Supporting Information

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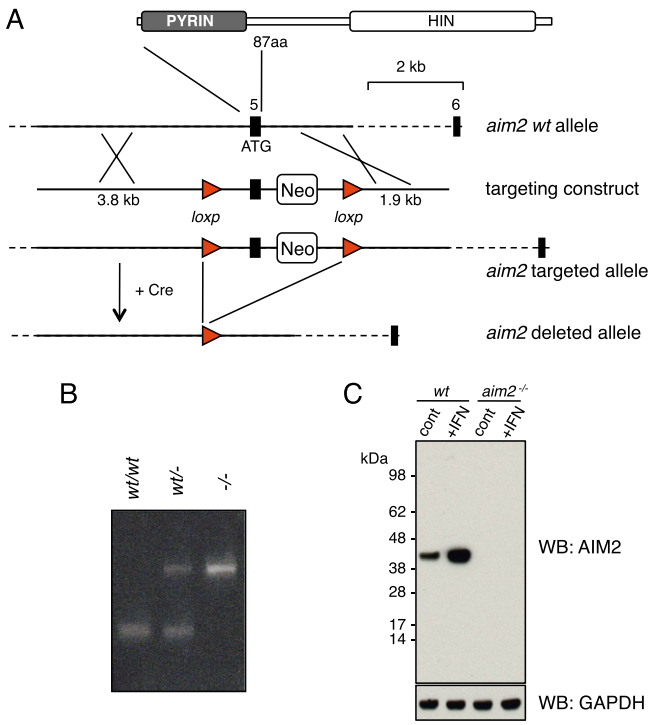

Fig. S1. Generation of absent in melanoma 2 (*Aim2*)$^{-/-}$ mice. (*A*) Strategy for deleting exon 5 of mouse *aim2*, which encodes the initiating methionine (ATG) and the entire PYRIN domain. Gene targeting was performed in C57BL/6 C2 embryonic stem cells. (*B*) PCR genotyping of *aim2*$^{-/-}$ mice. Primers 5′CCA GTG TTT CTC AAC TGT ACT GCT AT, 5′TAG GAG TGC CCT CCC TTA ATG, and 5′TTG GAG ACA GAC TCT GGT GAA G yield a 197-bp DNA fragment for the wild-type allele and a 397-bp DNA fragment for the knockout allele. (*C*) Bone marrow-derived macrophages (BMDMs) were incubated with 1,000 U/mL IFN-β for 5 h. AIM2 was Western blotted with 4G9.1.4 rat anti-mouse AIM2 monoclonal antibody.

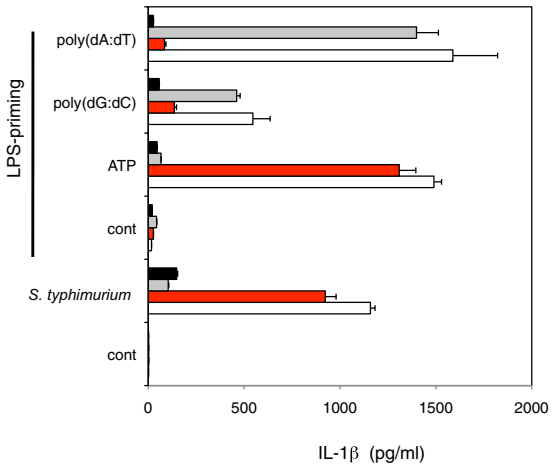

Fig. S2. AIM2 is essential for inflammasome activation in response to dsDNA in peritoneal macrophages. ■, *asc*$^{-/-}$; ■, *nlrp3*$^{-/-}$ *nlrc4*$^{-/-}$; ■, *aim2*$^{-/-}$; □, *wt*. Peritoneal macrophages were harvested 5 days after i.p. injection of 4% thioglycollate (DIFCO). These macrophages were primed with 500 ng/mL LPS for 5 h, and then, they were transfected with 1 μg/mL of the dsDNAs indicated for 16 h. As controls, LPS-primed cells were cultured in medium alone (cont) or stimulated with 5 mM ATP. Additional macrophages were not primed (cont) or were infected with *Salmonella typhimurium* (moi = 100). IL-1β secreted into the culture supernatant was measured by ELISA. Graphs show the mean ± SD of triplicate wells and are representative of three independent experiments.

