Validation data for A549-ASCoV2 cells

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Version 22H04-NJ

A549-ASCoV2 cells are designed as a control cell line for A549-ASCoV2-NLRP1 cells to study the activation of the NLRP1 inflammasome in real time, in the context of SARS-CoV-2 infection. They are derived from A549-ASC cells, a human A549 lung carcinoma epithelial cell line featuring an NF-κB-inducible ASC::GFP reporter gene. Additionally, A549-ASCoV2 cells stably express the SARS-CoV-2 receptors genes, human *ACE2* and *TMPRSS2*. The expression of *hTMPRSS2* was confirmed using quantitative RT-PCR **(Figure 1)** and of hACE2 using flow cytometry **(Figure 2)**. Western blot analysis confirmed stable ASC::GFP expression and absence of endogenous NLRP1 expression **(Figure 3)**. The formation of ASC specks upon NLRP1 inflammasome activation by SARS-CoV-2 viral infection was monitored using fluorescence microscopy **(Figure 4)**, and subsequent pyroptotic cell death using the LDH-release assay **(Figure 5)**. As expected, the lack of endogenous NLRP1 expression in A549-ASCoV2 cells precludes the formation of single ASC specks and cell death.

Validation of *TMPRSS2* overexpression

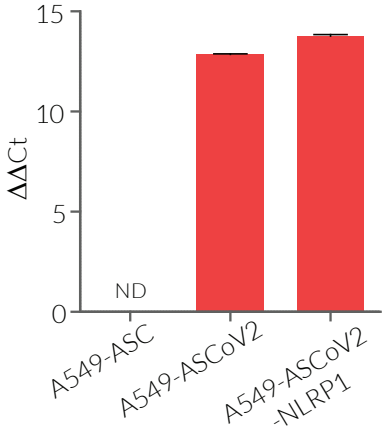

Figure 1: Human TMPRSS2 mRNA expression in A549-ASC, A549-ASCoV2, A549-ASCoV2-NLRP1 cells. Total mRNA was extracted from ~1x10^6 cells for each cell line. TMPRSS2 mRNA was amplified using quantitative RT-PCR. Data are represented as ΔΔCT comparing TMPRSS2 expression to a housekeeping gene. ND: not detected.

Validation of ACE2 surface expression

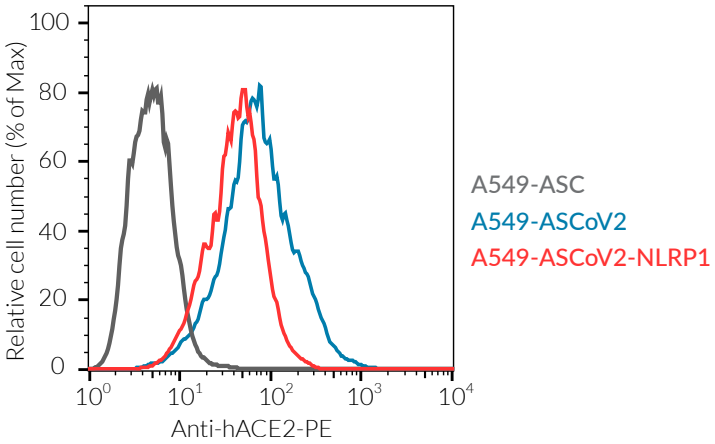

Figure 2: Surface expression of hACE2 by A549-ASC, A549-ASCoV2, A549-ASCoV2-NLRP1 cells. Each cell line was incubated with Anti-ACE2-PE antibody for 1 hour at 4°C. Cell surface staining was analyzed by flow cytometry.

Validation of NLRP1 and ASC::GFP expression

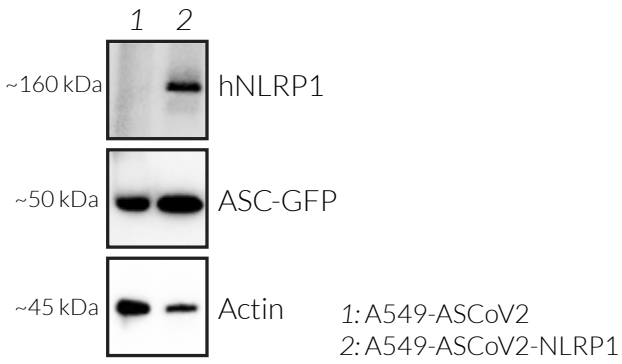

Figure 3: Validation of the expression of ASC::GFP and human NLRP1. Lysates from A549-ASCoV2 *(1)* and A549-ASCoV2-NLRP1 *(2)* cells were analyzed by western blot using an anti-human NLRP1 and an anti-human ASC antibody, followed by HRP conjugated secondary antibody. Actin was used as a loading control.

