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Supplemental information

Significance of interferon signaling based on mRNA-microRNA integration and plasma protein analyses in critically ill COVID-19 patients

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Table S1. Patient characteristics of COVID-19 patients in the IFN proteins profile cohort

	Non-critical	Critical		
		Overall	Survivor	Non-survivor
	(N=22)	(N=181)	(N=154)	(N=27)
Age, median, IQR	70 (61.5-73.3)	66 (56-74.5)	63.5 (55.75-73)	74 (70-80)
Sex, male (%)	19 (86.4)	125 (69.1)	109 (70.8)	16 (59.3)
BMI, median, IQR	24.2 (22.3-29.3)	25.2 (22.9-27.9)	25.2 (22.9-27.5)	25.0 (23.5-29.1)
Comorbidities, n (%)				
Diabetes	7 (31.8)	71 (39.2)	59 (38.3)	12 (44.4)
Hypertension	11 (50)	88 (48.6)	73 (47.4)	15 (55.6)
Hyperlipidemia	5 (22.7)	57 (31.4)	48 (31.2)	9 (33.3)
Hyperuricemia	4 (18.2)	19 (10.5)	16 (10.4)	3 (11.1)
Chronic heart disease	5 (22.7)	17 (9.4)	13 (8.4)	4 (14.8)
Chronic lung disease	4 (18.2)	22 (12.2)	17 (11.0)	5 (18.5)
Chronic kidney disease	2 (9.1)	21 (11.6)	17 (11.0)	4 (14.8)
Immunocompromised condition	1 (4.5)	5 (2.7)	4 (2.6)	1 (3.7)
Malignant neoplasm	1 (4.5)	15 (8.3)	12 (7.8)	3 (11.1)
Length of hospitalization	10 (6-20)	11 (8-17)	10 (7-16)	17.5 (11.8-34.5)
Acuity score				
1= Death	5 (22.7)	26 (14.4)	0 (0)	27 (100)
2= Intubated/ventilated, survived	5 (22.7)	155 (85.6)	154 (100)	0 (0)
3= Hospitalized, O_2 required, survived	12 (54.5)	0 (0)	0 (0)	0 (0)
4= Hospitalized, no O_2 required, survived	0 (0)	0 (0)	0 (0)	0 (0)
5= Discharged/Not hospitalized, survived	0 (0)	0 (0)	0 (0)	0 (0)
Intubation required, n (%)	9 (40.9)	181 (100)	154 (100)	27 (100)
Extracorporeal membrane oxygenation	1 (12.5)	8 (4.5)	6 (3.9)	2 (7.7)
SOFA score, median, IQR	2 (2-3)	5 (3-6)	5 (3-6)	6 (5-7)
APACHE II score, median, IQR	7 (4-12.5)	13 (10-15)	12 (9-15)	15 (12-20)
Clade, n (%)				
20A	0 (0)	1 (0.6)	1 (0.6)	0 (0)
20B	3 (13.6)	19 (10.5)	17 (11.0)	2 (7.4)
20I (Alpha, V1)	0 (0)	14 (7.7)	11 (7.1)	3 (11.1)
unknown	19 (86.4)	147 (81.2)	125 (81.2)	22 (81.5)

IQR, interquartile range; BMI, body mass index; SOFA, Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health Evaluation.
Data are shown as group number (percentage) or median (interquartile range).

Table S2. Significant canonical signaling pathways in COVID-19 mRNA identified in the RNA-seq derivation cohort

Ingenuity Canonical Pathways	-log (B-H p-value)	z-score
Interferon Signaling	10.1	3.962
PD-1, PD-L1 Cancer Immunotherapy Pathway	8.95	2.333
TREM1 Signaling	6.92	3.413
Neuroinflammation Signaling Pathway	6.91	2.018
MSP-RON Signaling in Macrophages Pathway	5.61	3.452
Role of PKR in Interferon Induction and Antiviral Response	5.22	3.124
Toll-like Receptor Signaling	5.22	3.838
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	5.19	2.746
Role of Hypercytokinemia/Hyperchemokinemia in the Pathogenesis of Influenza	4.83	4.382
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	4.42	2.48
Osteoarthritis Pathway	3.46	2.402
iNOS Signaling	3.35	3.357
p38 MAPK Signaling	3.18	3.772
Inflammasome Pathway	2.75	3.162
IL-8 Signaling	2.45	2.03
CREB Signaling in Neurons	2.39	-2.223
IL-6 Signaling	2.27	2.921
Acute Phase Response Signaling	2.16	3.528
Cyclins and Cell Cycle Regulation	2.12	2.236
Salvage Pathways of Pyrimidine Ribonucleotides	1.94	2.041
Estrogen-mediated S-phase Entry	1.91	2.53
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	1.89	2.041
GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells	1.86	-2.117
Ephrin Receptor Signaling	1.64	2.4
Salvage Pathways of Pyrimidine Deoxyribonucleotides	1.62	2
Nitric Oxide Signaling in the Cardiovascular System	1.49	-2.236
Autophagy	1.31	2.343

Table S3. Significant canonical signaling pathways in miRNA-targeted mRNA expressions in the RNA-seq derivation cohort

Ingenuity Canonical Pathways	-log (B-H p-value)	z-score
PD-1, PD-L1 Cancer Immunotherapy Pathway	3.92	2.5
MSP-RON Signaling in Macrophages Pathway	3.84	2.837
p38 MAPK Signaling	3.27	2.683
Interferon Signaling	2.88	2.53
Osteoarthritis Pathway	1.8	2.183
Role of Hypercytokinemia/Hyperchemokinemia in the Pathogenesis of Influenza	1.32	2.887

Table S4. Significant canonical signaling pathways in COVID-19 mRNA identified in the RNA-seq validation cohort

Ingenuity Canonical Pathways	-log (B-H p-value)	z-score
EIF2 Signaling	16	-4.025
Interferon Signaling	10.3	4.264
Neuroinflammation Signaling Pathway	8.77	3.491
PD-1, PD-L1 Cancer Immunotherapy Pathway	8.2	2.197
Pyroptosis Signaling Pathway	7.61	4.226
TREM1 Signaling	7.6	3.413
Role of PKR in Interferon Induction and Antiviral Response	7.53	3.042
STAT3 Pathway	6.63	2.6
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	6.52	3.307
Autophagy	6.19	3.641
Inflammasome Pathway	5.87	2.496
NAD Signaling Pathway	5.28	2.53
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	5.15	2.111
Phagosome Formation	4.86	2.025
IL-8 Signaling	4.64	3.395
Role of Hypercytokinemia/Hyperchemokinemia in the Pathogenesis of Influenza	4.44	4.426
MSP-RON Signaling in Macrophages Pathway	4.38	3.307
Toll-like Receptor Signaling	4.31	3.638
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	4.31	2.858
iNOS Signaling	4.2	3.357
Salvage Pathways of Pyrimidine Deoxyribonucleotides	3.87	2.449
Necroptosis Signaling Pathway	3.52	3.244
Tumor Microenvironment Pathway	3.26	2.03
p38 MAPK Signaling	3.17	2.746
Regulation of Actin-based Motility by Rho	2.95	2.294
Signaling by Rho Family GTPases	2.85	2.082
Ephrin Receptor Signaling	2.54	3.888
GM-CSF Signaling	2.38	2.183
Retinoic Acid Mediated Apoptosis Signaling	2.35	2.183
Endocannabinoid Cancer Inhibition Pathway	2.35	2.694
IL-6 Signaling	2.31	2.887
Kinetochore Metaphase Signaling Pathway	2.28	2.041
Phosphatidylglycerol Biosynthesis II (Non-plastidic)	2.26	2.53
Role of MAPK Signaling in Promoting the Pathogenesis of Influenza	2.19	3.138
Acute Phase Response Signaling	2	3.286

