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Supplementary Materials for

MAVI1, an endoplasmic reticulum–localized microprotein, suppresses antiviral innate immune response by targeting MAVS on mitochondrion

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

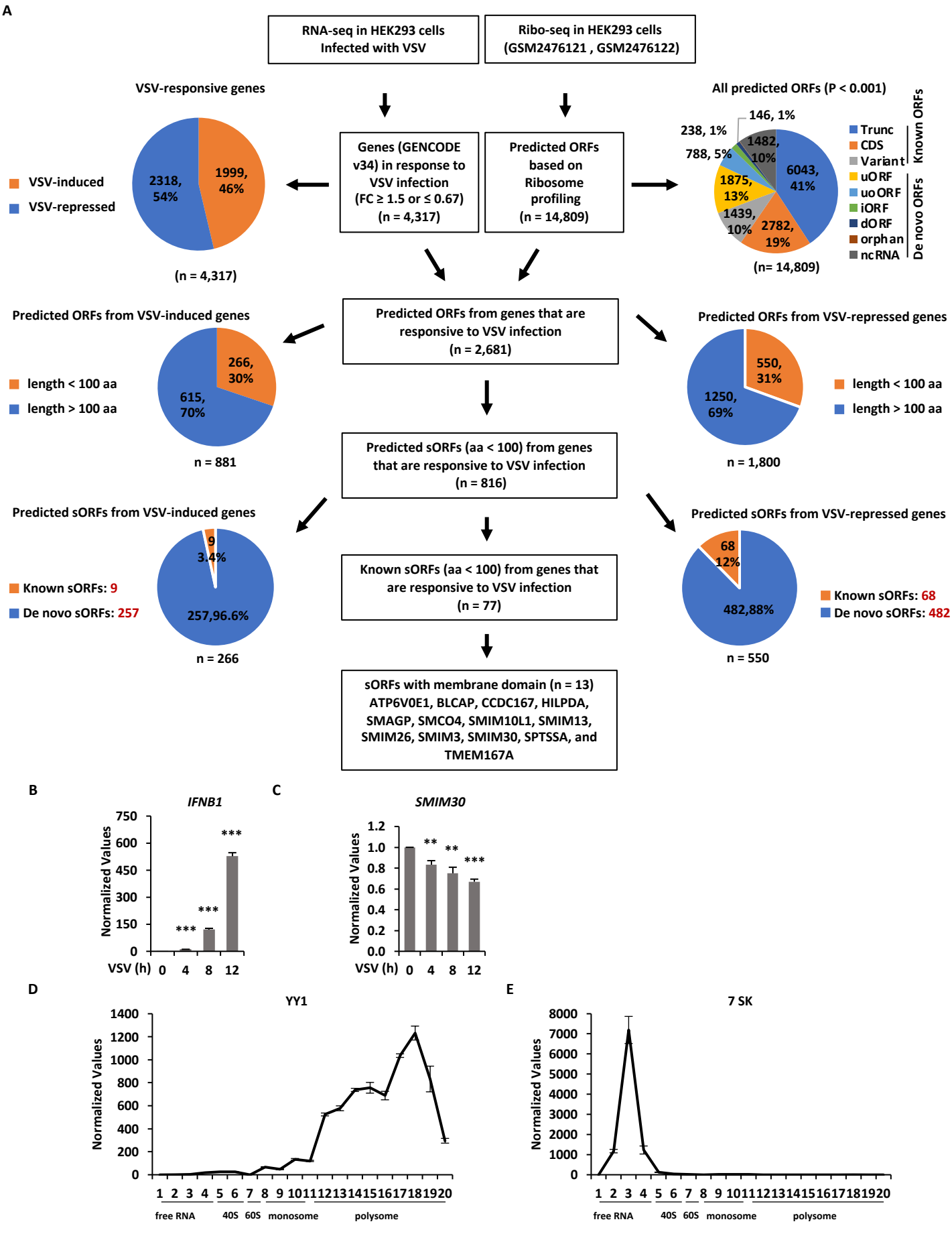
A
RNA-seq in HEK293 cells Infected with VSV
Ribo-seq in HEK293 cells (GSM2476121 , GSM2476122)
VSV-responsive genes
VSV-induced
VSV-repressed
2318, 54%
1999, 46%
(n = 4,317)
Genes (GENCODE v34) in response to VSV infection (FC ≥ 1.5 or ≤ 0.67) (n = 4,317)
Predicted ORFs based on Ribosome profiling (n = 14,809)
All predicted ORFs (P < 0.001)
146, 1%
238, 1%
788, 5%
1482, 10%
1875, 13%
1439, 10%
6043, 41%
2782, 19%
(n= 14,809)
Trunc
CDS
Variant
uORF
uoORF
iORF
dORF
orphan
ncRNA
Known ORFs
De novo ORFs
Predicted ORFs from genes that are responsive to VSV infection (n = 2,681)
Predicted ORFs from VSV-induced genes
length < 100 aa
length > 100 aa
266, 30%
615, 70%
n = 881
Predicted ORFs from VSV-repressed genes
550, 31%
1250, 69%
n = 1,800
Predicted sORFs (aa < 100) from genes that are responsive to VSV infection (n = 816)
Predicted sORFs from VSV-induced genes
Known sORFs: 9
De novo sORFs: 257
9 3.4%
257,96.6%
n = 266
Predicted sORFs from VSV-repressed genes
68 12%
482,88%
Known sORFs: 68
De novo sORFs: 482
n = 550
Known sORFs (aa < 100) from genes that are responsive to VSV infection (n = 77)
sORFs with membrane domain (n = 13)
ATP6V0E1, BLCAP, CCDC167, HILPDA, SMAGP, SMCO4, SMIM10L1, SMIM13, SMIM26, SMIM3, SMIM30, SPTSSA, and TMEM167A
B
IFNB1
Normalized Values
VSV (h) 0 4 8 12
C
SMIM30
Normalized Values
VSV (h) 0 4 8 12
D
YY1
Normalized Values
free RNA
40S
60S
monosome
polysome
E
7 SK
Normalized Values
free RNA
40S
60S
monosome
polysome

