Supplementary Materials

Supplementary Methods

Control group

We included 50 participants with respiratory symptoms and an elevated C-reactive protein (CRP >10 mg/dl), who presented to ED during similar period of time with negative SARS-CoV-2 test as control. Demographic information was reported in our prior study [1].

Unsupervised clustering

Principal components analysis (PCA) was performed using all proteins and all samples using the prcomp() function in R. Unsupervised clustering by UMAP was performed using all proteins, and either all samples or just Day 0 samples, using the umap() function in R. UMAP coordinates were plotted using the ggplot2 package.

SomaScan plasma proteomic profiling

The SomaScan Platform was reported in our previous study. Briefly, 4979 SOMAmer reagents, single-stranded DNA aptamers were used to detect 4776 unique human protein targets with standard controls [1].

Supplementary Tables

Supplementary Table S1. Association of SARS-CoV-2 viral load and clinical outcomes, detailed table including other clinical and laboratory variables.

	Univariate OR (95% CI)	P	Multivariate OR (95% CI)	P
Severe disease				
SARS-CoV-2 viremia				
Aviremic or below quantification range	Reference		Reference	
Viremic ≥ 2 log copies/ml	12.56 (5.96, 26.46)	<0.001	10.59 (4.40, 25.51)	**<0.001**
Age				
<50 years	Reference		Reference	
50-64 years	2.01 (1.00, 4.04)	0.049	1.06 (0.43, 2.59)	0.91
≥65 years	5.32 (2.82, 10.06)	<0.001	2.58 (1.02, 6.52)	0.045
Female (male as reference)	0.71 (0.44, 1.14)	0.16		
Non-Caucasian (Caucasian as reference)	1.42 (0.88, 2.29)	0.15		
Morbid obesity (BMI≥40 kg/m^2)				
No	Reference			
Yes	0.86 (0.40, 1.85)	0.69		
Unknown	0.43 (0.14, 1.32)	0.14		
Heart diseases	1.86 (0.98, 3.50)	0.06	2.05 (0.79, 5.29)	0.14
Lung diseases	0.49 (0.26, 0.92)	0.03	0.32 (0.13, 0.80)	**0.02**
Hypertension	2.09 (1.29, 3.38)	0.003	0.87 (0.41, 1.82)	0.70
Diabetes	1.97 (1.21, 3.21)	0.007	1.58 (0.80, 3.11)	0.19
Immunocompromised conditions	2.53 (1.11, 5.80)	0.03	1.93 (0.69, 5.41)	0.21
Lymphopenia <1000 cells/mm^3	2.83 (1.73, 4.64)	<0.001	2.00 (1.04, 3.84)	**0.03**
Creatinine elevation >1.20 mg/dl	3.93 (2.21, 7.00)	<0.001	2.12 (0.92, 4.90)	0.08
CRP>100 mg/dl				
No	Reference		Reference	
Yes	4.75 (2.80, 8.08)	<0.001	3.15 (1.60, 6.19)	**0.001**
Unknown	0.85 (0.18, 4.11)	0.84	1.72 (0.06, 46.51)	0.75
D-dimer>1000 ng/ml				
No	Reference		Reference	
Yes	3.21 (1.92, 5.39)	<0.001	1.40 (0.71, 2.76)	0.34
Unknown	0.79 (0.21 ,2.96)	0.73	0.63 (0.03, 12.03)	0.76
Troponin elevation within 72 hours	6.41 (2.46, 16.70)	<0.001	3.74 (1.01, 13.83)	**0.048**
Death within Day 28				
SARS-CoV-2 viremia				
Aviremic or below quantification range	Reference		Reference	
Viremic ≥ 2 log copies/ml	4.39 (2.15, 8.96)	<0.001	3.86 (1.47, 10.14)	**0.006**
Age				
<50 years	Reference		Reference	
50-64 years	3.43 (0.35, 33.65)	0.29	1.98 (0.16, 25.25)	0.60
≥65 years	43.39 (5.82, 323.31)	<0.001	22.61 (2.07, 246.40)	**0.01**
Female (male as reference)	0.83 (0.43, 1.60)	0.57		
Non-Caucasian (Caucasian as reference)	0.47 (0.24, 0.93)	0.03	0.73 (0.29, 1.84)	0.50
Morbid obesity (BMI≥40 kg/m^2)				
No	Reference			
Yes	0.86 (0.29, 2.61)	0.80		
Unknown	1.11 (0.31, 3.97)	0.88		
Heart diseases	4.24 (2.03, 8.88)	<0.001	2.97 (1.01, 8.70)	**0.048**
Lung diseases	1.04 (0.47, 2.32)	0.92		
Hypertension	3.52 (1.69, 7.34)	0.001	0.77 (0.28, 2.09)	0.61
Diabetes	1.16 (0.59, 2.29)	0.66		
Immunocompromised conditions	1.66 (0.59, 4.70)	0.34		
Lymphopenia <1000 cells/mm^3	7.42 (3.02, 18.25)	<0.001	6.62 (2.19, 20.00)	**0.001**
Creatinine elevation >1.20 mg/dl	5.98 (2.99, 12.01)	<0.001	2.36 (0.91, 6.08)	0.08

CRP>100 mg/dl				
No	Reference		Reference	
Yes	2.83 (1.35, 5.93)	0.006	1.78 (0.67, 4.68)	0.25
Unknown	2.38 (0.46, 12.26)	0.30	87.47 (1.52, 5038.05)	**0.03**
D-dimer>1000 ng/ml				
No	Reference		Reference	
Yes	3.42 (1.56, 7.50)	0.002	1.40 (0.49, 3.99)	0.53
Unknown	1.97 (0.39, 10.03)	0.42	0.15 (0.004, 6.51)	0.33
Troponin elevation within 72 hours	3.68 (1.46, 9.27)	0.006	0.91 (0.26, 3.23)	0.89

Supplementary Table S2. Association of SARS-CoV-2 viral load and clinical outcomes, sensitivity analyses.

	Univariate OR (95% CI)	P	Multivariate OR (95% CI)	P
Severe diseases[a]				
SARS-CoV-2 viral load accounting for detectable but non-quantifiable viremia				
Aviremic	Reference		Reference	
Viremic but below quantification range	1.91 (0.99, 3.66)	0.054	2.01 (0.91, 4.42)	0.08
Viremic ≥ 2 log copies/ml	14.66 (6.82, 31.53)	<0.001	11.73 (4.79, 28.74)	<0.001
SARS-CoV-2 viral load as continuous variable				
Viral load (per 1 log copy/ml increase)	2.57 (1.93, 3.43)	<0.001	2.49 (1.75, 3.54)	<0.001
Death at day 28[b]				
SARS-CoV-2 viral load accounting for detectable but non-quantifiable viremia				
Aviremic	Reference		Reference	
Viremic but below quantification range	1.58 (0.62, 4.05)	0.34	1.17 (0.36, 3.83)	0.79
Viremic ≥ 2 log copies/ml	4.92 (2.30, 10.53)	<0.001	4.01 (1.46, 11.06)	0.007
SARS-CoV-2 viral load as continuous variable				
Viral load (per 1 log copy/ml increase)	1.67 (1.33, 2.10)	<0.001	1.46 (1.08, 1.97)	0.01

a, multivariate models were adjusted for age groups, heart diseases, lung diseases, hypertension, diabetes, immunocompromised conditions, lymphopenia, creatinine elevation, C reactive protein elevation, D-dimer elevation and troponin elevation. Seen in Table 2.

b, multivariate models were adjusted for age groups, race, heart diseases, hypertension, lymphopenia, creatinine elevation, C reactive protein elevation, D dimer elevation, troponin elevation. Seen in Table 2.

Supplementary Table S3. Clinical factors associated with viremia.