Acute Phase Response Signaling	2	3.286
CDP-diacylglycerol Biosynthesis I	1.97	2.333
Colorectal Cancer Metastasis Signaling	1.95	2.214
Actin Cytoskeleton Signaling	1.94	2.333
Epithelial Adherens Junction Signaling	1.89	2.828
Chondroitin and Dermatan Biosynthesis	1.89	2
RHOGDI Signaling	1.88	-2.117
Remodeling of Epithelial Adherens Junctions	1.86	2.828
Gαi Signaling	1.84	2.294
3-phosphoinositide Degradation	1.84	2.335
HMGB1 Signaling	1.8	2.2
BEX2 Signaling Pathway	1.8	-2.357
IL-1 Signaling	1.64	2.111
Unfolded protein response	1.64	3
D-myo-inositol (1,4,5,6)-tetrakisphosphate Biosynthesis	1.61	2.268
D-myo-inositol (3,4,5,6)-tetrakisphosphate Biosynthesis	1.61	2.268
D-myo-inositol-5-phosphate Metabolism	1.54	2.191
Insulin Secretion Signaling Pathway	1.51	3.063
Ceramide Signaling	1.39	2.183
Oncostatin M Signaling	1.38	3.317
LPS-stimulated MAPK Signaling	1.34	2.668
Triacylglycerol Biosynthesis	1.31	2.714
Wound Healing Signaling Pathway	1.3	3.507
Semaphorin Neuronal Repulsive Signaling Pathway	1.3	2.041

Table S5. Significant canonical signaling pathways in miRNA-targeted mRNA expressions in the RNA-seq validation cohort

Ingenuity Canonical Pathways	-log (B-H p-value)	z-score
Autophagy	7.29	2.795
Interferon Signaling	5.51	3.464
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	5.48	3.273
Phagosome Formation	4.82	2.692
IL-8 Signaling	4.27	3.545
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	3.91	2.2
iNOS Signaling	3.6	2.333
MSP-RON Signaling In Macrophages Pathway	3.15	2.524
Integrin Signaling	2.87	2.558
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	2.86	2.84
Pyroptosis Signaling Pathway	2.54	2.84
Protein Kinase A Signaling	2.44	-2.263
Hypoxia Signaling in the Cardiovascular System	2.11	2
Salvage Pathways of Pyrimidine Deoxyribonucleotides	2.08	2
Signaling by Rho Family GTPases	1.96	2.828
Regulation of Actin-based Motility by Rho	1.82	2.309
Ephrin Receptor Signaling	1.79	3.9
IL-17A Signaling in Gastric Cells	1.78	2
LPS-stimulated MAPK Signaling	1.76	2.309
Toll-like Receptor Signaling	1.63	2.121
IL-6 Signaling	1.52	2.84
Actin Nucleation by ARP-WASP Complex	1.52	2.828
Insulin Secretion Signaling Pathway	1.52	2.746
Tumor Microenvironment Pathway	1.51	2.065
Ephrin B Signaling	1.48	2.121
Acute Phase Response Signaling	1.39	2.84
3-phosphoinositide Degradation	1.32	2.324
D-myo-inositol (1,4,5,6)-Tetrakisphosphate Biosynthesis	1.3	2.138
D-myo-inositol (3,4,5,6)-tetrakisphosphate Biosynthesis	1.3	2.138

Table S6. Top five statistically significant correlated studies in the BaseSpace Correlation Engine Analysis using mRNA gene expressions in the RNA-seq derivation cohort

Study name	Public Id
Study 1: Blood biomarker signature for the diagnosis of septicemic melioidosis	GSE13015
Study 2: Blood transcriptional diagnostic assay for septicemic melioidosis	GSE69528
Study 3: Systemic lupus erythematosus blood samples with different anti-Ro60 titers and interferon metrics	GSE72509
Study 4: Whole blood transcriptional modules for 9 different pathologies	GSE29536
Study 5: Blood gene expression profiles of tuberculosis patients and infected healthy donors	GSE28623

For details on each Study, see Table S6-2.

Table S7. Top five statistically significant correlated studies in the BaseSpace Correlation Engine Analysis using miRNA-targeted mRNA gene expressions in the RNA-seq derivation cohort

Study name	Public Id
Study 1: Blood biomarker signature for the diagnosis of septicemic melioidosis	GSE13015
Study 2: Blood transcriptional diagnostic assay for septicemic melioidosis	GSE69528
Study 3: Whole blood gene expression in response to dengue disease	GSE28405
Study 4: Whole blood of juvenile idiopathic arthritis and inflammatory bowel disease patients	GSE112057
Study 5: Whole blood transcriptional modules for 9 different pathologies	GSE29536

For details on each Study, see Table S7-2.

Table S8. Top five statistically significant correlated studies in the BaseSpace Correlation Engine Analysis using mRNA gene expressions in the RNA-seq validation cohort

Study name	Public Id
Study 1: Whole blood of sepsis survivors and nonsurvivors	GSE54514
Study 2: Blood biomarker signature for the diagnosis of septicemic melioidosis	GSE13015
Study 3: Blood transcriptional diagnostic assay for septicemic melioidosis	GSE69528
Study 4: Blood gene expression in sepsis patients compared to healthy subjects and patients post-surgery	GSE28750
Study 5: Whole blood gene expression in response to dengue disease	GSE28405

For details on each Study, see Table S8-2.

Table S9. Top five statistically significant correlated studies in the BaseSpace Correlation Engine Analysis using miRNA-targeted mRNA gene expressions in the RNA-seq validation cohort

Study name	Public Id
Study 1: Blood biomarker signature for the diagnosis of septicemic melioidosis	GSE13015
Study 2: Blood transcriptional diagnostic assay for septicemic melioidosis	GSE69528
Study 3: Whole blood of sepsis survivors and nonsurvivors	GSE54514
Study 4: Whole blood gene expression in response to dengue disease	GSE28405
Study 5: Systemic Lupus Erythematosus trial of Tabalumab	GSE88887

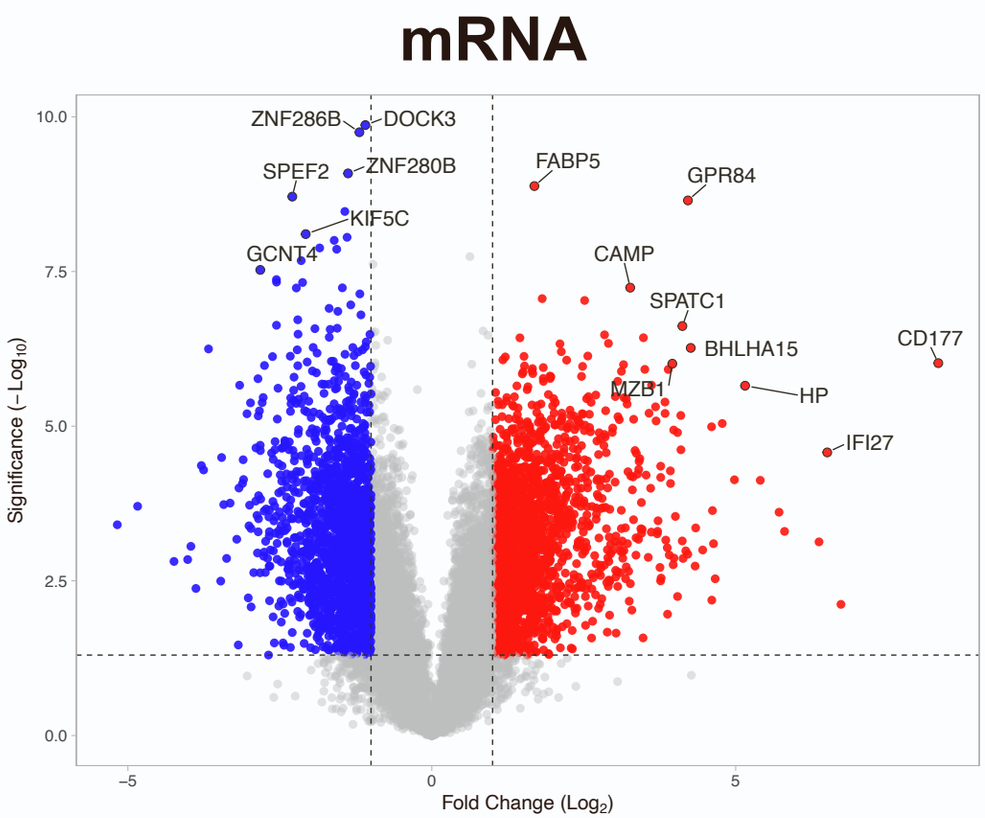
For details on each Study, see Table S9-2.

