THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Young BE, Fong S-W, Chan Y-H, et al. Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study. *Lancet* 2020; published online Aug 18. http://dx.doi.org/10.1016/S0140-6736(20)31757-8.

Multiplex microbead-based immunoassay

Kit analyte detection panel included granulocyte-macrophage colony-stimulating factor (GM-CSF), epidermal growth factor (EGF), brain-derived neurotropic factor, beta-nerve growth factor (bNGF), basic fibroblast growth factor (FGF-2), hepatocyte growth factor (HGF), monocyte chemoattractant protein (MCP) 1, macrophage inflammatory protein (MIP) 1α, MIP-1β, RANTES (regulated on activation, normal T cell expressed and secreted), chemokine (C-X-C motif) ligand (CXCL) 1 (GRO-α), stromal cell-derived factor 1 (SDF-1α), interferon (IFN) gamma-induced protein 10 (IP-10), eotaxin, IFN-α, IFN-γ, interleukin (IL) IL-1α, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, leukemia inhibitory factor (LIF), stem cell factor (SCF), tumor necrosis factor (TNF-α), TNF-β, vascular endothelial growth factors A and D (VEGF-A, VEGF-D), platelet derived growth factor (PDGF-BB), and placental growth factor (PLGF-1). Standards and plasma from COVID-19 patients and healthy controls were incubated with fluorescent-coded magnetic beads pre-coated with respective antibodies in a black 96-well clear-bottom plate overnight at 4˚C. After incubation, plates were washed 5 times with wash buffer (PBS with 1% BSA (Capricorn Scientific) and 0.01% Tween (Promega). Sample-antibody-bead complexes were incubated with Biotinylated detection antibodies for 1 hour and washed 5 times with wash buffer. Subsequently, Streptavidin-PE was added and incubated for another 30 mins. Plates were washed 5 times again before sample-antibody-bead complexes were re-suspended in sheath fluid for acquisition on the FLEXMAP® 3D (Luminex) using xPONENT® 4.0 (Luminex) software. Internal control samples were included in each Luminex assays to remove any potential plate effects. Readouts of these samples were then used to normalize the assayed plates. A correction factor was obtained from the differences observed across the multiple assays and this correction factor was then used to normalize all the samples. Standard curves were generated with a 5-PL (5-parameter logistic) algorithm, reporting values for mean florescence intensity (MFI) and concentration data. The concentrations were logarithmically transformed to ensure normality.

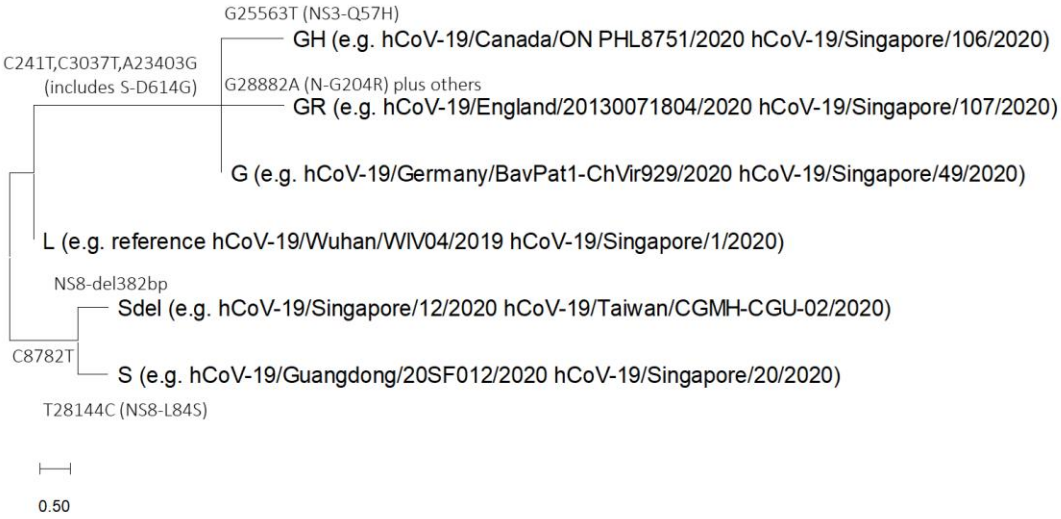
Table of 57 patients with virologic samples and complete sequence data: 12 deletion variant only detected, 7 mixed wildtype and deletion, 38 wildtype virus only

