Validation data for THP1-NLRC4 cells

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Version 21B25-NJ

THP1-NLRC4 cells were generated from the human monocytic THP1-Null2 cell line. Substantial Flagellin amounts are required for NLRC4 inflammasome activation in THP-1 cells. THP1-NLRC4 cells stably overexpress NLRC4 and naturally express TLR5. NLRC4 overexpression increases the cell line sensitivity to Flagellins from various Gram-negative bacteria, without impacting the inflammasome response to highly potent NLRC4 inducers, such as Needle-Tox (LFn-Needle from *B. thailandensis* combined with the anthrax protective antigen).

Inflammasome response in THP1-NLRC4 cells using different Flagellins

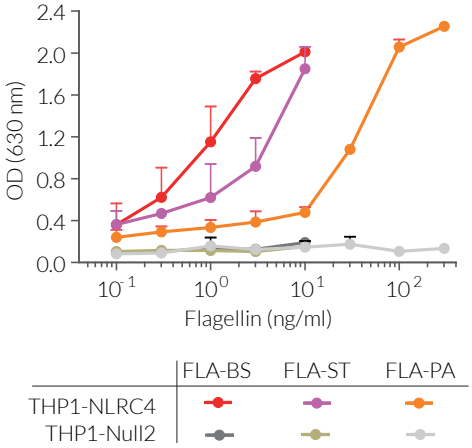

Figure 1: Secretion of mature IL-1β by THP1-NLRC4 cells and their parental THP1-Null2 cells upon inflammasome activation with Flagellins.
~3x10^5 THP1-Null2 and THP1-NLRC4 cells were incubated for 3h at 37°C with LPS-EK (1 µg/ml) (*priming*) and then incubated (*activation*) with increasing concentrations of Flagellin-BS (from *B. subtilis*), Flagellin-ST (from *S. typhimurium*), or Flagellin-PA (from *P. aeruginosa*). After 24h, the secretion of mature human (h)IL-1β was assessed in the culture supernatant using HEK-Blue™ IL-1β sensor cells which express an NF-κB-inducible SEAP reporter gene. QUANTI-Blue™ Solution was used to measure SEAP activity. Optical density (OD) was read at 630 nm.
Note: Ultrapure Flagellin grades were used.

Inflammasome response in THP1-NLRC4 cells using Needle-Tox

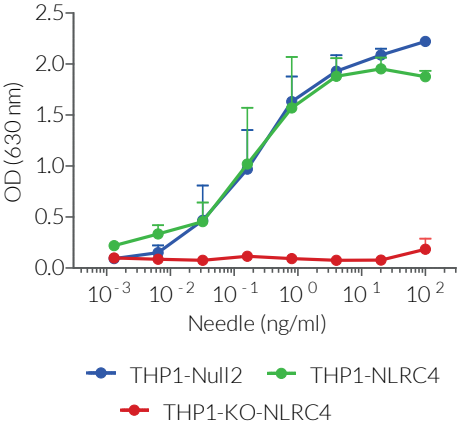

Figure 2: Secretion of mature IL-1β by THP1-NLRC4, THP1-KO-NLRC4, and their parental THP1-Null2 cells upon inflammasome activation with Needle-Tox.
~3x10^5 THP1-Null2, THP1-NLRC4, and THP1-KO-NLRC4 cells were incubated for 3h at 37°C with LPS-EK (1 µg/ml) (*priming*) and then incubated (*activation*) with increasing concentrations of Needle-Tox. After 24h, the secretion of mature human (h)IL-1β was assessed in the culture supernatant using HEK-Blue™ IL-1β sensor cells which express an NF-κB-inducible SEAP reporter gene. QUANTI-Blue™ Solution was used to measure SEAP activity. Optical density (OD) was read at 630 nm.
Note: Needle-Tox is a combination of LFn-Needle (4 ng/ml) with the anthrax protective antigen (PA, #171E) (20 ng/ml). PA allows LFn-Needle translocation into the cytosol.

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