Supplemental data

Supplemental methods

Patients

The diagnosis of SARS-CoV-2 infection was confirmed by real time PCR on material collected by nasal swabs. Citrated blood samples were collected for extended investigations of the plasmatic and cellular coagulation system.

Plasma coagulation assays

Platelet counts were determined with a Sysmex XN 9000 (Sysmex, Norderstedt, Germany). The following coagulation parameters were measured by the Sysmex CS-5100 system: aPTT (Dade Actin®), PT (Dade® Innovin®), D-Dimer (INNOVANCE D-Dimer) and fibrinogen (Dade Thrombin, quantitative, clot-based, functional assay, Clauss method). All reagents for coagulation tests were from Siemens Healthcare Systems (Siemens, Marburg, Germany).

Preparation of washed platelets to determine antibody-mediated apoptosis

Whole blood from healthy donors was centrifuged at 120g for 20 minutes (min*) without break at room temperature (RT). The supernatant platelet rich plasma (PRP) was gently collected and immediately supplemented with apyrase (5 µL/mL PRP, [Sigma-Aldrich, St. Louis, USA]) and pre-warmed anticoagulant-citrate dextrose solution A (ACD-A)(111 µL/mL PRP). Subsequently, platelets were separated from PRP via centrifugation (650g, 7 min*, RT, without brake), resuspended in 5 mL of wash-solution (modified Tyrode buffer: 5 mL bicarbonate buffer, 20 percent (%) bovine serum albumin, 10% glucose solution, 2.5 U/mL apyrase, 1 U/mL hirudin [Pentapharm, Basel, Swiss], pH 6.3) and allowed to rest for 15 min* at

37°C. Following a final centrifugation step (650g, 7 min*, RT, without brake) platelets were resuspended in 2 mL of resuspension-buffer (50 mL of modified Tyrode buffer, 0.5 mL of 1 mM $MgCl_2$, 1 mL of 2 mM $CaCl_2$, pH 7.2) and adjusted to $300x10^9$/L after cell count measurement at a Cell-Dyn Ruby hematological analyzer (Abbott Park, Illinois, USA).

Immunoglobulin G preparation

Immunoglobulin G (IgG) fractions were isolated from serum samples using a commercially available IgG purification kit (Melon™-Gel IgG Spin Purification Kit, Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions. Serum was diluted 1:10 in Melon Gel purification buffer and the purification was performed according to the manufacturer's instructions. IgG fractions were incubated with washed platelets as described for serum samples. In some experiments, Fc gamma receptor IIA (FcγRIIA) was blocked upon 45 min* of incubation at 37°C with a monoclonal antibody (mAb) anti-CD32 (mAb IV.3; stemcell™ technologies, Vancouver, Canada).

Western blot analysis

Protein levels of cleaved-caspase 9 from healthy donors and COVID-19 patients were determined by western blot. After isolation of platelets, cells were centrifuged for 5 min*, 700g at 4°C. Subsequently, the pellet was washed with ice-cold PBS and resuspended in 50 µL ice-cold RIPA lysis buffer (ThermoFisher Scientific, Paisley, UK) containing HALT™ protease and phosphatase inhibitor-cocktail (ThermoFisher Scientific, Paisley, UK). Protein concentration was determined using the NanoDrop One (VWR, Bruchsal, Germany). 250 µg of protein were solubilized in fluorescent compatible sample buffer (Invitrogen™, Carlsbad, USA) at 95ºC for 10 min*. The proteins were separated by electrophoresis for 60 to 90 min* using 12% SDS-PAGE gels in glycine-tris buffer. Thereafter, probes were transferred to polyvinylidene difluoride (PVDF) membranes (0.45 µm, Merck, Tullagreen, Irland). After blocking with 5% milk in tris-buffered saline (TBS-T) (20 mM Tris, 140 mM NaCl, 0.1% Tween, pH 7.6) at RT for 1

h*, the membranes were incubated with primary anti-rabbit cleaved-caspase 9 antibody (1:1000, Cell Signaling, Danvers, USA) and anti-mouse GAPDH (1:1000, Cell Signaling, Danvers, USA) at 4°C overnight. After washing with TBS-T buffer, the membranes were incubated with secondary anti-rabbit and anti-mouse antibody conjugated with IRDye®680 / IRDye®800 (1:3000, LI.COR®, Lincoln, USA) for 1 h* at RT. Protein bands were detected after additional washes (TBS-T) using infrared imaging system (Odyssey, LI.COR®, Lincoln, USA). Images were analysed by ImageJ software (NIH, Bethesda, USA). The results are shown as the ratio of total cleaved-caspase 9 to GAPDH normalized to the control group.