Deletion detected	Wildtype detected	Sequence name (GISIAD)	Clade
Yes	No	hCoV-19/Singapore/12Clin/2020	S
Yes	No	hCoV-19/Singapore/13/2020	S
Yes	No	hCoV-19/Singapore/14Clin/2020	S
Yes	Yes	hCoV-19/Singapore/15/2020	S
Yes	No	hCoV-19/Singapore/16/2020	S
Yes	Yes	hCoV-19/Singapore/18/2020	S
Yes	Yes	hCoV-19/Singapore/188/2020	Undefined
Yes	Yes	hCoV-19/Singapore/19/2020	S
Yes	Yes	hCoV-19/Singapore/21/2020	S
Yes	No	hCoV-19/Singapore/211/2020	Undefined
Yes	No	hCoV-19/Singapore/22/2020	S
Yes	No	hCoV-19/Singapore/23/2020	S
Yes	No	hCoV-19/Singapore/30/2020	S
Yes	Yes	hCoV-19/Singapore/315/2020	Undefined
Yes	Yes	hCoV-19/Singapore/383/2020	S
Yes	No	hCoV-19/Singapore/43/2020	S
Yes	No	hCoV-19/Singapore/467/2020	S
Yes	No	hCoV-19/Singapore/469/2020	Undefined
Yes	No	hCoV-19/Singapore/51/2020	S
No	Yes	hCoV-19/Singapore/1/2020	L
No	Yes	hCoV-19/Singapore/106/2020	GH
No	Yes	hCoV-19/Singapore/109/2020	L
No	Yes	hCoV-19/Singapore/11/2020	L
No	Yes	hCoV-19/Singapore/165/2020	L
No	Yes	hCoV-19/Singapore/17/2020	Undefined
No	Yes	hCoV-19/Singapore/175/2020	L
No	Yes	hCoV-19/Singapore/2/2020	L
No	Yes	hCoV-19/Singapore/20/2020	S
No	Yes	hCoV-19/Singapore/212/2020	L
No	Yes	hCoV-19/Singapore/220/2020	Undefined
No	Yes	hCoV-19/Singapore/24/2020	L
No	Yes	hCoV-19/Singapore/25/2020	L
No	Yes	hCoV-19/Singapore/26/2020	L
No	Yes	hCoV-19/Singapore/27/2020	V
No	Yes	hCoV-19/Singapore/28/2020	L
No	Yes	hCoV-19/Singapore/29/2020	L
No	Yes	hCoV-19/Singapore/3/2020	Undefined
No	Yes	hCoV-19/Singapore/31/2020	L
No	Yes	hCoV-19/Singapore/32/2020	L
No	Yes	hCoV-19/Singapore/33/2020	L
No	Yes	hCoV-19/Singapore/34/2020	L
No	Yes	hCoV-19/Singapore/35/2020	L
No	Yes	hCoV-19/Singapore/37/2020	GH
No	Yes	hCoV-19/Singapore/4/2020	Undefined

