Supporting Information

Yu et al. 10.1073/pnas.1414859111

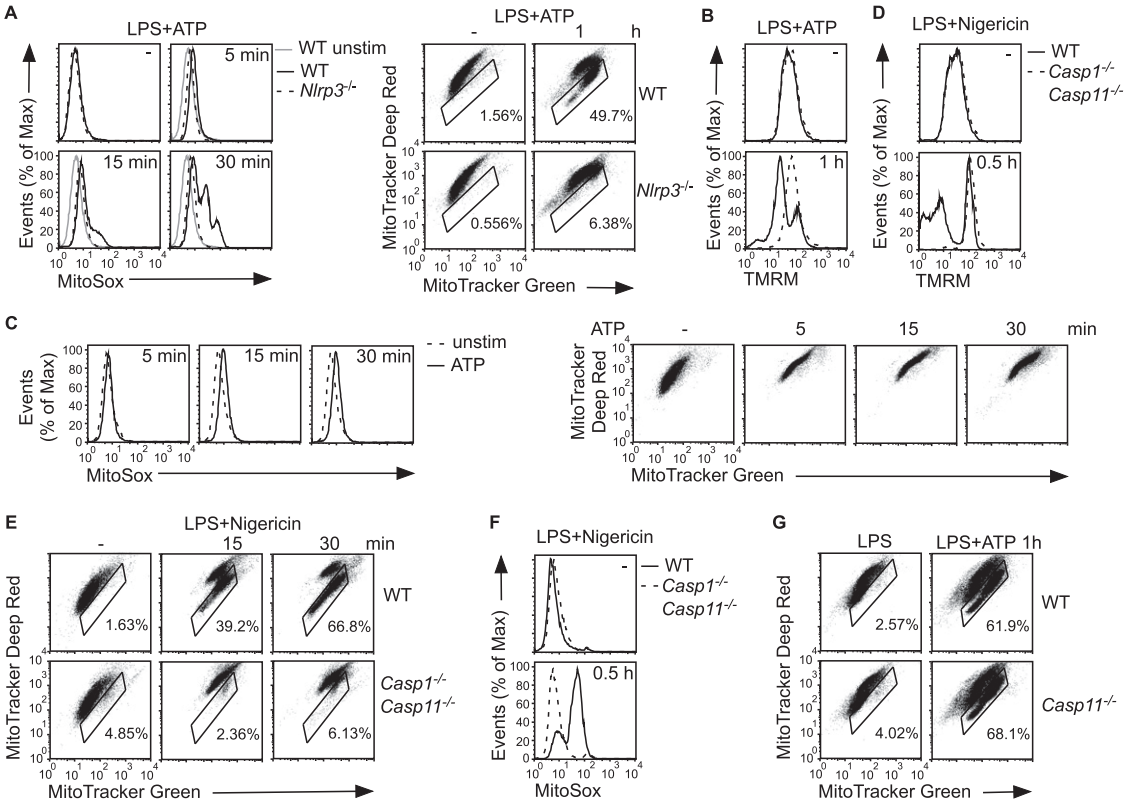

Fig. S1. The NLRP3 inflammasome amplifies mitochondrial damage. (*A*) WT and *Nlrp3*$^{-/-}$ BMDMs were stimulated as indicated, followed by MitoSox (*Left*) or Mitotracker Green and Deep Red (*Right*) staining. (*B*) WT and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ BMDMs stimulated as indicated were examined by TMRM staining. (*C*) WT BMDMs were stimulated with ATP followed by MitoSox (*Left*) or Mitotracker Green and Deep Red staining (*Right*). (*D–F*) WT and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ BMDMs were stimulated as indicated followed by TMRM (*D*), Mitotracker Green and Deep Red (*E*), and MitoSox (*F*) staining. (*G*) WT and *Casp11*$^{-/-}$ BMDMs stimulated as indicated were examined by Mitotracker Green and Deep Red staining.

