

Supplementary materials

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Manuscript Title: Endolysin NC5 improves early cloxacillin treatment in a mouse model of *Streptococcus uberis* mastitis

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Figure S1. Histopathological sections of murine mammary glands stained for Ly6G (neutrophils) in response to the intraductal inoculation with approximately 10^3 CFU of a clinical bovine mastitis-derived *S. uberis* isolate. Mammary glands were harvested 8, 12 and 24 h post-infection (p.i.). Pictures were captured at a 40 \times magnification, showcasing mammary glands of different mice. A scale bar, denoting 20 μ m, provides a reference for measurement.

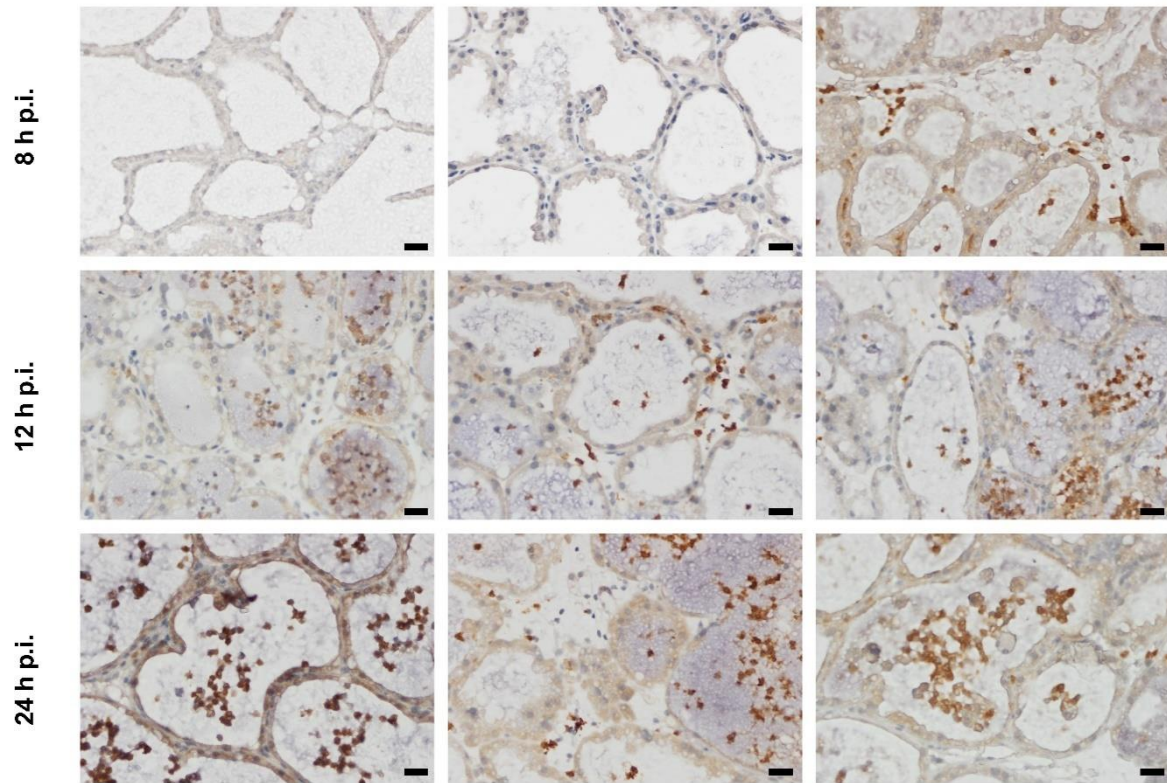


Figure S2. Histopathological sections of murine mammary glands stained for the polyhistidine tag of the endolysin after an intraductal inoculation with either 235.0 μg NC5 or endolysin buffer (phosphate buffered saline, PBS). Mammary glands were harvested 1 h post-inoculation. Pictures were captured at a 40 \times magnification, showcasing mammary glands of different mice. A scale bar, denoting 20 μm , provides a reference for measurement.

