Supplementary data

Supplementary Figure S1: The cartoon represents the reporter mRNA containing the β-globin 5'UTR. The sequence of β-globin 5'UTR mRNA is shown and labelled with a radioactive cap (*). The RNA degradation assay consists of incubating for 5 min at 30°C the radioactive RNAs in rabbit reticulocyte lysates (RRL) in the absence (Ø) or presence of 0.1 and 0.5 μM of wt SARS-CoV-2 NSP1 and wt SARS-CoV-1 NSP1. The black numbers indicate the position of G residues that are detected in the RNase T1 ladder (T1). The blue numbers indicate the position of the C residues from the spontaneous CA breaks that can be detected on the PAGE and the green numbers indicate the position of the U residues from the spontaneous UA breaks that can be detected in the PAGE. The cleavage sites from the SARS-CoV2 NSP1 are indicated by yellow arrows and from the SARS-CoV-1 NSP1 are indicated by blue arrows.

Supplementary Figure S2: The cartoon represents the secondary structure of the reporter mRNA containing the SARS-CoV-2 subgenomic N 5'UTR. The sequence of SARS-CoV-2 subgenomic N mRNA is shown and labelled with a radioactive cap (*). The RNA degradation assay consists of incubating for 5 min at 30°C the radioactive RNAs in rabbit reticulocyte lysates (RRL) in the absence (Ø) or presence of 0.1 and 0.5 μM of wt SARS-CoV-2 NSP1 and wt SARS-CoV-1 NSP1. The black numbers indicate the position of G residues that are detected in the RNase T1 ladder (T1). The blue numbers indicate the position of the C residues from the spontaneous CA breaks that can be detected on the PAGE and the green numbers indicate the position of the U residues from the spontaneous UA breaks that can be detected in the PAGE. The cleavage sites from the SARS-CoV2 NSP1 are indicated by yellow arrows and from the SARS-CoV-1 NSP1 are indicated by blue arrows.

Supplementary Figure S3: The cartoon represents the secondary structure of the reporter mRNA containing the CrPV IGR and the sequence of CrPV IGR is shown below. The RNA degradation assay consists of incubating for 5 min at 30°C the radioactive RNAs in rabbit reticulocyte lysates (RRL) in the absence (Ø) or presence of 0.1 and 0.5 μM of wt SARS-CoV-2 NSP1 and SARS-CoV-2 N-terminal domain (NTD). The black numbers indicate the position of G residues that are detected in the RNase T1 ladder

(T1). The blue numbers indicate the position of the C residues from the spontaneous CA breaks that can be detected on the PAGE and the green numbers indicate the position of the U residues from the spontaneous UA breaks that can be detected in the PAGE. No cleavage sites are detected.

Supplemental Figure S4: (**A**) The cartoon represents four types of RNA substrates. First, chimeric RNAs containing the 5'UTR of SARS-CoV-2 subgenomic RNA coding for the Nucleocapsid (CoV2-sub-N), second a truncated version in which SL1 has been deleted (ΔSL1-CoV2-subN), third chimeric RNA containing the 5'UTR of the SARS-CoV-2 genomic RNA (Cov2-gen), fourth a version of the chimeric RNA in which SL1 has been mutated in its apical part (MutSL1-Cov2-gen). (**B**) RNA degradation assay of the four reporter mRNAs. The full-length 5' labelled RNA transcripts are incubated for 5 min at 30°C in the presence of rabbit reticulocyte lysate (RRL) in absence (Ø) or in the presence of 0.1 and 0.5 μM of wt NSP1 protein and analysed by denaturing 12% PAGE.

