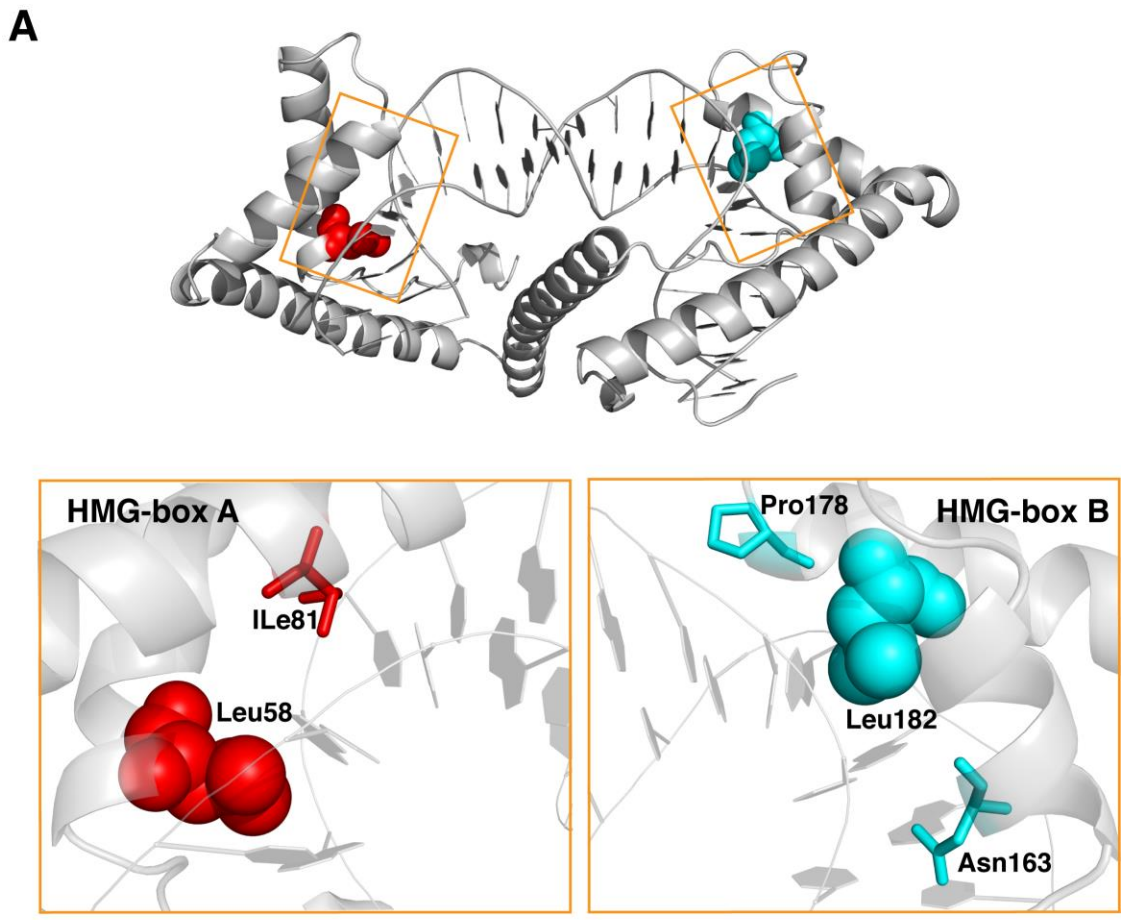
