SUPPLEMENTARY INFORMATION

ABBREVIATIONS

Ac……..……Acetyl

Ar…….……..Argon

C…….…….……Carbon

$CDCl_3$……..……....Deuterated chloroform

CD_3OD…….....Deuterated methanol

CFU…….Colony forming unit

CH_2CL_2.....Dichloromethane

CH_3CN……..Acetonitrile

Ci…….Curie

CT…...……..Computated tomography

CuI……..……...Copper iodide

DCM/CH_2Cl_2……Dichloromethane/Methylene chloride

DIPEA……..*N*,*N*-Diisopropylethylamine

DI water….....…….....Deionized water

DMF……..……..*N*, *N*-Dimethylformamide

D_2O……...…….Deuterium oxide

ESI..... Electrospray ionization

EtOAc…….....…….....Ethyl acetate

H……..…….....Proton

HCl……..…….....Hydrogen chloride

H_2O.....Water

HPLC.....High-performance liquid chromatography

HRMS…….…….....High resolution mass spectrometry

HSQC……..……...….....Heteronuclear single quantum coherence

Hz……..…….....……..…Hertz

KF.....Potassium fluoride

Kryptofix 2.2.2.....4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane

LB……. Luria-Bertani

LiOH……..……Lithium hydroxide

M…….…….…...……...Molar

MALDI……...Matrix assisted laser desorption ionization spectroscopy

MeOH/CH_3OH..……..…….…Methanol

MeV…… Megaelectron volt

mL……..Milliliter

$NaHCO_3$……..…….Sodium bicarbonate

NaOH…….....……...…….....Sodium hydroxide

$NaSO_4$…….....…….…….....Sodium sulfate

NMR……Nuclear magnetic resonance

N_2……...……...…….....Nitrogen

OD...…….Optical density

PBS...……...……Phosphate buffered PBS

PET……. Positron emission tomography

Py…..……..Pyridine

ROI…….Region of interests

s.e.m……...…Standard error of the mean

TEA……...Triethyl amine

TLC…...……Thin layer chromatography

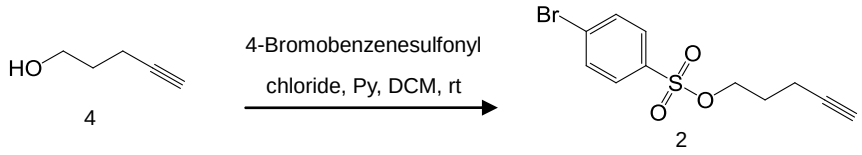
UV……...……Ultraviolet

µL…….……..Micro litter

EXPERIMENTAL PROCEDURES

^{1}H-NMR spectra were recorded in $CDCl_3$, CD_3OD or D_2O on a Varian 400 spectrometer equipped with a Sun workstation at 300K. TMS (δ *(ppm)*$_H$ = 0.00) was used as the internal reference. ^{13}C-NMR spectra were recorded in either $CDCl_3$, CD_3OD and D_2O at a 100MHz on a Varian 400 spectrometer, using the central resonances of $CDCl_3$ (δ *(ppm)*$_C$ = 77.0) and methanol (δ *(ppm)*$_C$ = 50.4) as the internal references. COSY and HSQC experiments were used to assist assignment of the products. Chemical shifts are reported in ppm and multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), and m (multiplet). Coupling constants, *J*, are reported in hertz (Hz). High-resolution mass spectra (HRMS) were obtained on a AB SCIEX TOF/TOF 5800 system and are reported as m/z (relative intensity). Accurate masses are reported for the molecular ion (M^+) or a suitable fragment ion. Chemicals were purchased from Aldrich or VWR and used without further purification. All solvents were purified using standard methods. Flash chromatography was carried out using silica gel (230-400 mesh). All reactions were performed under anhydrous conditions under N_2 or Argon and monitored by TLC on Kieselgel 60 F254 plates (Merck). Detection was accomplished by examination under UV light (254 nm) and by charring with 10 % sulfuric acid in methanol.

S1. Synthesis of MH^{18}F

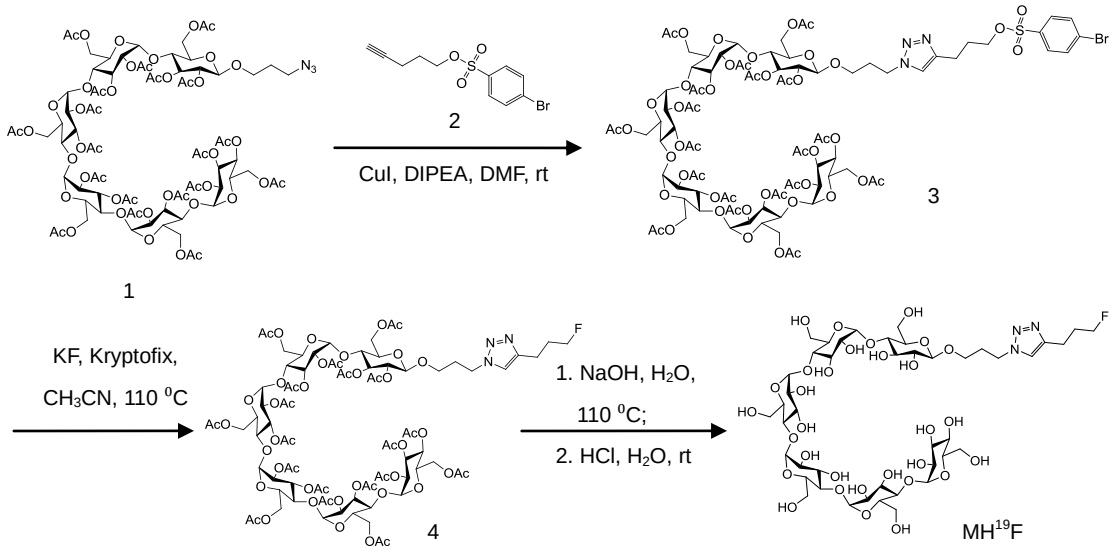


Supplementary Figure S1. Synthesis of pent-4-yn-1-yl 4-bromobenzenesulfonate **(2)**

S1.1. Synthesis of pent-4-yn-1-yl 4-bromobenzenesulfonate (2)

To a stirred solution of 4-pentyn-1-ol **4** (0.85 g, 10.0 mmol) in DCM (10 mL) was added

4-bromobenzenesulfonate chloride (2.93 g, 11.0 mmol) and pyridine (0.5 mL), and the reaction mixture was stirred vigorously at room temperature for 6 hours under argon. The reaction was quenched by adding ice water (10 mL) and the mixture was extracted with CH_2Cl_2 (15 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness under the reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc, 10:1) to afford pent-4-yn-1-yl 4-bromobenzenesulfonate **2** as yellow oil (2.1 g, 71.3%). 1H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.78 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 7.70 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 4.17 (t, 2 H, *J* = 4.0 Hz, CH_2O), 2.26 (m, 2 H, CH_2C), 1.88 (m, 2 H, CH_2CH_2C), 1.86 (t, 1 H, *J* = 4 Hz, alkyne). 13C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 134.9, 132.6, 129.4, 129.0, 81.8, 69.6, 69.1, 27.5, 14.6. HRMS (MALDI) m/z Found: 324.9489, calculated: 324.9510 for $C_{11}H_{116}BrNaO_3S$ [*M*+Na]$^+$.



