Validation data for THP1-KO-GSDMD cells

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THP1-KO-GSDMD cells were generated from the human monocytic THP1-Null2 cell line through the stable knockout of the gasdermin D *(GSDMD)* gene. The KO status has been verified by PCR **(Figure 1A)**, Western blot **(Figure 1B)**, and functional assays **(Figure 2)**. THP1-KO-GSDMD cells exhibit an impaired IL-1β secretion upon canonical and non-canonical inflammasome activation with Nigericin and *E. coli* outer membrane vesicles (OMVs), respectively **(Figure 2A)**. Although these cells are fully KO for the GSDMD protein, mature IL-1β secretion is detected with the AIM2 inducer Poly(dA:dT) **(Figure 2A)**, and with NLRP3 inducers such as MSU crystals or Alum Hydroxide (at 24 hours, data not shown). This observation illustrates the reported IL-1β secretion upon GSDMD-independent cell death. Importantly, pyroptosis is abrogated in THP1-KO-GSDMD cells upon NLRP3 activation with Nigericin **(Figure 2B)**.

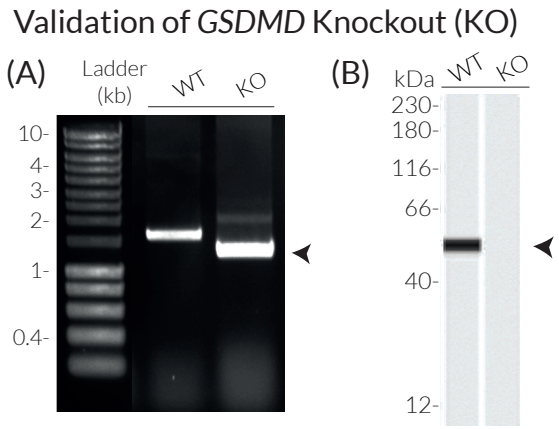

Figure 1: Validation of *GSDMD* KO in THP1-KO-GSDMD cells
(A) The targeted GSDMD region in THP1-Null2 (WT) and THP1-KO-GSDMD (KO) cells was amplified by PCR. THP1-KO-GSDMD cells feature a biallelic deletion (arrow). **(B)** Lysates from THP1-Null2 (WT) and THP1-KO-GSDMD (KO) cells were analyzed by Western blot (Wes™) using an anti-human GSDMD antibody and a HRP-conjugated anti-mouse secondary antibody. The arrow indicates the expected band for the GSDMD protein (48 kDa).

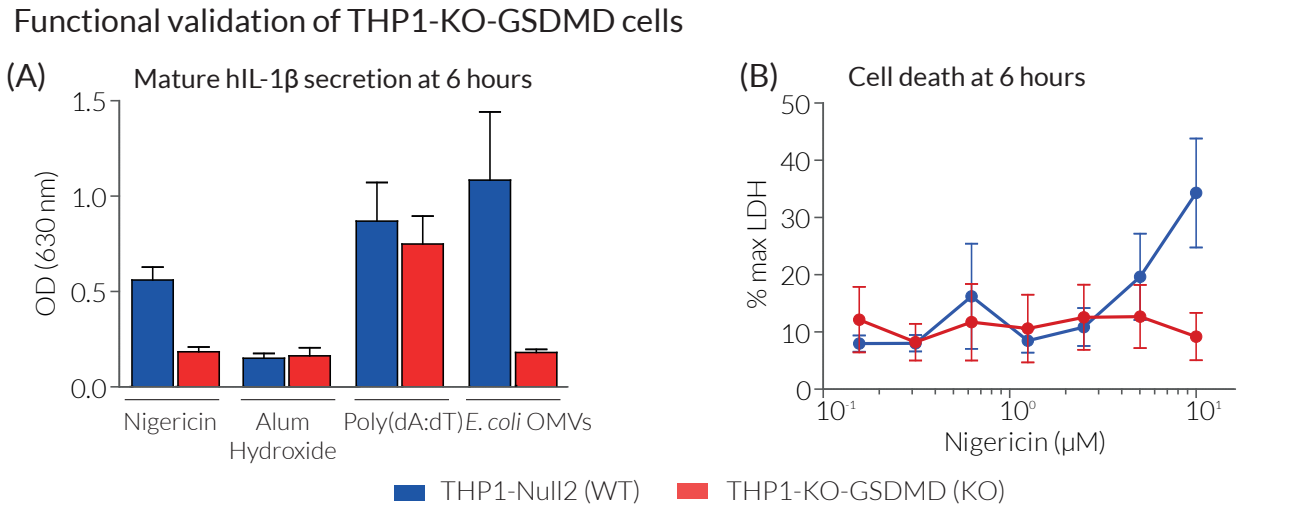

Figure 2: Altered mature IL-1β secretion and pyroptosis by THP1-KO-GSDMD cells upon inflammasome activation.
~3x10^5 THP1-Null2 (blue) and THP1-KO-GSDMD cells (red) were incubated for 3h at 37°C with LPS-EK (1 µg/ml) (*priming*) and then incubated (*activation*) with Nigericin (A: 5 µM; B: 0.15-10 µM), Alum Hydroxide (150 µg/ml), transfected Poly(dA:dT) (1 µg/ml), or *E. coli* outer membrane vesicles (OMVs) (100 µg/ml). After 6h, **(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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