Supplemental Material

Expanded Methods and Results

PCR and Western Blotting

TaqMan primers (Life Technologies) specific for mouse ADRB3 (Mm02601819_g1), FATP1 (Mm00449511_m1), SLC2A4 (Mm00436615_m1), PDK4 (Mm01166879_m1), CPT1B (Mm00487191_g1), ACADM (Mm01323360_g1), SLC2A1 (Mm00441480_m1), NPR1 (Mm00435309_m1), NPR3 (Mm00435329_m1), RPS18 (Mm02601777_g1) and TBP (Mm01277042_m1) were used. Data were normalized to reference genes RPS18 and TBP using the equation $2^{-(\text{CT target gene - CT reference genes})}$ and relative to control group.

UCP1 protein levels in BAT were measured. Equal amounts of total protein were separated by SDS-PAGE on 10% gels and transferred to PVDF membrane. UCP1 protein was detected using UCP1 primary antibody (Abcam) and normalized to αTubulin (Calbiochem). Protein-primary antibody complex was detected by using infrared-dye conjugated goat or donkey polyclonal antibody IRDye 680 or IRDye 800 (LICOR Biosciences) then scanned and quantified with LI-COR Odyssey Infrared Imaging System.

In Vitro Validation of Optical Signals

Calibration of the optical signals from ^{18}F-FDG and BODIPY-labeled free fatty acid (FFA) was performed by imaging a range of known concentrations of each probe *in vitro*. Optical detection of the ^{18}F-FDG signal was performed by CLI and detection of FFA by fluorescence reflectance imaging (FRI). Increasing activity of ^{18}F-FDG was prepared in separate wells in a black clear bottom well plate. Each well had a final fluid volume of 100 μL made up with PBS. Similarly, increasing concentrations of the FFA analog BODIPY® FL C_{16} (4,4-Difluoro-5,7-Dimethyl-4-Bora-3a,4a-Diaza-s-Indacene-3-Hexadecanoic Acid) (Thermo Fisher Scientific) (Bodipy C_{16} FFA) were prepared in PBS and 7% DMSO in separate wells in a black clear bottom well plate. Imaging was performed on the IVIS Spectrum imaging system (Perkin Elmer, Boston, MA). For

CLI of ^{18}F-FDG, no excitation was required and the emission filter was open to all wavelengths. For Bodipy C_{16} FFA, the excitation wavelength was 500 nm and the emission wavelength was 540 nm.

Ex Vivo Validation of Cerenkov Luminescence

Further validation of the Cerenkov luminescence signal was performed in mice (C57BL6, Jackson Laboratories, Bar Harbor, Maine) with myocardial infarction, providing very distinct areas of normal/abnormal myocardial metabolism. Myocardial infarction (MI) was induced in female C57BL6 mice (n=3) by coronary ligation, as previously described[1]. Briefly, permanent occlusion of the left coronary artery was performed with an 8-0 suture, and myocardial ischemia confirmed by tissue blanching and ECG monitoring. After occlusion, the chest was closed and animals were allowed to recover. Two days post myocardial infarction, the mice were injected via the intraperitoneal (i.p.) route with 0.05 mCi/g of ^{18}F-FDG. The ^{18}F-FDG was allowed to circulate for 1 hour while the animals were active, after which time they were euthanized. The heart was quickly excised, sliced into 2 mm short-axis sections and washed with a cold PBS solution. The sections were then immediately imaged for CLI using the IVIS Spectrum system. The following settings were used: Field of view (FOV) 13 x13 cm^2, excitation blocked, emission filters all open, exposure time 240 s, F-stop 1, binning 4. Following CLI, the sections were soaked at 37°C in 1% triphenyltetrazolium chloride (TTC) for 10 minutes, fixed in 1% paraformaldehyde and imaged for TTC staining 24 hours later using a conventional light stereomicroscope[2]. For each slice (n = 10), a manual region of interest was drawn outlining ^{18}F-FDG positive and ^{18}F-FDG negative areas. Likewise, in each slice, a manual region of interested was drawn outlining TTC positive and TTC negative areas. The percent viable area by ^{18}F-FDG was correlated with the percent viable area by TTC for each slice.

Tissue Specific Parameters for Multiplexed Optical Imaging of Substrate Uptake

The specific parameters used for imaging were optimized for the probe and tissues being imaged. For Cerenkov detection of ^{18}F-FDG, no excitation was required and the emission filter was open to all

