

Cell Reports, Volume 34

Supplemental information

***Sarbecovirus* ORF6 proteins hamper
induction of interferon signaling**

Izumi Kimura, Yoriyuki Konno, Keiya Uriu, Kristina Hopfensperger, Daniel Sauter, So Nakagawa, and Kei Sato

Supplementary Information

***Sarbecovirus* ORF6 proteins hamper the induction of interferon signaling**

Izumi Kimura, Yoriyuki Konno, Keiya Uriu, Kristina Hopfensperger, Daniel Sauter,
So Nakagawa, Kei Sato

Supplementary Figures S1-S3

Supplementary Tables S1-S5

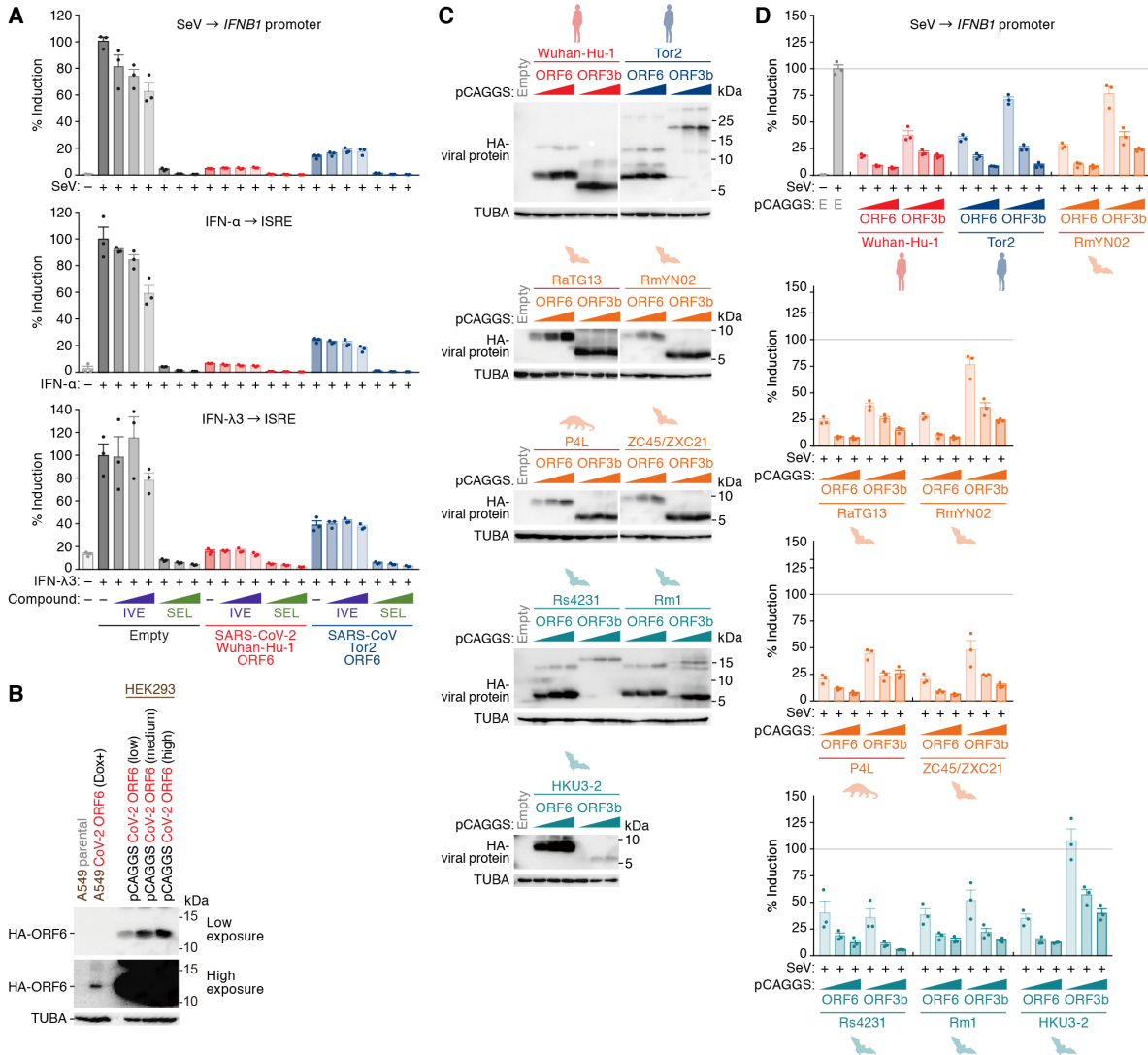


Figure S1. Anti-innate immune signaling activity of Sarbecovirus ORF6 (Related to Figure 2).

