Supplementary Figure legends

Figure S1 ***Generation of histone octamers fully acetylated on H4K16 by native chemical ligation***

ESI-MS analysis of unmodified histone H4 (**A**) and H4R21C,K16ac (**B**). The calculated mass of unmodified histone H4 is 11236 Da. The calculated mass of H4R21C,K16ac is 11225 Da (mass difference between Cys and Arg: -53 Da; acetylation: +42 Da). MS adducts indicate peaks in the spectra that are observed as noncovalent interaction pairs with ions (additions of masses as multiple of one species, i.e. SDS +288 Da). The presence of these peaks varies with sample preparation and MS conditions. (**C**) The indicated recombinant purified proteins were run on 15% SDS-PAGE and stained with Coomassie blue. H4(Δ1-20)R21C refers to the truncated H4 protein to which the modified N-terminal H4(1-20)K16ac peptide was fused by native protein ligation. H4R21C,K16ac refers to the ligated product (in the manuscript referred to as $H4K16^{ac}$). (**D**) Histone octamers reconstituted with the indicated H4 species were run on 15% SDS-PAGE and stained with Coomassie blue.

Figure S2 ***Sir2-dependent deacetylation of $H4K16^{ac}$ stabilizes the association of Sir2-3-4 to chromatin***

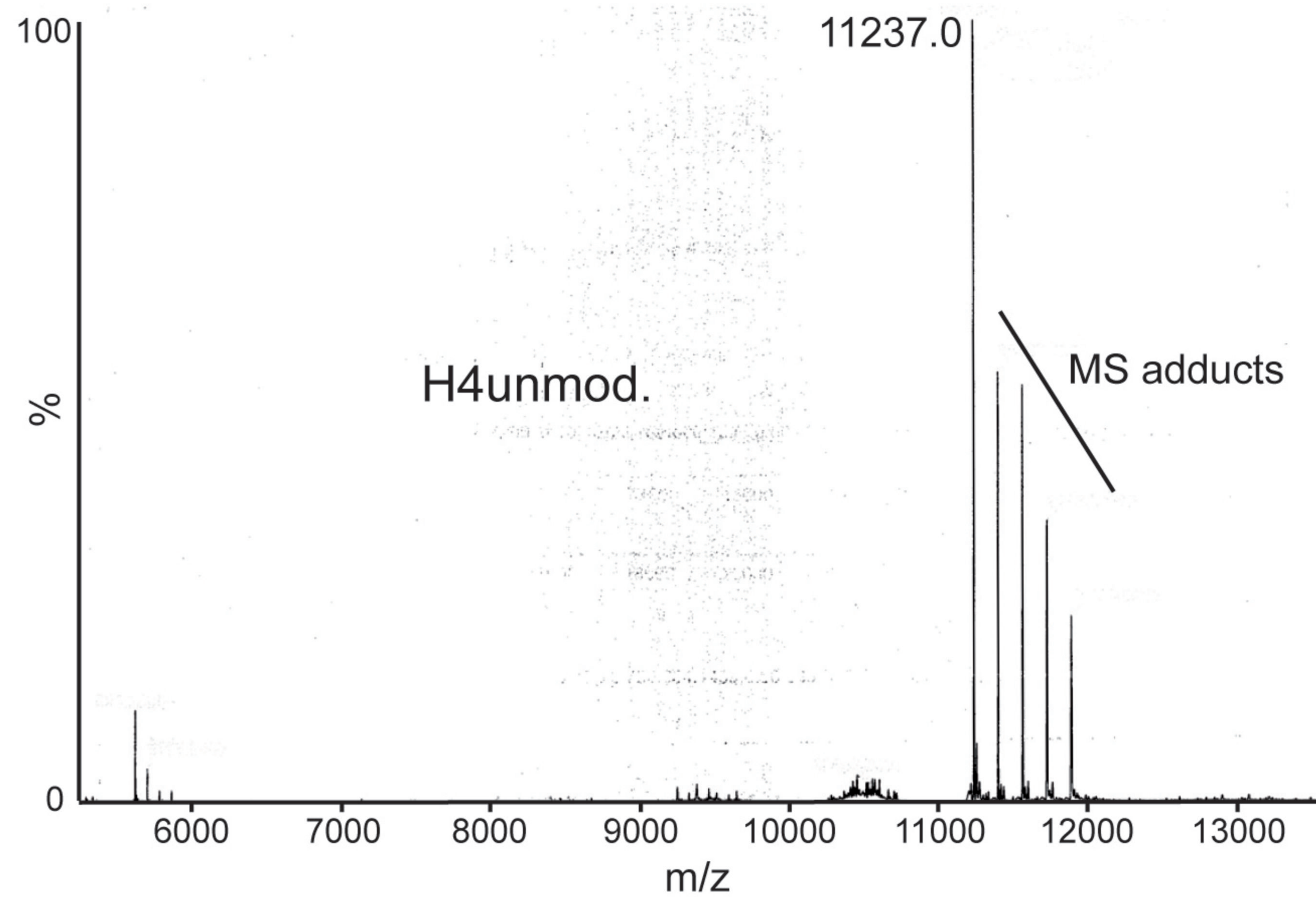
(**A**) The Sir2-3-4 complex was titrated into a constant amount of unmodified 6mer of 601 nucleosomes. Where indicated, 150 μM NAD were added to the samples. Scatter plot quantifications show the mean value ± SEM of the % of unbound chromatin compared to the input for at least three experiments. (**B**) Reconstituted chromatin fully acetylated on H4K16 was subjected to NAD-dependent deacetylation in presence of a 2.5-fold molar excess of the Sir2-3-4 complex. The acetylation state was then determined by immunoblotting using acetylation mark specific antibodies and H3 for loading.

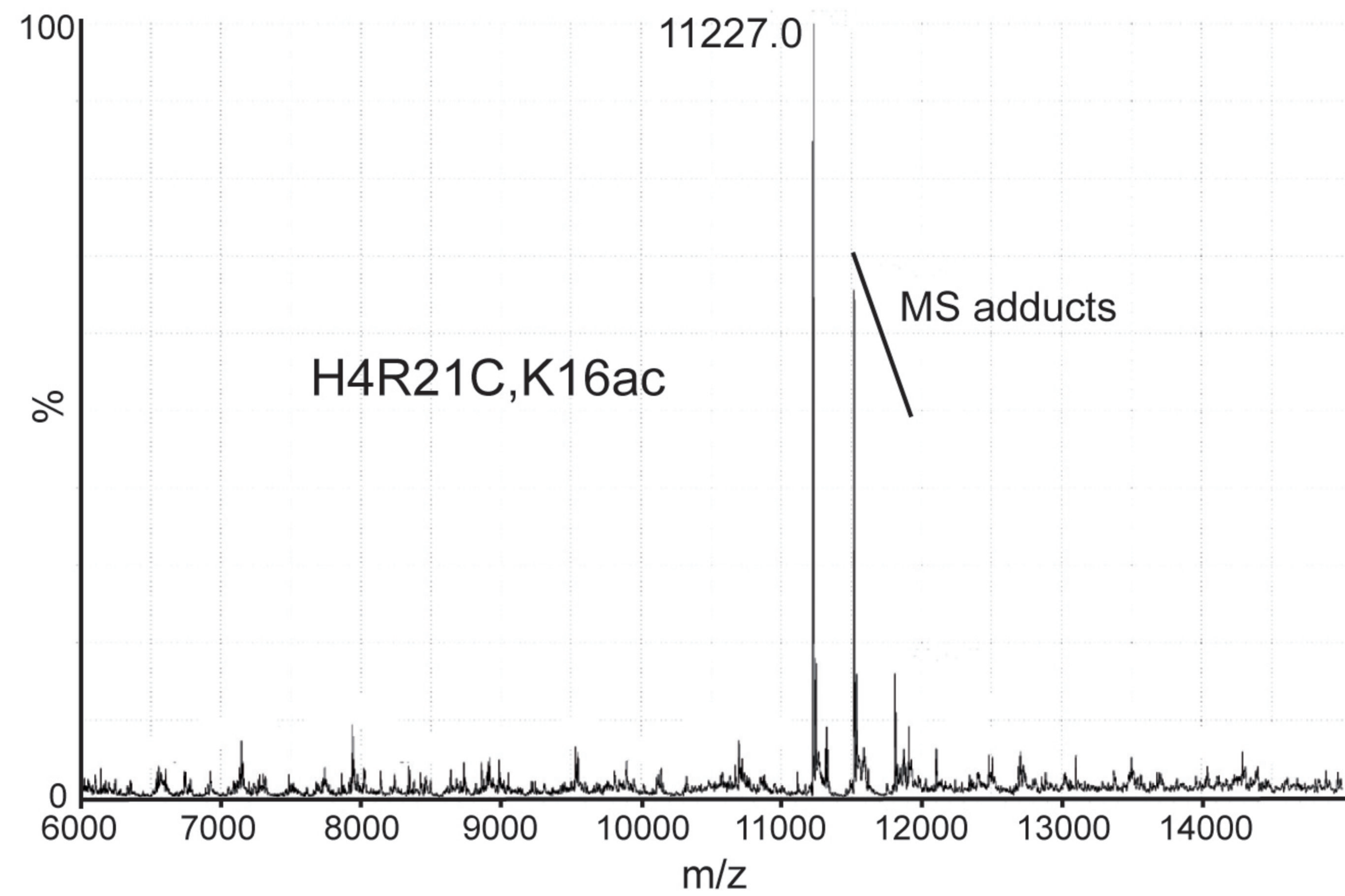
Figure S3 ***Sir2-3-4 loading on unmodified chromatin greatly protects linker DNA from enzymatic digestion independently on the presence of NAD***

