Supplemental figures

Figure S1

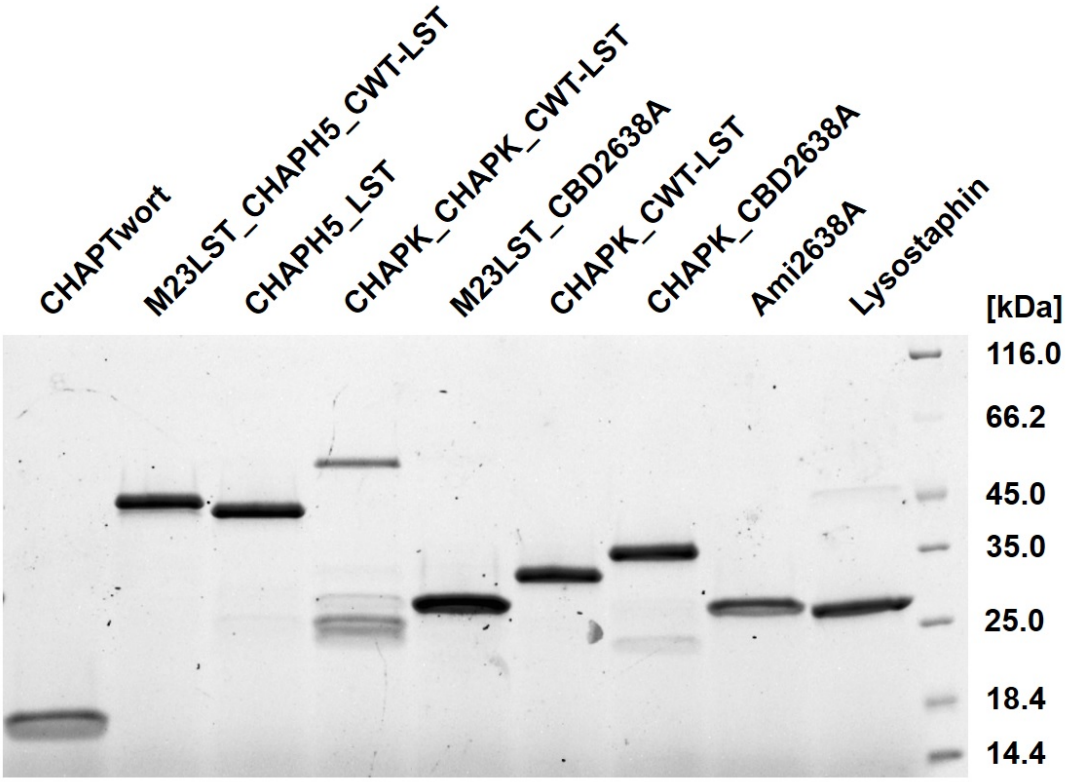

Figure S1. SDS-PAGE of PGH candidates. Expected molecular masses are listed in Table 2.

