Extended Data for

Human endogenous retrovirus K activation in the lower respiratory tract of severe COVID-19 patients associates with early mortality.

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[1]J.R.T. and N.F.-R. and contributed equally to this work.

Additional information

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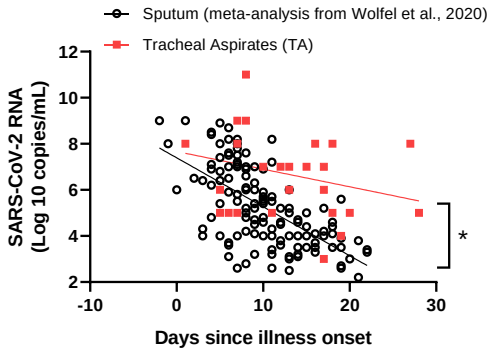
Extended Table 1 - **Demographic clinical and laboratorial aspects of the patients.**

Characteristics outcome[a]	Discharged (n=10)	Deceased (n=15)	*p*[c]
Age, years	57 (42.25 – 63.25)	56 (51 – 70)	0.4862
Sex, male	2 (20%)	8 (53.33%)	0.2107
SAPS3	63 (56 – 67)	68 (59 – 78.5)	0.1903
PaO_2/FiO_2 ratio	160.5 (114.1 - 208)	130 (80 – 176)	0.1776
Vasopressor	5 (50%)	9 (60%)	0.6968
Time from symptom onset to blood sample, days	12 (8 – 17)	13 (8 – 18)	0.7359
Comorbidities			
Obesity	2 (20%)	2 (13.33%)	>0.9999
Hypertension	5 (50%)	8 (53.33%)	>0.9999
Diabetes	2 (20%)	5 (33.33%)	0.6592
Cancer	2 (20%)	1 (6.67%)	0.5435
Heart disease[b]	1 (10%)	1 (6.67%)	>0.9999
Presenting symptoms			
Cough	7 (70%)	11 (73.33%)	>0.9999
Fever	9 (90%)	12 (80%)	0.6265
Dyspnea	8 (80%)	13 (86.66%)	>0.9999
Headache	3 (30%)	2 (13.33%)	0.3577
Anosmia	3 (30%)	4 (26.66%)	>0.9999
Laboratory findings on admission			
White blood cell	138.5 (91.75 – 149.5)	168 (123 – 275)	0.0534
Lymphocyte count, cells/mm^3	1238 (939 – 1527)	1136 (417 – 1765)	0.8609
Platelet count, x1000/mm^3	198 (137.5 – 336.5)	193 (131 – 240)	0.4373
C Reactive Protein, mg/L	11.98 (6.41 – 23.44)	20.33 (12.19 - 27.92)	0.1963
Fibrinogen, mg/dL	545.3 (332.8 – 591.7)	545.3 (453.1 – 621.6)	0.6505
D-dimer, IU/mL	3835 (2290 – 11410)	7401 (3373 – 17065)	0.3537
IL-6, pg/mL	23 (15.5 – 50.75)	56 (28 – 119)	0.0619
Viral load (RNA copies/mL)	196433 (3.369 – 3954576)	78911 (2.105 - 280815)	0.6540

[a] Numerical variables are represented as the median and the interquartile range, and qualitative variables are represented as the number and the percentage.
[b] Coronary artery disease or congestive heart failure
[c] Qualitative variables were compared using the two tailed Fisher exact test, and numerical variables using t test for parametric and Mann Whitney test for nonparametric distributions.

Extended Data

A



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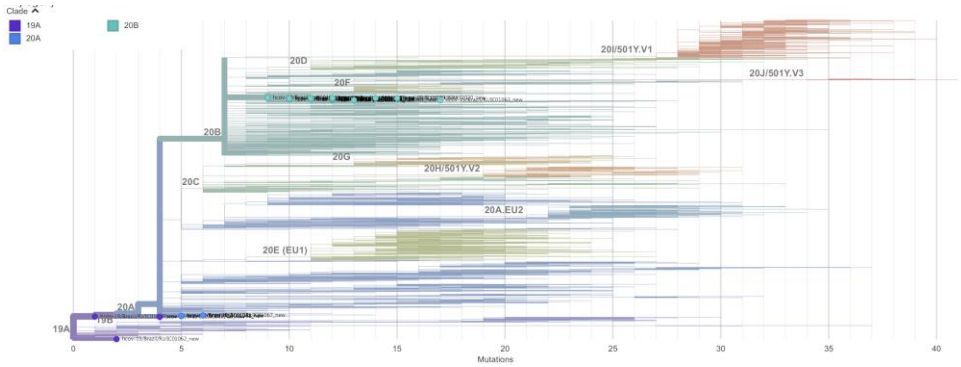

