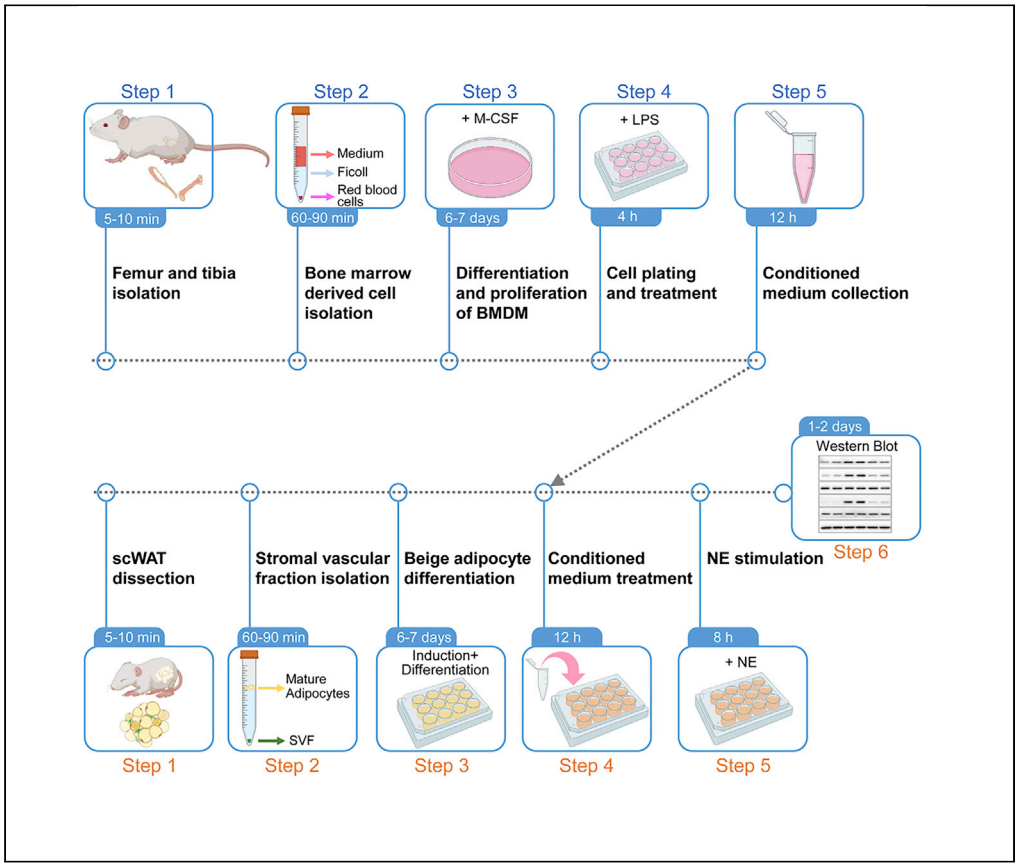
STAR Protocols

CellPress
OPEN ACCESS

Protocol

In vitro analyses of paracrine effects of murine classically activated macrophage on beige adipocyte metabolism



The communication between macrophage and adipocyte plays a critical role in the initiation and development of metabolic inflammation, which is difficult to study *in vivo*. Here, we provide a step-by-step protocol using differentiated cells to investigate the paracrine effects of classically activated macrophage on beige adipocyte metabolism *in vitro*. This protocol uses bone-marrow-derived macrophage and SVF-derived UCP1^{+} beige adipocyte in a culture model to study immune regulation of adipocyte metabolism by western blot analyses.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Jingfei Yao, Dongmei Wu, Yifu Qiu

yifu.qiu@pku.edu.cn (Y.Q.)
yaojf16@pku.edu.cn (J.Y.)

Highlights

Preparation and culture of murine bone-marrow-derived macrophage

Preparation and culture of murine SVF-derived UCP1^{+} beige adipocyte

An *in vitro* model analyzing paracrine effects of macrophage on adipocyte metabolism

Yao et al., STAR Protocols 3, 101480
September 16, 2022
https://doi.org/10.1016/j.xpro.2022.101480

STAR Protocols

CellPress
OPEN ACCESS

Protocol

In vitro analyses of paracrine effects of murine classically activated macrophage on beige adipocyte metabolism

Jingfei Yao,[1,3,*] Dongmei Wu,[1,2] and Yifu Qiu[1,2,4,*]

[1]Institute of Molecular Medicine, Beijing Key Laboratory of Cardiometabolic Molecular Medicine, College of Future Technology, Peking University, Beijing, China

