

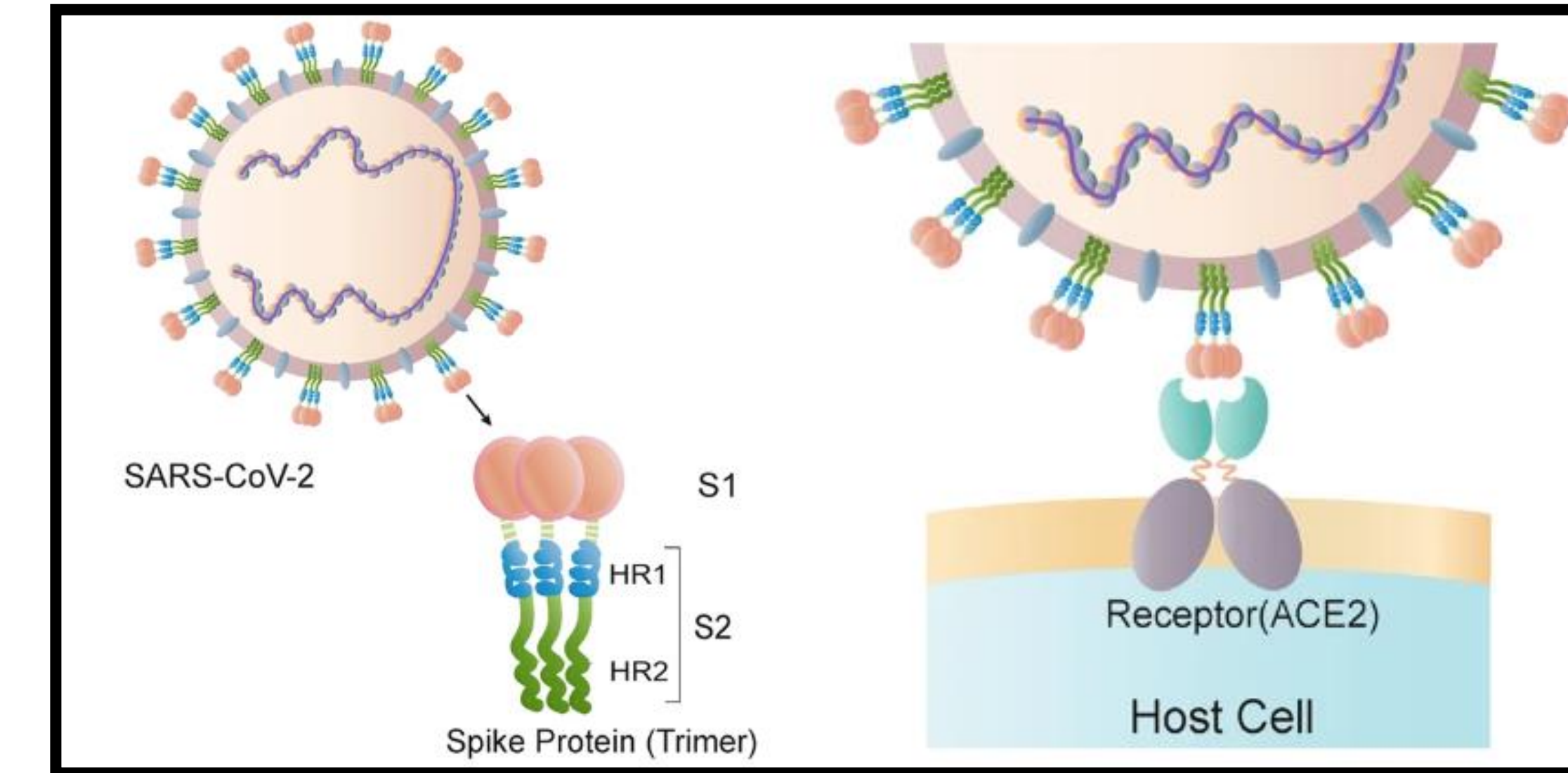
# Recombinant SARS-CoV-2 spike protein is not sufficient to initiate an inflammatory response in human alveolar epithelial cells in vitro

## Background

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## Hypothesis

Biosafety level-3 (BSL-3) facilities are a necessary safety measure to investigate the SARS-CoV-2 virus but also limit the pace of elucidating the mechanisms that lead to the hyperinflammatory response and acute lung injury in some patients with COVID-19. One solution is to develop a non-infectious model for COVID-19. The SARS-CoV-2 spike protein is a promising candidate for such a model as this viral component binds ACE2 to initiate endocytosis into the respiratory epithelium. Prior studies established the basis for this idea, showing that the spike protein alone can be internalized into cells expressing ACE2, and were shown to produce an inflammatory response in macrophages.



