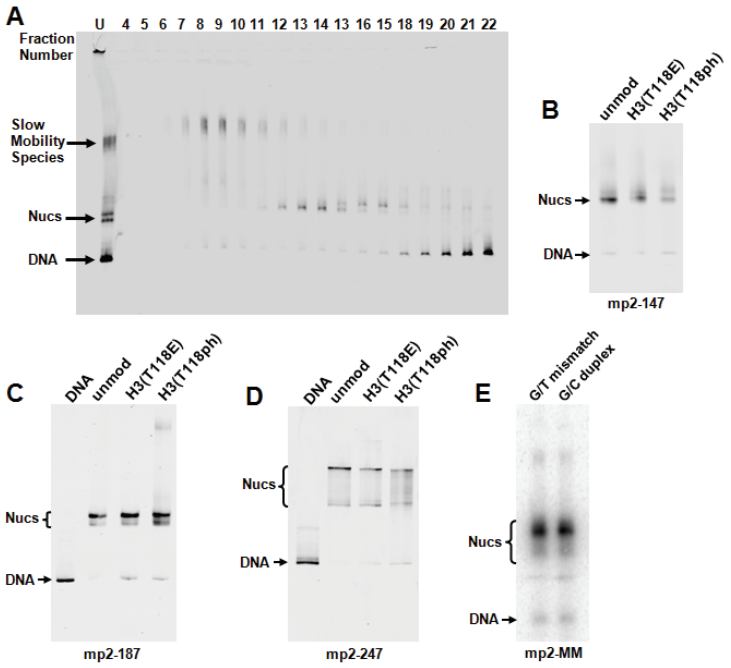
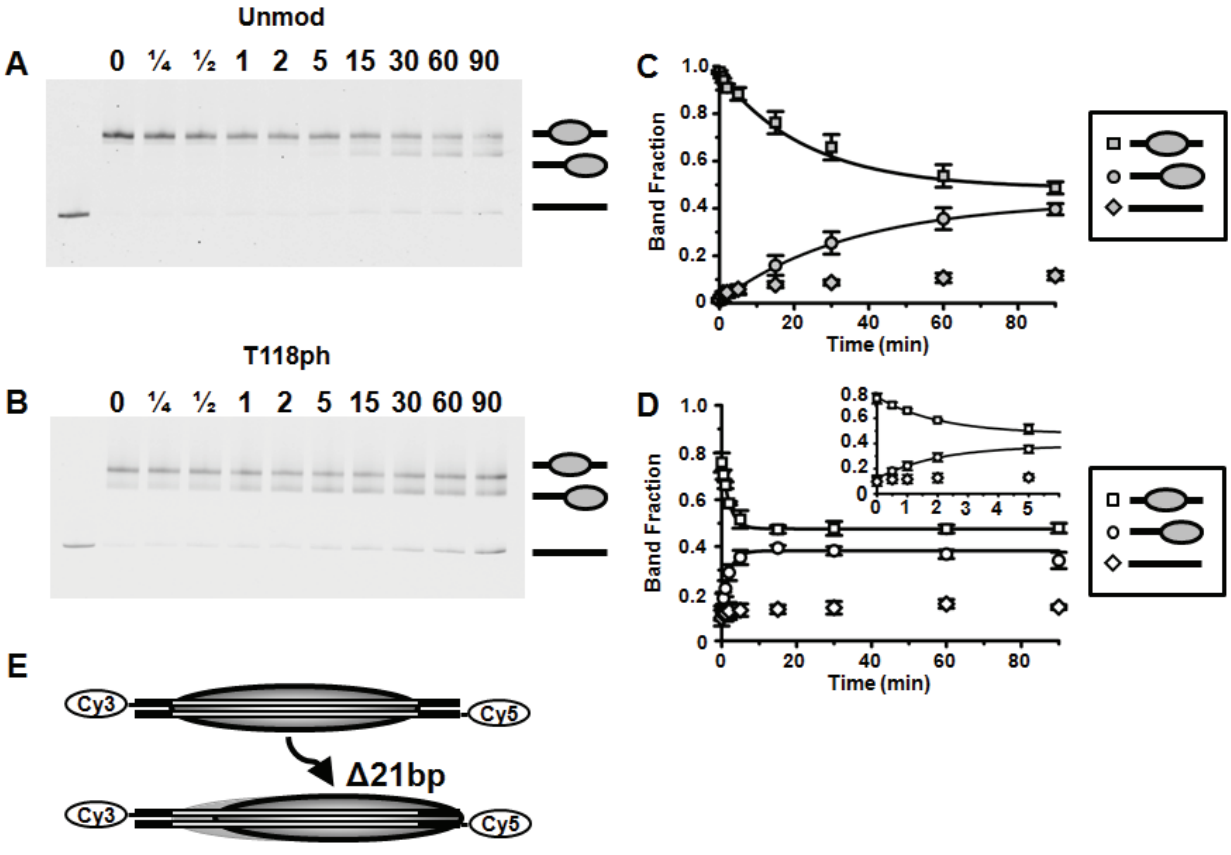
SUPPLEMENTARY DATA

