Supporting Information

Lamboy et al. 10.1073/pnas.1102226108

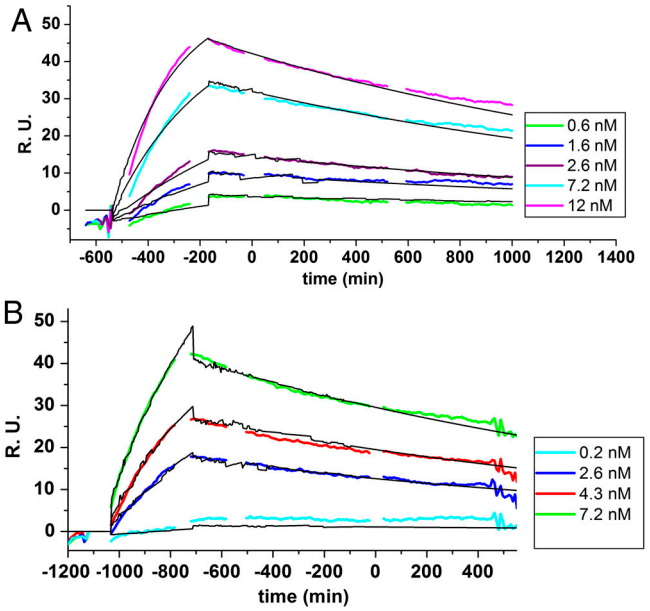

Fig. S1. SPR binding experiment in which a biotinylated NFκB(RelA:p50) was immobilized on a streptavidin chip, and the IκBα was bound. (*A*) Unlabeled IκBα containing the seven cysteines mutated to serine and two new cysteines introduced at 128 and 262. The kinetic constants were as follows: $k_a 3.2 \times 10^5$ M^{-1} s^{-1}, $k_d 5 \times 10^{-4}$ s^{-1}, R_{max} 64, $K_D 1.5 \times 10^{-9}$ M. (*B*) The same protein as in *A* but labeled with Alexa 555 and 647. The kinetic constants were as follows: $k_a 2.5 \times 10^5$ M^{-1} s^{-1}, $k_d 4.5 \times 10^{-4}$ s^{-1}, R_{max} 101, $K_D 1.9 \times 10^{-9}$ M.

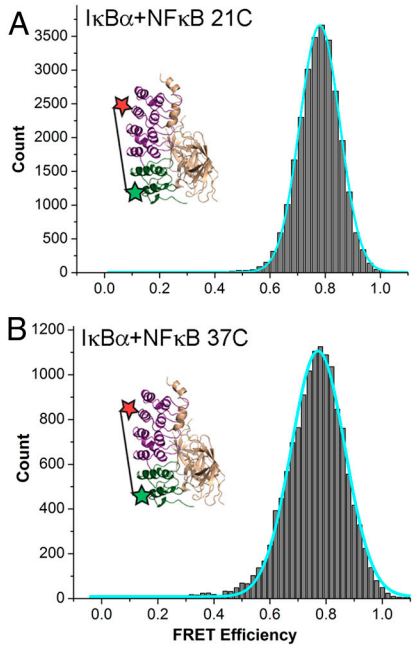

Fig. S2. Comparison of the single molecule FRET histograms of NFκB-bound IκBα at 21 °C (*A*) and 37 °C (*B*). The data for 37 °C is reproduced from Fig. 4*A*.

