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

**Validation of NLRP3 Knockout (KO)**

(A) Ladder (1 Kb) WT KO

(B) kDa WT KO

**Figure 1:** Validation of NLRP3 KO in THP1-KO-NLRP3 cells. (A) The targeted NLRP3 region in THP1-null (WT) and THP1-KO-NLRP3 (KO) cells was amplified by PCR. THP1-KO-NLRP3 cells feature a biallelic deletion (arrow). (B) Lysates from THP1-null (WT) and THP1-KO-NLRP3 (KO) cells were analyzed by Western blot (Wes™) using an anti-human NLRP3 antibody, followed by a HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the NLRP3 protein (110 kDa).

**Functional validation of THP1-KO-NLRP3 cells**

(A) Mature hIL-1β secretion

(B) Cell death

**Figure 2:** Absence of mature IL-1β secretion and pyroptosis by THP1-KO-NLRP3 cells upon inflammasome activation. ~3x10⁴ THP1-null (blue) and THP1-KO-NLRP3 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 - 0.1-10 µM), MSU crystals (MSU; 250 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 expressing an NF-κB SEAP reporter gene. QUANTI-Blue™ Solution was used to measure SEAP activity. Optical density (OD) was read at 630 nm. (B) Cell death was assessed using the lactate dehydrogenase (LDH) assay.

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TECHNICAL SUPPORT

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