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 ± 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\textit{lasB}::Tn5, abbreviated as \
\textit{lasB}) is functionally deficient in protease, elastase, and LasB activity. (A) Total protease activity of SN from PA parent strain, 
and \textit{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 \textit{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 \textit{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 LasBl post-infection reduces airspace neutrophil recruitment in Thbs1−/− mice. Thbs1−/− mice were inoculated with PA14 (10⁶ inoculum). At 2 h post-infection, mice were administered either vehicle (DMSO) or LasBl at 200 µg/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 OD$_{600}$. 5 µl of each bacteria was then inoculated in 5 mL LB and cultured in 37 °C at 250 RPM, and 180 µl of medium was measured at OD$_{600}$ 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 1.2x10⁶ CFU, lasB mutant inoculum 1.5x10⁶ 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 µg/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 1x10^6 CFU, lasB mutant inoculum 2.7 x10^6 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: PA14ΔpscD induces comparable bacterial burden, neutrophilic inflammation in WT mice lungs as PA14 parent strain. WT mice were inoculated with PA14 parent strain or ΔpscD and outcome measurements obtained 20 h post-infection (PA inoculum 3.5 x 10^6 CFU, ΔpscD mutant inoculum 2.3 x 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.