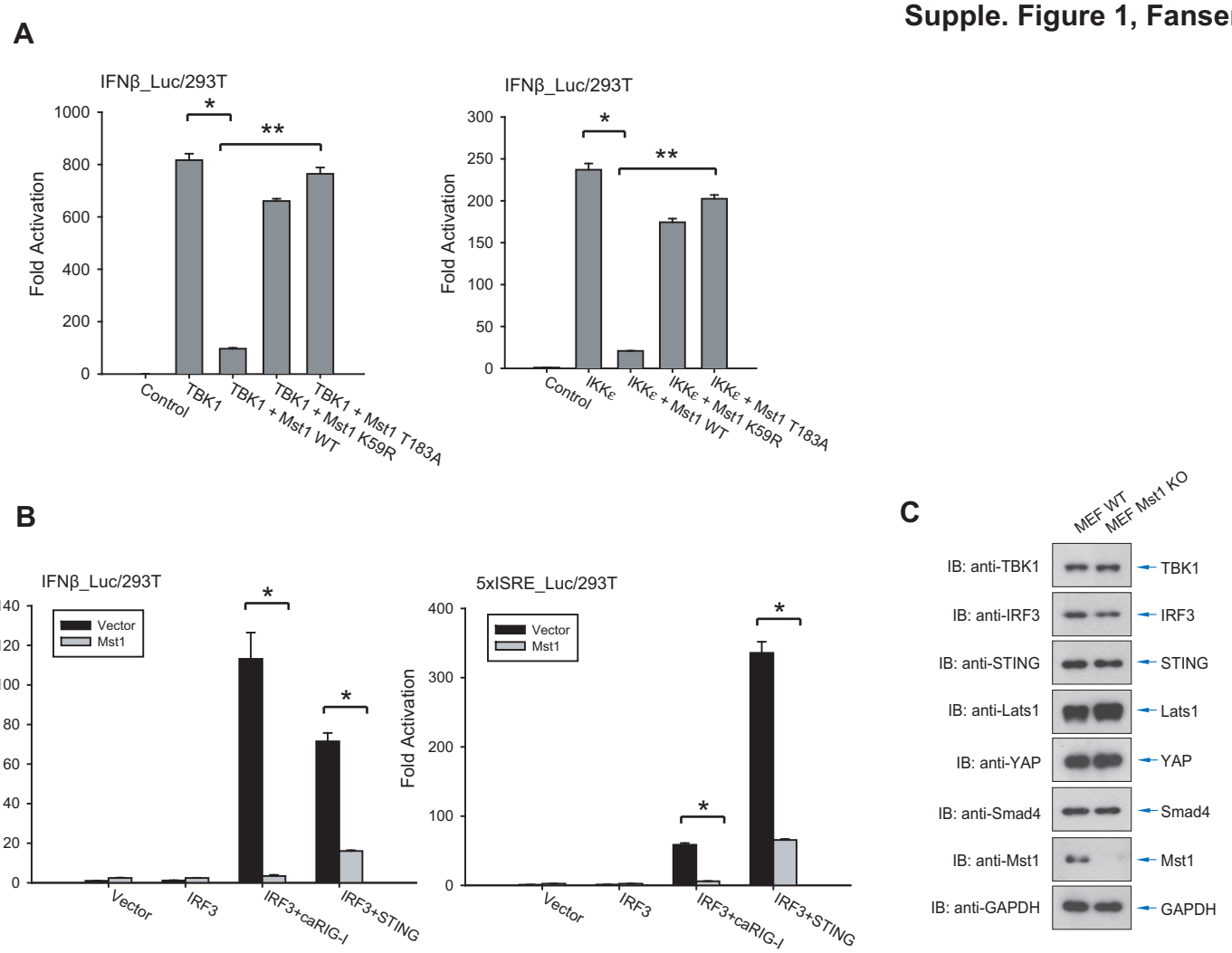
