Supplemental Figures

Fig. S1. Nuclease-independent activation and cytotoxicity of neutrophils after co-incubation with *S. aureus.*

Percentage of LDH release (as marker for cytotoxicity) and elastase release (as marker for activation) by PMA-stimulated neutrophils after co-incubation with *S. aureus* LAC wild type empty vector control (wt + pCM28), *nuc*-mutant empty vector control (*nuc* + pCM28) or complemented mutant strain *(nuc* + pCM28*nuc*). The results of n = 3 independent experiments were analyzed using a paired, one-tailed Student's t-test. P values of < 0.05 were considered to be significant. No significant differences were detected between the three different bacterial strains.

Fig. S2. Entrapment of bacteria by NETs.

Representative immuno-fluorescent micrograph showing entrapment of FITC-labelled bacteria within NETs. *S. aureus* LAC wild type empty vector control (wt + pCM28) or *nuc*-mutant empty vector control (*nuc* + pCM28) were co-incubated with PMA-stimulated neutrophils at a MOI of 2 for 90 min at 37°C in 5% CO_2. After washing and fixation, NETs were visualized with DNA-intercalating dye Dapi (blue). Bacteria are shown in green. Note that the remaining NETs that are not eliminated by the wild type strain have the same capability to capture bacteria compared to those NETs co-incubated with the *nuc*-mutant strain**.**

Fig. S3. Total (extra- and intracellular) antimicrobial activity of neutrophils against *S. aureus* strains.

Bacterial growth inhibition after co-incubation of *S. aureus* LAC wild type empty vector control (wt + pCM28), *nuc*-mutant empty vector control (*nuc* + pCM28) or complemented mutant strain *(nuc* + pCM28*nuc*) with PMA-stimulated neutrophils. Data are presented as percentage surviving bacteria compared to the respective bacterial growth control (100%). The results of n = 4 independent experiments were analyzed using a paired, one-tailed Student's t-test. P values of < 0.05 were considered to be significant (*** p<0.005).

Fig. S4. Nuc-mutation remains stable *in vivo*.

To confirm stability of the *nuc*-mutant (*nuc*) *in vivo*, bacteria were recovered from murine lungs at 24 h after infection. Harvested bacteria were grown overnight in BHI and culture supernatants were tested for nuclease activity in an agarose-gel-based nuclease assay. A representative agarose gel is shown, which demonstrates that bacteria recovered from wild type (wt)-infected mice show nuclease activity (DNA degradation), whereas the bacteria recovered from lungs after infection with the *nuc*-mutant (*nuc*) do not exhibit nuclease activity.