

Expanded View Figures

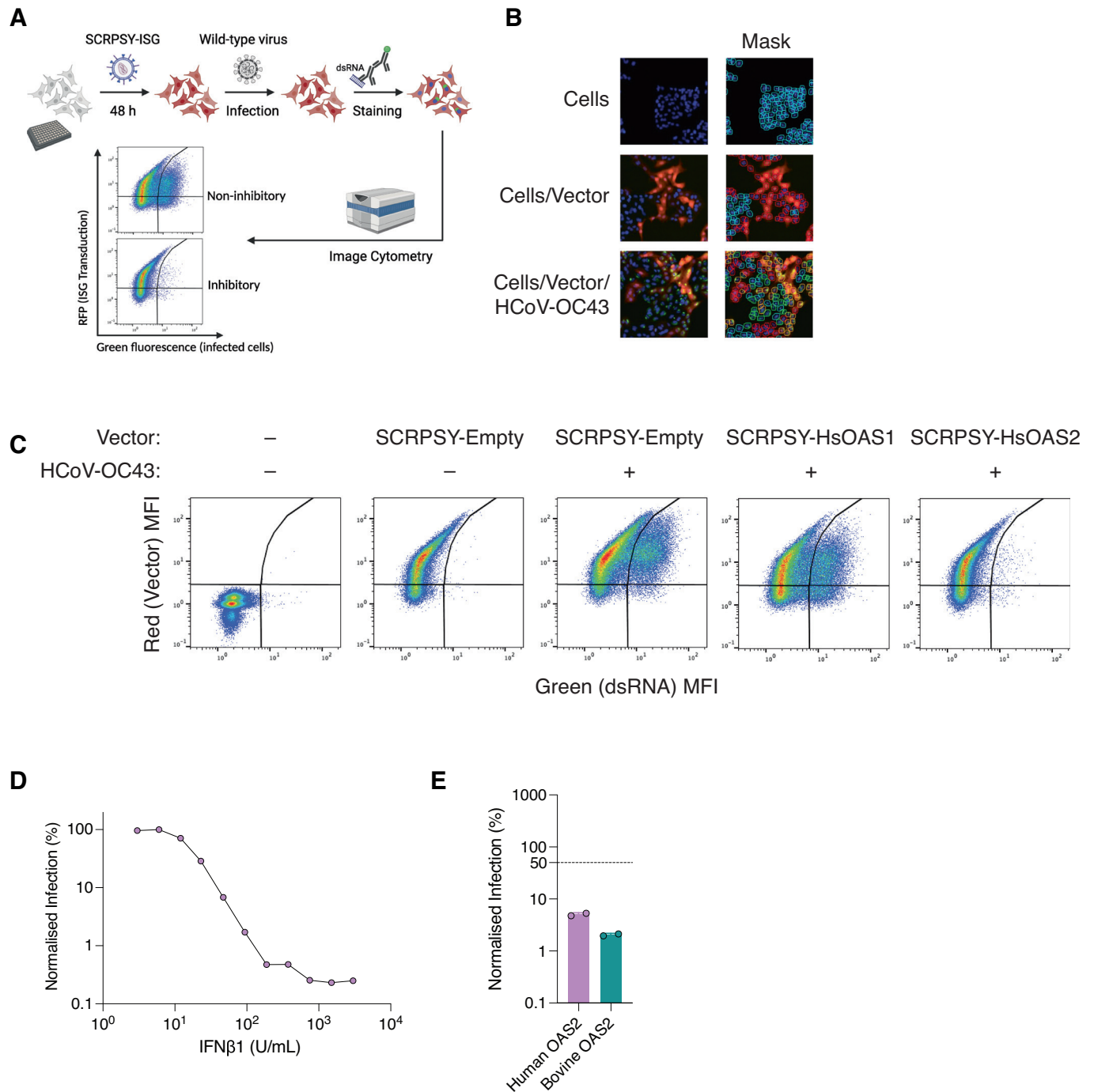


Figure EV1. Quantifying RNA virus infection by immunostaining.

