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# Supplementary Materials for

## HIPK2 is necessary for type I interferon–mediated antiviral immunity

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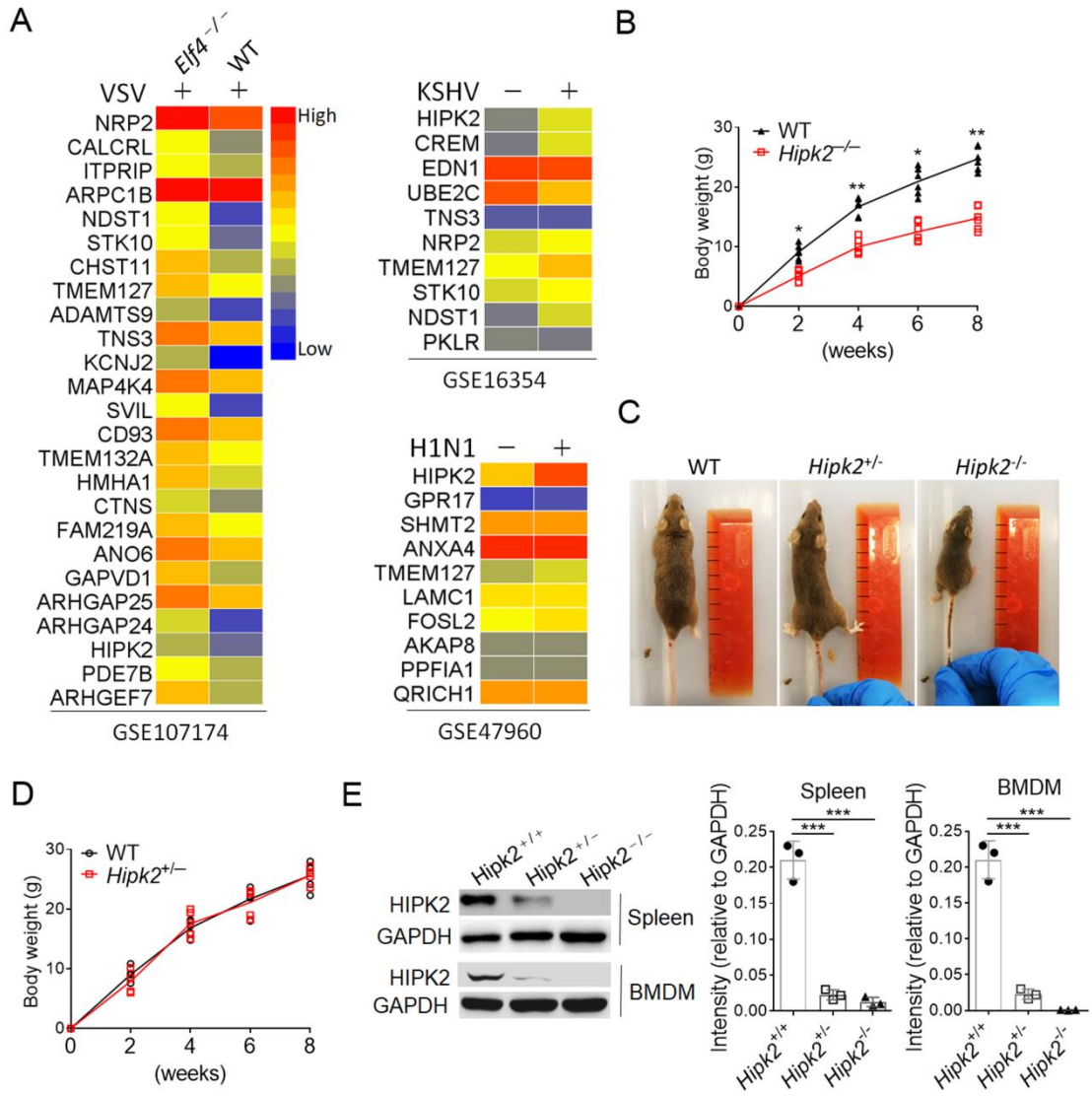