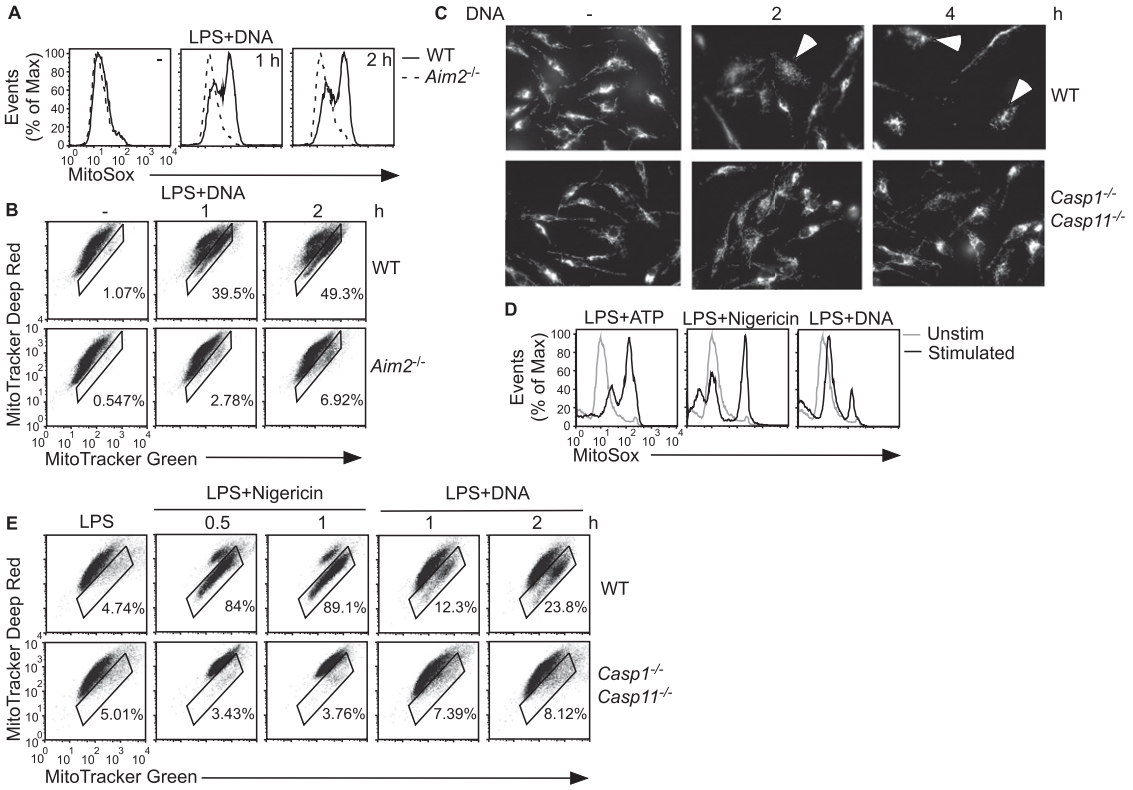

Fig. S2. The AIM2 inflammasome instigates mitochondrial damage. (*A* and *B*) WT and *Aim2*$^{-/-}$ BMDMs were stimulated as indicated followed by MitoSox (*A*) and Mitotracker Green and Deep Red (*B*) staining. (*C*) Mitochondrial morphology of DNA-transfected WT and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ BMDMs was examined by immunofluorescence by staining with an α-Tom20 antibody. (*D*) Thioglycollate-elicited peritoneal macrophages were stimulated as indicated followed by MitoSox staining. (*E*) MCSF-generated BMDMs were stimulated as indicated followed by MitoTracker Green and Deep Red staining.

