STAR★METHODS

KEY RESOURCES TABLE

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Carlos Bustamante (carlosb@berkeley.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Source organism

All proteins used herein were purified from the *Escherichia coli* Rosseta 2 cells as described below.

METHODS DETAILS

Optical tweezers

A home-built optical tweezers instrument was assembled as described previously (Gabizon et al., 2018; Righini et al., 2018). Briefly, an Nd:YAG 1,064-nm laser was used. The two traps were generated by switching a single beam at high rate using acoustic-optic deflector (AOD). The frequency of the AOD was controlled by a custom-made radio frequency board (Comstock et al., 2011), and the position of the traps were switched every 5 μs. In this case, detection of the bead positions in both traps was achieved on the same quadrant photodiode (QPD).

SRP RNA coded DNA template

A 114-bp DNA fragment coding for the *Escherichia coli* (*E. coli*) SRP RNA was inserted into a PGMZ plasmid at 42-bp downstream from T7A1 promotor. The 444-bp dsDNA template was amplified by PCR from the cloned plasmid such that the template ended with 39-bp downstream of the SRP RNA sequence. When an RNAP molecule reached the end of template DNA, the separation of an RNAP and RNA transcript was designed to be ~12-nt, so that the presence of RNAP would not interfere with the folding of SRP RNA. This DNA template design placed a stalled sequence (39-nt) between the transcription start site and T7A1 promotor. This stalled sequence comprises an AT-stretch where RNAP could be stalled by withholding GTP before reaching the SRP RNA sequence. Also, the first 30-nt of the stalled sequences were designed to hybridize with the cohesive end of the dsDNA handle, leaving a ~15-nt RNA linker region between the DNA handle and the SRP RNA structure after transcription. To prepare the DNA templates coding truncated as well as full length SRP RNAs, PCR reactions were performed with biotinylated oligo nucleotides (IDT) as the reverse primer for the force curve measurement in the presence of RNAP.

Purification of SRP RNA

A 114-bp DNA fragment coding for the *E. coli* SRP RNA was inserted into a PGMZ plasmid at 42-bp downstream from T7 promotor. The 448-bp dsDNA template was amplified by PCR from the cloned plasmid. The 5' sequences (30-bp) after T7 promotor as well as the 3' end sequences (23-bp) were designed to hybridize with the cohesive end of a dsDNA handle. Then the purified SRP RNA was synthesized by means of in vitro transcription using T7 RNA polymerase based on the Megascript protocol (Ambion). The SRP RNA was used after purification using MEGAclear Transcription Clean-Up Kit (ThermoFisher).

DNA handle

The DNA handles were prepared by PCR using lambda DNA as the template, with biotin or digoxigenin-labeled oligonucleotides (IDT) as primers. The constructs were used after standard PCR purification. For the handle to be attached to RNA transcript via complementary sequences, 1',2'-Dideoxyribose was introduced in the oligonucleotide primer (IDT).

Beads coating and passivation

Anti-digoxigenin coated bead 5% (w/v) 1 μm-diameter carboxylated polystyrene beads (50 μl) were washed with 1 ml Activation buffer (100 mM MES-NaOH pH = 6, 500 mM NaCl) three times, with centrifugation steps (3 min at 3000 rpm) between wash steps. EDC and sulfo-NHS were then added to the beads solution to final concentration of ~5 mM and ~10 mM, respectively. After incubation for 15 min at room temperature, the reaction was quenched by adding 2.8 μl 2-mercaptoethanol (40 mM). After washing the beads with Activation buffer, the anti-digoxigenin antibody was incubated with bead solution by tumbling at room temperature.

The reaction proceeded for 2 hours. The beads were washed with Quenching buffer (100 mM Tris pH = 7) and Storage buffer (40 mM HEPES-KOH pH = 7.5, 100 mM KCl), and stored at 4°C.

Oligo coated bead To prepare a double stranded oligo for coupling reaction, DNA amine oligo (5'-/5AmMC6/TTAATTCATTGCGTTCTGTACACG-3') was hybridized to DNA phosphorylated oligo (5'-/5Phos/CGGTCGTGTACAGAACGCAATGAATT- 3'). Annealing was performed by heating the mixture of DNA oligo (0.25 mM each) to 95°C for 10 min, followed by cooling to room temperature. 10% (w/v) 1 μm-diameter carboxylated polystyrene beads (10 μl) were washed four times with Coupling buffer (100 mM MES-NaOH pH = 4.7, 150 mM NaCl, 5% DMSO) by centrifugation (5 min at 4500 g). The prepared double stranded oligo and EDC were then added to final concentration of ~1.5 μM and ~300 mM, respectively. After incubation for 2 hours with vigorous shaking, more EDC (final concentration ~ 500 mM) was added, followed by overnight shaking at room temperature. The reaction was quenched by adding glycine (final concentration ~50 mM). Lastly the bead solution was washed with Stock buffer (20 mM Tris-pH = 8, 1 mM EDTA, 0.05% Tween 20, 5 mM NaN_3) four times and stored at

4°C.

Beads passivation The beads (1%, w/v) were passivated by diluting 10-fold in TE buffer (20 mM Tris-HCl pH = 8, 1 mM EDTA) and addition of casein to final concentration of 1 mg/ml. Then the beads solution was incubated with vortex for ~1 hour at room temperature and washed with TE buffer by centrifugation (3 min at 4500 g), and stored at 4°C. 0.82 µm-diameter streptavidin coated beads (Spherotech) were passivated with the same protocol and used without further processing.

Recombinant proteins

Preparation of Biotinylated E *coli* RNA polymerase. Biotinylated *E. coli* RNA polymerase was prepared as described previously (Righini et al., 2018). **Preparation of sigma 70.** Plasmid pIA1127 was transformed into Rosetta2 bacteria. The bacteria were grown in 2 L of 2YT medium supplemented with 1% glucose, NPS (25 mM $(NH_4)_2SO_4$, 50 mM KH_2PO_4, 50 mM Na_2HPO_4), 1 mM magnesium sulfate, 34 µg/mL chloramphenicol, and 50 µg/mL kanamycin. The culture was grown at 37°C and transferred to 17 °C. Then IPTG was added to 0.1 mM and incubated for 16 hours. For purification, the bacteria were dispersed in 80 mL of buffer A25 (20 mM Tris, pH 7.5, 0.5 M NaCl, 10% glycerol, 25 mM imidazole, 2 mM beta-mercaptoethanol) supplemented with 0.1 mg/mL lyzozyme and protease inhibitors (Roche). Then the bacteria were lysed by using French press, and the lysate was clarified by centrifugation and filtration and loaded on a 5 mL Ni-NTA column. The column was washed with 20 mL buffer A25 and 20 mL A50 (A25 + 50 mM imidazole), and the his-tagged sigma 70 was eluted in A300 (A25 + 300 mM imidazole). TEV protease (Tropea et al., 2009) was added at a molar ratio of 1:40, and reacted overnight at 4 °C while being dialyzed against A50. The protein was then passed again through a Ni-NTA column. The flow-through, containing non–his-tagged sigma 70 was collected, concentrated and purified by gel filtration on a sephacryl S300 column equilibrated with buffer B (20 mM Tris, pH 7.5, 0.5 M NaCl, 10% glycerol, 1 mM EDTA, 1 mM DTT). Aliquots were flash-frozen in liquid nitrogen and stored at −80 °C.