[2]Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China

[3]Technical contact

[4]Lead contact

*Correspondence: yifu.qiu@pku.edu.cn (Y.Q.), yaojf16@pku.edu.cn (J.Y.)
https://doi.org/10.1016/j.xpro.2022.101480

SUMMARY

The communication between macrophage and adipocyte plays a critical role in the initiation and development of metabolic inflammation, which is difficult to study *in vivo*. Here, we provide a step-by-step protocol using differentiated cells to investigate the paracrine effects of classically activated macrophage on beige adipocyte metabolism *in vitro*. This protocol uses bone-marrow-derived macrophage and SVF-derived UCP1^{+} beige adipocyte in a culture model to study immune regulation of adipocyte metabolism by western blot analyses.

For complete details on the use and execution of this protocol, please refer to Yao et al. (2021).

BEFORE YOU BEGIN

Institutional permissions

Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Peking University and conformed to the Guide for the Care and Use of Laboratory Animals (IMM-QiuYF-1).

Preparation of mice

CD-1(ICR) mice are used for the differentiation of bone marrow derived macrophage (BMDM), at the age of 8–12 weeks. CD-1(ICR) mice are used for stromal vascular fraction (SVF) isolation, at the age of 12–16 days.

Note: C57BL/6 mice can also be used for both BMDM and SVF isolation and differentiation.

Preparation of consumables and surgery tools

Timing: ~60 min (autoclave: 120 min)

1. For organ dissection.
   a. Scissors and forceps (autoclave).
   b. 75% ethanol.
   c. 23-gauge needle.
   d. 50 mL and 15 mL Falcon tubes.
2. For cell isolation and differentiation.
   a. 15 cm bacteriological petri dishes (Falcon) are needed for the differentiation of BMDM.
   b. Ficoll-Paque PLUS Media (GE Healthcare).

c. 12-well flat-bottom plates are needed for differentiated beige adipocytes and BMDM.
d. Subcutaneous white adipose tissue (scWAT) digestion buffer with 600 IU/mL collagenase type I (sterilized by 0.22 μm filter).
e. scWAT digestion buffer (sterilized by 0.22 μm filter).
f. PBS.
g. Beige adipocyte maintenance medium.
h. BMDM growth medium.

PBS, beige adipocyte maintenance medium, BMDM growth medium and scWAT digestion buffer are stored at 4°C and pre-warmed before usage. scWAT digestion buffer with 600 IU/mL collagenase type I is prepared freshly prior to usage.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-UCP1 [dilution (1:1000)]	Sigma-Aldrich	Cat. #U6382; RRID: AB_261838
Mouse anti-HSP90α/β [dilution (1:10000)]	Santa Cruz Biotech.	Cat. #sc-7947; RRID:AB_2121235
Rabbit anti-phospho-HSL (Ser660) [dilution (1:1000)]	Cell Signaling Technology	Cat. #4126; RRID:AB_490997
Rabbit anti-HSL [dilution (1:1000)]	Cell Signaling Technology	Cat. #4107; RRID:AB_2296900
Mouse anti-phospho-Perilipin1 (Ser522) [dilution (1:1000)]	Vala Sciences	Cat. #4856
Mouse anti- Perilipin1 [dilution (1:10000)]	Vala Sciences	Cat. #4854
Goat anti-mouse IgG:HRP [dilution (1:10000)]	Thermo Scientific	Cat. #32430
Goat anti-rabbit IgG:HRP [dilution (1:10000)]	Thermo Scientific	Cat. #31460
Chemicals, peptides, and recombinant proteins		
Norepinephrine	Sigma-Aldrich	Cat. #N5785
D-[+]-glucose	Sigma-Aldrich	Cat. #G7021
Lipopolysaccharides	Sigma-Aldrich	Cat. #L2630
DMEM	GE Healthcare	Cat. #SH30003.02
FBS	GE Healthcare	Cat. #SH30396.03
Penicillin/Streptomycin	Beyotime	Cat. #ST488-1/ST488-2
TRIzol Reagent	Invitrogen	Cat. #T9424
Collagenase Type I	Sigma-Aldrich	Cat. #V900891
Recombinant murine M-CSF	PeproTech	Cat. #315-02
BSA (fatty-acid free)	Yuanye Biotech.	Cat. #S25762
3-isobutyl-1-methylxanthine	Sigma-Aldrich	Cat. #I5879
Dexamethasone	Sigma-Aldrich	Cat. #D4902
T3 (3,3′,5-Triiodo-L-thyronine)	Sigma-Aldrich	Cat. #T2877
Indomethacin	Sigma-Aldrich	Cat. #I8280
Rosiglitazone	Sigma-Aldrich	Cat. #R2408
Insulin	Beyotime	Cat. # P3376
Ficoll-Paque™ PLUS Media	GE Healthcare	Cat. #45-001-749
HEPES	Sigma-Aldrich	Cat. #V900477
$NaHCO_3$	Sigma-Aldrich	Cat. #S5761
Tris base	Sigma-Aldrich	Cat. #V900483
Glycine	Sigma-Aldrich	Cat. #V900144
Acrylamide	Sigma-Aldrich	Cat. #V900845
Bisacrylamide	Sigma-Aldrich	Cat. #V900301
SDS	Sigma-Aldrich	Cat. #V900859
TEMED	Aladdin	Cat. #T105497
Critical commercial assays		
5× All-In-One RT MasterMix	ABM	Cat. #G492
2× qPCR mix	ABM	Cat. #MasterMix-R

