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Supplemental information

Multi-omics analysis of respiratory specimen characterizes baseline molecular determinants associated with SARS-CoV-2 outcome

Jaswinder Singh Maras, Shvetank Sharma, Adil Bhat, Sheetalnath Rooge, Reshu aggrawal, Ekta Gupta, and Shiv K. Sarin

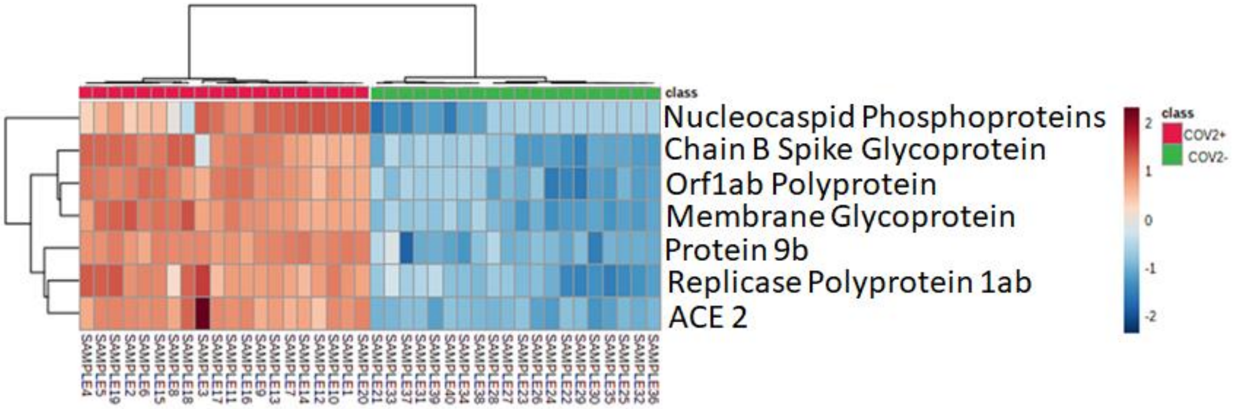
Multi-Omics analysis of respiratory specimen characterizes baseline molecular determinants associated with COVID-19 diagnosis and outcome.

Jaswinder Singh Maras[1*], Shvetank Sharma[1], Adil Bhat[1], Sheetalnath Rooge[2], Reshu aggrawal[2], Ekta Gupta[#2] and Shiv K Sarin[#1, 3*]

[1]Department of Molecular and Cellular Medicine, [2]Department of Virology and [3]Department of Hepatology, Institute of Liver and Biliary Sciences, New Delhi, India.

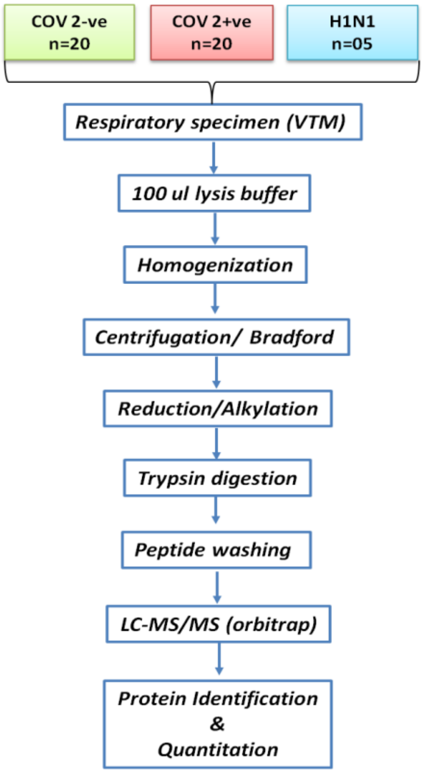
Supplementary Figures:

Supplementary Figure 1: Heat map and hierarchical cluster analysis for the viral proteins identified ($p<0.05$) show clear segregation of COVID-19 positive (Red bar) from COVID-19 negative (Green bar) patients. The expression is given in the range as Dark brown= upregulated, Blue= downregulated and white= unregulated. AUROC analysis shows clear distinction of SARS-COV2 proteins in Positive patients vs. negative patients. **(Related to Figure 1)**

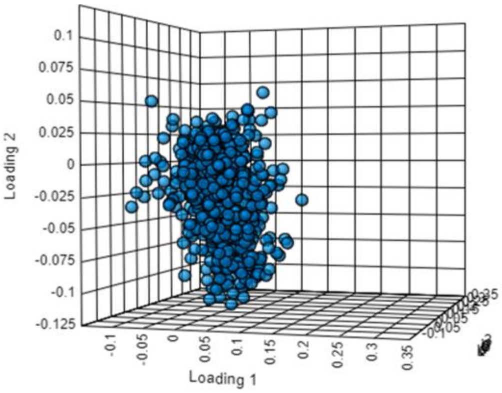


SARS-COV2 proteins	AUC	P-value	FC (LOG)
Membrane Glycoprotein	1.00	1.50E-25	11.790
Orf1ab polyprotein	0.99	3.22E-23	11.876
Neucelocapisid Phospoprotein	0.99	5.22E-25	11.783
Chain B SARS-CoV-2 spike glycoprotein	0.99	1.92E-20	11.644
ACE2	0.99	6.77E-23	11.605
Replicase polyprotein 1ab	0.98	1.90E-19	11.588
Protein 9b	0.98	4.17E-24	10.904

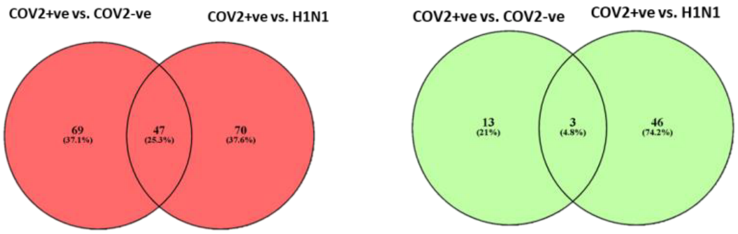
Supplementary Figure 2: Schema for proteomic sample preparation used in the study. **(Related to Figure 1)**



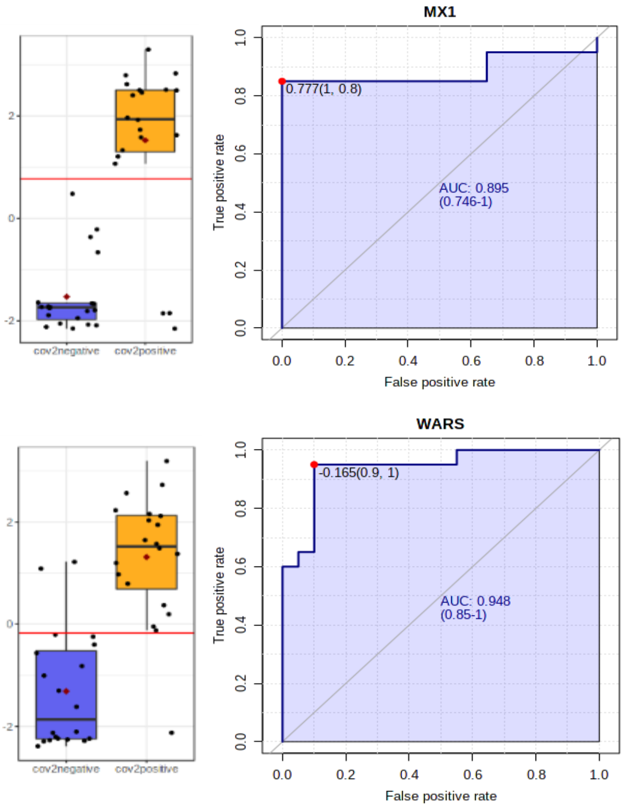
Supplementary Figure 3: Loading plot for the partial least square discriminant analysis showing clear segregation of COVID-19 positive patients from COVID-19 negative and Influenza A H1N1 pdm 2009 positive patients based on proteomic evaluation **(Related to Figure 1)**



