

## Supplementary Data for

### ***DNA repair factor APLF acts as a H2A-H2B histone chaperone through binding its DNA interaction surface***

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**Table S1. Structural quality of the APLF<sup>HBD</sup>-H2A-H2B model<sup>a</sup>.**

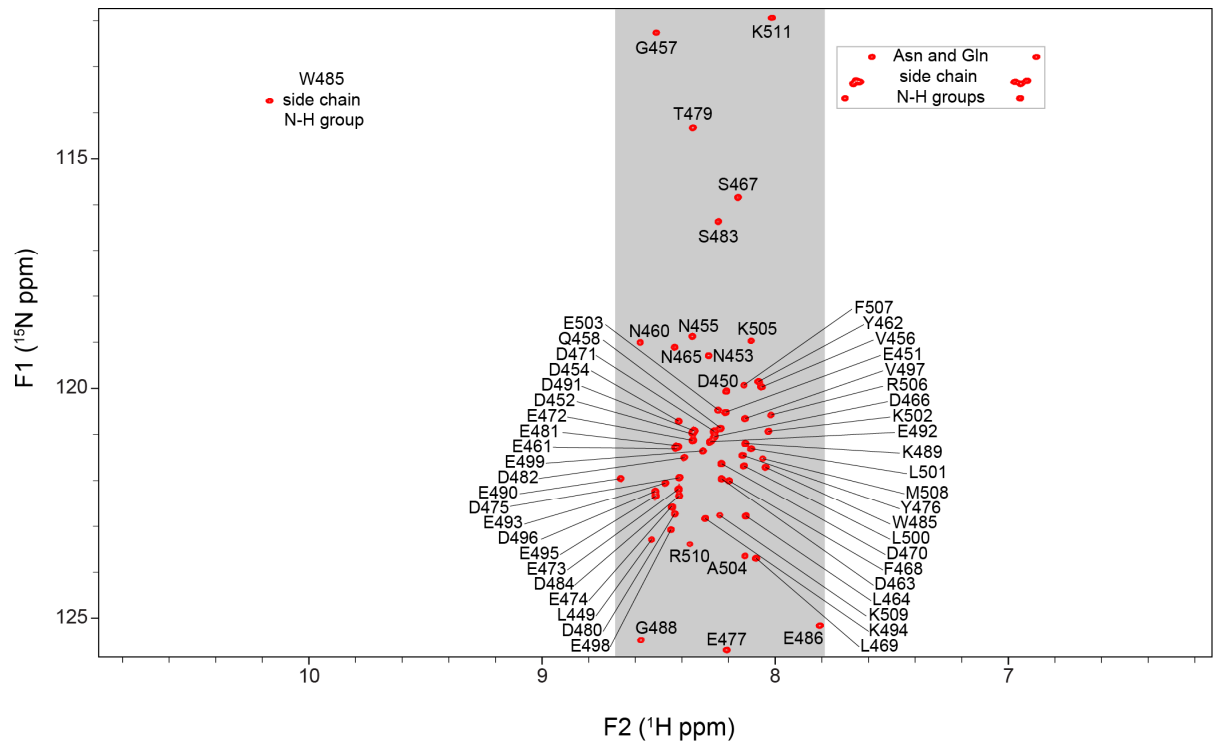
|                                      | <b>APLF<sup>HBD</sup></b> | <b>APLF<sup>HBD</sup>-H2A-H2B</b>   |
|--------------------------------------|---------------------------|-------------------------------------|
| <b>Ramachandran plot<sup>b</sup></b> | 93/7/0/0<br>(77/23/0/0)   | 92/6/2/0<br>(92/7/2/0)              |
| <b>number of clashes<sup>c</sup></b> | 1<br>(3)                  | 4 <sup>d</sup><br>(9 <sup>d</sup> ) |

<sup>a</sup> Scores and statistics are reported for only the APLF<sup>HBD</sup> chain and the full model for the lowest energy HADDOCK structure. Statistics for the top four structures are given between brackets.

