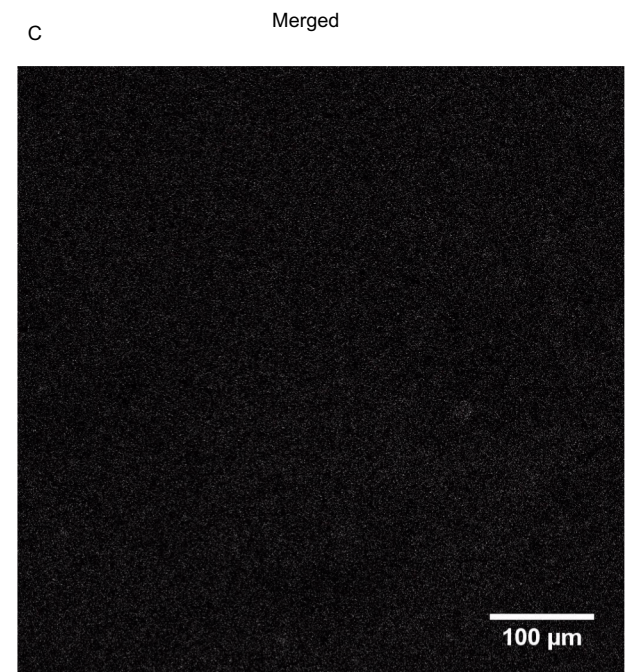
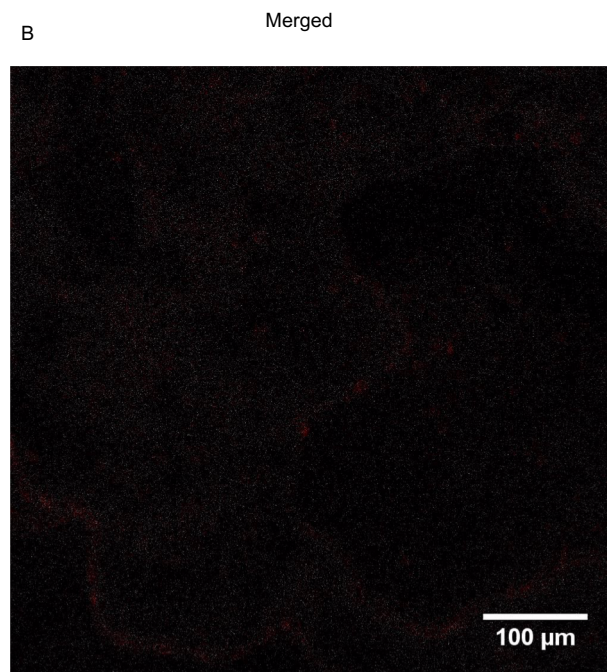
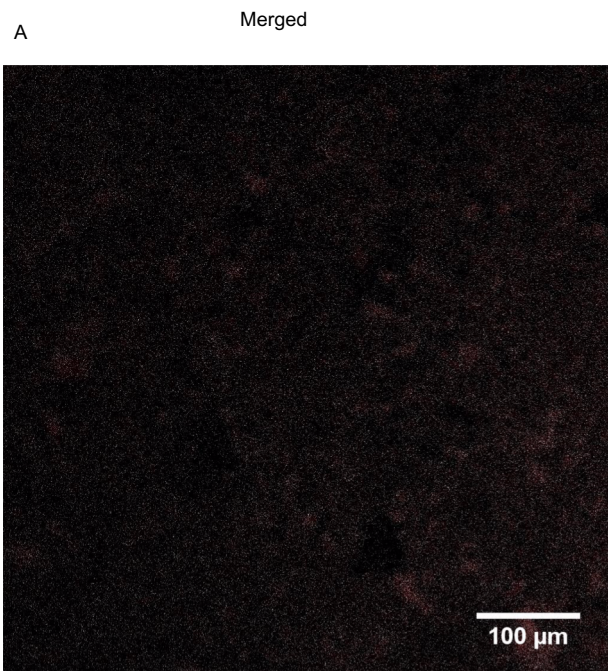
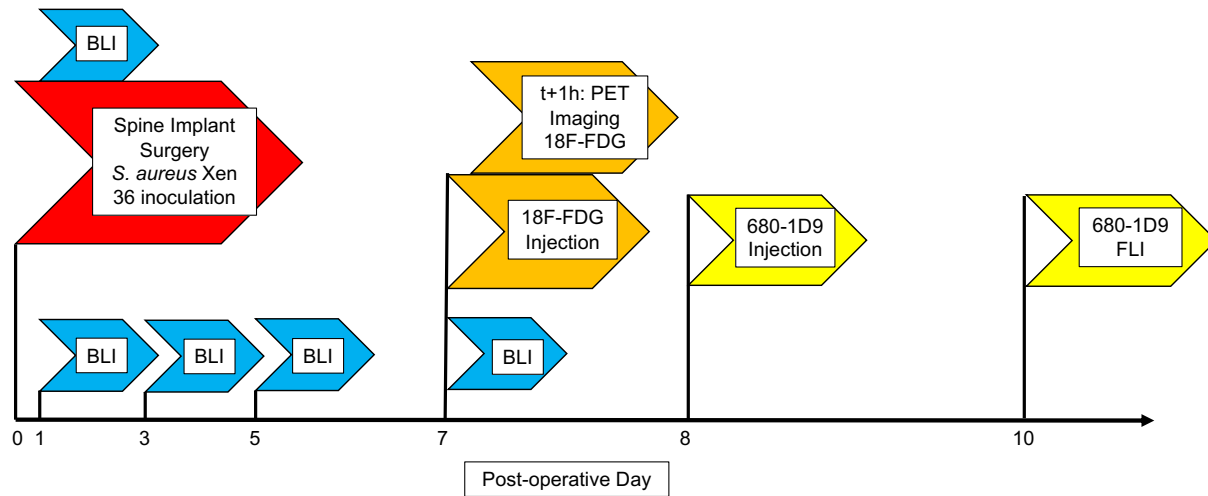


