

Supplemental Information for:

**Trigger-helix folding pathway and SI3 mediate catalysis and hairpin-stabilized pausing by *Escherichia coli* RNA polymerase**

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Supplemental information:

Figure S1. Elongation by wild-type, F937-736, and 6Ala RNAPs

Figure S2. Exonuclease digestion of ECs formed with wild-type and F937-736 RNAPs

Figure S3.  $\Delta$ SI3a and  $\Delta$ SI3b show increased elongation rate on longer templates.

Figure S4. Test of wild-type and PGPP RNAPs for possible misincorporation.

Table S1. Strains, plasmids, and oligonucleotides

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Table S3. Kinetics of pausing at the *his* pause signal

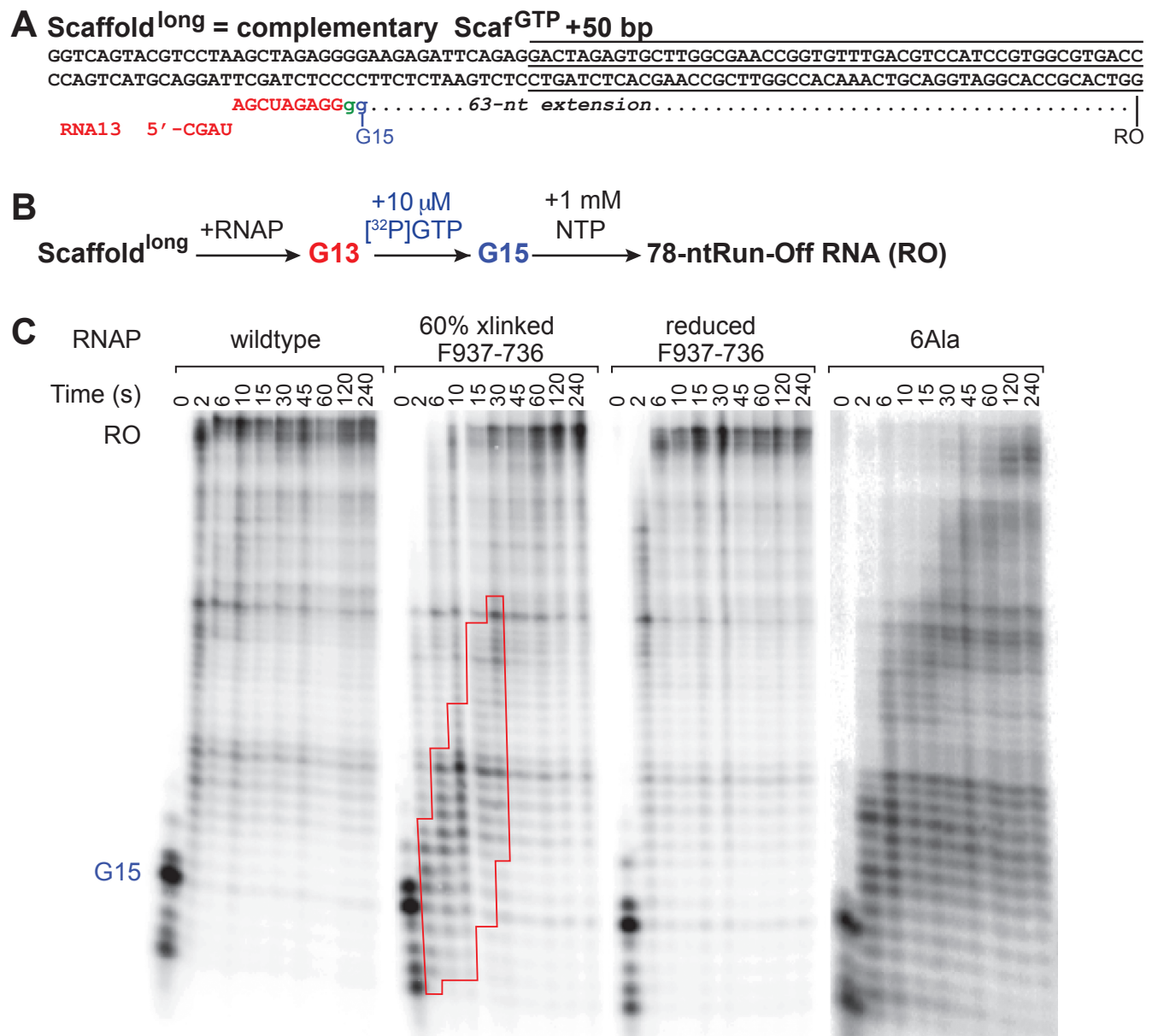


Fig. S1. Elongation by wild-type, F937-736, and 6Ala RNAPs

(A) Schematic of Scaffold<sup>Long</sup>, a derivative of Scaffold<sup>GTP</sup> with additional downstream DNA (42 basepairs) and a fully complementary NT strand.

