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Supplemental information

The N-terminal domain of SARS-CoV-2 nsp1 plays key roles in suppression of cellular gene expression and preservation of viral gene expression

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Figure S1.

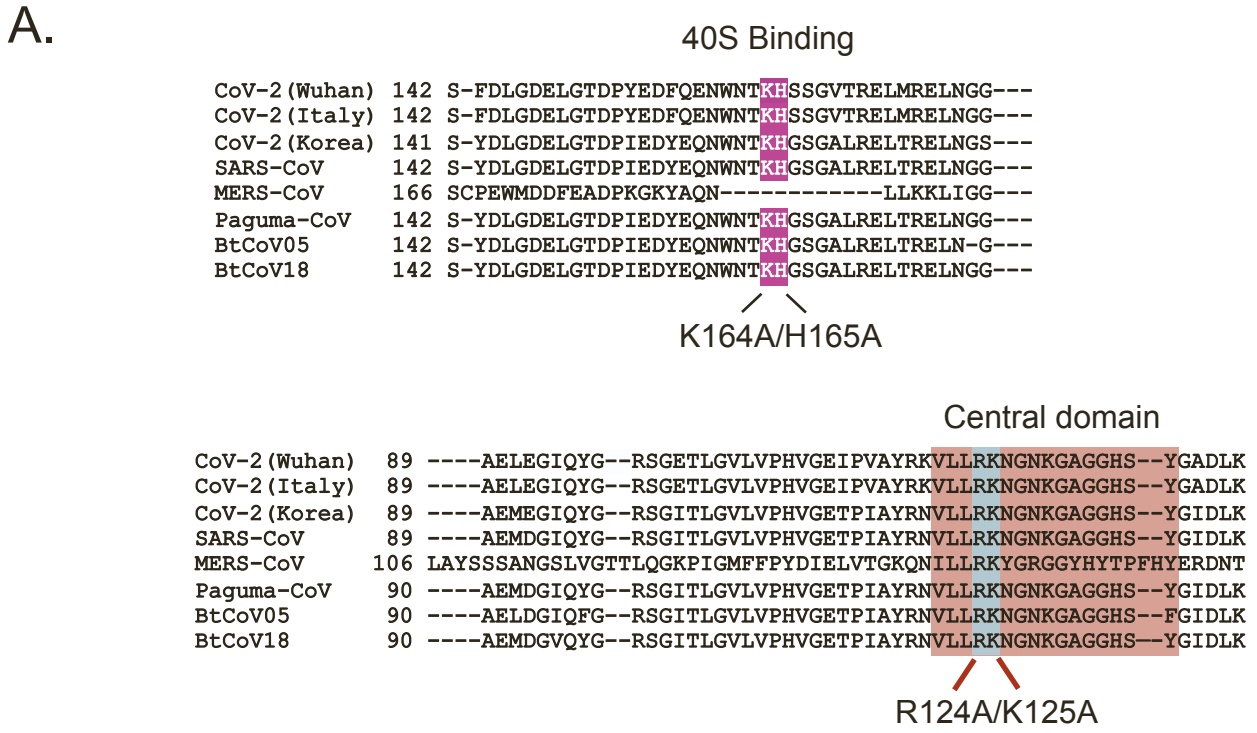

Figure S1. Sequence alignment of the nsp1 C-terminal and central domains and RRL translation assay. Related to Figure 1. (A) The purple box highlights conservation of the K164 and H165 residues and the red box shows the central domain containing conserved residues R124/K125 (blue) involved in mRNA destabilization across 8 betacoronavirus nsp1 proteins. (B) HBB-nLuc reporter RNA was incubated with rabbit reticulocyte lysate translation extracts alone or in the presence of increasing concentrations of purified WT, R124A/K125A or K164A/H165A nsp1. Translation of the reporter was then evaluated by luciferase assay and normalized to a GST protein control. Technical triplicates measurements were taken for each biological replicate. A total of at least three biological replicates were taken for each measurement. *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001, one-sample t test versus hypothetical value of 1.

Figure S2.