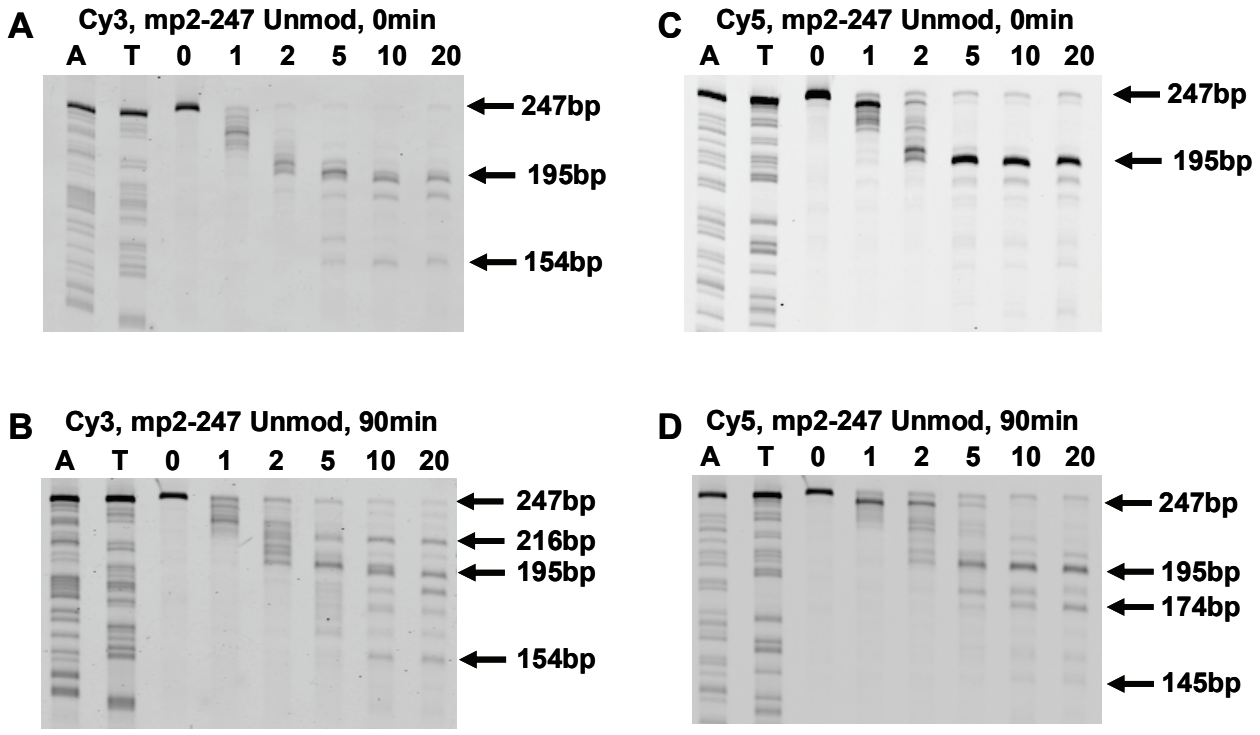
Supplementary Figures and Legends



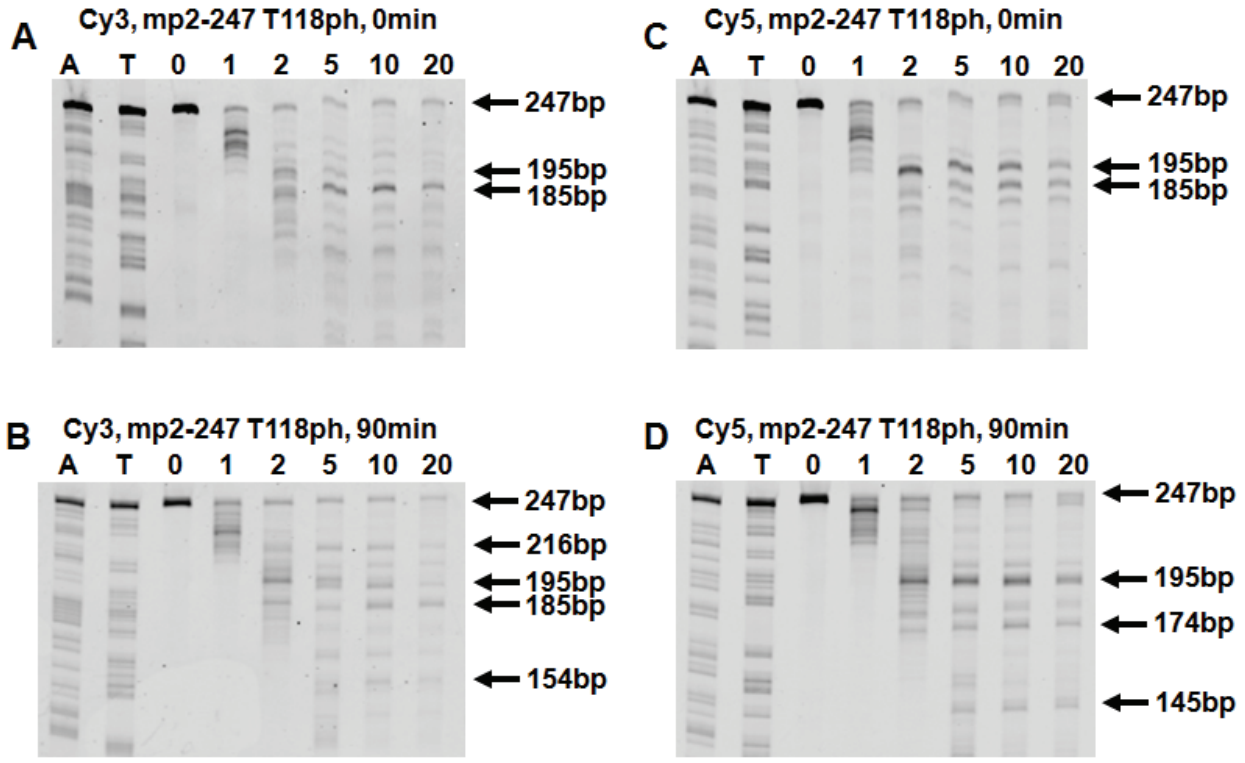
Supplementary Figure 1. Preparation of nucleosomes. (**A**) Cy5 fluorescence image of a native PAGE analysis of fractions from a sucrose gradient purification of a nucleosome reconstitution with histone octamer containing H3(T118ph). Lane U) is the sample before purification; Lanes 4-22) are the fraction numbers from the sucrose gradient. (**B**) Cy5 fluorescence image of an electrophoretic mobility shift analysis (EMSA) of purified nucleosomes containing mp2-147 with: Lane 1) unmodified, Lane 2) H3(T118E), and Lane 3) H3(T118ph) histone octamer. (**C**) Cy5 fluorescence image of EMSA of: Lane 1) free mp2-187 DNA, Lane 2) unmodified, Lane 3) H3(T118E) modified, and Lane 4) H3(T118ph) histone octamer. (**D**) Cy5 fluorescence image of EMSA of: Lane 1) free mp2-287 DNA, Lane 2) unmodified, Lane 3) H3(T118E) modified, and Lane 4) H3(T118ph) histone octamer. (**E**) Phosphor image analysis of purified H3(T118ph) containing nucleosomes with: Lane 1) mp2-GT mismatch NPS and Lane 2) mp2-GC duplex NPS.



