Supplemental figure legends

Figure S1. Methionine 142 of human MAVS is conserved among primates and other higher mammals

Related to Figure 1

Figure S1 shows an alignment of the region surrounding methionine 142 of human MAVS to several other species. Methionine 142 is highlighted in bold and conserved amino acids are highlighted with an asterisk.

Figure S2. miniMAVS inhibits FL MAVS-dependent interferon production over time and interacts with TRAF6

Related to Figure 4

Figure S2 A shows the level of FL and miniMAVS in 293T cells following SeV infection. Figure S2B and C demonstrate the MAVS dependent antiviral response by STAT1 phosphorylation following transient expression of several MAVS constructs. Cell lysates were probed for pSTAT1, actin, and MAVS. Figure S2D shows FL MAVS oligomerization following SeV infection. Crude mitochondrial extracts were separated by sucrose gradient centrifugation and probed for MAVS. Figure S2E shows co-immunoprecipitation of TRAF6 and miniMAVS. HA-TRAF6 and 3xFlag-miniMAVS were expressed in 293T cells and isolated with Flag specific affinity gel.

Figure S3. Ribosomal profiling identifies truncation variants of antiviral genes

Related to Figure 6

Figure S3 shows the pattern of ribosome initiation and elongation for several transcripts involved in antiviral immunity; IFIH1 (MDA5) (A), MX2 (B), IFITM2 (C), TRIM25 (D), DDX58 (RIG-I) (E), TMEM173 (STING) (F). The reading frames detected by harringtonine treatment are shown below each profile. Canonical reading frames are in grey, truncations in green, uORFs in red, internal out-of-frames in orange. These data highlight the idea that polycistronic messages may be more prevalent than previously appreciated.

Table S1. Start sites within the MAVS transcript vary in strength

Related to Figure 3

Table S1 lists the start sites at each ORF on the MAVS transcript. The strength of each start site was determined by adherence to the Kozak consensus sequence. Start sites with a purine at position -3 and a guanine at position +4 are considered

strong. Start sites with one of the above properties are considered medium, and those lacking both are considered weak.

Table S2. Genome-wide ribosomal profiling identifies active start sites

Related to Figure 6

Table S2 is a list of all the start sites that were identified in our ribosomal profiling study. The table includes transcript ID, start site location, and the classification of the predicted product.

Supplemental Procedures

None.