**Fig. S1. HIPK2 is involved in antiviral immunity. (A)** RNA-seq analysis of WT or *Elf4*$^{-/-}$ peritoneal macrophages that were infected with VSV for 4 hours. Heatmaps of differential gene expression are from 2 biological replicates. **(B)** Body weight of age- and sex-matched wild-type (WT) and *Hipk2*$^{-/-}$ mice. Data at the indicated times after birth are means of 6 mice/group from 3 experiments. **(C)** Body size of age- and sex-matched WT, *Hipk2*$^{+/-}$ and *Hipk2*$^{-/-}$ mice. Images are representative of the analysis of 6 mice/group from 3 experiments. **(D)** Body weight of age- and sex-matched WT and *Hipk2*$^{+/-}$ mice. Data are from 6 mice/group from 3 experiments. **(E)** Western blot analysis of HIPK2 in lysates of spleen and bone marrow derived macrophages (BMDM) from WT (*Hipk2*$^{+/+}$), *Hipk2*$^{+/-}$ or *Hipk2*$^{-/-}$ mice. Blots are representative of 3 independent experiments. Quantified band intensity values are pooled from all experiments. *P< 0.05, **P< 0.01, and ***P< 0.001.

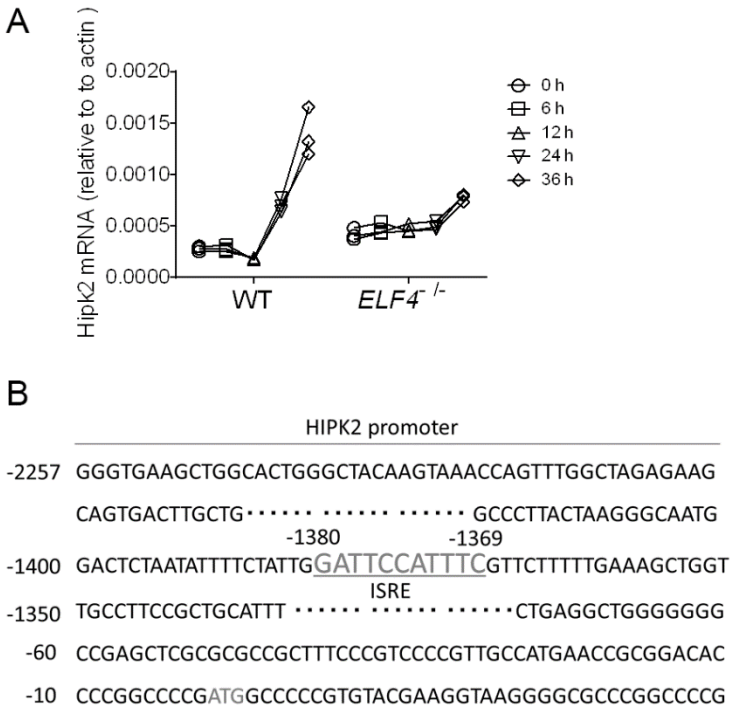
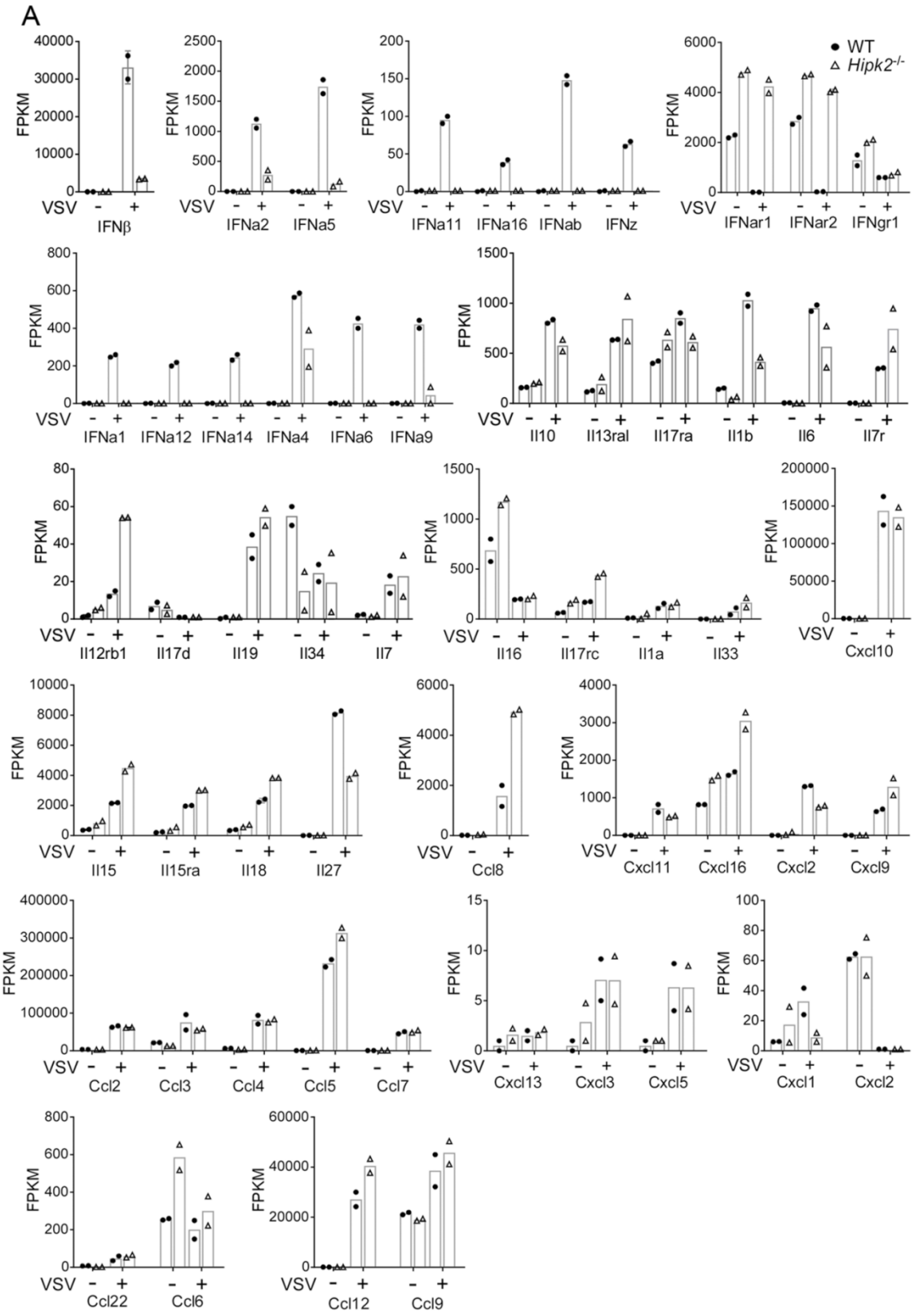



