

12N12

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5' CTTTCAGCTGAT AT CAAGATCCCG GTGCCGAGGC CGCTCAATTG GTCGTAGACA GCTCTAGCAC CGCTTAAACG CACGTACGCG C
3' GAAAGTCGACTA TA GTTCTAGGGC CACGGCTCCG GCGAGTTAAC CAGCATCTGT CGAGATCTGT GCGAATTGC GTGCATGCGC G

5' TGTCCCCCGC GTTTTAACCG CCAAGGGGAT TACTCCCTAG TCTCCAGGCA CGTGTGAGAT ATATACATCG AT ATCAGCTGAAAG
3' ACAGGGGGCG CAAAATGGC GGTTC CCTA ATGAGGGATC AGAGGTCCGT GCACAGTCTA TATATGTAGC TA TAGTCGACTTC
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16N16

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5' [Cy3] ACaatt gtgagcgtc ac aattATCCC GTGCCGAGGC CGCTCAATTG GTCGTAGACA GCTCTAGCAC CGCTTAAACG CACGTACGCG C
3' TGttaa cactcgcgag tg ttaaTTAGG CACGGCTCCG GCGAGTTAAC CAGCATCTGT CGAGATCTGT GCGAATTGC GTGCATGCGC G

5' TGTCCCCCGC GTTTTAACCG CCAAGGGGAT TACTCCCTAG TCTCCAGGCA CGTGTGAGAT ATATACATCC TG TGCATGTATT GAACAG
3' ACAGGGGGCG CAAAATGGC GGTTC CCTA ATGAGGGATC AGAGGTCCGT GCACAGTCTA TATATGTAGG AC ACGTACATAA CTTGTC [Cy5]
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12N80

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5' [Cy3] AC GCGGCCGCC TG GAGAATCCCG GTGCCGAGGC CGCTCAATTG GTCGTAGACA GCTCTAGCAC CGCTTAAACG CACGTACGCG C
3' TG CGCCGGCGGG AC CTCTTAGGGC CACGGCTCCG GCGAGTTAAC CAGCATCTGT CGAGATCTGT GCGAATTGC GTGCATGCGC G

5' TGTCCCCCGC GTTTTAACCG CCAAGGGGAT TACTCCCTAG TCTCCAGGCA CGTGTGAGAT ATATACATCC TG
3' ACAGGGGGCG CAAAATGGC GGTTC CCTA ATGAGGGATC AGAGGTCCGT GCACAGTCTA TATATGTAGG AC

5' TGCATGTATT GAACAGCGAC CTTGCCGGTG CCAGTCGGAT AGTGTTCGA GCTCCCACTC TAGAGGATCC CCGGGTACCG
3' ACGTACATAA CTTGTCGCTG GAACGGCCAC GGTGAGCCTA TCACAAGGCT CGAGGGTGAG ATCTCCTAGG GGCCCATGGC
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80N12

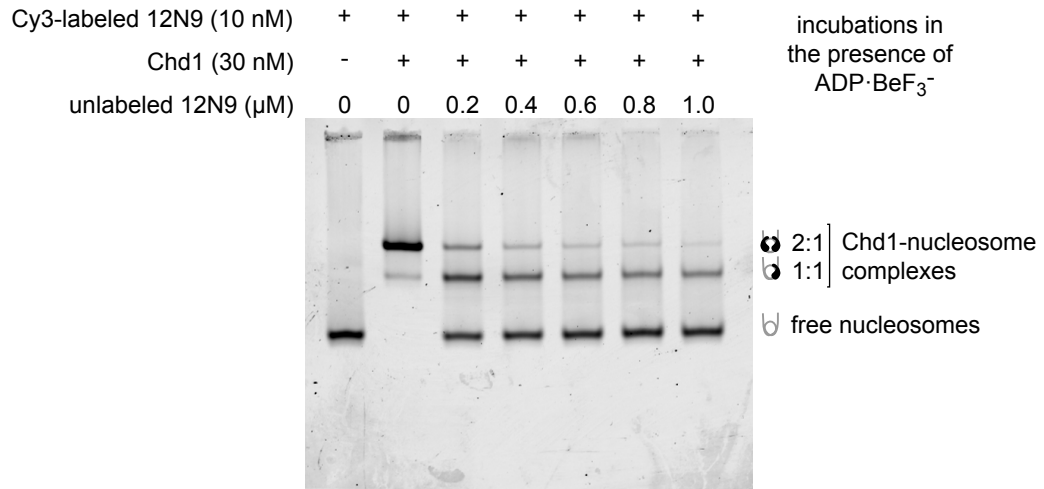
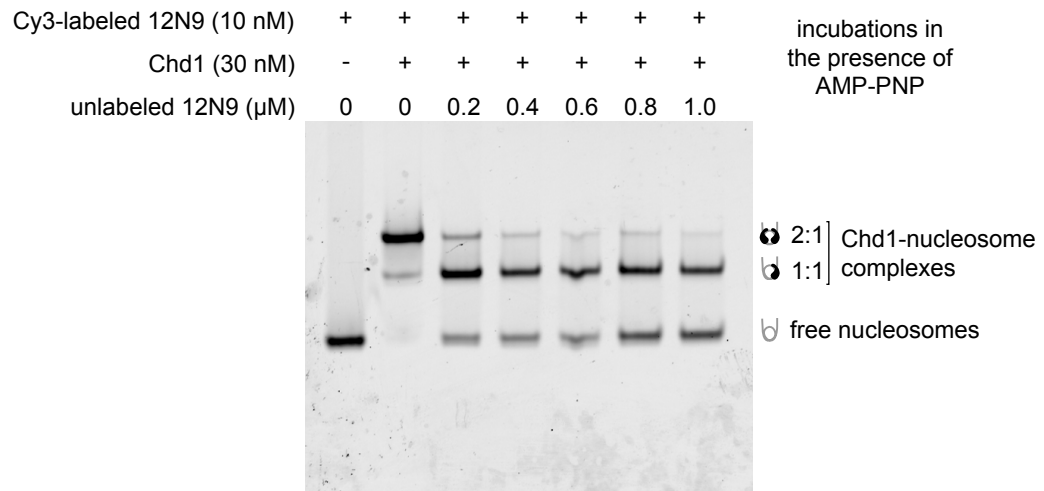
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5' GGGATCCTAA TGACCAAGGA AAGCATGATT CTTACACCG AGTTCATCC TTATGTGATG GACCCTATAC GCGGCCGCC
3' CCCTAGGATT ACTGGTTCCT TTCGTAATAA GAAGTGTGGC TCAAGTAGGG AATACACTAC CTGGGATATG CGCCGGCGGG

