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TIGIT Monoallelic Nonsense Variant in Patient with Severe COVID-19 Infection, Thailand

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A heterozygous nonsense variant in the *TIGIT* gene was identified in a patient in Thailand who had severe COVID-19, resulting in lower *TIGIT* expression in T cells. The patient's T cells produced higher levels of cytokines upon stimulation. This mutation causes less-controlled immune responses, which might contribute to COVID-19 severity.

To investigate SARS-CoV-2 genomic variants, we recruited 46 COVID-19 patients from King Chulalongkorn Memorial Hospital in Bangkok, Thailand, in January 2020. Recruited patients were 16–79 years of age and had moderate to severe COVID-19 symptoms according to World Health Organization interim guidelines (https://apps.who.int/iris/bitstream/handle/10665/331446/WHO-2019-nCoV-clinical-2020.4-eng.pdf). We performed whole-exome sequencing on peripheral blood samples as described (*1*). The institutional review board of the Faculty of Medicine, Chulalongkorn University, Bangkok, approved this study (COA no. 738/2020).

We filtered variants by using the following criteria. Variants had to pass the quality standards, have read depth >10, and be from the coding regions or canonical splice sites of 1,810 immune-related genes, including immune checkpoint genes (*2*). Variants also had to have <1% allele frequency in the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org), Exome Variant Server (University of Washington, https://evs.gs.washington.edu/EVS), 1000 Genomes Project Consortium (https://www.genome.gov), dbSNPs (https://www.ncbi.nlm.nih.gov/projects/SNP), and Thai Reference Exome (T-Rex) database (*3*). We called candidate variants novel pathogenic variants when they were not previously identified in patients in the literature.

In our patient cohort, exome sequencing identified no variants in type I interferon genes, which previously have been commonly observed in patients with severe COVID-19 (*4*). Of note, we identified a heterozygous nonsense variant (rs1386709957) in the T-cell immunoglobulin and ITIM domain (*TIGIT*) gene in 1 patient (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/29/11/22-0914-App1.pdf). We did not identify this nonsense variant among 3,742 persons in the T-Rex database but did observe it in 1 of 31,390 alleles in the gnomAD database, in an allele from a female patient from East Asia. This variant truncates the 245-amino acid residue proteins at residue 56 and is classified as a pathogenic variant American College of Medical Genetics guidelines (https://www.acmg.net).

We investigated *TIGIT* gene expression in T cells of the patient from our study (Co45), a 43-year-old man, and compared it with 2 other sex- and age-matched patients who had severe COVID-19 (Co6 and Co84) (Appendix). We collected peripheral blood mononuclear cells (PBMCs) from each of the patients 1 month after they recovered. We used RNA extracted from PBMCs for real-time reverse transcription PCR and found patient Co45 had the lowest *TIGIT* mRNA level (Figure, panel A). Because *TIGIT* is mainly expressed in T cells, we used flow cytometry to measure the mean fluorescence intensity of TIGIT expressed in the cytoplasmic domain (CD) T cells. Patient Co45 had lower TIGIT gene expression in all CD3+, CD4+, and CD8+ T cells than the other 2 patients, most remarkably in the CD8+ T cells (Figure, panels B–D). The percentages of CD3+, CD4+, and CD8+ T cells in patient Co45 were comparable those in the other 2 patients (Appendix Fig-

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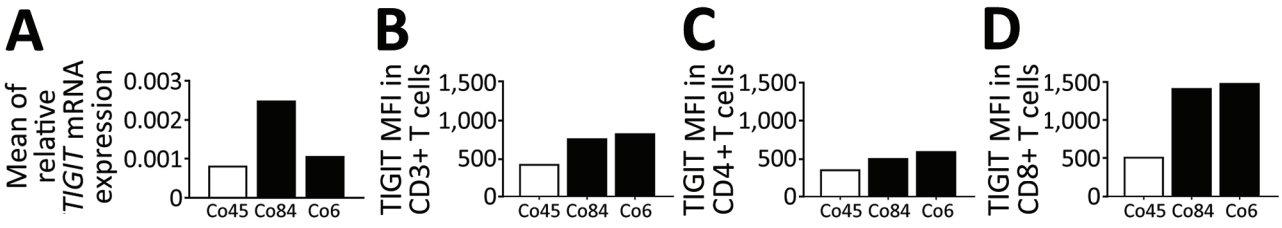