**Fig. S2. HIPK2 is an ISG. (A)** qRT-PCR analysis of HIPK2 expression in wild type and ELF4 deficient BMDM at the indicated times. Data are from 3 biological replicates. **(B)** The promoter sequence of HIPK2 used to construct the reporter plasmid and the ISRE (ISGF3-dependent) sequence in HIPK2 promoter.


A
WT
Hipk2-/-
FPKM
VSV
IFNβ
IFNa2
IFNa5
IFNa11
IFNa16
IFNab
IFNz
IFNar1
IFNar2
IFNgr1
IFNa1
IFNa12
IFNa14
IFNa4
IFNa6
IFNa9
Il10
Il13ral
Il17ra
Il1b
Il6
Il7r
Il12rb1
Il17d
Il19
Il34
Il7
Il16
Il17rc
Il1a
Il33
Cxcl10
Il15
Il15ra
Il18
Il27
Ccl8
Cxcl11
Cxcl16
Cxcl2
Cxcl9
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Ccl3
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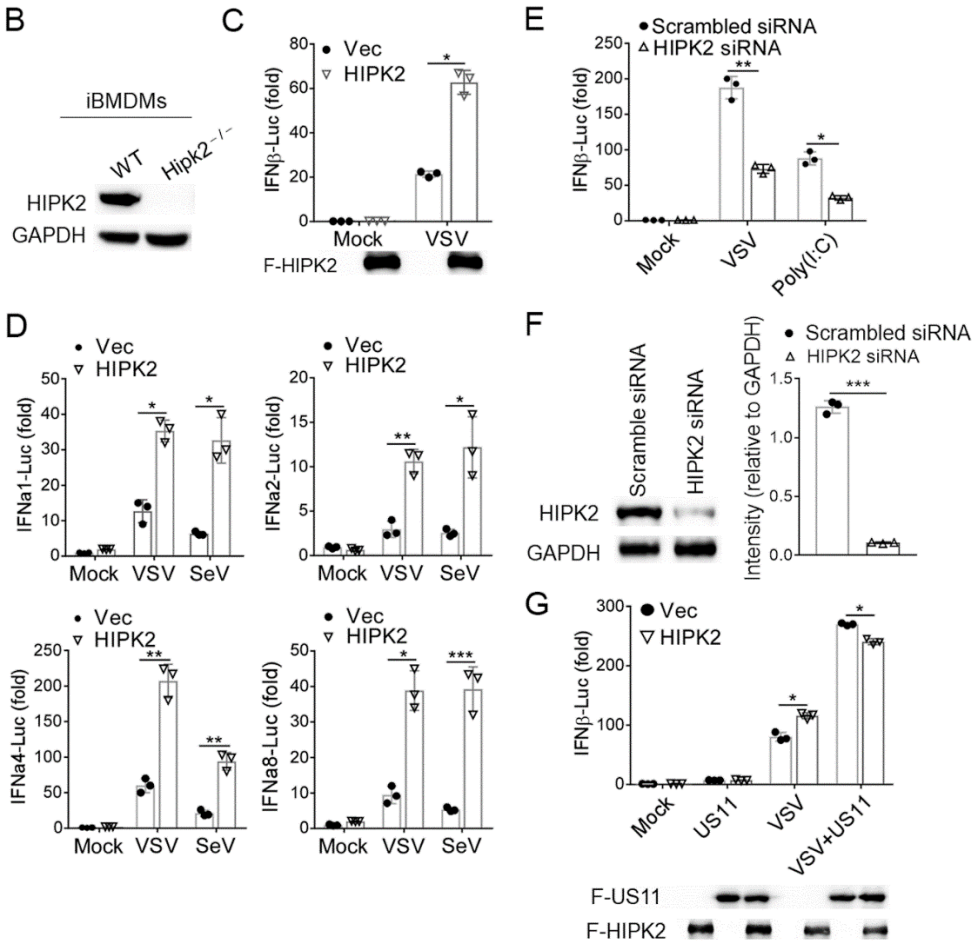


