Supplemental Material

Extended Methods

Human blood Collection

Blood from 10 STEMI, 10 NSTEMI, and 17 COVID-19 patients (Online Table I) was collected by phlebotomy into ACD Solution A (yellow top) tubes (Fisher Scientific, USA, cat#02-684-26). Blood from healthy donors was drawn only from people who were not on any medication for at least 7 days prior to blood draw. Blood from COVID-19 patients was drawn from adults who presented to UMass Memorial Medical Center from April to November of 2020 and tested positive for SARS-CoV-2 by RT-qPCR in the preceding 2 weeks (with the exception of one donor who tested positive 17 days before collection). Blood from STEMI or NSTEMI patients was drawn at the time of cardiac catheterization from April to September of 2020 from patients who presented to the UMass Memorial Medical Center. All procedures were approved by the University of Massachusetts Institutional Review Board (protocol #H00009277, 14268-10, H00017901) and participants signed informed consent as required by the IRB.

Platelet isolation

Platelets were isolated from venous blood that had been drawn into yellow top ACD Solution A tubes. Briefly, blood was centrifuged at 150 x g for 17 min at room temperature. Two-thirds of the platelet rich plasma was diluted with platelet wash buffer (10 mM sodium citrate, 150 mM sodium chloride, 1 mM EDTA, 1% (w/v) Dextrose, supplemented with 100 ng/mL PGE1), and centrifuged at 460 x g for 17 min at room temperature. The pellet was resuspended in HEPES-modified Tyrode's buffer (140 mM NaCl, 6.1 mM KCl, 2.4 mM $MgSO_4 \cdot 7H_2O$, 1.7 mM Na_2HPO_4, 5.8 mM Sodium HEPES, supplemented with 0.35% BSA and 0.1% Dextrose). Platelet number was determined by means of a blood cell analyzer (Beckman Coulter Ac.T8, CA, USA). Contamination of the platelet preparation was found to be <1 in 50,000.

Leukocyte isolation

The red blood cell fraction after complete removal of the platelet rich plasma (for platelet isolation) was lysed with 5 mL of RBC Lysis Buffer by Roche Diagnostics (Fisher Scientific, cat# 50-100-3296) that had been pre-warmed to 37 °C. The sample was gently mixed and lysed at 37 °C in a water bath for 10 min. Lysed cells were centrifuged at 350 × g for 5 min, low brake. The lysed solution was removed and the pellet was washed in 10 mL of 1× PBS at 350 × g for 5 min, low brake. The sample was resuspended in 1 mL 1X PBS and lysed once again with 1 mL of Lysis Buffer. The sample was topped off with 1X PBS and centrifuged at 250 × g for 5 min, low brake. The pellet was saved in Qiazol for sequencing.

Plasma isolation (double centrifugation)

1300 µL of ACD-blood from each patient was centrifuged at 500 × g for 10 min. The supernatant was removed, centrifuged at 2000 × g for 10 min and the plasma was immediately frozen at -80 °C.

Plasma RNA Isolation for SARS-CoV-2 Sequencing

Plasma (140 µL) was thawed and centrifuged at 6800 x g for 3 min to remove any cryoprecipitates. Viral RNA (vRNA) was isolated with the QIAamp® vRNA Mini Kit (Qiagen, Germany, cat#52904). vRNA was eluted in 60 µL of RNase-free water by eluting two times in 30 µL each.

Platelet or leukocyte RNA Isolation for Sequencing or RT-qPCR

Isolated platelets or leukocytes were lysed in 700 µL QIAzol® (included in miRNeasy Mini Kits—see manufacturer information below) and frozen at -80 °C. RNA isolation was performed as we have previously done. Briefly, frozen platelets in QIAzol® were thawed at room temperature for 60 min at 2000 rpm on an Eppendorf MixMate plate shaker (Eppendorf, Germany, cat#022674200). Leukocytes at $1x10^7$ in final volume of 700 uL were used for RNA isolation. Using the miRNeasy® Mini Kit (Qiagen, cat#217004), RNA was isolated following the manufacturer's instructions. To eliminate any presence of DNA, on-column DNA digestion was utilized with the RNase-Free DNase Set (Qiagen, cat#79254). RNA was eluted in 30 µL of RNase-free water. RNA concentration was determined via Fragment Analysis by the Molecular Biology Core Lab at the University of Massachusetts Medical School. The same platelet RNA was used to detect the SARS-CoV-2 genome by Artic V3 Sequencing (discussed below).

SARS-CoV-2 vRNA isolation from cell culture

SARS-CoV-2 vRNA was isolated from the supernatant of cell culture in TRIzol LS reagent following the manufacturer's instructions (ThermoFisher Scientific, cat#10296010). vRNA was dissolved in 50 µL of RNase-free water. vRNA was used for the control sequencing in Fig 1.

Sequencing of platelet RNA, platelet and leukocyte lncRNA

The RNA samples isolated from platelets were used to create libraries with the AmpliSeq Transcriptome Human Gene Expression Kit (ThermoFisher, CA, USA, cat#A26327) according to the manufacturer's protocol. Briefly, 10 ng of total platelet RNA was reverse transcribed, and complementary DNA (cDNA) was amplified for 12 cycles by adding PCR Master Mix and the AmpliSeq human transcriptome gene expression primer pool targeting 18,574 protein-coding messenger RNAs. Amplicons were digested with FuPa enzyme, then barcoded adapters were ligated onto the target amplicons. The library amplicons were bound to magnetic beads and residual reaction components were washed off. Libraries were eluted and individually quantitated by an Agilent Bioanalyzer (Agilent, CA, USA). Individual libraries were diluted to a 100 pM concentration, then combined in batches for RNA sequencing. Emulsion PCR, templating, and PI chip loading was performed with an Ion Chef Instrument (ThermoFisher). Sequencing was performed on an Ion Proton sequencer (ThermoFisher), with Ion PI Hi-Q sequencing chemistry.

Detection of SARS-CoV-2 genome by Artic V3 Sequencing

Platelet and plasma RNA were sequenced using the most current set of primers available which enrich for SARS-CoV-2 viral reads among the rest of the platelet RNA, using a tiled amplicon approach. The sequencing was performed by the Microbial Genomic Sequencing Center (MiGS), Pittsburg, PA, that specializes in SARS-CoV-2 sequencing.

Detection of SARS-CoV-2 RNA, ACE2 and TMPRSS2 by RT-qPCR

Platelet cDNA was synthesized using the High Capacity cDNA RT Kit (Applied Biosystems, CA, USA, cat#4368813) in a 10 µL reaction volume (10X Reverse Transcription Buffer [1 µL], 25X dNTPs [0.4 µL], 10X Random Primers [1 µL], and Multiscribe Reverse Transcriptase, 50 U/L [0.5 µL]). 7.1 µL of RNA was used. cDNA synthesis was performed on a thermal cycler (Applied Biosystems, ProFlex) under the following conditions: 25 °C for 10 min, 37 °C for 2 h, 4 °C hold.

cDNA was preamplified using TaqMan™ PreAmp Master Mix (Applied Biosystems, cat#4391128) in a 5 µL reaction volume (Master Mix [2.5 µL], 0.2X assay pool [1.25 µL]) with 1.25 µL of cDNA. Preamplification was performed on a thermal cycler as listed above, under the following conditions: 95 °C for 10 min, 14 cycles of 95 °C for 15 s and 60 °C for 4 min, 4 °C hold. The final preamplification product was diluted 1:9 with DNA Suspension Buffer (Teknova, CA, USA, cat#T0223), prior to qPCR.

SARS-CoV-2, ACE2, and TMPRSS2 gene expression in humans was quantified by RT-qPCR (ThermoFisher, QuantStudio3 Fast Real-Time PCR systems) using TaqMan™ Gene Expression Master Mix (Applied Biosystems, cat#4369016) and Gene Expression Assays (Online Table II) in a 10 µL reaction volume (Master Mix [5.0 µL], TaqMan™ Assay [0.5 µL], diluted pre-amplification product [4.5 µL]) under the following conditions: 50 °C for 2 min, 95 °C for 10 min, and 40 or 50 cycles of 95 °C for 15 min and 60 °C for 1 min.

Detection of transcripts related to programmed cell death by RT-qPCR in platelets

Platelet complementary DNA (cDNA) was synthesized using the High Capacity cDNA RT Kit (Applied Biosystems, CA, USA, cat#4368813) in a 10 µL reaction volume (10X Reverse Transcription Buffer [1 µL], 25X dNTPs [0.4 µL], 10X Random Primers [1 µL], and Multiscribe Reverse Transcriptase, 50 U/L [0.5 µL]). 7.1 µL of RNA was used. cDNA synthesis was performed on a thermal cycler (Applied Biosystems, ProFlex) under the following conditions: 25 °C for 10 min, 37 °C for 2 h, 4 °C hold.

cDNA was preamplified using TaqMan™ PreAmp Master Mix (Applied Biosystems, cat#4391128) in a 5 µL reaction volume (Master Mix [2.5 µL], 0.2X assay pool [1.25 µL]) with 1.25 µL of cDNA. Preamplification was performed on a thermal cycler as listed above, under the following conditions: 95 °C for 10 min, 14 cycles of 95 °C for 15 s and 60 °C for 4 min, 4 °C hold. The final preamplification product was diluted 1:9 with DNA Suspension Buffer (Teknova, CA, USA, cat#T0223). Transcripts related to programmed cell death (Online Table VI) were screened using RNA from patients by RT-qPCR using Dynamic Array 48.48 chip (Fluidigm Corp).

Measurements of plasma proteins by Olink Technology

Citrated plasma (double spun) was diluted according to Manufacturer's instructions and proteins were resolved using Antibody-qPCR method and the Immuno-Oncology panel (Olink Proteomics, Sweden). The Olink platform employs a quantitative polymerase chain reaction (PCR) technology called "Proximity Extension Assay" (PEA) for protein quantification (Olink, Uppsala, Sweden) according to the manufacturer's instructions. The primary output from the Olink method provides Cq values (quantification cycle number) corresponding to the amplicons that are specific for each protein. These raw Cq values are then imported to the Olink NPX Manager Software v2.2.2.322 and Normalized Protein Expression (NPX) values are calculated by normalizing the Cq values to inter-plate controls (included in triplicate in each run).

SARS-CoV-2 propagation and PEG purification

Infectious SARS-CoV-2 (NIAID/ATCC, VA, USA, USA-WA1/2020 Strain, BEI #NR-52281) was propagated by infecting Vero E6 (ATCC, cat#CRL-1586) cells at low multiplicity of infection (MOI) and then maintaining the infected cells in Eagle's Minimal Essential Medium (EMEM, Millipore Sigma, MO, USA) with 2% FBS (Atlas Biologicals, CO, USA). Cell-free virus supernatant was harvested when <70% cytopathic effects were evident and concentrated using the PEG Virus Purification kit (Abcam, MA, USA, cat#ab102538) according to the manufacturer's protocol. Briefly, cell-free virus supernatant was incubated overnight at 40 °C with PEG provided as a 5X solution in the kit, and the virus pellet obtained after centrifugation at 40 °C was resuspended in Virus Resuspension solution also provided in the kit. Aliquots of concentrated virus were stored at -80 °C, titered and used for infection of human donor platelets at 0.1 MOI (or 10 platelets to 1 infectious virus). All work with live SARS-CoV-2 was performed in a biosafety level 3 laboratory facility. Of note, no coagulation or aggregation was observed in the in vitro assays or in the TEM, suggesting reasonable viral purification. Control platelets were treated with the same volume of Virus Resuspension solution in which the virus was resuspended.

Confocal Microscopy and Antibodies

Whole blood from COVID-19 or STEMI/NSTEMI patients or healthy donors was lysed and fixed with 1X BD FACS™ lysing solution (BD Biosciences, CA, USA, cat #349202) for 10 min. The pellet was centrifuged at 2000 x g for 10 min and washed with 1X PBS. Samples were resuspended in 100 µL of HEPES-modified Tyrode's buffer supplemented with 2% FBS and blocked for 1 h at room temperature, then antibodies were added for an additional hour. At the end of incubation, samples were washed with 1 mL of 1X PBS and mounted on slides. To eliminate the effect of opsonization-mediated interactions, all microscopy staining was performed after fixation post-treatment. Mounted slides were resolved by fluorescent microscopy using a Spinning Disk Confocal Nikon TE2000E2 inverted microscope or Scanning Disk Nikon A1 confocal microscope. Images, in each figure, are representative of the patients listed and pictures are visual average of the tested phenomenon.

