Supplementary Information

SARS-CoV-2 RNAemia and proteomic biomarker trajectory inform prognostication in COVID-19 patients admitted to intensive care

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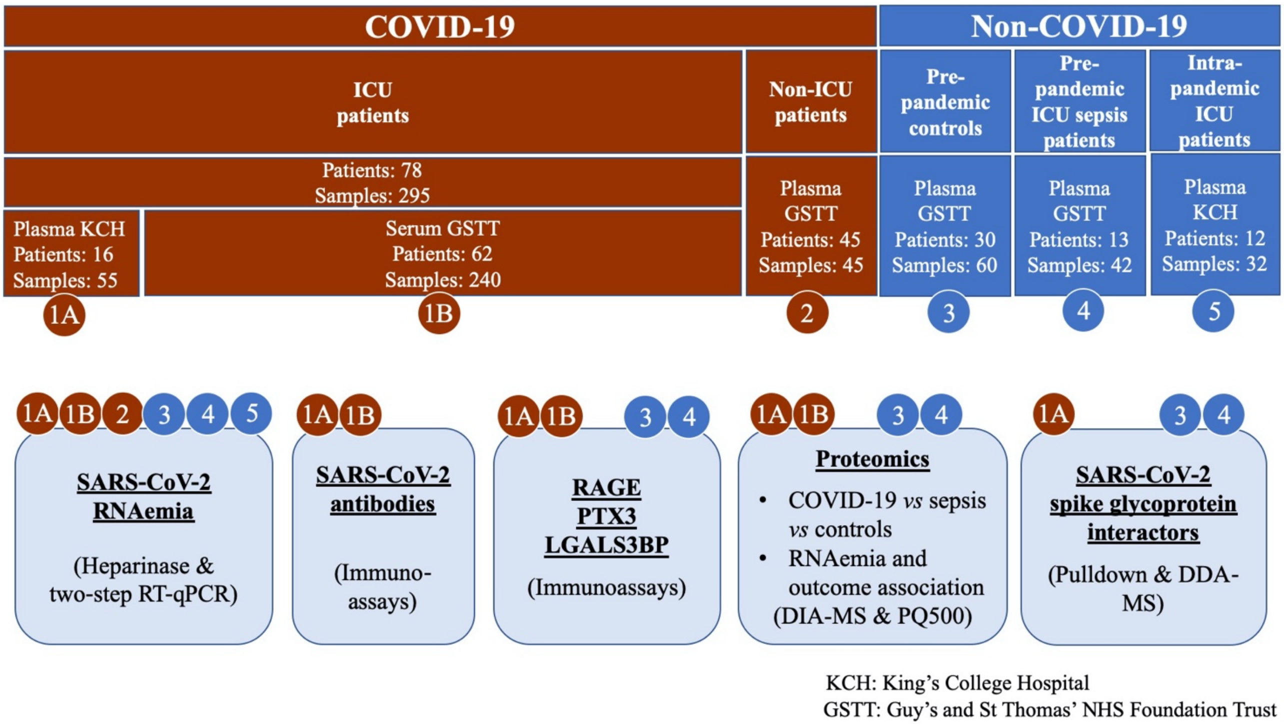
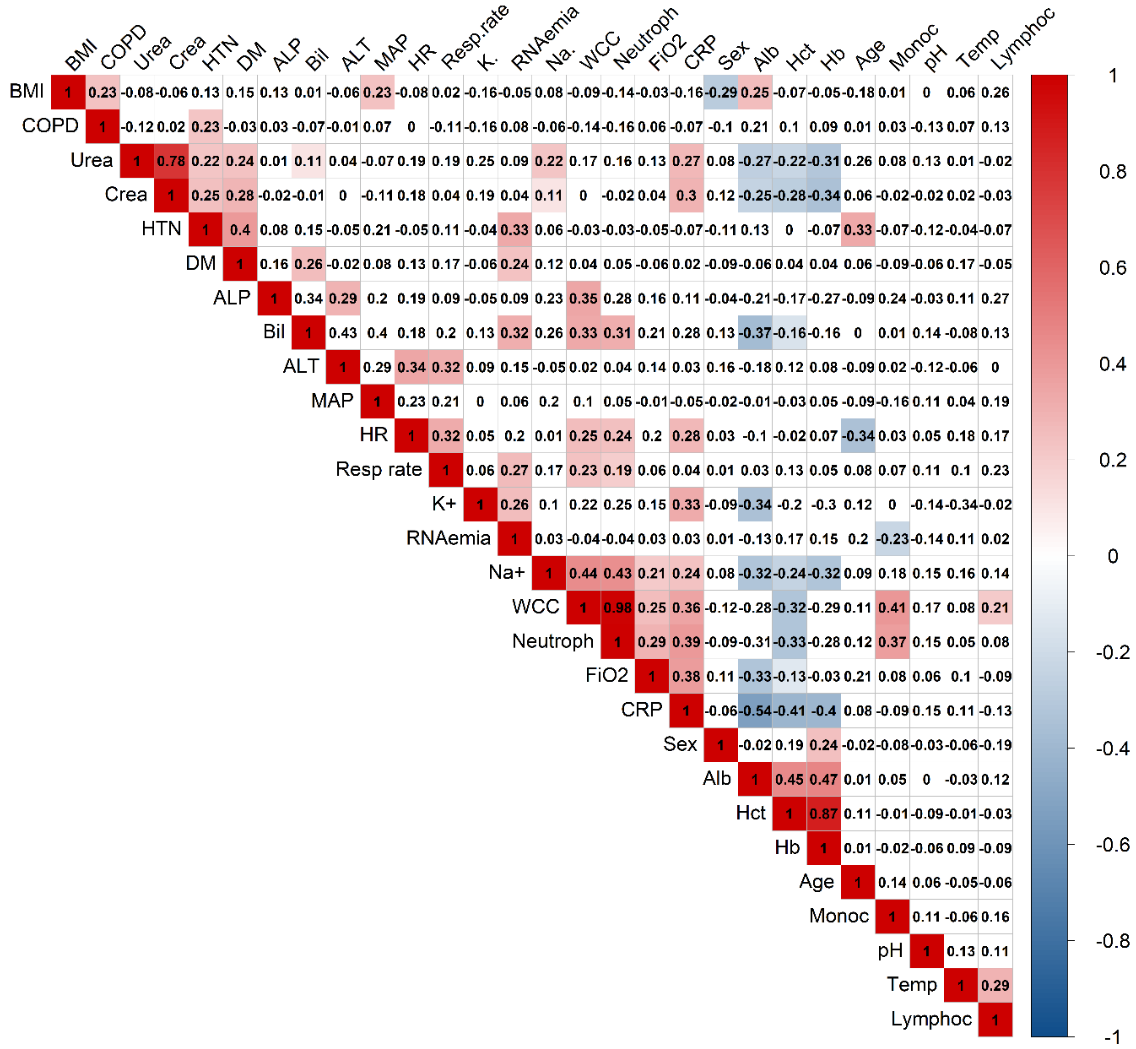
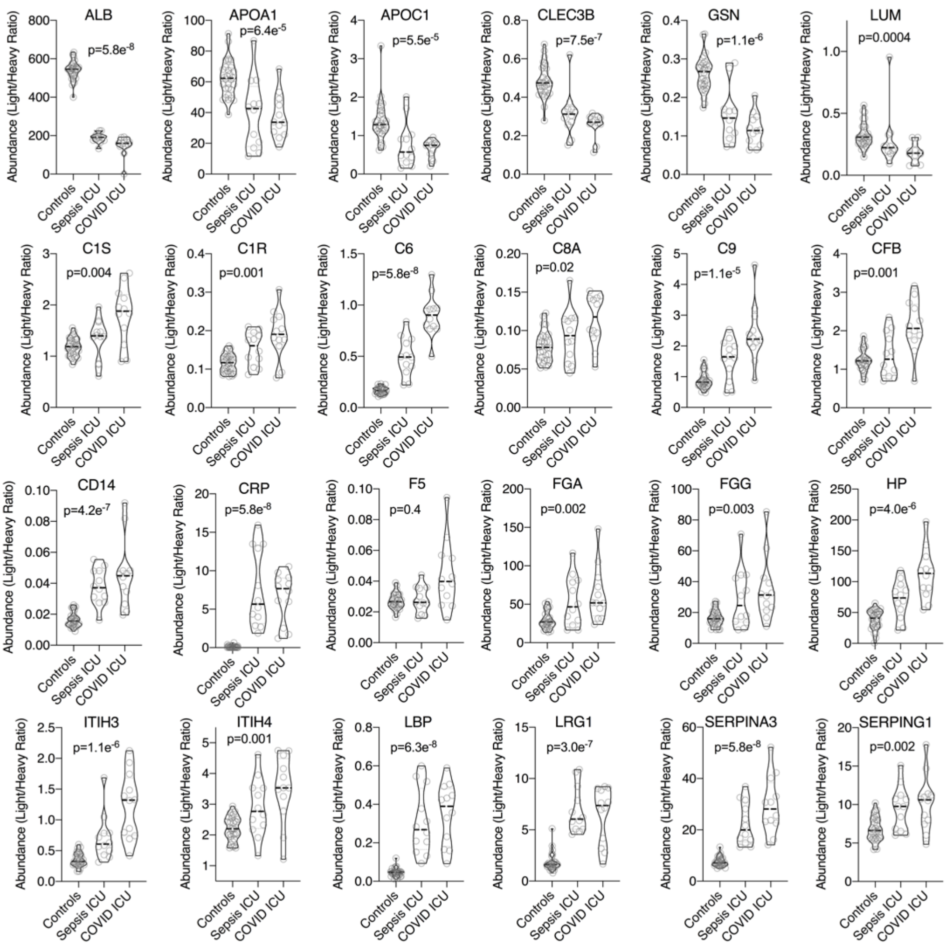


