Supplementary Table1: Bacterial strains used in this study.

Name of Bacterial strain	Relevant genotype
DH5α	F^- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*) U169, hsdR17(r_K^- m_K^+), λ
BL21 (DE3)	F^- ompT gal dcm lon $hsdS_B$(r_B^- m_B^-) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])
C43	F^- ompT gal dcm $hsdS_B$(r_B^- m_B^-)(DE3)

Supplementary Table 2: List of vectors used in this study.

Name of the vector	Source	Resistance Marker
pAcYc Duet	Novagen, USA	Chloramphenicol
pFPVmCherry	Addgene	Ampicillin
PCOLA Duet	Novagen, USA	Kanamycin
pET 28a	Novagen, USA	Kanamycin
PBAD Myc-HisA	Thermo Fisher Scientific	Ampicillin
pCDFDuet-1	Addgene	Streptomycin
pMIND	Dr. Sujay Dasgupta's lab	Kanamycin
pET Duet	Novagen, USA	Ampicillin

Supplementary Table 3: The different clones used in this study along with the cloning strategies applied for generating them.

Plasmid	Description	Reference
pFPV-*ppk1*-*mCherry*-*tetRO*-*mprAB*	The *ppk1* promoter (from -76 to +61 bases) was amplified from Mtb genomic DNA and inserted before *mCherry* gene using Xba1 and Nde1. The *mprAB* gene was PCR amplified from Mtb genomic DNA and incorporated using BamHI and SpeI (site created by SDM). The *tetRO* promoter from pMIND-*tetRO* vector (126 to 829) was PCR amplified and inserted using KpnI and BamHI before *mprAB* gene.	This study. Used for *in vivo* recombinant reporter assay.
pFPV-*ppk1*-mCherry- *tetRO*-*mprA*	As above except the *mprA* gene (Rv0981) was cloned in place of *mprAB* using BamHI and EagI.	This study. Used for *in vivo* recombinant reporter assay.
pCDF-*para*-*mprB*	The promoter region of pBAD vector (3198 to 458) was PCR amplified and cloned in pCDF Duet vector using XbaI and EcoRI. The *mprB* gene (Rv0982) PCR amplified from Mtb genomic DNA was incorporated using HindIII and KpnI.	This study. Used for *in vivo* recombinant reporter assay..
pFPV-*psigE*-mCherry-*tetRO*-*mprA*	As above except the promoter *ppk1* was replaced by *psigE* of *M. tuberculosis* (1, 2). The promoter was inserted into the plasmid using XbaI and NdeI, before insertion of the *tetRO* and *mprA* genes	This study. Used for *in vivo* recombinant reporter assay.
pFPV-*ppk1*-*mCherry*-*TetRO*-mut*mprA* (D48A)	The 48th amino acid (aa) of *mprA* gene, an aspartic acid residue, was mutated to alanine by site directed mutagenesis	This study. Used for *in vivo* recombinant reporter assay.
pAcYcDuet-*mprA*	The *mprA* gene PCR was cloned in pAcYc Duet vector using BamHI and KpnI.	This study. Used for protein purification.
pAcYcDuet-*mprB* (truncated)	A truncated *mprB* gene (aa 196 to 504) was PCR amplified and cloned into pAcYc Duet vector using BamHI and EcoRV. The purified protein MprB lacks the predicted periplasmic domain (aa 1-195).	This study. Used for protein purification.
pAcYcDuet-rpoA-sigE	The *rpoA* gene has been cloned in pAcYc Duet vector (using BamHI and NcoI) along with *sigE* gene using KpnI and NdeI.	Used for *In vivo*

		recombinant reporter assay.(3)
pCOLADuet rpoB-rpoC	The *rpoB* gene has been cloned in pAcYc Duet vector (using BamHI and NcoI) along with *rpoC* gene using EcoRV and NdeI.	Used for *in vivo* recombinant reporter assay.(3)
pET Duet- *ppk1*-mCherry- *tetRO-mprA*	The pFPV- *ppk1*-mCherry- *tetRO-mprA* plasmid was digested with KpnI and HindIII, and the whole insert of *tetRO-mprA*-*ppk1*-mCherry stretch of 2643bp was cloned in pET Duet vector.	This study. Used for *in vivo* recombinant reporter assay.