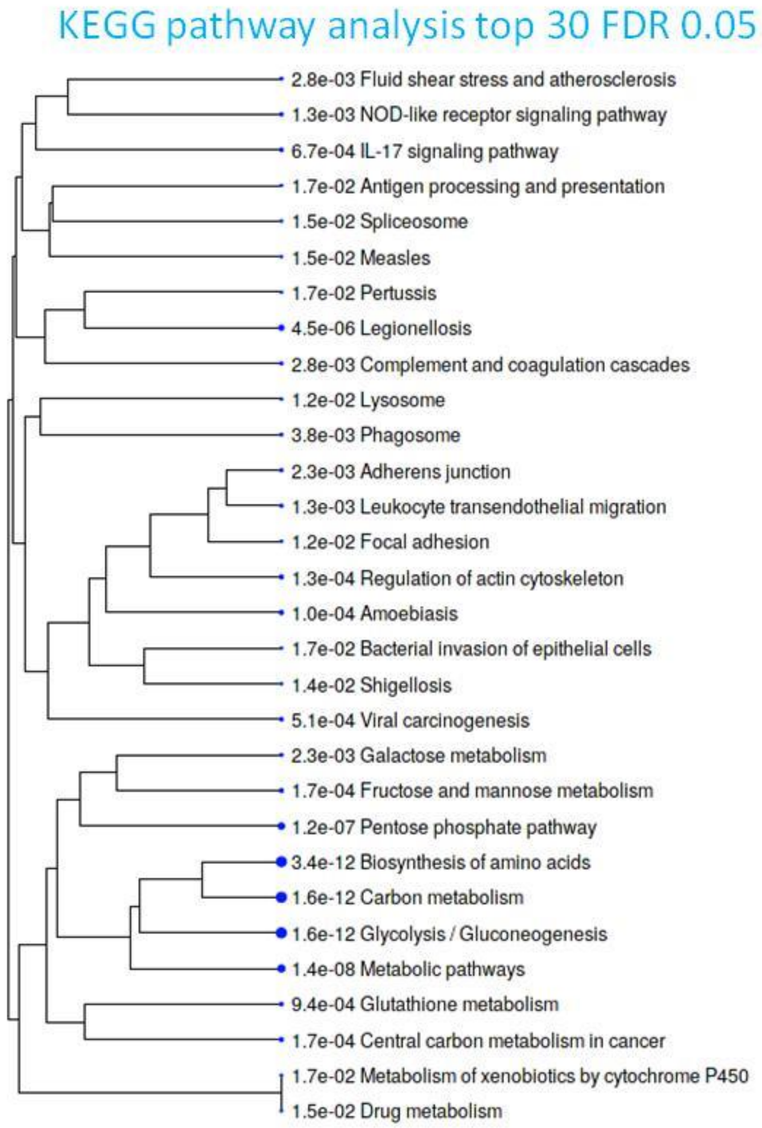
Supplementary Figure 4: Total no of proteins differentially regulated and their intersection in COV2+ vs. COV2- and COV2+ vs. Influenza A H1N1 pdm 2009 positive cases is shown. Red denotes proteins up regulated and green denoted the proteins down regulated. **(Related to Figure 1)**



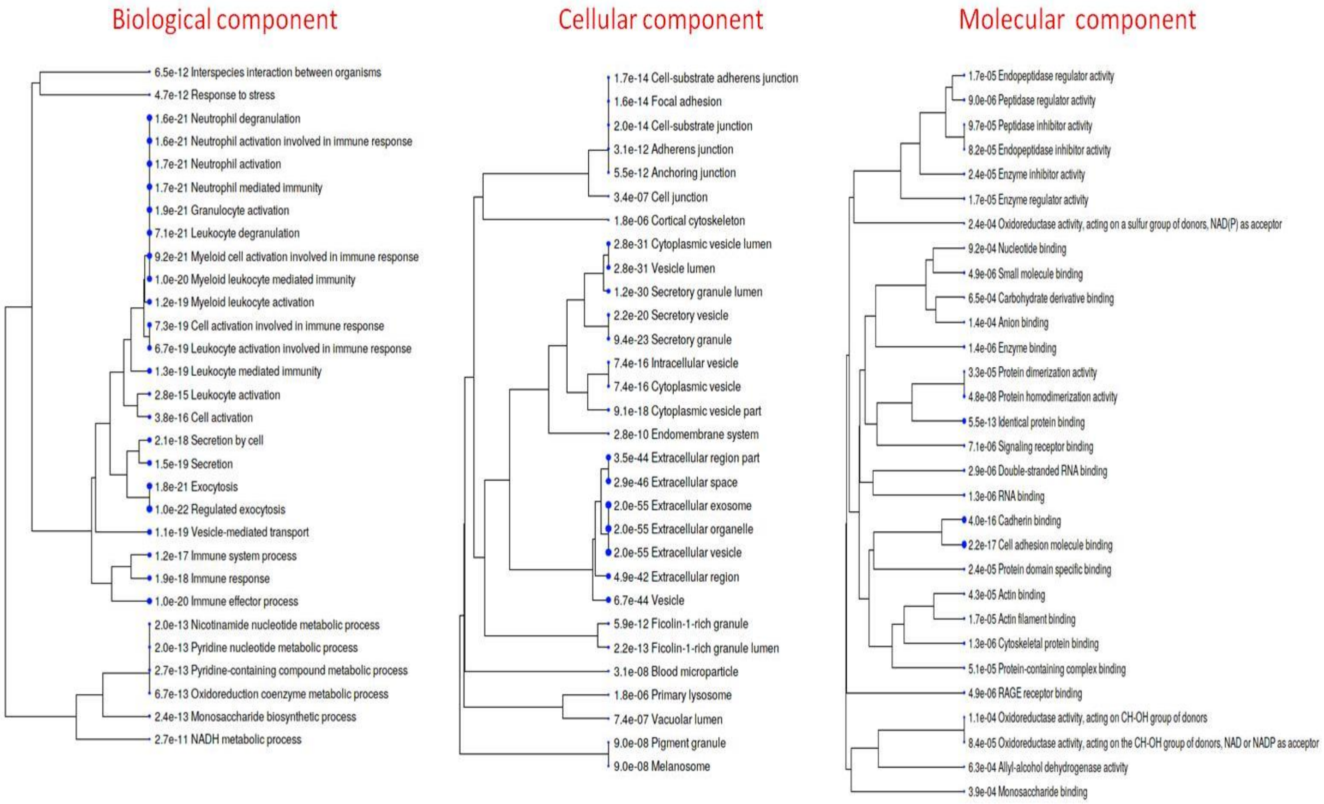
Supplementary Figure 5: Univariate area under the receiver operating curve analysis for MX1 (AUC=0.895 CI (0.75-1)) and WARS (AUC=0.948 CI (0.85-1)) **(Related to Figure 1)**



