Inflammatory response and pathogenic consequences from SARS-CoV-2 infection

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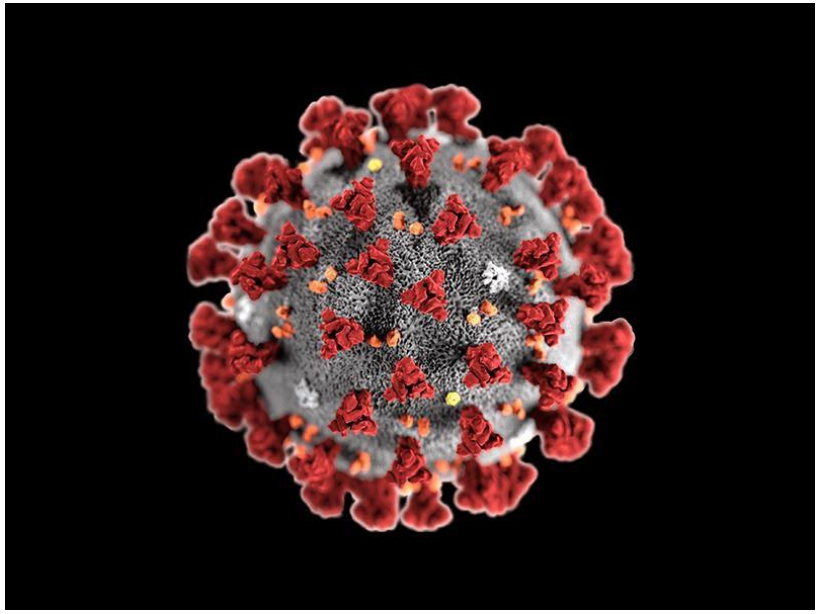
SARS-CoV-2



- Infects lung epithelial cells
- Mostly undetected in blood
- Risk factor for thrombosis and metabolic disorders
- Evidence for replicating in other organs?

How the virus infection initiates complications?

Clinical Course of COVID-19

- **Extremely variable** - some individuals are entirely asymptomatic, others experience fever, anosmia, diarrhea, severe respiratory distress, pneumonia, cardiac arrhythmia, blood clotting disorders, liver and kidney failure, enhanced cytokine release, and death of a small percentage of patients.
- **Clinical outcome depends** on the functionality of host immune system

Inflammatory Response

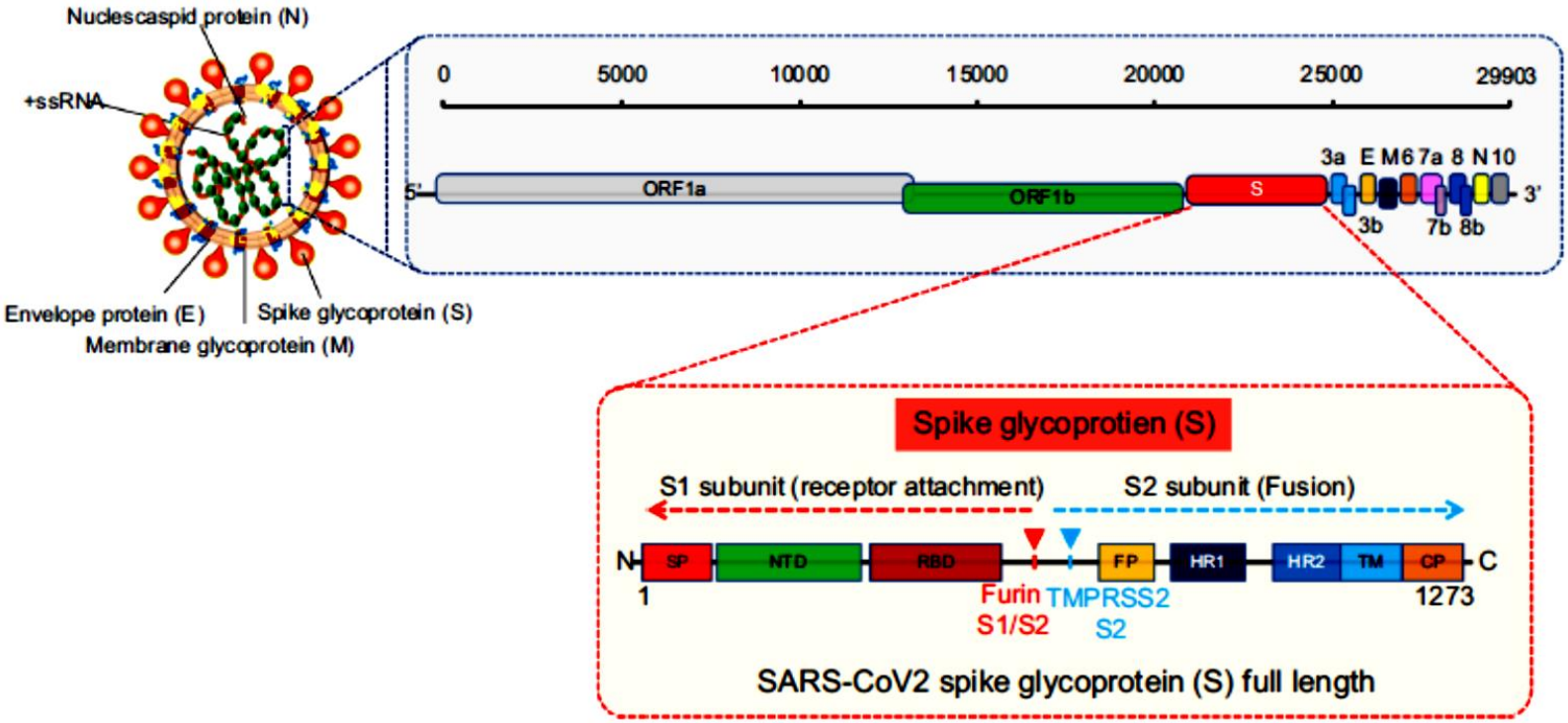
- Cytokine storm is typical of macrophage activation; which leads to tissue damage, lung injury, and acute respiratory distress syndrome.

- Lung epithelial cells; a primary host for SARS coronavirus infection, can generate a cytokine response leading to an expanded pathology.

- We aimed at understanding the mechanisms for SARS-CoV-2 induced inflammatory response

- We designed our experimental studies to understand SARS-CoV-2 and human epithelial cell interactions, by delineating their contribution to inflammatory cytokine systems with special emphasis on ectopic expression of the viral spike protein.

