Expanded View Figures

Figure EV1. DNA–H2A–H2B binding and electrostatics of the yNap1–H2A–H2B complex.
Related to Fig 2.

A, B $2F_O–F_c$ omit electron density map for the region comprising H2A–H2B contoured at 1σ in absence (A) or presence (B) of H2A/H2B in ribbon representation. Similar views as in Fig 2A–C are shown.
C–E DNA binding of the H2A–H2B heterodimer.
F View of the ternary complex with H2A–H2B in ribbon representation. APBS-calculated electrostatics (−7 kT to +7 kT) were mapped onto yNap1 dimer surface.
G HBR1.
H HBR2.

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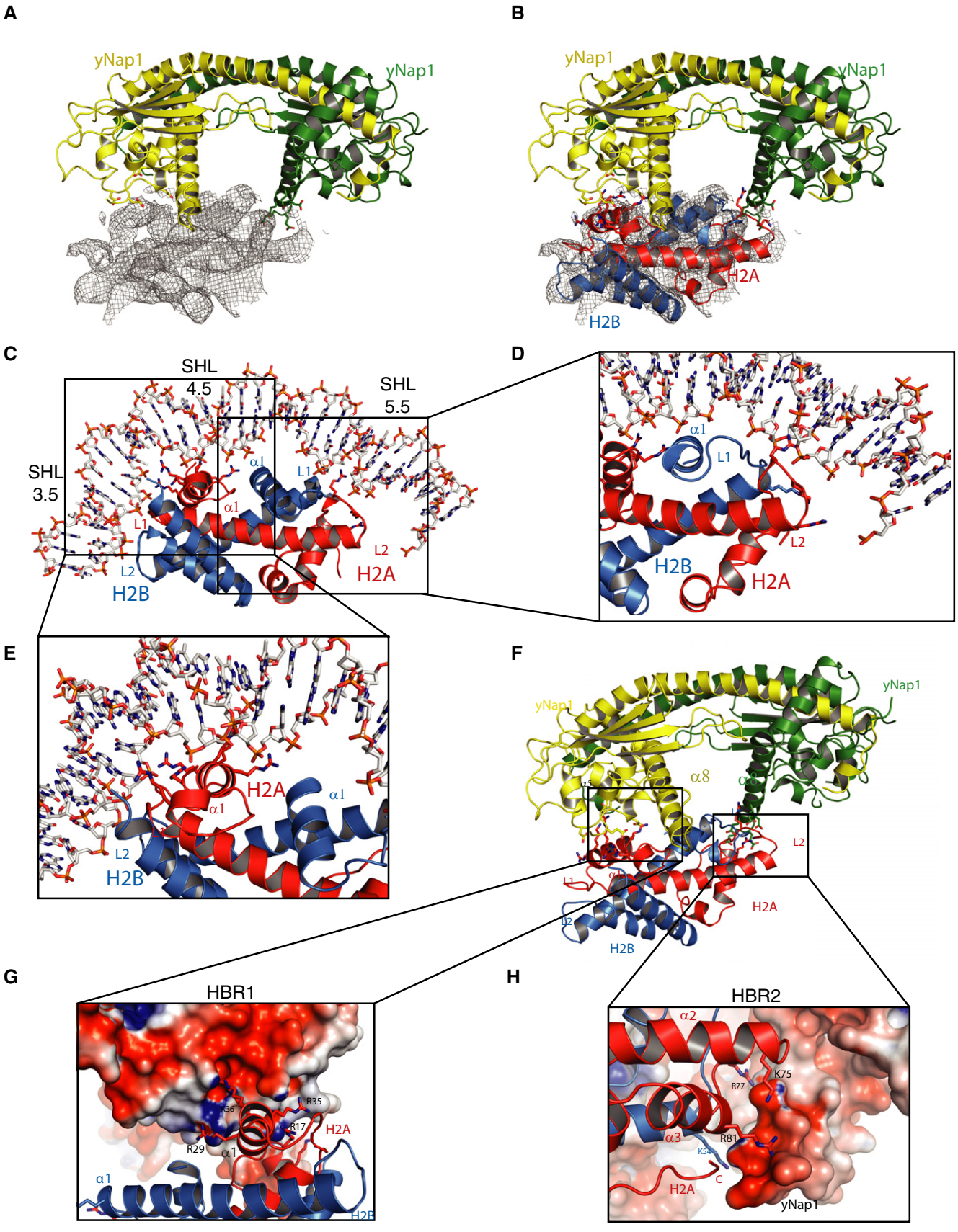

Figure EV1.

