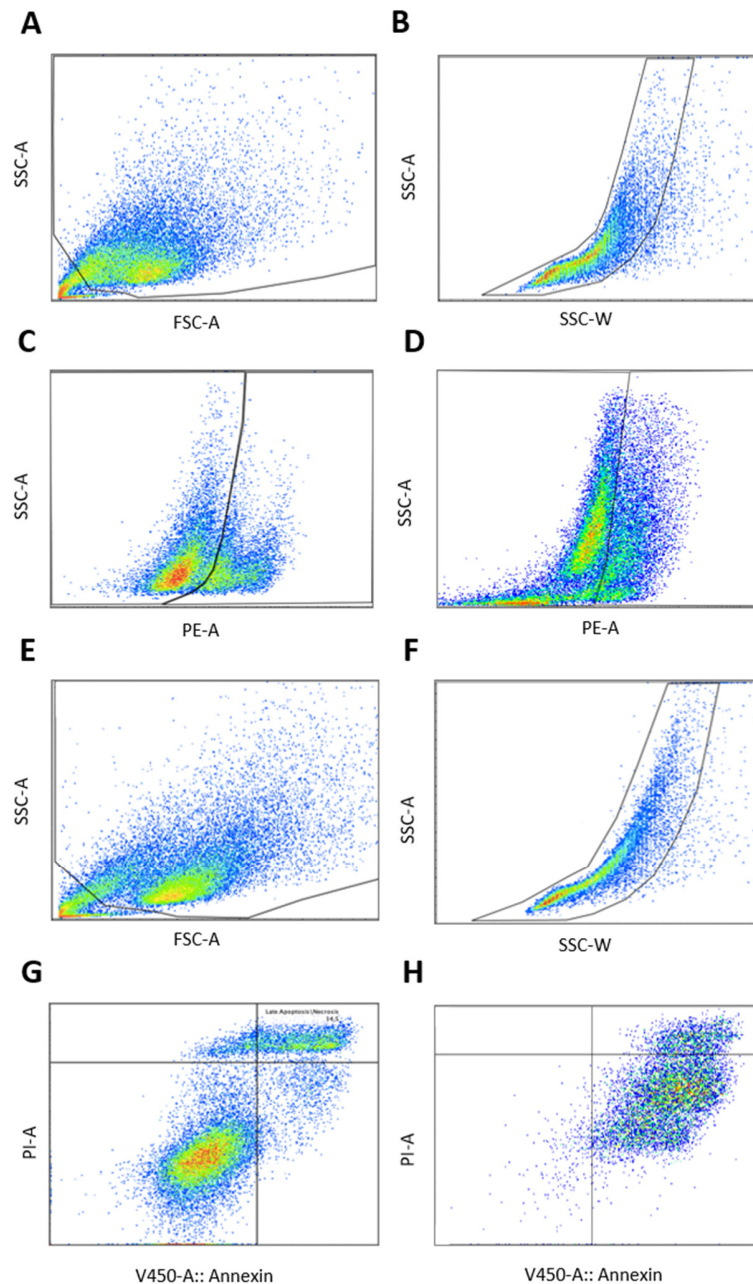


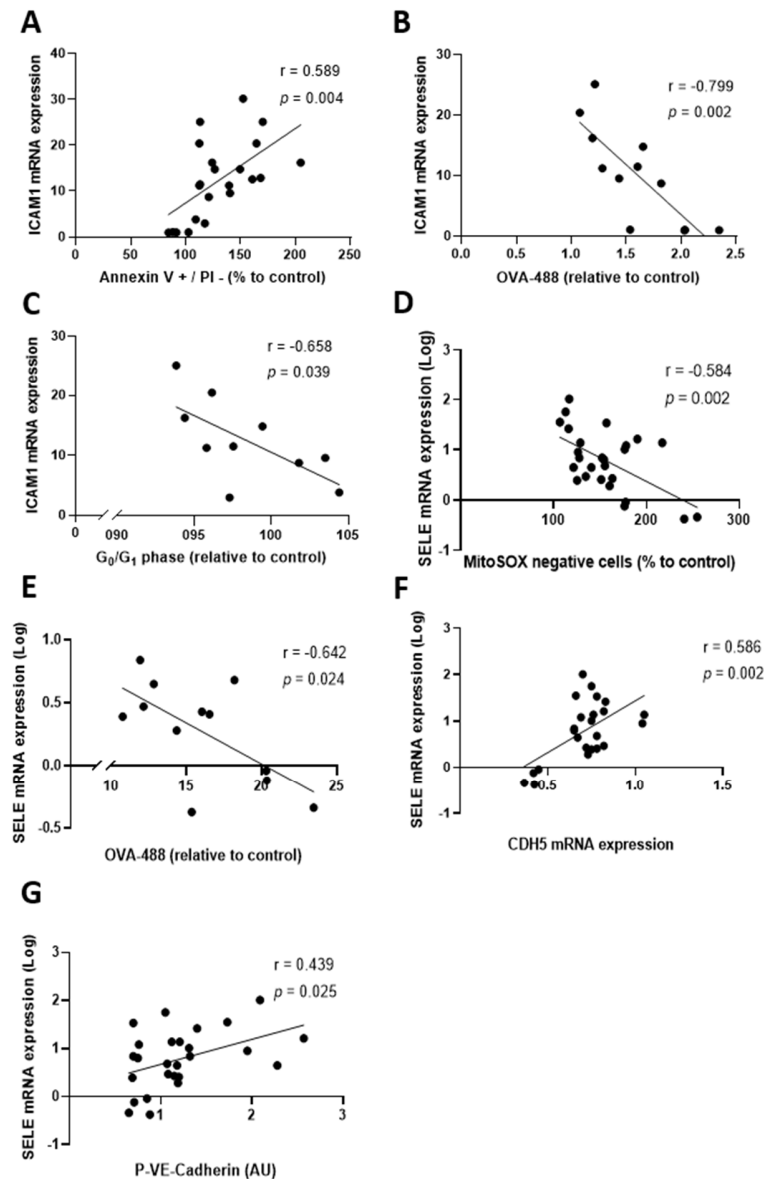
# **Use of Early Donated COVID-19 Convalescent Plasma Is Optimal to Preserve the Integrity of Lymphatic Endothelial Cells**

## **Supplementary material**



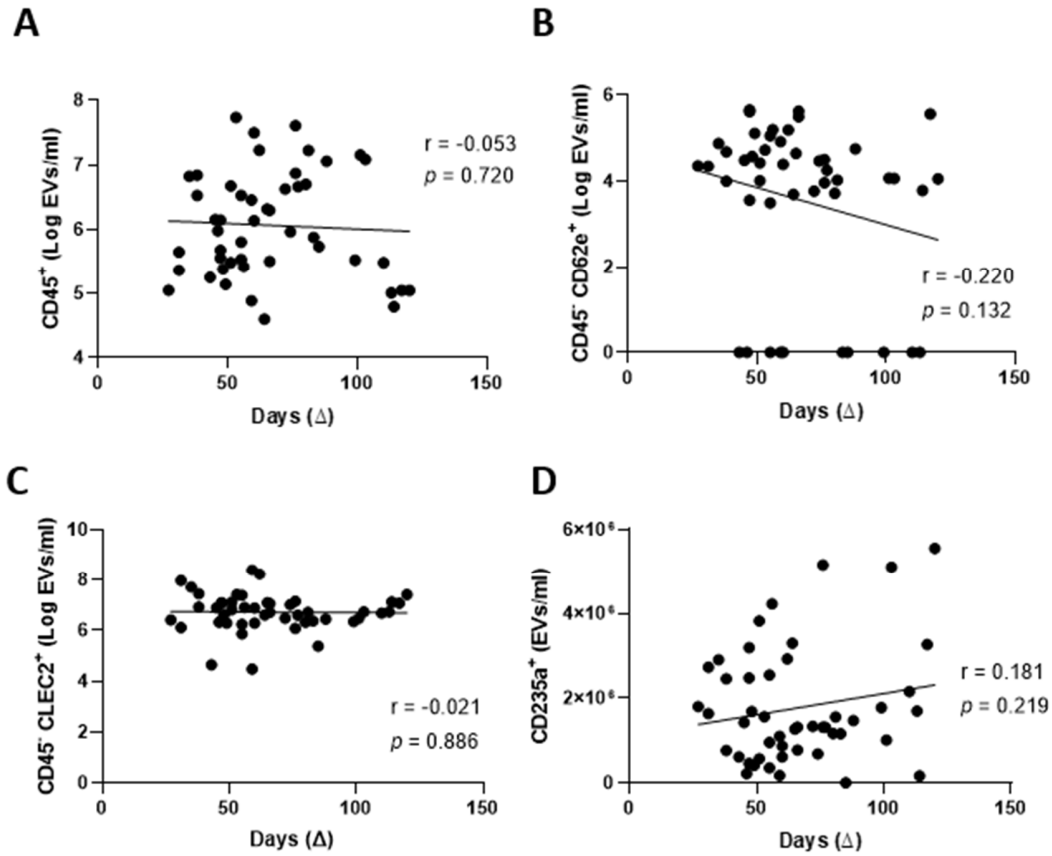
**Figure S1.** Gating strategy in flow cytometry for reactive oxygen species production and cellular viability. **(A – D).** MitoSOX™ Red gating strategy through viable cells (side scatter-area) **(A)**, single cells (side scatter-area) **(B)** MitoSOX™ Red negative and positive populations following incubation with CCP (side scatter-area) **(C)** and MitoSOX™ Red negative and positive populations following incubation with 1mM H<sub>2</sub>O<sub>2</sub> (side scatter-area) **(D)**. **(E – H)** Cellular viability gating strategy using Annexin V and PI through viable cells (side scatter-area) **(E)**, single cells (side scatter-area) **(F)** early apoptosis population (Annexin V positive and negative for PI) and necrosis (Annexin V positive and PI positive) following incubation with CCP (PI - area) **(G)** and MitoSOX™ Red negative and positive

