Supplementary Information

Regulation of polyphosphate glucokinase gene expression through co-transcriptional processing in *Mycobacterium tuberculosis* H37Rv

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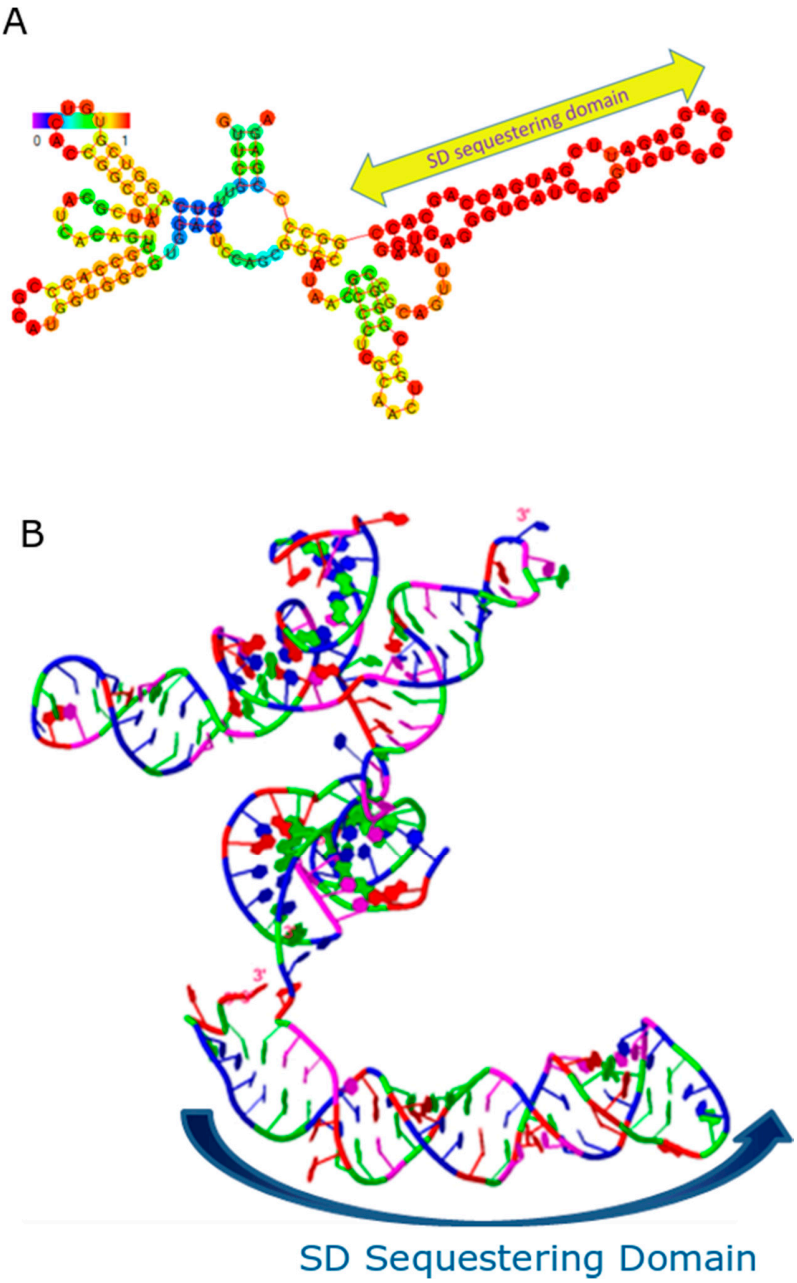
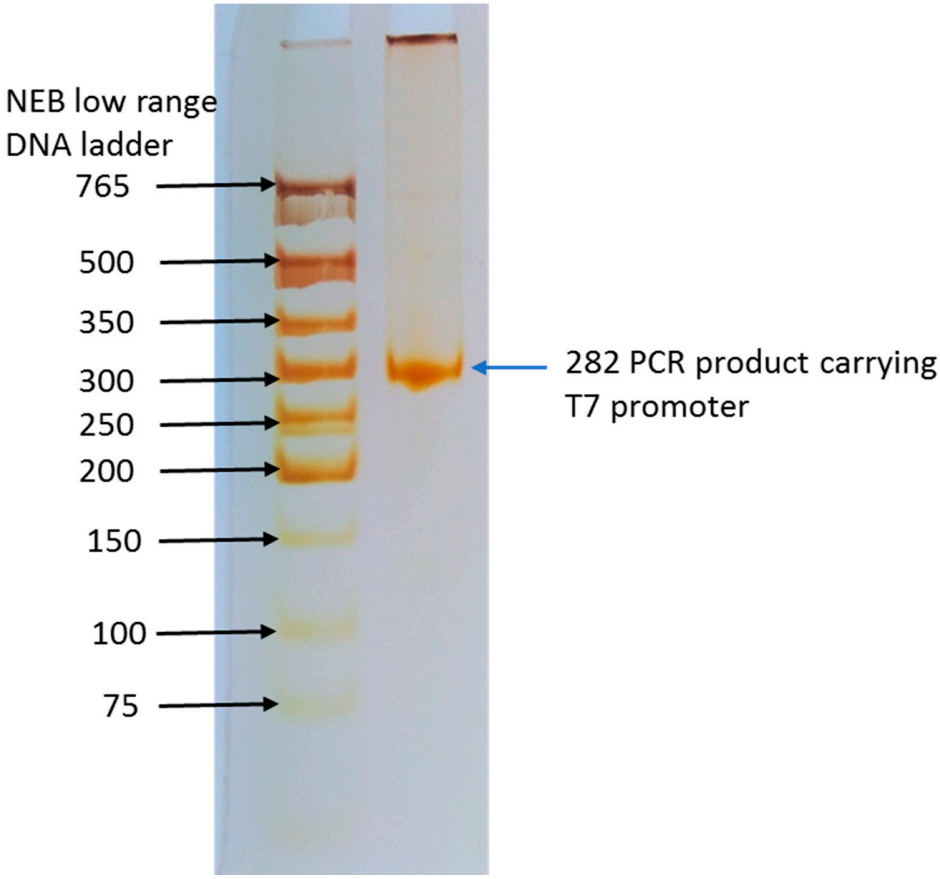
Supplementary Table 1. List of oligonucleotides used in this study

