
Longitudinal Analyses after COVID-19 Recovery or Prolonged Infection Reveal Unique Immunological Signatures after Repeated Vaccinations

Daisuke Hisamatsu ^{1,*}, Akari Ikeda ¹, Lisa Ito ¹, Yasushi Matsushita ², Makoto Hiki ³, Hirotake Mori ⁴, Yoko Tabe ⁵, Toshio Naito ⁴ and Chihiro Akazawa ^{1,*}

¹ Intractable Disease Research Center, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan

² Department of Internal Medicine and Rheumatology, Faculty of Medicine, Juntendo University School of Medicine, Tokyo 113-8421, Japan

³ Department of Cardiovascular Biology and Medicine, Juntendo University Faculty of Medicine, Tokyo 113-8421, Japan

⁴ Department of General Medicine, Juntendo University Faculty of Medicine, Tokyo 113-8421, Japan

⁵ Department of Clinical Laboratory Medicine, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan

* Correspondence: d.hisamatsu.ap@juntendo.ac.jp (D.H.); c.akazawa.gt@juntendo.ac.jp (C.A.)

Supplementary Materials

(A)

Severity	Healthy	WT Mild	WT Moderate I	WT Moderate II	Mut Moderate II
Number of participants	6	10	10	10	8
Age (mean \pm SD, years)	46.2 \pm 11.5	36.3 \pm 17.3	63.7 \pm 14.6	70.5 \pm 11.3	52.6 \pm 7.3
Sex (% male)	50	70	60	60	75
Time from symptom onset to blood collection on admission (mean \pm SD, days)	NA	2.9 \pm 1.7	5.8 \pm 1.2	8.3 \pm 4.1	8.0 \pm 2.8
Time from admission to discharge (mean \pm SD, days)	NA	7.8 \pm 5.6	11.1 \pm 2.4	14.5 \pm 6.3	18.3 \pm 5.0
Clinical factors on admission					
SpO2 (mean \pm SD, %)	NA	97.8 \pm 1.4	96.7 \pm 1.4	90.9 \pm 4.7	94.3 \pm 1.7
Fibrinogen (mean \pm SD, mg/dL)	NA	317.7 \pm 55.8	486.4 \pm 102.5	662.0 \pm 160.8	533.1 \pm 115.0
Ferritin (mean \pm SD, ng/mL)	NA	169.0 \pm 108.8	629.0 \pm 475.2	680 \pm 449.2	932.6 \pm 300.0
D-dimer (mean \pm SD, μ g/mL)	NA	1.6 \pm 0.4	1.6 \pm 0.3	2.5 \pm 0.8	1.5 \pm 0.2
IL-6 (mean \pm SD, pg/mL)	NA	3.6 \pm 2.6	22.1 \pm 18.3	42.4 \pm 32.6	29.3 \pm 11.2
Krebs von den Lungen-6 (KL-6) (mean \pm SD, U/mL)	NA	238.0 \pm 144.5	336.9 \pm 469.1	260.0 \pm 104.5	256.1 \pm 109.7
Serum Amyloid A (SAA) (mean \pm SD, μ g/mL)	NA	17.6 \pm 18.7	386.0 \pm 428.5	2029.5 \pm 1804.3	346.2 \pm 262.7
pulmonary Surfactant Protein-D (SP-D) (mean \pm SD, ng/mL)	NA	24.6 \pm 8.5	36.1 \pm 45.3	57.5 \pm 70.4	34.5 \pm 41.8
White Blood Cell (WBC) (mean \pm SD, $\times 10^3/\mu$ l)	NA	5.2 \pm 1.8	4.9 \pm 1.9	8.0 \pm 3.2	4.9 \pm 2.3
Hemoglobin (Hb) (mean \pm SD, g/dL)	NA	15.1 \pm 1.8	13.7 \pm 1.5	13.3 \pm 0.5	15.0 \pm 0.8
Neutrophil (NE) (mean \pm SD, %)	NA	59.9 \pm 11.5	68.7 \pm 9.8	81.5 \pm 11.3	71.5 \pm 9.7
Lymphocyte (Lym) (mean \pm SD, %)	NA	28.2 \pm 9.0	22.7 \pm 8.8	11.8 \pm 8.8	20.1 \pm 7.7
Monocyte (Mo) (mean \pm SD, %)	NA	8.7 \pm 3.1	7.7 \pm 2.4	5.3 \pm 3.2	7.8 \pm 2.5
Eosinophil (Eos) (mean \pm SD, %)	NA	2.8 \pm 2.7	0.7 \pm 0.8	0.2 \pm 0.3	0.1 \pm 0.2
C-Reactive Protein (CRP) (mean \pm SD, mg/dL)	NA	0.3 \pm 0.1	4.4 \pm 5.3	11.6 \pm 7.2	5.0 \pm 3.5

