

Supplementary Information

RNA with Azvudine Incorporated One Nucleotide Upstream of 3'-End Resists Cleavage by SARS-CoV-2 Proofreading Exonuclease

Ying Tong Yue^{1,2,#}, Kao Lin^{1,2,#}, Chuanjuan Tao^{1,2}, Xiaoxu Li^{1,2}, Shuxia Peng³, Marianna Teplova³, Shiv Kumar^{1,2}, Irina Morozova^{1,2}, Sergey Kalachikov^{1,2}, James J. Russo^{1,2}, Dinshaw J. Patel³, Jingyue Ju^{1,2,4,*}

1 - Center for Genome Technology and Biomolecular Engineering, Columbia University, New York, NY 10027.

2 - Department of Chemical Engineering, Columbia University, New York, NY 10027.

3 - Laboratory of Structural Biology, Memorial Sloan-Kettering Cancer Center, New York, NY 10065.

4 - Department of Molecular Pharmacology and Therapeutics, Columbia University, New York, NY 10032.

- These authors contributed equally

Supplementary Figures

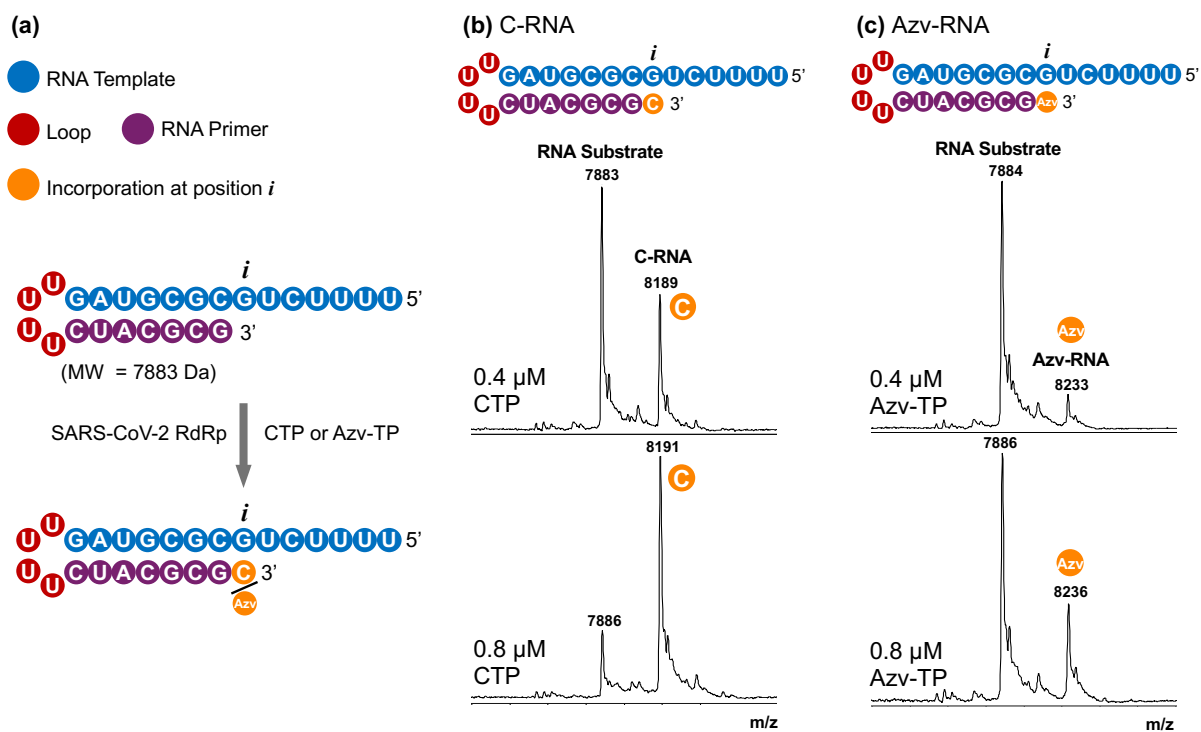


Fig. S-1 Incorporation of Azv-TP by SARS-CoV-2 RdRp. (a) Annealed RNA template-loop-primer substrate (top) and RNA products after RdRp reaction (bottom). (b) MS analysis of CTP incorporation by RdRp at 0.4 μ M and 0.8 μ M producing C-RNA (~8189 Da). (c) MS analysis of Azv-TP incorporation by RdRp at 0.4 μ M and 0.8 μ M producing Azv-RNA (~8233 Da). C-RNA and Azv-RNA are indicated by the peaks in the MS figure.