(A) Schematic diagram of the screening method used for the arrayed ISG expression screens described in (Fig. 1D–G). (B) Representative images of control wells in the ISG screen (Fig. 1D) were gated for RFP and dsRNA expression levels using an image cytometer. (C) Cell populations from (Fig. 1D) were gated using cell-only and SCRPSY-Empty mock-infected controls. Representative FlowJo analysis plots are shown, including OAS1 and OAS2 as examples of a non-candidate and candidate ISG, respectively. MFI, mean fluorescence intensity. (D) A549 cells were pre-treated with IFN β 1 for 24 h, infected with HCoV-OC43 for 72 h, and stained for dsRNA prior to quantifying dsRNA+ cells by image cytometry. (E) A549 expressing Human or Bovine OAS2, from the ISG libraries in (Fig. 1D), were infected with HCoV-OC43 for 72 h and stained for dsRNA prior to quantifying dsRNA+ cells by image cytometry. Data normalised to SCRPSY-EMPTY controls.

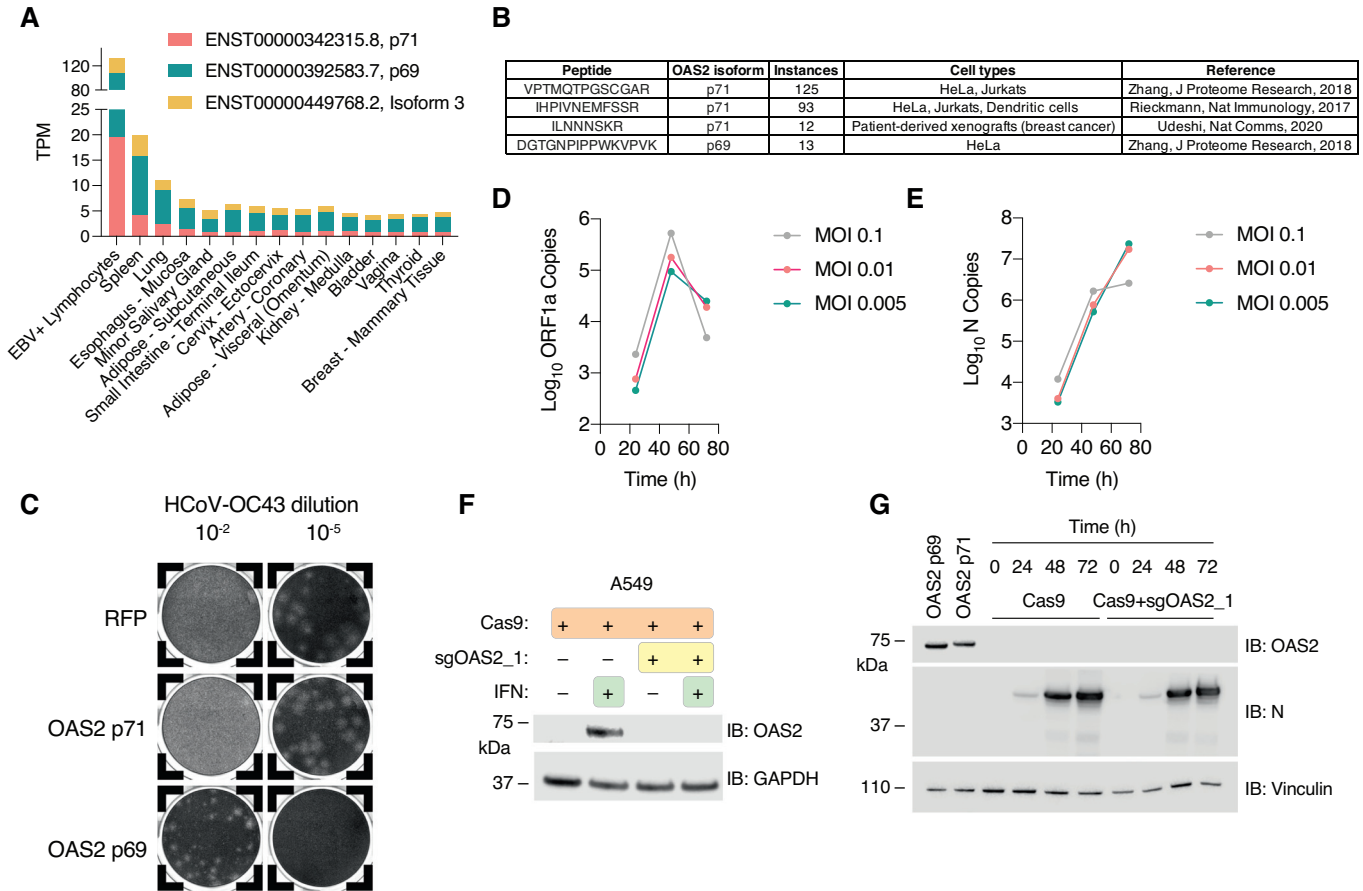


Figure EV2. Presence of endogenous OAS2 isoforms.

(A) Transcript expression of OAS2 isoforms across tissues analysed using the GTEx database. (B) The human peptide atlas (<https://peptideatlas.org/>) was searched for peptides from the OAS2 p71 and p69 C-terminus, generated by trypsin cleavage. (C) Representative images of HCoV-OC43 plaques formed in cell lines characterised in (Fig. 2C), 120 hpi. (D) HCoV-OC43 ORF1a transcript levels in A549 cells infected with HCoV-OC43 were quantified at multiple timepoints, by RT-qPCR. (E) HCoV-OC43 nucleocapsid transcript levels in A549 cells infected with HCoV-OC43 were quantified at multiple timepoints, by RT-qPCR. (F) Endogenous OAS2 levels in A549 cells transduced with a lentiviral vector-derived OAS2 sgRNA, with or without pre-treatment of 100 U/mL IFN β for 24 h. (G) Expression of endogenous OAS2 was monitored in A549 cells from (F), infected with HCoV-OC43 (MOI 0.01), at multiple timepoints, by Western blotting.

