Supplemental materials.

Methods

Generation of expression constructs.

This process involved using a multi-step PCR process. The first step involved amplification of the gene of interest using forward primers that contained a partial PreScission protease cleavage site followed by the desired h-mtTFA sequence, and reverse primers that contained the 3' AttB1 recombination site. The primers used are described below.

(mtTFA 1+)

5'CGCGGATCCTCATCTGTCTTGGCAAGTTGTCCAAAGAAACC3'

(mtTFA 204- REV)

5'AAAAGGAAAACGCCGGCGTCATTAACACTCCTCAGCACCATATTTTCGTTGTTTCT
TTATTGTGCG3'

(mtTFA 179-)

5'AAAAGGAAAACGCCGGCGTCATCATTGTTCTTCCCAAGACTTCATTTCATTATGAT
AACG3'

(mtTFA 109-)

5'AAAAGGAAAACGCCGGCGTCATCACAGTGTTAACTCTTTTTTTTTTGTCATAGC3'

(mtTFA 79-)

5'AAAAGGAAAACGCCGGCGTCATCATAGCTGTTCTTTAAATCTGCTTATCTC3'

(mtTFA 80+)

5'CGCGGATCCGCAAGTTGTCCAAAGAAACCTGTAAGTTCTTACC3'

(mtTFA 110+)

5'CGCGGATCCCCTGGAAAACCAAAAAGACCTCGTTCAGCTTATAACG3'

Following amplification and purification, a second PCR reaction was utilized using a primer that overlapped with the partial PreScission cleavage site and could be used for amplification of all of the gene constructs. In addition, the primer also contained the AttB1 recombination site at the 5' end. This primer is described below.

(UNIVERSAL PRIMER)

5'GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCTGGAAGTTCTGTTCCAGGGGCCC CT3'.

Following the second PCR reaction a product was generated that contained the 5' AttB1 recombination site, a PreScission protease cleavage site, the gene of interest and the 3' AttB1 recombination site. This fragment was purified and inserted into a pDONR vector (Invitrogen) that contained Zeocin resistance using a BP Clonase (Invitrogen) reaction.

Analytical Ultracentrifugation

The sample for Box B ($mtTFA^{110-179}$) of h-mtTFA was prepared and analyzed in the exact same method for the full-length protein with the exception of corrected sedimentation coefficients of Vbar = 0.738 and Rho = 0.9983 calculated from SEDNTRP.

Supplementary Figure Legends.

Figure S1. C-terminal tail of mtTFA is important for the sequence-specificity of human mtTFA. A) Competitive EMSA using LSP DNA. 75 nM mtTFA or $mtTFA^{1-179}$ were bound to 20 nM ^{32}P-radiolabeled 30 bp fragment of the LSP in the presence of varied amounts of an unlabeled 30 bp fragment of the LSP (LSP DNA). The concentrations of the competitor DNA were 0, 10 nM, 100 nM, 300 nM 600 nM, 1000 nM and 10 μM, respectively. **B)** Competitive EMSA using non-specific DNA. 75 nM mtTFA or $mtTFA^{1-179}$ were bound to 20 nM radiolabeled 30 bp fragment of the LSP in the presence of varied amounts of an unlabeled 30 bp scrambled non-specific DNA (NS DNA). The concentrations of the competitor DNA were 0, 10 nM, 100 nM, 300 nM 600 nM, 1000 nM and 10 μM, respectively.

