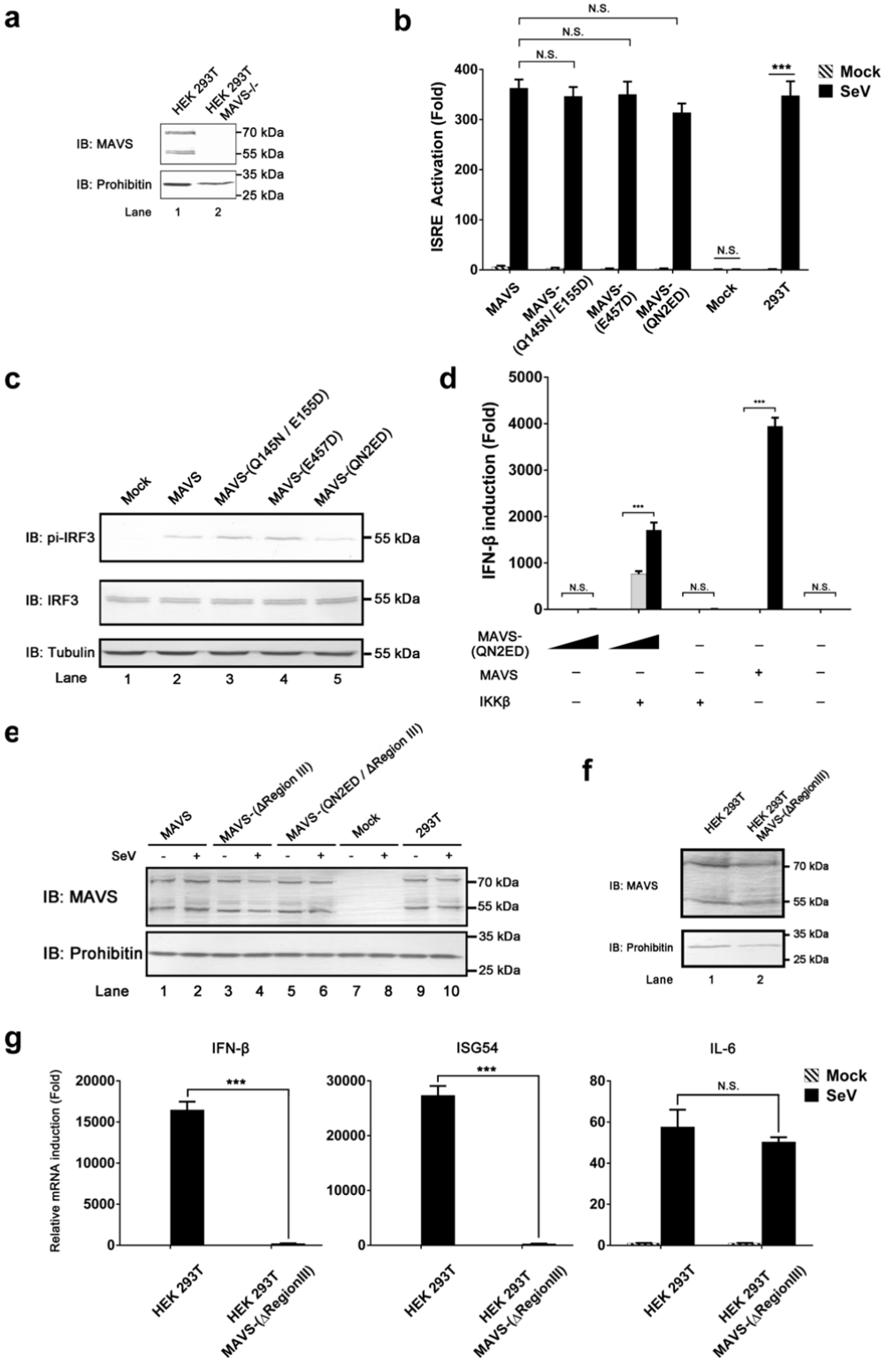
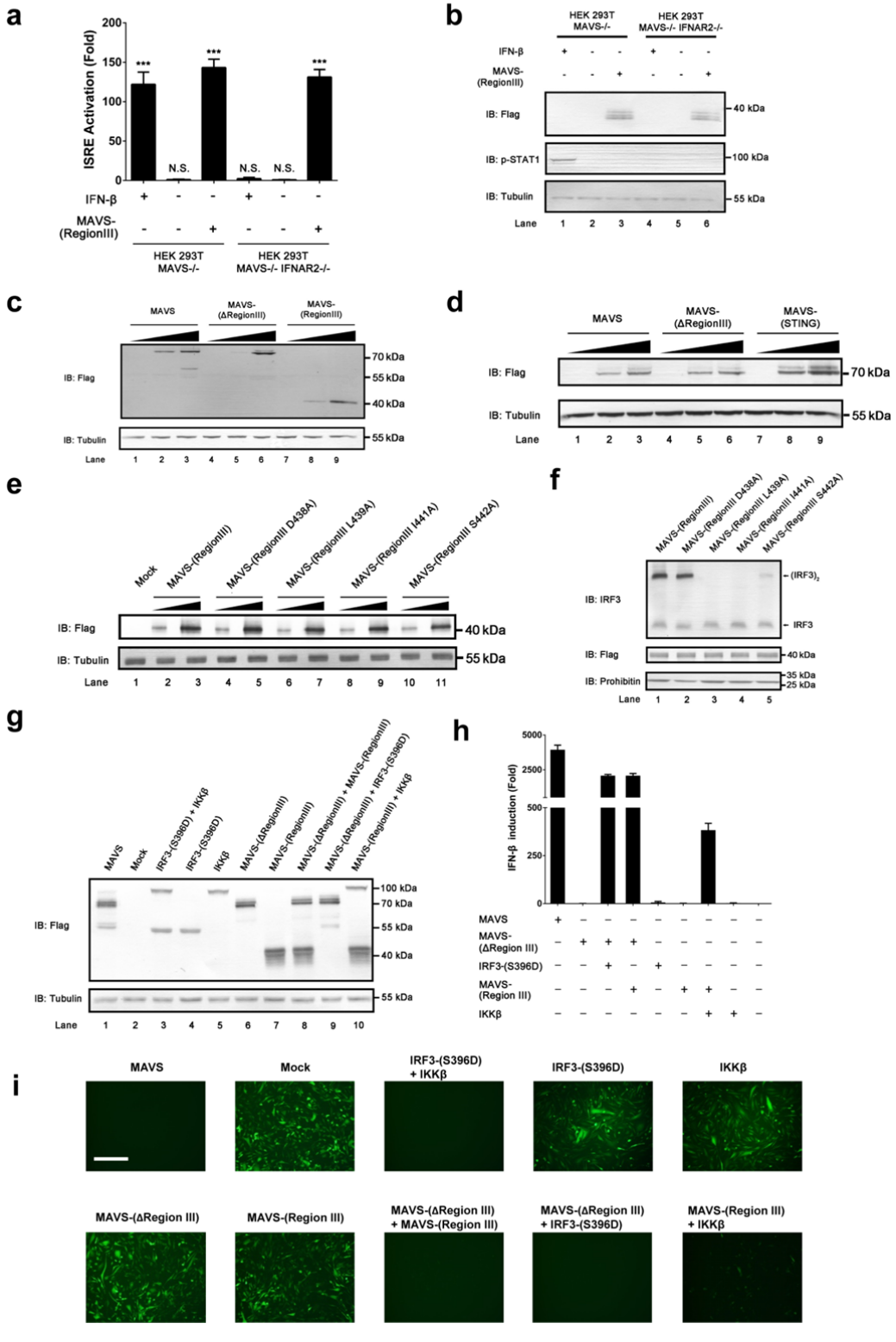
Supplementary Figure 1