Statistical analyses

Supplemental Table 1. Detailed information on ICU COVID-19, non-ICU and ICU control enrolled in this study.

#	demographic data						timing of PLT testing (days)		PLT-count †	D-dimer †	SOFA-Score on day of testing	COVID-AB (Ratio)	vasoactive agents	treatment on day of testing			outcome		
			potential risk factors											anti-platelet drug	anti-coagulation	vv-ECMO	thrombembolic events		Deceased within 30 days
	age	sex	diabetes	Hyper-tension	obesity*	coronary artery disease	from the first symptoms	from admission to the ICU									thrombosis /cerebral infarction	cardiac event	
ICU COVID-19 patients																			
1	29	f	no	yes	yes	no	13	6	332	1.6	6	7.45	none	none	UFH prophylactic	no	no	no	no
2	72	m	no	yes	no	yes	10	2	193	1.1	7	0.54	nor-epinephrine	aspirin	UFH prophylactic	no	yes	no	yes
3	70	m	yes	yes	no	no	4	2	161	1.1	8	2.55	nor-epinephrine	aspirin	UFH prophylactic	no	yes	no	no
4	56	m	no	yes	no	no	17	13	64	23.0	14	9.64	nor-epinephrine	none	UFH therapeutic	yes	yes	no	yes
5	88	m	no	yes	no	no	10	4	58	2.0	15	3.10	nor-epinephrine	none	UFH therapeutic	no	no	no	yes
6	44	m	no	no	no	no	17	1	223	42.0	8	6.48	nor-epinephrine	aspirin	UFH therapeutic	no	no	no	yes
7	33	m	yes	no	no	no	7	3	142	4.1	10	0.54	nor-epinephrine	none	UFH prophylactic	yes‡	no	no	no
8	79	m	yes	yes	no	yes	7	3	151	5.9	6	5.46	none	aspirin	UFH prophylactic	no	yes	no	yes
9	49	m	no	no	no	no	27	17	112	11.0	14	3.54	nor-epinephrine, vasopressine	none	UFH therapeutic	yes	no	no	yes
10	78	m	no	yes	no	no	21	18	121	2.3	8	5.35	nor-epinephrine	none	UFH therapeutic	no	no	no	no

11	65	f	no	yes	no	no	17	3	212	1.6	9	5.94	nor-epinephrine	none	UFH therapeutic	no	no	no	no
12	88	m	no	yes	no	yes	11	3	415	1.9	3	4.69	none	aspirin	UFH therapeutic	no	yes	no	no
13	70	m	yes	yes	yes	no	12	3	194	1.3	6	6.64	nor-epinephrine	aspirin	UFH therapeutic	no	no	no	no
14	44	f	no	yes	yes	no	34	12	263	3.6	2	9.82	none	aspirin	UFH prophylactic	no	yes	no	no
15	68	m	no	yes	no	yes	8	4	269	4.0	6	6.64	none	aspirin	UFH prophylactic	no	yes	yes	no
16	56	m	no	no	no	no	14	7	500	5.9	3	5.77	none	none	UFH prophylactic	no	no	no	no
17	59	m	no	no	no	no	27	11	126	14.0	10	6.20	nor-epinephrine	none	UFH therapeutic	yes	yes	no	no
18	33	m	no	no	no	no	36	20	134	24.0	9	6.86	nor-epinephrine	none	UFH prophylactic	yes	yes	no	no
19	49	m	no	yes	yes	no	59	23	62	3.2	14	4.54	nor-epinephrine	none	UFH prophylactic	yes	yes	no	yes
20	56	m	no	no	no	no	24	23	35	23.0	17	4.96	nor-epinephrine, vasopressine	none	UFH prophylactic	yes	yes	no	no
21	68	m	yes	yes	no	no	17	13	134	2.8	10	6.16	nor-epinephrine	none	UFH prophylactic	no	yes	no	no
non-ICU COVID-19 patients																			
22	88	m	no	yes	no	yes	28	-	468	n.t.	-	2.90	none	aspirin	LMH therapeutic	no	yes	no	no
23	82	m	yes	yes	no	yes	16	-	371	n.t.	-	6,99	none	aspirin	LMH prophylactic	no	no	no	no
24	84	f	yes	yes	yes	yes	18	-	180	1.1	-	0.12	none	aspirin	LMH prophylactic	no	no	no	no
25	77	m	yes	yes	no	no	3	-	191	3.3	-	2.67	none	none	LMH therapeutic	no	yes	no	no
ICU non-COVID-19 control																			
#	**age**	**sex**	Type of admission	isolated microorganism	**timing of PLT testing (days)**		**PLT-count †**	**D-dimer †**	**SOFA-Score on day of testing**	**COVID-AB (Ratio)**	**vasoactive agents**	**treatment on day of testing**			**outcome**		
					from the first symptoms	**from admission to the ICU**						**anti-platelet drug**	**anti-coagulation**	**vv-ECMO**	**thrombembolic events**		**Deceased within 30 days**
															thrombosis /cerebral infarction	**cardiac event**	
26	51	m	trauma	fungi	n.a.	18	229	19.0	8	n.t.	nor-epinephrine	none	argatroban	no	no	no	no
27	16	f	trauma	gram negative bacteria	n.a.	31	79	10.0	17	n.t.	nor-epinephrine	none	UFH prophylactic	no	yes	no	no
28	84	m	surgical	fungi	n.a.	1	137	3.4	7	n.t.	nor-epinephrine	aspirin	UFH prophylactic	no	no	no	no