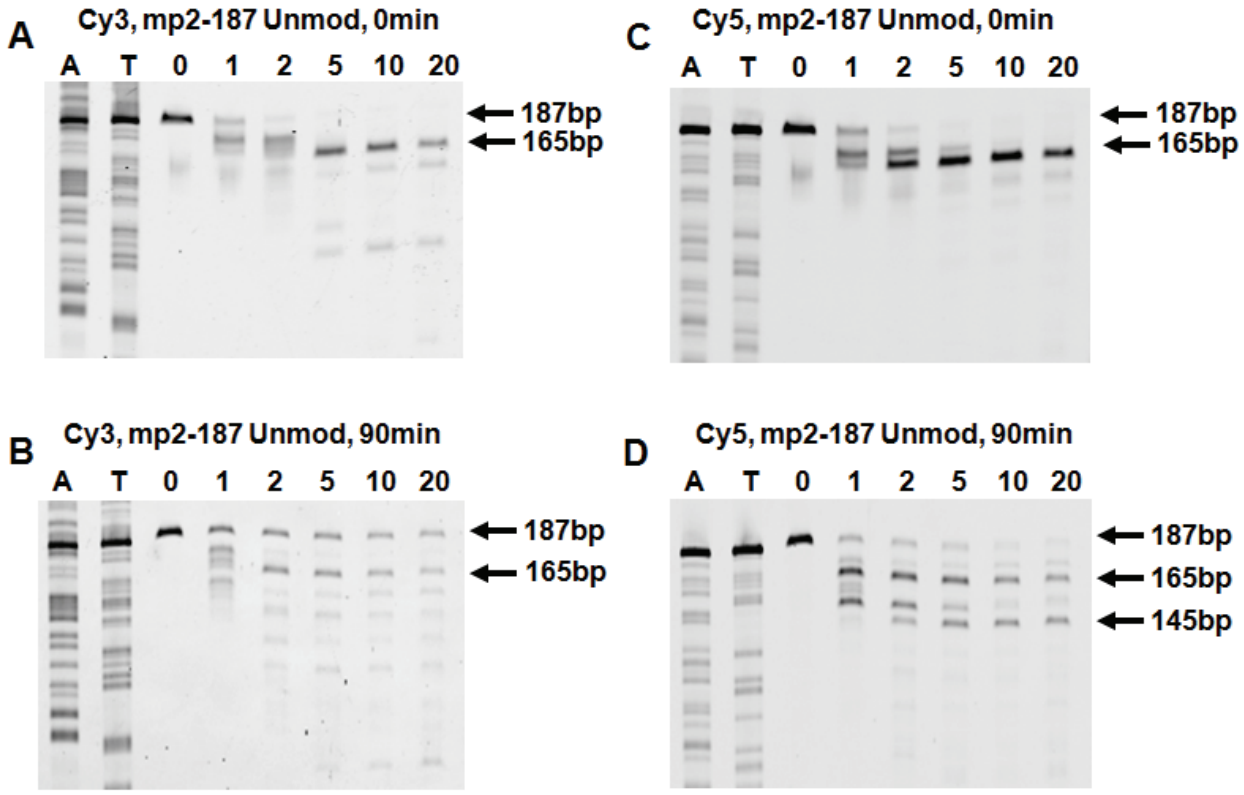
Supplementary Figure 2. H3(T118ph) increases the rate of nucleosome mobility. (**A**) and (**B**) Cy5 fluorescence images of thermally (53°C) induced shifts in unmodified and H3(T118ph) nucleosome positions within mp2-187 NPS, respectively. The top band is centrally positioned nucleosomes, the second band is nucleosomes positioned at the ends of the DNA and the bottom band is naked DNA. (**C**) and (**D**) Quantification of the fraction of center positioned nucleosomes (squares), end positioned nucleosomes (circles) and naked DNA (diamonds). The error bars were determined from the standard deviation from at least 3 separate experiments. The kinetic evolution of each nucleosome position was fit to a single exponential decay. The inset shows the first 6 minutes of plot (D). (**e**) Diagram of the predicted change in nucleosome positions at 53°C.



