Supplemental figures

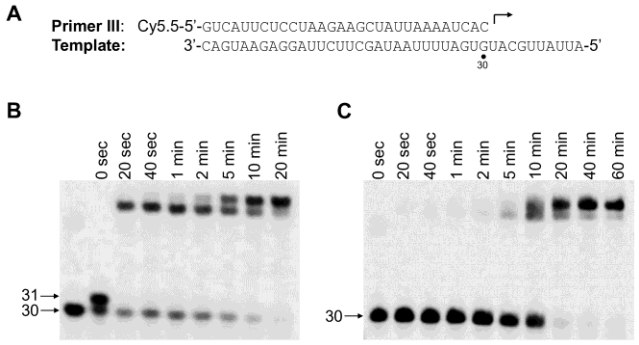


Fig. S1. Time-course analysis of RNA synthesis catalyzed by RdRp. (A) P/T used in this assay. (B) Time-course analysis of RNA synthesis with preformed RNA/RdRP complex. Nsp12 (50 nM) and nsp8-7 (2 μM) were incubated with 5 nM P/T and 0.1 μM ATP (the first nucleotide to be incorporated) in reaction buffer for 30 min at 37 °C to allow the first nucleotide incorporation, which promotes the complete formation of RNA/RdRp complex. After cool down to 22 °C, NTP (100 μM) was added to reaction mixture and aliquots (5 μl) was taken from the reaction mixture at different time indicated above each lane after adding NTP and mixed with 10 μl stopping solution. The products were resolved by denaturing PAGE. (C) Time-course analysis of RNA synthesis without preformed RNA/RdRp complex. Nsp12 (50 nM) and nsp8-7 (2 μM) were incubated with 5 nM P/T and 100 μM NTP in reaction buffer at 22 °C, and aliquots (5 μl) was taken from the reaction mixture at different time indicated above each lane after adding NTP and mixed with 10 μl stopping solution. The products were resolved by denaturing PAGE.

