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**TABLE S1 Primer pairs sequences used in the qRT Real time PCR analysis**

GENE		FORWARD PRIMER (5'→3')	REVERSE PRIMER (5'→3')
*++HERV W ENV	AF331500	GTATGTCTGATGGGGGTGGAG	CTAGTCCTTTGTAGGGGCTAGAG
#GUSB	NM_000181	CAGTTCCTCCAGCTTCAATG	ACCCAGCCGACAAAATGC
#IL-6	NM_000600.3	TGCAATAACCACCCCTGACC	ATTTGCCGAAGAGCCCTCAG
#IL-10	NM_000572.2	ACATCAAGGCGCATGTGAAC	CACGGCCTTGCTCTTGTGTTT
°IL-17	U32659.1	CGGACTGTGATGGTCAACCTGA	GCACTTTGCCTCCAGATCACA
§IL-17RA	NM_001289905.1	TCATCGTCTGCATGACCTGGAG	GGCTGAGTAGATGATCCAGACC
#TNF $\alpha$	NM_000594.3	CCCGAGTGACAAGCCTGTAG	TGAGGTACAGGCCCTCTGAT
#INF $\gamma$	NM_000619.2	TCAGCTCTGCATCGTTTTGG	GTTCCATTATCCGCTACATCTGAA
§MCP1	NM_002982.4	AGAATCACCAGCAGCAAGTGTC	TCCTGAACCCACTTCTGCTGG
§CXCR1	NM_000634.3	TCCTTTTCCGCCAGGCTTACCA	GGCACGATGAAGCCAAAGGTGT
§CXCL6	NM_002993.4	GGGAAGCAAGTTTGTCTGGACC	AAACTGCTCCGCTGAAGACTGG

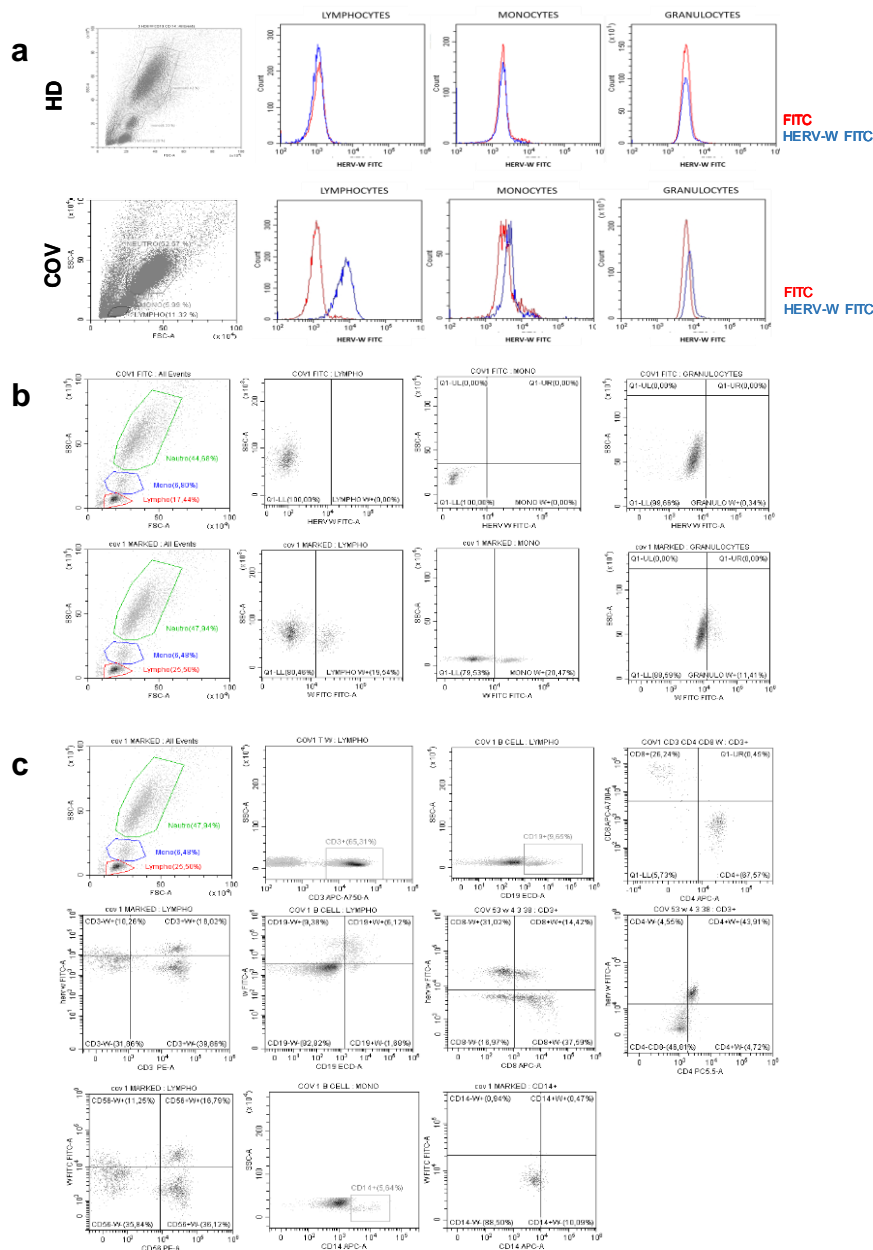
\*Levet S, Medina J, Joanou J, Demolder A, Queruel N, Réant K, Normand M, Seffals M, Dimier J, Germi R, Piofczyk T, Portoukalian J, Touraine JL, Perron H. An ancestral retroviral protein identified as a therapeutic target in type-1 diabetes. *JCI Insight*. 2017;2:e94387. doi: 10.1172/jci.insight.94387.

