SUPPLEMENTARY INFORMATION

Positron Emission Tomography Imaging with 2-[^{18}F]F-*p*-Aminobenzoic Acid Detects *Staphylococcus aureus* Infections and Monitors Drug Response

Zhuo Zhang[1¶], Alvaro A. Ordonez[2,3¶], Hui Wang[1], Yong Li[1], Kayla R. Gogarty[1], Edward A. Weinstein[2], Fereidoon Daryaee[1], Jonathan Merino[1], Grace E. Yoon [1,5], Alvin S. Kalinda[2,3], Ronnie C. Mease[4], James N. Iuliano[1], Peter M. Smith-Jones[5], Sanjay K. Jain[2,3,4*&], and Peter J. Tonge[1*&]

[1]Institute for Chemical Biology & Drug Discovery, Department of Chemistry and Radiology, Stony Brook University, Stony Brook, New York, USA.
[2]Center for Infection and Inflammation Imaging Research, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.
[3]Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.
[4]Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.
[5]The Facility for Experimental Radiopharmaceutical Manufacturing, Department of Psychiatry, Stony Brook University, Stony Brook, New York, USA.

¶These authors contributed equally to this work.
*S.K.J. and P.J.T. are joint senior authors.

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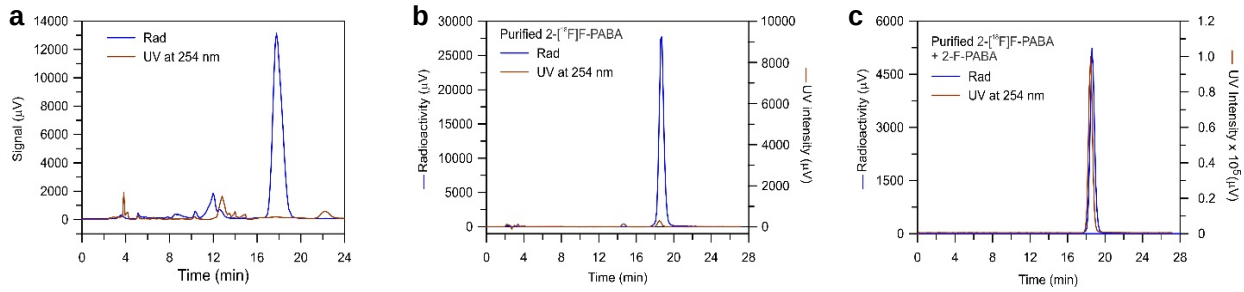

Figure S1. Analytical HPLC analysis of 2-[^{18}F]F-PABA. (**a**) Preparative HPLC chromatography of 2-[^{18}F]F-PABA using a Phenomenex Luna 10µm C18(2), 100Å 250 x 10 mm column with 5% EtOH, 0.5% AcOH, 5 mL/min as the eluent. The brown trace is the UV absorption of the eluent at 254 nm and the blue trace is the radioactive signal. (**b**) Analytical HPLC of purified 2-[^{18}F]F-PABA using a Phenomenex Luna 10µm C18(2), 100Å 250 x 4 mm column with 8% MeCN, 0.1 % TFA, 1 mL/min as the eluent. The brown trace is the UV absorption of the eluent at 254 nm and the blue trace is the radioactive signal. (**c**) Analytical HPLC of a co-injection of standard 2-F-PABA with purified 2-[^{18}F]F-PABA. The brown trace is the UV absorption of the eluent at 254 nm, and the blue trace is the radioactive signal.

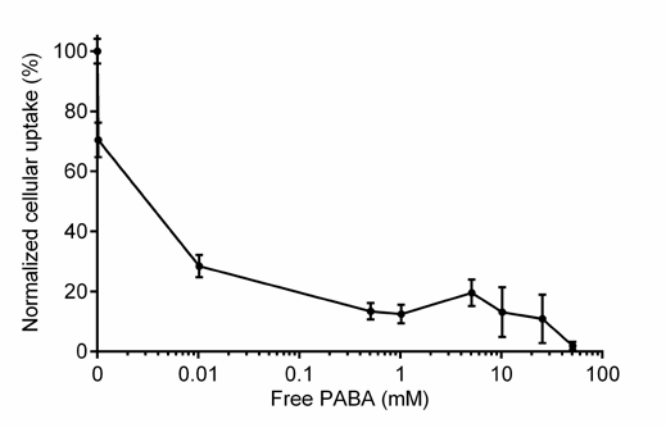

Figure S2. Concentration-dependent blocking of 2-[^{18}F]F-PABA accumulation in viable *S. aureus* cells by PABA. Co-incubation of 2-[^{18}F]F-PABA with multiple concentrations of PABA resulted in a dose-dependent inhibition of 2-[^{18}F]F-PABA uptake in *S. aureus*.

