

**Age-Dependent Susceptibility to Pulmonary Fibrosis is Associated with NLRP3  
Inflammasome Activation**

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ONLINE DATA SUPPLEMENT

## Supplemental Figure Legends

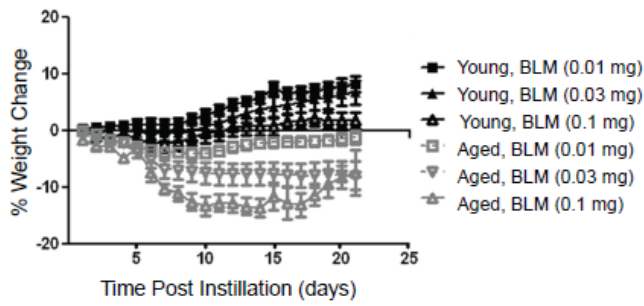
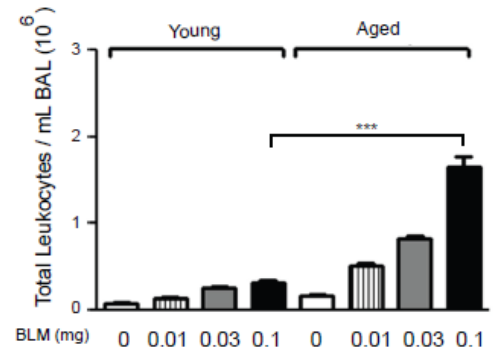
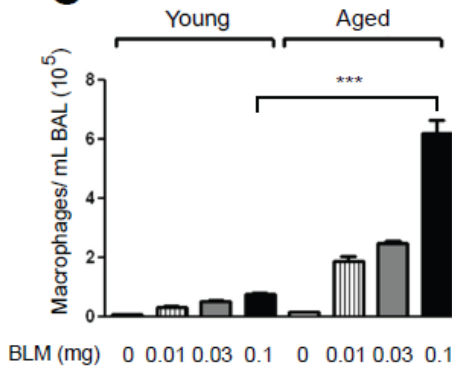
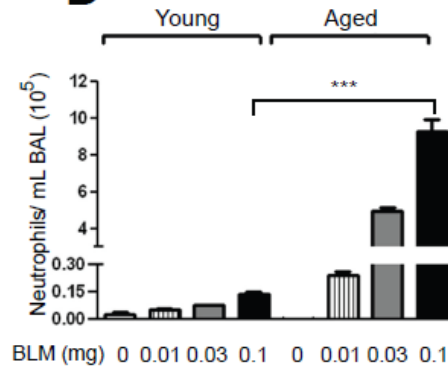
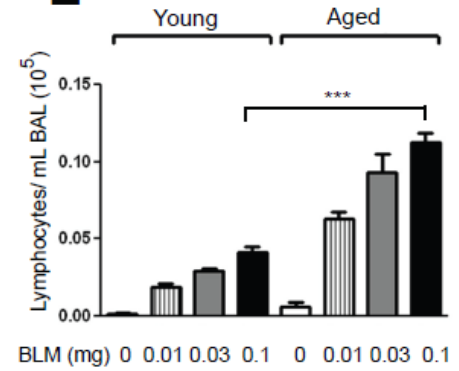
**Supplemental Figure 1. Enhanced leukocyte infiltration in aged lung with increasing doses of bleomycin.** (A) Weight change in young (2-4 months) and aged (17-18 months) male C57BL/6 mice post instillation with bleomycin (0.01 to 0.1 mg/mouse). (B-E) Bronchoalveolar (BAL) samples isolated from saline and bleomycin-instilled young and aged C57BL/6 mice on day 16 post administration. Similar results were obtained from at least two or more independent experiments with greater than N=10 per group and results are shown as the mean  $\pm$  SEM.

