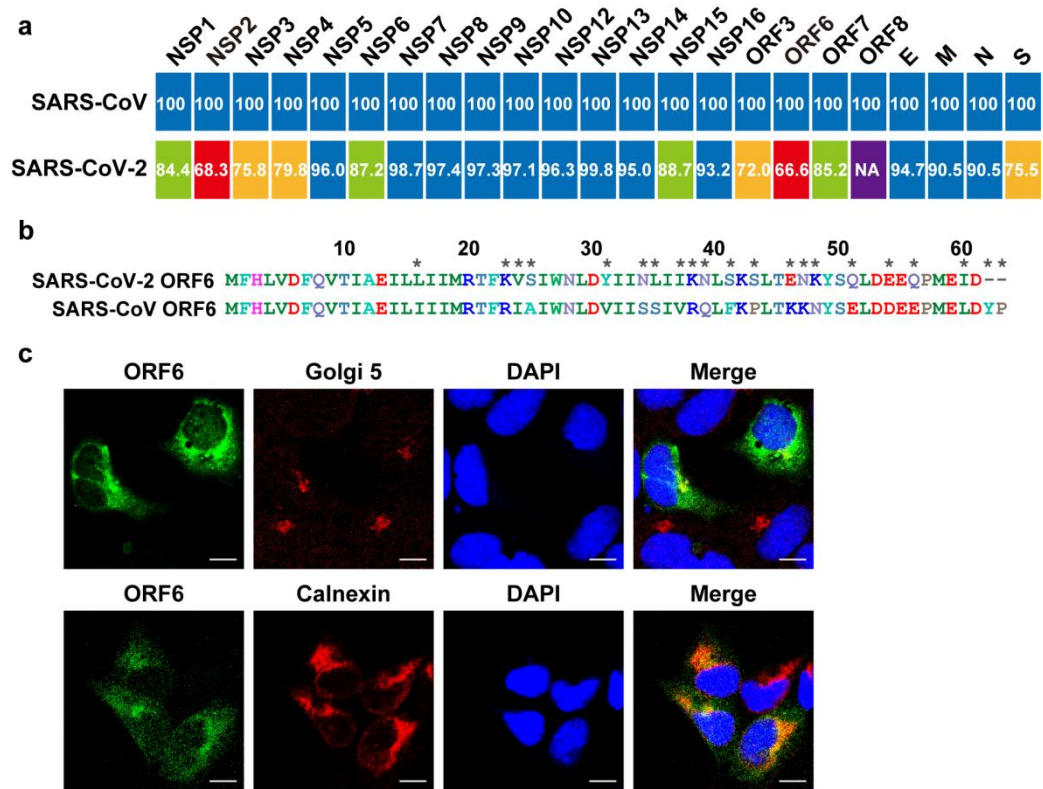


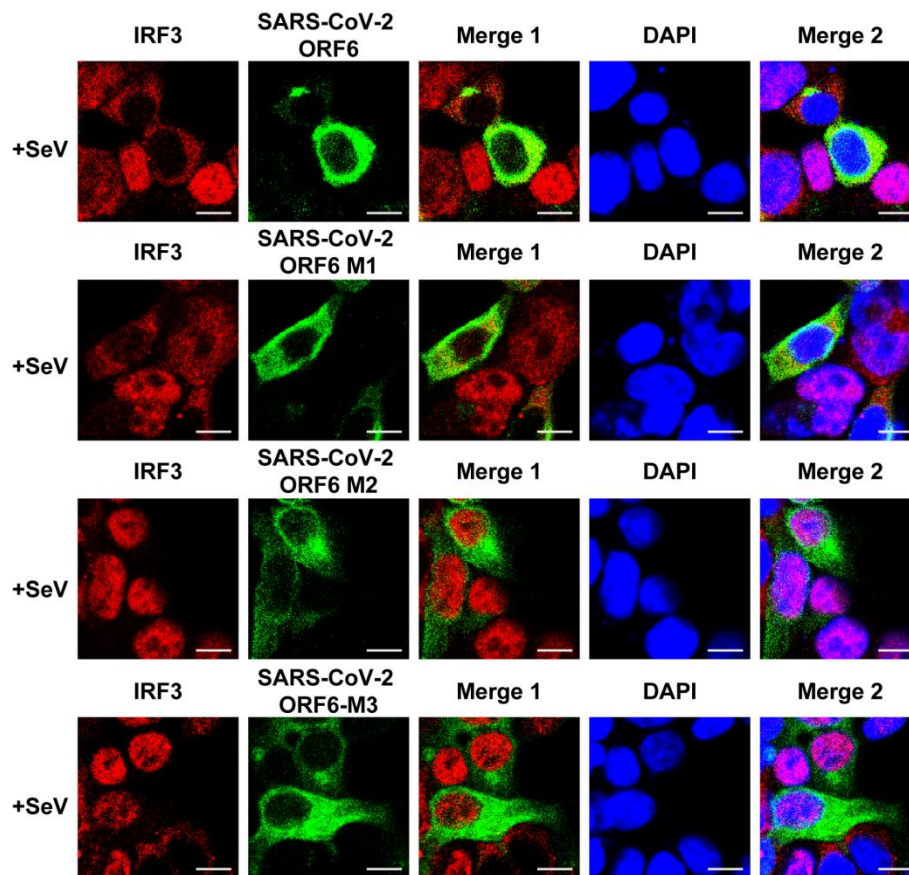
**SUPPLEMENTAL INFORMATION**

**Activation and Evasion of Type I Interferon Responses by SARS-CoV-2**

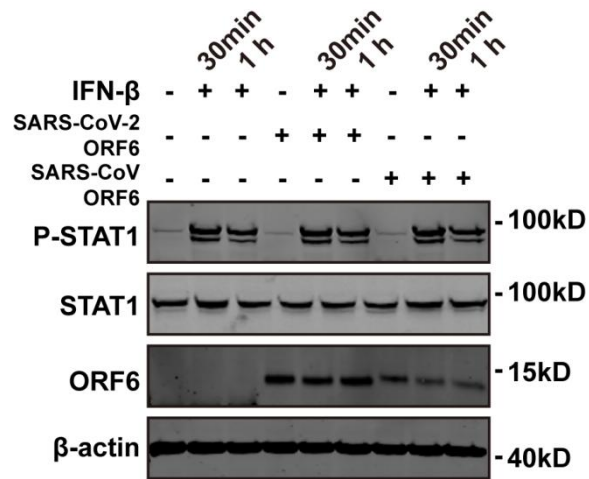
Lei et al.



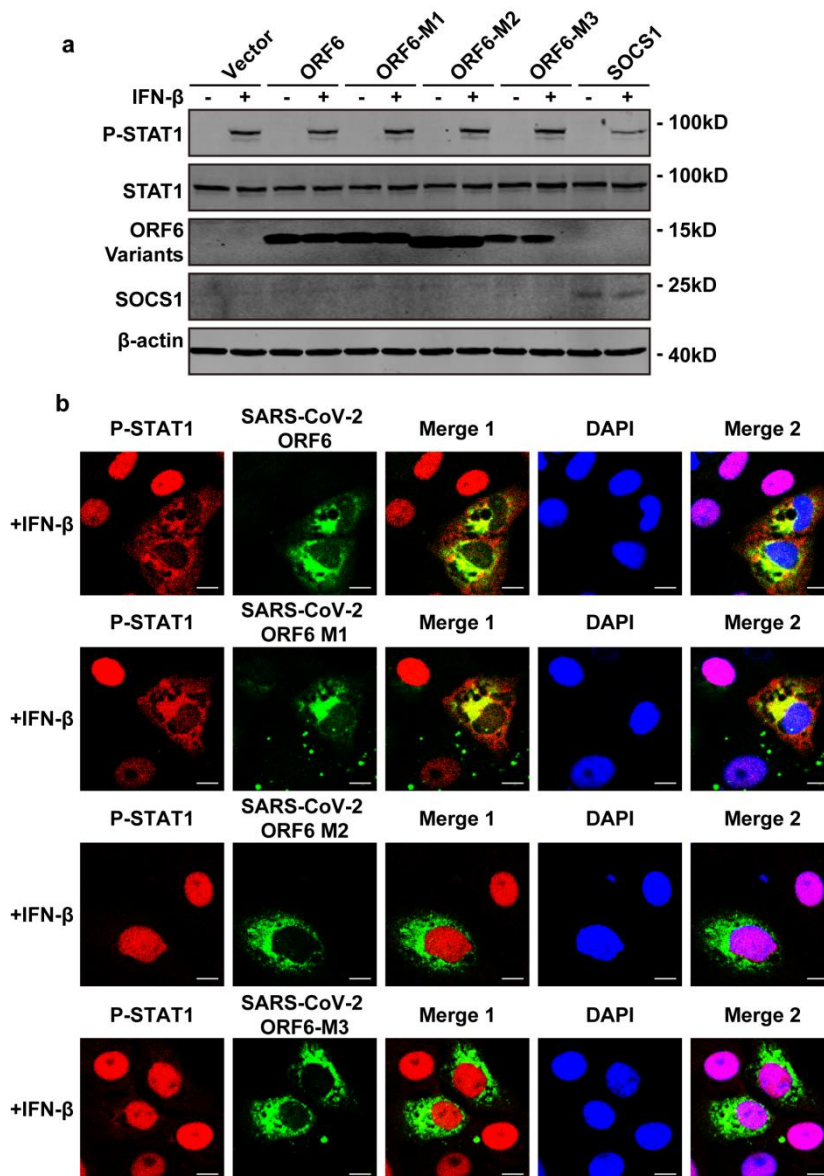
**Supplementary Figure 1. Amino acid identity between SARS-CoV-2 and SARS-CoV proteins, and cellular distribution of ORF6.** **a** Amino acid identity between proteins encoded by SARS-CoV and SARS-CoV-2. The identity value of all SARS-CoV proteins was set to 100. **b** Sequence alignment of SARS-CoV ORF6 and SARS-CoV-2 ORF6. **c** Cellular distribution of SARS-CoV ORF6. HEK293 cells were transfected with plasmids expressing Flag-tagged SARS-CoV-2 ORF6. After 24 h, cells were stained with anti-GolgiB1 (red), anti-calnexin (red) and anti-Flag (green) antibodies. Scale bar, 10  $\mu$ m. All experiments were done at least twice, and one representative is shown.