Supplementary Fig. 1: Study design.

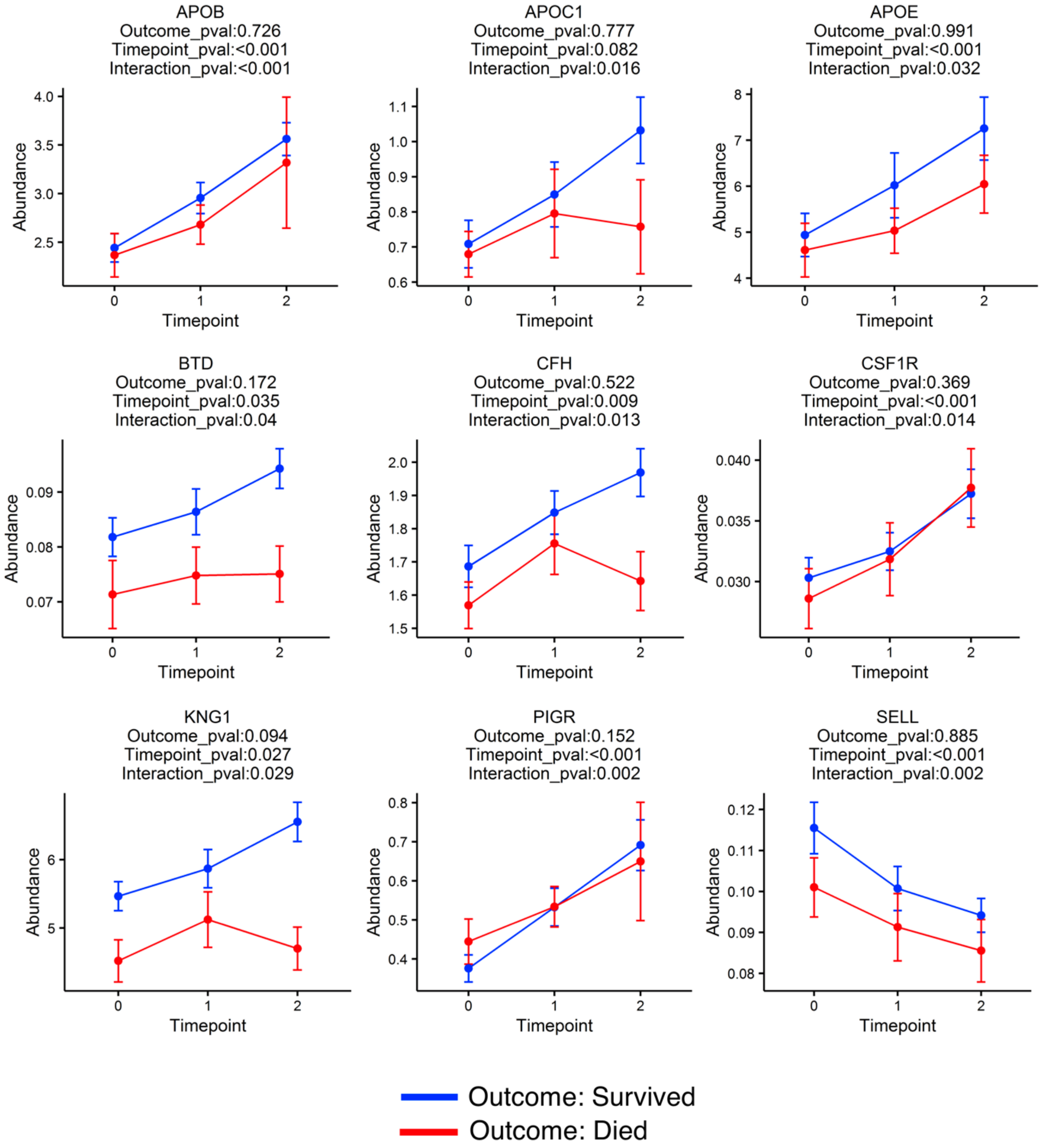


	BMI	COPD	Urea	Crea	HTN	DM	ALP	Bil	ALT	MAP	HR	Resp.rate	K.	RNAemia	Na.	WCC	Neutroph	FiO2	CRP	Sex	Alb	Hct	Hb	Age	Monoc	pH	Temp	Lymphoc
BMI	1	0.23	-0.08	-0.06	0.13	0.15	0.13	0.01	-0.06	0.23	-0.08	0.02	-0.16	-0.05	0.08	-0.09	-0.14	-0.03	-0.16	-0.29	0.25	-0.07	-0.05	-0.18	0.01	0	0.06	0.26
COPD		1	-0.12	0.02	0.23	-0.03	0.03	-0.07	-0.01	0.07	0	-0.11	-0.16	0.08	-0.06	-0.14	-0.16	0.06	-0.07	-0.1	0.21	0.1	0.09	0.01	0.03	-0.13	0.07	0.13
Urea			1	0.78	0.22	0.24	0.01	0.11	0.04	-0.07	0.19	0.19	0.25	0.09	0.22	0.17	0.16	0.13	0.27	0.08	-0.27	-0.22	-0.31	0.26	0.08	0.13	0.01	-0.02
Crea				1	0.25	0.28	-0.02	-0.01	0	-0.11	0.18	0.04	0.19	0.04	0.11	0	-0.02	0.04	0.3	0.12	-0.25	-0.28	-0.34	0.06	-0.02	-0.02	0.02	-0.03
HTN					1	0.4	0.08	0.15	-0.05	0.21	-0.05	0.11	-0.04	0.33	0.06	-0.03	-0.03	-0.05	-0.07	-0.11	0.13	0	-0.07	0.33	-0.07	-0.12	-0.04	-0.07
DM						1	0.16	0.26	-0.02	0.08	0.13	0.17	-0.06	0.24	0.12	0.04	0.05	-0.06	0.02	-0.09	-0.06	0.04	0.04	0.06	-0.09	-0.06	0.17	-0.05
ALP							1	0.34	0.29	0.2	0.19	0.09	-0.05	0.09	0.23	0.35	0.28	0.16	0.11	-0.04	-0.21	-0.17	-0.27	-0.09	0.24	-0.03	0.11	0.27
Bil								1	0.43	0.4	0.18	0.2	0.13	0.32	0.26	0.33	0.31	0.21	0.28	0.13	-0.37	-0.16	-0.16	0	0.01	0.14	-0.08	0.13
ALT									1	0.29	0.34	0.32	0.09	0.15	-0.05	0.02	0.04	0.14	0.03	0.16	-0.18	0.12	0.08	-0.09	0.02	-0.12	-0.06	0
MAP										1	0.23	0.21	0	0.06	0.2	0.1	0.05	-0.01	-0.05	-0.02	-0.01	-0.03	0.05	-0.09	-0.16	0.11	0.04	0.19
HR											1	0.32	0.05	0.2	0.01	0.25	0.24	0.2	0.28	0.03	-0.1	-0.02	0.07	-0.34	0.03	0.05	0.18	0.17
Resp rate												1	0.06	0.27	0.17	0.23	0.19	0.06	0.04	0.01	0.03	0.13	0.05	0.08	0.07	0.11	0.1	0.23
K+													1	0.26	0.1	0.22	0.25	0.15	0.33	-0.09	-0.34	-0.2	-0.3	0.12	0	-0.14	-0.34	-0.02
RNAemia														1	0.03	-0.04	-0.04	0.03	0.03	0.01	-0.13	0.17	0.15	0.2	-0.23	-0.14	0.11	0.02
Na+															1	0.44	0.43	0.21	0.24	0.08	-0.32	-0.24	-0.32	0.09	0.18	0.15	0.16	0.14