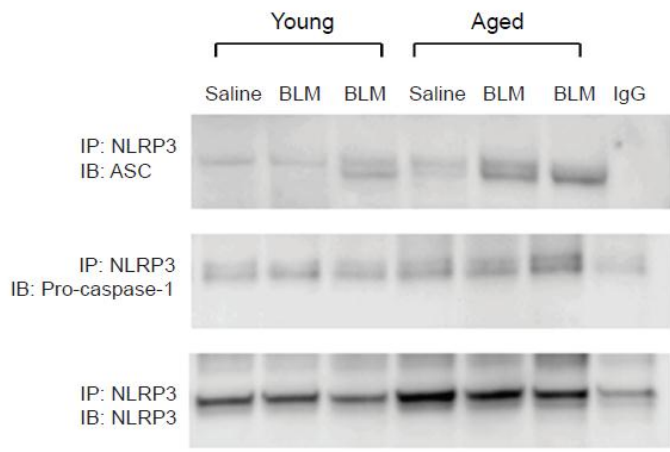
**Supplemental Figure 2. Reverse co-immunoprecipitation of young and aged lung samples day 3 post bleomycin administration.** Lung homogenate samples were isolated from young (2-4 months) and aged (17-18 months) C57BL/6 mice on day 3 post-bleomycin and co-immunoprecipitation was performed against anti-NLRP3.

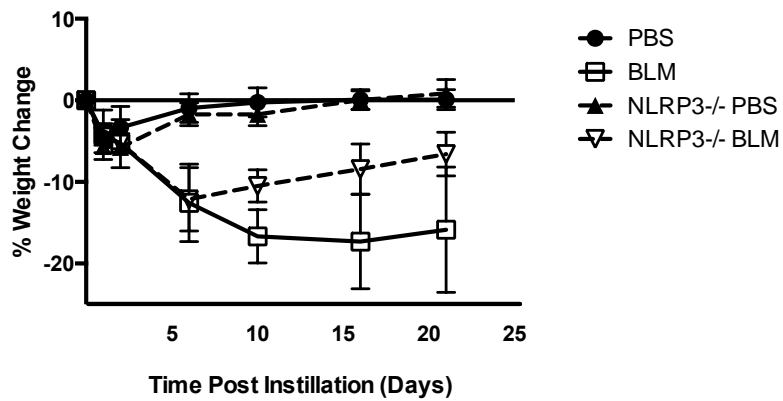
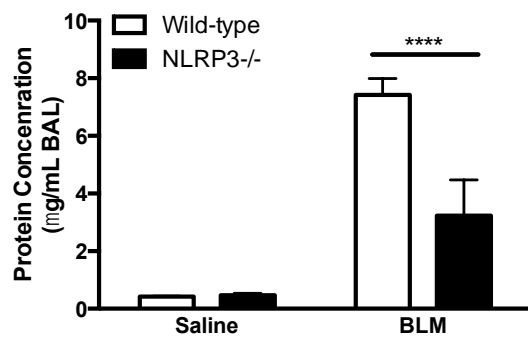
**Supplemental Figure 3. NLRP3<sup>-/-</sup> mice have reduced weight loss and BALF protein after bleomycin.** 13 month-old wild-type and NLRP3<sup>-/-</sup> mice were instilled with bleomycin (0.1 mg/mouse) via oral aspiration. (A) Weight loss (wild-type/bleomycin vs. NLRP3<sup>-/-</sup>/bleomycin,  $p < 0.0001$ , t-test) and (B) BALF protein concentration ( $p < 0.00001$ , t-test) were assessed at 21 days following bleomycin.