5' TG GAGAATCCCG GTGCCGAGGC CGCTCAATTG GTCGTAGACA GCTCTAGCAC CGCTTAAACG CACGTACGCG C
3' AC CTCTTAGGGC CACGGCTCCG GCGAGTTAAC CAGCATCTGT CGAGATCTGT GCGAATTGC GTGCATGCGC G

5' TGTCCCCCGC GTTTTAACCG CCAAGGGGAT TACTCCCTAG TCTCCAGGCA CGTGTGAGAT ATATACATCC TG TGCATGTATT GA
3' ACAGGGGGCG CAAAATGGC GGTTC CCTA ATGAGGGATC AGAGGTCCGT GCACAGTCTA TATATGTAGG AC ACGTACATAA CT [Cy3]
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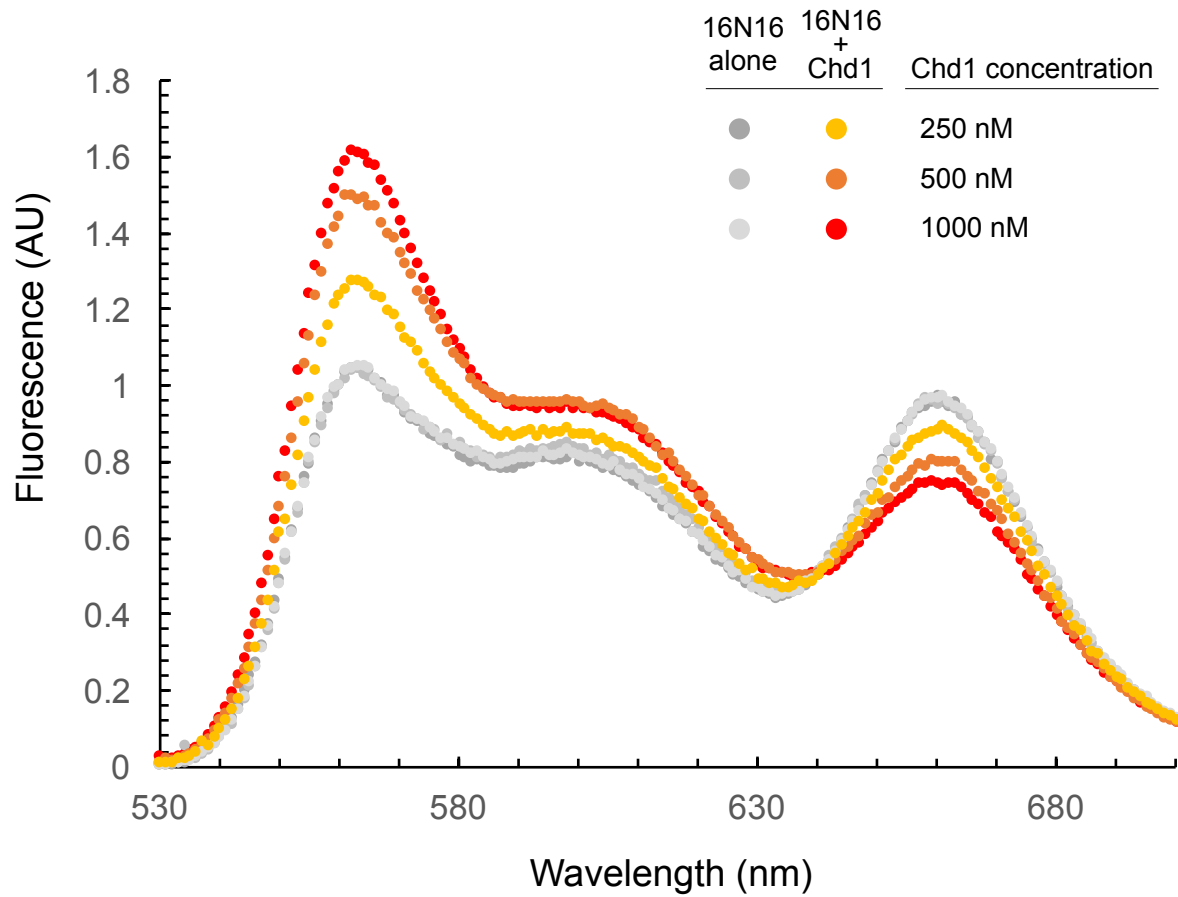
Supplementary Figure S1 Nucleosomal DNA sequences used in this study.

