Supplementary Information for

A translational riboswitch coordinates nascent transcription-translation coupling

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SUPPLEMENTARY FIGURES

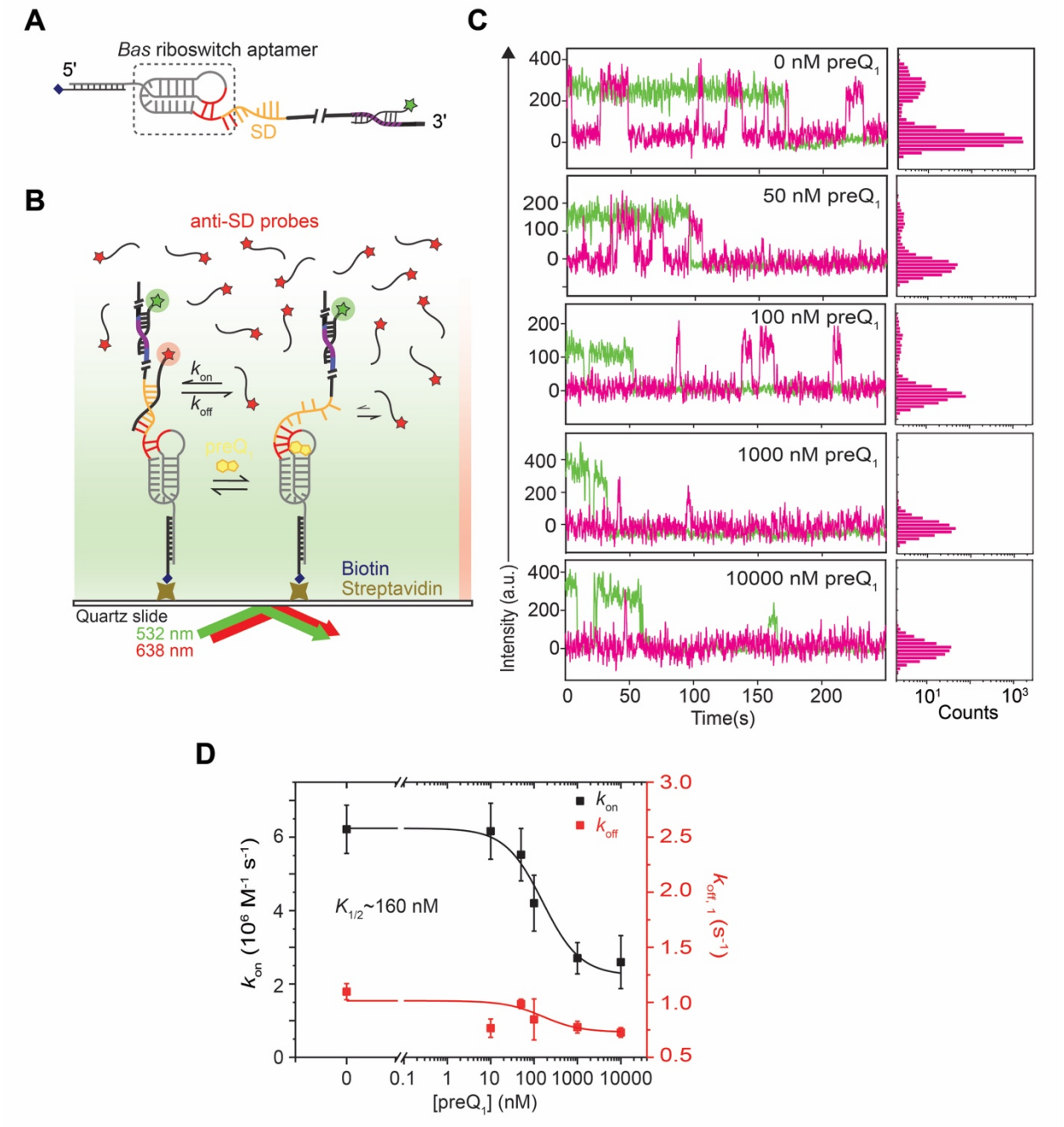

Fig. S1. Single-molecule measurements of anti-SD probe binding kinetics to *Bas* mRNA as a function of preQ$_1$ concentration. (A) The *Bas* mRNA complex used for monitoring anti-Shine-Dalgarno (anti-SD) probe binding to the SD. The full length mRNA was immobilized on the quartz slide via a biotinylated capture strand that was hybridized to the 5' end of the mRNA. A Cy3 labeled LNA was hybridized to the second

