Validation data for THP1-KO-NLRP3 cells

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

For research use only

Version 20F22-NJ

THP1-KO-NLRP3 cells were generated from the human monocytic THP1-Null2 cell line through the deletion of the N-terminal region of the *NLRP3* gene. Only an inactive NLRP3 C-terminal fragment is expressed. 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, MSU crystals, or Alum Hydroxide) 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 inducers of the AIM2 inflammasome (i.e. Poly (dA:dT)) and non-canonical inflammasome (i. e. *E. coli* outer membrane vesicles (OMVs)) **(Figure 2A)**. Furthermore, pyroptosis is abrogated in THP1-KO-NLRP3 cells upon NLRP3 activation **(Figure 2B)**.

Validation of *NLRP3* Knockout (KO)

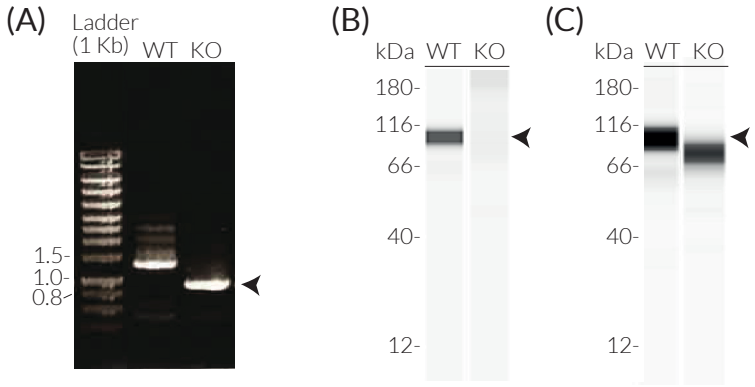

Figure 1: Validation of *NLRP3* KO in THP1-KO-NLRP3 cells. (A) The targeted NLRP3 region in THP1-Null2 (WT) and THP1-KO-NLRP3 (KO) cells was amplified by PCR. THP1-KO-NLRP3 cells feature a biallelic deletion (arrow). **(B and C)** Lysates from THP1-Null2 (WT) and THP1-KO-NLRP3 (KO) cells were analyzed 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

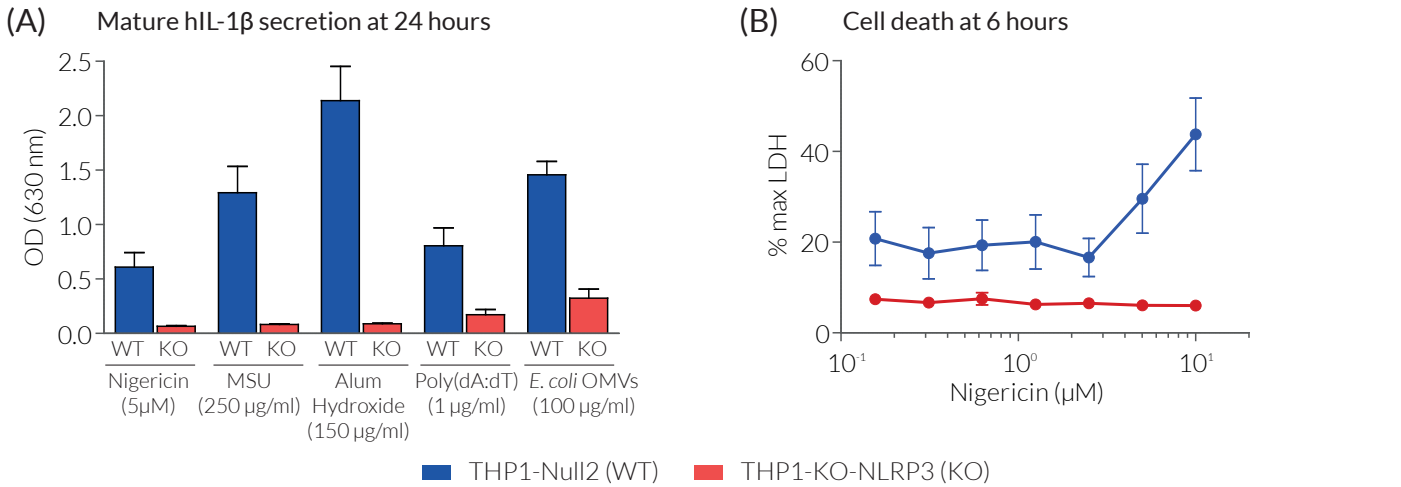

Figure 2: Absence of mature IL-1β secretion and pyroptosis by THP1-KO-NLRP3 cells upon inflammasome activation. ~3x10^5 THP1-Null2 (blue) and THP1-KO-NLRP3 cells (red) were incubated for 3h at 37°C with LPS-EK (1 μg/ml) (*priming*) and then incubated (*activation*) with Nigericin (A: 5 μM; B: 0.15-10 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.

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

FAQ Any questions about our cell lines? Visit our FAQ page.

InvivoGen
www.invivogen.com