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Supplementary Materials for

Cigarette Smoke Induction of Osteopontin (SPP1) Mediates T_H17 Inflammation in Human and Experimental Emphysema

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The PDF file includes:

Fig. S1

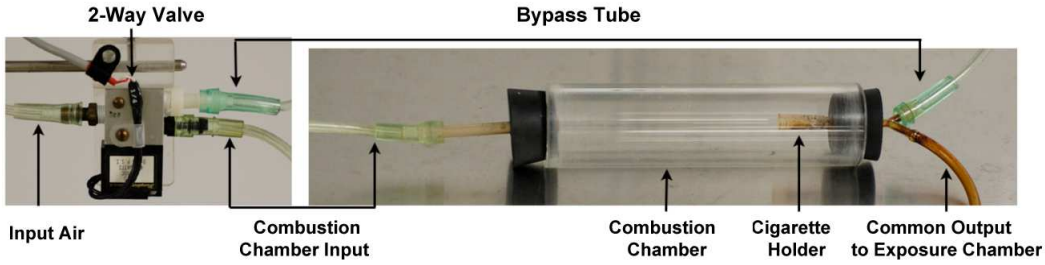

Fig. S1. Active cigarette smoke exposure assembly
Mice are exposed to the actively generated smoke of 4 cigarettes delivered over one hour, once per day, 5 days/week, for 4-6 months. Air (4L/min flow rate) feeds into a timer controlled 2-way valve that directs the flow of gas to a glass combustion chamber containing a lit cigarette (not shown) or to a bypass tube. Both paths feed into a common output leading to an exposure chamber capable of housing up to 10 mice. Smoke in the exposure chamber is directed through an exhaust port to the atmosphere.

Fig. S2

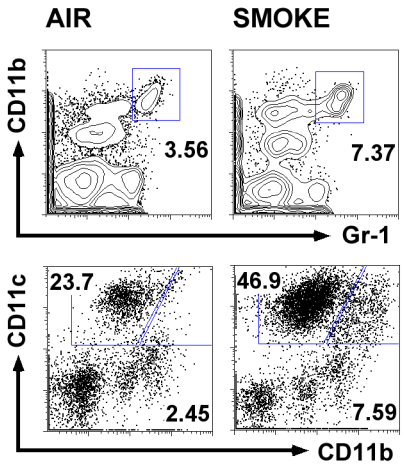

Fig. S2. Increased macrophage, dendritic cell, and neutrophil in response to cigarette smoke.
Total lung single cell suspensions were stained with Pacific Blue-B220, APC-CD11c, PE-CD11b, and APC-Cy7-Gr1 antibodies and analyzed by flow cytometry. Upper panel shows the percentage of $CD11b^{high}Gr1^{+}$ neutrophils gated from $B220^{-}CD11c^{-}$ population. Lower panel shows the percentage of $CD11c^{+}CD11b^{low}$ alveolar macrophages and $CD11c^{+}CD11b^{+}$ lung dendritic cells from B220- population. Data represent 3 different experiments with five mice each experiment.

Fig. S3

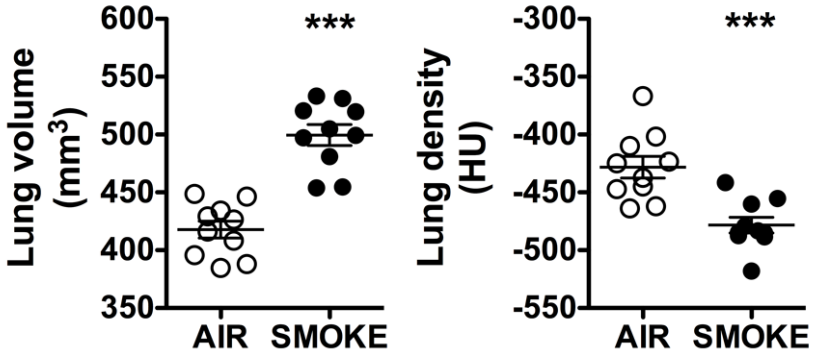

Fig. S3. Increased lung volume and decreased density in response to cigarette smoke.
Mouse micro-CT scan was obtained at Baylor College of Medicine mice phenotyping core facility using 35-micron resolution (n=10 in each group) protocol. Lung volume and average density were calculated using Amira 3.1.1 software. Data shown are mean±s.e.m. ***P<0.001, determined by student t test.