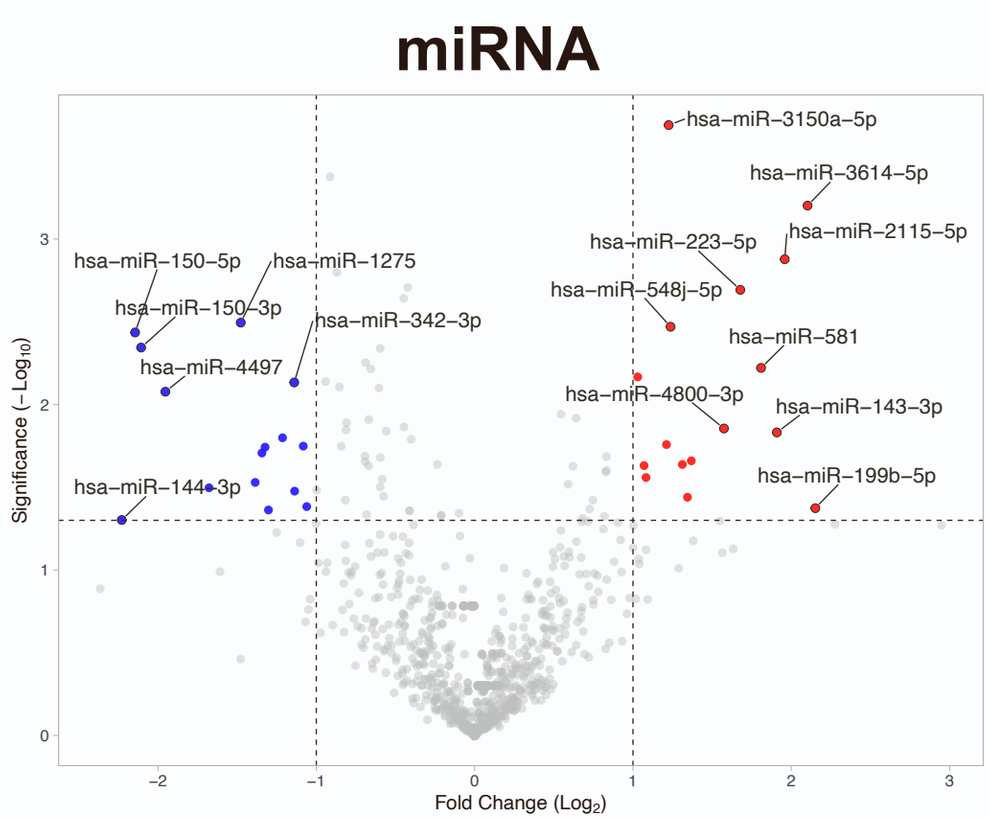
Table S10. List of qPCR primers used for technical validation.

Primer name	Sequence 5' to 3'
STAT1 forward	TGTATGCCATCCTCGAGAGC
STAT1 reverse	AGACATCCTGCCACCTTGTG
STAT3 forward	GGCCCCTCGTCATCAAGA
STAT3 reverse	TTTGACCAGCAACCTGACTTTAGT
IL-1β forward	CGCAGGACAGGTACAGATTCTT
IL-1β reverse	AAAAAGCTTGGTGATGTCTGGT
GADPH forward	CTCTGCTCCTCCTGTTCGAC
GADPH reverse	ACGACCAAATCCGTTGACTC

Figure S1

(A)

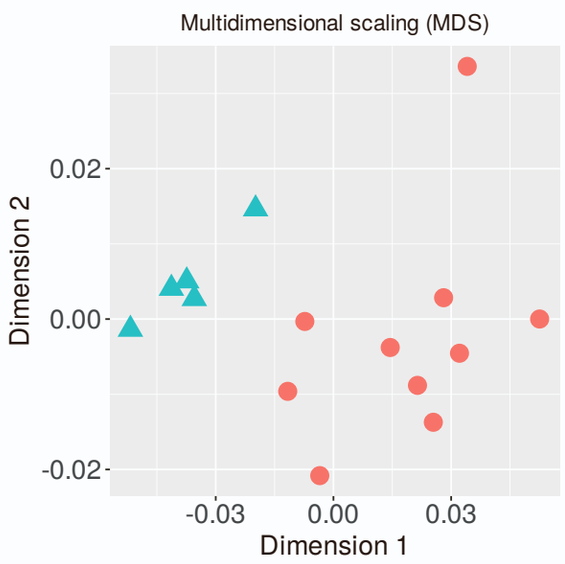


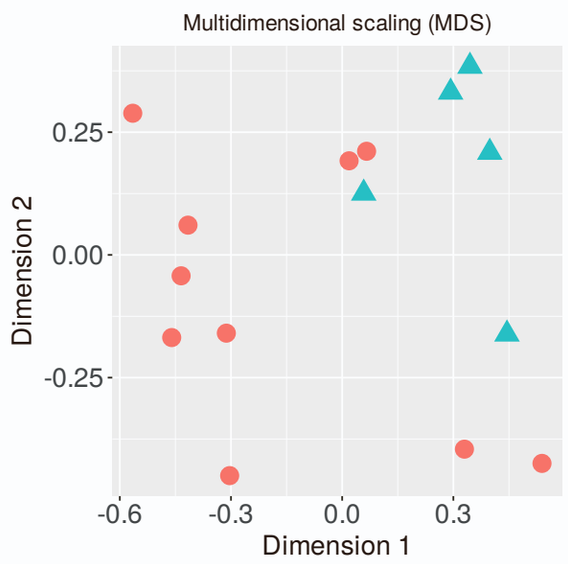


Up-regulation	1747
Down-regulation	1741

Up-regulation	16
Down-regulation	15

(B)





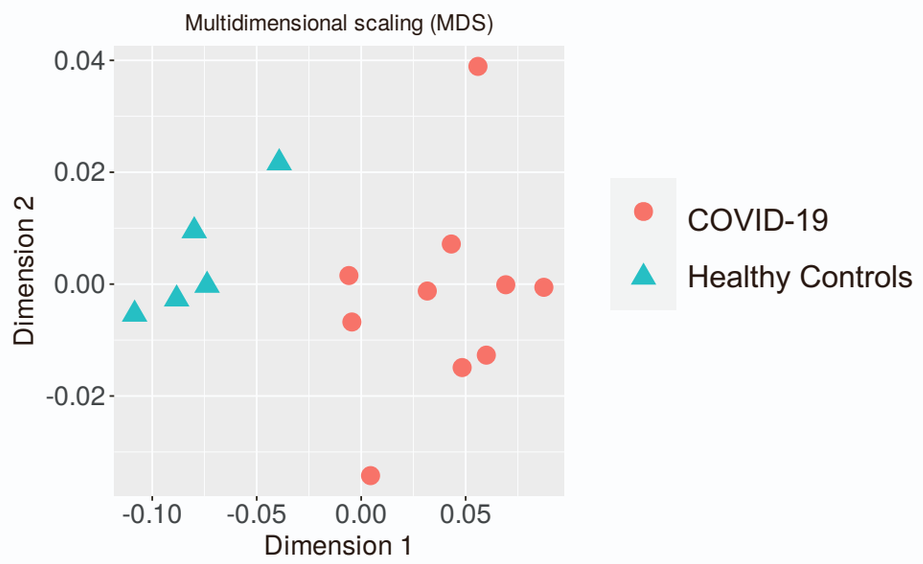
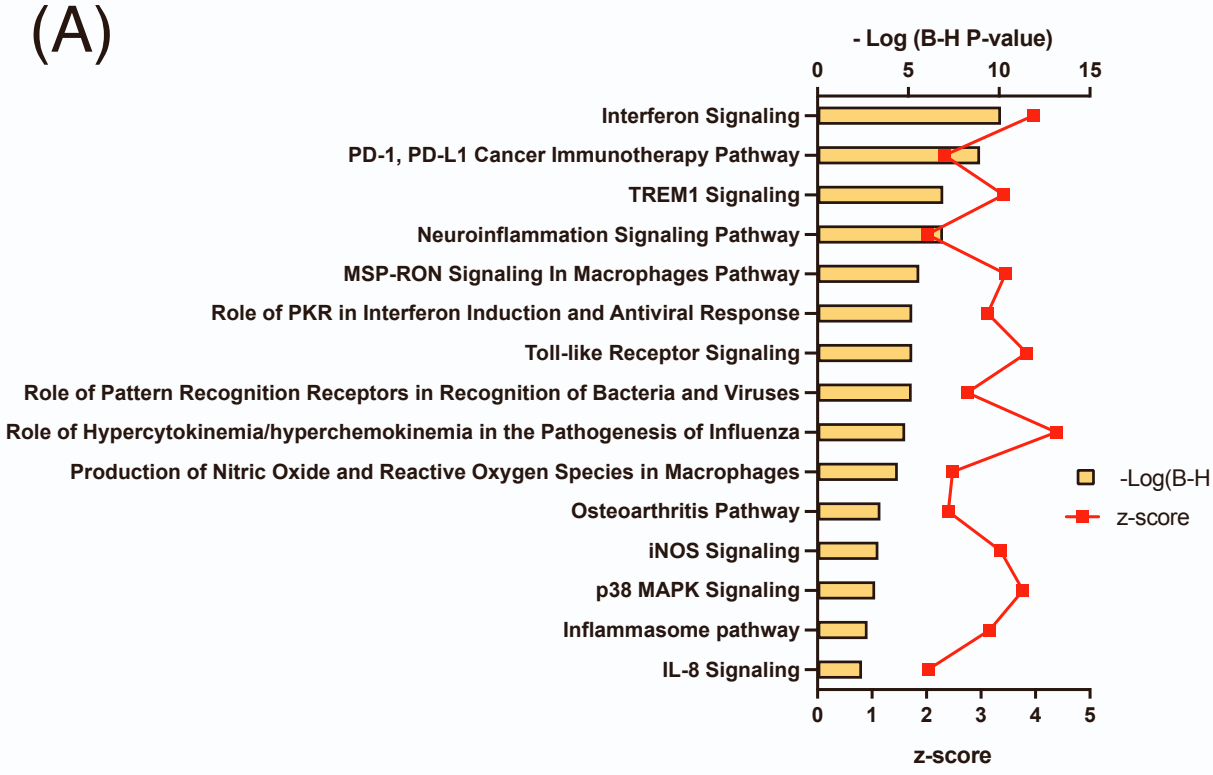


