SUPPLEMENTAL MATERIAL

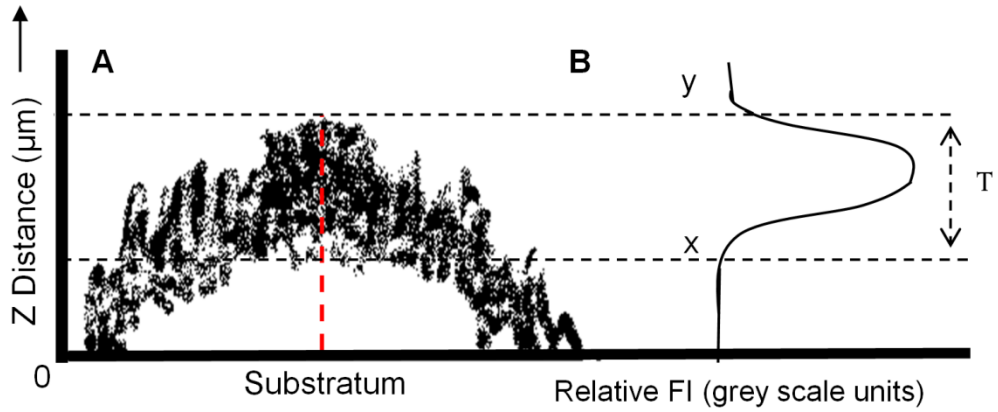
SUPPLEMENTARY METHODS

Skimmed milk casein assay

To determine whether DNase I contained contaminating proteases the skimmed milk casein assay was performed according to the following protocol. Skimmed milk agar (Sigma-Aldrich, UK) plates were prepared according to the manufacturer's instructions. DNase I (Sigma-Aldrich, UK) was prepared according to manufacturer's instructions and made up to 100 µg/ml - the same concentration that was used in the DNase experiments presented in the main text. Proteinase K (Sigma-Aldrich, UK) was also made up to 100 µg/ml and was used as a control. Aliquots of 20 µl of each enzyme were added onto two separate agar plates and the plates were incubated at 37^{o}C for 24 hours. The experiment was repeated three times and the results are presented in Fig. S5.

