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Supplemental information

Nucleic acid biomarkers of immune response and cell and tissue damage in children with COVID-19 and MIS-C

Conor J. Loy, Alicia Sotomayor-Gonzalez, Venice Servellita, Jenny Nguyen, Joan Lenz, Sanchita Bhattacharya, Meagan E. Williams, Alexandre P. Cheng, Andrew Bliss, Prachi Saldhi, Noah Brazer, Jessica Streithorst, William Suslovic, Charlotte J. Hsieh, Burak Bahar, Nathan Wood, Abiodun Foresythe, Amelia Gliwa, Kushmita Bhakta, Maria A. Perez, Laila Hussaini, Evan J. Anderson, Ann Chahroudi, Meghan Delaney, Atul J. Butte, Roberta L. DeBiasi, Christina A. Rostad, Iwijn De Vlaminck, and Charles Y. Chiu

Classification	Characteristics
Asymptomatic	This included patients with evidence of SARS-CoV-2 infection by nasopharyngeal RT-PCR but no symptoms of COVID-19, regardless of whether hospitalized for another cause or not hospitalized.
Mild	This included all outpatient cases (who did not require hospitalization for COVID-19) or if hospitalized, only upper respiratory symptoms, including fever, sore throat, cough, rhinorrhea, loss of sense of smell or taste from COVID-19 only.
Moderate	The patient must have been hospitalized due to COVID-19 respiratory disease and/or any systemic/non-respiratory symptoms attributed to COVID-19 (e.g., neonatal fever, dehydration, new diagnosis diabetes, acute appendicitis, necrosis of extremities, diarrhea, encephalopathy, renal insufficiency, mild coagulation abnormalities, etc.) and/or MIS-C.
Severe	The patient must have been hospitalized for COVID-19 or MIS-C with either high-flow oxygen requirement (high-flow NC, BIPAP, intubation with mechanical ventilation, or ECMO) and/or evidence of end-organ failure (acute renal failure requiring dialysis, coagulation abnormalities resulting in bleeding or stroke, DKA, hemodynamic instability requiring vasopressors) and/or dying from COVID-19 or MIS-C. These patients were almost always admitted to the ICU.

Table S1. Severity Classifications, Related to Table 1. Abbreviations: NC, nasal cannula; BIPAP, bilevel invasive positive airway pressure; ECMO, extra-corporeal membrane oxygenation; DKA, diabetic ketoacidosis; ICU, intensive care unit.

Variables	Group	Overall	COVID-19	MIS-C	Control	p-value
n		121	27	82	12	
Origin, n (%)	CNH	21 (17.4)		21 (25.6)		<0.001
	EMORY	100 (82.6)	27 (100.0)	61 (74.4)	12 (100.0)	
Disease Severity, n (%)	Moderate	31 (25.6)	9 (33.3)	22 (26.8)		0.62
	Severe	78 (64.5)	18 (66.7)	60 (73.2)		
Age, mean (SD)		11 (5)	14 (4)	10 (4)	15 (3)	
Gender, n (%)	Female	47 (38.8)	14 (51.9)	26 (31.7)	7 (58.3)	0.06
	Male	74 (61.2)	13 (48.1)	56 (68.3)	5 (41.7)	
Race, n (%)	American Indian	2 (1.7)			2 (16.7)	0.002
	Asian	1 (0.8)	1 (3.7)			
	Black/AA	68 (56.2)	18 (66.7)	48 (58.5)	2 (16.7)	
	White	35 (28.9)	7 (25.9)	22 (26.8)	6 (50.0)	
	Other/Declined	15 (12.4)	1 (3.7)	12 (14.6)	2 (16.7)	
Ethnicity, n (%)	Hispanic	26 (21.5)	4 (14.8)	18 (22.0)	4 (33.3)	0.86
	Non-Hispanic	95 (78.5)	23 (85.2)	64 (78.0)	8 (66.7)	

Table S2. Demographic and clinical characteristics of the cell-free RNA cohort, Related to Table 1. Demographic and clinical characteristics of the study cohort used in the cfRNA analysis. P-values calculated using a Fischer's Exact Test.

Variables	Group	Overall	COVID-19	MIS-C	Control	p-value
n		178	51	104	23	
Origin, n (%)	CNH	19 (10.7)		19 (18.3)		**<0.001**
	EMORY	142 (79.8)	37 (72.5)	82 (78.8)	23 (100.0)	
	UCSF	17 (9.6)	14 (27.5)	3 (2.9)		
Disease Severity, n (%)	Moderate	49 (27.5)	19 (37.3)	30 (28.8)		**0.36**
	Severe	106 (59.6)	32 (62.7)	74 (71.2)		
Age, mean (SD)		11 (5)	13 (6)	9 (5)	14 (2)	
Gender, n (%)	Female	79 (44.4)	27 (52.9)	37 (35.6)	15 (65.2)	0.013
	Male	99 (55.6)	24 (47.1)	67 (64.4)	8 (34.8)	
Race, n (%)	American Indian	2 (1.1)			2 (8.7)	<0.001
	Asian	8 (4.5)	3 (5.9)	1 (1.0)	4 (17.4)	
	Black/AA	92 (51.7)	21 (41.2)	66 (63.5)	5 (21.7)	
	White	46 (25.8)	12 (23.5)	24 (23.1)	10 (43.5)	
	Other/Declined	30 (16.9)	15 (29.4)	13 (12.5)	2 (8.7)	
Ethnicity, n (%)	Hispanic	39 (21.9)	16 (31.4)	18 (17.3)	5 (21.7)	0.63
	Non-Hispanic	139 (78.1)	35 (68.6)	86 (82.7)	18 (78.3)	

Table S3. Demographic and clinical characteristics of the whole blood RNA cohort, Related to Table 1. Demographic and clinical characteristics of the study cohort used in the wbRNA analysis. P-values calculated using a Fischer's Exact Test.

