Supporting Information

for

Site-Selective Tyrosine Phosphorylation in the Activation of the p50 Subunit of NF-κB for DNA Binding and Transcription

Shengxi Chen,* Xun Ji, Larisa M. Dedkova, Gal Reddy Potuganti and Sidney M. Hecht*

Biodesign Center for BioEnergetics, and School of Molecular Sciences, Arizona State University, Tempe, Arizona 85287, United States

Email addresses of corresponding authors: Shengxi Chen, Shengxi.chen.1@asu.edu; Sidney Hecht, sid.hecht@asu.edu

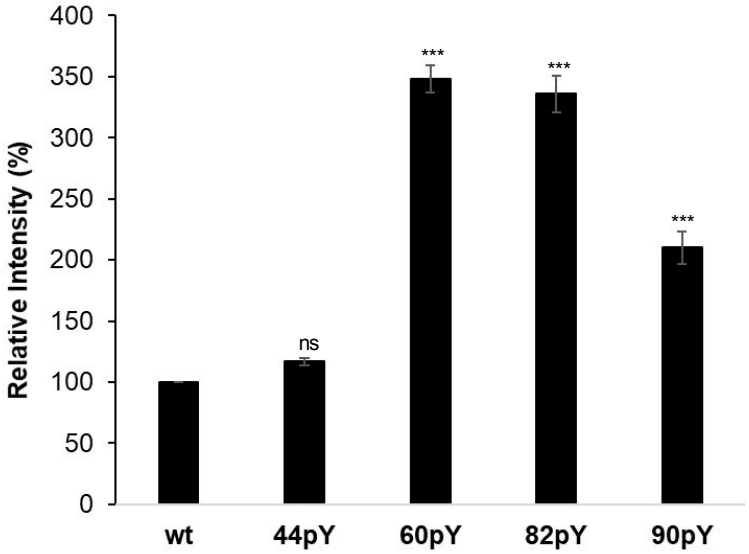

Figure S1. Statistical analysis of IL-2 promoter DNA binding of wild-type NF-κB in comparison with each modified NF-κBs. Statistical significance was calculated using the Student's t-test: $p < 0.001$ (***), $p > 0.05$ (not significant, ns).

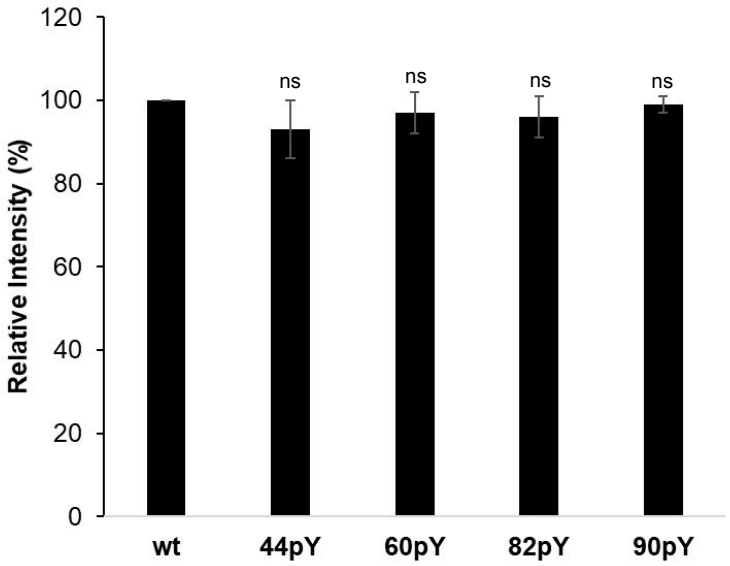

Figure S2. Statistical analysis of IL-2 promoter DNA binding of wild-type p50 in comparison with each modified p50. Statistical significance was calculated using the Student's t-test: $p > 0.05$ (not significant, ns).