Preparation of sortagged RNA polymerase holoenzyme. Plasmid pIA1234 was transformed into Rosetta2 bacteria. Sortag-RNAP was expressed using the same protocol as sigma 70, except that ampicillin was used instead of kanamycin. For purification, the cells were dispersed in 75 mL of lysis buffer (50 mM Tris, pH 6.9, 0.5 M NaCl, 5% glycerol) supplemented by 0.1 mg/mL lyzozyme and protease inhibitors and lysed via French press. The lysate was centrifuged and filtered, and imidazole was added to 20 mM. The protein was loaded on a 5-mL Ni-NTA column. The column was washed with 30 mL of lysis buffer plus 20 mM imidazole, and the his-tagged RNAP core enzyme was eluted in lysis buffer plus 250 mM imidazole. To form the holoenzyme, the sample was incubated with a twofold excess of purified sigma 70 overnight on ice and diluted 10-fold with buffer B0 (50 mM Tris, pH 6.9, 5% glycerol, 0.5 mM EDTA, 1 mM DTT) and loaded on a heparin 5-mL column. A gradient of 50 mM to 1 M NaCl was used to elute the protein. RNAP holoenzyme was separated clearly from excess sigma 70. The sample was dialyzed against buffer B50 (50 mM Tris, pH 6.9, 5% glycerol, 50 mM NaCl, 0.5 mM

EDTA, 1 mM DTT) and then purified further on a 1-mL monoQ column using a 50 mM to 1 M NaCl gradient (again, the sample was split into three portions loaded separately). Pure fractions were pooled, dialyzed against storage buffer (20 mM Tris, pH 7.5, 200 mM KCl, 0.2 mM EDTA, 0.2 mM DTT, 5% glycerol), aliquoted, flash-frozen, and stored at −80 °C.

Biotinylation of sortag-RNA polymerase. We obtained a peptide containing an N-terminal GGG tag with a biotin-modified lysine residue (Genscript): GGGGDGDY{K(biotin)}. We reacted 100 µL of 9.6 µM sortag-RNAP with a 200-fold excess of biotinylated peptide in 200 µL coupling buffer (50 mM Tris, pH 7.5, 5 mM CaCl2) containing 2 µM sortase (Chen et al., 2011). The reaction proceeded for 60 min. At this point, imidazole was added to 25 mM and NaCl to 350 mM, and the sample was passed through 70 µL Ni-NTA beads to remove sortase and unreacted RNAP. The excess peptide was removed by dialysis into storage buffer, and the biotinylated RNAP was stored in storage buffer at −80 °C.

Preparation of GreB. GreB was purified as described previously (Gabizon et al., 2018). The gene for GreB was cloned into a pET vector by ligation independent cloning (Addgene #29653). The plasmid was transformed in Rosetta 2 cells, the bacteria were grown in 1 liter of 2YT medium supplemented with 1% glucose, NPS (25 mM $(NH_4)_2SO_4$, 50 mM KH_2PO_4, 50 mM Na_2HPO_4), 1 mM magnesium sulfate, 34 µg/ml chloramphenicol and 50 µg/ml kanamycin. The culture was grown at 37°C to an OD600 of 0.6, IPTG was added to 0.5 mM and induction proceeded for 4 hours at 37°C. The bacteria were then centrifuged, and dispersed in 40 ml of lysis buffer (Tris 100 mM pH = 7.9, 25 mM imidazole, 1 M NaCl, 2 mM beta-mercaptoethanol) supplemented with 1 mM PMSF and 0.2 mg/ml lyzozyme. The bacteria were lysed by sonication, and the solution was centrifuged and filtered. The sample was loaded on a 2 ml Ni-NTA column, washed with 12 ml of lysis buffer, followed by 12 ml of lysis buffer with 50 mM imidazole, and finally eluted with lysis buffer with 300 mM imidazole. TEV protease was added at a 1:10 molar ratio, and the sample was incubated overnight at 4°C while dialyzing against lysis buffer. The sample was passed again over 1 ml Ni-NTA beads, concentrated to <3 ml and loaded on a sephacryl S100 gel filtration column equilibrated with Tris 25 mM pH = 8, 1 M NaCl, 1 mM EDTA, 1 mM DTT. Fractions containing clean GreB were pooled and concentrated to ~ 50 µM; glycerol was added to 50%; and the protein was flash frozen with liquid nitrogen and stored at -80°C. When performing experiments with GreB, the protein was dialyzed first into HEPES 25 mM pH = 8, 1 M KCl, 1 mM DTT and 1 mM EDTA so that it could be mixed into the experimental buffer in precalculated ratios in order to maintain the buffer composition.

Force curve measurement of truncated- and full length- SRP RNA with RNA polymerase

As a roadblock for RNAP, a streptavidin was attached to 3' end of template DNA in TB20 buffer (20 mM Tris (pH = 8), 20 mM NaCl, 10 mM $MgCl_2$, 20 mM DTT, 1 mg/ml BSA) by incubating for 1 hour at room temperature. Stalled complexes were prepared by incubating DNA/streptavidin complex with RNAP in TB20 buffer containing 5 µM ATP, CTP, 150 µM ApU and RNaseOut (ThermoFisher) for 20

minutes at 37°C. Stalled complexes were then incubated with heparin and digoxigenin-labeled 1.5 kb DNA handle harboring the 5' cohesive end which is complementary to the 5' sequence of transcripts for 20 minutes. 1 μm-diameter anti-digoxigenin coated beads were added to stalled complexes. After 20 min incubation, the mixture was incubated with NTPs (~50 μM) for 1 min and diluted in 1 ml TB130 buffer (20 mM Tris (pH = 8), 130 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT and 10 mM NaN_3). For the other side of the tether, 1 μm oligo-coated beads were ligated to biotin-labeled 1.5 kb DNA handle in TB20 including 0.4 units of T4 DNA ligase and 0.1 mM ATP for 1 hour at room temperature. Following the ligation, the mixture was diluted in 1 ml TB130 buffer. On the optical tweezers instrument, the stalled complex bead and oligo-biotin bead were trapped by laser beams, respectively. To allow tether formation, the two beads were moved in close proximity of each other, such that a tether could be formed through interaction of the streptavidin at the 3' end of template DNA with the biotin at DNA handle attached to oligo coated bead.

Force curve measurements of purified full length-SRP RNA

A purified SRP RNA was incubated with digoxigenin -labeled 1.5 kb DNA handle harboring the 5' cohesive end which is complementary to 5' transcripts in TB20 buffer for 20 minutes. 1 μm-diameter anti-digoxigenin coated beads were added to RNA/handle complexes. After 20 min incubation, the mixture was diluted in TB130 buffer. For the other beads, 0.82 μm-diameter streptavidin coated beads were attached to biotin-labeled 1.5 kb DNA handle harboring the 5' cohesive end which is complementary to 3' sequence of transcripts in TB20 buffer. After 20 min incubation, the mixture was diluted in TB130 buffer. Tether was formed between two beads through the complementary sequences of 3' RNA sequence and biotin-labeled DNA handle.

Real time observation of elongation and folding of nascent RNA chain

Stalled complexes were prepared by incubating template DNA with biotinylated RNAP in TB20 buffer containing 5 μM ATP, CTP, 150 μM ApU and RNaseOut (ThermoFisher) for 20 minutes at 37°C. Stalled complexes were then incubated with heparin and digoxigenin -labeled 1.5 kb DNA handle bearing the 5' cohesive end which is complementary to 5' sequence of transcripts for 20 min. 1 μm-diameter anti-digoxigenin coated beads were added to stalled complexes. After 20 min incubation, the mixture was diluted in 1 ml TB130 buffer. After preparation of DNA handle coated beads the same way as for force curve measurements in the presence of RNAP described above, the beads were incubated with streptavidin for 20 min and diluted in TB130 buffer. After formation of tether on the optical tweezers instrument, the extension of nascent transcript was measured with applying constant force by flowing TB130 buffer containing NTPs.

