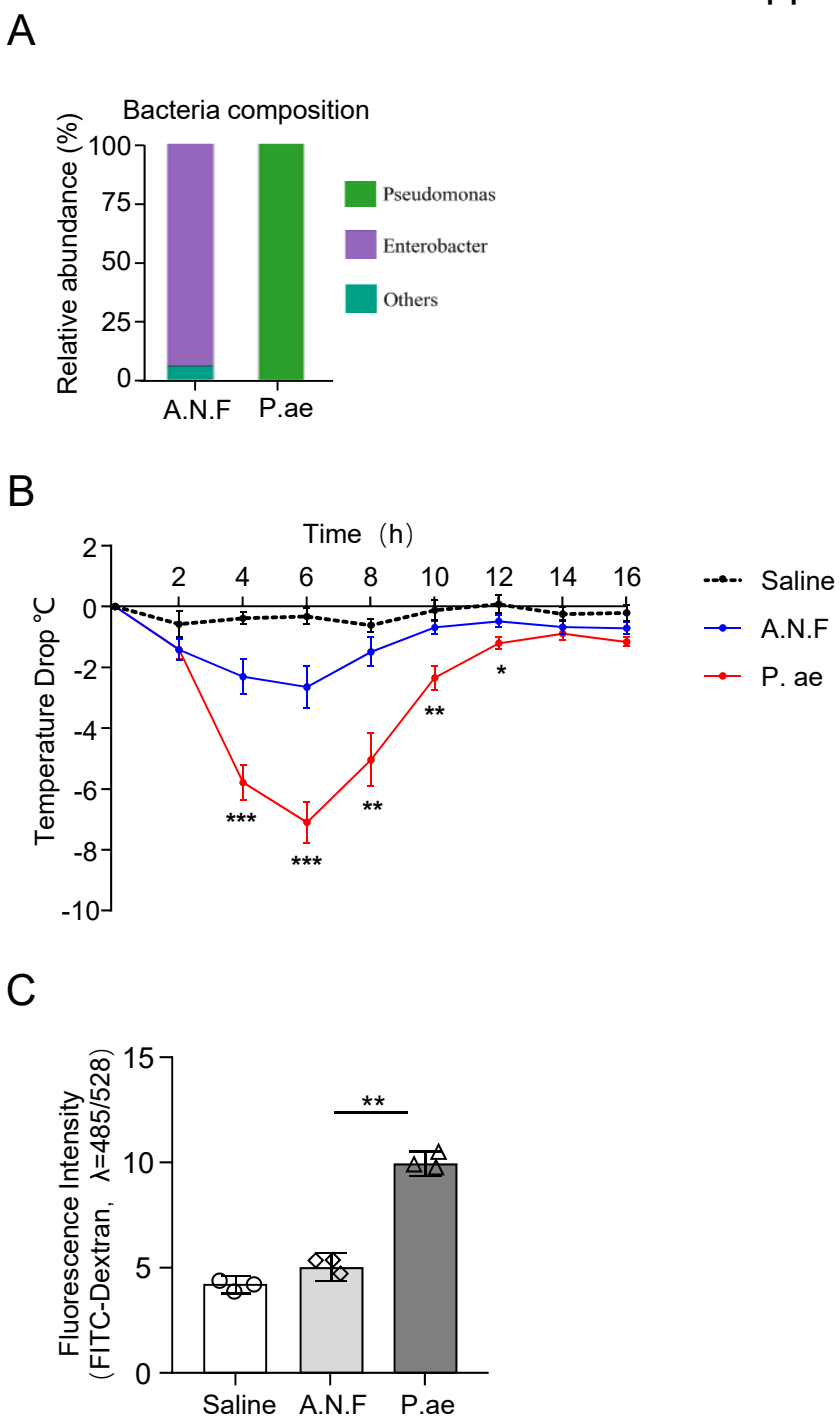

Figure S1. P. ae induced lung acute injury. (A) Bacteria composition in P. ae and A.N.F were detected by 16S rDNA sequencing. (B) C57BL/6 mice were intratracheally treated with P. aeruginosa (P. ae) or airway normal flora (A.N.F) (2×10^5 colony-forming units [CFUs] in a volume of 30 μl per animal, n = 5) or same amount of sterile saline as control (n = 3) for 16 hours. Rectal temperatures at every two hours were detected. (C) C57BL/6 mice were infected with bacteria same as (B) for 16 hours, and then intratracheally treated with FITC-Dextran (10mg/kg body weight in 30 μl PBS per animal) for 1 hours. Plasma samples were collected to detect fluorescence intensity (λ = 485/528, n = 3). Data in (B and C) are presented as means ± s.d. *** $p<0.001$, ** $p<0.01$, * $p<0.05$, as analyzed by two-tailed unpaired student's *t* test.