T7 282 *ppgk* transcription primer	Forward Primer	**TAATACGACTCACTATAGGG**CA AGGTTTTCGGCCACAGAG
	Reverse Primer	GACGATTCCGCCCTTGATG
Real time quantitative PCR, *ppgk* gene specific primer	Reverse primer for cDNA synthesis	AGCAGTACCACTAAGCCAGG
Primer pair C1 for *ppgk* mRNA I quantification (cleavage site I)	Forward Primer	CCGCAGTTTAAGGTGAGGGT
	Reverse Primer	CGACGATTCCGCCCTTGAT
Primer pair C2 for *ppgk* mRNA II quantification (cleavage site II)	Forward Primer	GGATAGGGACCAACGCACGC
	Reverse Primer	AGCCAGGGTTGTTCTTGCCG
Primer pair for full length *ppgk* mRNA quantification	Forward Primer	CCTATCGCATCACAGTCGCC
	Reverse Primer	ACGTGGATGACCCTCACCTT

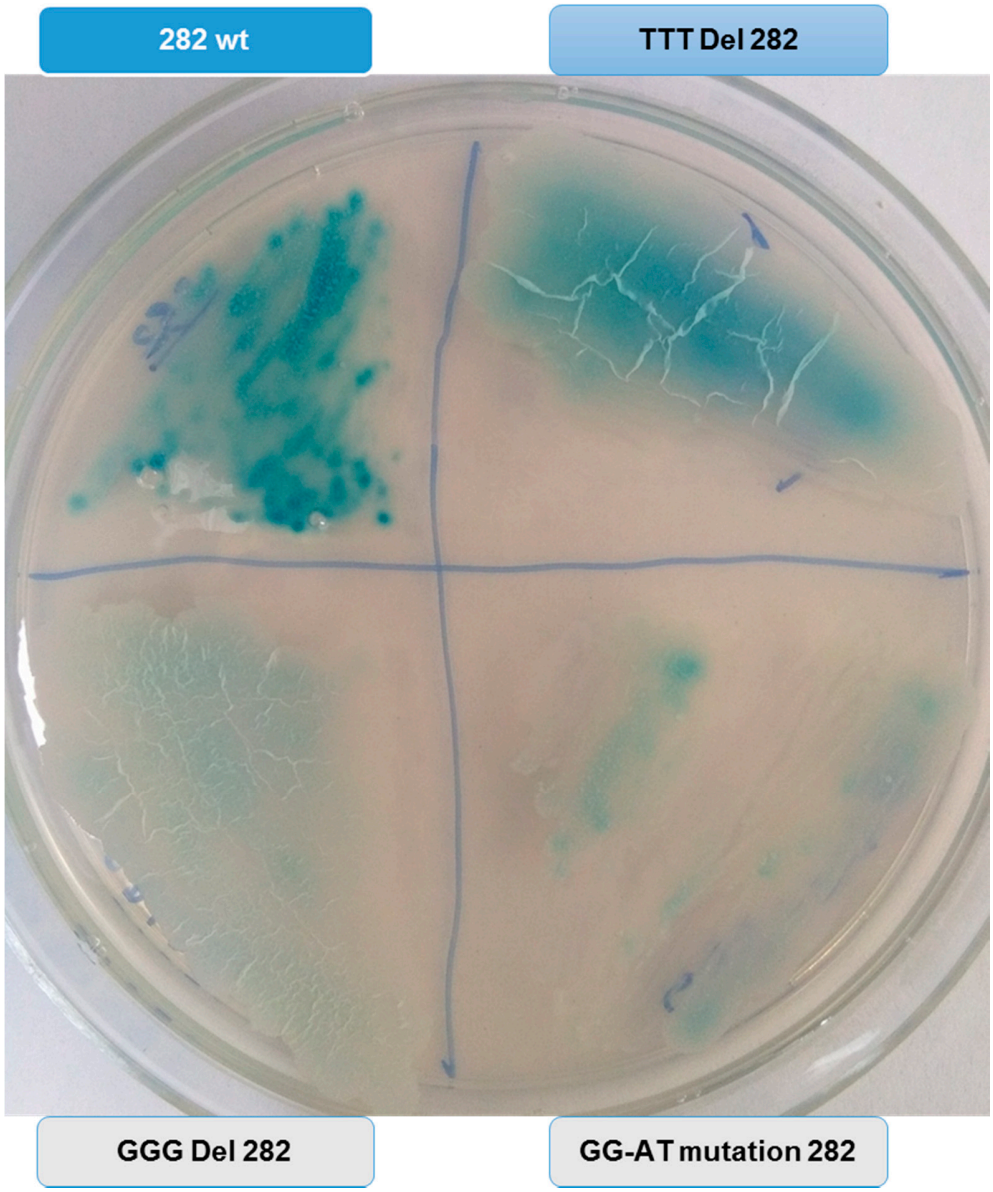
Real time quantitative PCR, 16s rRNA gene specific primer	Reverse primer for cDNA synthesis	CCGTATCTCAGTCCCAGTGT
16s rRNA (*rrs*) gene specific primer pair for quantitative PCR	Forward Primer	GGGATGCATGTCTTGTGGTG
	Reverse Primer	CCGTCGTCGCCTTGGTAG
Primer for cDNA synthesis for cleaved RNA fragment, eluted from denaturing PAGE	Reverse Primer	GACGATTCCGCCCTTGATG
Adapter and Primer for 118 nt fragment sequencing	5’ Adapter	5'GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCUUUGAUGAAA-3'
	5’ Adapter primer (will be complementary after first strand cDNA synthesis)	5'-GCTGATGGCGATGAATGAACACTG-3'