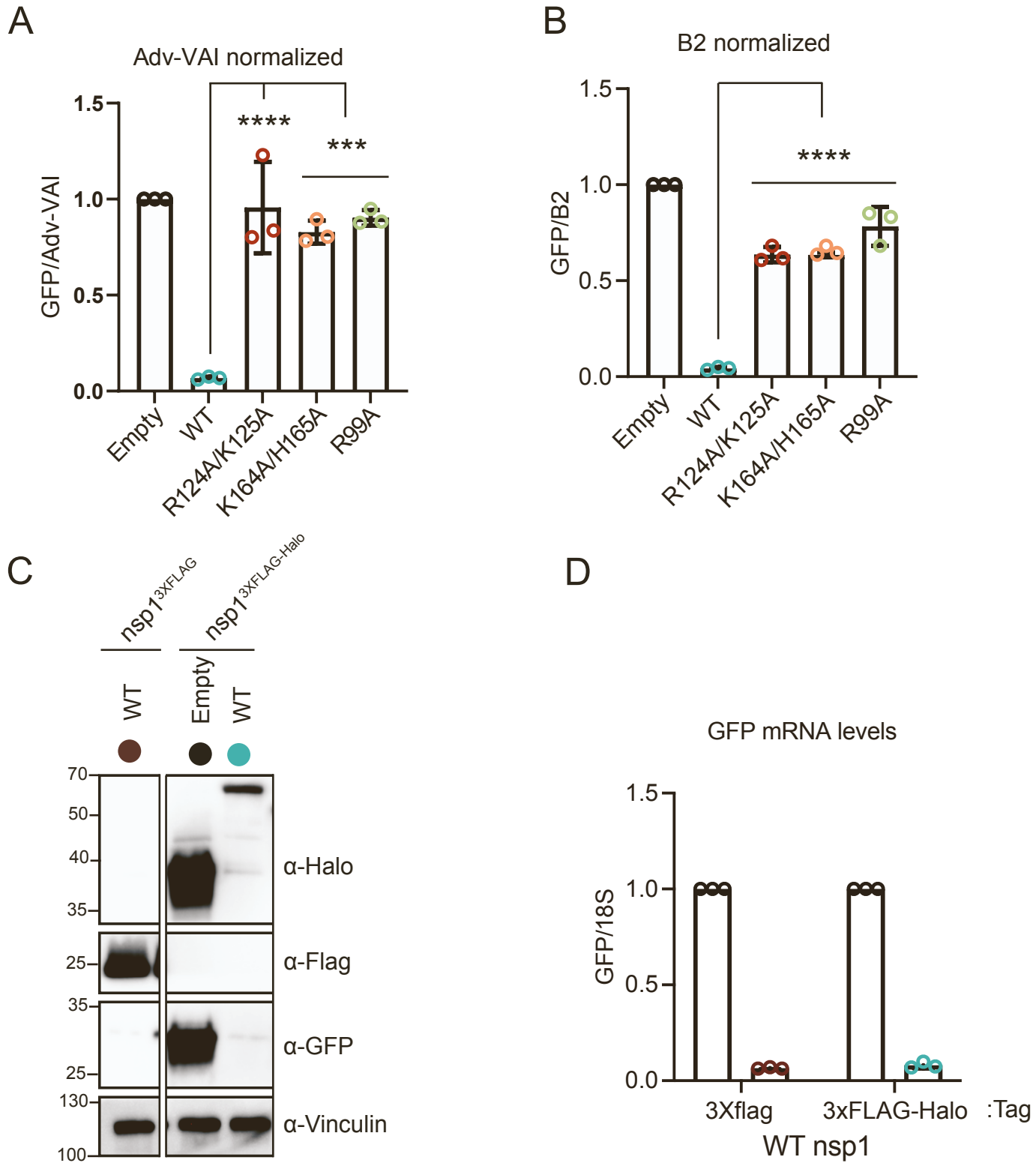

Figure S2. Western blot and RT-qPCR analysis of tagged nsp1 and transfection control experiments. Related to Figures 1, 2 and 3. (A-B) HEK293T cells were co-transfected for 24h with plasmids expressing GFP, WT or mutant nsp1 and either the Pol III transcribed Adenovirus (Adv)-VAI (*A*) or B2 SINE (*B*) transfection controls. GFP mRNA was quantified by RT-qPCR and normalized to Adv-VAI RNA, with the level of GFP mRNA in cells lacking nsp1 then set to 1. Each point represents an independent experiment, and a total of three biological replicates were completed for each sample. ***P≤0.001, ****P≤0.0001, one-way ANOVA followed by Dunnett's multiple comparisons test versus WT nsp1. (C-D) HEK293T cells were co-transfected with plasmids expressing GFP and either nsp1-3XFLAG or nsp1-3xFLAG-Halo and lysates were harvested for either protein (*C*) or RNA (*D*). Protein levels were measured by western blotting with antibodies against FLAG or Halo to detect nsp1 and antibodies against GFP as a marker of nsp1 host shutoff activity. Solid lines indicate lines cropped from the same gel. Vinculin was used as a loading control. GFP mRNA levels were determined by RT-qPCR and normalized to 18S rRNA, with the level of GFP mRNA in cells lacking nsp1 then set to 1. Each point represents an independent experiment, and a total of three biological replicates were completed for each sample.

Figure S3.