**Fig. S3. HIPK2 facilitates the induction of type I IFN. (A)** RNAseq analysis of transcript expression in WT and *Hipk2*$^{-/-}$ BMDMs infected with VSV for 6 hours. Data are from the analysis of 2 biological replicates. **(B)** Western blot for HIPK2 in lysates from WT and *Hipk2*$^{-/-}$ BMDMs. Blots are representative of 3 experiments. **(C)** Luciferase assay analysis of IFNβ promoter activity 24 hours after VSV infection of HEK293 cells transfected with IFNβ-Luc and empty vector (Vec) or HIPK2 plasmids. Data with means ± SEM are from 3 experiments. **(D)** Luciferase assay analysis of the indicated IFNα promoter activity at 24 hours after VSV or SeV infection of HEK293 cells expressing control (Vec) or HIPK2 constructs. Data with means ± SEM are from 3 experiments. **(E)** Luciferase assay analysis of IFNβ promoter activity at 24 hours after VSV infection or poly I:C transfection of HEK293 cells that were transfected with scrambled control or HIPK2 siRNA. Data with means ± SEM are from 3 experiments. **(F)** Western blot for HIPK2 in WT BMDM transfected with scramble control or HIPK2 siRNA. Blots (left) are representative of 3 independent experiments. Normalized band intensity values (right) are from all experiments. **(G)** Luciferase assay analysis of IFNβ promoter activity 24 hours after VSV infection or/and E6 transfection of HEK293 cells that transfected with IFNβ-Luc and Vec or HIPK2 plasmids. Data with means ± SEM are from 3 experiments. *P< 0.05, **P< 0.01, and ***P< 0.001, by Student's t-test.

