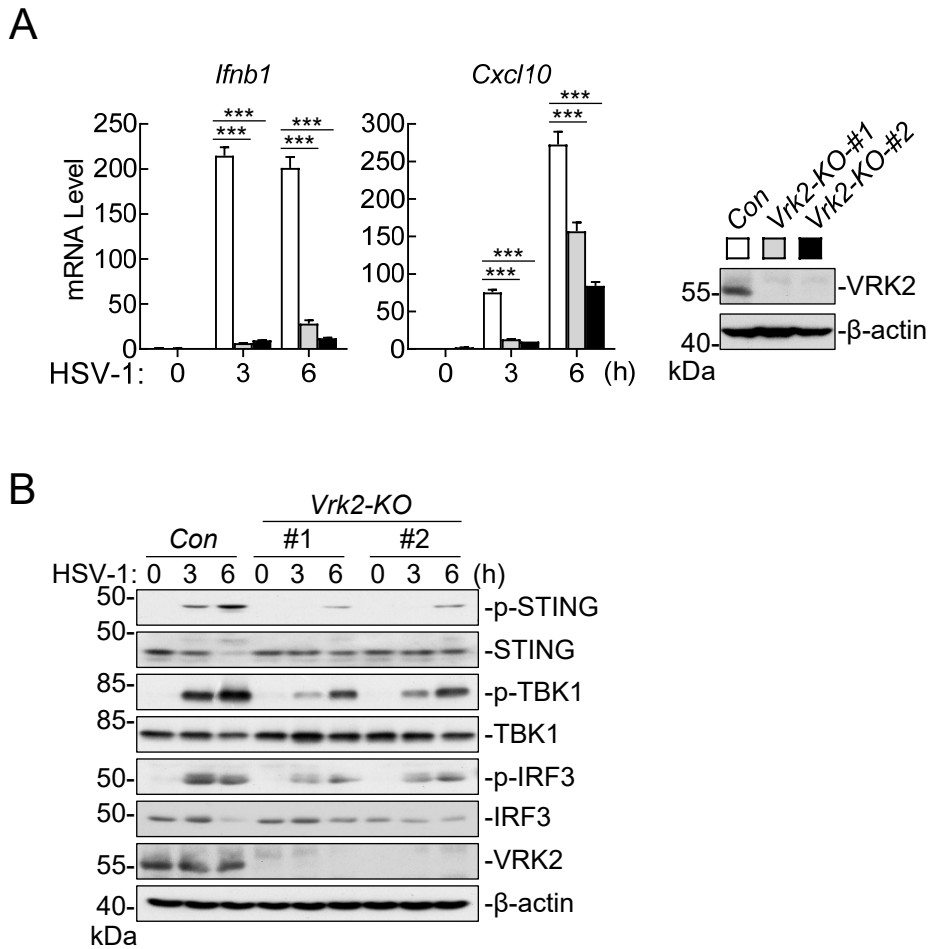


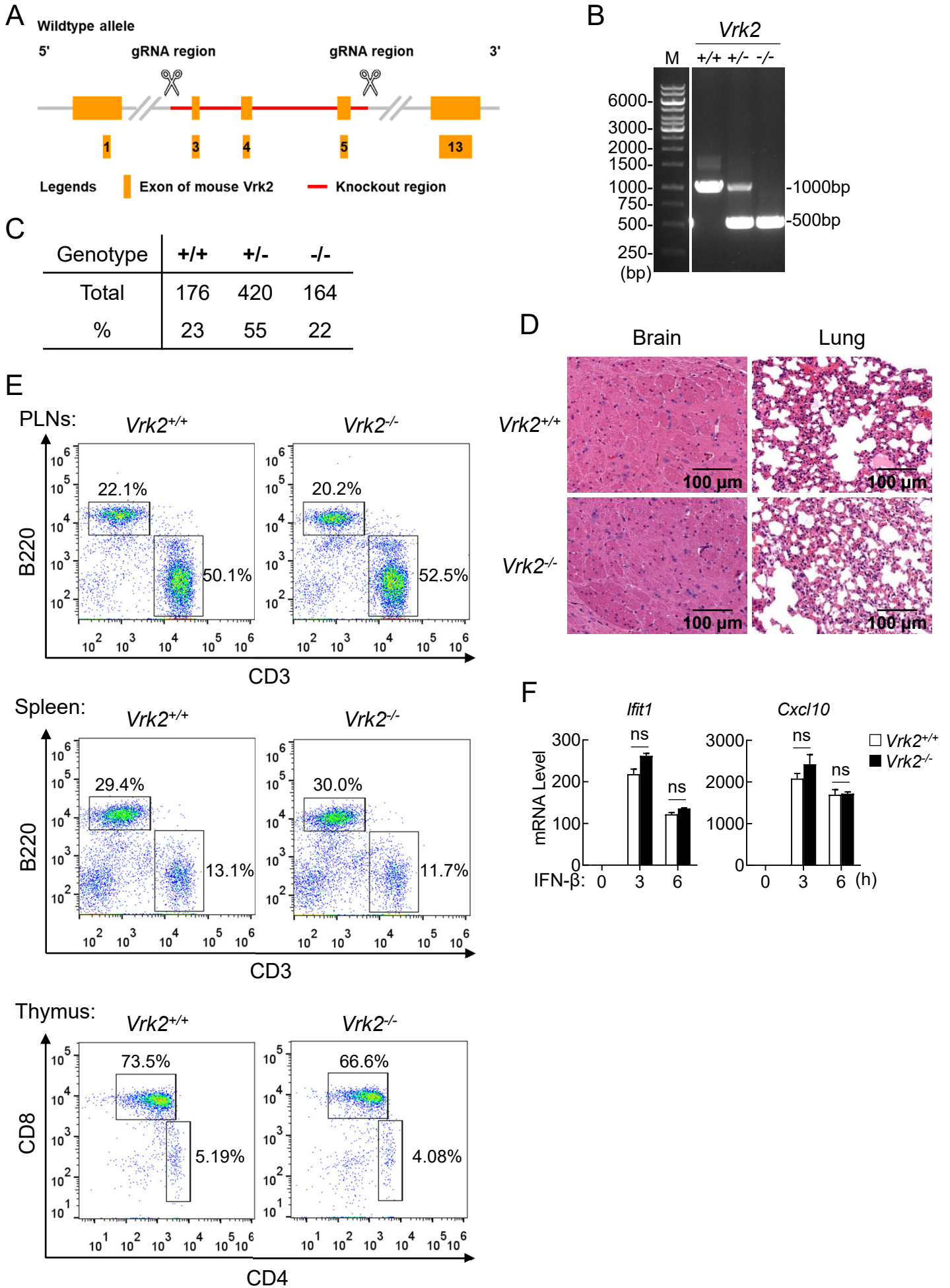
Supplementary information Fig. S1



Supplementary information Fig. S1. Effects of VRK2 knockout on innate antiviral signaling

- A. Effects of CRISPR-knockout of *Vrk2* on HSV-1 infection-triggered transcription of *Ifnb1* and *Cxcl10* genes. The control and *Vrk2*-gRNA stably transduced MLF cells (1×10^6) were left uninfected or infected with HSV-1 for the indicated times, followed by qPCR analysis of the indicated genes. The knockout efficiency of *Vrk2*-gRNA was shown on the right panel. Data shown are mean \pm SD from one representative experiment performed in triplicates. *** $P < 0.001$ (unpaired t-test).
- B. Effects of CRISPR-knockout of *Vrk2* on HSV-1-triggered phosphorylation of STING, TBK1 and IRF3. The control and *Vrk2*-gRNA stably transduced MLF cells (1×10^6) were left uninfected or infected with HSV-1 for the indicated times, followed by immunoblotting analysis with the indicated antibodies.

