Med, Volume *2*

Supplemental information

A monocyte/dendritic cell molecular signature of SARS-CoV-2-related multisystem inflammatory syndrome in children with severe myocarditis

Camille de Cevins, Marine Luka, Nikaïa Smith, Sonia Meynier, Aude Magérus, Francesco Carbone, Víctor García-Paredes, Laura Barnabei, Maxime Batignes, Alexandre Boullé, Marie-Claude Stolzenberg, Brieuc P. Pérot, Bruno Charbit, Tinhinane Fali, Vithura Pirabakaran, Boris Sorin, Quentin Riller, Ghaith Abdessalem, Maxime Beretta, Ludivine Grzelak, Pedro Goncalves, James P. Di Santo, Hugo Mouquet, Olivier Schwartz, Mohammed Zarhrate, Mélanie Parisot, Christine Bole-Feysot, Cécile Masson, Nicolas Cagnard, Aurélien Corneau, Camille Brunaud, Shen-Ying Zhang, Jean-Laurent Casanova, Brigitte Bader-Meunier, Julien Haroche, Isabelle Melki, Mathie Lorrot, Mehdi Oualha, Florence Moulin, Damien Bonnet, Zahra Belhadjer, Marianne Leruez, Slimane Allali, Christèle Gras-Leguen, Loïc de Pontual, Pediatric-Biocovid Study Group, Alain Fischer, Darragh Duffy, Fredéric Rieux-Laucat, Julie Toubiana, and Mickaël M. Ménager

Diagnosis		**Acute infection**		**Postacute hyperinflammatory illness**		
Groups of patients [1]		**Acute-Inf (CoV-2$^-$) n=4**	**Acute-Inf (CoV-2$^+$) n= 9**	**MIS-C (CoV-2$^+$) n=9**	**MIS-C_MYO (CoV-2$^+$) n=21**	**KD (CoV-2$^-$) n=13**
General						
	Male : Female	1:3	6:3	4:5	10:11	6:7
	Age; median (range)	5.8 (0.8-16.1)	0.3 (0.05-12)	5.1 (0.5-15)	8.4 (1.7-16.8)	2.3 (0.7-7.2)
	Comorbidities	None	1 (BMT)	None	None	None
Clinical features						
	Fever	4 (100)	9 (100)	9 (100)	21 (100)	13 (100)
	Respiratory manifestations	4 (100)	9 (100)	1 (11)	7 (33)	2 (15)
	Pneumonia	4 (100)	6 (67)	1 (11)	7 (33)	2 (15)
	Gastrointestinal symptoms	0	1 (11)	6 (67)	19 (95)	6 (46)
	Neurological symptoms	1 (25)	6 (67)	1 (11)	6 (29)	0
	KD criteria complete	N/A	N/A	4 (44)	10 (48)	6 (46)
	Admission to the intensive care unit	0	1 (11)	2 (22)	21 (100)	1 (8)
	Ventricular dysfunction [2]	N/A	N/A	0	21 (100)	0
	Coronary artery dilation or aneurysm [3]	N/A	N/A	1 (11)	3 (14)	1 (8)
Biological features [4]						
	Positive NP SARS-CoV-2 RT-PCR	0	9 (100)	4 (44)	10 (48)	0
	Positive serum SARS-CoV-2 IgG (Abbott)	0/1 (0)	N/D	9 (100)	21 (100)	0
	Troponin I; Median (range) (ref <26 ng/L)	N/D	N/D	10 (10-122) [5]	324 (30-6900)	51 (10-93) [5]
	Leukocytes x 10^9/L; Median (range) (ref 5.5-15.5)	N/D	N/D	12.3 (5.6-44)	18.9 (8-42.8)	13.7 (3.3-17.6)
	Neutrophils x 10^9/L; Median (range) (ref 1.8-8.0)	1.6 (1.2-14.5)	1.5 (0.6-7.5)	7.7 (0.2-39)	14.9 (6.0-36.4)	6.5 (0.8-6.4)
	Lymphocytes x 10^9/L; Median (range) (ref 1.5-6.5)	2.7 (2.1-3.4)	2.6 (0.3-7.6)	1.4 (0.6-5.6)	1.0 (0.4-3.7)	2.6 (0.8-6.4)
	CRP, mg/L; Median (range) (ref <6.0)	8 (4-16)	7 (4-32)	190 (90-287)	295 (159-448)	161 (31-309)
	PCT, ng/mL; Median (range) (ref <0.5)	0.2 (0.02-0.2)	0.16 (0.09-.22)	4.1 (0.2-152)	26.0 (1.7-299)	1.0 (0.1-5.5)
	Sodium, mmol/L; Median (range) (ref 136-146)	N/D	131 (130-136)	132 (124-136)	130 (116-135)	135 (133-136)
	Albumin, g/L; Median (range) (ref 35-50)	N/D	N/D	23 (15-41)	20 (16-26)	31 (25-40)
	ALT, U/L; Median (range) (ref 7-40)	N/D	N/D	28 (6-146)	70 (12-257)	18 (11-277)
	Ferritin µg/L; Median (range) (ref	N/D	N/D	336 (118-1400) [5]	1123 (172-4490) [5]	183 (139-390) [5]
Treatments						
	Vasoactive or inotropic agents	0	0	0	17 (81)	0
	Antibiotic therapy	3 (64)	3 (33)	5 (56)	21 (100)	4 (31)
	IVIG /IVIG before sampling	0	0	8 (89) / 7 (78)	21 (100) / 20 (95)	12 (92) / 11 (85)
	Corticosteroids / corticosteroids before sampling	0	0	2 (22) /1 (11)	10 (48) / 7 (33)	4 (31) / 4 (31)
	Other immunomodulatory agents	0	1 (tocilizumab)	0	0	0

[1] Values are numbers (percentages) unless stated otherwise; [2] ventricular dysunfunction observed at echocardiography; [3] Coronary artery abnormalities observed during hospitalization, Coronary artery dilation was defined as a coronary artery diameter z-score on echocardiography between 2.0 and 2.5, and aneurysm as a z-score ≥2.5 (McCrindle et al., 2017); [4] the most abnormal values before treatment or within 24 hours of treatment onset; [5] missing values; BMT: bone marrow transplantation; ALT: alanine transferase; IVIG: intravenous immunoglobulins; N/A : non applicable; N/D: not determined; Ref: reference interval.

Table S1. Clinical and biological features of pediatric patients enrolled. Related to Figure 1. All children and adolescents included in the study were suspected of SARS-CoV-2 illness between April 6, 2020 and May 30, 2020 and displayed either an acute respiratory infection or a postacute hyperinflammatory illness.