Figure S2

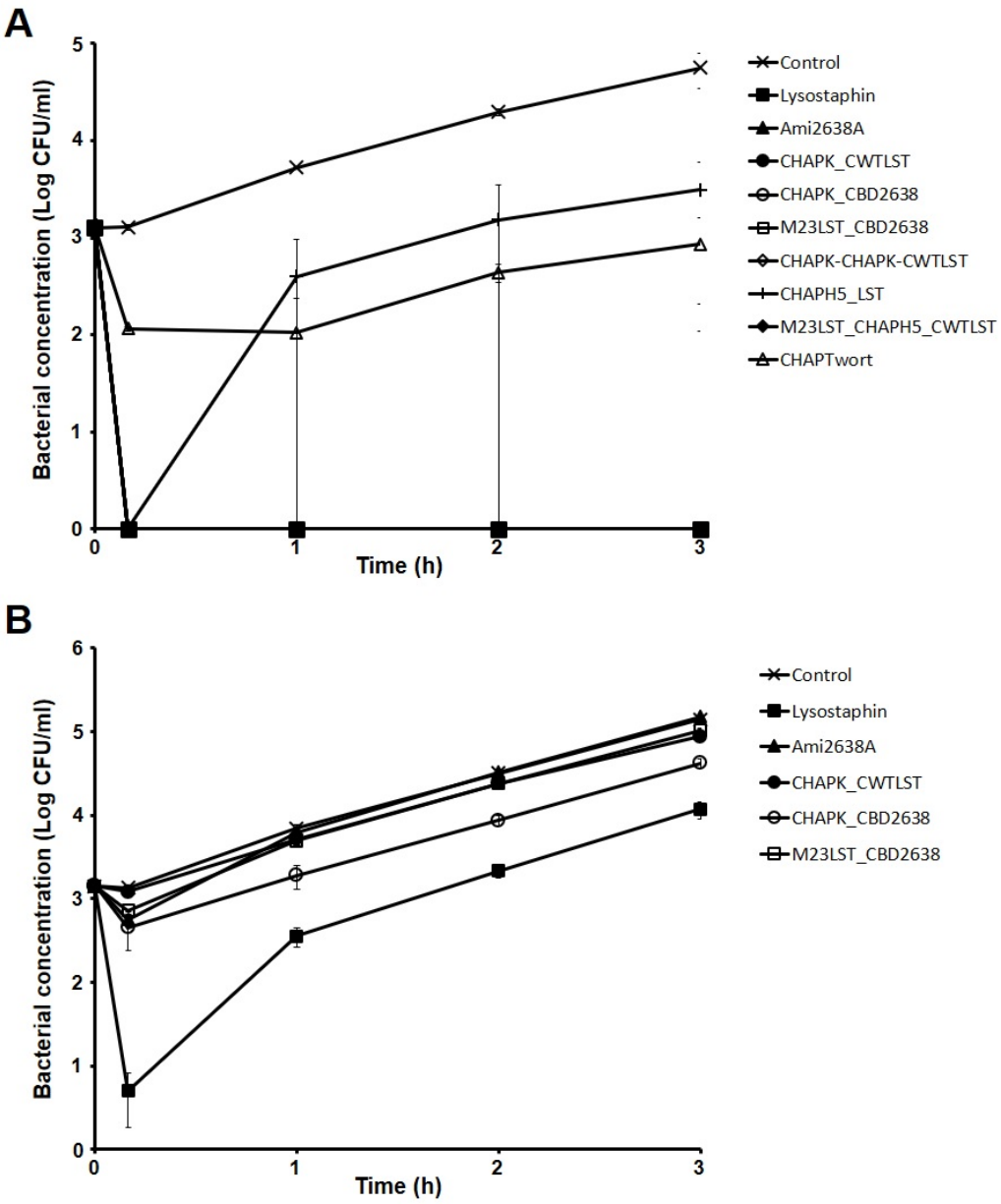

Figure S2. Time-kill assays with *S. aureus* Newbould 305 cells in UHT milk using purified PGH constructs at different concentrations or buffer as a negative control. Bacterial concentrations at different time points after enzyme/buffer addition were determined by serial dilution plating. **A.** Comparison of 9 constructs that had been identified as promising candidates by the screening (as shown in Fig. 1 A) at a concentration of 4 µM. Note that all constructs except for CHAPH5_LST

and CHAPTwort reduced bacterial numbers to undetectable levels within 10 min. **B.** Comparison of the 5 most effective PGH constructs (see Fig. 1 B, C) at a concentration of 36 nM.