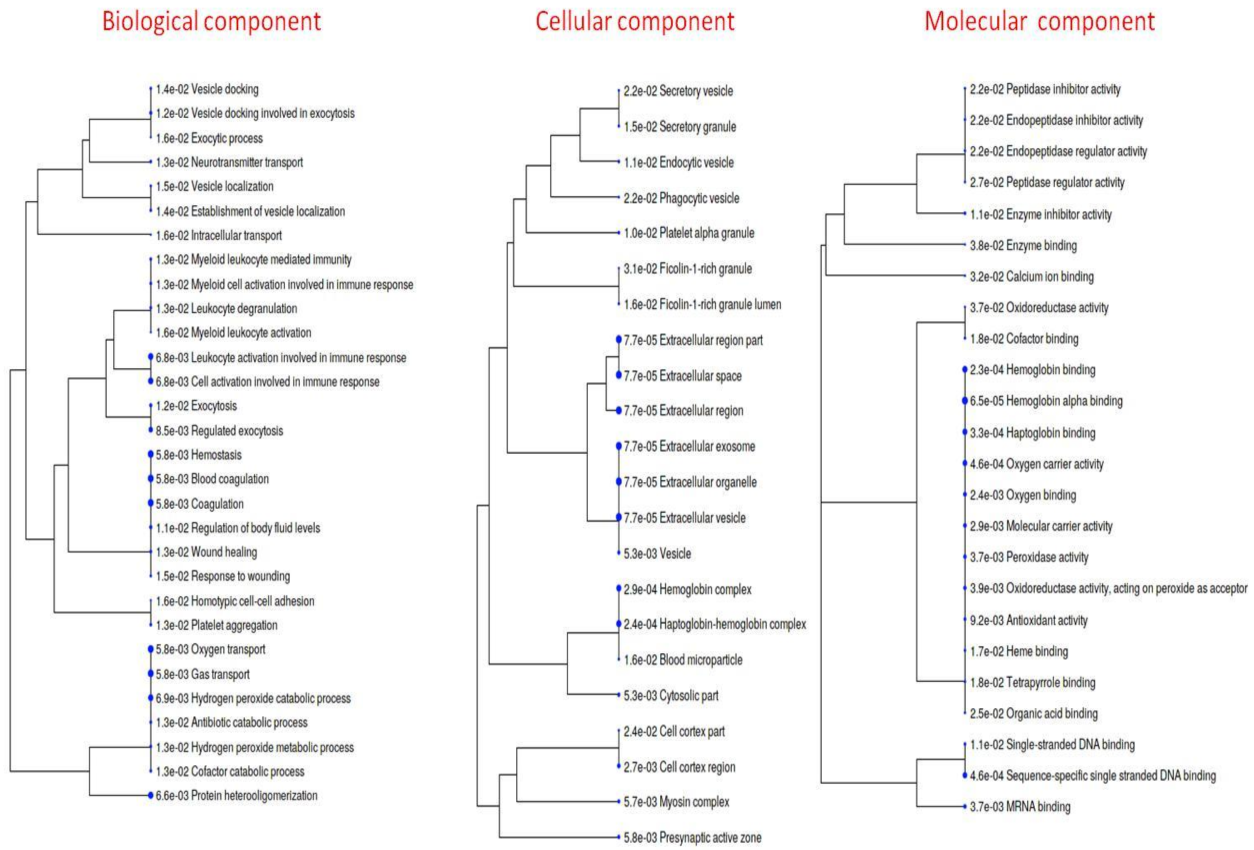
Supplementary Figure 6: A hierarchical clustering tree summarizing the correlation among significant pathways upregulated in COVID-19 positive respiratory specimen compared to COVID-19 negative or Influenza A H1N1 pdm 2009 positive cases. Pathways with many shared genes are clustered together. Bigger dots indicate more significant P-values. **(Related to Figure 1)**



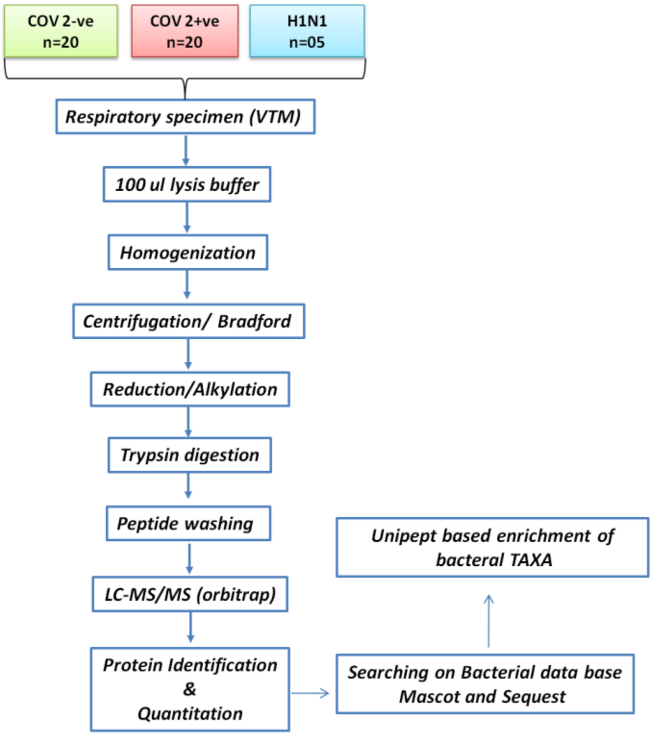
Supplementary Figure 7: A hierarchical clustering tree summarizing the correlation among significant pathways upregulated in COVID-19 positive respiratory specimen compared to COVID-19 negative or Influenza A H1N1 pdm 2009 positive cases. Pathways with many shared genes are clustered together. Bigger dots indicate more significant P-values. **(Related to Figure 1)**



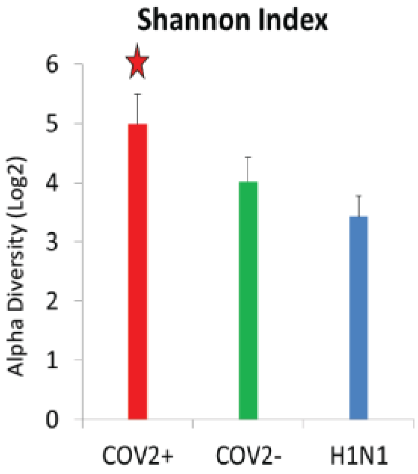
Supplementary Figure 8: A hierarchical clustering tree summarizing the correlation among significant pathways downregulated in COVID-19 positive respiratory specimen compared to COVID-19 negative or Influenza A H1N1 pdm 2009 positive cases. Pathways with many shared genes are clustered together. Bigger dots indicate more significant P-values. **(Related to Figure 1)**



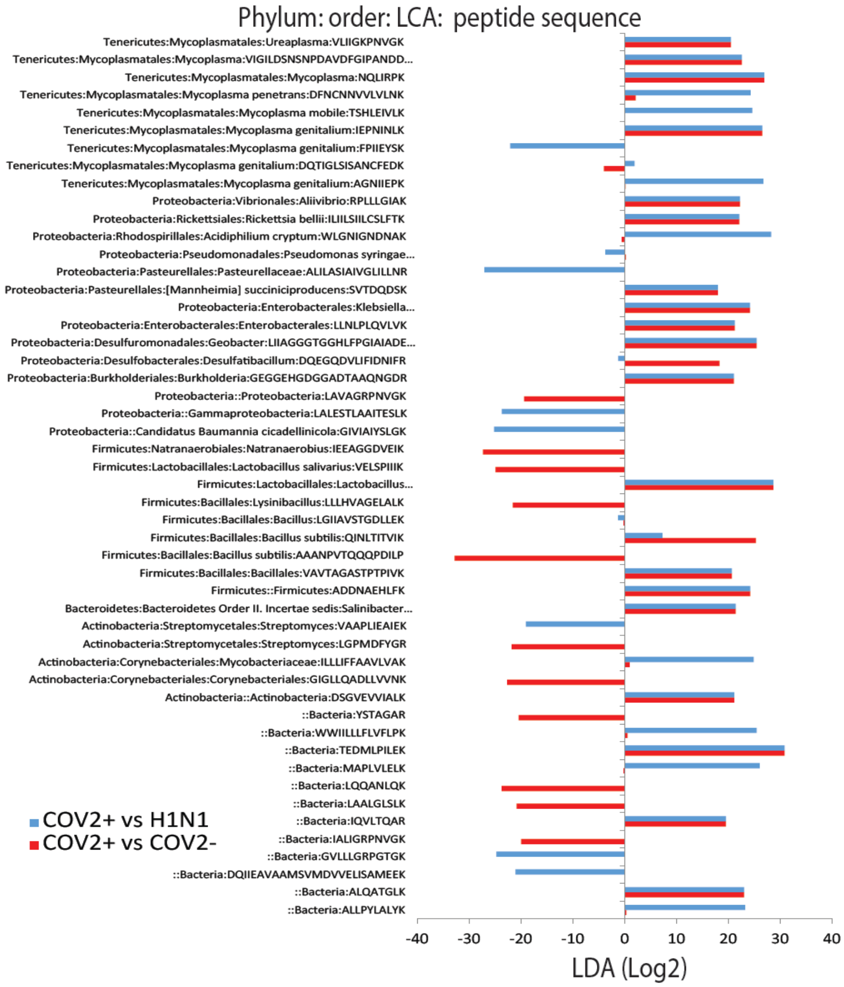
Supplementary Figure 9: Schema for metaproteomics analysis used in the study **(Related to Figure 1)**



