Supplementary Material

Long-term mitochondrial and metabolic impairment in lymphocytes of subjects who recovered after severe COVID-19

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Supplementary Table 1. Primary antibodies used for flow cytometry analyses

Antibody	Clone	Fluorochrome	Supplier (ref.)
CD3	UCHT1	APC/Fire™	BioLegend (300470)
CD3	HIT3a	PE	BioLegend (300308)
CD19	HIB19	APC	BioLegend (302212)
CD19	HIB19	PB	BioLegend (302232)
CD69	FN50	FITC	BioLegend (310904)

Supplementary Table 2. Primary antibodies used for Western blot analyses

Antibody	Host	Concentration	Dilution	Supplier (ref.)
DRP1	Mouse	0.25 µg/µL	1:500	BD Biosciences (611112)
FIS1	Rabbit	0.5 µg/µL	1:1000	Proteintech (10956-1-AP)
MFN2	Rabbit	1.5 µg/µL	1:500	Abcam (ab56889)
OPA1	Mouse	0.25 µg/µL	1:2000	BD Biosciences (612606)
OXPHOS	Mouse	1.5 µg/µL	1:500	Invitrogen (#45-8199)
p-DRP1 (Ser616)	Rabbit	1 µg/µL	1:500	Cell Signaling (#3455)
α-tubulin	Rabbit	1.1 µg/µL	1:5000	Tetubio (600-401-880)

Supplementary Table 3. Lipoprotein profile of serum samples from individuals who recovered of mild (CMC) and severe (CSC) COVID-19.

	CMC (n=12)	CSC (n=12)	p Value
Cholesterol			
Total Cholesterol (mg/dl)	219 [206;230]	205 [189;238]	0.419
VLDL-Cholesterol (mg/dL)	11.8 [9.38;15.2]	9.48 [6.50;12.7]	0.149
IDL-Cholesterol (mg/dL)	9.13 [8.32;11.0]	8.48 [6.56;11.0]	0.644
LDL-Cholesterol (mg/dL)	134 [129;149]	130 [116;153]	0.729
HDL-Cholesterol (mg/dL)	61.9 [57.1;66.2]	58.0 [55.0;62.5]	0.356
Triglycerides			
Total Triglycerides (mg/dL)	98.9 [85.3;123]	83.4 [73.6;98.0]	0.119
VLDL-Triglycerides (mg/dL)	57.7 [48.6;90.2]	50.9 [41.2;65.4]	0.149
IDL-Triglycerides (mg/dL)	9.79 [8.61;11.5]	9.09 [7.78;11.0]	0.525
LDL-Triglycerides (mg/dL)	14.0 [10.2;15.2]	11.5 [10.9;16.0]	0.954
HDL-Triglycerides (mg/dL)	11.2 [8.07;12.1]	9.56 [7.82;10.1]	0.248
VLDL particles			
VLDL-P (nM)	40.2 [34.5;60.9]	34.8 [29.6;45.2]	0.204
Large VLDL-P (nM)	1.02 [0.95;1.39]	0.98 [0.81;1.26]	0.225
Medium VLDL-P (nM)	5.06 [4.17;7.02]	4.06 [3.12;4.61]	0.038
Small VLDL-P (nM)	34.2 [29.6;51.1]	29.7 [25.5;39.2]	0.273
VLDL-Diameter (nm)	42.3 [42.2;42.4]	42.2 [42.1;42.3]	0.065
LDL particles			
LDL-P (nM)	1289 [1216;1482]	1285 [1182;1476]	0.862
Large LDL-P (nM)	214 [197;238]	201 [176;229]	0.686
Medium LDL-P (nM)	425 [298;469]	366 [286;512]	0.817
Small LDL-P (nM)	732 [643;783]	721 [663;742]	0.729
LDL-Diameter (nm)	21.1 [20.9;21.2]	21.1 [21.0;21.2]	0.908
HDL particles			
HDL-P (μmol/L)	29.2 [27.7;30.6]	27.5 [26.7;28.5]	0.133