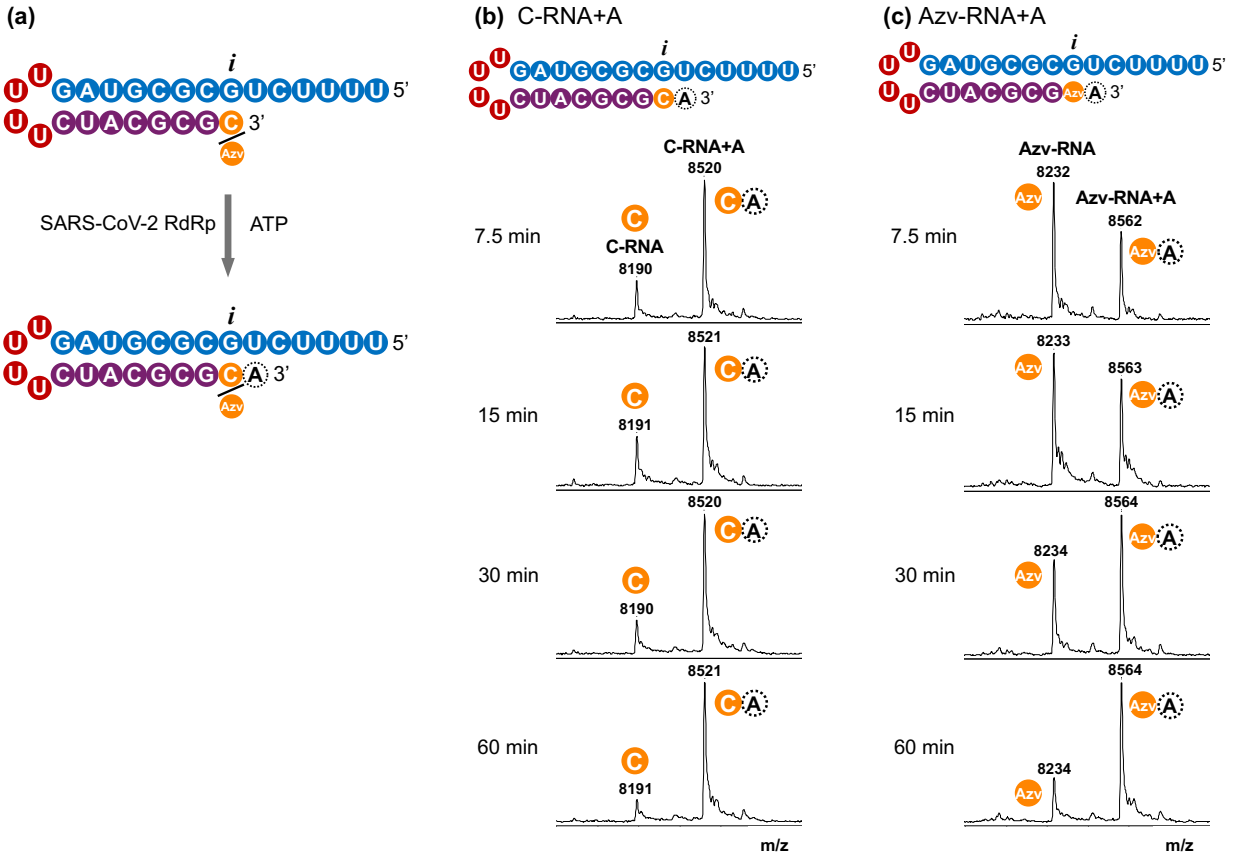


Fig. S-2 Azv hinders RNA extension by SARS-CoV-2 RdRp.

(a) Annealed RNA template-loop-primer substrates (top) and RNA products (bottom) after RdRp reaction. MS analysis of ATP incorporation by RdRp into C-RNA (b) or Azv-RNA (c) at different time points noted on the left of each figure. This result shows that Azv hinders next base incorporation 2-3-fold at all time points.