Supplementary Figure S2. Synthesis of MH^{19}F **(5)**

S1.2. Synthesis of brosylate-maltohexaose (3)

To a stirred solution of azidomaltohexaose **1** (57.0 mg, 0.03 mmol) and pent-4-yn-1-yl 4-bromobenzenesulfonate **2** (19.0 mg, 0.06 mmol) in DMF (5 mL) was added CuI (0.3

mg, 1.5 μmol) and DIPEA (1.2 mg, 0.01 mmol). The reaction mixture was stirred at room temperature for 24 hours under nitrogen and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and washed with water (2 x 5 mL) and brine (5 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/acetone, 1/1) to afford brosylate-maltohexaose **3** as a yellow powder (45.0 mg, 69.2%). 1H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.75 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 7.69 (d, 2 H, *J* = 8.0 Hz, Ar*H*), 7.45 (br, 1 H, triazole), 5.41−5.25 (m, 7 H), 5.05 (t, 1 H, *J* = 10.0 Hz, 4''-H), 4.85 (dd, 1 H, *J* = 4.0 and 10.0 Hz, α 2'-H), 4.71-4.68 (m, 3H), 4.47-3.52 (m, 34 H), 2.17-1.88 (m, 61 H), 1.55-1.44 (m, 4H, C*H*$_2$). 13C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 170.6, 170.6, 170.6, 170.5, 170.5, 170.4, 170.3, 170.3, 170.3, 170.0, 169.7, 169.6, 169.6, 169.5, 169.4, 169.4 (C=O), 155.3, 154.6, 152.3, 152.2, 145.4, 125.3, 121.8, 121.7, 101.3, 100.2 (β 1-C), 95.7 (1-C), 95.7 (1-C), 95.6 (1-C), 76.7, 75.2, 73.7, 73.2, 72.4, 72.3, 72.1, 71.7, 71.6, 71.3, 70.4, 70.3, 70.2, 70.1, 70.0, 69.6, 69.3, 68.9, 68.4, 67.9, 66.1, 62.7, 62.4, 62.3, 62.1, 61.7, 61.3, 49.0, 46.8, 41.4, 38.2, 37.8, 37.1, 37.0, 36.7, 29.3, 20.8, 20.7, 20.6, 20.5, 12.5. HRMS (MALDI) m/z Found: 2196.5301, calculated: 2196.5276 for $C_{88}H_{116}BrN_3NaO_{53}S$ $[M+Na]^+$.

S1.3. Synthesis of fluorinated maltohexaose (4)

To a stirred solution of brosylate-maltohexaose **3** (220 mg, 0.1 mmol) and *Kryptofix 222* (74.4 mg, 0.2 mmol) in anhydrous acetonitrile (2 mL) was added anhydrous KF (12 mg, 0.2 mmol). The reaction mixture was heated and kept at a 110 ^oC for 30 min under argon and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and washed with water (2 x 5 mL) and brine (5 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/ethyl

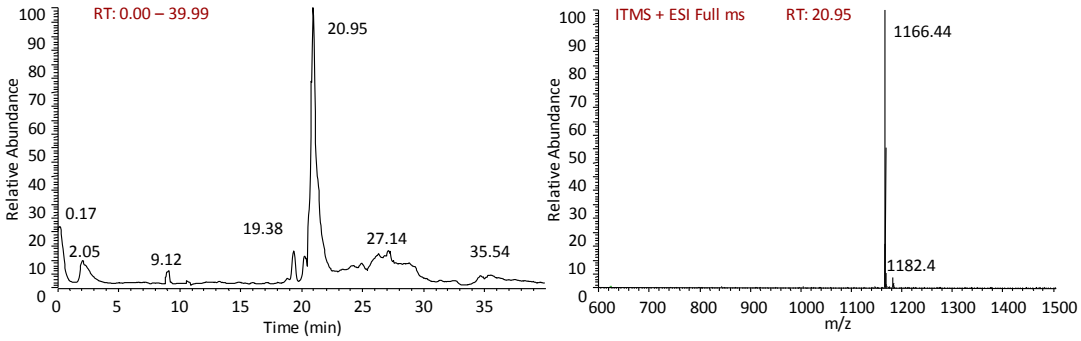
acetate, 1/3) to afford fluorinated maltohexaose **4** as a white powder (135 mg, 65.7%). 1H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.36 (br, 1 H, triazole), 5.41−5.25 (m, 7 H), 5.05 (t, 1 H, *J* = 10.0 Hz, 4''-H), 4.85 (dd, 1 H, *J* = 4.0 and 10.0 Hz, α 2'-H), 4.71-4.68 (m, 3 H), 4.47-3.52 (m, 34 H), 2.17-1.88 (m, 61 H), 1.55-1.44 (m, 4H, C*H*$_2$). 13C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 170.7, 170.6, 170.5, 170.4, 170.4, 170.3, 170.1, 170.0, 169.8, 169.6, 169.6, 169.5, 169.4, 169.4 (C=O), 145.3, 121.6, 100.3 (β 1-C), 95.7 (1-C), 95.6 (1-C), 95.6 (1-C), 83.9, 83.2, 76.7, 75.1, 73.6, 73.3, 72.3, 72.1, 71.6, 70.5, 70.4, 70.1, 70.0, 69.3, 68.9, 68.4, 67.9, 66.0, 62.7, 62.5, 62.3, 62.1, 61.3, 46.5, 30.2, 30.1, 29.8, 21.4, 21.3, 20.9, 20.8, 20.8, 20.7, 20.7, 20.6, 20.5. 19F NMR (376 MHz, $CDCl_3$): *δ (ppm)* -220.4. HRMS (MALDI) m/z Found: 1980.6205, calculated: 1980.6159 for $C_{82}H_{112}FN_3NaO_{50}$ $[M+Na]^+$.