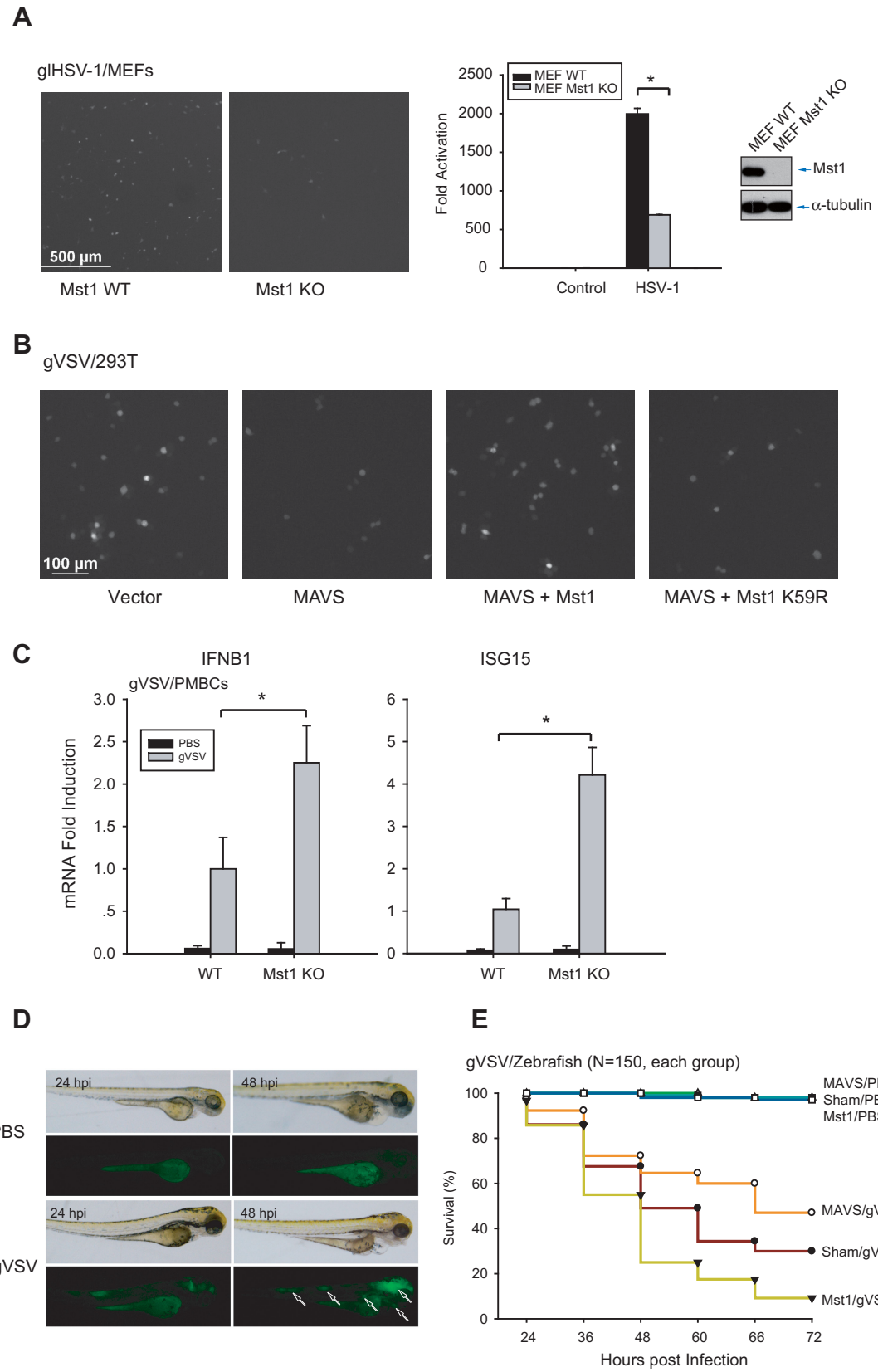
Supplementary Materials:

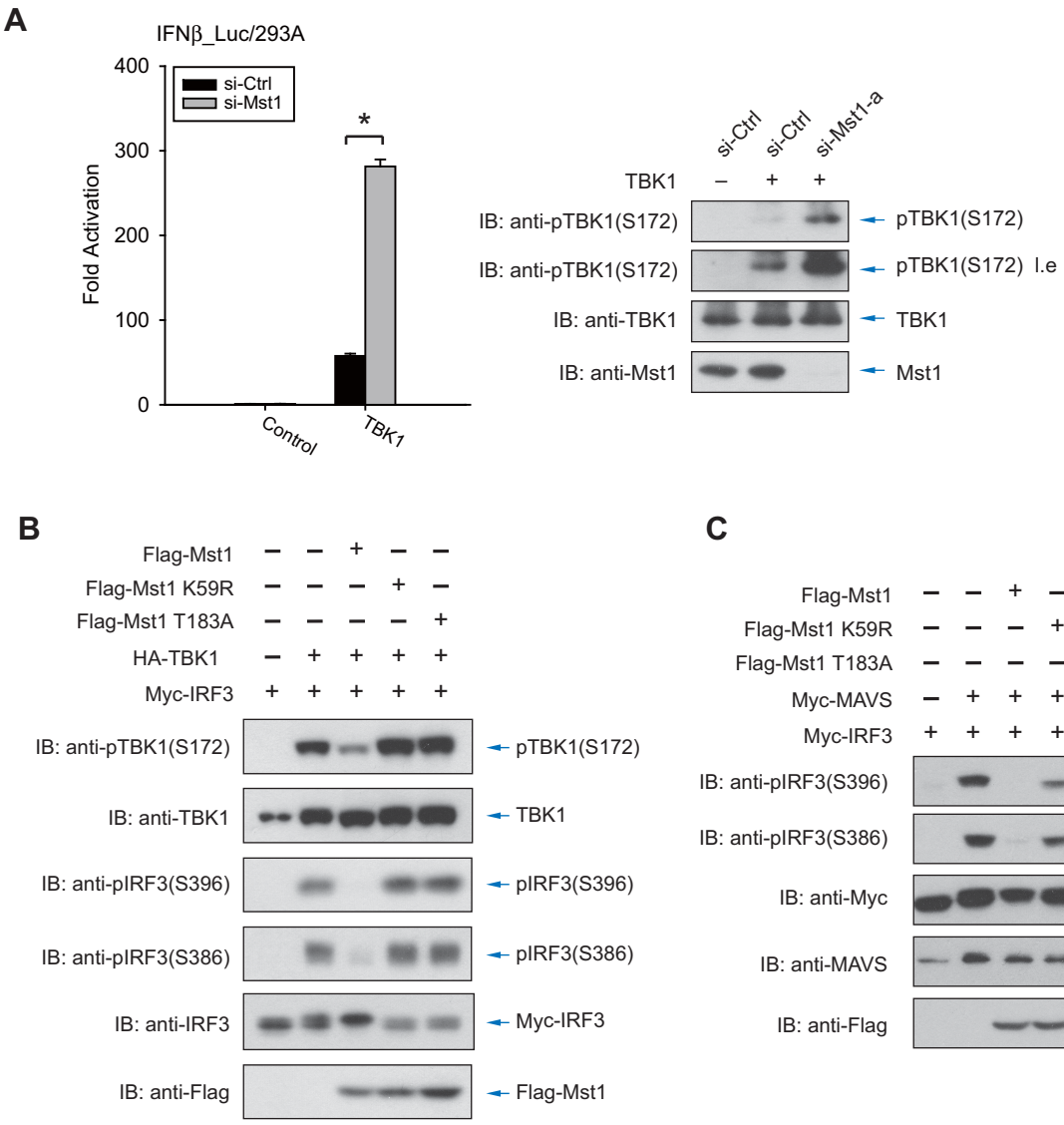
1) Supplemental Figures 1 - 4;