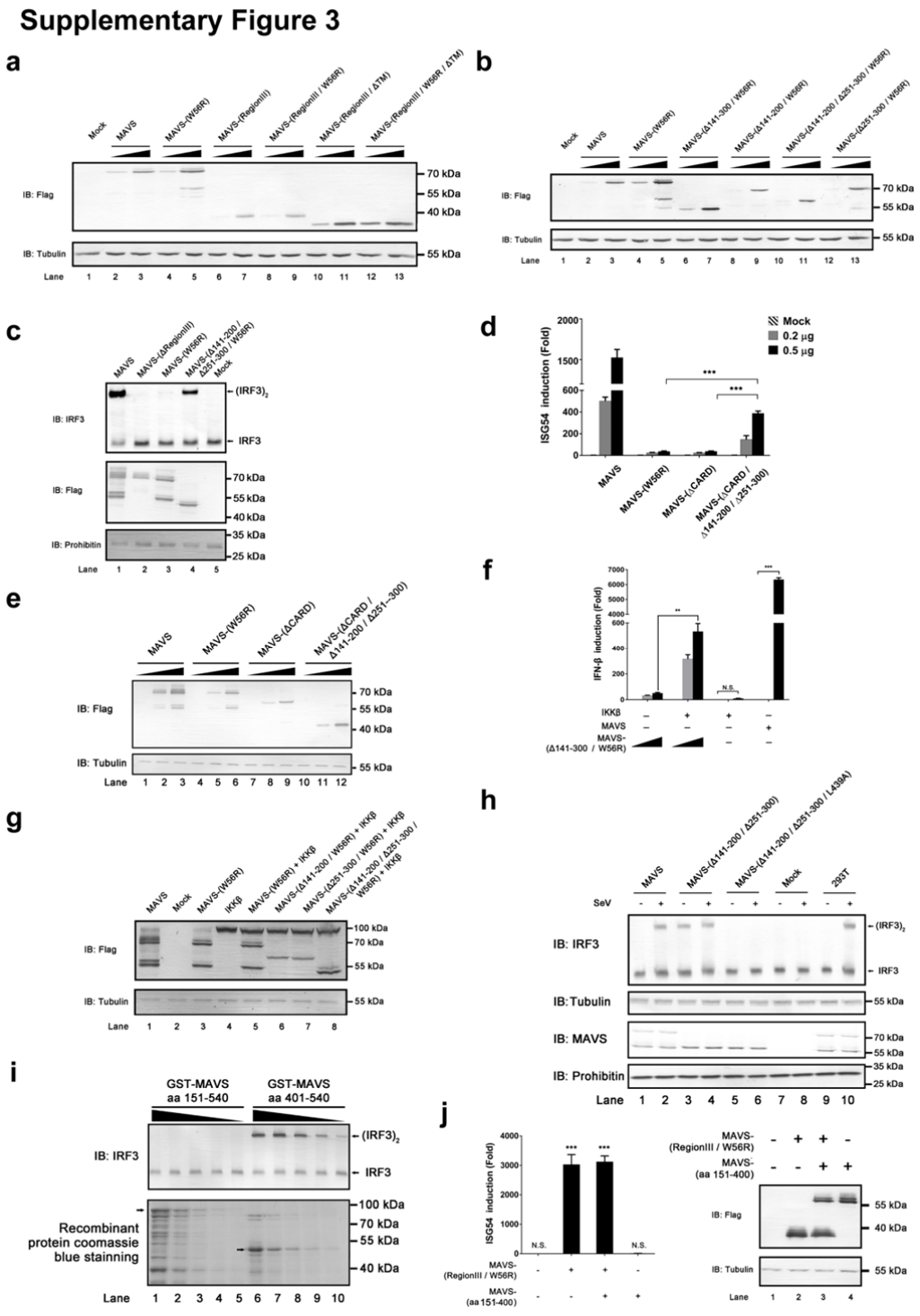
Supplementary figure 1 | MAVS harbors a region with TBK1/IRF3-stimulating activity other than its putative TRAF-binding sites . (**a**) P5 was isolated from HEK 293T or MAVS-deficient HEK 293T cells, which was used for western blot with anti-MAVS and anti-prohibitin antibodies. The original full blot can be found in Supplementary Fig. 6d. (**b**) Samples were made as described in Figure 1(**b**) and firefly luciferase inductions were measured. (**c**) Various MAVS forms were transduced into *MAVS-/-* MEF cells, which were harvested for following analysis thirty-six hours after transduction. IRF3 phosphorylation was examined by western blotting with anti-pi-IRF3 antibody. Total protein level of IRF3 was also examined. The original full blot can be found in Supplementary Fig. 6e. (**d**) MAVS with TRAF-binding motifs disrupted could still stimulate TBK1/IRF3. MAVS-(QN2ED) with or without IKKβ were expressed in *MAVS-/-* HEK 293T cells and IFN expressions were measured by qPCR. (**e**) MAVS expression level was detected for samples as described in Figure 1(**f**) (**g**) (**h**). The original full blot can be found in Supplementary Fig. 7a. (**f**) A cell line (*MAVS-(∆Region III)* 293T cells) was made with Region III of endogenous MAVS knocked-out. The original full blot can be found in Supplementary Fig. 7b. (**g**) *MAVS-(∆Region III)* 293T cells were infected with or without Sendai virus. Twelve hours after infection, ISG54, IL-6 and IFN induction were measured with qPCR.

