Supplementary Figures:

Structural characterization of interactions between transactivation domain 1 of the p65 subunit of NF-κB and transcription regulatory factors.

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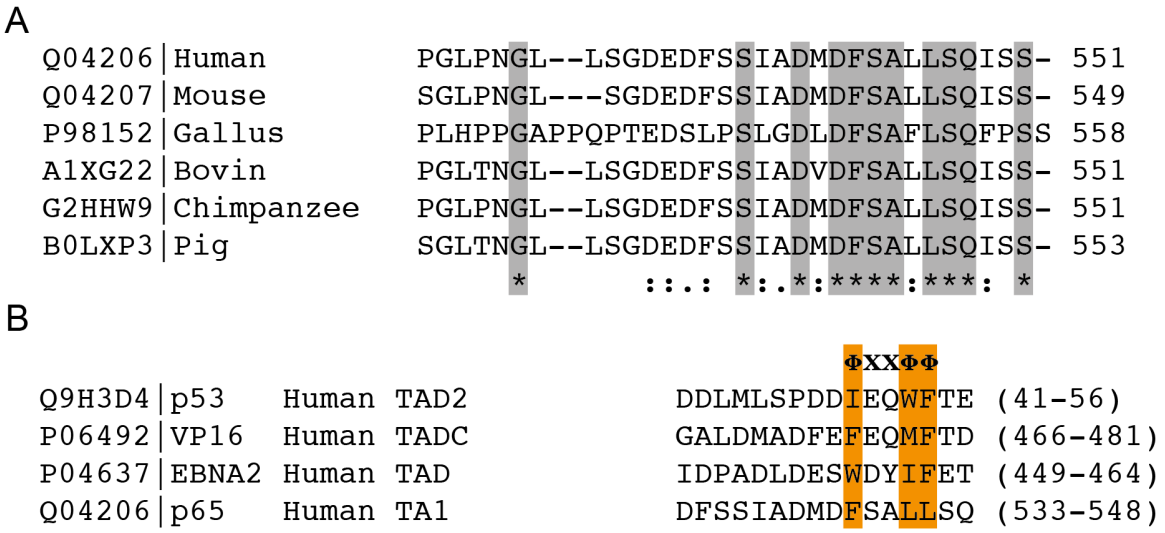
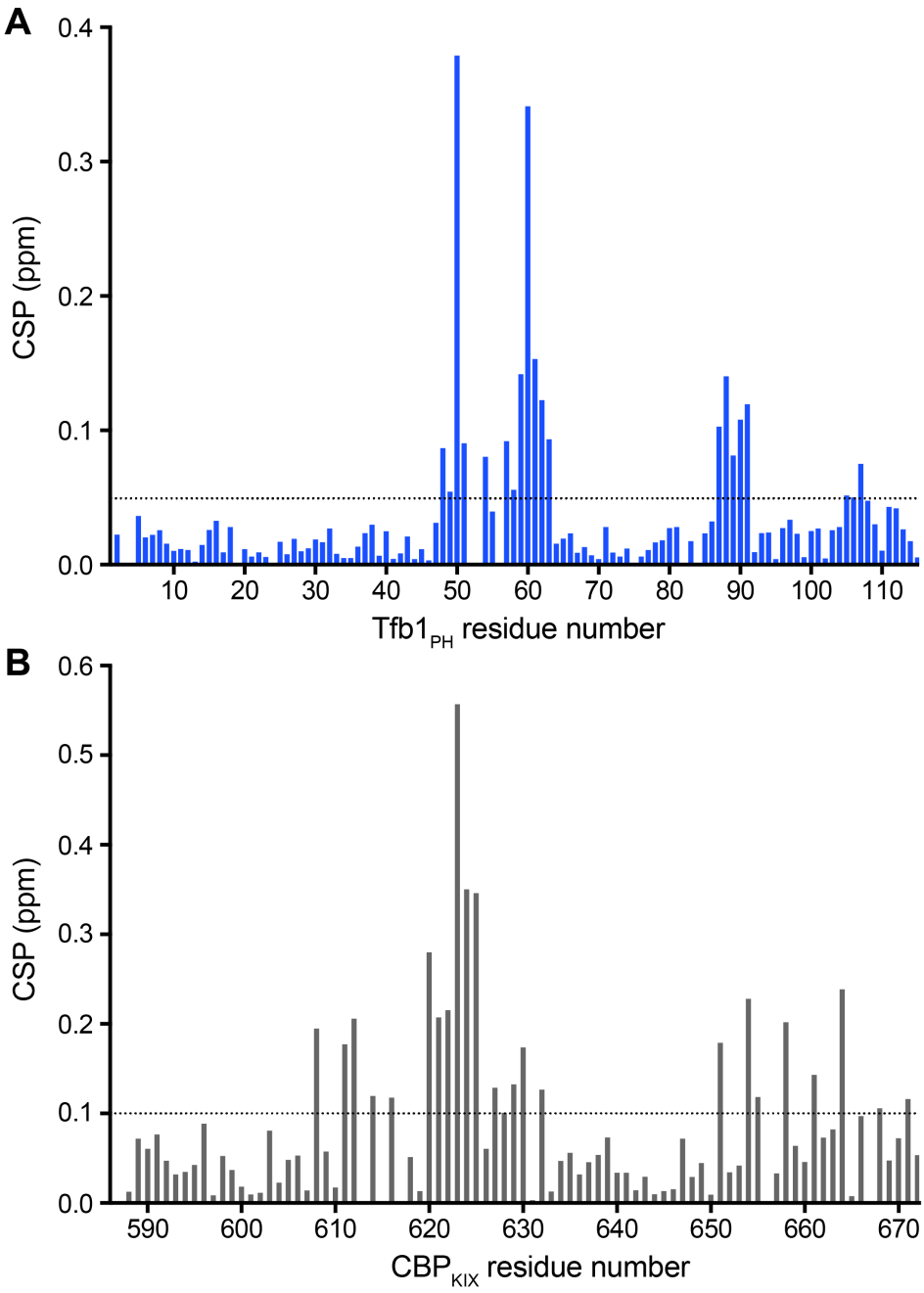