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Mouse *Il1b*-F: AGCTTCCTTGTGCAAGTGTCT	This paper	N/A
Mouse *Il1b*-R: GCAGCCCTTCATCTTTTGGG	This paper	N/A
Mouse *Il6*-F: TCCTCTCTGCAAGAGACTTCC	This paper	N/A
Mouse *Il6*-R: GTCACCAGCATCAGTCCCAA	This paper	N/A
Mouse *Tnf*-F: GTTCTATGGCCCAGACCCTCAC	This paper	N/A
Mouse *Tnf*-R: GGCACCACTAGTTGGTTGTCTTTG	This paper	N/A
Mouse *36B4*-F: AACGGCAGCATTTATAACCC	This paper	N/A
Mouse *36B4*-R: CGATCTGCAGACACACACTG	This paper	N/A
Experimental models: Organisms/strains		
Mouse: CD1(ICR) (Age Range: 8–12 weeks; Gender: Male)	Charles River	Strain Code: 201
Mouse: CD1(ICR) (Age Range: 12–16 days; Gender: Male)	Charles River	Strain Code: 201

MATERIALS AND EQUIPMENT

PBS		
Reagent	Final concentration	Amount
NaCl	137 mM	8.0 g
KCl	2.7 mM	0.2 g
Na_2HPO_4	10 mM	1.44 g
KH_2PO_4	1.8 mM	0.24 g
HCl	pH=7.4	
Total	**N/A**	**1 L**
Sterilize by autoclaving and store at 4°C for up to 6 months.		

scWAT digestion buffer			
Reagent	Stock concentration	Final concentration	Volume/weight
$CaCl_2$	1 M	1.1 mM	1.1 mL
KCl	1 M	2.7 mM	2.7 mL
NaCl	N/A	118 mM	6.9 g
$MgCl_2$	1 M	0.5 mM	0.5 mL
NaH_2PO_4	1 M	0.4 mM	0.4 mL
BSA (fatty-acid free)	N/A	1%	10 g
HEPES	1 M pH7.4	20 mM	5 mL
Glucose	N/A	5.5 mM	0.99 g
Penicillin-Streptomycin	100 kU/mL, 100 mg/mL	100 U/mL, 100 μg/mL	1 mL
ddH_2O	N/A	N/A	Bring up to 1 L
Total	**N/A**	**N/A**	**1 L**
Sterilize using a 0.22 μm filter and store at 4°C for up to 6 months.			

Beige adipocyte induction medium			
Reagent	Stock concentration	Final concentration	Volume
DMEM	N/A	N/A	8.8 mL
FBS	N/A	10% (v/v)	1 mL
HEPES	1 M pH7.4	20 mM	0.2 mL
Penicillin-Streptomycin	100 kU/mL, 100 mg/mL	100 U/mL, 100 μg/mL	10 μL
Insulin	10 mg/mL	5 μg/mL	5 μL

(*Continued on next page*)

Continued			
Reagent	Stock concentration	Final concentration	Volume
T3	1 μM	1 nM	10 μL
indomethacin	125 mM	125 μM	10 μL
Dexamethasone	2 mg/mL	1 μg/mL	5 μL
IBMX	250 mM	0.5 mM	20 μL
Rosiglitazone	1 mM	0.5 μM	5 μL
Total	**N/A**	**N/A**	**10 mL**

Always prepare freshly, do not store.

