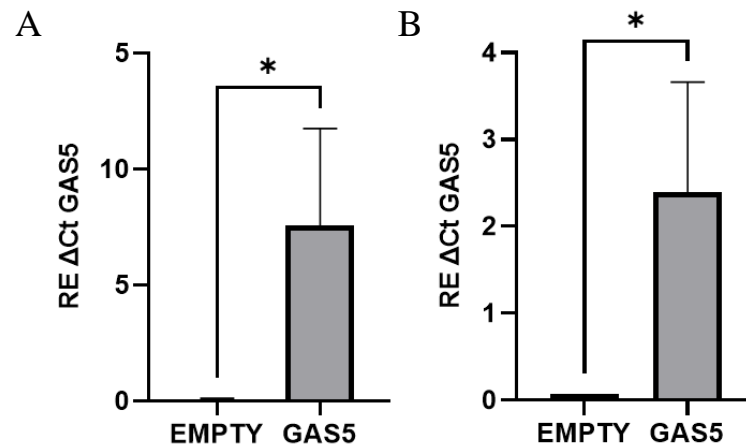


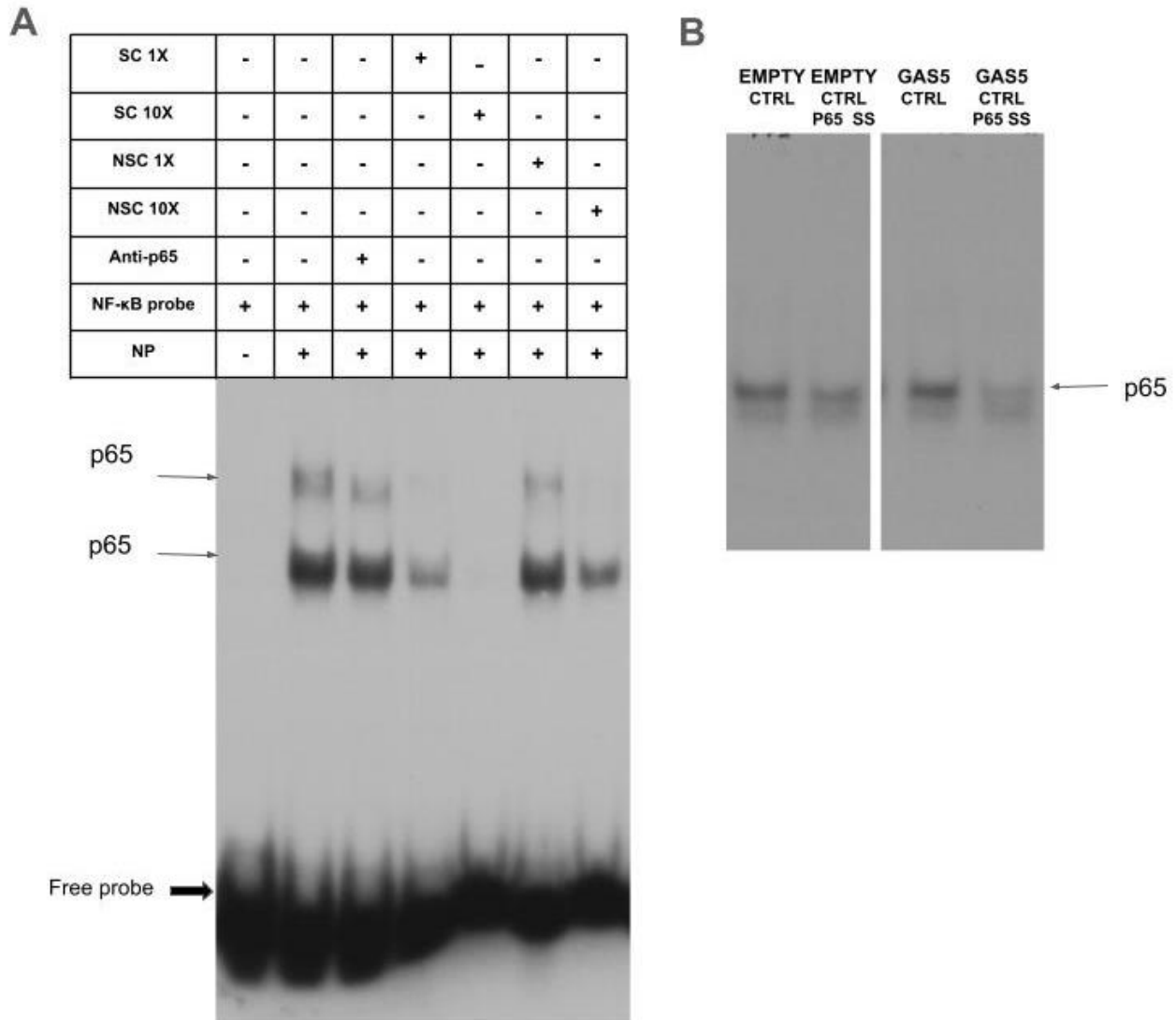
Supplementary Material

Supplementary Figures

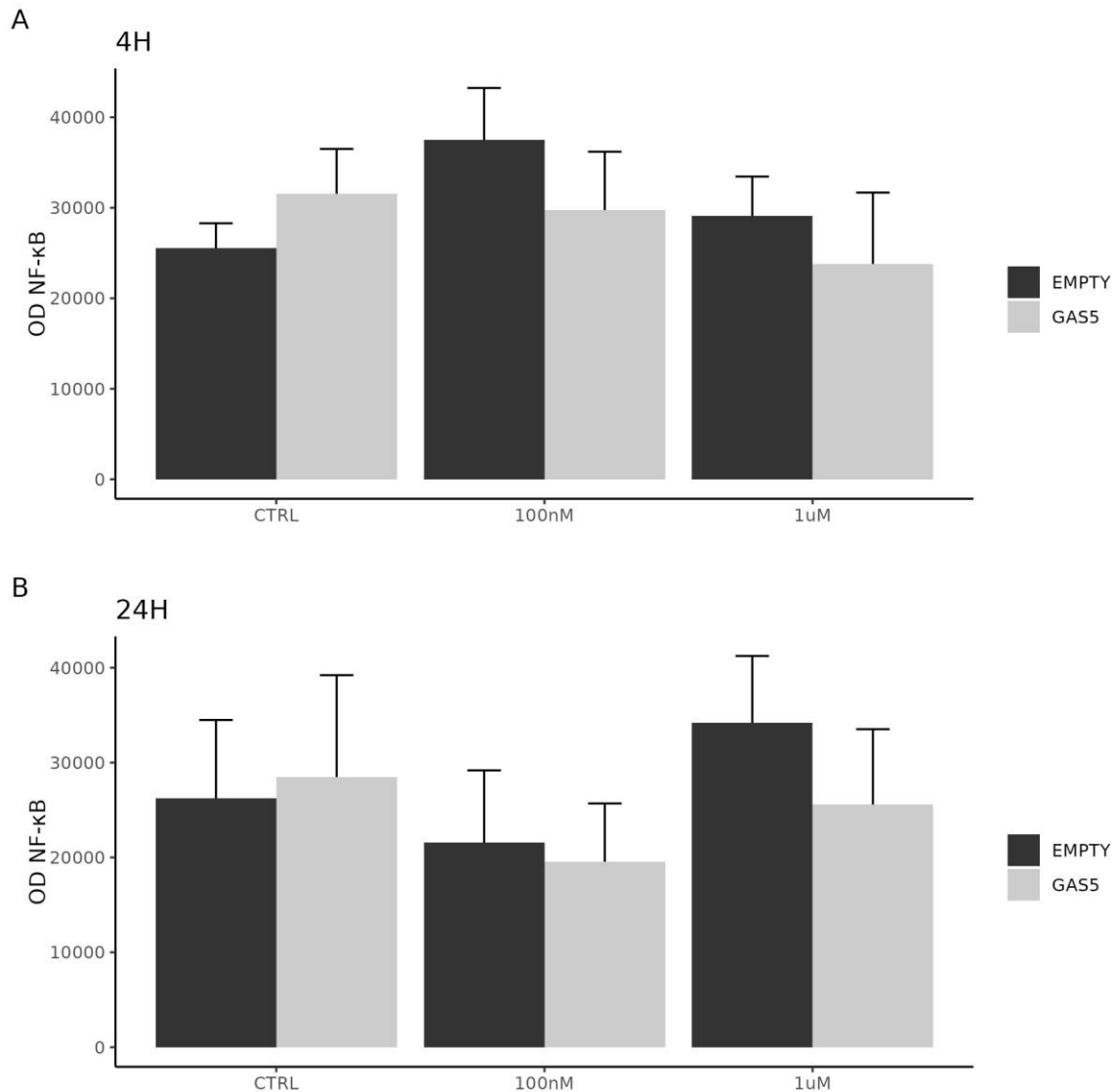


Supplementary Figure 1. Evaluation of GAS5 levels after transfection with pcDNA-GAS5 plasmid in HeLa cells after 4 (Panel A) and 24 hours (Panel B). GAS5 expression was normalized using GAPDH gene and relative expression (RE) values are expressed as $2^{-\Delta Ct}$. Unpaired t test * $p < 0.05$. The data are reported as means \pm SD of three independent experiments performed in triplicate.

Supplementary Material



Supplementary Figure 2. Competitive and supershift EMSA. (A) EMSA shows binding of 3 μ g of untransfected HeLa cells nuclear proteins to 32 P labeled DNA probes containing NF- κ B consensus sequence. For competitive assays, an unlabeled NF- κ B DNA probe (specific competitor) was used in 1X and 10X molar excess compared to labeled NF- κ B probe. An unlabeled probe containing NF- κ B binding unrelated sequence was used as a non-specific competitor in 1X and 10X molar excess compared to labeled NF- κ B probe. For supershift assay, 2 μ g of anti-p65 antibody was used to demonstrate presence of p65 in protein-DNA complexes. (B) Anti-p65 