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Correlated sequence signatures are present within the genomic 5'UTR RNA and NSP1 protein in coronaviruses



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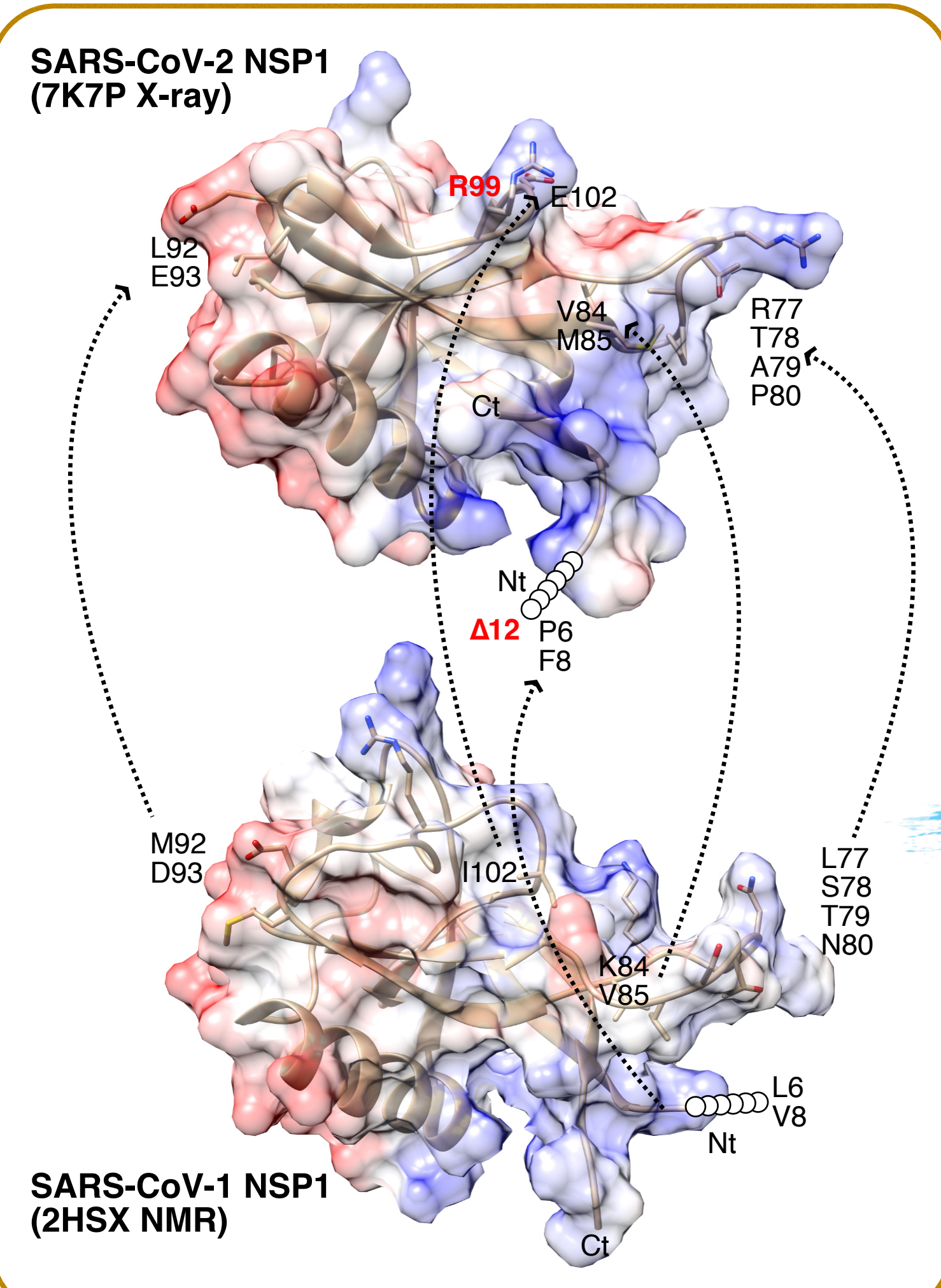
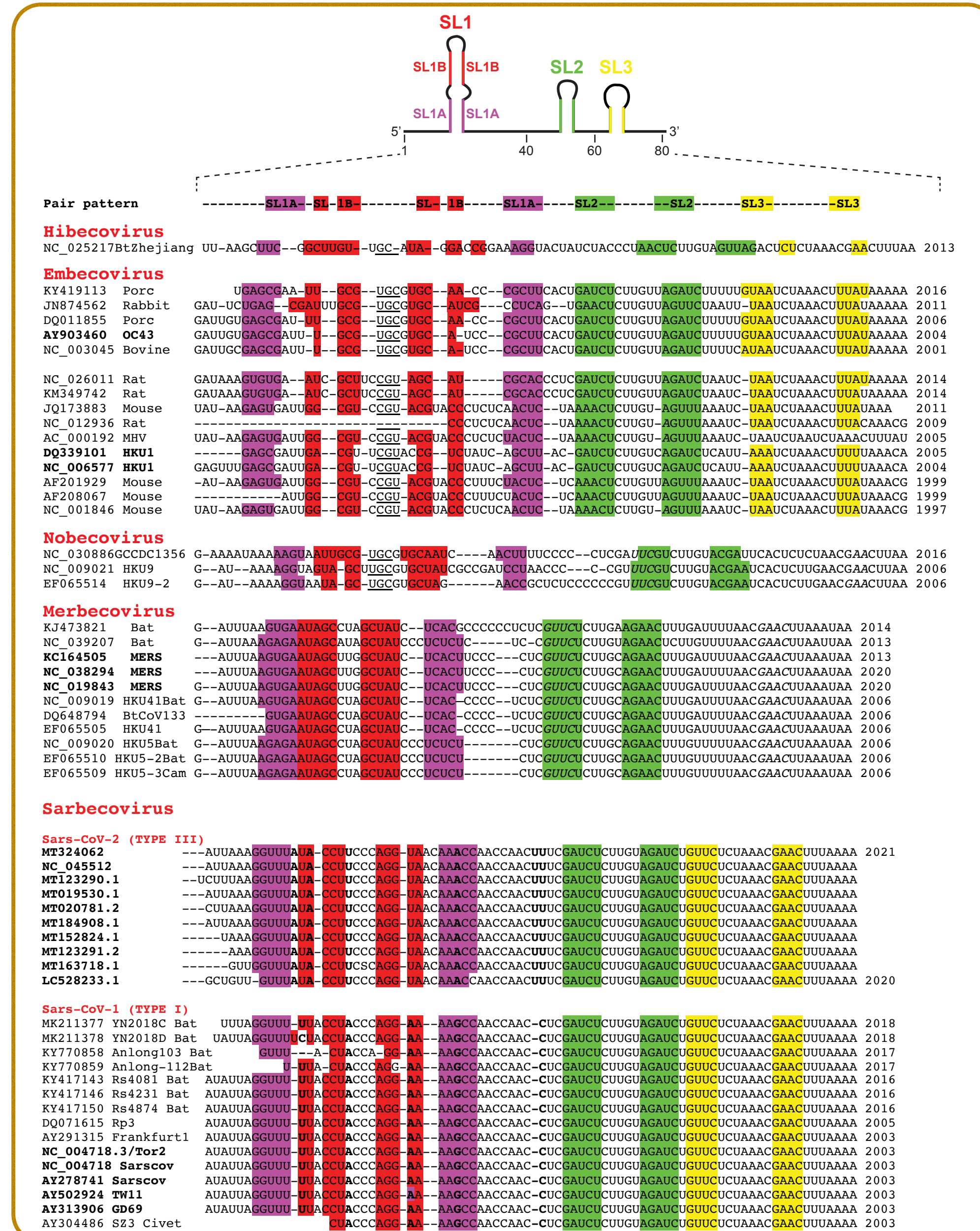
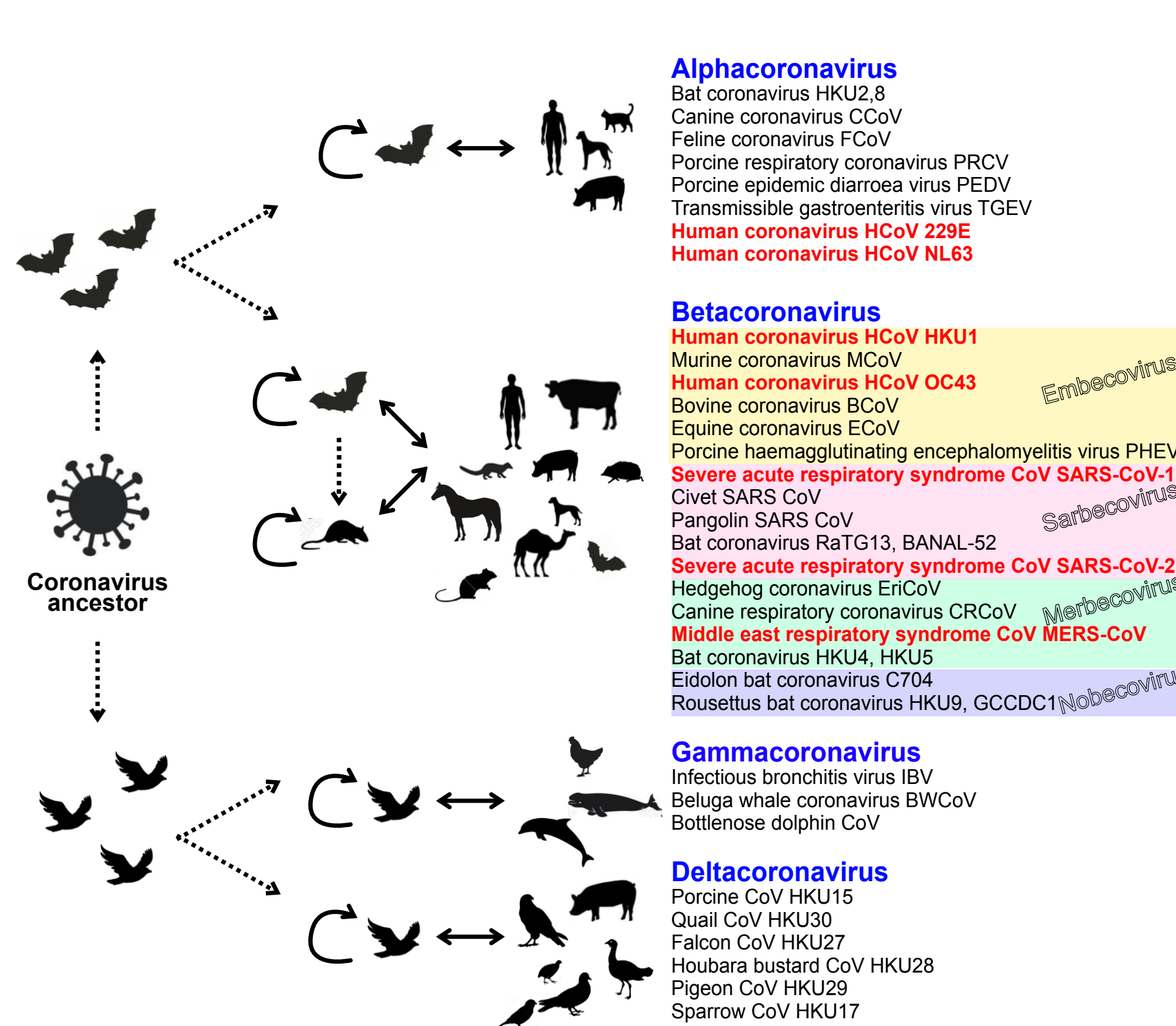
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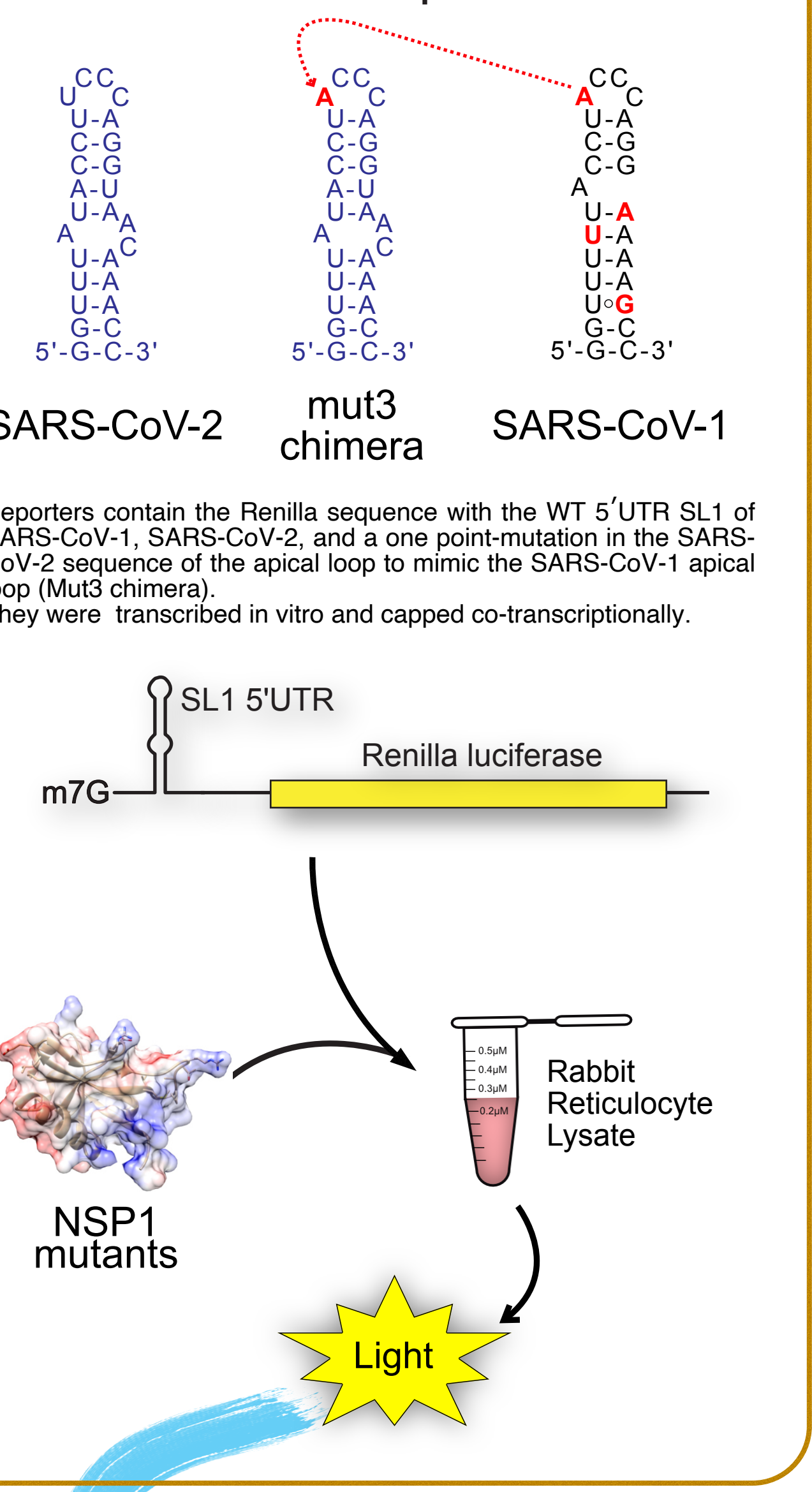
The 5'UTR part of coronavirus genomes plays key roles in the viral replication cycle and the translation of the viral mRNAs. The first 75-80 nucleotides, also called the leader sequence, are identical for the genomic mRNA and for the subgenomic mRNAs. Cooperative actions of a 5'UTR SL1 element and the non-structural protein NSP1 are essential for both the inhibition of host mRNAs and for specific translation of viral mRNAs¹⁻⁵. Sequence analyses of both the 5'UTR RNA segment and the NSP1 protein have been done for several coronaviruses with special attention to the betacoronaviruses. It appears that precise specific molecular signatures can be found in both the RNA and the NSP1 protein⁶. Indeed, definite sequence motifs in the RNA correlate with sequence motifs in the protein indicating a co-evolution between the 5'UTR and NSP1 in betacoronaviruses. In the Sarbecovirus subgenus, NSP1 sequences align very well. On the other hand, they do not align well with those of either the Merbecovirus or the Embecovirus⁶. Here we present mutational data on 5'UTR and NSP1 from SARS-CoV-2 and SARS-CoV-1 using cell-free translation extracts. We show that some conserved key residues in the N-terminal half of the NSP1 protein are essential for evasion to the inhibitory effect of NSP1 on translation⁶.

