SUPPLEMENTARY DATA

Group IIA Secreted Phospholipase A_2 is Associated with the Pathobiology Leading to COVID-19 Mortality

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table 1: Plasma sampling information

		COVID-19			
Variables	*Non COVID-19 (n=37)*	*Mild (n=30)*	*Severe (n=30)*	*Deceased (n=30)*	*p-value*
Length of stay (days)	2 (1-7)	5 (3-13)	46 (16.5-75.5)	27 (11 – 42)	<0.0001[A] <0.0001[B] 0.0005[C]
Sampling time (% of hospital stay)	75 (50 – 100)	6.25 (0 – 76.25)	47.36 (32.03 – 64.88)	37.57 (3.947 – 78.21)	0.0002[D] 0.0252[E] 0.0043[F]

Variables are reported as median (interquartile range). D'Agostino-Pearson normality test was used to assess continuous variables and determined that all had non-Gaussian distributions; Kruskal-Wallis test were then used to assess for equality of group variance. All p-values were corrected for multiplicity using Dunn's correction. [A]Non-COVID-19 patients vs. severe or deceased COVID-19 patients. [B]mild vs. severe COVID-19 patients. [C]mild vs. deceased COVID-19 patients. [D]non-COVID-19 patients vs. mild COVID-19 patients. [E]non-COVID-19 patients vs. severe COVID-19 patients. [F]non-COVID-19 patients vs. deceased COVID-19 patients.

Supplementary Table 2: Presenting signs and symptoms

		COVID-19			
Variables	*Non COVID-19 (n=37)*	*Mild (n=30)*	*Severe (n=30)*	*Deceased (n=30)*	*p-value*
Median NEWS2 score (IQR)	0 (0-1)	0 (0-1)	7 (5-9)	7 (5.75-9)	<0.0001
Median 7-category ordinal scale (IQR)	3 (1-3)	3 (3-3)	5 (5-5)	5 (4.75-5)	<0.0001
Pulmonary infiltration – no. of patients (%)	0 (0.0)	0 (0.0)	30 (100.0)	30 (100.0)	<0.0001
Bilateral pulmonary infiltration– no. of patients (%)	0 (0.0)	0 (0.0)	26 (86.7)	27 (90.0)	<0.0001
Oxygen saturation, % (IQR)[A]	98 (97-99)	98 (96.75-99)	92 (84.25-93)	90.5 (81.25-95)	<0.0001
Oxygen modality – no. of patients (%)					
Room air	33	30	17	21	<0.0001
Oxygen therapy	4	0	13	9	
Symptoms – no. of patients (%)					
Abdominal pain	4 (10.8)	5 (16.7)	0 (0.0)	2 (6.7)	0.1304
Loss of appetite	4 (10.8)	2 (6.7)	7 (23.3)	8 (26.7)	0.1009
Chest pain	8 (21.6)	5 (16.7)	5 (16.7)	4 (13.3)	0.8426
Chills/rigors	0 (0.0)	3 (10.0)	5 (16.7)	3 (10.0)	0.1080
Confusion/delirium	0 (0.0)	1 (3.3)	6 (20.0)	10 (33.3)	0.0002
Dry cough	0 (0.0)	1 (3.3)	5 (16.7)	10 (33.3)	0.0002
Cough with sputum	2 (5.4)	1 (3.3)	11 (36.7)	6 (20.0)	0.0008
Diarrhea	3 (8.1)	3 (10.0)	8 (26.7)	6 (20.0)	0.1399
Dizziness	5 (13.5)	1 (3.3)	0 (0.0)	2 (6.7)	0.1253
Shortness of breath	7 (18.9)	6 (20.0)	21 (70.0)	23 (76.7)	<0.0001
Fever	0 (0.0)	1 (3.3)	16 (53.3)	15 (50.0)	<0.0001
Headache	1 (2.7)	2 (6.7)	2 (6.7)	1 (3.3)	0.8090
Malaise	5 (13.5)	2 (6.7)	10 (33.3)	10 (33.3)	0.0157
Fatigue	3 (8.1)	2 (6.7)	10 (33.3)	11 (36.7)	0.0019
Nasal congestion	0 (0.0)	1 (3.3)	2 (6.7)	2 (6.7)	0.4356
Nausea/vomiting	4 (10.8)	3 (10.0)	4 (13.3)	3 (10.0)	0.9728
Other	22 (59.5)	21 (70.0)	8 (26.7)	12 (40.0)	0.0031

Categorical variables reported as proportions (%); continuous variables reported as median (interquartile range). D'Agostino-Pearson normality test was determined that all continuous variables had non-Gaussian distributions; Kruskal-Wallis test was then used to assess for equality of group variance. Categorical variables were compared using the chi-square test. P-values reflect comparisons of group variance; significant trends are reported in Figure S1. [A]Some oxygenation indices measured while on oxygen therapy (no baseline measurement on room air).

