Validation data for A549-ASC-NLRP1 cells

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Version 22H03-NJ

A549-ASC-NLRP1 cells are designed to study the activation of the NLRP1 inflammasome in real time using fluorescence microscopy. They stably express the human *NLRP1* gene. Additionally, these cells feature a reporter gene encoding an ASC::GFP fusion protein, driven by an NF-κB-inducible promoter. Western blot analysis confirmed NLRP1 expression **(Figure 1)**. The expression of ASC::GFP upon stimulation with human TNF-α, an NF-κB inducer, was confirmed by Western blot and flow cytometry **(Figures 1 and 2)**. The ASC speck formation upon NLRP1 activation in A549-ASC-NLRP1 cells was monitored using fluorescence microscopy and compared to the parental cell line, A549-ASC **(Figures 3A and 4A)**. The subsequent pyroptotic cell death was assessed using the LDH-release assay **(Figures 3B and 4B)**. As expected, Val-boroPro (VbP) and transfected Poly(I:C) triggered ASC speck formation and pyroptosis in A549-ASC-NLRP1 cells, but not in A549-ASC cells.

Validation of NLRP1 and inducible ASC::GFP expression

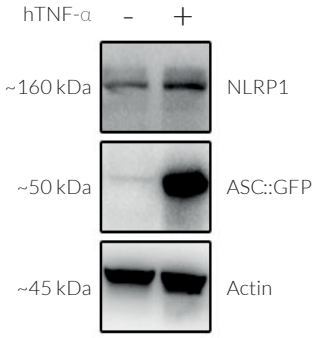

Figure 1. Expression of NLRP1 and ASC::GFP in A549-ASC-NLRP1 cells. Cells were either left untreated or incubated with the NF-κB-inducer, human TNF-α (4 ng/ml), overnight at 37°C. Cell lysates were analyzed by Western blot using anti-hNLRP1 and anti-hASC antibodies, and an HRP conjugated secondary antibody. Actin was used as a loading control.

Validation of inducible ASC::GFP expression

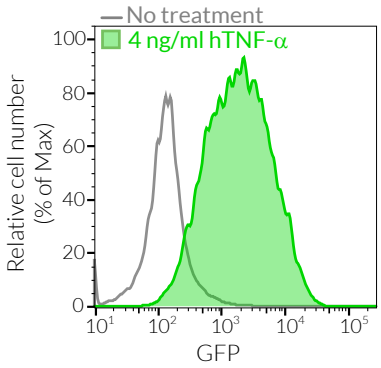

Figure 2. ASC::GFP inducible expression in A549-ASC-NLRP1 cells. 5 x 10^4 cells were incubated with or without 4 ng/ml hTNF-α overnight at 37°C in 5% CO_2. The next day, GFP expression was measured using flow cytometry and compared to untreated cells.

Monitoring of ASC speck formation and pyroptosis upon NLRP1 activation using Val-boroPro