SARS-CoV-2



SARS-CoV-2 is a positive sense RNA virus, genome length -29.9 kb
SARS-CoV-2 contains four structural proteins
S – spikes on the virus external surface; **mediates receptor binding**
M – membrane protein; assists viral assembly
N – nucleocapsid protein; safe guard viral genomic RNA, may interact with M protein during virus budding
E – small envelope protein; function necessary but not fully understood

IL-6/IL-6R Regulations

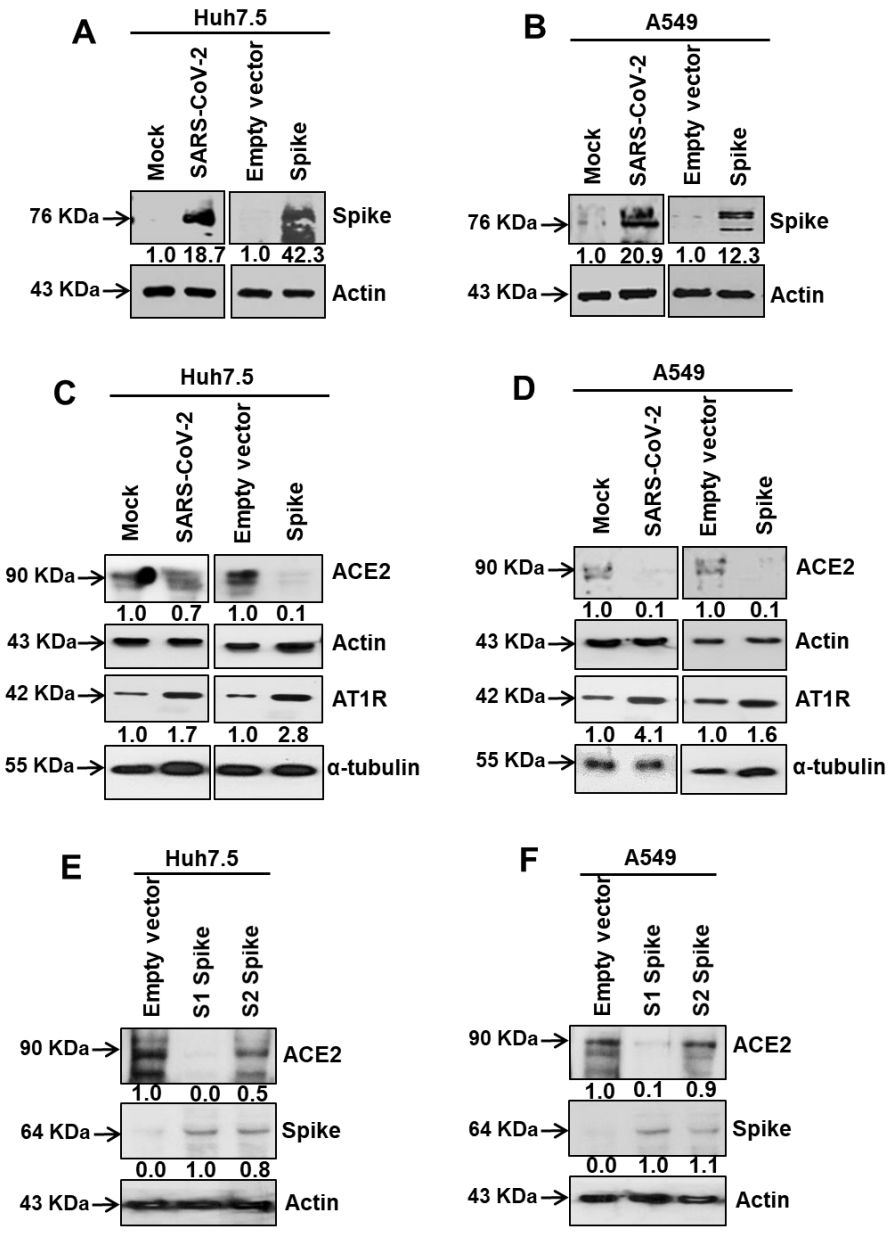
- IL-6 is a pleiotropic cytokine with biological functions that affect tissues beyond the immune system and the vasculature.

- Binding of IL-6 to the transmembrane receptor induces the homo-dimerization of a signal-transducing component, gp130, followed by tyrosine specific phosphorylation of STAT3, and its translocation to the nucleus for generation of inflammatory responses.

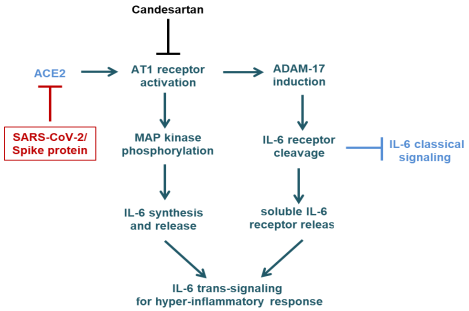
- Signaling via membrane-bound IL-6R (classic signaling) or via a soluble form of IL-6 receptor truncated at the integral membrane portion can bind IL-6 with a similar affinity as its transmembrane receptor.

- The complex of IL-6 and soluble IL-6R can bind to gp130 on cells, which do not express the IL-6R, and which are unresponsive to IL-6 is termed as IL-6 trans-signaling.

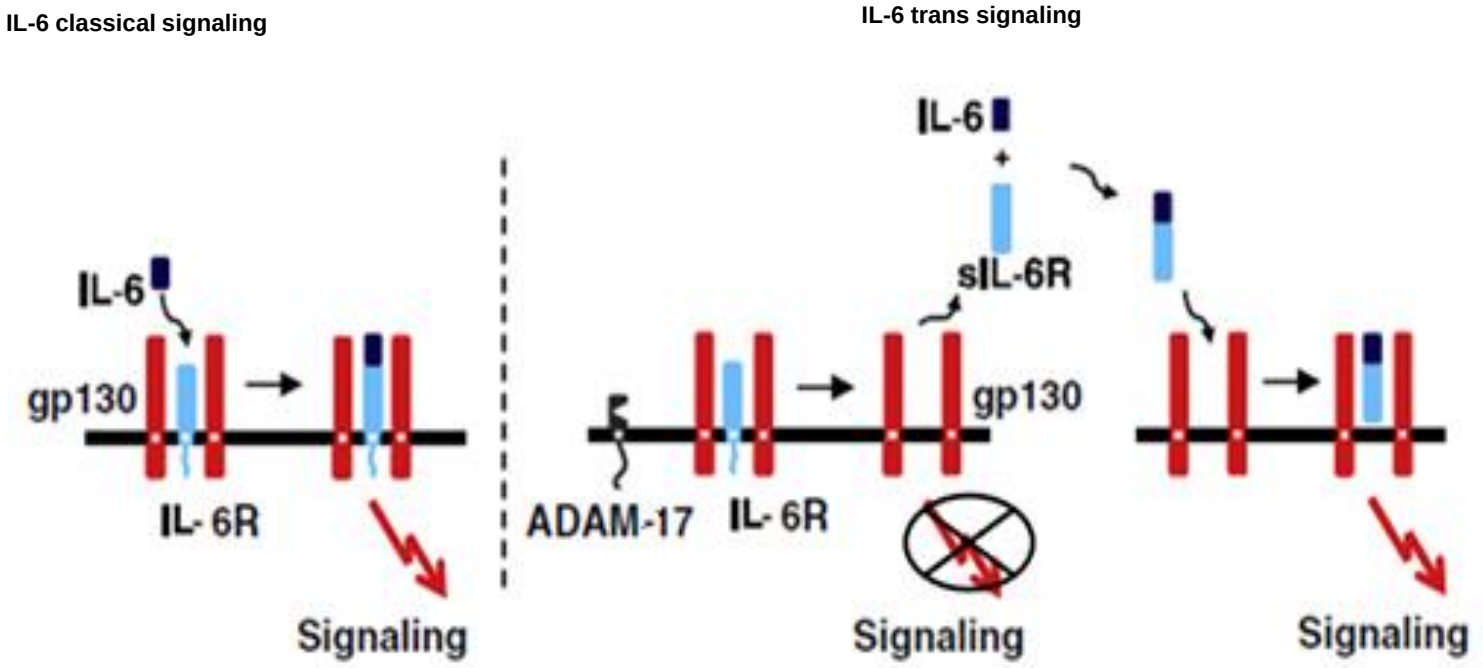
SARS-CoV-2 spike protein inhibits ACE2 expression