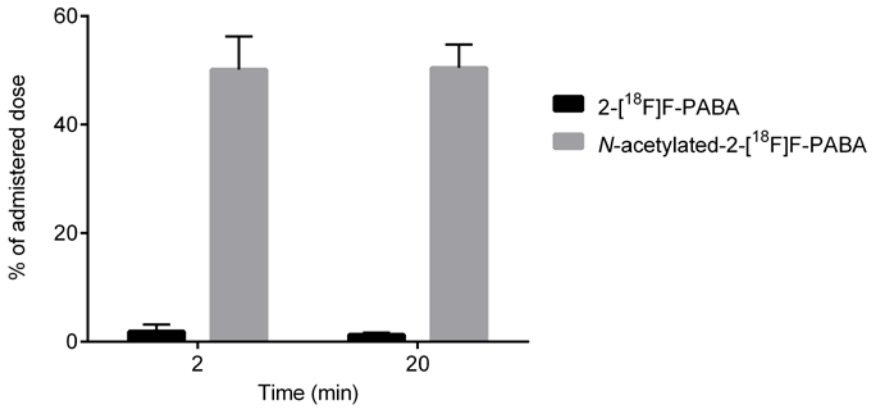

Figure S3. Analysis of metabolites in the plasma of rats after an IV dose of 2-[^{18}F]F-PABA. After receiving an IV dose of 2-[^{18}F]F-PABA, the animals were sacrificed and the blood collected through cardiac puncture for HPLC analysis. Two minutes after injection, 1.91% of the administered dose was 2-[^{18}F]F-PABA while 50.14% had been *N*-acetylated. Similarly, by 20 minutes post-injection 1.32% was the intact 2-[^{18}F]F-PABA and 50.47% was the *N*-acetylated compound. Data represented as the average percentage of the dose administered ± standard error of the mean (n=3 per time point).