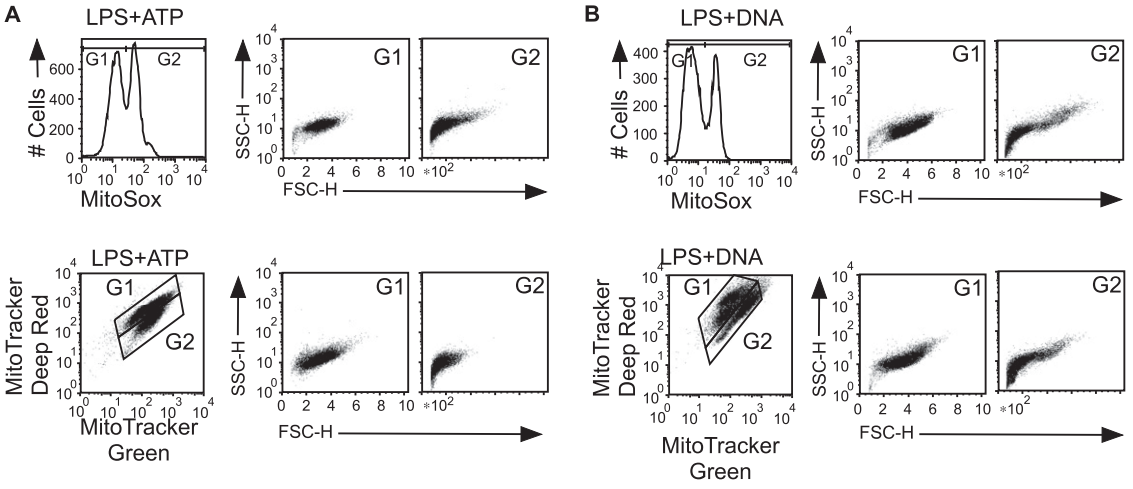

Fig. S3. Only cells with Caspase-1–dependent mitochondrial damage display the FSC/SSC profile of dead cells. LPS-primed BMDMs were stimulated with ATP (*A*) or transfected with DNA (*B*) followed by MitoSox staining (*Upper*) or Mitotracker Green and Deep Red (*Lower*) staining (reproduced from Figs.1 *A* and *B* and 2 *A* and *B*). Induction of Caspase-1–dependent mitochondrial damage (see comparison of WT and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ BMDMs in Figs. 1 *A* and *B* and 2 *A* and *B*) in some (G2 gate) but not other cells (G1 gate) correlates with a low FSC/SSC profile (*Right*) indicative of cell death.