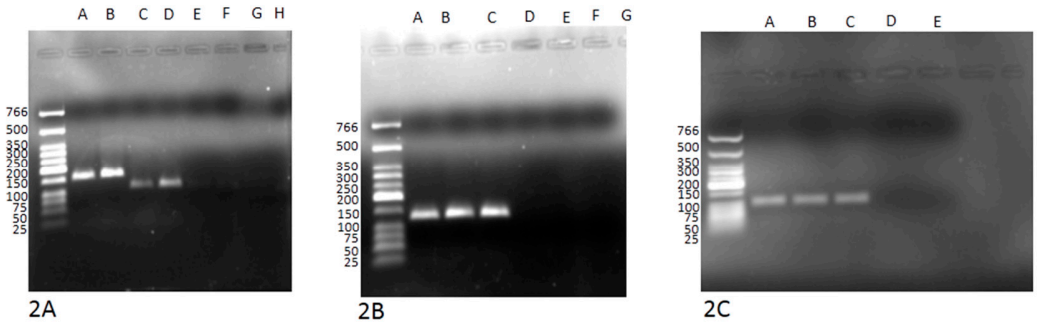
Supplementary Figure 1. (A) Secondary structure prediction of the specific region of ppgk gene. Probability of secondary structure formation is given from 0 to 1 (blue to red). Formation of SD sequestering domain has shown highest probability. This domain has also been predicted by other RNA folding webservers including Kinefold, mFold and Mathews Lab Webserver. (B) Three dimensional structure also exhibit the SD sequestering domain formation (blue arrow) that was simulated and predicted by simRNA webserver, resultant PDB file visualized by Jena 3D viewer.



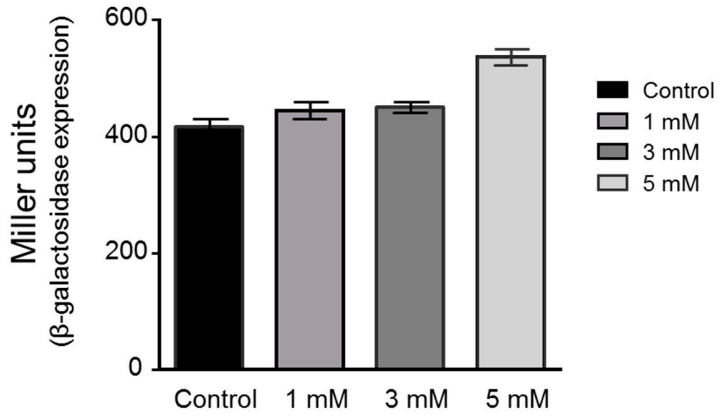
Supplementary Figure 2: PCR product was synthesized by using phusion DNA polymerase kit (GC buffer), that was optimized with addition of 1.4 µl DMSO, 1.4 µl formamide and 1.2 µl $MgCl_2$ in a total 50 µl reaction volume. The annealing temperature was set at 65.7^{o}C. 400 ng of PCR product was electrophoresed by native PAGE, and gel was silver stained.



Supplementary Figure 3. Effect of wild type 282-*ppgk* element and it's mutants on the β-galactosidase expression, constructs were transformed in *M. smegmatis* mc^2155 and streaked on 7H10 agar plates supplemented 20mg/ml X-gal. Plate was incubated at 37^oC for 30 hours and image was taken.