Huang et al., Nature 2020

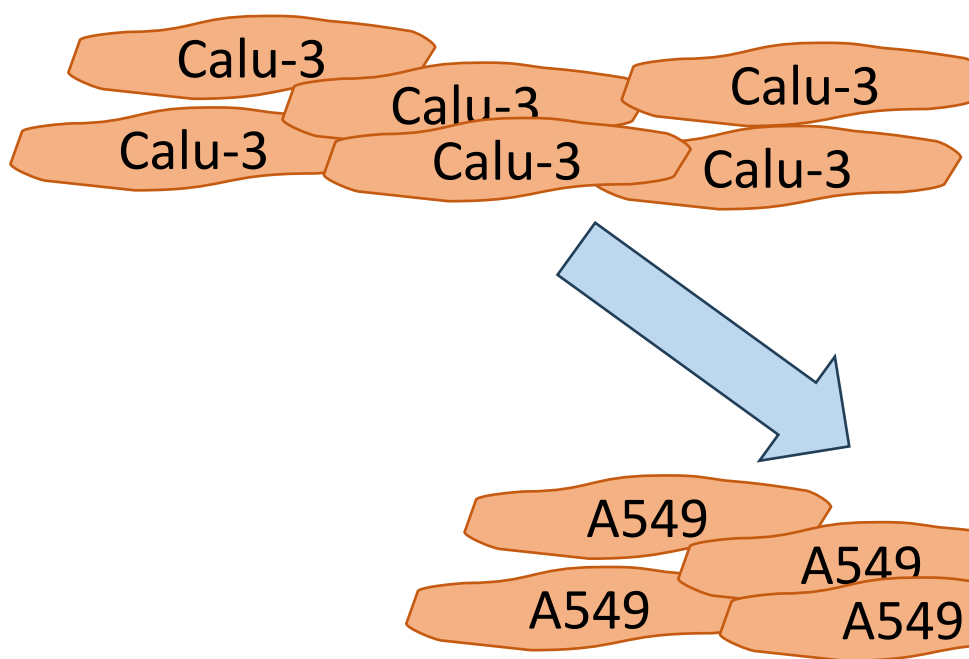
Challenging hACE2-expressing human alveolar epithelial cells in vitro with recombinant SARS-CoV-2 spike protein will cause an increase in the relative expression of the proinflammatory cytokines IL-6, IL-1 $\beta$ , and TNF- $\alpha$ .

## Results and Methodology

### Pulmonary epithelial cell line selection & cell culture methods

Began with Calu-3 (human airway epithelial cell line). Endogenously expresses ACE2... or so we thought.

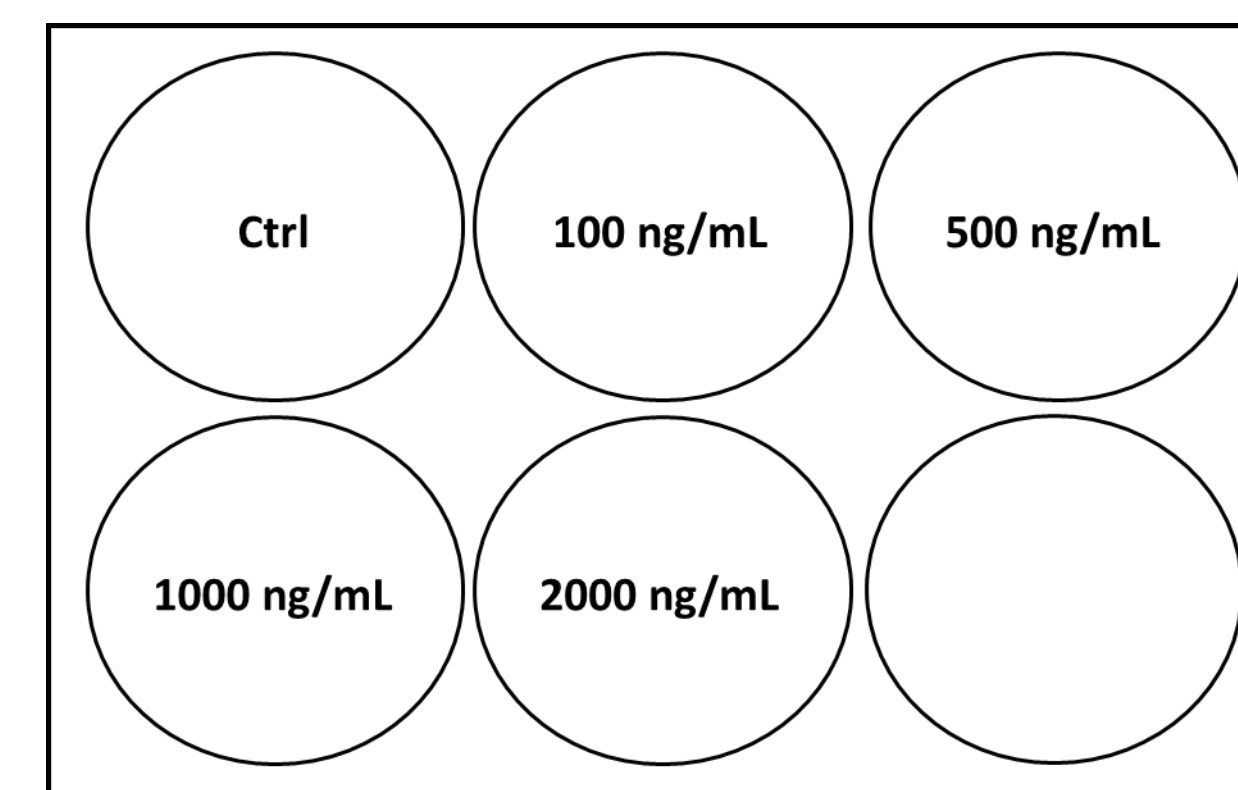
- No response to spike protein. Findings from other studies suggest Calu-3 cells do not express, or express lower levels of ACE2 than previously thought



