Supplementary Figure 1: Assembly of the ASC pyroptosomes requires TLR priming.

(A) Caspase-1 immunoblot of cell lysates (pro-caspase-1) and cell free supernatants (cleaved caspase-1) of C57BL/6, NLRP3-KO, and ASC-KO macrophages. (**B**) Immortalized wild type macrophages stably expressing ASC-YFP were primed as indicated with crude LPS from *E. coli*, re-purified LPS from *E. coli* or synthetic LPS, Pam2CysK4 (50 ng/ml), Pam3CysK4 (50 ng/ml) or R848 (0.5 μg/ml) for 4h. ATP was added for an additional 1h as indicated. Epifluorescence images of ASC-YFP wild type macrophages (a and b left) and calculated number of ASC pyroptosomes per visual field (**C** right and **D**). One representative experiment out of three (A) or four (B-D) is shown.

Supplementary Figure 2: Priming requirement for NLRP3 inflammasome activation for different NLRP3 inflammasome activators.

(A-C) LPS primed or resting macrophages were stimulated with nigericin (10 μM), ATP (5 mM) or monosodium uric acid crystals (MSU, 250 μg/ml) as indicated. Caspase-1 immunoblots of stimulated wild type macrophages or knock-out macrophages are shown. One representative experiment out of three independent experiments is depicted.

Supplementary Figure 3: IRAK4 deficiency impairs MyD88 dependent inflammasome formation.

(A) Wild type, MyD88-KO or IRAK4-KO macrophages were treated with Pam2CysK4 (50 ng/ml), R848 (2 μg/ml), poly(I:C) (1 μg/ml), MDP (10 μg/ml), LPS (200 ng/ml), or transfected with poly(dA-dT) for 4h or left untreated. ATP was added for an additional 1h as indicated. Immunoblot analysis of cleaved caspase-1 from supernatants is shown. **(B)** PBMCs of an IRAK4 mutant patient and a healthy volunteer were stimulated with Pam2CysK4 (5 ng/ml), R848 (100 ng/ml), iE-DAP (1 μg/ml), MDP (1 μg/ml), LPS (30 pg/ml) or transfected with poly(dA-dT) followed by addition of ATP (5 mM) for 1h. Caspase-1 activity in CD14 positive cells was assessed by flow cytometric analysis using the caspase-1 fluorescent inhibitor FAM-YVAD-FMK (FLICA). Percentage of FLICA peptide positive $CD14^+$ cells is shown. Data are from one representative experiment of three (A and B left). Data involving PBMCs from the IRAK4 mutant patient are from one single experiment.

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Supplementary Figure 4: NALP3 induction by LPS is dependent on LPS signaling and NF-κB

(A) Immunoblots for NLRP3, pro-IL-1β and β-actin in lysates from untreated or LPS-treated (200 ng/ml, 6 h) TLR4-KO, MyD88/TRIF-dKO or C57BL/6 macrophages. **(B)** Immunoblots for NLRP3, pro-IL-1β and β-actin from lysates of C57BL/6 macrophages treated as in A in the absence or presence of Bay11-7082. One representative experiment out of two independent experiments is depicted.