Variables	Group	Overall	COVID-19	MIS-C	Control	p-value
n		65	21	41	3	
Origin, n (%)	EMORY	56 (86.2)	14 (66.7)	39 (95.1)	3 (100.0)	0.01
	UCSF	9 (13.8)	7 (33.3)	2 (4.9)		
Disease Severity, n (%)	Asymptomatic	5 (7.7)	5 (23.8)			**0.99**
	Mild	5 (7.7)	5 (23.8)			
	Moderate	11 (16.9)	2 (9.5)	9 (22.0)		
	Severe	41 (63.1)	9 (42.9)	32 (78.0)		
Age, mean (SD)		11 (5)	12 (5)	10 (4)	15 (1)	
Gender, n (%)	Female	28 (43.1)	11 (52.4)	15 (36.6)	2 (66.7)	<0.001
	Male	37 (56.9)	10 (47.6)	26 (63.4)	1 (33.3)	
Race, n (%)	Asian	5 (7.7)	3 (14.3)	1 (2.4)	1 (33.3)	0.12
	Black/AA	33 (50.8)	6 (28.6)	26 (63.4)	1 (33.3)	
	White	18 (27.7)	7 (33.3)	10 (24.4)	1 (33.3)	
	Other/Declined	9 (13.8)	5 (23.8)	4 (9.8)		
Ethnicity, n (%)	Hispanic	13 (20.0)	6 (28.6)	7 (17.1)		**0.80**
	Non-Hispanic	52 (80.0)	15 (71.4)	34 (82.9)	3 (100.0)	

Table S4. Demographic and clinical characteristics of the cell-free DNA cohort, Related to Table 1. Demographic and clinical characteristics of the study cohort used in the cfDNA analysis. P-values calculated using a Fischer's Exact Test.

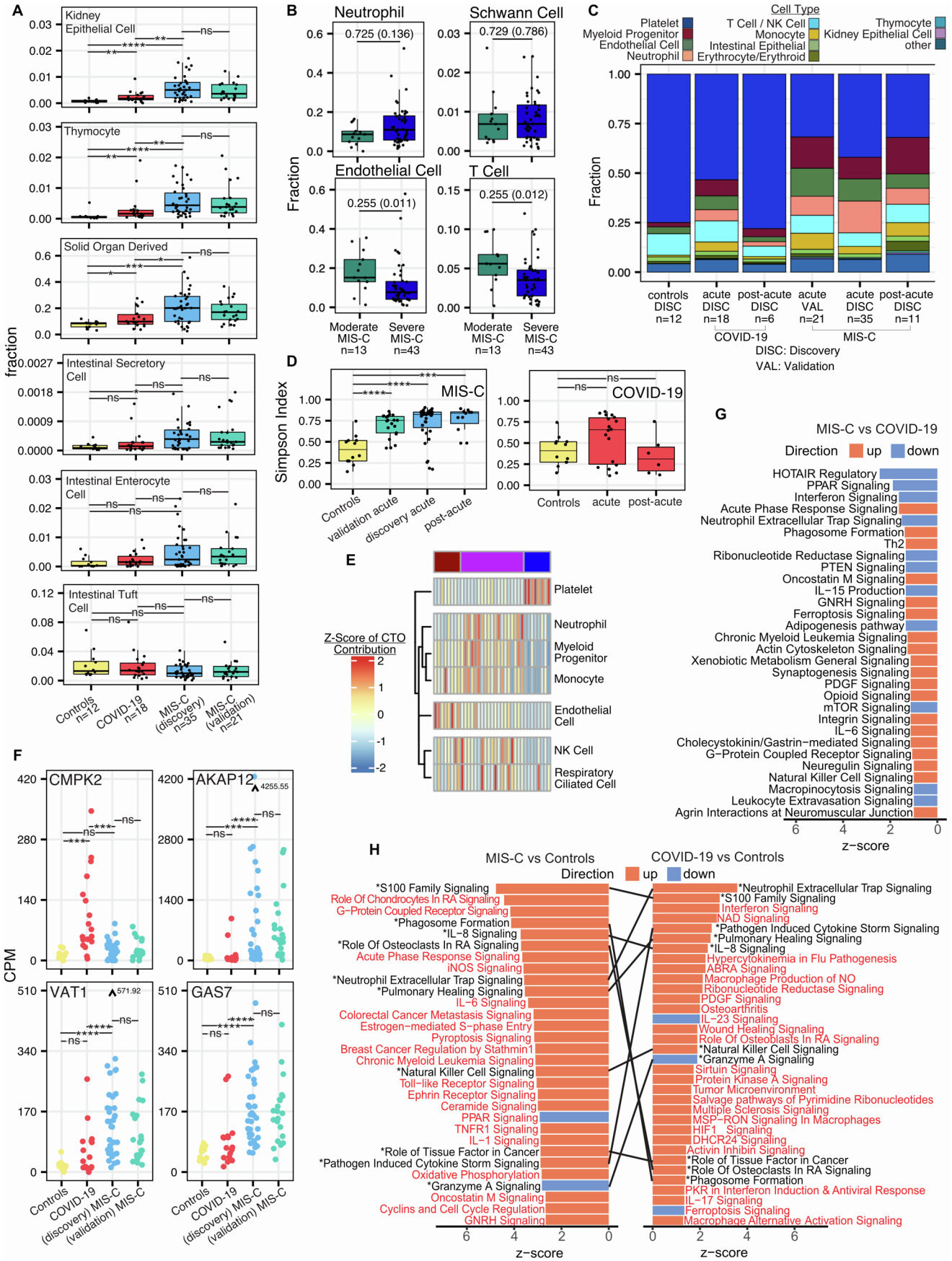


Figure S1. Plasma cell-free RNA profiling, Related to Figure 2. (A) Cell-free RNA (cfRNA) deconvolution results of kidney epithelial cell, thymocyte, solid-organ, intestinal secretory cell, intestinal enterocyte, and intestinal tuft cell derived cell-free RNA. **(B)** cfRNA deconvolution results of neutrophil, Schwann cell, endothelial cell, and T cell derived cell-free RNA between moderate and