S1.4. Synthesis of MH^{19}F (5)

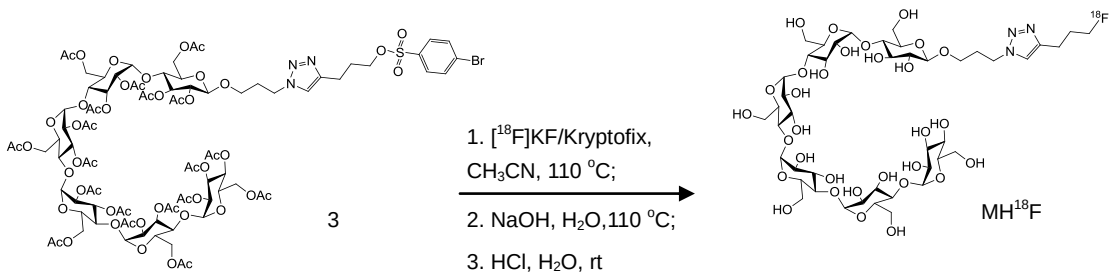
To a stirred solution of fluorinated maltohexaose **4** (100 mg, 0.05 mmol) in methanol (1 mL) was added aqueous 1N NaOH (1 mL) and the resulting mixture was heated and kept at a 110 ^oC for 30 min. After cooling, the mixture was neutralized with 1N HCl until pH 7 is reached. The aqueous solution was evaporated to dryness under reduced pressure, and the residue was purified by HPLC to afford MH^{19}F **5** (45 mg, 77.5%). 1H NMR (400 MHz, D_2O): *δ (ppm)* 7.67 (br, 1 H, triazole), 5.23-5.21 (m, 5 H), 4.59 (m, 5 H), 4.42 (t, 1 H, *J* = 4.0 Hz), 4.36-4.29 (m, 7 H), 4.24 (d, 1 H, *J* = 8.0 Hz, 1-H), 3.81-3.39 (m, 44 H), 3.24 (t, 2 H, *J* = 12.0 Hz, C*H*$_2$-F), 3.12 (t, 2 H, *J* = 6.0 Hz, C*H*$_2$), 2.66 (t, 2 H, *J* = 6.0 Hz, C*H*$_2$), 2.05 (m, 2 H, C*H*$_2$), 1.88 (m, 2 H). 13C NMR (100 MHz, D_2O): *δ (ppm)* 147.2, 123.5, 101.9 (β 1-C), 99.6, 99.5, 99.3 (1-C), 84.8, 83.2, 76.7, 76.6, 76.5, 76.0, 74.3, 73.1, 72.8, 72.7, 72.5, 71.6, 71.4, 71.3, 71.0, 69.1, 66.3, 60.2, 46.8, 29.3, 29.1, 20.4, 20.3. 19F NMR (376 MHz, D_2O): *δ (ppm)* -218.9. HRMS (MALDI) m/z Found: 1166.4471, calculated: 1166.4450 for $C_{44}H_{74}FN_3LiO_{31}$ $[M+Li]^+$.

S1.5. LC-ESI-MS Analyses of $MH^{19}F$ (5)

LC-ESI-MS analysis was performed on an Agilent 1200 LC/MSD instrument (Agilent Technologies, Inc. Wilmington, DE, USA) equipped with an Acquity UPLC BEH C18 column (75 μm i.d., 15 cm length, 3 μm particle size), 6300 ion trap and binary pump followed by a high-pressure UV detector. A manual micro-injection valve (Rheodyne 8125, Rohnert Park, CA) was used to inject samples directly onto the separation column. Two mobile phases were used in the LC-ESI-MS system, mobile phase A 0.1% (v/v) trifluoroacetic aicd in H_2O and mobile phase B 0.1% (v/v) trifluoroacetic acid in acetonitrile, and were run at a flow rate of 200 nL/min. The standard $MH^{19}F$ **6** was separated using a gradient of 5% (v/v) B for 4 min followed by linear ramping from 5% (v/v) to 35% (v/v) B in 20 min, 35% (v/v) to 60% (v/v) B in 5 min and 60% (v/v) to 90% (v/v) B in 25 min. The column effluents were continuously injected into the source of the ESI-MS for molecule detection and analysis. The electrospray interface was set in negative ionization mode with a skimmer potential of −40.0 V and a temperature of 350 ℃ to obtain maximum abundances of the ions in a full scan spectrum (200–1,500 Da)[1]. All analyses were performed in triplicate.



Supplementary Figure S3. LC-MS of $MH^{19}F$ (**5**). LC-ESI-MS analyses were performed to identify $MH^{19}F$ (**5**).

