Supporting Information

Insights into the NF-kappaB-DNA interaction through NMR spectroscopy

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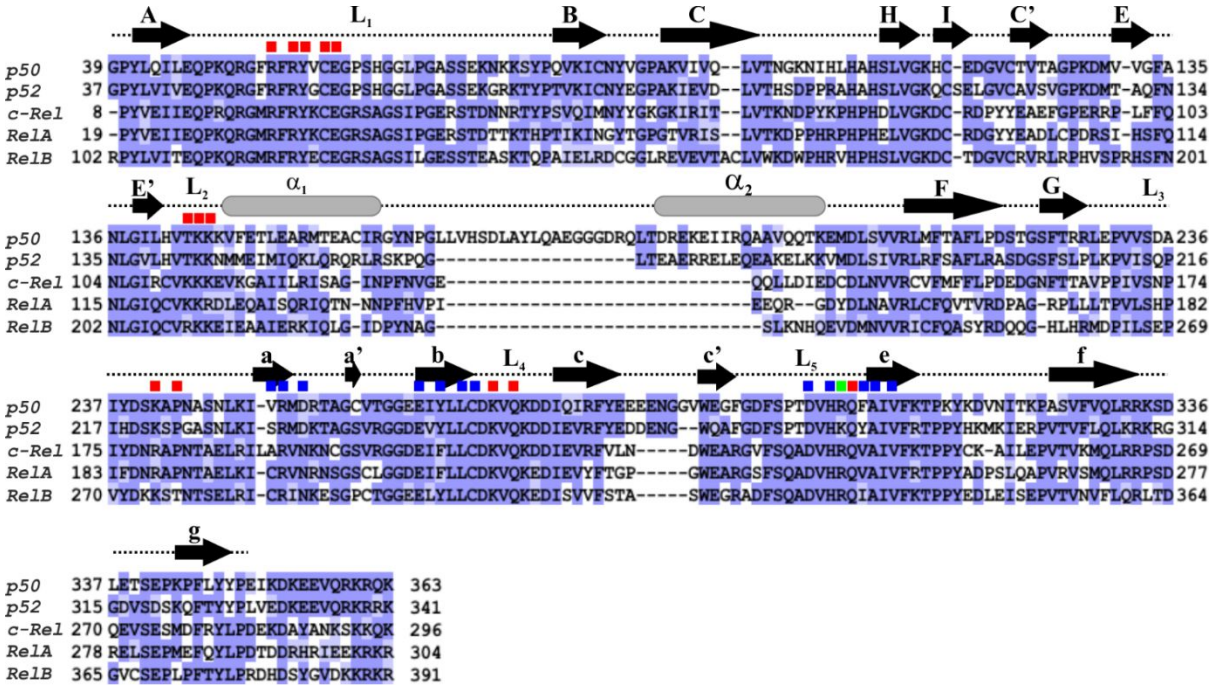


Figure S1. The NF-κB members share high sequence identity except for an additional loop present in p50 and p52 subunits. Sequence alignment of NF-κB members (mouse) using Jalview Version 2 software [1]. β-strand and α-helix are denoted by black arrow and grey horizontal cylinders, respectively, over the sequence. The DNA-contacting, dimer interfacial and both are marked with red, blue and green colored squares, respectively. The nomenclature of the secondary structure elements and loops are same as by Ghosh *et al* [2].

