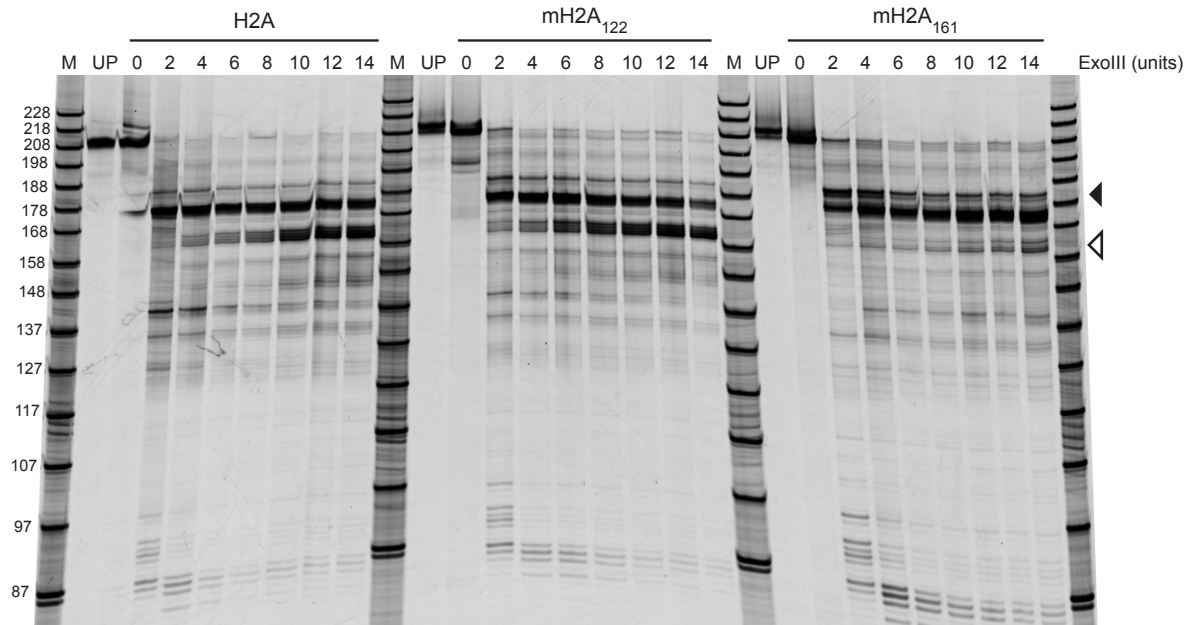


**Figure S1.** Nucleosomes containing the macro-linker are mobilized less efficiently by Chd1. **(A)** End-positioned nucleosomes (0-N-33, 150 nM) containing either mH2A<sub>122</sub> or mH2A<sub>161</sub> were incubated with Chd1 (10 nM) and monitored by nucleosome mapping. Shown are histogram traces of a sequencing gel at 0, 5, and 60 min time points, with the locations of the H2B-53C crosslink noted at 60 min. Note the absence of clear crosslinking at +36 bp and +49 bp for mH2A<sub>161</sub> compared with mH2A<sub>122</sub>. **(B)** Mapping nucleosomes sliding reactions as in (A), but using 0-N-80 nucleosomes.



**Figure S2.** The macro-linker extends protection of entry/exit DNA from exonuclease III digestion. Centrally-positioned 30-N-33 nucleosomes containing major-type H2A, mH2A<sub>122</sub>, or mH2A<sub>161</sub> were incubated in the presence or absence of exonuclease III as indicated for 5 min at 24°C, and the reactions quenched with 10 mM EDTA and 1% SDS. DNA fragments containing the 5'-FAM label, which was on the 30 bp side of the nucleosome, were resolved in an 8% polyacrylamide, 8 M urea sequencing gel, and visualized on a Typhoon 9410 imager. For mH2A<sub>161</sub>, increased cleavage is apparent at the outermost digestion site compared to H2A and mH2A<sub>122</sub>, corresponding to ~81 bp from the nucleosome dyad (closed triangle). Reduced digestion is observed more internal to the nucleosome, ~59 bp from the dyad (open triangle), likely due to increased exonuclease III pausing at ~81 bp and ~71 bp from the dyad. M = marker, UP = unprocessed.