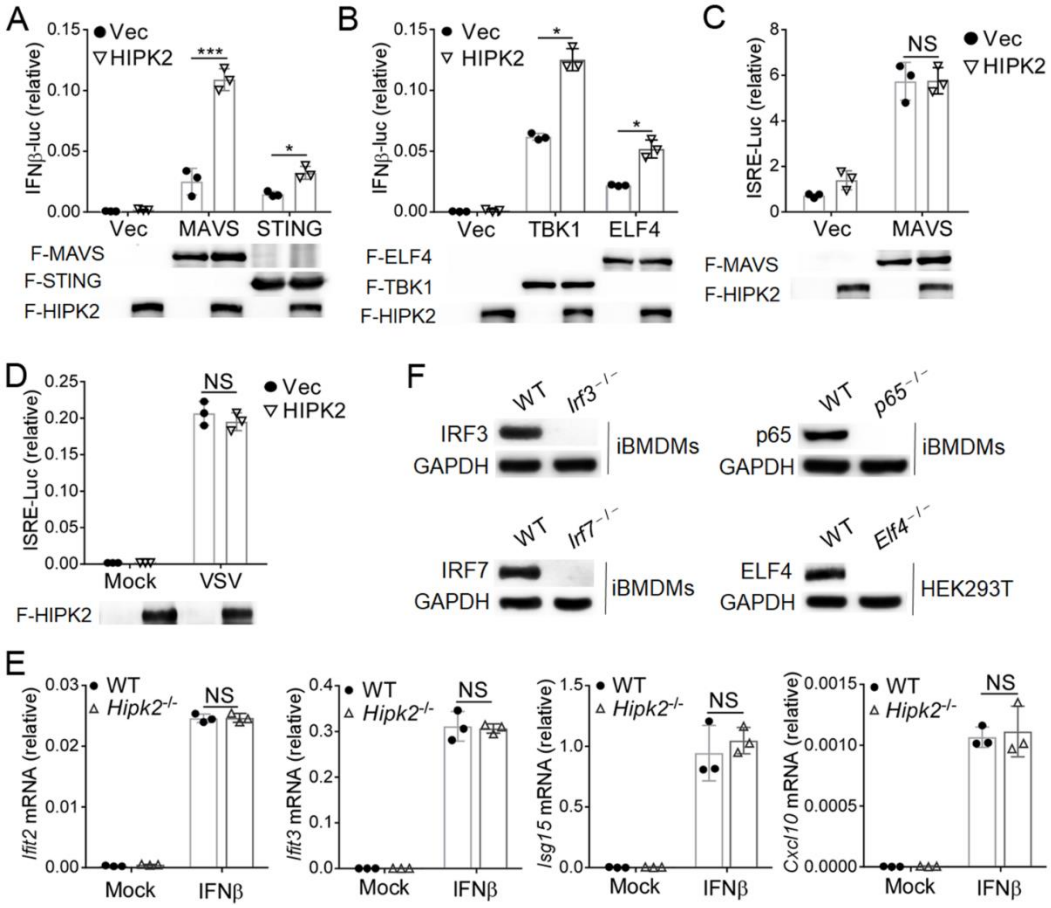


**Fig. S4. HIPK2 is involved in antiviral immune signaling. (A** and **B)** Luciferase assay analysis of IFN-β promoter activity at 24 hours after HEK293 cells expressing Vec control or HIPK2 constructs were transfected the indicated expression plasmids. Data with means ± SEM are from 3 experiments. **(C** and **D)** Luciferase assay analysis of ISRE promoter activity at 24 hours after HEK293 cells expressing Vec control or HIPK2 constructs were transfected with MAVS expression plasmids (C) or infected with VSV (MOI 1) (D). Data with means ± SEM are from 3 experiments. **(E)** qRT-PCR analysis of *Ifit2*, *Ifit3*, *Isg15* and *Cxcl10* mRNA expression after IFNβ treatment for 6 hours of WT or *Hipk2*$^{-/-}$ iBMDMs. Data with means ± SEM are from 3 experiments. **(F)** Western blot for the indicated proteins in lysates of WT, IRF3$^{-/-}$, IRF7$^{-/-}$, or p65$^{-/-}$ BMDMs, and WT and ELF4$^{-/-}$ HEK293 cells. Blots are representative of 3 independent experiments. *P< 0.05, **P< 0.01, and ***P< 0.001 by Student's t-test.

