Supplementary Table 1: List of Primers and plasmids used in this study.

Supplementary Table 2: Percent of active RNAP from each preparation as determined from primer extension (Figure 5B). Percent activity was calculated by dividing the sum of density for the 7-mer and 9-mer by the total density in the lane for each RNAP. Values are averages derived from three independent measurements.

Supplementary Figure 1: Sequence of source DNA template used in the study. Sequence is of native H37Rv genomic DNA (+1468189 to +1468605) -66 to +351 of the transcription start site of the *rrnA3* promoter. The -10 and -35 elements are highlighted and the transcription start site is in green. Sites of mutagenesis are also highlighted. Endogenous MscI restriction site used for pEC26 plasmid construction is not highlighted.

Supplementary Figure 2: SDS-PAGE of all RNAPs (left panel) and trans-acting transcription factors (right panel) used in this study.

Supplementary Figure 3: A) Phosphorimages of polyacrylamide gels used to determine open-complex stability for each RNAP in the presence and absence of CarD**. B)** Plots showing the decay of the open-promoter complex over time for each of the RNAPs in this study. All experiments are performed in triplicate.

Supplementary Figure 4: Phosphorimages of polyacrylamide gels of elongating MTB RNAPs. Time points for the βS450L mutant** (min): 2, 3, 4, 5, 7.5, 10, 12.5, 15, 20, 30, 45. Time points for all other RNAPs (min): 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 5, 15.

Supplementary Figure 5: Phosphorimages of polyacrylamide gels used to determine hydrolysis of RNA primer. **A)** RNA•DNA scaffold used in this study. **B)** Radiograph showing primer extension and hydrolysis of RNA8 primer.

Supplementary Figure 6: Phosphorimages of polyacrylamide gels used to determine open-complex stability for each RNAP in the presence and absence of CarD**.**

Supplementary Table 1

Primer Name	Sequence 5'-3'
Mt rrnA3 -66 AMP FOR	GATC**ACATGT**ATGGATATCTATGGATGACCGAACCTGG
Mt rrnA3 +351 AMP REV	GATC**GAATTC**CCAGTTTCCCAGGCTTATCCCGAAGTGC
SynB Terminator FOR	GACT**TCTAGA**AGAAAAAAAAAAGCGCCGCAACTGCGGCGCTTTTTTTTTT CAGGTATCTA**TCTAGA**GACT
SynB Terminator REV	AGTC**TCTAGA**TAGATACCTGAAAAAAAAAAGCGCCGCAGTTGCGGCGCT TTTTTTTTTCT**TCTAGA**AGTC
EC26 DNA Fragment FOR	G**ACATGT**AAATATTTGTTGTTAACTCTTGACAAAAGTGTTAAAAGCGGCTA GTATTTAAAGGGATGGATGAGATTTGAAGGTTGGGTCCCA**TGGCCA**GAT
EC26 DNA Fragment REV	ATC**TGGCCA**TGGGACCCAACCTTCAAATCTCATCCATCCCTTTAAATACT AGCCGCTTTTAACACTTTTGTCAAGAGTTAACAACAAATATTT**ACATGT**C
EC19 AMP FOR	ATGGATATCTATGGATGACCGAACCTGG
EC26 AMP FOR	TTGTTGTTAACTCTTGACAAAAGTGTTAAAAGCGG
EC AMP REV	GCCTGCAGGTCGACTCTAGAGG
rrnA3 EC19 FOR	GACTGGCAGGGTTGCCCCGTTGCGGGC0GG
rrnA3 EC19 REV	CCGCCCGCAACGGGGCAACCCTGCCAGTC
CarD AMP FOR	GGGAATTC**CATATG**ATTTTCAAGGTCGGAGACACCGTTGTC
CarD AMP REV	GATCCC**GGATCC**TCAAGACGCGGCGGCTAAAACCTCGTCAAG
NusA AMP FOR	CCGG**CATATG**AACATCGACATGGCTGCTCTGCATGCC
NusA AMP REV	ACGC**GGATCC**TTAGCGGTCGTGCGCCATACCGC
NusG AMP FOR	GCGC**CATATG**GTGACTACCTTCGACGGT
NusG AMP REV	GCGC**CTCGAG**CTAGATCTTGGAGACTTG

