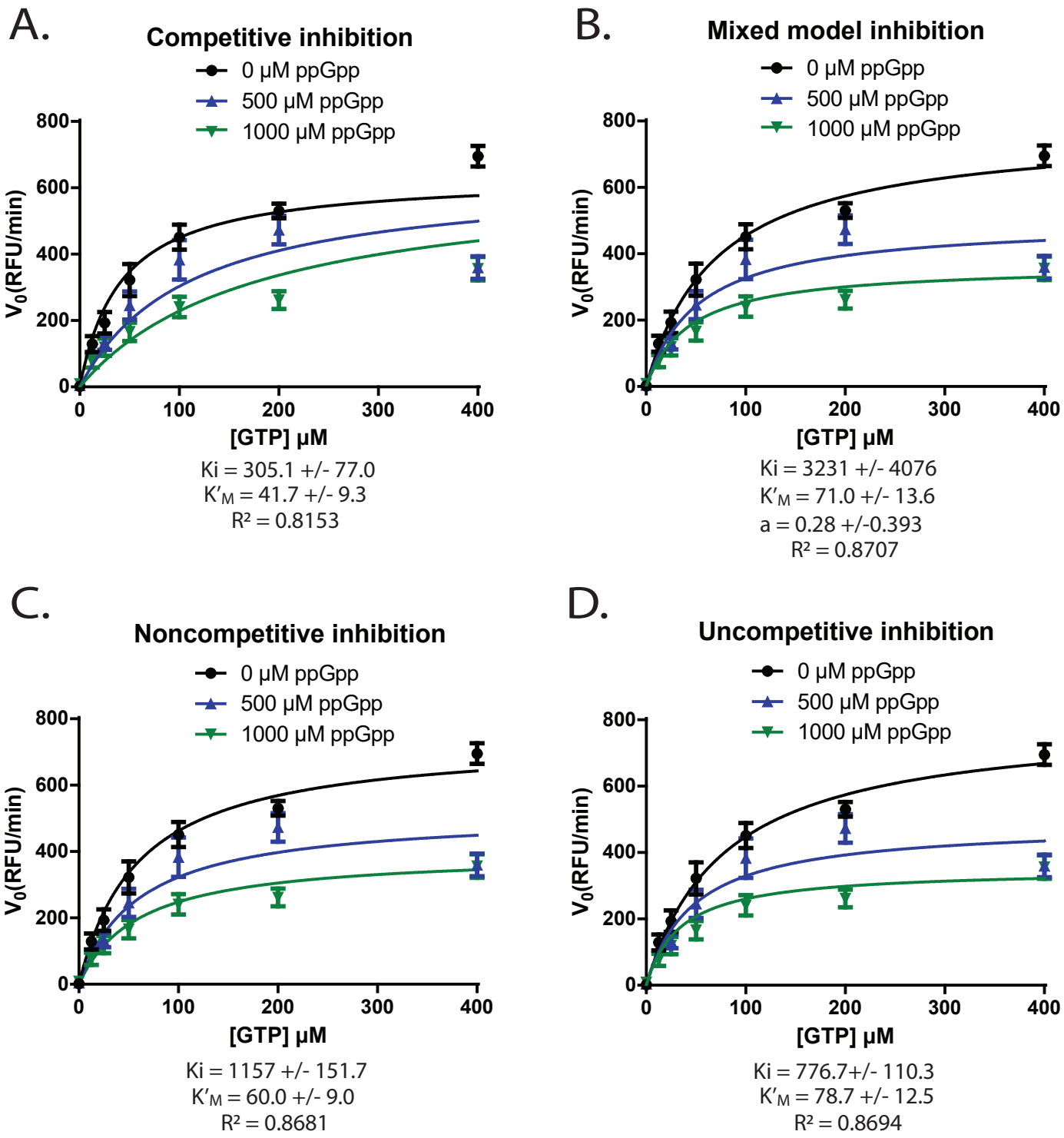
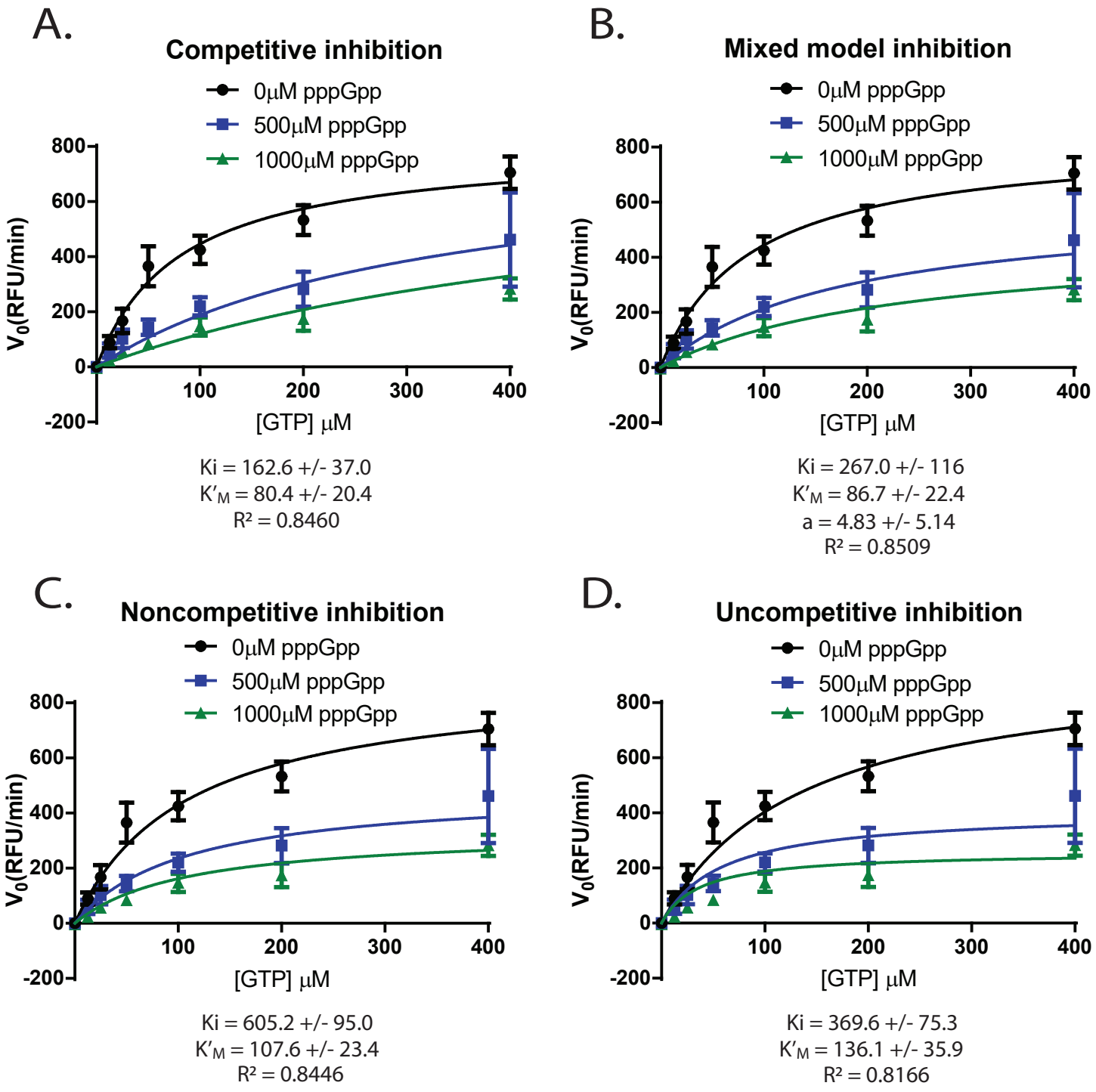
