SUPPLEMENTAL FIGURES AND LEGENDS

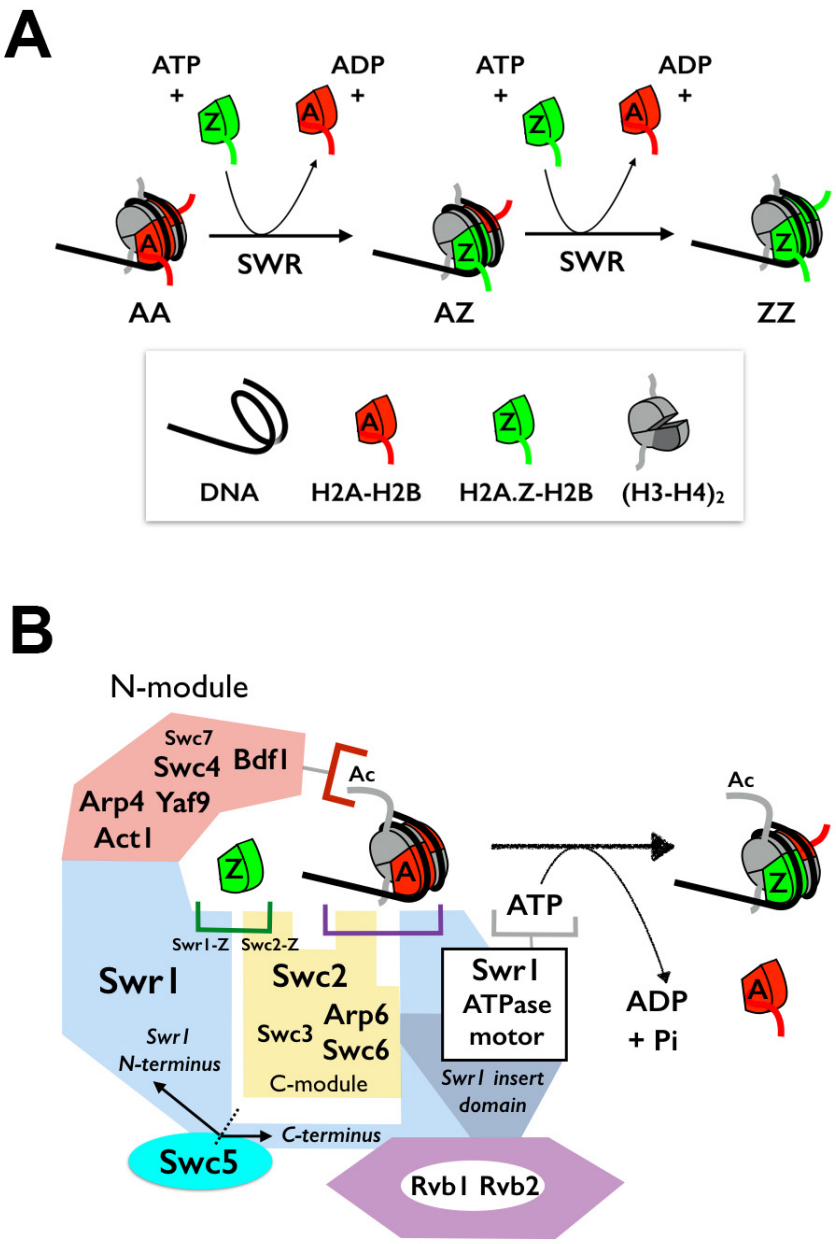

Figure S1. Cartoon depiction of the SWR-mediated histone exchange reaction and the roles of the SWR subunits in the process. (**A**) The chromatin remodeling reaction catalyzed by SWR. *Red*: H2A-H2B dimer. *Green*: H2A.Z-H2B dimer. *Gray* $(H3\text{-}H4)_2$ tetramer. (**B**) The Swr1 polypeptide is in *blue*. The N- and C-modules (*pink* and *yellow*, respectively) are associated with the N- and C-terminal halves of Swr1 (1). Bdf1 contains tandem bromodomains (*red* half bracket) that preferentially bind the tetra-acetylated H4 tail (2). Swc2 and Swr1 both contribute to the binding sites of the histone substrates, i.e. H2A.Z-H2B dimer and the H2A-

containing nucleosome (*green* and *purple* half brackets, respectively) (3, 4). The heterohexameric Rvb1-Rvb2 ring requires the Swr1 ATPase domain for binding (4). The EM structure of SWR suggests the Swr1 core, the N- and C-modules are organized around one face of the Rvb1-2 ring (not shown for simplicity) (5). Swc5 (oval in *cyan*) requires both the N- and C-terminal halves of Swr1 for stable interaction (1). "Ac" indicates acetylation; "Pi" indicates inorganic phosphate. The Swr1 insert domain is highlighted in dark blue.