Inhibition of ACE2 expression, leads to increased angiotensin II and AT1 receptor expression.

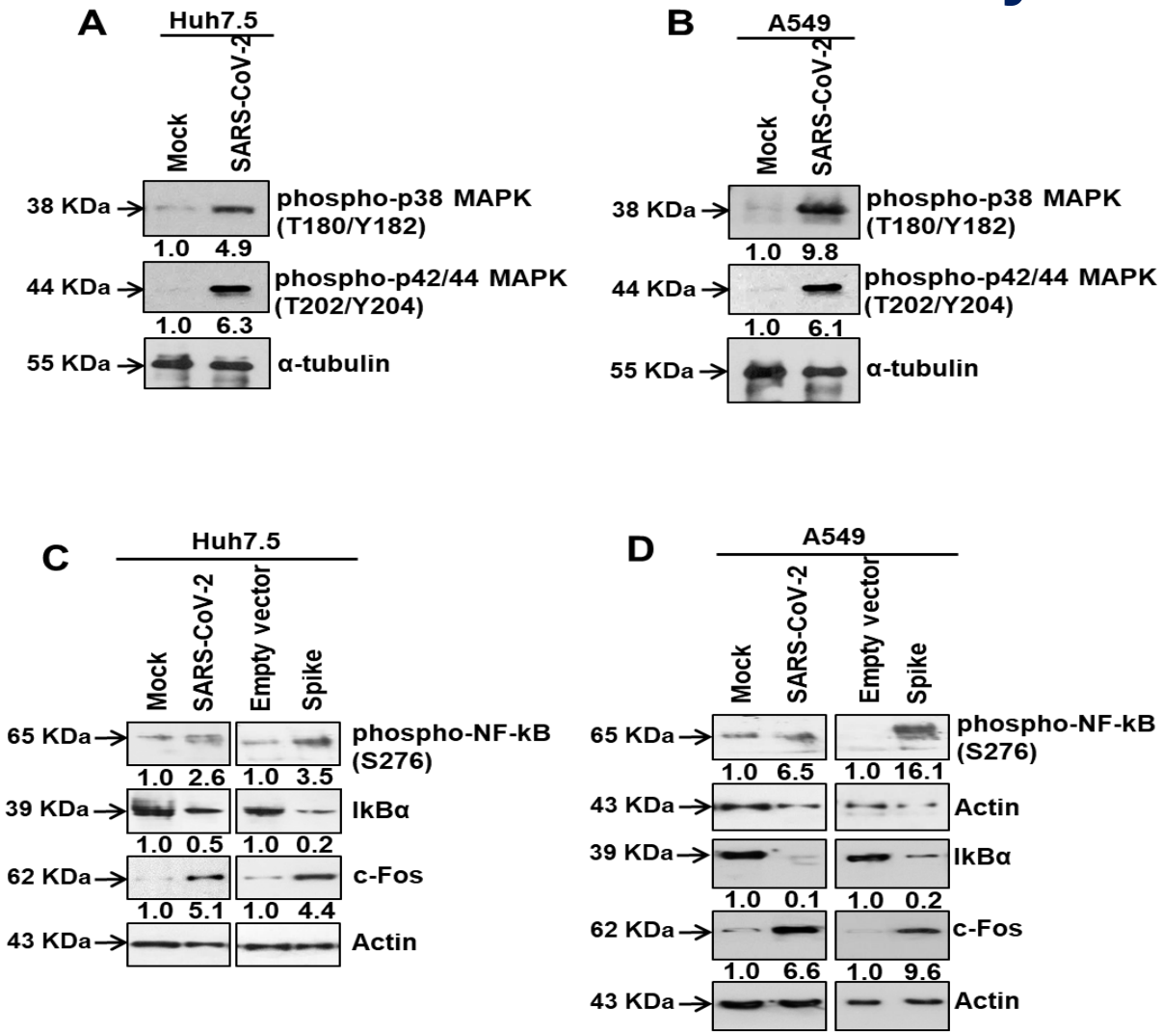


Overview of IL-6 mediated classical and trans-signaling mechanisms

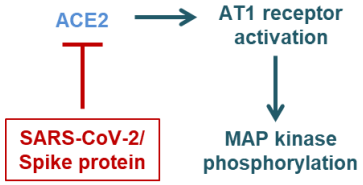


NIH recommends the use of Tocilizumab or Sarilumab for severely ill COVID-19 patients who require only supplemental oxygen, but not high-flow oxygen, invasive mechanical ventilation, or admission to an ICU (Salama *et al*., 2020)