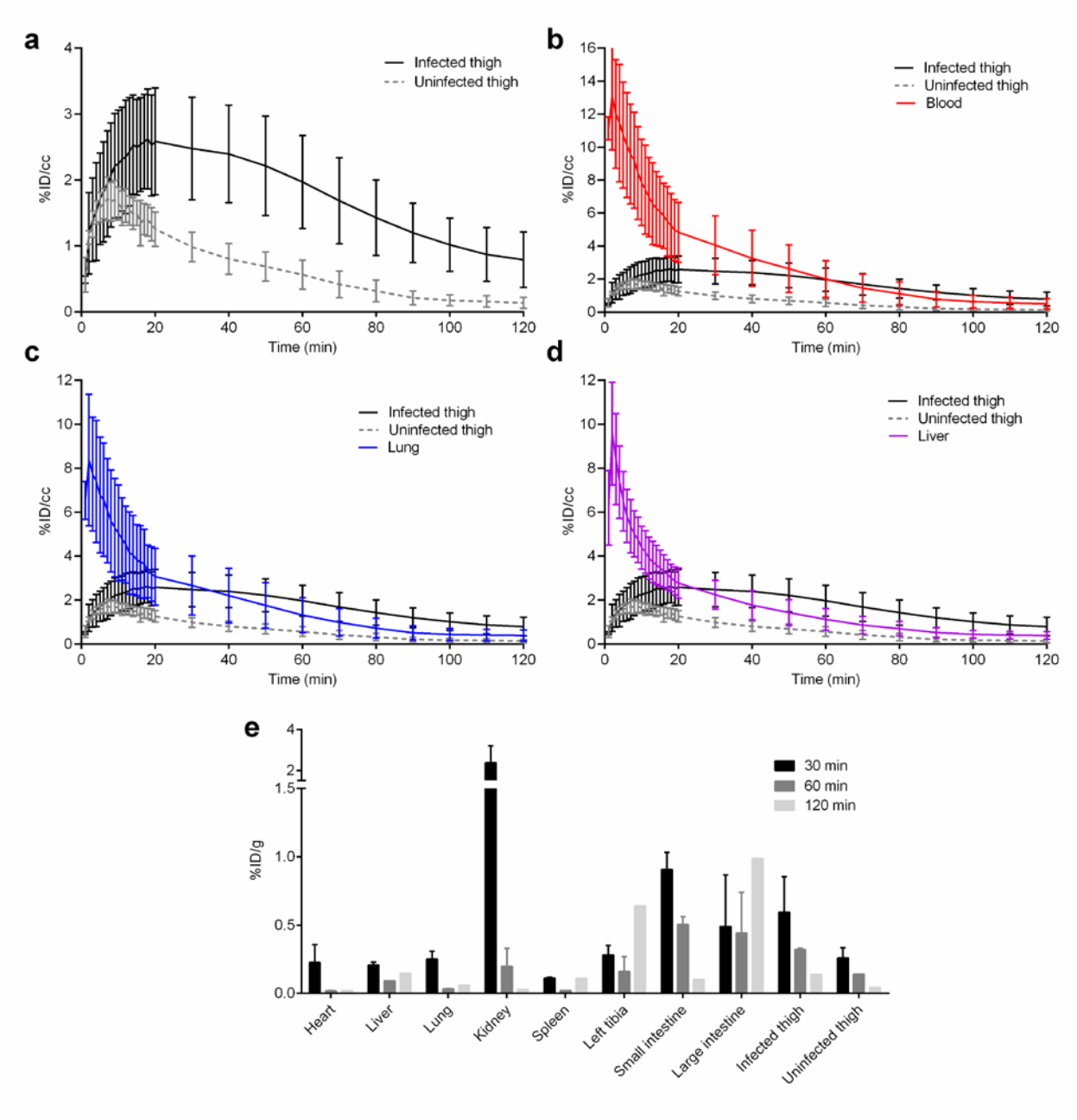

Figure S4. 2-[^{18}F]F-PABA biodistribution in *S. aureus*-infected mice. Mice were infected with *S. aureus* in the right thigh and no infection in the opposite thigh. After injection with 2-[^{18}F]F-PABA, the animals were PET scanned for 120 min and time-activity curves (TAC) were calculated. (**a**) The TAC of infected thighs showed a higher signal over time compared to uninfected thighs. The TAC were also calculated for blood, measured in the left ventricle of the heart (**b**), lung (**c**), and liver (**d**), and compared to the infected and uninfected muscle. (**e**)

After IV injection of 2-[^{18}F]F-PABA, a group of mice was sacrificed 30, 60 or 120 minutes and their organs harvested for tissue biodistribution. Tissues were weighed, and radioactivity was acquired using a gamma counter (Wizard 2480, Perkin Elmer). Data represented as mean and standard error of the mean (n=3).

