Supplementary Material for:

Identifying inhibitors of −1 programmed ribosomal frameshifting in a broad spectrum of coronaviruses

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Table S1: Frameshift signal sequences for panel used in testing inhibitors. Slippery sequence is underlined, spacer is shown in italic font.

Name	Sequence
KU182958	TT<u>TTTAAAC</u>*GAGTCA*GGGGTACTAGTGGAGTAGCCCGTCTAGTACCCCTAGGTTCTGGTGTAGAACCAGATATTGTATTAAGGGCTTT
LC469308	TT<u>TTTAAAC</u>*GAGTCC*GGGGTTCTATTGTAAATGCCCGAATAGAACCCTGTGCTAGTGGACTTTCCACTGATGTCGTTTTTAGGGCATT
KY770854	TT<u>TTTAAAC</u>*GAGTGC*GGGGCTCTAGTGATGCTCGACTAGAGCCCCTTAATGGCACTGACACTGACAAGTGTGTTCGTGCTTTTGACGTTTACAACAAGGATGTTGCATGTATTGG
KF294282	TT<u>TTTAAAC</u>*GAGTGC*GGGGCTCTAGTGCAGCTCGACTAGAGCCCTGTAATGGTACTGAACCTGAGCATGTGATTCGCGCCTT
MERS-CoV	TT<u>TTTAAAC</u>*GAGTCC*GGGGTTCTATTGTAAATGCCCGAATAGAACCCTGTTCAAGTGGTTTGTCCACTGATGTCGTCTTTAGGGCATT
SARS-CoV-2	TT<u>TTTAAAC</u>*GGGTTT*GCGGTGTAAGTGCAGCCCGTCTTACACCGTGCGGCACAGGCACTAGTACTGATGTCGTATACAGGGCTT
PEMV	TT<u>TTTAAAC</u>*GGGTAAT*TCCGGTCGACTCCGGAGAAACAAAGTCAATA
HIV	AA<u>TTTTTTA</u>*GGGAAGAT*CTGGCCTTCCCACAAGGGAAGGCCAGGGAATTTTCTTC

Table S2: Hits from initial screen of drug library.

Hits altering only −1 PRF		Hits altering −1 PRF and general translation	
Febuxostat	Fostamatinib	Plerixafor	Azatadine
Tranilast	Erdafitinib	Idebenone	Ketoconazole
Bepridil	10-undecenoic acid	Ibudilast	Abemaciclib
Tafamidis	Santonin	Cariprazine	Ibrutinib
Brexpiprazol	Garenoxacin	Pasireotide	Maraviroc
Penbutolol	Cefmenoxime	Bremelanotide	Nelfinavir
Benzocaine	Tetracycline	Diclofenac	Ribostamycin
Omargliptin	Piromidic acid	Desloratadine	Streptomycin
Nafamostat	Dapsone		Azithromycin
Indinavir	Demeclocycline		
Nitazoxanide	Valnemulin		
Palbociclib	Raltitrexed		

Table S3: Oligonucleotides for bi-fluorescent reporter construction. Underlined bases in gBlock denote HIV-1 −1 PRF signal, lower-case bases in α-helix spacer denote linkers complementary to restriction sites for cloning.

Oligo name	Sequence
AcGFP1, mCherry, inteins, and HIV-1 −1 PRF gBlock	GCATCAGAGCAGATTGTACTGAGAGTGCACCATCTAGGGCGCGCCGGTACCGGTTCCAAGCTTGTTGACGCTAGCCTGCAGGCGGCCGCTAATACGACTCACTATAGGGCCACCATGGTGAGCAAGGGCGCCGAGCTGTTCACCGGCATCGTGCCCATCCTGATCGAGCTGAATGGCGATGTGAATGGCCACAAGTTCAGCGTGAGCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCTGTGCCCTGGCCCACCCTGGTGACCACCCTGAGCTACGGCGTGCAGTGCTTCTCACGCTACCCCGATCACATGAAGCAGCACGACTTCTTCAAGAGCGCCATGCCTGAGGGCTACATCCAGGAGCGCACCATCTTCTTCGAGGATGACGGCAACTACAAGTCGCGCGCCGAGGTGAAGTTCGAGGGCGATACCCTGGTGAATCGCATCGAGCTGACCGGCACCGATTTCAAGGAGGATGGCAACATCCTGGGCAATAAGATGGAGTACAACTACAACGCCCACAATGTGTACATCATGACCGACAAGGCCAAGAATGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGATGGCAGCGTGCAGCTGGCCGAC

