Supplemental Information

Figures

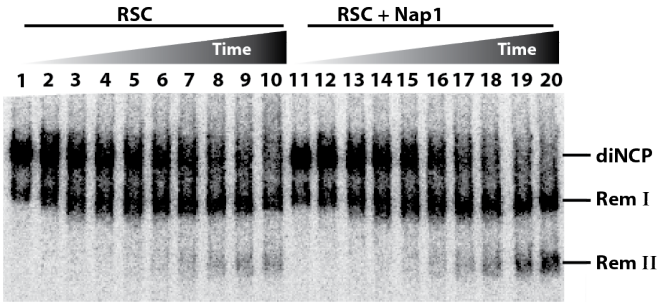

Figure S1. The extent of dinucleosomes disassembled were tracked by gel shift assays and reactions contained 20 nM 601-603 dinucleosomes, 20 nM RSC, and in lanes 11-20 300 nM Nap1. Reactions were stopped as described and ranged from 5 s to 20 min.

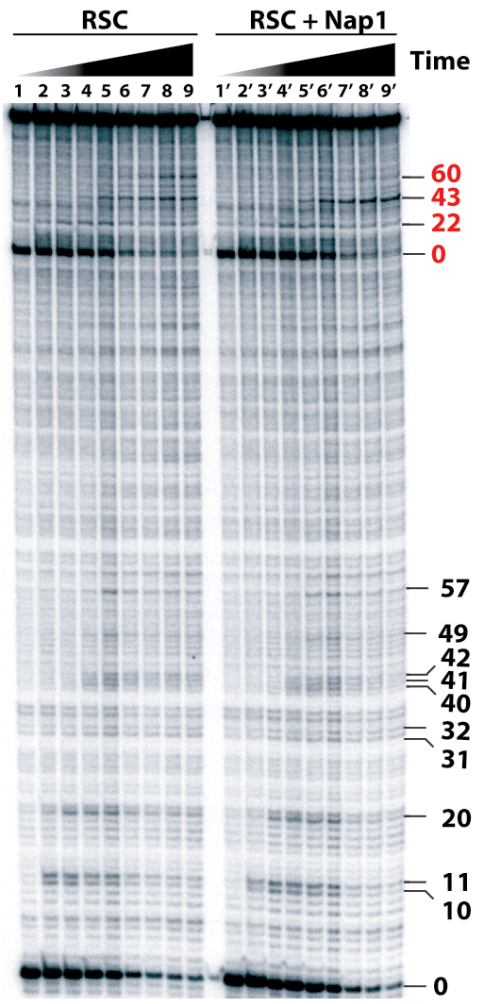

Figure S2. Nap1 changes the displacement of nucleosomes by RSC in a dinucleosome. DNA movement inside of dinucleosome was monitored from the H2A-H2B dimer interface and residue 53 of histone H2B was modified for tracking changes in DNA contacts. DNA cleavage due to the histone modification was followed on a denaturing 6% polyacrylamide gel and visualized by phosphorimaging as shown. Nucleosomes were remodeled with RSC in the absence (lanes 1-9) and presence of Nap1 (lanes 1'-9') for specified times (0, 5, 10, 20, 40, 80, 160, 300 and 600 s). Numbers on the right side of the gel image refer to number of nucleotides (nt) moved from the starting cleavage position (0) due to remodeling for N1 (black) and N2 (red) nucleosome.