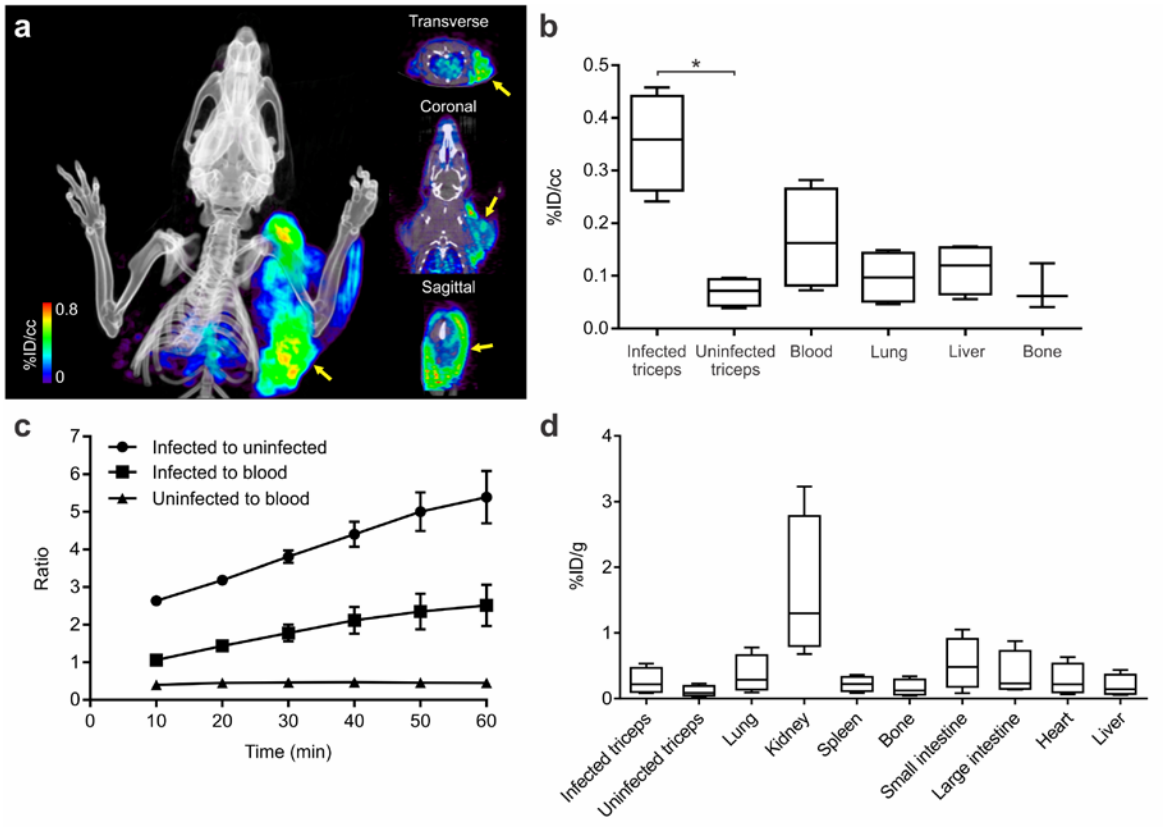

Figure S5. Accumulation of 2-[^{18}F]F-PABA in *S. aureus*-infected rat triceps. (**a**) PET/CT images of 2-[^{18}F]F-PABA biodistribution in rats in which an *S. aureus* infection has been induced in the right triceps (yellow arrow). The images are a representative three-dimensional projection, transverse, coronal and sagittal views 60 min post tracer injection. (**b**) The comparison of 2-[^{18}F]F-PABA accumulation in infected triceps, uninfected triceps, blood (left heart ventricle), lungs, liver, and bone 60 min after tracer injection. Data are medians with interquartile and ranges are shown. **P*=0.028 from a two-tailed Mann-Whitney *U* Test (n=4). (**c**) The time-dependent comparison of 2-[^{18}F]F-PABA accumulation in infected and uninfected triceps and in the blood (left heart ventricle). Data represented as mean and standard error of the mean (n=6). (**d**) Post-mortem analysis of 2-[^{18}F]F-PABA distribution. After the animals were injected IV with 2-[^{18}F]F-PABA and scanned for 60 min, they were sacrificed and the tissues weighed and harvested for automated gamma counting. Data are medians with interquartile and ranges are shown (n=4).