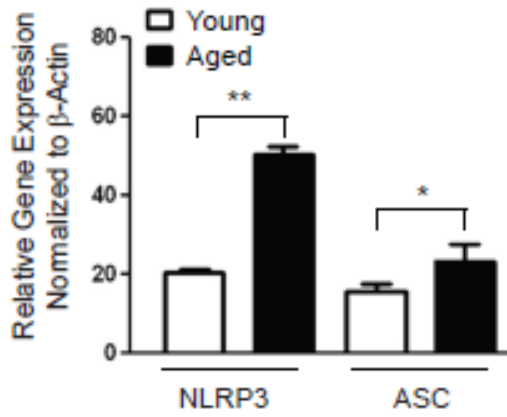
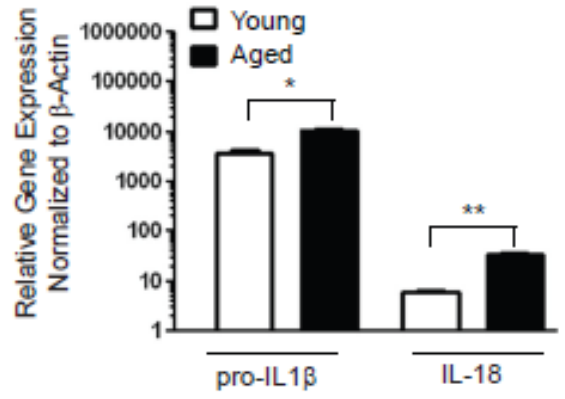
**Supplemental Figure 4. NLRP3, ASC, IL-1 $\beta$  and IL-18 gene expression is elevated in aged LPS-primed macrophages treated with bleomycin.** Young (2-4 months) and aged (17-18 months) bone marrow cells were cultured with murine M-CSF (10 ng/mL) for 7 days in 37°C, 5% CO<sub>2</sub>. Macrophages were then primed with LPS (100 ng/mL) for 4 hours prior to treatment with containing bleomycin (0.1 U) for 24 hours. (A) NLRP3 and ASC and (B) pro-IL-1 $\beta$  and pro-IL-18 gene expression was then quantified by real time PCR.

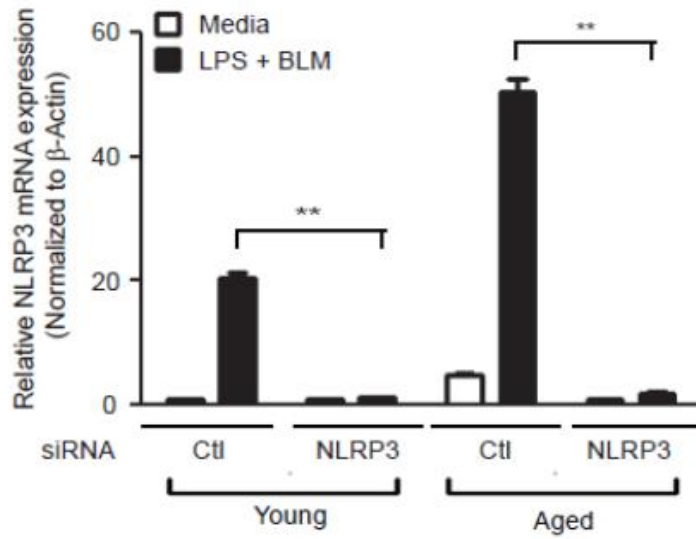
**Supplemental Figure 5. NLRP3 protein in aged macrophages is reduced in response to NLRP3 siRNA.** Young (2-4 months) and aged (17-18 months) bone marrow cells were cultured with murine M-CSF (10 ng/mL) for 7 days in 37°C, 5% CO<sub>2</sub>. Cells were treated with missense or NLRP3-specific siRNA for 24 hours prior. (A) RNA was isolated from cells and NLRP3 gene expression was quantified by real time PCR (>90% reduction in young: p=0.0013, t-test; >96% reduction in aged: p=0.0022, t-test). (B) Protein was isolated from elderly BMMs and 30  $\mu$ g of protein was electrophoresed through a 4-12% SDS-PAGE prior to western blot analysis with mouse-specific anti-NLRP3 and anti- $\beta$ -actin. Similar results were obtained from three or more independent experiments with an N=3 or greater per experiment. NLRP3 mRNA expression is expressed as the mean  $\pm$  SEM.

**A****B****C****D****E**



**A****B**

**A****B**

**A****B**