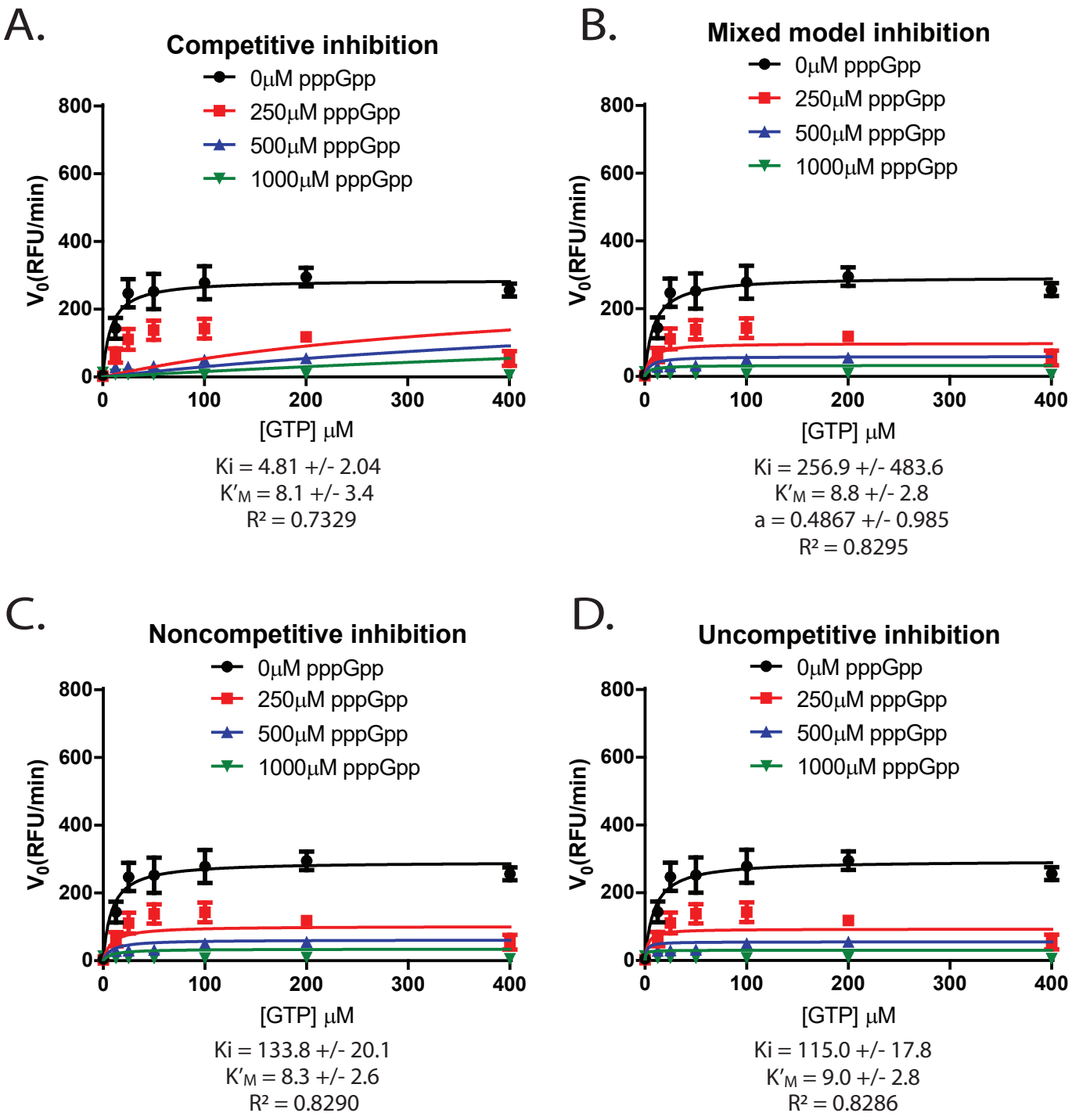


