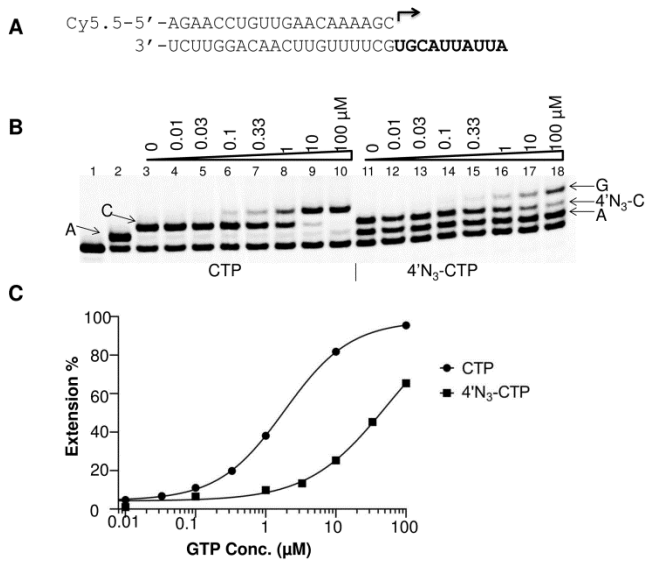
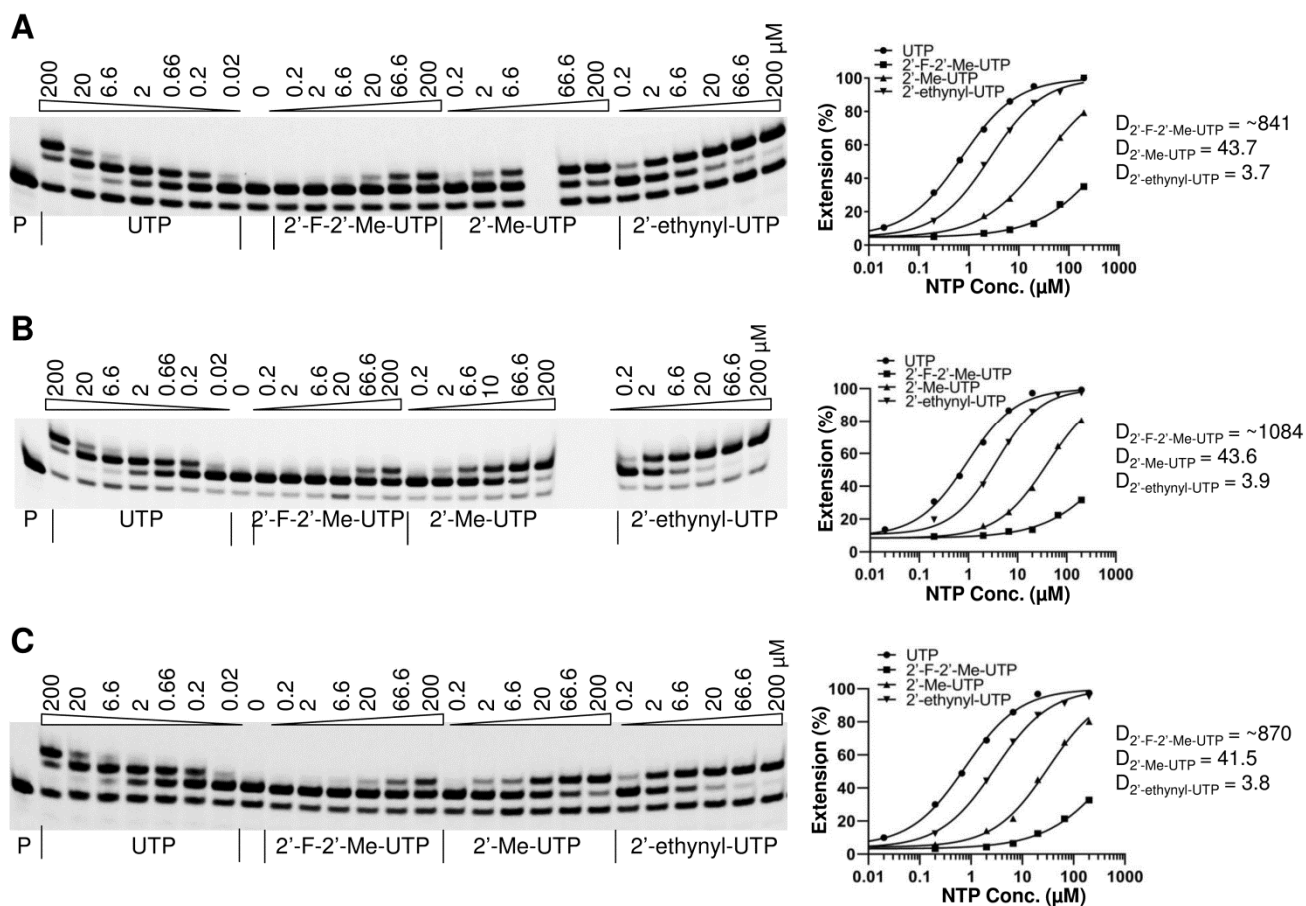