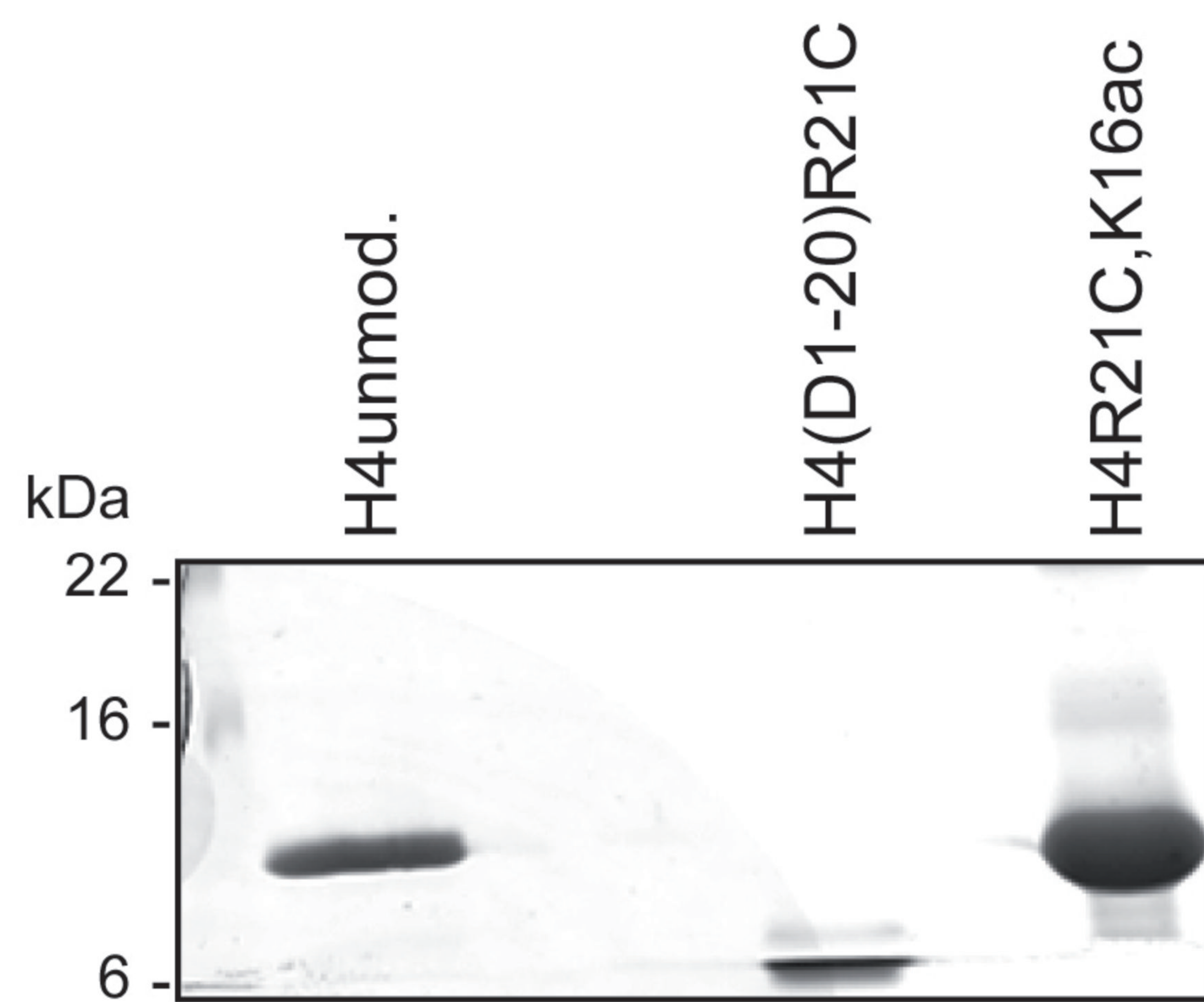
Unmodified 6mers of nucleosomes incubated with buffer or the Sir2-3-4 complex were challenged with increasing amounts of MNase (**A**) or the restriction enzyme *Ava*I (**B**). After protein digestion the denatured DNA was separated by electrophoresis and visualized by SYBR® Safe staining. The reaction with *Ava*I was carried out essentially as described for MNase, but 1.5 mM $MgCl_2$ replaced 1.5 mM $CaCl_2$ and 0.1 mM TCEP was added to the reaction. The band corresponding to the intact 6mer (**A**) or 6-3mer (**B**), shown by a black arrow, were quantified and normalized to input. An aliquot of undigested sample was separated by native agarose gel electrophoresis to control for proper binding. (**C**) Unmodified 6mer of nucleosomes (50 nM) were incubated with the indicated amount of Sir2-3-4 and challenged with increasing amounts of MNase. Where indicated, SIR-bound chromatin was supplemented with 150 μM NAD and incubated 15 min at 30°C prior to MNase digestion. Deproteinated samples were separated by electrophoresis and the amount of intact 6mer DNA (black arrow) was quantified and normalized to input. Quantification of at least three experiments were used to generated the vertical bar charts, data represent mean value ± SEM.