SD (purple) to locate the mRNA molecules on the slide surface. (B) Experimental set-up for monitoring binding of Cy5 labeled anti-SD probes to the surface-immobilized single mRNA molecules using TIRF microscopy. (C) Representative fluorescence versus intensity versus time traces showing repeated anti-SD probe binding (magenta spikes) to individual mRNA molecules (green) as a function of $preQ_1$ concentration. Cy5 intensity histogram corresponding to each trace are shown on the right-hand side. (D) Association (k_{on}) and dissociation (k_{off}) rates were calculated from exponential fits of the dwell times in the unbound and bound states, respectively. The half-saturation point ($K_{1/2}$) value from the global saturation curve fit of the anti-SD probe is indicated. Values represent the average ± standard error of the mean of at least three independent experiments.

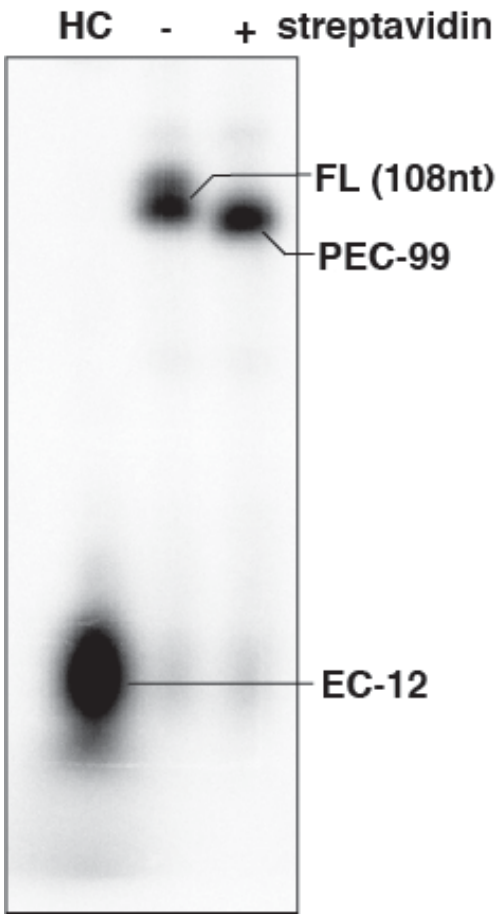

Fig. S2. mRNA synthesis after rNTPs addition to the halted complex (HC). RNAP resumes transcription from HC and transcribes 108 nt long RNA-only transcripts in the absence of streptavidin roadblock. In the presence of streptavidin, RNAP is stalled at the C99 position to form PEC-99.

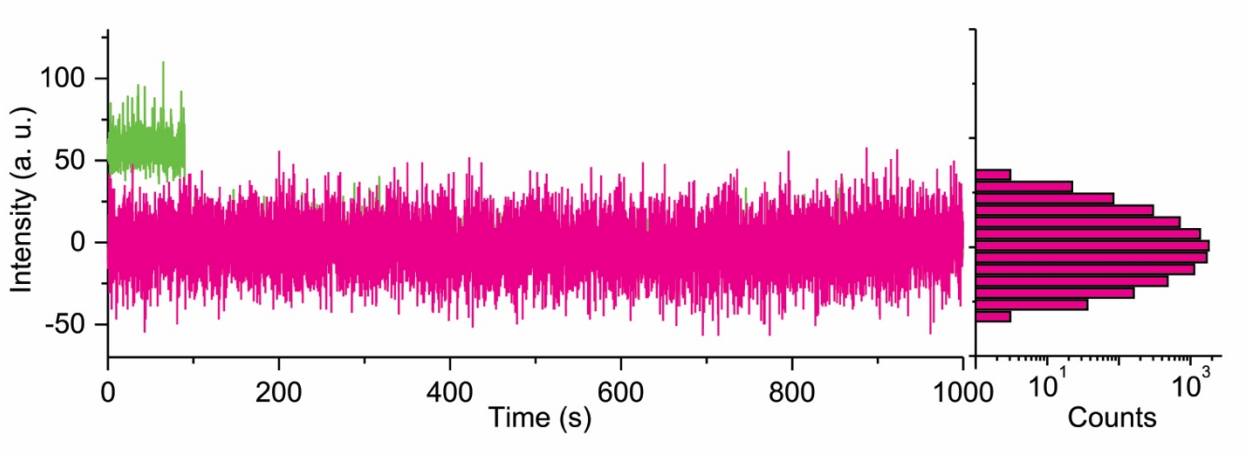

Fig. S3. 30S binding to the surface immobilized halted complex. Single-molecule trajectory representative of 99% of traces from a 30S binding measurement with the halted complex (HC), showing exceptionally rare binding events.

