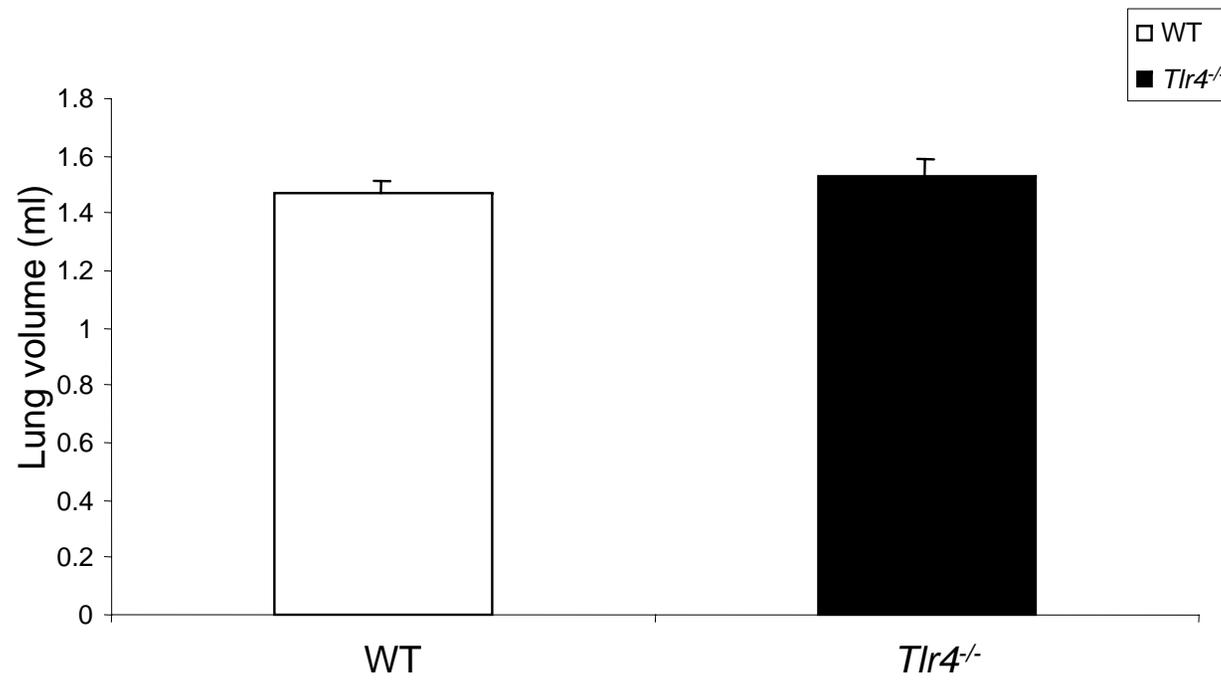
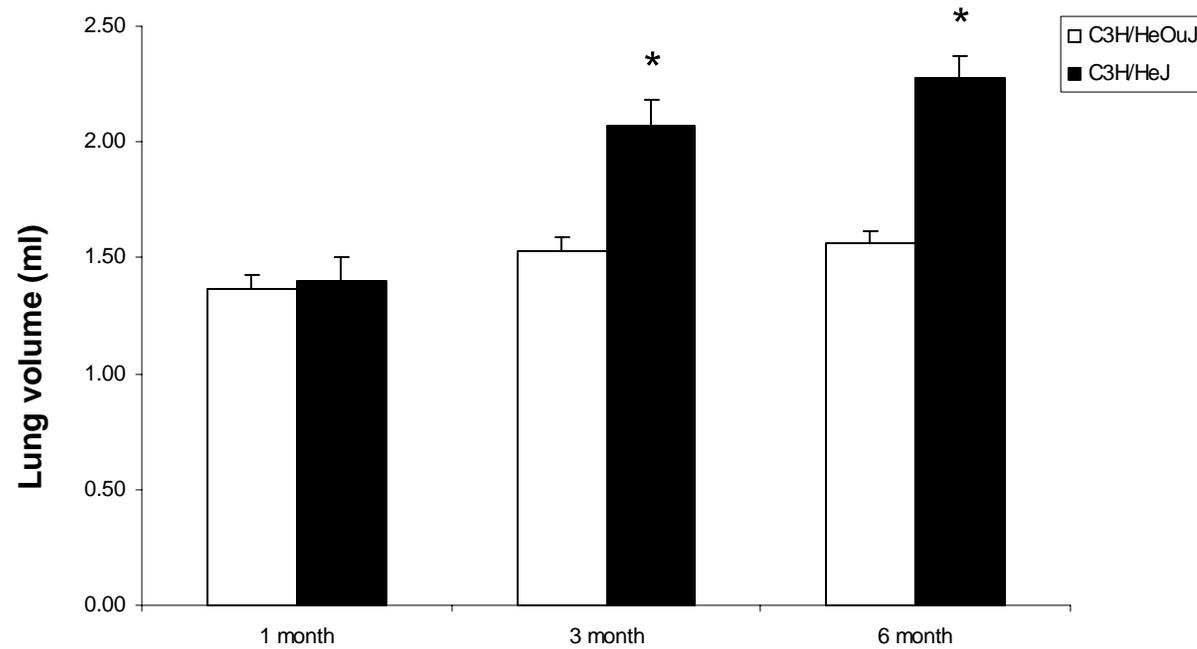