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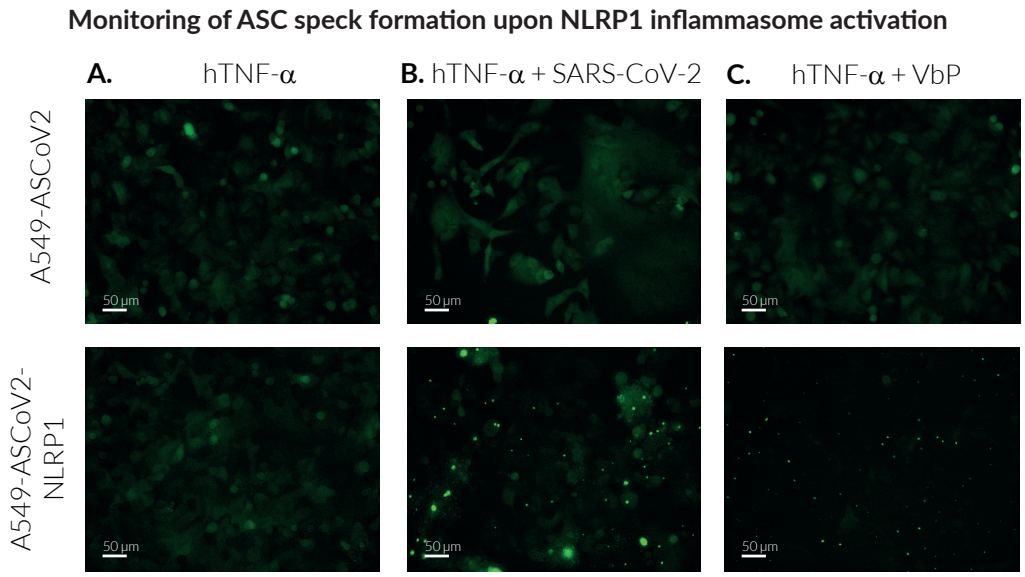

Figure 4: SARS-CoV-2 virus and Val-boroPro induce ASC speck formation in A549-ASCoV2-NLRP1 cells. A549-ASCoV2 cells and A549-ASCoV2-NLRP1 cells were incubated with 0.5 ng/ml human TNF-α overnight at 37°C, 5% CO_2 **(A-C)**. The following day, the cells were incubated in test medium containing SARS-CoV-2 particles (MOI of 0.1) for 1 hour, then for 24 hours in fresh test medium **(B)**. Alternatively, cells were incubated in test medium containing 10 µM Val-boroPro (VbP) for 24 hours **(C)**. The ASC::GFP expression and ASC speck formation were monitored using fluorescence microscopy. Scale bar: 50 µm.

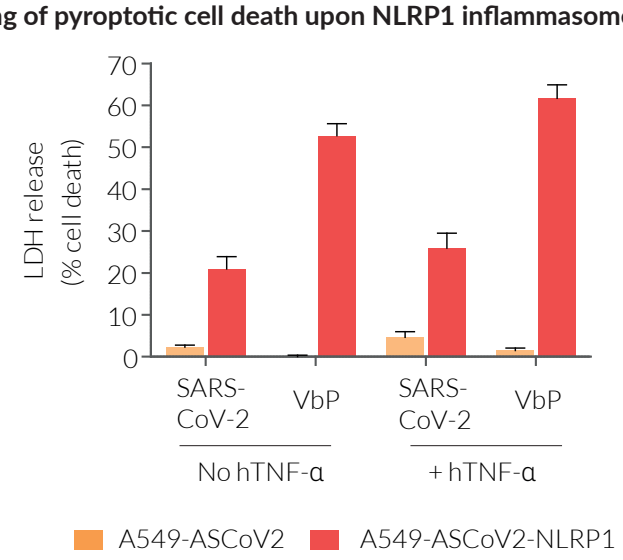

Figure 5: SARS-CoV-2 virus and Val-boroPro induce pyroptotic cell death in A549-ASCoV2-NLRP1 cells. A549-ASCoV2 cells and A549-ASCoV2-NLRP1 cells were incubated with/without 0.5 ng/ml human TNF-α overnight at 37°C, 5% CO_2. The following day, the cells were incubated in test medium containing SARS-CoV-2 particles (MOI of 0.1) for 1 hour, then for 24 hours in fresh test medium. Alternatively, cells were incubated in test medium containing 10 µM Val-boroPro (VbP) for 24 hours. Cell death was assessed using the lactate dehydrogenase (LDH) assay. Data is shown as percentage of cell death (mean ± SEM).