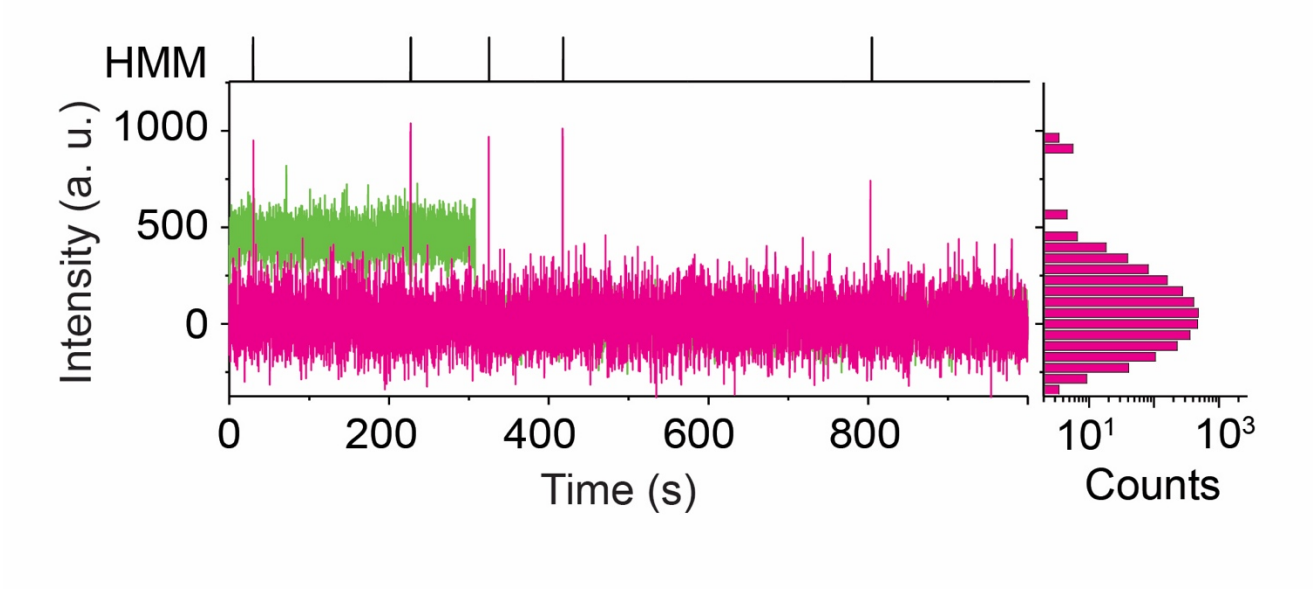

Fig. S4. 30S binding to the PEC-99 is specific to the RBS region of the nascent mRNA. Representative single-molecule trajectory as in Fig. 2C, but in the presence of a blocking strand that competes with the 30S for binding to the SD of the nascent mRNA in PEC-99.