Figure S1. The landscape of microproteins in response to VSV infection in HEK293 cells as detected by RNA-seq and Ribo-seq

(A) HEK293 cells infected with VSV (2×10^6 p.f.u, 12 h) were subjected to RNA-seq, and differential expression analysis was then performed. Genes that are responsive to virus infection (n = 4,317) were integrated with genes with open reading frames (ORFs) (n = 14,809) detected based on Ribo-seq in HEK293 cells (GSM2476121 and GSM2476121), which led to the discovery of 2,681 genes with detected ORFs that are responsive to virus infection. Among these 2,681 genes, 816 contains predicted short ORFs (sORFs) that have less than 100 amino acids (aa), with 77 sORFs are from known coding genes. Further domain searching analysis revealed that 13 protein products from these 77 sORFs contain membrane domain.

(B, C) HeLa cells infected with VSV (2×10^6 p.f.u) for 0, 4, 8, or 12 h were subjected to RT-qPCR analysis to examine the expression of *IFNB1* (B) and *SMIM30* (C) (mean ± SEM, ** $P < 0.01$, *** $P < 0.001$).

(D, E) Fractions as described in Fig. 1G were subjected to RNA extraction and RT-qPCR analysis to examine the expression of YY1 (D) and 7SK snRNA (E).

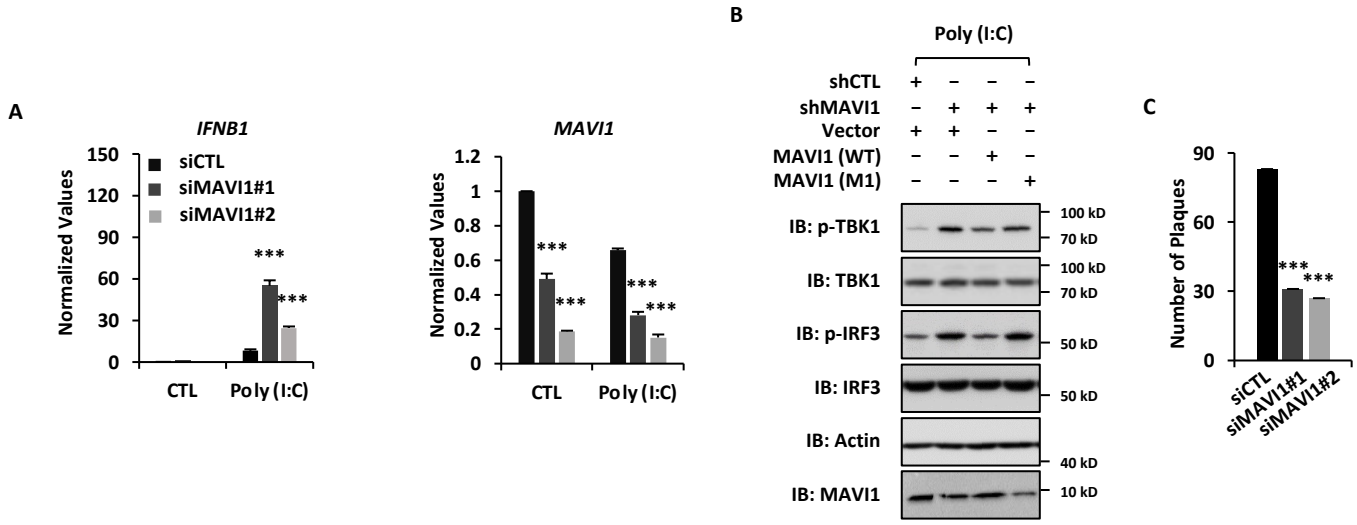

Figure S2. MAVI1 negatively regulates RLR-mediated antiviral immune responses *in vitro*

(A) Hela transfected with siCTL, siMAVI1#1, or siMAVI1#2 were treated with Poly I:C (5 mg/mL, 6 h), followed by RNA extraction and RT-qPCR analysis to examine the expression of *IFNB1* (left panel) and *MAVI1* (right panel) (mean ± SEM, *** $P < 0.001$).

(B) HeLa cells were infected with lentivirus expressing shCTL or shMVAI in the presence or absence of empty vector, Flag-tagged MAVI1 (WT), or Flag-tagged MAVI1 (M1), and treated with or without Poly I:C (5 mg/mL, 6 h), followed by IB using antibodies as indicated.

(C) BHK21 cells were incubated with supernatant from cells infected VSV-GFP as described in Fig. 2F, followed by plaque assay (mean ± SEM, *** $P < 0.001$).

