SUPPLEMENTAL INFORMATION for

Multimodal Imaging Approach to Monitor Browning of Adipose Tissue *In Vivo*

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SUPPLEMENTAL MATERIALS AND METHODS

MSOT Imaging

Briefly, a tunable optical parametric oscillator (OPO) pumped by an Nd:YAG laser provides excitation pulses with a duration of 9 ns at wavelengths from 660 nm to 1300 nm at a repetition rate of 10 Hz with a wavelength tuning speed of 10 ms and a peak pulse energy of 100 mJ at 730 nm. Ten arms of a fiber bundle provide even illumination of a ring-shaped light strip of approx. 8 mm width. For ultrasound detection, 512 toroidally-focused ultrasound transducers with a center frequency of 5 MHz (60% bandwidth), organized in a concave array of 270 degree angular coverage and a radius of curvature of 4 cm, were used. In addition to optoacoustic imaging, the system is also capable of interleaved reflection-mode ultrasound computed tomography (R-UCT) image acquisition (1). For pulse-echo image generation in the R-UCT acquisition mode, an ultrasound imaging platform was used that consolidates a 128-channel beam former and a function of triggered acquisition for synchronizing ultrasound and optoacoustic image streams. The pulser was programmed to generate bipolar 1-cycle pulse trains with a peak-to-peak voltage of 20V and frequency of 6 MHz, and the reflected signals were digitized at 20MS/s sampling rate.

The optoacoustic signals of the cell-expressed iRFP720 were measured in cylindrical polyurethane phantoms (diameter = 2 cm), customized to mimic the characteristics of a mouse model. There are 2 channels of 3 mm width within the phantom, one to contain the cell suspension and the other, control aqueous solvent (180 μL). MSOT in vivo images were

acquired at multiple wavelengths – 680, 685, 690, 695, 700, 705, 710, 720, 730, 760, 800, 850, 900, 930, 950, 1000, 1050, 1100, 1200, 1210, 1220 and 1250 nm. Multiple transverse slices across the phantom was defined using 10 average frames per wavelength. The animals were anesthetized by 2-3% isoflurane mixed with 100% medical air. The animals were shaved; with residual hair removed with a depilatory cream and clear ultrasound gel was applied on the skin for better acoustic coupling, prior to MSOT imaging. The animals were placed horizontally in a holder covered with a thin polyethylene membrane and immersed in deuterium oxide (Cambridge Isotope Laboratories, Inc, USA) maintained at 34°C, for acoustic coupling.

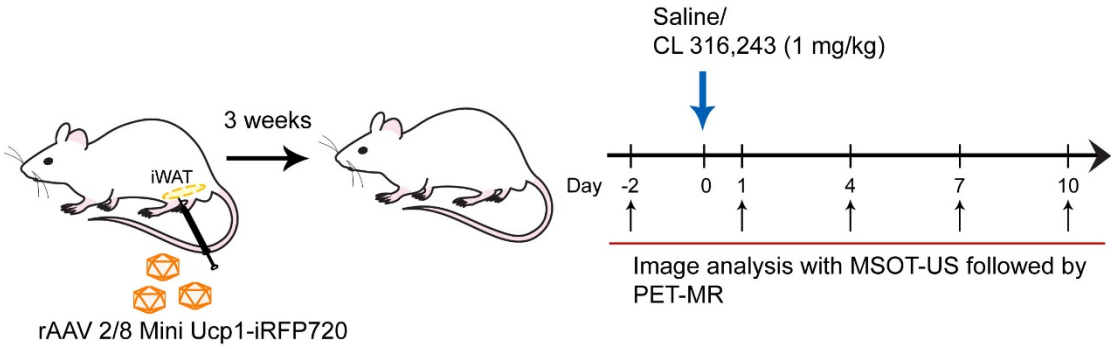
SUPPLEMENTAL REFERENCES

1. Mercep, E., G. Jeng, S. Morscher, P. C. Li, and D. Razansky. 2015. Hybrid optoacoustic tomography and pulse-echo ultrasonography using concave arrays. *IEEE Trans Ultrason Ferroelectr Freq Control* **62**: 1651-1661.

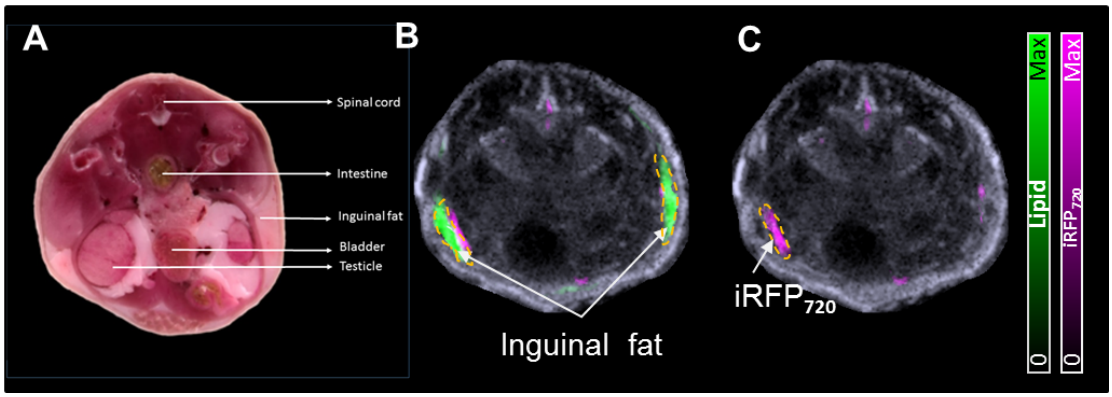
2. Sarantopoulos, A., G. Themelis, and V. Ntziachristos. 2011. Imaging the bio-distribution of fluorescent probes using multispectral epi-illumination cryoslicing imaging. *Mol Imaging Biol* **13**: 874-885.

