Supplementary Table 1: Primers used in this study:

Rv1222 fw(NdeI)	GGATCCATATGGCCGACCCCGGAAGCGT
Rv1222 rv(HindIII)	AAGCTTCTAGCGACGCACCCGCGATTG
Rv1222 rv(EcoRI)	ATGAATTCATGGCCGACCCCGGAAGCG
Rv1222 C70/73A fw	CCTTTCGCTGGCTGCCCAAGCCGCGGCCGAAG
Rv1222 C70/73A rv	CTTCGGCCGCGGCTTGGGCAGCCAGCGAAAGG
Rv1222 C109G fw	CCGAGATCCCGCGTGGTCCACCTGAAGG
Rv1222 C109G rv	CCTTCAGGTGGACCACGCGGGATCTCGG
Rv1222 A23C fw	CCCGGCGCAGTTCTGCTCCCAGAGTGAC
Rv1222 A23C rv	GTCACTCTGGGAGCAGAACTGCGCCGGG
Rv1222 G133C fw	GGGCTGCGGCATGCTTCGGCGAC
Rv1222 G133C rv	GTCGCCGAAGCATGCCGCAGCCC
Rv1222ΔC fw	GCTTCGCTGACGGCTGATGGCGGGAATC
Rv1222ΔC rv	GATTCCCGCCATCAGCCGTCAGCGAAGC
***lacCONS* fw**	ATGGTACCTAGGCACCCCAGGCTTG
***lacCONS* rv**	ATGGATCCTGTGTGAAATTGTTATCCGC
DNA pol cy5 rv	GAAGGTGAAAGGCCTTTCG
***abrB* fw**	AAGAATTCGTTTCCAAGACATTACTGACTATAAG
***abrB* rv**	TTCTCCTCCCAAGAGATACTTATTTG
***Bpr* fw**	CGTCTGTTGGCCGGCG
***Bpr* rv**	CCCTTGTGGGTGCATCGG
***sinP3* fw**	CAGCCAGAAGTCATACCG
***sinP3* rv**	GCCAAGCTTGCATGCCT
***rrnA* fw**	TCTGGTACCTCGTGGAGAACCTGGTGAGTC
***rrnA* rv**	TCTGGATCCTACGCCGCCAGCGTTCGT
sigE fw(EcoRV)	ATGATATCATGGAACTCCTCGGCGGACC
SigE rv(XhoI)	TACTCGAGTCAGCGAACTGGGTTGACGTG
T7 fw	CCCGCGAAATTAATACGACTCACTATAGGGG
T7 rv	GCTAGTTATTGCTCAGCGGTGGC
***T7A1* fw**	CGGAATTCGGATCCAGATCCCG
***T7A1* rv**	CCTTTTTCACAGGTTTATAACCC

Supplementary Table 2: Templates used in this study:

Tailed-template	Used in *in vitro* transcription assay with Mtb RNAPcore	CCTTGAGTTTCAGGCCGAAACAGCAATTTGGGGTACTGTCACGTTCGGCTAGCCCTCCAGCCCCCCCCCC GGAACTCAAAGTCCGGCTTTGTCGTTAAACCCCAT**G**ACAGTGCAAGCCGATCGGGAGGTC
Bpr	Used in EMSA and *in vitro* transcription assay with RNAP-σ^Eholo	TAGCCGTCTGTTGGCCGGCGTTCCGGGTTGTCGGCCACTGGCCACACTTCTCAGGACTTTCTCAGGTCTT CGGCAGATTCCTGCACGTCACAGGGCGTCAGATCACTGCTGGGTGGGAACTCAAAGTCCGGCTTTGTCGT TAAACCCCAT**G**ACAGTGCAAGCCGATCGGGAGGTCGCTATGGCCGATGCACCCACAAGGG
lacCONS15	Used in *in vitro* transcription assay with Ec RNAP-σ^{70}holo.	CACCCCAGGCTTGACACTTTATGCTTCGGCTCGTATAATGTGTGGA**A**TTGTGAGGAGAGGCG GATAACAA TT TCACACAGG
lacCONS	Used in *in vivo* reporter assay	TAGGCACCCCAGGCTTGACACTTTATGCTTCGGCTCGTATAATGTGTGGA**A**TTGTGAGCGGATAACAATTT CACACAGG
sinP3	Used in *in vitro* transcription assay with Mtb RNAP-σ^Aholo (time kinetics)	CAGCCAGAAGTCATACCGTAAATCCTTTCTGAATGTGCTATAATATCACA**A**TTGCTCGATGAGAAACATGAA ACCGAATACGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGC
rrnA	Used in *in vitro* transcription assay with Mtb RNAP-σ^Aholo	TCGTGGAGAACCTGGTGAGTCTCGGTGCCGAGATCGAACGGGTATGCTGTTAG**G**CGACGGTCACCTATG GATATCTATGGATGACCGAACCTGGTCTTGACTCCATTGCCGGATTTGTATTAGACTGGCAGGGTCGCCC CGAAGCGGGCGGAAACAAGCAAGCGTGTTGTTTGAGAACTCAATAGTGTGTTTGGTGGTTTCACATTTTT GTTGTTATTTTTGGCCATGCTCTTGATGCCCCGTTGTCGGGGGCGTGGCCGTTTGTTTTGTCAGGATATTT CTAAATACCTTTGGCTCCCTTTTCCAAAGGGAGTGTTTGGGTTTTGTTTGGAGAGTTTGATCCTGGCTCAG GACGAACGCTGGCGGCGT
abrB	Used in *in vitro* transcription assay with Bs RNAP.	AAGAATTCGTTTCCAAGACATTACTGACTATAAGAACTAATTCTTACAATCAATAGTAAACAAAATGATTG ACGATTATTGGAAACCTTGTTATGCTATGAAG**G**TAAGGATTTTGTCGAATAATGACGAAGAAAAATATAAT TTAAACAAATAAGTATCTCTTGGGAGGAGAATGTTTATGAAATCTACTGGTATTGTACGTAAAGTTGATGA ATTAGGACGTGTAGTTATTCCTATCGGGATCCTT
TEMPLATE for *in vitro* replication	Used in *in vitro* replication assay with Klenow fragment	CGAAGTTGACTTTTCACTGGTTTTTTTCACTTAACAAAACAGAAGGGAAAACGAAAGGCCTTTCA
Promoter-less DNA	Used in anisotropy assay	GTGAGTATCTCGCAGTCCGACGCGTCGTTGGCCGCCGTCCCCGCCGTGGATCAGTTCGATCCGTCGTC AGGTGCATCAGGTGGCTACGACACCCCGCTGGGCATCACCAATCCGCCCATCGACGAGTTGCTGGACC GCGTCTCGAGCAAATACGCCCTCGTGATCTATGCGGCAAAGCGTGCCCGGCAGATCAACGACTACTACA ACCAGCTTGGCGAGGGCATCCTCGAATATGTCGGTCCGCTGGTTGAGCCGGGGTTGCAAGAGAAGCCG TTGTCCATCGCGTTGCGCGAGATCCACGCCGATCTGCTCGAGCACACCGAGGGCGAGTAG
T7	Used in *in vitro* transcription assay with T7 RNAP	TTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAAC TTTAGAAGGAGATATACATATGCACCATCATCATCATCATTCTTCTGGTCTGGTGCCACGCGGTTCTG GTATGAAAGAAACCGCTGCTGCTAAATTCGAACGCCAGCACATGGACAGCCCAGATCTGGGTACCGA CGACGACGACAAGGCCATGGCTGATATCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCG CACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTT GGCTGCTGCCACCGCTGAGCAATAACTAGC
T7A1	Used in *in vitro* transcription assay with EcRNAPholo	CGGAATTCGGATCCAGATCCCGAAAATTTATCAAAAAGAGTATTGTAAAGTCTAACCTATAGGATACTTA CAGCC**A**TCGAGAGGGACACGGGCGAAGCTTGGATCGAATCGAAGAAGCTACTCGTAATTTCACGGGTT ATAAACCTGTGAAAAAGG

