Supplementary Figure Legends

MOV10 provides antiviral activity against RNA viruses by enhancing RIG-I-MAVS independent IFN induction

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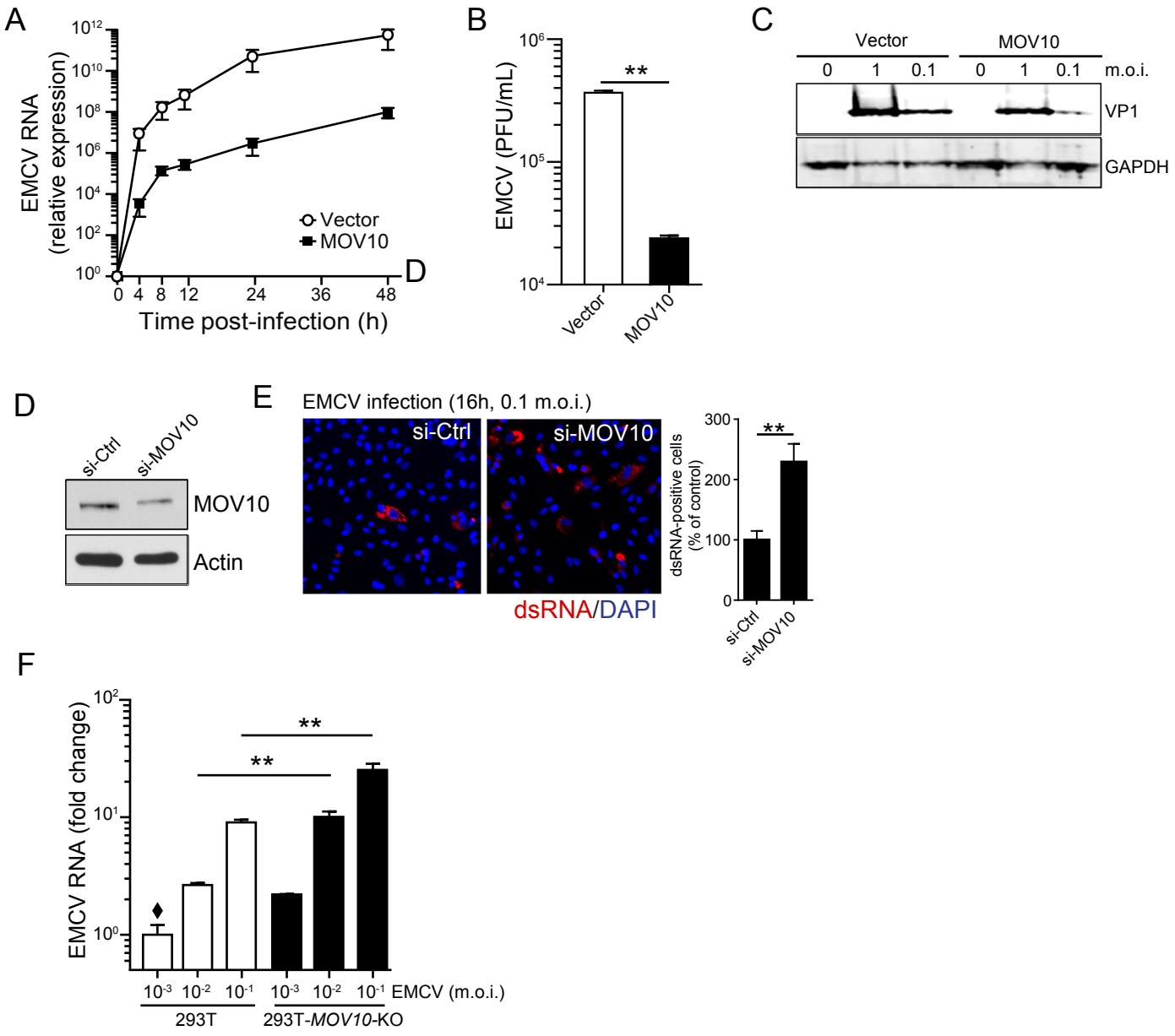


Supplementary Fig. S1, Cuevas *et al.*

Fig. S1: Antiviral activity of MOV10.