severe MIS-C. Numbers indicate above lines indicate statistical significance (Benjamini=Hochberg correct p-value), those in parenthesis are not adjusted for multiple comparisons. (**C**) Average cfRNA deconvolution results for COVID-19, MIS-C, and controls during acute and post-acute timepoints. (**D**) Diversity of cell type contributions to the cell-free transcriptome as measured by Simpson's Index during acute and post-acute timepoints and in controls. **(E)** Scaled CTO values of cell types with statistically significant variation across sample groups (ANOVA, Benjamini-Hochberg corrected p-value <0.05). Cell types clustered based on correlation. Samples ordered based on clustering from differential abundance analysis (Fig 2F). (**F**) Normalized CPM values of CMPK2, AKAP12, VAT1, and GAS7 across sample groups. (**G**) Top 20 differential pathways between MIS-C and COVID-19 ranked by activation z-score. (**H**) Top 30 differential pathways between Controls and MIS-C or COVID-19 ranked by activation z-score. Lines connect matching pathways. Pathways in red are not in the other comparison's top 30 differential pathways. Panels Fig. 2A and Fig. 2B show the number of samples in each group. Outliers are indicated with arrows and values. Asterisks indicate statistical significance by Mann-Whitney U test using Benjamini-Hochberg adjusted p-values as follows: ns, non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, ****, $p < 0.001$.

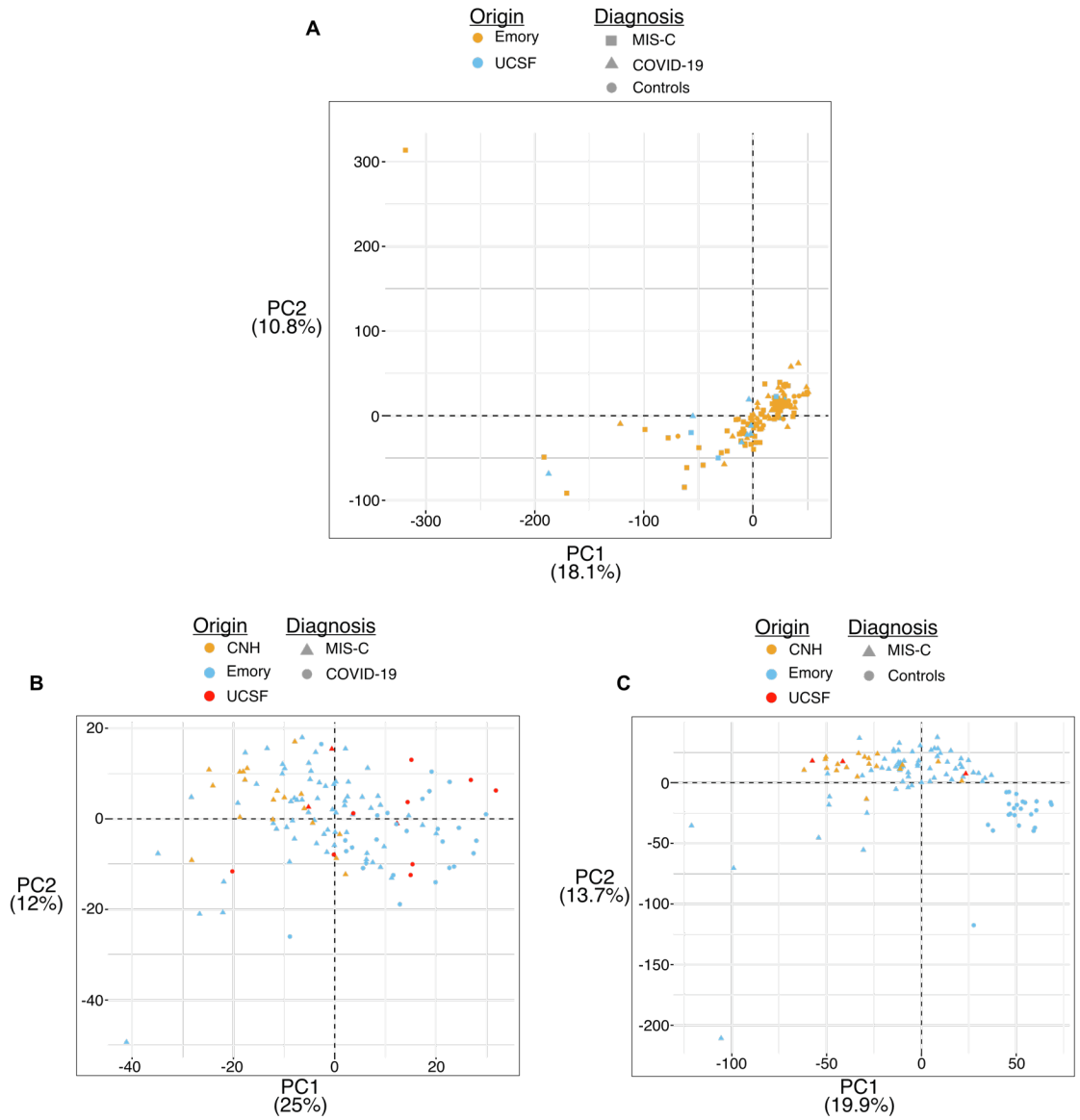


Figure S2. Whole blood RNA clustering, Related to Figure 3. (A) PCA plot of samples from UCSF and Emory used in the differential expression analysis. Samples clustered by CPM values of all genes. (**B**) PCA plot of acute moderate to severe MIS-C and COVID-19 samples from CNH, Emory, and UCSF. Samples clustered by CPM values of DEGs discovered using Emory and UCSF samples. (**C**) PCA plot of acute moderate to severe MIS-C and donor control samples from CNH, Emory, and UCSF. Samples clustered by CPM values of DEGs discovered using Emory and UCSF samples.

