IL-18 Induces Emphysema, and Airway and Vascular Remodeling via IFN-γ, IL-17A and IL-13

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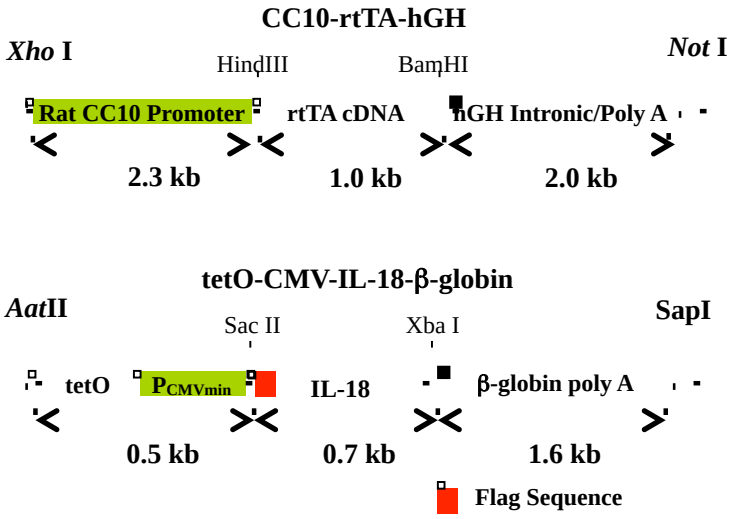
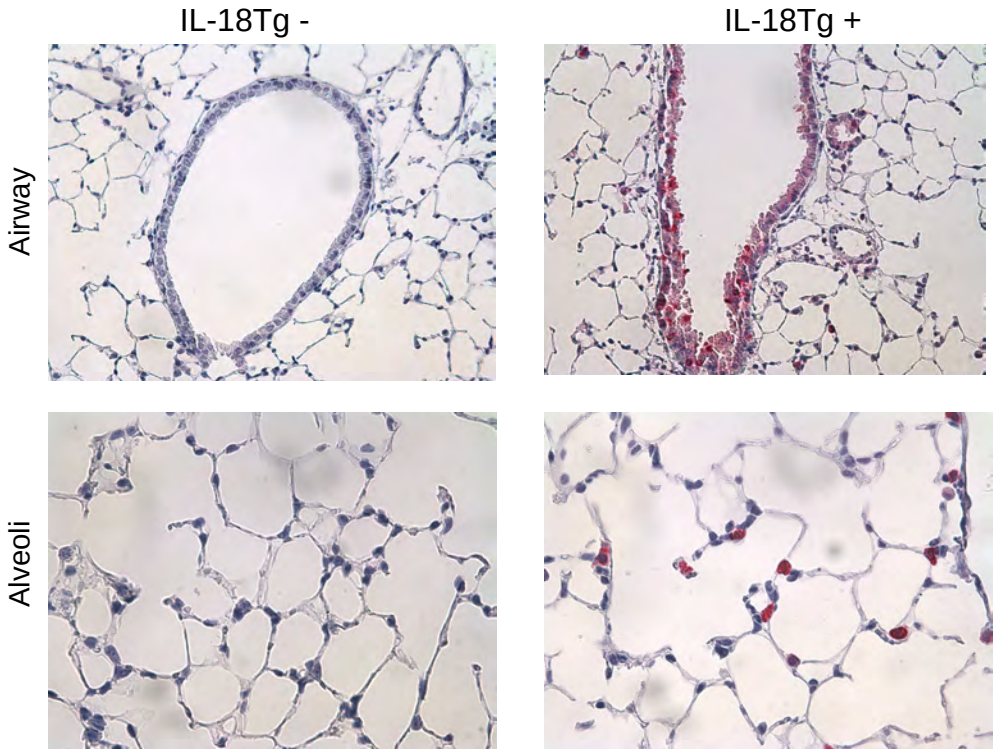
ONLINE DATA SUPPLEMENT

