Supporting Information

Cannon et al. 10.1073/pnas.1309816110

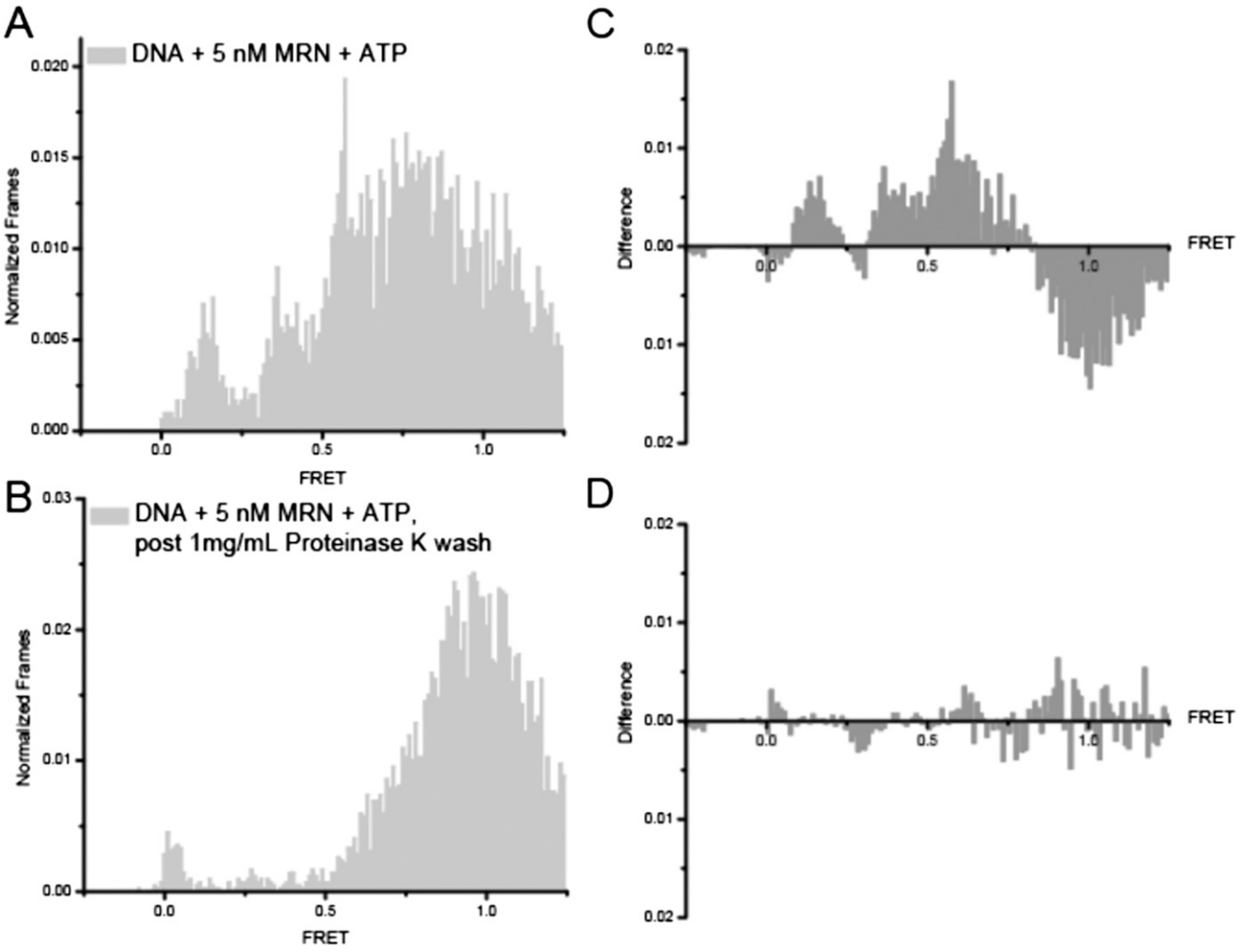

Fig. S1. Proteinase K treatment restores a high-FRET state for duplex DNA after Mre11/Rad50/Nbs1 (MRN) binding. (*A*) FRET histogram for the fully base-paired DNA duplex in the presence of 5 nM MRN and ATP, as in Fig. 1*E*. (*B*) After incubation with MRN, the duplex was further incubated with proteinase K (1 mg/mL) for 30 min. The FRET histogram was restored to the pre-MRN distribution, indicating that duplex unwinding by MRN is reversible and that MRN does not degrade or irreversibly change the DNA. (*C*) Difference plot showing the FRET values of the DNA duplex subtracted from the FRET values of the DNA with MRN, as in Fig. 1*G*. (*D*) Difference plot as in Fig. 1*E*, but with the FRET values of the DNA duplex subtracted from the FRET values of the DNA duplex incubated first with MRN and then with proteinase K.

