Supplemental Methods

Transmission Electron Microscopy

Murine neutrophils were harvested from the peritoneum as described above. Cell suspensions were fixed in 2.5% glutaraldehyde in PBS then immediately pelleted in a 1.5 ml microfuge tube at 300 x G. After 1 hour fixation, the supernatant was removed and the cell pellets were washed 3x in PBS then post-fixed in 1% OsO_4, 1% $K_3Fe(CN)_6$ for 1 hour. Following 3 additional PBS washes, the pellet was dehydrated through a graded series of 30-100% ethanol, 100% propylene oxide then infiltrated in 1:1 mixture of propylene oxide:Polybed 812 epoxy resin (Polysciences, Warrington, PA) for 1 hr. After several changes of 100% resin over 24 hrs, pellet was embedded in a final change of resin, cured at 37^{o}C overnight, followed by additional hardening at 65^{o}C for two more days. Ultrathin (70 nm) sections were collected on 200 mesh copper grids, stained with 2% uranyl acetate in 50% methanol for 10 minutes, followed by 1% lead citrate for 7 min. Sections were imaged using a JEOL JEM 1011 transmission electron microscope (Peabody, MA) at 80 kV fitted with a bottom mount AMT 2k digital camera (Advanced Microscopy Techniques, Danvers, MA).

Neutrophil in vitro phagocytosis assay

Neutrophil in vitro phagocytosis was assessed using the Vybrant phagocytosis kit (Molecular Probes, Grand Island, NY). Briefly, neutrophils were isolated from WT and *thbs1*$^{-/-}$ mice 6 hours after intraperitoneal injection of thioglycollate, and 0.5x10^{6} cells were seeded in a 96-well plate. Non-adherent cells were gently washed off after 30 minutes. Next, 100 µl of fluorescein labeled *E. coli* (strain K-12) bioparticles (1 mg/ml) was added to each well. After incubation for 90 minutes, non-cell-associated bioparticles were removed by aspiration. The remaining fluorescence from extracellular bioparticles was quenched by trypan blue according to the

manufacturer's protocol. After removing trypan blue, the fluorescence intensity was determined by a fluorescence plate reader using 480nm excitation and 520 nm emission. Fluorescence intensity of background (neutrophils in the absence of fluorescently-labeled bioparticles) was subtracted from each sample.

Neutrophil microbial killing assay

In vivo neutrophil microbial killing was assessed utilizing a modification of a protocol previously reported[1-3]. Briefly, mice were i.p. injected with 2 mL of 3% thioglycollate to recruit neutrophils. Six hours later, mice were inoculated with 1×10^8 *K. pneumoniae* intraperitoneally. In select experiments, 10 µg recombinant TSP-1 (rTSP-1) or vehicle (PBS) was administered intraperitoneally 30 minutes prior to the inoculation of *K. pneumoniae.* In other experiments, mice were injected intraperitoneally with peptide DV-9 (1.5 mg) or vehicle DMSO 10 minutes prior to the inoculation of *K. pneumoniae*. Either immediately or 30 minutes following *K. pneumoniae* inoculation, mice were euthanized and neutrophils were harvested from the peritoneum with sterile HBSS containing 100 µg/ml gentamicin (3 mL x 4) to kill extracellular, attached bacteria[1,2]. Cell counts were manually obtained using a standard hemocytometer. Cytospins confirmed >90% neutrophils. $1x10^6$ neutrophils/sample were either lysed to obtain the initial time point or incubated ex vivo in HBSS + gentamicin at 37 ^oC for additional time and subsequently lysed by 0.1% Triton X-100, followed by serial plating for bacterial CFU quantification. CFU/10^6 PMN was obtained for each sample.

In vitro neutrophil microbial killing assay was conducted similarly to the *in vivo* microbial killing assay described above, except that neutrophils were stimulated with *K. pneumoniae in*

vitro. Briefly, mice were i.p. injected with 2 mL of 3% thioglycollate to recruit neutrophils. Six hours later, mice were euthanized and neutrophils were immediately harvested from the peritoneum. Cell counts were manually obtained using a standard hemocytometer. Cytospins confirmed >90% neutrophils. *K. pneumoniae* were pre-opsonized in 20 % mouse serum on ice for 15 minutes. Neutrophils in RPMI were plated onto 24 well plates at $1x10^6$ neutrophils/well and infected with equal volume of *K. pneumoniae* in serum at a multiplicity of infection of 50 bacteria: 1 neutrophil. Following a 30 min incubation, cells were washed with HBSS + gentamicin at 100 μg/mL and neutrophils lysed to obtain the initial CFU, or incubated further at 37 ^oC for additional time and subsequently lysed by 0.1% Triton X-100, followed by serial plating for bacterial CFU quantification. CFU/10^6 PMN was obtained for each sample.

