[^{11}C]PABA: A PET tracer targeting bacterial folic acid biosynthesis

Christopher A. Mutch[1], Alvaro Ordonez[2], Hecong Qin[1], Matthew Parker[1], Lauren Bambarger[2], Javier E. Villanueva-Meyer[1], Yong Seok Chang[2], Joseph Blecha[1], Valerie Carroll[1], Celine Taglang[1], Robert Flavell[1], Renuka Sriram[1], Henry VanBrocklin[1], Oren Rosenberg[3], Michael A. Ohliger[1,4], Sanjay K. Jain[2]*, Kiel D. Neumann[5]*, David M. Wilson[1*]

[1]Department of Radiology and Biomedical Imaging
University of California, San Francisco
San Francisco, CA 94158, USA

[2]Center for Infection and Inflammation Imaging Research
Johns Hopkins University School of Medicine
Baltimore, MD 21287, USA

[3]Department of Medicine
University of California, San Francisco
San Francisco, CA 94158, USA

[4]Department of Radiology
Zuckerberg San Francisco General Hospital
San Francisco CA 94110, USA

[5]Department of Radiology and Medical Imaging
University of Virginia
Charlottesville, VA 22903, USA

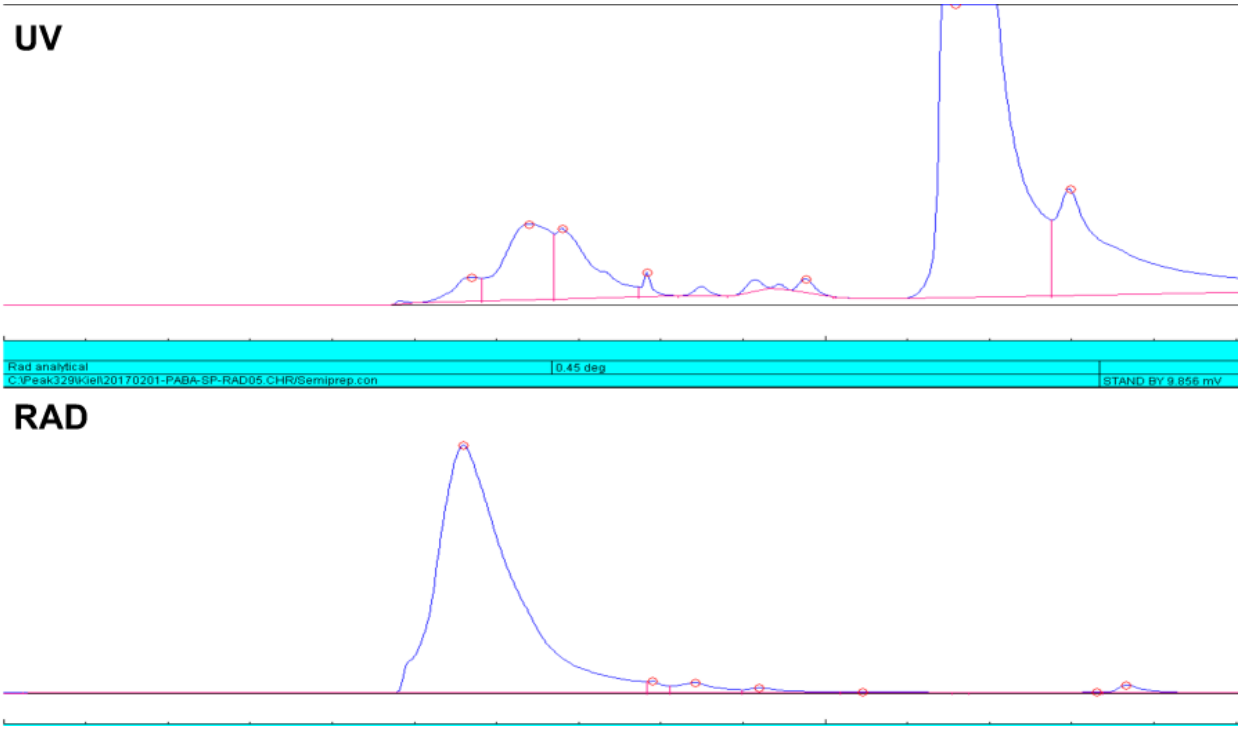
SUPPLEMENTARY INFORMATION:

A. Synthetic Procedures for [α-^{11}C]PABA and [α-^{13}C]PABA:

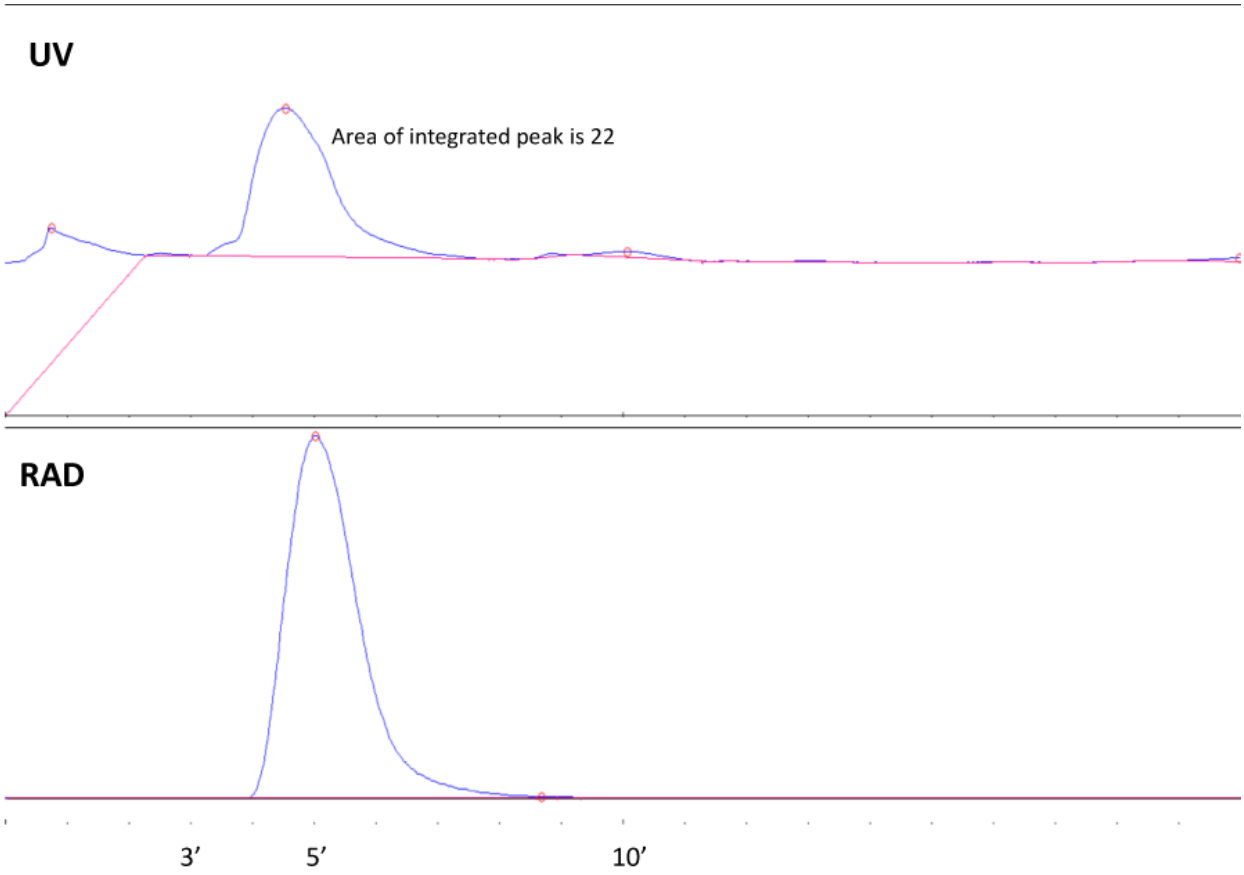
Procedure for the radiosynthesis and purification of [^{11}C]PABA: A 300 µL solution of 4-[Bis(trimethylsilyl)amino]phenylmagnesium bromide (0.5 M in THF, Sigma Aldrich) was dispensed into a 5 mL Wheaton V-vial. The vial was capped with a PTFE-lined silicone septum and a 20-gauge needle was inserted to the bottom. [^{11}C]CO_2 was delivered into the solution from the cyclotron at a rate of 50 mL/min with argon carrier gas for 2 minutes at room temperature. The