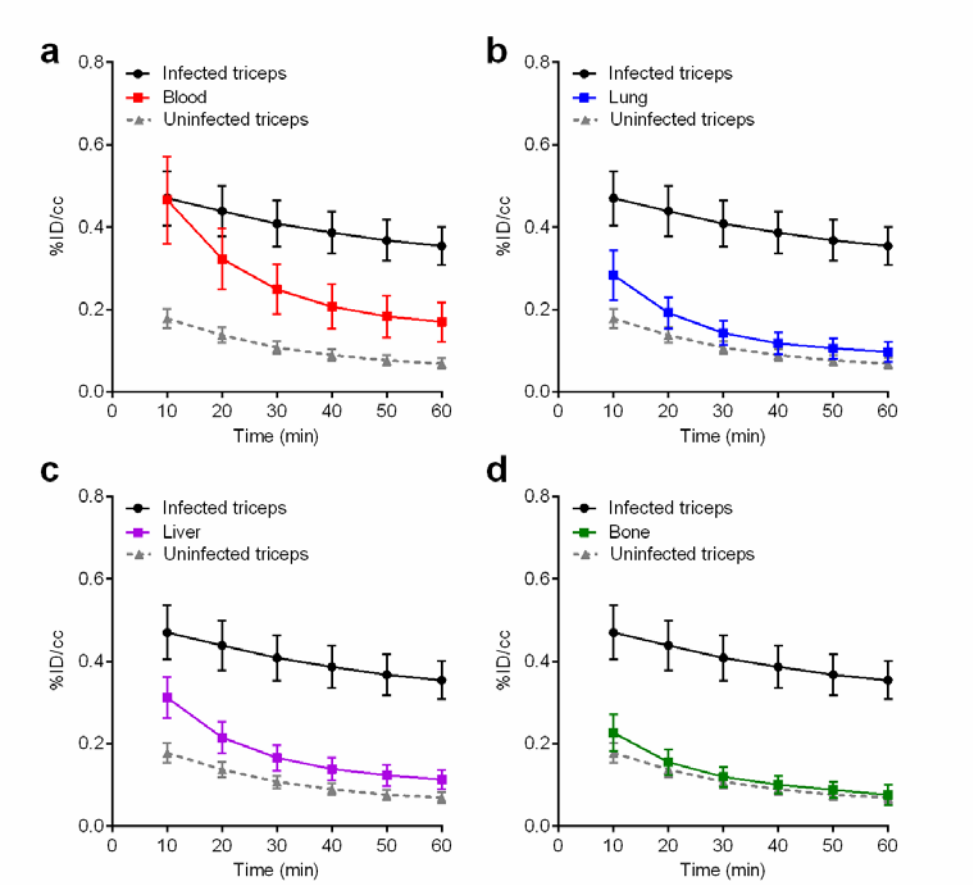

Figure S6. Time-activity curve (TAC) of 2-[^{18}F]F-PABA in infected triceps, uninfected triceps, lungs, livers and bones of rats with *S. aureus* infection in the right triceps. (**a**)TAC of infected (right), uninfected (left) triceps and heart. (**b**) TAC of infected (right), uninfected (left) triceps and lung. (**c**) TAC of infected (right), uninfected (left) triceps and liver. (**d**) TAC of infected (right), uninfected (left) triceps and bone. The rats were scanned by PET/CT for 60 min starting from 10 min after tracer administration. The 60-min PET scan was broken into six 10-min frames. Quantitative analysis was performed using Amide version 1.0.4 (http://www.amide.sourceforge.net). Spherical ROIs were drawn manually using CT as a guide. Data represented as mean with standard error of the mean (n=4).

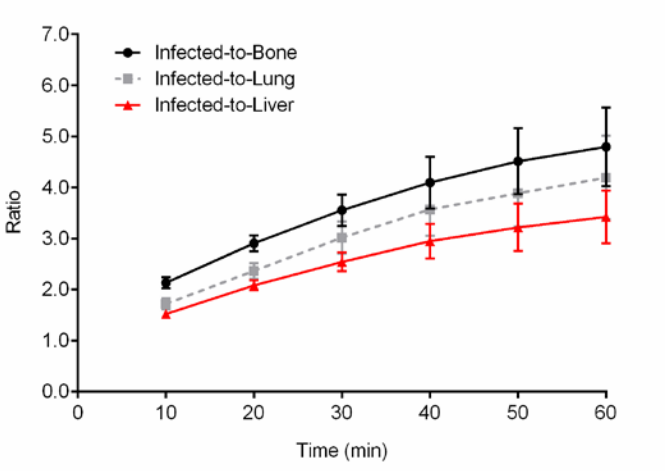

Figure S7. Time-dependent comparison of 2-[^{18}F]F-PABA accumulation in infected triceps with lung, liver, and bone of rats. The ratios were computed by dividing the activity (%ID/cc) in infected triceps by the activities in bone (black), lung (grey) and liver (red), respectively. Data represented as mean with standard error of the mean (n=4).