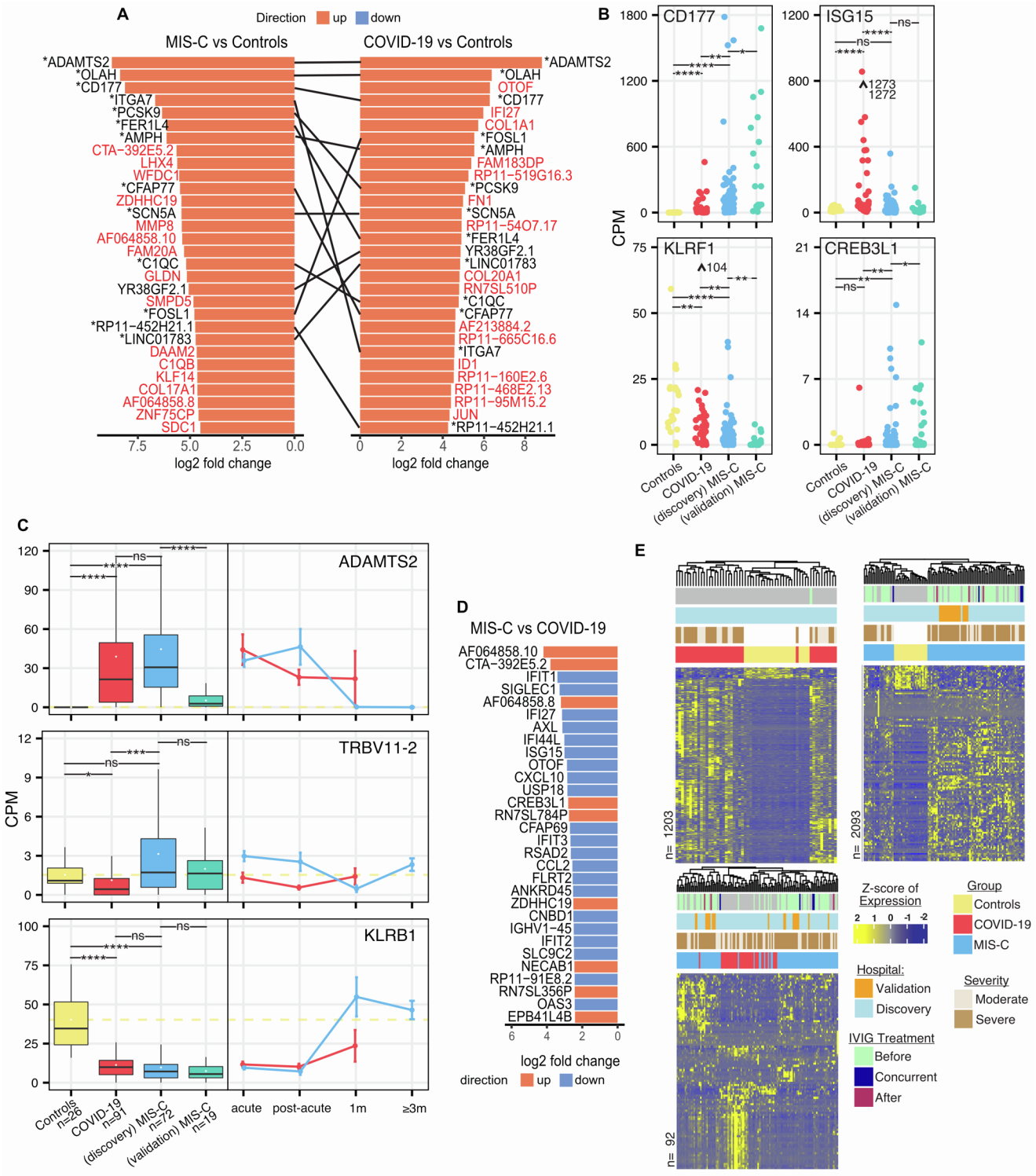


Figure S3. Whole blood RNA profiling, Related to Figure 3. (A) Top 30 differentially expressed genes between controls and acute MIS-C or acute moderate-to-severe COVID-19 ranked by log2 fold change. Lines connect matching genes. Labels in red are genes not in the other comparison's top 20 differential genes. **(B)** CPM of CD177, ISG15, KLRF1, and CREB3L1 in controls, acute MIS-C, and acute moderate-to-severe COVID-19. **(C)** CPM of ADAMTS2, TRBV11-2, and KLRB1 across sample groups and timepoints. Boxplots show counts per million (CPM) distribution of controls and acute timepoint MIS-C and moderate-to-severe COVID-19. Points represent average CPM and bars represent standard error. **(D)** Top 30 differentially expressed genes between acute MIS-C and acute moderate-to-severe COVID-19 ranked by log2 fold change. (**E**) Scaled counts per million (CPM) values of significantly differentially expressed genes (DEGs) using while blood cell counts as a covariate (DESeq2, Benjamini-Hochberg adjusted p-value < 0.01, |Log2FoldChange| > 1.5). Number of DAGs indicated to the left of the heatmap. Samples and genes are clustered based on correlation. Panels Fig. 3A show the number of samples in each group. Outliers are indicated with arrows and values. Asterisks

indicate statistical significance by Mann-Whitney U test using Benjamini-Hochberg adjusted p-values as follows: ns, non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, ****, $p < 0.001$