THF/Grignard solution was subsequently quenched with a hydrochloric acid solution (150 µL of 1 M HCl diluted with 3 mL of 15 mM phosphate buffer) and bubbling was continued for 60 seconds. The entire solution was injected and purified on a semi-prep High-Performance Liquid Chromatography (HPLC) column (Synergi Hydro-RP 10 x 250 mm, 10 µm; mobile phase = 5% ethanol + 20 mM phosphate buffer @ 3 mL/min, pH = 8; Rt = 6 min). The final product was analyzed on a Synergi Hydro-RP 3 x 250 mm, 4 µm analytical HPLC; mobile phase = 20mM phosphate buffer, pH = 8, flow = 0.4 mL/min, R_t = 5 min.

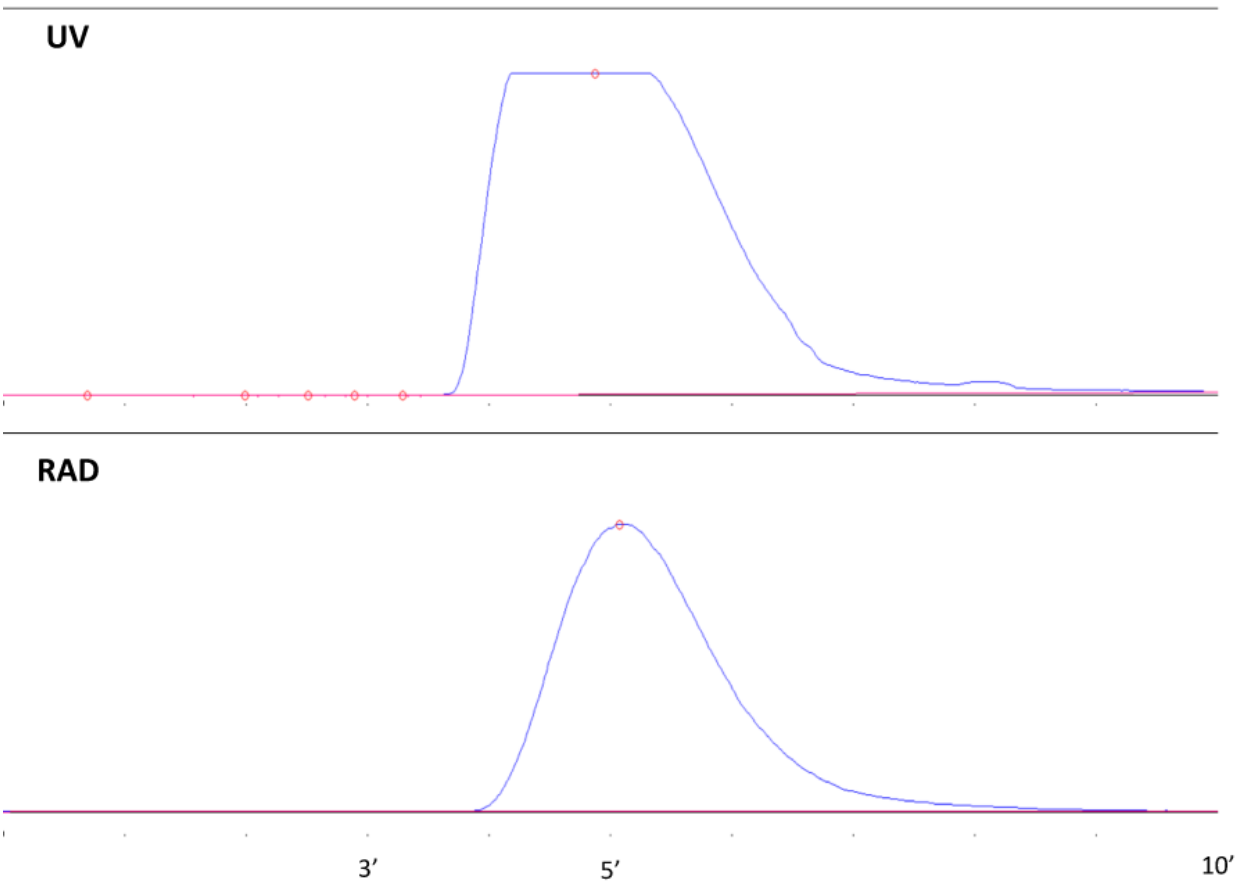
Phosphate buffers: *15 mM phosphate buffer* was prepared by dissolving 200 mg of monosodium phosphate in 100 mL of deionized water. *20 mM (pH = 8) phosphate buffer* was prepared by dissolving 0.16 g of sodium phosphate monobasic and 5.0 g of sodium phosphate dibasic in 1 L of deionized water).



