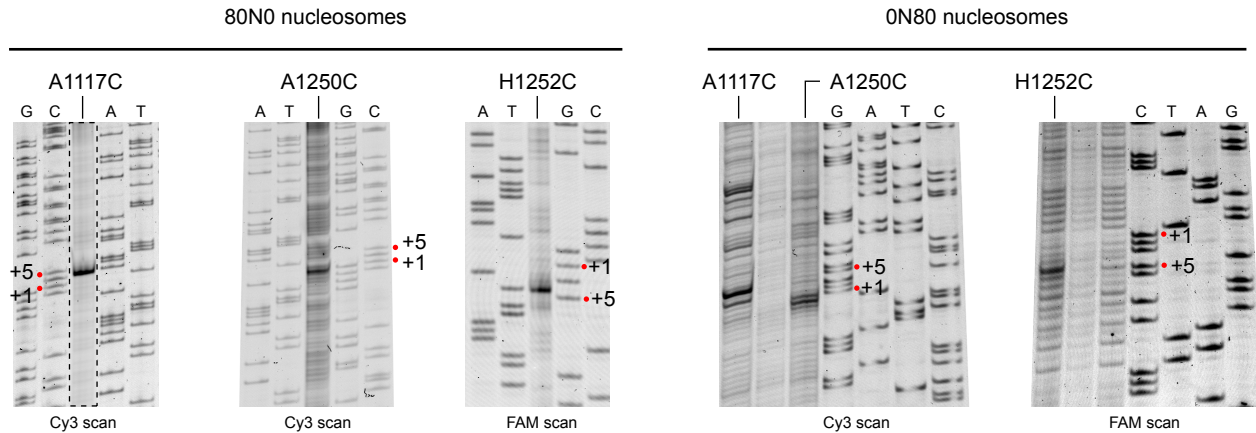
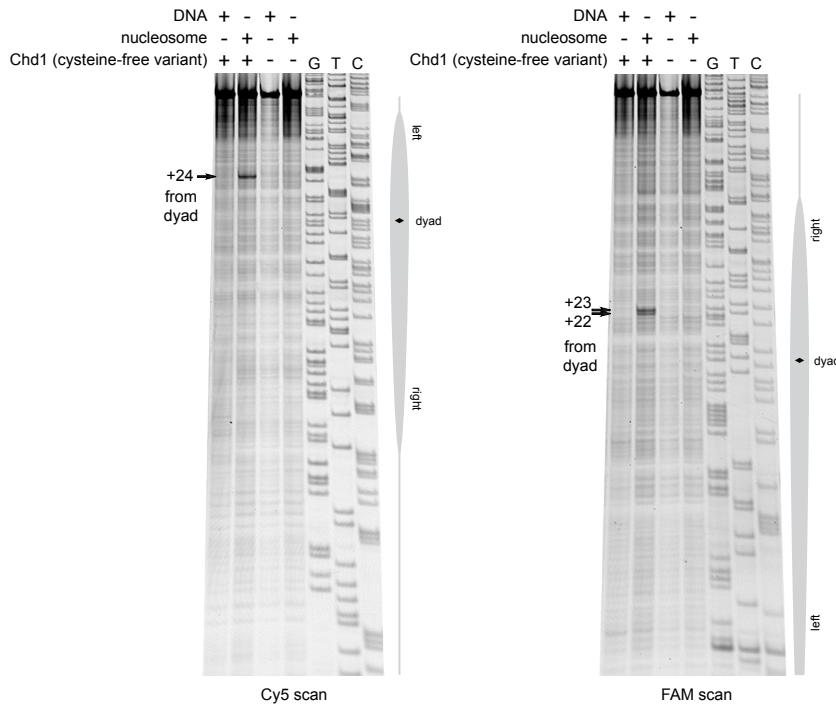