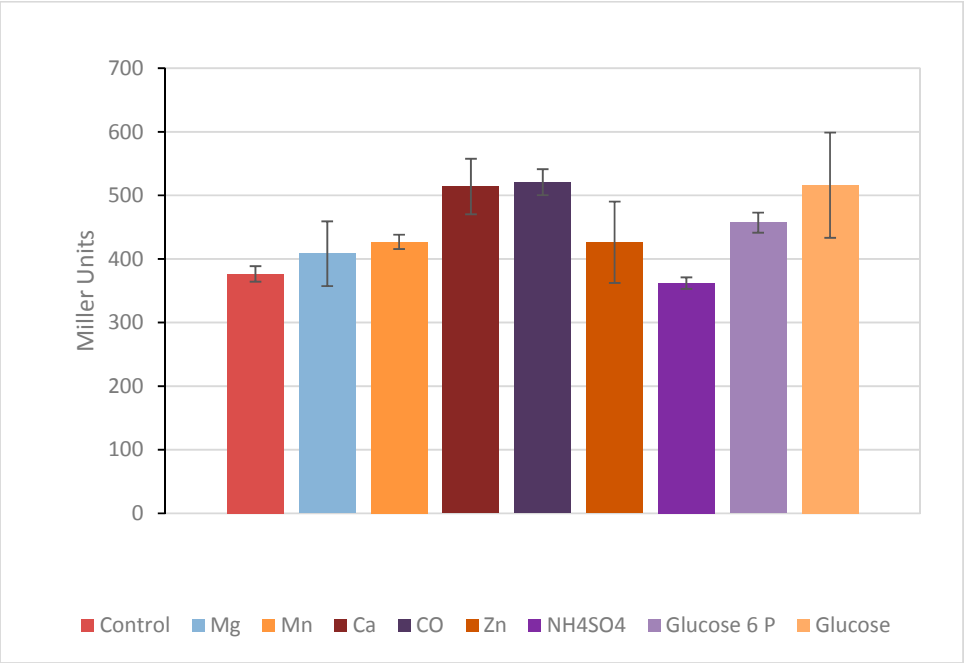


Supplementary Figure 4. Agarose gel electrophoresis of real time quantitative PCR products. 5µl of each sample analyzed and confirmed for the single product (along with specific single melting peak) of respective size. Gel image 2A, shows the separation of 152bp (lanes A and B) and 102bp real time PCR products (lanes C and D), quantified by primer pair C1 and primer pair FL respectively. Lanes E and F shows no specific amplification (Cq value~34) from primer controls (no template), and lanes G and H shows the No RT control from both primer pairs (Cq value~32). Gel image 2B shows the separation of 129bp real time PCR product quantified by primer pair C2. No RT and primer control samples are loaded in Lanes D and E respectively. Gel image 2C shows the separation of 96bp PCR product quantified (Cq value~14) from the 16srRNA reference gene.

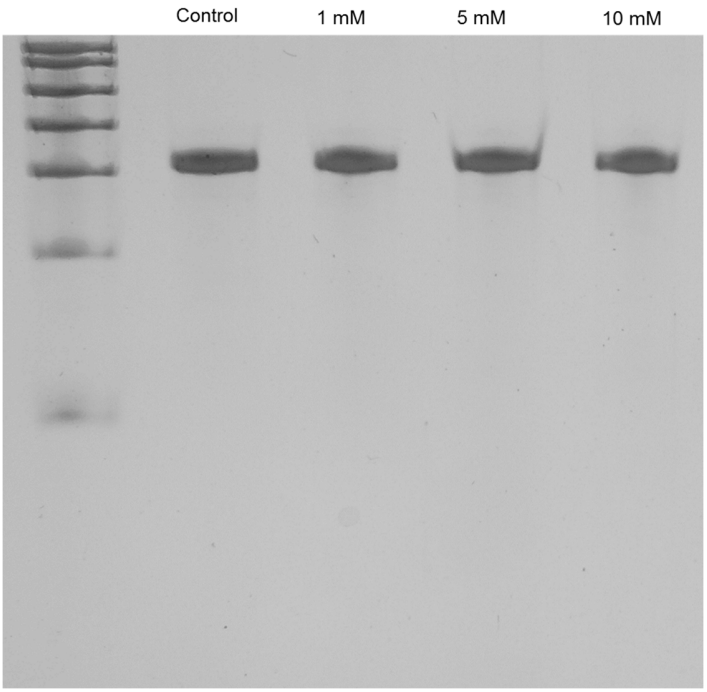


Supplementary Figure 5. A, Analysis of β-galactosidase expression with the increasing concentration of MgCl2. Exogenous MgCl2 was added when O.D. reached 0.2, and allow to grow for next ~30 hours (O.D.~0.7-0.8). Expression becomes increased and observed to be maximum at 5mM. Higher concentration of MgCl2 (10mM- 20mM)