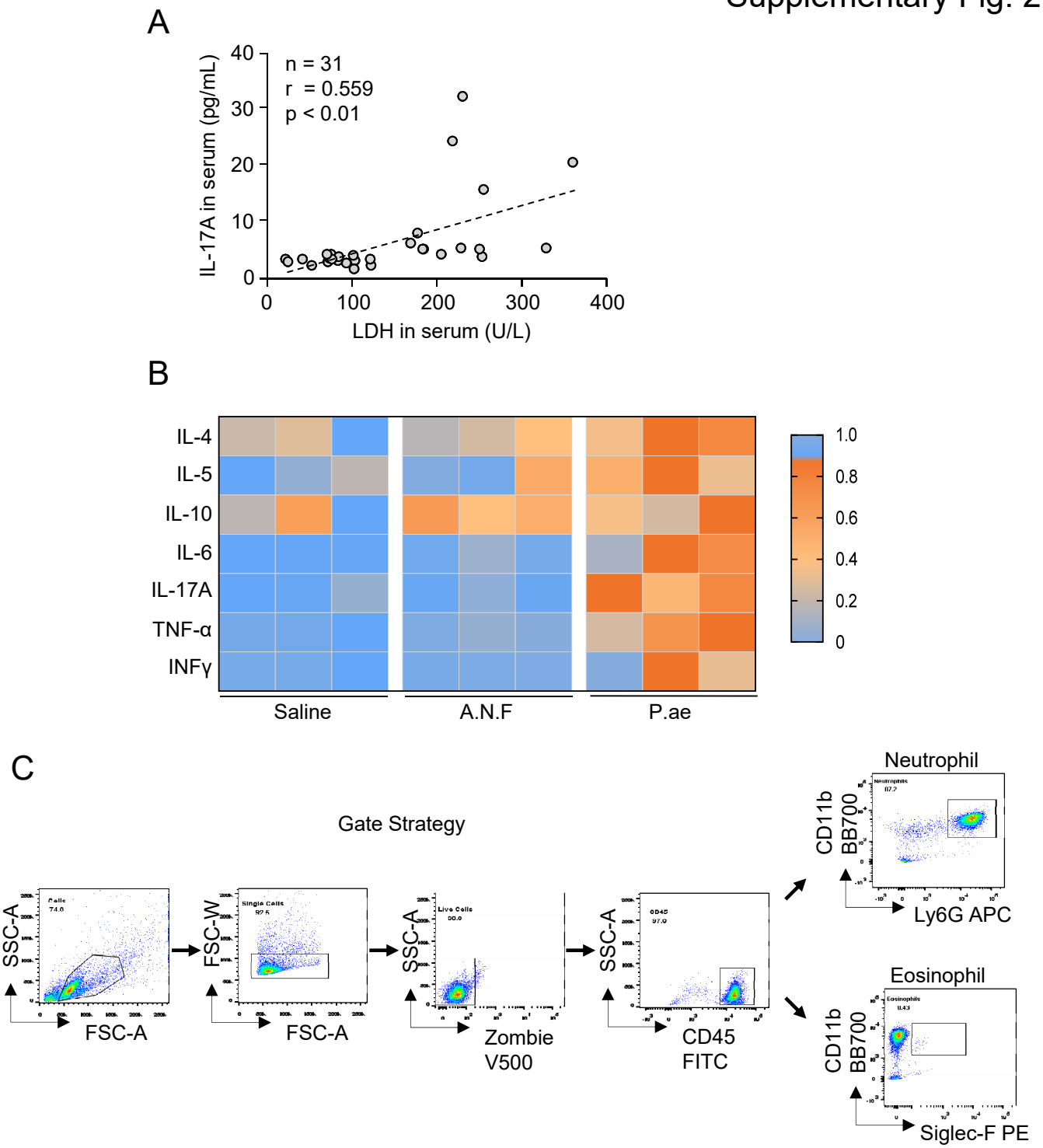

Figure S2. **P. ae mediated lung inflammation**. (A) The level of IL-17A and LDH in the serum of patients were detected by ELISA as mentioned in (Figure 1A and 2A). Linear correlation between IL-17A and LDH were analyzed by Pearson correlation coefficients test. (B and C) C57BL/6 mice were intratracheally treated with P. ae, A.N.F or sterile saline same as (Figure S1). Multiple cytokines (IL-4, IL-5, IL-10, IL-6, IL-17A, TNF-α, IFNγ) in BALF were detected through multi-analyte flow assay and presented in the Hot-map (n = 3/group) (B); Gate strategies of neutrophil and eosinophil through flow cytometry analysis (C).