	CMC (n=12)	CSC (n=12)	p Value
Large HDL-P (μM)	0.29 [0.28;0.31]	0.29 [0.27;0.33]	0.954
Medium HDL-P (μM)	10.2 [9.47;11.0]	9.95 [8.75;10.8]	0.386
Small HDL-P(μM)	18.1 [17.1;18.8]	17.2 [16.0;18.8]	0.386
HDL-Diameter (nm)	8.28 [8.23;8.30]	8.26 [8.25;8.30]	0.954
Lipoprotein composition			
VLDL-TG/VLDL-C	5.27 [4.70;6.17]	5.91 [5.00;6.35]	0.356
IDL-TG/IDL-C	1.08 [0.96;1.20]	1.04 [1.00;1.24]	0.773
LDL-TG/LDL-C	0.10 [0.09;0.11]	0.10 [0.09;0.11]	0.773
HDL-TG/HDL-C	0.16 [0.14;0.21]	0.16 [0.14;0.17]	0.603

VLDL: Very Low-Density Lipoprotein; IDL: Intermediate-Density Lipoprotein; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein; P: Particles (referring to lipoprotein particles). Statistical significance was evaluated by using the Kruskal-Wallis test.

Supplementary Table 4. Glycoprotein profile of serum samples from individuals who recovered of mild (CMC) and severe (CSC) COVID-19.

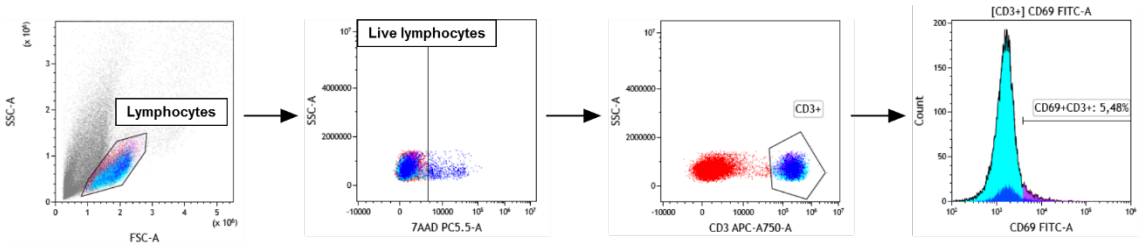
	CMC (n=12)	CSC (n=12)	p Value
Glycoprotein profile			
Glyc-B (μmol/L)	309 [296;345]	334 [305;354]	0.248
Glyc-A (μmol/L)	641 [572;694]	657 [599;707]	0.729
H/W Glyc-B	3.89 [3.72;4.36]	4.20 [3.83;4.45]	0.386
H/W Glyc-A	15.6 [13.6;16.3]	16.2 [14.3;17.2]	0.356

Glyc: Glycoprotein. Statistical significance was evaluated by using the Kruskal-Wallis test.