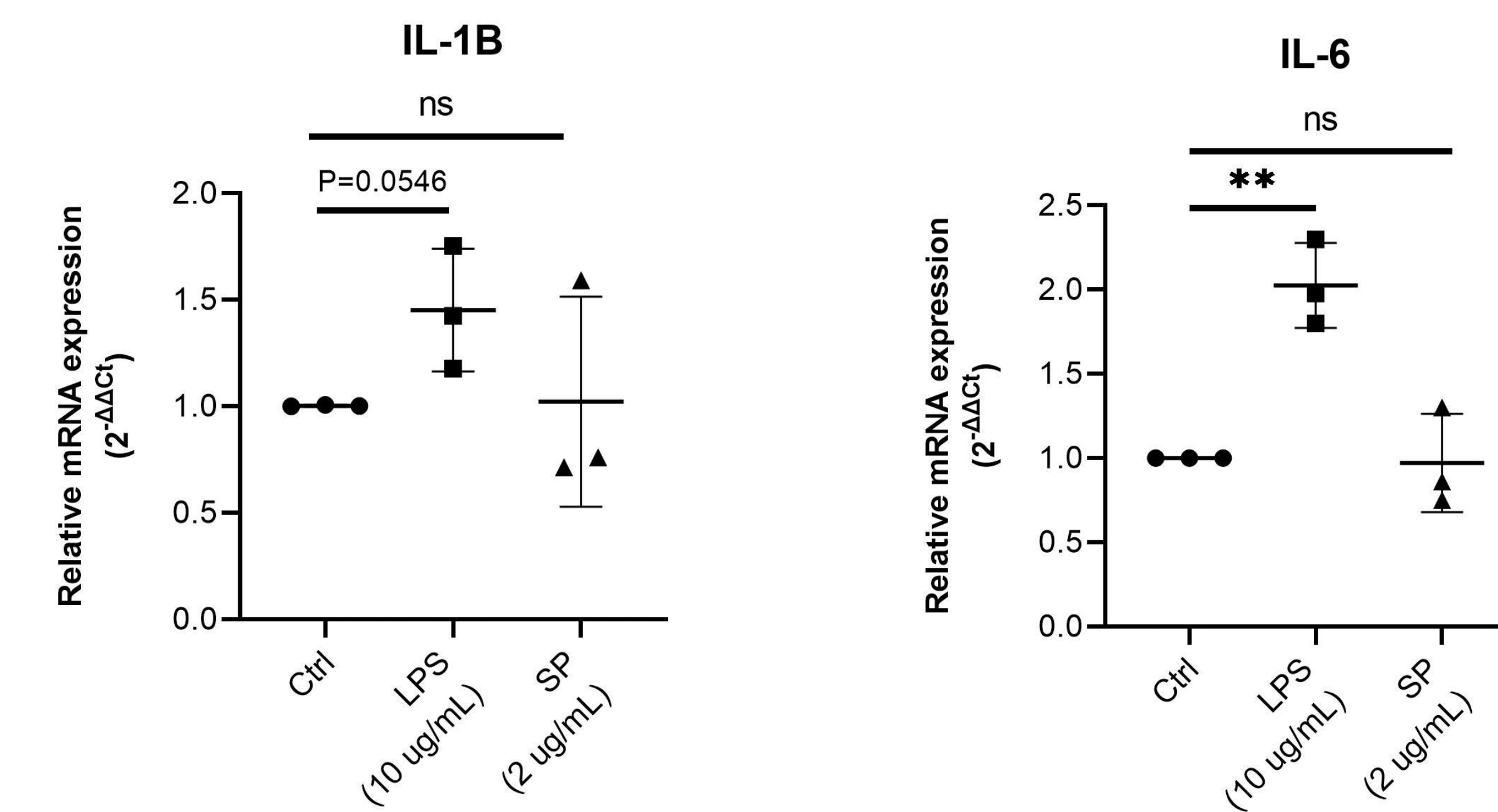
hACE2 stable transfection of A549 human alveolar epithelial cell line

