

Supplemental Information

Upregulated Proteasome Subunits in COVID-19 Patients: A Link with Hypoxemia, Lymphopenia and Inflammation

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Table S1: Primers used for qPCR

PSMA4	Forward primer	CTTGTGAGCAGTTGGTTACAGCG
	Reverse primer	AGCCATAGTGCTTATCCCAGCC
PSMA 5	Forward primer	TGCCATGTCTCGTCCCTTG
	Reverse primer	TTTGTTCATTCAGCTTCTCCT
PSMB1	Forward primer	TGCAATGCTGTCTACAATCC
	Reverse primer	TCTCTGGTAAGACCCTACTG
PSMB2	Forward primer	GACACTGTACAGTTTGCAGA
	Reverse primer	GGAGGTTACATGATATGGG
PSMB3	Forward primer	GAGCATGGTGGCCAACCTCT
	Reverse primer	GCAGGTGCCACTGACCACAA
PSMB4	Forward primer	CGACTACGCTGATTTCCAG
	Reverse primer	GGTGTTCACAAAAGGGTTCA
PSMB5	Forward primer	TGTAGCAGCTGCCTCCAAAC
	Reverse primer	CCCTGAAATCCGGTTCCCTT
PSMB6	Forward primer	AGACTGGGAAAGCCGAGAAG
	Reverse primer	GCAGAAAATGCGGTCTGAA
PSMB7	Forward primer	AAACTGGCACGACCATCGCT
	Reverse primer	CAGCTGTCCCAGCACCACAA
PSMB8	Forward primer	ACCCCGCGTGACACTACT
	Reverse primer	GGGACTGGAAGAATTCTGTGG
PSMB9	Forward primer	CGTTGTGATGGGTTCTGATTCC
	Reverse primer	GACAGCTTGTCAAACACTCGGTT
PSMB10	Forward primer	GGTTCAGCCGAACATGA
	Reverse primer	GCCCAGGTCACCCAAGAT
PSMD11	Forward primer	TAACTTCTGCTCGAACCACA
	Reverse primer	GTCGATGGAGTCATAACCCT
PSMD14	Forward primer	GTGGATATCAAACTCAGCA
	Reverse primer	TGGTTCATGTCCTAAGACCA
PSME1	Forward primer	GCGCTTGAAGCCTGAGATCA
	Reverse primer	CCTTCTCCTGGACAGCCACT
PSME2	Forward primer	CTTTCCAGGAGGCTGAGGAAT
	Reverse primer	GGAGGGAAGTCAAGTCAGCC
PSME3	Forward primer	CCAGACCTAAGCTGCCTTCT
	Reverse primer	GATAGCAGCCTCTACTGGCA
HIF-1 α	Forward primer	TTCCAGTTACGTTCCCTCGATCA
	Reverse primer	TTTGAGGACTTGGCCTTTCA
NF- κ B	Forward primer	GGTGCGGCTCATGTTTACAG
	Reverse primer	GATGGCGTCTGATACCACGG
NLRP3	Forward primer	TGCCCCGTCTGGGTGAGA
	Reverse primer	CCGGTGCTCCTTGATGAGA
ASC	Forward primer	AACCCAAGCAAGATGCGGAAG
	Reverse primer	TTAGGGCCTGGAGGAGCAAG
CAS-1	Forward primer	GGAAACAAAAGTCGGCAGAG
	Reverse primer	ACGCTGTACCCCAGATTTTG
GSDMD	Forward primer	GTGTGTCAACCTGTCTATCAAGG
	Reverse primer	CATGGCATCGTAGAAGTGAAG
STAT3	Forward primer	CAGCAGCTTGACACACGGTA
	Reverse primer	AAACACCAAAGTGGCATGTGA
c-FOS	Forward primer	GTGGGAATGAAGTTGGCACT
	Reverse primer	CTACCACTACCCGCAGACT
c-JUN	Forward primer	GTCTTCTTCTTTCGCTGG
	Reverse primer	GGAGACAAGTGGCAGAGTCC
18S	Forward primer	CGGCGACGCCATTTCGAAC
	Reverse primer	GAATCGAACCTGATTCCTCCGTC

Table S2. Clinical parameters of COVID-19 patients grouped by proteasome subunits expression.