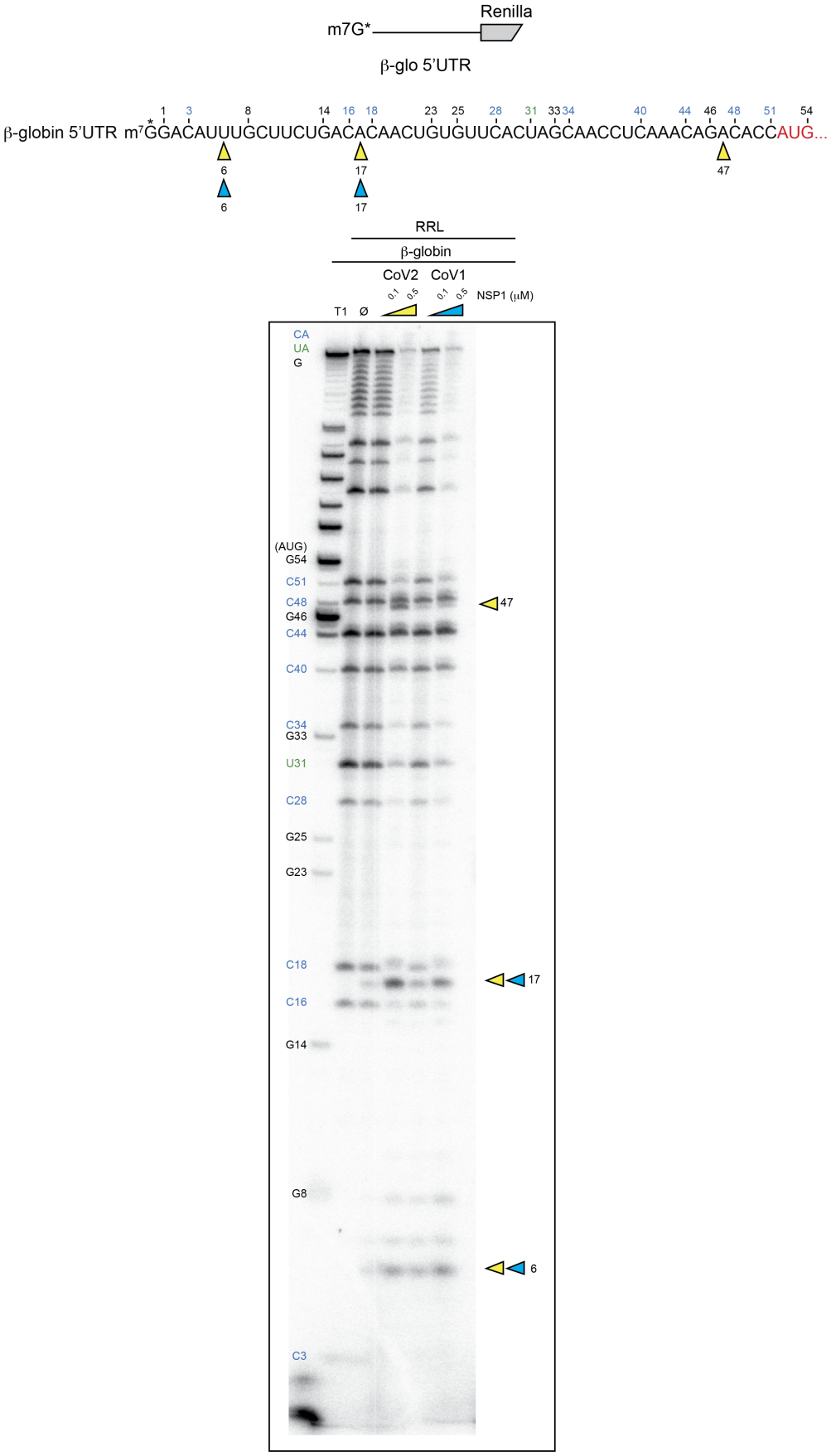
Supplemental Figure S5: The cartoon represents the reporter mRNA containing the ΔSL1-CoV2-sub-N 5'UTR. The sequence of ΔSL1-CoV2-sub-N mRNA is shown and labelled with a radioactive cap (*). The RNA degradation assay consists of incubating for 5 min at 30°C the radioactive RNAs in rabbit reticulocyte lysates (RRL) in absence (Ø) or in the presence of 0.1 and 0.5 μM of wt or R99A SARS-CoV-2 NSP1. The black numbers indicate the position of G residues that are detected in the RNase T1 ladder (T1). The blue numbers indicate the position of the C residues from the spontaneous CA breaks that can be detected on the PAGE and the green numbers indicate the position of the U residues from the spontaneous UA breaks that can be detected in the PAGE. The mutated residues are boxed. The cleavage sites are indicated by yellow arrows, the cleavage sites that are detected in wt subgenomic N SARS-CoV-2 5'UTR are shown by dashed line arrows.

