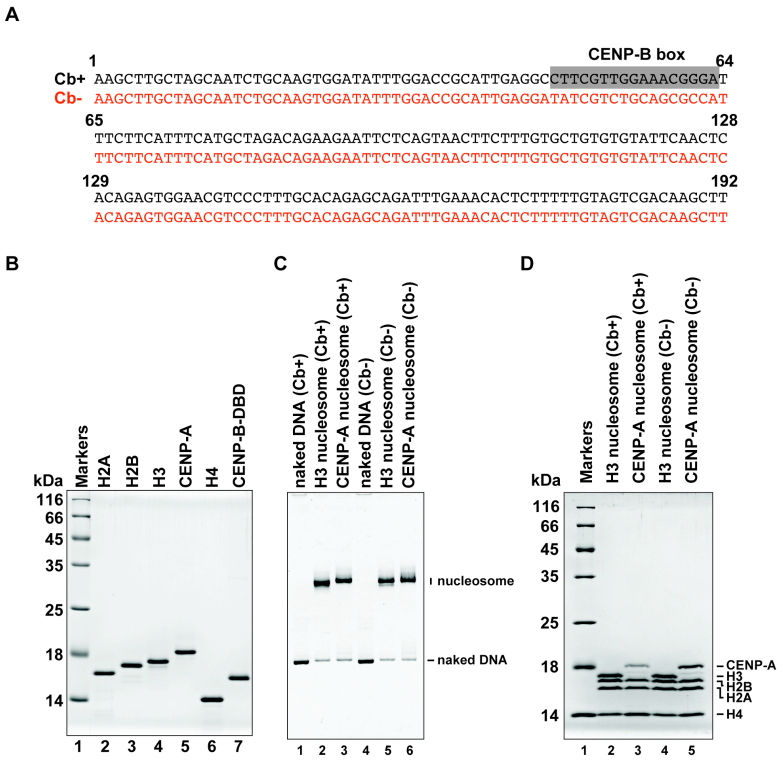


**Figure S1.** Preparation of histones, the CENP-B DBD, and the CENP-A or H3 nucleosomes. (**A**) Sequences of 192 base-pair alphoid DNAs used in the CENP-A nucleosome formation. A gray box indicates the CENP-B box sequence. The Cb+ and Cb- DNA sequences are represented with black and red letters, respectively. (**B**) SDS-polyacrylamide gel electrophoresis of purified histone H2A (lane 2), histone H2B (lane 3), histone H3 (H3.1) (lane 4), CENP-A (lane 5), histone H4 (lane 6), and CENP-B DBD (lane 7). Lane 1 indicates molecular mass markers. (**C**) Reconstitution of nucleosomes. The H3 nucleosomes were reconstituted with the 192 base-pair DNA with (lane 2) or without (lane 5) the CENP-B box sequence. The CENP-A nucleosomes were reconstituted with a 192 base-pair DNA with (lane 3) or without (lane 6) the CENP-B box sequence. The samples were analyzed by non-denaturing 6% polyacrylamide gel electrophoresis with ethidium bromide staining. Lanes 1 and 4 indicate 192 base-pair DNAs with and without the CENP-B box sequence, respectively. (**D**) Histone composition of the reconstituted CENP-A or H3 nucleosomes. The CENP-A or H3 nucleosomes were purified, and the histones were analyzed by SDS-18% polyacrylamide gel electrophoresis with Coomassie Brilliant Blue staining.

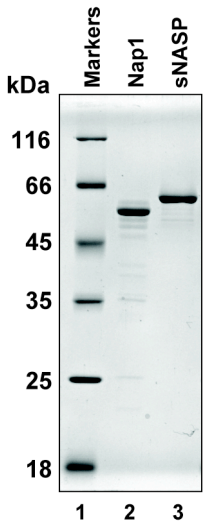


**Figure S2.** Preparation of human Nap1 and sNASP. Purified Nap1 and sNASP were analyzed by SDS-12% polyacrylamide gel electrophoresis with Coomassie Brilliant Blue staining (lanes 2 and 3, respectively). Lane 1 indicates molecular mass markers.