Fig. S4

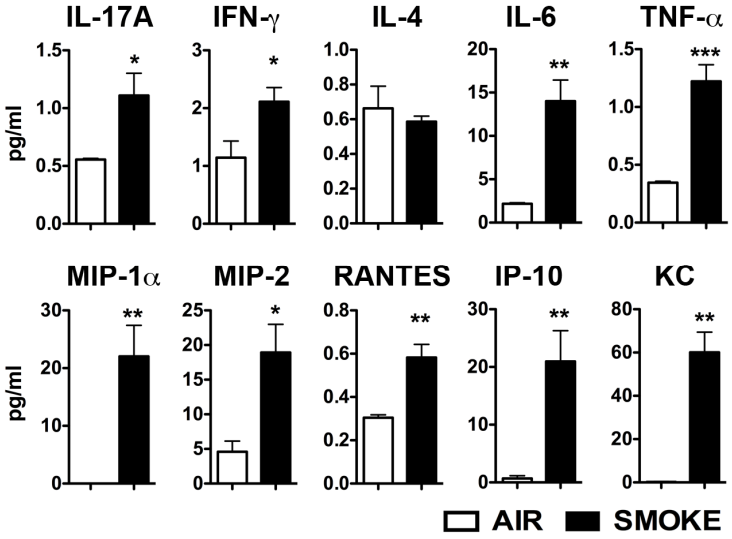

Fig. S4. Cytokine profile in BALF of air- or smoke-exposed mice.
Cytokine concentration of BAL fluid from 4-month air or cigarette smoke exposed mice were measured using Milliplex kit for (n=5 in each group). Data shown are mean±s.e.m.. *P<0.05, **P<0.01, ***P<0.001, determined by student t test.

Fig. S5

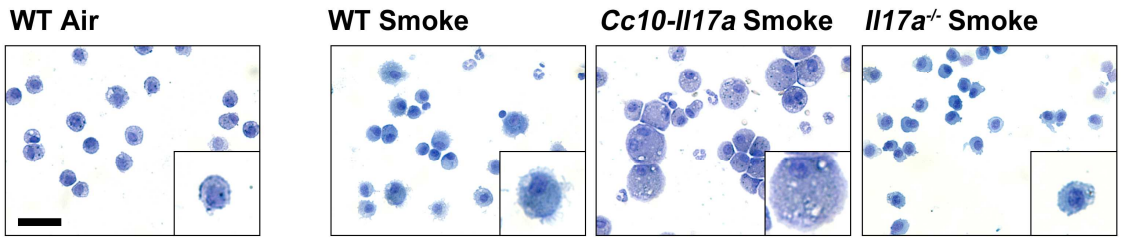

Fig. S5. Morphology of alveolar macrophage in response to smoke.
Representative HEMA3 stain of BALF cells that were collected following 4 months of air or smoke exposure in wild type (WT), IL-17A-overexpressing (*Cc10-Il17a*) mice, and IL-17A deficient (*Il17a*$^{-/-}$) mice. Data represents at least two different experiments with 4-8 mice each experiment. Scale bar: 50μm.