SARS-CoV-2 spike protein activates the transcription factors for IL-6 synthesis

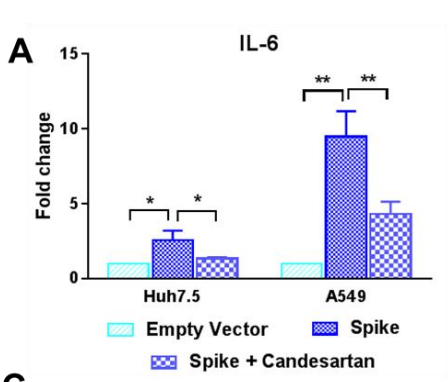


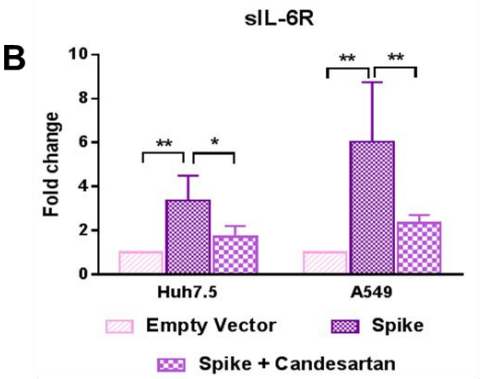
MAPK regulates NF-κB activation for cytokine synthesis

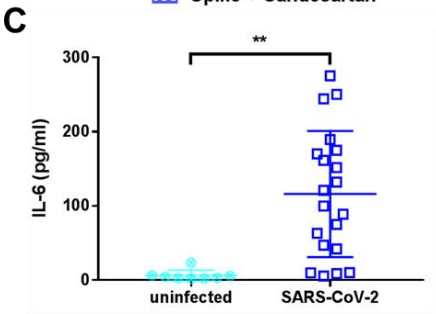


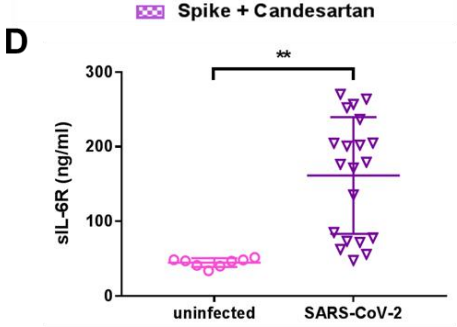
- Angiotensin II receptor blocker medications were associated with a reduced risk of hospitalization and admission to the ICU in Covid-19 patients (Rubin *et al*., 2020)
- Dilmapimod and Losmapimod are the novel p38 MAPK inhibitors and a phase II clinical trial with these inhibitors suggested prevention of acute respiratory distress syndrome.

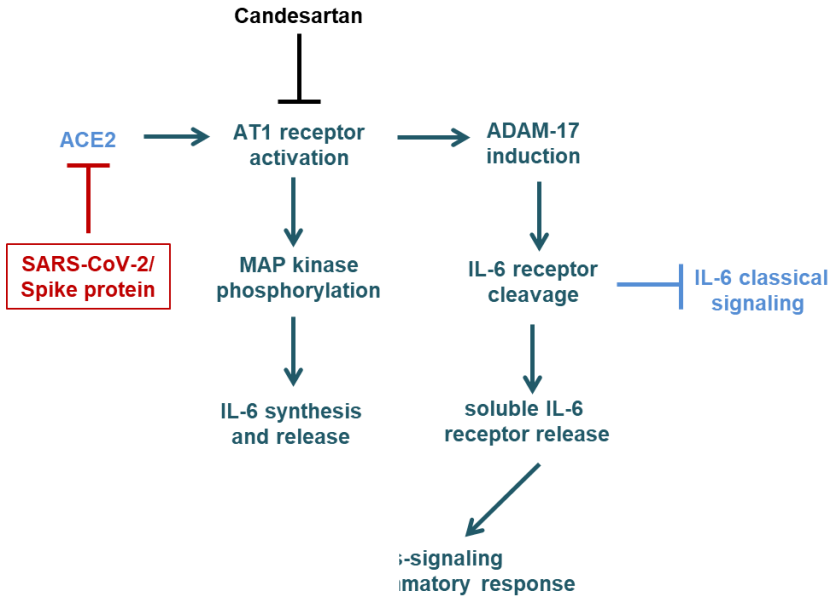
SARS-CoV-2 spike protein stimulates IL-6 and soluble IL-6R production