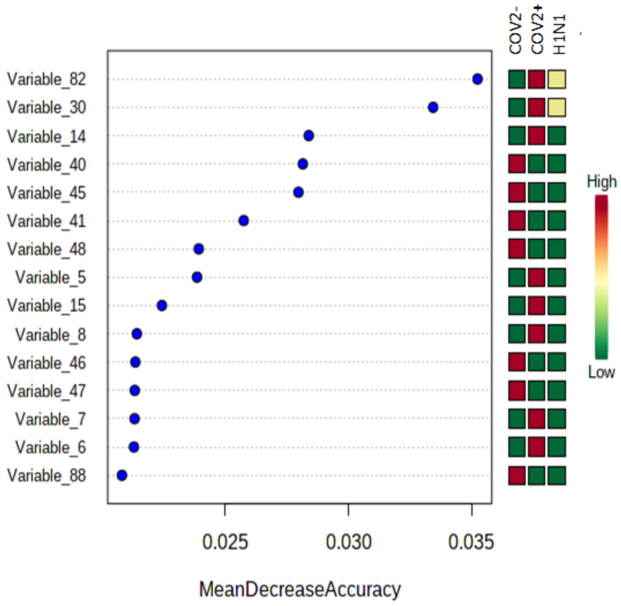
Supplementary Figure 10: Alpha diversity index calculated based on the relative abundance of bacterial peptides identified in COVID-19 positive respiratory specimen as compared to COVID-19 negative or Influenza A H1N1 pdm 2009 positive cases (p-value *=<0.05). **(Related to Figure 1)**



Supplementary Figure 11: Linear discriminant analysis showing log2 levels of the identified bacterial peptides in the respiratory specimen of COVID-19 positive compared to COVID-19 negative (Red) and COVID-19 positive compared to Influenza A H1N1 pdm 2009 positive cases (Blue). The Linear discriminating analysis showed that COVID-19 positive respiratory specimen has significantly increased bacterial peptide linked to the (phylum: LCA); Bacteroidetes: Bacteroidetes Order II. Incertae sedis, Firmicutes: Bacillales, Bacillus subtilis, Lactobacillus plantarum, Proteobacteria: Burkholderiales, Enterobacterales, Klebsiella pneumonia and others, Tenericutes: Mycoplasma genitalium, Mycoplasma mobile and others whereas bacterial peptide linked to Actinobacteria: Corynebacteriales, Streptomycetales, Firmicutes: Bacillus subtilis, Lysinibacillus, Lactobacillus salivarius and others, Proteobacteria : Gamma proteobacteria, pseudomonadales and others and Tenericutes:Mycoplasmatales were significantly reduced as compared to other groups **(Related to Figure 1)**

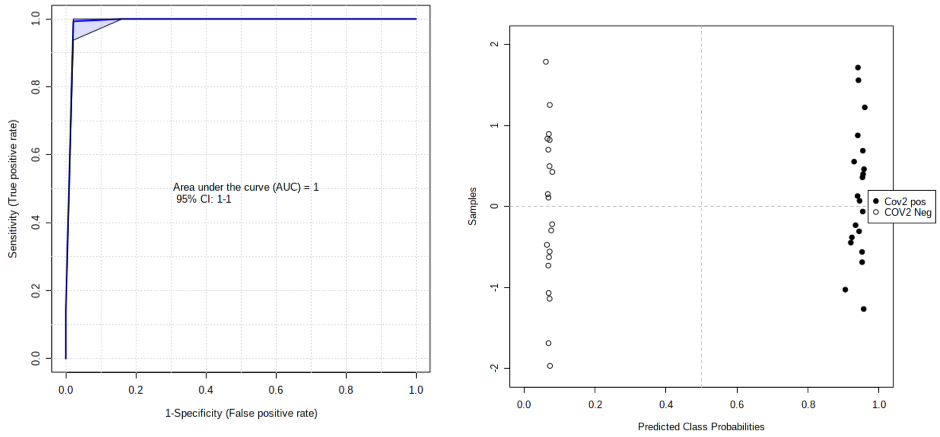


Supplementary Figure 12: Random forest analysis and mean decrease in accuracy plot showing the mean decrease in accuracy of the metaproteome along with their expression status Red= upregulated and Green= downregulated and yellow= unchanged in COVID-19 positive as compared to COVID-19 negative or Influenza A H1N1 pdm 2009 positive cases patients **(Related to Figure 1)**

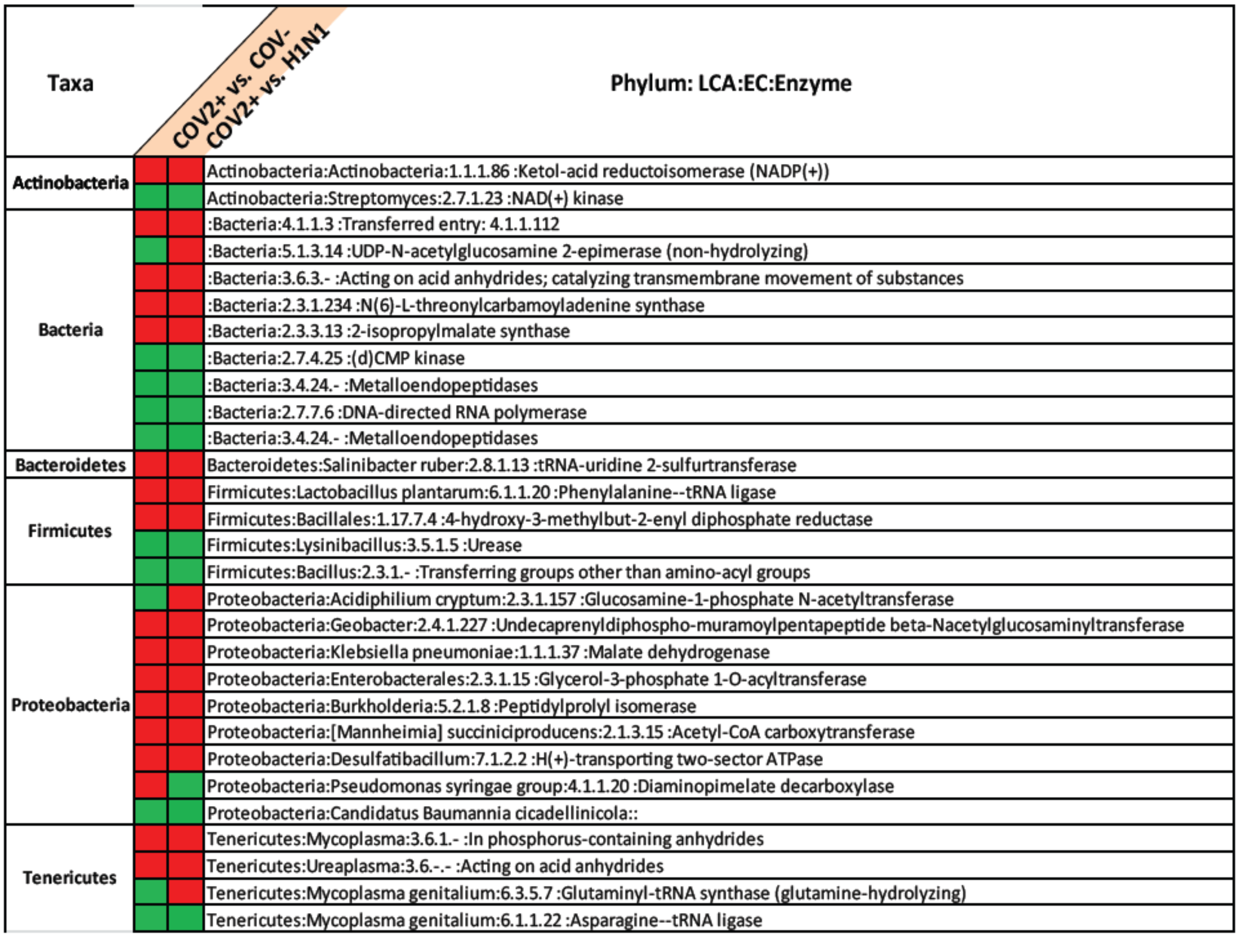


Variable ID	Identification status	LCA
Variable 82	Common COV2 pos and H1N1	Bacillus subtilis
Variable 30	Common Cov 2 Positive and Negative	Burkholderia
Variable 15	Unique to Cov2 positive	Aliivibrio
Variable 14	Unique to Cov2 positive	Mycoplasma
Variable 8	Unique to Cov2 positive	Rickettsia bellii
Variable 7	Unique to Cov2 positive	Mycoplasma genitalium
Variable 6	Unique to Cov2 positive	Lactobacillus plantarum
Variable 5	Unique to Cov2 positive	Salinibacter ruber