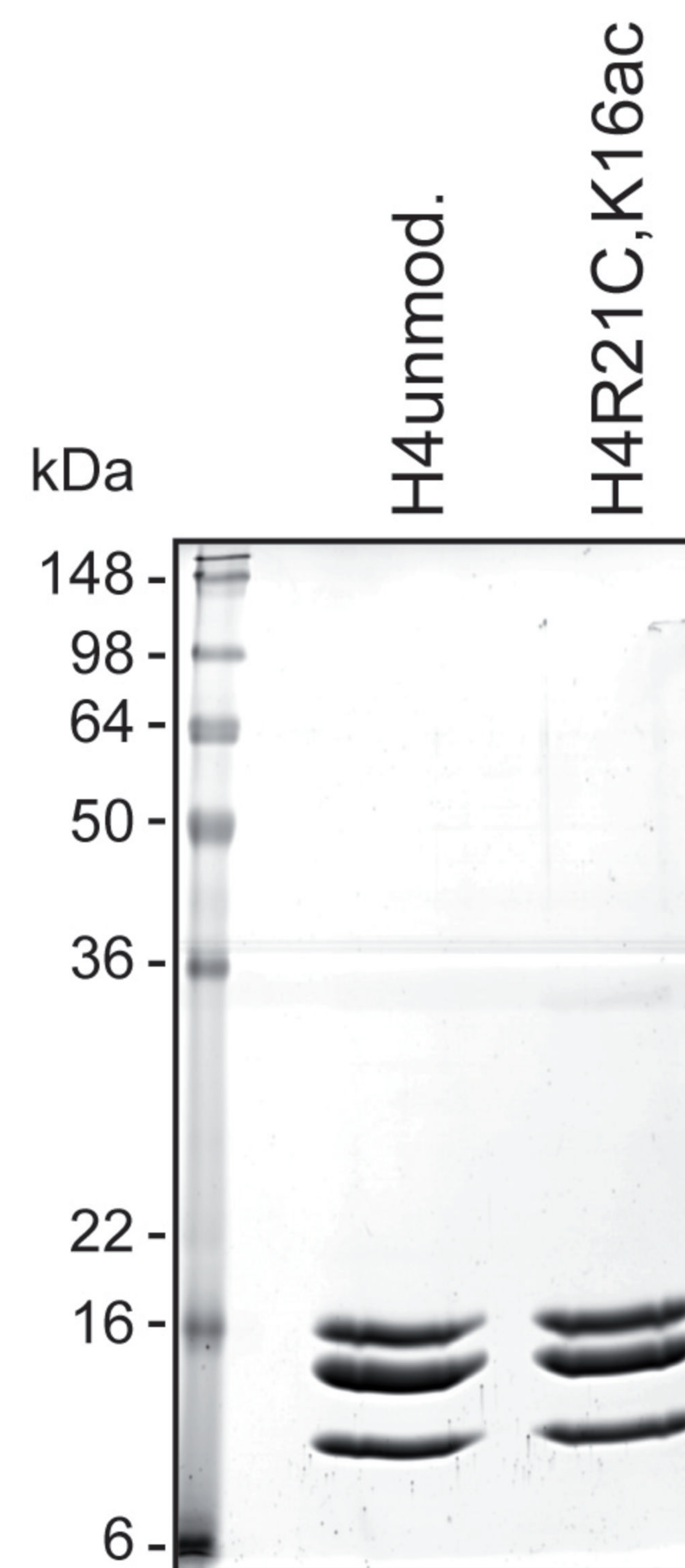
Figure S4 Gel shift analysis of the SIR-bound chromatin use for MNase assays in Figure 4 and Figure 5

Samples of the material analyzed in Figure 4G (**A**), Figure 5D (**B**) and Figure 5E (**C**) were run in a native agarose gel to demonstrate that the SIR-bound chromatin was undersaturated (**B**) or saturated (**A**) and (**C**) at the indicated concentration of the SIR complex.



