Structural basis of ALC1/CHD1L autoinhibition and the mechanism of activation by the nucleosome

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Chromatin remodeler ALC1 (amplification in liver cancer 1) is crucial for repairing damaged DNA. It is autoinhibited and activated by nucleosomal epitopes. However, the mechanisms by which ALC1 is regulated remain unclear. Here we report the crystal structure of human ALC1 and the cryoEM structure bound to the nucleosome. The structure shows the macro domain of ALC1 binds to lobe 2 of the ATPase motor, sequestering two elements for nucleosome recognition, explaining the autoinhibition mechanism of the enzyme. The H4 tail competes with the macro domain for lobe 2-binding, explaining the requirement for this nucleosomal epitope for ALC1 activation. A dual-arginine-anchor motif of ALC1 recognizes the acidic pocket of the nucleosome, which is critical for chromatin remodeling in vitro. Together, our findings illustrate the structures of ALC1 and shed light on its regulation mechanisms, paving the way for the discovery of drugs targeting ALC1 for the treatment of cancer.
Packaging the genome into chromatin within the nucleus blocks access to the DNA. Chromatin remodelers alter the positions and compositions of nucleosomes, regulating the chromatin structure and nuclear transactions. ALC1, also known as CHD1L (chromodomain helicase/ATPase DNA-binding protein 1-like), is an ATP-dependent chromatin remodeler that relaxes chromatin and plays an important role in the poly(ADP-ribose) polymerase 1 (PARP1)-mediated DNA repair pathway. Defect in ALC1 regulation is associated with the development of hepatocellular carcinoma (HCC). The loss or inactivation of ALC1 in many other proteins suggest that the macro domain interacts with the preceding segment forming a helix that packs against a nearby molecule in the crystals.

Mechanism of ALC1 autoinhibition. The macro domain interacts with lobe 2 and sequesters two elements that are important for nucleosome recognition (Fig. 2a and Suppl. Fig. 2), which provides the structural basis of ALC1 autoinhibition. The macro domain of ALC1 adopts a highly conserved macro domain fold, with Dali Z-scores of 14.7 and 10.2 compared with the macro domains of DarG and macroH2A1.1, respectively (Suppl. Fig. 3). The macro domain of ALC1 binds to a saddle-shaped surface of lobe 2 formed by the conserved helicase motif IV and the H4-binding sites, with the ADP-ribose binding pocket exposed to the solvent. Lobe 2-macro domain binding occurs through mixed hydrophobic and H-bond interactions, covering an area of ~750 Å².

The β5-α4 loop, referred to as the P-loop (because the equivalent sequences in canonical macro domains bind to the phosphate group of ADP-ribose (Suppl. Fig. 3)), adopts an extended conformation and interacts with the long side chain of Arg402 of motif IV (Fig. 2b). The side chain of Tyr874 (the equivalent residues in DarG and macroH2A1.1 pack against the adenosine ring of ADP-ribose (Suppl. Fig. 3)) interacts with Ser396 (Fig. 2b). The bulky side of Trp852 of the macro domain is buried by the binding interface, contacting Arg402 and Arg398 of motif IV. In support of the structure, relative to the protein with

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**Fig. 1 Structure of ALC1 in the autoinhibited state. a** Domain architecture of ALC1. **b** Two different views of the crystal structure of ALC1 in the autoinhibited state. Bound nucleotide are colored magenta spheres. The boxed region is enlarged for analysis in Fig. 2. The bound scFv is colored blue.
The macro domain engages lobe 2 through a second element, the H4-binding surface (Fig. 2c). Arg857 and Arg860 at the C-terminus of α4 form H-bonds and charge–charge interactions with Glu332, Asp377, and Asp381. Tyr853 at the N-terminus of α4 makes hydrophobic contacts with the side chains of Phe336 and Val338 of lobe 2. In support of the structure, the D381A mutation notably increased the PARP1-independent ATPase activities (Fig. 2d). This structure is also supported by the cancer-associated mutations at Arg857 and Arg860, which release the autoinhibition of ALC1. This macro-domain-binding interface is in line with the HDX2 region detected with the H/D exchange assays. Interestingly, the acidic pocket formed by Glu332, Asp377, and Asp381 of ALC1 is similar to those of ISWI and Snf2 (Suppl. Fig. 1c), which bind to the H4 tails. The H4 tails are important for ALC1 activation. The structure suggests that the macro domain sequesters the H4-binding sites, which provides a second mechanism of ALC1 autoinhibition.