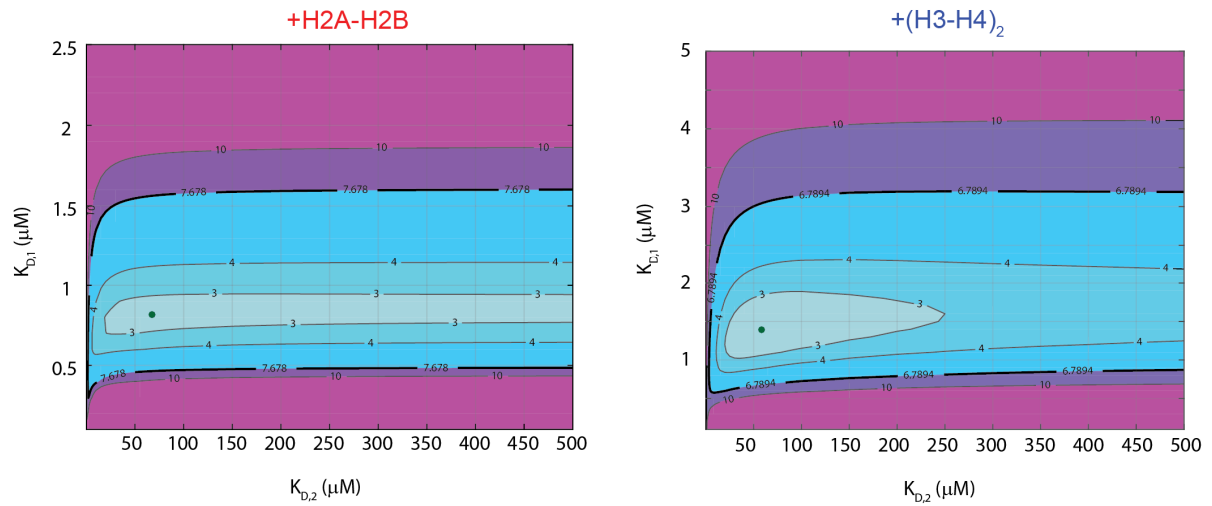
<sup>b</sup> percentage core/allowed/generously allowed/outliers, as reported by PROCHECK (1).

<sup>c</sup> number of clashes > 0.4 Å, reported by MolProbity webserver (2).

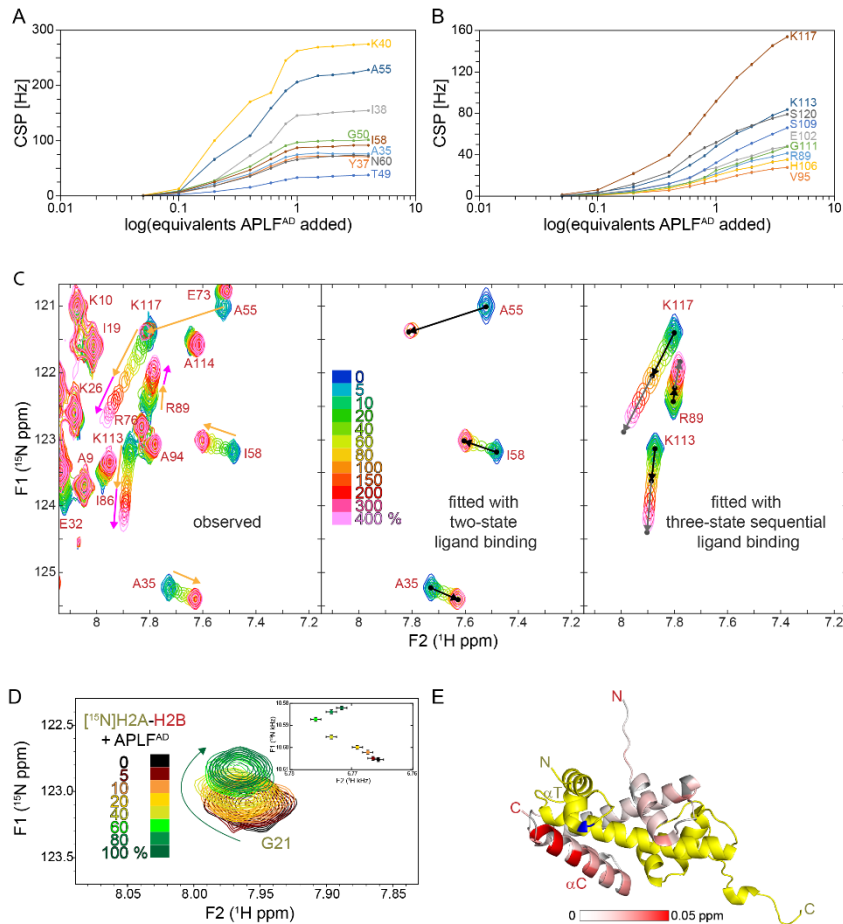
<sup>d</sup> intermolecular clashes only.



