

Figure S1. Pyroptosis, caspase-1 activation and IL-1β secretion in RAW264.7, J774 and their derived cell lines. A. Pyroptosis, processing of caspase-1 (CASP1) and secretion of IL-1β in RAW264.7 and RAW-asc cell lines. RAW-asc is a RAW264.7-derived cell line with ectopic expression of ASC. The supernatants of the cultures of RAW264.7 and RAW-asc cells were collected after they were primed with LPS (100 ng/ml) for 4 hours followed by nigericin (10 µM) treatment for 2 hours. LDH release was used to measure pyroptosis. ELISA was used to measure the secretion of IL-1β in the supernatants. Pro-caspase-1 and p20 caspase-1 in the supernatants were TCA precipitated followed by immunoblotting with anti-casp1 antibody. B. Pyroptosis, processing of caspase-1 and IL-1β in J774 derived cell lines. The supernatants of the cultures of wildtype (WT), *NIrp3-/-* and NLRP3-Flag-reconstituted (NLRP3-Flag) J774 cells were collected after they were treated as in A. zVAD (10 µM) was included in some samples to block caspase-1 auto-cleavage. Pyroptosis was determined by measuring LDH release in the media. Supernatants were analyzed to determine LDH activity, and were TCA-precipitated and then analyzed by anti-CASP1 and anti-IL-1β immunoblotting. Graphs show mean ± s.d of triplicate wells and represent two independent experiments.