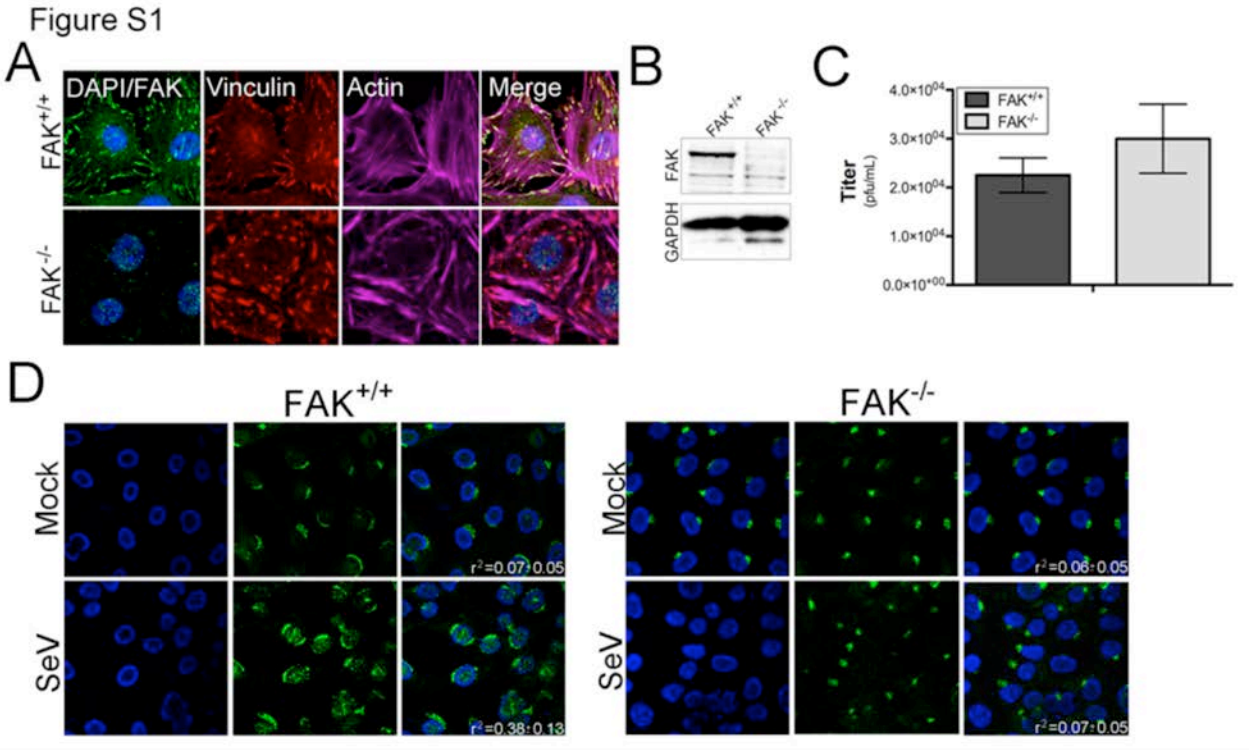

Figure S1, related to Figure 1: Characterization of FAK$^{-/-}$ MEFs. (A), Immunofluorescence microscopy for FAK (green), vinculin (red), and actin (purple) in FAK$^{+/+}$ or FAK$^{-/-}$ MEFs. (**B)**, Lysates from FAK+/+ or FAK-/- MEFs were immunoblotted for FAK (top) or GAPDH (bottom). **(C),** Vaccinia virus titers (as assessed by plaque assays) in FAK$^{+/+}$ and FAK$^{-/-}$ MEFs. Data are shown as mean ± standard deviation. **(D),** FAK+/+ or FAK-/- MEFs were infected with SeV (100HAU/mL) or PBS (mock) for 16hrs, fixed, and stained for IRF3 (in green). Blue, DAPI-stained nulcei. Values shown in white at bottom right hand corner indicate Pearson's correlation coefficients (r^2) ± standard deviation for IRF3 and DAPI-stained nuclei