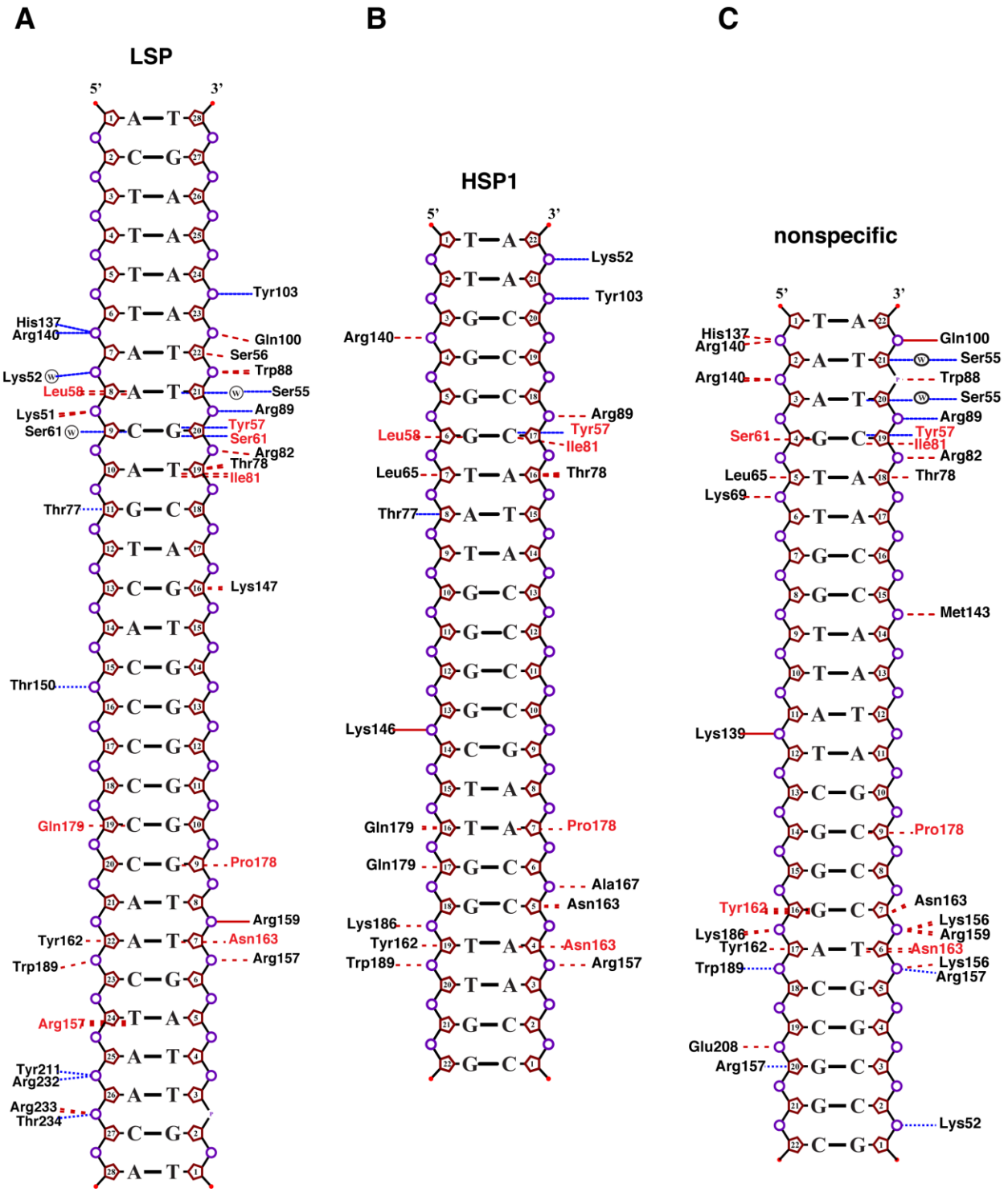
SUPPLEMENTARY INFORMATION