**A**



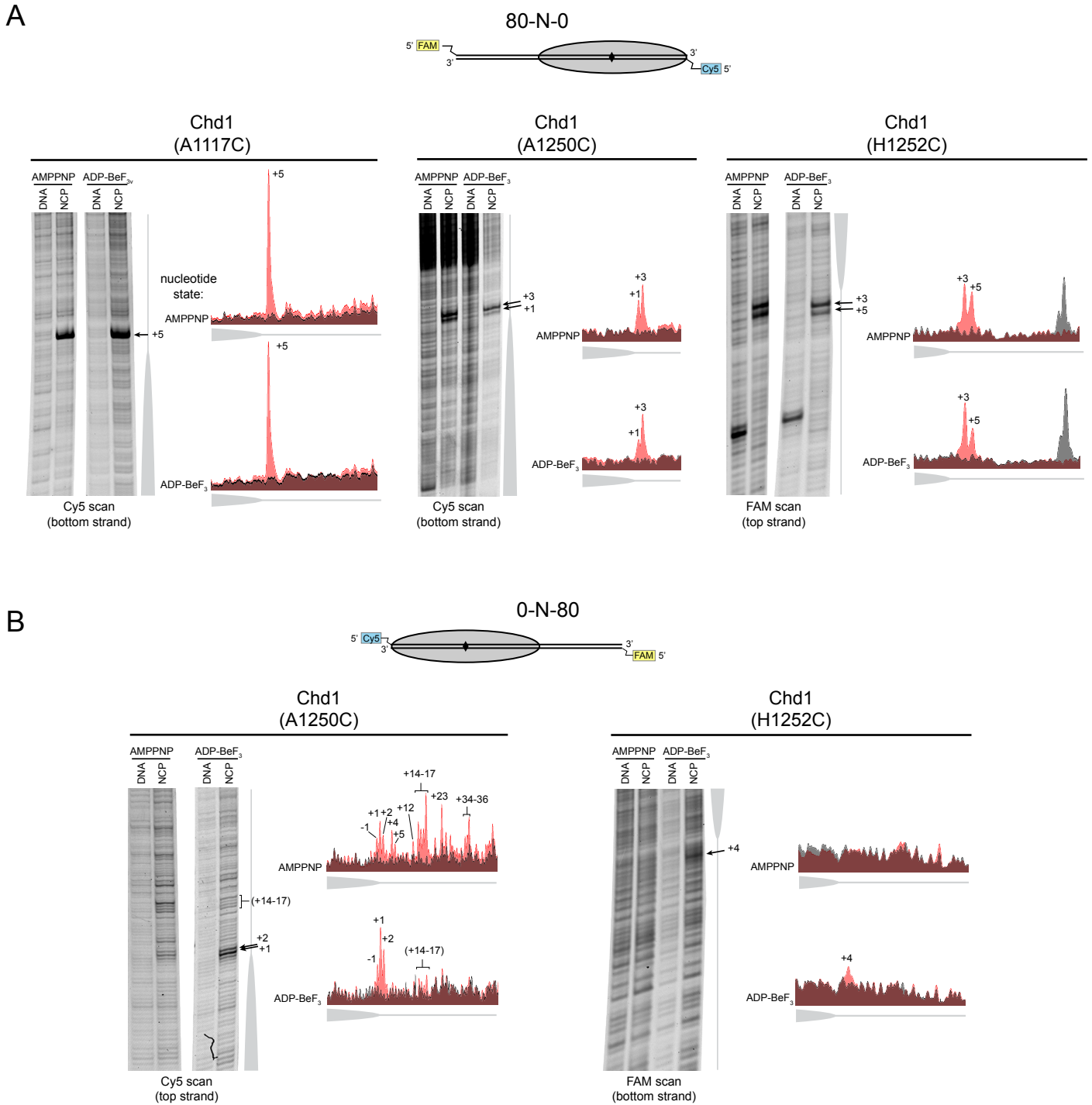
**B**



**Figure S1. Related to Figure 1.** Identification of cross-linking positions using dideoxy sequencing ladders.

(A) Cross-linking sites from cysteines introduced on the Chd1 DNA-binding domain. The 80N0 nucleosomes had a LacO site spanning the nucleosome edge, starting at position -11, whereas the ON80 nucleosomes had no changes to the core 601 sequence. The dotted box indicates part of the gel contrasted differently to more easily identify band position. Red dots mark the first and fifth nucleotides immediately outside the nucleosome. The sequencing ladders were calibrated using potassium permanganate-generated cleavage reactions.