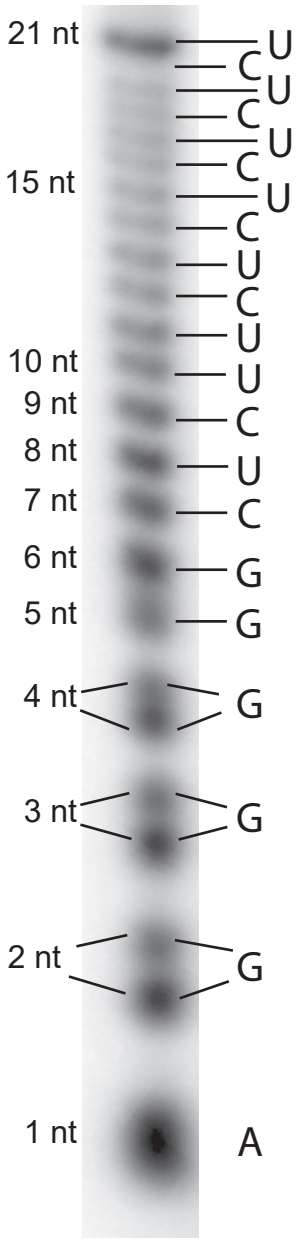

**Supplemental Figure 1. Various inhibition models for reactions with ppGpp and full-length DnaG in Figure 1.** The initial velocity ($V_0$) of full-length DnaG activity at indicated concentrations of ppGpp. Reactions were run with 0, 12.5, 25, 50, 100, 200, or 400 μM GTP. Results are normalized to the enzyme concentration and are reported as relative fluorescent units per minute (RFU/min). Points represent averages of ≥ 3 replicates. Error bars represent ± SEM for ≥ 3 replicates. Curves were fit with a competitive inhibition (A), mixed model inhibition (B), noncompetitive inhibition (C) and an uncompetitive inhibition model (D). The best fit model is uncompetitive inhibition for full-length DnaG with ppGpp.




**Supplemental Figure 2. Various inhibition models for reactions with pppGpp and full-length DnaG in Figure 1.** The initial velocity ($V_0$) of full-length DnaG activity at indicated concentrations of pppGpp. Reactions were run with 0, 12.5, 25, 50, 100, 200, or 400 μM GTP. Results are normalized to the enzyme concentration and are reported as relative fluorescent units per minute (RFU/min). Points represent averages of ≥ 3 replicates. Error bars represent ± SEM for ≥ 3 replicates. Curves were fit with a competitive inhibition (A), mixed model inhibition (B), noncompetitive inhibition (C) and an uncompetitive inhibition model (D). The best fit model is competitive inhibition for full-length DnaG with pppGpp.

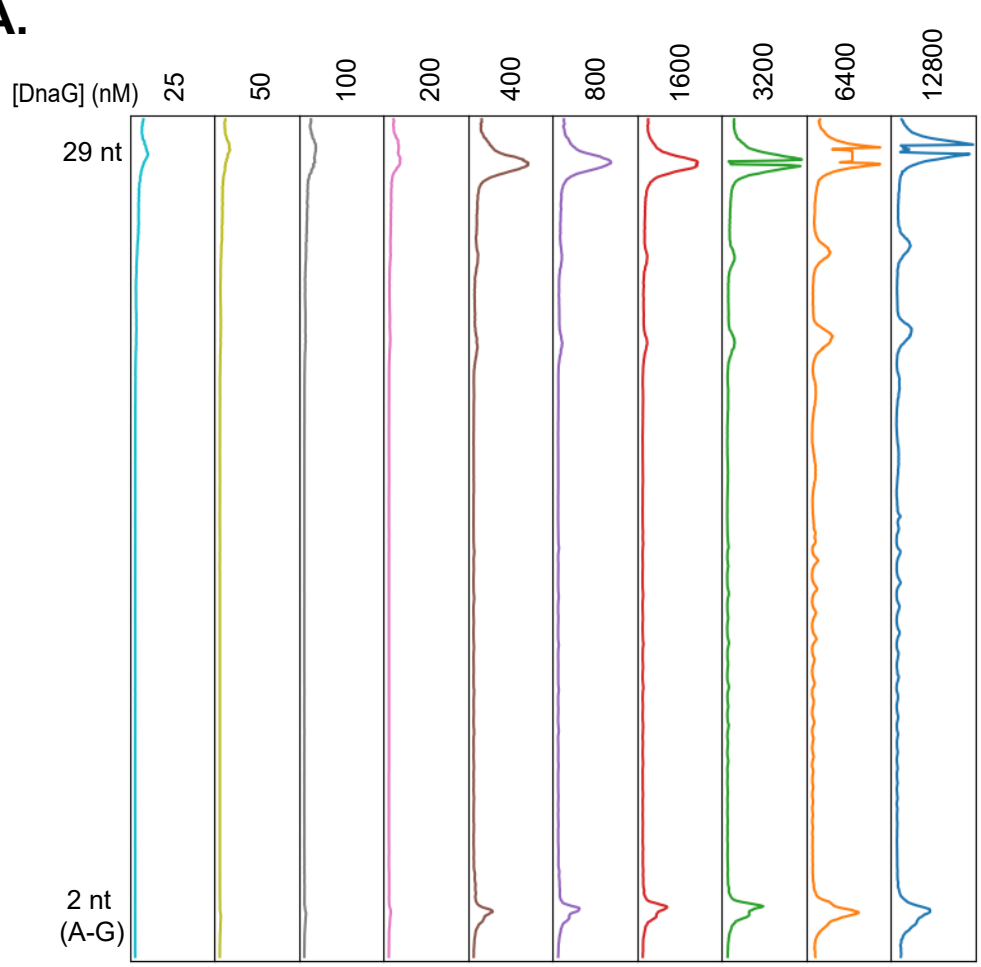



**Supplemental Figure 3. Various inhibition models for reactions with pppGpp and DnaG(ZBD+RPD) in Figure 1.** The initial velocity ($V_0$) of DnaG(ZBD+RPD) activity at indicated concentrations of pppGpp. Reactions were run with 0, 12.5, 25, 50, 100, 200, or 400 μM GTP. Results are normalized to the enzyme concentration and are reported as relative fluorescent units per minute (RFU/min). Points represent averages of ≥ 3 replicates. Error bars represent ± SEM for ≥ 3 replicates. Curves were fit with a competitive inhibition (A), mixed model inhibition (B), noncompetitive inhibition (C) and an uncompetitive inhibition model (D). The best fit model is uncompetitive inhibition for DnaG(ZBD+RPD) with pppGpp.