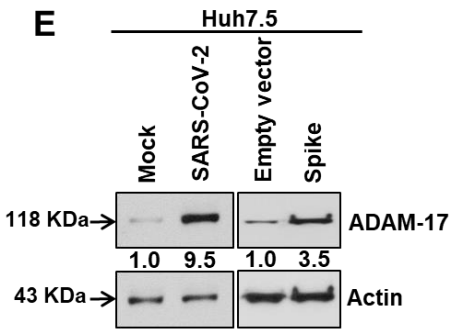


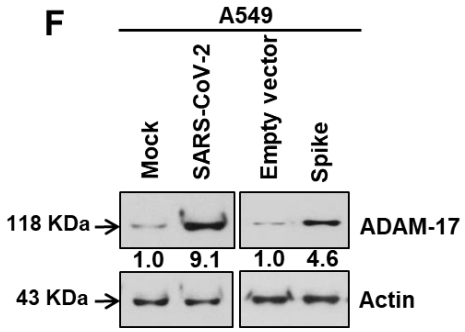






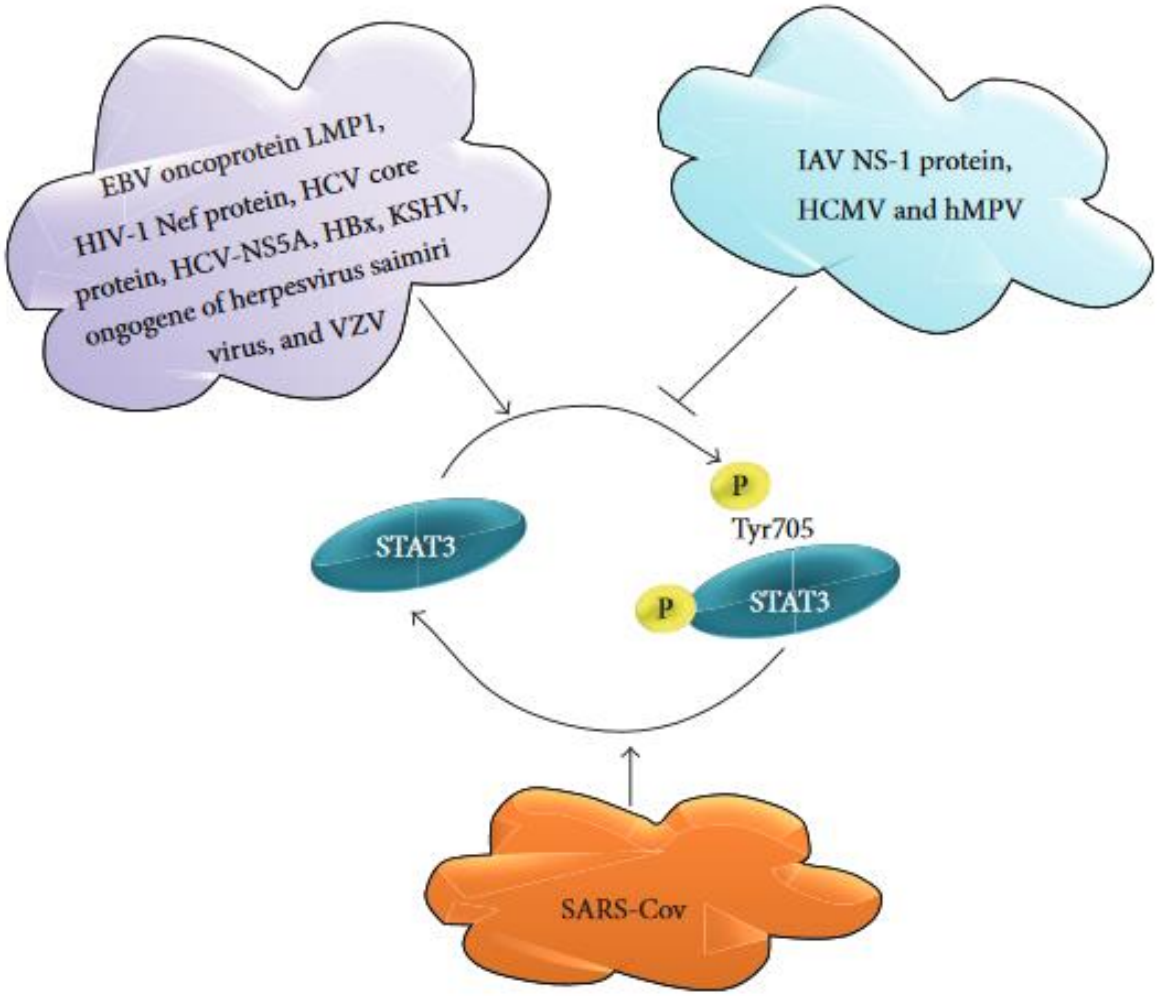






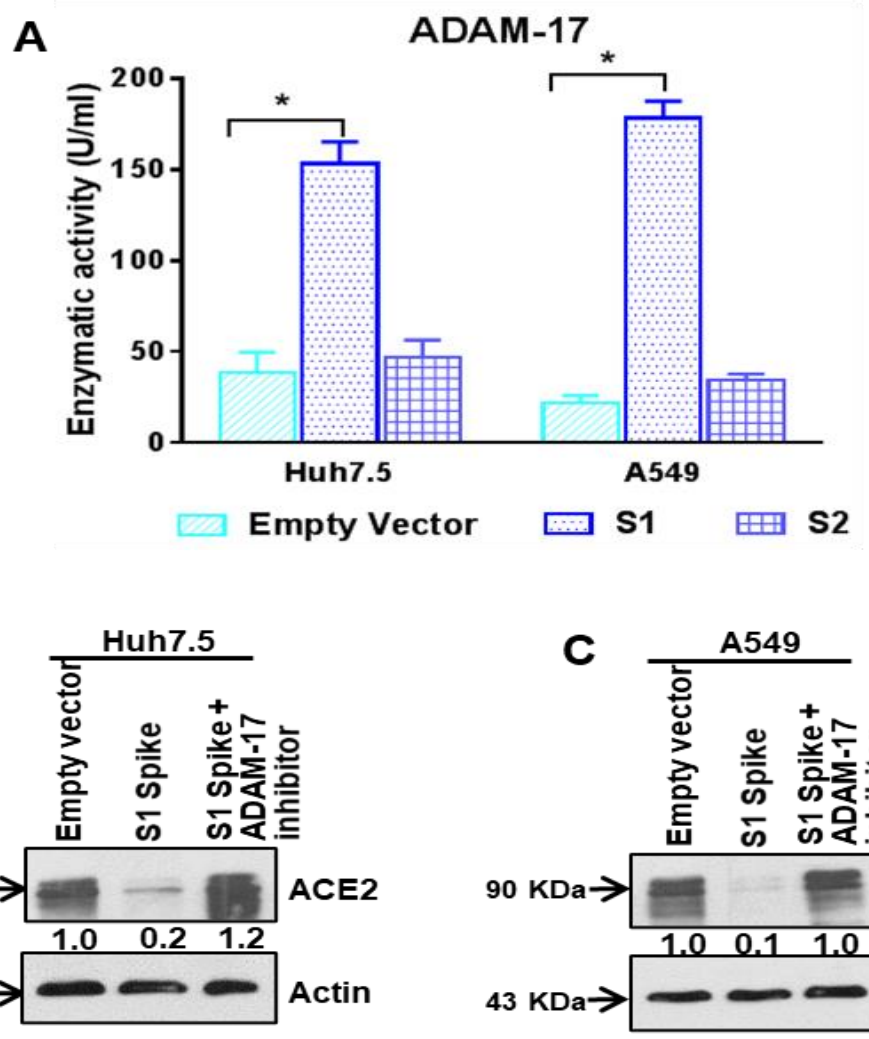
ADAM-17 metalloprotease is responsible for generation of soluble IL-6R by cleavage of transmembrane domain

STAT3 Regulation in Viral Infection

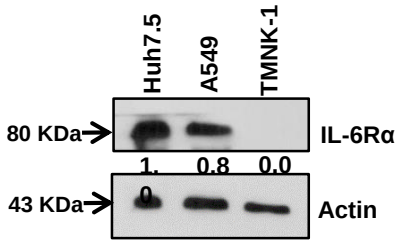


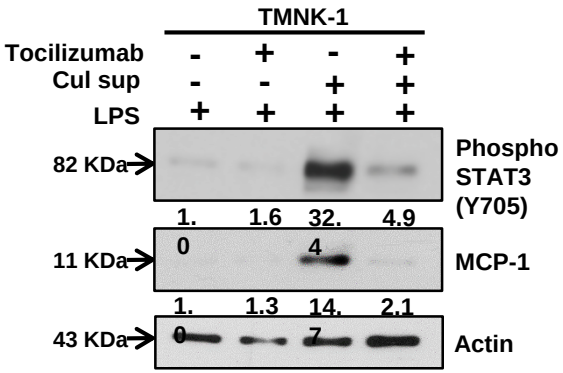
SARS-CoV-2 spike protein causes inhibition of tyrosine phosphorylation of STAT3

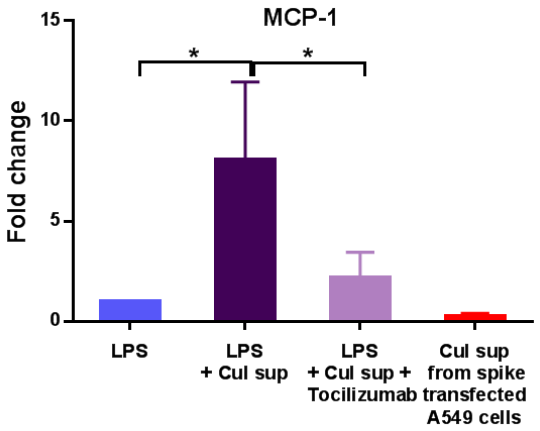
SARS-CoV-2 spike protein regulates ADAM-17 activity

