Proteomic and phosphoproteomic analyses reveal hyperactive phenotype of platelets in humanized K18-ACE2 mice infected with SARS-CoV-2

Saravanan Subramaniam[1], Ryan Matthew Hekman[2], Archana Jayaraman[1], Aoife Kateri O'Connell[3,4], Paige Montanaro[3,5], Devin Kenney[3,4], Maria Ericsson[6], Sarah Walachowski[1], Benjamin Blum[2], Katya Ravid[7,8], Nicholas A Crossland[3,5], Florian Douam[3,4], Andrew Emili[2], Markus Bosmann[1,3,9]

[1]Boston University, Department of Medicine, MA, USA; [2]Center for Network Systems Biology, Boston University, Boston, MA, USA; [3]Boston University, National Emerging Infectious Diseases Laboratories (NEIDL), Boston MA, USA; [4]Boston University, Department of Microbiology, Boston, MA, USA [5]Boston University, Department of Pathology and Laboratory Medicine, Boston, MA, USA; [6]Electron Microscopy Core Facility, Harvard Medical School, Boston, MA, USA; [7]Department of Medicine, Boston University School of Medicine, Boston, MA, USA;[8]Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, MA, USA;[9]Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg- University, Mainz, Germany

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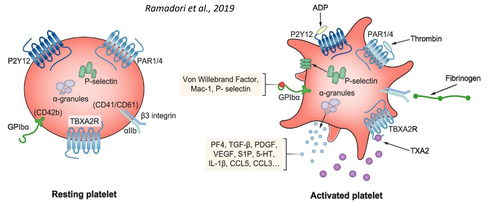
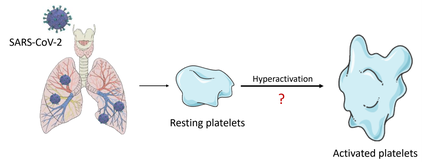
INTRODUCTION

- Coronavirus disease 2019 (COVID-19) is associated with a hypercoagulable state and increased incidence of cardiovascular complications.
- Platelets are effectors of hemostasis and play a major role in coordinating immune and inflammatory activities.
- Suitable animal models are needed to study COVID-19-associated coagulopathy (CAC) and platelet effector functions in COVID-19, which remain largely unclear.
- In our current study, we aimed to characterize alterations in platelets isolated from K18-hACE2 transgenic mice infected with SARS-CoV-2.



Schematic representation of a platelet in its resting state and upon activation: Platelets present membrane G-protein coupled receptors on their surface that can bind several ligands resulting in decreased intracellular cAMP, mobilization of Ca2+ stores and subsequent changes of cell morphology. Upon activation, soluble proteins retained in the granules are released via exocytosis, exerting their biological functions in an autocrine or paracrine manner. Similarly, membrane proteins retained in the granules are mobilized and presented at the cellular surface where they can bind related ligands. 5HT, 5-hydroxytryptamine; CCL2, chemokine ligand 2; CCL5, chemokine ligand 5; GPIbα, glycoprotein Ibα; IL-1β, interleukin-1β; PDGF, platelet-derived growth factor; PF4, platelet factor 4; S1P, sphingosine-1-phosphate; TGF-β, transforming growth factor-β; TXA2, thromboxane A2; VEGF, vascular endothelial growth factor.

AIM(S)



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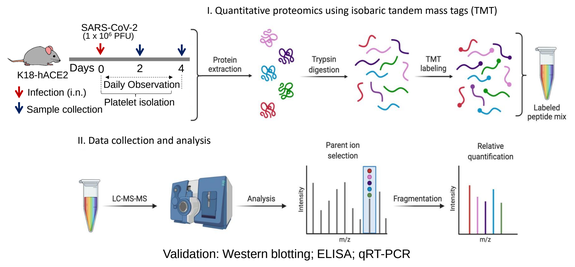
METHOD(S)



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- Abundance of SARS-CoV-2 spike protein in lungs of infected K18-hACE2 mice, but not in platelets and other organs (kidneys), suggests that platelet re-programming towards activation-degranulation-aggregation is likely attributable to a pneumonia-induced elevated circulatory factors (e.g., cytokines and thrombin)-driven response rather than direct platelet infection.
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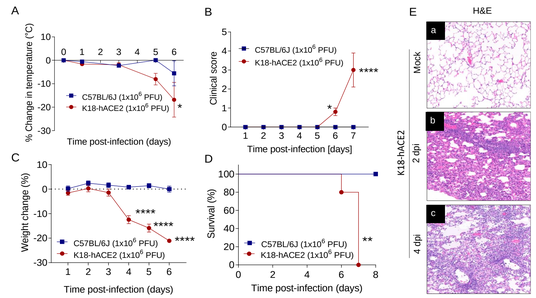
Clinical decline of SARS-CoV-2-infected humanized ACE2 mice



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CD61 aggregates in SARS-CoV-2-infected humanized ACE2 mice

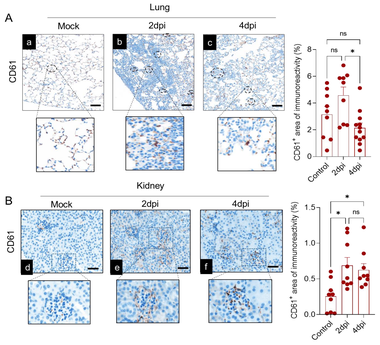

Figure 2. Microvascular platelet aggregates humanized ACE2 mice. SARS-CoV-2 spike protein was detectable in the lungs of inoculated mice, immunohistochemistry. ($A^{a,b,c}$) Lung capillaries contained increased CD61+ platelet aggregates in SARS-CoV-2-infected mice with no overt thrombosis. 100x, each image is representative of n=3 mice/group, scale bar=50 µm. Whole-slide quantifications of platelet aggregates in lungs of SARS-CoV-2-infected mice. ($B^{d,e,f}$) Renal capillaries contained increased platelet aggregates in SARS-CoV-2-inoculated mice with no overt thrombosis or edema. Quantification of platelet aggregates in renal capillaries. Data are shown as the mean ± SEM, n=3 mice/group, biological and technical triplicates; *P < .05; **P < .01; ****p < .0001; ns: not significant.

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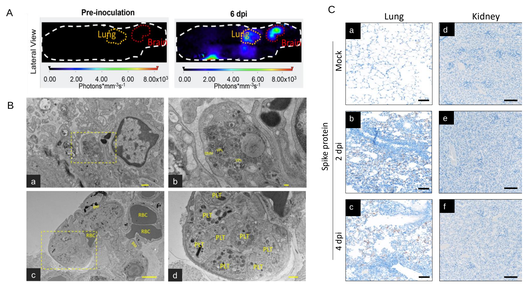

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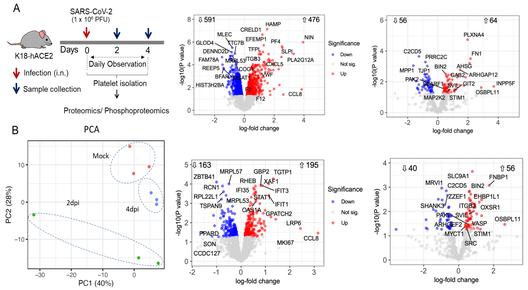
Platelet Proteome Analysis