Addition of transcription factor GreB or antisense oligo in real-time transcription

experiments

Real-time transcription experiments in the presence of GreB or antisense oligo were performed in the same manner as in its absence, except the transcription buffer was supplemented with 1 μM of GreB or 10 μM HPLC purified antisense oligo (IDT).

Conversion from length of transcriptional trajectories to nucleotide

In order to convert the observed transcription extension to nucleotides, we take into account the force-dependent characteristics determined from the extensible worm-like chain (WLC) model (Bustamante et al., 1994):

$$\frac{FP}{k_B T} = \frac{1}{4}\left(1 - \frac{x}{L}\right)^{-2} - \frac{1}{4} + \frac{x}{L} - \frac{F}{K} \qquad \text{(Equation 1)}$$

where k_B is the Boltzmann constant, P is the persistence length, K is the elastic modulus and L is the counter length. Among the parameters in WLC model, persistence length represents the stiffness of RNA chain, which has big impact on the conversion factor between nanometers and nucleotides. It has been shown that persistence length is altered with buffer condition such as ionic strength (Chen et al., 2012) and RNA sequence (Seol et al., 2007), such that NTP concentration can affect the extensional change observed by optical tweezers. To determine the persistence length in our experimental condition, we observed unfolding/refolding behavior of a long RNA hairpin (method following Yan et al., 2015); the value determined was also verified using SRP RNA in the absence of RNAP. The reference RNA comprises 180-bp long hairpin structure, which produces three unfolding rips by mechanical pulling process. From the extensional change upon unfolding, the persistence lengths were estimated in different buffer conditions, and the conversion factors were calculated at each experimental condition (i.e. applied force and NTP concentration) as listed in Table S1.

Table S1. Persistence lengths and conversion factors (nt/nm) in the optical tweezers experiments. These values were determined by force extension curve measurements described above.

NTP concentration	persistence length (nm)	conversion factor (nt/nm)
1 mM	1.31	2.77 (F = 8.6 pN)
50 uM	1.34	2.75 (F = 8.6 pN), 2.27 (F = 22 pN)
10 uM	1.39	2.71 (F = 8.6 pN)
nucleotide free	1.40	———

Plasmid constructs for cell viability experiments

pZE21MCS (MCS: multiple cloning sites; sold by Expressys; a gift from A. Flamholz) and customized

gblocks (~500 bp long double-stranded DNAs, which sequences from 5' to 3' contain: $P_{LtetO-1}$ promoter, specific SRP RNA variant sequence, rrnC terminator, and a second $P_{LtetO-1}$; purchased from IDT) were digested with BbsI and AvrII (R3539 and R0174, NEB), ligated with quick T4 ligase kit (M2200, NEB) following NEB protocol, and transformed into an *E. coli* cloning strain, DH5alpha-Z1 (Expressys). A series of plasmids (pZE21_SRP_rrnC) were constructed: pZE21_wtSRP_FL_rrnC, pZE21_SRP_100_rrnC, pZE21_U18C_100_rrnC, pZE21_U3234C_100_rrnC, pZE21_empty_rrnC, and pZE21_SRP_100_rrnC_read-through. The plasmids harboring full-length SRP RNA sequence mutants (U18C and U32C/U34C) were prepared from pZE21_wtSRP_FL_rrnC using the Q5 site-directed mutagenesis kit (E0554, NEB), and following protocols therein for appropriate primer designs and sequential procedures to obtain the modified plasmids (pZE21_U18C_FL_rrnC and pZE21_U3234C_FL_rrnC). All constructed plasmids are sequenced (Sequetech) and verified to carry the correct SRP variant designs.

Complete plasmid sequence of pZE21_wtSRP_FL_rrnC

Notations:

ori (colE1) / Kan^R / $P_{LtetO-1}$ / wtSRP_FL / rrnC / point mutation for U18C and U32C/U34C / transcript truncation to 100-bp long

CTAGGCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCAC
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ATAAAAAAAATCCTTAGCTTTCGCTAAGGATGATTTCTAC

Cell viability plate assay

BL21 (non-T7 expression, C2530H from NEB) were transformed with purified, sequencing-verified plasmids (pZE21_SRP_rrnC) that carry specific SRP RNA variants (following protocol from NEB; 50 ng plasmid per 50 µl competent cell) and plated on kanamycin (40 µg/ml) LB agar plates. Images of the plates, showing the emergence of colonies that carry plasmids encoding with specific SRP RNA variants, were taken after 12-16 hours of growth at 37 °C. At least 5 colonies were picked from each transformation experiments and sent for sequencing (Sequetech) to confirm the plasmid constructs therein.

Recovery/post-relaxation growth rate

BL21 (non-T7 expression, C2530H from NEB) were transformed with purified, sequencing-verified plasmids (pZE21_SRP_rrnC) that carry specific SRP RNA variants (following protocol from NEB; 50 ng plasmid per 50 µl competent cell) and plated on kanamycin (40 µg/ml) LB agar plates. A single

colony was picked and inoculated into 10 mL of LB (antibiotic-free), and let grow at 37 °C with shaking at 180 rpm for 6 hours. We then split each culture sample in half, where 5 ml continues to grow in LB (i.e. permissive condition), and the other 5 ml grows in the presence of kanamycin (final conc. 40 μg/ml), for another 10 hours. After a total of 16 hours (6+10) overnight growth, aliquot from each culture is diluted with LB plus kanamycin (final conc. 40 μg/ml) to OD_{600} = 0.02 as the starting point of recovery growth (t_0). We then record OD_{600} every 30-45 min interval, to monitor the rate of growth at 37 °C, for a total of 3-4 hours until the culture reaches the mid-log phase (OD_{600} = 0.6-0.8).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical details of individual experiments, including numbers of observations and replicates, dispersion and precision measures, are as described in the manuscript text, figure legends, and the figures themselves.

DATA AND SOFTWARE AVAILABILITY

The full dataset will be made available upon request.