(A) No effects of Ivermectin and Selinexor on the anti-innate immune signaling activity of ORF6. HEK293 cells were cotransfected with plasmids expressing HA-tagged ORF6 proteins of SARS-CoV-2 and SARS-CoV and either p125Luc (top) or pISRE-luc (middle and bottom). 24 h post transfection, cells were infected with SeV (MOI 10) (top), or treated with IFN- α (middle) or IFN- λ 3 (bottom) together with three doses of Ivermectin (IVE; 0.2, 1 and 5 μ M) or Selinexor (SEL; 0.4, 2 and 10 μ M). 24 h post infection or treatment, cells were harvested for luciferase assay.

(B) Comparison of ORF6 expression levels in HEK293 cells and A549 cells. To directly compare the expression levels of HA-ORF6 (SARS-CoV-2 strain Wuhan-Hu-1) in HEK293 cells transiently transfected with pCAGGS expression plasmids and those in A549 cells treated with Dox, the cell lysates used in **Figures 2B** and **2F**

were analyzed by Western blotting on the same membrane. To better visualize the bands representing ORF6, blots with lower (top) and higher (bottom) exposures are shown.

(C and D) Potent anti-IFN activity of *Sarbecovirus* ORF6 and ORF3b. HEK293 cells were cotransfected with three different amounts of plasmids expressing HA-tagged *Sarbecovirus* ORF6 (left) or ORF3b (right) and p125Luc. 24 h post transfection, cells were infected with SeV (MOI 10). 24 h post infection, cells were harvested for Western blotting **(C)** and luciferase assay **(D)**.

For Western blotting **(B and C)**, the input of cell lysate was normalized to TUBA, and one representative result out of three independent experiments is shown. kDa, kilodalton. For the luciferase assay **(A and D)**, the value was normalized to the unstimulated, empty vector-transfected cells (no SeV infection). E, empty vector.

pISRE-luc. 24 h post transfection, cells were treated with IFN- α (top) or IFN- λ 3 (bottom). 24 h post treatment, cells were harvested for luciferase assay.

For the luciferase assay (**B-D**), values were normalized to unstimulated, empty vector-transfected cells (no IFN treatment). In **B**, statistically significant differences ($P < 0.05$) compared to SARS-CoV-2 (Wuhan-Hu-1) ORF6 WT-transfected cells (*) are shown. In **C**, statistically significant differences ($P < 0.05$) compared to SARS-CoV (Tor2) ORF6 WT-transfected cells (*) are shown. E, empty vector.

