Supplemental Data

yFACT Induces Global Accessibility of Nucleosomal DNA without H2A-H2B Displacement

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Sequences of DNA variants used for restriction endonuclease tests.

The sequences of the 181 bp 5S rDNA nucleosome positioning sequence variants used in these studies are listed below. 146 bp versions include only the expected 5S rDNA NPS as indicated in upper case letters. The original WT template (Dral-78) was mutated to convert the Dral site to an AseI site (colored pink with the two bases mutated in lower case) in Dral-0. Other variants were derived from Dral-0 to introduce the Dral sites shown in red (with mutated bases shown in lower case letters).

The sea urchin 5S rDNA sequence used here (Simpson and Stafford, 1983) was expected to position histone octamers over the central 146 bp NPS in the 181 bp DNA fragment. However, nucleosomes that were formed with Dral-152 and Dral-157 were readily digested even in the absence of yFACT, but they were resistant to AseI digestion indicating normal formation and stability of the nucleosomes. It therefore appears that the positioning of yeast histones is more strongly influenced by DNA ends than expected, resulting in a tendency for the histone cores to preferentially occupy the left end of these linear fragments, as shown in Fig 1 of the main text. Additional tests with multiple endonucleases on these and other variants showed that histone octamers were distributed at multiple sites after deposition from high salt at 4°C, but shifted principally to the left end of the fragments after heating to 42°C. For example, the fraction of nucleosomes assembled with the Dral-152 template that were sensitive to Dral digestion increased upon heating, but the fraction sensitive to AseI digestion decreased, consistent with a shift of the histone octamers to the left end of the fragment (not shown). This translocation of histone cores during heating was expected (Luger et al., 1999), but the preferential positioning at the end was not.

Seven previously described nucleosome positioning sequence variants that contain two TG rotational phasing sequences (Shrader and Crothers, 1989) and recognition sites for PstI in various positions (Fan et al., 2003) were obtained from G. Narlikar (UCSF). The 189 bp PCR fragment used
here with the original sequence is shown below as PstI-73. PstI-0 was derived from PstI-73 by mutating the PstI site to the EagI site shown below in pink (with the mutated bases shown in lower case letters). Two additional variants were derived from PstI-0 by introducing the mutations shown in lower case letters in red. The MluI site found in all variants except PstI-24 is underlined.

Restriction endonuclease tests with nucleosomes assembled with these fragments showed that the principal products have the histone cores at the left end of the DNA molecules as noted for the DraI variant set. The 5S rDNA and TG sequences used here primarily set the rotational phase of nucleosomes, so this altered translational positioning is not entirely unexpected (Shrader and Crothers, 1989) but may reflect a stronger tendency for yeast histones to abut the end of a linear DNA molecule.

**DraI-0**
```
cgcggattccgggaattccACCGATAAACCTTCAGGGAGATTATAGCCGATCGTCATAAATCCCTGACCCaTTAA
  TAGCTTAACCTTTTCAAGCAAGCCTAGCCATACCATGCTGAATATACCGGTTCTCGTCCGATCACCGAAGTCAA
  GCAGATatcgggcctgcgttagt
```

**DraI-29**
```
cgcggattccgggaattccACCGATAACCTTTTCAAGCAAGCCTAGCCATACCATGCTGAATATACCGGTTCTCGTCCGATCACCGAAGTCAA
  TAGCTTAACCTTTTCAAGCAAGCCTAGCCATACCATGCTGAATATACCGGTTCTCGTCCGATCACCGAAGTCAA
  GCAGATatcgggcctgcgttagt
```

**DraI-58**
```
cgcggattccgggaattccACCGATAAACCTTCAGGGAGATTATAGCCGATCGTCATAAATCCCTGACCCaTTAA
  TAGCTTAACCTTTTCAAGCAAGCCTAGCCATACCATGCTGAATATACCGGTTCTCGTCCGATCACCGAAGTCAA
  GCAGATatcgggcctgcgttagt
```