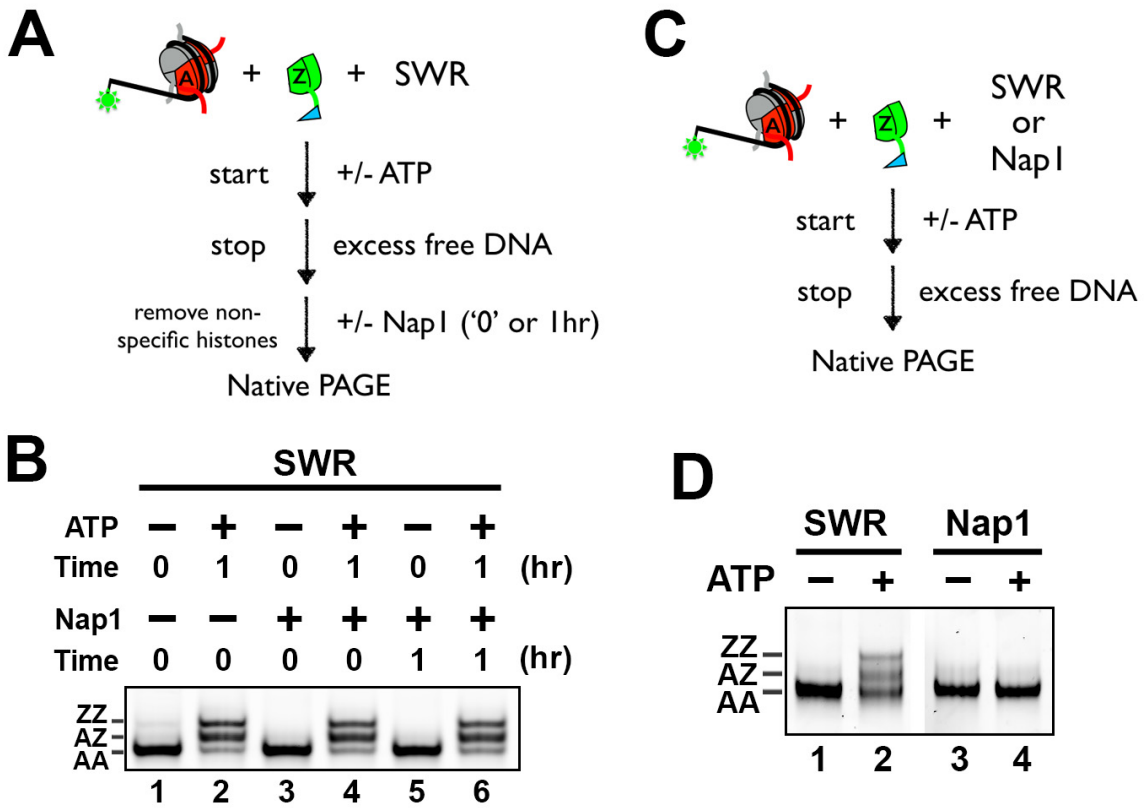

Figure S2. ***Nap1 prevents non-specific binding of H2A.Z-H2B^{FL} to nucleosome and does not contribute to histone exchange.*** (**A, C**) Cartoon depictions of the histone exchange assay with Nap1 at different points during the process. (**B**) *Lanes 1-2*: No Nap1 was added. *Lanes 3-4*: Nap1 was added after quenching, and the mixture was immediately separated by native PAGE. *Lanes 5-6*: After quenching, Nap1 was added and the mixture was incubated for an additional 1 hour before native PAGE. The gel was visualized by SYBR Green I staining. (**D**) *Lanes 1-2*: Same as *lanes 1-2* in **B**. *Lanes 3-4*: Nap1-only control. The lanes in **D** were cropped from the same gel.

