Supporting Information

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SI Materials and Methods

Reagents. *Pseudomonas* exotoxin A was purchased from List Biological Laboratories, and FP59 was a generous gift from S. Leppla (National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda). Anti-capn2 antibody was purchased from Cell Signaling Technology. The mouse calpain-2 (capn2) cDNA construct (pCMV6-capn2) was purchased from OriGene.

Overexpression of Capn2. The mouse capn2 ORF was amplified from the pCMV6-capn2 cDNA construct (OriGene) using primers 5′-AACCGGATCGCTACCATGGCGGGCAT and 5′-TCCAGACTAGTAACTTCAGAGT and was cloned into lentiviral vector, pLenti-CMV-BSD. For capn2 overexpression, RAW264.7 cells were infected with the lentivirus containing pLenti-CMV-BSD-capn2 or empty vector pLenti-CMV-BSD, followed by selection using 2 μg/mL of blasticidin S (Invitrogen).

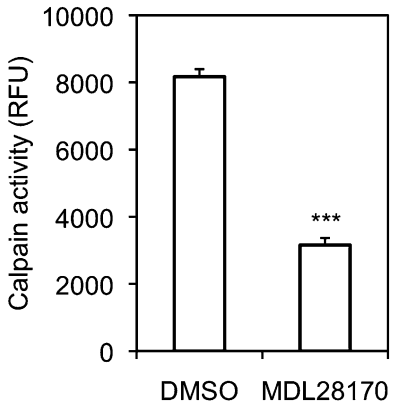

Fig. S1. Effect of MDL28170 on calpain activity. RAW264.7 cells were treated with 40 μM MDL28170 or 0.1% (vol/vol) DMSO for 2 h. Calpain activity was determined in total-cell lysates using synthetic fluorogenic substrates as described in *Materials and Methods*. The bar graph shows the mean ± SD for values obtained in three independent experiments. ***$P < 0.001$. RFU, relative fluorescence units.

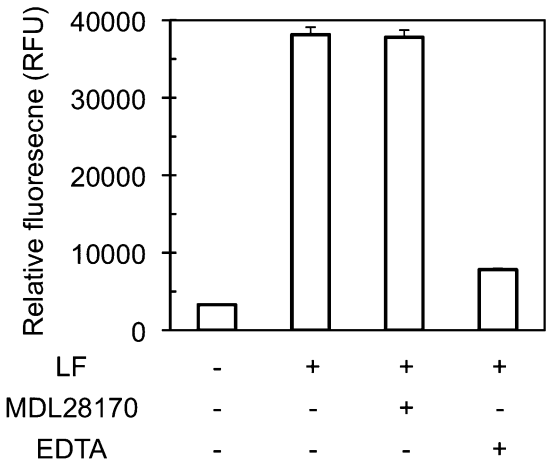

Fig. S2. Absence of effect of MDL28170 on lethal factor (LF) enzymatic activity. LF enzymatic activity was determined using a FRET-based substrate, MAPKKide (List Biological Laboratories). Cleavage of the quenched fluorescent substrate by LF is proportional to an increase in fluorescence intensity (1). LF was preincubated with 40 μM MDL28170 or 5 mM EDTA (an LF inhibitor) at room temperature for 30 min. Then 30 μM MAPKKide (o-Abz/Dnp) was incubated with or without 10 nM LF in 20 mM Hepes (pH 8.2) at 37 °C for 30 min, and the fluorescence signal was measured at 420 nm following excitation at 320 nm. Data shown are the mean ± SD from three independent experiments.

1. Kocer SS, Walker SG, Zerler B, Golub LM, Simon SR (2005) Metalloproteinase inhibitors, nonantimicrobial chemically modified tetracyclines, and ilomastat block *Bacillus anthracis* lethal factor activity in viable cells. *Infect Immun* 73(11):7548–7557.

