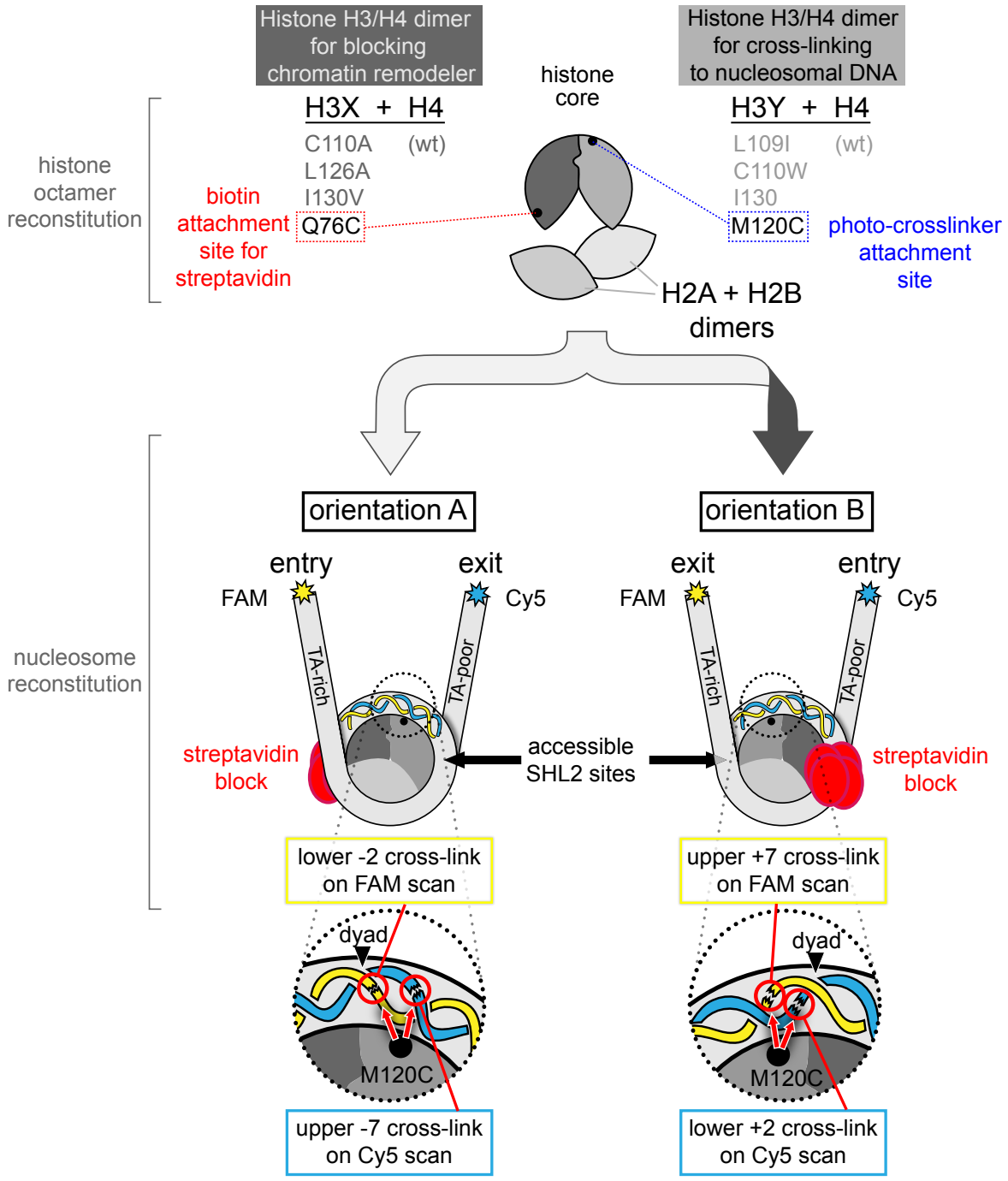


Figure S1. Design for making asymmetrically blocked nucleosomes.
Following the design for generating an asymmetric H3/H4 tetramer, described by Ichikawa et al., (2017, 2018), two copies of H3 were uniquely altered to create a heterodimeric H3X-H3Y interface. In this background, cysteines were introduced to allow for biotinylation on one side (H3X[Q76C]) and DNA cross-linking on the other (H3Y[M120C]). Note that here the Xenopus H3 sequence was used, which unlike yeast H3, has an isoleucine at position 130. Therefore, no change was needed to match the original design for yeast H3Y, which had an L130I substitution. Reconstitution into nucleosomes yields two distinct orientations of H3X/H3Y, denoted A and B. The Widom 601 sequence is asymmetric, with characteristic "TA-rich" and "TA-poor" sides, which here are identified by end-labeling with FAM and Cy5, respectively. Each orientation of H3X/H3Y places H3Y-M120C on opposite sides of the dyad, yielding unique single cross-links on either strand (yellow for FAM-labeled and blue for Cy5-labeled).

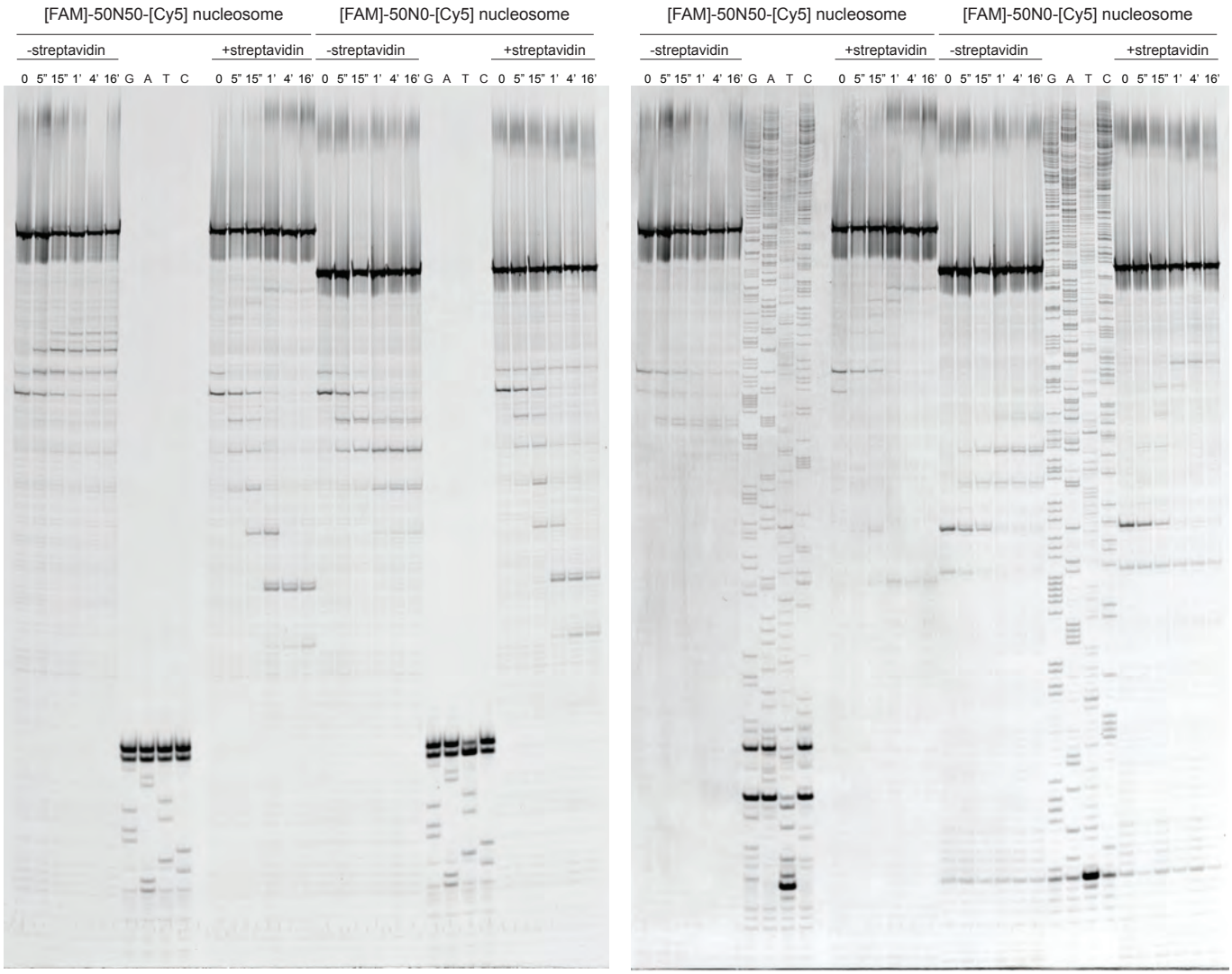


Figure S2. Extended gel and Cy5 scan for panels B and C in Figure 1.
The Chd1 remodeler shifts nucleosomes unidirectionally in the presence of the asymmetric biotin/streptavidin block. Experiments were carried out as described in Methods and Legend of Figure 1.