	CACTACCAGCAGAATACCCCCATCGGCGATGGCCCTGTGCTGCTGCCCGATAACCACTAC CTGTCCACCCAGAGCGCCCTGTCCAAGGACCCCAACGAGAAGCGCGATCACATGATCTAC TTCGGCTTCGTGACCGCCGCCGCCATCACCCACGGCATGGATGAGCTGTACAAGTATTTC TACACCTCGAGAGAAGCCAGACACAAACAGAAAATTGTGGCACCGGTGAAACAGACTTTG AATTTTGACCTTCTTAAGCTGGCGGGAGACGTCGAGTCCAACCCCGGGCCCtcGTCGAC<u>TT TTTTAGGGAAGATCTGGCCTTCCCACAAGGGGAGGCCAGGGAATTTTCTTCCGAGCTCGA AGACGCCAAAAACATAAAGAAAGGCCCGGCGCCATTCTATCCTCTAGAGGATGGAACCGC TGGAGAGCAACTGCATAAGGCT</u>GGATCCGAGGCACGGCATAAGCAAAAGATCGTAGCCCC AGTAAAGCAAACACTCAACTTCGATCTACTCAAACTCGCAGGTGATGTGGAATCTAATCCA GGACCTTTCGGATCTGCCACCATTATCGTGAGCAAGGGCGAGGAGGATAACATGGCCATC ATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTC GAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGA AGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGT ACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCT TCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACC GTGACCCAGGACTCCTCCCTCCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGG CACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAGGCCT CCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTG AAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAA GCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAA CGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCG GCATGGACGAGCTGTACAAGTAGTTAATTAAAATAAAATATCTTTATTTTCATTACATCTGTG TGTTGGTTTTTTGTGTGGTTTAGAGCGAGATTCCGTCTCAA
α-helix spacer top	tcgactcGAAGCAGCCGCTAAAGAGGCTGCGGCAAAAGCTg
α-helix spacer bottom	GATCCAGCTTTTGCCGCAGCCTCTTTAGCGGCTGCTTCGAG

Table S4: Library used for drug screening. Details of the drugs included in the screening library are available in the attached spreadsheet (TableS4.xlsx).

Table S5: Effects of −1 PRF inhibitors on general translation. The change in *Renilla* luciferase signal with drug present compared to that with drug absent was used to assay the effect of the inhibitors on general translation. Errors represent s.e.m. from 4 replicates.

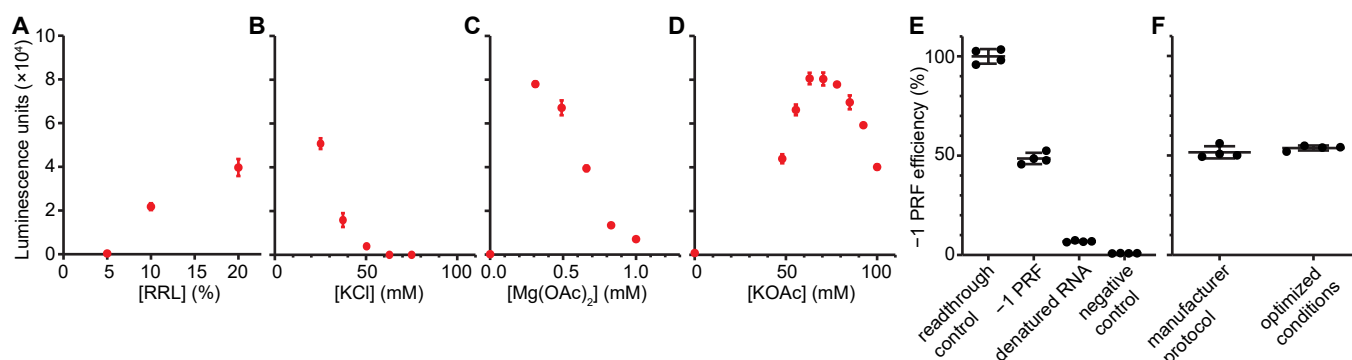| Compound	Change in *Renilla* luciferase signal (%)
Nafamostat	−47 ± 9
Abemaciclib	−29 ± 5
Palbociclib	4 ± 15
Valnemulin	−28 ± 7