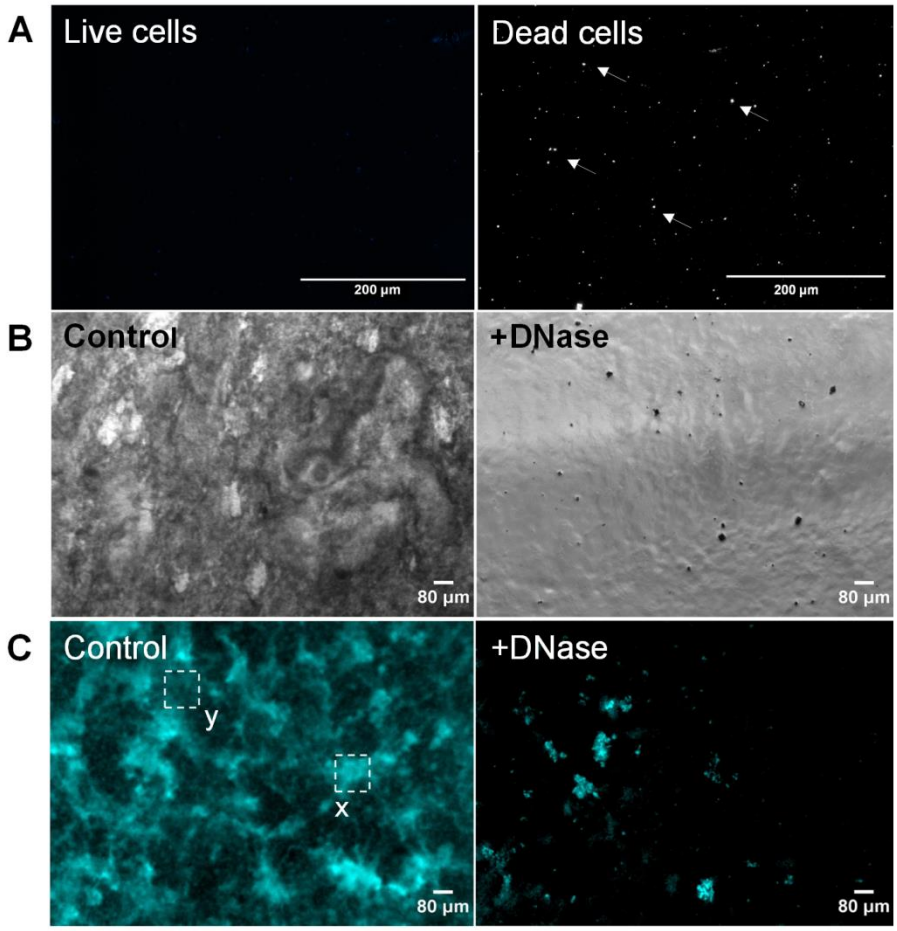
SUPPLEMENTARY FIGURES

SUPPLEMENTAL FIGURE S1



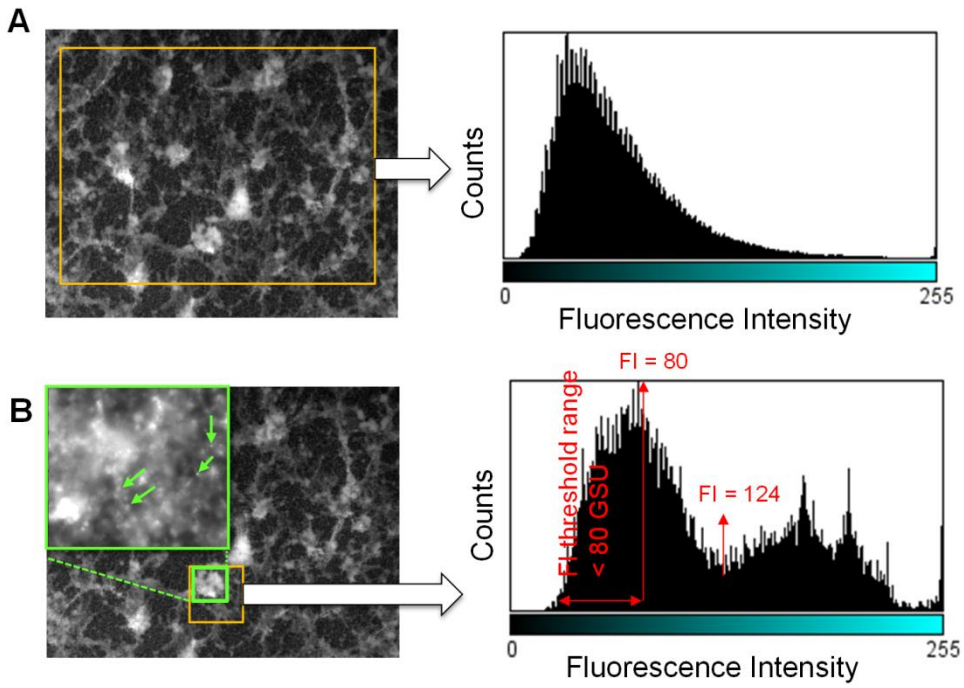
Supplemental Figure S1: Schematic of the quantification protocol of biofilm fluorescence. A) A hypothetical CLSM image depicting a fluorescent layer in a biofilm. B) A line fluorescence profile acquired from a specific location in the biofilm (red dashed line in A) illustrating how the fluorescence intensity changes as a function of biofilm depth. The depth of each fluorescent layer (T) was taken as y- x.

SUPPLEMENTAL FIGURE S2



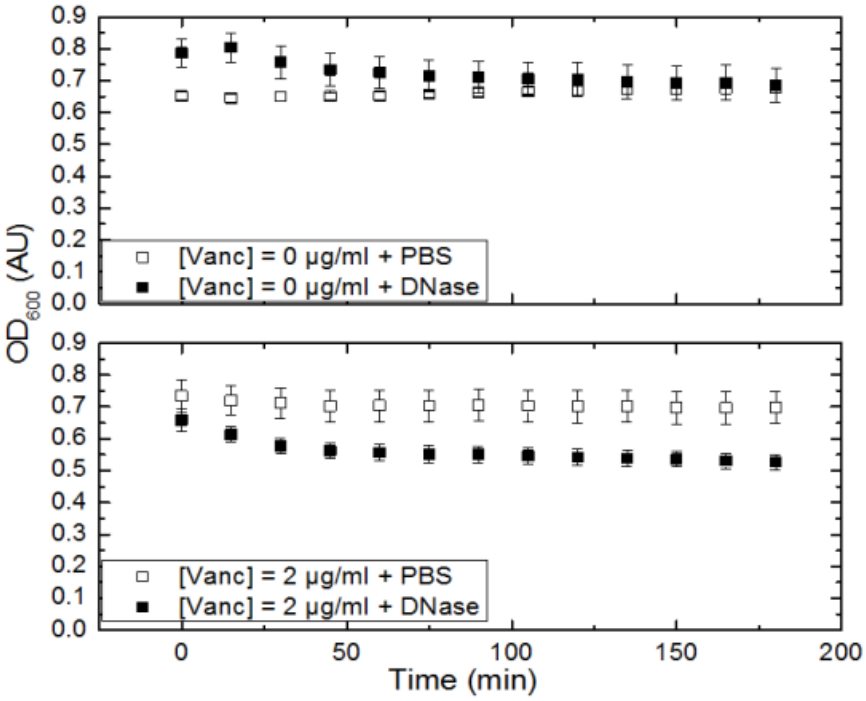
Supplemental Figure S2: Characterization of the DAPI-staining protocol used for the visualisation of biofilm eDNA. A) Fluorescence images of DAPI stained exponentially growing *S. epidermidis* planktonic cells. Live cells were exposed to 30 min PBS treatment. Dead cells were acquired by 30 min exposure to ethanol. Fluorescence was observed only from dead cells (white arrows). Scale bar = 200 µm. B) Bright-field microscopy images of 48-h control and DNase I treated *S. epidermidis* biofilms. Scale bar = 80 µm. C) DAPI stained 48-h control and DNase I treated *S. epidermidis* biofilms. Areas x and y indicate high and low fluorescence intensity regions attributed to cytoplasmic dead cell DNA and eDNA respectively. Scale bar = 80 µm.