The core Widom 601 nucleosome positioning sequences (145 bp), which are wrapped around the histone core, are highlighted in gray. The dyad is indicated by a triangle, and TA steps are indicated in boldface type. For this orientation of the Widom 601, the left side is TA rich and right side is TA poor. For the 16N16 sequence, lowercase letters indicate the location of the LacO site on one side.

A**B****Supplementary Figure S2****Chd1 binds preferentially to nucleosomes that are Cy3-labeled at H4(A15C)**

(A) Competition experiments in the presence of ADP·BeF₃⁻, where Cy3-labeled 12N9 nucleosomes were incubated with Chd1 in the presence of increasing amounts of unlabeled 12N9 nucleosomes. Samples were separated by native PAGE and visualized on a Typhoon scanner.

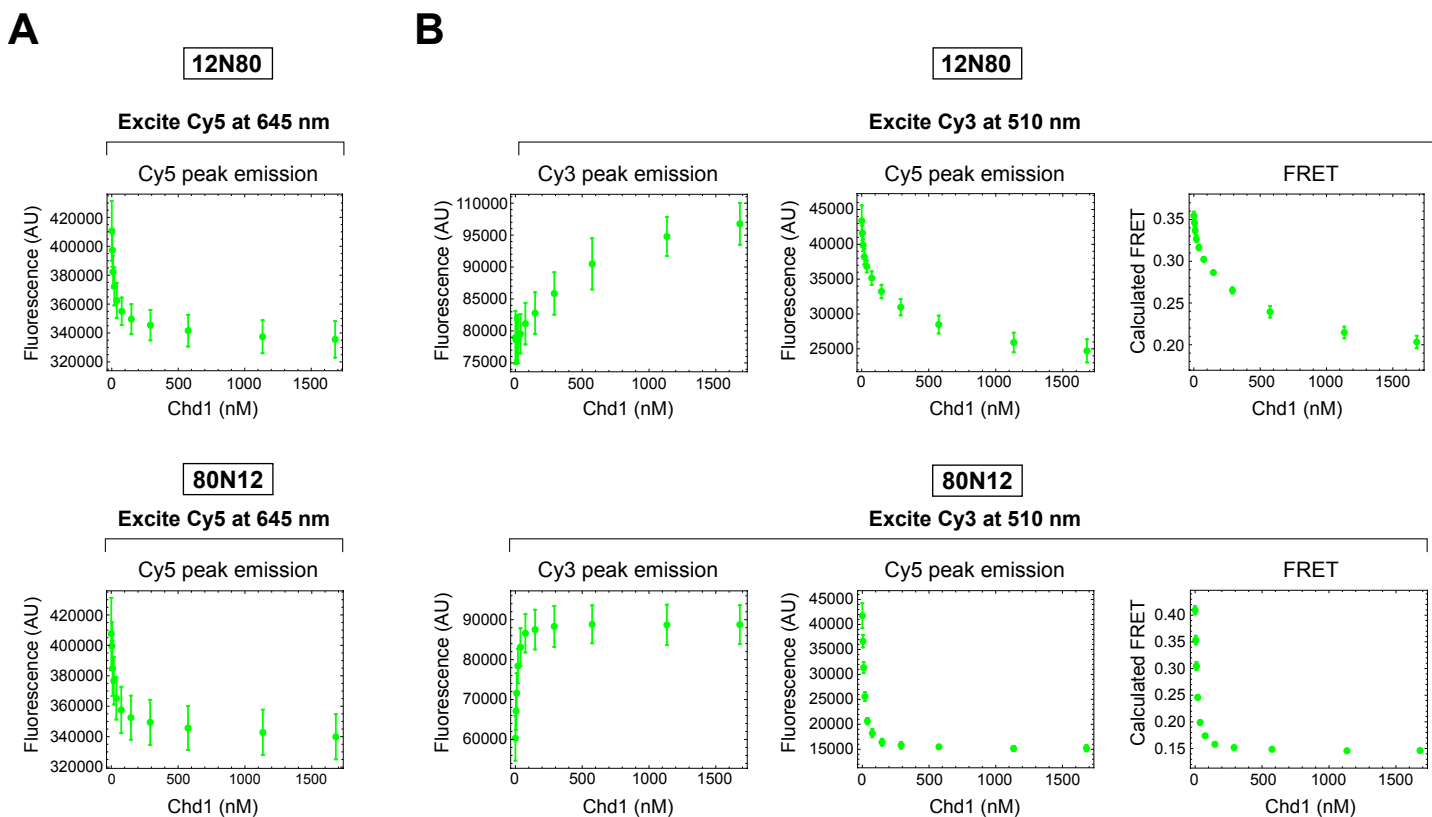
(B) Similar experiment to (A), except incubation conditions contained AMP-PNP. The gels shown in (A) and (B) are representative of three independent experiments.



