Supporting Online Material

Materials and Methods:

Immunohistochemistry: Tissue sections were deparaffinized and rehydrated in an ethanol series. Sections were blocked for non-specific binding with 3% normal serum from chicken and incubated with the primary antibodies for 1 hour at room temperature. For immunofluorescence, sections were then incubated with secondary antibodies at 1:200 for 30 minutes at room temperature (Molecular Probes). Sections were counterstained with 4',6'-diamidinio-2-phenylindole (DAPI) and mounted with Vectashield hard set mounting medium (Vector Labs).

ELISA Analysis: We measured the active mature fragment of TGFβ using the R&D Duoset assay (Cat#DY1679). Polystyrene plates (Maxisorb; Nunc) were coated with capture antibody in PBS overnight at 25°C. The plates were washed 4 times with 50mM Tris, 0.2% Tween-20, pH 7.0-7.5 and then blocked for 90 minutes at 25°C with assay buffer (PBS containing 4% BSA (Sigma) and 0.01% Thimerosal, pH 7.2-7.4). The plates were washed 4 times and 50µl assay buffer was added to each along with 50µl of sample or standard prepared in assay buffer and incubated at 37°C for 2h. The plates were washed 4 times and 100µl of biotinylated detecting antibody in assay buffer was added and incubated for 1h at 25°C. After washing the plate 4 times strepavidin-peroxidase polymer in

casein buffer (RDI) was added and incubated at 25°C for 30min. The plate was washed 4 times and 100µl of commercially prepared substrate (TMB; Neogen) was added and incubated at 25°C for approximately 10-30 min. The reaction was stopped with 100µl 2N HCl and the A450 (minus A650) was read on a microplate reader (Molecular Dynamics). A curve was fit to the standards using a computer program (SoftPro; Molecular Dynamics) and cytokine concentration in each sample was calculated from the standard curve equation. Levels below the assay range should be interpreted as "Low" (below the lower detection limit). Because of the shape of the standard curve, negative values are occassionally calculated for some samples. These should also be interpretted as "undetectable." Values above the range are calculated by extrapolation and thus may not be accurate. We have marked those samples that are above or below the range in the "Inrange" column of the results as "High".

Zymography: Lung tissue lysates were prepared in a cold room at 4C. Tissue was homogenized in 50µL PBS and centrifuged at 14000 RPM for 20 min. The supernatant was removed and used as sample lysates. Fifty µg of lung lysates were loaded on a10% Criterion Zymography Precast Gel (Biorad) and run at 120V. Twenty-five µL of recombinant mouse MMP9 protein (R&D Systems, Minneapolis, MN) was loaded as a positive control. The gel was soaked in 1X Renaturing Buffer (Biorad) twice for 30 minutes each at room temperature and

incubated in 1X Development Buffer (Biorad) overnight at 37C. The gels were stained with Coomassie Brilliant Blue R-250 Staining Solution (Biorad), followed by 1X Destain Coomassie R-250 Solution (Biorad) until a clear band appeared against a blue background.

Measurement of mouse lung mechanics. After being connected they were paralyzed with Succinylcholine (75 mg/kg) and ventilated with a tidal volume of 0.2 mL of 100% oxygen at a rate of 150 breathes/min, with a positive end expiratory pressure (PEEP) of 3 cm H_2O. A deep inspiration (to 30 cmH_2O for 5 sec) was given and then the animal was returned to normal ventilation. One minute later Rrs and Ers were measured(1). After determination of Rrs and Ers, ventilation was stopped, and the tracheal cannula was occluded for 4 min, which led to complete degassing of the lungs by absorption atelectasis. Quasi-static PV curves were performed as previously reported(2). Quasistatic compliance of the respiratory system was computed from the P-V relationships as the slope of the deflation limb between 3 and 8 H_2O, which is where the curves are most linear.

Real-Time PCR: Total RNA isolated from lung tissues was treated with DNase and reverse-transcribed using a first-strand DNA sysnthesis kit from Invitrogen. The PCR was performed on an ABI Fast 7500 System (Applied Biosystems, Foster City, CA). TaqMan probes for the respective genes were custom-generated by Applied Biosystems based on the sequences in the Illumina array and used per

manufacturer's instructions. The expression levels of target genes were determined in triplicate from the standard curve and normalized to *Gapdh* mRNA level.