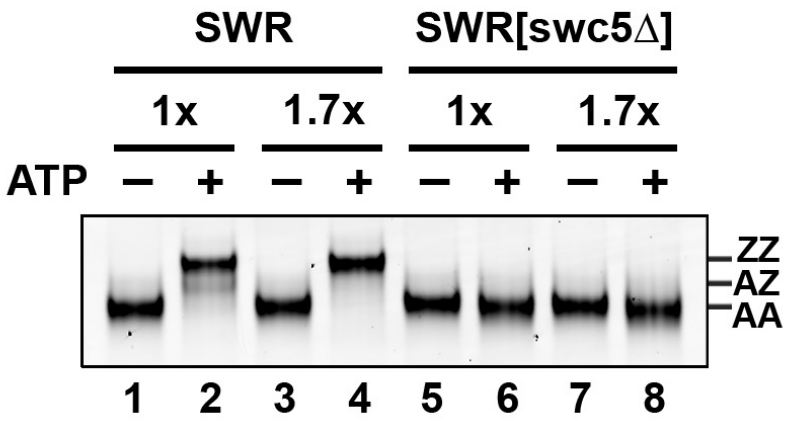

Figure S3. In vitro histone exchange activity of SWR and SWR[swc5Δ]. The histone exchange assay was performed under the same condition as described in Figure 1C, except that 1.7-fold more SWR and SWR[swc5Δ] were used in lanes 3-4 and 7-8 and that the gel was visualized with SYBR Green staining.

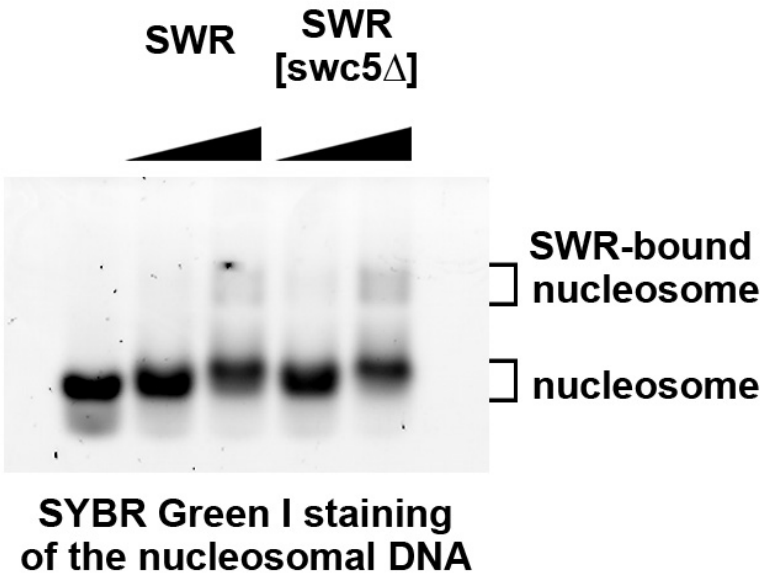

Figure S4. ***Swc5 is not required for SWR binding to AA nucleosome.*** Four nanomolar of AA nucleosomes were incubated with or without SWR and SWR[swc5Δ]. The tapered bars above the lanes indicate that either 4 or 8 nM of SWR was used. SWR-bound nucleosomes and free nucleosomes were separated on a 1.3% agarose gel. SYBR Green I was used to stain the nucleosomal DNA.