Supplementary Figure S3

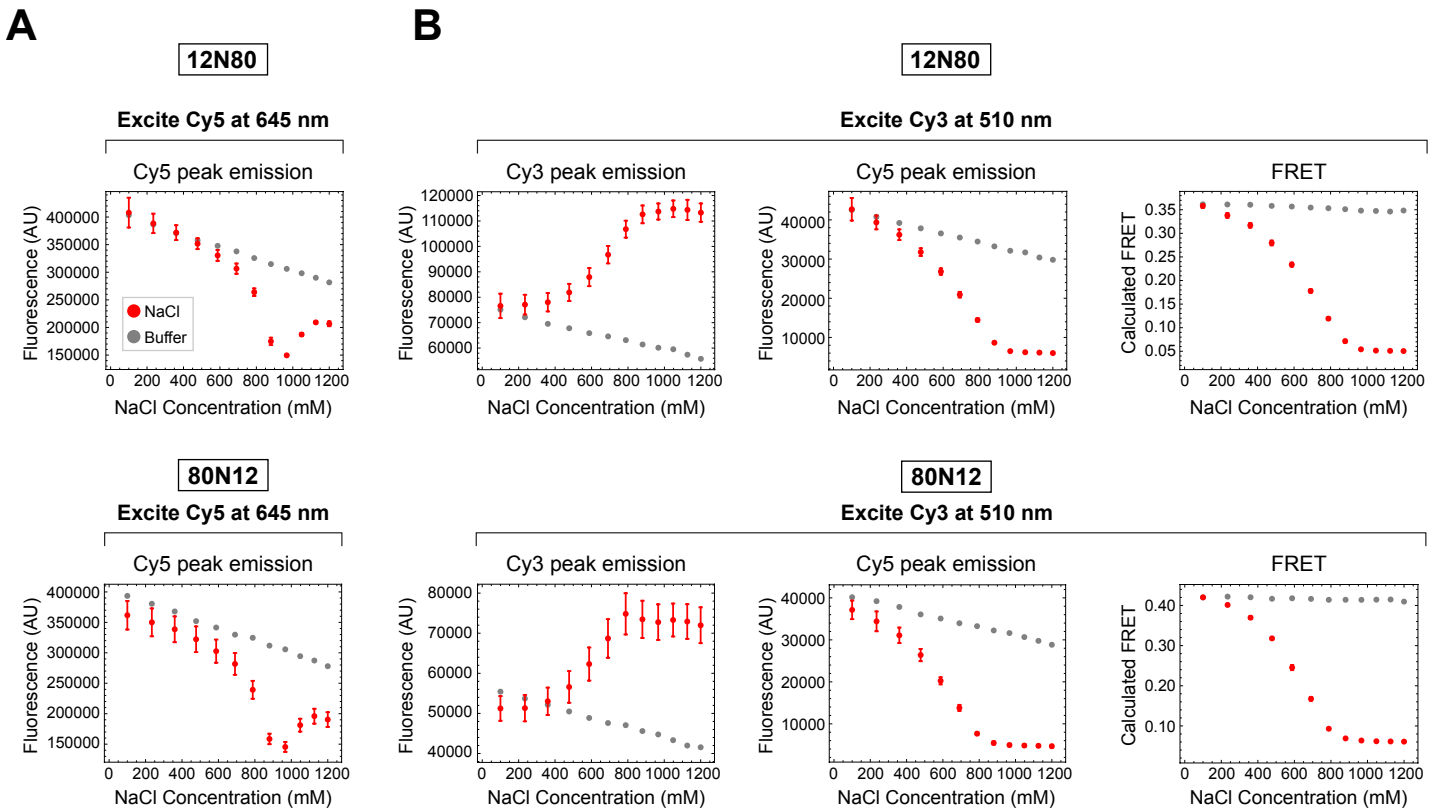
Chd1 unwraps 16N16 nucleosomes in the absence of nucleotide.

Shown are wavelength scans of six independent samples containing either FRET-labeled 16N16 nucleosomes alone (gray) or 16N16 nucleosomes plus Chd1 in the absence of nucleotide (colored). In each case, addition of Chd1 yielded increased intensity of the Cy3 peak (560 nm) and decreased intensity of the Cy5 peak (660 nm), indicative of reduced FRET. Each pair of measurements was scaled to 560 nm intensity for nucleosome alone.



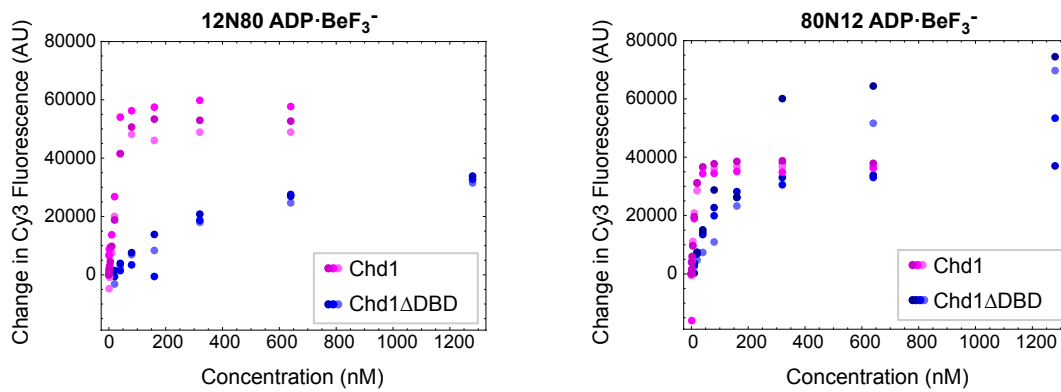
Supplementary Figure S4. Both Cy3 and Cy5 dye positions on the nucleosome are sensitive to the presence of Chd1.