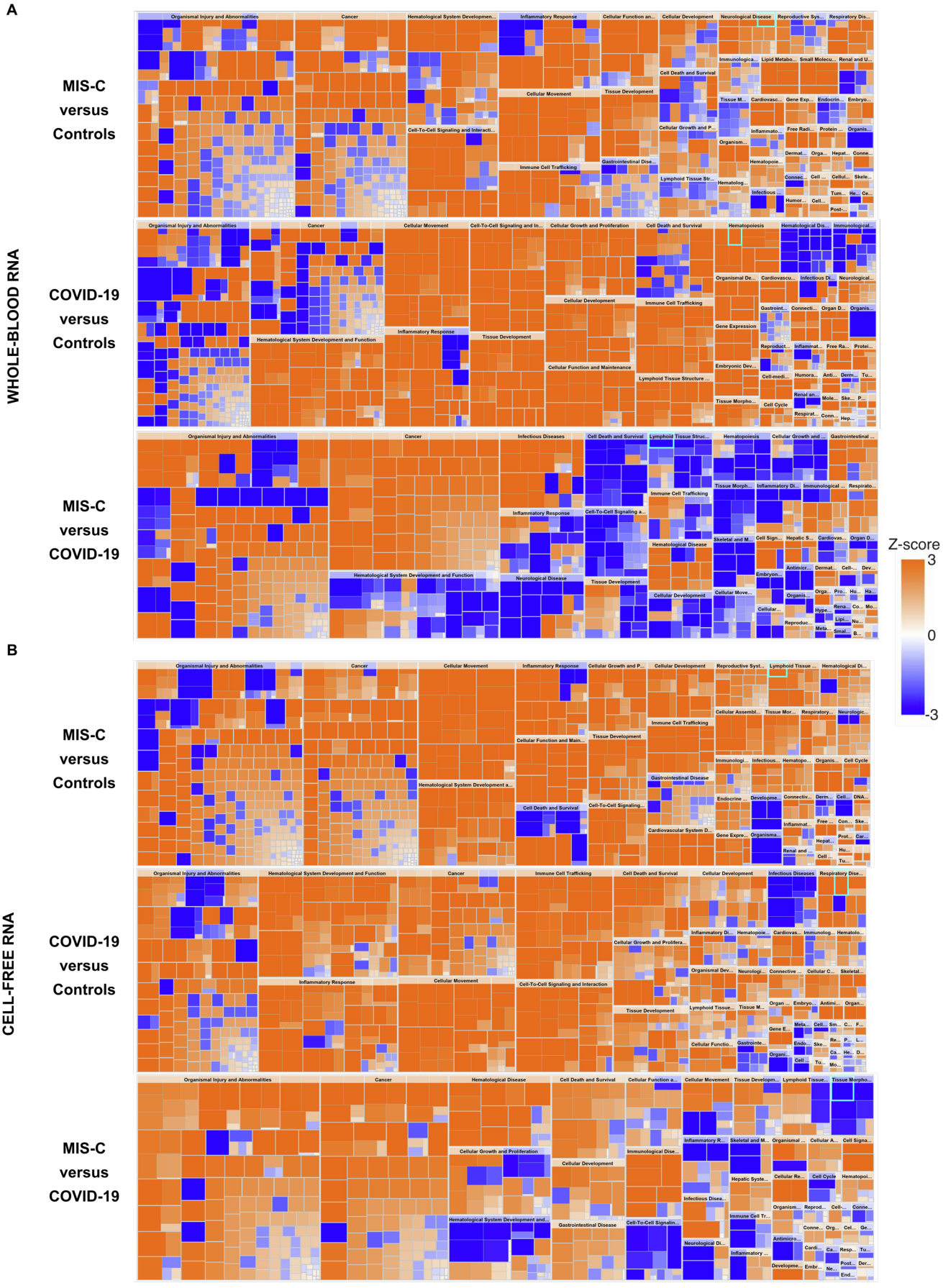

A
WHOLE-BLOOD RNA
MIS-C versus Controls
COVID-19 versus Controls
MIS-C versus COVID-19
B
CELL-FREE RNA
MIS-C versus Controls
COVID-19 versus Controls
MIS-C versus COVID-19
Z-score
3
0
-3

Figure S4. Whole blood RNA and cell-free RNA disease and biological function associations, Related to Figure 3. Diseases and biological functions associated with differentially expressed pathways between MIS-C and controls (top row), COVID-19 and controls (middle row), and MIS-C and COVID-19 (bottom row). **(A)** Whole blood RNA. **(B)** Cell-free RNA.