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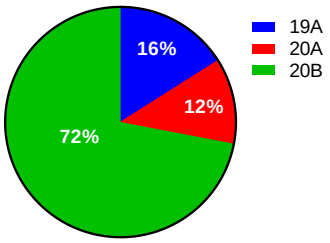
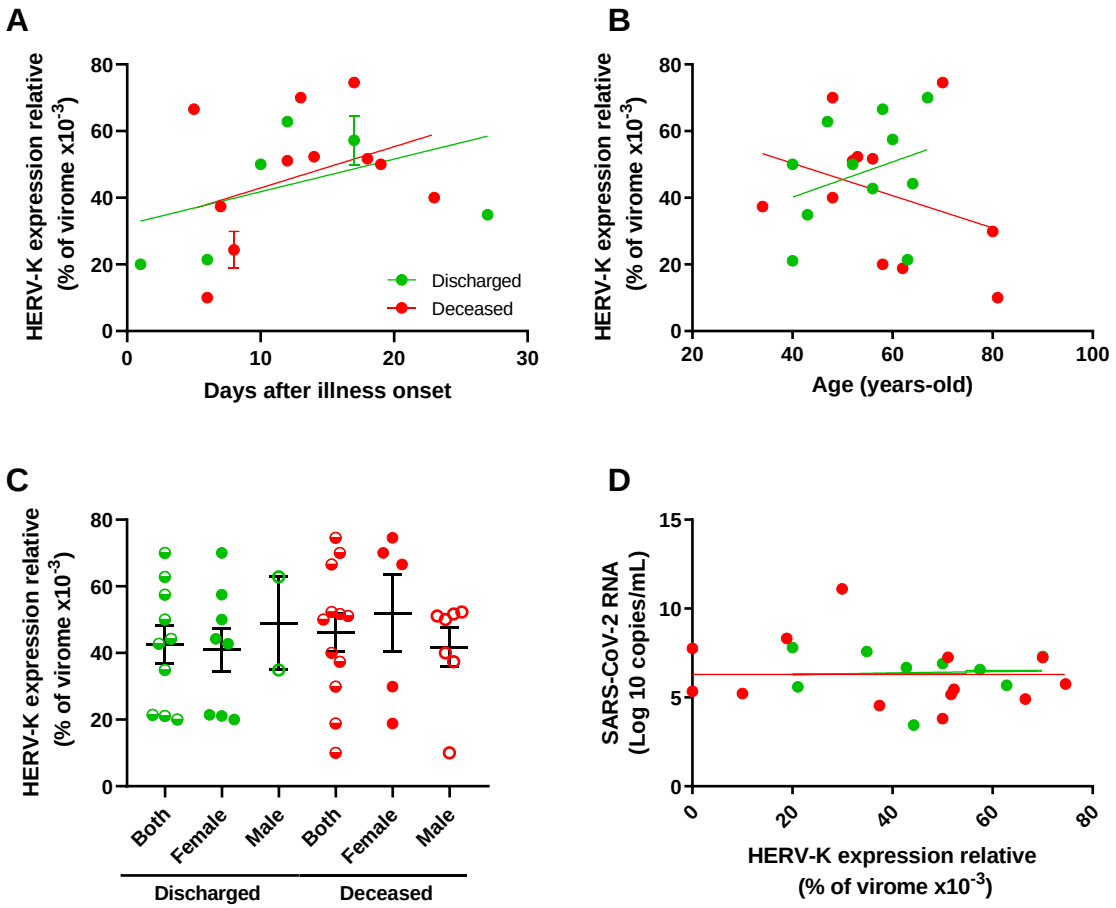
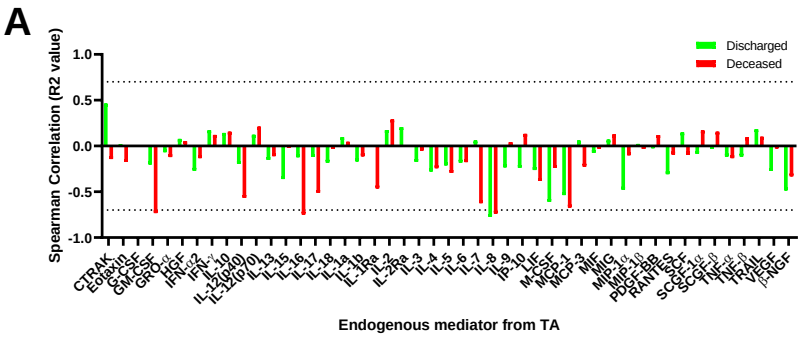
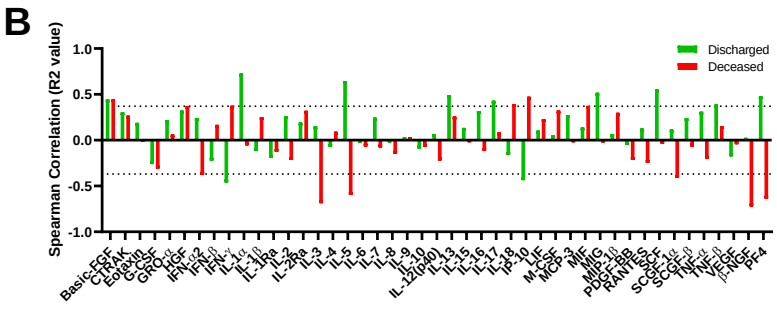


Extended Figure 1 - Characteristics of the SARS-CoV-2 detected in the tracheal aspirates of patients under the invasive mechanical ventilation. A) Linear regression (± 90% bands) of SARS-CoV-2 RNA levels from tracheal aspirates (TA) of patients under mechanical ventilation (red), compared to meta-analysis of the sputum from Wolfel et al., 2020[1]. **B)** Phylogenetic tree of the full-length consensus SARS-CoV-2 genomes, generated with Atoplex kit using a MGI-2000 sequencer, from TA (circles) was constructed through NextClade[2] and colored according to the emerging clade default definitions. **C)** Distribution of SARS-CoV-2 emerging clades among the patients.



Extended Figure 2. HERV-K expression and social-demographic indicators of the cohort. HERV-K expression is presented as a function of **(A)** days since onset of illness **(B)** age, **(C)** gender and **(D)** SARS-CoV-2 RNA levels.