(A) Direct Cy5 excitation was used to determine whether changes in Cy5 fluorescence occur independently of Cy3. For both 12N80 (top) and 80N12 (bottom) nucleosomes, titration of Chd1 correlated with a decrease in peak Cy5 emission. With Cy5 excitation, Cy5 emission for both nucleosomes showed similar profiles. These reactions were carried out in triplicate in 1 mM AMP-PNP conditions, with error bars indicating standard deviations. (B) Cy5 emission after Cy3 excitation yields distinct profiles for 12N80 (top) and 80N12 (bottom) nucleosomes. These data were collected on the same reactions carried out in (A) but with the emissions recorded during Cy3 excitation at 510 nm. For 12N80 nucleosomes, the Cy5 emission peak showed more dramatic changes at lower Chd1 concentrations than the Cy3 emission peak. The sensitivity at low Chd1 concentrations likely reflects the intrinsic changes in Cy5 fluorescence, which means that the calculated FRET profile is not strictly reporting FRET between Cy3 and Cy5, but also environmental changes around Cy5. FRET was calculated as the ratio of the fluorescence intensity (I) of donor (Cy3) and acceptor (Cy5) emission peaks: $I_A/(I_A + I_D)$.



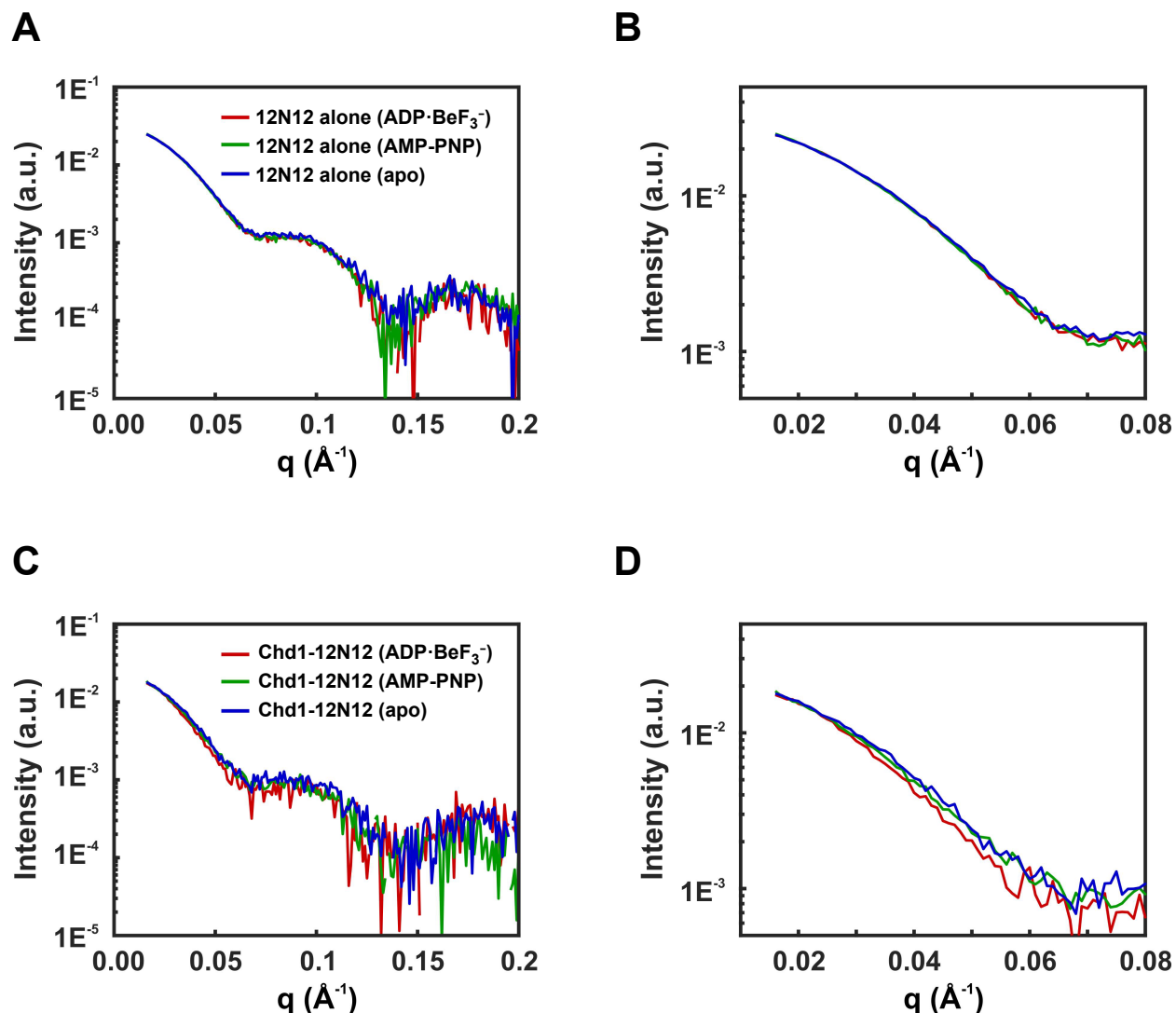
Supplementary Figure S5. Both Cy3 and Cy5 report on salt-induced unwrapping of the nucleosome.