**DraI-78 (WT)**
```
cgcggattccgggaattccACCGATAAACCTTCAGGGAGATTATAGCCGATCGTCATAAATCCCTGACCCaTTAA
  TAGCTTAACCTTTTCAAGCAAGCCTAGCCATACCATGCTGAATATACCGGTTCTCGTCCGATCACCGAAGTCAA
  GCAGATatcgggcctgcgttagt
```

**DraI-93**
```
cgcggattccgggaattccACCGATAAACCTTCAGGGAGATTATAGCCGATCGTCATAAATCCCTGACCCaTTAA
  TAGCTTAACCTTTTCAAGCAAGCCTAGCCATACCATGCTGAATATACCGGTTCTCGTCCGATCACCGAAGTCAA
  GCAGATatcgggcctgcgttagt
```

**DraI-125**
```
cgcggattccgggaattccACCGATAAACCTTCAGGGAGATTATAGCCGATCGTCATAAATCCCTGACCCaTTAA
  TAGCTTAACCTTTTCAAGCAAGCCTAGCCATACCATGCTGAATATACCGGTTCTCGTCCGATCACCGAAGTCAA
  GCAGATatcgggcctgcgttagt
```
DraI-130
cgcgcggatccgggaattcCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATCCCTGACCCaTTAA
\textup{}\textsubscript{ATAGCTTTACATCAAGCAAGAGCCTACGACCATAACCAGACTGCTGAAATACCCGGTTATTCgGcGGTTAACCGATACCGTACCATACATCCCTGACCCaTTAA
\textup{}\textsubscript{GCAGATatcggctcggttagt}

DraI-140
cgcgcggatccgggaattcCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATCCCTGACCCaTTAA
\textup{}\textsubscript{ATAGCTTTACATCAAGCAAGAGCCTACGACCATAACCAGACTGCTGAAATACCCGGTTATTCgGcGGTTAACCCCGATACCGTACCATACATCCCTGACCCaTTAA
\textup{}\textsubscript{GCAGATatcggctcggttagt}

DraI-152
cgcgcggatccgggaattcCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATCCCTGACCCaTTAA
\textup{}\textsubscript{ATAGCTTTACATCAAGCAAGAGCCTACGACCATAACCAGACTGCTGAAATACCCGGTTATTCgGcGGTTAACCGATACCGTACCATACATCCCTGACCCaTTAA
\textup{}\textsubscript{GCAGATatcggctcggttagt}

DraI-157
cgcgcggatccgggaattcCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATCCCTGACCCaTTAA
\textup{}\textsubscript{ATAGCTTTACATCAAGCAAGAGCCTACGACCATAACCAGACTGCTGAAATACCCGGTTATTCgGcGGTTAACCGATACCGTACCATACATCCCTGACCCaTTAA
\textup{}\textsubscript{GCAGATatcggctcggttagt}

DraI-(Hydroxyl Radical Template)

CAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATCCCTGACCCTTTAAATAGCTTTACATCAAGCAAGAGCCTACGACCATAACCAGACTGCTGAAATACCCGGTTATTCgGcGGTTAACCGATACCGTACCATACATCCCTGACCCaTTAA
\textup{}\textsubscript{GCAGATatcggctcggttagt}

This 161 bp PCR product was labeled with PNK then digested with EcoRV (blue) to yield a 146 bp DNA fragment labeled at the 5' end on one strand.

PstI-0
gaccatgattacgcaagctgACGCGTCGGGTGTGTTAGAGCCTGTAAACTCGGTGTAGAGCCTGTAAACTAGTCgGcGGTTAACCGATACCGTACCATACATCCCTGACCCaTTAA
\textup{}\textsubscript{ACACTGTCTAGAAGATTactggccgtcg}

PstI-24
gaccatgattacgcaagctgctGcagCGGTGTTAGAGCCTGTAAACTCGGTGTAGAGCCTGTAAACTAGTCgGcGGTTAACCGATACCGTACCATACATCCCTGACCCaTTAA
\textup{}\textsubscript{ACACTGTCTAGAAGATTactggccgtcg}