Compared with the WT protein under the same conditions, deletion of the macro domain in ALC1 (1-673) dramatically stimulated the basal and DNA-dependent ATPase activities by factors of ~7 and ~35, respectively (Fig. 2d and Suppl. Fig. 7a). Lobe 1 and lobe 2 of ALC1 underwent large structural transitions, with the ATP-binding/hydrolysis motifs I and VI realigning in close proximity to one another (Suppl. Fig. 7a). In the activated state, the macro domain dislodged from lobe 2 extended one more helical turn and interacted with lobe 1 (Suppl. Fig. 7c). These conformational changes are consistent with ALC1 in the activated state. In the cryoEM samples, some nucleosomes were bound by two copies of ALC1 symmetrically, the structure of which was refined to 3.3 Å (Suppl. Figs. 5c, 6i, and 7d). In this double-binding model, ALC1 interacted with the nucleosome in a manner similar to that of the high resolution single-binding structure (Suppl. Fig. 7e), which is the focus of this study.

Structure of ALC1 bound to the nucleosome. To gain insights into ALC1 activation by the nucleosome, we determined the cryoEM structure of the R857Q mutant bound to the nucleosome in the presence of a stable ATP analog, ADP-BeF₃. The R857Q mutant is known to release the autoinhibition of ALC1, bypassing the requirement for the PAR chain. The structure of the ALC1-nucleosome complex was determined at an overall resolution of 2.8 Å, with local resolutions of 2.8 Å and 3.1 Å at the nucleosome bound with the ALC1 linker and the ALC1 motor domain, respectively (Suppl. Figs. 5 and 6 and Suppl. Table 2). ALC1 bound to the nucleosome at superhelical location 2 (SHL 2), in a manner conserved with Snf2 and other chromatin remodelers (Fig. 3a and Suppl. Fig. 7a). Lobe 1 and lobe 2 of ALC1 underwent large structural transitions, with the ATP-binding/hydrolysis motifs I and VI realigning in close proximity to one another (Suppl. Fig. 7b). The brace helix of lobe 2 extended one more helical turn and interacted with lobe 1 (Suppl. Fig. 7c). These conformational changes are consistent with ALC1 in the activated state. In the cryoEM samples, some nucleosomes were bound by two copies of ALC1 symmetrically, the structure of which was refined to 3.3 Å (Suppl. Figs. 5c, 6i, and 7d). In this double-binding model, ALC1 interacted with the nucleosome in a manner similar to that of the high resolution single-binding structure (Suppl. Fig. 7e), which is the focus of this study.

In the activated state, the macro domain dislodged from lobe 2 and its structure could not be resolved, suggesting that the macro domain did not bind stably to the nucleosome or the ATPase motor domains. This is in line with the previous findings that the macro domain is not essential for chromatin relaxation in cells. Consistent with this notion, the macro domain deletion mutant ALC1 (1-673) showed a greater remodeling activity than the WT
Different from the WT protein, the remodeling activity of the mutant was, however, not further enhanced in the presence of PARP1. These data indicated that the macro domain inhibits the ATPase activity of ALC1 in the nucleosome-free state, but is not essential for the remodeling reaction in the activated state.