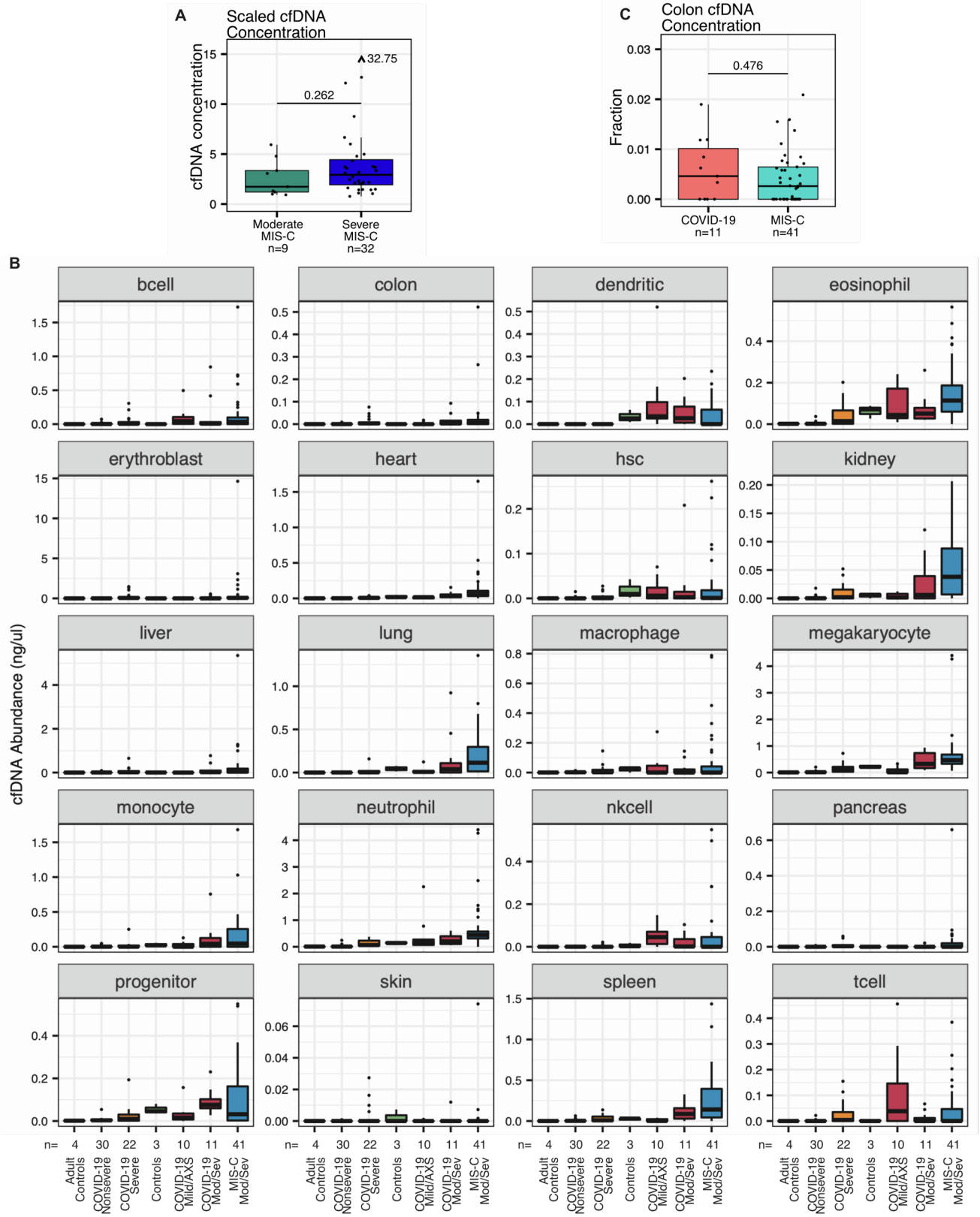
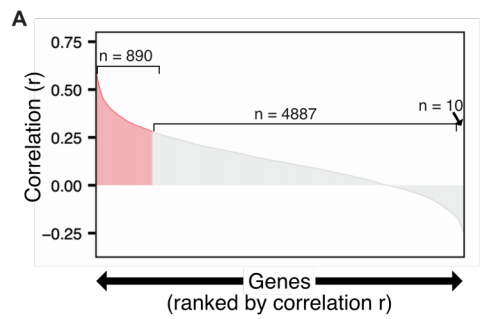
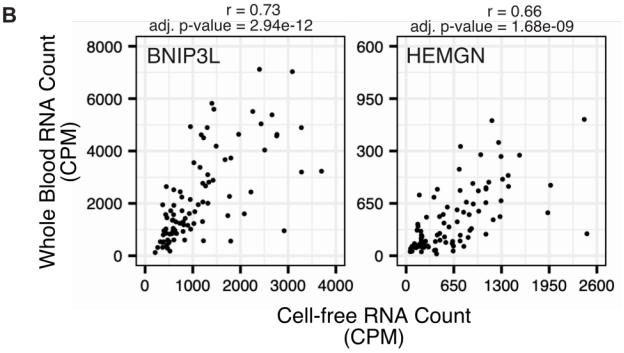
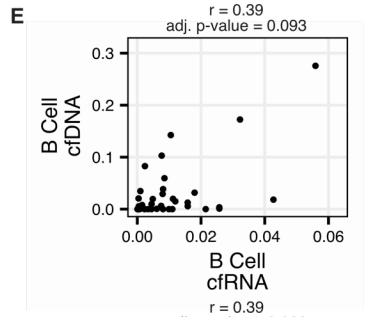
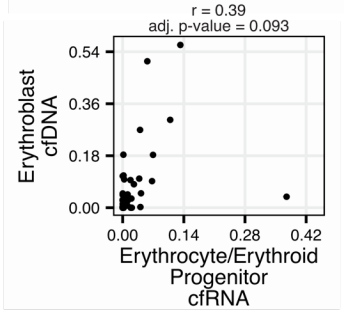

A
Scaled cfDNA Concentration
cfDNA concentration
0.262
32.75
Moderate MIS-C n=9
Severe MIS-C n=32
C
Colon cfDNA Concentration
Fraction
0.476
COVID-19 n=11
MIS-C n=41
B
cfDNA Abundance (ng/ul)
bcell
colon
dendritic
eosinophil
erythroblast
heart
hsc
kidney
liver
lung
macrophage
megakaryocyte
monocyte
neutrophil
nkcell
pancreas
progenitor
skin
spleen
tcell
n= 4 30 22 3 10 11 41
Adult Controls
COVID-19 Nonsevere
COVID-19 Severe
Controls
COVID-19 Mild/AXS
COVID-19 Mod/Sev
MIS-C Mod/Sev

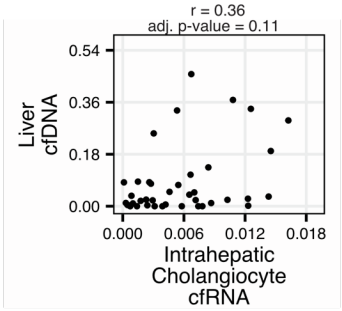
Figure S5. Plasma cell-free DNA tissues-of-origin by methylation profiling, Related to figure 4. (**A**) Total cell-free DNA (cfDNA) concentration in samples from acute moderate and severe MIS-C patients. Bar and number indicate statistical significance as measured with a Mann-Whitney U test. (**B**) Cell type concentration derived from top 20 most abundant cell types in deconvolution reference. Samples in the Adult Control, COVID-19 Non-severe, and COVID-19 severe group are from a previously published adult COVID-19 cohort (Cheng et al., 2021). Abbreviations: ASX, asymptomatic. (**C**) Colon derived cell-free DNA (cfDNA) concentration in samples from acute moderate to severe MIS-C and COVID-19 patients. Bar and number indicate statistical significance as measured with a Mann-Whitney U test.