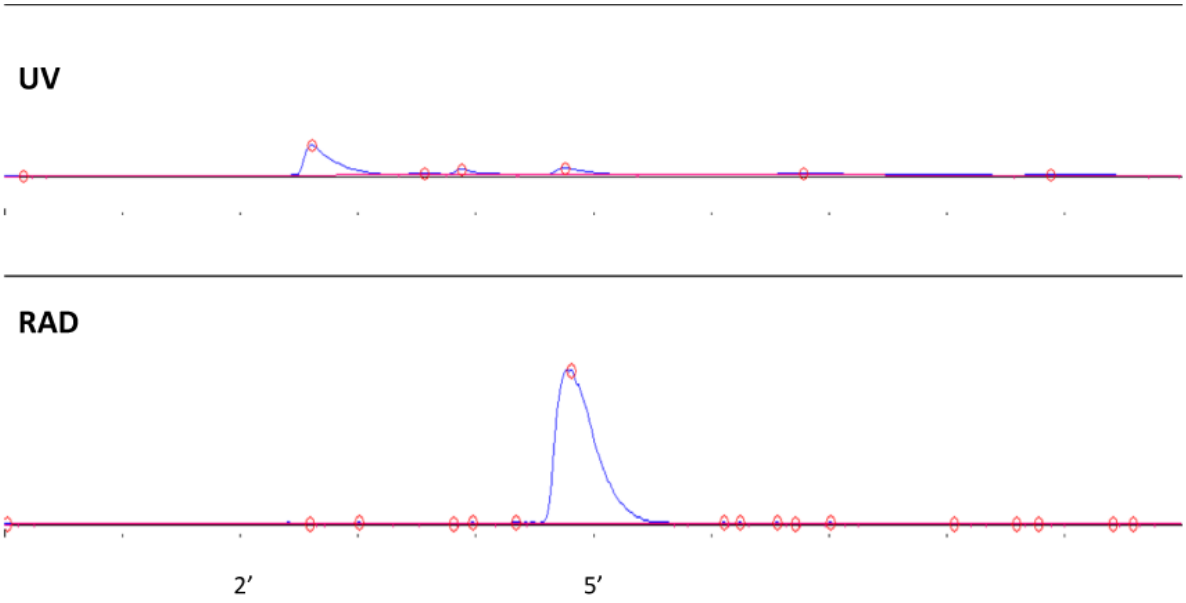
Supplementary Figure 1. Typical semi-preparative HPLC trace from purification of [^{11}C]PABA. UV trace is above, RAD trace below.



Supplementary Figure 2. Typical trace of HPLC-purified [^{11}C]PABA (no cold standard injection), solid/ stationary phase condition 1, Hydro-RP @ pH = 8.0 . This is example of data point used for specific activity calculation. UV trace is above, RAD trace below.



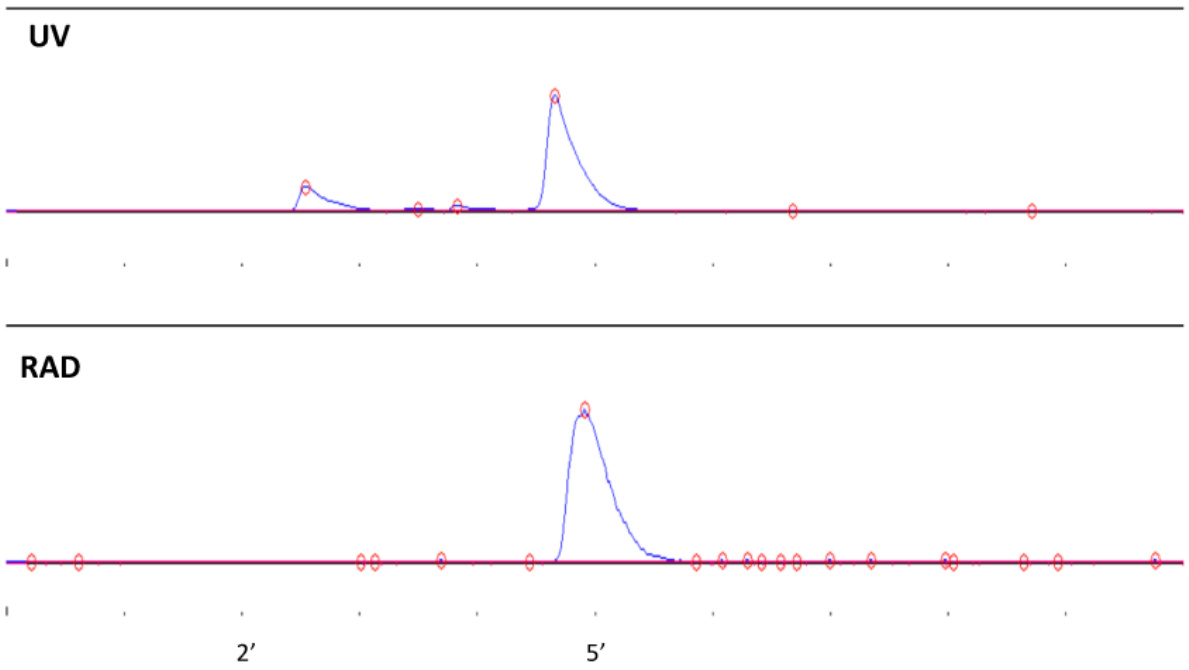
Supplementary Figure 3. Typical trace of HPLC-purified [^{11}C]PABA with cold standard injection, solid/ stationary phase condition 1, Hydro-RP @ pH = 8.0. UV trace is above, RAD trace below.



Quality control sample on a C18 column at pH 3.0. Delay is 0.15’ as calculated.

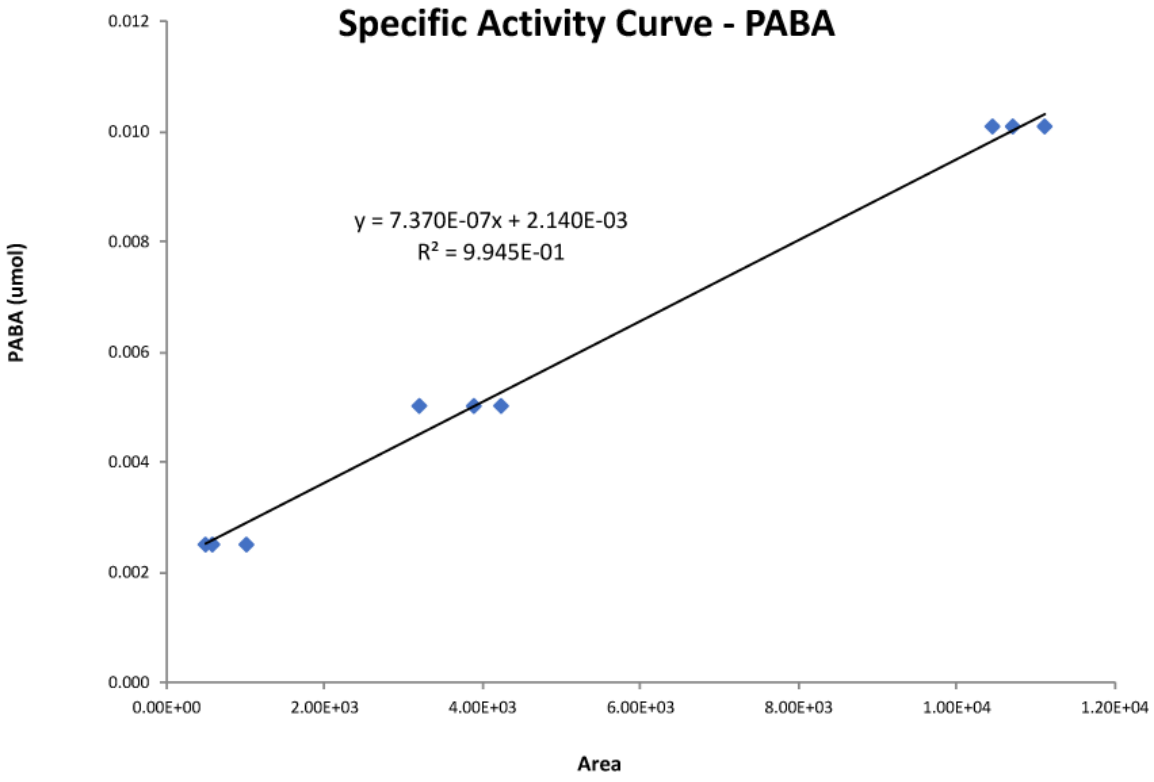
Luna C18(2) 250 x 4.6 mm, 30% ACN/ 20mM KH_2PO_4 pH 3.0, 1 mL/ min, 254 nm