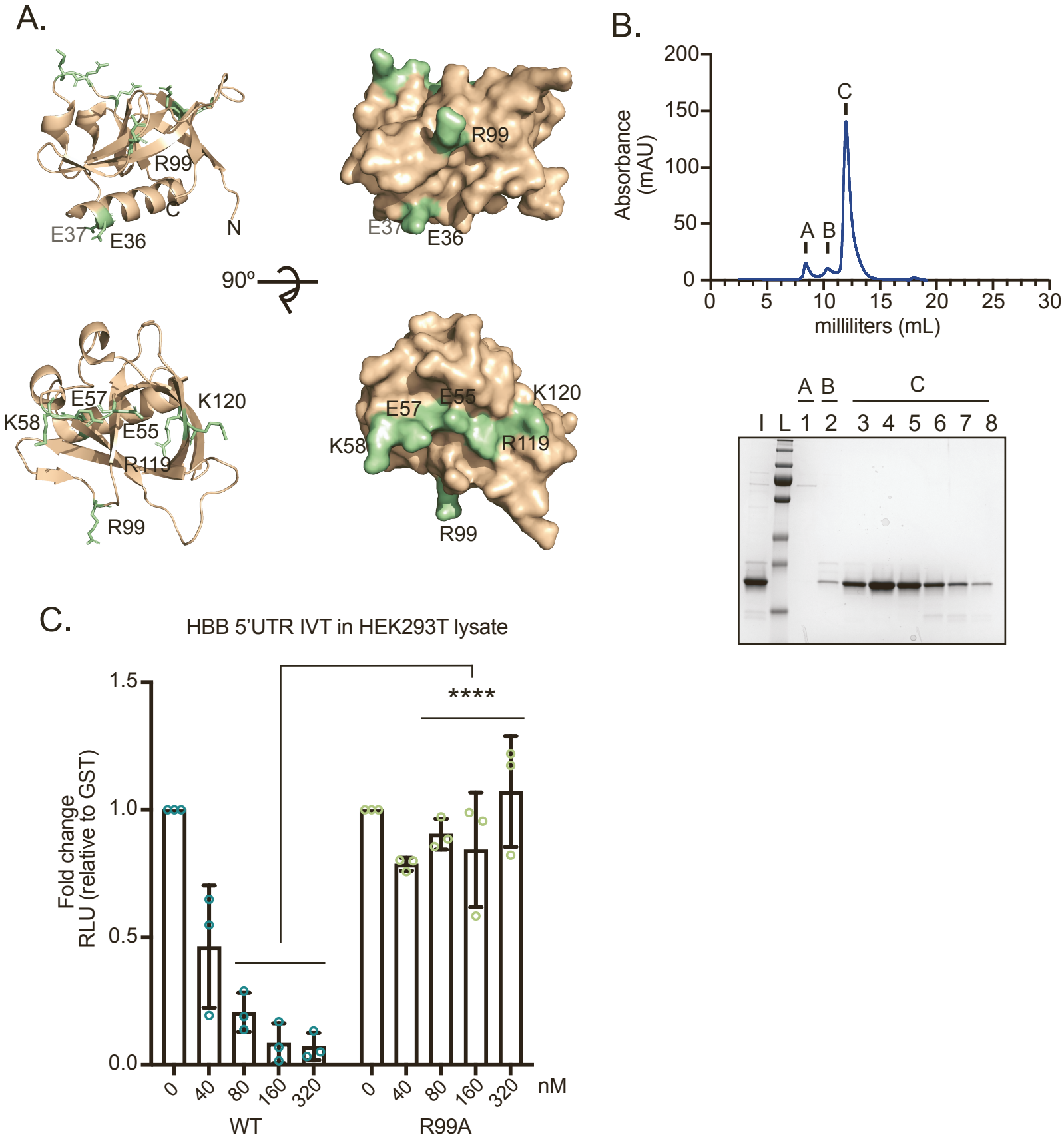

Figure S3. Mutations to the N-terminal globular domain of nsp1. Related to Figure 3. (A) Structure of the nsp1 N-terminal domain (PDB: 7K7P) (Clark et al. 2021). Residues selected for mutation are highlighted in green. (B) Size exclusion run of the purified nsp1 R99A protein. (I) indicates input sample and peaks A and B represent higher molecular weight contaminants. Peak C corresponds to the expected molecular weight of the nsp1 R99A mutant. (L) corresponds to the prestained ladder. (C) HEK293T translation extracts were used to monitor the effect of increasing concentrations of WT nsp1 versus the R99A mutant on translation of HBB-nLuc reporter, as measured by luciferase assay. Technical