Supplementary information Fig. S2

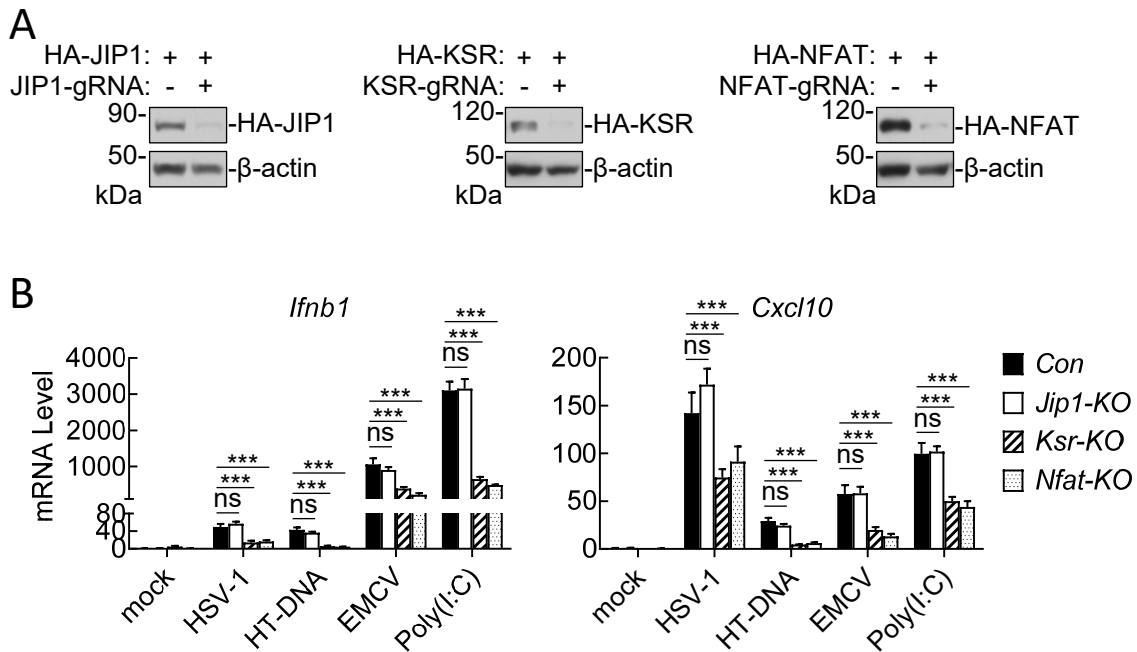


Supplementary information Fig. S2

Supplementary information Fig. S2. Generation of *Vrk2*-knockout mice and characterization analysis

- A. Strategy for knockout of *Vrk2* by the CRISPR-Cas9 method.
- B. Genotyping of *Vrk2*-knockout mice.
- C. Genotypes of the offspring from the breeding of *Vrk2* heterozygous mice.
- D. The lung and brain sections of sex- and age-matched *Vrk2*^{+/+} and *Vrk2*^{-/-} mice were used for histological analysis (H&E staining). Scale bars, 100 μ m.
- E. Cells from peripheral lympho nodes (PLNs), spleen and thymus were analyzed by FACS after staining with the indicated antibodies.
- F. Effects of VRK2-deficiency on IFN- β -triggered transcription of *Ifit1* and *Cxcl10* genes. The primary MLF cells (1×10^6) from WT and *Vrk2*^{-/-} mice were left unstimulated or stimulated with IFN- β (100 ng/ml) for the indicated times, followed by qPCR analysis of the indicated genes. Data shown are mean \pm SD from one representative experiment performed in triplicates. ns, no significance, $P > 0.05$ (unpaired t-test).