- Kindly provided by the Morrison Lab

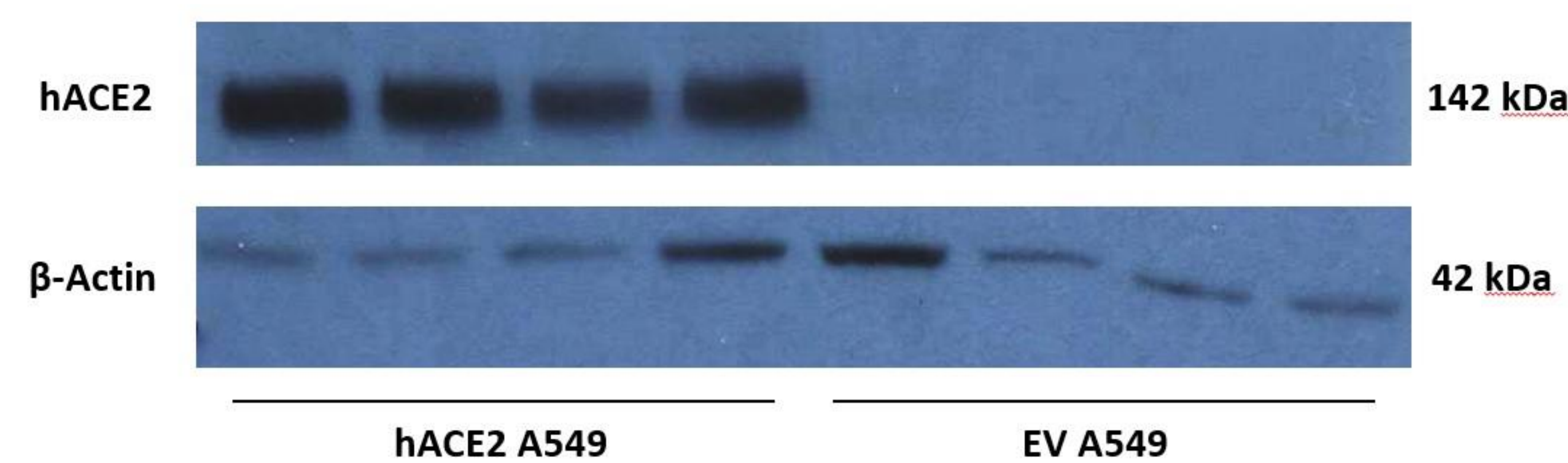
Cells challenged in with increasing concentration of recombinant SARS-CoV-2 spike protein in vitro for 24 hr. time course