Figure S2

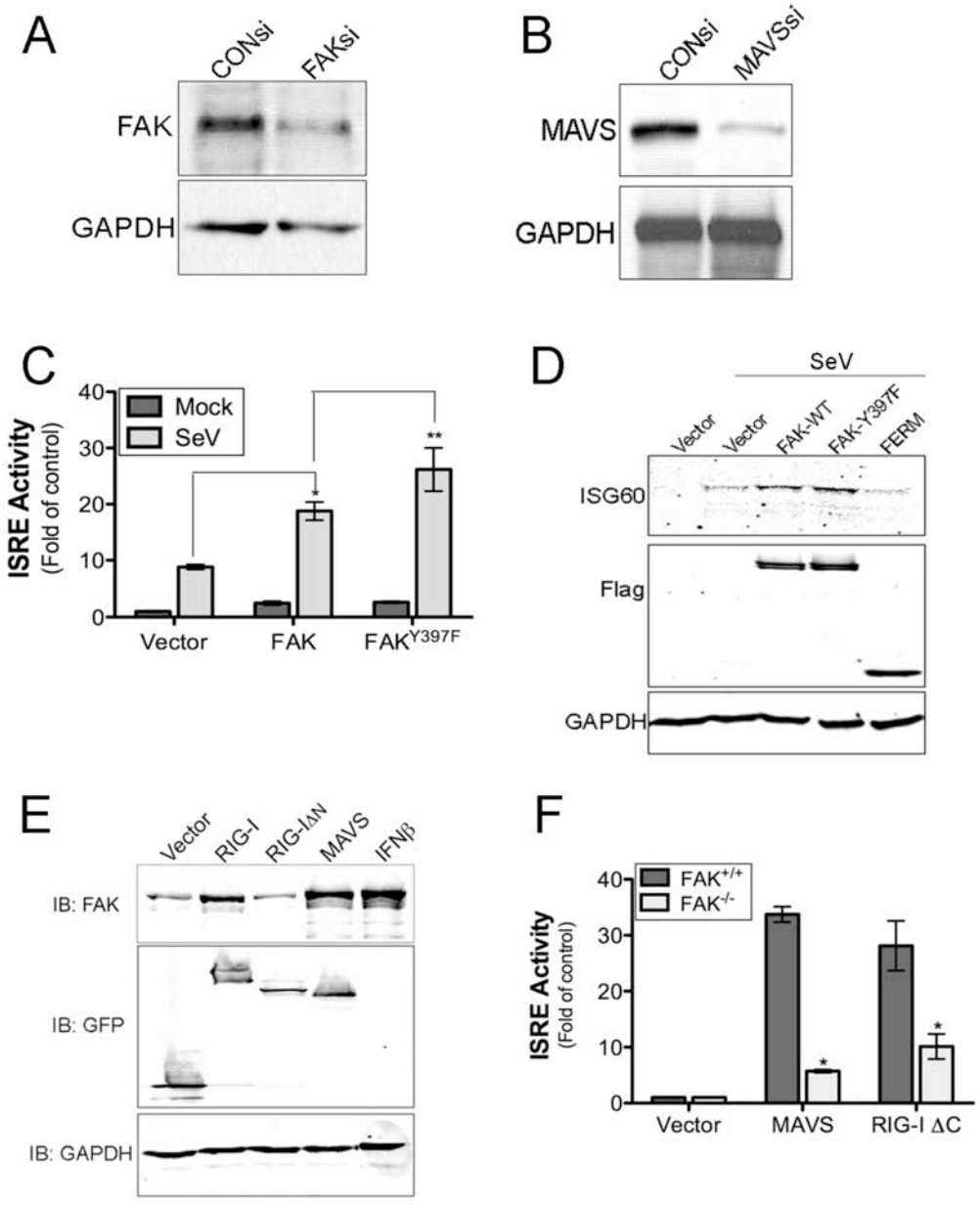

Figure S2, related to Figure 2 and 3. (A, B) Immunoblot analysis from HEK293 cells transfected with FAK (A) or MAVS (B) siRNAs. GAPDH is shown as a loading control. **(C),** Luciferase assays from HEK293 cells transfected with vector and either wild-type (WT) or FAK^{Y397F} and an ISRE-driven luciferase reporter. Cells were infected with SeV (25HAU/mL) or PBS (mock) for 16hr following transfection (~24hrs). **(D),** Immunoblots

