Francois et al. Supplemental Material

SUPPLEMENTAL METHODS

Differential white blood cell counts in the BALF by flow cytometry.

After cyto-centrifugation of collected BALFs, 20 µl of reconstituted cell pellets were pre-mixed for 20 min at room temperature in the dark, with a cocktail of antibodies: V500 conjugated rat anti-mouse CD45, Alexa Fluor 700 conjugated rat anti mouse Ly-6G, Alexa Fluor 488 conjugated CD11b, and PE conjugated rat anti mouse CD115 (all from BD Bioscience). After fixation in 1% paraformaldehyde for 10min, cells were diluted in 300 µl PBS to block fixation. Before flow cytometry analysis (BD LSR II), 10 µl of flow-count fluorospheres (Beckman Coulter) was added to determine the concentration of subpopulations. Results were analyzed by the gating analysis of $CD45^+$ cells (leukocytes) and separation of three subpopulations : 1) $CD11^-$ cells (lymphocytes), 2) $CD11b^+Ly6G^+CD115^{low}$ (neutrophils) and 3) $CD11b^+Ly6G^-CD115^+$ (monocytes/macrophages).

Determination of haemoglobin content in BALF

After cyto-centrifugation of collected BALFs, hemoglobin content of cell pellet was measured by absorbance reading at 405 nm after a 4 fold-dilution in formic acid.

SUPPLEMENTAL FIGURE LEGENDS

Figure 1: Differential white blood cell counts in the BALF by flow cytometry.

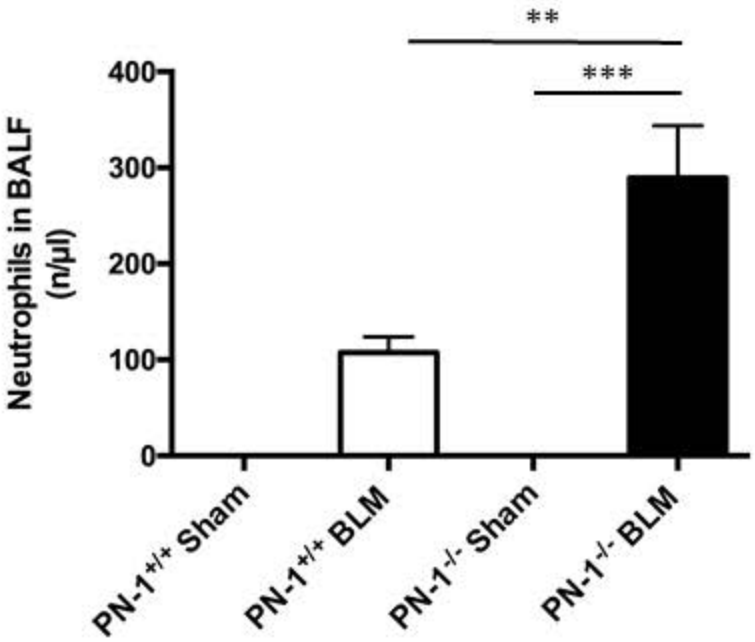
Collected BALFs from the various groups of mice were pre-mixed with a cocktail of antibodies to detect neutrophils, lymphocytes and monocytes/macrophages, and 10 µl of flow-count fluorospheres to determine the concentration of each type of white blood cells. Data (mean ± SEM; n= 9-14 per group; ***P*<0.01, ****P*<0.001) were analyzed by 1-way ANOVA with a Tukey's multiple comparison test.

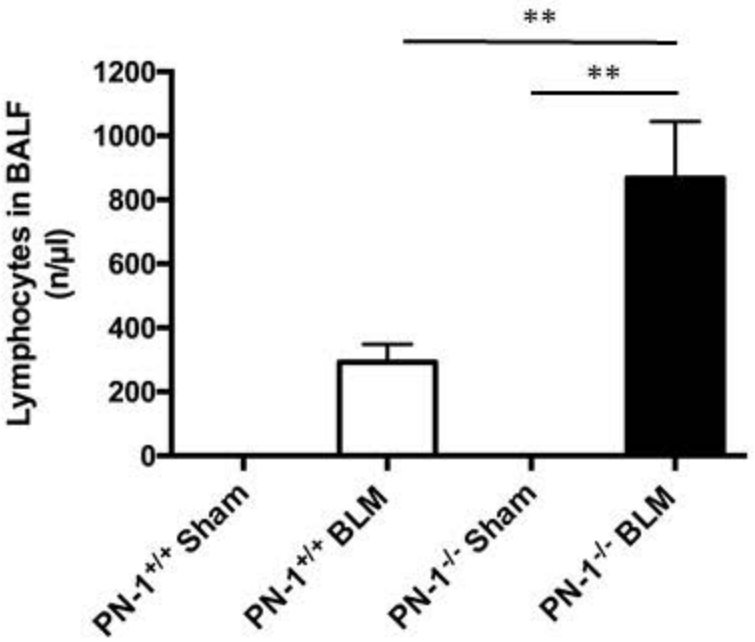
Figure 2. Hydroxyproline contents in lung tissues from *PN-1*$^{+/+}$ mice.

