Auto-inhibitory regulation of DNA binding by the C-terminal tails of the mitochondrial transcription factors Mtf1 and TFB2M

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Supplementary Information

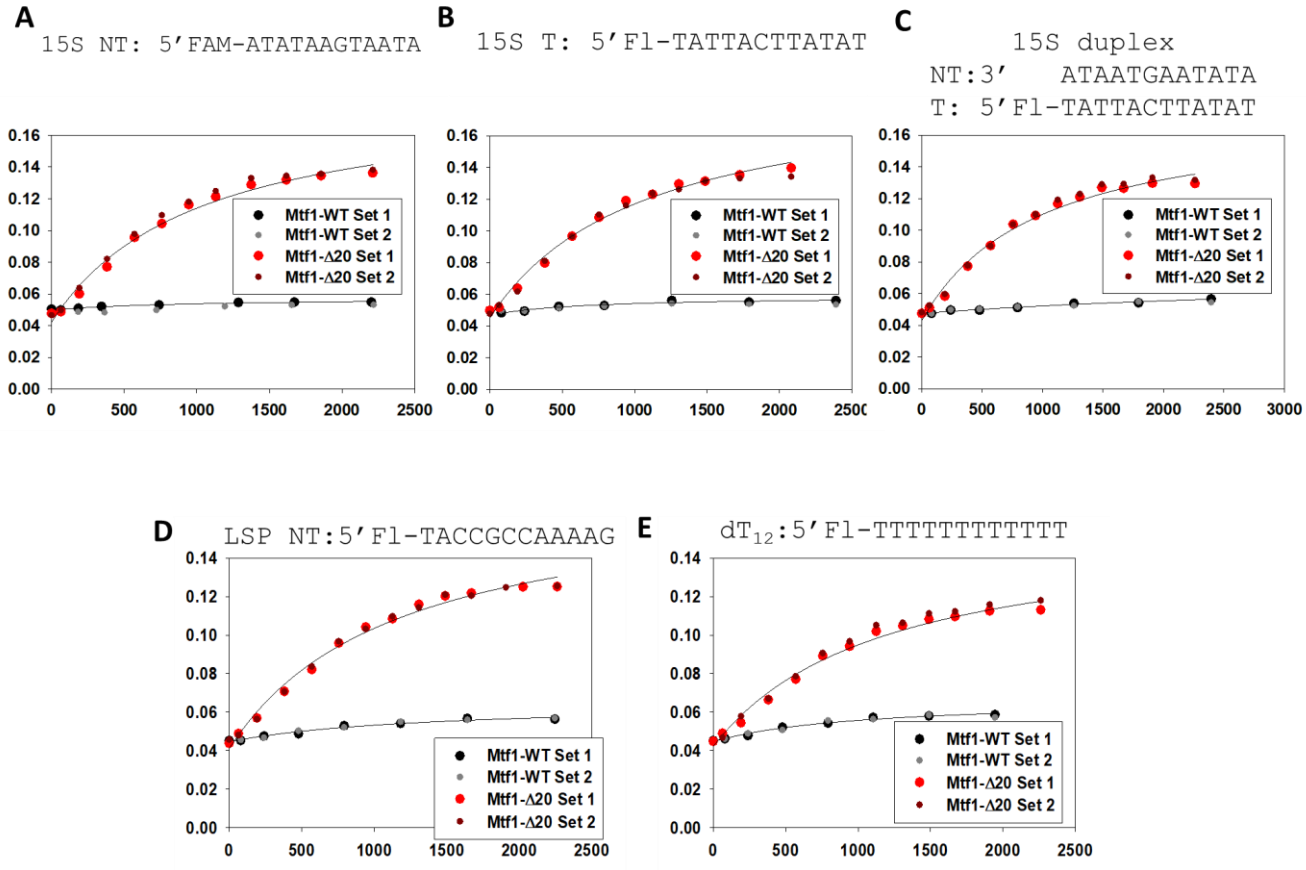

Fig S1: Titration of DNA substrates with Mtf1 proteins. DNA substrates (5 nM) were titrated with Mtf1-WT (black) or Mtf1-Δ20 (red). Repeats are shown in dark gray circles (Mtf1-WT repeats) or dark red circles (Mtf1-Δ20 repeats). The data were fit to a hyperbola (solid line) to obtain the K_d values shown in Fig. 1F. The various substrates tested are shown: *15S* NT (A), *15S* T (B), *15S* duplex (C), LSP NT (D) and $(dT)_{12}$ (E).

