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# 1 Infection of lung megakaryocytes and platelets by SARS-CoV-2 2 anticipate fatal COVID-19

3

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41 Keywords: Platelets; SARS-CoV-2; COVID-19; Megakaryocytes; Lung; Macrophages

42 **Short title:** Platelets carry infectious SARS-CoV-2 in fatal COVID-19

43

44 **Abstract**

45

46 SARS-CoV-2, although not being a circulatory virus, spread from the respiratory tract resulting in multiorgan  
47 failures and thrombotic complications, the hallmarks of fatal COVID-19. A convergent contributor could be  
48 platelets that beyond hemostatic functions can carry infectious viruses. Here, we profiled 52 patients with  
49 severe COVID-19 and demonstrated that circulating platelets of 19 out 20 non-survivor patients contain  
50 SARS-CoV-2 in robust correlation with fatal outcome. Platelets containing SARS-CoV-2 might originate from  
51 bone marrow and lung megakaryocytes (MKs), the platelet precursors, which were found infected by SARS-  
52 CoV-2 in COVID-19 autopsies. Accordingly, MKs undergoing shortened differentiation and expressing anti-  
53 viral IFITM1 and 3 RNA as a sign of viral sensing were enriched in the circulation of deadly COVID-19.  
54 Infected MKs reach the lung concomitant with a specific MK-related cytokine storm rich in VEGF, PDGF and  
55 inflammatory molecules, anticipating fatal outcome. Lung macrophages capture SARS-CoV-2-containing  
56 platelets *in vivo*. The virus contained by platelets is infectious as *in vitro* capture of platelets carrying SARS-  
57 CoV-2 propagate infection to macrophages in a process blocked by an anti-GPIIb/IIIa drug. Altogether,  
58 platelets containing infectious SARS-CoV-2 infect COVID-19 pathogenesis and provide a powerful fatality  
59 marker. Clinical targeting of platelets might prevent viral spread, thrombus formation and exacerbated  
60 inflammation at once and increase survival in COVID-19.

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## **Introduction**

Since December 2019, the world has experienced an outbreak of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although the epidemiological and clinical characteristics of patients with COVID-19 have been reported, biological risk factors for mortality are needed. Critical cardio-vascular, as well as multifactorial thrombotic complications in patients with COVID-19 are frequent, even in individuals without a history of cardio-vascular disease (1). Furthermore, patients with severe COVID-19 admitted into intensive care unit (ICU) have increased cumulative thrombotic complications compared with patients not admitted to ICU (31% versus 1.3%) (2). Microthrombotic events are especially frequent in the lungs where MKs, the platelet precursors, are found to accumulate atypically in COVID-19 patients (3), suggesting abnormal behaviour (4). However anticoagulant treatment of COVID-19 patients is of limited efficacy, and any benefit may be patient-specific (5, 6). Despite clinical evidence of a link between COVID-19 and haemostatic disorders, the underlying mechanisms of thrombosis remain uncertain.

The  $\beta$ -coronavirus SARS-CoV-2 is a single strand RNA (+) enveloped virus (7). The viral spike protein-1 binds to its main receptors, the surface expressed angiotensin-converting enzyme-2 (ACE-2) and TMPRSS2 (8), through which the virus infects target cells. Virus replication involves the production of double-stranded (positive- (+)/negative- (-) strand) complexes in the cytosol of the infected cells initiating viral component production. SARS-CoV-2 infected cells are found not only in the lung, but the virus is also widely found in other tissues (9). The means of viral dissemination remains unknown. Since there is a lack of significant blood viremia, but only occasional detection of viral RNA, since blood cells are rarely infected (10-12), and since no studies have so far demonstrated the presence of viruses and their infectiousness, our results indicate that the major route of SARS-CoV-2 dissemination is not blood.

Platelets have a critical role in hemostasis and thrombosis (13). Their interaction with the subendothelium during viral infection results in platelet hyperactivity and in turn, arterial thrombus producing end-organ ischemia. In particular, the influenza virus can directly activate platelets (14, 15) with consequent uncontrolled coagulation cascade resulting in lung injury. Besides their role in hemostasis, platelets have immunological function contributing to the immune response and inflammation (13, 16, 17). Platelets can also harbor pathogens including viruses (18, 19) where some, such as Dengue virus, may even replicate (20). Furthermore, HIV as well as Dengue and influenza virus can infect MKs (21), the cell producing platelets (21-23). Accordingly, platelets can also shelter viruses such as HIV *in vivo* as we have recently shown (22), thereby participating in the propagation of the infection and altering the viral pathology. Platelets are hyperactivated in COVID-19 and transcriptomics found N and more often E genes associated with platelets in some patients irrespective of disease severity (24, 25). Whether MK infection occurs and replication-competent SARS-CoV-2 is contained in platelets with a possible role in both COVID-19 thrombophilia and virus spread have not been addressed.

## **Material and Methods**

### **Patients and ethical statement**

106 This non-interventional study was approved by the institutional review board of the ethical committee for  
107 research (CER) of the University of Paris-Saclay (CER-Paris-Saclay-2020-050) and conformed to the  
108 principles outlined in the Declaration of Helsinki. Accordingly, all participants were informed in writing about  
109 the study and allowed not to participate. We studied prospectively samples from 76 COVID-19 patients  
110 admitted at the Cochin (Paris, France), Ambroise Paré (Boulogne-Billancourt, France), and Raymond  
111 Poincaré (Garches, France) Hospitals between March and May 2020. All patients had COVID-19 diagnosis  
112 confirmed by SARS-CoV-2 RNA RT-qPCR in nasopharyngeal swabs at the hospital. For this study, blood  
113 (n= 52), bronchoalveolar lavages (BAL) (n=19) and autopsy (n=5) unpaired samples were obtained from  
114 severe COVID-19 patients.

115

116 Additional methods are detailed in supplementary material.

117

## 118 **Results**

119

### 120 **Platelets from COVID-19 non-survivors harbor SARS-CoV-2**

121

122 Platelet samples from randomly chosen individuals with confirmed severe COVID-19 diagnosis (n=30)  
123 (Figure S1, Table 1, S1-2) were screened for SARS-CoV-2 by RT-qPCR (ORF, S and N genes). SARS-CoV-  
124 2 RNA was detected in 7 out of 30 patients. Strikingly, 6 out of the 7 platelet positive patients died within the  
125 week following sampling (mean days [95%CI]: 8.3 [5-14]), thus referred as non-survivors. Among the group  
126 of surviving patients (survivors), only 1 out of 24 platelet samples contained the viral RNA (Figure 1A, mean  
127 [95%CI] RNA copies/million platelets in samples from non-survivors versus survivors, for ORF1 gene: 580  
128 [160-1200] vs 2.2 [1.8-8.2],  $p<0.001$ ; for S gene: 840 [160-1600] vs 1.3 [1.1-5],  $p<0.001$ ; for N gene: 1200  
129 [320-2000] vs 1.6 [1.3-5.6],  $p<0.001$ ). Using 11 of these samples and additional ones from 28 patients, we  
130 then evaluated if the RNA detected in platelets corresponded to full viral particles, using a flow cytometry  
131 technique for combined detection of SARS-CoV-2 RNA and spike proteins in platelets, referred to as FISH-  
132 Flow (22) we now validated for SARS-CoV-2 (Figure S2 and Table S3). Detection of both viral RNA and  
133 protein was remarkably more frequent in platelets from non-survivors than from survivors (Figure 1B, Figure  
134 S2, mean % [95%CI] of RNA<sup>+</sup>/spike<sup>+</sup> platelets in samples from non-survivors versus survivors, 0.22 [0.06-  
135 0.42] vs 0.02 [0.01-0.07],  $p=0.001$ ).

136

137 Taken RT-qPCR and FISH-Flow analyses together, SARS-CoV-2 virus was detected within circulating  
138 platelets in 20 patients out of 52, from which 19 died (Figure 1C) in direct correlation with fatal outcome that  
139 persisted after adjustment for age (OR 63.4 [95% CI 6.6 to 610.1],  $p=0.0003$ ). Of note, the time from first  
140 symptoms to sampling was similar between survivors and non-survivors with mean value of 10 days [95% CI  
141 6;26] and 7 [95% CI 5;12.5], respectively ( $p=0.22$ ), and thus did not introduce a bias in the analysis. The  
142 presence of SARS-CoV-2 in platelets was the strongest factor associated with fatal outcome with  $p < 0.0001$   
143 in a multivariate analysis based on all patients' clinical data collected (Table 1).

144

145 In non-survivor patients, the virus resided inside platelets and was not associated to platelet surface: this  
146 was demonstrated by confocal microscopy after viral (+) and (-) strands RNA *in situ* hybridization using  
147 RNAscope technology (22) we validated (Figure S3A), coupled to immunostaining of the platelet marker

148 CD41 (Figure 1D-E and Figure S3B-C). In contrast, no viral signal was found in platelets from survivors. At  
149 the ultrastructural level, spherical crowned structures with a diameter of 50-80nm and visible spikes at their  
150 periphery typical of SARS-CoV-2 (26-30) were detected inside non-survivor platelets (Figure 1F and Figure  
151 S3D). These viral structures were found in subcompartments of the open canalicular system (OCS) (31) as  
152 well as in another type of compartment similar to the intra-platelet localization of dengue virus (32), whereas  
153 HIV (22), and Influenza viruses (33) are exclusively found in the lumen of OCS. Immunolabeling of SARS-  
154 CoV-2 spike proteins on cryosections from these non-survivor platelets (Figure 1G and Figure S3E)  
155 confirmed the SARS-CoV-2 nature of the viral spherical crowned images observed above (Figure 1F and  
156 Figure S3D). Immunolabeled viruses were again exclusively detected within platelets, with no virus detected  
157 at the platelet surface. No such crowned viral structures nor immunolabelling were observed in survivor  
158 platelets. Platelets from non-survivors appeared also hyperactivated as indicated by the increase in  
159 frequency of platelets surface stained with Von Willebrand factor (vWF) in non-survivors versus survivors or  
160 healthy donors (Figure S4A-B) (34).