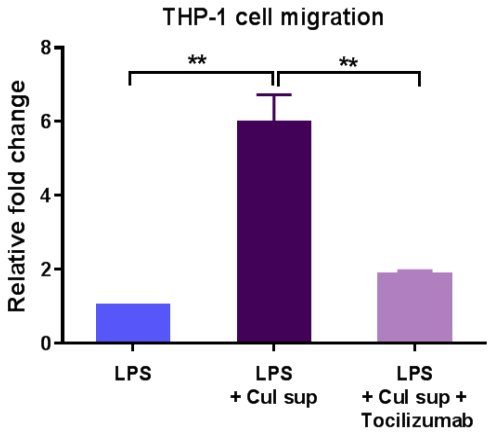


Activation of IL-6 trans-signaling from SARS-CoV-2 spike protein expressing epithelial cells forms an inflammatory circuit in endothelial cells



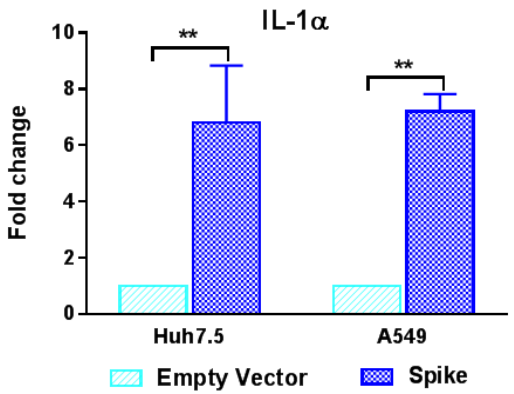


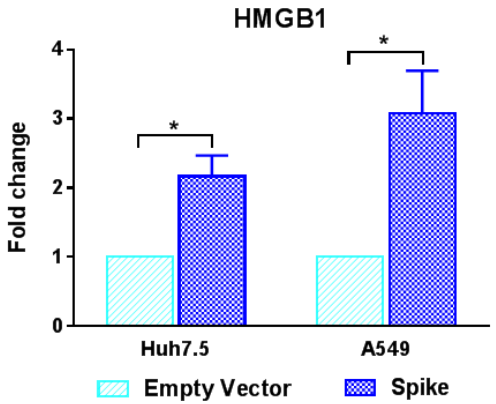




TMNK-1 liver endothelial cells poorly express IL-6Rα. TMNK-1 cells stimulated by LPS were treated with or without culture supernatant from SARS-CoV-2 spike expressing A549 cells in the presence or absence of an AT1 receptor antagonist. The results show an increased phosphoStat3 (Tyr705) expression in TMNK-1 cells which was not inhibited by AT1 receptor blockage. Tocillizumab significantly suppressed Stat3 phosphorylation. The results suggested SARS-CoV-2 spike protein activates IL-6 trans-signaling, but not classical signaling in epithelial cells leading to a hyper-inflammatory response.

SARS-CoV-2 spike expressing epithelial cells release alarmin molecules for surrounding or distant effects

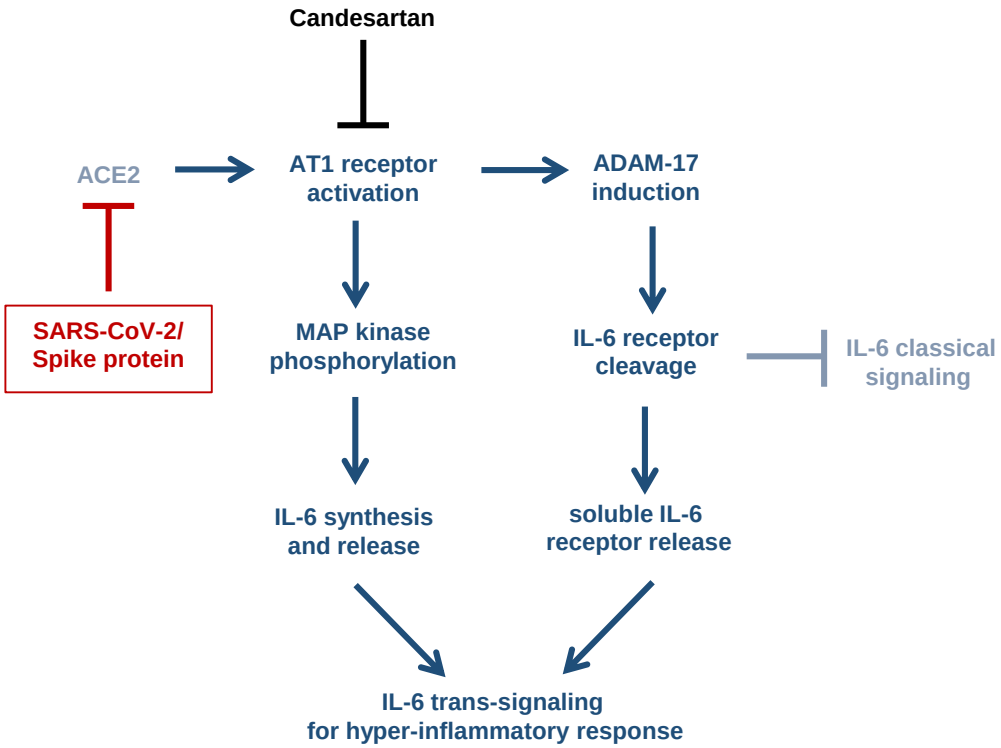




Alarmins are released from deleterious response in the host provoking an uncontrolled inflammatory activity. This group of proteins includes IL-1α and the high-mobility group box 1 protein (HMGB1).