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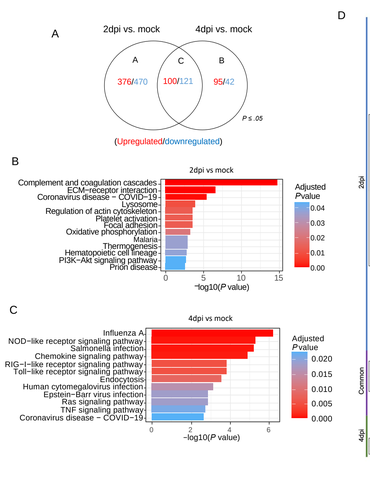
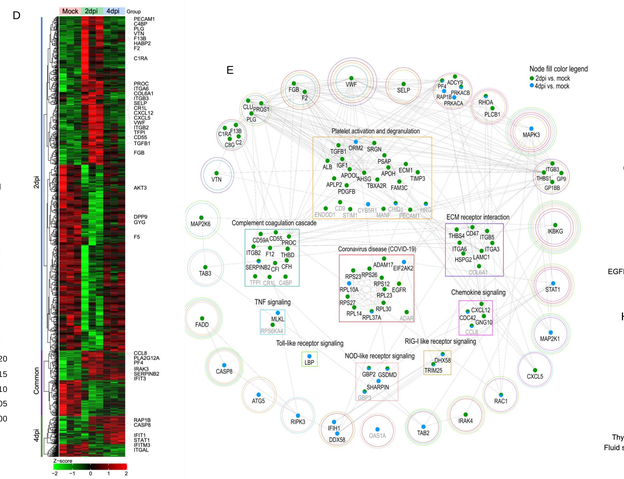


STRING-based protein-protein interaction network



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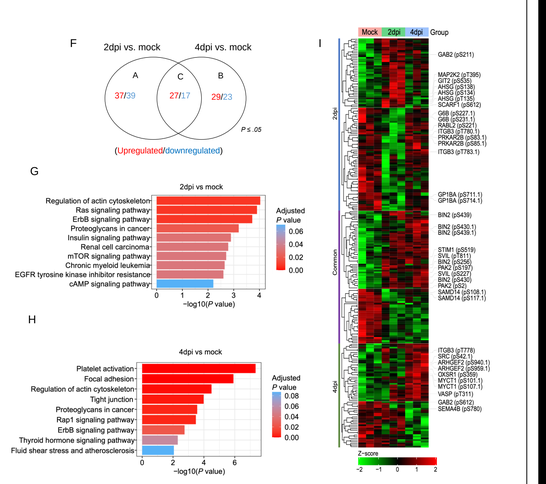

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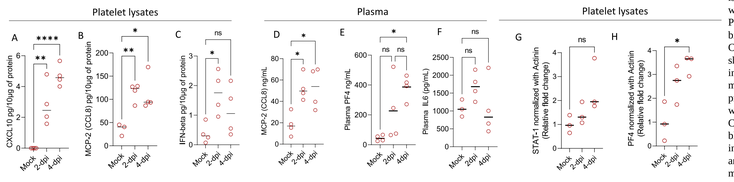

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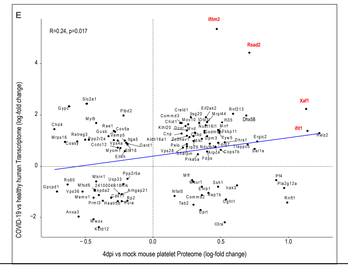

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Saravanan Subramaniam, PhD

Pulmonary Center, Department of Medicine, School of Medicine, Boston University MA, USA

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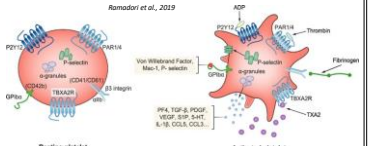

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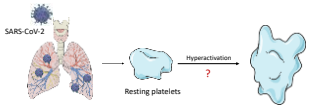

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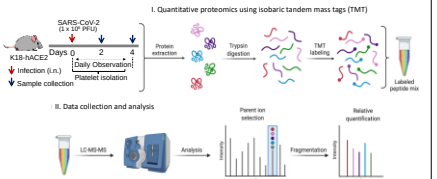

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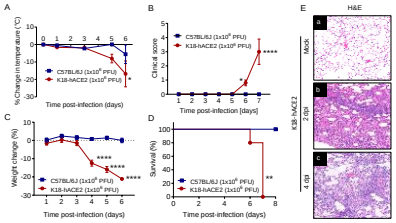

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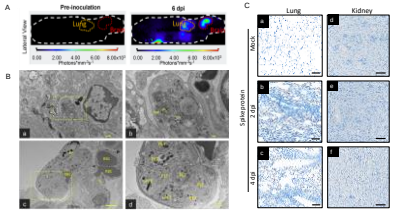

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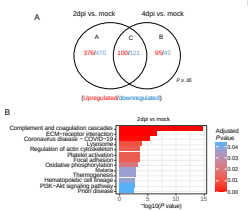

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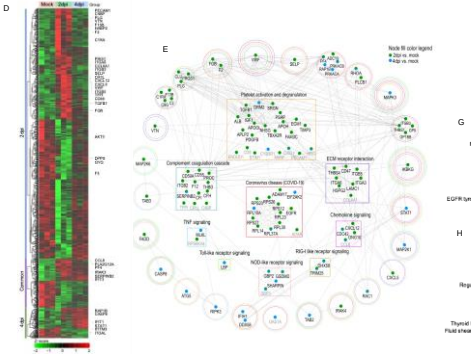
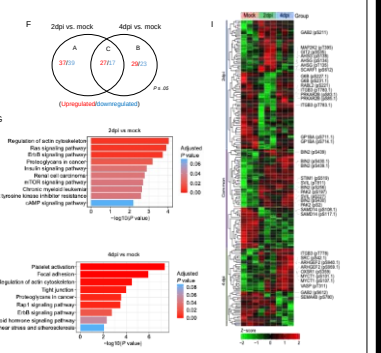


Phosphoproteome



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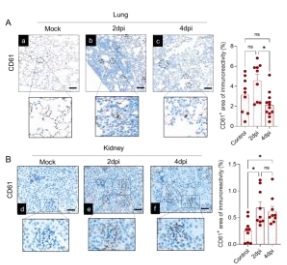

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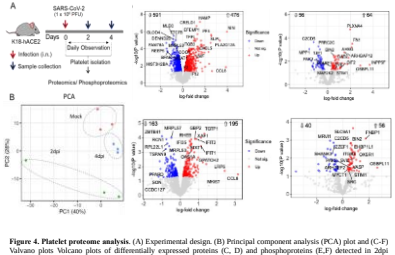
Platelet Proteome Analysis



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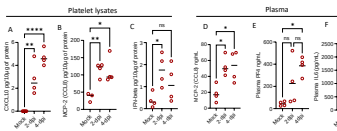