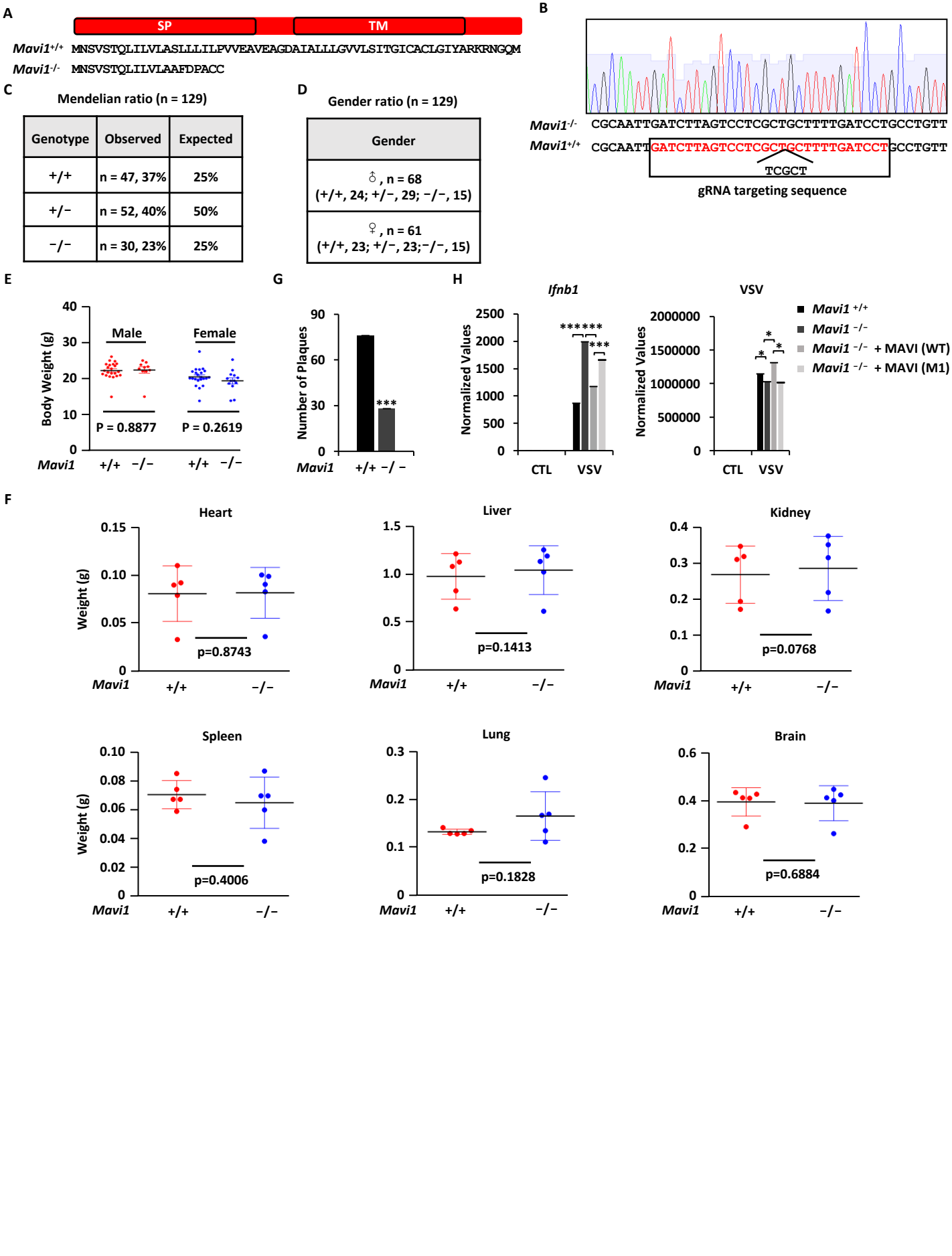
A
SP
TM
Mavi1+/+ MNSVSTQLILVLASLLLILPVVEAVEAGDAIALLLGVVLSITGICACLGIYARKRNGQM
Mavi1-/- MNSVSTQLILVLAAFDPACC
B
Mavi1-/- CGCAATTGATCTTAGTCCTCGCTGCTTTTGATCCTGCCTGTT
Mavi1+/+ CGCAATTGATCTTAGTCCTCGCTGCTTTTGATCCTGCCTGTT
TCGCT
gRNA targeting sequence
C
Mendelian ratio (n = 129)
Genotype | Observed | Expected
+/+ | n = 47, 37% | 25%
+/− | n = 52, 40% | 50%
−/− | n = 30, 23% | 25%
D
Gender ratio (n = 129)
Gender
♂, n = 68 (+/+, 24; +/−, 29; −/−, 15)
♀, n = 61 (+/+, 23; +/−, 23;−/−, 15)
E
Body Weight (g)
Male
Female
P = 0.8877
P = 0.2619
Mavi1 +/+ −/− +/+ −/−
G
Number of Plaques
***
Mavi1 +/+ −/ −
H
Ifnb1
Normalized Values
******
***
CTL VSV
VSV
Normalized Values
*
*
*
CTL VSV
Mavi1 +/+
Mavi1 −/−
Mavi1 −/− + MAVI (WT)
Mavi1 −/− + MAVI (M1)
F
Heart
Weight (g)
p=0.8743
Mavi1 +/+ −/−
Liver
p=0.1413
Mavi1 +/+ −/−
Kidney
p=0.0768
Mavi1 +/+ −/−
Spleen
Weight (g)
p=0.4006
Mavi1 +/+ −/−
Lung
p=0.1828
Mavi1 +/+ −/−
Brain
p=0.6884
Mavi1 +/+ −/−

Figure S3. MAVI1 negatively regulates RLR-mediated antiviral immune responses *in vivo*

(A) The expected protein sequence from both *Mavi1*$^{+/+}$ and *Mavi1*$^{-/-}$ mice is shown.

(B) Genomic DNA was extracted from *Mavi1*$^{-/-}$ mice, followed by PCR using specific primer sets spanning gRNA targeting region (black box). The resultant PCR products were subjected to Sanger sequencing.

(C, D) The genotype (C) and gender (D) ratio of the progeny from heterozygous mouse are shown.

(E, F) The weight of body (E) as indicated as well as the organs weight (F) of both *Mavi1*$^{+/+}$ and *Mavi1*$^{-/-}$ mice are shown.