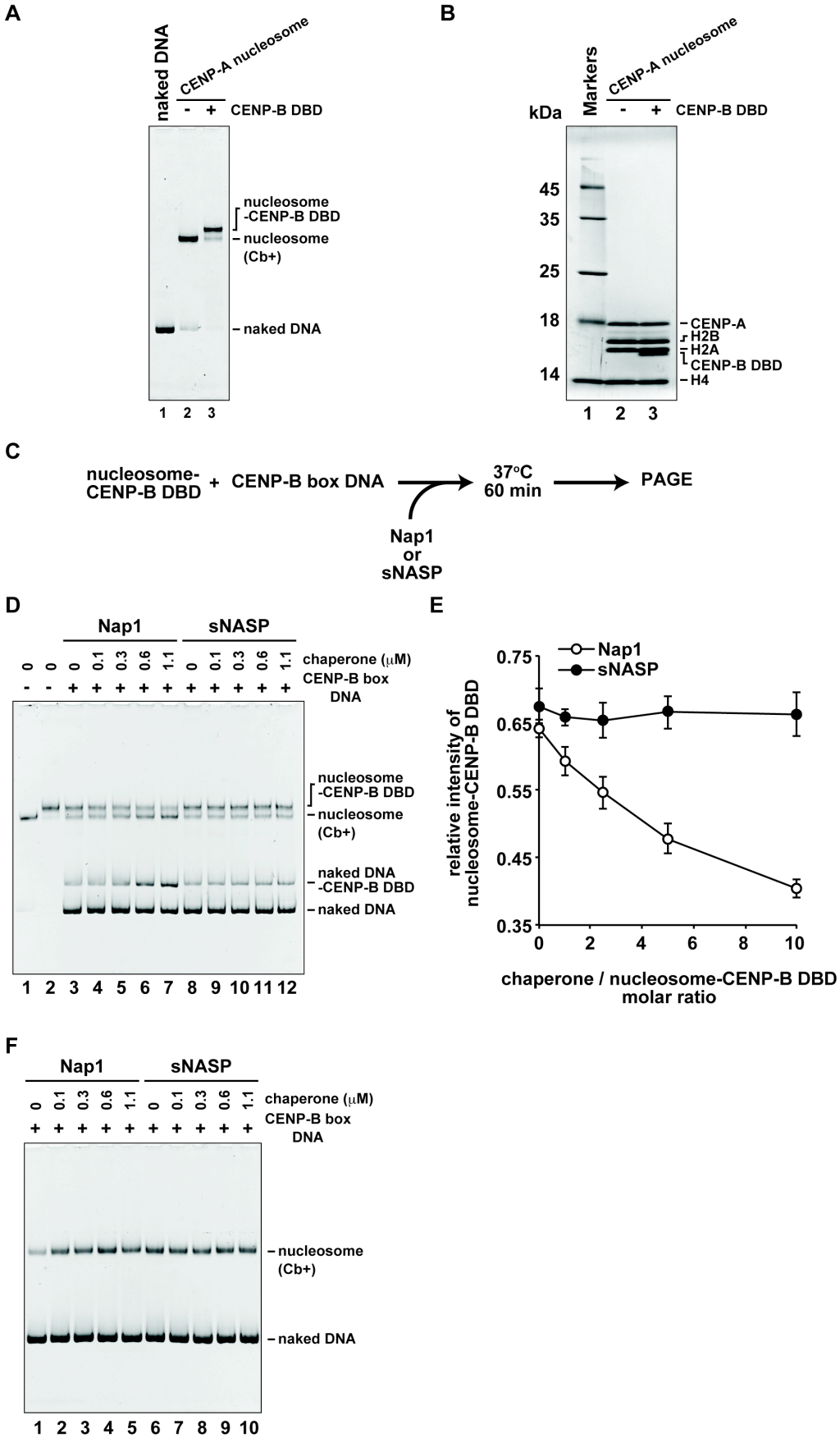
A
naked DNA
CENP-A nucleosome
- + CENP-B DBD
nucleosome -CENP-B DBD
nucleosome (Cb+)
naked DNA
1 2 3
B
kDa
Markers
CENP-A nucleosome
- + CENP-B DBD
45
35
25
18
14
CENP-A
H2B
H2A
CENP-B DBD
H4
1 2 3
C
nucleosome-CENP-B DBD + CENP-B box DNA
37°C 60 min
PAGE
Nap1 or sNASP
D
Nap1
sNASP
chaperone (μM)
0 0 0 0.1 0.3 0.6 1.1 0 0.1 0.3 0.6 1.1
CENP-B box DNA
- - + + + + + + + + + +
nucleosome -CENP-B DBD
nucleosome (Cb+)
naked DNA -CENP-B DBD
naked DNA
1 2 3 4 5 6 7 8 9 10 11 12
E
Nap1
sNASP
relative intensity of nucleosome-CENP-B DBD
0.75
0.65
0.55
0.45
0.35
0 2 4 6 8 10
chaperone / nucleosome-CENP-B DBD molar ratio
F
Nap1
sNASP
chaperone (μM)
0 0.1 0.3 0.6 1.1 0 0.1 0.3 0.6 1.1
CENP-B box DNA
+ + + + + + + + + +
nucleosome (Cb+)
naked DNA
1 2 3 4 5 6 7 8 9 10