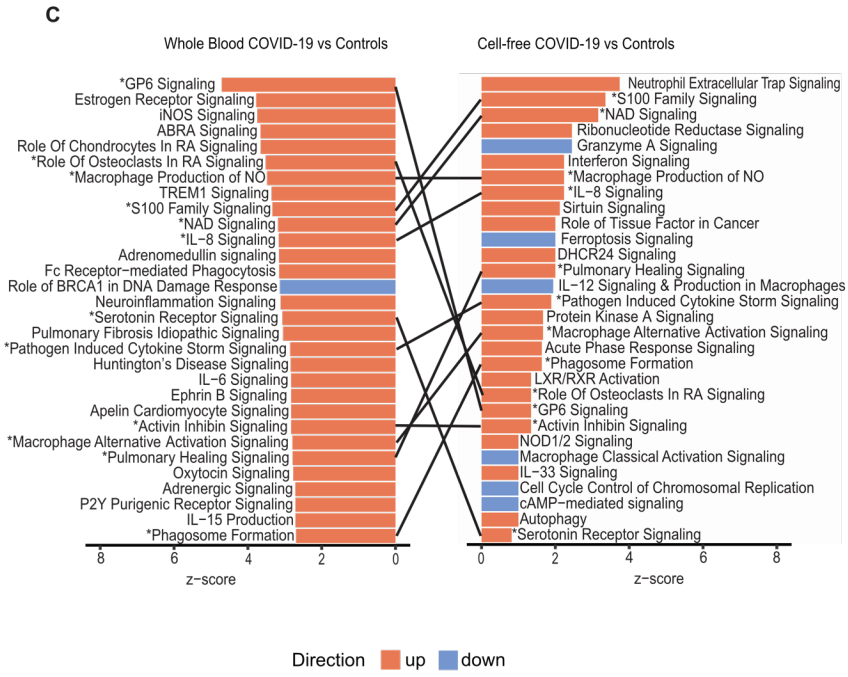

A
n = 890
n = 4887
n = 10
Correlation (r)
Genes
(ranked by correlation r)

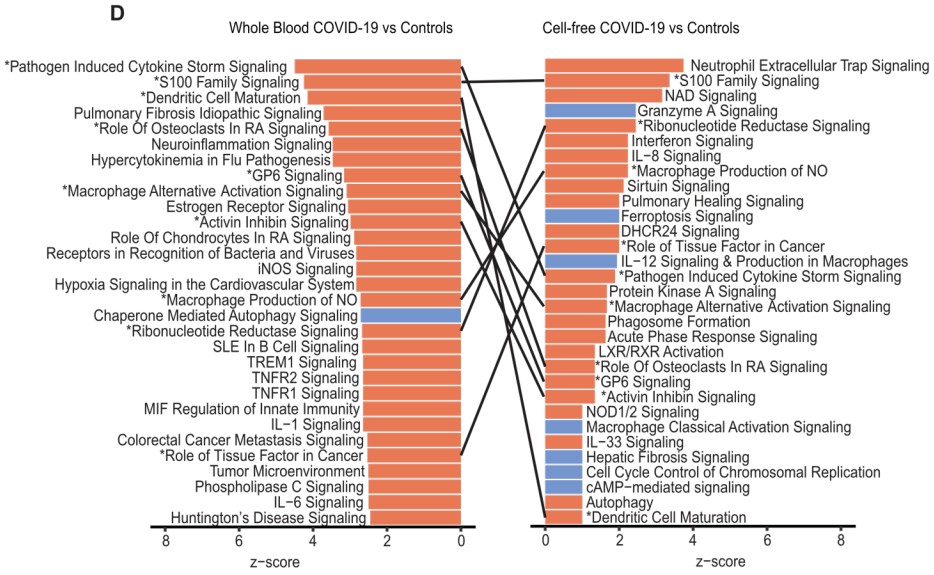

B
r = 0.73
adj. p-value = 2.94e-12
BNIP3L
r = 0.66
adj. p-value = 1.68e-09
HEMGN
Whole Blood RNA Count (CPM)
Cell-free RNA Count (CPM)


E
r = 0.39
adj. p-value = 0.093
B Cell cfDNA
B Cell cfRNA


r = 0.39
adj. p-value = 0.093
Erythroblast cfDNA
Erythrocyte/Erythroid Progenitor cfRNA