WCC																1	0.98	0.25	0.36	-0.12	-0.28	-0.32	-0.29	0.11	0.41	0.17	0.08	0.21
Neutroph																	1	0.29	0.39	-0.09	-0.31	-0.33	-0.28	0.12	0.37	0.15	0.05	0.08
FiO2																		1	0.38	0.11	-0.33	-0.13	-0.03	0.21	0.08	0.06	0.1	-0.09
CRP																			1	-0.06	-0.54	-0.41	-0.4	0.08	-0.09	0.15	0.11	-0.13
Sex																				1	-0.02	0.19	0.24	-0.02	-0.08	-0.03	-0.06	-0.19
Alb																					1	0.45	0.47	0.01	0.05	0	-0.03	0.12
Hct																						1	0.87	0.11	-0.01	-0.09	-0.01	-0.03
Hb																							1	0.01	-0.02	-0.06	0.09	-0.09
Age																								1	0.14	0.06	-0.05	-0.06
Monoc																									1	0.11	-0.06	0.16
pH																										1	0.13	0.11
Temp																											1	0.29
Lymphoc																												1

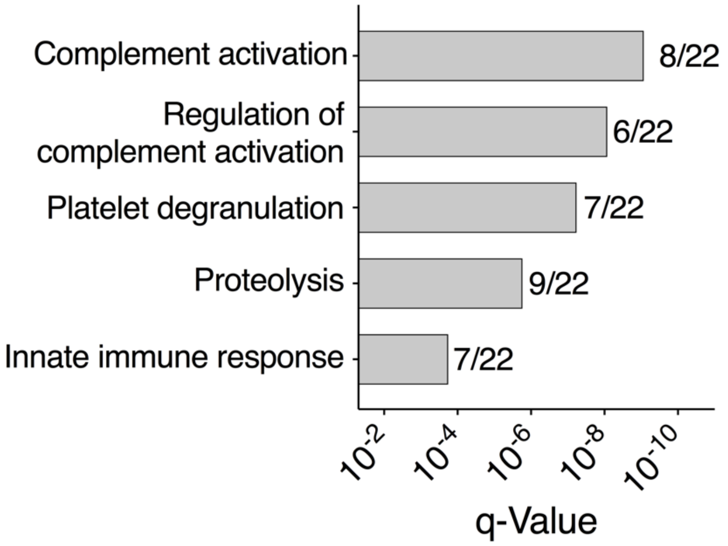
Supplementary Fig. 2: Pairwise Spearman correlation for continuous variables and point-biserial correlation for binary variables including SARS-CoV-2 RNAemia. *Hierarchical clustering analysis and heat-map matrix illustrates positive and negative co-expression and clusters. Three distinct clusters emerge: One comprising of albumin, hematocrit and hemoglobin, second cluster with urea, creatinine, hypertension, and type 2 diabetes and third cluster with sodium, WCC, neutrophils, Fi*O_2 *and CRP. White indicates no significant correlation (P value >0.05). Red indicates positive and blue negative correlation with P value <0.05. Abbreviations: Alb: albumin, ALP: alkaline phosphatase, ALT: alanine aminotransferase, Bil: bilirubin, Crea: creatinine, CRP: C-reactive protein, DM: type 2 diabetes, Hct: hematocrit, Hb: hemoglobin, HR: heart rate, HTN: hypertension, Lymphoc: lymphocytes, MAP: mean arterial pressure, Monoc: monocytes, Neutroph: neutrophils, K: potassium, Resp. rate: respiratory rate, Na.: sodium, Temp: body temperature, WCC: white cell count.*



