Supplemental Data

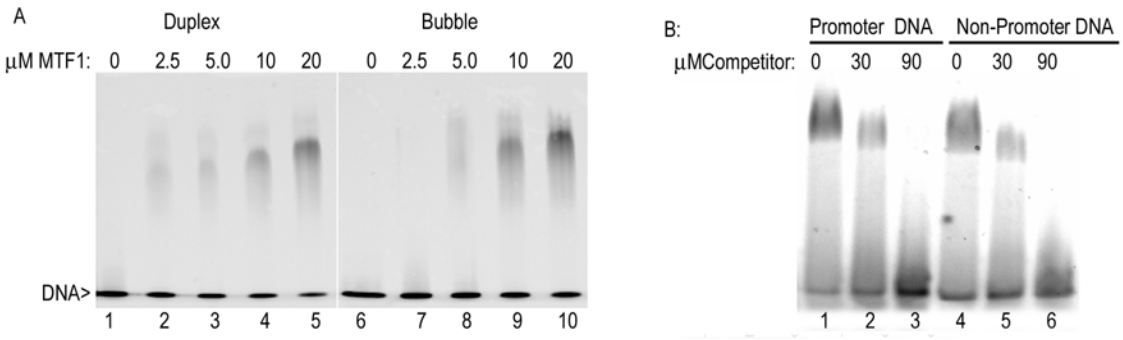

Fig. S1. *MTF1 alone has weak, non-specific DNA binding activity*. A: EMSA experiments carried out with 1 μM fluorescently labeled DNA and the indicated concentrations of MTF1 reveal formation of complex migrating as a diffuse band with an apparent K_d of ~10-20 μM with either a 33 bp duplex (lanes 1-5) or pre-melted ('Bubble') promoter (lanes 6-10). B. EMSA experiments carried out with 10 μM MTF1 and 1 μM fluorescently labeled 33 bp pre-melted promoter and with the indicated μM concentrations of either unlabeled bubble promoter (lanes 2, 3) or non-promoter DNA (lanes 5, 6) show that promoter or non-promoter DNA are equally effective at competing for MTF1 binding to promoter.

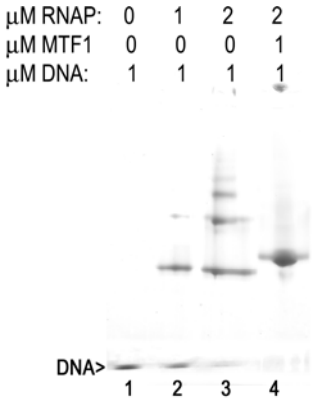

Fig. S2. *Excess MtRNAP alone forms heterogeneous complexes with a pre-melted promoter*. EMSA experiments carried out with the indicated mM concentrations of MtRNAP, fluorescently labeled pre-melted promoter DNA and MTF1 show that, when MtRNAP is in molar excess of DNA, multiple shifted bands are observed (lane 3). Addition of MTF1 results in a single major species (lane 4) which is super-shifted relative to the most rapidly migrating complex in the reactions without MTF1.