In line with the structures of Snf2 and ISWI, the acidic pocket of lobe 2 formed by Glu332, Asp377, Asp381, and Asp384 of ALC1 interacts with the basic patch K16R17H18R19 of the H4 tail of the nucleosome (Fig. 3b and Suppl. Fig. 6b). The breadth of the pocket is increased by the hydrophobic packing of Phe336 of ALC1 to His18 of the H4 tail. In agreement with the requirement of H4 for ALC1 activation, disruption of the H4-binding interface by the D381A mutation diminished the remodeling activity (Suppl. Fig. 9a). Therefore, the functions of Asp381 are twofold. It interacts with the macro domain in the nucleosome-free state (Fig. 2c), inhibiting the enzyme in the absence of the substrate, whereas it interacts with H4 tail in the nucleosome-bound state, promoting the remodeling activity. To get insights into the loss of the remodeling activity caused by the D381A mutation at the pocket.
the activated state, we performed the experiments at multiple enzyme/substrate concentrations to do a $K_{\text{m}}/K_{\text{cat}}$ analysis using the construct without the macro domain, which bypassed the complication due to the autoinhibition and requirement for PARP1 activation (Fig. 3c). The data showed that the D381A mutation increased the $K_{\text{m}} \sim 2$-fold, while it slightly decreased the catalytic efficacy ($K_{\text{cat}}$), suggesting the defect was mainly because of the compromised nucleosome binding. The competition of the H4 tails with the inhibitory macro domain for binding to the same surface of lobe 2 explains the requirement for this nucleosomal epitope for ALC1 activation (Fig. 3d).

Likewise, motif IV underwent a loop-to-helix conformational change (Fig. 3e), with the side chain of Arg402 binding to the phosphate backbone of nucleosomal DNA, as typically observed in the activated state of chromatin remodelers. Therefore, the release of the macro domain frees the H4-binding surface and motif IV to interact with the nucleosome, providing the mechanism of ALC1 activation.

**Recognition of the acidic pocket of H2A-H2B by ALC1.** Notably, we found that a segment of the linker region (residues 610–617) adjacent to lobe 2 binds to the H2A-H2B acidic patch of the nucleosome (Figs. 3a and 4a, b). In particular, two arginine residues, Arg611 and Arg614, play critical roles. Arg611 interacts with Gln44 of H2B and Glu56 of H2A, whereas Arg614 forms a network of H-bonds with Glu61, Asp90, and Glu92 of H2A. Arg611 and Arg614 are highly conserved in vertebrate forms a network of H-bonds with Glu61, Asp90, and Glu92 of H2A. Arg611 and Arg614 are highly conserved in vertebrate.

To test the function of the linker region-nucleosome interaction, we mutated the key arginine residues and measured the ATPase and remodeling activities in vitro. The mutations R611E and R614E did not alter the ATPase activity (Suppl. Fig. 9b), but caused notable losses of the remodeling activity (Fig. 4d and Suppl. Fig. 9c). The double mutant R611E/R614E showed a defect similar to that observed with the single mutants, suggesting that the two arginine residues work in a conjugated manner to facilitate the remodeling reaction.

The binding of Arg611 and Arg614 to the acidic pocket of the nucleosome is unexpected. We did the reciprocal experiment with mutations of the acidic patch to further validate these findings. Consistent with our model, disruption of the acidic patch reduced the remodeling activity of the WT enzyme (Fig. 4d and Suppl. Fig. 9d). Moreover, the R611E and R614E mutations were not worse on the acidic patch mutant nucleosomes. The data support that the arginine anchors of ALC1 and the acidic patch of the nucleosome work through the same mechanism to regulate the activity of the enzyme.

The loss of the remodeling activity was probably not because of thermal instability, as addition of more mutant protein at a later time point, and lowering the reaction temperature, did not...
the data indicated the remodeling rate constants were ~0.1 and ~0.05 min
lines indicating the means (open symbols) of PARP1. Error bars indicate standard deviations for three independent measurements and the measure of center for the error bars is the mean value.

ALC1 dysregulation by cancer-associated mutations. In addition to gene amplification, over 200 missense mutations of ALC1 are reported in the COSMIC database24. Many of these occur in bioactive regions, disturbing the varied mechanisms of ALC1 regulation (Fig. 5a). As discussed above (Fig. 2), the mutations at Trp852, Arg857, or Arg860 of the macro domain release the ALC1 autoinhibition. The Arg857 and Arg860 mutants were previously shown to be constitutively active69, and we evaluated the impact of the W852C mutation on the chromatin remodeling activity, Fig. 5b. Consistent with the ATPase activity (Fig. 2d), the W852C mutant was hyperactive in the absence of PARP1, whereas it displayed an activity similar to WT in the presence of PARP1, suggesting Trp852 is important for autoinhibition of ALC1, and dispensable for the remodeling activity when the enzyme is activated by PARP1 in vitro.