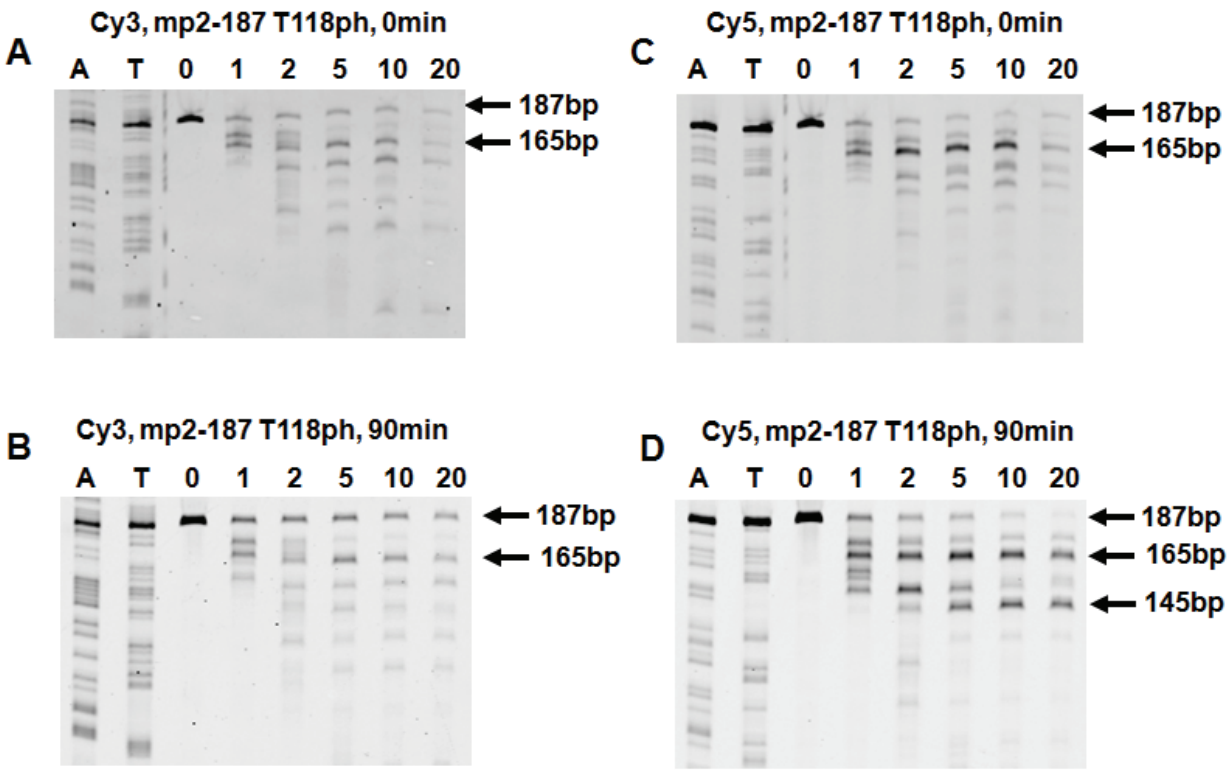
Supplementary Figure 3. Exonuclease III mapping of the position of unmodified nucleosomes within the mp2-247 NPS. Denaturing PAGE analysis of exonuclease III mapping of unmodified nucleosomes reconstituted with the mp2-247 NPS before (**A**) and after (**B**) heating to 53 °C for 90 min and visualized using the Cy3 fluorescent label. Lanes are labeled with the time at which the exonuclease digestion was quenched. (**C**) and (**D**), visualization of the gels from (**A**) and (**B**) using the Cy5 fluorescent label, which illustrates digestion from the other end of the DNA; a positioned nucleosome will protect 147 bp of DNA between the two digests. The new position that appears after heating is shifted 20 bp toward the Cy5-labeled end of the DNA relative to the mp2 NPS.