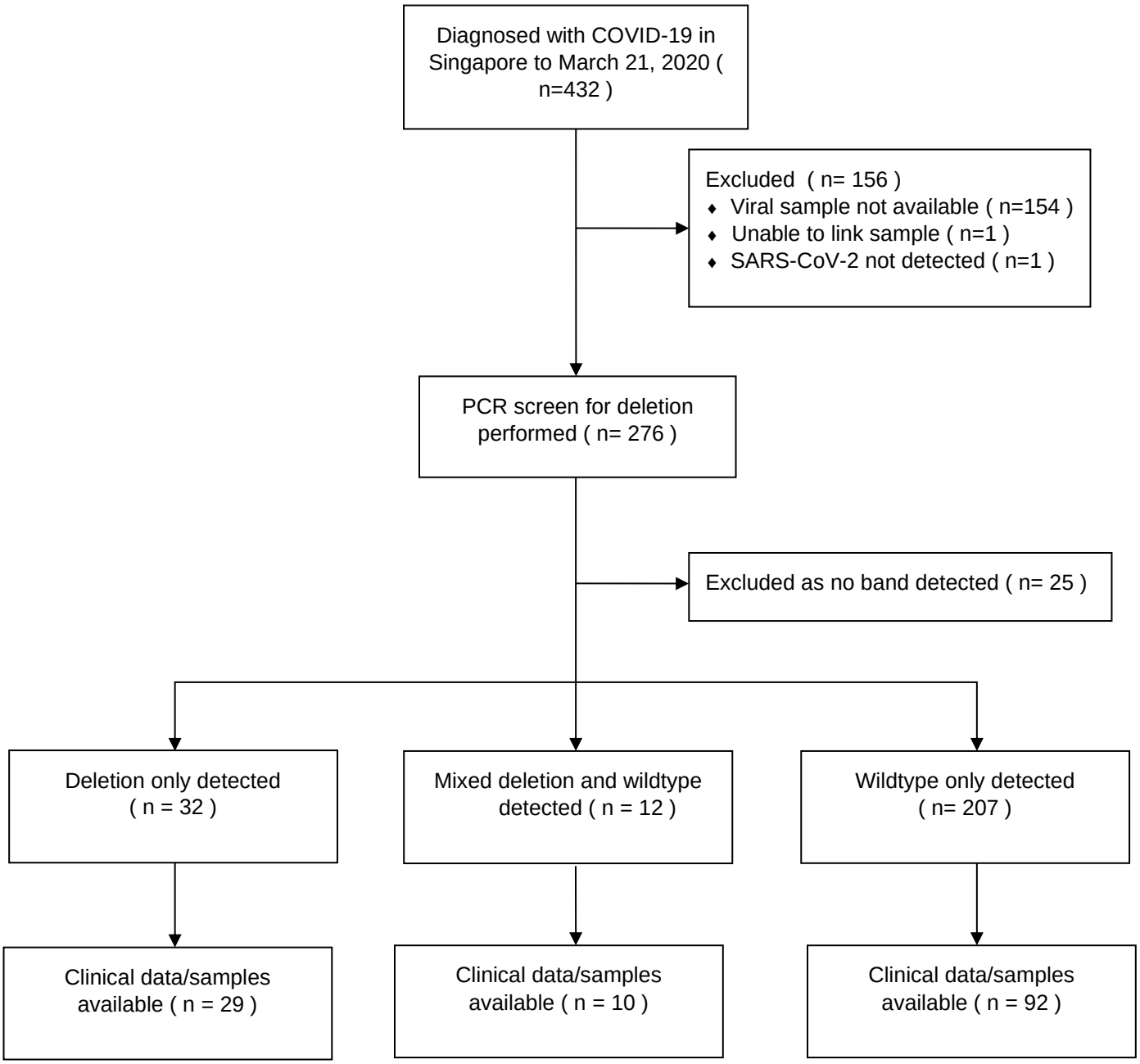
No	Yes	hCoV-19/Singapore/434/2020	Undefined
No	Yes	hCoV-19/Singapore/466/2020	S
No	Yes	hCoV-19/Singapore/471/2020	G
No	Yes	hCoV-19/Singapore/472/2020	L
No	Yes	hCoV-19/Singapore/474/2020	L
No	Yes	hCoV-19/Singapore/476/2020	Undefined
No	Yes	hCoV-19/Singapore/479/2020	Undefined
No	Yes	hCoV-19/Singapore/482/2020	G
No	Yes	hCoV-19/Singapore/483/2020	G
No	Yes	hCoV-19/Singapore/49/2020	G
No	Yes	hCoV-19/Singapore/5/2020	Undefined
No	Yes	hCoV-19/Singapore/6/2020	L
No	Yes	hCoV-19/Singapore/9/2020	Undefined

All deletion variant and mixed infections mapped to Clade S. For the control group with wildtype infection, caldes were L: 20, G: 4, GH: 2, S: 2, V: 1, Undefined: 9.

Phylogenetic tree based on 9 marker mutations that define early and current major genotypes and clades including Clade S which contains the 382-variant (NS8-del382bp) and Clade G which includes the D614G variant