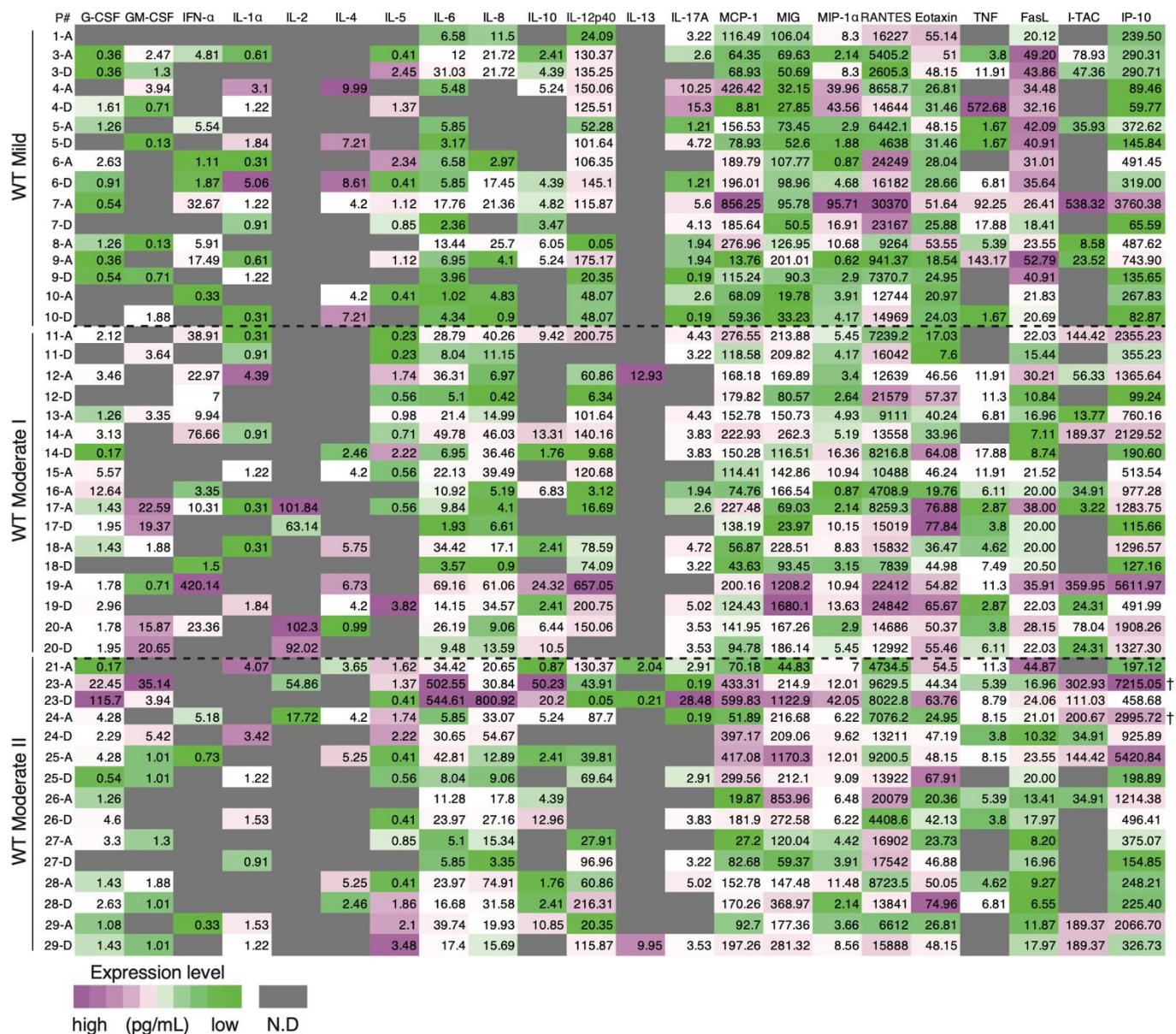
(B)