++Charvet et al. Human Endogenous Retrovirus Type W Envelope from Multiple Sclerosis Demyelinating Lesions Shows Unique Solubility and Antigenic Characteristics *Virologica Sinica*, 2021. DOI 10.1007/s12250-021-00372-0

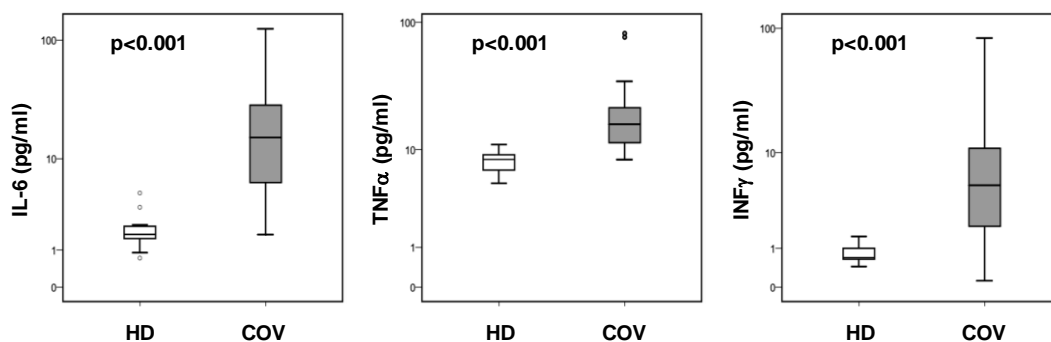
#Balestrieri E, Cipriani C, Matteucci C, et al. Children With Autism Spectrum Disorder and Their Mothers Share Abnormal Expression of Selected Endogenous Retroviruses Families and Cytokines. *Front Immunol*. 2019;10:2244. Published 2019 Sep 26. doi:10.3389/fimmu.2019.02244

° Deng J, Fan C, Gao X, Zeng Q, Guo R, Wei Y, Chen Z, Chen Y, Gong D, Feng J, Xia Y, Xiang S, Gong S, Yuan L, Shen W, Shen W, Lin L, Jiang T, He D, Lu L, Chen X, Yu D. Corrigendum: Signal Transducer and Activator of Transcription 3 Hyperactivation Associates With Follicular Helper T Cell Differentiation and Disease Activity in Rheumatoid Arthritis. *Front Immunol*. 2019 Aug 22;10:2008. doi: 10.3389/fimmu.2019.02008.

§ Matteucci C, Minutolo A, Balestrieri E, Petrone V, Fanelli M<sup>1</sup>, Malagnino V, Ianetta M, Giovinazzo A, Miele MT, di Francesco P, Mastino A, Sinibaldi Vallebona P, Bernardini S, Rogliani P, Sarmati L, Andreoni M, Grelli and Garaci. Thymosin alpha 1 mitigates cytokine storm in blood cells from COVID-19 patients. *Open Forum Infectious Disease* 2020. DOI: 10.1093/ofid/ofaa588



**Figure S1. Gating strategy for flow cytometry analysis.** (a) FSC vs SSC dot plots of a representative healthy donor (HD, upper panels) and a COVID-19 patient (COV, lower panels), analysis of the mean intensity of HERV W ENV fluorescence in lymphocytes, monocytes, and granulocytes. HD were used as controls for the expression of HERV-W ENV (b), Analysis of HERV-W ENV-positive cells (%) in a representative COV patient: cells stained only with FITC-labelled secondary antibody (upper panels), cells marked with anti HERV-W ENV antibody and FITC-labelled secondary antibody (lower panels). (c) Analysis of positive HERV-W ENV cells (%) in T cells (CD3+/CD4+, CD3+/CD8+), B cells (CD19+), Natural Killer cells (CD3-CD56+), and monocytes (CD14+). The analysis was done by acquiring at least 450000 events in the Leukocyte gate.