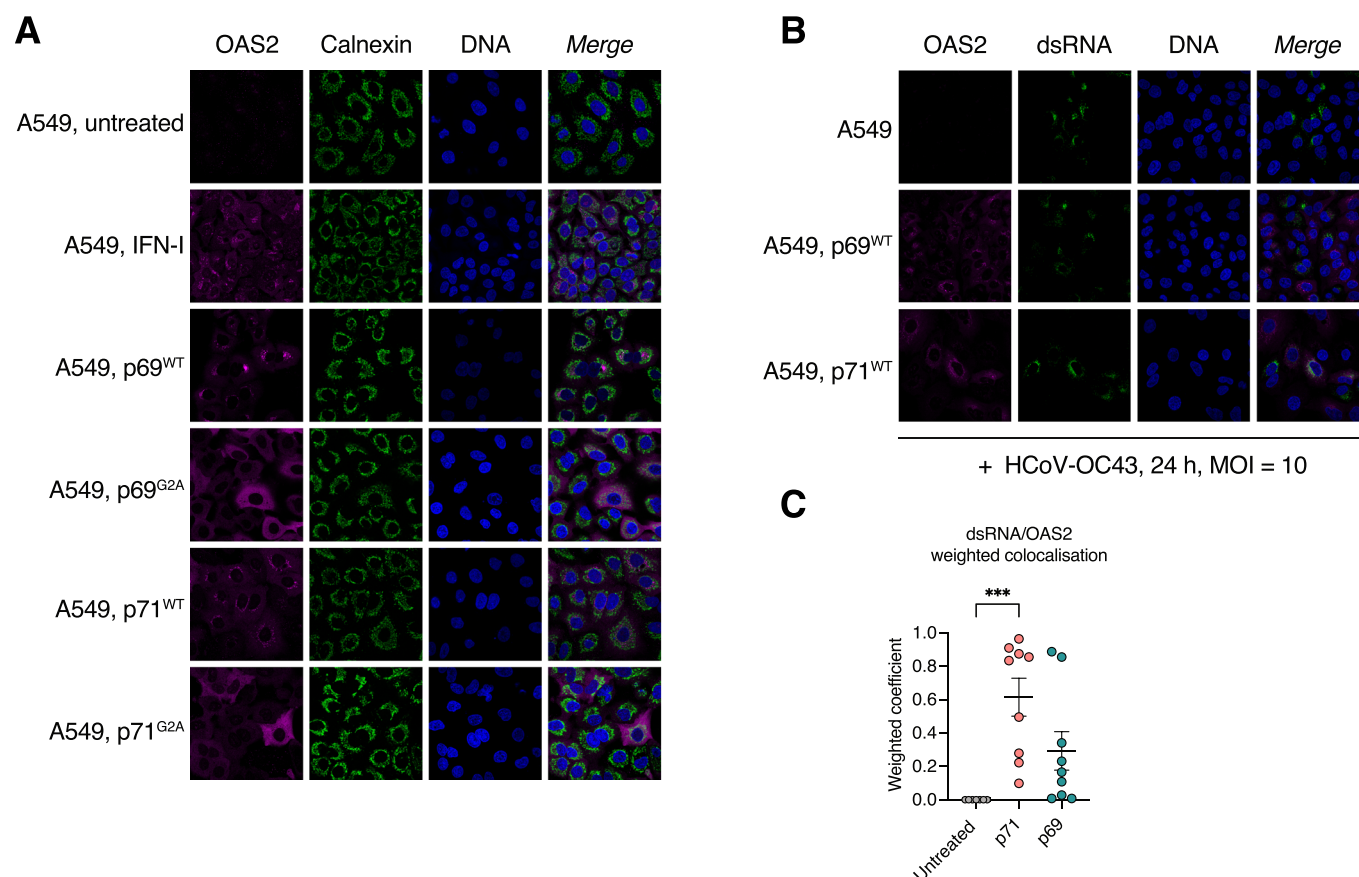


Figure EV3. N-terminal myristoylation activity is required for antiviral activity.

(A) A549 cells stimulated with 1000 U/mL IFN β or A549 cells expressing p71, p69, p71^{G2A} or p69^{G2A}, were immunostained for OAS2 (magenta), calnexin (green) and DNA (blue). (B) Representative immunofluorescence images of A549 cells expressing p71 or p69 infected with HCoV-OC43 (MOI = 10) for 24 h and immunostained for OAS2 (magenta), dsRNA (green) and DNA (blue). (C) Weighted coefficients between p71 and p69 isoforms and dsRNA. Each data point represents a separate region of interest from a representative experiment, $n = 2$. Data were presented as the mean $\pm$ SEM and analysed by one-way ANOVA with Dunnett's multiple comparison test, *** $p = 0.0008$.