161  
162 Although SARS-CoV-2 genes are scarcely found in blood (10, 11, 35), viral genes were more frequently  
163 detected in plasma (here in platelet-poor plasma (PPP)) from non-survivors than survivors (66% vs 25%)  
164 (Figure S4C). Furthermore, viral RNA copy number detected per million platelets in PRP was not  
165 proportional to that detected per ml of PPP using RT-qPCR (Figure S4D), indicating that viral genes detected  
166 in the plasma is not the source of virus detected in platelets. In addition, platelets from healthy donor  
167 incubated with infectious SARS-CoV-2 were unable to internalize SARS-CoV-2 (Figure S4E-G), confirming  
168 that the source of SARS-CoV-2 in patient platelets was not by endocytosis of virus possibly present in the  
169 plasma.

170  
171 **In COVID-19 non-survivors, megakaryocytes are produced following a shortened megakaryopoiesis**  
172 **and express viral sensing genes**

173  
174 Alternative to virus endocytosis, platelets could have acquired SARS-CoV-2 in the bone marrow during  
175 maturation of their precursors, the megakaryocytes (MKs) that would be infected. This has been observed in  
176 Dengue and Influenza virus infection (21). After entering the cava vein, bone marrow MKs reach the  
177 pulmonary circulation and then the lung (36). These large MKs are filtrated in the pulmonary capillary bed  
178 producing platelets locally but also releasing MKs with monolobed nucleus that are found later on in the  
179 peripheral circulation (37, 38). Such monolobed MKs present in PBMCs were thus used as easily accessible  
180 surrogates for bone marrow MKs (4, 39). Accordingly, monolobed MKs were detected at low frequencies in  
181 PBMCs from non-COVID-19 healthy individuals and COVID-19 survivors but their frequency strikingly  
182 increases in COVID-19 non-survivor samples (Figure 2A and Figure S5A).

183  
184 To further compare MK characteristics in COVID-19 non-survivors versus survivors and healthy controls, we  
185 integrated two PBMC single-cell RNA sequencing (scRNA-seq) data sets (4, 39) including healthy donor,  
186 COVID-19 survivor and non-survivor samples. Clustering analysis revealed that MK transcriptome profile  
187 was more diverse in severe COVID-19 than in healthy controls (Figure 2B-C and Figure S5B-D).  
188 Furthermore, genes associated with megakaryopoiesis in severe COVID-19 pointed to a shortened MK  
189 differentiation process consistent with the non-classical pathway recently described that bypasses the MK-

190 erythroid progenitors (MEP) step from the classical differentiation route (40) and associated to inflammatory  
191 conditions (41) (Figure 2D and Figure S5E).

192  
193 Strikingly, when compared with healthy donors and survivors, a unique MK profile (cluster 3) was enriched in  
194 non-survivors, both when cells were analyzed altogether (Figure 2B-C) and by individuals (Figure 2E).  
195 Resulting differentially expressed genes (DEG) in Cluster 3 (Figure S6A-B) comprised platelet secreted  
196 molecules upon activation such as PF4, inflammatory genes such as S100A8, a large set of IFN-stimulated  
197 genes (IFI6, IFI27, IFTIM3) that attest viral sensing, and genes driving megakaryopoiesis (PF4, MYL9, and  
198 histone associated genes). Accordingly, Cluster 3 specifically associated pathways included not only rapid  
199 MK development and platelet functions but also viral sensing by INF-stimulated genes and inflammation  
200 (Figure S6B-C). Cluster 3-enriched transcription factors such as JUND, GATA1, and RUNX1 were  
201 characteristic of megakaryopoiesis while NFKB1, RELA and STAT3 characterized an inflammatory immune  
202 response (Figure S6B). Inferred cluster 3 transcription factors targets were also enriched in IFN regulatory  
203 factors (Figure S6B). Among all trajectories rooted on cluster 0 (Figure S6D) predominant in MKs from  
204 healthy donors (Figure 2B-C), the one transitioning MKs from cluster 0 to 3 (Figure S6D) confirmed the  
205 significant increase of histone-associated and inflammatory genes but also that of antiviral IFN-stimulated  
206 genes (Figure 2F).

207

#### 208 **Infected bone marrow MKs in COVID-19 patients as a source of platelets containing SARS-CoV-2**

209

210 Altogether, the scRNA-seq results indicated that MKs from COVID-19 non-survivors have matured faster  
211 than usual and have sensed the virus, thus being likely infected. In addition, MKs at an abnormally higher  
212 density were detected in bone marrow from COVID-19 versus non-COVID-19 autopsy cases (Table 2 and  
213 Figure 2G,  $49.89 \pm 14.37$  cells per  $\text{mm}^2$  versus  $13 \pm 3$  cells per  $\text{mm}^2$ ,  $p=0.0001$ ), with an increased diameter  
214 size ( $103.36 \pm 42.36$   $\mu\text{m}$  versus  $30-100$   $\mu\text{m}$ ). Furthermore, these bone marrow MKs recurrently contained  
215 replicative viral (-) RNA (Figure 2H) with  $14.99 \pm 9.58$  % of the bone marrow MKs positive to (-) SARS-CoV-2  
216 RNA (Figure S7A), establishing that MKs are infected in the bone marrow in COVID-19 non-survivors and  
217 constitute the likely source for producing platelets containing SARS-CoV-2.

218

#### 219 **Infected MKs are retained in the lung in COVID-19 non-survivors**

220

221 Bronchoalveolar lavages (BAL) from severe COVID-19 patients provide an easily accessible fluid to probe  
222 the lung thrombotic and inflammatory environment during the progression of the disease. Thus, among a  
223 panel of factors implicated in hemostasis, inflammation and tissue repair analyzed, PF4/CXCL4 (platelet  
224 activation) and S100A8 (neutrophil and macrophage inflammatory activation) and VEGF-A and PDGF-BB  
225 (tissue repair and angiogenesis) were the only ones significantly increased in BAL from non-survivors  
226 compared to survivors (Figure 3A, CXCL4 mean pg/ml,  $1487.8$  [ $459.8-2798.7$ ] vs  $334.7$  [ $111.9-630.6$ ],  
227  $p=0.048$ ; VEGF-A mean pg/ml,  $3481.8$  [ $1308.7-6077.6$ ] vs  $335$  [ $68.9-826.4$ ],  $p=0.006$ ; PDGF-BB mean pg/ml,  
228  $24$  [ $6.7-43.5$ ] vs  $0$ ,  $p=0.037$ ; and S100A8 mean pg/ml,  $5004.7$  [ $1481.4-8762.9$ ] vs  $242.2$  [ $63.53-484.4$ ],  
229  $p=0.01$ ). This set of cytokines issued from or implicated with platelet/MK lineage which was enriched in non-  
230 survivor BAL pointed to a virus-mediated MK impairment and retention in the lung, as already observed in  
231 the bone marrow in deadly COVID-19.

232  
233 MKs appear specifically recruited into or retain within the lung in COVID-19 autopsy cases (3) together with  
234 endothelial damages (42, 43). We therefore searched for MKs in BAL from patients with severe COVID-19  
235 (Table 3). In line with the infection that we observed in bone marrow MKs, pulmonary MKs, identifiable  
236 through their large size and multilobed nucleus harboring cytoplasmic SARS-CoV-2 (+) RNA were detected  
237 in the BAL of COVID-19 patients irrespective of outcome, signing their viral infection (Figure 3B and Figure  
238 S7B). When quantified by flow cytometry, a trend, although statistically non-significant, towards increase  
239 frequency of MKs and platelets among BAL cells from non-survivors compared to survivors was observed  
240 (Figure 3C, Figure S7C). The frequencies of both infected spike<sup>+</sup> MKs and virus-containing spike<sup>+</sup> platelets in  
241 total MK and platelet populations were significantly increased by 4 and 29-fold in BAL from non-survivors  
242 versus survivors, respectively (Figure 3D, Figure S7C) (mean % spike<sup>+</sup> MKs and platelets in BAL from non-  
243 survivors versus survivors, 9.5 [7.1-12.3] vs 2.3 [0.8-4.7],  $p=0.016$ , and 2.9 [0.8-6.5] vs 0.1 [0.07-0.2],  
244  $p=0.029$ , respectively).

245  
246 The lack of statistically significant correlation between the frequency of spike<sup>+</sup> platelets among total platelets  
247 recovered in BAL measured by flow cytometry and the viral load measured per ml of BAL by RT-qPCR  
248 (Figure S7D), further indicates that detection of virus in platelets is not a result of virus endocytosis by the  
249 latter.

250  
251 Upon pathological examination of lung autopsy cases, a high density of MKs, identified by their size and the  
252 caterpillar appearance of their large nuclei, surrounded by fibrin webs, was detected by histochemistry  
253 (Table 2, Figure 3E upper). Furthermore, capillary walls were highly damaged lacking the usual covering  
254 endothelial layer. MKs were not only found within the pulmonary vessels but also entering the alveoli (Figure  
255 3E lower) (Figure 3E lower inset) and in the alveolar space as confirmed after specific vWF immunolabelling  
256 (Figure 3F and Figure S7E) in line with the unexpected presence of MKs in the BAL. When quantified in non-  
257 COVID-19 (n=6) and COVID-19 (n=3) lung autopsies as described (44, 45), the quantities of MKs increased  
258 by almost fourfold (from  $5.78 \pm 4.97$  to  $20.25 \pm 6.99$  cells per section ( $5 \text{ cm}^2$  and  $5\text{-}7 \text{ }\mu\text{m}$  thickness) in non-  
259 COVID-19 vs COVID-19 autopsies:  $p=0.0015$ ), as well as their mean diameter ( $52.98 \pm 21.98 \text{ }\mu\text{m}$  in non-  
260 COVID-19 vs  $79.68 \pm 19.45 \text{ }\mu\text{m}$  in COVID-19 autopsies:  $p=0.05$ ) and nucleus size ( $26.6 \pm 1.1 \text{ }\mu\text{m}$  in non-  
261 COVID-19 vs to  $30.3 \pm 1.2 \text{ }\mu\text{m}$  in COVID-19 autopsies,  $p=0.044$ , Figure S7F).