Supplemental Figure 1



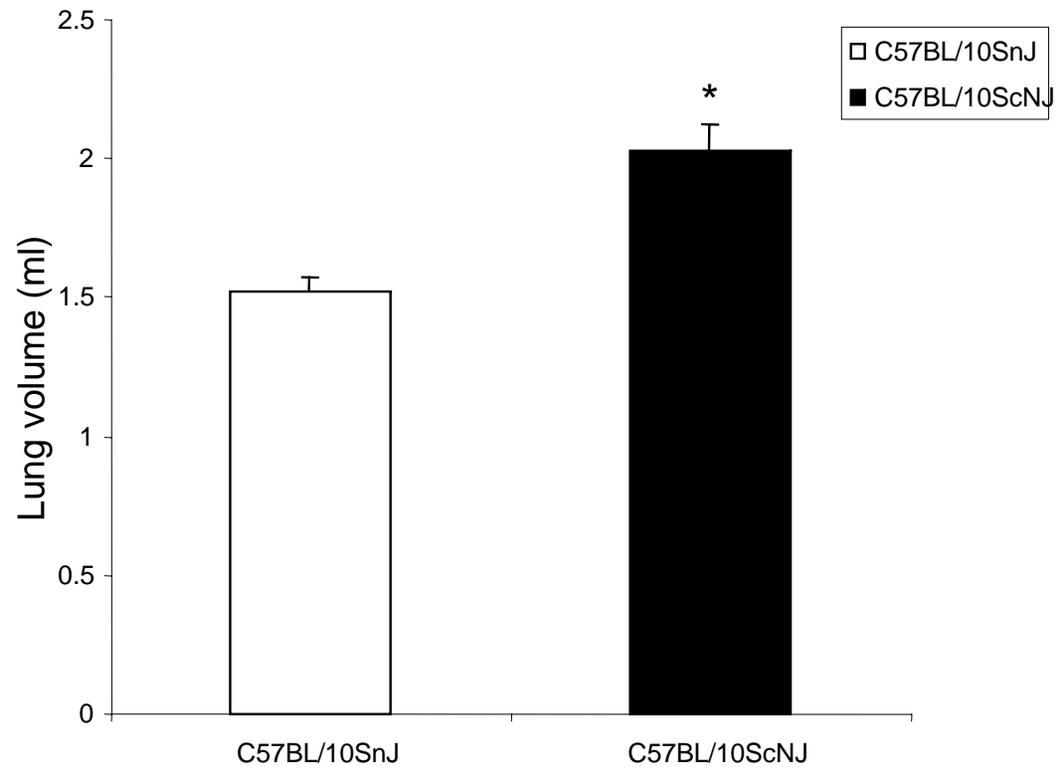
Lung volumes in 2-month-old wildtype (WT) and *Tlr4*^{-/-} mice (n=5). Data are shown as mean ± SEM.

Supplemental Figure 2



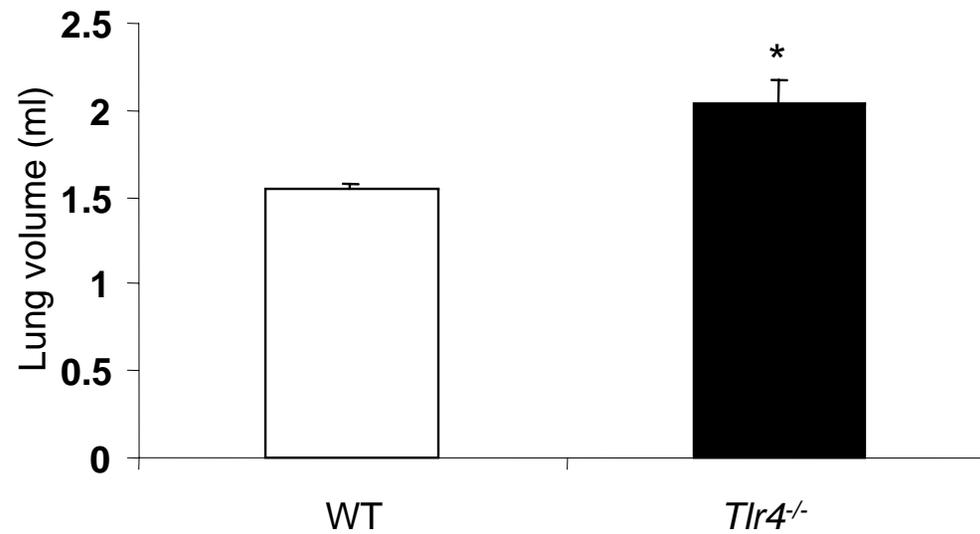
Progressively increased lung volumes were detected in C3H/HeJ (*Tlr4*^{-/-}) (n=5-7). Data are shown as mean \pm SEM. * P <0.05 versus corresponding C3H/HeOuJ (WT) mice.