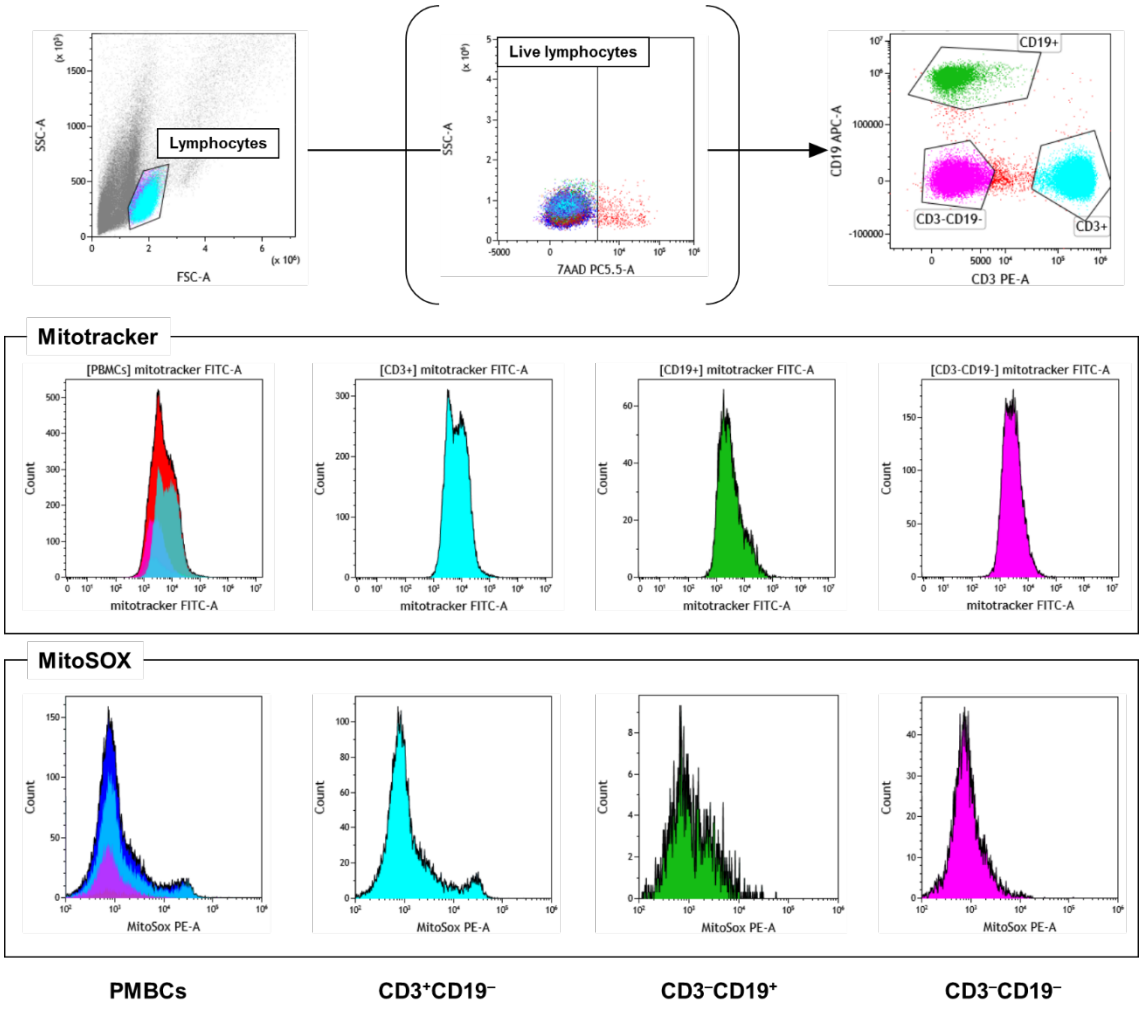
Supplementary Table 5. Lipid profile of serum samples from individuals who recovered of mild (CMC) and severe (CSC) COVID-19.

	CMC (n=12)	CSC (n=12)	p Value
Lipid profile			
Esterified cholesterol (mM)	2.57 [2.34;3.05]	2.60 [2.12;2.79]	0.644
Free cholesterol (mM)	2.45 [2.16;2.64]	2.37 [2.19;2.46]	0.326
Triglycerides (mM)	1.03 [0.72;1.37]	0.72 [0.56;0.91]	0.149
Glycerophospholipids (mM)	3.72 [3.36;3.91]	3.43 [3.12;3.74]	0.299
Phosphatidylcholine (mM)	3.10 [2.83;3.26]	2.90 [2.62;3.20]	0.453
Sphingomyelin (mM)	0.93 [0.90;0.99]	0.93 [0.90;1.02]	1.000
Lysophosphatidylcholine (mM)	0.86 [0.78;0.92]	0.76 [0.70;0.84]	0.083
PUFA (mM)	15.2 [13.0;16.6]	15.1 [11.4;16.3]	0.729
Linoleic (mM)	4.32 [3.98;4.81]	4.31 [3.99;4.61]	0.773
SFA (mM)	10.0 [9.73;10.6]	9.58 [9.14;11.3]	0.644
w6 & w7 fatty acids (mM)	6.10 [5.24;7.45]	6.10 [5.62;6.47]	0.954
w9 fatty acids (mM)	3.10 [2.38;4.54]	3.03 [1.88;3.41]	0.453
w3 fatty acids (mM)	0.27 [0.21;0.30]	0.27 [0.25;0.32]	0.386
DHA (mM)	0.15 [0.14;0.16]	0.14 [0.11;0.18]	0.326
AA + EPA (mM)	1.83 [1.61;2.04]	1.62 [1.35;1.88]	0.386