Beige adipocyte differentiation medium			
Reagent	Stock concentration	Final concentration	Volume
DMEM	N/A	N/A	8.8 mL
FBS	N/A	10% (v/v)	1 mL
HEPES	1 M pH7.4	20 mM	0.2 mL
Penicillin-Streptomycin	100 kU/mL, 100 mg/mL	100 U/mL, 100 μg/mL	10 μL
Insulin	10 mg/mL	5 μg/mL	5 μL
T3	1 μM	1 nM	10 μL
Rosiglitazone	1 mM	0.5 μM	5 μL
Total	**N/A**	**N/A**	**10 mL**

Always prepare freshly, do not store.

Beige adipocyte maintenance medium			
Reagent	Stock concentration	Final concentration	Volume
DMEM	N/A	N/A	8.8 mL
FBS	N/A	10% (v/v)	1 mL
HEPES	1 M pH7.4	20 mM	0.2 mL
Penicillin-Streptomycin	100 kU/mL, 100 mg/mL	100 U/mL, 100 μg/mL	10 μL
Total	**N/A**	**N/A**	**10 mL**

The medium can be stored at 4°C for a couple of weeks.

BMDM growth medium			
Reagent	Stock concentration	Final concentration	Volume
DMEM	N/A	N/A	9 mL
FBS	N/A	10% (v/v)	1 mL
Penicillin-Streptomycin	100 kU/mL, 100 mg/mL	100 U/mL, 100 μg/mL	10 μL
M-CSF	100 μg/mL	20 ng/mL	2 μL
Total	**N/A**	**N/A**	**10 mL**

Always prepare freshly, do not store.

STEP-BY-STEP METHOD DETAILS

Preparation and culture of bone-marrow-derived macrophage

Timing: 6–7 days

1. Euthanize 8–12 weeks old CD-1 mice according to an approved IACUC protocol.
2. Isolate the femurs and tibias at joint, and remove skin and muscle as clear as possible.
3. Wash 3 times with PBS, then transfer to a new 10 cm dish with PBS at room temperature (RT: 18°C–24°C).
4. Cut off the ends of the bones and flush with PBS using a 23-gauge needle. Collect bone marrow in a 50 mL Falcon tube.
5. Centrifuge at 400 × *g* for 5 min at RT (18°C–24°C) and aspirate supernatant.

6. Resuspend pellet in 4 mL of BMDM growth medium.
7. Overlay 4 mL of bone marrow (per mouse) over 3 mL of Ficoll-Paque PLUS Media in a 15 mL tube.

⚠ CRITICAL: As slowly as possible, to form a separate and distinct layer.

8. Centrifuge for 20 min at 600 × *g* at RT (18°C–24°C), no brake (deceleration rate is 0). Take ~45 min.

⚠ CRITICAL: Do not centrifuge at cold condition because the density of Ficoll will change.

9. Collect all the supernatant (both Ficoll and BMDM medium, but not the pellet) into a 50 mL tube.
10. Add 25 mL pre-warmed PBS and mix well.
11. Centrifuge for 5 min at 200 × *g*.
12. Resuspend the cell pellet in BMDM growth medium and plate into 2 Falcon dishes (15 cm).

⚠ CRITICAL: Do not use cell culture dish, which will make it difficult to digest differentiated BMDM.

13. On day 3, 5 and 6, aspirate medium gently and add fresh BMDM growth medium.

⚠ CRITICAL: It is very important to change the medium at day 5 and 6 for the differentiation of BMDM. (Troubleshooting 1)

14. By day 7, cells should be fully differentiated and are ready to be plated.
15. Wash cells with 5 mL PBS once, and then incubate with 5 mL 5 mM EDTA (in PBS) for 5 min in cell incubator.

Note: Do not use trypsin for digestion, which will activate the macrophage, and the digestion should not be longer than 10 min. (Troubleshooting 3)

16. View cells under microscope to ensure that half of cells are detached.
17. Add another 5 mL pre-warmed BMDM growth medium to stop the digestion by EDTA and re-suspend the cells.
18. Centrifuge for 5 min at 200 × *g*.
19. Resuspend the cell pellet with 5 mL BMDM growth medium.
20. Count and adjust the final cell density to 4–6 × 10^5/mL.
21. Plate 1 mL cell suspension per well into a 12-well cell culture plate.
22. After 8–16 h, the plated mature macrophages are stimulated with PBS or 100 ng/mL lipopolysaccharide (LPS) for 4 h (Figure 1).

Optional: You can also polarize the BMDM to classically activated macrophage or alternatively activated macrophage by 10 ng/mL LPS or 10 ng/ml IL-4 treatment for 24–48 h.