Circulating IL-1α and HMGB1 level in COVID-19 patients were observed (Del Valle et al., 2020; Chen et al., 2020)

Summary



The presence of SARS-CoV-2 spike protein in epithelial cells promotes IL-6 trans-signaling (upon endothelial cells) by activation of the AT1 axis to initiate co-ordination of a hyper-inflammatory response

(Patra et al., PLoS Pathogens, 2020)

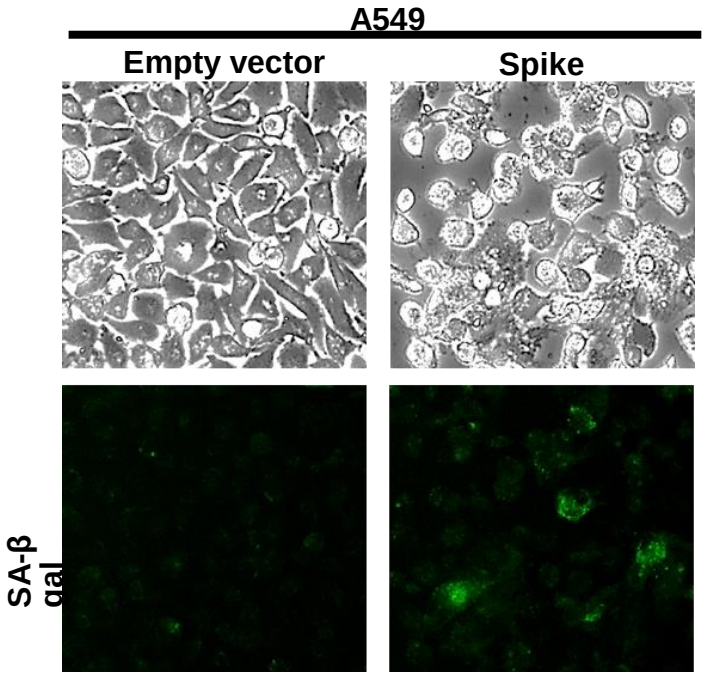
SARS-CoV-2 spike protein causes endothelial senescence initiating leukocyte extravasation

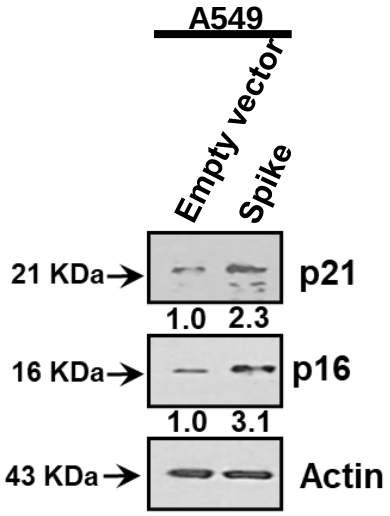
- We observed an increased IL-6, IL-1α and HMGB1 from SARS-CoV-2 spike expressing epithelial cells

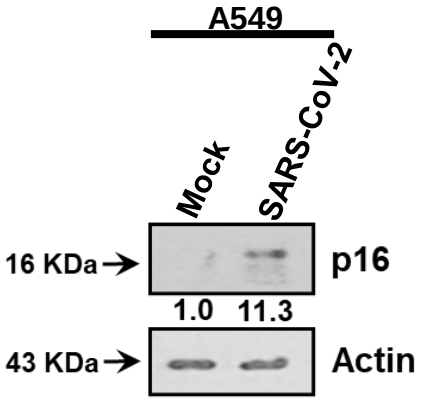
- The inflammatory molecules are released by senescence-associated secretory phenotype (SASP)

We asked whether spike expressing epithelial cells undergo senescence mechanism?

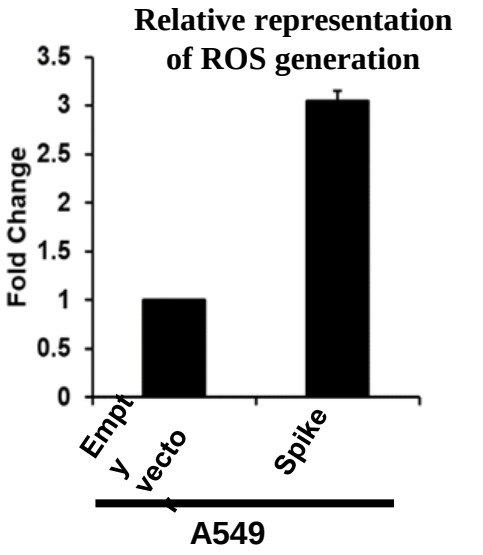
SARS-CoV-2 spike protein induces senescence markers in A549 lung epithelial cells

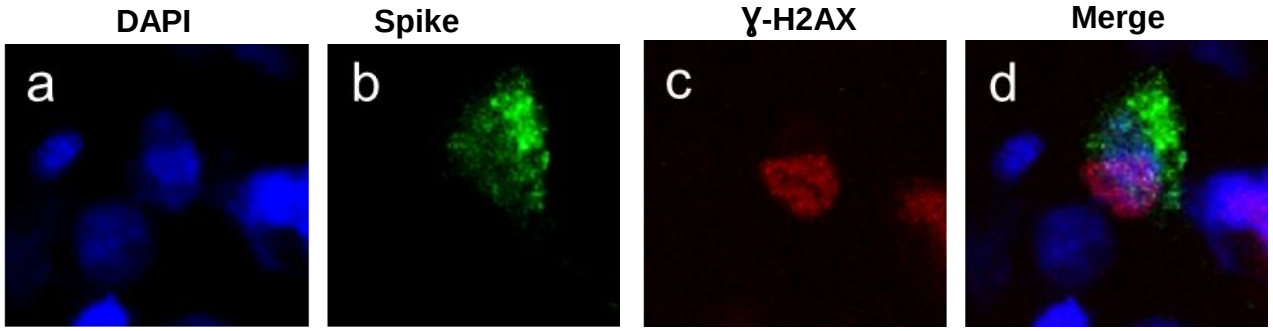




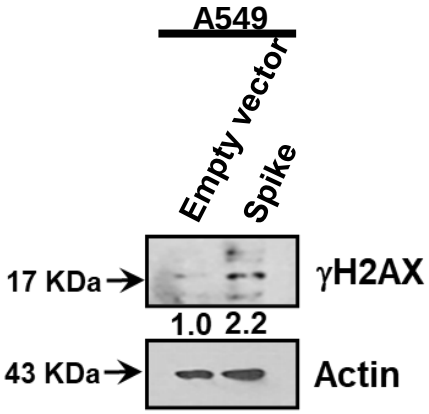


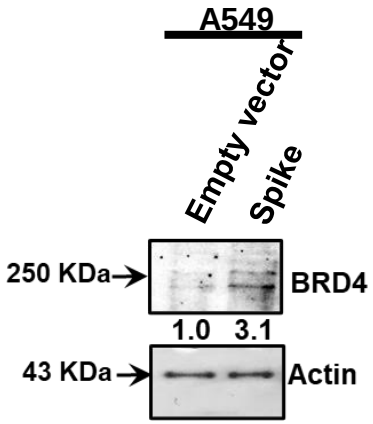
Spike protein expression generates oxidative stress in A549 cells



