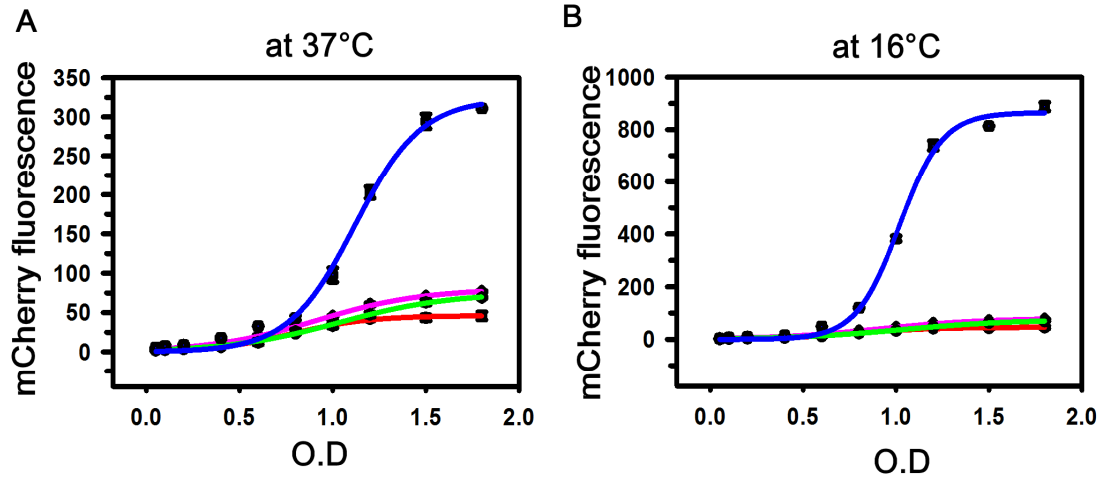


SUPPLEMENTARY DATA:

Supplementary Figures:



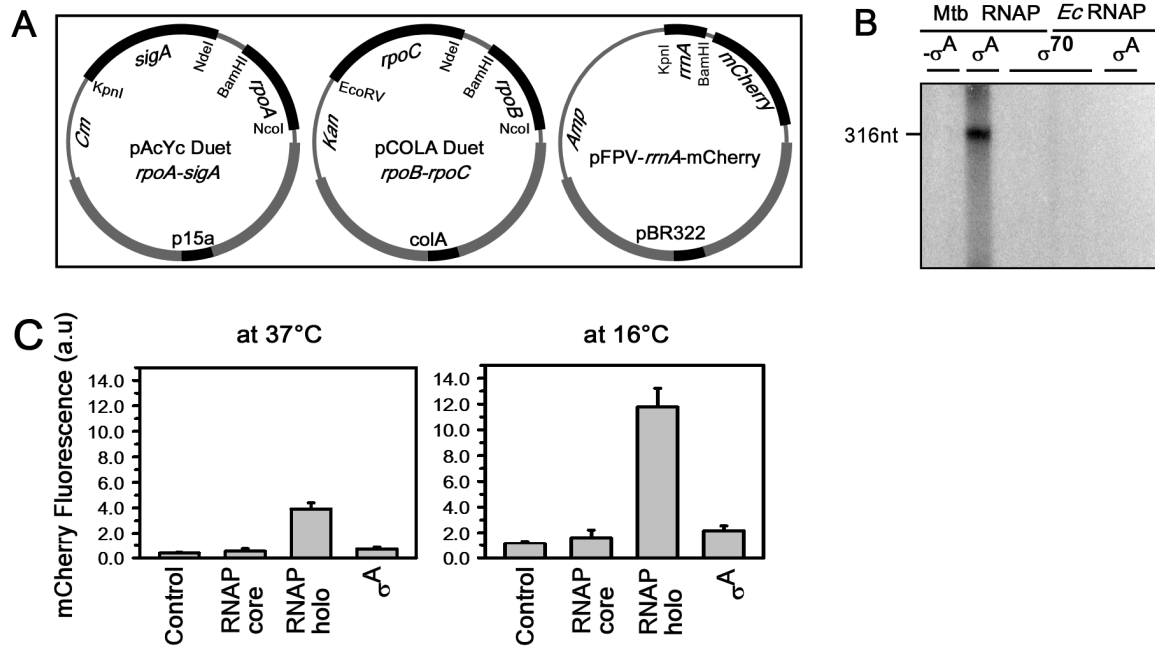
Supplementary Fig. S1, Mukhopadhyay

Supplementary Fig. S1: *In vivo* recombinant Mtb reporter assay: Dependence of mCherry expression as a function of *E. coli* growth:

A. Fluorescent intensity of mCherry was measured at regular intervals and plotted as a function of OD. Fluorescent intensity gets saturated at OD (595) = 1.5. Red Line: pFPVmCherry *sinP3*, Green Line: RNAP core + pFPVmCherry *sinP3*, Pink line: Mtb σ^A + pFPVmCherry *sinP3*, Blue line: RNAP-holo + pFPVmCherry *sinP3*. Assays were conducted at 37°C for 6 hours.