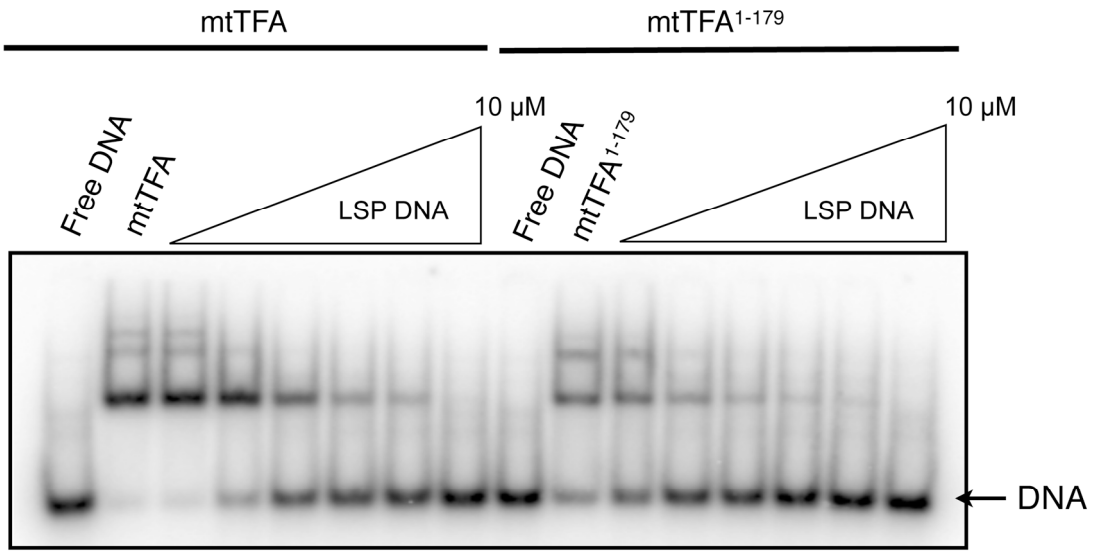
Figure S2. Human mtTFA box B competes weakly with full-length mtTFA for DNA binding. Competitive EMSA experiment of mtTFA box B and full-length mtTFA. All lanes have 20 nM radiolabeled 30 bp fragment of the LSP. Lane 2 includes 40 nM of mtTFA box B. Lane 3 includes 40 nM of mtTFA alone. Lanes 4-6 include 40 nM mtTFA with increasing concentrations of box B at 40 nM, 400 nM and 4 μM.

Figure S3. Crystal structure of mtTFA box B shows dimerization through helix α3. Crystal structure contacts of box B suggest mechanism of dimerization between molecules. The mechanism occurs through an anti-parallel interaction between helix α3 with key interactions occurring between Lys^{163}, Glu^{166} and His^{170}. The figure was made using PYMOL.

Figure S4. Box B of human mtTFA does not dimerize independently. A) Sedimentation velocity profiles for the raw data acquired at different time points and the residuals after fittings had been performed using SEDFIT in 50 mM HEPES-Na pH 7.0, 150 mM NaCl, 1 mM EDTA, and 1 mM DTT. **B)** Calculated sedimentation coefficient distributions for box B of human mtTFA (amino acids 110-179).

Figure S1.

A.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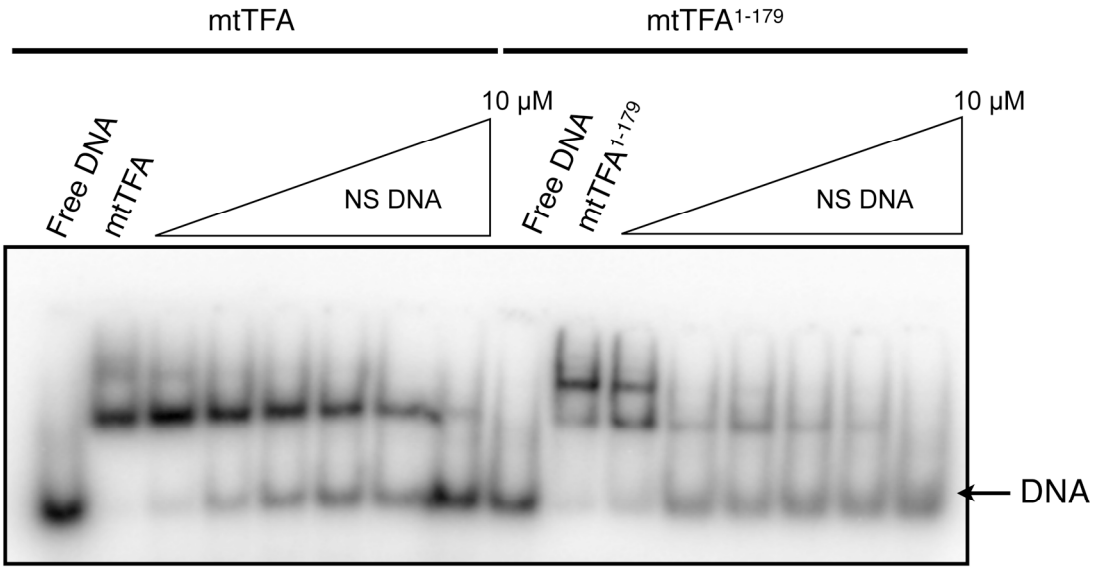
Free DNA
mtTFA
10 μM
LSP DNA
Free DNA
mtTFA1-179
10 μM
LSP DNA
DNA