Lung Histology Inflammation Scoring

Random images at 60x objective magnification were taken from H&E sections of lung tissue from 3 WT and 3 *thbs1*$^{-/-}$ mice at 72 h post-bacterial inoculation with i.t. *K. pneumoniae*. A total of 60 images (10 images from each lung tissue section) were saved as TIF files and blinded by operator (SS). The images were then scored by 3 blinded reviewers (MH, MY, JL) using the following histology scoring system: (1) normal; (2) inflammatory cell infiltration encompassing < 50% of airspaces and/or interstitium, minimal patchy disease severity; (3) inflammatory cell infiltration encompassing < 50% of airspaces and/or interstitium, more than minimal patchy disease severity; (4) inflammatory cell infiltration encompassing > 50% of airspaces and/or interstitium, moderate patchy disease severity; (5) diffuse inflammatory cell infiltration, encompassing >75% of airspaces and/or interstitium involvement. The average score from 3

blinded reviewers were then calculated for all images to yield lung histology inflammation score for WT and *thbs1*$^{-/-}$ mice.

Supplemental Figure Legend

Figure S1. Kinetics of pulmonary host response in WT and *thbs1*$^{-/-}$ *mice* following intratracheal *K. pneumoniae* inoculation. (A) Colony forming units (CFU) obtained from lung tissue homogenate cultures of WT and *thbs1*$^{-/-}$ mice 24, 48, 72, and 96 hours after i.t. inoculation with *K. pneumoniae* (1.8 x 10^3 CFU inoculum). (B) CFU obtained from splenic homogenate cultures of WT and *thbs1*$^{-/-}$ mice 24, 48, 72, and 96 h after i.t. inoculation with *K. pneumoniae.* Each data point shown reflects individual mouse. Kruskal-Wallis test with Dunn's multiple comparisons for *in vivo* studies, *$p<0.05$. Note $\log_{10}$ scale. (C) BAL total cell counts (x 10^6/mL), and (D) BAL total PMN cell counts (x 10^6/mL) are reported for each of the time point studied. (E) Total BAL protein was measured in mcg/mL, n= 6 mice/group at 24 and 48 h time point, n=7-8 mice/group at 72 h time point, n=3-4 mice/group at 96 h time point. (F) BAL IL-10 concentrations were measured by ELISA and reported as pg/mL, n=7-8 mice/group at 72 h time point, n=3-4 mice/group at 96 h time point. Mann-Whitney U rank sum test, *$p<0.05$, **$p<0.01$.

Figure S2. Representative H&E sections of R lung tissue obtained from WT and *thbs1*$^{-/-}$ mice at 72 hours post-*K. pneumoniae* instillation. Experimental mice underwent bronchoalveolar lavage of the lung prior to tissue fixation. Red arrows indicate polymorphonuclear cells. Scale bar = 100 μm.