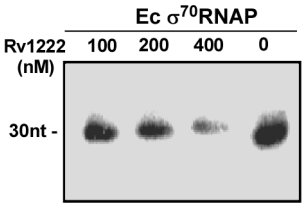
Supplementary Table 3: List of vectors used in this study

Name of the vector	Source	Resistance Marker
pAcYc Duet	Novagen, USA	Chloramphenicol
pET Duet	Novagen, USA	Ampicillin
pET 28a(+)	Novagen, USA	Kanamycin
pUC19	New England Biolabs, UK	Ampicillin
pBluescript II SK(+)	Agilent technologies, USA	Ampicillin
pLAM12	Addgene, USA	Kanamycin
pFPV mcherry	Addgene, USA	Ampicillin

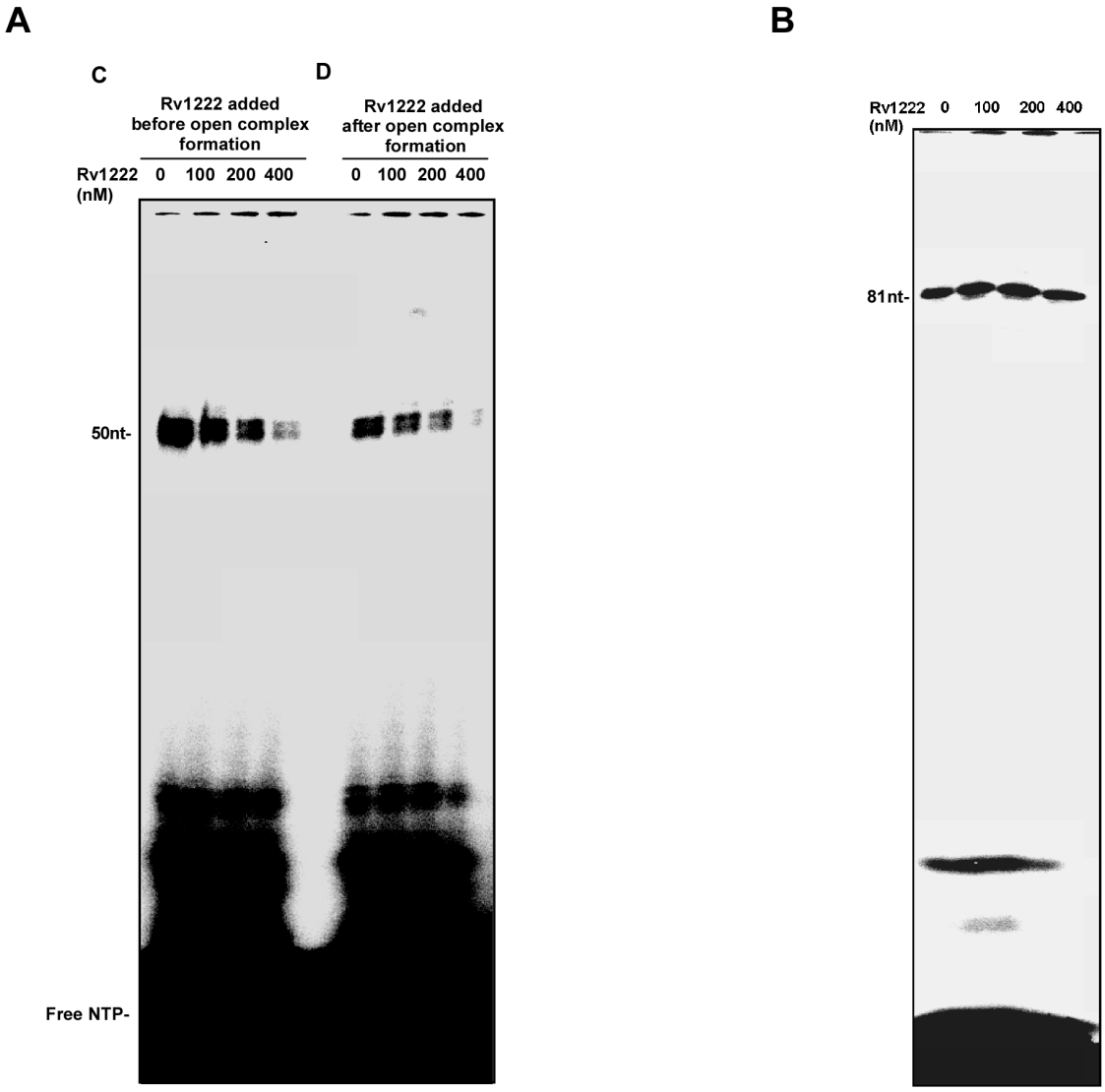
Supplementary Table 4: List of plasmids

pETDuet-*rpoB-rpoC*	Banerjee et al.
pAcYc-*rpoA-sigE*	This study
pET28a-rv1222	This study
pAcYc-rv1222	This study
pAcYc-rv1222ΔC	This study
pFPV-mcherryUV5	This study
pLAM12-Rv1222	This study
pUC19- *lacCONS*	This study
pET29a-*sigA*	Banerjee et al.
pET30a-*sigE*	Rodrigue et al.
pET16b-*rpoA*	Rodrigue et al.