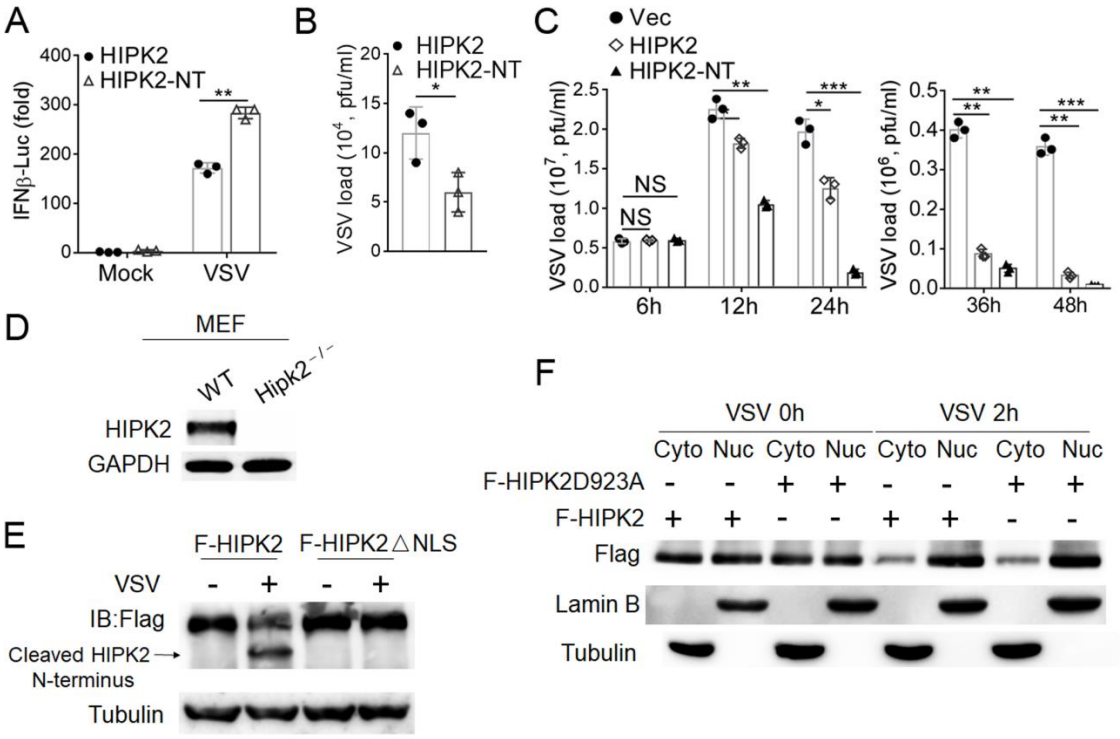


**Fig. S5. HIPK2 translocates to nucleus and is cleaved after virus infection. (A** and **B)** Luciferase assay analysis of IFN-promoter activity at 24 hours after HEK293 cells expressing HIPK2 or N-terminal HIPK2 (HIPK2-NT, 1-923) were infected with VSV (A); and the VSV abundance was detected by plaque assay (B). Data with means ± SEM are from 3 experiments. **(C)** Plaque assay analysis of VSV abundance in the supernatants of HEK293 cells expressing HIPK2 or HIPK2-NT were infected with VSV for indicated times. Data with means ± SEM are from 3 experiments. **(D)** Western blot for the indicated proteins in lysates of wild-type and *Hipk2*$^{-/-}$ MEFs. Blots are representative of 3 independent experiments. **(E)** Western blot analysis of HIPK2 in lysates of HEK293 cells transfected with WT Flag-HIPK2 or HIPK2△NLS (deletion of nuclear localization sequence) and infected with VSV for 2 hours. Blots are representative of 3 independent experiments. **(F)** HEK293 cells expressing HIPK2 or HIPK2D923A were infected with VSV for 2 hours, and nuclear–cytoplasmic fractionations showing the cell localization of indicated proteins. Blots are representative of 3 independent experiments. *P< 0.05, **P< 0.01, and ***P< 0.001 by Student's t-test.

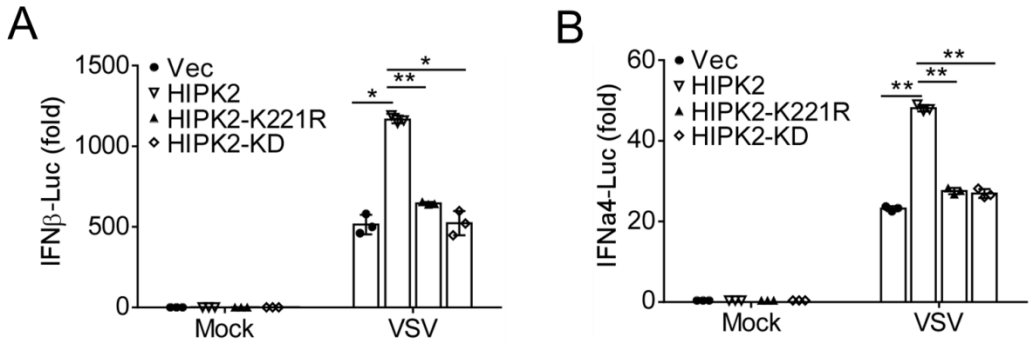


**Fig. S6. HIPK2 phosphorylates ELF4 at Ser$^{369}$ and contributes to its binding to type I IFN promoters. (A** and **B)** Luciferase assay analysis of IFNβ or Ifnα4 promoter activity 24 hours after VSV infection of HEK293 cells that transfected with IFNβ-Luc (A) or Ifnα4-Luc (B) and Vec control, HIPK2, the kinase-defective mutant HIPK2-K221R and the amino-terminal deletion HIPK2-KD. All data are means ± SEM of 3 experiments. *$P$< 0.05 and **$P$< 0.01.

