SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Single-molecule FRET histograms and time traces for reconstituted nucleosome and tetrasome for the 601- ED1 and INT scheme, Related to Figure 1

(**A**) FRET histogram of Nucleosome (blue) and Tetrasome (magenta) reconstituted with the ED1 labeling scheme. The peak at ~0.1 corresponds to free DNA, the peak at ~ 0.75 corresponds to intact nucleosome. FRET peak of the tetrasome sample, which is broader and lower then nucleosome peak, corresponds to the mixture of free DNA and tetrasome.

(**B**) Representative single molecule time traces of the ED1 nucleosome at zero force show stable FRET over 1 min. (donor (Cy3, in green), acceptor (Cy5, in red) and corresponding FRET efficiency (blue)).

(**C**) FRET histogram of Nucleosome (blue) and Tetrasome (magenta) reconstituted from DNA construct in INT scheme. The FRET peak at ~0.1 corresponds to free DNA and the peak at ~ 0.95 corresponds to intact nucleosome (blue). The FRET peak of the tetrasome sample corresponds to the mixture of free DNA and tetrasome.

(**D**) Representative single molecule time traces under force free conditions at zero force. Cy3 donor (green), Cy5 acceptor (red) and corresponding FRET efficiency (blue) for the INT nucleosome.

(**E**) Averaged curves (23 traces) for FRET as a function of force during stretching and relaxation of INT tetrasomes.

Figure S2. Effect of Pulling Speed on Nucleosome Unwrapping at Higher Force, Related to Figure 2

(A) Illustration of labeling schemes in Figure 2G.

(B) The loading rate of pulling experiments at constant velocity of 233 nm/s (red, closed circle) and 455 nm/s (black, closed square). The loading rate increases monotonically as force increases. The maximum loading rate at high force is ~ 11 pN/s and 7 pN/s for two constant velocity experiments of 455 nm/s and 233 nm/s, respectively.

(C) Examples of single molecule stretching traces for ED2 construct at constant velocity of 233 nm/s.

(**D**) Averaging stretching curves for INT (I28, J28) (15 traces) and ED2 (I9, J58) (8 traces). ED2 unwraps at higher forces than INT, which was also the case at twice the pulling speed as discussed in the main text.

Figure S3. Ensuring the Correct Translational Positioning of Nucleosomes on the 601 Sequence, Related to Figure 3

(A) Illustration of labeling schemes ED1-12 and ED2-12. The acceptor dye at the -12 position on the extrachromosomal handle allows for probing for differences in translational positions of the nucleosome.

(**B and C**) Single-molecule FRET histograms for both ED1-12 and ED2-12 display similar high FRET peaks consistent with the correct translational frame for the nucleosome on the 601 sequence.

Figure S4. **Probing the Early Unwrapping Process on the 'Strong' Side, Related to Figure 4**

(A) Illustration of the ED2-12 construct which places Cy5 acceptor in the extra-nucleosomal handle of the strong side.

(**B**) Representative force-fluorescence single-molecule time traces for ED2-12 construct. At low force (≤5 pN), FRET initially decreases, but then increases again and maintains a high FRET value (between approximately 5-15pN) until it fully drops at high force (16-20 pN).

(**C**) Representative FRET-force stretching for ED2-12 curve (light grey) with overlay by averaged (21 traces) curve (magenta).

Figure S5. Stretching Nucleosomes Forming on 601 Derivative Sequences, Related to Figure 5

A: The LL8-24 construct is derived by replacing 17 nts from position 8-24 on the right (ED1) side by corresponding region on the left (ED2) side such that the sequence of the outer quarter from 8-24 nucleotides (orange) on the both sides becomes identical.

B: Looping time of the two halves of the LL8-24 construct.

C: Force-fluorescence stretching curves for LL8-24 nucleosome reconstituted with ED1 (7 traces) and ED2 (21 traces). The GC-richness of the outer regions does not affect pulling behavior of either side.

Figure S6. Nucleosome Positioning on 601 Derivative Templates with Reconstituted Labeling Scheme IJ-12, Related to Figure 6

(**A**) Cartoon of labeling scheme IJ-12 in which donor and acceptor are placed at 12 nts upstream and downstream, respectively from the 601 nucleosome positioning frame.

(**B**) Nucleosomes with IJ-12 labeling scheme reconstituted on 601, 601-MF, and 601-RTA templates migrate at identical positions on 5% Native PAGE.

(**C**) Single-molecule FRET histogram for 601, 601MF and 601RTA (IJ-12) nucleosomes display the identical FRET peak at ~0.9, indicating positioning is maintained on all three sequences.