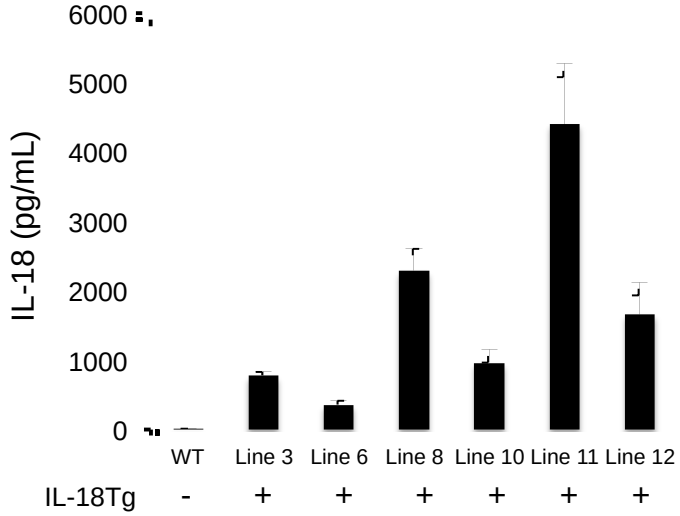
S1. Transgenic constructs.



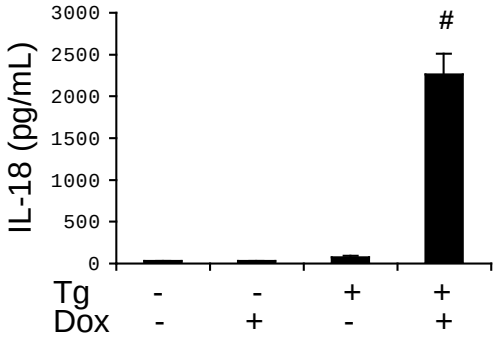
S2. Immunohistochemistry evaluation of IL-18 expression. IL-18 positive cells in airway (X20) and alveoli (X40) are stained in red color.



S3. Levels of IL-18 in BAL fluids from different lines of transgenic mice as assessed by ELISA.



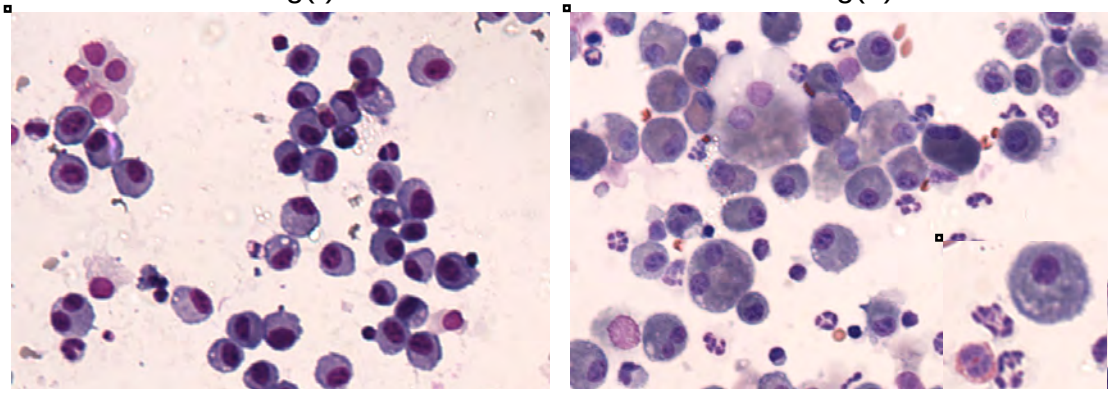
S4. . Levels of IL-18 in BAL fluids from Line 8 mice on normal water and dox as assessed by ELISA.



S5. For flowcytometric analysis, antibodies against CD4, CD8, CD19, NK1.1, IFN-γ, IL-13 and IL-17A were purchased from eBioscience (San Diego, CA). For single cell preparation of lung tissues, Ficoll-Paque Premium® (#17-5442-02) was purchased from GE Healthcare (Piscataway, NJ). For intracellular cytokine staining, fixation buffer and permeabilization buffer were purchased from eBioscience (San Diego, CA). The antibody against IL-18 were from Santa Cruz Biotechnology Inc (Santa Cruz, CA) and the antibodies against β-actin and perforin were from Cell Signaling Technology (Danvers, MA).

