SUPPLEMENTAL MATERIAL

Peptide. PXL150 was synthesised using Fmoc solid phase technology (Bachem AG, Bubendorf, Switzerland). Aqueous solutions of PXL150 were prepared by dissolving the peptide in ultra pure water to the final concentrations 0.5, 2, 4 and 8.3 mg/g of PXL150. PXL150 in 1.5% hydroxypropyl cellulose (HPC) gel in 10 mM acetate buffer, 25% propylene glycol, 0.1% EDTA, pH 4.9 (Galenica AB, Malmö, Sweden) was prepared to the final concentrations 5, 8 and 10 mg/g of the peptide.

Preparation of bacteria. A few colonies of *S. aureus* (ATCC 29213, American Type Culture Collection, Manassas, VA) cultured on horse blood agar plates (Columbia agar, Oxoid, Basingstoke, UK) supplemented with 5% defibrinated horse blood (Swedish National Veterinary Institute, Uppsala, Sweden) were transferred into 10 ml 3.7% brain heart infusion (BHI) broth (Difco, BD Diagnostics, Franklin Lakes, NJ) and incubated at +37°C, 250 rpm over night. The bacterial suspension was centrifuged for 10 minutes at 900 × *g*. The supernatant was discarded and the pellet was resuspended in 1 ml 0.037% BHI. This suspension was further diluted in 0.037% BHI to reach 2×10^9 CFU/ml, as estimated by measuring optical density at 600 nm. Eight milliliters of bacterial suspension were transferred into a 15 ml test tube and 3-0 silk sutures (684G, Ethicon, Sollentuna, Sweden) were soaked for 30 minutes in the suspension. The sutures were subsequently dried on filter paper at +4°C and thereafter kept at +4°C until use (a maximum of 4 hours). During this preparation, approximately 5×10^3 cells were adsorbed per cm suture, as measured by using the same analysis protocol as described for tissue samples below.

Mouse model of surgical site infections (SSIs). All animal experiments were performed after prior approval from the local Ethics Committee for Animal Studies at the Administrative Court of Appeals in Gothenburg, Sweden. The animals were kept in a 12-hours light–dark cycle with free access to water and pellets (Lab For, Lantmännen, Malmö, Sweden), and were cared for in accordance with regulations for the protection of laboratory animals. Briefly, female mice of CD1 strain (25-30 g, Charles River, Sulzfeldt, Germany) were anaesthetized with isoflurane (Isobavet, Shering-Plough Animal Health, Farum, Denmark). The back of the mouse was shaved with a clipper, washed with 70% ethanol and a 1 cm full-thickness incision wound was placed centrally on the back of the mouse at the neck region with a scalpel. Approximately 1 cm of the infected suture was placed into the wound and secured in the skin by knotting. To avoid the mice from tearing up the wound and/or pulling out the infected suture, a single nylon suture 5-0 Ethilon*II (EH7800H, Ethicon, Sollentuna, Sweden) was attached over the middle of the incision (Fig. 1A). Buprenorfin (48 µg/kg, Temgesic, Shering-Plough, Brussels, Belgium) was given preoperatively by intraperitoneal injection for post-surgical pain relief. Thirty microliters of placebo or active treatment were applied to the wound with a micropipette two hours post-infection. The viscosity and bioadhesiveness of the HPC gel ensured that the product stayed well at the site of application, even when the mice were actively moving. At different time points post-infection (see figure legends for details), the mice were euthanized by cervical dislocation and an area of 2 × 1 cm around the wound was excised and homogenized with a rotor stator homogenizer (T10 basic ULTRA-TURRAX, IKA-WerkeGmbH & Co. KG, Staufen, Germany) in 2 ml ice cold 0.037% BHI. The homogenate was centrifuged at 900 × *g* for 10 minutes, the supernatant

was discarded and the pellet was resuspended in ice cold 0.037% BHI. The centrifugation and resuspension step was repeated twice, and at the last step the pellet was resuspended in 2 ml phosphate buffer (0.05% Triton X-100 in 0.0375 M phosphate buffer). This suspension was diluted in five 10-fold steps by transferring 22.2 µl to 200 µl phosphate buffer in a 96 well plate. Fifty microliters of each dilution, including the original suspension, were transferred to horse blood agar plates and incubated at +37°C over night. The colonies on the plates containing 30-300 CFU were counted and the number of CFU/wound was determined. Previously, the tissue homogenization technique used in this study to quantify bacteria in the wound has been shown to yield the largest bacterial counts, since both surface and tissue-associated organisms are quantified, in contrast to the various types of surface-sampling techniques such as the surface rinse technique or the moist swabs, and the suture culture techniques (1). The data are presented as % of the control group ± SEM. The results were analysed with Mann-Whitney U-test and $p<0.05$ was considered statistically significant.

References:

1. McRipley RJ, Whitney RR. 1976. Characterization and quantitation of experimental surgical-wound infections used to evaluate topical antibacterial agents. Antimicrob. Agents Chemother. **10:**38-44.