Supplementary Figure 2



Supplementary figure 2 | Characterization of the TBK1/IRF3-stimulating region of MAVS. (**a**, **b**) ISRE-luciferase reporter, together with or without MAVS-(Region III) were transfected into *MAVS-/-* HEK 293T cells or *IFNAR2-/- & MAVS-/-* HEK 293T cells, which were then treated with or without IFN-β (10 U per ml). The cells were harvested for following analysis twelve hours after treatment. Firefly luciferase inductions were measured (**a**). Protein expression levels were analyzed (**b**). The original full blot can be found in Supplementary Fig. 7c. (**c**) Protein expression levels were shown for samples described in Figure 2b. The original full blot can be found in Supplementary Fig. 7d. (**d**) Protein expression levels were shown for samples described in Figure 2d. The original full blot can be found in Supplementary Fig. 7e. (**e**) Protein expression levels were indicated for samples described in Figure 2e. The original full blot can be found in Supplementary Fig. 7f. (**f**) Various amounts of pcDNA3-flag-MAVS-(Region III) mutants were transfected into *MAVS-/-* HEK 293T cells. Thirty-six hours after transfection, P5 fractions were isolated from the cells and IRF3 dimerization was performed *in vitro*. The original full blot can be found in Supplementary Fig. 8a. (**g**) Protein expression levels were detected for samples described in Figure 2f. The original full blot can be found in Supplementary Fig. 8b. (**h**) Expression vectors as indicated were transfected into *MAVS-/-* HEK 293T cells and IFN expression were measured by qPCR. (**i**) MAVS without Region III is defective in its antiviral function, which could be rescued by coexpression of MAVS (Region III) or a

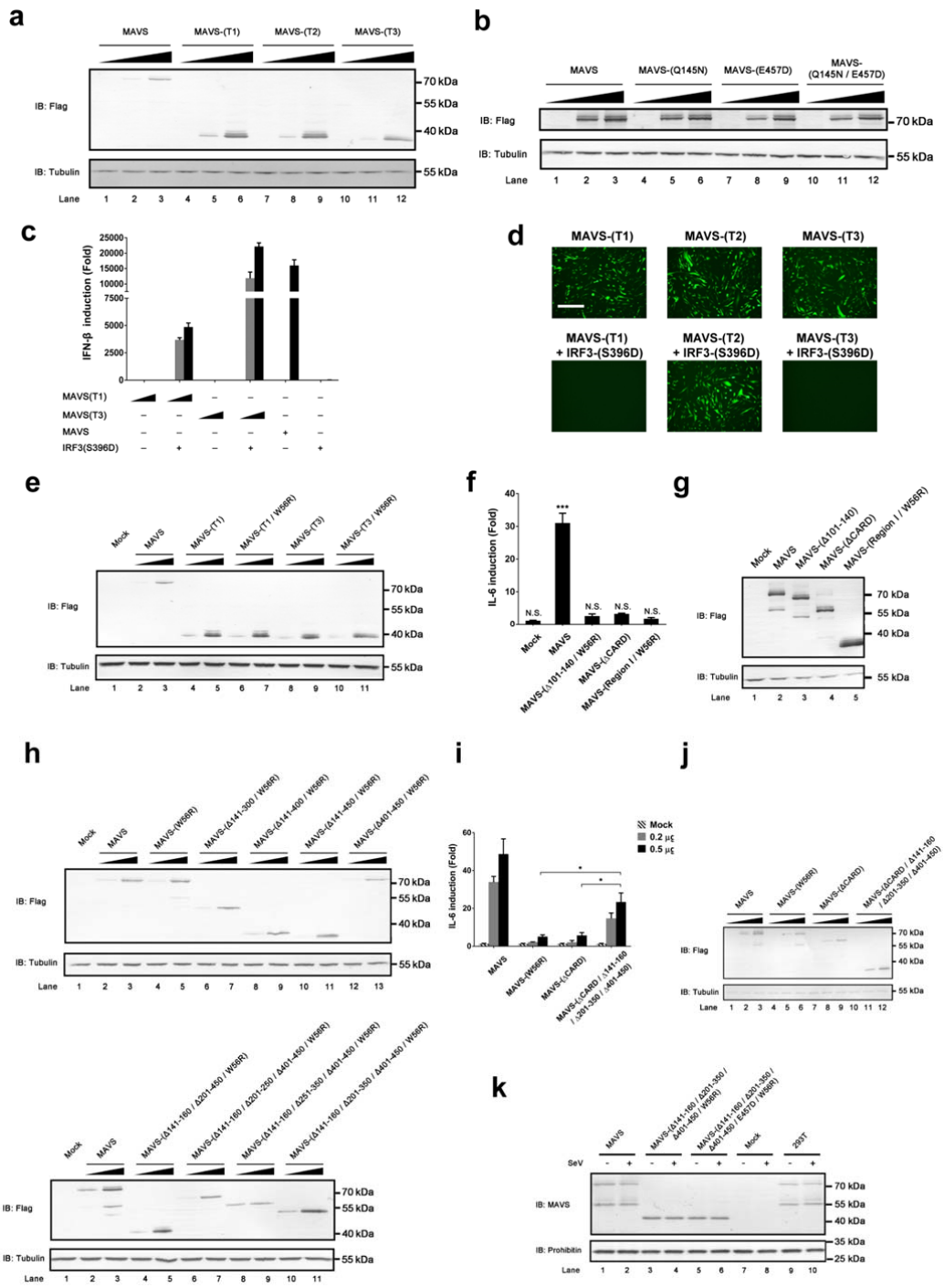
constitutively active form of IRF3-(S396D). Various MAVS forms were transfected into *MAVS-/-* HEK 293T cells. Thirty-six hours after transfection, cells were infected with VSV-ΔM51-GFP. Fluorescent images were taken eight hours after VSV infection to visualize GFP-positive cells, indicating VSV proliferation. Scale bar represents 500 micrometers.



