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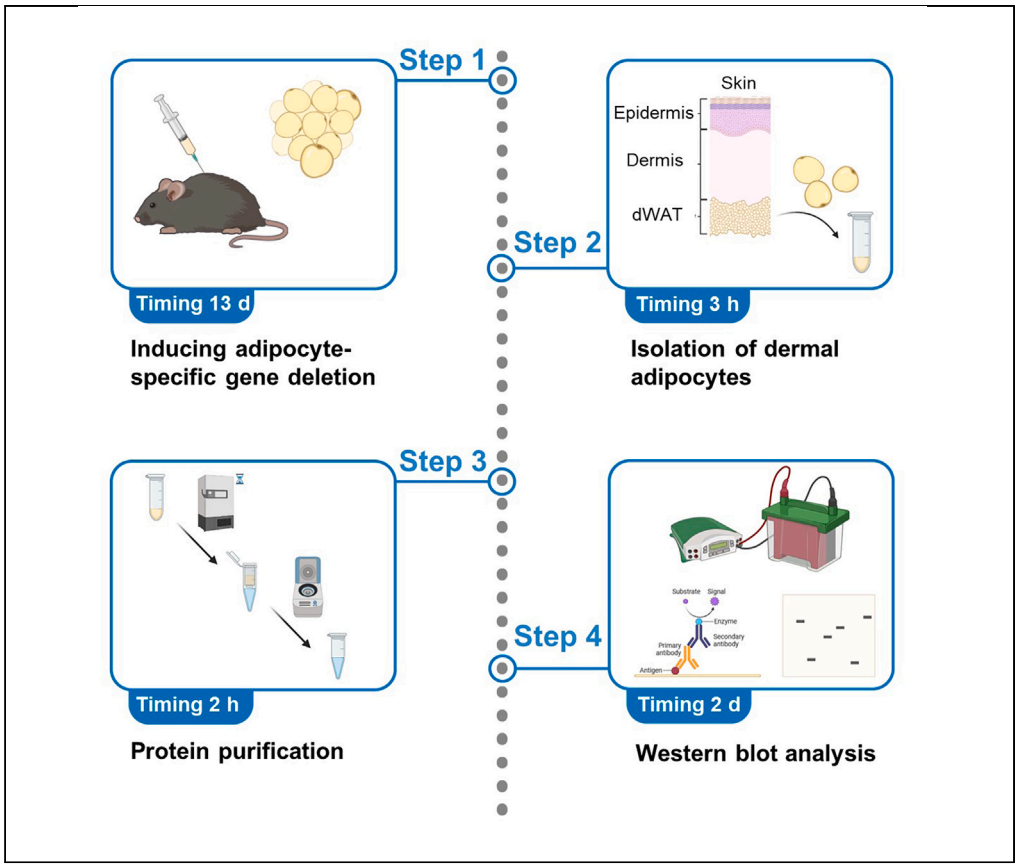
# Isolation of dermal adipocytes from mouse skin for biochemical analysis




The role of dermal white adipose tissue in regulating skin homeostasis and self-renewal processes has recently attracted interest. However, the isolation of proteins from dermal adipocytes for biochemical analysis is challenging. Here, we provide a protocol for the isolation of murine dermal adipocytes. We describe steps for inducing adipocyte-specific gene deletion, adipocyte isolation, protein purification, and western blot analysis. The reliability of the protocol is demonstrated by verifying efficient adipocyte-specific *Atgl* gene deletion in a tamoxifen-inducible Cre/loxP-based mouse model.

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

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## Highlights

Procedure for tamoxifen-induced adipocyte-specific gene deletion in mice

Isolation of dermal adipocytes from mouse skin

Procedure for protein purification and analysis from dermal adipocytes

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# Isolation of dermal adipocytes from mouse skin for biochemical analysis

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## SUMMARY

**The role of dermal white adipose tissue in regulating skin homeostasis and self-renewal processes has recently attracted interest. However, the isolation of proteins from dermal adipocytes for biochemical analysis is challenging. Here, we provide a protocol for the isolation of murine dermal adipocytes. We describe steps for inducing adipocyte-specific gene deletion, adipocyte isolation, protein purification, and western blot analysis. The reliability of the protocol is demonstrated by verifying efficient adipocyte-specific *Atgl* gene deletion in a tamoxifen-inducible Cre/loxP-based mouse model.**

**For complete details on the use and execution of this protocol, please refer to Zhang et al. (2019).[1]**

## BEFORE YOU BEGIN

### Institutional permissions

Institutional permission for the animal study should be obtained. All animal experiments in this study were in accordance with the German Law for Protection of Animals and approved by the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Germany.