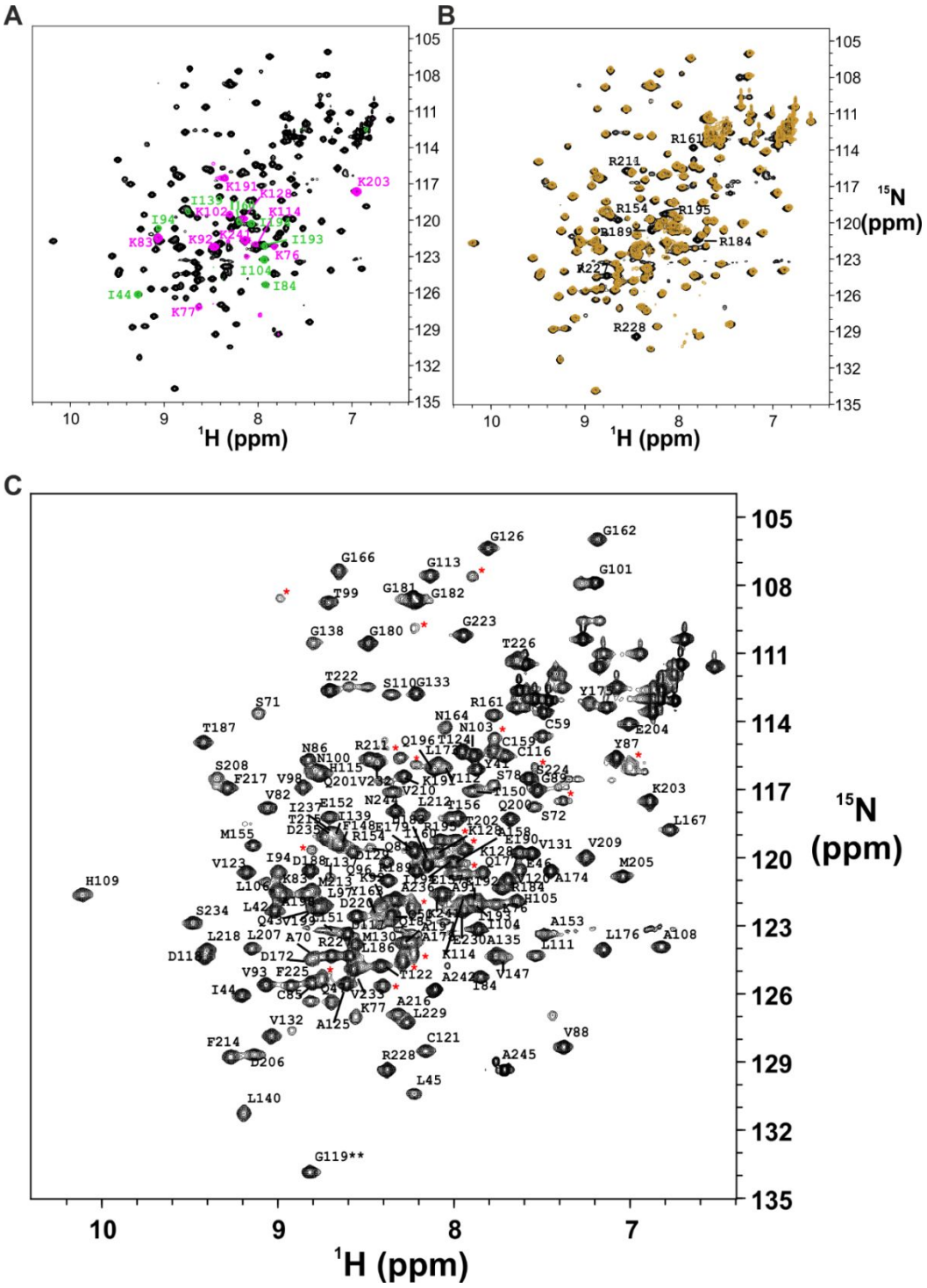
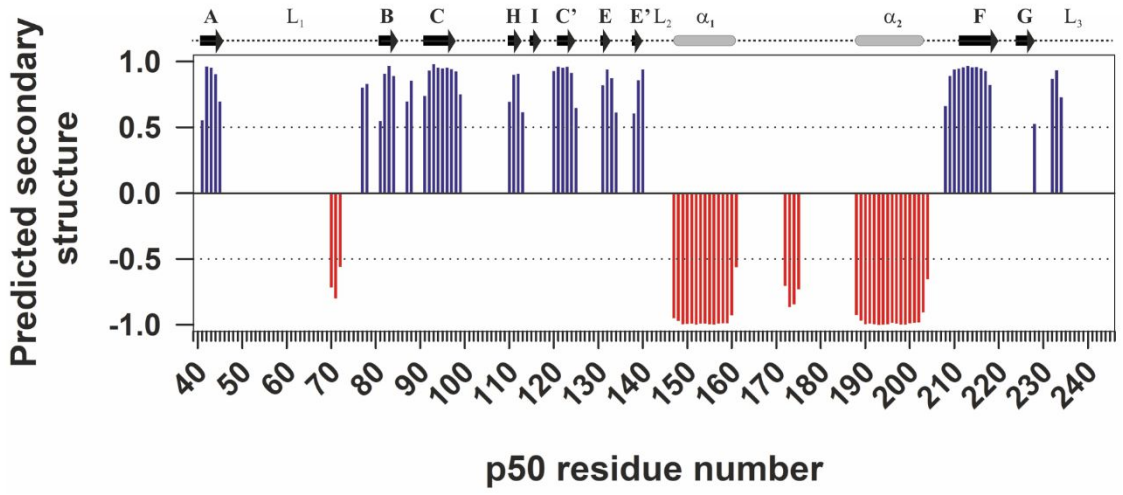