Supplementary figure 3 | The activity of MAVS Region III is inhibited by leading region aa-141-300 in its quiescent state. (**a**) Protein expression levels were detected for samples described in Figure 3b. The original full blot

can be found in Supplementary Fig. 8c. (**b**) Protein expression levels were detected for samples described in Figure 3c. The original full blot can be found in Supplementary Fig. 8d. (**c**) pcDNA3-flag-MAVS (wild type and mutants) were transfected into *MAVS-/-* HEK 293T cells. P5 was isolated thirty-six hours after transfection to perform IRF3 dimerization assay *in vitro*. The original full blot can be found in Supplementary Fig. 8e. (**d**, **e**) Various amounts of constructs as indicated were transfected into *MAVS-/-* HEK 293T cells. Gene inductions were measured thirty-six hours after transfection (**d**). Protein expression levels are shown (**e**). The original full blot can be found in Supplementary Fig. 8f. (**f**) Coexpression of MAVS-(∆aa-141-300 / W56R) and IKKβ induced IFN expression. Various expression vectors as indicated were transfected into *MAVS-/-* HEK 293T cells and IFN inductions were measured with qPCR. (**g**) Protein expression levels were detected for samples described in Figure 3e. The original full blot can be found in Supplementary Fig. 9a. (**h**) Protein expression levels were detected for samples described in Figure 3f. The original full blot can be found in Supplementary Fig. 9b. (**i**) Recombinant proteins GST-MAVS-(aa-151-400) and GST-MAVS-(aa-401-540) were expressed and purified from *E. coli*. Increasing amounts of GST-MAVS-(aa-151-400) or GST-MAVS-(aa-401-540) were used for IRF3 dimerization assay *in vitro*. The original full blot can be found in Supplementary Fig. 9c. (**j**) Constructs as indicated were transfected into *MAVS-/-* HEK 293T cells. Gene inductions were measured thirty-six hours after transfection (left).

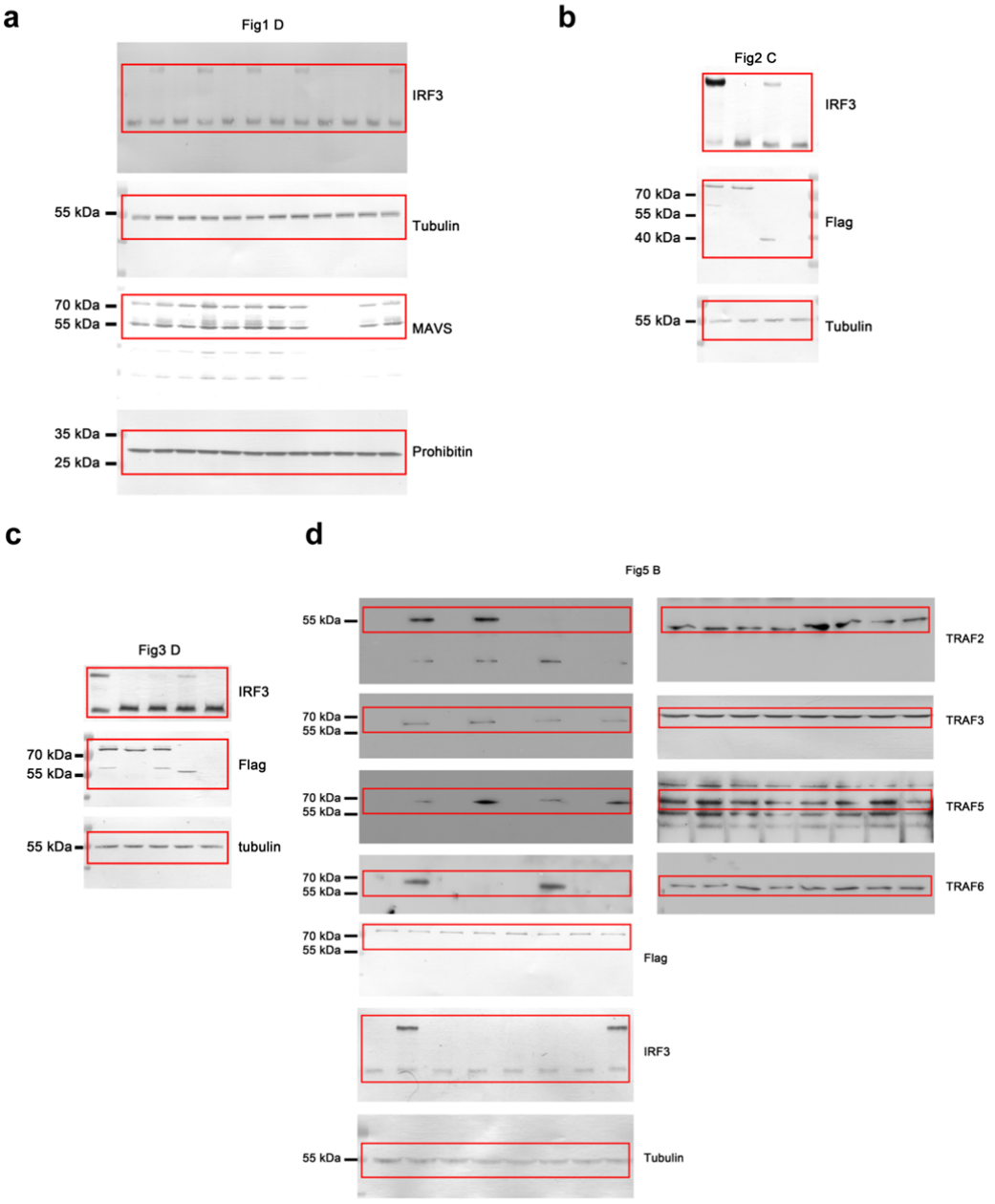
Protein expression levels are shown (right). The original full blot can be found in Supplementary Fig. 9d.



Supplementary figure 4 | Identification of two NF-κB-stimulating regions in MAVS. (**a**) Protein expression levels were detected for samples described in Fig. 4b. The original full blot can be found in Supplementary Fig. 9e. (**b**) Protein expression levels were detected for samples described in Fig.