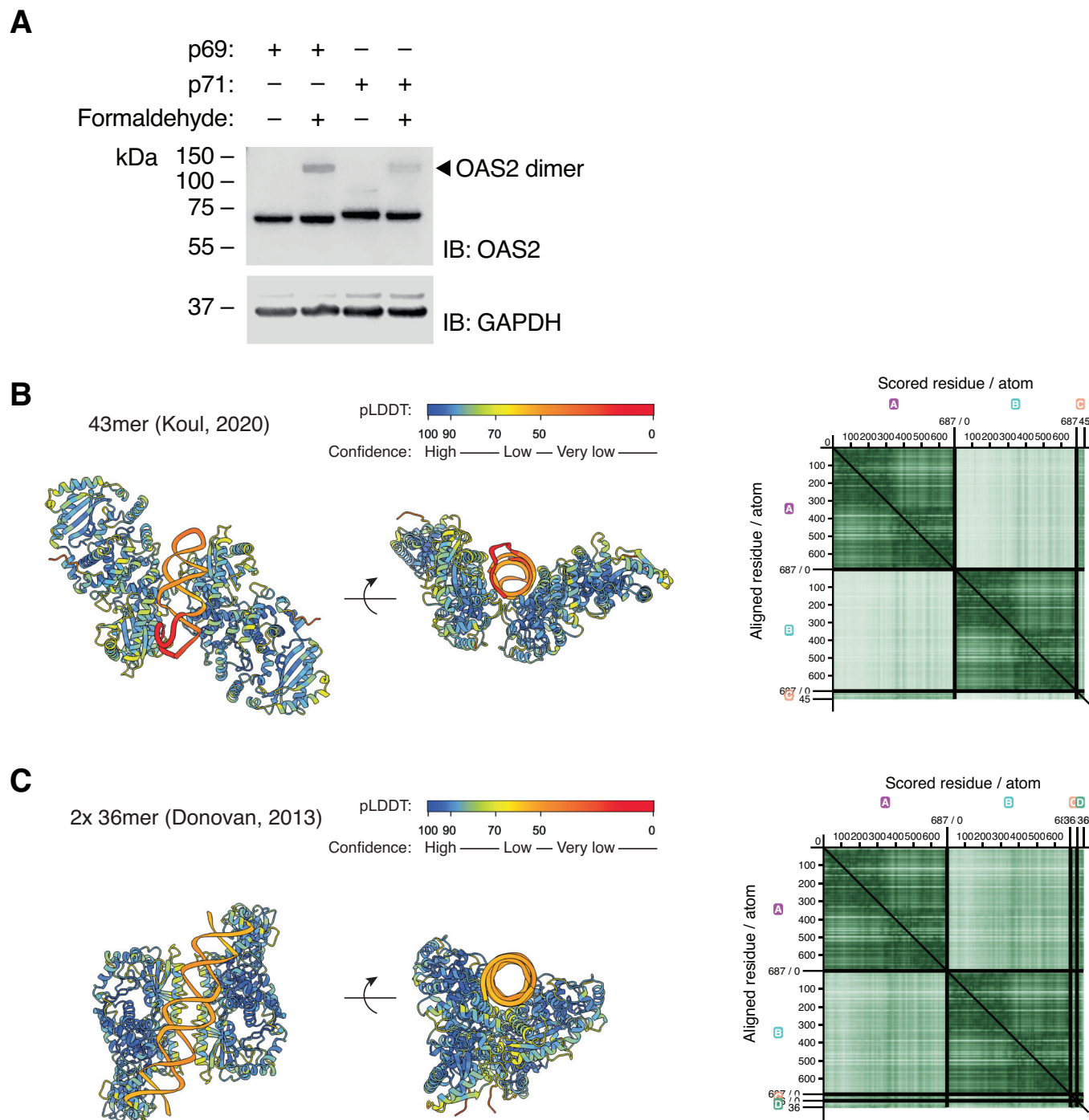


Figure EV4. AlphaFold 3 models of OAS2:dsRNA.

(A) A549 cells expressing OAS2 p71 or p69, treated with or without 0.5% formaldehyde. OAS2 expression assessed by Western blotting. (B, C) Top-ranking AlphaFold3 predictions of human OAS2 p69 in complex with dsRNA 43mer palindromic RNA (Koul et al, 2020a) (B) or 2x36mer RNA (Donovan et al, 2013) (C), coloured according to pLDDT scores as indicated by key. Predicted Aligned Error (PAE) plots for the top-ranking structures are shown, which indicate high confidence in the conformation of individual OAS2 structures, and lower confidence in the relative position of one OAS2 chain to the other.