23. Aspirate medium gently, then wash with pre-warmed PBS twice and add fresh BMDM growth medium.
24. After 12 h, the conditioned medium is collected for cell treatment or stocked at −80°C.
25. For extracting RNA of BMDM, add 350 μL TRIzol per well and transfer to 1.5 mL tubes. Then perform qRT–PCR to analyze proinflammatory gene expression in BMDM (Figure 2).

Preparation and culture of SVF-derived beige adipocyte

Timing: 9–10 days

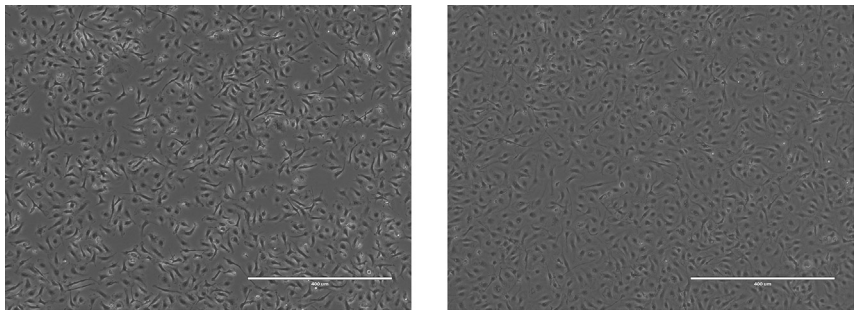


Figure 1. Bright-field images illustrating the differentiated BMDM before (left) and after (right) 100 ng/mL LPS treatment for 4 h

Scale bar represents 400 μm.

26. Add 5 mL pre-chilled scWAT digestion buffer to a 6 cm dish.
27. Euthanize 12–16 days old CD-1 mice according to an approved IACUC protocol.
28. Soak mice with 70% ethanol.
29. Place mouse ventral side up, and make central incision from the base of the tail extending to the sternum.

Note: Make sure not to penetrate the peritoneal cavity.

30. Pull ventral skin away from both flanks gently and slowly.
31. Identify the scWAT depot, then use scissor to trace the edges of the tissue and cut along the way until the entire depot is detached from the skin (Figure 3).
32. Transfer dissected scWAT into pre-chilled scWAT digestion buffer in a 6 cm dish immediately.
33. Wash scWAT twice with pre-chilled scWAT digestion buffer.
34. Transfer the tissues to a 1.5 mL tube with 200 μL scWAT digestion buffer supplemented with 600 IU/mL collagenase type I.
35. Mince the tissues by using sterile surgical scissors. Tissue pieces should be ~1–2 mm thick.
36. Transfer tissues and scWAT digestion buffer into a 50 mL sterile conical tube containing 15 mL scWAT digestion buffer supplemented with 600 IU/mL collagenase type I.
37. Place the tube horizontally and digest tissues at 37°C with shaking at 100 rpm for 40–60 min.

Note: Digestion time depends on disappearance of visible pieces of adipose tissue, but no more than 60 min.

38. Add 15 mL scWAT digestion buffer to stop the digestion.
39. Suspend tissues promptly by 10 mL pipette until tissue pieces are no longer visible.
40. Centrifuge the cells for 5 min at 200 × *g*.

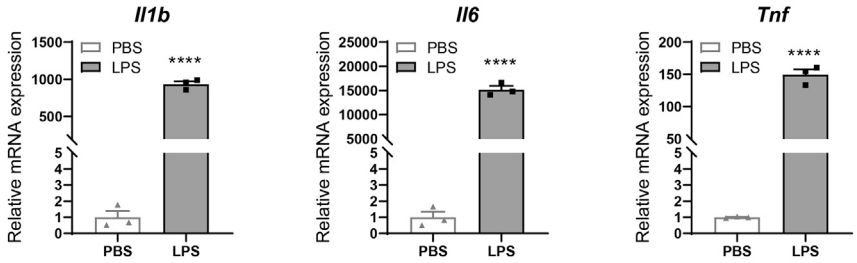


Figure 2. qRT–PCR analysis of proinflammatory gene expression in BMDM treated by 100 ng/mL LPS for 4 h

Data are represented as mean ± SEM. ****$P < 0.0001$ by two-tailed Student's t test.