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Figure EV2. Sequence conservation.
Related to Fig 3.

A The yNap1 dimer is color-coded according to the sequence conservation.
B HBR2.
C HBR1. The higher the conservation, the darker the color. Similar views as in Fig 3A–C are shown.
D Nap1-like histone chaperons with highlighted conserved residues. Secondary structure elements are shown above the amino acid sequences. The histone binding region 1 or 2 (HBR1 or HBR2) as well as the oligomerization interface (IF1) of yNap1 are highlighted in green. Key residues in the HBR1 and HBR2 (●) as well as IF1 (△) are indicated. The structure-based sequence alignment was performed using Pymol (Schrodinger, 2010) and ClustalW (Thompson *et al*, 1994). The figure was generated using Espript (Gouet *et al*, 2003).

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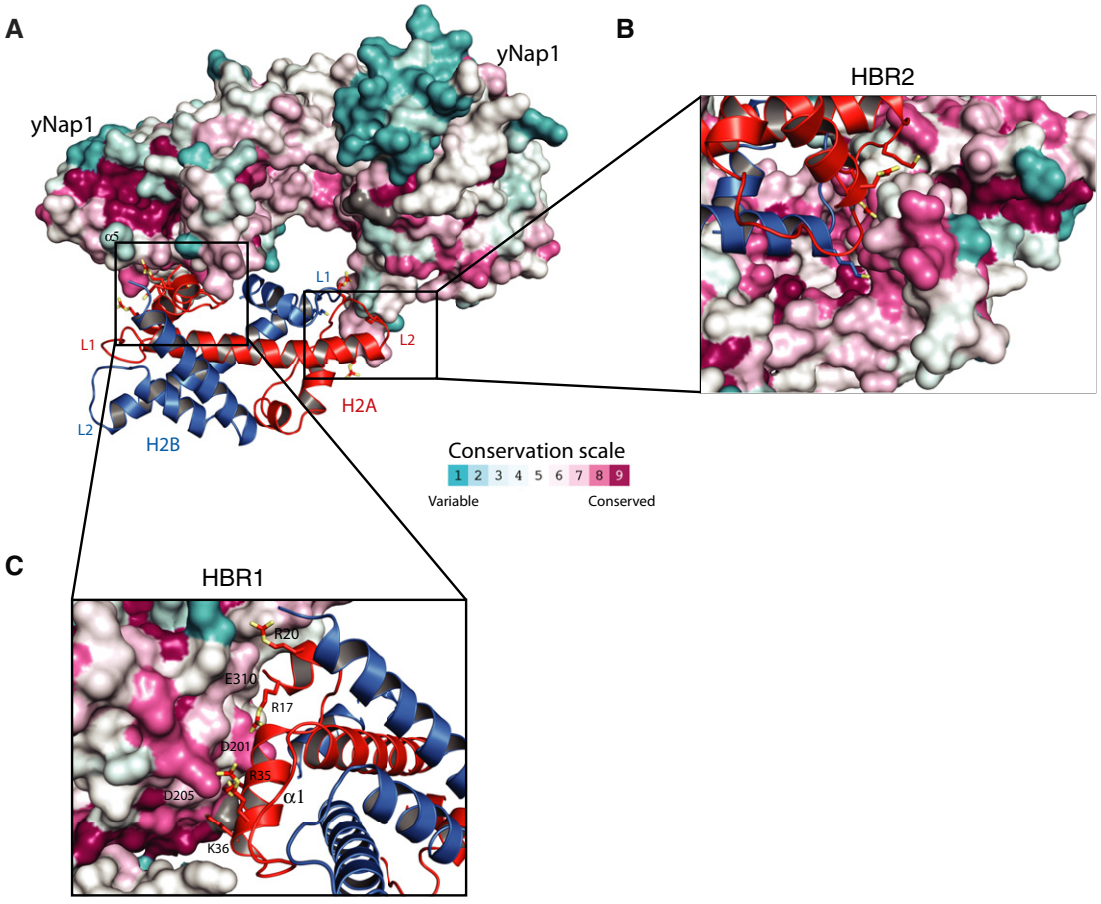