References:

1. **Dona V, Rodrigue S, Dainese E, Palu G, Gaudreau L, Manganelli R, Provvedi R.** 2008. Evidence of complex transcriptional, translational, and posttranslational regulation of the extracytoplasmic function sigma factor sigmaE in Mycobacterium tuberculosis. J Bacteriol **190:**5963-5971.
2. **He H, Hovey R, Kane J, Singh V, Zahrt TC.** 2006. MprAB is a stress-responsive two-component system that directly regulates expression of sigma factors SigB and SigE in Mycobacterium tuberculosis. J Bacteriol **188:**2134-2143.
3. **Banerjee R, Rudra P, Saha A, Mukhopadhyay J.** 2015. Recombinant reporter assay using transcriptional machinery of Mycobacterium tuberculosis. J Bacteriol **197:**646-653.

Supplementary Table 4: List of primers used in this study

mprAfwd (BamHI)	ATCGCGGATCCGTGCGAATTCTTGTCGTTGA
mprArev (KpnI)	ATACTGGTACCTCAGGGTGGTGTTTCACGTA
mprB(t)fwd (BamHI)	TTAAGGATCCATGACCGAAGCGGCCGAG
mprB(t)rev (EcoRV)	ATATGATATCCTAGGTTGCGCGCGTGGACT
***ppk1*fwd (XbaI)**	AATCTAGATGAACGGCAGACCAACGG
***ppk1*rev (NdeI)**	AAGCTTCATATGGATACCACGCATGCTCCTC
pFPVseqfwd	GGATACATATTTGAATGTATTTAGAAA
pFPVseqrev	TCAGTTCATGTACGGCTCCAA
seqprimer	GACATTAACCTATAAAAATAGGCG
mprBfwd (HindIII)	TAATAAGCTTATGACCGAAGCGGCCGAGCG
mprBrev (KpnI)	TTGCGCGCGTGGACTGAGAGGTACCAATT
pBADfwd (XbaI)	AAGTTCTAGATTATGACAACTTGACGGCTA
pBADrev (EcoRI)	AAAACAGCCAAGCTTCGAATTCCCACTAGT
***ppk1*fwd200 (HpaI)**	TTAACTAGTTAACTGCGATGTCGACACCCCC
***ppk1*rev200 (NdeI)**	ATATATTAACATATGCCGCACCTCTGTGACGG
pBADnewrev (BclI)	TTGATGATCAGAGCTCGGATCCATGGTTAA
psigEfwd (XbaI)	AATCTAGATATTCCCTGGACAGCGCA
psigErev (NdeI)	AAAGCTTCATATGGGAATTACCGTCGCGTA
psigBfwd	TTAAACTAGTTAACTATTCGCCGACCACGGTTAGC
psigBrev	TATATTAACATATGACGAGGTCCGCCGCG
mprAmutD48Afwd	GCGATCGCCCCGCCGCGTTGGTCCTG
mprAmutD48Arev	CAGGACCAACGCGGCGGGGCGATCGC
p2+p3fwd (1)	TTAAACTAGTTAACTCTAAGCCAAAGCTCAGATTGC
p2+p3fwd (2)	TTAAACTAGTTAACTGCTCATATATGGCCCATACG
p3onlyfwd	TTAAACTAGTTAACTGGAACTCCTCGGCGGA
***TetRO*fwd (KpnI)**	TTAATATAAGGTACCTCTAGATCACGATTCGCTCG
***TetRO*rev (BamHI)**	TATAAATATGGATCCTGTCAGGATTCCACGA
***mprAB*fwd (BamHI)**	TGGATCCACTGATGTCCGTGCGAATTCTTG
***mprAB*rev (SpeI)**	ATACTAGTCTAGGTTGCGCGCGTGGACTG
mprAfwd (BamHI)	ATCGCGGATCCGTGCGAATTCTTGTCGTTGA
mprArev (EagI)	ATACTCGGCCGTCAGGGTGGTGTTTCACGTA

