Supplementary data

Mycobacterium tuberculosis RbpA is a new type of transcriptional activator stabilizing the σ^A containing RNA polymerase holoenzyme

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MATERIALS AND METHODS

Promoter DNA fragments. The -35 and -10 consensus promoter elements are underlined and the start sites are in bold red.

The 150 bp *rrnA*P3 fragment (5'-3'): (amplifed from *M. tuberculosis* genomic DNA)

ATCGAACGGGTATGCTGTTAGGCGACGGTCACCTATGGATATCTATGGATGACCGAACCT

GGTCTTGACTCCATTGCCGGATTTGTATTAGACTGGCAGG**G**TTGCCCCGAAGCGGGCGG

AAACAAGCAAGCGTGTTGTTTGAGAACTCAA

The 150 bp *usfX*P1 fragment (5'-3'): (amplified from *M. tuberculosis* genomic DNA)

AAGTCCGGCGGCAGCGGCTGGCCGGCTAGACATCCTAGTGCGGCTGGAAATCCCGGCAT

CGCGGGGTTTCACCGGCAGCTGCGAATGGGTATCACGGGTA**C**ACCATGATGAATCCCGA

CCATGTTGCGTTAGATCCCCACTACCAGCAGG

The 106 bp *sigA*P fragment (5'-3'): (amplified from *M. tuberculosis* genomic DNA)

CACTGAAACTTGCCCGCTCGGGCTGTACTCGTGCGCAGTAAAGTTACAATGGTCAG**C**GG

CGGCCGCCCGACCGATAGCGCGCGAGTATTCACGCTGATATCAACGC

The 127 bp *sin*P3 fragment (5'-3'): (amplified from *B. subtilis* genomic DNA)

TTTAAACAAAAAGTCTGCTCATCCTGGTCCGGCAGCCAGAAGTCATACCGTAAATCCTTT

CTGAATGTGCTATAATATCAC**A**AGGAAGGTGATGACATTGATTGGCCAGCGTATTAAACA

ATACCGT

Assembly of the Transcription Elongation Complexes (TEC) and the RNA primer extension. Assembly of TECs from the synthetic DNA templates and the 16nt RNA primer was done essentially as described (51). In brief, 200 nM template strand DNA oligonucleotide (TEC_tcon) and 400 nM of 5'-fluorescein labeled RNA primer (pRNA16) were annealed in reaction buffer, and incubated with 250 nM of *E. coli* core or 500 nM of *M. tuberculosis* core for 30 min at 24°C. Then the 200 nM non-template strand DNA oligonucleotide (TEC_ntcon) was added and incubated at 37°C for 20 min. Transcription was initiated by the addition of 15 μM NTPs. The reactions were stopped by adding the equal volume of stop buffer (2×TBE, 8 M urea). The RNA products were analyzed on 20% PAGE-7M urea denaturing gel.

FeBABE conjugation and RNAP cleavage.

Purified RbpA (~2,5 nmole) was dialyzed at 4°C for 24 h against metal-removal buffer: 30 mM MOPS, 4 mM EDTA, pH 8.2 and then exchanged to conjugation buffer: 30 mM MOPS, 100 mM NaCl, 1 mM EDTA, 5% glycerol, pH 8.2. The 20-fold molar excess of FeBABE (PIERCE) was added to initiate conjugation reactions. After 1 h incubation at 37 °C, an excess of FeBABE was removed by dialysis against cutting buffer: 50 mM MOPS, 120 mM NaCl, 0.1 mM EDTA, 10 mM $MgCl_2$, 10% glycerol, pH 8.0. RNAP core or holoenzyme was also dialyzed against cutting buffer and then mixed with 2-fold molar of FeBABE-RbpA and incubated at 30°C for 30 min. Cleavage reactions were initiated by the rapid sequential addition of ascorbic acid (5 mM final), H_2O_2 (5 mM final) and EDTA (5 mM final) and allowed to proceed for 30 s at 30°C. Control reactions using nonconjugated RbpA were treated identically. Reaction mixtures were quenched by the addition of 1 vol of sample loading buffer: 60 mM Tris-HCl pH 6.8, 2% SDS, 50% glycerol, 5% 2-mercaptoethanoly, 0.02% bromophenol blue.