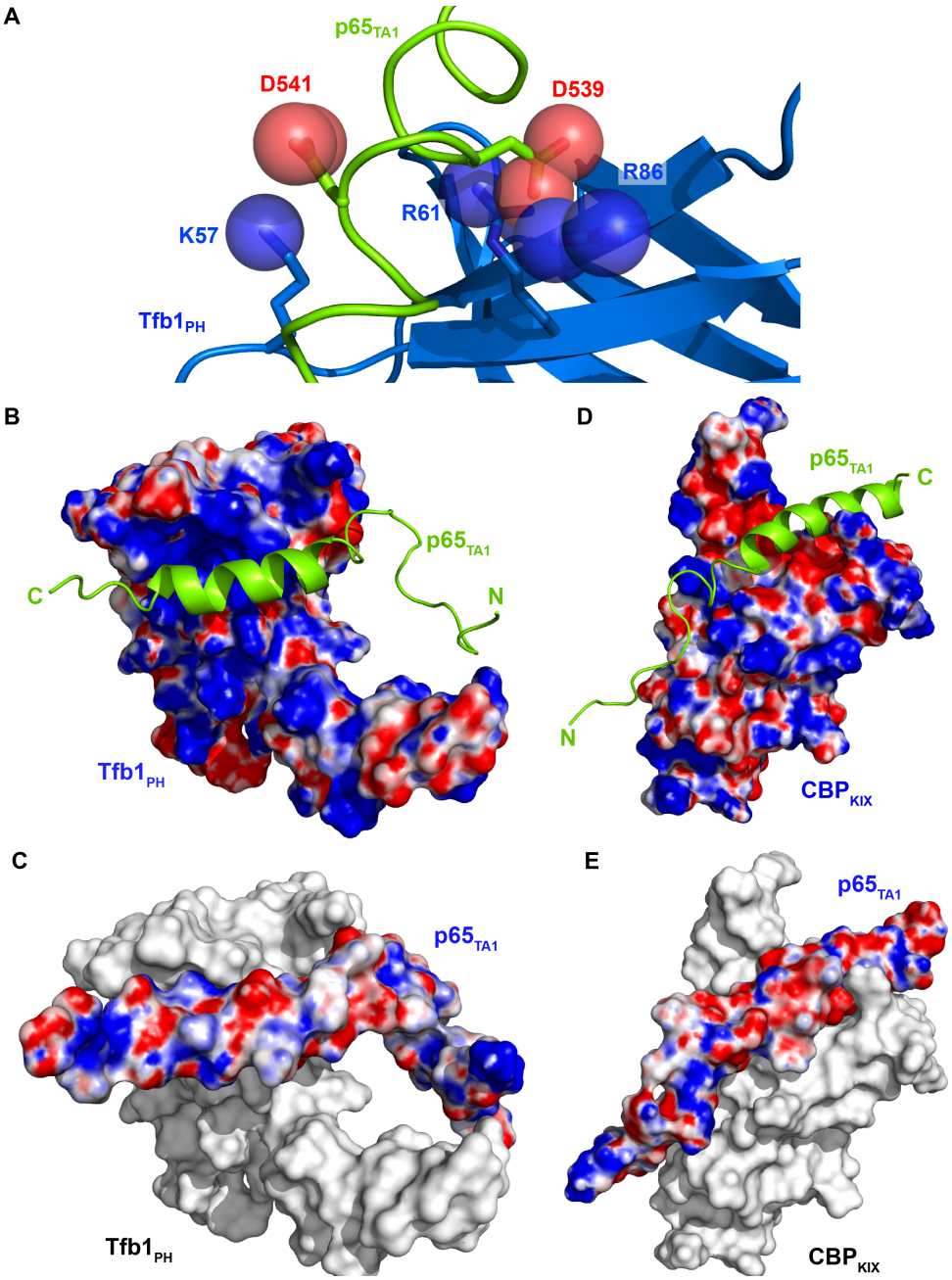


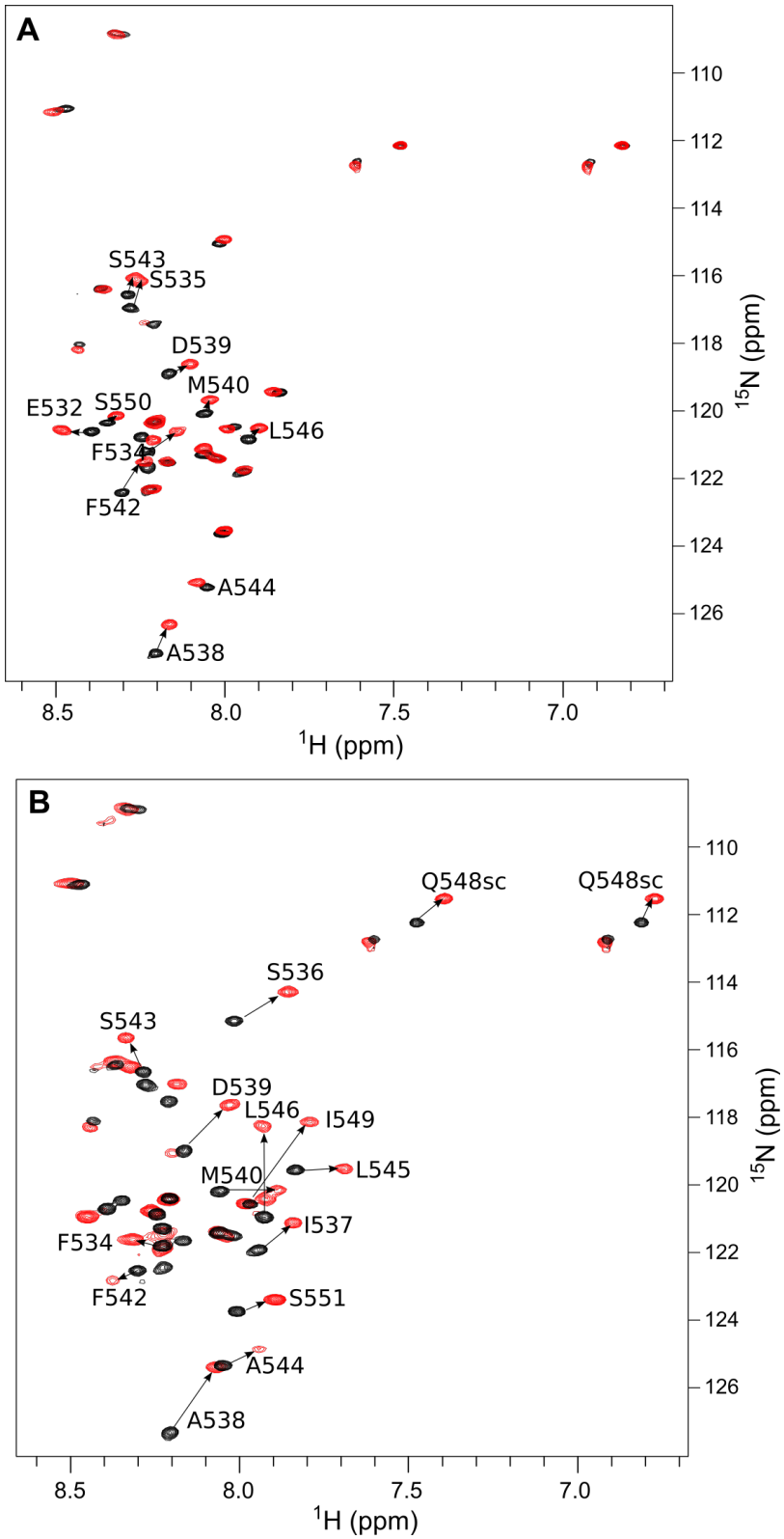
Supplementary Figure 1. Conservation of the ΦXXΦΦ motif among species and transactivation domains. (**A**) Alignment of $p65_{TA1}$ sequences from six different species. Grey color together with the stars indicates full sequence conservation, while the dots indicate moderate sequence conservation. UniProt numbers are indicated on the left. (**B**) Sequence alignment of the acidic transactivation domains of p53, VP16, EBNA2 and $p65_{TA1}$. The alignment is based on the ΦXXΦΦ motif indicated at the top. Φ hydrophobic residues are highlighted in orange.