WT Moderate II
Day 6

N501Y Moderate II
Day 5

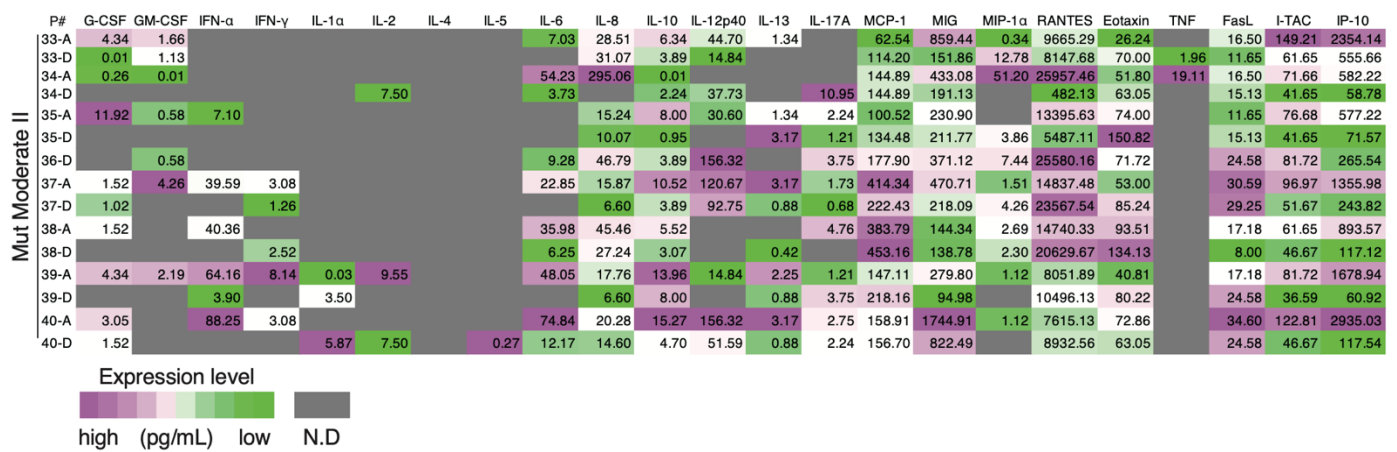
L452R Moderate II
Day 3

Supplementary Figure S1. Study cohort. (A) Clinical information of all participants. WT indicates the original SARS-CoV-2 strain, and Mut indicates the VOCs strain, including Alpha and Delta strains. (B) Representative images of COVID-19 pneumonia in moderate II patients. Red circles show lesions.