Supplemental Figure 3



Increased lung volumes in 3-month-old C57BL/10ScNJ (*Tlr4*^{-/-}) mice (n=5-7). Data are shown as mean \pm SEM. * $P < 0.05$ versus C57BL/10SnJ (WT) mice.

Supplemental Figure 4

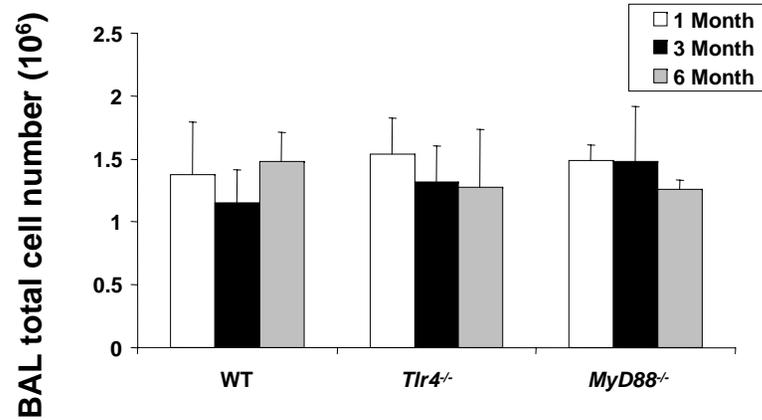


Increased lung volumes in 3-month-old C57BL/6J (*Tlr4*^{-/-}) mice (n=5). Data are shown as mean \pm SEM. * $P < 0.05$ versus C57BL/6J littermate wildtype (WT) mice.

Supplemental Figure 5

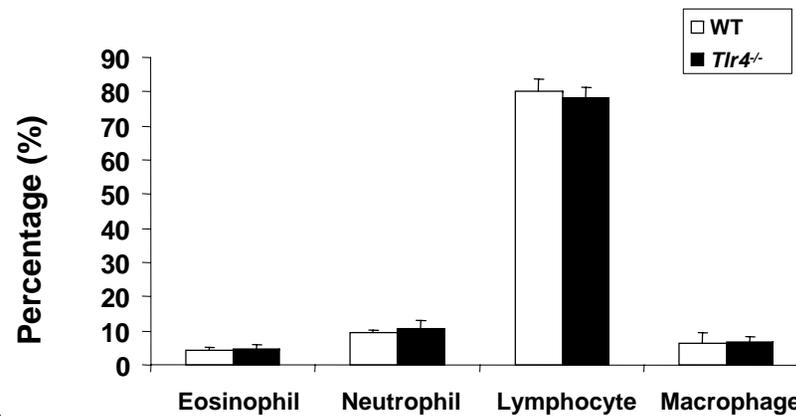
Zhang et al. TLR4 deficiency

A



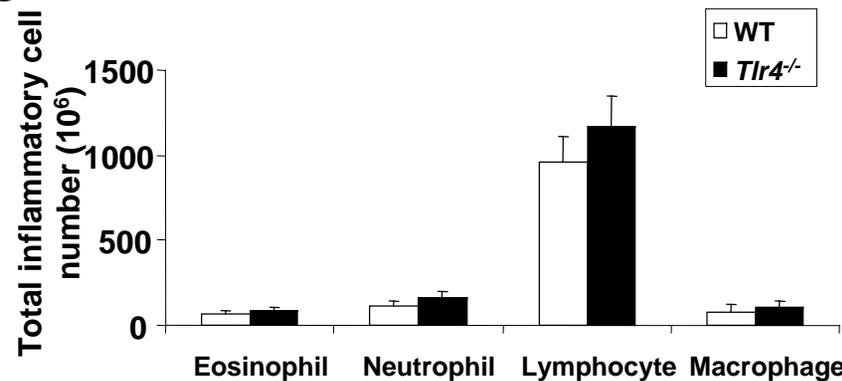
A. BAL total cell numbers in 1- month-old (1m), 3- month-old (3m), and 6- month-old (6m) wildtype (WT), *Tlr4*^{-/-}, and *MyD88*^{-/-} mice (n=3-11). Data are shown as mean ± SEM.

B



B. Percentage of total lung inflammatory cells in lungs of 3-month-old WT and *Tlr4*^{-/-} mice (n=5). Data are shown as mean ± SEM.