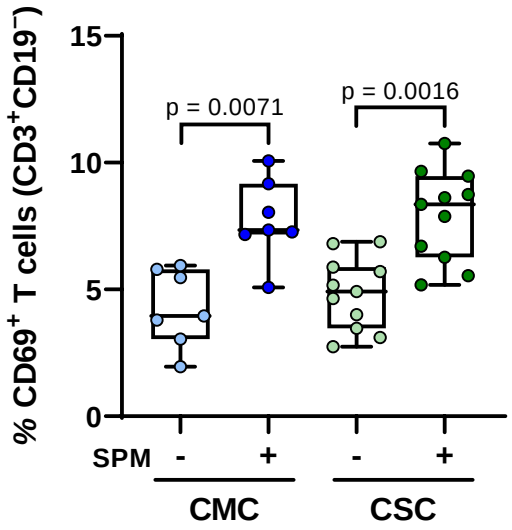
PUFA: Polyunsaturated Fatty Acid; SFA: Saturated Fatty Acid; w3: Omega-3 Fatty Acid; w6: Omega-6 Fatty Acid; w7: Omega-7 Fatty Acid; w9: Omega-9 Fatty Acid; DHA: Docosahexaenoic Acid; AA: Arachidonic Acid; EPA: Eicosapentaenoic Acid. Statistical significance was evaluated by using the Kruskal-Wallis test.



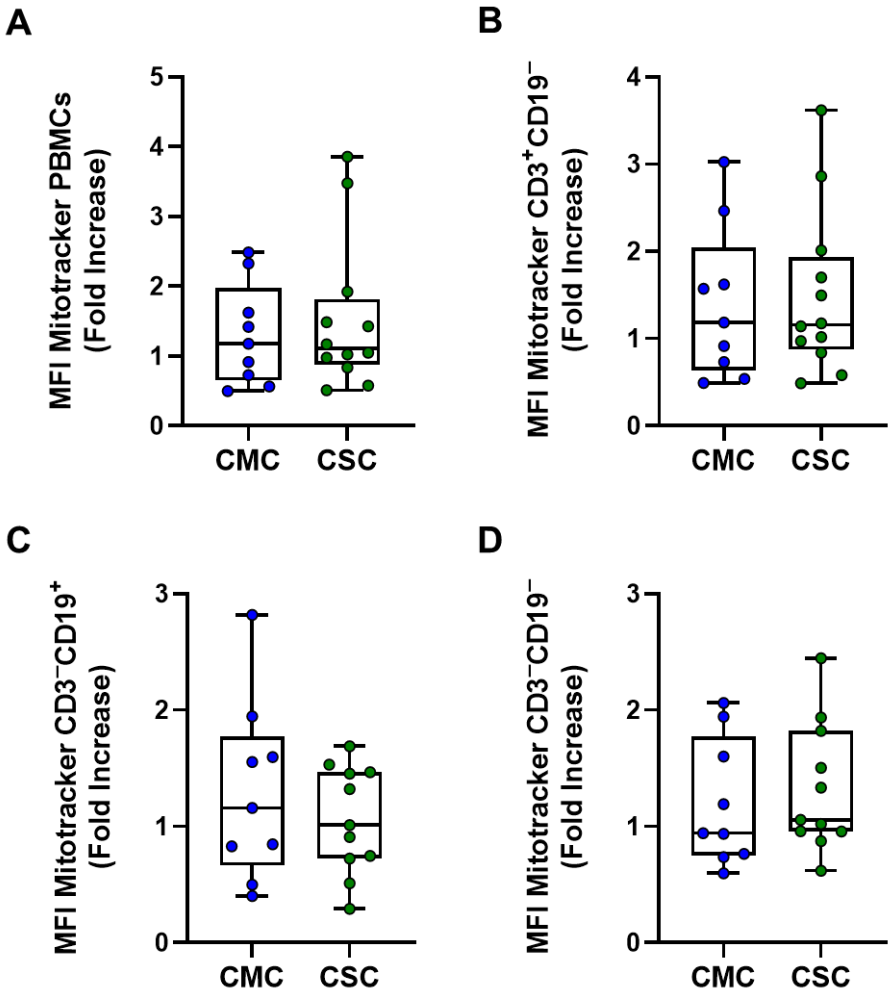
Supplementary Figure S1. Gating strategy of the CD69 flow cytometry. Gating strategy performed with $CD3^{+}CD19^{-}$ lymphocytes from CMC and CSC individuals to analyse the immune cell activation through CD69 surface expression by flow cytometry.