pBluescriptSK-*Bpr*	This study

*.:



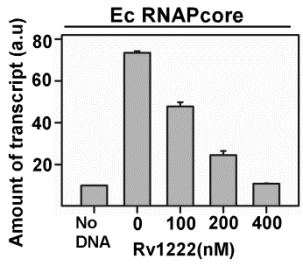
Supplementary Figure 1: Inhibition of transcription by Rv1222 purified from soluble fraction: Open complex was formed with 100nM Ec RNAP holo and 50nM *lacCONS* DNA. Rv1222 was added after open complex formation. Transcripts were produced using P^{32} labelled NTPs.



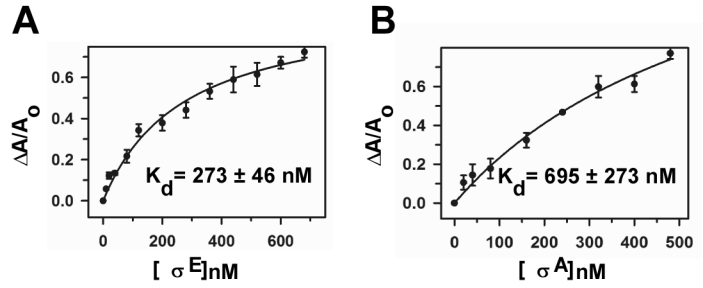
Supplementary Figure 2A: Full gel of SigE transcription assay with Rv1222.

Supplementary Figure 2B: Nuclease assay: P^{32} labelled transcripts (81nt) were formed using *in vitro* transcription assay with 100nM Ec RNAP and 50nM *T7A1* template DNA. The samples were

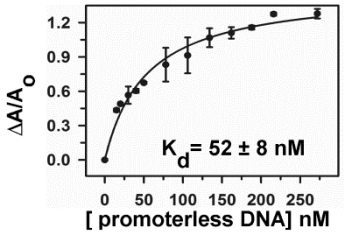
treated with increasing concentrations of Rv1222 for 5mins at 37℃ and loaded in denaturing PAGE and scanned by autoradiography.