Figure S1. Volcano plot and multidimensional scaling analyses for mRNA, miRNA and miRNA-targeted mRNA expressions in the RNA-seq derivation cohort

(A) Volcano plot representing the differentially expressed mRNA and miRNA expressions in COVID-19 as compared to the healthy controls. Among the differentially expressed RNA expressions, 1747 mRNA and 16 miRNA expressions were up-regulated and 1741 mRNA and 15 miRNA expressions were down-regulated. The significant differentially expressed RNA expressions are indicated. The vertical dotted lines represent |log2 fold change| > 1. The horizontal dotted line represents the threshold for $p < 0.05$. Red dots indicate up-regulated RNA expressions, and blue dots, down-regulated RNA expressions. (B) Multidimensional scaling plot of all mRNA, miRNA and miRNA-targeted mRNA expressions in COVID-19 as compared to the healthy control. COV, COVID-19 patients; HC, healthy controls.

Figure S2

(A)



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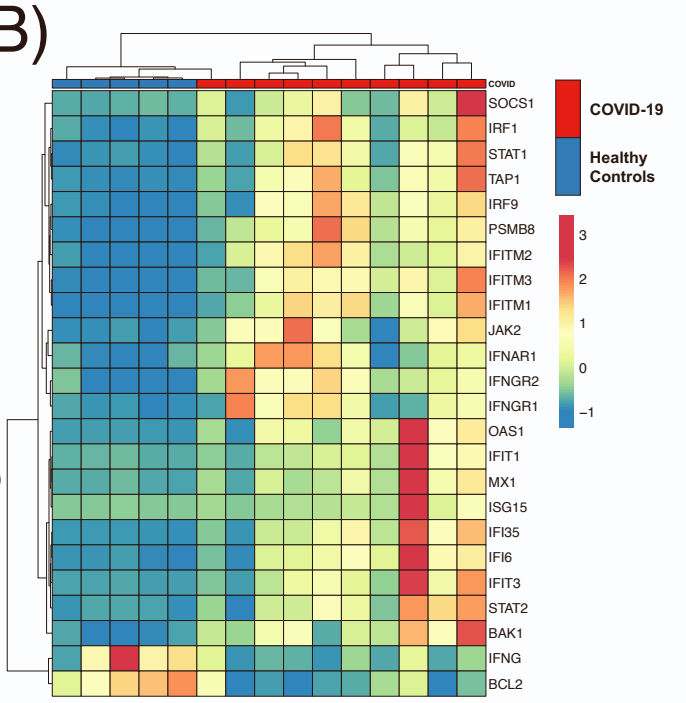

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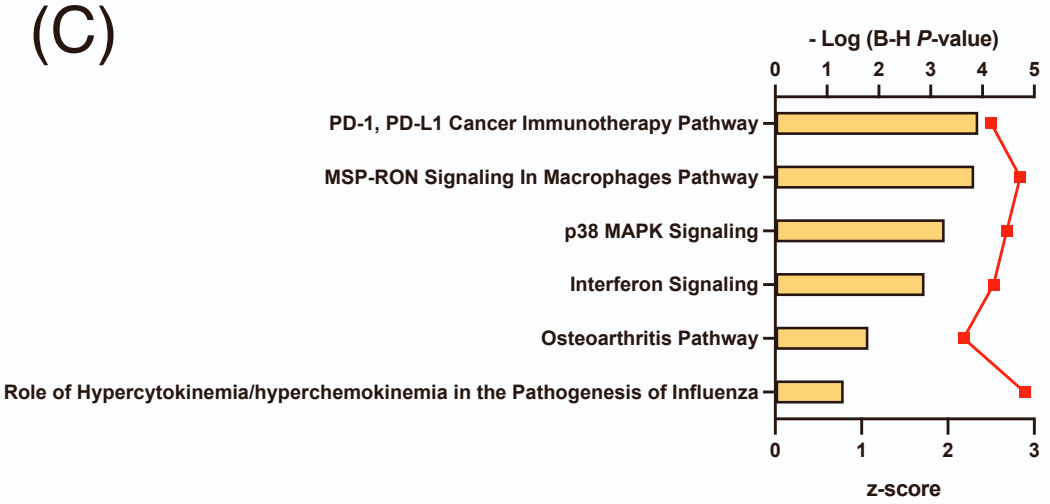

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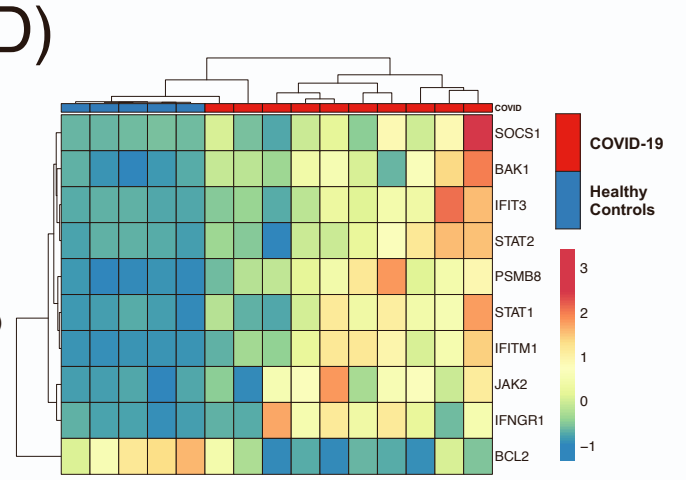