A
Spearman Correlation (R2 value)
Discharged
Deceased
Endogenous mediator from TA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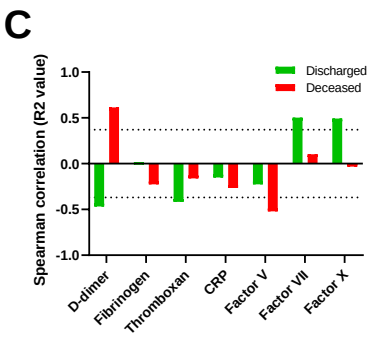
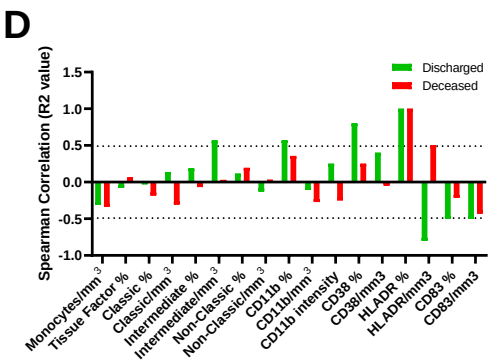
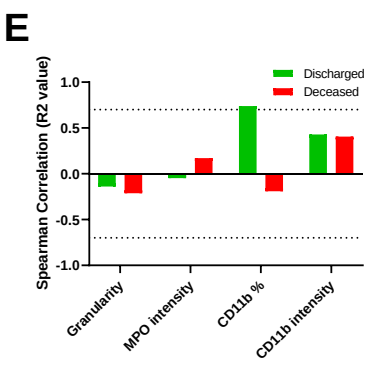
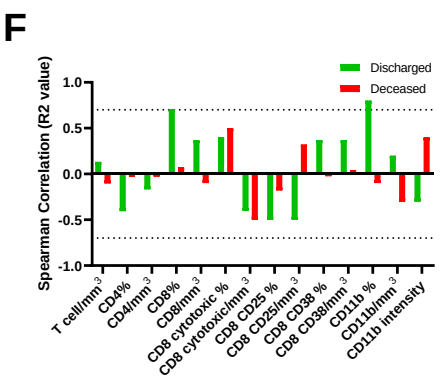
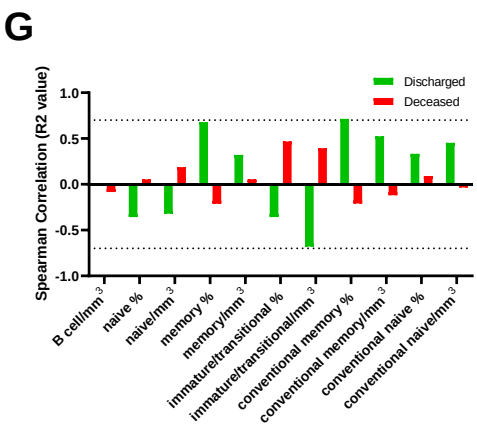
Spearman Correlation (R2 value)
Discharged
Deceased
Endogenous Mediators from Plasma

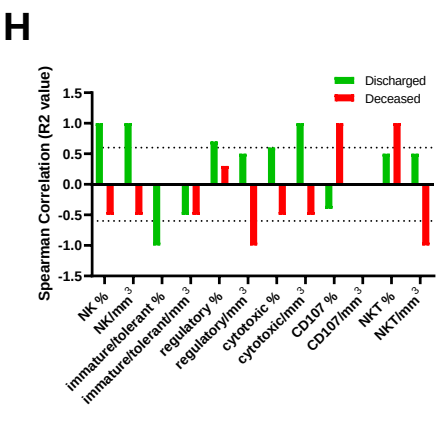

C
Spearman correlation (R2 value)
Discharged
Deceased
D-dimer
Fibrinogen
Thromboxan
CRP
Factor V
Factor VII
Factor X


D
Spearman Correlation (R2 value)
Discharged
Deceased


E
Spearman Correlation (R2 value)
Discharged
Deceased
Granularity
MPO intensity
CD11b %
CD11b intensity


F
Spearman Correlation (R2 value)
Discharged
Deceased


G
Spearman Correlation (R2 value)
Discharged
Deceased


H
Spearman Correlation (R2 value)
Discharged
Deceased

Extended Figure 3 - Spearman Correlation between HERV-K and severity markers in COVID-19. Endogenous mediators in the TA **(A)**, in the plasma from peripheral blood **(B)**, coagulation marker in the plasma **(C)**, monocytes **(D)**, neutrophiles **(E)**, T cell **(F)**, B cells **(G)** and natural killers (NK) cells **(H)** from peripheral blood mononuclear cells (PBMCs) were plotted as function of HERV-K expression. One-way Spearman Correlation R2 was plotted and statistical significance with p values <0.05 are presented by the bars that cross the dots lines. Gate strategy for PBMCs is presented in the **Supplementary Figure 2**.

Extended Table 2 - Association of HERV-K transcripts and human transcripts.