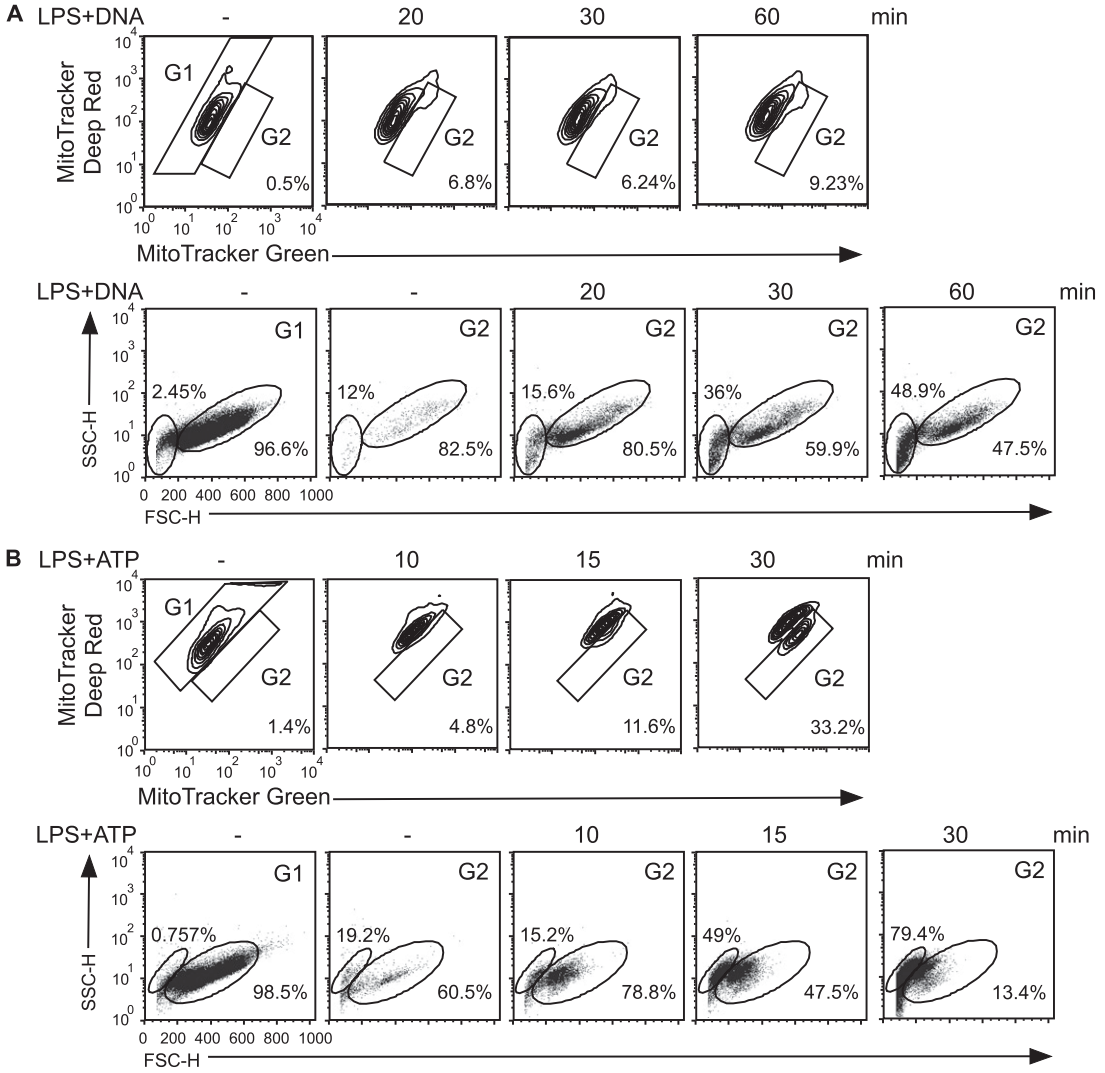

Fig. S4. Caspase-1–dependent mitochondrial damage precedes cell death. LPS-primed BMDMs were transfected with DNA (*A*) or stimulated with ATP (*B*) for varying times as indicated followed by Mitotracker Green and Deep Red staining. Cells are gated based on Caspase-1–dependent mitochondrial swelling or depolarization (*Upper*), followed by analysis of FSC/SSC in gated populations (*Lower*). Caspase-1–dependent mitochondrial swelling (*A*) or depolarization (*B*) in a subpopulation of cells precedes the appearance of dead cells with low FSC/SSC profile.

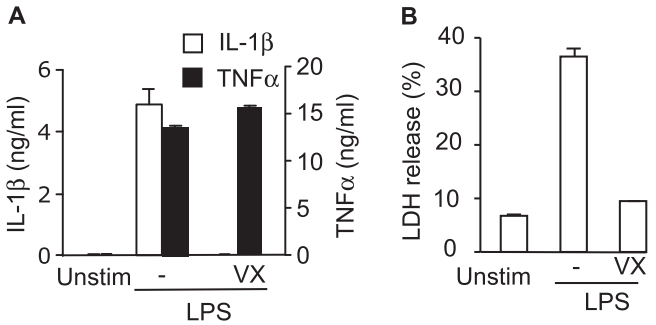

Fig. S5. Mitochondrial damage in CAPS macrophages. The Caspase-1 inhibitor VX765 (VX) reduces LPS-induced IL-1β production (*A*) and LDH release (*B*) in *Nlrp3*$^{L351P+/-}$*/CreT* BMDMs.