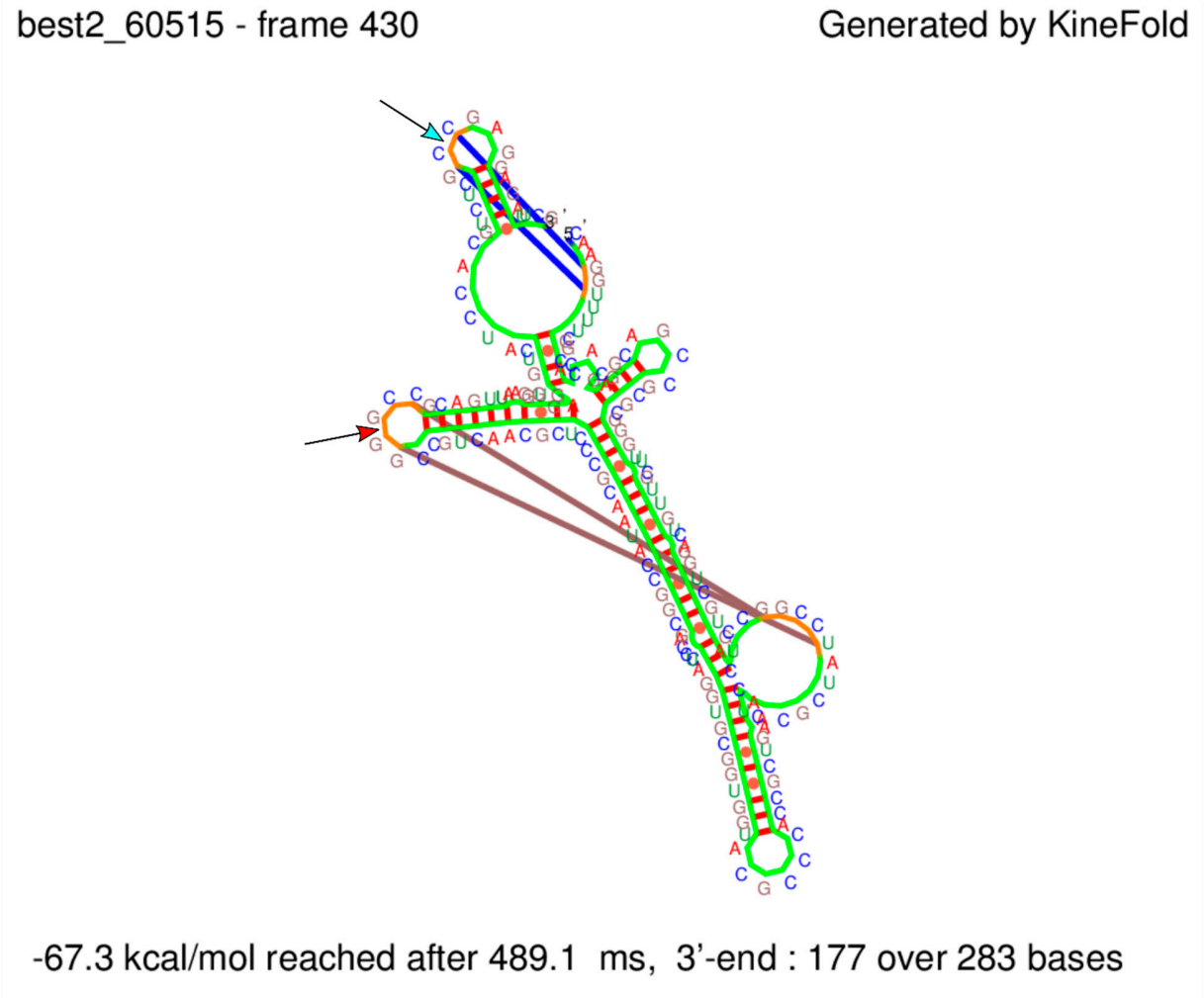
caused the variation in O.D., therefore expression was not measured from these cultures.



Supplementary Figure 6: Analysis of β-galactosidase expression from the constructs (plasmid pML163+282nt insert) grown in Sauton's fluid medium base. Specific metabolite or salts were added at final concentration of 5mM, when $O.D._{600}$ reached at ~0.3, followed by cultures were incubated further for 24 hours. *M. smegmatis* cultures were sonicated and processed for β-galactosidase measurement as described in Methods section. As data indicates there is no significant difference of LacZ expression between control and experimental samples.



Supplementary Figure 7. Incubation of PAGE purified 282-*ppgk* RNA element at different concentrations of Mg^{2+} ions. No specific cleaved fragment was observed after incubation at 37^{o}C for 45 minutes.



Supplementary Figure 8. RNA Structure of 177 bases over 282 bases that is generated by kinefold webserver. Pseudoknot formation is displayed by straight lines. Predicted pseudoknots highlighting the significance of these structures during co-transcriptional folding and processing at these sites.