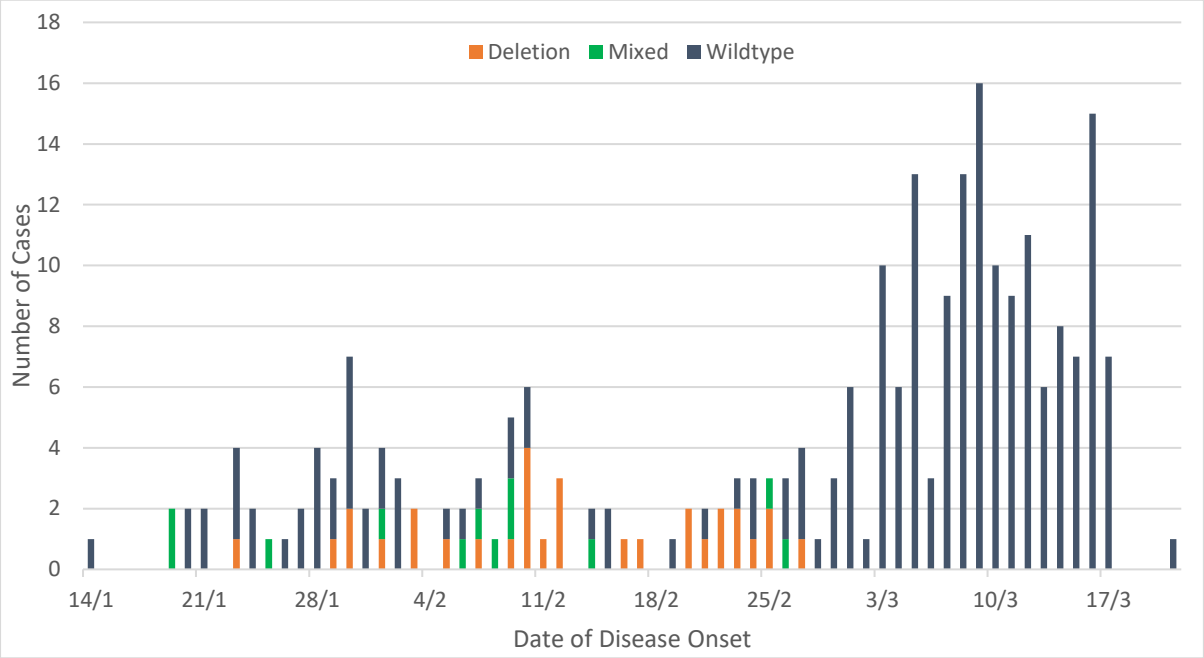
Flow diagram of study.



Stored respiratory samples available and valid result obtained from ORF8 PCR for 251 patients. Of these, 131 were recruited to the cohort study ('PROTECT"), while 120 were either not approached due to resource constainsts or declined to participate.

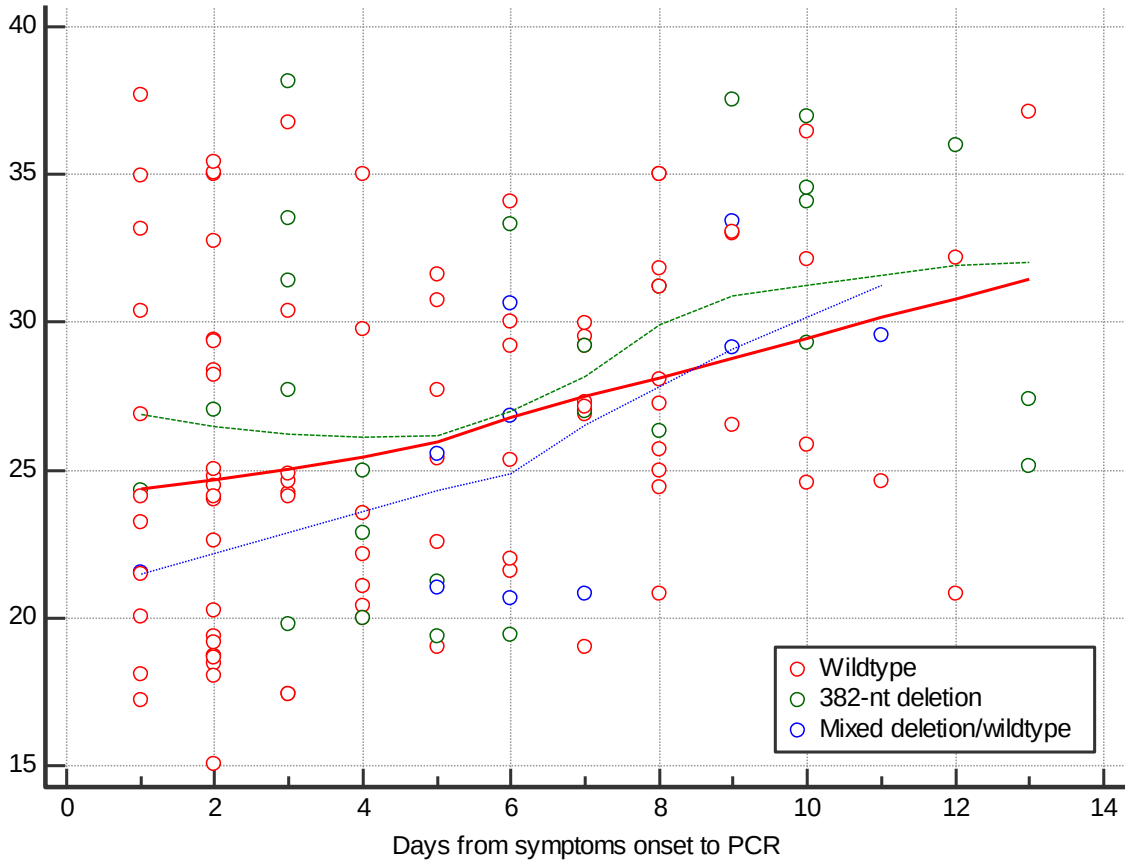
Recruitment was conducted at eight public hospitals in Singapore; numbers in brackets indicate number of patients from each hospital enrolled in the study [National Centre for Infectious Diseases (115), Singapore General Hospital (6), National University Hospital (3), Ng Teng Fong General Hospital (3), Changi General Hospital (2), Alexandra Hospital (1), Khoo Teck Puat Hospital (1)]