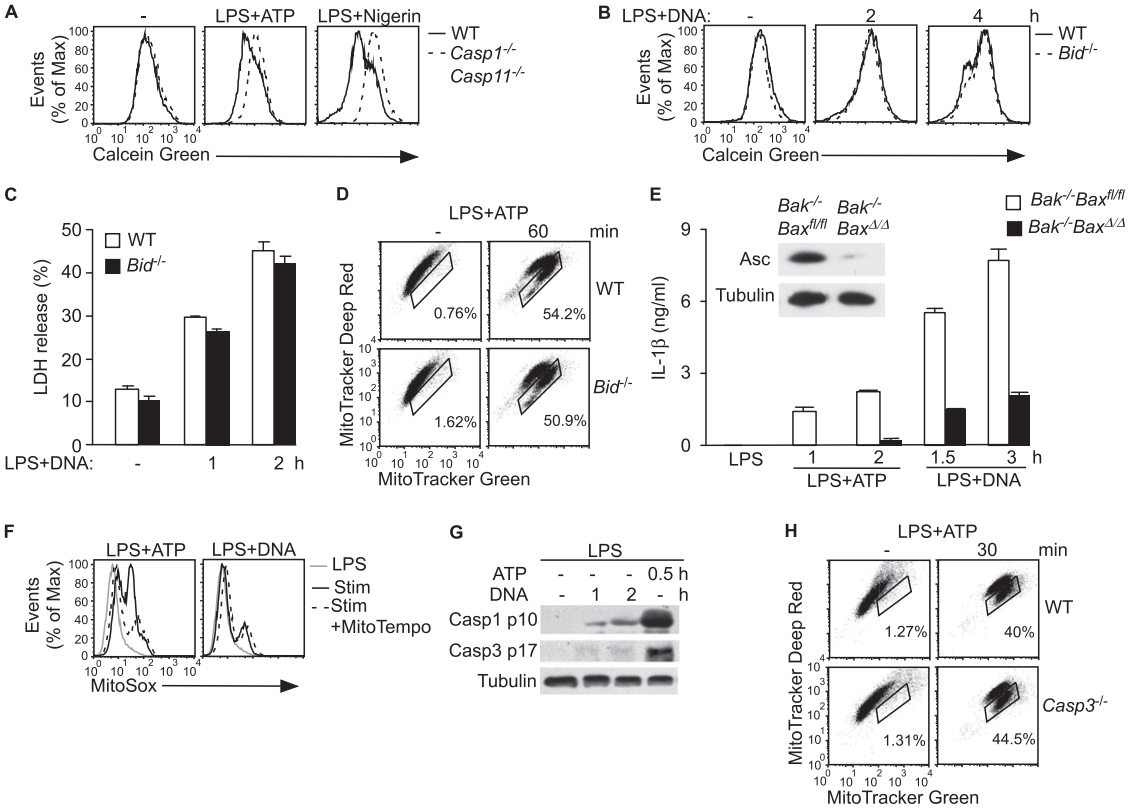

Fig. S6. Role of Bid in inflammasome-mediated mitochondrial damage. (*A*) WT and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ BMDMs were stimulated as indicated, followed by analysis in calcein green quenching assay. (*B–D*) WT and *Bid*$^{-/-}$ BMDMs were stimulated as indicated, followed by analysis in calcein green quenching (*B*), LDH release assay (*C*), and Mitotracker Green and Deep Red staining (*D*). (*E*) *Bak*$^{-/-}$*Bax*$^{fl/fl}$ and *Bak*$^{-/-}$*Bax*$^{\Delta/\Delta}$ macrophage cell lines were examined by Western blotting of whole-cell lysates with α-Asc antibody (*Upper*) or IL-1β ELISA of culture free supernatants (*Lower*). (*F*) Analysis of MitoSox staining in WT BMDMs stimulated as indicated ± MitoTempo cotreatment. (*G*) WT BMDMs were stimulated as indicated followed by analysis of Caspase-1 and Caspase-3 processing to the active subunits. (*H*) WT and *Casp3*$^{-/-}$ BMDMs were stimulated as indicated and analyzed by Mitotracker Green and Deep Red staining.