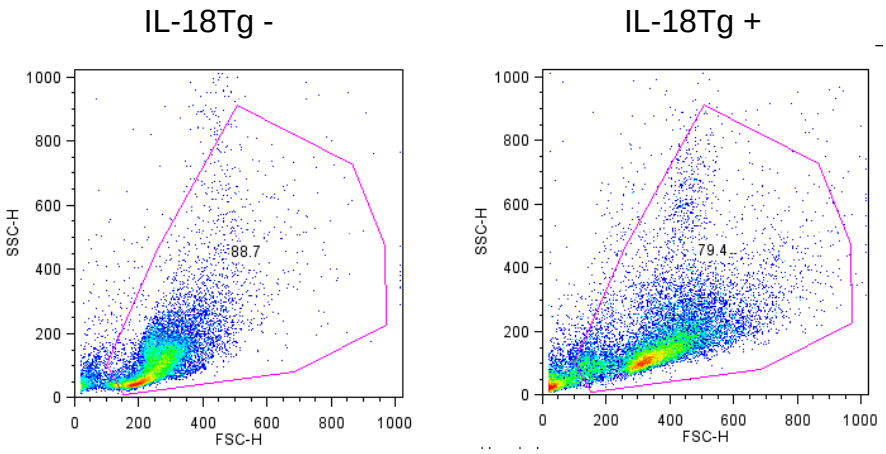
S6. Representative BAL cells. Compared to the cells from control mice (Tg-), activated and enlarged macrophages are readily seen from IL-18 Tg(+) mice (X20). In addition, neutrophils, lymphocytes and eosinophils are seen in cells from IL-18 Tg(+) mice, which are rarely seen from control mice. Inserted figure is taken in X40 magnification.

Tg(-) Tg(+)