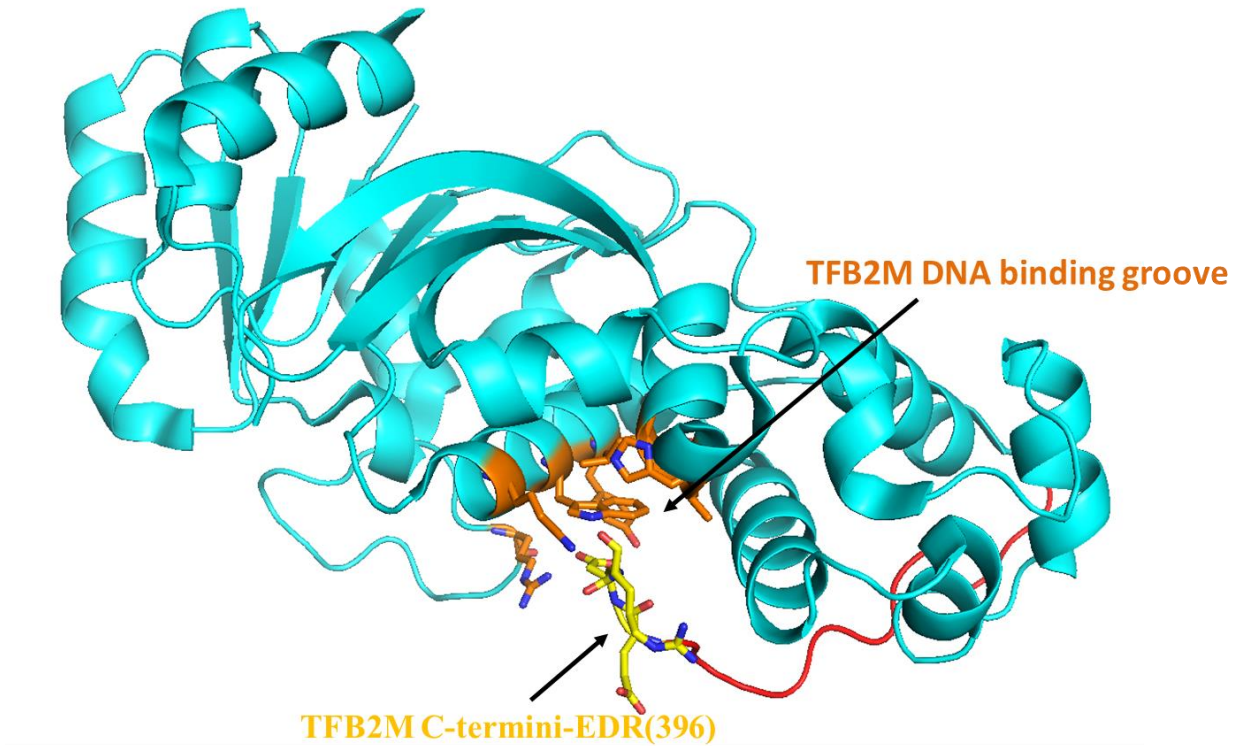

Fig. S2. Intramolecular interactions of the TFB2M C-tail with the DNA binding groove. TFB2M structure (6ero, chain A) is shown in cyan with some of the DNA binding groove residues in orange. The C-tail is shown in red and the C-tail termini residues interacting with the DNA binding groove are shown in yellow.

