

# Validation data for Val-boroPro

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Version 22H03-AK

Val-boroPro (VbP) is a non-selective inhibitor of various dipeptidyl peptidases (DPPs) including DPP4, DPP8 and DPP9. It weakens the inhibitory interaction between DPP8/9 and the NLRP1 inflammasome sensor. The ability of VbP to trigger NLRP1 inflammasome responses was validated in A549-ASC-NLRP1 cells expressing NLRP1 and an NF- $\kappa$ B-inducible ASC::GFP fusion protein. The ASC speck formation was monitored using fluorescence microscopy (Figure 1), and the pyroptosis was measured using the LDH release assay (Figure 2).

## Val-boroPro-induced ASC speck formation

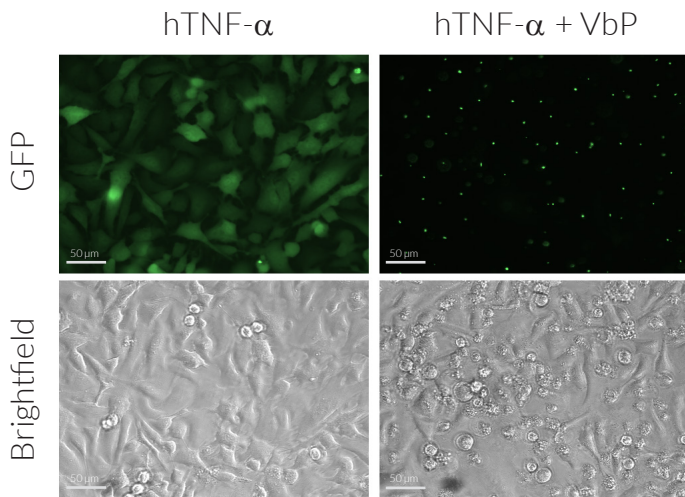


Figure 1. Monitoring of ASC speck formation upon NLRP1 inflammasome activation by Val-boroPro.

A549-ASC-NLRP1 cells were cultured with 4 ng/ml hTNF- $\alpha$  overnight at 37°C, 5% CO<sub>2</sub>. The following day, the cells were further incubated with 10  $\mu$ M of Val-boroPro (VbP) for 8 hours at 37°C, 5% CO<sub>2</sub>. The ASC::GFP expression and speck formation were monitored using fluorescence microscopy. Scale bar: 50  $\mu$ m.

## Val-boroPro-induced pyroptosis

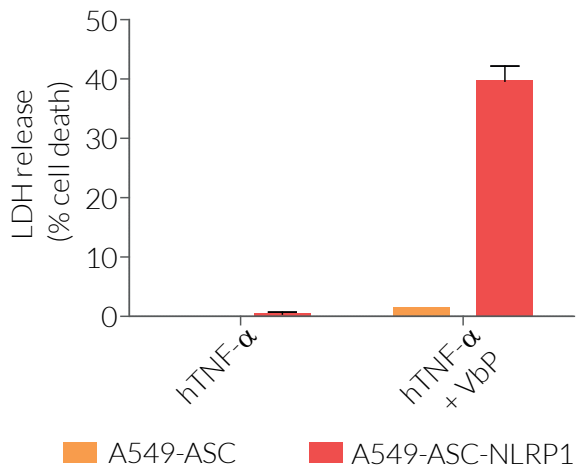


Figure 2. Pyroptotic cell death in A549-ASC-NLRP1 cells upon treatment with Val-boroPro.

A549-ASC-NLRP1 cells and A549-ASC control cells were cultured with 4 ng/ml hTNF- $\alpha$  overnight at 37°C, 5% CO<sub>2</sub>. The following day, the cells were further incubated with 10  $\mu$ M Val-boroPro (VbP) for 8 hours at 37°C, 5% CO<sub>2</sub>. Pyroptotic cell death was assessed using the lactate dehydrogenase (LDH) assay (mean  $\pm$  SEM).

### TECHNICAL SUPPORT

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