Date of disease onset by genotype, January 14, 2020 to March 21, 2020



Excludes 4 of 251 cases where date of disease onset was unknown

Scatter plot of first PCR Cycle threshold (Ct) value by day of symptom onset and infection group (wildtype vs. 382-nucleotide(nt) deletion variant vs. mixed deletion/wildtype)



Line plotted using locally weighted scatterplot smoothing (LOWESS), data limited to first PCR Ct value within first 14 days from symptoms onset.

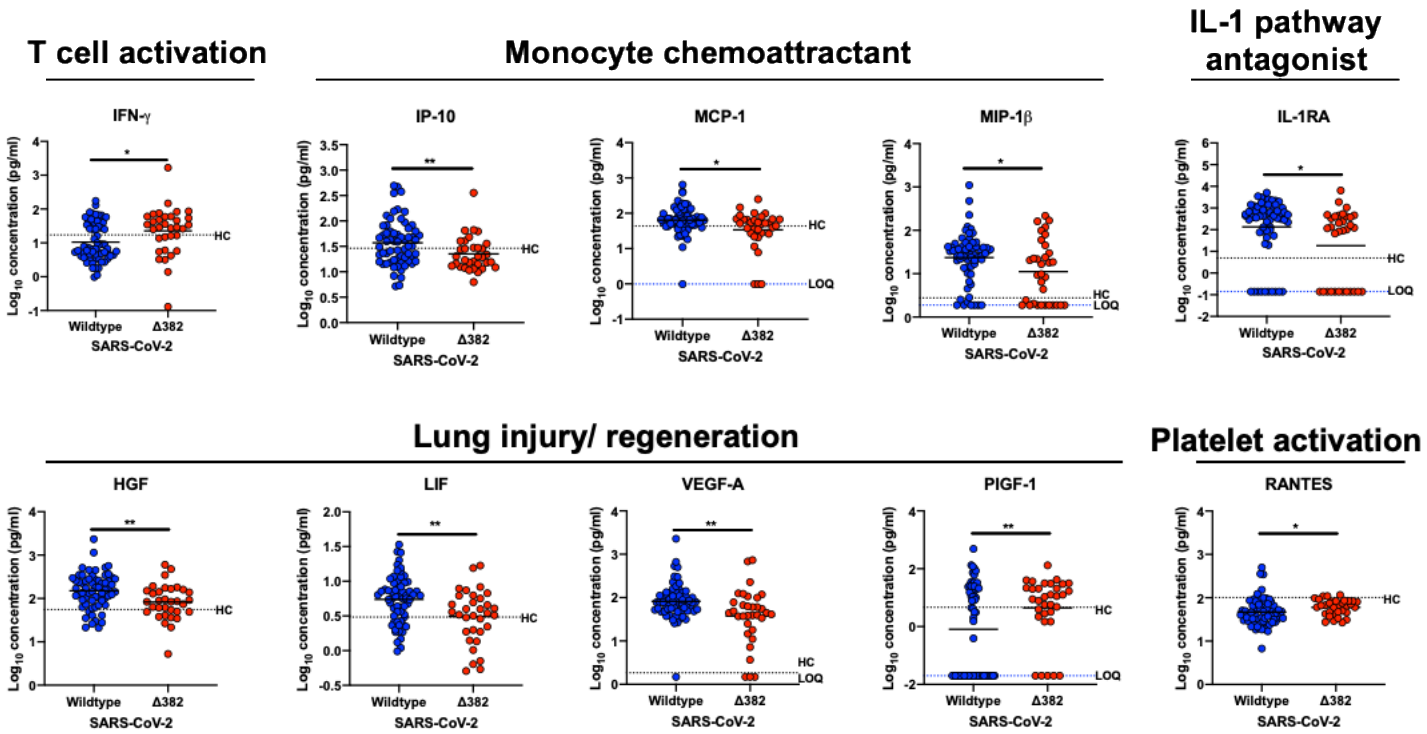
Concentrations of immune mediators in COVID-19 patients (n = 97)