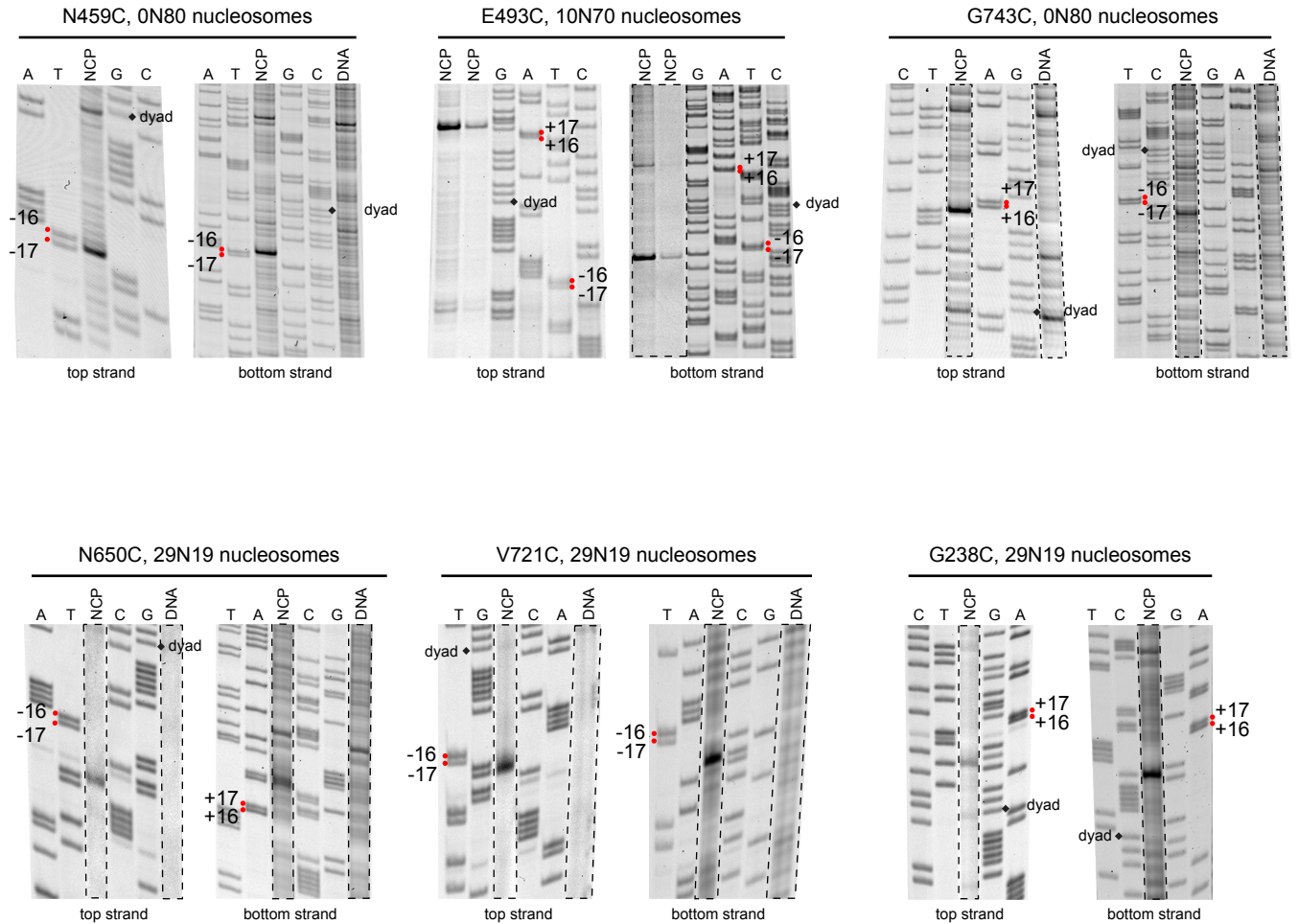
(B) Cross-linking from the cysteine-free variant of Chd1. These reactions used FAM-11N70-Cy5 nucleosomes containing a LacO site starting at position -11 on the left side. As indicated in the figure, the APB-dependent cross-link was only apparent when Chd1 was included in the reaction. This cross-link was strongest in ADP-BeF<sub>3</sub> conditions and observed with different nucleosome constructs.



