SUPPLEMENTARY INFORMATION

The Chd1 chromatin remodeler shifts nucleosomal DNA bidirectionally as a monomer

Yupeng Qiu[1, 2], Rob Levendosky[1], Srinivas Chakravarthy[3], Ashok Patel[4], Gregory Bowman[1], Sua Myong[1, 2, 5]

1. Department of Biophysics, Johns Hopkins University, Baltimore, Maryland 21218, United States
2. Department of Bioengineering, University of Illinois, Urbana, Illinois 61801, United States
3. Biophysics Collaborative Access Team, Argonne National Laboratory, Argonne, Illinois 60439, United States
4. Kusuma School of Biological Sciences, Indian Institute Of Technology Delhi, Hauz Khas, New Delhi 110016, India.
5. Physics Frontier Center (Center for Physics of Living Cells), University of Illinois, Urbana, Illinois 61801, United States

Supplementary Information includes:

- Figure S1
- Figure S2
- Figure S3
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- Figure S6
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- Supplemental Table S1

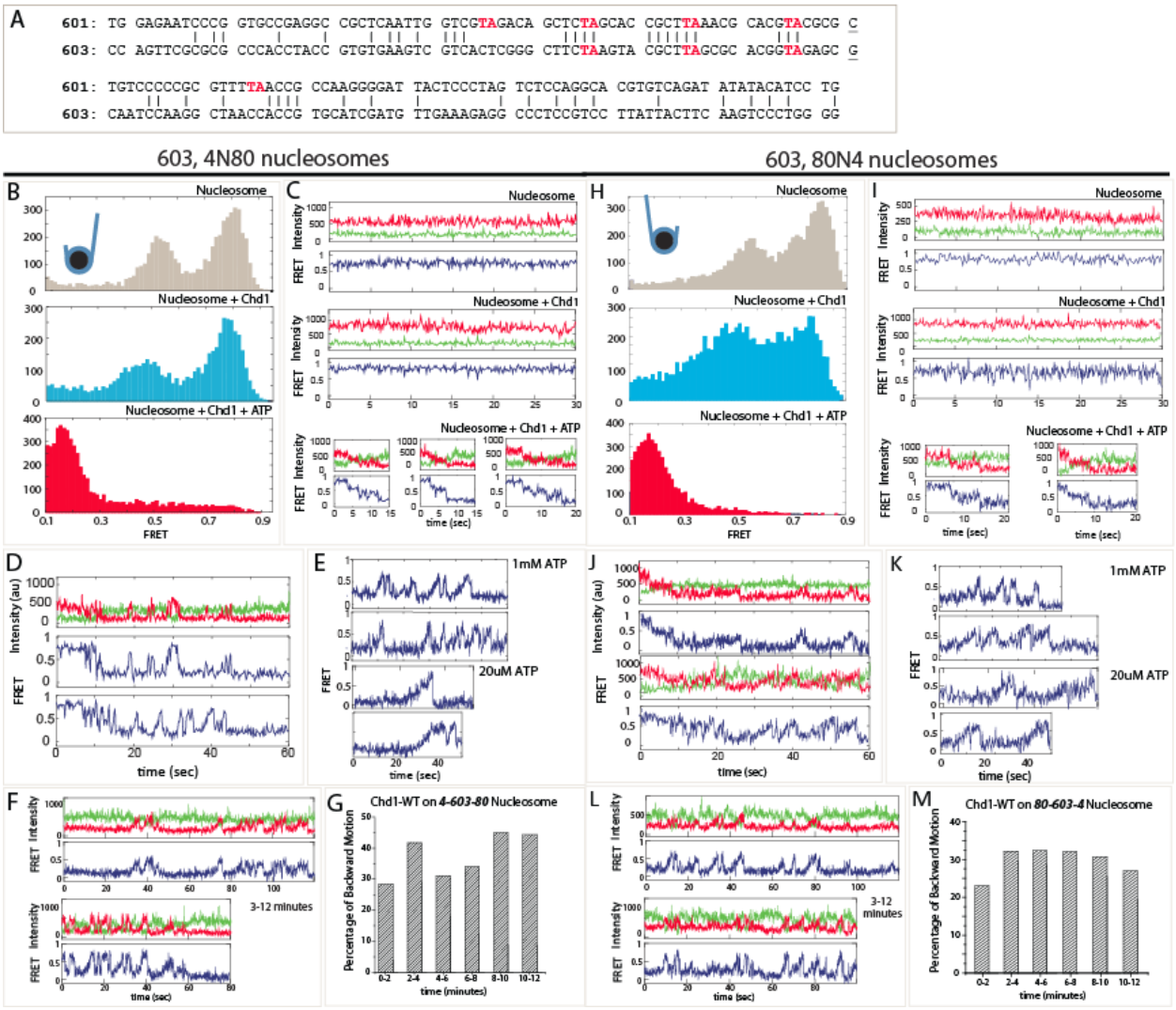

Figure S1. Related to Figure 4. Chd1 exhibits similar remodeling behaviors for 603 and 601 nucleosomes. (A) Sequence alignment of 601 and 603 nucleosome positioning sequences. (B, H) FRET histograms taken for nucleosome only, with Chd1 and with ATP (1mM) for 603 based nucleosome in two different configurations, 4N80 and 80N4. (C, I) Representative smFRET traces for each corresponding conditions to B, H. (D, J) smFRET traces showing the initial FRET decrease followed by successive FRET increase and decrease, similar to 601 case. (E, K) smFRET traces taken at high (1 mM) and low (20 μM) ATP, demonstrating the highly asymmetric pattern of FRET entailing gradual FRET increase and rapid FRET decrease, suggesting ATP dependent FRET increase and ATP independent FRET drop, in agreement with the 601 data. (F, L) smFRET traces taken from movies taken after 3-12 minutes after adding ATP, showing continuous FRET fluctuation exhibited by many Chd1 molecules. (G, M) Statistics of percent dynamic FRET traces in 0-12 minute post ATP addition, again signifying that the repetitive nature of the back and forth shifting of DNA.