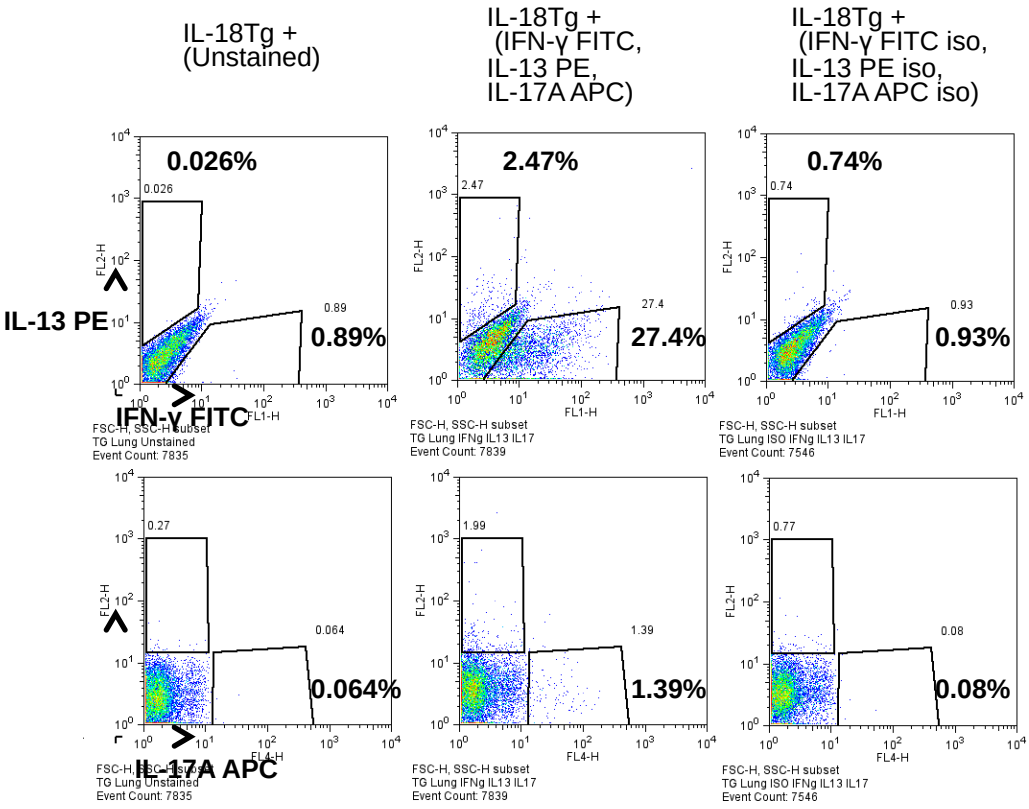


S7. For flow cytometric analysis, whole lung tissues were utilized. Lungs were perfused with PBS through the main pulmonary artery to eliminate blood cells in the pulmonary circulation and harvested en bloc. In these assessments bronchoalveolar lavage was not undertaken to be sure that intraalveolar cells were included in this analysis. Perihilar and mediastinal lymph nodes were then removed, isolated lungs were cut into small pieces (1-2mm^2), mechanically disintegrated and filtered through a 70-μm strainer. To enrich for tissue infiltrating inflammatory cells and eliminate red blood cells and cellular debris the cell suspensions were subjected to Ficoll-Paque density gradient centrifugation. For intracellular cytokine staining, cells were stimulated with phorbol myristate acetate (PMA, 50ng/ml) and ionomycin (1μg/ml) for 4 hours. Intracellular cytokine staining was undertaken using intracellular staining kit (eBioscience) as per the manufacturer's instruction.

S8. Representative gating (FSC/SSC) employed in flow cytometry



S9. Representative iso-type control staining utilized in evaluations of intracellular cytokines.



S10. The primers for the noted genes are detailed below;

GAPDH; Forward, 5’ – AGG TCG GTG TGA ACG GAT TTG– 3’
Reverse, 5’ – TGT AGA CCA TGT AGT TGA GGT CA– 3’

IFN-γ; Forward, 5’ – ATG AAC GCT ACA CAC TGC ATC – 3’
Reverse, 5’ – CCA TCC TTT TGC CAG TTC CTC – 3’

IL-13; Forward, 5’ – CCT GGC TCT TGC TTG CCT T – 3’
Reverse, 5’ – GGT CTT GTG TGA TGT TGC TCA – 3’

IL-17A; Forward, 5’ – TTT AAC TCC CTT GGC GCA AAA – 3’
Reverse, 5’ – CTT TCC CTC CGC ATT GAC AC – 3’

Elastin; Forward, 5’ – TTG CTG ATC CTC TTG CTC AAC – 3’
Reverse, 5’ – GCC CCT GGA TAA TAG ACT CCA C – 3’

Fibronectin; Forward, 5’ – GCA GTG ACC ACC ATT CCT G – 3’
Reverse, 5’ – GGT AGC CAG TGA GCT GAA CAC – 3’

Collagen 1A1; Forward, 5’ – GCT CCT CTT AGG GGC CAC T – 3’
Reverse, 5’ – CCA CGT CTC ACC ATT GGG G – 3’

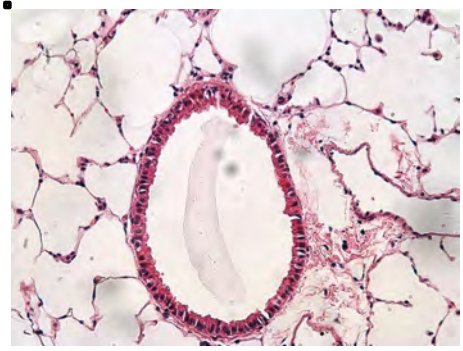
Raet-1; Forward, 5’ – AGG GCC AAA TTC CTA GTG CC – 3’
Reverse, 5’ – TGT CTG CAT TCG GGT ATC AAG A – 3’

Perforin; Forward, 5’ – AGC ACA AGT TCG TGC CAG G – 3’
Reverse, 5’ – GCG TCT CTC ATT AGG GAG TTT TT – 3’