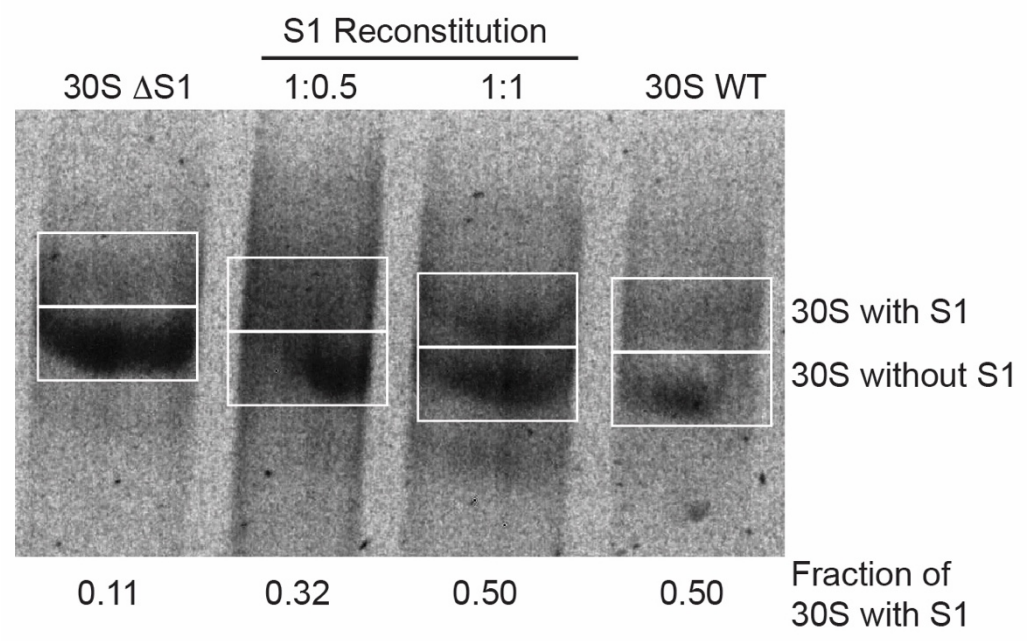

Fig. S5. Assessment of the fraction of purified 30S that contains S1 using a composite non-denaturing 3% polyacrylamide:0.5% agarose gel. 30S depleted of S1 (30S ΔS1, first lane) shows only little (~11%) remaining S1. Addition of half-stoichiometric (second lane) and fully stoichiometric purified S1 (third lane) resulted in 32% and 50% reconstitution, respectively, into a slower moving band for 30S with S1 (top band). Our “wild-type” 30S preparation used for all experiments (30S WT, fourth lane) also shows two bands for the 30S with (top band) and without S1 (bottom band), with a similar 50% fraction in the top band as the stoichiometric reconstitution. The quantification areas are indicated.

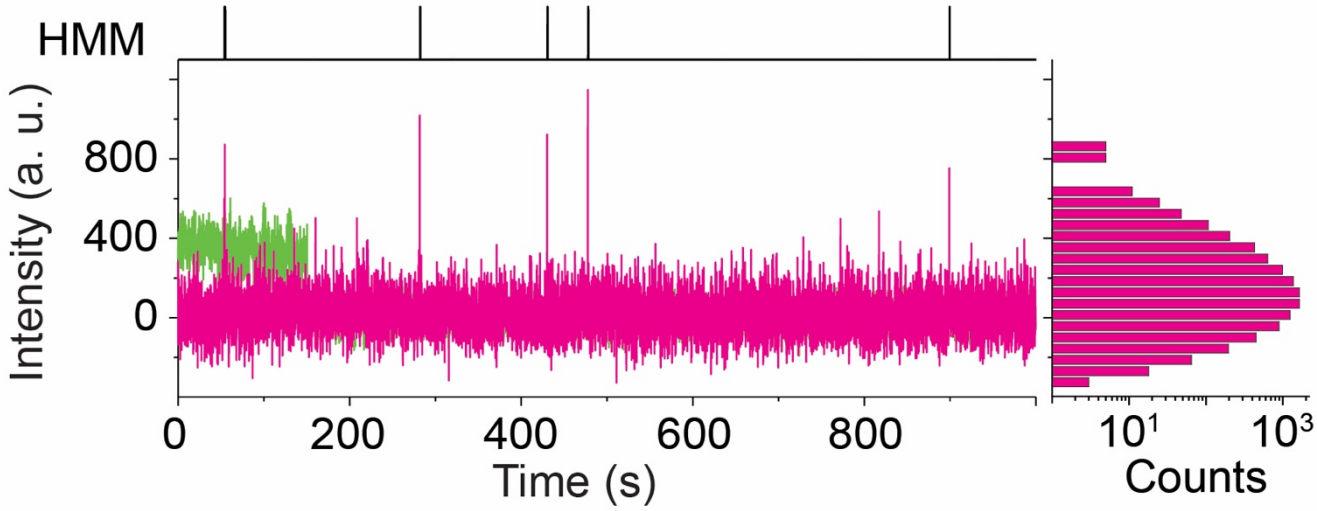

Fig. S6. 30S binding to the nascent mRNA alone is less frequent compared to binding to the PEC-99 embedded mRNA. Representative single-molecule time trace, HMM idealization (top panel) and Cy5 intensity histogram (right panel) showing less frequent 30S binding events to the corresponding mRNA alone compared to PEC-99 shown in Fig 2C.