### Preparation on the first day of tamoxifen injections

Timing: 3 h

1. Preheat 10 mL sterile corn oil at 65°C.
2. Dissolve 300 mg tamoxifen in 200 µL sterile DMSO in a 15 mL conical tube.

*Note:* The tamoxifen will not fully dissolve and rather create a heterogeneous, viscous mix.

3. Add 10 mL 65°C corn oil to the tamoxifen/DMSO mix.
4. Place the conical tube at 65°C under constant circular shaking at 400 rpm until the tamoxifen is fully dissolved.

⚠ CRITICAL: Wrap the tube in tin foil to avoid light exposure.

5. Store tamoxifen solution in 1.5 mL Eppendorf tubes at −20°C for up to 1 week.

*Note:* These are single use aliquots; avoid repeated thaw and freeze cycles.

### Preparation one day before isolation of dermal adipocytes

Timing: 1 h

6. Prepare 10 mg/mL Dispase stock solution in sterile PBS.
   a. Dissolve the powdered enzyme under slow stirring at 18°C–25°C.
   b. Filter sterilize the enzyme solution through a 0.22 μm filter and aliquot in 1.5 mL Eppendorf tubes.
   c. Store the reconstituted enzyme at −20°C for up to 1 month; avoid repeated thaw and freeze cycles.
7. Prepare 20 mg/mL Collagenase D stock solution in sterile PBS.
   a. Dissolve the powdered enzyme under slow stirring at 18°C–25°C.
   b. Filter sterilize the enzyme solution through a 0.22 μm filter and aliquot in 1.5 mL Eppendorf tubes.
   c. Store the reconstituted enzyme at −20°C for up to 1 week; avoid repeated thaw and freeze cycles.
8. Prepare 20 mg/mL DNase stock solution in sterile PBS.
   a. Dissolve the powdered enzyme under slow stirring at 18°C–25°C.
   b. Filter sterilize the enzyme solution through a 0.22 μm filter and aliquot in 0.2 mL Eppendorf tubes.
   c. Store the reconstituted enzyme at −20°C for up to 18 months; avoid repeated thaw and freeze cycles.

### Preparation on the day of dermal adipocyte isolation

Timing: 30 min

9. Prepare 70% ethanol solution in sterile distilled $H_2O$.
10. Preheat water bath to 37°C.
11. Prepare sterile working surface such as culture hood or dissection pad.
12. Preheat centrifuges to 37°C.
13. Ensure sufficient battery charges on electronic devices such as shaver or automatic pipette.

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Antibodies | | |
| ATGL (30A4) rabbit mAb | Cell Signaling Technology | Cat# 2439 |
| β-actin (AC-74) mouse mAb | Sigma-Aldrich | Cat# A5316 |
| Rabbit anti-mouse immunoglobulins/HRP | Dako | Cat# P0260 |
| Swine anti-rabbit immunoglobulins/HRP | Dako | Cat# P0399 |
| Chemicals, peptides, and recombinant proteins | | |
| Collagenase D | Roche | Cat# 11088858001 |
| Corn oil | Sigma-Aldrich | Cat# C8267 |
| $ddH_2O$ | Roth | Cat# T143 |
| Dispase | Gibco | Cat# 17105-041 |
| DNase | Roche | Cat# 10104159001 |
| DMEM/F12 media | Gibco | Cat# 11320-033 |
| Ethanol | Roth | Cat# 9065 |

(*Continued on next page*)