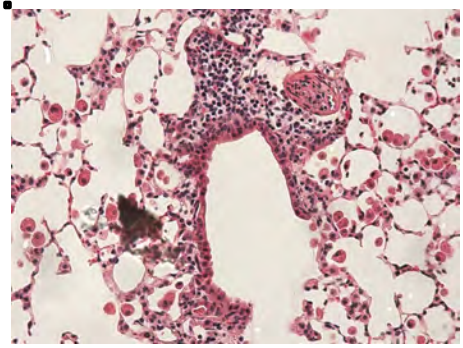
Granzyme-B; Forward, 5’ – CCA CTC TCG ACC CTA CAT GG – 3’
Reverse, 5’ – GGC CCC CAA AGT GAC ATT TAT T – 3’

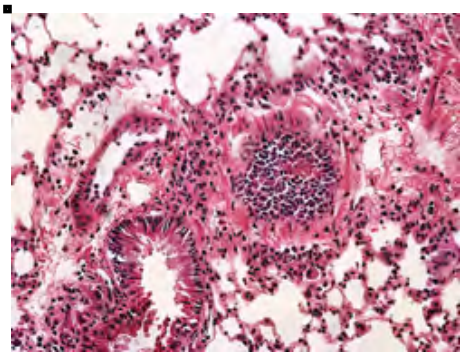
S11. Peribronchial & perivascular mononuclear cell infiltration in lung tissues from WT and IL-18 Tg (+) mice (H&E stain, X40)

IL-18Tg -



IL-18Tg +



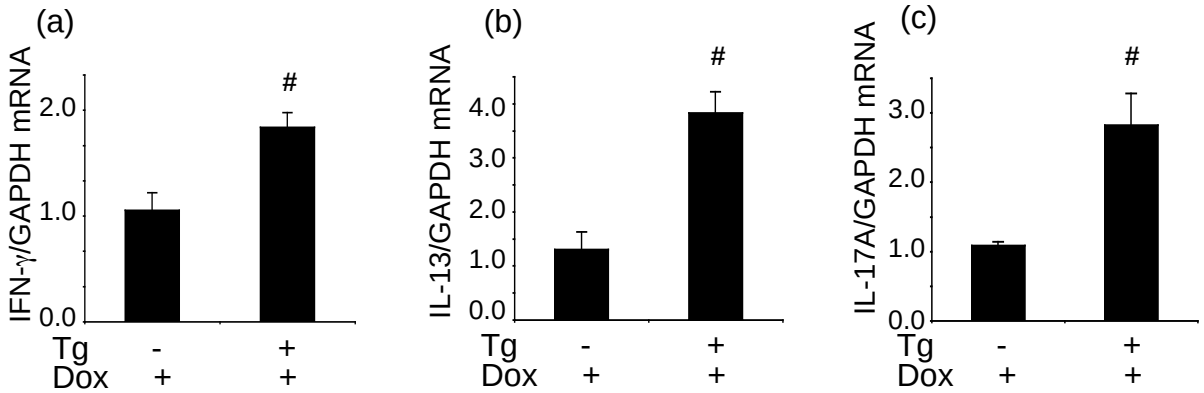


S12. Results of Intracelluar cytokine staining of single cell suspension from whole lung tissues. The values represent the % of the cells in flow cytometry analysis (mean ± standard error mean (SEM) of a minimum of 10 mice).