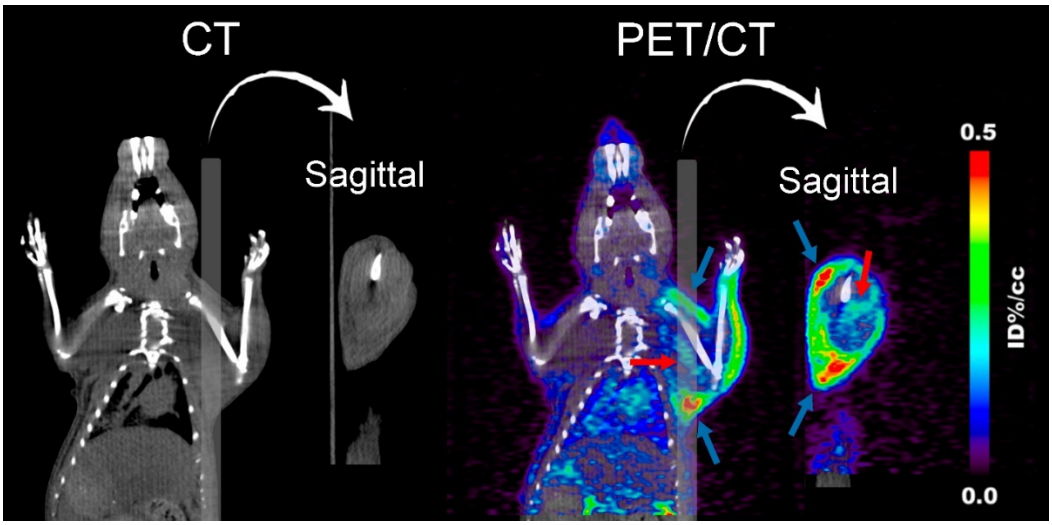

Figure S8. 2-[^{18}F]F-PABA distribution in the infected triceps. Activity was found both in the center of the infected muscle (red arrow) and in the soft tissues surrounding the infected muscle (blue arrows). The PET/CT image is a 2D section which only shows activity in this plane. The image shows 2-[^{18}F]F-PABA distribution in the rats 60 min after tracer administration and is a representative of 4 rats with *S. aureus* infection in the right triceps while the left triceps were uninfected.

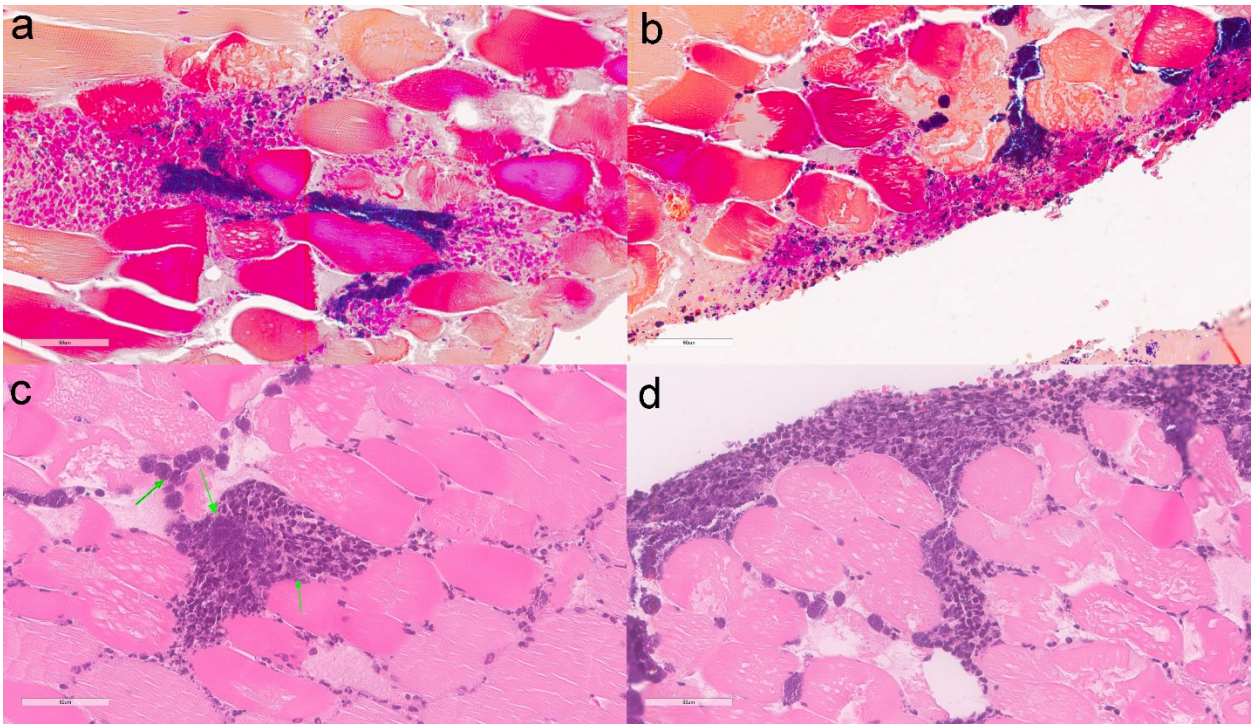

Figure S9. Histology of *S. aureus* infection and sterile inflammation in the rat model. (**a**) H&E staining of *S. aureus*-infected triceps. (**b**) H&E staining of triceps with sterile inflammation (heat-killed *S. aureus*). (**c**) Gram stain of infected triceps showing Gram-positive cocci in clusters, found in the center of the triceps (green arrows). (**d**) Gram stain of infected triceps showing Gram-positive cocci found at the edge of the infected muscle.