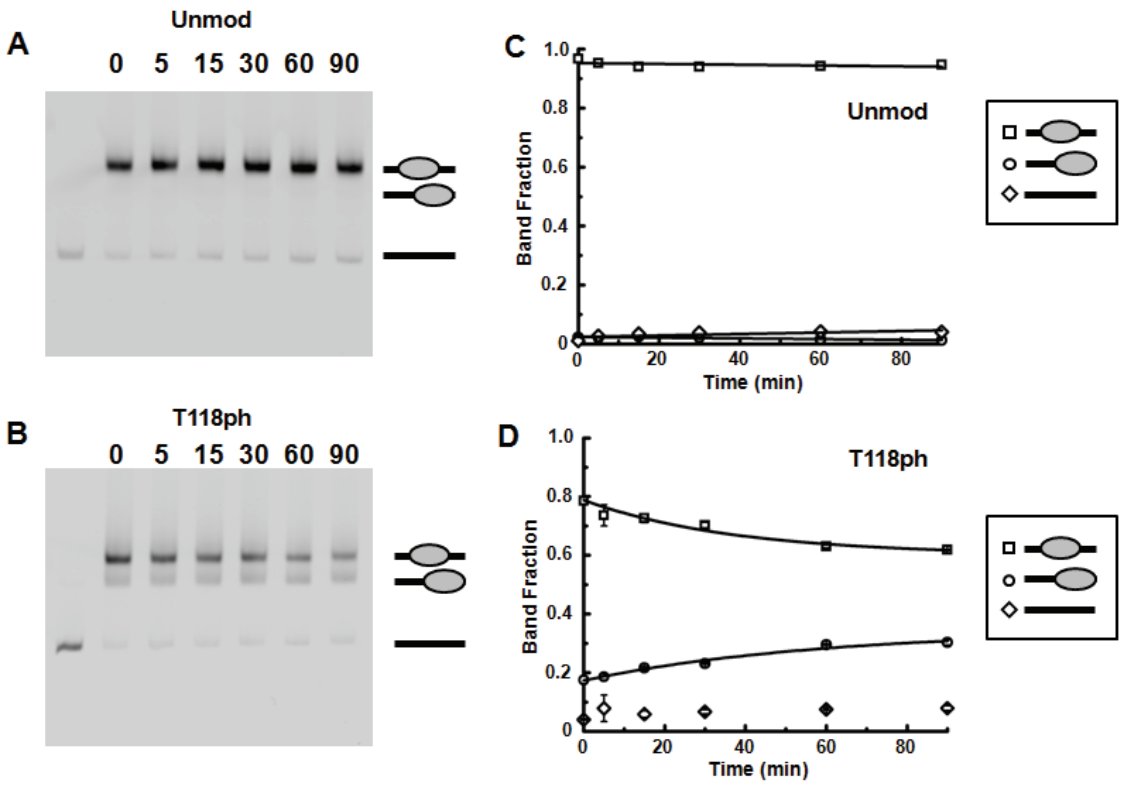
Supplementary Figure 4. Exonuclease III mapping of H3(T118ph) containing nucleosomes on mp2-247. Denaturing PAGE analysis of exonuclease III mapping of H3(T118ph) containing nucleosomes reconstituted with the mp2-247 NPS before (**A**) and after (**B**) heating to 53 °C for 90 min and visualized using the Cy5 fluorescent label. Lanes are labeled with the time at which the exonuclease digestion was quenched. (**C**) and (**D**), visualization of the gels from (**A**) and (**B**) using the Cy3 fluorescent label, which illustrates digestion from the other end of the DNA; a positioned nucleosome will protect 147 bp of DNA between the two digests. The new position that appears after heating is shifted 20 bp toward the Cy5-labeled end of the DNA relative to the mp2 NPS.



