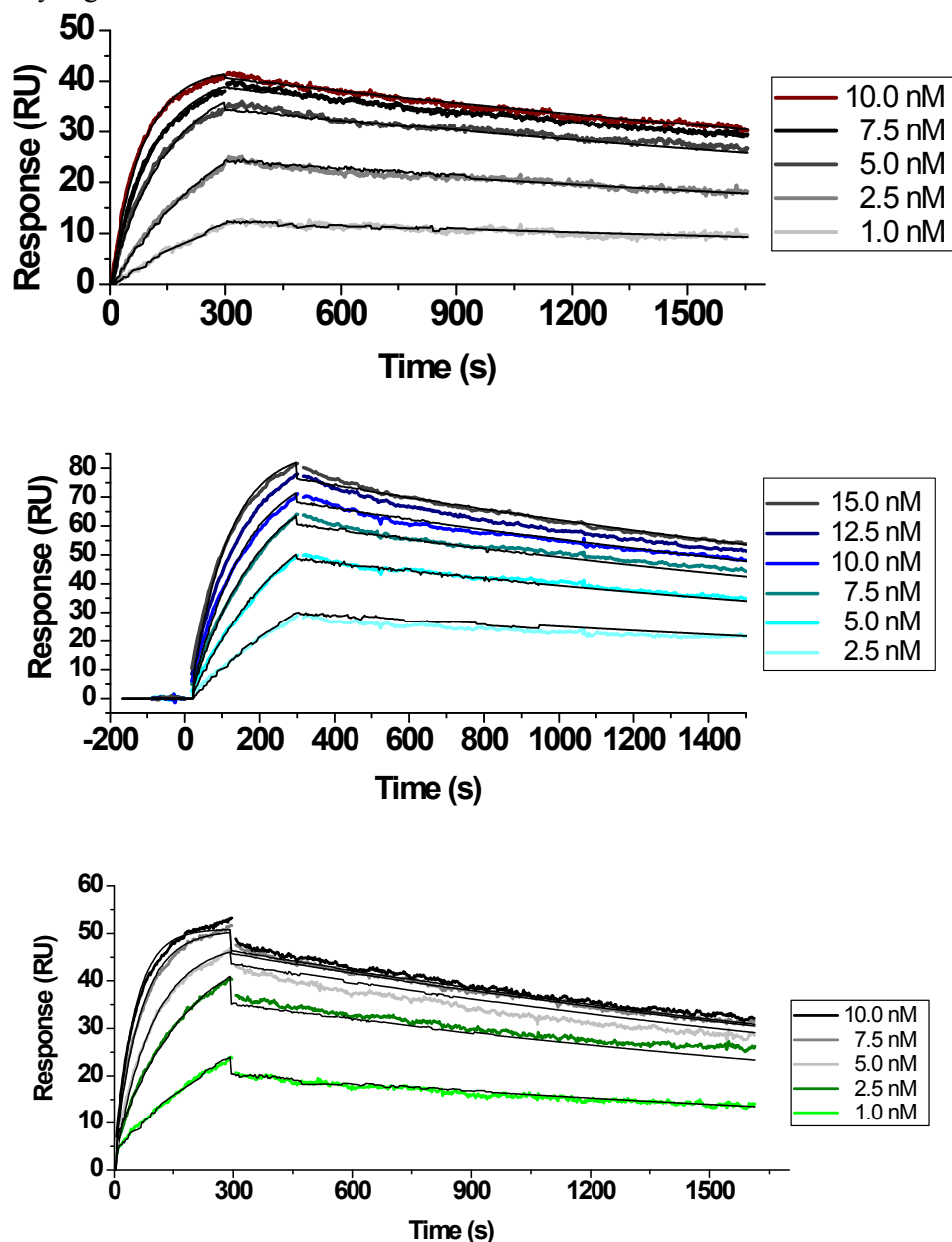


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Supplementary Figure 1

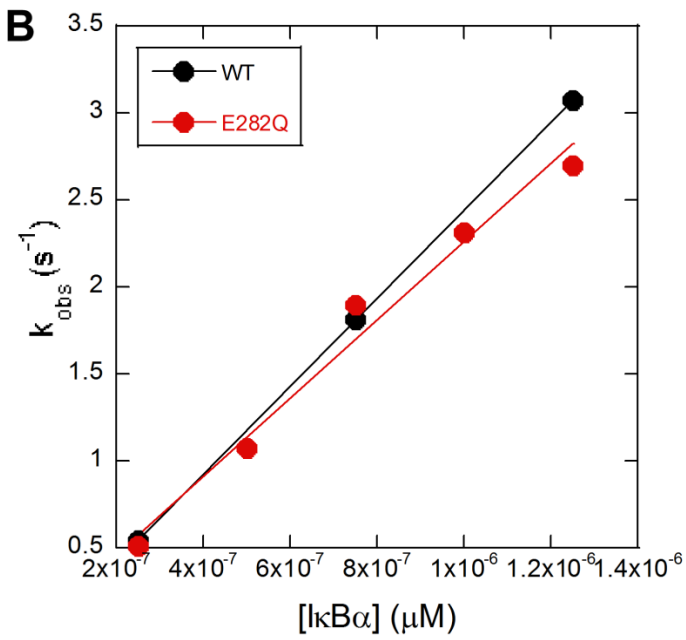
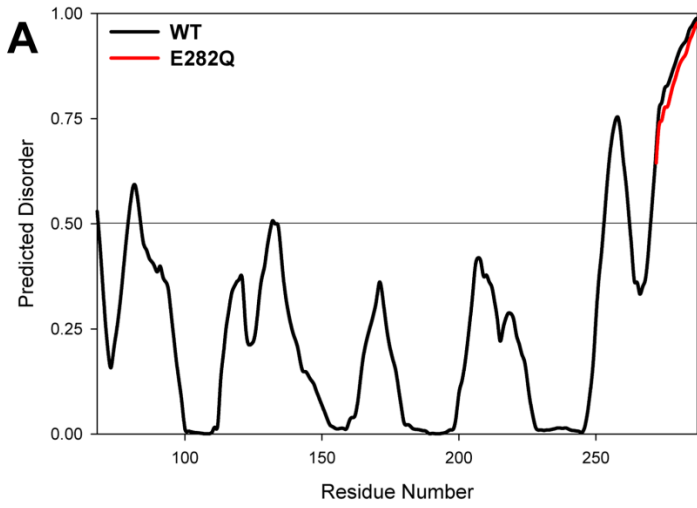


Supplementary Figure 1. SPR traces for binding of the various D→O mutants to NFκB. The binding constants derived from these plots are given in Supplementary Table 1. The traces are colored according to the scheme used throughout the manuscript with wild type (black), the P261F mutant (blue) and the E282W mutant (green).

Supplementary Table 1.

| SPR | | | | | | | | | |
|-------------|-----------|---------|---------|-----------|---------|---------|-----------|---------|---------|
| Data | ka | | | kd | | | KD | | |
| | Average | Stdev | % Error | Average | Stdev | % Error | Average | Stdev | % Error |
| P261F | 6.9E+05 | 3.4E+04 | 4.9 | 3.2E-04 | 1.4E-05 | 4.5 | 4.6E-10 | 8.2E-12 | 1.8 |
| E282W | 2.0E+06 | 1.0E+05 | 5.1 | 3.0E-04 | 7.2E-06 | 2.4 | 1.6E-10 | 1.4E-11 | 9.2 |
| WT | 1.2E+06 | 3.1E+04 | 2.5 | 2.1E-04 | 6.7E-06 | 3.2 | 1.7E-10 | 4.0E-12 | 2.4 |
| E282Q | 5.5E+06 | 6.2E+05 | 11.2 | 5.0E-04 | 2.0E-05 | 4.0 | 9.2E-11 | 1.2E-11 | 13.4 |

Supplementary Figure 2



Supplementary Figure 2. Control studies were performed with the E282Q mutant of $I\kappa B\alpha$. A) This mutation was not predicted to impart order. B) the E282Q mutant had a second order rate constant for dissociating NF κ B from DNA that was equivalent to that of the wild type $I\kappa B\alpha$.