Gene	Lymphocytes (x10 ³ cells/mm ³)			PaO ₂ /FiO ₂ ratio			C-reactive protein (mg/L)			Ferritin (mg/mL)		
	Low	High	P	Low	High	P	Low	High	P	Low	High	P
PSMB3	0.885±0.345	0.889±0.430	0.987	309.0±91.03	232.0±77.67	0.127	53.08±38.75	38.74±27.97	0.462	699.3±691.8	882±689.3	0.633
PSMB5	0.997±0.357	0.742±0.339	0.213	290.6±98.11	257.1±88.33	0.503	47.99±32.83	43.08±32.03	0.775	867.4±620.8	699.0±713.6	0.633
PSMB6	0.873±0.317	0.976±0.471	0.623	302.0±85.14	244.4±88.27	0.209	52.13±35.46	38.77±26.52	0.423	804.9±690.5	707.1±639.2	0.777
PSMB7	0.873±0.317	0.976±0.471	0.623	302.0±85.14	244.4±88.27	0.209	52.13±35.46	38.77±26.52	0.423	804.9±690.5	707.1±639.2	0.777
PSMD11	0.884±0.295	0.979±0.509	0.675	295.8±80.78	243.5±94.32	0.254	57.05±35.66	32.18±18.89	0.110	758.5±652.6	741.3±674.5	0.959
PSMD14	0.884±0.295	0.921±0.498	0.858	295.8±80.78	240.3±88.77	0.197	57.05±35.66	42.19±34.85	0.400	758.5±652.6	667.0±669.1	0.780

Clinical parameters (Lymphocyte count, PAFI ratio, C-reactive protein concentration and ferritin concentration) comparison in COVID-19 patients grouped by high or low expression of proteasome subunits (PSMB3/5/6/7 and PSMD11/14). Data are mean±standard deviation. P-values are shown. Differences were considered significant p<0.05.