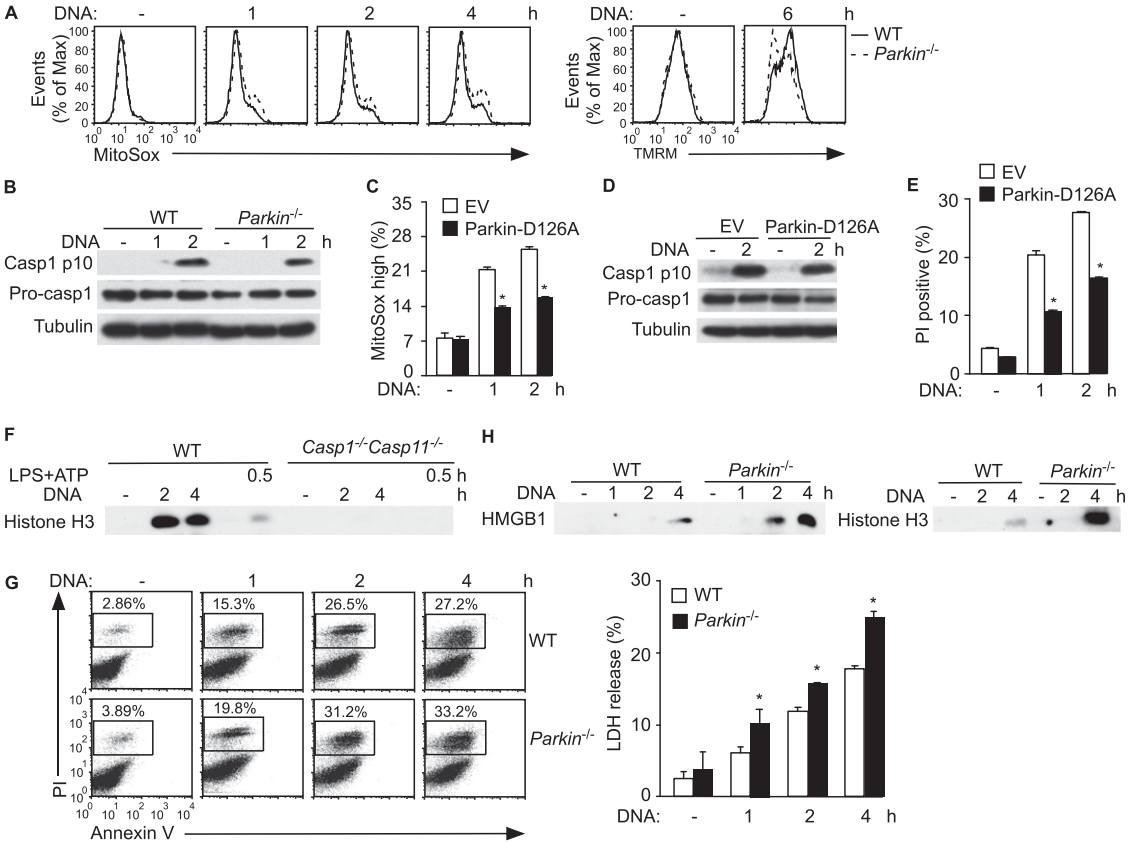

Fig. S7. Role of Parkin in inflammasome-mediated mitochondrial damage. (*A*) WT and *Parkin*$^{-/-}$ BMDMs were stimulated as indicated, followed by analysis by MitoSox (*Left*) and TMRM (*Right*) staining. (*B*) Whole-cell lysates from WT and *Parkin*$^{-/-}$ BMDMs were analyzed for Caspase-1 processing by Western blotting. (*C*) Quantitation of $n > 3$ experiments represented in Fig. 6*B*. *$P < 0.05$ relative to cells expressing EV. (*D*) Whole-cell lysates from Parkin-deficient macrophage cell lines reconstituted with empty vector (EV) or Parkin-D126A were analyzed for Caspase-1 processing by Western blotting. (*E*) Quantitation of $n > 3$ experiments represented in Fig. 6*D*. *$P < 0.05$ relative to cells expressing EV. (*F*) WT and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ BMDMs stimulated as indicated were analyzed for histone release by immunoblotting of cell-free culture supernatants. (*G* and *H*) WT and *Parkin*$^{-/-}$ BMDMs transfected with DNA were analyzed for plasma-membrane permeabilization by AV/PI staining (*G*, *Left*) and LDH release (*G*, *Right*), or for extracellular release of histone H3 and HMGB1 by immunoblotting of cell-free culture supernatants (*H*).

