## **Supporting Information**

## ATP enhances the error-prone ribonucleotide incorporation by the SARS-CoV-2 RNA polymerase

Yasin Pourfarjam<sup>1,2</sup>, Zhijun Ma<sup>1</sup>, and In-Kwon Kim<sup>1,\*</sup>

From the <sup>1</sup> Department of Chemistry, University of Cincinnati, 301 Clifton Ct, Cincinnati, OH 45221, USA.

Running title: ATP-dependent error-prone RNA replication by SARS-CoV-2

<sup>2</sup>Present address: Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer, New York, NY 10065, USA.

\* To whom correspondence should be addressed: In-Kwon Kim, Department of Chemistry, University of Cincinnati, 301 Clifton Ct, Cincinnati, OH 45221, USA; E-mail: kimiw@ucmail.uc.edu; Tel.: (513) 556-1909; Fax.: (513) 556-9239.

## Supporting information contents:

Figure S1. Purified SARS-CoV-2 nsp12, nsp7, and nsp8 proteins used in this study.

**Figure S2.** ATP-dependent low-fidelity nucleotide incorporation at [NTP] = 200  $\mu$ M, in the presence and absence of RNA proofreading nuclease.

**Figure S3.** ATP-dependent low-fidelity nucleotide incorporation at [NTP] = 2  $\mu$ M, in the presence and absence of RNA proofreading nuclease.

Table S1. RNA and DNA oligonucleotides used in this study.

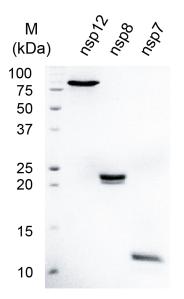
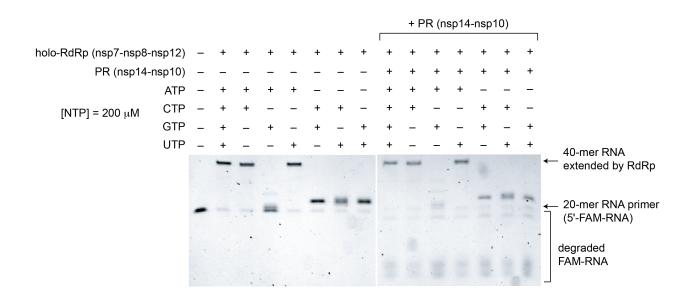
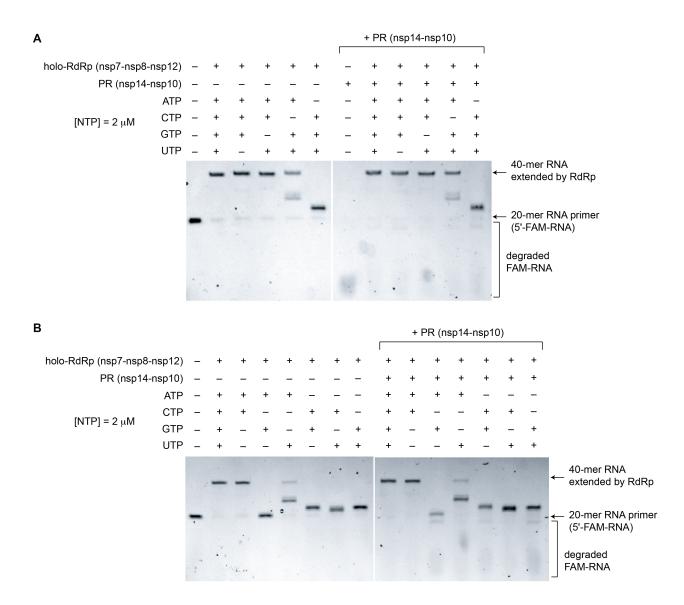


Figure S1. Purified SARS-CoV-2 nsp12, nsp7, and nsp8 proteins used in this study.



**Figure S2.** ATP-dependent low-fidelity nucleotide incorporation at [NTP] =  $200 \mu$ M, in the presence and absence of RNA proofreading nuclease. PR indicates proofreading nuclease (nsp14-nsp10).



**Figure S3.** ATP-dependent low-fidelity nucleotide incorporation at [NTP] = 2  $\mu$ M, in the presence and absence of RNA proofreading nuclease. PR indicates proofreading nuclease (nsp14-nsp10).

 Table S1. RNA and DNA oligonucleotides used in this study.

Oligo name	Sequence 5′ → 3′
5'-FAM-20mer-RNA primer	5'-FAM-GUCAUUCUCCUAAGAAGCUA-3'
40mer-RNA template	5'-CUAUCCCCAUGUGAUUUUAAUAGCUUCUUAGGAGAAUGAC-3'
5'-FAM-20mer-DNA primer	5'-FAM-GTCATTCTCCTAAGAAGCTA-3'
40mer-DNA template	5'-CTATCCCCATGTGATTTTAATAGCTTCTTAGGAGAATGAC-3'