Supplemental Information

Supplemental Experimental Procedures

Plasmid Construction. FLAG-MDA5, GST-MDA5 2CARD, MAVS-CARD-PRD-FLAG, HA-PP1α, HA-PP1β, and HA-PP1γ were previously described (Gack et al., 2007; Wies et al., 2013). pCR3-FLAG-PIV5-V and pCR3-FLAG-MV-V (Schwarz strain) were kindly provided by Karl-Klaus Conzelmann (LMU, Munich) and have been described (Schuhmann et al., 2011). pCAGGS-HA-NiV-V was provided by Chris Basler (Mount Sinai) and has been described (Shaw et al., 2005). FLAG- and V5-tagged PP1γ were subcloned into the pIRES-FLAG and pIRES-V5 vectors, respectively, between *MluI* and *XbaI*. HA-tagged PIV5-V and MV-V genes were subcloned into the pCAGGS plasmid between *EcoRI* and *XhoI*. FLAG-tagged NiV-V was subcloned into pEF-Bos vector containing an N-terminal FLAG tag between *NotI* and *SalI*. GST-MV-V_N (aa 1-231), GST-MV-V_C (aa 232-299), GST-NiV-V_N (aa 1-407), and GST-NiV-V_C (aa 408-456) were constructed by subcloning into the pEBG vector between *BamHI* and *ClaI*. GST-MV-V_C and HA-MV-V AIAA mutants were generated through site-directed mutagenesis. GST-MV-V_C and HA-MV-V Δtail mutants were generated through subcloning residues 232-283 or 1-283 into the pEBG or pCAGGS vector, respectively. All constructs were sequenced to verify 100% agreement with the original sequence.

Cell Culture and Transfection. HEK293T, HeLa, 2fTGH, and immortalized MDA5-deficient MEF cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM L-glutamine, and 1% (w/v) penicillin-streptomycin (Pen-Strep; Gibco-BRL). Immortalized MDA5-deficient MEFs were described previously (Wies et al., 2013). A549-hCD150 and Vero-hCD150 were cultured in Advanced Modified Eagle’s

Medium (MEM) supplemented with 10% (v/v) FBS and 2 mM GlutaMAX (Gibco-BRL). Stable expression of CD150 was maintained in A549-hCD150 and Vero-hCD150 using 500 μg/μL Zeocin (Invitrogen) and 400 μg/μL G418 (Sigma) respectively. Transient transfections were performed with calcium phosphate (Clontech) or Lipofectamine LTX and Plus Reagent (Invitrogen) according to the manufacturer's instructions.

Immature monocyte-derived DCs were cultured as described before (Gringhuis et al., 2009). In short, peripheral blood mononuclear cells, obtained from buffy coats of healthy donors, were isolated by a Lymphoprep (Axis-shield) gradient step and monocytes were subsequently isolated by a Percoll (Amersham biosciences) gradient step. Purified monocytes were differentiated into immature DCs in the presence of 500 U/ml interleukin-4 and 800 U/ml granulocyte-macrophage colony-stimulating factor (Schering-Plough, Brussels, Belgium). DCs were used for experiments at day 6-7.

Viruses. MV^{Ed} and EMCV (strain EMC) were purchased from ATCC. DenV serotype 2 (strain 16681) was kindly provided by Lee Gehrke (Harvard/MIT). SeV (Cantell strain) was purchased from Charles River Laboratories.

Generation of an rMV expressing EGFP and a truncated V protein. rMV^{KS}EGFP(3) was generated by insertion of EGFP as an additional transcription unit (Lemon K, 2011; Ludlow et al., 2013a; Ludlow et al., 2013b). The plasmid template for rMV^{KS}EGFP(3) was further modified to generate rMV^{KS}EGFP(3)VΔtail by exchanging the *AfeI*/*AscI* fragment with one containing two in-frame stop codons in the sequence encoding the unique C-terminus of the V protein. Sequence changes to introduce the stop codons were designed to be silent in the overlapping P

reading frame. The first stop codon terminates the V protein just before the RIWY motif. Plasmid and primer sequences are available on request. Recombinant viruses were recovered from fowlpox-T7-infected Vero-hCD150 cells transfected with the full-length plasmids along with plasmids expressing MV N, P and L. Virus stocks were grown in B-LCL and tested negative for contamination with mycoplasma species. Virus titers were determined by endpoint titration in Vero-hCD150 cells, and expressed in 50% tissue culture infectious dose ($TCID_{50}$/ml).

Reagents. HMV-poly(I:C) complexed with LyoVec was purchased from Invivogen. The following phosphatase inhibitors were used to test MV-V phosphorylation: Calyculin A (25 nM) (Sigma), Cantharidic acid (100 nM, 500 nM, and 1 μM) (Abcam), Endothall (10 μM) (Millipore), and (-)-p-bromotetramisole oxalate (50 μg/mL) (Enzo Life Sciences). Silencing of endogenous MDA5 in A549-hCD150 cells was achieved by transduction of lentiviral particles expressing MDA5-specific shRNAs, or non-targeting control shRNAs (Santa Cruz Biotechnology) following the manufacturer's instructions.

Quantitative Real-time PCR in DCs. RNA isolation from primary human DCs was performed by using the mRNA capture kit (Roche Diagnostic Systems). cDNA was synthesized with a reverse transcriptase kit (Promega). For real-time PCR analysis, PCR amplification was performed in the presence of SYBR green in a 7500 Fast Real-time PCR System (ABI). Transcription of the target gene was adjusted for *GAPDH* transcription with Nt = 2Ct(GAPDH)-Ct(target). Primers for IFN-β, MxA and ISG15 were kindly provided by C.L. Verweij, VUmc, Amsterdam.

Confocal Microscopy. For determining the ability of MV-V WT and mutant proteins to prevent STAT2 nuclear translocation, 2fTGH cells were transfected with Lipofectamine 2000 (Life Technologies) according to the manufacturer's instructions. At 24 h post-transfection, cells were treated with 1000 U/ml IFNα2 (PBL Biomedical Laboratories) for 30 min. Cells were fixed with 1% paraformaldehyde for 15 min and permeabilized in ice-cold 1:1 methanol/acetone for 10 min at -20 °C. Samples were washed with PBS and blocked with 5% bovine serum in PBS for 30 min. For immunostaining of HA-tagged MV-V proteins and endogenous STAT2, mouse anti-HA (Sigma) and rabbit anti-STAT2 (clone C-20, Santa Cruz) antibodies were used, followed by incubation with goat anti-mouse Alexa-fluor-594 and donkey anti-rabbit Alexa-fluor-488 (Life Technologies), respectively. Laser scanning images were taken on an Olympus IX8I confocal microscope.

Supplemental References

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