¹ Miao Z, Tidu A, Eriani G and Martin F (2020) Secondary structure of the SARS-CoV-2 5'UTR. RNA Biology, 23, 1-10.
² Tidu A, Janvier A, Schaeffer L, Sosnowski P, Kuhn L, Hammann P, Westhof E, Eriani G and Martin F (2021) The viral protein NSP1 acts as a ribosome gatekeeper for shutting down host translation and fostering SARS-CoV-2 translation. RNA, 27, 253-264.
³ Schubert K, Karousis ED, Jomaa A, Scaiola A, Echeverria B, Gurlzer LA, Leibundgut M, Thiel V, Mühlemann O, Ban N (2020) SARS-CoV-2 Nsp1 binds the ribosomal mRNA channel to inhibit translation. Nat Struct Mol Biol 27, 959-966.
⁴ Thoms M, Buschauer R, Amelmeier M, Koepke L, Denk T, Hirschenberger M, Kratzat H, Hayn M, MacKens-Kiani T, Cheng J, Straub JH, Stürzel CM, Fröhlich T, Berninghausen O, Becker T, Kirchhoff F, Sparrer KMJ, Beckmann R (2020) Structural basis for translational shutdown and immune evasion by the Nsp1 protein of SARS-CoV-2. Science 369, 1249-1256.
⁵ Mendez AS, Ly M, González-Sánchez AM, Hartenian E, Ingolia NT, Cate JH, Glaunsinger BA. 2021. The N-terminal domain of SARS-CoV-2 nsp1 plays key roles in suppression of cellular gene expression and preservation of viral gene expression. Cell Rep 37, 109841.