(G) BHK21 cells were incubated with supernatant from cells infected VSV-GFP as described in Fig. 2K, followed by plaque assay (mean ± SEM, *** $P < 0.001$).

(H) Wild-type (*Mavi1*$^{+/+}$) and *Mavi1* knockout (*Mavi1*$^{-/-}$) MEFs treated with or without VSV (2×10^6 p.f.u, 12 h) in the presence or absence of empty vector, MAVI1 (WT), or MAVI1 (M1) were subjected to RT-qPCR analysis to examine the expression of genes as indicated. (mean ± SEM, * $P < 0.05$, *** $P < 0.001$).

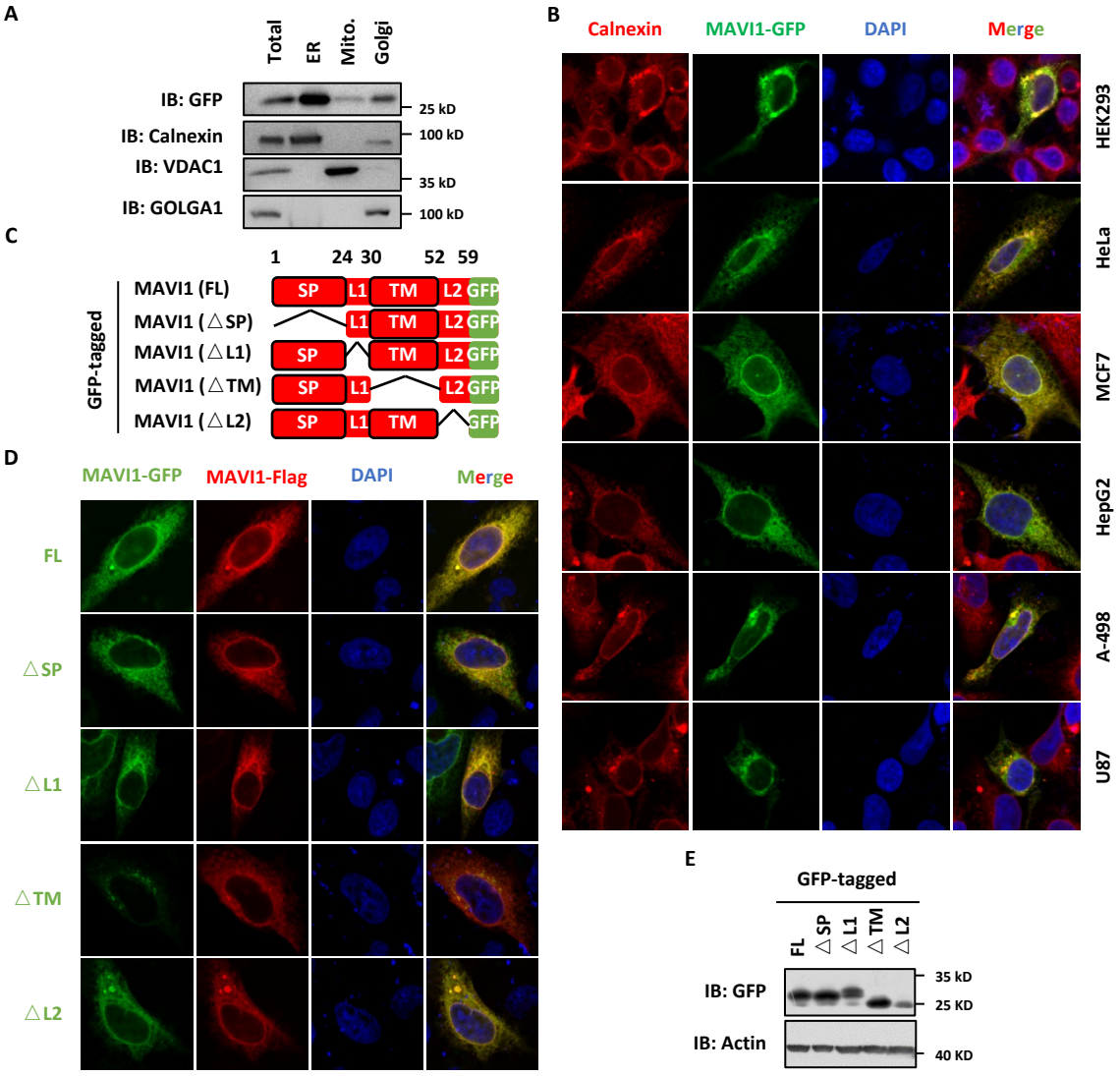

Figure S4. MAVI1 is an endoplasmic reticulum transmembrane protein

(A) HEK293 cells transfected with GFP-tagged MAVI1 were subjected to ER, Mitochonria (Mito.), Golgi apparatus isolation, followed by IB analysis with antibodies as indicated.