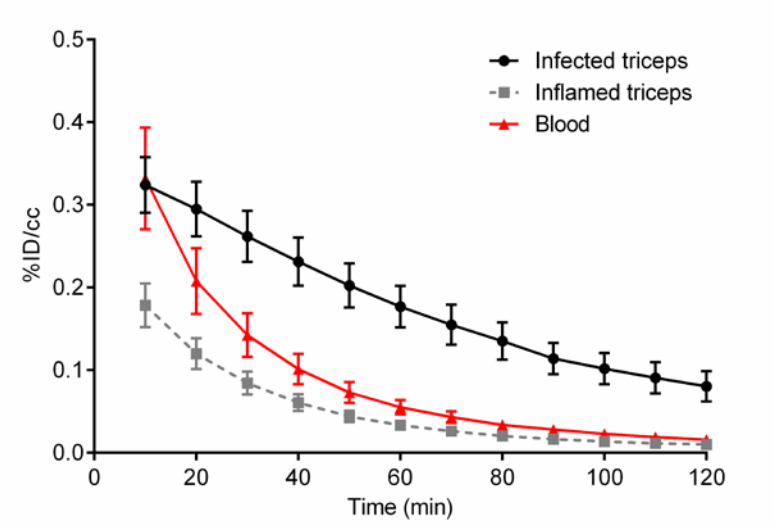

Figure S10. Time-activity curve (TAC) of 2-[^{18}F]F-PABA in triceps and blood of rats. The right triceps of the rats were infected with *S. aureus* infection. The inflamed triceps were injected with 10-fold higher burden of heat-killed *S. aureus* cells to induce sterile inflammation. TAC of infected (black), inflamed triceps (grey) and blood (left heart ventricle, red). The rats were scanned by PET/CT for 120 min (rats with sterile inflammation in the left triceps) starting from 10 min after tracer administration. The PET scan was broken into twelve or six 10-min frames. Data represented as mean with standard error of the mean (n=7).

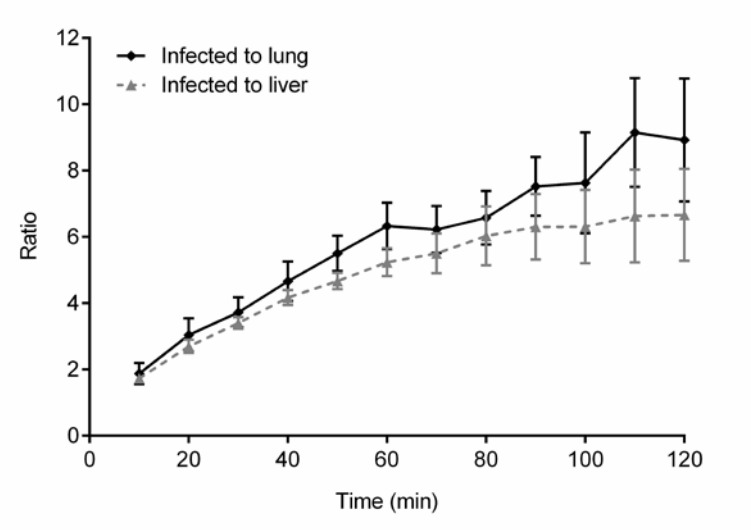

Figure S11. Time-dependent comparison of 2-[^{18}F]F-PABA accumulation in infected triceps with lung and liver in the *S. aureus* myositis rat model. The right triceps of the rats were infected with *S. aureus* infection. The ratios were computed by dividing the activity (%ID/cc) in infected triceps by the activities in the lung (black) and liver (grey dotted), respectively. Data represented as mean with standard error of the mean (n=3).