SUPPLEMENTAL FIGURE S3



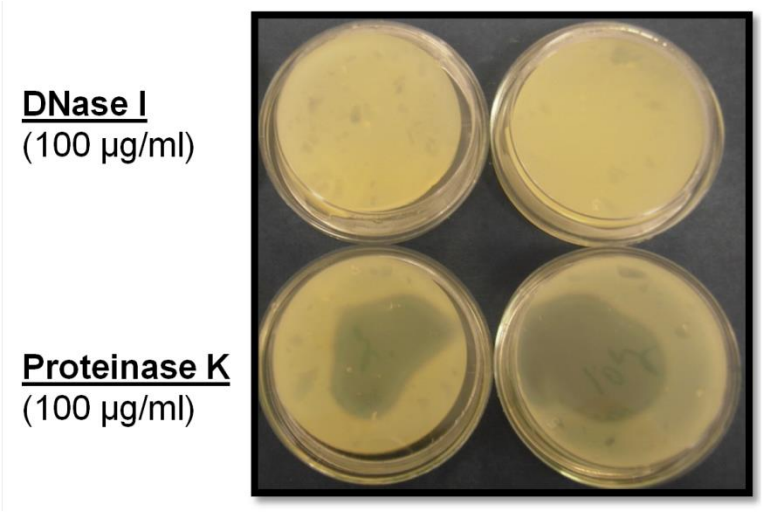
Supplemental Figure S3: Regions containing fluorescence showed a binary distribution of fluorescence. A) Histogram of the overall fluorescence from DAPI stained control *S. epidermidis* biofilms. B) Histogram of a localized region of interest shows a binary distribution of high and low fluorescence intensity corresponding to cytoplasmic DNA in dead cells and eDNA respectively.

SUPPLEMENTAL FIGURE S4



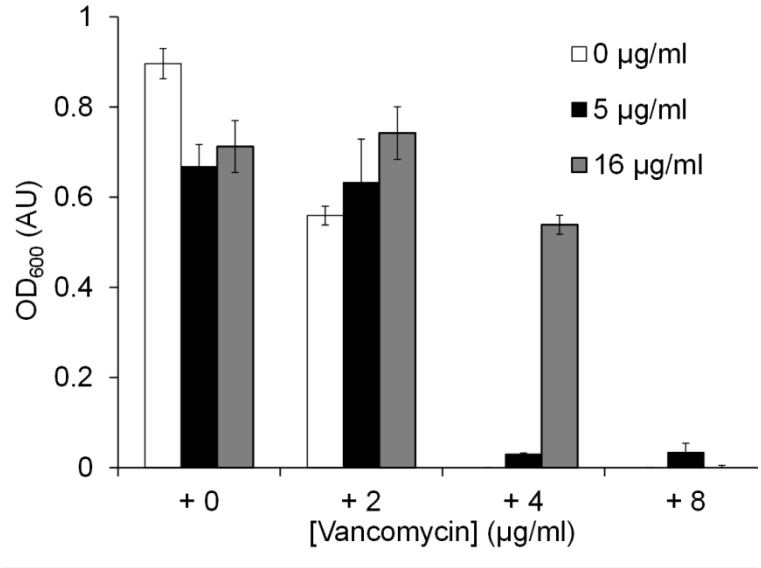
Supplemental Figure S4: DNase I removes sub-MIC vancomycin treated biofilms. Reduction in OD_{600} of control (top panel) and sub-MIC vancomycin treated (bottom panel) *S. epidermidis* biofilms as a result of DNase I treatment. PBS incubation was used as a control for DNase I. All error bars represent standard error of the mean (n = 10). Baseline not subtracted.

SUPPLEMENTAL FIGURE S5



Supplemental Figure S5: DNase I does not exhibit protease activity. Photographs of duplicate skimmed milk agar plates post incubation, demonstrating the protease activity of Proteinase K as indicated by the translucent zone of casein clearance.

SUPPLEMENTAL FIGURE S6



Supplemental Figure S6: Salmon sperm DNA hinders vancomycin activity. Average 24-h OD of *S. epidermidis* planktonic culture exposed to a combined treatment of salmon sperm DNA and vancomycin, as well as individual treatments of vancomycin and salmon sperm DNA. Error bars; standard error of the mean, n=12.