Supplement 1. Materials and Methods.

Microarray experiment.

RNA isolation and quality evaluation. Isolated RNA was purified using RNeasy mini column purification (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. Integrity of RNA was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The purity/concentration was determined using a GeneSpec III (Miraibio). All RNA samples used for hybridization had an OD260/280 and OD260/230 ratio >1.8 and total RNA concentration > 1μg/ml.

Target preparation and microarray hybridization. All gene array hybridizations were performed at the Functional Genomics Facility, University of Chicago. The target preparation protocol followed the Affymetrix GeneChip Expression Analysis Manual (Santa Clara, CA). Briefly, 10 μg of total RNA was used to synthesize double-stranded cDNA using the Superscript Choice System (Life Technologies). First strand cDNA synthesis was primed with a T7-(dT24) oligonucleotide. From the phase-log gel-purified cDNA, biotin-labeled antisense cRNA was synthesized using BioArray High Yield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY). After precipitation with 4 M Lithium Chloride, 20 μg of cRNA was fragmented in fragmentation buffer (40 mM Tris-Acetate, pH 8.1, 100 mM KOAc, 30 mM MgOAc) for 35 minutes at 94°C and then 12 μg of fragmented cRNA was hybridized to U133 Plus 2.0 Arrays (Affymetrix) for 16 hours at 45°C and 60 rpm in an Affymetrix Hybridization Oven 640. The arrays were washed and stained with streptavidin phycoerythrin in Affymetrix Fluidics Station 450 using the Affymetrix GeneChip protocol and scanned using the Affymetrix GeneChip Scanner 3000.

Data acquisition and analysis. The acquisition and initial quantification of array images was performed using the GCOS (Affymetrix). Subsequent data analyses were performed using DNA-Chip Analyzer 1.3 with the CEL files. We used a PM-MM model to estimate gene expression level and the invariant set approach for data normalization. For comparison analyses, thresholds for selecting significant genes were set at a relative difference > 1.5 fold, absolute difference > 100 signal intensity and statistical difference at $p<0.05$.

Nuclei isolation and western blot analysis. Mock- or tributyrin (TB)-treated PC-3 cells were harvested using a cell lifter (Fisher Scientific), centrifuged for 5 sec at maximum speed using a bench top mini centrifuge and resuspended in 1 ml of PBS. Cell pellets were resuspended in buffer A (500µl 1M HEPES, pH 7.9, 75µl 1M MgCl2, 500µl 1M KCl, 25µl 1M DTT, 100µl 0.1M PMSF, 48.2 ml dH_2O) containing proteinase inhibitor mix (Sigma). Cells were set on ice for 10 minutes and lyzed with a glass/Teflon homogenizer (50 strokes). The efficiency was monitored by staining with trypan-blue. Lysed cells were centrifuged at maximum speed for 1 minute. Supernatants containing soluble cytosolic proteins were stored at -80°C. Pellets were resuspended in 0.1 ml of buffer B (100µl 1M HEPES, pH 7.9, 2.5ml glycerol, 840µl 5M NaCl, 15µl 1M MgCl2, 4µl 0.5M EDTA, 5µl 1M DTT, 6.4ml dH_2O) containing proteinase inhibitor mix (Sigma). Nuclei were incubated on ice for 15 minutes and centrifuged at maximum speed at 4°C for 10 minutes. Aliquots were store at -80°C. To reduce salt content in the samples, extracts were acetone precipitated and solubilized in ice-cold radioimmunoprecipitation assay buffer (50mM Tris, pH 7.4, 1% Nonidet P-40, 0.25%

sodium deoxycholate, and 150 mM NaCl). SDS was added to a final concentration of about 2%. Concentrations of extracted proteins were measured using a Bio-Rad kit (Bio-Rad, Hercules, CA). 30 μg of proteins extracted from nuclei were mixed with 5x sample lysis buffer (0.3 mM Tris, pH 6.8, 50% glycerol, 25% β-mercaptoethanol, 20% SDS, 0.01% bromophenol blue), boiled for 5 minutes and loaded into a 10-well 10% polyacryamide gel. Separated proteins were transferred to a PVDF membrane using a semi-dry blotter (Bio-Rad). The PVDF membrane was blocked with 2% milk in PBS + 0.05% Tween 20 (MPT) for 10 minutes and incubated with primary antibody anti-p65 (Santa Cruz Biotechnology, Santa Cruz, CA) at dilution 1:500 for 60 min at room temperature. The membrane was washed three times for 5 minutes with MPT. The secondary antibody (goat anti-mouse labeled with horse radish peroxidase, Southern Biotechnology Group, Birmingham, AL) was used at dilution 1:20,000 for 60 minutes at room temperature. The membrane was washed three times for 5 minutes with MPT and developed using SuperSignal West Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL).

