Supplemental Figure 1. Visualization of NETs by ex vivo immunofluorescence and intravital lung microscopy. (A-C) BAL from mice challenged i.t. with MIP-2. Ex vivo, BAL was treated with (A) PMA (NETosis control), (B) PBS (untreated control) or (C) repeated freeze and thaw cycles (necrosis control). BAL was stained with Sytox green (green), neutrophil elastase antibody (red), and histone H2B antibody (blue). Scale bar=10μm. (D,E) Lung 2-photon intravital microscopy. (D) MRP8-mTmG mice (green neutrophils) were challenged with SytoRed-stained MRSA (2 x 10^7 cfu, i.t., pseudo-colored in blue). Bacteria (circles) were observed from 0 to 1h after the infection. Arrowheads indicated bacteria internalized by neutrophils. Scale bar=10 μm (E) MRP8-nTnG mice (red vasculature, blue neutrophils) were challenged i.t. with 5 x 10^6 cfu mcherry-PAO1 (pink, arrows) and observed from 3 to 5h after the infection. Extracellular DNA was stained with Sytox green. Scale bar=20 μm
Supplemental Figure 2. NETs in plasma, BAL cell counts, and cytokines in PAD4-impaired mice after MRSA infection. *PAD4+/+, PAD4+-, or PAD4-- littermates were challenged in vivo with MRSA (5 x 10^7 cfu, i.t.). BAL, blood and lung were collected at 24h. (A) NETs (NE-DNA complexes) and (B) CihH3-DNA complexes were quantified in plasma. (C) BAL WBCs, (D) neutrophils, and (E) macrophages. (F) IL-12 and (G) IFN-γ concentration in BAL. n=11-19. Data were analyzed and compared to the WT mice group using one-way ANOVA (*p<0.05, ** p<0.01).
Supplemental Figure 3. Inhibition of NETs with Cl-amidine treatment after MRSA infection. (A) WT mice were challenged in vivo with MRSA (5 x 10^7 cfu, i.t) and treated with Cl-amidine (50 mg/kg, i.p.) 3 hours after infection (n=12). BAL, blood, and lung were collected at 24h. (B) CitH3-DNA complexes and (C) NETs (NE-DNA complexes) in BAL fluid were quantified. (D) NETs (NE-DNA complexes) in plasma. (E) BAL total protein concentration, (F) BAL WBCs, (G) bacterial counts in lung, (H) body temperature loss, and (I-O) BAL cytokines: IL-6, IL1β, MCP-1, TNF-α, IL-12, IFN-γ and IL-10. Data were analyzed using Student’s t-test (*p<0.05, ** p<0.01, ****p<0.0001).
Supplemental Figure 4. DNasel concentration in human plasma. DNasel concentration in plasma from patients with (A) ARDS (n=104) or with acute cardiac conditions (n=40), p=0.003; (B) pneumonia ± ARDS (n=24 and 14), p=0.7; (C) non-pulmonary sepsis ± ARDS (n=73 and 21), p=0.07; (D) mild, moderate or severe ARDS according to the Berlin definition (n=19, 30, 25), p=0.6. (E) Association of NETs with ARDS mortality (n=64, 40), p=0.8 and (F) mortality in sepsis/pneumonia (n=102, 47), p=0.8. Data were analyzed using the Mann-Whitney-Wilcoxon test.