(B) Schematic of reaction. RNAPs were reconstituted at G13, elongated to G15 with addition of 10 $\mu$ M <sup>32</sup>P-GTP, and given 1 mM all four NTPs at time zero.

(C) Reaction products were halted at the times indicated and separated by denaturing polyacrylamide electrophoresis. The red boxes indicate the apparent products from the crosslinked F937-736 RNAP.

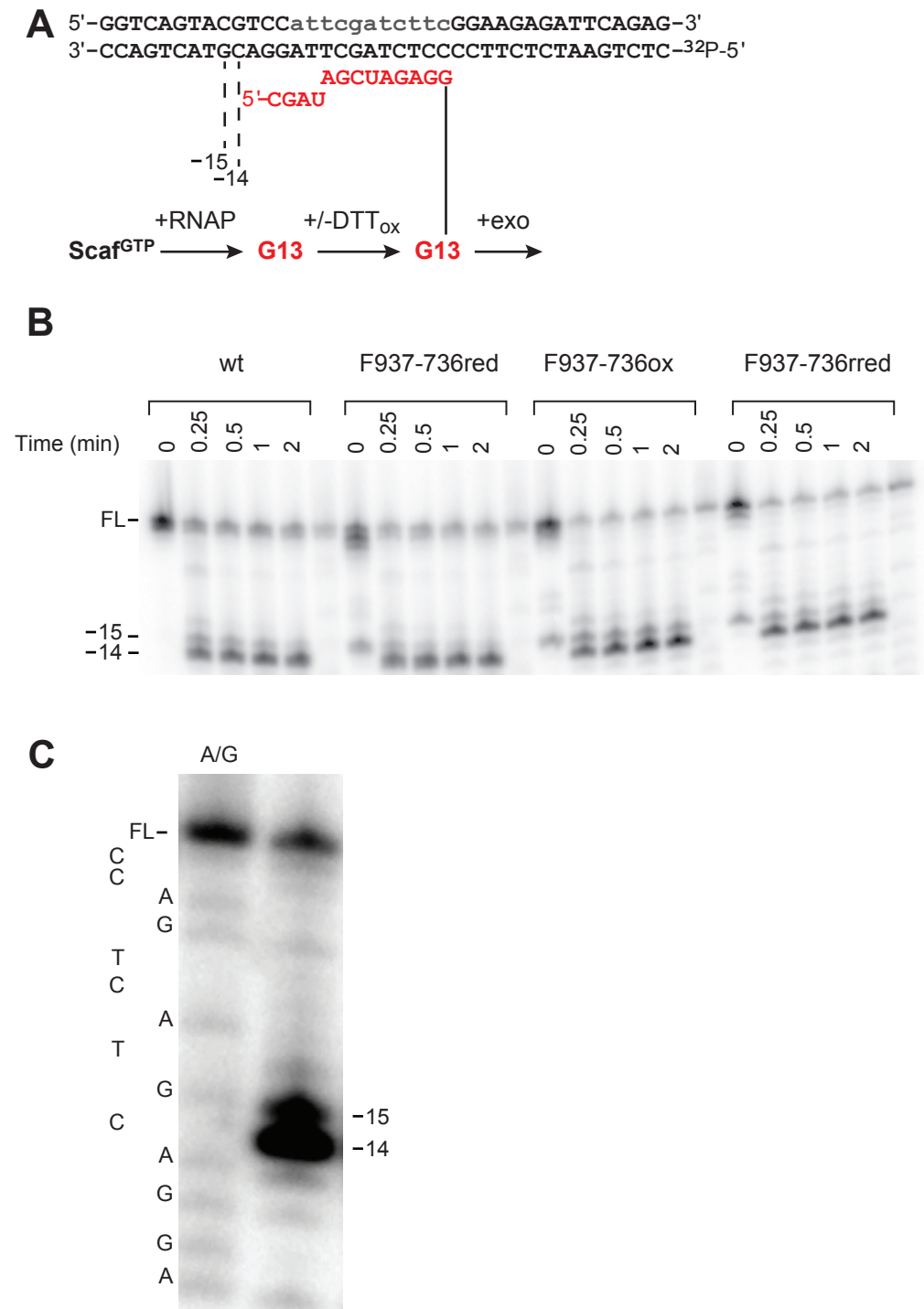


Fig. S2. Exonuclease digestion of ECs formed with wild-type and F937-736 RNAPs.  
 (A) Schematic of exonuclease assay. After reconstitution of complexes and crosslinking, exonuclease is added at time zero, with samples removed during digestion.  
 (B) Samples separated on 20% PAGE, time points as indicated.  
 (C) Mapping of cleavage products by separation alongside an A/G marker.

