Supplementary Materials and Methods

Bacterial Strains and Culture Conditions

NTHi strain 12 was used in the present studies. Both live bacteria and bacterial lystaes were used as treatment. Live NTHi was used at a concentration of 10-100 bacteria/cell (m.o.i) *in vitro*. For *in vivo* experiment, mice were intratracheally inoculated with NTHi ($3X10^7$ cfu/mouse). For making NTHi crude extract, NTHi were harvested from a plate of chocolate agar after overnight incubation and incubated in 30 ml of brain-heart infusion broth supplemented with 3.5 μg/ml nicotinamide adenine dinucleotide. After overnight incubation, NTHi were centrifuged at 10,000 x g for 10 min, and the supernatant was discarded. The resulting pellet of NTHi was suspended in 10 ml of PBS and sonicated. Subsequently, the lysate was collected and stored at -70°C. We chose to also use NTHi lysates because of the following reasons. NTHi has been shown to be highly fragile, and it has the tendency to autolyse. NTHi undergo spontaneous autolysis *in vivo* and the autolysis can also be triggered *in vivo* under various conditions, including antibiotic treatment. Therefore, the use of lysates of NTHi represents a common clinical condition *in vivo*, especially after antibiotic treatment.

Real-Time Quantitative RT-PCR Analysis of TNF-α and IL-1α

Total RNA was isolated by using TRIzol reagent (Invitrogen) by following the manufacturer's instructions. For the reverse transcription reaction, TaqMan reverse transcription reagents (Applied Biosystems) were used. Briefly, the reverse transcription reaction was performed for 60 min at 37°C, followed by 60 min at 42°C by using oligo(dT) and random hexamers. PCR amplification was performed by using TaqMan Universal Master Mix. Predeveloped TaqMan assay reagents (probe and primer mixture of human TNF-α and IL-1α were used to detect expression of each gene. In brief, reactions were performed in duplicate containing 2x Universal Master Mix, 2 μl of template cDNA, 200 nM primers, and 100 nM probe in a final volume of 25 μl, and they were analyzed in a 96-well optical-reaction plate (Applied Biosystems). Probes include a fluorescent reporter dye, 6-carboxyfluorescein (FAM), on the 5' end and labeled with a fluorescent quencher dye, 6-carboxytetramethyl-rhodamine (TAMRA), on the 3' end to allow direct detection of the PCR product. Reactions were amplified and quantified by using an ABI 7700 sequence detector and the manufacturer's corresponding software (Applied Biosystems). Relative quantity of TNF-α and IL-1α mRNA was obtained by using the comparative Ct Method (for details, see User Bulletin 2 for the ABI PRISM 7700 sequence-detection system) and was normalized by using predeveloped TaqMan assay reagent human cyclophilin as an endogenous control (Applied Biosystems).

Plasmids, Transfection, and Luciferase Assay

The reporter construct NF-κB luc was generated as described (Shuto *et al*, 2001). It contains three copies of the NF-κB site from the IL-2 receptor (α) promoter by using the following oligonucleotides: 5'-TCGAGACGGCAGGGGAATCTCCCTCTCCG-3' and 3'-CTGCCGTCCCCTTAGAGGGAGAGGCAGCT-5'. The reporter construct was sequenced to verify the number and orientation of inserted oligonucleotides. Smad3DN and WT, Smad4DN and WT were previously described (Jono *et al*, 2003). WT-p65, p65-KR, p65-K218R, p65-K221R, p65-K310R, WT-p300 and p300 HAT mutant were previously described (Chen *et al*, 2002, 2005). All transient transfections were performed in triplicate by using TransIT-LT1

reagent (Mirus) by following the manufacturer's instructions. Luciferase activity was normalized with respect to β-galactosidase.