(B) HEK293, HeLa, MCF7, HepG2, A-498, and U87 cells transfected with GFP-tagged MAVI1 were stained with Calnexin (Red). Nuclei was stained with DAPI (Blue).

(C) Schematic representation of GFP-tagged, full length (FL), SP-deleted(△SP), linker region 1-deleted (△L1), TM domain-deleted (△L2), and linker region 2-deleted (△L2) is shown.

(D) HEK293 cells transfected with GFP-tagged MAVI1 expression vectors as described in (C) and Flag-tagged MAVI1 were stained with anti-Flag antibody (Red). Nuclei was stained with DAPI (Blue).

(E) HEK293 cells as described in (D) were subjected to IB analysis using antibodies as indicated.

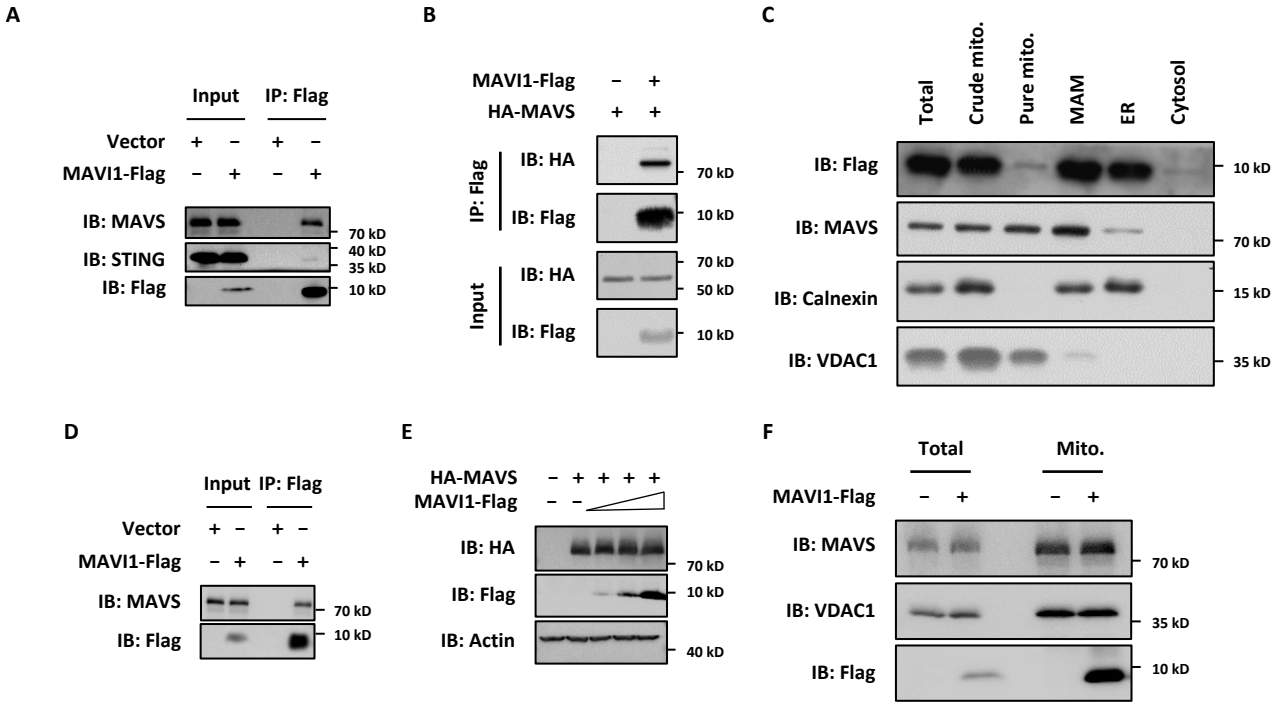

Figure S5. MAVI1 interacts with MAVS

(A) HEK293 cells stably expressing Flag-tagged MAVI1 were subjected to IP with anti-Flag antibody followed by IB analysis using antibodies as indicated.