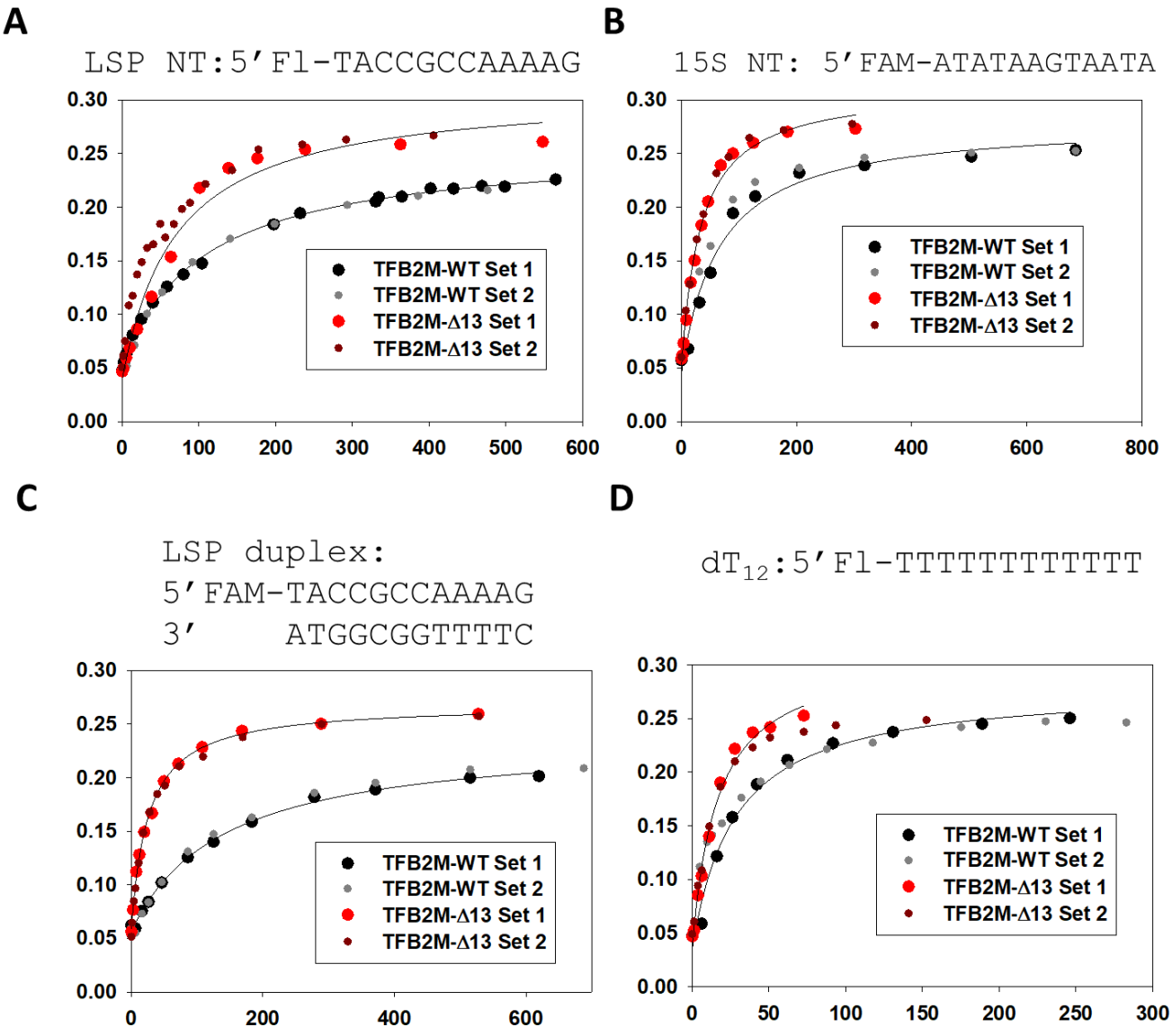

Fig S3: Titration of DNA substrates with TFB2M. DNA substrates (5 nM) were titrated with TFB2M-WT (black) or TFB2M-Δ13 (red). Repeats are shown in dark gray circles (TFB2M-WT repeats) or dark red circles (TFB2M-Δ13 repeats). The data were fit to a hyperbola as shown by the solid lines to obtain the K_d values. The various substrates tested are shown: LSP NT (A), *15S* NT (B), LSP duplex (C) and $(dT)_{12}$ (D).