⁶ Sosnowski P, Tidu A, Eriani G, Westhof E and Martin F (2022) Correlated sequence signatures are present within the genomic 5'UTR RNA and NSP1 protein in coronaviruses. RNA 28, 729-741.

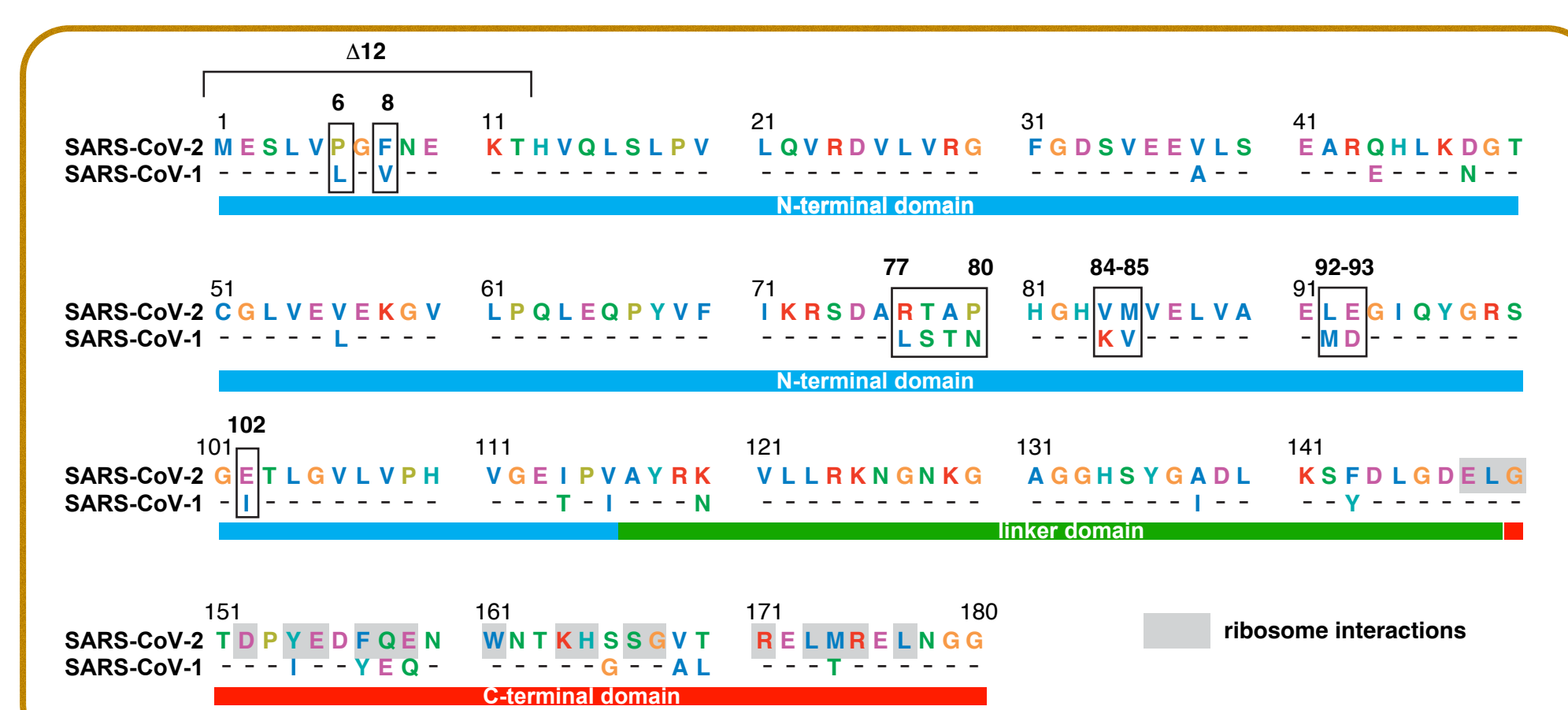


Amino acid residues specific of SARS-CoV-1 that were swapped into a SARS-CoV-2 background. They were chosen on the 'front' side of NSP1 where is found the previously studied R99A mutation (Mendez et al. 2021). R99A abrogates viral translation evasion while reducing the translation inhibition activity of NSP1 against cellular mRNA. This suggested that SL1 might interact by contacts with residues located on the same front side.
 Ten NSP1 proteins were overproduced and purified, corresponding to 11 amino acid residues located on the 'front' side.

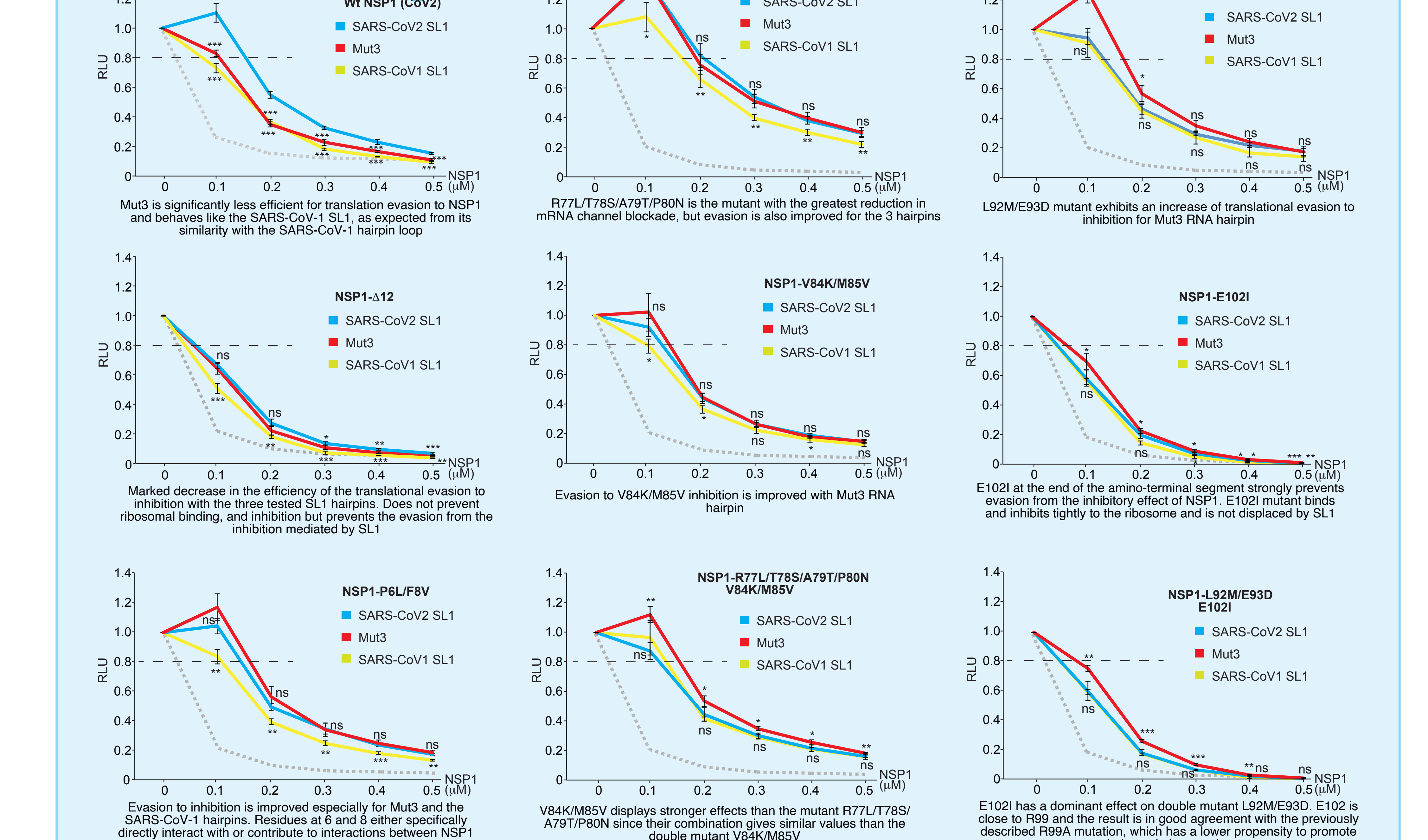
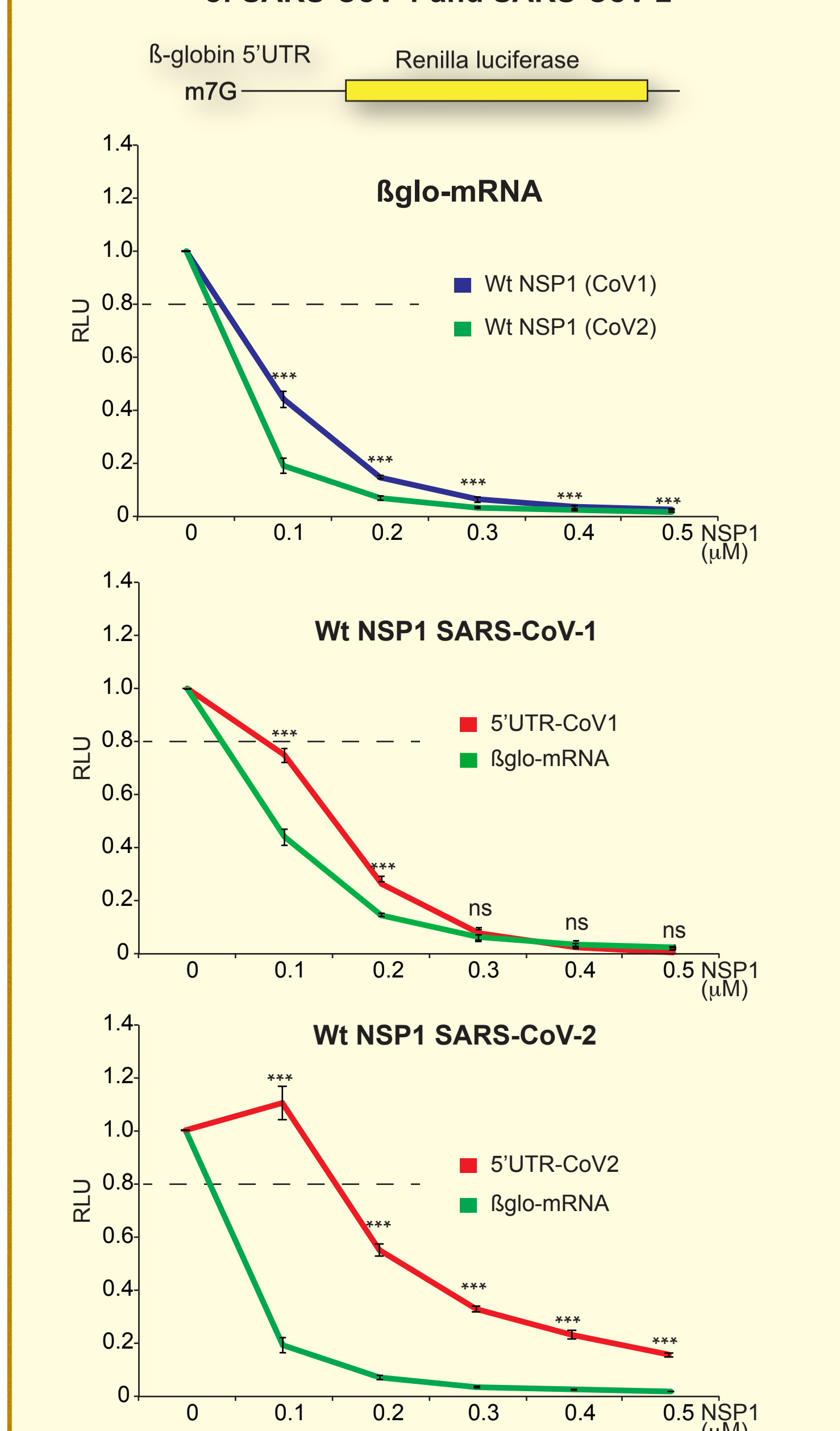
Three SL1 tested in the 5' UTR of the renilla luciferase reporter



Hibecovirus subgenus contains a single sequence and its SL1 apical loop has similarities (UGC) with that of the Nobeovirus. These two subgenera have been observed only in bats. The Embecovirus subgenus