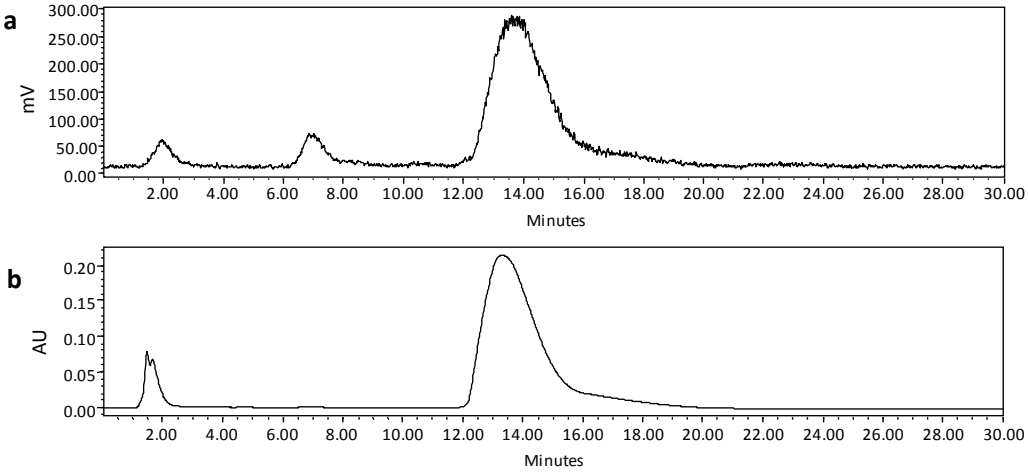


Supplementary Figure S4. Synthesis of $MH^{18}F$

S1.6. Synthesis of $MH^{18}F$

The labeling of ^{18}F-labeled maltohexaose ($MH^{18}F$) was accomplished as depicted in Scheme 1 with no-carrier-added (NCA) cyclotron-produced [^{18}F]-fluoride using a modified single vessel automated method as previously described.[2] Briefly, ^{18}F-fluoride adsorbed on an anion exchange resin cartridge was released by washing with the cartridge with a solution of potassium carbonate (0.9 mg), dissolved in water (0.6 mL), to a glass-reaction vessel containing Kryptofix 222 (5mg) dissolved in acetonitrile (1 mL). Water was evaporated at 110 °C azeotropically with additional acetonitrile (3 mL) with N_2 gas bubbling for 15 min. A solution of brosylate precursor **3** (10 mg, 4.6 μmole) in dry acetonitrile (0.5 mL) was then added to the reaction vessel. The resulting mixture was heated at 110 °C for 30 min. After cooling to room temperature, a mixture of aqueous sodium hydroxide solution (1 M, 1 mL) and acetonitrile (1 mL) was added to the reaction vessel and heated at 110 °C for 30 min. For the last 10 min of the 30 min period, N_2 was bubbled into the reaction mixture to remove the acetonitrile. Aqueous hydrochloric acid (1 N, 0.9 mL) was then added in the reaction mixture to neutralize the base, and mixture was then diluted with saline (10 mL) for transferring. Unreacted [^{18}F]-fluoride and radiolabeled ionic byproducts in the crude mixture were removed by passing the reaction mixture through an ion-retardation resin column followed by an alumina N Sep-Pak. The purified final product $MH^{18}F$ was collected into three fractions 6 mL, 4 mL, and 6 mL, respectively. The 4 mL fraction containing 10 to 20 mCi was used

for the rodent studies. In a representative synthesis, a total of 33 mCi of $MH^{18}F$ was obtained from 1360 mCi of [^{18}F]-fluoride in a synthesis time of approximately 100 min. The brosylate precursor 3 provides an average 4.2 % (n=11) decay corrected yield (DCY) of $MH^{18}F$ in 87 ± 4 % (n=11) radiochemical purity based on radiometric HPLC. The specific activity of $MH^{18}F$ doses in saline (4 mL) was 1.7 ± 0.6 Ci/μmole (n=3) and the concentration of the MHOH in $MH^{18}F$ doses in saline (4 mL) was 0.6 ± 0.06 mg/mL (n=3).



Supplementary Figure S5. Sample chromatograms of the radio-HPLC analysis of formulated $MH^{18}F$. $MH^{18}F$ was identified by co-injection of $MH^{18}F$ and non-radiolabeled $MH^{19}F$ onto a RP-HPLC system. (a) radioactivity (b) UV absorbance at 220 nm.

S2.1. *In vitro* uptake of $MH^{19}F$ in bacteria

The uptake of $MH^{19}F$ was investigated in *E.coli* (ATCC 33456) and *E.coli* LamB mutants (JW3992-1). Bacteria were cultured overnight in Luria-Bertani (LB) medium at 37 ℃ under 5% CO_2 under shaking. Bacteria (100 μL from the overnight culture) were re-suspended in 30 mL of fresh LB medium and cultured in a 250mL flask, as described above, to an optical density of 0.5 at 600 nm. The bacterial culture solution was transferred into 6 well plates, with each well containing 2 mL of bacteria, and 50 μL of

$MH^{19}F$ stock solution (20 mM in PBS) was added, generating a 500 µM $MH^{19}F$ concentration. The bacteria were incubated with the $MH^{19}F$ for 1 hour at 37 ℃ under 5% CO_2 under shaking. The bacteria were harvested by centrifuging the bacterial suspensions at 10,000 rpm for 15 minutes in 15 mL centrifuge tubes, using a Microfuge® 18 centrifuge (Beckman Coulter, Brea, CA). The resulting pellets were washed 3 times with 10 mL PBS by re-suspending the pellets in PBS and centrifuging. The washed bacterial pellets were transferred into a centrifuge tube (BD Falcon Centrifuge Tube, BD Biosciences), placed in an ice bath and lysed by sonication for 20 seconds in 0.5 mL DI water. The bacterial supernatant was isolated by centrifuging at 10,000 rpm for 10 minutes. The $MH^{19}F$ concentration of the supernatant was determined by ^{19}F-NMR using the electronic reference to access *in vivo* concentrations (ERETIC) method [3], and was normalized to the bacterial protein content, determined by the BCA assay[4].

S2.2. Competitive inhibition of $MH^{19}F$ transport by maltohexaose

The ability of maltohexaose to inhibit the uptake of $MH^{19}F$ in *E.coli* was investigated. *E.coli* (ATCC 33456) were grown in LB medium at 37 °C under 5% CO_2 to an OD_{600} = 0.5. The bacterial suspensions (2 mL) were transferred to 6 well plates, preincubated at 37 ℃ for 5 minutes under shaking (as described above), and 50 µL of maltohexaose stock solutions (2 M in PBS) were added, generating a 50 mM final concentration of maltohexaose. The bacteria were incubated with maltohexaose for 5 minutes and 50 µL of $MH^{19}F$ stock solutions (20 mM in PBS) were added, generating a 500 µM $MH^{19}F$ concentration. The bacteria were incubated at 37 ℃ under 5% CO_2 for 1 hour and the bacterial suspensions were rapidly filtered through 0.45-µm-pore-size nitrocellulose filters (diameter, 25 mm; Schleicher & Schuell GmbH, Dassel, Germany). The filtered bacteria were recovered and washed three times with 3 mL PBS buffer, via centrifugation (as described above). The washed bacterial pellets were transferred into a centrifuge tube (BD Falcon Centrifuge Tube, BD Biosciences), placed in an ice bath and lysed by

sonication for 20 seconds in 0.5 mL DI water. The bacterial supernatant was isolated by centrifuging at 10,000 rpm for 10 minutes. The $MH^{19}F$ concentration of the supernatant was determined by F-19 NMR using the electronic reference to access *in vivo* concentrations (ERETIC) method [3], and was normalized to the bacterial protein content, determined by the BCA assay[4].