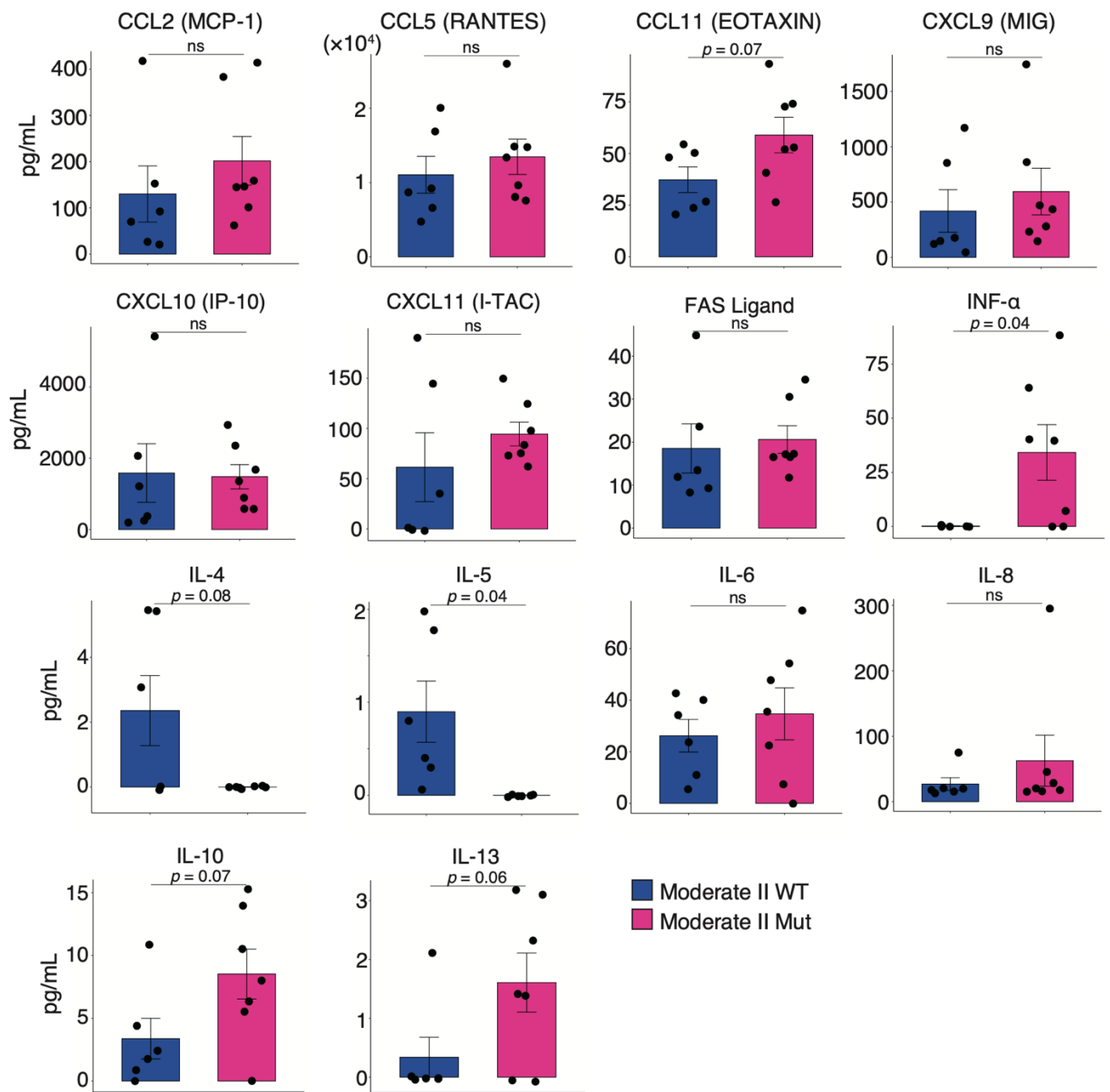


Supplementary Figure S2. Comprehensive serum cytokine analysis in COVID-19 patients infected with

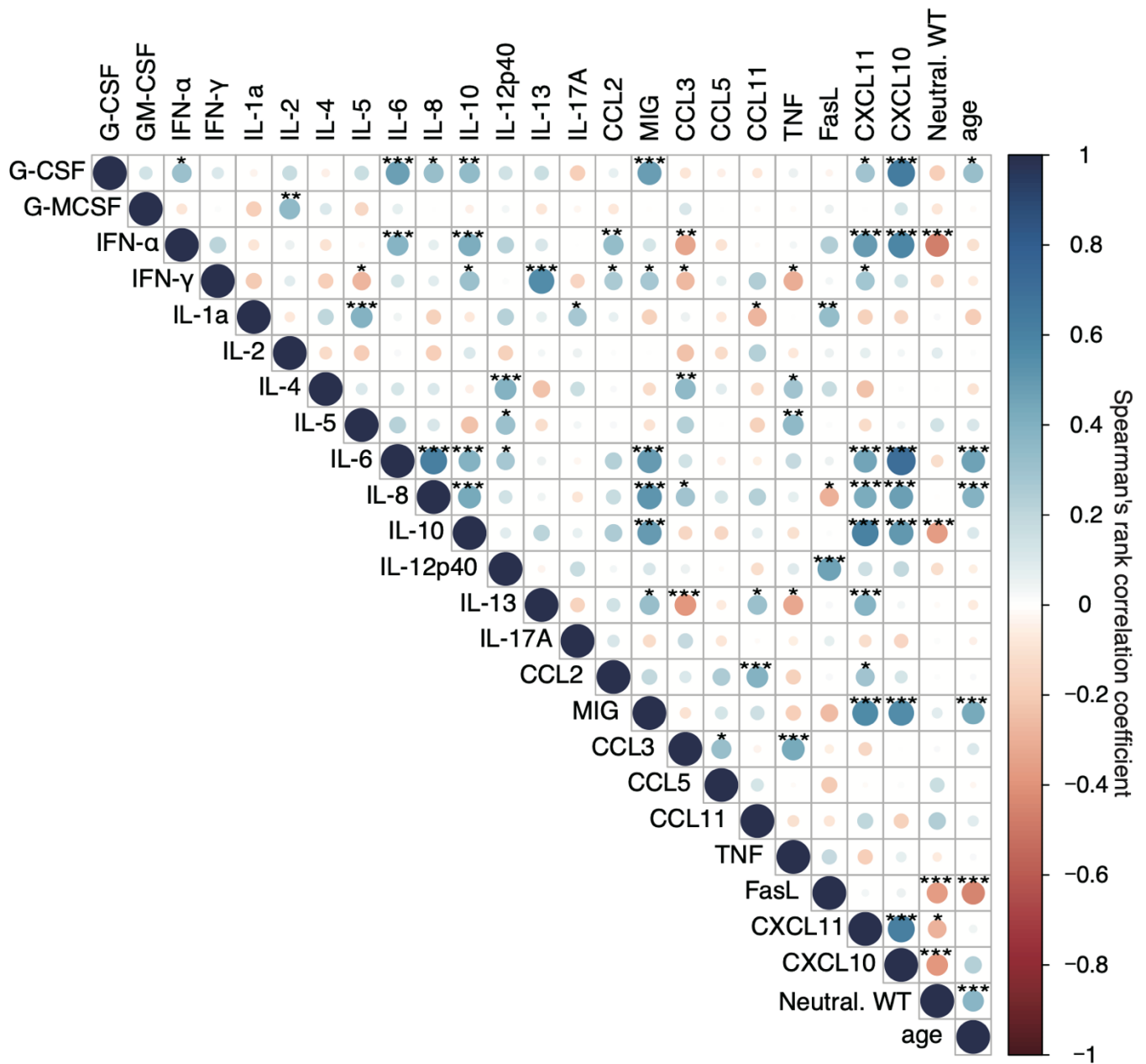
WT. Table shows the concentration of each serum cytokine. P# indicates patient identification numbers ($n = 9$ patients/WT-infected mild patient group; 10 patients/WT-infected moderate II patient group; 8 patients/WT-infected moderate II patient group). A indicates admission and D indicates discharge. Crosses show deceased patients.