Supplementary Figure 2. ^{1}H-^{15}N chemical shift perturbations (CSP) induced by the binding of p65$_{TA1}$ to Tfb1$_{PH}$ (A) and CBP$_{KIX}$ (B). In both cases, 3 equivalents of p65$_{TA1}$ were added to 700 µM of Tfb1$_{PH}$/CBP$_{KIX}$ in 20 mM $NaPO_4$ buffer at pH 6.5 in 10% D_2O / 90% H_2O. ^{1}H and ^{15}N chemical shift changed were monitored in 2D ^{1}H-^{15}N HSQC spectra at 25 °C. ^{1}H-^{15}N CSPs were calculated as: $\Delta\delta = \sqrt{(\Delta\delta_H)^2 + (0.17\,\Delta\delta_N)^2}$ where $\Delta\delta_H$ and $\Delta\delta_N$ represent the respective variation of ^{1}H and ^{15}N chemical shifts after the addition of p65$_{TA1}$. Missing data correspond to proline residues which don't provide signal in the ^{1}H-^{15}N HSQC spectrum, as well as H3 and A53 in Tfb1$_{PH}$ and G586, V587 and K667 in CBP$_{KIX}$. CSPs are considered as significant when their value is above the dotted lines, *i.e.* 0.05 ppm for Tfb1$_{PH}$-p65$_{TA1}$ complex and 0.1 ppm for CBP$_{KIX}$-p65$_{TA1}$ complex.



Supplementary Figure 3. Electrostatic interactions in $Tfb1_{PH}$-$p65_{TA1}$ and CBP_{KIX}-$p65_{TA1}$ complexes. (**A**) Close-up view of potential electrostatic interactions between D539 of $p65_{TA1}$ and either R61 or R86 of $Tfb1_{PH}$, as well as between D541 of $p65_{TA1}$ and K57 of $Tfb1_{PH}$. (**B**) Electrostatic surface potential of $Tfb1_{PH}$ in the $Tfb1_{PH}$-$p65_{TA1}$ complex, where $p65_{TA1}$ is represented as cartoon for clarity. This electrostatic surface potential shows that the cleft where F542 of $p65_{TA1}$ anchors is highly positive on $Tfb1_{PH}$. The color scale is set from -10 kT/e (red) to 10 kT/e (blue), and electrostatic potentials were calculated with the default parameters on http://www.charmm-gui.org/, using the Poisson-Boltzmann equation. (**C**) Electrostatic surface potential of $p65_{TA1}$ in the $Tfb1_{PH}$-$p65_{TA1}$ complex, where $Tfb1_{PH}$, is represented as a neutral surface for clarity. (**D**) Electrostatic surface potential of CBP_{KIX} in the CBP_{KIX}-$p65_{TA1}$ complex, where $p65_{TA1}$ is represented as cartoon, showing that $p65_{TA1}$ is surrounded by both negatively and positively charged regions. (**E**) Electrostatic surface potential of $p65_{TA1}$ in the CBP_{KIX}-$p65_{TA1}$ complex, where CBP_{KIX}, is represented as a neutral surface.



Supplementary Figure 4. Overlay of the 2D ^{1}H-^{15}N-HSQC NMR spectra of ^{13}C-^{15}N- p65$_{TA1}$ either in the absence (black) or in the presence (red) of 2 equivalents of (**A**) Tfb1$_{PH}$ and (**B**) CBP$_{KIX}$. Residues with significant chemical shift perturbation upon binding are labeled and the shift is indicated by arrow.