufacturer's instruction) and loaded onto the system with other
637 reagents, while RNA was extracted from 200 µL of sample and eluted in 100 µL; the final reaction
638 volume consisted of 5 µL of RNA plus 15 µL of reagents mix. The RT-PCR set up according to
639 manufacturer's instructions were: 50° C for 20 minutes, 95° C for five minutes plus 45 cycles at 95° C for
640 15 seconds and 58° C for 60 seconds. Three target genes, RNA-dependent RNA polymerase (RdRP),
641 nucleocapsid protein (N), and Envelope membrane protein (E) were simultaneously amplified and
642 tested. A cycle threshold value (Ct-value) less than 40 was defined as a positive test result, and a Ct-
643 value greater than 40 was defined as a negative outcome according to our criteria. The quality of
644 nasopharyngeal swabs was checked using the CELL Control r-gene kit (bioMérieux). Primer and probes
645 used for SARS-CoV-2 rRT-PCR are reported in Table S1.
646
647 IL-6 levels were determined on the fully automated immunochemistry platform COBAS e601 (Roche
648 Diagnostics) by the proprietary electrochemiluminescent immunoassay (ref. 05109442190, lot
649 43676101) using 30 µL of serum. SARS-CoV-2 serum IgG and IgM were qualitatively assessed using the
650 immunochromatographic COVID-19 IgG/IgM RAPID TEST, following instructions (PRIMA Lab SA, Balerna,
651 Switzerland). Briefly, 10 µL of EDTA anticoagulated plasma were loaded into the well of each cassette.
652 80 µL of buffer were subsequently added to the well. The presence of SARS-CoV-2 IgG and IgM were
653 visually determined after an incubation of 10 minutes.

Immunoglobulin levels (IgG, IgA, IgM) were measured on the automated platform AU 480 (Beckman Coulter) by their respective proprietary immunoturbidimetric assays; complete blood cell count was performed on XN-9000 Automated Hematology System (Sysmex); coagulation tests including D-dimer levels were carried out on the fully automated hemostasis testing analyzer ACL 750 TOP (Werfen) using proprietary reagents; ALT and AST activity, C-reactive protein (CRP) and serum ferritin concentrations were determined on the fully automated platform for Clinical Chemistry and Immunoassay Alinity ci (Abbott Diagnostics) again with the proprietary reagents. For measuring AST and ALT the assays with the addition of pyridoxal-5-phosphate (P-5'-P) traceable to the Reference Measurement System, for CRP the high sensitive immunoturbidimetric assay, and for ferritin levels the chemiluminescent microparticle immunoassay, were respectively used.

On the wards, standard laboratory and clinical management according to two treating physicians' discretions (MC and GC) were used. The chest CT shown was performed using a single inspiratory phase in One commercial multidetector CT scanner (General Electric Healthcare Revolution64) with a breath-holding protocol (tube voltage 120 kVp, thickness 1.4 mm, increment of 1.4 mm, mean CTDIvol 19 mGy).

Statistical analysis

IC_{50} values for cytokine signaling

IC_{50} values were determined by analyzing the mean fluorescence intensity (MFI) of cytokine-stimulated samples in the presence of the designated concentration of compound. For a given case (stimulation, cell type, and pSTAT combination), the MFI for unstimulated and stimulated cells was determined for each donor. To ensure that a biologically relevant signal was induced, concentration-response curves (CRCs) were only analyzed when a consistent response to stimulus was observed as described below.

678 Data for baricitinib were analyzed with a mixed effect model having compound as a fixed effect and
679 donor as a random effect.
680
681 *Selection of cases for analysis, fitting, and selection of CRC curves for anti-cytokine activity of baricitinib*
682 Two sets of criteria for reporting an IC_{50} value and computing a steady state activity (SSACTIVITY) curve
683 were used: one at the case level and another at the individual curve level. At the case level, the median
684 across the 6 donors of the minimum stimulated to unstimulated ratio over two replicates is computed,
685 and it is required that the median minimum ratio be above 1.5. Out of a total of 85 cases, 43 were
686 selected according to these criteria. Once a case met this criterion, four-parameter logistic curves were
687 fit to the curve response concentration data. If an individual curve had top outside of the 80%-120%
688 activity range or bottom outside of the (-20%, 20%) range, the 4PL was refitted with these constraints.
689 After a CRC was fit, the following quality criteria was evaluated: (1) R2 above 0.8; (2) SE of ln(I IC_{50})
690 below 10; (3) estimated IC_{50} is within a five-fold difference of the minimum and maximum experimented
691 concentrations. A curve failing any of these criteria was discarded.
692
693 *Estimation of daily percent inhibition*
694 The individual 4PL CRCs were combined with population pharmacokinetic (PK) curves to calculate
695 the average steady state daily percent inhibition. The PK profiles of baricitinib were estimated from a
696 two-compartment model with zero-order absorption that was developed using data from healthy
697 volunteers from three Phase 1 clinical trials with once-daily 2-20 mg or twice-daily 5-mg. Protein binding
698 effects were accounted for by replacing the in vitro IC_{50} with an adjusted IC_{50} value computed by dividing
699 the IC_{50} value for each donor by the proportion of compound unbound. Protein-bound adjusted CRCs
700 were constructed by replacing the in vitro IC_{50} value with the adjusted value. The average daily percent
701 inhibition for a subject was obtained by entering the steady-state PK concentrations into the adjusted