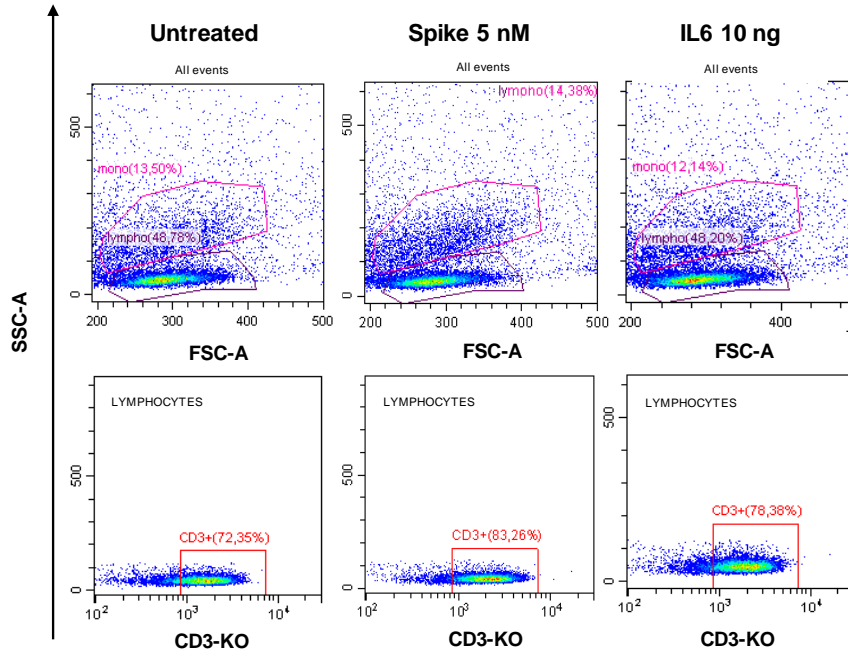
**Figure S2. Cytokine levels (protein) in plasma samples of COVID-19 patients and Healthy Donors.**  
 The concentration of cytokines (pg/ml) including IL-6, TNF $\alpha$ , and INF $\gamma$  has been evaluated in plasma samples of COVID-19 patients and healthy donors by ELISA analysis, and results are represented as box plots. Non-parametric Mann–Whitney test was used to compare groups and statistically significant values were considered when  $p < 0.050$ .

**Table S2. Median values, interquartile range, and Mann–Whitney U test of cytokine expression (proteins) in plasma samples of COVID-19 patients and HDs.**

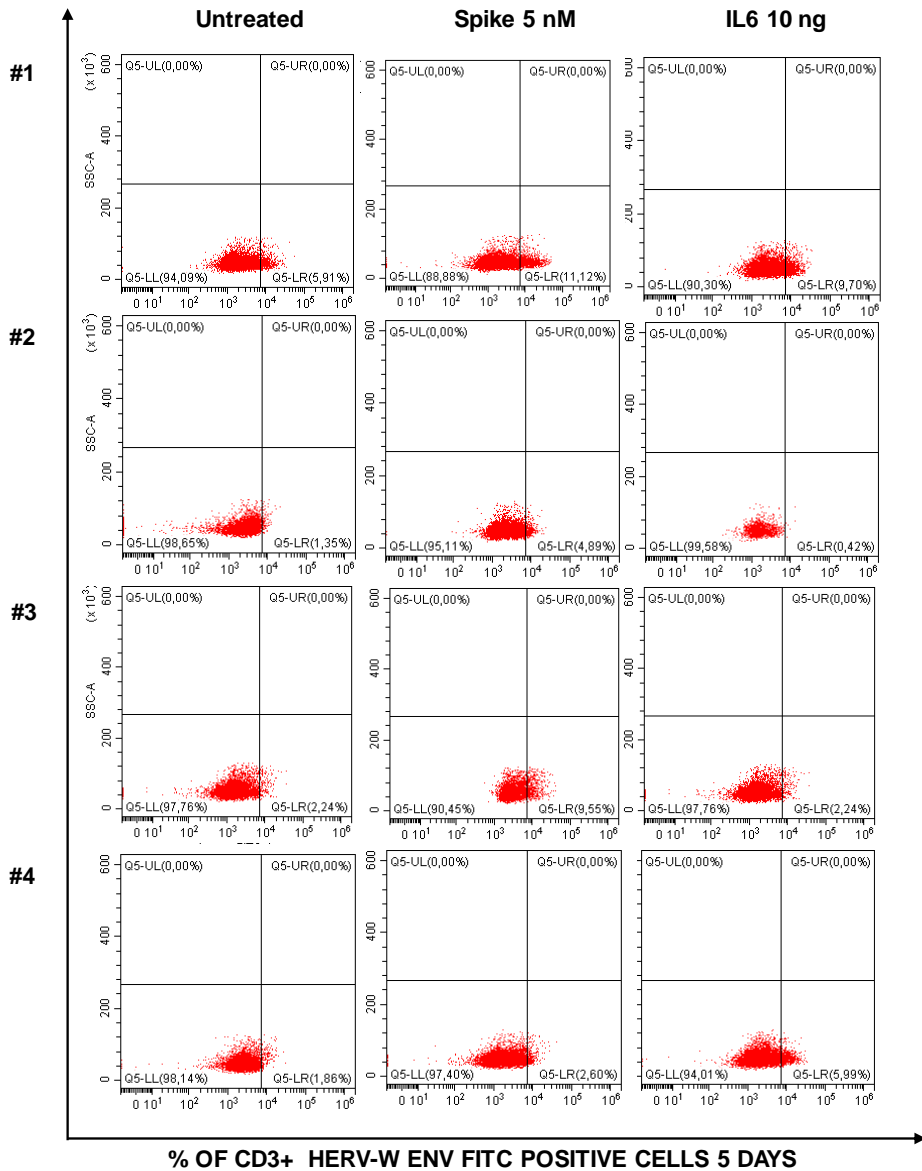
	IL-6	TNF $\alpha$	INF $\gamma$
<b>COVID-19 PATIENTS Number</b>	29	29	29
Median	15.400	16.100	5.150
Percentiles			
25	5.500	11.350	1.945
50	15.400	16.100	5.150
75	29.200	21.850	11.150
<b>HEALTHY DONORS Number</b>	17	17	17
Median	1.680	8.260	0.694
Interquartile Range (IQR)			
25	1.470	6.580	0.625
50	1.680	8.260	0.694
75	2.145	9.259	1.025
<b>Healthy donors vs COVID-19 patients</b>	* $<0.001$	$<0.001$	$<0.001$