PstI-33
gaccatgattacgcaagctgACGCGTCGGGTGTGTTAGAGCCTGTAAACTCGGTGTAGAGCCTGTAAACTAGTCgGcGGTTAACCGATACCGTACCATACATCCCTGACCCaTTAA
\textup{}\textsubscript{ACACTGTCTAGAAGATTactggccgtcg}

PstI-73
gaccatgattacgcaagctgACGCGTCGGGTGTGTTAGAGCCTGTAAACTCGGTGTAGAGCCTGTAAACTAGTCgGcGGTTAACCGATACCGTACCATACATCCCTGACCCaTTAA
\textup{}\textsubscript{ACACTGTCTAGAAGATTactggccgtcg}
Table S1: Restriction endonuclease digestion rate summary.

<table>
<thead>
<tr>
<th></th>
<th>DNA</th>
<th>DNA + Nhp6 or yFACT</th>
<th>Nuc</th>
<th>Nuc + Nhp6</th>
<th>Nuc + yFACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dra I rate (%/min/U)</td>
<td>3.8</td>
<td>0.35</td>
<td>0.008</td>
<td>0.027</td>
<td>0.22</td>
</tr>
<tr>
<td>Std Dev</td>
<td>+/- 1.4</td>
<td>+/- 0.27</td>
<td>+/- 0.006</td>
<td>+/- 0.031</td>
<td>+/- 0.14</td>
</tr>
<tr>
<td>(n)</td>
<td>(18)</td>
<td>(52)</td>
<td>(28)</td>
<td>(21)</td>
<td>(35)</td>
</tr>
<tr>
<td>Normalized Rate</td>
<td>470</td>
<td>44</td>
<td>1.0</td>
<td>3.4</td>
<td>27</td>
</tr>
<tr>
<td>Pst I rate (%/min/U)</td>
<td>8.9</td>
<td>1.7</td>
<td>0.008</td>
<td>0.067</td>
<td>0.87</td>
</tr>
<tr>
<td>Std Dev</td>
<td>+/- 4.9</td>
<td>+/- 1.5</td>
<td>+/- 0.005</td>
<td>+/- 0.051</td>
<td>+/- 0.43</td>
</tr>
<tr>
<td>(n)</td>
<td>(7)</td>
<td>(23)</td>
<td>(15)</td>
<td>(13)</td>
<td>(32)</td>
</tr>
<tr>
<td>Normalized Rate</td>
<td>1100</td>
<td>210</td>
<td>1.0</td>
<td>8.2</td>
<td>110</td>
</tr>
<tr>
<td>Ase I rate (%/min/U)</td>
<td>2.3</td>
<td>0.009</td>
<td>.005</td>
<td>.002</td>
<td>.003</td>
</tr>
<tr>
<td>Std Dev</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>(1)</td>
<td>(3)</td>
<td>(3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normalized Rate</td>
<td>270</td>
<td>1.0</td>
<td></td>
<td></td>
<td>0.6</td>
</tr>
</tbody>
</table>

Digestion rates were determined from initial slopes as in Fig 1B from n independent measurements.

The values presented here are cumulative for all variant templates used under the conditions noted; the values for individual templates are presented in Table S2 and plotted in Fig 1. Normalized rates were obtained by dividing each rate by the rate observed with nucleosomes alone.
Table S2: Restriction endonuclease digestion rates for each template variant.

Rate measurements (determined as described in the main text and reported as % digested/min/unit of enzyme with approximately 100 fmol nucleosome samples) from n independent experiments with each variant template are averaged, with the standard deviation calculated for cases with 3 or more measurements. No significant differences were observed among the variant templates when tested as free DNA fragments, so the data for all are combined here. These data are plotted in Fig 1 (panels C and F) of the main text. Spt16-Pob3 had no effect in this assay in the absence of Nhp6 (see Fig S1 below), so this condition was usually omitted.