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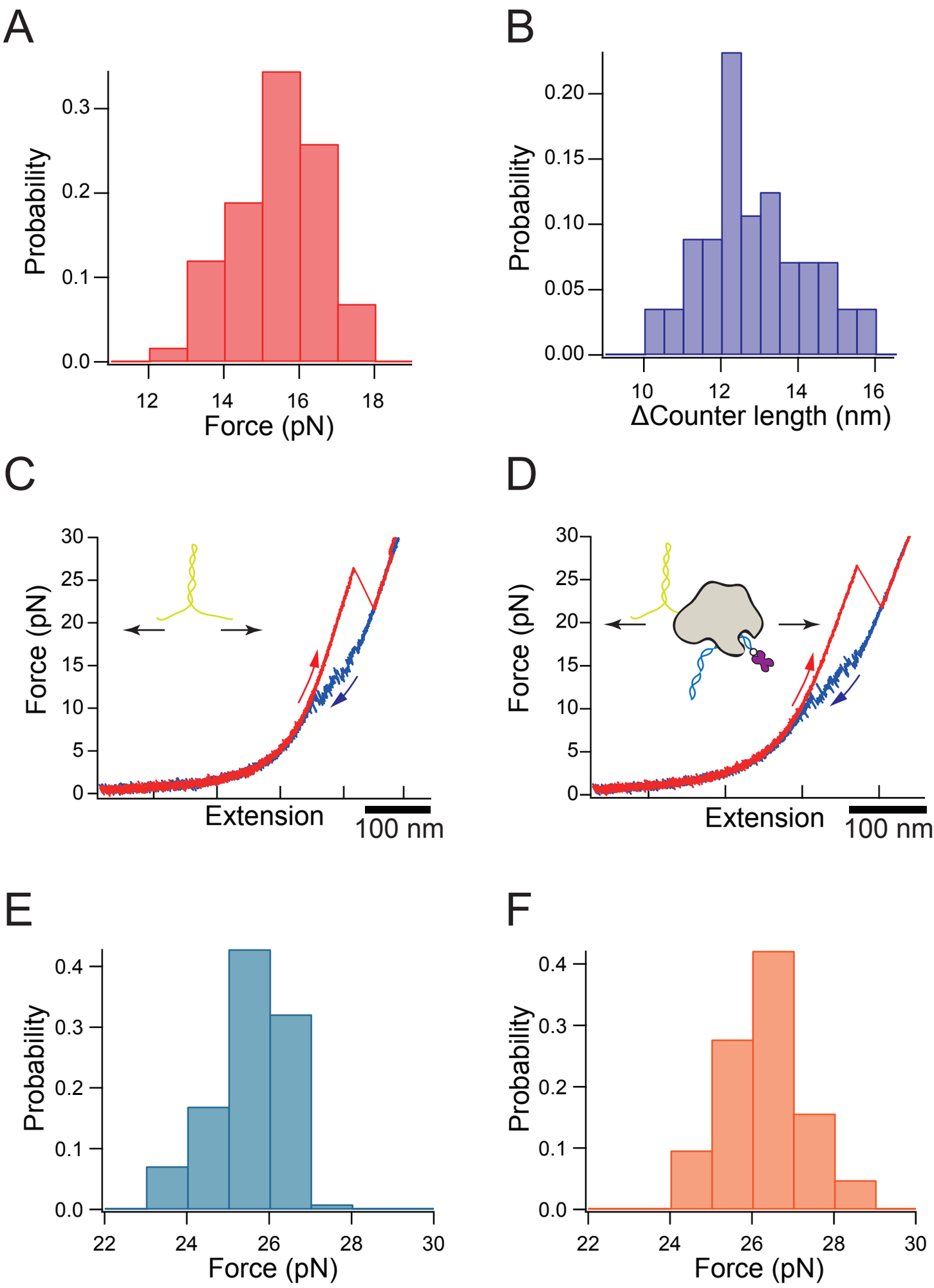
Yan, S., Wen, J. Der, Bustamante, C., and Tinoco, I. (2015). Ribosome Excursions during mRNA Translocation Mediate Broad Branching of Frameshift Pathways. Cell *160*, 870–881.

Tropea JE, Cherry S, Waugh DS (2009) Expression and purification of soluble His 6-tagged TEV protease. High Throughput Protein Expression and Purification: Methods and Protocols, ed Doyle SA (Humana, New York), pp 297–307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0.2
0.1
0.0
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14
16
18
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B
Probability
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0.15
0.10
0.05
0.00
10
12
14
16
ΔCounter length (nm)
C
Force (pN)
30
25
20
15
10
5
0
Extension
100 nm
D
Force (pN)
30
25
20
15
10
5
0
Extension
100 nm
E
Probability
0.4
0.3
0.2
0.1
0.0
22
24
26
28
30
Force (pN)
F
Probability
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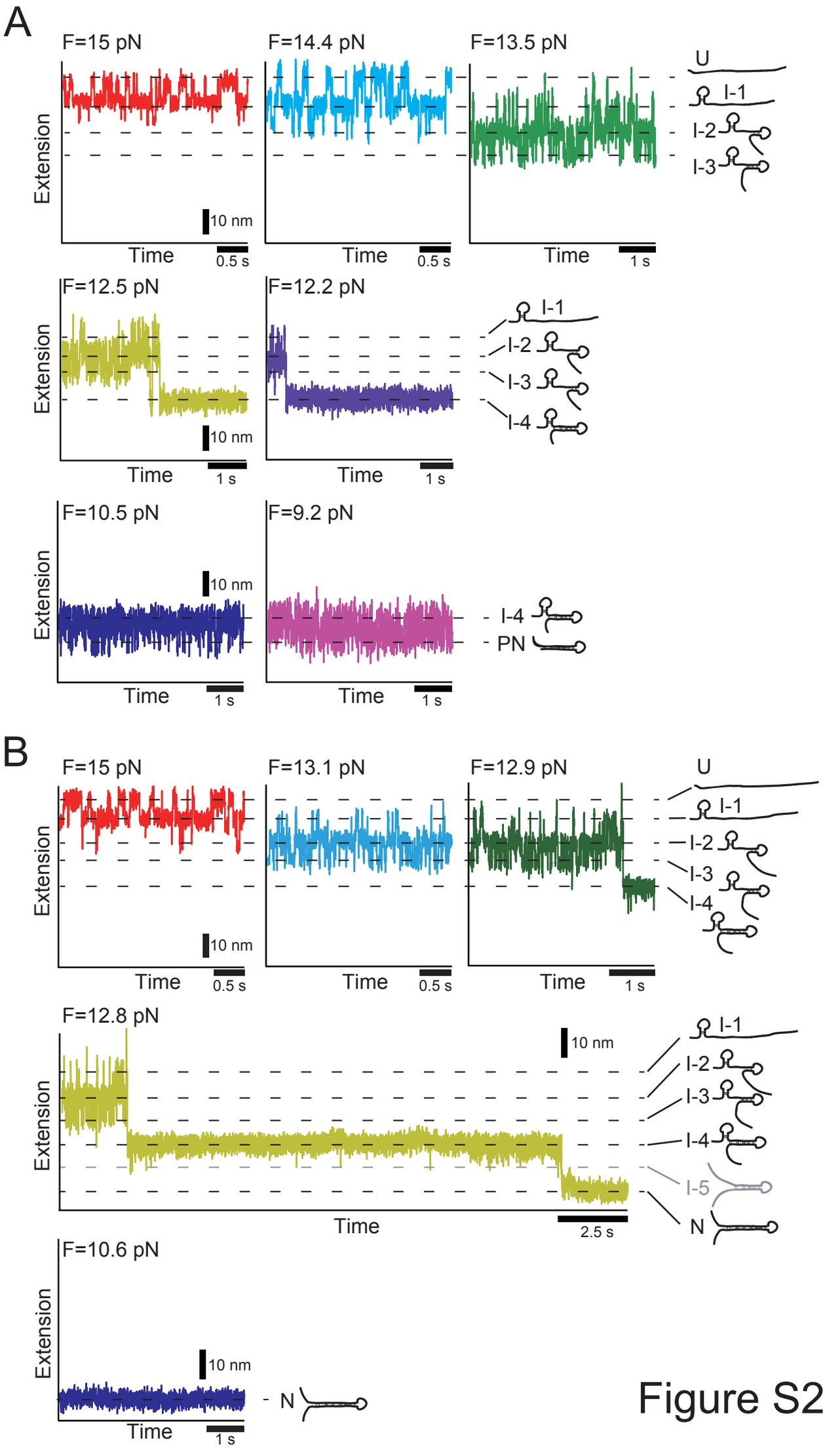
0.0
22
24
26
28
30
Force (pN)