Figure S1: Optimization of dual-luciferase assay for high-throughput screening of −1 PRF in SARS-CoV-2. The following were sequentially optimized: (A) RRL content, (B) potassium chloride concentration, (C) magnesium acetate concentration, and (D) potassium acetate concentration. Each condition was measured in triplicate. (E) Using optimized conditions, ~50% −1 PRF efficiency was measured for the SARS-CoV-2 frameshift signal. −1 PRF was dependent on RNA structure: thermally denaturing RNA for 5 min at 95 °C followed by snap cooling on ice reduced −1 PRF efficiency to near zero. (F) Optimized conditions with 20% RRL have the same −1 PRF efficiency as reactions using the manufacturer protocol with 70% RRL (unpaired t-test with Welch's correction, $p = 0.26$).
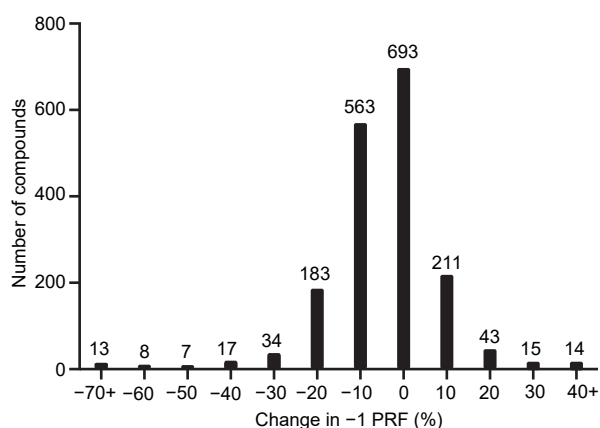

Figure S2: Results of drug-library screening. Distribution of effect sizes for change in −1 PRF using the SARS-CoV-2 frameshift reporter.
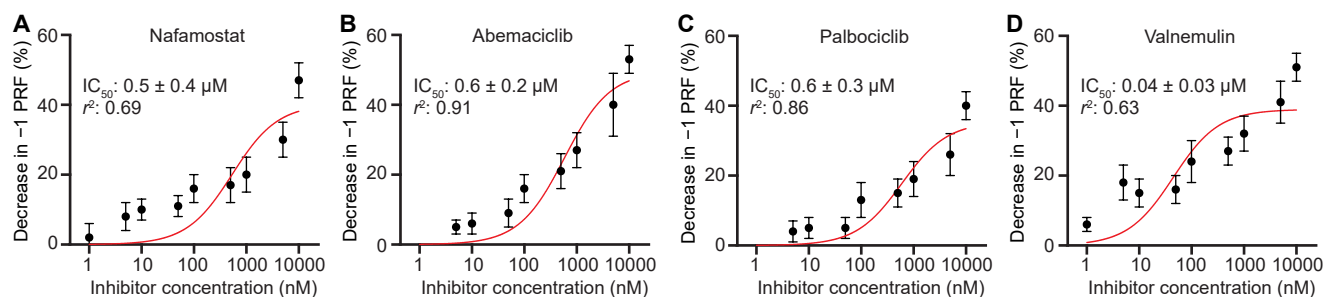