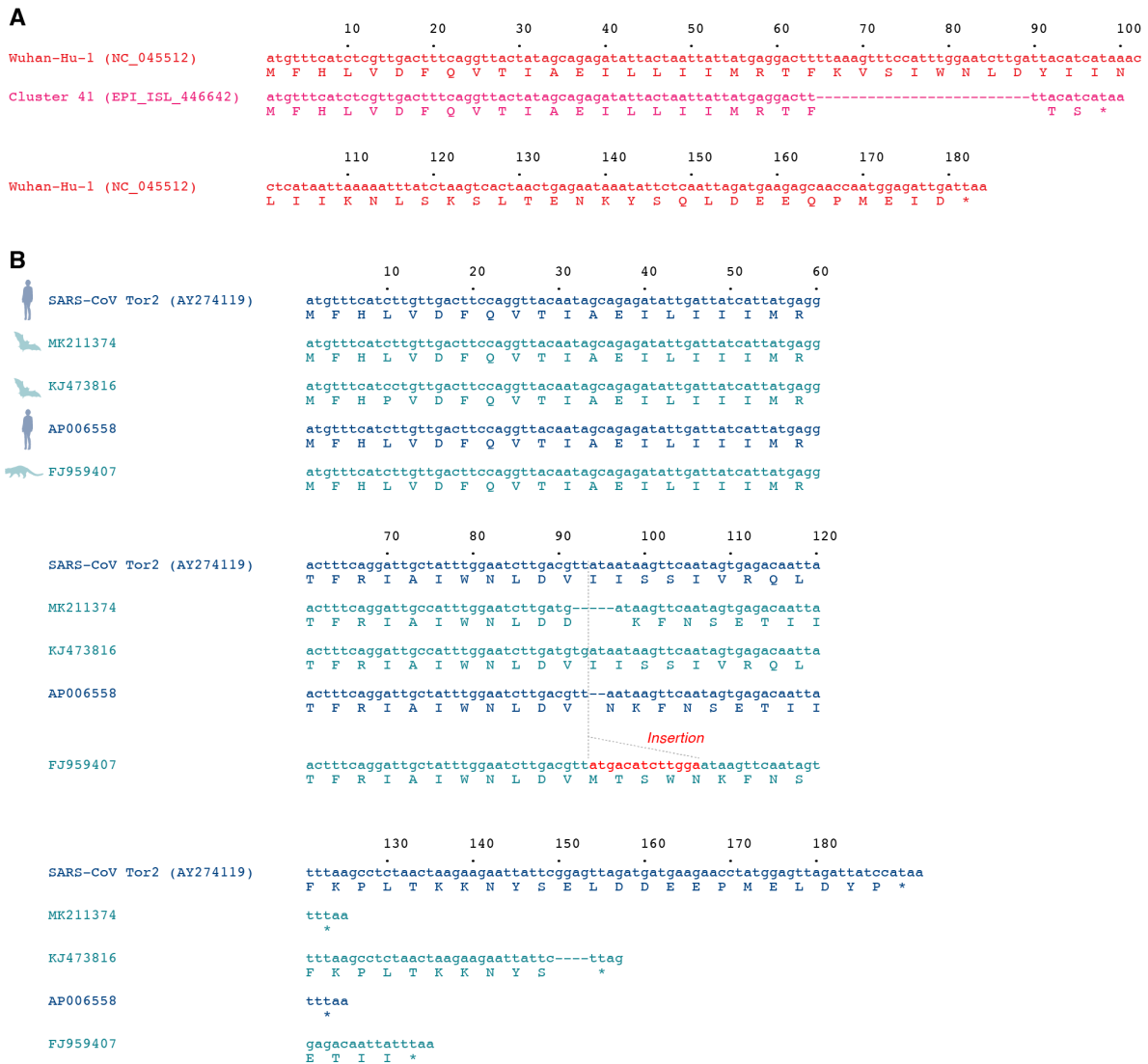


Figure S3. Nucleotide and amino acid sequences of truncated ORF6 (Related to Figure 4)

(A) 25-bp deletion of the *ORF6* gene observed in cluster 41. The nucleotide (top) and amino acid sequence (bottom) of SARS-CoV-2 *ORF6* (strain Wuhan-Hu-1, GenBank accession number: NC_045512) and that of the truncated *ORF6* encoded by the viruses belonging to cluster 41 (Figure 4B) are shown. Since all *ORF6* sequences of the viruses belonging to cluster 41 are identical, only one representative sequence (GISAID ID: EPI_ISL_446642) is shown.

(B) Four frameshift *ORF6* mutants the SARS-CoV lineage. The nucleotide (top) and amino acid (bottom) sequence of SARS-CoV *ORF6* (strain Tor2, GenBank accession number: AY274119, as the reference) and those of the truncated *ORF6* encoded by viruses belonging to the SARS-CoV lineage are shown. For the four

ORF6 frameshift mutants, GenBank accession numbers are indicated. Three of these ORF6 proteins (GenBank accession numbers MK211374, KJ473816 and AP006558) are truncated because of deletions at different regions, while the fourth ORF6 protein (GenBank accession number FJ959407) is truncated due to a frameshift caused by an insertion. MK211374 and KJ473816 are SARS-CoV-related viruses isolated from *Rhinolophus* sp. and *Rhinolophus sinicus*, respectively, while FJ959407 is a SARS-CoV-related virus isolated from a palm civet. AP006558 (strain TWJ) is a human SARS-CoV.