**Supplemental Figures:**

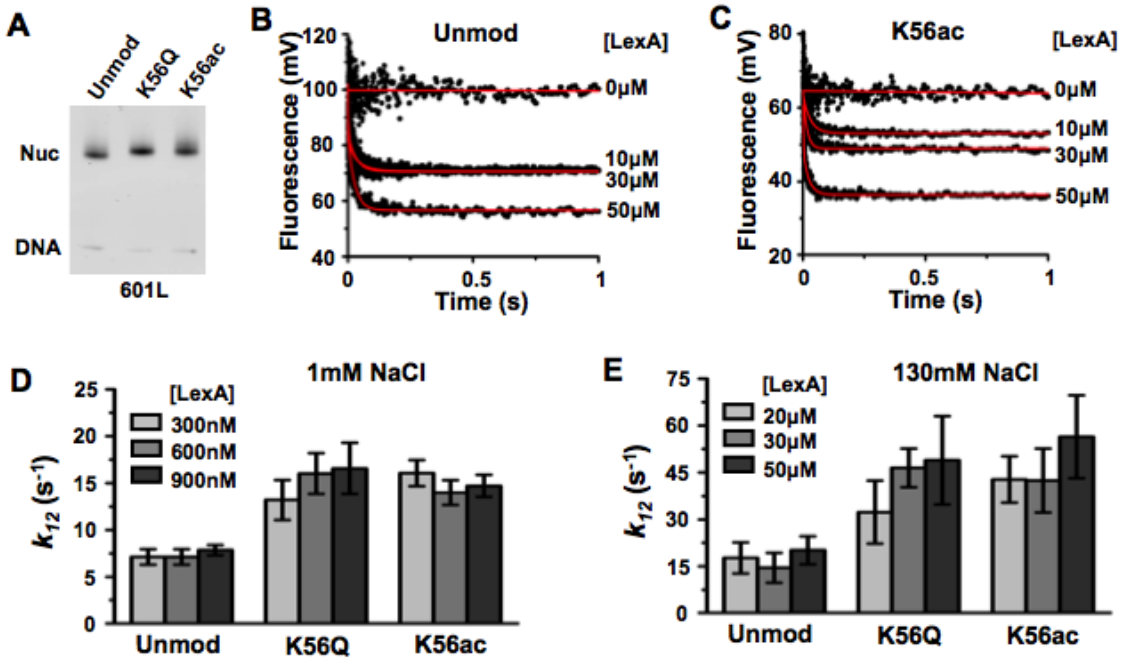


**Figure S1. Nucleosome unwrapping rate is [LexA]-independent at high-LexA concentration –** (**A**) Cy3 fluorescence image of native PAGE analysis of purified FRET-labeled nucleosomes containing the 601 NPS and unmodified H3, H3(K56Q), or H3(K56ac). (**B**) and (**C**) Stopped Flow Cy5 emission versus time of 601L nucleosomes containing unmodified H3 or H3(K56Ac) nucleosomes, respectively, at 130mM NaCl mixed with 0-50μM LexA. (**D**) and (**E**) Apparent unwrapping rate, $k_{12}^{app}$, for nucleosomes in 1mM NaCl and 130mM NaCl, respectively as a function of [LexA]. At 1mM NaCl in the presence of 300-900nM LexA and at 130mM NaCl in the presence of 20-50μM LexA, the apparent unwrapping rate is independent of [LexA], indicating that in this regime the apparent unwrapping rate is the actual nucleosome unwrapping rate, $k_{12}$.