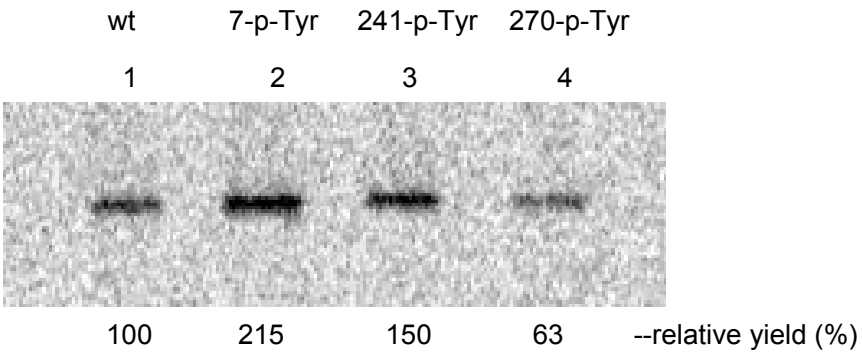

Figure S3. Cell free expression of modified p50 subunits of NF-κB containing phosphorylated tyrosine at positions 7, 241 and 270, each synthesized by suppression of nonsense codon UAG in an *E. coli* protein biosynthesizing system using phosphotyrosyl-$tRNA_{CUA}$. Visualization was accomplished using the ^{35}S-methionine incorporated into the proteins. Following *in vitro* synthesis, each protein was purified on a Strep-tactin column. The purified proteins were analyzed by 15% SDS-PAGE. The isolated yields of the proteins relative to wild type are listed below each lane.

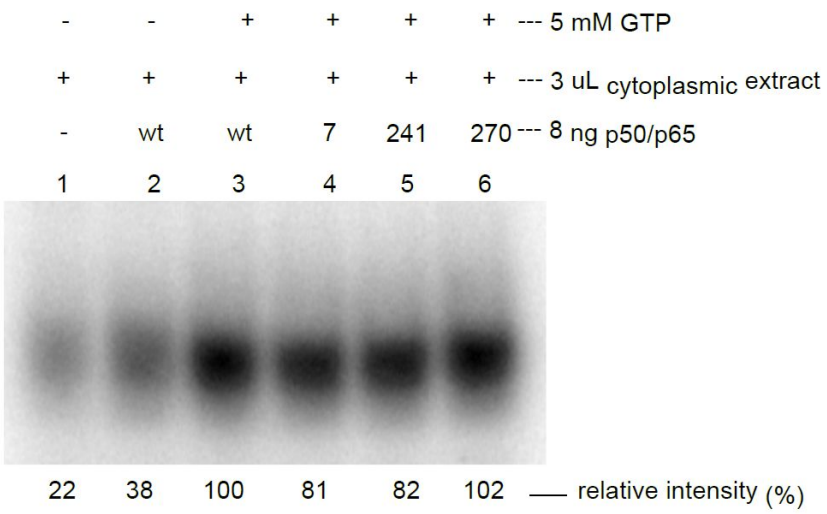

Figure S4. DNA binding assay of modified NF-κBs expressed in a cell free *E. coli* protein biosynthesizing system following treatment with a cytoplasmic lysate from activated Jurkat cells in the presence GTP. The cytoplasmic lysatewas incubated at 37 ^{o}C for 6 h. The extract was then cooled on ice and combined with cold samples of NF-κB and IL-2 promoter DNA. The incubation was carried out at 4 ^{o}C for 10 min. Controls included the cytoplasmic extract alone (lane 1), wild-type NF-κB in the absence and presence of GTP (lanes 2 and 3), and modified NFκBs containing p50 subunits having pTyr at positions 7, 241 or 270 (lanes 4, 5 and 6, respectively). The analysis of samples was carried out by 5% native PAGE.