Patient code	GAG-like chromosome region	POL-like chromosome region	ENV-like chromosome region
1	HML-2 3q27.2	HML-2 11q22.1	HML-2 3q13.2
2	HML-2 6q14.1	HML-2 11q22.1	HML-2 3q13.2
3	HML-2 19p12b	HML-2 19p12b	HML-2 19p12b
4	HML-2 3q27.2	HML-2 11q22.1	HML-2 3q13.2
5	HML-2 19p12b	HML-2 19p12b	HML-2 19p12b
6	HML-2 3q27.2	HML-2 11q22.1	HML-2 3q13.2
7	HML-2 6q14.1	HML-2 11q22.1	HML-2 3q13.2
8	HML-2 3q27.2	HML-2 11q22.1	HML-2 3q13.2
9	HML-2 19p12b	HML-2 3q24	HML-2 19p12b
10	HML-2 6q14.1	HML-2 11q22.1	HML-2 3q13.2
11	HML-2 19p12b	HML-2 3q24	HML-2 19p12b
12	HML-2 19p12b	HML-2 11q22.1	HML-2 3q13.2
13	HML-2 19p12b	HML-2 19p12b	HML-2 3q13.2
14	HML-2 3q27.2	HML-2 19p12b	HML-2 10q24.2 / HML-2 8q24.3a
15	HML-2 6q14.1	HML-2 3q24	HML-2 10q24.2 / HML-2 8q24.3a
16	HML-2 19p12b	HML-2 10q24.2	HML-2 3q13.2
17	HML-2 19p12b	HML-2 3q24	HML-2 19p12b
18	HML-2 3q27.2	HML-2 11q22.1	HML-2 3q13.2
19	ND	ND	HML-2 12q24.11
20	HML-2 19p12b	HML-2 3q24	HML-2 3q13.2
21	HML-2 19p12b	HML-2 11q22.1	HML-2 3q13.2
22	HML-2 19p12b	AY037928	HML-2 3q13.2
23	ND	ND	HML-2 19p12b
24	HML-2 10q24.2	HML-2 11q22.1	HML-2 3q13.2

25	HML-2 12q13.2	HML-2 12q13.2	ND
MC01	HML-2 6q14.1	ND	HML-2 3q13.2
MC02	HML-2 6q14.1	HML-2 11q22.1	HML-2 3q13.2
MC03	HML-2 6q14.1	HML-2 11q22.1	HML-2 3q13.2
MC04	ND	HML-2 3q24	ND
MC05	HML-2 19p12b	HML-2 3q24	HML-2 3q13.2

*MC = Mild COVID-19 patients, ND – not determined and to be assigned

SUPPLEMENTARY MATERIAL

Supplementary Table 1 - Quality control of SARS-CoV-2 sequences

Patient code	Emerging clade	GenBank code	Q score SARS-CoV-2	SARS-CoV-2 reads (mean)	SE (±)	Coverage (% mean)	SE (±)	Depth coverage (mean)	SE (±)
1	20B	SUB9550672	>30	484 051	21 986.16	1 561	0.012	100	7.672
2	20B	SUB9550672	>30	3 966	31.752	12	7.492	82	0.120
3	20B	SUB9550672	>30	23 362	86.417	67	1.550	97	1.082
4	20A	SUB9550672	>30	5 376	84.828	23	7.201	82	3.492
5	20B	SUB9550672	>30	91 687	499.756	272	0.183	100	1.647
6	20B	SUB9550672	>30	1 294 397	2 751.61	2 968	0.023	100	431.237
7	19A	SUB9550672	>30	963	7.720	4	0.430	77	0.037
8	20B	SUB9550672	>30	25 960	199.854	77	0.971	98	0.633
9	20B	SUB9550672	>30	14 342	65.626	43	0.014	100	0.198
10	20B	SUB9550672	>30	17 966 706	4 252 024	9 669	0.023	100	30.902
11	20B	SUB9550672	>30	4 095	62.077	13	9.425	64	0.145
12	20B	SUB9550672	>30	950 508	234 655.2	3 987	0.133	100	32.832