**Figure S3.** The CENP-B disassembly assay. (**A**) Reconstitution of the CENP-A nucleosomes. Lane 1 indicates the 166 base-pair naked DNA. The CENP-A nucleosomes were reconstituted with the 166 base-pair DNA without (lane 2) or with (lane 3) the CENP-B DBD by the salt dialysis method, in the absence of Nap1. The samples were then purified by the Prep Cell apparatus, and were analyzed by non-denaturing 6% polyacrylamide gel electrophoresis with ethidium bromide staining. (**B**) Protein composition of the CENP-A nucleosomes reconstituted with the 166 base-pair DNA, with (lane 2) or without (lane 3) the CENP-B DBD. Proteins were analyzed by SDS-18% polyacrylamide gel electrophoresis with Coomassie Brilliant Blue staining. (**C**) A schematic representation of the disassembly assay. (**D**) The CENP-B DBD, complexed with the CENP-A nucleosomes (114 nM) containing the CENP-B box sequence, was incubated with Nap1 (lanes 3-7) or sNASP (lanes 8-12) for 1 h at 37°C in the presence of the naked 166 base-pair DNA (114 nM), as a competitor DNA. The Nap1 and sNASP concentrations were 0 μM (lanes 3 and 8), 0.1 μM (lanes 4 and 9), 0.3 μM (lanes 5 and 10), 0.6 μM (lanes 6 and 11), and 1.1 μM (lanes 7 and 12). The samples were then analyzed by non-denaturing 6% polyacrylamide gel electrophoresis with ethidium bromide staining. (**E**) Graphic representation of the experiments shown in panel (**D**). Averages of three independent experiments were plotted with the standard deviations. (**F**) Nap1 and sNASP did not disrupt the CENP-A nucleosomes under the experimental conditions used in this study. The CENP-A nucleosomes (114 nM) containing the CENP-B box sequence were incubated with Nap1 (lanes 1-5) or sNASP (lanes 6-10) for 1 h at 37°C. The Nap1 and sNASP concentrations were 0 μM (lanes 1 and 6), 0.1 μM (lanes 2 and 7), 0.3 μM (lanes 3 and 8), 0.6 μM (lanes 4 and 9), and 1.1 μM (lanes 5 and 10). The samples were then analyzed by non-denaturing 6% polyacrylamide gel electrophoresis with ethidium bromide staining.