	Univariate OR (95% CI)	P	Multivariate OR (95% CI)	P
Age				
<50 years	Reference		Reference	
50-64 years	1.82 (0.77, 4.29)	0.17	1.33 (0.53, 3.34)	0.54
≥65 years	2.68 (1.23, 5.84)	0.01	1.75 (0.69, 4.44)	0.24
Female (male as reference)	0.60 (0.33, 1.11)	0.10		
Non-Caucasian (Caucasian as reference)	1.52 (0.83, 2.76)	0.18		
Morbid obesity (BMI≥40 kg/m^2)				
No	Reference			
Yes	1.24 (0.51, 3.04)	0.64		
Unknown	0.51 (0.11, 2.29)	0.38		
Heart diseases	0.66 (0.26, 1.65)	0.37		
Lung diseases	0.42 (0.17, 1.02)	0.06	0.40 (0.15, 1.07)	0.07
Hypertension	1.29 (0.71, 2.33)	0.41		
Diabetes	2.34 (1.28, 4.27)	0.006	2.15 (1.13, 4.10)	**0.02**
Immunocompromised conditions	1.18 (0.42, 3.31)	0.75		
Lymphopenia <1000 cells/mm^3	1.86 (1.01, 3.43)	0.045	1.38 (0.70, 2.71)	0.35
Creatinine elevation >1.20 mg/dl	2.01 (1.04, 3.88)	0.04	1.29 (0.58, 2.87)	0.53
CRP>100 mg/dl				
No	Reference		Reference	
Yes	3.22 (1.66, 6.25)	0.001	2.29 (1.10, 4.75)	**0.03**
Unknown	0.83 (0.10, 6.93)	0.86	0.41 (0.02, 8.45)	0.47
D-dimer>1000 ng/ml				
No	Reference		Reference	
Yes	1.98 (1.05, 3.75)	0.04	1.12 (0.54, 2.32)	0.76
Unknown	0.97 (0.20, 4.67)	0.98	2.36 (0.24, 23.28)	0.46
Troponin elevation within 72 hours	2.57 (1.04, 6.36)	0.04	1.90 (0.64, 5.68)	0.25

Supplementary Table S4. Differentially expressed proteins related to peripheral blood cells.

List of protein derived from Monaco et al., Cell Rep 2019 [2].

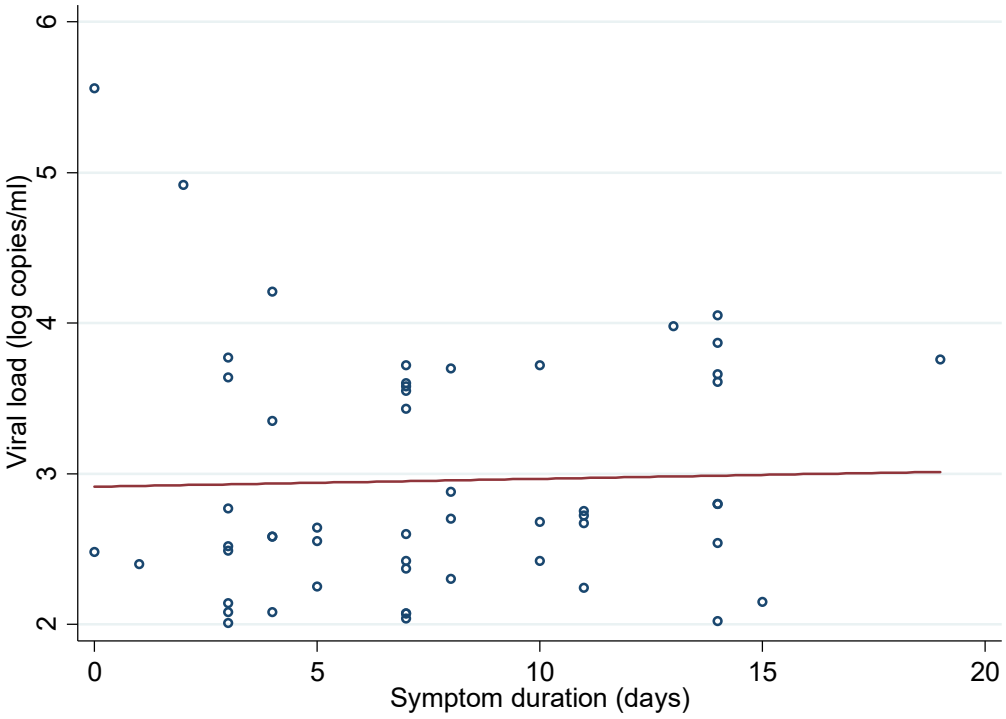
Protein	NPX difference (Viremic- Aviremic)	Adjusted P value (without severity as covariate)
Monocytes		
IL6	1.71	2.12E-15
CCL7	1.31	4.00E-14
CCL20	1.12	1.06E-09
CXCL10	0.96	1.99E-10
VSIG4	0.95	1.06E-13
CTSL	0.65	2.80E-13
PTX3	0.61	3.61E-09
CD300E	0.59	4.00E-08
CXCL11	0.58	3.59E-06
PVR	0.55	1.81E-10
VCAN	0.52	2.10E-07
CLEC5A	0.52	7.25E-09
CCL3	0.50	4.30E-06
CD14	0.50	8.22E-07
CLEC4D	0.50	3.81E-05
CCL8	0.48	4.89E-05
VMO1	0.45	9.60E-05
SIGLEC10	0.44	5.34E-08
CXCL16	0.43	4.09E-08
CES1	0.42	1.18E-03
HMOX1	0.40	2.13E-04
LRP1	0.40	1.31E-09
SIGLEC1	0.39	1.00E-07
NID1	0.39	1.09E-07
CLEC1B	0.38	1.10E-02
TNFRSF8	0.37	4.84E-05
MSR1	0.36	4.37E-06
CD300LF	0.36	1.41E-02
CCL13	0.36	4.50E-04
TYMP	0.35	6.65E-04
SPINT1	0.31	3.22E-06
CST3	0.30	2.30E-03
CD163	0.29	2.50E-05
CD93	0.28	1.52E-05
LILRA5	0.27	5.57E-06
PLXNB2	0.26	1.88E-06
OSCAR	0.24	1.91E-04
CD302	0.22	3.73E-02
SLAMF8	0.21	1.77E-03
HBEGF	0.21	2.34E-02
LILRB2	0.20	3.71E-04
C1QA	0.18	1.37E-02
CPVL	0.18	3.48E-02
Neutrophils		
CHI3L1	1.23	4.82E-14
IL1RN	0.92	6.62E-11
ADM	0.73	1.91E-10
CD177	0.51	1.18E-05
TNFSF13B	0.50	1.54E-08
S100A12	0.48	3.66E-09
VNN2	0.45	1.20E-05
SIRPB1	0.41	5.11E-06
PRTN3	0.41	9.02E-07

S100P	0.37	5.76E-04
SIGLEC5	0.37	1.50E-02
LCN2	0.35	6.28E-04
MMP9	0.28	4.88E-03
DEFA1	0.24	3.28E-02
Plasmablasts		
CD138/SDC1	0.83	4.73E-06
RRM2	0.59	6.86E-07
MZB1	0.46	1.09E-04
TXNDC5	0.44	1.71E-04
CD4+T cells		
TNFRSF4	0.26	7.89E-03
NK cells		
NCR1	0.17	2.35E-02

Bold font indicates statistical significance after further adjustment for severity. Of note, NPX is already a Log_2 transformed index.