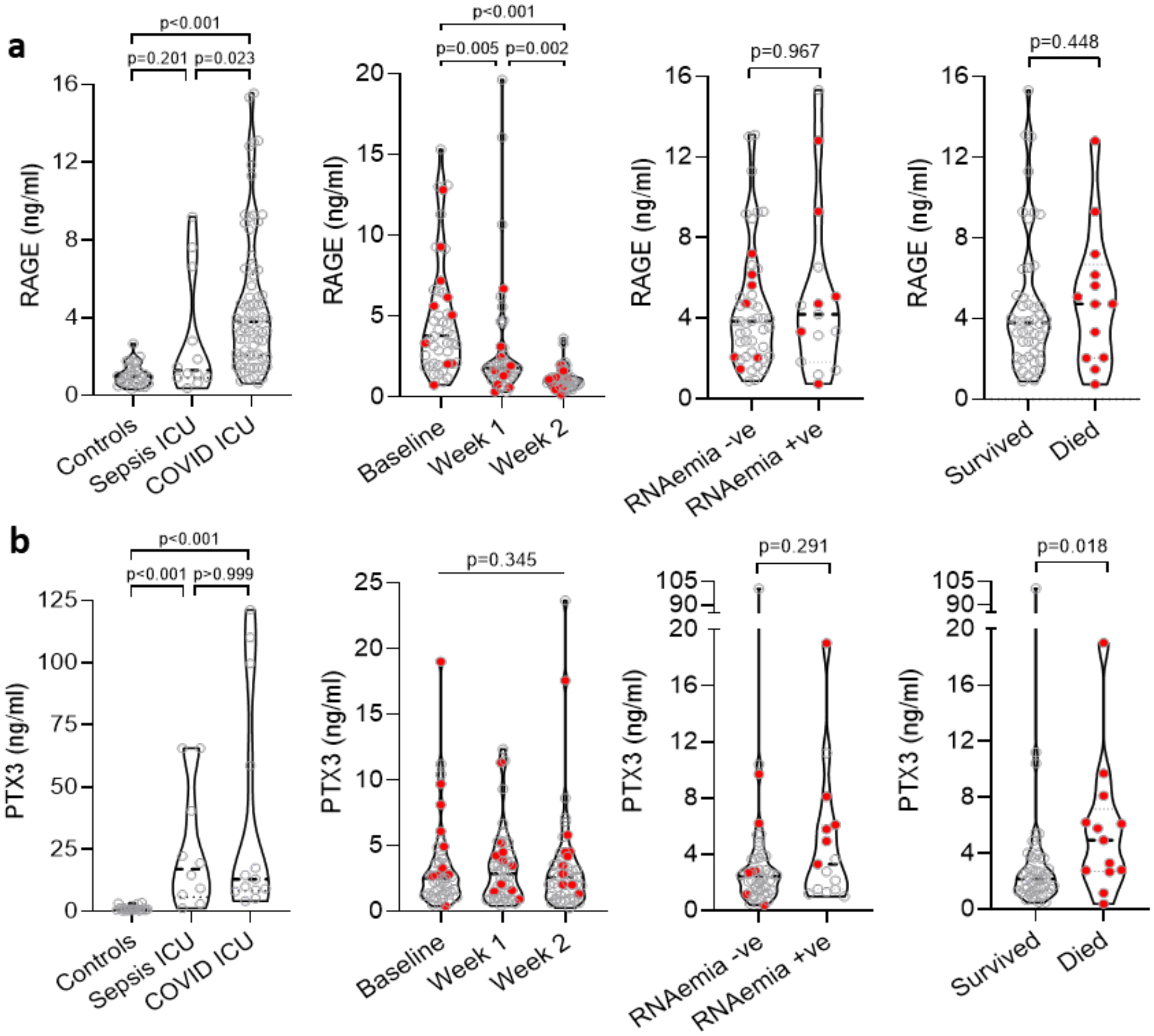
Supplementary Fig. 3: COVID-19 plasma proteome signature, common to published reports. *Plasma proteome profiling was conducted using a DIA-MS approach with spiked authentic heavy standards. 100 proteins were significantly different in the plasma proteome of COVID-19 ICU patients, when compared to control and sepsis ICU patients, and this panel was cross-referenced against two previously published proteomic studies, exploring circulating protein markers of COVID-19 severity, to generate a panel of 29 common proteins. These 29 common proteins are each individually represented to highlight their variation across disease phenotype. Significance was determined through the Kruskal-Wallis test with Benjamini and Hochberg's FDR correction*



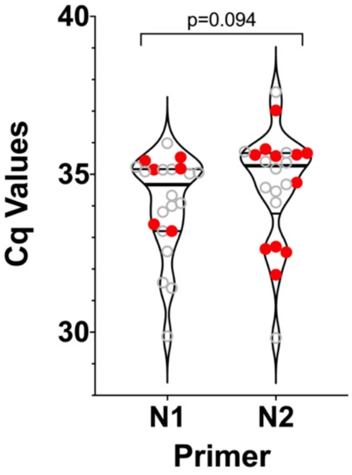
Supplementary Fig. 4: Comparison of the trajectories of individual proteins in COVID-19 ICU patients. *Listed are the proteins that show a significant interaction between survival and time of measurement (0 = ICU admission; 1 = first week after ICU admission; 2 = second week after ICU admission). Linear Mixed Models analysis was performed to calculate the P values, correcting for age and sex. Abbreviations: ApoB, apolipoprotein B; ApoC1, apolipoprotein C1; ApoE, apolipoprotein E; BTD, biotinidase; CFH, complement factor H; CSF1R, macrophage colony-stimulating factor 1 receptor; KNG1, kininogen; SELL, L-selectin.*



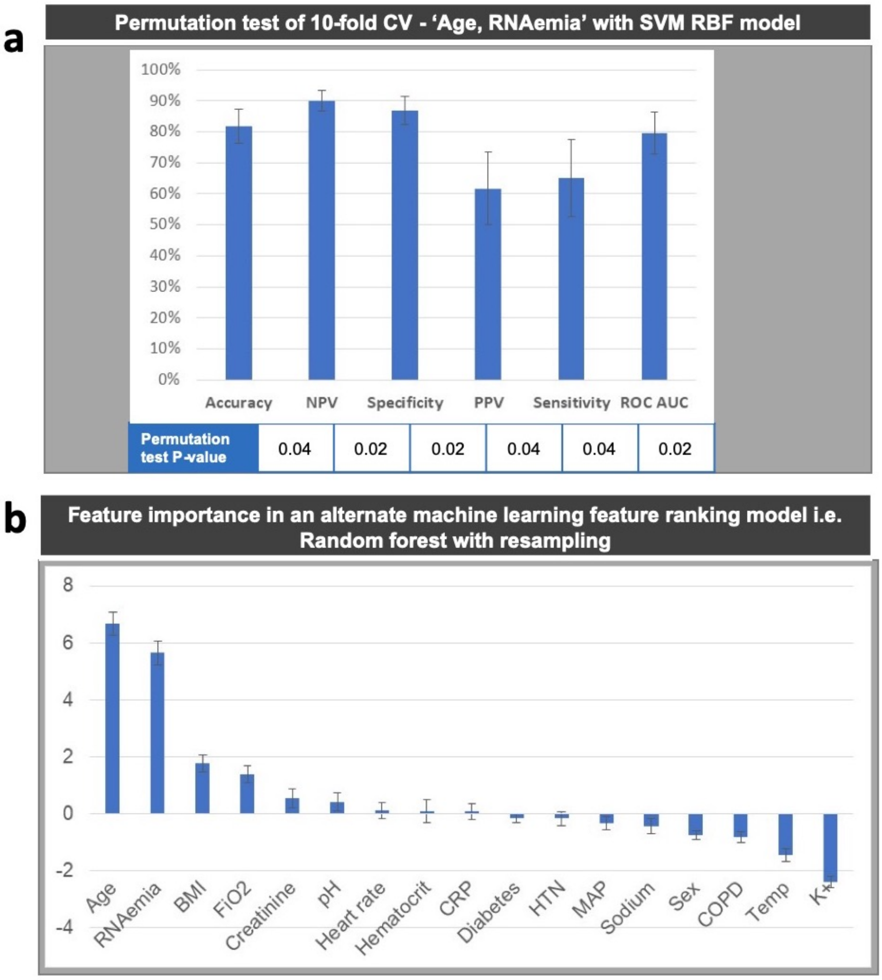
Supplementary Fig. 5: LGALS3BP enriched functional pathways. *Proteins that correlated with LGALS3BP levels as determined by proteomics with a greater than 0.6 Spearman coefficient were analyzed to determine enriched functional pathways. Gene ontology analysis revealed an enrichment of protein pathways related to the complement system, platelet degranulation, proteolysis and the innate immune response.*