populations following incubation with 1  $\mu$ M Staurosporine (side scatter-area) (**H**). SSC-A, side scatter-area; FSC-A, forward scatter-area; PI, propidium iodide; CCP, COVID-19 convalescent plasma.

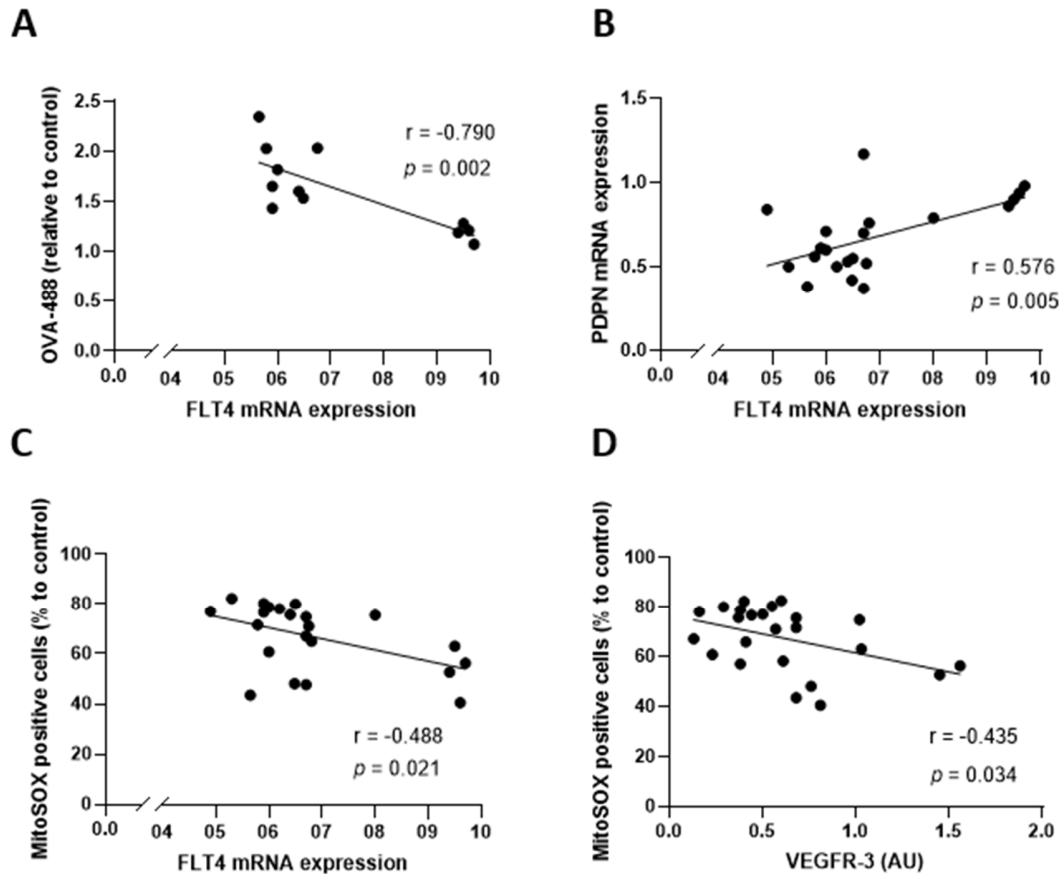


**Figure S2.** Endothelial activation markers are predictors of preserved endothelial integrity. (A – C) Treated aHDLEC (convalescent plasma for 4h and cytokines for 20h) were harvested and mRNA expression of activation marker *ICAM1* was assessed by RT-qPCR and correlated with cells in early apoptosis (positive for Annexin V and negative for propidium iodide (PI) (A), concentration of ovalbumin-488 following the permeability transwell test (B) and cells in the G<sub>0</sub>/G<sub>1</sub> phase of the cell cycle, determined by DNA fragmentation using PI (C). (D – G). Treated aHDLEC were harvested and mRNA expression of activation marker E-selectin (*SELE*) was determined by RT-qPCR and correlated with cells not labeled by the MitoSOX<sup>TM</sup> Red probe (D), concentration of ovalbumin-488 (E), VE-Cadherin mRNA expression (F) and phosphorylated VE-Cadherin expression measured by immunoblot and normalized to the expression of non-phosphorylated VE-Cadherin (G). Significance was determined by a Pearson correlation

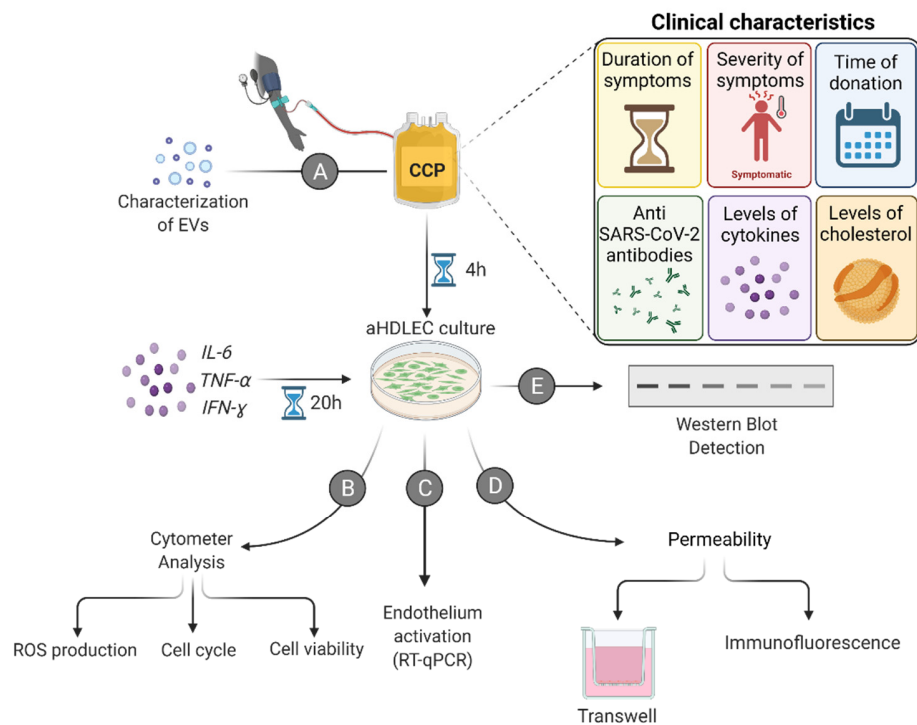