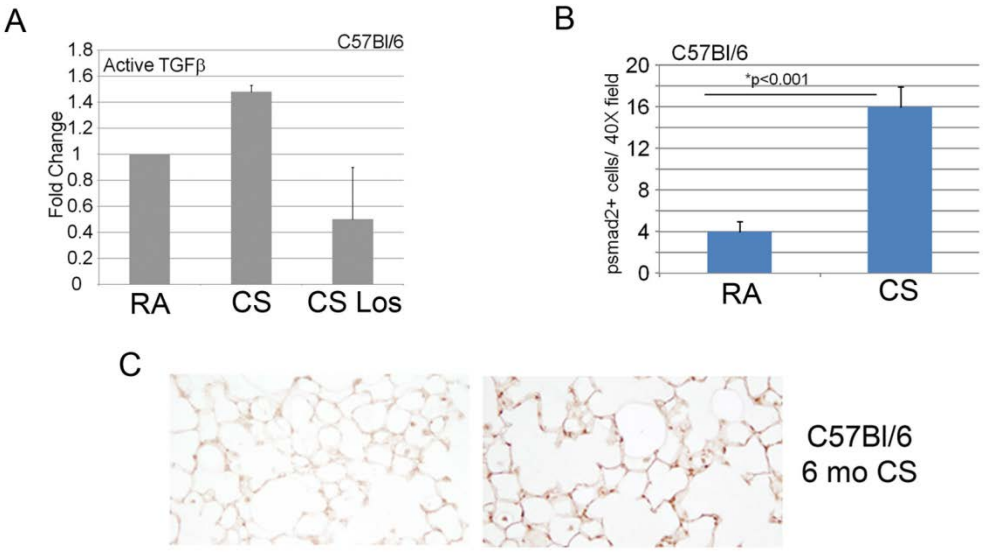
RNA Extraction and Illumina Chip Hybridization: Total RNA was extracted from the designated murine lungs, six in each treatment group, using the Trizol Reagent method (Invitrogen, Carlsbad, California 92008, cat. no. 15596-026). Additional purification was performed on RNAeasy columns (Qiagen, Valencia, CA 913555, cat. no. 74104). The quality of total RNA samples was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The six RNA samples from each time point were pooled into two groups comprised of three murine specimens. RNA samples were labeled according to the chip manufacturers recommended protocols. In brief, for Illumina, 0.5 μg of total RNA from each sample was labeled by using the Illumina TotalPrep RNA Amplification Kit (Ambion, Austin, TX 78744-1832, cat. no. IL1791) in a process of cDNA synthesis and *in vitro* transcription. Single stranded RNA (cRNA) was generated and labeled by incorporating biotin-16-UTP (Roche Diagnosics GmbH, Mannheim, Germany, cat. no. 11388908910). 0.85 ugs of biotin-labeled cRNA was hybridized (16 hours) to Illumina's Sentrix MouseRef-8 Expression BeadChips (Illumina, San Diego, CA 92121-1975, cat.no. BD-26-201). The hybridized biotinylated cRNA was detected with streptavidin-Cy3 and quantitated using

Illumina's BeadStation 500GX Genetic Analysis Systems scanner. *The complete data set has been submitted and is currently available in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/; accession number: GSE33561).*