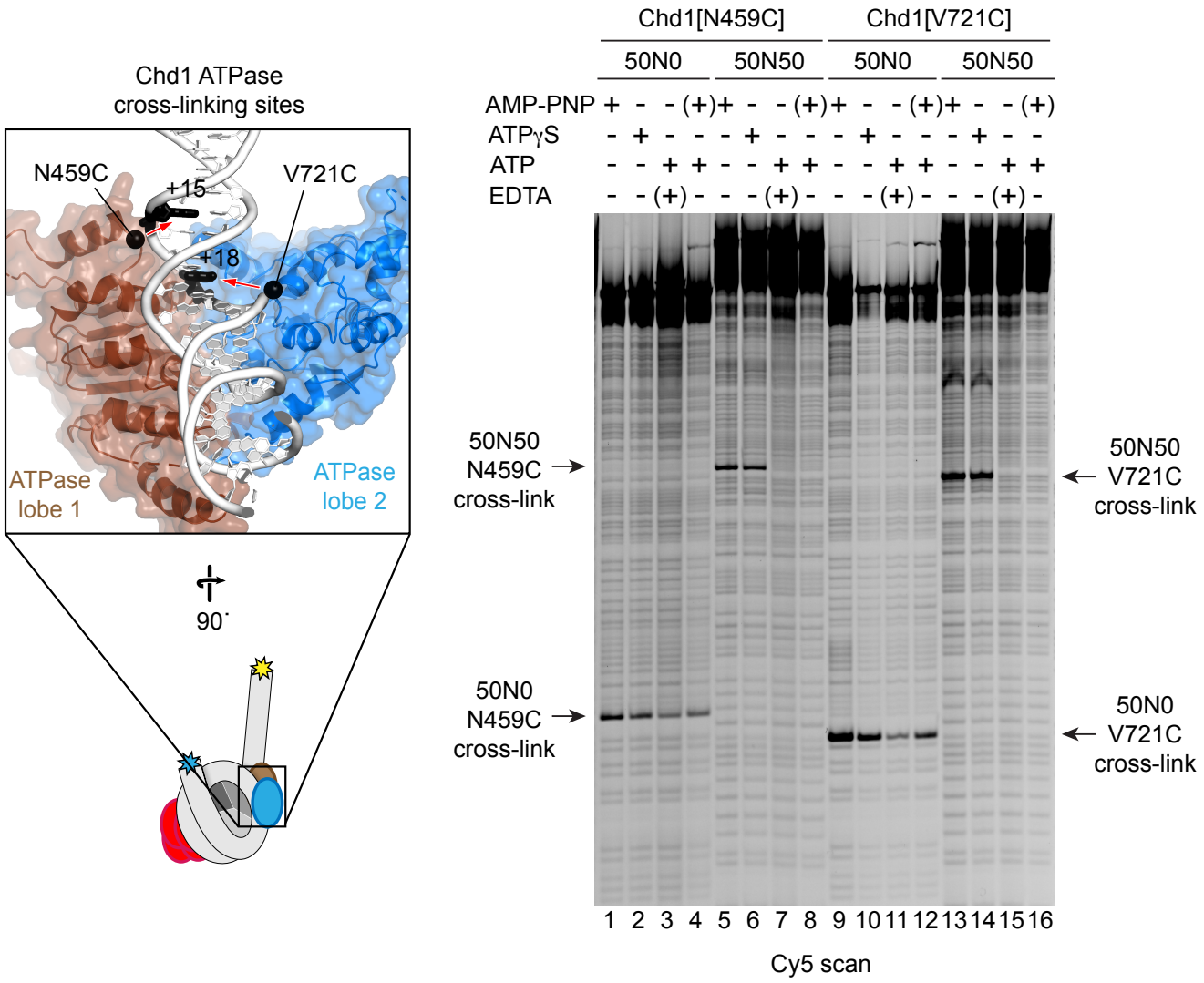


Figure S3. Cross-linking of Chd1 to streptavidin/biotin-H3X/H3Y nucleosomes shows that entry DNA is not required for engagement of the ATPase motor at SHL2.
A. Molecular representation of the Chd1 ATPase motor contacting DNA at SHL2, using the Chd1-nucleosome cryo-EM complex, 5O9G. The residues targeted by the photo-cross-linker APB (N459C for lobe 1 and V721C for lobe 2) are highlighted with black spheres; cross-linking sites on DNA are shown as black sticks.
B. Chd1 cross-linking reactions, separated by urea denaturing gel. Reactions contained 2 mM nucleotide, 450 nM Chd1, 150 nM nucleosome, and 10 μM streptavidin. After 30 min in the presence of ATP, either 20 mM EDTA or 2 mM AMP-PNP was added where indicated by (+). Note that the cross-links for 50N50 are expected to disappear upon addition of ATP, since a shift of the DNA relative to the histone core would no longer allow the ATPase motor to cross-link to the same location. In contrast, the cross-links for 50N0 nucleosomes (where the zero side is entry DNA) remain after ATP addition, a further indication that Chd1 specifically interacts with nucleosomes even though DNA is not shifted.

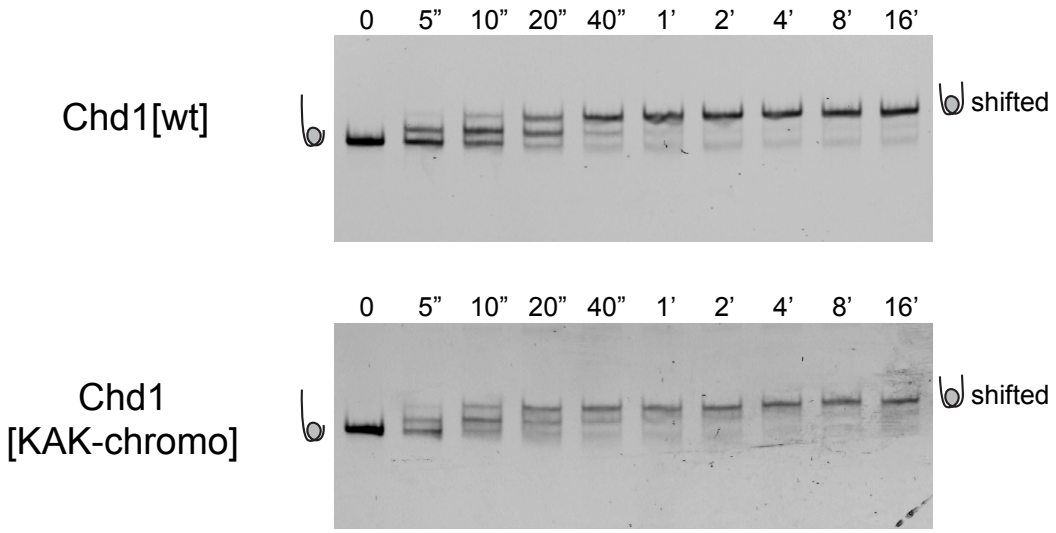


Figure S4. Disruption of the chromodomains does not impair nucleosome centering activity of Chd1.
Native gel sliding experiments with Chd1[wt] and Chd1[KAK-chromo], performed with 100 nM Chd1, 40 nM biotin-H3X/H3Y 50N0 nucleosomes, without streptavidin. Sliding reactions were stopped at the indicated time points by mixing 1 µl of a master mix with 8 µl of quench buffer containing 25 mM EDTA plus 2 µg/ml salmon sperm DNA. Shown are FAM scans of native acrylamide gels.