TABLES

Table S1. Oligos used in this study

Name	Sequence 5'-3'	Description
PrpoZ-NF	CAGCATATGAGTATCTCGCAGTCCG	*rpoZ* cloning
PrpoZ-HR	TTTAAGCTTCTCGCCCTCGGTGTG	
PrpoB-tag-F	AACCATATGGATTACAAGGATGACGACGATAAGTT GGCAGATTCCCGCCAGAGCA	Tagged *rpoB* amplification
PrpoB-tag-R	TTTAAGCTTTTAAGCGTAGTCTGGGACGTCGTATGG GTACGCAAGATCCTCGACACTTGCGGA	
PrbpA-NF	CTGCATATGGCTGATCGTGTCCTG	*rbpA* cloning
PrbpA-HR	TTTAAGCTTGCCGCGCCGACGTGA	
PrrnP-F*	ATCGAACGGGTATGCTGTTAG	*rrnA*P3 promoter amplification
PrrnP-R	TTGAGTTCTCAAACAACACGCT	
PusfXP-F*	AAGTCCGGCGGCAGCGG	*usfX*P1 promoter amplification
PusfXP-R	CCTGCTGGTAGTGGGGATCT	
PsigAP-F*	CACTGAAACTTGCCCGCTC	*sigA* promoter amplification
PsigAP-R	GCGTTGATATCAGCGTGAAT	
PsinP-F	GGTAAACAAAAAGTCTGCTCATCC	*sin*P3 promoter amplification
PsinP-R	ACGGTATTGTTTAATACGCTGG	
TEC_tcon	GAATTCTGTTTCCTGTGTGAAATTGTTATCCGCTCA CTATACCACACATCGCGTC	TEC assembly
pRNA16*	CUCAAUUGUGAGCGGA	
TEC_ntcon	GACGCGATGTGTGGTATAGTGAGCGGATAACAATT TCACACAGGAAACAGAATTC	

* oligos labeled at the 5'-end by fluorescein

FIGURE LEGENDS

Fig. S1. *M. tuberculosis* RNAP purification and activity test. *(A)* The purified RNAP subunits (**lanes 2-7**), reconstituted core enzyme (**lane 8**) and purified RbpA (**lane 9**) resolved on 15% SDS-PAGE. Lane 1: broad range molecular weight marker (Fisher BioReagents). *(B)* A scheme of the assembled TEC is shown on the top of the panel. Template DNA stand is shown in black, non-template DNA strand in blue and the RNA primer is in red. *(C)* The 16nt RNA primer extension performed by the *E. coli* core RNAP (Ec core) and *M. tuberculosis* RNAP core enzymes assembled

either with wildtype β (Mt core1) or the FLAG-tagged β (Mt core2) subunits. The RNA products were separated on 20% PAGE-7MUrea. *(D)* The σ^A -dependent abortive transcription from the *rrnA*P3 promoter (25) initiated by the addition of 0.6 μM [^{32}P]-UTP, 50μM CTP and 100μM GTP. Transcription from the *rrnA*P3 starts from G followed by two U and should generate abortive products from 2 to 9 nt. in length. Short RNA's (indicated by asterisk) observed even during initiation performed by core RNAP alone appeared to be due to the initiation at the DNA fragment ends. The σ^F-dependent transcription on *usfX*P1 promoter (26) initiated by the addition of 0.6 μM [^{32}P]-UTP, 50μM ATP and CTP. The first occurrence of U is at the +6 position followed by G that is omitted in the reaction. Therefore, the shortest and the only labeled abortive RNA product formed during abortive initiation is the 6 nt RNA. The minor abortive products observed in the reaction may arise due to misincorporation.