Supplemental Table S1. Real time primer sequences used

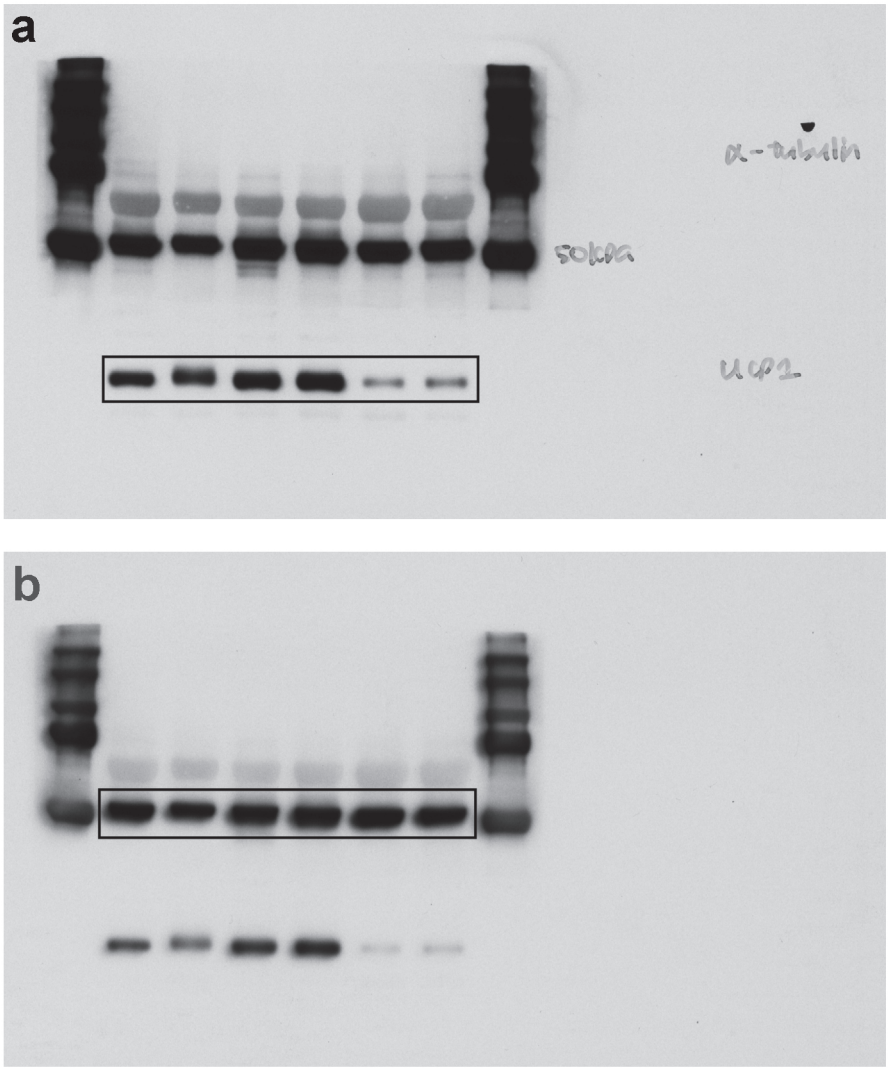
Gene Id	Forward	Reverse
Fabp4	5'-GAT GCC TTT GTG GGA ACCT	5'-CTG TCG TCT GCG GTG ATT T
Adipoq	5'-CAC CGC AGA CGA CAG GAA G	5'-GCA CCT GCA CCA GGG C
Ucp1	5'-GGC ATT CAG AGG CAA ATC AGC T	5'-CAA TGA ACA CTG CCA CAC CTC
Cebp/b	5'-ACG ACT TCC TCT CCG ACC TCT	5'-CGA GGC TCA CGT AAC CGT AGT
Dio2	5'-GAT GCT CCC AAT TCC AGT GT	5'-TGA ACC AAA GTT GAC CAC CA
18s rRNA	5'-GTA ACC CGT TGA ACC CCA TT	5'-CCA TCC AAT CGG TAG TAG C



Supplemental Figure S1. Schematic illustration of imaging approach for detection of beige adipose tissue. rAAV encoding the iRFP720 reporter under control of the Ucp1 promoter was administered into the iWAT of mice. 3 weeks after vector administration, mice were then treated daily with CL-316,243 (1 mg/kg) or vehicle (saline). MSOT-US and PET/MR imaging was performed on individual animals in both groups at Days 1, 4, 7 and 10 during adrenergic stimulation.



Supplemental Figure S2. MSOT data analysis to quantify iRFP and lipid signals. (A) Representative cryosection slice of a male mouse was used as anatomical reference. ROIs (demarcated by dotted line) inclusive of lipid (B) and $iRFP_{720}$ (C) signals were drawn separately using Image J. Fig S2A was obtained from CryoMouse™, iThera Medical, with permission of the owner. The method of animal preparation and imaging is as previously described (2).



Supplemental Figure S3. Original Western blots shown in Fig. 3f. (a) The area highlighted in black line of the original blot with 30 seconds exposure was used to show UCP1. (b) The area highlighted in black line of the original blot with exposure of 5 seconds was used to show α tubulin.