(A) To determine whether the Cy5 label on H3(V35C) was sensitive to nucleosome unwrapping, the Cy5 emission peak was collected during Cy5 excitation at 645 nm for both 12N80 (top) and 80N12 (bottom) nucleosomes in the presence of increasing NaCl (red symbols). To account for reduced fluorescence from dilution, titrations were also performed with buffer alone (gray symbols), which show a roughly linear decrease in intensity, as expected. Nucleosomes are well documented to unwrap at elevated salt, suggesting that the movement of entry/exit DNA is responsible for altering the environment of Cy5. Such a response is not unexpected since a fully wrapped nucleosome restricts the positioning of the H3 tail between the two DNA gyres, and unwrapping would allow for a wider range of positions for the labeled V35C residue. The salt titration was repeated in triplicate, with error bars showing standard deviations. The buffer-alone titration was performed twice with similar results, and one experiment is shown. (B) DNA unwrapping experiments monitored with Cy3 excitation. Data were derived from the same reactions as those shown in (A), differing only in excitation and emission wavelengths. FRET was calculated as the ratio of the fluorescence intensity (I) of donor (Cy3) and acceptor (Cy5) emission peaks: $I_A/(I_A + I_D)$.



Supplementary Figure S6. Titrations of Chd1 and Chd1 Δ DBD in ADP·BeF₃⁻ produce comparable changes in Cy3 fluorescence.

Shown are the intensity changes in Cy3 peak emission in the presence of increasing Chd1 (magenta) or Chd1 Δ DBD (blue) in ADP·BeF₃⁻ conditions. Three or four independent titrations are shown for each protein for the 12N80 (left) and 80N12 (right) nucleosomes. Note that the Chd1 titrations saturate at significantly different fluorescence levels for the two nucleosomes. Though the Chd1 Δ DBD titrations also stimulated comparable changes in fluorescence, there was significant variation between trials.



Supplementary Figure S7

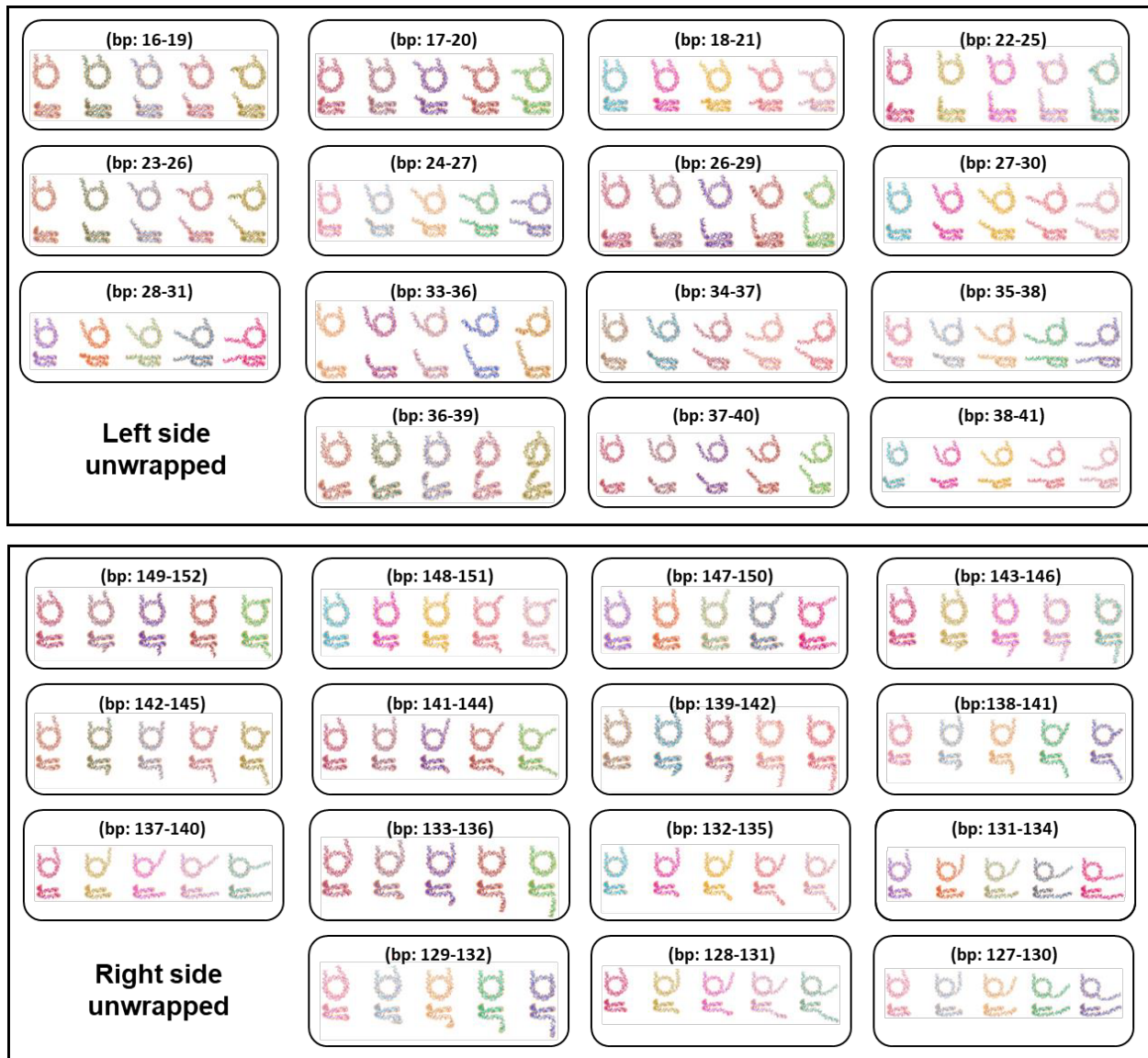
Comparison of SAXS profiles measured with 60% sucrose in the presence or absence of nucleotides.