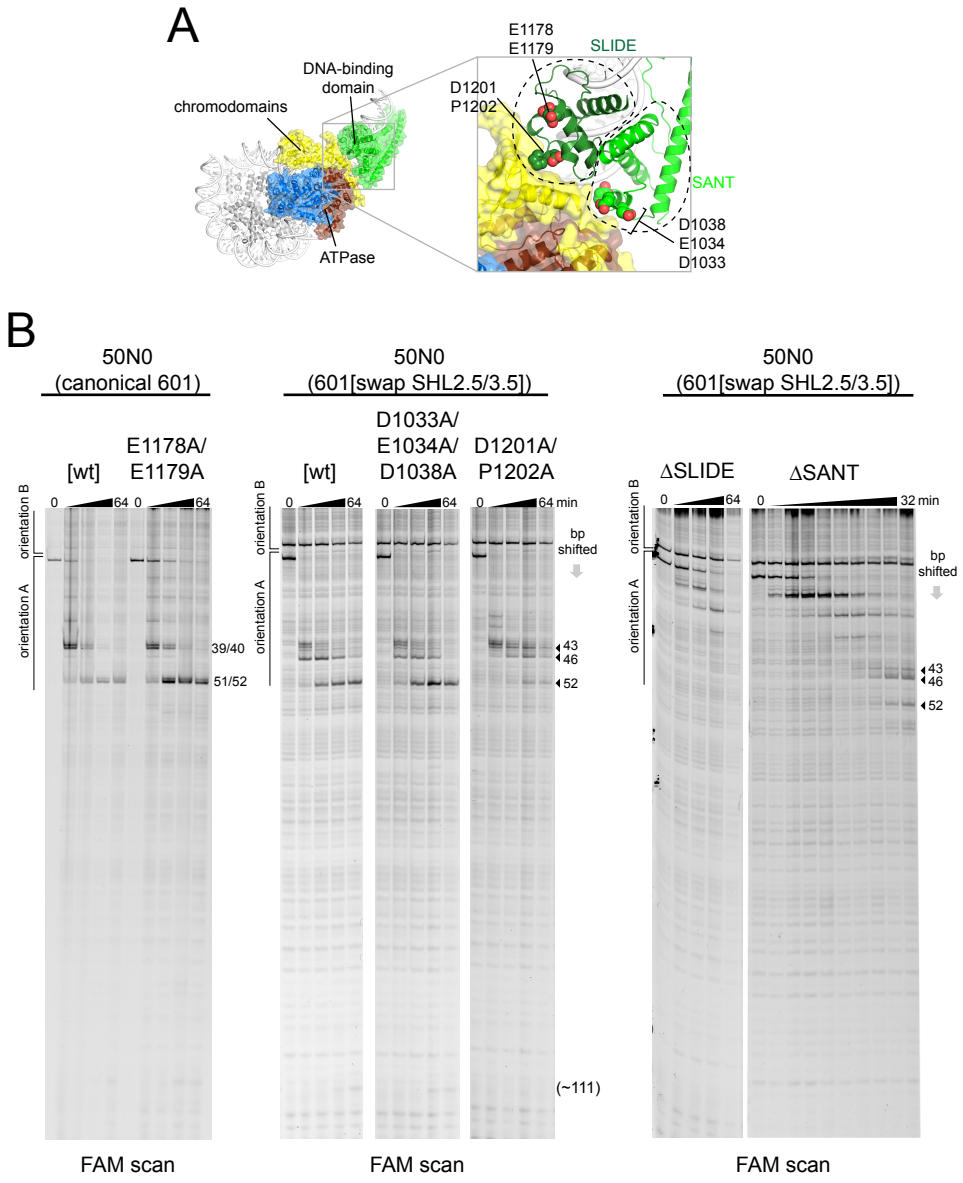


Figure S5. Disruption of the interface between the DNA-binding domain and chromodomains does not relieve exit-side inhibition.

A. Molecular representation of residues at and close to the interface between the Chd1 chromodomains and DNA-binding domain (pdb code 5O9G).

B. Sliding activity of Chd1 variants on half-blocked 50N0 nucleosomes. 50N0 nucleosomes made with the canonical 601 or 601[swap SHL2.5/3.5] variant (150 nM) were incubated with Chd1 at either 1 μM (Chd1[wt] (left), [E1178A/E1179A], ΔSANT, ΔSLIDE) or 150 nM (Chd1[wt] (right), [D1033A/E1034A/D1038A], [D1201A/P1202A]) with 2 mM ATP for the indicated time points prior to UV cross-linking. The FAM scans show that orientation A does not move farther than ~50 bp and orientation B does not significantly move from its starting position.

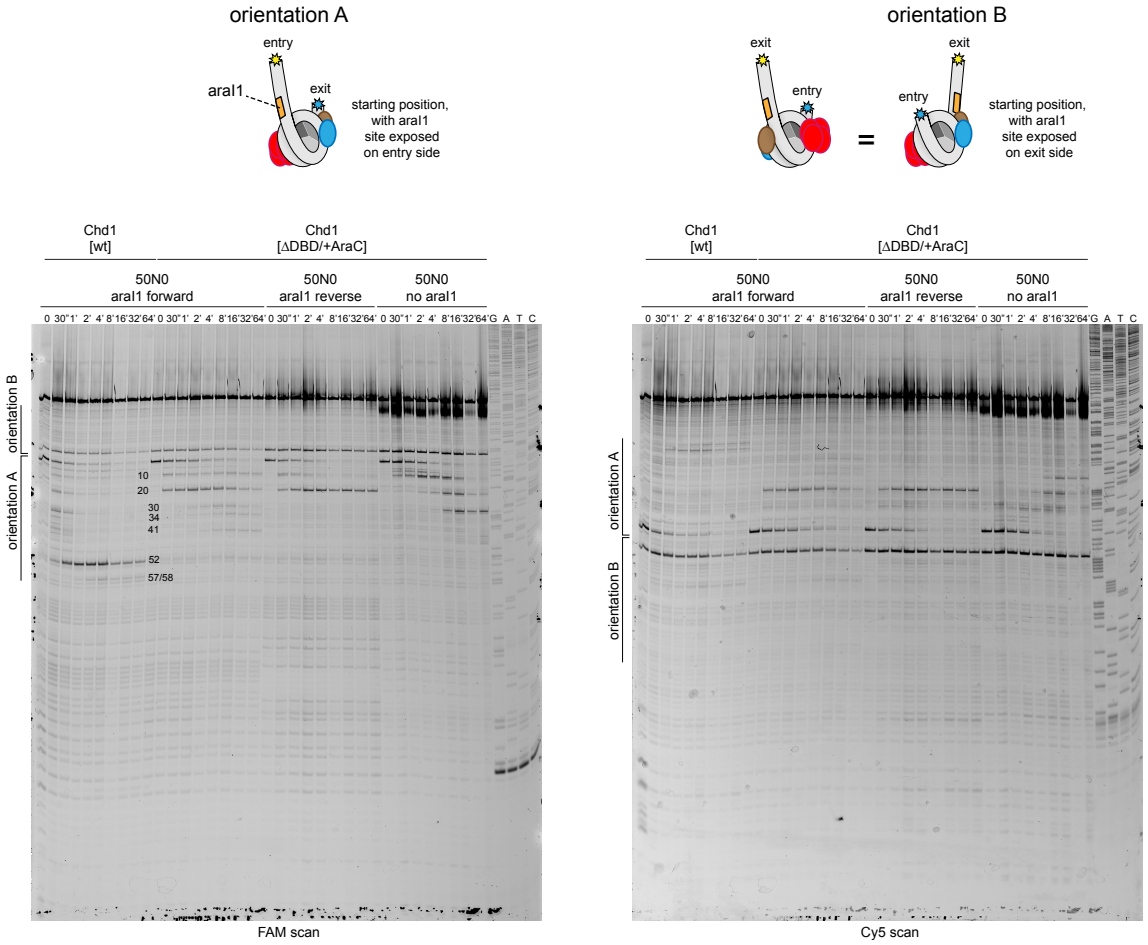


Figure S6. Chd1[ΔDBD/+AraC] repositions nucleosomes when the ara1 binding site is on entry but not exit DNA.
Nucleosome sliding reactions were carried out on 50N0 nucleosomes, made with the 601[swap SHL2.5/3.5], that either contained or lacked the 17 bp ara1 sequence, 3 bp away from the nucleosome edge. The ara1 sequence was in one of two orientations (forward, reverse). Biotin-H3X/H3Y nucleosomes (150 nM) were incubated with Chd1[wt] or Chd1[ΔDBD/+AraC] (1 μM) for the indicated times in the presence of 10 μM streptavidin and 1 mM ATP, then quenched, UV irradiated, and processed. Reactions were analyzed with a urea denaturating gel, scanned for FAM (TA-rich side) or Cy5 (TA-poor side). Sequencing ladder was made using the 601[swap SHL2.5/3.5] template without an ara1 site. These scans include data shown in Figure 2.