Supplementary Table 3: COVID treatments

Variables	*Severe (n=30)*	*Deceased (n=30)*	p-value[H]
Supplemental oxygen therapy[A]	23 (76.7)	26 (86.7)	0.5062
High flow nasal cannula oxygen therapy	26 (86.7)	27 (90)	>0.9999
Non-invasive mechanical ventilation[B]	14 (46.7)	18 (60)	0.4379
Invasive mechanical ventilation (endotracheal intubation)	17 (56.7)	22 (73.3)	0.2789
Extracorporeal membrane oxygenation	0 (0)	1 (3.3)	>0.9999
Renal replacement therapy	5 (16.7)	10 (33.3)	0.2326
Antibiotic therapy[C]	29 (96.7)	30 (100)	>0.9999
Corticosteroid therapy[D]	21 (70)	24 (80)	0.5520
Immunosuppressant therapy (tocilizumab)	11 (36.7)	8 (26.7)	0.5796
Antiviral therapy[E]	8 (26.7)	10 (33.3)	0.7787
Anti-malarial therapy (hydroxychloroquine)	22 (73.3)	15 (50)	0.1102
Convalescent plasma therapy	2 (6.7)	4 (13.3)	0.6707
Nitric oxide therapy	5 (16.7)	5 (16.7)	>0.9999
Vasopressors[F]	15 (50)	25 (83.3)	0.0127
Other[G]	1 (3.3)	0 (0)	>0.9999

Variables are reported as number of patients (%). [A]Venturi mask, non-rebreather mask.[B]bilevel positive airway pressure (BiPAP) and continuous positive airway pressure (CPAP).[C]amikacin, amoxicillin, ampicillin, augmentin, Avycaz, azithromycin, Bactrim, cefazolin, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, colistin, daptomycin, doxycycline, ertapenem, erythromycin, gentamicin, levofloxacin, linezolid, meropenem, metronidazole, sulfamethoxazole, tobramycin, trimethoprim, vancomycin, Zerbaxa, and Zosyn. [D]dexamethasone, hydrocortisone, methylprednisolone, and prednisone. [E]Lopinavir/Ritonavir and Remdesivir. [F]epinephrine, midodrine, norepinephrine, phenylephrine and vasopressin. [G]ivermectin and colchicine. [H]Proportions were compared using Fisher's exact test.

Supplementary Table 4 List of LASSO-selected variables

Age	0.002
BMI	-0.003
Glucose (mg/dL)	0.004
BUN (mg/dL)	0.580
Cardiac arrest	0.024
sPLA2 (ng/mL)	0.604

LASSO-selected variables in a fitted logistic regression model to classify severe and deceased COVID patients.