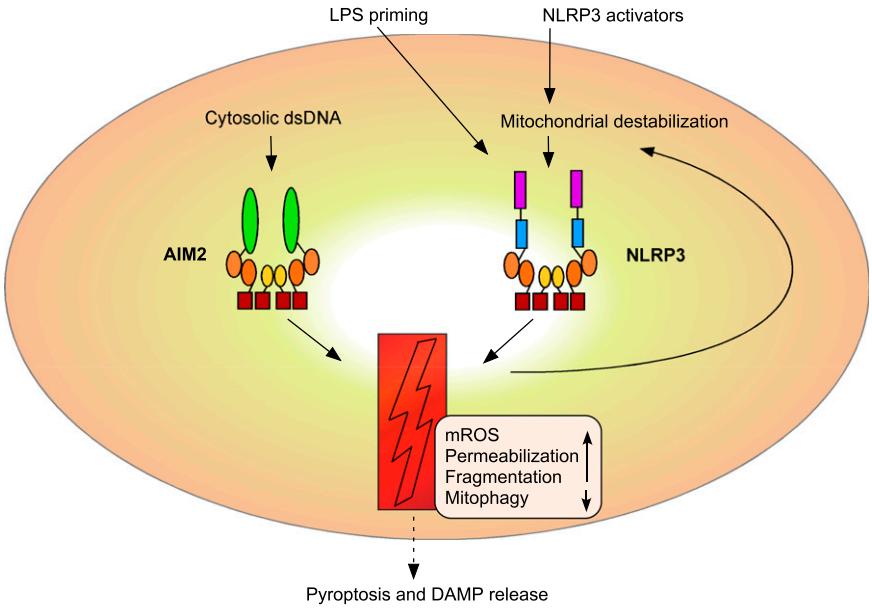

Fig. S8. Working model. Activation of the AIM2 inflammasome by cytosolic double-strand DNA leads to Caspase-1–dependent instigation of mitochondrial damage. NLRP3 inflammasome activators may perturb mitochondria directly to activate the NLRP3 inflammasome, resulting in Caspase-1–dependent amplification of mitochondrial damage. Caspase-1–mediated mROS production, mitochondrial permeabilization, fragmentation of the mitochondrial network, and inhibition of mitophagy may contribute to pyroptosis and DAMP release.

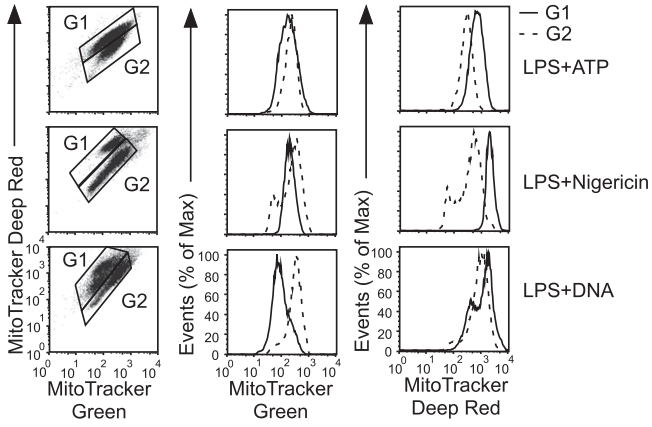

Fig. S9. Nonidentical mitochondrial damage profiles triggered by the AIM2 and NLRP3 inflammasome pathways. Analysis of Mitotracker Green and Deep Red staining in LPS-primed WT BMDMs stimulated with ATP for 30 min, nigericin for 30 min, or transfected with DNA for 2 h (taken from Fig. 1*B*, Fig. S1*E*, and Fig. 2*B*, respectively, and reproduced here in *Left*). Cells in G2 but not G1 display inflammasome-mediated mitochondrial damage. Overlay of G1 and G2 populations indicate prominent NLRP3 inflammasome-mediated mitochondrial depolarization (decreased Mitotracker Deep Red staining, *Right*) and AIM2-inflammasome-mediated mitochondrial swelling (increased Mitotracker Green staining, *Center*).