and  $p < 0,05$  was considered significant. *ICAM1*, gene coding for intercellular adhesion molecule 1; *SELE*, gene coding for E-selectin; PI, propidium iodide; *CDH5*, gene coding for VE-Cadherin; OVA, ovalbumin; P-VE-Cadherin, phosphorylated VE-Cadherin; aHDLEC, adult human dermal lymphatic endothelial cells.



**Figure S3.** No significant correlations between extracellular vesicles and time of donation of COVID-19 convalescent plasma. (A – D) Correlation between the duration between the onset of symptoms and the donation and the concentration of CD45<sup>+</sup> EVs (A), CD45<sup>+</sup> CD62e<sup>+</sup> EVs (B), CD45<sup>-</sup> CLEC2<sup>+</sup> EVs (C) and CD235a<sup>+</sup> EVs in CCP (D). Significance was determined by a Pearson correlation after a logarithmic transformation of CD45<sup>+</sup> EVs, CD45<sup>-</sup> CD62e<sup>+</sup> EVs and CD45<sup>-</sup> CLEC2<sup>+</sup> EVs concentration to reach a normal distribution.  $p < 0,05$  was considered significant.  $\Delta$ , time of donation since onset of symptoms; EVs, extracellular vesicles; CLEC2, C-type lectin-like type II; CCP, COVID-19 convalescent plasma.