**Supplementary Figure 2. ORF6 inhibits IRF3 nuclear translocation via its C-terminus.** HEK293 cells were transfected with plasmids expressing Flag-tagged wildtype ORF6 or indicated ORF6 variants. At 24 h post-transfection, cells were infected with SeV for 4 h. Cells were then stained with anti-IRF3 (red), anti-Flag (green), and DAPI (blue). Merge 1 and Merge 2 indicate the merged red and green channels and the merged red, green, and blue channels, respectively. Scale bar, 10  $\mu$ m. All experiments were done at least twice, and one representative is shown.



**Supplementary Figure 3. ORF6 does not inhibit STAT1 phosphorylation in Vero cells.** Vero cells were transfected a control plasmid, or with plasmids expressing SARS-CoV ORF6 or SARS-CoV-2 ORF6. At 24 h post-transfection, cells were treated with 1000 U/ml IFN- $\beta$  for 30 min or 1 h. Phosphorylation of STAT1 was detected by Western blot using a phospho-STAT1-specific antibody. All experiments were done at least twice, and one representative is shown. Source data are provided as a Source Data file.



**Supplementary Figure 4. The SARS-CoV-2 ORF6 or its variants does not inhibit STAT1 phosphorylation.** **a** Effect of SARS-CoV-2 ORF6 and its variants on IFN- $\beta$ -induced phosphorylation of STAT1. HEK293T cells were transfected with plasmids expressing wildtype ORF6 or indicated ORF6 variants, or with a plasmid expressing SOCS1. At 24 h post-transfection, cells were treated with 1000 U/ml IFN- $\beta$  for 30 min. The phosphorylation of STAT1 was detected by Western blot. **b** Effect of SARS-CoV-2 ORF6 and its variants on nuclear translocation of phosphorylated STAT1. Vero cells were transfected with plasmids expressing Flag-tagged wildtype ORF6 or indicated ORF6 variants. At 24 h post-transfection, cells were treated with 1000 U/ml IFN- $\beta$  for 30 min. Cells were then stained with anti-phospho-STAT1 (red), anti-Flag (green), and DAPI (blue). Merge 1 and Merge 2 indicate the merged red and green channels and the merged red, green, and blue channels, respectively. Scale bar, 10  $\mu$ m. All experiments were done at least twice, and one representative is shown. Source data are provided as a Source Data file.

Supplementary Table 1 – Primers used in this study

Gene name	Primers	Reference
<i>IFN<math>\beta</math></i>	Forward primer TAGCACTGGCTGGAATGAG Reverse primer GTTTCGGAGGTAACCTGTAAG	1
<i>ISG56</i>	Forward primer TACAGCAACCATGAGTACAA Reverse primer TCAGGTGTTTCACATAGGC	1
<i>ISG54</i>	Forward primer CTGCAACCATGAGTGAGAA Reverse primer CCTTTGAGGTGCTTTAGATAG	1
<i>P gene of Sendai virus</i>	Forward primer CTCTGGGAGAACAAGCAAGC Reverse primer TCGCCAGATCCTGAGATAC	2

1. Lei, X. et al. The 3C Protein of Enterovirus 71 Inhibits Retinoid Acid-Inducible Gene I-Mediated Interferon Regulatory Factor 3 Activation and Type I Interferon Responses. *J Virol* **84**, 8051–8061 (2010).
2. Liu, B. et al. The C-Terminal Tail of TRIM56 Dictates Antiviral Restriction of Influenza A and B Viruses by Impeding Viral RNA Synthesis. *J Virol* **90**, 4369–4382 (2016).