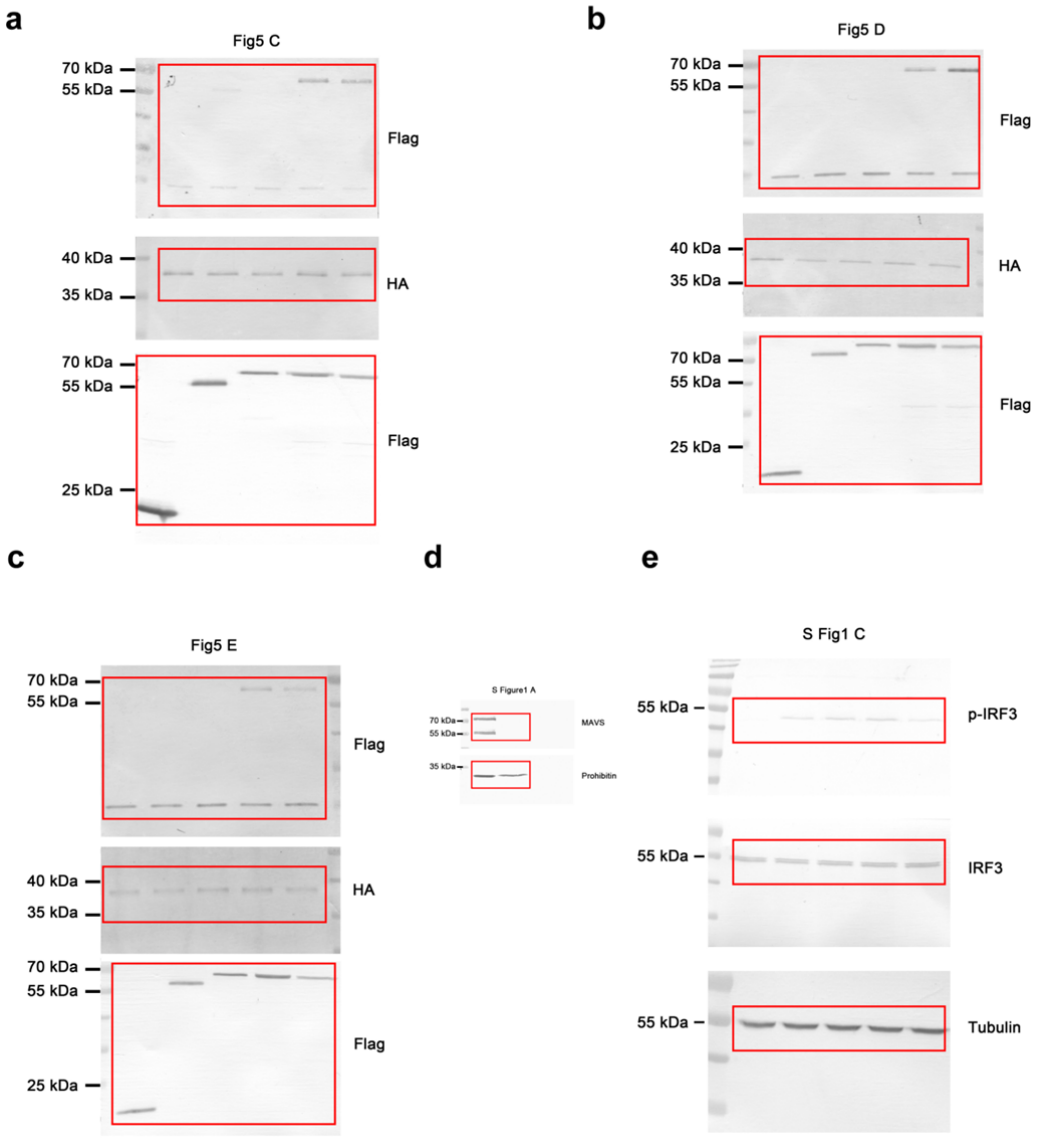
4c. The original full blot can be found in Supplementary Fig. 9f. (**c**) Coexpression of MAVS (T1) and MAVS (T3) with IRF3 (S396D) induced IFN expression. A combination of plasmids as indicated were transfected into *MAVS-/-* HEK 293T cells and IFN inductions were measured with qPCR thirty-six hours after transfection. (**d**) In the presence of IRF3 (S396D), both MAVS (T1) and MAVS (T3) could provide antiviral activity. Expression vectors as indicated were transduced into *MAVS-/-* MEF cells followed by VSV-ΔM51-GFP infection. Fluorescent images were taken twenty-four hours post virus infection to examine VSV proliferation. Scale bar represents 500 micrometers. (**e**) Protein expression levels were detected for samples described in Fig. 4d. The original full blot can be found in Supplementary Fig. 10a. (**f, g**) Constructs as indicated were transfected into *MAVS-/-* HEK 293T cells. Gene inductions were measured thirty-six hours after transfection (**f**). Protein expression levels are shown (**g**). The original full blot can be found in Supplementary Fig. 10b. (**h**) Protein expression levels were detected for samples described in Fig. 4e. The original full blot can be found in Supplementary Fig. 10c-d. (**i, j**) Various amounts of constructs as indicated were transfected into *MAVS-/-* HEK 293T cells. Gene inductions were measured thirty-six hours after transfection (**i**). Protein expression levels are shown (**j**). The original full blot can be found in Supplementary Fig. 10e. (**k**) Protein expression levels were detected for samples described in Fig. 4f. The original full blot can be found in Supplementary Fig. 10f.

Supplementary Figure 5



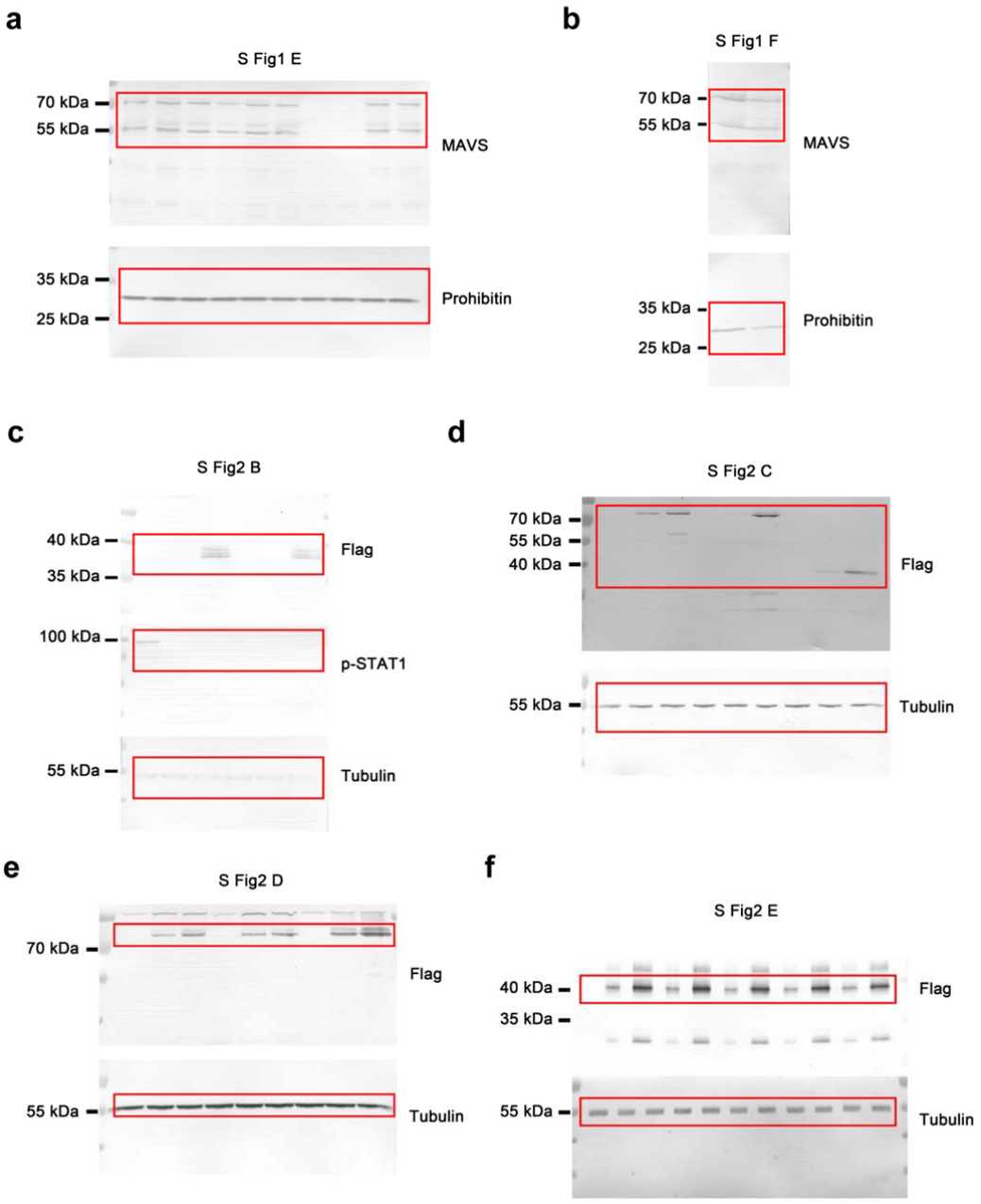
Supplementary figure 5 | **Full blot.** (**a**) For Fig. 1d. (**b**) For Fig. 2c. (**c**) For Fig. 3d. (**d**) For Fig. 5b.