*Continued*

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| HCl (37%) | Th. Geyer GmbH & Co. KG | Cat# 836.1000 |
| Methanol | PanReac AppliChem | Cat# 131091.1212 |
| Milk powder | Roth | Cat# T145.3 |
| NaCl | Roth | Cat# 3957.1 |
| PhosSTOP phosphatase inhibitor | Roche | Cat# 4906845001 |
| Tamoxifen | Sigma-Aldrich | Cat# T5648 |
| PageRuler Plus prestained protein ladder | Thermo Fisher Scientific | Cat# 26619 |
| Protease inhibitor cocktail | Sigma-Aldrich | Cat# P8340 |
| Tris/tris(hydroxymethyl)aminomethane | Sigma-Aldrich | Cat# T5030 |
| Tween 20 | Roth | Cat# 9127.2 |
| β-Mercaptoethanol | Sigma-Aldrich | Cat# M7522 |
| **Critical commercial assays** | | |
| Minute Total Protein Extraction Kit for Adipose Tissue/Cultured Adipocytes | Invent Biotechnologies | Cat# AT-022 |
| Pierce BCA Protein Assay Kit | Thermo Fisher Scientific | Cat# 23225 |
| Ponceau staining solution | Sigma-Aldrich | Cat# P7170 |
| Western Lightning Ultra ECL | PerkinElmer | Cat# NEL112001EA |
| **Experimental models: Organisms/strains** | | |
| B6.129-Tg(Adipoq-cre/Esr1*)1Evdr/J (*Mus musculus*)[2] Genotype: *AdipoqCre$^{ER}$* | Jackson Laboratory | 024671 |
| B6N.129S-Pnpla2tm1Eek/J (*Mus musculus*)[3,4] Genotype: *Atgl$^{fl/fl}$* | Jackson Laboratory | 024278 |
| Experimental control mice (*Mus musculus*) Genotype: *Atgl$^{fl/fl}$* Age: 8–16 weeks Sex: male or female | | N/A |
| Experimental mice with adipocyte-specific *Atgl* gene deletion (*Mus musculus*) Genotype: *AdipoqCre$^{ER}$ Atgl$^{fl/fl}$* Age: 8–16 weeks Sex: male or female | | N/A |
| **Software and algorithms** | | |
| Microsoft Office | Microsoft Corporation | 16.0.5366.1000 |
| VICTOR Nivo control software | PerkinElmer | 5.0.0 |
| **Other** | | |
| 1 mL syringe | B. Braun | Cat# 9166254 |
| 150 μm filter | pluriSelect | Cat# 43-50150-50 |
| 6 cm cell culture plate | Falcon | Cat# 353004 |
| 96-well plate | Falcon | Cat# 353072 |
| Curix 60 X-ray development machine | AGFA | Type 9462/106 |
| Cannula Microlance 3 | BD | Cat# 302200 |
| Centrifuge | Thermo Fisher Scientific | Cat# 75004230 |
| CL-XPosure film | Thermo Fisher Scientific | Cat# 34090 |
| Depilatory cream | Veet | GTIN:4002448090649 |
| Electrophoresis/transfer chamber XCell II | Thermo Fisher Scientific | Cat# EI9051 |
| Eppendorf Safe-Lock tubes 1.5 mL | Eppendorf AG | Cat# 0030123611 |
| Eppendorf Safe-Lock tubes 2.0 mL | Eppendorf AG | Cat# 0030123620 |
| Eppendorf PCR tubes 0.2 mL | Eppendorf AG | Cat# 0030124707 |
| Filter/blotting paper | GE Healthcare | Cat# RPN6101M |
| Freezer (−20°C) | Liebherr | IFNe 3924 Plus |
| Mixer Mill Retsch 200 | Merck | Cat# Z313955 |
| NuPAGE 20× SDS running buffer | Invitrogen | Cat# NP0001 |
| NuPAGE 20× transfer buffer | Invitrogen | Cat# NP0006 |
| NuPAGE Bis-Tris 4%–12% precast PA gel | Invitrogen | Cat# NP0321BOX |
| NuPAGE LDS sample buffer | Invitrogen | Cat# NP0007 |
| Power supply | Pharmacia LKB | GPS 200/400 |
| PVDF membrane | Merck Millipore | Cat# IPVH00010 |

