## Validation data for Bay11-7082

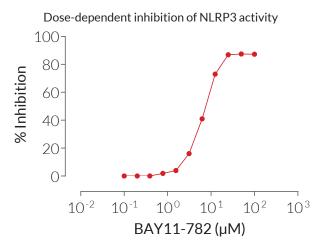
https://www.invivogen.com/bay11-7082

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Version 22F07-MM

Bay11-7082 is a potent inhibitor of the transcription factor NF- $\kappa$ B (nuclear factor kappa-light-chain-enhancer of activated B cells) and the NLRP3 (NOD-like receptor (NLR) pyrin domain-containing protein 3) inflammasome. NF- $\kappa$ B regulates multiple aspects of innate and adaptive immune functions and serves as a pivotal mediator of inflammatory responses. While the NLRP3 inflammasome is an innate immune sensor that is activated by a two-step process; a first signal ('priming') is provided by microbial molecules such as lipopolysaccharide (LPS), and the second signal is provided by a wide array of stimuli including endogenous molecules or crystalline substances such as monosodium urate (MSU) crystals. The ability of Bay11-7082 to inhibit the NLRP3 inflammasome was validated using InvivoGen's THP-1/HEK-Blue<sup>TM</sup> II-16 assay. This assay uses the

The ability of Bay11-7082 to inhibit the NLRP3 inflammasome was validated using InvivoGen's THP-1/HEK-Blue<sup>M</sup> IL-1 $\beta$  assay. This assay uses the secretion of IL-1 $\beta$  by THP1-Null2 cells as an indicator of NLRP3 inflammasome induction. The IL-1 $\beta$  production by these cells is measured using HEK-Blue<sup>M</sup> IL-1 $\beta$  cells. Treatment with Bay11-7082 inhibited IL-1 $\beta$  secretion in a dose-dependent manner (Figure 1).



## Figure 1: Bay11-7082 inhibits the NLRP3 inflammasome response in a dose-dependent manner.

THP1-Null2 cells were primed with LPS-EK (1  $\mu$ g/ml) for 3 h and then stimulated with MSU (150  $\mu$ g/ml) and increasing concentrations of Bay11-7082. After overnight incubation, IL-1 $\beta$  secretion was analyzed by adding 50  $\mu$ l of supernatant from treated THP1-Null2 cells to HEK-Blue<sup>TM</sup> IL-1 $\beta$  cells. IL-1 $\beta$ -induced activation of NF-KB was assessed by measuring the levels of SEAP in the supernatant of HEK-Blue<sup>TM</sup> IL-1 $\beta$  cells using QUANTI-Blue<sup>TM</sup> Solution, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm. Data are shown as percentage (%) inhibition.