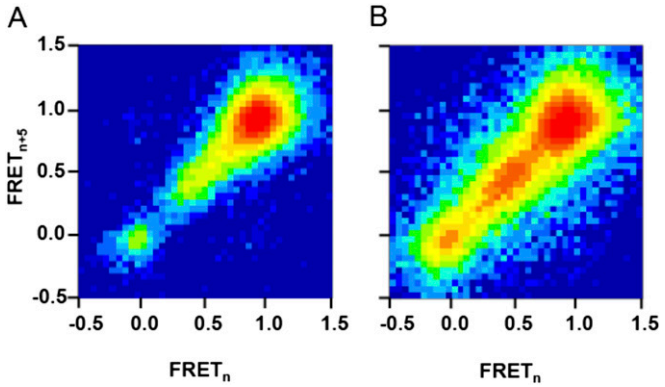

Fig. S2. Transition density plots do not show transitions between discrete states. Transition density plots are shown for DNA (N_{mol} = 403) (*A*) and DNA + WT (MRN) with ATP (N_{mol} = 554) (*B*). These plots show how the FRET value changes between the *n*th frame and the *n* + 5 frame. The time difference between the *n*th and *n* + 5 frames was 250 ms. A five-frame offset was selected to eliminate any suppression of off-axis populations in the plot owing to the three-point data smoothing.

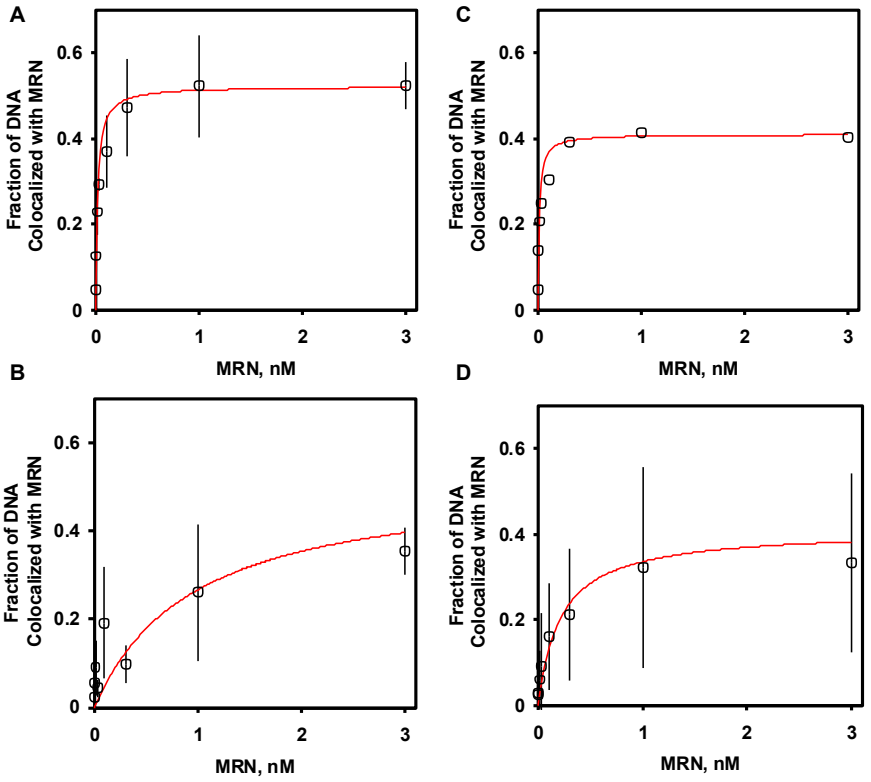

Fig. S3. Binding curves for MRN to immobilized DNA substrates. The fraction of immobilized Cy5-labeled DNA substrate that colocalized with mOrange-MRN was measured at each MRN concentration. (*A* and *B*) Binding of mOrange-MRN to immobilized duplex Cy5-labeled DNA in the presence of ATP (*n* = 3) (*A*) and absence of ATP (*n* = 3) (*B*). (*C* and *D*) Binding of mOrange-MRN to immobilized 15-nt frayed duplex labeled with Cy5 in the presence of ATP (*n* = 1) (*C*) and absence of ATP (*n* = 2) (*D*). The open symbols and error bars represent the mean and SD of the indicated number of experiments (*n*). The red lines are hyperbolic fits to the data that were weighted by the SDs of the data points.