(A) SAXS profiles for 12N12 nucleosomes in ADP·BeF₃⁻, AMP-PNP, and no nucleotide (apo).

(B) Closer look at low-q region in (A), showing no significant differences in scattering for nucleosomes alone.

(C) SAXS profiles for Chd1 + 12N12 nucleosomes in ADP·BeF₃⁻, AMP-PNP, and apo states.

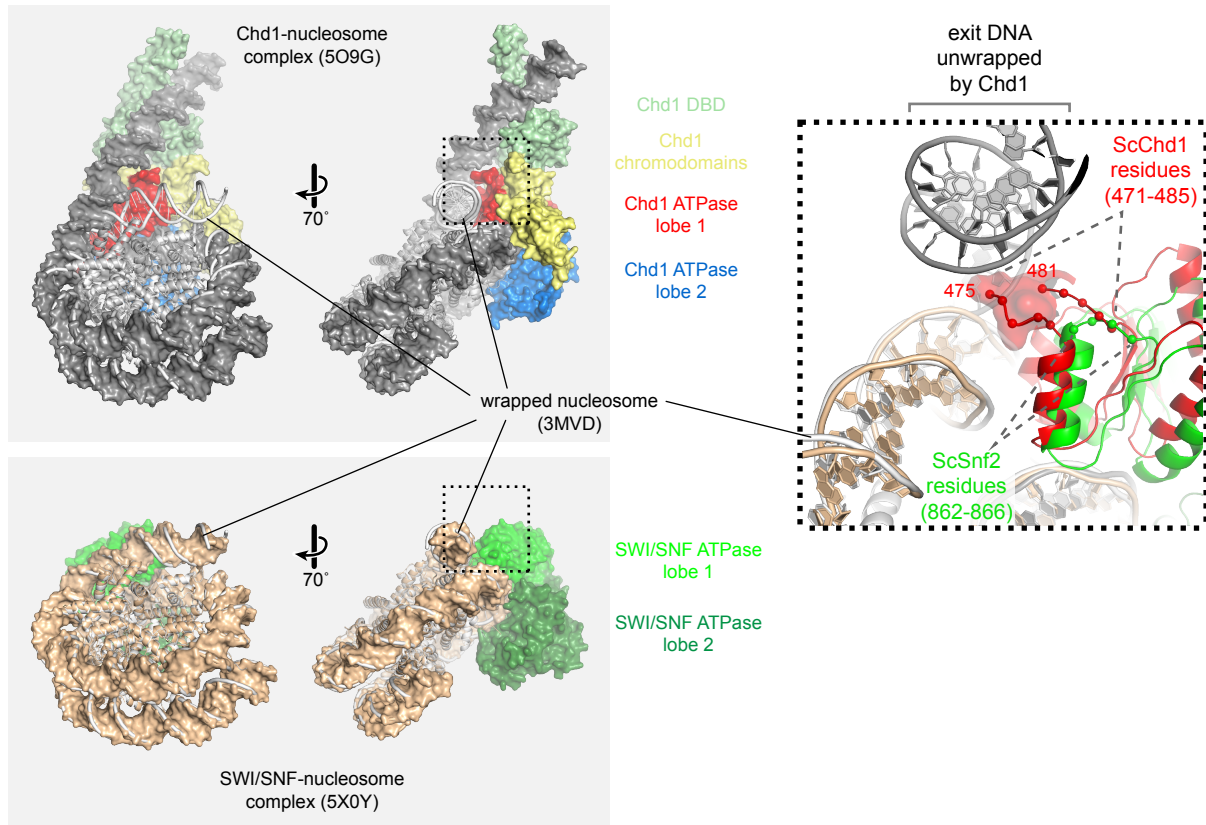
(D) Closer look at low-q region in (C), showing slight but significant variations in scattering for nucleosome plus Chd1 samples.



Supplementary Figure S8

Variations of DNA bends used to generate kinked DNA pool with both in-plane and out-of-plane unwrapping.

DNA bends were introduced into 12N12 nucleosomes using the 3D-DART server (36) on either the left or right side of the nucleosome. Bending angles of 10° , 30° , 50° , 70° , or 90° were applied as depicted in the 5 structures shown in each box. Each of these bends were applied across 4 basepairs as indicated. Note: some basepair ranges lead to varying degrees of out-of-plane unwrapping. Each variation of left- and right-side unwrapping was combined (by stitching left and right halves of structures together at the dyad) to produce 5,625 total variations of kinked DNA structures.