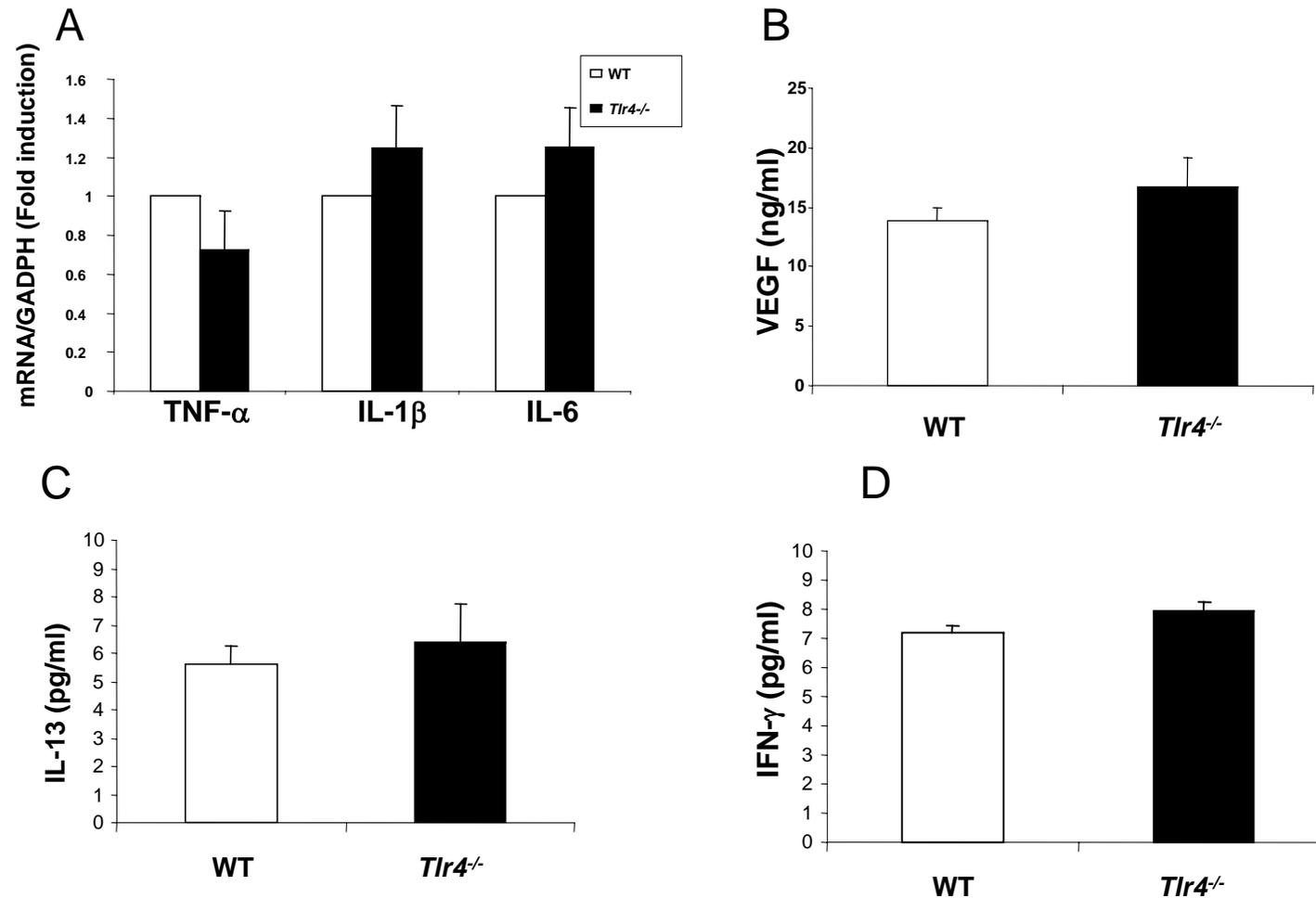
C



C. Differential of total lung inflammatory cells in lungs of 3-month old WT and *Tlr4*^{-/-} mice (n=5). Data are shown as mean ± SEM.

Supplemental Figure 6

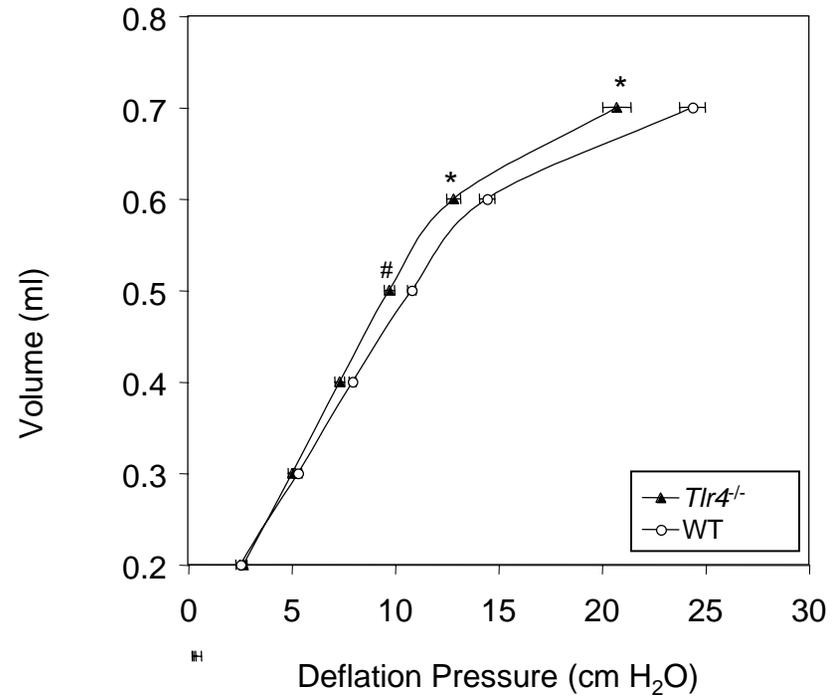
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A. TNF- α , IL-1 β , and IL-6 mRNA expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by real time RT-PCR (n=5). Data are shown as mean \pm SEM. **B, C, and D.** Contents of VEGF, IL-13, and IFN- γ in the BAL of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by ELISA (n=5-10). Data are shown as mean \pm SEM.

