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Supplemental Information

Omics-Driven Systems Interrogation of Metabolic Dysregulation in COVID-19 Pathogenesis

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Figure S1

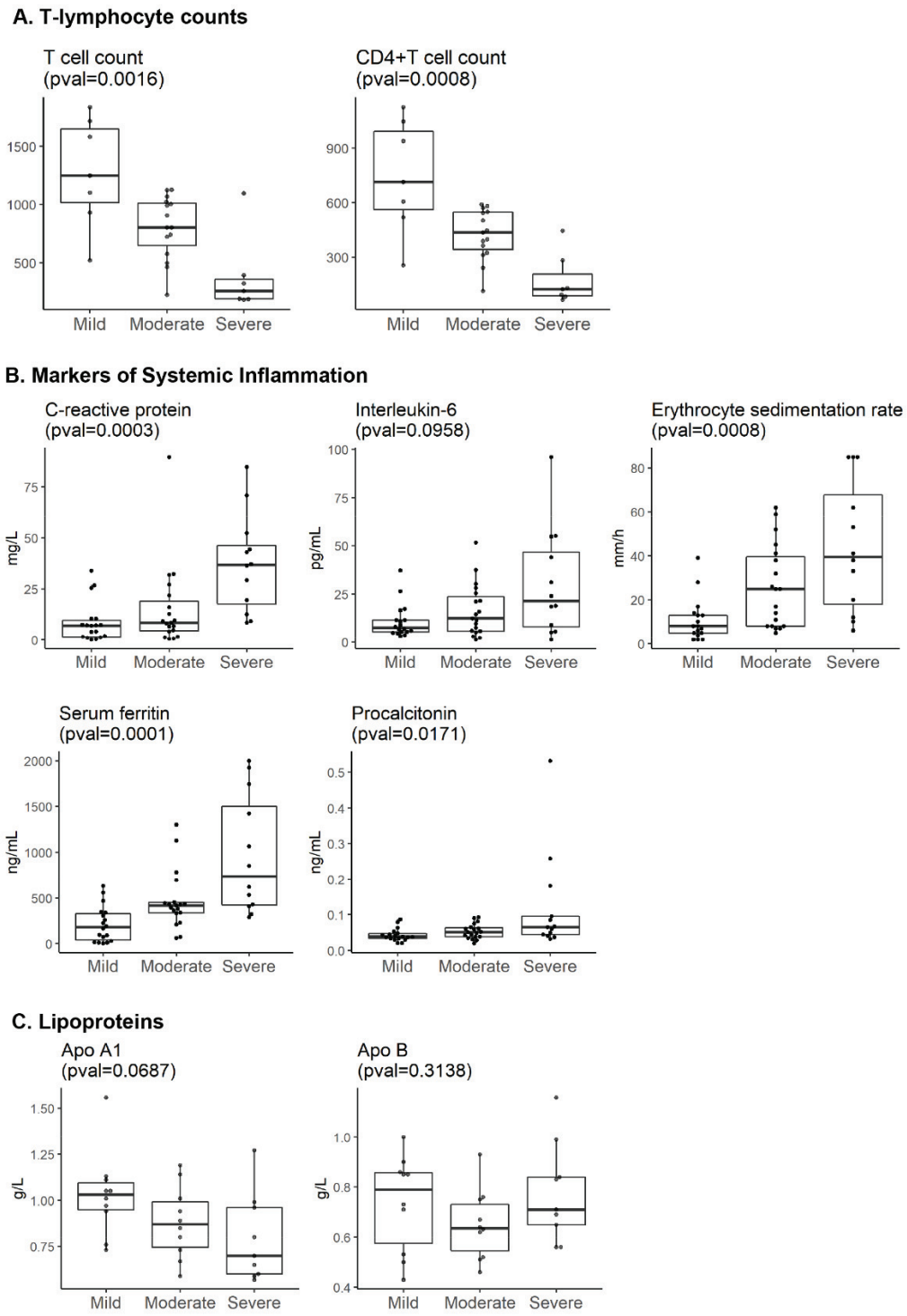

Figure S1. Changes in clinical indices and laboratory findings across COVID-19 patients of different severity, Related to Table 1. Boxplots of T-cell count (A), circulating lipoprotein content (B) and selected clinical indices relevant to systemic inflammation (C).