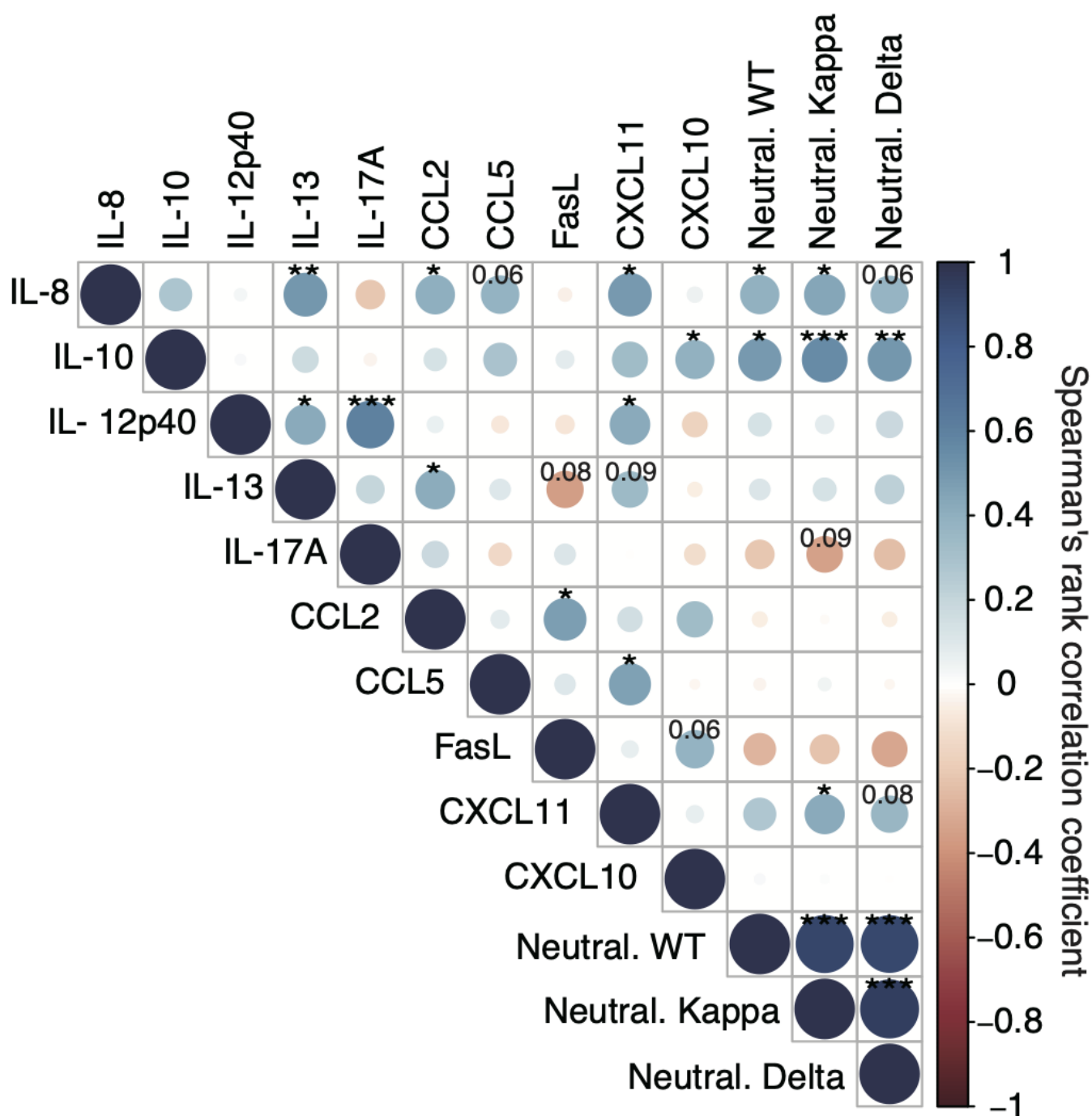
Supplementary Figure S3. Comprehensive serum cytokine analysis in COVID-19 patients infected with VOCs. Table shows the concentration of each serum cytokine. P# indicates patient identification numbers ($n = 8$ patients/VOC-infected moderate II patient group). A indicates admission and D indicates discharge.