Figure S1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F=14.4 pN
F=13.5 pN
U
I-1
I-2
I-3
Extension
10 nm
Time
0.5 s
1 s
F=12.5 pN
F=12.2 pN
I-4
F=10.5 pN
F=9.2 pN
PN
B
F=13.1 pN
F=12.9 pN
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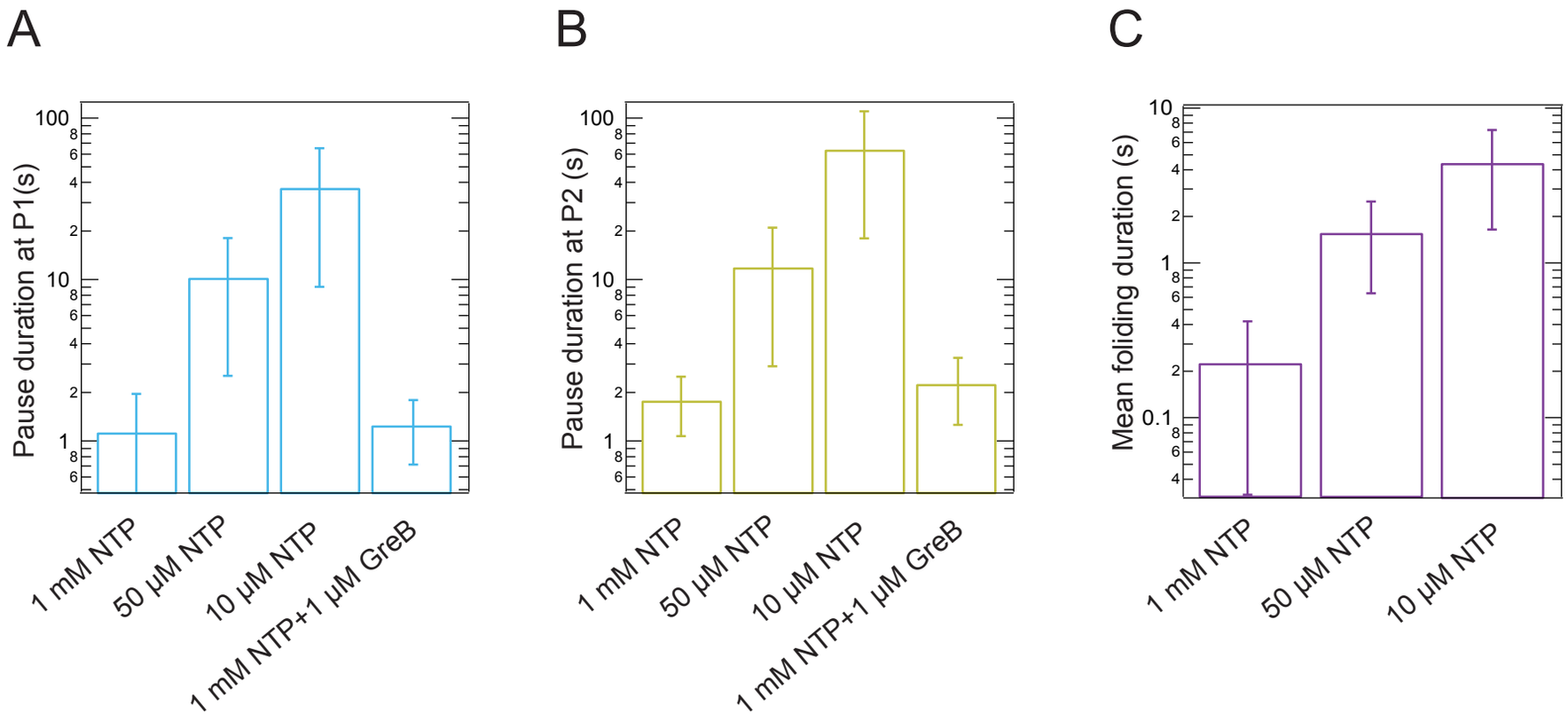
I-5
N
F=10.6 pN

Figure S2


A
B
C
Pause duration at P1(s)
Pause duration at P2 (s)
Mean foliding duration (s)
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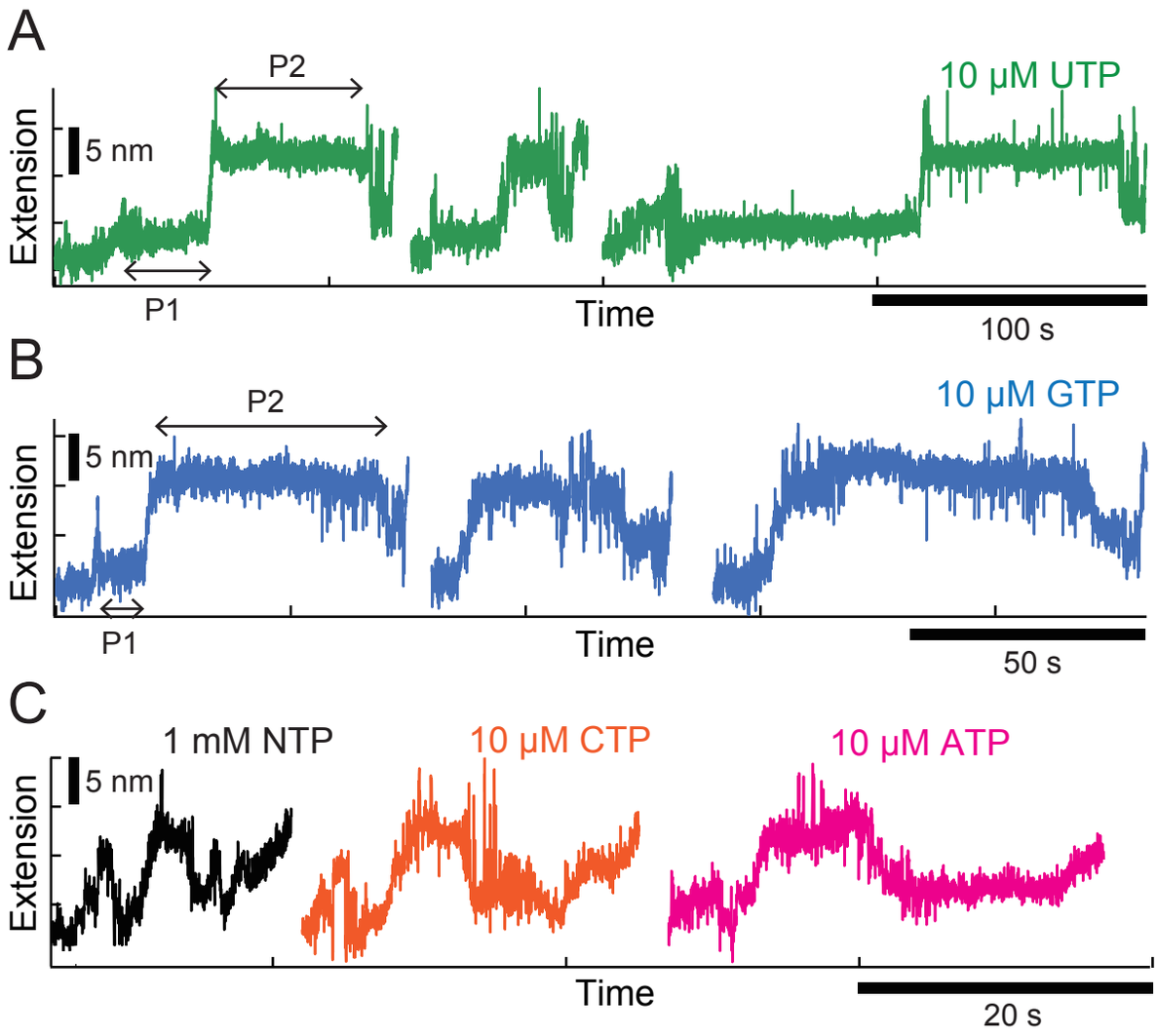
50 μM NTP
10 μM NTP
1 mM NTP+1 μM GreB