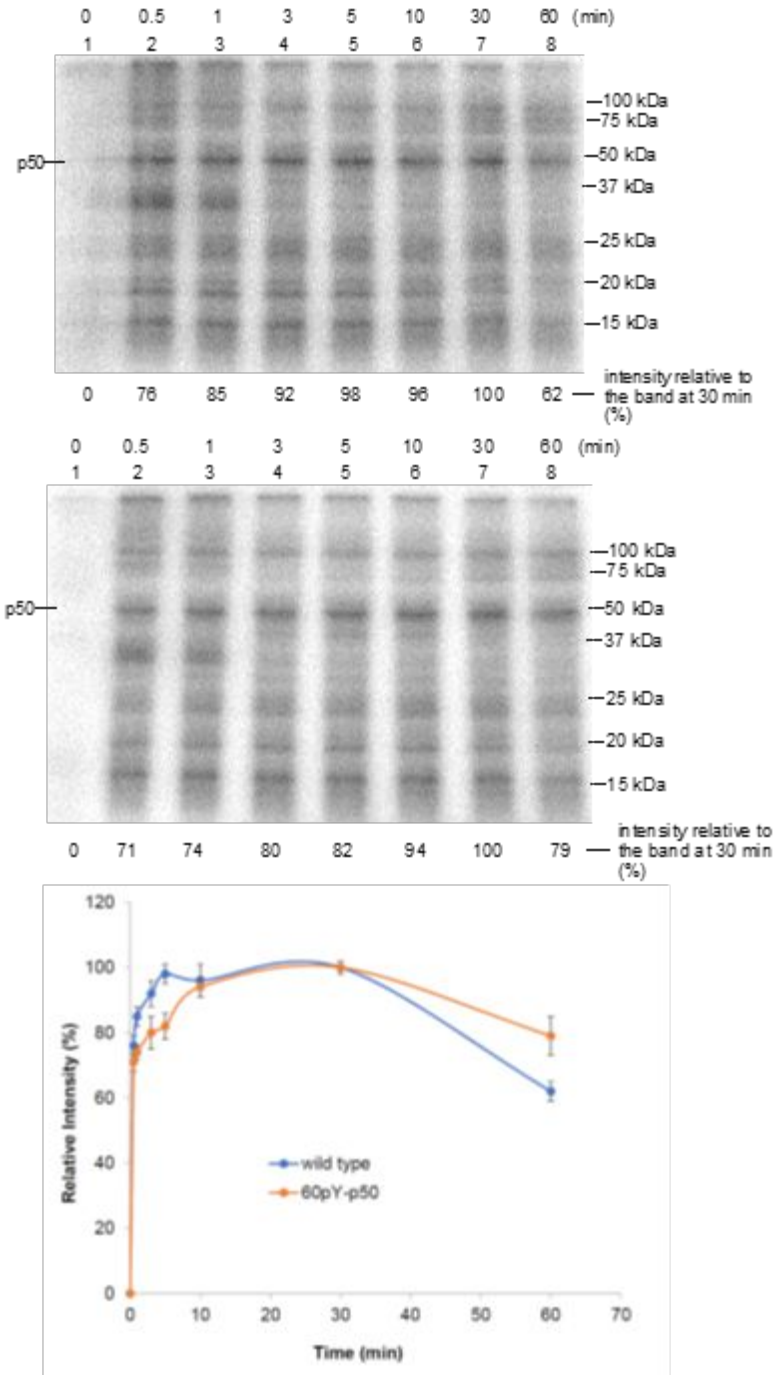

Figure S5. Phosphorylation of wild-type p50 (upper panel) and modified p50 (containing pY60) (middle panel) in the presence of Jurkat cell extracts. The *in vitro* expressed p50 proteins (80 ng) were added into the solution containing 15 μL of activated Jurkat cell extract in the presence of 80 nM γ-^{32}P-GTP (6000 Ci/mmol). The reaction mixture was incubated at room temperature. The results were analyzed by 15% SDS-PAGE. 0 min: no Jurkat cell extract was added; 0.5 – 60 min after Jurkat cell extract addition. (lower panel) Time course of phosphorylation of the wild-type and modified p50s in the presence of cell extract from activated Jurkat cells. The band at 30 min was defined as 100%.