Supplementary Figure 4. Typical trace of HPLC-purified [^{11}C]PABA (no cold standard injection), solid/ stationary phase condition 2, Luna C18 @ pH = 3.0. UV trace is above, RAD trace below.



Mass spiked sample of previous trace. Delay is 0.15' as calculated.
Luna C18(2) 250 x 4.6 mm, 30% ACN/ 20mM KH_2PO_4 pH 3.0, 1 mL/ min, 254 nm

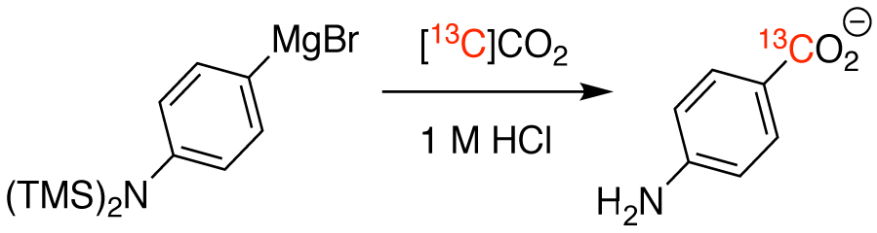
Supplementary Figure 5. Typical trace of HPLC-purified [^{11}C]PABA (with cold standard injection), solid/ stationary phase condition 2, Luna C18 @ pH = 3.0. UV trace is above, RAD trace below.



run	area	PABA (µmol)	activity (mCi) EOS	SA (mCi/µmol) @ EOS	ave SA	St.dev
1	38	0.002168006	1.40	645.7546704	819.7771	517.1937
2	21	0.002155477	2.10	974.2623095		
3	15	0.002151055	3.10	1441.153295		
4	22.49	0.002156575	0.47	217.9381527		

Supplementary Figure 6. Specific activity standard curve and data for HPLC-purified [^{11}C]PABA.

Procedure for the synthesis and purification of [^{13}C]PABA:

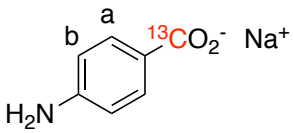


A 20 mL Wheaton V-vial charged with a teflon stir bar was capped with a teflon-lined silicone septum and vented in connection with a reservoir of mineral oil. The Wheaton vial was flushed with He gas for 15 minutes. The He gas supply was removed from the vial and the vial was diluted with 5 mL of anhydrous THF. A 5 mL solution of 4-[Bis(trimethylsilyl)amino]phenylmagnesium bromide (0.5 M in THF) was dispensed into the 20 mL Wheaton V-vial and allowed to stir for 2 minutes. A lecture bottle containing [^{13}C]CO_2 was connected to the Wheaton V-vial using a 20 gauge 6" needle. The needle was inserted to the bottom of the vial and the [^{13}C]CO_2 was allowed to flow through the solution at a rate of 10 mL/min**.** After approximately 10 minutes, the solvent was visibly removed. The [^{13}C]CO_2 gas was removed and the vial was purged with He for an additional 10 minutes. The residue is quenched with a hydrochloric acid solution (2.5 mL of 1 M HCl diluted with 10 mL of 0.5 M phosphate buffer, pH 3.6*). The aqueous layer was removed from the vial and extracted with (3 x 20 mL) chloroform. The chloroform was separated, dried with sodium sulfate, and filtered by gravity. The solution was stirred with a Teflon stir bar and the pH of the solution was slowly adjusted to 1.5 using ethereal-HCl, at which time, a tan solid had precipitated. The highly crystalline solid was collected by vacuum filtration and dried overnight under dynamic vacuum. The crystalline solid was confirmed by [^{1}H] and [^{13}C] NMR to be *p*-[^{13}C]aminobenzoic acid (250 mg, 73% yield).