Supplemental Figure S6: The cartoon represents the reporter mRNA containing the MutSL1-CoV2-gen 5'UTR. The sequence of MutSL1-CoV2-gen mRNA is shown and labelled with a radioactive cap (*) The positions of the mutated nucleotides in SL1 are shown in bold and red. The RNA degradation assay consists of incubating for 5 min at 30°C the radioactive RNAs in rabbit reticulocyte lysates (RRL) in absence (Ø) or in the

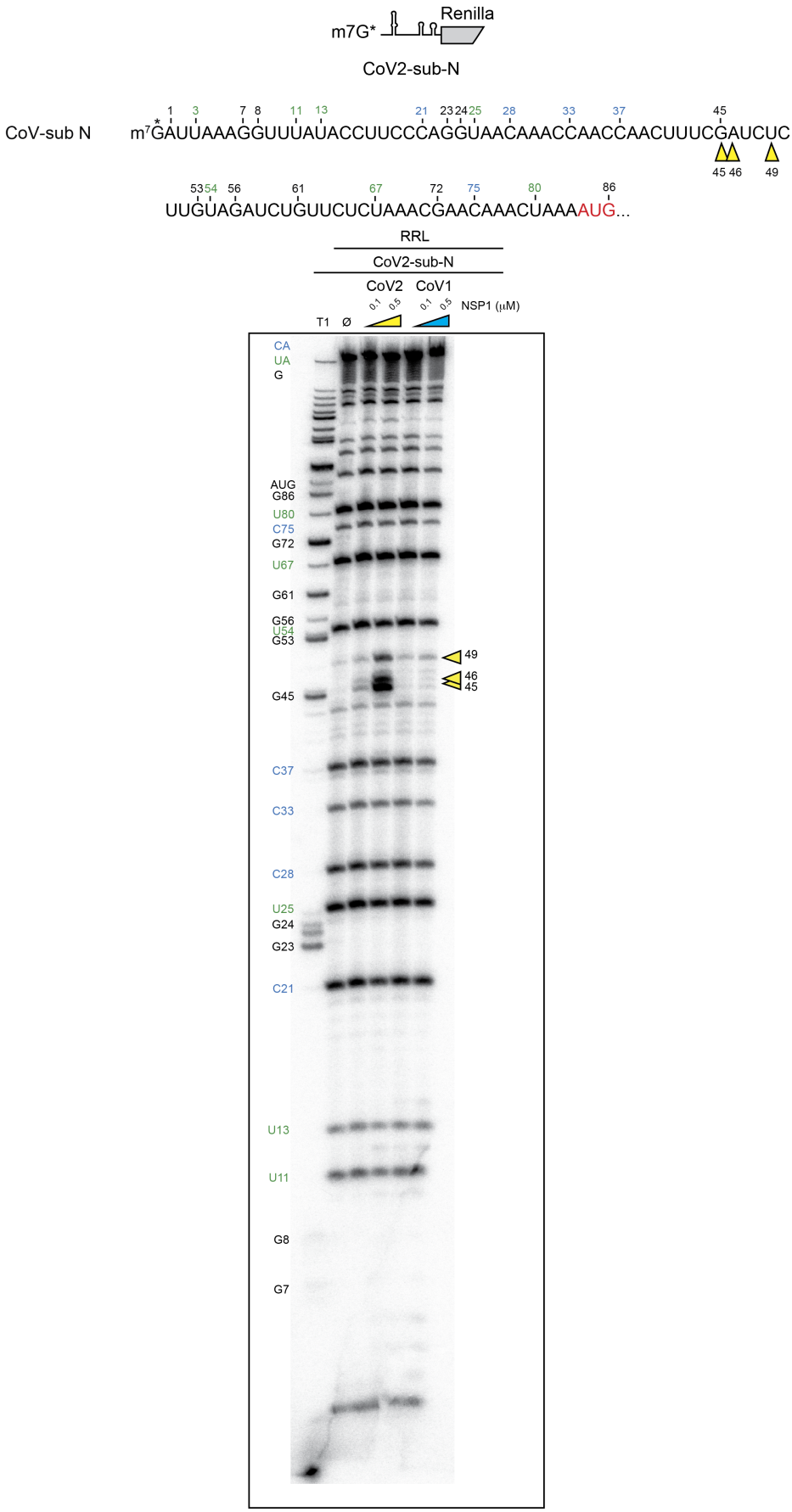
presence of 0.1 and 0.5 μM of wt or R99A SARS-CoV-2 NSP1. The black numbers indicate the position of G residues that are detected in the RNase T1 ladder (T1). The blue numbers indicate the position of the C residues from the spontaneous CA breaks that can be detected on the PAGE and the green numbers indicate the position of the U residues from the spontaneous UA breaks that can be detected in the PAGE. The mutated residues are boxed. The cleavage sites are indicated by yellow arrows, the cleavage sites that are detected in the wt SARS-CoV-2 5'UTR are shown by dashed line arrows.