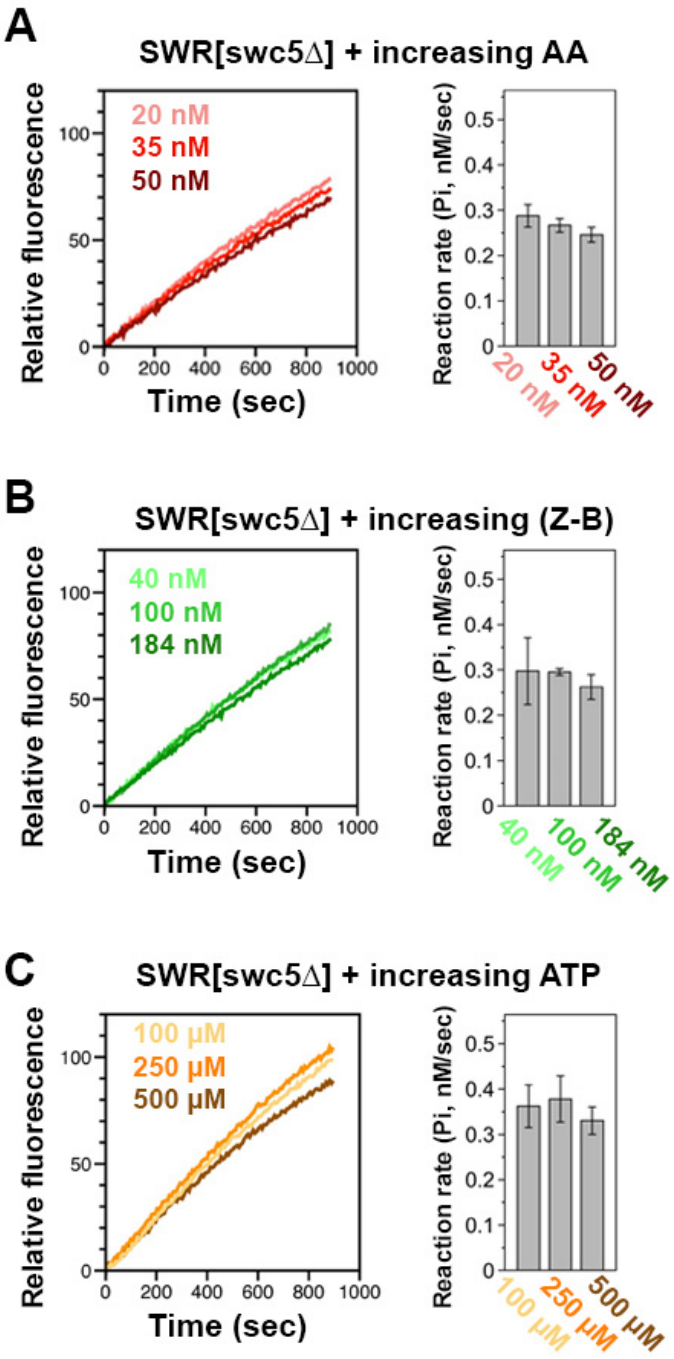

Figure S5. ***Swc5 is not required for substrate binding.*** (**A**) ATPase activity of SWR[swc5Δ] with increasing amount of AA nucleosome in the presence of excess H2A.Z-H2B^{FL} (40 nM) and ATP (100 µM). (**B**) Same as **A**, except that increasing concentration of H2A.Z-H2B^{FL} dimer was used while AA nucleosome (20 nM) and ATP (100 µM) were held constant. (**C**) Same as **A**, except that increasing concentration of ATP was used while AA (20 nM) nucleosome and H2A.Z-H2B^{FL} (40 nM) were held constant.