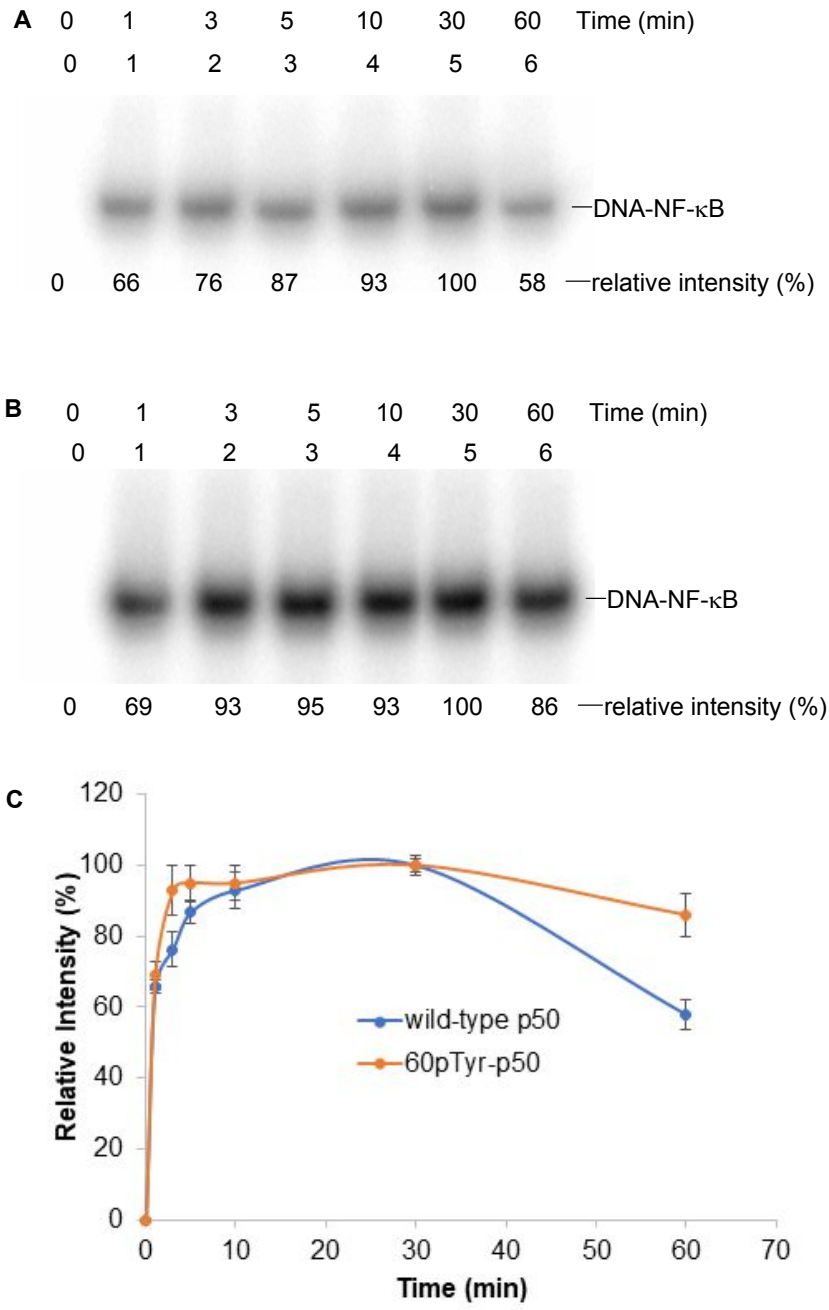

Figure S6. Time dependent DNA binding of phosphorylated wild-type p50 (**A**) and modified p50 (**B**, p50 containing pY60) in the presence of Jurkat cell extracts. The *in vitro* expressed p50 protein (8 ng) was mixed with 10 ng of wild-type p65 protein and added into (1.5 μL) extract from activated Jurkat cells in the presence of 80 nM GTP. The reaction mixture was incubated at room temperature. At the determined time, ^{32}P-labeled DNA (IL-2, 2 pmol) was added to the reaction mixture, which was incubated at room temperature. The results were analyzed using 5% native PAGE. 0 min: no activated Jurkat cell extract was added; 1 – 60 min after Jurkat cell extract addition. (**C**) Time course of binding of the wild-type and modified p50s to IL-2 promoter DNA in the presence of cell extract from activated Jurkat cells. The band having the highest intensity for each protein was defined as 100.

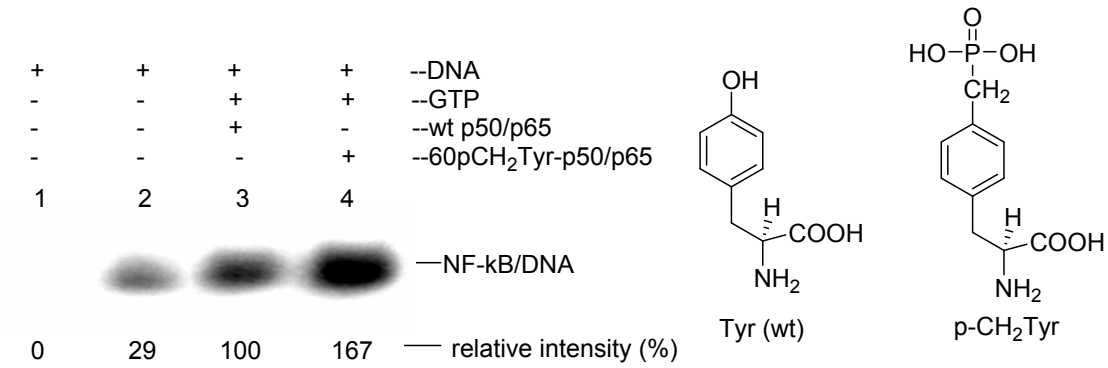

Figure S7. The *in vitro* expressed wild-type or modified (60-pCH_2Tyr) p50 protein (8 ng) was combined with 10 ng of wild-type p65 protein and was preincubated with 1.5 μL of lysate from activated Jurkat cells in the presence of 5 mM GTP. ^{32}P-labeled IL-2 DNA (2 pmol) was added to the reaction mixture, which was incubated at 0 ^{o}C for 5 min. The results were analyzed using 5% native PAGE.