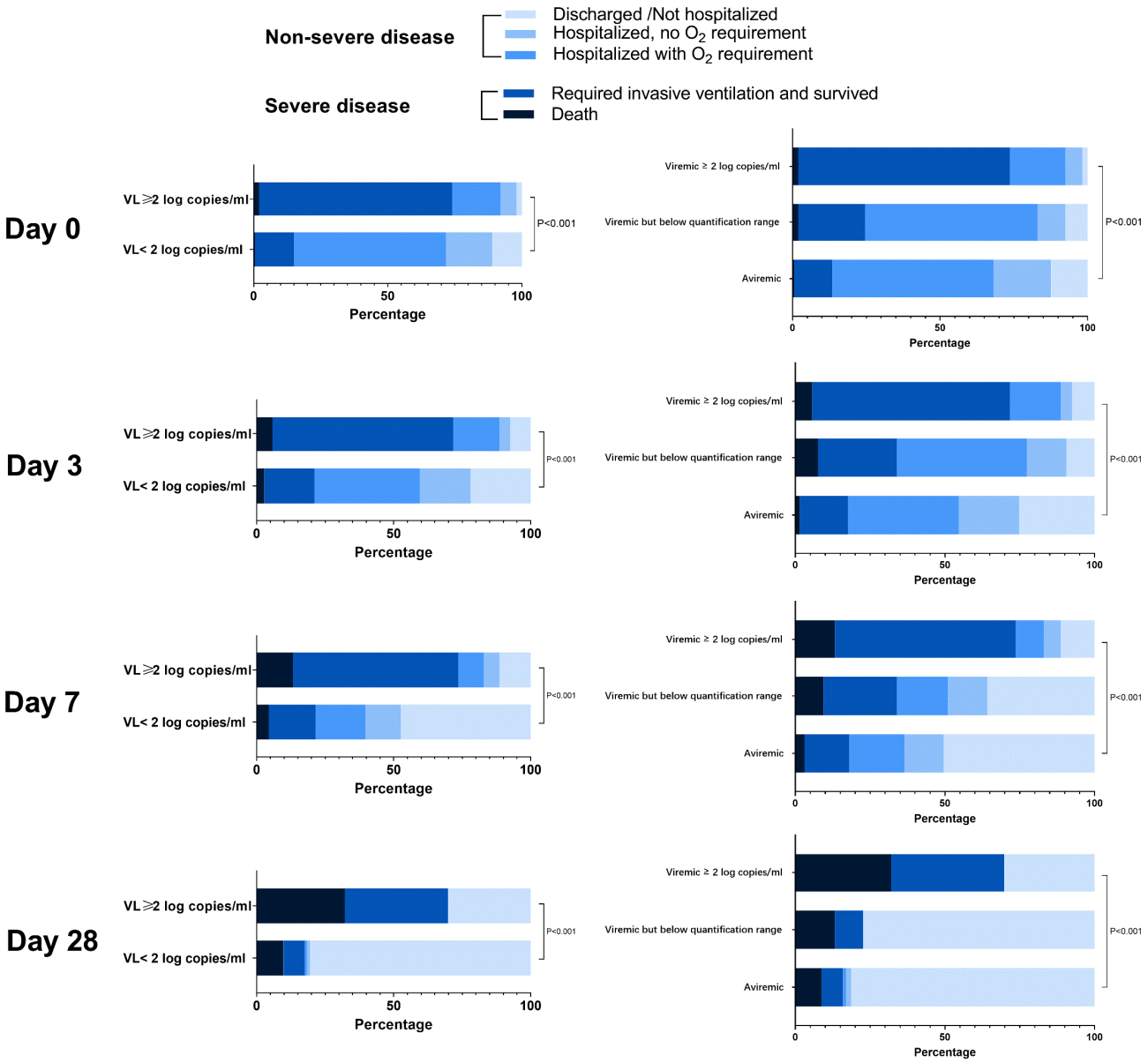
Supplementary Figures

Supplementary Figure S1. Correlation between viral load and duration between symptom onset and ED presentation.

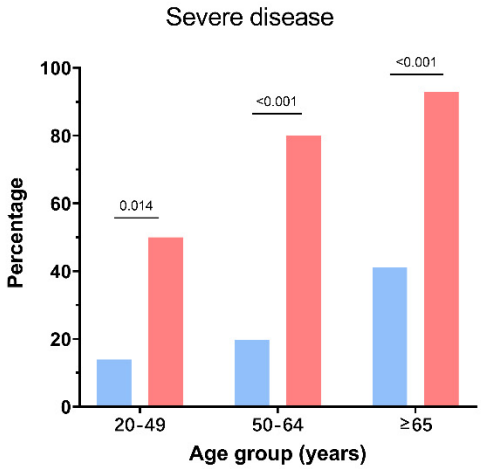


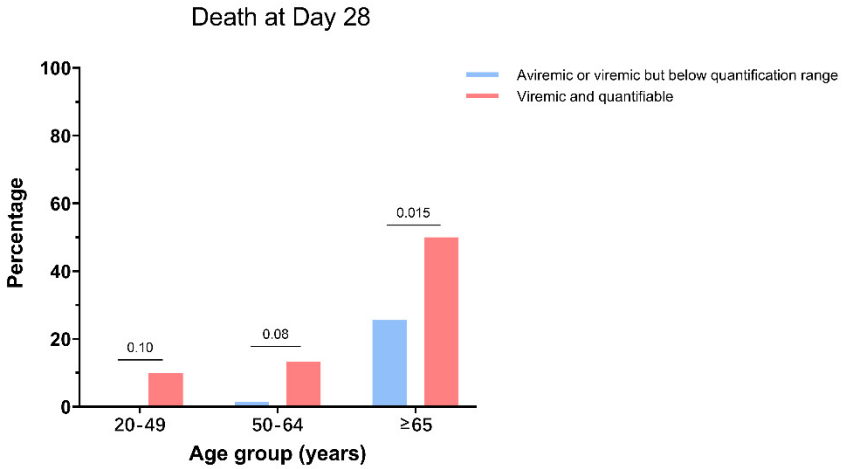
Supplementary Figure S2. Snapshots of disease severity at Day 0, 3, 7 and 28.

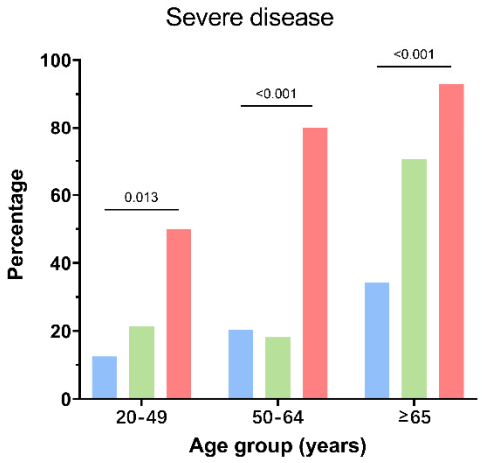
The left column was grouped by quantifiable viremia (≥ 2 log copies/ml) and undetectable or unquantifiable viremia (< 2 log copes/ml). The right column was grouped by quantifiable viremia, unquantifiable viremia (detectable but < 2 log copies/ml) and undetectable viremia (aviremic). Chi-squared test or Fisher's exact test was used.

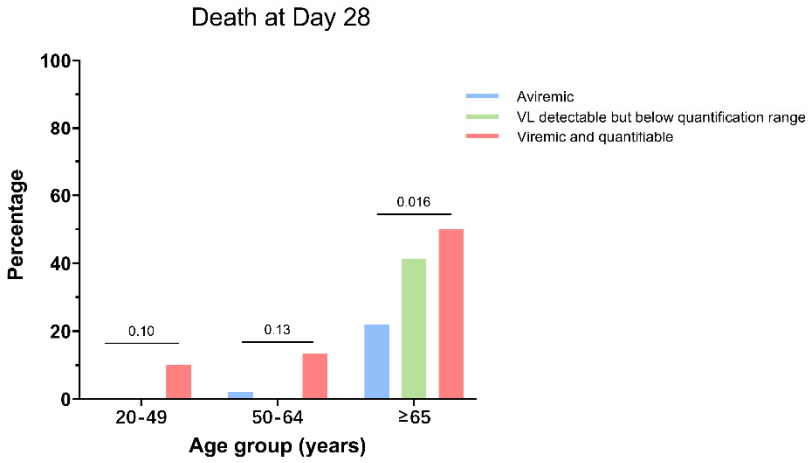


Supplementary Figure S3. Association of viremia and disease severity stratified by age groups.
P values were calculated by either chi-squared test or Fisher's exact test.