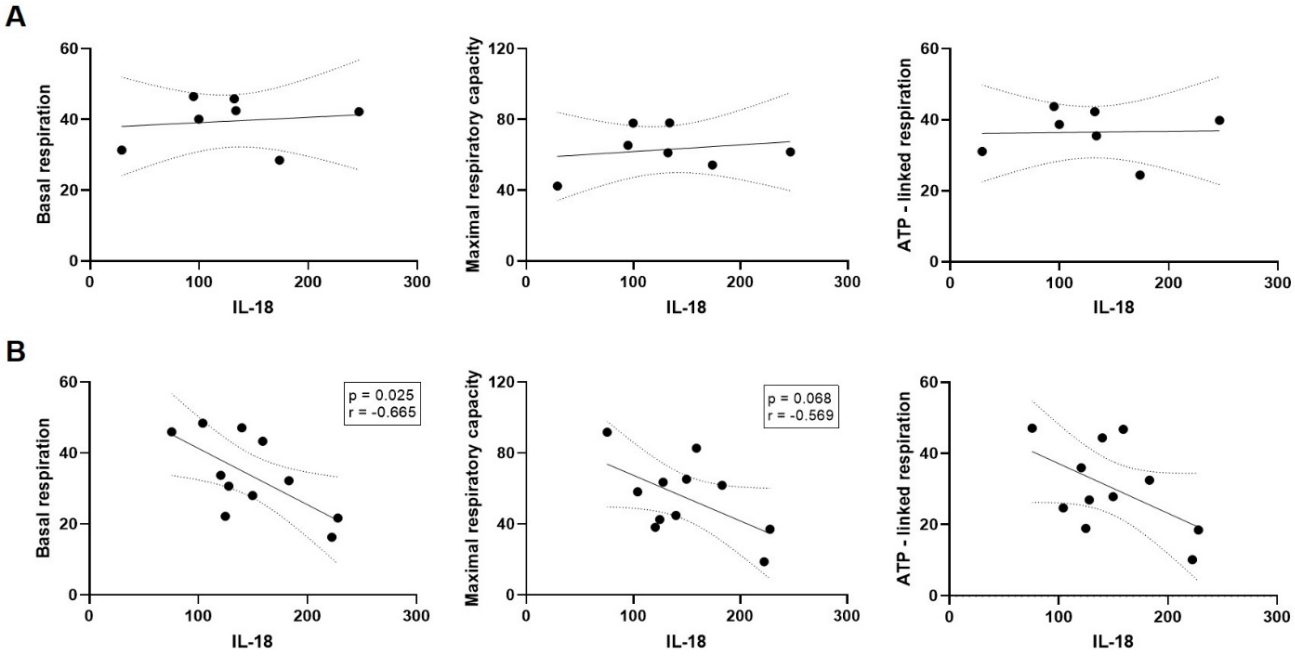
Supplementary Figure S2. Gating strategy of Mitotracker™ and MitoSOX™ flow cytometry. Gating strategies of flow cytometry analyses to assess the differences of mitochondrial mass and superoxide content between PBMCs and lymphocyte subpopulations from CMC and CSC samples. As opposed to Mitotracker™ cytometry, the addition of 7-AAD is not performed in the case of MitoSOX™ assay.



Supplementary Figure S3. Immune cell activation upon spike peptide mix (SPM) stimulation in samples from cases who recovered of mild/ severe COVID-19. CD69 surface expression of $CD3^{+}CD19^{-}$ lymphocytes (n= 7-11) stratified according to the group severity: mild (CMC) and severe (CSC) cases. Lines represent median values, boxes the 25 and 75 percentiles, and whiskers mark maximum and minimum values. Statistical significance was assessed by two-tailed unpaired Student's and Paired t-tests.