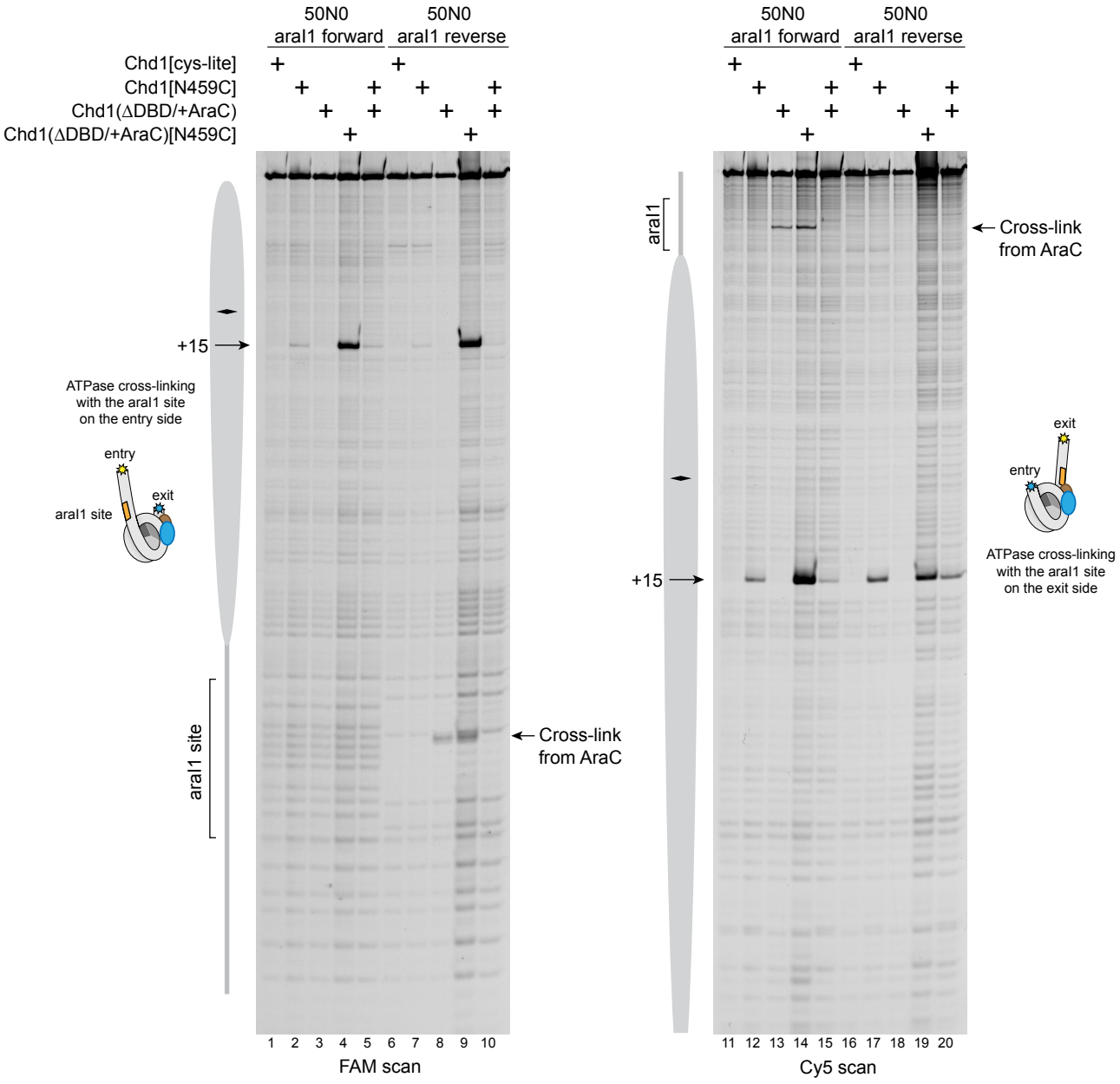


Figure S7. The ATPase motor of Chd1[ΔDBD/+AraC] strongly engages with both SHL2 sites on the nucleosome.

UV cross-linking reactions between the indicated APB-labeled Chd1 constructs (600 nM) with 50N0 nucleosomes (150 nM) in the presence of 2 mM AMP-PNP. Nucleosomes were made with 601[swap SHL2.5/3.5] and contained an ara1 site (forward or reverse) starting at +3 bp from the nucleosome edge. Chd1[cys-lite] is a negative control where all native cysteines were mutated to alanine. Note that the AraC DBD cross-links to its binding site in a sequence- and strand-specific manner.

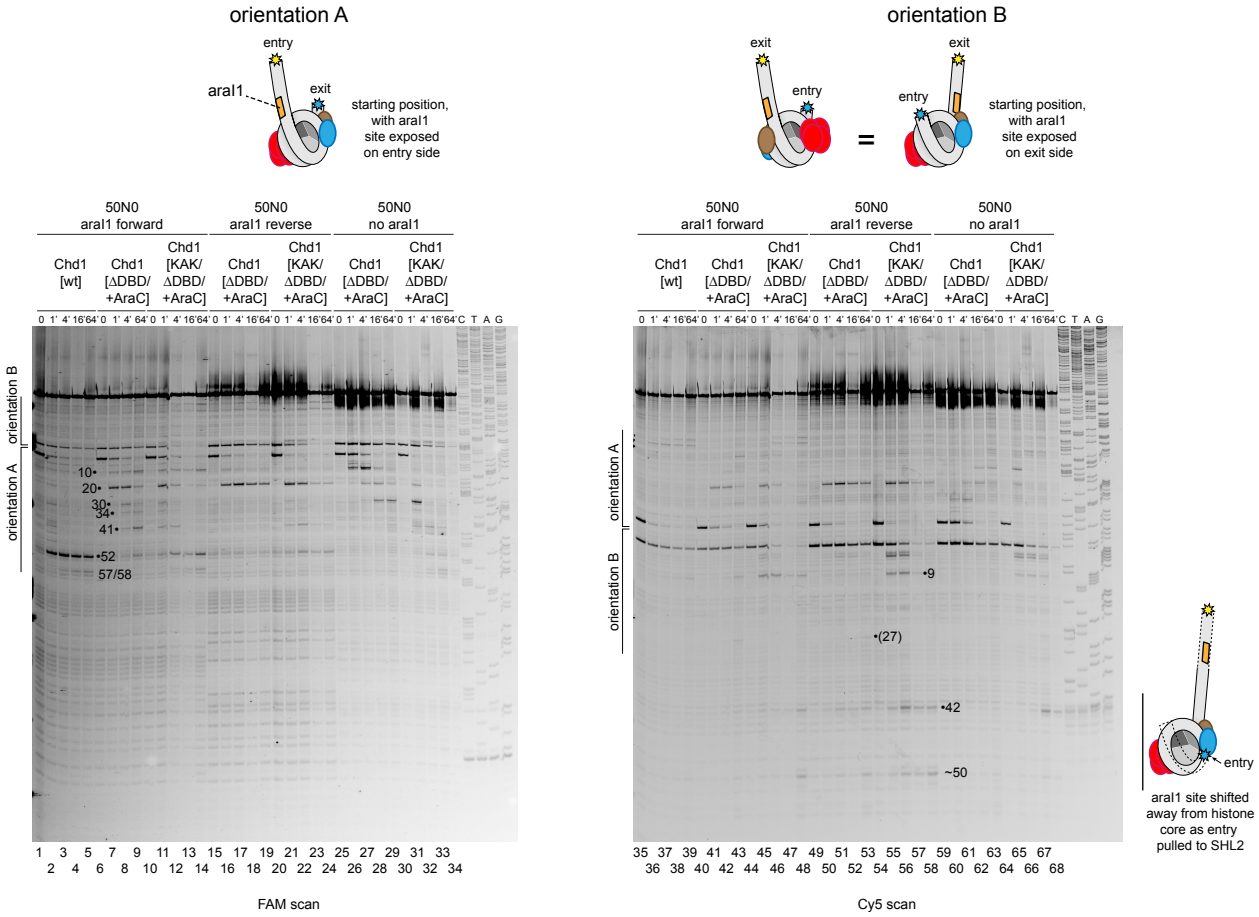


Figure S8. Disruption of the chromodomains allows Chd1[KAK/ΔDBD/+AraC] to reposition nucleosomes even with the ara1 binding site on exit DNA.
Nucleosome sliding reactions were carried out on 50N0 nucleosomes, made with the 601[swap SHL2.5/3.5], that either contained or lacked the 17 bp ara1 sequence, 3 bp away from the nucleosome edge. The ara1 sequence was in one of two orientations (forward, reverse). Biotin-H3X/H3Y nucleosomes (150 nM) were incubated with Chd1[wt] or Chd1[ΔDBD/+AraC] (1 μM) for the indicated times in the presence of 10 μM streptavidin and 2 mM ATP, then quenched on ice in buffer containing 25 mM EDTA, UV irradiated, and processed. Reactions were analyzed with a urea denaturating gel, scanned for FAM (TA-rich side) or Cy5 (TA-poor side). Sequencing ladder was made using the 601[swap SHL2.5/3.5] template without an ara1 site.

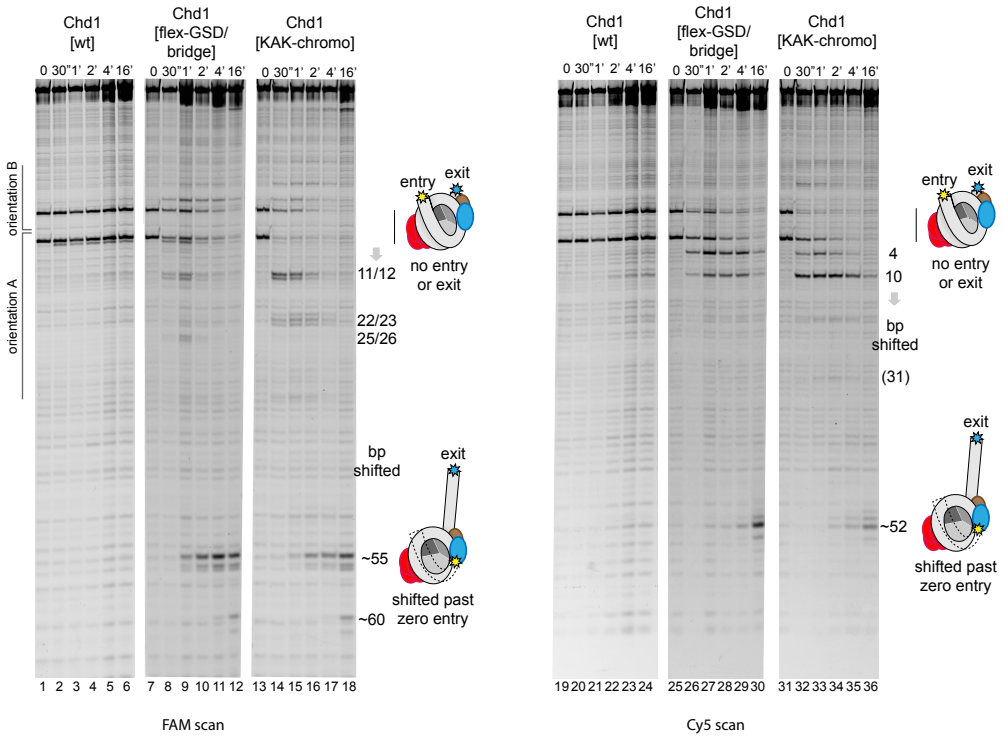


Figure S9. Entry DNA is required for nucleosome sliding by Chd1.
Shown are nucleosome sliding reactions using streptavidin/biotin-H3X/H3Y nucleosomes (150 nM), made with 601[SHL2.5/3.5 swap], incubated with 1 µM of indicated Chd1 variants and 2 mM ATP. Reactions were quenched on ice with buffer containing 25 mM EDTA, then UV cross-linked and processed before separation on urea denaturing gels.