Figure S2

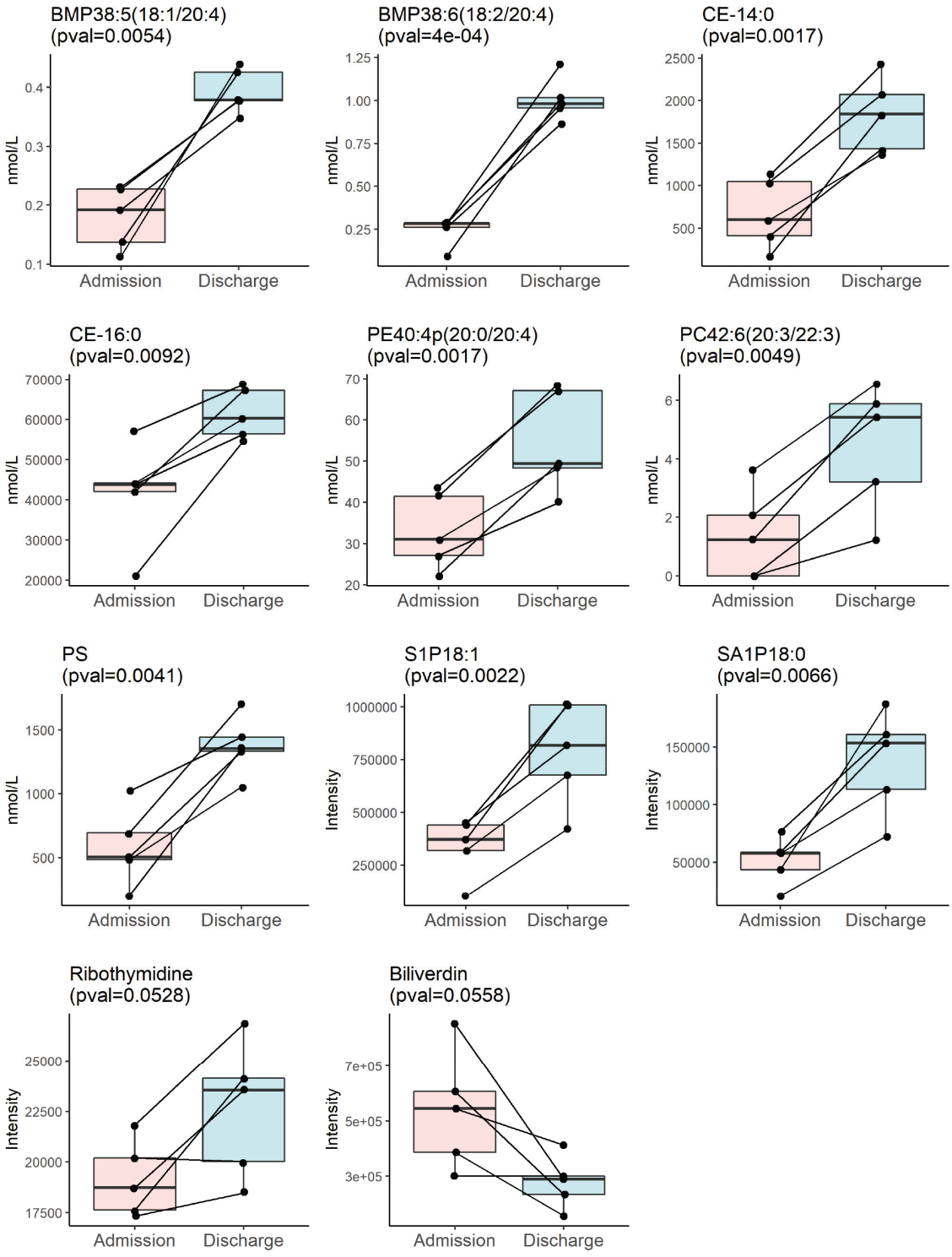

Figure S2. Longitudinal changes in plasma lipids and metabolites in patients from hospital admission to discharge, Related to Figure 1. Changes in metabolite levels in plasma samples collected at hospital admission and prior to discharge from five patients were shown in boxplots, with line segments connecting samples from the same patient. P value of paired t-test was indicated in each plot title.