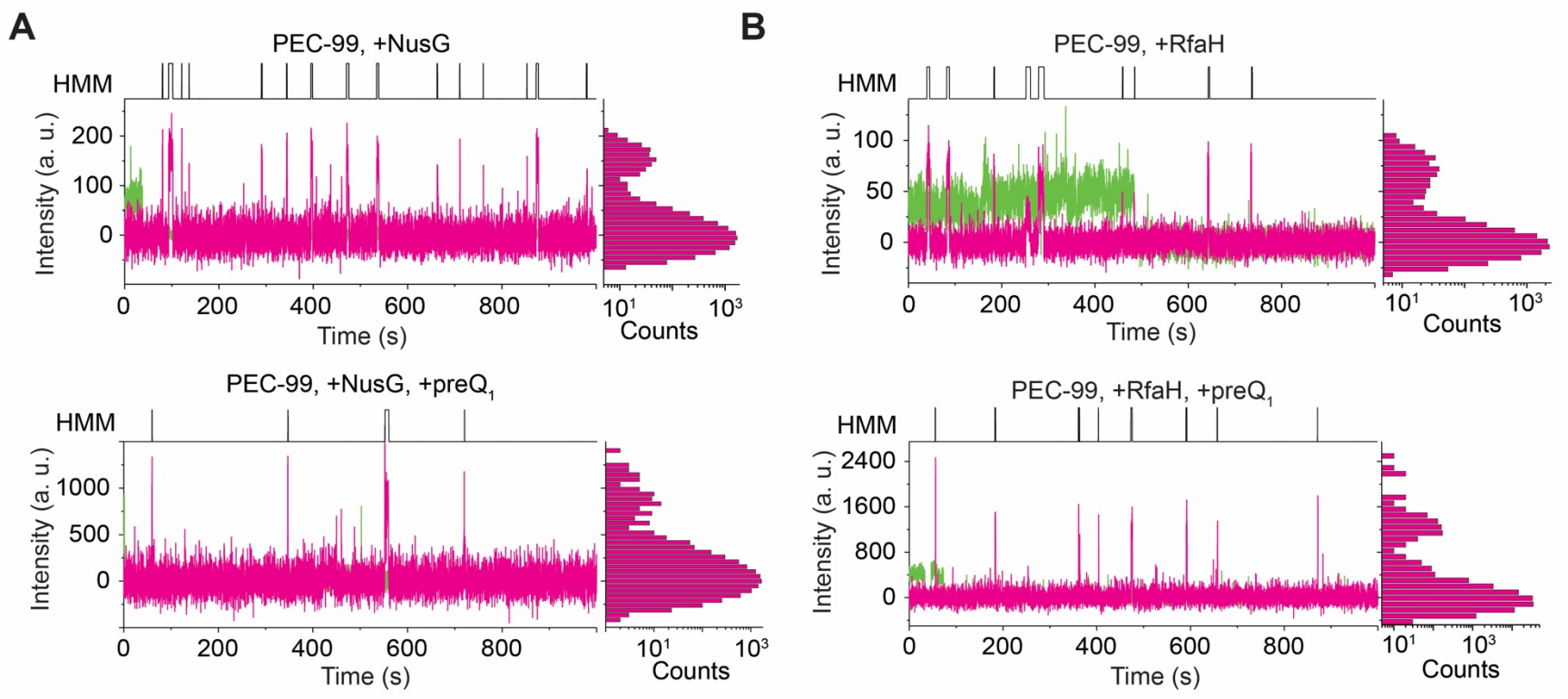

Fig. S7. 30S binding to PEC-99 is assisted and stabilized by transcription factors NusG and RfaH, respectively. (A) Representative single-molecule trajectories as in Fig. 2C,D (top and bottom, respectively), but in the presence of NusG. (B) Representative single-molecule trajectories as in Fig. 2C,D (top and bottom, respectively), but in the presence of RfaH.

