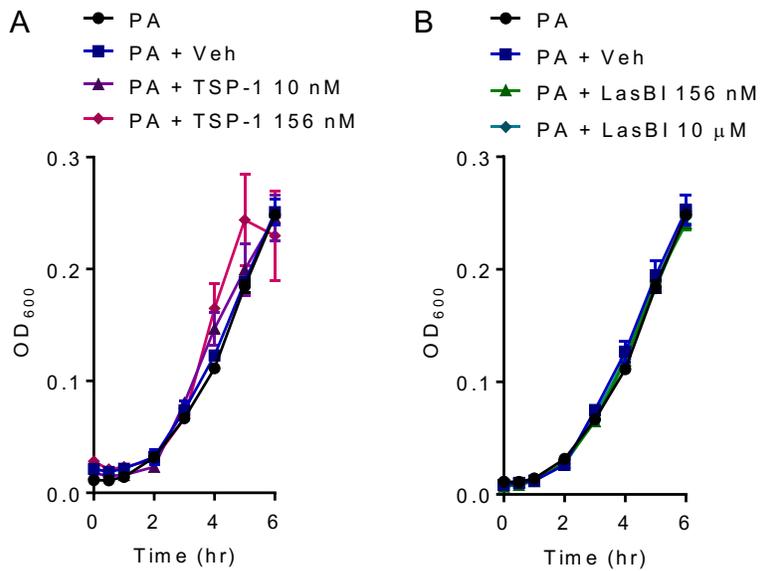
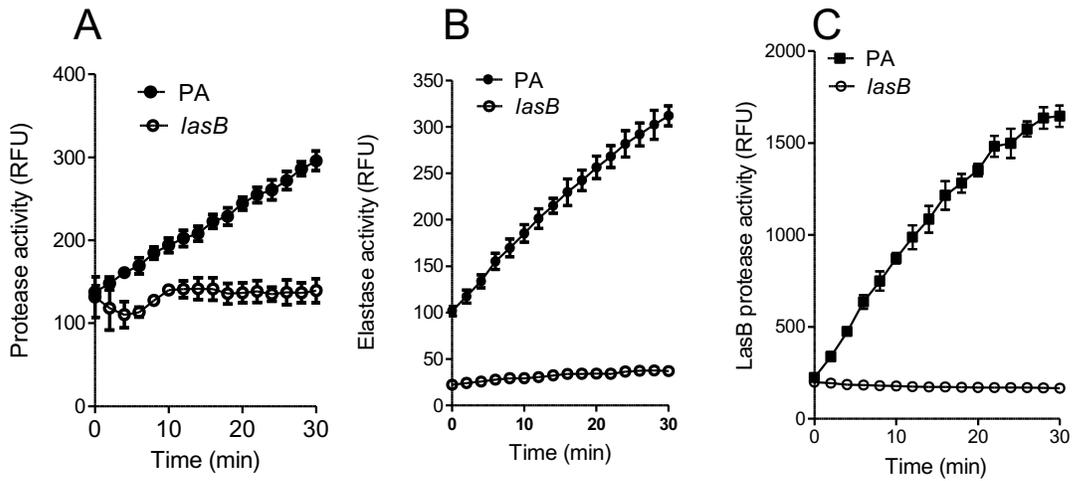


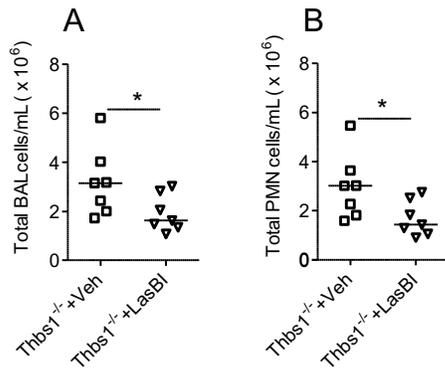
SUPPLEMENTARY INFORMATION



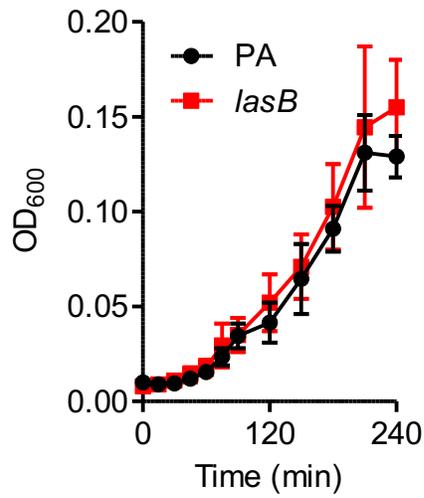
Supplementary Figure 1: Neither purified TSP-1 nor di-peptide compound LasBI possess direct antibacterial activity. PA14 was cultured in Luria broth in the presence or absence of (A) purified TSP-1 or (B) LasBI at the indicated concentrations. Growth curve was obtained by measuring optical density at 600 nm in duplicates over 6 hours. Data is presented as mean \pm SEM. Representative study of 2 independent experiments is shown for (A) and one experiment for (B).