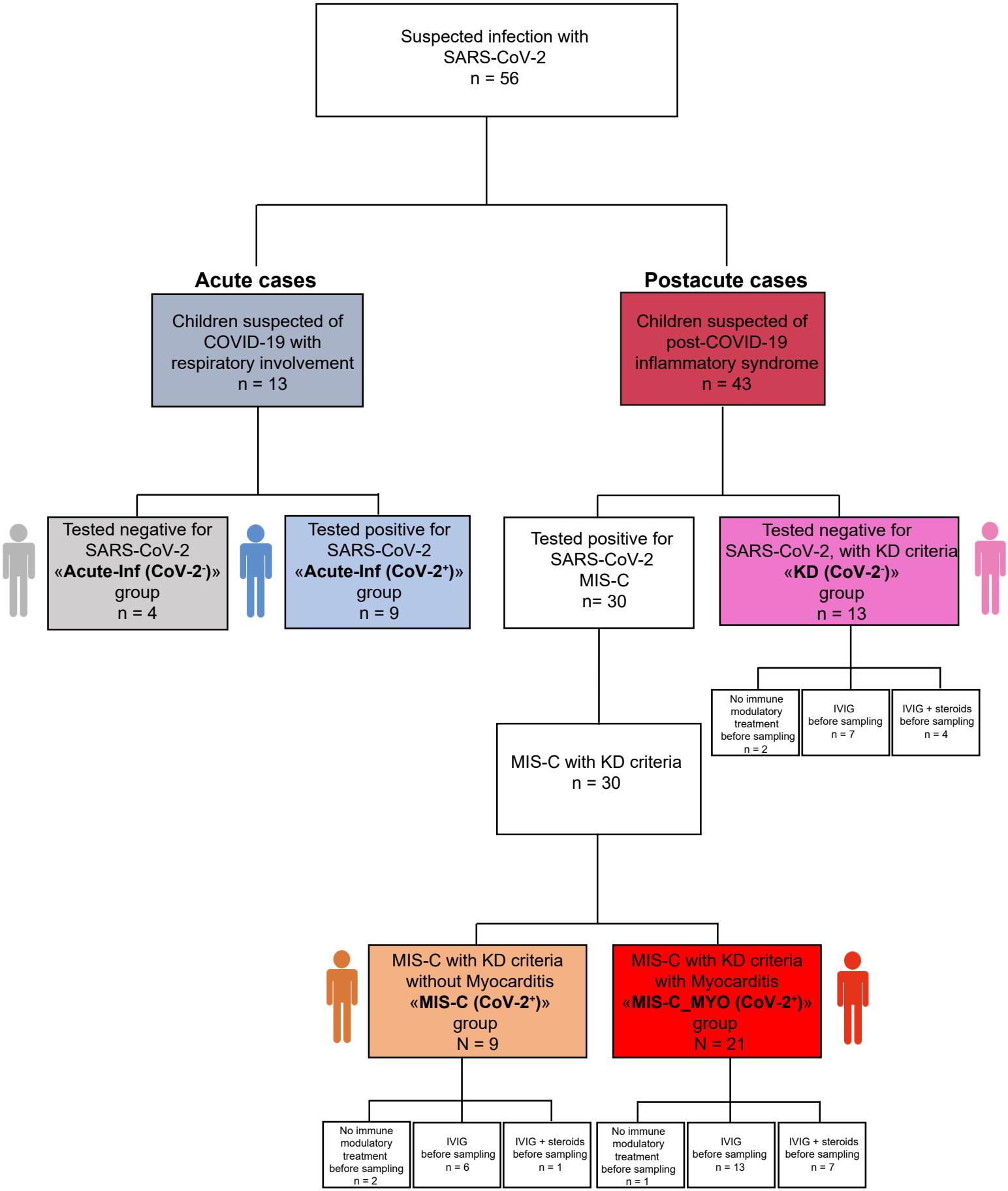
Suspected infection with SARS-CoV-2 n = 56
Acute cases
Postacute cases
Children suspected of COVID-19 with respiratory involvement n = 13
Children suspected of post-COVID-19 inflammatory syndrome n = 43
Tested negative for SARS-CoV-2 «Acute-Inf (CoV-2-)» group n = 4
Tested positive for SARS-CoV-2 «Acute-Inf (CoV-2+)» group n = 9
Tested positive for SARS-CoV-2 MIS-C n= 30
Tested negative for SARS-CoV-2, with KD criteria «KD (CoV-2-)» group n = 13
No immune modulatory treatment before sampling n = 2
IVIG before sampling n = 7
IVIG + steroids before sampling n = 4
MIS-C with KD criteria n = 30
MIS-C with KD criteria without Myocarditis «MIS-C (CoV-2+)» group N = 9
MIS-C with KD criteria with Myocarditis «MIS-C_MYO (CoV-2+)» group N = 21
No immune modulatory treatment before sampling n = 2
IVIG before sampling n = 6
IVIG + steroids before sampling n = 1
No immune modulatory treatment before sampling n = 1
IVIG before sampling n = 13
IVIG + steroids before sampling n = 7

Figure S1

Figure S1: Flowchart describing the patients enrolled. Related to Figure 1. Patients were initially divided into an "acute" and a "postacute" group. Each group was then divided based on SARS-CoV-2 positivity. Each group with a name in bold and a color associated corresponds to a group of patients analyzed in this study. Names and colors are used throughout the manuscript. "n" represents the number of patients enrolled in each group. Number of immune modulator-treated patients is indicated when applicable.

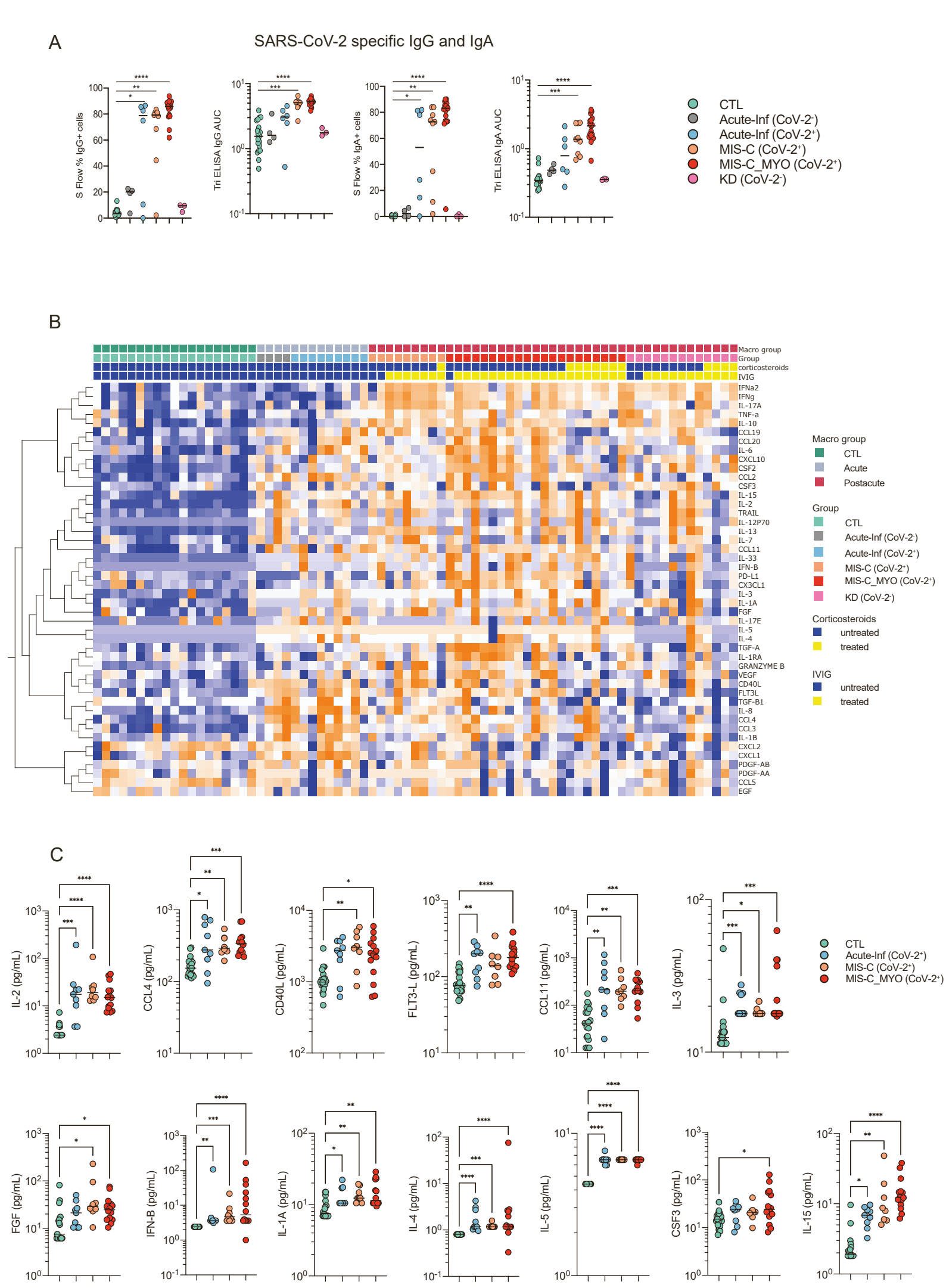
A
SARS-CoV-2 specific IgG and IgA
S Flow % IgG+ cells
Tri ELISA IgG AUC
S Flow % IgA+ cells
Tri ELISA IgA AUC
CTL
Acute-Inf (CoV-2-)
Acute-Inf (CoV-2+)
MIS-C (CoV-2+)
MIS-C_MYO (CoV-2+)
KD (CoV-2-)
B
Macro group
Group
corticosteroids
IVIG
IFNa2
IFNg
IL-17A
TNF-a
IL-10
CCL19
CCL20
IL-6
CXCL10
CSF2
CCL2
CSF3
IL-15
IL-2
TRAIL
IL-12P70
IL-13
IL-7
CCL11
IL-33
IFN-B
PD-L1
CX3CL1
IL-3
IL-1A
FGF
IL-17E
IL-5
IL-4
TGF-A
IL-1RA
GRANZYME B
VEGF
CD40L
FLT3L
TGF-B1
IL-8
CCL4
CCL3
IL-1B
CXCL2
CXCL1
PDGF-AB
PDGF-AA
CCL5
EGF
Macro group
CTL
Acute
Postacute
Group
CTL
Acute-Inf (CoV-2-)
Acute-Inf (CoV-2+)
MIS-C (CoV-2+)
MIS-C_MYO (CoV-2+)
KD (CoV-2-)
Corticosteroids
untreated
treated
IVIG
untreated
treated
C
IL-2 (pg/mL)
CCL4 (pg/mL)
CD40L (pg/mL)
FLT3-L (pg/mL)
CCL11 (pg/mL)
IL-3 (pg/mL)
FGF (pg/mL)
IFN-B (pg/mL)
IL-1A (pg/mL)
IL-4 (pg/mL)
IL-5 (pg/mL)
CSF3 (pg/mL)
IL-15 (pg/mL)
CTL
Acute-Inf (CoV-2+)
MIS-C (CoV-2+)
MIS-C_MYO (CoV-2+)