Supplementary Figures:

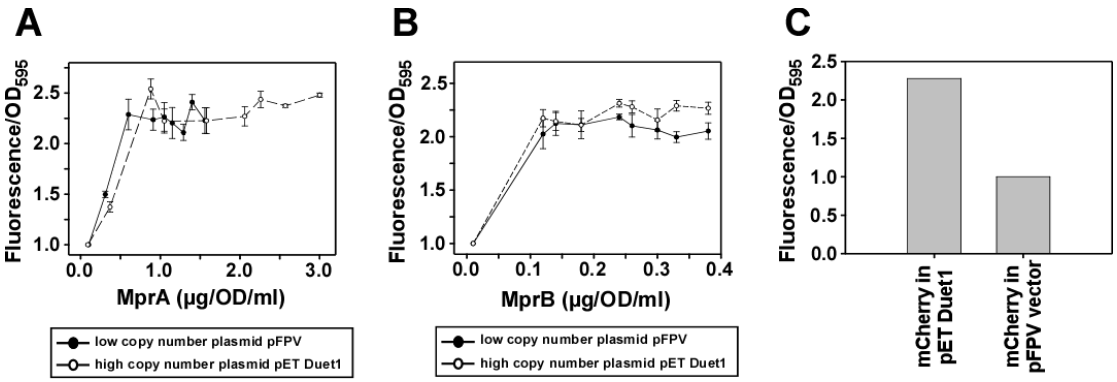


Figure S1. Comparative studies of *Invivo* Recombinant Reporter Assay

(A) The assay as in Fig 2 was repeated with a higher copy number plasmid pET Duet1, where the gene cassette of *tetRO promoter- mprA- ppk1 promoter- mCherry* as shown in Fig 2B were cloned in pET Duet1 as a control study for promoter saturation. Relative fluorescence intensities of mCherry expression with respect to control for 1 OD cells (average of 3 replicates with standard error) were plotted against varying concentration (0.09, 0.37, 0.88, 1.05, 1.58, 2.06, 2.26, 2.57, 3.00 µg/OD/ml) of MprA keeping MprB fixed at 0.14 µg/OD/ml (dashed lines, open circles), control: cells with no induction of MprA, but in presence of MprB (0.14 µg/OD/ml). For comparison the curve of Fig 2C upper panel (solid line, closed circles), was shown as a solid line where the concentration of MprB was 0.14µg/OD/ml (closed circles).

(B) Same as in (A), but with varying concentration (0.06, 0.12, 0.14, 0.18, 0.24, 0.26, 0.30, 0.33, 0.38 µg/OD/ml) of MprB keeping MprA fixed at 0.96 µg/OD/ml (dashed lines, open circles), control: cells with no induction of MprB, but in presence of MprA (0.96 µg/OD/ml). For comparison the curve of Fig 2D upper panel (solid line, closed circles), was shown as a solid line where the concentration of MprA was 0.96µg/OD/ml (closed circles).

(C) Average of 3 replicates corresponding to the control sets (no induction of either MprA or MprB) of pET Duet1 plasmid having *tetRO promoter- mprA- ppk1 promoter- mCherry* cassette and pFPV plasmid having *tetRO promoter- mprA- ppk1 promoter- mCherry* cassette were taken and their fold changes were plotted as a bar graph.