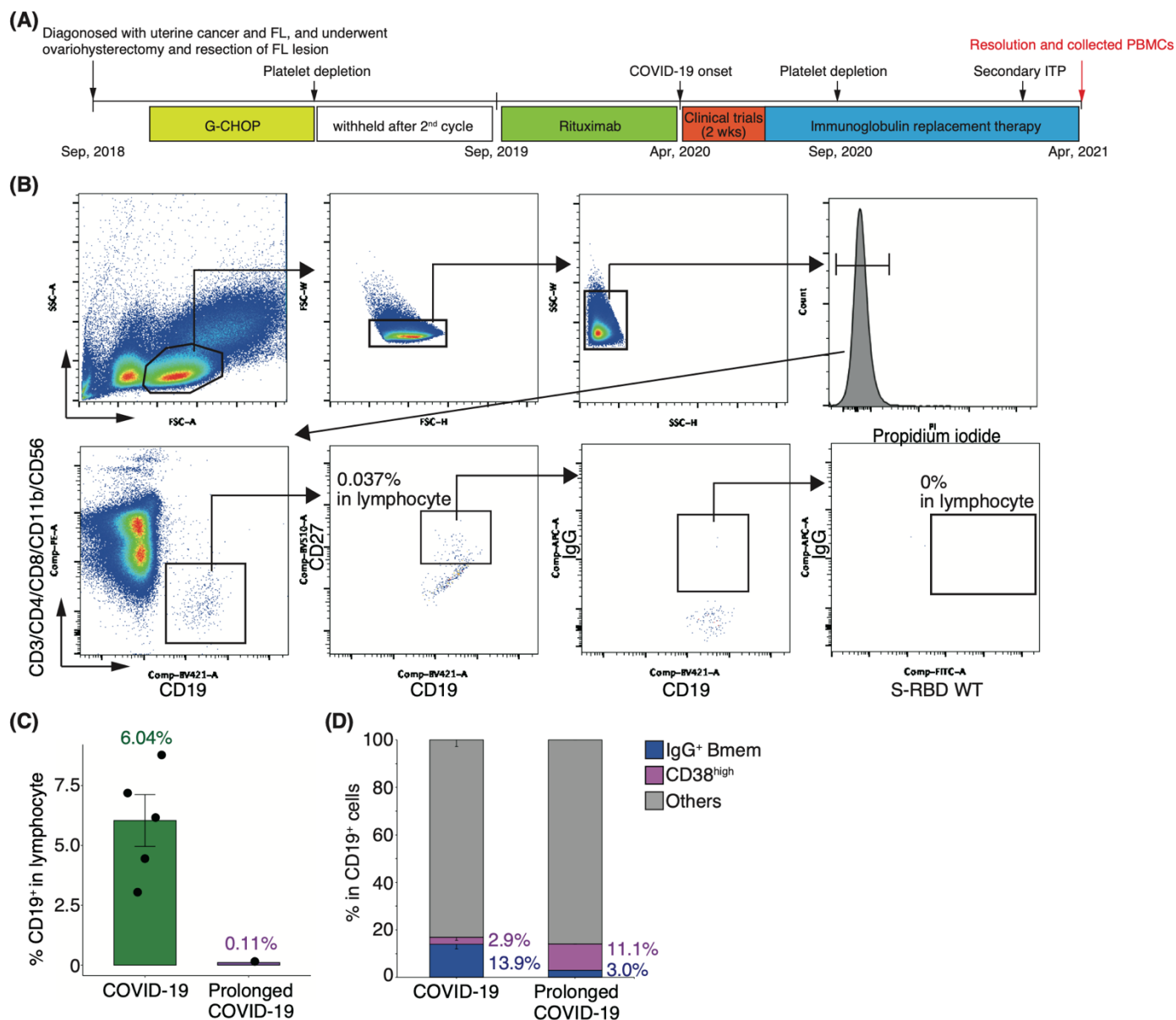
Supplementary Figure S4. Comparison of serum cytokine levels in moderate II patients infected with the WT strain or VOCs on admission. Graphs show the concentration of each cytokine ($n = 6$ patients/WT-infected moderate II patient group; 7 patients/VOC-infected moderate II patient group). Statistical significance was determined using Welch's t-test. ns, not significant.