262  
263 Furthermore, as observed in bone marrow and in BAL, lung tissue MKs were found actively infected  
264 harboring replicative viral (-) RNA (Figure 3G).  $21.33 \pm 12.1 \%$  (n=3) of lung MKs are positive for SARS-CoV-2  
265 RNA, and infected lung MK are  $2.5 \pm 0.8$  (n=3) times more frequently detected in alveoli than in blood vessels  
266 in lung tissues (Figure S7A). This suggests that platelets containing SARS-CoV-2 could also be produced in  
267 the lung of deadly COVID-19 patients.

268  
269 Accordingly, a rough estimation of (-) SARS-CoV-2 positive platelet in tissue autopsies was obtained by  
270 measuring the Pearson's correlation coefficients (46) for colocalization of SARS-CoV-2 RNA with CD41 after  
271 excluding the signal from large CD41<sup>+</sup> polylobed MKs and including CD41 signal from anucleated bodies  
272 only. As a result, correlation coefficients are significantly higher in both lung and bone marrow tissues from  
273 COVID-19 individuals (Pearson's coefficient:  $0.739 \pm 0.252$  and  $0.747 \pm 0.632$ , respectively) as compared with



274 non-COVID-19 individuals (Pearson's coefficient for lung and bone marrow tissues:  $0.160 \pm 0.093$  and  
275  $0.122 \pm 0.056$ , respectively) ( $p < 0.005$ ,  $n=3$ ).

### 276 277 **Platelets containing SARS-CoV-2 are captured by pulmonary macrophages**

278  
279 The rupture of the endothelium from the lung capillaries and the epithelium covering the alveolar surface  
280 observed here in COVID-19 lung autopsies and as we reported (56) might let platelets penetrate the alveolar  
281 tissue and space. In turn macrophages could capture these platelets by hemophagocytosis (47-49),  
282 establishing a route to lung macrophage infection mediated by SARS-CoV-2-containing platelets and  
283 inflammatory modulation (50). Indeed, hemophagocytosed platelets were detected in macrophages in the  
284 alveolar tissue and alveolar space of COVID-19 autopsies (Figure 4A, Table 2). Furthermore, in BAL from  
285 additional severe COVID-19 patients (Table 3) those from non-survivors contained also platelets containing  
286 SARS-CoV-2 RNA (+) (Figure 4B).

287  
288 When quantified by flow cytometry, BAL from non-survivors had increased frequencies of platelet-  
289 macrophage conjugates than survivors (Figure 4C and Figure S7C), mean frequency of platelet-macrophage  
290 conjugates among total macrophage in BAL from non-survivors versus survivors was  $4.6 [3.5-5.8]$  vs  $2.4$   
291  $[1.5-3.3]$ , respectively,  $p=0.011$ ). However, the frequency of macrophages (Figure S7G left) and spike<sup>+</sup> lung  
292 macrophages (Figure S7G right) conjugated or not with platelets did not differ in BAL from non-survivors and  
293 survivors.

### 294 295 **SARS-CoV-2 contained in platelets of non-survivors is infectious**

296  
297 Whereas circulating SARS-CoV-2 free particles are not usually detected in infected patients (10), SARS-  
298 CoV-2 infected cells are found not only in the lung, but also at extrapulmonary sites in deadly COVID-19 (9).  
299 We thus hypothesized that in these patients, infection could be spread by platelets containing SARS-CoV-2  
300 present in the circulation and at the pulmonary level.

301  
302 We first investigated whether SARS-CoV-2 contained by platelets could be titrated on non-phagocytic Vero  
303 cells using the very sensitive FISH-flow assay we validated using different concentrations of SARS-CoV-2  
304 (Figure S8).

305  
306 To measure the titer of SARS-CoV-2 within platelets, we had first to force the virus to exit platelets from  
307 within, namely purging, by activating platelet with thrombin receptor activating peptide (TRAP) (51) inducing  
308 virus release in the medium without platelet aggregation (52). Platelets from COVID-19 non-survivors, with  
309 confirmed detection of SARS-CoV-2, were thus treated or not with TRAP, and centrifuged to collect platelet  
310 supernatants referred to as releasates that were then titrated on Vero cells (Figure S9A). In paired samples  
311 of 3 different non-survivors, the percentage of SARS-CoV-2 RNA<sup>+</sup>/dsRNA<sup>+</sup> Vero cells infected with TRAP-  
312 treated compared to untreated platelet releasates was statistically significantly higher ( $p=0.042$ ), the later  
313 falling below the limit of detection established using healthy donor platelet controls (Figure 4D and Figure  
314 S9B). Estimating Vero cell infection in PFU using the PFU/FISH-flow standard curve (Figure S8D), the mean

315 titer of SARS-CoV-2 virus sheltered in platelets and released by activating platelets was 35 [CI: 19-50]  
316 SARS-CoV-2 PFU per million platelets from COVID-19 non-survivors (n=3) (Figure 4D).

317

### 318 **SARS-CoV-2 trans-infection from platelets to macrophages is blocked by anti-platelet drug** 319 **abciximab**

320

321 Platelets are short-lived and are mainly eliminated following their capture by tissue macrophages (53).  
322 Furthermore, lung macrophages express the main receptors for the virus necessary for infection (ACE2 and  
323 TMPRSS2) (54-56). Lung macrophages are found infected in COVID-19 and support SARS-CoV-2  
324 replication *in vitro* as we and others have demonstrated recently (57-60), being a key cell in promoting viral  
325 spread (57-60), and the cytokine storm driving severe COVID-19 (50). Primary macrophages derived from  
326 blood monocytes (MDM) cultivated with M-CSF, interleukin (IL)-4 and IL-13 mimic tissue macrophages (22,  
327 61) express ACE2 and TMPRSS2, support SARS-CoV-2 replication and self-limiting release of infectious  
328 viruses (Figure S10A-D). These MDM were cultivated with platelets from non-survivors for 2 hours (pulse),  
329 extensively washed and further cultivated for 24 hours (chase). Both viral (+) and (-) strands RNA indicative  
330 of virus replication were detected in macrophage cytosol after *in situ* hybridization and confocal microscopy  
331 analysis (Figure 4E). In contrast, after the pulse, only the (+) RNA signal corresponding to the platelet  
332 inoculum was detected in MDM (Figure 4E left). Viral RNA signal was also undetectable after incubation with  
333 platelets from survivors (not shown). Replication-competent virus was detected in the supernatant of the  
334 MDM-platelet co-culture 24 hours after inoculation with virus-containing platelets (Figure 4F left image and  
335 middle graph) using Vero cells as in (62). In contrast, no viral signal was detected after the pulse (Figure 4F  
336 middle, % of SARS-CoV-2 RNA<sup>+</sup>/dsRNA<sup>+</sup> Vero cells infected by the supernatant of MDM incubated with  
337 platelets samples from non-survivor after the pulse vs the chase, 0 vs 1.07 % [0.6-1.5],  $p=0.029$ ). When  
338 titrated on Vero reporter cells, plasma deprived of platelets (Platelet-poor plasma: PPP) from survivors and  
339 non-survivors were not infectious (Figure S10E left) revealing the absence of infectious virus in the  
340 circulation despite viral gene detection. In contrast, plasma containing SARS-CoV-2 -positive platelets added  
341 to macrophages resulted in the production of infectious viral particles, titrated on Vero reporter cells (Figure  
342 S10E right).

343

344 Finally, the SARS-CoV-2-containing platelet to MDM trans-infection process was blocked when platelets  
345 were pre-incubated with an anti-platelet GPIIb/IIIa drug, abciximab, prior to MDM inoculation. In the presence  
346 of abciximab, the production of replication-competent virus by MDM after 24hrs infection was reduced by  
347  $98\pm 0.9\%$  ( $p<0.001$ ) (Figure 4F right graph).

348

### 349 **Discussion**

350

351 Platelet activation, thrombophilia and hypercoagulability have emerged as crucial pathological characteristics  
352 in severe COVID-19 that can lead to fatal outcome (1). Hence, the frequency of thrombotic events in critical  
353 COVID-19 cases is particularly high, with increased frequency of venous and arterial thrombosis resulting in  
354 clinical complications including pulmonary embolism, ischemic stroke, and myocardial infarction (2, 6).  
355 Increased platelet activation has been shown as a poor prognostic factor (34). Previous studies have shown  
356 the presence of SARS-CoV-2 components in platelets in the context of COVID-19 platelet hyperactivation

357 (24) (25).

358

359 Here we not only demonstrate for the first time the presence of replication-competent functional virus in  
360 platelets but also correlate the detection of virus-containing platelets with patient outcome. In addition to  
361 contribution to life-threatening thrombotic disorders described in COVID (1, 2), we now show that platelets  
362 can also harbor infectious SARS-CoV-2 detected as early as three weeks prior to death (median [IQR] days  
363 from sampling to death: 7 [5;10] [from 1 to 20]) (Table 1). Furthermore, in patients with COVID-19, the  
364 presence of infectious SARS-CoV-2 in platelets is a strong predictive marker of fatal outcome. The detection  
365 of SARS-CoV-2 in platelets by RT-qPCR or FISH-Flow, easily implementable as a routine analysis, could  
366 serve as a diagnostic useful tool to foresee as early as possible poor prognosis and take appropriate medical  
367 action.

368

369 The present results prompt three questions: how does the virus enter platelets, what is the possible causal  
370 effect of the presence of virus in platelets in COVID-19 pathology, and which type of treatment might prevent  
371 these effects.

372

373 As we found MKs, the platelet precursors, actively infected in bone marrow and the lung in fatal forms of the  
374 disease, SARS-CoV-2 might likely associate with platelet during thrombopoiesis, in a process we and other  
375 demonstrated for HIV (22, 63), Dengue and Influenza virus (23) (21). Alternatively, platelets could  
376 endocytose the virus in the circulation as they express SARS-CoV-2 receptors such as ACE2 (64) and DC-  
377 SIGN that participate in virus endocytosis, as we have shown *in vitro* (18, 65, 66). It is however unlikely as  
378 free SARS-CoV-2 in platelet-poor plasma is not infectious and as platelets could not internalize SARS-CoV-  
379 2 *in vitro*.