Figure S2. Specific labeling/unlabeling of amino acids improved the resonance assignment of p50-NTD. A. 2D [^{15}N-^{1}H]-HSQC spectrum of the uniformly-^{15}N-labeled (black) N-terminal DNA binding domain of p50 subunit of NF-κB (p50NTD) at 298 K superimposed on ^{15}N-Lys-labeled (pink) and ^{15}N-Ile-labeled (green) p50NTD. **B.** 2D [^{15}N-^{1}H]-HSQC spectrum of the u-[^{15}N-labeled] p50NTD (black) at 298 K superimposed by u-[^{15}N-labeled plus Arg-unlabeled] p50NTD (golden yellow). **C.** 2D [^{15}N-^{1}H]-HSQC spectrum of the N-terminal DNA binding domain of p50 subunit of NF-κB at 298 K with assignments.

A



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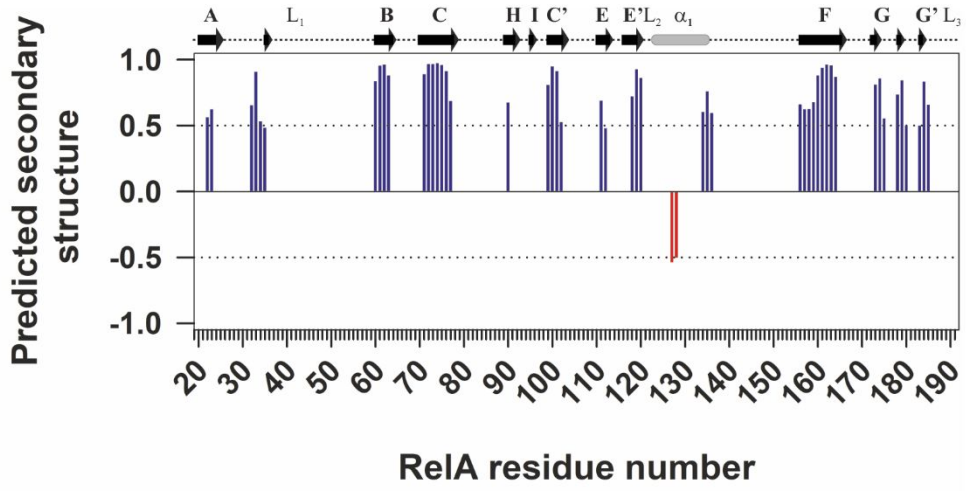

Figure S3. Secondary structure for DNA free form of (A) p50-NTD and (B) RelA-NTD predicted using TALOS-N. α-helix shown with negative and β-strands with positive probability values. Loop regions are given by values between -0.3 to 0.3. The secondary structure as observed in the DNA bound form of p50-RHR (PDB id 1NFK) is illustrated as cartoon over the top of the prediction with β-strands depicted as solid black arrows and helical turns as grey cylinders.