displays a larger variation in sequence types (first sequence dates to 1997). Two subgroups exist depending on the apical loop of SL1, either UGC (like in the Nobe- and Hibecovirus) or CGU. The sequences of the Merbecovirus subgenus display a much larger conservation with a 3-nt loop, often -CUA. Two subgroups can be distinguished in the Sarbecovirus subgenus: the SARS-CoV-1 and SARS-CoV-2. The sequence conservation is high and for SARS-CoV-1, it extends over 15 years. The similarities between the two subgroups are extensive: SL2 and SL3 are identical, the stem of SL1 presents only a change from a UoG to a U-A pair. The main differences are in SL1B, the first nucleotide of the SL1 hairpin loop (A-U), two positions 3' of following SL1, after the conserved stretch AACCAAC, there is a conserved UU in SARS-CoV-2 that is replaced by a single C in SARS-CoV-1.



Translation inhibitory properties of WILD-TYPE NSP1 of SARS-CoV-1 and SARS-CoV-2



The level of translation inhibition of SARS-CoV-1 NSP1 is significantly lower than that with SARS-CoV-2 NSP1. Similarly, the translation evasion of cognate viral mRNA constructs was also less efficient with SARS-CoV-1 NSP1 than with SARS-CoV-2 NSP1.

CONCLUSION

SARS-CoV NSP1 inhibits host mRNA translation by binding to the 40S ribosomal subunit and blocking the mRNA channel while promoting translation of viral mRNA. We show here that mutations in the N-terminal domain of NSP1 can induce distinct types of effects on mRNA translation. Several mutations such as the deletion of the first residues of the N-terminal end or E102I mutant abrogates escape of SARS-CoV-2 leader mRNA. A partial loss of inhibitory capacity was observed for several mutants suggesting decreased stability on the ribosome. We also show that the presence of an A in the apical loop of SL1 confers the capacity for recognition by NSP1 originating from SARS-CoV-1 and that identity swapping can be achieved by substitution of this residue. Finally, residues specific to SARS-CoV-1 or -2 have been identified allowing recognition switches of the corresponding SL1. Altogether, data show that both the 5'UTR and the NSP1 protein are linked functionally and therefore are forced to coevolve to interact cooperatively with highly conserved elements of the ribosomal machinery and initiation cofactor proteins.