Figure. Results of rRT-PCR assay and flow cytometry of *TIGIT* nonsense variant in a patient with severe COVID-19 infection, Thailand. Co45 is the patient with *TIGIT* nonsense variant; Co84 and Co6 are age- and sex-matched patients who also had severe COVID-19 infection. A) Mean relative mRNA levels of *TIGIT* expression from rRT-PCR assay. B) TIGIT expression MFI on CD3+ T cells. C) TIGIT expression MFI on CD4+ T cells. D) TIGIT expression MFI on CD8+ T cells. CD, cytoplasmic domain; MFI, mean fluorescence intensity; rRT-PCR, real-time reverse transcription PCR; *TIGIT*, T cell immunoglobulin and ITIM domain gene.

ure 2, panel A), demonstrating that the truncated *TIGIT* variant reduced *TIGIT* expression in CD3+, CD4+, and CD8+ T cells.

TIGIT is known to exert immune suppressive functions, such as inhibiting T cell activation, proliferation, and functions that inhibit inflammation and anti-tumor responses. Thus, we investigated the effect of this monoallelic *TIGIT* variant on T cell functions by examining activation markers and cytokine-secreting T cells after stimulation with anti-CD3/CD28 coupled beads for 24 hours. We then assessed activation by using flow cytometry. We found no differences in frequencies of CD69-expressing CD3+, CD4+, and CD8+ T cells among the 3 patients (Appendix Figure 2, panel B); however, patient Co45 had higher interferon gamma (IFNγ), tumor necrosis factor alpha (TNF-α), and interleukin (IL) 2–producing CD3+, CD4+, and CD8+ T cells than the other 2 patients (Appendix Figure 3).

We believe this patient's heterozygous nonsense *TIGIT* variant contributed to the increased inflammatory cytokine functions we observed. His serum cytokine levels at acute illness onset did not differ from the other 2 COVID-19 patients (Appendix Figure 4), but some of his cytokine levels, including IL-10, IL-12p70, IL-4, and IL-7, remained high 1 month after recovery. Upregulation of co-inhibitory receptors, including cytotoxic T-lymphocyte–associated protein 4, programmed cell death protein 1, lymphocyte-activation gene 3, and T-cell immunoglobulin mucin-3, including *TIGIT*, has been reported in COVID-19 patients in other studies (*5*). These co-inhibitory receptors upregulated after T-cell activation to regulate immune responses and limit immunopathology (*6*,*7*). *TIGIT* can modulate expression of proinflammatory cytokines in acute lymphocytic choriomeningitis virus infection, in which the *TIGIT* blockage increased TNF-α expression by CD8+ T cells (*8*). *TIGIT* -deficient mice displayed increased IFNγ and IL-17+CD4+ T-cell frequencies (*9*). Similarly, *TIGIT* knockdown can increase IFNγ expression in human T cells (*10*). We hypothesize that the nonsense *TIGIT* variant led to low *TIGIT* expression and hyperactive T responses in patient Co45 and might have contributed to his severe inflammation and symptoms. Unfortunately, the patient refused follow-up, so we could not perform further investigations to confirm our hypothesis.

In conclusion, we identified a patient with severe COVID-19 and a *TIGIT* monoallelic nonsense variant. He had lower TIGIT expression in CD3+, CD4+, and CD8+ T cells and produced higher cytokine expression, including IFNγ, TNF-α, and IL-2 upon stimulation. Our findings suggest *TIGIT* could be involved in COVID-19 severity.

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SARS-CoV-2 Omicron BA.1 Challenge after Ancestral or Delta Infection in Mice

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We assessed cross-reactivity to BA.1, BA.2, and BA.5 of neutralizing antibodies elicited by ancestral, Delta, and Omicron BA.1 SARS-CoV-2 infection in mice. Primary infection elicited homologous antibodies with poor cross-reactivity to Omicron strains. This pattern remained after BA.1 challenge, although ancestral- and Delta-infected mice were protected from BA.1 infection.