The following antibodies were used throughout this study: anti-human: CD41-FITC, CD41-APC, or CD41-SB436 (clone HIP8, eBioscience, CA, USA, cat#11-0419, cat#17-0419, cat#62-0419-42); ACE2-AF647 (Novus Biologicals, CO, USA, cat#NBP2-72117AF647); TMPRSS2-AF488 (Santa Cruz Biotechnology, Inc., TX, USA, cat#sc-515727); SARS-CoV-2-Spike1 AF488 (R&D Systems, MN, USA, cat#FAB105403G); SARS-CoV-2 Nucleocapsid AF647 (Novus Bio, cat#NBP2-90967AF647); SARS-CoV-2 Spike Protein RBD Recombinant Human Monoclonal Antibody (P05DHu), Alexa Fluor 488 (eBioscience, CA, USA, cat# 53-6490-82) ; Caspase-3 DyLight405 (Novus Biologicals, cat#NB100-56708V); phospho-MLKL DyLight 405 (Novus Biologicals, cat#MAB91871V). of note, all antibody staining done throughout this manuscript is after fixation. After fixation of platelets multiple isotype controls are not appropriate for low expressed proteins as they can interfere with each other leading to misinterpretation of results. Instead, we utilized negative biological controls as well as single antibody and no stain controls. Spike and N1 were adjusted above the negative controls of healthy individuals and healthy individuals were adjusted above the no stain control of platelets from COVID-19 patients. Links to all antibodies are provided in the Major Resource Table in Online data.

A549$^{ACE2+TMPRSS2}$ clonal cell line generation

A549 cells (ATCC, cat#CCl-185) were cultured in 1X DMEM medium (Gibco, ThermoFisher Scientific, cat#11965-092) supplemented with 10% Fetal bovine serum (Gibco, cat#10437-028), 1x MEM NEAA (Gibco, cat#11140-050), 100U/mL of penicillin-streptomycin (Corning, MA, USA, cat#30–002-CI), 1X Sodium pyruvate (Gibco, cat# 11360-070), and 1X Glutamax (Gibco, cat#35050-061) at 37 ºC and 5% CO_2. Cells were fed with fresh media every 2-3 days, and routinely checked for mycoplasma contamination. Lentivirus for ACE2 and TMPRSS2 was produced in HEK293T/17 (ATCC, cat#CRL-11268) with 3rd generation lentiviral packaging plasmids pHDM-G, pHDM-Hgpm2, pHDM-tat1b, and pRC/CMV-rev1b (all packaging plasmids are from Dr. Rene Maher's lab) using TransIT-293 transfection reagent (Mirus, WI, USA, cat#2700) according to the manufacturer's recommendations. After 48h of transfection, viral supernatant was collected, filtered, and stored at -80ºC for later use. The A549$^{ACE2+TMPRSS2}$ clonal cell line was generated by transducing lentivirus expressing ACE2 and TMPRSS2 genes into the parental A549 cells. Transduced cells were selected with 1 µg/mL puromycin (Invitrogen, MA, USA, cat#A1113803) and screened for high SARS-CoV-2 infection clone as a result of the expression of ACE2 and TMPRSS2. In this manuscript A549$^{ACE2+TMPRSS2}$ clonal cell line was used as a control to account for the specificity of the antibodies used to detect ACE2 and TMPRSS2 in platelets.

Western Blot Analysis

Washed platelets from COVID-19 patients or from non-infected controls, were sex and age matched. Platelets were lysed in RIPA buffer (20mM Tris-HCl, 24.1 mM sodium deoxycholate, 150 mM sodium

chloride, 0.5 mM EGTA, 1% Triton X100; or purchased from Millipore Sigma, MO, USA, cat# R0278) supplemented with protease inhibitors (Roche, Switzerland, Complete™, cat#11697498001 or Complete™ Mini, cat#11836153001,) and phosphatase inhibitors (Roche, PhosStop™, cat#4906845001). Protein (20-56 μ) was separated using Mini PROTEAN Tris-Glycine eXtended Stain-Free Gels, 4-20% (BioRad, CA, USA, cat# 4568093) and probed with the following primary anti-human antibodies: Caspase-1 (Novus Biologicals, cat#NBP1-45433), Caspase-3 (clone 31A1067, Novus Biologicals, cat#NB100-56708), Caspase-11 (clone 17D9, Novus Biologicals, cat#NB-120-10454), MLKL [pThr357] (clone 9547241, Novus Biologicals, cat#MAB91871), MLKL (clone 3B2, Novus Biologicals, cat#H00197259-M02), GSDMDC1 (Novus Biologicals, cat#NBP2-33422), cleaved N-Terminal GSDMD [EPR20829-408] (Abcam, cat#ab215203), Actin (C-2, Santa Cruz Biotechnology, Inc., cat#sc-8432). The following secondary antibodies were used (Santa Cruz Biotechnology, Inc.): mouse anti-rabbit IgG-HRP (cat#sc-2357), goat anti-mouse IgG-HRP (cat#sc-2005), chicken anti-rat IgG-HRP (cat#sc-2956). ACE2 and TMPRSS2 detection was performed in washed platelets using the following primary anti-human antibodies: ACE2 (Abcam, cat#15348); TMPRSS2 (Abcam, cat#92323); tubulin (Cell Signaling Technology, MA, USA cat#3873S), and secondary antibodies (GE Healthcare, IL, USA). Human brain lysate is commercially available (Novus, cat#NB820-59177).

Transmission Electron Microscopy of COVID-19 platelets and platelets incubated with SARS-CoV-2

Platelets from COVID-19 patients and non-infected donors: Isolated washed platelets were resuspended in HEPES-modified Tyrode's buffer. 300 μL of platelet solution was fixed with Karnovsky's fixative (a mixture of 2.0% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M Sorensen's phosphate buffer, pH 7.4) for 10 min at 37 °C and constant rotation of 1000 rpm in 7.25 x 55mm Siliconized Micro Test Tubes (BioDataCorp, PA, USA, cat#101521). Platelets were then processed for TEM.

Platelets incubated with SARS-CoV-2 in vitro: Isolated platelets were incubated with purified infectious virus in a proportion of 1 virion to 10 platelets for various lengths of time at $2x10^5$ platelets/μL of HEPES-modified Tyrode's buffer, at constant rotation of 1000 rpm, in 7.25 x 55mm Siliconized Micro Test Tubes (BioDataCorp) with a stir bar (BioDataCorp, cat#105990) at the bottom at 37 °C. In the cases where ACE2-inhibitor DX600 (Cayman Chemical, MI, USA, cat#22186, dissolved in PBS) was used, platelets were pretreated for 10 min before the virus was added. After incubation, the solution was brought to 500 μL with 1X PBS. Immediately after, platelets were fixed with 500 μL Karnovsky's fixative for 10 min at 37 °C and left in the fixative for an hour before processing. TEM of fixed platelets was resolved by a Philips CM10 electron microscope (Eindhoven, Netherlands) with the help of the UMass Electron Microscopy Core. Quantitation of morphological changes observed by TEM was done using an image of a grid taken at 1100X magnification. The image was split into sections, platelet changes of budding and vacuolization were counted and normalized per total platelet number.

Blinding Procedures:

No specific blinding procedures were utilized in this study. COVID-constraints and the limited personnel allowed to work with these samples made us unable to design assessment of samples/findings utilizing multiple investigators over longer periods. Enrollment of patients was done in an unblinded fashion as we studied patients as they presented and agreed to be part of the study. Sequencing and enrichment was done in an unbiased fashion as it is analyzed by software that are not person-dependent.

Statistical analysis

RNA-seq FASTQ files were uploaded on the NIH Genboree platform (https://www.genboree.org/site/) to generate read counts (PMID: 25080362; PMID: 23320832). Samples were categorized in two different groups (MI and Covid-19 positive samples). Differential expression analysis, including raw and adjusted p-values, was performed with DESeq2 at https://www.genboree.org/site/. All other statistical tests were

done using GraphPad Prism 7; details for each test can be found in the legend of each figure. Correlations between platelet transcripts and plasma proteins was established using Spearman correlation coefficient and two-tail p-values generated by Prism Graph Pad 7. Of note, no wide/across-test with multiple correction was applied throughout the manuscript.

Online Figure Legends:

Online Figure I: SARS-CoV-2 in platelets from COVID-19 patients. A-C: RNA from platelets from COVID-19 patients and non-infected controls was sequenced using Artic V3 sequencing specifically enriching for SARS-CoV-2 genome. For positive control we used RNA isolated from SARS-CoV-2 (USA-WA1/2020 Strain, BEI). Sequencing results were analyzed using Nucleotide Blast NCBI server against SARS-CoV-2 NC_045512_Wuhan-Hu-1 genome. Alignment of viral RNA in platelets to the viral gene for **A.** Spike glycoprotein, **B.** Nucleocapsid protein, and C. Envelop protein. Immunofluorescent staining of whole blood from non-infected controls, treated the same way as in Figure 2A and 2B does not show presence of SARS-CoV-2 **D.** Spike or **E.** Nucleocapsid proteins in platelets. Transmission electron micrographs (TEM) of platelets isolated from three different donors that have tested positive for SARS-CoV-2. TEMS are showing **F.** Potential virion in phagocytic granule, **G.** and **H.** Potential digested virus. Presence of intact virions was not possible to detect with the exception of A. Images are representative of n=6 donors.

Online Figure II: SARS-CoV-2 internalization leads to fast virion-digestion and platelet content release. Washed platelets from healthy donors were incubated with SARS-CoV-2 for 15 min, fixed and resolved by TEM. Representative images of **A.** Donor 1 (F, 45y), **B.** Donor 2, (F, 59y) **C.** Donor 3 (M, 58y) and show the entire platelet from which images in Fig 4H were generated. D. Donor 1 showing platelet morphological changes and content release.

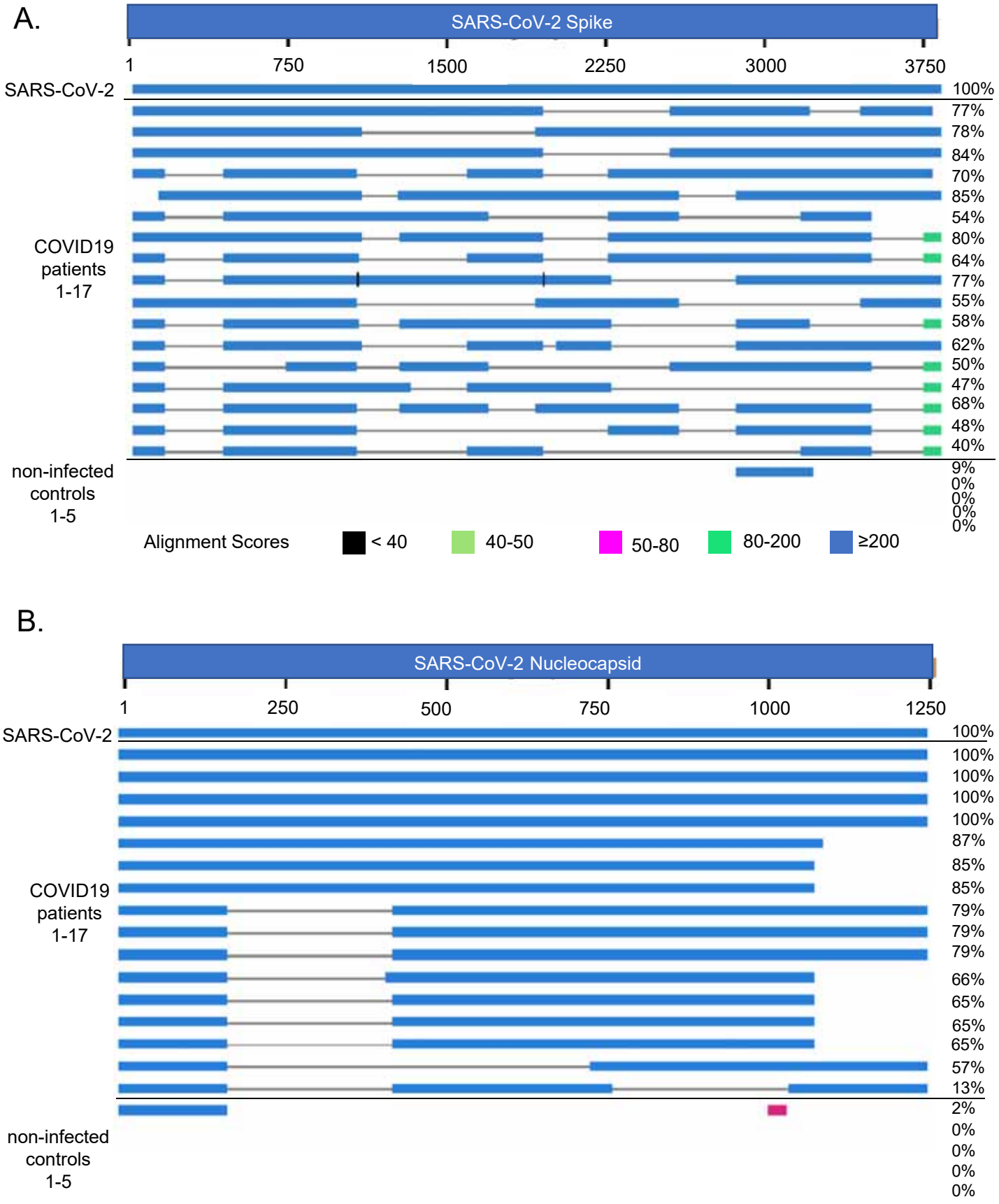
Online Figure III: COIVD19 patients have platelets bigger than 5 μm and some of those express **ACE2.** Citrated whole blood was RBC-lysed and fixed with 4% PFA immediately after draw. Platelets were stained with the indicated antibodies and resolved by spinning disk confocal microscopy and 100X lens as indicated in Methods. A. Platelets from patients stained with ACE2 and CD41; B. Platelets from COVID patients showing size bigger than 5 μm and smaller than 2 μm.