Supplementary Figures 1-8



Supplementary Figure 1. TFAM residues contacting the DNA minor groove
(A) TFAM contacts on nonspecific DNA. Leu58, Ile81, Asn163, Pro178, and Leu182 are shown binding to the DNA minor groove. (B) Buried surface area of the dimerization interface.

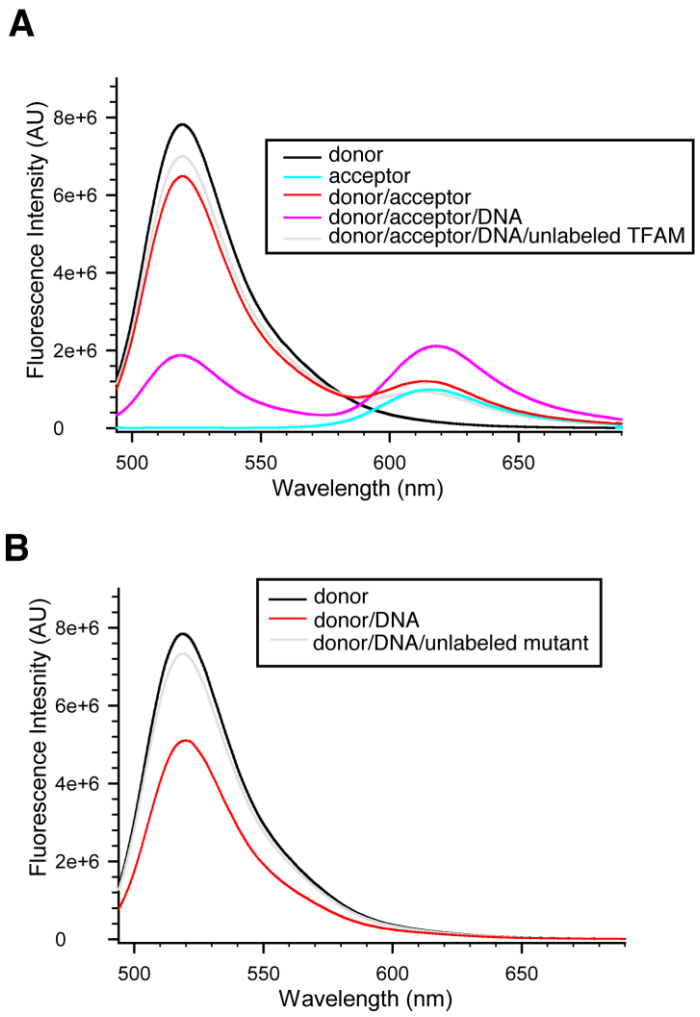


Supplementary Figure 2. Analysis of TFAM residues that contact DNA

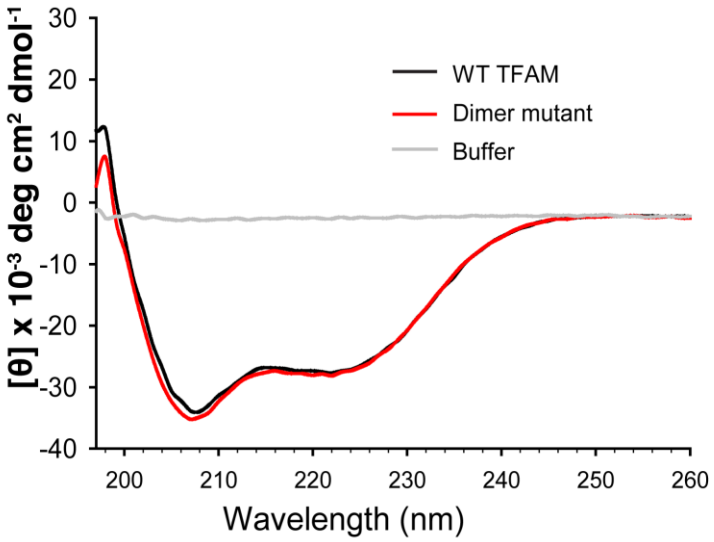
Diagram of contacts between TFAM with LSP (A), HSP1 (B), or nonspecific DNA (C), analyzed by NUCPLOT. Blue (dotted) and red (dashed) lines represent hydrogen-bonded and nonbonded contacts (<3.35 Å) to DNA, respectively. Red amino acid residues are in direct contact with DNA bases. Circles labeled W indicate water-mediated interactions with DNA.

Supplementary Figure 3. Protein sequence alignment of TFAM

Partial sequence alignment of TFAM from mammalian species. Amino acid residues colored in red are in the dimerization interface and are mutated in the dimer mutant. The alignments were made with the program ClustalW.