Supplemental Figure 7

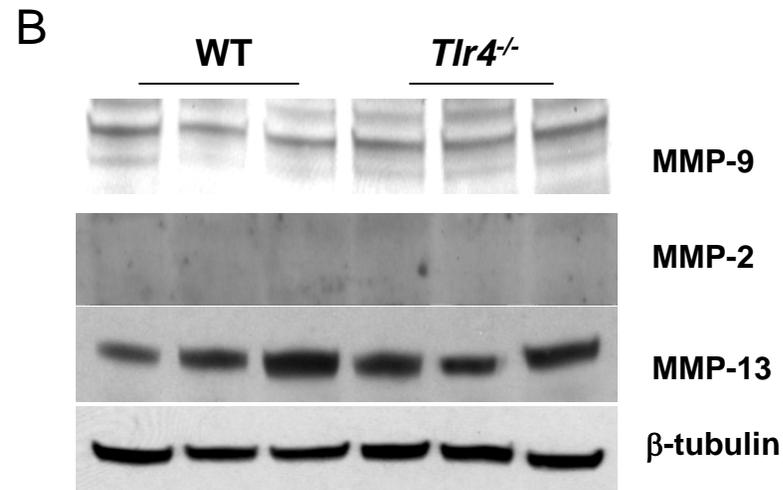
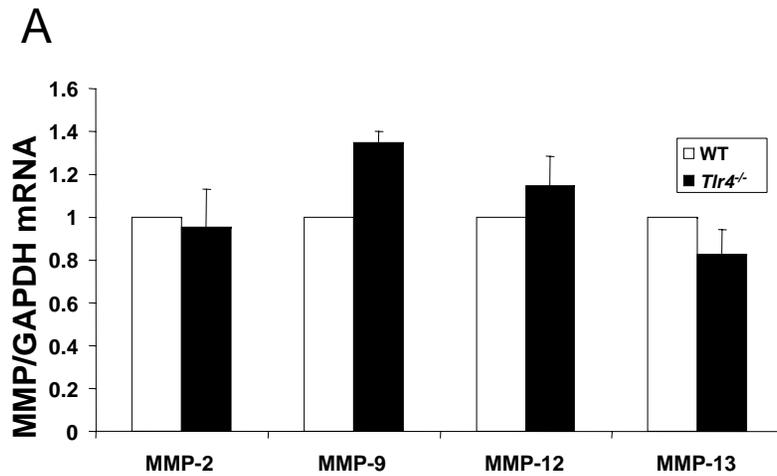
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Increased lung compliance in 3-month-old *Tlr4*^{-/-} mice (n=3-5). Data are shown as mean \pm SEM. *P<0.05 versus WT mice.

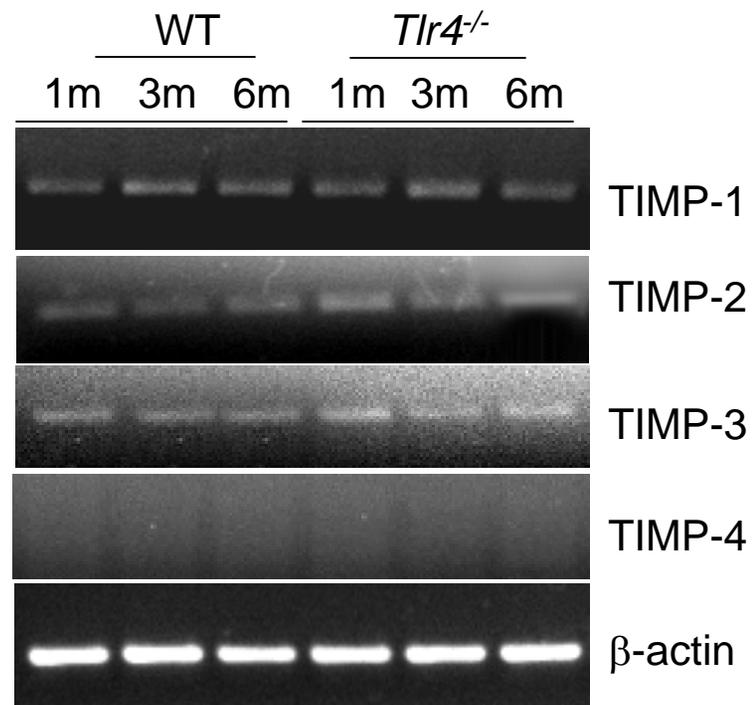
Supplemental Figure 8

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A. MMP-2, 9, 12, and 13 mRNA expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by real-time RT-PCR (n=6). Data are shown as mean \pm SEM. **B.** MMP-9, 2 and 13 protein expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by Western blots (n=3).