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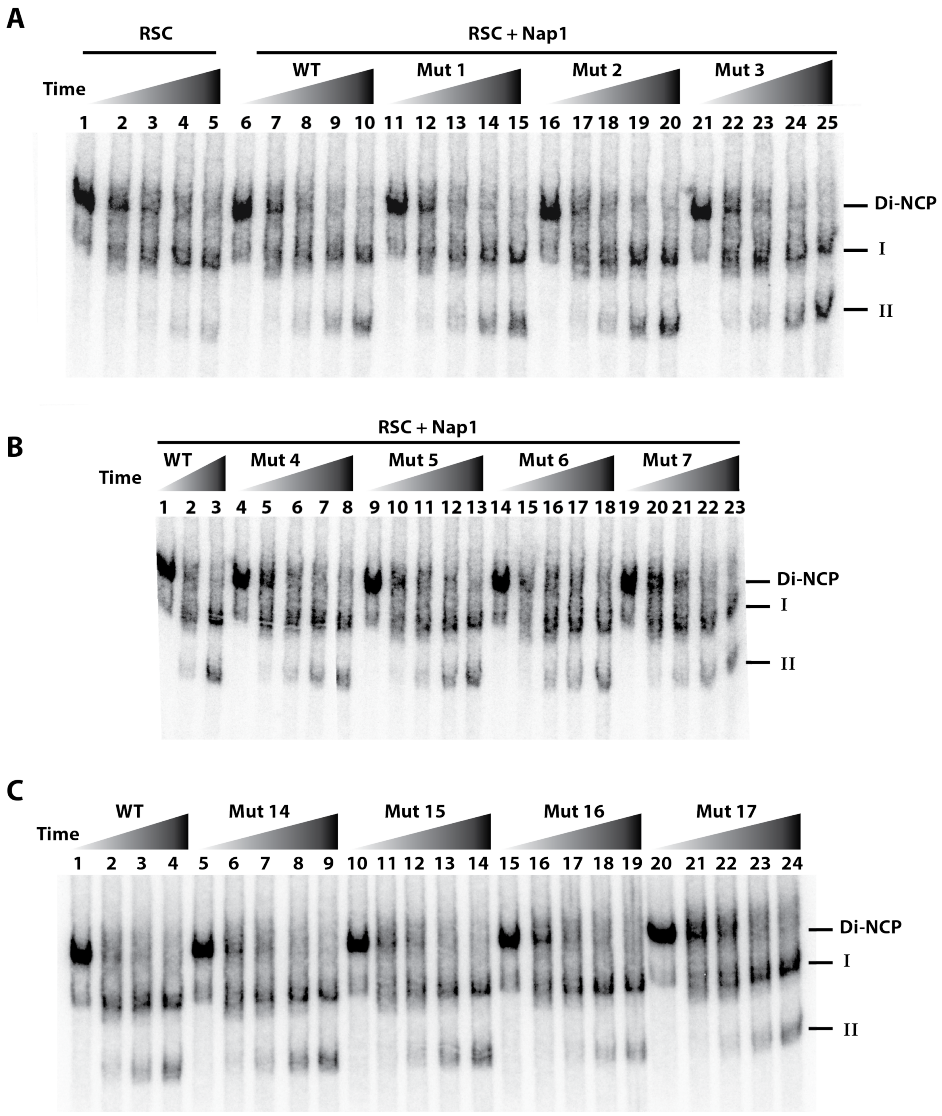

Figure S3 Many but not all of the Nap1 mutants do not affect dinucleosome disassembly by RSC.
Remodeling time course experiments with RSC and Nap1 mutants were analyzed by gel shift assays as shown here and the data plotted in Figure 6. The Nap1 mutants shown here are (A) mutants 1-3; (B) mutants 4-7; and (C) mutants 14-17.

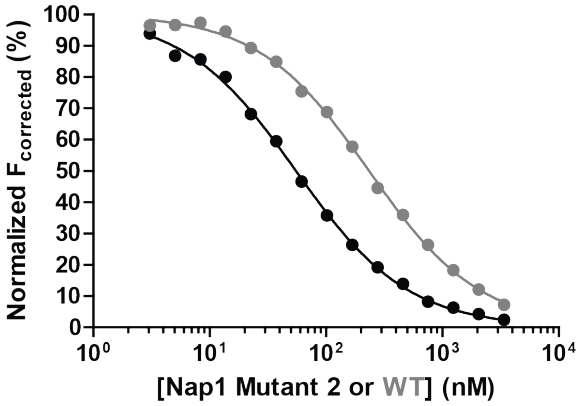

Figure S4. The Nap1 mutant 2 has a higher affinity for H2A-H2B than does wild type Nap1.

The affinity of Nap1 was measured by FRET between residue 118 of H2B (10 nM 488H2A-H2BT118C) and a site on Nap1 (50 nM 647NNap) and competing with unlabeled Nap1 WT (grey) or Mutant 2 (black). Each curve is representative of at least two independent experiments and each data point is the mean of duplicate measurements within a replicate. Errors bars are plus/minus one standard error of the mean and are too small to be visible. All fits have $R2 \geq 0.99$.

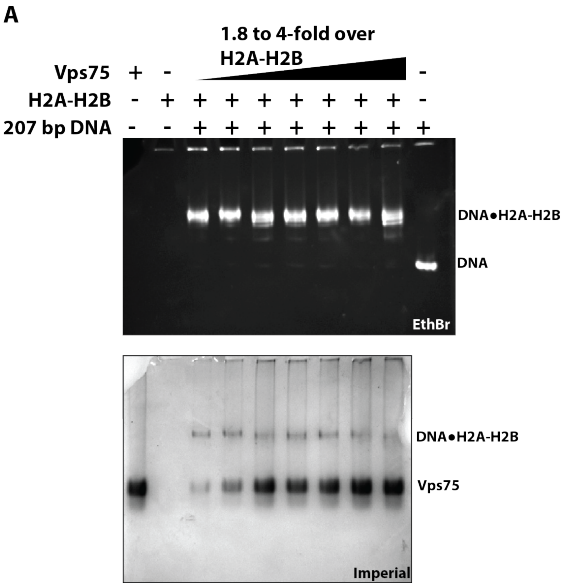

Figure S5. Vps75 does not compete for H2A-H2B binding to DNA.