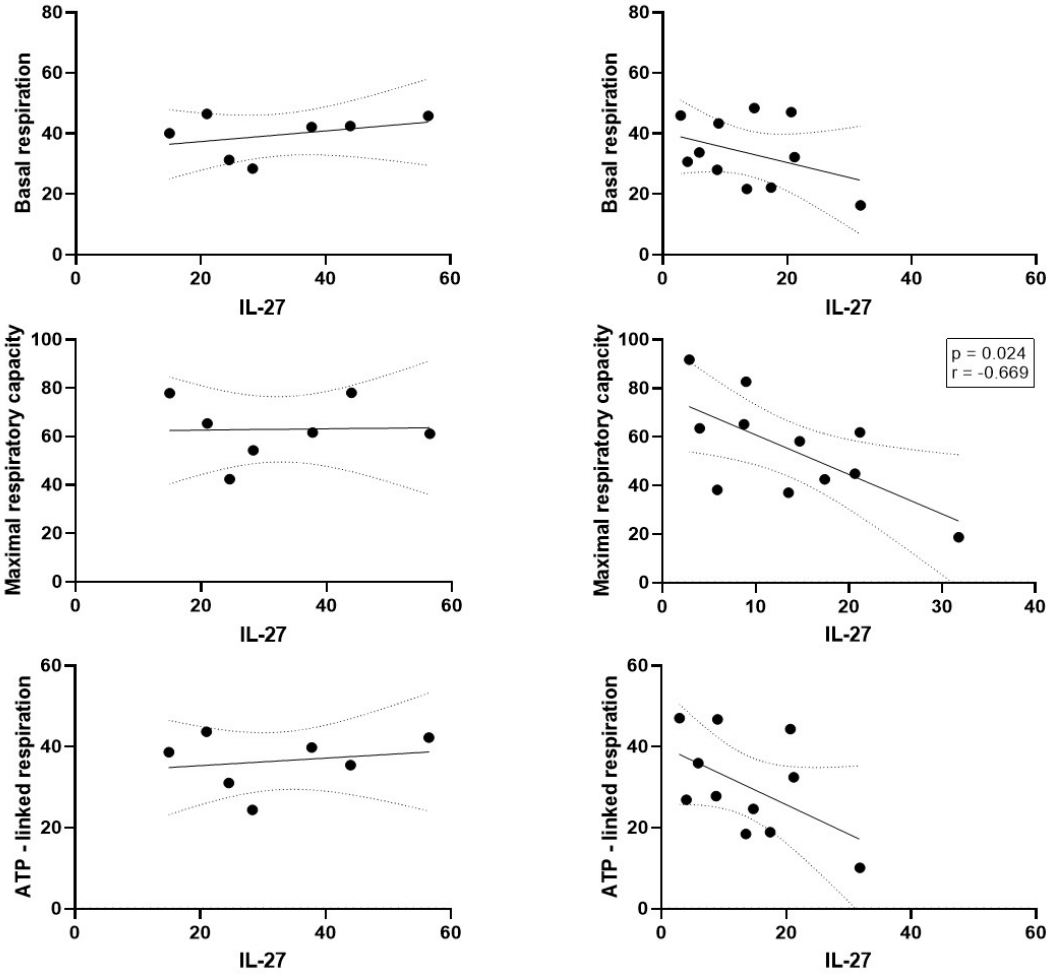


Supplementary Figure S4. Mitochondrial mass in the lymphocyte subpopulations of individuals recovered of mild COVID-19 (CMC) and severe COVID-19 (CSC). Diagrams display the fold increase in mitochondrial mass before and after stimulation with spike peptide mix (SPM) measured by flow cytometry (MitoTrackerTM Green FM probe). The MFI ratio in the presence/absence of SPM was calculated for: (A) PBMCs, and (B) $CD3^{+}CD19^{-}$, (C) $CD3^{-}CD19^{+}$, (D) $CD3^{-}CD19^{-}$ lymphocyte subpopulations from CMC (n= 9) and CSC (n= 10-11) groups. Results are expressed using box and whiskers diagrams, where lines represent median values, boxes the 25 and 75 percentiles, and whiskers mark maximum and minimum values. PBMCs from the CSC group were evaluated by unpaired non-parametric Mann-Whitney U test, and the statistical significance of the resting populations was assessed by two-tailed unpaired Student's t-test.