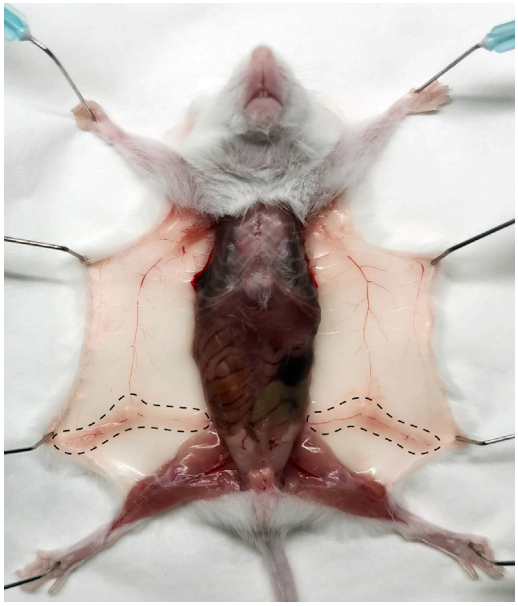


Figure 3. Illustration of scWAT dissection

41. Completely remove mature adipocyte layer and aspirate supernatant gently.

    Optional: The mature adipocytes at the upper layer can be collected for analysis.

42. Resuspend SVF cell pellet in 5 mL scWAT digestion buffer.
43. Filter the cells by 75 μm cell strainer.
44. Rinse the cell strainer with another 5 mL scWAT digestion buffer.
45. Centrifuge the cells for 5 min at 200 × *g*.
46. Aspirate supernatant and resuspend SVF cell pellet with beige adipocyte maintenance medium. (SVF cells from every mouse (two pieces of scWAT) resuspended by 2 mL medium).
47. Plate 1 mL cell suspension per well into a 12-well flat-bottom plate.

    Note: The SVF cells from one mouse can be seeded to 2 wells of 12-well flat-bottom plate.

48. After 12–24 h, remove the beige adipocyte maintenance medium carefully and wash the cells with pre-warmed PBS for twice.
49. Change beige adipocyte maintenance medium every 2 days until the cells reach 90% confluent.
50. When the cells reach 90% confluent (day 0), remove beige adipocyte maintenance medium and add 1 mL beige adipocyte induction medium per well.

    ⚠ CRITICAL: Beige adipocyte induction medium has to be prepared freshly prior to usage.

51. On day 2, remove beige adipocyte induction medium and add 1 mL beige adipocyte differentiation medium per well.

    ⚠ CRITICAL: Beige adipocyte differentiation medium has to be prepared freshly prior to usage.

52. On day 4, remove the medium and add 1 mL fresh beige adipocyte differentiation medium per well.

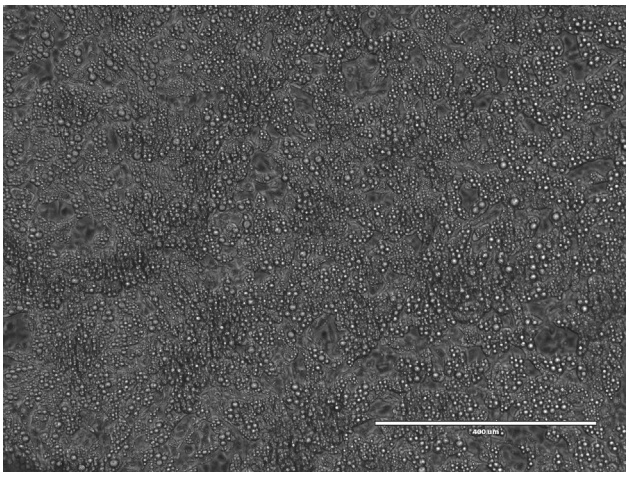


Figure 4. Bright-field images illustrating the differentiated beige adipocyte
Scale bar represents 400 μm.

53. Change beige adipocyte differentiation medium every day until the cells are fully differentiated to beige adipocyte on day 7–8 (Figure 4).

BMDM conditioned medium treatment for western blot analysis

Timing: ~24 h

54. Remove beige adipocyte differentiation medium of beige adipocyte and add 0.5 mL beige adipocyte maintenance medium per well.
55. Add 0.5 mL conditioned medium from PBS- or LPS-treated BMDM per well.
56. Culture at 37°C 5% CO_2 for 12 h.
57. Add vehicle (Veh) or 50 nM norepinephrine to differentiated beige adipocyte directly without changing the medium.
58. Culture at 37°C 5% CO_2 for 8 h.
59. Remove the medium and wash cells twice with pre-chilled PBS.
60. Remove as much of the PBS as possible.
61. Add 300 μL RIPA supplemented with protease inhibitor cocktail per well.
62. Resuspend the cells and transfer to 1.5 mL tubes.
63. Lyse the cells for 10 min on ice and then vortex.
64. Centrifuge for 20 min at 15,000 × g and transfer 200 μL supernatant to 1.5 mL tubes.

Note: Avoid to aspirate or contact the sediment as carefully as possible. (Troubleshooting 5).