380

381 Small MKs that reach the peripheral circulation after fragmentation from bone marrow and lung MKs (37, 38,  
382 67), especially in inflammatory lung diseases (41), are present at low levels in PBMCs and can thus serve as  
383 surrogates in the analysis of human lung MKs, the access of which is challenging. Some of these circulating  
384 MKs harbor immunomodulatory functions in healthy donors (41, 68, 69). Similarly, we found in the blood  
385 from COVID-19 non-survivors a significant increase of circulating MKs carrying type I IFN and inflammatory  
386 gene signatures and an upregulation of genes conditioned by NFKB1, characteristics of a response to viral  
387 infection including that to SARS-CoV-2 (70). The expression of inflammatory genes typically involved in MK  
388 anti-viral response, such as IFITM1 and IFTIM3 (21), together with the non-classical MK differentiation  
389 pathway that we observed support the development of an emergency megakaryopoiesis in severe COVID-  
390 19 avoiding the MEP commitment (71). Such shortened pathway might be followed by an increase in MK  
391 number and size as we observed in bone marrow and lung tissues in COVID-19 compared to non-COVID-19  
392 autopsies. Furthermore, we found in the lung of non-survivors an increase of CCL5, IL-1beta and IL6,  
393 cytokines inducing the production of thrombopoietin (TPO) that in turn speed megakaryopoiesis up, through  
394 the direct non-classical pathway (71). Additionally, such increased CCL5, known to stimulate MK ploidy (71)  
395 and in turn speed megakaryopoiesis, could explain the increase in MK nucleus size we found in COVID-19  
396 compared with non-COVID-19 lung autopsies.

397

398 Altogether, in line with the emergency myelopoiesis that we reported earlier (72), these results point to a MK  
399 response to pathogenic stimuli, i.e., infection by SARS-CoV-2 of MKs in the bone marrow, before entry in the  
400 circulation. Infected MKs would then migrate from bone marrow to the lung and produce platelets containing  
401 SARS-CoV-2 at both sites as we specifically detected in non-survivors: this finding being likely connected to  
402 COVID-19 fatal immunothrombosis.

403  
404 However, patient platelet counts monitored during the following 15 days or until discharge for the non-  
405 survivors (Figure S1A) did not show the appearance of thrombocytopenia or significant variation in platelets  
406 counts in both patient sets. Of note, the increase in megakaryocyte numbers observed in autopsy tissues  
407 from COVID versus non COVID patients is not accompanied by an increase in platelet numbers during  
408 deadly COVID. This observation suggests either an inefficient thrombopoiesis due to shortened  
409 megakaryopoiesis; or alternatively, a retention of megakaryocytes in the lung of patients with an inefficient  
410 platelet production and, as megakaryocytes are known to contain high amounts of growth factors and  
411 cytokines (73), they could in turn participate to a local cytokine storm.

412  
413 MKs might also exert immunological functions by using the set of histone-associated genes that we found  
414 specifically increased in non-survivor inflammatory MKs. This may generate extra-nuclear histones that  
415 serve as damage-associated molecular patterns (DAMP) (74, 75), and participate in severing systemic  
416 inflammation and immunothrombosis in COVID-19 as it has been shown in sepsis (76, 77).

417  
418 Infected MKs themselves would also participate to the cytokine storm by upregulation of interferon type I  
419 gene, as in other viral infections (21, 33, 78) and contribute to differential expression of virus-induced lung  
420 cytokines implicated in hemostasis and inflammation in COVID-19 non-survivors. Hence, PF4/CXCL4 is  
421 synthesized by MK and packaged within platelets alpha-granules during platelet production (13). Increase in  
422 PF4 in BAL from non-survivors might reflect both MK destruction in pulmonary vessels and platelet  
423 activation, shown to occur in COVID-19 patients (34). In turn, PF4 could intensify inflammation by polarizing  
424 lung tissue macrophages to the M4 inflammatory subtype (79) resulting in S100A8 secretion, thereby fueling  
425 inflammation (72, 79). VEGF-A and PDGF-BB, expressed in particular by myeloid cells including MKs and  
426 macrophages (80) could represent stress responses to pulmonary endothelium damage (81, 82) that we  
427 observed in pulmonary autopsy tissues. VEGF-A binds to Neuropilin 1 (NRP1) on endothelial cells  
428 contributing to the integrity of the vessel wall and to the inhibition of platelet aggregation in blood vessels (80,  
429 83). SARS-CoV-2 spike protein that binds also to NRP1 (84, 85), may displace VEGF-A resulting in the  
430 increased levels of VEGF-A that we detected in COVID+ BAL and meanwhile facilitate small vessel  
431 thrombosis by inhibiting endothelial function both systemically and in the pulmonary circulation. In sum, the  
432 uncontrolled cytokine release may contribute to endothelial wall injuries (86) that would allow entrance of  
433 MKs and platelets to the alveolar space as assessed by bronchoalveolar lavage observation. This process  
434 appears specific to COVID-19, increased in non-survivors, although remaining limited in survivors.

435  
436 Fully functional SARS-CoV-2 virus found within circulating platelets of non-survivors is infectious as in other  
437 viral infections (20, 22) indicating that SARS-CoV-2 viruses, protected by the platelet membrane from  
438 degradation by assault of antibodies or complement, might disseminate to other tissues. Virus would then  
439 propagate infection contributing to rapid multiple organ failure (87, 88). Accordingly, non-survivors in this

440 study reach a fatal issue within two weeks after symptom onset (Table 1) when virus is still replicating (10).  
441 We demonstrate that platelets harboring SARS-CoV-2 are capable to trans-infect macrophages *in vitro*, a  
442 process abrogated by the anti-platelet GPIIb/IIIa drug abciximab. We have already shown that blocking  
443 platelet GPIIb/IIIa with abciximab fully prevents platelets internalization by macrophages (22) although by a  
444 mechanism that is not yet demonstrated. We can hypothesize that platelet GPIIb/IIIa interacts with either  
445 macrophage CD40 (89) or the integrin Mac1 ( $\alpha$ M $\beta$ 2) in the presence of fibrinogen (90, 91) likely associated  
446 to platelets in our experimental conditions (Platelet Rich Plasma), promoting an outside-in GPIIb/IIIa  
447 mechanism of activation that in turn promotes platelet uptake by macrophages (92) in a  $\beta$ 2-mediated  
448 phagocytosis process (93). Abciximab would impair such interactions and in turn, platelet induced  
449 macrophage infection and/or possibly activation. This hypothesis remains to be verified.

450  
451 In COVID-19 patients lung tissue macrophages that we and other have found infected (57-60) may act as a  
452 Trojan horse, capable of transferring SARS-CoV-2 to nearby lung regions and in turn slowly propagating  
453 SARS-CoV-2 infection and spreading hyperinflammation across the lung (57-60) as witnessed by the  
454 increase in lung inflammatory cytokines we reported here. Upon phagocytosis by macrophages, SARS-CoV-  
455 2-containing platelets might transfer infection to macrophages that would produce IFN (94). Incoming viral  
456 RNA could also signal PRR to enhance phagocytosis, increase oxidative burst and release of pro-  
457 inflammatory cytokines and chemokines, resulting in inflammation. This would promote an influx of  
458 monocytes HLA-DR low S100A8+ into the lung, sustaining inflammation and tissue damage (60).  
459 Furthermore, platelet hyperactivation might be induced by virus or viral components within platelets resulting  
460 in exposure of vWF (this study) and P-Selectin (34) followed by platelet aggregation, which could contribute  
461 to lung inflammation as in influenza virus infection (95), and also been prevented by anti-GPIIb/IIIa drug.  
462 Therefore, therapeutic targeting of the platelet surface protein GPIIb/IIIa could help blocking the process of  
463 viral spread in addition to its anti-thrombotic effect.

464  
465 The following features we observed in non-survivors, namely increased phagocytosis of virus-containing  
466 platelets, endothelial cell wall injury and the infection of MKs themselves, also occurring in other viral  
467 infection (21, 33, 78), may likely result in exacerbated macrophage-dependent cytokine production,  
468 increasing thrombotic risk and complications (86), a hallmark of severe COVID-19. These deleterious effects  
469 of platelets harboring SARS-CoV-2 are summarized and schematized altogether in Figure 5. Drugs targeting  
470 specifically the platelet receptor GPIIb/IIIa may offer an alternative to scantily efficient anticoagulant treatment  
471 (6, 96): indeed, its direct and indirect anti-thrombotic effect, prevention of macrophage infection and  
472 activation limiting their contribution to the cytokine storm, in addition to impairing the spread of infection to  
473 other tissue represent a valuable therapeutic recourse. Accordingly, a clinical trial using an anti-GPIIb/IIIa  
474 drug in critically ill patients with COVID-19 has shown a beneficial effect on respiratory functions and clinical  
475 outcome (97). In any case, the strategy of modifying platelet behavior needs careful consideration in the light  
476 of the results presented here. Indeed, it appears that platelets from severe COVID-19 patients bear a dual  
477 prothrombotic effect: via virally induced platelet activation and via decrease in endothelial function caused by  
478 the virus blocking the effect of VEGF-A at the NRP1 receptor which may be considered as an appropriate  
479 therapeutical target.

480

481 The present study may likely apply to patients infected with various SARS-CoV-2 variants. Our study was  
482 performed in France during the first wave of COVID-19 between April and June 2020 with samples from  
483 patients infected by the ancestral Wuhan virus SARS-CoV-2 before the appearance of the following variants  
484 (98). SARS-CoV-2 variants still use ACE2/TMPRSS2 for infection, although with different affinities (99).  
485 Thus, variants might likely infect megakaryocytes resulting in the production of platelets containing SARS-  
486 CoV-2 that would likewise trans-infect macrophages. We cannot rule out the possibility that infection by  
487 different variants would not affect megakaryocyte transcriptome (regulation) and in turn the genes  
488 transferred to platelets during thrombopoiesis and downstream effects.