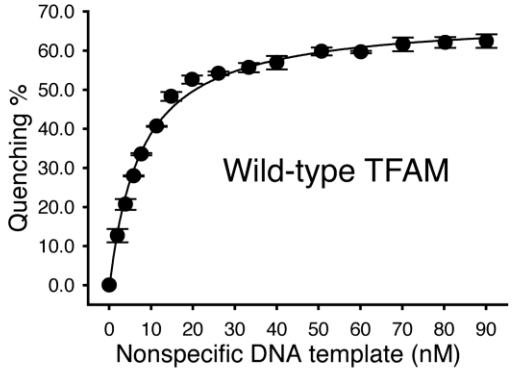
Supplementary Figure 4. Controls for the FRET assay for TFAM dimerization
(A) Competition of FRET signal by excess unlabeled TFAM. In the chase experiment (gray line), the FRET signal observed between 400 nM TFAM-Alexa Fluor 488 (donor) and 100 nM TFAM-Alexa Fluor 594 (acceptor) in the presence of DNA was competed by 5 μM unlabeled TFAM. (B) Decrease in donor fluorescence upon DNA binding. In Fig. 5A, the dimer mutant does not show FRET upon DNA addition, and yet there is a decrease in donor emission. To explain this phenomenon, the dimer mutant was labeled with the donor fluorophore alone, and its response to DNA was monitored. Donor emission (400 nM dimer mutant labeled with Alexa Fluor 488, black line) was decreased upon addition of 10 nM DNA (red line). This effect was chased off by addition of 5 μM unlabeled mutant (grey line). This environmental sensitivity of the donor fluorophore explains why donor emission of the dimer mutant is decreased upon addition of DNA, even though no FRET signal is produced (Fig 5A).

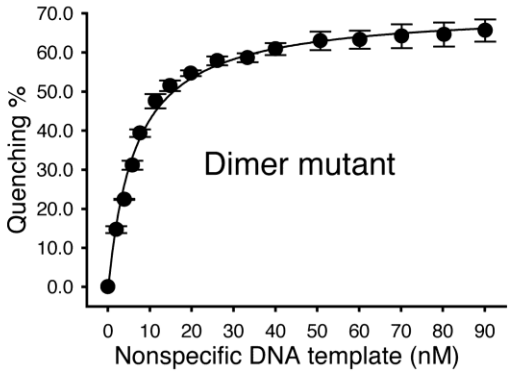


Supplementary Figure 5. Circular dichroism spectra of TFAM and the dimer mutant
CD spectra of 10 μM TFAM or the dimer mutant. Total ellipticity is plotted from 197 nm to 260 nm.

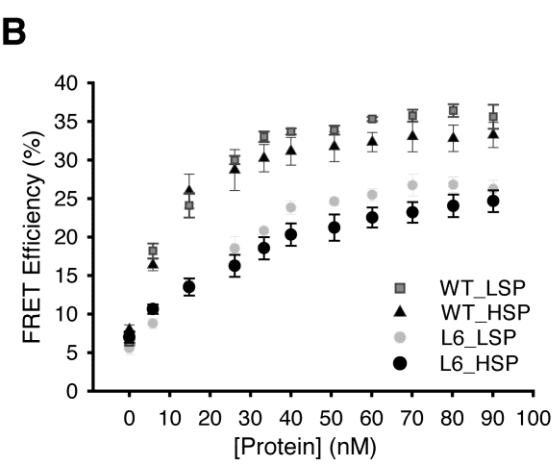
A

Protein	LSP Kd (nM)	HSP1 Kd (nM)	Nonspecific Kd (nM)
Wild-type	6.3 ± 0.9	7.1 ± 1.1	7.4 ± 1.3
Dimer mutant	6.1 ± 1.2	6.9 ± 0.8	6.4 ± 0.7



