Supplemental Information

SARS-CoV-2 ORF3b Is a Potent Interferon Antagonist

Whose Activity Is Increased by a Naturally

Occurring Elongation Variant

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Supplementary Information

SARS-CoV-2 ORF3b is a potent interferon antagonist whose activity is further increased by a naturally occurring elongation variant

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Supplementary Figures S1-S3
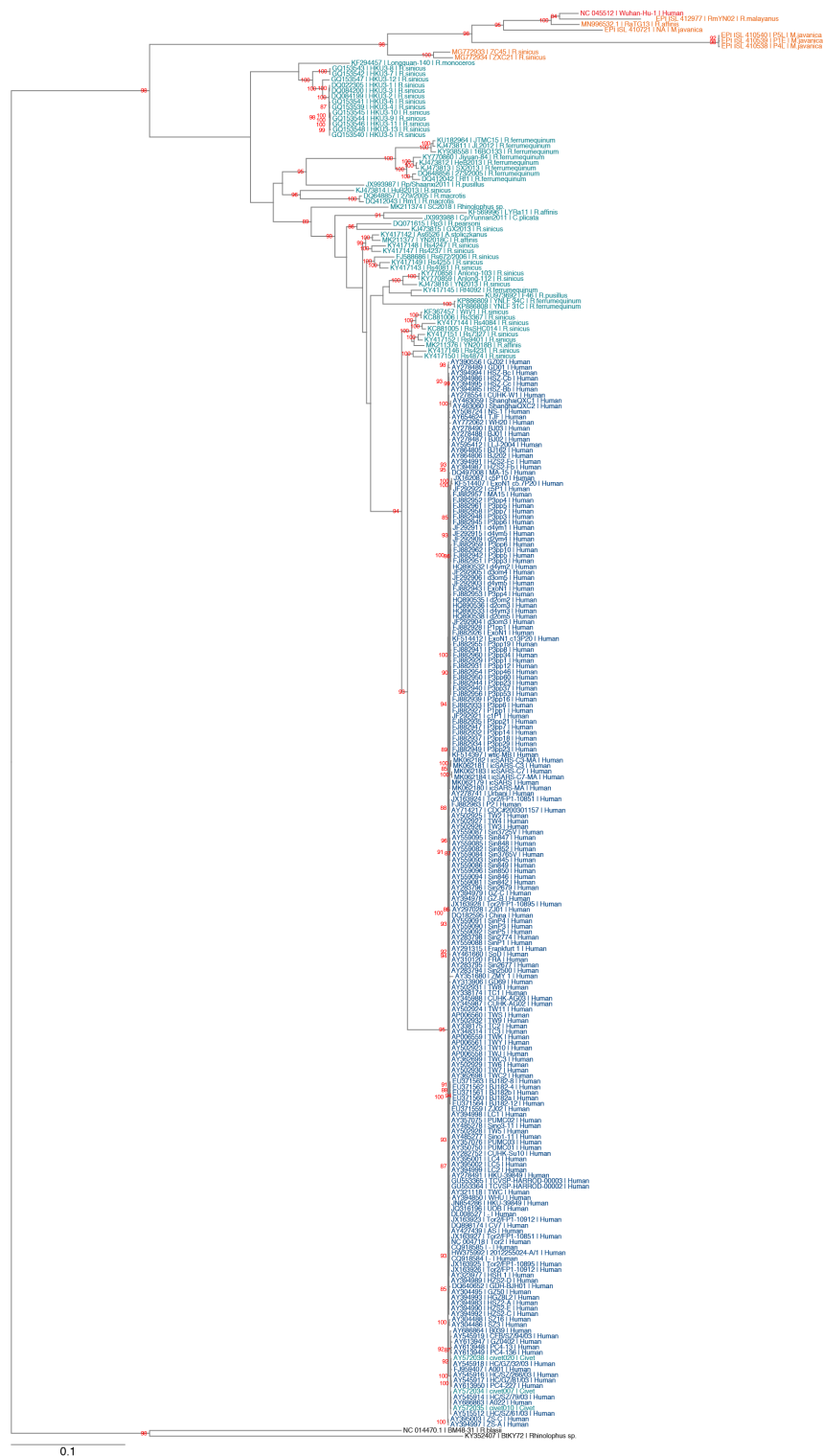Supplementary Tables S1-S5

Figure S1. Uncollapsed maximum likelihood phylogenetic tree of the full-length *Sarbecovirus* sequences (Related to Figure 1)