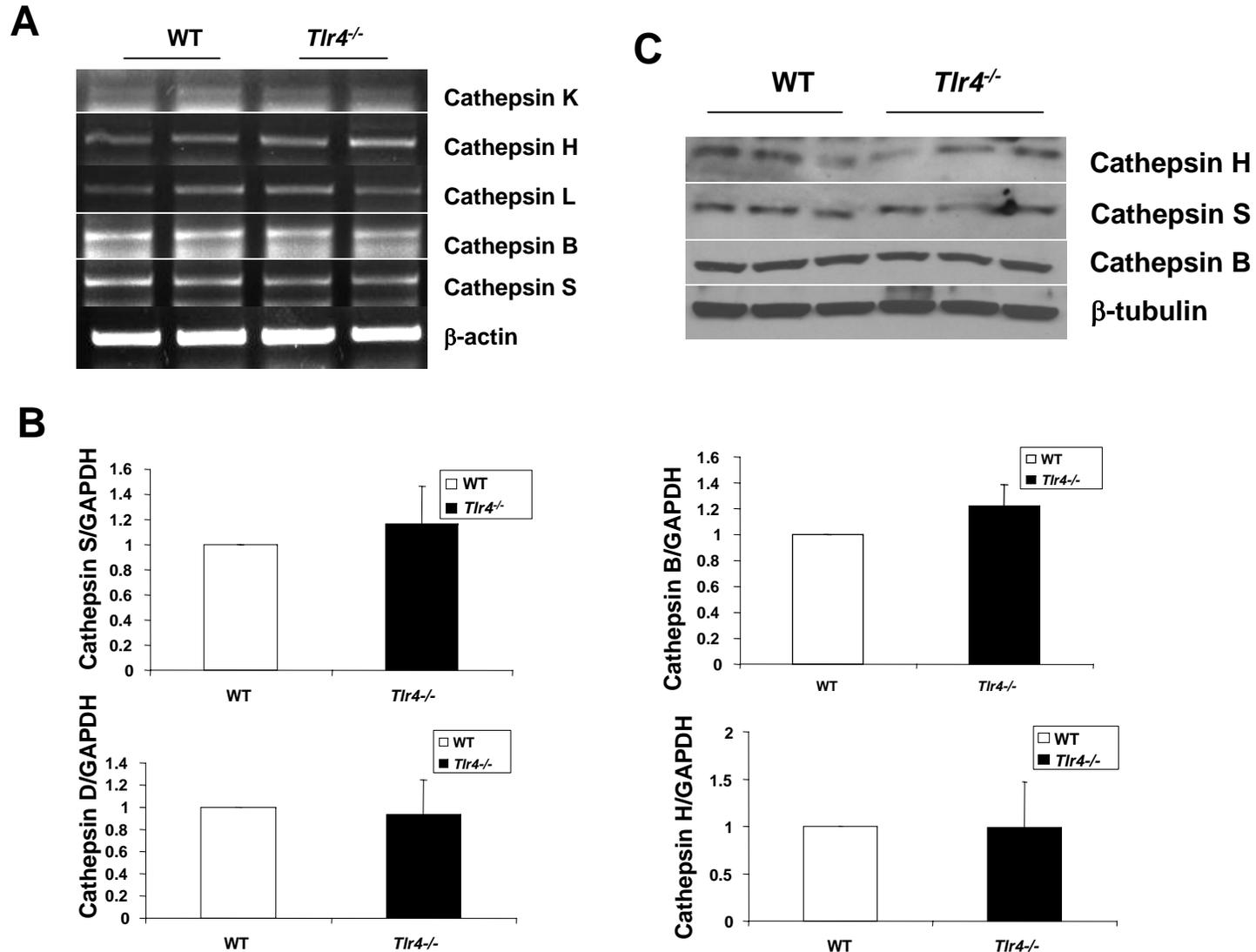
Supplemental Figure 9



TIMP-1, 2, 3, and 4 mRNA expression in the lungs of 1-month-old (1m), 3-month-old (3m), and 6-month-old (6m) WT and *Tlr4*^{-/-} mice analyzed by RT-PCR.