\*Mann–Witney U Test

a)



b)



**Figure S3. Gating strategy for flow cytometry analysis of Healthy Donors expose to SARS-CoV2 Spike and IL6 proteins.** (a) FSC vs SSC dot plots of a representative healthy donor (HD, upper panels) in untreated (left panel) or treated with SPIKE (5 nM, central panel) or IL6 (10ng/ml, right panel); in the lower panel CD3-KO vs SSC dot plots gated of Lymphocytes. (b) Analysis of HERV-W ENV-positive cells (%) in CD3+ cells of 4 Healthy Donors after 5 days of exposure.

**Table S3** Bonferroni's multiple comparison \*p≤0.05 of HERVW-ENV and IL-6 expression in HDs stimulated with SARS-CoV2 Spike protein and IL-6 protein for 3,24 hours and 5 days.

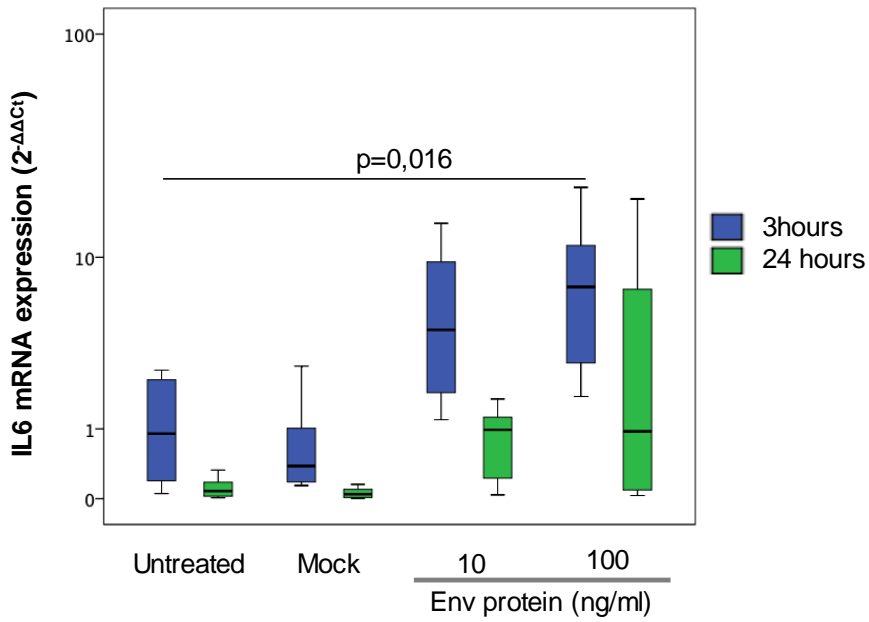
Variabile dipendente			Mean of Difference	std. error	Sign.	Confidence 95%	
						low	up
HERVW ENV 3h	CTR	IL6	-1,86971	9,25801	1,000	-28,1526	24,4132
		SPIKE1	-40,85878*	9,25801	<b>,001</b>	-67,1417	-14,5759
		SPIKE5	-26,20205	9,25801	,051	-52,4849	,0808
	IL6	CTR	1,86971	9,25801	1,000	-24,4132	28,1526
		SPIKE1	-38,98907*	9,25801	<b>,001</b>	-65,2719	-12,7062
		SPIKE5	-24,33234	9,25801	,083	-50,6152	1,9505
	SPIKE1	CTR	40,85878*	9,25801	<b>,001</b>	14,5759	67,1417
		IL6	38,98907*	9,25801	<b>,001</b>	12,7062	65,2719
		SPIKE5	14,65673	9,25801	,748	-11,6261	40,9396
	SPIKE5	CTR	26,20205	9,25801	,051	-,0808	52,4849
		IL6	24,33234	9,25801	,083	-1,9505	50,6152
		SPIKE1	-14,65673	9,25801	,748	-40,9396	11,6261
HERV W ENV 24h	CTR	IL6	-4,80439	30,13272	1,000	-90,3492	80,7404
		SPIKE1	-48,50968	30,13272	,712	-134,0545	37,0351
		SPIKE5	-110,39406*	30,13272	,006	-195,9388	-24,8493
	IL6	CTR	4,80439	30,13272	1,000	-80,7404	90,3492
		SPIKE1	-43,70529	30,13272	,948	-129,2501	41,8395
		SPIKE5	-105,58967*	30,13272	,009	-191,1344	-20,0449
	SPIKE1	CTR	48,50968	30,13272	,712	-37,0351	134,0545
		IL6	43,70529	30,13272	<b>,948</b>	-41,8395	129,2501
		SPIKE5	-61,88438	30,13272	,297	-147,4292	23,6604
	SPIKE5	CTR	110,39406*	30,13272	,006	24,8493	195,9388
		IL6	105,58967*	30,13272	<b>,009</b>	20,0449	191,1344
		SPIKE1	61,88438	30,13272	,297	-23,6604	147,4292
HERV W ENV 5DAYS	CTR	IL6	-17,39140	14,46794	1,000	-58,4649	23,6821
		SPIKE1	-55,62558*	14,46794	<b>,004</b>	-96,6991	-14,5521
		SPIKE5	-36,33136	14,46794	,108	-77,4049	4,7421
	IL6	CTR	17,39140	14,46794	1,000	-23,6821	58,4649
		SPIKE1	-38,23418	14,46794	,080	-79,3077	2,8393
		SPIKE5	-18,93996	14,46794	1,000	-60,0135	22,1335
	SPIKE1	CTR	55,62558*	14,46794	<b>,004</b>	14,5521	96,6991
		IL6	38,23418	14,46794	,080	-2,8393	79,3077
		SPIKE5	19,29422	14,46794	1,000	-21,7793	60,3677
	SPIKE5	CTR	36,33136	14,46794	,108	-4,7421	77,4049
		IL6	18,93996	14,46794	1,000	-22,1335	60,0135
		SPIKE1	-19,29422	14,46794	1,000	-60,3677	21,7793