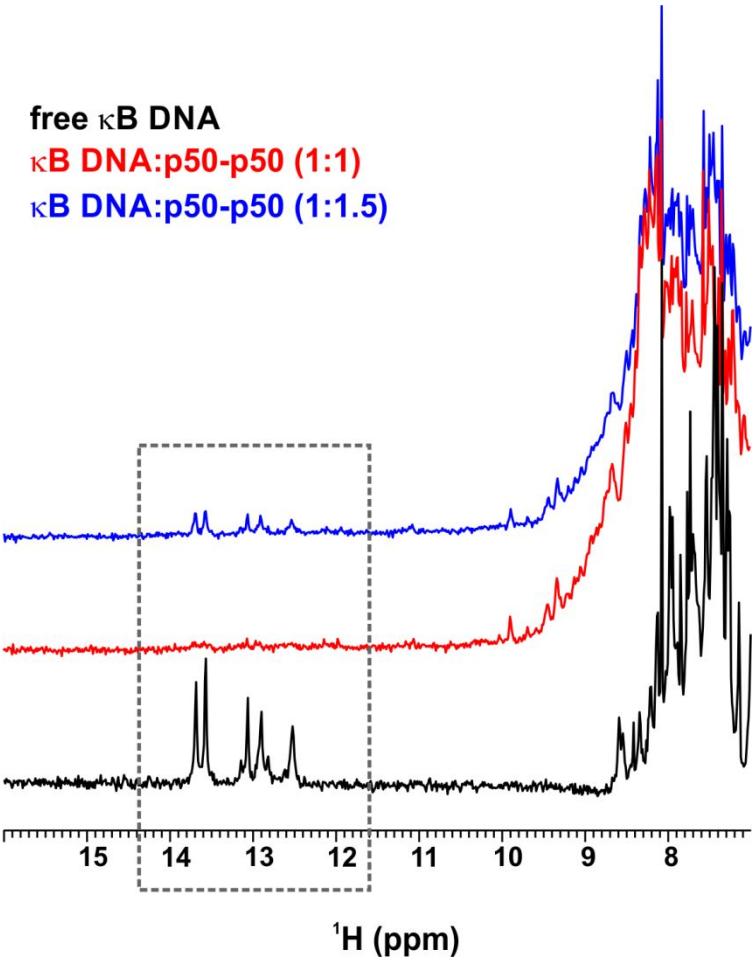

Figure S4. Characteristic imino regions (enclosed in the dashed grey box) of the 1D ^{1}H spectrum of unlabeled duplex κB DNA in free form (black), protein bound in 1:1 stoichiometric protein:full κB DNA ratio (red) and protein bound in 1:1.5 ratio (blue). Formation of complex with the protein leads to broadening of the imino peaks as seen in the red spectrum. DNA was added till excess of the free DNA was observed (blue spectrum). This ensured complete binding of the protein to the DNA.