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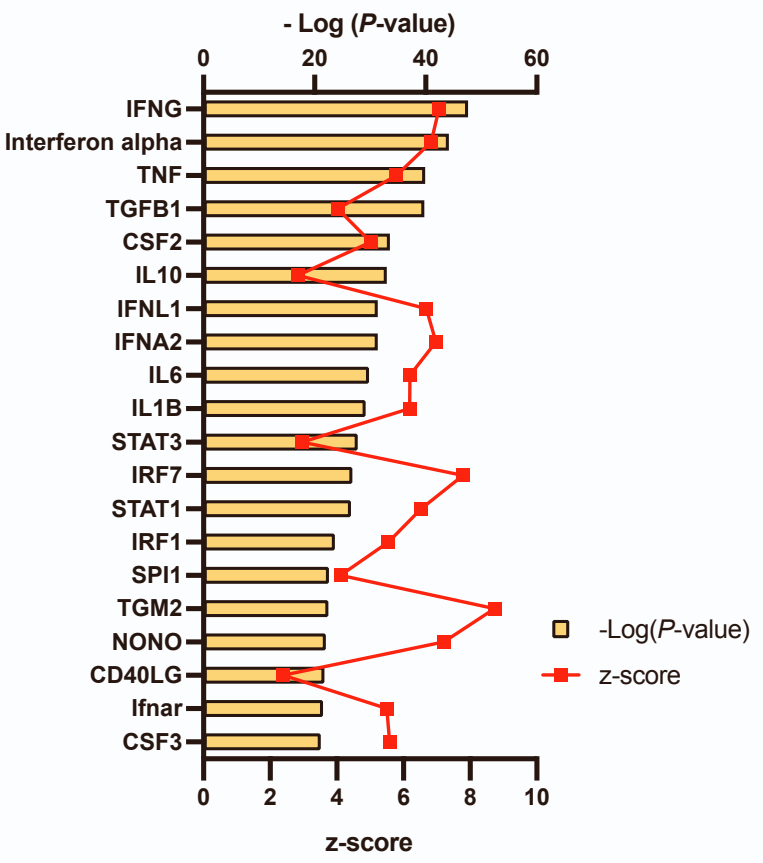

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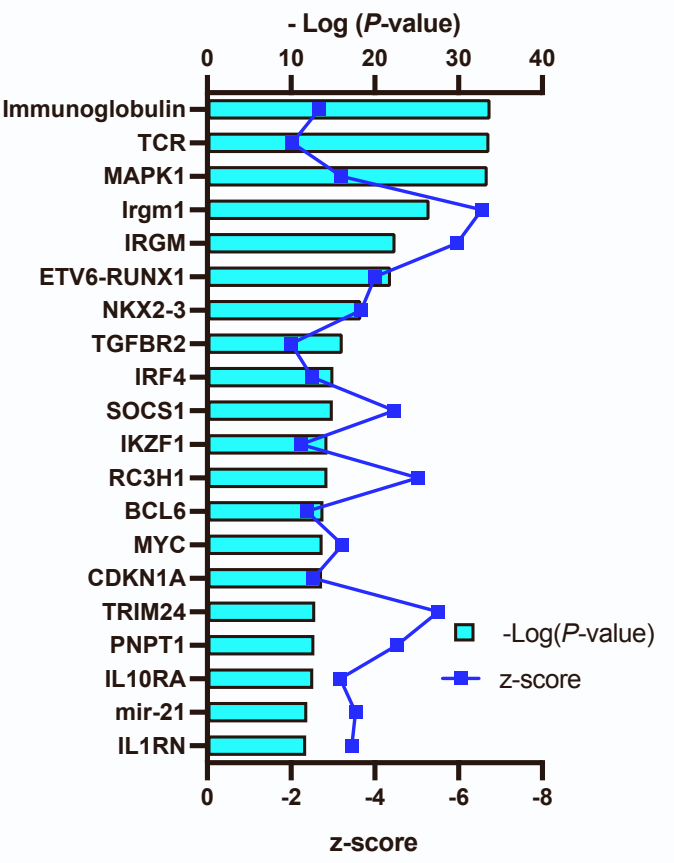


Figure S2. Canonical pathway and upstream analysis in the RNA-seq derivation cohort

(A) Top 15 activated canonical signaling pathways in COVID-19 mRNA identified using Ingenuity Pathway Analysis. (B) Heatmap of gene expression as calculated through RNA-seq involved in the interferon signaling pathway of the samples. (C) Five activated canonical pathways in miRNA-targeted mRNA expressions. (D) Heatmap of gene expression of miRNA-targeted mRNA expressions involved in the interferon signaling pathway of the samples. (E) Top 20 activated upstream regulators. (F) Top 20 inhibited upstream regulators. PD-1, programmed death-1; PD-L1, programmed death-ligand 1; TREM1, triggering receptor expressed on myeloid cells 1; MSP-RON, macrophage-stimulating protein-recepteur d'origine nantais; PKR, protein kinase R; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; IL, interleukin.

Figure S3

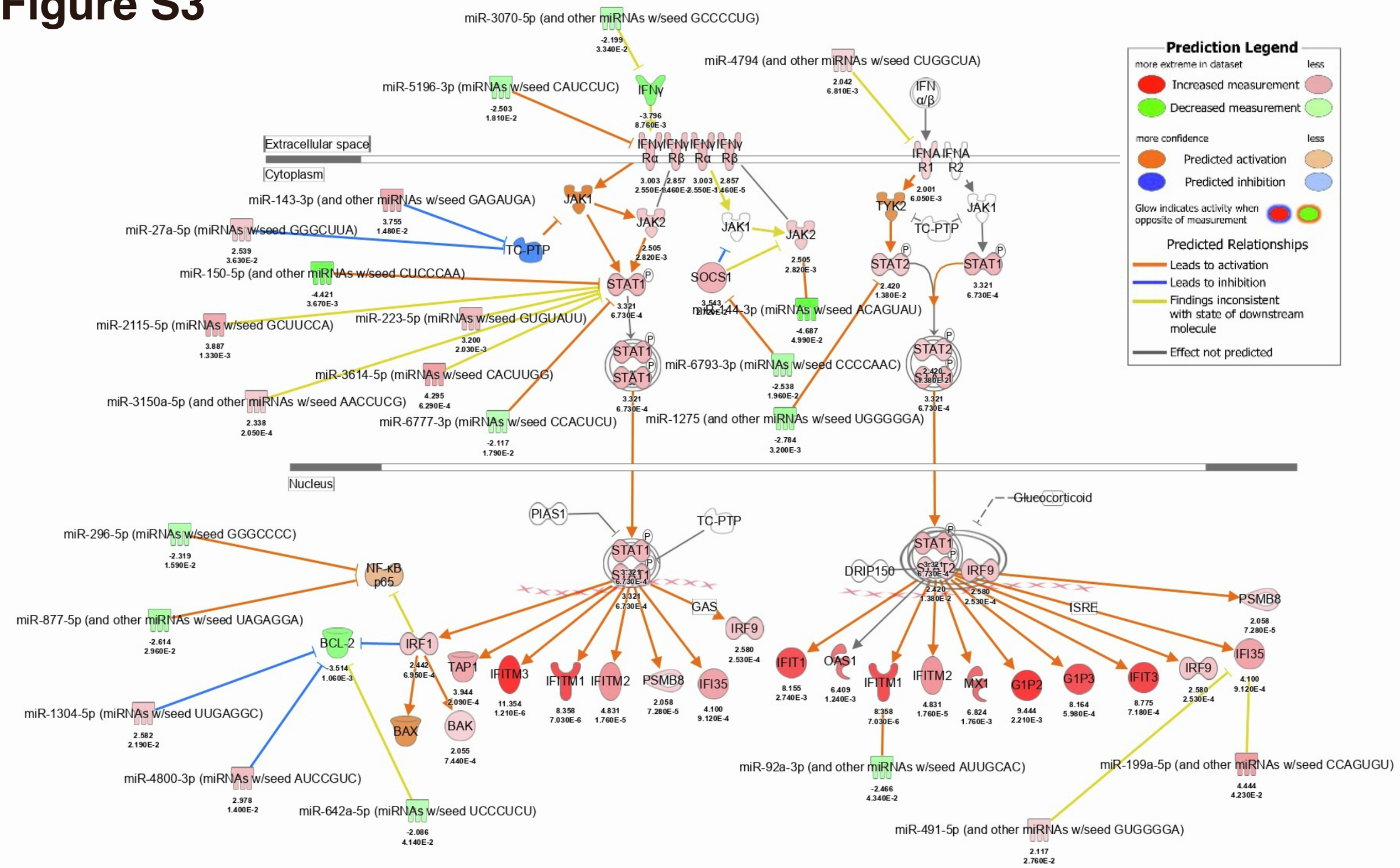
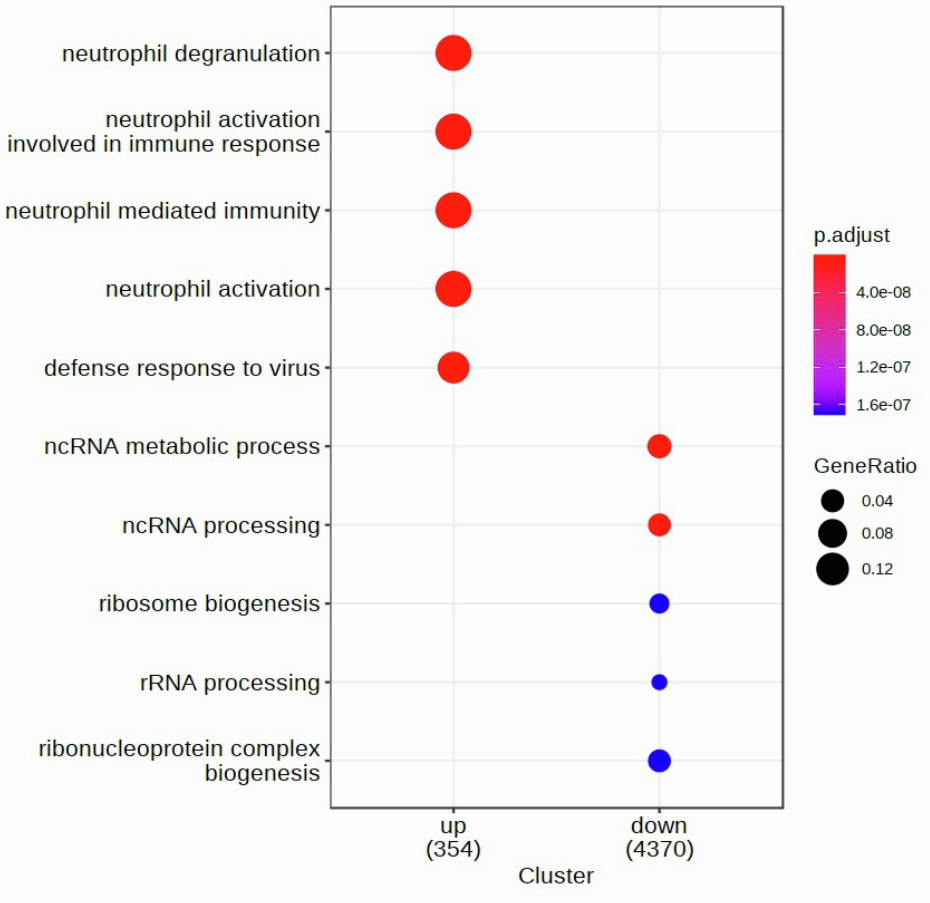