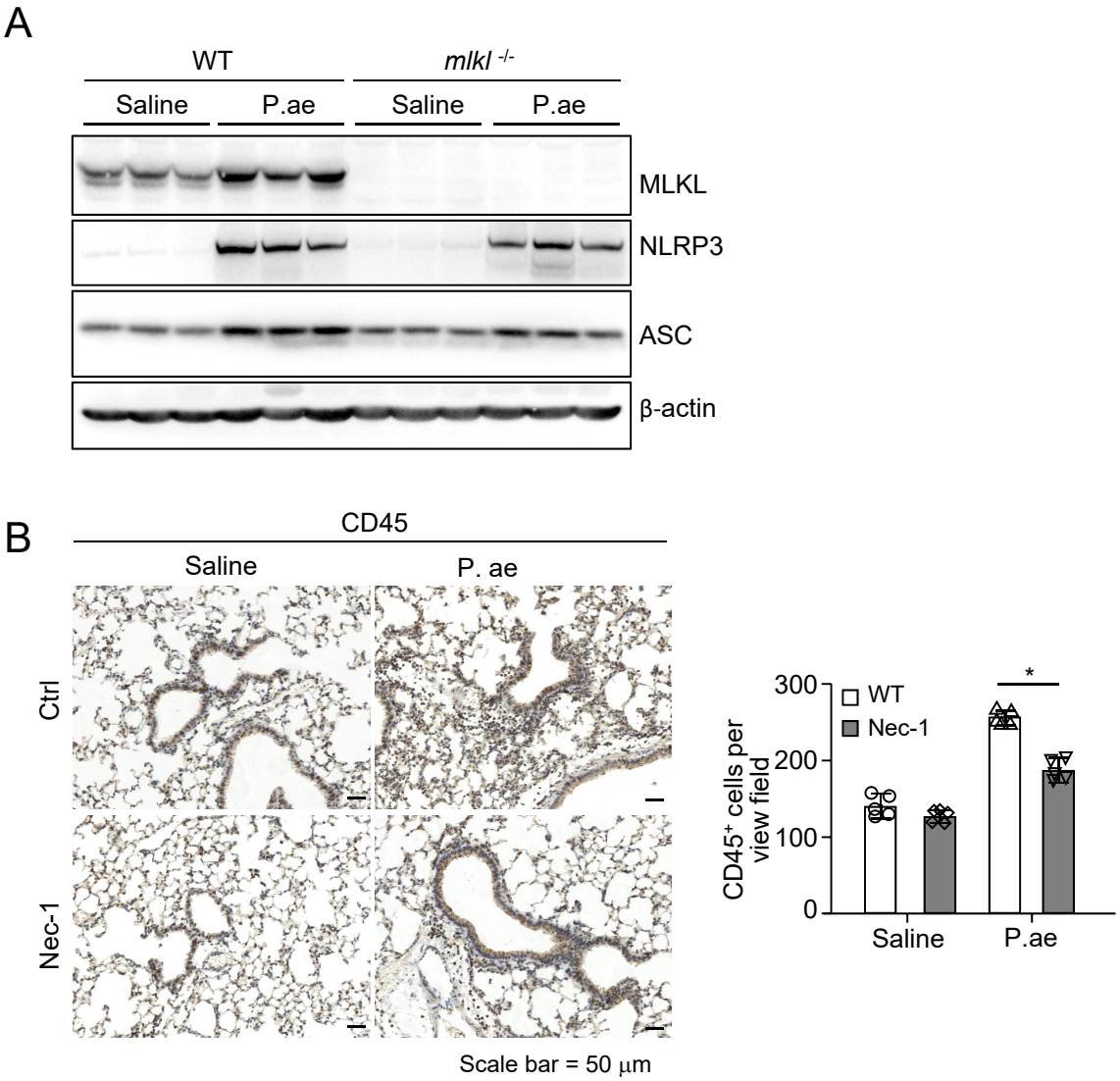

Figure S3. **Pharmacological and genomic inhibition of necroptosis signaling ameliorates P. aeruginosa mediated acute lung injury and pulmonary inflammation**. (A) *mlkl* deficient mice (*mlkl*$^{-/-}$) and WT littermates were intratracheally treated with P. ae (2×10^5 CFUs in 30 μl per animal) or same amount of sterile saline as control for 16 hours. Immunoblot analysis of MLKL, NLRP3 and ASC level in the lungs as indicated, with β-actin as internal control. (B) C57BL/6 mice were intratracheally treated with Nec-1 (4 mg/kg body weight in 30 μl 10 % DMSO solution per animal) or same amount of 10 % DMSO solution as control for 6 hours, and then intratracheally treated with P. ae (2×10^5 CFUs in 30 μl per