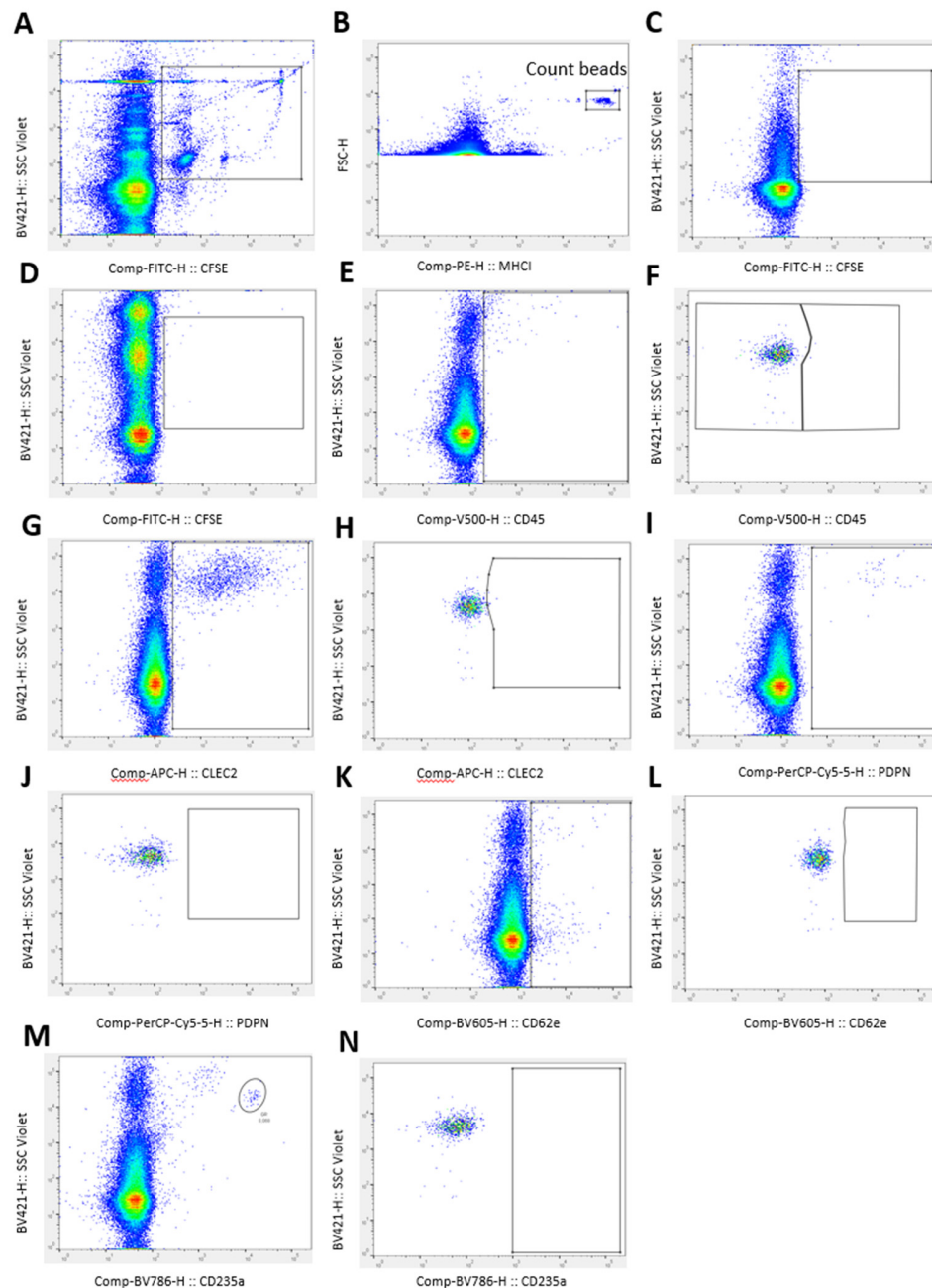


**Figure S4.** VEGFR-3 mRNA expression correlates to a preserved endothelial integrity. (A) Treated cells (convalescent plasma for 4h and cytokines for 20h) were harvested and mRNA expression of *FLT4* was assessed by RT-qPCR and correlated with the concentration of ovalbumin-488 measured following the permeability transwell assay. (B) The mRNA expression of FLT-4 was correlated with the mRNA expression of podoplanin in treated cells (C) The mRNA expression of FLT4 was correlated with cells labeled by the MitoSOX<sup>TM</sup> Red probe (relative to cells treated with control plasma) (D) Proteins from treated cells were extracted and the expression of VEGFR-3 was assessed by immunoblot and correlated with cells labeled by the MitoSOX<sup>TM</sup> Red probe (relative to cells treated with control plasma). Significance was determined by a Pearson correlation and  $p < 0,05$  was considered significant. OVA, ovalbumin; FLT4, gene coding for VEGFR-3; VEGFR-3, vascular endothelial growth factor receptor 3; PDPN; gene coding for podoplanin.



**Figure S5.** Experimental design. (A – E) Clinical characteristic of COVID-19 convalescent plasma donors were assessed. EVs within CCP were characterized (A). Following the incubation of CCP on aHDEC, cells were treated with a pro-inflammatory cocktail of cytokines (IFN $\gamma$  [10 ng/ml], IL-6 [20 ng/ml] and TNF $\alpha$  [20 ng/ml]). Flow cytometry analysis for cell viability, mitochondrial ROS production and cell cycle were performed (B). Endothelial activation (C), permeability (D), and protein expression (E) were also analyzed. CCP, COVID-19 convalescent plasma; EVs, extracellular vesicles; aHDEC, adult human dermal lymphatic endothelial cells; ROS, reactive oxygen species; IFN $\gamma$ , interferon gamma; IL-6, interleukin 6; TNF $\alpha$ , tumor necrosis factor alpha. *Created with BioRender.com*





**Figure S6.