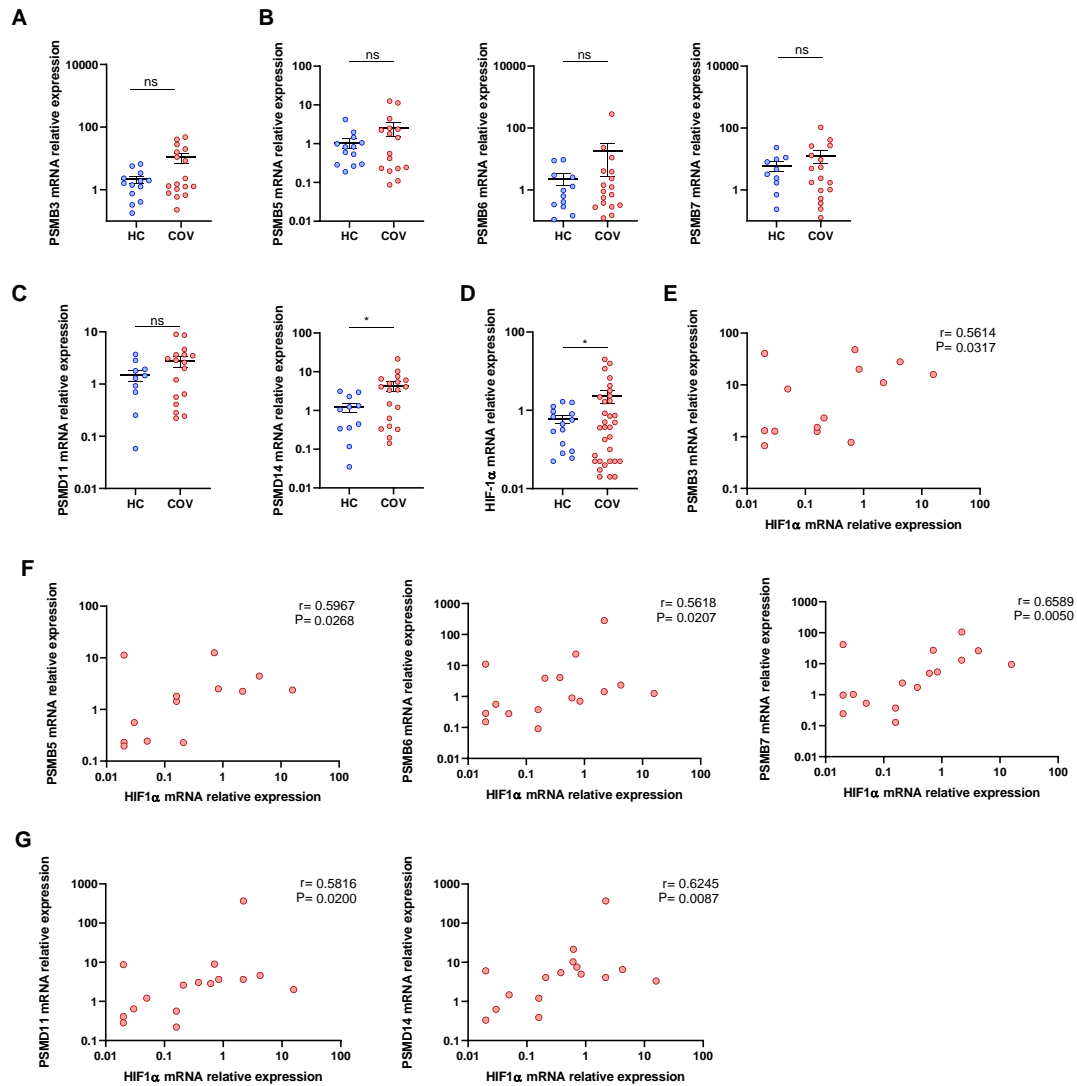


Figure S1. Proteasome and HIF-1 α upregulation in COVID-19 patients. (A-C) mRNA expression in PBMCs from healthy controls (HC) compared to COVID-19 patients (COV) of (A) 20s proteasome beta ring non-catalytic subunits: PSMB3 (HC n=13; COV n=17); (B) 20S proteasome beta ring catalytic subunits: PSMB5 (HC n=13; COV n=16), PSMB6 (HC n=12; COV n=19), PSMB7 (HC n=11; COV n=19); (C) 19S proteasome subunits: PSMD11 (HC n=10; COV n=17), PSMD14 (HC n=11; COV n=19); and (D) HIF-1 α (HC n=15; COV n=32). Mean differences were analysed using unpaired Student's t-test analysis with Welch's correction. Error bars: standard error of the mean. *:p<0.05. (E-G) Correlation of hypoxia-inducible factor 1-alpha (HIF-1 α) mRNA expression and mRNA expression of (E) PSMB3 (n=15), (F) PSMB5 (n=14), PSMB6 (n=17), PSMB7 (n=17); and (G) PSMD11(n=16), PSMD14(n=17). Correlations were analysed using Spearman's analysis. P-value and Spearman's correlation coefficient (ρ) are shown.