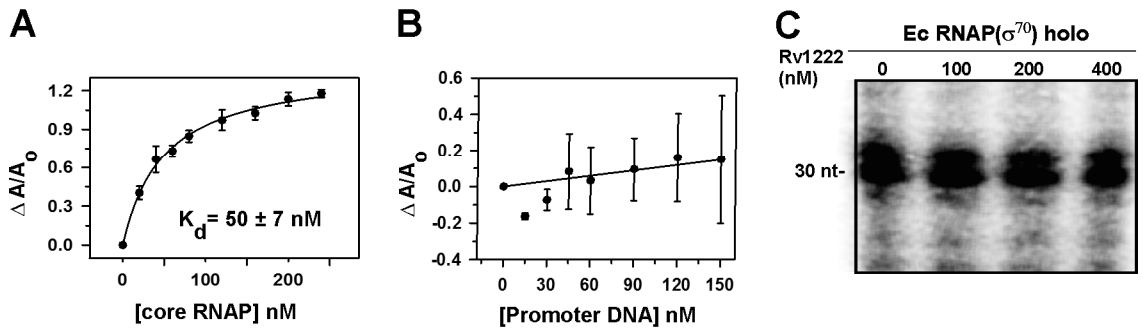
Supplementary Figure 3: *In vitro* transcription assay with 100nM Ec RNAP core, Rv1222 and 28ng Kool NC-45 template (14ng/µl) (Epicenter, USA). Lane 1: Control, no DNA. Lane 2-5: increasing concentrations of Rv1222. Rv1222 was incubated with the core enzyme before the open complex formation. RNA transcript produced were monitored by fluorescence with Ex at 490nM; Em at 530nM.



Supplementary Figure 4: Binding of TMR labelled Rv1222 (20nM) to Mtb σ^E and Mtb σ^A :Fluorescence anisotropy assay.



Supplementary Figure 5: Binding of TMR labelled Rv1222 (20nM) to promoter-less DNA fragment. Sequence of DNA was shown in Supplementary table 1.

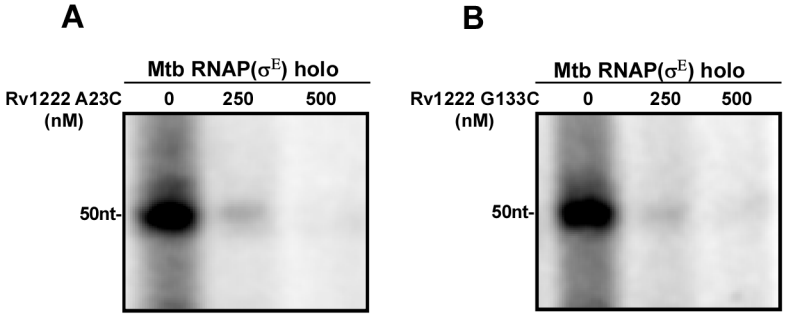


Supplementary Figure 6: Effect of salt concentration on Rv1222 activity.

A. Binding assay with RNAP: 20nM TMR labelled Rv1222 was titrated with Mtb RNAPcore in a transcription buffer containing 200mM KCl. Fluorescence anisotropy of the labelled protein were monitored at Ex 530 nm and Em 580 nm.