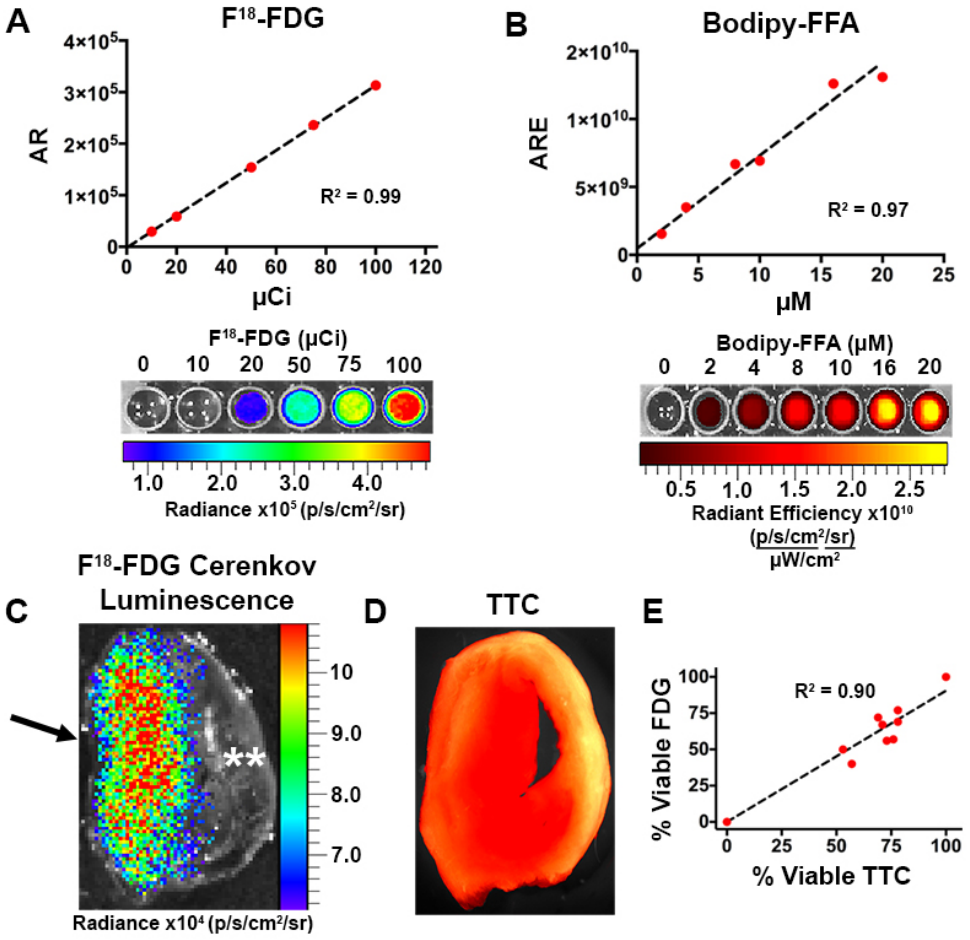
wavelengths. The exposure time ranged from 60 s (BAT) and 240 s (myocardium and skeletal muscle) to 360 s (WAT), while the binning setting ranged from 4 (BAT and myocardium) to 8 (skeletal muscle and WAT). A FOV of 13 x 13 cm^2 and F-Stop of 1 were used for all tissues. For Bodipy C_{16} FFA, the excitation wavelength was set to 500 nm and emission detection was set to 540 nm. The exposure times ranged from 1 s (WAT) and 5 s (BAT, skeletal muscle) to 15 s (myocardium). FOV was 13 x 13 cm^2, binning was 4 and F-stop was 2 for all tissues.

References

1. Huang S, Chen HH, Yuan H, Dai G, Schuhle DT, Mekkaoui C, Ngoy S, Liao R, Caravan P, Josephson L, Sosnovik DE. Molecular MRI of Acute Necrosis With a Novel DNA-Binding Gadolinium Chelate: Kinetics of Cell Death and Clearance in Infarcted Myocardium. *Circulation: Cardiovascular Imaging*. 2011;4:729–737.

2. Chen HH, Feng Y, Zhang M, Chao W, Josephson L, Shaw SY, Sosnovik DE. Protective effect of the apoptosis-sensing nanoparticle AnxCLIO-Cy5.5. *Nanomedicine*. 2012;8:291–298.

Supplemental Figure 1



Supplemental Figure 1: Calibration and Validation of Cerenkov Luminescence. (A) Calibration curve with corresponding well plate images showing an excellent correlation between the emission of Cerenkov luminescence and radioactivity from ^{18}F-FDG. (B) Calibration curve with corresponding well plate images showing an excellent correlation between the intensity of the emitted fluorescence and concentration of Bodipy-FFA. (C) Mid-ventricular slice from a mouse heart 2 days after ligation of the left coronary artery and 1 hour after i.p. injection of ^{18}F-FDG showing glucose uptake via Cerenkov luminescence in the remote zone (arrow) but not in the infarct zone (**), confirmed by TTC staining (D). A strong correlation was seen between viable tissue area by Cerenkov imaging and TTC (E). AR = Average radiance; ARE = Average radiant efficiency.

Supplemental Table 1. Tissue Substrate Uptake 15 Weeks Post Banding. AR = Average Radiance; ARE = Average Radiant Efficiency. (*p < 0.05, **p < 0.01, *** p < 0.001).

		Control	Banded
Heart	Glucose (AR/g)	$1.7 \times 10^5 \pm 0.9 \times 10^5$	$3.0 \times 10^5 \pm 0.8 \times 10^5$*
	FFA (ARE/g)	$2.1 \times 10^8 \pm 0.9 \times 10^8$	$1.8 \times 10^8 \pm 0.7 \times 10^8$
BAT	Glucose (AR/g)	$2.7 \times 10^5 \pm 0.3 \times 10^5$	$7.2 \times 10^5 \pm 1.7 \times 10^5$**
	FFA (ARE/g)	$1.0 \times 10^9 \pm 0.1 \times 10^9$	$1.9 \times 10^9 \pm 0.2 \times 10^9$***
Skeletal Muscle	Glucose (AR/g)	$0.9 \times 10^4 \pm 0.2 \times 10^4$	$1.2 \times 10^4 \pm 0.2 \times 10^4$*
	FFA (ARE/g)	$1.8 \times 10^9 \pm 1.5 \times 10^9$	$3.7 \times 10^9 \pm 2.0 \times 10^9$*
WAT	Glucose (AR/g)	$3.7 \times 10^4 \pm 2.2 \times 10^4$	$6.2 \times 10^4 \pm 3.3 \times 10^4$
	FFA (ARE/g)	$7.3 \times 10^{10} \pm 5.1 \times 10^{10}$	$5.8 \times 10^{10} \pm 2.5 \times 10^{10}$