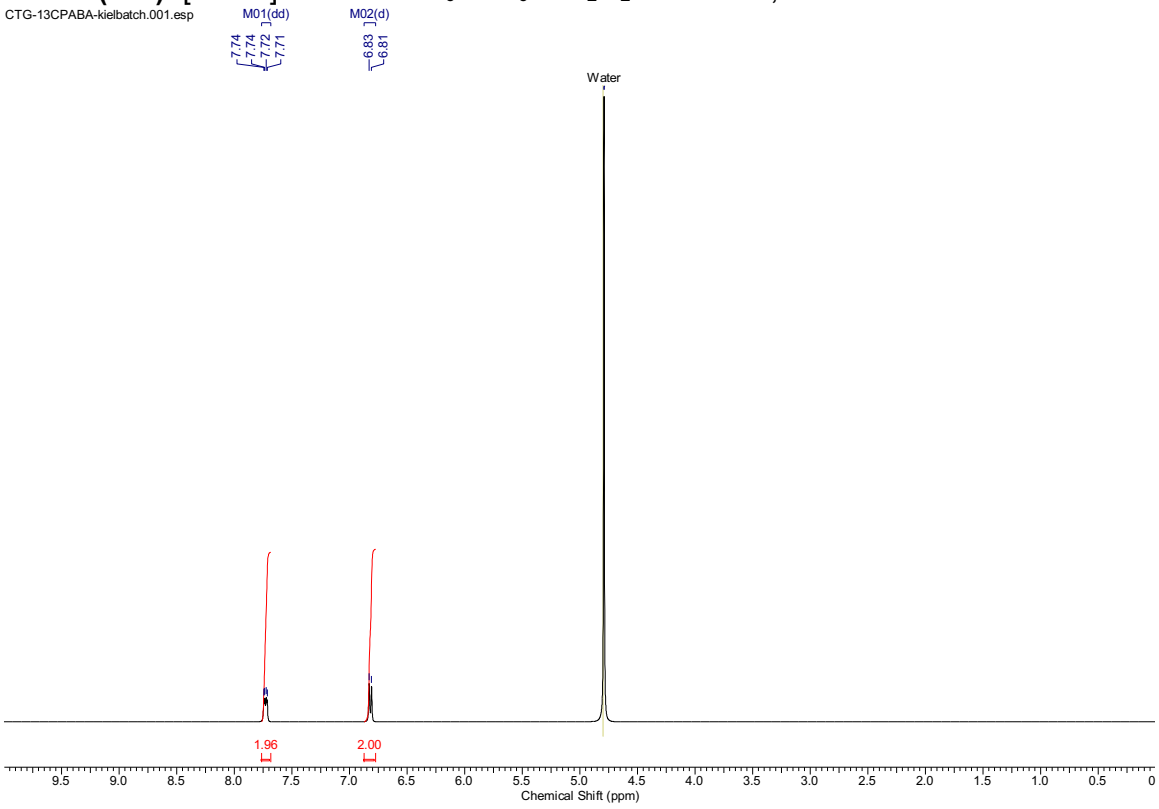
The sodium salt, which was used for [^{13}C]hyperpolarized magnetic resonance spectroscopy, was synthesized by dissolving 250 mg of zwitterion in 1.7 mL of 1 M NaOH. The aqueous solution was then lyophilized overnight to yield a tan crystalline solid.

*0.5 M phosphate buffer (pH = 3.6) is prepared by dissolving 6.9 g of monosodium phosphate, monohydrate and 8.3 mg of disodium phosphate, heptahydrate in 100 mL of deionized water.

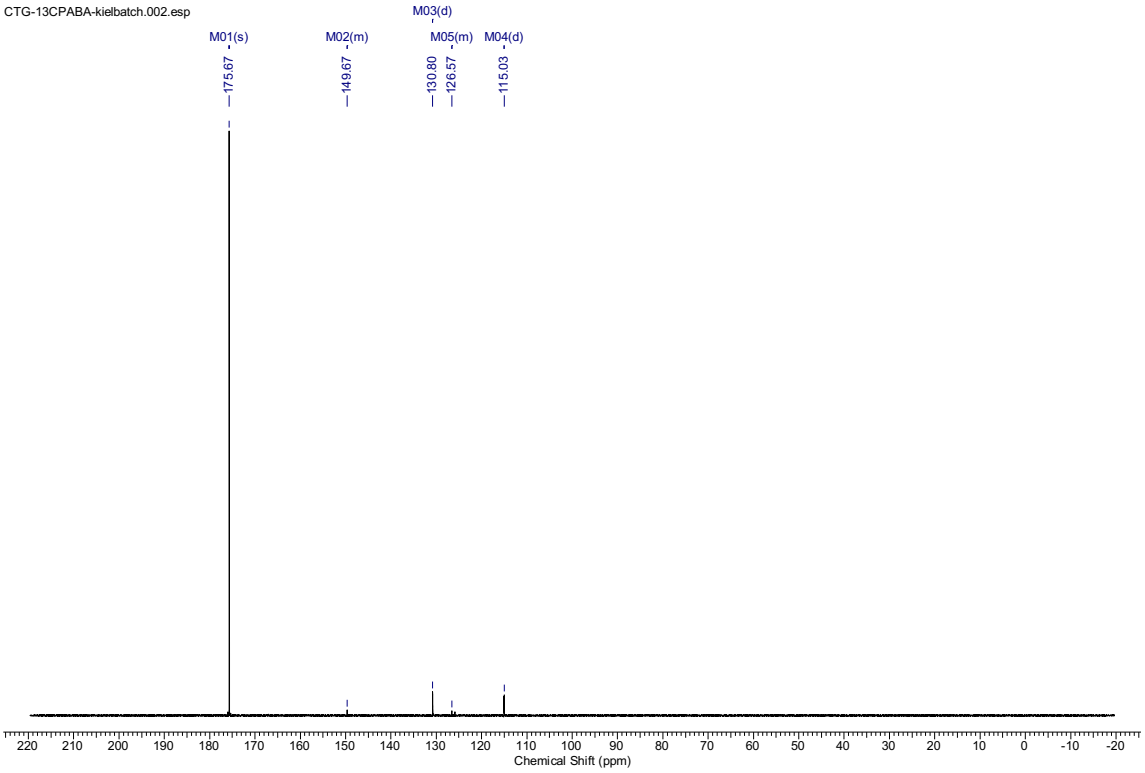
[^{13}C]PABA characterization:



^{1}H NMR (400 MHz, D_2O) δ ppm 6.82 (d, *J*=8.28 Hz, 2H_b) 7.73 (dd, *J*=8.28, 3.17 Hz, 2H_a)
^{13}C NMR (101 MHz, D_2O) δ ppm 115.03, 126.57, 130.80, 149.67, 175.67 (enriched)
HRMS (ESI): [M+Na]$^+$ calcd. for $C_6{}^{13}CH_6NNa_2O_2$: 183.0222, found: 183.0247.