Figure S3: Dose-response curves for −1 PRF inhibition by compounds found in screen. Dose-response curves for (A) nafamostat, (B) abemaciclib, (C) palbociclib, and (D) valnemulin were fit to the Hill equation with slope 1 to find IC_{50}. Error bars represent standard error on the mean from 6 replicates.
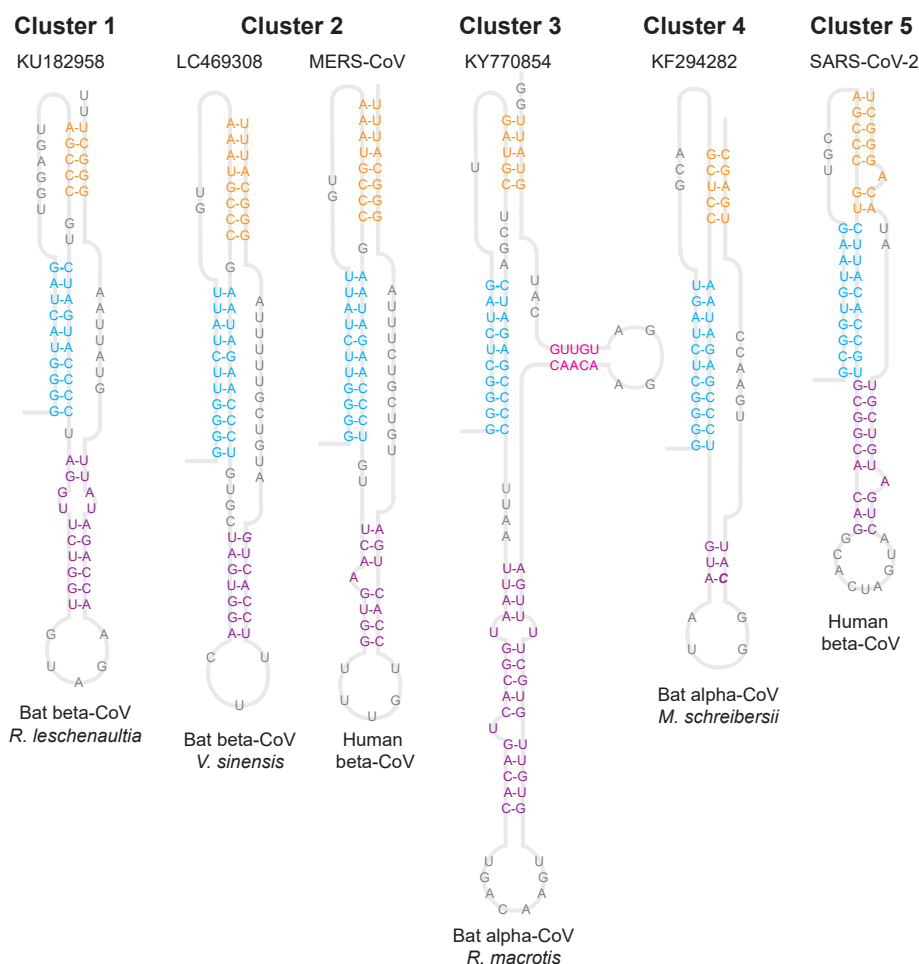
Figure S4: Secondary structure predictions for pseudoknots. Lowest-energy consensus structures homologous to the structures of the SARS-CoV and SARS-CoV-2 pseudoknots. Stem 1: cyan, stem 2: orange, stem 3: purple, stem 4: magenta, loops: grey.
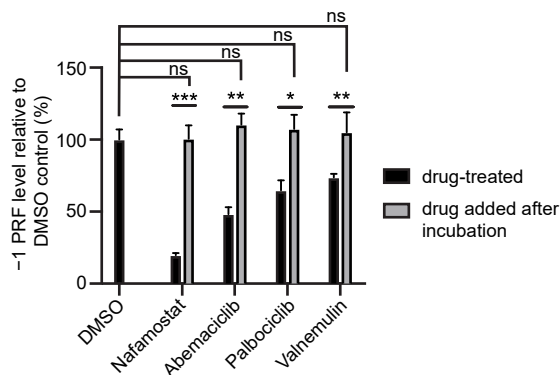

Figure S5: Testing for selective inhibition of firefly luciferase. The compounds from the drug screen that were used in testing against the panel of CoV frameshift signals did not selectively inhibit firefly luciferase: adding the drugs after incubating the translation product with luciferase assay reagents did not alter the firefly:*Renilla* luminescence ratio compared to the untreated case (unpaired t-test with Welch's correction, $p = 0.17$–0.95) and yielded a significantly different −1 PRF level compared to adding the drugs before translation ($p = 0.019$ to less than 0.0001).