Arg260 maps to lobe 1 and is in close contact with Ile494 and Glu495 of the brace helix at the lobe 1-lobe 2 binding interface (Fig. 5c); Arg319 is close to the nucleosomal DNA (Fig. 5d); and Arg457 is one of the “arginine fingers” that catalyze ATP hydrolysis (Fig. 5e). As expected, the catalytic R457H mutation abolished the ATPase (Fig. 5f) and remodeling activities (Fig. 5g and Suppl. Fig. 9f) in vitro. The cancer-associated mutations R260M and R319Q did not alter the ATPase activities much (Fig. 5f), but markedly diminished the remodeling activities. The R260M mutation seemed to perturb the thermal stability, as the mutant enzyme remodeled a higher amount of the nucleosome at a lower temperature, to a level similar to WT under the same conditions (Fig. 5h and Suppl. Fig. 9g). Fitting the data indicated that the remodeling rate constant of the mutant enzyme was about half of the value of the WT protein, suggesting the R260M mutation impacts both the stability and remodeling efficiency of the enzyme. The data supported that the remodeling activity of ALC1 is regulated through multiple fashions, and our findings provide a framework to analyze the functions of the ALC1 mutants in cancer cells.

Discussion

Our studies reveal how ALC1 is autoinhibited and provide the mechanisms by which it is regulated by the nucleosomale epitopes. Chromatin remodelers are often autoinhibited to avoid futile ATP hydrolysis in the absence of the nucleosome substrate. Snf2 is inhibited through direct stacking of the two RecA-like lobes27. The ATPase motor of Chd1 is inhibited by the N-terminal double chromodomain28, which binds lobe 2 and sequesters the DNA-binding elements, motif V in particular, with the H4-binding surface exposed. ISWI is tightly autoinhibited by the AutoN domain, which sequesters motif V and the H4-binding surface25. The regulatory mode of ALC1 shows analogy to the autoinhibitory mechanism of ISWI, in that the macro domain binds to lobe 2, inhibiting the enzyme by sequestering motif IV and the H4-binding surface. Different from ISWI, in which the AutoN domain remains binding to the lobe1 of the enzyme at the activated state29, the macro domain of ALC1 dislodges from the motor when activated by the nucleosome. Disruption of the macro-lobe 2 interaction of ALC1 by cancer-associated mutations, or binding of the PAR chain, allows the enzyme to realign the ATPase lobes and engage the nucleosome. In the activated state, the H4 tails and the acidic patch of the nucleosome interact with lobe 2 and the linker region of ALC1, respectively.

The previous studies supported the importance of the linker sequence of ALC1 in cells30. Our data suggest that binding of the linker region through the arginine anchors to the H2A-H2B acidic pocket of the nucleosome stabilizes the activated conformation and/or prevents the enzyme from zipping back to the autoinhibited state, providing the mechanisms of ALC1
regulation by the nucleosomal epitopes. The residues neighboring the H2A-H2B acidic pocket are subjected to modifications, and the acidic pocket is believed to function as a tunable hub for many chromatin proteins. Several chromatin remodelers, including Snf2 and ISWI, are also regulated by the H2A-H2B acidic pocket, although the mechanisms are not completely clear. Poly-basics motifs are found in ISWI enzymes and the SnAc domain of Snf2. The interaction mode between the dual arginine "RxxR" motif of ALC1 and H2A-H2B of the nucleosome provides a framework for understanding the regulation mechanisms of other chromatin remodelers. While our manuscript was under review, a study by Lehmann et al. reported the structure of the isolated ALC1 linker peptide bound to the acidic patch of the nucleosome, which, however, shows an interaction mode different from our structure. The Lehmann’s structure revealed only one of the arginine anchors. More importantly, the isolated ALC1 linker peptide bound to the nucleosome with Arg611 interacting Glu64, Glu61, Asp90, and Glu92 of H2A, whereas Arg614 bound to this acidic pocket in our structure. We noticed that the EM density of the side chain of Leu613 in our structure (Fig. 4b), could not easily be distinguished by the smaller side chain of Ser612 in the Lehmann’s structure.