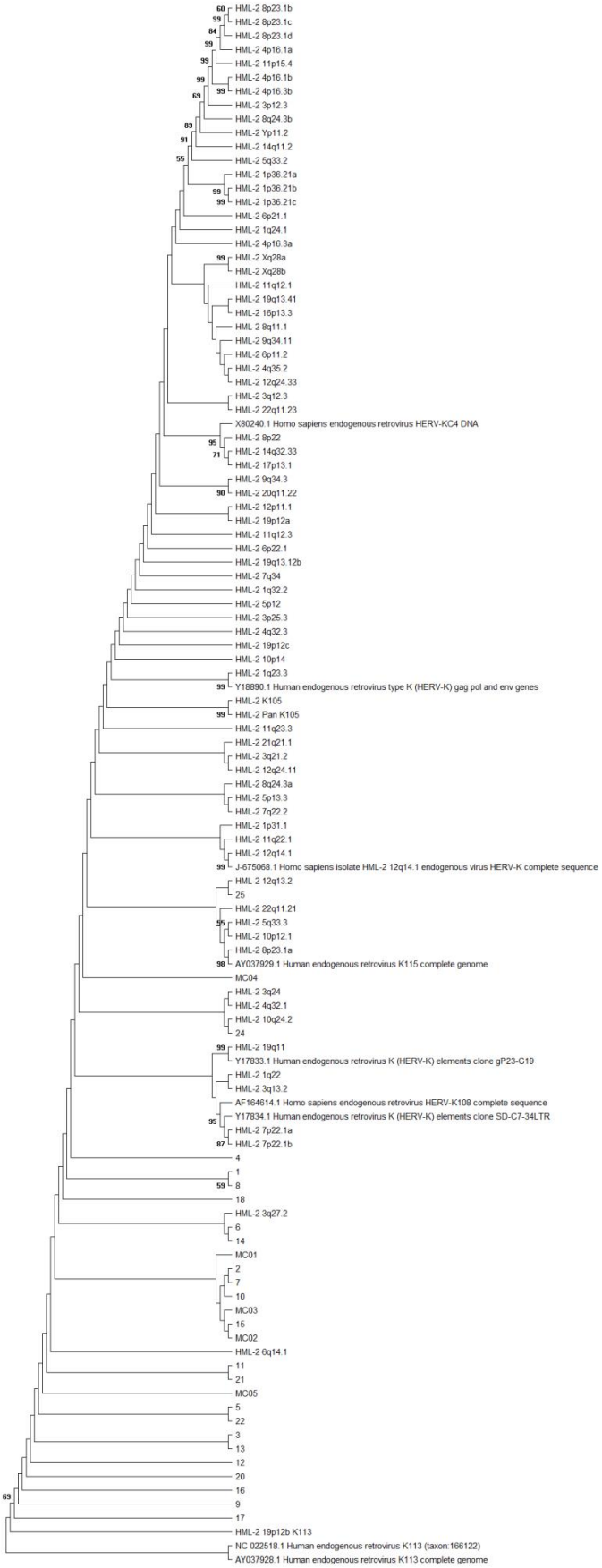
13	20B	SUB9550672	>30	13 085	84.196	42	3.080	93	1.801
14	20B	SUB9550672	>30	5 544	45.642	17	0.347	98	0.112
15	20B	SUB9550672	>30	2 587 016	12 847.09	7 653	0.023	100	37.941
16	19A	SUB9550672	>30	1 577	23.355	21	2.612	83	7.837
17	20B	SUB9550672	>30	38 487	117.565	135	4.511	88	8.872
18	20B	SUB9550672	>30	201 370	654.369	668	0	100	38.121
19	20A	SUB9550672	>30	7 682	85.488	23	0.032	99	0.254
20	20A	SUB9550672	>30	724 200	2 139.85	2 143	0.014	100	6.556
21	20B	SUB9550672	>30	8 220	51.036	25	0.126	99	0.139
22	19A	SUB9550672	>30	4 098	36.522	13	0.181	98	0.102
23	19A	SUB9550672	>30	6 363	61.782	19	0.088	99	0.196
24	20B	SUB9550672	>30	55 489	139.733	165	0	100	0.415
25	20B	SUB9550672	>30	4 767 728	221 803.1	28 208	9.642	67	3.969

Supplementary Table 2 - Quality control of the HERV-K sequences

Patient code	Biosample code	Q score HERV-K	HERV-K reads (mean)	SE (±)	Coverage (% mean)	SE (±)	Depth coverage (mean)	SE (±)
1	SAMN19068909	>30	2 493.75	67.50	97.9	0.119	22.6	0.582
2	SAMN19068910	>30	7 322	207.90	64.95	1.847	65.05	1.835
3	SAMN19068911	>30	591.5	36.42	28.725	5.754	38.75	8.874
4	SAMN19068912	>30	6 039	152.85	100	0	53.575	1.348
5	SAMN19068913	>30	1 101.75	38.92	35.75	7.293	60.525	14.087
6	SAMN19068914	>30	5 854.25	112.90	100	0	51.925	1.006
7	SAMN19068915	>30	4 492	111.86	85.9	6.734	53.45	7.629
8	SAMN19068916	>30	5 357.75	169.76	99.95	0.025	47.55	1.494
9	SAMN19068917	>30	1 472	55.85	64.2	1.877	20.625	1.068
10	SAMN19068918	>30	4 938.5	113.46	100	0	43.8	1.006
11	SAMN19068919	>30	2 820.5	43.41	94.075	0.654	26.55	0.236
12	SAMN19068920	>30	1 234.75	30.80	89.825	0.814	12.175	0.275
13	SAMN19068921	>30	1 031.25	39.88	67.175	2.113	13.6	0.320
14	SAMN19068922	>30	841	29.15	69.65	2.666	10.75	0.185

15	SAMN19068923	>30	862.5	17.43	81.65	0.794	9.4	0.167
16	SAMN19068924	>30	1 091.75	58.15	45.65	5.765	38.525	11.630
17	SAMN19068925	>30	1 363	38.33	87.9	0.657	13.8	0.437
18	SAMN19068926	>30	8 974.5	199.80	100	0	79.575	1.773
19	SAMN19068927	>30	24	3.5	7.4	3.4	3.8	1.13
20	SAMN19068928	>30	410.5	17.20	44.85	5.735	13.975	3.921
21	SAMN19068929	>30	2 591.5	180.94	88.225	4.916	25.8	0.576
22	SAMN19068930	>30	562.25	29.895	8	0.671	64.675	2.686
23	SAMN19068931	>30	950	50.3283	63.6	1.988	12.975	0.384
24	SAMN19068932	>30	2 830.25	74.293	99.175	0.092	20.2	3.32
25	SAMN19068933	>30	165.5	5.88	16.525	5.394	25.3	0.649
MC01	SAMN19070690	>30	4 728.25	104.442	99.825	0.012	42.025	0.933
MC02	SAMN19070691	>30	2 718.5	50.267	98.575	0.100	24.45	0.462
MC03	SAMN19070692	>30	1 662.5	37.604	92.875	0.532	15.875	0.391
MC04	SAMN19070693	>30	191.5	9.012	15.5	4.122	18.075	3.999
MC05	SAMN19070694	>30	1 756.25	31.993	92.4	0.507	16.85	0.275