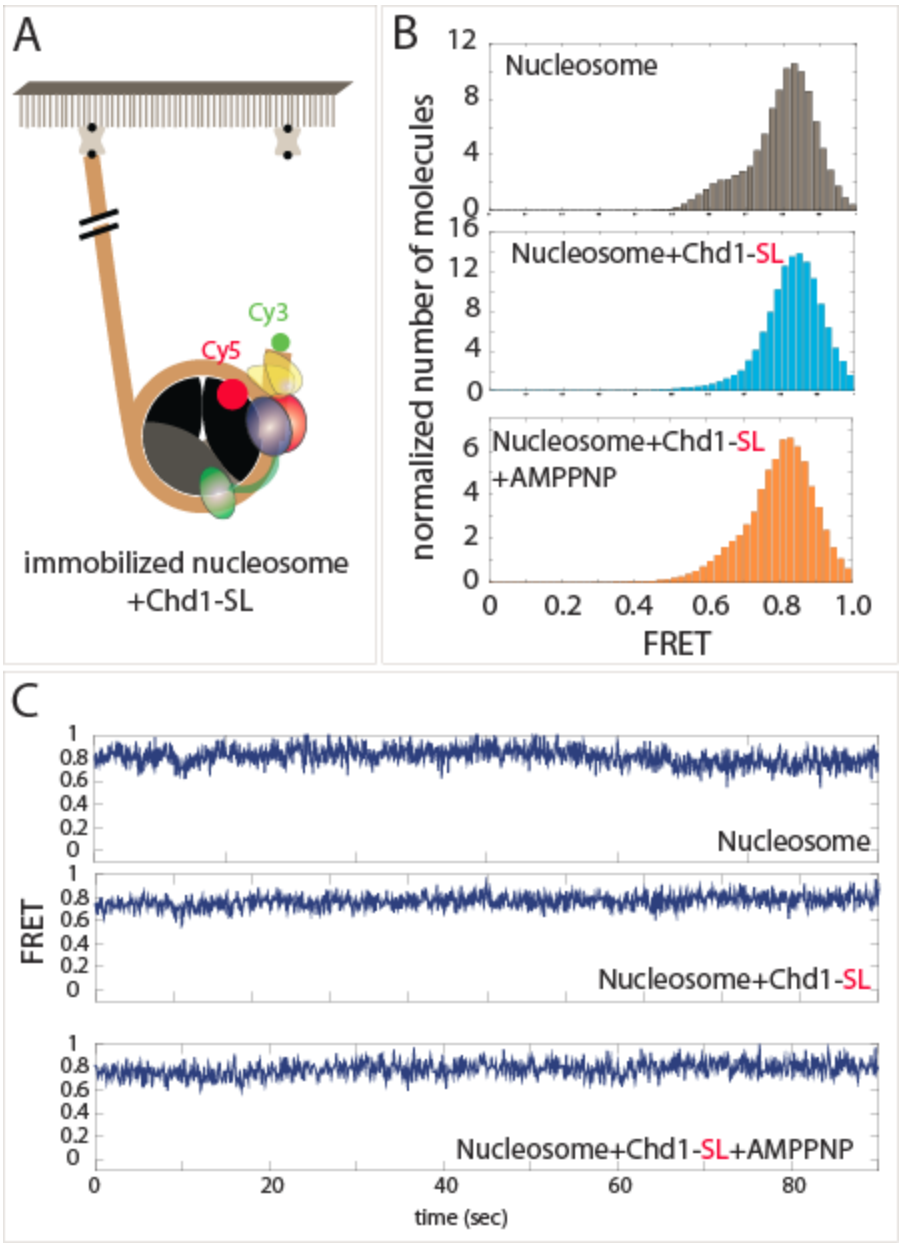

Figure S2. Related to Figure 5. FRET fluctuations of nucleosomes were not observed with Chd1-SL in AMP-PNP (A) Schematic of the smFRET experiment. (B) FRET histogram taken at each condition as stated. (C) smFRET traces in each condition. Unlike in ATP, Chd1-SL did not yield FRET fluctuation, indicating that FRET fluctuations seen in Figure 5B (bottom) is ATP hydrolysis dependent.

