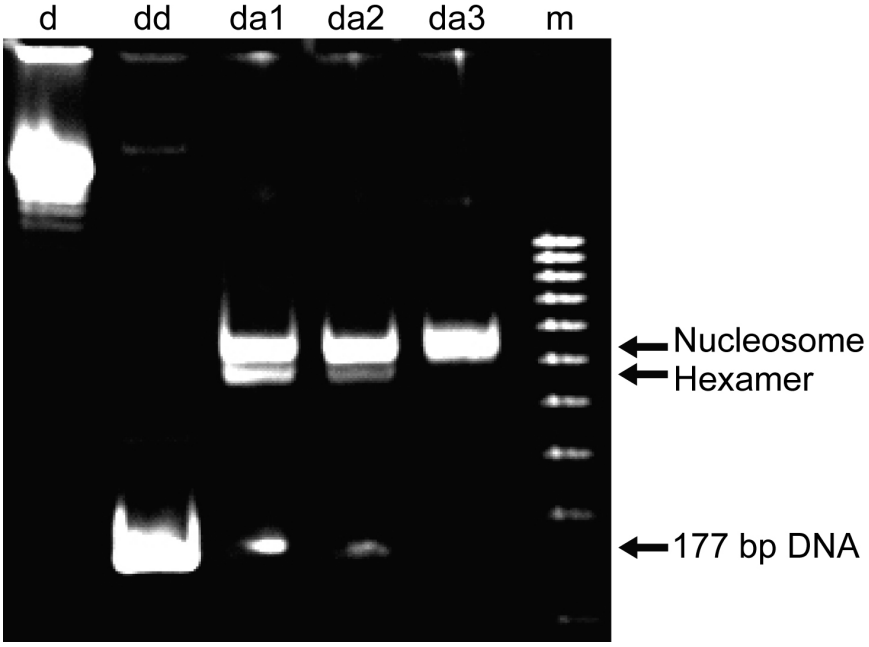
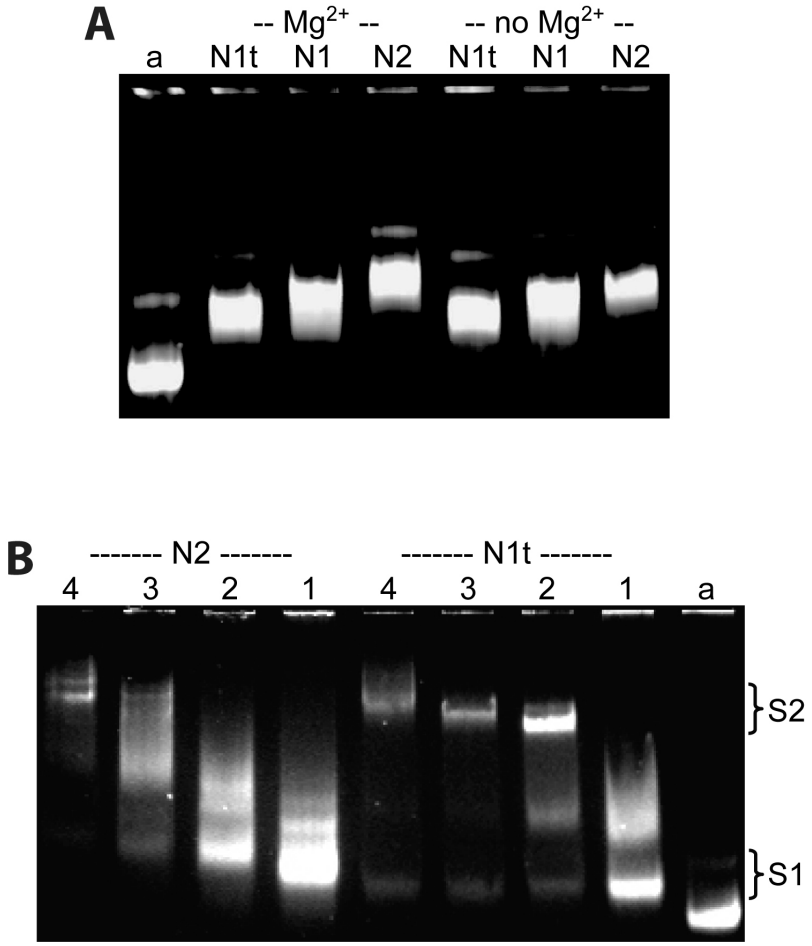
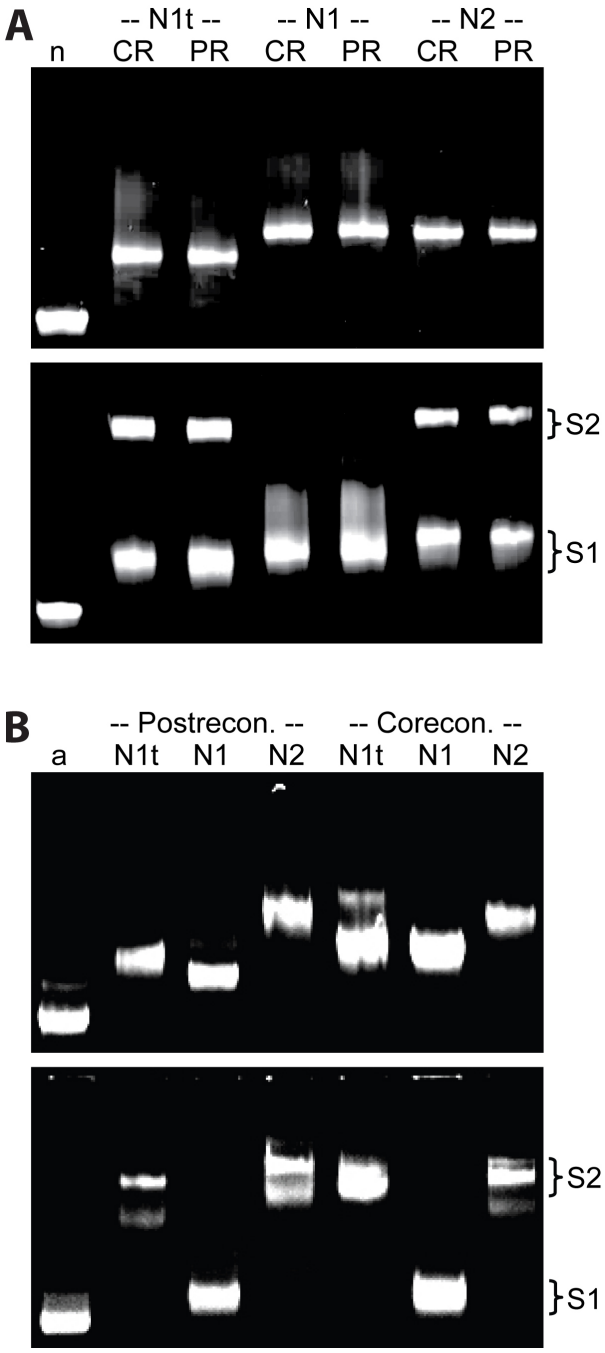


