

Validation data for RAW-ASC-KO-GSDMD cells

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RAW-ASC-KO-GSDMD cells were generated from the murine RAW-ASC macrophage cell line through the stable knockout of the murine gasdermin D (*GSDMD*) gene. The KO status has been verified by Western blot (Figure 1) and functional assays (Figure 2). Mature IL-1 β secretion is abolished after activation of canonical (NLRP3 and AIM2) and non-canonical (caspase-11) inflammasomes (Figure 2A-B).

Validation of *GSDMD* Knockout (KO)

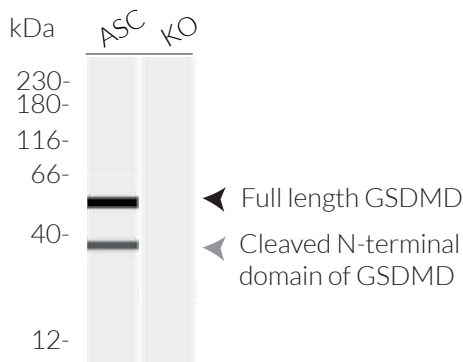


Figure 1: Validation of *GSDMD* KO in RAW-ASC-KO-GSDMD cells by Western blot (WES™). Lysates from RAW-ASC (ASC) and RAW-ASC-KO-GSDMD (KO) cells were analyzed using an anti-mouse *GSDMD* antibody, followed by a HRP-conjugated anti-rabbit secondary antibody. The arrows indicate the expected bands for the full length *GSDMD* protein (black arrow; 53 kDa) and the cleaved N-terminal domain (grey arrow; 33 kDa).

Functional validation of RAW-ASC-KO-GSDMD cells

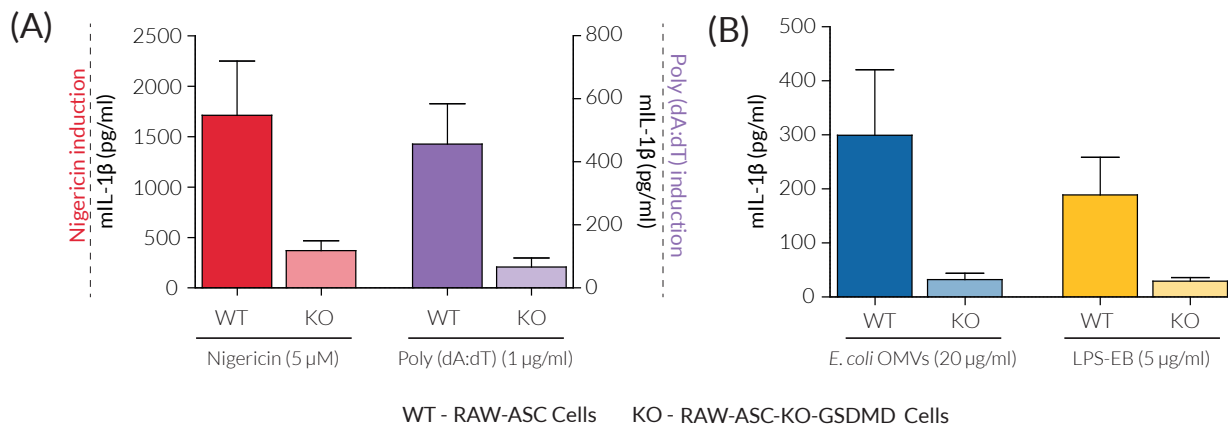


Figure 2: Secretion of mature IL-1 β by RAW-ASC-KO-GSDMD cells and their parental RAW-ASC cells upon inflammasome activation. $\sim 1 \times 10^6$ RAW-ASC (WT) and RAW-ASC-KO-GSDMD (KO) cells were incubated for 3h at 37°C with Pam3CSK4 (100 ng/ml) (priming) and then incubated (activation) with (A) canonical inflammasome inducers Nigericin (5 μ M) or transfected Poly (dA:dT) (1 μ g/ml), and (B) non-canonical inflammasome inducers, *E. coli* outer membrane vesicles (OMVs) (20 μ g/ml) or transfected LPS-EB (5 μ g/ml). After 6h, the secretion of mature IL-1 β 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.

TECHNICAL SUPPORT

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