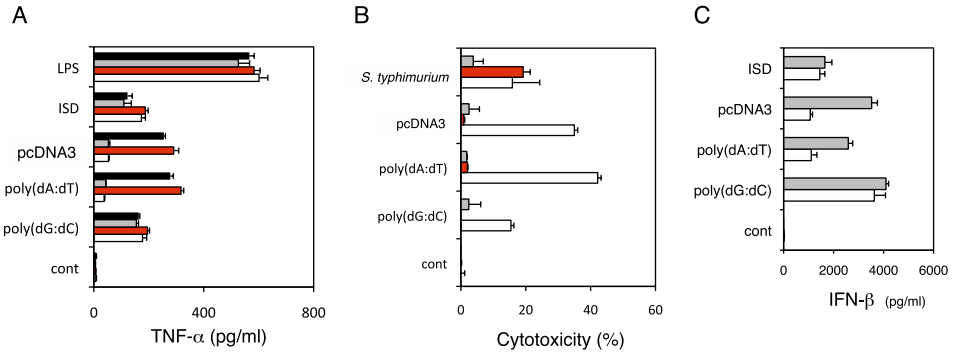

Fig. S3. AIM2 is dispensable for IFN-β and TNF-α production in response to dsDNA. BMDMs were unstimulated (cont), treated with 1 μg/mL LPS, or transfected with 1 μg/mL of the dsDNAs indicated for 16 h. (*A*) ■, *asc*$^{-/-}$; ■, *nlrp3*$^{-/-}$ *nlrc4*$^{-/-}$; ■, *aim2*$^{-/-}$; □, *wt*. TNF-α secreted into the culture supernatant was measured by Bio-Plex Cytokine Assay (Bio-Rad). (*B*) ■, *casp-1*$^{-/-}$; ■, *aim2*$^{-/-}$; □, *wt*. Cytotoxicity was measured by lactate dehydrogenase (LDH) release. (*C*) ■, *casp-1*$^{-/-}$; □, *wt*. IFN-β secreted into the culture supernatant was measured by ELISA. Graphs show the mean ± SD of triplicate wells and are representative of three independent experiments.

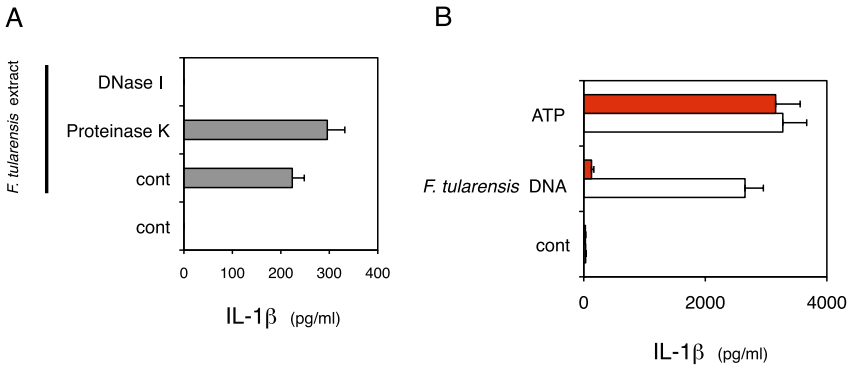

Fig. S4. *Francisella tularensis* DNA triggers IL-1β secretion. (*A*) Extract from *F. tularensis* ssp. *novicida* strain U112 was prepared in 10 mM Tris-HCl (pH 7.5) with a French press. Extract corresponding to 2 × 10^6 cfu was treated with nothing (cont), proteinase K (Qiagen) for 16 h at 55 °C, or DNase I (Qiagen) for 2 h at 25 °C, and then, it was transfected into BMDMs primed with 500 ng/mL Pam3CSK4 for 5 h. IL-1β secreted into the culture supernatant was assayed 3 h later. (*B*) ■, *aim2*$^{-/-}$; □, *wt*. Pam3CSK4-primed BMDMs were transfected with 1 μg/mL *F. tularensis* DNA, which was isolated from bacteria with a Qiagen DNeasy Blood and Tissue Kit. IL-1β secreted into the culture supernatant was assayed 16 h later. Graphs show the mean ± SD of triplicate wells and are representative of three independent experiments.

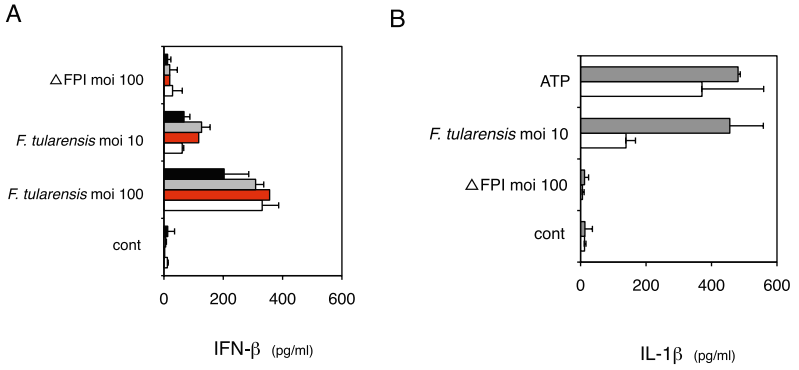

Fig. S5. IFN-1 facilitates formation of the AIM2-containing inflammasome. (*A*) ■, *asc*$^{-/-}$; ■, *caspase-1*$^{-/-}$; ■, *aim2*$^{-/-}$; □, *wt*. IFN-β secretions by BMDMs were untreated (cont) or infected with *F. tularensis* ssp. *novicida* strain U112 and isogenic mutant ΔFPI for 5 h. (*B*) ■, *ifnar*$^{-/-}$ + AIM2/GFP; □, *ifnar*$^{-/-}$ + GFP. *ifnar*$^{-/-}$ bone marrow was transduced with pMSCV2.2-IRES-GFP–encoding mouse AIM2 or the empty parental vector after retroviral particles were generated with the Phoenix.Eco packaging cell line. Three days later, macrophages were differentiated with macrophage-colony stimulating factor (M-CSF)–containing medium, and GFP-positive cells were sorted in a FACS Aria (Becton Dickinson). These BMDMs were primed with 500 ng/mL Pam3CSK4 for 16 h and then infected with *F. tularensis* or treated with 5 mM ATP. IL-1β secreted into the supernatant was assayed after 5 h. Graphs show the mean ± SD of triplicate wells and are representative of three independent experiments.