Supplementary Figure 13: Area under the receiver operating curve analysis for Variable 80 and Variable 30 together show AUC=1 CI (1-1) p<0.05 and Prediction class probability with a predictive accuracy of 100%. **(Related to Figure 1)**

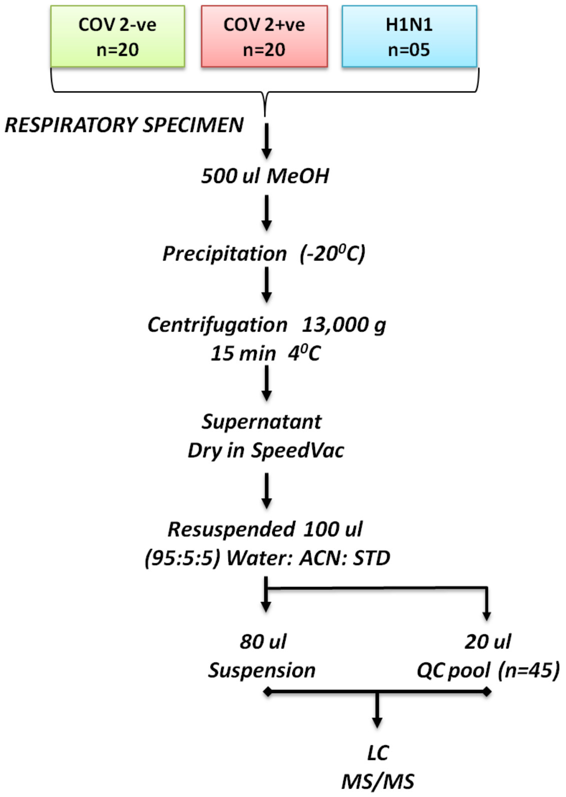


Supplementary Figure 14: Enzyme coded by the bacterial peptides and their abundance in COVID-19 positive compared to COVID-19 negative and COVID-19 positive compared to Influenza A H1N1 pdm 2009 positive cases (Red shows upregulated and Green shows down regulated). Functionality analysis of the COVID-19 positive respiratory microbiome highlighted significant increase in enzymes linked to pantothenate and CoA biosynthesis in actinobacteria (EC1.1.1.86), aminoacyl-trna biosynthesis and terpenoid backbone biosynthesis in fermicutes (EC6.1.1.20, EC1.17.7.4), amino-nucleotide sugar metabolism, peptidoglycan biosynthesis, fatty acid biosynthesis and glycerophospholipid metabolism, energy metabolism and others (EC2.3.1.157, EC2.4.1.227, EC1.1.1.37, EC2.3.1.15, EC2.1.3.15, EC7.1.2.2, EC4.1.1.20) in proteobacteria and others showing a clear indication of bacterial pathogenic thrust in presence of CoV-2. Whereas enzyme linked to nicotinate and nicotinamide metabolism (EC2.7.1.23) in actinobacteria, arginine biosynthesis (EC3.5.1.5) in fermicutes, and Aminoacyl-tRNA biosynthesis linked to aspartate and asparagine metabolism in tenericute were decreased (**(Related to Figure 1)**

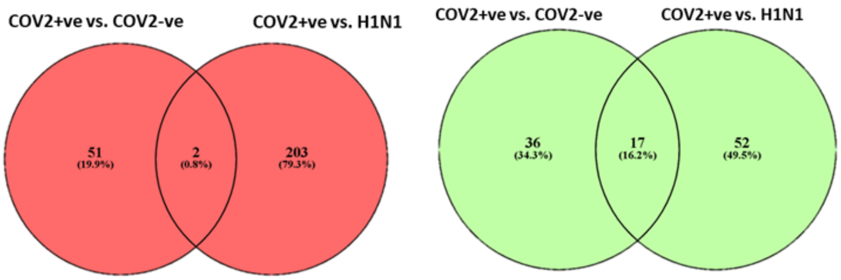


Taxa	COV2+ vs. COV-	COV2+ vs. H1N1	Phylum: LCA:EC:Enzyme
Actinobacteria	🟥	🟥	Actinobacteria:Actinobacteria:1.1.1.86 :Ketol-acid reductoisomerase (NADP(+))
	🟩	🟩	Actinobacteria:Streptomyces:2.7.1.23 :NAD(+) kinase
Bacteria	🟥	🟥	:Bacteria:4.1.1.3 :Transferred entry: 4.1.1.112
	🟩	🟥	:Bacteria:5.1.3.14 :UDP-N-acetylglucosamine 2-epimerase (non-hydrolyzing)
	🟥	🟥	:Bacteria:3.6.3.- :Acting on acid anhydrides; catalyzing transmembrane movement of substances
	🟥	🟥	:Bacteria:2.3.1.234 :N(6)-L-threonylcarbamoyladenine synthase
	🟥	🟥	:Bacteria:2.3.3.13 :2-isopropylmalate synthase
	🟩	🟩	:Bacteria:2.7.4.25 :(d)CMP kinase
	🟩	🟩	:Bacteria:3.4.24.- :Metalloendopeptidases
	🟩	🟩	:Bacteria:2.7.7.6 :DNA-directed RNA polymerase
	🟩	🟩	:Bacteria:3.4.24.- :Metalloendopeptidases
Bacteroidetes	🟥	🟥	Bacteroidetes:Salinibacter ruber:2.8.1.13 :tRNA-uridine 2-sulfurtransferase
Firmicutes	🟥	🟥	Firmicutes:Lactobacillus plantarum:6.1.1.20 :Phenylalanine--tRNA ligase
	🟥	🟥	Firmicutes:Bacillales:1.17.7.4 :4-hydroxy-3-methylbut-2-enyl diphosphate reductase
	🟩	🟩	Firmicutes:Lysinibacillus:3.5.1.5 :Urease
	🟩	🟩	Firmicutes:Bacillus:2.3.1.- :Transferring groups other than amino-acyl groups
Proteobacteria	🟩	🟥	Proteobacteria:Acidiphilium cryptum:2.3.1.157 :Glucosamine-1-phosphate N-acetyltransferase
	🟥	🟥	Proteobacteria:Geobacter:2.4.1.227 :Undecaprenyldiphospho-muramoylpentapeptide beta-Nacetylglucosaminyltransferase
	🟥	🟥	Proteobacteria:Klebsiella pneumoniae:1.1.1.37 :Malate dehydrogenase
	🟥	🟥	Proteobacteria:Enterobacterales:2.3.1.15 :Glycerol-3-phosphate 1-O-acyltransferase
	🟥	🟥	Proteobacteria:Burkholderia:5.2.1.8 :Peptidylprolyl isomerase
	🟥	🟥	Proteobacteria:[Mannheimia] succiniciproducens:2.1.3.15 :Acetyl-CoA carboxytransferase
	🟥	🟥	Proteobacteria:Desulfatibacillum:7.1.2.2 :H(+)-transporting two-sector ATPase
	🟥	🟩	Proteobacteria:Pseudomonas syringae group:4.1.1.20 :Diaminopimelate decarboxylase
	🟩	🟩	Proteobacteria:Candidatus Baumannia cicadellinicola::
Tenericutes	🟥	🟥	Tenericutes:Mycoplasma:3.6.1.- :In phosphorus-containing anhydrides
	🟥	🟥	Tenericutes:Ureaplasma:3.6.-.- :Acting on acid anhydrides
	🟩	🟥	Tenericutes:Mycoplasma genitalium:6.3.5.7 :Glutaminyl-tRNA synthase (glutamine-hydrolyzing)
	🟩	🟩	Tenericutes:Mycoplasma genitalium:6.1.1.22 :Asparagine--tRNA ligase