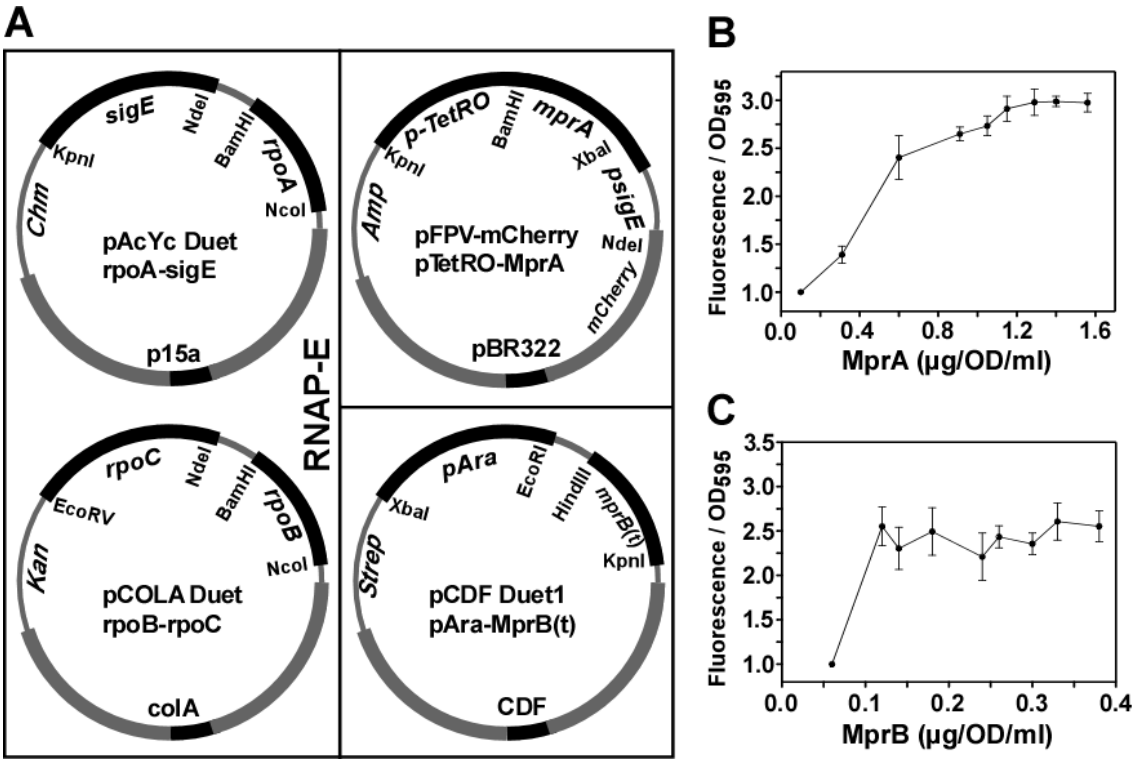
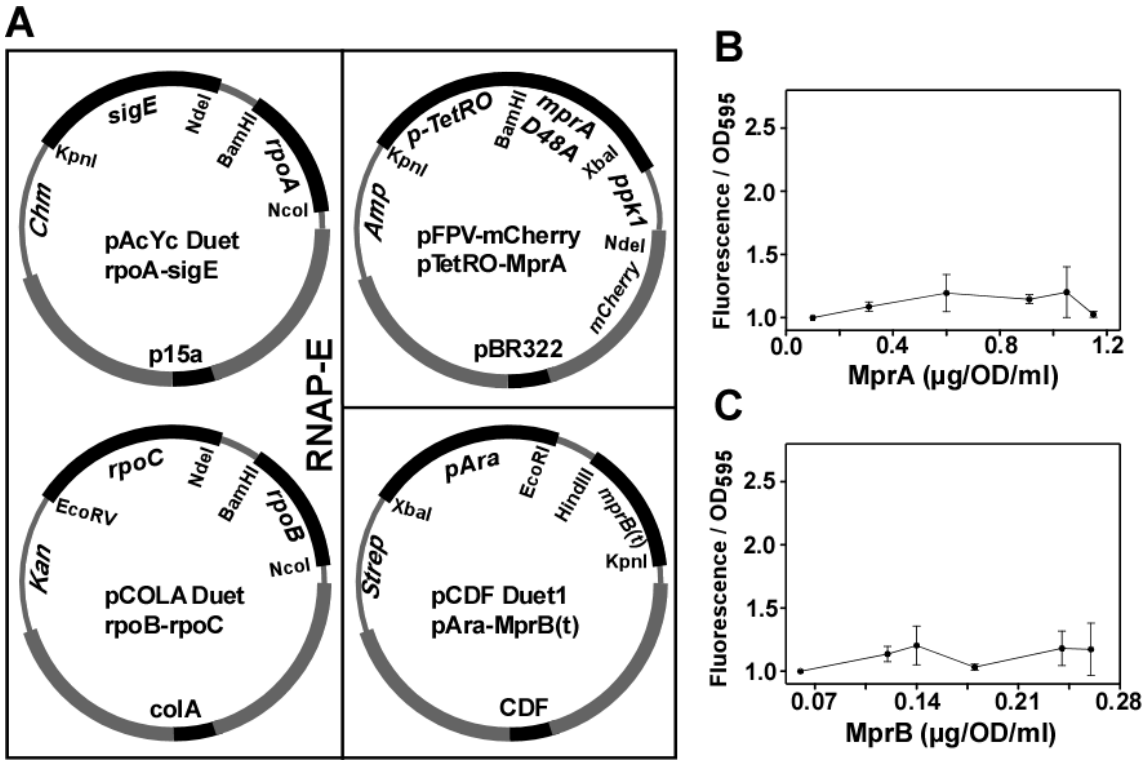