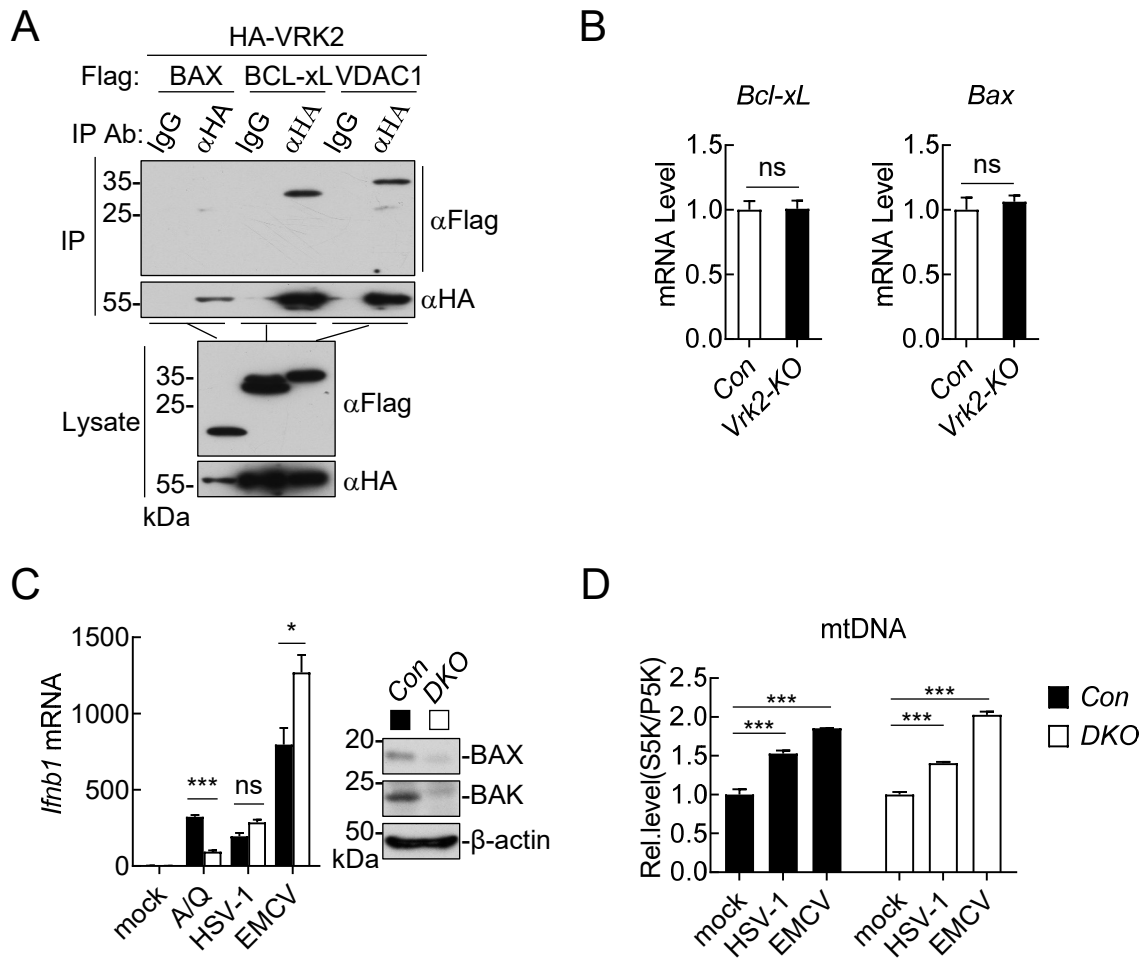
Supplementary information Fig. S3



Supplementary information Fig. S3. Effects of JIP1-, KSR- or NFAT-deficiency on innate antiviral immune responses .

- A. Knockout efficiency of the indicated gRNAs. HEK293 cells were transfected with the indicated plasmids for 24 hours before cells were lysed, followed by immunoblotting analysis with the indicated antibodies.
- B. Effects of JIP1-, KSR- or NFAT-deficiency on transcription of *Ifnb1* and *Cxcl10* genes induced by HSV-1, EMCV, or transfected HT-DNA or poly(I:C). The control and indicated knockout MLFs (1×10^6) were infected with HSV-1 or EMCV, or transfected with HT-DNA (2 μ g) or poly(I:C) (2 μ g) by Fugene (4 μ g) for 6 hours, followed by qPCR analysis of the indicated genes. Data shown are mean \pm SD from one representative experiment performed in triplicates. ns, no significance, $P > 0.05$; . *** $P < 0.001$ (unpaired t-test).

