Validation data for A549-ASC cells

https://www.invivogen.com/a549-ascg-nlrp1

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Version 22I12-AK

A549-ASC cells are designed as a control cell line for A549-ASC-NLRP1 cells to study the activation of the NLRP1 inflammasome in real time using fluorescence microscopy. They feature a reporter gene encoding an ASC::GFP fusion protein, driven by an NF-κB-inducible promoter. The expression of ASC::GFP upon stimulation with human TNF-α, an NF-κB inducer, was confirmed by Western blot and flow cytometry **(Figure 1)**. 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. As expected, the lack of endogenous NLRP1 expression in A549-ASC cells precludes the formation of single ASC::GFP specks **(Figure 2)**.

Validation of inducible ASC::GFP expression

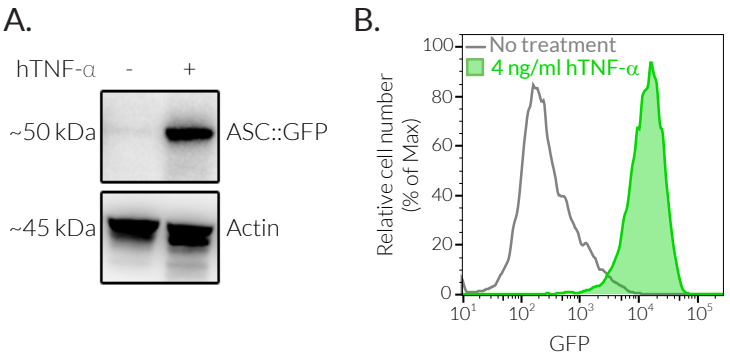

Figure 1. Validation of NF-κB-inducible expression of ASC::GFP in A549-ASC cells.
A549-ASC cells were either left untreated or incubated with the NF-κB-inducer, human TNF-α (4 ng/ml), overnight at 37°C. Cell lysates were analyzed by Western blot using an anti-human ASC antibody and an HRP conjugated secondary antibody. Anti-human actin was used as a loading control **(A)**. Alternatively, the induction of ASC::GFP expression in A549-ASC cells upon human TNF-α treatment was assessed by flow cytometry **(B)**.

Monitoring of ASC speck formation upon NLRP1 inflammasome activation

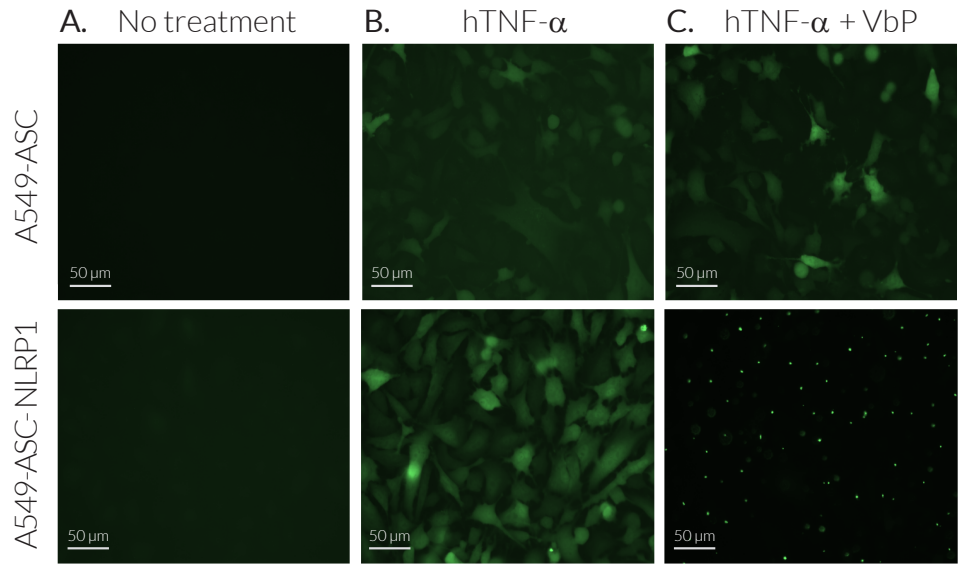

Figure 2. Val-boroPro induces ASC speck formation in A549-ASC-NLRP1 cells. A549-ASC and A549-ASC-NLRP1 cells were either left untreated **(A)** or cultured with 4 ng/ml human TNF-α overnight at 37°C, 5% CO_2 **(B, C)**. The following day, the cells were further incubated with 10 µM of the NLRP1 inducer Val-boroPro (VbP) for 8 hours at 37°C, 5% CO_2 **(C)**. The ASC::GFP expression and speck formation were monitored using fluorescence microscopy. Scale bar: 50 µm.

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