(A-B) Inhibition of EMCV replication by MOV10 expression. HEK293 cells with stable expression of either MOV10 or control vector were infected with EMCV (1 m.o.i.) and analyzed by qRT-PCR for EMCV-specific RNA (A). Supernatants from infected cells were collected at 24 h post infection, and virus titers were determined by plaque assay on Vero cells as described before (27) (B).

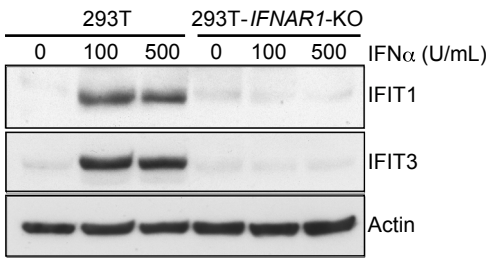
(C) MOV10 expression reduces CVB replication as measured by viral capsid protein VP1 expression. HEK293 cells with stable expression of either MOV10 or control vector were infected with CVB as indicated, and analyzed by immunoblotting for viral capsid protein VP1.

(D) siRNA mediated silencing of MOV10 in primary human fibroblasts (HFF). HFF were transfected with either si-MOV10 or si-Ctrl for 72 h. Silencing efficiency was examined by analyzing MOV10 levels by immunoblotting. Two different siRNA targeting two different regions of MOV10 were tested showing similar silencing.

(E) MOV10 silencing enhances EMCV replication in primary human fibroblasts (HFF). Primary human foreskin fibroblasts were transfected either with control (si-Ctrl) or MOV10-specific (si-MOV10) siRNA for 72 h. Cells were subsequently infected with EMCV as indicated, followed by immunofluorescence analysis using anti-dsRNA sera (J2) to estimate virus infection (27). Representative micrographs with quantitation of the % of infected cells are shown as in Fig. 2A.

(F) Genomic loss of MOV10 enhances EMCV replication. MOV10-deficient 293T cells (293T-*MOV10*-KO) along with control 293T were infected with EMCV at the indicated virus concentration for 16 h followed by qRT-PCR analysis of EMCV RNA. Plots show mean with standard error bars, where * and ** are $P < 0.05$ and $P < 0.01$, respectively, by two-tailed Student's *t* test analysis. Sample (♦) was set as 1 for comparison.

A



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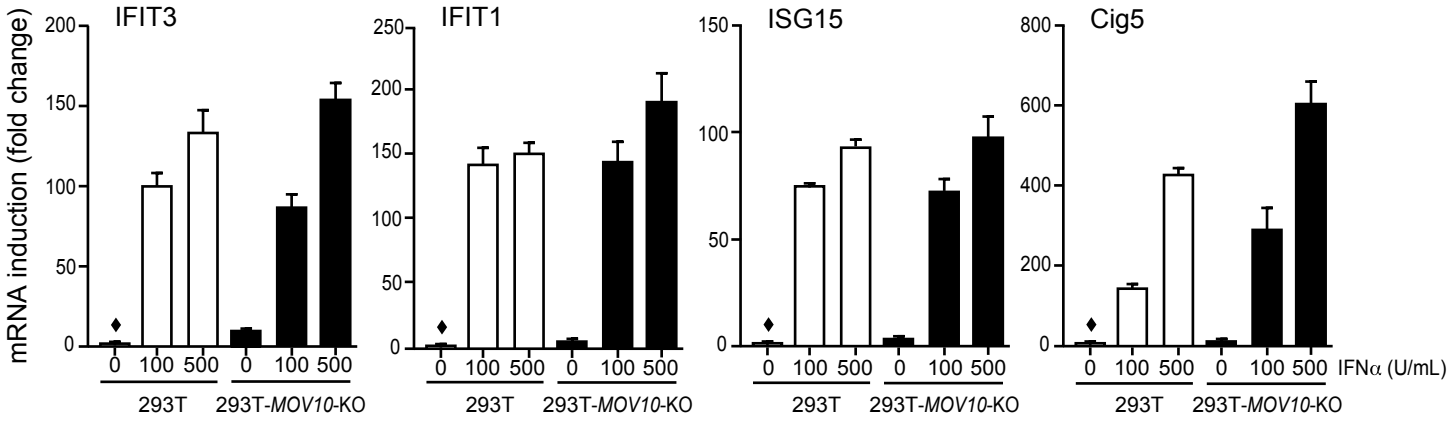