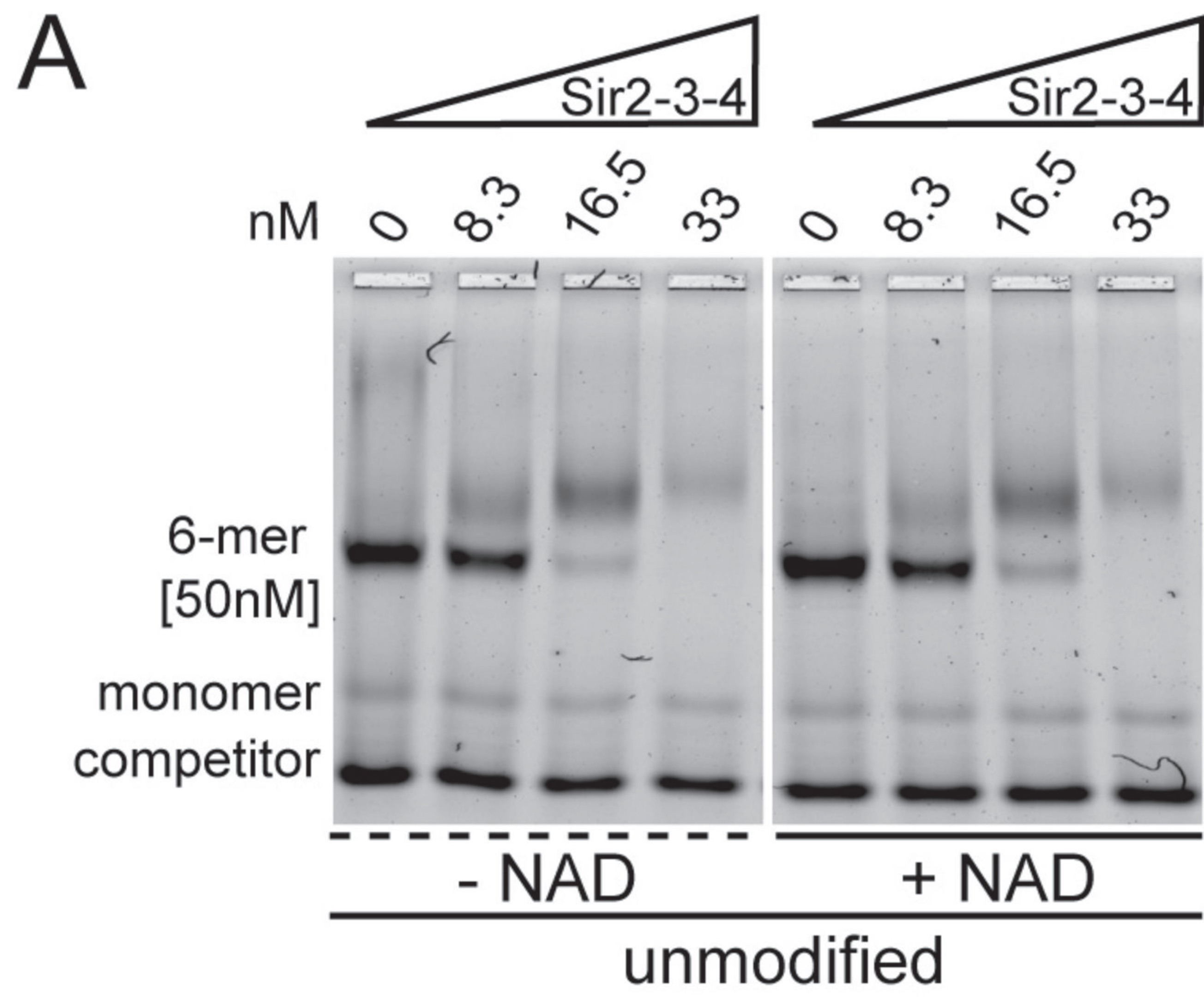


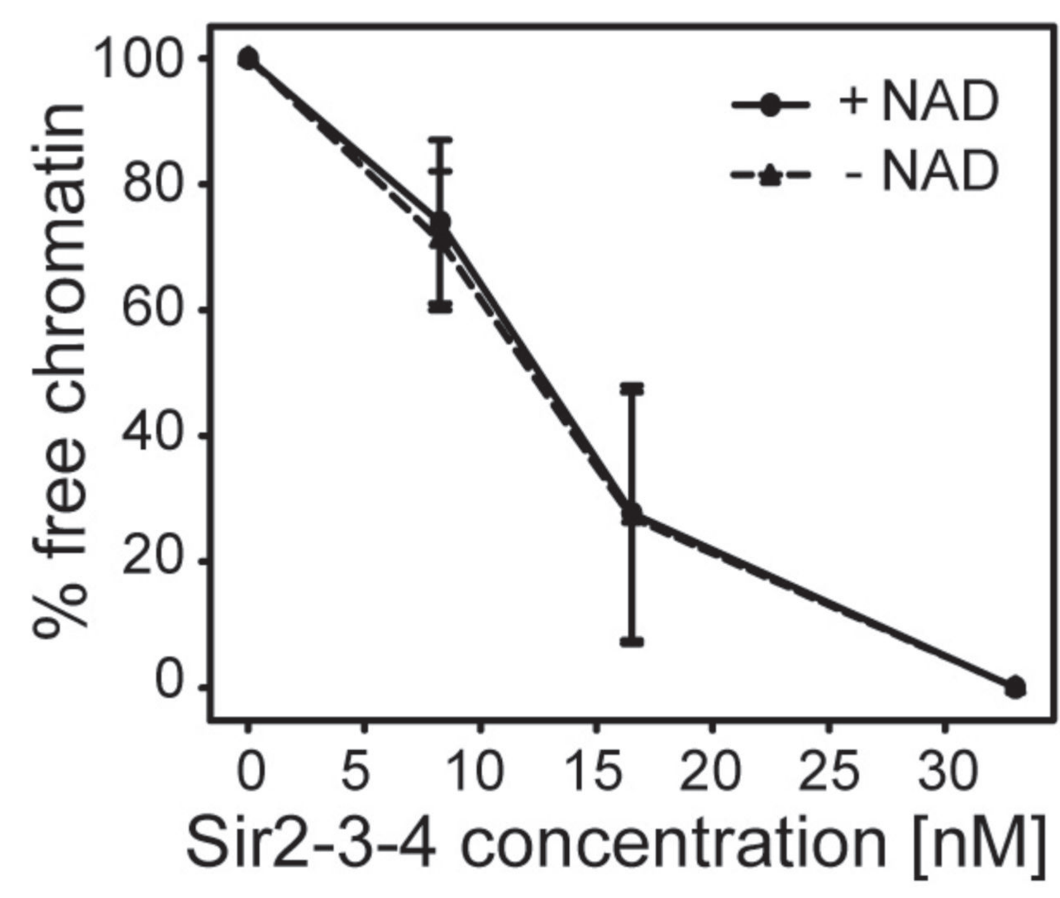




Oppikofer et al. Fig_S1

A





