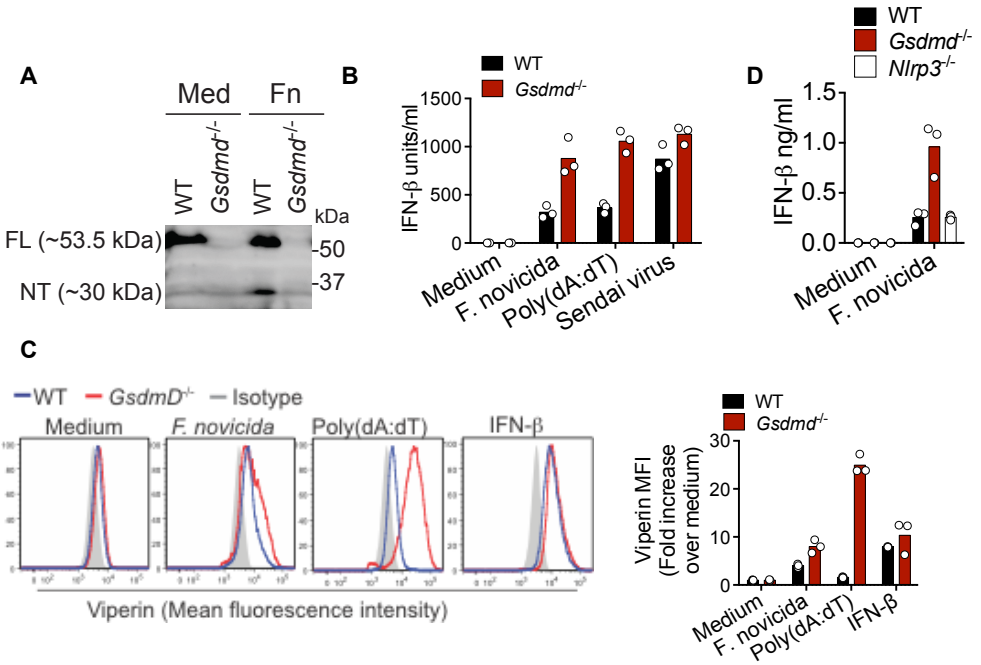

Fig. S1. Gasdermin D deficiency enhances cGAS-induced IFN-β response in primary BMDMs. Related to Figure 2.

(A) Immunoblot showing full length (FL) and cleaved N terminal fragment (NT) of gasdermin D in the lysates of wild-type and *Gsdmd*$^{-/-}$ iBMDMs untreated (Med, medium) or infected with *F. novicida* (Fn) for 6 h.

(B) IFN-β in the supernatants of wild-type and *Gsdmd*$^{-/-}$ primary BMDMs stimulated with poly(dA:dT), *F. novicida* or Sendai virus. IFN-β data are presented as units/ml or ng/ml depending upon the recombinant IFN-β standard used in the ELISA.

(C) Histograms displaying fluorescence intensities of viperin in WT and *Gsdmd*$^{-/-}$ iBMDMs stimulated with *F. novicida*, poly(dA:dT) or IFN-β. The fold increase in mean fluorescence intensity (MFI) of viperin in *F. novicida*-, poly(dA:dT)-, or IFN-β-treated over untreated cells (medium) is also shown.

(D) IFN-β in the supernatants of wild-type, *Gsdmd*$^{-/-}$, and *Nlrp3*$^{-/-}$ primary BMDMs stimulated with *F. novicida* for 6 h.

Data presented as mean are from one experiment representative of three. In the bar graphs, each dot represents a technical replicate.