**Table S3 continued**

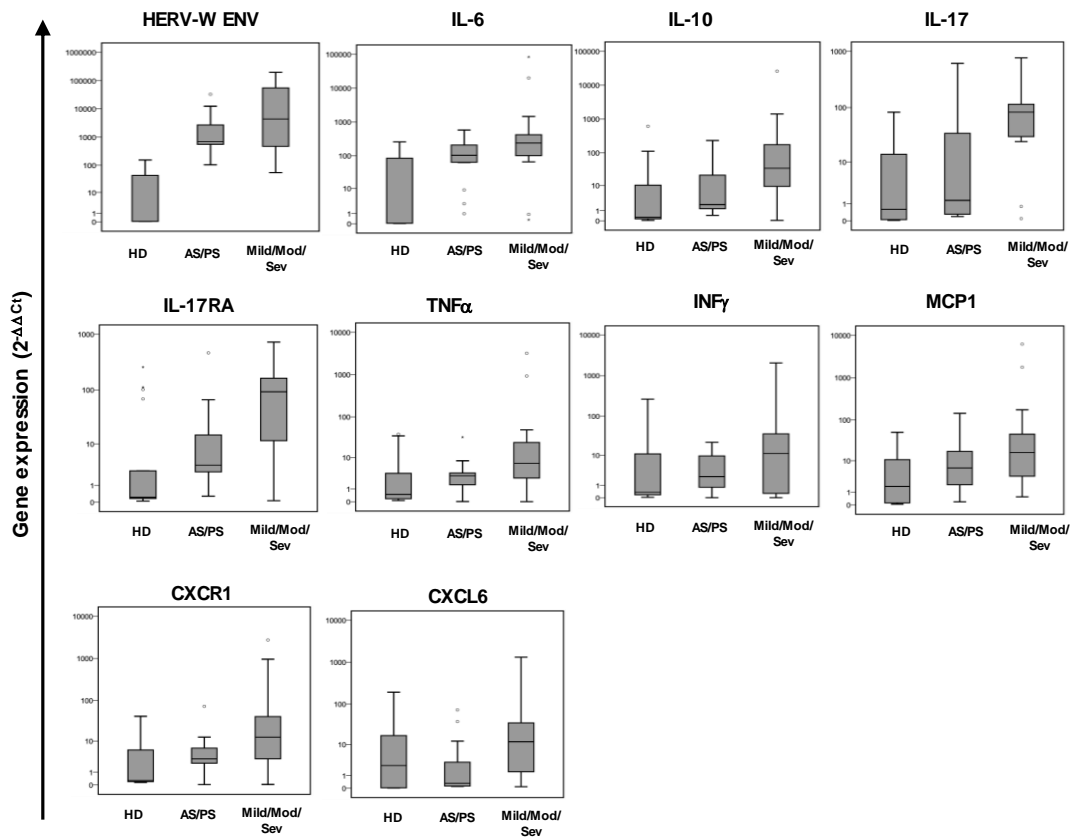
Independent			Mean of Difference	std. error	Sign.	Confidence 95%	
						low	up
IL6_3h	CTR	IL6	1,02678	,76073	1,000	-1,1329	3,1864
		SPIKE1	,83211	,76073	1,000	-1,3275	2,9918
		SPIKE5	-1,45729	,76073	,394	-3,6169	,7024