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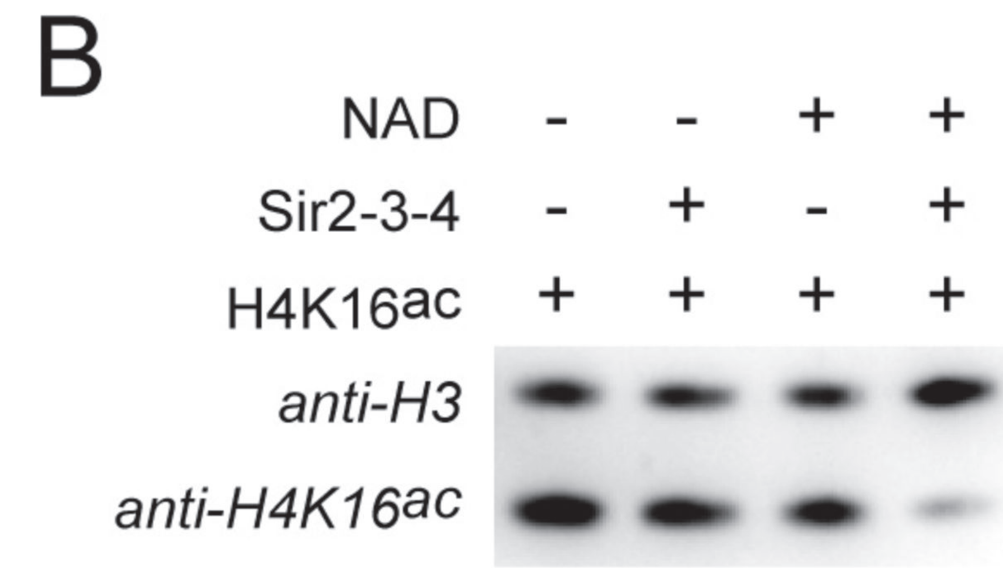
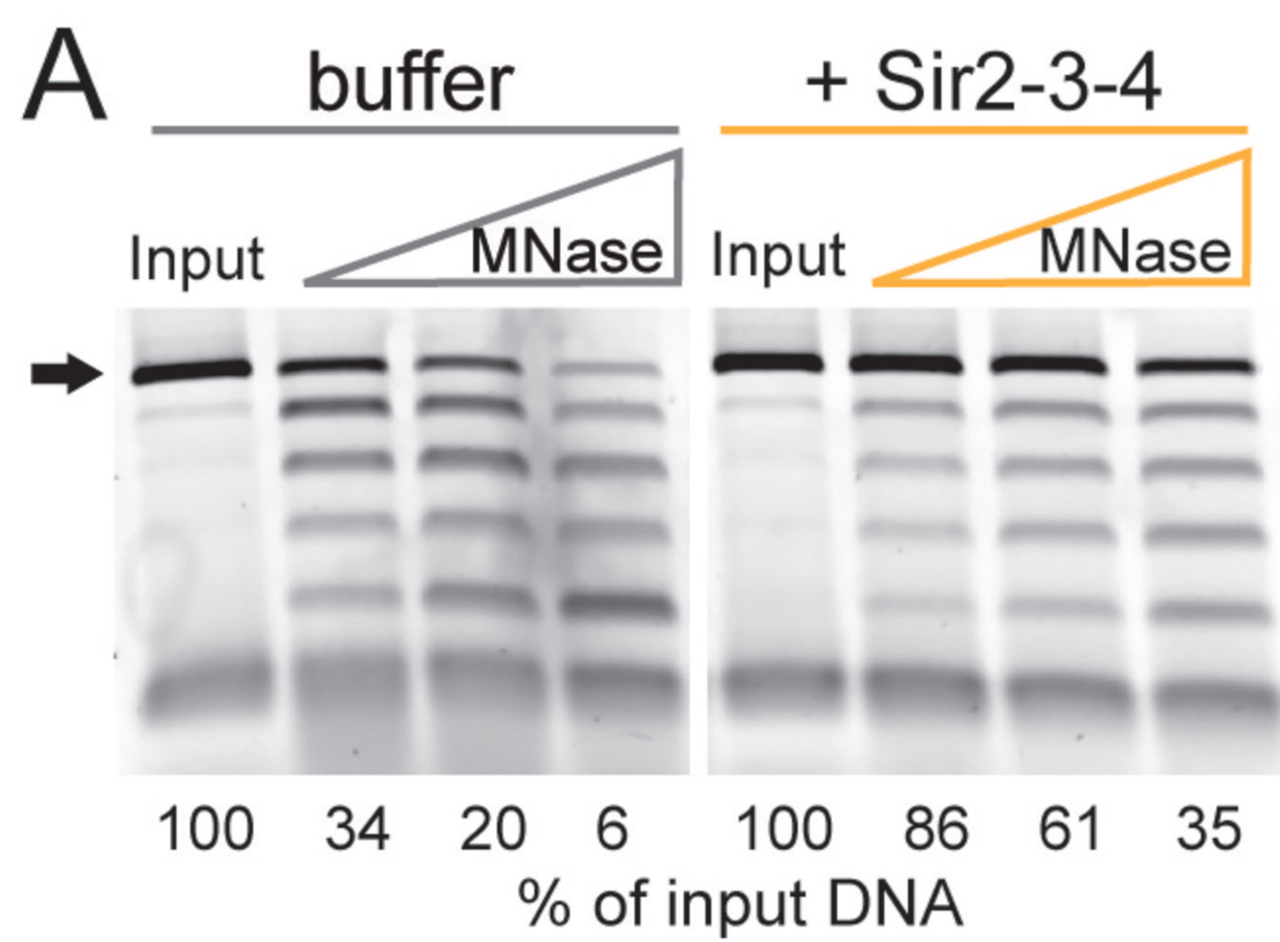