(*Continued on next page*)

| *Continued* | | |
|---|---|---|
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
| Scalpel | Feather | Cat# No22 |
| Shaver (electric) | Aesculap Isis | Cat# GT420 |
| Sponges | Novex | Cat# EI9052 |
| Sterile hood | Thermo Fisher Scientific | Safe 2020 |
| Tin foil | Avantor by VWR | Cat# 293-4186 |
| VICTOR Nivo multimode plate reader | Revvity/PerkinElmer | Cat# HH35000500 |

## MATERIALS AND EQUIPMENT

| **TBST buffer** | | |
|---|---|---|
| Reagent | Final concentration | Amount |
| Tris | 20 mM | 2.42 g |
| NaCl | 150 mM | 87.66 g |
| Tween 20 | 0.1% | 1 mL |
| $ddH_2O$ | N/A | Fill up to 1 L |
| **Total** | **N/A** | **1 L** |
| Adjust pH to 7.5 with HCl. Store at 18°C–25°C; stable for up to 6 months. | | |

| **SDS running buffer** | | |
|---|---|---|
| Reagent | Final concentration | Amount |
| NuPAGE 20× MOPS SDS running buffer | 1× | 25 mL |
| $ddH_2O$ | N/A | 475 mL |
| **Total** | **N/A** | **500 mL** |
| Store at 18°C–25°C; stable for up to 6 months; reusable. | | |

| **Transfer buffer** | | |
|---|---|---|
| Reagent | Final concentration | Amount |
| NuPAGE 20× transfer buffer | 1× | 12.5 mL |
| Methanol | 10% | 25 mL |
| $ddH_2O$ | N/A | 212.5 mL |
| **Total** | **N/A** | **250 mL** |
| Seal container to minimize evaporation. Prepare at 18°C–25°C; single use. | | |

| **Digestion-Mix** | | |
|---|---|---|
| Reagent | Final concentration | Amount |
| Dispase (10 mg/mL) | 1.8 U/mL | 460 µL |
| Collagenase D (20 mg/mL) | 1 U/mL | 850 µL |
| DNase (20 mg/mL) | 0.1% | 50 µL |
| DMEM/F12 | N/A | 3640 µL |
| **Total** | **N/A** | **5 mL** |
| Store at 18°C–25°C until use; mix carefully directly before use. | | |

## STEP-BY-STEP METHOD DETAILS

*Note:* The same protocol can be used for the isolation of dermal adipocytes from male or female mice aged 8–12 weeks.

### Tamoxifen treatment to induce adipocyte-specific *Atgl* gene deletion

Timing: 13 days

This initial segment of the protocol describes the treatment of *AdipoqCre*$^{ER}$*Atgl*$^{fl/fl}$ mice with tamoxifen to induce adipocyte-specific *Atgl* gene deletion (iAAKO).

1. Thaw individual single use corn oil tamoxifen aliquots.
2. Heat up thawed corn oil tamoxifen aliquots at 37°C for 20 min.

⚠ CRITICAL: Avoid light exposure by wrapping the tubes in tin foil.

3. Draw up 100 μL of corn oil tamoxifen solution into 1 mL syringes with 0.4 × 19 mm cannula.

*Note:* Use individual syringes for each experimental mouse.

4. Inject 100 μL of corn oil tamoxifen solution intraperitoneally (i.p.) in each experimental mouse for five consecutive days (Figure 1).

⚠ CRITICAL: The corn oil tamoxifen solution is quite viscous. Inject slowly and properly tighten the cannula on the syringe.

5. Monitor health and maintain documentation over the entire time span of the experiment.

*Note:* Please note that this tamoxifen application regime for *AdipoqCre*$^{ER}$ *Atgl*$^{fl/fl}$ mice worked efficiently in our laboratory under the given circumstances and legal framework. Variable tamoxifen dosage and recombination efficiency has been reported in different mouse models, and eventually requires optimization of the tamoxifen protocol in specific CreER and floxed allele mouse lines.

### Isolation of dermal adipocytes

Timing: 3 h

This second step focuses on the isolation of dermal adipocytes, reaching from the living animal to snap frozen adipocytes for later usage. This step of the protocol is modified from Zhang et al., 2019.[1]

6. Sacrifice the experimental mouse.
7. Shave the dorsal skin using an electric shaver.
8. Chemically depilate the dorsal skin with depilatory cream.

