SUPPORTING INFORMATION

Protein co-factors are essential for high affinity DNA binding by the nuclear factor κB RelA subunit

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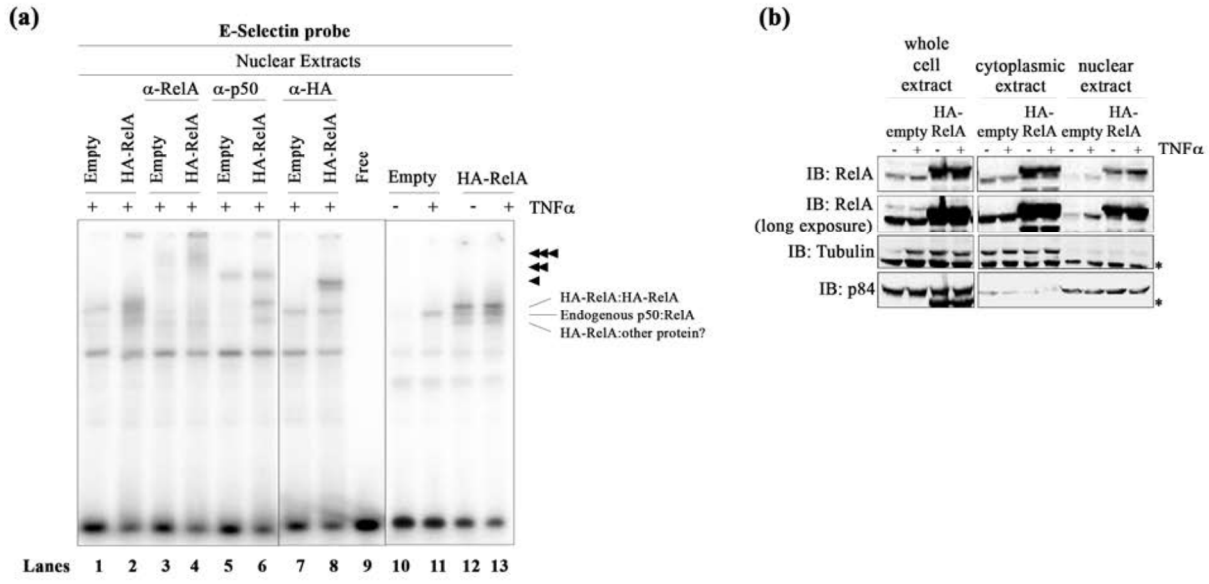
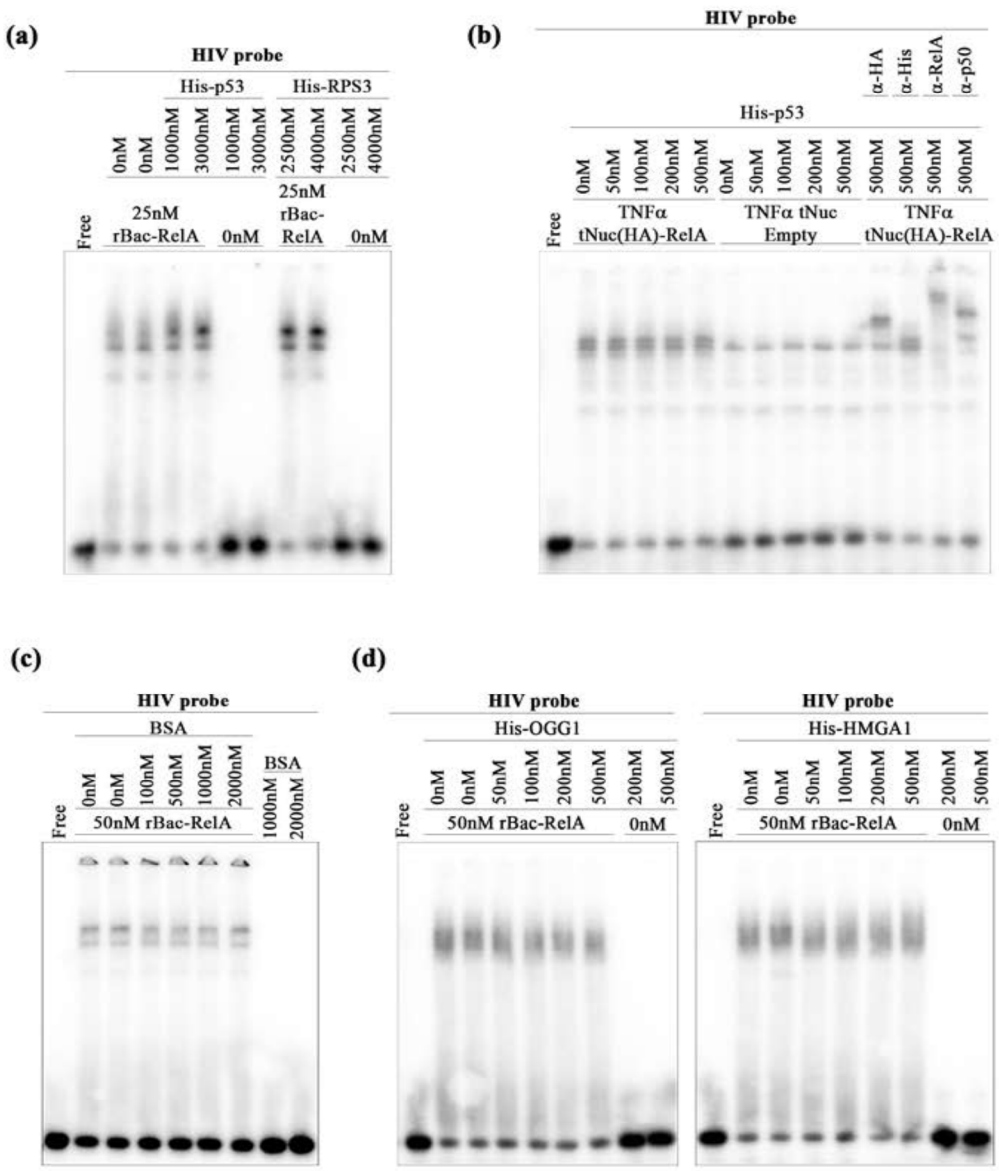
SUPPLEMENTARY FIGURE S1