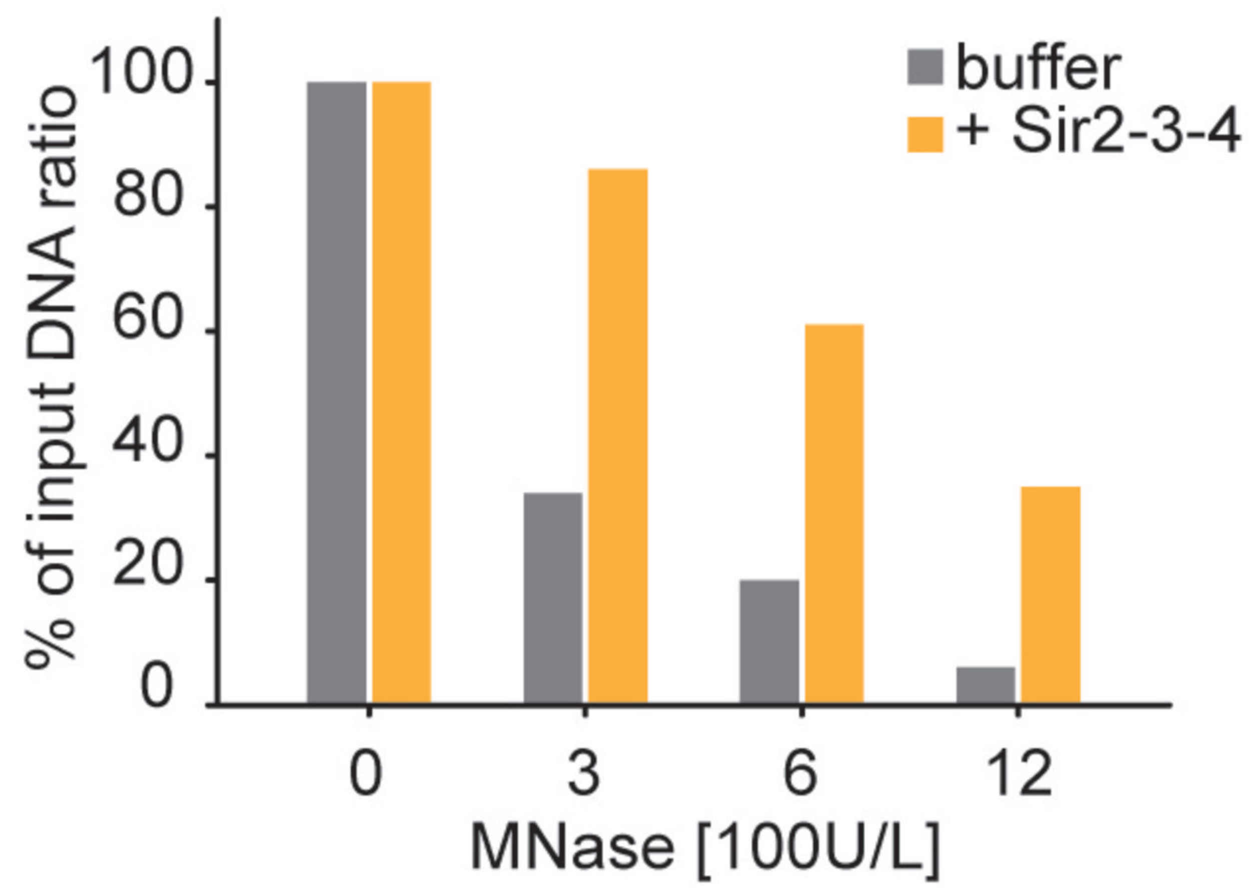
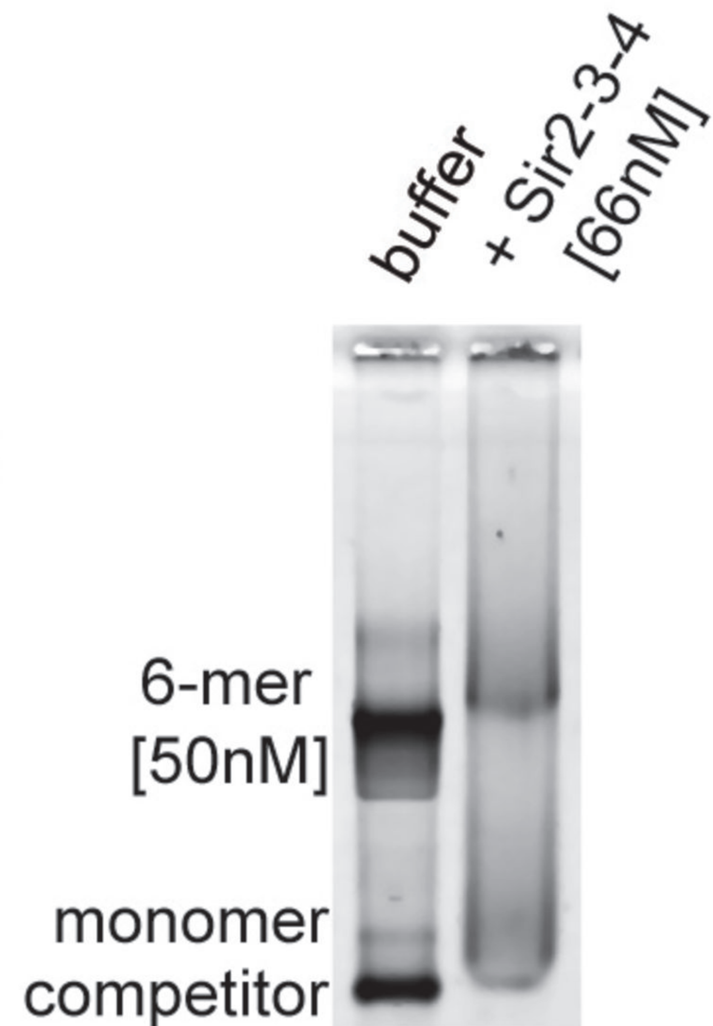
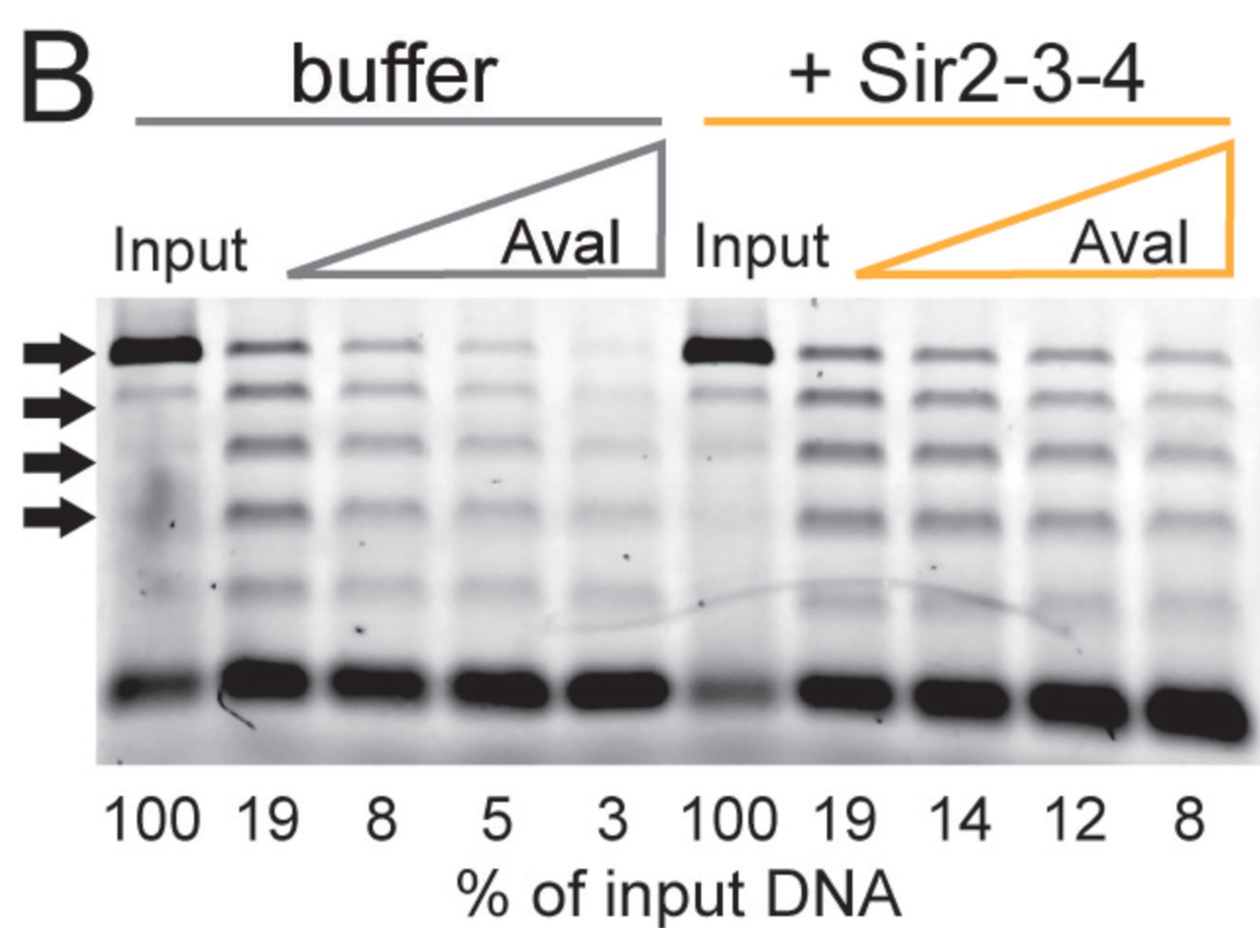