Supplemental References

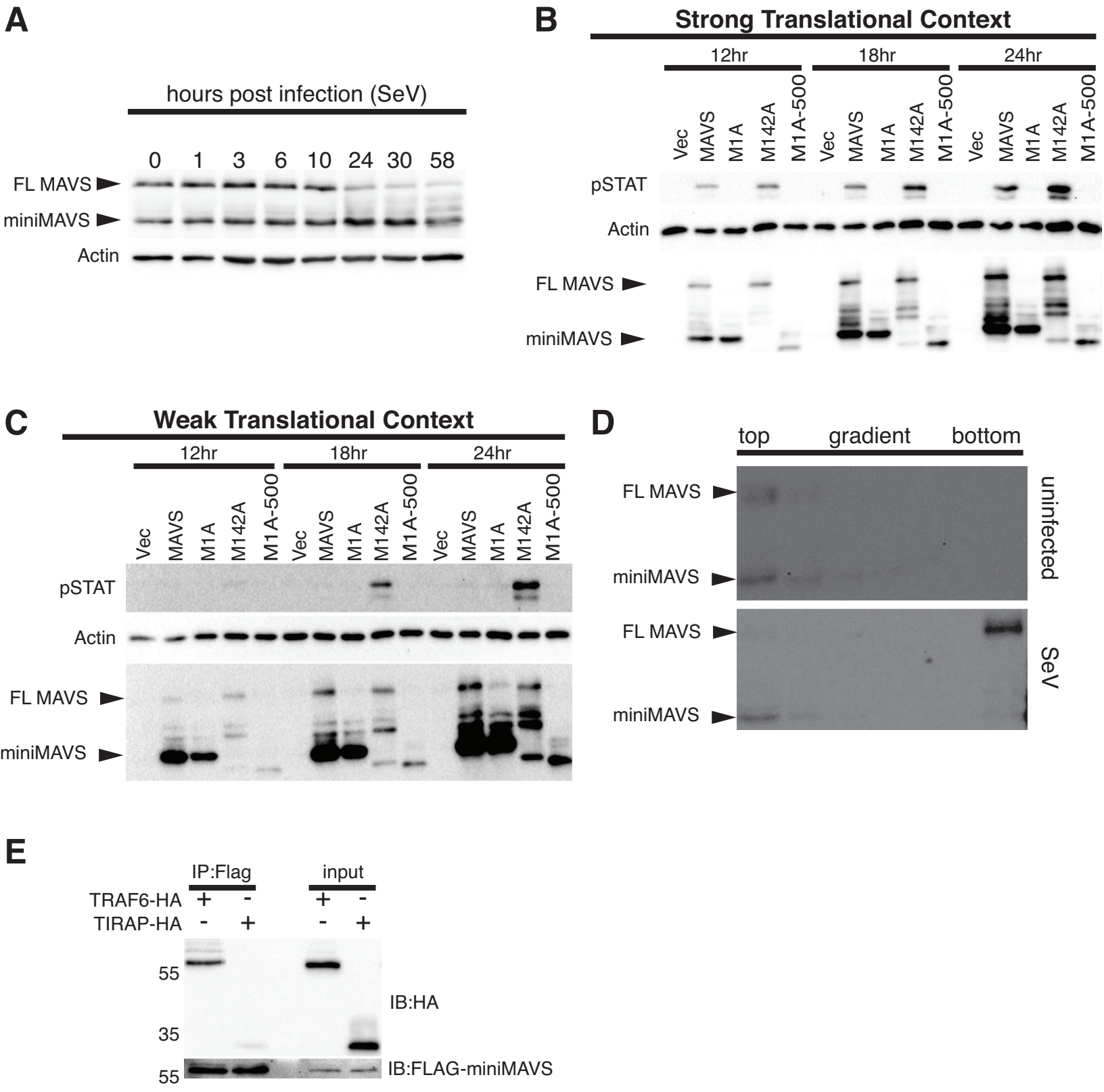
None

Figure S1

A

```
                          M 142
                           ▼
Human           SCREKEPSYPMPVQETQAPES    152
Chimpanzee      SCREKEPSYPMPVQETQVPES    152
Gorilla         SCREKEPSYPMPVQETHAPES    151
Macaque         GYREKEPSYPMPVQETQAPDS    152
Panda           GYREEEPSFPMPVQDTQPPES    153
Horse           GYRGEEPSYPMPVQDTRPPEP    152
Pig             GYREDEPSYPMPVQDTQSPEA    152
Dolphin         GYREDEPSYPMPVQDTQSPES    152
Ferret          GYREEEPSFPLPVQDTQPAES    153
Mouse           GLRE-TPSCPKPVQDTQPPES    152
Rat             GFQD-KPGYPKPVQDTQPPKS    152
Guinea          GYRE-EPSYPRPVQDTQPPMS    152
Squirrel        GYRE-DPSYPRPVQDTQPPKS    151
                       *  * *** *
```

Figure S2


A
hours post infection (SeV)
0 1 3 6 10 24 30 58
FL MAVS
miniMAVS
Actin
B
Strong Translational Context
12hr
18hr
24hr
Vec
MAVS
M1A
M142A
M1A-500
pSTAT
Actin
FL MAVS
miniMAVS
C
Weak Translational Context
12hr
18hr
24hr
Vec
MAVS
M1A
M142A
M1A-500
pSTAT
Actin
FL MAVS
miniMAVS
D
top
gradient
bottom
FL MAVS
miniMAVS
uninfected
FL MAVS
miniMAVS
SeV
E
IP:Flag
input
TRAF6-HA + - + -
TIRAP-HA - + - +
55
35
55
IB:HA
IB:FLAG-miniMAVS

Supplemental Figure 3

Figure S3