Figure S3

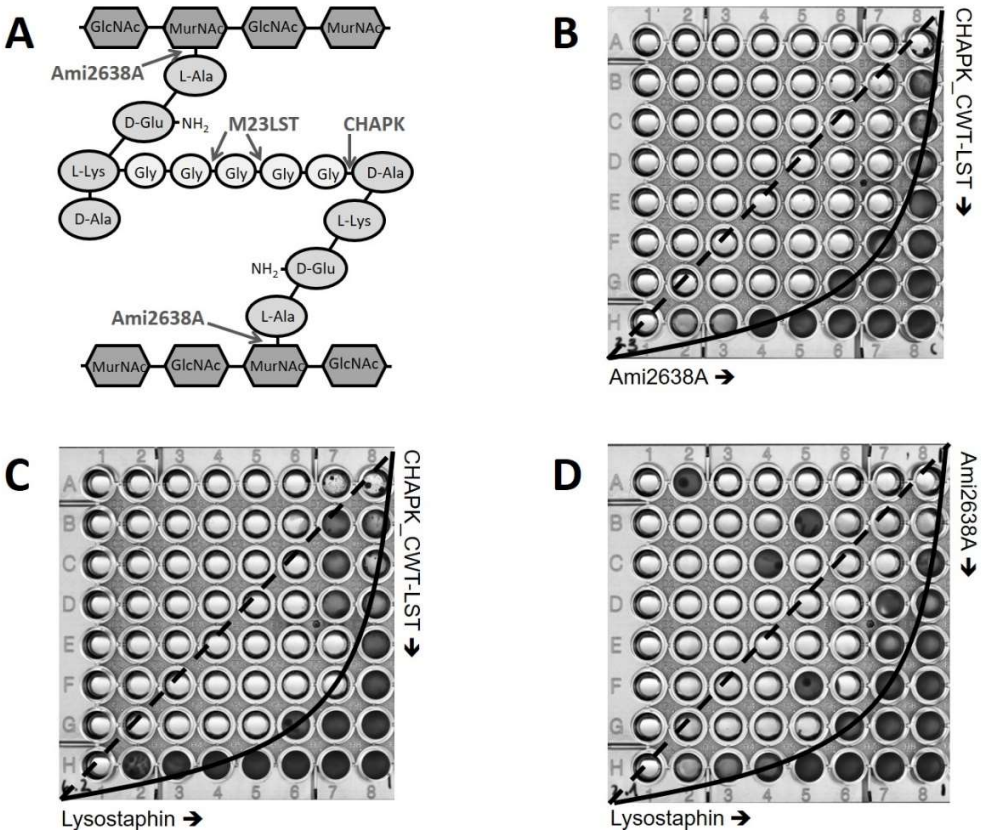

Figure S3. Peptidoglycan cleavage sites and synergistic effects of lysostaphin, Ami2638A, and CHAPK_CWT-LST. **A.** Schematic representation of *S. aureus* peptidoglycan, with sugar units depicted as hexagons, amino acids of the stem peptide as ovals, and glycines within the pentaglycine bridge as circles. Arrows indicate cleavage sites of the M23 endopeptidase domain of lysostaphin (M23LST), the amidase domain of the 2638A endolysin (Ami2638A), and the CHAP domain of LysK (CHAPK, i.e. the catalytic domain of the CHAPK_CWTLST construct). Modified from (1). **B – D.** Checkerboard assays (broth microdilution method) demonstrating synergistic effects of pairwise combinations of lysostaphin, Ami2638, and CHAPK_CWT-LST against *S. aureus* Newbould 305. Dark wells indicate bacterial growth and light wells show no growth after incubation at 37°C for 20 h. Dashed and solid black curves illustrate theoretical additive and synergistic effects, respectively. Tested combinations include Ami2638A and CHAPK_CWTLST

(**B**), lysostaphin and CHAPK_CWTLST (**C**), and lysostaphin and Ami2638A (**D**). For all assays, row A and column 1 contained the respective PGH constructs at their MICs (100% MIC); the following wells along the concentration gradients contained 86% MIC (B/2), 71% MIC (C/3), 57% MIC (D/4), 43% MIC (E/5), 29% MIC (F/6), 14% MIC (G/7), and 0% MIC (H/8).