The SARS-CoV-2 Omicron variant (B.1.1.529, BA.1 sublineage) emerged nearly 2 years after the ancestral strain was identified (*1*). The Omicron BA.1 variant contains ≈50 mutations in the spike protein (*2*), resulting in substantial antigenic change. The strain was more infectious than prior variants of concern (VOCs) and escaped immunity, causing infections in persons who were previously vaccinated with ancestral strain–based vaccines (*3*) or infected with the ancestral virus or Delta (B.1.617.2) VOC. Since January 2022, additional Omicron sublineages (BA.2 to BA.5) have been detected worldwide. BA.4/BA.5 have identical spike proteins, most similar to BA.2, with additional spike mutations (*4*).

We sought to mimic the human scenario and selected a mouse model from available animal models (*5*) to assess the cross-reactivity of neutralizing antibody elicited by ancestral, Delta, and BA.1 viruses and to assess the effect of primary homologous and heterologous infection on secondary infection with the Omicron BA.1 strain. We also compared antibody cross-reactivity to BA.2 and BA.5 in serum samples from mice infected with ancestral, Delta, and BA.1 strains.

We first compared the associated illness, mortality rates, and kinetics of replication of 10^4 50% tissue culture infectious dose ($TCID_{50}$) of SARS-CoV-2/Australia/Vic/01/20 (ancestral strain–like),

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Appendix

Materials and Methods

Subject Recruitment and Sample Collection

In January 2020, we recruited 46 patients with moderate to severe COVID-19 from the King Chulalongkorn Memorial Hospital who were confirmed by standard real-time reverse transcription PCR for SARS-CoV-2 from nasopharyngeal swabs. COVID-19 severity was classified as follows: mild for mild symptoms or only upper respiratory tract infection; moderate for pneumonia without oxygen desaturation; or severe for pneumonia treated with oxygen support. This study was reviewed and approved by the institutional review board of the Faculty of Medicine, Chulalongkorn University (COA no. 738/2020). In addition, written informed consent was obtained from all patients.

We enrolled 1 patient (Co45) with NM_173799.4:c.166C>T (hg19, chr3:114014496 C>T, p.Gln56Ter, rs1386709957) variant in the T cell immunoglobulin and ITIM domain (*TIGIT*) gene and 2 patients (Co6 and Co84) without the variant, all of whom had severe COVID-19 symptoms. We collected serum samples at the acute illness onset or diagnosis, on various hospitalization days (day 4, 5, or 6), and again 1 month after recovery or hospital discharge. In addition, we also collected peripheral blood mononuclear cells (PBMCs) from these 3 patients (Co45, Co6, Co84) at 1-month post-recovery to examine the phenotyping and function of T cells. For healthy controls, we collected blood from 40 healthy donors from the Thai Red Cross Society (IRB approval no. 426/63). All healthy donors were negative for the IgG test kit for SARS-CoV-2.

Clinical Data of Patient with *TIGIT* Variant

A 43-year-old male patient with *TIGIT* nonsense variant (patient Co45) had a history of pulmonary tuberculosis 10 years prior and presented with cough, nasal congestion, and myalgia. At admission, his physical examination revealed body mass index of 24 kg/m^2, body temperature of 36.5°C, respiratory rate 18/min, SpO_2 99%, with normal breath sounds. Chest x-ray showed minimal and faint reticular opacity in both upper lungs, and no cavity was noted. Complete blood count revealed hematocrit of 49.6%, white blood count 5,710 cells/mm^3 (PMNs 59%, lymphocytes 30.8%), and normal platelet count (222,000/mm^3). After 5 days of admission, a new low-grade fever developed with dyspnea (SpO2 95% on room air), and new patchy opacity appeared in the right lower lung field. He was treated with favipiravir 1,600 mg twice per day for the first day and 600 mg twice per day for the next 9 days, oral azithromycin 500 mg daily for the first day, then 250 mg daily for the next 4 days, and hydroxychloroquine 200 mg 3 times per day for 10 days according to a regimen for pneumonia in March 2020. Three days later, his symptoms improved and was afebrile and had normal oxygen saturation during the 6-minute walk test.