for ISG60 in HEK293 cells infected with SeV (10HAU/mL) and transfected with vector, WT or Y397F FAK, or the FERM domain of FAK alone. Uninfected vector-transfected cells are shown in the left lane. Lysates were collected and immunobloted for ISG60, Flag (to control for transfection), and GAPDH to control for protein loading. **(E),** Immunoblot analysis of endogenous FAK, GFP (to control for expression), and GAPDH (to control for equal loading) from HEK293 cells transfected with the indicated constructs for 48 hr or exposed to purified IFNβ (100U/mL) for 18 hr. **(F),** Luciferase assays from $FAK^{+/+}$ and $FAK^{-/-}$ MEFs transfected with vector, MAVS, or RIG-I ΔC and an ISRE-driven luciferase reporter.

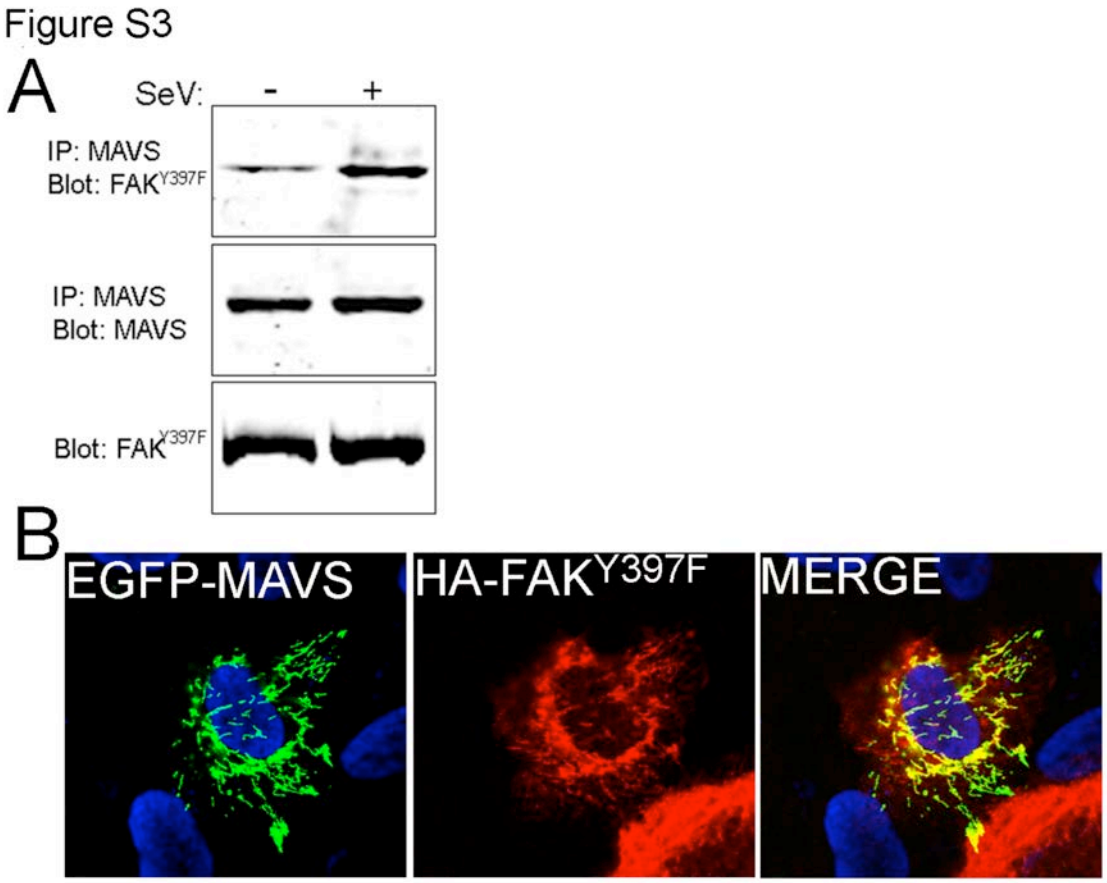

Figure S3, related to Figure 3. Association of FAK^{Y397F} with MAVS. (A) Co-immunoprecipitation studies from HEK293 cells transfected with HA-FAK-Flag Y397F and EGFP-MAVS. Following transfection (24 hr), cells were infected