The ability of the histone chaperone to compete H2A-H2B from DNA was determined using a native gel shift competition assay where Vps75 is titrated against H2A-H2B•207 bp 601 DNA. H2A-H2B and 207 bp 601 DNA are held constant at a ratio of 7.5:1 in 20 mM Tris pH 7.5, 5 mM DTT, 200 mM NaCl. H2A-H2B•DNA and free DNA are visualized by staining with EthBr (top), and protein is visualized by staining with Imperial (bottom). In the same assay, Nap1 is able to compete H2A-H2B off the DNA (For Nap1 data, see D'Arcy et al.)

Tables

Mutant	Mutations	Location	H2A-H2B Binding compared to WT	REF
1	C200A, C249A, C272A	Cysteine mutant background	Same	1
2	Q71A, D72A, S76A, Q80A C200, 249, 272A	Short helix before dimerization helix	Tighter	1
3	F113A, Q114A, M117A, E121A C200, 249, 272A	Underside of dimerization helix	Same, but region changes upon H2A-H2B binding	1
4	K123A, K127A, E133A, Q134A, R137A C200, 249, 272A	Top of dimerization helix	Same, but region changes upon H2A-H2B binding	1
5	P130A C200, 249, 272A	Kink in dimerization helix	Same, but region changes upon H2A-H2B binding	1
6	P130G C200, 249, 272A	Kink in dimerization helix	Same, but region changes upon H2A-H2B binding	1
7	V115A, E116A, E119A, N122A, K123A, Q126A, K129A, E133A, R137A C200, 249, 272A	Dimerization helix	Same, but region changes upon H2A-H2B binding	1
8	V115A, E116A, E119A, N122A, K123A C200, 249, 272A	Dimerization helix (Beginning)	Same, but region changes upon H2A-H2B	1

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			binding	
9	Q126A, K129A, E133A, R137A C200, 249, 272A	Dimerization helix (End)	Same, but region changes upon H2A-H2B binding	[1]
10	V115A, E119A, N122A, Q126A, K129A, E133A, R137A C200, 249, 272A	Dimerization helix (All)	Same, but region changes upon H2A-H2B binding	[1]
11	Q142A, Q144A, E148A, K152A, E155A, E158A C200, 249, 272A	Loop and helix of accessory domain	Same	[1]
12	E162A, E164A, D168S, E169A, E170G, E171S, K172A C200, 249, 272A	Disordered loop/quasi helix	Same	[1]
13	D176A, E178G, E179G, E180S, K183A C200, 249, 272A	Disordered loop	Same	[1]
14	K230A, R234A C200, 249, 272A	Beta-sheet – second strand	Same	[1]
15	H268A, E270A C200, 249, 272A	Beta-sheet – fourth strand	Same	[1]
16	E310A, N314A, D317A C200, 249, 272A	3_{10} helix	Same	[1]-
17	K320S, E324S, D325A, D327S, E328G, E329A C200, 249, 272A	Extended loop not conserved in Vps75	Same	[1]
18	E331A, E332A, D333A, E335A, E336A, R337A C200, 249, 272A	Inside helix	Same	[1]
19	S343A, E346A, Q347A, K349A, D350A, K351A, R355A C200, 249, 272A	Removes side-chains on alpha helix 6	Reduced 50-60-fold	[1]
20	W359A, L352A C200, 249, 272A	Abolishs hydrophobic 'knuckle'	Reduced 50-60-fold	[1]
21	Includes 74-417	No N-terminal tail	Slightly reduced	[2]
22	Includes 1-365	No C-terminal tail	Slightly reduced	[2]
23	ΔE288-K305	No β-hairpin	Same and unable to form Nap1 tetramer	Data not shown and [3]
24	181-417	No N-terminal	Reduced and	Data not

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		tail &dimerization helix	unable to form Nap1 dimer	shown

Table S1. List of mutations in Nap1 and their properties

References

1 D'Arcy, S. *et al.* Chaperone Nap1 shields histone surfaces used in a nucleosome and can put H2A-H2B in an unconventional tetrameric form. *Mol Cell* **51**, 662-677, doi:10.1016/j.molcel.2013.07.015 (2013).

2 Hieb, A. R., D'Arcy, S., Kramer, M. A., White, A. E. & Luger, K. Fluorescence strategies for high-throughput quantification of protein interactions. *Nucleic Acids Res* **40**, e33, doi:10.1093/nar/gkr1045 (2012).

3 Park, Y. J., McBryant, S. J. & Luger, K. A beta-hairpin comprising the nuclear localization sequence sustains the self-associated states of nucleosome assembly protein 1. *J Mol Biol* **375**, 1076-1085, doi:10.1016/j.jmb.2007.11.031 (2008).