⚠ CRITICAL: Limit the time of cream exposure to 1 min to avoid damaging the skin.

9. Wash the skin with warm water and dry with paper towels.
10. Clean the depilated skin using 70% ethanol.
11. Carefully cut out and excise the total dorsal skin (Figure 2A).

⚠ CRITICAL: Avoid subcutaneous adipose tissue and remove visible blood vessels or hairs with tweezers.

12. Wash the skin twice by placing it in a 50 mL conical tube containing DMEM/F-12 media (37°C) (Figure 2B).

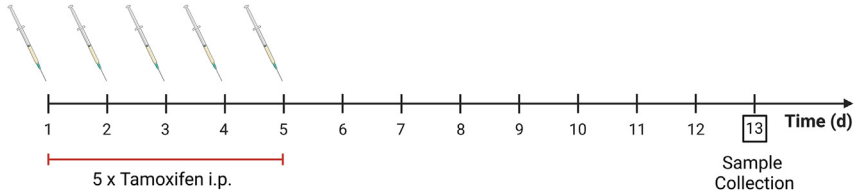


**Figure 1. Tamoxifen injection regime to induce adipocyte-specific *Atgl* gene deletion in *AdipoqCre$^{ER}$Atgl$^{fl/fl}$* mice (iAAKO)**

d, days; i.p., intraperitoneal.

13. Cut the skin into large coin-sized pieces, remove any extra tissues attached to the skin, and float the skin on 5 mL digestion-mix in a 6 cm culture dish with the epidermal side up (Figure 2C).
14. Incubate the floating skin in a 37°C cell incubator for 90–120 min.

*Note:* A good indicator for a proceeding enzymatic digestion is the color change of the media from red/pink to yellow/orange. The viscosity of the digestion solution will most likely increase. The digestion can be completed when the dermal adipocyte layer can be dissociated from the dermis without having to use explicit force. Spot check after 90 min in 15 min intervals.

15. Carefully remove the skin pieces from the digestion medium and transfer the pieces to a new 6 cm dish containing 3 mL 37°C DMEM/F-12 medium.

*Note:* This step as well as the following are to be performed under a sterile culture hood.

16. Gently dissociate the dWAT layer into the medium using a curved scalpel, being cautious not to disturb the dermis (Figure 2D).
17. Carefully pipet the cell suspension up and down using a pipette with truncated 1 mL tip.

*Note:* Mature adipocytes are prone to rupture when floated in solution. By using a truncated 1 mL pipette tip, we can minimize shear stress on the cells and increase the final yield.

*Note:* The size of adipocytes depends on the amount of triglycerides they contain and fluctuates over the course of hair follicle cycling. The physical fragility depends heavily on adipocyte size thus it is assumed that this protocol works best during the telogen phase as this is hallmarked by a small average size of dermal adipocytes and subsequent maximized stability. In our laboratory we were able to successfully apply this protocol on mouse skin during the telogen hair follicle cycle phase and on mouse skin with asynchronized phases of hair follicle cycle phases. In the major reference for this work, Zhang et al.,[1] this slightly modified protocol was successfully used to isolate dermal adipocytes during the anagen phase of hair follicle cycling, so we assume that this protocol is applicable independently of the hair follicle cycle.

18. Filter the dissociated cells through a 150 μm cell strainer into a new 50 mL conical tube.

⚠ CRITICAL: Use truncated 1 mL pipette tips for transfer and carefully stir the solution by pipetting up and down in between transfers.

⚠ CRITICAL: Wash the cell strainer with 2 mL DMEM/F12 before filtering the cell suspension. Ensure complete wetting of the entire sieve!