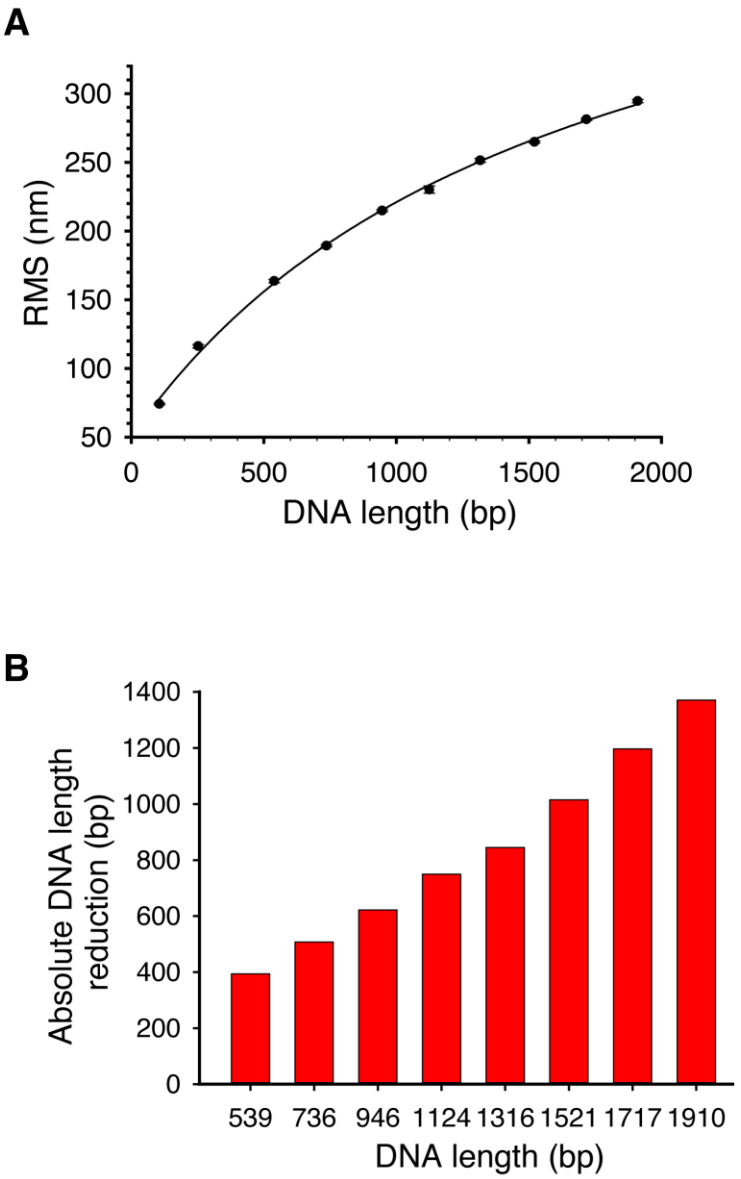


B

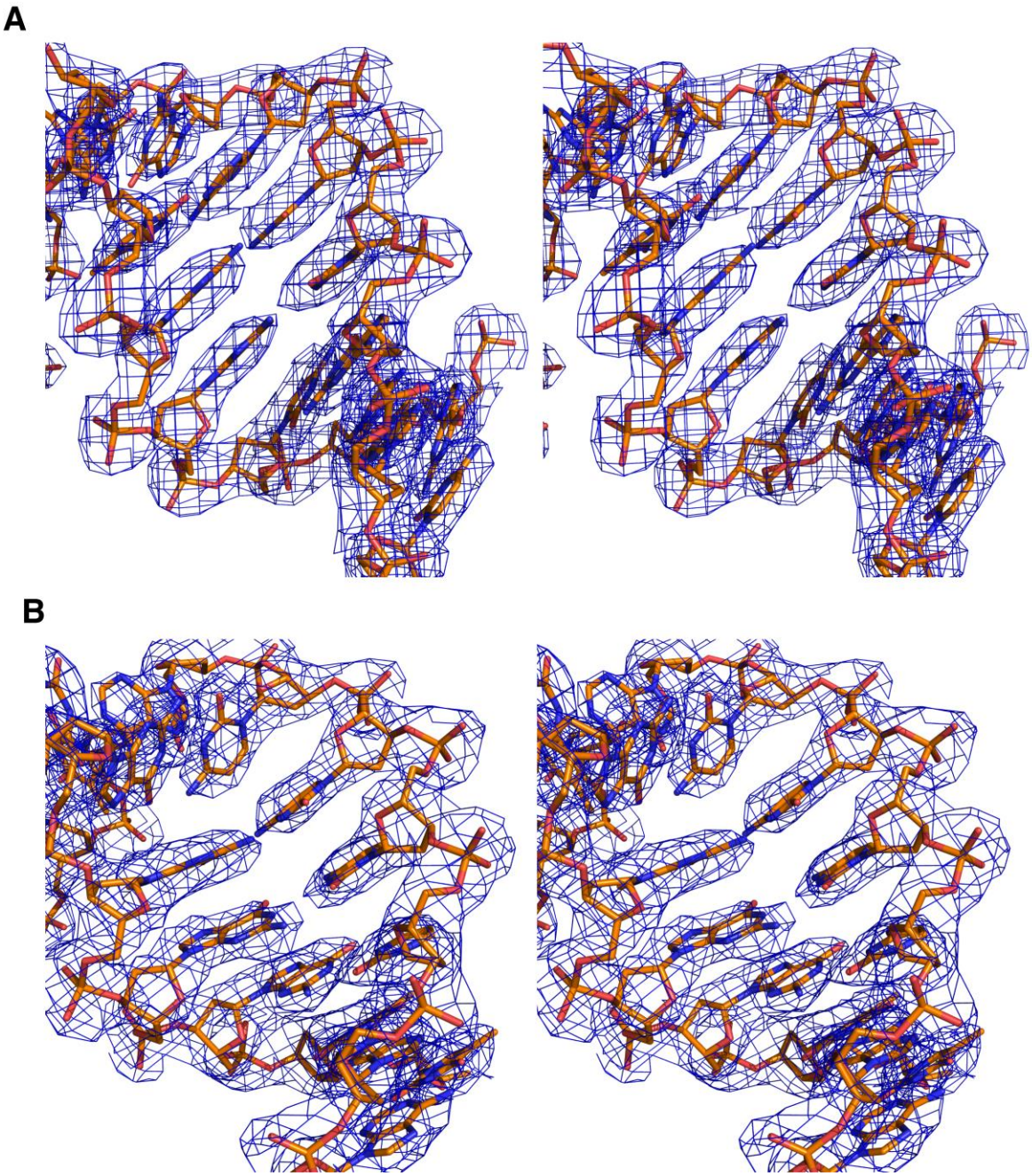


Supplementary Figure 6. DNA binding and bending of TFAM and mutants

(A) DNA binding measurements of TFAM and the dimer mutant. The table shows the affinities to LSP, HSP1, or nonspecific DNA, as monitored by the change in intrinsic tryptophan fluorescence upon DNA binding. Binding titration curves with nonspecific DNA are shown, with error bars representing standard deviations from three independent experiments. As DNA is bound, strong quenching of the intrinsic fluorescence of tryptophan is observed. (B) Analysis of L6 mutant. The left panel shows a FRET assay for DNA bending. The right panel shows a transcription assay using the LSP promoter. Data points are the average of three independent experiments, with error bars representing standard deviations.



Supplementary Figure 7. DNA compaction in the TPM assay
(A) Calibration curve for linear DNA. The plot was generated by measuring RMS values for naked DNA of various lengths (105, 252, 539, 736, 946, 1124, 1316, 1521, 1717, and 1910 bp). (B) Reduction of DNA length by TFAM for DNA of various lengths. For each DNA length, RMS values were measured with and without TFAM. The reduction in apparent DNA length was obtained by fitting RMS values to DNA lengths, using the DNA calibration curve in (A).



Supplementary Figure 8. Stereo images of electron density
Stereo images showing the final weighted $2F_o$-F_c electron density map, contoured at 1.4 σ, of (A) TFAM/nonspecific DNA (amino acids E15-E22 and DNA bases F1-F8) and (B) TFAM/HSP1 (amino acids J15-J22 and DNA bases I1-I8).