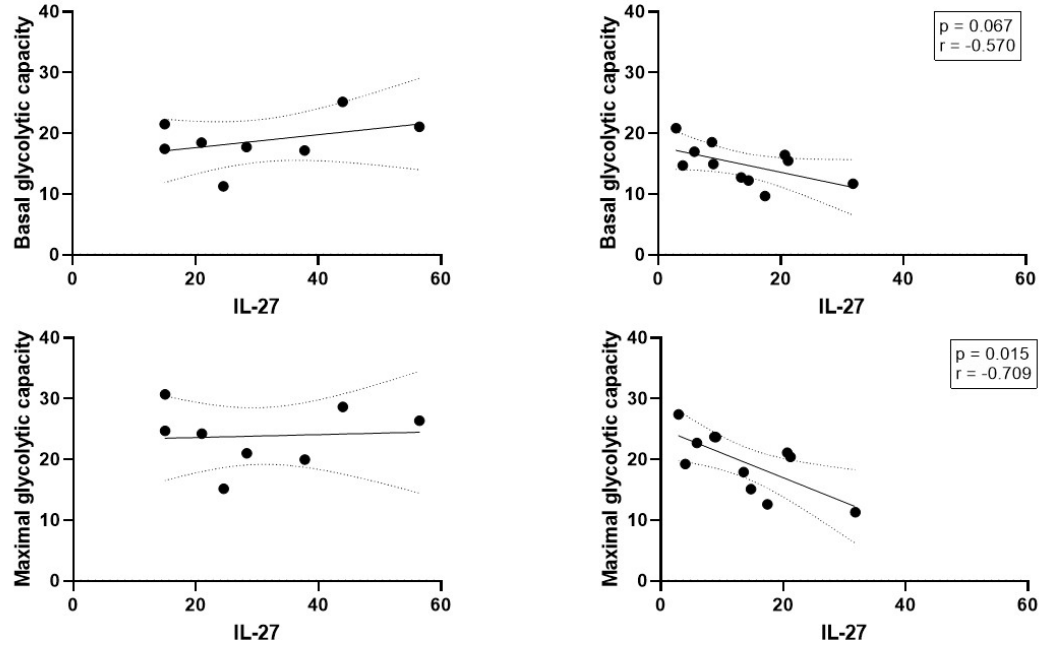


Supplementary Figure S5. Correlation between IL-18 and mitochondrial parameters in subjects who recovered from mild (CMC) and severe (CSC) COVID-19. Scatter plots showing the correlation between IL-18 and the mitochondrial OCR parameters in the CMC **(A)** and CSC **(B)** subjects. Linear correlation was assessed by using Spearman's rank correlation coefficient test.

