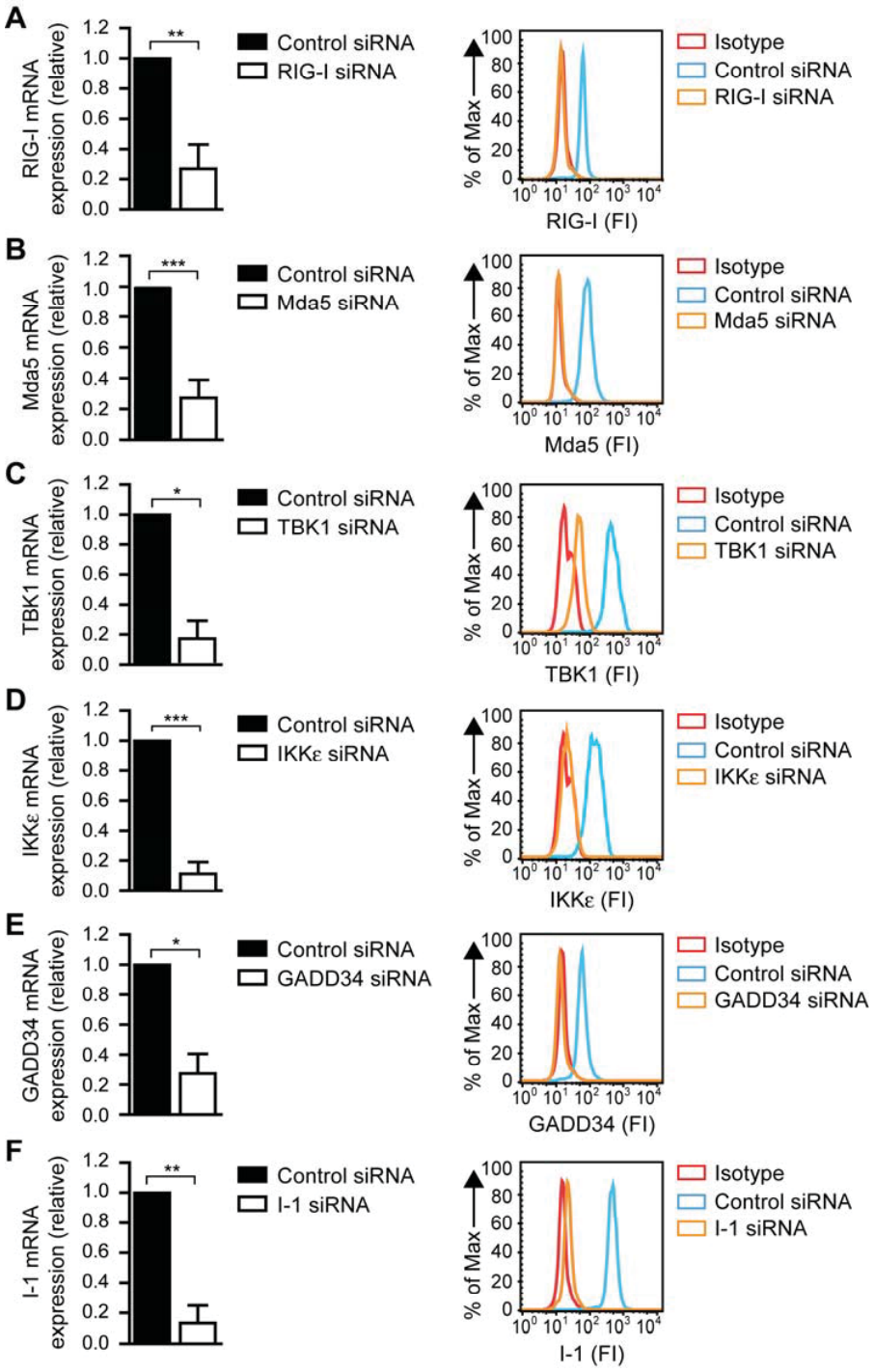

Figure S1, related to Figures 2, 6 and 7. Silencing of RIG-I, Mda5, TBK1, IKKε, GADD34, and I-1 in human primary DCs by RNA interference

(**A-F**) Silencing of indicated proteins using specific SMARTpools and non-targeting siRNA as a control. Silencing was confirmed by real-time PCR (left panels) or flow cytometry (right panels; FI, fluorescence intensity). During real-time PCR analysis, mRNA expression was normalized to GAPDH and set at 1 in control-silenced cells. Antibodies used for staining are anti-RIG-I (3743; Cell Signaling) (**A**), anti-Mda5 (4109; Cell Signaling) (**B**), anti-TBK1 (3504; Cell Signaling) (**C**), anti-IKKε (2905; Cell Signaling) (**D**), anti-GADD34 (ab131402; Abcam)

(**E**), and anti-I-1 (ab40877; Abcam) (**F**). Data are presented as mean ± SD (real-time PCR). *P < 0.05, **P < 0.01, ***, P < 0.001. Data are representative of at least three independent experiments.

