Validation data for THP1-KO-CASP4 cells

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THP1-KO-CASP4 cells were generated from the human monocytic THP1 (WT) cell line through the stable knockout of the caspase-4 (*CASP4*) gene. The KO status has been verified by DNA sequencing, PCR **(Figure 1A),** and Western blot **(Figure 1B).** In THP1-KO-CASP4 cells, mature IL-1β secretion is not affected upon canonical inflammasome activation **(Figure 2A)**, however, it is severly impaired after non-canonical inflammasome activation by *E. coli* outer membrane (OMVs) or transfected LPS **(Figure 2B)**. Of note, due to the presence of CASP5 (functional homolog of CASP4), the secretion of IL-1β is not completely abolished.

Validation of *CASP4* Knockout (KO)

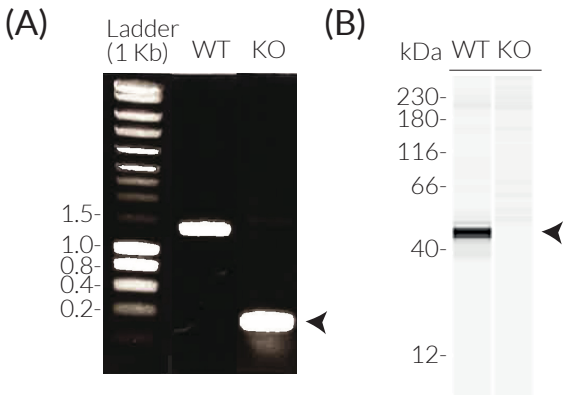

Figure 1: Validation of *CASP4* knockout in THP1-KO-CASP4 cells. **(A)** The targeted CASP4 region in THP1-null (WT) and THP1-KO-CASP4 (KO) cells was amplified by PCR. THP1-KO-CASP4 cells feature a biallelic deletion (arrow). **(B)** Lysates from THP1-null (WT) and THP1-KO-CASP4 (KO) cells were analyzed by Western blot (Wes™) using an anti-human CASP4 antibody, followed by a HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the CASP4 protein (48 kDa).

Functional validation of THP1-KO-CASP4 cells

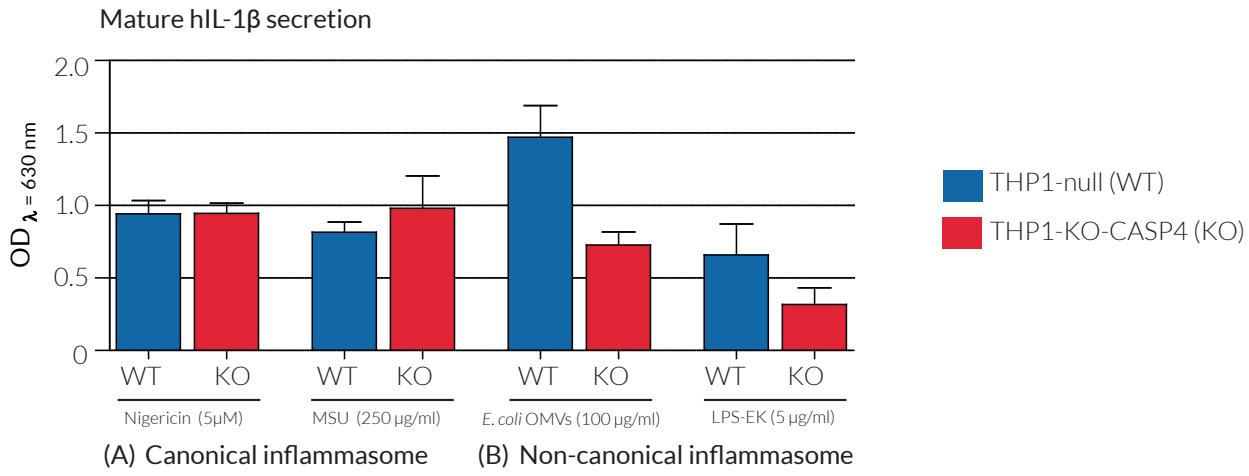

Figure 2: Secretion of mature IL-1β by THP1-KO-CASP4 cells upon inflammasome activation. ~3x10^5 THP1-null (blue) and THP1-KO-CASP4 (red) cells were incubated for 3h at 37°C with LPS-EK (1µg/ml) (*priming*) and then incubated (*activation*) with **(A)** canonical inflammasome inducers, Nigericin (5 µM) or MSU crystals (MSU; 250 µg/ml), and **(B)** non-canonical inflammasome inducers, *E. coli* outer membrane vesicles (OMVs) (100 µg/ml) or transfected with LPS-EK (5 µg/ml). After 6h, the secretion of mature human (h)IL-1β was assessed in the culture supernatant using HEK-Blue™ IL-1β sensor cells expressing an NF-κB SEAP reporter gene. QUANTI-Blue™ Solution was used to measure SEAP activity. Optical density (OD) was read at 630 nm.

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