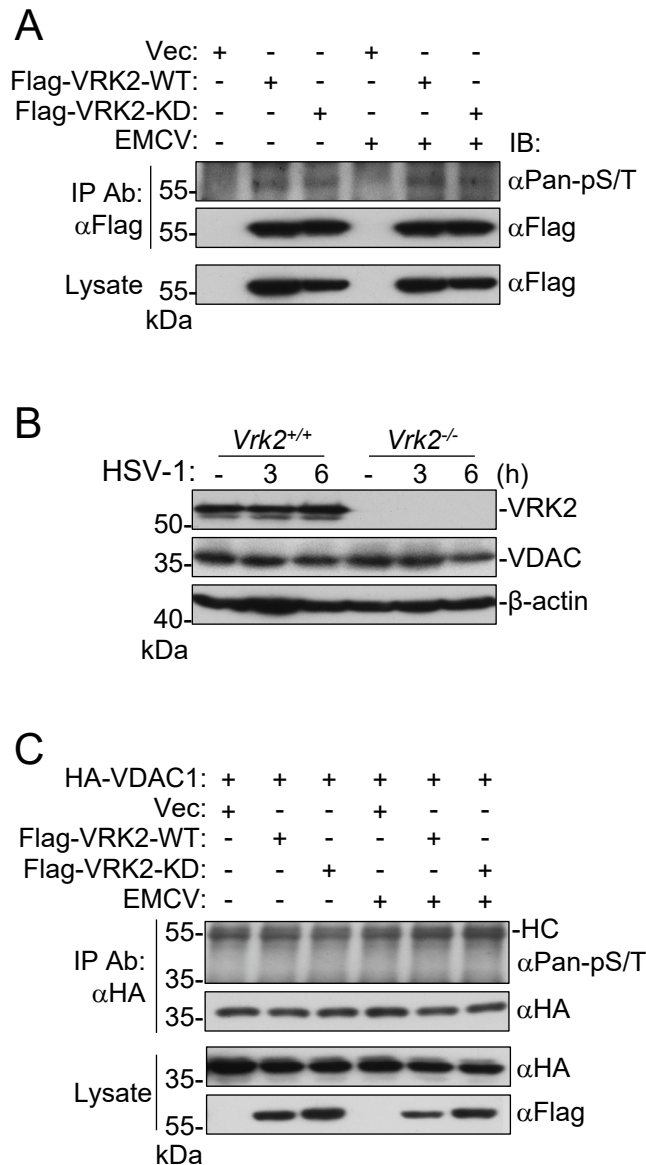
Supplementary information Fig. S4



Supplementary information Fig. S4. Effects of BAX/BAK-deficiency on virus-induced expression of antiviral genes and mtDNA release

- A. Association of VRK2 with BAX and BCL-xL. HEK293 cells were transfected with the indicated plasmids for 20 hours, and then lysed for coimmunoprecipitation with IgG or anti-HA, followed by immunoblotting analysis with the indicated antibodies.
- B. Effects of VRK2-deficiency on transcription of *Bax* and *Bcl-xL*. The control and *Vrk2* knockout Raw264.7 cells (1×10^6) were lysed for qPCR analysis of the indicated genes.
- C. Effects of BAX/BAK-deficiency on transcription of antiviral genes. The control and BAX/BAK-deficient MLFs (1×10^6) were left un-stimulated or stimulated with A/Q ($10 \mu\text{M}$ each), infected with HSV-1 or EMCV (MOI=1) for 6 hours, followed by qPCR analysis of the indicated genes. The knockout efficiency of was shown by immunoblots.
- D. Effects of BAX/BAK-deficiency on virus-induced mtDNA release. The control and BAX/BAK-deficient MLFs (2×10^7) were left uninfected or infected with HSV-1 or EMCV (MOI=2) for 1 hour, followed by subcellular fractionation and mtDNA measurement. P5K, mitochondrial fraction; S5K, non-mitochondrial cytosolic fraction. Data shown are mean \pm SD from one representative experiment performed in triplicates. ns, no significance, $P > 0.05$; . *** $P < 0.001$ (unpaired t-test).