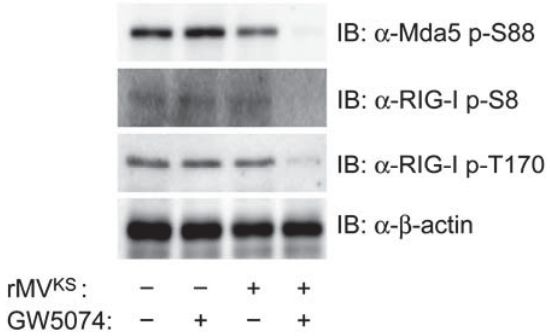

Figure S2, related to Figure 4. MV inhibits dephosphorylation of RIG-I and Mda5 via Raf-1

Mda5 phosphorylation at Ser88, and RIG-I phosphorylation at Ser8 or Thr170 in whole cell lysates of DCs 8 h after infection with rMV^{KS}EGFP(3), in the absence or presence of Raf inhibitor GW5074, determined by immunoblotting. β-actin (lower panel) served as a loading control.

Data are representative of two independent experiments.

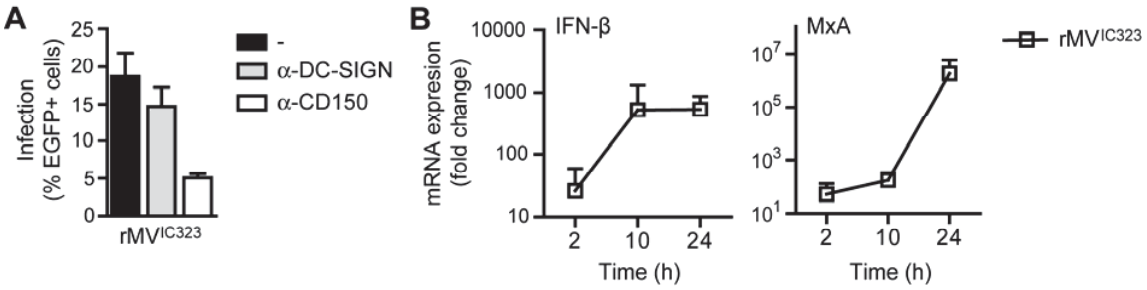

Figure S3, related to Figure 3. Infection and type I IFN responses in DCs in response to rMV^{IC323}EGFP(1)

(**A**) Infection of DCs 24 h after infection with rMV^{IC323}EGFP(1) in the absence (*black bar*) or presence of blocking DC-SIGN (*grey bar*) or CD150 (*white bar*) antibodies, determined by flow cytometry by measuring % of EGFP$^+$ cells. Data are presented as mean ± SD.

(**B**) IFN-β and MxA mRNA expression by DCs 2, 10, and 24 h after infection with rMV^{IC323}, measured by real-time PCR and normalized to GAPDH. Data are presented as mean ± SD.

Data are representative of at least three (**A**) or four (**B**) independent donors.

Gene product	Forward primer	Reverse primer
MV-N [1]	GACATTGACACTGCATC	GATTCCTGCCATGGCTTGCAGCC
IFN-β (Homo sapiens)	ACAGACTTACAGGTTACCTCCGAAAC	CATCTGCTGGTTGAAGAATGCTT
IFN-β (Macaca fascicularis)	ACAAACTTACAGGTTACCTCCGAAAC	CATCTGCCGGTTGAAGAATGCTT
MxA	TTCAGCACCTGATGGCCTATC	GTACGTCTGGAGCATGAAGAACTG
ISG15	TTTGCCAGTACAGGAGCTTGTG	GGGTGATCTGCGCCTTCA
RIG-I	CCAAGCCAAAGCAGTTTTCAAG	CATGGATTCCCCAGTCATGG
Mda5	TGAGAGCCCTGTGGACAACC	CGCTGCCCACTTAGAGAAGC
TBK1	TTACAGGAAAGCCTTCTGGTGC	TCCACTCCAGTCAATTGGTCC
IKKε	TTGGAGTGACCTTGTACCATGC	CATGATCTCCTTGTTCCGCC
Raf-1	GGTGATAGTGGAGTCCCAGCA	TCAGATGAGGGACTGGAGGTG
GADD34	GATGATGGCATGTATGGTGAGC	CCATCTGCAAATTGACTTCCC
I-1	CTGAAGCATGTGGTACAGAGGC	GGCTCATAGTAGCTGCATGGC
GAPDH	CCATGTTCGTCATGGGTGTG	GGTGCTAAGCAGTTGGTGGTG

Table S1, related to Experimental Procedures. Primer sequences

[1] Druelle, J., Sellin, C.I., Waku-Kouomou, D., Horvat, B., and Wild, F.T. (2008). Wild type measles virus attenuation independent of type I IFN. *Virol. J.* *5*, 22.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cells, stimuli, inhibitors and RNA interference. Peripheral blood mononuclear cells were isolated from buffy coats of healthy donors (Sanquin) by a Lymphoprep (Axis-shield) gradient step, and monocytes were subsequently isolated by a Percoll (Amersham biosciences) gradient step. Immature monocyte-derived DCs were cultured for 6–7 days from monocytes obtained from buffy coats of healthy donors (Sanquin) in the presence of IL-4 and GM-CSF (500 and 800 U/ml, respectively; Biosource/Invitrogen). DCs were cultured in RPMI supplemented with 10% FCS, pen/strep (10 U/ml and 10 µg/ml, respectively; Invitrogen) and 2 mM L-glutamine (Lonza).