489  
490 The scarce availability of platelet samples and the relative low frequency of virus-containing platelets,  
491 hindering detection by traditional techniques such as plaque assay is a limitation of this study. Hence, we  
492 applied the more sensitive single-cell FISH-flow technique (22) to approach infectious SARS-CoV-2.  
493 Importantly, the estimated quantity of infectious virus sheltered by platelets is non-negligible, being of 35 [CI:  
494 19-50] SARS-CoV-2 PFU per million platelets. Severe COVID-19 patients with fatal outcome appears to  
495 harbor 3.7-3.9 log<sub>10</sub> platelet-associated SARS-CoV-2 PFU per ml of blood in the circulation. In addition, the  
496 contribution of the lung, the main site affected in severe COVID-19, has recently been estimated to half of  
497 the total platelet production with the lung producing 10 million platelets per hour (37). This would translate  
498 into a lung production of around 2 log<sub>10</sub> platelet-associated SARS-CoV-2 PFU/hour.

499  
500 In sum, the presence of infectious SARS-CoV-2 in platelet combined with the intricate relationship between  
501 hemostasis, inflammation and the spread of infection has major consequences on COVID-19 pathogenesis  
502 and can turn out fatal. Anti-platelet drugs might be explored to develop anti-inflammatory coupled to pro-  
503 thrombotic treatment against severe SARS-CoV-2.

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527

## 528 **List of Supplementary Materials**

529

### 530 **Supplementary Material and Methods**

531 **Figure S1. Supplementary information of platelet samples.**

532 **Figure S2. FISH-flow experimental validation.**

533 **Figure S3. Additional confocal microscopy and electron microscopy images of platelets containing**  
534 **SARS-CoV-2 localized exclusively inside platelets and not at their surface.**

535 **Figure S4. Virus found in the plasma is non-infectious and does not quantitatively correlate with virus**  
536 **found within platelets that are unable to endocytose SARS-CoV-2.**

537 **Figure S5. FACS gating strategies and scRNA-seq analytical approach to assess PBMC-derived MKs.**

538 **Figure S6. Gene expression of MKs employed in clustering analysis.**

539 **Figure S7. Infected and abnormal lung MKs accessed in BAL by FACS and confocal microscopy, and in**  
540 **tissues by histology**

541 **Figure S8. FISH-flow validation using Vero cell infection with titrated primary SARS-CoV-2.**

542 **Figure S9. Titration of SARS-CoV-2 within platelets using TRAP-treated releasates.**

543 **Figure S10. Validation of macrophages as host cells for SARS-CoV-2 replication**

544 **Table S1: Patient medications according to the hospital outcome (PRP samples)**

545 **Table S2: Patient death causes**

546 **Table S3. List of FISH-flow RNA probe sequences**

547

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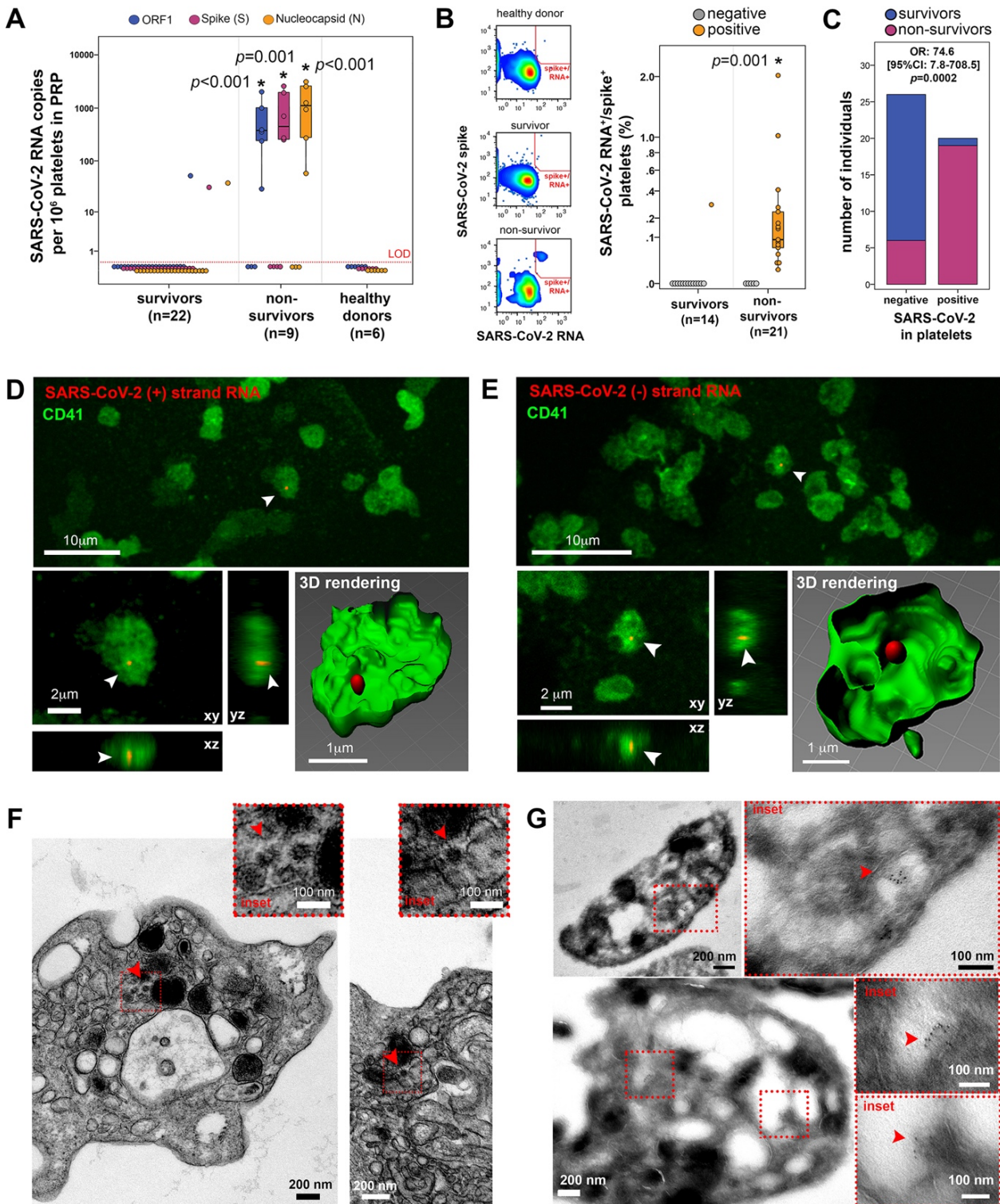
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## Figures and Figure legends



**Figure 1: Platelets from non-survivor patients with COVID-19 harbor SARS-CoV-2.**

A) Copies of SARS-CoV-2 ORF1 (blue), Spike (S, magenta) and Nucleocapsid (N, orange) RNA per million platelets detected by RT-qPCR, from COVID-19 survivors, COVID-19 non-survivors and healthy donor samples. Asterisk indicates statistical significance in the comparison between survivors and non-survivors per detected gene target (Kruskal-Wallis between the three groups). LOD=limit of detection.

B) Combined detection of SARS-CoV-2 spike protein and SARS-CoV-2 RNA by flow cytometry (FISH-flow). On the left, SARS-CoV-2 spike<sup>+</sup>/RNA<sup>+</sup> detection gate (red) showing an example of healthy donor, COVID-19

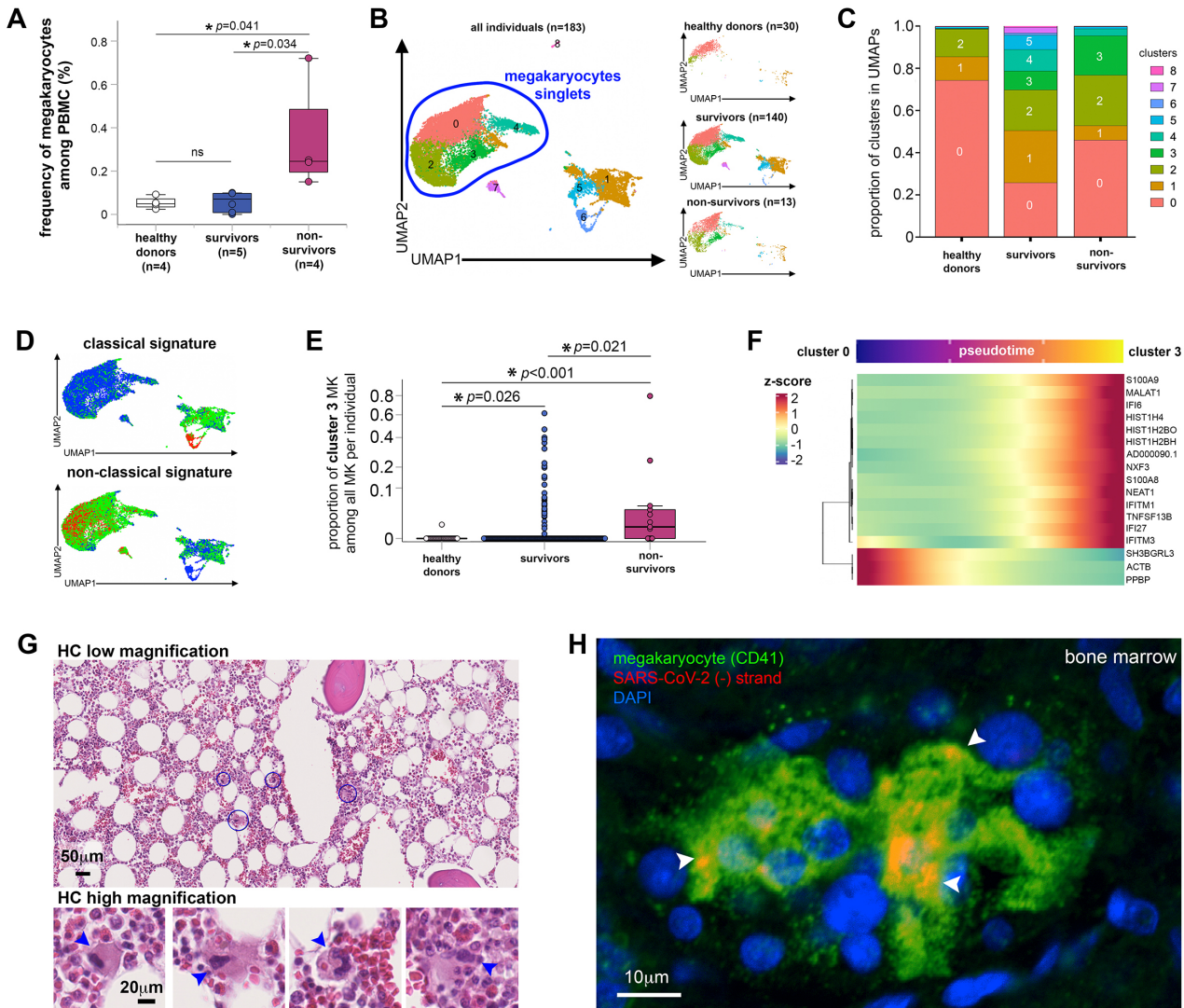
survivor, and COVID-19 non-survivor. On the right, the percentage of SARS-CoV-2 spike<sup>+</sup>/RNA<sup>+</sup> platelets among platelets from COVID-19 survivors and non-survivors, normalized by detected events in healthy donor samples. Samples were classified as negative (gray) or positive (orange) for the presence of SARS-CoV-2 in platelets. Asterisk indicates statistical significance in the comparison between survivors and non-survivors (Mann-Whitney between the two groups).