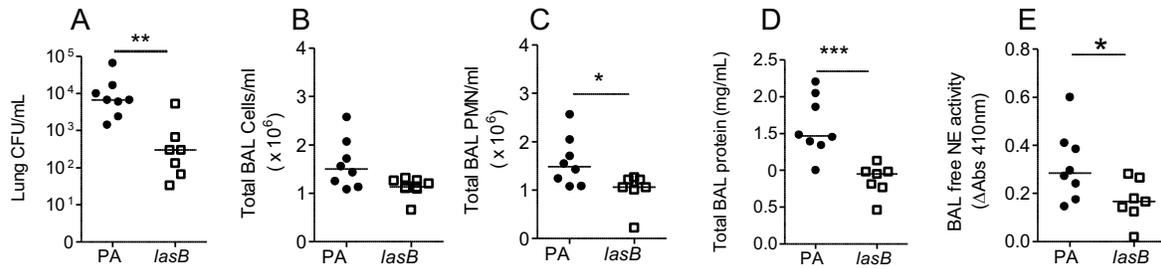
Supplementary Figure 2. PA14 mutant containing a transposon insertion in the *LasB* gene (*PA_{LasB}::Tn5*, abbreviated as *lasB*) is functionally deficient in protease, elastase, and LasB activity. (A) Total protease activity of SN from PA parent strain, and *lasB* mutant was measured by the cleavage of fluorogenic casein substrate in relative fluorescence units (RFU) over time. (B) Total elastase activity of PA and *lasB* was measured by the cleavage of elastin substrate in relative fluorescence units (RFU) over time. (C) total LasB activity of SN from PA parent strain, and *lasB* mutant was measured by the cleavage of LasB substrate aminobenzoyl-Ala-Gly-Leu-Ala-p-nitro-benzyl-amide in relative fluorescence units (RFU) over time. Assays were performed in triplicates, and a representative study of 3 independent experiments is shown.



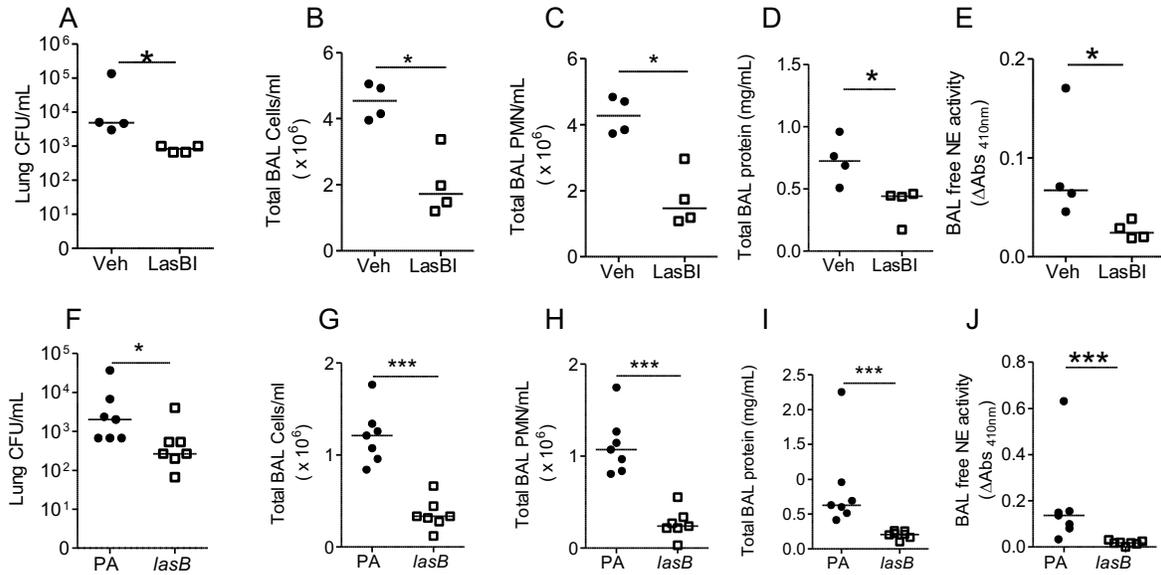
Supplementary Figure 3. Administration of di-peptide compound LasBI post-infection reduces airspace neutrophil recruitment in *Thbs1*^{-/-} mice. *Thbs1*^{-/-} mice were inoculated with PA14 (10^6 inoculum). At 2 h post-infection, mice were administered either vehicle (DMSO) or LasBI at 200 μ g/mouse by i.p. injection. Outcome measurements were obtained 20 h-post infection. (A) Total BAL cells counts/mL, (B) total BAL PMN counts/mL were obtained. Each data point represents an individual mouse, n=7 mice per group. Lines indicate the median. Mann Whitney U two-tailed test, *p < 0.05.