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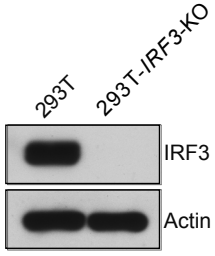


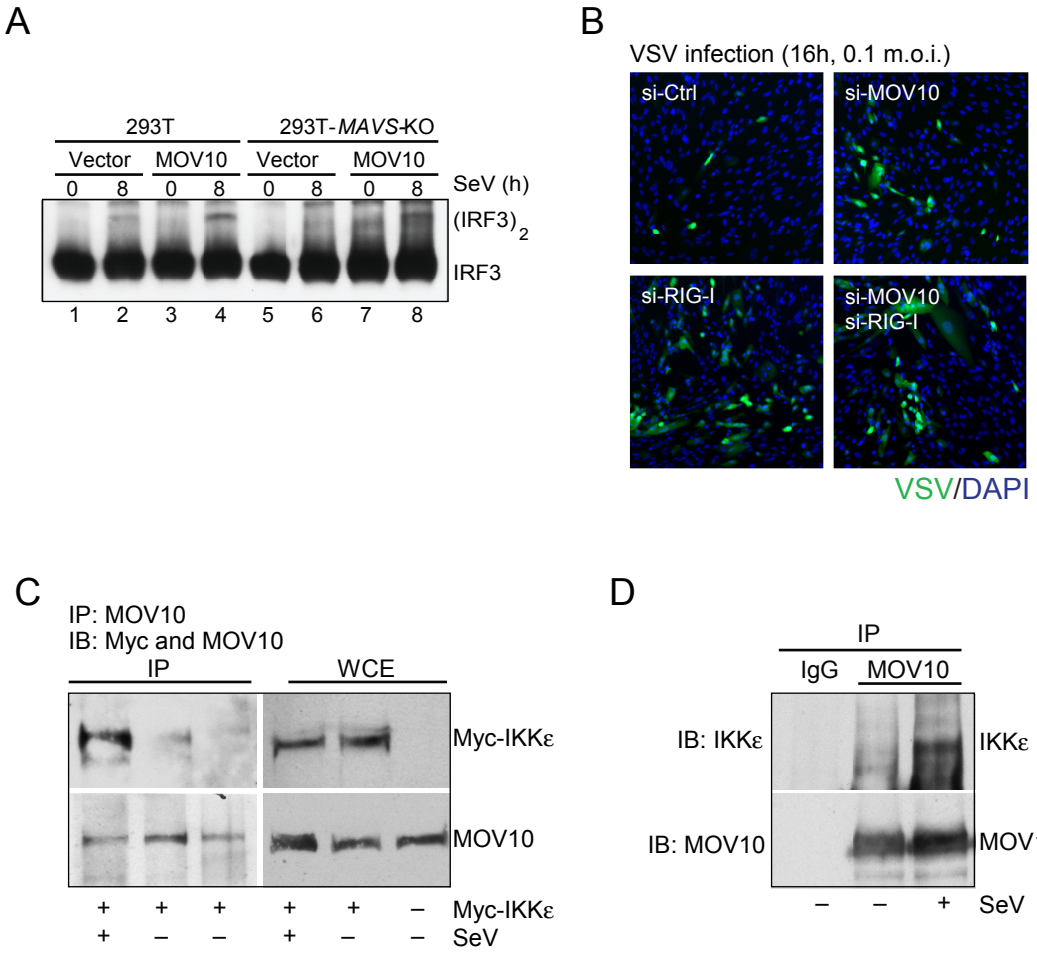
Supplementary Fig. S2, Cuevas *et al.*

Fig. S2: Antiviral activity of MOV10 is mediated through IRF3 signaling.

(A) Characterization of *IFNAR1* targeted 293T cells. Cells were treated with IFN (100 and 500 U/ml, 16 h) and analyzed by immunoblotting to detect induction of endogenous IFIT1 and IFIT3.