Exome Sequencing

Peripheral blood was obtained for genomic DNA extraction, then whole-exome sequencing (WES) of all 46 patients was performed, as previously described (*1*). In brief, the sequencing libraries were enriched by using a SureSelect Human All Exon V7 Kit (Agilent Technologies, https://www.agilent.com) and were sequenced using HiSeq 4000 (Illumina, https:www.illumina.com). The variants were filtered with the following criteria: 1) passed the quality filters; 2) had read depth >10; 3) located in the coding regions and canonical splice sites of 1,810 immune-related genes, including genes related to an abnormality of the immune system (HP:0002715) and immune checkpoint genes (*2*); 4) had <1% allele frequency in the Genome Aggregation Database (gnomAD), Exome Variant Server, 1000 Genomes Project Consortium, dbSNPs; and 5) absent from the Thai reference exome (T-Rex) variant database (*3*). The candidate variants were called novel if they were not noted in the 1000 Genomes Project, gnomAD, and in-house databases.

PBMC Isolation

PBMCs from whole blood were isolated by density gradient centrifugation by using Lymphoprep (STEMCELL Technologies, https://www.stemcell.com) at 1,500 rpm for 30 min at

room temperature (no deceleration force). Isolated PBMCs were washed with RPMI 1640 Medium (GIBCO, Thermo Fisher–Life Technologies Corporation, https://www.thermofisher.com) supplemented with 10% fetal bovine serum (FBS) and cryopreserved in 10% dimethyl sulfoxide (DMSO) in FBS for further experiments.

T Cell Phenotyping

PBMCs were stained with monoclonal antibodies, including 7-AAD viability staining solution, anti-CD3-PE/Cy7, anti-CD4-AF700, anti-CD8-APC, anti-TIGIT- PE/Dazzle 594, and anti-CD69-BV650 (Biolegend, https://www.biolegend.com). After washing twice, cells were fixed and acquired by CytoFLEX flow cytometer (Beckman Coulter Life Sciences, https://www.beckmancoulter.com). All data were analyzed using Flowjo X software (https://www.flowjo.com).

T cell Functional Analysis

PBMCs were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin (GIBCO). One million PBMCs were seeded in U-bottomed 96 well-plate (Thermo Fisher Scientific) and stimulated with or without anti-CD3/CD28-coupled beads (Thermo Fisher Scientific) for 24 hours. Brefeldin A (Biolegend) was added for the final 5 hours of incubation for intracellular cytokine detection. After the incubation period, cells were harvested and stained with antibodies for surface markers, including 7-AAD viability staining solution, anti-CD3-PE/Cy7, anti-CD4-AF700, anti-CD8-APC, anti-TIGIT-PE/Dazzle 594, and anti-CD69-BV650. Next, cells were fixed and permeabilized by BD Cytofix/Cytoperm (BD Biosciences, https://www.bdbiosciences.com), the intracellular staining was performed with fluorescently labeled antibodies including anti-TNFα-pacific blue, anti-IFNγ-PE, and anti-IL2-BV605 for 1 hour on ice. After washing twice, cells were fixed and acquired by CytoFLEX flow cytometer (Beckman Coulter Life Sciences, https://www.beckmancoulter.com), and all data were analyzed by using Flowjo X software. All antibodies were purchased from Biolegend.

Cytokine Measurement by Bio-Plex Multiplex Immunoassay System

Bio-Plex Pro Human Cytokine 27-plex Assay (Bio-Rad, https://www.bio-rad.com) was performed to investigate cytokine and chemokine levels, including FGF basic, Eotaxin, G-CSF, GM-CSF, IFN-γ, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1 (MCAF), MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, and

VEGF (Appendix Figure 4), in blood serum of healthy subjects and patients. Fifty microliters of diluted serum were used for the assay following the manufacturer's instructions. Levels of cytokines were measured by Bio-Plex 200 Systems (BioRad), and the data were analyzed using Bio-Plex Manager software (BioRad).