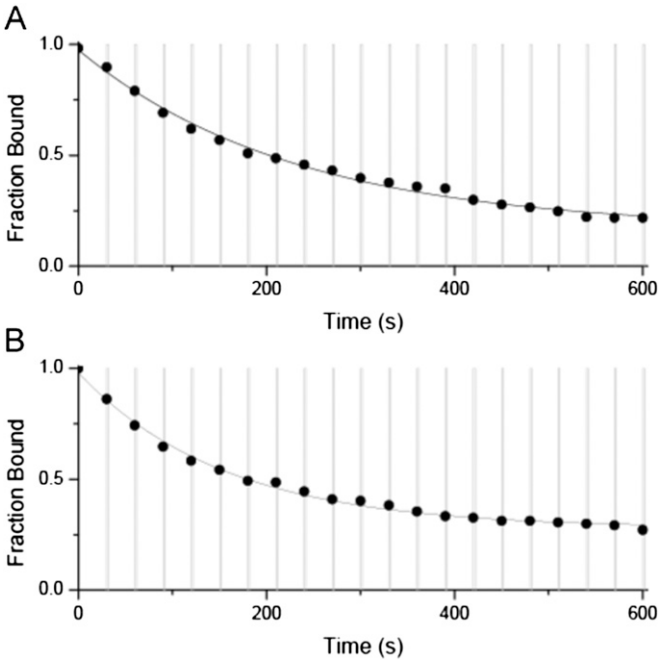

Fig. S4. MRN dissociation from duplex DNA. (*A*) Dissociation of mOrange-MRN from immobilized Cy5-labeled DNA for a single field of view using pulsed-laser excitation. The data were fit by a single exponential function, giving a dissociation rate of 4.3×10^{-3} s^{-1}. (*B*) Dissociation of Cy5-labeled DNA from mOrange-MRN immobilized through a 6X histidine tag to the surface of Cu-chelated quartz slides. The rate of dissociation was 6.4×10^{-3} s^{-1}. The data were collected from a single field of view using the pulsed-laser excitation method. The gray bars indicate the time and duration (2 s) of the pulses.

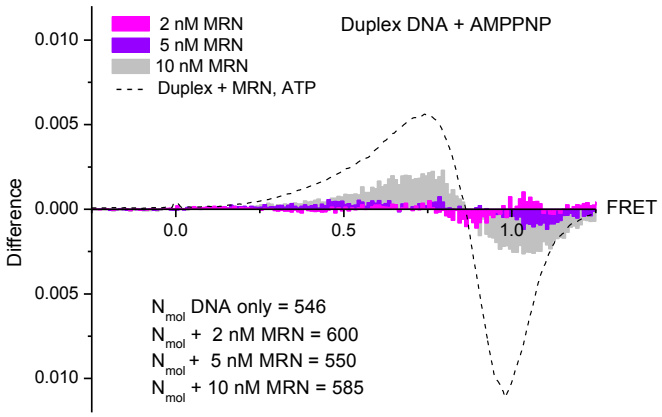

Fig. S5. MRN does not induce DNA end unwinding in the presence of AMP-PNP. Difference plot showing a histogram for the DNA duplex in the absence of MRN subtracted from data in the presence of various concentrations of MRN, performed as in Fig. 1*A* but with AMP-PNP instead of ATP.