for 18 h with SeV (10 HAU/mL) and then lysed. Lysates were then subject to immunoprecipitation with antibodies directed against GFP and lysates immunoblotted for HA-FAK-Flag Y397F (top panel), or GFP (middle panel). In parallel, lysates were immunoblotted for HA-FAK-Flag Y397F to control for the level of expression (bottom panel). (**B)** U2OS cells were transfected with EGFP-MAVS and HA-FAK-Flag Y397F and then infected with SeV (25 HAU/mL) for 18 hr. Following infection, cells were fixed and stained for FAK (Flag, red) and confocal microscopy performed. Blue, DAPI-stained nuclei.

Figure S4

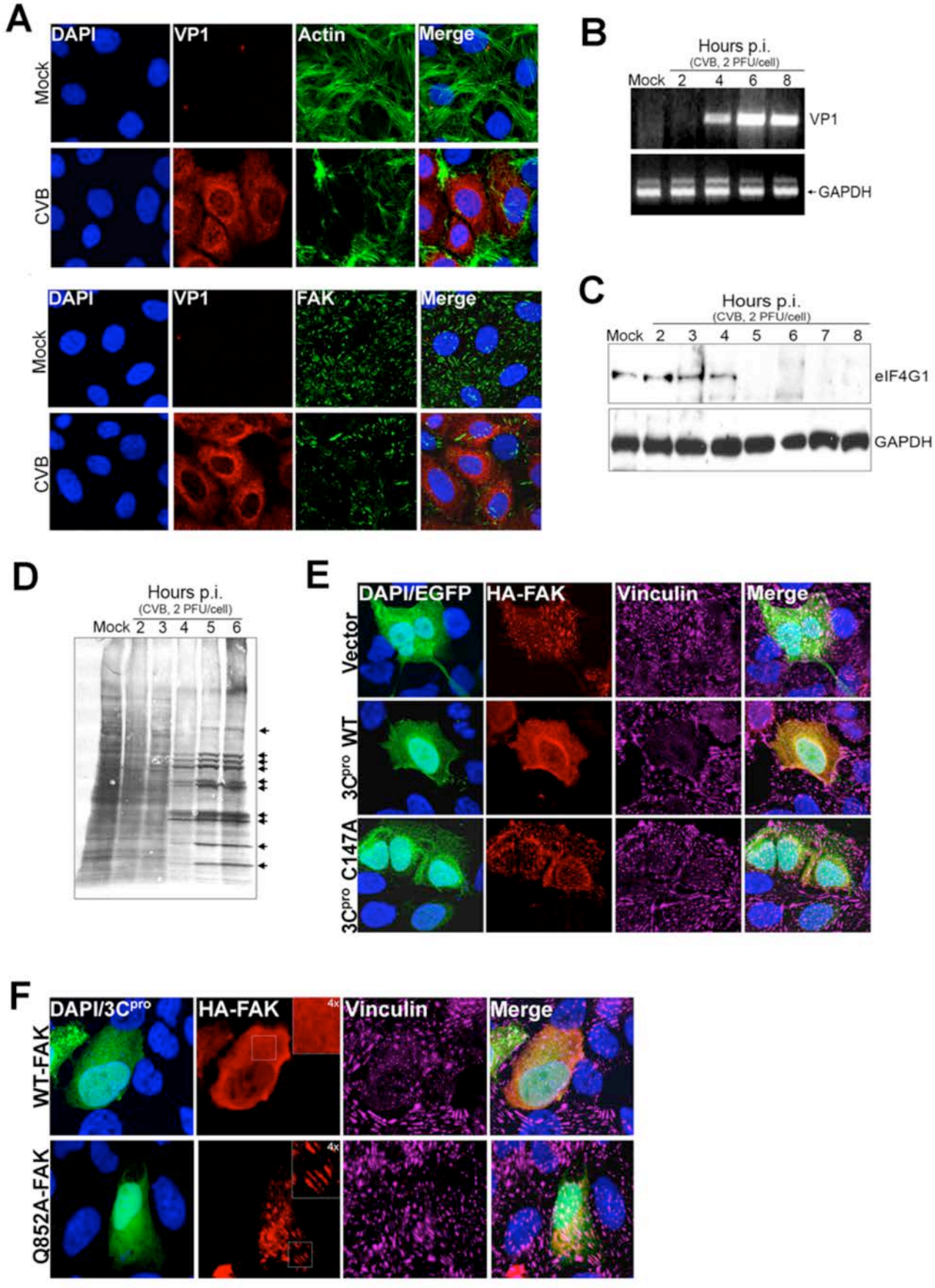
A
DAPI
VP1
Actin
Merge
Mock
CVB
DAPI
VP1
FAK
Merge
Mock
CVB
B
Hours p.i.
(CVB, 2 PFU/cell)
Mock 2 4 6 8
VP1
GAPDH
C
Hours p.i.
(CVB, 2 PFU/cell)
Mock 2 3 4 5 6 7 8
eIF4G1
GAPDH
D
Hours p.i.
(CVB, 2 PFU/cell)
Mock 2 3 4 5 6
E
DAPI/EGFP
HA-FAK
Vinculin
Merge
Vector
3Cpro WT
3Cpro C147A
F
DAPI/3Cpro
HA-FAK
4x
Vinculin
Merge
WT-FAK
Q852A-FAK
4x

