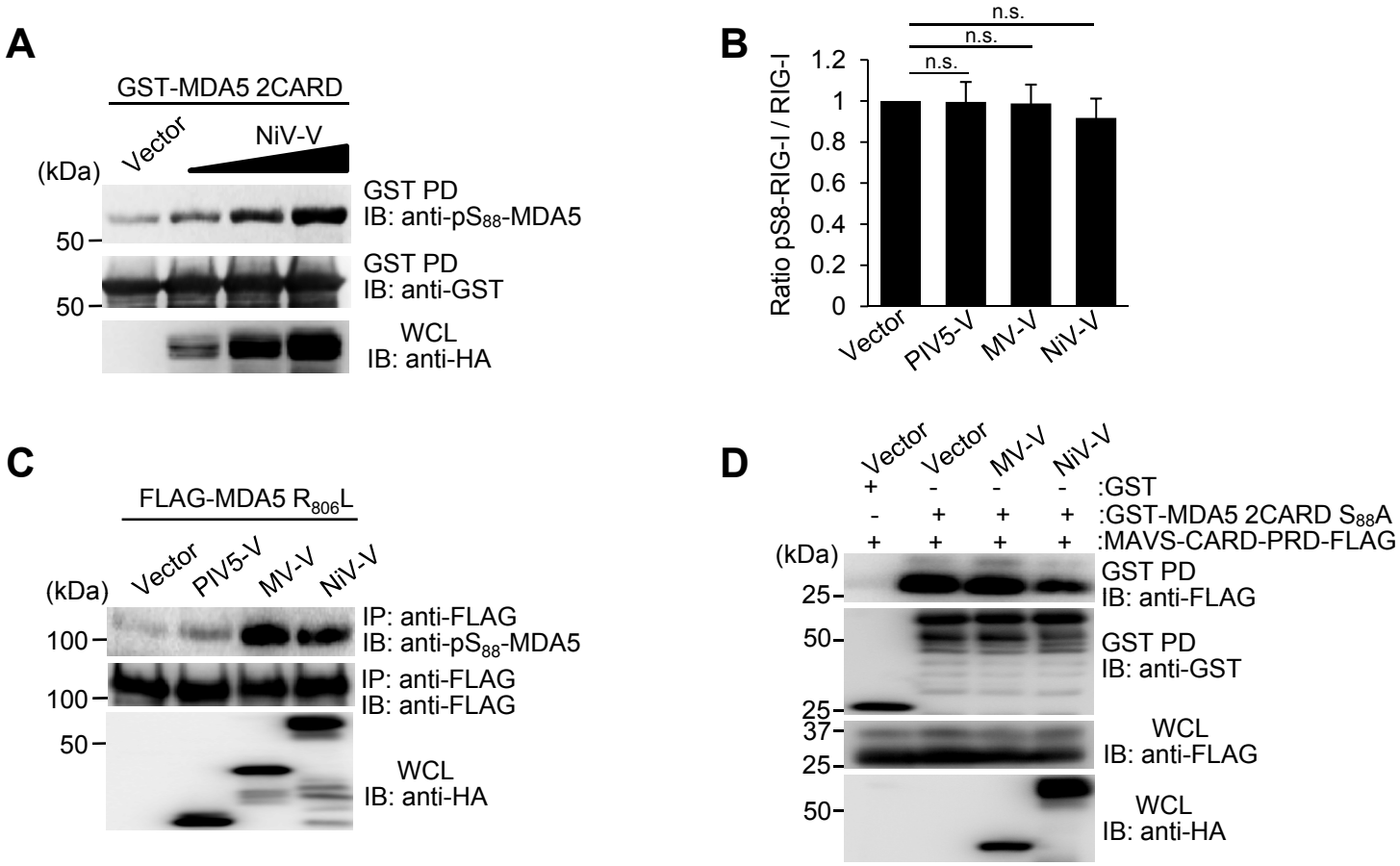

Figure S1 related to Figure 1. (A) NiV-V inhibits MDA5 dephosphorylation in a dose-dependent manner. HEK293T cells were transfected with GST-MDA5 2CARD and increasing amounts of HA-tagged NiV-V. WCLs were subjected to GST pull-down (GST PD), followed by IB with anti-pS_{88}-MDA5 or anti-GST antibody. **(B) Densitrometric quantification of RIG-I S8 phosphorylation upon V protein expression.** The intensities of the bands from the Western blots shown in Figure 1F (first and second panels) were quantified by densitometric analysis. The extent of RIG-I S8 phosphorylation was calculated as the ratio of the amount of phosphorylated FLAG-RIG-I (first panel) to the amount of total FLAG-RIG-I (second panel). Values were normalized to control (FLAG-RIG-I cotransfected with vector). Data are the means +/- s.d. of three independent experiments. n.s., statistically not significant**. (C) MV-V and NiV-V robustly enhance the phosphorylation of MDA5 $R_{806}L$.