triplicates measurements were taken for each biological replicate. A total of at least three biological replicates were taken for each measurement. ****P≤0.0001, one-way ANOVA followed by Dunnett's multiple comparisons test versus WT nsp1.

Figure S4.

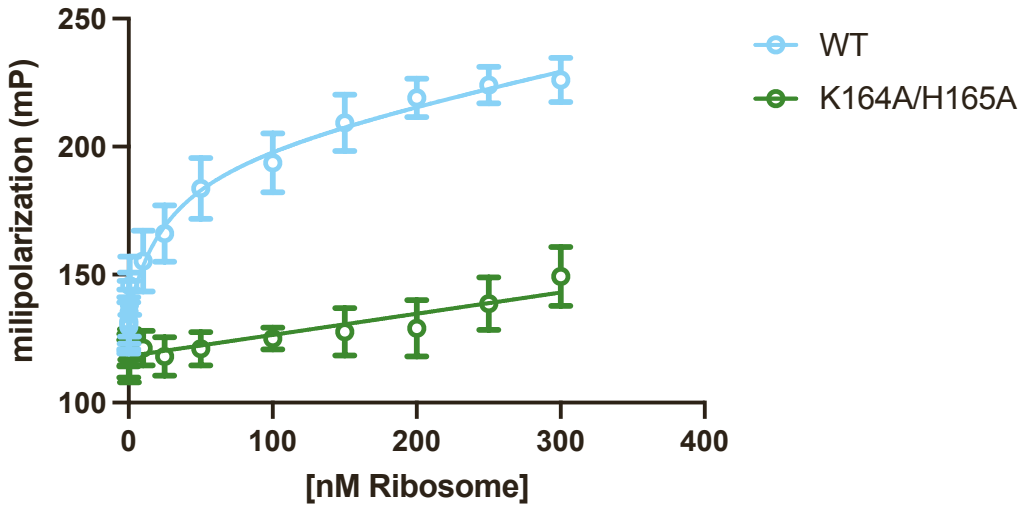

Figure S4. Equilibrium binding of fluorescently labeled K164A/H165A nsp1. **Related to Figure 4.** Binding experiments were performed with nsp1 WT (blue circles) and K164A /H165A (green circles) and purified ribosomes. Raw millipolarization units are shown on the Y axis (mP). Technical triplicates measurements were taken for each biological replicate. A total of at least three biological replicates were taken for each measurement.