Figure S3

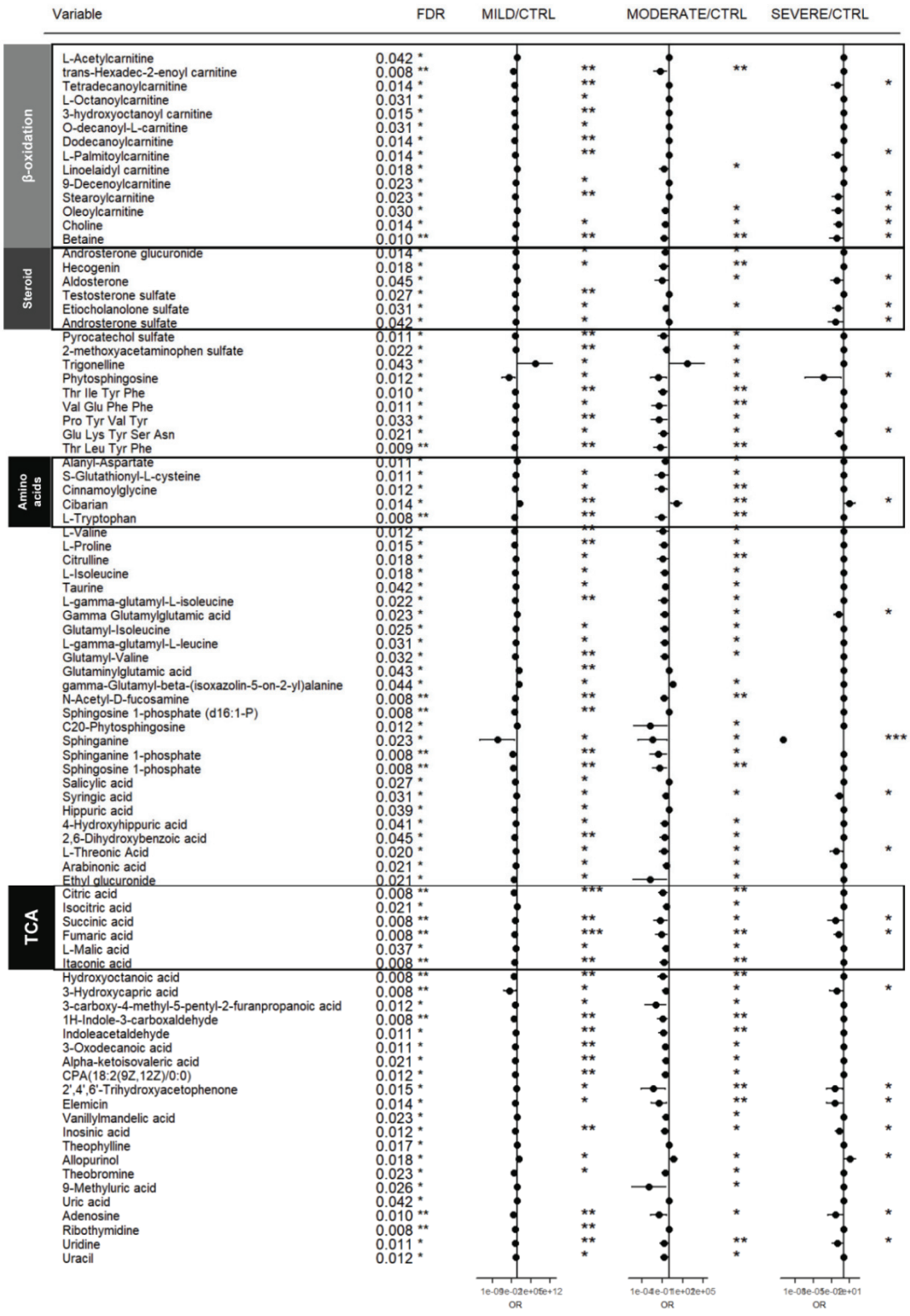

Figure S3. Plasma polar metabolites associated with disease severity, Related to Figure 1. Logistic regression model with covariates BMI, age and sex was built with each metabolite to search for significant variables that could predict disease severity of subjects (*i.e.* healthy control, mild, moderate, severe COVID-19). Only lipids with false discovery rate (fdr) < 0.05 were shortlisted and presented. Forest plots illustrate the magnitude of odds ratios with indicator of significance of the estimate in the model with *** representing p <0.001, ** representing p<0.01 and * representing p<0.05. For non-significant species, the estimates were plotted as zero. TCA: tricarboxylic acid cycle. Metabolites implicated in β-oxidation, TCA cycle, as well as those belonging to the subclasses of steroids and amino acids were boxed up.