Fig. S2. Effect of RbpA on transcription from the *sigA*P and *sin*P3 promoters. *(A)* [^{32}P]-RNA products produced during single round run-off transcription from the *sigA*P promoter in the absence or in the presence of 1.2 μM RbpA. *(B)* Quantification of the experiment on panel A. The run-off RNA amounts were normalized to the amount of the run-off RNA synthesized without RbpA after the 10 min transcription. *(C)* Transcription from the *sin*P3 promoter performed either in the absence or in the presence of 1.2 μM RbpA. The inset on the right shows overexposure of the bands corresponding to run-off RNA.

Fig. S3. Probing of the RbpA effect on transcription elongation rate. *(A)* Kinetics of the 16nt RNA primer extension in assembled TECs performed in the presence or absence of 1.2 μM RbpA. *(B)* Quantification of the experiment shown on panel A. The run-off RNA amounts were normalized to the amount of the run-off RNA synthesized without RbpA after the 90 s transcription.

Fig. S4. Probing the effect of RbpA on the hybrid RNAP assembled from the *E. coli* core and *M. tuberculosis* σ^A. *(A)* Abortive transcription from the *rrnA*P3 promoter carried out for 10 min in the presence of 0.6 μM [^{32}P]-UTP, 50 μM CTP and 100 μM GTP. RbpA (1.2 μM) was added to the reactions when indicated. Transcription was performed in the absence or presence of 10 or 100 nM of rifampicin, to explore if RbpA changes the sensitivity of the hybrid RNAP to the antibiotic *(B)* EMSA of the complexes formed by the hybrid RNAP and the end-labeled *rrnA*P3 promoter fragment either in the absence or presence of the increasing concentrations of RbpA (0.15, 0.3, 0.6 and 1.2 μM). The RNAP-promoter complex is indicated as "**RP**" and non-bound DNA is indicated as "**Free DNA**".

Fig.S5. FeBABE-conjugated RbpA is active. *(A)* EMSA of the promoter complexes formed between the σ^A-containing RNAP and *rrnA*P3 promoter and chased by the addition of poly(dA-dT). Complexes were formed in the presence of the unmodified RbpA or FeBABE-RbpA. *(B)* The run-off [^{32}P]-RNA synthesized during single-round transcription from the *rrnA*P3 promoter in the presence or absence of the unmodified RbpA or the FeBABE-RbpA.

Fig. S6. Probing of the interactions between FeBABE-conjugated RbpA and σ^A. (A) A scheme of the σ^A subunit with the N-terminal 6×His-tag marked in black. The evolutionarily conserved regions 1.2, 2, 3, and 4 are colored. The triangles beneath the scheme mark the positions of the Trp residues cleaved by the Trp-specific reagent, N-bromosuccinimide. The cleavage was performed as described (52). *(B & C)* Western blotting analysis of the FeBABE cleavage reactions. FeBABE-RbpA was incubated either with RNAP holoenzyme *(B)* or σ^A alone *(C)*. The control cleavage reactions were performed with unmodified RbpA. Blots were stained using anti-N-terminal His-tag antibodies that allows detection of the His-tagged σ^A and α subunits. Protein ladder was generated by the Trp-specific cleavage producing two fragments marked as "a" and "b".

SUPPLEMENTARY REFERENCES

51 Toulokhonov, I. & Landick, R. (2006) The role of the lid element in transcription by E. coli RNA polymerase. *J Mol Biol* **361**, 644-658.