(B) MOV10 does not affect IFN-stimulated gene induction. 293T-*MOV10*-KO and 293T cells were treated with IFN (100 and 500 U/ml, 16 h) and analyzed by qRT-PCR to detect mRNA induction of the indicated ISGs.

(C) Characterization of IRF3 targeted 293T cells. Indicated cell lysates were analyzed by immunoblotting for IRF3 and Actin expression. Plots show mean with standard error bars, ♦ set as 1 for comparison.



Supplementary Fig. S3, Cuevas *et al.*

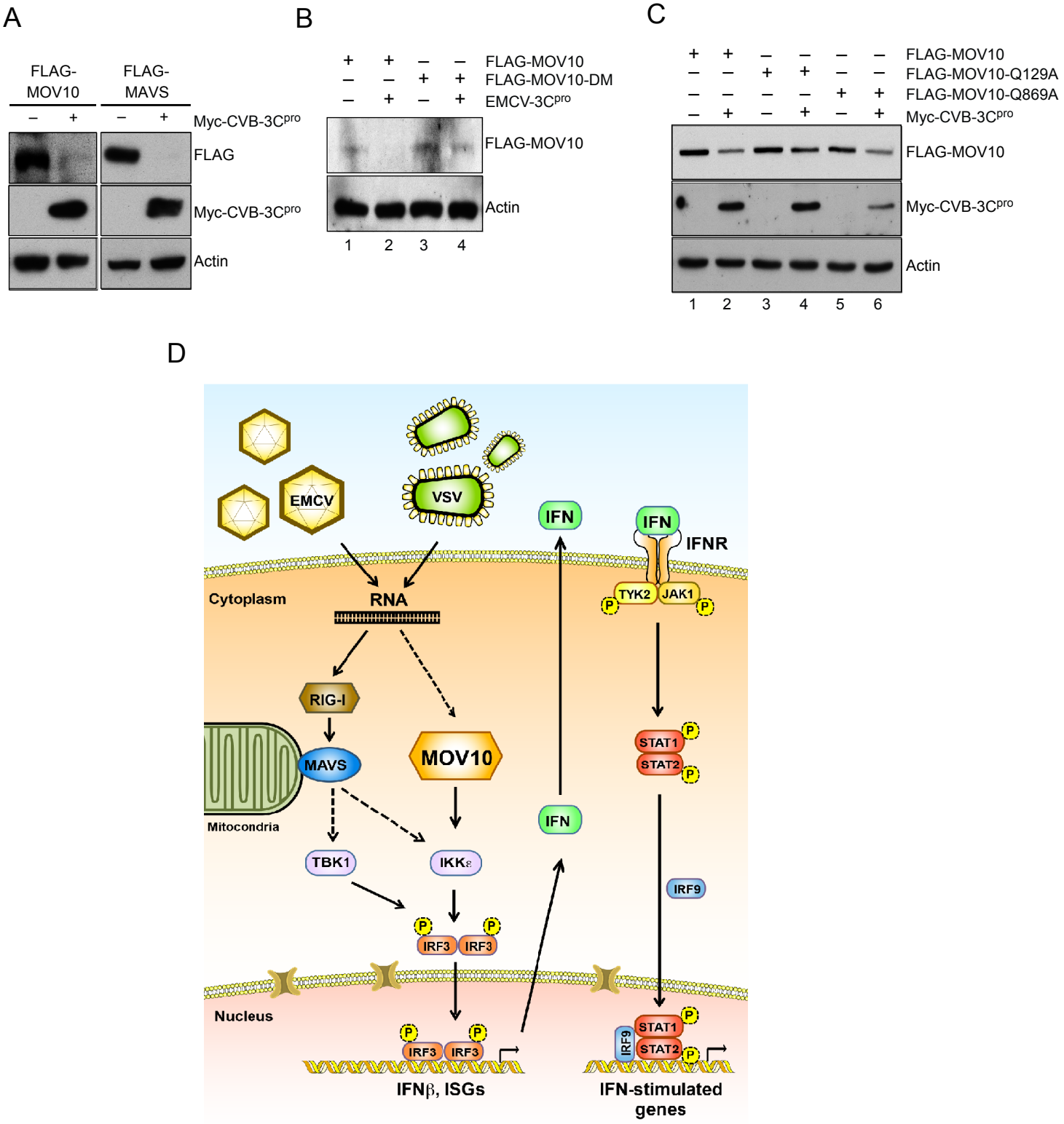
Fig. S3: MOV10 enhances IKKε–dependent IRF3 signaling independent of RIG-I-MAVS.