Fig. S6

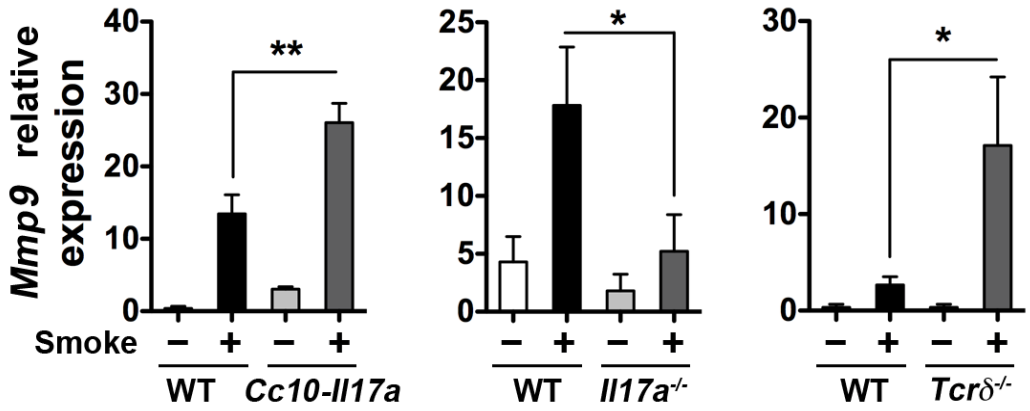

Fig. S6. *Mmp9* expression in BALF of smoke-exposed mice.
Mmp9 mRNA expression from total BALF cells of air and smoke exposed mice (n = 4-8 / group; data normalized to 18S expression). Data shown are mean±s.e.m.
**P<0.01,*P<0.05, determined by one-way ANOVA test.

Fig. S7

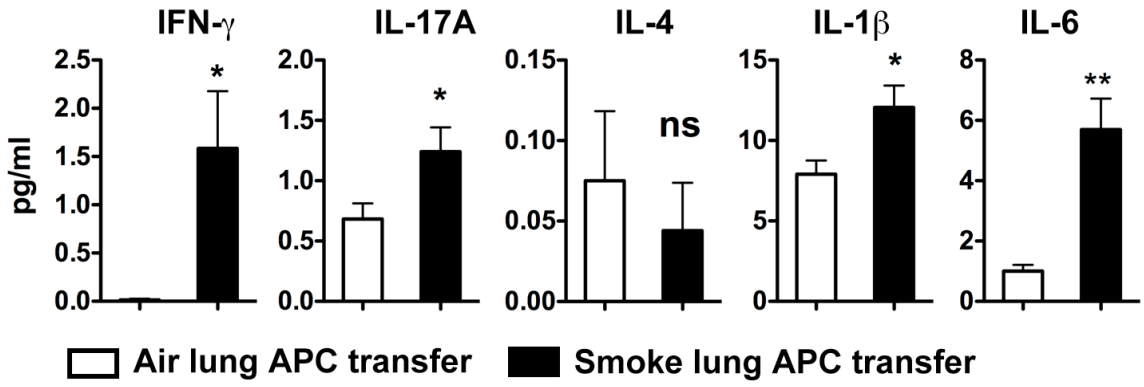

Fig. S7. Inflammatory cytokine production after lung APC adoptive transfer. Cytokine concentration in BALF of WT recipient mice following adoptive transfer of air or smoke exposed lung APCs; cytokines were measured by Milliplex kit. (n = 4~5 / group). Data shown are mean±s.e.m. *P<0.05, **P<0.01, ns, no significance, determined by student t test.

Fig. S8

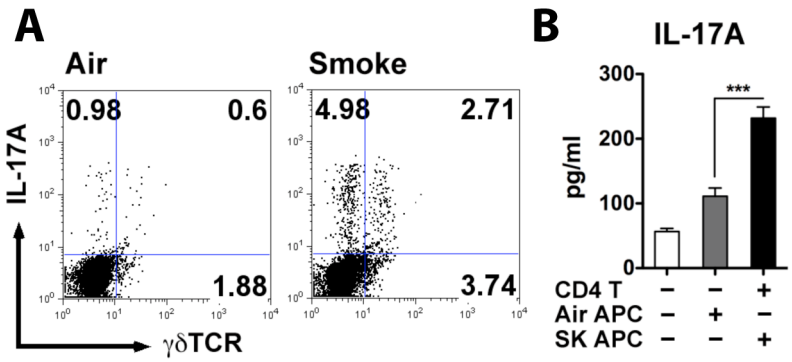

Fig. S8. Short-term cigarette smoke exposure activates APC and IL-17A production. **(A)** Representative IL-17A intracellular staining of T cells isolated from lungs of mice exposed to 6-8 weeks of cigarette smoke. Data are representative of 2 independent studies, n=4 / group / study. **(B)** Splenic CD4 T cells were cultured for 3 days with mouse lung APCs isolated from air- or smoke-exposed animals in the presence of soluble anti-CD3 antibody (1μg/ml). IL-17A was quantified from culture supernatants. (n = 4, data represent two independent experiments). SK, smoke. Data shown are mean±s.e.m. ***P<0.001, determined by one-way ANOVA test.

