Supplementary Data

INO80 prefers to bind nucleosomes with extranucleosomal DNA

Extranucleosomal DNA is required for efficient binding of INO80 as shown by gel shift analysis. Nucleosomal substrates with increasing lengths of extranucleosomal DNA at one entry site were bound with varying amounts of INO80 and analyzed on a 4% native gel with 1 x TE buffer. The percent of nucleosomes bound was plotted versus the concentration of INO80 (nM) in the binding reaction for each nucleosomal substrate (Figure S1). The observed K_d or the concentration of INO80 required for binding 50% of the nucleosomes is shown in Table S1.

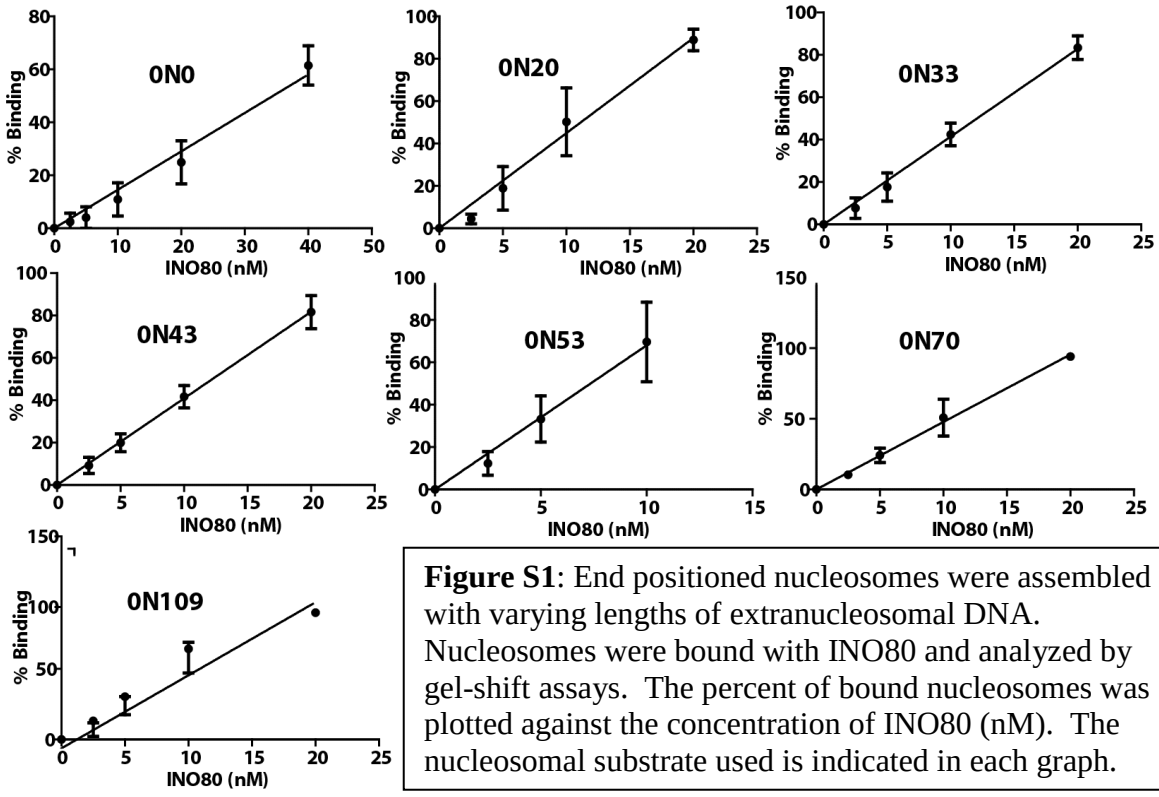

Figure S1: End positioned nucleosomes were assembled with varying lengths of extranucleosomal DNA. Nucleosomes were bound with INO80 and analyzed by gel-shift assays. The percent of bound nucleosomes was plotted against the concentration of INO80 (nM). The nucleosomal substrate used is indicated in each graph.

Although INO80 does not remodel centrally positioned nucleosomes, INO80 binding to these nucleosomes was not impaired. INO80 bound readily to 53N53 or 70N70 nucleosomes with an observed K_d value of 7.3 and 8.1 respectively (Table S1 and Figure S2).