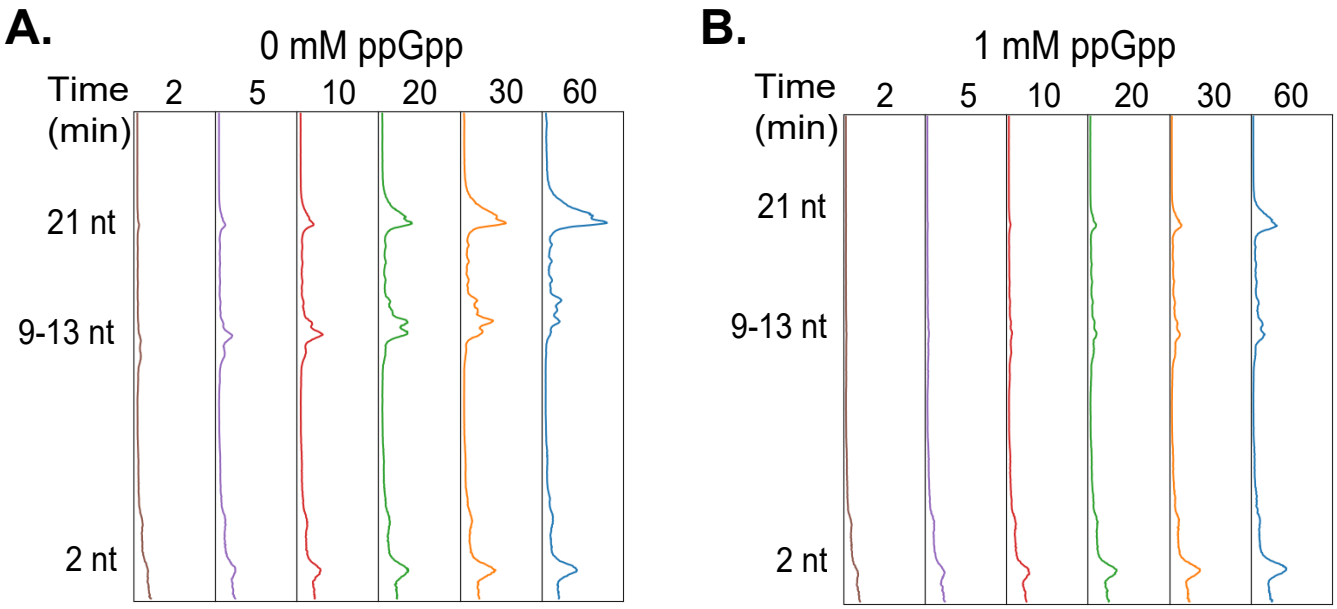



**Supplemental Figure 4. RNA standard ran alongside primase reactions.** Standard was generated by labeling the 5' end of an RNA with a sequence identical to that of the primase reaction product. The labeled RNA was subjected to NaOH hydrolysis to generate the RNAs of different lengths. The hydrolysis was able to occur on either the 2' or 3'-OH which is why on the very short RNAs (2-4) there are two different species.