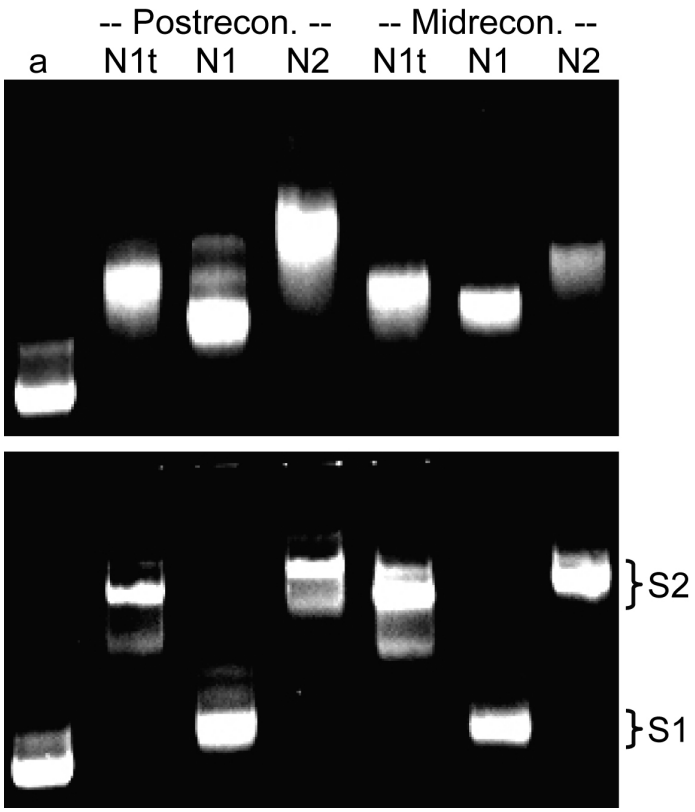
Supplementary Figure 1. Restriction digestion analysis to assess histone octamer saturation of 12-array. 12-array naked DNA (undigested, d; digested, dd) and 12-array reconstituted with increasing histone octamer:DNA stoichiometry (da1, da2, da3) were subjected to digestion by ScaI, whereby cleavage occurs at the linker DNA midpoint between nucleosome sites. When the 12-array is unsaturated, one observes in addition to 177 bp nucleosome, H2A-H2B dimer-depleted nucleosome (hexamer) and naked DNA as digest products (da1 and da2). A 100 bp DNA ladder (m) is shown for reference.