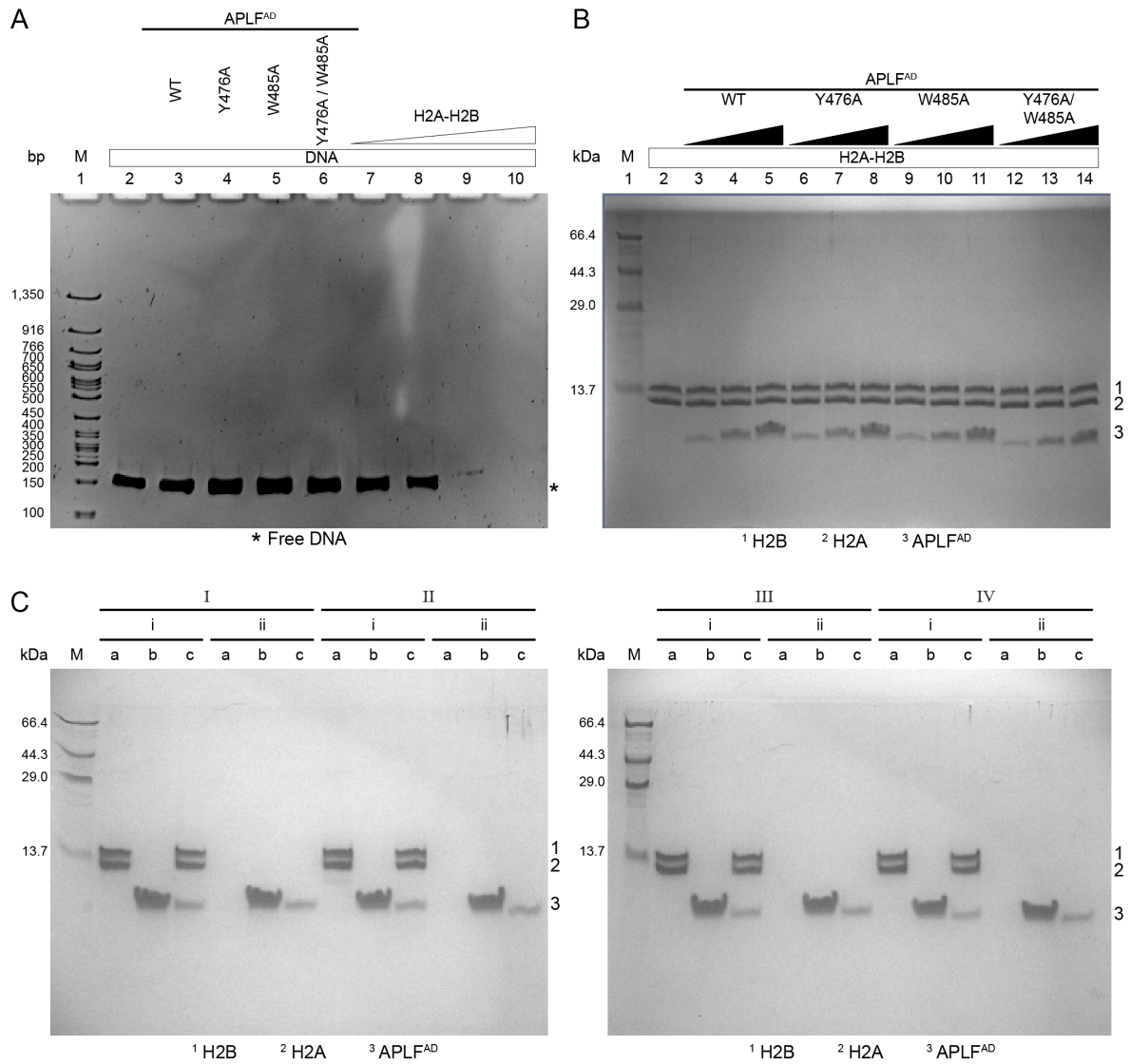
**Figure S1.**  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum of APLF<sup>AD</sup> showing all backbone and side chain amide resonances with their assignments. Spectrum recorded at 22 °C, 25 mM NaPi, pH 6.6, 300 mM NaCl, 900 MHz  $^1\text{H}$  Larmor frequency. Assignments of the main-chain amide resonances are indicated by the single-letter amino acid code and residue number, asparagine/glutamine side chain amine resonances and side chain indole resonance of W485 are indicated. In total, 60 out of a possible 62 ( $^1\text{H}$ ,  $^{15}\text{N}$ ) backbone cross-peaks are resolved with only the N-terminal 2 residues from the thrombin-cleavage site missing. All resolved backbone cross-peaks are in the spectral region indicated in gray.



**Figure S2.** Error estimation in best-fit  $K_D$  values obtained from MST. MST-derived binding curves were fit to a sequential binding model and quality of the fit was evaluated using the reduced  $\chi^2$ . The plots show the reduced  $\chi^2$ -surface in contour-mode as function of the high-affinity ( $K_{D,1}$ ) and the low-affinity ( $K_{D,2}$ ) dissociation constants for H2A-H2B (left) and (H3-H4)<sub>2</sub> (right) binding to APLF<sup>AD</sup>. The best-fit values are indicated by a green dot (H2A-H2B: 0.8/68  $\mu\text{M}$   $K_{D,1}/K_{D,2}$  with reduced  $\chi^2$  2.7; H3-H4: 1.4/58  $\mu\text{M}$   $K_{D,1}/K_{D,2}$  with reduced  $\chi^2$  2.4). The 95% confidence critical value of the reduced  $\chi^2$  is indicated with a thick black line.