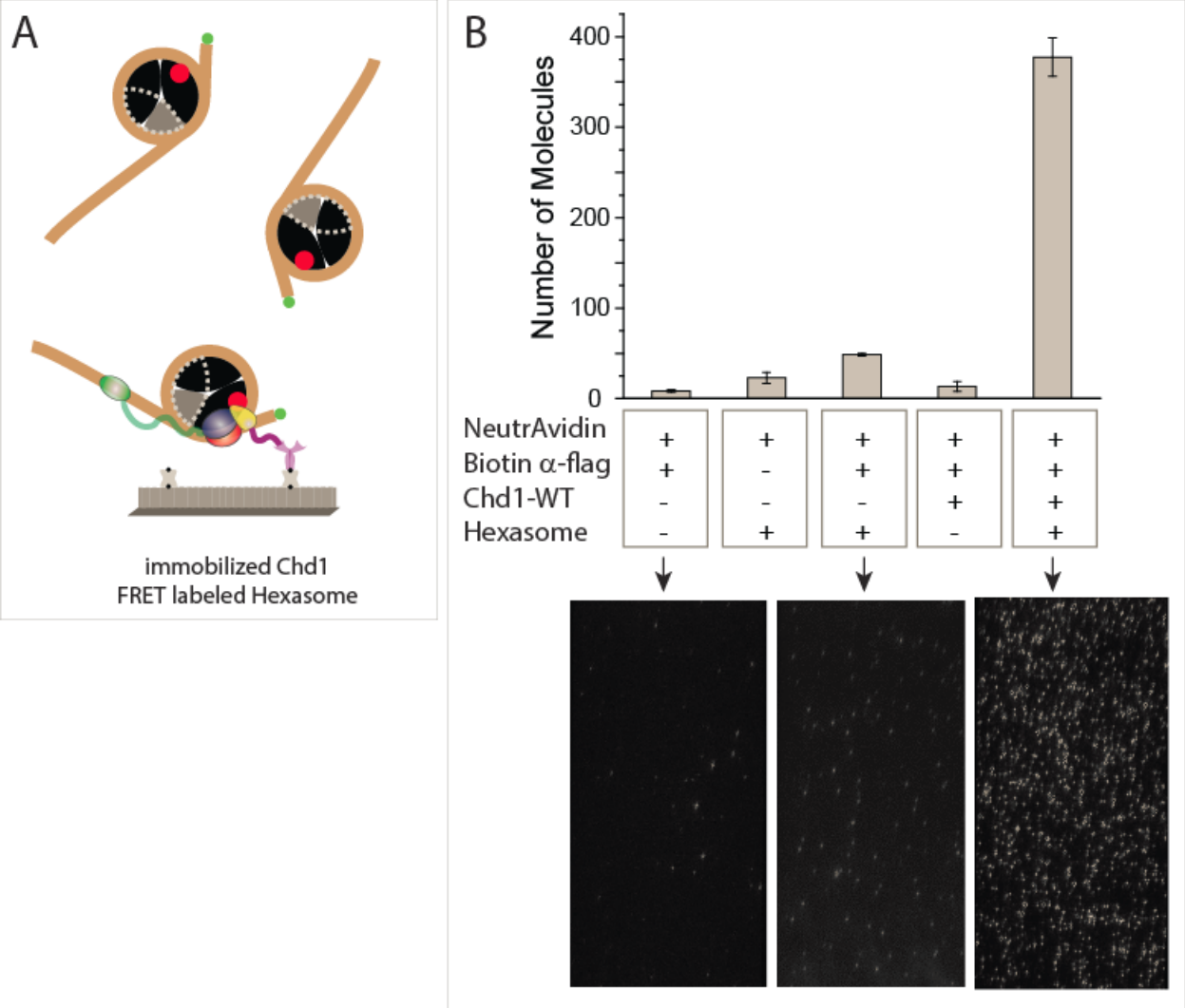

Figure S3. Related to Figure 6. Hexasomes bind to surface immobilized Chd1 (A) Schematic of fluorescently labeled hexasome applied to Chd1 bound surface. (B) Quantification of fluorescent surface-bound particles in varying conditions, which indicates that hexasomes did not bind to the surface coated with NeutrAvidin, anti-flag antibody, but specifically to Chd1.

