SUPPLEMENTARY MATERIAL

ATP-dependent Nucleosome Unwrapping Catalyzed by human RAD51

Justin A. North, Ravindra Amunugama, Marcelina Klajner, Aaron N. Bruns, Michael G. Poirier and Richard Fishel

Supplemental Table 1: PCR Primers

601D forward:	CTGGAGAATCCCGGTGCCGA
601D reverse:	ACAGGATGTATATATCTGACACGTGCCTGGAGACTAGGG AGTAATCCCCTTGGCGGTTAAAACGCGG(T-Cy3)GGACA
601E forward:	Cy3-CTGGAGAATCCCGGTGCCGA
601E reverse:	ACAGGATGTATATATCTGACACGTGCCTGGAGACTA
5SD forward:	GTACTAATACTGTATGAGCATACAGTACTTCCG
5SD reverse:	CTGGCATGGGGAGGAGCTGGGCCCCCCCCCAGAAGGCAGCA CAAGGGGAGGAAAAGTCAGCCTTGTGC(TCy3)CGC
5SE forward:	Cy3-GTACTAATACTGTATGAGCATACAGTACTTCCG
5SE reverse:	CTGGCATGGGGAGGAGCTGG
601-linker forward:	CTGGAGAATCCCGGTGCCGAGGC
601D-linker reverse:	GCACCCCACTGTCTATACACAGGATGTATATATCTGACACGTG CCTGGAGACTAGGGAGTAATCCCCTTGGCGGTTAAAACGCGG(T-Cy3)GG
601E-linker reverse:	TCGCCTCCGCACCCCACTGTCTATACACA(T-Cy3)GATGTATA
linker forward:	CCTGTGTATAGACAGTGGGGTGCGGAGGCG
linker reverse:	CACGTGTGGGGGCCCGAGCTCGCGGC

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Assembly of Nucleosomes. A) Agarose gel analysis of assembled nucleosomes using 601E, 601D, 5SE, 5SD, 601E-linker, and 601D-linker nucleosome positioning DNAs (see Fig. 1A). Nucleosome (Nuc) mobility and free DNA (DNA) mobility are marked on the left. **B)** Crystal structure of a nucleosome (Lugar et al., *Nature* 389:6648, 1997) showing the relative positions of the entry-exit (601E, left) and dyad (601D, right) Cy3 fluorophore relative to the Cy5 fluorophore linked to H2A(K119C) within the histone octamer.

Supplementary Figure 2. Nucleosome disassembly by HsRAD51(D316K). A) Representative fluorescence emission spectra of 601E-linker nucleosomes (2 nM) excited at 510nM in the presence 130mM KCl, ATP (250 μM) and 2mM $MgCl_2$ (Mg^{+2}) with HsRAD51(D316K) at 0nM (black), 100nM (blue), 200nM (green), 500nM (orange) and 900nM (purple). **D)** Representative fluorescence emission spectra of 601D-linker nucleosomes (2 nM) excited at 510 nM in the presence 130 mM KCl, ATP (250 μM) and 2 mM $CaCl_2$ (Ca^{+2}) with HsRAD51 at 0 nM (black), 100 nM (blue), 200 nM (green), 500nM (orange) and 900nM (purple).