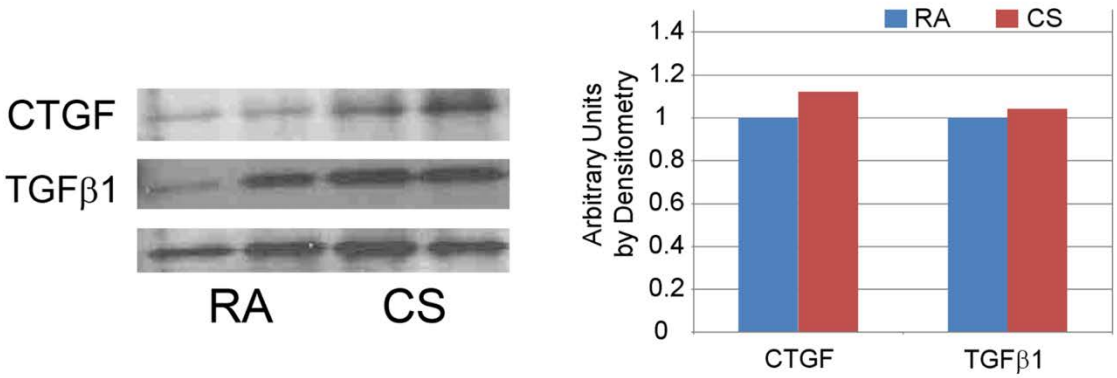
Microarray Analysis: DAVID Analysis (NIAID) was used to analyze expression profile pathway data from the various treatment groups(3). DAVID provides typical batch annotation and gene-GO term enrichment analysis to highlight the most relevant GO terms associated with a given gene list. Extended annotation includes GO terms, protein-protein interactions, protein functional domains, disease associations, bio-pathways, sequence general features, homologies, gene functional summaries, gene tissue expressions, literatures, etc. In the DAVID annotation system, the Fisher Exact test is adopted to measure the gene-enrichment in annotation terms and generate significance estimates (p-values).

1. Ewart, S., Levitt, R., and Mitzner, W. 1995. Respiratory system mechanics in mice measured by end-inflation occlusion. *J Appl Physiol* 79:560-566.
2. Soutiere, S.E., and Mitzner, W. 2004. On defining total lung capacity in the mouse. *J Appl Physiol* 96:1658-1664.
3. Huang da, W., Sherman, B.T., and Lempicki, R.A. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4:44-57.