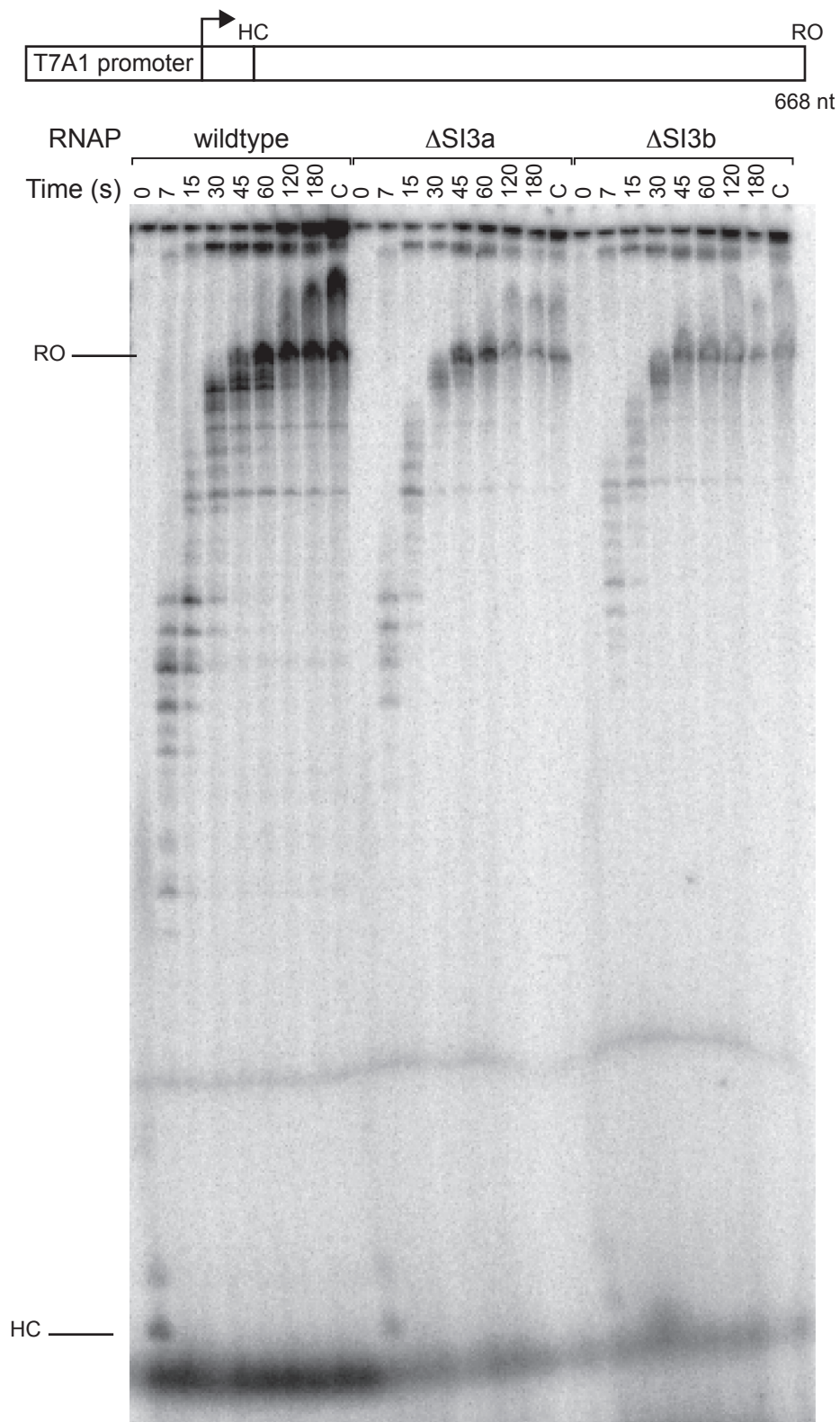


Fig. S3.  $\Delta SI3a$  and  $\Delta SI3b$  show increased elongation rate on longer templates. Top. Schematic of promoter-driven template containing a large fragment of *rpoB* to allow transcription of a 668-nt transcript (pIA146; Table S1).

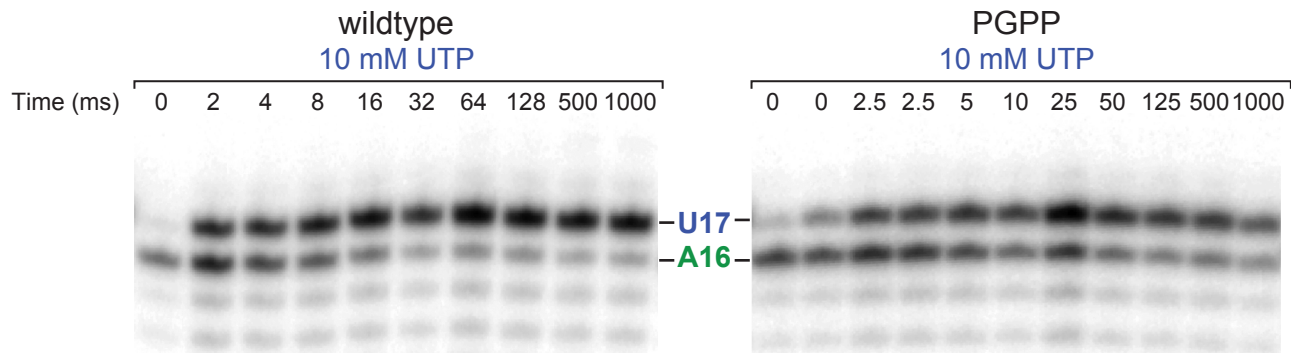


Fig. S4. Test of wild-type and PGPP RNAPs for possible misincorporation. Transcription reactions with wild-type or PGPP RNAPs were performed on ScaffoldGTP, as in Figure 4. Reactions were incubated with 10 mM of both ATP and UTP up to 1 second but no extension beyond U17 (to misincorporate a G) was detected. Thus, we see no evidence for purine-for-purine misincorporation.

Table S1. Strains, Plasmids, and Oligonucleotides

Stock #	Name	Description	Source or Note
<b>Strains</b>			
1607	DH10b	<i>F- mcrAΔ(mrr-hsdRMS-mcrBC) φ80 lacZΔM15 ΔlacX74 deoR recA1 araD139D(araA-leu)7697 galU galK rpsL endA1 nupG</i>	(Ref. 1)
	C2566	C2566,T7 Express. <i>fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--TetS)2 [dcm] R(zgb-210::Tn10--TetS) endA1 Δ(mcrC-mrr)114::IS10. recA-</i>	New England Biolabs
2657	RL2657	<i>recA</i> derivative of C2566	This work
<b>Plasmids</b>			
2956	pRM756	Expresses wild-type <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) with His <sub>10</sub> tag on the $\beta'$ C-terminus	This work
2995	pRM795	Expresses wild-type <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) with His <sub>10</sub> and HMK tags on the $\beta'$ C-terminus	(Ref. 2)
4963	pDJ124	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ I937C Q736C	(Ref. 2)
4291	pMTF028	pRM756 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ G1136P, G1137P (GGPP)	This work
4954	pDJ115	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ I937C P1139C	(Ref. 2)
2961	pRM761	pRM756 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ L930P, T931P (LTPP)	This work
5126	pRM826	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ (KDPP)	This work
5127	pRM827	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ (DIPP)	This work
5132	pRM832	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ (PGPP)	This work