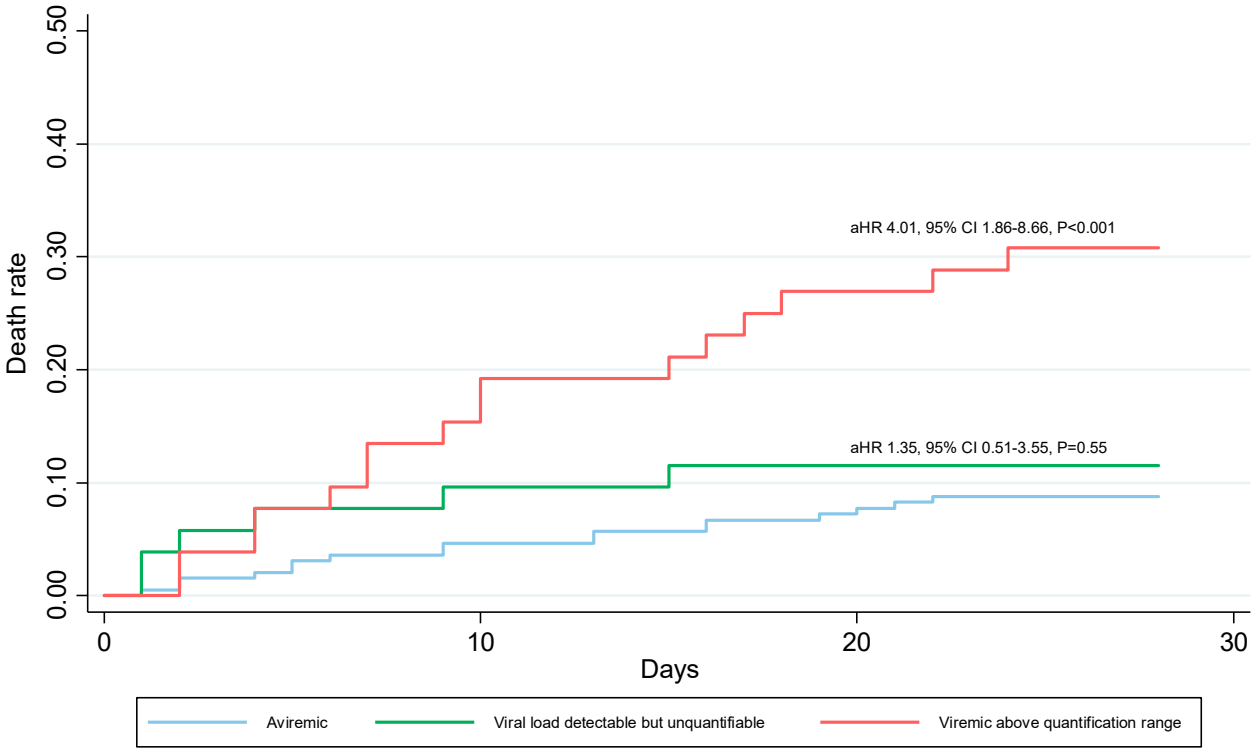






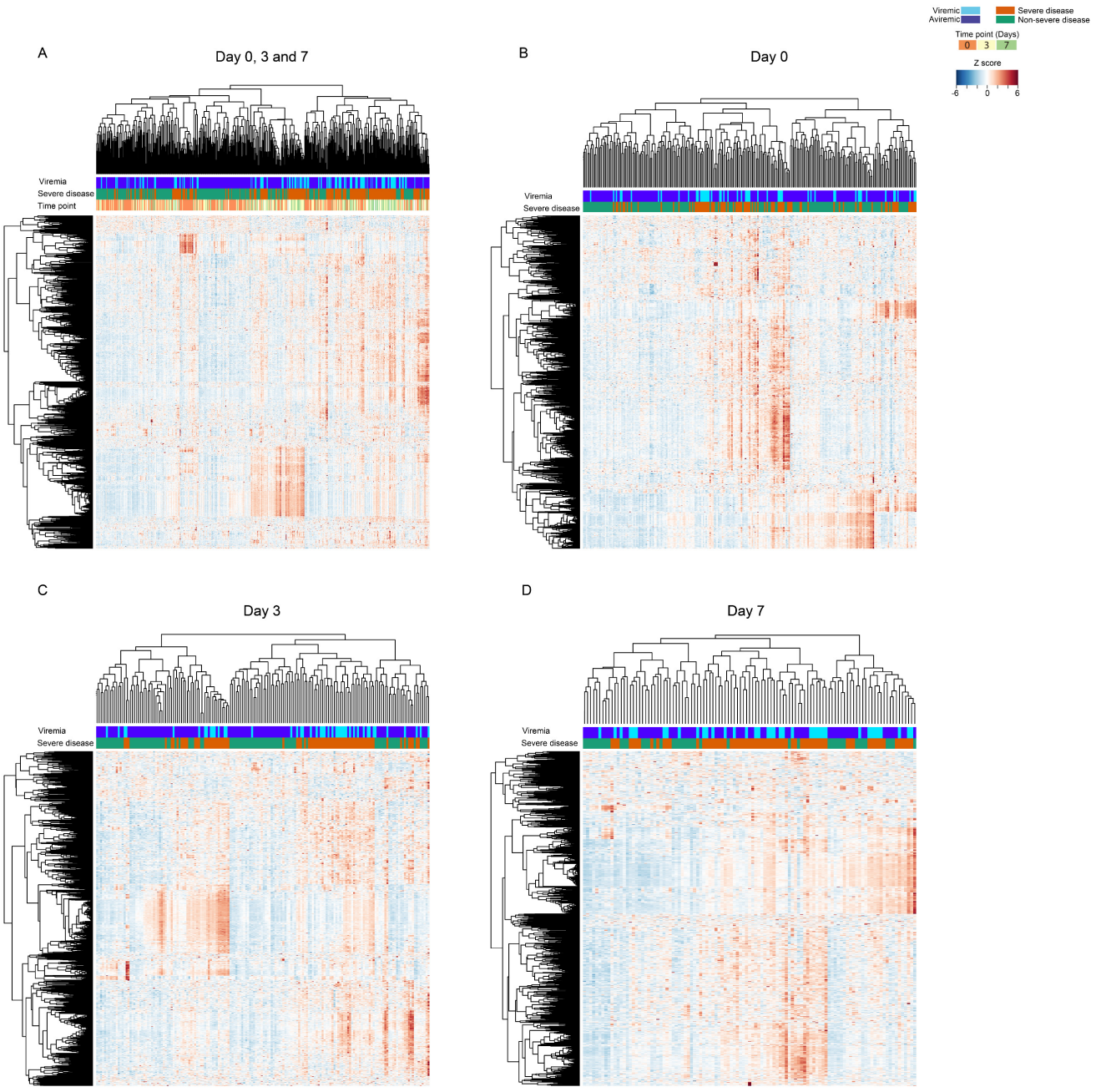
Supplementary Figure S4. 28-day mortality among different viremic groups.

Adjusted hazard ratio (aHR) was calculated using Cox Proportional regression adjusting for baseline characteristics including age, sex, race, BMI and laboratory values. Aviremic group served as reference.



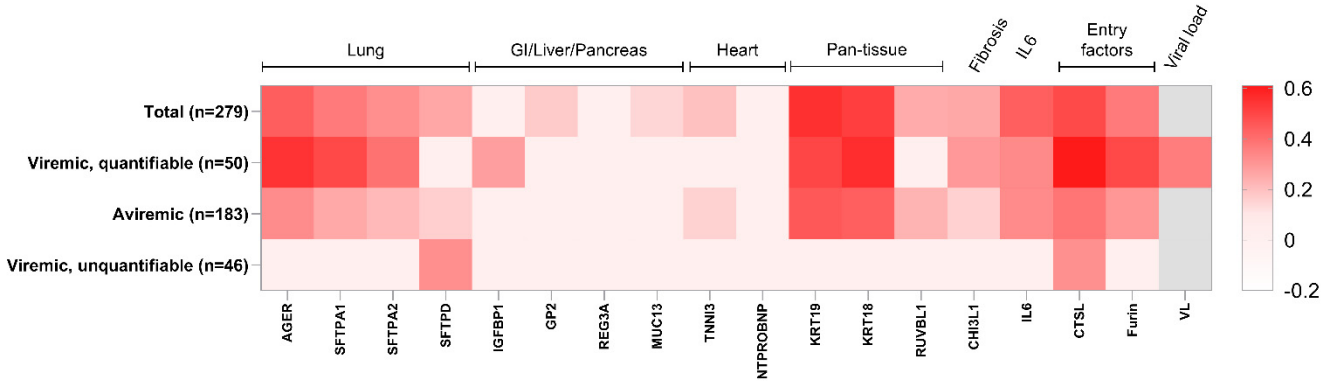
Supplementary Figure S5. Heatmap highlighting differentially expressed proteins between viremic and aviremic participants.