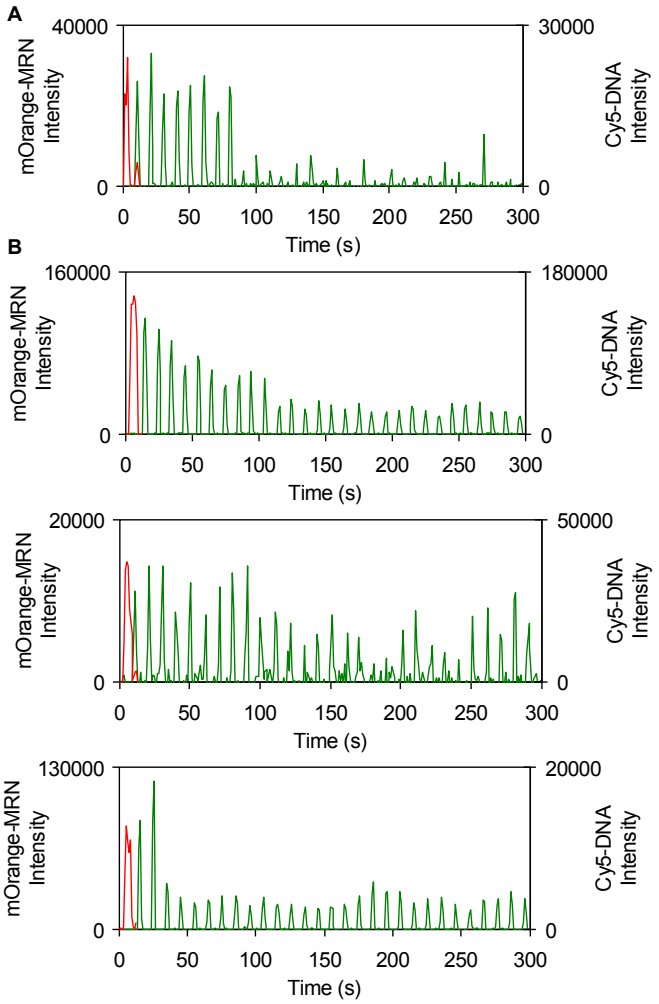

Fig. S6. Representative pulsed-excitation time traces for mOrange-MRN. The time traces for DNA-colocalized, mOrange-MRN exhibited single-step (*A*) and multistep (*B*) decay in fluorescence resulting from photobleaching. The presence of multistep photobleaching suggests the presence of multiple MRN molecules associated with a single DNA molecule. The green and red lines correspond to mOrange-MRN and Cy5 fluorescence, respectively. The pulse frequency was 0.1 Hz, with a duration of 1 s.

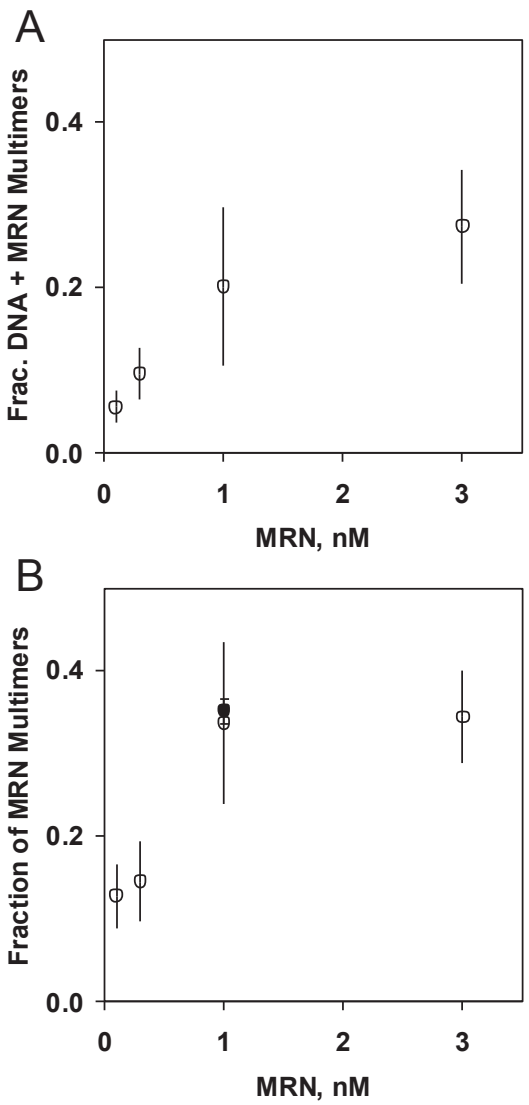

Fig. S7. MRN oligomers bind to DNA and increase with increasing MRN concentration. (*A*) The fraction of immobilized Cy5-labeled duplex DNA showing co-localization with mOrange-MRN oligomers, based on multistep photobleaching, in the presence of 5 mM Mg^{2+} and 1 mM ATP. (*B*) The fraction of DNA-bound mOrange MRN that was present as MRN oligomers (open circles) and the fraction of mOrange MRN protein that was oligomeric when the protein was immobilized to the surface of copper-chelated slides in the absence of DNA (filled circle; one data point at 1 nM). MRN oligomers were identified by the presence of multistep photobleaching in the time traces.