Validation data for RAW-ASC cells

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RAW-ASC cells were generated by stable transfection of the murine *ASC* gene into the murine RAW 264.7 macrophage cell line which is otherwise ASC-deficient. The expression of ASC in RAW-ASC cells was verified by Western blot **(Figure 1)** and functional assays **(Figure 2)**. RAW-ASC cells produce and secrete mature IL-1β upon activation of canonical (NLRP3 and AIM2) **(Figure 2A)** and non-canonical (caspase-11) inflammasomes **(Figure 2B).**

Validation of RAW-ASC cells

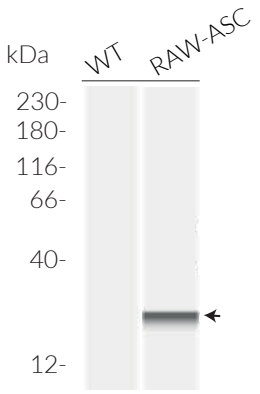

Figure 1: Validation of *ASC* expression in RAW-ASC cells by Western blot (WES™). Lysates from RAW 264.7 (WT) and RAW-ASC cells were analyzed using an anti-mouse ASC antibody, followed by a HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the ASC protein (27 kDa).

Functional validation of RAW-ASC cells

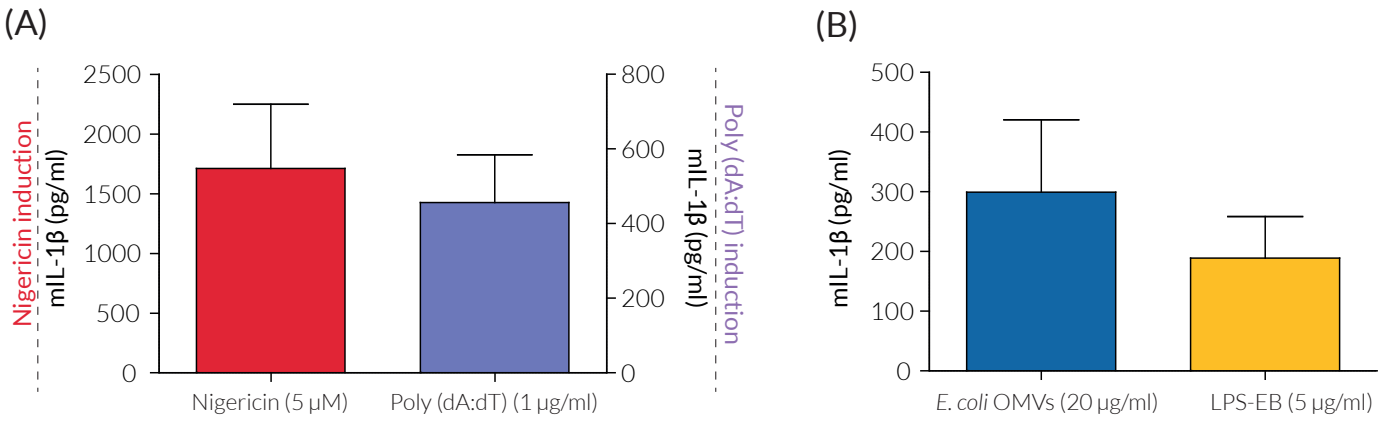

Figure 2: Secretion of mature IL-1β by RAW-ASC cells upon inflammasome activation. ~1x10^6 RAW-ASC cells were incubated for 3h at 37°C with Pam3CSK4 (100 ng/ml) (*priming*) and then incubated (*activation*) with **(A)** canonical inflammasome inducers Nigericin (5 µM) or transfected Poly (dA:dT) (1 µg/ml), and **(B)** non-canonical inflammasome inducers, *E. coli* outer membrane vesicles (OMVs) (20 µg/ml) or transfected LPS-EB (5 µg/ml). After 6h, the secretion of mature 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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