S.2.3. *In vitro* uptake of $MH^{19}F$ in mammalian cells

Hepatocytes (ACBRI 3716) were cultured in DMEM at 37 °C in a humidified atmosphere containing 5% CO_2 and sub-cultured before confluence. Cells were seeded into 6-well plates (5×10^4 cells/well) and grown to 80% confluence. At the start of each experiment, the culture medium was removed and replaced with 2 mL of DMEM, and 50 µL of $MH^{19}F$ stock solution (20 mM in PBS) were added, generating a 500 µM $MH^{19}F$ concentration. The cells were incubated with $MH^{19}F$ in a humidified atmosphere of 5% CO_2 at 37 °C for 1 h. The medium was removed from each well, and washed 3 times with 3 mL of PBS. The cells were removed from the wells by adding cell dissociation buffers, followed by centrifugation at 5000 rpm for 10 min, using a Microfuge® 18 centrifuge (Beckman Coulter, Brea, CA). The resulting pellets were washed 3 times with 10 mL PBS by resuspending the pellets in PBS and centrifuging. The washed cell pellets were transferred into a centrifuge tube (BD Falcon Centrifuge Tube, BD Biosciences), placed in an ice bath and lysed by sonication for 20 seconds in 0.5 mL DI water. The cell supernatant was isolated by centrifuging at 10,000 rpm for 10 minutes. The $MH^{19}F$ concentration of the supernatant was determined by ^{19}F-NMR using the electronic reference to access *in vivo* concentrations (ERETIC) method [3], and was normalized to the cell protein content, determined by the BCA assay[4].

S.2.4. Kinetics of $MH^{19}F$ uptake in bacteria

E.coli were cultured overnight in LB medium at 37 ℃ under 5% CO_2 under shaking.

E.coli (100 µL from the overnight culture) were re-suspended in 30 mL of fresh LB medium and cultured in a 250 mL flask to an OD_{600} = 0.5, as described above, and stored on ice until use. The bacterial culture solution (2 mL) was transferred into 6 well plates and preincubated at 37 ℃ for 5 minutes under shaking. 50 µL of $MH^{19}F$ stock solutions (20 mM in PBS**)** were added to the *E.coli* suspension, generating a 500 µM $MH^{19}F$ concentration. The *E.coli* were incubated at 37 ℃ under 5% CO_2, and at various time points (5, 20, 40 and 60 min), a small aliquot of the bacterial culture was plated to determine the CFUs/mL and the approximate total intracellular volume of bacteria was estimated, following literature procedures [5]. Bacterial suspensions were rapidly filtered through 0.45-µm-pore-size nitrocellulose filters (diameter, 25 mm; Schleicher & Schuell GmbH, Dassel, Germany). The filtered bacteria were recovered and washed three times with 5 mL PBS buffer, via centrifugation. The washed bacterial pellets were transferred into a centrifuge tube (BD Falcon Centrifuge Tube, BD Biosciences), placed in an ice bath and lysed by sonication for 20 seconds in 0.5 mL DI water. The bacterial supernatant was isolated by centrifuging at 10,000 rpm for 10 minutes. The $MH^{19}F$ concentration of the supernatant was determined by ^{19}F-NMR using the electronic reference to access *in vivo* concentrations (ERETIC) method [3], and was normalized to intracellular concentration by estimating the bacterial volume following literature procedures. [5]

S3. *In vivo* imaging of bacterial infections by PET

S3.1. Preparation of bacteria for *in vivo* PET imaging of soft tissue infections

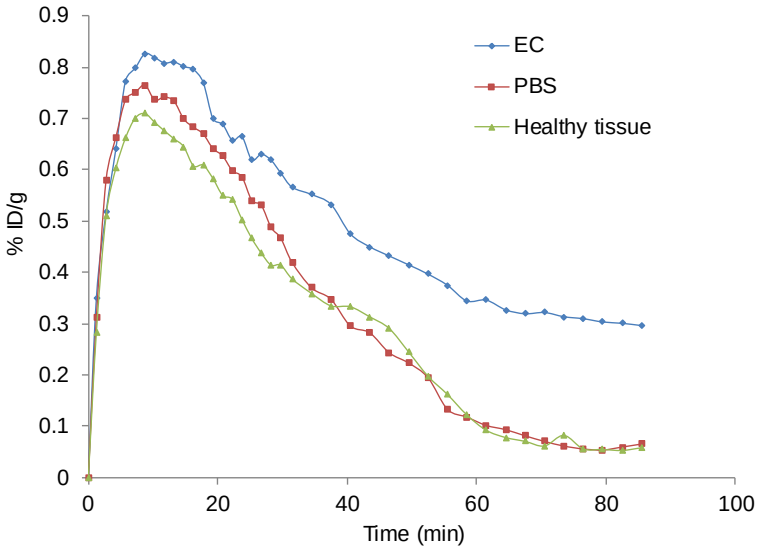
E.coli (ATCC 33456) were cultured overnight in Luria-Bertani (LB) medium at 37 ℃ under 5% CO_2 in an incubator shaker (Innova™ 4230, New Brunswick Scientific, Edison, NJ). Bacteria (100 µL from the overnight culture) were re-suspended in 30 mL of fresh LB medium, cultured to an OD_{600} of 0.5 in an incubator shaker, and harvested by centrifuging. The resulting pellet was washed 2 times with 10 mL of PBS by

re-suspending the pellets in PBS and centrifuging. The washed pellets were re-suspended in PBS, and were used for *in vivo* experiments.

S3.2. *In vivo* imaging of bacterial infections with $MH^{18}F$

The ability of $MH^{18}F$ to detect bacteria *in vivo* was investigated using an Inveon microPET/CT scanner (Siemens). Female Sprague Dawley rats (10 weeks, 200-250 g, Charles River Lab, Inc.) were anaesthetized by administration of ketamine (60 mg/kg), atropine (0.1 mg/kg) and xylazine (10 mg/kg) through IM injection. A suspension of *E.coli* (10^5 CFUs) in 250 μL of PBS was injected into the left triceps muscle (injection depth 5 mm), and 250 μL of PBS was injected into right triceps muscle as a control (injection depth 5 mm). After 2 hours the rats were injected with $MH^{18}F$ (250 μCi in 100 μL of PBS) via a tail vein. PET images were acquired for 90 minutes using a micro PET/CT Preclinical Scanner (Siemens) right after administration of $MH^{18}F$. All the PET images in the manuscript were generated from dynamic PET scans taken between 40 and 70 minutes, and images in the figures represent the averaged value over this time period. At the end of the imaging procedure rats were sacrificed by injecting sodium pentobarbital (100 mg/kg) via a tail vein. The radioactivity from the region of interest (ROI) including the infected muscles (target) and PBS injection areas (control) and healthy areas (background) were integrated using a ASI Pro VM™ micro PET analysis software. PET Images were reconstructed using a 2-dimensional ordered-subsets expectation maximum (OSEM) algorithm, the frame rates were 60×30s and 20×180s. To quantify the ability of $MH^{18}F$ to target bacteria *in vivo*, the target or control to background ratio of $MH^{18}F$ (ROI ratio) was calculated and defined as the mean radioactivity in the target/the mean radioactivity in the background. Kinetic analysis of regional time activity curves (TACs) were performed with a one-tissue (two-compartment) model (see Figure S6), following literature procedures[6] . Four rats were used for each experimental group.