Supplementary Fig. 6: Existing biomarkers for ARDS and sepsis: *RAGE (**a**) and PTX3 (**b**) were measured by ELISA. Comparisons of control (RAGE: n = 30, PTX3: n = 23), sepsis ICU (RAGE: n = 12, PTX3: n = 10) and COVID-19 ICU (RAGE: n = 74, PTX3: n = 13) patients using a Kruskal-Wallis and Dunn's multiple comparisons test. Longitudinal comparisons at baseline, week 1 and week 2 after admission to ICU (n = 46). Red dots highlight patients who died, white dots represent patients who survived. Friedman test with Dunn's multiple comparisons were used to determine statistical significance. Comparisons according to RNAemia status (n = 46 negative: "-ve"; n = 15 positive: "+ve"). Significance was determined by Mann-Whitney U test. Comparisons according to according to 28-day mortality (n = 13 died, n = 48 survived). Mann-Whitney U test were used for statistical comparisons.*



***Supplementary Fig. 7: Abundance of SARS-CoV-2 RNA in plasma or serum**. Cq values of all samples in which SARS-CoV-2 RNA was detectable (Cq <40) are shown. Mann-Whitney U test. Red - died, white - survived.*



Supplementary Fig. 8: Technical validation of machine learning model. a, Permutation test of SVM classifier performance: *10-fold cross-validation (CV) of the best predictor showing mean performance and standard error of mean. 10-fold CV is comparable with leave-one-out metrics shown in Supplementary table 4. Significance test of 10-fold CV performed using permutation test with 50 permutes i.e. repeating the classification procedure after random permuting of the outcome labels. This returned a significant P value i.e. <0.05, thus indicating that the classifier has found a real class structure (pattern) in the data. Hence, rejecting the null hypothesis that the classifier performance is by chance i.e. input variables and outcome labels are independent.* ***b, Stability of feature importance under a Random Forest (RF) Model showing mean variable importance*** *and standard error of mean across 100 resampling cycles of sensitivity analysis. Each resampling cycle takes equal proportions of the two outcome classes from the complete training data. Top 17 (of the 28) features are shown based on mean importance. The features with negative scores make the prediction worse and should be excluded from the model. The technical validation of feature importance stability using this* *alternate machine learning method i.e. RF with resampling reinforces the importance of best predictor i.e. 'Age, RNAemia' that are ranked among the top 2 most important features. Abbreviations: CRP: C-reactive protein, HTN: Hypertension, MAP: Mean arterial pressure,* K^+*: potassium, Temp: Body temperature.*

Supplementary Table 1: Baseline characteristics for COVID-19 ICU patients.

COVID-19 Clinical Characteristics	COVID-19 ICU Patients (*n* = 78)	COVID-19 'Survivors' (*n* = 60)	COVID-19 'Non-Survivors' (*n* = 18)	*P* value
SARS-COV-2 RNAemia (%)	23.08%	13.33%	55.56%	<0.001
Days POS until ICU Admission	7.00 (6.25, 10.00)	7.00 (6.00, 10.00)	9.50 (7.00, 14.00)	0.099
Days POS until Death	-	-	22.00 (19.00, 34.00)	-
Days from Admission to Death	-	-	13.50 (11.00, 15.50)	-
Demographics				
Age (Years)	54.00 (46.25, 64.01)	52.01 (44.00, 61.01)	65.01 (57.51,77.04)	<0.001
Sex (% Male)	71.79%	70.00%	77.78%	0.766
BMI (kg/m^2)	28.05 (24.71, 34.28)	28.10 (24.80, 34.90)	25.87 (24.55, 31.46)	0.252
Comorbidities				
COPD (%)	16.67%	15.00%	22.22%	0.483
Diabetes (%)	25.64%	25.00%	27.78%	0.769
Hypertension (%)	37.18%	33.33%	50.00%	0.267
Acute Care Parameters				
APACHE II Score	15.00 (11.00,19.00)	14.00 (11.00, 18.00)	17.00 (13.75, 20.50)	0.126
SOFA Score	6.00 (4.00, 8.00)	6.00 (4.00, 7.00)	8.00 (4.00, 9.00)	0.171
FiO_2 (Fraction of 1)	0.50 (0.35, 0.60)	0.47 (0.35, 0.56)	0.52 (0.50, 0.60)	0.113
Heart rate (bpm)	96.01 (63.27, 112.49)	96.50 (63.01, 114.99)	93.00 (86.00, 101.51)	0.767
MAP (mmHg)	65.51 (61.09, 79.75)	65.17 (61.09, 80.75)	65.51 (61.33, 70.50)	0.749
Respiratory rate (bpm)	22.00 (16.25, 28.07)	20.7 (16.00, 28.00)	23.83 (22.00, 28.77)	0.138
Temperature (°C)	38.19 (36.85, 39.07)	38.44 (36.95, 39.02)	37.94 (36.85, 38.97)	0.656
Blood Biochemistry				
Albumin (g/L)	30.00 (27.01, 33.00)	30.00 (27.01, 33.00)	29.50 (26.26, 30.28)	0.167
ALP (U/L)	64.49 (48.99, 94.49)	64.49(48.74, 97.24)	61.00 (50.25, 80.75)	0.981
ALT (IU/L)	36.01 (27.02, 51.92)	35.49 (25.02, 47.31)	41.68 (30.47, 55.86)	0.171
Bilirubin (µmol/L)	11.49 (8.00, 25.91)	10.99 (8.00, 21.89)	18.02 (8.00, 34.75)	0.313
Creatinine (µmol/L)	94.49 (72.02, 170.03)	89.38 (69.77, 174.54)	124.03 (91.24, 153.75)	0.265
C-reactive protein (mg/L)	226.21 (143.02, 328.21)	237.71 (103.56, 336.98)	215.68 (167.97, 299.57)	0.981
Hemoglobin (g/L)	115.63 (103.26, 123.75)	114.50 (100.51, 121.25)	120.00 (107.76, 125.74)	0.224
pH	7.36 (7.31, 7.41)	7.36 (7.31, 7.42)	7.32(7.31, 7.38)	0.233
Potassium (mmol/L)	4.50 (4.30, 4.80)	4.50 (4.30, 4.80)	4.35 (4.12, 4.85)	0.668
Sodium (mmol/L)	140.00 (137.00, 143.99)	140.00 (137.00, 143.24)	140.50 (137.25, 144.99)	0.426
Urea (mmol/L)	7.11 (5.01, 12.37)	6.55 (4.87, 10.77)	10.70 (6.36, 15.27)	0.039
Cellular Evaluation				
Hematocrit (%)	37.20 (30.26, 39.82)	36.00 (29.71, 39.30)	39.30 (35.25, 41.10)	0.069
Lymphocytes (10^9/l)	0.86 (0.63, 1.20)	0.88 (0.69, 1.20)	0.83 (0.45, 1.09)	0.498
Monocytes (10^9/l)	0.40 (0.25, 0.50)	0.36 (0.28, 0.51)	0.40 (0.24, 0.48)	0.743
Neutrophils (10^9/l)	7.38 (4.90, 9.68)	7.27 (4.90, 9.53)	7.50 (4.29, 9.83)	0.934
White cell count (10^9/l)	8.50 (6.53, 11.28)	8.50 (6.60, 11.00)	8.53 (5.95, 12.12)	0.962