** HEK293T cells were transfected with FLAG-MDA5 $R_{806}L$ together with empty vector or the indicated HA-tagged paramyxovirus V proteins. WCLs were subjected to FLAG-IP, followed by IB with anti-pS_{88}-MDA5 or anti-FLAG antibody**. (D) MV-V and NiV-V do not inhibit MAVS binding of a MDA5 $S_{88}A$ mutant.** HEK293T cells were transfected with MAVS-CARD-PRD-FLAG and GST or GST-MDA5 2CARD $S_{88}A$ together with vector, MV-V, or NiV-V. WCLs were subjected to GST PD, followed by IB with anti-FLAG or anti-GST antibody.

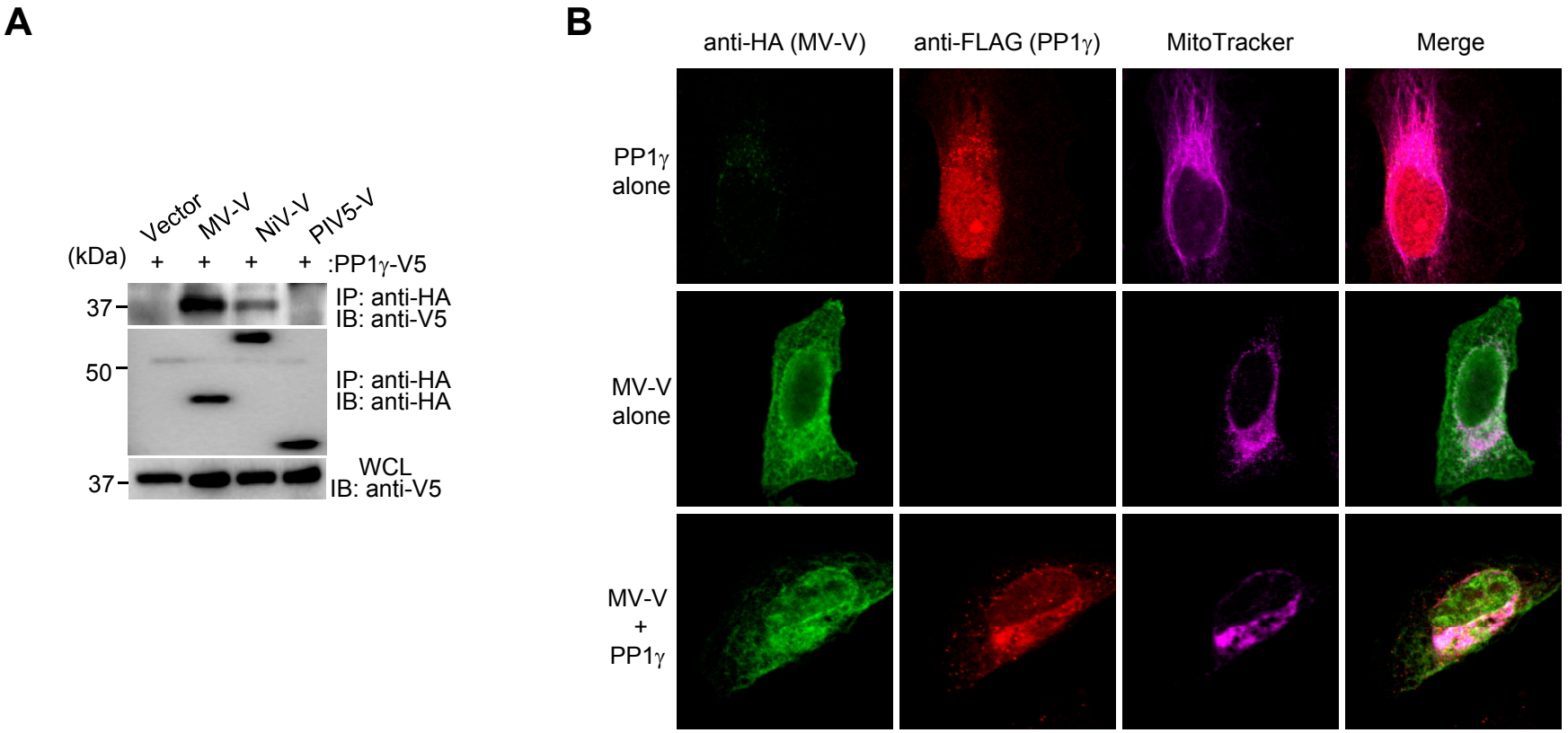

Figure S2 related to Figure 2. PP1 binding of the paramyxovirus V proteins. (A) The V proteins of measles and Nipah virus, but not that of PIV5, bind to PP1γ. HEK293T cells were transfected with V5-tagged PP1γ together with vector, HA-tagged MV-V, NiV-V, or PIV5-V protein. PP1γ binding was