**Figure S3.** (A,B) CSP of H2B resonances selected for two-dimensional NMR lineshape analysis of the high (A) and the low (B) affinity binding site plotted against equivalents of APLF<sup>AD</sup> added. (C) Observed and fitted 2D NMR lineshapes for binding analysis. Zoomed region of overlaid <sup>1</sup>H-<sup>15</sup>N-TROSY spectra of H2A-[<sup>15</sup>N]-H2B (left panel) with increasing concentrations of APLF<sup>AD</sup>. Data recorded at 750 MHz <sup>1</sup>H Larmor frequency in 25 mM NaPi buffer, pH 7 with 300 mM NaCl, 35°C. Peak shifts of selected resonances up to one (four) equivalents added are indicated with orange (pink) arrows. Results of two-dimensional lineshape analysis of the high (middle panel) and the low (right panel) affinity binding site for the titration of H2A-[<sup>15</sup>N]-H2B with APLF<sup>AD</sup>. The chemical shifts of free and 1<sup>st</sup> (2<sup>nd</sup>) bound states determined by the fitting procedure are marked by the black (gray) dots. The black (gray) arrows indicate the displacements for the high (low) affinity interactions. Color coding of spectra is indicated in the figure. (D) Zoomed H2A G21 region (note: peak is aliased) of overlaid <sup>1</sup>H-<sup>15</sup>N-TROSY spectra from the [<sup>15</sup>N]-H2A-H2B titration with APLF<sup>AD</sup> (see Figure 5). Color coding of spectra is indicated in the figure. Data recorded at 850 MHz <sup>1</sup>H Larmor frequency in 25 mM NaPi buffer, pH 7 with 300 mM NaCl, 35°C. Direction and curvature of the peak shift is indicated with a green arrow. Inset: Plot showing the peak displacement of H2A G21; black bars – standard deviation. (E) Residue H2A G21 (blue) is located close to the H2B  $\alpha$ C helix. Observed CSPs from the titration of H2A-[<sup>15</sup>N]-H2B between one and four molar equivalents of APLF<sup>AD</sup> added color coded on the cartoon representation of H2A-H2B (PDB ID code 2PYO; yellow – H2A; gray – residues without titration data) with labeling of relevant secondary structure elements (see Figure 6).



**Figure S4.** (A) Native PAGE analysis of 167 base-pair (bp) DNA (1 μM) with the 601 nucleosome positioning sequence in presence or absence of APLF<sup>AD</sup> WT or mutants, and H2A-H2B. Lane 1: 50 bp DNA ladder (M). Lane 2: free DNA. Lanes 3-6: DNA upon addition of 90 μM APLF<sup>AD</sup> WT and mutants, showing no effect. Lanes 7-10: DNA upon addition of 6, 9, 12, and 15 μM H2A-H2B, showing loss of free DNA due to precipitation. (B) Tris-Tricine SDS PAGE analysis of protein mixes used in the chaperone assay (see Figure 8A). Lane 1: Protein molecular weight marker (M). Lane 2: H2A-H2B. Lanes 3-14: H2A-H2B with increasing concentrations (1, 3, and 6 molar equivalents) of APLF<sup>AD</sup> WT, Y476A, W485A, and Y476A/W485A. (C) Tris-Tricine SDS PAGE analysis of protein mixes used in the ITC experiments of H2A-H2B with WT or mutant APLF<sup>AD</sup> (see Figure 8B). I, II, III, and IV stand for the reactions with APLF<sup>AD</sup> WT, Y476A, W485A, and Y476A/W485A, respectively. i and ii stand for the reactions of H2A-H2B with APLF<sup>AD</sup> and control experiments without H2A-H2B, respectively. a, b, and c stand for the component in the cell, the syringe, and the reaction mixture at the end of the titration, respectively. M: Protein molecular weight marker.

## Supplementary References

1. Laskowski, R.A., MacArthur, M.W., Moss, D.S. and Thornton, J.M. (1993) PROCHECK - a program to check the stereochemical quality of protein structures. *J Appl Cryst*, **26**, 283-291.
2. Davis, I.W., Leaver-Fay, A., Chen, V.B., Block, J.N., Kapral, G.J., Wang, X., Murray, L.W., Arendall, W.B., 3rd, Snoeyink, J., Richardson, J.S. *et al.* (2007) MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res*, **35**, W375-383.