Table S1 – Extranucleosomal DNA length vs. INO80 affinity									
NCP	0N0	0N20	0N33	0N43	0N53	0N70	0N109	53N53	70N70
K_d	35±4.1	14±2.9	13±0.2	12±1.1	9.8±0.1	10±0.9	9.1±1.7	7.3±0.5	8.1±0.4

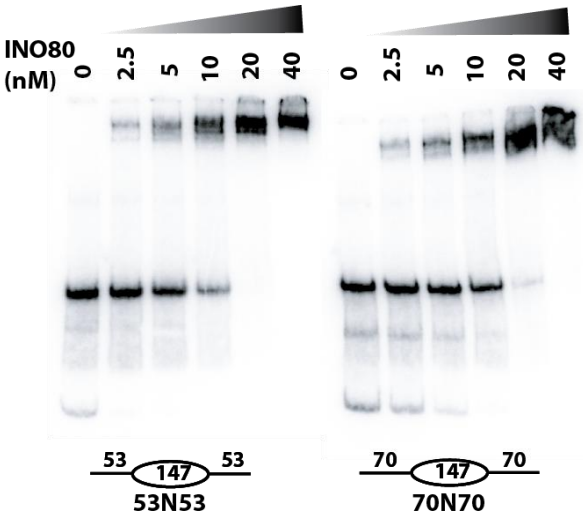

Figure S2. Centrally positioned nucleosomes with 53bp (53N53) and 70bp (70N70) extranucleosomal DNA on either side of the nucleosome was bound with increasing amounts of INO80 (2.5 nM, 5 nM, 10 nM, 20 nM and 40 nM) and 30 nM nucleosomes. INO80:Nucleosome complex was analyzed on a 4% native PAGE with 1 x TE buffer and the reactions did not contain salmon sperm DNA.

INO80 did not require histone tails for its remodeling activity

Nucleosomes missing different histone tails or combinations of N-terminal histone tails were assembled with 0N70 DNA and remodeled with INO80. Gel shift assays corresponding to data presented in Figure 8 are shown in Figure S3. Nucleosomes with one or two histone tails missing did not adversely affect nucleosome movement by INO80 as seen by changes in the electrophoretic mobility of labeled nucleosomes (Figure S3). These data were used to calculate the rates of INO80 remodeling as shown in Figure 8 with the one exception of nucleosomes missing all N-terminal histone tails that did not show any change in electrophoretic mobility after remodeling with INO80.

Presence of H2A-H2B dimer is required for INO80 remodeling

The importance of the H2A-H2B dimer for nucleosome binding and remodeling by INO80 was determined with tetrasomes assembled on 601 positioning sequence with 70 bp extranucleosomal DNA. INO80 bound to tetrasomes readily as seen by gel shift analysis at a level comparable to nucleosome binding (Figure S4A), but no significant changes were observed by gel-shift analysis upon INO80 remodeling (Figure S4B). The ability of tetrasomes to be remodeled by INO80 was examined further by restriction

enzyme accessibility assays with NotI and PmlI. INO80 cannot mobilize tetrasomes as shown by no significant change in the cleavage pattern with NotI or PmlI upon addition of INO80 and ATP (Figure S4C).