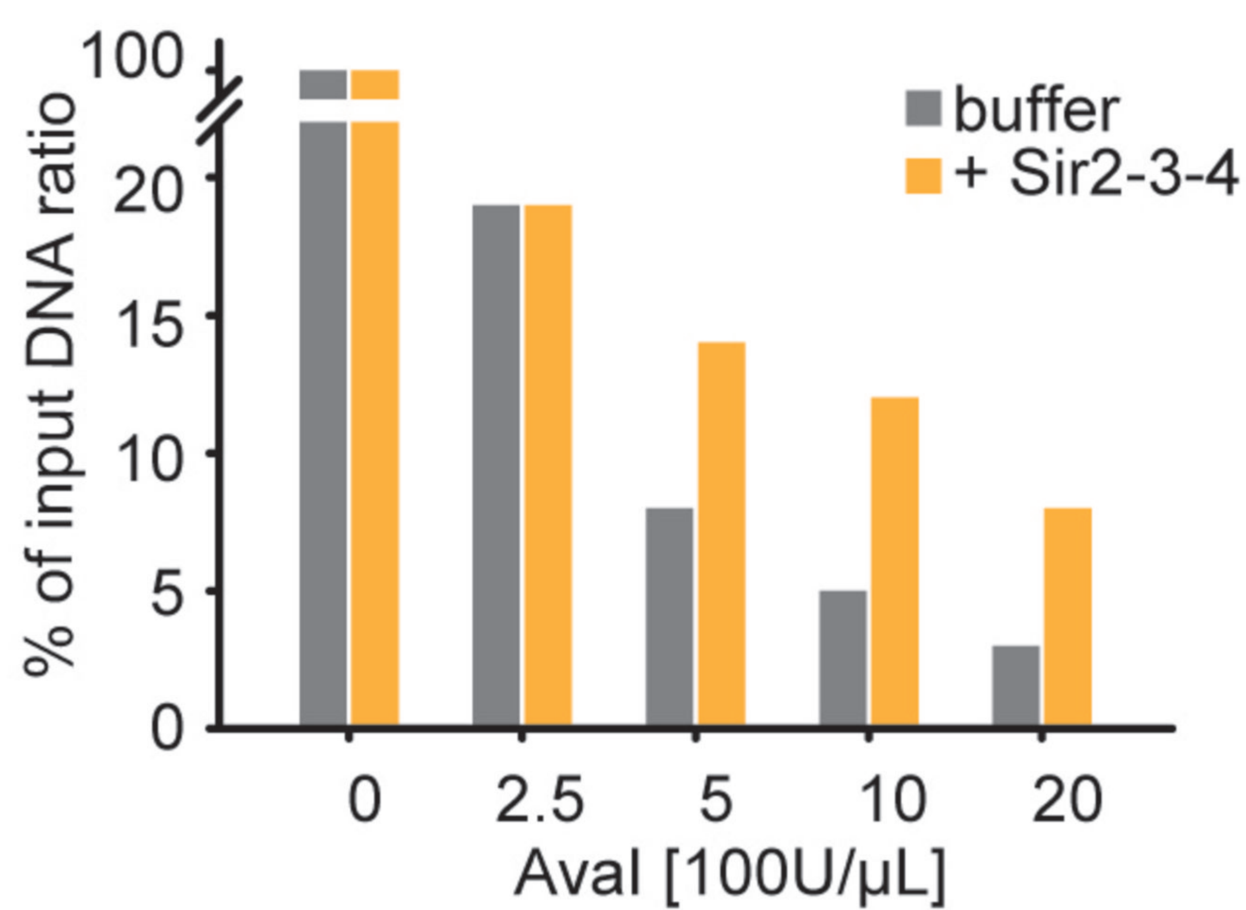
Oppikofer et al. Fig_S2

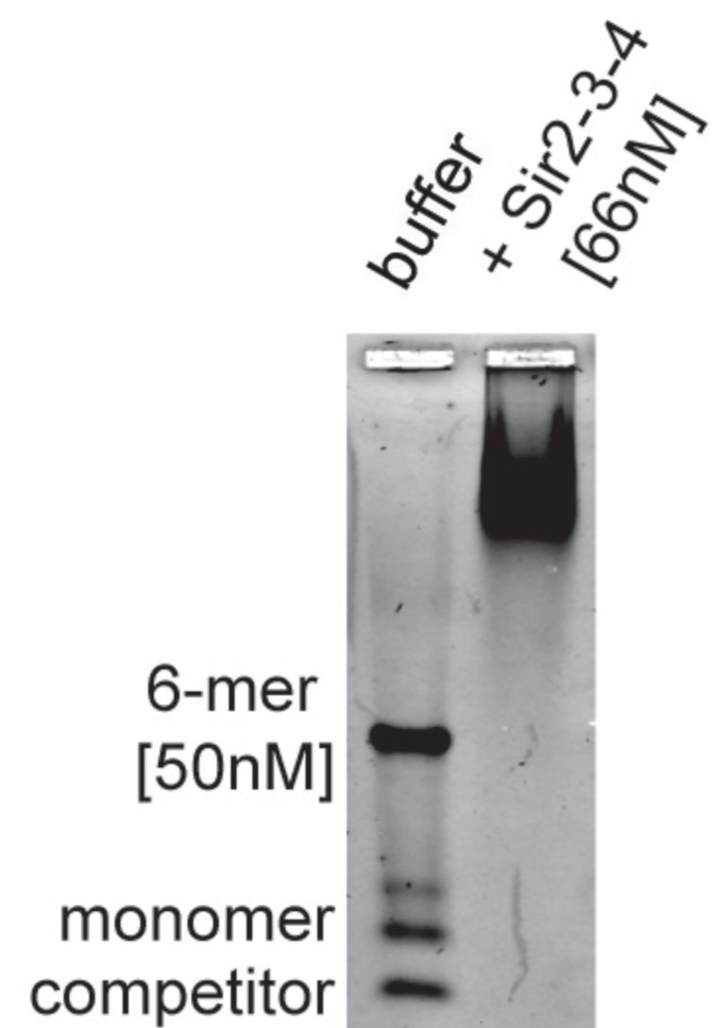


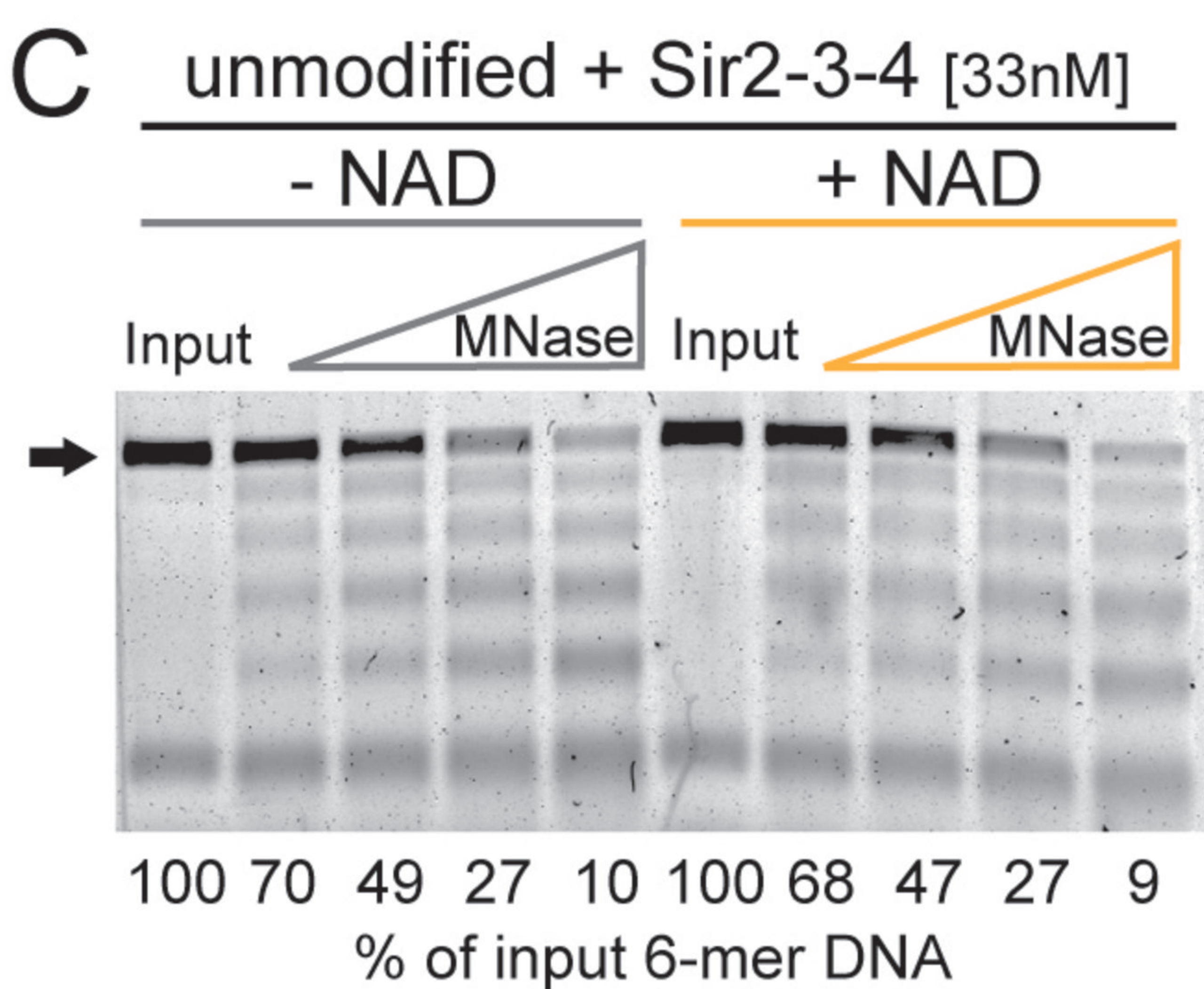