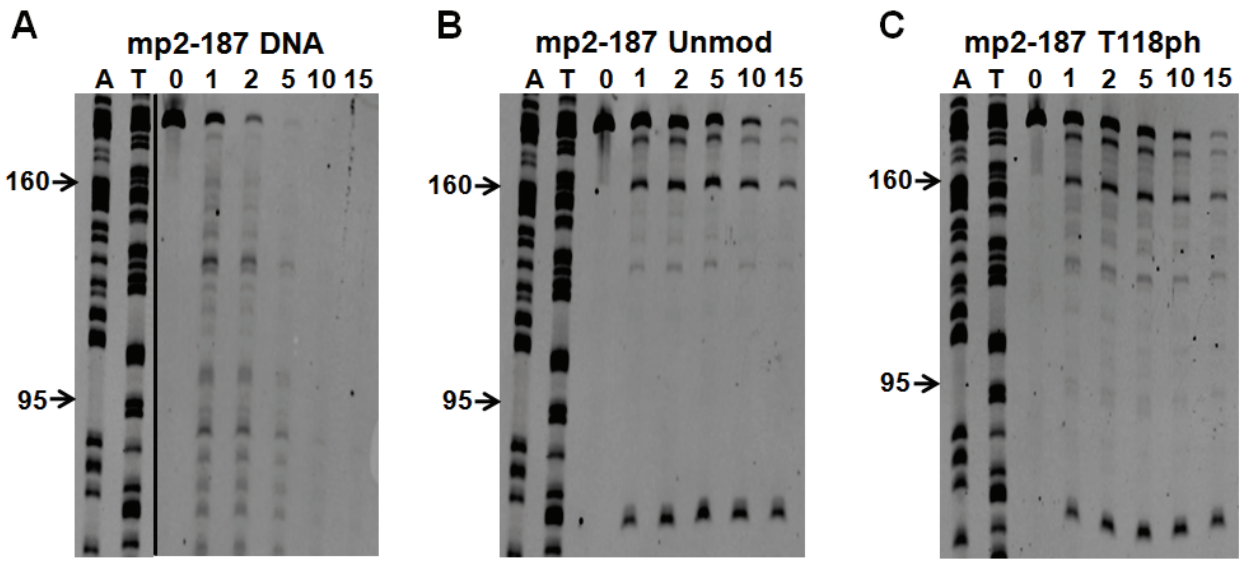
Supplementary Figure 5. Exonuclease III mapping of unmodified nucleosomes on mp2-187. Denaturing PAGE analysis of exonuclease III mapping of unmodified nucleosomes reconstituted with the mp2-187 NPS before (**A**) and after (**B**) heating to 53 °C for 90 min and visualized using the Cy3 fluorescent label. Lanes are labeled with the time at which the exonuclease digestion was quenched. (**C**) and (**D**), visualization of the gels from (**A**) and (**B**) using the Cy5 fluorescent label, which illustrates digestion from the other end of the DNA; a positioned nucleosome will protect 147 bp of DNA between the two digests. The new position that appears after heating is shifted 20 bp to the Cy5-labeled end of the DNA relative to the mp2 NPS.



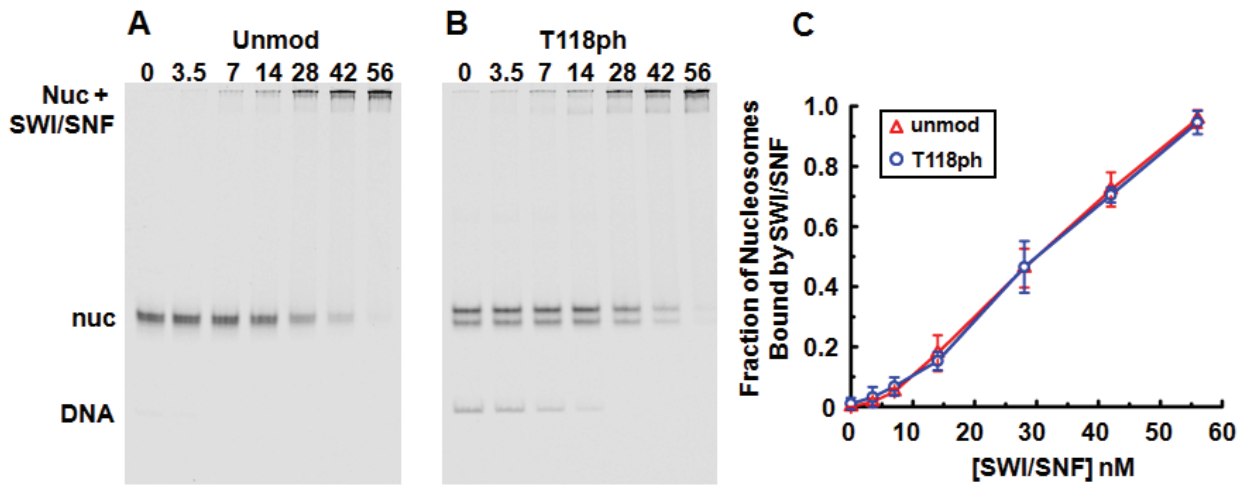
Supplementary Figure 6. Exonuclease III mapping of of H3(T118ph) containing nucleosomes on mp2-187. Denaturing PAGE analysis of exonuclease III mapping of H3(T118ph) containing nucleosomes reconstituted with the mp2-187 NPS before (**A**) and after (**B**) heating to 53 °C for 90 min and visualized using the Cy3 fluorescent label. Lanes are labeled with the time at which the exonuclease digestion was quenched. (**C**) and (**D**), visualization of the gels from (**A**) and (**B**) using the Cy5 fluorescent label, which illustrates digestion from the other end of the DNA; a positioned nucleosome will protect 147 bp of DNA between the two digests. The new position that appears after heating is shifted 20 bp to the Cy5-labeled end of the DNA relative to the mp2 NPS.