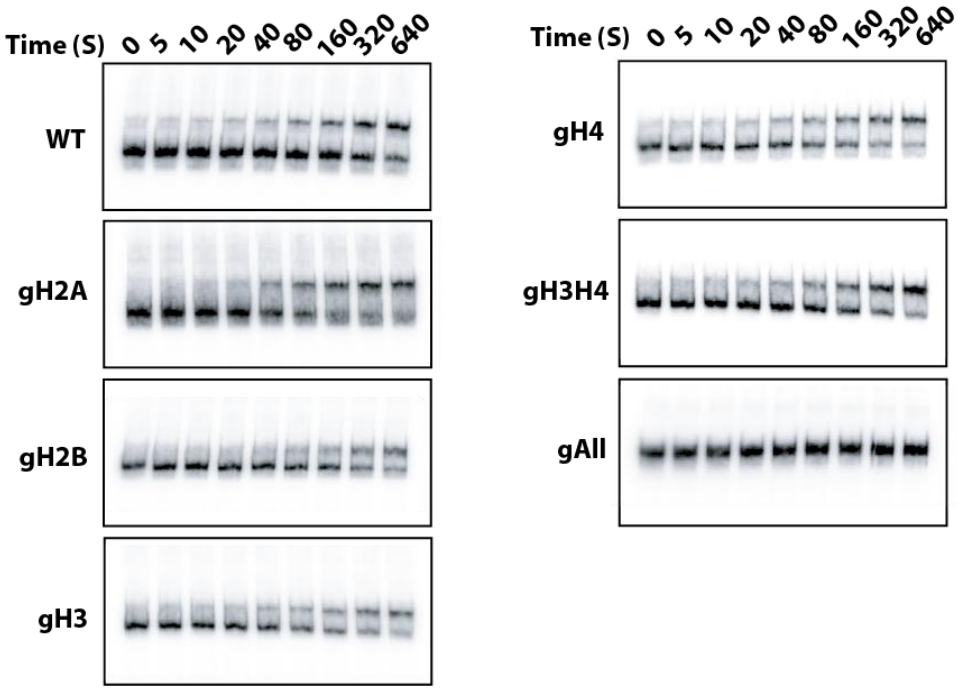

Figure S3. Remodeling kinetics of INO80 was performed with wild type (WT), H2A tail-less (gH2A), H2B tail-less (gH2B), H3 tail-less (gH3), H4 tail-less (gH4), H3 and H4 tail-less (gH3H4) and all-tails deleted (gAll) nucleosomes (33 nM). Nucleosomes were prebound with excess of INO80 (40 nM) to achieve full binding conditions. ATP was added to a final concentration of 800 µM and incubated for a desired time. Reactions were stopped with excess gamma thio ATP and sonicated salmon sperm DNA. Remodeled products were analyzed on a 5% native PAGE with 0.5 x TBE buffer.

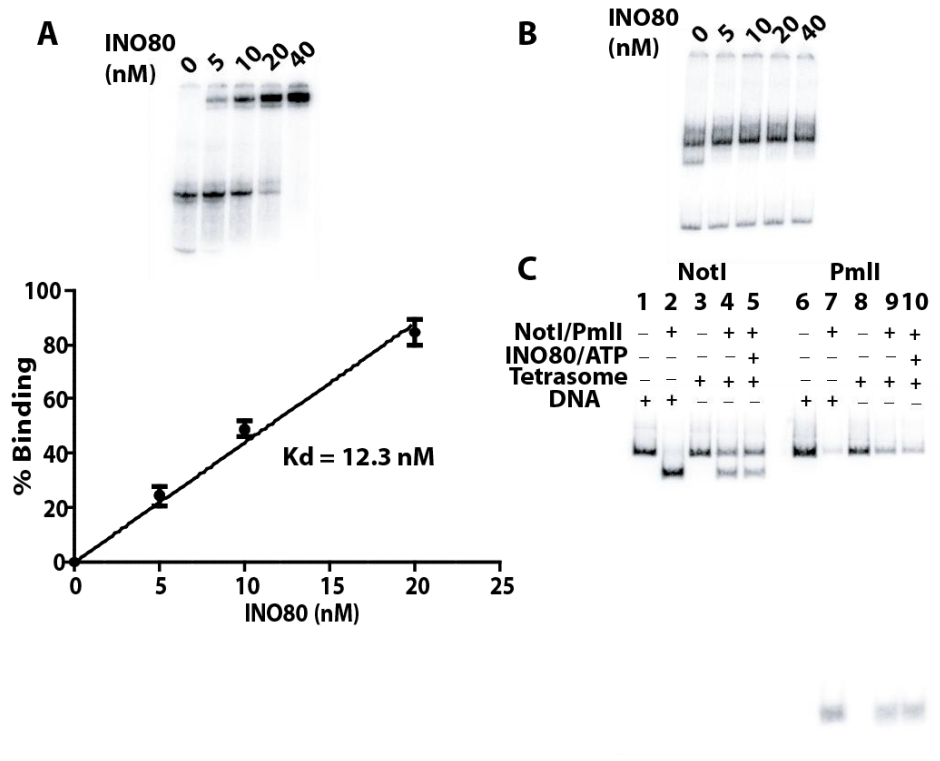

Figure S4. (A) Ability of INO80 binding to tetrasomes was tested with increasing amounts of INO80 (5 nM, 10 nM, 20 nM and 40 nM) and 37 nM tetrasomes. Binding reactions were carried out for 30 min at 30ºC and analyzed on a 4% native PAGE with 1 x TE buffer. The percent of nucleosomes bound was plotted against the concentration of INO80 (nM). Observed K_d value was calculated to be 12.3 nM**.** (B) Remodeling of tetrasomes was carried out with 37 nM tetrasomes and increasing amounts of INO80 (5 nM, 10 nM, 20 nM and 40 nM) in the presence of 800 µM of ATP. Reactions were incubated for 30 min at 30ºC and were stopped by the addition of excess gamma thio ATP and the enzyme was competed off with sonicated salmon sperm DNA. Remodeled nucleosomes were analyzed on a 5% native PAGE (60:1). (C) Remodeling of tetrasomes by INO80 was analyzed by restriction enzyme accessibility assay using NotI (lanes 1 – 5) and PmlI (6 – 10). After restriction enzyme digestion, DNA was analyzed on a 6% native PAGE.