Supplementary Figure S7: (A) The cartoon represents the secondary structure of the reporter mRNA containing the SARS-CoV-2 genomic 5'UTR. The NSP1-mediated cleavage sites in SL2 are indicated by yellow arrows. **(B)** The cartoon represents the reporters mRNAs obtained with the cleavage products of SARS-CoV-2 genomic 5'UTR and their putative secondary structure Δ45, Δ46 and Δ49. **(C)** Translation assays in the absence (Ø) or presence of 0.1 and 0.2 μM of wt SARS-CoV-2 NSP1. **(D)** The histogram represents the quantification of Renilla luciferase activity of Δ45, Δ46 and Δ49 relative to wt Cov2-gen.

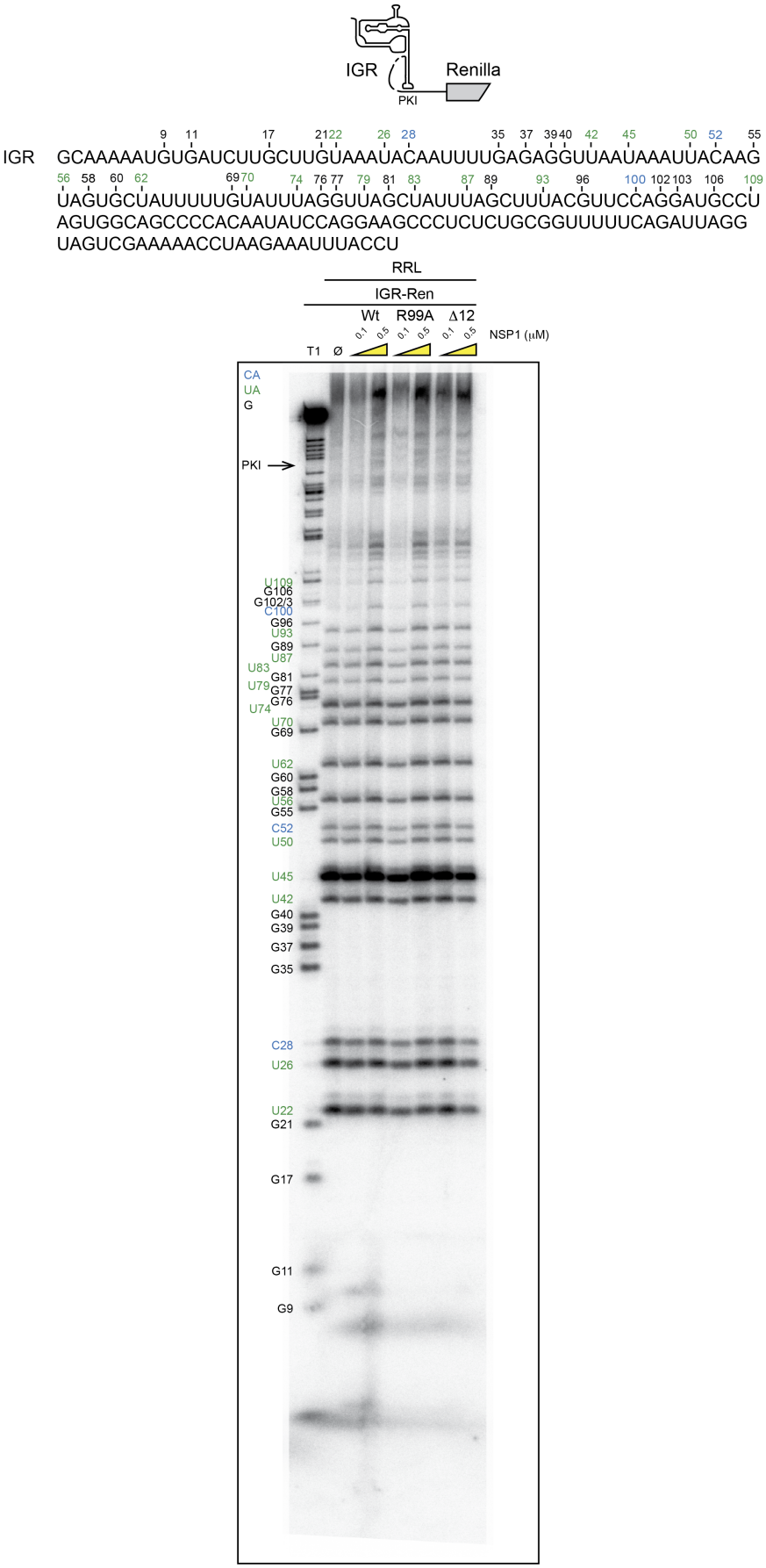
Supplemental Figure S1



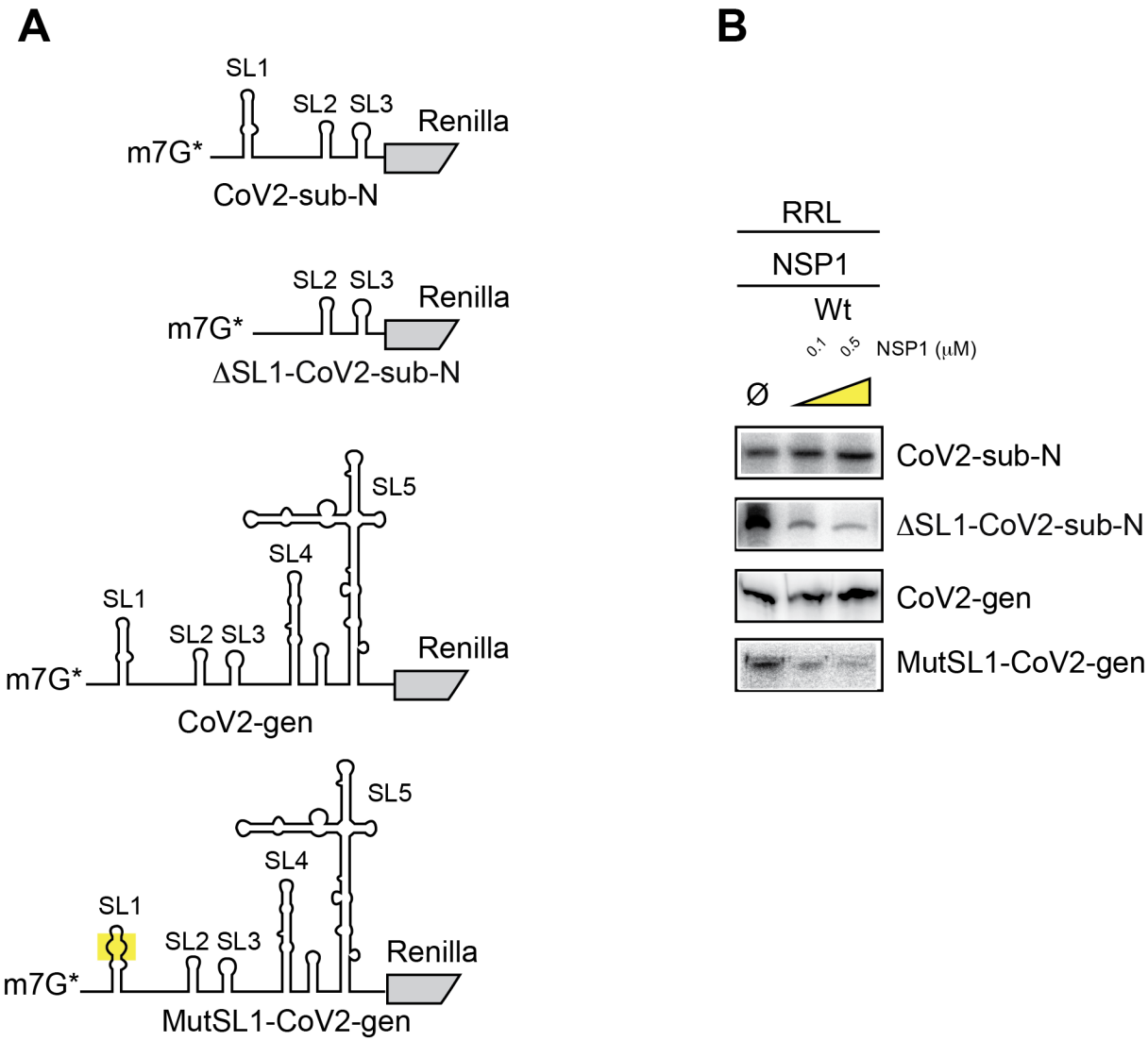
Supplemental Figure S2