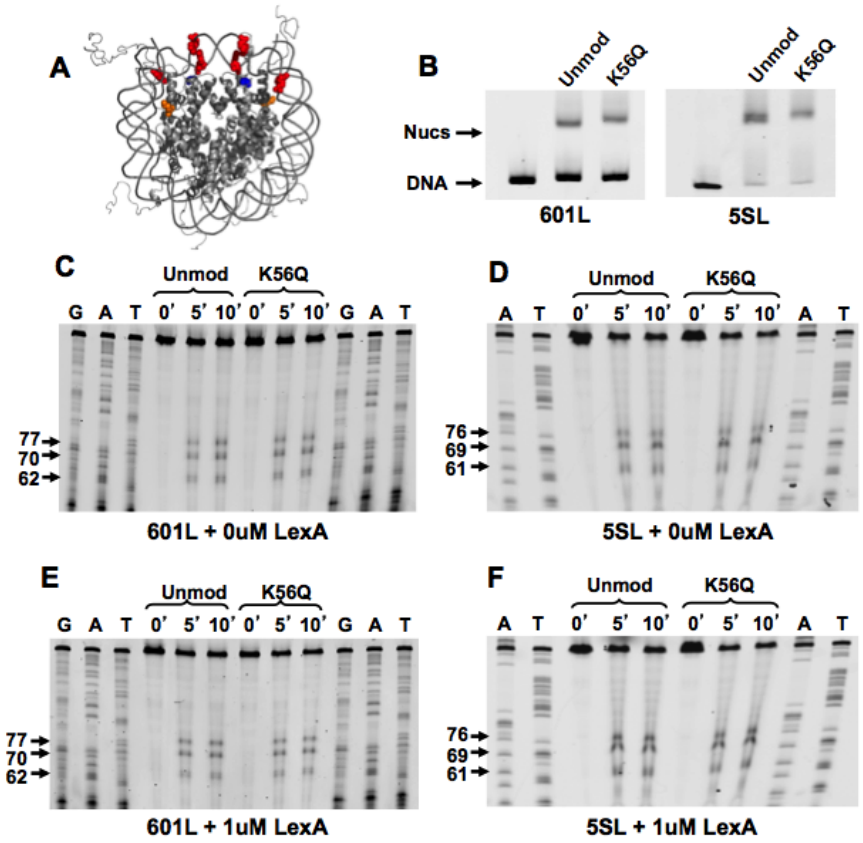


**Figure S2. Hydroxyl Radical mapping of nucleosome position.** (**A**) The crystal structure of the nucleosome[1]. H3(K56) is shown in orange. H4(S47), which is replaced with a cysteine and labeled with FeBABE, is shown in blue. The bases that are cleaved by FeBABE are shown in red. (**B**) EMSA of nucleosomes labeled with FeBABE at H4(S47C) and containing 601L (left) or 5SL (right) DNA. Lane 1 contains the DNA substrate, Lane 2 contains nucleosomes with unmodified H3 and Lane 3 contains nucleosome with H3(K56Q). (**C**) and (**D**) Cy3 image of denaturing PAGE of 601L and 5SL nucleosomal DNA, respectively, cleaved by FeBABE for 0, 5 and 10 minutes. Within each gel, lanes 1-3 and 10-12 contain sequencing tracks terminated with ddGTP,ddATP and ddTTP respectively, lanes 4-6 contain nucleosomes with unmodified H3 and lanes 7-9 contain nucleosomes with H3(K56Q). (**E**) and (**F**) Cy3 image of denaturing polyacrylimide gel electorphoresis of 601L and 5SL nucleosomal DNA, respectively, cleaved by FeBABE for 0, 5 and 10 minutes after pre-incubation with 1μM LexA. Lanes are the same as in (C) and (D). The cleavage pattern is identical to that of nucleosome in the absence of LexA, indicating that LexA binding does not reposition nucleosomes.