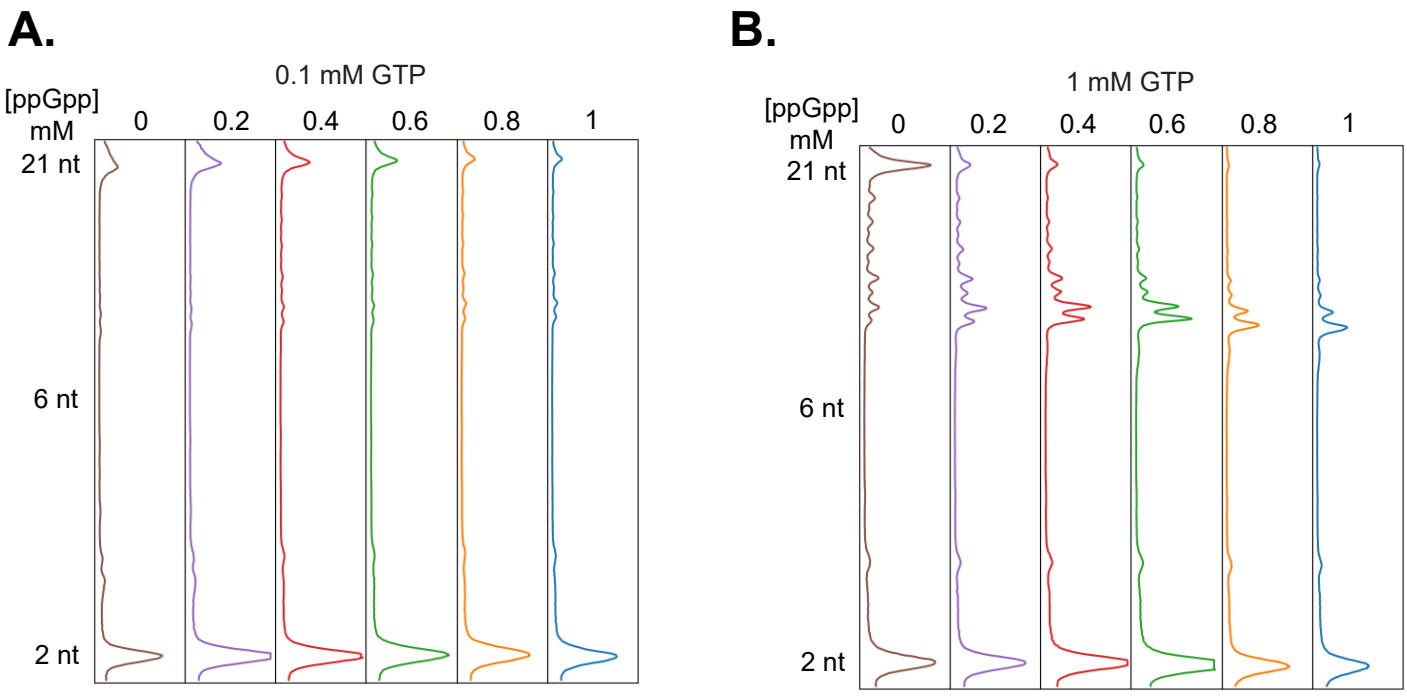
**A.**



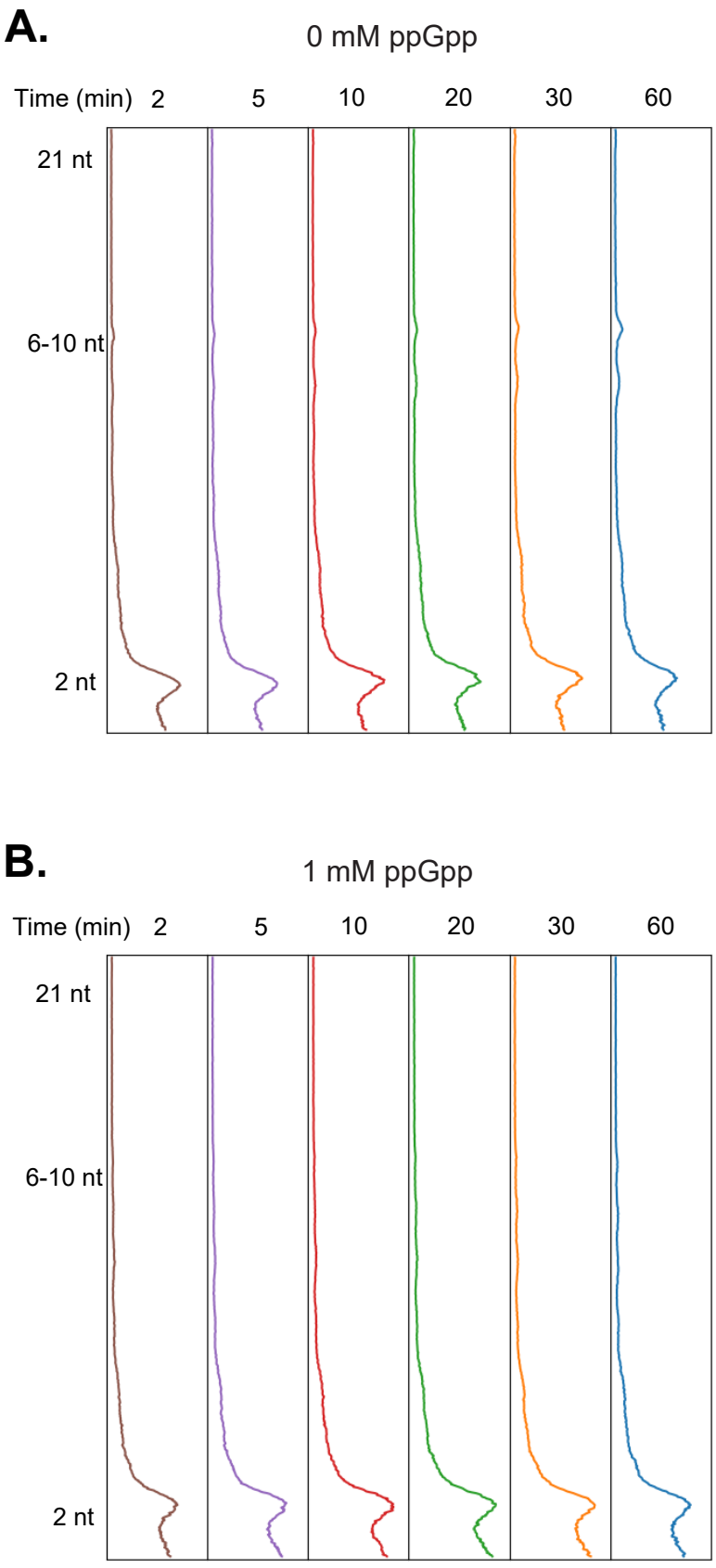

**Supplemental Figure 5. Chromatograms representing band intensity from the gel in Figure 2.** Reactions titrated DnaG with 500 nM ssDNA, 0.4 mM NTPs (CTP, UTP, GTP) and ~0.4 μM [γ-$^{32}$P]-ATP. Primers from 2 nucleotides (bottom) up to intermediate and full-length (29 nucleotides) primers were observed. A small amount of primers longer than 29 nucleotides are due to impurity of the DNA template (Supplemental Figure 12) and result in disrupted peaks within the chromatograms near 29 nucleotides for concentrations of DnaG above 1600 nt.