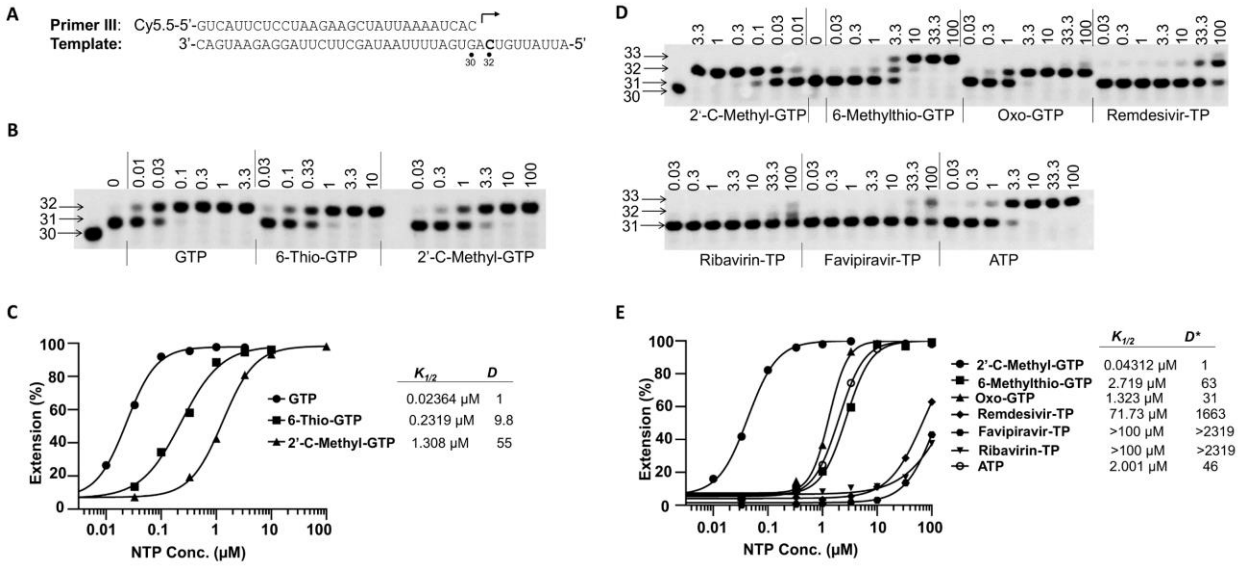


Fig. S2. Measurement of the discrimination value of GTP analogs. (A) The primer and template used to assay GTP analog shown in panel B and D. **(B)** A representative image of the results of the analysis of $D_{6\text{-Thio-GTP}}$ and $D_{2'\text{-C-methyl-GTP}}$ values. Nsp12 (50 nM), and nsp8-7 (2 μM) were incubated with 5 nM P/T and 0.1 μM UTP (the first nucleotide to be incorporated) in reaction buffer for 30 min at 37 °C and then rapidly mixed with different concentrations (in micromolar) of GTP, 6-Thio-GTP or 2'-C-methyl-GTP, as indicated above each lane. The reactions were continued at 22 °C for 20 s before the addition of stopping solution, and the products were resolved by denaturing PAGE. The identity of the tested nucleotide is indicated at the bottom of the gel. The location of 30-mer primer and 31-mer and 32-mer (first and second nucleotide extension products, respectively) are indicated on the left of the gel. **(C)** Quantitative analysis of GTP, 6-Thio-GTP, and 2'-C-methyl-GTP incorporation in the assay whose results are shown in panel B. The incorporation efficiency was evaluated based on the extension of 31-mer to 32-mer products. The measured $K_{1/2}$ values for GTP, 6-Thio-GTP, and 2'-C-methyl-GTP in this experiment were 0.02364 μM, 0.2319 μM and 1.308 μM, respectively. The discrimination values were calculated using the equation as $K_{1/2,\text{ GTP analog}}/K_{1/2,\text{ GTP}}$ and are shown on the right of the graph. **(D)** A representative image of the result of the analysis of GTP analogs. Primer extension reactions were performed using a similar method described in Panel B. After adding different concentrations of GTP analog, the reactions were continued at 22 °C for 15 min before adding stopping solution. **(E)** Quantitative analysis of GTP analogs incorporation in the assay whose results are shown in panel D. $K_{1/2}$ analysis is as same as described in panel B and C. The discrimination between GTP analog and 2'-C-Methyl-GTP, $D^*_{\text{GTP analog}}$, was calculated as $K_{1/2,\text{ GTP analog}}/K_{1/2,\text{ 2'-C-methyl-GTP}}$, and the values are shown on the right of the graph. The discrimination between GTP analogs and natural GTP, $D^{cal}_{\text{GTP analog}}$ was calculated as $D^*_{\text{GTP analog}} \times D_{2'\text{-C-methyl-GTP}}$. $D_{2'\text{-C-Methyl-GTP}}$ is 61.7 ± 9.0 (average of 2 independent experiments, one is shown in panel C). For 6-methylthio-GTP, Oxo-GTP, remdesivir-TP, ribavirin-TP, favipiravir-TP and ATP (mis-incorporated as GTP) calculated values of D^{cal}_{analog} were 4516 ± 884, 2317 ± 600, 110222 ± 10726, >143089, >143089 and 3427 ± 797, respectively (average of 2 independent experiments, one is shown in this figure).