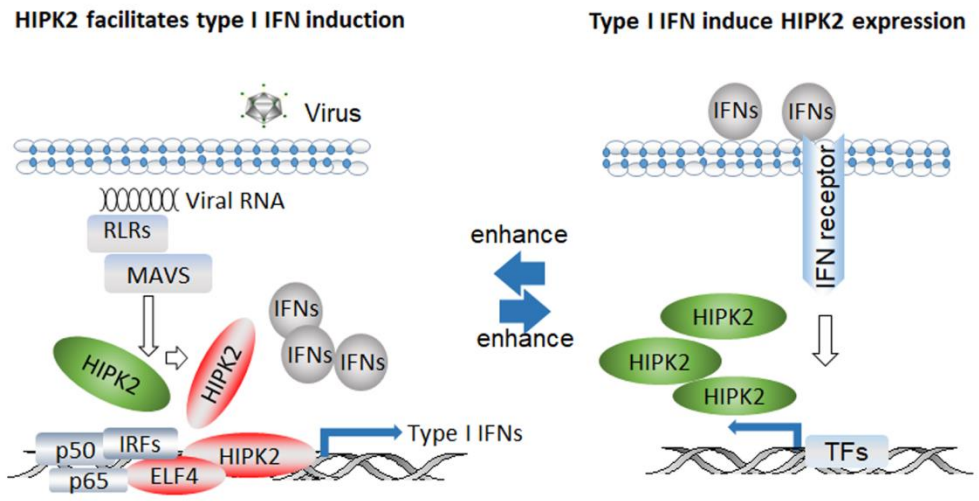


**Fig. S7. HIPK2 promotes IFN-αβ transcription, and type I IFNs augment HIPK2 expression.** After RNA virus infection, HIPK2 enters the nucleus and is cleaved by caspase into its hyperactive form. Then HIPK2 interacts with ELF4 to promote type I IFN enhancesome formation and transcription. To amplify this antiviral response in a feed-forward loop, the expression of HIPK2 is also stimulated by type I IFN.

**Table S1. Guide RNA used in this study.**

| Gene | Guide RNA sequence (5'—3') |
|---|---|
| Murine *Irf3* | GAACGAGGTTCAGGATCCCG |
| Murine *Irf7* | GGGGTCCAGCGAGTGCTGTT |
| Murine *Tbk1* | AGAGCACCTCCAACCATCTG |
| Murine *p65* | CAGAGCCAGCCCAGGCTTCT |
| Murine *Hipk2* | AAGTAGAGCCAAGTTCCAAC |
| Human *Elf4* | ATCTTTGAGTTCGCAAGCAA |

**Table S2. Primers used in this study.**

| Gene | Forward Primer (5'-3') | Reverse Primer (5'-3') |
|---|---|---|
| Murine *Ifnβ* | TCCGAGCAGAGATCTTCAGGAA | TGCAACCACCACTCATTCTGAG |
| Murine *Gapdh* | ATTCAACGGCACAGTCAAGG | GCAGAAGGGGCGGAGATGA |
| Murine *Isg15* | AGCAATGGCCTGGGACCTA | AGCCAGAACTGGTCTTCGTG |
| Murine *Ifng* | CGGCACAGTCATTGAAAGCC | TGCATCCTTTTTCGCCTTGC |
| Murine *Hipk2* | ACCCCACTCCCTTCTCTACC | CACATGTGAGGCCATACCTTTA |
| Human *HIPK2* | ACTGTCCACAACCAGCCCTC | GCATTTTCCATTCGCACCGA |
| Human *GAPDH* | GAAGGTGAAGGTCGGAGTC | GAAGATGGTGATGGGATTTC |
| VSV | ACGGCGTACTTCCAGATGG | CTCGGTTCAAGATCCAGGT |