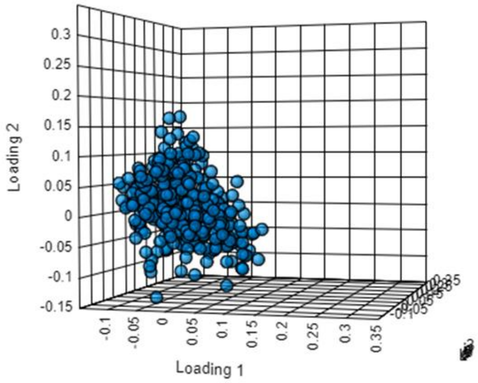
Supplementary Figure 15: Schema for metabolomics analysis used in the study **(Related to Figure 2)**



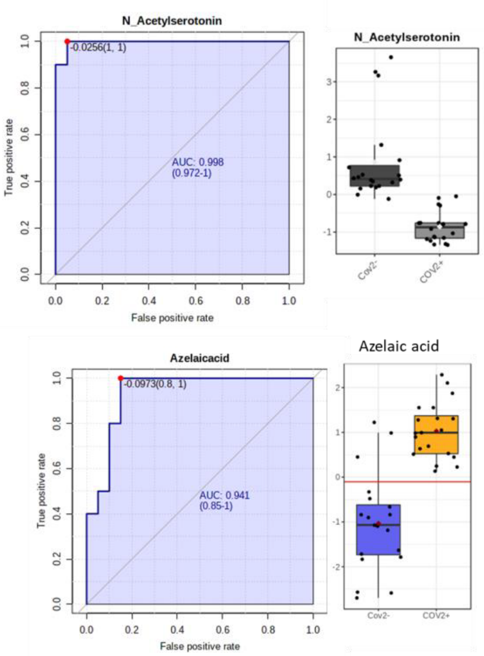
Supplementary Figure 16: Total no of metabolites differentially regulated and their intersection in COV2+ vs. COV2- and COV2+ vs. Influenza A H1N1 pdm 2009 positive cases is shown. Red denotes proteins up regulated and green denoted the proteins down regulated. **(Related to Figure 2)**



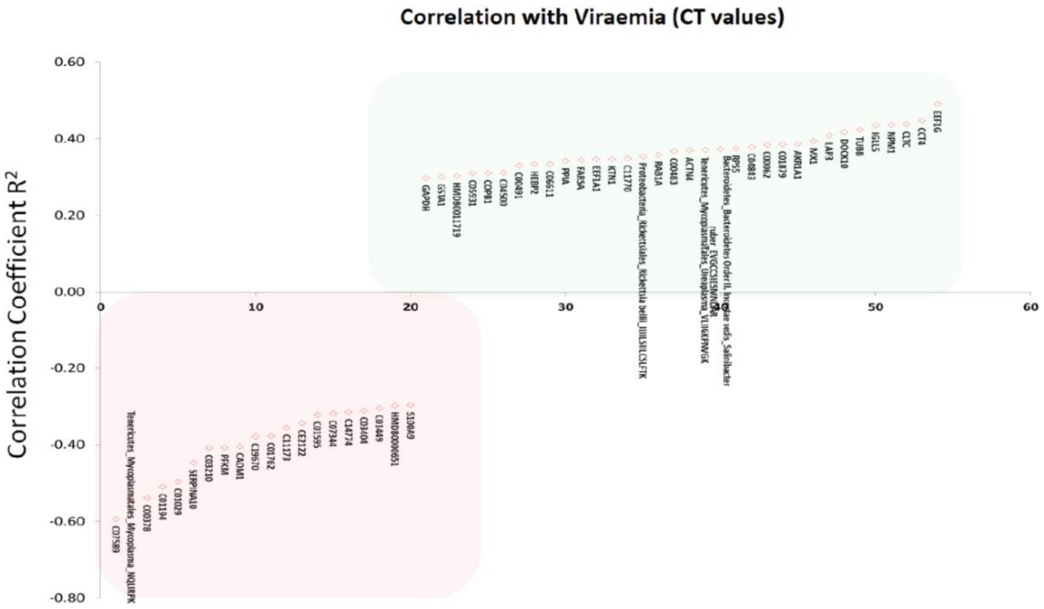
Supplementary Figure 17: Loading plot for the partial least square discriminant analysis showing clear segregation of COVID-19 positive patients from COVID-19 negative and Influenza A H1N1 pdm 2009 positive cases patients based on metabolomics evaluation **(Related to Figure 2)**



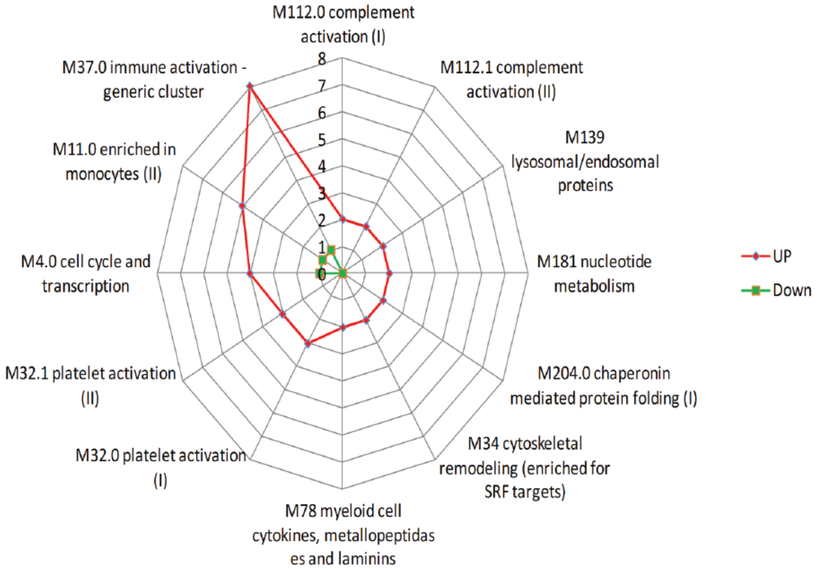
Supplementary Figure 18: Univariate area under the receiver operating curve analysis for N-Acetylserotinin(AUC=0.99 CI (0.975-1)) and Azelaic acid (AUC=0.941 CI (0.85-1)) **(Related to Figure 2)**



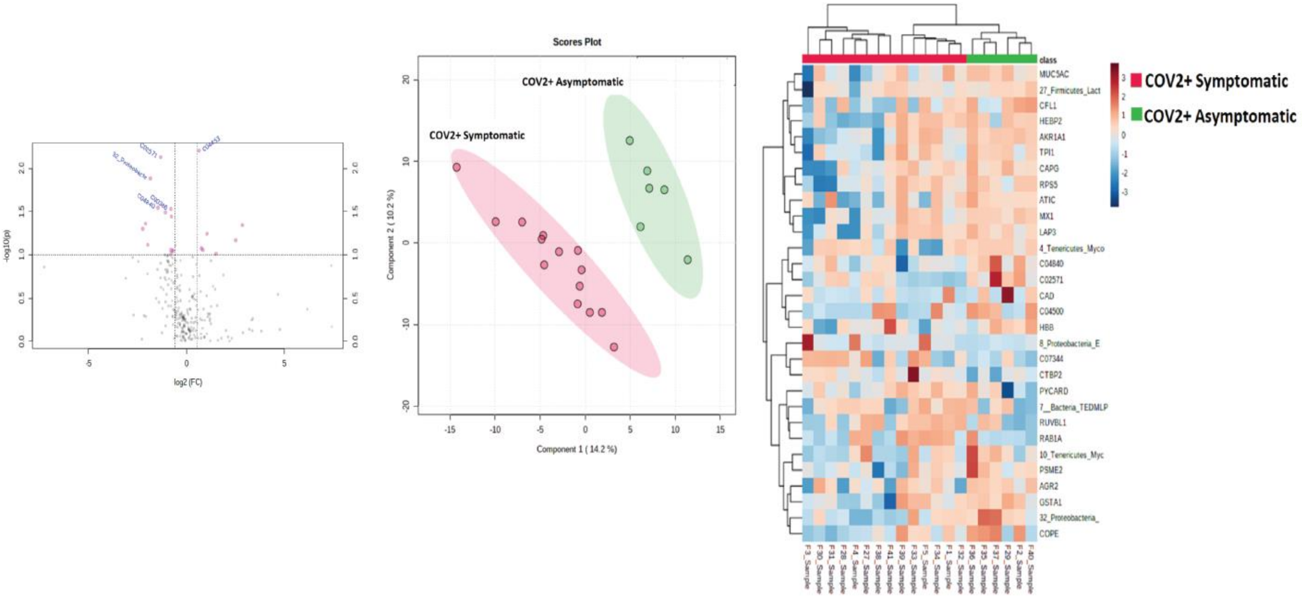
Supplementary Figure 19: Correlation plot showing top 54 proteins and metabolites which showed significant correlation with the CT values of COVID-19 positive patients. Green highlight on the molecules are positively correlated to the CT values and Red color highlight on the molecules denotes negative correlation with the CT values (RT-PCR). **(Related to Figure 3)**