SUPPLEMENTARY FIGURES

Figure S1

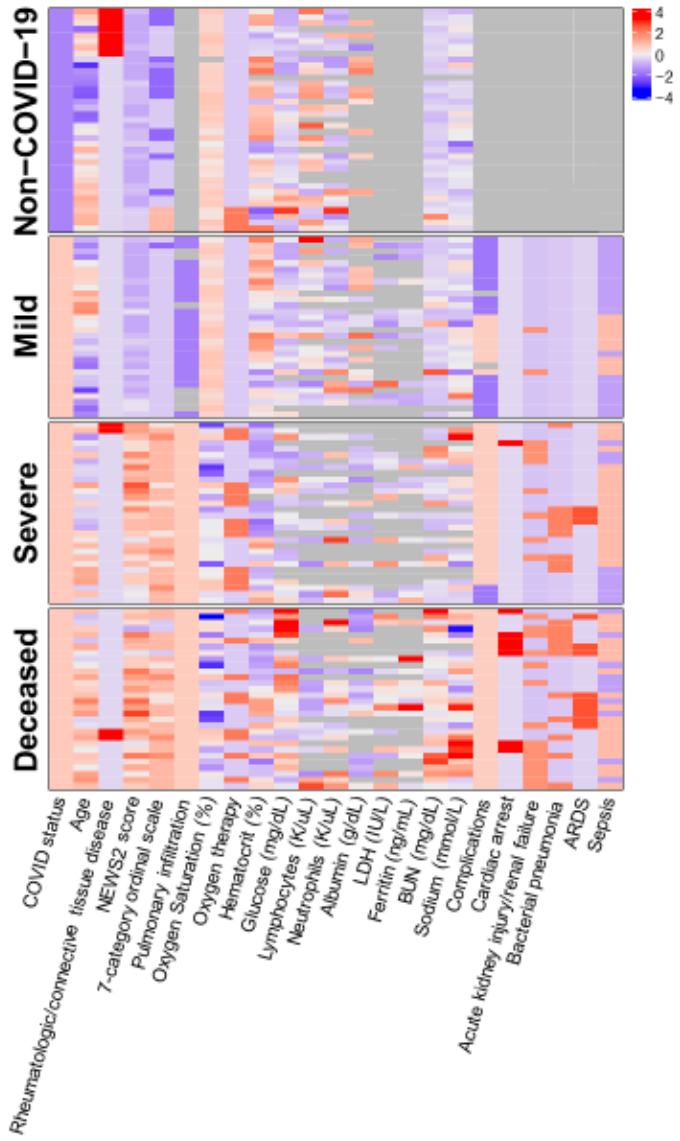

Figure S1. Clinical indices and COVID-19 status. Clinical indices that vary significantly (FDR<0.05, F-test) across 4 patient groups are shown in a heatmap, with blue to red representing low to high values of each index. Color intensity represents the magnitude of value (mean-centered and scaled by the standard deviation). Missing values are shown in grey.