Supplementary Figures 1-4

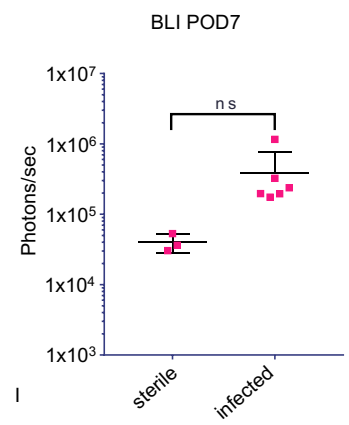
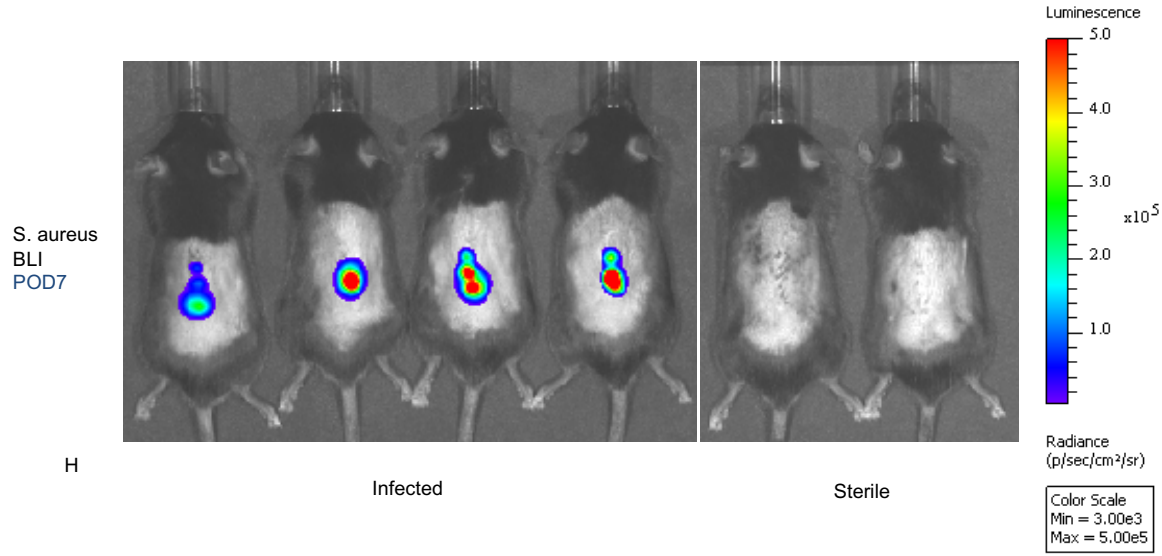
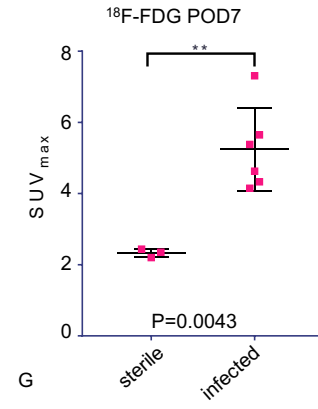
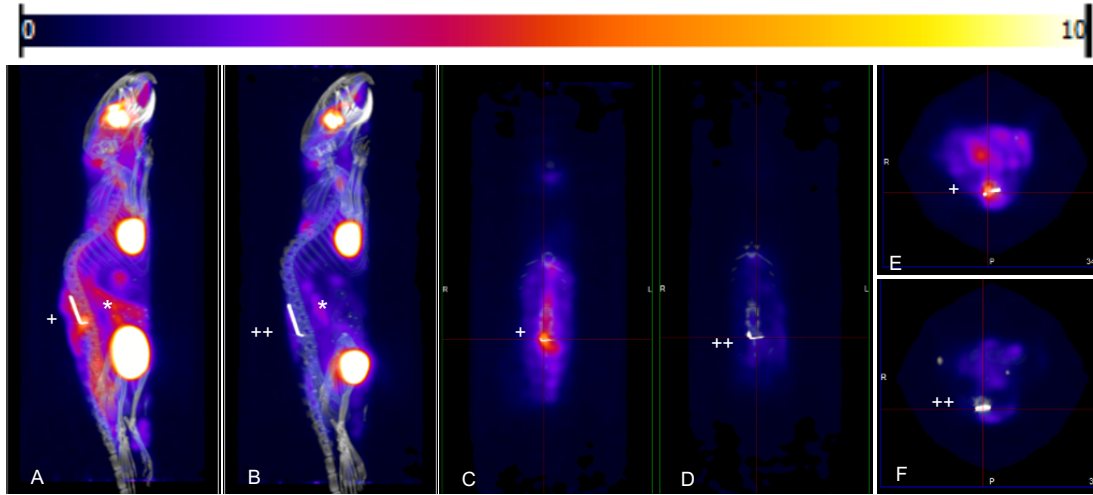
Supplementary Figure 1. Three-dimensional reconstructions from stacks of two-dimensional confocal microscopy images of *S. aureus* biofilms stained with DAPI and 1D9-Alexa Fluor 555. Biofilms of (a) *S. aureus* Xen-36, (b) *S. aureus* SH1000, and (c) *S. aureus* MS001 ($\Delta isaA$) were grown on coverslips for microscopy and, subsequently, stained with DAPI and the IsaA-specific monoclonal antibody 1D9 labelled with Alexa Fluor 555. Note that *S. aureus* MS001 ($\Delta isaA$) shows some residual binding of 1D9-Alexa Fluor 555 due to the presence of the IgG-binding proteins Spa and Sbi.(26)