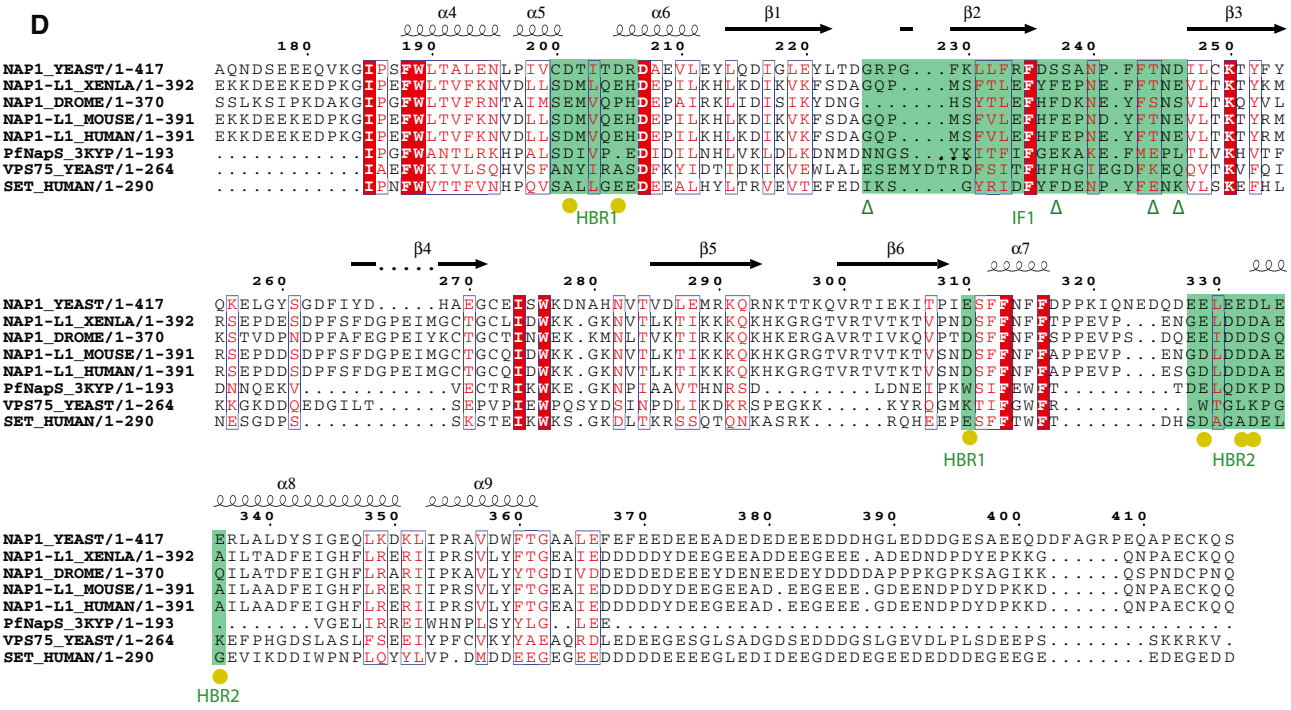

Figure EV2.

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Figure EV3. yNap1–H2A–H2B oligomerization.
Related to Fig 4.

A Native mass spectra of yNap1c against varying concentrations of H2AΔ14–H2BΔ28.
B Native PAGE assay of yNap1 binding to H2A–H2B (lanes 1–5), yNap1c binding to H2A–H2B (lanes 6–10), or of yNap1_IF1 to H2A–H2B (lanes 11–15).
C Native PAGE assay of H2AΔ14_IF2–H2BΔ28 binding to yNap1 (lanes 1–5) or yNap1c (lanes 6–10) or yNap1_IF1 (lanes 11–15). Samples were separated by 6% native PAGE and visualized by Coomassie staining.
D, E EM analysis of the full-length yNap1–H2A–H2B complex.