animal) or same amount of sterile saline as control for 16 hours (n = 5). IHC staining with anti-CD45 antibody in the representative lung sections as indicated; At least five random fields of each mouse were performed to analyzed the number of CD45^{+} cells through ImageJ program, and the scatter plot represents the average value of each sample. Data is presented as means ± s.d. * $p<0.05$, as analyzed by two-tailed unpaired student's *t* test.

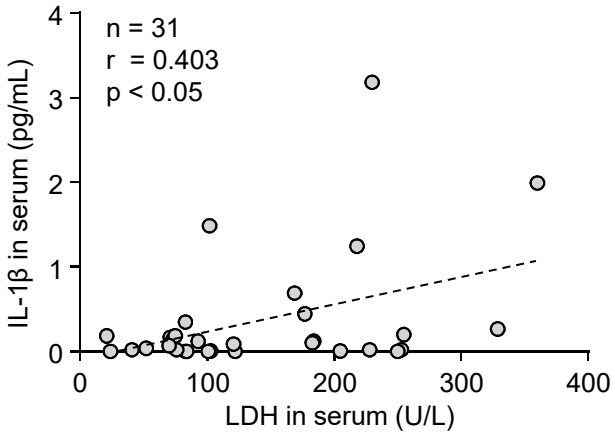

Figure S4. **P. ae mediated NLRP3 inflammasome activation**. The level of IL-1β and LDH in the serum of patients were detected by ELISA as mentioned in (Figure 1A and 4A). Linear correlation between IL-1β and LDH were analyzed by Pearson correlation coefficients test.