Figure S4

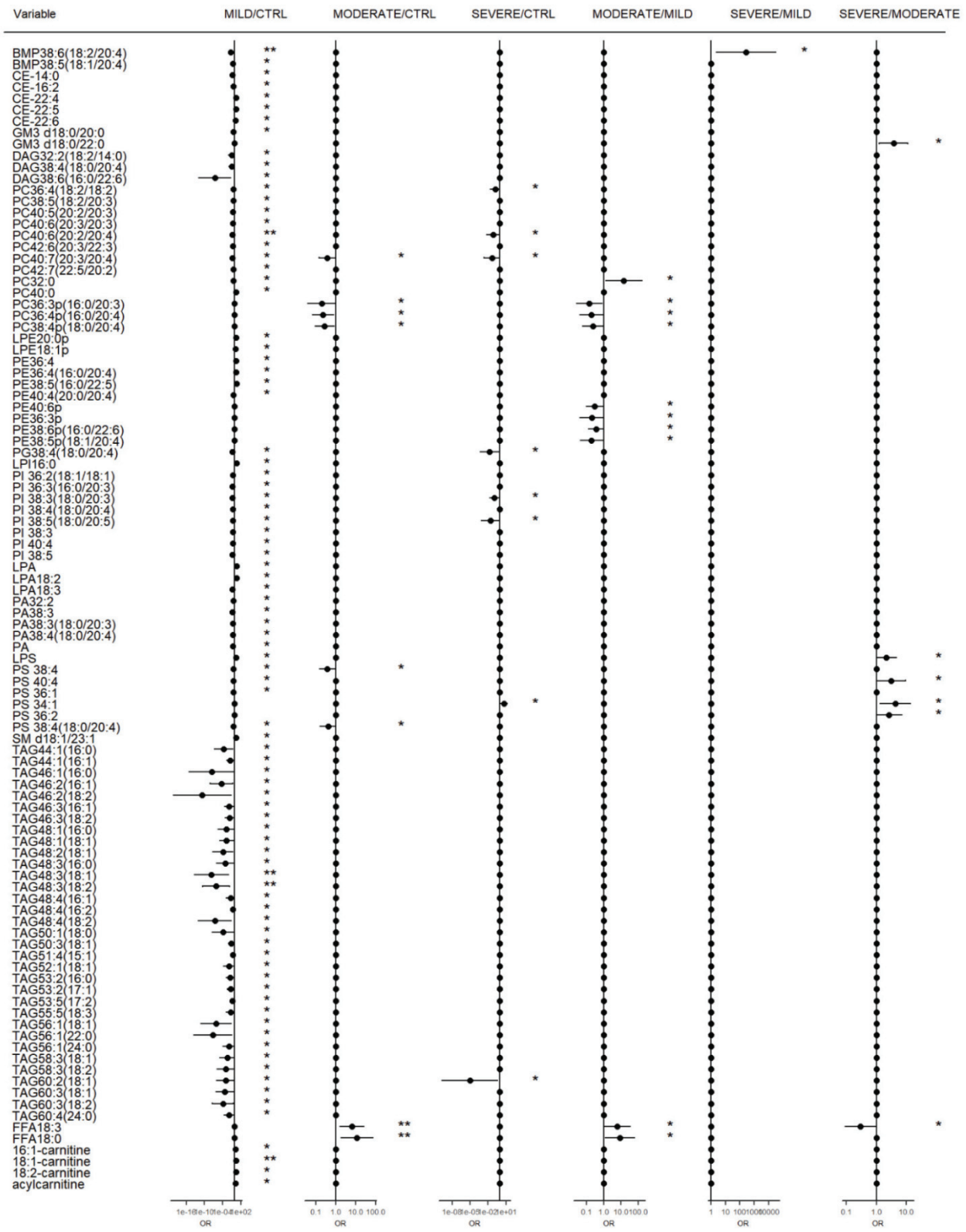

Figure S4. Plasma lipids associated with disease severity, Related to Figure 2. Logistic regression model with covariates age and sex was built with each lipid to search for significant variables that could predict disease severity of subjects (*i.e*. mild, moderate, severe COVID-19). The p-value of the variable estimate in the model was extracted and those $p < 0.05$ were shortlisted and presented. Forest plot displays the magnitude of odds ratios with ***p<0.001, **p<0.01, *p<0.05; For non-significant variables, estimates were plotted as zero.