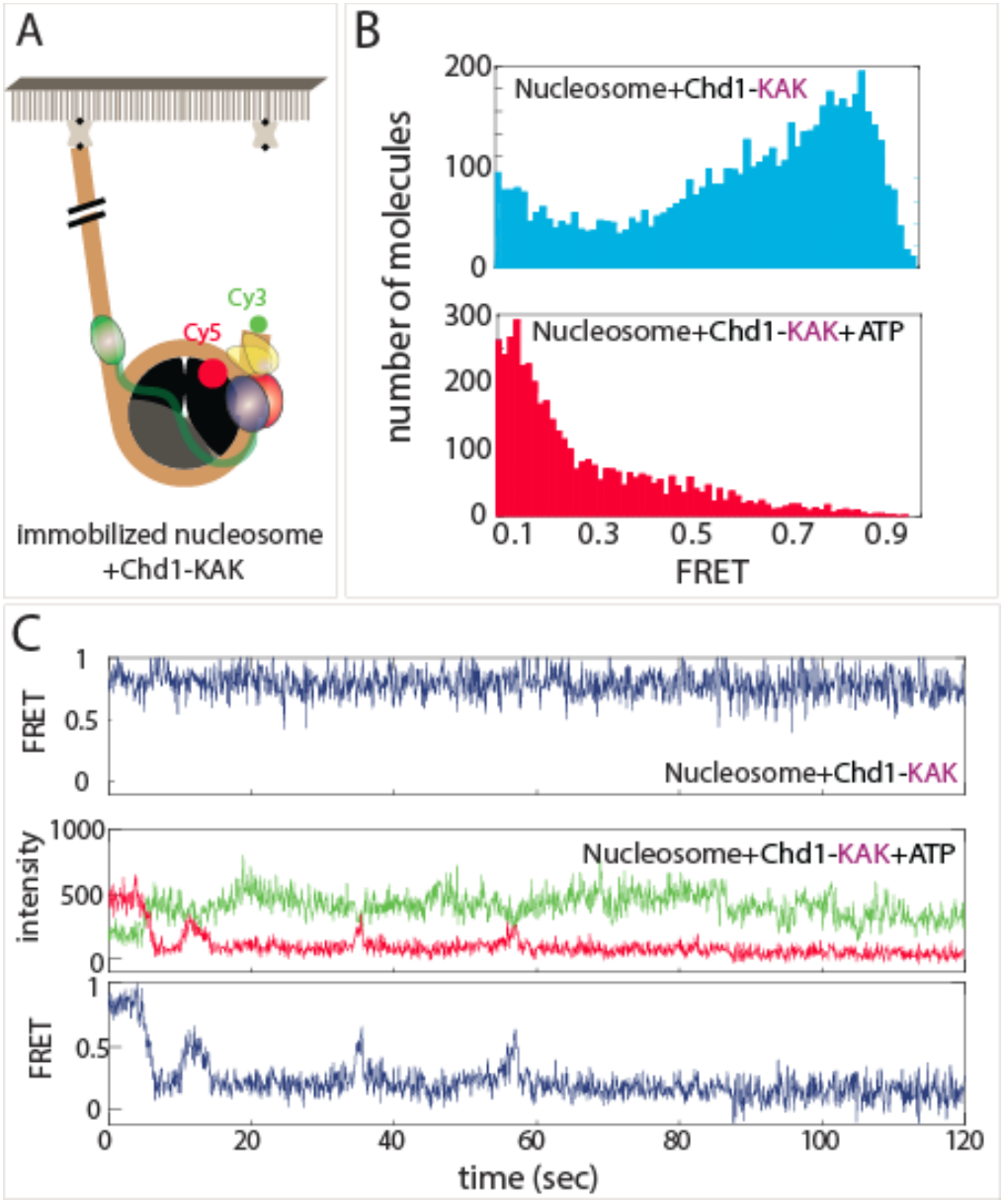

Figure S4. Related to Figure 6. Chd1-KAK mutant repositions nucleosomes (A) Schematic for the experiment in which FRET labeled nucleosome was immobilized to the surface to which the Chd1-KAK mutant was added with and without ATP. (B) FRET histograms of nucleosome + Chd1 before (light blue) and after (red) addition of ATP. (C) Representative FRET traces for conditions in (B).