supershift assay was assessed in EMPTY and GAS5 CTRL transfected cells (3 μ g of NP, 2 μ g anti-p65 antibody) (B). SC - specific competitor, NSC - non-specific competitor, NP - nuclear proteins, SS – supershift.



Supplementary Figure 3. Level of NF-kB DNA binding activity in HeLa cells transfected with EMPTY or GAS5-constructed pcDNA3.1 vector and treated with dexamethasone (100 nM and 1 μ M) for 4H (A) and 24H (B). Level of NF-kB DNA binding activity was determined using EMSA and presented in optical density units (OD) using ImageJ. Differences within samples in different treatment groups (control, 100 nM, 1 μ M) were assessed using a non-parametric Friedman test for repeated measurements, followed by post-hoc pairwise comparisons by Wilcoxon sign-rank test and Benjamini–Hochberg multiple comparison correction: (A) Friedman test EMPTY 4H, CTRL vs 100 nM vs 1 μ M, $p=0.049$, no significant difference between treatments and control after pairwise comparisons; Friedman test GAS5 4H, CTRL vs 100 nM vs 1 μ M, $p=0.038$, no significant difference between treatments and control after pairwise comparisons. (B) Friedman test EMPTY 24H, CTRL vs 100 nM vs 1 μ M, $p=1$; Friedman test GAS5 24H, CTRL vs 100 nM vs 1 μ M, $p=0.37$.