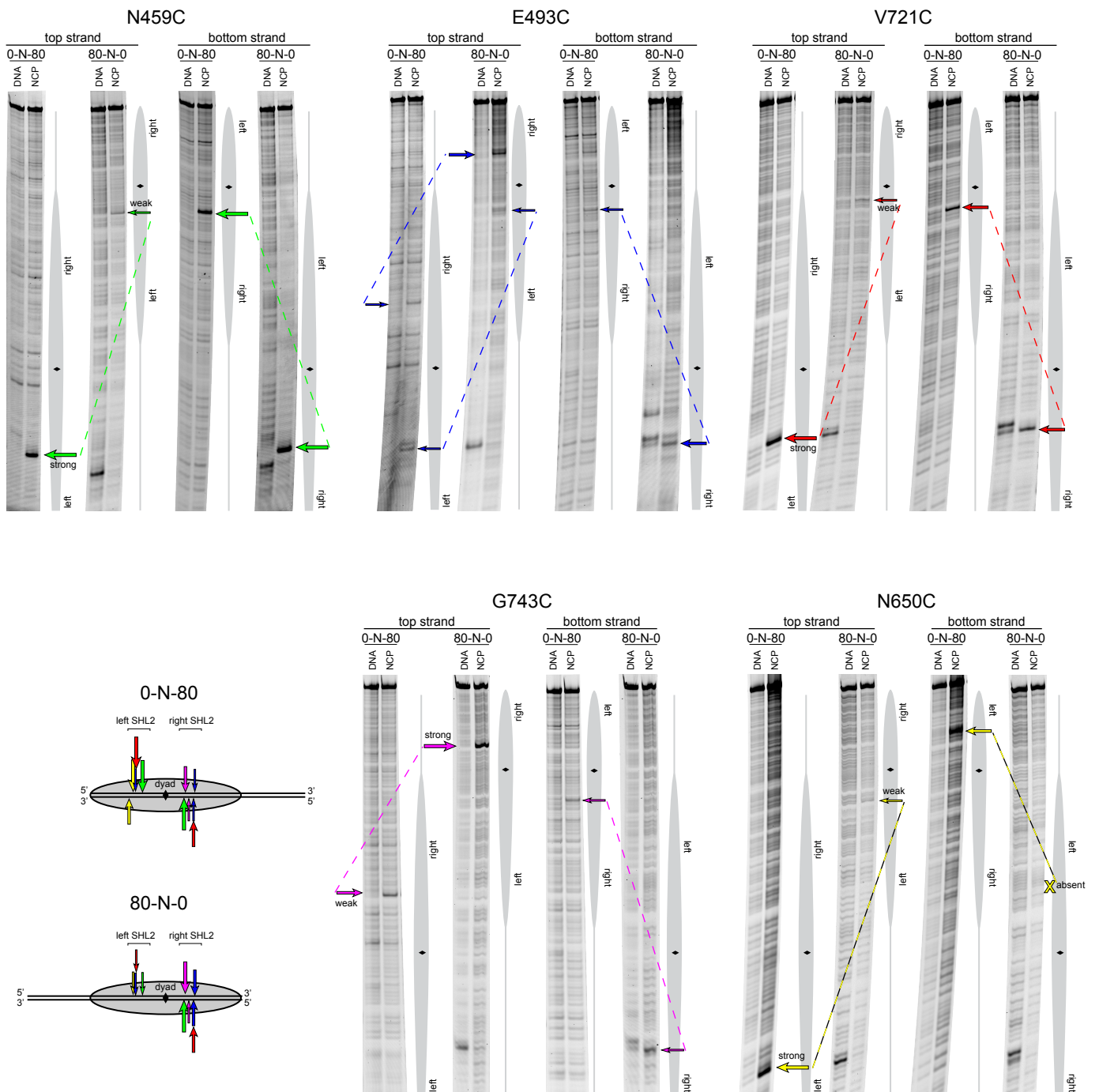
**Figure S2. Related to Figure 2.** Nucleotide dependence of cross-linking the Chd1 DNA-binding domain.