Figure S5

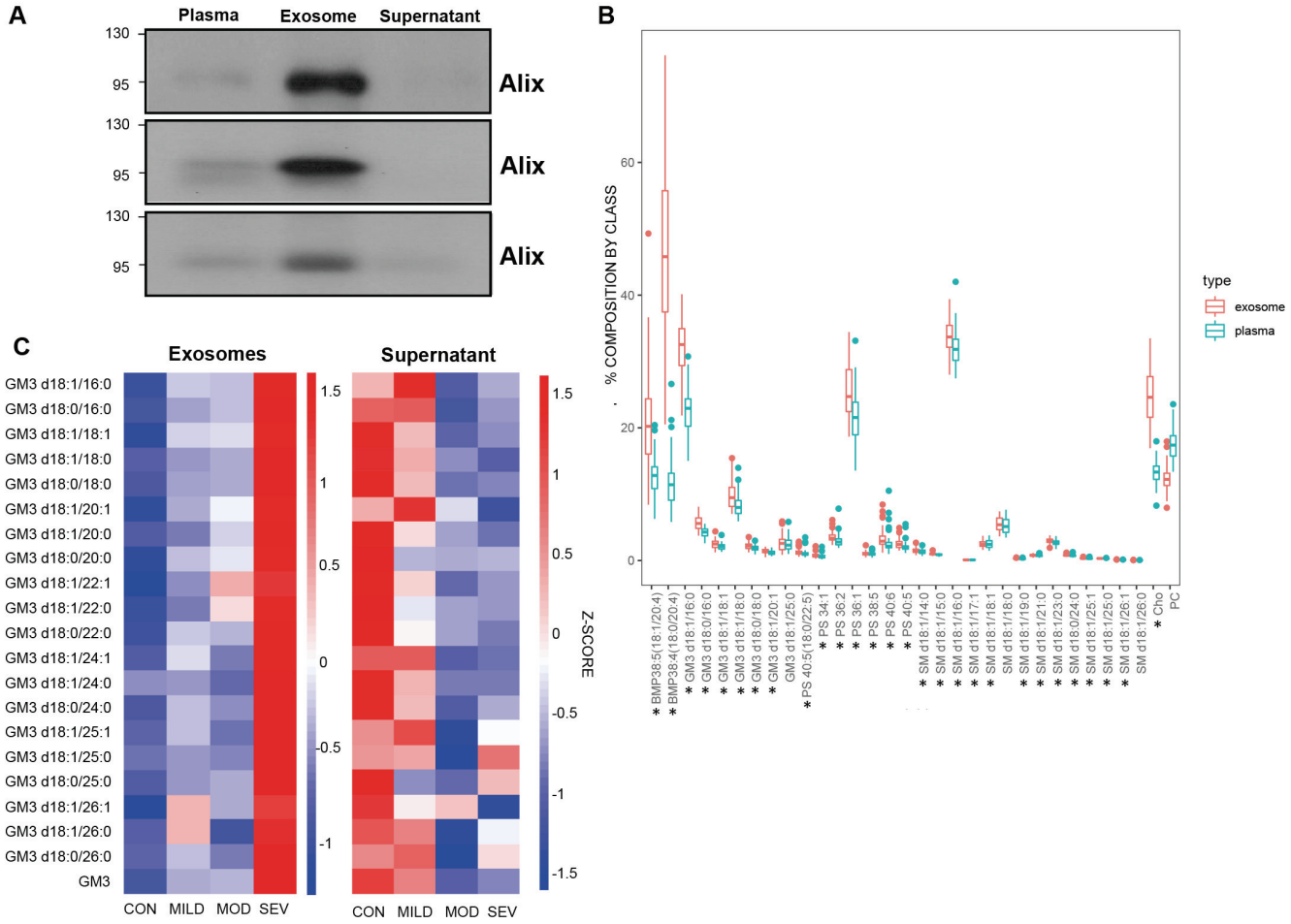

Figure S5. Protein markers and lipid composition of isolated exosomes, Related to Figure 5.

A. Exosomes were isolated from 500 µL of plasma using total exosome isolation kit (Thermofisher Scientific, Invitrogen™ 4478360) according to the manufacturer's protocol. Isolated exosome pellet and supernatant (without exosomes) were collected separately. Proteins were extracted from plasma, isolated exosomes and supernatant samples using RIPA lysis buffer with protease inhibitor cocktail (Sigma). An equal amount of total protein (50 µg) was loaded for each type of samples for electrophoresis and subsequently blotted to nitrocellulose membrane. After blocking with 5% skimmed milk, the membrane was incubated with an anti-Alix primary antibody (Abcam 1/1000) and an HRP-conjugated anti-rabbit secondary antibody (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd) successively. An ECL chemiluminescence system (Thermofisher Scientific) was applied to develop membrane blots, followed by X-ray detection. Digital images were captured using a scanner and presented. Images from three experimental repeats were shown. Alix: ALG-2-interacting protein X

B. To investigate enrichment in specific lipid species in exosome relative to plasma, % composition of each lipid was calculated relative to its class sum total. T-test was performed to identify lipids with significant change in composition between exosome and plasma (n = 75). Boxplots for selected