Figure S4, related to Figure 5. Time course of CVB infection of Caco-2 cells. (A), Caco-2 cells were infected with CVB for 8 hrs and fixed and stained for actin (green, top) or FAK (green, bottom) and VP1 (red). NoV, no virus control**.** Blue, DAPI-stained nuclei. Images were acquired at the basal surfaces of cells. **(B),** RT-PCR for VP1 (top) or GAPDH (bottom) in Caco-2 cells infected with CVB (2 PFU/cell) for the indicated time. **(C),** Immunoblot analysis for eIF4G1 and GAPDH from Caco-2 cells infected with CVB (2 PFU/cell) for the indicated time. **(D),** Kinetics of shut off of host protein synthesis in Caco-2 cells infected with CVB. Caco-2 cell monolayers were infected with CVB (2 PFU/cell) in methionine-free medium and at the indicated times post-infection, cells were pulsed with 60μCi of ^{35}S-methionine for 30min. Cells were then washed and harvested and lysates anayzed by SDS-PAGE and dried gels exposed to film for 24 h. **(E),** U2OS cells were transfected with vector control or EGFP-3C^{pro} wild-type (WT) or C147A mutant and HA-FAK and immunofluorescence microscopy performed for FAK (red) and vinculin (purple) 48 h following transfection. **(F),** Confocal micrographs of U2OS cells transfected with either WT FAK or Q852A FAK and WT 3C^{pro} stained for HA-FAK (red) and vinculin (magenta).

Supplemental Material and Methods

Cells. Caco-2 and HeLa cells were cultured in MEM supplemented with 10% FBS and 1x penicillin/streptomycin. HEK293 cells stably expressing HA-tagged human TLR3 were purchased from Invivogen.

Antibodies. Rabbit polyclonal and mouse monoclonal antibodies directed against GFP (FL, B-2), GAPDH, HA (Y-11, F-7), and FAK (A-17, C-20) were purchased from Santa Cruz Biotechnology. Mouse monoclonal anti-Flag (M2) was purchased from Sigma. Mouse anti-enterovirus VP1 (Ncl-Entero) was obtained from Novocastra Laboratories (Newcastle upon Tyne, United Kingdom). Mouse ant-vinculin was purchased from Sigma and mouse anti-phosphotyrosine PY20 was purchased from Thermo Scientific. Alexa Fluor-conjugated secondary antibodies and phalloidin were purchased from Invitrogen.