B. Binding assay with DNA: Same as A except Rv1222 was titrated with promoter DNA(*lacCONS* DNA).

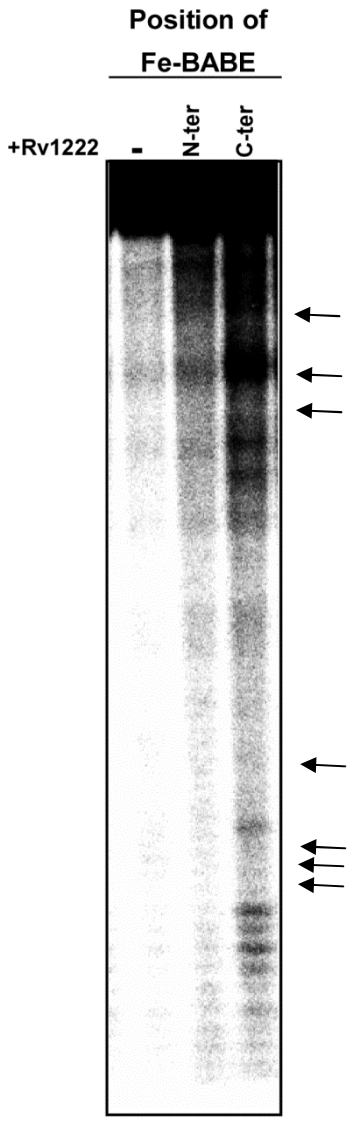
C. *In vitro* transcription assay: Open complex was formed with 100nM Ec RNAP holo and 50nM *lacCONS* DNA in above transcription buffer and incubated with Rv1222 before transcription initiation.



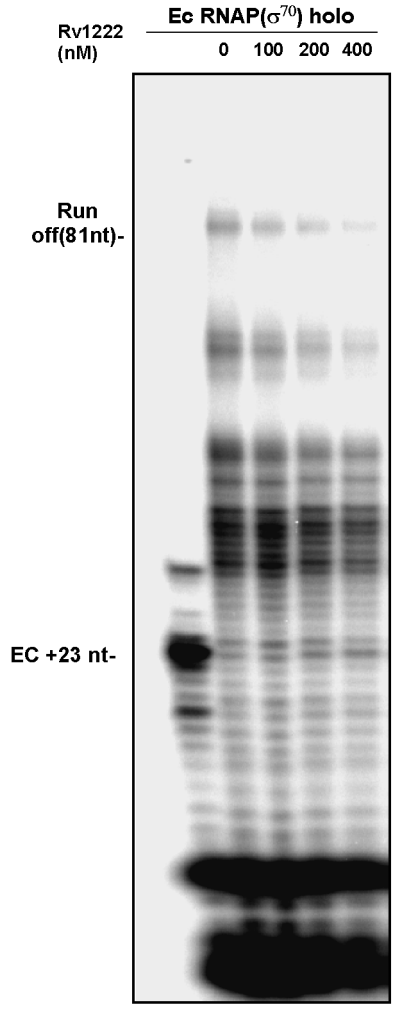
Supplementary Figure 7: A. *In vitro* transcription assay with Fe-BABE labelled Rv1222 derivatives. 100nM Mtb RNAP(σ^E)holo and 50nM *Bpr* promoter DNA was used to form the open complex. Rv1222 derivatives was added and incubated for 30mins at 37℃ followed by addition of NTP (125µM ATP, GTP, CTP, and 20 µM α^{32P}-CTP (0.4 µCi)). After 5 min, the reactions were terminated by addition of 2.5 µl of FLB dye.

A. Result with Rv1222 A23C derivative.

B. Result with Rv1222 G133C derivative.



Supplementary Figure 8: DNA-Protein footprinting with Fe-BABE labelled Rv1222.Lane 1: No Rv1222; Lane 2: labelled Rv1222 at residue A23C (N-ter); Lane 3: labelled Rv1222 at residue G133C (C-ter). Arrows indicate the site of cleavage.



Supplementary Figure 9: Rv1222 inhibits transcription elongation. Stalled elongation complex (EC +23) were formed by *in vitro* transcription assay with 100nM Ec RNAP holo and 50nM *T7A1* promoter that had the first template-strand adenine residue at position +23, with 5 µM of ATP, GTP and 2.5 µM of α^{32P}-CTP (0.2 µCi). After the formation of EC, the stalled complexes were incubated with increasing concentrations of Rv1222 for 10min at 37℃ and chased with 5 µM UTP for 5mins.