ALC1 is regulated by diverse mechanisms, for example, by modulating macro inhibition, disrupting the lobe 1-lobe 2 interaction, perturbing H4 binding, and interfering ATP hydrolysis. Tight regulation of ALC1 action, chromatin remodelers in general, is critical for the function in vivo. We speculate that the constitutively active mutants, such as W852C and R857Q, probably excessively relaxes the chromatin even in the absence of DNA damage, leading to oncogenic activities as found during ALC1 overexpression. In contrast, mutations of Arg611 and Arg614 cause loss of ALC1 function, which would sensitize cells to DNA damages in a manner similar to that seen in the ALC1-deficient cells. More studies are needed to understand the in vivo effects. ALC1 is a promising target for cancer treatment, and small-molecule inhibitors targeting the ATPase activity have been found, although they showed low affinities. In addition to the common strategy of inhibiting the ATPase activity, the sensitivity of the remodeling activity and the varied regulation modes of ALC1 provide rich opportunities and a large chemical space to control the function of this enzyme in cells. The structures and regulation mechanisms revealed in this study pave the way for the future discovery of drugs that target ALC1 in cancer treatments.

Methods

ALC1 expression and purification. The gene of ALC1 (residues 1–880) was cloned from Homo sapiens complementary DNA (cDNA), and inserted into a modified pET-28b vector containing a SUMO tag. The ALC1 mutants were generated through Quikchange mutagenesis or Gibson assembly, and confirmed by DNA sequencing. ALC1 was overexpressed in the Esherichia coli expression strain Rosetta (DE3). Cells were grown in LB media at 37 °C until the absorbance at 600 nm (OD600) reached 0.8. The temperature of the culture was reduced to 16 °C before addition of 0.5 mM isopropyl-beta-D-thiogalactoside (IPTG). Cells were cultured overnight, and harvested by centrifugation at 4000 r.p.m. (Beckman, Rotor JA10) for 15 min at 4 °C. The pellets were resuspended in 20 mM HEPES, 150 mM NaCl, 10 mM DTT, pH 7.5. The supernatant was loaded on a Ni-NTA column and allowed to flow through by gravity. After a washing step, the protein was eluted with 20 mM HEPES, 150 mM NaCl and 250 mM imidazole, pH 7.5. The elution was further purified by gel-filtration chromatography using a Superdex 75 column (GE Healthcare) pre-equilibrated with elution buffer without the 250 mM imidazole. Fractions containing scFv were collected and then concentrated to 1 mg/ml and stored at −80 °C.

The purified scFv and ALC1 were incubated on ice for 1 h at a ratio of 1:1 in the binding buffer 20 mM HEPES, 150 mM NaCl, 10 mM DTT, pH 7.5. The mixture was purified by gel-filtration chromatography using a Superdex 200 column (GE Healthcare) pre-equilibrated with the same binding buffer. Fractions containing the complex were collected and were concentrated to 12 mg/ml and stored at −80 °C.

Crystallization and data collection. Crystals of ALC1-scFv complex were grown at 4 °C by hanging drop vapor diffusion above a reservoir solution from 35% (v/v) 2-methyl-2,4-pentanediol (MPD), 100 mM acetic acid (pH 4.5), 10 mM DTT, with equal volumes of protein and reservoir buffer. Crystals were optimized by increasing MPD to 80% MPD, 100 mM HEPES (pH 7.0), 10 mM DTT, 0.5 mM ADP, 2 mM MgCl2, and harvested in cryo-protectant containing extend 5–15% MPD and then flash-frozen in liquid nitrogen. Diffraction data from crystals were collected at −170 °C at the beamline BL17U of Shanghai Synchrotron Radiation Facility.

Data processing and structure solution. The data were processed with HKL2000 suit. The structure of ALC1-scFv complex was solved by molecular replacement using lobe 1 of Chd1 (Protein Data Bank accession number 3MWY), lobe 2 of ISWI (Protein Data Bank accession number 5XR6), macro domain (Protein Data Bank accession number 2FG1), and VH and VL domain of a human neutralizing antibody (SYX) as the initial searching models. The rest of the model was built manually using Coot. Refinement was performed with Phenix. The final structure was refined to 3.5 Å, with Rwork/Rfree = 0.268/0.316, Rama-chandran outlier 0.10%, allowed 8.84%, and favored 91.06%.