r = 0.36
adj. p-value = 0.11
Liver cfDNA
Intrahepatic Cholangiocyte cfRNA


C
Whole Blood COVID-19 vs Controls
Cell-free COVID-19 vs Controls
*GP6 Signaling
Estrogen Receptor Signaling
iNOS Signaling
ABRA Signaling
Role Of Chondrocytes In RA Signaling
*Role Of Osteoclasts In RA Signaling
*Macrophage Production of NO
TREM1 Signaling
*S100 Family Signaling
*NAD Signaling
*IL-8 Signaling
Adrenomedullin signaling
Fc Receptor-mediated Phagocytosis
Role of BRCA1 in DNA Damage Response
Neuroinflammation Signaling
*Serotonin Receptor Signaling
Pulmonary Fibrosis Idiopathic Signaling
*Pathogen Induced Cytokine Storm Signaling
Huntington's Disease Signaling
IL-6 Signaling
Ephrin B Signaling
Apelin Cardiomyocyte Signaling
*Activin Inhibin Signaling
*Macrophage Alternative Activation Signaling
*Pulmonary Healing Signaling
Oxytocin Signaling
Adrenergic Signaling
P2Y Purigenic Receptor Signaling
IL-15 Production
*Phagosome Formation
Neutrophil Extracellular Trap Signaling
*S100 Family Signaling
*NAD Signaling
Ribonucleotide Reductase Signaling
Granzyme A Signaling
Interferon Signaling
*Macrophage Production of NO
*IL-8 Signaling
Sirtuin Signaling
Role of Tissue Factor in Cancer
Ferroptosis Signaling
DHCR24 Signaling
*Pulmonary Healing Signaling
IL-12 Signaling & Production in Macrophages
*Pathogen Induced Cytokine Storm Signaling
Protein Kinase A Signaling
*Macrophage Alternative Activation Signaling
Acute Phase Response Signaling
*Phagosome Formation
LXR/RXR Activation
*Role Of Osteoclasts In RA Signaling
*GP6 Signaling
*Activin Inhibin Signaling
NOD1/2 Signaling
Macrophage Classical Activation Signaling
IL-33 Signaling
Cell Cycle Control of Chromosomal Replication
cAMP-mediated signaling
Autophagy
*Serotonin Receptor Signaling
z-score
Direction
up
down


D
Whole Blood COVID-19 vs Controls
Cell-free COVID-19 vs Controls
*Pathogen Induced Cytokine Storm Signaling
*S100 Family Signaling
*Dendritic Cell Maturation
Pulmonary Fibrosis Idiopathic Signaling
*Role Of Osteoclasts In RA Signaling
Neuroinflammation Signaling
Hypercytokinemia in Flu Pathogenesis
*GP6 Signaling
*Macrophage Alternative Activation Signaling
Estrogen Receptor Signaling
*Activin Inhibin Signaling
Role Of Chondrocytes In RA Signaling
Receptors in Recognition of Bacteria and Viruses
iNOS Signaling
Hypoxia Signaling in the Cardiovascular System
*Macrophage Production of NO
Chaperone Mediated Autophagy Signaling
*Ribonucleotide Reductase Signaling
SLE In B Cell Signaling
TREM1 Signaling
TNFR2 Signaling
TNFR1 Signaling
MIF Regulation of Innate Immunity
IL-1 Signaling
Colorectal Cancer Metastasis Signaling
*Role of Tissue Factor in Cancer
Tumor Microenvironment
Phospholipase C Signaling
IL-6 Signaling
Huntington's Disease Signaling
Neutrophil Extracellular Trap Signaling
*S100 Family Signaling
NAD Signaling
Granzyme A Signaling
*Ribonucleotide Reductase Signaling
Interferon Signaling
IL-8 Signaling
*Macrophage Production of NO
Sirtuin Signaling
Pulmonary Healing Signaling
Ferroptosis Signaling
DHCR24 Signaling
*Role of Tissue Factor in Cancer
IL-12 Signaling & Production in Macrophages
*Pathogen Induced Cytokine Storm Signaling
Protein Kinase A Signaling
*Macrophage Alternative Activation Signaling
Phagosome Formation
Acute Phase Response Signaling
LXR/RXR Activation
*Role Of Osteoclasts In RA Signaling
*GP6 Signaling
*Activin Inhibin Signaling
NOD1/2 Signaling
Macrophage Classical Activation Signaling
IL-33 Signaling
Hepatic Fibrosis Signaling
Cell Cycle Control of Chromosomal Replication
cAMP-mediated signaling
Autophagy
*Dendritic Cell Maturation
z-score

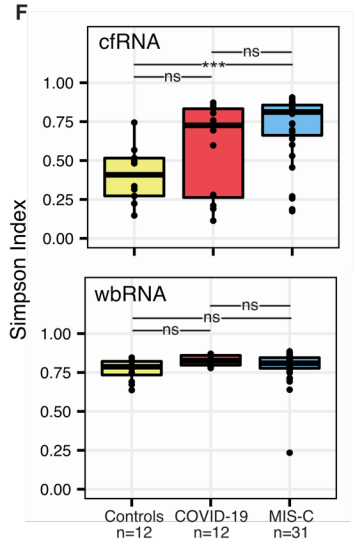

F
cfRNA
wbRNA
Simpson Index
ns
***
Controls n=12
COVID-19 n=12
MIS-C n=31

Figure S6. Comparison of paired whole blood RNA-seq, cell-free RNA, and cell-free DNA sequencing data, Related to Figure 5. (A) Pearson correlation of gene counts between paired whole blood RNA and cfRNA samples (log-transformed CPM, mean CPM > 10 in both cfRNA and whole blood RNA). Genes ordered by Pearson correlation. Genes with significant correlation are shaded in red (Benjamini-Hochberg corrected p-value <0.05). (**B)** wbRNA and cfRNA counts (CPM) of BNIP3L and HEMGN from paired samples. Pearson correlations and Benjamini-Hochberg adjusted p-values calculated using log transformed CPM values. (**C**) cfRNA and cfDNA deconvolution results from paired samples. Pearson correlations and Benjamini-Hochberg adjusted p-values calculated using calculated cell type of origin / tissue of origin fractions. (**D)** Diversity of cell type contributions in whole blood RNA and cell-free RNA as measured by Simpson's Index. Analysis performed using paired samples. Asterisks indicate statistical significance by Mann-Whitney U test using Benjamini-Hochberg adjusted p-values as follows: ns, non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.001$. (**E)** Top 30 differential pathways between acute MIS-C and Controls in whole blood RNA (left) and cfRNA (right) ranked by activation z-score (QIAGEN Ingenuity Pathway Analysis). Lines connect matching pathways. Analysis performed using paired samples. (**F)** Top 30 differential pathways between acute moderate-to-severe COVID-19 and Controls in whole blood RNA (left) and cfRNA (right) ranked by activation z-score (QIAGEN Ingenuity Pathway Analysis). Lines connect matching pathways. Analysis performed using paired samples.