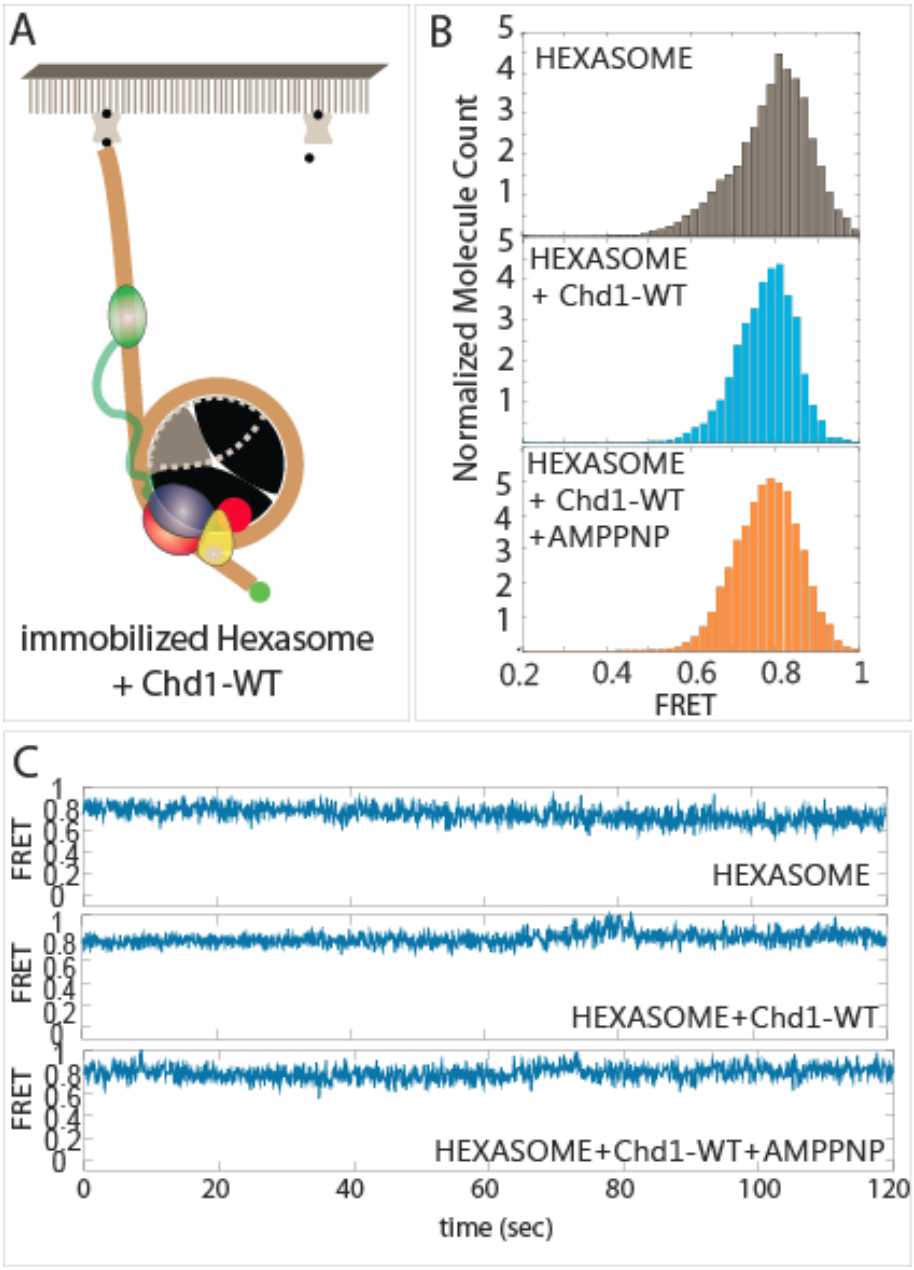

Figure S5. Related to Figure 6. Chd1-WT on hexasomes failed to promote FRET fluctuations in AMP-PNP (A) Schematic of the smFRET experiment. (B) FRET histogram taken at each condition as stated. (C) smFRET traces in each condition. Unlike in ATP, Chd1-WT did not yield FRET fluctuations.