(A) Cross-linking of Chd1 variants to 80-N-0 nucleosomes in the presence of either AMP-PNP or ADP-BeF<sub>3</sub>. The 80-N-0 nucleosome for the A1117C reaction contained a LacO-11 site.

(B) Cross-linking of A1250C and H1252C variants to 0-N-80 nucleosomes in the presence of AMP-PNP or ADP-BeF<sub>3</sub>. Cross-linking for the A1117C variant is shown in Figure 2.

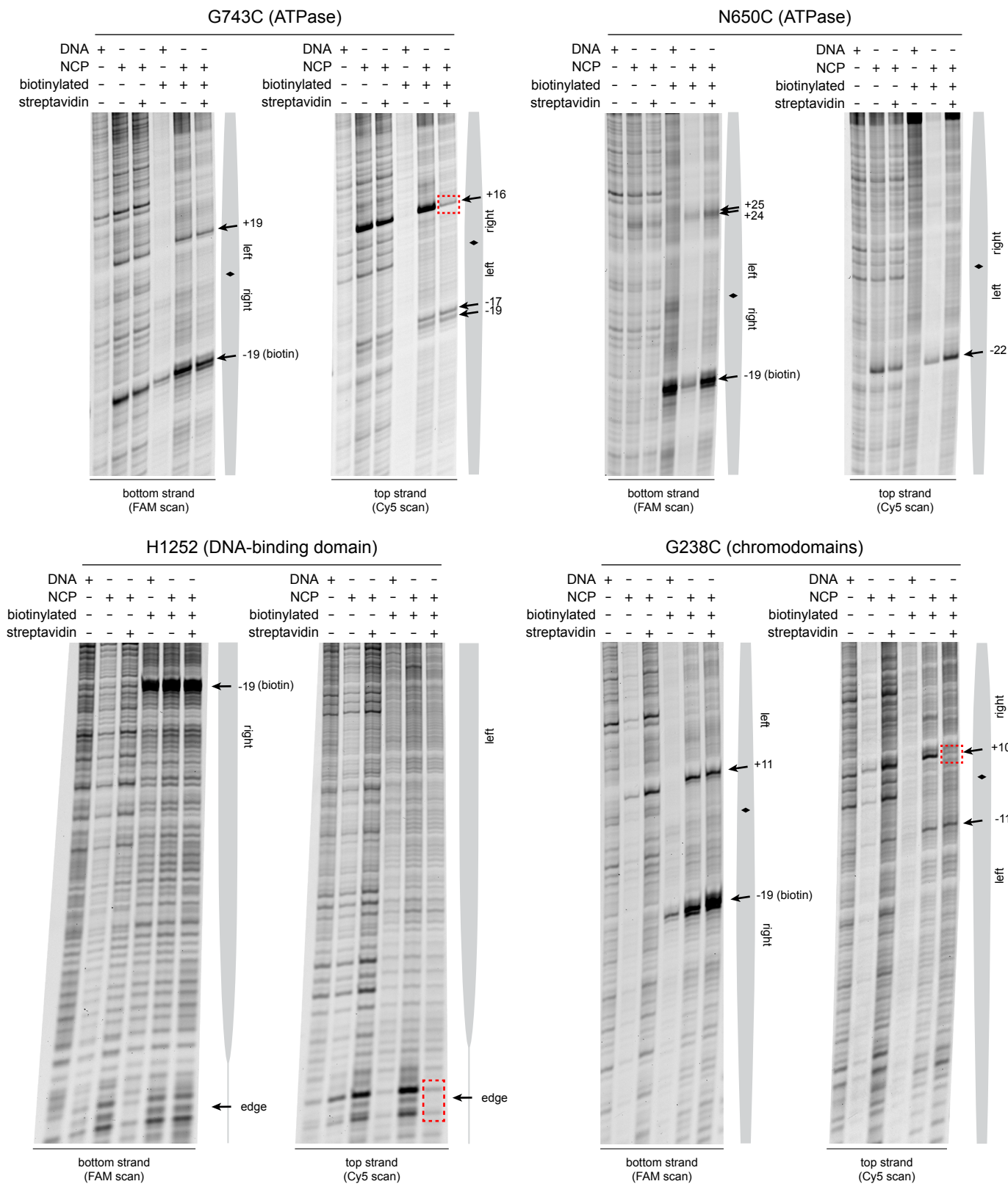


**Figure S3. Related to Figure 4.** Identification of cross-linking positions of the Chd1 ATPase motor and chromodomains using dideoxy sequencing ladders. Negative values indicate nucleotides 5' relative to dyad, positive values are 3' from the dyad. The sequencing ladders were calibrated using potassium permanganate cleavage reactions to obtain final cross-linking positions. The dotted boxes indicate regions of the gels given different contrast.



**Figure S4. Related to Figure 4.** The presence of extranucleosomal DNA affects cross-linking from the Chd1 ATPase motor. Shown are side-by-side cross-linking reactions in ADP-BeF<sub>3</sub> for 0N80 and 80N0 nucleosomes for each of the cysteine variants of the Chd1 motor. The dotted lines indicate positions that are equivalent in sequence between the two nucleosome substrates. Schematic on the left summarizes the relative strength of cross-links for each nucleosome substrate.