Fig. S9

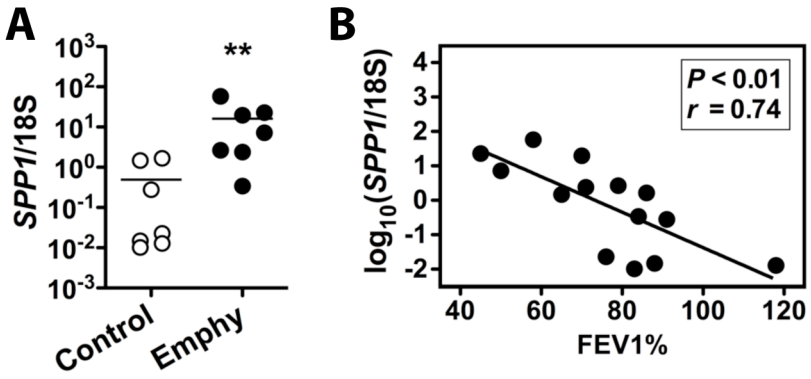

Fig. S9. Enhanced SPP1 expression in human lung macrophage.
(A) SPP1 mRNA expression in control (n=7) and emphysema lung macrophages (n=7) as determined by quantitative PCR (normalized to 18S expression). **P<0.01, determined by Mann-Whitney test. **(B)** Correlation of lung macrophages SPP1 mRNA expression as shown in (A) and emphysema disease severity (FEV1%) (control n=7; emphysema n=7). Statistics are derived by linear regression.

Fig. S10

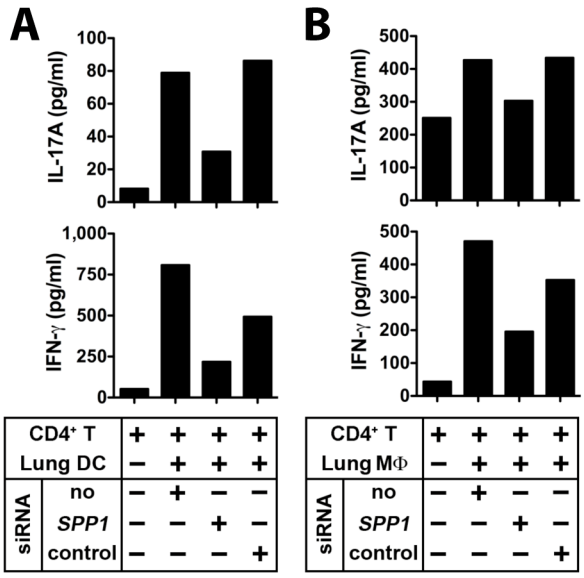

Fig. S10. SPP1 siRNA inhibits IL-17A and IFN-γ cytokine production.
(A) Emphysema lung DCs or **(B)** macrophages were transfected with 10ng of SPP1 or scramble control siRNA. After 24 hrs, lung DCs and macrophages were cultured with allogenic human PBMC CD4 T cells plus 1μg/ml anti-CD3 for 3 days. The concentration of IFN-γ and IL-17 present in the cell culture supernatant was measured by Milliplex. Data are representative of three independent experiments.

Table S1

Characteristics	Controls	Emphysema
No.	13	13
Age (mean ± SEM)	63±4	66±2
Male (No.)	11	11
Lung Function		
%FEV1 (mean ± SEM)	92±5	66±4
GOLD stages	0	I-III
Smoking Status, No.		
Never	1	-
Former	6	7
Current	6	6
PPY, (mean ± SEM)	32±6	65±7
Quitting years, (mean ± SEM)	13±6	5±3

Table S1. Demographics of study participants.
No., number; SEM, Standard Error; %FEV1, Forced Expiratory Volume in 1 second % predicted; GOLD, Global initiative Obstructive Lung Disease; PPY, pack-year of smoking where one pack/day/year equals 1 PPY; Quitting years, the number of years since smoke cessation.