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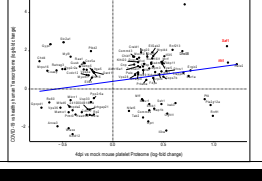
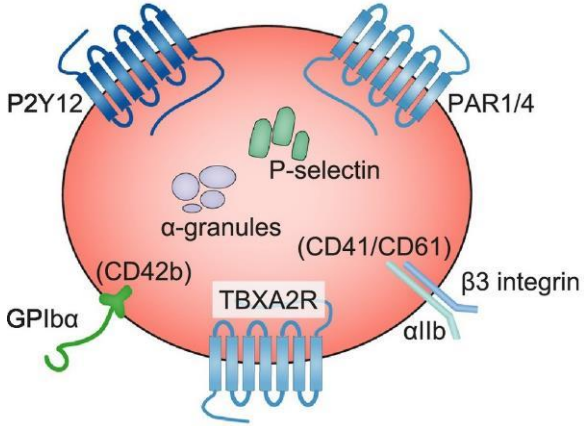
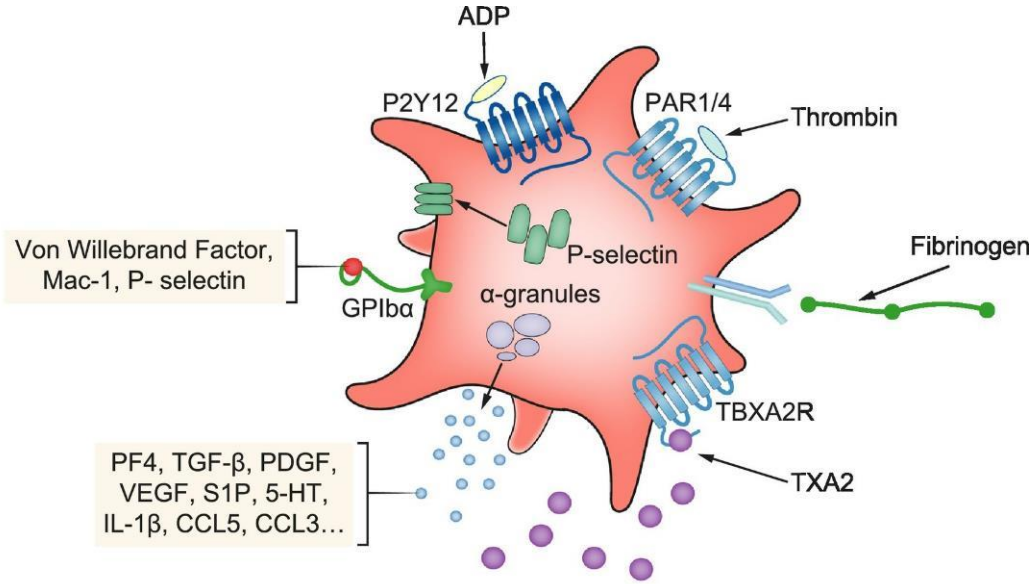


Figure 8. Correlation analysis of mouse proteome with human transcriptomic data from COVID-19 patients) We compared the SARS-CoV-2 infected mouse circulating platelets proteome data with human circulating platelets transcriptomic data from COVID-19 patients (published data set; *Manne et al., Blood 2020*). (E) Pearson correlation analysis of 4-dpi mouse platelet proteome with human COVID-19 patients' platelets transcriptomic data set.

Platelets



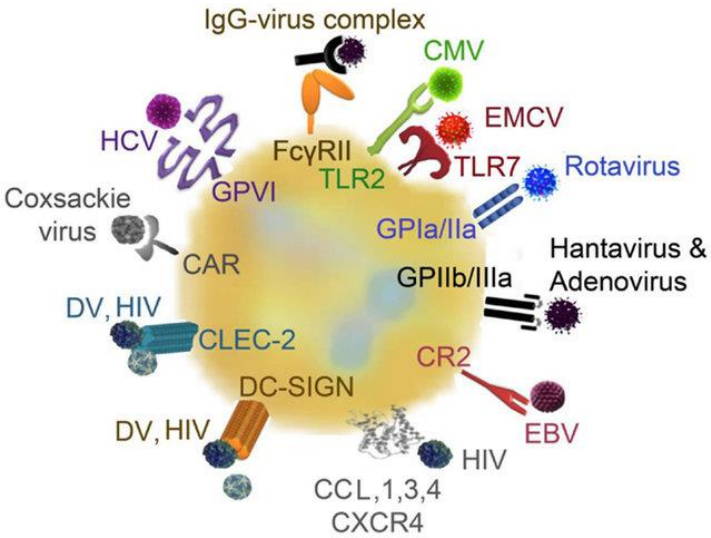
Resting platelet



Activated platelet

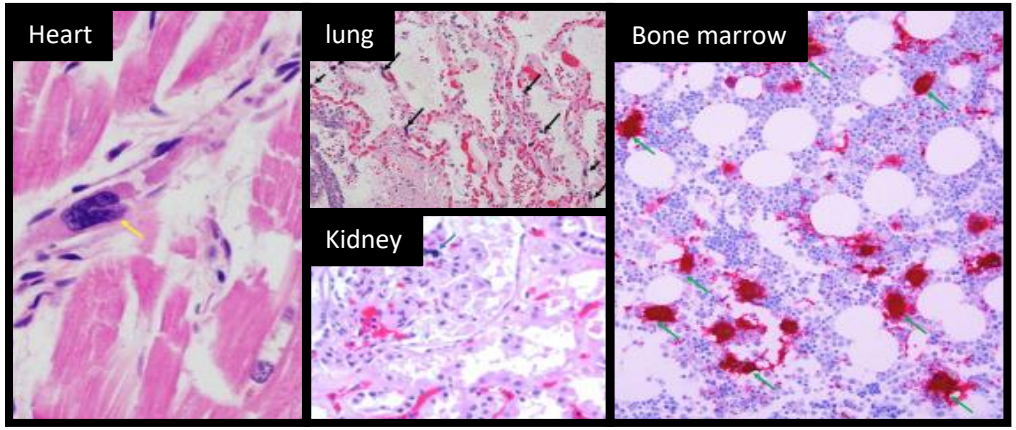
Ramadori et al., 2019

Platelets and viruses



Autopsies (females) – COVID-19

Rapkiewicz, et al., LANCET, 2020



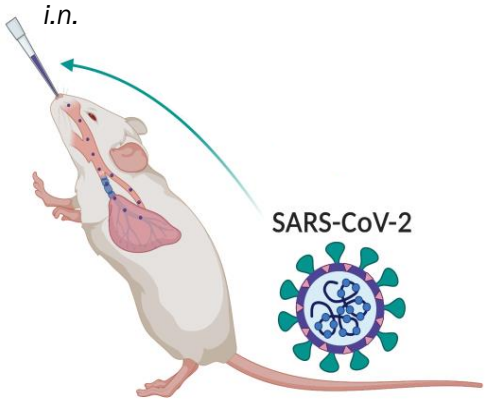
Hematoxylin and Eosin staining (Megakaryocytes and platelets are highlighted by CD61 staining)

1. Does SARS-CoV-2 infect platelets?
2. Understand the phenotypic changes in circulating platelets after SARS-CoV-2 infection

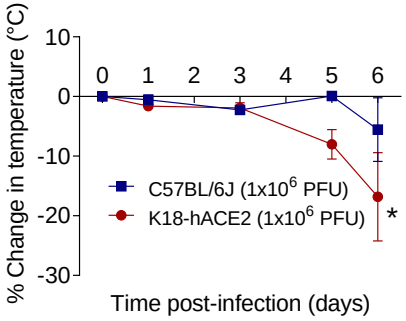
Assinger, 2014

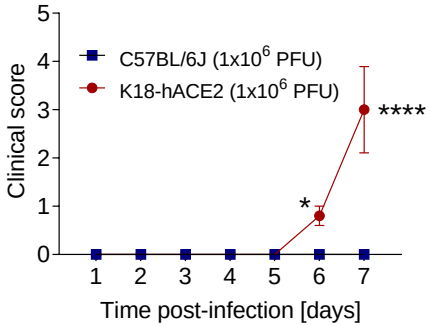
Clinical decline of SARS-CoV-2-infected humanized ACE2 mice