2959	pRM759	pRM756 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ $\Delta$ 943-1130 ( $\Delta$ SI3a)	(This work and Ref. 3)
2993	pRM793	pRM756 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ $\Delta$ 945-1132 ( $\Delta$ SI3b)	(This work and Ref. 4)
5140	pRM840	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ (DIPP) $\Delta$ 945-1132 ( $\Delta$ SI3b)	This work
5154	pRM854	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ L930P, T931P (LTPP) 937C 736C	This work
5144	pRM844	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ (DIPP) 937C 736C	This work
4967	pDJ128	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ 937C 736C $\Delta$ 945-1132 ( $\Delta$ SI3b)	This work
5162	pRM862	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ (DIPP) $\Delta$ 945-1132 ( $\Delta$ SI3b) 937C 736C	This work
5155	pRM855	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ (LTPP) $\Delta$ 945-1132 ( $\Delta$ SI3b) 937C 736C	This work
4288	pMTF025	pRM756 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ 6Ala (924A, 926A, 927A, 11336A, 1137A, 1139A)	This work
5226	pRM926	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ 6Ala 937C 736C $\Delta$ 945-1132 ( $\Delta$ SI3b)	This work
5227	pRM927	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ 6Ala 937C 736C	This work
2996	pRM796	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ 6Ala $\Delta$ 945-1132 ( $\Delta$ SI3b)	This work
5225	pRM925	pRM795 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'\omega$ ) containing $\beta'$ 6Ala 937C 1139C (924A, 926A, 927A, 11336A, 1137A, but 1139C)	This work
3737	pIA423	Expresses wild-type <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'$ ) with CBP/intein	(Ref. 3)

		on the $\beta'$ C-terminus	
3798	pIA373	pIA423 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'$ $\Delta$ 1149-1190; $\Delta$ jaw) with CBP/intein on the $\beta'$ C-terminus	(Ref. 5)
47512	pJZ1	pIA423 derivative that expresses mutant <i>E. coli</i> RNAP ( $\alpha_2\beta\beta'$ $\Delta$ 1149-1190, $\Delta$ jaw and $\Delta$ 943-1130, $\Delta$ SI3a) with CBP/intein on the $\beta'$ C-terminus	(This work and Ref. 3)
761	pIA146	pT7A1-668nt template plasmid	(Ref. 5)
775	pIA171	pT7A1- <i>his</i> P template plasmid	(Ref. 5)
3774	pIA349	pT7A1-ops P- <i>his</i> P - <i>his</i> T template plasmid	(Ref. 6)
<b>Oligonucleotides (5'→3')</b>			
5069	NT, Scaffold <sup>PEC</sup> and Scaffold <sup>UTP</sup>	GGTCAGTACGTCCATTTCGATCTTCGGAAGAGATTCAGAG	
5420	T, Scaffold <sup>PEC</sup>	CTCTGAATCTCTTCCAGCACACATCAGGACGTACTGACC	
4903	T, Scaffold <sup>UTP</sup>	CTCTGAATCTCTTCCCCTCTAGCTTAGGACGTACTGACC	
7636	RNA13, Scaffold <sup>GTP</sup> and Scaffold <sup>Long</sup>	CGAUAGCUAGAGG	
8079	RNA13 (EC-dG)	CGAUAGCUAGAG3'dG	
4865	RNA29, Scaffold <sup>PEC</sup>	CCUGACUAGUCUUUCAGGCGAUGUGUGCU	
4867	RNA27, Scaffold <sup>PEC</sup>	CCUGACUAGUCUUUCAGGCGAUGUGUGCU	
7712	NT, Scaffold <sup>UTP</sup>	GGTCAGTACGTGGCTtcgatcttcTGACAGCAATCAGAGTACC	
7713	T, Scaffold <sup>UTP</sup>	GGTACTCTGATTGCTGTACATGCCCGGCTAGCCACGTACTGACC	
7718	RNA14, Scaffold <sup>UTP</sup>	UUUUUUUAGCCGGG	
8576	NT, Scaffold <sup>Long</sup>	GGTCAGTACGTCCCTAAGCTAGAGGGGAAGAGATTCAGAGGACTAG AGTGCTTGGCGAACC GGTGTTTGACGTCCAGGAATGTCAAATCCGT GGCGTGACC	
8493	T, Scaffold <sup>Long</sup>	GGTCACGCCACGGATTTGACATTCTGGACGTCAAACACCGGTTTCG	