	IL6	CTR	-1,02678	,76073	1,000	-3,1864	1,1329
		SPIKE1	-,19466	,76073	1,000	-2,3543	1,9650
		SPIKE5	-2,48406*	,76073	,017	-4,6437	-,3244
	SPIKE1	CTR	-,83211	,76073	1,000	-2,9918	1,3275
		IL6	,19466	,76073	1,000	-1,9650	2,3543
		SPIKE5	-2,28940*	,76073	<b>,033</b>	-4,4490	-,1297
	SPIKE5	CTR	1,45729	,76073	,394	-,7024	3,6169
		IL6	2,48406*	,76073	<b>,017</b>	,3244	4,6437
		SPIKE1	2,28940*	,76073	,033	,1297	4,4490
IL6_24h	CTR	IL6	,09539	108,13983	1,000	-306,9063	307,0971
		SPIKE1	-172,88139	108,13983	,727	-479,8831	134,1203
		SPIKE5	-323,52919*	108,13983	<b>,034</b>	-630,5309	-16,5275
	IL6	CTR	-,09539	108,13983	1,000	-307,0971	306,9063
		SPIKE1	-172,97678	108,13983	,726	-479,9785	134,0249
		SPIKE5	-323,62458*	108,13983	<b>,034</b>	-630,6263	-16,6229
	SPIKE1	CTR	172,88139	108,13983	,727	-134,1203	479,8831
		IL6	172,97678	108,13983	,726	-134,0249	479,9785
		SPIKE5	-150,64780	108,13983	1,000	-457,6495	156,3539
	SPIKE5	CTR	323,52919*	108,13983	<b>,034</b>	16,5275	630,5309
		IL6	323,62458*	108,13983	<b>,034</b>	16,6229	630,6263
		SPIKE1	150,64780	108,13983	1,000	-156,3539	457,6495
IL6_5DAYS	CTR	IL6	-3,68067	279,37395	1,000	-796,8045	789,4432
		SPIKE1	-938,50588*	279,37395	<b>,014</b>	-1731,6297	-145,3820
		SPIKE5	-536,50671	279,37395	,390	-1329,6306	256,6172
	IL6	CTR	3,68067	279,37395	1,000	-789,4432	796,8045
		SPIKE1	-934,82521*	279,37395	<b>,014</b>	-1727,9491	-141,7013
		SPIKE5	-532,82604	279,37395	,401	-1325,9499	260,2978
	SPIKE1	CTR	938,50588*	279,37395	<b>,014</b>	145,3820	1731,6297
		IL6	934,82521*	279,37395	<b>,014</b>	141,7013	1727,9491
		SPIKE5	401,99917	279,37395	,968	-391,1247	1195,1230
	SPIKE5	CTR	536,50671	279,37395	,390	-256,6172	1329,6306
		IL6	532,82604	279,37395	,401	-260,2978	1325,9499
		SPIKE1	-401,99917	279,37395	,968	-1195,1230	391,1247
Bonferroni's multiple comparison *p≤0.05							





**Figure S4.** PBMCs from Healthy donors (n=4) stimulated with HERV W ENV protein (10 or 100 ng/ml) for 3, and 24 hours. Box plots of IL-6 mRNA levels, obtained by qRT-PCR analysis. ANOVA and Bonferroni's multiple comparison tests were performed \*p≤0.05



**Figure S5. HERV-W ENV and cytokines expression in leukocytes of COVID-19 patients according to clinical status.**

The COVID-19 patients were stratified based on clinical status as asymptomatic/pauci-symptomatic (AS+PS, n=15), mild, moderate and severe (Mild/Mod/Sev, n=15). The expression of HERV-W ENV, cytokines and related receptors were analysed in leukocytes, lymphocytes, monocytes, granulocytes, and T cell, B cell, and NK lymphocyte subtypes. Data are represented as box plots (white box: healthy donors, HD; grey box: all patients positive for SARS-CoV-2), the dashed lines separate the first two groups consisting of HD and SARS-CoV-2 positive asymptomatic patients that were hospitalized for comorbidities. Non-parametric Mann-Whitney test was used to compare groups and statistically significant values were considered when  $p < 0.050$  (Table S2).

**Table S4. Kruskal-Wallis test of HERV-W ENV, cytokines and receptors expression (mRNA) in blood samples of COVID19 and HD according to clinical status.**

	HERV-W ENV	IL-6	IL-10	IL-17	IL-17RA	TNF $\alpha$	INF $\gamma$	MCP1	CXCR1	CXCL6
HD vs Asymp/Paucisympt	<0.001*	0.030	0.327	0.567	0.246	0.082	0.447	0.401	0.683	0.083
HD vs Mild/Mod/Sev	<0.001	<0.001	0.003	<0.001	0.001	0.082	0.447	0.028	0.021	0.083
Asymp/Paucisympt vs Mild/Mod/Sev	1.000	0.740	0.304	0.076	0.265	0.082	0.447	0.860	0.440	0.083