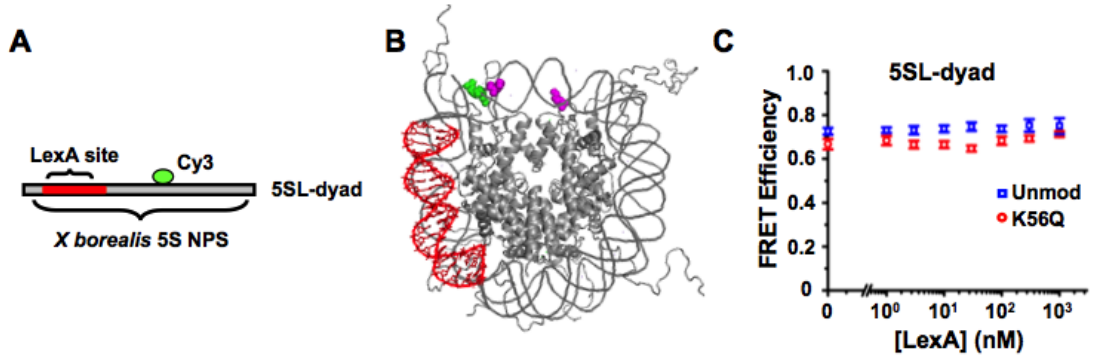


**Figure S3. Nucleosomal DNA is not repositioned upon LexA binding**. (**A**) DNA constructs for FRET measures of LexA-induced nucleosome repositioning. The 601 NPS contains a LexA protein binding site from bases 8-27 and a Cy3 molecule on the 80$^{th}$ base. (**B**) Structure of FRET-labeled nucleosome[1] containing 601L-dyad DNA; the LexA binding site in red, Cy3 in green, and Cy5 on H2A(K119C) in magenta. (**C**) Steady state FRET efficiency, as determined by the $(ratio)_A$ method[2], versus LexA concentration for nucleosomes containing unmodified H3 (blue) or H3(K56Q) (red). Plots are the average of three LexA titrations and the error bars were determined from the standard deviation of the three measurements. The FRET is constant over the range of [LexA] in which the LexA site is occupied within the nucleosome, indicating that the DNA is not repositioned by LexA binding.