<table>
<thead>
<tr>
<th>Nucleosome</th>
<th>n</th>
<th>Nuc + Nhp6</th>
<th>n</th>
<th>Nuc + yFACT</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dral-29</td>
<td>4</td>
<td>0.0091 +/- .0039</td>
<td>3</td>
<td>0.12 +/- .02</td>
<td>6</td>
</tr>
<tr>
<td>Dral-58</td>
<td>4</td>
<td>0.0082 +/- .0070</td>
<td>3</td>
<td>0.21 +/- .06</td>
<td>5</td>
</tr>
<tr>
<td>Dral-78</td>
<td>7</td>
<td>0.0036 +/- .0036</td>
<td>5</td>
<td>0.22 +/- .10</td>
<td>8</td>
</tr>
<tr>
<td>Dral-93</td>
<td>4</td>
<td>0.0046 +/- .0024</td>
<td>3</td>
<td>0.26 +/- .07</td>
<td>4</td>
</tr>
<tr>
<td>Dral-125</td>
<td>5</td>
<td>0.014 +/- .010</td>
<td>3</td>
<td>0.19 +/- .05</td>
<td>5</td>
</tr>
<tr>
<td>Dral-130</td>
<td>4</td>
<td>0.0057 +/- .0021</td>
<td>2</td>
<td>0.12 +/- .06</td>
<td>5</td>
</tr>
<tr>
<td>Dral-140</td>
<td>3</td>
<td>0.014 +/- .0014</td>
<td>3</td>
<td>0.60 +/- .04</td>
<td>3</td>
</tr>
<tr>
<td>Dral-152</td>
<td>3</td>
<td>2.1 +/- .21</td>
<td>2</td>
<td>0.11 +/- .02</td>
<td>6</td>
</tr>
<tr>
<td>Dral-157</td>
<td>1</td>
<td>1.0</td>
<td>2</td>
<td>0.10 +/- .03</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DNA</th>
<th>DNA + Nhp6</th>
<th>DNA + yFACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>All DNAs</td>
<td>3.8 +/- 1.4</td>
<td>0.23 +/- .10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nucleosome</th>
<th>n</th>
<th>Nuc + Nhp6</th>
<th>n</th>
<th>Nuc + yFACT</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>PstI-24</td>
<td>2</td>
<td>0.015</td>
<td>1</td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>PstI-33</td>
<td>3</td>
<td>0.0044 +/- .0004</td>
<td>3</td>
<td>0.98 +/- .08</td>
<td>6</td>
</tr>
<tr>
<td>PstI-69</td>
<td>1</td>
<td>0.0090</td>
<td>1</td>
<td>0.031</td>
<td>1</td>
</tr>
<tr>
<td>PstI-73</td>
<td>3</td>
<td>0.010 +/- .003</td>
<td>3</td>
<td>1.7 +/- .26</td>
<td>5</td>
</tr>
<tr>
<td>PstI-83</td>
<td>1</td>
<td>0.0090</td>
<td>1</td>
<td>0.031</td>
<td>1</td>
</tr>
<tr>
<td>PstI-94</td>
<td>2</td>
<td>0.0072</td>
<td>2</td>
<td>0.89 +/- .24</td>
<td>6</td>
</tr>
<tr>
<td>PstI-105</td>
<td>3</td>
<td>0.0027 +/- .0029</td>
<td>2</td>
<td>1.1 +/- .3</td>
<td>4</td>
</tr>
<tr>
<td>PstI-116</td>
<td>3</td>
<td>0.0087 +/- .0038</td>
<td>4</td>
<td>0.61 +/- .37</td>
<td>9</td>
</tr>
<tr>
<td>PstI-158</td>
<td>8.4</td>
<td>1</td>
<td>1.2</td>
<td>1</td>
<td>1.1 +/- .2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DNA</th>
<th>DNA + Nhp6</th>
<th>DNA + yFACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>All DNAs</td>
<td>8.9 +/- 4.9</td>
<td>1.2 +/- .22</td>
</tr>
</tbody>
</table>
Methods for hydroxyl radical mapping of DNA accessibility.