Figure S2

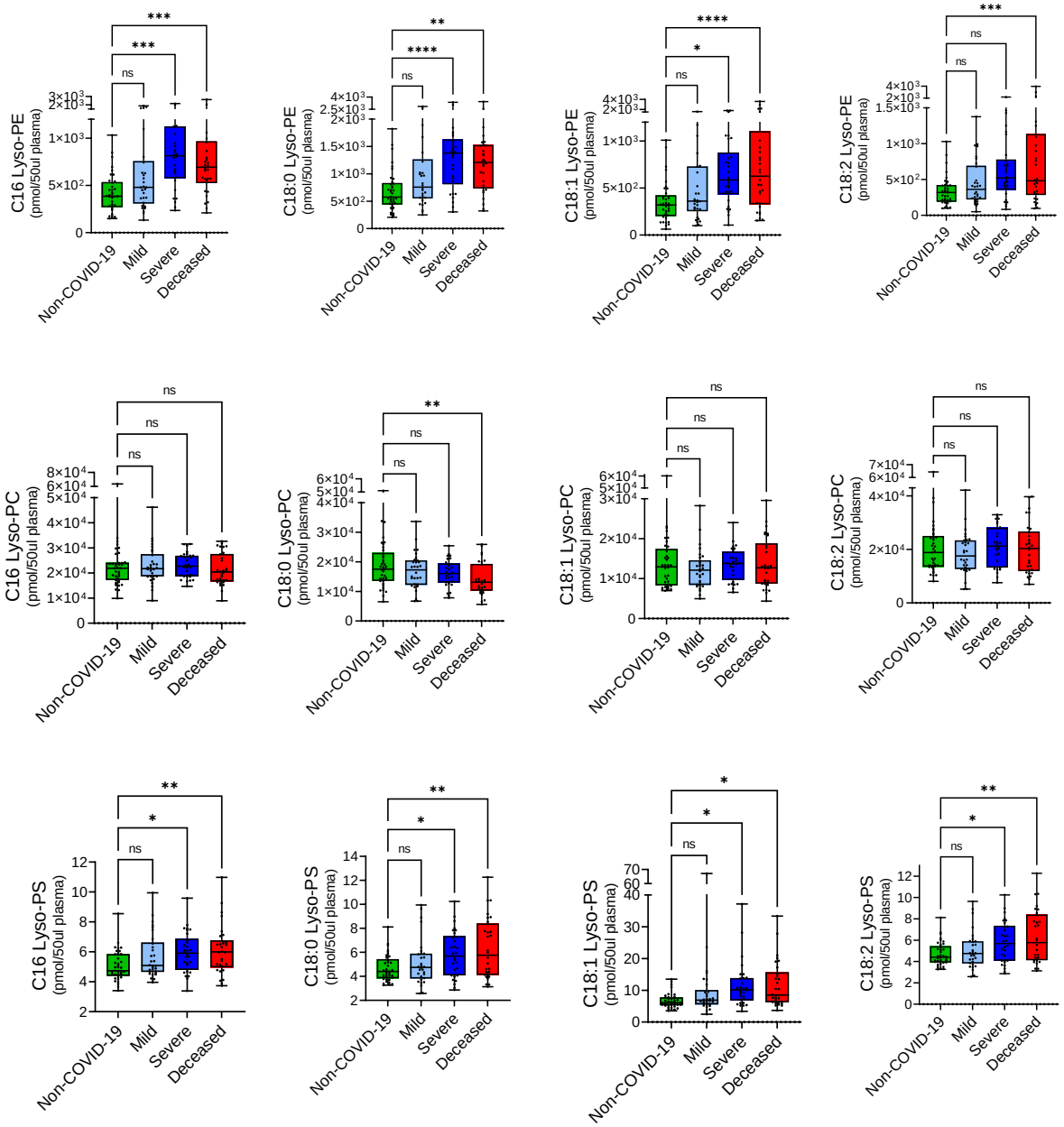

Figure S2. Targeted lyso-phospholipid (Lyso-PL) analysis. Lyso-PLs were identified as top molecules of interest from the qualitative untargeted lipidome data set. Samples were re-analyzed utilizing lyso-PL standards in a targeted lyso-PL method. Upper and lower bounds indicate the 75th (Q3) and 25th (Q1) percentile, respectively; the line within the box indicates the median value; whiskers extend to the minimum and maximum points. Asterisks indicate significance by ANOVA (1-way ANOVA with Dunnet's multiple comparison): * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