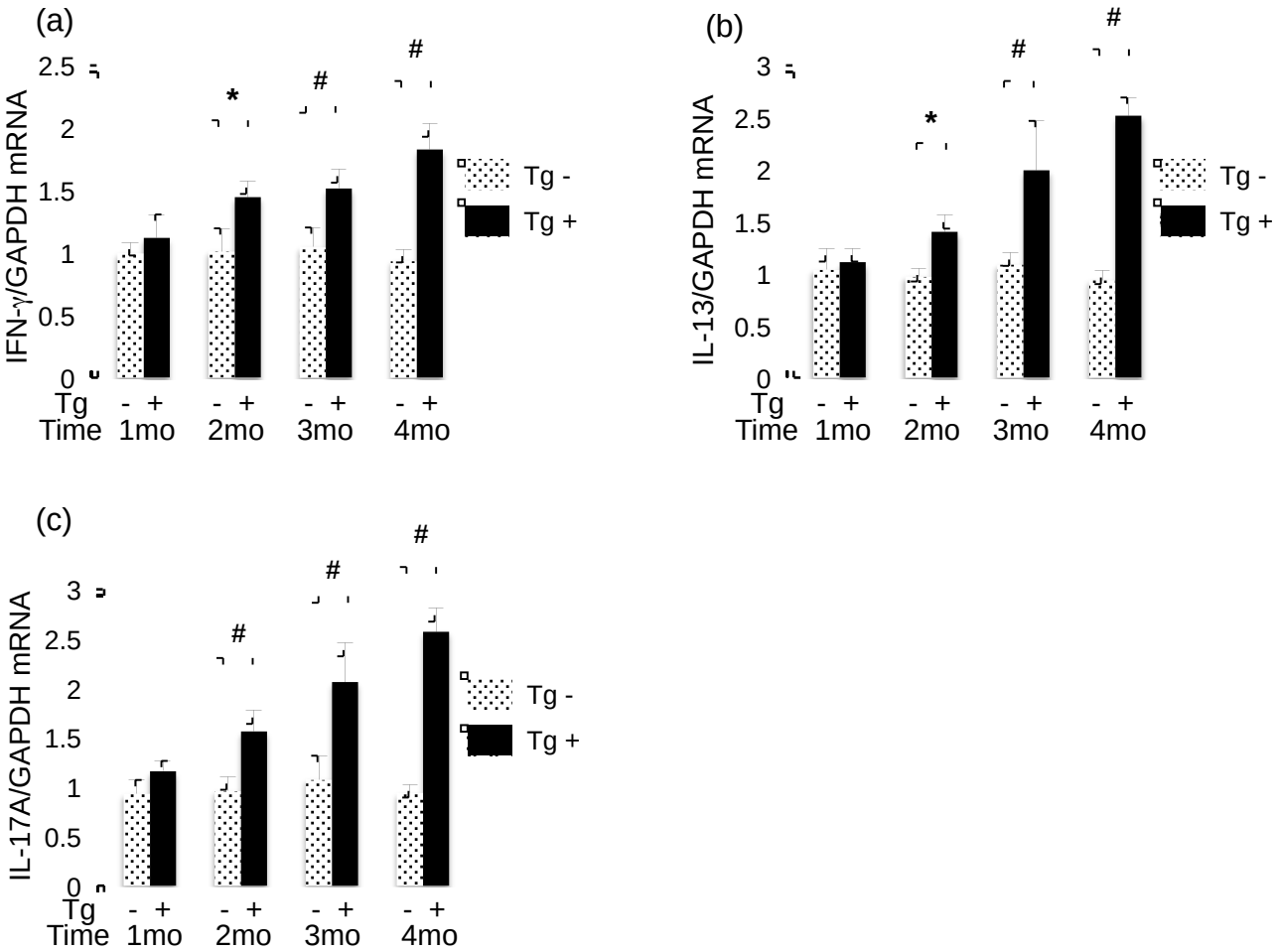
* Intracellular Cytokines in CD4+ cells					
Cell Population	WT	IL-18 Tg	IL-18 Tg/ IFN-g (-/-)	IL-18 Tg/ IL-17A (-/-)	IL-18 Tg/ IL- 13 (-/-)
CD4+, IFN-g+	1.70(±0.24)*	6.77(±1.80)	N.D.	10.18(±2.70)	9.50(±1.89)
CD4+, IL-17A+	0.08(±0.03)	2.39(±0.32)	3.62(±0.63)	N.D.	1.94(±0.57)
CD4+, IL-13+	0.31(±0.12)	2.57(±0.26)	4.44(±0.41)	2.03(±0.75)	N.D.

* % of cells in flow cytometry
N.D. = none detected

S13. . Levels of IFN-γ, IL-13 and IL-17A mRNA in Tg- and Tg+ mice



S14. Kinetics of IFN-γ, IL-13 and IL-17A mRNA accumulation in IL-18 Tg mice assessed via real-time RT PCR



S15. IL-6, CCL2 and CCL3 mRNA expressions evaluated by real-time RT PCR