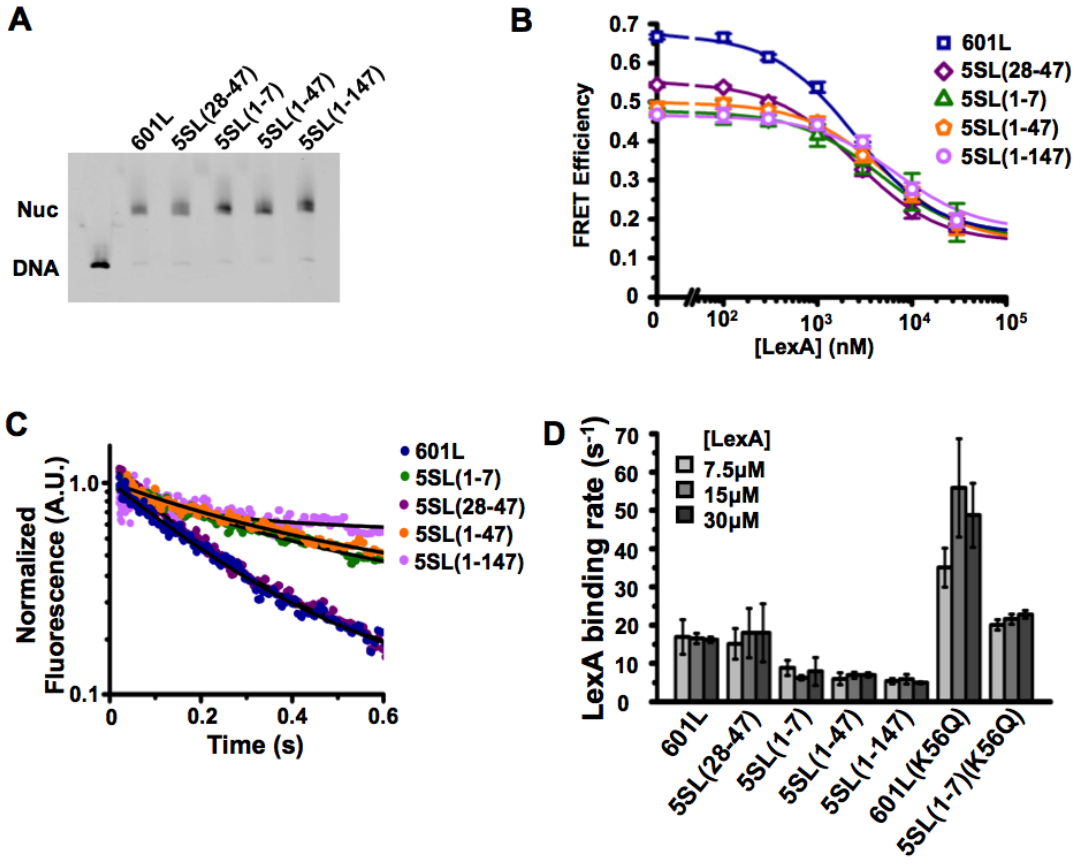


**Figure S4. DNA sequence between the nucleosome entry-exit and the TF binding site impacts nucleosome unwrapping.** (**A**) Cy3 fluorescence image of native PAGE analysis of purified FRET-labeled containing unmodified H3 and the 601L, 5SL(28-47), 5SL(1-7), 5SL(1-47), or 5SL(1-147) NPS. (**B**) Steady state FRET efficiency, as determined by the $(ratio)_A$ method[2], versus LexA concentration for nucleosomes containing unmodified H3 and 601L (blue), 5SL(28-47) (purple), 5SL(1-7) (green), 5SL(1-47) (orange), and 5SL(1-147) (violet) at 75mM NaCl. Plots are the average of three LexA titrations and the error bars were determined from the standard deviation of the three measurements. The data were fit to a non-cooperative binding curve, which determines $S_{0.5\text{-}nuc}$, the LexA concentration at which 50% of the nucleosomes are bound by LexA. (**C**) Normalized stopped flow Cy5 emission versus time at 15μM LexA for nucleosomes containing unmodified H3 and 601L (blue), 5SL(28-47) (purple), 5SL(1-7) (green), 5SL(1-47) (orange), and 5SL(1-147) (violet) at 75mM NaCl. (**D**) The LexA binding rate for nucleosomes in 75mM NaCl as a function of [LexA]. In the presence of 7.5-30μM LexA, the apparent unwrapping rate is independent of [LexA], indicating that in this regime the LexA binding rate is the equal to the nucleosome unwrapping rate, $k_{12}$.

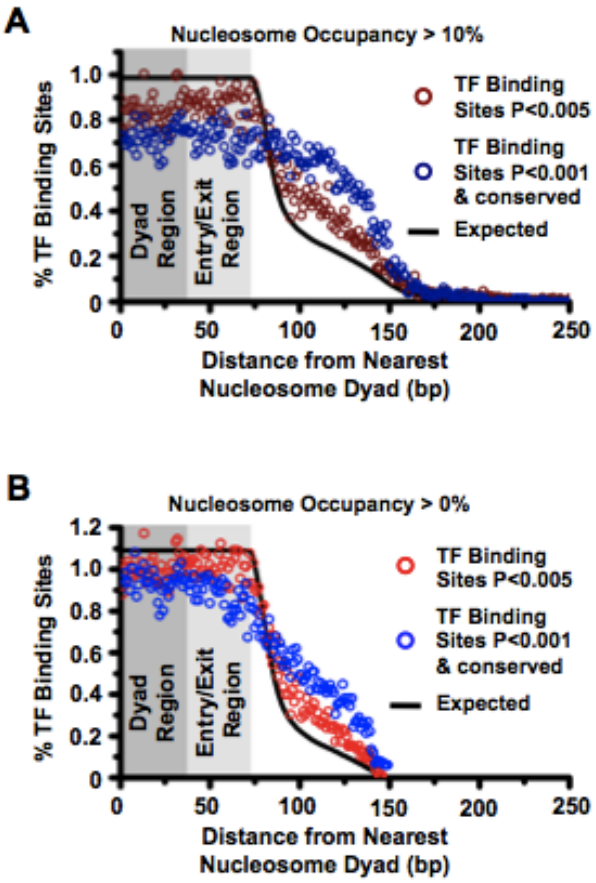


**Figure S5. Transcription factor binding site distances from nearest nucleosome dyad.** (**A**) and (**B**) Distribution of nearest nucleosome dyad distance from a TF binding site for only nucleosomes with occupancy >10% or for all nucleosomes in the consensus map of nucleosome positions reported by Jiang and Pugh[3], respectively. The black line represents the distribution of TF binding site distances to the nearest nucleosome dyad if TF binding sites are randomly distributed. Red circles represent all mapped TF binding sites with P<0.005, while blue circles represent only mapped TF binding sites with P<0.001, which are evolutionarily conserved[4]. These data sets have been normalized such that they have the same integral as the random distribution.

