Supplemental Methods

Reagents

Ac-YVAD-CHO (ALX-260-027) was purchased from Enzo Life Sciences (Farmingdale, NY, USA). Recombinant human TNFα (#210-TA) and IL-1β (#201-LB) were purchased from R&D Systems (Minneapolis, MN, USA). Actinomycin D (A9415) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Quantifying mRNA decay

mRNA decay quantification was performed as previously described. Specifically, Actinomycin D (5 μg/mL) was added to cells pretreated for 18 h with 30 μM R848, with or without the presence of BIRB 796 (50 nM) for the latter 12 h. Total RNA was prepared at regular time intervals thereafter. IL-1β mRNA was quantified using real-time qRT-PCR.

Primers and TaqMan probes for the 3′ UTR sequences of IL-1β and TNFα were designed using Primer Express Version 3.0 software (Life Technologies, Carlsbad, CA, USA) and were as follows: IL-1β: probe 6-FAM-CGGCCACATTTGG-MGB; 5′ primer TTAAAGCCCGCCTGACAGA; 3′ primer GCGAATGACAGAGGGTTTCTTAGA; and TNFα: probe 6-FAM-CCGTGAAAACGGAGCT-MGB; 5′ primer TGCCTTGGCTCAGACATGTTT; 3′ primer GCTACATGGGAACAGCCTATTGT. Primers and probe were purchased from Life Technologies.

Immunoprecipitation

Immunoprecipitations were performed as previously described.
**BIO treatment**

A total of 200 000 cells/200 μL (T-shNT or T-shFC) were cultured in 96-well plates, pretreated with BIO (6-bromoindirubin-3’-oxime; 0.2-2 μM) for 2 h, and then treated with R848 (30 μM) for 24 h, after which culture supernatants were assayed for IL-1β using Quantikine ELISA Kits (R&D Systems).
Supplemental Figures

Figure S1. TLR-induced overproduction of IL-1β by FANCC-deficient cells is suppressed by the caspase-1 inhibitor YVAD. T-shNT and T-shFC cells were plated at a concentration of $10^6$/mL, pretreated with Ac-YVAD-CHO (YVAD; 50 μM) for 6 h, and stimulated with R848 (30 μM) for 24 h. Secreted IL-1β (A) and TNFα (B) were measured in the conditioned media by ELISA.

A

![IL-1β graph]

B

![TNFα graph]
Figure S2. TLR-induced overproduction of IL-1β by FA patient CD14+ cells is suppressed by the inflammasome inhibitor glyburide. CD14+ cells from a FA complementation group A patient were isolated from peripheral blood mononuclear cells using magnetic microbeads. Cells were plated at a concentration of 50,000/mL, pretreated with glyburide (Glyb; 50 μM) for 6 h, and stimulated with the indicated doses (μM) of R848 for 24 h. Secreted IL-1β was measured in the conditioned media by ELISA. R1 indicates R848 1 μM, R3 indicates R848 3 μM, R5 indicates R848 5 μM, and R10 indicates R848 10 μM.
Figure S3. Inhibition of p38 MAP kinase does not reduce the half-life of IL-1β and TNFα mRNA. T-shFC cells were plated at a concentration of 10^6/mL and treated with R848 (30 μM) for 6 h before addition of BIRB 796 (BIRB; 50 nM) for 12 h. Actinomycin D (5 μg/mL) was then added to the cultures. Total RNA was harvested at 0, 30, 60, 120, and 180 minutes after actinomycin D treatment. IL-1β (A) and TNFα (B) mRNA were quantified using real-time qRT-PCR and normalized to levels of 18S rRNA.

A

B
Figure S4. TNFα enhances R848-induced IL-1β in THP-1 cells, but IL-1β does not enhance R848-induced TNFα. (A) T-shNT and T-shFC cells were plated at a concentration of 10⁶/mL and treated with R848 (30 μM), with and without recombinant human TNFα (rhTNFα; 2 ng/mL) for 24 h. Secreted IL-1β was measured in the conditioned media by ELISA. P values were calculated using a paired Student t test. (B) T-shNT and T-shFC cells were plated at a concentration of 10⁶/mL and treated with R848 (30 μM), with and without the indicated doses of recombinant human IL-1β (rhIL-1β; 0, 1, 10, or 100 ng/mL) for 24 h. Secreted TNFα was measured in the conditioned media by ELISA.