Figure S2

Figure S2: Immunoglobulin and cytokine/chemokine analyses. Related to Figure 2. A. SARS-CoV-2 specific immunoglobulins (IgG, left panels; IgA, right panels) were dosed in the plasma of the different groups, by two methods: S Flow and Tri ELISA. **B.** Heatmap of all the cytokines/chemokines measured in the different clinical groups, including the SARS-CoV-2 negative groups: CTL, green; Acute-Inf (CoV-2$^-$), gray; Acute-Inf (CoV-2$^+$), blue; MIS-C (CoV-2$^+$), orange; MIS-C_MYO (CoV-2$^+$), red; KD (CoV-2$^-$), pink. On the x axis, blood donors are organized by groups and immune modulatory treatments (untreated, blue; treated, yellow) and on the y axis, cytokines/chemokines are displayed following hierarchical clustering. Cytokines/chemokines were expressed as pg/mL and log transformed with blue to orange colors representing lower to higher expression respectively. **C.** Dot plots of cytokines/ chemokines increased in all SARS-CoV-2$^+$ groups compared to healthy blood donors (CTL). ρ-values are calculated by Kruskal-Wallis test for multiple comparisons, followed by a post-hoc Dunn's test. * ($p < 0.05$), **($p < 0.01$), ***($p < 0.001$)

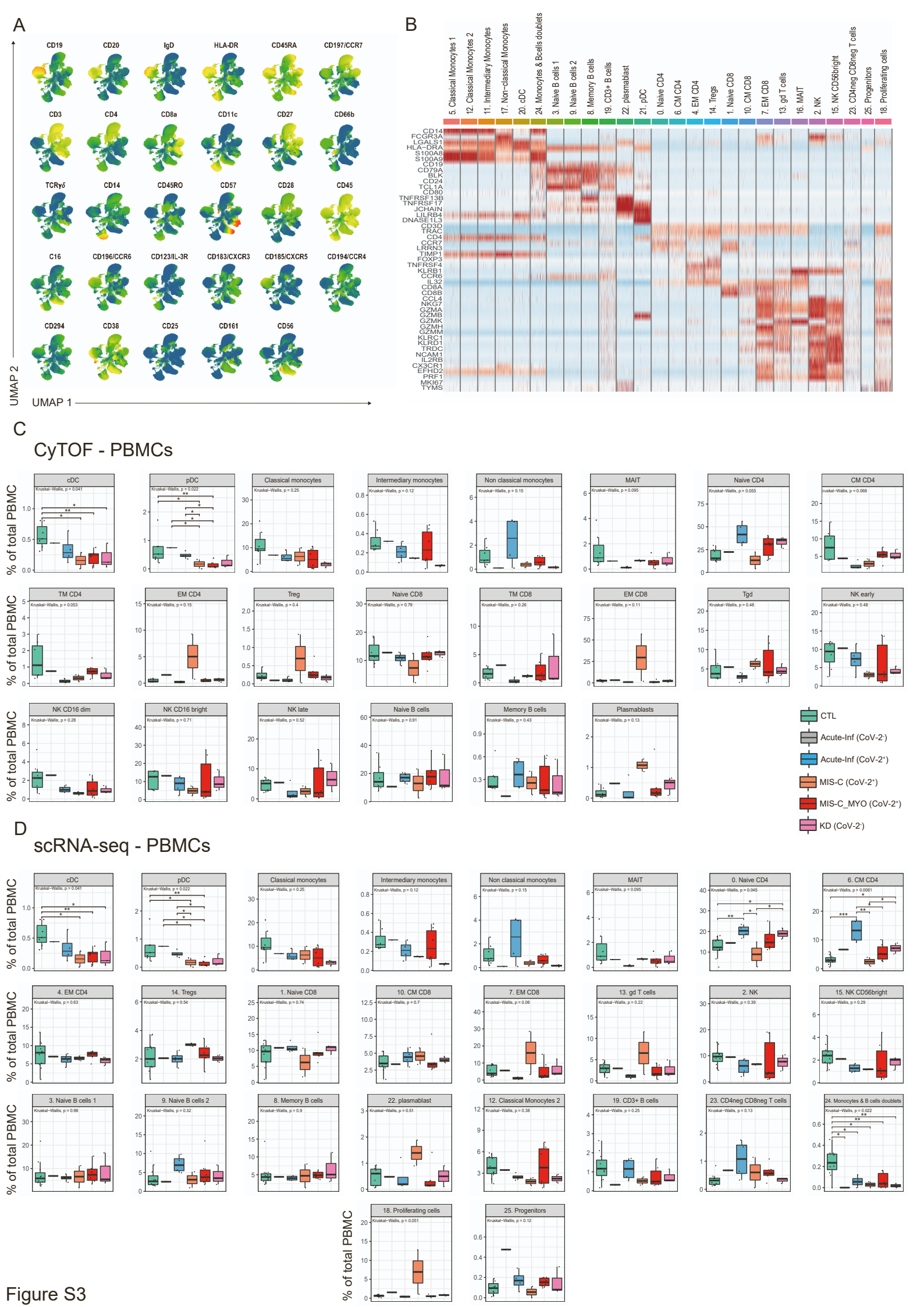
A
CD19
CD20
IgD
HLA-DR
CD45RA
CD197/CCR7
CD3
CD4
CD8a
CD11c
CD27
CD66b
TCRγδ
CD14
CD45RO
CD57
CD28
CD45
C16
CD196/CCR6
CD123/IL-3R
CD183/CXCR3
CD185/CXCR5
CD194/CCR4
CD294
CD38
CD25
CD161
CD56
UMAP 2
UMAP 1
B
5. Classical Monocytes 1
12. Classical Monocytes 2
11. Intermediary Monocytes
17. Non−classical Monocytes
20. cDC
24. Monocytes & Bcells doublets
3. Naive B cells 1
9. Naive B cells 2
8. Memory B cells
19. CD3+ B cells
22. plasmablast
21. pDC
0. Naive CD4
6. CM CD4
4. EM CD4
14. Tregs
1. Naive CD8
10. CM CD8
7. EM CD8
13. gd T cells
16. MAIT
2. NK
15. NK CD56bright
23. CD4neg CD8neg T cells
25. Progenitors
18. Proliferating cells
CD14
FCGR3A
LGALS1
HLA−DRA
S100A8
S100A9
CD19
CD79A
BLK
CD24
TCL1A
CD80
TNFRSF13B
TNFRSF17
JCHAIN
LILRB4
DNASE1L3
CD3D
TRAC
CD4
CCR7
LRRN3
TIMP1
FOXP3
TNFRSF4
KLRB1
CCR6
IL32
CD8A
CD8B
CCL4
NKG7
GZMA
GZMB
GZMK
GZMH
GZMM
KLRC1
KLRD1
TRDC
NCAM1
IL2RB
CX3CR1
EFHD2
PRF1
MKI67
TYMS
C
CyTOF - PBMCs
% of total PBMC
cDC
pDC
Classical monocytes
Intermediary monocytes
Non classical monocytes
MAIT
Naive CD4
CM CD4
TM CD4
EM CD4
Treg
Naive CD8
TM CD8
EM CD8
Tgd
NK early
NK CD16 dim
NK CD16 bright
NK late
Naive B cells
Memory B cells
Plasmablasts
CTL
Acute-Inf (CoV-2⁻)
Acute-Inf (CoV-2⁺)
MIS-C (CoV-2⁺)
MIS-C_MYO (CoV-2⁺)
KD (CoV-2⁻)
D
scRNA-seq - PBMCs
% of total PBMC
cDC
pDC
Classical monocytes
Intermediary monocytes
Non classical monocytes
MAIT
0. Naive CD4
6. CM CD4
4. EM CD4
14. Tregs
1. Naive CD8
10. CM CD8
7. EM CD8
13. gd T cells
2. NK
15. NK CD56bright
3. Naive B cells 1
9. Naive B cells 2
8. Memory B cells
22. plasmablast
12. Classical Monocytes 2
19. CD3+ B cells
23. CD4neg CD8neg T cells
24. Monocytes & B cells doublets
18. Proliferating cells
25. Progenitors