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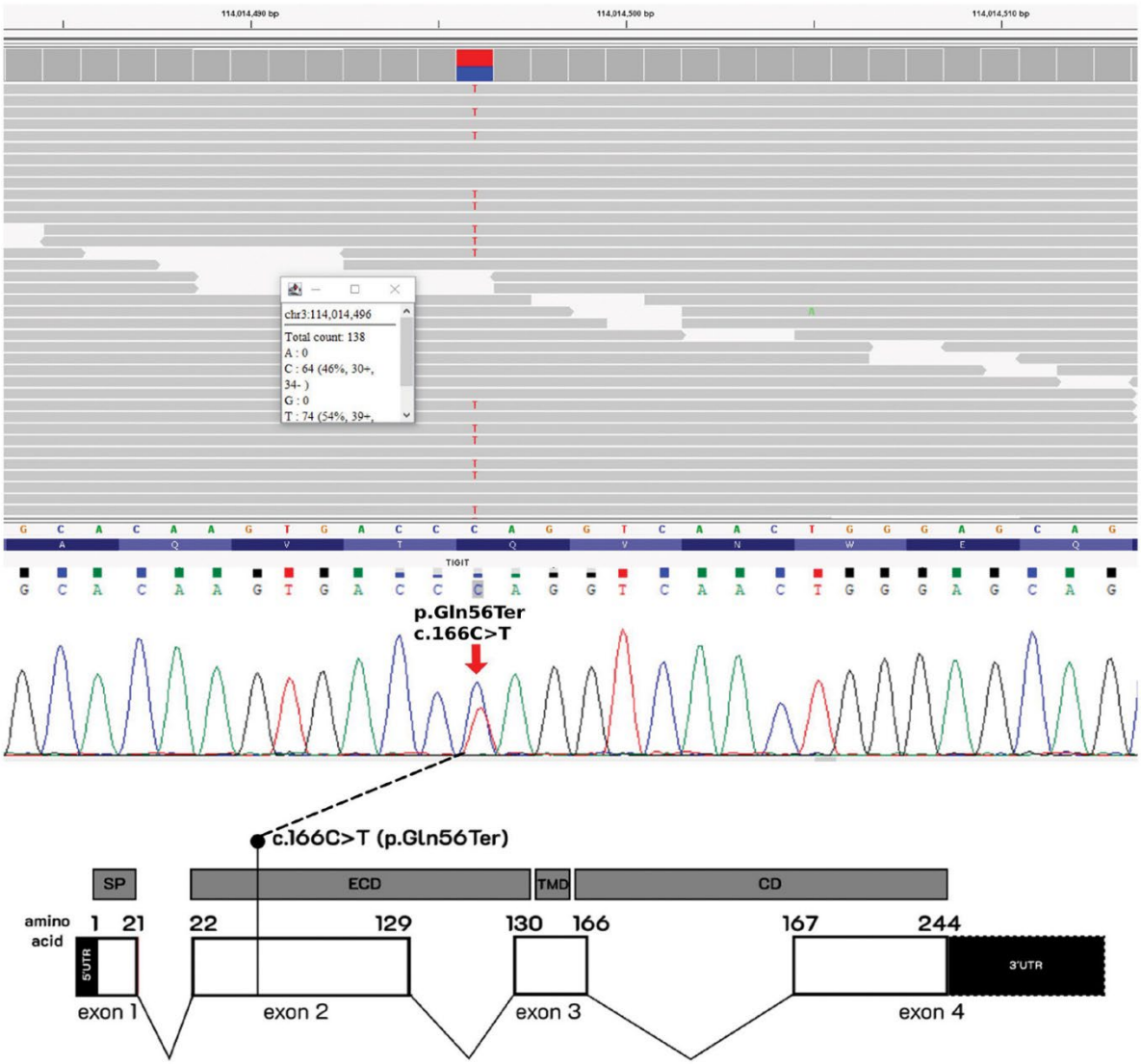