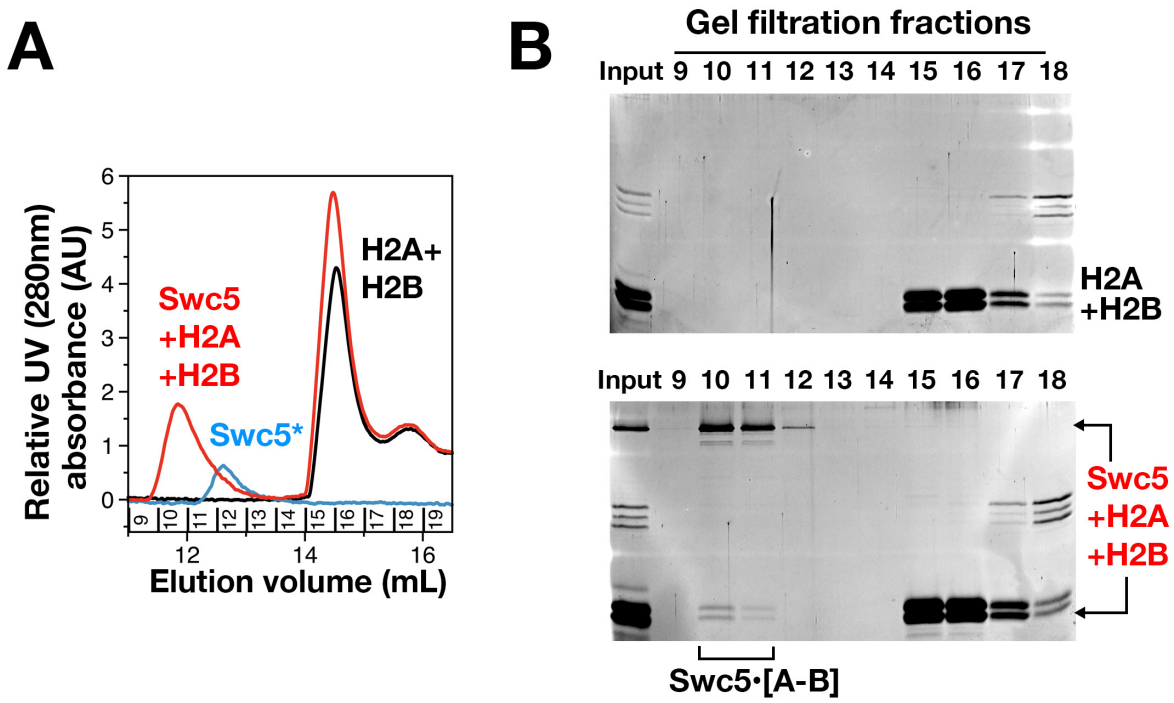

Figure S6. ***Refolding individual H2A, H2B and Swc5 polypeptides into a stable complex.*** (**A**) The gel filtration profile revealed by UV absorption at 280 nm of refolding reactions containing H2A and H2B (200 µg each) with or without Swc5 (22.8 µg) (bottom panel and top panel, respectively). (**B**) SDS-PAGE and SYPRO Ruby analysis of the eluted proteins. The asterisk indicates that the elution profile of Swc5 (*blue*) from **Figure 3** was re-plotted here for comparison.

