Validation data for LFn-Needle

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Version 21A04-NJ

LFn-Needle is a model of NLRC4/NAIP inflammasome agonist. Needle is a component of the type III secretion systems (T3SS) of intracellular bacteria described as an NLRC4/NAIP ligand. It is fused to the amino-terminal domain of *B. anthracis* lethal factor (LFn). This fusion system, when co-administred with the anthrax toxin's protective antigen (PA), allows intracellular delivery of the bacterial ligand. The combination of LFn-Needle with the anthrax protective antigen (PA) is named Needle-Tox. LFn-Needle specificity for NLRC4 has been verified using THP1-KO-NLRC4 cells **(Figure 1)**. It induces NLRC4-dependent IL-1β secretion and pyroptosis in a dose-dependent manner **(Figure 2)**.

Functional validation of LFn-Needle using THP1-derived cells

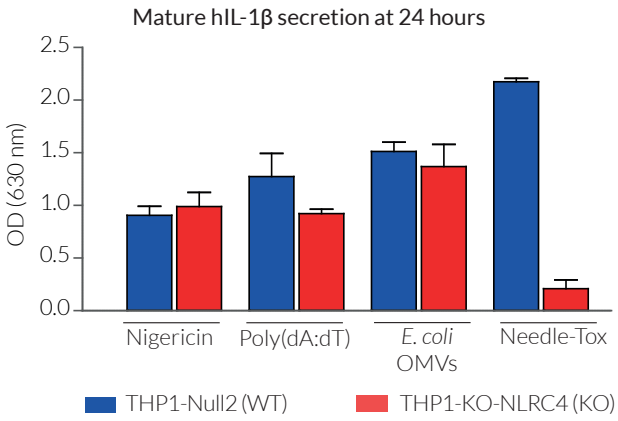

Figure 1: Secretion of mature IL-1β by THP1-KO-NLRC4 cells and their parental THP1-Null2 cells upon inflammasome activation.
~3x10^5 THP1-Null2 (WT) and THP1-KO-NLRC4 (KO) cells were incubated for 3h at 37°C with LPS-EK (1 µg/ml) (*priming*) and then incubated (*activation*) with inflammasome inducers: Nigericin (5 µM), transfected Poly (dA:dT) (1 µg/ml), *E. coli* outer membrane vesicles (OMVs) (100 µg/ml), or Needle-Tox (4 ng/ml). 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 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) (20 ng/ml). PA allows LFn-Needle translocation into the cytosol.*

Human NLRC4 inflammasome response to LFn-Needle

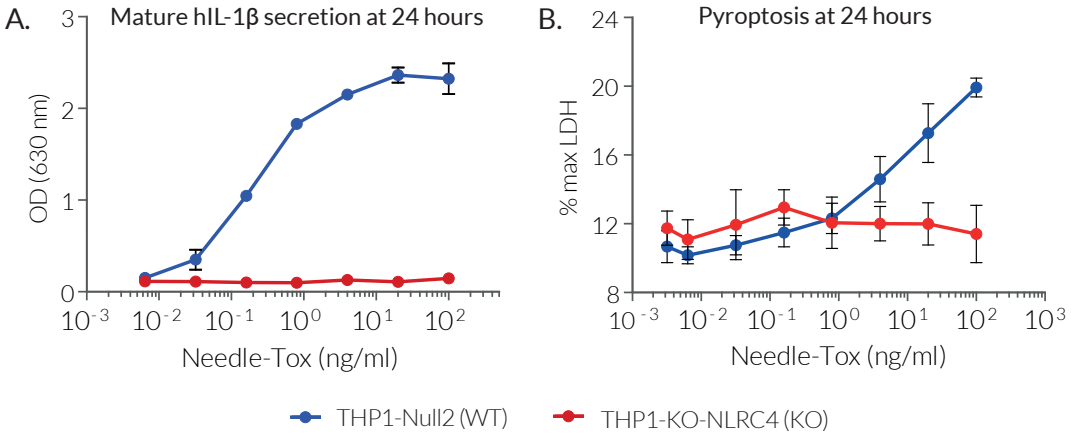

Figure 2: Secretion of mature IL-1β and pyroptosis upon THP1-derived cell activation using LFn-Needle.
~3x10^5 THP1-Null2 (blue) and THP1-KO-NLRC4 cells (red) were incubated for 3h at 37°C with LPS-EK (1 µg/ml) (priming) and then incubated (activation) with Needle-Tox (0.006-100 ng/ml). After 24h, mature human (h) IL-1β was assessed in the culture supernatant using HEK-Blue™ IL-1β sensor **(A)**, and cell death was assessed using the lactate dehydrogenase (LDH) assay **(B)**.

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