ATPase assays. The ATPase activities were measured with an EnzChek Phosphate Assay Kit. The assays were performed with 0.1 μM ALC1 in the buffer of 3 mM ATP, 50 mM Tris-HCl (pH 7.5), 1 mM MgCl2, 0.2 mM sodium azide, 0.2 mM DTT, 0.05 mM glutathione, and 0.05 mM reduced glutathione. After high-speed centrifugation, the supernatant was loaded on a Ni-NTA column and allowed to flow through by gravity. After a washing step with 25 mM imidazole, the protein was eluted with 20 mM HEPES, 150 mM NaCl and 250 mM imidazole, pH 7.5. The His6-SUMO tag was cleaved by Ulp at 4 °C overnight. The protein was further purified on an ion-exchange column (Source-15S, GE Healthcare), and then subjected to gel-filtration chromatography (Superdex 200, GE Healthcare) in buffer containing 10 mM HEPES, 150 mM NaCl and 10 mM dithiothreitol (DTT), pH 7.5. The purified protein was concentrated to 13 mg/ml and stored at −80 °C. The ALC1 mutants were purified similarly, and concentrated to 5–10 mg/ml.

We cloned the gene of PARP1 from Homo sapiens cDNA, and inserted it into a modified pET-28b vector. The construct was confirmed by DNA sequencing. PARP1 was overexpressed and purified using the similar protocol as described above. PARP1 was concentrated to 10 mg/ml for assays. All primers were used were provided in Suppl. Table 3.

Single-chain antibodies screening and purification. Single-chain variable fragments (scFv) against ALC1 were obtained from a yeast-display library carrying non-immunized human scFv using a protocol as described before. Briefly, two purified ALC1 proteins with different tags, ALC1-6His and ALC1-biotin, were used as antigens for screening the yeast-display library. ALC1-6His was constructed and inserted into a modified pET-28b vector, and purified similarly as described above. ALC1-biotin was obtained by coexpression of ALC1-avi (in a pET-28b vector) and BirA enzyme (in a pAACYC vector) in Escherichia coli BL21 (DE3) strain. Streptavidin microbeads (200 μl, Milltenyi Biotec) were mixed with ALC1-biotin (100 mM) and 1 × 1010 yeast cells in 5 ml PBS, and the bound candidate scFv cells were selected through a LS column, and then were amplified. In the sequential sorting, ALC1-biotin pre-mixed with Anti-His-Biotin was used as the antigen for screening using Anti-Biotin microbeads in a similar way.

The isolated candidate scFv cells were then used for three rounds of flow cytometry sorting. In the first round of sorting, 100 mM biotinylated ALC1 was mixed with 2 × 107 candidate scFv cells and the relevant antibodies (chicken anti-c-Myc IgY and Alexa Fluor 488-goat anti-chicken IgG; streptavidin, R-phycoerythrin) was used. The third round of sorting was performed similarly, and concentrated by Anti-His-Biotin was used as the antigen for screening using Anti-Biotin microbeads in a similar way.

Cells were grown in LB media at 37 °C until the absorbance at 600 nm (OD600) was 0.5, and harvested by centrifugation at 4000 r.p.m. (Beckman, Rotor JA10) for 15 min at 4 °C. The pellets were resuspended in 20 mM HEPES, 150 mM NaCl and 250 mM imidazole, pH 7.5. The elution was further purified by gel-filtration chromatography using a Superdex 75 column (GE Healthcare) pre-equilibrated with elution buffer without the 250 mM imidazole. Fractions containing scFv were collected and then concentrated to 1 mg/ml and stored at −80 °C.