B. Same as (A), assays were conducted at 16°C for 16 hours.

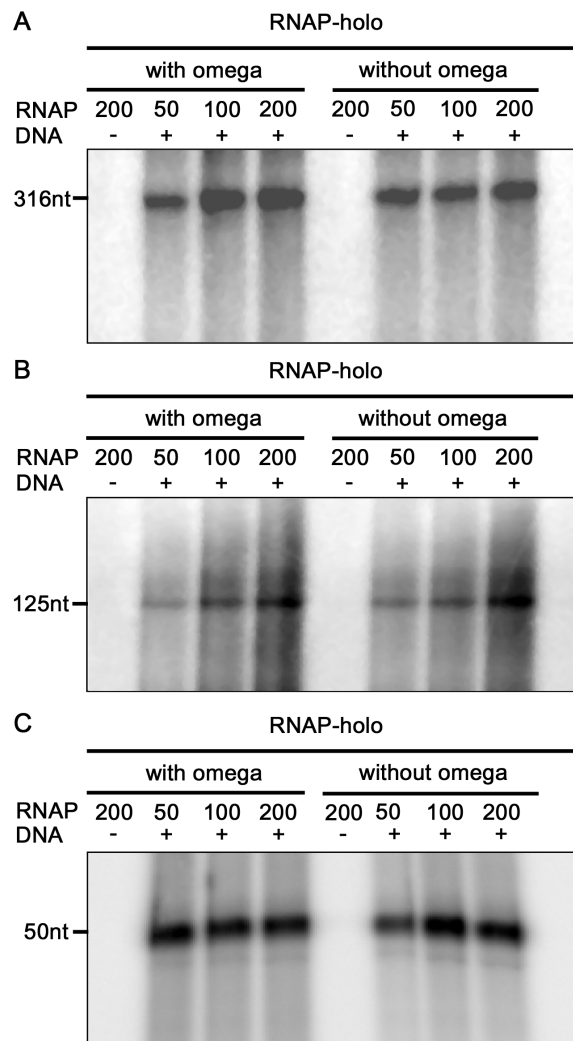
The data represents the mean of three replicates and error bars represent standard deviations.



Supplementary Fig. S2, Mukhopadhyay

Supplementary Fig. S2: Recombinant *in vivo* Mtb reporter assay in *E. coli* using Mtb σ^A specific promoter *rrnA*:

- A. Strategy: three plasmid expression system, dual plasmid (first two plasmids) for expression of Mtb RNAP holo and the third plasmid containing a σ^A dependent promoter element *rrnA* for expression of mCherry. All three plasmids were transformed in *E. coli* B121 (DE3) cells and then the cells were grown at 37°C up to OD (at 595 nm) 0.4 and induced with 0.5 mM IPTG at different growth conditions.
- B. *In vitro* transcription assay to assess the interactions between Mtb core and *E. coli* σ^{70} and *E. coli* RNAP core with Mtb σ^A . *rrnA* was used as a DNA fragment. Run-off transcripts of 316 nucleotides (nt) were produced. Lane 1: Mtb RNAP core, Lane 2: Mtb RNAP core + Mtb σ^A , Lane 3: Mtb RNAP core + *E. coli* σ^{70} , Lane 4: *E. coli* RNAP + *E. coli* σ^{70} , Lane 5: *E. coli* RNAP + Mtb σ^A .
- C. Results for recombinant Mtb reporter assays: The bars represent mCherry fluorescence of *E. coli* cells containing pFPVmCherry-*rrnA* plasmid in presence of the following; 1. No Mtb RNAP; 2. Mtb RNAP core (pAcYc Duet *rpoA-rpoZ* + pCOLA Duet *rpoB-rpoC*); 3. Mtb RNAP holo (as in Fig. S2); 4. σ^A : (pAcYc Duet-*sigA*). The data represents the mean of three replicates and error bars represent standard deviations.
- Left panel: cells were induced with 0.5 mM IPTG at 37°C for 6 hrs.
 Right panel: cells were induced with 0.5 mM IPTG at 16°C for 16 hrs.
 The data represents the mean of three replicates and error bars represent standard deviations.



Supplementary Fig. S3, Mukhopadhyay

Supplementary Fig. S3: Effect of ω subunit on *in vitro* transcriptional activity of Mtb RNAP:

Assays were performed with the purified Mtb RNAP holo (with and without ω subunit) samples.

- A. Assays with RNAP- σ^A holo and *rrnA* promoter DNA fragment. Lane 1, 5: No DNA, Lane 2-4: RNAP holo with ω subunit; Lane 6-8: with RNAP holo without ω subunit. Run-off transcripts were 316 nt.
- B. Same as (A) with Mtb CRP dependent promoter *WhiB1*. Mtb CRP was incubated with RNAP holo before open complex formation. Run-off transcripts were 125 nt.
- C. Same as (A) with RNAP-*sigE* holo enzyme and *sigE* dependent promoter *SigBpr*. Run-off transcripts were 50 nt.