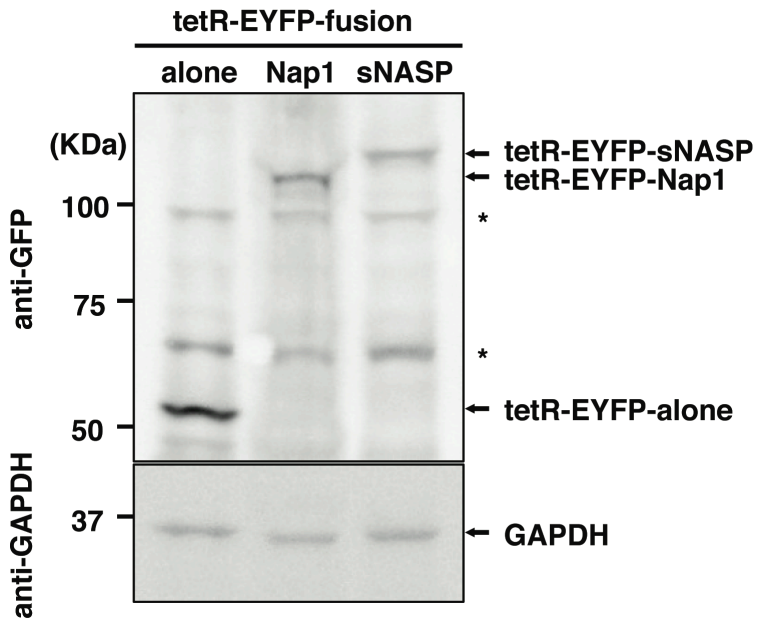


**Figure S4.** Expression of tetR-EYFP-fusion proteins in HeLa cells. HeLa-HAC-2-4 cells were transfected with a set of tetR-EYFP-fusion expressing plasmids. Comparable signals of the fusion proteins were detected upon western blotting with an anti-GFP antibody. An anti-GAPDH antibody was used for a loading control.

The cells were collected two days after transfection, and $1\times10^5$ cells were used for SDS-PAGE. The proteins were transferred to a PVDF membrane. The membrane was blocked with PBS containing 10% skim milk (Wako) and in 0.1% Tween20 for 60 min at room temperature, incubated with primary antibodies for 60 min at room temperature, washed 5 times with PBS containing 0.1% Tween20, and incubated with HRP-conjugated secondary antibodies for 60 min at room temperature. After 5 washes, chemiluminescence was detected using the Pierce ECL Western Blotting Substrate (Thermo). The anti-GFP polyclonal antibody (ab290: Abcam) was used at a 1:5,000 dilution. The anti-GAPDH antibody (HRP) (ab9385: Abcam) was used at 0.5 μg/ml. Goat Anti-Rabbit IgG, HRP-conjugate (Nacalai Tesque) was used at 0.5 μg/ml. The antibodies were diluted in PBS containing 0.5% skim milk and 0.1% Tween20.