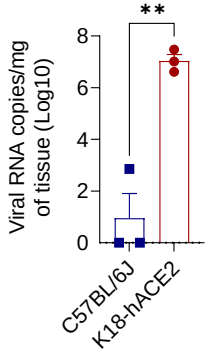
C57BL6J vs. K18-hACE2

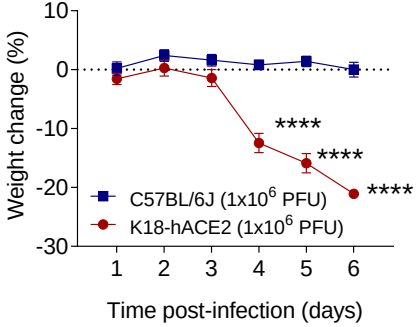


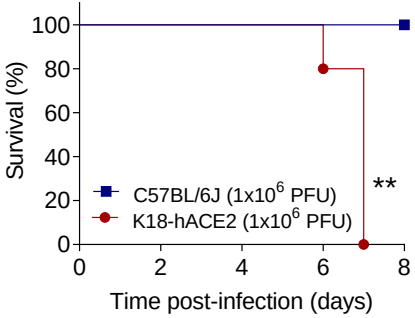
1x10^6 plaque-forming units (PFU)









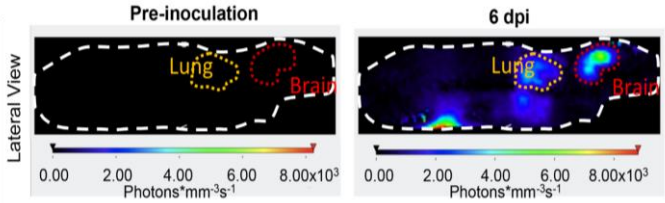


"hACE2 is required for SARS-COV-2 infection in mice"

Subramaniam, et al., BioRxiv, 2022

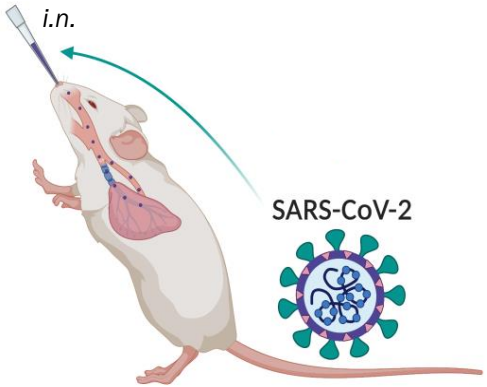
Invasive viral load and ultrastructural findings of lung cross sections

In vivo 3D-imaging



Carossino, et al., Viruses, 2022

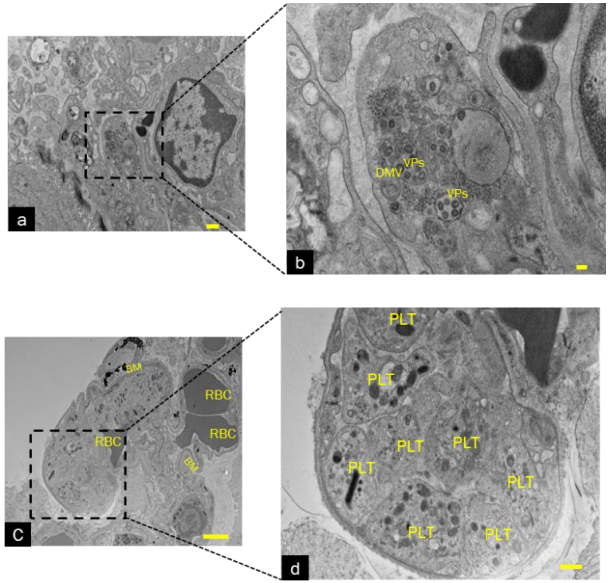
K18-hACE2



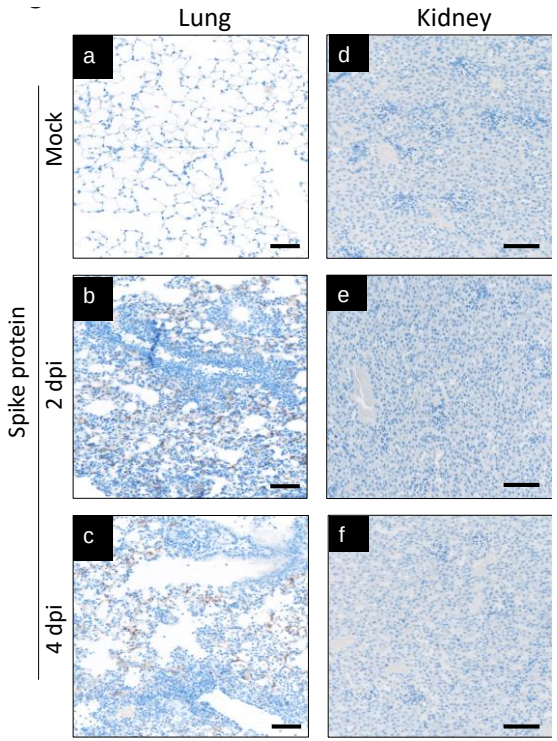
$1x10^6$ plaque-forming units (PFU)

Mock – 2 dpi – 4 dpi – 6 dpi

Transmission electron microscopy (TEM)