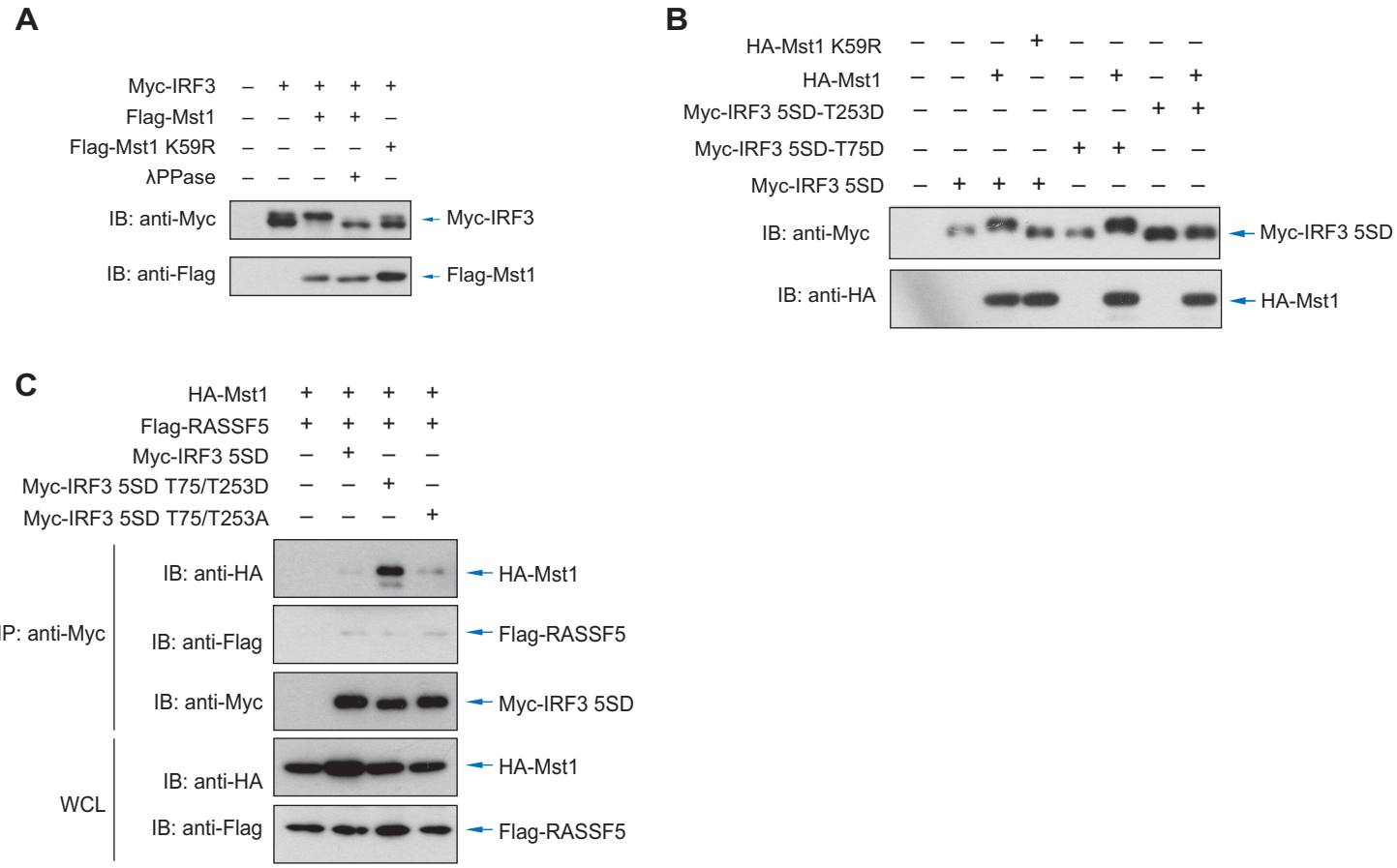
2) Supplemental Figure Legends;