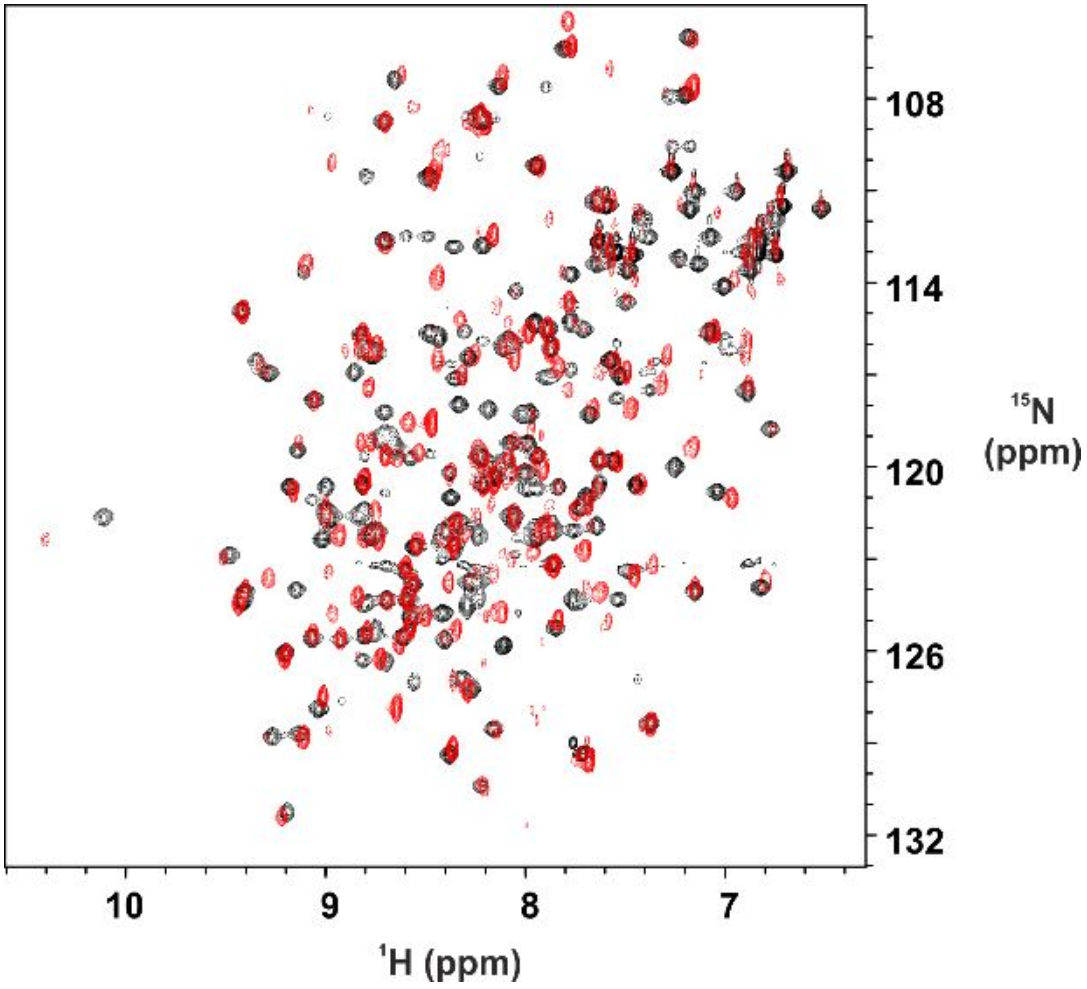

Figure S5. [^{15}N,^{1}H]-HSQC spectra of DNA bound and free p50-NTD is markedly distinct. Superimposed [^{15}N,^{1}H]-HSQC spectra of p50-NTD in free (black) and [^{15}N,^{1}H]-TROSY spectra of κB DNA bound form (red). The DNA bound form has markedly distinct chemical shift signatures.