Supplementary Figure S5. Correlation analysis among serum cytokine level, neutralizing antibody titer, and age in COVID-19 patients during hospitalization period. Correlation matrices were created using the Spearman's correlation coefficient ($n = 33$ patients). Neutral. WT, neutralizing potency for WT strain; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

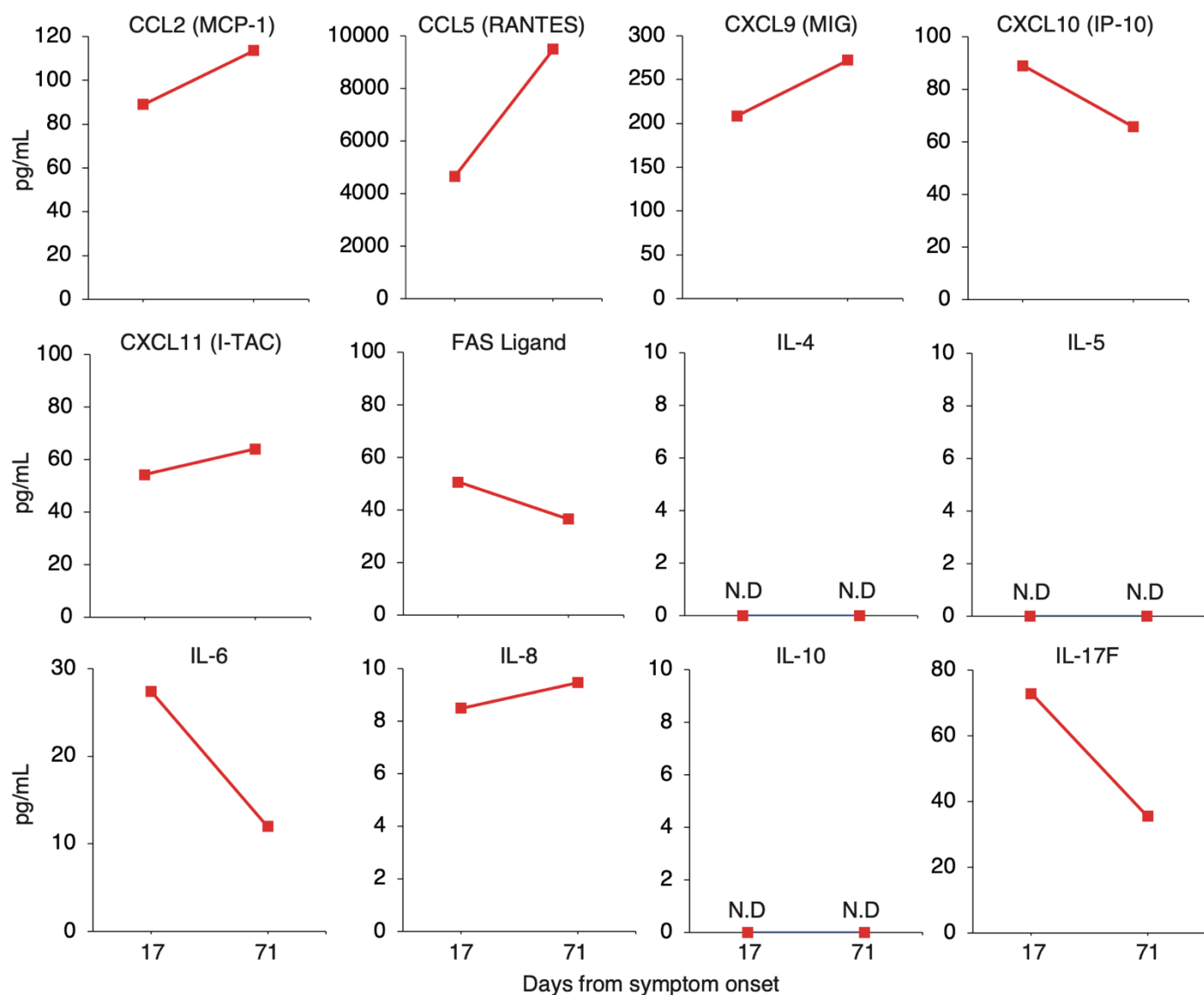


Supplementary Figure S6. Correlation analysis of serum cytokine level and neutralizing antibody titer in all participants, including naïve and recovered individuals. Correlation matrices were created using the Spearman's correlation coefficient ($n = 10$ participants). Neutral. WT, neutralizing potency for WT strain; Neutral. Kappa, neutralizing potency for Kappa strain; Neutral. Delta, neutralizing potency for Delta strain; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.



Supplementary Figure S7. Prolonged SARS-CoV-2 infection in a patient with follicular lymphoma undergoing B-cell depletion therapy. (A) Graph showing the clinical course and treatment strategy. FL, follicular lymphoma; ITP, immune thrombocytopenia; G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone therapy. (B) Representative FACS plots of the cell population are shown in panels. The number indicates the positive rate. (C) The graph shows the proportion of CD19⁺ cells in each group during recovery ($n = 5$ patients/COVID-19 group). (D) Graph showing the frequencies of IgG⁺ Bmems

and CD38^{high} cell populations during recovery ($n = 5$ patients/COVID-19 group). Each number indicates the mean value.



Supplementary Figure S8. Serum cytokine level change in a patient with prolonged COVID-19. Graphs show representative cytokine concentrations. N.D, not detected.

Supplementary Table S1. Primer sets of single-cell RT-PCR and immunoglobulin gene sequencing.

Forward Primer

Target	Sequence
IGH_variable region (v) 1	5-ACAGGTGCCCCACTCCCAGGTGCAG-3
IGH_v3	5-AAGGTGTCCAGTGTGARGTGCAG-3
IGH_v4/6	5-CCCAGATGGGTCCTGTCCCAGGTGCAG-3
IGH_v5	5-CAAGGAGTCTGTTCCGAGGTGCAG-3
IGL(κ)_v1/2	5-ATGAGGSTCCCYGCTCAGCTGCTGG-3
IGL(κ)_v3	5-CTCTTCCTCCTGCTACTCTGGCTCCCAG-3
IGL(κ)_v4	5-ATTTCTCTGTTGCTCTGGATCTCTG-3
IGL(λ)_v1	5-GGTCCTGGGCCAGTCTGTGCTG-3
IGL(λ)_v2	5-GGTCCTGGGCCAGTCTGCCCTG-3
IGL(λ)_v3	5-GCTCTGTGACCTCCTATGAGCTG-3
IGL(λ)_v4/5	5-GGTCTCTCTCSCAGCYTGTGCTG-3
IGL(λ)_v6	5-GTTCTTGGGCCAATTTTATGCTG-3
IGL(λ)_v7	5-GGTCCAATTCYCAGGCTGTGGTG-3
IGL(λ)_v8	5-GAGTGGATTCTCAGACTGTGGTG-3

Reverse Primer

Target	Sequence
IGH_constant region (c) 1	5-CGCCTGAGTTCCACGACACC-3
IGH_c for nested PCR	5-TCGGGGAAGTAGTCCTTGAC-3
IGL(κ)_c1	5-GAGGCAGTTCCAGATTTCAA-3
IGL(κ)_c for nested PCR	5-GGGAAGATGAAGACAGATGGT-3
IGL(λ)_c1	5-GCTTGAAGCTCCTCAGAGG-3
IGL(λ)_c for nested PCR	5-GGGCGGGAACAGAGTGACC-3