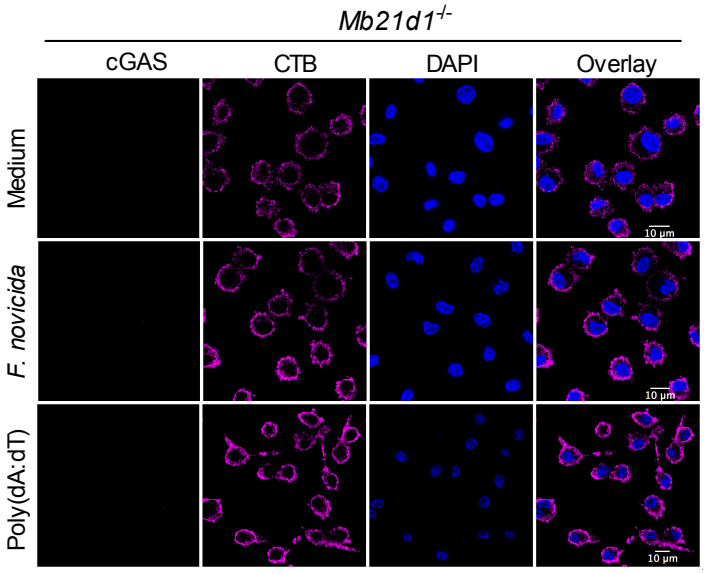

Fig. S2. Validation of cGAS antibody for immunofluorescence staining. Related to Figure 3.

Confocal images of cGAS-deficient BMDMs stimulated with *F. novicida* or poly(dA:dT) for 5 h. Cells were stained with an antibody for cGAS (green), cholera toxin B for plasma membrane (magenta), and DAPI for nucleus and DNA (blue).Scale bar, 10 μM. Corresponding confocal images of WT cells (positive controls) are shown in Fig. 3E.

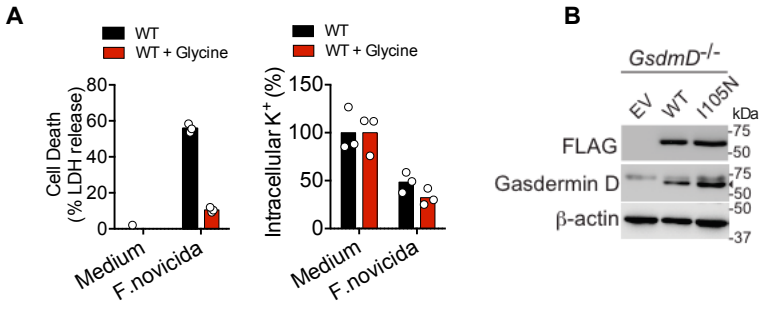

Fig. S3. Glycine treatment prevents pyroptotic lysis without altering K^+ efflux. Related to Figure 4.

(A) Cell death (% LDH release) and intracellular K^+ in uninfected (medium) or *F. novicida*-infected wild-type (WT) BMDMs in the presence or absence of 50 mM glycine (assessed at 6 h post-infection). Note that glycine treatment reduces LDH release without affecting K^+ efflux.
(B) Immunoblots of FLAG, gasdermin D and β-actin in the lysates of *Gsdmd*$^{-/-}$ iBMDMs reconstituted with the empty vector (EV), WT gasdermin D, or I105N mutant gasdermin D. Arrowhead indicates gasdermin D band.
Data presented as mean are from one experiment representative of three. In the bar graphs, each dot represents a technical replicate.