Figure S3. The activated interferon pathway predicted by Ingenuity Pathway Analysis in the RNA-seq derivation cohort

Twenty-five mRNAs and 17 miRNAs with $p < 0.05$, $|\log2 \text{ fold change}| > 1$ were included in the activated interferon pathway.

Figure S4

(A)



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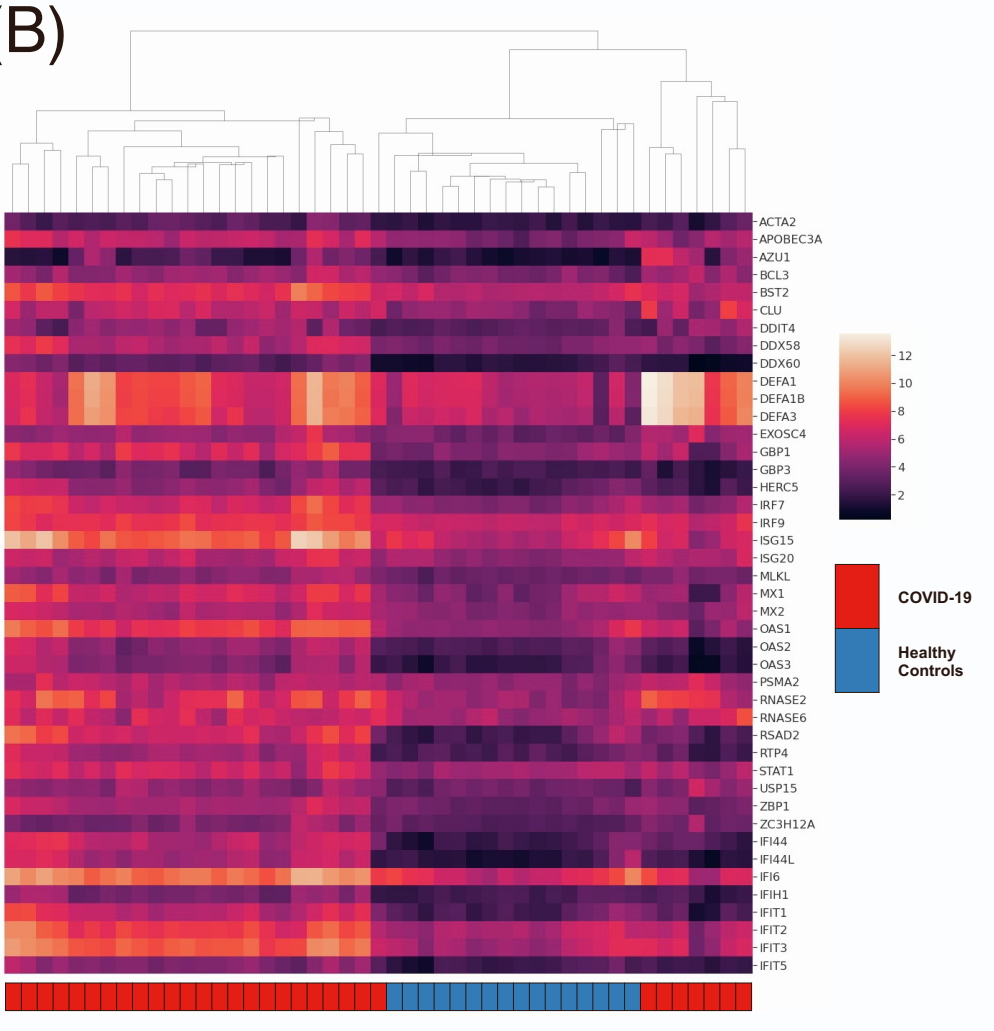

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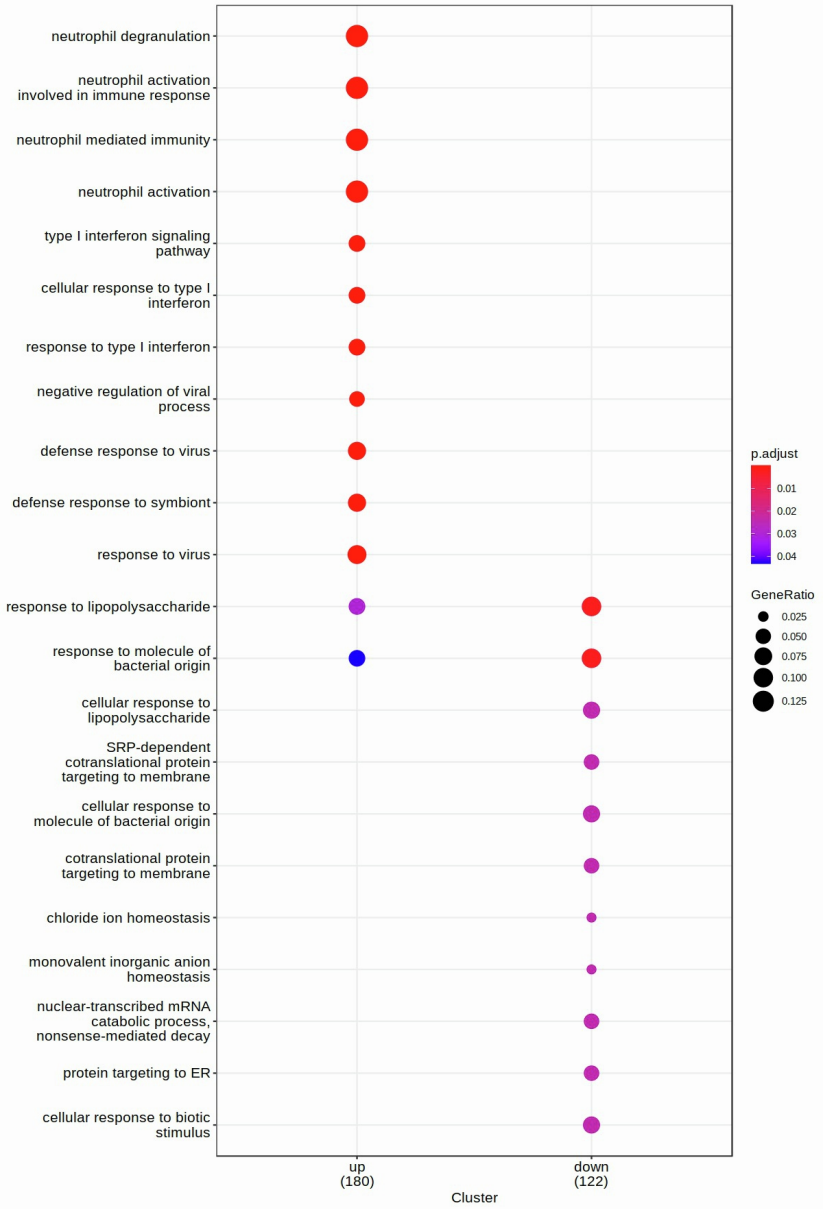