29	77	m	surgical	gram negative bacteria	n.a.	35	388	n.t.	11	n.t.	nor-epinephrine	none	argatroban	no	no	no	no
30	76	m	surgical	gram positive bacteria	n.a.	6	139	0.84	7	n.t.	nor-epinephrine	aspirin	UFH prophylactic	no	no	no	no

AB indicates antibodies; ICU, intensive care unit; LMH, low molecular weight heparin; PLT, platelet; SOFA, sequential organ failure assessment score; UFH, unfractionated heparin; vv-ECMO, venous venous extracorporeal membrane oxygenation; * obesity (body mass index, BMI>30); † reference range PLT-count (150-450 $x10^9$/L, D-Dimer (<0.5 µg/ml); ‡ blood samples were drawn before vv-ECMO implantation; ICU COVID-19 patients: #1 until #21, non-ICU COVID-19 patients #22 until #25 and ICU control patients #26 until #30.

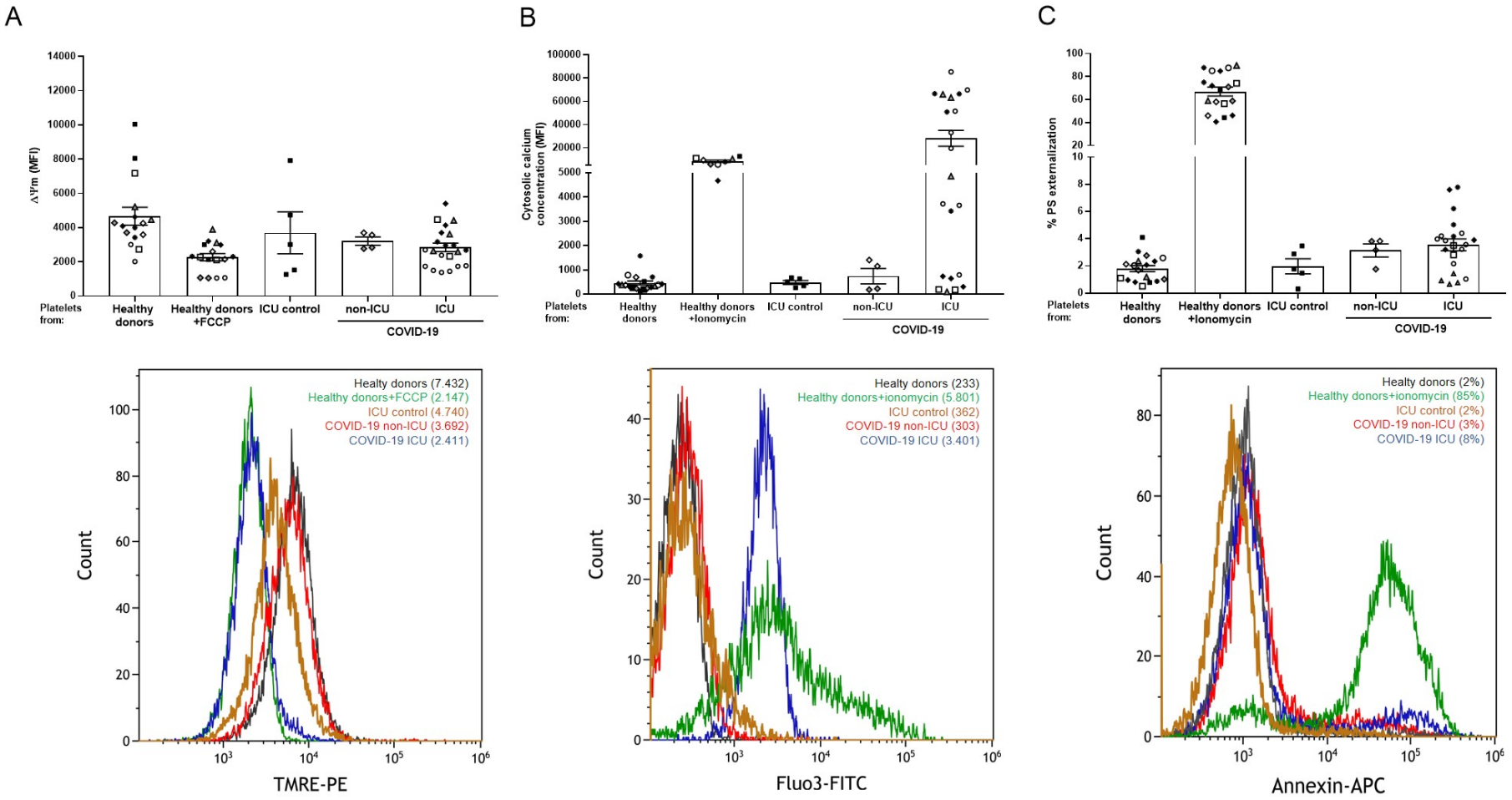
Supplemental Table 2: Detailed information on ΔΨ depolarization, cytosolic calcium and phospatidylserine externalization