Figure S4

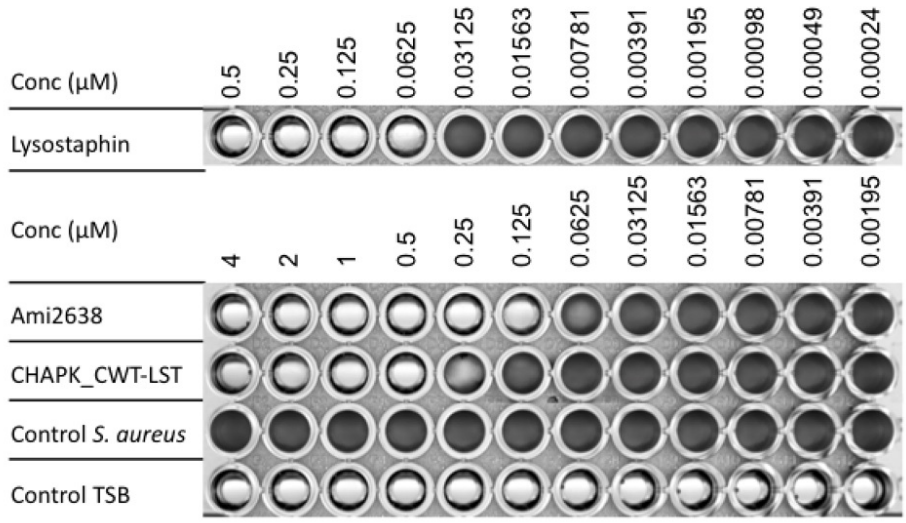

Figure S4. Determination of MICs of PGH constructs against *S. aureus* Newbould 305. Dilution series of constructs in TSB were mixed with *S. aureus*. Dark and light wells indicate bacterial growth and absence of growth after overnight incubation, respectively. *S. aureus* control wells contained bacteria without any enzyme. TSB control wells contained TSB medium without *S. aureus*.

Figure S5

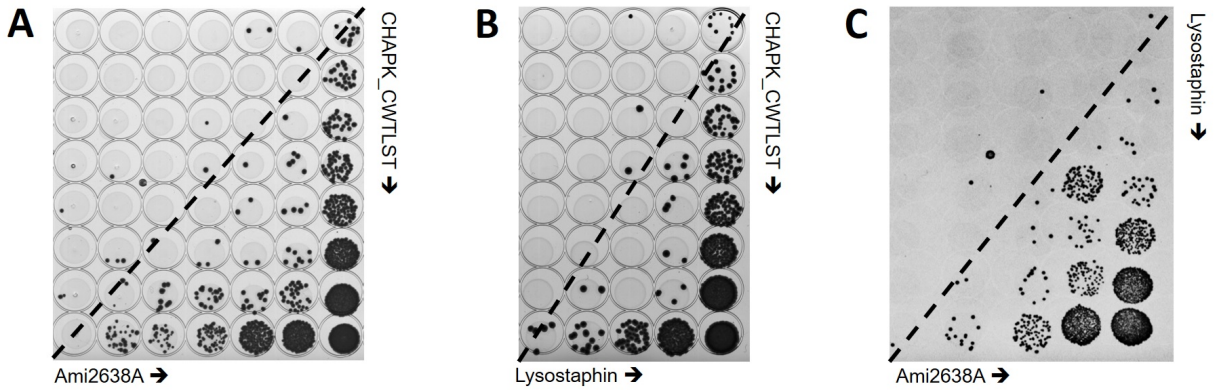

Figure S5. Modified checkerboard assays showing combined effects of lysostaphin, Ami2638, and CHAPK_CWT-LST against *S. aureus* in milk. Assays were performed essentially like classical broth microdilution checkerboard assays (see. Fig. S3) but using UHT milk instead of TSB and spotting 5 µL aliquots from each well on a TSB agar plate after incubation for 2h, followed by overnight incubation of the agar plate. Dashed lines indicate theoretical additive effects.

References

1. **Schmelcher M, Donovan DM, Loessner MJ.** 2012. Bacteriophage endolysins as novel antimicrobials. Future Microbiol **7:**1147-1171.