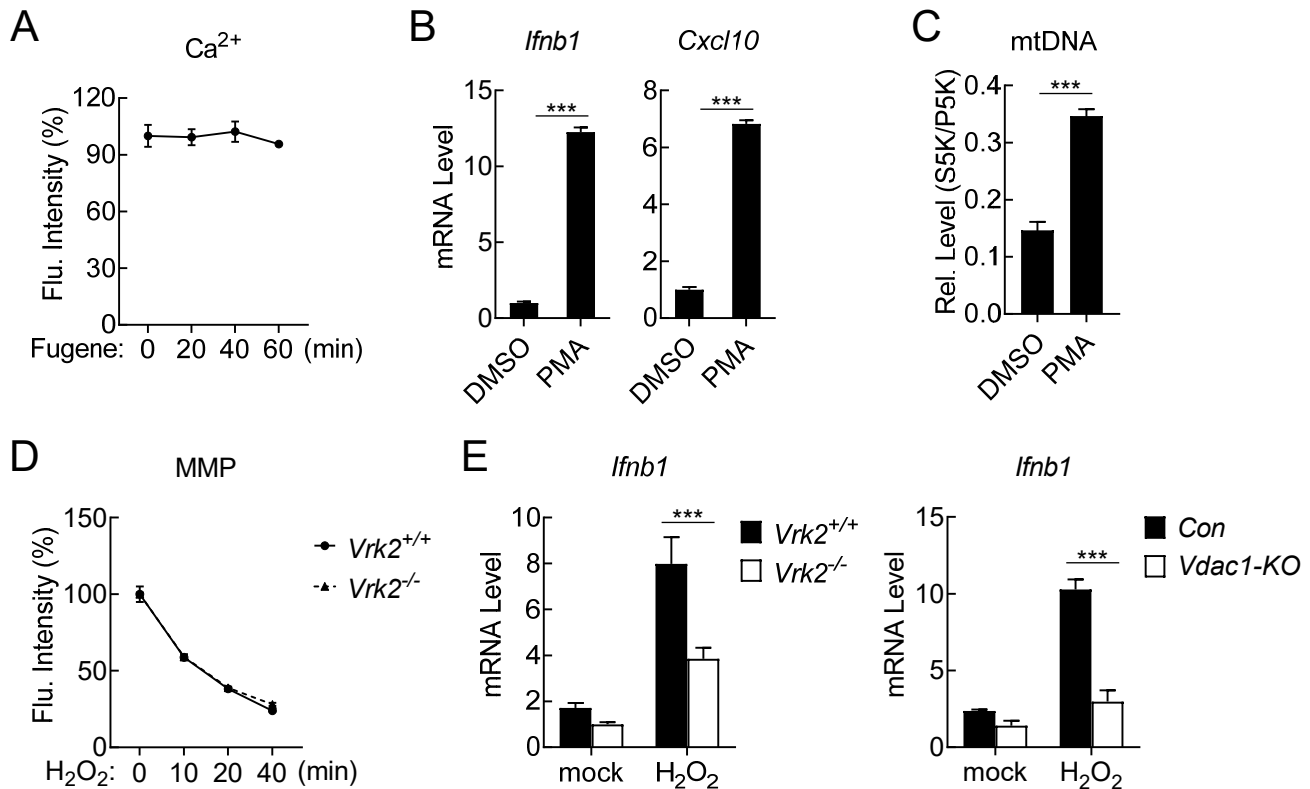
Supplementary information Fig. S5



Supplementary information Fig. S5. VRK2 regulates innate immune response independent of its kinase activity

- Phosphorylation of VRK2 and VRK2-KD. HEK293 cells were transfected with the indicated plasmids for 18 hours, infected with EMCV (MOI=2), and lysed for immunoprecipitation with anti-Flag, followed by immunoblotting analysis with the indicated antibodies.
- Effects of VRK2-deficiency on expression of VDAC. WT and *Vrk2*^{-/-} MLFs (1×10^6) were left uninfected or infected with HSV-1 (MOI=1) for the indicated times, followed by immunoblotting analysis with the indicated antibodies.
- Effects of VRK2 on phosphorylation of VDAC1. HEK293 cells were transfected with the indicated plasmids for 18 hours, infected with EMCV (MOI=2), and lysed for immunoprecipitation with anti-HA, followed by immunoblotting analysis with the indicated antibodies. HC, IgG heavy chain.

Supplementary information Fig. S6



Supplementary information Fig. S6. VRK2 is essential for mtDNA-mediated innate immune responses triggered by non-viral factors

- Fugene-triggered changes of cytosolic Ca^{2+} levels. Raw264.7 cells (2×10^6) were left untreated or treated with Fugene (20 $\mu\text{g}/\text{ml}$) for the indicated times before stained with Fluo 3-AM (5 μM) for 20 minutes, followed by fluorescence detection for cytosolic Ca^{2+} levels.
- PMA-induced transcription of *Ifnb1* and *Cxcl10* genes in MLF cells. MLFs (1×10^6) were treated with PMA (20 μM) for 4 hours, followed by qPCR analysis of the indicated genes.
- PMA-induced mtDNA release. MLFs (2×10^7) were left untreated or treated with PMA (20 μM) for 2 hours, followed by subcellular fractionation and mtDNA measurement. P5K, mitochondrial fraction; S5K, non-mitochondrial cytosolic fraction.
- Effects of H_2O_2 on MMP. WT and *Vrk2*^{-/-} MLFs (2×10^6) were left untreated or treated with H_2O_2 (400 μM) for the indicated times before stained with JC-10 (4 μM) for 20 minutes, followed by fluorescent detection for MMP.
- Effects of VRK2- or VDAC1-deficiency on H_2O_2 -induced transcription of *Ifnb1* gene. The indicated MLFs (1×10^6) were treated with H_2O_2 (400 μM) for 4 hours, followed by qPCR analysis of the indicated gene. Data shown are mean \pm SD from one representative experiment performed in triplicates (B, C, E). ***P < 0.001 (unpaired t-test).