Figure S5.

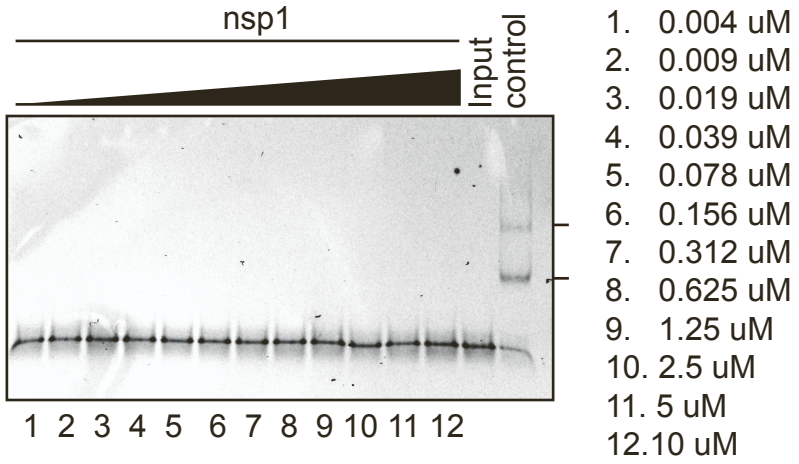

Figure S5. Nsp1 does not directly bind CoV2 leader RNA. Related to Figure 4. To test for direct nsp1-RNA binding, an electrophoretic mobility shift assay was conducted by incubating increasing amounts of purified nsp1 protein (0.004-10uM) with 5' Cy5 labeled SARS-CoV-2 leader RNA for 30 min. The Kaposi's associated sarcoma herpesvirus RNA binding protein ORF37 (5uM) was included as a control to show RNA binding under these conditions.

Figure S6.