Online Figure IV: **Platelets from COVID-19 patients exhibit similar morphological changes and various forms of extracellular vesicle release to platelets treated with SARS-CoV-2.** Washed platelets were isolated from COVID-19 patients and immediately fixed with Karnovsky's fixative. Transmission electron micrographs (TEM) showed: **A.** Microparticles in platelet vacuoles of various sizes and cytoplasmic origin. **B.** Microparticles within platelet vacuoles and forming near a platelet, **C.** Platelets showing content release from broken membrane. **D.** Platelet interaction with an exosome (tilting images of the same exosome), Images are representative of n=6 different COVID-19 patients. **E. F.** Recalculation of the data in Fig 4H and 4I showing no significant effect of DX600 on the observed morphological changes. Data was evaluated using Mann-Whitney two-tailed test.

Online Figure V: **RNAseq of platelets from COVID-19 patients as compared to healthy subjects and those with MI**. RNAseq data provided in Supplemental Table VII was sorted by Log2FoldChange (large to small) and by p-adjusted (small to large). The top 40 upregulated and 40 downregulated transcripts in platelets were used for pathway enrichment analysis utilizing https://metascape.org/ **A.** upregulated and **B.** downregulated genes; when platelets from COVID19 patients are compared to platelets from MI patients. Pathway enrichment analysis of 40 upregulated and 40 downregulated transcripts in platelets sorted by the same criteria and **C.** Using published data from Manne et al, comparing transcripts in healthy platelets to those from COVID-19 patients.

Online Figure VI: Platelets from COVID-19 patients evaluated for markers for pyroptosis. Washed platelets from COVID-19 patients were subjected to Western Blot Analysis and screened for markers for pyroptosis. Of note, since these patients are at steady state, we may not be able to accurately evaluate if the virus itself leads to pyroptosis.

Online Figure I



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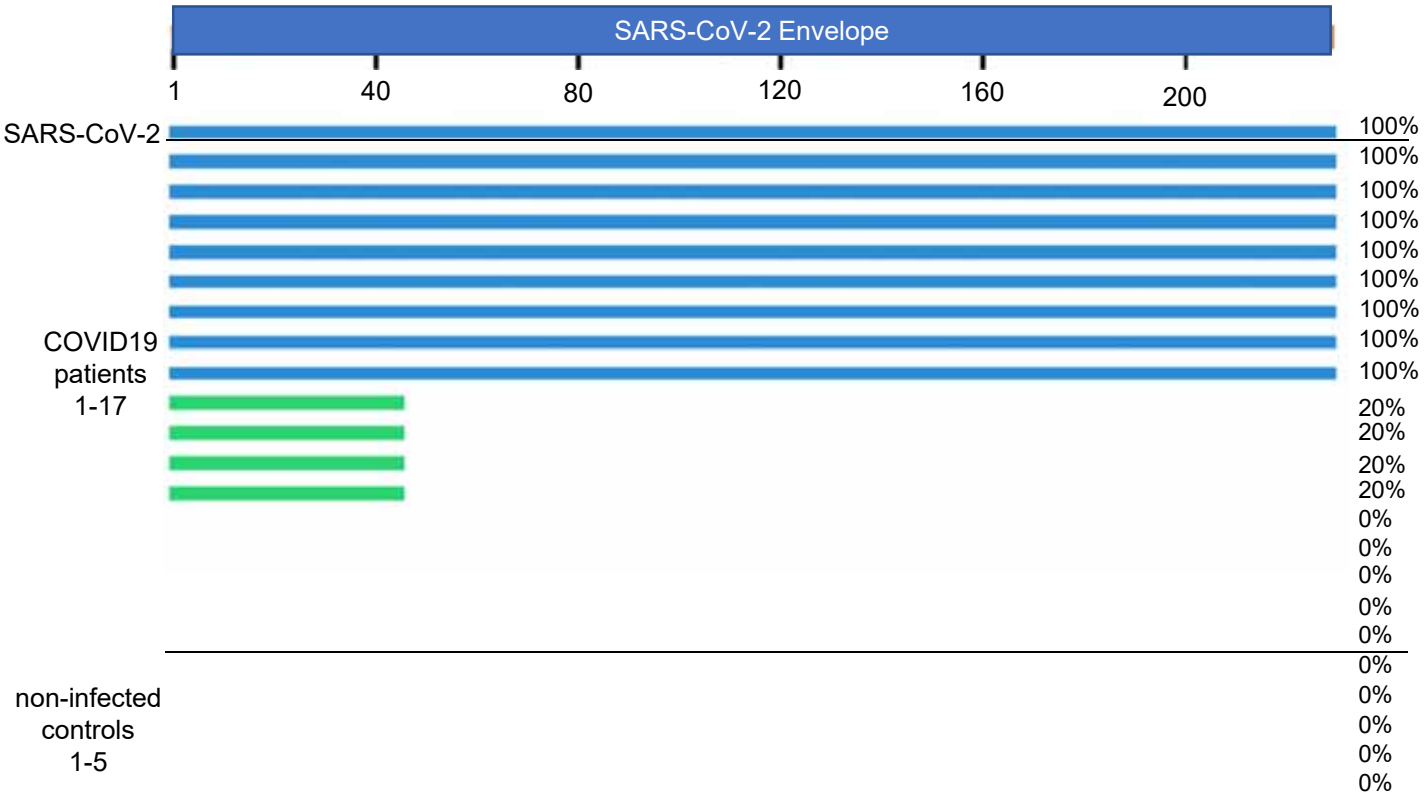
SARS-CoV-2 Envelope
1
40
80
120
160
200
SARS-CoV-2
COVID19 patients 1-17
non-infected controls 1-5
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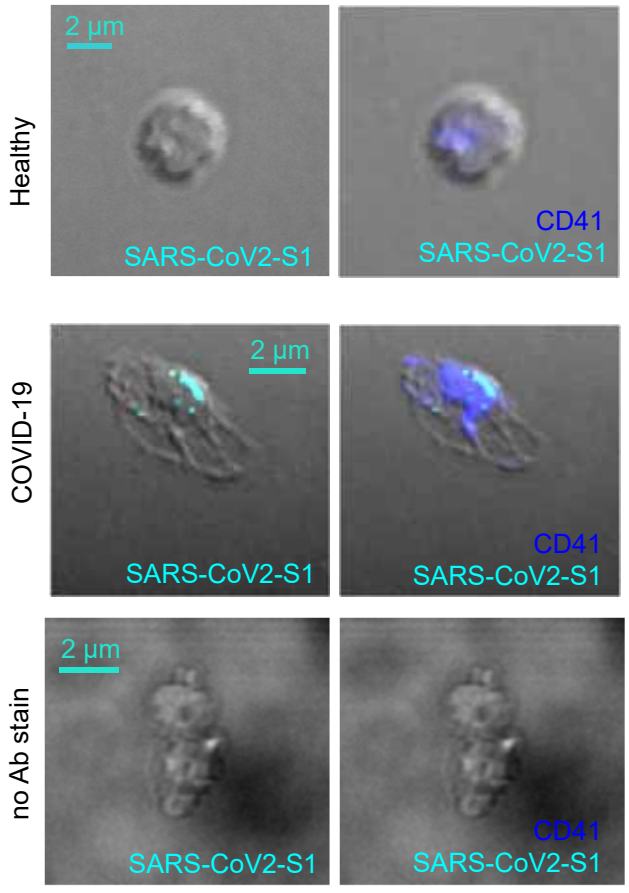
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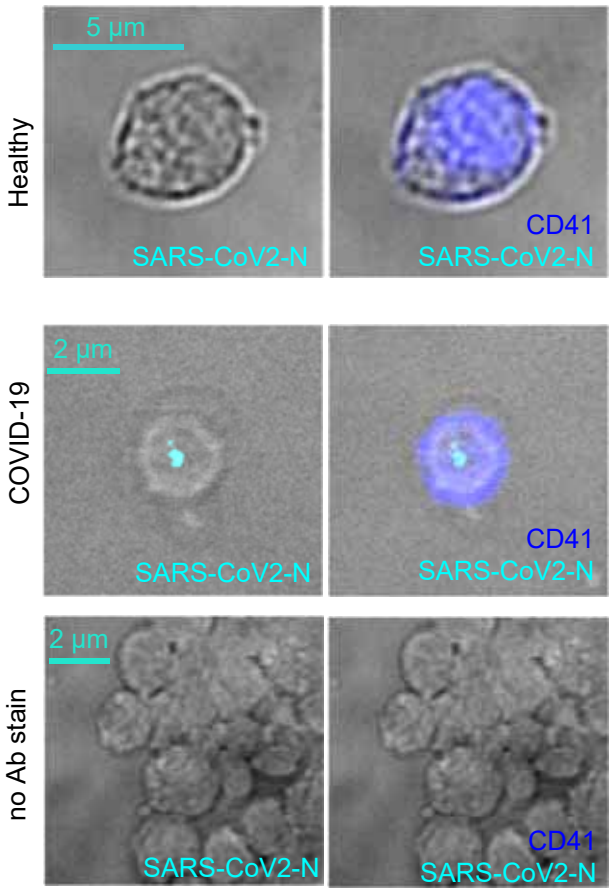
SARS-CoV2-S1
CD41
SARS-CoV2-S1

E.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CD41
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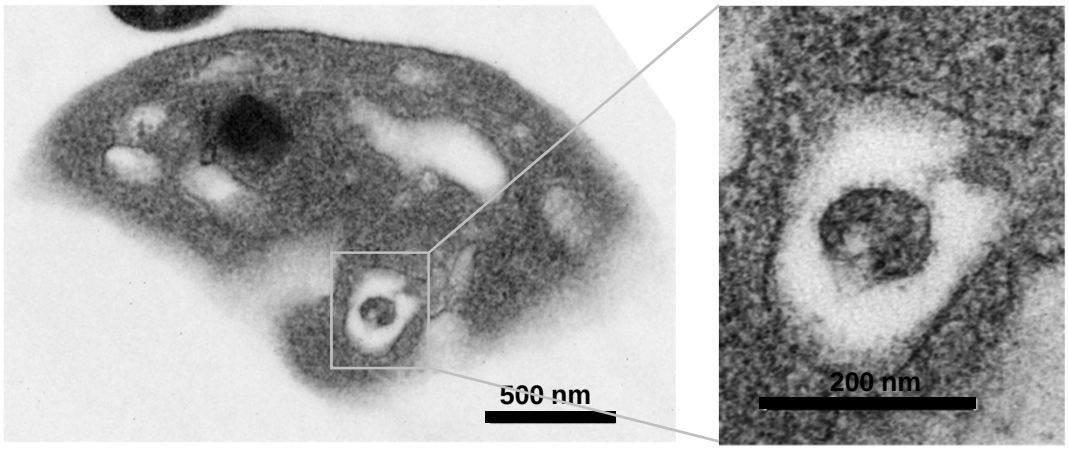
Online Figure I cont.

F.