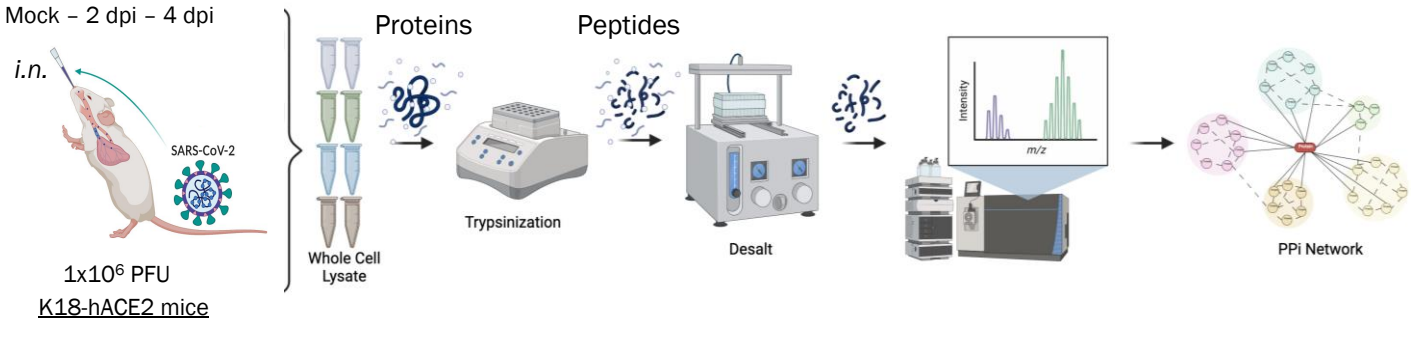
Immunohistochemistry

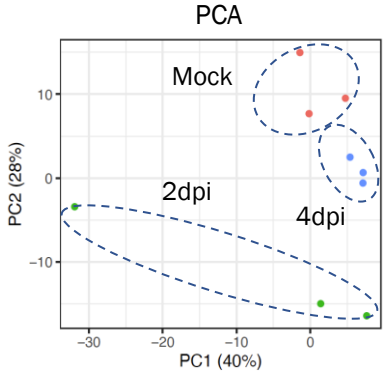


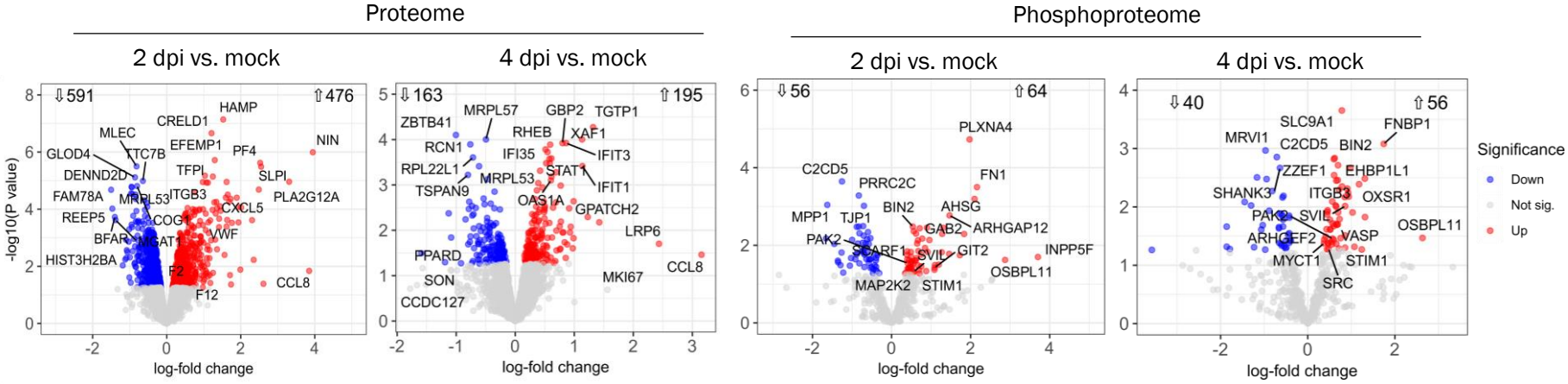
Subramaniam, et al., BioRxiv, 2022

Live virus in lungs and brain, but not in other major organs

Proteome and Phosphoproteome analyses of SAR-CoV-2 infected K18-hACE2 mice

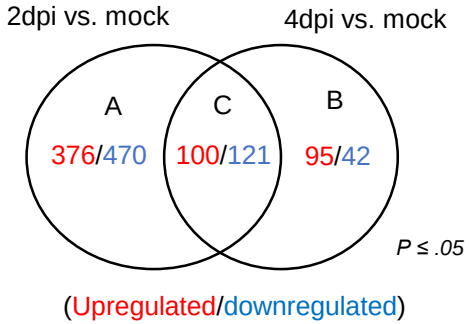


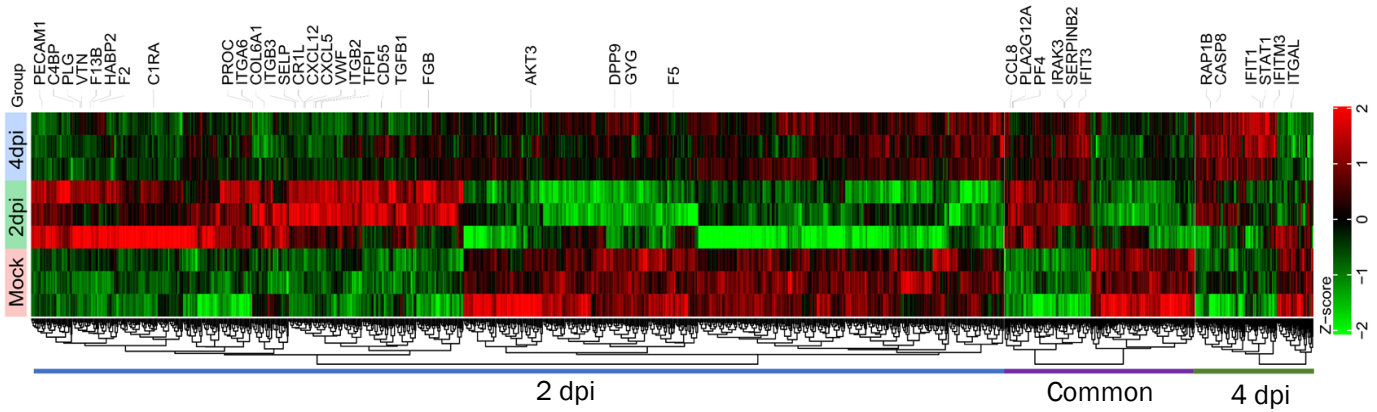


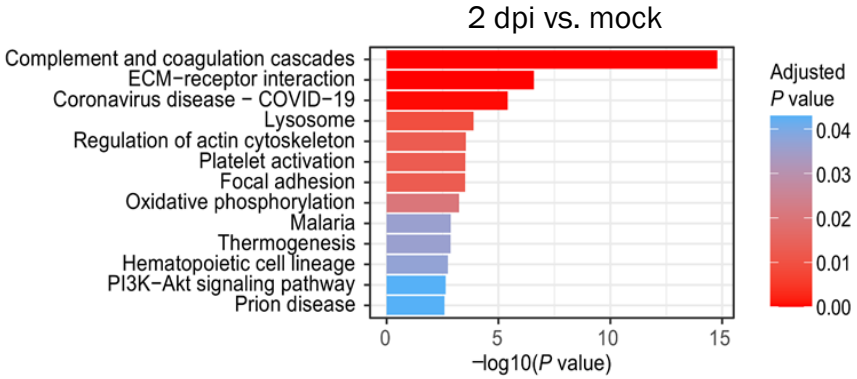


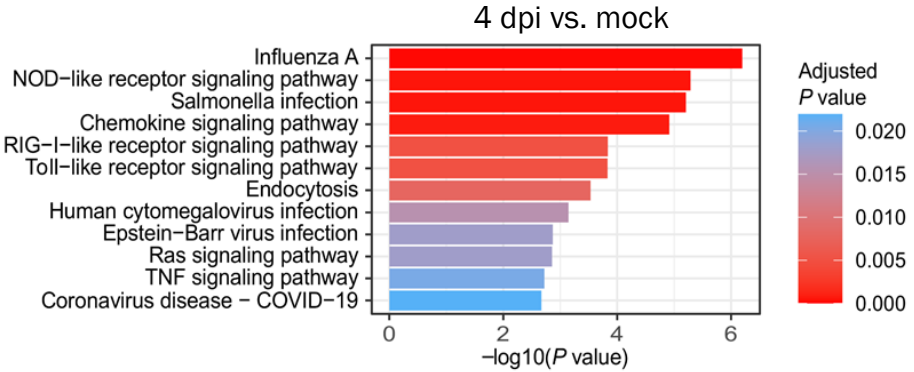
Subramaniam, et al., BioRxiv, 2022

Distinct expression of proteins in circulating platelets after SARS-CoV-2 infection