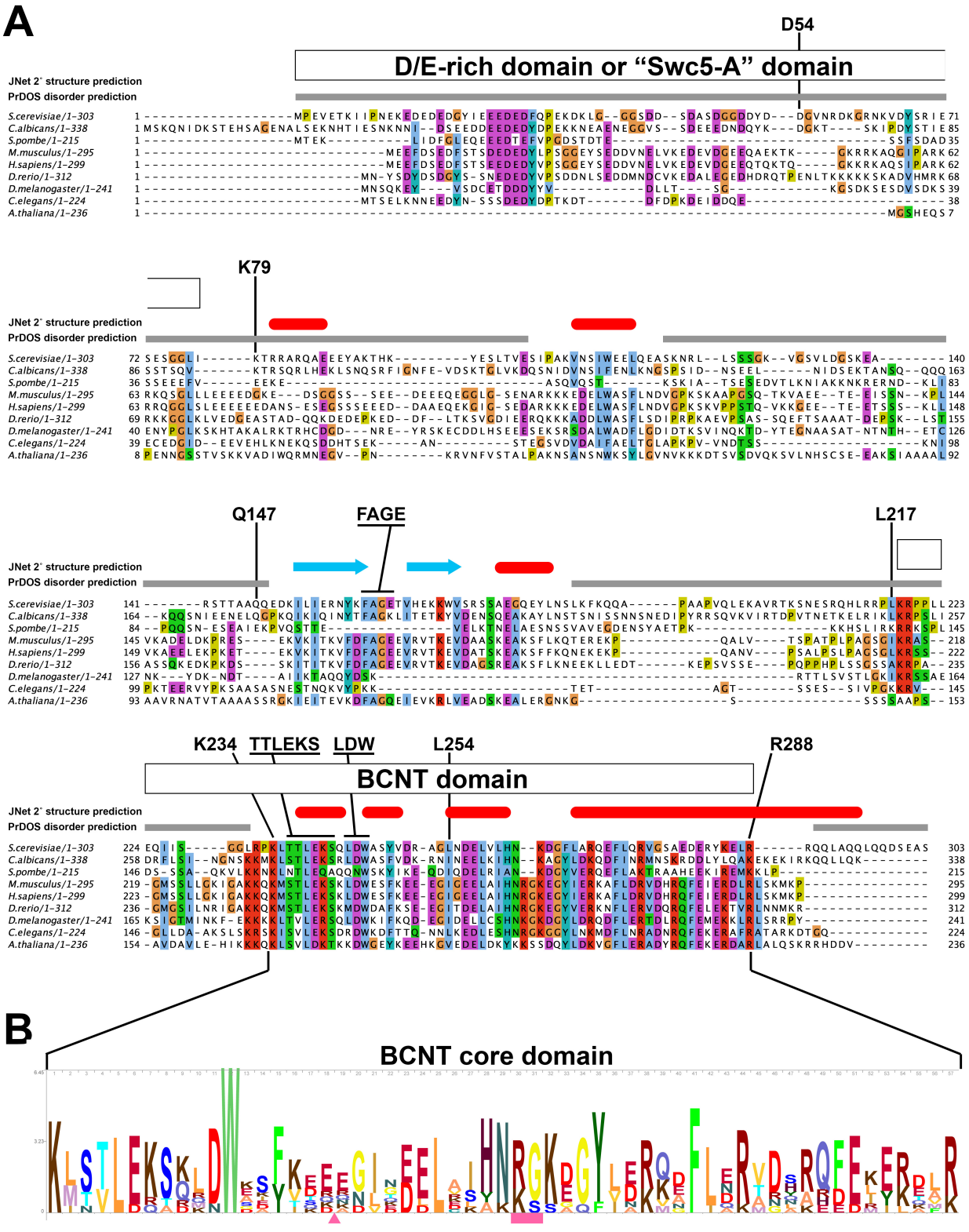

Figure S7. ***Protein sequence analysis of yeast Swc5 and its homologs.***
(**A**) Multiple sequence alignment of budding yeast Swc5 and its homologs from other yeasts, mammals, fish, nematodes and plants. The alignment was generated in Jalview (version 2.10.1)

using the ClustalO algorithm with default settings (6, 7). Secondary structures were predicted using JNet with a confidence level cutoff at 5 (8). Red bars and blue arrows indicate helices and sheets, respectively. Disordered regions (gray lines) were calculated using PrDOS with the false positive rate set at 5%. The highlighted sequence motifs are based on the yeast Swc5. (**B**) Logo plot of the BCNT core domain of the sequences in (**A**) was generated by Skylign (9). The pink bar indicates two amino acids missing in the yeast sequences. The arrowhead indicates a missing residue only found in some plants.

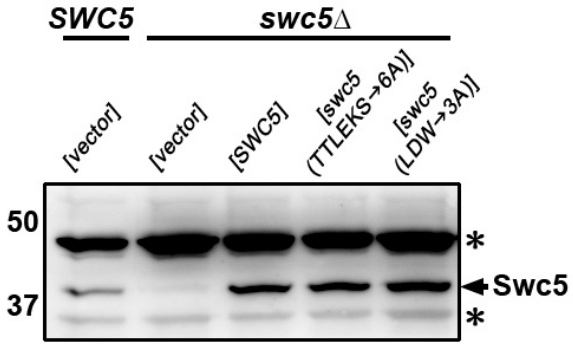

Figure S8. ***Western analysis of ectopically expressed Swc5 and alanine mutants.*** (**A**) Yeast total lysates of *SWC5* and *swc5*Δ cells transformed with the indicated vectors were analyzed on a 14% polyacrylamide gel followed by western blotting analysis using a polyclonal antibody directed against Swc5 (gift of Carl Wu). The asterisks indicate non-specific bands.

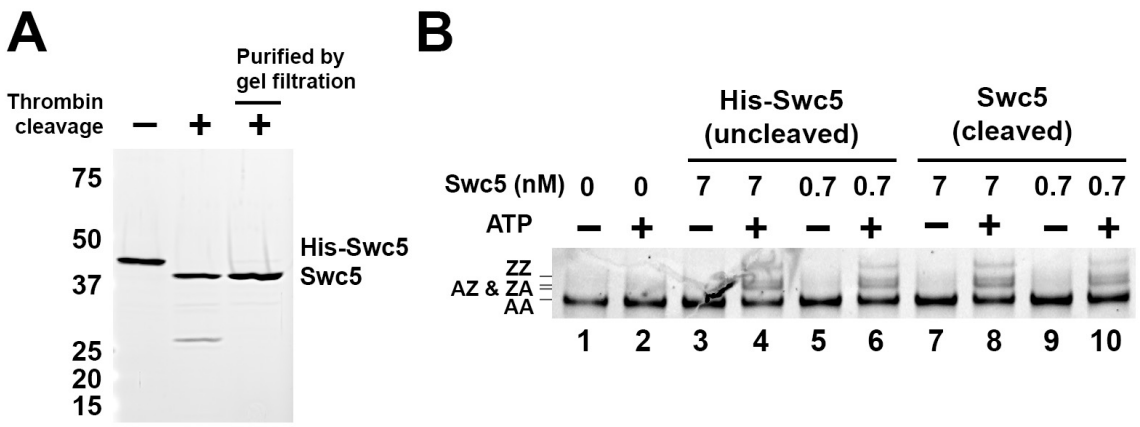

Figure S9. ***The 6xHis tag on Swc5 does not appear to interfere with Swc5 function.*** (**A**) Swc5 proteins before and after cleavage with thrombin. The cleaved Swc5 in lane 3 was further purified by Superdex 200 gel filtration. (**B**) Histone exchange assay conducted as described in **Figure 5C**. Gel filtered Swc5 without the His-tag was used in lanes 7-10.