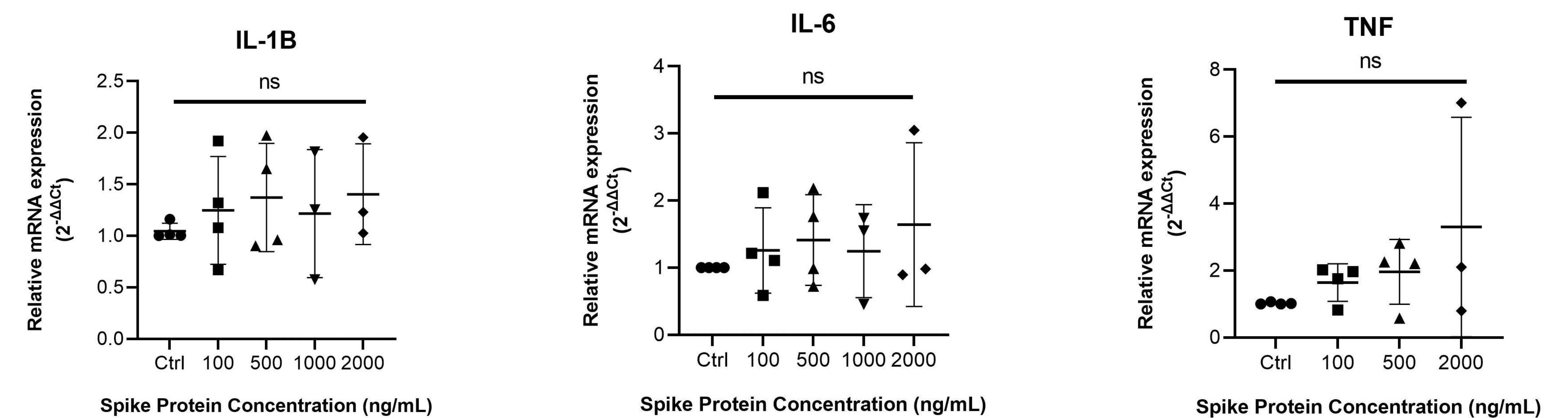
### LPS positive control increased proinflammatory markers in ACE2 A549 cells



### ACE2 expression was confirmed in the modified A549 cell line



### Recombinant spike protein did not increase proinflammatory markers in ACE2 A549 cells



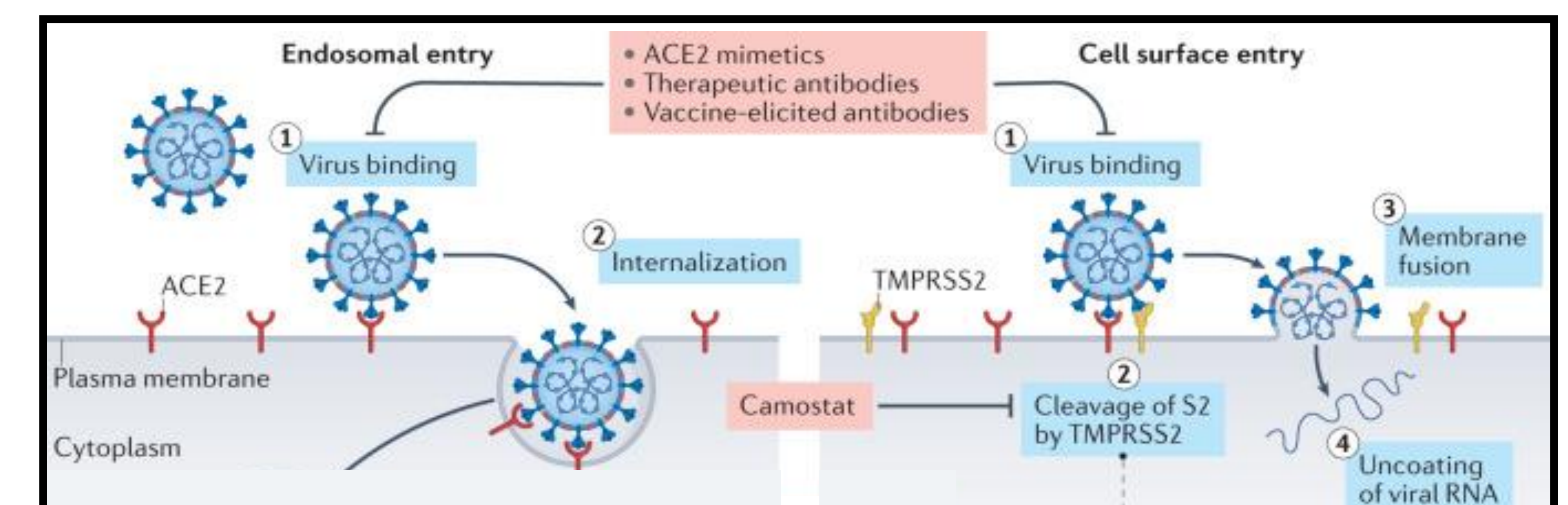
## Conclusion

Recombinant, full-length, SARS-CoV-2 spike protein is not sufficient to induce an inflammatory response in human alveolar epithelial cells in vitro

- And therefore, would not be an appropriate agent on its own to serve as a non-infectious model of SARS-CoV-2 infection of the alveolar epithelium.

Additional cofactors for viral binding, like TMPRSS2, have been found to be essential to efficient infection by SARS-CoV-2.

- An effective non-infectious model may need to account for this complexity to better resemble the inflammatory response that occurs during infection



Jackson et al., Nature 2022