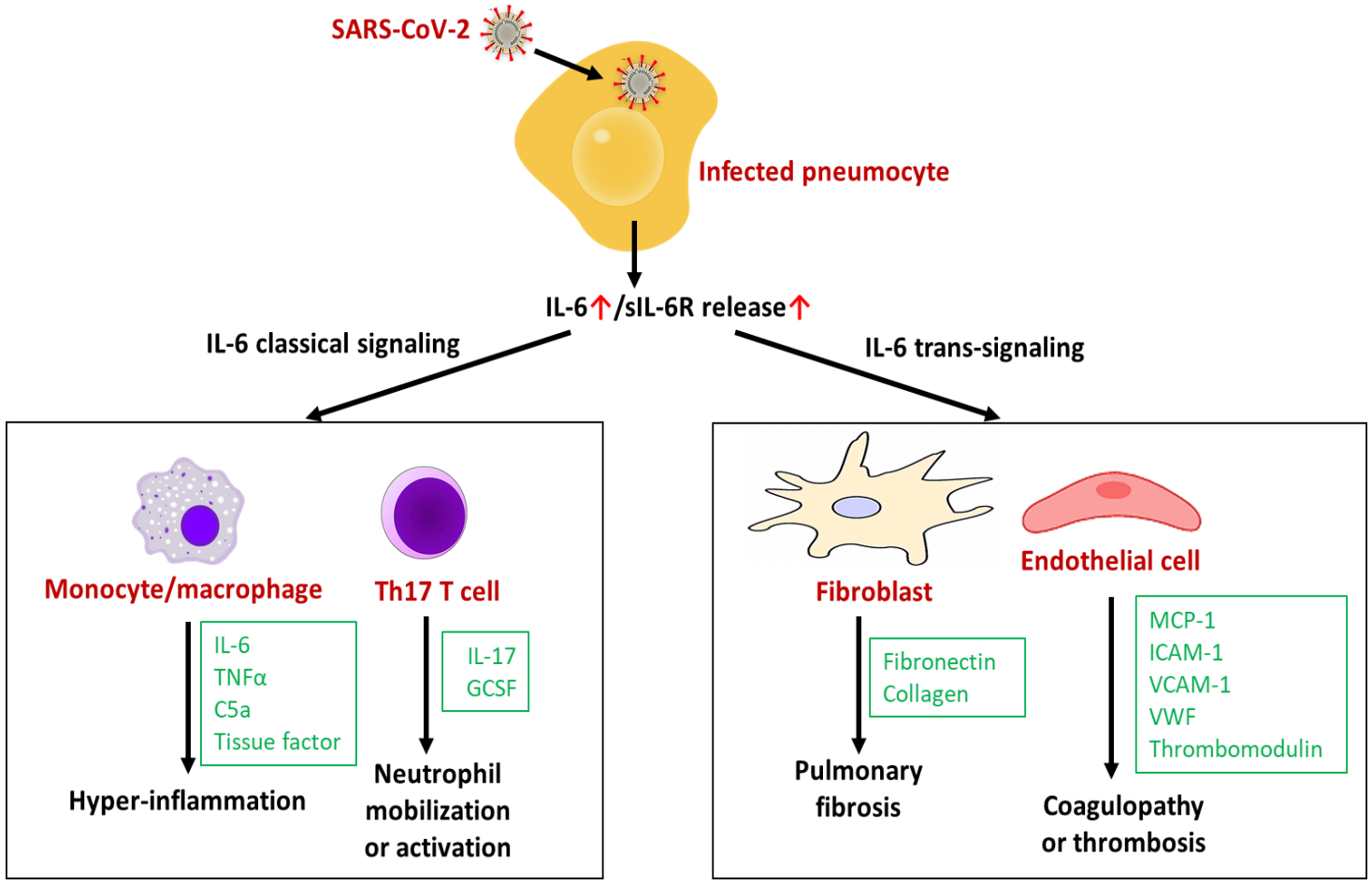


Localization of ɣ-H2AX and spike protein in A549 cells

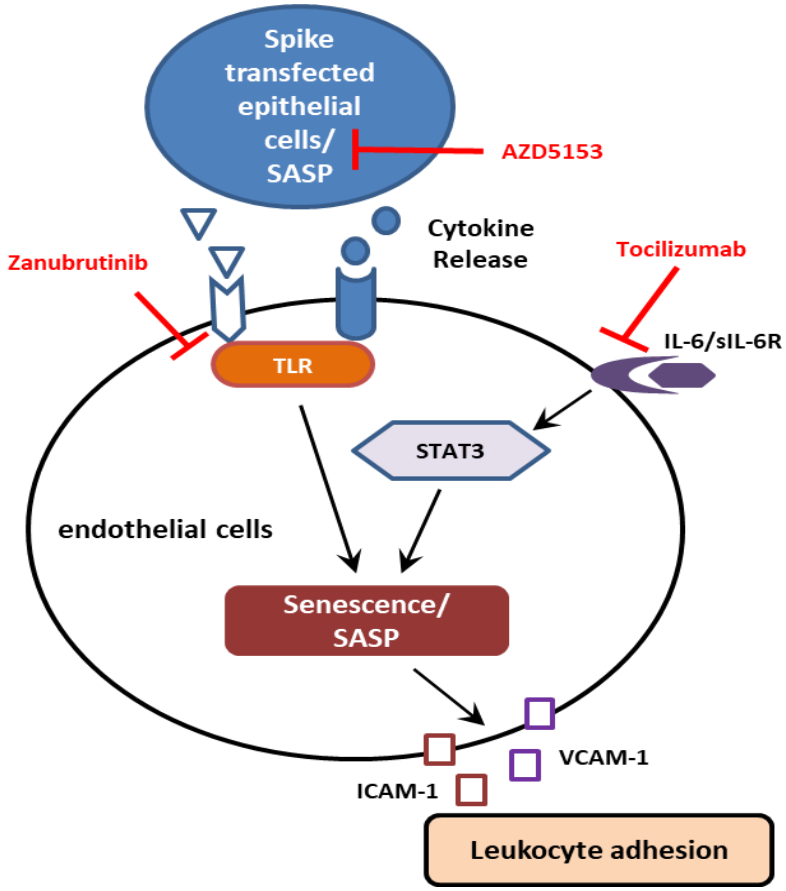




IL-6 mediated pathogenesis in SARS-CoV-2 infection of pneumocytes and potential consequences on other cells



Summary:



Epithelial cells expressing SARS-CoV-2 spike protein induces a paracrine mode of endothelial senescence which ultimately leads to enhanced leukocyte adhesion with microvascular complications

(Meyer et al., J. Virol., 2021)

How SARS-CoV-2 infected cells communicate with distant organs?

We have shown circulatory exosomes from COVID-19 patients trigger NLRP3 inflammasome in endothelial cells.

(Sur et al., mBio. 2022)

Acknowledgments

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