*Note:* This step represents one of the most critical and yield determining steps of this protocol. The cell suspension is viscous and might not wash through the strainer easily. Work fast

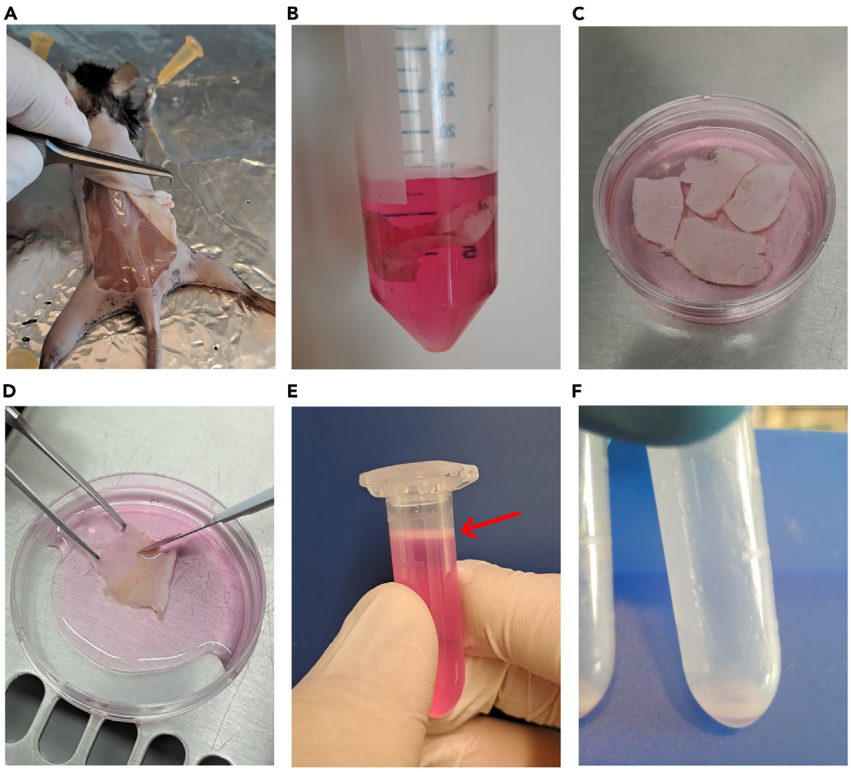


**Figure 2. Isolation of dermal adipocytes**

(A) Excision of total dorsal skin.
(B) Washing of excised dorsal skin in DMEM/F12.
(C) Coin-sized pieces of dorsal skin floating on top of digestion media with the epidermal side upwards.
(D) Dissociation of the dermal adipocyte layer into solution.
(E) Floating dermal adipocytes after centrifugation.
(F) Dermal adipocytes after removing the infranatant.

but handle the suspension with care. Continuously rinse through the strainer with fresh DMEM/F12 but do not exceed the volume of 15 mL.

19. Carefully transfer the cell mixture into a new 15 mL conical tube using truncated 1 mL pipette tips.
20. Centrifuge the tube at 600 × *g* for 5 min at 37°C. Dermal adipocytes will float on top of the medium as a white layer.

***Note:*** It is to be expected that some free lipid will also be included in this layer, but this does not pose a major issue in small quantities.

21. Carefully transfer the floating dermal adipocytes to a 2 mL tube using a truncated 1 mL pipette tip.

⚠ CRITICAL: Hold the pipette vertical and only insert the tip 1–2 mm lower than the lipid/adipocyte layer. Take up only a small volume at a time.

⚠ CRITICAL: The fluid level in the 2 mL tube must not reach the lid when centrifuging in the next step so do not fill up the whole 2 mL.

22. Centrifuge at 200 × *g* at 37°C for 2 min (Figure 2E).

23. Remove the underlying medium using a pipette with thin tip (Figure 2F).
24. Snap freeze the dermal adipocytes for future use or immediately proceed with major step three of this protocol for protein isolation.

### Isolation of protein from isolated dermal adipocytes

Timing: 2 h

The third segment of this protocol describes the isolation of water-soluble proteins from the freshly isolated dermal adipocytes using the 'Minute TOTAL Protein Extraction Kit for Adipose Tissue/Cultured Adipocytes' with an adapted protocol.

25. Cool down centrifuge to 4°C.
26. Thaw the previously frozen dermal adipocyte samples at 4°C.
27. Prepare protease inhibitor and phosphatase inhibitor mix in buffer A (provided by the Minute TOTAL Protein Extraction Kit for Adipose Tissue/Cultured Adipocytes):
    a. Protease inhibitor cocktail: dilute 1:100.
    b. Phosphatase inhibitor tablets: dissolve 1 tablet in 10 mL.
28. Add 250 μL of the enzyme inhibitor mix prepared in step 27 to the thawed dermal adipocyte sample and homogenate for 3 min at 30 Hz using the MixerMill system.