52 Zenkin, N., Kulbachinskiy, A., Yuzenkova, Y., Mustaev, A., Bass, I., Severinov, K. & Brodolin, K. (2007) Region 1.2 of the RNA polymerase sigma subunit controls recognition of the -10 promoter element. *EMBO J* **26**, 955-964

Figure S1

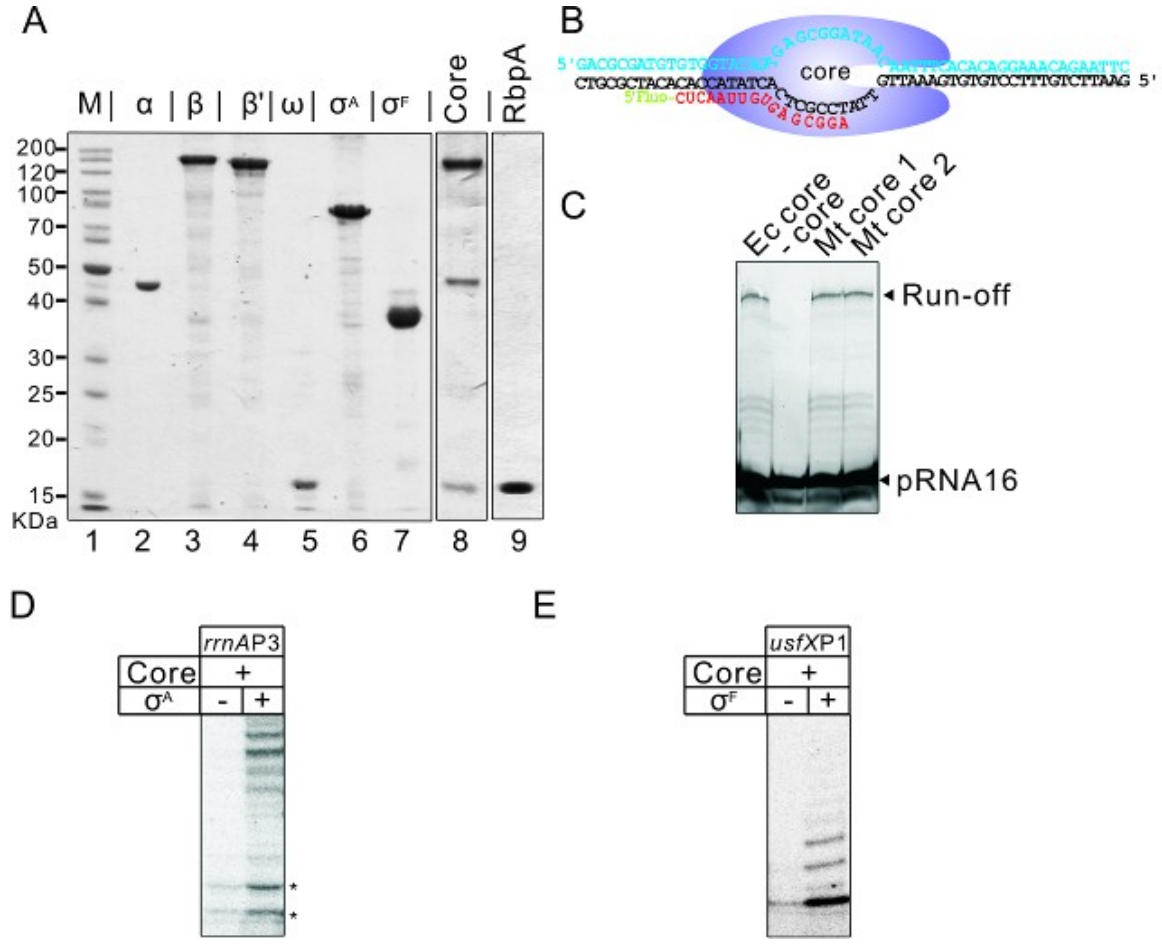
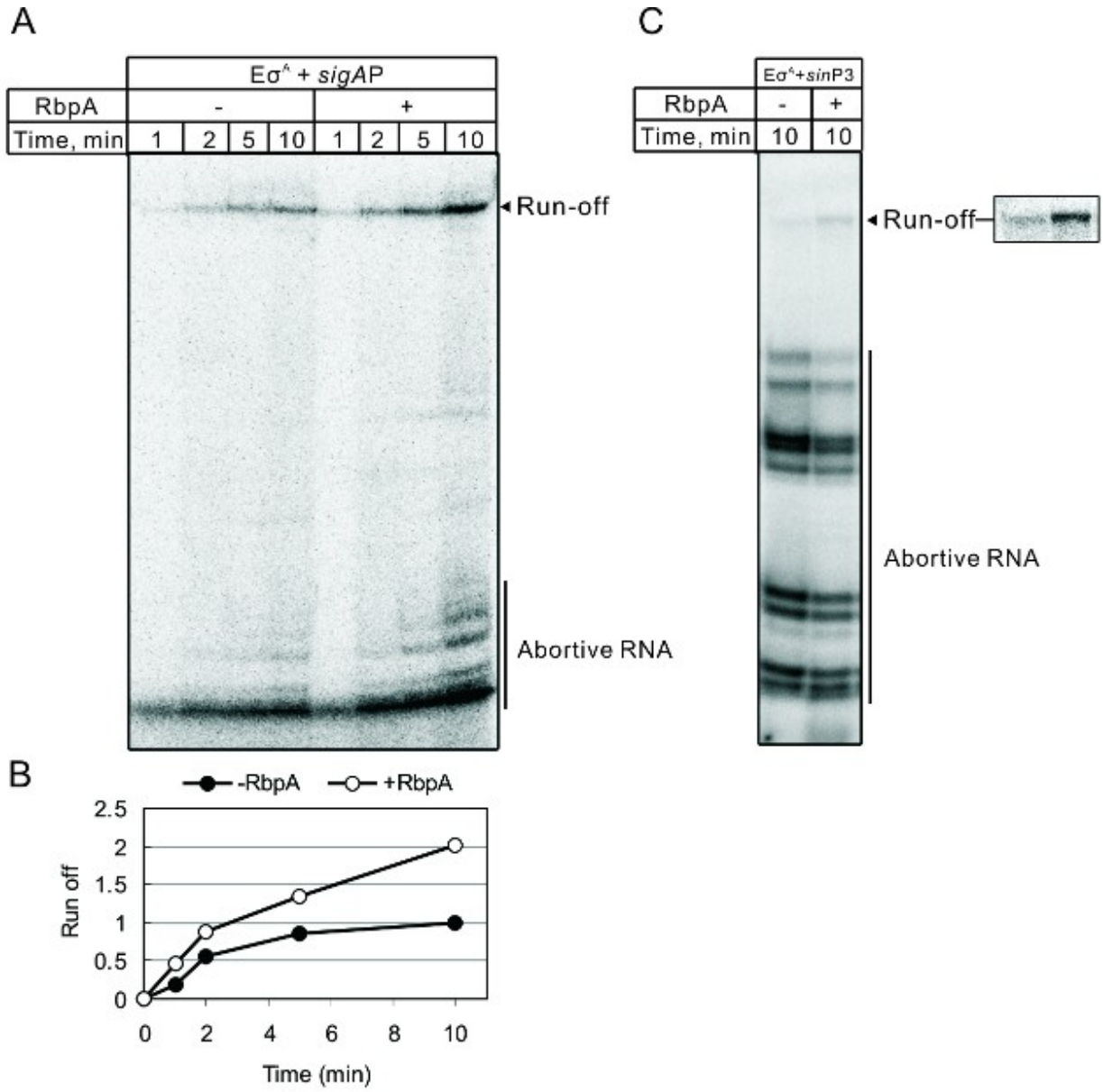