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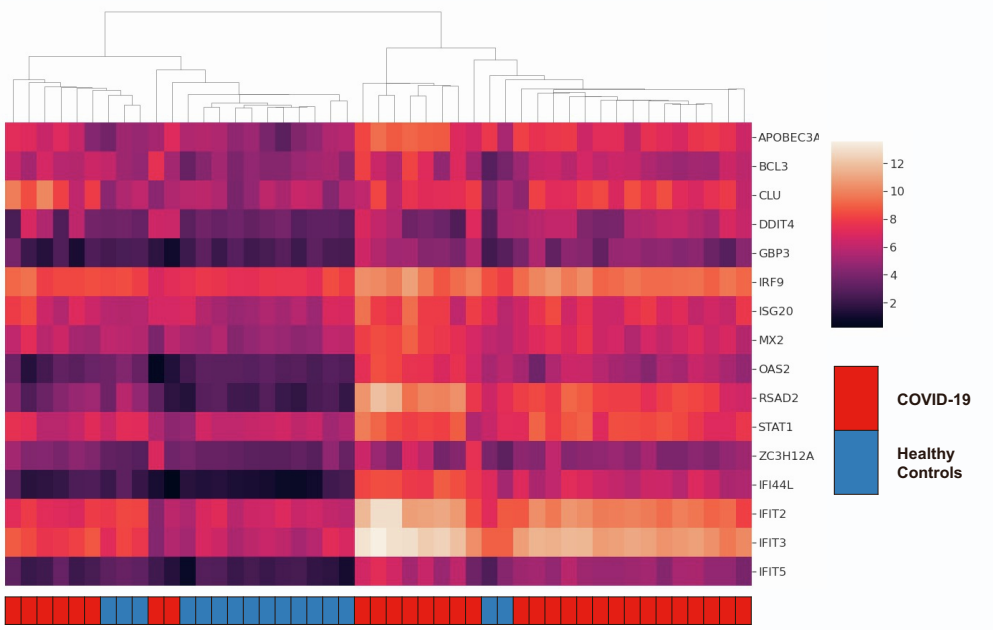
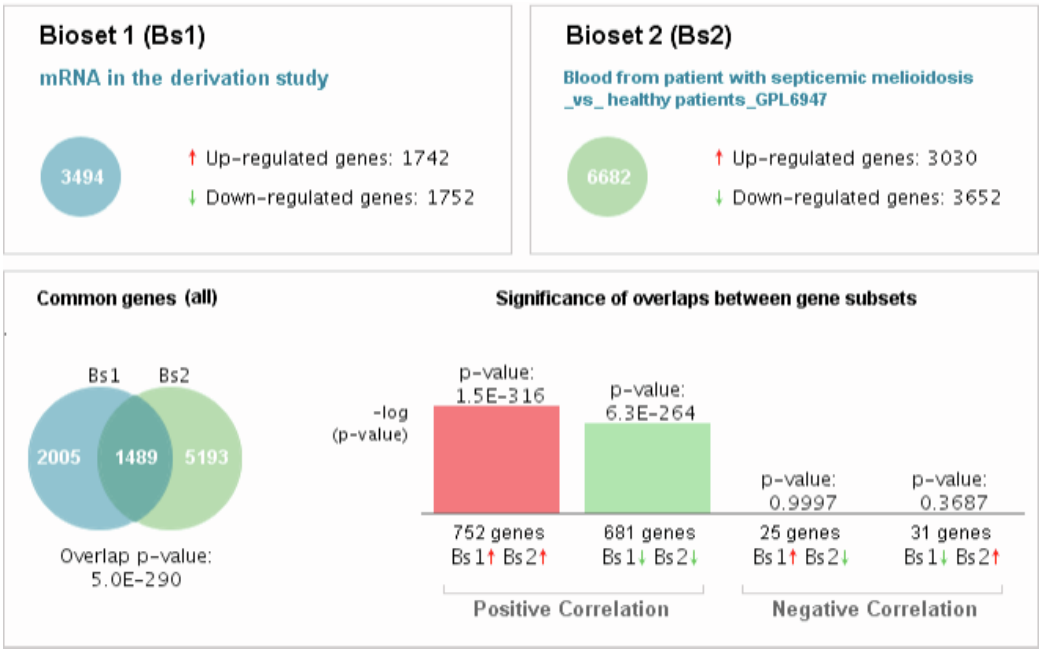


Figure S4. Gene Set Enrichment Analysis (GSEA) in the RNA-seq validation cohort

(A) Dot plot of top five most enriched Gene Ontology (GO) terms for significantly upregulated and downregulated in mRNAs. (B) Heatmap of gene expression of mRNA expressions involved in response to virus. (C) Dot plot of enriched GO terms for significantly upregulated and downregulated in miRNA-targeted mRNAs. (D) Heatmap of gene expression of miRNA-targeted mRNA expressions involved in response to virus.

Figure S5

(A)


Bioset 1 (Bs1)
mRNA in the derivation study
3494
↑ Up-regulated genes: 1742
↓ Down-regulated genes: 1752
Bioset 2 (Bs2)
Blood from patient with septicemic melioidosis _vs_ healthy patients_GPL6947
6682
↑ Up-regulated genes: 3030
↓ Down-regulated genes: 3652
Common genes (all)
Bs1
Bs2
2005
1489
5193
Overlap p-value: 5.0E-290
Significance of overlaps between gene subsets
-log (p-value)
p-value: 1.5E-316
p-value: 6.3E-264
p-value: 0.9997
p-value: 0.3687
752 genes Bs1↑ Bs2↑
681 genes Bs1↓ Bs2↓
25 genes Bs1↑ Bs2↓
31 genes Bs1↓ Bs2↑
Positive Correlation
Negative Correlation

(B)