**Figure S5. Related to Figure 5.** Analysis of cross-linking from the ATPase motor, DBD, and chromodomains of Chd1 in the presence and absence of a streptavidin-biotin block. Cross-linking reactions were performed in ADP-BeF<sub>3</sub> with 3:1 Chd1:nucleosome stoichiometry, using biotinylated and non-biotinylated 29-N-19 nucleosomes. Dotted red boxes indicate positions where cross-linking was reduced due to the presence of the streptavidin-biotin block. Consistent with across-the-gyre interactions, the cross-links from the DBD are reduced on the left side, whereas cross-links from the ATPase and chromodomains are reduced on the right side, where the biotin is located.

**Table S1. Related to Figure 3.****Measured affinities for nucleosomes**

	nucleotide state	nucleosome	KCl (mM)	affinity ( $K_{1/2}$ , nM)
Chd1	ADPBeF <sub>3</sub>	2N61	100	3.5 ± 0.4
	ADPBeF <sub>3</sub>	2N61	150	4.3 ± 1.4
	AMP-PNP	0N63	100	8.4 ± 1.6
	AMP-PNP	0N63	150	159 ± 20
	ADPBeF <sub>3</sub>	0N0	100	5.9 ± 1.3
	ADPBeF <sub>3</sub>	0N0	150	12 ± 4
	AMP-PNP	0N0	100	143 ± 10
	AMP-PNP	0N0	150	610 ± 60
Chd1(ΔDBD)	ADPBeF <sub>3</sub>	2N61	100	10 ± 3
	ADPBeF <sub>3</sub>	2N61	150	21 ± 5
	AMP-PNP	0N63	100	465 ± 8
	AMP-PNP*	0N63	150	2400 ± 700

\* note that these measurements did not reach saturation and therefore likely underestimate  $K_{1/2}$ .