Figure S3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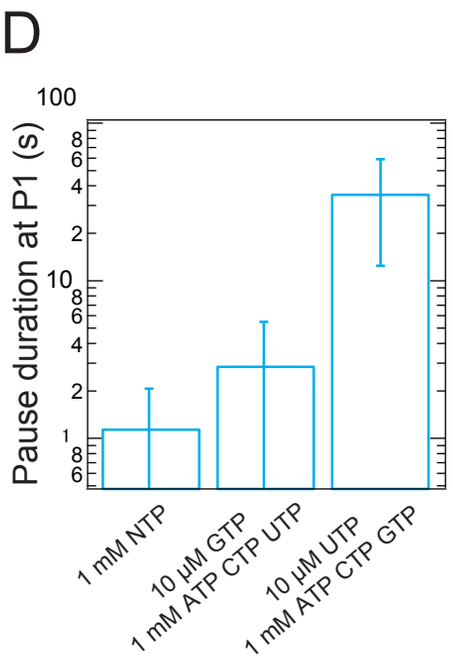
P2
10 μM UTP
Extension
5 nm
P1
Time
100 s
B
P2
10 μM GTP
Extension
5 nm
P1
Time
50 s
C
1 mM NTP
10 μM CTP
10 μM ATP
Extension
5 nm
Time
20 s


D
Pause duration at P1 (s)
100
8
6
4
2
10
8
6
4
2
1
8
6
1 mM NTP
10 μM GTP
1 mM ATP CTP UTP
10 μM UTP
1 mM ATP CTP GTP

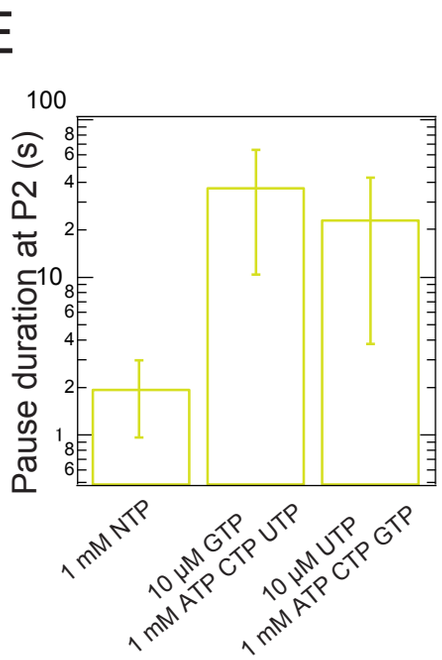

E
Pause duration at P2 (s)
100
8
6
4
2
10
8
6
4
2
1
8
6
1 mM NTP
10 μM GTP
1 mM ATP CTP UTP
10 μM UTP
1 mM ATP CTP GTP

Figure S4

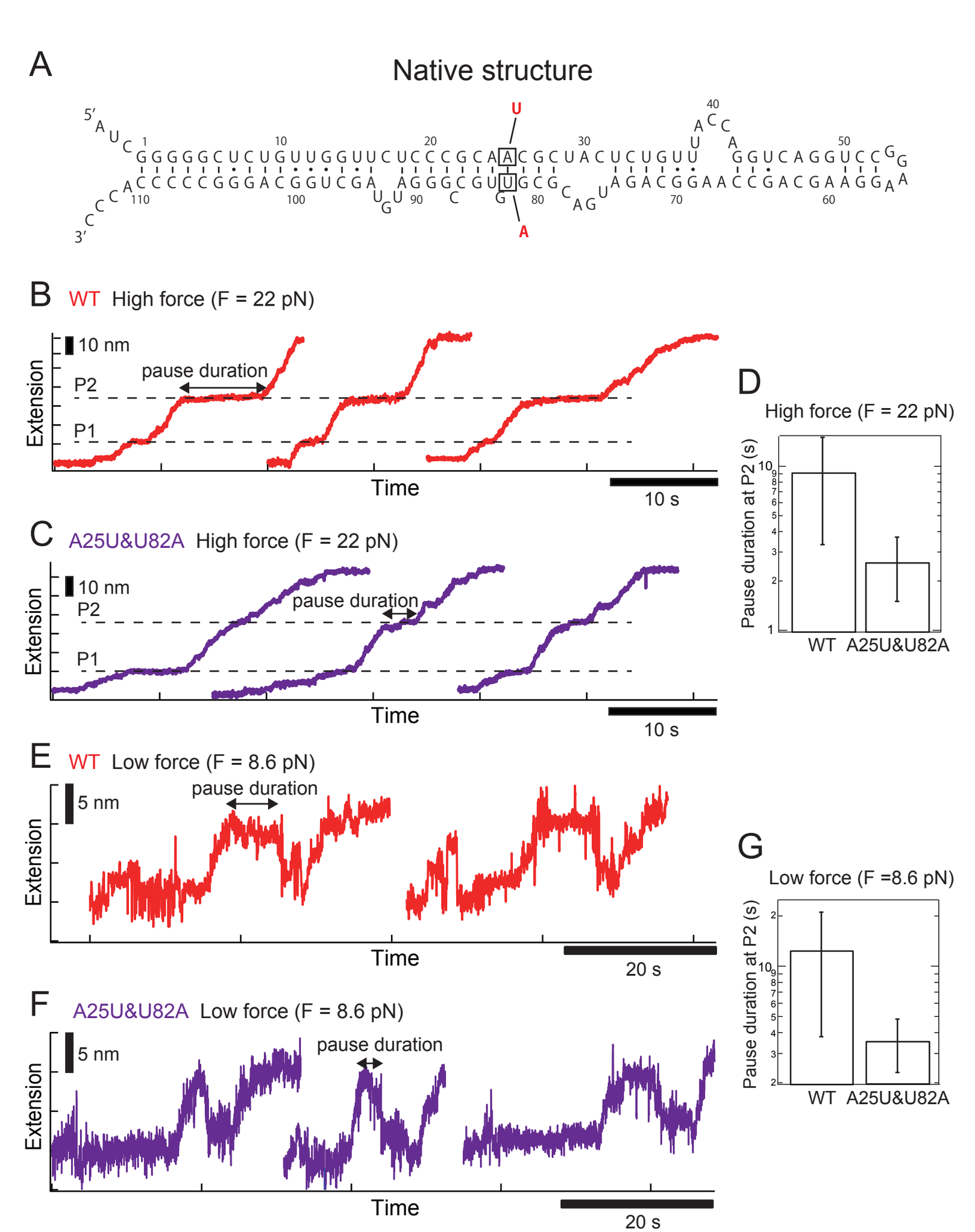

A
Native structure
B WT High force (F = 22 pN)
10 nm
pause duration
P2
P1
Extension
Time
10 s
C A25U&U82A High force (F = 22 pN)
10 nm
pause duration
P2
P1
Extension
Time
10 s
D
High force (F = 22 pN)
Pause duration at P2 (s)
WT
A25U&U82A
E WT Low force (F = 8.6 pN)
5 nm
pause duration
Extension
Time
20 s
F A25U&U82A Low force (F = 8.6 pN)
5 nm
pause duration
Extension
Time
20 s
G
Low force (F =8.6 pN)
Pause duration at P2 (s)
WT
A25U&U82A