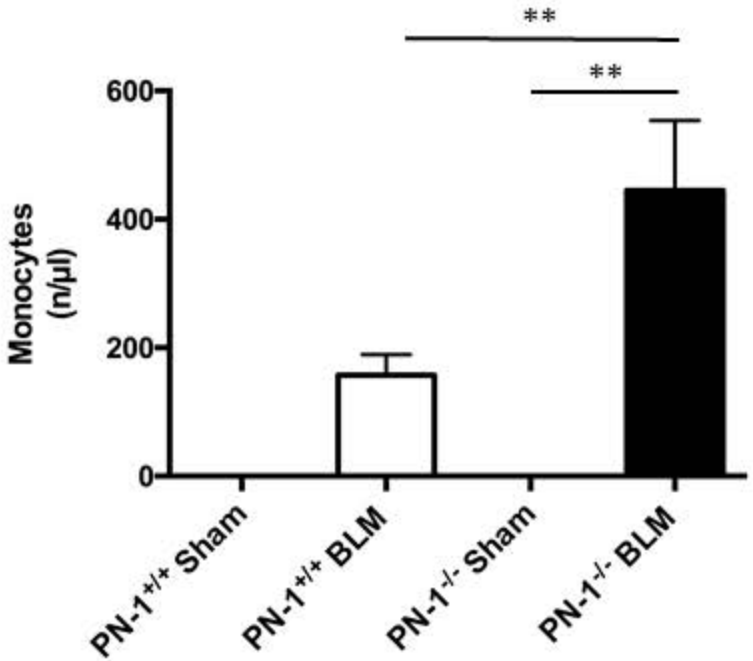
Hydroxyproline contents in lung tissues from *PN-1*$^{+/+}$ mice were measured 3, 9, and 14 days after bleomycin (BLM) treatment *vs* saline treatment (Sham). Data (mean ± SEM; n= 5-7 per group) were analyzed by 2-tailed Mann-Whitney U test. ***P*<0.01 vs sham.

Figure 3. Effect of thrombin inhibition by argatroban in bleomycin-injured *PN-1*$^{+/+}$ mice.

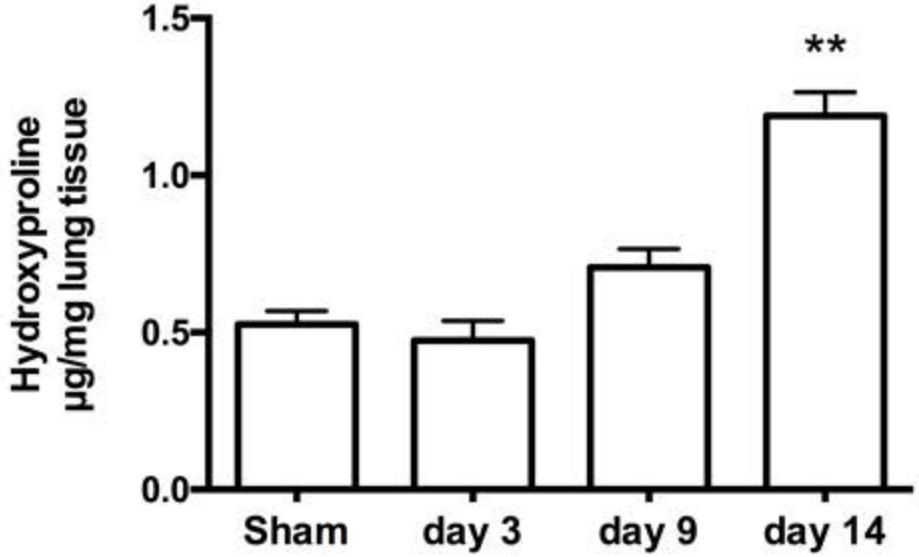
PN-1$^{+/+}$ mice were subjected to bleomycin-induced lung injury (BLM) (2 mg/kg) and treated daily with Argatroban (9 mg/kg i.p.). (A) Percentages of surviving *PN-1*$^{+/+}$ mice were plotted over a 14-days period. Log-rank test was used to compare the difference between *PN-1*$^{+/+}$ mice with BLM and *PN-1*$^{+/+}$ mice with BLM plus argatroban (*PN-1*$^{+/+}$ BLM: n= 6, *PN-1*$^{+/+}$ BLM + Argatroban: n= 11). (B) After cyto-centrifugation of collected BALFs from the various groups of mice, hemoglobin content was measured by absorbance reading at 405 nm after a 4 fold-dilution in formic acid.