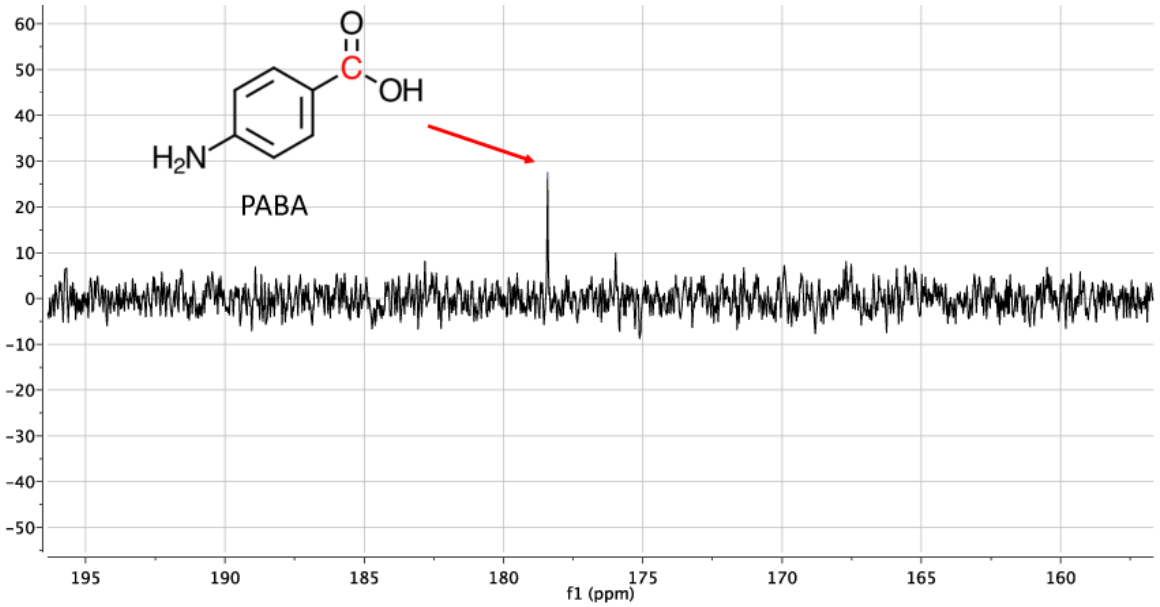
^{1}H NMR spectrum of [^{13}C]PABA



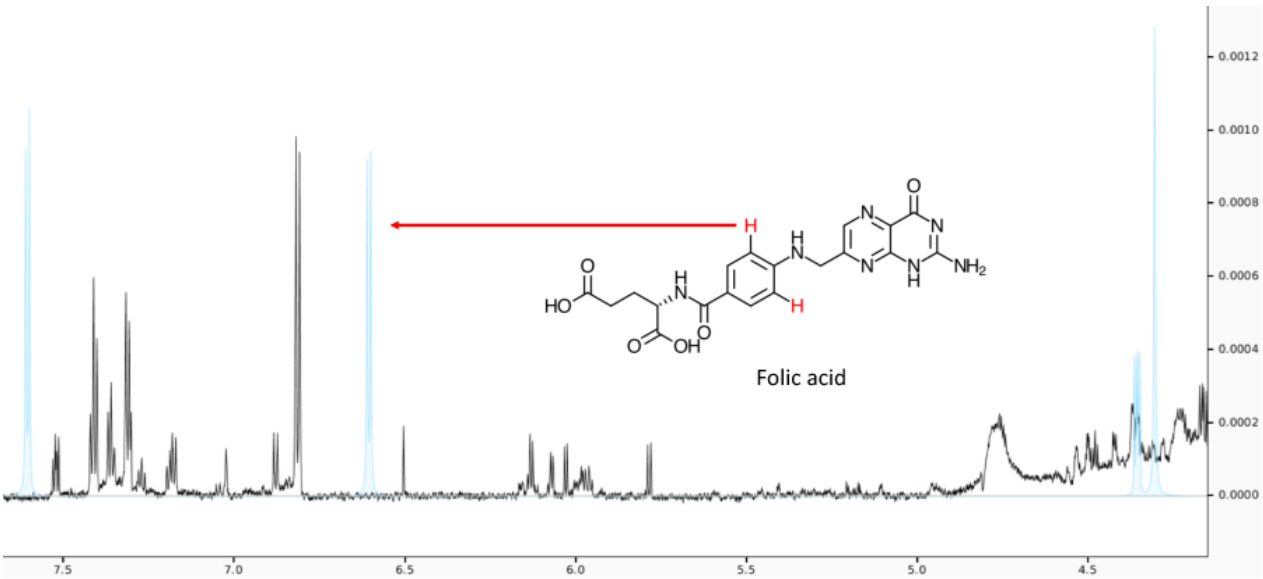
^{13}C NMR spectrum of [^{13}C]PABA

Supplementary Figure 7. Characterization of [^{13}C]PABA via (a) high resolution mass spectrometry (b) ^{1}H NMR (c) ^{13}C NMR.

B. *In vitro* evaluation of [^{13}C]PABA in *E. coli*:

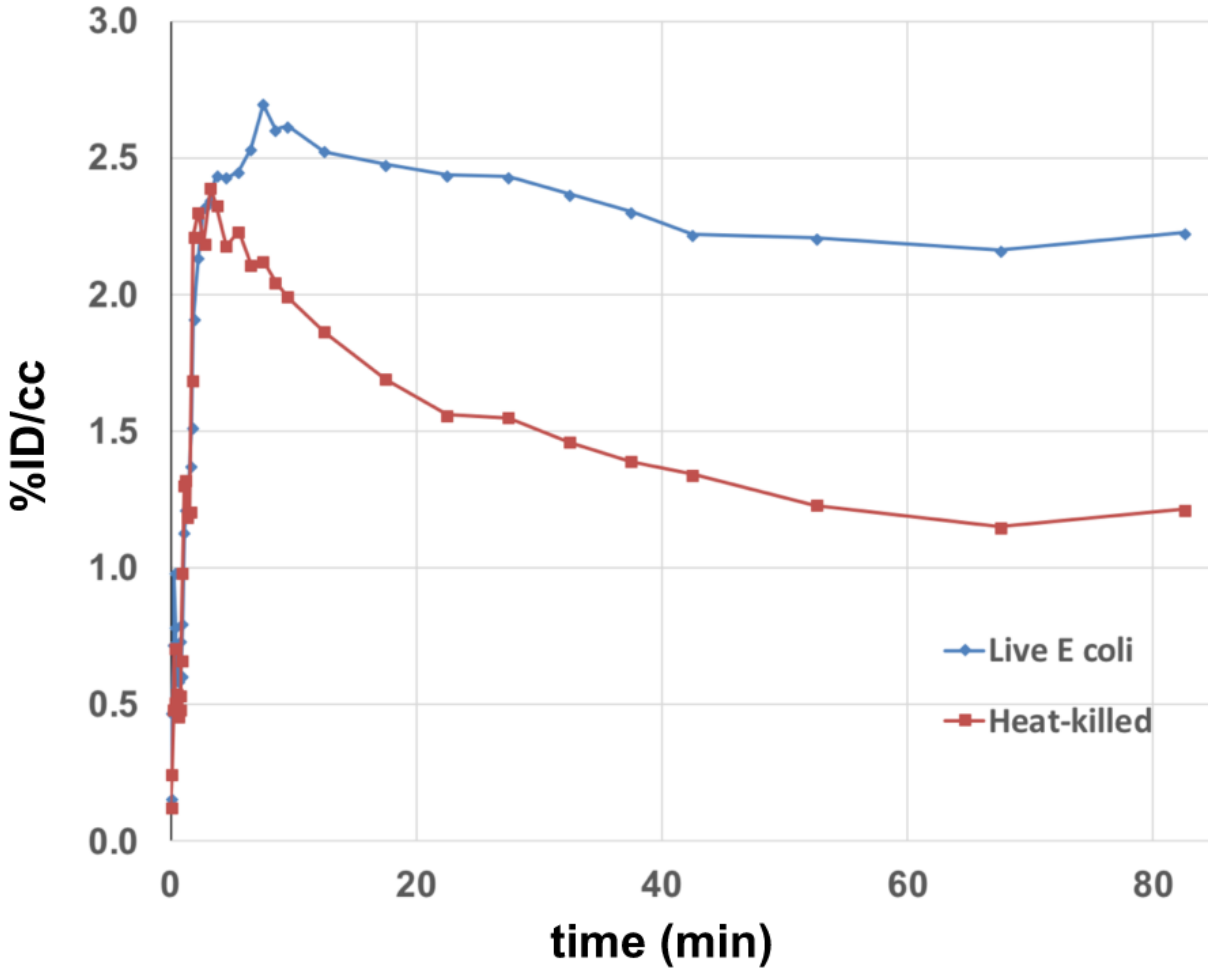


Supplementary Figure 8. ^{13}C NMR characterization of intracellular *E. coli* contents following incubation with [^{13}C]PABA. The sole ^{13}C-enriched resonance observed is [^{13}C]PABA itself.