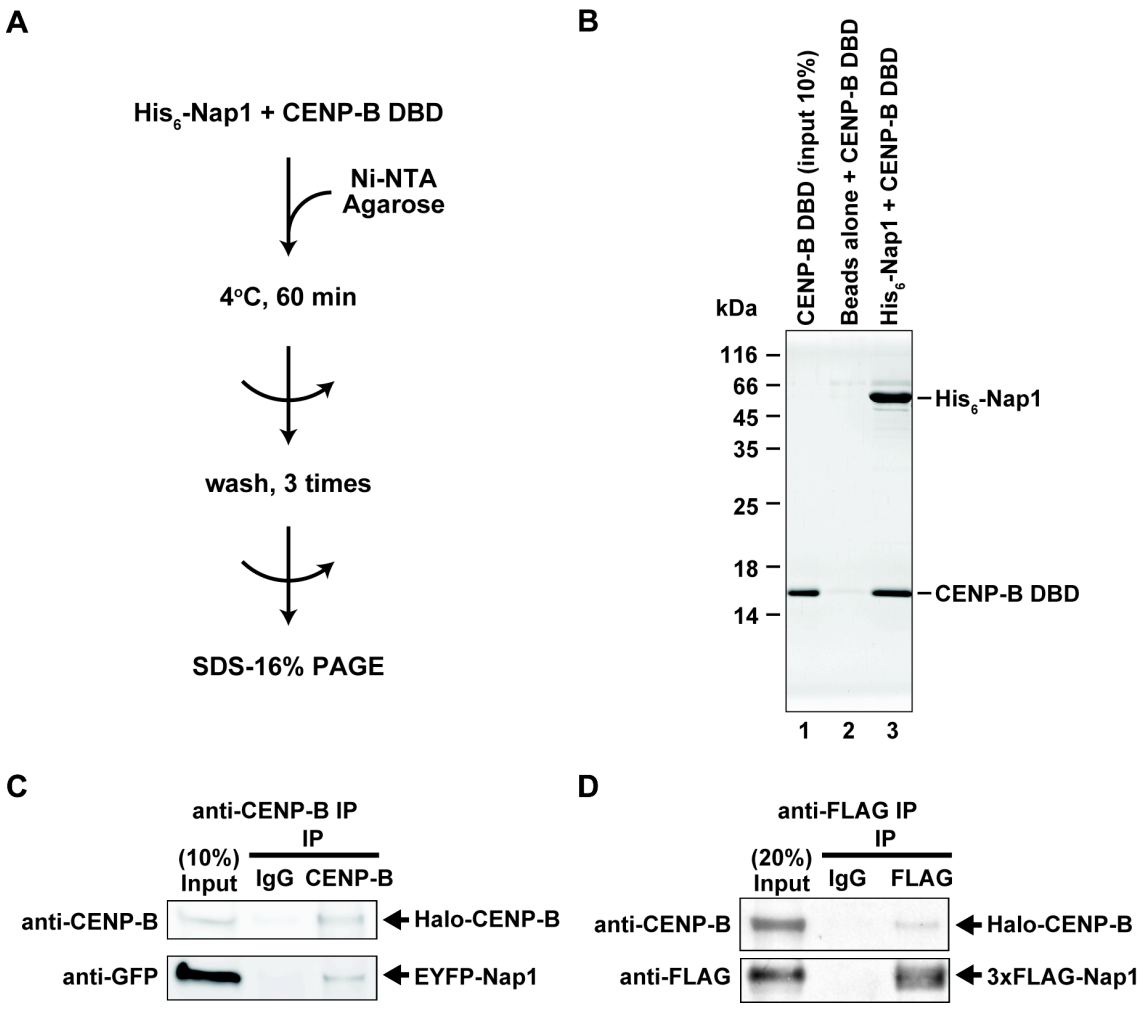
A
His6-Nap1 + CENP-B DBD
Ni-NTA Agarose
4°C, 60 min
wash, 3 times
SDS-16% PAGE
B
CENP-B DBD (input 10%)
Beads alone + CENP-B DBD
His6-Nap1 + CENP-B DBD
kDa
116
66
45
35
25
18
14
His6-Nap1
CENP-B DBD
1 2 3
C
anti-CENP-B IP
IP
(10%) Input
IgG
CENP-B
anti-CENP-B
anti-GFP
Halo-CENP-B
EYFP-Nap1
D
anti-FLAG IP
IP
(20%) Input
IgG
FLAG
anti-CENP-B
anti-FLAG
Halo-CENP-B
3xFLAG-Nap1