lipids were illustrated to visualize compositional changes. Vertical axis was plotted on a logarithmic scale. Note: To visualize Cho enrichment in lipid membranes, % free Cho was calculated as sum of Cho + PC. * indicate lipid with $p<0.05$ for exosome-specific enrichment. Cho: free cholesterol; BMP: bis(monoacylglycero)phosphates; PS: phosphatidylserines; DAG: diacylglycerols; PC: phosphatidylcholines.

C. Plasma samples (100 µL each) were pooled from four subjects from each of the four clinical categories (control, mild, moderate, severe) and exosomes were isolated using total exosome isolation kit. Both exosome pellets and supernatant samples were collected. Lipids were extracted from exosome and supernatant samples respectively, and the levels of GM3s (expressed in nmol/g total protein) present in exosomes and supernatant of control, mild, moderate and severe COVID-19 patients were illustrated in the heatmaps. CON: healthy controls: MILD: mild; MOD: moderate; SEV: severe COVID-19 patients. GM3: monosiaolodihexosyl gangliosides

Table S1. Laboratory findings of hospitalized COVID-19 patients, Related to Table 1.

Data are median (IQR). P values comparing mild, moderate and severe patients were computed using Kruskal-Wallis H test (leucocytes count, neutrophils count, D-dimer, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, interleukin-6, procalcitonin, erythrocyte sedimentation rate, serum ferritin) and One-way ANOVA (all other indices). COVID-19: coronavirus disease 2019.