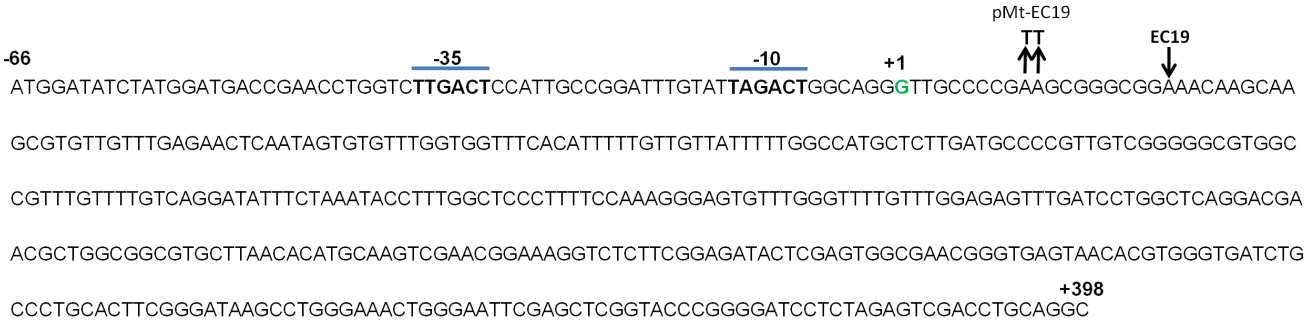
- Restriction sites are in bold and mutagenesis sequences are underlined

Plasmid	Purpose
pET19bpps-CarD	Expression of MTB CarD
pET19bpps-NusA	Expression of MTB NusA
pET19bpps-NusG	Expression of MTB NusG
pMt-rrnA3	Template manipulation vector for assays
pMt-rrnA3-synB	Open-promoter half-life determination
pEC19	Amplification of EC19 template
pEC26-Tuf	Amplification of Tuf terminator containing template
pEC26-MetK	Amplification of MetK terminator containing template

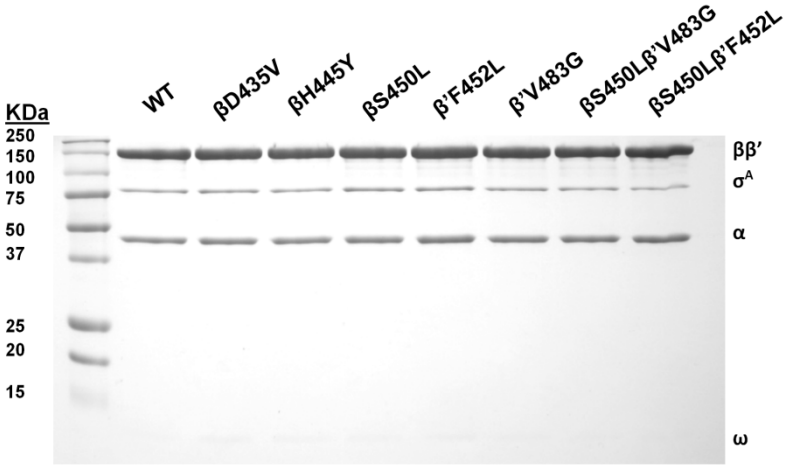
Supplementary Table 2

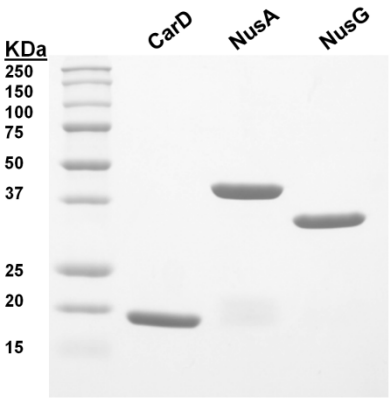
	% Active RNAP
WT	55
βD435V	73
βH445Y	49
βS450L	53
β'F452L	67
β'V483G	64
βS450Lβ'F452L	47
βS450Lβ'V483G	47