(B) HEK293 cells transfected with Flag-tagged MAVI1 and HA-tagged MAVS were subjected to IP with anti-Flag antibody, followed by IB analysis using antibodies as indicated.

(C) HEK293 cells stably expressing Flag-tagged MAVI1 were subjected to crude Mitochondria (Mito.), pure Mito, Mitochondria-associated Membrane (MAM), ER, and cytosol isolation, followed by IB analysis with antibodies as indicated.

(D) MAM fraction isolated from control or HEK293 cells stably expressing Flag-tagged MAVI1 were subjected to IP analysis with anti-Flag antibody, followed by IB analysis with antibodies as indicated.

(E) HEK293 cells were transfected with Flag-tagged MAVI1 and HA-tagged MAVS, followed by IB analysis using antibodies as indicated.

(F) HEK293 cells transfected with or without Flag-tagged MAVI1 were subjected to Mitochondria isolation, followed by IB analysis with antibodies as indicated.

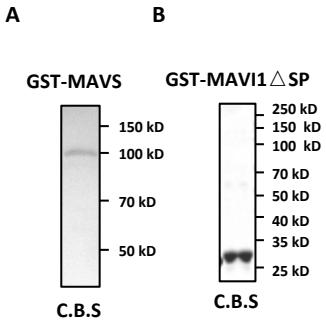

Figure S6. MAVI1 interacts with MAVS through the transmembrane domain

(A, B) The expression of purified, bacterially expressed GST-tagged MAVS (A) and MAVI1 (△SP) (B) was examined by Coomassie Blue Staining (C.B.S).

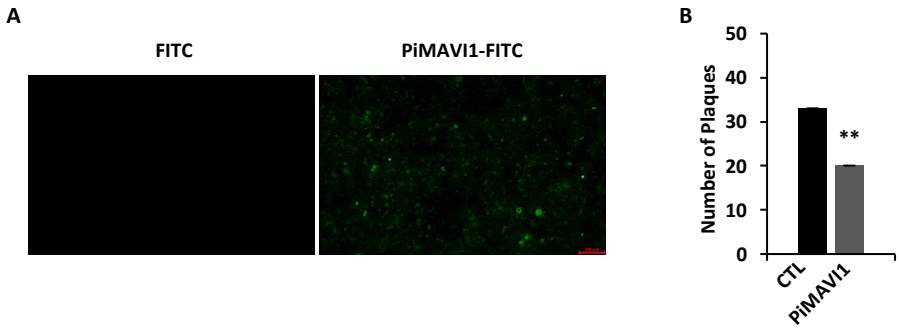

Figure S7. PiMAVI1 is potent in activating type I IFN signaling and antiviral immune responses

(A) HEK293 cells were treated with or withoutPiMAVI1 conjugated with FITC (FITC-PiMAVI1) (10 mM, 12 h), followed by microscopy imaging analysis.

(B) BHK21 cells were incubated with supernatant from cells infected VSV-GFP as described in Fig. 6E, followed by plaque assay (mean ± SEM, ** $P < 0.01$).

Supplementary Table Legend

Table S1. List of genes in response to VSV infection detected from RNA-seq (sheet 1), ORFs predicted from Ribo-seq (sheet 2), ORFs from genes that were induced by VSV (sheet 3), ORFs from genes that were repressed by VSV (sheet 4), short ORFs (sORFs) predicted to have less than 100 amino acids (AAs) (sheet 5), known sORFs (sheet 6), de novo ORFs (sheet 7), and known sORFs with transmembrane domain in HEK293 cells as described in Fig. S1A are shown.

Table S2. List of proteins that were identified to be associated with MAVI1 through IP-MS (sheet 1), that have a SAINT score > 0.95 in REPRINT (sheet 2), and that have a FC-B score > 4 in REPRINT (sheet 3) as described in Fig. 4A are shown.

Table S3. Sequences information for all PCR primers used in the current study are shown. Sequence information of all standard PCR and qPCR primers are shown. F: forward; R: reverse.