(A) Heatmap derived from Olink proteomics from all three time points. (B)-(D) Heatmap from Day 0, 3, and 7, respectively.



Supplementary Figure S6. Correlation between LDH, tissue-enriched protein levels, fibrosis markers, IL6, entry factors and viral load.

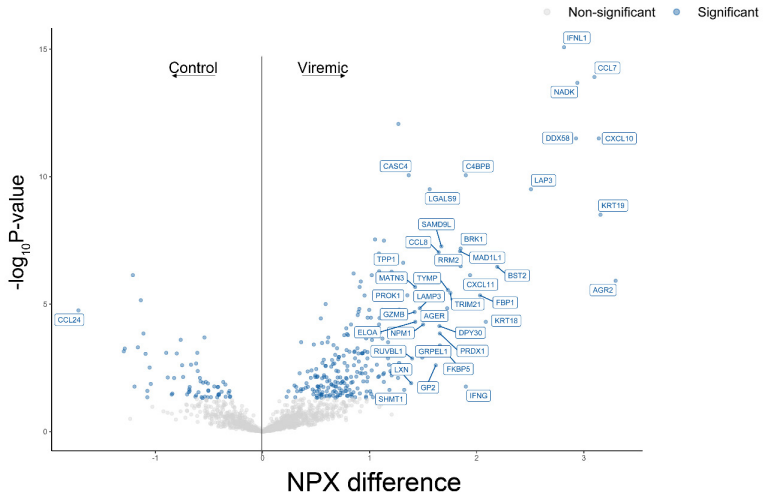
Spearman correlation (rho) with P value <0.05 was demonstrated in this figure.



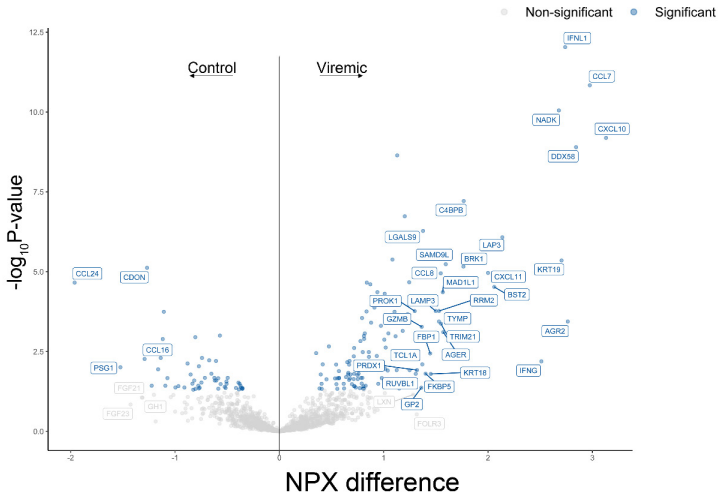
Supplementary Figure S7. Volcano plots showing NPX differences in protein levels between viremic and COVID-19 negative control participants at Day 0.

(A) Volcano plot generated by linear model without severity as a covariate. (B) Overlap of differentially expressed proteins derived from viremic vs. aviremic group comparisons and viremic vs. control group comparison, without severity as a covariate. (C) Volcano plot generated by linear model with severity as a covariate. (D) Overlap of differentially expressed proteins derived from viremic vs. aviremic group comparisons and viremic vs. control group comparison, with severity as a covariate.