Supplementary Figure 2. Validation experiment. Full details provided in Methods. Flaps listed are procedures performed on mice on given post-operative day. n = 6 infected mice and 3 sterile mice. BLI = bioluminescence imaging. FLI = fluorescence imaging. 18F-FDG = Fluorodeoxyglucose. PET = positron emission tomography. 1D9 = Staphylococcus antibody probe. 680-1D9 = Near infrared 680nm fluorophore-Staphylococcus antibody probe.



Supplementary Figure 3. Validation 18F-FDG PET Imaging. Validation 18F-FDG imaging was performed on POD7 in n=6 infected mice and n=3 sterile mice. PET images performed on the G8 PET/CT imaging system (Perkin Elmer, Hopkinton, MA). A) Sagittal view of infected mouse. B) Sagittal view of sterile mouse. C) Coronal view of infected mouse D) Coronal view of sterile mouse E) Axial view of infected mouse. F) Axial view of sterile mouse. G) SUVmax for infected and sterile mice. H) Representative bioluminescence imaging of infected and sterile mice on POD7. I) BLI for infected and sterile mice. * = spleen. + = infected steel implant. ++ = sterile steel implant. BLI = bioluminescence imaging. SUV = standard uptake value. 18F-FDG = fluorodeoxyglucose. p/s/cm²/sr = photons/second/square centimeter/steradian. Error bars are standard error of the mean (SEM). Statistical test used was two-tailed student's t-test.



Supplementary Figure 4. Bioluminescence of Xen36 on Blood Agar plate. In vivo demonstration of Xen36 bioluminescence captured on IVIS Spectrum (Caliper LS, Hopkinton, MA, USA) at an exposure time of 10 seconds.