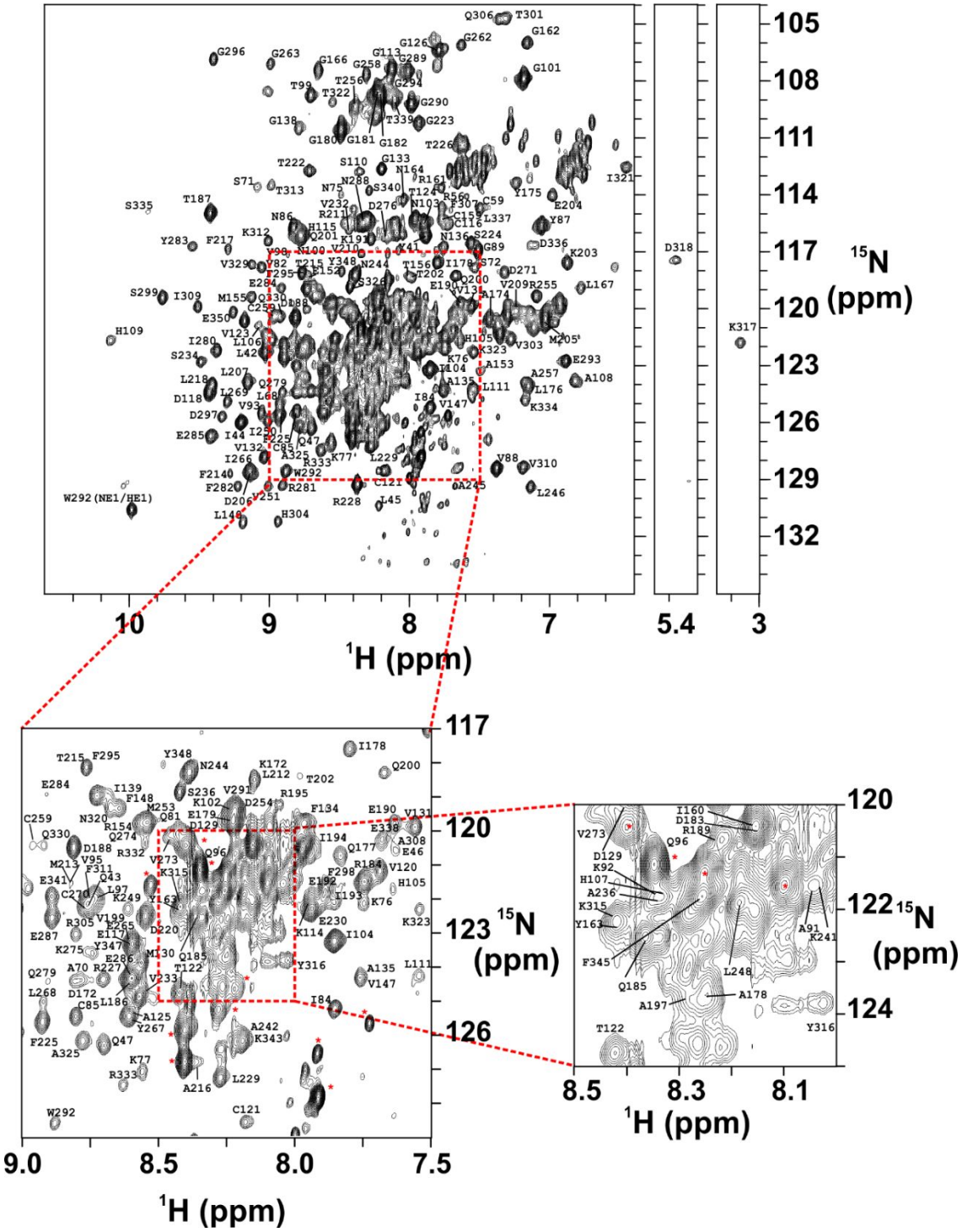

Figure S6. 2D [^{15}N-^{1}H]-TROSY spectrum of the rel-homology region of the 73.1 kDa of p50 subunit of NF-κB at 298 K. The assignments are depicted as one letter amino acid code followed by the sequence number in the full-length protein only for well-dispersed peak. The p50 in this study (residues 39-363) had 13 extra residues in the C-terminal end as compared to p50-DD fragment (245-350) which remained unassigned. Peaks marked with red asterisk (*) remained unassigned. The portion of TROSY spectrum enclosed in a red box is zoomed in the respective insets connected with the marked boxes.