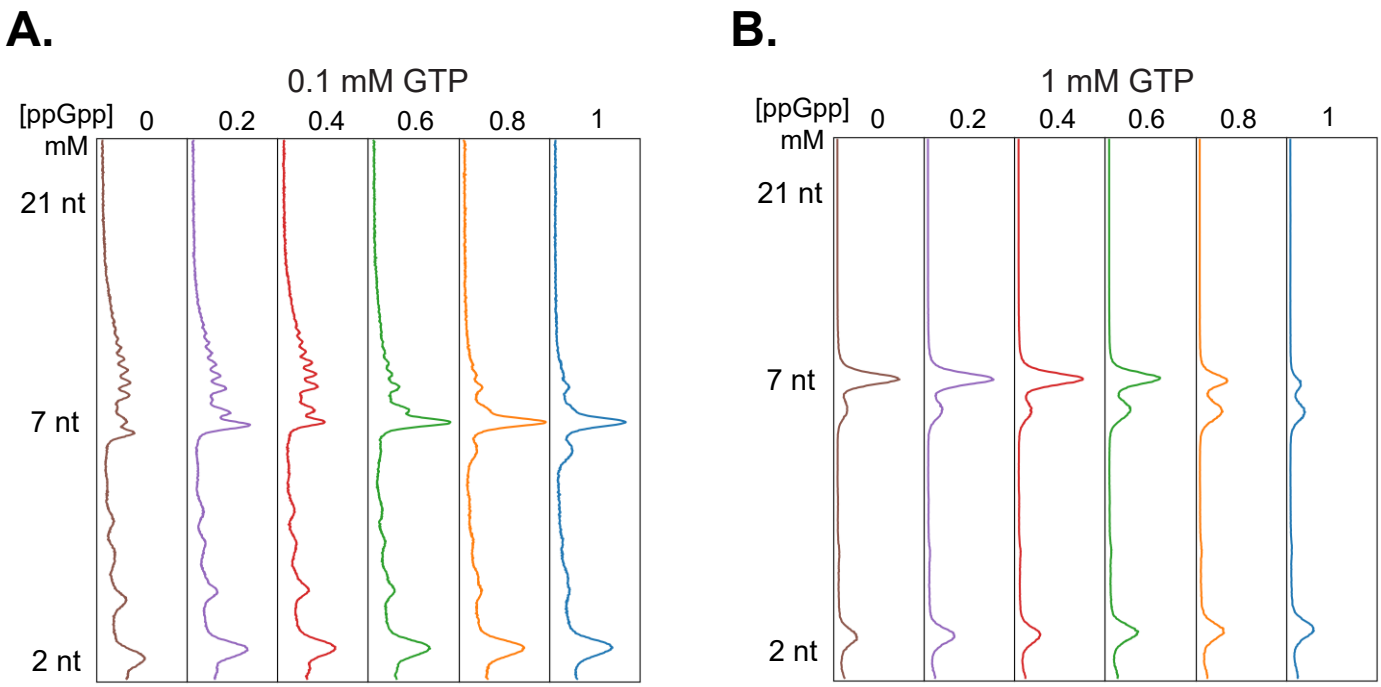

**Supplemental Figure 6. Chromatograms representing band intensity from the gel in Figure 3.** Primer synthesis was measured over the course of one hour (60 min) without ppGpp (A) or with 1 mM ppGpp (B). Time points were taken at 2, 5, 10, 20, 30, and 60 minutes. Reactions contained 400 nM DnaG, 5 μM ssDNA template, ~0.4 μM [γ-$^{32}$P]-ATP, and 1 mM NTPs (GTP, CTP, UTP). Diminished chromatogram peak size over time for primers longer than the dinucleotide show reduction in primer extension with 1 mM ppGpp (B) compared to no ppGpp (A).



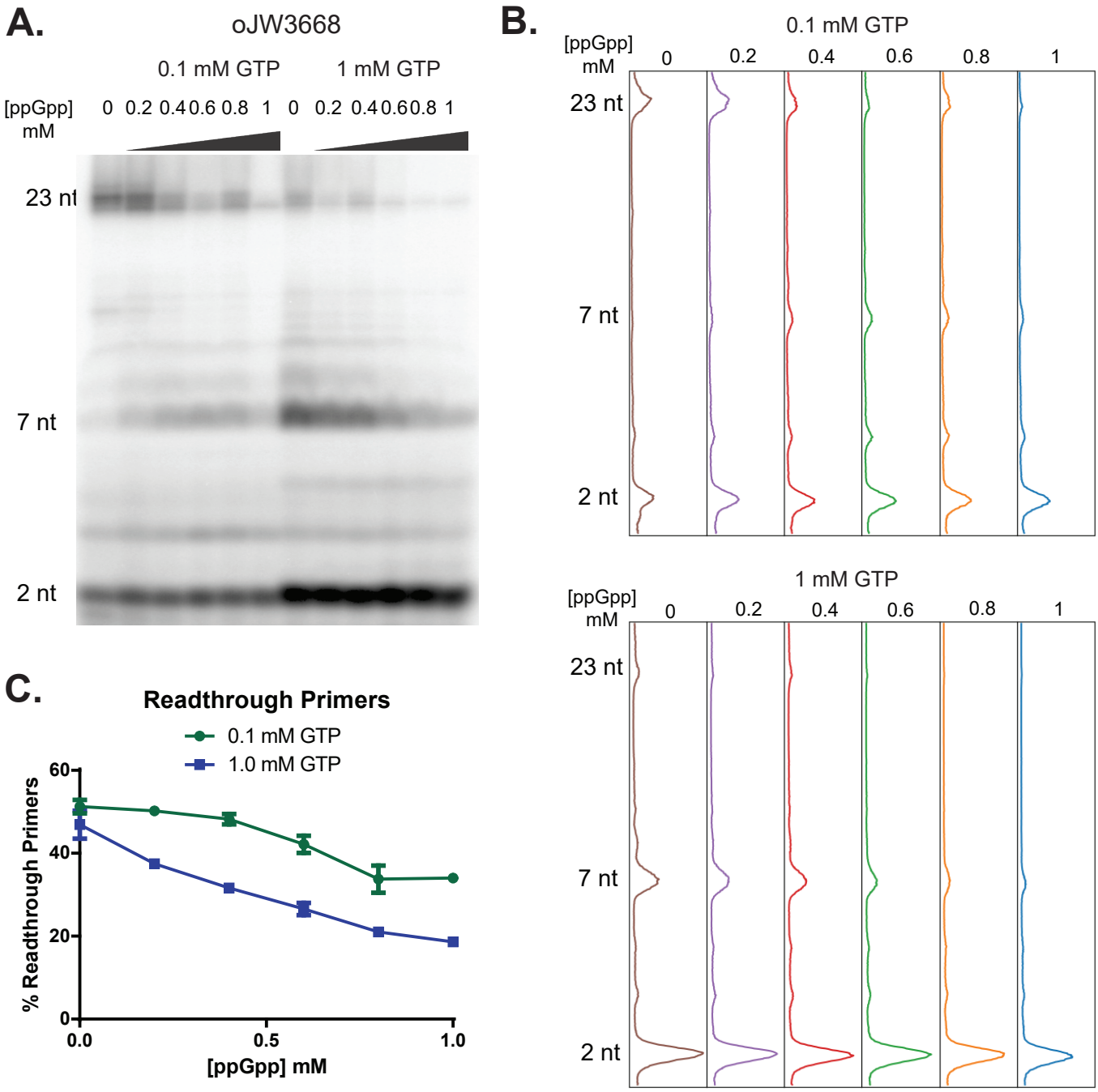

**Supplemental Figure 7. Chromatograms representing band intensity from the gel in Figure 4.** Primer synthesis reactions contained 6.4 μM DnaG, 500 nM oJW3319, ~0.4 μM [γ-$^{32}$P]-ATP, 1 mM each of CTP and UTP, and either 0.1 mM GTP (A) or 1 mM GTP (B). Reactions incubated 30 minutes after addition of NTPs. Higher GTP concentrations promote synthesis of priming products above 6 nucleotides, while higher ppGpp concentrations inhibit synthesis of full-length (21 nucleotide) products. Dinucleotide formation appears to be unaffected by GTP or ppGpp concentration. Together, this data suggests that primer extension is regulated by GTP and ppGpp concentrations.