Figure S5

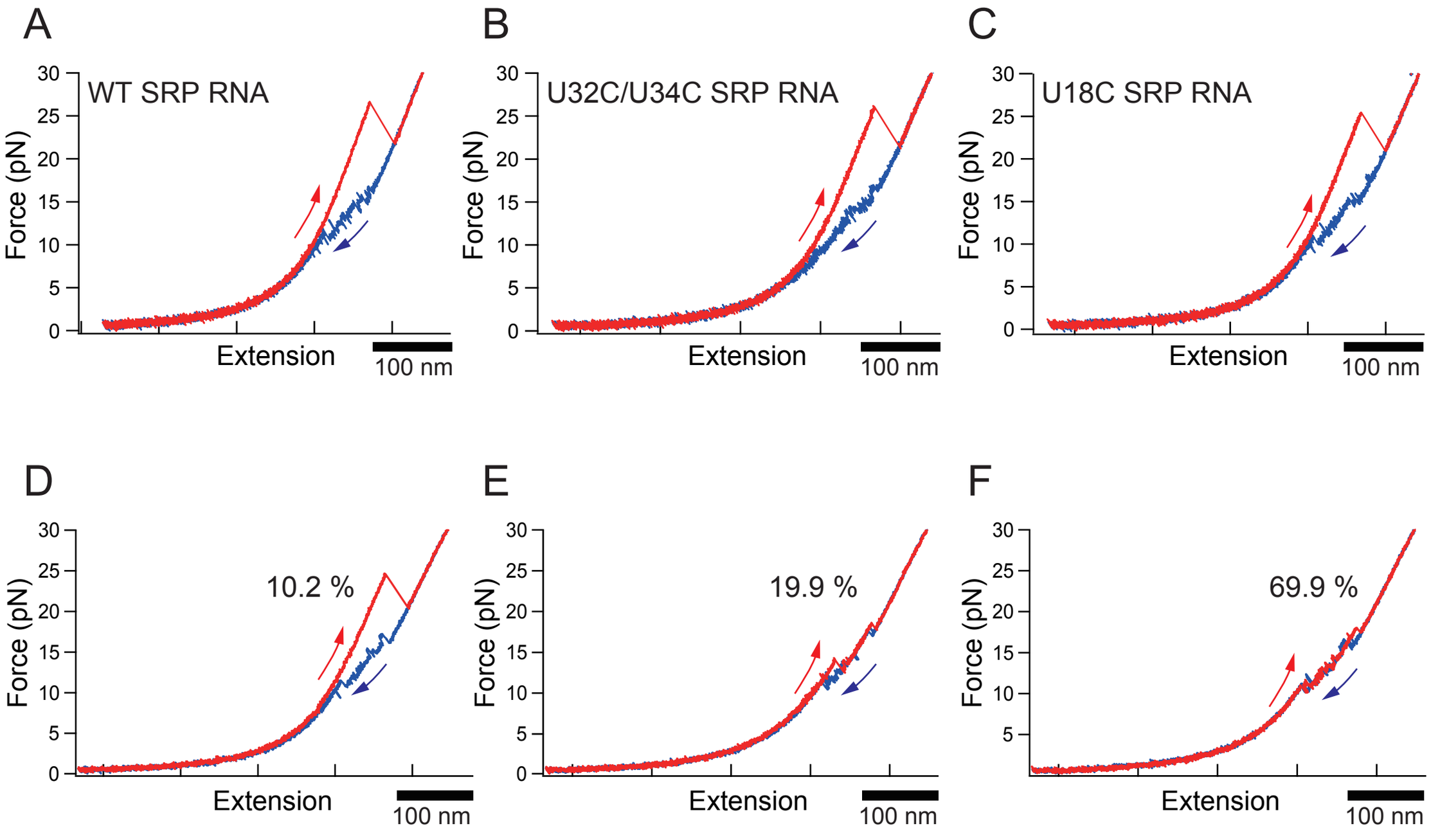
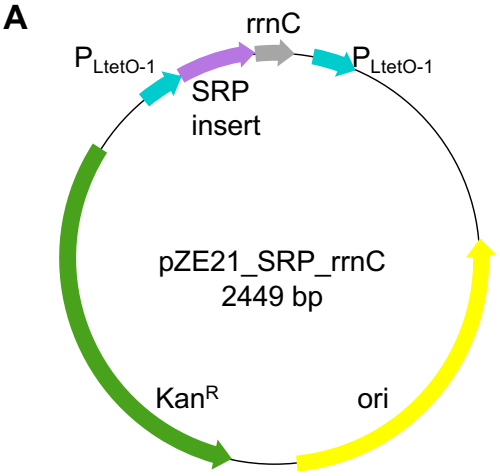
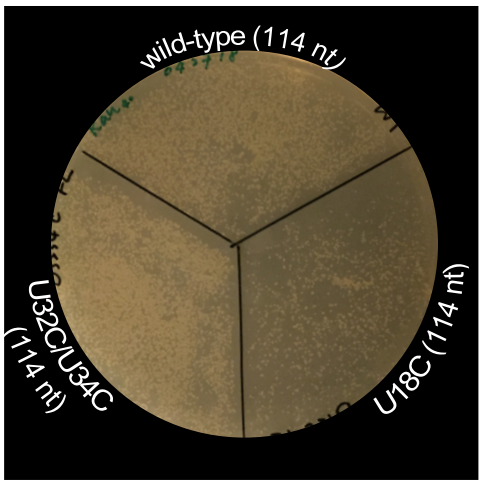

A
WT SRP RNA
B
U32C/U34C SRP RNA
C
U18C SRP RNA
D
10.2 %
E
19.9 %
F
69.9 %
Force (pN)
Extension
100 nm

Figure S6





B

Modifications in plasmids harbored in surviving colonies (N=5 for each variant)				
SRP RNA variant	truncated/impaired promoter (1^{st} $P_{LtetO-1}$)	loss of SRP insert	truncated/impaired terminator (rrnC)	termination read-through (2^{nd} $P_{LtetO-1}$)
SRP_100	# 4	# 4	# 4	# 1, 2, 3, 5
U18C_100	# 2, 4, 5	# 2, 5	# 2, 5	# 1, 3
U3234C_100	# 1, 3, 5	# 1, 2, 3, 5	# 1, 2, 3, 5	# 2, 4

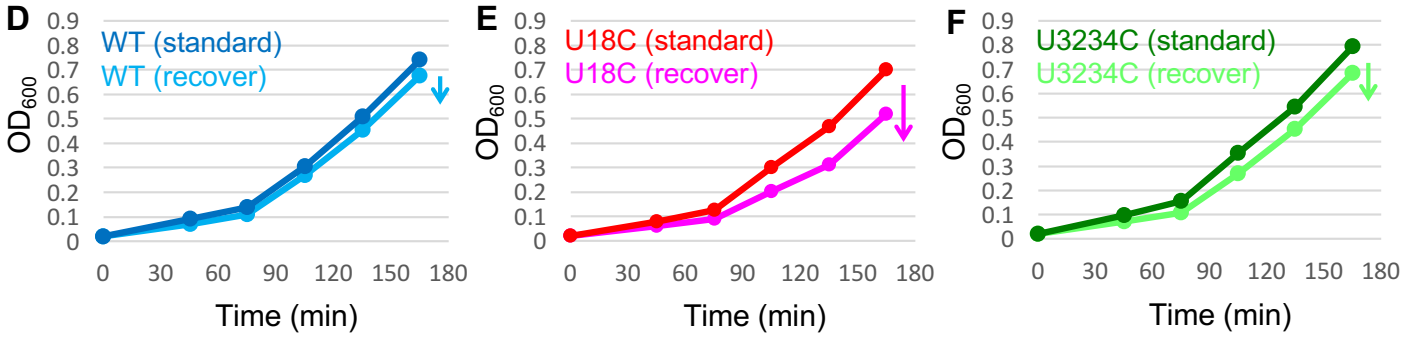


Figure S7

SYPPLEMENTARY FIGURE LEGENDS

Figure S1. Unfolding Force and Contour Length Change of Truncated SRP RNA (~57-nt) and Force Extension Curve of Full-SRP RNA with and without RNAP, Related to Figure 2

(A) Histograms for unfolding force of intermediate SRP RNA in the presence of RNAP (N = 80 unfolding events).