Supplementary Figure 7. H3(T118ph) provokes nucleosome repositioning at physiological temperatures. (**A**) and (**B**) Cy5 fluorescence images of thermally (37°C) induced shifts in unmodified and H3(T118ph) nucleosome positions within the mp2-187 NPS, respectively. The top band is centrally positioned nucleosomes, the second band is nucleosomes positioned at the ends of the DNA and the bottom band is naked DNA. (**C**) and (**D**) Quantification of the fraction of center positioned nucleosomes (squares), end positioned nucleosomes (circles) and naked DNA (diamonds). The error bars were determined from the standard deviation from at least 3 separate experiments. The kinetic evolution of each nucleosome position was fit to a single exponential decay. The decay of the unmodified nucleosomes is so slow that it is immeasurable, while the time decay of the H3(T118ph) nucleosomes was 40 ± 10 minutes.



Supplementary Figure 8. H3(T118ph) increases DNase I accessibility near the dyad symmetry axis. (**A**) Cy3 fluorescence image of a denaturing PAGE analysis of a DNase digestion with naked mp2-187 DNA. The first 2 lanes are A and T ladders of the mp2-187 DNA molecule. Lanes 3 through 8 are labeled with the time in min. (**B**) Cy3 fluorescence image of a denaturing PAGE analysis of a DNase digestion with unmodified nucleosomes containing the mp2-187 DNA molecule. The labels 95 and 160 denote the length of the DNA molecule in base pairs. The 160 and 95 base pair bands indicate cleavage at the 160th and 95th base pair positions within the mp2-197 DNA molecule, which are located in the nucleosome DNA entry-exit and dyad regions, respectively. (**C**) Cy3 image of a denaturing PAGE analysis of a DNase digestions with H3(T118ph) containing nucleosomes.



Supplementary Figure 9. H3(T118ph) does not influence SWI/SNF binding to nucleosomes. (**A**) and (**B**) are native PAGE analysis of SWI/SNF binding to unmodified and H3(T118ph) nucleosomes, respectively. The lanes are labeled with the concentration of SWI/SNF (nM). (**C**) Quantification of the fraction of unmodified (red triangles) and H3(T118ph) (blue circles) nucleosomes bound by SWI/SNF. The error bars were determined from the standard deviation of 3 separate experiments.

Supplementary Tables

Experiment	Temperature
Binding Free Energy	12°C, 25°C, 33°C
Thermal Mobility	37°C, 53°C
RE Accessibility	37°C (65°C for Taq$^{\alpha}$I)
DNase Accessibility	16°C
hMSH2-hMSH6 Remodeling	37°C
SWI/SNF Binding	30°C
SWI/SNF Remodeling	18°C

Supplementary Table 1: Temperatures used for the different nucleosome dynamics and remodeling measurements.

Supplementary Methods

Exonuclease III Mapping: The nucleosome positions within the mp2-187 and mp2-247 DNA molecule were determined with ExoIII mapping. Reactions were carried out in an initial volume of 50 ul with 10 nM nucleosomes and 50U/ml of ExoIII (NEB) in 20mM Tris pH8, 0.5mM MgCl2 at 16C to prevent H3(T118ph) nucleosome disassembly (data not shown). At each time point, a 7 ul aliquot of the reaction was quenched with EDTA to a final concentration of 20 mM. A final concentration of 1 mg/ml of proteinase K and 0.02% of SDS was added to each time point to remove the histone octamer from the DNA and samples were separated by 8% denaturing PAGE in 7 M Urea and 1x TBE. The sequence markers were prepared with a SequiTherm Excel II DNA sequencing kit (Epicentre) using cy3 or cy5 labeled primers, an mp2-187 or mp2-247 DNA template and either ddATP or ddTTP. Results were imaged by a Typhoon 8600 variable mode imager (GE Healthcare), which detects cy3 and cy5 separately in the same gel. The cy3 and cy5 ladders could be loaded in the same lanes to increase accuracy of the mapping gel readout. In parallel we carried out ExoIII digestions with naked DNA to confirm that none of the positions observed with nucleosomal DNA were due to exonuclease pause sites (Data not shown).