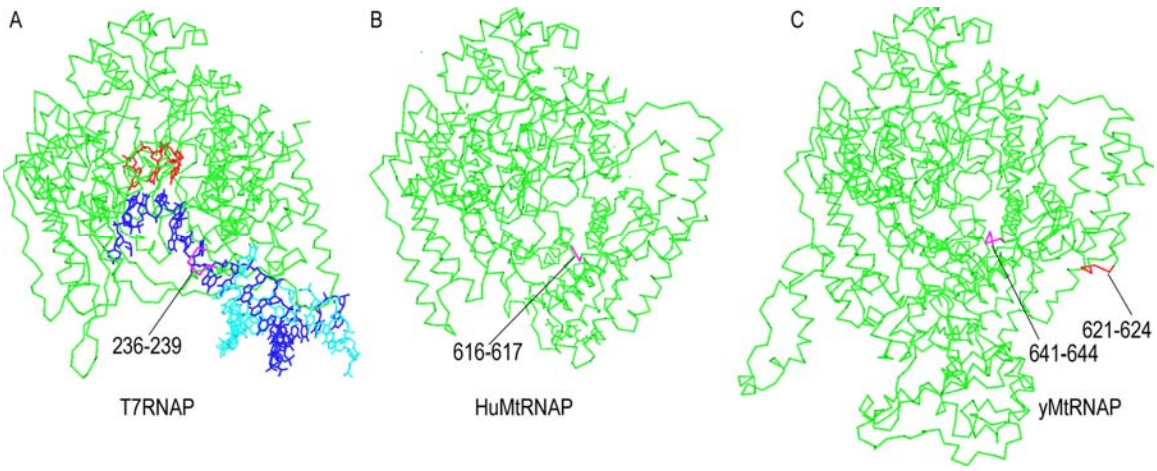

Fig. S3. Sequences identified as functionally important for promoter melting occupy similar positions in 3-D (a-carbon models) structures of T7RNAP (A; 1QLN.pdb), human MtRNAP (B; 3SPA.pdb) and a homology model of yeast MtRNAP based on the human MtRNAP structure (C). The residues aligning with the T7RNAP intercalating hairpin in the 3-D structures are in magenta (for HuMtRNAP only 2 residues are colored because resides 612-615 are disordered and invisible in the crystal structure). In the yeast MtRNAP the residues suggested by an alternate sequence alignment to correspond to the T7 RNAP intercalating hairpin are in red.

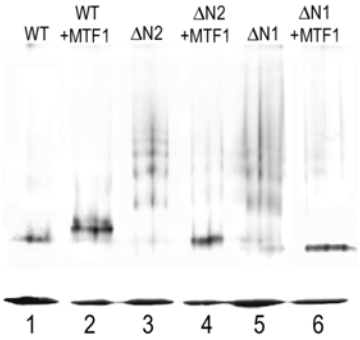

Fig. S4: EMSA with ΔN1 and ΔN2 RNAP ICs shows that they bind MTF1. EMSA experiments run with fluorescently labeled bubble promoters as in fig. S2. The WT RNAP IC runs as a single-band in the absence of MTF1 (lane 1) that supershifts upon MTF1 addition (lane 2). In the absence of MTF1 the ΔN1 and ΔN2 form heterogeneous complexes (lanes 3 and 5), similar to what is seen with the smaller deletions in the putative intercalating hairpin (DNA is in 2-fold excess of RNAP). However, addition of MTF1 results in formation of a predominant single band (lanes 4 and 6), indicating that MTF1 does bind to the ΔN1 and ΔN2 ICs.

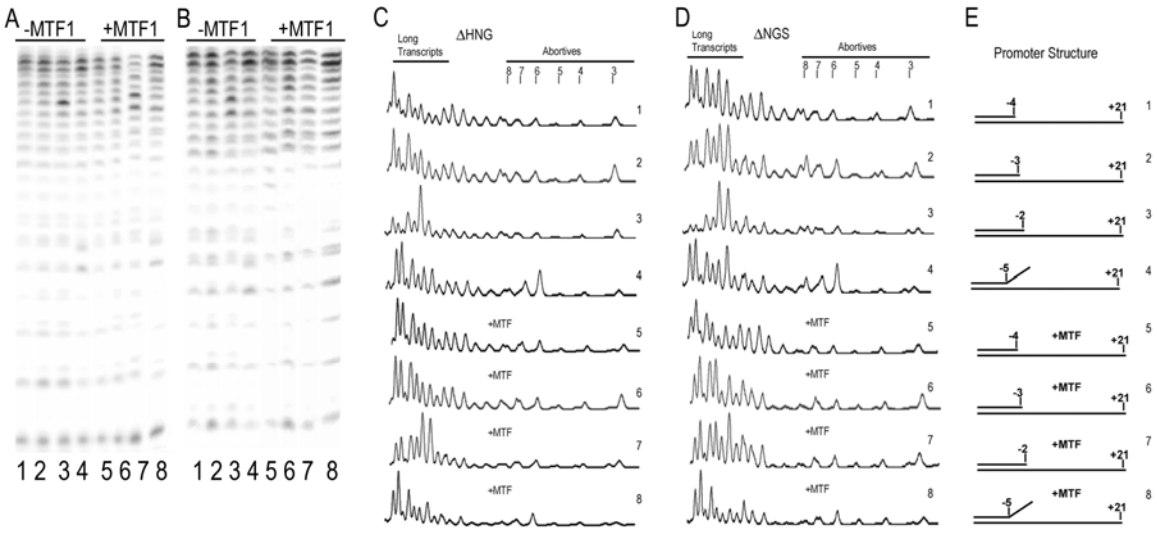

Fig. S5. Effect of MTF1 on deletion mutant transcription from promoters with varying NT strand lengths. A: Transcription by ΔHNG MtRNAP in either the absence (lanes 1-4) or presence (lanes 5-8) of MTF1 and on promoters whose structures are as shown in panel E. B: As in A, but with ΔNGS MtRNAP. C: Scans of lanes 1-8 in panel A. D: Scans of lanes 1-8 in panel B. E. Structure of promoters used in lanes 1-8 of panel A-D.