**Figure S5.** Interaction between Nap1 and CENP-B *in vitro* and *in vivo*. (**A**) A schematic diagram of the Ni-NTA bead pull-down assay. (**B**) The CENP-B DBD (1.5 μg, 200 nM) was mixed with $His_6$-tagged Nap1 (14 μg, 600 nM) (lane 3), and the proteins bound to the Ni-NTA beads were analyzed by SDS-15% polyacrylamide gel electrophoresis with Coomassie Brilliant Blue staining. Lane 1 indicates the input (10%) of the CENP-B DBD. Lane 2 indicates a negative control experiment without $His_6$-tagged Nap1. (**C**) HeLa cells were transfected with the Halo-CENP-B and EYFP-Nap1 expression plasmids. Cell extracts were immunoprecipitated with either an anti-CENP-B C terminus antibody (5E6C1) or normal IgG (IgG). Immunoprecipitated samples were analyzed by western blotting with an anti-CENP-B N-ter polyclonal antibody (BN1) or an anti-GFP antibody. Lanes labeled "Input" contain the equivalent of 10% of the input protein. (**D**) HeLa cells were transfected with the Halo-CENP-B and 3×FLAG-Nap1 expression plasmids. Cell extracts were immunoprecipitated with either the anti-FLAG antibody (FLAG) or normal IgG (IgG).

HeLa cells expressing Halo-CENP-B and EYFP-Nap1 (**C**) or 3×FLAG-Nap1 (**D**) were collected two days after transfection. For lysate preparation, $1.1\times10^6$ or $3\times10^6$ cells were sonicated with a Bioruptor (Cosmobio) for 5 min in sonication buffer (20 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 1% Sigma protease inhibitor cocktail for mammalian cell). After centrifugation, the supernatant was incubated with IP buffer (20 mM Tris-HCl (pH 7.4), 1 mM EDTA, 150 mM NaCl, 1% Triton X-100, 0.1% SDS and 20% glycerol), containing 1% BSA overnight at 4°C. For immunoprecipitation, 5 μg anti-CENP-B C terminus antibody (5E6C1) or anti-FLAG M2 antibody (Sigma-Aldrich) and normal mouse IgG (Santa Cruz Biotechnology) with Protein G Sepharose 4 Fast Flow (GE Healthcare) were used. The beads were pelleted and washed three times with IP buffer. The proteins bound to the beads were analyzed by western blotting. The anti-CENP-B N terminus antibody (BN1) was used at a 1:2,000 dilution. The anti-GFP polyclonal antibody (ab290: Abcam) was used at a 1:2,000 dilution. The monoclonal anti-FLAG M2-HRP antibody (Sigma-Aldrich) was used at a 1:1,000 dilution.

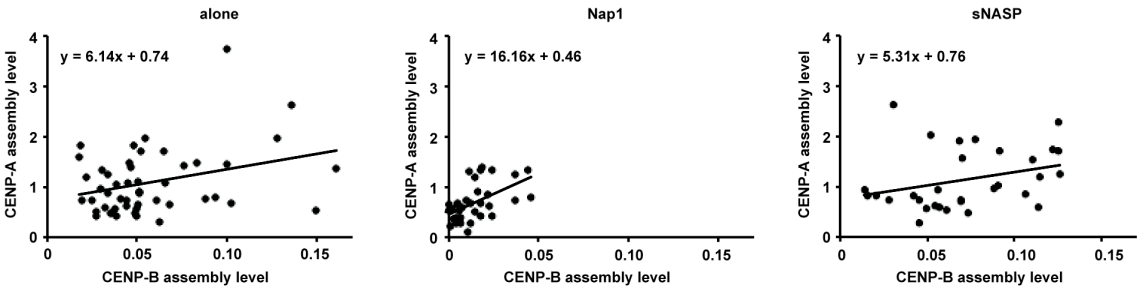


**Figure S6.** Positive correlation between CENP-B and CENP-A assembly levels on the alphoid$^{tetO}$ HAC tethered by Nap1 fusions. The data sets of Figure 4 (C) and (D) were analyzed and replotted. The CENP-A assembly level positively correlates with the CENP-B assembly level, and the correlation is stronger when CENP-B assembly was reduced by Nap1 tethering. Linear approximation equations are shown on each graph.