Continuous variables are presented as median (25th and 75th percentile). P value computed for survivors vs non-survivors using Mann-Whitney test for continuous variables and Fisher exact test for binary variables. SARS-CoV-2 RNAemia: positive blood test within first six days of admission to ICU; Abbreviations: Days POS: days post onset of symptoms; APACHE II score: acute physiology and chronic health evaluation II score; SOFA score: the sequential organ failure assessment score; MAP: mean arterial pressure; ALP: alkaline phosphatase, ALT: alanine aminotransferase.

Supplementary Table 2: Baseline characteristics for non-ICU, hospitalized COVID-19 patients.

Clinical Characteristics	**Non-ICU COVID-19 patients (*n* = 45)**
SARS-CoV-2 RNAemia (%)	4.44%
Non-Survivors 28 days after hospitalization (%)	11.11%
Demographics	
Age (years)	60.82 (45.95, 71.58)
Sex (% Male)	66.66%
BMI (kg/m^2)	26.32 (21.32, 31.50)
Comorbidities	
COPD – no. (%)	8 (17.77%)
Diabetes – no. (%)	12 (26.66%)
Hypertension – no. (%)	21 (46.66%)
Acute Care Parameters	
FiO$_2$ (Fraction of 1)	0.21 (0.21, 0.28)
Heart rate (bpm)	103 (87, 118)
Temperature (°C)	37.6 (36.85, 38.3)
Blood Biochemistry	
Albumin (g/L)	37.00 (32.75, 40.00)
ALT (IU/L)	30.50 (16.50, 52.00)
Bilirubin (µmol/L)	8 (4.5, 11)
Creatinine (µmol/L)	71 (62, 89)
C-reactive protein (mg/L)	58 (29, 138)
Cellular Evaluation	
Lymphocytes (10^9/l)	1.05 (0.63, 1.30)
Monocytes (10^9/l)	0.50 (0.40, 0.70)
Neutrophils (10^9/l)	5.15 (3.33, 7.00)
Platelets (10^9/l)	261.5 (192.0, 334.5)
White cell count (10^9/l)	6.90 (4.65, 9.28)

Continuous variables are presented as median (25th and 75th percentile). Abbreviations: ALT: alanine aminotransferase.

Supplementary Table 3: Baseline characteristics for COVID-19 ICU patients with and without RNAemia.

COVID-19 Clinical Characteristics	**COVID-19 ICU Patients (*n* = 78)**	**RNAemia Negative (*n* = 60)**	**RNAemia Positive (*n* = 18)**	***P* value**
Non-Survivors (%)	23.08%	13.33%	55.56%	<0.001
Days POS until ICU Admission	7.00 (6.25, 10.00)	7.00 (6.00, 10.00)	7.00 (6.25, 10.00)	0.690
Days POS until Death	22.00 (19.00, 34.00)	26.00 (19.00, 36.50)	21.50 (18.50, 25.25)	0.434
Days from Admission to Death	13.50 (11.00, 15.50)	13.00 (12.00, 17.00)	14.00 (11.00, 15.00)	0.641
Demographics				
Age (Years)	54.00 (46.25, 64.01)	53.01 (45.75, 62.26)	61.01 (48.25, 66.01)	0.107
Sex (% Male)	71.79%	71.67%	72.22%	1.000
BMI (kg/m^2)	28.05 (24.71, 34.28)	28.05 (24.65, 34.68)	29.38 (24.93, 31.70)	0.845
Comorbidities				
COPD (%)	16.67%	15.00%	22.22%	0.483
Diabetes (%)	25.64%	20.00%	44.44%	0.062
Hypertension (%)	37.18%	28.33%	66.67%	0.005
Acute Care Parameters				
APACHE II Score	15.00 (11.00, 19.00)	14.50 (11.00, 17.75)	17.00 (11.75, 23.00)	0.114
SOFA Score	6.00 (4.00, 8.00)	5.00 (4.00, 8.00)	7.00 (4.00, 9.00)	0.363
FiO$_2$ (Fraction of 1)	0.50 (0.35, 0.60)	0.50 (0.35, 0.60)	0.50 (0.46, 0.59)	0.306
Heart rate (bpm)	96.01 (63.27, 112.49)	93.50 (62.76, 114.25)	99.50 (92.50, 108.25)	0.140
MAP (mmHg)	65.51 (61.09, 79.75)	63.50 (59.08, 80.08)	67.67 (65.34, 70.76)	0.057
Respiratory rate	22.00 (16.25, 28.07)	20.75 (13.80, 28.00)	26.00 (22.00, 30.00)	0.023
Temperature (°C)	38.19 (36.85, 39.07)	38.29 (36.05, 39.09)	38.19 (37.75, 38.99)	0.622
Blood Biochemistry				
Albumin (g/L)	30.00 (27.01, 33.00)	30.50 (27.01, 33.00)	29.50 (27.01, 30.00)	0.178
ALP (U/L)	64.49 (48.99, 94.49)	63.49 (48.99, 94.98)	65.51 (49.24, 88.49)	0.896
ALT (IU/L)	36.01 (27.02, 51.92)	33.00 (25.02, 43.31)	51.62 (41.02, 60.75)	0.003
Bilirubin (µmol/L)	11.49 (8.00, 25.91)	10.99 (8.00, 17.27)	30.47 (10.14, 44.17)	0.011
Creatinine (µmol/L)	94.49 (72.02, 170.03)	93.49 (71.53, 166.00)	100.51 (81.03, 168.03)	0.817
C-reactive protein (mg/L)	226.21 (143.02, 328.21)	237.71 (138.78, 336.88)	189.52 (153.54, 320.12)	0.910
Hemoglobin (g/L)	115.63 (103.26, 123.75)	113.85 (100.51, 121.25)	119.50 (114.00, 126.00)	0.104
pH	7.36 (7.31, 7.41)	7.36 (7.31, 7.42)	7.32 (7.30, 7.38)	0.217
Potassium (mmol/L)	4.50 (4.30, 4.80)	4.40 (4.30, 4.72)	4.65 (4.27, 4.98)	0.118
Sodium (mmol/L)	140.00 (137.00, 143.99)	140.00 (137.00, 143.99)	139.50 (137.25, 144.49)	0.794
Urea (mmol/L)	7.11 (5.01, 12.37)	6.55 (4.80, 11.77)	8.42 (6.63, 14.55)	0.141
Cellular Evaluation				
Hematocrit (%)	37.20 (30.26, 39.82)	36.00 (29.63, 39.30)	38.55 (35.85, 40.57)	0.078
Lymphocytes (10^9/l)	0.86 (0.63, 1.20)	0.80 (0.64, 1.19)	0.88 (0.62, 1.23)	0.784
Monocytes (10^9/l)	0.40 (0.25, 0.50)	0.40 (0.30, 0.59)	0.30 (0.21, 0.40)	0.064
Neutrophils (10^9/l)	7.38 (4.90, 9.68)	7.34 (4.90, 9.53)	7.69 (4.16, 10.27)	0.896
White cell count (10^9/l)	8.50 (6.53, 11.28)	8.50 (6.58, 11.00)	9.40 (5.36, 12.16)	0.995