Figure S2. ***In vivo*** **Recombinant Reporter Assay with *psigE*:**
(A) A four plasmid construct has been designed as in Fig 2(B) with the third plasmid having promoter *sigE* in place of *ppk1* promoter.

(B) Fluorescence intensities of mCherry expression for 1 OD cells (average of 3 replicates with standard error) were plotted against varying concentration (0.10, 0.31, 0.60, 0.91, 1.05, 1.15, 1.29, 1.40 and 1.56 µg/OD/ml) of MprA keeping MprB fixed at 0.14µg/OD/ml.

(C) Same as above, but with varying concentration (0.06, 0.12, 0.14, 0.18, 0.24, 0.26, 0.30, 0.33 and 0.38 µg/OD/ml) of MprB keeping MprA fixed at 0.96µg/OD/ml.



FigureS3. ***In vivo*** **Recombinant Reporter Assay with phosphorylation deficient mutant of MprA (D48A).**

(A) The aspartic acid residue at position 48 is mutated to alanine using site directed mutagenesis and cloned in place of wild type *mprA* gene in the pFPV-*ppk1*-mCherry-*tetRO-mprA* vector keeping all other component of the four plasmid construct intact.

(B) The mCherry fluorescence per OD is plotted against varying concentrations (0.10, 0.31, 0.60, 0.91, 1.05, and 1.15 µg/OD/ml) of MprA keeping MprB fixed at 0.14µg/OD/ml. No significant increase in mCherry fluorescence has been observed which proves that phosphorylated MprA is required for the regulation of *ppk1* promoter and also verifies the functionality of our synthetic circuit.

(C) Same as above but with varying concentrations (0.06, 0.12, 0.14, 0.18, 0.24, 0.26 µg/OD/ml) of MprB keeping MprA fixed at 0.96 µg/OD/ml.