Supplementary Figure 1

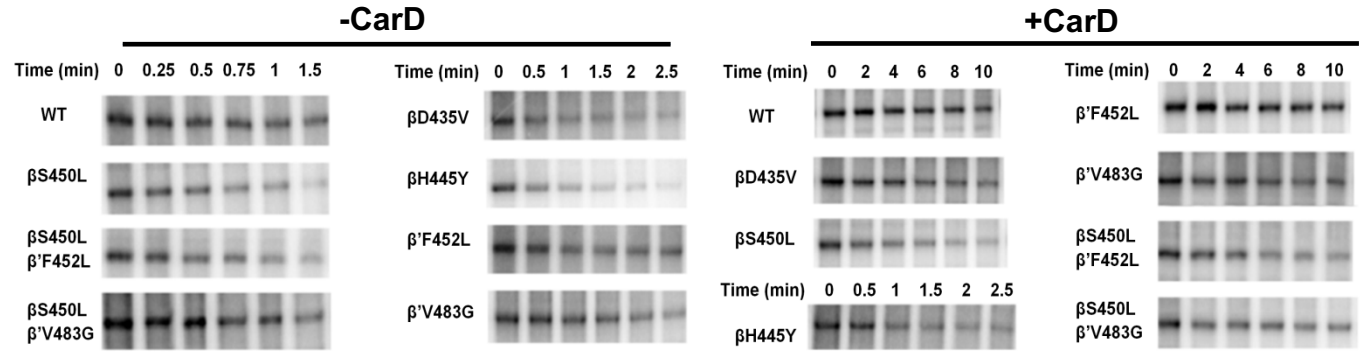


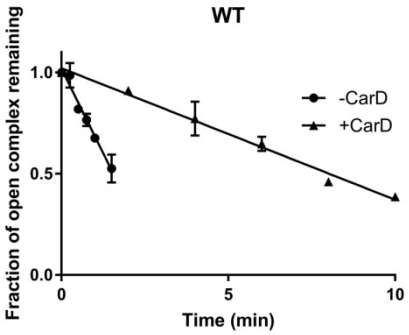
Supplementary Figure 2

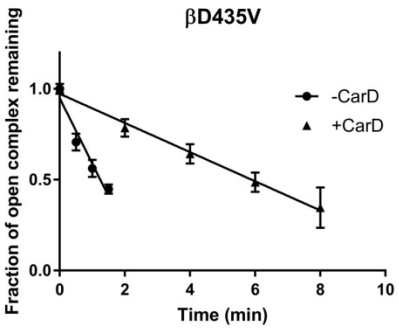


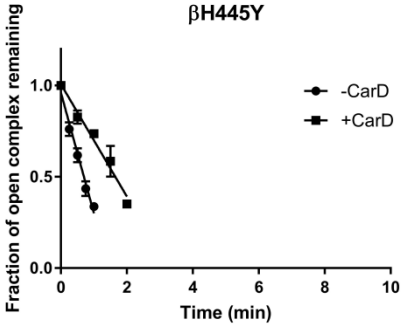


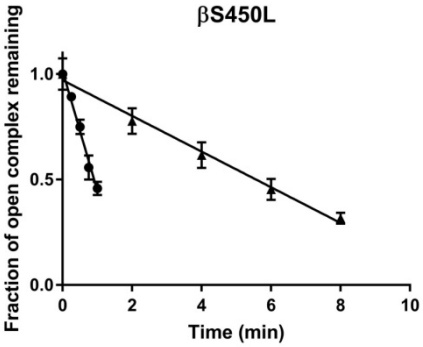
Supplementary Figure 3

