Supplemental:

Cells: An alveolar macrophage cell line (MH-S cells: ATCC no. CRL-2019) were cultured in RPMI medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. Cells were maintained at 37°C with 5% CO_2.

Reagents: Mouse recombinant IFN-gamma was obtained from R&D systems (Minneapolis, MN); MG132, 17-AAG, ERK1/2 inhibitors (PD98059) were purchased from EMD Biosciences (San Diego, CA); Hsp70 siRNA was obtained from Ambion (Austin, TX) and Fugene from Roche Laboratories (Indianapolis, IN). *Antibodies*: The following list of antibodies indicates the dilution used in the western blotting experiments: STAT1 1:1000 in 3% dried milk (BD Biosciences, San Diego CA). Phosphorylated STAT1 (P-STAT1) 1:1000 in 3% BSA and ERK1/2 and PhhosphoERK1/2 antibodies 1:500 in 3% BSA (Cell Signaling, Beverly MA). Hsp70 and Hsp90 1:1,000 in 3% dried milk (Stressgen, San Diego CA). iNOS 1:1000 in 3% BSA (EMD Biosciences, San Diego CA). Secondary antibodies, horse radish peroxidase conjugated goat anti-mouse (HRP-GAM) and HRP-goat anti-rabbit (HRP-GAR) were diluted in 3% milk at 1:2000 (Cappell, Aurora OH).

SPR activation using heat (Heat Shock): The incubation time at 43°C was determined based on the absence of cell death, using the Alamar blue assay, and the efficiency of SPR activation by measuring the intracellular increase in inducible Hsp70 protein.

Western Blotting: Cells were seeded and cultured for 24 hours prior to any treatment. After treatment with IFN-γ (10 ng/ml) for the indicated time, the cells were washed three times with phosphate buffered saline (PBS) on ice, and lysed with 25 mM Tris buffer (pH 7.5) containing 100 mM NaCl, 5mM $MgCl_2$, 1 mM EDTA, 1% Triton, 1 mM sodium orthovanadate, 1 mM NaF, and protease inhibitors. Nuclei were pelleted at 14,000 x g for 5 minutes. The protein concentrations of the samples were determined using the BioRAD protein assay (Hercules, CA). Equal amounts of protein were separated by gel electrophoresis using SDS-polyacrylamide. Proteins were transferred to a nitrocellulose or PVDF membrane. The membrane was blocked in either 5% dried

milk or 3% bovine serum albumin (BSA) in Tris buffered saline containing 0.1% Tween-20 (TBST). Membranes were incubated overnight in primary antibody, washed and incubated in HRP-conjugated secondary antibody for 1 hour. Proteins were detected by chemiluminescence (Supersignal, Pierce, Rockford, IL).

EMSA: MH-S cells were plated in 35-mm culture dishes at a density of 2 x 10^6 per well and were cultured for 24h. Cells were subjected to heat shock treatment (as described above) before their exposure to IFN-γ. For nuclear protein isolation, cells were washed three times with cold PBS and then harvested by scraping. Cells were collected by centrifugation, and the cell pellets were resuspended in lysis buffer (10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.5 mM DTT, 0.4% Nonidet P-40, and 0.5 mM PMSF). After incubation on ice for 10 min, crude nuclei were isolated by centrifugation at 10,000 x *g* for 10 min at 4°C. The supernatant was removed, three cell pellet volumes of a high salt extraction buffer (20 mM HEPES (pH 7.9), 420 mM NaCl, 0.5 mM DTT, 1 mM EDTA, 0.5 mM PMSF) were added, and the suspension was incubated on a rotary shaker for 30 min at 4°C. The sample was centrifuged at 16,000 x *g* for 30 min at 4°C, the supernatant (containing extracted nuclear proteins) was collected, protein concentration was determined, and the lysate was stored at $^{-}$70°C until further use. EMSA was performed using a STAT1 consensus oligonucleotide probe (5'-ATG TGA GGG GAC TTT CCC AGG C-3') that was end labeled with [γ-^{32}P]ATP. Nuclear protein (5 µg) was incubated with 100,000 cpm of ^{32}P-labeled STAT1 consensus nucleotide for 20 min in a binding buffer consisting of 10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 50 mM NaCl, 0.5 mM DTT, 0.5 mM EDTA, 4% glycerol, and 1 µg of poly(dI-dC). The specificity of the DNA/protein binding was determined by competition reactions in which a 100-fold molar excess of unlabeled STAT1 oligonucleotide was added. After incubation, the samples were analyzed by nondenaturing electrophoresis, and the bands were visualized by autoradiography.

Isolation of membrane enriched fraction: MH-S cells were scraped into a hypotonic buffer (10 mM HEPES, 10 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, protease inhibitors, phosphatase inhibitors). Cells were allowed to swell by rotation for 30 minutes at 4°C. Cells were broken open by dounce using a loose fitting pestle and 18

strokes. Nuclei were removed by centrifugation 1,000 rpm for 3 minutes at 4^oC. The supernatant was subjected to centrifugation at 15,000 x g for 15 minutes at 4^oC to pellet an enriched membrane fraction [1]. This fraction was resuspended in Laemmli sample buffer (LSB) and further analyzed by SDS-PAGE and western blot.

Immunoprecipitations: MH-S cells were lysed as described for western blots with the addition of 40mM sodium molybdate for the immunoprecipitation of STAT1/Hsp90 complexes. The lysates were incubated with primary antibodies overnight at 4^oC. Antigen-antibody (Ag-Ab) complexes are captured using protein A sepharose. The protein A sepharose-Ag-Ab complex was pelleted at 3,000 rpms for 4 seconds. The Ag-Ab complexes were washed three times and subjected to SDS-PAGE and western blot as described above.

Adenovirus infection: Adenovirus infection of macrophages was performed according the published protocol of Fasbender and colleagues [2]. Briefly, calcium phosphate precipitates containing adenovirus were formed by mixing adenovirus in 1 ml of RPMI media and adding calcium to a final concentration of 2 mM. The mixture was incubated at room temperature for 60 minutes and then added to the macrophages for 60 minutes. The cells were then washed and placed in RPMI containing 10% heat inactivated FBS and pen/strep for 24 hours then stimulated with IFN-γ for 24 hours prior to analysis of NO production using the extracellular media. The cells were lysed and Hsp70i expression determined by western.

Statistics: All of the data are summarized as mean +/- SEM. One-way ANOVA and the Fisher's exact *t* test were used to compare experimental with control groups. A p value of <0.05 was considered statistically significant.

References:

1. Shah M, Patel K, Fried VA, Sehgal PB. Interactions of STAT3 with caveolin-1 and heat shock protein 90 in plasma membrane raft and cytosolic complexes. Preservation of cytokine signaling during fever. J Biol Chem 2002; 277:45662-9.
2. Fasbender A, Lee J, L, Walters R, W, Moninger T, O, Zabner J, Welsh M, J. Incorporation of Adenovirus in Calcium Phosphate Precipitates Enhances Gene Transfer to Airway Epithelia In Vitro and In Vivo. J. Clin. Invest. 1998; 102:184-193.