Statistical Analysis - Analysis was performed with Prism statistical software (version 4). Student's *t*-test was used to compare the accumulation of $MH^{18}F$ in infected muscles and the uninfected muscles, and $P<0.05$ was regarded as significant. Data are shown as mean ± s.e.m.



Supplementary Figure S6. Time activity curves of decay-corrected $MH^{18}F$ activity in *E.coli* infected, PBS injected (control) and healthy muscles of a representative rat (see Figure 3b).

S.3.3. Preparation of methylene blue labeled *E.coli* for biodistribution studies

E.coli were cultured in 30 mL of LB to an OD_{600} = 0.5, and 300 µL of methylene blue stock solution (1 M in ethanol) was added, generating a 10 mM methylene blue concentration. The *E.coli* were incubated with methylene blue for 10 minutes at 25 ℃ in an incubator shaker, and harvested by centrifuging. The resulting pellets were washed 3 times with 10 mL PBS by resuspending the pellets in PBS and centrifuging. The washed pellets were re-suspended in 1 mL of saline, and were used for *in vivo* experiments

S3.4. Whole-body biodistribution of MH^{18}F and ^{18}FDG in bacterial infected rats

Female Sprague Dawley rats (10 weeks, 200-250 g, Charles River Lab, Inc.) were anaesthetized by intramuscular (IM) injection of ketamine (60 mg/kg), atropine (0.1 mg/kg) and xylazine (10 mg/kg). A suspension of methylene blue dye labeled *E.coli* (10^9 CFUs) in 250 μL of PBS was injected into the left rear thigh muscle (injection depth 5 mm), and a 250 μL of PBS was injected into the right rear thigh muscle (injection depth 5 mm). After 2 hours the rats were injected with either MH^{18}F (50 μCi in 100 μL of PBS) or ^{18}FDG (50 μCi in 100 μL of PBS) via a tail vein. One hour after administration of MH^{18}F or ^{18}FDG, the rats were implanted with a catheter in the renal vein and the blood was collected. The cardiovascular system was perfused through the renal vein with 50 mL of PBS until the liver turned from reddish pink to pale white. Infected tissues were harvested based on methylene blue staining, and other rat organs including muscle, heart, liver, lung, kidney, small intestine, large intestine, spleen, bone and brain were harvested and weighed. The radioactivity of all the harvested tissues was determined using a γ-counter (Wallac 1480; Perkin Elmer). The radioactive decay of each sample was corrected. The percentage injected dose of MH^{18}F or ^{18}FDG per gram of tissues was determined based on the radioactivity, and results are expressed as % injected dose/gram tissue ± s.e.m (standard error of the mean). Four rats were used for each experimental group.

Statistical Analysis - Analysis was performed with Prism statistical software (version 4). Student's *t*-test was used to compare the accumulation of MH^{18}F or ^{18}FDG in the infected muscles and the uninfected muscles, and $P<0.05$ was regarded as significant. Data are shown as mean±s.e.m.

S3.5. Preparation of metabolically inactive *E.coli*

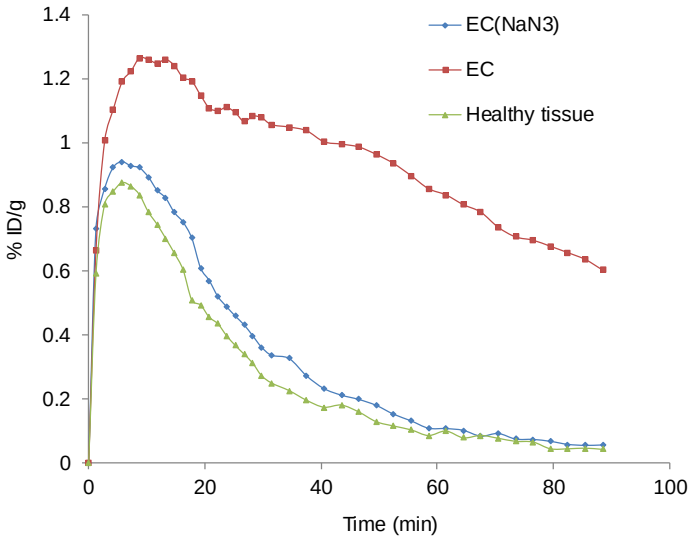
Metabolically inactive *E.coli* were prepared by treating *E.coli* (ATCC 33456) with sodium azide[7]. *E.coli* were cultured in 30 mL of LB to an OD_{600} of 0.5, and 300 μL of

sodium azide stock solution (1 M in PBS) was added, generating a 10 mM concentration of sodium azide. The *E.coli* were incubated with sodium azide for 1 hour at 37 ℃ in an incubator shaker, and harvested by centrifuging. The resulting pellet was washed 3 times with 10 mL of PBS by re-suspending the pellets in PBS and centrifuging. The washed pellet was re-suspended in PBS, and was used for *in vivo* experiments.

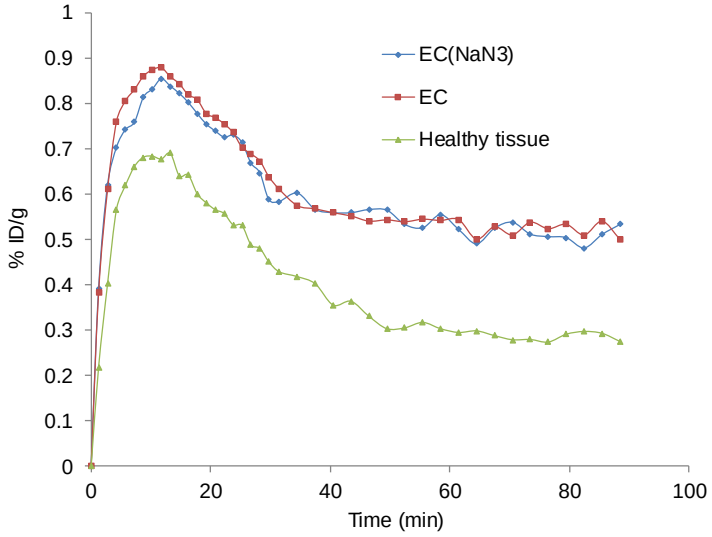
S3.6. Distinguishing bacterial infections from inflammation *in vivo* with $MH^{18}F$