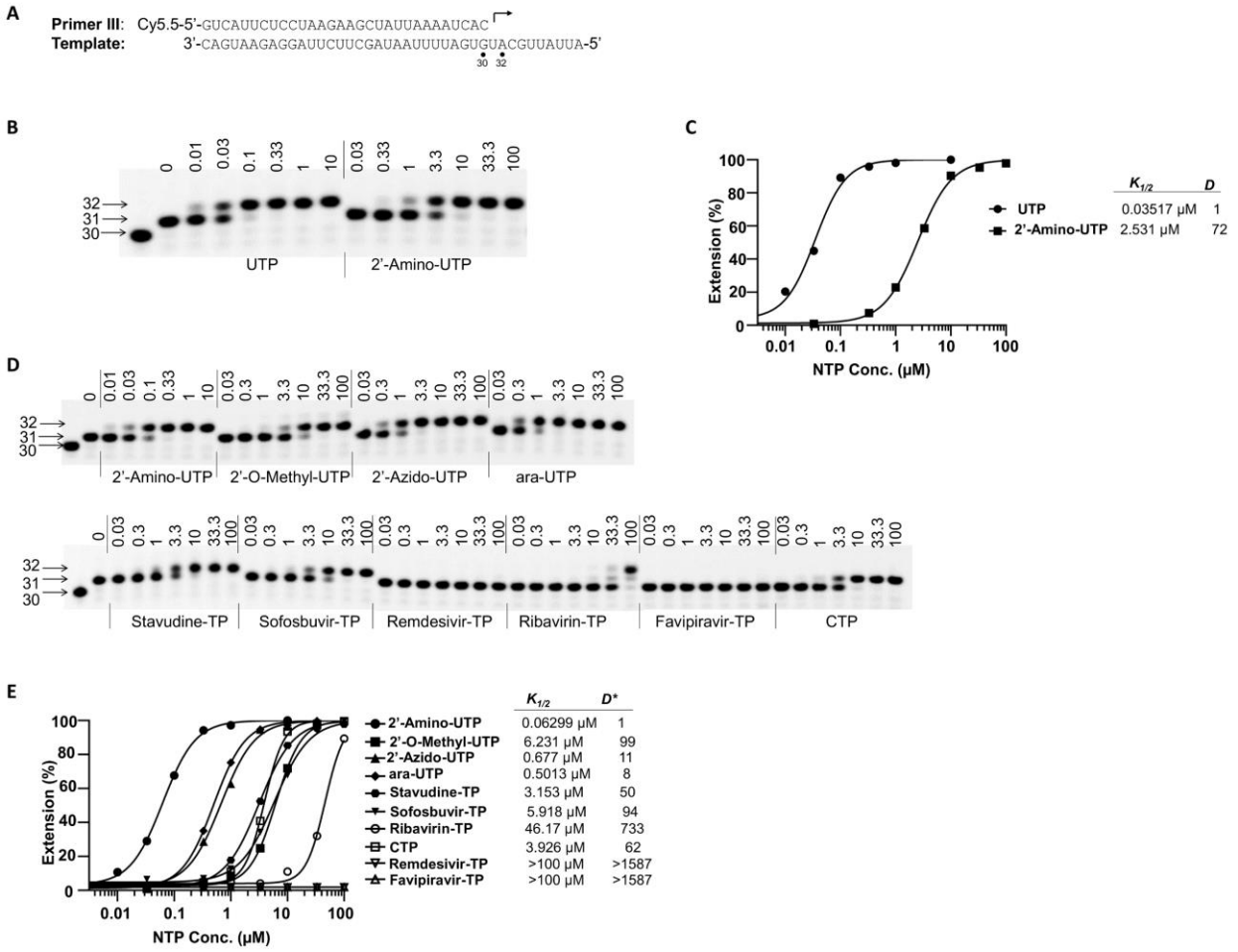


Fig. S3. Measurement of the discrimination value of UTP analogs. (A) The primer and template used to assay UTP analog shown in panel B and D. **(B)** A representative image of the results of the analysis of $D_{2'\text{-amino-UTP}}$ values. Nsp12 (50 nM), and nsp8-7 (2 μM) were incubated with 5 nM P/T and 0.1 μM ATP (the first nucleotide to be incorporated) in reaction buffer for 30 min at 37 °C and then rapidly mixed with different concentrations (in micromolar) of UTP, 2'-Amino-UTP, as indicated above each lane. The reactions were continued at 22 °C for 20 s before the addition of stopping solution, and the products were resolved by denaturing PAGE. The identity of the tested nucleotide is indicated at the bottom of the gel. The location of 30-mer primer, and 31-mer and 32-mer (first and second nucleotide extension products, respectively) are indicated on the left of the gel. **(C)** Quantitative analysis of UTP, 2'-amino-UTP incorporation in the assay whose results are shown in panel B. The incorporation efficiency was evaluated based on the extension of 31-mer to 32-mer products. The measured $K_{1/2}$ values for UTP, 2'-amino-UTP in this experiment were 0.03517 μM and 2.531 μM, respectively. The discrimination values were calculated using the equation as $K_{1/2,\text{ UTP analog}}/K_{1/2,\text{ UTP}}$ and are shown on the right of the graph. **(D)** A representative image of the result of the analysis of UTP analogs. Primer extension reactions were performed using a similar method described in Panel B. After adding different concentrations of UTP analog, the reactions were continued at 22 °C for 15 min before adding stopping solution. **(E)** Quantitative analysis of UTP analogs incorporation in the assay whose results are shown in panel D. $K_{1/2}$ analysis is as same as described in panel B and C. The