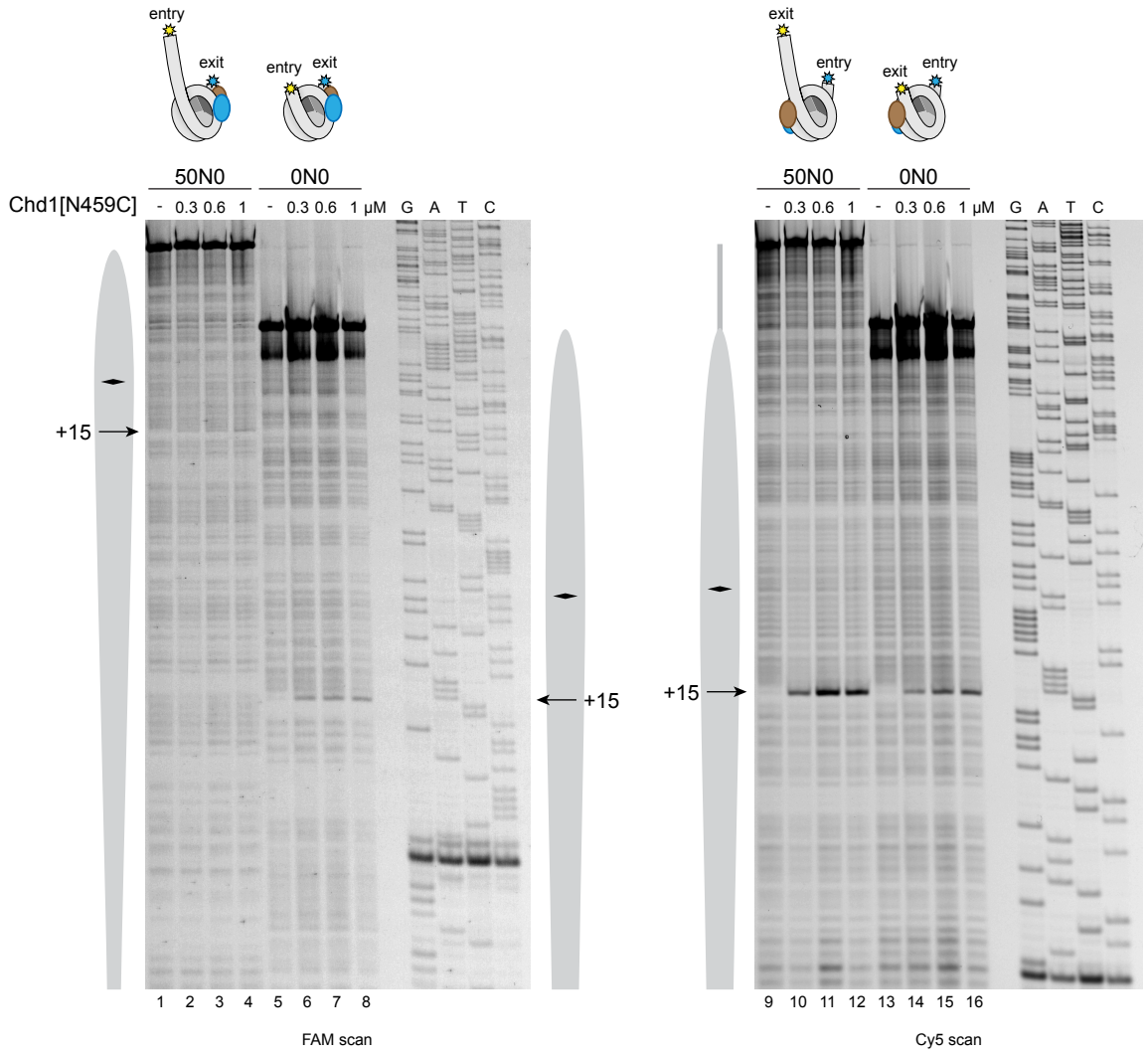


Figure S10. The Chd1 ATPase cross-links to SHL2 in the absence of flanking DNA. Shown are cross-linking reactions using either 1 µM Chd1[cys-lite] (lanes 1, 5, 9, 13) or indicated amounts of Chd1[N459C] with 150 nM 601[swap SHL2.5/3.5] nucleosomes in the presence of 2 mM AMP-PNP. The sequencing ladder used 0N0 as a template.

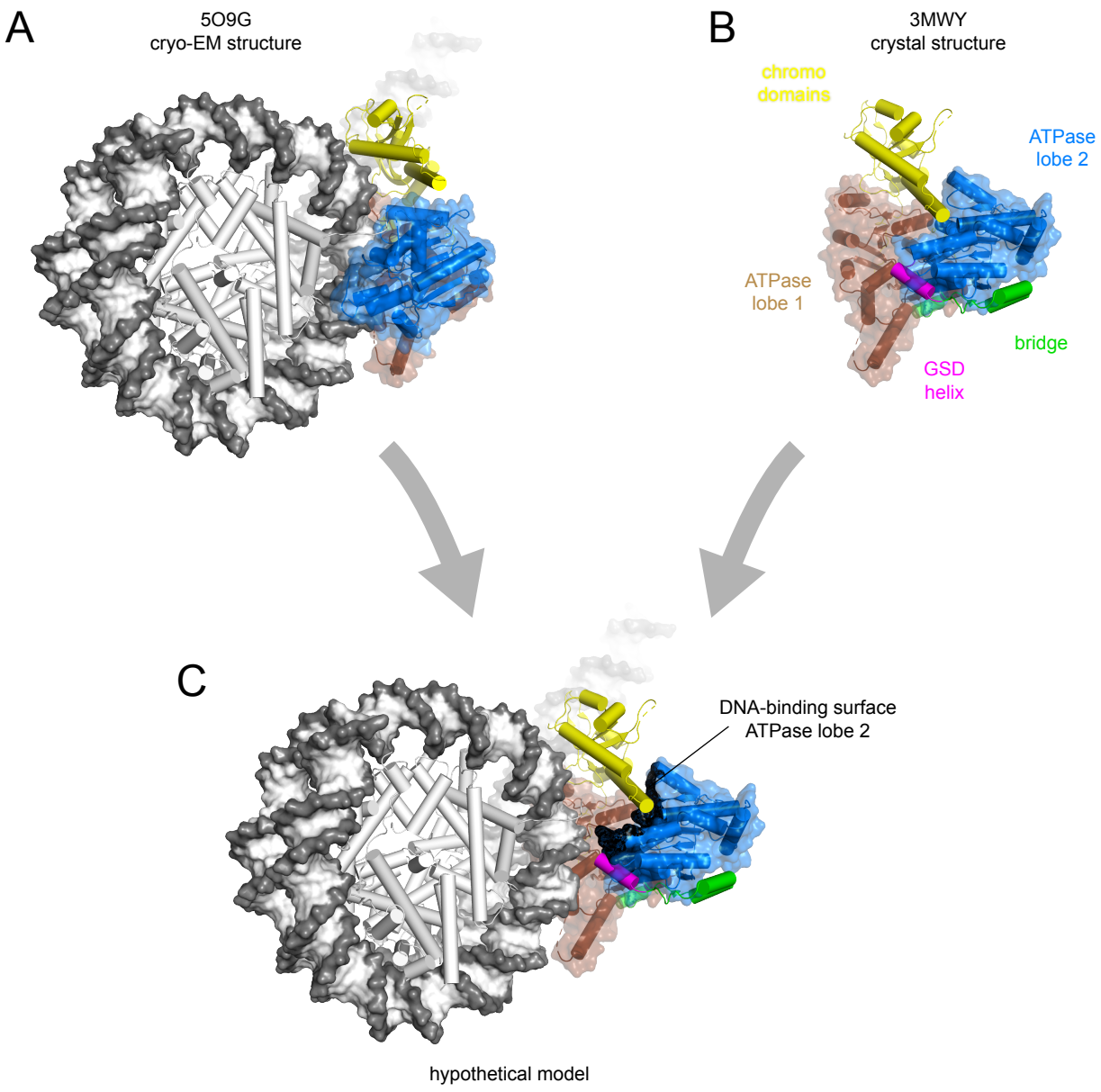


Figure S11. A model for an autoinhibited state of Chd1 on the nucleosome.
A. Structure of the Chd1 remodeler in a nucleosome-bound state (5O9G), where the ATPase motor is bound to ADP•BeF_3^- and is fully engaged with nucleosomal DNA.
B. Structure of Chd1 in a nucleosome-free state (3MWY), where the ATPase is an opened, autoinhibited conformation.
C. Docking the autoinhibited structure onto the nucleosome by superimposing ATPase lobe 1 of each structure places the chromodomains in a similar location at SHL1. Relative to the fully engaged state, ATPase lobe 2 is swung out in the autoinhibited state with its DNA-binding surface instead bound to the chromodomains and GSD helix.