Figure S3

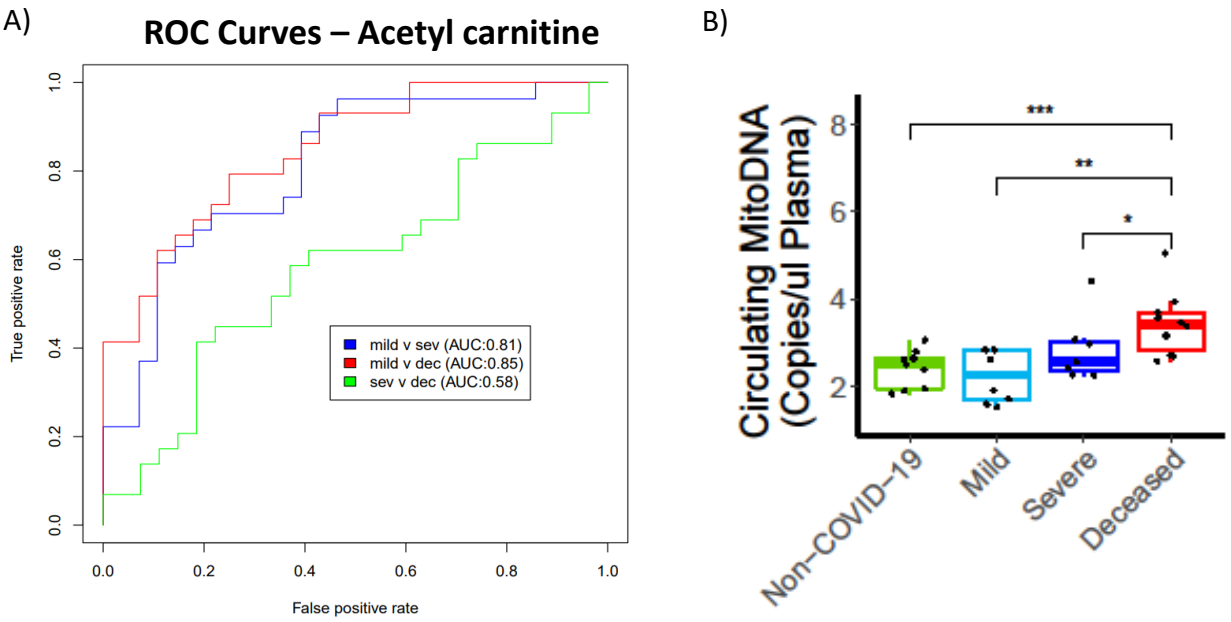

Figure S3. Markers of mitochondrial dysfunction. A) ROC curves demonstrate the performance of acetylcarnitine as a predictor of disease status. B) Mitochondrial DNA was quantified in a selected subset of patients (n=34; 9 non-COVID-19, 8 mild, 7 severe, and 10 deceased COVID-19 patients). Summed copy numbers of genes for human cytochrome C and cytochrome C oxidase subunit III are shown. Data were log transformed and compared using a one-sided Wilcoxon test with Holm correction for multiple comparisons. Significance is indicated as: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Figure S4

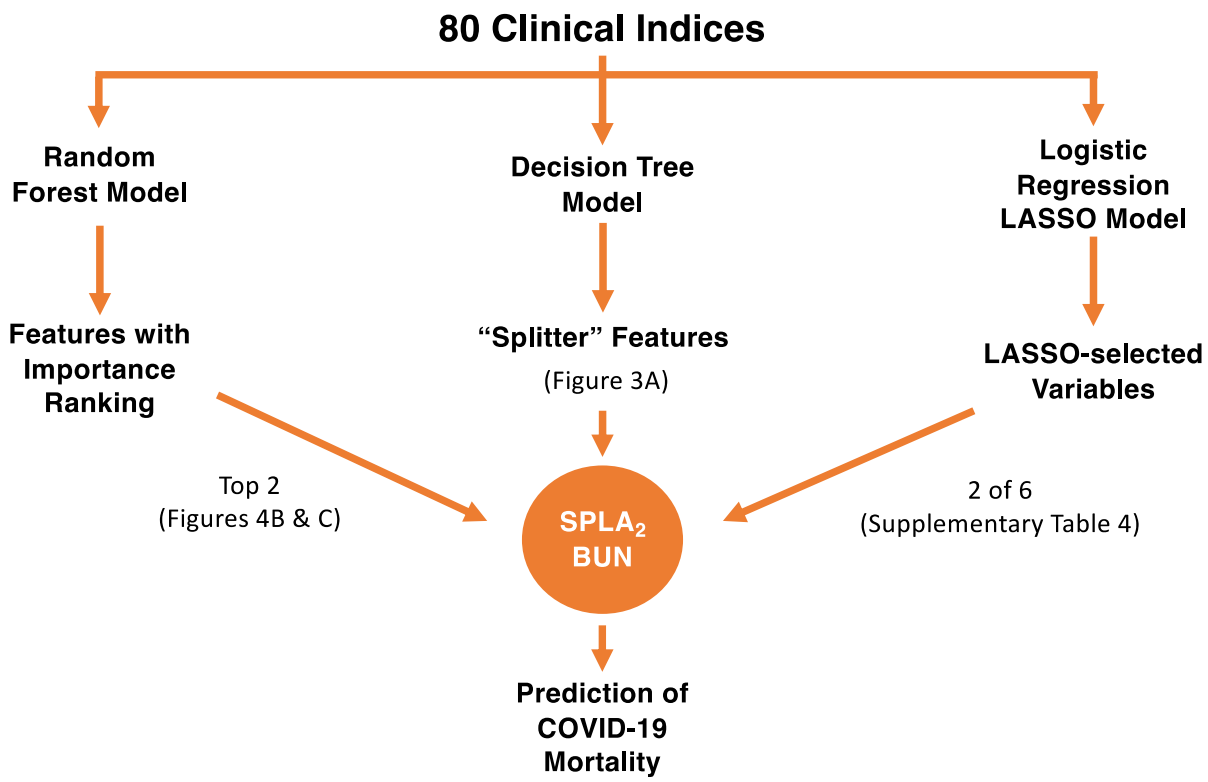

Figure S4. Three independent machine learning approaches identified sPLA2 and BUN as key features for predicting COVID-19 mortality. Additional information about each approach can be found in the Methods section of the main text and the referenced figures.

