Supplemental Data

Inhibition of NF-κB-dependent transcription by MKP-1: Transcriptional repression by glucocorticoids occurring via p38 MAPK

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ADDITIONAL EXPERIMENTAL PROCEDURES

Electrophoretic mobility shift assay (EMSA) – Nuclear proteins were extracted from BEAS-2B and A549 cells and subjected to binding reactions using a radioactively labeled NF-κB consensus probe (Promega) (5′-AGT TGA GGG GAC TTT CCC AGG-3′ (NF-κB consensus underlined)) as previously described (1). Specificity of binding was determined by prior addition of 100 x excess unlabeled consensus oligonucleotide. Reactions were separated on 8 % acrylamide gels prior to vacuum drying and autoradiography.

SUPPLEMENTAL FIGURES

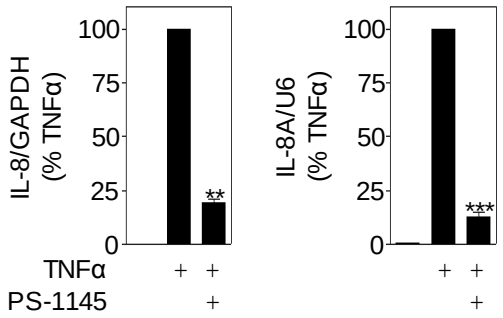

Figure S1. **NF-κB-dependence of IL-8 expression.** BEAS-2B cells were pre-treated with the selective IKK2 inhibitor, PS-1145 (10 μM), for 1 h prior to stimulation with TNFα (10 ng/ml) for 1 h. Cells were then harvested for total RNA which was converted to cDNA and subject to analysis by real-time PCR for IL-8, GAPDH, nIL-8A and U6. Data (n = 6), normalized to GAPDH (IL-8) or U6 (nIL-8A) and expressed as percentage of TNFα stimulated cells, are plotted as means ± SE. Significance, relative to TNFα stimulated cells, using a paired t-test is indicated; ** $p < 0.01$, *** $p < 0.001$.

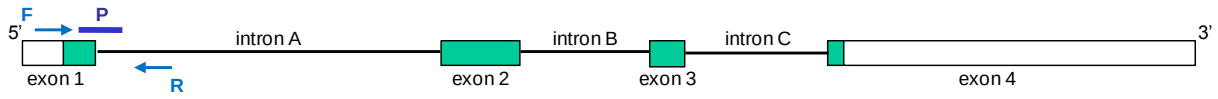

Figure S2. **Schematic diagram showing design of nuclear RNA probe and primer sets to allow analysis of unspliced nuclear IL-8 RNA.** The unspliced RNA structure of IL-8 (NC_000004, unspliced RNA/genomic DNA; NM_000584, mRNA) is shown (not to scale) in diagramatic form with introns and exons indicated. The coding region is depicted in green and the untranslated region in white. The TaqMan probe (P) was designed to cross the intron A/exon 1 boundary and forward (F) and reverse (R) primers were designed within exon 1 and intron A respectively. This combination of probe and primers results in detection of unspliced RNA and genomic DNA.