(A) MOV10 enhances IRF3 dimerization in MAVS-deficient cells. MOV10 was expressed in 293T-*MAVS*-KO cells and assayed for IRF3 dimerization after 8 h stimulation with SeV (240 HAU/ml). Cell lysates were analyzed by immunoblotting for IRF3 in non-denaturing PAGE.

(B) Additive effect of MOV10 and RIG-I double silencing on virus replication. Representative micrographs for experiment described in Fig. 5C are shown.

(C) Interaction of endogenous MOV10 with ectopically expressed IKKε. 293T cells were transfected with Myc-IKKε plasmid and infected with SeV (50 HAU/ml) for 8 h. Cell lysates from infected and un-infected cells were immunoprecipitated with MOV10 antibody and immunoblotted with MOV10 and Myc antibodies. Whole cell extracts (WCE) were from each sample were also analyzed for expression.

(D) Co-immunoprecipitation of endogenous IKKε with MOV10 after SeV infection. 293T cells were infected with SeV as above, followed by immunoprecipitation with MOV10 antibody or control IgG. Immunoprecipitated proteins were analyzed by immunoblotting with IKKε or MOV10 antibodies.



Supplementary Fig. S4, Cuevas *et al.*

Fig. S4: MOV10 is targeted for degradation by picorna viral proteases. (related to Fig. 7)

(A) FLAG-tagged MOV10 was co-expressed along with Myc-tagged CVB $3C^{pro}$ or vector plasmids in 293T cells. Cells lysates were analyzed by immunoblotting for MOV10 and Myc expression. FLAG-tagged MAVS was included as a positive control as previously described in (24).

(B) MOV10 is targeted by EMCV protease $3C^{pro}$. 293T cells were co-transfected with Wt or MOV10-DM along with EMCV $3C^{pro}$ as indicated followed immunoblotting. Mutant Q129A/Q869A is resistant to degradation by EMCV $3C^{pro}$.

(C) Partial protection of MOV10 mutants Q129A and Q869A from $3C^{pro}$-mediated cleavage. MOV10 single-mutants Q129A and Q869A were co-expressed along with CVB $3C^{pro}$ in 293T cells and analyzed as in (A).

(D) A working model for MOV10-mediated antiviral activity. Our data suggest MOV10 is able to signal downstream to activate the IFN induction in a MAVS/RIG-I-independent manner.