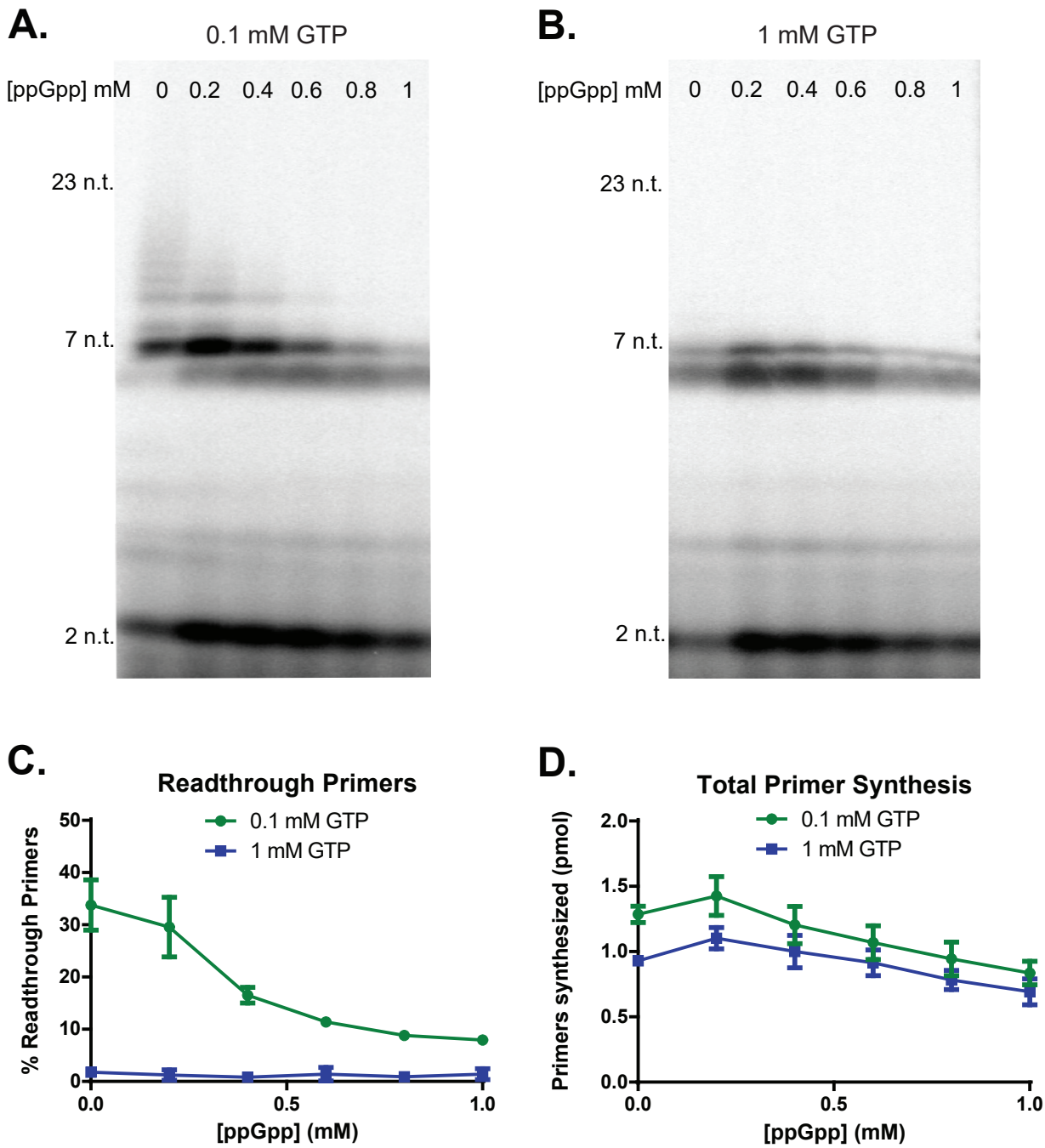

**Supplemental Figure 8. Chromatograms representing band intensity from the gel in Figure 5.** Primer synthesis was measured over the course of one hour (60 minutes) in the presence of heparin (~5 µg/mL) either without ppGpp (A) or with 1 mM ppGpp (B). Time points were taken at 2, 5, 10, 20, 30, and 60 minutes. Reactions contained 400 nM DnaG, 5 µM ssDNA template, ~0.4 µM [γ-32P]-ATP, and 1 mM NTPs (GTP, CTP, UTP). Synthesis of 7 nucleotide and longer priming products is slightly reduced in the presence of ppGpp.