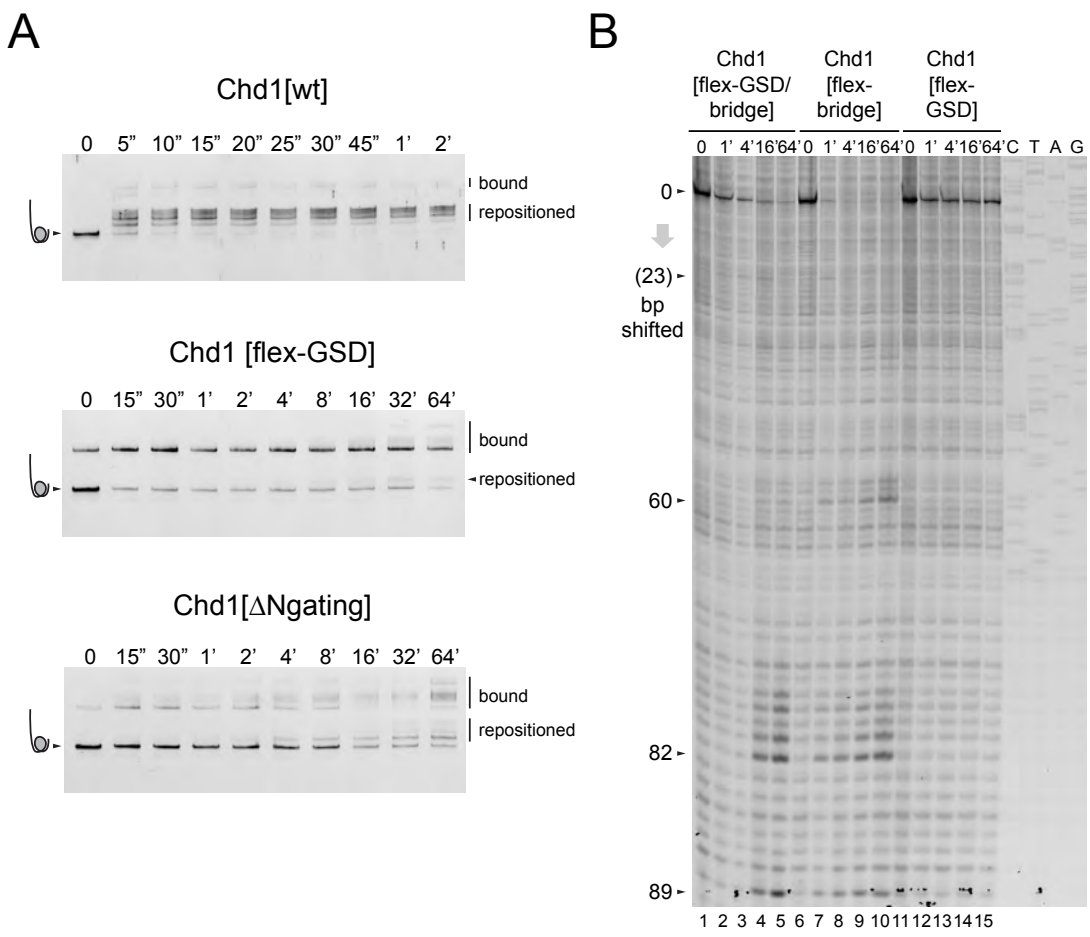
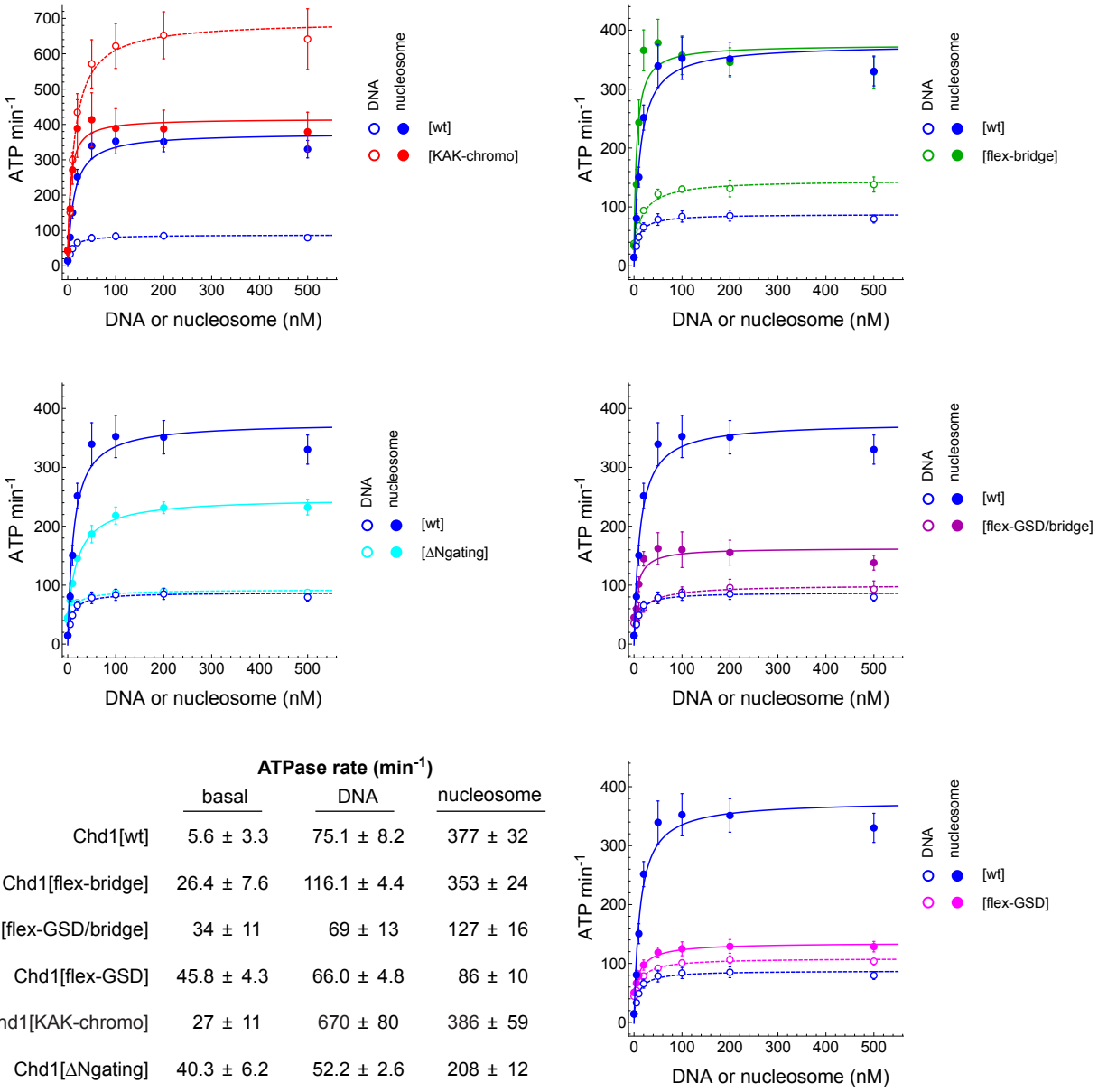


Figure S12. Disruption of the GSD helix or N-terminal deletion of the gating helix greatly reduce nucleosome sliding activity of Chd1.

A. Native gel nucleosome sliding assays. Experiments were carried out with 40 nM 0N80 nucleosomes (canonical 601 sequence) and 100 nM Chd1, initiated with addition of ATP.

B. Disruption of the GSD helix of Chd1 decreases the rate of nucleosome sliding, both alone and in the context of a disrupted bridge. Nucleosome sliding reactions were carried out with 150 nM 80N0 nucleosome (canonical 601 sequence) and 300 nM Chd1 and monitored by UV cross-linking to APB-labeled H2B(S53C). Reactions were resolved on a urea denaturing gel.



ATPase rate (min^{-1})

	basal	DNA	nucleosome
Chd1[wt]	5.6 ± 3.3	75.1 ± 8.2	377 ± 32
Chd1[flex-bridge]	26.4 ± 7.6	116.1 ± 4.4	353 ± 24
Chd1[flex-GSD/bridge]	34 ± 11	69 ± 13	127 ± 16
Chd1[flex-GSD]	45.8 ± 4.3	66.0 ± 4.8	86 ± 10
Chd1[KAK-chromo]	27 ± 11	670 ± 80	386 ± 59
Chd1[ΔNgating]	40.3 ± 6.2	52.2 ± 2.6	208 ± 12

Figure S13. Nucleosome- and DNA-stimulated ATP hydrolysis rates.
Nucleosome titrations used unlabeled 61N2 Widom constructs. DNA titrations used the same DNA construct as the nucleosomes. Solid/dotted lines show Michaelis-Menton fits to each nucleosome/DNA titration. The basal, DNA- and nucleosome-stimulated k_{cat} values along with standard deviations are given in the table. The number of replicates for DNA and nucleosome titrations were as follows: Chd1[wt]: n=8 (DNA), n=8 (nucleosome); Chd1[flex-bridge]: n=4 (DNA), n=6 (nucleosome); Chd1[flex-GSD/bridge]: n=5 (DNA), n=4 (nucleosome); Chd1[flex-GSD]: n=6 (DNA), n=7 (nucleosome); Chd1[KAK-chromo]: n=6 (DNA), n=9 (nucleosome); Chd1[ΔNgating]: n=5 (DNA), n=6 (nucleosome). Each variant is plotted alongside Chd1[wt] for comparison.