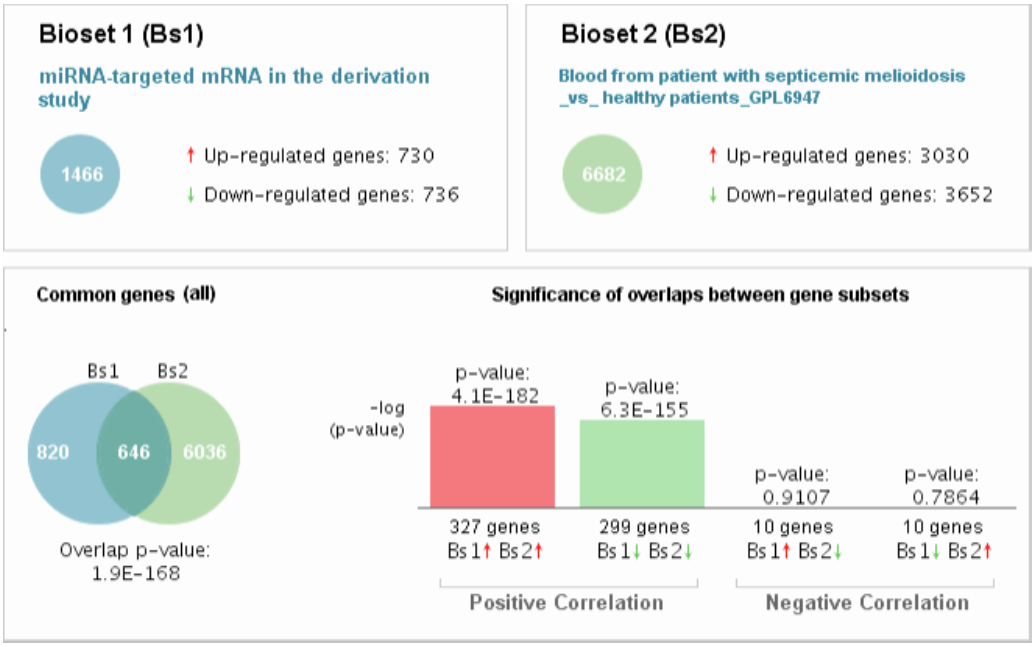
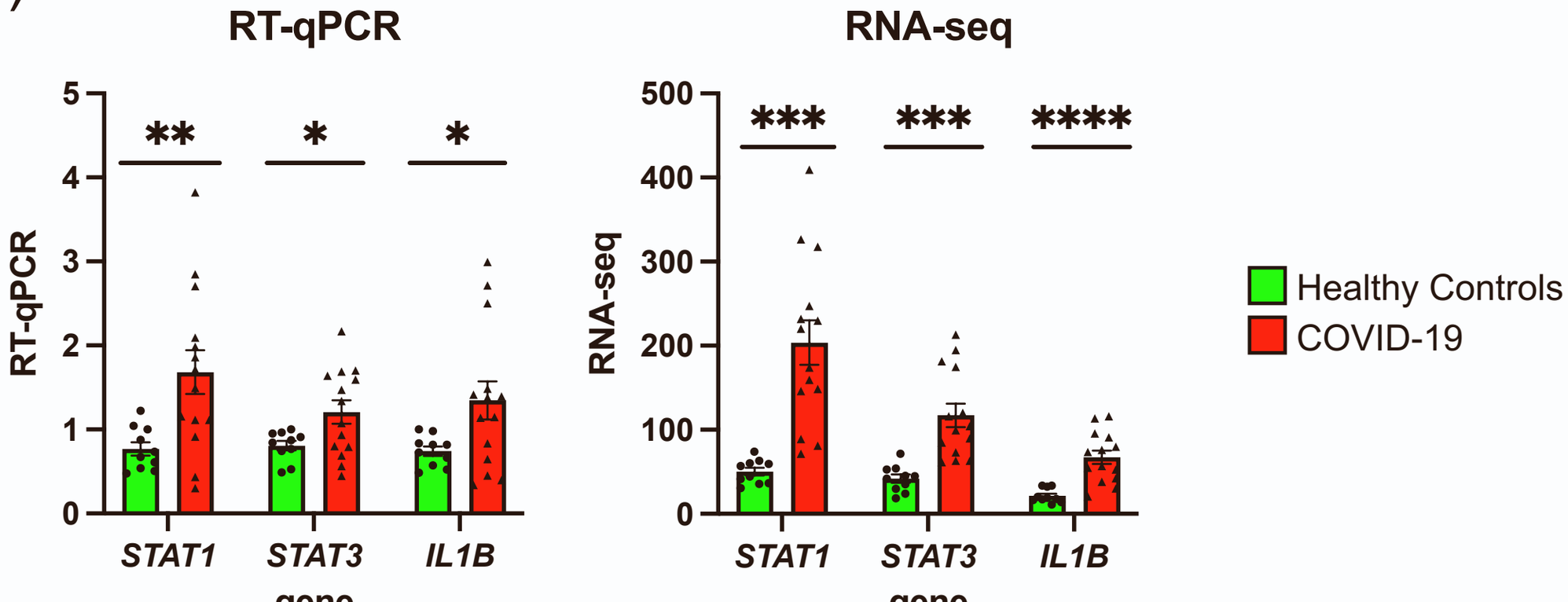

Bioset 1 (Bs1)
miRNA-targeted mRNA in the derivation study
1466
↑ Up-regulated genes: 730
↓ Down-regulated genes: 736
Bioset 2 (Bs2)
Blood from patient with septicemic melioidosis _vs_ healthy patients_GPL6947
6682
↑ Up-regulated genes: 3030
↓ Down-regulated genes: 3652
Common genes (all)
Bs1
Bs2
820
646
6036
Overlap p-value: 1.9E-168
Significance of overlaps between gene subsets
-log (p-value)
p-value: 4.1E-182
p-value: 6.3E-155
p-value: 0.9107
p-value: 0.7864
327 genes Bs1↑ Bs2↑
299 genes Bs1↓ Bs2↓
10 genes Bs1↑ Bs2↓
10 genes Bs1↓ Bs2↑
Positive Correlation
Negative Correlation

Figure S5. BaseSpace Correlation Engine analysis of the expressions of mRNA and miRNA-targeted mRNA genes in the RNA-seq derivation cohort as compared to the healthy control

The patterns of changes in the expression of genes in the COVID-19 patients in this study [(A) mRNA gene expressions and (B) miRNA-targeted mRNA gene expressions] are compared to the patients with melioidosis. Venn diagrams illustrate the overlap in genome-wide changes in gene expression between the COVID-19 patients and the patients with melioidosis. Bar graphs depict the -log of the overlap P values for up-regulated (red arrows) or down-regulated (green arrows) genes.

Figure S6

(A)


RT-qPCR
RNA-seq
RT-qPCR
RNA-seq
**
*
*
***
***
****
STAT1
STAT3
IL1B
gene
gene
Healthy Controls
COVID-19

(B)

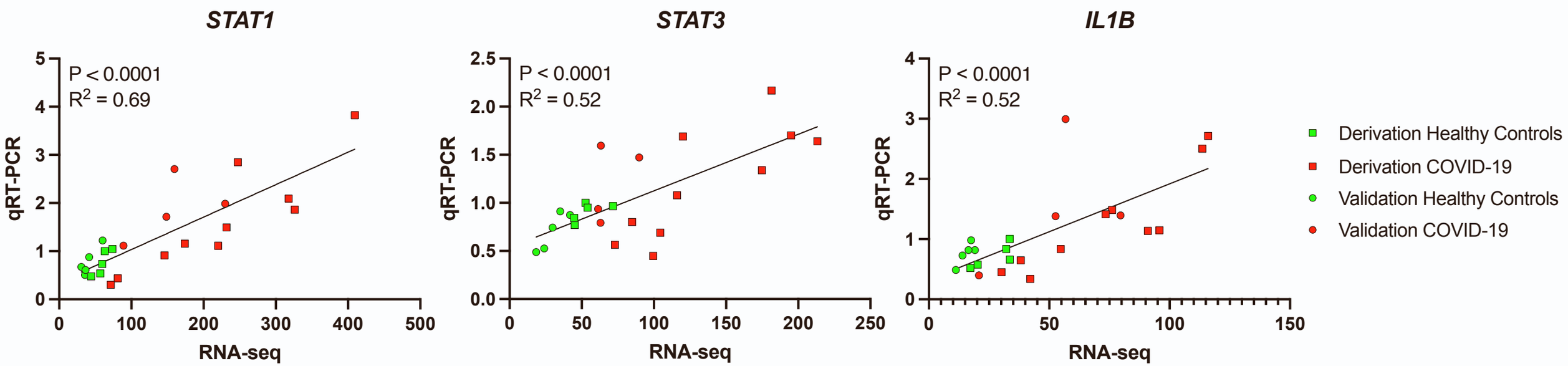

STAT1
STAT3
IL1B
P < 0.0001
R2 = 0.69
P < 0.0001
R2 = 0.52
P < 0.0001
R2 = 0.52
qRT-PCR
RNA-seq
Derivation Healthy Controls
Derivation COVID-19
Validation Healthy Controls
Validation COVID-19

Figure S6. Validation of RNA-Sequencing results by quantitative RT-PCR

(A) Gene expression levels determined by RNA-seq and RT-PCR of three selected upstream regulators (*STAT1, STAT3, IL1B*) in healthy control subjects and COVID samples. (B) Correlation between RNA-Seq and RT-PCR gene expression (*STAT1*; $R^2 = 0.69$, $p < 0.0001$. *STAT3*; $R^2 = 0.52$, $p < 0.0001$. *IL1B*; $R^2 = 0.52$, $p < 0.0001$).