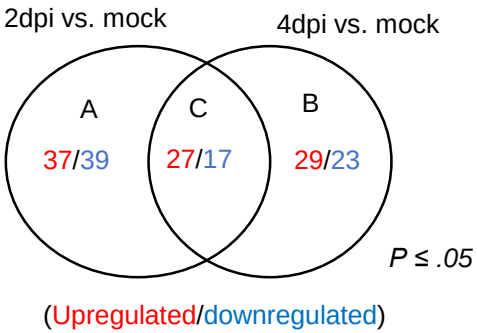


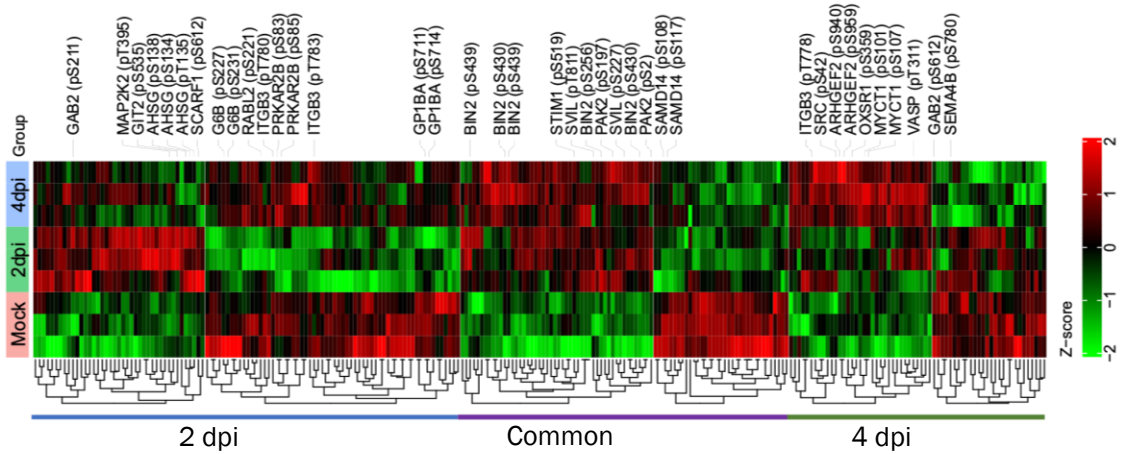


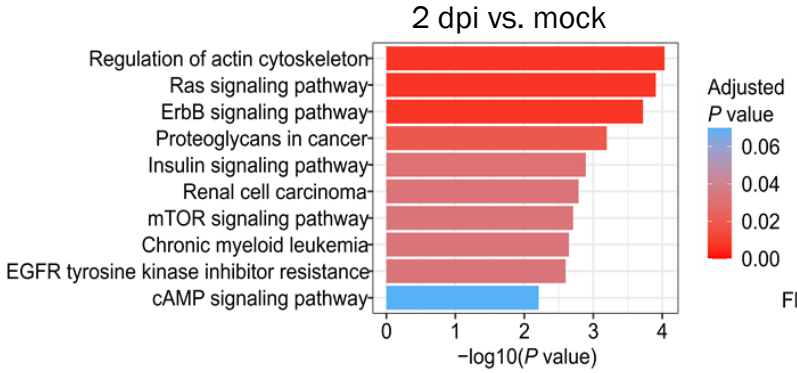


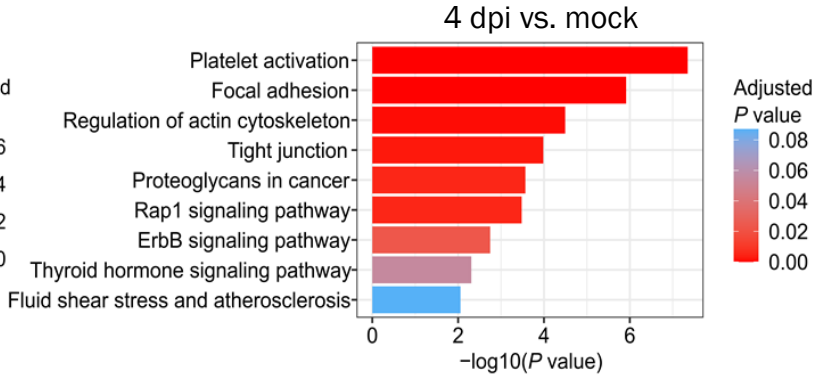
Subramaniam, et al., BioRxiv, 2022

Distinct expression of Phosphoproteins in circulating platelets after SARS-CoV-2 infection



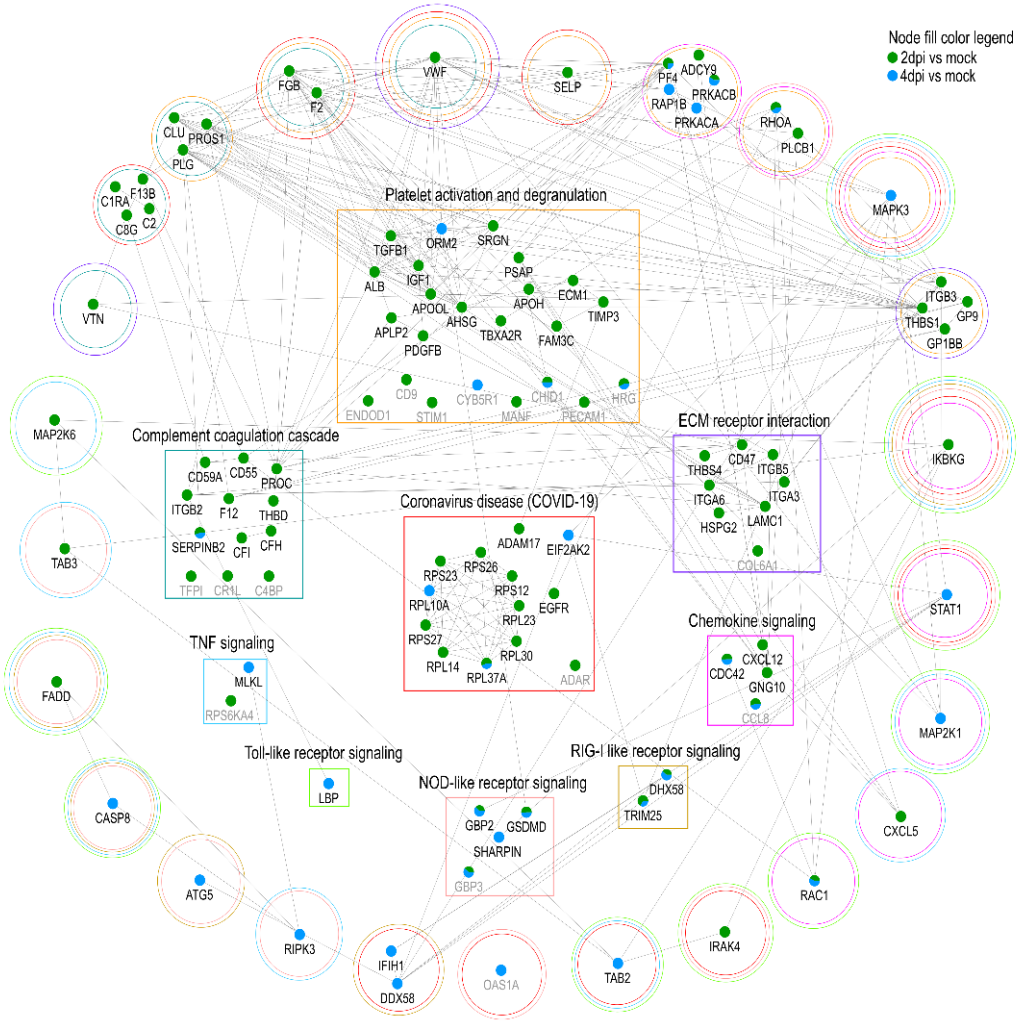






Subramaniam, et al., BioRxiv, 2022

Protein interactome (node map)