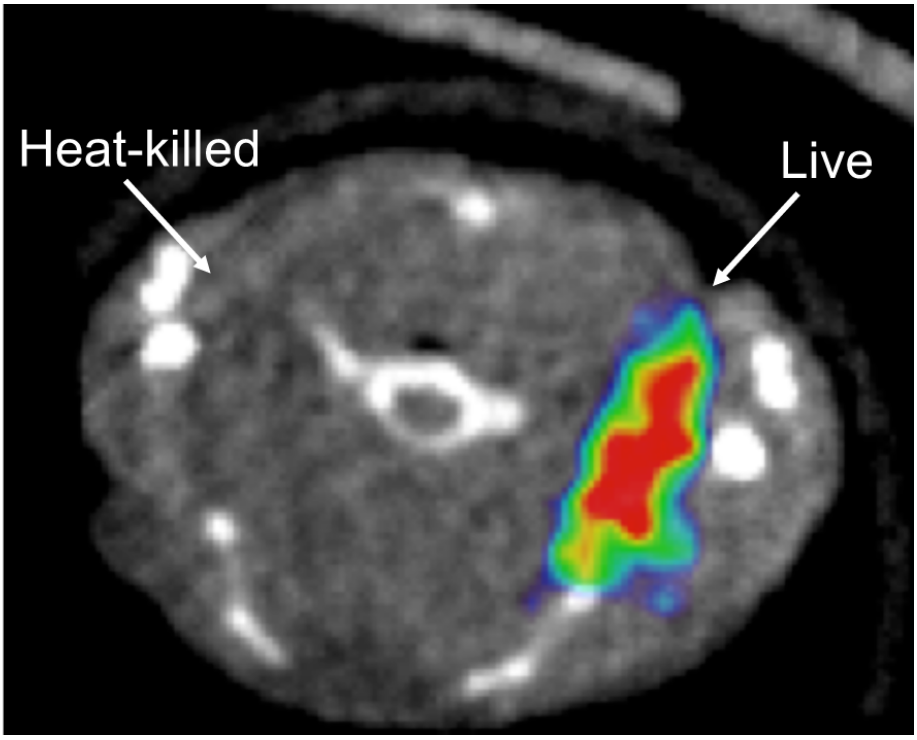


Supplementary Figure 9. ^{1}H NMR characterization of intracellular *E. coli* contents following incubation with [^{13}C]PABA. A referenced spectrum of folic acid is shown in light blue highlighting expected resonances if folic acid (or structurally similar H_2PteGlu) are present. These resonances are NOT observed in *E. coli* extracts following administration of [^{13}C]PABA in cell culture. A key expected resonance is at 6.7 ppm and the corresponding protons of folic acid are highlighted.

C. *In vivo* evaluation of [^{13}C]PABA:



Supplementary Figure 10. Dynamic [^{11}C]PABA PET data performed via spherical ROI analysis as for static data. Several similar studies were conducted to determine the static imaging time point employed.

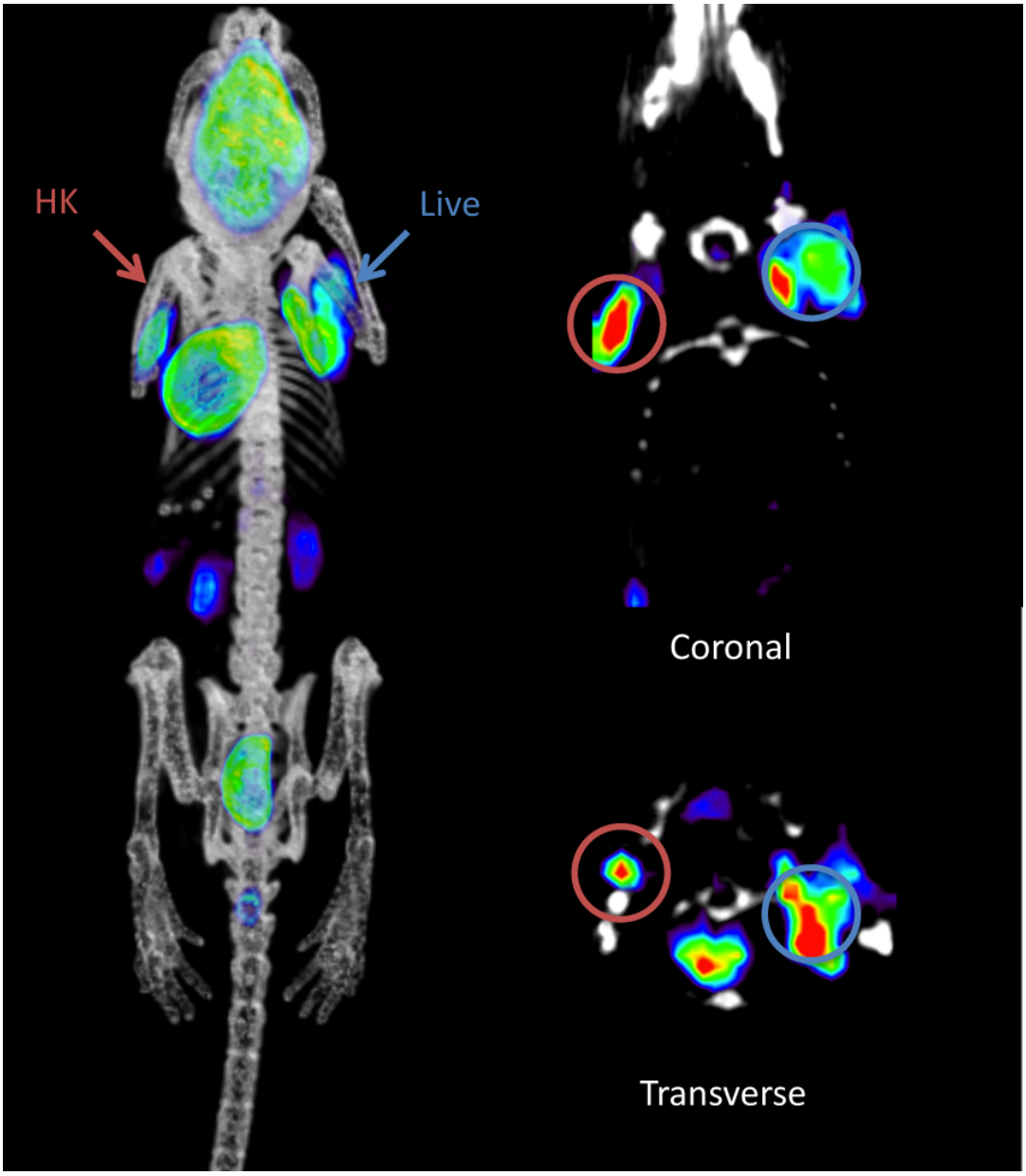


Supplementary Figure 11. Axial[^{11}C]PABA PET image of an *E. coli* infected mouse.