Figure S2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Eσ^A + sigAP
RbpA
-
+
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2
5
10
1
2
5
10
Run-off
Abortive RNA
B
-RbpA
+RbpA
Run off
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
Time (min)
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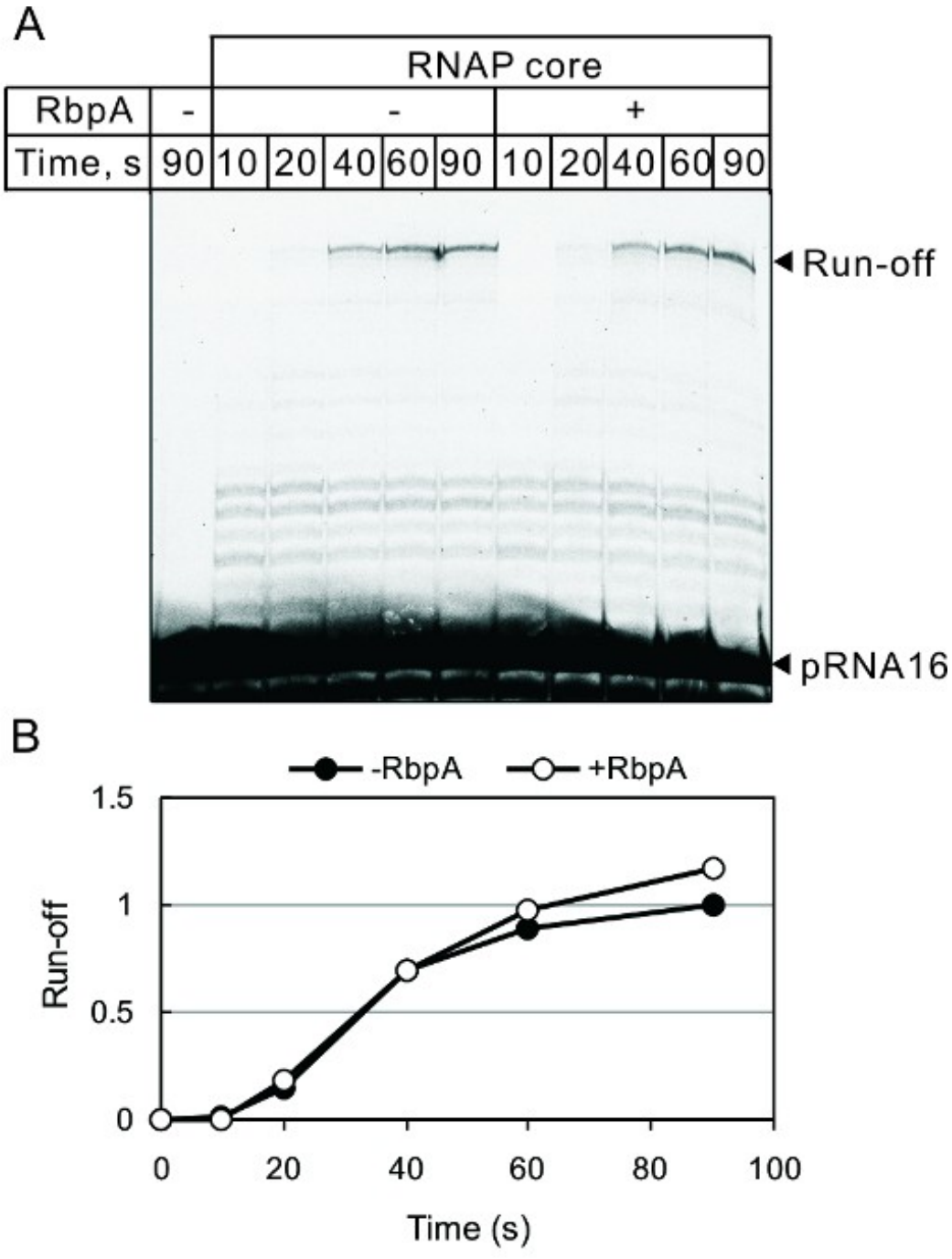
RbpA
-
+
Time, min
10
10
Run-off
Abortive RNA