CRCs, computing the area under this curve, and dividing it by 24 hours. Using the individual donor values for average daily percent inhibition, a mixed effect model having compound as a fixed effect and donor as a random effect was fit. The reported estimates for population average SSACTIVITY are taken as the least squares means from this model. No transformations were undertaken to keep the estimates of SSACTIVITY within the 0–100% range.

SUPPLEMENTARY TABLES

Table S1. Primer and probe sequences used for SARS-CoV-2 rRT-PCR

Assay	Oligonucleotide	Sequence
N	Forward primer (HKU-NF)	TAATCAGACAAGGAACTGATTA
N	Reverse primer (HKU-NR)	CGAAGGTGTGACTTCCATG
N	Probe (HKU-NP)	FAM-GCAAATTGTGCAATTTGCGG-TAMRA
E	Primer E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT
E	Primer E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA
E	Probe E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
RdRP	Primer RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG
RdRP	Primer RdRP_SARSr-R1	CARATGTTAAASACACTATTAGCATA
RdRP	Probe RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ
RdRP	Probe RdRP_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ

Primer and probe sequences for gene N are from the Hong Kong protocol[42]. Primer and probe sequences for genes E and RdRp are from the Berlin protocol[43]. E, envelope membrane; N, nucleocapsid protein; RdRp, RNA-dependent RNA polymerase; rRT-PCR, reverse transcription polymerase chain reaction

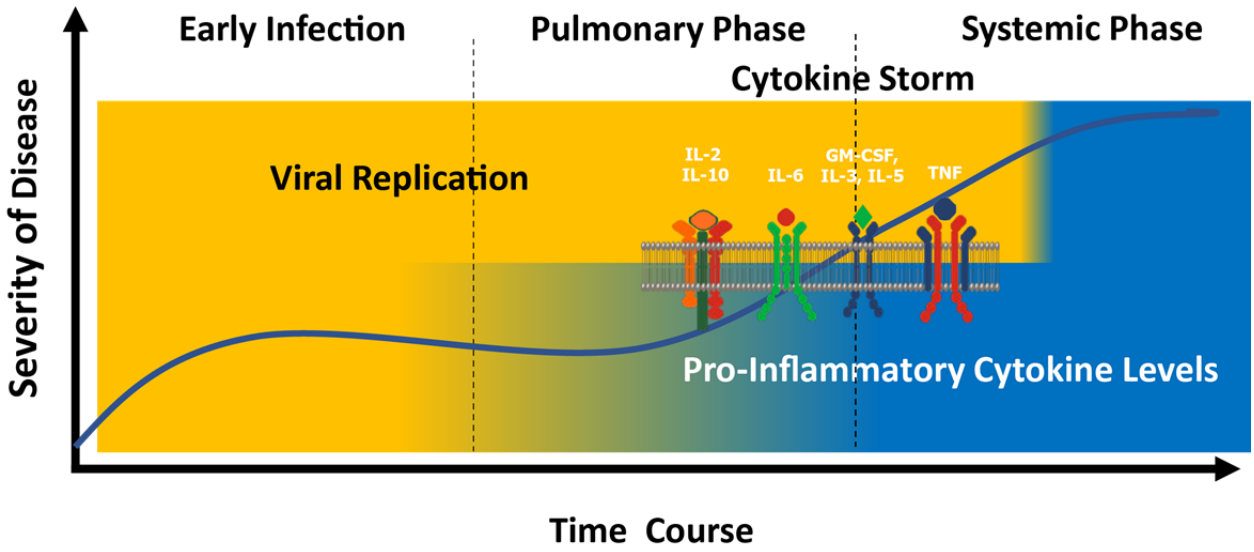
716 **Table S2. Detection of SARS-CoV-2 by rRT-PCR in the upper respiratory tract and peripheral blood**