** Flow cytometry plots gating for the identification of extracellular vesicles. **(A)** Megamix (180- to 1300-nm silica beads and 110- and 500-nm fluorescent latex Apogee beads) and filter of 405/40 gated with CFSE (side scatter-height). **(B)** Count Beads (3- $\mu$ m-diameter MP-CB) (forward scatter-height). **(C)** Background sample used to eliminate background noise (side scatter-height). **(D)** Unlabeled sample used as unstained sample for gating strategy (side scatter-height). Single stained samples were used for gating strategy. As controls, detergent Triton X-100 was added to the antibody mix to verify the membrane of EVs. Single stained **(E)** and depletion using Triton X-100 **(F)** of CD45<sup>+</sup> EVs. Single stained **(G)** and depletion **(H)** of CLEC2<sup>+</sup> EVs. Single stained **(I)** and depletion **(J)** of

podoplanin<sup>+</sup> EVs. Single stained (**K**) and depletion (**L**) of CD62e<sup>+</sup> EVs. Single stained (**M**) and depletion (**N**) of CD235a<sup>+</sup> EVs. SSC, side scatter; FSC-H, forward scatter-height; CFSE, carboxyfluorescein succinimidyl ester; MHCI, major histocompatibility complex I; PDPN, podoplanin; CLEC2, C-type lectin-like type II; EVs, extracellular vesicles.

Table S1 List of conjugated antibodies and markers used for flow cytometry

Antibodies/ Proteins	Fluoro.	Excitation (nm)	Emission (nm)	Company	Catalogue	Dilution or Conc.
CFSE	FITC	488	530/30	Cedarlane, Burlington, Ontario, Canada,	S8269- 10MG	1/5000
MHC I	PE	488	575/25	BD Biosciences, Franklin Lakes, NJ, USA	555553	1/200
CD235a	BV786	405	780/60	BD Biosciences, Franklin Lakes, NJ, USA	740984	1/500
CD45	V500	405	525/50	BD Biosciences, Franklin Lakes, NJ, USA	560777	1/100
CLEC2	APC	640	670/30	BioLegend, San Diego, CA, USA	372005	1/50
CD62e	BV605	405	610/20	BD Biosciences, Franklin Lakes, NJ, USA	563359	1/75
Podoplanin	PerCP-Cy5.5	488	695/40	BioLegend, San Diego, CA, USA	337011	1/100
Annexin V	V450	405	450/40	BD Biosciences,	560506	1µL/test