	Normal Range	Total n=50	Mild n=18	Moderate n=19	Severe n=13	P value
Blood routine						
Leucocytes count, × 10⁹/L	3.97-9.15	4.34 (3.61-5.43)	5.05 (3.99-5.83)	4.02 (3.36-4.59)	4.33 (3.53-5.40)	0.193
Neutrophils count, × 10⁹/L	2.00-7.00	2.74 (1.95-3.42)	2.86 (2.12-3.35)	2.26 (1.90-2.85)	3.27 (1.96-3.91)	0.186
Lymphocytes count, × 10⁹/L	0.80–4.00	1.31 (0.85-1.61)	1.55 (1.33-2.28)	1.30 (1.01-1.67)	0.77 (0.41-1.14)	<0.0001
Platelets count, × 10⁹/L	85.0-303.0	168.5 (149.0-211.5)	169.5 (151.0-214.8)	167.0 (153.0-195.5)	162.0 (131.0-234.0)	0.762
Haemoglobin, g/L	131.0–172.0	137.0 (129.3-149.5)	140.5 (129.3-150.0)	137.0 (133.0-149.5)	135.0 (123.0-147.0)	0.477
Coagulation function						
Activated partial thromboplastin time, s	23.0–42.0	32.5 (29.5-36.0)	30.7 (28.6-34.4)	34.0 (30.4-37.0)	32.3 (30.6-35.2)	0.333
Prothrombin time, s	10.20–14.30	12.1 (11.4-12.6)	12.3 (11.8-12.5)	12.2 (11.3-12.8)	11.8 (11.4-12.1)	0.984
D-dimer, μg/L	0.00–0.55	0.24 (0.17-0.53)	0.23 (0.17-0.28)	0.23 (0.18-0.47)	0.61 (0.31-2.72)	0.032
Blood biochemistry						
Albumin, g/L	35.0–55.0	40.0 (36.0-43.0)	41.0 (38.3-43.0)	41.0 (36.5-43.0)	36.0 (34.0-40.0)	0.005
Alanine aminotransferase, U/L	5.0-40.0	26.5 (15.0-43.8)	26.5 (14.3-43.8)	26.0 (17.5-34.5)	31.0 (15.0-69.0)	0.4
Aspartate aminotransferase, U/L	8.0–40.0	27.0 (22.0-48.0)	27.0 (21.8-33.3)	24.5 (23.3-30.0)	38.0 (22.0-70.0)	0.578
Total bilirubin, μmol/L	3.40-20.50	10.85 (8.53-16.15)	10.15 (7.00-14.68)	12.00 (9.10-17.25)	10.70 (9.20-13.10)	0.437
Serum creatinine, μmol/L	62.0-115.0	79.5 (69.0-85.8)	76.0 (69.0-85.8)	84.0 (72.0-88.5)	79.0 (69.0- 82.0)	0.707
Lactate dehydrogenase, U/L	109.0–245.0	212.0 (190.0-257.0)	212.5 (198.5-242.3)	194.5 (165.0-242.3)	305.0 (209.0-418.0)	0.028
Interleukin-6, pg/mL	0.0-7.0	11.5 (5.6-24.0)	7.5 (5.4-11.6)	12.5 (5.8-23.6)	21.4 (8.1-46.7)	0.096
C-reactive protein, mg/L	0.068-8.200	8.91 (4.20-27.76)	7.05 (1.58-9.68)	8.50 (4.55-19.00)	36.70 (17.76-46.28)	0.0005
Procalcitonin, ng/mL	0.0-0.5	0.046 (0.037-0.065)	0.039 (0.034-0.047)	0.051 (0.039-0063)	0.065 (0.044-0.095)	0.017
Erythrocyte sedimentation rate, mm/h	0.0-15.0	14.0 (7.8-38.3)	8.0 (5.0-13.0)	25.0 (8.0-39.5)	39.5 (18.0-67.8)	0.001
Serum ferritin, ng/mL	30.0-400.0	379.4 (212.8-556.1)	180.4 (41.7-331.0)	418.8 (335.7-449.7)	738.1 (425.2-1502.8)	0.0001
Lactic acid, mmol/L	0.6-2.2	1.81 (1.52-2.36)	1.85 (1.54-2.16)	1.62 (1.42-1.93)	2.55 (1.67-3.36)	0.118
LDLC, mmol/L	2.1-3.1	2.5 (1.9-3.0)	2.5 (1.7-3.2)	2.3 (1.9-2.6)	3.0 (2.8-3.0)	0.460
HDLC, mmol/L	1.16-1.42	0.81 (0.77-1.09)	1.05 (0.80-1.17)	0.77 (0.76-0.94)	0.90 (0.83-0.91)	0.804
cholesterol, mmol/L	2.8-5.2	3.5 (2.8-4.2)	3.5 (2.7-3.5)	3.2 (2.7-3.6)	4.5 (4.0-4.8)	0.600
glucose, mmol/L	3.9-6.1	5.3 (4.6-7.4)	4.9 (4.6-5.3)	5.3 (4.5-5.7)	8.8 (8.3-9.9)	0.007
triglyceride, mmol/L	0.56-1.7	1.24 (0.82-1.74)	1.51 (1.23-1.84)	0.78 (0.71-1.07)	1.64 (1.40-1.73)	0.927
apolipoprotein A1, g/L	1.05-1.75	0.94 (0.73-1.05)	1.03 (0.95-1.10)	0.87 (0.75-1.00)	0.70 (0.60-0.96)	0.075
apolipoprotein B, g/L	0.6-1.4	0.71 (0.56-0.85)	0.79 (0.58-0.86)	0.64 (0.55-0.73)	0.71 (0.65-0.84)	0.293
lipoprotein, mg/L	0-300	73 (31-134)	41 (21-111)	64 (46-161)	104 (43-134)	0.976