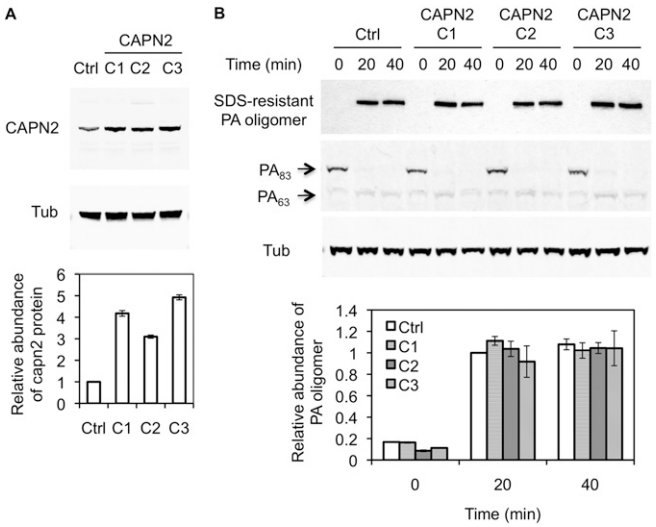

Fig. S3. Effect of adventitious expression of capn2 on protective antigen (PA) endocytosis. RAW264.7 cells were infected with lentivirus containing pLenti-CMV-BSD-capn2 or the empty vector as a control. Three clones (CAPN2 C1–C3) in which capn2 protein expression was more than threefold higher than in the control cells (Ctrl) were tested. (*A*) (*Upper*) The abundance of capn2 protein was assessed by Western blotting using anti-capn2 antibody and normalized against tubulin detected using rabbit anti-tubulin antibody (Abgent). (*Lower*) Mean ± SD of relative levels of capn2 from three independent experiments. (*B*) PA endocytosis. The cells were exposed to 1 μg/mL PA at 4 °C for 1 h, washed, and shifted to 37 °C for the indicated time. Cell lysates were analyzed by Western blotting using anti-PA and anti-tubulin antibodies. The mean ± SD of the level of SDS-resistant PA oligomer relative to tubulin from three independent experiments is shown.

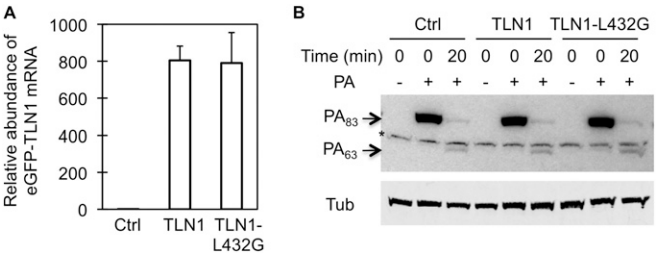

Fig. S4. Effect of adventitious expression of talin-1 (TLN1) on binding and processing of PA. RAW264.7 cells were transfected with pEGFP-C1 vector expressing wild-type TLN1 or the mutant form TLN1-L432G. (*A*) Abundance of mRNA encoding EGFP-tagged TLN1 was assessed in parental cells (Ctrl) and in transfected cells by quantitative RT-PCR using EGFP-specific primers and was normalized to that of β-actin. Data represent mean ± SD of three independent experiments. (*B*) Binding and processing of PA. The cells were exposed to 1 μg/mL PA at 4 °C for 1 h and then were shifted to 37 °C for 20 min. Cell lysates were analyzed by Western blotting using anti-PA and anti-tubulin antibodies. The asterisk indicates a band that was determined to be nonspecific as noted in the legend of Fig. 6.

