Validation data for RAW-ASC KO-CASP11 cells

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Version 20G27-NJ

RAW-ASC KO-CASP11 cells were generated from the murine RAW-ASC macrophage cell line through the stable knockout of the murine caspase-11 (*CASP11)* gene. The KO status has been verified by PCR, Western blot **(Figure 1A-B)**, and functional assays **(Figure 2)**. While mature IL-1β secretion in RAW-ASC KO-CASP11 cells is comparable to the parental cell line upon canonical inflammasome (NLRP3 and AIM2) activation **(Figure 2A)**, this response is impaired upon activation of the non-canonical caspase-11 inflammasome using *E. coli* outer membrane vesicles (OMVs) or transfected LPS-EK **(Figure 2B)**.

Validation of *CASP11* Knockout (KO)

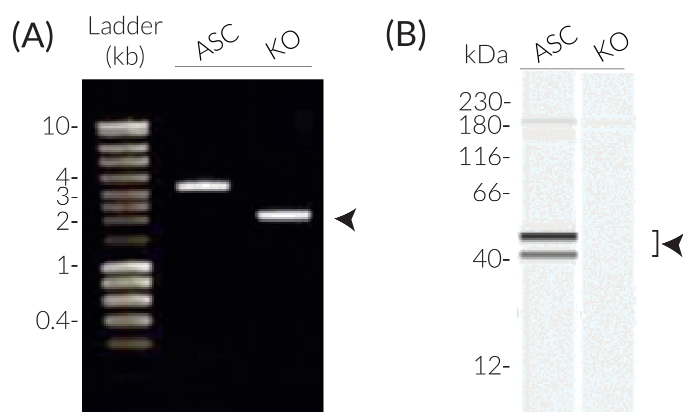

Figure 1: Validation of *CASP11* KO in RAW-ASC KO-CASP11 cells by PCR and Western blot (WES™).
(A) The targeted CASP11 region in RAW-ASC (WT) and RAW-ASC KO-CASP11 (KO) was amplified by PCR. RAW-ASC KO-CASP11 feature a biallelic deletion (arrow). **(B)** Lysates from RAW-ASC (ASC) and RAW-ASC KO-CASP11 (KO) cells were analyzed using an anti-mouse CASP11 antibody, followed by a HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the bands for the pro-caspase 11 protein isoforms (expected sizes ~38 and 43 kDa).

Functional validation of RAW-ASC KO-CASP11 cells

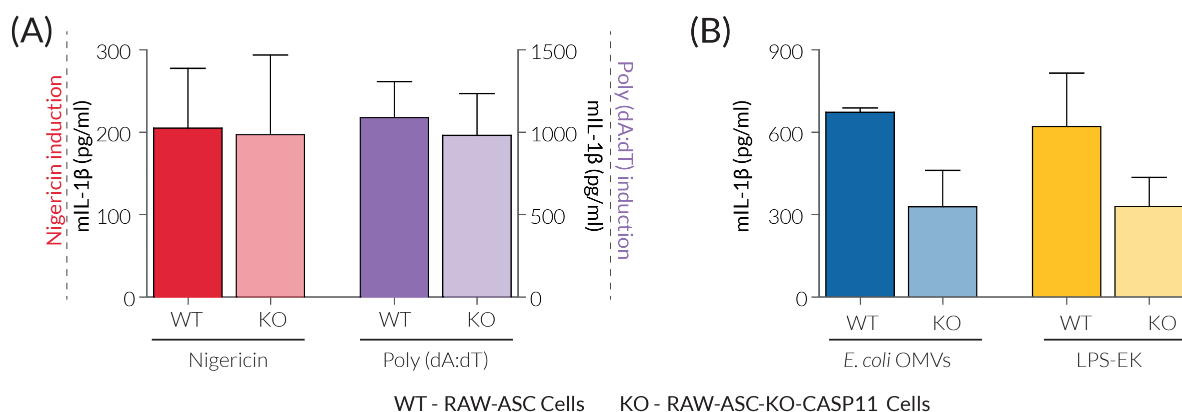

Figure 2: Secretion of mature IL-1β by RAW-ASC KO-CASP11 cells and their parental RAW-ASC cells upon inflammasome activation.
~$2x10^5$ RAW-ASC (WT) and RAW-ASC-KO-CASP11 (KO) cells were incubated for 3h at 37°C with Pam3CSK4 (100 ng/ml) (*priming*) and then incubated (*activation*) with **(A)** canonical inflammasome inducers Nigericin (5 μM) or transfected Poly(dA:dT) (1 μg/ml), and **(B)** non-canonical inflammasome inducers, *E. coli* outer membrane vesicles (OMVs) (100 μg/ml) or transfected LPS-EK (5 μg/ml). After 24h, the secretion of mature murine (m)IL-1β was assessed in the culture supernatant using an ELISA assay.
Note: For non-canonical inflammasome activation, cells were pre-primed with recombinant murine IFN-γ (10 ng/ml) overnight at 37°C before priming.

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