Chromatin immunoprecipitation. PC-3 cells ($5x10^6$ total cells) were either mock-treated or treated with 1 mM TB for 3 h. Chromatin immunoprecipitation was performed by the following modifications of existing protocols {Kuo, 1999 #600; Weinmann, 2001 #601}. Cells were exposed to 1% formaldehyde in growth medium for 10 min. The formaldehyde was neutralized by the addition of glycine to 125 mM and the cells were scrapped and washed with PBS by centrifugation (800 rpm, 5 min at room temperature). The cytoplasm was removed with Cell Lysis Buffer (5 mM Hepes, pH 8.0, 85 mM KCl,

0.5% NP-40). Nuclei were recovered by centrifugation and lysed in Nuclei Lysis Buffer (50 mM Tris pH8.0, 10 mM EDTA, 1% Triton-X-100, sigma protease inhibitor cocktail, 1 mM PMSF). Chromatin was sheared by application of 5 x 20 sec pulses with a Kontes Micro-Disrupter tip sonicator. Affinity matrices were prepared and blocked by equilibrating ProteinA-agarose (Sigma) in buffer and then incubating with 20 μg/ml sheared salmon sperm DNA for 3h followed by extensive washing in Dilution buffer (1.1% Triton-X-100, 1.2 mM EDTA, 16.7 mM Tris, pH 8.0, 167 mM NaCl). Beads were resuspended in 100 μl Dilution buffer. To precipitate the cross-linked p65 protein, 4 μg of anti-p65 rabbit polyclonal antibody (Santa Cruz) was added to the prepared chromatin from mock- and TB-treated cells in Dilution buffer, and the reactions were rocked overnight at 4°C. A mock reaction was assembled by combining equal amounts of chromatin from the samples but including no primary antibody. Blocked protein A-agarose was added and the reactions were rocked at 4°C for 0.5 h. The beads were then recovered by centrifugation and washed extensively with 2 washes of dialysis buffer and 6 washes in wash buffer (100 mM Tris, pH 8.0, 500 mM LiCl, 1% NP-40). Bound material was removed with 3 washes (15 min each) in freshly prepared elution buffer (50 mM $NaHCO_3$, 1% SDS) and RNA and crosslinks removed by addition of RNAaseA to 1 μg/ml, NaCl to 300 mM, and heating at 70°C for 6 h. Nucleic acids were then precipitated with ethanol and the resultant pellet dissolved in 10 mM Tris, pH 7.5, 5 mM EDTA, 0.25% SDS, and Proteinase K added (100 μg/ml) and the reactions subsequently incubated at 45°C for 2h. The reactions were extracted with phenol/chloroform (1:1), chloroform, and then the supernatants were ethanol-precipitated. The pellet was dried and resuspended in 50 μl of TE. Two μl of the precipitate was analyzed for specific promoter

sequences by PCR using primer pairs for the human CCL20 (5' CTTCGCACCTTCCCAATATG 3' and 5' TGTACACAGAAGGCGTGTTG 3'), CXCL3 (5' AGTGCACGGGGGTTACTCT 3' and 5' GATCGGCGAACCCTTTTTAT 3') and IL-8 (5' CATCAGTTGCAAATCGTGGA 3' and 5' GAAGCTTGTGTGCTCTGCTG 3'). PCR products were analyzed by horizontal agarose gel electrophoresis.

Transfection of siRNA oligonucleotides. PC-3 cells were plated at 1×10^6 cells per 100 mm plate a day prior the transfection in antibiotics-free RPMI 1640 media (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum. Transfection of the siRNA oligonucleotides (siRNA for p65: sense 5' GCCCUAUCCCUUUACGUCA dTdT 3', non-specific control siRNA: sense 5' AGGUAGUGUAAUCGCCUUG dTdT 3') was performed using Oligofectamine (Invitrogen) according manufacture's protocol to result in a final RNA concentration of 50 nM. siRNA duplexes were purchased from MWG-Biotech and diluted as a 200 μM stock solution. Two days after transfection, cells were treated with 1 mM TB for 12 h. At the end of treatment, cells were harvested, washed in cold PBS and resuspended in 900 μl of PBS. Cells in 300 μl of PBS were used for Western blotting (rabbit anti-human GAPDH antibody, Abcam, Cambridge, MA, was used at 1:2500 dilution to monitor loading) and the rest was used for RNA isolation in order to perform a real-time PCR assay as described above. Western blots were quantified by the software Quantity One (Bio-Rad).