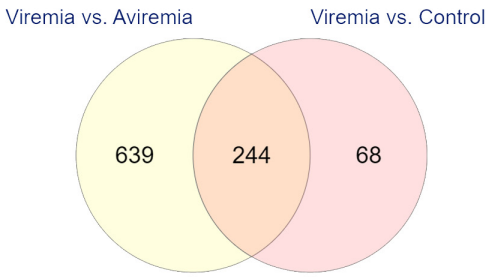
A



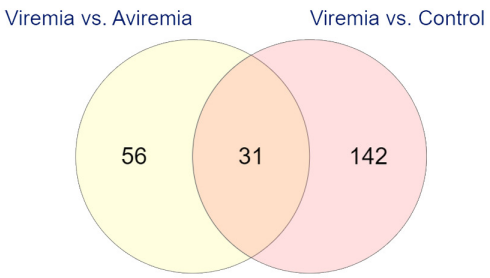
C



B

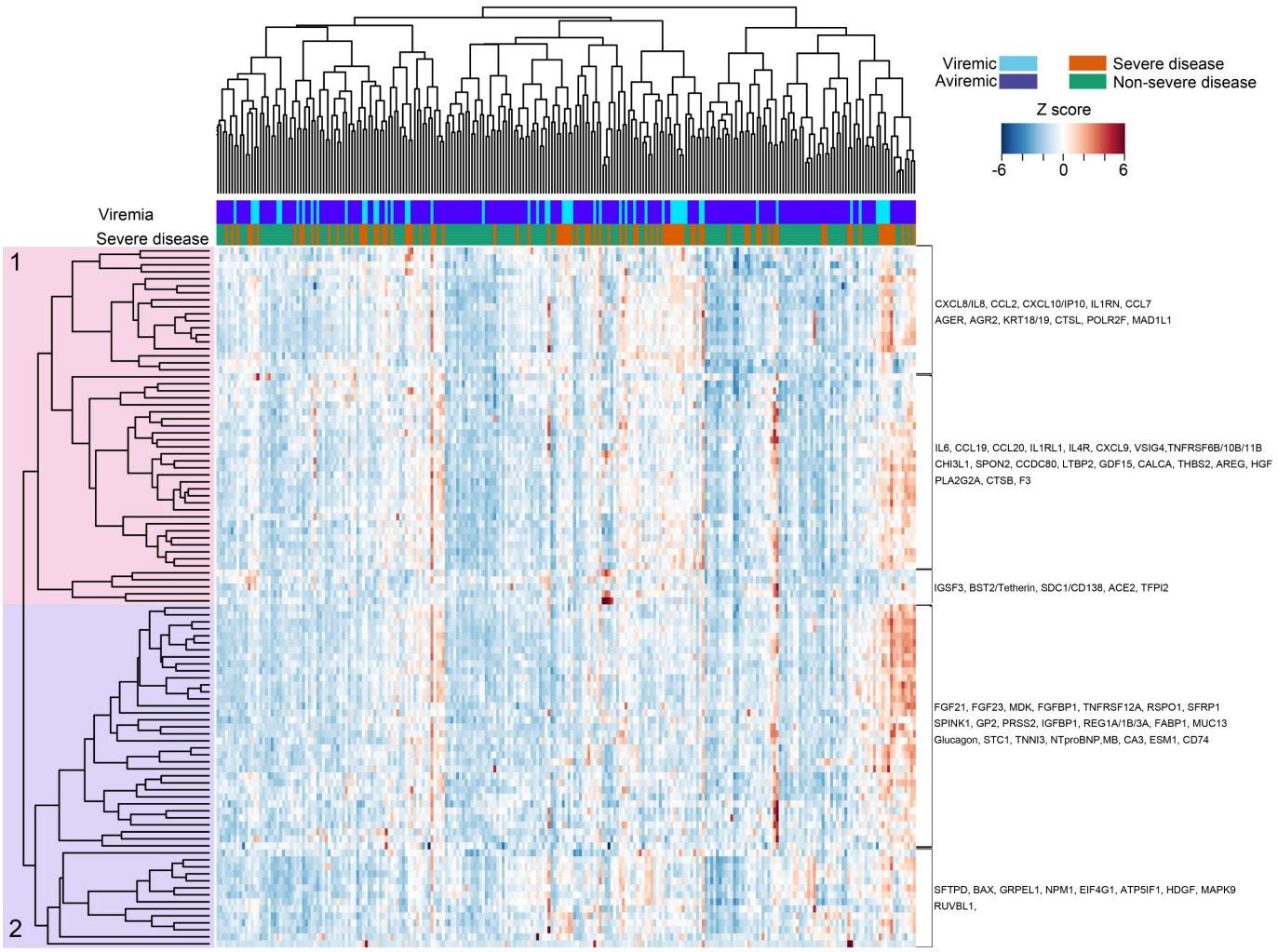


D



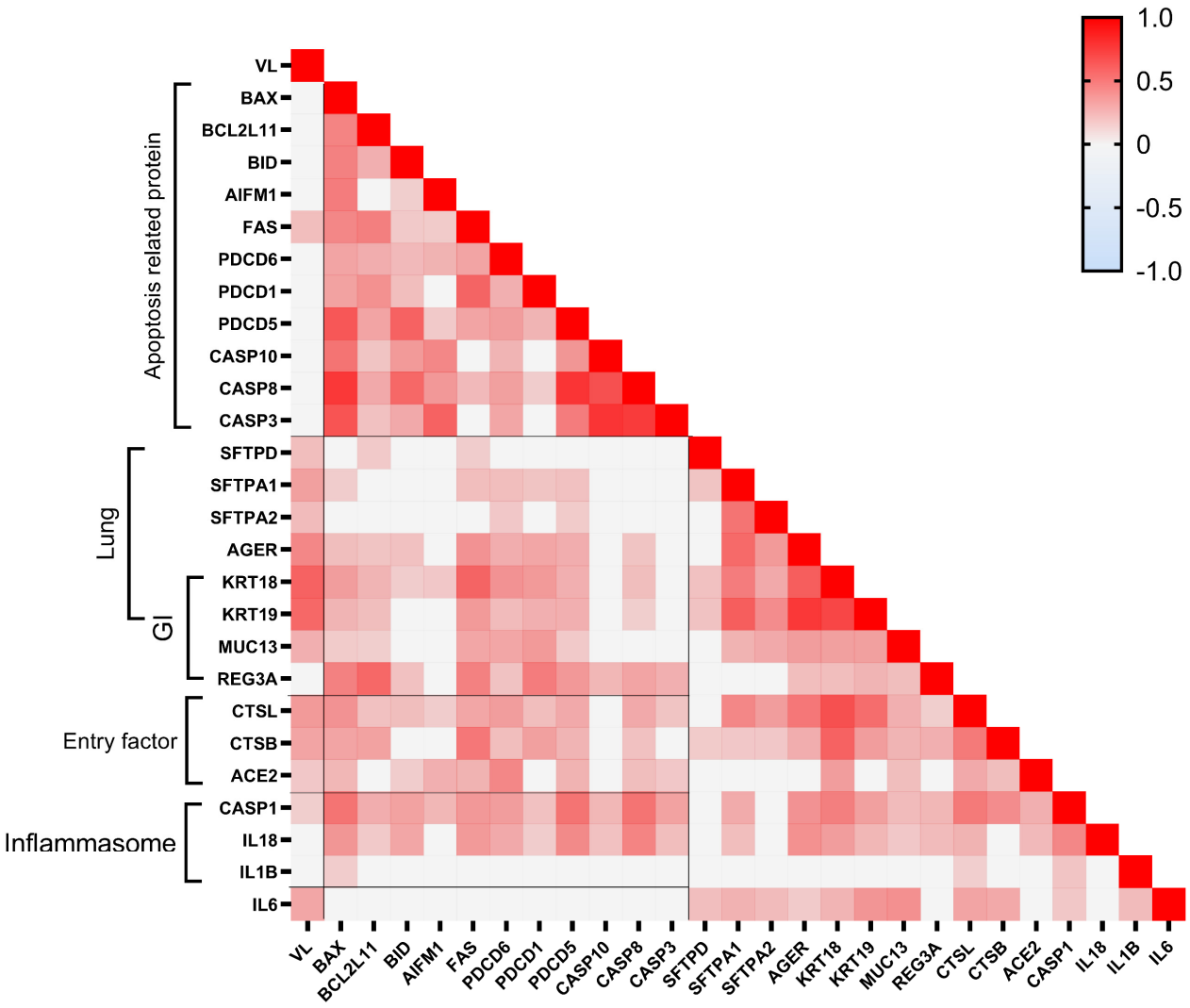
Supplementary Figure S8. Heatmap of the top 100 differentially expressed proteins between viremic and aviremic participants at Day 0.

Each row represents expression of an individual protein over the entire cohort; each cell represents the Z score of protein expression for all measurements across a row. Selected proteins are indicated.



Supplementary Figure S9. Correlation between apoptosis-related protein, lung/GI tract related protein, pyroptosis-related protein and IL6.

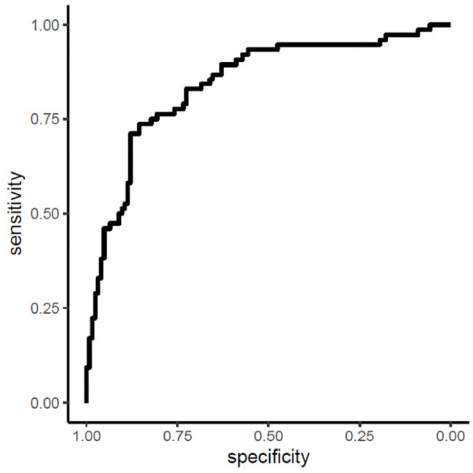
Spearman correlation was performed in this analysis and only correlation value (rho) with a P<0.05 were shown in this graph in color (rho with P>0.05 was left in blank).



Supplementary Figure S10. Receiver operating characteristic (ROC) curve showing predictive performance of an elastic net logistic regression classifier of disease severity and viremia for Olink proteins.

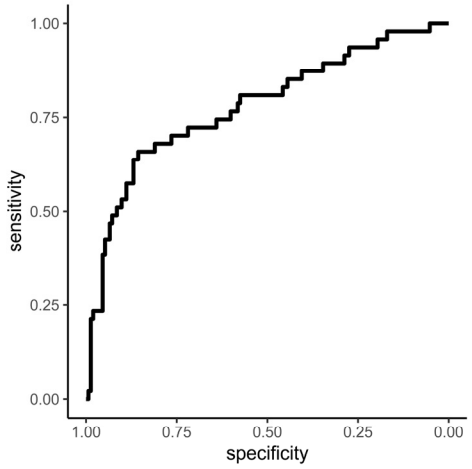
Only participants with viremia above quantification range and undetectable were included in this analysis. Performance was evaluated using 100 repeats of 5-fold cross validation. (A) Predictors for severe disease. Mean area under the curve (AUC) with 95% confidence intervals (CI) was 0.83, 95%CI (0.80, 0.86). Top ten predictors are listed here. (B) Predictors for viremia. Mean AUC with 95% CI was 0.81, 95% CI (0.78, 0.83). Top ten predictors are listed here.