Figure S3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+
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90
Run-off
pRNA16
B
-RbpA
+RbpA
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Run-off
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60
80
100
Time (s)

Figure S4

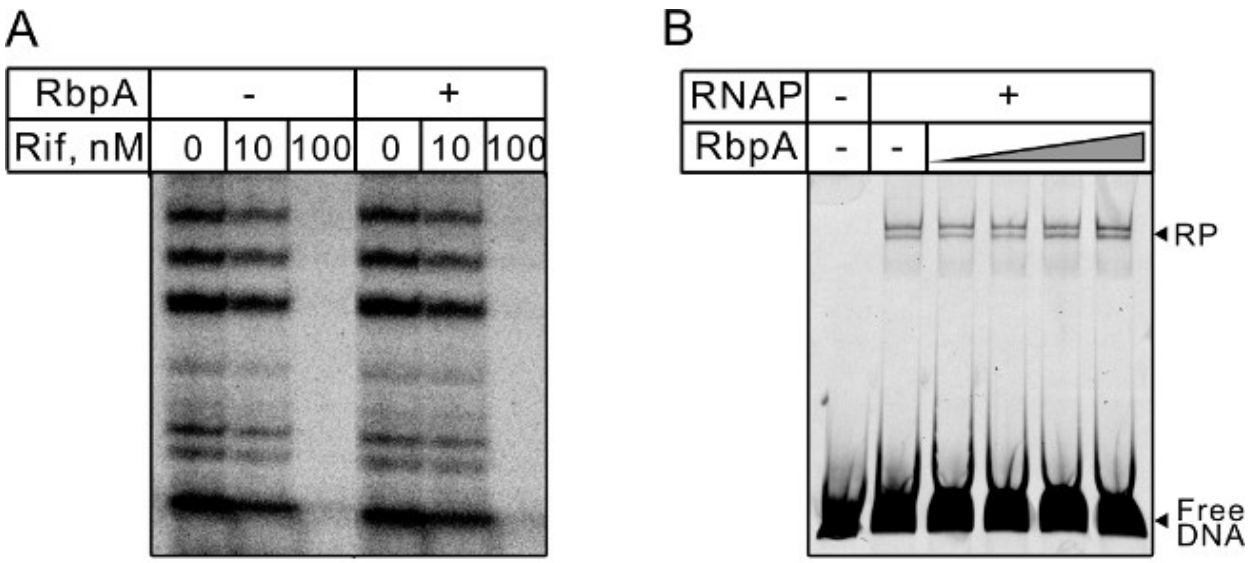


Figure S5

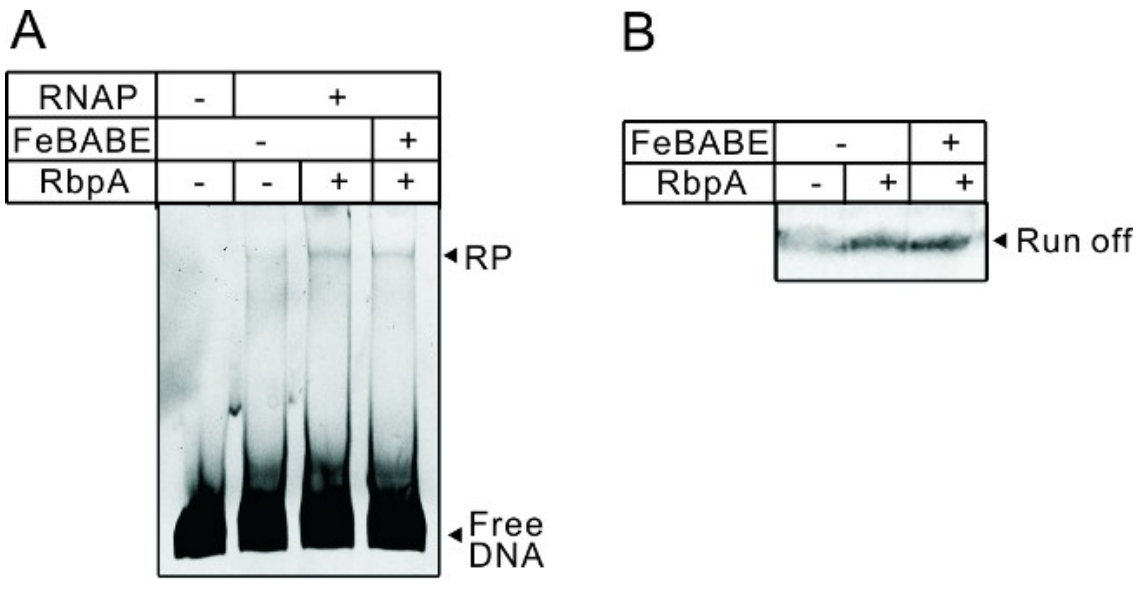
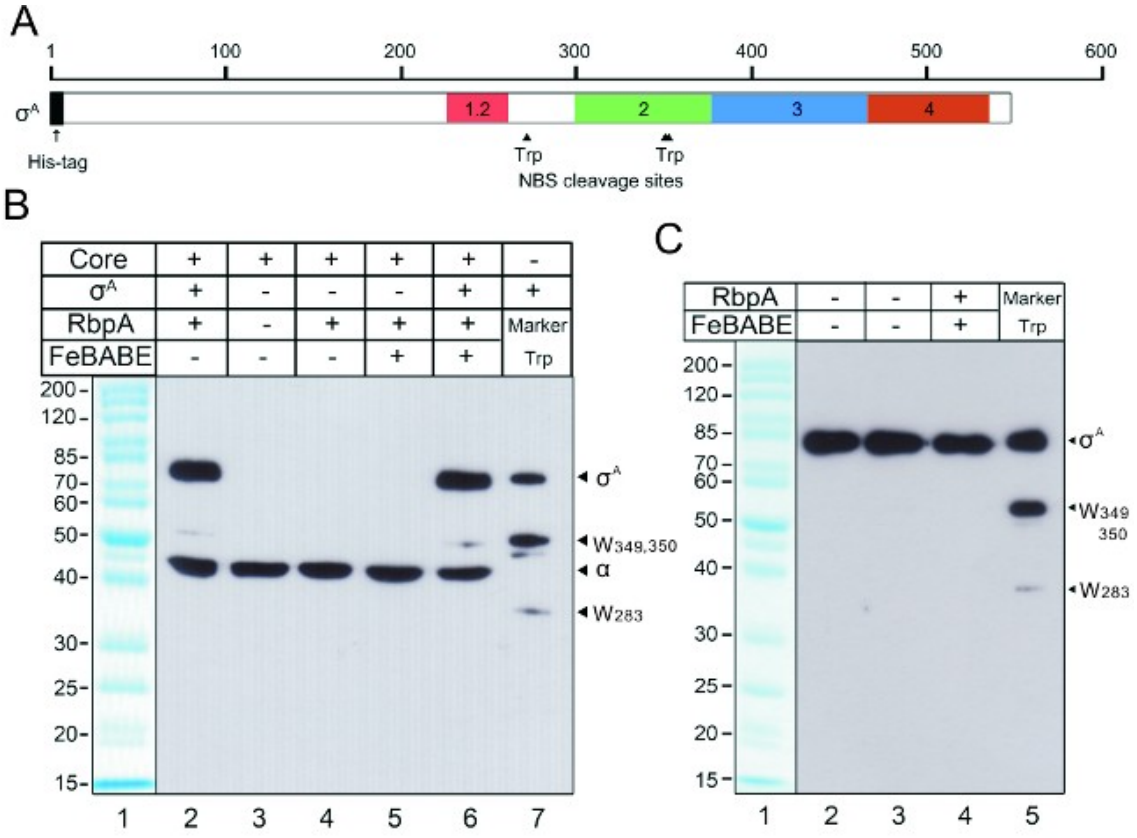


Figure S6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300
400
500
600
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2
3
4
His-tag
Trp
Trp
NBS cleavage sites
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Core + + + + + -
σA + - - - + +
RbpA + - + + + Marker
FeBABE - - - + + Trp
200
120
85
70
60
50
40
30
25
20
15
σA
W349,350
α
W283
1 2 3 4 5 6 7
C
RbpA - - + Marker
FeBABE - - + Trp
200
120
85
70
60
50
40
30
25
20
15
σA
W349
350
W283
1 2 3 4 5