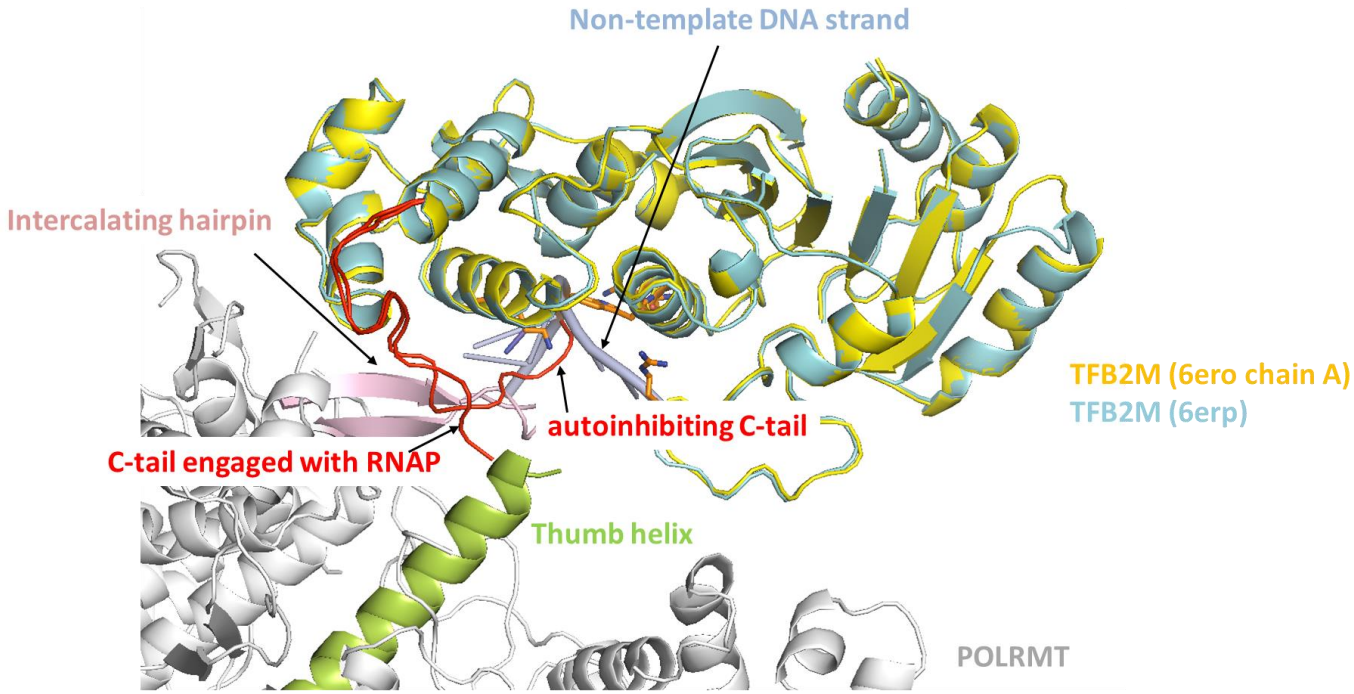

Fig S4. Structural comparison of the C-tail conformation in free TFB2M and in the presence of POLRMT in the initiation complex. TFB2M (chain A in 6ero) is aligned with TFB2M (6erp) in the initiation structure. The autoinhibited C-tail is clashing with the non-template DNA strand in the DNA binding groove of TFB2M in the initiation structure. The C-tail of TFB2M is relocated in the initiation structure and interacting with the intercalating hairpin and thumb helix of POLRMT relieving autoinhibition.

