Validation data for THP1-KO-ASC cells

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THP1-KO-ASC cells were generated from the human monocytic THP1-Null2 (WT) cell line through the stable knockout of the *ASC* gene. The KO status has been verified by DNA sequencing, PCR, Western blot **(Figure 1)**, and functional assays **(Figure 2).** Mature IL-1β secretion is abolished in THP1-KO-ASC cells (KO) after activation with inducers of the ASC-dependent NLRP3 (i. e. Nigericin, MSU crystals, or Alum Hydroxide) and AIM2 (i.e. Poly (dA:dT)) canonical inflammasomes, when compared to the parental (WT) cell line **(Figure 2A)**. The NLRP3-caspase-1 dependent maturation of IL-1β is abolished upon indirect activation of NLRP3 by induction of the non-canonical inflammasome by *E. coli* outer membrane vesicles (OMVs) **(Figure 2A)**. Furthermore, pyroptosis is abrogated in THP1-KO-ASC cells as shown by an LDH assay **(Figure 2B).**

Validation of *ASC* Knockout (KO)

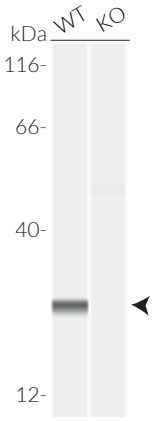

Figure 1: Validation of *ASC* KO in THP1-KO-ASC cells by Western blot (WES™).
Lysates from THP1-Null2 (WT) and THP1-KO-ASC (KO) cells were analyzed using an anti-human ASC antibody, followed by a HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the ASC protein (27 kDa).

Functional validation of THP1-KO-ASC cells

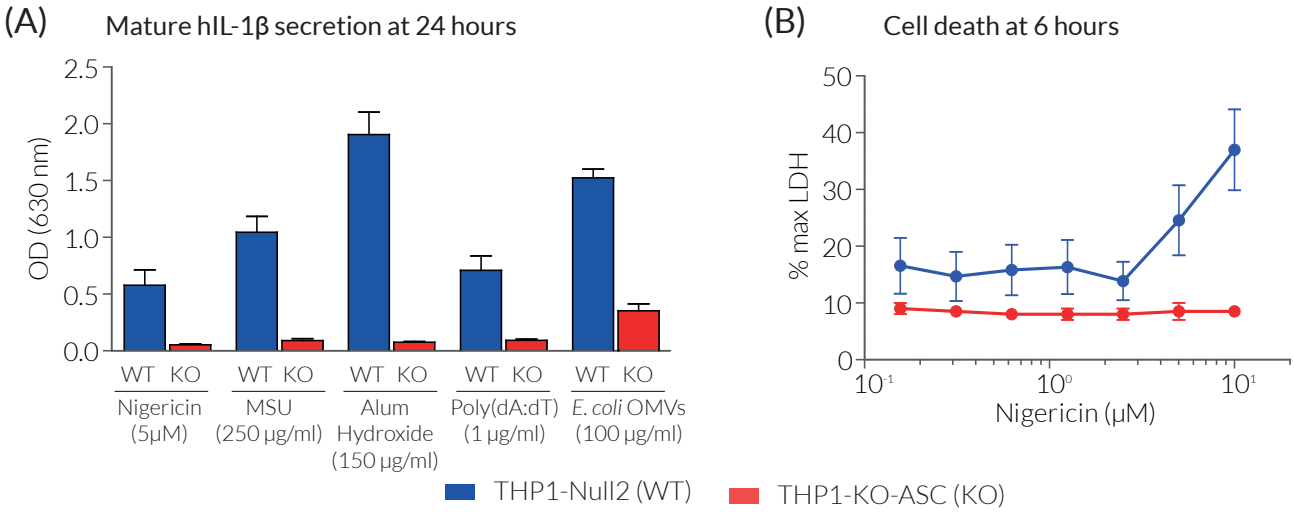

Figure 2: Absence of mature IL-1β secretion and pyroptosis by THP1-KO-ASC cells upon inflammasome activation.
~3x10^5 THP1-Null2 (blue) and THP1-KO-ASC cells (red) were incubated for 3h at 37°C with LPS-EK (1 µg/ml) (*priming*) and then incubated (*activation*) with Nigericin (A - 5 µM; B - 0.15-10 µM), MSU crystals (MSU; 250 µg/ml), Alum Hydroxide (150 µg/ml), transfected Poly (dA:dT) (1 µg/ml), or *E. coli* outer membrane vesicles (OMVs) (100 µg/ml). After 24h, **(A)** 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. **(B)** After a 6 hour incubation with Nigericin, cell death was assessed using the lactate dehydrogenase (LDH) assay.

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