Cells were stimulated with poly(I:C)-LyoVec (LMW) (1 µg/ml; Invivogen), while DC-SIGN crosslinking was performed by coating 10 µg/ml goat-anti-mouse (Jackson), followed by 20 µg/ml anti-DC-SIGN (AZN-D1; Geijtenbeek et al., 2000) or IgG1 isotype control, before addition of cells. Cells were preincubated with inhibitors for 2 h or blocking antibodies for 30 min with Raf inhibitor GW5074 (Lackey et al., 2000) (1 µM; Sigma), guanabenz acetate salt (Tsaytler et al., 2011) (5-50 nM; Sigma), anti-DC-SIGN (20 µg/ml; AZN-D1), or anti-CD150 (20 µg/ml; MCA2251XZ; SBD Serotec). Cells were cocultured with Z-D-Phe-Phe-Gly-OH fusion inhibitory peptide (FIP) (0.2 mM; Bachem) or recombinant neutralizing soluble IFNAR (Vaccinia Virus-Encoded Neutralizing Type I Interferon Receptor B18R; eBioscience).

DCs were transfected with 25 nM siRNA using transfection reagens DF4 (Dharmacon) as described (Gringhuis et al., 2009). SMARTpool siRNAs used were: RIG-I (M-012511-01), Mda5 (M-013041-00), TBK1 (M-003788-02), IKKε (M-003723-02), Raf-1 (M-003601-02), GADD34 (M-004442-01), I-1 (M-017092-01), and non-targeting siRNA (D-001206-13) as a control (Dharmacon).

RIG-I, Mda5, TBK1, IKKε and Raf-1 phosphorylation. For flow cytometry analysis, cells were first fixed in 3% *para*-formaldehyde for 10 min and permeabilized in 90% methanol at 4°C for 30 min. Primary antibody incubation with phospho-RIG-I(S8) (PAB15905; Abnova), phospho-RIG-I(T170) (PAB15906; Abnova), phospho-Mda5(S88) (generated by M.E.D. and M.U.G (Wies et al., 2013)), phospho-c-raf(Ser338) (9427S; Cell Signaling), c-raf(pTyr340/341) (553009; Calbiochem), phospho-TBK1/NAK(Ser172) (5483S; Cell Signaling) and phospho-IKK-epsilon(Ser172) (06-1340; Millipore) was followed by incubation with PE-conjugated anti-rabbit (711-116-152; Jackson Immunoresearch). Phosphorylation was analyzed on a FACS Calibur (BD).

For detection by immunoblotting, whole cell extracts were prepared using RIPA lysis buffer (Cell Signaling), proteins resolved by SDS-PAGE and detected by immunoblotting with phospho-RIG-I(S8), phospho-RIG-I(T170) and phospho-Mda5(S88). Membranes were also probed with anti-RNAPII (clone CTD4H8; Millipore) or anti-β-actin (sc-81178; Santa Cruz) to

ensure equal protein loading. Primary antibody incubation was followed by incubation with HRP-conjugated secondary antibody (rabbit: 21230; Pierce, or mouse: P0161, DAKO) and ECL detection (Pierce).

Cellular localization of IRF3. Nuclear and cytoplasmic extracts were resolved by SDS-PAGE, and detected by immunoblotting with anti-IRF3 (sc-9082; Santa Cruz). Membranes were also probed with anti-RNAPII (clone CTD4H8; Millipore) or anti-β-actin (sc-81178; Santa Cruz) to ensure equal protein loading among cytoplasmic and nuclear extracts, respectively. Detection was done as described above.

I-1 phosphorylation and association with PP1. I-1 immunocomplexes were resolved by SDS-PAGE and phosphorylation of I-1 or I-1-associated proteins were detected by immunoblotting with anti-phosphoserine (ab9332; Abcam), anti-phosphothreonine (9381; Cell Signaling), anti-PP1alpha (2582; Cell Signaling) or anti-PP1Cgamma (ab169976; Abcam). Detection was done as described above.

PP1 phosphatase activity. PP1 activity in lysates was measured using ProFluor Ser/Thr PPase assay (Promega), in the presence of 4 μM okadaic acid to block PP2 activity. GADD34-PP1 specific activity in lysates was measured after capturing GADD34 in anti-GADD34 (ab131402; Abcam)-coated black-walled high-binding 96-wells plates. The detected R110 fluorescence is a measure for PP1 activity.

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