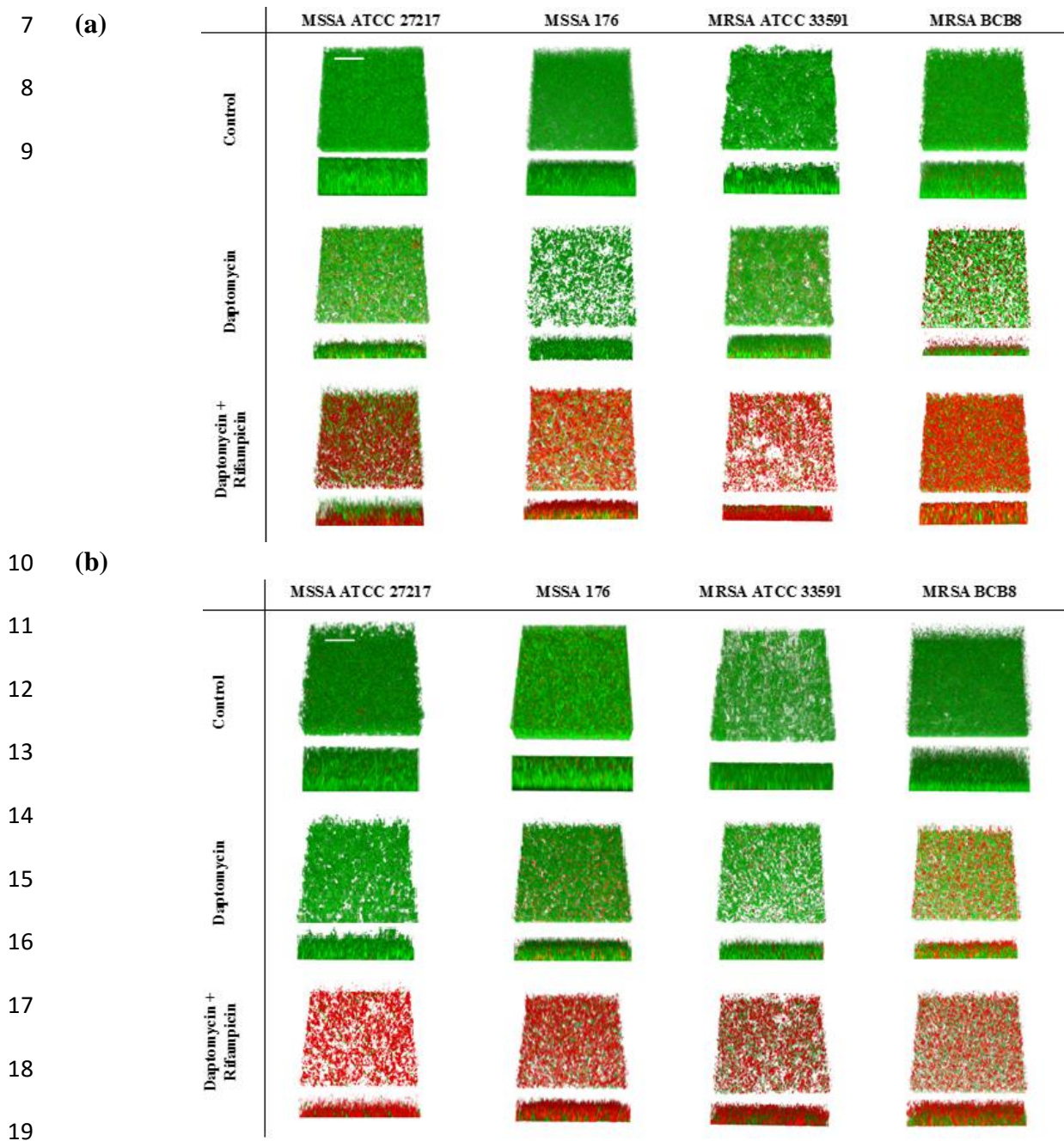
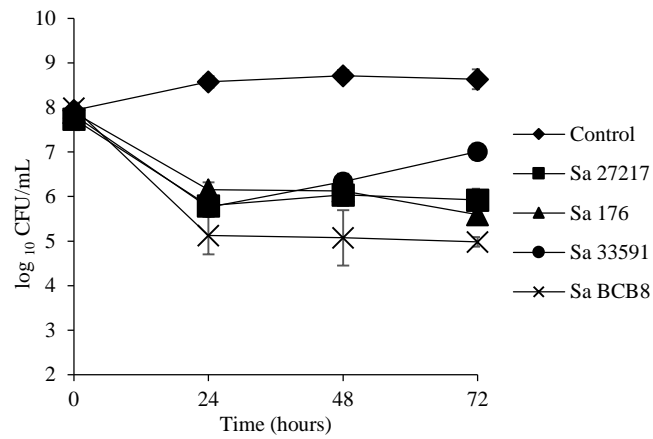


2 **FIG S1** Time course of BODIPY-FL®-daptomycin penetration measured at the bottom of (a) *S.*
3 *aureus* 27217, 176 and 33591 and (b) *S. aureus* BCB8 biofilms. The relative intensity corresponds to
4 a normalization of the maximum intensity value to one. Time zero corresponds to the moment at
5 which BODIPY-FL®-daptomycin was added above biofilm.

6



20 **FIG S2** Visualization of MSSA and MRSA biofilms using 3D reconstruction to observe biofilm
 21 thickness. Images were collected without any drug exposure (control) and after (a) 24 h and (b) 48 h
 22 exposure to daptomycin (20 $\mu\text{g}/\text{mL}$) alone and in association with rifampicin (20 $\mu\text{g}/\text{mL}$). Dead cells
 23 were stained red with PI and all bacteria were stained green with Syto9[®]. The acquisition was
 24 performed on the whole biofilm thickness with an axial displacement of 1 μm . Images dimension is
 25 82x82 μm^2 . The scale bar corresponds to 20 μm .



26

27 **FIG S3** Time-kill curves of daptomycin (40 µg/mL) against the four MSSA and MRSA biofilms.

28 Since no statistically significant difference was observed between the four controls ($P > 0.05$), only

29 one curve was represented. Filled diamonds: controls, filled squares: *S. aureus* 27217, filled triangles:

30 *S. aureus* 176, filled circles: *S. aureus* 33591, crosses: *S. aureus* BCB8. Error bars represent the

31 standard deviation.

32

33 **Movie S4.** Movie sequence of BODIPY-FL®-daptomycin penetration inside *S. aureus* 27217 biofilm
34 obtained by time-lapse imaging showing the fluorescence intensity evolution over time.

35