LDLC: low density lipoprotein cholesterol, HDLC: high density lipoprotein cholesterol.

Table S2. Baseline characteristics of healthy controls and COVID-19 patients, Related to Experimental Model and Subject Details (Study Participants and Data Collection).

Data are median (IQR). P values comparing between controls, mild, moderate and severe patients were computed using One-way ANOVA for age and BMI, and the chi-square test for sex. COVID-19: coronavirus disease 2019.

Baseline characteristics of study cohort					
	Control (n=26)	Mild (n=18)	Moderate (n=19)	Severe (n=13)	*p*
Age, years	30 (29-35)	32.0 (22.3-40.0)	45.0 (38.0-53.5)	50.0 (40.0-78.0)	<0.001
Sex					0.525
Men	15 (58%)	9 (50%)	12 (63%)	9 (69%)	
Women	11 (42%)	9 (50%)	7 (37%)	4 (31%)	
BMI	21.25 (19.68-24.00)	25.75 (23.25-27.27)	24.62 (23.35-26.60)	25.99 (24.32-26.89)	0.013

Table S3. Summary of treatments prescribed to COVID-19 patients prior to blood collection, Related to Table 1.

Treatment of COVID-19 patients before sampling				
	All patients (n =50)	Mild (n =18)	Moderate (n =19)	Severe (n =13)
Kaletra	33/50 (66%)	10/18 (55.56%)	16/19 (84.21%)	7/13 (53.85%)
Arbidol	5/50 (10%)	0/18 (0%)	2/19 (10.53%)	3/13 (23.08%)
Interferon therapy	40/50 (80%)	14/18 (77.78%)	17/19 (89.47%)	9/13 (69.23%)
Antibiotic therapy	17/50 (34%)	0/18 (0%)	5/19 (26.32%)	12/13 (92.31%)
Glucocorticoid	12/50 (24%)	0/18 (0%)	4/19 (21.05%)	8/13 (61.54%)
Mechanical ventilation	2/50 (4%)	0/18 (0%)	0/19 (0%)	2/13 (15.38%)