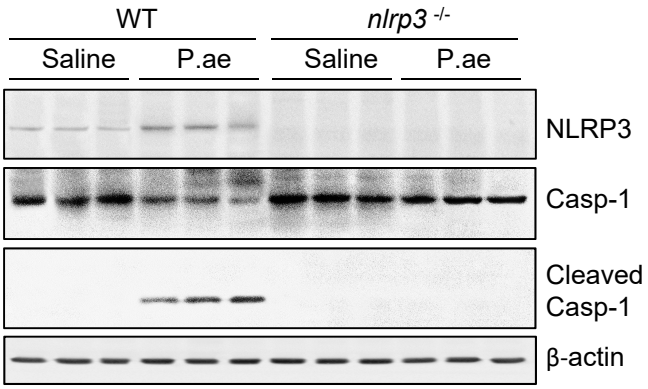

Figure S5. ***nlrp3* ablation suppresses P. aeruginosa induced inflammasome activation**. *nlrp3* deficient mice (*nlrp3*$^{-/-}$) and WT littermates were intratracheally treated with P. ae (2×10^5 CFUs in 30 μl per animal) or same amount of sterile saline as control for 16 hours. Immunoblot analysis of NLRP3, Caspase-1 and cleaved caspase-1 level in the lungs as indicated, with β-actin as internal control.

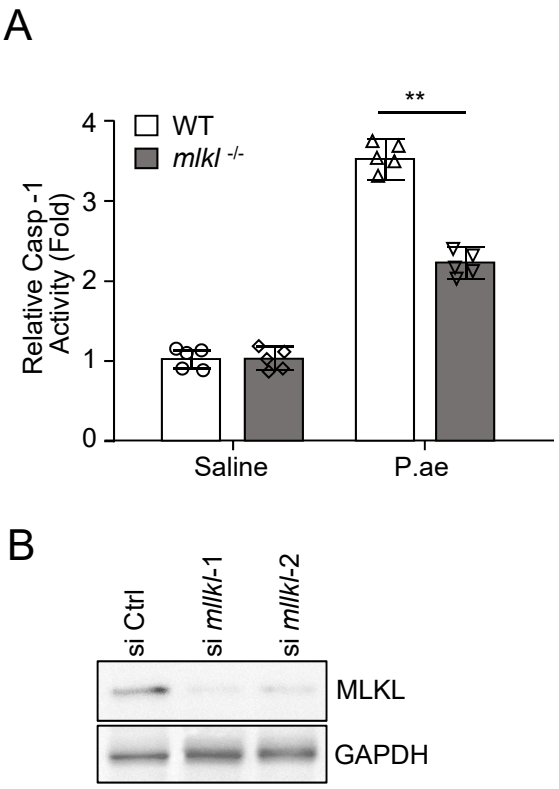

Figure S6. ***mlkl*** **ablation suppresses P. aeruginosa induced inflammasome activation.** (A) *mlkl* deficient mice (*mlkl*$^{-/-}$) and WT littermates were intratracheally treated with P. ae (2×10^5 CFUs in 30 μl per animal) or same amount of sterile saline as control for 16 hours. Representative lung lysis were subjected to caspase 1 activity analysis. Data is presented as means ± s.d. ** $p<0.01$, as analyzed by two-tailed unpaired student's *t* test. (B) A549 cells were transfected with scramble siRNA (si Ctrl) or two different target sites of si *mlkl* for 48 h. Protein level of MLKL was detected by immunoblotting, with GAPDH as internal control.