Hydroxyl radical damage was induced essentially as described (Hampel and Burke, 2001). 100 fmole of nucleosome was incubated with yFACT or its individual components as in the main text in a volume of 8 µl. 0.7 µl each of Fe-EDTA solution (50 mM Na3EDTA, 25 mM Fe(NH4)2(SO4)2), 200 mM sodium ascorbate, and 30% H2O2 were mixed and incubated at room temperature (~22° C) for 2 minutes, then 10 µl of 30 mM thiourea was added to stop the reaction. Samples were then prepared for separation on 7M urea-6% PAGE as described (Rhoades et al., 2004). About 63% of the signal remained in the undamaged 146 bp band after this treatment. The concentrations of hydroxyl radical generating reagents were increased significantly over the protocol described (Hampel and Burke, 2001 Hampel and Burke, 2001), because large amounts of the free radical scavengers glycerol and sucrose were present in the nucleosome and protein preps used here.

Methods for ChIP measurements of histone occupancy in vivo

Strains from the W303 genetic background were used as listed below:

DY13736  MATa HIS3::GAL1::YLR454w hta1-htb1::NatMX hta2-htb2::HphMX YCp-TRP1-HTA1-(Flag)HTB1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3

DY13744  MATa pob3(L78R) HIS3::GAL1::YLR454w hta1-htb1::NatMX hta2-htb2::HphMX YCp-TRP1-HTA1-(Flag)HTB1 ade2 can1 his3 leu2 lys2 trp1 ura3

The YCp-TRP1-HTA1-(Flag)HTB1) plasmid expressing a native Hta1 and an N-terminally Flag tagged Htb1 gene was isolated from a strain provided by Mary Ann Osley. Chromatin immunoprecipitations (ChIPs) were performed as described (Bhoite et al., 2001; Voth et al., 2007) with anti-Flag (M2, Sigma) monoclonal antibody to the FLAG epitope, and anti-histone H3 (07-
antibodies and antibody coated magnetic beads (Rabbit and Pan Mouse IgG beads, Dynal Biotech). ChIP assays were analyzed by real time PCR as described (Eriksson et al., 2004). PCR primers for the YLR454w gene were described previously (Biswas et al., 2006). Each ChIP sample was first normalized to an input DNA sample to and then to the ChIP signal for a control region on chromosome I control (Biswas et al., 2008).
Fig S1: Spt16-Pob3 without Nhp6 does not affect the rate of nucleosomal Dral digestion.

A 181 bp 5S rDNA fragment (Dral-78) incorporated into nucleosomes using yeast histones was digested with 20 U of Dral as described in the main text. Reactions contained nucleosomes alone, 10 μM Nhp6, 200 nM Spt16-Pob3, or 10 μM Nhp6 and 200 nM Spt16-Pob3 (yFACT). In this experiment, yFACT induced digestion at an initial rate of about 0.25%/min/U while the other three conditions resulted in uniformly low rates of digestion of about 0.005%/min/U, typical of background digestion rates for this substrate. We conclude that Spt16-Pob3 has no effect on the rate of Dral digestion in the absence of Nhp6. Similar results were obtained with other combinations of templates and enzymes (not shown).
**Fig S2; Measuring H2A-H2B dimer loss by the dimer/DNA ratio method.**

The method used in the main text to quantify removal of H2A-H2B dimers from nucleosomes involves measuring the amount of dimer that is displaced from the nucleosomes and therefore is unaccompanied by DNA in a region of a native polyacrylamide gel that is not associated with nucleosomes. An alternative method is to measure the amount of dimer signal that remains in the nucleosomal region after yFACT treatment. In this case, the ratio of dimer signal to DNA signal is used to infer the composition of the nucleosomal remnants, and could reveal the amount of hexasome or tetrasome formation that occurs during yFACT binding if these migrate as discrete species.