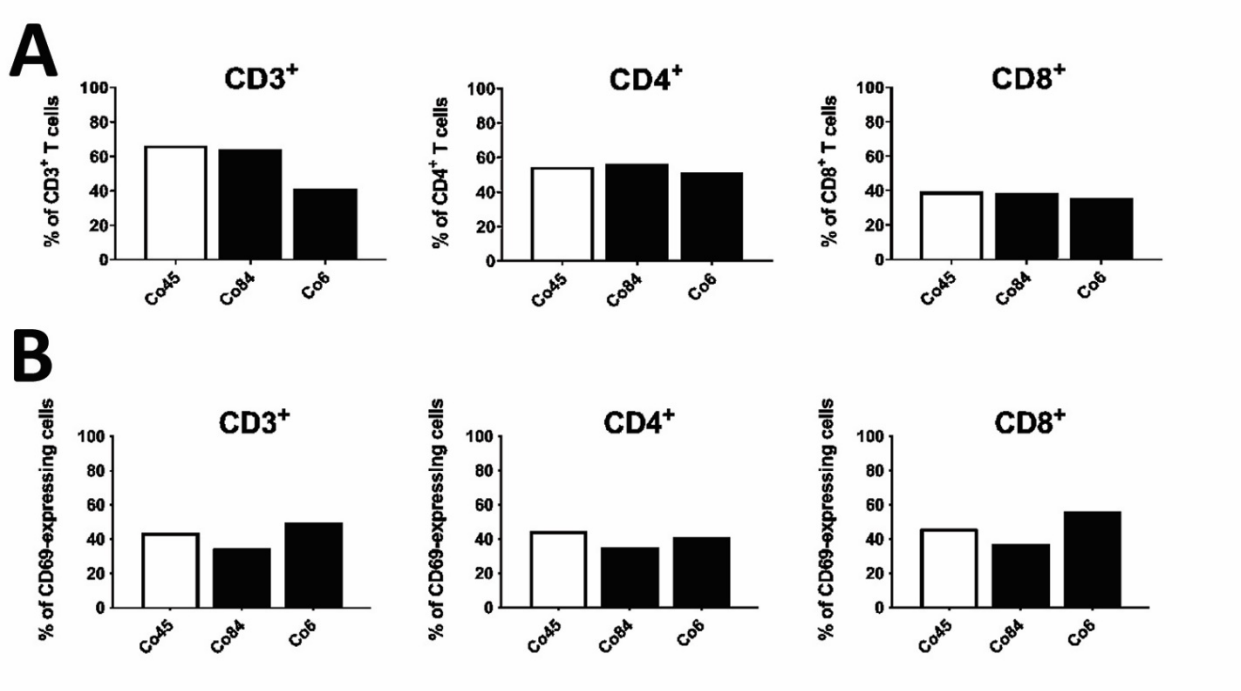
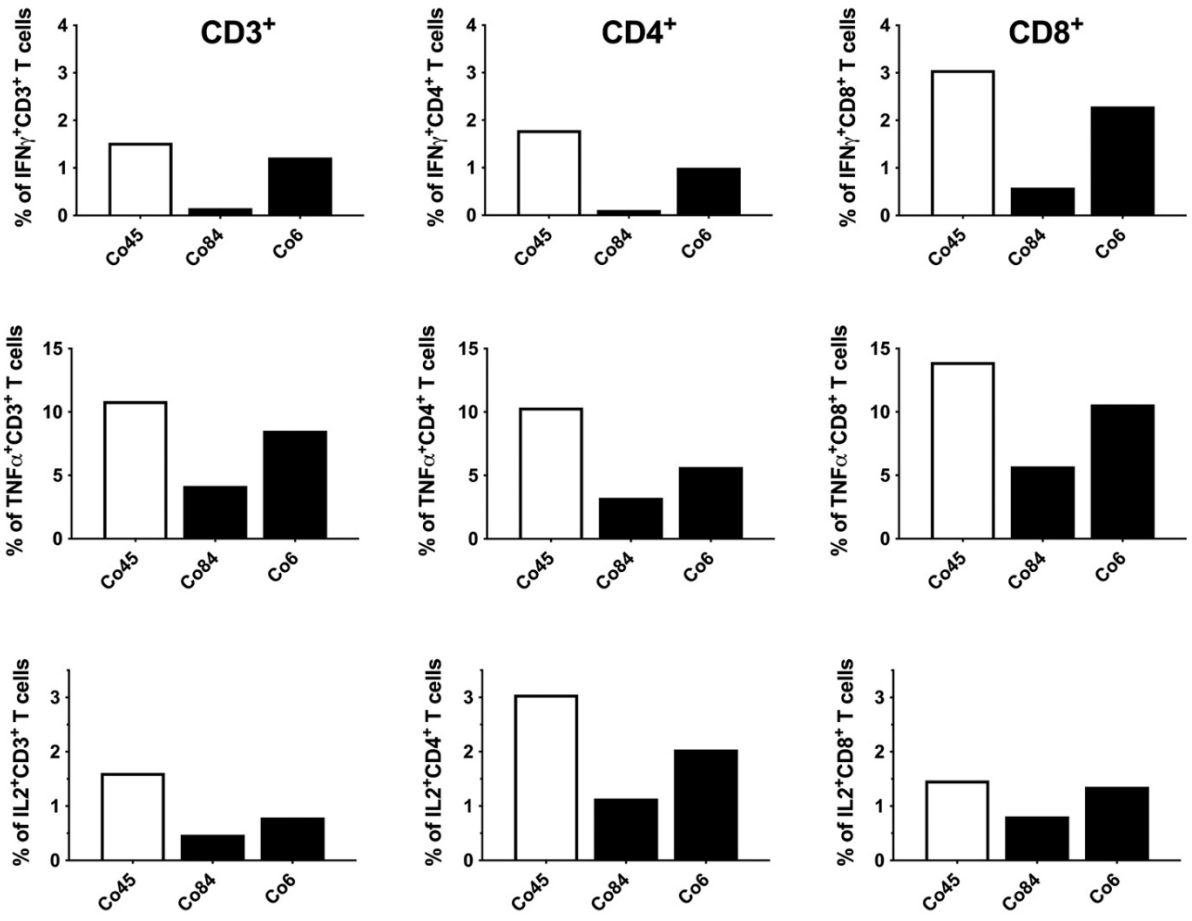