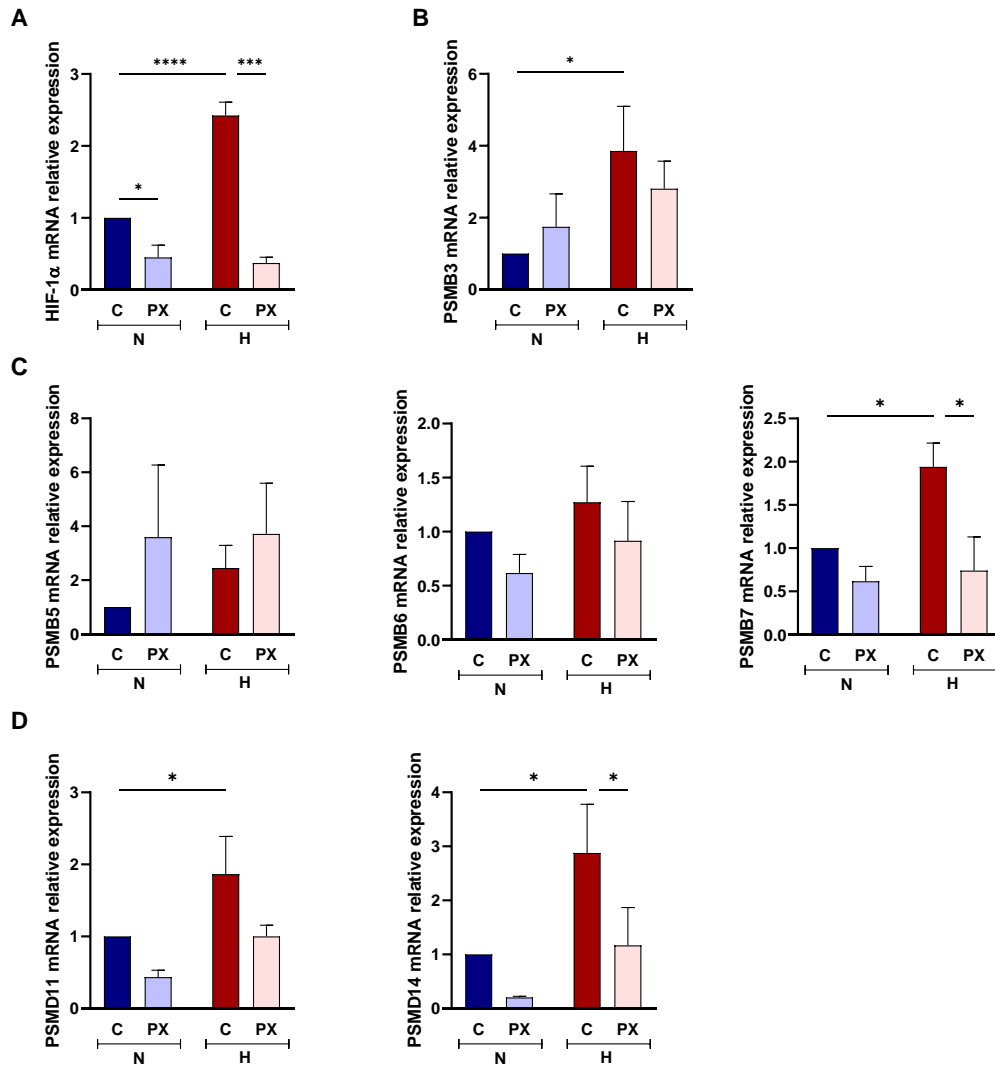


Figure S2. *In vitro* model of the effect of hypoxia on proteasome subunits expression. mRNA expression from healthy volunteers PBMCs (n=5) cultured in normoxia (N) or hypoxia (H) and treated with PX478 (PX) or untreated (C) of (A) HIF-1 α ; (B) 20S proteasome beta ring non-catalytic subunits: PSMB3; (C) 20S proteasome beta ring catalytic subunits: PSMB5, PSMB6, PSMB7; and (D) 19S proteasome subunits: PSMD11, PSMD14. Mean differences were analysed using mixed-effects analysis with Bonferroni's multiple comparison test. Error bars: standard error of the mean. *:p<0.05, ***:p<0.001, ****:P<0.0001.