## Table S2, related to STAR Methods.

### Primers used for generating nucleosomal DNA

**40-601 5' primer (FAM)**

5' ATCCGACTGGCACCGCAAGGTCGCTGTTC

**601-40 3' primer (Cy5)**

5' AGTTCATCCCTTATGTGATGGACCCTATAC

**80-601 5' primer (FAM)**

5' CGGTACCCGGGGATCCTCTAGAGTGGGAGC

**601-0 3' primer (Cy3 or unlabeled)**

5' TGGAGAATCCCGGTGCCGAGGCCGCTC

**0-601 5' primer (FAM or Cy3)**

5' CAGGATGTATATATCTGACACGTGCCTGG

**601-80 3' primer (FAM)**

5' CGGGATCCTAATGACCAAGGAAAGCATGATTC

**601-63 3' primer**

5' GGAAAGCATGATTCTTCACACCGAGTTC

**11-601(LacO-11L) 5' (FAM)**

5' FAM CA aattgtgag cgctcacaatt T ATCTGACAC

**601-70 3' primer (Cy5)**

5' TGACCAAGGAAAGCATGATTCTTCACACCG

**29-601 5' (Cy5)**

/5Cy5/ACCGCAAGGTCGCTGTTCAATACATGC

**601-19[-19biotinT] 3' (FAM)**

/56-FAM/ accctatac gggccgccc TG GAGAATCCCG GTGCCGAGGC CGCTCAATTG  
GTCGTAGacA GCTCTAGCAC C/[iBiodT](#)/CTTAAACG CACGTACG

**601-19 3' (FAM)**

/56-FAM/ accctatac gggccgccc TG GAGAATCCCG GTGCCGAGGC CGCTCAATTG  
GTCGTAGacA GCTCTAGCAC CTCTTAAACG CACGTACG

## Table S3, related to STAR Methods.

### Templates used for PCR amplification of nucleosomal DNA

Widom 601 (Nucleosome positioning sequence capitals; dyad underlined):

```
      +80      +70      +60      +50      +40      +30      +20      +10
      |        |        |        |        |        |        |
5'  cggta+cccg+ ggatc+ctcta+ g+agtg+gggagc+ tcgga+a+ca+ct+ atccg+a+ctg+g+ caccg+gca+ag+ gtcg+tgttc+ aata+catgca+

      -70      -60      -50      -40      -30      -20      -10      0
      |        |        |        |        |        |        |
5'  CA GGATGTATAT ATCTGACACG TGCCTGGAGA CTAGGGAGTA ATCCCCTTGG CGGTTAA+AAC GCGGGGGACA G

      10      20      30      40      50      60      70
      |        |        |        |        |        |
5'  CGCGTACGTG CGTTTAAGCG GTGCTAGAGC TGTCTACGAC CAATTGAGCG GCCTCGGCAC CGGGATTCTC CA

      +10      +20      +30      +40      +50      +60      +70      +80
      |        |        |        |        |        |        |
5'  gggcg+gccgc+ g+tatagg+gtc+ catcacataa+ g+g+gatga+act+ cggtgtga+ag+ aatcatg+c+tt+ tccttg+gtca+ ttaggatccc+
```

601 (LacO-11L) (LacO highlighted in yellow)

```
      +80      +70      +60      +50      +40      +30      +20
      |        |        |        |        |        |
5'  cggta+cccg+ ggatc+ctcta+ g+agtg+gggagc+ tcgga+a+ca+ct+ atccg+a+ctg+g+ caccg+gca+ag+ gtcg+tgttc+

      +10      +1      -70      -60      -50      -40      -30      -20      -10      0
      |        |        |        |        |        |        |        |
5'  aaattgtgag cg ctca+caatt ATCTGACACG TGCCTGGAGA CTAGGGAGTA ATCCCCTTGG CGGTTAA+AAC GCGGGGGACA G

      10      20      30      40      50      60      70
      |        |        |        |        |        |
5'  CGCGTACGTG CGTTTAAGCG GTGCTAGAGC TGTCTACGAC CAATTGAGCG GCCTCGGCAC CGGGATTCTC CA
```