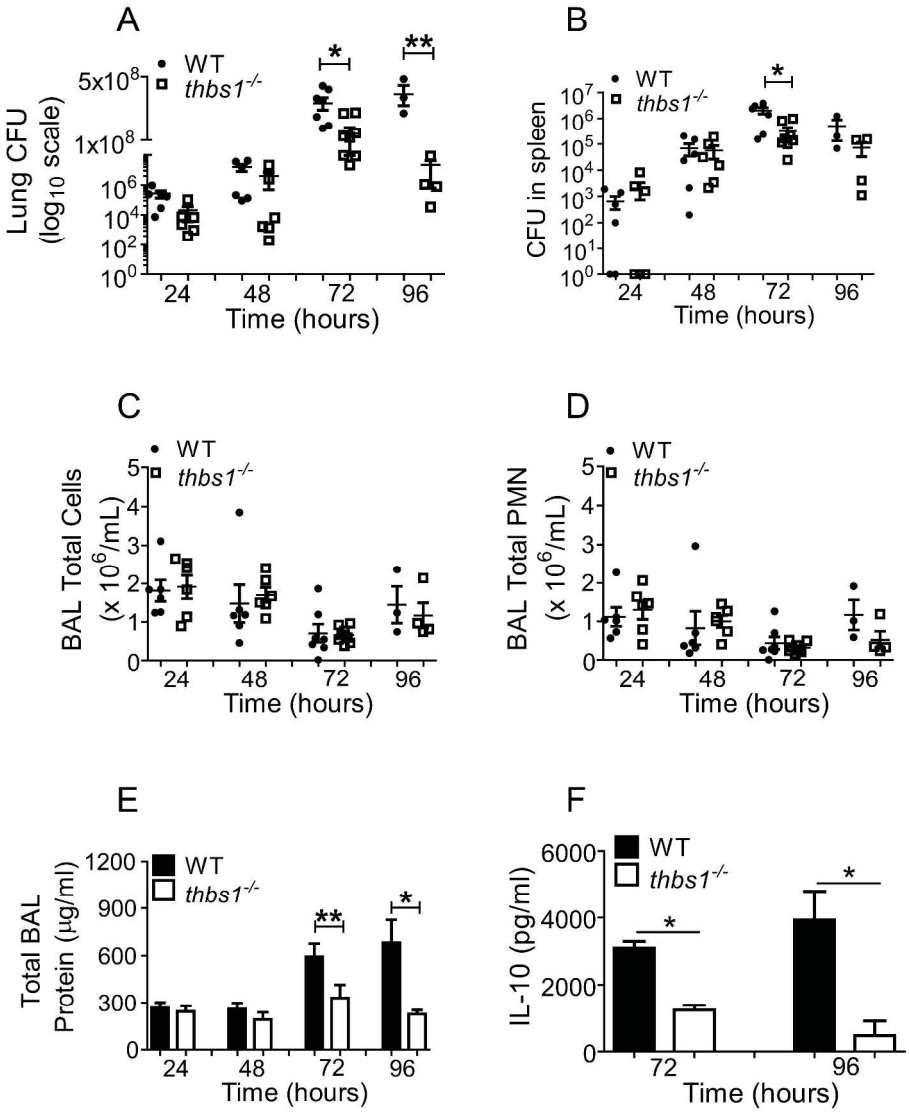
Online Reference Citations

1 Ledford, J. G., Kovarova, M. & Koller, B. H. Impaired host defense in mice lacking ONZIN. *J Immunol* 178, 5132-5143 (2007).

2 Madenspacher, J. H. *et al.* p53 Integrates host defense and cell fate during bacterial pneumonia. *J Exp Med* 210, 891-904, doi:10.1084/jem.20121674 (2013).

3 Hirche, T. O., Gaut, J. P., Heinecke, J. W. & Belaaouaj, A. Myeloperoxidase plays critical roles in killing Klebsiella pneumoniae and inactivating neutrophil elastase: effects on host defense. *J Immunol* 174, 1557-1565 (2005).

Supplemental Figure1



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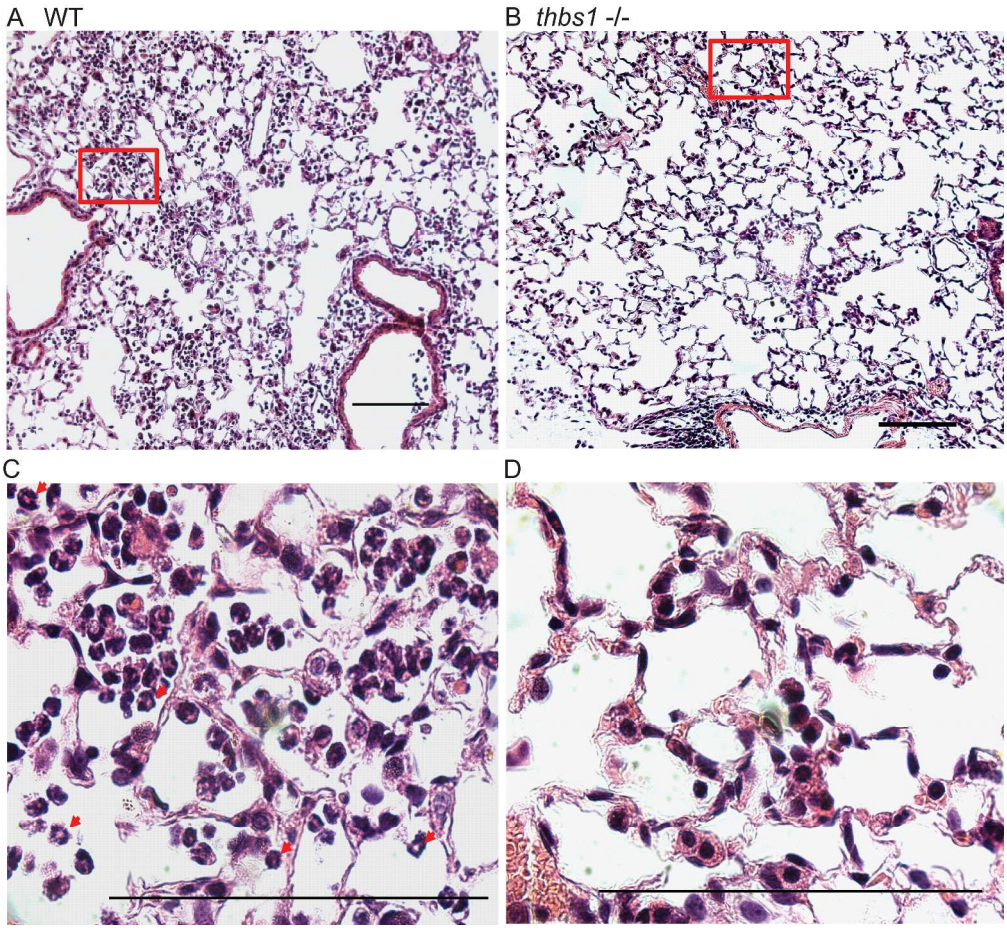
Supplemental Figure 2



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