Figure S3

Figure S3: Identification of cell clusters and PBMCs distribution. Related to Figure 3. A. UMAP showing all cell surface proteins measured by CyTOF to identify clusters displayed in Figure 3A. **B.** Heatmap of the genes (y axis) used in scRNA-seq to identify clusters of the UMAP in Figure 3B. Color scale represents scaled expression of the genes. Red and blue indicate high and low expression respectively. **C & D.** Boxplots showing the percentage of cell populations from CyTOF **(C)** and scRNA-seq **(D)** for both SARS-CoV-2 positive and negative groups. Among the MIS-C (CoV-2^{+}) group, one patient was diagnosed with a primary Epstein Barr viral infection, characterized at the cellular level by an increase of CD4, CD8 effector memory T cells, Tregs and proliferating cells.(CTL, green; Acute-Inf (CoV-2^{-}), gray; Acute-Inf (CoV-2^{+}), blue; MIS-C (CoV-2^{+}), orange; MIS-C_MYO (CoV-2^{+}), red; KD (CoV-2^{-}), pink). In the boxplots, each dot represents a sample. Boxes range from the 25th to the 75th percentiles. The upper and lower whiskers extend from the box to the largest and smallest values respectively. Any samples with a value at most x1.5 the inter-quartile range of the hinge is considered an outlier and plotted individually. ρ values are calculated by Kruskal-Wallis test for multiple comparison, followed by a post hoc Dunn's test. *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