No	Immune mediator	Mean Concentration, pg ml^{-1}			
		Wildtype (n = 64)	**Δ382 (n = 33)**	**Difference (Wildtype vs. Δ382)**	**Healthy controls (n = 23)**
1	BDNF	22·7	21·8	0·9	11·1
2	EGF	0·7	1·2	0·5	0·4
3	Eotaxin	17·4	17·5	0·1	26·9
4	FGF-2	0·4	0·4	0	0·2
5	GM-CSF	1	0·9	0·1	1·1
6	GRO-α	0·2	0·2	0	2·5
7	HGF	150·9	83	67·9	55·5
8	IFN-α	0·2	0·1	0·1	0·1
9	IFN-γ	10·6	22·7	12·1	17·2
10	IL-1α	0·1	0·1	0	0·3
11	IL-1β	1·3	1·1	0·2	0·8
12	IL-10	0·1	0·1	0	0·1
13	IL-12p70	0·3	0·3	0	0·1
14	IL-13	0·5	0·6	0·1	0·6
15	IL-15	6·3	1·7	4·6	1·9
16	IL-17A	0·6	0·2	0·4	0·2
17	IL-18	53·3	46·2	7·1	29·2
18	IL-1RA	135·1	18·5	116·6	5
19	IL-2	6·6	10·8	4·2	12·1
20	IL-21	1·1	0·9	0·2	1·2
21	IL-22	1·8	1·7	0·1	9·6
22	IL-23	0·4	0·4	0	0·4
23	IL-27	2	1·7	0·3	2
24	IL-31	2·7	2·7	0	2·7

25	IL-4	0·3	0·5	-0·2	5·3
26	IL-5	0·2	0·4	-0·2	0·1
27	IL-6	1·2	0·4	0·8	0·4
28	IL-8	0·2	0·2	0	0·2
29	IP-10	37·2	22·5	14·7	29
30	IL-7	0·3	0·2	0·1	0·3
31	LIF	5·5	3·2	2·3	3·1
32	MCP-1	64·2	34	30·2	43·6
33	MIP-1α	1·1	1·2	0·1	2
34	MIP-1β	23·5	11·2	12·3	2·8
35	PDGF-BB	46·9	49·7	2·8	91·4
36	PIGF-1	0·9	4·5	3·6	4·7
37	RANTES	46·9	60·2	13·3	100·9
38	SCF	4·1	4·2	0·1	4
39	SDF-1α	526·2	641·1	114·9	645·9
40	TNF-α	1·7	3·5	1·8	1·3
41	VEGF-A	82·5	37·7	44·8	1·9
42	VEGF-D	0·3	0·2	0·1	1·4