65. Add 1/5 volume of 6 × Loading buffer (E.g. add 40 uL 6 × loading buffer into 200 uL supernatant), and boil for 10 min at 95°C.
66. Store at -20°C until western blot analysis.

EXPECTED OUTCOMES

In preparation and culture of bone marrow-derived macrophage, it shows that many non-adherent cells detach from day 2–3, which are erythrocytes, neutrophils and other bone marrow-derived cells. They can be removed when changing the medium at day 3. The adherent cells will be very sparse before day 3 and increase rapidly from day 4–5. For bone marrow cell isolation from one male CD-1 mouse (2 femurs and 2 tibias), it can generate 5–10 × 10^7 macrophages at day 6–7. The

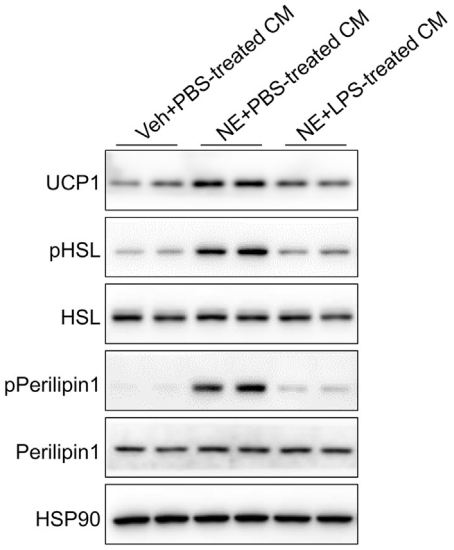


Figure 5. Western blot analyses of thermogenic and lipolytic protein

Western blot analysis of beige adipocyte treated with PBS- or LPS-treated conditioned medium (CM) of BMDM, followed by a stimulation of 50 nM NE for 8 h. anti-UCP1 (1:1,000), anti-HSP90α/β (1:10,000), anti-phospho-perilipin 1 (1:1,000), anti-perilipin 1 (1:10,000), anti-phospho-HSL (1:1,000), anti-HSL (1:1,000), goat anti-mouse IgG:HRP (1:10,000), goat anti-rabbit IgG:HRP (1:10,000).

differentiated BMDM can be classically activated by LPS treatment (Figure 1) and show increased proinflammatory gene expression (Figure 2).

In preparation and culture of SVF-derived beige adipocyte, about 12–24 h after plating, the confluence of preadipocytes from scWAT SVF should be 20%–50%. The cell confluence will increase to 90% after 1–3 days. After changing to beige adipocyte induction medium, cells will drastically alter their morphological appearance and lipid droplets will appear from day 3–4. Then, the differentiating cells will increase the number and size of lipid droplets as the differentiation process goes on. Eventually, the differentiation efficiency will be 70%–90% at day 7 (Figure 4).

Proinflammatory cytokines, including IL-1β and TNF, play an important role in the inhibition of adipocyte lipolysis and thermogenesis (Goto et al., 2016; Mowers et al., 2013). Thus, the lipolysis and thermogenesis of beige adipocyte can be inhibited by the LPS-treated BMDM medium when compared with the PBS-treated (Figure 5).

LIMITATIONS

Firstly, differentiated mature beige adipocytes are viable for only 2–4 days. Thus, the cells will float and fall off once they are cultured for more than 2 days. Secondly, BMDM and beige adipocyte generated using this protocol are useful for analyzing their common functions, but they cannot reflect the heterogeneity of cells *in vivo*. For example, there are different types of macrophage in different tissues, and even many different types in one tissue (Epelman et al., 2014; Felix et al., 2021; Lavin et al., 2015). Thirdly, these experiments cannot completely mimic the interactions between macrophage and beige adipocyte *in vivo* by using this protocol. In adipose tissue, macrophage can interact with beige adipocyte both directly and indirectly (Cohen and Kajimura, 2021). The treatment of beige adipocyte by conditioned BMDM medium can only mimic the effects of indirect interaction, but not the direct one via physical contact.

TROUBLESHOOTING

Problem 1

Differentiated BMDM count is very low. (step 20 in "preparation and culture of bone marrow-derived macrophage")

Potential solution

It can be expected to generate at least 5 × 10^7 macrophages from one mouse (2 femurs and 2 tibias). If the total cell number is less than 1 × 10^7, the following improvements should be considered. Firstly, make sure that the mice are no more than 16 weeks old. The perfect age is from 8 to 12 weeks. Secondly, rinse the bone marrow out as much as possible, until the bone turns white. Thirdly, the BMDMs should be changed to fresh medium strictly every day after day 5.