discrimination between UTP analog and 2'-amino-UTP, $D^*_{\text{UTP analog}}$, was calculated as $K_{1/2,\ \text{UTP analog}}/K_{1/2,\ 2'\text{-amino-UTP}}$, and the values are shown on the right of the graph. The discrimination between UTP analogs and natural UTP, $D^{cal}_{\text{UTP analog}}$ was calculated as $D^*_{\text{UTP analog}} \times D_{2'\text{-amino-UTP}}$. $D_{2'\text{-amino-UTP}}$ is 75 ± 5 (average of 2 independent experiments, one is shown in panel C). For 2'-O-methyl-UTP, 2'-azido-UTP, ara-UTP, stavudine-TP, sofosbuvir-TP, ribavirin-TP and CTP (mis-incorporated as UTP) calculated values of $D^{cal}_{\text{UTP analog}}$ were 7688 ± 380, 831 ± 35, 625 ± 40, 4109 ± 502, 7307 ± 368, 61933 ± 9843 and 5131 ± 645, respectively (average of 2 independent experiments, one is shown in this figure).

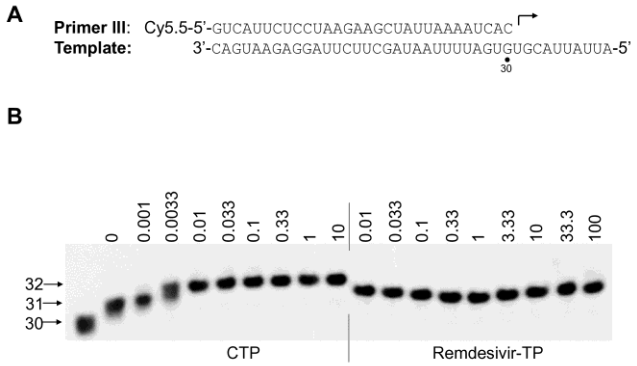
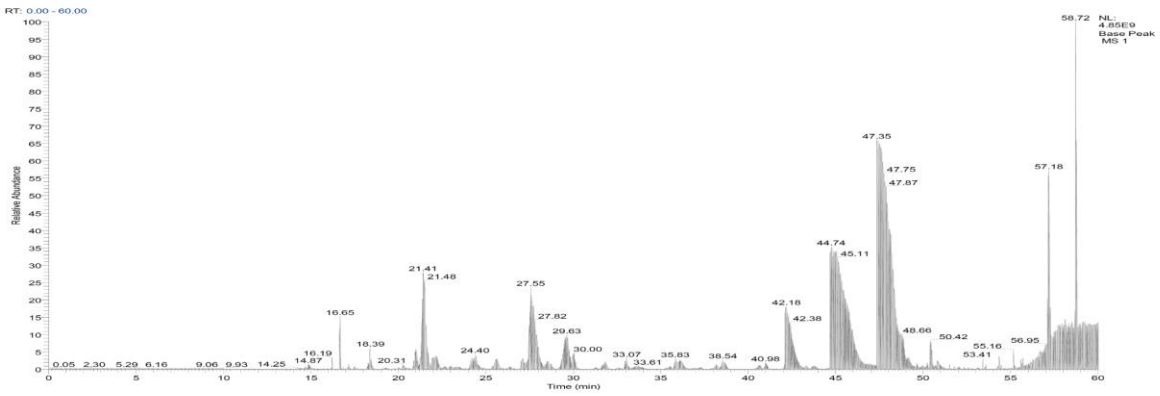