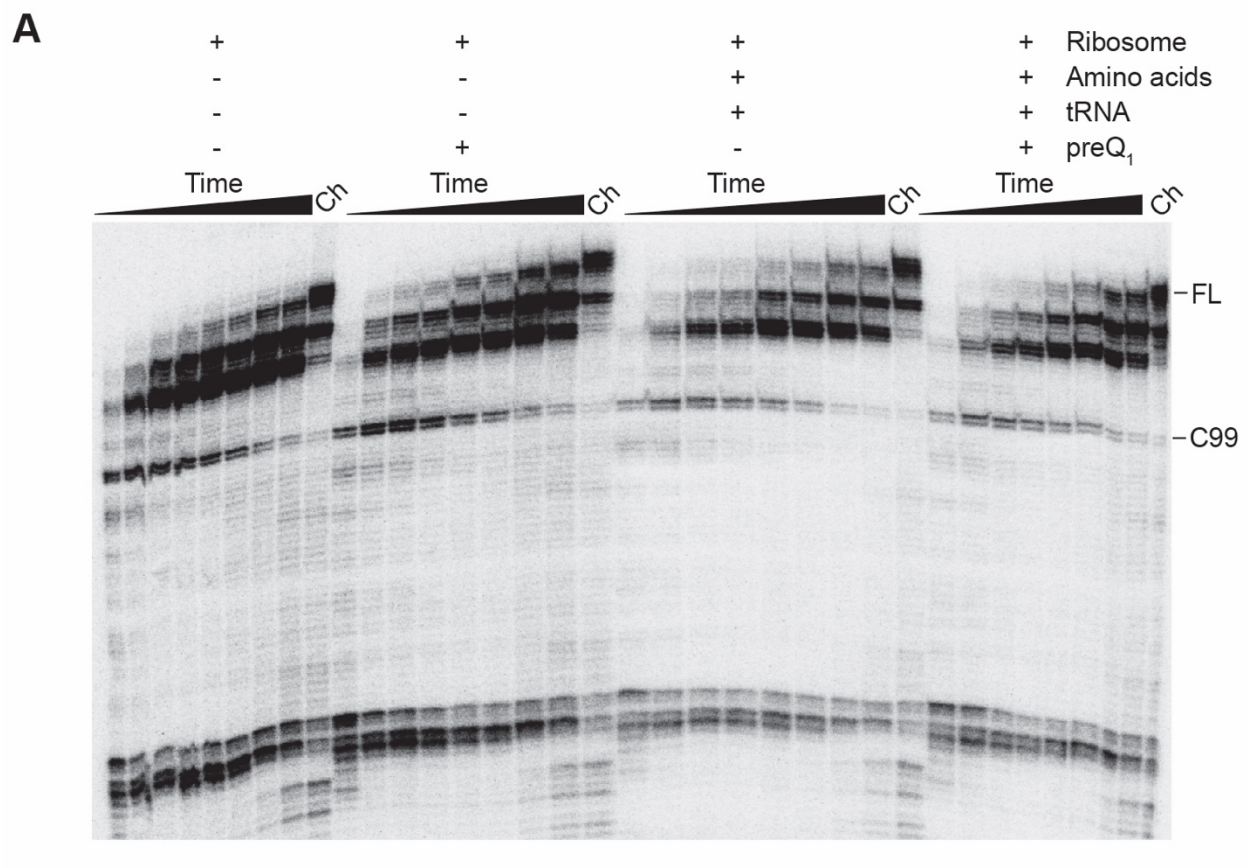

Fig. S8. Effect of the 30S binding and translation on the C99 pause half-life. (A) *In vitro* transcription assay of *Bas* mRNA (as in Fig. 1D) in the absence and presence of $preQ_1$ under non-translating (-tRNA, -amino acids) and translating (+tRNA, + amino acids) ribosome conditions (conditions 2 and 3, respectively).