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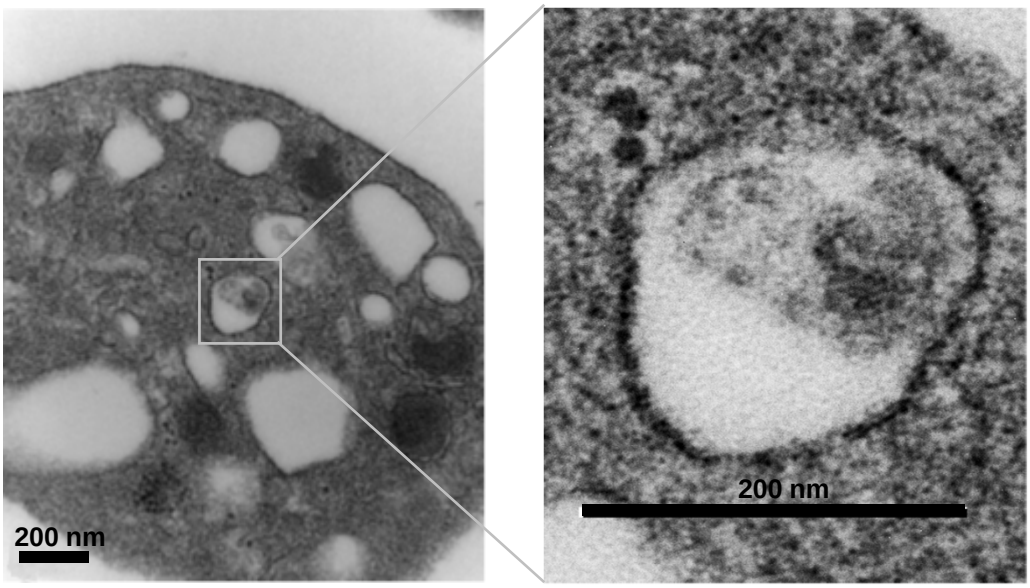

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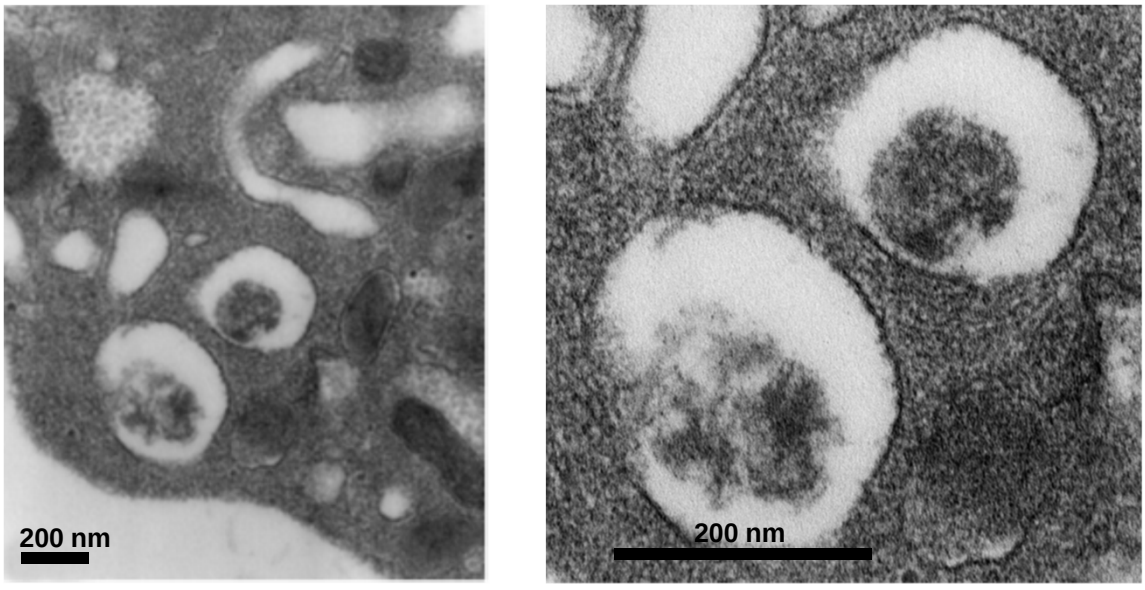

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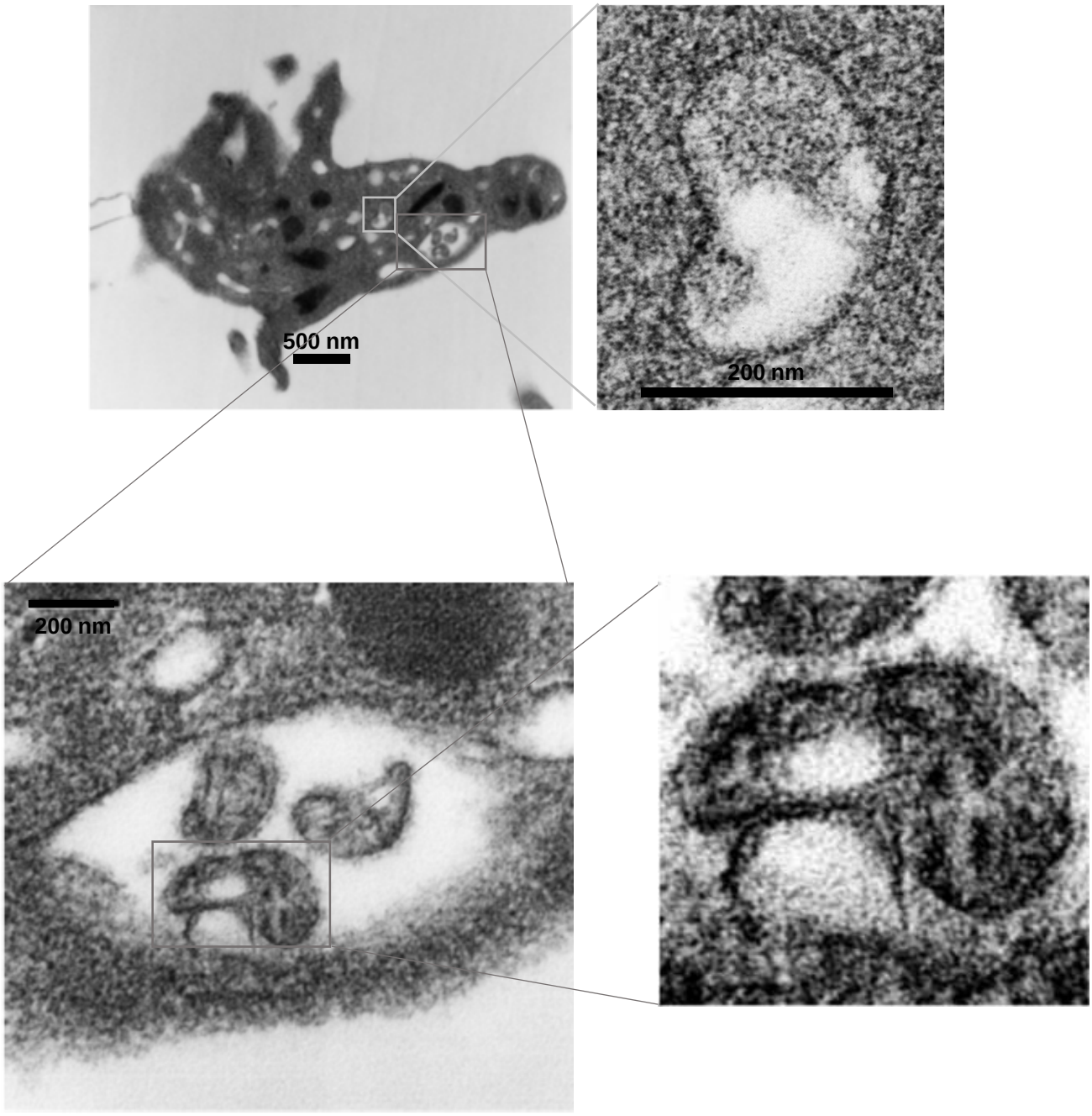
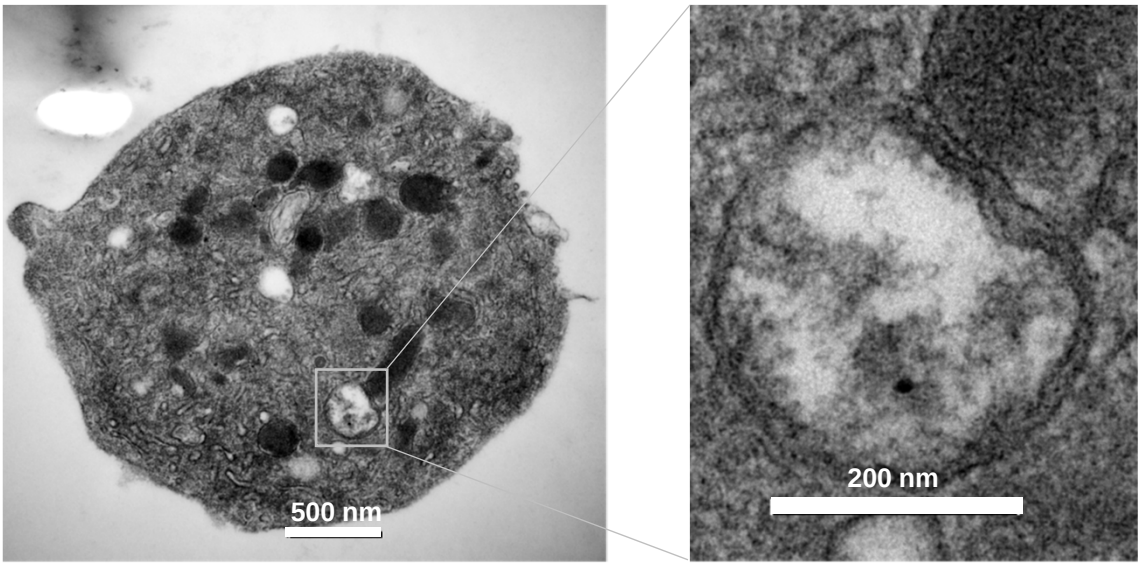
I.



Online Figure II

A.



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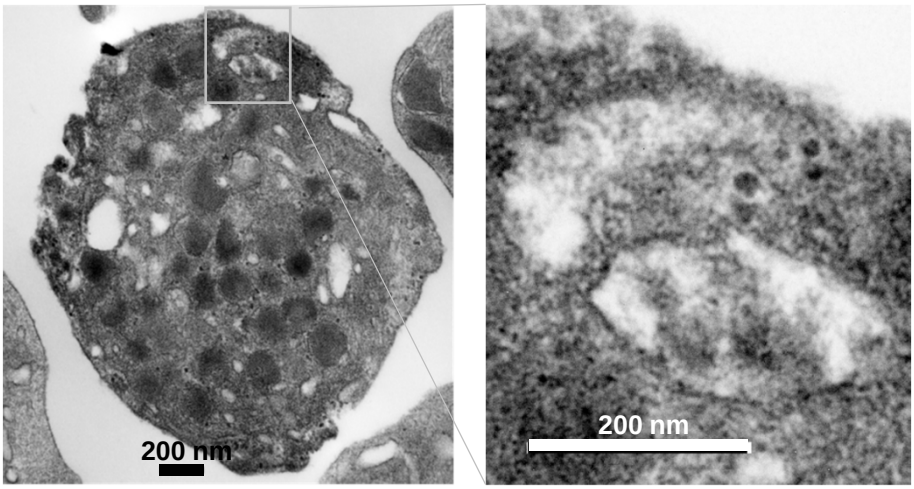