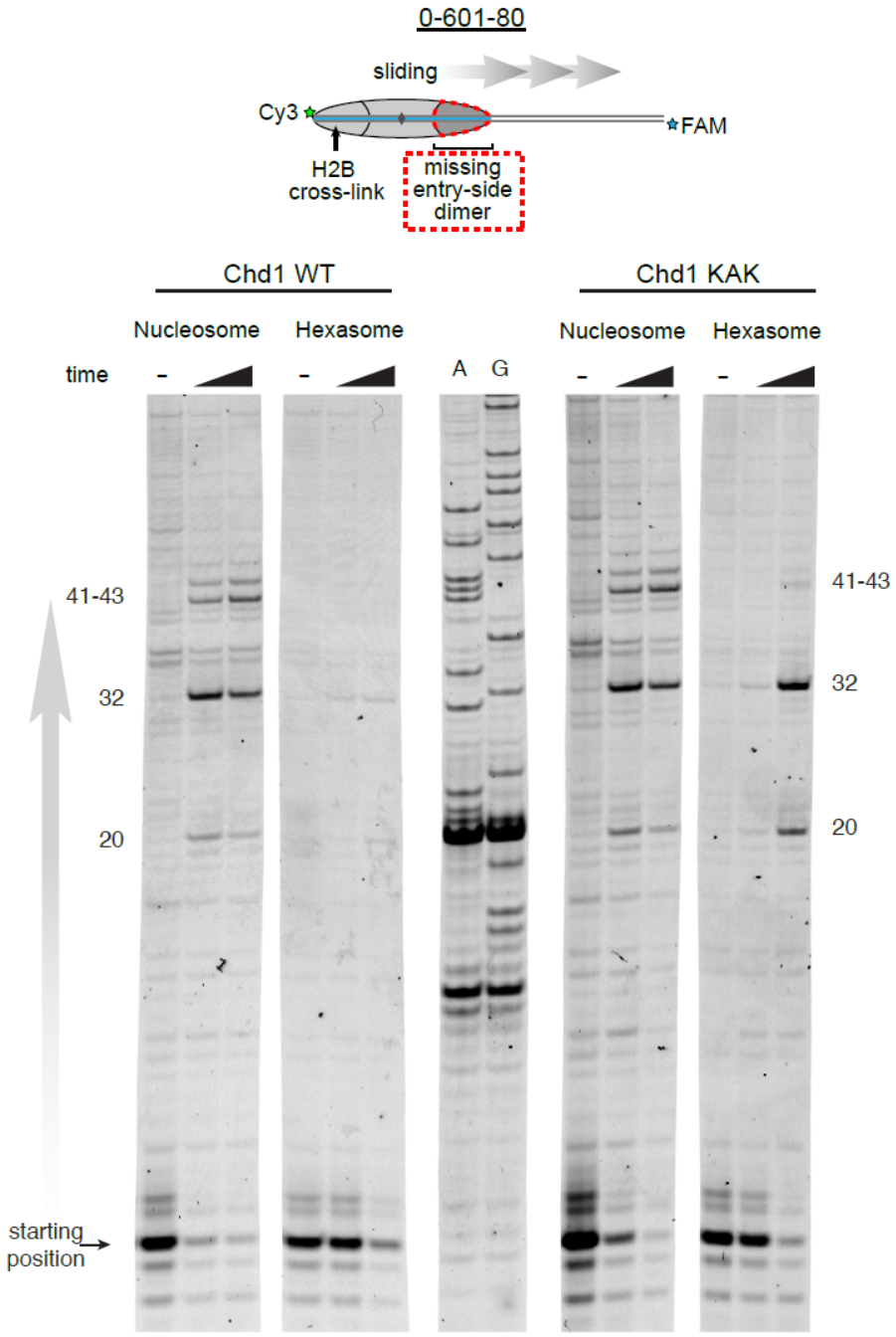

Figure S6. Related to Figure 6. Disruption of the chromo-ATPase interface enables hexasome repositioning.
Nucleosome repositioning by Chd1 was monitored by histone mapping at H2B-S53C. Chd1 protein possessing a wild type interface between the chromodomains and ATPase motor (WT) robustly repositioned nucleosomes but mobilized hexasomes poorly (left). With a E265K/D266A/E268K substitution on the chromodomains (KAK), the ability to shift hexasomes was markedly improved. Gels show H2B-S53C cross-linking to the Cy3-labeled DNA.

