Validation data for RAW-ASC KO-NLRC4 cells

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RAW-ASC KO-NLRC4 cells were generated from the murine RAW-ASC macrophage cell line through the stable knockout of the murine NLRC4 (*NLRC4*) gene. The KO status has been verified by PCR and Western blot **(Figure 1)**, and functional assays **(Figure 2)**. While mature IL-1β secretion in RAW-ASC KO-NLRC4 cells is comparable to the parental cell line upon NLRP3 and AIM2 inflammasome activation, this response is impaired upon activation of the NLRC4 inflammasome using Rod-Tox (LFn-Rod from *S. typhimurium* combined to the antrax protective antigen) **(Figure 2)**.

Validation of *NLRC4* Knockout (KO)

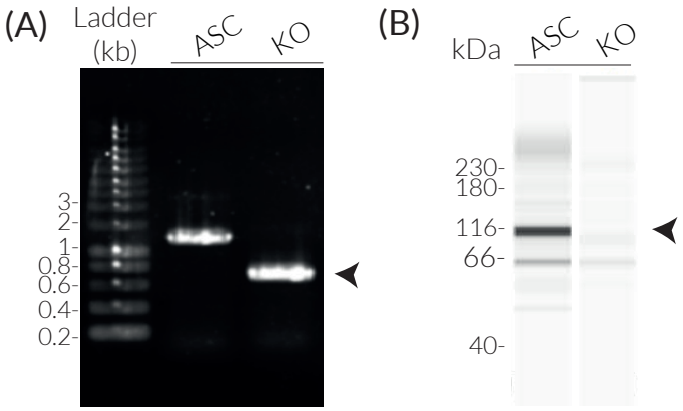

Figure 1: Validation of *NLRC4* KO in RAW-ASC KO-NLRC4 cells by PCR and Western blot (WES™).
(A) The targeted NLRC4 region in RAW-ASC (WT) and RAW-ASC KO-NLRC4 (KO) cells was amplified by PCR. RAW-ASC KO-NLRC4 cells feature a biallelic deletion (arrow).
(B) Lysates from RAW-ASC (ASC) and RAW-ASC KO-NLRC4 (KO) cells were analyzed by Western blot (Wes™) using an NLRC4 antibody (Abcam, ref 201792), followed by a HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the band for the NLRC4 protein (expected size ~115 kDa).

Functional validation of RAW-ASC KO-NLRC4 cells

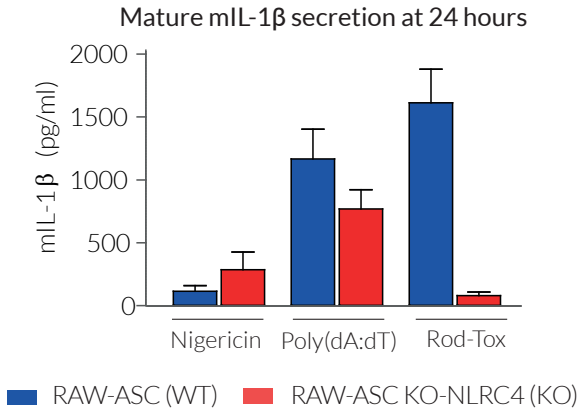

Figure 2: Secretion of mature IL-1β by RAW-ASC KO-NLRC4 cells and their parental RAW-ASC cells upon inflammasome activation. ~2x10^5 RAW-ASC (WT) and RAW-ASC KO-NLRC4 (KO) cells were incubated for 3h at 37°C with Pam3CSK4 (100 ng/ml) (*priming*) and then incubated (*activation*) with inflammasome inducers: Nigericin (5 µM), transfected Poly (dA:dT) (1 µg/ml), Rod-Tox (2 µg/ml). After 24h, the secretion of mature IL-1β was assessed in the culture supernatant using an ELISA assay.
Note: Rod-Tox is a combination of LFn-Rod (2 µg/ml) with the anthrax protective antigen (PA) (1 µg/ml). PA allows LFn-Rod translocation into the cytosol.

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