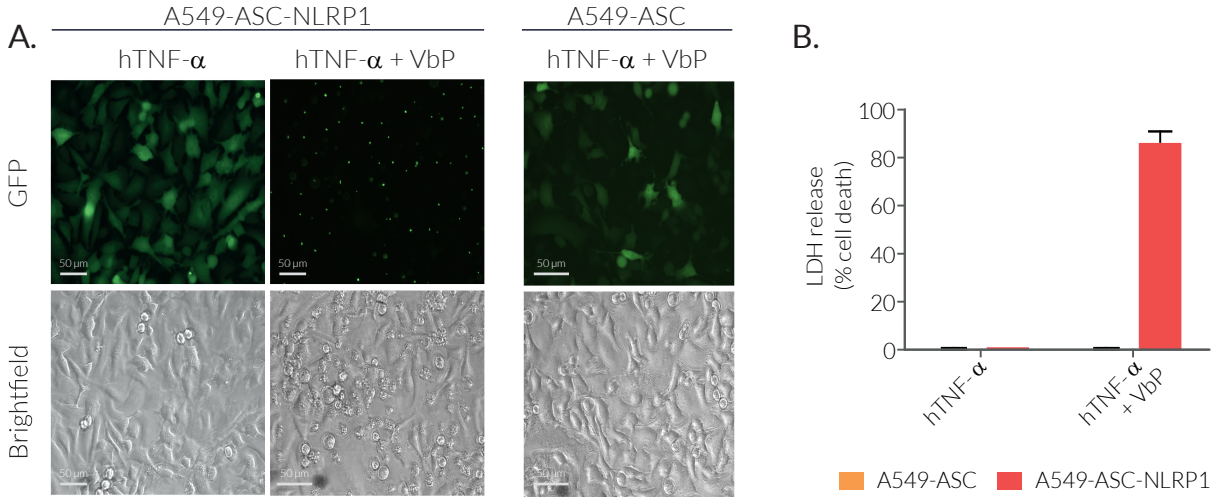

Figure 3. Functional validation of Val-boroPro-induced NLRP1 inflammasome activation. A549-ASC-NLRP1 and A549-ASC cells were cultured with 4 ng/ml human TNF-α overnight at 37°C, 5% CO_2. The following day, the cells were further incubated, or not, with 10 µM of the NLRP1 inducer Val-boroPro (VbP) for 8 hours. The ASC::GFP expression and ASC speck formation were monitored using fluorescence microscopy. Scale bar: 50 µm **(A)**. Pyroptosis was assessed using the lactate dehydrogenase (LDH) assay. Data is shown as percentage of cell death (mean ± SEM) **(B)**.

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Monitoring of ASC speck formation and pyroptosis upon NLRP1 activation using Poly(I:C)

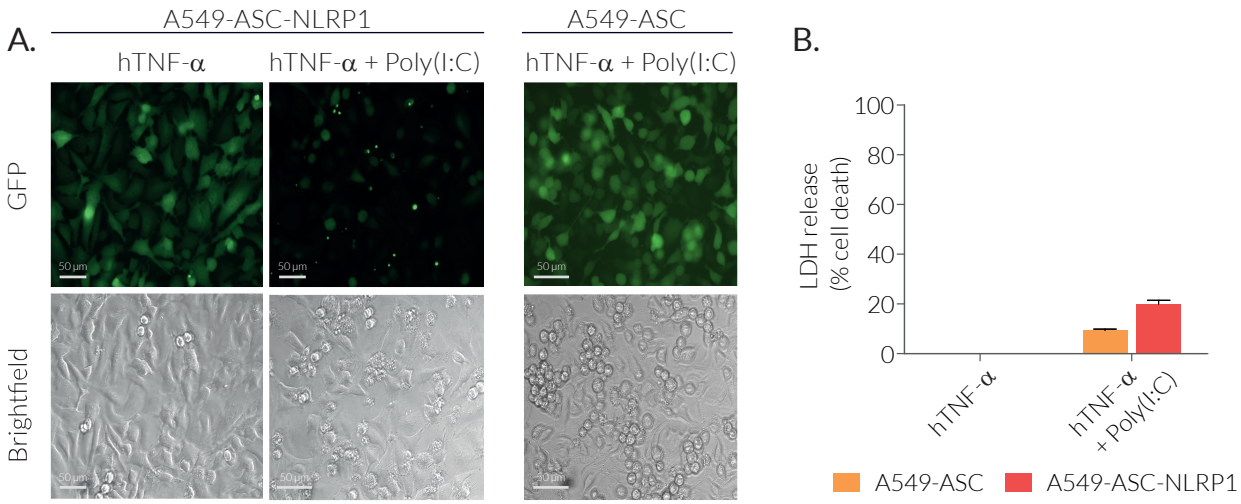

Figure 4. Functional validation of Poly(I:C) HMW-induced NLRP1 inflammasome activation. A549-ASC-NLRP1 and A549-ASC cells were cultured with 4 ng/ml human TNF-α overnight at 37°C, 5% CO_2. The following day, the cells were further incubated, or not, with 0.5 µg/ml transfected Poly(I:C) HMW overnight. The ASC::GFP expression and ASC speck formation were monitored using fluorescence microscopy. Scale bar: 50 µm **(A).** Pyroptosis was assessed using the lactate dehydrogenase (LDH) assay. Data is shown as percentage of cell death (mean ± SEM) **(B)**.

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