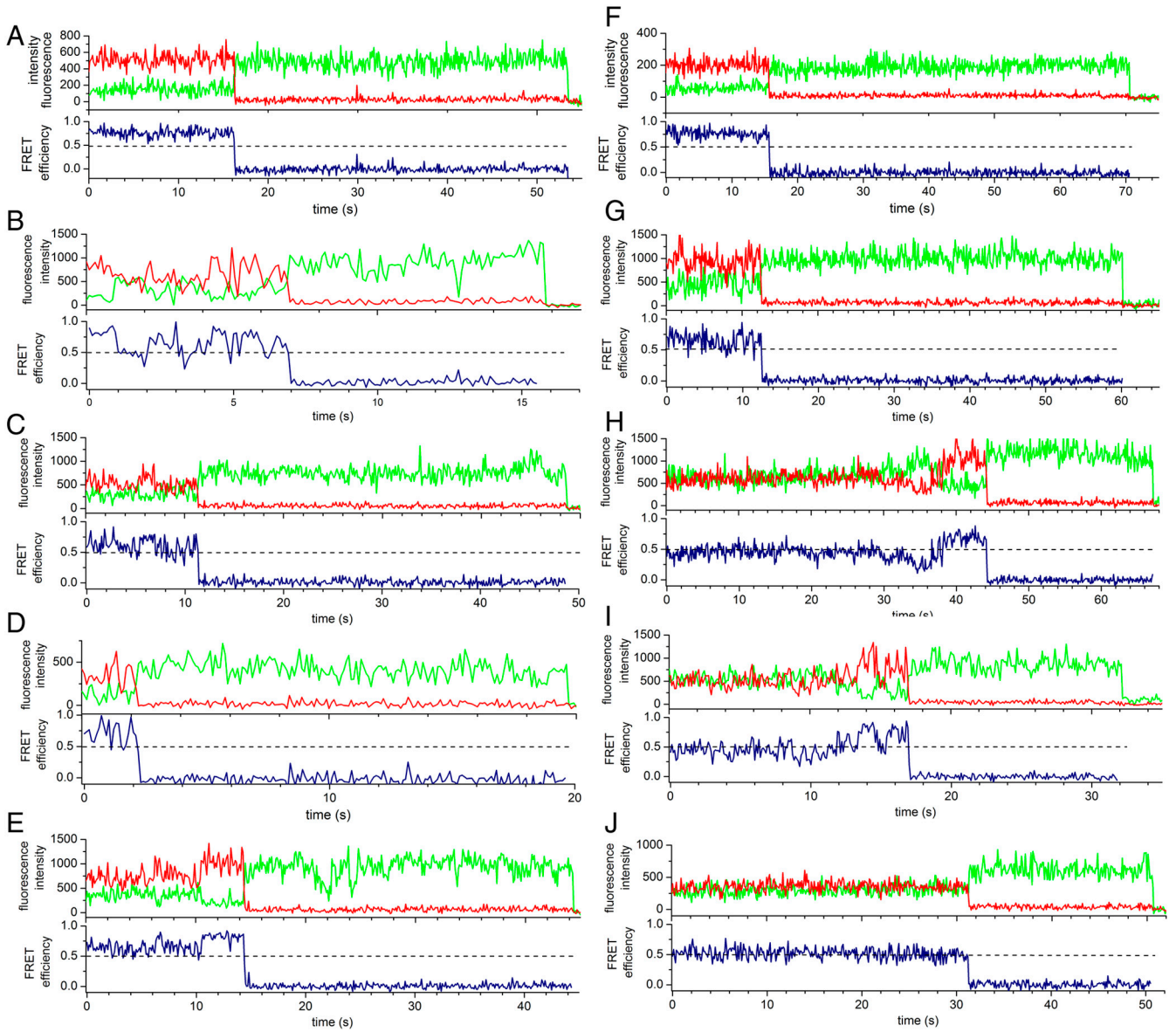

Fig. S3. Sample traces of N-His (*A–E*) and C-His (*F–J*) IκBα. Traces *A* and *F* represent the most prevalent traces in which the molecules have stable high FRET. The rest of the traces are examples of some of the many different types of fluctuating traces that were observed. Trace *J* is an example of a stable mid-FRET trace. Both the N-His and the C-His IκBα had some molecules with stable mid FRET.

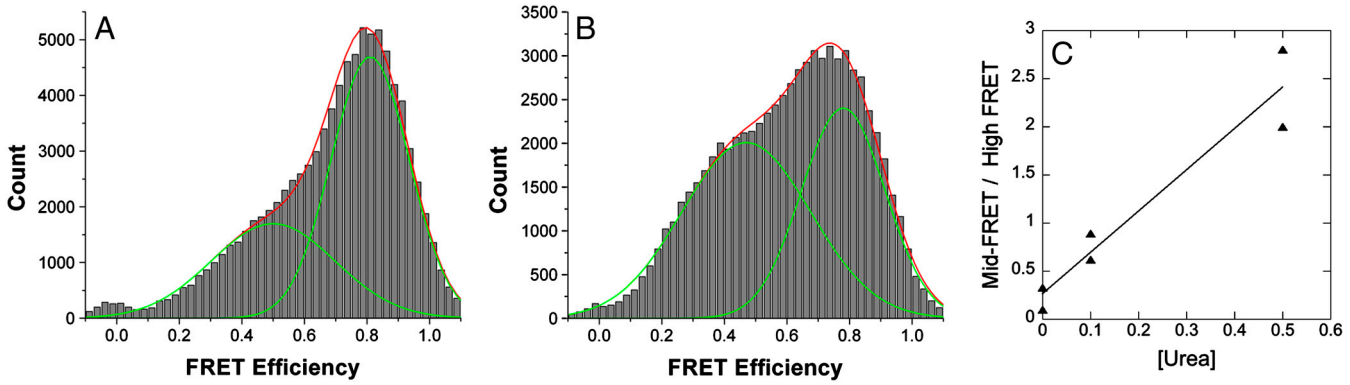

Fig. S4. Single molecule FRET histogram of YLTA IκBα in 0.1 M (*A*) and 0.5 M urea (*B*). The YLTA mutant demonstrated similar destabilization of ARs 5–6 as wild-type IκBα under the same urea concentrations. (*c*) Plot of the ratio of mid-FRET peak area to high-FRET peak area as a function of urea concentration for both wild type and the YLTA mutant.