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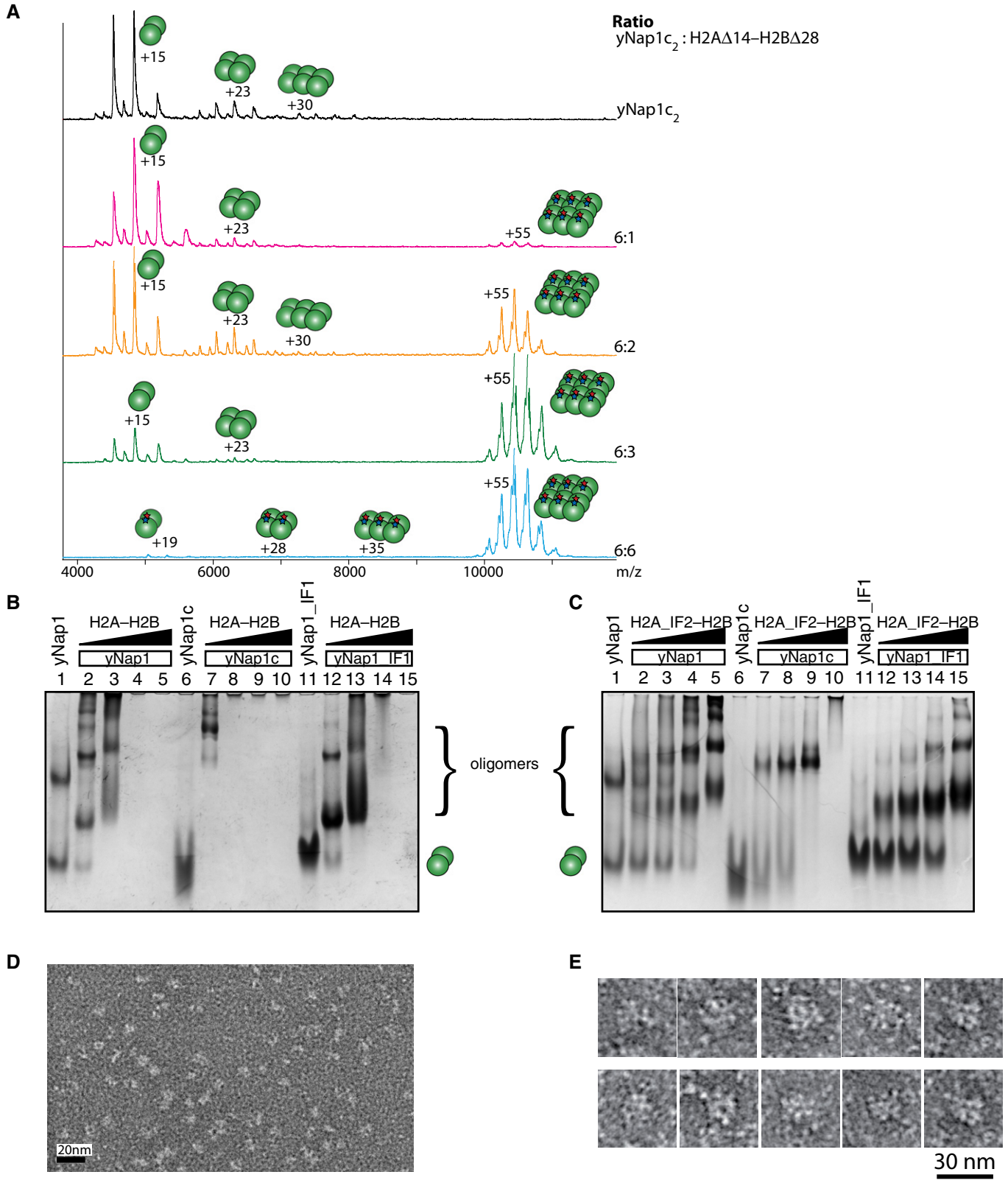

Figure EV3.

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Figure EV4. Nucleosome assembly *in vitro*.
Related to Fig 6.

A Native PAGE gel showing the result of a multiple fluorescence relative affinity shift assay in the absence of yNap1; 31 nM–8 μM H2A–H2B (lanes 4–12) were incubated with a mixture of 0.7 μM AF488-labeled H3–H4–DNA tetrasome complexes and 0.7 μM AF647-labeled DNA. The gel was scanned to detect AF488- (left) or AF647-labeled DNA (right). Lane 1 shows migration of tetrasomes (tetra). Appearance of nucleosomes (nucl) is indicated.

B To quantify nucleosome assembly, the intensity of the band (nucl) was plotted as a function of H2A–H2B concentration. To measure non-specific DNA binding, the disappearance of the free DNA AF647 band was plotted. The value in the absence of H2A–H2B (lane 1) was set to 100% intensity. The curve is representative of three independent experiments performed on different days. Standard deviations are indicated.

C, D Same experiment as in (A) for the yNap1_IF1–H2A–H2B and for the yNap1c–H2A–H2B.

E Nucleosome assembly rescue assay. Increasing amounts 1 μM–4 μM H2A–H2B were added to 0.7 μM AF488-labeled H3–H4–DNA tetrasome complexes (lane 1) and resulted in non-specific aggregation at 2–4 μM H2A–H2B (lanes 2–4). Incubation of 4 μM H2A–H2B with increasing amounts 0.31 μM–5 μM of yNap1 (lanes 5–9) resulted in a rescue of nucleosome assembly and prevented non-specific DNA binding of H2A–H2B. The yNap1_HBR1 + 2 (lanes 10–14) show a defect in this rescue assay.