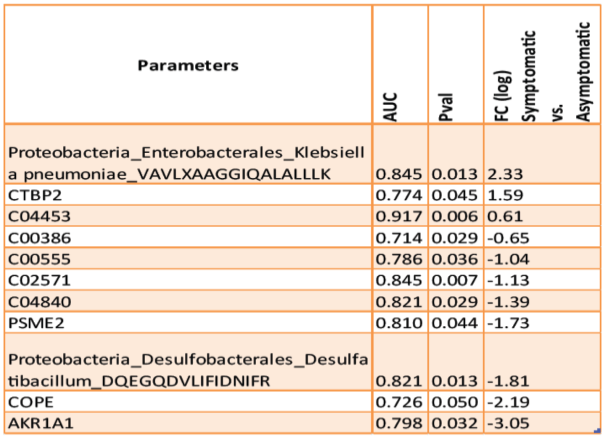
Supplementary Figure 20: BTM enrichment analysis of respiratory proteome identifies immune clusters represented as web plot showing the number of proteins present in each module, red= upregulated in COVID-19 respiratory specimen and green= downregulated in COVID-19 respiratory specimen. **(Related to Figure 3)**



Supplementary Figure 21: Volcano plot showing differentially expressed multi-omics data in asymptomatic COVID-19 as compared to symptomatic COVID-19 respiratory specimen. Pink dots are significant ($p<0.05$, FC>1.5), Partial least square discriminant analysis showing clear segregation of symptomatic COVID-19 patients (Red dots) from asymptomatic COVID-19 patients (Green dots) based on Multi-omics estimations, Heat map and hierarchical cluster analysis of the multi-omics identified ($p<0.05$) show clear segregation of COVID-19 symptomatic (Red bar) from asymptomatic COVID-19 (Green bar) patients. The expression is given in the range as Dark brown= upregulated, Blue= downregulated and white= unregulated. **(Related to Figure 3)**

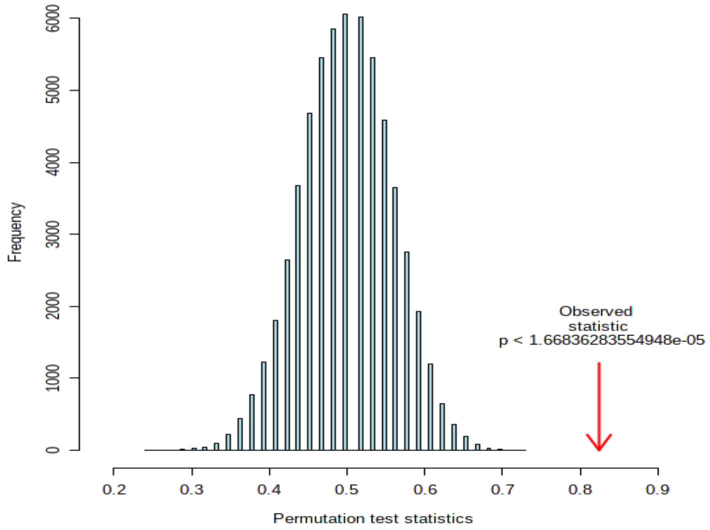


Supplementary Figure 22: Area under the receiver operating curve analysis along with fold change and significance of bio-molecules capable of segregating COVID-19 symptomatic from asymptomatic COVID-19 patients. **(Related to Figure 3)**

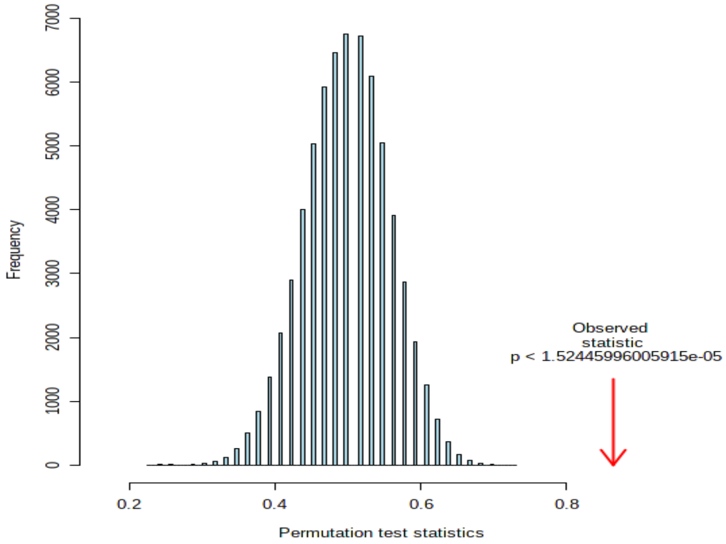


Parameters	AUC	Pval	FC (log) Symptomatic vs. Asymptomatic
Proteobacteria_Enterobacterales_Klebsiella pneumoniae_VAVLXAAGGIQALALLLK	0.845	0.013	2.33
CTBP2	0.774	0.045	1.59
C04453	0.917	0.006	0.61
C00386	0.714	0.029	-0.65
C00555	0.786	0.036	-1.04
C02571	0.845	0.007	-1.13
C04840	0.821	0.029	-1.39
PSME2	0.810	0.044	-1.73
Proteobacteria_Desulfobacterales_Desulfatibacillum_DQEGQDVLIFIDNIFR	0.821	0.013	-1.81
COPE	0.726	0.050	-2.19
AKR1A1	0.798	0.032	-3.05

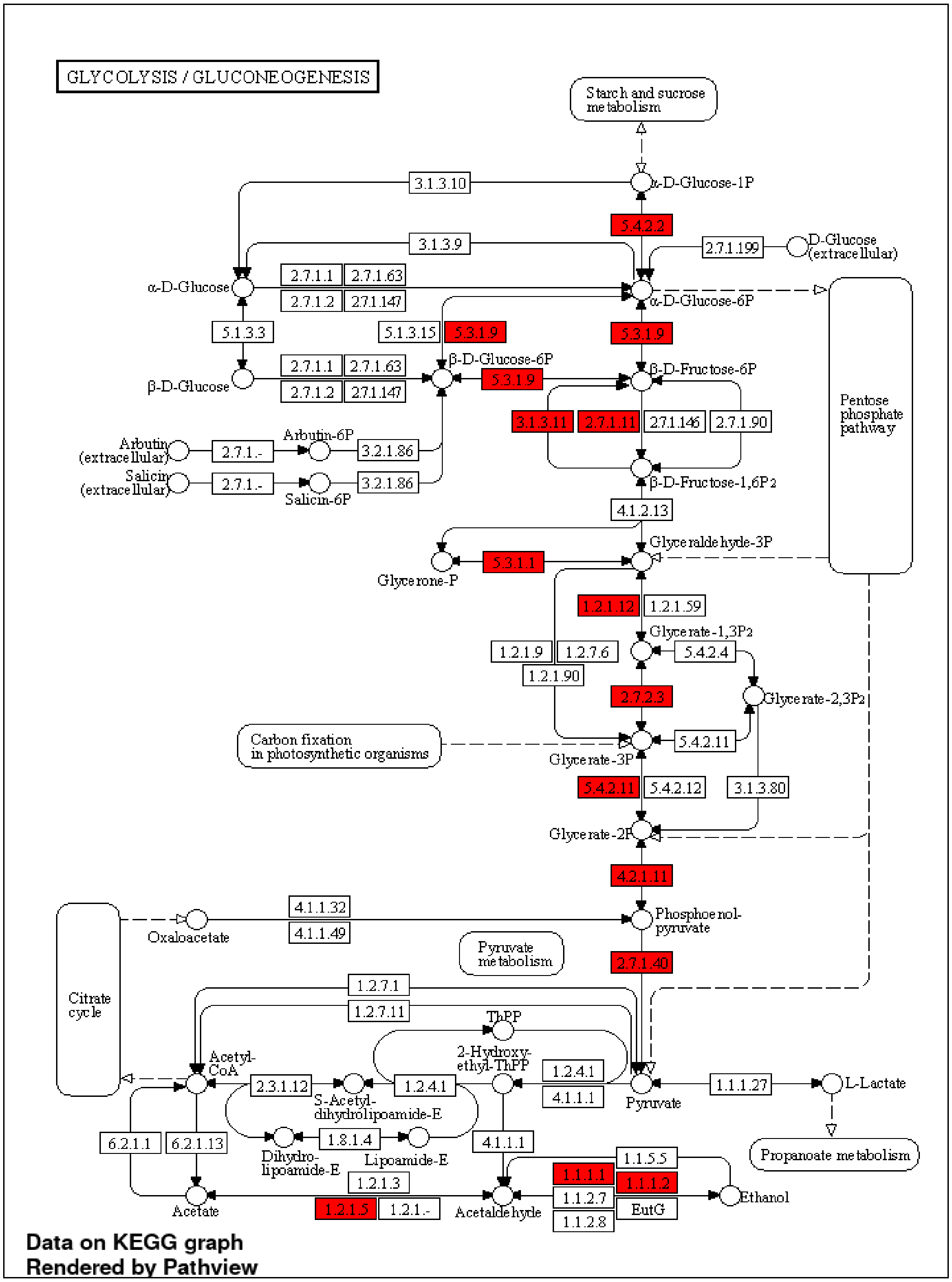
Supplementary Figure 23: Permutation statistical analysis for prediction class probability of MX1 (1000 permutation) show p<0.01. **(Related to Figure 4)**