The full-length sequences (~30,000 bp) of SARS-CoV-2 (Wuhan-Hu-1 as a representative), SARS-CoV-2-related viruses from bats (n=4) and pangolins (n=4), SARS-CoV (n=190), SARS-CoV-related viruses from civets (n=3) and bats (n=54), and outgroup viruses (n=2; BM48-31 and BtKY72) were analyzed. Accession number, strain name, and host of each virus are indicated for each branch. The collapsed tree is shown in **Figure 1A**, and the sequences used are summarized in **Table S1**. The red numbers on the nodes indicates the bootstrap values (>85%). A scale bar indicates 0.1 nucleotide substitutions per site.
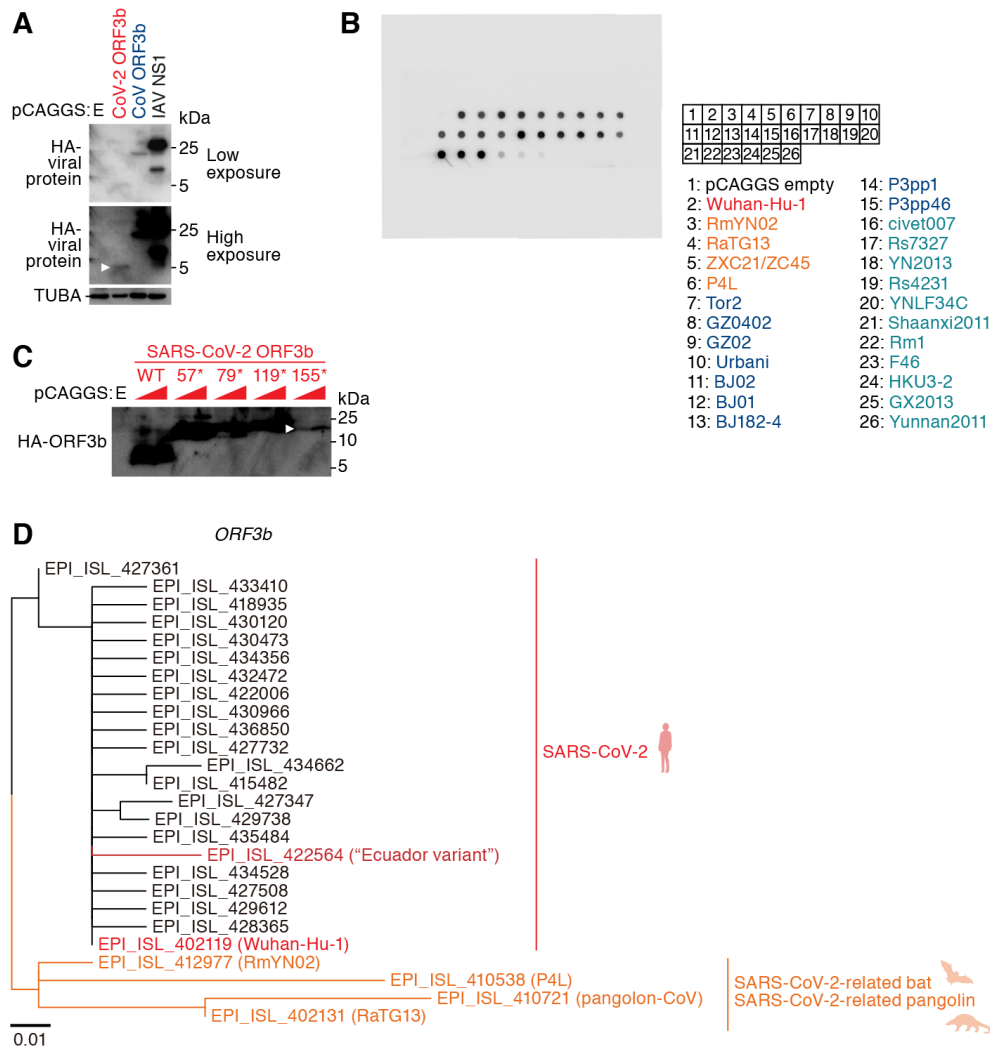
Figure S2. Comparison of *Sarbecovirus* ORF3b (Related to Figures 1-3)

(**A**) Expression of the HA-tagged viral proteins in A549 cells. To visualize Western blot bands of the ORF3b proteins as well as IAV NS1 in the Western blotting, the blots with lower (top) and higher (bottom) exposures are shown. The band of SARS-CoV-2 ORF3b is indicated by a white arrowhead. kDa, kilodalton.

(**B**) Uncropped dot blots of **Figure 2C** (middle). The individual dots are labeled on the right.

(**C**) Western blotting with a higher exposure. To visualize the band of the SARS-CoV-2 ORF3b 155* mutant, a higher exposure of the Western blot in **Figure 3C** is shown. The band of the SARS-CoV-2 ORF3b 155* mutant is indicated by a white arrowhead. kDa, kilodalton.

(**D**) Maximum likelihood phylogenetic tree of the *ORF3b* genes of SARS-CoV-2 during current pandemic. The sequences used are listed in **Table S4**, and SARS-CoV-2-related viruses from bats (n=2) and pangolins (n=2) are outgrouped. GISAID accession ID and strain name (in parenthesis) are indicated for each branch. A scale bar indicates 0.01 nucleotide substitutions per site.

Figure S3. Comparison of the cryptic SARS-CoV *ORF3b*-like sequence in the SARS-CoV-2 genome and SARS-CoV *ORF3b* (Related to Figure 3)

(**A**) The nucleotide (top) and amino acid (bottom) sequences of SARS-CoV-2 Wuhan-Hu-1 (GenBank accession no. NC_045512.2, nucleotides 25814-26281) and SARS-CoV Tor2 (GenBank accession no. AY274119.3, nucleotides 25689-26153) are shown in red and blue, respectively. The stop codons in the cryptic SARS-CoV *ORF3b*-like sequence in the SARS-CoV-2 genome are indicated in black. The authentic SARS-CoV-2 *ORF3b* is highlighted in yellow (i.e., the first stop codon is the authentic stop codon of SARS-CoV-2 *ORF3b*).

(**B**) The nucleotide (top) and amino acid (bottom) sequences of the cryptic ORF3b of SARS-CoV-2-related viruses are shown. The premature stop codons in the cryptic SARS-CoV *ORF3b*-like sequences encoded in the genomes of SARS-CoV-2-related viruses are indicated in red. The authentic *ORF3b* is highlighted in yellow (i.e., the first stop codon is the authentic stop codon of authentic *ORF3b*).

(**C**) The nucleotide (top) and amino acid (bottom) sequences of Ecuador variant ORF3b (GISAID accession IDs: EPI_ISL_422564 and EPI_ISL_422565) are shown. Codons and amino acids differing from the SARS-CoV-2 Wuhan-Hu-1 ORF3b 57* derivative are indicated in red.