Supplementary Figure 6



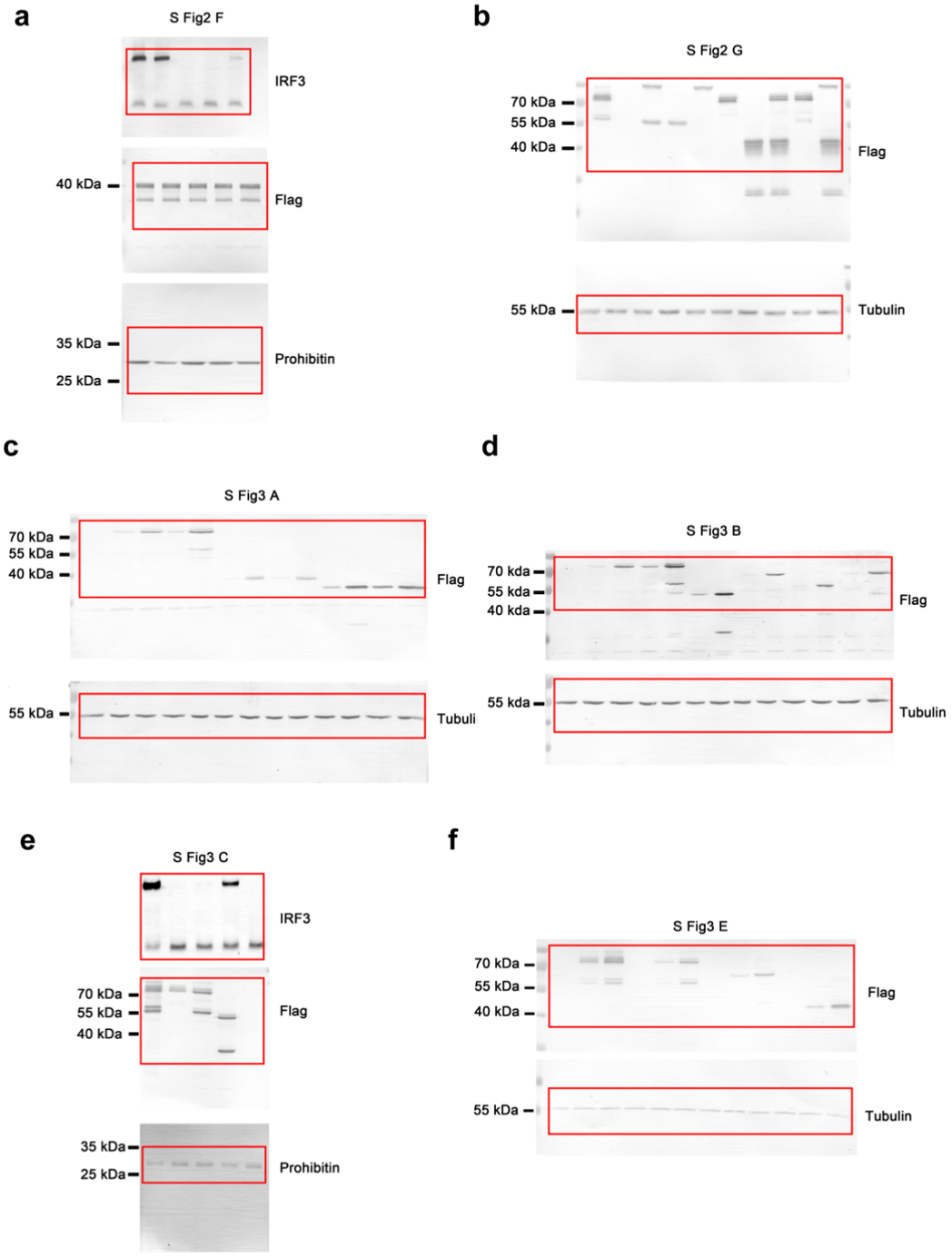
Supplementary figure 6 | **Full blot.** (**a**) For Fig. 5c. (**b**) For Fig. 5d. (**c**) For Fig. 5e. (**d**) For Supplementary Fig. 1a. (**e**) For Supplementary Fig. 1c.