Fig S2 below shows results obtained using this "dimer/DNA ratio" method. In S2A, 181 bp 5S rDNA yeast nucleosomes were incubated for 10 minutes at 30° without or with 10 mM Mg++ in either acetate or chloride salts in the absence or presence of yFACT, as indicated. Unlabeled genomic DNA was then added to reverse the formation of yFACT-nucleosome complexes prior to native PAGE analysis (lanes 3, 4, 7, and 8 here are the same as lanes 5, 6, 7, and 8 in Fig 3 of the main text, with the same labeling and color coding as described in that figure). Fig S2B shows the amount of signal for DNA and H2A labels in the nucleosome region for lanes 1 and 5, normalized by subtracting a background value for the gel, then dividing by the total signal for lane 1. Fig S2C shows the dimer/DNA ratio for each gel segment, normalized to a value of 1.0 for the ratio obtained with intact nucleosomes on the same gel. A reading of 1.0 therefore should correspond to a standard histone octamer with 2 H2A molecules per dsDNA molecule. In Fig S2C, the signal in segments 55-62 for lane 1 were summed to get an average ratio for the entire untreated nucleosome band.
If the nucleosome band in lane 1 represents a single species, then the ratio of dimer/DNA should be constant across the band. Fig S2C shows the unexpected result that the dimer/DNA ratio varies in this region, with a reproducible inflection point near the center of the band (near segment 59 in S2C without yFACT). This can be observed in Fig S2B as well, as the peaks for the DNA and dimer signals are slightly offset, leading to a change in the ratio for different segments. Because the average value for the band was defined to be 1.0, the dimer/DNA ratio is less than 1 in the region to the left of the peak, and greater than 1 to the right of it. Essentially the same result is obtained with lanes 2-4 from this experiment, as well as with analysis of untreated nucleosomes in other experiments (not shown). This result is therefore both typical and reproducible. This suggests that even untreated nucleosomes that appear to migrate in a single band by native PAGE are actually a mixed population of different forms, and that species lacking the normal complement of H2A-H2B dimers tend to migrate slightly more slowly than full octameric nucleosomes in this gel system. This is consistent with the observed migration of tetrasomes in Fig 3 in the main text, and with previously reported properties of hexasomes (Kireeva et al., 2002). If this analysis is correct, then the dimer/DNA ratio should be normalized to the peak segment (number 60 in S2C), as this represents the maximal ratio obtainable, which should be 2 dimers per dsDNA molecule.
normalized this way, segments 52-58 without yFACT produce an average dimer/DNA ratio of 0.60, and the entire nucleosome band gives a value of 0.75 (not shown). Even untreated nucleosomes therefore display significant complexity using this method of analysis, and suggest that what appears to be a single band on the native gel may be comprised of nucleosomes with different compositions or fluorescence properties.

Examination of the nucleosomal remnants after yFACT treatment using the dimer/DNA ratio method provides a similarly complex picture. Fig S2C shows that, as expected, the ratio of dimer/DNA is lower than it is for the untreated sample for all segments in the nucleosome band. The overall average for segments 52-66 is 76% of the value for the untreated sample, suggesting loss of 24% of the dimer molecules due to yFACT binding. When the standard "dimer displacement" method used in the main text is applied to this sample, a similar but not identical value of 18% dimer loss due to yFACT is obtained. The nucleosome band for the sample treated with yFACT migrates more rapidly than the untreated sample, but has essentially the same characteristics, with lower dimer/DNA ratios to the left of the peak and higher ratios to the right. The dimer/DNA ratio therefore shows that yFACT causes dimer displacement, but does not reveal a specific set of discrete products. Dimer loss does not appear to be responsible for the increased migration rate, because the analysis above indicated that loss of dimers leads to slower migration, not faster. Possible explanations for the migration are discussed below.

As noted in the main text, yFACT treatment also causes accumulation of both DNA and H2A with a peak near segment 34 (Figs 3C and S2D). Analysis of the dimer/DNA ratio in this region suggests that this form has a slightly higher ratio than the average for the nucleosome band (1.2, Fig S2E), not the value of 0.5 expected for a hexasome. This is complicated by the presence of free dimers in this region of the gel (see Fig 4B in the main text), which will inflate the dimer/DNA ratio. While at least some of the products of dimer loss are likely to be hexasomes, and the peak
near segment 34 may represent hexasomes, the migration of DNA-containing forms is too complex to draw firm conclusions about composition using the dimer/DNA ratio measurements.