A

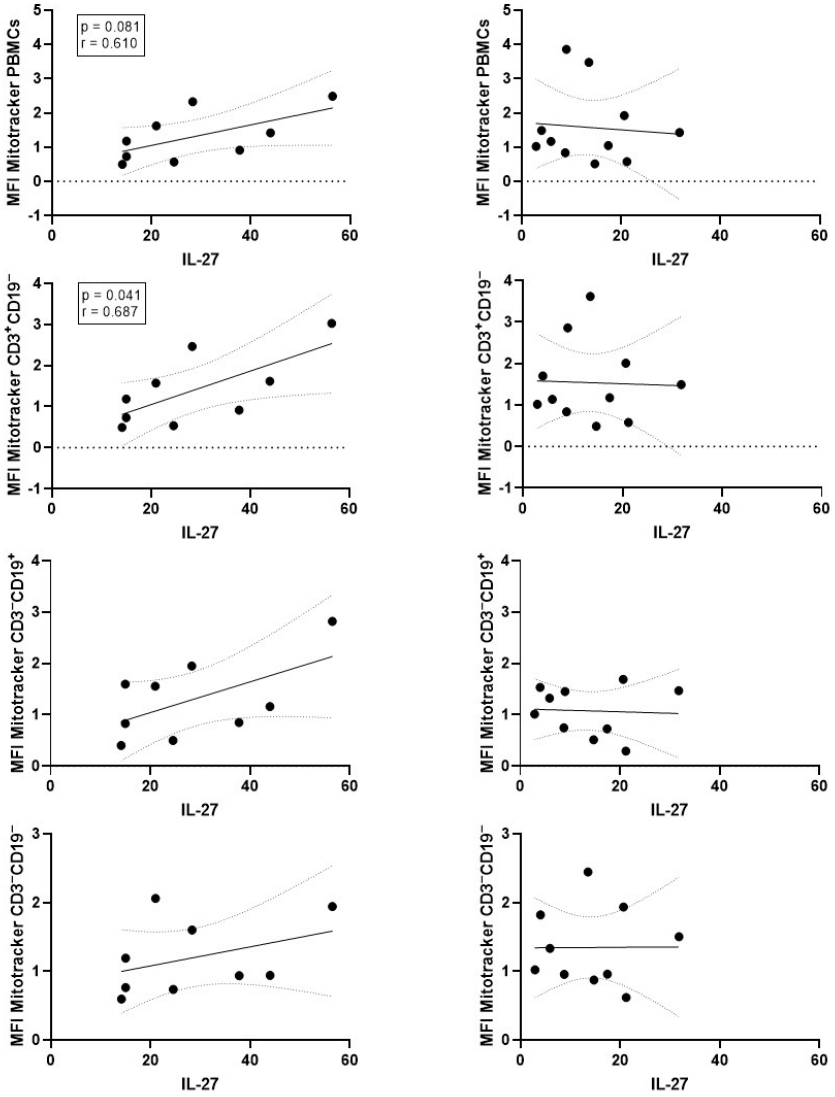

Basal respiration
IL-27
Maximal respiratory capacity
IL-27
p = 0.024
r = -0.669
ATP - linked respiration
IL-27

B

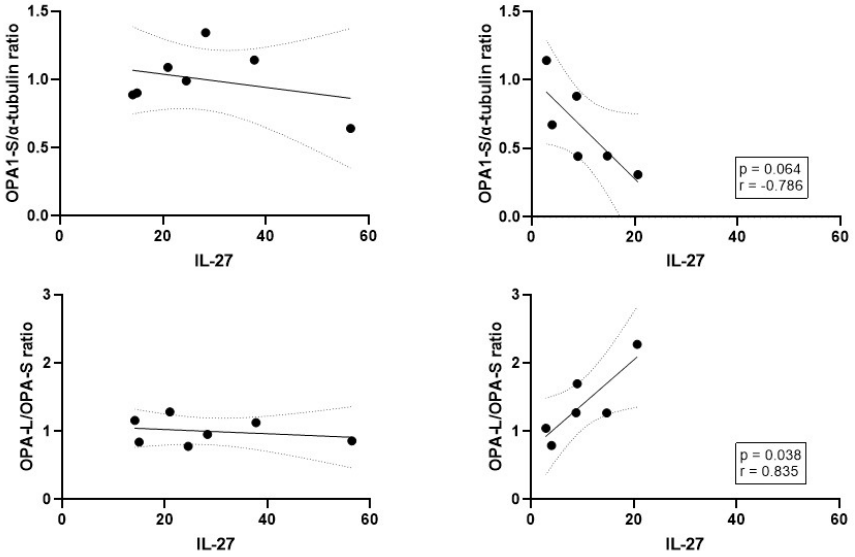

Basal glycolytic capacity
IL-27
p = 0.067
r = -0.570
Maximal glycolytic capacity
IL-27
p = 0.015
r = -0.709

Supplementary Figure S6. Correlation between IL-27 and mitochondrial parameters in subjects who recovered from mild (CMC) and severe (CSC) COVID-19. Scatter plots showing the correlation between IL-27 and (A) the mitochondrial OCR parameters and (B) the ECAR profile in the CMC (Left) and CSC (Right) individuals. Linear correlation was assessed by using Spearman's rank correlation coefficient test.

A


p = 0.081
r = 0.610
MFI Mitotracker PBMCs
IL-27
p = 0.041
r = 0.687
MFI Mitotracker CD3+CD19−
MFI Mitotracker CD3−CD19+
MFI Mitotracker CD3−CD19−

B


OPA1-S/α-tubulin ratio
p = 0.064
r = -0.786
OPA-L/OPA-S ratio
p = 0.038
r = 0.835
IL-27

Supplementary Figure S7. Correlation between IL-27 and mitochondrial mass and fusion in subjects who recovered from mild (CMC) and severe (CSC) COVID-19. Scatter plots showing the correlation between IL-27 and **(A)** mitochondrial mass measured with MitoTrackerTM Green FM probe, and **(B)** protein expression of the fusion protein OPA1-S and the OPA1-L/OPA1-S ratio quantification. Both evaluations were shown in the CMC **(Left)** and CSC **(Right)** individuals. Linear correlation was assessed by using Spearman's rank correlation coefficient test.