Continuous variables are presented as median (25th and 75th percentile). P value computed for SARS-CoV-2 RNAemia positive vs negative patients within first six days of admission to ICU using Mann-Whitney U test for continuous variables and Fisher exact test for binary variables. Abbreviations: Days POS: days post onset of symptoms; APACHE II score: acute physiology and chronic health evaluation score; SOFA score: the sequential organ failure assessment score; MAP: mean arterial pressure; ALP: alkaline phosphatase, ALT: alanine aminotransferase.

Supplementary Table 4: Baseline characteristics for non-COVID-19 ICU patients.

Clinical Characteristics	**Pre-pandemic non-COVID-19 ICU sepsis patients ($n = 13$)**	**Intra-pandemic non-COVID-19 ICU patients ($n = 12$)**
SARS-CoV-2 RNAemia (%)	0%	0%
Non-Survivors at day 28 (%)	0%	41.66%
Demographics		
Age (Years)	64 (38, 73)	70 (59.25, 78)
Sex – % Male	53.85%	58.33%
BMI (kg/m^2)	26.12 (23.88, 27.55)	30.30 (26.77, 34.09)
Comorbidities		
COPD (%)	3 (23.08%)	3 (25%)
Diabetes (%)	2 (15.39%)	6 (50%)
Hypertension (%)	7 (53.85%)	5 (41.66%)
Acute Care Parameters		
APACHE II score	16 (15, 21)	-
FiO_2 (Fraction of 1)	0.4 (0.27, 0.65)	0.28 (0.25, 0.31)
Heart rate (bpm)	115 (69, 136)	80 (78, 93)
MAP (mmHg)	65.67 (60, 99.33)	81.5 (72, 85.75)
Respiratory rate	23.50 (19.00, 28.75)	20.5 (19.75, 25.25)
SOFA score	-	3.5 (1.75, 6.25)
Temperature (°C)	37.7 (35.8, 38.5)	36.9 (36.8, 37.0)

Continuous variables are presented as median (25th and 75th percentile). Abbreviations: APACHE II score: acute physiology and chronic health evaluation II score, MAP: mean arterial pressure. SOFA score: sequential organ failure assessment score. Intra-pandemic, non-COVID-19 ICU patients repeatedly tested negative for nasopharyngeal SARS-CoV-2 by RT-qPCR. SARS-CoV-2 RNAemia was assessed in COVID-19-negative patients to determine assay specificity.

Supplementary Table 5: Baseline characteristics for control patients before elective cardiac surgery.

Clinical Characteristics	**Non-COVID-19, non-ICU controls (*n* = 30)**
SARS-CoV-2 RNAemia (%)	0%
Demographics	
Age (Years)	70 (64.00, 74.75)
Sex (% Male)	73.33%
BMI (kg/m^2)	28.22 (25.02, 30.77)
Comorbidities	
COPD (%)	6 (20%)
Diabetes (%)	7 (23.33%)
Hypertension (%)	17 (56.66%)

Continuous variables are presented as median (25th and 75th percentile). SARS-CoV-2 RNAemia was assessed in COVID-19-negative patients to determine assay specificity.

Supplementary Table 6: External validation data based on proximity-extension assays.

Protein	Log2 Fold Change Non-Survivors vs Survivors	Average Relative Quantity	*P* value	FDR
PTX3	0.89784	5.79471	<0.00001	**<0.0001**
IL17C	0.96308	2.67565	<0.00001	0.0001
IL6	1.42029	5.81313	<0.00001	0.0003
PROC	-0.37773	3.21418	<0.00001	**0.0005**
IL1RN	0.86502	2.69615	0.00001	0.001
CX3CL1	0.56603	4.56926	0.00003	0.002
IL1RL1	0.81686	2.75287	0.00004	0.003
CCL2	0.60728	6.13895	0.00007	0.003
IL15	0.47248	3.49095	0.0001	0.004
CXCL8	0.7077	3.27201	0.00014	0.004
CCL20	0.92856	5.23471	0.00032	0.007
IL4R	0.60584	3.31738	0.00054	0.009
IL19	0.67502	2.16278	0.00061	0.010
CXCL13	0.58454	3.52346	0.00094	0.013
CRLF1	0.31295	1.68468	0.00188	0.019
F7	-0.29250	3.56072	0.002	**0.021**
CXCL10	0.72777	7.41412	0.002	0.021
CCL7	0.76166	4.81714	0.003	0.025
CCL19	0.49678	3.5662	0.007	0.044
IL18R1	0.3364	6.64045	0.008	0.048
LBP	0.34828	4.96949	0.014	0.068
CD14	0.14460	7.28775	0.309	0.523
CDH5	0.06908	1.67967	0.343	0.554
ITIH3	-0.00439	2.16440	0.967	0.978

Differential expression analysis of proteins in survivors and non-survivors 28-days after hospitalization. This analysis was performed on a publicly available proximity-extension assay proteomics-based dataset (data provided by the MGH Emergency Department COVID-19 Cohort (Filbin, Goldberg, Hacohen) with Olink Proteomics: https://www.olink.com/mgh-covid-study/). Only proteins that were also quantified in our study; reported in our main findings; and cytokines and interleukins significantly associated with 28-days mortality were analyzed. Statistical analysis was conducted using the Ebayes method of the limma package, correcting for age and adjusting for multiple testing using Benjamini and Hochberg's FDR correction.

Supplementary Table 7: Plasma proteins binding to SARS-CoV-2 spike glycoprotein.