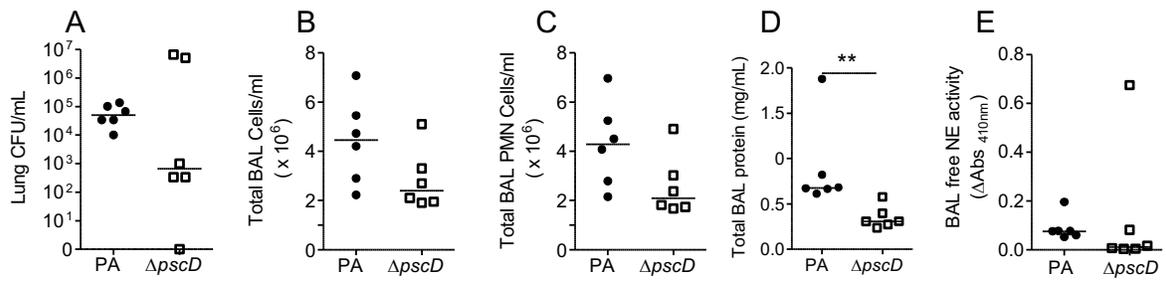
Supplementary Figure 4: PA14 and *lasB* in vitro growth pattern. A growth curve showing the growth of two strains of bacteria over time. PA14 parent strain and *lasB* were cultured overnight for 18 h, and the concentration was adjusted to the same value at OD₆₀₀. 5 μ l of each bacteria was then inoculated in 5 mL LB and cultured in 37 °C at 250 RPM, and 180 μ l of medium was measured at OD₆₀₀ over time. The result of one representative experiment out of 3 is shown. Assays were performed in duplicate wells.



Supplementary Figure 5: *LasB* mutant induces less bacterial burden, neutrophilic inflammation, and lung microvascular leak than parent PA strain in *Thbs1*^{-/-} mice. *Thbs1*^{-/-} mice were inoculated with PA14 parent strain or *lasB* mutant and outcome measurements were obtained 20 h post-infection (PA inoculum 1.2×10⁶ CFU, *lasB* mutant inoculum 1.5×10⁶ CFU). (A) Lung CFU/mL, (B) total BAL cell counts/ mL, (C) total BAL PMN counts/ mL, (D) total BAL protein concentrations, and (E) BAL free NE activity. Each data point represents an individual mouse, n=7-8 mice/group. Lines indicate the median. Mann-Whitney U two-tailed test, *p < 0.05, **p<0.01, ***p < 0.001.



Supplementary Figure 6: Effect of LasB inhibitor and PA mutant *lasB* in the lungs following acute intrapulmonary infection in WT mice. (A-E) WT mice were intratracheally instilled with PA14 (10^6 CFU inoculum). At 2h post-infection, mice were administered either vehicle or LasBI at 200 μ g/mouse by i.p. injection. Outcome measurements were obtained 20 h post-infection. (A) Lung CFU/mL, (B) total BAL cell counts/mL, (C) total BAL PMN counts/mL, (D) total BAL protein concentrations, (E) BAL free NE activity. (F-J) WT mice were inoculated with PA14 or *lasB* mutant and outcome measurements obtained 20 h post-infection (PA inoculum 1×10^6 CFU, *lasB* mutant inoculum 2.7×10^6 CFU). (F) Lung CFU/mL, (G) total BAL cell counts/ mL, (H) total BAL PMN counts/ mL, (I) total BAL protein concentrations, and (J) BAL free NE activity. Each data point represents an individual mice. Lines indicate the median. Mann-Whitney U two-tailed test, * $p < 0.05$, *** $p < 0.001$.



Supplementary Figure 7: PA14 $\Delta pscD$ induces comparable bacterial burden, neutrophilic inflammation in WT mice lungs as PA14 parent strain. WT mice were inoculated with PA14 parent strain or $\Delta pscD$ and outcome measurements obtained 20 h post-infection (PA inoculum 3.5×10^6 CFU, $\Delta pscD$ mutant inoculum 2.3×10^6 CFU). (A) Lung CFU/mL, (B) total BAL cell counts/mL, (C) total BAL PMN counts/mL, (D) total BAL protein concentrations, and (E) BAL free NE activity were measured. Each data point represents an individual mouse. Lines indicate the median. Mann-Whitney U two-tailed test, ** $p < 0.01$.