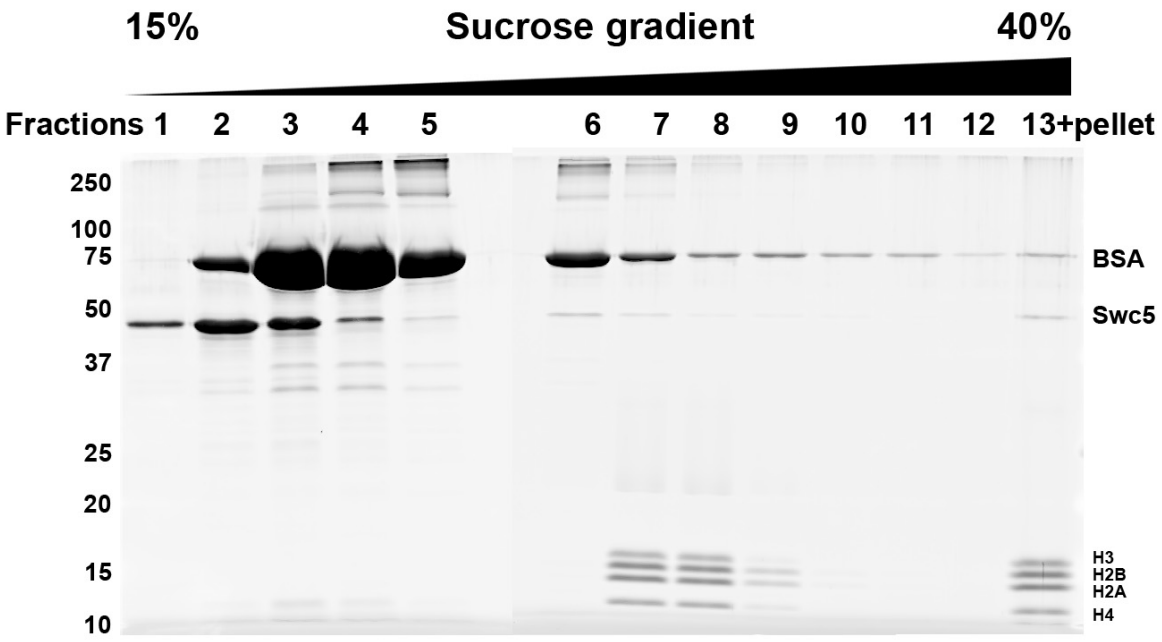

Figure S10. ***Recombinant Swc5 does not appear to bind H2A nucleosome.*** Recombinant Swc5 and AA nucleosome assembled by dialysis were mixed and then separated by sucrose gradient sedimentation. Bovine serum albumin (BSA) was added in the binding reaction to stabilize the nucleosome. The proteins in each fraction were concentrated by TCA precipitation before analyzed by SDS-PAGE and SYPRO Ruby staining.

