Supplemental Materials

Nsp12 expression and purification

The codon-optimized SARS-CoV-2 virus nsp12 gene was cloned into the pFastBac vector, and the baculovirus was prepared using the Bac-to-Bac system (Invitrogen). The N-terminal 6xHis-TEV-nsp12 containing a TEV cleavage site was expressed in baculovirus-mediated transduction of Sf21 suspension cell cultures. Cells were lysed by Dounce homogenization in lysis buffer **1A** (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 6 mM $MgCl_2$, 10% glycerol, and EDTA-free protease inhibitor), followed by Ni^{2+}-NTA agarose beads (GoldBio), washed with wash buffer **1B** (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 6 mM $MgCl_2$, 25 mM imidazole, and 10% glycerol), and eluted with elution buffer **1C** (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 6 mM $MgCl_2$, 500 mM imidazole, and 10% glycerol). The eluted sample was applied to the HiTrap Q HP column (buffer **D**: 50 mM Tris-HCl pH 8.0, 50 mM NaCl, 6 mM $MgCl_2$, and 10% Glycerol, and buffer **E**: 50 mM Tris-HCl pH 8.0, 6 mM $MgCl_2$, 1.5 M NaCl, and 10% Glycerol). The 6xHis-TEV-nsp12 sample was collected from HiTrap Q HP flow-through and treated with TEV enzyme in TEV cleavage buffer **F** (25 mM Tris-HCl pH 8.0, 300 mM NaCl, 10% glycerol, and 1.4 mM 2-mercaptoethanol) overnight at 4°C and then applied to Ni^{2+}-NTA agarose beads. The flow-through of TEV cleaved no-tagged nsp12 sample was applied to size-exclusion chromatography using the gel filtration buffer **G** (25 mM HEPES pH 7.4, 200 mM NaCl, 6 mM $MgCl_2$, and 0.5 mM TCEP) with a Superose 6 Increase 10/300 GL (GE Healthcare). The quality of purified proteins was analyzed by SDS-PAGE gel. The proteins were tested active for RNA synthesis activity. The purified proteins were flash-frozen in liquid nitrogen and stored in 30 µL aliquots at −80 °C for further use.

Nsp7 and nsp8 expression and purification

The codon-optimized SARS-CoV-2 virus nsp7 and nsp8 genes were cloned into the pET-28a and pET-15b(+) vectors, respectively. The N-terminal 6xHis-MBP-TEV-nsp7 and 6xHis-thrombin-nsp8 were expressed in *E. coli* BL21(DE3) strain. Cell cultures were grown at 30°C in LB (Miller's Luria Broth) medium until A600 reached 0.8, and expression was induced with 0.5 mM isopropyl 1-thio-β-D-galactopyranoside (IPTG) and incubated at 30°C for 4 h. (1) For nsp7: cells were lysed by sonication in lysis buffer **2A** (50 mM sodium phosphate pH7.4, 300 mM NaCl, 5 mM imidazole, and 10% Glycerol) for 10 minutes with 3-second pulses, followed by centrifugation. The supernatant was loaded on a pre-equilibrated Ni^{2+}-NTA column with the lysis buffer 2A. The nickel column was washed with wash buffer **2B** (50 mM sodium phosphate pH7.4, 300mM NaCl, 50 mM imidazole, and 10% Glycerol). The protein was eluted from the beads in elution buffer **2C** (50 mM

sodium phosphate pH7.4, 300mM NaCl, 500mM imidazole, and 10% Glycerol). The protein was further purified by a HiTrap Q HP column (Buffers **D** & **E**). TEV cleaved nsp7 was purified from 6xHis-MBP-TEV-nsp7 by treated with TEV enzyme in TEV cleavage buffer **F** overnight at 4°C. The protein sample was further purified with Nickel and amylose beads to remove TEV enzyme and His-MBP tag before applied to Superdex 200 column with buffer **G**. (2) For nsp8: cells were lysed by sonication in lysis buffer **3A** (50 mM Tris-HCl pH 8.5, 300 mM NaCl, 5 mM imidazole, and 10% Glycerol) for 10 min with 3-sec pulses, followed by centrifugation. The supernatant was loaded on a pre-equilibrated column with the lysis buffer **3A**. The nickel column was washed with the wash buffer **3B** (50 mM Tris-HCl pH 8.5, 300mM NaCl, 50 mM imidazole, and 10% Glycerol). The protein was eluted from the beads in the elution buffer **3C** (50 mM Tris-HCl pH 8.5, 300 mM NaCl, 500 mM imidazole, and 10% Glycerol). The protein was further purified by a HiTrap Q HP column (Buffers **D** & **E**) and then isolated by the superdex 200 column equilibrated with gel filtration buffer **G**. The qualities of purified proteins were analyzed by SDS-PAGE gel. The purified proteins were flash-frozen in liquid nitrogen and stored at -80 °C for further use.

SARS-CoV-2 RNA polymerase inhibition assay

The inhibitory effects of triphosphate forms of sofosbuvir and remdesivir on SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) activities were analyzed via an *in vitro* RNA synthesis assay, using SARS-CoV-2 RdRp complex consisting of the purified nsp7, nsp8 and nsp12 as described above. Briefly, all three purified proteins were mixed on ice for 15 mins to allow polymerase complex formation. In 10 uL reaction mixtures, SARS-CoV-2 RdRp complex was mixed with each compound (10 or 1μM) or nuclease-free water on ice, in the presence of TRIS-HCl (25 mM, pH8), NTPs (50 mM ATP, CTP and TTP; 25 mM GTP), [a-^{32}P]-GTP (0.1 mM), RNA primer (200 mM), RNA template (2 mM). After 10 mins incubation at 30°C, $MnCl_2$ (5 mM) was added into the reactions which were further incubated at 30°C for 30 mins. The RdRp reactions were stopped by adding formamide containing 40 mM EDTA and heated at 95°C for 10 mins. Using 20% polyacrylamide-urea denaturing gels (SequaGel, National Diagnostics), 6 μL of respective reaction mixtures was resolved, and the resulting 14-nucleotide primer extended RNA products were visualized using the Amersham Typhoon IP (Cytiva) before the data were analyzed using ImageQuant TL 8.2 (Cytiva).