**Fig S3: Analysis of just the center of the lane by the dimer/DNA ratio method.**

We considered the possibility that the analysis above was limited by the resolution of the process of dividing the gel into segments. For example, Fig S2A reveals evidence for two distinct species in the nucleosome region after yFACT treatment (see lanes 5a and 5b, for example), but the quantitation in S2B seems to show only a single broad peak. One explanation is that separation into two bands is only visible in the centers of the lanes, but the entire width of each lane was included in the quantitation in Fig S2. We tested this by limiting the analysis to the center of the lane in Fig S3A. Here, a 146 bp 5S rDNA yeast nucleosome was used, as the separation of the two apparent products is more distinct with a shorter DNA fragment. In this case, 50 segments were measured over just the nucleosome band, so the segments are spaced more closely to provide maximal resolution. Fig S3B shows the total signal for lanes 5 and 9, with two bands now easily discriminated after yFACT treatment, especially in the dimer analysis. However, the quantitative
analysis of the dimer/DNA ratio presented in Fig S3C fails to provide a simple interpretation of the bands. The dimer/DNA ratio still varies across the untreated nucleosome band, and also across each of the bands detected after yFACT treatment. Neither of the bands detected after yFACT binding displays the twofold difference in dimer/DNA ratio expected for a distinct hexasome band. Instead, the dimer/DNA ratio is once again lower for the yFACT sample across the range of the nucleosomal region, suggesting loss of dimers, but comigration of forms with varying compositions.

There are several ways that yFACT could alter the migration and fluorescence intensity of nucleosomal species. Even though yFACT complexes with nucleosomes are disrupted by the addition of genomic DNA, the yFACT protein is still in the sample and could exert an effect on electrophoretic mobility or the fluorescence of dyes during scanning. Mapping with exonuclease III indicated that nucleosomes do not translocate along the DNA in the presence of yFACT (Rhoades et al., 2004), but it is possible that hexasomes or tetrasomes are either inherently more mobile or can translocate in the presence of yFACT. The dimer/DNA ratio method also assumes that detection of fluorescent dyes is unaffected by changes in the composition or translational position of the histones on the DNA fragment, but either of these changes could alter the environment of the dye molecules and therefore the efficiency of fluorescence detection. It may be, for example, that hexasomes do not produce one half the dimer signal of nucleosomes, or that translocation of histones to different positions along the DNA affects the fluorescence yield from the dye molecules. Given the significant ambiguity encountered in the interpretation of the dimer/DNA ratio, we cannot use the native gel system to assess the amount of nucleosomes, hexasomes, and tetrasomes individually. The efficiency of fluorescence seems less likely to be variable when dimers are not accompanied by DNA or other histones, making the dimer displacement method the favored one for quantitation. We routinely used both methods, and obtained qualitatively similar conclusions with each (see, for
example, Fig S4 below), but we consider the dimer displacement results to be more quantitatively reliable.

**Fig S4: The effect of temperature and DNA fragment size on dimer displacement.**

Variables affecting dimer loss

Inclusion of 10 mM Mg$^{++}$ ions significantly reduces the amount of H2A-H2B displacement that occurs during yFACT binding (Fig 3, Fig S2A). Fig S4A shows that temperature also affects dimer loss, with increased temperature correlating with greater loss. In the example shown, 181 bp 5S rDNA yeast nucleosome samples were incubated in triplicate in acetate salts in the presence of Mg$^{++}$ ions at either 30° C or 37° C for 10 minutes prior to addition of excess unlabeled genomic DNA and analysis by native PAGE. 11% of the dimer was displaced as the result of yFACT binding at 30° and 22% at 37°. Analysis using the dimer/DNA ratio method suggests a similar conclusion, but the magnitude of the difference is larger, with 16% loss at 30° and 31% at 37° (Fig S4B). Fig S4C shows that the size of the DNA fragment also alters the amount of dimer loss. Here, yeast nucleosomes assembled with 5S rDNA fragments of 146 bp or 181 bp were incubated in acetate
salts with Mg\(^{++}\) ions for 8-32 minutes at 37\(^{\circ}\) and the results from each time point were averaged. About 20% of the dimers were lost from the 181 bp nucleosomes due to yFACT treatment, and 32% from the 146 bp nucleosomes. Shorter DNA fragments therefore form nucleosomes that are more prone to dimer loss during yFACT binding.