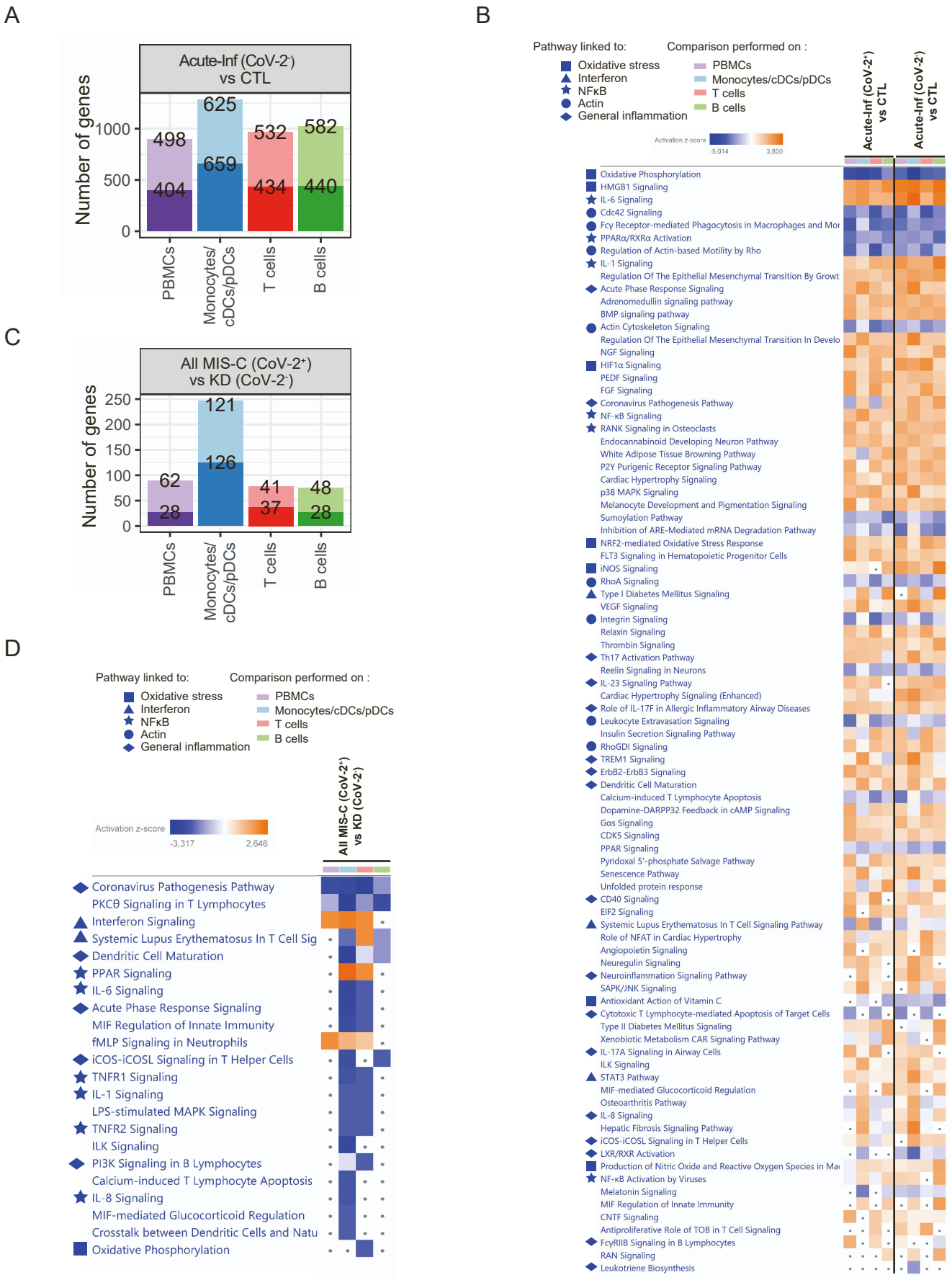
A
Acute-Inf (CoV-2-) vs CTL
Number of genes
1000
500
0
498
404
625
659
532
434
582
440
PBMCs
Monocytes/ cDCs/pDCs
T cells
B cells
C
All MIS-C (CoV-2+) vs KD (CoV-2-)
Number of genes
250
200
150
100
50
0
62
28
121
126
41
37
48
28
PBMCs
Monocytes/ cDCs/pDCs
T cells
B cells
D
Pathway linked to:
Oxidative stress
Interferon
NFκB
Actin
General inflammation
Comparison performed on :
PBMCs
Monocytes/cDCs/pDCs
T cells
B cells
Activation z-score
-3,317
2,646
All MIS-C (CoV-2+) vs KD (CoV-2-)
Coronavirus Pathogenesis Pathway
PKCθ Signaling in T Lymphocytes
Interferon Signaling
Systemic Lupus Erythematosus In T Cell Sig
Dendritic Cell Maturation
PPAR Signaling
IL-6 Signaling
Acute Phase Response Signaling
MIF Regulation of Innate Immunity
fMLP Signaling in Neutrophils
iCOS-iCOSL Signaling in T Helper Cells
TNFR1 Signaling
IL-1 Signaling
LPS-stimulated MAPK Signaling
TNFR2 Signaling
ILK Signaling
PI3K Signaling in B Lymphocytes
Calcium-induced T Lymphocyte Apoptosis
IL-8 Signaling
MIF-mediated Glucocorticoid Regulation
Crosstalk between Dendritic Cells and Natu
Oxidative Phosphorylation
B
Pathway linked to:
Oxidative stress
Interferon
NFκB
Actin
General inflammation
Comparison performed on :
PBMCs
Monocytes/cDCs/pDCs
T cells
B cells
Activation z-score
-5,014
3,800
Acute-Inf (CoV-2+) vs CTL
Acute-Inf (CoV-2-) vs CTL
Oxidative Phosphorylation
HMGB1 Signaling
IL-6 Signaling
Cdc42 Signaling
Fcγ Receptor-mediated Phagocytosis in Macrophages and Mor
PPARα/RXRα Activation
Regulation of Actin-based Motility by Rho
IL-1 Signaling
Regulation Of The Epithelial Mesenchymal Transition By Growt
Acute Phase Response Signaling
Adrenomedullin signaling pathway
BMP signaling pathway
Actin Cytoskeleton Signaling
Regulation Of The Epithelial Mesenchymal Transition In Develo
NGF Signaling
HIF1α Signaling
PEDF Signaling
FGF Signaling
Coronavirus Pathogenesis Pathway
NF-κB Signaling
RANK Signaling in Osteoclasts
Endocannabinoid Developing Neuron Pathway
White Adipose Tissue Browning Pathway
P2Y Purigenic Receptor Signaling Pathway
Cardiac Hypertrophy Signaling
p38 MAPK Signaling
Melanocyte Development and Pigmentation Signaling
Sumoylation Pathway
Inhibition of ARE-Mediated mRNA Degradation Pathway
NRF2-mediated Oxidative Stress Response
FLT3 Signaling in Hematopoietic Progenitor Cells
iNOS Signaling
RhoA Signaling
Type I Diabetes Mellitus Signaling
VEGF Signaling
Integrin Signaling
Relaxin Signaling
Thrombin Signaling
Th17 Activation Pathway
Reelin Signaling in Neurons
IL-23 Signaling Pathway
Cardiac Hypertrophy Signaling (Enhanced)
Role of IL-17F in Allergic Inflammatory Airway Diseases
Leukocyte Extravasation Signaling
Insulin Secretion Signaling Pathway
RhoGDI Signaling
TREM1 Signaling
ErbB2-ErbB3 Signaling
Dendritic Cell Maturation
Calcium-induced T Lymphocyte Apoptosis
Dopamine-DARPP32 Feedback in cAMP Signaling
Gαs Signaling
CDK5 Signaling
PPAR Signaling
Pyridoxal 5'-phosphate Salvage Pathway
Senescence Pathway
Unfolded protein response
CD40 Signaling
EIF2 Signaling
Systemic Lupus Erythematosus In T Cell Signaling Pathway
Role of NFAT in Cardiac Hypertrophy
Angiopoietin Signaling
Neuregulin Signaling
Neuroinflammation Signaling Pathway
SAPK/JNK Signaling
Antioxidant Action of Vitamin C
Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells
Type II Diabetes Mellitus Signaling
Xenobiotic Metabolism CAR Signaling Pathway
IL-17A Signaling in Airway Cells
ILK Signaling
STAT3 Pathway
MIF-mediated Glucocorticoid Regulation
Osteoarthritis Pathway
IL-8 Signaling
Hepatic Fibrosis Signaling Pathway
iCOS-iCOSL Signaling in T Helper Cells
LXR/RXR Activation
Production of Nitric Oxide and Reactive Oxygen Species in Ma
NF-κB Activation by Viruses
Melatonin Signaling
MIF Regulation of Innate Immunity
CNTF Signaling
Antiproliferative Role of TOB in T Cell Signaling
FcγRIIB Signaling in B Lymphocytes
RAN Signaling
Leukotriene Biosynthesis

Figure S4

Figure S4: Genes and pathways differentially regulated from scRNA-seq in acute infection and postacute hyperinflammation when comparing patients with or without evidence of SARS-CoV-2 infection. Related to Figure 4. A. Bar charts of the number of up- and downregulated genes in Acute-Inf (CoV-2$^-$) compared to CTL, in PBMCs, monocytes/cDCs/pDCs, T and B cell clusters obtained following scRNA-seq experiments as displayed in Figure 3B. **B.** Heatmap of the canonical pathways obtained with IPA from DEGs of Acute-Inf (CoV-2$^+$), as shown in 4A or of Acute-Inf (CoV-2$^-$), S4A, compared to CTL in PBMCs, monocytes/cDCs/pDCs, T and B cells. **C.** Bar charts of the number of up- and downregulated genes in All MIS-C (CoV-2$^+$) compared directly to KD (CoV-2$^-$), in PBMCs, monocytes/cDCs/pDCs, T and B cells. **D.** Heatmap of the canonical pathways obtained with IPA from DEGs of All MIS-C (CoV-2$^+$) compared directly to KD (CoV-2$^-$) in PBMCs, monocytes/cDCs/pDCs, T and B cells.

A & C. The top value on the light-colored bars represent the upregulated genes and the bottom dark represent the downregulated genes.

B & D. Symbols are used in front of the pathways to represent pathways belonging to the same functional groups. Pathways with an absolute Z-score ≤ 2 or adjusted p-value > 0.05 in all conditions were filtered out. Z-score > 2 means that a function is significantly increased (orange) whereas a Z-score < −2 indicates a significantly decreased function (blue). Grey dots indicate non-significant pathways ($p > 0.05$).