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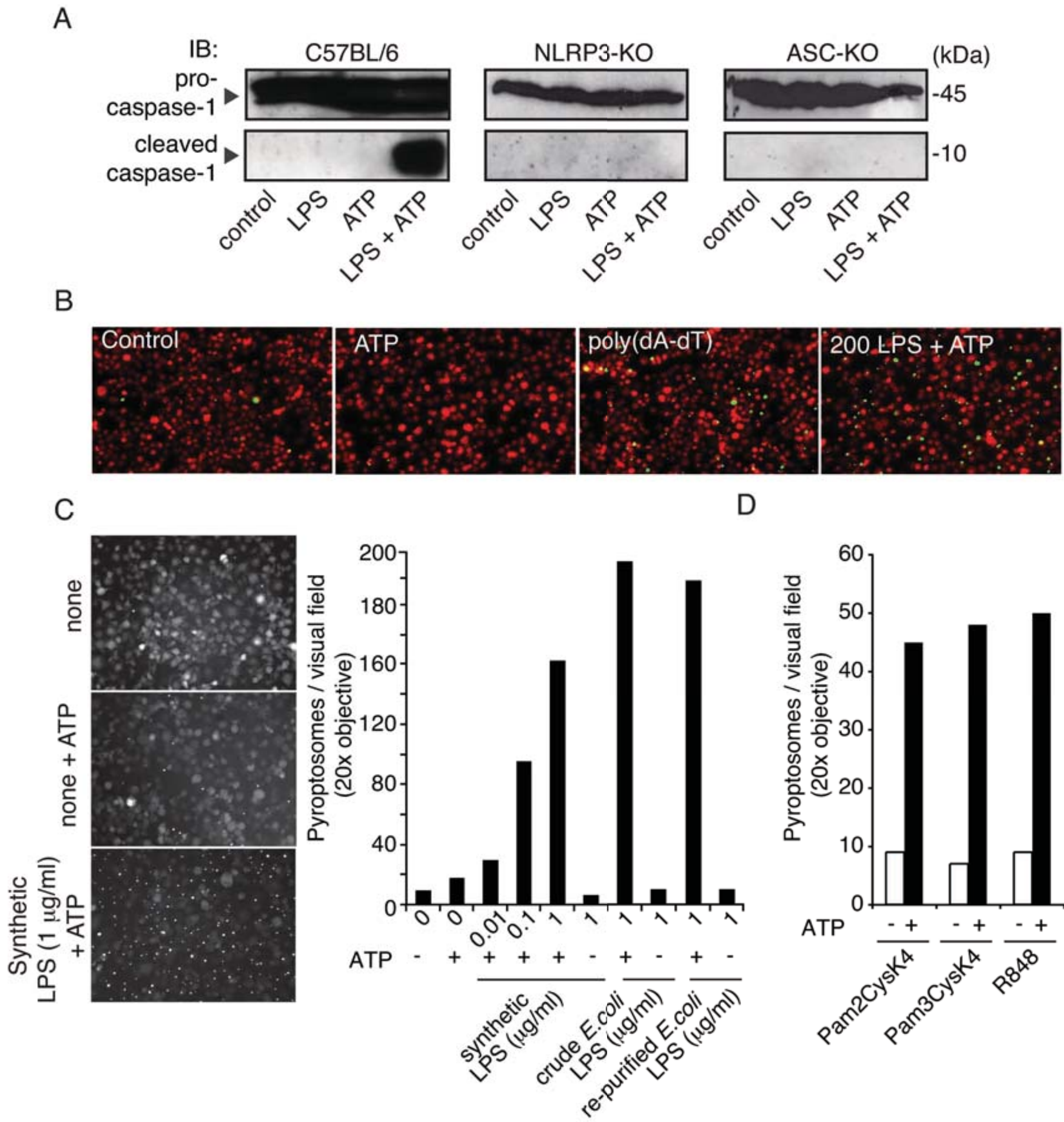

Supplementary Figure 1

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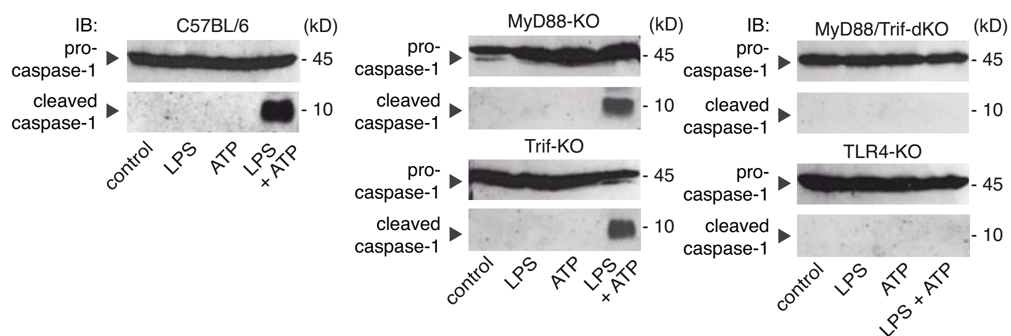

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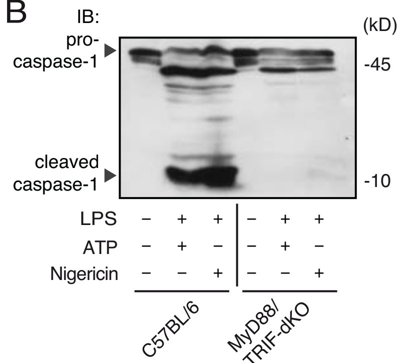

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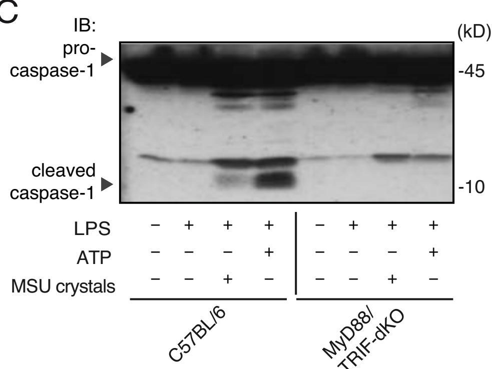