Supplementary Figure S1. (a) an EMSA showing E-Selectin κB DNA binding by exogenous HA-RelA homodimer (upper band), endogenous p50:RelA heterodimer (middle band), and additional HA-RelA:protein complex (bottom band) present in transfected HA-RelA nuclear extracts unstimulated or stimulated with TNF-α. Single, double and triple arrowheads indicate the supershifted complexes when using anti-RelA, anti-p50 and anti-HA antibodies. (b) a Western blot using whole cell extracts as well as nuclear and cytoplasmic fractions obtained from HEK 293T cells transfected either with empty vector or HA-RelA. Cells were unstimulated or stimulated with TNF-α, as indicated in the figure. There are two panels corresponding to the anti-RelA antibody, a short and long exposure, to highlight the differences observed in the cytoplasmic and nuclear fractions when cells were stimulated with TNF-α. Tubulin was used as the cytoplasmic marker whereas p84 was the nuclear marker. The asterisks indicate unspecific bands detected by these antibodies.

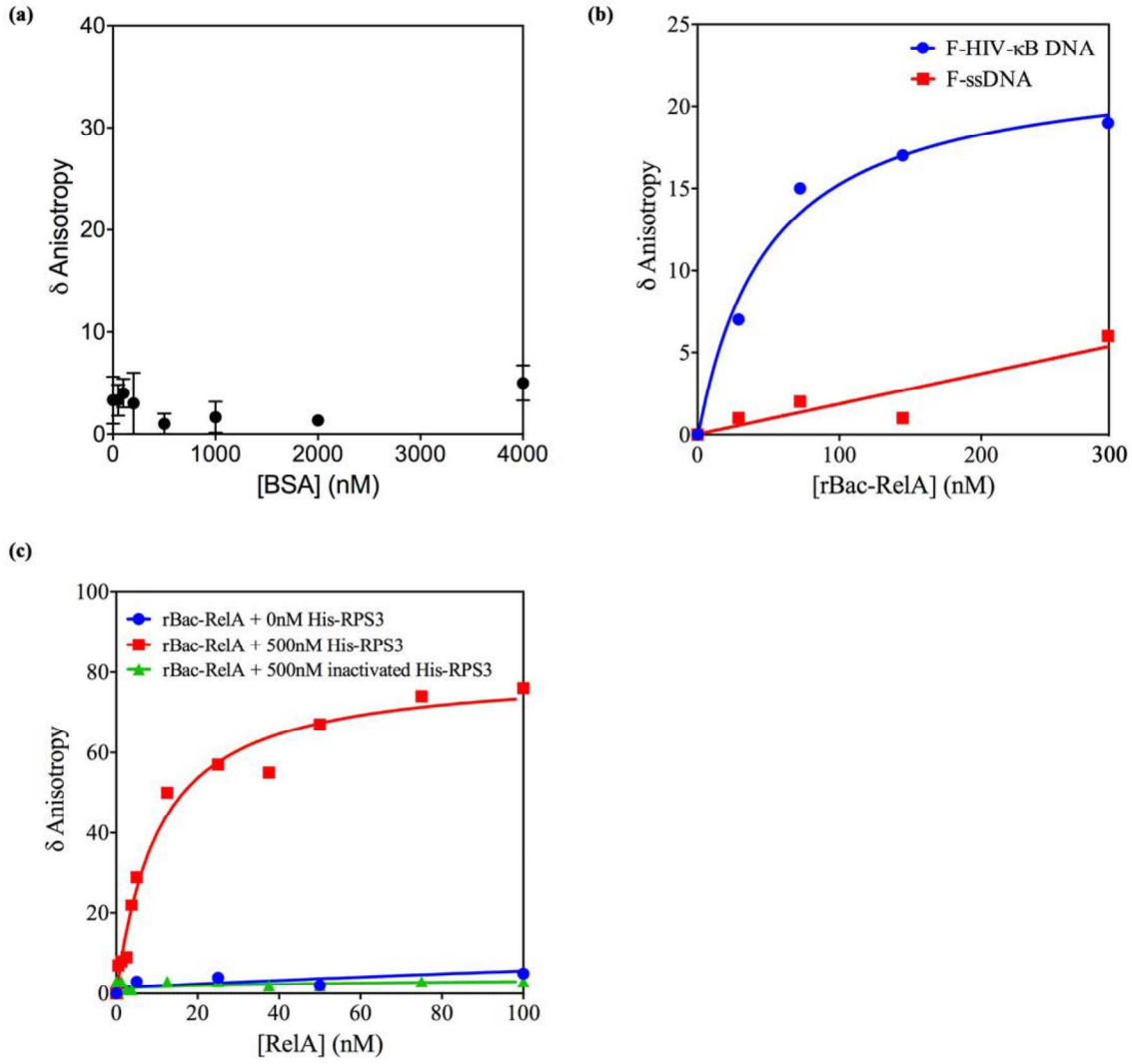
SUPPLEMENTARY FIGURE S2



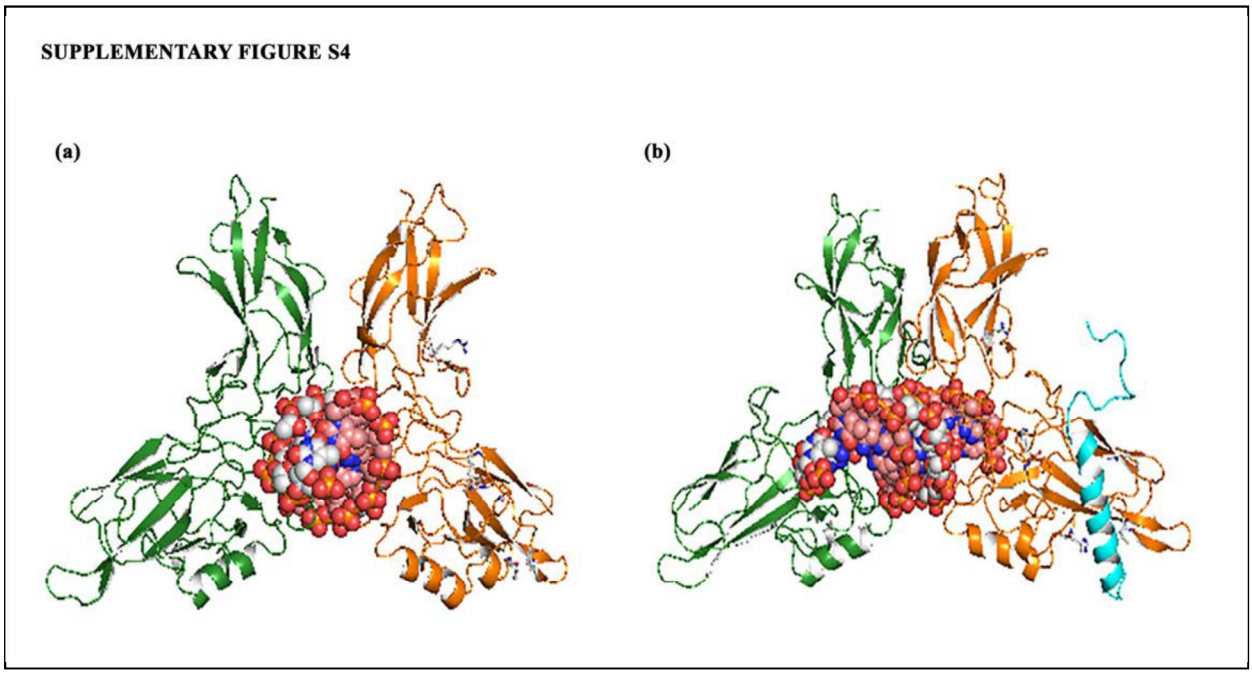
Supplementary Figure S2. (a) an EMSA showing that neither His-p53 nor His-RPS3 directly binds to the HIV-κB DNA sequence used throughout this paper. (b) an EMSA showing that HIV-κB DNA binding by TNF-α treated tNuc(HA)-RelA extracts in the presence of increasing concentrations of His-p53 is not affected. DNA:protein complexes