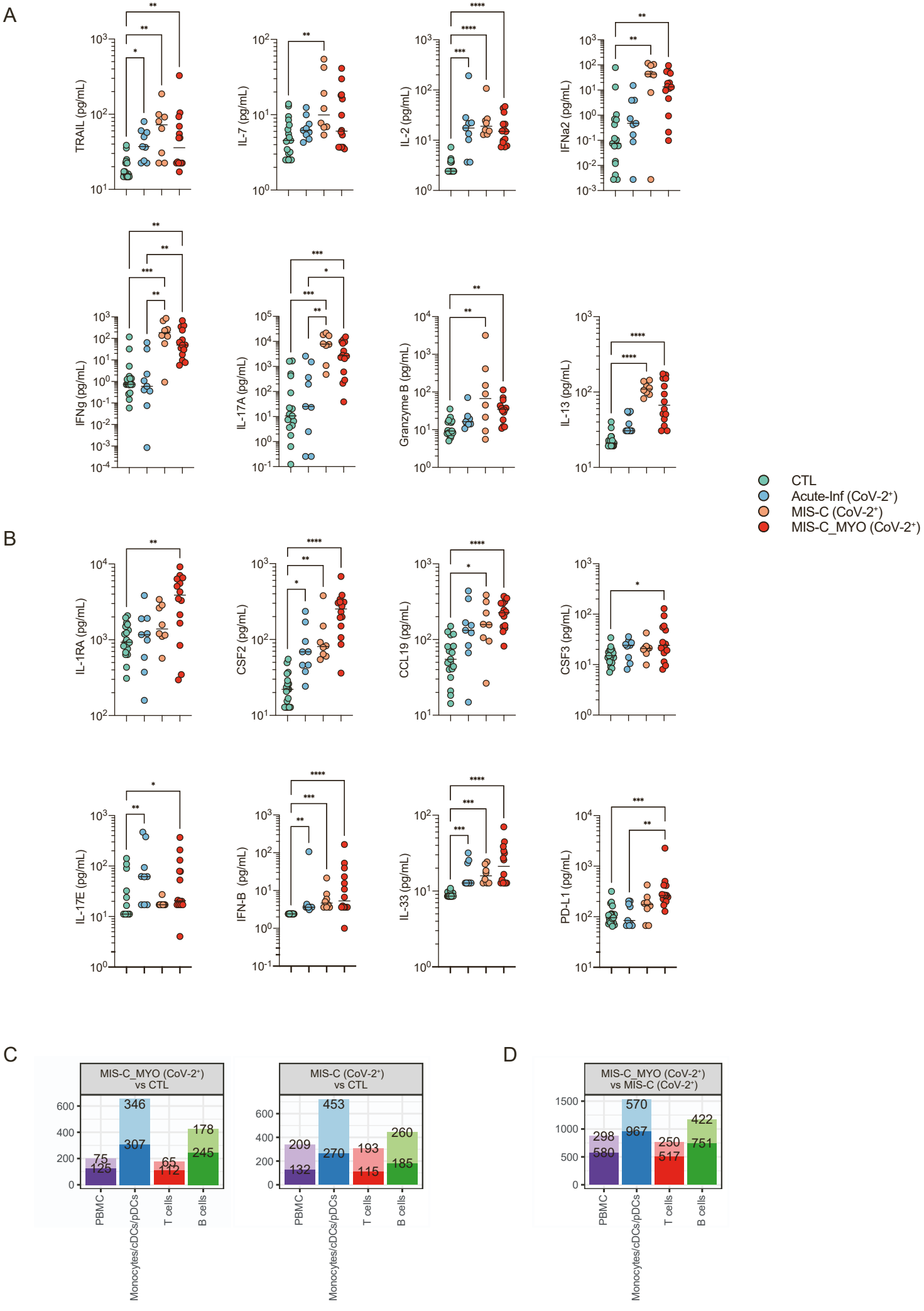
A
TRAIL (pg/mL)
IL-7 (pg/mL)
IL-2 (pg/mL)
IFNa2 (pg/mL)
IFNg (pg/mL)
IL-17A (pg/mL)
Granzyme B (pg/mL)
IL-13 (pg/mL)
B
IL-1RA (pg/mL)
CSF2 (pg/mL)
CCL19 (pg/mL)
CSF3 (pg/mL)
IL-17E (pg/mL)
IFN-B (pg/mL)
IL-33 (pg/mL)
PD-L1 (pg/mL)
CTL
Acute-Inf (CoV-2+)
MIS-C (CoV-2+)
MIS-C_MYO (CoV-2+)
C
MIS-C_MYO (CoV-2+) vs CTL
MIS-C (CoV-2+) vs CTL
D
MIS-C_MYO (CoV-2+) vs MIS-C (CoV-2+)
PBMC
Monocytes/cDCs/pDCs
T cells
B cells

Figure S5

Figure S5: Cytokine/chemokine and gene expression analyses between MIS-C_MYO (CoV-2$^+$) and MIS-C (CoV-2$^+$). Related to Figure 5. A. Dot plots of cytokines/chemokines upregulated in the MIS-C (CoV-2$^+$) group compared to MIS-C_MYO (CoV-2$^+$). **B.** Dot plots of cytokines/chemokines upregulated in the MIS-C_MYO (CoV-2$^+$) group compared to MIS-C (CoV-2$^+$), and not related to TNF-α or NF-κB signalings. **C**. Bar charts of the number of up- and downregulated genes in MIS-C_MYO (CoV-2$^+$) (left panel) and MIS-C (CoV-2$^+$) (right panel), compared to CTL, in PBMCs, monocytes/cDCs/pDCs, T and B cells clusters obtained following scRNA-seq experiments as displayed in Figure 3B. **D**. Bar chart of the number of up- and downregulated genes between MIS-C_MYO (CoV-2$^+$) and MIS-C (CoV-2$^+$) in PBMCs, monocytes/cDCs/pDCs, T and B cells clusters obtained following scRNA-seq experiments as displayed in Figure 3B.

A & B. ρ values are calculated by Kruskall-Wallis test for multiple comparisons, followed by a post hoc Dunn's test. *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$)

C & D. The top value on the light-colored bars represent the upregulated genes and the bottom dark represent the downregulated genes. Median age for each group: CTL, 15 years; MIS-C (CoV-2$^+$), 3.7 years; MIS-C_MYO (CoV-2$^+$), 8.4 years