F Same experiment as in (E) for the yNap1_HBR1 (lanes 5–9) and yNap1_HBR2 (lanes 10–14).

G Same experiment as in (E) for the yNap1c (lanes 5–9) and yNap1_IF1 (lanes 10–14).

H Quantification of the "rescue" activity of yNap1 variants. Each data point is the mean of three independent measurements. The error bar indicates the standard deviation of the data.

I To show the composition of the salt assembled tetrasome and nucleosome preparations, the same sample was run on a native and SDS–PAGE gel.

J Expression controls for yNap1 mutants. Nap1 pool in BY4741, Nap1Δ, and Nap1 mutants compared with TBP (TATA binding protein) as loading control.

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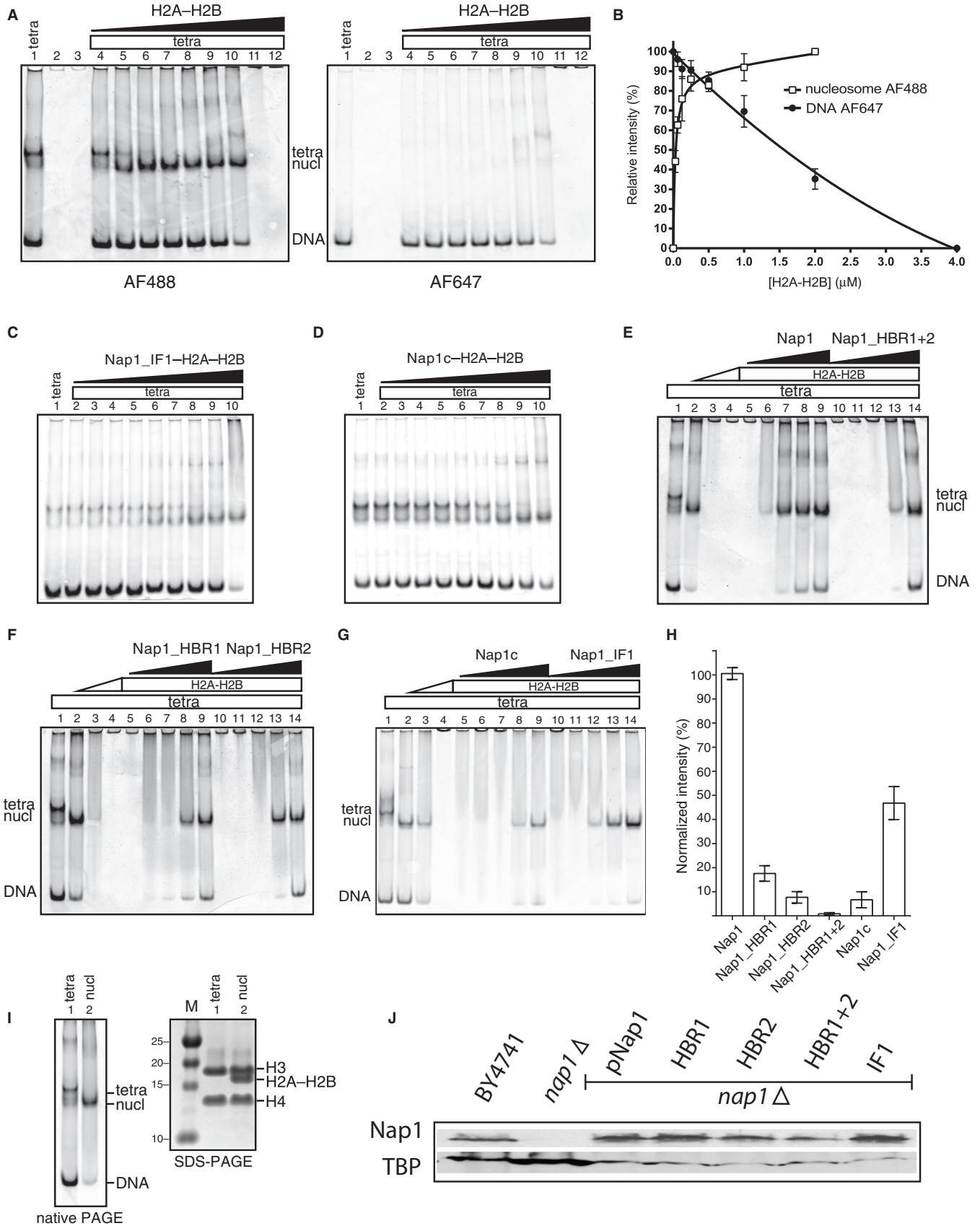

Figure EV4.

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