\* Kruskal-Wallis Test

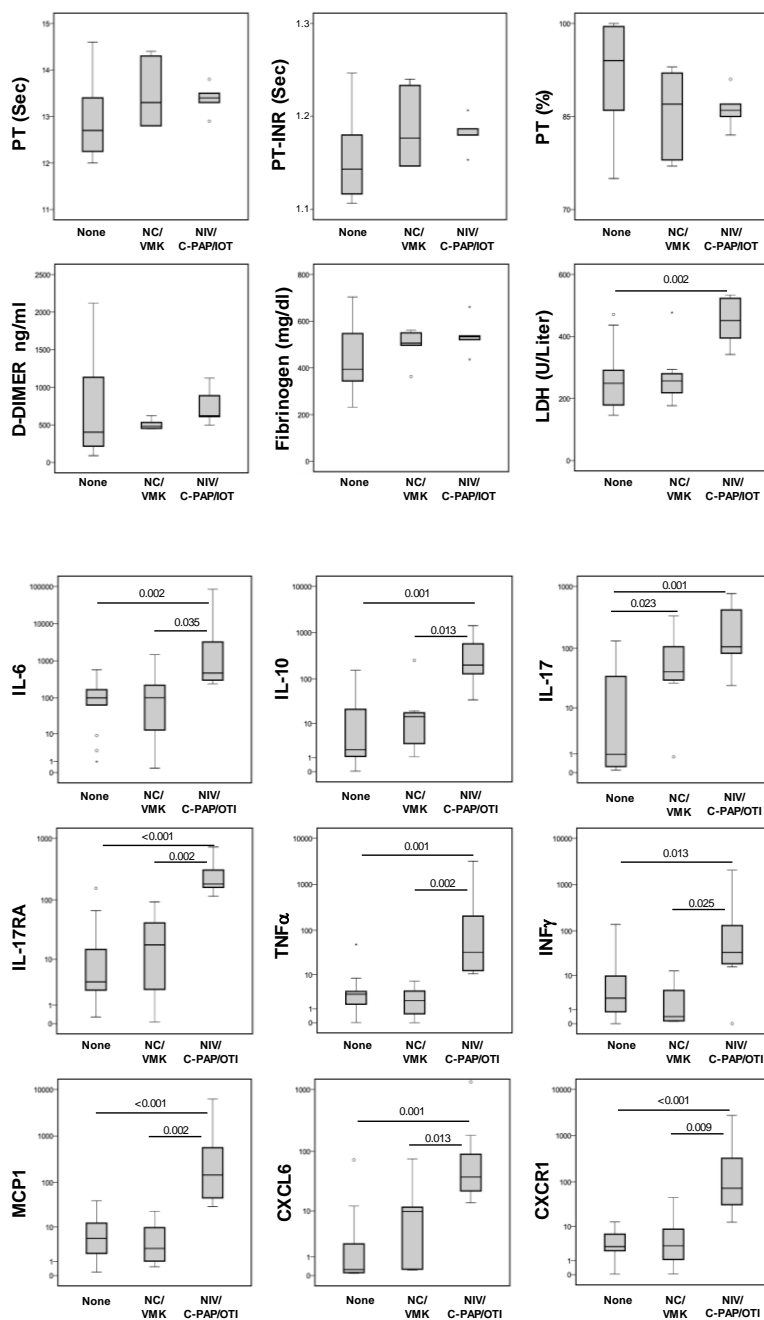
**Table S5. Median values, interquartile range and Kruskal-Wallis test of HERV-W ENV, cytokines and receptors expression (mRNA) in blood samples of COVID19 and HD according to clinical status.**

	HERV-W ENV	IL-6	IL-10	IL-17	IL-17RA	TNF $\alpha$	INF $\gamma$	MCP1	CXCR1	CXCL6
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<b>Healthy donors number</b>	<b>17</b>										
Median		0.045	0.040	0.269	0.594	0.225	0.490	0.354	1.717	0.274	2.474
Interquartile Range (IQR)	25	0.039	0.028	0.111	0.044	0.164	0.159	0.177	0.100	0.188	0.021
	50	0.045	0.040	0.269	0.594	0.225	0.490	0.354	1.717	0.274	2.474
	75	45.339	92.546	11.544	25.985	35.813	4.614	12.233	13.349	7.589	26.096
<hr/>											
<b>Asymptomatic and</b>											
<b>Paucisymptomatic patients number</b>	<b>15</b>										
Median		684.997	103.630	2.000	1,292	3.598	3.104	2.287	6.382	3.158	0.323
Interquartile Range (IQR)	25	498.451	63.360	1.255	0.260	2.342	1.396	0.744	1.198	2.221	0.115
	50	684.997	103.630	2.000	1.292	3.598	3.104	2.287	6.382	3.158	0.323
	75	9421.437	325.770	45.422	39.068	36.705	3.842	10.522	17.867	11.029	6.956
<b>Mild, moderate and severe patients number</b>	<b>15</b>										
Median		4376.194	240.628	34.432	82.683	92.885	7.019	11.163	16.268	12.542	11.773
Interquartile Range (IQR)	25	357.968	100.991	5.916	26.721	7.401	2.001	0.147	2.827	3.132	0.268
	50	4376.194	240.628	34.432	82.683	92.885	7.019	11.163	16.268	12.542	11.773
	75	56563.819	469.597	198.821	124.940	171.080	44.286	38.627	50.997	45.354	43.816

**Table S6 Median values and interquartile range of HERV-W ENV percentage in Lymphocytes according to Pulmonary involvement**

		None+P	MiP	BiP	BiP+Bac
Median		24,84	43,64	78,86	66,95
Interquartile Range (IQR)	25	8,31	35,43	44,00	60,93
	50	24,84	43,64	78,86	66,95
	75	47,43	61,41	90,37	



**None:** no oxygen support

**NC/VMK:** nasal Cannula/Venturi Mask

**NIV/C-PAP/OTI:** non invasive ventilation or orotracheal intubation

**Figure S6. Analysis of biochemical inflammatory markers and cytokine mRNA expression according to respiratory outcome.** The COVID-19 patients were stratified based on respiratory outcome during hospitalization as follows: no oxygen support needed (None; n=16), oxygen support with nasal cannula or ventimask (NC/VMK; n=8), oxygen support by non-invasive ventilation, continuous positive airway pressure or orotracheal intubation (NIV/C-PAP/OTI; n=6). The biochemical inflammatory marker levels and cytokine mRNA expression according to respiratory outcome are represented as box plots. Non-parametric Kruskal Wallis test with Bonferroni's correction was used to compare groups and statistically significant values were considered when  $p < 0.050$