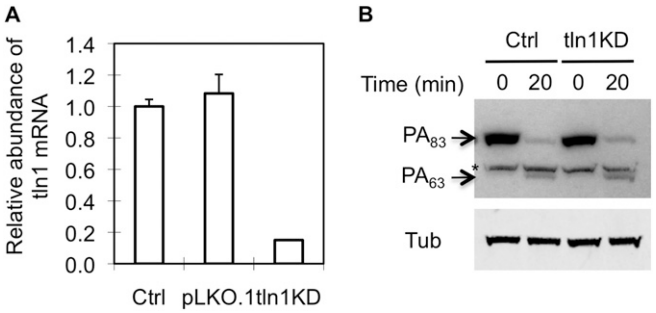

Fig. S5. Effect of shRNA-mediated knockdown of TLN1 on binding and processing of PA. RAW264.7 cells were infected with lentivirus expressing TLN1 shRNA (tln1KD) or empty vector (pLKO.1). (*A*) Talin-1 mRNA abundance was assessed in parental cells (Ctrl) and lentivirus-infected cells by quantitative RT-PCR using primers specific to TLN1 and normalized to values obtained for β-actin mRNA. Values are shown as mean ± SD from three independent experiments. (*B*) Binding and processing of PA. The cells were exposed to 1 μg/mL PA at 4 °C for 1 h and then shifted to 37 °C for 20 min. Cell lysates were analyzed by Western blotting using anti-PA and anti-tubulin antibodies. The asterisk indicates a band that was determined to be nonspecific as noted in the legend to Fig. 6.

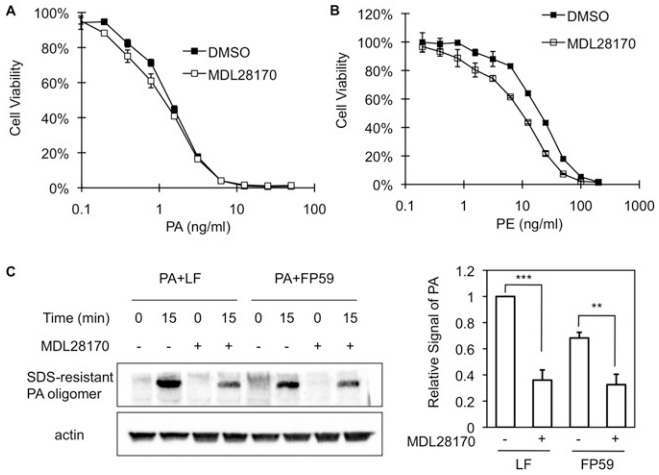

Fig. S6. Effect of MDL28170 on macrophage susceptibility to PA-FP59 and *Pseudomonas aeruginosa* exotoxin A (PE toxin). (*A* and *B*) Effect of MDL28170 on cellular susceptibility to PA-FP59 or to PE toxin. RAW264.7 cells were pretreated with 40 μM MDL28170 or 0.1% DMSO for 1 h before exposure to serially diluted PA in the presence of 50 ng/mL FP59 (*A*) or serially diluted PE toxin (*B*). After 1 d of toxin treatment, cell viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Data are from one representative experiment ($n = 3$) carried out in triplicate. LD_{50} values for PA of DMSO-treated cells and MDL28170-treated cells in the presence of FP59 are 1.36 ± 0.02 ng/mL and 1.12 ± 0.07 ng/mL, respectively ($P > 0.05$). LD_{50} values for PE in DMSO-treated cells and MDL28170-treated cells are 18.93 ± 0.01 ng/mL and 8.94 ± 0.21 ng/mL, respectively ($P < 0.01$). (*C*) Effect of MDL28170 on PA endocytosis in the presence of LF or FP59. Cells were incubated with 80 MDL28170 for 1 h, followed by exposure to 0.5 μg/mL PA in the presence of 0.25 μg/mL LF or FP59 at 4 °C for 1 h and then were shifted to 37 °C. After 15 min, the cells were collected and analyzed by Western blotting using anti-PA antibody as probe. Relative amounts of SDS-sensitive PA oligomer were determined by normalization against β-actin in two independent experiments. $^{**}P < 0.01$; $^{***}P < 0.001$.