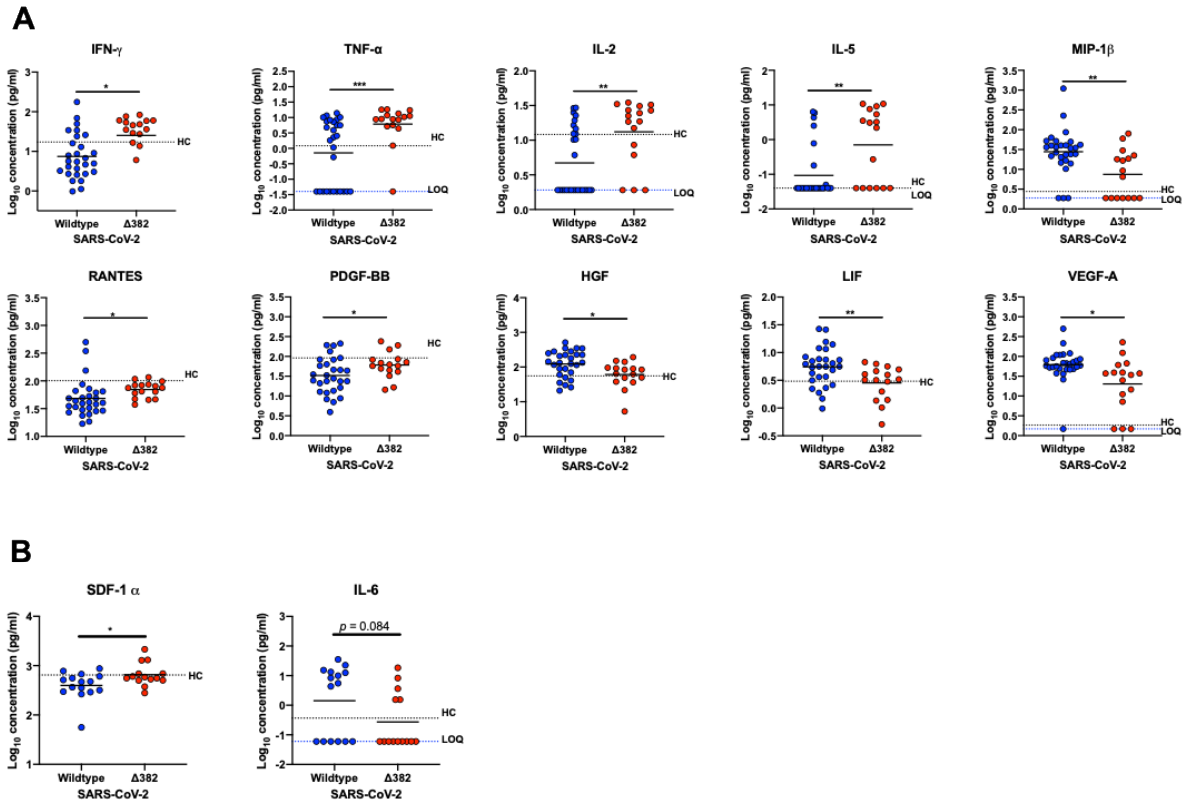
Majority of the samples have readings of IL-9 and TNF-beta below limit of quantitation (LOQ), thus IL-9 and TNF-beta are excluded in the analysis. granulocyte-macrophage colony-stimulating factor (GM-CSF); epidermal growth factor (EGF); brain-derived neurotropic factor (BDNF); beta-nerve growth factor (bNGF); basic fibroblast growth factor (FGF-2); hepatocyte growth factor (HGF); monocyte chemoattractant protein (MCP) 1; macrophage inflammatory protein (MIP) 1α, MIP-1β; RANTES (regulated on activation, normal T cell expressed and secreted); chemokine (C-X-C motif) ligand (CXCL) 1 (GRO-α); stromal cell-derived factor 1 (SDF-1α); interferon (IFN) gamma-induced protein 10 (IP-10); interferon alpha (IFN-α), interferon gamma (IFN-γ), interleukin (IL) IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31; interleukin-1 receptor antagonist (IL-1RA); leukemia inhibitory factor (LIF); stem cell factor (SCF); tumor necrosis factor-alpha (TNF-α); tumor necrosis factor-beta (TNF-β); vascular endothelial growth factors A and D (VEGF-A, VEGF-D); platelet derived growth factor (PDGF-BB); and placental growth factor (PLGF-1).

Plasma immune mediator levels of COVID-19 patients infected with 382-nt deletion variant of SARS-CoV-2 (Δ382).