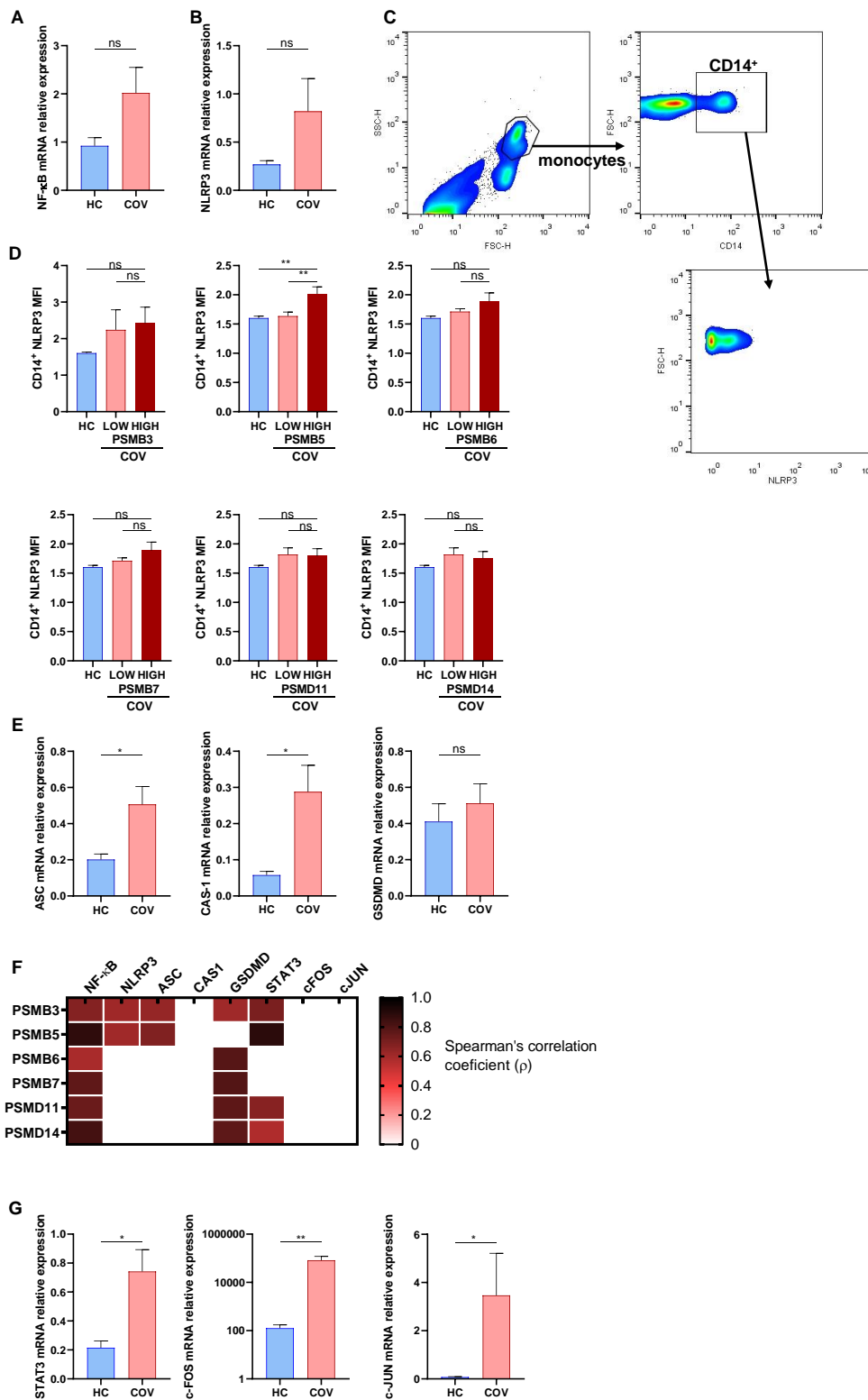


Figure S3. Elevated mRNA expression of genes related to inflammation or STAT3 pathway in COVID-19 patients. (A) NF- κ B mRNA expression in healthy control (HC, n=12) and COVID-19 patients (COV, n=40), (B) NLRP3 mRNA expression in HC (n=18) and COV (n=44). (C) Monocyte gating strategy: representative plots demonstrating the used gating strategy to compare the NLRP3 expression. (D) NLRP3 expression in monocytes analysed by flow cytometry in monocytes from healthy controls (HC),

and COVID-19 patients (COV) presenting LOW or HIGH expression of proteasome subunits. Mean differences were analysed using one-way ANOVA analysis with Tuckey's multiple comparisons test. Error bars: standard error of the mean. *: $p < 0.05$, **: $p < 0.01$. **(E)** Comparison of NLRP3 inflammasome components mRNA expression in healthy control (HC) and COVID-19 patients (COV): ASC (HC, $n=20$; COV, $n=44$), CAS-1 (HC, $n=20$; COV, $n=44$) and GSDMD (HC, $n=12$; COV, $n=43$). **(F)** Heatmap represents Spearman's correlation coefficient (ρ) between proteasome subunits mRNA expression and genes related to inflammation or STAT-3 pathway mRNA expression. White gaps represent non-significant correlations, on the contrary the rest of correlations are significant ($p < 0.05$). **(G)** STAT3 mRNA expression from PBMCs of healthy control (HC, $n=14$) and COVID-19 patients (COV, $n=39$), c-FOS mRNA expression in HC ($n=14$) and COV ($n=39$), c-JUN mRNA expression in HC ($n=14$) and COV ($n=37$). Mean differences were analysed using unpaired Student t-test analysis with Welch correction. Error bars: SEM. *: $p < 0.05$, **: $p < 0.01$.