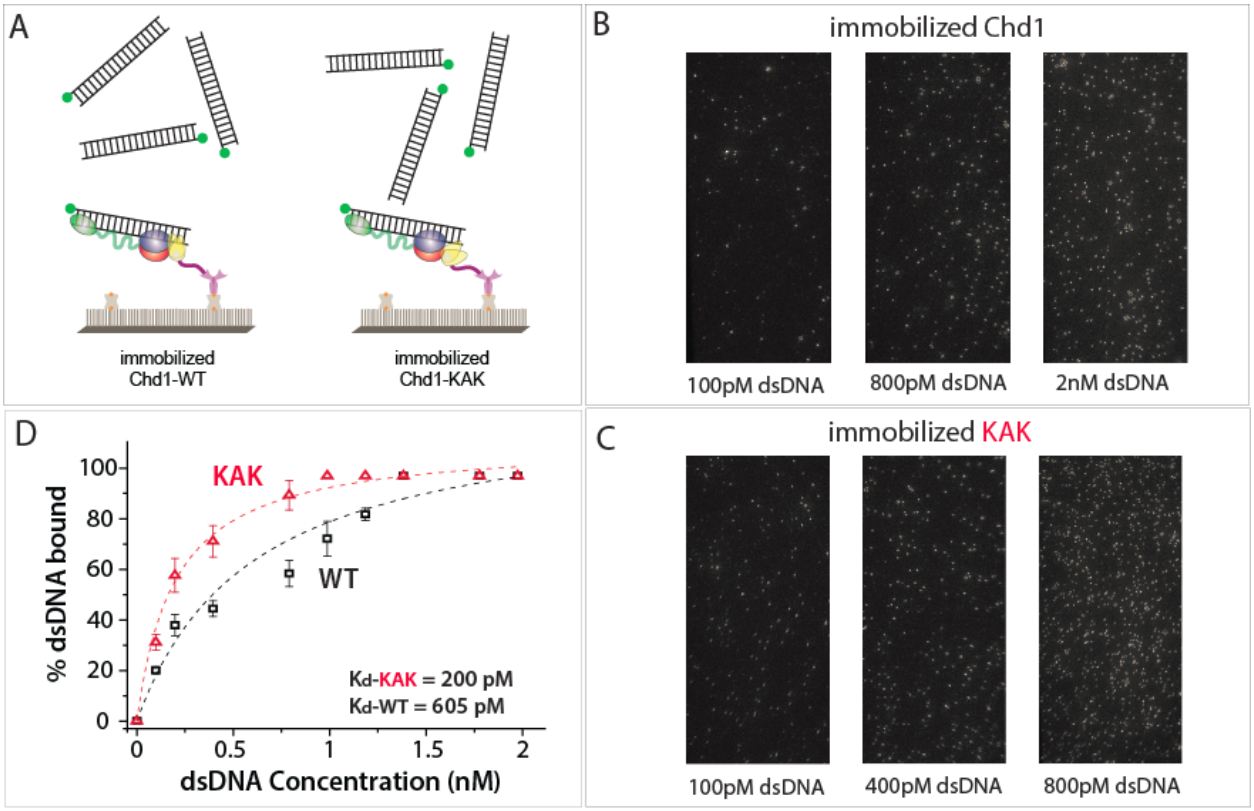

Figure S7. Related to Figure 6. KAK mutant binds naked DNA better than wildtype Chd1 (A) Surface immobilized Chd1 and KAK mutant was applied to the single molecule surface to which Cy3 labeled 40 bp double stranded DNA was added. (B, C) Fluorescence spots appeared upon addition of Cy3 DNA. (D) Calculated $K_{1/2}$ of 40 bp DNA to wildtype Chd1 and KAK mutant, which suggests that disruption of the chromo-ATPase interface improved binding to DNA.

Table S1. All DNA olilgonucleotide sequences used in single molecule experiments and nucleosome reconstitution. Related to Figures 1- 6.

Oligonucleotides
40mer sequence: 5'- ATC CGC GAA GGG CGG CGG CGT GGC GCG TGA ATT CGC GCC C – 3' 5'- GGG CGC GAA TTC ACG CGC CAC GCC GCC GCC CTT CGC GGA T – 3'
60mer sequence: 5'- ATC CCG GGA TCG CCG ACC GGG TGT TCC TGA AGC GCG GGC GTG GCG CGT GAA TTC GCG CCC – 3' 5'- GGG CGC GAA TTC ACG CGC CAC GCC CGC GCT TCA GGA ACA CCC GGT CGG CGA TCC CGG GAT – 3'