Gene	Ctrl average	Spike average	Fold enrichment	*P* value
SPIKE	3.35E+07	7.67E+09	228.8	0.00006
MYH9	2.37E+06	1.61E+07	6.7	0.0063
FLNA	1.10E+07	5.40E+07	4.9	0.015
C1QA	1.85E+07	7.78E+07	4.2	0.007
C1QC	4.29E+07	1.73E+08	4	0.004
LGALS3BP	1.27E+06	4.59E+06	3.6	0.008
C1QB	2.87E+07	1.00E+08	3.4	0.022
LSM4	2.46E+06	8.50E+06	3.4	0.015
C4BPB	2.27E+07	7.05E+07	3.1	0.022
APOD	6.18E+06	1.58E+07	2.5	0.037
ACTB	1.23E+08	2.86E+08	2.3	0.037
THAP5	1.72E+07	3.91E+07	2.2	0.028
C4BPA	3.89E+08	8.76E+08	2.2	0.045
KIF20B	7.46E+08	1.66E+09	2.2	0.011
APCS	1.03E+07	2.17E+07	2.1	0.022
KIF4B	8.84E+09	1.75E+10	1.9	0.03
SELENOP	1.00E+09	1.89E+09	1.8	0.02
HIST2H2AC	5.42E+06	1.02E+07	1.8	0.021
HRG	4.48E+10	8.18E+10	1.8	0.038
ZW10	7.39E+07	1.35E+08	1.8	0.027
HABP2	2.02E+07	3.65E+07	1.8	0.017
CDA	2.12E+06	3.58E+06	1.6	0.021
ERP44	9.64E+05	1.48E+06	1.5	0.035
CPN1	3.04E+07	4.39E+07	1.4	0.024

List of the 24 non-immunoglobulin proteins detected at significantly higher levels by LC-MS/MS in pull-downs of His-tagged SARS-CoV-2 spike glycoprotein mixed with COVID-19 plasma. Control experiments were conducted to exclude: 1) co-isolates binding to the solid phase of the His-tagged pull-down metal affinity beads; 2) proteins present in the His-tagged SARS-CoV-2 preparation prior to mixing with plasma (i.e. carryover from HEK293 cells used for production of the recombinant Spike glycoprotein).

Supplementary Table 8: Comparison of plasma protein binding to SARS-CoV-2 spike glycoprotein in patients with and without COVID-19.

Gene	Non-COVID-19 average	COVID-19 average	Fold enrichment	*P* value
LGALS3BP	1.23E+06	4.59E+06	3.7	0.0025
APOD	4.95E+06	1.58E+07	3.1	0.0036

Listed are the two non-immunoglobulin proteins detected at significantly higher levels by LC-MS/MS in pull-down samples of His-tagged spike glycoprotein mixed with plasma from COVID-19 compared to non-COVID-19 ICU patients.

Supplementary Table 9: Machine learning signatures using the SVM RBF model.

Single Signature	Accuracy	NPV	Specificity	PPV (A)	Sensitivity (B)	ROC AUC (C)	Average of A+B+C
RNAemia	79.49%	86.67%	86.67%	55.56%	55.56%	85.37%	65.49%
Age	73.08%	86.79%	76.67%	44.00%	61.11%	71.11%	58.74%
PTX3	78.21%	82.09%	91.67%	54.55%	33.33%	69.35%	52.41%
Urea	65.38%	78.95%	75.00%	28.57%	33.33%	54.63%	38.84%

Binary Signature	Accuracy	NPV	Specificity	PPV (A)	Sensitivity (B)	ROC AUC (C)	Average of A+B+C
Age, PTX3	87.18%	90.32%	93.33%	75.00%	66.67%	78.52%	73.40%
Age, RNAemia	83.33%	91.23%	86.67%	61.90%	72.22%	79.81%	71.31%
Age, FiO$_2$	76.92%	90.38%	78.33%	50.00%	72.22%	78.70%	66.98%
Age, Albumin	82.05%	89.66%	86.67%	60.00%	66.67%	74.07%	66.91%
Age, ALT	82.05%	89.66%	86.67%	60.00%	66.67%	72.41%	66.36%
Age, Respiratory rate	80.77%	89.47%	85.00%	57.14%	66.67%	74.72%	66.18%
COPD, RNAemia	79.49%	86.67%	86.67%	55.56%	55.56%	86.39%	65.83%
Diabetes, RNAemia	79.49%	86.67%	86.67%	55.56%	55.56%	86.30%	65.80%
pH, RNAemia	73.08%	91.49%	71.67%	45.16%	77.78%	69.91%	65.30%
Hypertension, RNAemia	79.49%	86.67%	86.67%	55.56%	55.56%	82.13%	64.41%

Triplet Signature	Accuracy	NPV	Specificity	PPV (A)	Sensitivity (B)	ROC AUC (C)	Average of A+B+C
Age, FiO$_2$, RNAemia	84.62%	91.38%	88.33%	65.00%	72.22%	85.93%	74.38%
Age, RNAemia, PTX3	80.77%	90.91%	83.33%	56.52%	72.22%	82.59%	70.45%
Age, HR, Diabetes	87.18%	89.06%	95.00%	78.57%	61.11%	76.67%	72.12%
Age, FiO$_2$, PTX3	83.33%	91.23%	86.67%	61.90%	72.22%	81.85%	71.99%
Age, Sodium, PTX3	87.18%	87.88%	96.67%	83.33%	55.56%	76.20%	71.70%
Age, Lymphocytes, RNAemia	84.62%	90.00%	90.00%	66.67%	66.67%	81.20%	71.51%
Age, CRP, RNAemia	83.33%	91.23%	86.67%	61.90%	72.22%	78.98%	71.04%
Age, HR, PTX3	85.90%	87.69%	95.00%	76.92%	55.56%	80.65%	71.04%
Age, pH, RNAemia	82.05%	91.07%	85.00%	59.09%	72.22%	81.67%	70.99%
Age, Bilirubin, Hb	83.33%	91.23%	86.67%	61.90%	72.22%	78.06%	70.73%

Single markers were filtered for prediction model based on statistical significance (P value <0.05). The top 10 combinations were selected from 114 binary and 885 triplet combinations based on average score i.e. average of PPV, Sensitivity and ROC. Signatures are shown in descending order based on average score and using leave-one-out validation. Note that PTX3 levels are different in serum and plasma. The best triplet signature 'Age, FiO$_2$, RNAemia' provides nominal gain in prediction probability as reflected in PPV with no uplift to specificity when compared to 'Age, RNAemia' suggesting the binary combination to be an optimal signature to choose. Abbreviations: NPV: negative predictive value; PPV: positive predicted value; Temp: body temperature; Hb: Hemoglobin, HR: Heart rate.

Supplementary Table 10: Isolation windows used in DIA-MS methods.

Precursor ion [m/z]	Isolation Window [m/z]
360.5	61
405.5	31
431, 452, 473	22
492, 509, 526, 543, 560, 577, 594, 611, 628, 645	18
664, 685, 706, 727	22
752, 781, 810, 839	30
871	36
908, 947	40
991, 1040	50
1095	62
1162.5	75

Supplementary Table 11: Features with missing data in the combined COVID-19 ICU cohorts.

Feature	Missing Count	Missing %	Imputed
Creatinine	1	1.3%	Yes
C-reactive protein	2	2.6%	Yes
Albumin	3	3.8%	Yes
Bilirubin	4	5.1%	Yes
Lymphocytes	3	3.8%	Yes
Monocytes	3	3.8%	Yes
Hemoglobin	3	3.8%	Yes
ALP (alkaline phosphatase)	3	3.8%	Yes
Respiratory rate	5	6.4%	Yes
White cell count	5	6.4%	Yes
Neutrophil	5	6.4%	Yes
SOFA score	7	9.0%	Yes
Urea	9	11.5%	Yes
ALT (alanine aminotransferase)	16	20.5%	Yes

List of features with missing data in the combined cohort i.e. GSTT and KCH cohort. Features with missing percentage less than 30% were imputed using KNN impute with K=5. Eosinophil counts and basophil counts with missing percentage greater than 30% were not imputed and excluded from data analysis.