Figure S5

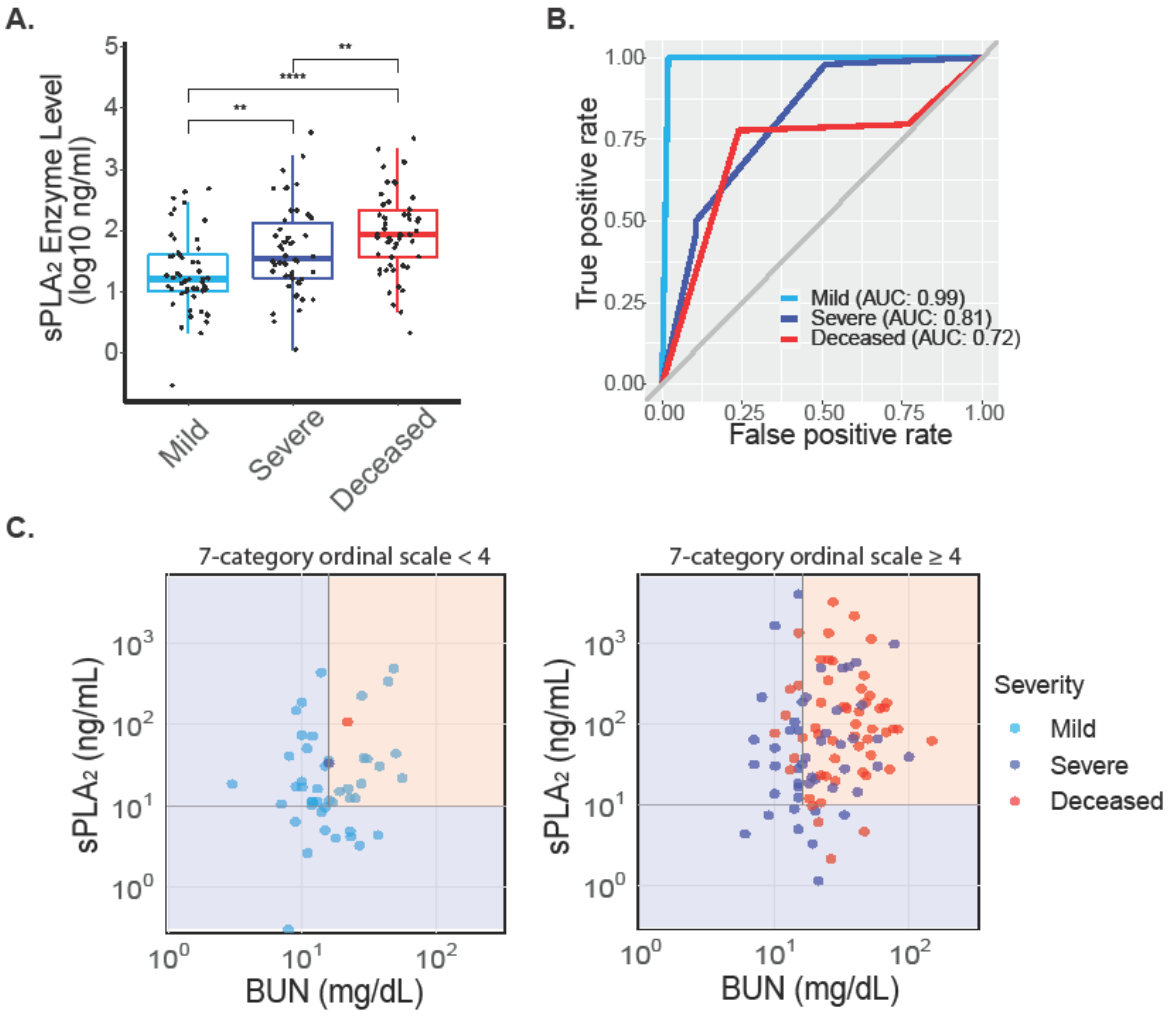

Figure S5. Predicting COVID-19 severity and mortality in a second independent test cohort. A) $sPLA_2$-IIA levels were determined in 154 additional plasma samples. Levels were compared with a one-sided Wilcoxon test with Holm correction for multiple comparisons. Significance is indicated as: ** p<0.01; **** p<0.0001. B) The area under the ROC curve, AUC, of the decision tree (Figure 3A) in determining each patient group membership in the second independent test cohort. C) Decision surface plot. Left and right graphs show the results of applying the sPLA2 and BUN boundary conditions in Figure 3A to the L and R subsets of patients in the tree (split following the 7-category ordinal scale) in the second independent test cohort.