Supplementary Figure 7



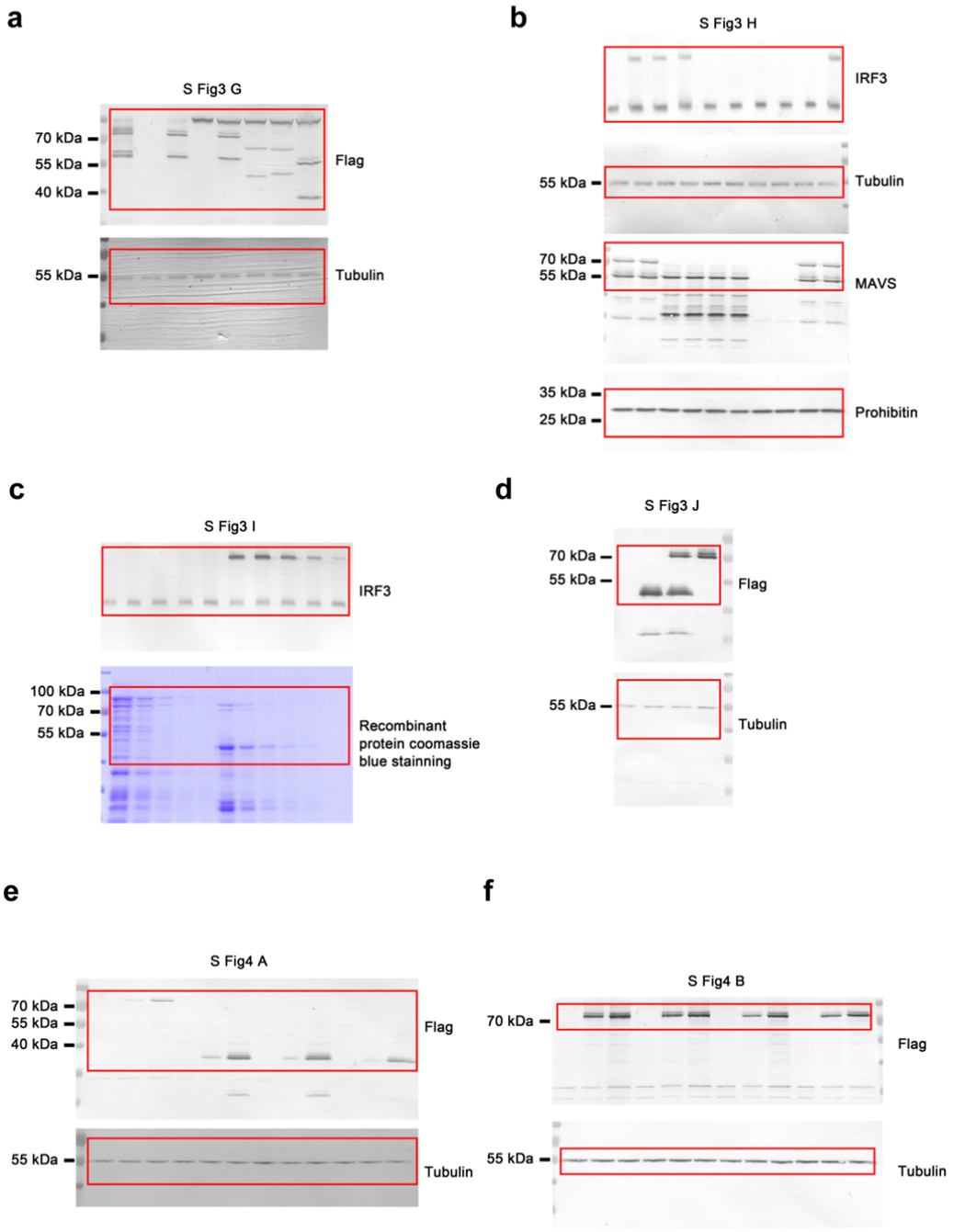
Supplementary figure 7 | **Full blot.** (**a**) For Supplementary Fig. 1e. (**b**) For Supplementary Fig. 1f. (**c**) For Supplementary Fig. 2b. (**d**) For Supplementary Fig. 2c. (**e**) For Supplementary Fig. 2d. (**f**) For Supplementary Fig. 2e.

Supplementary Figure 8



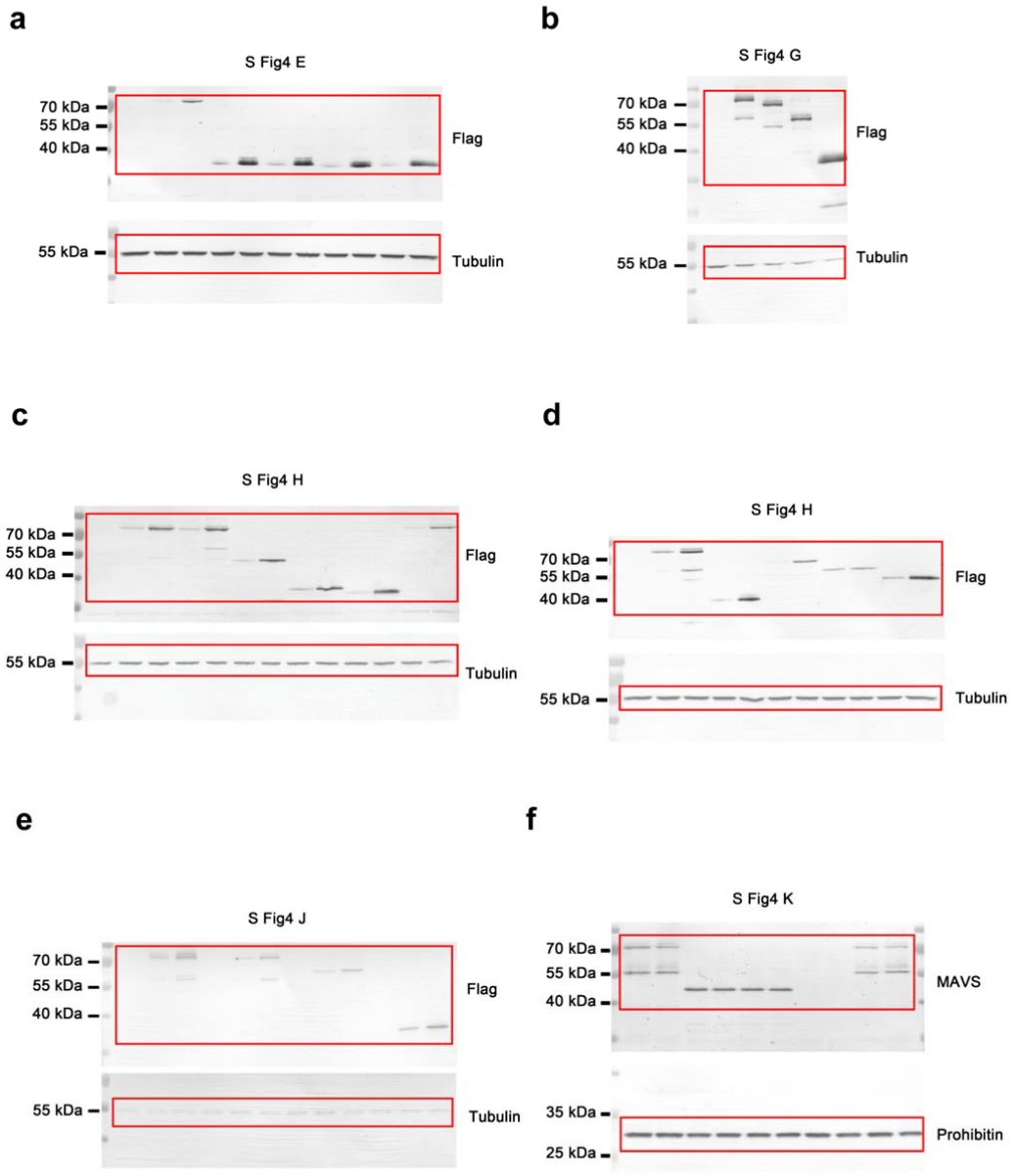
Supplementary figure 8 | **Full blot.** (**a**) For Supplementary Fig. 2f. (**b**) For Supplementary Fig. 2g. (**c**) For Supplementary Fig. 3a. (**d**) For Supplementary Fig. 3b. (**e**) For Supplementary Fig. 3c. (**f**) For Supplementary Fig. 3e.