Problem 2

BMDM is contaminated by bacteria. (steps 1–25 in "preparation and culture of bone marrow-derived macrophage")

Potential solution

Firstly, Penicillin-Streptomycin should be added into the BMDM growth medium to prevent bacterial contamination. Secondly, skin and muscle attached to bone should be removed as completely as possible. Thirdly, bone should be washed as least twice before flushing bone marrow.

Problem 3

BMDM is activated after digestion. (step 15 in "preparation and culture of bone marrow-derived macrophage")

Potential solution

The digestion time is very important, which should be limited strictly between 3-10 min. If it is longer than 10 min, the viability of BMDM will decrease. If it is shorter than 3 min, the BMDM has to be pipetted harder, which may cause cell damage and activation.

Problem 4

Low differentiation efficiency of beige adipocyte. (step 53 in "preparation and culture of SVF-derived beige adipocyte")

Potential solution

Usually, the differentiation efficiency of beige adipocyte can reach over 80%. If it is less than 70%, the following improvements should be considered. Firstly, the beige adipocyte induction medium and differentiation medium should be prepared freshly before usage. Secondly, the cell confluence should be limited strictly between 90%–100% when changing to beige adipocyte induction medium. Thirdly, digestion time for scWAT tissue should not be too long and restrict the time to no more than 60 min. Fourthly, the mice used for scWAT SVF isolation should not be older than 16 days.

Problem 5

It is difficult to get pure protein samples for western blot analysis when extracted from beige adipocytes. (step 64 in "BMDM conditioned medium treatment for Western Blot analysis")

Potential solution

It is mainly due to an incomplete removal of lipids. The cell lysate of beige adipocytes contains a lot of lipids, which cannot be precipitated and thus spread in the supernatant or aggregate in the top as a low-density and insoluble layer. To make the supernatant purer, one should add more RIPA or centrifuge it for several additional times.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yifu Qiu (yifu.qiu@pku.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate any unique datasets or code.

ACKNOWLEDGMENTS

This work was supported by grants from National Key R&D Program of China (2018YFA0800702), National Natural Science Foundation of China (31671227 and 91642113), and the Thousand Young Talents Program of the Chinese government (to Y.Q.).

AUTHOR CONTRIBUTIONS

J.Y., D.W., and Y.Q. conceptualized the study and designed experiments. J.Y. performed the experiments and analyzed the data. J.Y., D.W., and Y.Q. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Cohen, P., and Kajimura, S. (2021). The cellular and functional complexity of thermogenic fat. Nat. Rev. Mol. Cell Biol. *22*, 393–409. https://doi.org/10.1038/s41580-021-00350-0.

Epelman, S., Lavine, K.J., and Randolph, G.J. (2014). Origin and functions of tissue macrophages. Immunity *41*, 21–35. https://doi.org/10.1016/j.immuni.2014.06.013.

Félix, I., Jokela, H., Karhula, J., Kotaja, N., Savontaus, E., Salmi, M., and Rantakari, P. (2021). Single-cell proteomics reveals the defined heterogeneity of resident macrophages in white adipose tissue. Front. Immunol. *12*, 719979. https://doi.org/10.3389/fimmu.2021.719979.

Goto, T., Naknukool, S., Yoshitake, R., Hanafusa, Y., Tokiwa, S., Li, Y., Sakamoto, T., Nitta, T., Kim, M., Takahashi, N., et al. (2016). Proinflammatory cytokine interleukin-1beta suppresses cold-induced thermogenesis in adipocytes. Cytokine *77*, 107–114. https://doi.org/10.1016/j.cyto.2015.11.001.

Lavin, Y., Mortha, A., Rahman, A., and Merad, M. (2015). Regulation of macrophage development and function in peripheral tissues. Nat. Rev. Immunol. *15*, 731–744. https://doi.org/10.1038/nri3920.

Mowers, J., Uhm, M., Reilly, S.M., Simon, J., Leto, D., Chiang, S.H., Chang, L., and Saltiel, A.R. (2013). Inflammation produces catecholamine resistance in obesity via activation of PDE3B by the protein kinases IKKepsilon and TBK1. Elife *2*, e01119. https://doi.org/10.7554/elife.01119.

Yao, J., Wu, D., Zhang, C., Yan, T., Zhao, Y., Shen, H., Xue, K., Huang, X., Wang, Z., and Qiu, Y. (2021). Macrophage IRX3 promotes diet-induced obesity and metabolic inflammation. Nat. Immunol. *22*, 1268–1279. https://doi.org/10.1038/s41590-021-01023-y.