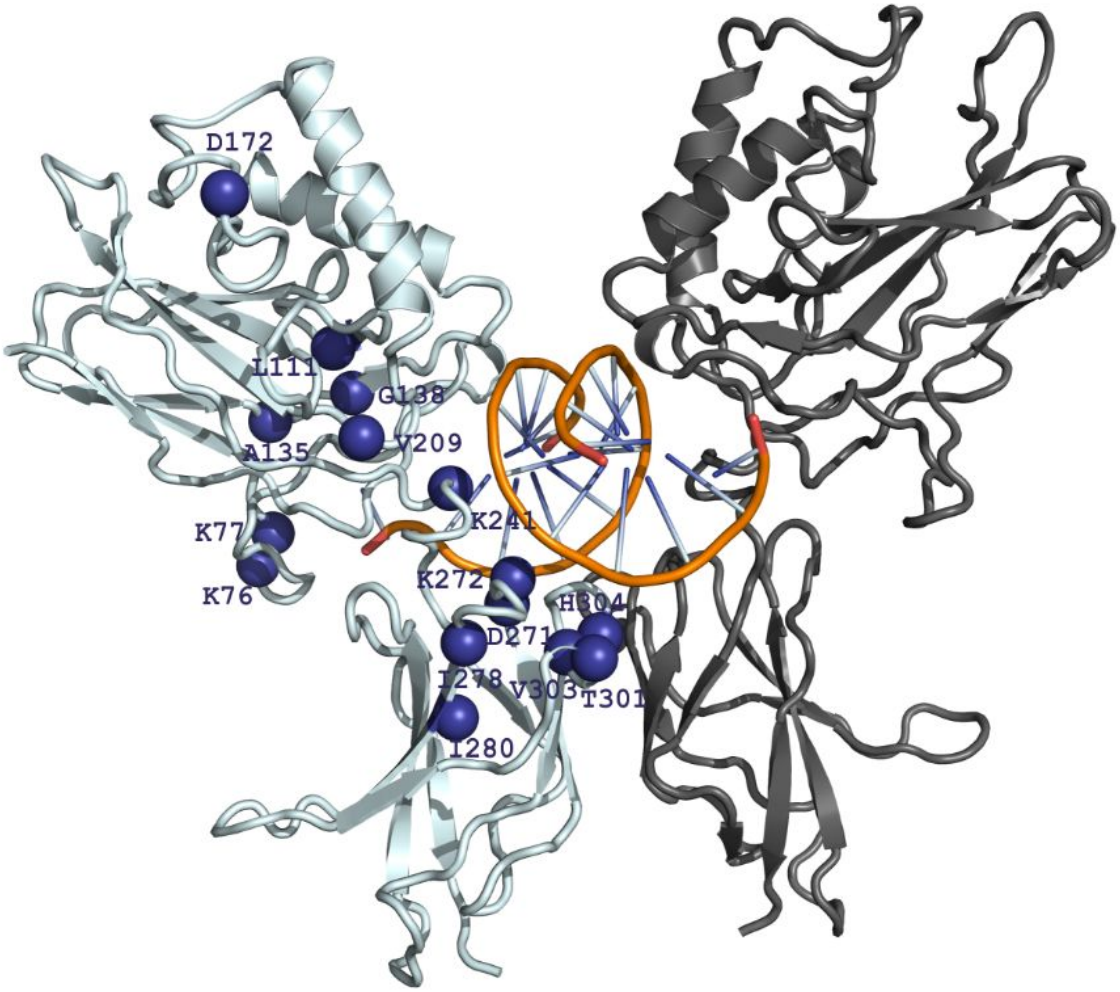

Figure S7. Cartoon representation of p50 homodimer (PDB id 1NFK) highlighting the residues that undergo significantly high CSP (blue sphere) upon binding to full-κB DNA.

Supplementary Tables

Table S1. Protein sample description

Protein sample	Labeling Strategy
p50-NTD	u-[^{15}N]
p50-NTD	u-[^{15}N, ^{13}C] partially deuterated
p50-NTD (^{15}N-Lys)	[^{15}N-Lys]
p50-NTD (^{15}N-Ile)	[^{15}N-Ile]
p50-NTD (Arg unlabeled)	[^{15}N, ^{13}C] + Arg-unlabeled
p50-NTD (Ile, Leu unlabeled)	[^{15}N, ^{13}C] + Ile, Leu-unlabeled
p50-NTD (Lys unlabeled)	[^{15}N, ^{13}C] + Lys-unlabeled
p50-DD	u-[^{15}N, ^{13}C, ^{2}H]
p50RHR	u-[^{15}N, ^{13}C, ^{2}H]

Supplementary References

1. Waterhouse, A. M.; Procter, J. B.; Martin, D. M.; Clamp, M.; Barton, G. J., Jalview Version 2--a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **2009,** *25* (9), 1189-91.
2. Ghosh, G.; van Duyne, G.; Ghosh, S.; Sigler, P. B., Structure of NF-kappa B p50 homodimer bound to a kappa B site. *Nature* **1995,** *373* (6512), 303-10.