		#	**Figure 1:** platelet apotosis in COVID-19 patients (patients`platelets)			**Figure 3:** impact of sera from COVID-19 patients on platelet apotosis (patients`sera incubated with platelets from healthy donors)			**Figure 4:** IgG fraction from ICU COVID-19 patients induce platelet apoptosis via cross linking Fc gamma RIIA								
			(A) ratio of ΔΨ depolariza-tion (MFI normalized to control)	(B) cytosolic calcium concentration (MFI normalized to control)	(C) PS externaliza-tion (% normalized to control)	(A) ratio of ΔΨ depolariza-tion (MFI normalized to control	(B) cytosolic calcium concentration (MFI normalized to control)	(C) PS externaliza-tion (% normalized to control)	(A) ratio of ΔΨ depolariza-tion (MFI normalized to control	(B) cytosolic calcium concentra-tion (MFI normalized to control)	(C) PS externaliza-tion (% normalized to control)	(D) ratio of ΔΨ depolarization (MFI normalized to control		(E) cytosolic calcium concentra-tion (MFI normalized to control)		(F) PS externalization (% normalized to control)	
												-VI.3	+VI.3	-VI.3	+VI.3	-VI.3	+VI.3
COVID-19	ICU	1	1.4	1.4	0.5	1.3	n.m.	1.6	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		2	1.7	4.8	1.7	1.2	1.5	1.1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		3	1.3	1.7	1.4	1.3	n.m.	1.4	1.52	1.71	1.73	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		4	1.5	3.7	1.7	1.3	1.5	1.8	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		5	0.9	5.7	1.8	1.0	1.7	1.5	0.96	1.25	0.91	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		6	1.1	4.6	1.6	1.7	1.2	1.8	1.19	1.26	0.89	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		7	1.5	1.4	0.9	1.1	1.1	1.6	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		8	1.8	2.7	1.9	1.1	1.7	1.4	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		9	1.1	3.7	0.8	0.9	1.2	1.1	1.82	1.33	2.39	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		10	1.3	3.5	0.4	1.8	1.3	1.7	1.60	1.24	2.38	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		11	1.8	0.9	0.5	1.1	1.2	1.1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		12	1.9	1.6	0.4	2.2	1.3	1.7	1.76	1.32	2.02	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		13	1.3	1.8	0.6	2.3	1.2	1.9	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		14	1.2	2.1	0.7	1.3	1.3	1.8	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		15	1.3	1.2	1.1	2.1	1.4	1.6	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		16	1.4	0.6	0.9	1.1	1.2	1.0	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		17	1.1	4.0	4.4	3.1	0.9	2.8	n.t.	n.t.	n.t.	1.9	1.0	2.0	0.6	7.8	1.8
		18	2.1	3.9	3.5	1.7	1.3	3.4	n.t.	n.t.	n.t.	2.7	1.4	2.0	0.7	12.6	2.0
		19	0.9	3.3	4.9	1.5	2.3	1.5	2.38	1.40	3.0	2.0	1.3	1.5	0.5	8.3	2.5
		20	1.2	3.2	9.5	1.3	1.8	0.9	1.37	1.32	1.39	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		21	1.3	1.6	4.0	1.5	1.3	1.4	1.23	1.38	0.6	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	non-ICU	1	0.6	1.2	1.0	n.t.	n.t.	n.t.	1.22	0.99	1.17	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		2	1.1	1.0	1.1	n.t.	n.t.	n.t.	1.15	0.98	0.90	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		3	0.9	1.1	1.1	n.t.	n.t.	n.t.	0.94	0.88	0.73	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		4	0.8	0.9	0.8	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
ICU non-COVID-19		1	1.6	1.8	0.8	1.0	1.1	1.4	1.1	1.2	0.9	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		2	1.7	1.0	0.3	1.1	1.3	1.1	1.1	1.2	1.1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		3	1.2	1.8	0.7	1.0	1.1	0.7	1.0	1.3	1.2	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		4	1.0	1.0	0.6	1.0	1.0	0.5	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		5	1.3	1.6	1.9	1.3	0.9	1.1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.