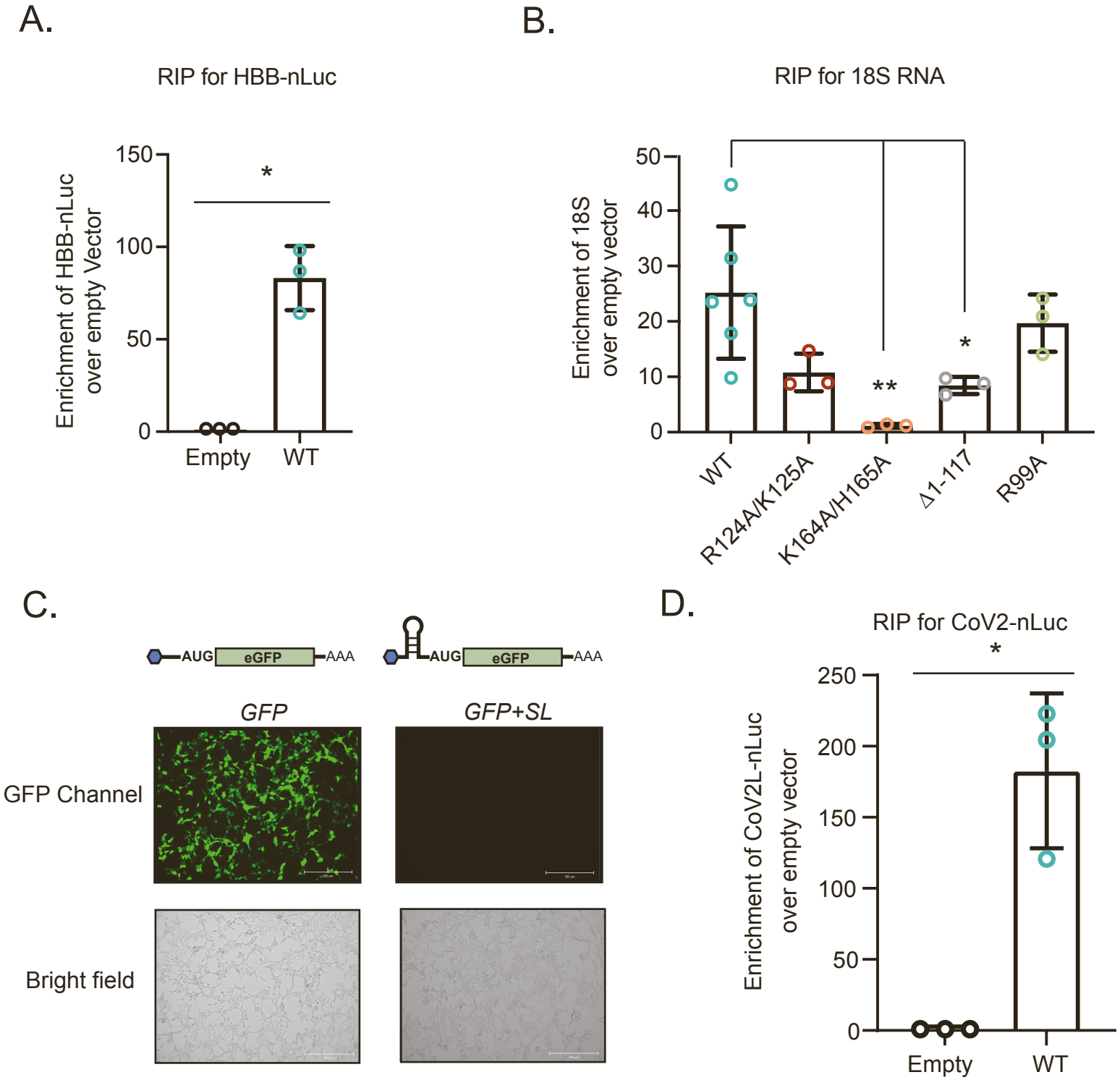

Figure S6. Binding of reporter mRNAs and 18S rRNA to WT and/or mutant nsp1. Related to Figures 4 and 5. (A) RIP data showing the enrichment value of HBB-nLuc mRNA for WT nsp1 compared to empty vector, using the data from the experiments in Figure 4C. Each point represents an independent experiment, and a total of three biological replicates were completed for each sample. *P≤0.05, one-sample t test versus hypothetical value of 1. (B) RT-qPCR was performed to quantify 18S levels in the RIP experiment shown in Figure 4C, with the RNA values then normalized to the RNA values obtained from the empty vector control. Each point represents an independent experiment, and a total of at least three biological replicates were completed for each sample. *P≤0.05, **P≤0.01, one-way ANOVA followed by Dunnett's multiple comparisons test versus WT nsp1. (C) Plasmids encoding GFP mRNA or GFP mRNA containing a cap-proximal stem loop structure (*GFP+SL*) were transfected into HEK293T cells, and GFP fluorescence from each cell was assessed by fluorescence microscopy. Bright field images show cell density; all images were taken at 10X magnification. (D) RIP data showing the enrichment value of CoV2L-nLuc mRNA for WT nsp1 compared to empty vector, using the data from the experiments in Figure 5B. Each point represents an independent experiment, and a total of three biological replicates were completed for each sample. *P≤0.05, one-sample t test versus hypothetical value of 1.

Supplemental Table 1. Oligos used in this study. Related to STAR Methods.