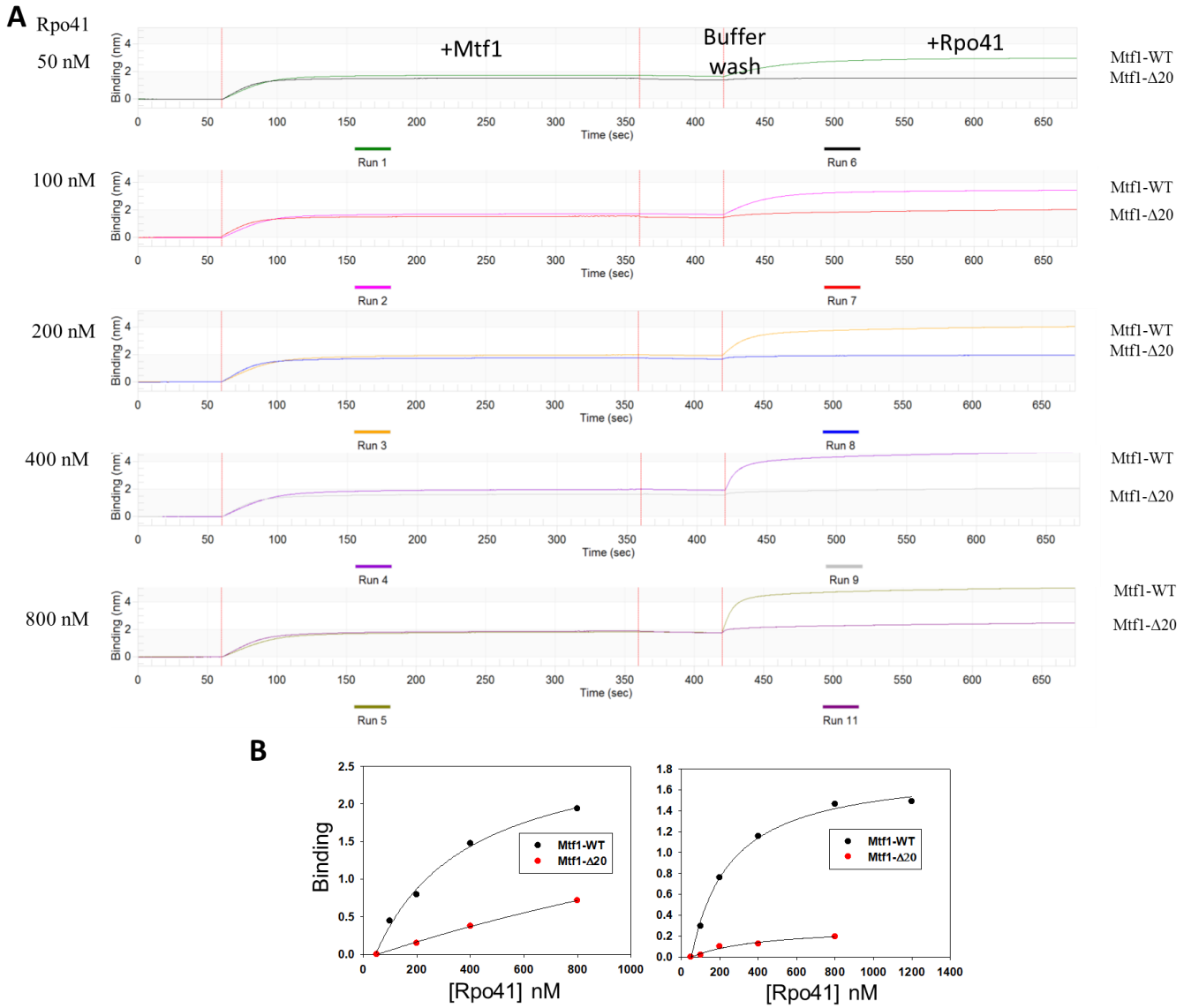

Fig. S5: **Bio-layer Interferometry assays using BLItz instrument (ForteBio) showing increased binding in Mtf1-WT compared to Mtf1-Δ20 mutant.** (A) HIS1K probes were soaked in water overnight and yeast transcription buffer for 10 min, then equilibrated with Buffer A for 60 s. His-tagged Mtf1 or Mtf1-Δ20 (0.4 µM) was loaded on the probes for 300 s followed by washing with buffer for 60 s. The probes were then dipped in various concentrations of Rpo41 for 300 s. The resulting binding curves are shown in the different panels. (B) The amplitudes of change upon addition of various concentrations of Rpo41 are shown for the two repeats.