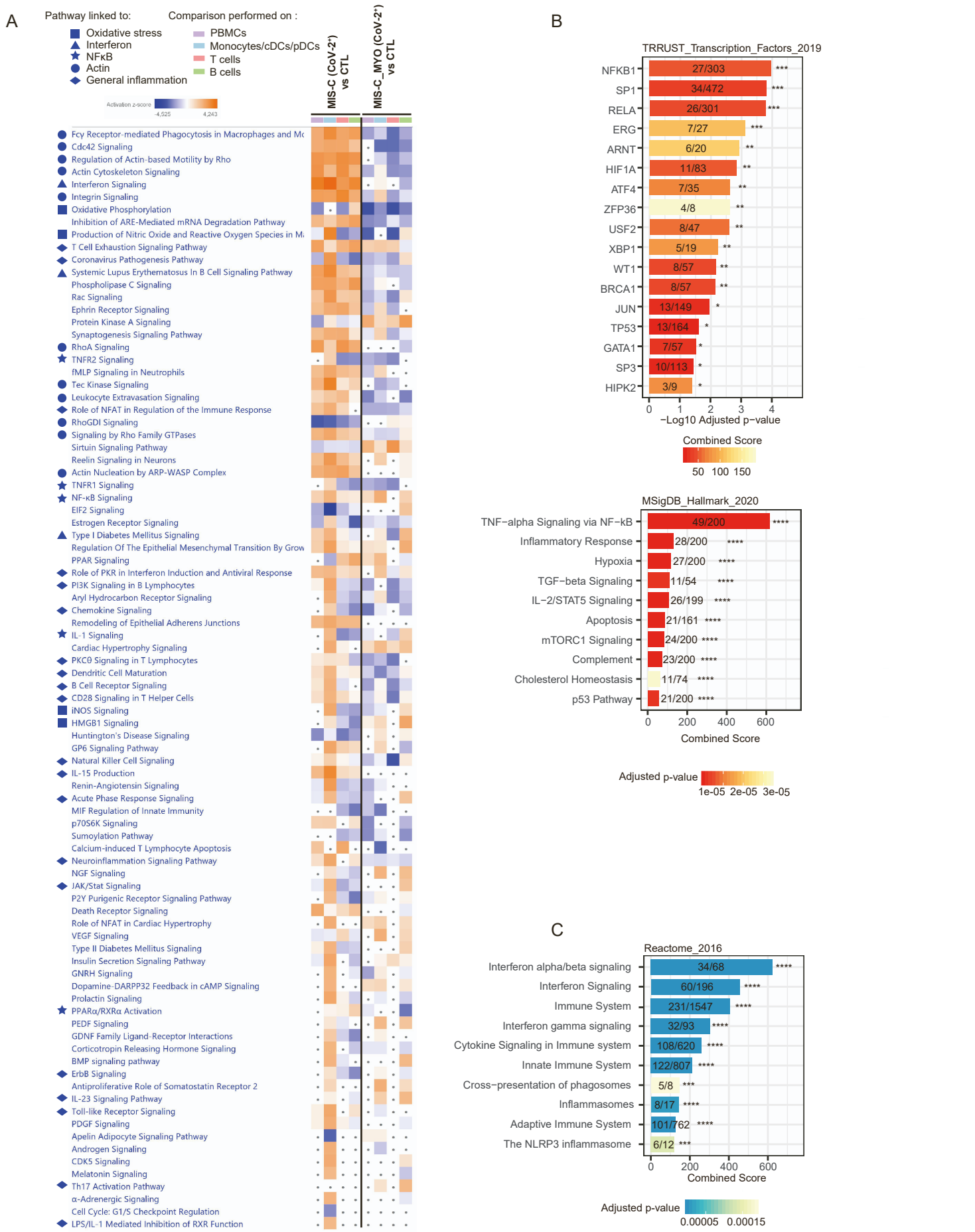
A
Pathway linked to:
Oxidative stress
Interferon
NFκB
Actin
General inflammation
Comparison performed on :
PBMCs
Monocytes/cDCs/pDCs
T cells
B cells
Activation z-score
-4,525
4,243
MIS-C (CoV-2+) vs CTL
MIS-C_MYO (CoV-2+) vs CTL
Fcγ Receptor-mediated Phagocytosis in Macrophages and M
Cdc42 Signaling
Regulation of Actin-based Motility by Rho
Actin Cytoskeleton Signaling
Interferon Signaling
Integrin Signaling
Oxidative Phosphorylation
Inhibition of ARE-Mediated mRNA Degradation Pathway
Production of Nitric Oxide and Reactive Oxygen Species in M
T Cell Exhaustion Signaling Pathway
Coronavirus Pathogenesis Pathway
Systemic Lupus Erythematosus In B Cell Signaling Pathway
Phospholipase C Signaling
Rac Signaling
Ephrin Receptor Signaling
Protein Kinase A Signaling
Synaptogenesis Signaling Pathway
RhoA Signaling
TNFR2 Signaling
fMLP Signaling in Neutrophils
Tec Kinase Signaling
Leukocyte Extravasation Signaling
Role of NFAT in Regulation of the Immune Response
RhoGDI Signaling
Signaling by Rho Family GTPases
Sirtuin Signaling Pathway
Reelin Signaling in Neurons
Actin Nucleation by ARP-WASP Complex
TNFR1 Signaling
NF-κB Signaling
EIF2 Signaling
Estrogen Receptor Signaling
Type I Diabetes Mellitus Signaling
Regulation Of The Epithelial Mesenchymal Transition By Grow
PPAR Signaling
Role of PKR in Interferon Induction and Antiviral Response
PI3K Signaling in B Lymphocytes
Aryl Hydrocarbon Receptor Signaling
Chemokine Signaling
Remodeling of Epithelial Adherens Junctions
IL-1 Signaling
Cardiac Hypertrophy Signaling
PKCθ Signaling in T Lymphocytes
Dendritic Cell Maturation
B Cell Receptor Signaling
CD28 Signaling in T Helper Cells
iNOS Signaling
HMGB1 Signaling
Huntington's Disease Signaling
GP6 Signaling Pathway
Natural Killer Cell Signaling
IL-15 Production
Renin-Angiotensin Signaling
Acute Phase Response Signaling
MIF Regulation of Innate Immunity
p70S6K Signaling
Sumoylation Pathway
Calcium-induced T Lymphocyte Apoptosis
Neuroinflammation Signaling Pathway
NGF Signaling
JAK/Stat Signaling
P2Y Purigenic Receptor Signaling Pathway
Death Receptor Signaling
Role of NFAT in Cardiac Hypertrophy
VEGF Signaling
Type II Diabetes Mellitus Signaling
Insulin Secretion Signaling Pathway
GNRH Signaling
Dopamine-DARPP32 Feedback in cAMP Signaling
Prolactin Signaling
PPARα/RXRα Activation
PEDF Signaling
GDNF Family Ligand-Receptor Interactions
Corticotropin Releasing Hormone Signaling
BMP signaling pathway
ErbB Signaling
Antiproliferative Role of Somatostatin Receptor 2
IL-23 Signaling Pathway
Toll-like Receptor Signaling
PDGF Signaling
Apelin Adipocyte Signaling Pathway
Androgen Signaling
CDK5 Signaling
Melatonin Signaling
Th17 Activation Pathway
α-Adrenergic Signaling
Cell Cycle: G1/S Checkpoint Regulation
LPS/IL-1 Mediated Inhibition of RXR Function
B
TRRUST_Transcription_Factors_2019
NFKB1 27/303 ***
SP1 34/472 ***
RELA 26/301 ***
ERG 7/27 ***
ARNT 6/20 **
HIF1A 11/83 **
ATF4 7/35 **
ZFP36 4/8 **
USF2 8/47 **
XBP1 5/19 **
WT1 8/57 **
BRCA1 8/57 **
JUN 13/149 *
TP53 13/164 *
GATA1 7/57 *
SP3 10/113 *
HIPK2 3/9 *
0 1 2 3 4
−Log10 Adjusted p−value
Combined Score
50 100 150
MSigDB_Hallmark_2020
TNF−alpha Signaling via NF−kB 49/200 ****
Inflammatory Response 28/200 ****
Hypoxia 27/200 ****
TGF−beta Signaling 11/54 ****
IL−2/STAT5 Signaling 26/199 ****
Apoptosis 21/161 ****
mTORC1 Signaling 24/200 ****
Complement 23/200 ****
Cholesterol Homeostasis 11/74 ****
p53 Pathway 21/200 ****
0 200 400 600
Combined Score
Adjusted p-value
1e-05 2e-05 3e-05
C
Reactome_2016
Interferon alpha/beta signaling 34/68 ****
Interferon Signaling 60/196 ****
Immune System 231/1547 ****
Interferon gamma signaling 32/93 ****
Cytokine Signaling in Immune system 108/620 ****
Innate Immune System 122/807 ****
Cross−presentation of phagosomes 5/8 ***
Inflammasomes 8/17 ****
Adaptive Immune System 101/762 ****
The NLRP3 inflammasome 6/12 ***
0 200 400 600
Combined Score
Adjusted p-value
0.00005 0.00015

Figure S6

Figure S6: Pathway enrichment analysis in MIS-C (CoV-2$^+$) with or without severe myocarditis. Related to Figure 6. A. Heatmap of the canonical pathways obtained with IPA from DEGs of MIS-C (CoV-2$^+$) or MIS-C_MYO (CoV-2$^+$) compared to CTL in PBMCs, monocytes/cDCs/pDCs, T and B cells. Symbols are used in front of the pathways to represent pathways belonging to the same functional groups. Pathways with an absolute z-score ≤ 2 or adjusted p-value > 0.05 in all conditions were filtered out. Z-score > 2 means that a function is significantly increased (orange) whereas a Z-score < −2 indicates a significantly decreased function (blue). Grey dots indicate non-significant pathways (p > 0.05). **B.** Bar charts of the Transcription factors from TRRUST database (top) and the top 10 pathways from MSigDB_Hallmark_2020 (bottom) predicted in EnrichR to be modulated by the genes upregulated in monocytes/cDCs/pDCs of MIS-C_MYO (CoV-2$^+$) compared to MIS-C (CoV-2$^+$). Pathways are ranked based on combined score; Transcription factors are ranked based on adjusted p-values. **C.** Bar charts of the top 10 pathways from Reactome_2016, predicted in EnrichR to be modulated by the downregulated genes in the monocytes/cDCs/ pDCs cells of MIS-C_MYO (CoV-2$^+$) compared to MIS-C (CoV-2$^+$). Pathways are ranked based on combined score.