A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HML-2 3p12.3
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HML-2 Xq28b
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HML-2 19q13.41
HML-2 16p13.3
HML-2 8q11.1
HML-2 9q34.11
HML-2 6p11.2
HML-2 4q35.2
HML-2 12q24.33
HML-2 3q12.3
HML-2 22q11.23
X80240.1 Homo sapiens endogenous retrovirus HERV-KC4 DNA
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HML-2 17p13.1
HML-2 9q34.3
HML-2 20q11.22
HML-2 12p11.1
HML-2 19p12a
HML-2 11q12.3
HML-2 6p22.1
HML-2 19q13.12b
HML-2 7q34
HML-2 1q32.2
HML-2 5p12
HML-2 3p25.3
HML-2 4q32.3
HML-2 19p12c
HML-2 10p14
HML-2 1q23.3
Y18890.1 Human endogenous retrovirus type K (HERV-K) gag pol and env genes
HML-2 K105
HML-2 Pan K105
HML-2 11q23.3
HML-2 21q21.1
HML-2 3q21.2
HML-2 12q24.11
HML-2 8q24.3a
HML-2 5p13.3
HML-2 7q22.2
HML-2 1p31.1
HML-2 11q22.1
HML-2 12q14.1
J-675068.1 Homo sapiens isolate HML-2 12q14.1 endogenous virus HERV-K complete sequence
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25
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HML-2 5q33.3
HML-2 10p12.1
HML-2 8p23.1a
AY037929.1 Human endogenous retrovirus K115 complete genome
MC04
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HML-2 4q32.1
HML-2 10q24.2
24
HML-2 19q11
Y17833.1 Human endogenous retrovirus K (HERV-K) elements clone gP23-C19
HML-2 1q22
HML-2 3q13.2
AF164614.1 Homo sapiens endogenous retrovirus HERV-K108 complete sequence
Y17834.1 Human endogenous retrovirus K (HERV-K) elements clone SD-C7-34LTR
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HML-2 7p22.1b
4
1
8
18
HML-2 3q27.2
6
14
MC01
2
7
10
MC03
15
MC02
HML-2 6q14.1
11
21
MC05
5
22
3
13
12
20
16
9
17
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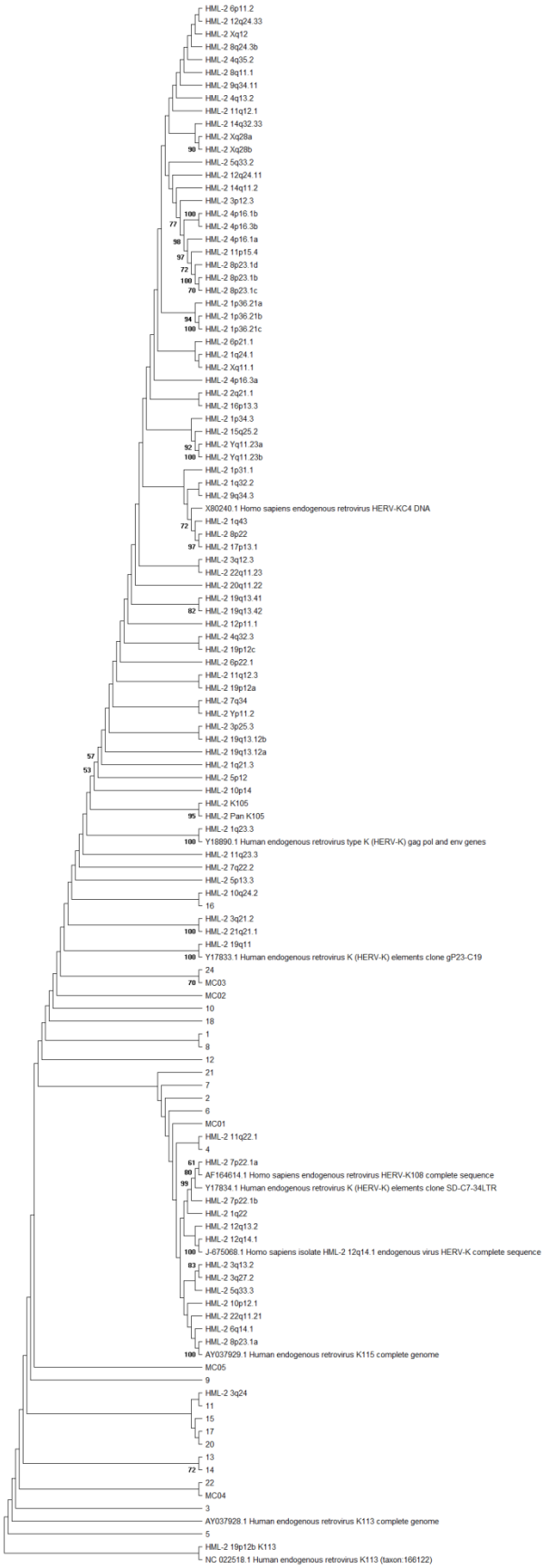
NC 022518.1 Human endogenous retrovirus K113 (taxon:166122)
AY037928.1 Human endogenous retrovirus K113 complete genome