C) Number of COVID-19 survivors (blue) and non-survivors (magenta) among individuals tested for the presence of SARS-CoV-2 in platelets (negative or positive). OR: odds ratio.

D-E) Confocal microscopy images after CD41 (green) immunolabelling and SARS-CoV-2 RNA *in situ* hybridization (red) for SARS-CoV-2 (+) RNA (D) or SARS-CoV-2 (-) RNA (E) in platelet samples from a COVID-19 non-survivor. Images show low magnification (upper, bar=10µm), three-dimensional projections (lower left - xy, xz and yz, bar=2µm) and three-dimensional rendering (lower right, bar=1µm). Arrowheads indicate SARS-CoV-2 RNA, showing definite intracellular localization of the virus within the platelets.

F) Representative electron microscopy images of platelets with spherical crowned SARS-CoV-2 particles of 50-80nm in diameter (arrowheads) located in the lumen of OCS in platelets of non-survivors (bars= 200 nm). Dotted line indicates area magnified as shown in the insets (bar= 100 nm).

G) Immunogold labeling with a polyclonal anti-spike antibody of spike protein of non-survivor platelets otherwise tested positive for the presence of SARS-CoV-2 by FISH-Flow techniques (two examples, upper and lower images). Dotted squares point magnified regions where spike proteins were immunolocalized (red arrowhead). No spike immunolabeling was observed on platelet surface. Bar=100, 200 or 500 nm.



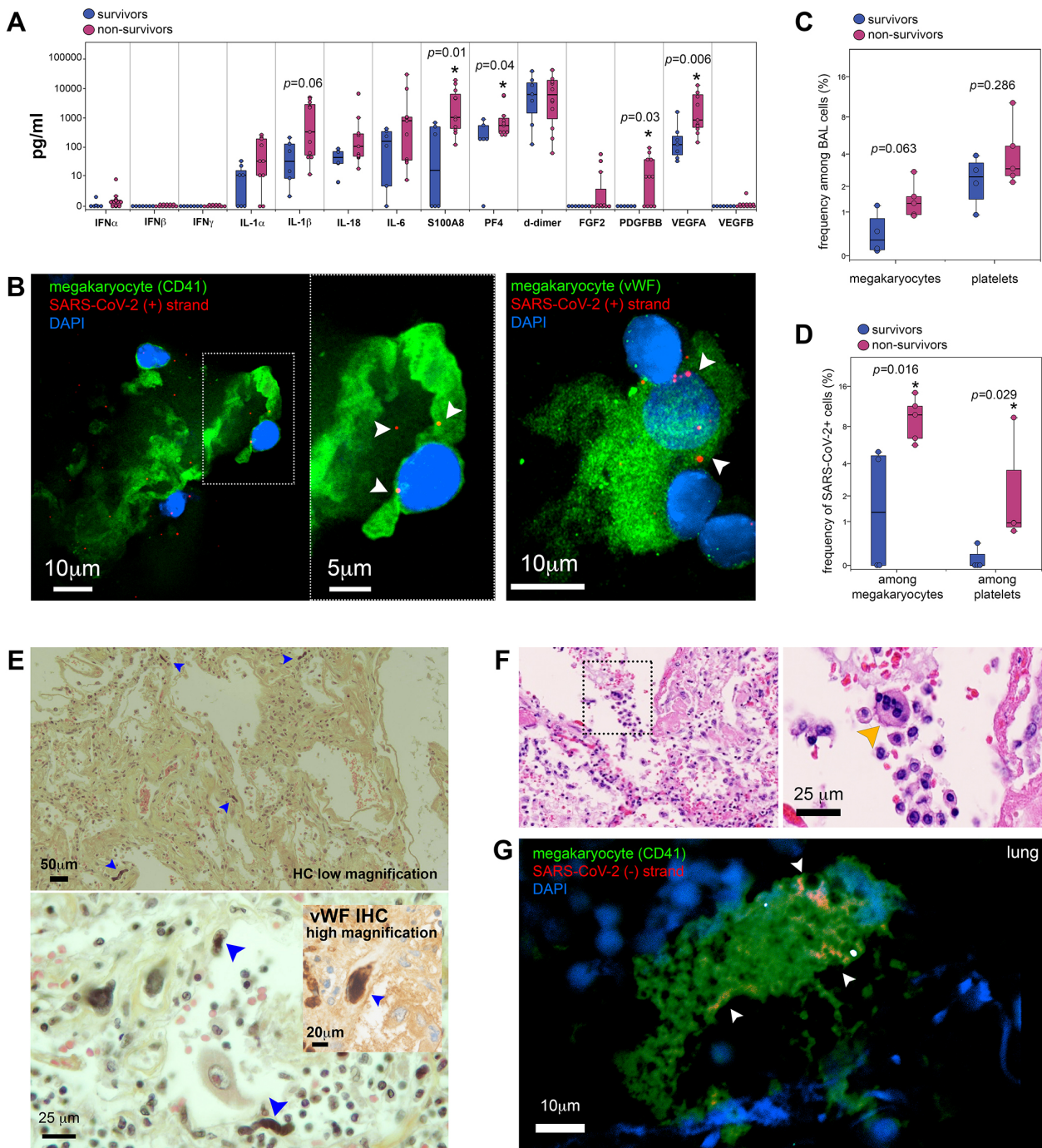
**Figure 2: In COVID-19 non-survivors, MKs are infected and express viral sensing genes.**

A) Frequency of MKs detected among PBMC from healthy donors and COVID-19 survivors and non-survivors as assessed by flow cytometry. Asterisks indicate statistical significance (Kruskal-Wallis test).

B-F) Transcriptional identity of MKs in COVID-19 patients by single-cell RNA sequencing reveals distinct phenotypes in non-survivor patients. B) UMAP of single-cell transcriptomic data of MKs detected among PBMC from non-COVID-19 healthy donors (n=30), COVID-19 survivors (n=140) and COVID-19 non-survivors (n=13). Unsupervised clustering detected 9 different clusters (0 to 8) of all cells analyzed. MK singlets are indicated by blue region. C) Proportion of each cluster in healthy donors, COVID-19 survivors and non-survivors. D) Scored gene signature expression of classical (upper) and non-classical (bottom) MK differentiation. E) Fraction of cells from cluster 3 in comparison to all other clusters in individual patient samples categorized as healthy donors, COVID-19 survivors and non-survivors. F) Heatmap of the genes that significantly change along pseudotime trajectory of MK development ( $p < 0.05$  and Morans I score  $> 0.25$ ).

G) Hematoxylin/eosin histology of bone marrow tissue obtained after COVID-19 non-survivor autopsy (low magnification (bar=50 $\mu$ m)) in which some MKs surrounded by blue circles are shown in high magnification insets (bar=20 $\mu$ m). Arrowheads indicate MKs.

H) Confocal microscopy images after CD41 (green) immunolabelling and replicative SARS-CoV-2 (-) RNA strand *in situ* hybridization (red) in bone marrow samples obtained from COVID-19 non-survivors autopsies (bar=10 $\mu$ m). Arrowheads indicate SARS-CoV-2 (-) RNA inside.



**Figure 3: SARS-CoV-2 in platelets and MKs in lung from non-survivor COVID-19 patients.**

A) Quantification of different cytokines/chemokines in bronchoalveolar lavage samples from COVID-19 survivors (blue) and non-survivors (magenta). Asterisk indicates statistical significance in the comparison between survivors and non-survivors (Mann-Whitney).

B) Confocal microscopy images after SARS-CoV-2 RNA *in situ* hybridization (red) for positive (+) RNA strand and immunolabelling of either CD41 (upper row) or vWF (lower row), both in green, in bronchoalveolar lavage samples from two different COVID-19 non-survivors (bar=10 $\mu$ m or 5 $\mu$ m for the inset in upper image). Arrowheads indicate SARS-CoV-2 RNA inside MKs.

C) Frequency of MKs and platelets among the cell population detected in bronchoalveolar lavage samples from COVID-19 survivors (blue) and non-survivors (magenta) detected by flow cytometry.