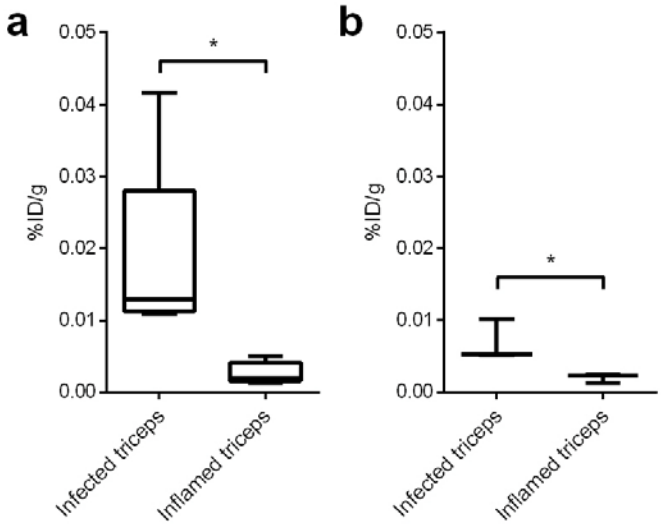

Figure S12. Effect of unlabeled F-PABA on the accumulation of 2-[^{18}F]F-PABA in infected and inflamed triceps. (**a**) 2-[^{18}F]F-PABA (0.8-1 mCi; 29.6-37 MBq) containing 2mg of ^{19}F-PABA was injected via the tail vein into rats that had been infected with *S. aureus* in the right triceps and with heat-killed *S. aureus* in the left triceps 24 hours prior to the study. Two hours after injection of the tracer the animals were sacrificed, the tissues harvested, and the radioactivity quantified by gamma counting. The signal in infected triceps was 9.38 times higher compared to the inflamed muscle (P<0.01, n=7) (**b**) A separate group of similarly infected rats were injected only with 2-[^{18}F]F-PABA and used as controls. The signal in infected triceps was 3.5 times higher compared to inflamed triceps (P=0.04, n=3). In each case, the bacterial load was also quantified by plating an enumeration of CFUs.

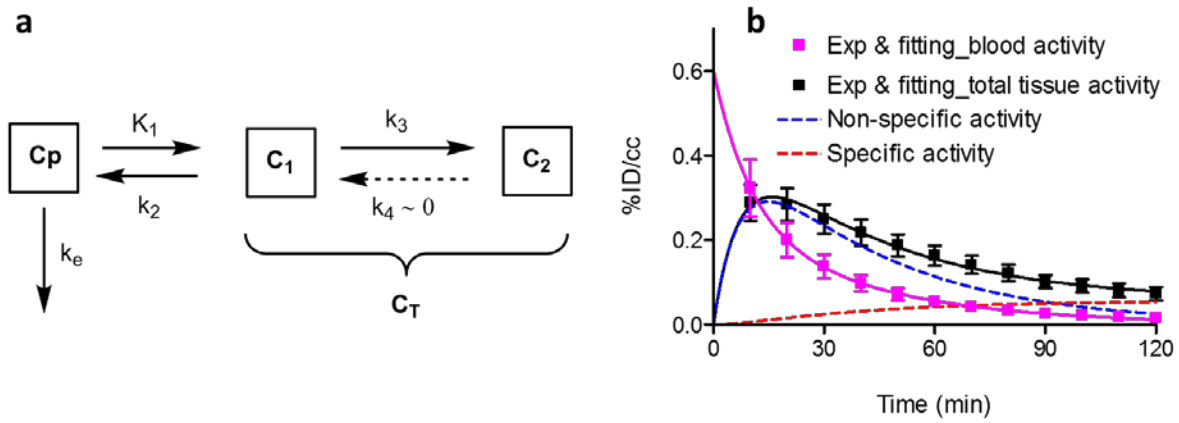

Figure S13. Pharmacokinetic (PK) compartment modeling of 2-[^{18}F]F-PABA distribution in blood and infected triceps. (**a**) Two-compartment PK model which includes irreversible incorporation of the tracer. (**b**) Experimental and simulated tracer distribution. Experimental data for tracer levels in the blood (pink squares) and infected tissue (black squares) have been fit to the PK model (solid lines). Dashed-lines represent a simulation of non-specific activity (blue dashed line) and specific activity (red dashed line) using the rate constants generated from fitting the experimental data to the model (0.099, 0.053 and 0.003 min^{-1} are respective values of K_1, k_2, and k_3).