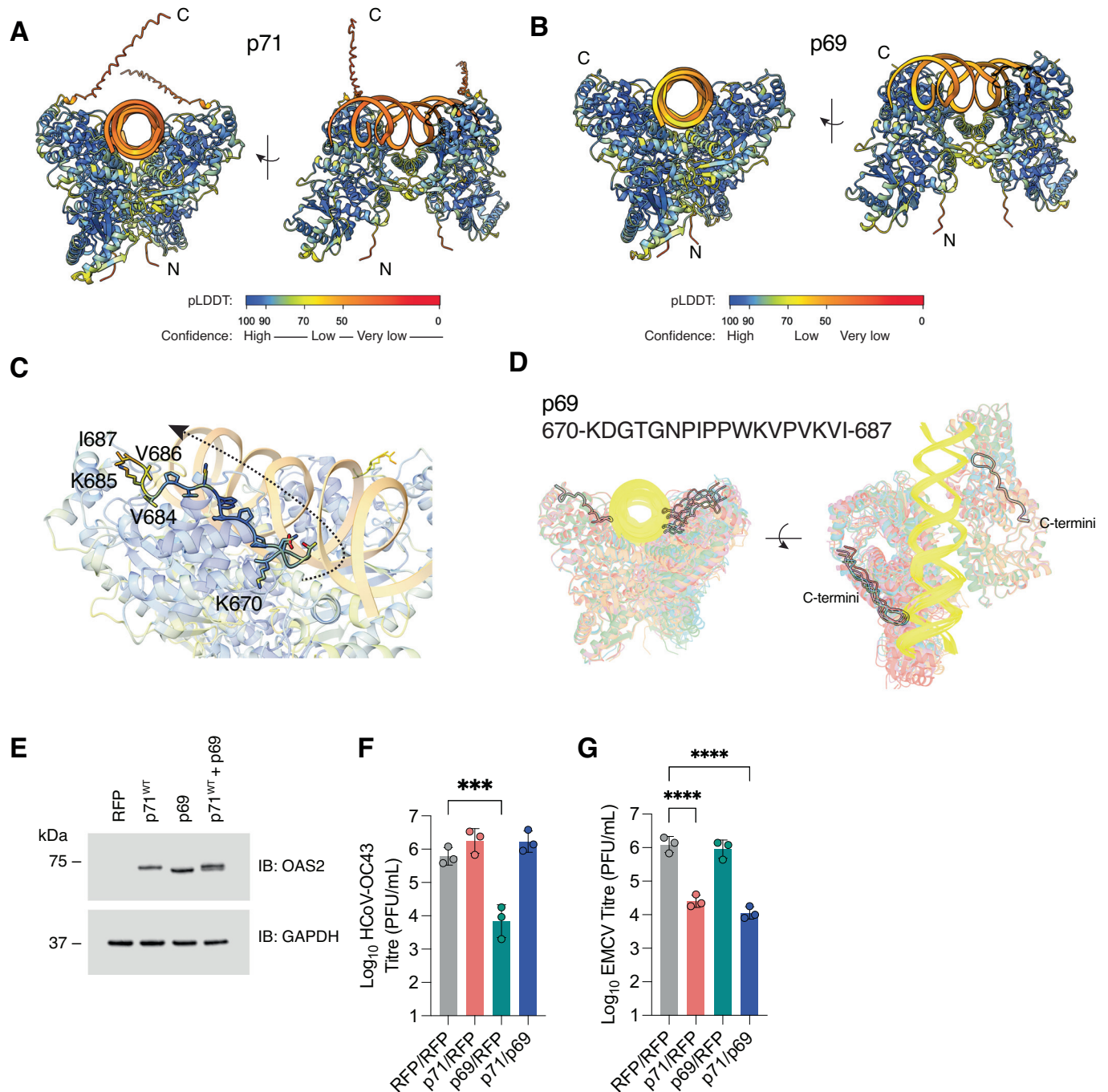


Figure EV5. OAS2 C-terminal tails shape antiviral activity.

(A, B) AlphaFold3 top-ranked models from (Fig. EV4) of p71 (A) or p69 (B) with dsRNA (Donovan et al, 2013) coloured by pLDDT score, indicated in colour key. OAS2 N- and C-termini are labelled N and C, respectively. (C) Close up of OAS2 p69 from structure presented in (B), showing side chains of peptide beginning at K670, until terminal residue I687, coloured as in (B). The dashed arrow indicates the sequence direction from 670-687. pLDDT scores are high between residues 670-683, and low between residues 684-687. (D) Superposition of all 5 OAS2 p69 AlphaFold3 structure predictions in complex with dsRNA (Donovan et al, 2013). The highlighted tail peptides begin at residues K670, as indicated; note that sequence K670-P683 is shared between p69 and p71 isoforms, thus the region highlighted for p69 is also partly present in p71. (E) A549 were modified to co-express both p71^{WT} and p69 isoforms. Single isoforms were co-expressed with RFP to control for transgene dosage. OAS2 expression was confirmed by Western blotting. (F) Infectious titre of HCoV-OC43 in cells characterised in (E), determined by plaque assay at 120 hpi. (G) Infectious titre of EMCV in cells characterised in (E), determined by plaque assay at 30 hpi. Data information: Data were presented as the mean $\pm$ SD. (F, G) Data were analysed by one-way ANOVA with Dunnett's multiple comparison test (vs. RFP), where * p < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001. (F) *** p = 0.0005. (G) **** p < 0.0001.