601 (LacO-11R) (LacO highlighted in yellow)

```
      -70      -60      -50      -40      -30      -20      -10      0
      |        |        |        |        |        |        |
5'  CA GGATGTATAT ATCTGACACG TGCCTGGAGA CTAGGGAGTA ATCCCCTTGG CGGTTAA+AAC GCGGGGGACA G

      10      20      30      40      50      60      70      +10
      |        |        |        |        |        |        |
5'  CGCGTACGTG CGTTTAAGCG GTGCTAGAGC TGTCTACGAC CAATTGAGCG GCCTCGGCAC Caattgtgag cg ctca+caatt

      +20      +30      +40      +50      +60      +70      +80
      |        |        |        |        |        |
g+tatagg+gtc+ catcacataa+ g+g+gatga+act+ cggtgtga+ag+ aatcatg+c+tt+ tccttg+gtca+ ttaggatccc+
```

601 (LacO-6R) (LacO highlighted in yellow)

```
      -70      -60      -50      -40      -30      -20      -10      0
      |        |        |        |        |        |        |
5'  CA GGATGTATAT ATCTGACACG TGCCTGGAGA CTAGGGAGTA ATCCCCTTGG CGGTTAA+AAC GCGGGGGACA G

      10      20      30      40      50      60      70      +10
      |        |        |        |        |        |        |
5'  CGCGTACGTG CGTTTAAGCG GTGCTAGAGC TGTCTACGAC CAATTGAGCG GCCTCGGCAC GGGATTaat+t+ gt gagc+gctcac

      +20      +30      +40      +50      +60      +70      +80
      |        |        |        |        |        |
aat+t+gggtcc+ atcacataa+ g+g+atga+act+ ggtgtga+aga+ atcatg+c+tt+ ccttg+gtcat+ taggatccc+
```

## Table S4, related to STAR Methods.

### Array plasmid inserts used for nucleosomal DNA

#### pJ201-601array-208bpx34 (Widom 601, 34 repeats)

digestion with **EcoRI** yields 33N26

digestion with **EcoRV** yields 2N61 (Nucleosome positioning sequence capitals; dyad underlined)

```
5' gaattcggg atgaactcgg tgtgaagaa agatcc  
gat atcAGGATGTATATATCTGACACGTGCCTGGAGACTAGGGAGTAATCCCCTTGGCGGTTAAAACGCGGGGGACAG  
CGCGTACGTGCGTTTAAAGCGGTGCTAGAGCTGTCTACGACCAATTGAGCGGCCTCGGCACCGGGATTCTCCA  
ggg cggccgcgta tagggtccat cag aattcgg atgaactcgg tgtgaagaa agatccgat at
```

#### pJ201-601array-169bpx16 (Widom 601, 16 repeats)

digestion with **EcoRV** yields 145 bp fragment for 0N0 nucleosomes

```
ATC GATGTATAT ATCTGACACG TGCCTGGAGA CTAGGGAGTA ATCCCCTTGG CGGTTAAAAC GCGGGGGACA G  
CGCGTACGTG CGTTTAAAGCG GTGCTAGAGC TGTCTACGAC CAATTGAGCG GCCTCGGCAC CGGGATCTT GAT
```

#### pJ201-601array-178bpx12 (Widom 601, 12 repeats)

digestion with **EcoRV** yields 178 bp fragment for 0N33 nucleosomes

```
ATCGATGTATAT ATCTGACACG TGCCTGGAGA CTAGGGAGTA ATCCCCTTGG CGGTTAAAAC GCGGGGGACA G  
CGCGTACGTG CGTTTAAAGCG GTGCTAGAGC TgtCTACGAC CAATTGAGCG GCCTCGGCAC CGGGATTCTC CA  
ggcggcgcgcatagcaagatcctccataaggat
```