Figure S6

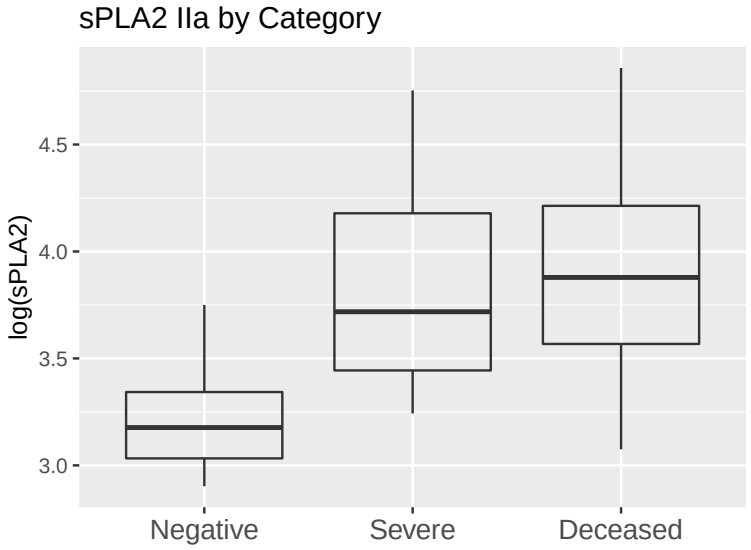

Figure S6. Relative Levels of $sPLA_2$-IIA using the SomaLogic Somascan® Proteomics Platform. Levels are shown in relative fluorescent units (RFU) on the log scale for 82 patients from Banner-University Medical Center Tucson representing non-COVID-19 patients (n=21), severe COVID-19 patients (n=30), and deceased COVID-19 patients (n=31). Groups were compared using a linear model of the log-transformed data vs group, adjusted for Age and Sex. Pairwise comparisons were made using estimated marginal means with Tukey's adjustment for multiple testing. Results: non-COVID-19 vs. severe mean, $p=1.4x10^{-5}$; non-COVID-19 vs. deceased, $p=7.25x10^{-7}$; severe vs. deceased, $p>0.05$. Both severe and deceased COVID-19 patients showed high levels of $sPLA_2$-IIA.

Figure S7.

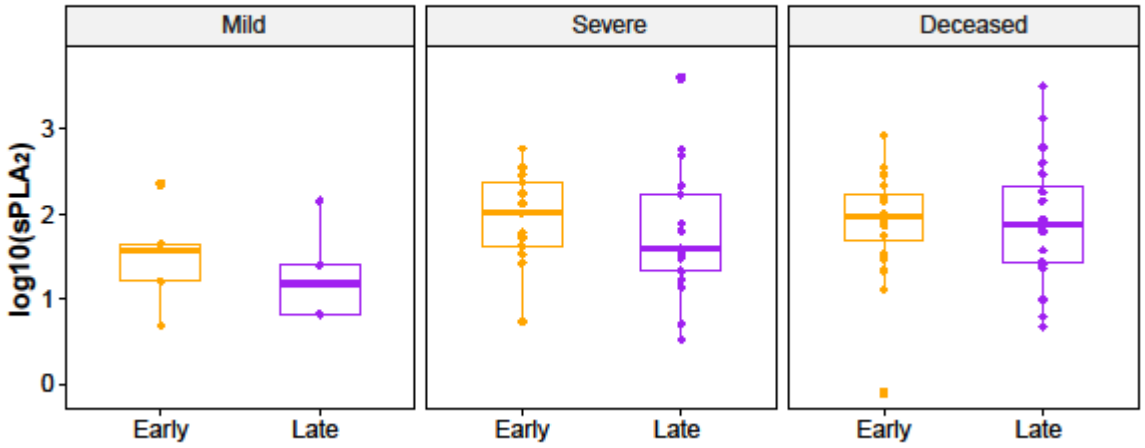

Figure S7. Changes in $sPLA_2$ levels in sequential samples. $sPLA_2$-IIA levels were determined in 46 sets of sequential samples (5 mild, 17 severe, and 24 deceased). The median separation between the late (2^{nd}) and early (1^{st}) time points are as follows: mild, 61 days (late time, post-discharge during revisit); severe, 6 days (27.5% of hospital-stay duration); deceased, 7 days (36.7% of hospital-stay duration).