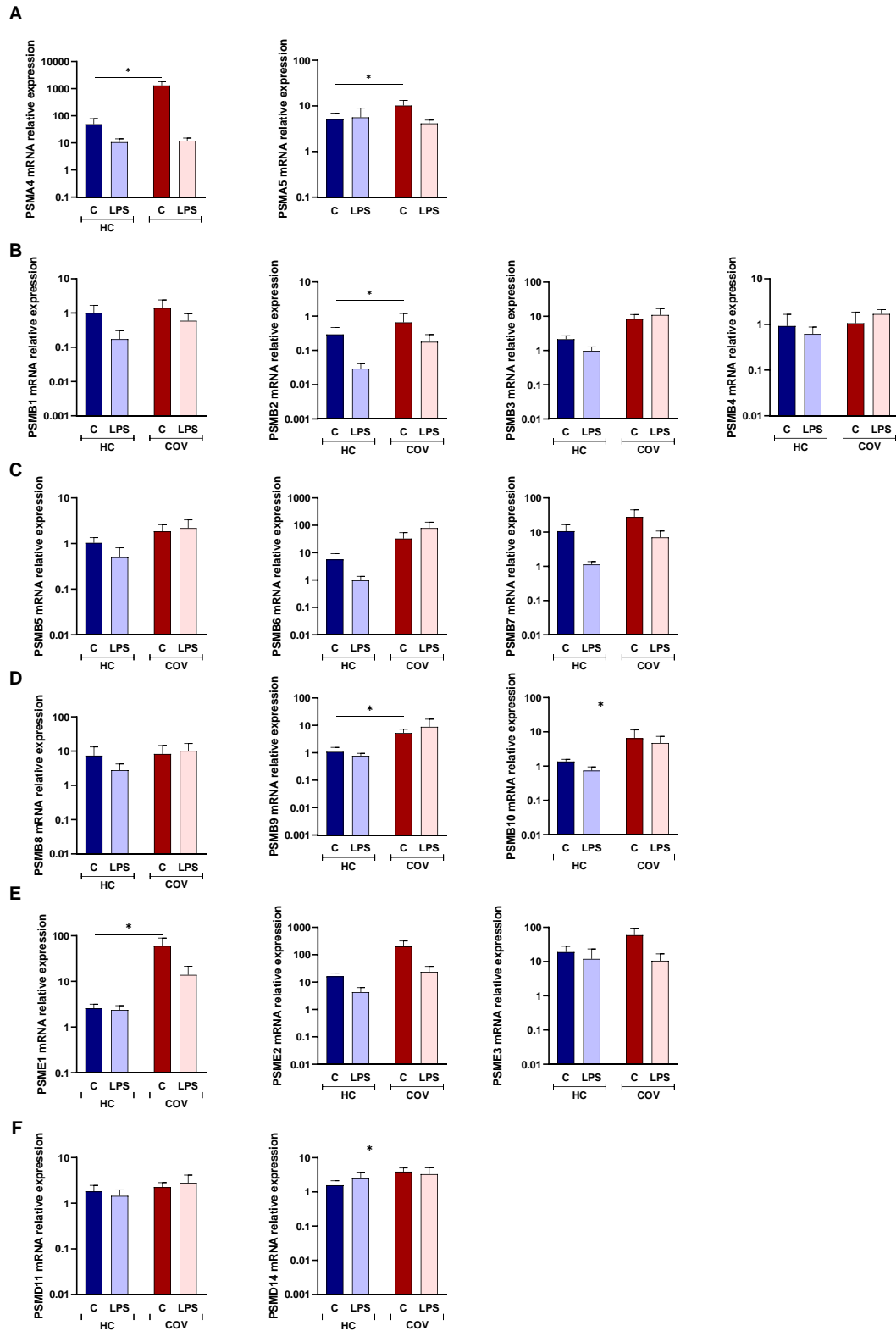


Figure S4. *In vitro* model of the effect of inflammatory response is proteasome subunits expression. Proteasome subunits mRNA expression in PBMCs from healthy controls (HC) (n=12) or COVID-19 patients (COV) (n=24) cultured for 16h under control conditions (C) or treated with 10ng/ml of LPS (LPS). (A) 20S proteasome alpha ring subunits: PSMA4, PSMA5; (B) 20S proteasome beta ring non-catalytic subunits: PSMB1, PSMB2, PSMB3, PSMB4; (C) 20S proteasome beta ring catalytic subunits: PSMB5, PSMB6, PSMB7; (D) 20S immunoproteasome beta ring catalytic subunits: PSMB8, PSMB9, PSMB10;

(E) 11S proteasome subunits: PSME1, PSME2, PSME3; and (F) 19S proteasome subunits: PSMD11, PSMD14. Mean differences were analysed using mixed-effects analysis with Bonferroni's multiple comparison test. Error bars: standard error of the mean. *:p<0.05.