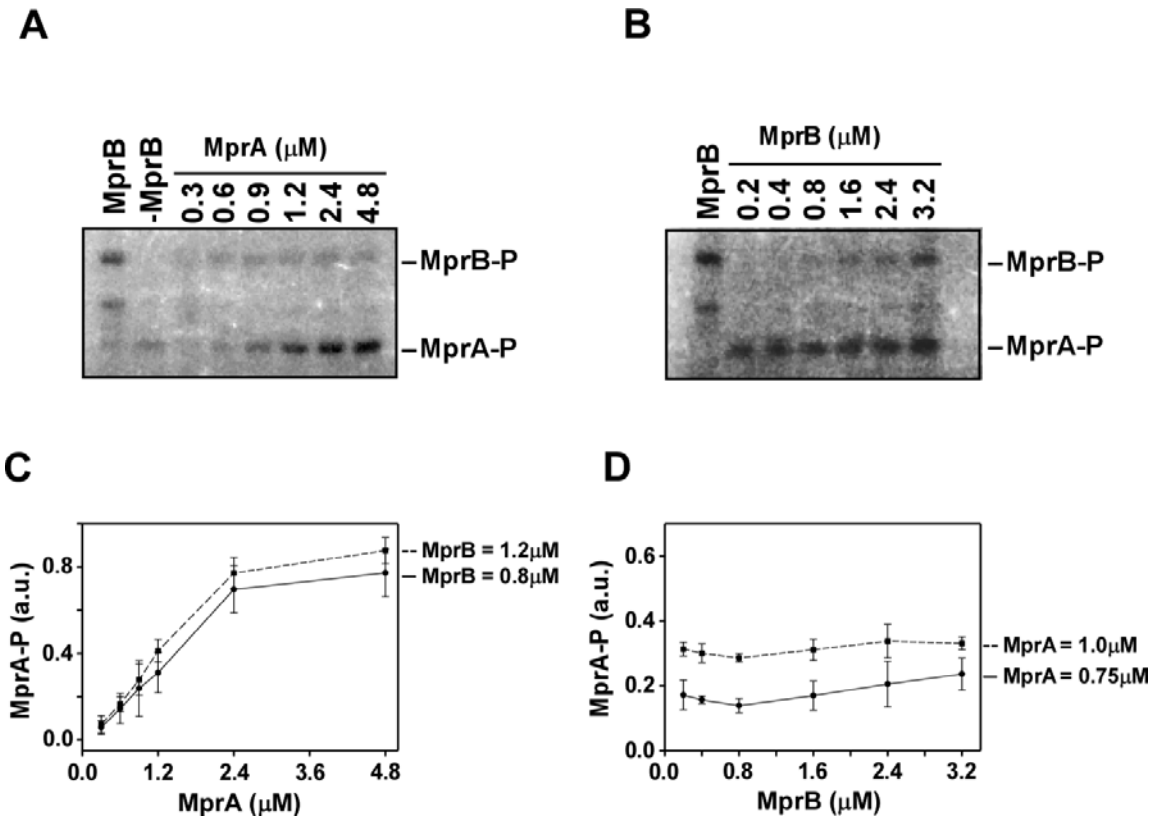


Figure S4. ***In vitro*** **Phosphorylation Assay with higher concentration of MprB and MprA respectively.**

(A) A varying concentration of MprA was reacted with 1.2 μM MprB (dashed line). For comparison the curve of Fig 3C was shown as a solid line where the concentration of MprA was 0.8 μM.

(B) A varying concentration of MprB was reacted with MprA (initially fixed at 0.75 μM as in Fig3B) kept constant at 1.0 μM.

(C) Amount of Phosphorylated MprA from three replicates was plotted in dashed lines. For comparison the curve of Fig 3C was shown as a solid line where the concentration of MprB was 0.8 μM.

