Validation data for LFn-Rod

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Version 21A04-NJ

LFn-Rod is a model of NLRC4/NAIP inflammasome agonist. Rod is a component of the type III secretion systems (T3SS) of intracellular bacteria described as an NLRC4/NAIP ligand. It is fused to the amino-terminal domain of *B. anthracis* lethal factor (LFn). This fusion system, when co-administred with the anthrax toxin's protective antigen (PA), allows intracellular delivery of the bacterial ligand. The combination of LFn-Rod with the anthrax protective antigen (PA) is named Rod-Tox. LFn-Rod specificity for NLRC4 has been verified using RAW-ASC KO-NLRC4 cells **(Figure 1)**. It induces NLRC4-dependent IL-1β secretion and pyroptosis in a dose-dependent manner **(Figure 2).**

Functional validation of LFn-Rod using RAW-derived cells

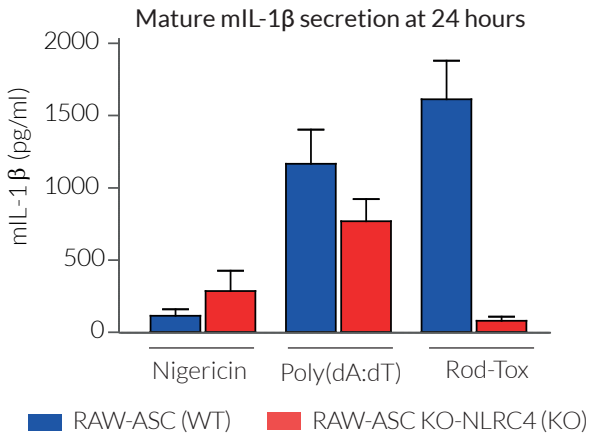

Figure 1: Secretion of mature IL-1β by RAW-ASC KO-NLRC4 cells and their parental RAW-ASC cells upon inflammasome activation.
~2x10^5 RAW-ASC (WT) and RAW-ASC KO-NLRC4 (KO) cells were incubated for 3h at 37°C with Pam3CSK4 (100 ng/ml) (*priming*) and then incubated (*activation*) with inflammasome inducers: 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.*

Murine NLRC4 inflammasome response to LFn-Rod

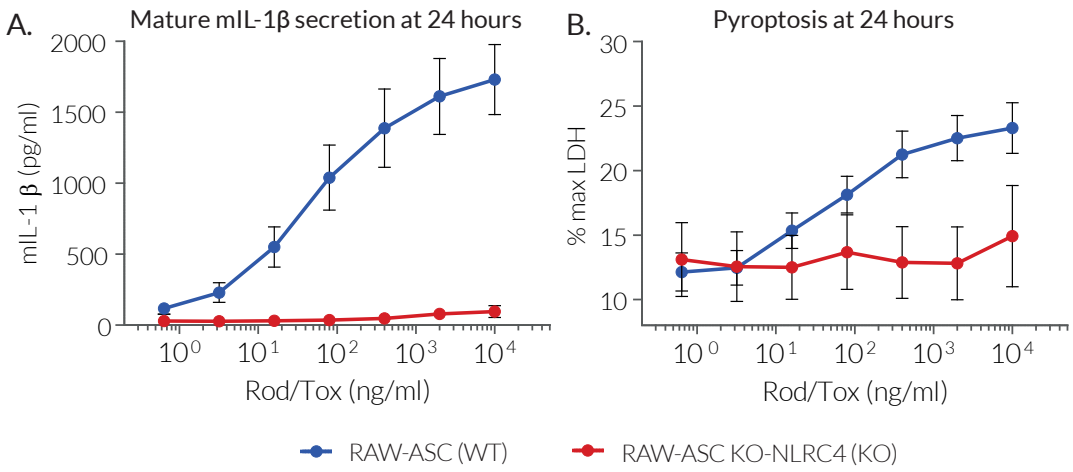

Figure 2: Secretion of mature IL-1β and pyroptosis upon RAW-derived cell activation using LFn-Rod.
~2x10^5 RAW-ASC (WT) parental and RAW-ASC KO-NLRC4 (KO) cells were incubated for 3h at 37°C with Pam3CSK4 (100 ng/ml) (priming) and then incubated (activation) with LFn-Rod (0.64 ng -10 µg/ml). After 24h activation, the secretion of mature IL-1β was assessed in the culture supernatant using an ELISA assay **(A)**, and cell death was assessed using the lactate dehydrogenase (LDH) assay **(B)**.

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