Validation data for THP1-KO-NLRP3 cells

https://www.invivogen.com/thp1-ko-kd-nlrp3

For research use only

Version 20E28-NJ

THP1-KO-NLRP3 cells were generated from the human monocytic THP1 cell line through the stable knockout of the N-terminal region of the *NLRP3* gene. The KO status has been verified by DNA sequencing, PCR **(Figure 1A),** Western blot **(Figure 1B, C)**, 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)

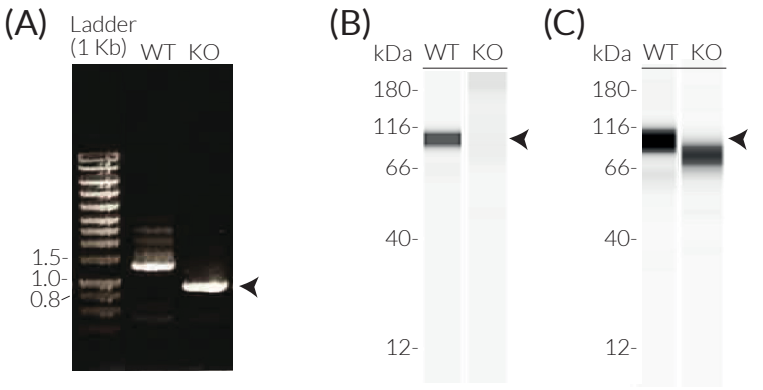

Figure 1: Validation of *NLRP3* KO in THP1-KO-NLRP3 cells. (A) The targeted NLRP3 region in THP1-null (WT) and THP1-KO-NLRP3 (KO) cells was amplified by PCR. THP1-KO-NLRP3 cells feature a biallelic deletion (arrow). **(B and C)** Lysates from THP1-null (WT) and THP1-KO-NLRP3 (KO) cells were analyzed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

Functional validation of THP1-KO-NLRP3 cells

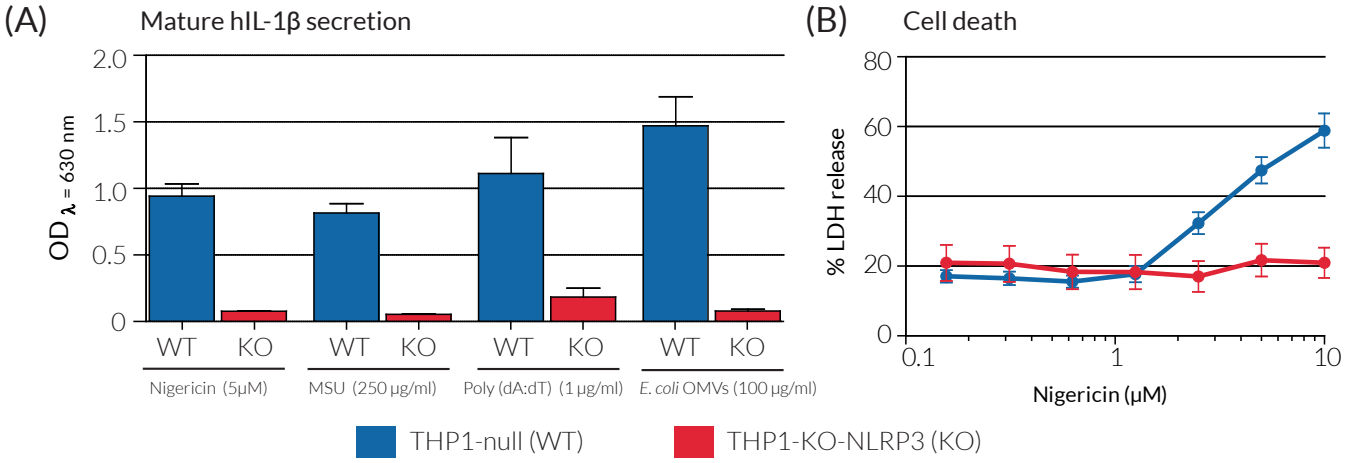

Figure 2: Absence of mature IL-1β secretion and pyroptosis by THP1-KO-NLRP3 cells upon inflammasome activation. ~3x10^5 THP1-null (blue) and THP1-KO-NLRP3 cells (red) were incubated for 3h at 37°C with LPS-EK (1 µg/ml) (*priming*) and then incubated (*activation*) with Nigericin (A - 5 µM; B - 0.1-10 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.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com