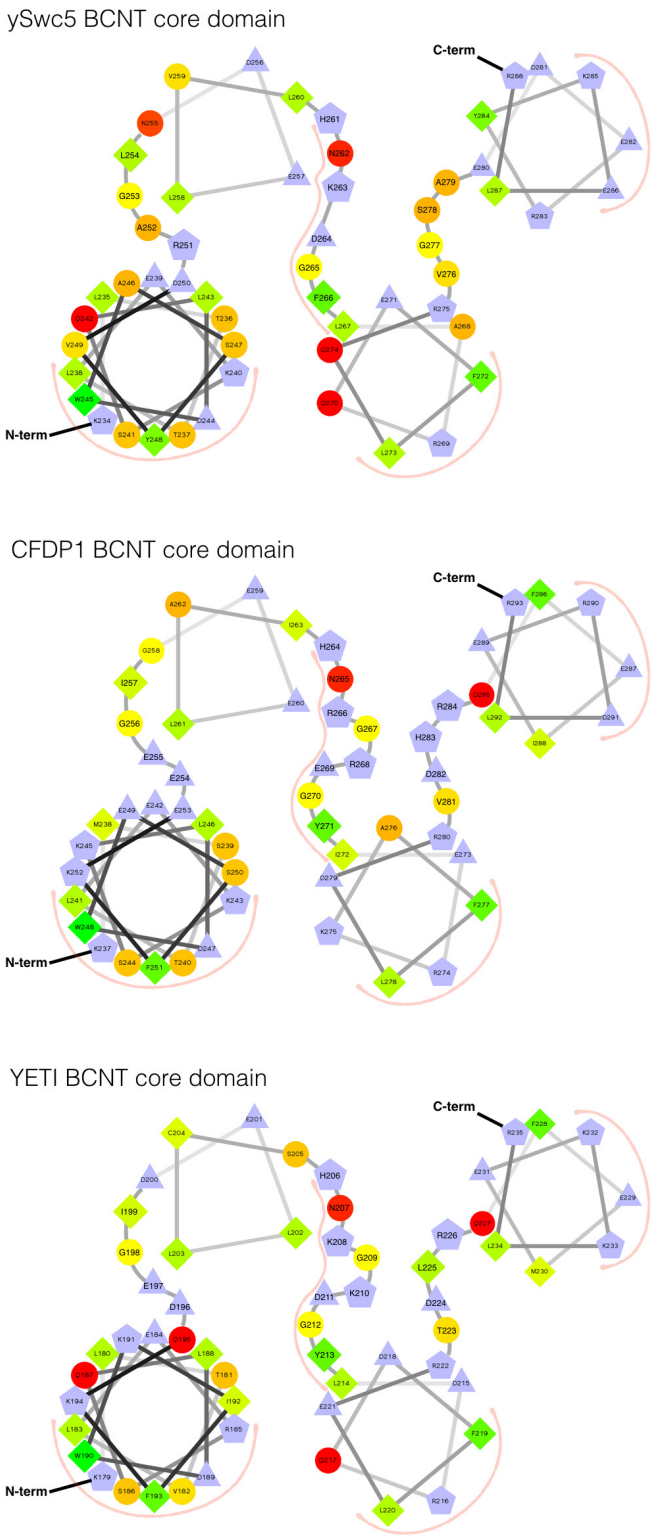

Figure S11. ***Helical wheel projections analysis of the BCNT core domain.*** The BCNT core domains of yeast Swc5, human CFDP1, and fly YETI were plotted as helical wheel projections using a program developed by Armstrong and Zidovetzki (10). Highlighted in pink are the highly conserved residues within the BCNT core.

Table S1: Yeast strains

Strain	Genotype	Source/reference
yEL190	*W1588-4C swr1::SWR1-3Flag-P-kanMX-P htz1Δ::natMX*	Gift of Carl Wu
yEL291	*W1588-4C swr1::SWR1-3Flag-P-kanMX-P swc5Δ::hphMX htz1Δ::natMX*	Gift of Carl Wu
BY4741	*MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Standard Strain
yEL274	*BY4741 swc5Δ::kanMX*	GE Dharmacon (Clone ID: 3371)

Table S2: Plasmids

Plasmid ID	Description	Precursor	Source/reference
pRS416	*URA3 CEN ARS*	--	(11)
pEL460	*pRS416-SWC5*	*pRS416*	This study
pEL467	*pRS416-swc5(1-254)*	*pEL460*	This study
pEL468	*pRS416-swc5(1-232)*	*pEL460*	This study
pEL469	*pRS416-swc5(FAGE→4A)*	*pEL460*	This study
pEL470	*pRS416-swc5(TTLEKS→6A)*	*pEL460*	This study
pEL477	*pRS416-swc5(79-303)*	*pEL460*	This study
pEL478	*pRS416-swc5(147-303)*	*pEL460*	This study
pEL479	*pRS416-swc5(LDW→3A)*	*pEL460*	This study
pEL483	*pRS416-swc5(EcoRI site at +1)*	*pEL460*	This study
pEL340	*pET28c(+)-SWC5*	*pEL28c(+)*	Gift of Carl Wu
pEL472	*pET28c(+)-swc5(1-254)*	*pEL340*	This study
pEL473	*pET28c(+)-swc5(1-232)*	*pEL340*	This study
pEL474	*pET28c(+)-swc5(FAGE→4A)*	*pEL340*	This study
pEL475	*pET28c(+)-swc5(TTLEKS→6A)*	*pEL340*	This study
pEL481	*pET28c(+)-swc5(79-303)*	*pEL340*	This study
pEL482	*pET28c(+)-swc5(147-303)*	*pEL340*	This study
pEL484	*pET28c(+)-swc5(LDW→3A)*	*pEL340*	This study

Supporting material S1

Widom-601 sequence

Nucleosome positioning sequence in cyan

TCTTCACACCGAGTTCATCCCTTATGTGATGGACCCTATACGCGGCCGCCCTGGAGAATCCCGG
TGCCGAGGCCGCTCAATTGGTCGTAGCAAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTC
CCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACAT
CCTGTGCATGTA

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