B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HML-2 4q13.2
HML-2 11q12.1
HML-2 14q32.33
HML-2 Xq28a
HML-2 Xq28b
HML-2 5q33.2
HML-2 12q24.11
HML-2 14q11.2
HML-2 3p12.3
HML-2 4p16.1b
HML-2 4p16.3b
HML-2 4p16.1a
HML-2 11p15.4
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HML-2 8p23.1b
HML-2 8p23.1c
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HML-2 1q24.1
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HML-2 1p31.1
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HML-2 3q12.3
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MC02
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MC01
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HML-2 3q27.2
HML-2 5q33.3
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MC05
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HML-2 3q24
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13
14
22
MC04
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5
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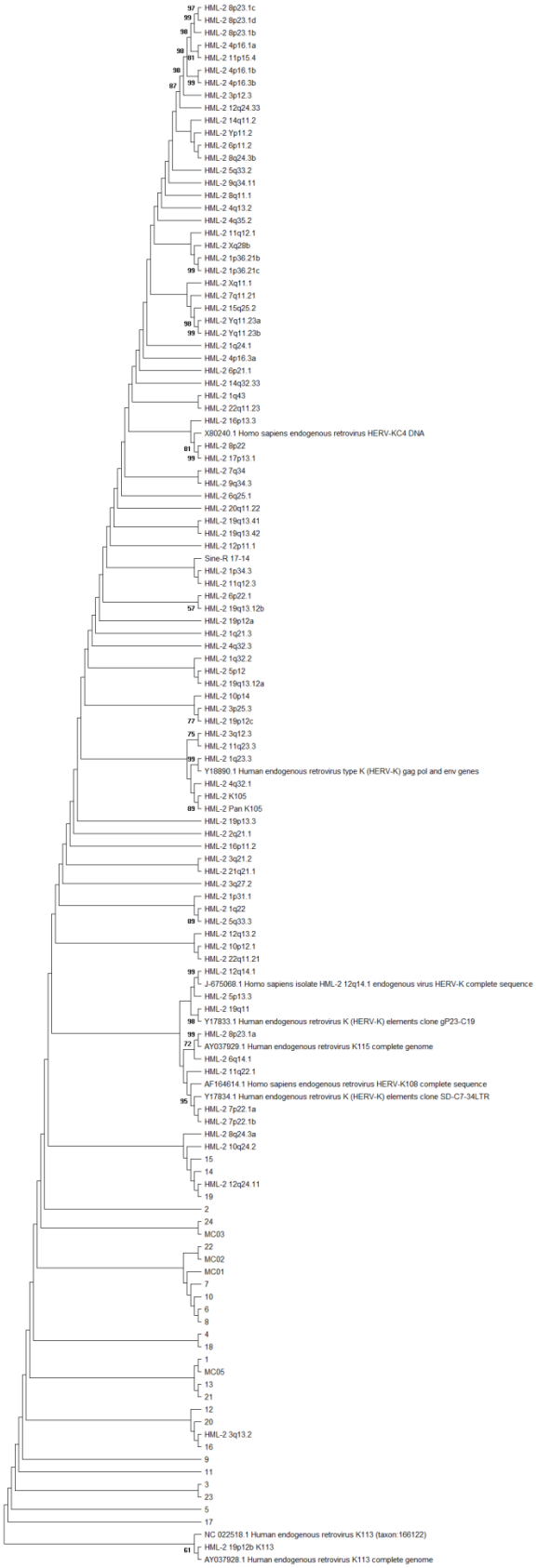
NC 022518.1 Human endogenous retrovirus K113 (taxon:166122)

C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MC05
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HML-2 3q13.2
16
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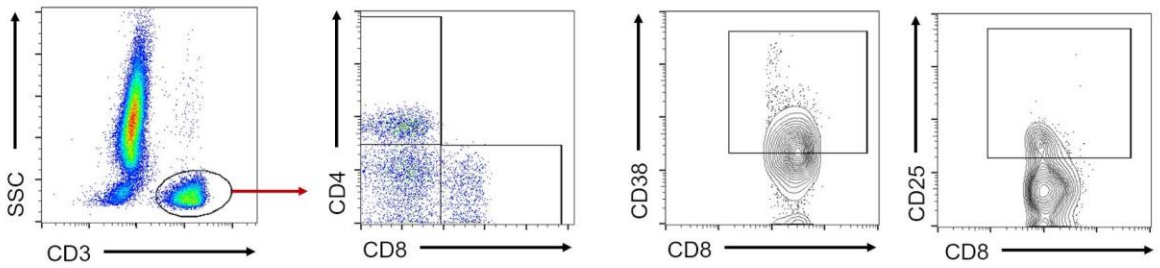
NC 022518.1 Human endogenous retrovirus K113 (taxon:166122)
HML-2 19p12b K113
AY037928.1 Human endogenous retrovirus K113 complete genome

Supplementary Figure 1 – **Representative phylogenetic trees of Gag, Pol and Env of HERV-K.** Representative genomes deposited in GenBank were compared with sequences from HERV-K polymerase, gag and envelope genome found in the tracheal aspirate samples from severe COVID-19 patients and nasal swabs from mild COVID-19. Three condensed phylogenetic trees rooted by reference genomes (AY037928.1 and NC 022518.1) were created with a total of 100 bootstraps. Evolutionary analyses were conducted in MEGA X[3]. **(A)** Gag evolutionary history was inferred using the maximum-likelihood method and Tamura-Nei model using a discrete Gamma distribution to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1,4126)). The tree with the highest log likelihood (-27455.55) is shown. This analysis involved 113 nucleotide sequences and there were a total of 2651 positions in the final dataset. **(B)** Pol evolutionary history was inferred using the maximum-likelihood method and General Time Reversible model using a discrete Gamma distribution to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1.1361)). The tree with the highest log likelihood (-31587.82) is shown. This analysis involved 122 nucleotide and there were a total of 2719 positions in the final dataset. **(C)** Env evolutionary history was inferred using the maximum-likelihood method and Hasegawa-Kishino-Yano model using a discrete Gamma distribution to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1.7307)). The tree with the highest log likelihood (-31664.88) is shown. This analysis involved 125 nucleotide sequences and there were a total of 2382 positions in the final dataset.