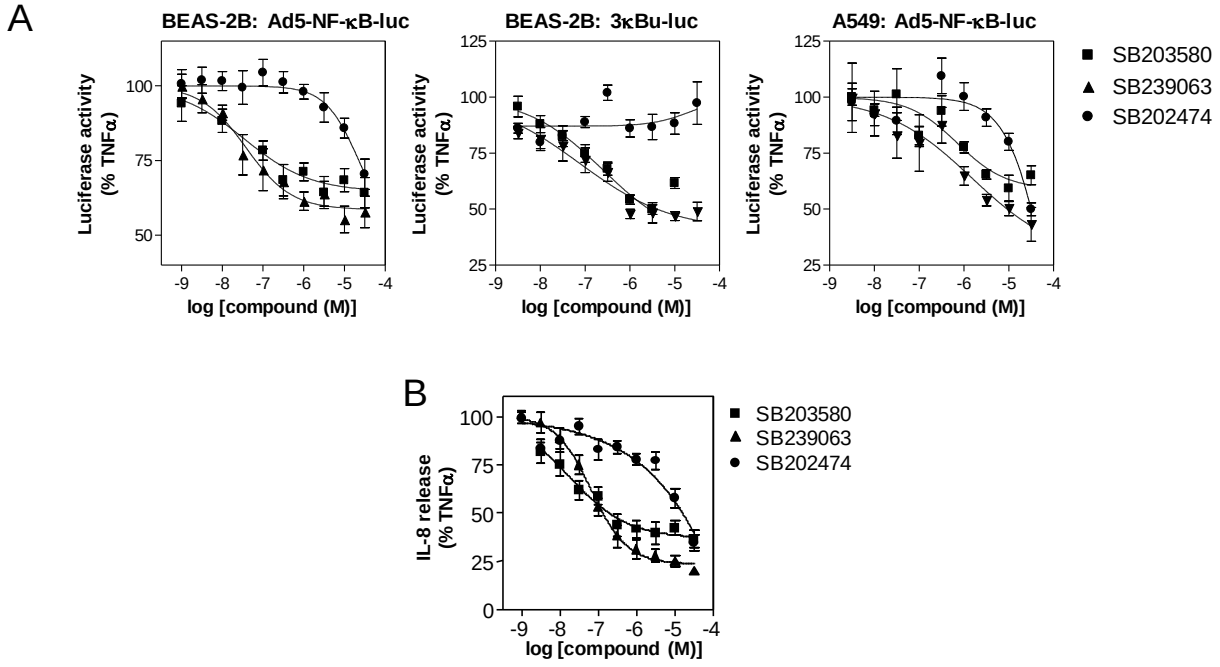

Figure S3. **Effect of p38 inhibitors on NF-κB-dependent transcription and IL-8 release.** *A,* BEAS-2B cells stably harboring the NF-κB-dependent reporter, 3κBu-luc, BEAS-2B and A549 cells infected with the NF-κB-dependent reporter Ad5-NF-κB-luc were incubated with various concentrations of SB203580, SB239063 or SB202474 for 30 min. Cells were then stimulated for 6 h with TNF-α (10 ng/ml) before harvesting for luciferase activity determination. Data, (n = 4 – 8), expressed as % TNF-α stimulated cells are plotted as means ± SE. *B,* BEAS-2B cells were treated as in *A* and supernatants harvested for analysis of IL-8 release by ELISA. Data (n = 5 – 6) are expressed as % TNF-α stimulated cells, are plotted as means ± SE.

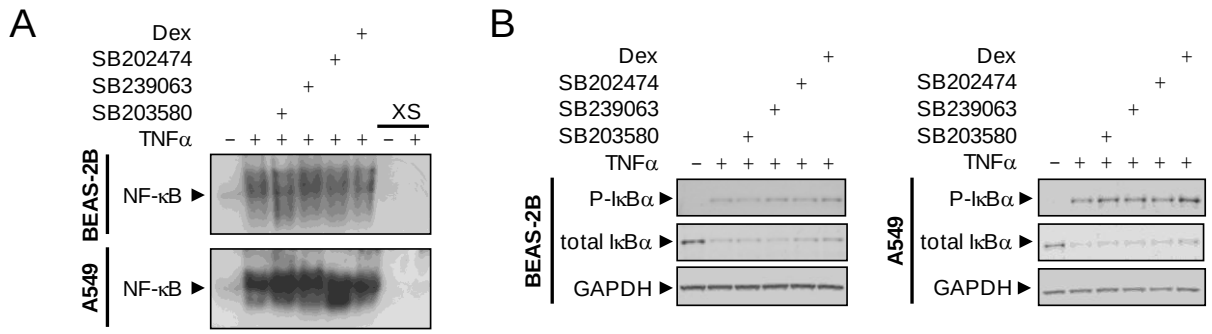

Figure S4. **Effect of dexamethasone and p38 inhibitors on NF-κB DNA binding and signaling pathway.** *A*, BEAS-2B and A549 cells were pre-treated with dexamethasone (1 μM), SB203580, SB239063 or SB202474 (all 10 μM) for 1 h prior to stimulation with TNFα (10 ng/ml). Cells were then harvested and nuclear proteins extracted for analysis by EMSA. Specificity of binding to the NF-κB consensus probe is indicated by competition with 100 × excess cold competitor probe (XS). Blots representative of two such experiments are shown. *B*, BEAS-2B and A549 cells were treated as in *A*, harvested for protein after 4 min and then subject to western blot analysis for serine 32/36 phosphorylated IκBα (P-IκBα), total IκBα and GAPDH. Blots representative of two such experiments are shown.

REFERENCES

1. Nasuhara, Y., Adcock, I. M., Catley, M., Barnes, P. J., and Newton, R. (1999) *J. Biol. Chem.* **274,** 19965-19972