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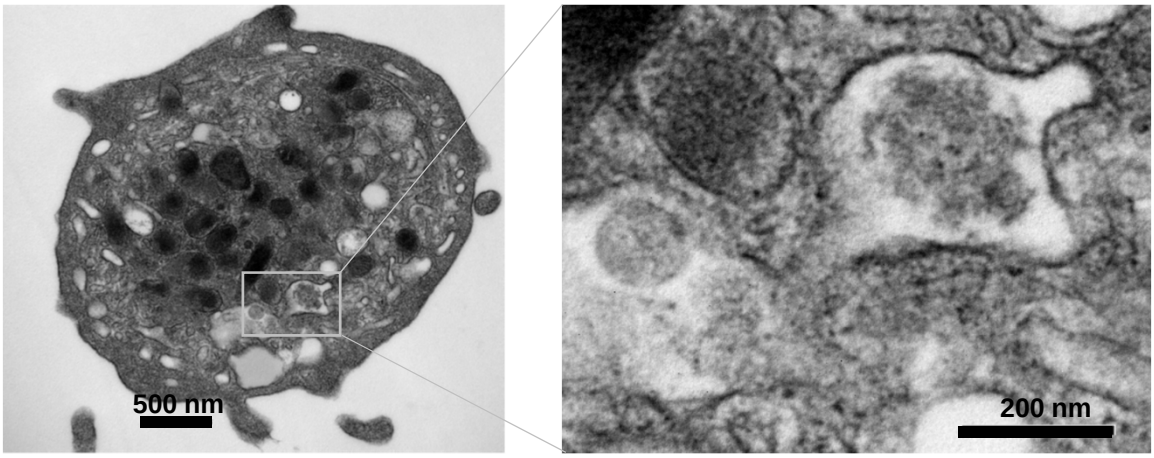
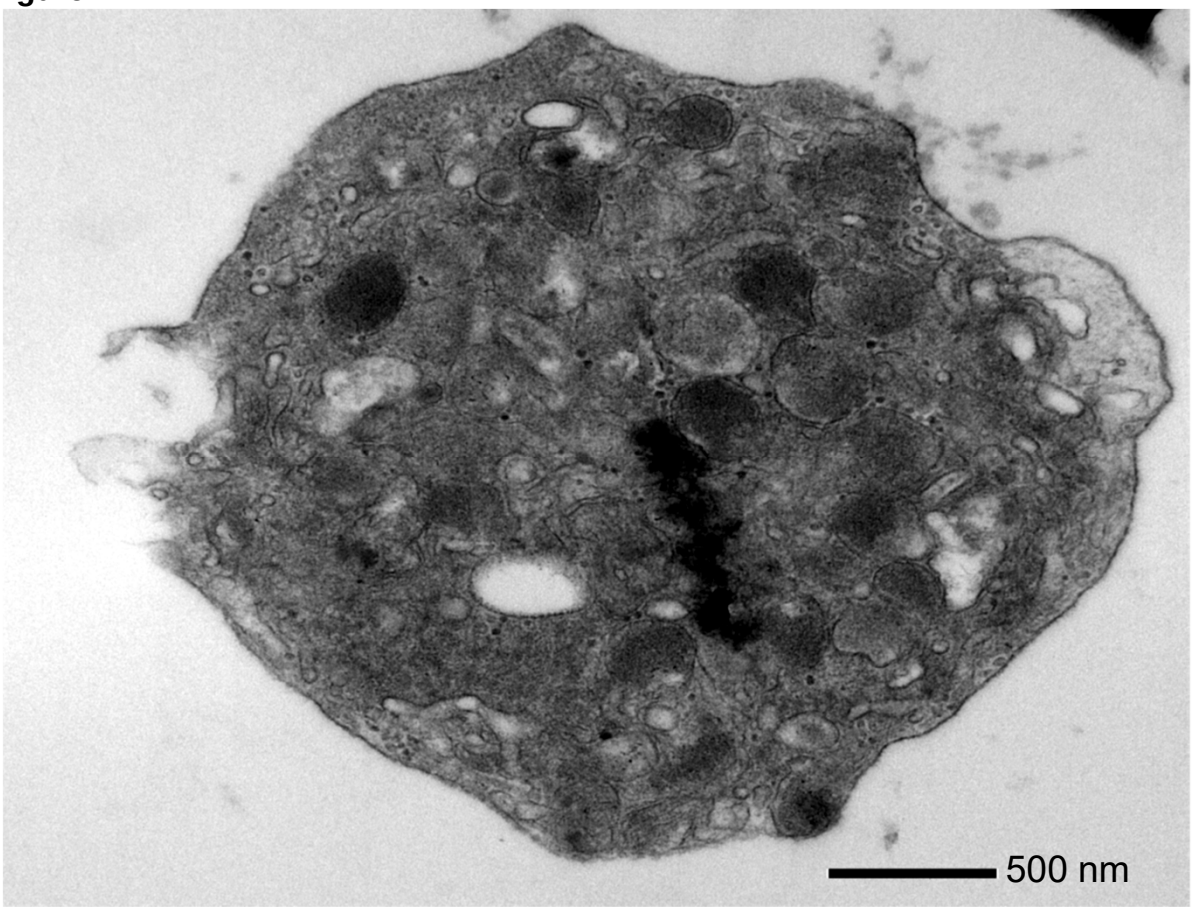
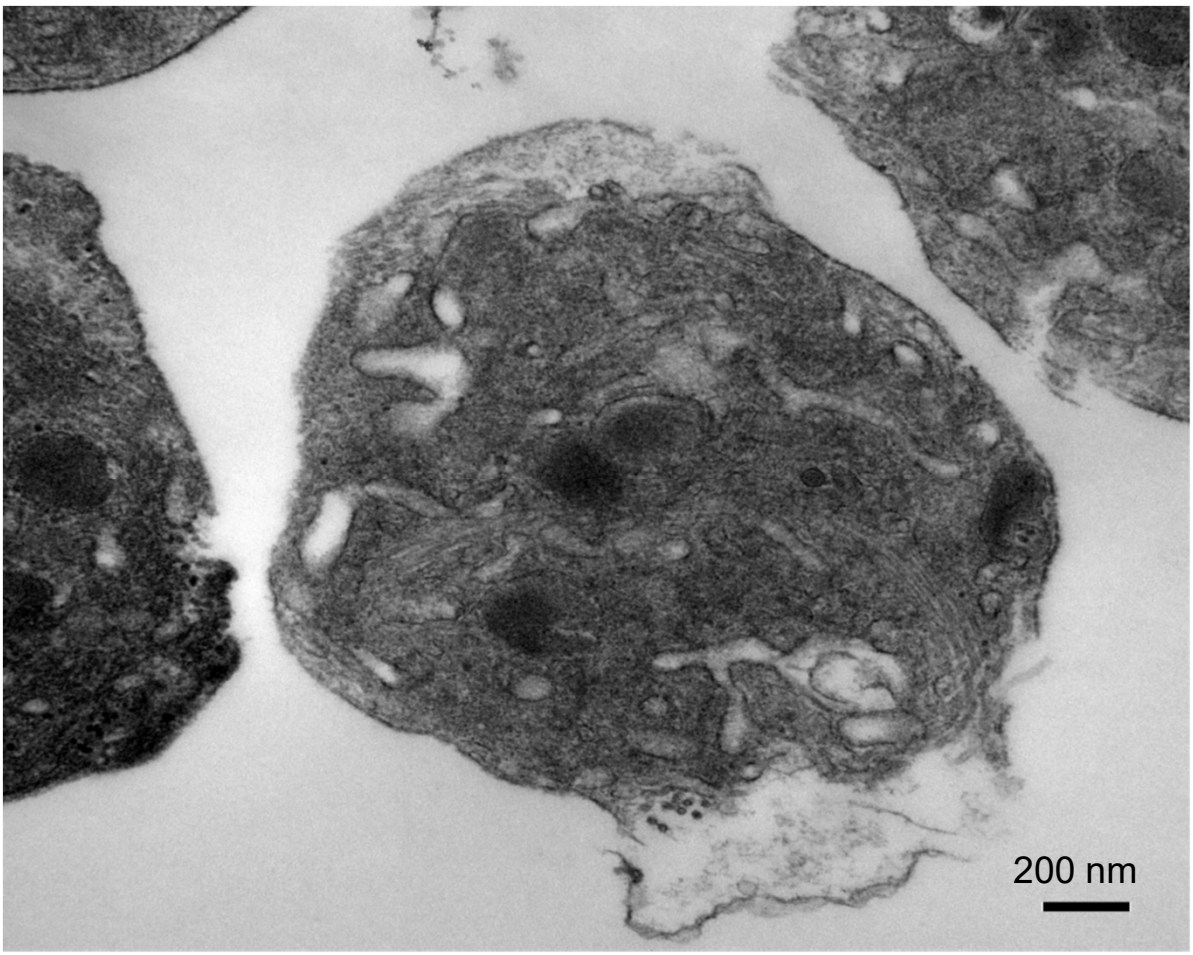


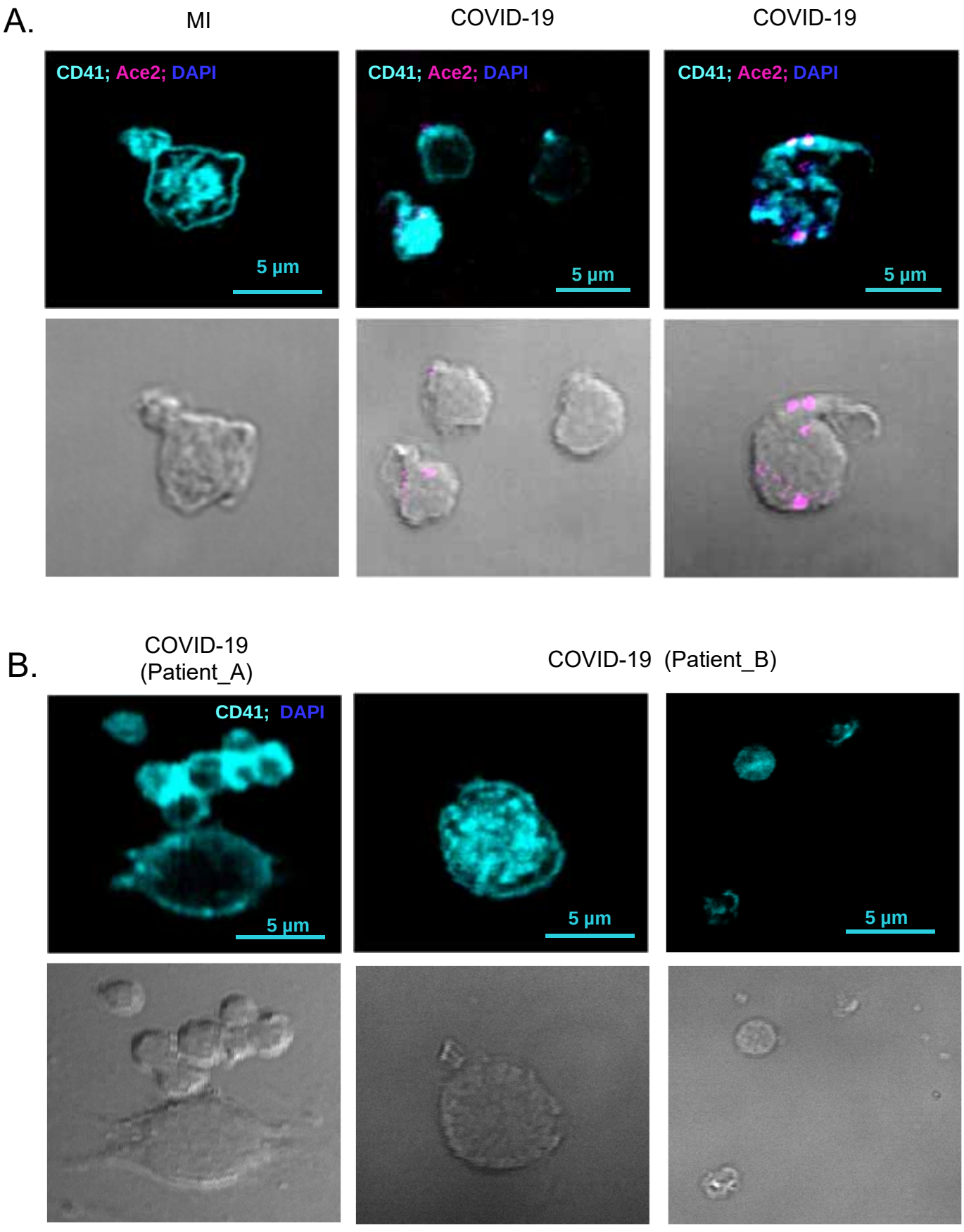
Online Figure II

D.