A**B**

| | 450 | 460 | 470 | 480 | 490 | 500 | 510 | 520 |
|----------|----------------|--------------|-------------|---------------|---------------|-----------|-----------------------|------|
| ScChd1 | DLNLCICMGNQKSR | TIREYEFYTNPR | AKGKKTMKFN | VLLTTYEYILKDR | AELGSIKWC | MAVDEA | HRLKN | 520 |
| YlChd1 | GINYLAYLGNTE | SRKALRDE | FYN | KTGNKKPKFN | VLLTTYEYILKDR | AELGSIKWC | YLADEA | 439 |
| SpHrp1 | DLNSICYTGNT | ESRANIRE | YEFYLS | TNSRRLKFN | NLLTTYEYILKDR | QELNNIR | WYLADEA | 531 |
| SpHrp3 | DMNCISYLGNT | TTSRQVIR | YEFYV | G | TQKIKFN | NLLTTYEYV | LKDRSVLSN | 515 |
| GgCHD1Z | QMNNAVYVLG | HTSRNMIR | THEWHP | QTKRLKFN | NLLTTYEILLKDR | KSFLGGLN | WAFIGVDEA | 618 |
| HsCHD1 | QMNNAVYVLG | HTSRNMIR | THEWTH | QTKRLKFN | NLLTTYEILLKDR | KAFLGGLN | WAFIGVDEA | 621 |
| HsCHD2 | EINVVVYIG | DLMSRNT | IREYEWHS | QTKRLKFN | NLLTTYEILLKDR | KTVLGSIN | WAFIGVDEA | 624 |
| DmCHD1 | DMNVVTYLGD | IKSR | LIQYEWQ | SSKRLKFN | CILTTEIVL | KQFLG | TLOWAALLVDEA | 668 |
| ScIsw1 | DVNAFIILOG | DKER | RAELIQKLLGC | FDVVIAS | YEII | IREKSP | LKINWYIIIDEA | 331 |
| ScIsw2 | NVNVLVLHG | DKDTRAD | IVRNII | EA | FDVLLITS | YEMVIRE | KNALKRLAWCYIVIDEA | 319 |
| HsISWI | TLRSVCLIG | KEQRAAF | VRDVL | EG | FDVVCVTS | YEMLIKES | VSFKFNWR | 315 |
| DmISWI | SLRAVCLIG | QDTRNT | FIRDV | LM | EG | FDVVCVTS | YEMCIRE | 263 |
| ScSth1 | SLNTIYKGT | PNQRHS | LQHQIR | VG | FDVLLTTYE | YI | IKRSLLSKHDWA | 604 |
| ScSnf2 | TLRTISFG | SPN | RRKAKQAK | IRAG | FDVLLTTYE | YI | IKERALLSKV | 901 |
| HsCHD3 | KFYVVTYTG | DKDSRAI | IRENEFS | EDNAIKGGKKA | FKMKRE | EAQVK | FHVLLTSYELITID | 890 |
| HsCHD4 | DMYVVTYTG | DKDSRAI | IRENEFS | EDNAIKGGKKA | SRMKRE | EAQVK | FHVLLTSYELITID | 880 |
| HsCHD5 | DFYVVTYTG | DKESR | VIRENEFS | EDNAIR | SCKRV | ERMKE | EVQIKFHVLLTSYELITID | 854 |
| HsCHD6 | EMNAIVYHG | SQISR | OMIQYEM | YRDAQ | GL | NFLSGV | KFFHVITTFEMILAD | 603 |
| HsCHD7 | ELNVVVYHG | SQASR | TIQLYEM | YKDP | OG | RV | LKGSYKFAHAIITTFEMILTD | 1112 |
| HsCHD8 | EMNTIVYHG | SLASR | OMIQYEM | YCKDS | RG | RLIP | GAYRFDALITTFEMILS | 676 |
| HsCHD9 | DINVVVYHG | SGLISR | OMIQYEM | YRDSQ | GL | RIIR | GAYRFQAIITTFEMILGG | 1004 |
| ScSwr1 | CFKVLTYYG | SPQQR | KKRKC | WPK | PF | AFHVCIV | SYQLVVQDQHSF | 831 |
| DmDomino | GFKILTYYS | GSQKR | RKLKRV | GWTK | PN | AFHVCITS | YKLVVQDQ | 1049 |
| HsSRCAP | SFKILTYYS | GAQKR | RKLKRV | GWTK | PN | AFHVCITS | YKLVVQDQ | 753 |
| HsINO80 | KFKVLPYWG | NPHDR | KVIR | FW | SQ | LT | YQDAPFHVVITSY | 659 |
| ScIno80 | QFKILPYWG | NAND | DRKVL | RK | FW | DR | KNLLRYSKNAP | 854 |

Figure S9. The ATPase motor of Chd1 possesses a basic loop that interacts with unwrapped DNA.

(A) Different views of Chd1-nucleosome (5O9G; Farnung et al., 2017) and SWI/SNF-nucleosome (5X0Y; Liu et al., 2017) cryoEM complexes, comparing the length and placement of the basic loop that protrudes from the Chd1 ATPase motor. For reference, both complexes are shown superimposed with a fully wrapped 601 nucleosome structure (3MVD; Makde et al., 2010). The residues highlighted in the zoomed window correspond to the gray bracket in (B). Note that residues 476-480 in the Chd1 structure were not visible due to disorder.

(B) Sequence alignment of several remodeler families, centered on the loop (gray bracket) of the Chd1 ATPase motor that contacts unwrapped exit DNA in the cryoEM structure (5O9G). Abbreviations: *Saccharomyces cerevisiae* (Sc); *Yarrowia lipolytica* (Yl); *Schizosaccharomyces pombe* (Sp); *Gallus gallus* (Gg); *Homo sapiens* (Hs); *Drosophila melanogaster* (Dm).