assessed by IP with anti-HA, followed by IB with anti-V5 antibody. **(B)** The measles V protein and PP1 co-localize at mitochondria-associated membranes (MAM). Confocal scanning laser images of HA-MV-V (green) and FLAG-PP1γ (red) in transfected HeLa cells (as shown in Figure 2C). MAM were stained with MitoTracker (purple).

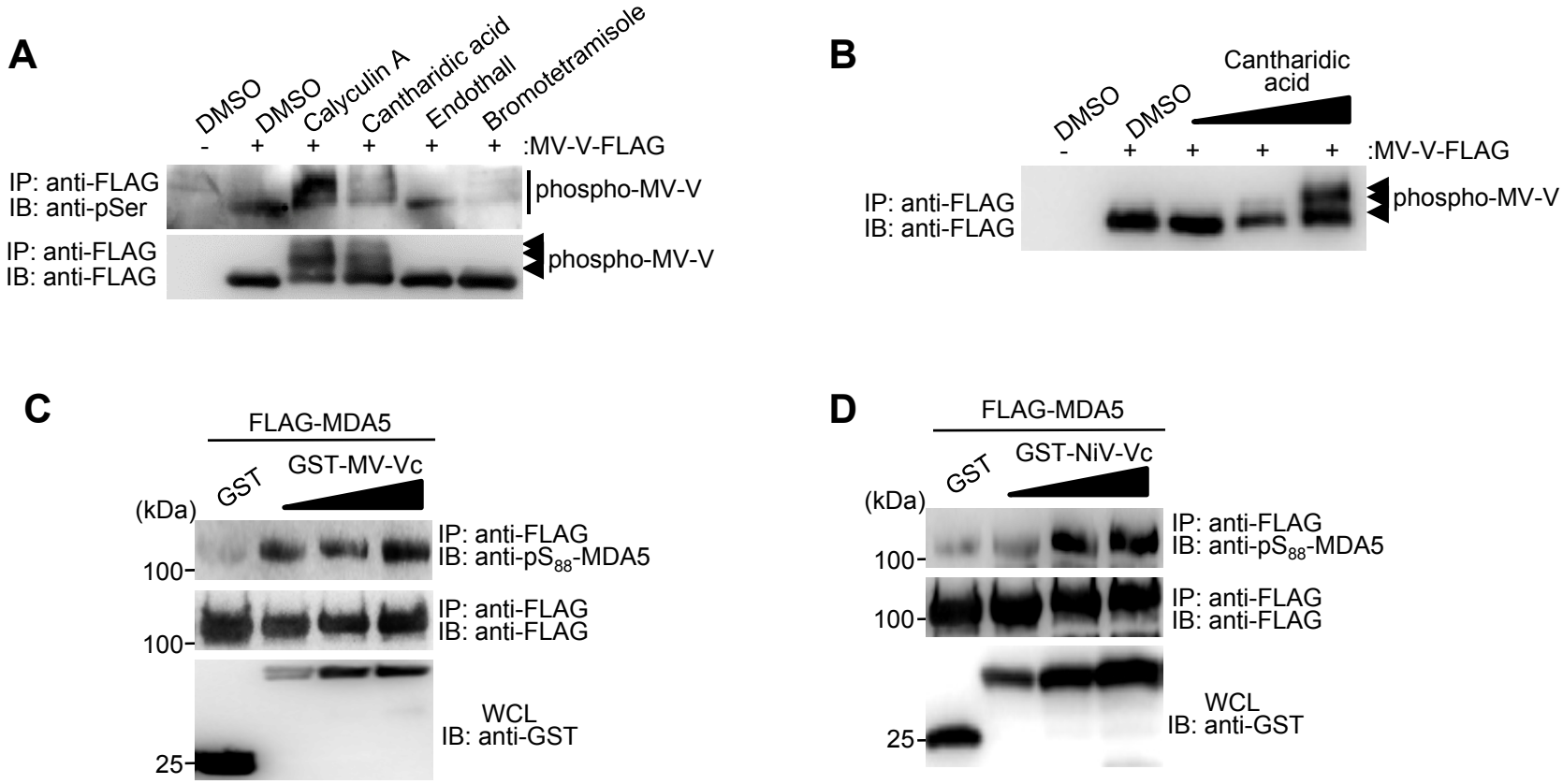

Figure S3 related to Figures 3 and 4. (A and B) Phosphorylation of the MV-V protein is enhanced by chemical inhibition of PP1. (A) Phosphorylation of MV-V-FLAG in transfected HEK293T cells treated with the indicated inhibitors (or DMSO control) for 4 h, determined by IB using anti-pan-phospho-Ser antibody (anti-pSer). **(B)** IB analysis of MV-V-FLAG phosphorylation upon treatment with 100 nM, 500 nM, or 1 μM of Cantharidic acid for 4 h in transfected HEK293T cells. Arrow heads indicate phosphorylated forms of MV-V. **(C and D) The C-terminal domain of the V protein (V_C) of measles and Nipah virus is sufficient for preventing MDA5 S88 dephosphorylation.