3) Oligo sequence of qPCR.


A
IFNβ_Luc/293T
Fold Activation
1000
800
600
400
200
0
*
**
Control
TBK1
TBK1 + Mst1 WT
TBK1 + Mst1 K59R
TBK1 + Mst1 T183A
IFNβ_Luc/293T
Fold Activation
300
250
200
150
100
50
0
*
**
Control
IKKε
IKKε + Mst1 WT
IKKε + Mst1 K59R
IKKε + Mst1 T183A
B
IFNβ_Luc/293T
Vector
Mst1
Fold Activation
140
120
100
80
60
40
20
0
*
*
Vector
IRF3
IRF3+caRIG-I
IRF3+STING
5xISRE_Luc/293T
Vector
Mst1
Fold Activation
400
300
200
100
0
*
*
Vector
IRF3
IRF3+caRIG-I
IRF3+STING
C
MEF WT
MEF Mst1 KO
IB: anti-TBK1
TBK1
IB: anti-IRF3
IRF3
IB: anti-STING
STING
IB: anti-Lats1
Lats1
IB: anti-YAP
YAP
IB: anti-Smad4
Smad4
IB: anti-Mst1
Mst1
IB: anti-GAPDH
GAPDH


A
gIHSV-1/MEFs
500 μm
Mst1 WT
Mst1 KO
MEF WT
MEF Mst1 KO
Fold Activation
2500
2000
1500
1000
500
0
*
Control
HSV-1
MEF WT
MEF Mst1 KO
Mst1
α-tubulin
B
gVSV/293T
100 μm
Vector
MAVS
MAVS + Mst1
MAVS + Mst1 K59R
C
IFNB1
gVSV/PMBCs
mRNA Fold Induction
PBS
gVSV
3.0
2.5
2.0
1.5
1.0
.5
0.0
*
WT
Mst1 KO
ISG15
6
5
4
3
2
1
0
*
WT
Mst1 KO
D
24 hpi
48 hpi
PBS
24 hpi
48 hpi
gVSV
E
gVSV/Zebrafish (N=150, each group)
Survival (%)
100
80
60
40
20
0
MAVS/PBS
Sham/PBS
Mst1/PBS
MAVS/gVSV
Sham/gVSV
Mst1/gVSV
* P=0.022
* P=0.024
24
36
48
60
66
72
Hours post Infection
Sham
MAVS-Flag
MAVS-Flag
Non-specific
Sham
Mst1-Flag
Mst1-Flag
Non-specific


A
IFNβ_Luc/293A
si-Ctrl
si-Mst1
Fold Activation
400
300
200
100
0
*
Control
TBK1
si-Ctrl
si-Ctrl
si-Mst1-a
TBK1 – + +
IB: anti-pTBK1(S172)
pTBK1(S172)
IB: anti-pTBK1(S172)
pTBK1(S172) l.e
IB: anti-TBK1
TBK1
IB: anti-Mst1
Mst1
B
Flag-Mst1 – – + – –
Flag-Mst1 K59R – – – + –
Flag-Mst1 T183A – – – – +
HA-TBK1 – + + + +
Myc-IRF3 + + + + +
IB: anti-pTBK1(S172)
pTBK1(S172)
IB: anti-TBK1
TBK1
IB: anti-pIRF3(S396)
pIRF3(S396)
IB: anti-pIRF3(S386)
pIRF3(S386)
IB: anti-IRF3
Myc-IRF3
IB: anti-Flag
Flag-Mst1
C
Flag-Mst1 – – + – –
Flag-Mst1 K59R – – – + –
Flag-Mst1 T183A – – – – +
Myc-MAVS – + + + +
Myc-IRF3 + + + + +
IB: anti-pIRF3(S396)
pIRF3(S396)
IB: anti-pIRF3(S386)
pIRF3(S386)
IB: anti-Myc
Myc-IRF3
IB: anti-MAVS
MAVS
IB: anti-Flag
Flag-Mst1


A
Myc-IRF3 – + + + +
Flag-Mst1 – – + + –
Flag-Mst1 K59R – – – – +
λPPase – – – + –
IB: anti-Myc
Myc-IRF3
IB: anti-Flag
Flag-Mst1
B
HA-Mst1 K59R – – – + – – – –
HA-Mst1 – – + – – + – +
Myc-IRF3 5SD-T253D – – – – – – + +
Myc-IRF3 5SD-T75D – – – – + + – –
Myc-IRF3 5SD – + + + – – – –
IB: anti-Myc
Myc-IRF3 5SD
IB: anti-HA
HA-Mst1
C
HA-Mst1 + + + +
Flag-RASSF5 + + + +
Myc-IRF3 5SD – + – –
Myc-IRF3 5SD T75/T253D – – + –
Myc-IRF3 5SD T75/T253A – – – +
IP: anti-Myc
IB: anti-HA
HA-Mst1
IB: anti-Flag
Flag-RASSF5
IB: anti-Myc
Myc-IRF3 5SD
WCL
IB: anti-HA
HA-Mst1
IB: anti-Flag
Flag-RASSF5

SUPPLEMENTAL FIGURE LEGENDS

sFig. 1: (A), Transfection of TBK1 (left panel) or IKKε (right panel) induced robust activation of IRF3-responsive IFNβ promoter, which was similarly blocked by cotransfection of wild-type Mst1. Kinase-dead mutants of Mst1 had only marginal effects on TBK1/IKKε-induced IRF3 activation. N=3 experiments. * and **, $P < 0.001$, compared with control or wild-type Mst1, by Student's t-test. **(B)**, Transfection of activated RIG-I (caRIG-I or RIG-I-N) or STING exerted a robust activation of IRF3-responsive promoters. Mst1 cotransfection, while strongly suppressed the stimulated IRF3 transactivation, did not inhibit both IFNβ (left panel) and 5xISRE (right panel) promoters in resting cells. N=3 experiments. *, $P < 0.001$, compared with control, by Student's t-test. **(C)**, Immunoblotting revealed protein levels of endogenous IRF3, TBK1, STING, Lats1, YAP and Smad4 in wild-type and Mst1 KO MEFs, without showing profound changes with Mst1 ablation.

