# Validation data for THP1-KO-CASP4 cells

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THP1-KO-CASP4 cells were generated from the human monocytic THP1-Null2 (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).** Although these cells are fully KO for the CASP4 protein, they exhibit some IL-1β secretion after non-canonical inflammasome activation by transfected LPS **(Figure 2)** or *E. coli* outer membrane vesicles (OMVs) (data not shown). This remnant activity may be attributed to a partial rescue of the non-canonical inflammasome response by CASP5 (functional homolog of CASP4). As expected, the mature IL-1β secretion is not affected upon canonical inflammasome activation **(Figure 2)**.

## Validation of *CASP4* Knockout (KO)

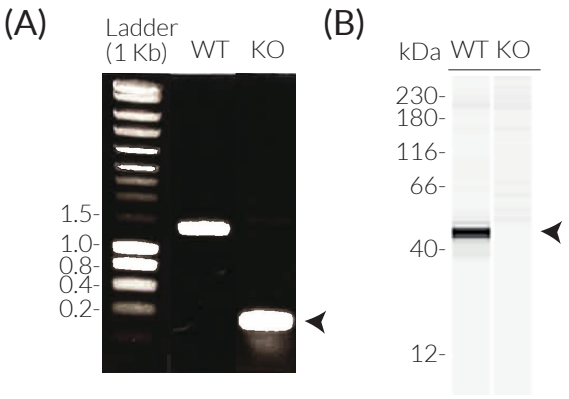


**Figure 1: Validation of *CASP4* knockout in THP1-KO-CASP4 cells.**
**(A)** The targeted CASP4 region in THP1-Null2 (WT) and THP1-KO-CASP4 (KO) cells was amplified by PCR. THP1-KO-CASP4 cells feature a biallelic deletion (arrow). **(B)** Lysates from THP1-Null2 (WT) and THP1-KO-CASP4 (KO) cells were 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

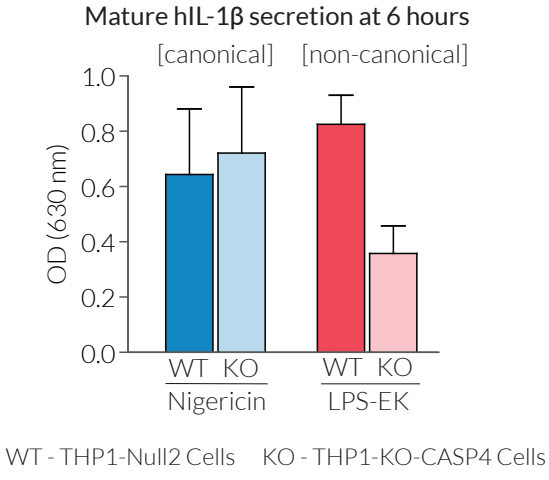


**Figure 2: Altered mature IL-1β secretion by THP1-KO-CASP4 cells upon non-canonical inflammasome activation.**
~3x10$^5$ THP1-Null2 (WT) and THP1-KO-CASP4 cells (KO) were incubated for 3h at 37°C with LPS-EK (1 µg/ml) (*priming*) and then incubated (*activation*) with Nigericin (5 µM), or transfected 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 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.


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