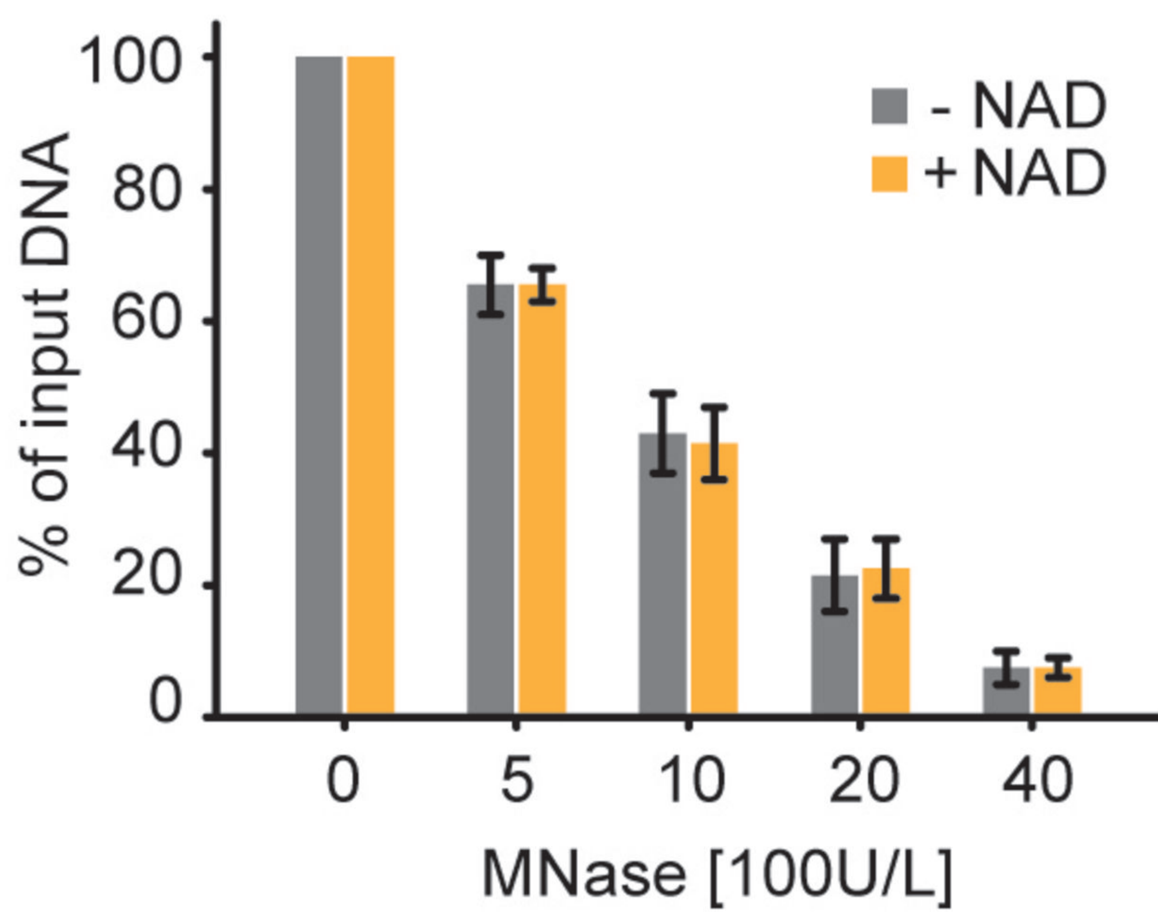




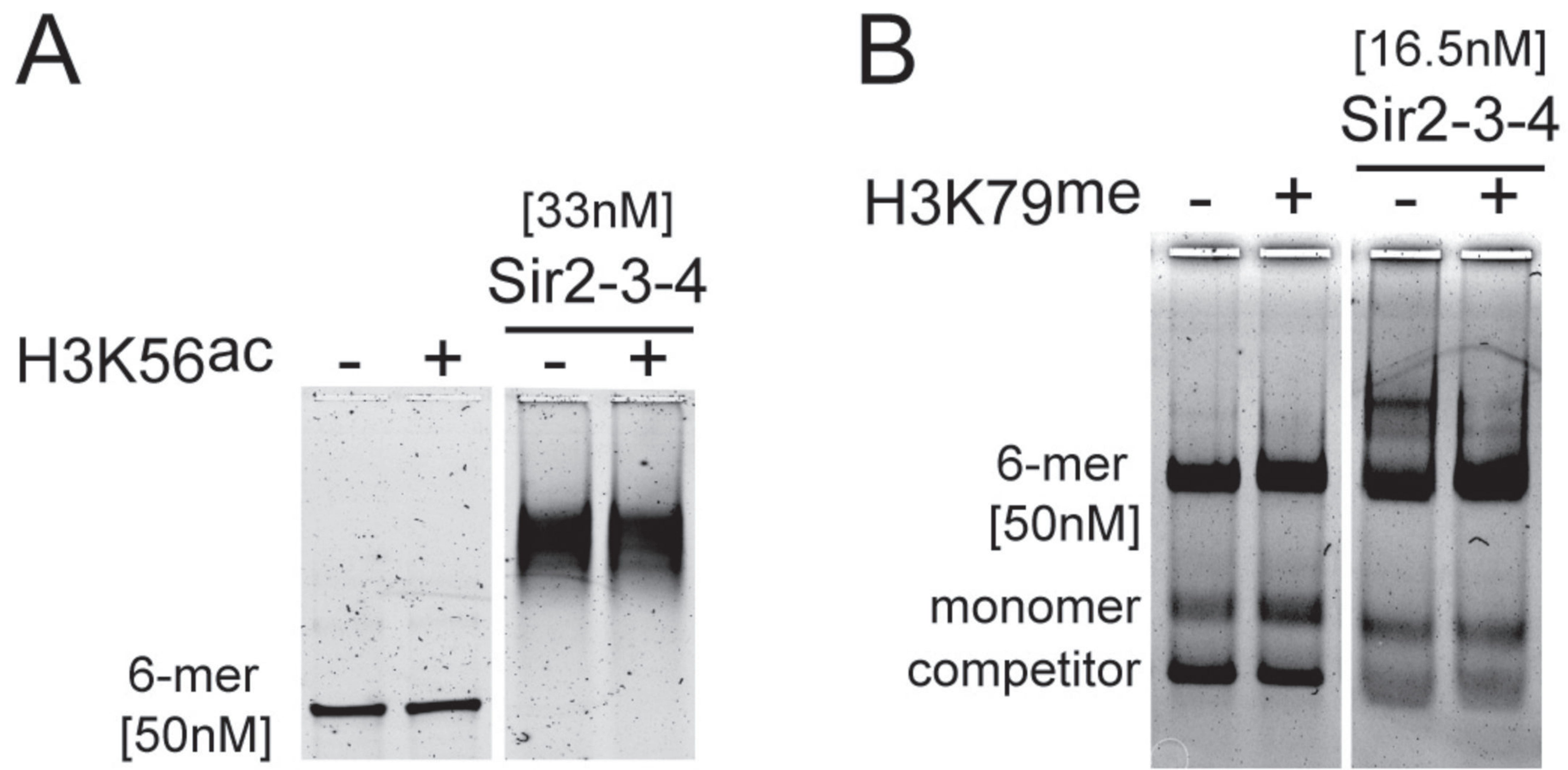


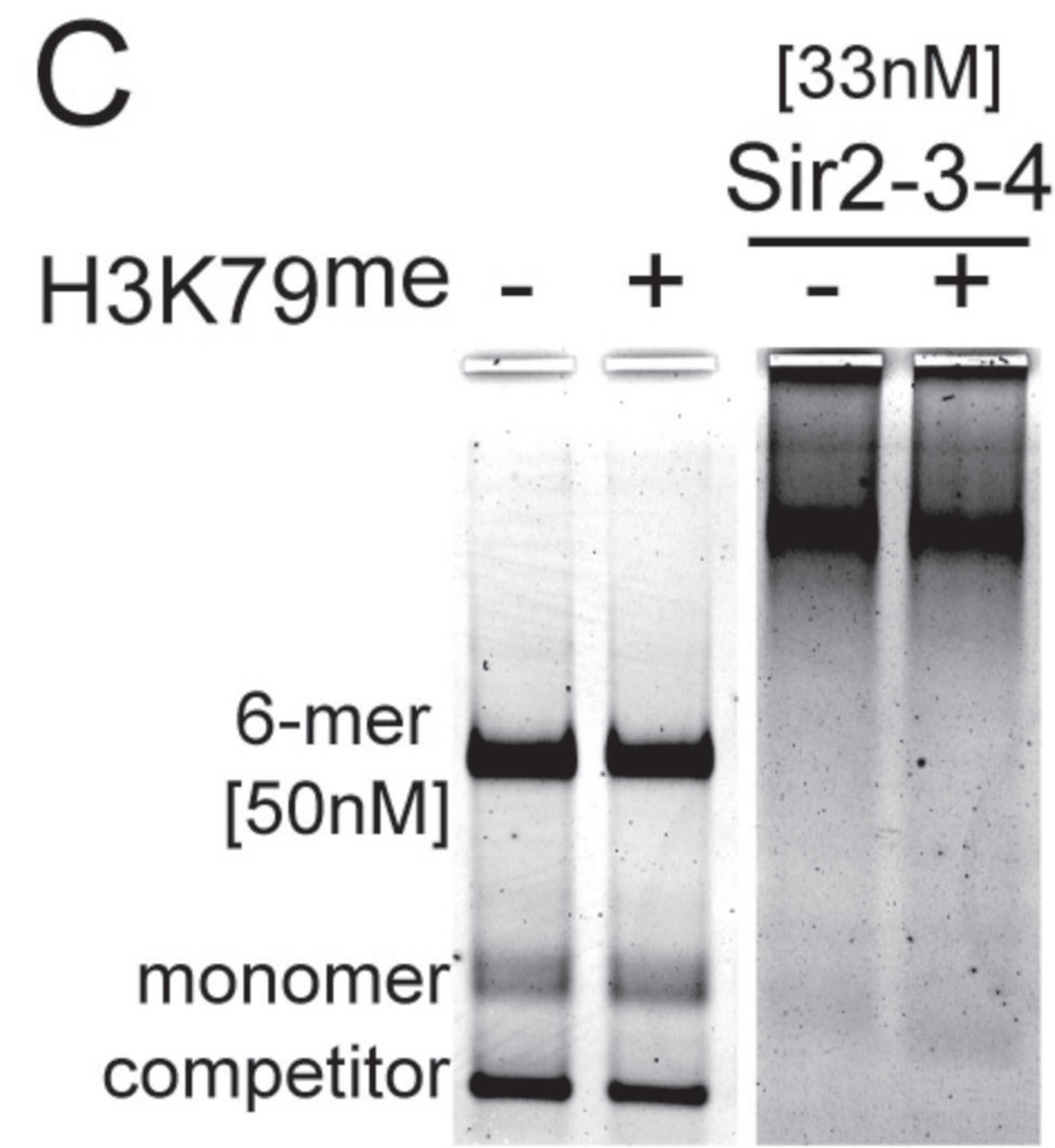






Oppikofer et al. Fig_S3





Oppikofer et al. Fig_S4