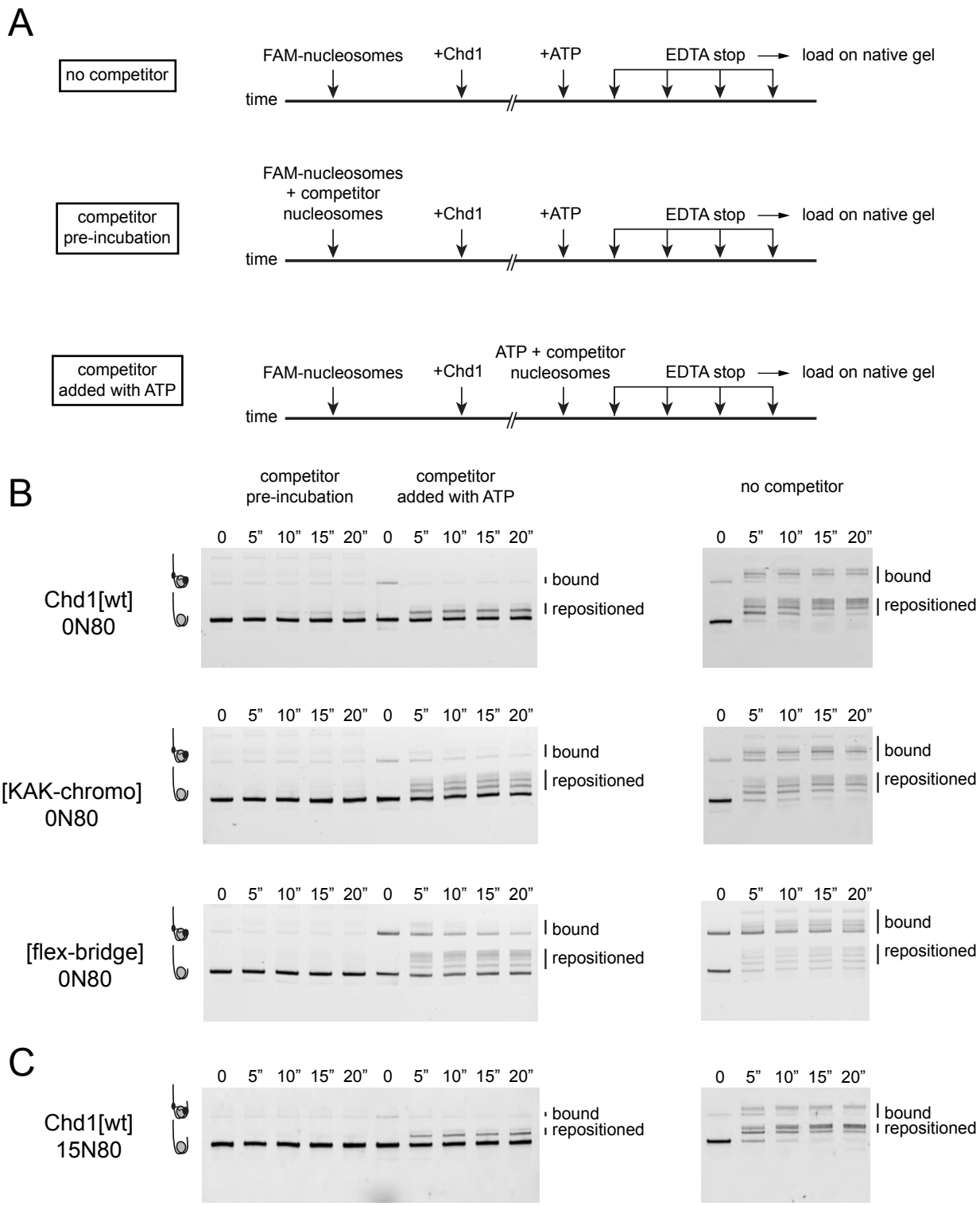


Figure S14. Autoinhibitory elements reduce sliding processivity of Chd1.
A. Schematics for processivity experiment and related controls.
B. Processivity experiments for Chd1[wt], Chd1[KAK-chromo], and Chd1[flex-bridge], using 30 nM 0N80 FAM-labeled nucleosomes, in the presence and absence of 3 μM unlabeled 0N80 nucleosomes. The data for "competitor added with ATP" are equivalent to those shown in Figure 4B. Times points are given in seconds.
C. Processivity experiments for Chd1[wt] using 30 nM 15N80 FAM-labeled nucleosomes, in the presence and absence of 3 μM unlabeled 0N80 nucleosomes.

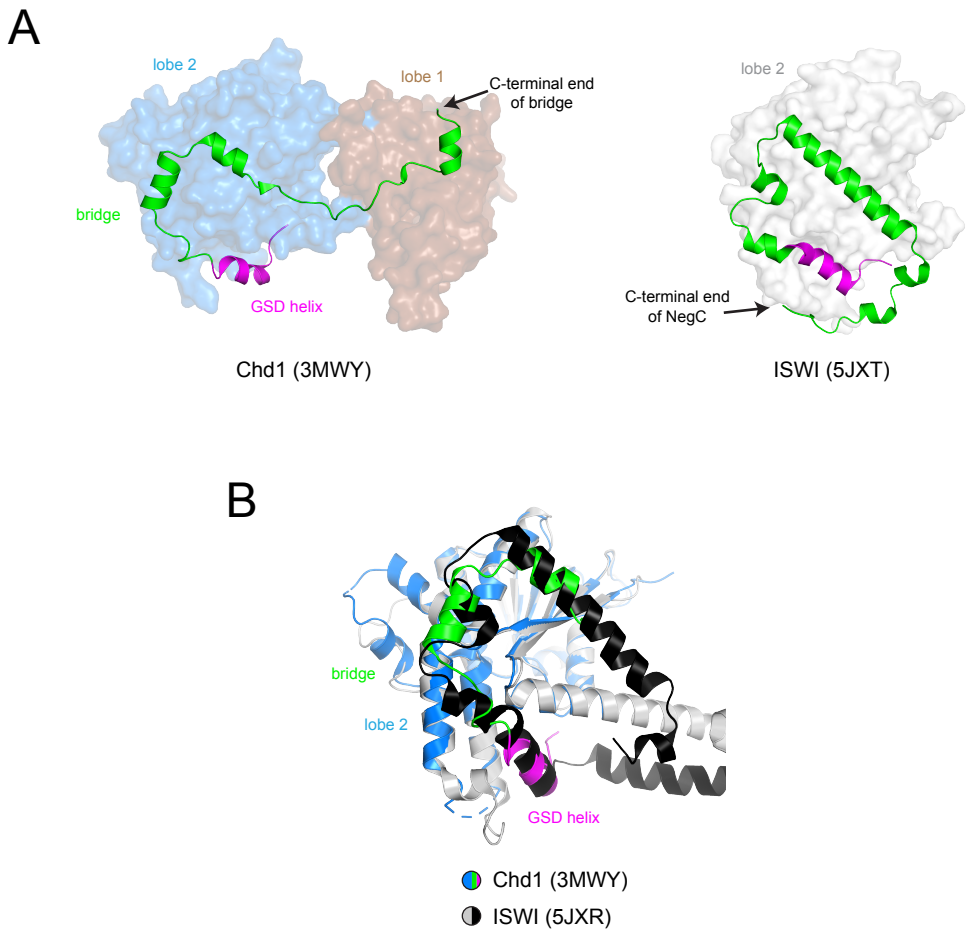


Figure S15. The bridge and GSD helix of Chd1 have structural equivalents in ISWI. **A**. Surface rendering of ATPase lobes 1 and 2 of Chd1 and ATPase lobe 2 of ISWI, showing how the bridge of Chd1 extends from lobe 2 to lobe 1, whereas NegC of ISWI begins in a similar fashion but is more helical and maintains contacts with ATPase lobe 2. **B**. Superpositioning ATPase lobe 2 for Chd1 and ISWI reveals that ISWI has a helix in the same location as the Chd1 GSD helix, and that NegC of ISWI has a different structure yet follows a similar path as the Chd1 bridge along most of ATPase lobe 2. Note that for ISWI, the black and white backbone represent two domain-swapped proteins in the crystal asymmetric unit.