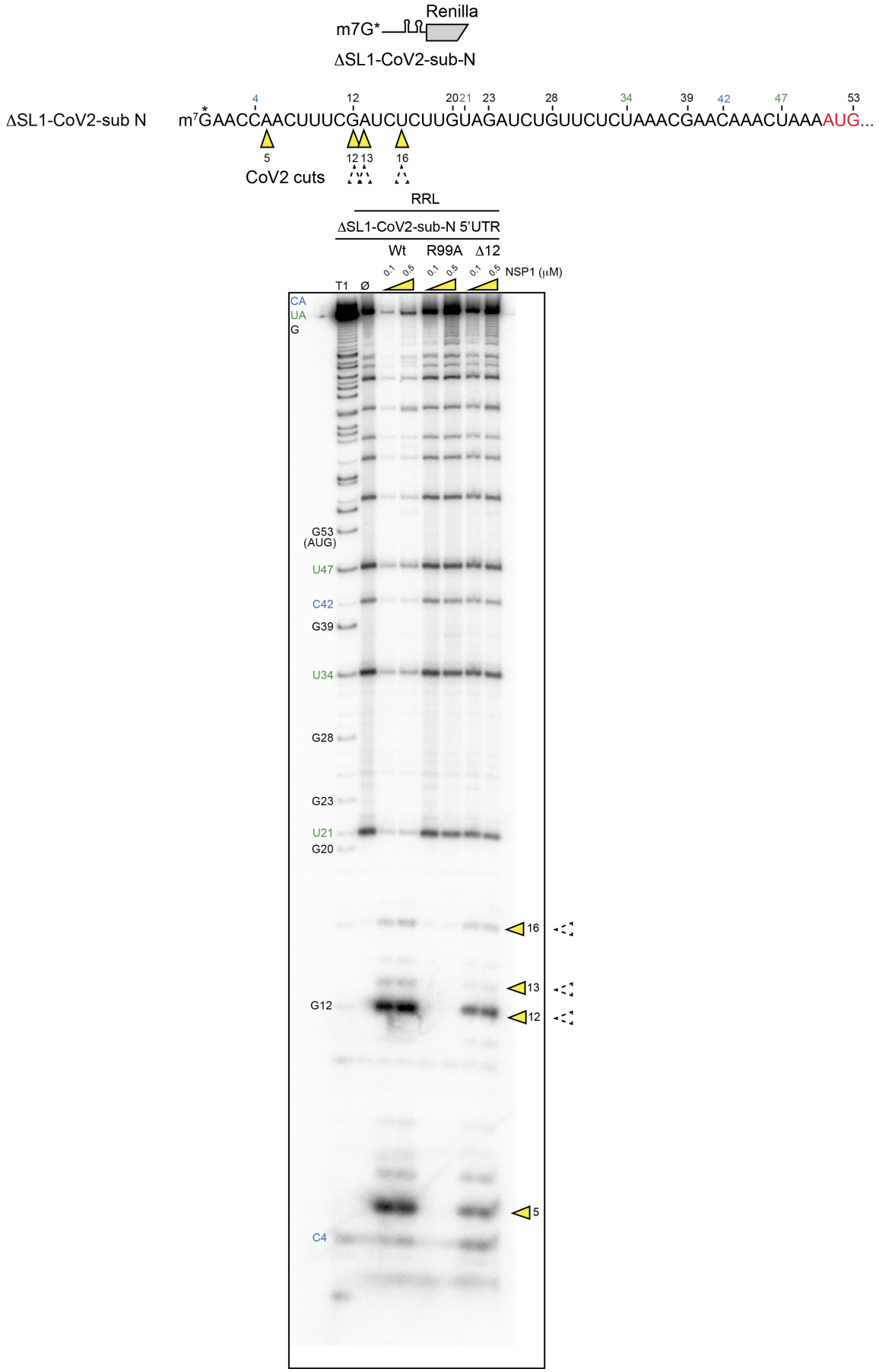
Supplemental Figure S3



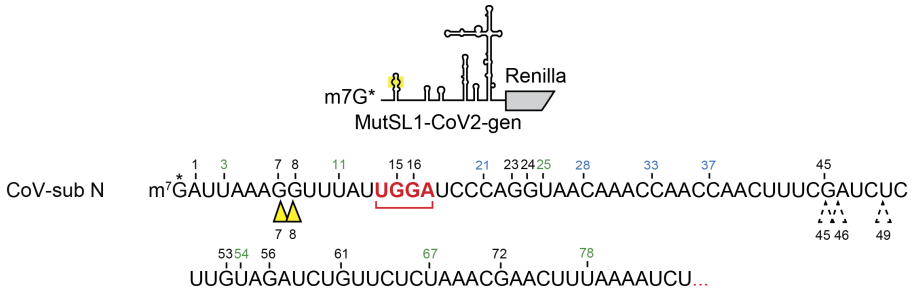
Supplemental Figure S4

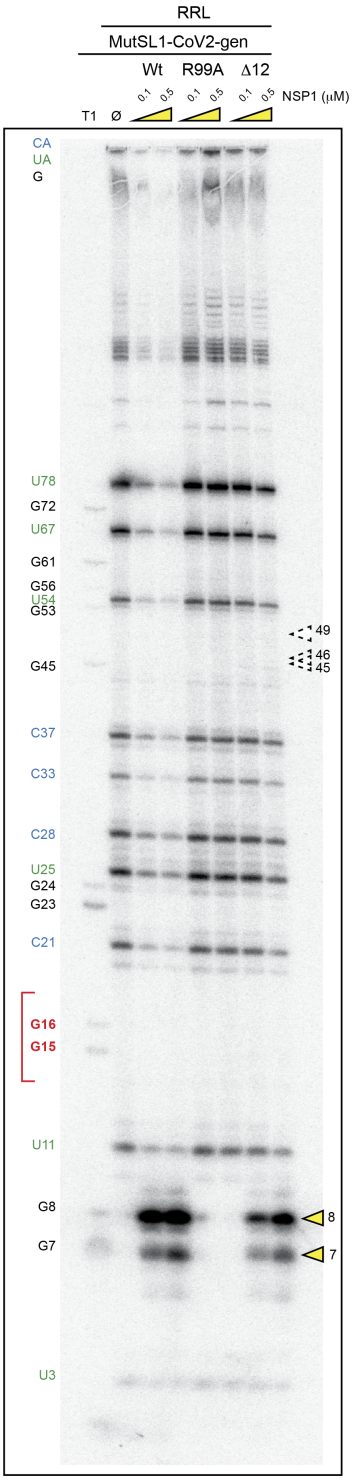


Supplemental Figure S5



Supplemental Figure S6





Supplemental Figure S7