were analyzed by supershift using anti-HA, anti-His, anti-RelA and anti-p50 antibodies. (c) an EMSA showing that HIV-κB DNA binding by rBac-RelA in the presence of increasing concentrations of BSA is not affected. The last two lanes show that BSA does not directly bind

to the DNA probe tested. (d) an EMSA showing that HIV-κB DNA binding by rBac-RelA is not enhanced in the presence of His-OGG1 (left panel) or His-HMGA1 (right panel).

SUPPLEMENTARY FIGURE S3



Supplementary Figure S3. (a) a graphical representation of the binding of 1nM F-HIV-κB DNA to increasing amounts of BSA. Data, given as normalized anisotropy units, were analyzed with the GraphPad PRISM 4.0 program and fitted using a non-linear regression model to a hyperbolic dose-response curve. (b) a graphical representation of increasing amounts of rBac-RelA with either 1nM F-HIV-κB DNA (in blue) or 1nM F-ssDNA (single stranded negative control DNA, in red). (c) a graphical representation of the binding of 1nM F-HIV-κB DNA to His-RPS3 before and after heat inactivation of His-RPS3 for 10min at 90°C. In blue, rBac-RelA; In red, rBac-RelA plus His-RPS3; In green, rBac-RelA plus His-RPS3 previously heat inactivated



Supplementary Figure S4. (a) a model of the RelA homodimer:DNA complex bound to RelA-AD-TA1. The DNA is represented by the space-filling model and the proteins are presented as ribbons. (b) same as (a) but viewed after rotating the complex by 30 degrees around the short DNA axis.