RT-qPCR primers		
Target gene	Forward primer	Reverse primer
GFP	GAACCGCATCGAGCTGAA	TGCTTGTCGGCCATGATATAG
Nanoluciferase	GGAGGTGTGTCCAGTTTGTT	ATGTCGATCTTCAGCCCATTT
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Adv-VAI	TGGTCTGGTGGATAAATTCG	GTTGTCTGACGTCGCACA
B2 SINE	GGGGCTGGAGAGATGGCT	CCATGTGGTTGCTGGGAT
Primer extension oligo		
Target gene	Sequence	
Nanoluciferase	CGCCAGAATGCGTTCGCACAGCCGCCAGCCGGTC	
Oligos used for cloning		
Name	Sequence	Description
pGEX-CoV2nsp1_Fw	GGATCCCCAGGAATTGAGAGCCTTGTCCCTGGTTTC	Forward primer to amplify CoV-2 nsp1 and clone into pGEX vector via InFusion reaction for protein expression
pGEX-CoV2nsp1_Rv	AGTCACGATGCGGCCTTACCCTCCGTTAAGCTCACGC	Reverse primer to amplify CoV-2 nsp1 and clone into pGEX vector via InFusion reaction for protein expression
pCDNA4-3xFLAG-HaloTEV-CoV2_Fw	GACAAGGGGGCGGCCGCAGAAATCGGTACTGGCTTTCC	Forward primer to amplify CoV-2 nsp1 and generate N-terminally 3xFLAG-HaloTEV nsp1 via InFusion reaction
pCDNA4-3xFLAG-HaloTEV-CoV2_Rv	TAGACTCGAGCGGCCCTACCCTCCGTTAAGCTCACG	Reverse primer to amplify CoV-2 nsp1 and generate N-terminally 3xFLAG-HaloTEV nsp1 via InFusion reaction
pCDNA4-CoV2-FLAG_Fw	CACAGTGGCGGCCGCATGGAGAGCCTTGTCCCTGGTTTC	Forward primer to amplify CoV-2 nsp1 and generate C-terminally 3xFLAG tagged

		nsp1 via restriction enzyme cloning
pCDNA4-CoV2-FLAG_Rv	CTCGAGCGGCCGCCACCCTCCGTTAAGCTCACGCATGAG	Reverse primer to amplify CoV-2 nsp1 and generate C-terminally 3xFLAG tagged nsp1 via restriction enzyme cloning
pCDNA4-CoV2-delta118-180_Fw	GACAAGGGGGCGGCCGCAGAAATCGGTACTGGCTTTCC	Forward primer to amplify the N-terminal half of CoV-2 nsp1 and generate CoV-2 nsp1 Δ 118-180 via InFusion reaction
pCDNA4-CoV2-delta118-180_Rv	TAGACTCGAGCGGCCCTAAGCCACTGGTATTTCGCCC	Reverse primer to amplify the N-terminal half of CoV-2 nsp1 and generate CoV-2 nsp1 Δ 118-180 via InFusion reaction
pCDNA4-CoV2-delta1-117_Fw	GACAAGGGGGCGGCCGCAGAAATCGGTACTGGCTTTCC	Forward primer to amplify the C-terminal half of CoV-2 nsp1 and generate CoV-2 nsp1 Δ 1-117 via InFusion reaction
pCDNA4-CoV2-delta1-117_Rv	TAGACTCGAGCGGCCCTACCCTCCGTTAAGCTCACG	Reverse primer to amplify the C-terminal half of CoV-2 nsp1 and generate CoV-2 nsp1 Δ 1-117 via InFusion reaction
CoV-2 nsp1 R124A/K125A	CGCAAGGTTCTTCTTGCTGCGAACGGTAATAAAGGA	Mutagenesis primers to generate R124A/K125A mutant
CoV-2 nsp1 K164A/H165A	GAAAACTGGAACACTGCAGCTAGCAGTGGTGTTACC	Mutagenesis primers to generate K164A/H165A mutant
CoV-2 nsp1 E36A/E37A	GGCTTTGGAGACTCCGTGGCAGCAGTCTTATCAGAGGCAC	Mutagenesis primers to generate E36A/E37A mutant
CoV-2 nsp1 E55A/E57A/K58A	GGCACTTGTGGCTTAGTAGCAGTTGCAGCGGGCGTTTTGCCTCAAC	Mutagenesis primers to generate E55A/E57A/K58A mutant
CoV-2 nsp1 R99A	CGAAGGCATTCAGTACGGTGCAAGTGGTGAGACACTTGG	Mutagenesis primers to generate R99A mutant
CoV-2 nsp1 R119A/K120A	GAAATACCAGTGGCTTACGCAGCGGTTCTTCTTCGTAAGAAC	Mutagenesis primers to generate R119A/K120A mutant
CoV-2 nsp1 Δ 122-130	CAGTGGCTTACCGCAAGGTTGCTGGTGGCCATAGTTACG	Mutagenesis primers to generate Δ 122-130 mutant