ICU indicates intensive care unit; MFI, mean fold increase; n.m., no material; n.t., not tested; PS, phosphatidylserine

Supplemental Figures

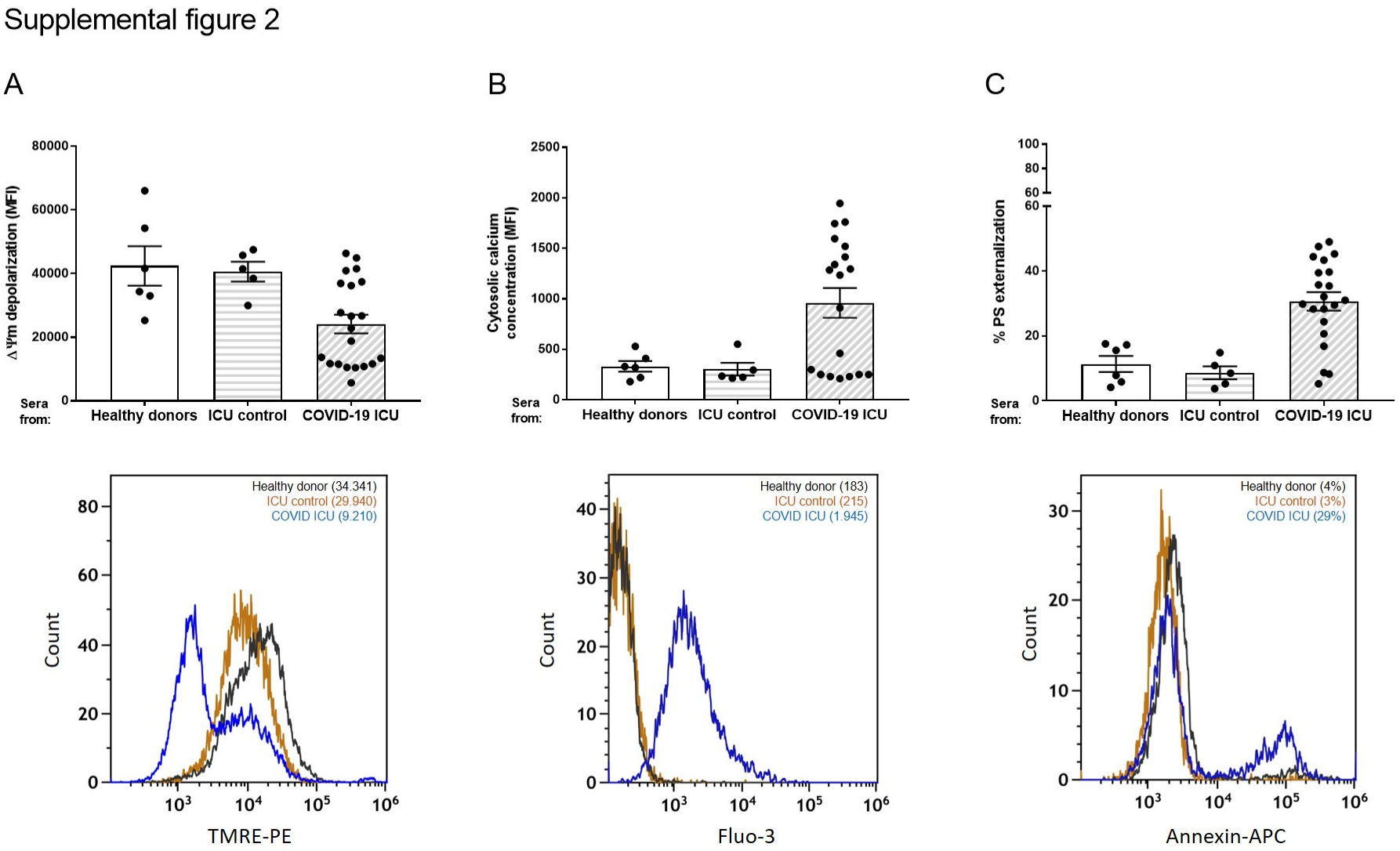
Supplemental Figure 1



Supplemental figure 1. Platelet apoptosis in COVID-19 patients and representative histograms of flow cytometry.

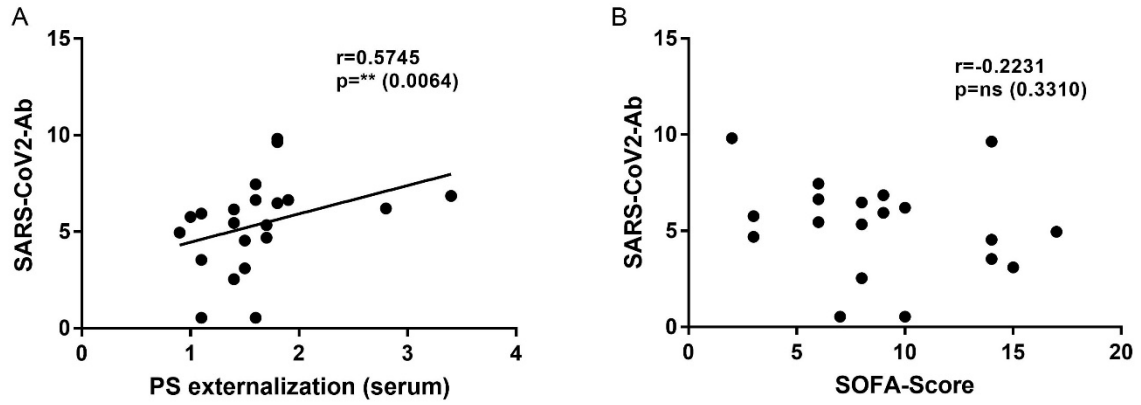
Changes in apoptosis pathways were analyzed by assessing the depolarization of the mitochondrial inner transmembrane potential (ΔΨm) (A), cytosolic calcium concentration (B), and phosphatidylserine (PS) externalization (C) in platelets from intensive care unit (ICU) COVID-19 (n=21) patients, non-ICU patients (n=4), ICU control patients (n=5) as well as healthy donors (n=14), respectively. Lower panels, representative flow cytometry histograms (black: healthy donors; green: positive control; brown: ICU control; red: non-ICU COVID-19 and blue: ICU COVID-19). Ionomycin and carbonyl cyanide 4-trifluoromethoxy phenylhydrazone (FCCP) were used as positive controls to induce apoptosis. Data are presented as mean ± standard error of the mean (SEM) of the mean fluorescence intensity (MFI) or percentage (%), not significant, *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

Supplemental figure 2



Supplemental figure 2. Impact of sera from COVID-19 ICU patients on platelet apoptosis and representative histograms of flow cytometry. Changes in apoptosis pathways induced by sera from intensive care unit (ICU) COVID-19 (n=21) patients and ICU control patients (n=5) patients as well as healthy donors (n=6) were analyzed by assessing the depolarization of the mitochondrial inner transmembrane potential (ΔΨm) (A), cytosolic calcium concentration (B, n=19 due to the lack of biomaterial for 2 patients), and phosphatidylserine (PS) externalization (C). Lower panels, representative flow cytometry histograms (black: sera from healthy donors, blue: sera from ICU COVID-19 and brown sera from ICU control patients). Data are presented as mean ± standard error of the mean (SEM) of the mean fluorescence intensity (MFI) or percentage (%), not significant, *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

Supplemental Figure 3



Supplemental figure 3: Correlation between SARS-CoV2-antibodies (AB) and phosphatidylserine (PS) externalization (A). SARS-CoV2 IgG antibodies are not correlated with SOFA-Score (B).