The effects of different anions were complex, as illustrated above in Fig S2A. For example, the level of dimer loss due to yFACT binding was slightly lower in acetate salts than in chloride salts in the absence of Mg\(^{++}\) ions (compare S2A lanes 5b and 7b) but higher in the presence of Mg\(^{++}\) ions (compare lanes 6b and 8b). The effects of different histone types were also complex (Fig S5). Here, two nucleosomes were assembled using a 181 bp 5S rDNA fragment and either yeast or *X. laevis* histones. Parallel triplicate samples were incubated for 10 minutes at 30\(^{\circ}\) under NaCl-HSA conditions without or with MgCl\(_2\) and without or with yFACT, as indicated, and analyzed by native PAGE after addition of excess unlabeled genomic DNA. Displaced dimer was measured for both nucleosomes, and displaced H4 was measured for the *X. laevis* samples (Fig S5A is the same as Fig 4D in the main text). The *X. laevis* samples with yFACT contained 500 nM Spt16-Pob3 whereas the yeast samples contained 200 nM, as *X. laevis* nucleosomes do not respond to the lower concentration of yFACT (HX, unpublished results). *X. laevis* nucleosomes were more prone to dimer loss under the same reaction conditions lacking Mg\(^{++}\) ions, with 44% displacement due to yFACT (Fig S5A) whereas yeast nucleosomes lost 16% (Fig S5B). However, this reflects both greater dimer loss for *X. laevis* nucleosomes when yFACT was present (52% versus 30%) and lower spontaneous loss in the absence of yFACT (8.2% versus 14%). Also, Mg\(^{++}\) was somewhat less effective at stabilizing yeast nucleosomes against dimer loss in the presence of yFACT than it was with *X. laevis* nucleosomes. Fig S5C shows analysis of the ratio of H4/DNA. Supporting the conclusion that yFACT binding does not lead to displacement of (H3-H4)\(_2\) tetramers, the ratio of H4/DNA is not significantly altered even under conditions that cause severe loss of dimers. Both
the anion used and the nature of the histones tested therefore alter the stability of the association of H2A-H2B dimers with nucleosomes during yFACT binding, but each of these variables produces complex effects.

**Fig S5: Comparison of dimer loss induced by yFACT from nucleosomes formed with yeast histones and *X. laevis* histones.**

The amount of sensitivity to restriction endonucleases induced during yFACT binding exceeds the amount of H2A-H2B dimer loss (Fig 5 in the main text) indicating that dimer loss is not responsible for the sensitivity. Consistent with this conclusion, other conditions that affect the amount of dimer loss do not produce corresponding changes in nuclease sensitivity. For example, nucleosomes formed with a 146 bp DNA fragment experience more dimer loss than nucleosomes
formed with 181 bp DNA fragments during yFACT binding (Fig S4C), but the rate of Dral digestion is lower with the 146 bp nucleosomes (Fig S6A). Similarly, nucleosomes formed with X. laevis histones are much more prone to dimer loss during yFACT treatment than nucleosomes formed with yeast histones, but are reproducibly less prone to digestion (Fig S6B). Enhanced dimer loss and nuclease sensitivity therefore both appear to be features of nucleosomes reorganized by yFACT, but they respond independently to altered reaction conditions.

**Fig S6: Comparing rates of Dral digestion with the level of H2A-H2B dimer loss.**

![Graph showing Dral digestion rates and dimer loss](image)

Initial digestion rates shown are derived from the slopes of the curves at early time points and dimer loss is reported using the dimer displacement method for the 32 minute time point. Panel A shows a Dral digestion performed in chloride salts instead of the recommended acetate salts (New England Biolabs buffer 2 instead of buffer 4) which causes a slight decrease in the digestion rate. The yeast histones used in panel B contain an H2A-A60V mutation (yeast*) that causes a mild
decrease in the amount of dimer loss in response to yFACT treatment but does not affect sensitivity to DraI.

**Supplemental References**