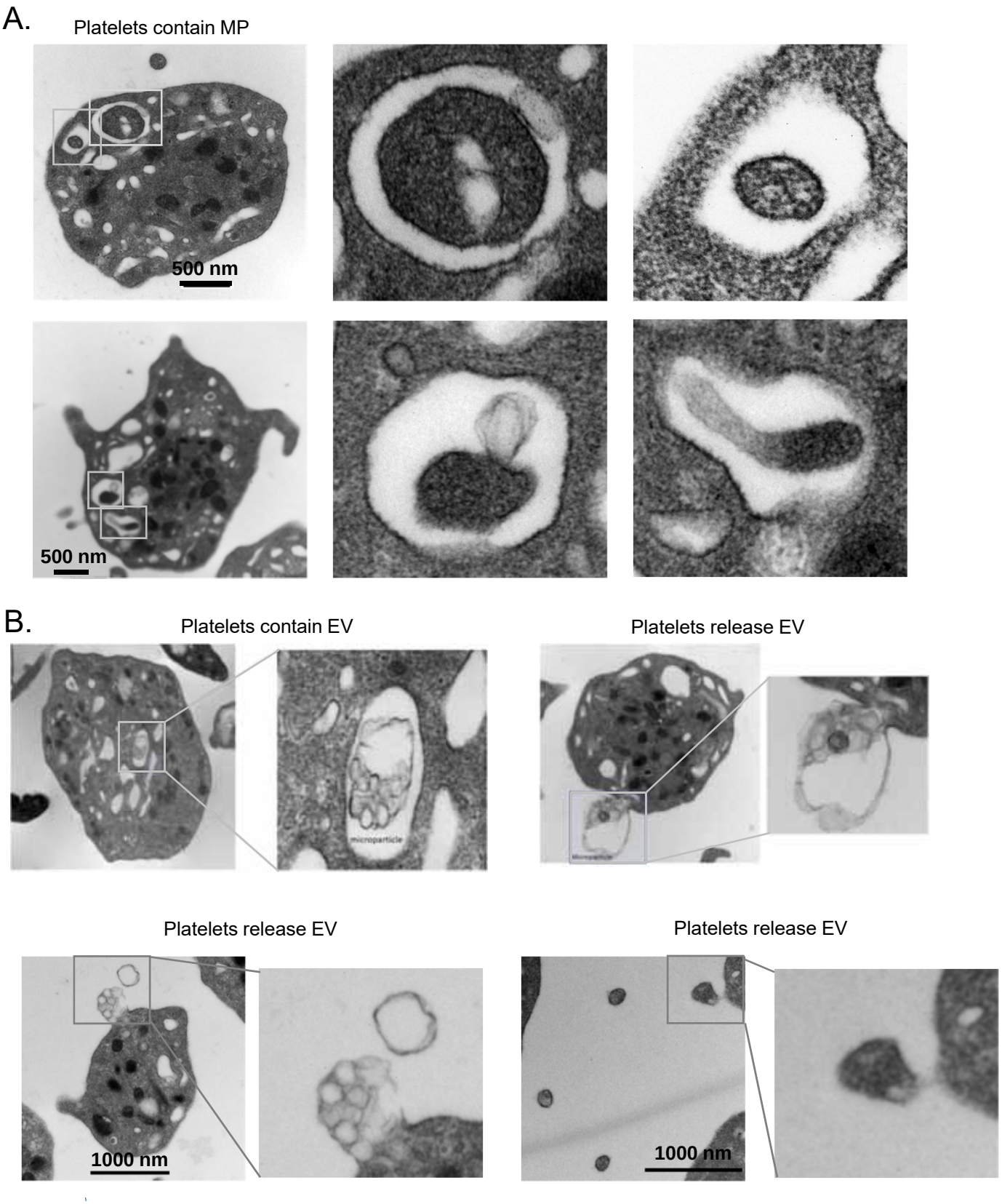




Online Figure III

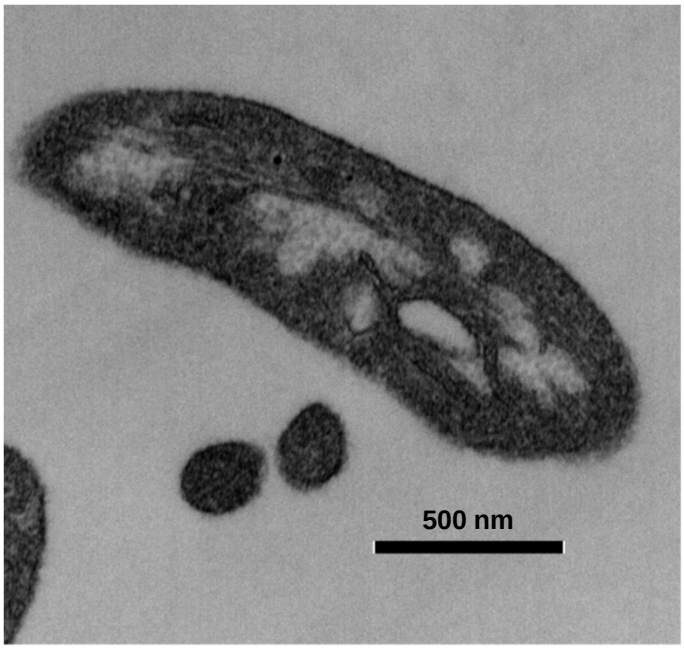


Online Figure IV

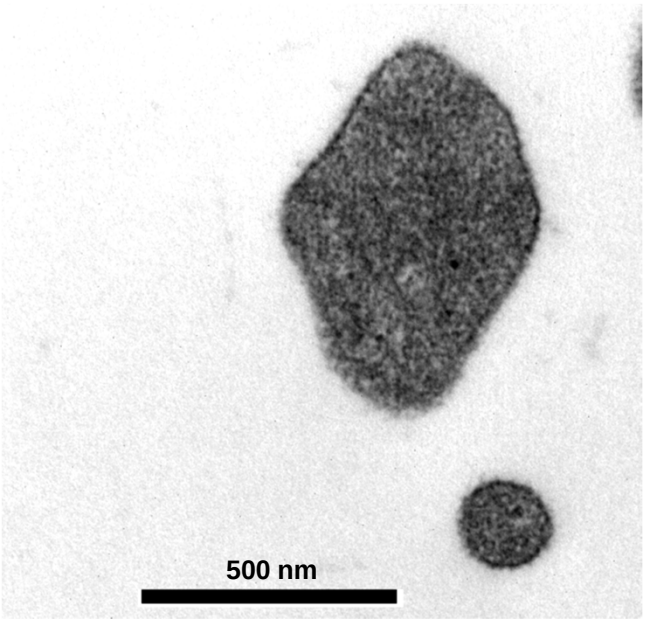


Online Figure IV cont.

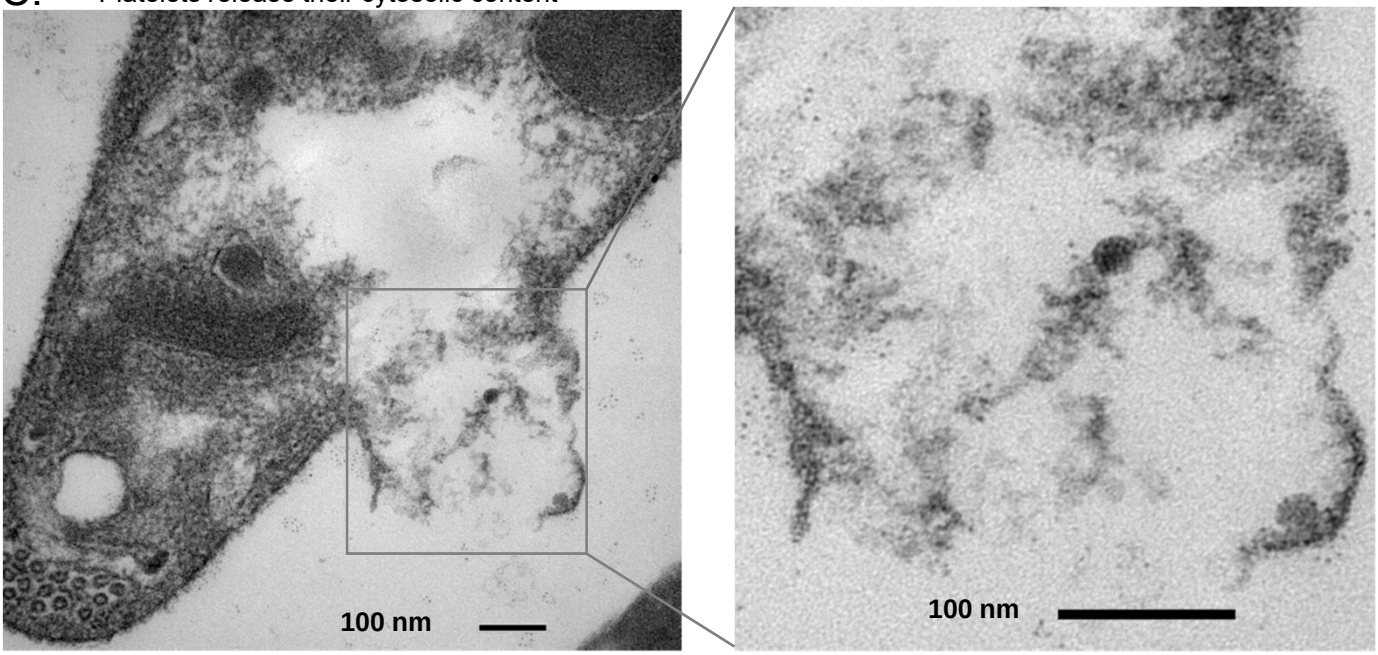
Platelets release EV



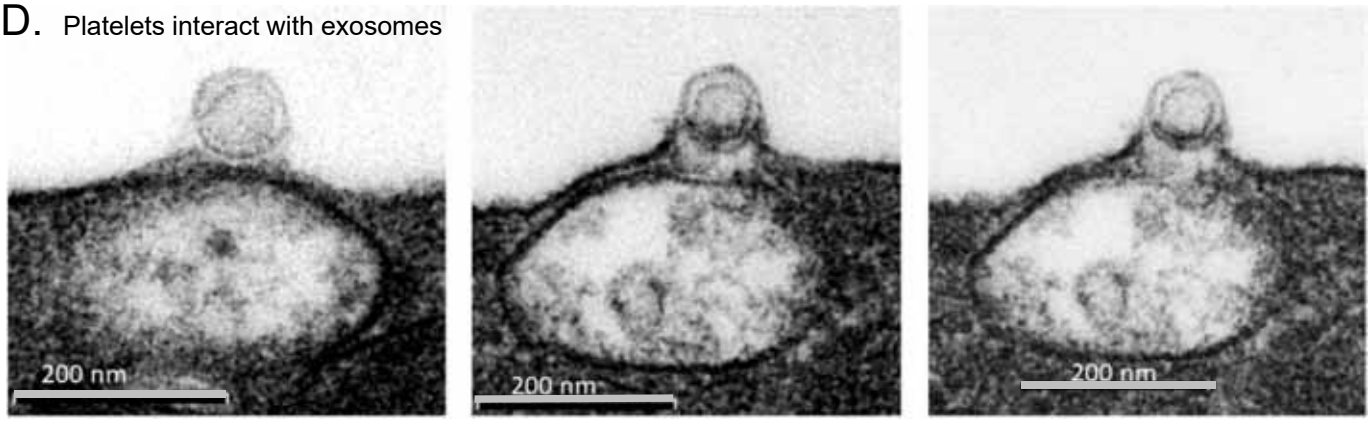
Platelet-derived EV



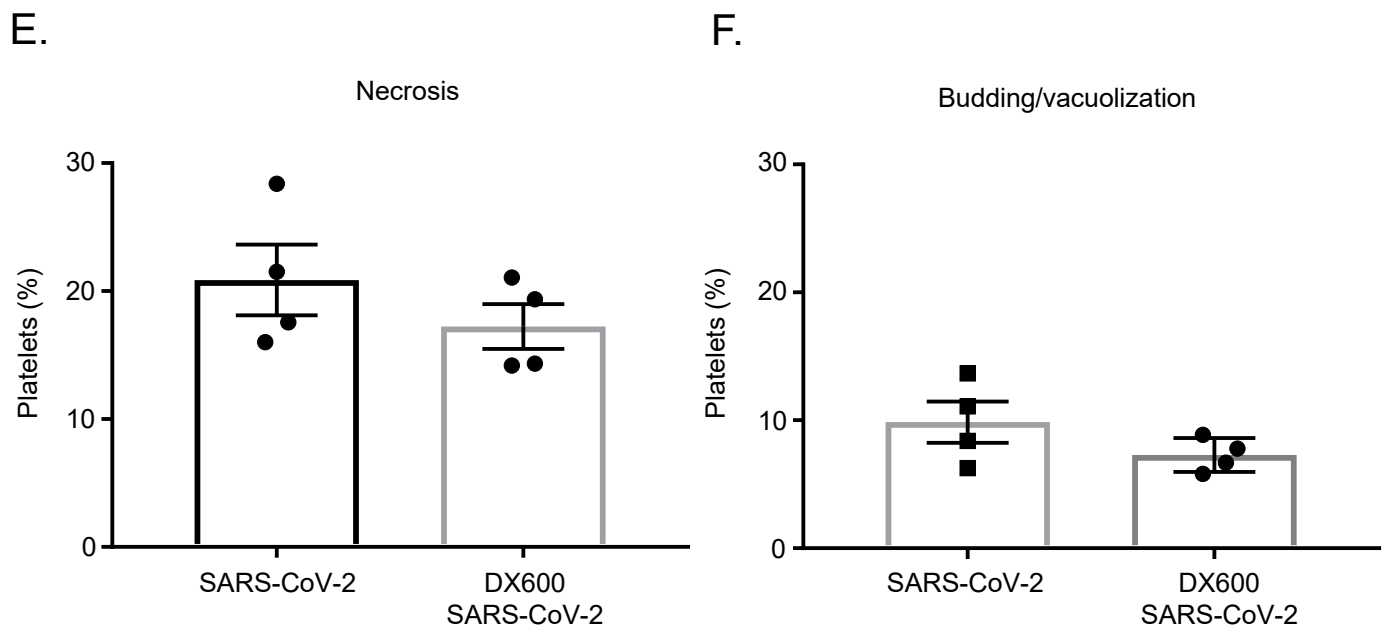
C. Platelets release their cytosolic content