			Franklin Lakes, NJ, USA		
<b>PI*</b>	488	610/20	Biotium, Fremont, CA, USA	40017	2,5µg/mL
<b>PI**</b>	488	610/20	Sigma, St. Louis, Missouri, USA	P4170- 25MG	50µg/mL
<b>MitoSOX™ Red</b>	510	575/25	Thermo Fisher, Waltham, MA, USA	M36008	1,67µM

Fluoro, fluorochrome; Conc, concentration; CFSE, carboxyfluorescein succinimidyl ester; MHC I, major histocompatibility complex I; CLEC2, C-type lectin-like type II; PI\*, propidium iodide used for cell viability; PI\*\*, propidium iodide used for cell cycle distribution.

Table S2 List of sequences for PCR primers used throughout the study

Genes	Primers Sequence (5' → 3')
<i>ACTB</i>	F : GACGACATGGAGAAAATCTG R : ATGATCTGGGTCATCTTCTC
<i>ICAM1</i>	F : ACCATCTACAGCTTTCCG R : TCACACTTCACTGTCACC
<i>SELE</i>	F : GAGAATTCACCTACAAGTCC R : AGGCTTGAACATTTTACCAC
<i>FLT4</i>	F : GAGCAGATAGAGAGCAGGCAT R : ACATTCTGGCCAGGTCCTTTAC
<i>PDPN</i>	F : AAGATGGTTTGTCAACAGTG R : GTACCYYCCCGACATTTTTC
<i>CDH5</i>	F : CGCAATAGACAAGGACATAAC R : TATCGTGATTATCCGTGAGG

F, forward; R, reverse; *ACTB*, gene coding for  $\beta$ -actin; *ICAM1*, gene coding for intercellular adhesion molecule 1; *SELE*, gene coding for E-selectin; *FLT4*, gene coding for vascular endothelial growth factor receptor 3; *PDPN*, gene coding for podoplanin; *CDH5*, gene coding for VE-Cadherin.

Questionnaire

**Multicentre Study to Evaluate the Safety and Efficacy of Convalescent Plasma in the Treatment of COVID-19: Convalescent Plasma Donor Component.**

<b>Participant ID number</b>	_____
<b>Origin of infection</b>	
Travel abroad	<input type="checkbox"/>
Community transmission	<input type="checkbox"/>
Unknown	<input type="checkbox"/>
<b>Symptoms</b>	
Date of onset of symptoms	____/____/____
Date of positive test result	____/____/____
End date of symptoms	____/____/____
<b>Symptoms: types and severity</b>	
Overall health condition	<input type="checkbox"/> Fever <input type="checkbox"/> Fatigue
Respiratory	<input type="checkbox"/> Cough <input type="checkbox"/> Difficulty breathing
Digestive	<input type="checkbox"/> Nausea <input type="checkbox"/> Stomach ache <input type="checkbox"/> Diarrhea
ENT	<input type="checkbox"/> Loss of smell/taste <input type="checkbox"/> Nasal congestion
Severity of symptoms	<input type="checkbox"/> Mild <input type="checkbox"/> Moderate <input type="checkbox"/> Strong

Hospitalization	
<p>Were you hospitalized for COVID-19?</p> <p>If you answered Yes :</p> <p style="text-align: right;">Date of hospitalization</p> <p style="text-align: right;">Date of discharge from hospital</p>	<p><input type="checkbox"/> Yes</p> <p><input type="checkbox"/> No</p> <p style="text-align: right;">____/____/____</p> <p style="text-align: right;">____/____/____</p>
Intensive Care	
<p>Were you admitted to intensive care?</p> <p>If yes, how many days were you in intensive care?</p>	<p><input type="checkbox"/> Yes</p> <p><input type="checkbox"/> No</p> <p>_____</p>
<p>Did you require mechanical ventilation/Were you intubated?</p>	<p><input type="checkbox"/> Yes</p> <p><input type="checkbox"/> No</p>
Post-test	
<p>Were you tested for the virus after symptoms ceased/disappeared?</p> <p>If yes, what was the result of this test?</p>	<p><input type="checkbox"/> Yes</p> <p><input type="checkbox"/> No</p> <hr/> <p><input type="checkbox"/> Positive</p> <p><input type="checkbox"/> Negative</p>
<p><b>Completed by:</b></p> <p>_____</p>	<p><b>DATE :</b></p> <p>____/____/____</p> <p><b>DATE:</b></p> <p>____/____/____</p>