Female Sprague Dawley rats (10 weeks, 200-250 g, Charles River Lab, Inc.) were anaesthetized by administration of ketamine (60 mg/kg), atropine (0.1 mg/kg) and xylazine (10 mg/kg) via IM injection, and a catheter was placed in the tail vein. A suspension of *E.coli* (10^9 CFUs) in 250 μL of PBS was injected into the left rear thigh muscle (injection depth 5 mm), and a suspension of NaN_3 treated *E.coli* (10^9 CFUs) in 250 μL of PBS was injected into the right rear thigh muscle (injection depth 5 mm). After 2 hour the rats were injected with $MH^{18}F$ (250 μCi in 100 μL of PBS) via the tail vein. PET images were acquired for 90 minutes using an Inveon micro PET/CT Preclinical Scanner (Siemens) right after $MH^{18}F$ injection. At the end of the imaging procedure rats were sacrificed by injecting sodium pentobarbital (100 mg/kg) via the tail vein. The radioactivity from the region of interest (ROI) including the bacterial injection areas (target) and healthy areas (background) were integrated using a ASI Pro VM™ micro PET analysis software. PET Images were reconstructed using a 2-dimensional ordered-subsets expectation maximum (OSEM) algorithm, the frame rates were 60×30s and 20×180s. To quantify the ability of $MH^{18}F$ to target bacteria *in vivo*, the target or control to background ratio of $MH^{18}F$ (ROI ratio) was calculated and defined as the mean radioactivity in the target/the mean radioactivity in the background. Kinetic analysis of regional time activity curves (TACs) were performed with a one-tissue (two-compartment) model (see Figures S7 and S8), following literature procedures[6] . Four rats were used for each experimental group.

Statistical Analysis - Analysis was performed with Prism statistical software (version 4). Student's *t*-test was used to compare the infected muscles and the inflammatory muscles, and $P<0.05$ was regarded as significant. Data are shown as mean±s.e.m.



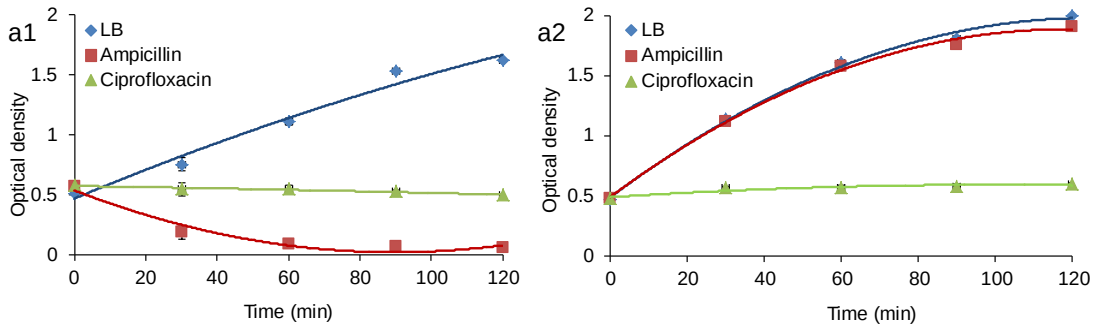
Supplementary Figure S7. Time activity curves of decay-corrected MH^{18}F activity in *E.coli* infected, dead *E.coli* infected (EC (NaN3)) and healthy muscles of a representative rat (see Figure 5a).



Supplementary Figure S8. Time activity curves of decay-corrected ^{18}FDG activity in the *E.coli* infected, dead *E.coli* infected (EC (NaN3)) and healthy muscles of a representative rat (see Figure 5b).

S3.7. Susceptibility of wild-type and drug-resistant *E.coli* to antibiotics

The susceptibility of wild-type *E.coli* (ATCC 33456) and ampicillin-resistant *E.coli* (ATCC 6538) to antibiotics was investigated. Bacteria were cultured overnight in Luria-Bertani (LB) medium at 37 ℃ under 5% CO_2 in an incubator shaker (Innova™ 4230, New Brunswick Scientific, Edison, NJ), set at 220 rpm. Bacteria (100 µL from the overnight culture) were re-suspended in 30 mL of fresh LB medium and cultured in a 250 mL flask, as described above, to an OD_{600} of 0.5. The bacterial culture solution at steady-state growth was transferred into a 6 well plate, containing 3 mL of bacteria in each well, and 30 µL of either ampicillin or ciprofloxacin stock solutions (20 mg/mL in PBS) were added, generating a 200 µg/mL antibiotic concentration. The bacteria were incubated with the antibiotics at 37 ℃ under 5% CO_2, and 500 µL aliquots of the bacterial solutions were taken at various time points (30, 60, 90 and 120 minutes) , and OD_{600} was measured to determine bacterial viability. Bacterial culture solutions without antibiotics were used as the control for this experiment.

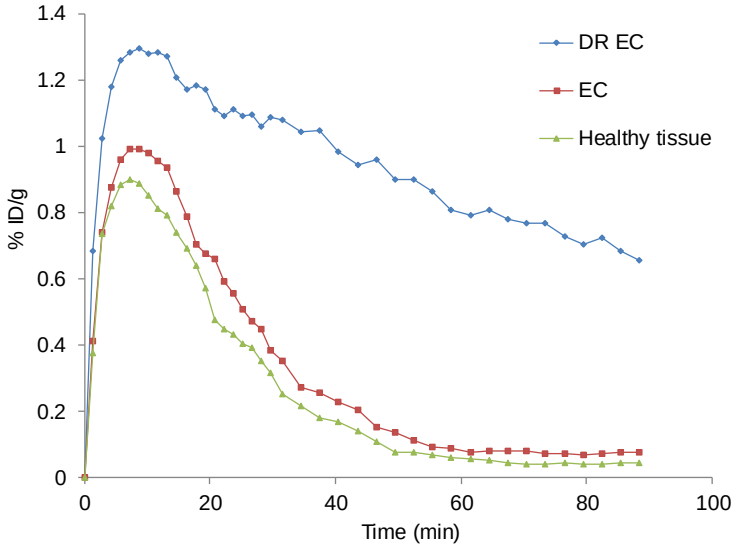


Supplementary Figure S9. Susceptibility of bacteria to different antibiotics. Wild-type and ampicillin-resistant *E.coli* were treated with either ampicillin or ciprofloxacin, and antibiotic susceptibility was determined. (**a1)** Susceptibility of wild-type *E.coli* to antibiotics. (a2) Susceptibility of ampicillin-resistant *E.coli* to antibiotics.