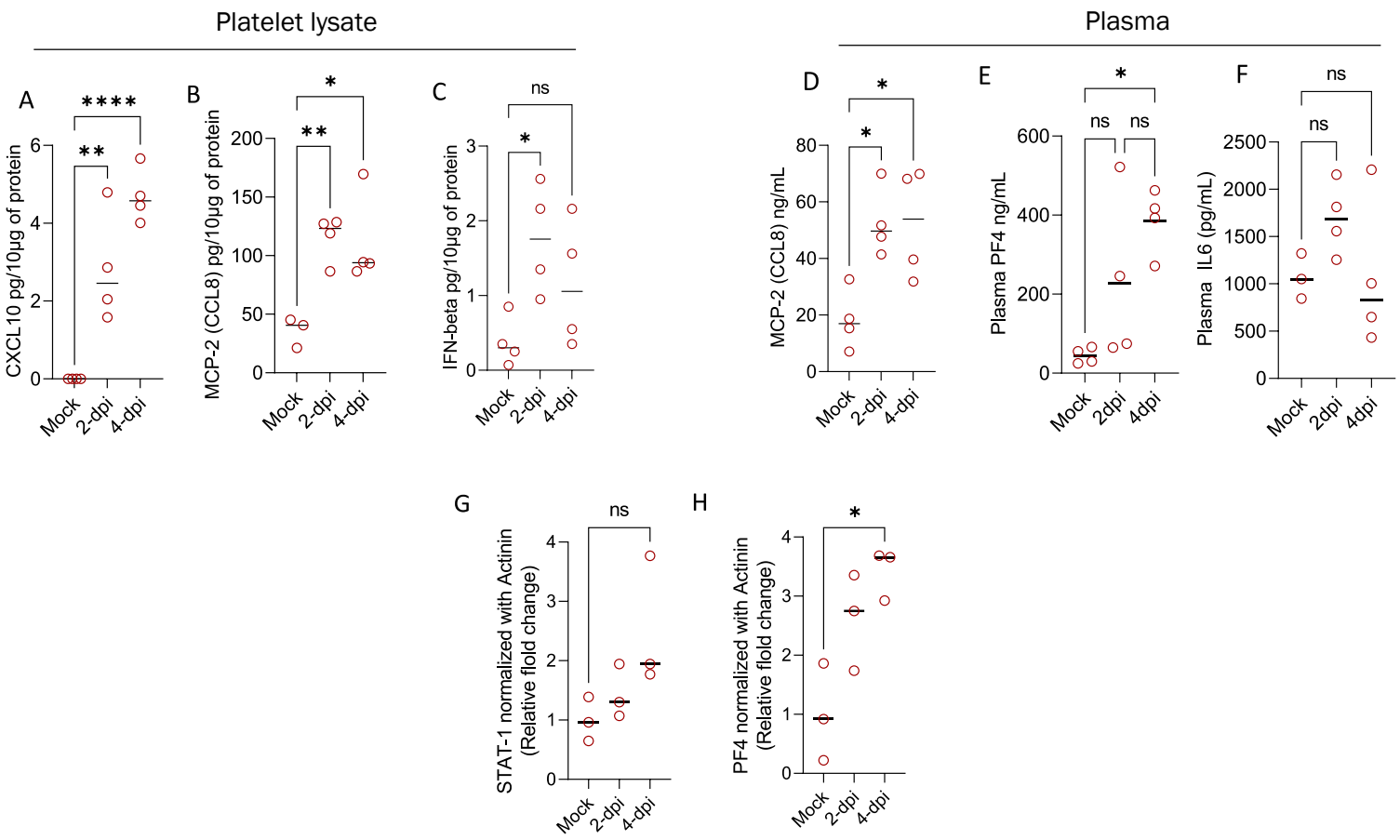


Node fille color legend

- **2dpi vs mock**
- **4dpi vs mock**

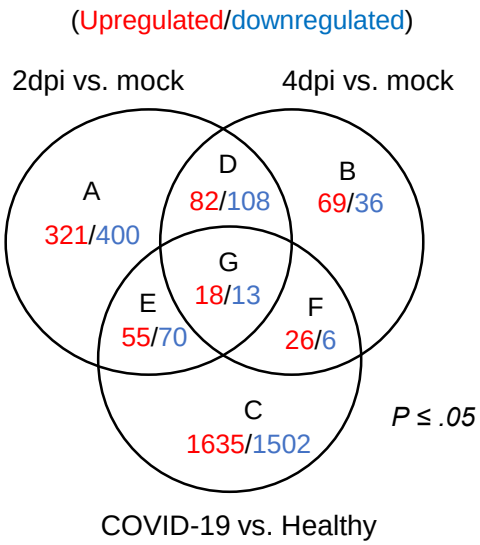
Subramaniam, et al., BioRxiv, 2022

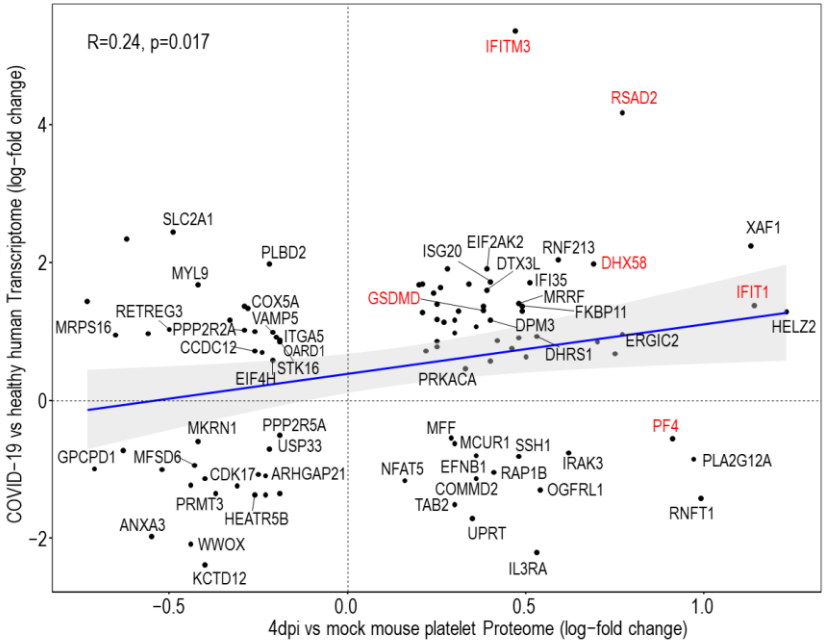
Validation: Platelet lysate/plasma from SARS-CoV-2 infected mice

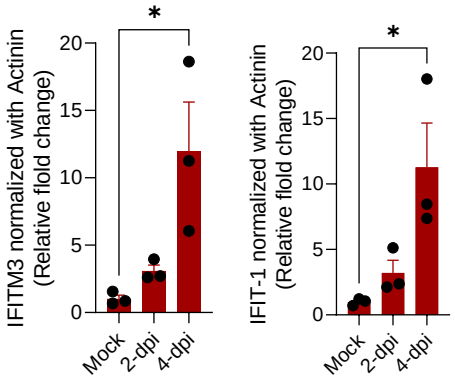


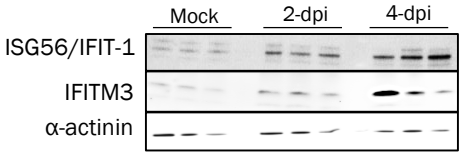
Subramaniam, et al., BioRxiv, 2022

Correlation: Mouse proteome Vs. Human transcriptome







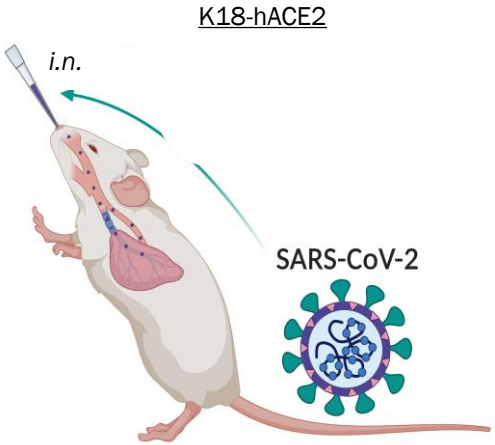


K18-hACE2 mouse can serve as a good animal model to study COVID-19 associated coagulation and immune responses.