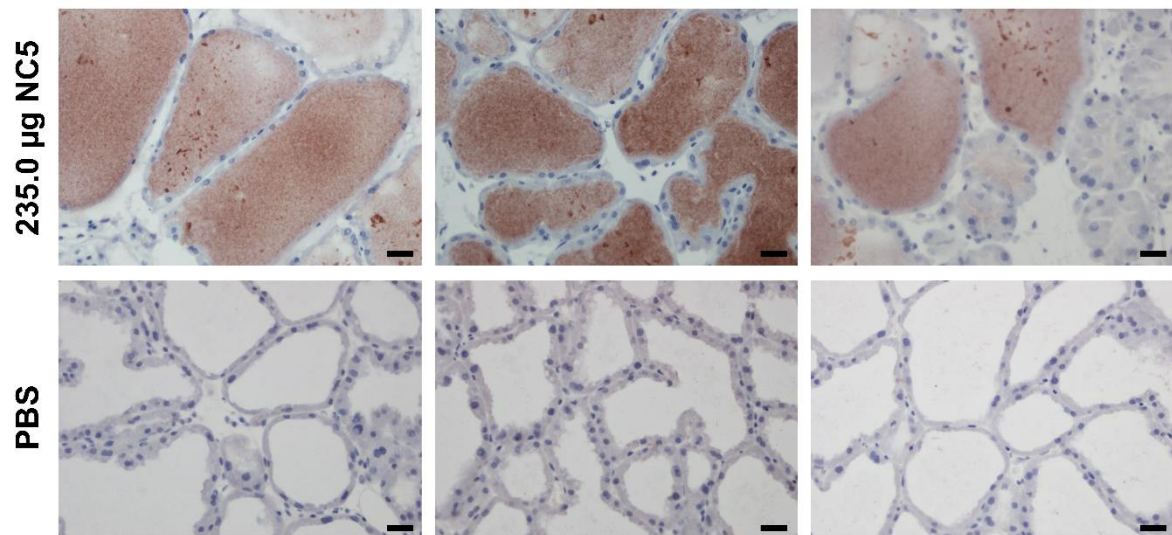


Figure S3. Histopathological sections and quantification of murine mammary glands stained for Ly6G (neutrophils) in response to the intraductal inoculation with approximately 10^3 CFU *S. uberis* and treatment administered 12 h post-infection, consisting of either placebo (PBS), 30.0 μ g cloxacillin as stand-alone antibiotic therapy, and addition of NC5 in a low (23.5 μ g) and high (235.0 μ g) dose to 30.0 μ g cloxacillin as a combination therapy. Pictures were captured at a 40 \times magnification, showcasing mammary glands of different mice. A scale bar, denoting 20 μ m, provides a reference for measurement.