B.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mtTFA1-179
Free DNA
mtTFA
10 μM
NS DNA
Free DNA
mtTFA1-179
10 μM
NS DNA
DNA

Figure S2.

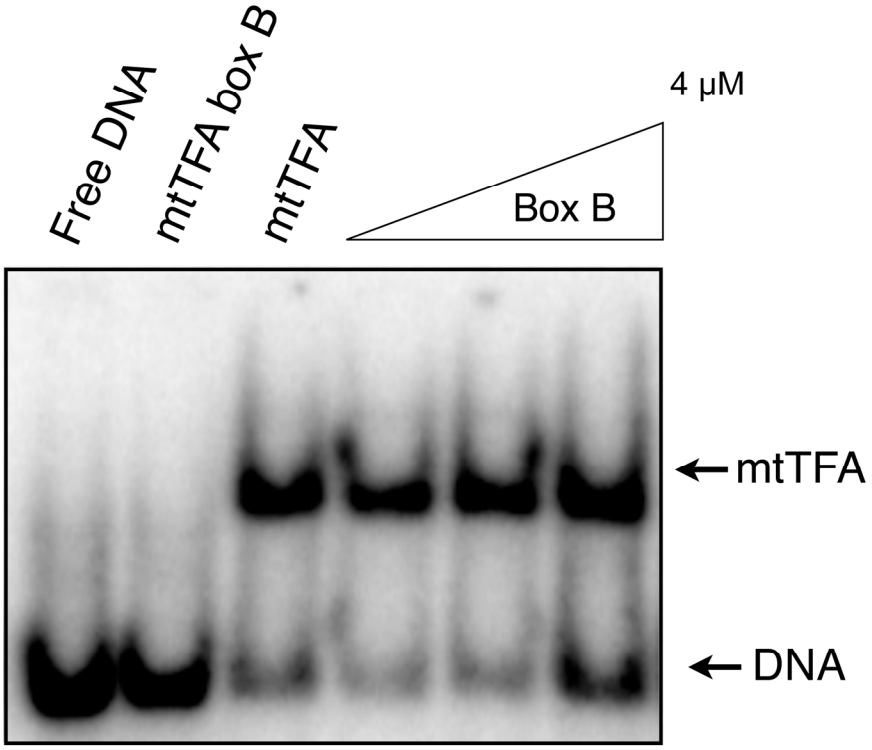


Figure S3.