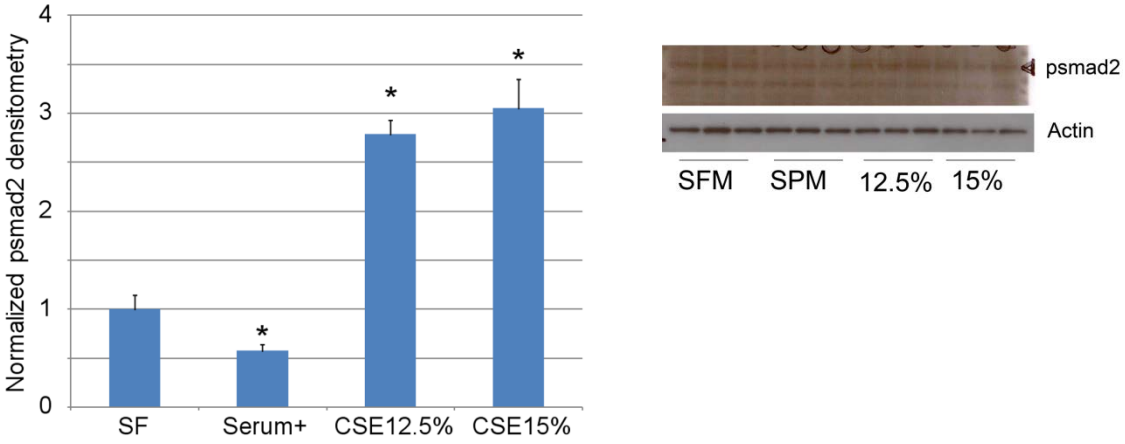
Supplementary Data



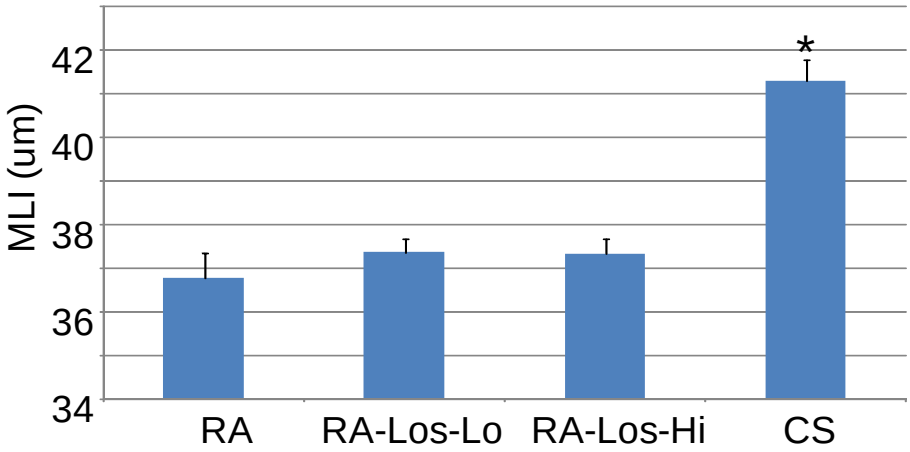
Supplementary Figure 1: Chronic cigarette smoke induces TGFβ expression and signaling in C57Bl/6 lungs. A. TGFβ induction profile by ELISA analysis in lung lysates from C57Bl/6 mice exposed to two weeks of CS. *p<0.01, **p<0.05, CS versus RA (room air) or CS+Los versus CS. N=3-5 mice per treatment group. B. Representative histologic sections of lungs from adult C57Bl/6 mice exposed to room air (RA), right panel, or chronic CS, left panel, subjected to immunohistochemical staining for psmad2. 20X magnification. N=4-8 mice per treatment group. C. Quantitative immunohistochemistry of psmad2 staining in room air and CS-exposed C57Bl/6 mice depicted in S1B.



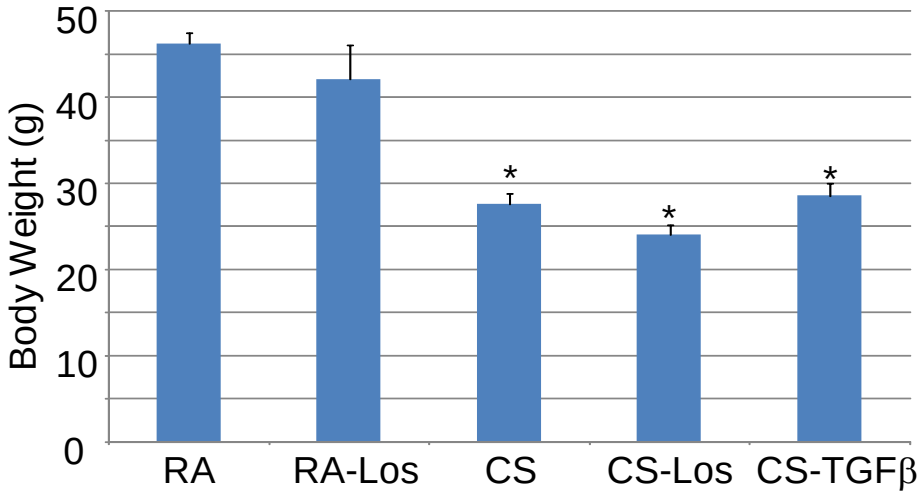
Supplementary Figure 2. Cigarette smoke induced alterations in the expression of TGFb signaling mediators. A. Left, representative immunoblot analysis of CTGF and TGFb1 expression in lung lysates from AKR/J mice exposed to 4 mos CS. Right, densitometric quantitation of designated immunoblots. N=6-8 mice per condition.