80mer sequence: 5'- ATC GCA TCG CAT CGC ATC GGG CCC GGG ATC CCG ACC GGG TGT TCC TGA AGG CGG GCG GGC TGG CGC GTG AAT TCG CGC CC – 3' 5'- GGG CGC GAA TTC ACG CGC CAG CCC GCC CGC CTT CAG GAA CAC CCG GTC GGG ATC CCG GGC CCG ATG CGA TGC GAT GCG AT – 3'
Cy3-3-601 5'/5Cy3/cccTGGAGAATCCCGGTGCCGAGGCCGCTC
Cy3-6-601 5'/5Cy3/ccgcccTGGAGAATCCCGGTGCCGAGGCCG
Cy3-9-601 5'/5Cy3/cggccgcccTGGAGAATCCCGGTGCCGAGG
Cy3-12-601 5'/5Cy3/acgcggccgcccTGGAGAATCCCGGTGCCG
601-80-biotin 5'/5biosg/cggtacccggggatcctctagagtgggagc
601-80-digoxygenin 5'/5digN/cggtacccggggatcctctagagtgggagc
Cy3-4-603 5'/Cy3/tgccCAGTTCGCGCGCCCACCTACCGT
603-80-biotin 5'/5Biosg/tagagctgtctacgaccaattgagcggcct
biotin-80-603 5'/5Biosg/cacaggaaacagctatgaccatgattacgccaag
603-4-Cy3 5'/5Cy3/tgtaCCCCAGGGACTTGAAGTAATAAGGAC
FLAG Insert Nterm ScChd1 (169) sense 5'gaggaaaatgtgcatgaagcatctgccGATTACAAGGAT GACGACGATAAGaatcctcaaccagaggacttccac
FLAG Insert Nterm ScChd1(169) anti 5'gtggaagtcctctggttgaggattCTTATCGTCGTCATCCTTGTAATCggcagatgcttcatgcacattttcctc
Chd1 KAK sense 5'gattagatccatacgttaccgccAaagCtataAagatcatggacatggaacgtg
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