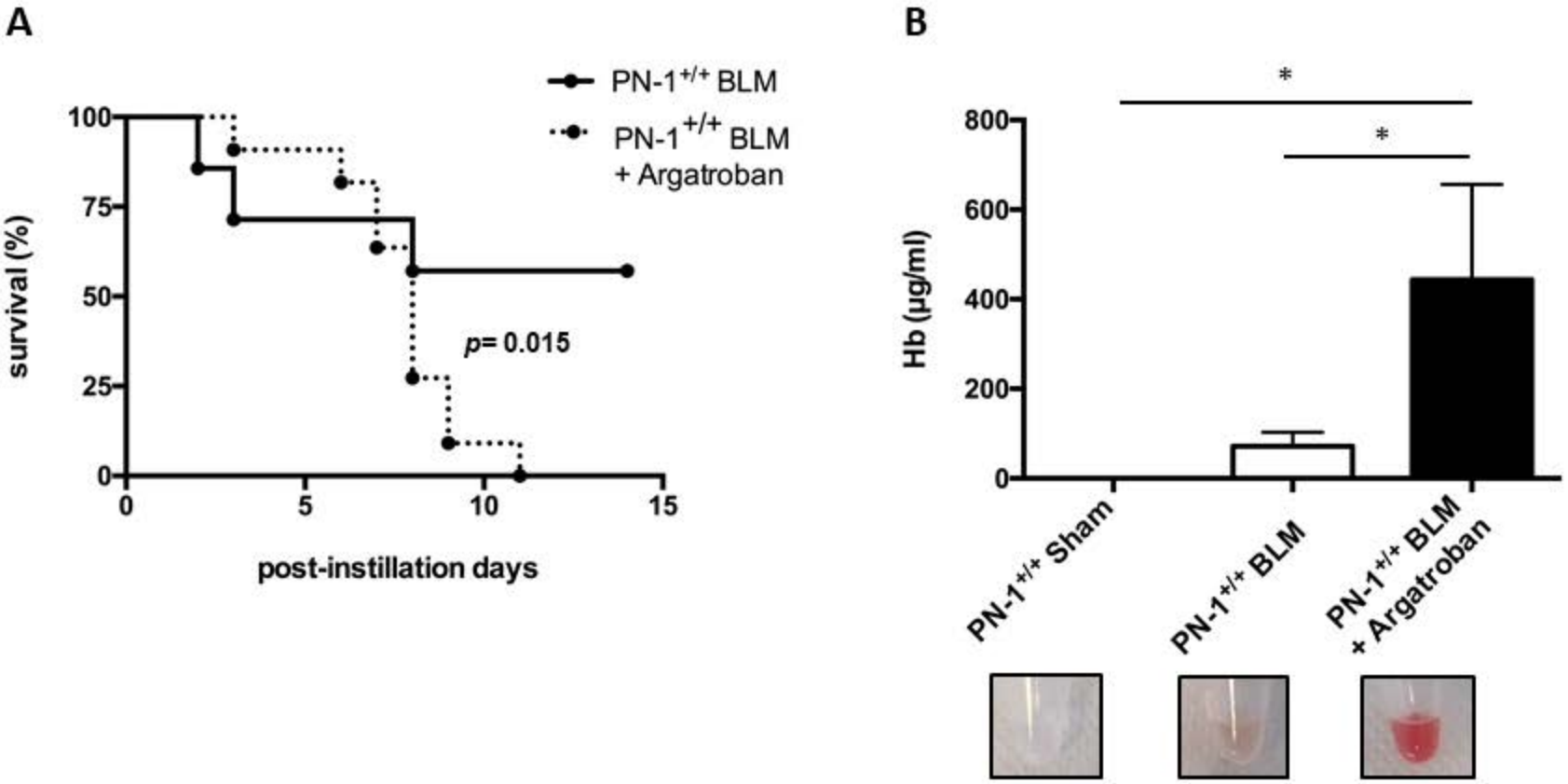




Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3