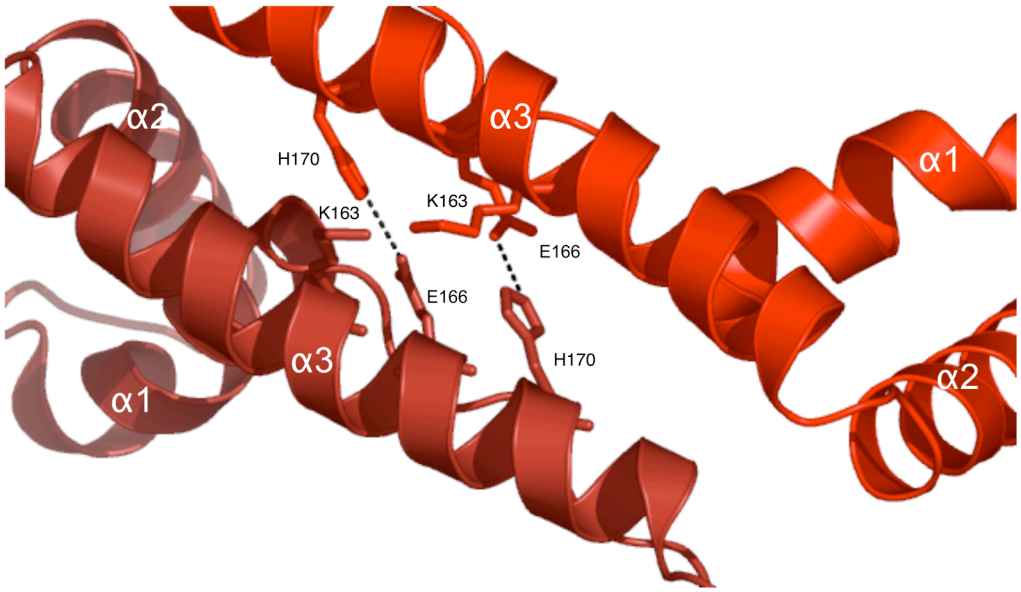
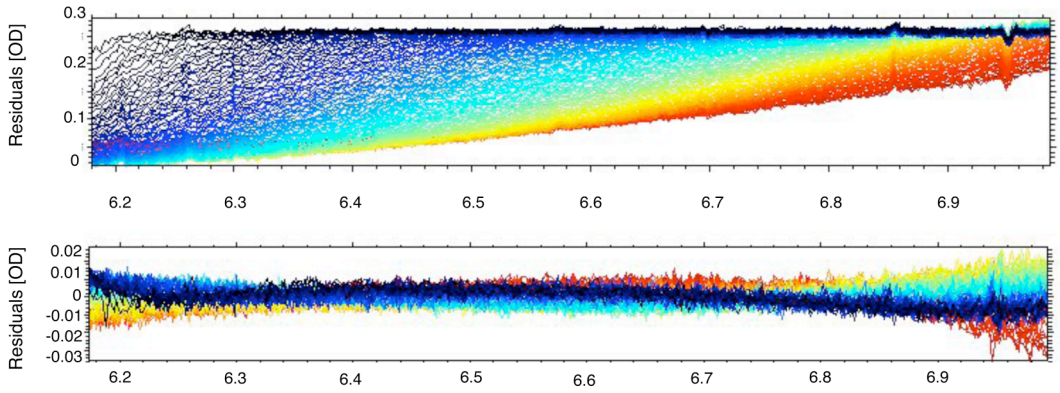


Figure S4.

A.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6.7
6.8
6.9
Residuals [OD]
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0.01
0
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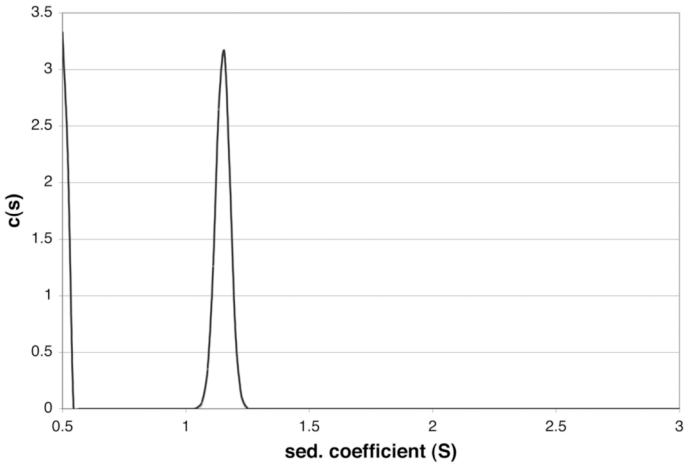
-0.03
6.2
6.3
6.4
6.5
6.6
6.7
6.8
6.9

B.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2
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1
0.5
0
0.5
1
1.5
2
2.5
3
sed. coefficient (S)