Supplementary Figure 24: Permutation statistical analysis for prediction class probability of MX1 and WARS (1000 permutation) together show p<0.01. **(Related to Figure 4)**



Supplementary Figure 25: KEGG pathway enrichment analysis for proteins upregulated in the respiratory specimen of COVID-19 positive patients as compared to COVID-19 negative patients showing significant enrichment of proteins lined to Glycolysis/ Gluconeogenesis. **(Related to Figure 1)**



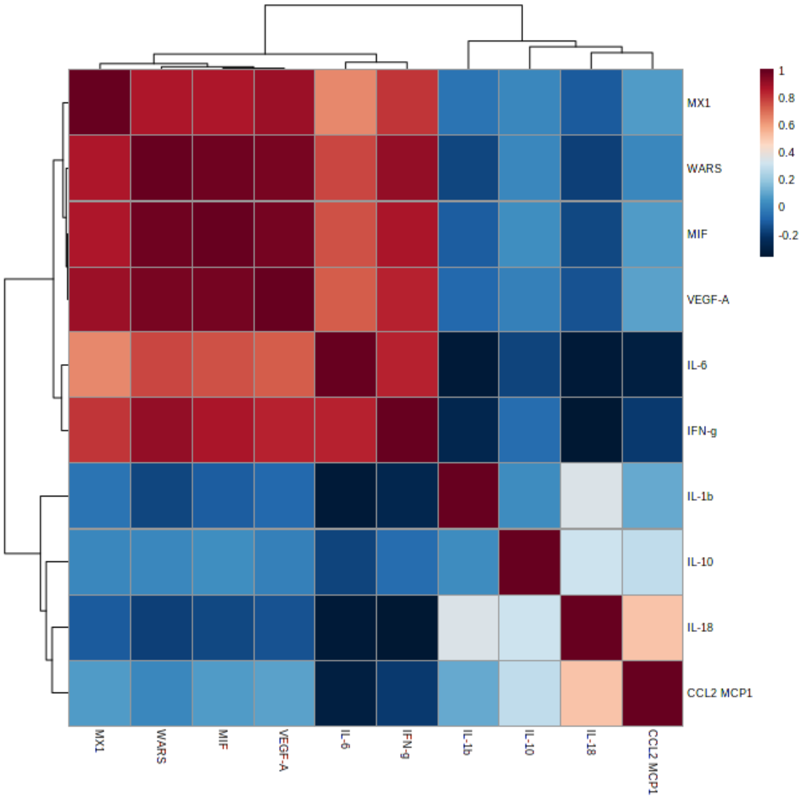
Supplementary Figure 26: Correlation of MX1 and WARS Elisa levels with the respiratory rate and SOFA score of COVID 19 patients. **(Related to Figure 4)**

Parameters	MX1	WARS
Respiratory Rate	r=0.65, p=0.0024	r=0.48, p=0.041
SOFA Score	r=0.71, p=0.0001	r=0.53, p=0.047

Supplementary figure 27: **(Related to Figure 4)**

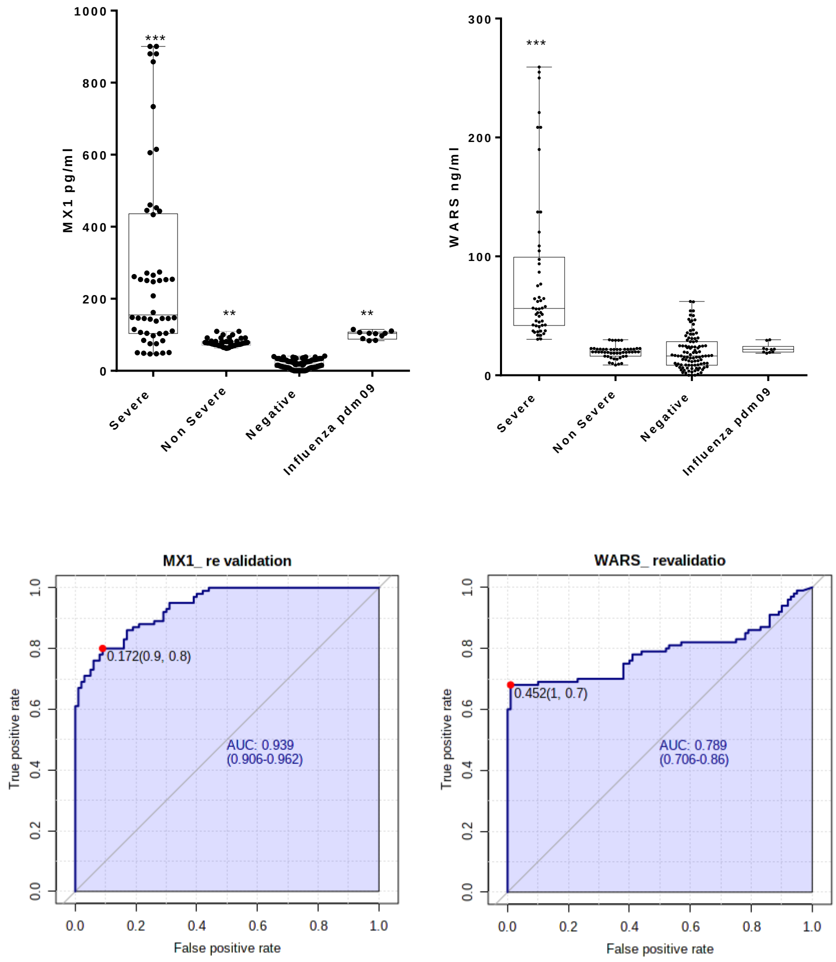
Correlation Plot OF MX1 and WARS with MIF, VEGF-A, IL-6 and Immune interferon gamma

Red documents p<0.05 and R>0.5



Supplement Figure 28 (Related to Figure 4)

Quantitative assessment of MX1 and WARS in the respiratory specimen of the test cohort (n=200; severe infection: 50, non-severe infection: 50 and RTPCR based COVID-19 positive specimen. COVID-19 positive specimen show significant increase in MX1 and WARS levels in COVID-19 positive particularly in patients with severe infection as compared to COVID-19 negative (upper panel; FC> 2, p<0.05). Lower panel documents AUROC value for prediction of COV2+ for MX1 and WARS in COVID-19 patients.



Supplementary Figure 29 (Related to Figure 4)

Combined AUROC of MX1 and WARS and 2000 samples bootstraping analysis in the test cohort (n=200; severe infection: 50, non-severe infection: 50 and RTPCR based COVID-19 positive specimen).