⚠ CRITICAL: For homogenization using MixerMill, use one stainless steel body per sample/tube.

29. Centrifuge at 350 × *g* at 4°C for 2 min and transfer the supernatant to a kit provided pre-chilled −20°C filter cartridge with a collection tube.
30. Incubate the filter cartridge with the cap open at −20°C for 15–20 min.

⚠ CRITICAL: Ensure precise incubation to decisively separate the lipid and aqueous fractions. This timing likely varies even between different freezer units but the technical notes provided in the manual of the protein extraction kit can help pinpoint this time point.

31. After incubation, immediately centrifuge at 350 × *g* for 2 min at 4°C with the cap open. The flow-through contains total proteins from dermal adipocytes.
32. Perform BCA analysis to determine protein concentration.
    a. Prepare protein standards according to BCA datasheet.
    b. Prepare working reagent by mixing 50 parts of reagent A with 1 part of reagent B.
    c. Pipette 25 μL of each standard and sample into a 96-well plate.
    d. Add 200 μL of working reagent and mix gently.
    e. Incubate for 30 min at 37°C.
    f. Cool plate to 18°C–25°C.
    g. Measure absorbance at 570 nm on a plate reader.
33. Add 1/10 of buffer B provided by the Minute TOTAL Protein Extraction Kit for Adipose Tissue/Cultured Adipocytes to the protein solution resulting in a denatured protein solution.

*Note:* Do not add buffer B if your downstream applications involve ELISA or immune precipitation.

### Gel electrophoresis and western blotting

Timing: 2 days

34. Add LDS Sample buffer 1:4 to the sample volume for loading.

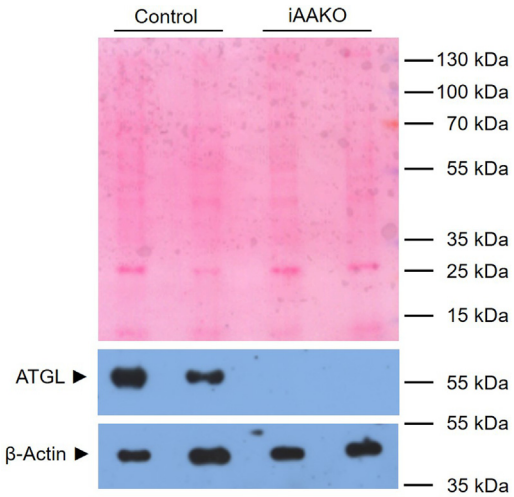


**Figure 3. Western blot analysis of ATGL and β-actin in dermal adipocytes isolated from tamoxifen-treated *Atgl*$^{fl/fl}$ mice (control) and tamoxifen-treated *AdipoqCreERAtgl*$^{fl/fl}$ mice (iAAKO)**

The figure shows successful isolation of proteins from dermal adipocytes and efficient ATGL deletion in iAAKO mice compared to controls.

35. Add 4% β-mercaptoethanol to the sample.
36. Heat up for 10 min at 75°C.
37. Cool on ice and spin down at 4°C to collect evaporated fluid.
38. Freeze sample at −80°C or continue with SDS-PAGE.
39. Centrifuge 25 min at 4°C maximum speed to remove cell debris.

⚠ CRITICAL: This centrifugation step will most likely result in the formation of a pellet and potentially even a small lipid layer on top of the solution. Avoid both when drawing up sample with your pipette.

40. Prepare 4%–12% Bis-Tris NuPAGE gel by removing the white stripe.
41. Prepare 500 mL of SDS running buffer.
42. Put gel into the device and completely fill the Upper-Buffer-Chamber with SDS running buffer. Fill the rest of the buffer into the Lower-Buffer-Chamber.
43. Remove the comb and wash pockets with SDS running buffer.
44. Load 20 μg protein in up to 40 μL sample per lane.
45. Load 10 μL of protein ladder.
46. Run gel at 130 V for approximately 70 min. The 10 kDa band should be at the lower edge of the gel.
47. Soak 4 sponges and 8 sheets of filter paper in transfer buffer.
48. Activate PVDF-membrane in methanol (15–60 s) and soak in transfer buffer.
49. Put into the blotting chamber:
    a. 2 Sponges.
    b. 4 Filters.
    c. Gel.
    d. Membrane.
    e. 4 Filters.
    f. 2 Sponges.