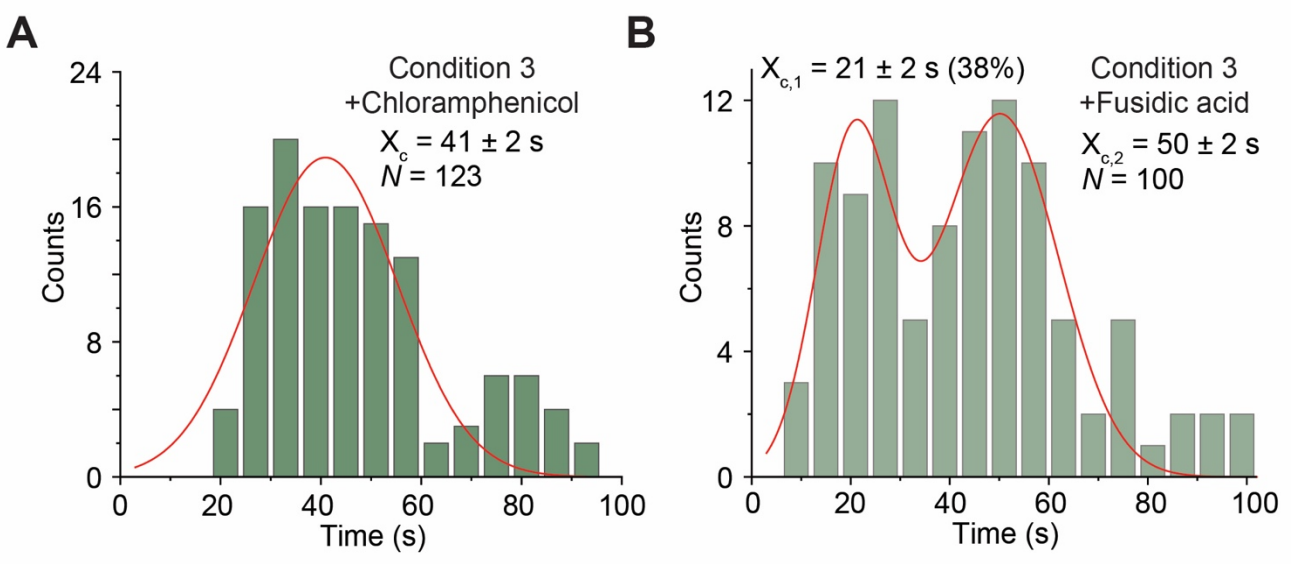

Fig. S9. Monitoring transcription speed under translating ribosome conditions in the presence of translation inhibitors chloramphenicol and fusidic acid. Histogram of transcription times constructed from *N* number of molecules for the translating condition (condition 3 in Fig. 4A) in the presence of translation inhibitors chloramphenicol (A) or fusidic acid (B). X_c represents the mean transcription time ± standard deviation from the Gaussian fitting.

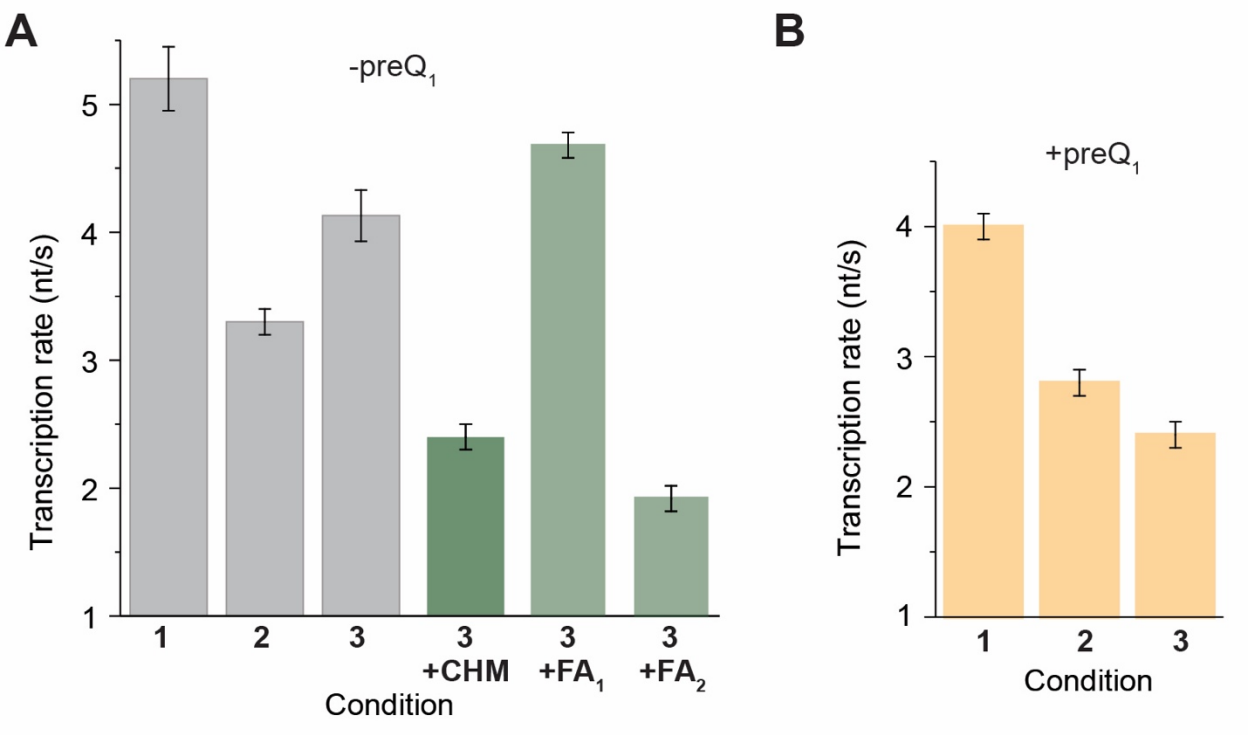

Fig. S10. Monitoring transcription rates by real-time transcription assay. (A) Changes in transcription rates for RNAP alone (condition 1 in Fig. 4A), and in the presence of ribosome under non-translating (condition 2 in Fig. 4A) and translating conditions (condition 3 in Fig. 4A) in the absence and presence of chloramphenicol (CHM) or fusidic acid (FA) as indicated. (B) Changes in transcription rates under conditions 1, 2, and 3 in the presence of $preQ_1$.