sFig. 2: (A), $Mst1^{-/-}$ MEFs displayed a resistance to HSV-1 infection, seeing by reduced virus-containing GFP^{+} cells (left panel), and lower amount of luciferase activity (right panel). Both GFP and luciferase ORFs were integrated into viral genome of HSV-1 (glHSV). N=3 experiments, *, $P < 0.01$, compared with control MEF, by Student's t-test. **(B)**, Expression of MAVS enhanced cellular resistance against gVSV infection in 293T cells, as displayed by reduced level of GFP^{+} cells. Cotransfection of wild-type Mst1, but not its kinase-dead form K59R, negated the MAVS-endowed viral resistance. **(C)**, Enhanced antiviral response was detected in PBMCs from gVSV-infected $Mst1^{-/-}$ mice, revealing by induction of antiviral proteins including IFNB1 and ISG15 at 6 hpi. N=5 mice for each group. *, $P < 0.05$, compared with control $Mst1^{+/+}$ group, by Student t-test. **(D)**, gVSV was microinjected into yolk of zebrafish embryos, which elicited a robust virus infection, occurring strongly at brain and also visible at muscle and gut tissues. **(E)**, Zebrafish embryos were microinjected with mammal MAVS or Mst1 mRNA to gain ectopic expression of MAVS or Mst1 protein, as detected by immunoblottings (right panel). Zebrafish were then infected with VSV, and a vulnerable phenotype of Mst1 expressing embryos and

a resistance phenotype of MAVS expressing embryos were detected by VSV challenge. N=150 embryos for each group, * and **, $P < 0.05$, compared with sham group, by paired Student t-test.

sFig. 3: (A), siRNA-mediated depletion of Mst1 in HEK293A cells potentiated TBK1-induced IRF3 transactivation (left panel), and the enhanced TBK1 activation that is evidenced by TBK1 phospho-Ser172 immunoblotting (right panel). N=3 experiments, *, $P < 0.01$, compared with control siRNA, by Student's t-test. **(B)**, IRF3 mobility shift by Mst1 cotransfection was revealed by immunoblotting with anti-IRF3 antibody, similar as experiment in Fig. 3C. **(C)**, Loss of IRF3 phosphorylation and IRF3 mobility shift were also observed when IRF3 was cotransfected with MAVS in the presence of wild-type Mst1.

sFig. 4: (A), Mst1-induced IRF3 mobility shift was eliminated by λ phosphatase treatment, suggesting it is caused by the phosphorylation modification(s). **(B)**, SDS-PAGE of phospho-mimetic mutants of IRF3 5SD showed that phospho-Thr253 was main modification by Mst1 to change IRF3 migration. **(C)**, Co-immunoprecipitation assay by differential tags showed a weak interaction between IRF3 and wild-type or T75A/T253A Mst1, while an enhanced association with T75D/T253D mutant, implying its conformational changes after phosphorylation.

Oligo sequence:

Q-PCR primer sequence (mouse) 5'->3'	
IFIT1-Forward	GCCTATCGCCAAGATTTAGATGA
IFIT1-Reverse	TTCTGGATTTAACCGGACAGC
ISG15-Forward	GGTGTCCGTGACTAACTCCAT
ISG15-Reverse	CTGTACCACTAGCATCACTGTG
IFNB1-Forward	AGCTCCAAGAAAGGACGAACA
IFNB1-Reverse	GCCCTGTAGGTGAGGTTGAT
IRF7-Forward	GCGTACCCTGGAAGCATTTC
IRF7-Reverse	GCACAGCGGAAGTTGGTCT

Q-PCR primer sequence (VSV) 5'->3'	
VSV -Forward	GATAGTACCGGAGGATTGACGACTA
VSV -Reverse	TCAAACCATCCGAGCCATTC