CoV-2 nsp1 G-linker	CAGTGGCTTACCGCAAGGTTGG AGGAGGAGGAAGCGGAGGAGG AGGAGCTGGTGGCCATAGTTAC G	Mutagenesis primers to insert Glycine linker into the central domain
N-terminal cysteine/lysine CoV-2 nsp1	GAAGTTCTGTTCCAGGGGCCCT GTAAAGAGAGCCTTGTCCCTGG	Primers used to add cysteine and lysine to CoV-2 nsp1 for fluorescence polarization
pJP-HBB-nLuc_Fw	GTCAGATCCGCTAGCGCTA CATTTGCTTCTGAC	Forward primer to amplify HBB-nLuc and clone into pJP vector via InFusion reaction
pJP-HBB-nLuc_Rv	TTCTCTAGAGATATCTTAC GCCAGAATGCGTTCGCA	Reverse primer to amplify HBB-nLuc and clone into pJP vector via InFusion reaction
CoV-2 leader geneblock	AAATGGACTATCATATGCC AAGTACGCCCCCTATTGAC GTCAATGACGGTAAATGGC CCGCCTGGCATTATGCCCA GTACATGACCTTATGGGAC TTTCCTACTTGGCAGTACA TCTACGTATTAGTCATCGC TATTACCATGGTGATGCGG TTTTGGCAGTACATCAATG GGCGTGGATAGCGGTTTG ACTCACGGGGATTTCCAAG TCTCCACCCCATTGACGTC AATGGGAGTTTGTTTTGGC ACCAAAATCAACGGGACTT TCCAAAATGTCGTAACAAC TCCGCCCCATTGACGCAAA TGGGCGGTAGGCGTGTAC GGTGGGAGGTCTATATAAG CAGAGCTGGTTTAGTGAAC CGACCTTCCCAGGTAACAA ACCAACCAACTTTCGATCT CTTGTAGATCTGTTCTCTAA ACGAACATGGTCTTCACAC TCGAAGATTTCGTTGGGGA CTGGCGACAGACAGCCGG CTACAACCTGGACCAAGTC CTTGAACAGGGAGGTGTGT CCAGTTTGTTTCAGAATCT CGGGGTGTCCGTAACTCC GATCCAAAGGATTGTCCTG AGCGGTGAAAATGGGCTG AAGATCGACATCCATGTCA	CoV-2 leader geneblock was synthesized by IDT and cloned into pJP vector via InFusion reaction

	TCATCCCGTATGAAGGTCT GAGCGGCGACCAAATGGG CCAGATCGAAAAAATTTTTA AGGTGGTGTACCCTGTGG ATGATCATCACTTTAAGGT GATCCTGCACTATGGCACA CTGGTAATCGACGGGGTTA CGCCGAACATGATCGACTA TTTCGGACGGCCGTATGAA GGCATCGCCGTGTTCGAC GGCAAAAAGATCACTGTAA CAGGGACCCTGTGGAACG GCAACAAAATTATCGACGA GCGCCTGATCAACCCCGA CGGCTCCCTGCTGTTCCGA GTAACCATCAACGGAGTGA CCGGCTGGCGGCTGTGCG AACGCATTCTGGCGTAGGA ATTCTCGACCTCGA	
T7+HBB5'UTR+Koz+Nluc_F	TAATACGACTCACTATAGGA CATTTGCTTCTGACACAACT GTGTTCACTAGCAACCTCAA ACAGACACCGCCACCATGG TCTTC	Forward primer to generate HBB-nLuc template for in-vitro transcription
NLuc_R_60T	TTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTACGC CAGAATGCGTTCGCAC	Reverse primer to generate HBB-nLuc template for in-vitro transcription