Supplementary Table S1: Oligonucleotides used in this study:

Name of the primer	Sequences
pFPV-mCherry <i>sinP3</i> fwd	GAATTCCAGCCAGAAGTCATACCGTA
pFPV-mCherry <i>sinP3</i> rv	GGATCCGTATTTCGGTTTCATGTTTCT
pFPVmcherry <i>rrnA</i> fwd	TCTGGTACCTCGTGGAGAACCTGGTGAGTC
pFPVmcherry <i>rrnA</i> rv	TCTGGATCCTACGCCGCCAGCGTTCGT
pBlueScriptII <i>sigBpr</i> fwd	CGTCTGTTGGCCGCG
pBlueScriptII <i>sigBpr</i> rv	CCCTTGTGGGTGCATCGG
pBSII- <i>WhiB1</i> fwd	GCTCTAGAGCAAGAAAGCGGATCTG
pBSII- <i>WhiB1</i> rv	GCAAGCTTGCCTTGTGGCGCCAATC
pET 28a-Mtb CRP fwd	CATCCGGAATTCGTGGACGAGATCCTGGCC
pET 28a-Mtb CRP rv	CATCATACTCGAGCACTATTACCTCGCTCGGCGGGC
pET 28a-<i>E. coli</i> CRP fwd	CGAAACATATGGTGCTTGGCAAACCGCA
pET 28a-<i>E. coli</i> CRP rv	GCGTTGGATCCAATTTAACGAGTGCCGT
pFPV mCherry-CRP fwd	CCCGCGAAATTAATACGACTCACTATAGGGG
pFPV mCherry-CRP rv	TTAAAGCTTTTACCTCGCTCGGCGG
pAcYc Duet <i>sigA</i> fwd	GGAATTCGTGGCAGCGACCAAAG
pAcYc Duet <i>sigA</i> rv	ATAAGAATGCGGCCGCTCAGTCCAGGTA
pAcYc Duet <i>sigE</i> fwd	CCAGGATCCGAATTCCATCATGGAACCTCCTCGG
pAcYc Duet <i>sigE</i> rv	CCGAGGAGTTCCATGATGGAATTCGGATCCTGG

Supplementary Table S2: Cloning strategies:

Name of the plasmid	Restriction enzymes used and cloning strategies	Source of gene
pCOLA <i>rpoB-rpoC</i>		Banerjee <i>et al</i> (24)*
pAcYc Duet <i>rpoA-rpoZ</i>		Banerjee <i>et al</i> (24)*
pAcyC Duet <i>rpoA- sigA</i>		Banerjee <i>et al</i> (24)*
pCDF 1b <i>rpoZ</i>		Banerjee <i>et al</i> (24)*
pAcYc Duet <i>rpoA- sigE</i>	<i>rpoA</i> :NcoI & BamHI; <i>sigE</i> : EcoRV &XhoI	Genomic DNA
pFPVmcherry- <i>sinP3</i>	<i>sinP3</i> : EcoRI & BamHI	Synthetic DNA
pFPVmcherry- <i>rrnA</i>	<i>rrnA</i> : KpnI & BamHI	Verma <i>et al</i> (21)**
pFPV mcherry- <i>sigBpr</i>	<i>sigBpr</i> : KpnI & BamHI	Genomic DNA
pFPV-mcherry- <i>WhiB1</i>	<i>WhiB1</i> : KpnI & BamHI	Genomic DNA
pFPV-mcherry- <i>WhiB1</i> -CRP	<i>Rv3676</i> : EcoRV & Hind III	Genomic DNA
pET 28a-Mtb CRP	<i>Rv3676</i> : EcoRI & XhoI	Genomic DNA
pET 28a- <i>E. coli</i> CRP	<i>CRP</i> : NdeI & BamHI	Genomic DNA
pAcyC Duet- <i>sigA</i>	<i>sigA</i> : EcoRI & NotI;	Genomic DNA
pAcyC Duet- <i>sigE</i>	<i>sigE</i> : EcoRV &XhoI	Genomic DNA
pBluescript II Sk(+)- <i>WhiB1</i>	<i>WhiB1</i> :SmaI	Genomic DNA
pBluescript II Sk(+)- <i>rrnA</i>	<i>rrnA</i> : SmaI	Genomic DNA
pBluescript II Sk(+)- <i>sigBpr</i>	<i>SigBpr</i> :SmaI	Genomic DNA

Supplementary Reference:

* **Banerjee R, Rudra P, Prajapati RK, Sengupta S, Mukhopadhyay J.** 2014. Optimization of recombinant Mycobacterium tuberculosis RNA polymerase expression and purification. *Tuberculosis* (Edinb) **94**:397-404.

****Verma A, Sampla AK, Tyagi JS.** 1999. Mycobacterium tuberculosis *rrn* promoters: differential usage and growth rate-dependent control. *Journal of bacteriology* **181**:4326-4333.