T cells



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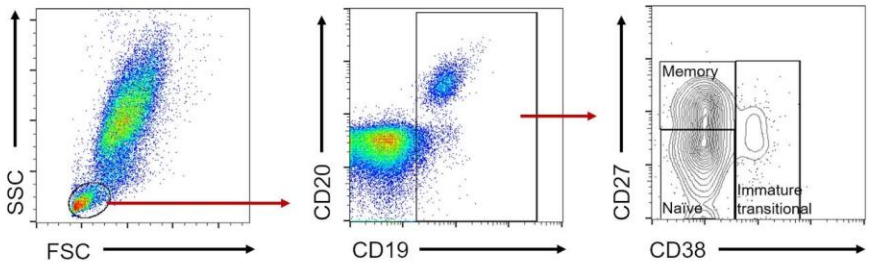
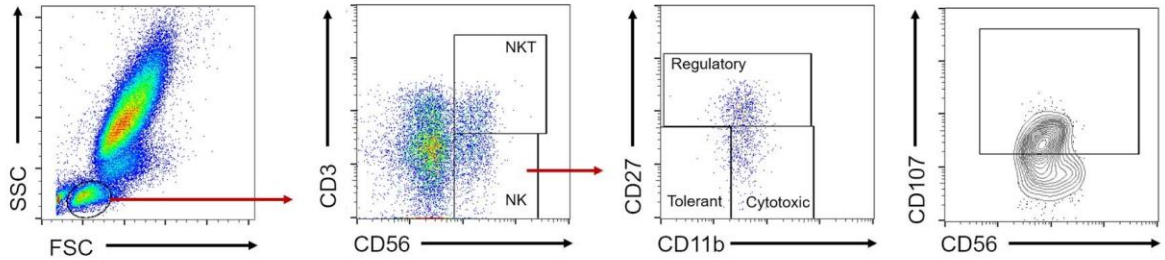
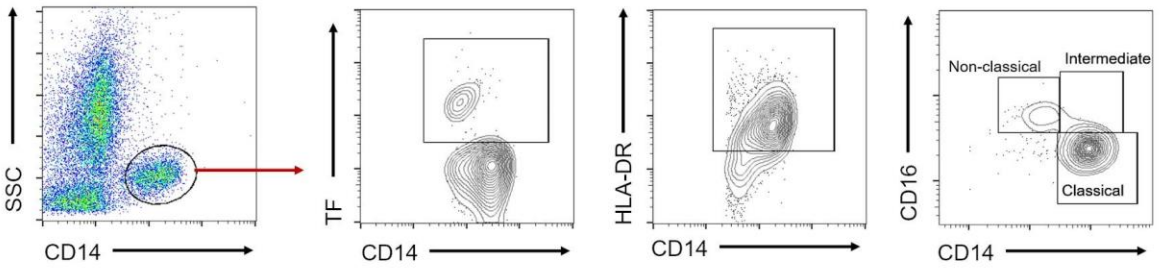


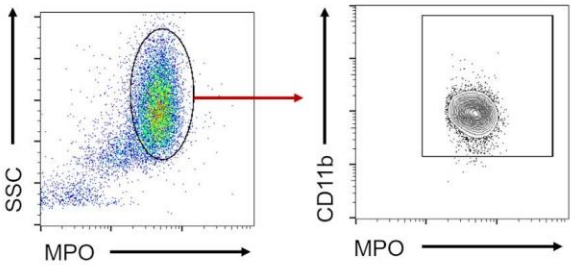
NK cells



Monocytes



Neutrophils



Supplementary Figure 2 - Gate strategy for the immune profiling of severe COVID-19 patients. (A) T cells were gated based on their characteristic forward and side scatters and CD3 positivity. T cells were phenotyped according to CD4 and CD8 expression. **(B)** B cells were gated by their characteristic forward and side scatters and the expression of CD19 and/or CD20, and then classified as naïve, immature/transitional or memory phenotypes based on the CD38 and CD27 expression patterns. **(C)** NK and NKT cells were identified by their characteristic forward and side scatters and the expression of CD56 only or CD56 plus CD3, respectively. NK cells were classified as tolerant, regulatory or cytotoxic phenotypes according to the CD11b and CD27 expression patterns. Cytotoxic activity was confirmed by CD107 surface expression. **(D)** Monocytes were identified according to their characteristic forward and side scatters and CD14 expression, and then classified as classical, intermediate or nonclassical phenotypes according to the CD14 and CD16 expression patters. TF and HLA-DR expression were evaluated. **(E)** Neutrophils were identified by to their characteristic forward and side scatters and MPO positivity. CD11b expression was evaluated.

References

1. Wölfel, R. *et al.* Virological assessment of hospitalized patients with COVID-2019. *Nature* **581**, 465–469 (2020).
2. Hadfield, J. *et al.* Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* **34**, 4121–4123 (2018).
3. Kumar, S., Nei, M., Dudley, J. & Tamura, K. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Br. Bioinform* **9**, 299–306 (2008).