		CCAAGCACTCTAGTCCTCTGAATCTCTTCCCCTCTAGCTTAGGACG TACTGACC
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## References

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Table S2. Nucleotide addition rate measurements

<b>G14 and G15 nucleotide addition rates</b>		
<b>RNAP</b>	<b><math>V_{\max}^{\text{app}}</math> G14 <math>\text{s}^{-1}</math></b>	<b><math>V_{\max}^{\text{app}}</math> G15 <math>\text{s}^{-1}</math></b>
WT	560 ± 10	170 ± 30
F937-736ox	12 ± 1	15 ± 1
F937-736red	300 ± 70	240 ± 30
WTox	530 ± 120	140 ± 30
6Ala	1.7 ± 0.3	7.3 ± 1.1
LTPP	0.031 ± 0.006	0.081 ± 0.013
DIPP	0.36 ± 0.06	0.99 ± 0.18
PGPP	580 ± 50	26 ± 4
$\Delta$ SI3	550 ± 50	88 ± 3
<b>U17 nucleotide addition rates</b>		
<b>RNAP</b>	<b><math>V_{\max}^{\text{obs}}</math> <math>\text{s}^{-1}</math></b>	<b><math>k_{\text{UTP}}^{\text{obs}}</math> mM</b>
WT	360 ± 100	1.4 ± 0.6
LTPP	0.008 ± 0.002	1.3 ± 1.0
$\Delta$ TL	0.008 ± 0.000	2.3 ± 0.1
6Ala	0.41 ± 0.06	0.35 ± 0.11
KDPP	0.32 ± 0.02	0.27 ± 0.04
DIPP	0.18 ± 0.01	0.17 ± 0.10
GGPP	0.21 ± 0.01	0.28 ± 0.06
PGPP	480 ± 50	0.45 ± 0.13
$\Delta$ SI3	750 ± 70	1.2 ± 0.2
DIPP $\Delta$ SI3	0.61 ± 0.03	0.13 ± 0.02
6Ala $\Delta$ SI3	0.31 ± 0.02	0.62 ± 0.14
$\Delta$ SI3	550 ± 50	88 ± 3
<b>U17 nucleotide addition, 10 mM UTP</b>		
<b>RNAP</b>	<b><math>V_{\max}^{\text{app}}</math> <math>\text{s}^{-1}</math>, 10 mM UTP</b>	
$\Delta$ SI3a	680 ± 70 ( $V_{\max}^{\text{obs}} = 750 \pm 70 \text{ s}^{-1}$ )	
$\Delta$ SI3b	560 ± 100	

Table S3. Kinetics of pausing at the *his* pause signal

<b>A. <i>his</i> pause escape, elongating through the pause</b>			
<b>RNAP</b>	<b>Bypass fraction</b>	<b>Pause fraction, E</b>	<b>Pause escape rate, <math>k_e</math> <math>s^{-1}</math></b>
F937-736red	$0.08 \pm 0.03$	$0.75 \pm 0.01$	$0.010 \pm 0.005$
F937-736ox	$0.68 \pm 0.10$	$0.21 \pm 0.02$	$0.002 \pm 0.001$
F937-736rred	$0.10 \pm 0.03$	$0.87 \pm 0.03$	$0.002 \pm 0.001$
<b>B. <i>his</i> pause escape, elongating through the pause</b>			
<b>RNAP</b>	<b>Pause fraction, E</b>	<b>Pause escape rate, <math>k_e</math> <math>s^{-1}</math></b>	
WT	$0.75 \pm 0.01$	$0.010 \pm 0.001$	
$\Delta$ SI3a	$0.65 \pm 0.09$	$0.016 \pm 0.02$	
$\Delta$ SI3b	$0.67 \pm 0.01$	$0.020 \pm 0.04$	
<b>C. <i>his</i> pause escape, starting at the pause</b>			
<b>RNAP</b>	<b>Fast escape rate, <math>s^{-1}</math></b>	<b>Pause fraction, E</b>	<b>Pause escape rate, <math>k_e</math> <math>s^{-1}</math></b>
WT	NA	$0.94 \pm 0.02$	$0.015 \pm 0.002$
PGPP	$0.20 \pm 0.07$	$0.26 \pm 0.03$	$0.025 \pm 0.002$
F773V	$0.27 \pm 0.01$	$0.24 \pm 0.02$	$0.020 \pm 0.004$