Condition	k_{on} (10^6 M^{-1} s^{-1})	k_{off} (s^{-1})
PEC-99[a]	3.4 ± 0.4	2.7 ± 0.3 (92%) 0.2 ± 0.05 (8%)
PEC-99 + $preQ_1$[a]	1.8 ± 0.3	2.3 ± 0.9 (81%) 0.2 ± 0.2 (19%)
PEC-99 + blocking strand[b]	2.0 ± 0.3	2.7 ± 0.3 (93%) 0.3 ± 0.2 (7%)
mRNA alone[b]	2.0 ± 0.3	3.3 ± 0.3 (95%) 0.02 ± 0.01 (5%)
mRNA alone + $preQ_1$[b]	1.4 ± 0.3	3.2 ± 0.5 (88%) 0.4 ± 0.1 (12%)
PEC-99 + NusG[a]	4.3 ± 0.1	2.4 ± 0.03 (88%) 0.2 ± 0.04 (12%)
PEC-99 + NusG + $preQ_1$[a]	2.6 ± 0.3	1.8 ± 0.2 (85%) 0.14 ± 0.1 (15%)
PEC-99 + RfaH[a]	3.4 ± 0.2	1.1 ± 0.1 (86%) 0.04 ± 0.01 (14%)
PEC-99 + RfaH + $preQ_1$[a]	3.9 ± 0.3	1.4 ± 0.2 (86%) 0.06 ± 0.03 (14%)
PEC-99 + IF mix[a]	3.0 ± 0.2	2.0 ± 0.1 (88%) 0.1± 0.01 (12%)

[a.] Values represent the average ± the standard error of the mean of three independent experiments.

[b.] The reported error was estimated by bootstrapping using a custom MATLAB script.

Table S1. Kinetic parameters extracted from the single-molecule fluorescence co-localization experiments for 30S binding to PEC-99 and the corresponding nascent mRNA alone under different experimental conditions.

Recombinant DNA	Source
pKK3535	Gift from Dr. Joseph Puglisi
BAS1509	This study

Table S2. List of plasmids used in this study.

Oligonucleotides	Source
T7A1-*Bas*_preQ$_1$-Fwd2: TCCAGATCCCGAAAATTTATCAAAAAGAGT ATTGACTTAAAGTCTAACCTATAGGATACTTACAGC	IDT
Bas-preQ$_1$-rev21cd: CATAGAAACAGCAATATATAATGCCGCTAA AATACC	IDT
Bas-EC99: /5Biosg/TATAATGCCGCTAAAATACCATTACCGACT	IDT
Bas-Tr99: AGACCACGTTGAAAGATTGGGTTACGCTAAAATACCATTACCGACTAA TGTTCTAATATTCAC	IDT
Anchor_bio: /5Biosg/AGACCACGTTGAAAGATTGGGTTAC	IDT
Hp5extn_ribo_5Cy5_3Cy5: /5Cy5/AAAGGGAGATCAGGATATAAAG /3Cy5Sp/	IDT
Bas-109-Cy3rev: /5Cy3/ATATAATGCCGCTAAAATACCATTACCGACT	IDT
Bas-blocking Strand: CTAATGTTCTAATATTCACGACAAAATCTCCTTAG	IDT

Table S3. Sequences for primers and oligonucleotides used in this study.

Note S1. Full-length *Bas* mRNA sequence for monitoring anti-SD probe binding:

ggguguugcuuaaaaaacgaauaacgugguucgaaaccaucccacguaaaaaaacuaaggagauuuuguc
GUGAAUAUUAGAACAUUAGUCGGUAAUGGUAUUUUAGCGGCAUUAUAUAUUGCU
GUUUCUAUGCUUAUUCAGCCAUUUGGCUUUACGAAUGUACAGUUUCGUAUUUCA
GAGAUGUUUAAUCAUCUCGUUGUAUUUAAUAAGAAAGCAAUUUACGGAAUUGUA
UUAGGUGUAUUUUUAACGAAUCUCUUUUUCUCACCUAUGAUUGCUUACGAUUUA
GUAUUUGGAGUAGGGCAAUCUAUUCUUGCAUUAGUUGCAACCAUUAUUUCUAUG
CGAUUCAUUAAAGGUGUUUGGGCUCGUAUGAUUUUUAAUACAGUUAUCUUUACA
AUUACAAUGUUUAUGAUUGCAAUUGAACUUCAUCUUGCAUUUGAUUUACCAUUU
AUGUUGACUUGGUUAACAUGUGCAGUCGGUGAAUUUGUUGUCAUGGCCAUUGG
UAUGCCUGUAAUGUACUGGAUUAAUAAACGAGUACAAUUUGAAAGAUUUAUGUA
Auagaugaaagagcuauuccuauagggauagcucuuuuuuaaagcu