D. Platelets interact with exosomes

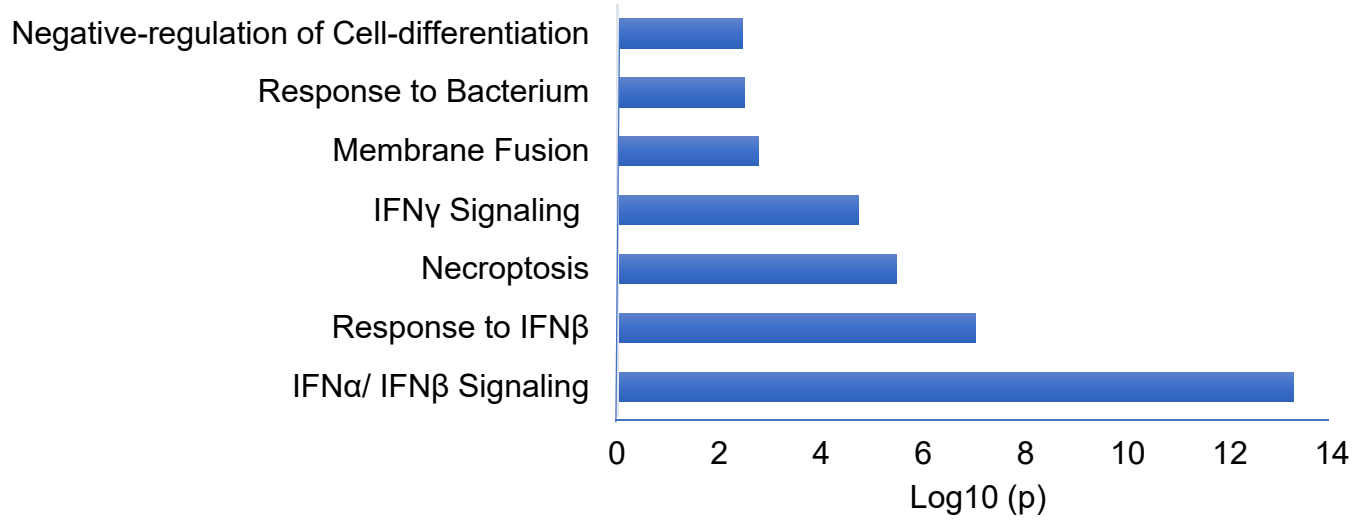


Online Figure IV cont.

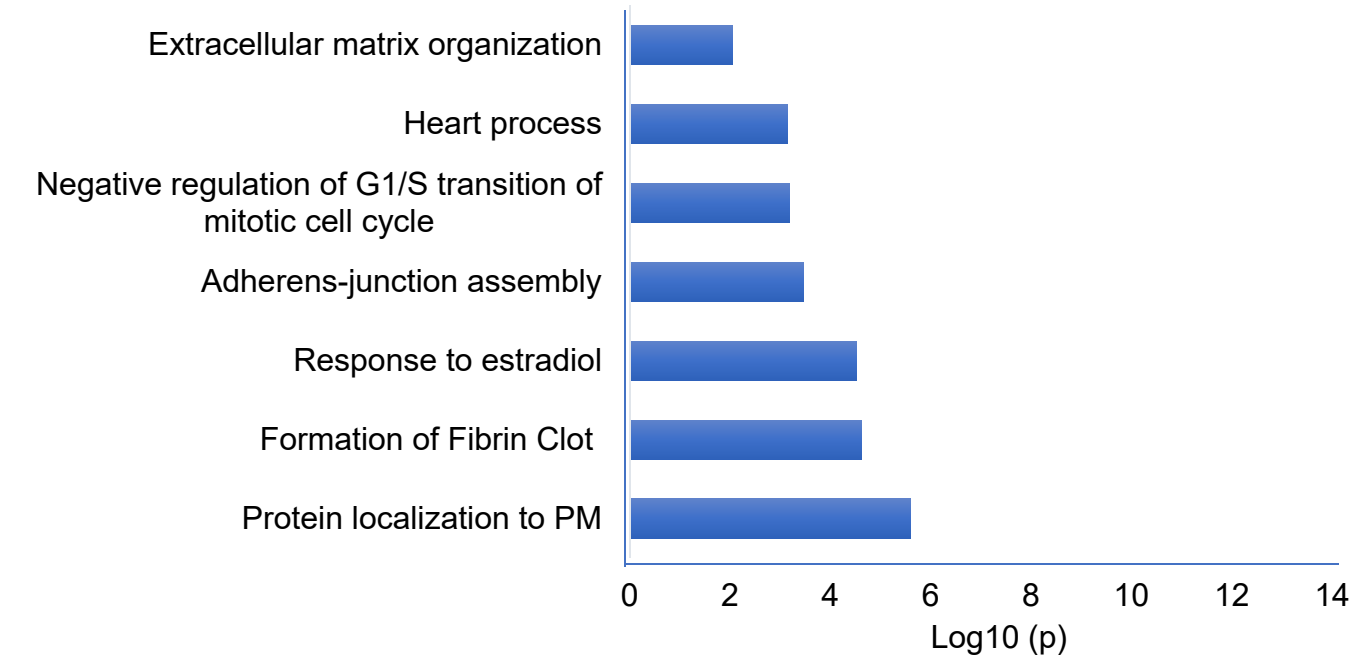


Online Figure V

A.

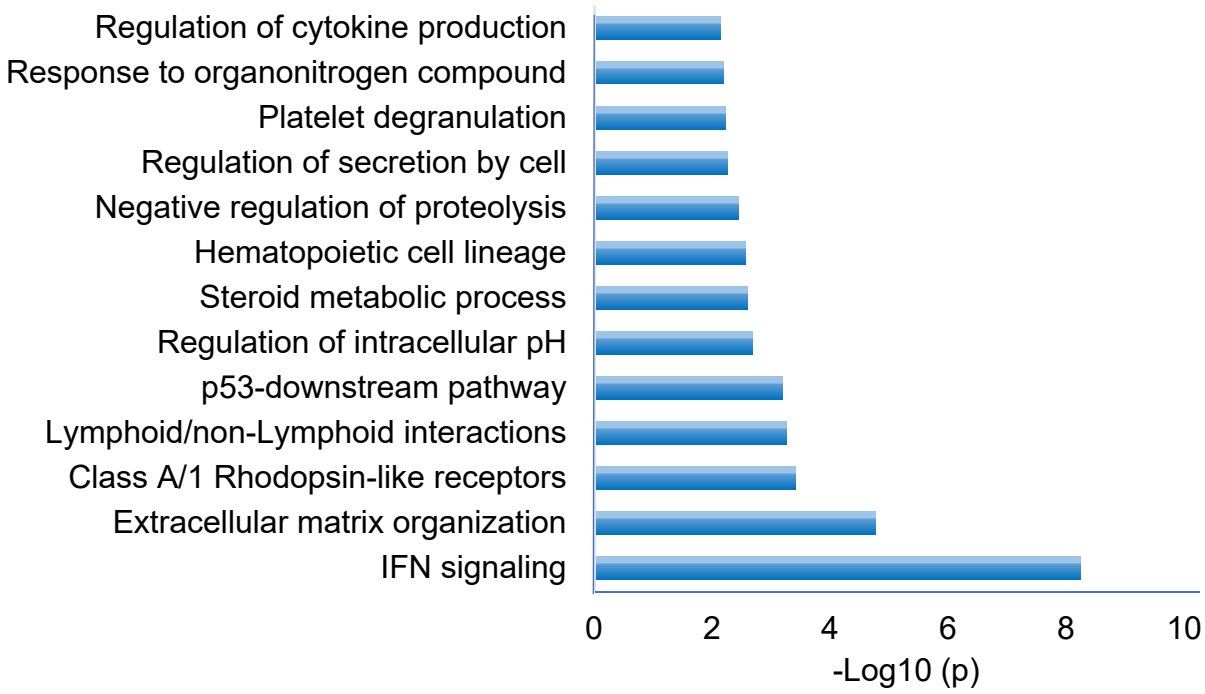


B.

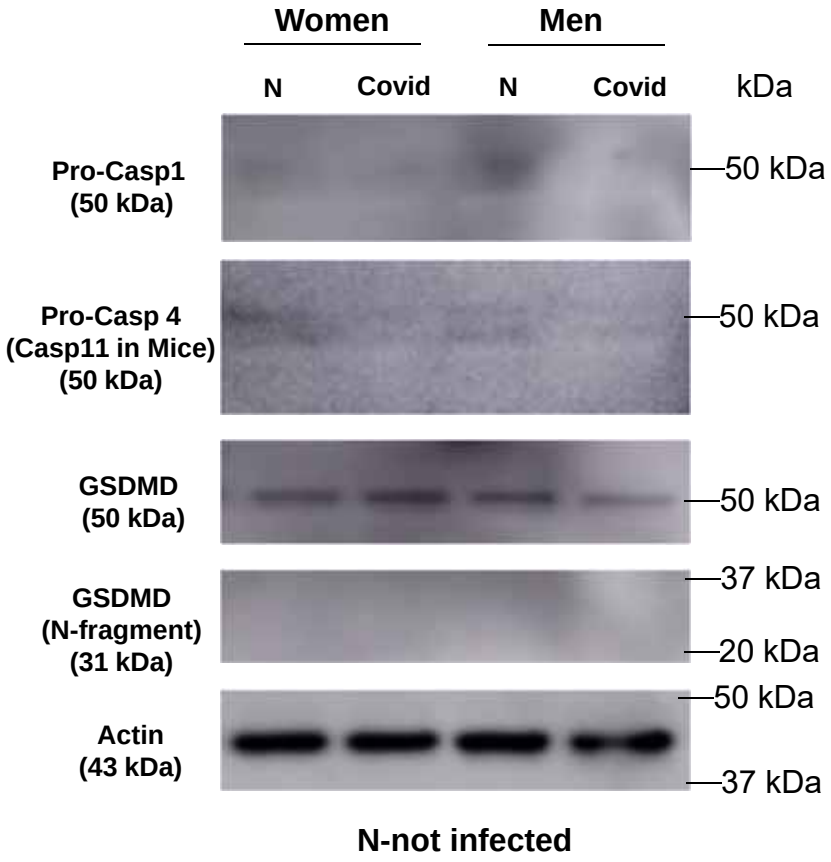


Online Figure V cont.

C.



Online Figure VI



N-not infected

Online Table I: Characteristics of patients used throughout this study including the RNAsequencing and Artic V3 SARS-CoV-2 RNAsequeinicng

Variable	Myocardial Infarction	COVID-19
n	20	17
Age	66.5 ± 3	60.3 ± 3
Sex (F%)	50%	33%
Race/Ethnicity	Asian 1 Multiracial 1 Hispanic 3 White 16	Asian 2 Multiracial 0 Hispanic 7 White 9
Time post COVID-19 diagnosis (days)*	0	5.4 ± 1
Ordinal Score**	--	2-3 (n=2) 4 (n=5) 5 (n=2) 6 (n=5) 7 (n=2) 8 (n=2)
RBC (10^6/µl)	3.6 ± 0.1	3.6 ± 0.1
WBC (10^3/ µl)	7.7 ± 0.7	7.2 ± 0.8
Platelets (10^3/µl)	211 ± 22	239 ± 26
Aspirin (%)	100%	23%
ACE inhibitor (%)	35%	30%
ADP receptor antagonist	90%	11%
Troponin	2.95 ± 0.93 (n=15) >70 (n=5); nd (n=1)	0.29 ± 0.2 (n=14) >70 (n=1); nd (n=4)
AST	74.3 ± 26 (n=9)	32.1 ± 3 (n=17)
ALT	85.6 ± 24 (n=9)	43.7 ± 10 (n=17)
D-dimer	<0.19 (n=1)	4.04 ± 3 (n=12)
Fibrinogen	419 (n=1)	165 (n=1)
TAT	nd	nd
INR	1 ± 0.03 (n=14)	4.2 ± 3 (n=13)

Values are shown as mean ± SEM; nd-not determined; RBC-red blood cells; WBC-white blood cells; TAT-Thrombin-Antithrombin (TAT) Complex; AST-aspartate aminotransferase; ALT-alanine aminotransferase; INR-international normalized ratio; all listed medications are at time of blood draw.

*Positive diagnosis refers to positive test by nasal swab; all MI patients were tested for COVID-19 at time of admission. The criteria for COVID-19 patient selection was hospitalization after testing positive by qPCR nasal swab test, with the exception of one individual who did not become hospitalized. We aimed to have a broad representative group and did not exclude based on duration since positive test.