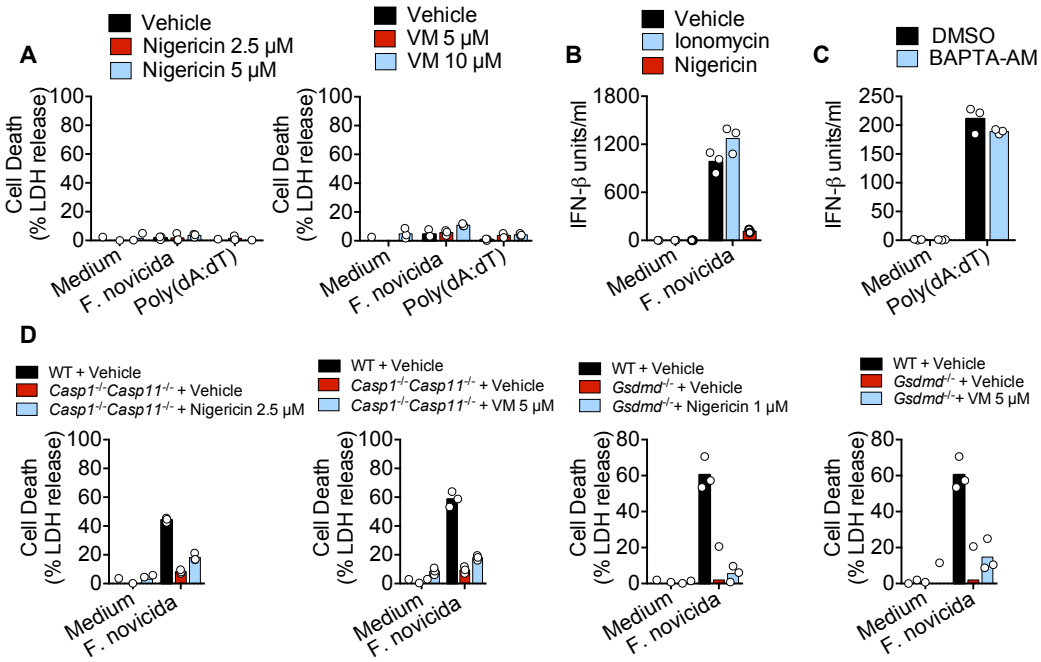

Fig. S4. Cytosolic DNA-induced IFN-β was not affected by the calcium ionophore (ionomycin) and calcium chelator (BAPTA-AM). Related to Figure 5.

(A) LDH release (at 6 h post-stimulation) by RAW macrophages stimulated with *F. novicida* (MOI of 50) or poly(dA:dT) and treated at 2 h after stimulations with vehicle (ethanol), 2.5 or 5 μM nigericin or 5 or 10 μM valinomycin (VM).
(B) IFN-β secretion by RAW macrophages (measured at 6 h post-infection) infected with *F. novicida* and treated with vehicle (ethanol), 1 μM ionomycin, or 2.5 μM nigericin.
(C) IFN-β secretion by BMDMs (measured at 6 h post-infection) transfected with poly(dA:dT), and treated with vehicle or 10 μM BAPTA-AM.
(D) LDH release by BMDMs of indicated genotypes (measured at 6 h post-infection) infected with *F. novicida* and treated with vehicle (ethanol), nigericin, or valinomycin (VM) 2 h post-infection at the indicated concentrations.
Data presented as mean are from one experiment representative of three, and each dot represents a technical replicate.