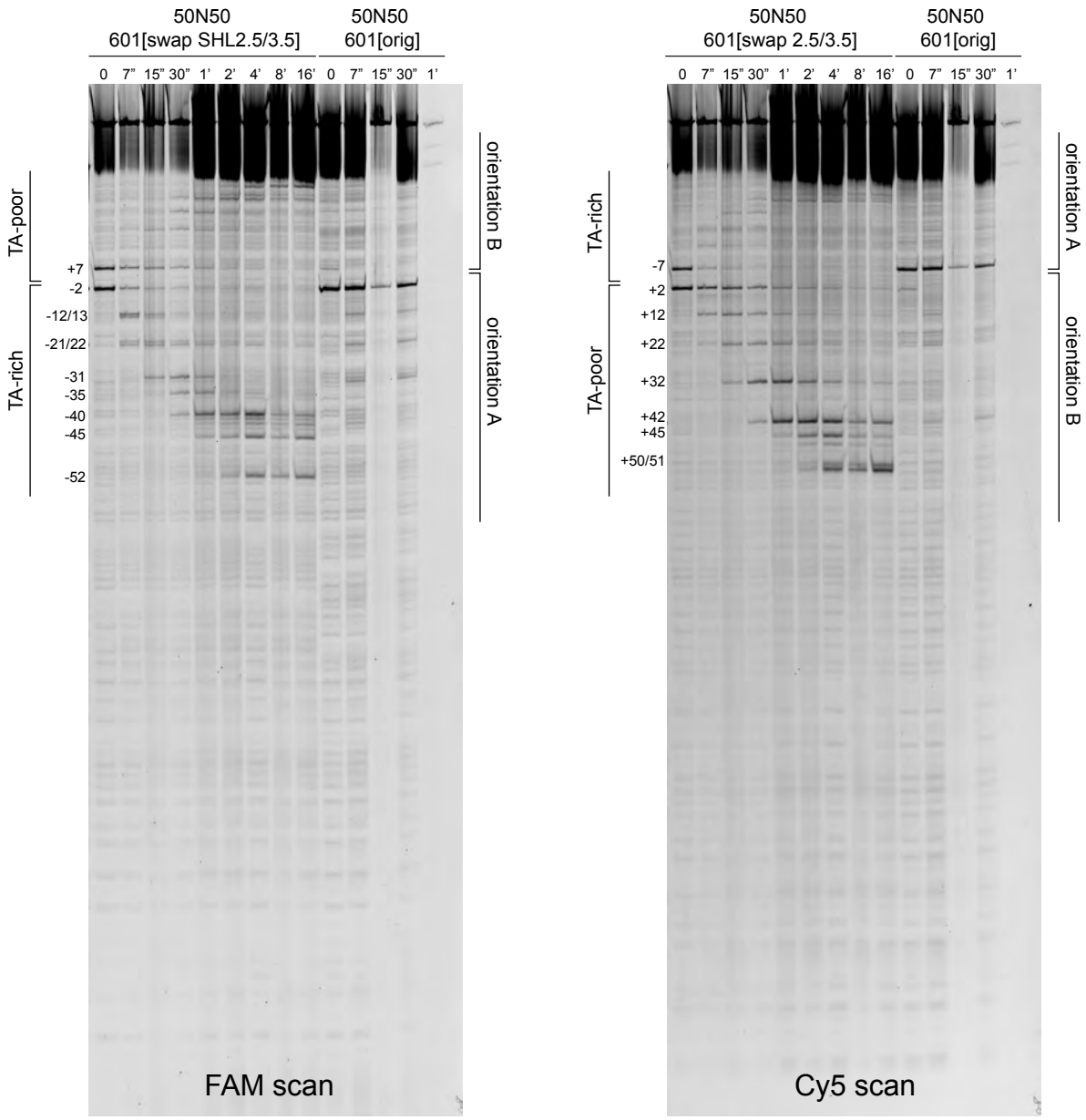


Figure S16. Comparison of half-blocked nucleosomes on the canonical Widom 601 versus the 601[swap SHL2.5/3.5].
Nucleosomes containing the H3X/H3Y heterodimer (biotinylated at H3X-Q76C; APB labeled at H3Y-M120C) were reconstituted with the canonical Widom 601 sequence (601[orig]) or a 601 variant (601[swap SHL2.5/3.5]), and repositioned by Chd1. Reactions contained 150 nM nucleosome, 1 μM Chd1, 50 μM ATP, and 10 μM streptavidin, and were quenched at the indicated time points. Note how orientation A is dominant for 50N50 601[orig] compared with a more even distribution of orientation A and B for 601[swap SHL2.5/3.5], and also how much more poorly the starting material for 601[orig] is shifted compared to 50N50 601[swap SHL2.5/3.5] under these conditions.

Table S1. Primers for generating Chd1 variants

Chd1[flex-GSD].sense
5′ cactaagaagaatgaaccaaatgccggtGGCGGTTCCGGCGGTTCTGGAGGCggtgcaggcaatatgttcactgccacag

Chd1[flex-GSD].anti
5′ ctgtggcagtgaacatattgcctgcaccGCCTCCAGAACCGCCGGAACCGCCaccggcatttggttcattcttcttagtg

G-Block, Chd1[flex-GSD/bridge,864-902] 178bp
5′cactaagaagaatgaaccaaatgccggtGGCGGTTCCGGCGGTTCTGGAGGCGGTGGAAGCGGTGGGTCCGGCGGATCTGGTGG
GTCGGGCGGATCCGGTGGATCGGGGGGCTCTGGAGGTTCTGGCGGGTCGGGCGGTTCCGGTactactccagacttaggagagtcgc
atcttggc

G-Block, Chd1[flex-bridge,884-902] 130bp:
5′gcaggcaatatgttcactgccacagacaaccagGGTGGAAGCGGTGGGTCCGGCGGATCTGGTGGGTCGGGCGGATCCGGTGGA
TCGGGGactactccagacttaggagagtcgcatcttggcggtgaag

ScChd1-1178/79a.fwd (1178,1179 to Ala)
5′ cag aat tgg tca tct aat tgg acg aaa gCg gCa gat gag aag cta ttg att ggt gtt ttc

ScChd1-1178/79a.rev (1178,1179 to Ala)
3′ gaa aac acc aat caa tag ctt ctc atc tGc cGc ttt cgt cca att aga tga cca att ctg

ScChd1-1201/02a.fwd (1201,1202 to Ala)
5′ ggt tcc tgg aca caa ata aga gac gCt Gca ttt cta ggc att act gat aaa ata ttc

ScChd1-1201/02a.rev (1201,1202 to Ala)
5′ gaa tat ttt atc agt aat gcc tag aaa tgC aGc gtc tct tat ttg tgt cca gga acc

ScChd1-1033/38a.fwd (1033,1034,1038 to Ala)
5′ ggc aac cta aag gag att ttg gCc gCg ttg att gca gCt gga acc ctg ccg gtc aaa tc

ScChd1-1033/38a.rev (1033,1034,1038 to Ala)
5′ ga ttt gac cgg cag ggt tcc aGc tgc aat caa cGc gGc caa aat ctc ctt tag gtt gcc

ScChd1-ΔSANT(1006-1125).fwd
5′ CT TCT AGA AGA AGA GCA AGA GCT AAT aaa tct tta aac gcc gaa tct tta c

ScChd1-ΔSANT(1006-1125).rev
5′ g taa aga ttc ggc gtt taa aga ttt ATT AGC TCT TGC TCT TCT TCT AGA AG

Chd1[ΔDBD/+AraC].sense
5' ct aga aga aga gca aga gct aat gac atg GAT AAT CGG GTA CGC GAG GC

Chd1[ΔDBD/+AraC].anti
5' GC CTC GCG TAC CCG ATT ATC cat gtc att agc tct tgc tct tct tct ag

Chd1-Asn459C.sense
5′ GAT TTG AAT TGT ATA TGC TAT ATG GGC TGC CAA AAA TCA AGA GAT ACC ATT CGA G

Chd1-Asn459C.anti
5′ C TCG AAT GGT ATC TCT TGA TTT TTG GCA GCC CAT ATA GCA TAT ACA ATT CAA ATC

Table S2. Primers for nucleosomal DNA

601-40-FAM (for 40N40 nucleosomes, dissociation assay)
5′ /56-FAM/AT CCG ACT GGC ACC GGC AAG GTC GCT GTT C

40-601 (for 40N40 nucleosomes for dissociation assay)
5′ AG TTC ATC CCT TAT GTG ATG GAC CCT ATA C

601-80-FAM (for 0N80 and 15N80 nucleosomes, non-biotin block sliding)
5′ /56-FAM/CGGTACCCGGGGATCCTCTAGAGTGGGAGC

15-601 (for 15N80 nucleosomes, non-biotin block sliding)
5′ tatac gcggccgccc TG GAGAATCCCG GTG

Cy5-0-601 (for 0N80 nucleosomes, non-biotin block sliding)
5′ /5Cy5/CAGGATGTATATATCTGACACGTGCCTGGA

FAM-50-601 (for 50N50 and 50N0 nucleosomes)
5′ /56-FAM/CTTCACACCGAGTTCATCCCTTATGTGATG

FAM-0-601 (for 0N0)
5′ /56-FAM/TGGAGAATCCCGGTGCCGAGGCCGCTC

601-50-Cy5 (for 50N50 nucleosomes)
5′ /5Cy5/TCGGAACACTATCCGACTGGCACCGGCAAG

601-0-Cy5 (for 0N0,50N0,50N0/araI1 nucleosomes)
5′ /5Cy5/CAGGATGTATATATCTGACACGTGCCTGGA

FAM-50-601-araI1-fwd (for 50N0/araI1-forward nucleosomes)
5′ /56-FAM/CTTCACACCGAGTTCATCCCTTATGTGATG**TATGGATAAAAATGCTA**CCCTGGAGAATCCCGGTGCCG

FAM-50-601-araI1-rev (for 50N0/araI1-reverse nucleosomes)
5′ /56-FAM/CTTCACACCGAGTTCATCCCTTATGTGATG**TAGCATTTTTATCCATA**CCCTGGAGAATCCCGGTGCCG