**Rebuttal Letter**

Please rephrase the Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

*Here we report an optimized approach by which we have been able to successfully label and quantitatively compare the densities of blood vessels as well as nerve fibers in different adipose tissues.*

Expand?

*expand*

Please remove this phrase “On the other hand”

*It’s already removed.*

Need a one liner about what angiogenesis is and how it is a key step.

*Angiogenesis is a process of new blood vessel formation. Since blood vessels provide oxygen, nutrients, hormones as well as growth factors to the tissues, angiogenesis has been considered to be a key step during adipose tissue remodeling.*

Please use imperative tense and complete sentence throughout. Use 6week old C57BL/6 male mice.

*Use 6 week old C57BL/6J male mice*

Amount and concentration.

*the mice with isoflurane (4 - 5% for induction then 1 – 2% for maintenance).*

What do you look for? Please include – and look for the lack of pedal reflexes.

*perform a toe-pinch reflex to determine the anesthetic depth judging by the lack of pedal reflexes.*

Do you sterilize the surgical area before starting the surgery? Do you shave the hair? Do you use vet ointment for the eyes? Please provide every specific detail of performing your experiment. How big is the cut?

*Sterilize the blunt scissors and the surgical area of the chest (no need to shave the hair). Open the chest by cutting diaphragm and ribs along the lateral surface with the size of ~ 2 cm using the blunt scissors. Clamp the chest flag with hemostat and reflect it by putting the hemostat over the head.*

How small?

*Make a small incision into the left ventricle (~0.5 cm) with iris scissors.*

Open how? Please provide details.

*Open the right atrium by section-cutting with iris scissors as the perfusion outlet.*

Transfer how?

*Transfer the small chunks by the sterilized tweezers*

How is done,? Do you use OCT? What is meant by proper labeling?

*into tissue embedding cassettes with proper labeling of the tissue samples on the cassettes.*

We cannot have paragraphs of text in the protocol section. Please consider moving some parts to the discussion.

*The “Note a” following Step 2.5 is now moved to representative results.*

*“The distal region of the epididymal white adipose tissue (eWAT), the medial region of dorsolumbar - subcutaneous white adipose tissue (sWAT) as well as the medial region of interscapular brown adipose tissue (BAT) were collected. The locations for collecting these tissues are indicated in Figure 1.”*

*The “Note b” following step 2.5 is now removed.*

What volume?

*1X PBS buffer three times (0.5 mL/wash).*

We cannot have paragraphs of text in the protocol section. Please consider making this an action step and/or a one liner note.

*Note: The tissues can be stored in 1 X PBS at 4 °C for further process.*

Cut how?

*Cut the fixed tissue samples into approximately 2 mm3 cubes with the sterilized scissors.*

Remove how by aspiration? Wash how? Do you add PBS directly to the same tube? Do you use different tube?

*Carefully remove the 1x PBS-TX by aspiration. Wash the samples three times by adding the 1XPBS directly into the same tubes (no need to change the tubes). During each wash, invert the tubes several times.*

maker or marker? Needs citation

*(the marker of blood vessels)5*

Maker or marker please check. Needs citation here.

*the marker of nerve fibers5*

Is this correct? Please check. Not sure if 50 microlitres will cover the whole tissue?

*Add 100 μL of primary antibody solution prepared in step 3.4 into tubes*

Volume?

*Wash the samples with 1 X PBST (pH 7.4, 100 µL/wash)*

Please do not use commercial term in the manuscript and use generic term instead.

*fluorophore (wave length 495 nm) conjugated anti-goat IgG (1:200) and 1 μL of fluorophore (wave length 650 nm) conjugated anti-rabbit IgG (1:200) (see the Table of Materials)*

Please consider making this an action step and rewrite in imperative tense. “Pause point: The mounted slides can be stored at 4 °C in darkness for about 2 weeks.”

*Now it is removed.*

How do you acquire the image?

*Acquire Z-stack images (refer to 4.2 to 4.7).*

Please do not use commercial terms in the manuscript. Please use generic term instead.

*confocal microscope/software.*

How is this done?

*Among the WATs, the sWAT exhibited more blood vessel density than the eWAT (****Figure. 5, compare the middle to the top lane****).*

???

*Changed to “collecting the samples”.*

Please remove the commercial term from the table in Figure 4.

*Done!*