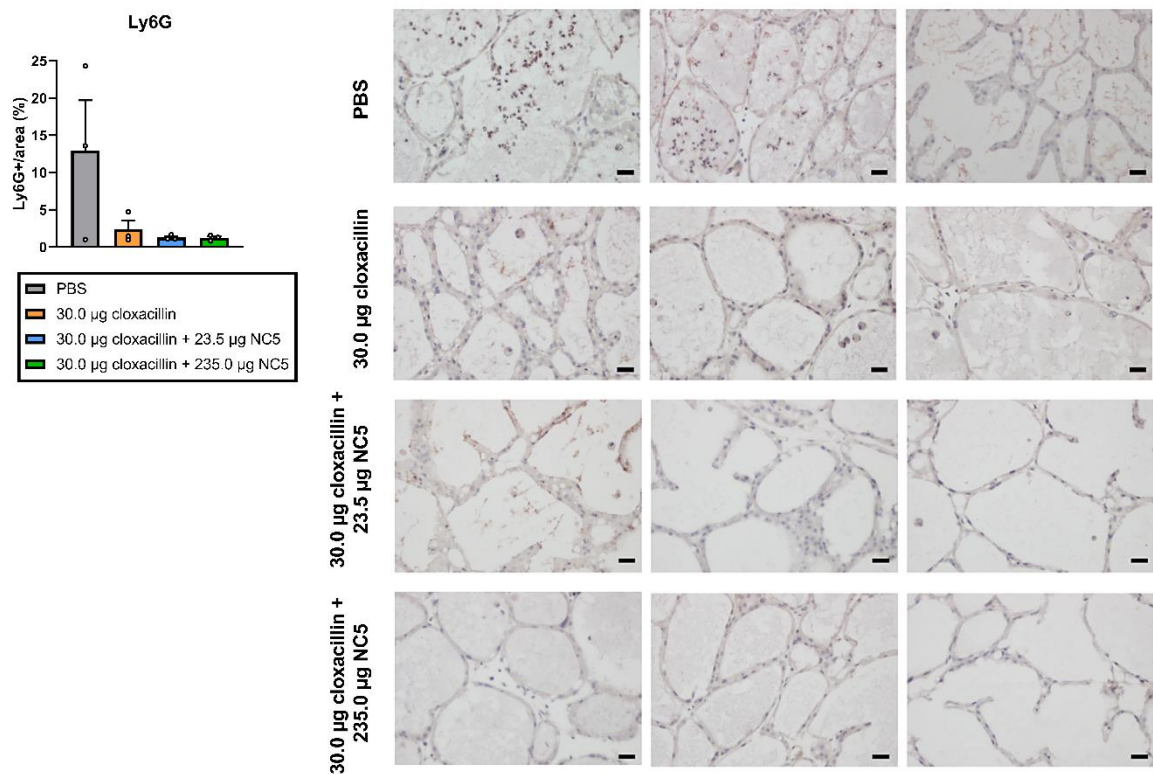


Figure S4. Comparative evaluation of the inflammatory protein profile between the different treatment groups of this study after exclusion of the slow-responding mice. Data are shown as individual points representing each mouse with a bar indicating the mean and an error bar representing the standard error of the mean. One mouse in the group that received 30.0 µg cloxacillin supplemented with 235.0 µg NC5 consistently had outliers and was removed from the dataset. A single (*) asterisk indicates $p < 0.05$.

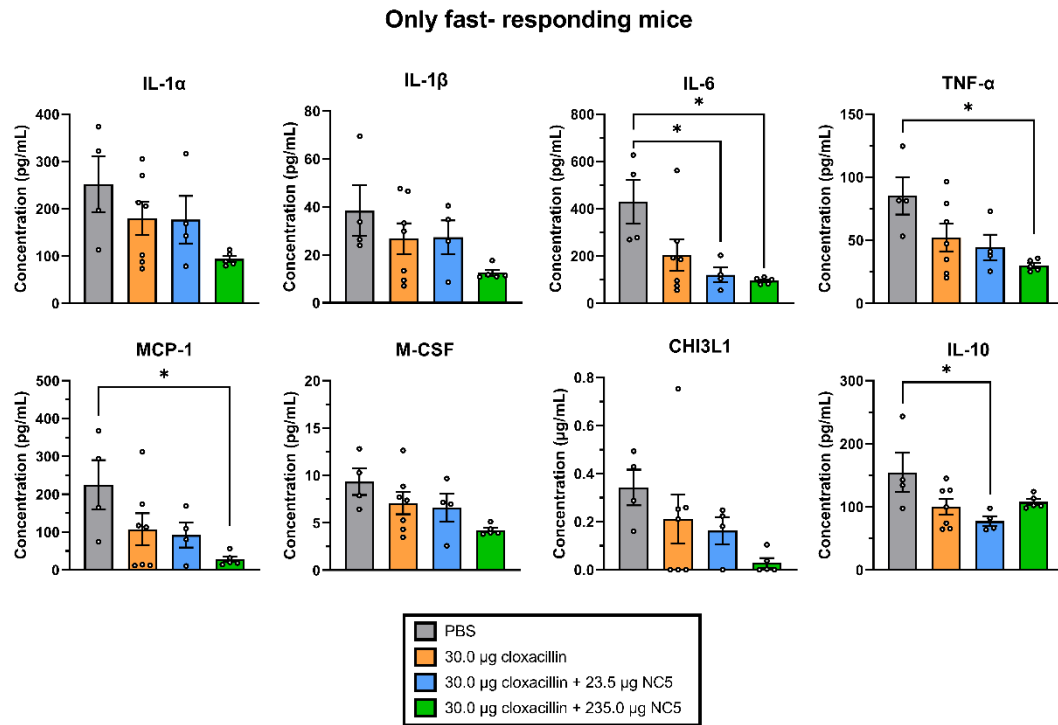


Figure S5. Histopathological sections and quantification of murine mammary glands stained for Iba-1 (macrophages) in response to an intraductal inoculation with 10^3 CFU *S. uberis* and treatment administered 12 h post-infection, consisting of either placebo (PBS), 30.0 μ g cloxacillin as stand-alone antibiotic therapy, and NC5 in a low (23.5 μ g) and high (235.0 μ g) dose to 30.0 μ g cloxacillin as a combination therapy. Pictures were captured at a 40 \times magnification, showcasing mammary glands of different mice. A scale bar, denoting 20 μ m, provides a reference for measurement.