Supplementary Fig. 7

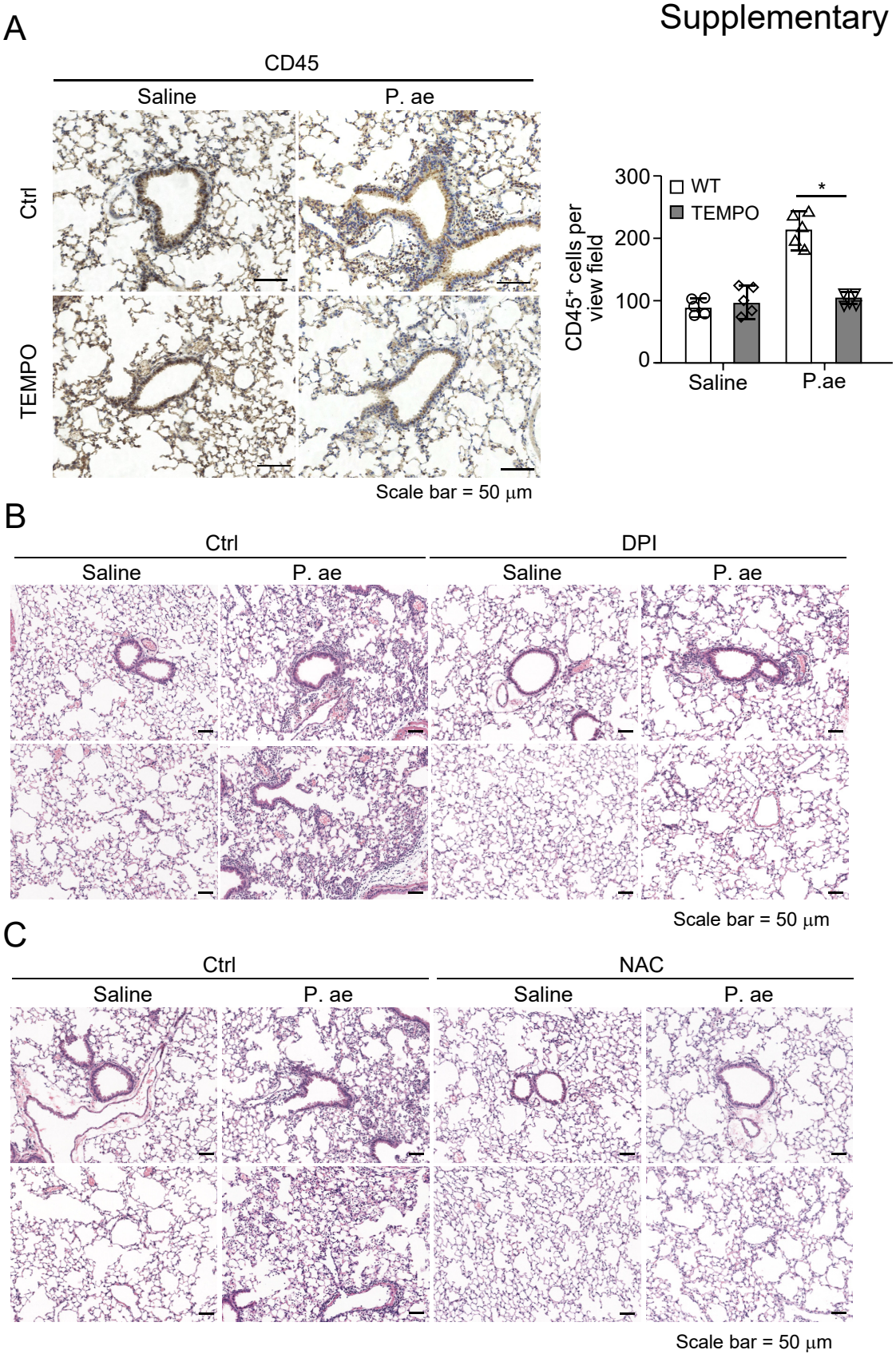

Figure S7. **Antioxidants restrain P. aeruginosa induced acute lung injury**. (A) C57BL/6 mice were intratracheally treated with Mito-TEMPO (20 mg/kg body weight in 30 μl PBS per animal) or same amount of sterile saline as control for 6 hours, and then intratracheally treated with P. ae (2×10^5 CFUs in 30 μl per animal) or same amount of sterile saline as control for 16 hours (n = 5). IHC staining with anti-CD45 antibody in the representative lung sections as indicated; At least five random fields of each mouse were performed to analyzed the number of $CD45^+$ cells through ImageJ program, and the scatter plot represents the average value of each sample. Data is presented as means ± s.d. * $p<0.05$, as analyzed by two-tailed unpaired student's *t* test. (B and C) C57BL/6 mice were intratracheally treated with DPI (1 mg/kg body weight in 10 % DMSO solution per mouse, B), NAC (125 mg/kg body weight in 30 μl PBS per animal, C) or same amount of resolving buffer (10% DMSO or PBS) as control for 6 hours, followed with intratracheally treatment with P. ae (2×10^5 CFUs in 30 μl per animal) or same amount of sterile saline as control for 16 hours (n = 5). H&E staining of the representative lung sections (Upper panel, bronchial area; Lower panel, alveolar area) from different treated mice as indicated.