Fig. S4. Remdesivir-TP cannot be incorporated as CTP analog. **(A)** The primer and template used to assay CTP analog shown in panel B. **(B)** Image of the analysis of remdesivir-TP as CTP analog. Nsp12 (50 nM), and nsp8-7 (2 μM) were incubated with 5 nM P/T and 0.1 μM ATP (the first nucleotide to be incorporated) in reaction buffer for 30 min at 37 °C and then rapidly mixed with different concentrations (in micromolar) of CTP or remdesivir-TP, as indicated above each lane. The reactions were continued at 22 °C for 20 min before the addition of stopping solution, and the products were resolved by denaturing PAGE. The identity of the tested nucleotide is indicated at the bottom of the gel. The location of 30-mer primer, and 31-mer and 32-mer (first and second nucleotide extension products, respectively) are indicated on the left of the gel.

A



B

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 61   DEDDNLIDSY FVVKRHTFSN YQHEETIYNL LKDCPAVAKH DFFKFRIDGD MVPHISRQRL
121   TKYTMADLVY ALRHFDEGNC DTLKEILVTY NCCDDDYFNK KDWYDFVENP DILRVYANLG
181   ERVRQALLKT VQFCDAMRNA GIVGVLTLDN QDLNGNWYDF GDFIQTTPGS GVPVVDSYYS
241   LLMPILTLTR ALTAESHVDT DLTKPYIKWD LLKYDFTEER LKLFDRYFKY WDQTYHPNCV
301   NCLDDRCILH CANFNVLFST VFPPTSFGPL VRKIFVDGVP FVVSTGYHFR ELGVVHNQDV
361   NLHSSRLSFK ELLVYAADPA MHAASGNLLL DKRTTCFSVA ALTNNVAFQT VKPGNFNKDF
421   YDFAVSKGFF KEGSSVELKH FFFAQDGNAA ISDYDYYRYN LPTMCDIRQL LFVVEVVDKY
481   FDCYDGGCIN ANQVIVNNLD KSAGFPFNKW GKARLYYDSM SYEDQDALFA YTKRNVIPTI
541   TQMNLKYAIS AKNRARTVAG VSICSTMTNR QFHQKLLKSI AATRGATVVI GTSKFYGGWH
601   NMLKTVYSDV ENPHLMGWDY PKCDRAMPNM LRIMASLVLA RKHTTCCSLS HRFYRLANEC
661   AQVLSEMVMC GGSLYVKPGG TSSGDATTAY ANSVFNICQA VTANVNALLS TDGNKIADKY
721   VRNLQHRLYE CLYRNRDVDT DFVNEFYAYL RKHFSMMILS DDAVVCFNST YASQGLVASI
781   KNFKSVLYYQ NNVFMSEAKC WTETDLTKGP HEFCSQHTML VKQGDDYVYL PYPDPSRILG
841   AGCFVDDIVK TDGTLMIERF VSLAIDAYPL TKHPNQEYAD VFHLYLQYIR KLHDELTGHM
901   LDMYSVMLTN DNTSRYWEPE FYEAMYTPHT VLQHHHHHHH HHH
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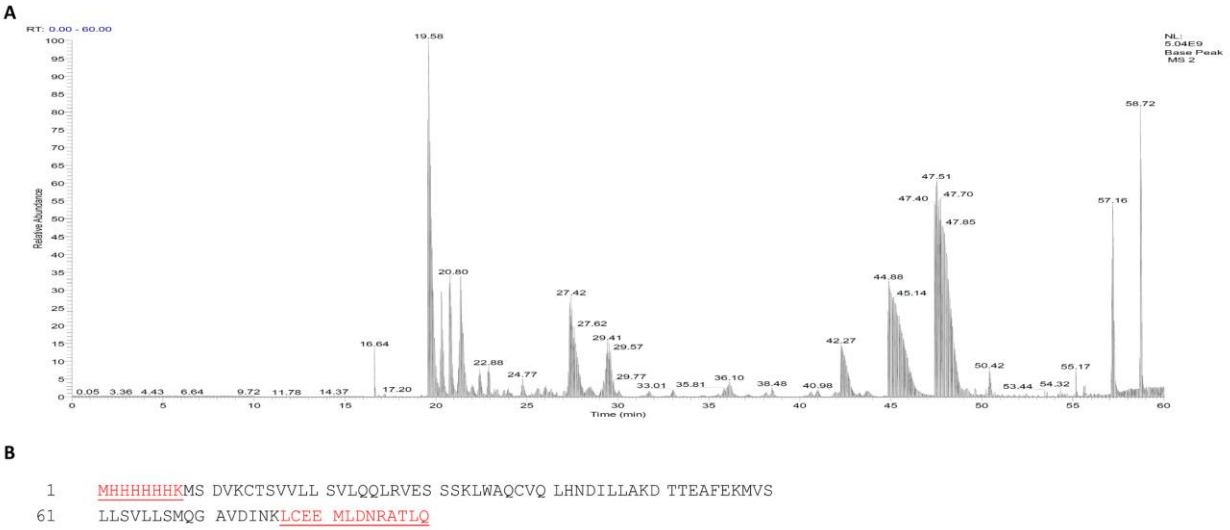
Fig. S5. LC-MS/MS identification of nsp12. (A) Fragment ion spectra of nsp12 after in-gel trypsin digestion. **(B)** Sequence coverage of nsp12. The identified sequence was labeled with red color. Total coverage rate of nsp12 sequence was 45.8%.