A



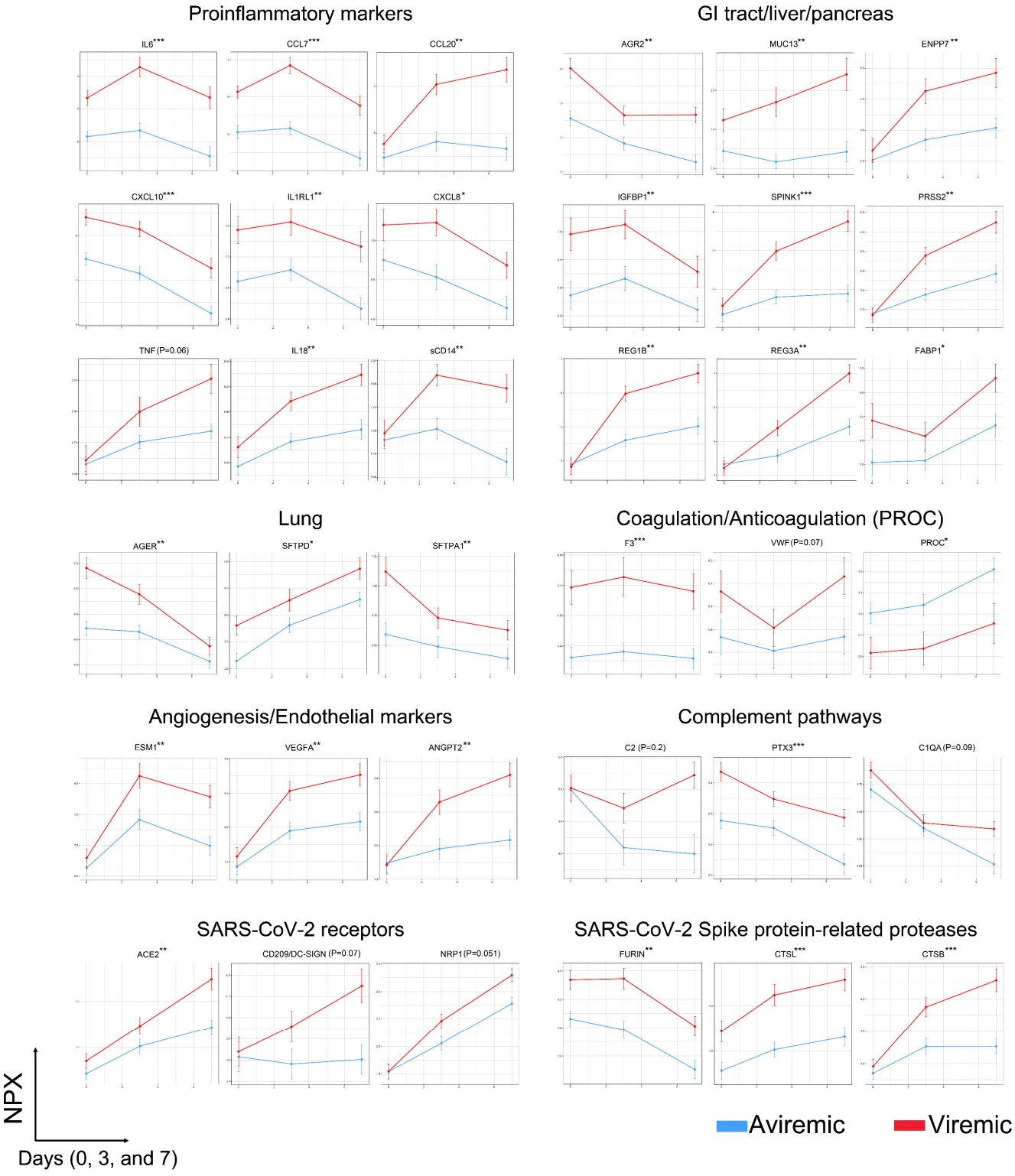
Assay	Mean Coefficient	UniProt
Viremia	88.0	
SEMA4D	39.8	Q92854
ADGRG1	21.0	Q9Y653
NDRG1	19.6	Q92597
GBP2	10.4	P32456
DNER	10.1	Q8NFT8
TNFRSF11B	8.1	O00300
KRT19	7.9	P08727
FASLG	7.8	P48023
IL6	7.7	P05231

B



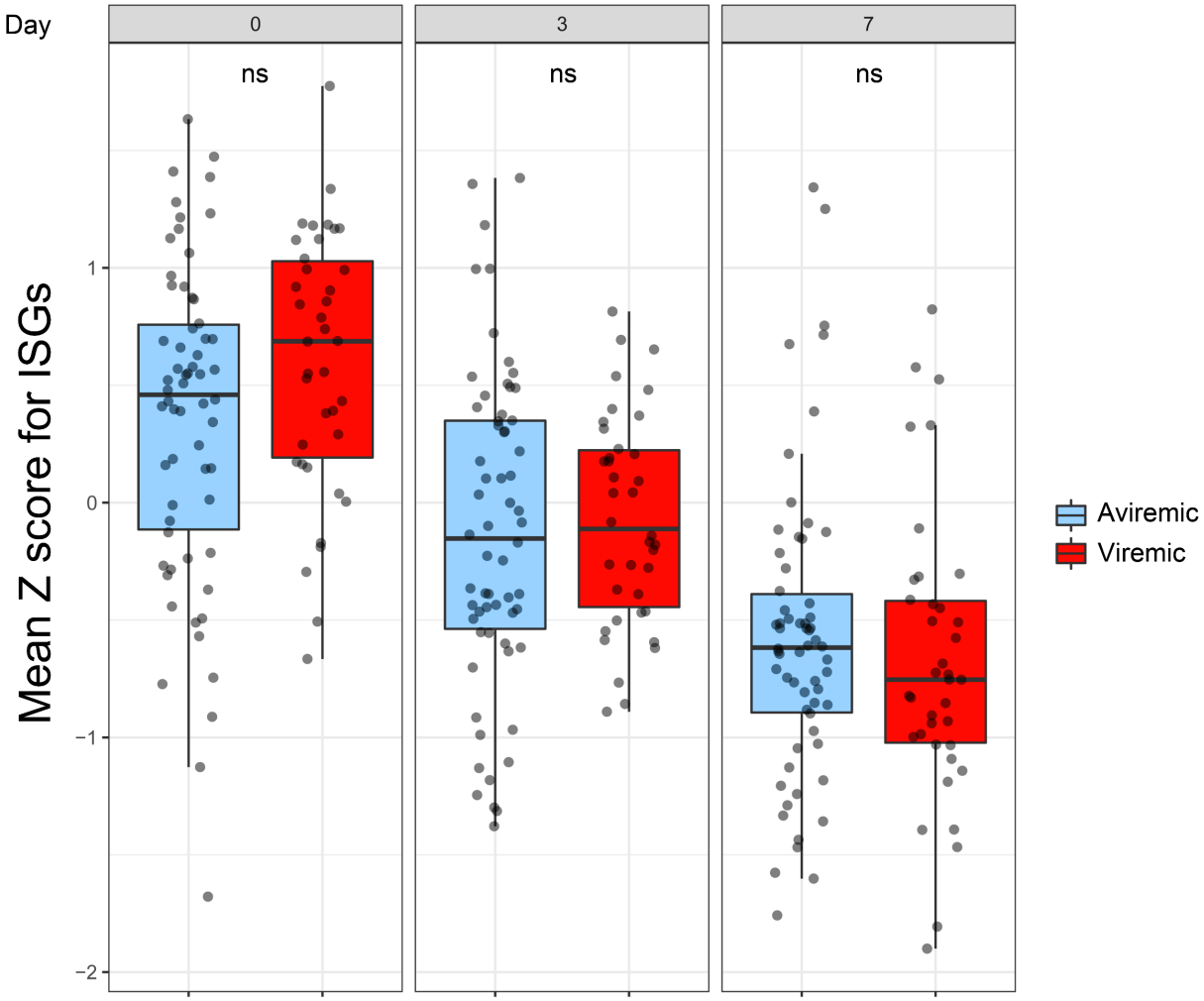
Assay	Mean Coefficient	UniProt
PAMR1	80.0	Q6UXH9
AMN	53.9	Q9BXJ7
BAG3	41.5	O95817
AGER	35.4	Q15109
KRT19	28.6	P08727
NADK	17.3	O95544
IL6	13.0	P05231
ANG	11.8	P03950
PRDX1	11.0	Q06830
IL7R	10.4	P16871

Supplementary Figure S11. Temporal trend of differentially expressed protein identified at Day 0.
Protein levels (NPX) at Day 0, 3, and 7 were demonstrated. Differences between viremic and aviremic groups were evaluated using the linear mixed model. Error bars indicate standard error of mean. *, P<0.05; **, P<0.01; ***, P<0.001.