**Table S7. Median values and interquartile range of HERV-W env transcriptional activity and cytokines and receptors expression (mRNA) in blood samples from COVID19, respect to respiratory outcome**

		HERV-W ENV	IL-6	IL-10	IL-17	IL-17RA	TNF $\alpha$	INF $\gamma$	MCP1	CXCR1	CXCL6
<b>No oxygen support</b>	<b>16</b>										
Median		667.673	100.328	1.959	0.989	3.743	3.147	2.564	5,29531	3.018	0.251
Interquartile Range (IQR)	25	527.741	63.748	1.077	0.244	2.415	1.456	0.775	1,66552	2.251	0.110
	50	667.673	100.328	1.959	0.989	3.743	3.147	2.564	5,29531	3.018	0.251
	75	2.407.388	191.030	36.465	36.883	28.956	3.801	10.097	14,8452	9.182	3.148
<b>Nasal Cannula/Venturi Mask</b>	<b>7</b>										
Median		690.906	101.616	14.404	41.207	17.827	2.006	0.416	2.826	3.206	9.804
Interquartile Range (IQR)	25	102.260	0.882	1.344	26.721	0.529	0.038	0.146	0.918	0.093	0.258
	50	690.906	101.616	14.404	41.207	17.827	2.006	0.416	2.826	3.206	9.804
	75	6201.429	380.793	19.539	124.940	80.566	4.189	11.163	16.268	12.636	11.733
<b>Non invasive ventilation or orotracheal intubation</b>	<b>7</b>										
Median		56563.819	469.597	198.821	105.356	181.462	32.423	33.618	143.850	72.372	37.714
Interquartile Range (IQR)	25	32713.820	249.303	124.257	82.683	149.817	12.111	15.729	40.844	26.783	17.521
	50	56563.819	469.597	198.821	105.356	181.462	32.423	33.618	143.850	72.372	37.714
	75	154766.801	20053.152	1420.835	610.367	464.042	928.520	433.114	1767.423	955.226	180.598

**Table S8. Median values and interquartile range of biochemical markers in blood samples from COVID19, respect to respiratory outcome**

		PT (Sec)	PT - INR (Sec)	PT (%)	D-DIMER (ng/ml)	Fibrinogen (mg/dl)	LDH (U/liter)
<b>No oxygen support</b>	<b>12</b>						
Median		12.700	1.065	94	402.5	394	249
Interquartile Range (IQR)	25	12.225	1.022	86	210.5	330	173.5
	50	12.700	1.065	94	402.5	394	249
	75	13.400	1.120	99.750	1192.25	588	309
<b>Nasal Cannula/Venturi Mask</b>	<b>6</b>						
Median		13.300	1.115	87	481	506	257
Interquartile Range (IQR)	25	12.800	1.070	77.750	452.5	463.5	207
	50	13.300	1.115	87	481	506	257
	75	14.325	1.202	92.250	554.5	552.75	294
<b>Non invasive ventilation or orotracheal intubation</b>	<b>5</b>						

Median		13.400	1.120	86	622	533	451.5
Interquartile Range (IQR)	25	13.100	1.100	83.5	552.5	478.5	381.75
	50	13.400	1.120	86	622	533	451.5
	75	13.650	1.145	89	1005	599	525.75

**Table S9. Median values and interquartile range of the percentage of leucocytes and different subpopulations of leucocytes in blood samples from COVID19, respect to respiratory outcome**

		Leukocytes	Lymphocytes	Monocytes	Granulocytes	CD3	CD4	CD8	CD19	CD56	CD14
<b>No oxygen support</b>		<b>16</b>									
Median		8.430	42.485	34.825	2.145	47.040	8.565	15,1	2,155	4,225	6,53
Interquartile Range (IQR)	25	1.540	21.63	8.387	1.102	24.435	5.230	5,835	1,0025	2,0225	1,675
	50	8.430	42.485	34.825	2.145	47.040	8.565	15,1	2,155	4,225	6,53
	75	49	61.537	58.582	12.652	59.857	21.802	22,37	33,66	9,285	20,3975
<b>Nasal Cannula/Venturi Mask</b>		<b>7</b>									
Median		55	66.950	60.050	51.100	61.670	25.090	5,05	3,33	11,77	4,46
Interquartile Range (IQR)	25	16.400	32.580	23.250	11.860	46.500	11.700	2,25	1,96	10,86	1,09
	50	55	66.950	60.050	51.100	61.670	25.090	5,05	3,33	11,77	4,46
	75	71.220	90.070	89.700	91.170	88.670	39.310	10,82	10,62	89,38	10,2
<b>Non invasive ventilation or orotracheal intubation</b>		<b>7</b>									
Median		9.930	78.860	5.290	3.360	28.900	56.800	25,31	17,33	4,2	3,85
Interquartile Range (IQR)	25	4.710	45.540	2.100	0.120	15.690	35.760	1,91	1,04	1,73	1
	50	9.930	78.860	5.29	3.360	28.900	56.800	25,31	17,33	4,2	3,85
	75	40.900	90.370	23.550	5.170	92.860	76.670	57,45	96,88	14,02	42,63