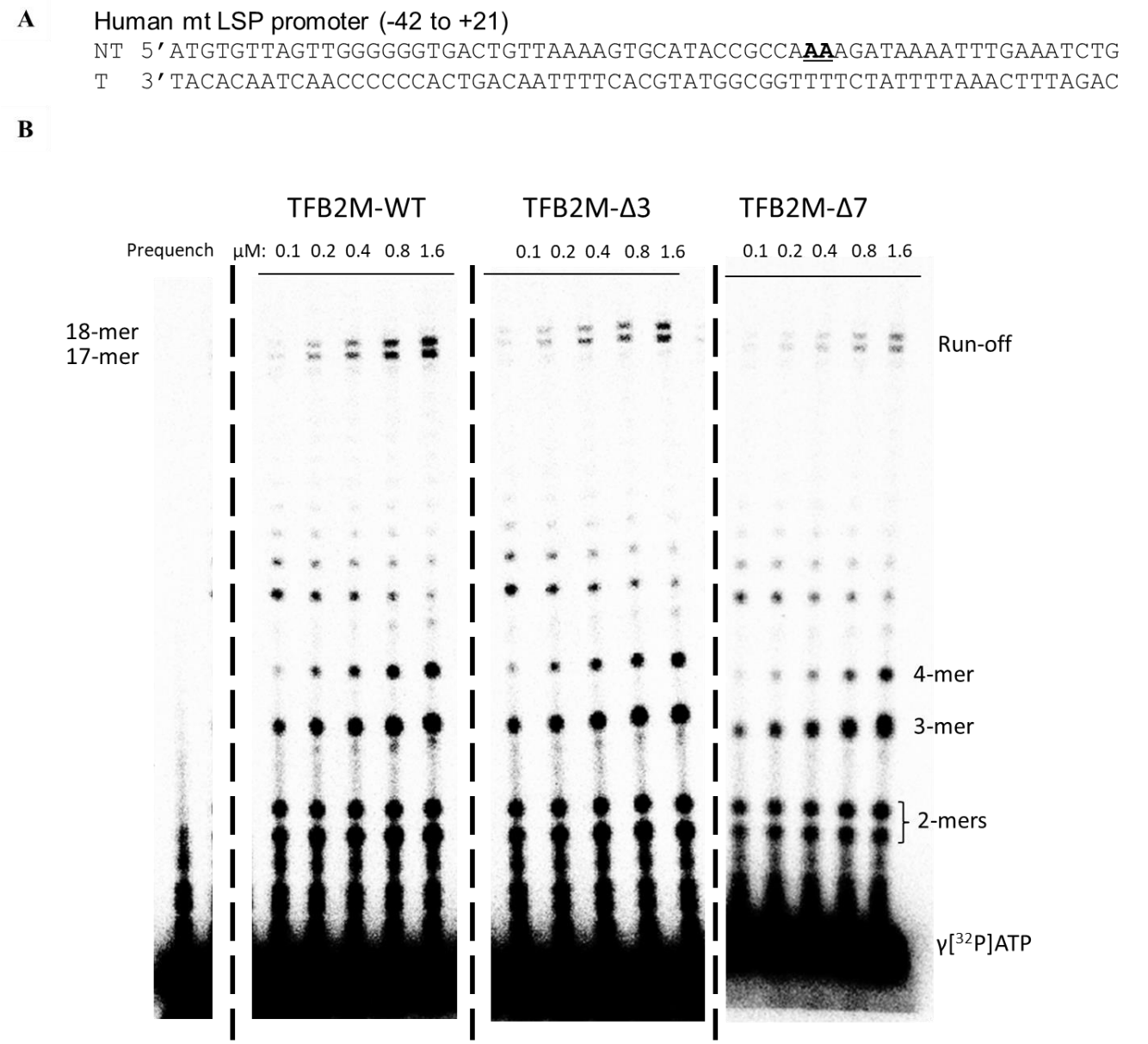

Fig S6: The C-tail deletion in TFB2M affects initiation complex formation as seen from the transcription runoff synthesis. (A) The promoter fragment LSP used in the study is shown. (B) Full transcription profiles of TFB2M-WT and C-tail deletion mutants TFB2M-Δ3 and TFB2M-Δ7 on the LSP promoter are shown. Reactions were carried out with 0.6 μM each of POLRMT, TFAM and promoter duplex and increasing concentration of TFB2M-WT or C-tail mutant and 250 μM ATP, UTP, GTP for LSP and γ[^{32}P]ATP for 15 min at 25^{o}C in the human transcription buffer.