Supplemental Figure 10

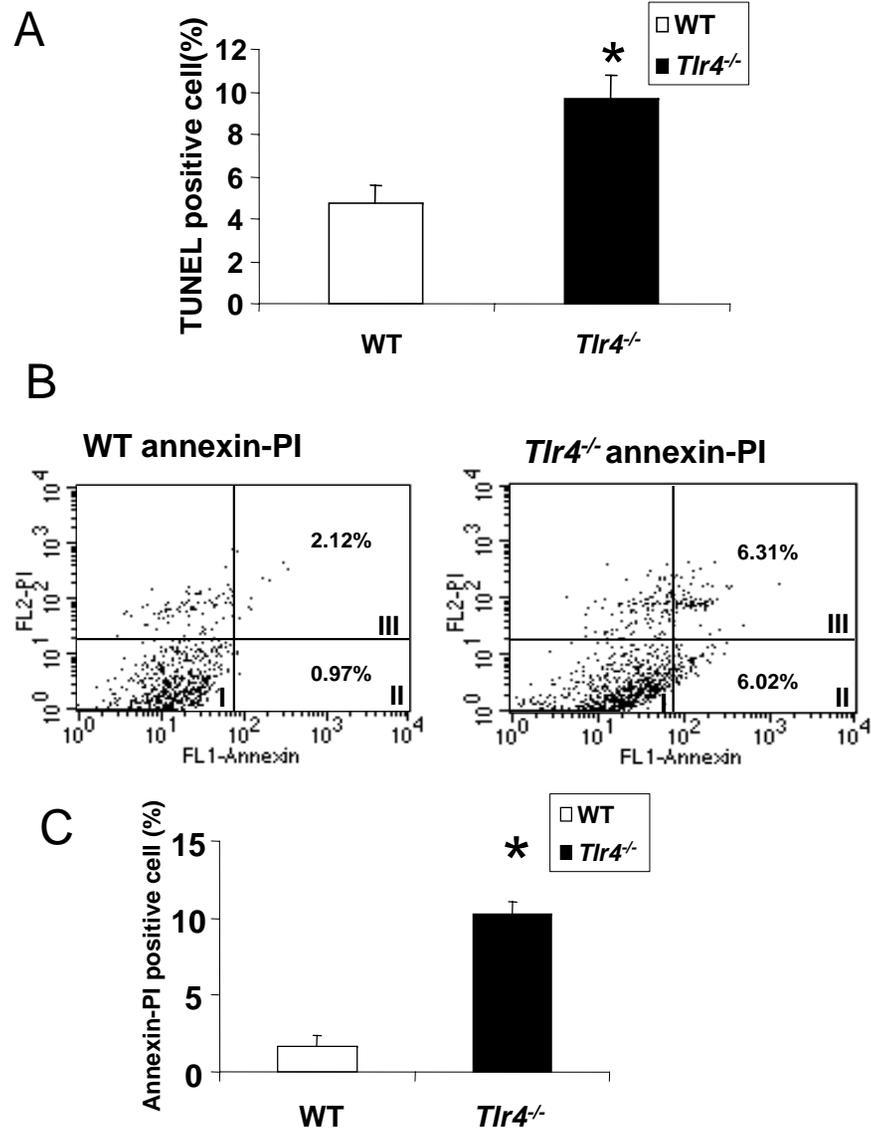
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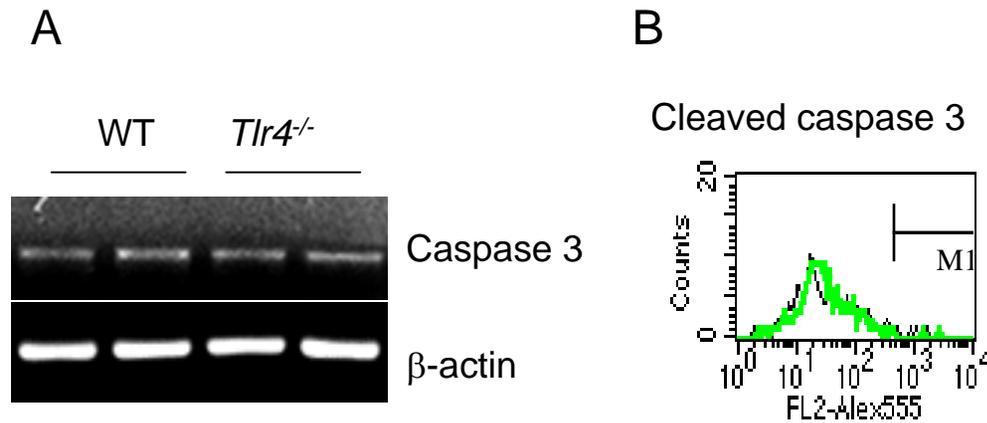
A. Cathepsin K, H, L, B, and S mRNA expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by RT-PCR. **B.** Cathepsin S, D, B, and H mRNA expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by real time RT-PCR (n=3-5). Data are shown as mean ± SEM. **C.** Cathepsin H, S, and B protein expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by Western blots (n=3).