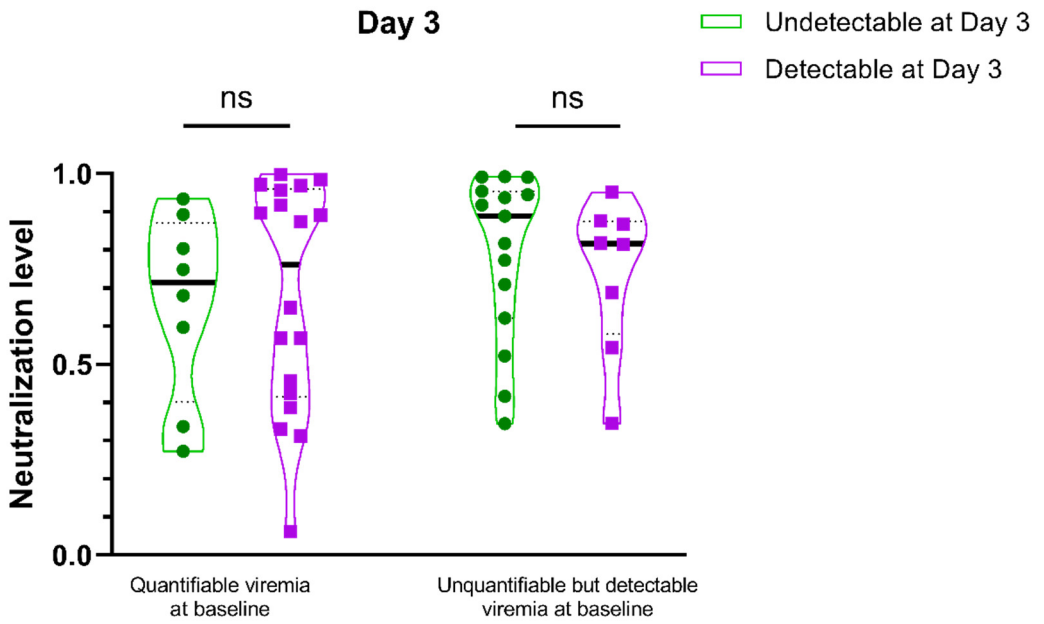
Supplementary Figure S12. Mean Z scores of interferon-stimulated genes (ISGs) based on SomaScan results.

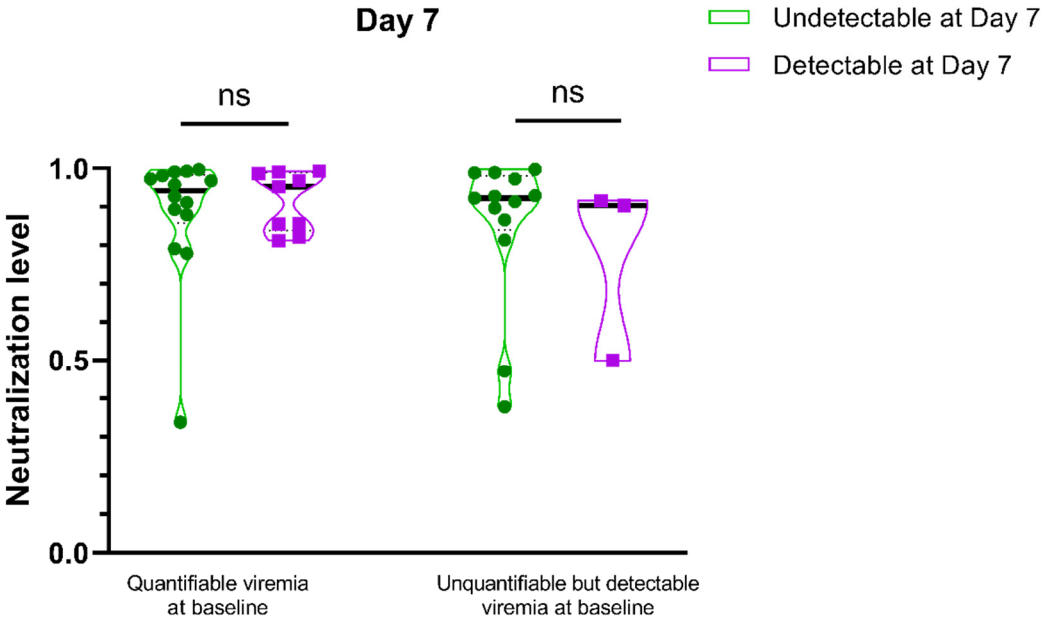
ns, not significant.



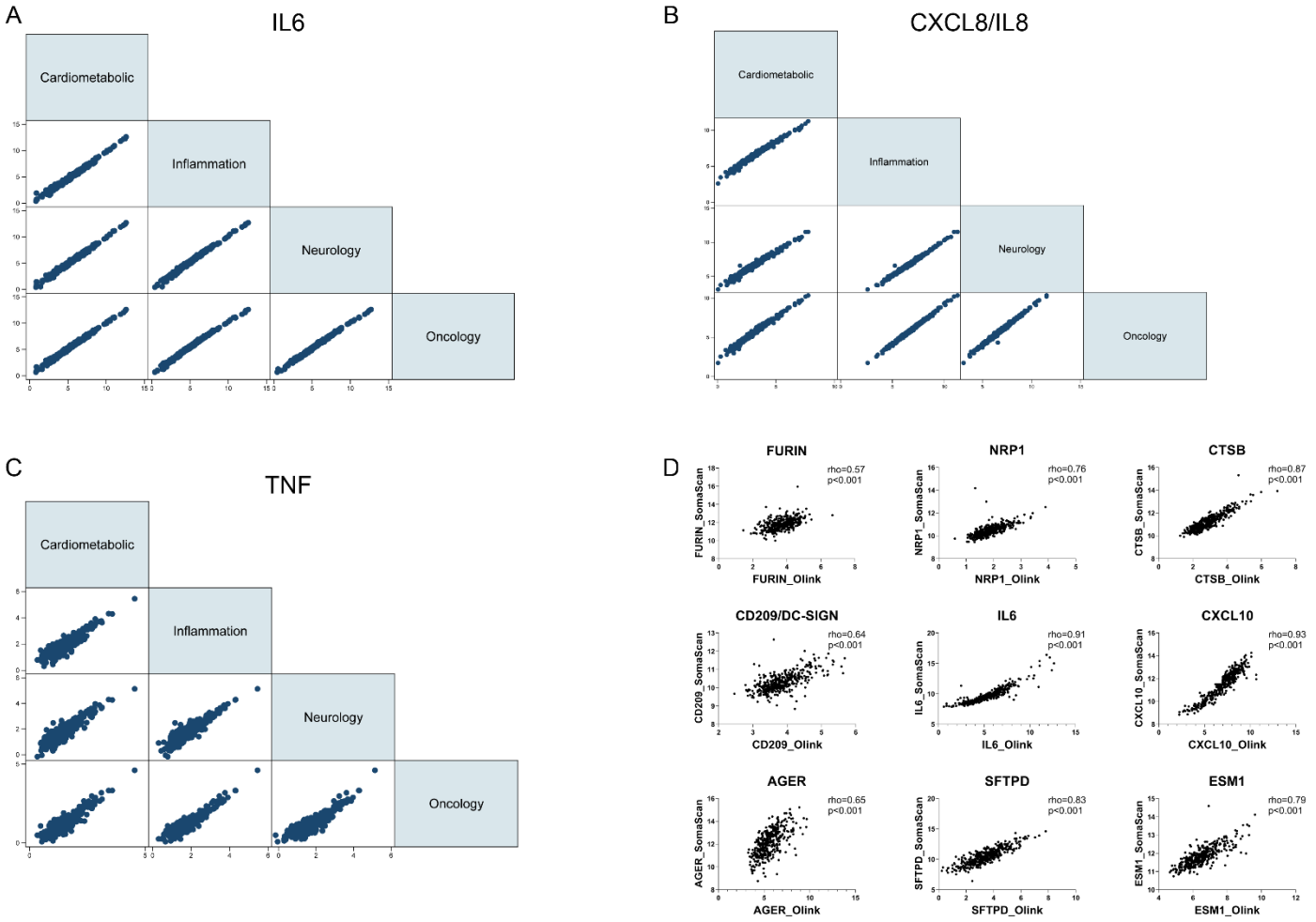
Supplementary Figure S13. Neutralization level at Day 3 and Day 7.

A subgroup of participants with undetectable viral load or detectable viral load (quantifiable or unquantifiable) at Day 3 (n=49) or Day 7 (n=39) were included in this analysis. Mann-Whitney test was used to compare the difference between two different groups. ns, not significant.





Supplementary Figure S14. Correlation of IL6, CXCL8/IL8, and TNF levels from four different Olink panels. (A-C) IL6, CXCL8/IL8 and TNF levels were repeated in four different Olink panels. Day 0 levels were displayed here and showed great correlation among Cardiometabolic, Inflammatory, Neurology and Oncology Olink panels. (D) Correlation between Olink proteomic panel and SomaScan proteomic panel.



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