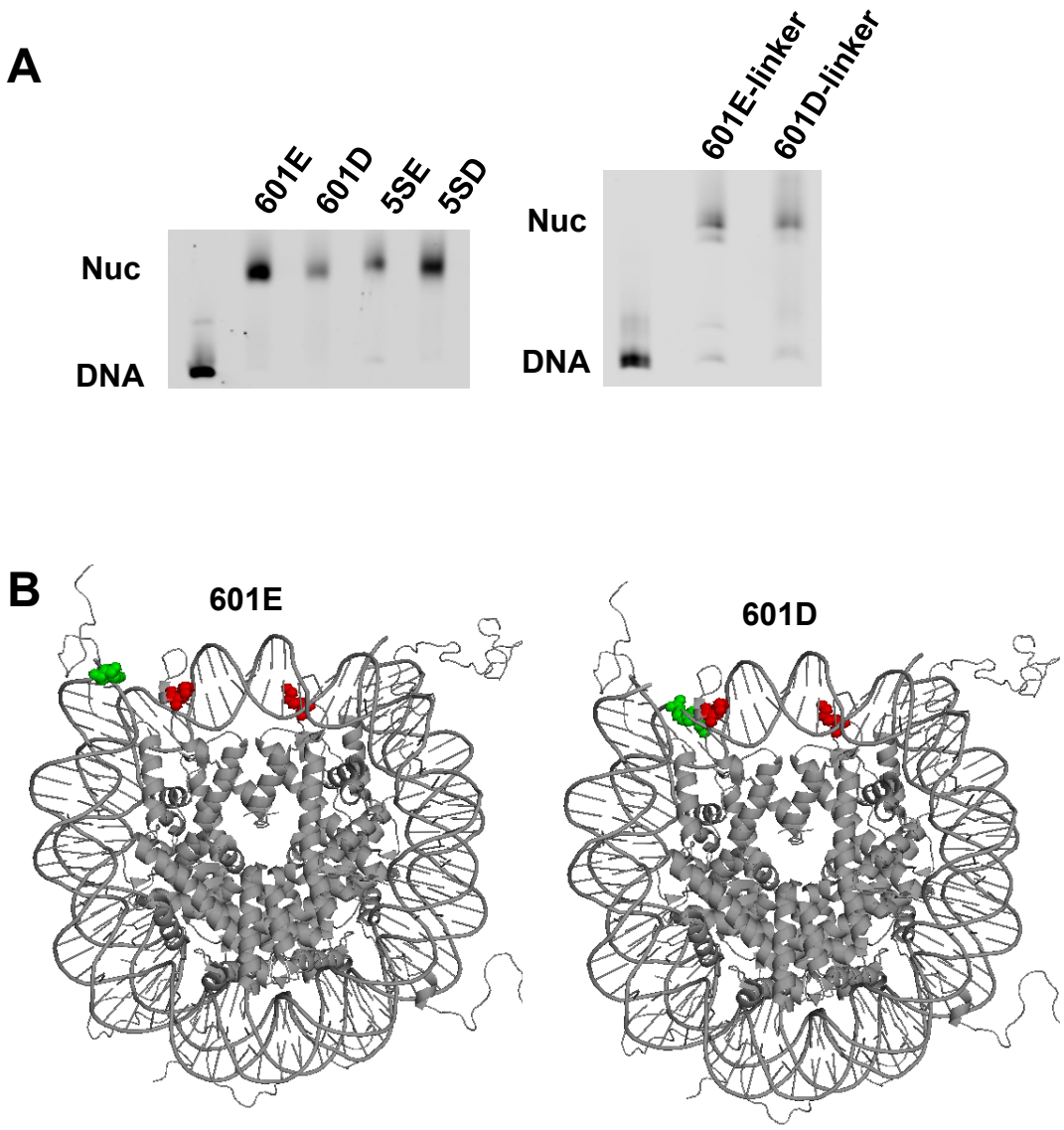
A
601E
601D
5SE
5SD
Nuc
DNA
601E-linker
601D-linker
Nuc
DNA
B
601E
601D

Figure S1

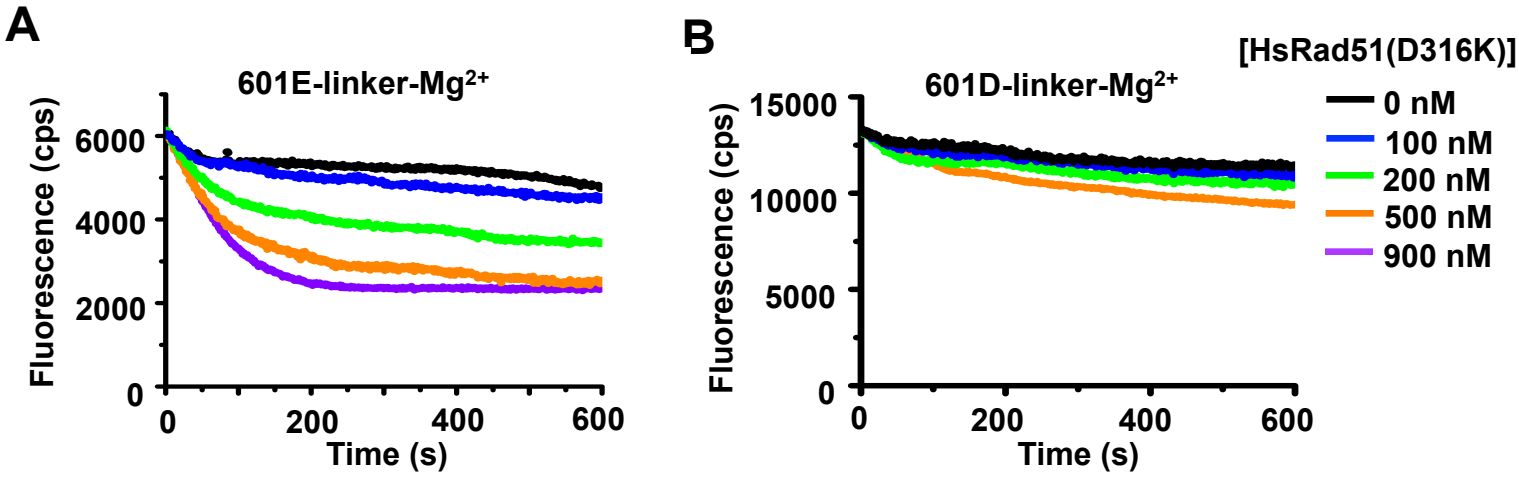
A
601E-linker-Mg2+
Fluorescence (cps)
6000
4000
2000
0
0
200
400
600
Time (s)
B
601D-linker-Mg2+
Fluorescence (cps)
15000
10000
5000
0
0
200
400
600
Time (s)
[HsRad51(D316K)]
0 nM
100 nM
200 nM
500 nM
900 nM

Figure S2