Supplementary Figure 9



Supplementary figure 9 | **Full blot.** (**a**) For Supplementary Fig. 3g. (**b**) For Supplementary Fig. 3h. (**c**) For Supplementary Fig. 3i. (**d**) For Supplementary Fig. 3j. (**e**) For Supplementary Fig. 4a. (**f**) For Supplementary Fig. 4b.

Supplementary Figure 10



Supplementary figure 10 | Full blot. (**a**) For Supplementary Fig. 4e. (**b**) For Supplementary Fig. 4g. (**c**) For Supplementary Fig. 4h (upper). (**d**) For Supplementary Fig. 4h (lower). (**e**) For Supplementary Fig. 4j. (**f**) For Supplementary Fig. 4k.

Supplementary Table 1 | Primers

Primer Name	Primer sequence (from 5' to 3')
MAVS E457D 5'	gccatggcccagaggataatgagtataagtccgaaggcaccttt
MAVS E457D 3'	atcctctgggccatggcaagg
MAVS Δ 401-450 5'	ccggcgccactggaggaccctgtcatggacctgaggagaatgag
MAVS Δ 401-450 3'	tcctccagtggcgccggcgg
MAVS 401-450 5'	tttggtaccatggactacaaggacgacgatgacaagagttcggcc tggctagacagcag
MAVS 401-450 3'	tttgaattcccccatgcccaaggaggtgc
MAVS W56R 5'	cagggaaccgggacacactcaggcatctcttcaataccccttcagc
MAVS W56R 3'	tgagtgtgtcccggttccctgagagtg
IKK-beta 5'	tacggtaccatggactacaaggacgacgatgataagatgagctg gtcaccttccctg
IKK-beta 3'	atcgtctagactatgaggcttgctccaggc
MAVS Q145N,E155D 5'	aatgaaacccaagcgccagagtcccccggagacaattc
MAVS Q145N,E155D 3'	gtctccgggggactctggcgcttgggtttcattgac
MAVS Δ141-400 5'	gcagagagaaggaaccaagttacagctcagcctggctagacag cag
MAVS Δ141-400 3'	gtaacttggctccttctctctgcagc
MAVS Δ251-300 5'	cccacaggatcagttgtatctactaccttgatgcccgtgaacacagt gg
MAVS Δ251-300 3'	agtagatacaactgatcctgtgggtcc
MAVS Δ101-140 5'	ctaccagcctcggacctcgcccatgcctgtgcaggagacc
MAVS Δ101-140 3'	cgaggtccgaggctggtagctctc
MAVS Δ141-150 5'	cagagagaaggagccaagttacgagtccccaggagagaattca gag
MAVS Δ141-150 3'	gtaacttggctccttctctctgcagc
MAVS Δ141-300 5'	cagagagaaggagccaagttacacgttgatgcctgtgaacacag tgg
MAVS Δ201-300 5'	gggcaccaggaaaaggacactactctcatgcctgtgaacacagt gg
MAVS Δ201-300 3'	agtgtccttttcctggtgcccgctg
MAVS Q145N 5'	caagttaccccatgcctgtcaatgagacccaagcgccagag
MAVS Q145N 3'	attgacaggcatggggtaacttggctc
MAVS Δ141-450 3'	ctctgggccgtggcaggggtaacttggctccttctctctgcag
MAVS Δ201-450 3'	ctctgggccatgacacggtgtgtccttctcctgatgcccgc
MAVS Δ301-450 3'	ctctgggccatggcaggggtagggactttggagggcagag
MAVS Δ251-350 5'	cccacaggatcagttgtatctactatcaattcaacccgtgctggc
MAVS Δ201-250 5'	gggcaccaggaaaaggacacaggcacatcattctcctcctcatc c
MAVS Δ201-350 5'	gggcaccaggaaaaggacactatcaactcaacccgtgctggc
MAVS Δ141-200 5'	cagagagaaggagccaagttacgaactcggaagtacccacac agcag

MAVS-500 FLAG 5'	gactacaaggacgacgatgacaagtttcaggagagggaggtgc catgc
MAVS CARD FLAG 3'	cttgtcatcgtcgtccttgtagtcagaggtccgaggctggtagctc
MAVS 500 XhoI 3'	atactcgagcttccggtctgcttgtggcctgg
MAVS STING SWAP 3'	ctctgggccatggcaggggagaaatccgtgcggagaggg
MAVS STING SWAP 5'	gccggcgcaactggaggagttactgtgggcagcttgaagacc
MAVS 450 5'	ccctgccatggcccagaggag
MAVS 400 3'	tcctccagttgcgccggcgg
Region III aa437 3'	ttcaaagcagcccgagaacg
Region III D438A 5'	cgttctcgggctgctttgaagctcttgccatcagcgccagcac
Region III L439A 5'	cgttctcgggctgctttgaagacgctgccatcagtgctagcacctcc
Region III I441A 5'	cgttctcgggctgctttgaagatcttgcggcaagtgctagcacctcct tgggc
Region III S442A 5'	cgttctcgggctgctttgaagatcttgccatcgctgctagcacctcctt gggcatgg
Region III S442Y 5'	cgttctcgggctgctttgaagatcttgccatctacgccagcacctcctt gggcatgg
MAVS aa-151-540 5'	ttaggatccgagtccccaggagagaattcagag
MAVS aa-401-540 5'	tttggatccagctcagcatggctagacagcag
MAVS aa-540 3'	tttctcgagctagtgcagacgccgccggtac
TRAF2 5'	ctgaggtaccatggcagcagctagcgtgac
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IRF3 S396D 3'	ggtggctgtcaatatgcaggtccacagtatt