**Ordinal Scores are as follows: 2=not hospitalized; 3=hospitalized, not requiring supplemental oxygen and no longer requiring ongoing medical care; 4=hospitalized, not requiring supplemental oxygen but requiring ongoing medical care; 5= hospitalized, requiring any supplemental oxygen;6, hospitalized, requiring noninvasive ventilation or use of

high-flow oxygen devices; 7= hospitalized, receiving invasive mechanical ventilation or extracorporeal membrane oxygenation; 8=death

Online Table II: Primer Assays used for RT-qPCR in this study

Gene Name	**Taqman Assay ID#**	**Manufacturer**
ACTB	Hs99999903_m1	ThermoFisher Scientific
ACE2	Hs01085333_m1	
ACE2	Hs01085335_m1	
ACE2	Hs00222343_m1	
CASP1	Hs00354836_m1	
CASP3	Hs00234387_m1	
CASP4	Hs01031951_m1	
CASP7	Hs00169152_m1	
CASP8	Hs01018151_m1	
CASP9	Hs00962278_m1	
GSDMD	Hs00988209_m1	
MLKL	Hs04188505_m1	
TMPRSS2	Hs00237175_m1	
Gene Name	**Catalog Number**	**Manufacturer**
2019-nCoV_N1	10006713	Integrated DNA Technologies
2019-nCoV_N2		

Supplemental Table III: Distinct signatures of highly abundant lncRNA in total blood leukocytes that are not present in platelets.

n	lncRNA	baseMean	log2FoldChange	lfcSE	stat	p-value	p-adjusted	present in platelets
1	CTD-2139B15.2	50114.07521	-0.275037938	0.193672244	-1.420120574	0.155572582	0.431431542	**no**
2	MALAT1	25913.53608	-0.230726127	0.13618183	-1.694250451	0.090217702	0.32099541	**no**
3	RMRP	11973.79835	0.140705649	0.162117798	0.867922285	0.385436879	0.668543615	**no**
4	RP4-669L17.10	2036.38431	-0.134425572	0.243859415	-0.551242083	0.581467735	0.809437311	**no**
5	NEAT1	1445.531211	-0.337810481	0.170821096	-1.977568873	0.047977364	0.225377807	**no**
6	RP11-324E6.6	1398.637654	-0.04376979	0.206715527	-0.211739245	0.832310469	0.937418496	**no**
7	LINC00265	755.6946743	-0.091169612	0.113799599	-0.801141768	0.423049578	0.699465583	**no**
8	FAM157B	429.3631197	-0.228955612	0.242291729	-0.944958429	0.344680099	0.636117426	**no**
9	SNHG8	347.1799152	-0.619164179	0.119915218	-5.163349463	2.4257E-07	0.000103112	**no**
10	AC013394.2	304.6807548	-0.362017859	0.174629789	-2.073059021	0.038166787	0.197691713	**no**
11	RP11-34P13.13	223.4429819	-0.442296936	0.23718724	-1.864758557	0.062215255	0.260093811	**no**
12	LINC00338	158.2137766	-0.774563923	0.149578624	-5.178306256	2.23909E-07	0.000101889	**no**
13	FTX	141.1820447	-0.023215603	0.136666941	-0.169869924	0.865112434	0.950345549	**no**
14	CAHM	139.0057631	-0.412692122	0.168469481	-2.449655102	0.014299311	0.112102314	**no**
15	MIR4435-1HG	134.6552303	0.423076028	0.199241125	2.12343726	0.033717228	0.18499843	**no**
16	LINC00152	114.2014624	0.14384694	0.208377499	0.690318967	0.489993622	0.748632365	**no**
17	RP11-220I1.1	109.2240802	-0.35022436	0.110294598	-3.175353703	0.00149654	0.028246679	**no**
18	CTA-217C2.1	83.55311995	-0.366843612	0.22142648	-1.656728736	0.09757433	0.33493373	**no**
19	SNORA71B	64.32802259	-0.737694298	0.175049889	-4.214194621	2.50671E-05	0.001972574	**no**
20	LINC00623	60.26443939	0.336116367	0.144493977	2.326161783	0.020009922	0.136832069	**no**
21	SNHG16	59.69837697	-0.472340531	0.234486326	-2.014362796	0.043971457	0.214299586	**no**
22	LINC00672	51.0523433	0.375113864	0.189721795	1.97717855	0.048021451	0.225377807	**no**
23	LINC01001	3448.056155	-0.055028872	0.233755882	-0.23541171	0.813889198	0.929524699	**yes**
24	AC138035.2	1196.529798	-0.268245953	0.245674895	-1.091873685	0.274888626	0.567496749	**yes**
25	SNHG15	506.7474273	-0.442403081	0.147480023	-2.999749199	0.00270202	0.040660446	**yes**
26	SNHG5	242.8274784	-0.004595589	0.263827198	-0.017418936	0.986102403	0.994892104	**yes**

Supplemental Table III: Distinct signatures of highly abundant lncRNA in total blood leukocytes that are not present in platelets.

27	SNHG6	128.8563454	0.129156887	0.256425576	0.503681767	0.614485023	0.82679183	**yes**
28	LINC00493	111.0790353	0.151200396	0.189782403	0.796703981	0.425622971	0.700995654	**yes**
29	GAS5	68.2086558	-0.076715532	0.213972617	-0.35852967	0.719946968	0.887510597	**yes**
30	RNU12	63.79923873	-0.187240822	0.276786057	-0.676482133	0.498734586	0.753646575	**yes**

*Fold change indicates changes in leukocytes of COVID19 patients when compared to MI patients.

Supplemental Table IV Detection of SARS-CoV-2 in the plasma of 3 of the 17 COVID-19 patients, using the two CDC primers toward the nucleocapsid gene of the virus

Patient	Assay	CT	Ordinal Score
B	SARS-CoV-N2	28.96786	8
C	SARS-CoV-N1	27.93075	6
D	SARS-CoV-N2	27.95515	8

*Ordinal Scores are as follows: 6=hospitalized, requiring noninvasive ventilation or use of high-flow oxygen devices; 8=death; Patients B-D are the same patients in Fig. 1B.

**Citrated plasma from all patients was screened with the 2 primes only the patients listed in the table tested positive

Supplemental Table V: RT-qPCR for ACE2 in platelets from MI and COVID-19 patients using 3 different TaqMan primers

Donors	n total	ACE2_a			ACE2_b			ACE2_c			Total positive
		n positive	CT	ΔCT	n positive	CT	ΔCT	n positive	CT	ΔCT	
Healthy	3	0			0			0			0
MI	20	2	28±2	19.5±1.2	4	31.5±0.4	23.4±0.5	3	29.5±1.5	21.0±1.1	6
COVID	17	2	28.4±0.6	20.0±2.4	1	31.078	23.593	2	29.5±0.1	21.0±1.8	4

*Assay number for each ACE2 primer are listed in Supplemental Table II; From the patients that tested positive for ACE2 n=1 was positive with ACE2_a and ACE2_b; n=1 was positive with ACE2_a and ACE2_c; n=1 was positive with ACE2_b and ACE2_c; no patient was ever positive with all 3 primers. Healthy donors here were (F,60 y, white; F, 46 y, white; M, 56 y; white)

Online Table VI: Correlation of platelet transcripts (qPCR) related to programmed cell death with plasma cytokines measured by Olink in the COVID-19 patients

RNA → / Plasma ↓	MLKL	Casp3	Casp1	GSDMD	Casp7	Casp8	Casp4	Casp9
IFNg	**0.5074**	**0.5343**	0.1667	0.1818	0.3456	0.3652	0.2794	0.1544
P (two-tailed)	0.0397	0.0291	0.5214	0.4833	0.1744	0.1500	0.2765	0.5530
GZMA	**0.6985**	**0.6961**	**0.5368**	0.05897	**0.6569**	0.4804	0.4485	**0.5441**
P (two-tailed)	0.0024	0.0026	0.0283	0.8236	0.0052	0.0529	0.0727	0.0259
ANGPT2	**0.5735**	**0.6863**	0.2745	0.2236	**0.6275**	0.4436	0.3088	**0.6103**
P (two-tailed)	0.0179	0.0031	0.2853	0.3867	0.0083	0.0762	0.2273	0.0108
GP6	0.2228	0.1567	-0.0210	0.2411	0.2948	0.0922	0.2353	0.2767
P (two-tailed)	0.3901	0.5481	0.9363	0.3512	0.2507	0.7248	0.3633	0.2823
GP1bA	0.0349	-0.1169	-0.1214	-0.0844	-0.0921	0.1028	-0.3207	-0.1024
P (two-tailed)	0.8943	0.6551	0.6426	0.7473	0.7252	0.6945	0.2095	0.6957
P-selectin	0.1667	0.3529	0.3946	0.2924	**0.5025**	0.4461	**0.6054**	0.2623
P (two-tailed)	0.5214	0.1650	0.1180	0.2539	0.0419	0.0744	0.0115	0.3080
vWF	-0.2475	-0.1789	-0.1078	0.0270	-0.0588	-0.0294	-0.0539	**0.5368**
P (two-tailed)	0.3367	0.4907	0.6803	0.9207	0.8241	0.9134	0.8389	0.0283
MCP1	0.3015	0.4755	0.0662	**0.5160**	0.3578	0.1691	0.3603	0.0172
P (two-tailed)	0.2390	0.0557	0.8020	0.0361	0.1589	0.5152	0.1559	0.9510
ADA	0.4387	-0.0931	-0.0662	**-0.5233**	-0.1299	0.0564	-0.2647	0.2745
P (two-tailed)	0.0798	0.7226	0.8020	0.0332	0.6187	0.8315	0.3034	0.2853
TGFb1	**0.6569**	0.3505	0.03676	0.0737	0.3505	0.3922	0.1005	0.4608
P (two-tailed)	0.0052	0.1681	0.8909	0.7795	0.1681	0.1205	0.7013	0.0645
FASLG	**0.6005**	0.4706	0.4608	-0.2899	0.3284	0.2868	0.3333	0.0662
P (two-tailed)	0.0124	0.0585	0.0645	0.2580	0.1979	0.2636	0.1910	0.8020
CCL4	0.1985	**0.5809**	-0.1054	-0.0713	0.2132	0.3456	-0.03922	0.3971
P (two-tailed)	0.4435	0.0162	0.6873	0.7868	0.4097	0.1744	0.8835	0.1156

HMOX1	0.0417	-0.4314	**-0.5637**	-0.1941	-0.4387	-0.4363	**-0.5515**	-0.0392
P (two-tailed)	0.8760	0.0854	0.0203	0.4537	0.0798	0.0816	0.0237	0.8835
CCL23	0.1005	0.1250	-0.0784	0.3415	0.1250	0.1593	-0.0049	**0.4877**
P (two-tailed)	0.7013	0.6322	0.7657	0.1797	0.6322	0.5402	0.9887	0.0490
CD5	**0.6127**	0.4583	0.1103	-0.0885	0.4044	0.1373	0.2108	**0.5466**
P (two-tailed)	0.0104	0.0661	0.6733	0.7361	0.1086	0.5986	0.4153	0.0251
MMP12	0.2721	**0.5368**	0.1740	0.1425	0.4828	0.1765	0.4020	**0.5196**
P (two-tailed)	0.2897	0.0283	0.5028	0.5843	0.0516	0.4967	0.1109	0.0346
VEGFA	0.1789	0.1985	-0.1618	0.0074	0.1446	0.1520	0.0098	**0.6054**
P (two-tailed)	0.4907	0.4435	0.5339	0.9811	0.5789	0.5594	0.9736	0.0115
CCL20	0.2892	**0.5833**	0.1814	0.1744	**0.5441**	0.4020	0.3676	0.4314
P (two-tailed)	0.2594	0.0157	0.4846	0.5016	0.0259	0.1109	0.1471	0.0854
KLRD1	**0.5588**	0.2328	0.3480	0.0442	0.3186	0.3039	-0.0417	0.4657
P (two-tailed)	0.0216	0.3670	0.1712	0.8682	0.2123	0.2350	0.8760	0.0615
CSF-1	0.2206	0.4314	0.0294	0.2457	0.3652	0.3799	0.2157	**0.6520**
P (two-tailed)	0.3934	0.0854	0.9134	0.3403	0.1500	0.1333	0.4043	0.0056
TIE2	0.2132	0.2696	0.3652	0.0737	0.2696	**0.5343**	0.0490	0.0833
P (two-tailed)	0.4097	0.2942	0.1500	0.7795	0.2942	0.0291	0.8537	0.7512
IL18	0.3873	0.1176	-0.1176	0.0049	-0.0368	0.1789	-0.1103	0.1912
P (two-tailed)	0.1255	0.6526	0.6526	0.9886	0.8909	0.4907	0.6733	0.4609
IL7	0.1520	0.0931	-0.0662	0.2875	0.0343	0.3284	-0.1176	0.1446
P (two-tailed)	0.5594	0.7226	0.8020	0.2622	0.8984	0.1979	0.6526	0.5789

*Spearman Correlation coefficients were generated in Prism Graph Pad. Multiple correction was not applied as these transcripts and proteins are specifically selected to address programmed cell death and inflammation.