D) Frequency of SARS-CoV-2<sup>+</sup> MKs and platelets among the population of MKs and platelets detected in bronchoalveolar lavage samples from COVID-19 survivors (blue) and non-survivors (magenta) as detected

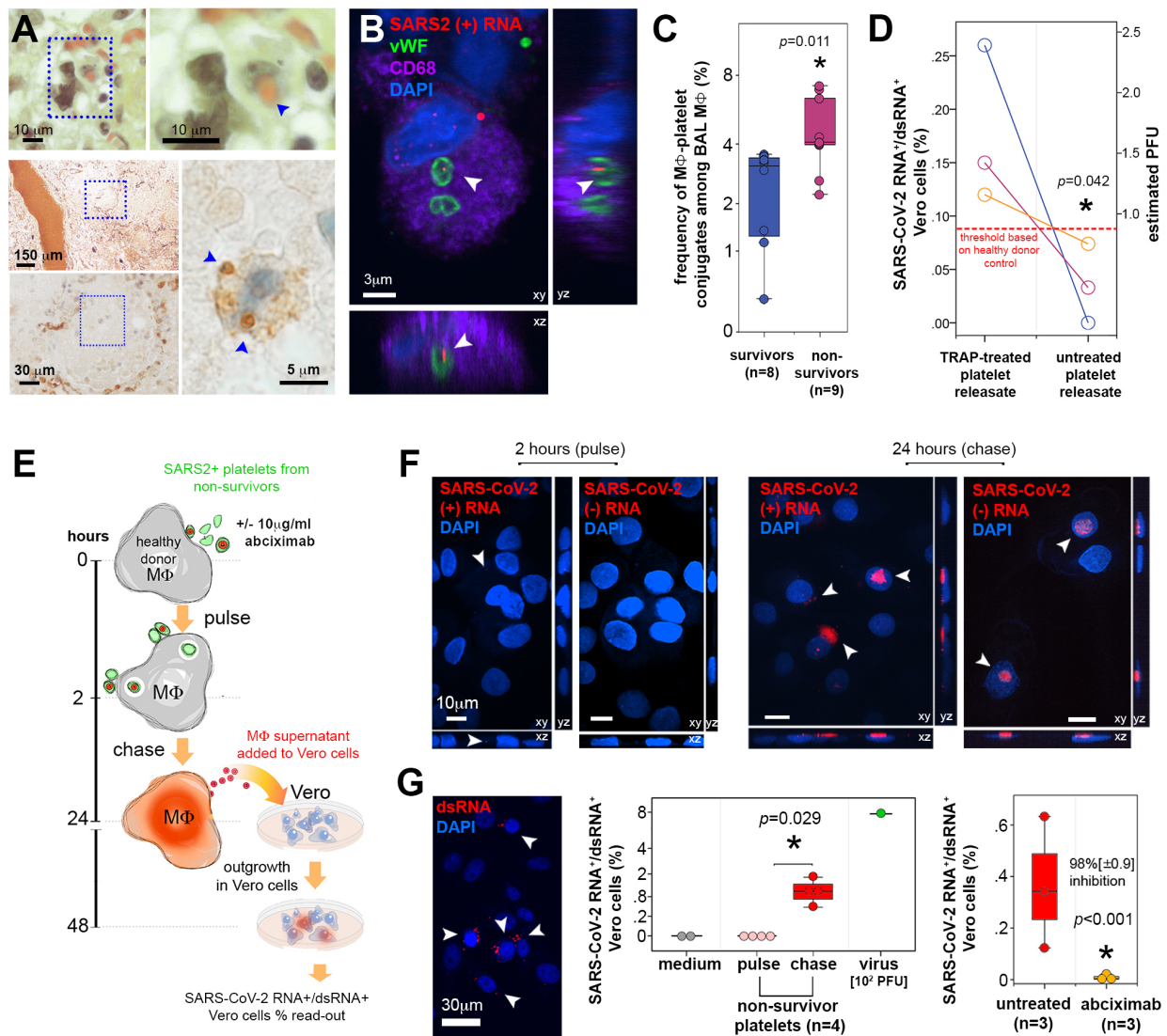


by flow cytometry. Asterisk indicates statistical significance in the comparison between survivors and non-survivors (Mann-Whitney).

E) Hematoxylin/eosin/saffron histology and vWF immunohistochemistry (lower inset) of lung tissue from COVID-19 autopsy, showing low (bar=50 $\mu$ m) and high magnification (bar=20 and 25  $\mu$ m) images. Blue arrowheads indicate MKs.

F) Hematoxylin/eosin histology of lung tissue in which some MKs indicated by dotted square region (left column), resided to reside inside alveolar space (right column) (bar=25  $\mu$ m). Orange arrowheads indicate MKs.

G) Confocal microscopy images after CD41 (green) immunolabelling and replicative SARS-CoV-2 (-) RNA strand *in situ* hybridization (red) in lung samples obtained from COVID-19 non-survivors autopsies (bar=10 $\mu$ m). Arrowheads indicate SARS-CoV-2 (-) RNA inside MKs.



**Figure 4: SARS-CoV-2 sheltered by platelets from non-survivor patients with COVID-19 is infectious to macrophages.**

A) Upper row: Hematoxylin/eosin/saffron stain histology of lung tissue from a COVID-19 autopsy showing a macrophage, indicated by dotted square region (left) and shown in higher magnification (right) phagocytosing a red blood cell (blue arrowhead). Bar=10 $\mu$ m. Lower panel: Immunohistochemistry for vWF of alveoli from non-survivor lung autopsy (indicated by blue dotted square at low magnification, bar=150 $\mu$ m) where a macrophage (indicated by blue dotted square at middle magnification, bar=30 $\mu$ m) contained hemophagocytosed vWF<sup>+</sup> platelets (blue arrowheads in high magnification image, bar=5 $\mu$ m).

B) Representative confocal microscopy images after vWF (green) and CD68 (purple) immunolabelling and SARS-CoV-2 RNA *in situ* hybridization (red) for positive (+) RNA strand in bronchoalveolar lavage samples from COVID-19 non-survivors. Images show three-dimensional projections (xy, xz and yz, bar=3 $\mu$ m). Arrowheads indicate SARS-CoV-2 RNA inside platelets engulfed by macrophages (representative of n=3 different individuals).

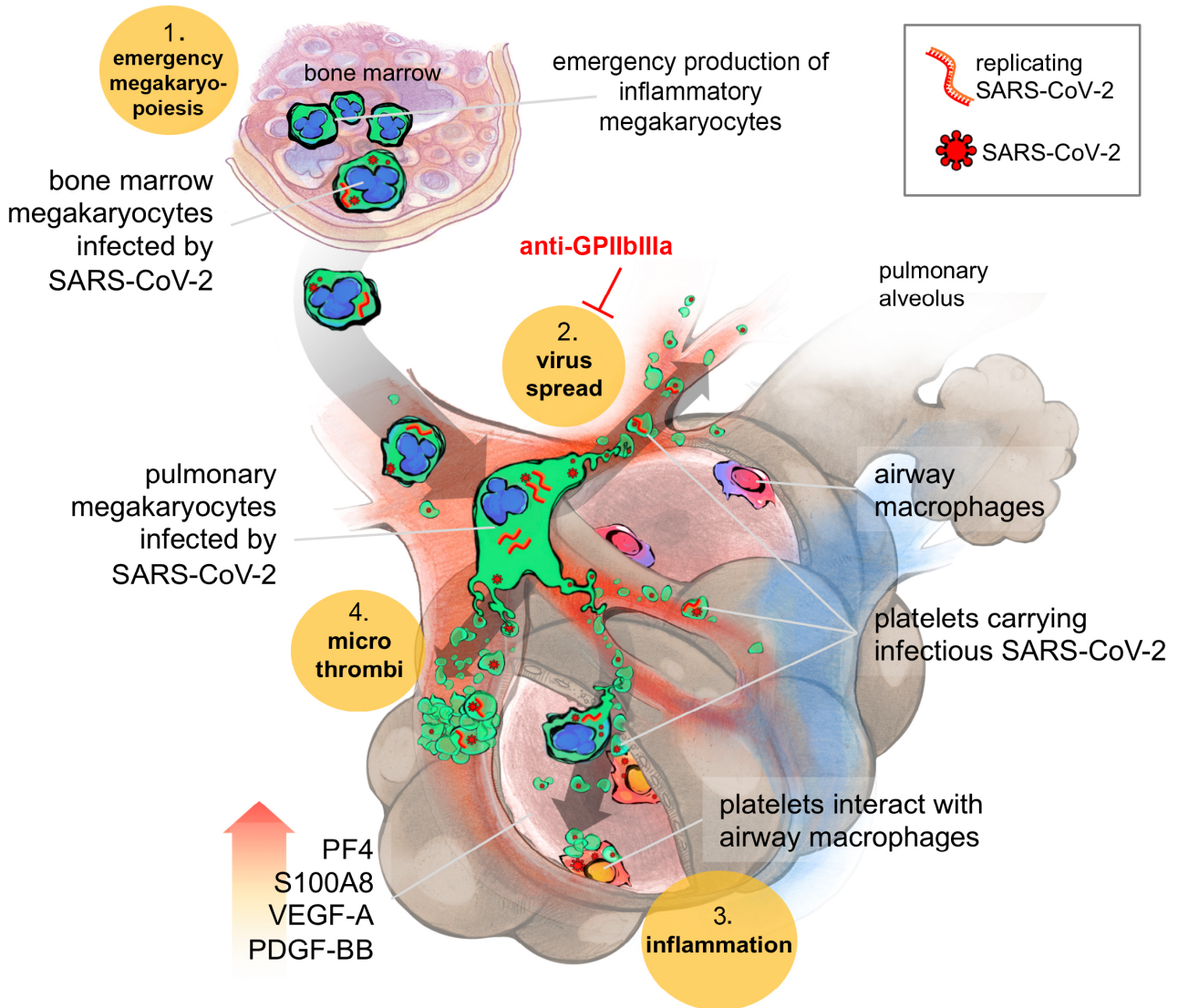
C) Frequency of macrophage-platelet conjugates among macrophages in bronchoalveolar lavage samples from COVID-19 survivors (blue) and non-survivors (magenta) detected by flow cytometry. Asterisk indicates statistical significance in the comparison between survivors and non-survivors (Mann-Whitney).