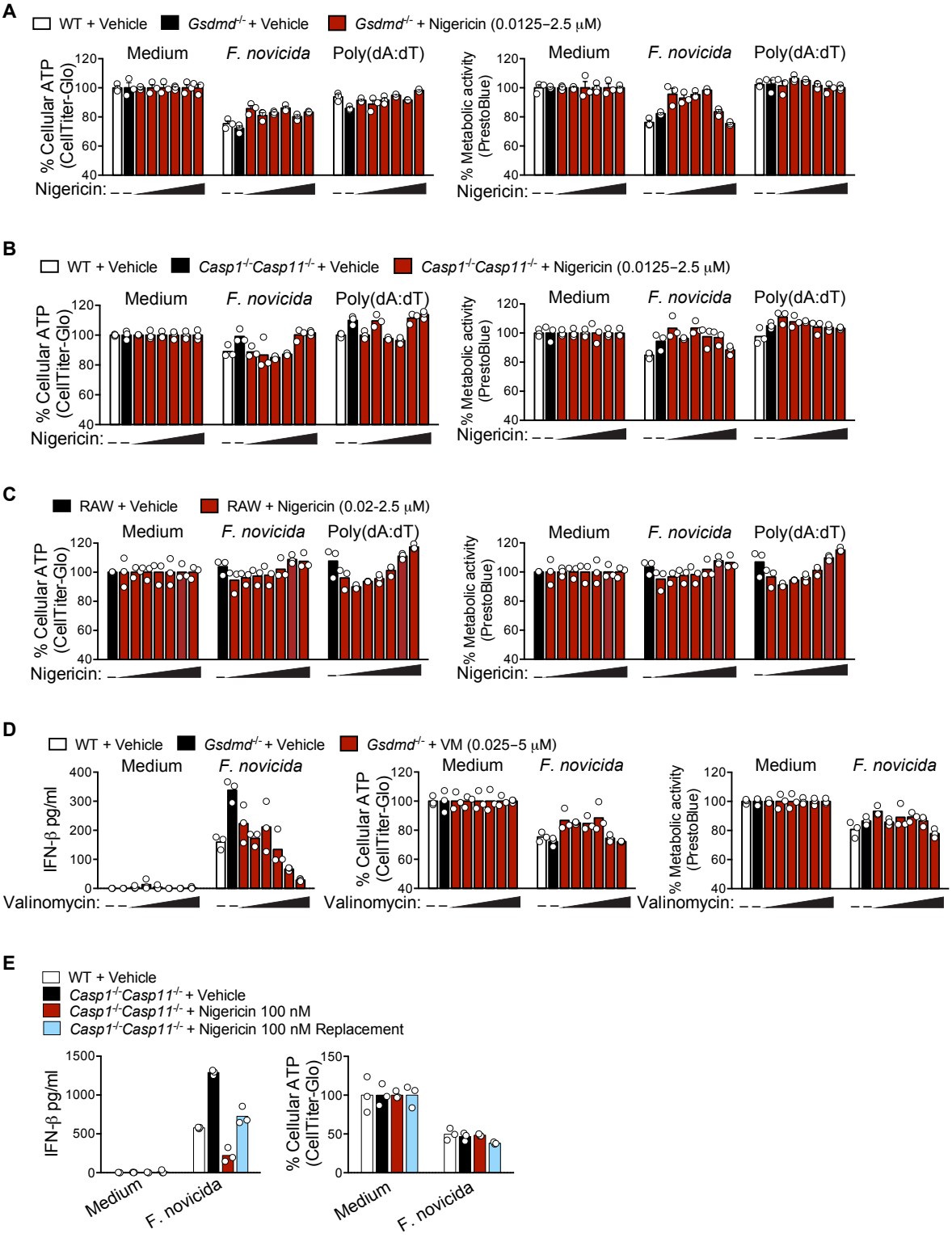
A
WT + Vehicle
Gsdmd-/- + Vehicle
Gsdmd-/- + Nigericin (0.0125–2.5 μM)
Medium
F. novicida
Poly(dA:dT)
% Cellular ATP (CellTiter-Glo)
% Metabolic activity (PrestoBlue)
Nigericin:
B
WT + Vehicle
Casp1-/-Casp11-/- + Vehicle
Casp1-/-Casp11-/- + Nigericin (0.0125–2.5 μM)
C
RAW + Vehicle
RAW + Nigericin (0.02-2.5 μM)
D
WT + Vehicle
Gsdmd-/- + Vehicle
Gsdmd-/- + VM (0.025–5 μM)
IFN-β pg/ml
Valinomycin:
E
WT + Vehicle
Casp1-/-Casp11-/- + Vehicle
Casp1-/-Casp11-/- + Nigericin 100 nM
Casp1-/-Casp11-/- + Nigericin 100 nM Replacement
IFN-β pg/ml
% Cellular ATP (CellTiter-Glo)
Medium
F. novicida

Fig S5. Induction of K^+ efflux in inflammasome-deficient macrophages is sufficient to reduce cytosolic DNA-induced type I interferon response. Related to Figure 5.

(A-C) Percent cellular ATP levels and metabolic activity as measured by CellTiter-Glo and PrestoBlue assays, respectively, in indicated macrophages (at 6 h post-stimulation) stimulated with *F. novicida* (MOI of 50) or poly(dA:dT) and treated at 1.5 – 2 h after stimulations with vehicle (ethanol) or increasing concentrations of nigericin (0.0125–2.5 μM).

(D) IFN-β in the supernatants of, cellular ATP, and metabolic activity as measured by ELISA, CellTiter-Glo, and PrestoBlue assays, respectively, in indicated macrophages (at 6 h post-stimulation) stimulated with *F. novicida* (MOI of 50) and treated at 1.5 – 2 h after stimulations with vehicle (ethanol) or increasing concentrations of valinomycin (0.025–5 μM).

(E) IFN-β in the supernatants of and cellular ATP in *Casp1*$^{-/-}$ *Casp11*$^{-/-}$ BMDMs (at 6 h post-stimulation) stimulated with *F. novicida*. At 1.5 h p.i., cells were treated with vehicle or nigericin (100 nM), and 1 h later, media was replaced with media without nigericin.

Data presented as mean are from one experiment representative of three, and each dot represents a technical replicate.