**Figure S1. Time course of the primer extension reaction.** (A) Sequences of the primer/template used in the experiment. (B) Primer extension reaction without pre-incubation. NS5 (30 nM) was incubated with 10 nM P/T in the polymerase reaction buffer containing 3 mM MnCl<sub>2</sub>. The reactions were initiated by addition of 100 μM each of rNTPs. At different times after addition of rNTPs, 3 μl aliquots were withdrawn and mixed with 6 μl quenching/loading buffer. (C) Primer extension reaction after pre-incubation with ATP. NS5 was pre-incubated with P/T in the reaction buffer containing 3 mM MnCl<sub>2</sub> and 10 μM ATP for 30 min, and then all rNTPs were added to the reaction to 100 μM. At different times after addition of rNTPs, 3 μl aliquots were withdrawn and mixed with 6 μl quenching/loading buffer. The products were analyzed by denaturing PAGE. (D) Graphical representation of the results in panels B and C. The percent of the full-length products in B and C were plotted against the reaction time.



**Figure S2. Effect of incorporation of 4'-C-azido-CTP on the incorporation efficiency of next ribonucleotide by ZIKV NS5 polymerase. (A)** Sequence of the P/T duplex used in the reactions. **(B)** Polymerase reactions were initiated by addition of 10 μM ATP, either 10 μM CTP or 100 μM 4'-C-azido-CTP, and increasing concentrations of GTP. Lane 1, unextended primer; lane 2, reaction contains ATP only; lane 3-10, reactions contain ATP, CTP and GTP at indicated concentrations; lanes 11-18, reactions contain ATP, 4'-C-azido-CTP (4'N<sub>3</sub>-CTP) and GTP at indicated concentrations. Location of individual products of extension are indicated on the left and on the right. 4'N<sub>3</sub>C, 4'-C-azido-C. **(C)** Quantitative analysis of GTP incorporation. Percent of extension of the C-product to G-product in lanes 3-10 and of the 4'N<sub>3</sub>-C product to G-product in lanes 11-18 were plotted against GTP concentrations, and the results were fitted to a sigmoidal dose-response curves to generate  $K_{1/2}$ .  $K_{1/2(C:G)} = 1.85 \mu\text{M}$ ,  $K_{1/2(4'N_3C:G)} = 52.09 \mu\text{M}$ .



**Figure S3. Effect of  $\text{Mg}^{2+}$  on the efficiency of UTP analog incorporation by ZIKV NS5.** Incorporation of UTP, 2'-F-2'-Me-UTP, 2'-Me-UTP and 2'-ethynyl-UTP were measured as described in Figure 6 legend, except that 3 mM  $\text{MnCl}_2$  was used in panel A, 3 mM  $\text{MnCl}_2$  plus 3 mM  $\text{MgCl}_2$  were used in panel B, and 6 mM  $\text{MnCl}_2$  was used in panel C.