The seven genes of scFvs were cloned, and inserted into the pVRC-8400 vector, which contains an N-terminal signal peptide (MGWCSIKLFLVATATCYS) for secretion and a C-terminal 6xHis tag for purification. The scFv plasmids were transfected into HEK293F cells (2 × 106 cells per ml) by PEI at a ratio 1:3. After 72 h transfection, the supernatant of cell culture containing the secreted scFv was collected, concentrated and buffer-exchanged to 20 mM HEPES, 500 mM NaCl, 1 mM TCEP, pH 7.5. The supernatant was loaded on a Ni-NTA column and allowed to flow through by gravity. After a washing step, the protein was eluted with 20 mM HEPES, 150 mM NaCl and 250 mM imidazole, pH 7.5. The elution was further purified by gel-filtration chromatography using a Superdex 200 column (GE Healthcare) pre-equilibrated with elution buffer without the 250 mM imidazole. Fractions containing scFv were collected and then concentrated to 1 mg/ml and stored at −80 °C.

DNA sequencing. The seven genes of scFvs were cloned, and inserted into the pET-28b vector containing a SUMO tag. The ALC1 mutants were generated through Quikchange mutagenesis or Gibson assembly, and confirmed by DNA sequencing.
Nucleosome remodeling assays. Mononucleosome restriction enzyme accessibility assays were performed as described before.\textsuperscript{2} Cpf1-matured mononucleosome (5 nM) and 0.1 \(\mu\)M of various ALC1 proteins (0.04 \(\mu\)M proteins in Suppl. Fig. 8) were incubated at 37°C at 100 °C of H2A at the remodeling buffer (20 mM HEPES, pH 7.5, 50 mM NaCl, 5 mM MgCl\textsubscript{2}, 5% glycerol, and 0.1 mg/ml bovine serum albumin). After adding 0.1 \(\mu\)M PARP1 (pre-incubated with 50 \(\mu\)M NAD at 37 °C for 5 min), 3 mM ATP was added to initiate the reaction. Fractions were taken at various time points and quenched with 2\(\times\)Stop buffer (20 mM Tris, pH 8.0, 1.2% sodium dodecyl sulfate (SDS), 80 mM EDTA, and 0.2 mg/ml proteinase K). The reaction mixtures were incubated at 55 °C for 20 min to deproteinate the samples. The remodeling activities of ALC1 proteins (WT, R611E, R614E, R614E/601 (WT(604–623), R611E and R614E) purchased from Scilight Biotechnology (95% purity), the mutant NCP assembled with H2A (E56K/E61K/D908R/E92K) were obtained in the same way. To analyze the temperature dependence/thermal stability, the reactions were performed at 25 °C. To analyze the thermal stability of R611E, extra R611E (0.1 \(\mu\)M) was added at 32 °C and reaction continued for another 32 °C.

Fractions were running on 8% native TBE polyacrylamide gels in 0.25\(\times\)TBE for 100 min at 120 V on ice. Gels were imaged using a Typhoon FLA9500 variable mode imager (GE Healthcare). Band intensities were quantified in Quantity One software. The reaction rate constants were fit to a single exponential decay using GraphPad Prism 8.4.3.

To compare the values of Km and Kmax of ALC1(1–673) and mutant D381A, different concentrations (10–640 nM) were titrated into the reaction mixtures. The measured 

\[ K_a \]

were fit to a Hyperb model (OriginPro 8.5).

Microscale thermophoresis. MST analysis was performed using a Nano Temper Monolith NT.115 instrument (NanoTemper Technologies GmbH). H2A–H2B dimer was reconstituted as before.\textsuperscript{4} It was fluorescently labeled with the RED-NHS 2nd Generation kit (NanoTemper Technologies GmbH), and desalted to the reaction buffer (10 mM MES, 175 mM NaCl, 0.05% Tween 20, pH 6.0). Peptides 167NCP and 0.1 \(\mu\)M of unlabeled peptides before being loaded into standard glass capillaries (Monolith NT.115 Capillaries) at room temperature. The difference of the thermophoretic properties was measured at 645 nm with the laser power 40% (20% with the WT peptide was used) and medium MST power with the MO. Control software. The dissociation constant was measured with the MO. Affinity Analysis software. Each experiment was repeated three times.

CryoEM sample preparation and data collection. The nucleosome core particles (167NCPs) were constituted with 167 bp DNA containing the ‘601’ positioning sequence (5’- strand: CCGGCGCGCGCGCGCGGCGCGCCTGAGATCGGCGCGCGTTAGCAAGCCTGACGCTCGAGTTGCTCAGAAGCCGACGGCAGACTGGTGCC

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