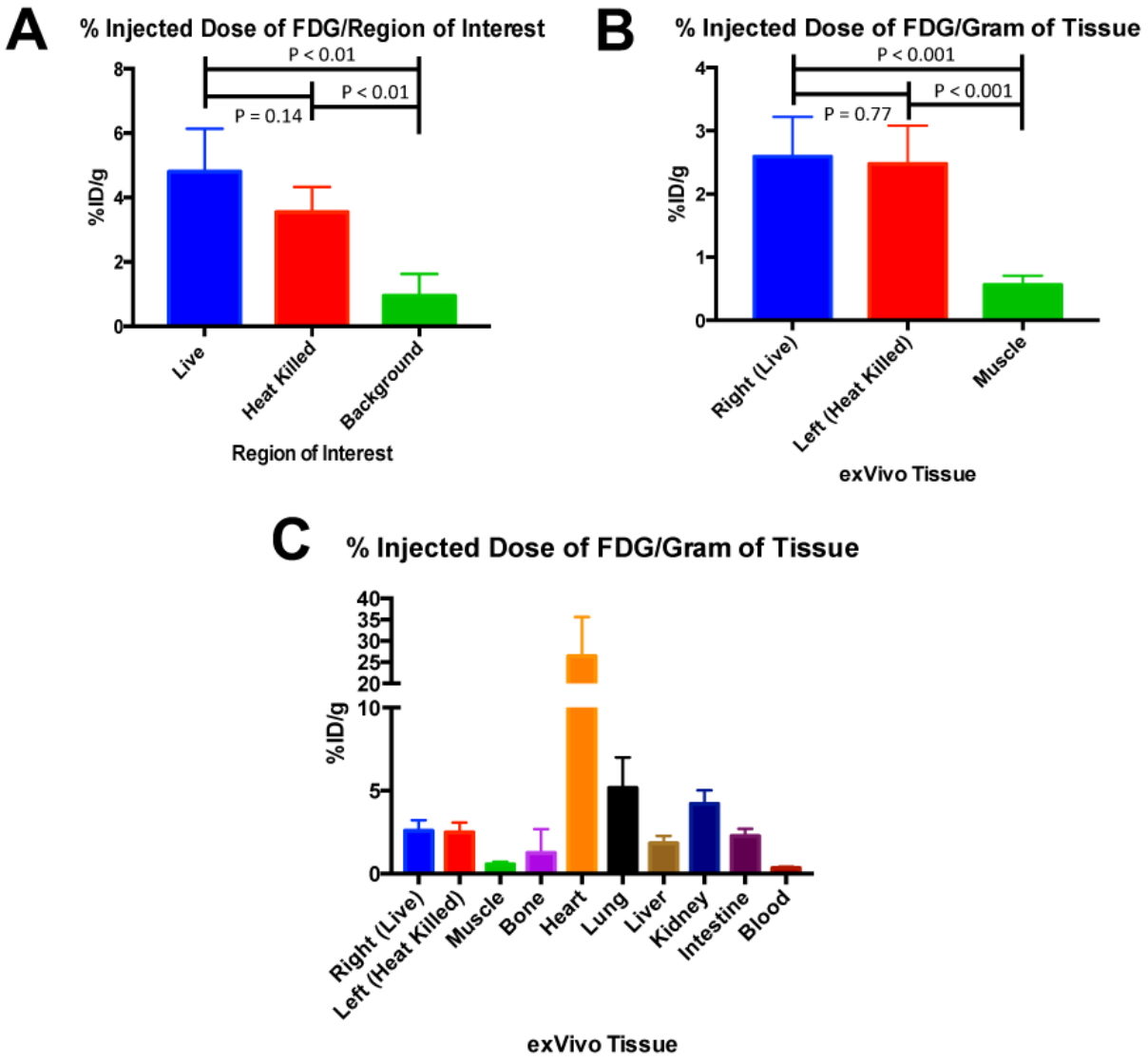
[^{18}F]FDG study of *E. coli* infected mice:

50 mL cultures of *E. coli* in LB Broth were incubated until OD_{600} = 1 at which point a 10 mL portion was centrifuged to pellet the bacteria and suspended in 1mL of LB broth. This portion was transferred to a sealed vial and heated to 130°C for 30 min. CBA/J mice, female, 6-8 weeks old (2 cages, n = 10) were injected with 50 uL of Live E. Coli into the right shoulder and 50 uL of heat killed E. Coli into the left shoulder. At this injection time, food was removed and the animals were monitored for 11 hours. 100 uCi of F^{18}-FDG was inject via tail vein. Injection times for each cage was spaced 25 mins. 1 hour post injection, 4 mice from each cage was imaged in a hotel for a 15 min static PET scan followed by a 10 min CT. At the time of imaging, the 5th mouse from the

cage was sacrificed and the right and left shoulders were taken for CFU determination. After completion of the imaging, the animals were sacrificed and the tissues were collected for direct gamma counting (biodistribution, n = 8) and for histology (n = 2). The imaging data is representative of the n = 8 that was imaged. The biodistribution data represents cohorts on n = 6 as well as the ROI analysis.



Supplementary Figure 12. Images from [^{18}F]FDG PET image of an *E. coli* infected mouse (static study). Similar probe accumulation is seen in muscle inoculate with live versus heat-killed bacteria.



Supplementary Figure 12. Region of interest (ROI) and *ex vivo* (biodistribution) analysis of [^{18}F]FDG studies on infected mice. (A) ROI analysis of live versus heat-killed inoculations. There was no statistically significant difference between tracer accumulation in live versus heat-killed tissues, but both were elevated with respect to background (normal muscle) (B) confirmation of these findings via harvested tissue analysis (gamma counting). (C) Biodistribution analysis in

other organs. These data are highly consistent with those of previously published work in this model using identical *E. coli* (Weinstein et al., 2014).