B & C. *(p < 0.05), **(p < 0.01), ***(p < 0.001), ****(p < 0.0001)

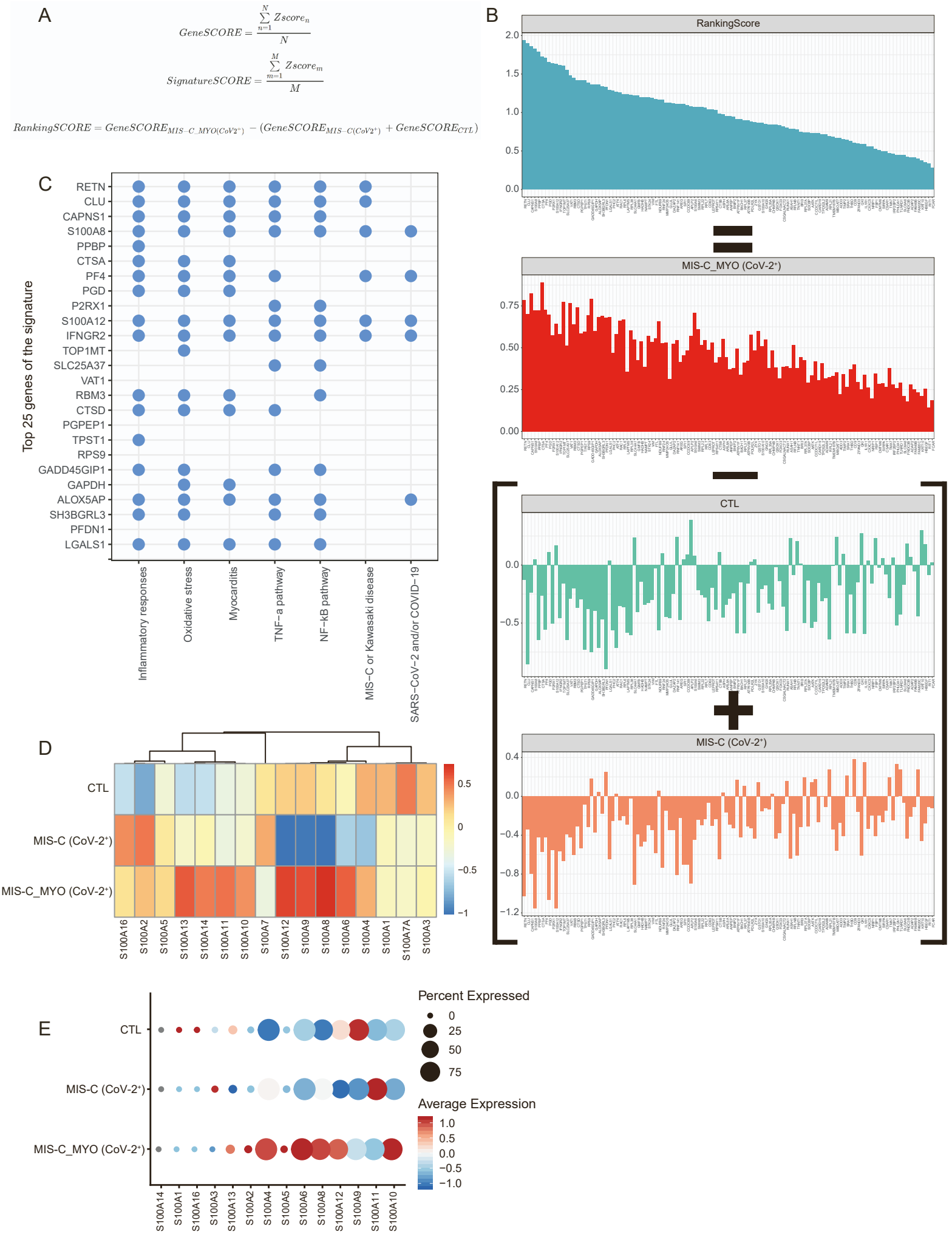
A
$GeneSCORE = \frac{\sum_{n=1}^{N} Zscore_n}{N}$
$SignatureSCORE = \frac{\sum_{m=1}^{M} Zscore_m}{M}$
$RankingSCORE = GeneSCORE_{MIS-C\_MYO(CoV2^+)} - (GeneSCORE_{MIS-C(CoV2^+)} + GeneSCORE_{CTL})$
B
RankingScore
MIS-C_MYO (CoV-2+)
CTL
MIS-C (CoV-2+)
C
Top 25 genes of the signature
RETN
CLU
CAPNS1
S100A8
PPBP
CTSA
PF4
PGD
P2RX1
S100A12
IFNGR2
TOP1MT
SLC25A37
VAT1
RBM3
CTSD
PGPEP1
TPST1
RPS9
GADD45GIP1
GAPDH
ALOX5AP
SH3BGRL3
PFDN1
LGALS1
Inflammatory responses
Oxidative stress
Myocarditis
TNF−a pathway
NF−kB pathway
MIS−C or Kawasaki disease
SARS−CoV−2 and/or COVID−19
D
CTL
MIS-C (CoV-2+)
MIS-C_MYO (CoV-2+)
S100A16
S100A2
S100A5
S100A13
S100A14
S100A11
S100A10
S100A7
S100A12
S100A9
S100A8
S100A6
S100A4
S100A1
S100A7A
S100A3
E
CTL
MIS-C (CoV-2+)
MIS-C_MYO (CoV-2+)
S100A14
S100A1
S100A16
S100A3
S100A13
S100A2
S100A4
S100A5
S100A6
S100A8
S100A12
S100A9
S100A11
S100A10
Percent Expressed
Average Expression

Figure S7

Figure S7: SignatureScore and top genes correlated with the occurrence of myocarditis in MIS-C (CoV-2$^+$). Related to Figure 7. **A.** MIS-C_MYO (CoV-2$^+$)-specific genes were scored (GeneSCORE) by adding their expression values (as z-scores) in each sample, divided by the total number of samples. Similarly, a SignatureSCORE was derived for each sample as the mean z-score of these samples. From these two scores, a RankingScore was calculated by subtracting the GeneScore of the MIS-C (CoV-2$^+$) and the CTL groups to the GeneSCORE of the MIS-C_MYO (CoV-2$^+$) group. **B.** Top: Barplot of the RankingScore of each gene. Red, green and orange barplots: GeneScore of the MIS-C_MYO (CoV-2$^+$)-specific genes in the MIS-C_MYO (CoV-2$^+$), CTL and MIS-C_MYO (CoV-2$^+$) groups respectively. Genes are ranked based on their RankingScore as explained in S7A **C.** Curated analyses based on literature regarding the known links between the top 25 genes, and inflammatory response, myocarditis, oxidative stress, TNF-α, NF-κB, MIS-C or Kawasaki disease, SARS-CoV-2 and/or COVID-19. On the Y axis, genes are positioned from top to bottom following their rank (Top = ranked 1). A blue dot means strong links between the gene and the pathway (on the X axis) were found in peer-reviewed scientific publications. **D.** Heatmap of the expression of the genes of the S100A family, extracted from Bulk-RNA-SEQ, from PBMCs. Color scale indicate the scaled GeneSCORE (mean z-score of the gene in all samples of a group), with red and blue representing the highest and lowest expressions respectively. Hierarchical clustering of the genes was computed with a Pearson's correlation as a distance. **E.** Dot plot of the calcium binding genes of the S100 family in CTL, MIS-C (CoV-2$^+$) and MIS-C_MYO (CoV-2$^+$) as measured in monocytes/cDCs/pDCS in the scRNA-seq dataset. Color scale shows scaled average expression in all cells with red and blue being the highest and lowest expression respectively. Size of dot show the percentage of cells that express the gene.