(D) Same as C but with fixed amount MprA. Solid line as in Fig 3D

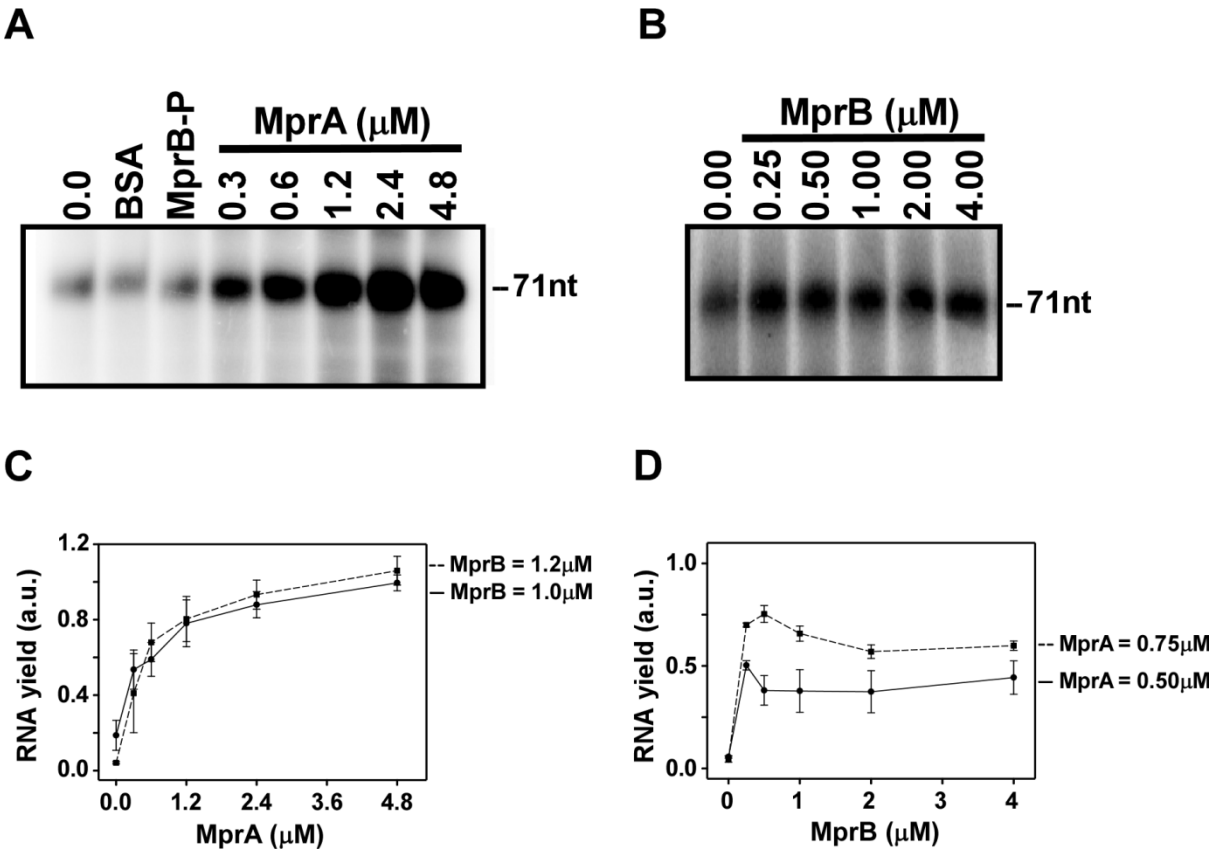


Figure S5. ***In vitro*** **Transcription Assay with higher concentration of MprB and MprA respectively.**

(A) Assay was done using varying concentration of MprA keeping MprB fixed at 1.2 μM lane1: RNAP only, lane 2: RNAP + MprB-P+BSA, lane 3: RNAP + MprB-P, lane 4-8: RNAP + MprB-P + MprA. In the control experiment (lane 2), upon addition of MprB-P or BSA did not increase (two-tailed Mann-Whitney U Test, p-value < 0.05) the yield of the transcript.

(B) Assay was done using varying concentration of MprB keeping MprA fixed at 0.75μM.

(C) Relative RNA yield from three replicates were plotted as dashed lines. The data were compared with the assay performed at lower MprB concentration (solid line) as in Fig 4D.

(D) Same as C but assay was performed with fixed MprA shown by solid line as in Fig 4E.

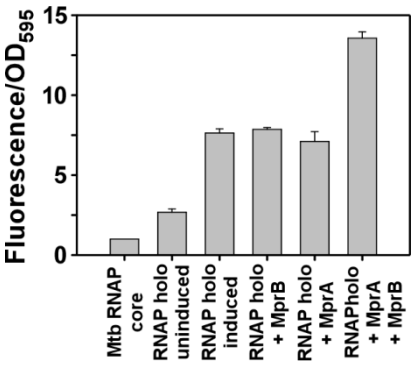


Figure S6. Control experiments showing that there are no cross-talks among MprAB and *E.coli* TCS.

Relative fluorescence intensities of mCherry expression in the *in vivo* recombinant reporter assays (as in Fig 2) in the presence of different proteins as shown