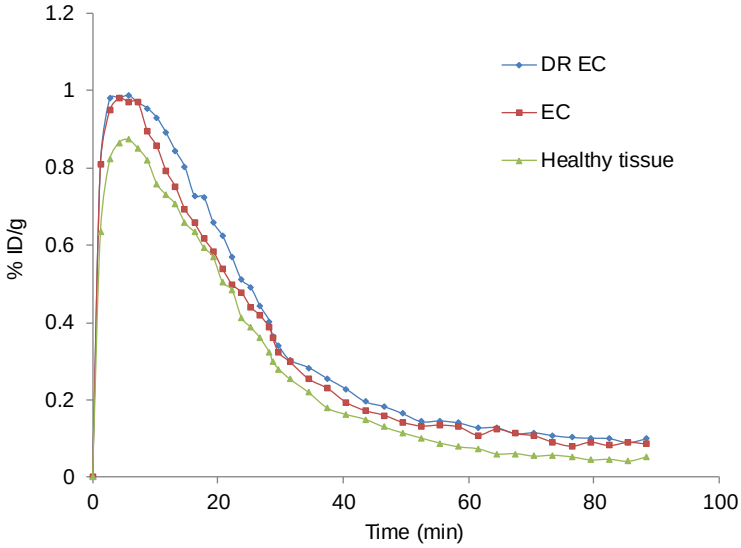
S3.8. *In vivo* imaging of drug-resistance and drug efficacy in bacteria

The ability of $MH^{18}F$ to monitor therapeutic effect of antibiotic was investigated. Female Sprague Dawley rats (10 weeks, 200-250 g, Charles River Lab, Inc) were anaesthetized with IM injection of ketamine (60 mg/kg), atropine (0.1 mg/kg) and xylazine (10 mg/kg). A suspension of ampicillin-resistant *E.coli* (10^9 CFUs) in 250 μL of PBS was injected into the left triceps muscle (injection depth 5 mm), and a suspension of wild-type *E.coli* (10^9 CFUs) in 250 μL of PBS was injected into the right triceps muscle (injection depth 5 mm). After 1 hour the rats were given a 500 μL solution of either ampicillin (20 mg/mL) or ciprofloxacin (20 mg/mL) via the tail vein. Two hours after administration of antibiotics, the rats were injected with $MH^{18}F$ (250 μCi in 100 μL of PBS) via the tail vein. PET images were acquired for 90 minutes using an Inveon micro PET/CT Preclinical Scanner (Siemens) right after the injection of $MH^{18}F$. At the end of the imaging procedure rats were sacrificed by injecting sodium pentobarbital (100 mg/kg) via the tail vein. The radioactivity from the region of interest (ROI) including the bacterial injection areas (target) and healthy areas (background) were integrated using a ASI Pro VM™ micro PET analysis software. PET Images were reconstructed using a 2-dimensional ordered-subsets expectation maximum (OSEM) algorithm, the frame rates were 60×30s and 20×180s. To quantify the ability of $MH^{18}F$ to target bacteria *in vivo*, the target or control to background ratio of $MH^{18}F$ (ROI ratio) was calculated and defined as the mean radioactivity in the target/the mean radioactivity in the background. Kinetic analysis of regional time activity curves (TACs) were performed with a one-tissue (two-compartment) model (see Figures S10 and S11), following literature procedures[6] . Four rats were used for each experimental group.

Statistical Analysis - Analysis was performed with Prism statistical software (version 4). Student's *t*-test was used to compare the two infected muscles, and $P<0.05$ was regarded as significant. Data are shown as mean±s.e.m.



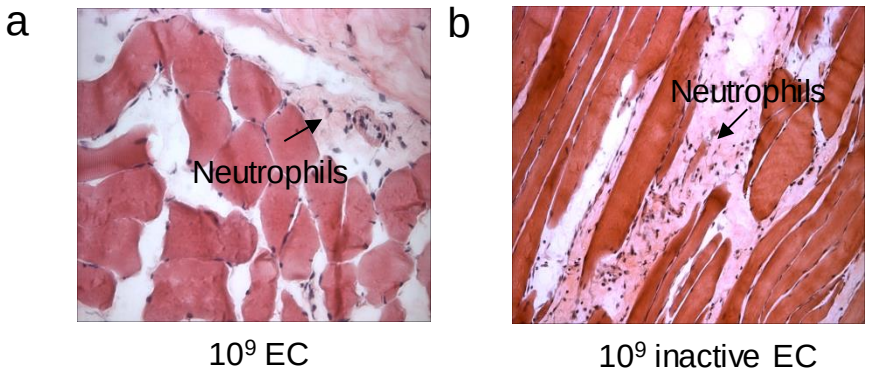
Supplementary Figure S10. Time activity curves of decay-corrected MH^{18}F activity in the *E.coli* infected, drug-resistant *E.coli* infected (DR EC) and healthy muscles of a representative rat (see Figure 6a).



Supplementary Figure S11. Time activity curves of decay-corrected MH^{18}F activity in the *E.coli* infected, drug-resistant *E.coli* infected (DR EC) and healthy muscles of a representative rat (see Figure 6b).

S3.9. Histology of bacterial infected muscles

Female Sprague Dawley rats (10 weeks, 200-250 g, Charles River Lab, Inc.) infected with *E.coli* were sacrificed, and the infected and healthy muscles were harvested. Tissues were cut to 3 x 4 x 5 mm pieces, fixed in PBS containing 4% formaldehyde, washed in PBS, embedded in OCT compound (Tissue Tek, Miles, Elkhart, IN), rapidly frozen in liquid nitrogen, and kept at -80 ℃ until examined. Tissues were cut with a rotary microtome (AO 820 microtome; American Optical, Buffalo, NY) at an 8 μm thickness and mounted on poly-L-lysine-coated glass slides. Randomly selected sections were stained with hematoxylin and eosin (H&E) to detect neutrophils and macrophages[8].



Supplementary Figure S12. Histological analysis of infected muscles (× 20 magnification). Both live *E.coli* (a) and dead *E.coli* (b) induce inflammation as evidenced by the presence of neutrophils.

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