Validation data for THP1-KO-NLRC4 cells

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THP1-KO-NLRC4 cells were generated from the human monocytic THP1-Null2 cell line through a deletion encompassing the nucleotide-binding domain (NBD) in the gene encoding NLRC4. NBD is essential for the NLRC4 polymerization and inflammasome assembly. Thus, THP1-KO-NLRC4 cells express an inactive NLRC4 protein, unable to form a functional inflammasome. The KO status has been verified by PCR, Western blot **(Figure 1)**, and functional assays **(Figure 2).** These cells do not produce mature IL-1β upon NLRC4/NAIP inflammasome activation using Needle-Tox (LFn-Needle from *B. thailandensis* combined to the antrax protective antigen). However, they retain the ability to respond to other canonical inflammasome inducers, as well as to non-canonical inflammasome inducers **(Figure 2)**.

Validation of *NLRC4* Knockout (KO)

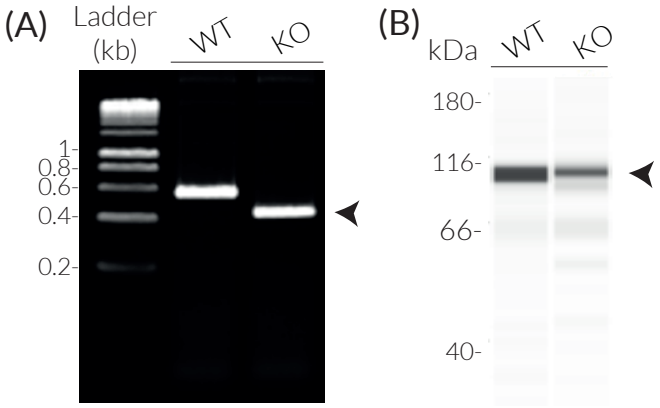

Figure 1: Validation of *NLRC4* KO in THP1-KO-NLRC4 cells by PCR and Western blot (WES™).
(A) The targeted NLRC4 region in THP1-Null2 (WT) and THP1-KO-NLRC4 (KO) cells was amplified by PCR. THP1-KO-NLRC4 cells feature a biallelic deletion (arrow).
(B) Lysates from THP1-Null2 (WT) and THP1-KO-NLRC4 (KO) cells were analyzed by Western blot (Wes™) using an NLRC4 antibody (Abcam, ref 201792), followed by a HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the band for the NLRC4 protein (expected size ~115 kDa).
Note: THP1-KO-NLRC4 cells feature a deletion encompassing the NLRC4 nucleotide binding domain, which explains the presence of a band in the KO lane.

Functional validation of THP1-KO-NLRC4 cells

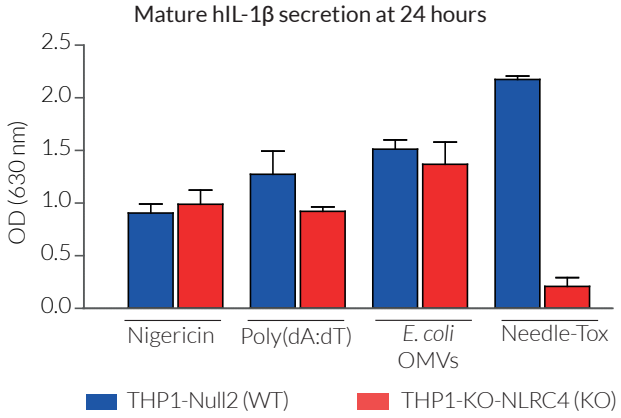

Figure 2: Secretion of mature IL-1β by THP1-KO-NLRC4 cells and their parental THP1-Null2 cells upon inflammasome activation.
~3x10^5 THP1-Null2 (WT) and THP1-KO-NLRC4 (KO) cells were incubated for 3h at 37°C with LPS-EK (1 µg/ml) (*priming*) and then incubated (*activation*) with inflammasome inducers: 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.

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