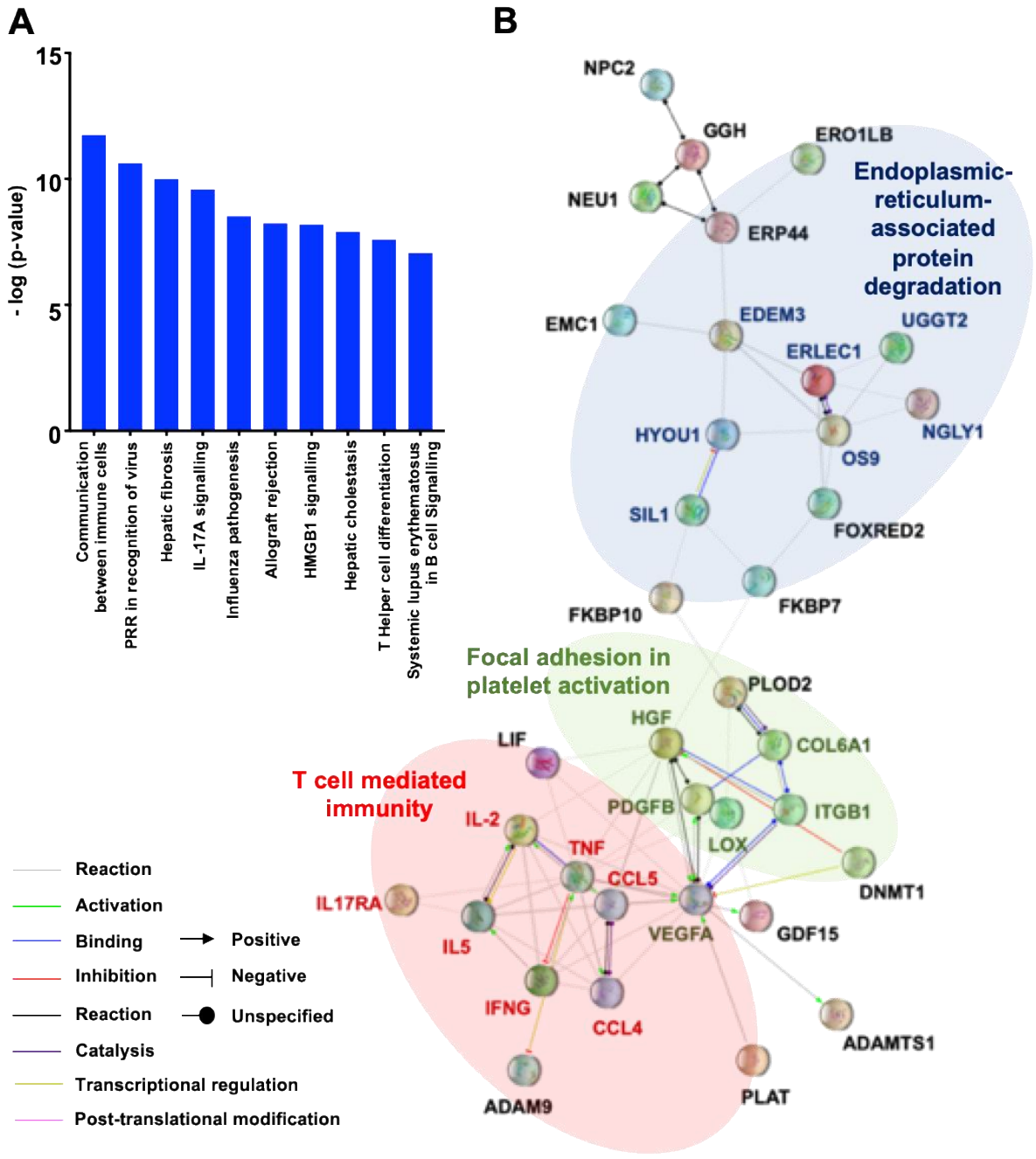
Concentrations of 45 immune mediators were quantified using a 45-plex microbead-based immunoassay. Immune mediator levels in plasma samples of patients infected with either wildtype (n = 64) or **Δ**382 (n = 33) SARS-CoV-2 during first collection timepoint upon hospital admission (median PIO of 8 days). Profiles of significant immune mediators, including monocyte chemoattractants, IL-1 pathway antagonist, growth factors involved in lung injury or regeneration, soluble mediators that regulate T cells and platelet activities, are illustrated as scatter plots. Unpaired t-tests were conducted on the logarithmically transformed concentration (*$P < 0{\cdot}05$; **$P < 0{\cdot}01$). Immune mediator levels for healthy controls (n = 23) are indicated by the black dotted line. Patient samples with concentration out of measurement range are presented as the value of logarithm transformation of Limit of Quantification (LOQ), indicated by the blue dotted line.

Plasma immune mediator levels of COVID-19 patients infected with 382-nt deletion variant of SARS-CoV-2 (Δ382) stratified by infection severity



(A) Profiles of significant immune mediators of COVID-19 patients without pneumonia infected with either wildtype (n = 28) or **Δ**382 (n = 16) SARS-CoV-2 are illustrated as scatter plots. **(B)** Profiles of immune mediators of COVID-19 patients with pneumonia infected with either wildtype (n = 15) or **Δ**382 (n = 14) SARS-CoV-2 are illustrated as scatter plots. Unpaired t-tests were conducted on the logarithmically transformed concentrations (*$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$). Immune mediator levels for healthy controls (n = 23) are indicated by the black dotted line. Patient samples with concentration out of measurement range are presented as the value of logarithm transformation of Limit of Quantification (LOQ), indicated by the blue dotted line.

Network analysis of immune mediators associated with 382-nt deletion variant of SARS-CoV-2



(A) Ingenuity Pathway Analysis (IPA) of the ten significant immune mediators associated with 382-nt deletion in SARS-CoV-2. The chart represents the top ten significantly associated canonical pathways with the immune mediators. **(B)** Interactive relationships between specific immune mediators and host proteins were determined by STRING (Search Tool for the Retrieval of Interacting Genes/ Proteins) analysis, with a confidence threshold of 0·5.