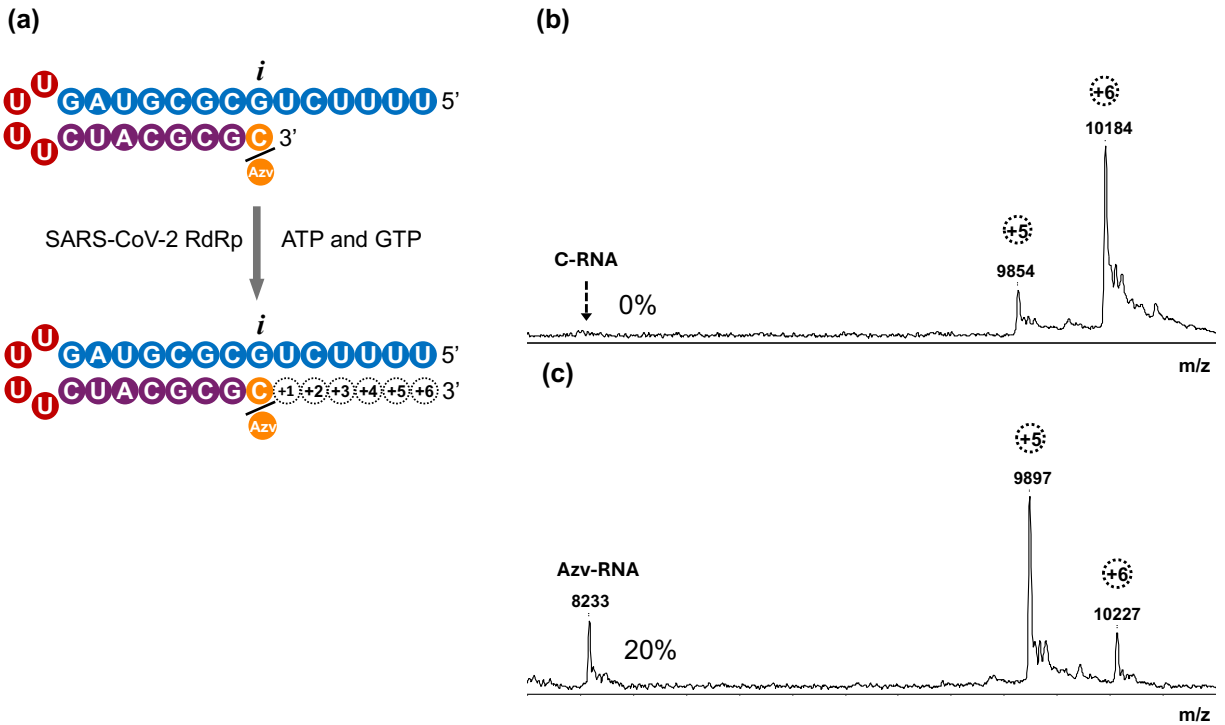


Fig. S-3 The incorporated Azv reduces RNA synthesis by SARS-CoV-2 RdRp.

(a) Annealed Azv- or C-extended RNA substrate (top) and the fully extended RNA products (bottom) after RdRp reaction. MS analysis of continuous nucleotide incorporation of natural RNA (b) or Azv-RNA (c) by RdRp with the presence of excess amount of ATP and GTP. Incorporation of Azv reduces further RNA elongation when compared to natural RNA elongation, shown by the remaining 20% Azv-RNA (~8233 Da) after the RdRp reaction. Percentage of remaining starting material is shown by the corresponding peaks of each spectrum.

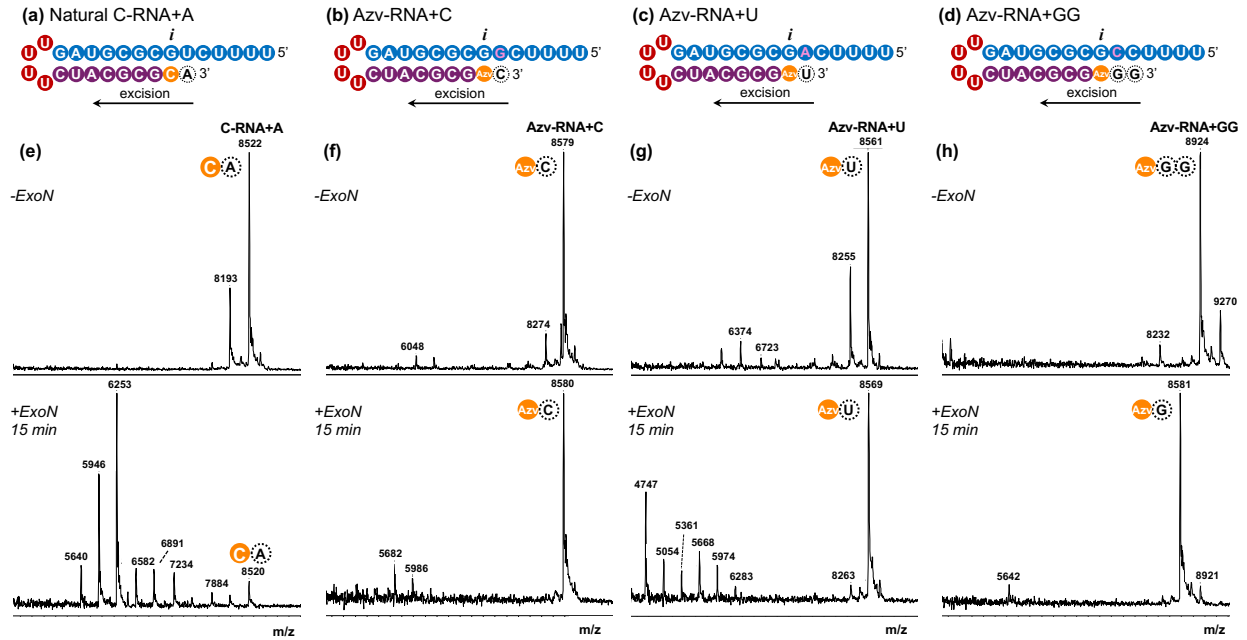


Fig. S-4 SARS-CoV-2 ExoN resistance of Azv-embedded RNA (Azv-RNA+1) is not sequence specific.

(a) Natural C-RNA+A used as control. (b) Azv-RNA+C substrate. (c) Azv-RNA+U substrate. (d) Azv-RNA+GG substrate. (e) MS analysis of natural C-RNA+A (~8522 Da) before (-ExoN) and after ExoN reaction. (f) MS analysis of Azv-RNA+C before (-ExoN) and after ExoN reaction. (g) MS analysis of Azv-RNA+U before (-ExoN) and after ExoN reaction. (h) MS analysis of Azv-RNA+GG before (-ExoN) and after ExoN reaction. All three Azv-RNA+1 showed strong ExoN resistance.