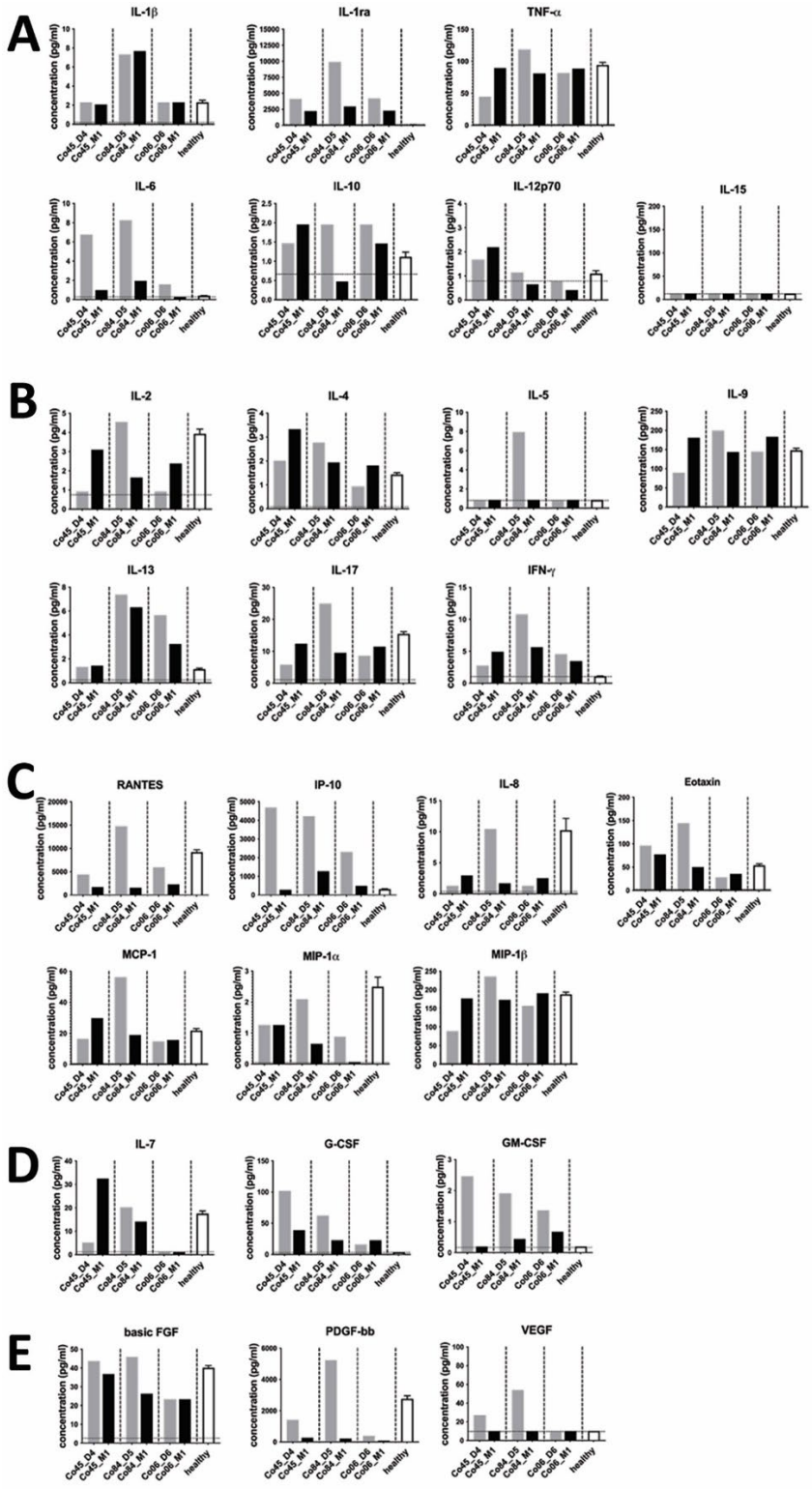
Appendix Figure 1. Whole-exome sequencing of *TIGIT* nonsense variant in a patient with severe COVID-19 infection, Thailand. Whole-exome sequencing of 138 read depths showed a nonsense *TIGIT*:c.166C>T (p.Gln56Ter) variant (64 reads with the reference C allele and 74 reads with alternative T allele) (Upper panel), compared with the direct sequencing electropherogram (middle panel). This variant was in exon 2 of the *TIGIT* gene, which causes truncation from the ECD to the entire polypeptide (bottom panel). ECD; extracellular domain; *TIGIT*, T cell immunoglobulin and ITIM domain gene.