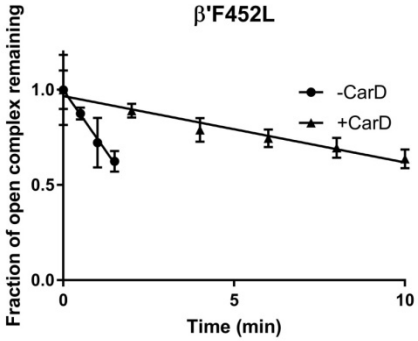


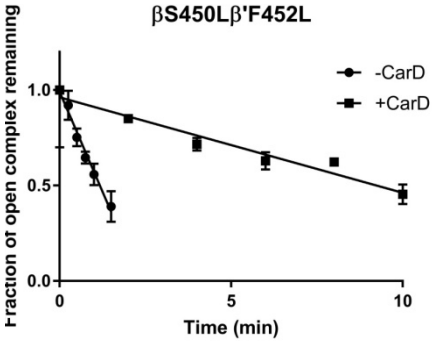


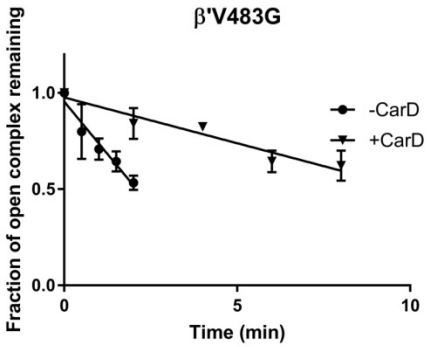


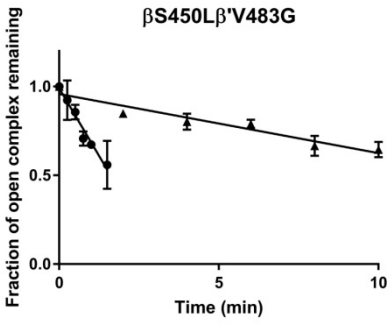




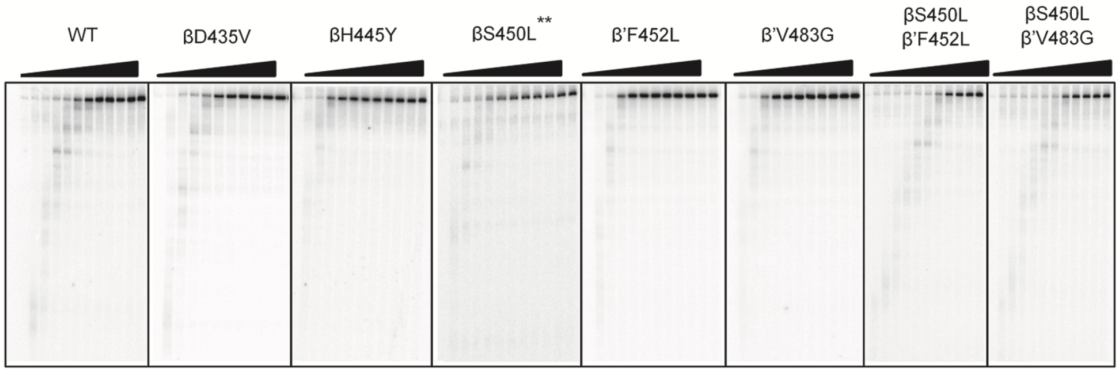




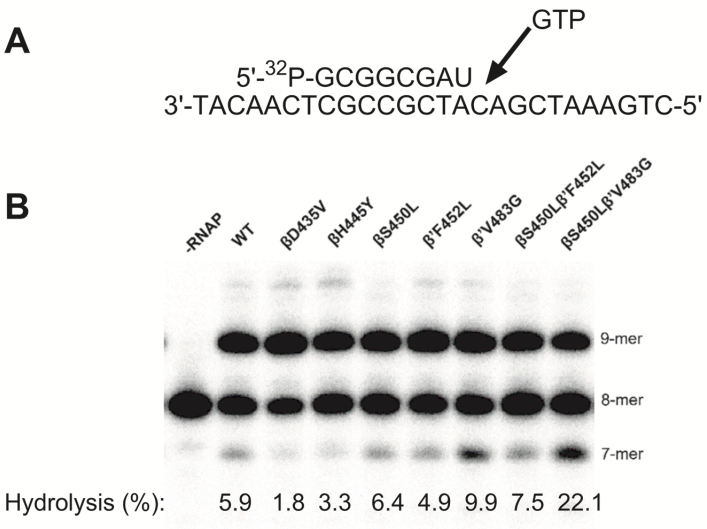




Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6