Patient ID	Days post symptom onset	Source	RdRp (Ct value)	N (Ct value)	E (Ct value)
Patient A	4	Nasopharyngeal swab	26	24	24
Patient A	7	Blood	31	31	> 40
Patient A	9	Nasopharyngeal swab	> 40	36	> 40
Patient A	9	Blood	> 40	36	> 40
Patient A	11	Nasopharyngeal swab	> 40	35	> 40
Patient A	11	Blood	> 40	36	> 40
Patient A	14	Nasopharyngeal swab	> 40	37	40
Patient A	14	Blood	> 40	> 40	> 40
Patient A	16	Nasopharyngeal swab	> 40	> 40	> 40
Patient A	16	Blood	> 40	> 40	> 40
Patient A	18	Nasopharyngeal swab	> 40	> 40	> 40
Patient A	21	Nasopharyngeal swab	> 40	34	> 40
Patient A	21	Blood	> 40	38	> 40
Patient A	23	Nasopharyngeal swab	> 40	37	> 40
Patient A	23	Blood	> 40	> 40	> 40
Patient A	25	Nasopharyngeal swab	> 40	37	> 40
Patient A	25	Blood	> 40	> 40	> 40
Patient B	1	Nasopharyngeal swab	24	21	21
Patient B	4	Blood	> 40	> 40	> 40
Patient B	6	Nasopharyngeal swab	31	29	29
Patient B	6	Blood	> 40	> 40	> 40
Patient B	8	Nasopharyngeal swab	32	29	30
Patient B	8	Blood	> 40	> 40	> 40
Patient B	11	Nasopharyngeal swab	36	31	33
Patient B	11	Blood	> 40	> 40	> 40
Patient B	13	Nasopharyngeal swab	> 40	> 40	> 40
Patient B	13	Blood	> 40	> 40	> 40
Patient B	15	Nasopharyngeal swab	> 40	35	> 40

Patient B	15	Blood	> 40	> 40	> 40
Patient B	18	Nasopharyngeal swab	> 40	37	> 40
Patient B	18	Blood	> 40	> 40	> 40
Patient B	20	Nasopharyngeal swab	> 40	> 40	> 40
Patient B	20	Blood	> 40	> 40	> 40
Patient B	22	Nasopharyngeal swab	> 40	38	> 40
Patient B	22	Blood	> 40	> 40	> 40
Patient C	8	Nasopharyngeal swab	35	32	33
Patient C	9	Blood	> 40	37	> 40
Patient C	11	Nasopharyngeal swab	> 40	35	38
Patient C	11	Blood	> 40	38	> 40
Patient C	13	Nasopharyngeal swab	34	29	30
Patient C	13	Blood	> 40	37	> 40
Patient C	16	Nasopharyngeal swab	26	23	23
Patient C	16	Blood	> 40	38	> 40
Patient C	18	Nasopharyngeal swab	> 40	> 40	> 40
Patient C	18	Blood	> 40	37	> 40
Patient C	20	Nasopharyngeal swab	> 40	> 40	> 40
Patient C	20	Blood	> 40	> 40	> 40
Patient C	23	Nasopharyngeal swab	> 40	> 40	> 40
Patient C	23	Blood	> 40	> 40	> 40
Patient C	25	Nasopharyngeal swab	> 40	> 40	> 40
Patient C	25	Blood	> 40	> 40	> 40
Patient C	27	Nasopharyngeal swab	> 40	> 40	> 40
Patient C	27	Blood	> 40	> 40	> 40
Patient D	7	Nasopharyngeal swab	> 40	31	38
Patient D	8	Nasopharyngeal swab	> 40	32	34
Patient D	9	Blood	> 40	> 40	> 40
Patient D	11	Nasopharyngeal swab	> 40	35	> 40
Patient D	11	Blood	> 40	> 40	> 40
Patient D	14	Nasopharyngeal swab	> 40	36	> 40

Patient D	14	Blood	> 40	38	> 40
Patient D	16	Nasopharyngeal swab	> 40	> 40	> 40
Patient D	16	Blood	> 40	> 40	> 40
Patient D	18	Nasopharyngeal swab	> 40	> 40	> 40
Patient D	18	Blood	> 40	> 40	> 40

Three viral target genes, RNA-dependent RNA polymerase (RdRp), nucleocapsid protein (N) and envelope membrane (E)[22], together with the housekeeping gene GAPDH were simultaneously amplified. Negative results for SARS-CoV-2 detection were defined as those with Ct values > 40. This is a stringent cut-off compared to other studies[23] In addition, the most sensitive target gene (N gene) was chosen for detection. (Study note: according to eligibility criteria, Patient A tested negative for pregnancy and all 4 individuals tested negative for HIV-1/2, and Tuberculosis using QuantiFERON-TB Gold Plus.)



724

725 **Supplementary Figure 1. Classification of COVID-19 disease states and presence of cytokine storm**.

726 Escalating phases of disease progression with COVID-19, with associated signs, symptoms, and presence

727 of cytokine storm are shown. Baricitinib is a potent inhibitor of several cytokines implicated in COVID-19

728 (except TNF shown in figure). *Adapted from Siddiqi, et al[12].

729