Appendix Figure 2. Measurement of T cell frequencies and activation of *TIGIT* nonsense variant in a patient with severe COVID-19 infection, Thailand. Co45 is the patient with *TIGIT* variation; Co84 and Co6 are the patients without the variation. A) We evaluated PBMCs for frequencies of $CD3^+$, $CD4^+$, and $CD8^+$ T cells. B) We stimulated PBMCs of the 3 patients with anti-CD3/CD28-coupled beads for 24 hours and measured the frequencies of $CD69^+CD3^+$, $CD69^+CD4^+$, and $CD69^+CD8^+$ T cells by flow cytometry. CD, cytoplasmic domain; PBMCs, peripheral blood mononuclear cells; *TIGIT*, T cell immunoglobulin and ITIM domain gene.



Appendix Figure 3. Measurement of T cell frequencies and activation of *TIGIT* nonsense variant in a patient with severe COVID-19 infection, Thailand. Co45 is the patient with *TIGIT* variation; Co84 and Co6 are the patients without the variation. We stimulated PBMCs with beads coupled with anti-CD3/CD28 for 24 hours and assessed by measuring IFN_{γ} (upper panel), TNF_{α} (middle panel), and IL-2 production (lower panel) in $CD3^{+}$, $CD4^{+}$, and $CD8^{+}$ T cells. CD, cytoplasmic domain; IFN_{γ}, interferon gamma; IL-2, lymphokine, interleukin 2; *TIGIT*, T cell immunoglobulin and ITIM domain gene; TNF_{α}, tumor necrosis factor alpha.



Appendix Figure 4. Measurement of cytokine and chemokine levels in a patient with *TIGIT* nonsense variant and severe COVID-19 infection, Thailand. We compared levels from the patient (Co45) with *TIGIT* variation against patients without the variation (Co84 and Co06) and against 40 healthy controls. Cytokine

and chemokine levels in serum of patients at acute COVID-19 infection phase (day 4, 5, or 6) and at 1 month after recovery were assessed comparing to healthy controls by using Bio-Plex Pro Human Cytokine 27-plex Assay (Lumninex, https://www.luminexcorp.com). Dotted lines indicate assay sensitivity, limit of detection (LOD). A) Cytokines in innate immunity; B) cytokines in adaptive immunity; C) chemokines; D) hematopoietic cytokines; E) growth factors. D4, day 4; D5, day 5; D6, day 6; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN_{γ}, interferon gamma; IL, lymphokine, interleukin; M1, month 1; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; *TIGIT*, T cell immunoglobulin and ITIM domain gene; VEGF, vascular endothelial growth factor.