(B) Histogram for contour length change upon unfolding (N = 56 unfolding events).

(C and D) Force extension curve (red, unfolding; blue, refolding) of full-SRP RNA on the RNAP (D) and free in solution (C). These RNAs unfold in one cooperative transition near 25 pN.

(E and F) Rapture force histograms for unfolding of the RNAP-bound (F) and free (E) RNA. The RNA bound with RNAP unfolds at mean force (F_{Unf}) of 26.2 ± 0.9 pN (N = 110 unfolding events). The contour length increase upon unfolding (ΔL_c) of 64.3 ± 1.0 nm (N = 39 unfolding events) in good agreement with the value expected for folded part of full-length SRP RNA (110 nt × 0.59 nm per nucleotide – 2.2 nm corresponding to the folded RNA end-to-end distance = 62.7 nm). The free energy difference between the folded and unfolded forms of SRP RNA, obtained from the area under the reversible force extension curves is ΔG =273.1 ± 30.1 kJ/mol (N =10 molecules), consistent with a free energy calculation (ΔG =250.6 kJ/mol) obtained by Mfold. The free SRP RNA in the absence of RNAP shows similar unfolding characteristics: F_{Unf} = 25.5 ± 0.9 pN (N = 112 unfolding curves), ΔL_C =64.8 ± 1.1 nm (N = 34 unfolding events), ΔG = 270.5 ± 50.3 kJ/mol (N = 10 molecules).

Figure S2. All Refolding Trajectories of Intermediate and Full SRP RNA, Related to Figure 3

(A and B) All refolding trajectories of ~103-nt intermediate (A) and full SRP RNA (B) at different forces.

Figure S3. Pause and Folding Durations from Real Time Transcriptional Traces, Related to Figure 4

(A and B) Pause durations at three NTP concentrations (1 mM, 50 μM and 10 μM), and in the presence of GreB, for first (A) and second pausing (B).

(C) Duration of folding after second pausing at three NTP concentrations (1 mM, 50 μM and 10 μM).

Figure S4. Real Time Transcription of SRP RNA with Specific Nucleotide Depletion, Related to Figure 4

(A) Representative traces of SRP RNA transcription under condition of UTP depletion. The NTP condition is 10 μM UTP and 1 mM ATP, CTP and GTP. Both pause durations are prolonged.

(B) Representative traces of SRP RNA transcription under condition of GTP depletion. The NTP condition is 10 μM GTP and 1 mM ATP, CTP and UTP. Second pause duration is prolonged.

(C) Representative traces of SRP RNA transcription under condition of CTP (orange) and ATP (magenta) depletion. The trace with saturated NTP condition (1 mM NTPs) is also shown (black) for comparison.

(D) Average pause duration at first pause site with GTP-limited (N = 12 molecules), UTP-limited (N = 10

molecules) and saturated NTP (N = 11 molecules) conditions.

(E) Average pause duration at second pause site with GTP-limited (N = 12 molecules), UTP-limited (N = 10 molecules) and saturated NTP (N =11 molecules) conditions.

Figure S5. Real Time Transcription of SRP RNA Mutant with Applying High and Low Force, Related to Figure 4.

(A) Secondary structure of full-length (native) SRP RNA. A25/U82 are mutated to their complementary (U25/A82) to eliminate the U82 pause site.

(B and C) Representative traces of transcriptional extension of WT (B) and A25U/U82A (C) SRP RNA with 50 μM NTP when applying high force (22 pN).

(D) Average pause duration at second pause site during transcription of WT (N = 12 molecules) and A25U/U82A (N = 12 molecules) SRP RNA with 50 μM NTP when applying high force (22 pN).

(E and F) Representative traces of transcriptional extension of WT (E) and A25U/U82A (F) SRP RNA with 50 μM NTP when applying low force (8.6 pN).

(G) Average pause duration at second pause site during transcription of WT (N = 15 molecules) and A25U/U82A (N = 13 molecules) SRP RNA with 50 μM NTP when applying low force (8.6 pN).

Figure S6. Force Extension Curves of Mutants and Wild Type SRP RNA, Related to Figure 7

(A-C) Force extension curve (red, unfolding; blue, refolding) of WT (A), U32C/U34C (B) and U18C (C) full-length SRP RNA. These RNAs unfold in one cooperative transition near 25 pN. These force extension curves show long hairpin structure, which are consistent with structural prediction by Mfold.

(D-F) Representative force extension curve (red, unfolding; blue, refolding) showing full-length like curve (D), two unfolding rips curve (E), and several unfolding rips curve (F). The relative fractions of each curve to total number of curve are 10.2 %, 19.9 % and 69.9 % for full-length like curve, two unfolding rips curve and several unfolding rips curve, respectively (N =156 unfolding events). Although the unfolding curve shows three distinct features, the refolding curves are not distinguishable (blue traces).

Figure S7. *In Vivo* Response toward SRP RNA Variant-carrying Plasmids, Related to Figure 7

(A) The architecture of plasmid, pZE21_SRP_rrnC, designed for precise transcription of SRP RNA variants inserted therein (see Methods for plasmid sequence). $P_{LtetO-1}$: operon-embodying and tightly repressible promoter; *rrnC*: strong terminator; Kan^R: kanamycin resistant; ori: high-copy-number colE1 origin of replication.

(B) Modifications—such as impaired promoter, loss of SRP variant insert, and/or inefficient termination—were found in plasmids harbored in colonies surviving from the otherwise lethal transformation (Figure 7A, bottom half). These modifications compromise the precise transcription of the 3'-end-truncated SRP RNA variants.

(C) Under stress condition—e.g. high salt (2x LB) and stiff growth medium (2x agar), as compared to normal LB agar plates (Figure 7B), the difference in toxicity among plasmids carrying different full-length SRP RNA variants is further accentuated: an even greater reduction in surviving colonies is seen with the transformation of U18C_FL SRP variant (lower right).

(D-F) The more toxic an SRP RNA variant-carrying plasmid is, the higher fraction of cell population would reject the plasmid under relaxed/antibiotic-free growth condition, and lead to a larger slowdown in recovery/post-relaxation growth rate under restrictive condition, compared to that of a never-relaxed/standard culture. While the U18C_FL variant shows the largest drop in growth rate (magenta arrow)—and hence is the most toxic, the mildly toxic U32C/U34C_FL variant exhibits a noticeable decrease (green arrow) compared to that of wild-type_FL (blue arrow).