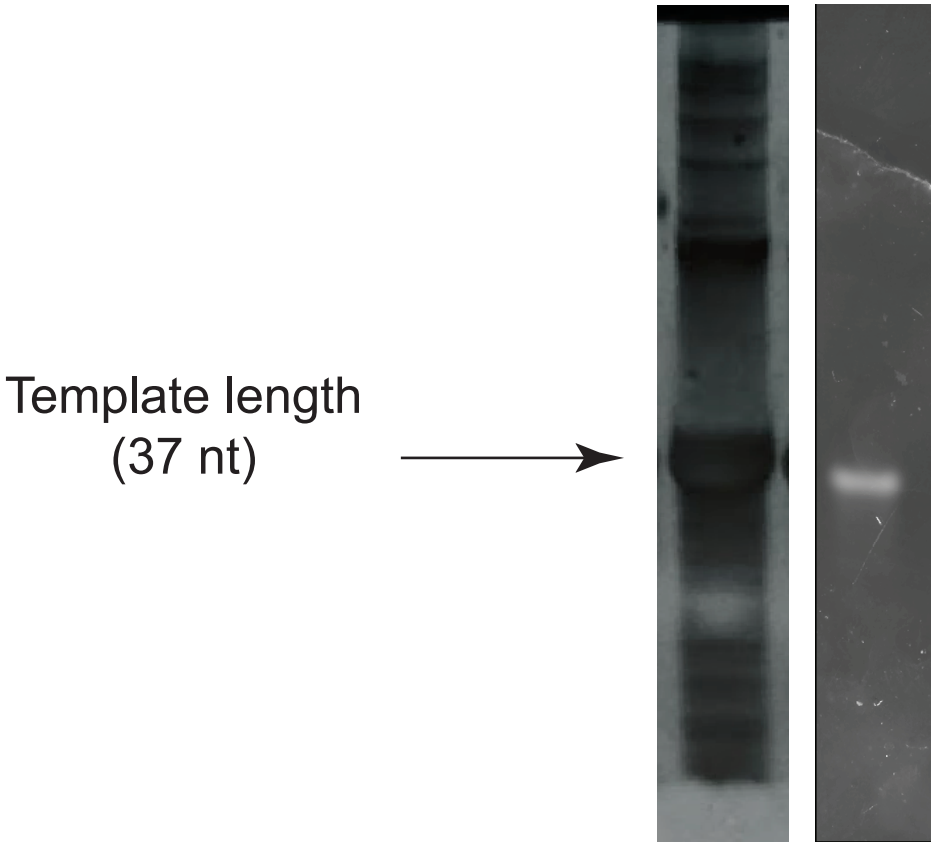

**Supplemental Figure 9. Chromatograms representing band intensity from the gel in Figure 6.** Primer synthesis reactions contained 6.4 μM DnaG, 500 nM oJW3319, ~0.4 μM [γ-$^{32}$P]-ATP, 1 mM UTP, and either 0.1 mM GTP (A) or 1 mM GTP (B). Since CTP is withheld in these reactions, priming products should be 7 nucleotides long. Readthrough is observable at low GTP but diminishes as ppGpp concentrations increase.




**Supplemental Figure 10. DNA primase reads through a starved site in an alternative oligonucleotide template.** (A) Representative gel of primase reactions with 6.4 μM DnaG and 500 nM oJW3668 with CTP-starved site (expected product is 7 nucleotides). Oligo oJW3668, unlike oJW3319 in Figure 6, lacks a CCCC repeat in its sequence. (B) Chromatograms representing band intensity from (A) at 0.1 mM GTP (top) and 1 mM GTP (bottom) with increasing concentrations of ppGpp. (C) Quantification of readthrough primers as a percentage of total primers synthesized at 0.1 mM GTP (green line) and 1 mM GTP (blue line). Points represent averages of n = 3, and the SEM is indicated by error bars. (D) Quantification of total primers synthesized over increasing concentrations of ppGpp at 0.1 mM GTP (green line) and 1 mM GTP (blue line). Points represent averages of n = 3, and the SEM is indicated by error bars.




**Supplemental Figure 11. ppGpp reduces readthrough of a starved site for the DnaG(ZBD+RPD) variant.** (A) Representative gel of primase activity of the DnaG(ZBD +RPD) variant (6.4 μM, 500 nM oJW3319) shows that priming readthrough is still observed at 0.1 mM GTP and decreases upon addition of ppGpp. (B) Representative gel of primase activity of the DnaG(ZBD+RPD) variant (6.4 μM, 500 nM oJW3319) at 1 mM GTP shows that no priming readthrough is observed. (C) Quantification of readthrough primers as a percentage of total primers synthesized at 0.1 mM GTP (green line) and 1 mM GTP (blue line). Points represent averages of n = 3, and the SEM is indicated by error bars. (D) Quantification of total primers synthesized over increasing concentrations of ppGpp at 0.1 mM GTP (green line) and 1 mM GTP (blue line). Points represent averages of n = 3, and the SEM is indicated by error bars.




**Supplemental Figure 12. Oligonucleotide used in initial fluorescent based primase assay.** (Left) Run on 7M Urea 20% PAGE, the template used is not pure, but instead a heterogenous population. (Right) PAGE purified template shows the template is now pure and a purely homogenous population.

| Name | Sequence | Purpose |
|---|---|---|
| oJW2646 | TACTTCCAATCCAATGCAATGGGAAATCGGATACCAGATGA | Forward primer to amplify full-length *dnaG* and *dnaG*(ZBD+RPD) |
| oJW702 | TTATCCACTTCCAATGTTATTATTATTTTAAAGATCGGTTCAATG | Reverse primer to amplify full-length *dnaG* |
| oJW2649 | TTATCCACTTCCAATGTTATTACTGCTCAGTTAAAGACTCCT | Reverse primer to amplify *dnaG*(ZBD+RPD) |
| oJW2384 | CAGACACACACACACACACACACACACA**CTA**CACACA | Template used in primase assays in Figures 1 & 2 |
| oJW3319 | A*G*A*G*A*G*A*G*AA*G*AG<u>CCCC</u>**CTA**GAGAGA | Template used in primase assays in Figures 3-6 |
| oJW3668 | CACACACCAACAACCA*G*CACA**CTA**GAGAA/3InvdT/***** | Alternative template used in primase fidelity assay in supplemental Figure 8 |
| oJW4337 | AGUGUGUGUGUGUGUGUGUGUGUGUCUG | RNA standard used in primase assay in Figures 1 & 2 |
| oJW4338 | AGGGGGCUCUUCUCUCUCUCU | RNA standard used in primase assays in Figures 3-6 |

**Supplemental Table 1. List of oligonucleotides. For oligos used as templates in primase assays, the CTA start site is bolded and the pause site G is italicized.**

***** /3InvdT/ is a 3'-3' linkage synthesized by Integrated DNA Technologies (IDT) that inhibits both degradation by 3' exonucleases and extension from the 3' end.

**Supplemental Table 2. List of plasmids with corresponding genotypes.**

| **Plasmids** | |
|---|---|
| **Name** | **Genotype** |
| pJW269 | pLICtrPC-HA amp$^R$ |
| pJW743 | pLICtrPC-HA *dnaG* amp$^R$ |
| pJW744 | pLICtrPC-HA *dnaG*(ZBD+RPD) amp$^R$ |