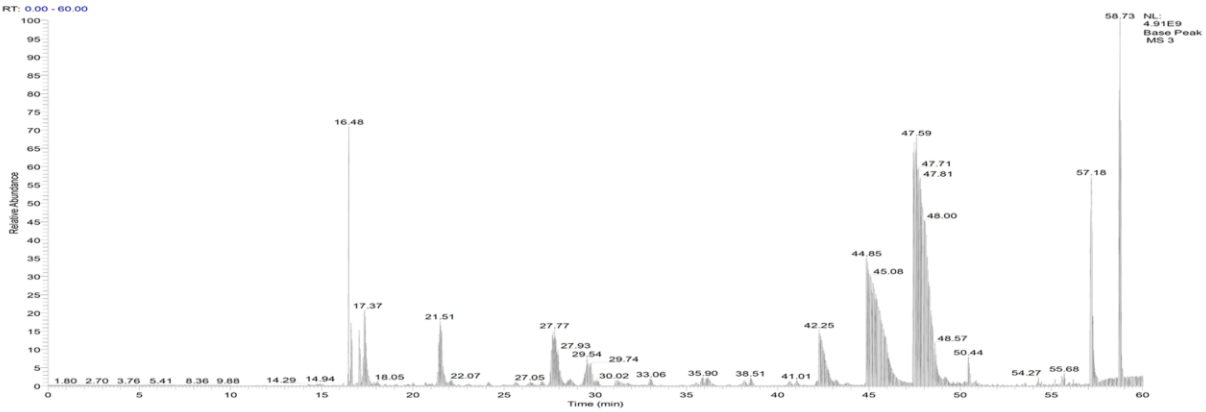
B

1 MHHHHHHKMS DVKCTSVVLL SVLQQLRVES SSKLWAQCVQ LHNDILLAKD TTEAFEKMVS

61 LLSVLLSMQG AVDINKLCEE MLDNRATLQ

Fig. S6. LC-MS/MS identification of nsp7. (A) Fragment ion spectra of nsp7 after in-gel trypsin digestion. **(B)** Sequence coverage of nsp7. The identified sequence was labeled with red color. Total coverage rate of nsp7 sequence was 23.6%.

A



B

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  1    MHHHHHHAIA SEFSSLPSYA AFATAQEAYE QAVANGDSEV VLKKLKKSLN VAKSEFDRDA
 61    AMQRKLEKMA DQAMTQMYKQ ARSEDKRAKV TSAMQTMLFT MLRKLDNDAL NNIINNARDG
121    CVPLNIIPLT TAAKLMVVIP DYNTYKNTCD GTTFTYASAL WEIQQVVDAD SKIVQLSEIS
181    MDNSPNLAWP LIVTALRANS AVKLQ
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Fig. S7. LC-MS/MS identification of nsp8. (A) Fragment ion spectra of nsp8 after in-gel trypsin digestion. **(B)** Sequence coverage of nsp8. The identified sequence was labeled with red color. Total coverage rate of nsp8 sequence was 3.9%.