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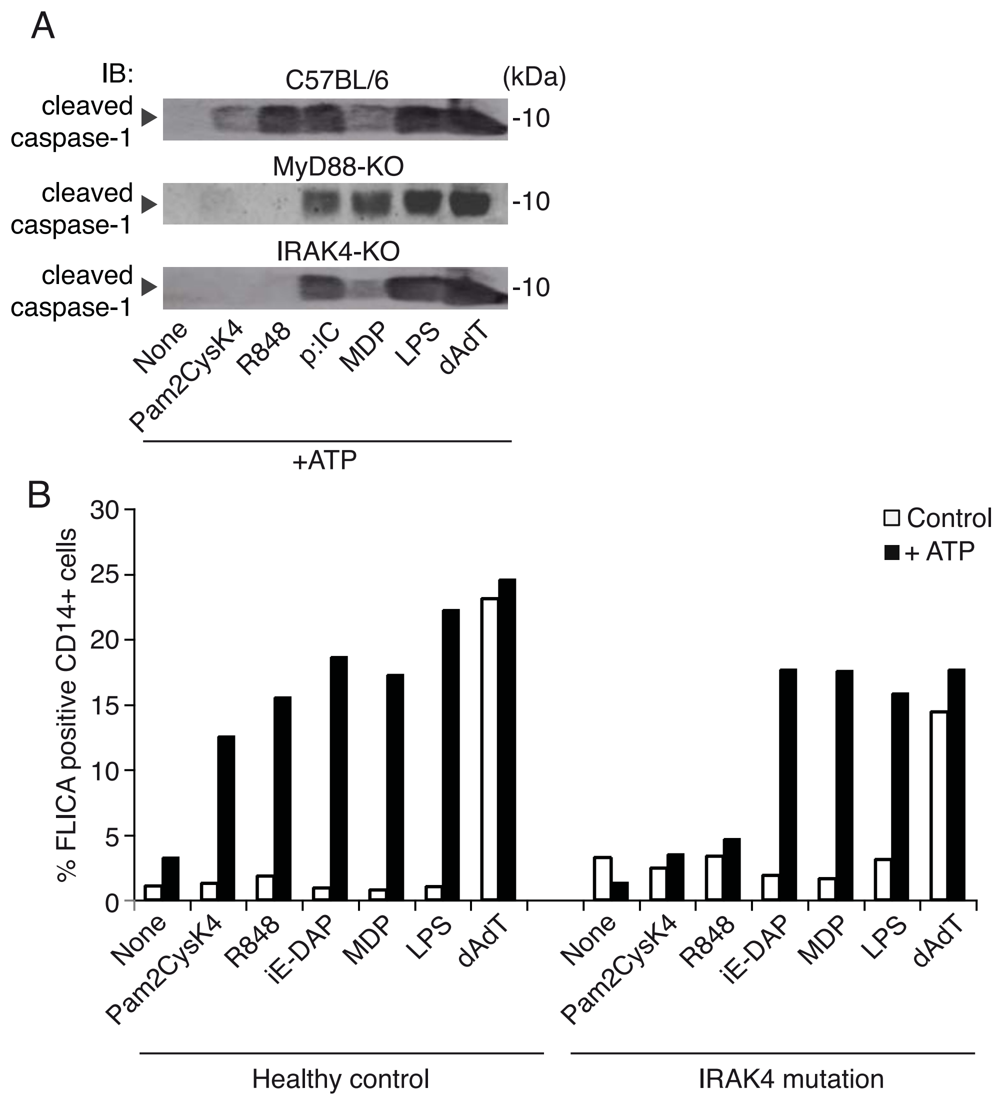
Supplementary Figure 2



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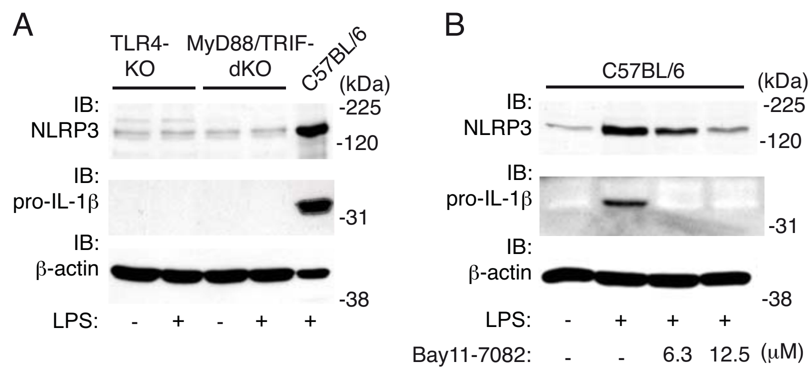
Supplementory Figure 3:



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Supplementary Figure 4