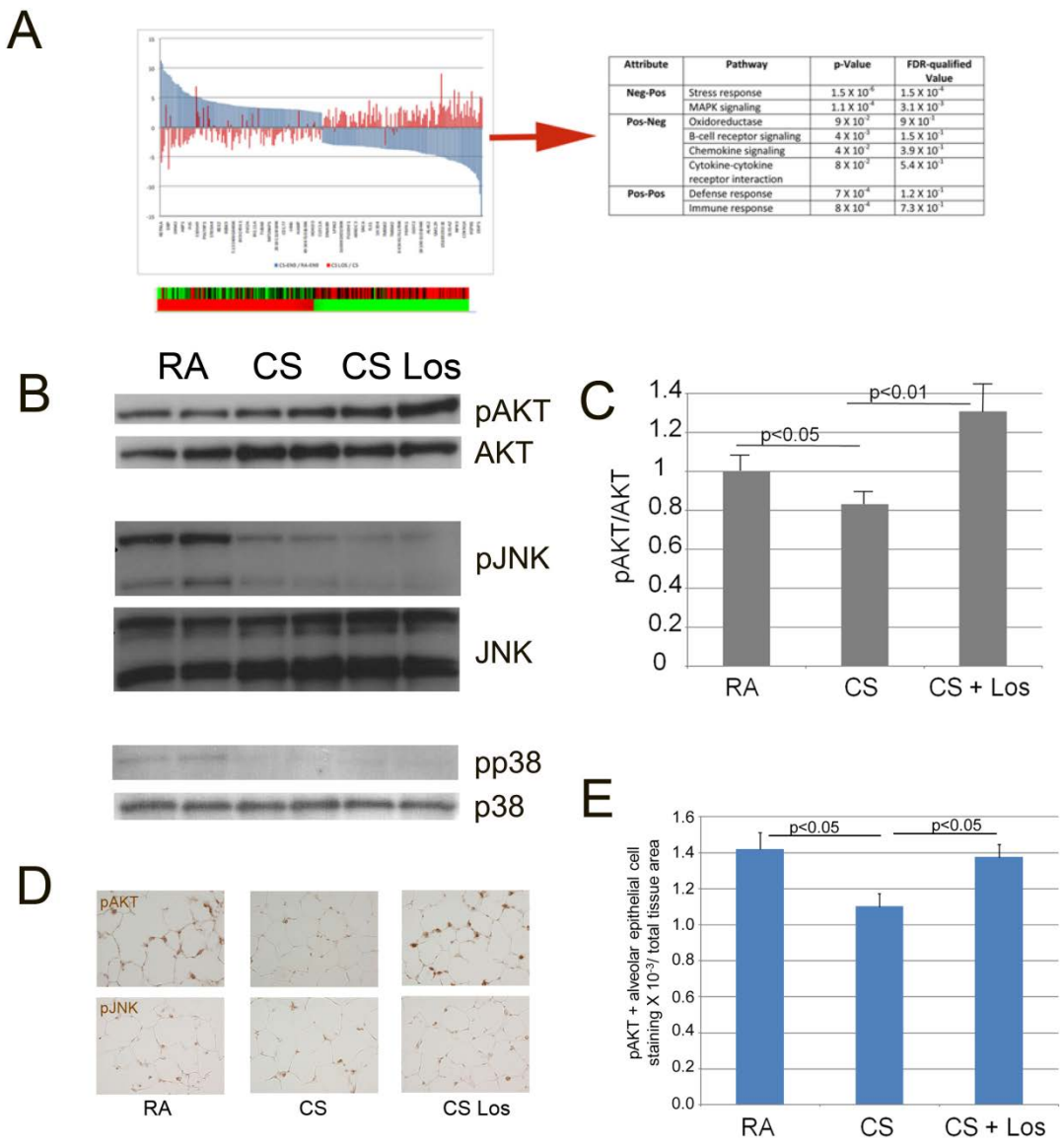
Supplementary Figure 3. Cigarette smoke extract treatment of MLE12 cells induces TGFb signaling. Densitometric analysis of psmad2 expression in MLE12 cells upon exposure to 72 h of cigarette smoke extract. SF-serum free media, CSE-cigarette smoke extract.



Supplementary Figure 4. Effect of Losartan treatment on airspace dimension. Two months of low or high dose Losartan treatment does not affect airspace dimension. *p<0.05 compared with all other treatment groups. N=6-8 mice per condition.



Supplementary Figure 5. Effect of Losartan treatment on body weight. Two months of Losartan treatment does not rescue CS-induced weight loss. *p<0.05 compared RA treatment. N=6-8 mice per condition.



Supplementary Figure 6. Transcriptional analysis of protective effect of Losartan in CS-exposed mice. Graphic depiction of proportion of genes dysregulated with CS (blue) and corrected with concurrent Losartan treatment (red) utilizing expression profile analysis of whole lung RNA. Selected genes are identified. Bottom panel shows heatmap of dataset. Red-induced genes. Green-repressed genes. B. Representative immunoblotting of activated and total Akt, JNK and p38 in lung lysates from mice in designated groups. C. Densitometric analysis of pAKT normalized to Akt in lung lysates. N=4-6 mice per group. D. Representative immunohistochemistry of activated Akt (top) and activated JNK (bottom) of murine lungs with designated exposures. N=5-8 mice per treatment and per exposure. E. Quantitative immunohistochemistry of

pAKT staining in airspaces of mice normalized to tissue area. Data are represented as means plus SEM. N=4-6 mice per condition or per treatment.