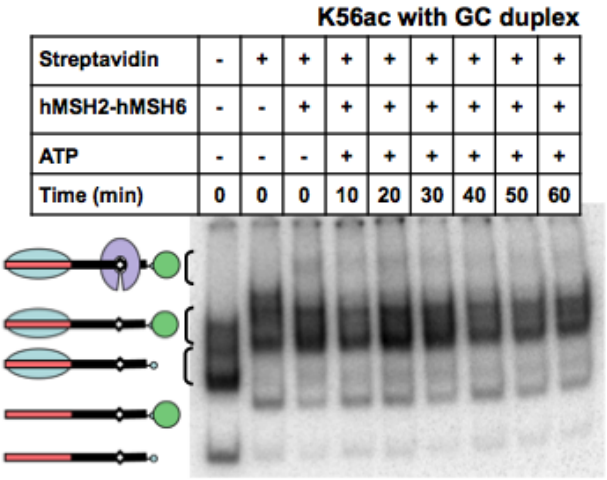


**Figure S6. Nucleosome disassembly is hMSH2-hMSH6 dependent.** Electrophoretic mobility shift analysis of H3(K56ac) nucleosomes with CG duplex incubated with hMSH2-hMSH6. Lane 1: sucrose gradient purified nucleosomes, Lane 2: nucleosomes bound by streptavidin, Lane 3: nucleosomes bound by streptavidin and hMSH2-hMSH6, Lanes 4 – 9: kinetic analysis of streptavidin-bound nucleosome disassembly by hMSH2-hMSH6 in the presence of 1 mM ATP. No disassembly is observed, indicating nucleosome disassembly is hMSH2-hMSH6 dependent.

| DNA | Primer | Sequence |
|---|---|---|
| 601 | 601-F* | Cy3-CTGGAGAATCCCGGTGCCGA |
| | 601-R | ACAGGATGTATATATCTGACACGTGCCTGGAGACTA |
| 601L | 601L-F* | Cy3-CTGGAGATACTGTATGAGCATACAGTACAATTGGTC |
| | 601-R | ACAGGATGTATATATCTGACACGTGCCTGGAGACTA |
| 601L-dyad | 601-F | CTGGAGATACTGTATGAGCATACAGTACAATTGGTC |
| | 601R* | ACAGGATGTATATATCTGACACGTGCCTGGAGACTAGGGAGT AATCCCCTTGGCGGTTAAAACGCGG(T-Cy3)GGACA |
| 5S | 5S-F | Cy3-GTACTAACCAGGCCCGACCCTGC |
| | 5S-R | CTGGCATGGGGAGGAGCTGG |
| 5SL | 5SL-F* | Cy3-GTACTAATACTGTATGAGCATACAGTACTTCCG |
| | 5S-R | CTGGCATGGGGAGGAGCTGG |
| 5SL-dyad | 5SL-F | GTACTAATACTGTATGAGCATACAGTACTTCCG |
| | 5S-R* | CTGGCATGGGGAGGAGCTGGGCCCCCCCCAGAAGGCAGCAC AAGGGGAGGAAAAGTCAGCCTTGTGC(T-Cy3)CGCC |
| 5SL(1-7) | 5SL(1-7)-F* | Cy3-GTACTAATACTGTATGAGCATACAGTACAATTGGTCG |
| | 601-R | ACAGGATGTATATATCTGACACGTGCCTGGAGACTA |
| 5SL(28-47) | 601L(1-7)-F* | Cy3-CTGGAGATACTGTATGAGCATACAGTACTTCCGAGAT |
| | 601-R | ACAGGATGTATATATCTGACACGTGCCTGGAGACTA |
| 5SL(1-47) | 5SL-F* | Cy3-GTACTAATACTGTATGAGCATACAGTACTTCCG |
| | 601-R | ACAGGATGTATATATCTGACACGTGCCTGGAGACTA |
| 5S-*Sv* | 5S-*Sv*-F* | Cy3-TTCCAGGGATTTATAAGCCGATGACGTCAT |
| | 5S-Sv-R | AACCGAGCCCTATGCTGCTTG |

**Table S1.** List of DNA oligos used for preparing the nucleosome positioning sequences. The 601 sequence is in blue; the *X. borealis* 5S sequence is in pink; the *L. variegatus* 5S sequence is in black; the LexA target sequence is in red.

References


1. Richmond, T.J. & Davey, C.A. The structure of DNA in the nucleosome core. *Nature* **423**, 145-50 (2003).

2. Clegg, R.M. Fluorescence resonance energy transfer and nucleic acids. *Methods Enzymol* **211**, 353-88 (1992).

3. Jiang, C. & Pugh, B.F. A compiled and systematic reference map of nucleosome positions across the Saccharomyces cerevisiae genome. *Genome Biol* **10**, R109 (2009).

4. MacIsaac, K.D. et al. An improved map of conserved regulatory sites for Saccharomyces cerevisiae. *BMC bioinformatics* **7**, 113 (2006).