Plasmids and siRNAs

EGFP-$3C^{pro}$ wild-type and C147A have been previously described (Mukherjee et al., 2011). Myc-tagged $3C^{pro}$ and C147A were constructed by inserting PCR-amplified BamHI/EcoRI fragments into the corresponding sites of pRK6-Myc. N-terminal HA-tagged FAK (HA-FAK, pKH3-FAK) was provided by Dr. Jun-Lin Guan (University of Michigan). Dual HA- and Flag-tagged FAK was generated by amplification of FAK cDNA with primers encoding N-terminal HA and C-terminal Flag epitope tags and cloned into a pcDNA-TOPO fusion vector (Invitrogen). Mutagenesis of FAK was performed using a Quickchange mutagenesis kit following the manufacturer's protocol (Stratagene). EGFP-fusion constructs expressing the cleavage fragments of FAK were generated by PCR amplification and subsequent cloning into the NT-GFP-TOPO fusion vector (Invitrogen). FAK FERM domain and FRNK cDNAs were generated by PCR amplification and cloned into pcDNA3.1 using BamHI/XhoI and BamHI/EcoRi sites, respectively. EGFP-fused

MAVS (full length and N- or C-terminal constructs) and RIG-I and CFP-fused TRIF have been described (Mukherjee et al., 2011; Mukherjee et al., 2009). EGFP-STING and HA-RIP1 were constructed by amplification from cDNA (purchased from Open Biosystems) and cloned into EGFP-C2 or pcDNA3.1, respectively, following PCR amplification. DsRed-Mito was purchased from Clontech. Primer sequences are available from the corresponding author on request.

SiRNAs against MAVS (ON-TARGETplus) were purchased from Thermo Scientific. SiRNA against FAK were purchased from Integrated DNA Technologies (target sequence, 5'-AAGCAUGUGGCCUGCUAUGGA-3').

Transfections Plasmid transfections into HEK293 or U2OS cells were performed using XtremeGENE 9 according to the manufacturer's protocol (Roche Applied Science). Plasmid transfections into MEFs were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocols. In both cases, cells were plated as described above and used 24-72 hours later. Transfections with siRNAs were performed using Hiperfect (Qiagen) according to the manufacturer's protocol.

qRT-PCR

Total RNA was isolated from cells by TRI reagent (Ambion), and cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad). For each sample, 1/10th of the cDNA synthesized from 500 ng of RNA was subjected to real-time PCR using iQ SYBR Green Supermix (Bio-Rad) in a StepOnePlus real-time PCR machine according to the manufacturer's instructions. The primers used were IκBα (5′-TCGCTCTTGTTGAAATGTGG-3′; 5′-TCATAGGGCAGCTCATCCTC -3′) and IFNβ (5′-CCGAGCAGAGATCTTCAGGAA-3′; 5′- CCTGCAACCACCACTCATTCT -3′). Each PCR

amplification was normalized to GAPDH (5'-TCACCACCATGGAGAAGGC-3'; 5'-GCTAAGCAGTTGGTGGTGCA-3'), and ΔCT and ΔΔCT values were calculated.

Immunoprecipitations

HEK293 cells transiently transfected with the indicated plasmids were lysed with NETN buffer (150 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl [pH 7.8], 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, and 0.5 μg/ml pepstatin), and insoluble material was cleared by centrifugation. Lysates were incubated with the indicated antibodies for 1-2 hr at 4°C followed by the addition of Sepharose G beads for an additional 1-2 hr at 4°C. After centrifugation, the beads were washed with RIPA buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% NP40, 0.25% Na-deoxycholate, 1 mm phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, and 0.5 μg/ml pepstatin) a minimum of five times and heated at 95 °C for 10 min in Laemmli sample buffer. Following a brief centrifugation, the supernatant was immunoblotted with various antibodies as described. Recombinant Flag-MAVS for use in *in vitro* binding assays was purified as described previously (Mukherjee et al., 2011).