** HEK293T cells were transfected with FLAG-MDA5 together with GST or 4μg, 8μg, or 12 μg of GST-fused MV-V_C **(C)** or NiV-V_C **(D).** WCLs were subjected to IP with anti-FLAG antibody, followed by IB with anti-pS_{88}-MDA5 or anti-FLAG antibody. Expression of GST and GST-fused V_C were determined by IB with anti-GST antibody.

Figure S4 related to Figure 4. The PP1-binding motif RIWY is conserved among different measles virus strains. Vc protein sequence alignment of different MV strains. Alignment was performed using ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/). Asterisks (*) indicate positions which have a single, fully conserved residue. Colons (:) indicate conservation between groups of strongly similar properties, and periods (.) indicate conservation between groups of weakly similar properties. The PP1-binding motif is indicated in red. Numbers indicate amino acids.

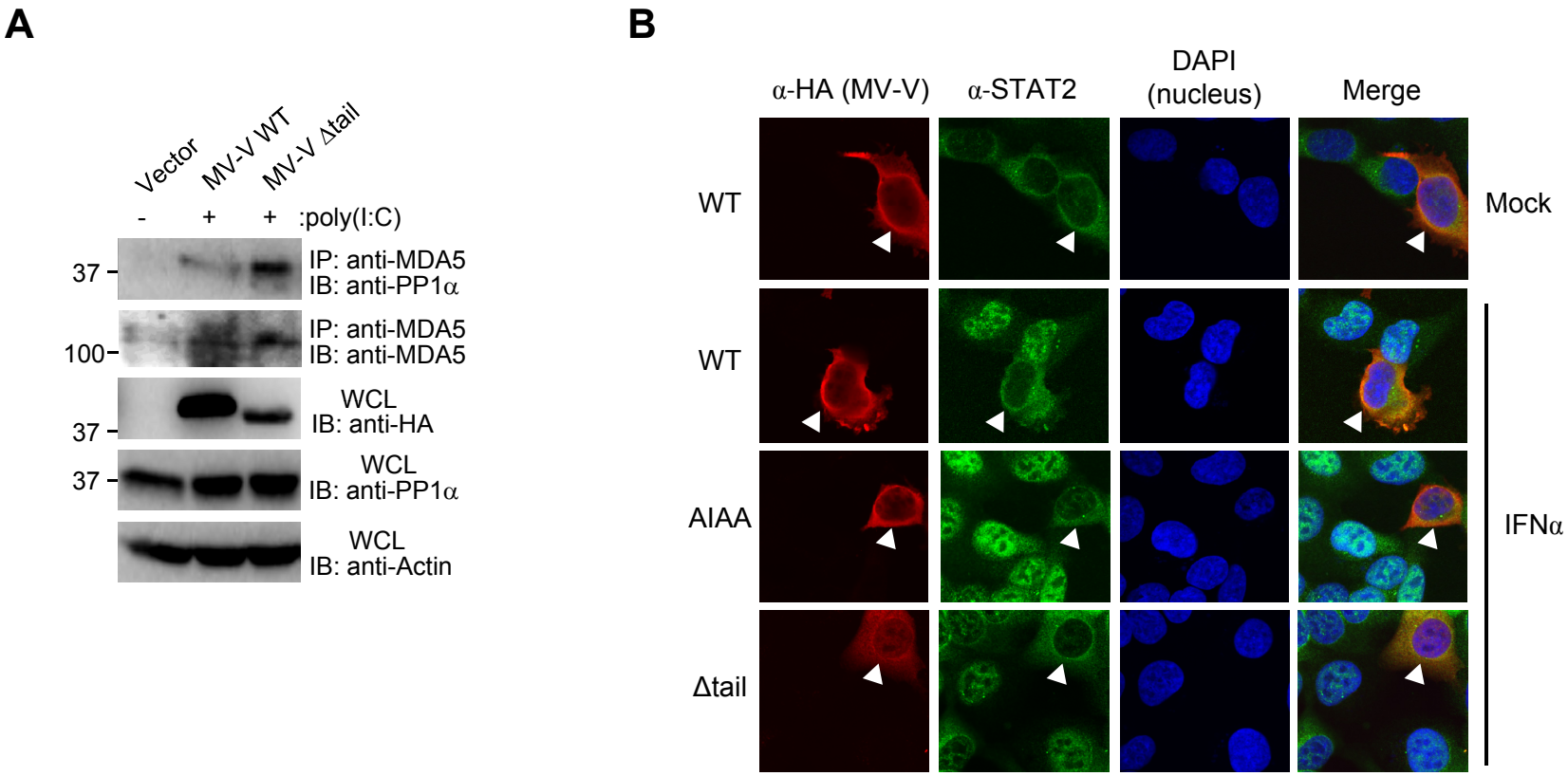

Figure S5 related to Figure 4. (A) Effect of the MV-V WT and Δtail proteins on endogenous MDA5-PP1 binding stimulated by poly(I:C). Endogenous MDA5-PP1 binding in Mock-treated or poly(I:C)-stimulated HEK293T cells, transfected with vector or HA-tagged MV-V WT or Δtail, assessed by IP with anti-MDA5, followed by IB with anti-PP1α. **(B) Mutation or deletion of the PP1-binding motif in the measles V protein does not affect its ability to inhibit the nuclear translocation of STAT2.** Confocal images of 2fTGH cells, that had been transfected with either HA-tagged MV-V WT, MV-V AIAA, or MV-V Δtail, were treated with IFNα for 30 min, or left untreated. Cells were then stained for endogenous STAT2 (green) or HA (MV-V, red). Nuclei were stained with DAPI (blue). Arrow heads indicate cells that show expression of MV-V.