Western Blot Analysis
Antibodies against phospho-IκBα (Ser32), IκBα, phospho-p65 NF-κB (Ser276) and phospho-p65 NF-κB (Ser536) were purchased from Cell Signaling Technology, p65, p300 and TFIIB from Santa Cruz Biotechnology, Acetyl-Lysine from Upstate. Phosphorylation of IκBα, p65 NF-κB (Ser536) and p65 NF-κB (Ser276) were detected as described and by following the manufacturer's instructions (Shuto *et al*, 2001).

Immunoprecipitation
For immunoprecipitation, 800 μl of lysates were incubated for 1 hour at 4 °C with control mouse IgG antibody and protein A/G-agarose (Santa Cruz Biotechnology). After centrifugation, p65 antibody (Santa Cruz Biotechnology) was incubated with supernatant for 1 h at 4°C, followed by incubation overnight with protein A/G-agarose. Immunoprecipitates were washed twice with RIPA buffer, resuspended in 2x SDS loading buffer, and separated on 8% SDS-PAGE, followed by Western blot analysis.

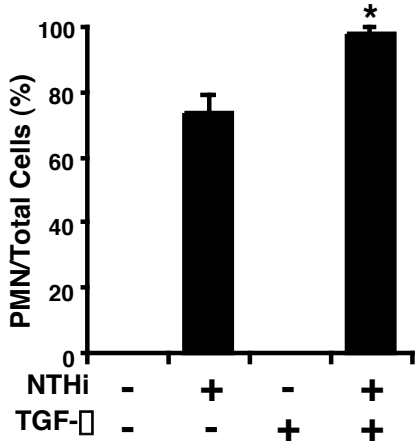
Electrophoretic Mobility Shift Assay (EMSA)
5-7μg of nuclear extracts was prepared and non-radioactive EMSA was performed using an EMSA kit according to the manufacturer's instructions (Pierce). Oligonucleotide (oligo) 5'-AGTTGAGGGGACTTTCCCAGGC-3' was used as the consensus κB site-containing probe. TNF-α promoter derived NF-κB probe was previously described (Yao *et al*, 1997). Oligo was obtained from Integrated DNA Technologies and end-labeled with biotin-N4-CTP using Terminal Deoxynucleotidyl transferase (Pierce). For supershift assays, nuclear extracts were preincubated with antibodies p65, p50, Smad3, Smad4, Smad1/2/3, (Santa Cruz Biotechnology) for 1 h on ice before the addition of reaction mixtures with the labeled probes.

Chromatin immunoprecipitation (ChIP) assays.
ChIP was performed using an EZ ChIP kit (Upstate Biotechnology). HeLa cells (2 X 10^6 cells) were crosslinked in 1% formaldehyde for 10 min. The cross-linking reaction was stopped by the addition of 0.125 M glycine for 5 min. Cells were washed with ice-cold PBS containing protease inhibitors and lysed in SDS lysis buffer containing protease inhibitors. Samples were sonicated on ice to an average size of 200–1,000 bp and centrifuged for 10 min at 14,000 rpm at 4°C to remove insoluble material. Chromatin was precleared with salmon sperm DNA/protein G agarose for 1 h at 4°C, and 1% of this solution was saved (input chromatin) and processed with the eluted immunoprecipitates beginning with the cross-link reversal step. Samples were incubated overnight at 4°C with anti-p65 antibody (Santa Cruz Biotechnology) or isotype-matched control IgG (negative control) with rotation. Immune complexes were collected by incubating samples with salmon sperm DNA/protein G agarose for 1 h at 4°C with rotation. The beads were pelleted by brief centrifugation and washed as per the manufacturer's instructions. The immunocomplexes were eluted from the agarose beads by elution buffer (1% SDS and 0.1 M $NaHCO_3$). Input and immunoprecipitated chromatin were incubated with 200 mM NaCl at 65°C for 4 h to reverse DNA–protein cross-links. Immunoprecipitated DNA was purified as per the manufacturer's instructions (Upstate Biotechnology) and analyzed by PCR using specific primers: TNF-α (forward: 5'-CCCTCCAGTTCTAGTTCTATC-3'; reverse: 5'-