Supplemental Figure 11

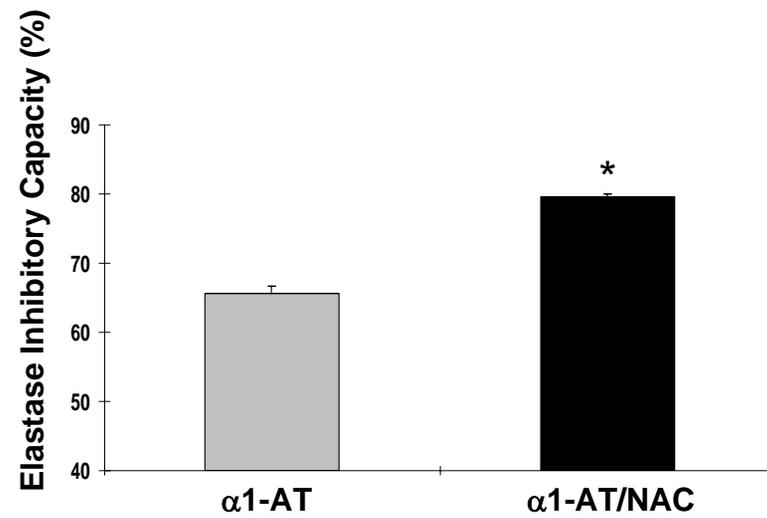
Zhang et al. TLR4 deficiency



A. Quantitation of flow cytometric results of TUNEL positive cells from TUNEL fluorescence staining in total lung cells isolated from 3-month-old WT and *Tlr4*^{-/-} mice (n=3). Data are shown as mean ± SEM. *P<0.05 versus WT mice. **B.** Representative flow cytometry analysis of cell death by annexin V-propidium iodide staining in total lung cells isolated from 3-month-old WT and *Tlr4*^{-/-} mice (n=3). Quadrant I, viable cells; Quadrant II, early apoptotic cells; Quadrant III, late apoptotic/early necrotic cells. **C.** Graphical quantitation of quadrants II and III combined. Data are shown as mean ± SEM. *P<0.05 versus WT mice.

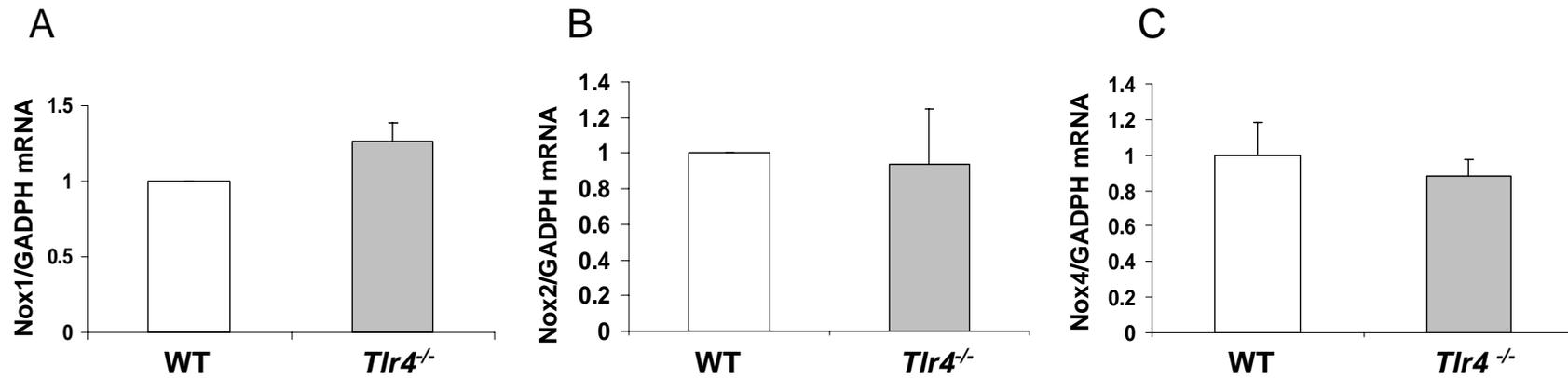


A. Caspase 3 mRNA expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by RT-PCR. **B.** Representative flow cytometric histograms of cleaved caspase 3 by fluorescence staining in total lung cells isolated from 3-month-old WT and *Tlr4*^{-/-} mice. Green: WT; Black: *Tlr4*^{-/-}.



Serum from 3-month-old *Tlr4*^{-/-} mice was incubated with 100 nM $\alpha 1$ -AT alone or with NAC and the elastase inhibitory capacity measured (n=3). Data are shown as mean \pm SEM. *P<0.05 versus $\alpha 1$ -AT alone.

Supplemental Figure 14



A. Nox3 mRNA expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by RT-PCR. **B.** Nox3 mRNA expression in WT and *Tlr4*^{-/-} lung endothelial cells (MLEC) analyzed by RT-PCR. **C.** Nox1 mRNA expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by real time RT-PCR. **D.** Nox2 mRNA expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by real-time RT-PCR. **E.** Nox3 mRNA expression in the lungs of 3-month-old WT and *Tlr4*^{-/-} mice analyzed by real-time RT-PCR. (n=3-5). Data are shown as mean \pm SEM.