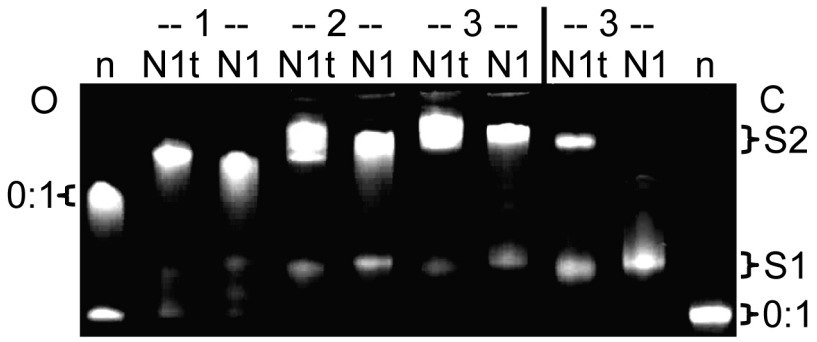
Supplementary Figure 2. The HMGN binding-induced S2 transition is fully reversible. EMSA gel labels indicate HMGN identity (N1t, HMGN1t; N1, HMGN1; N2, HMGN2; a, 12-array alone) and numbers designate HMGN:nucleosome molar stoichiometry. (**A**) 12-array samples were pre-incubated with 4:1 HMGN:nucleosome molar stoichiometry in 1X TB buffer containing either 1 mM Mg^{2+} (S2-forming conditions) or 5 mM EDTA, prior to electrophoresis under near physiological ionic strength buffer without divalent metal (1X TBE). (**B**) 12-array stock samples in 1X TB buffer with 1 mM Mg^{2+} were pre-incubated with 4:1 HMGN:nucleosome molar stoichiometry (4), prior to electrophoresis under near physiological ionic strength with 1 mM Mg^{2+}. To these stock samples additional 12-array was added to maintain equimolar nucleosome concentration while reducing HMGN:nucleosome stoichiometry (3, 2, 1).



Supplementary Figure 3. HMGN-binding induced transitions are not influenced by HMGN presence during nucleosome assembly. EMSA gel labels indicate HMGN identity (N1t, HMGN1t; N1, HMGN1; N2, HMGN2; n, NCP alone; a, 12-array alone). (**A**,**B**) Comparison of HMGN-NCP-601 (A) and HMGN-12-array (B) assemblies in which HMGN was either present at the onset of nucleosome reconstitution (CR, Corecon.) or was added subsequent to complete assembly (PR, Postrecon.). HMGN:nucleosome molar stoichiometry was 2.2 (NCP) or 4.2 (12-array) and 3.0 (NCP) or 2.3 (12-array) for co-reconstitution and post-reconstitution trials, respectively. Electrophoresis was conducted in running buffers of near physiological ionic strength (top) and near physiological ionic strength with 1 mM Mg^{2+} (bottom).



Supplementary Figure 4. HMGN-binding induced transitions are not influenced by HMGN introduction during nucleosome assembly. EMSA gel labels indicate HMGN identity (N1t, HMGN1t; N1, HMGN1; N2, HMGN2; a, 12-array alone). Comparison of HMGN-12-array assemblies in which HMGN was introduced either at the midpoint of nucleosome reconstitution (Midrecon.) or was added subsequent to complete assembly (Postrecon.). HMGN:nucleosome molar stoichiometry was 4.0 and 2.3 for mid-reconstitution and post-reconstitution trials, respectively. Electrophoresis was conducted in running buffers of near physiological ionic strength (top) and near physiological ionic strength with 1 mM Mg^{2+} (bottom).



Supplementary Figure 5. Off-centered NCP undergoes a transition in the presence of divalent metal and associates with HMGN1 and HMGN1t to form an S2-like state. EMSA of HMGN binding to centered (C; right) versus off-centered (O; left) NCP146b under near physiological ionic strength buffer with 1 mM Mg^{2+}. Gel labels indicate HMGN identity (N1t, HMGN1t; N1, HMGN1; n, NCP alone) and numbers designate HMGN:nucleosome molar stoichiometry. Off-centered samples (left 7 lanes) also contain a fraction of faster migrating centered NCP.