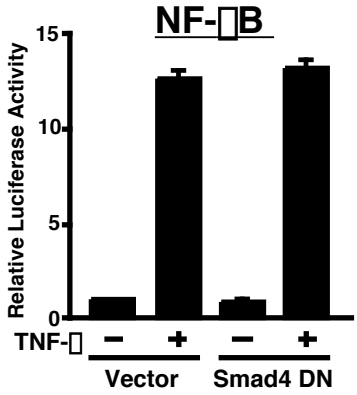
GGGGAAAGAATCATTCAACCA-3') and β-actin (5'-GTCGACAACGGCTCCGGC-3'; reverse: 5'-GGTGTGGTGCCAGATTTTCT-3') (Chen et al, 2005; Taggart *et al*, 2005).

Mouse and Animal Experiments

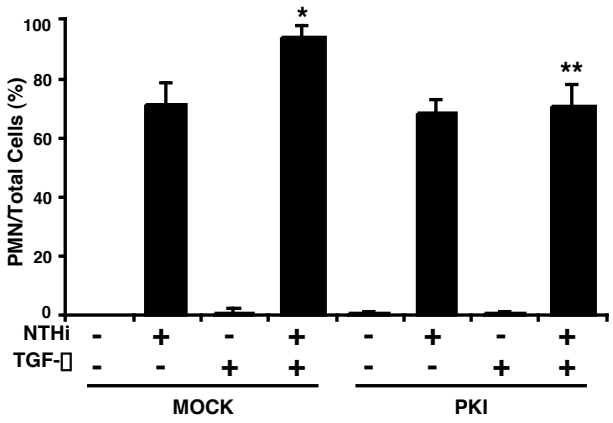
Animal studies were performed as described (Mikami et al. 2006). BALB/cJ and C57BL/6 mice were purchased from Jackson Laboratories. For the mouse model of NTHi-induced pneumonia, anesthetized mice were intratracheally inoculated with NTHi ($3X10^7$ cfu/mouse) with or without intratracheal inoculation of TGF-β for 3 h for mRNA expression and 6 h for polymorphonuclear neutrophil (PMN) recruitment analysis. For PKI experiment, PKI (5 mg/kg) was first inoculated intraperitoneally 2 h prior to intratracheal inoculation of NTHi and TGFβ. For mRNA expression analysis, total RNA was extracted from whole lung tissues of NTHi-inoculated mice with or without TGF-β (100ng/mouse) by using TRIzol, 3 h after NTHi inoculation or saline for control, and real-time quantitative PCR was performed as described above. For PMN recruitment analysis, broncho-alveolar lavage (BAL) was performed by cannulating the trachea with sterilized PBS, 6 h after intratracheal inoculation of NTHi with or without TGF-β (50ng per mouse), and cells from BAL was stained with Hemacolor (EM Science) after cytocentrifugation (Shandon Cytospin4, Thermo Electronic Co.). All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at University of Rochester.



Supplementary Fig. S1. **TGF-β1 synergistically enhanced NTHi-induced PMN infiltration in lung of BALB/c mice *in vivo*.** Ratio of the PMN number/Total cell number (%) was shown. Values are mean ± S.D. (n=3). *$p<0.05$ compared with NTHi inoculation alone.



***Supplementary Fig. S2*. Overexpressing dominant-negative mutant of Smad4 did not inhibit TNF-α-induced NF-κB activation in HeLa cells.** Values are mean ± S.D. (n=3).



Supplementary Fig. S3. PKI markedly inhibited the TGF-β-mediated enhancement of PMN accumulation in BAL fluids from the lungs of the NTHi-inoculated mice. Ratio of the PMN number/Total cell number (%) was shown. Values are mean ± S.D. (n=3). *$p<0.05$ compared with NTHi inoculation alone in Mock group; **$p<0.05$ compared with NTHi plus TGF-β in Mock group.