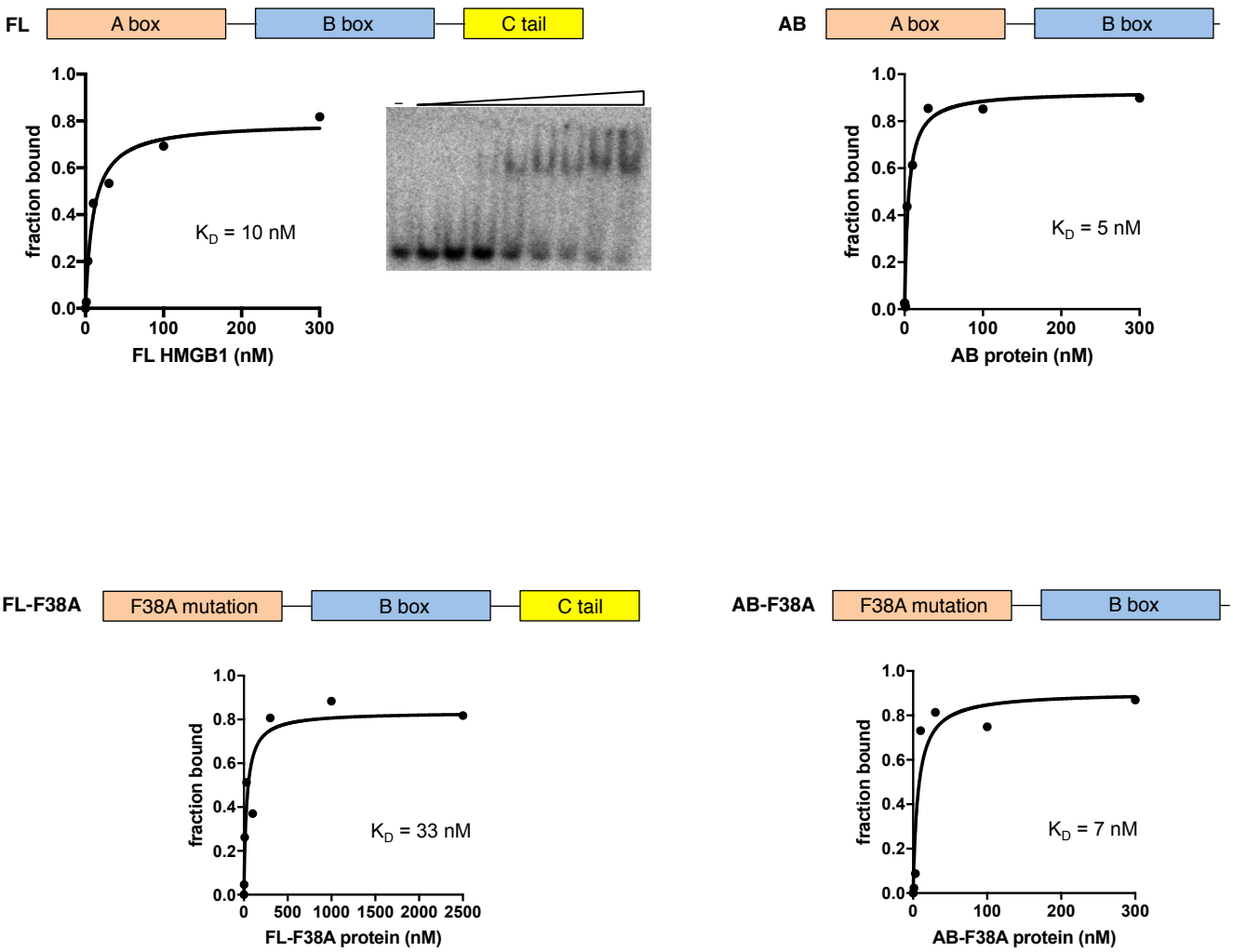
Supplemental Figure



Supplemental Figure. Determining the DNA binding affinities for HMGB1 proteins that contain two HMG boxes. EMSAs (electrophoretic mobility shift assays) were performed and quantified as described below. For FL protein, AB, and AB-F38A the protein titrations were: 0, 0.1, 0.3, 1, 3, 10, 30, 100, 300 nM. For FL-F38A the protein titration was: 0, 1, 3, 10, 30, 100, 300, 2500 nM. A representative gel image is shown for the FL protein.

Supplemental Method. Binding reactions were set up as follows. ^{32}P-labeled 18 bp dsDNA (0.1 nM) was incubated with protein (at the concentrations indicated in the Figure legend) in 10% (v/v) glycerol, 25 mM Tris (pH 7.9), 50 mM KCl, 1 mM DTT, 0.05 mg/mL BSA, 5 mM $MgCl_2$, and 0.1% NP-40. Incubation was at room temperature for 45 min. Reactions were loaded on a 7% native polyacrylamide (29:1 acrylamide:bis ratio) gel containing 0.33X TBE. The gel had been pre-run at 125 V for 45 min; after samples were loaded the gel ran for and additional 45 min at 125 V. The gels were dried, visualized using phosphorimagery, and the bands were quantified using ImageJ software. The fraction of DNA bound at each concentration of protein was calculated and plotted. The data were fit with a single site binding equation using Prism to obtain K_D values.