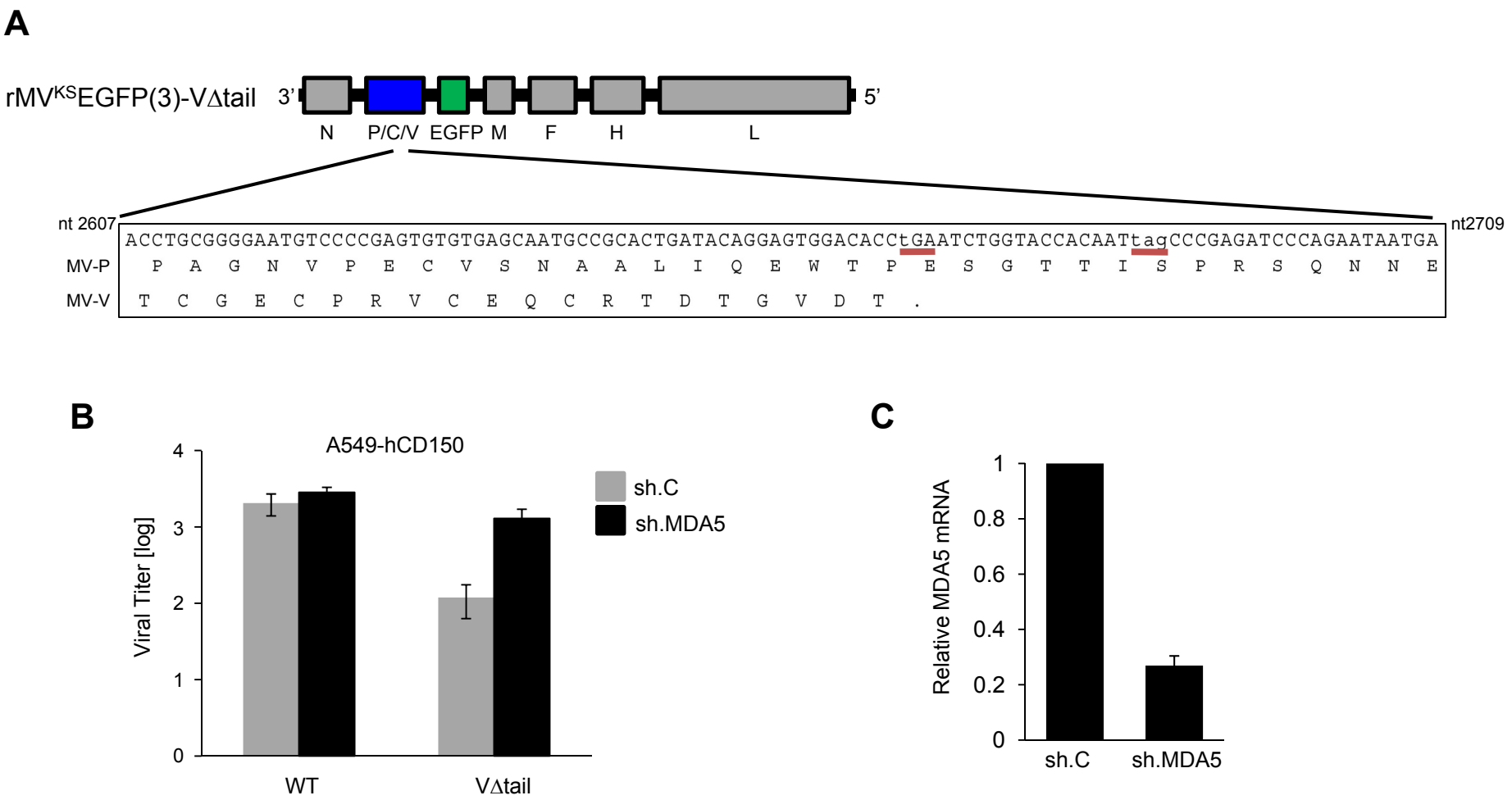

Figure S6 related to Figure 5. (A) Cloning strategy for the generation of the rMV^{KS}EGFP(3)-VΔtail virus. Schematic of rMV^{KS}EGFP(3)VΔtail (lower inset) nucleotide sequence of mutated region with P and V protein translations showing uninterrupted P sequence and in-frame V stop codons (underlined in red). Mutated nucleotides are shown as lowercase letters. **(B and C) The growth defect of the VΔtail virus in A549-hCD150 cells is dependent on its inability to antagonize MDA5. (B)** Viral titers of rMV^{KS}EGFP(3) WT and VΔtail were determined 48 h.p.i. in A549-hCD150 cells, that had been transduced with lentiviral particles expressing MDA5-specific shRNA (sh.MDA5) or non-targeting control shRNA (sh.C). Data are expressed as mean $TCID_{50}$ +/- s.d. (n=2) and are representative of 2 independent experiments. **(C)** Confirmation of MDA5 knockdown from the experiment shown in (B) as determined by qRT-PCR normalized to *gapdh* mRNA levels. The results are expressed as means +/- s.d. (n=2).