Subramaniam, et al., BioRxiv, 2022

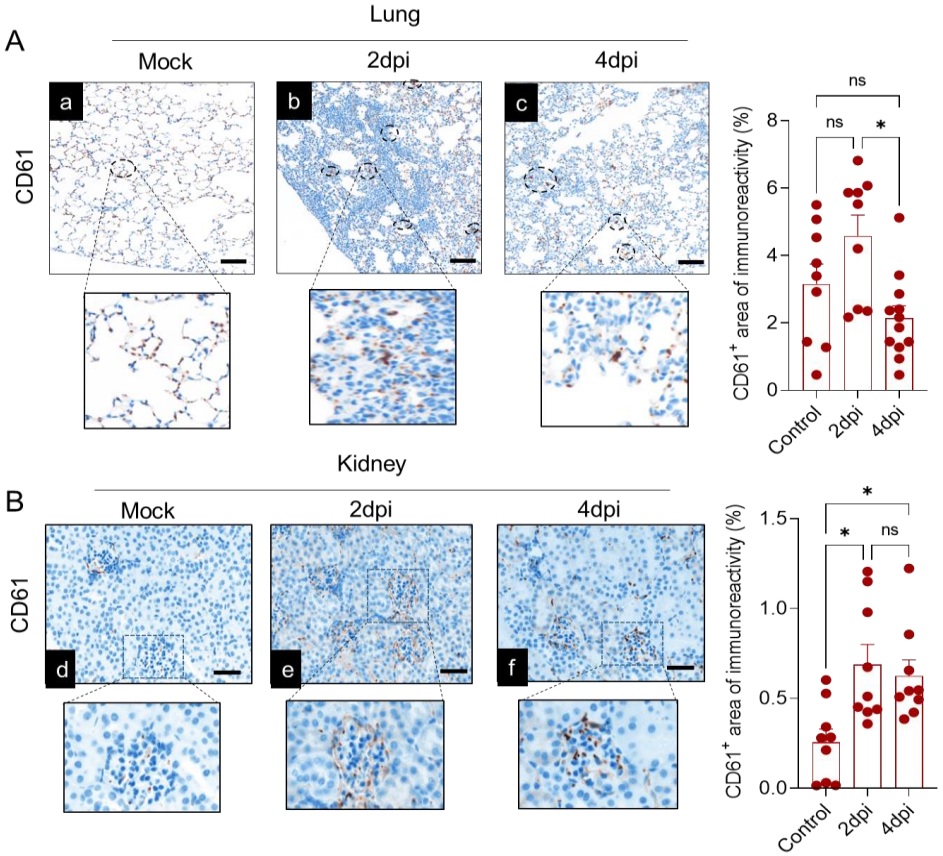
Human COVID-19 patients data set → Manne et al., Blood 2020

CD61 (Integrin B3) aggregates in SARS-CoV-2-infected humanized ACE2 mice



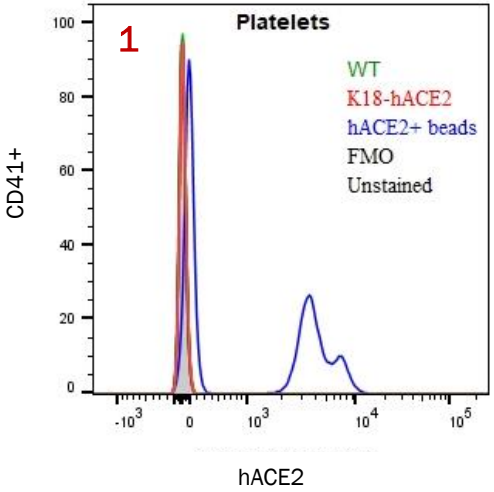
$1x10^6$ plaque-forming units (PFU)

Mock – 2 dpi – 4 dpi

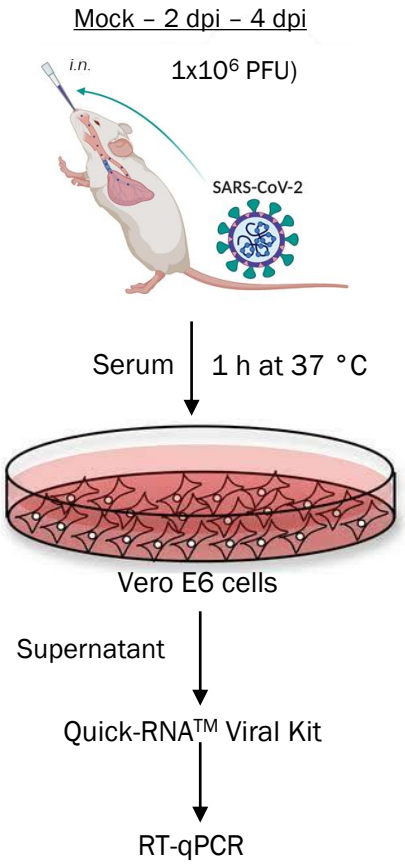


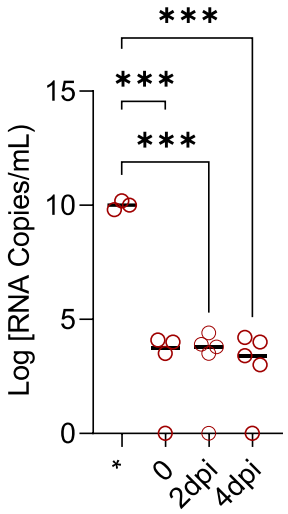
Subramaniam, et al., BioRxiv, 2022

Serum infectivity assay of Vero E6 cells



K18-hACE2 mouse platelets do not express hACE2 receptor





"No detectable infectious viral particles in the serum"

Carossino et al., Viruses, 2022

Subramaniam, et al., BioRxiv, 2022

Conclusions

- our in vivo proteome analysis showed no detectable direct SARS-CoV-2 infection of platelets (early time points) in K18-hACE2 mice.
- SARS-CoV-2 infected K18-hACE2 mice showed early platelet activation-adhesion-degranulation markers.
- Complement-coagulation cascades and hyperactive platelet phenotype were dominant at 2-dpi and interferon signaling was dominant at 4-dpi.
- Abundance of SAR-CoV-2 spike protein in lungs but not in platelets and other organs (kidney), as well as lack of hACE2 on K18-hACE2 mouse platelets, suggests that platelet re-programming towards activation-degranulation-aggregation is likely attributable to pneumonia-induced other factors (such as, cytokines, thrombin)-driven response rather than direct platelet infection.

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