D) Paired comparison of percentages of SARS-CoV-2 RNA<sup>+</sup>/dsRNA<sup>+</sup> Vero cells treated with releasate from platelets treated or not with TRAP, from 3 different non-survivors. The detection threshold (dotted red line) was established with healthy donor platelets treated equally. The percentages were converted in PFU per million platelets using the standard curve established. Mann-Whitney test. The estimated mean PFU per million platelets is shown in blue, with 95% confidence intervals.

E) Scheme of the experiments evaluating platelet-mediated SARS-CoV-2 trans-infection of macrophages *in vitro*. SARS-CoV-2 -containing platelets from non-survivors interacted with macrophages in the presence or absence of abciximab (anti-GpIIb/IIIa) for 2 hours (pulse) followed by 24-hour chase. At these time-points, macrophages positive for (+) and (-) SARS-CoV-2 RNA were assessed by *in situ* hybridization and macrophage supernatants were collected and further evaluated for infectious virus content in reporter Vero cells. SARS-CoV-2 RNA<sup>+</sup>/dsRNA<sup>+</sup> Vero cells were assessed by *in situ* hybridization and quantified by FISH-flow.

F) Confocal microscopy images of SARS-CoV-2 RNA *in situ* hybridization (red) for positive (+) and negative (-) strand RNA in macrophages that interacted *in vitro* with platelets samples from COVID-19 non-survivors. Images were acquired after 2 hours of interaction with platelets (pulse, left) or 24 hours after interaction (chase, right). Images show three-dimensional projections (xy, xz and yz, bar=10µm). Arrowheads indicate SARS-CoV-2 RNA. Macrophage nuclei are stained with DAPI (blue).

G) Outgrowth in Vero cells of SARS-CoV-2 produced by macrophages after platelet-mediated infection. On the left, confocal microscopy image of double-strand RNA (dsRNA, red) in Vero cells cultivated with macrophage supernatants for 24 hours. Arrowheads indicate infected Vero cells (bar=30µm), Vero cells nuclei are stained with DAPI (blue). On the middle graph, the number of infected Vero cells were quantified by FISH-flow and expressed as % of SARS-CoV-2 RNA<sup>+</sup>/dsRNA<sup>+</sup> Vero cells, comparing negative controls (medium, gray=non-infected Vero), positive controls (virus, green=primary SARS-CoV-2 obtained from patient bronchoalveolar lavage) and COVID-19 non-survivors platelets after pulse (2 hours interaction with macrophages, light red) and chase (24 hours after interaction with macrophages, red). Asterisk indicates statistical significance in the comparison between pulse and chase (Mann-Whitney). The right graph shows the outgrowth of SARS-CoV-2 in Vero cells incubated with macrophages that interacted with COVID-19 non-survivors' platelets in the presence or not of abciximab (10µg/ml) also quantified by FISH-Flow. Results are expressed as % of SARS-CoV-2 RNA<sup>+</sup>/dsRNA<sup>+</sup> Vero cells in the two conditions. The % inhibition of Vero cell infection in the presence of abciximab is indicated with asterisk corresponding to statistical significance in the comparison between the two groups (Student T-test).



**Figure 5: Scheme: Platelets harboring SARS-CoV-2 offer a convergent therapeutical target in severe COVID-19 with multiple manifestations.**

1- SARS-CoV-2 favors emergency inflammatory megakaryopoiesis. SARS-CoV-2 infected MKs in the bone marrow (containing both viruses and replicating SARS-CoV-2 (-) RNA) migrate to the lungs where they contribute to thrombopoiesis, and produce SARS-CoV-2-containing platelets in the pulmonary circulation.

2- These infectious platelets will then spread the virus and contribute to the systemic inflammatory component of severe COVID-19. As platelets sheltering SARS-CoV-2 are coated with Von Willebrand Factor, indicating their highly activated status, they will also contribute to thrombus formation typical of COVID-19 complications.

3- Increase in lung VEGF-A and PDGF-BB participates to alveolar endothelial destruction and effraction allowing for platelets carrying SARS-CoV-2 to reach and infect alveolar macrophages.

4- Increased lung PF4 released by platelets and S100A8 likely contributes to the maintenance of a highly inflammatory environment, macrophage activation, and cytokine storm.

These three platelet-mediated components of severe COVID-19 suggest that targeting platelets, with the use of anti-platelet drugs like anti-GPIIb/IIIa, might be an efficient strategy to block viral spread, thrombus formation and exacerbated inflammation at once, increasing the chance of survival.

## Tables

**Table 1: Patient characteristics according to the hospital outcome (PRP samples)**

Median [IQR]; N(%)	Survivors, n=27	Non-survivors, n=25	p-value
<b>Patients</b>			
Age, years	61.6 [52.0;76.2]	72.7 [68.4;87.9]	<b>0.009</b>
Male sex	17 (63%)	16 (64%)	1.00
At least one comorbidity	22 (81%)	23 (92%)	0.42
Obesity	6 (19%)	2 (8%)	0.27
High Blood pressure	8 (30%)	10 (40%)	0.56
Cardiovascular disease	7 (26%)	8 (32%)	0.76
Diabetes	6 (22%)	10 (40%)	0.23
Active malignancy	5 (19%)	5 (20%)	1.00
Chronic renal failure	1 (4%)	2 (8%)	0.60
Chronic respiratory failure	4 (15%)	3 (12%)	1.00
<b>At Hospital admission</b>			
Days since first symptoms	10 [6.5;25.5]	8 [5;12.5]	0.23
Need of oxygen supply	2 (7%)	5 (20%)	0.24
O2 saturation, %	91 [88;98]	91.5 [82;95.75]	0.40
ICU admission	11 (41%)	12 (48%)	0.78
IGSII*	32 [30;39]	45 [34.25;57]	0.16
SOFA*	4 [3; 5]	4 [3.75; 8.75]	0.49
<b>At Sampling time</b>			
Days since first symptoms	10 [6;26]	7 [5;12.5]	0.22
WBC count, Giga/L	7.4 [5.3;9.65]	9.2 [6.4;13.8]	0.26
Platelet count, Giga/L	238 [138.5; 334]	189 [160;255]	0.40
Fibrinogen in plasma, g/L	6.5 [5.175;7.525]	6.6 [4.9;8.1]	0.72
D-dimer in plasma, mg/L	4.4 [2.5;5.6]	3.5 [1.2;4.3]	0.091
vWF in plasma, µg/ml**	9.6 [7.448;14.35]	18.0 [12.56;27.33]	<b>0.015</b>
%vWF <sup>+</sup> platelets**	5.9 [3.6;7.8]	14.5 [8.6;19.5]	<b>0.001</b>
SARS-CoV-2 in platelets	1 (4%)	19 (76%)	<b>&lt;0.0001</b>
<b>At Hospital discharge</b>			
Days from first symptoms to discharge	25 [18;72]	15 [12.5;28]	<b>0.01</b>
Days from sampling to discharge	13 [10;44]	7 [5;10]	<b>0.001</b>

\*only available in those patients admitted to ICU (11 survivors and 12 deceased patients)

\*\* only measured in 16 survivors and 14 non-survivor patients

**Table 2: Patient characteristics according to the hospital outcome (autopsy samples)**

Patient	Sex	Age	Sample Origin	ICU days	Days until demise	BMI index	Cardio Vascular	Pre-existing condition	Treatment	Cause of death	Lung sampling	Bone Marrow sampling
A1	F	82	France	11	17	24.1	0	N.A.	N.A.	Multivisceral failure	Yes	Yes
A2	M	51	France	10	20	34.5	AHT, AVB1	Small airways obstruction/ Leukemia	AINS	Pulmonary embolism	Yes	No
A3	F	59	France	13	18	28.4	AHT	Asthma	Corticoids	Pulmonary embolism	Yes	Yes
A4	M	51	France	11	19	38	AHT	Small airways obstruction/ Arthritis	N.A.	Massive Pulmonary embolism	Yes	Yes
A5	M	71	France	0	N.A.	N.A.	Low grade coronary illness	Marked hepatic steatosis	N.A.	major pulmonary edema	Yes	No

Abbreviations: COVID-19: Coronavirus disease 19; LC: Lung Carcinoma; TB: tuberculosis; N.A.: not applicable; N/R: not register; F: Female; M: Male; AINS: non-steroidal anti-inflammatory drugs. AHT: Arterial Hypertension), AVB1: Atrioventricular block level 1.

**Table 3: Patient characteristics according to the hospital outcome (BAL samples)**

Median [IQR]; N(%)	Survivors, n=6	Non-survivors, n=13	p-value
<b>Patients</b>			
Age, years	54 [42;66]	69 [63;77]	<b>0.046</b>
Male sex	5 out of 6 (83%)	7 out of 13 (53%)	0.33
At least one comorbidity*	5 out of 6 (83%)	5 out of 11 (45%)	0.62
Obesity*	1 out of 6 (16%)	1 out of 11 (9%)	1.00
Cardiovascular disease*	2 out of 6 (33%)	4 out of 11 (36%)	1.00
Diabetes*	2 out of 6 (33%)	2 out of 11 (18%)	0.58
<b>At Hospital admission</b>			
Days since first symptoms*	9 [6;9]	4.5 [3;7]	0.49
PPP	1 [1;4.5]	1 [1;1.5]	0.83
ICU admission	6 out of 6 (100%)	13 out of 13 (100%)	-
Need of oxygen supply at ICU admission*	5 out of 6 (83%)	10 out of 12 (83%)	1.00
<b>At Sampling time</b>			
SARS-CoV-2 in BAL fluid	4 out 6 (66%)	6 out 13 (46%)	0.62
<b>At Hospital discharge</b>			
Days from first symptoms to discharge*	46.5 [38.5;66]	41.5 [23;51.5]	0.57
Days from sampling to discharge*	21 [20;74]	25 [12;27]	0.89

\*only available indicated number of patients