⚠ CRITICAL: Make sure there are no bubbles, wear gloves and handle the membrane with forceps.

50. Close blotting chamber, put it into the device and fill the blotting chamber with transfer buffer. Put device into a box with ice and fill the Lower-Buffer-Chamber with ice.
51. Blot for 105 min at 30 V.
52. After blotting, perform Ponceau staining of the membrane (Figure 3):

a. Immerse the membrane in Ponceau-staining-solution and stain for 5 min.
b. Rinse membrane with distilled water until the background is clear.

53. If required, cut membrane into stripes to detect different sized proteins.

*Note:* Cutting the membrane is optional. In this protocol, we cut the membrane at ~ 50 kDa to allow simultaneous detection of ATGL (~ 54 kDa) and β-actin (~ 42 kDa).

54. Incubate in 5% milk in TBST (blocking solution) for 1 h at 18°C–25°C or for 14 h at 4°C with shaking.
55. Incubate membrane with primary antibody according to manufacturer's instructions in blocking solution for 2 h at 18°C–25°C or 14 h at 4°C with shaking:
    a. Upper part of membrane (> 50 kDa): ATGL (1:2000).
    b. Lower part of membrane (< 50 kDa): β-actin (1:2000).

*Note:* The ATGL antibody here is used to validate the gene knock-out. To verify the adipocyte identity of the sample we suggest the use of a control marker for adipocytes such as adiponectin, perilipin-1 or FABP4. To probe for potential presence of stomal vascular cells in the sample we suggest the control markers CD45, CD31, CD34 and vimentin.

56. Wash membrane 3 × 10 min with TBST at 18°C–25°C.
57. Incubate membrane with secondary antibody in blocking solution for 1 h at 18°C–25°C:
    a. Upper part of membrane (> 50 kDa): swine anti-rabbit HRP (1:2000).
    b. Lower part of membrane (< 50 kDa): goat anti-mouse HRP (1:2000).
58. Wash membrane 1 × 15 min and 3 × 10 min with TBST at 18°C–25°C.
59. Prepare 1 mL of ECL solution by mixing 500 μL of each solution.

⚠ CRITICAL: Use different pipette tips for each solution.

60. Transfer membrane onto a clean surface (e.g., plastic foil).
61. Pipette ECL solution onto the membrane, distribute evenly and incubate for 1 min.
62. Wrap the membrane into a clean plastic bag and fixate it into the film-cassette.

*Note:* Remove excessive ECL solution.

63. Detect the HRP-generated signal by applying a CL-XPosure Film on the membrane for 10 s to 30 min.

⚠ CRITICAL: Protect the undeveloped film from light.

64. Develop the film using a Curix 60 X-ray development machine (Figure 3).
65. Draw protein ladder bands onto the film, before removing the membrane from the film-cassette.

*Note:* The membrane can be air dried and stored at −20°C for later repeated use.

## EXPECTED OUTCOMES

The expected outcome of this protocol is a protein sample of water-soluble proteins from dermal adipocytes that is relatively pure and free from lipid contaminants. One can expect a sample with a volume of around 250 μL and a protein concentration in the range of 0.5–3 μg/μL. The protein yield may be affected by animal size, experimental model or the skin area from where the dermal adipocytes were initially isolated with the dorsal area generally being the most profitable. The resulting sample might appear to be slightly cloudy due to water-insoluble cellular components. The adjoined western blot analysis is poised to include a clear separation of proteins in the polyacrylamide gel and

successful transfer onto the blotting membrane. Primary and secondary antibodies should aggregate properly, allowing for clear and specific visualization of proteins at the expected molecular weight.

## LIMITATIONS

Despite the successful implementation of this protocol on single-mouse-samples, the process itself is fairly straining for the adipocytes of interest and often results in a significant portion of dermal adipocytes being lost before the final snap freezing of the cell sample. Adipocytes breaking down, adhering to various surfaces or being removed together with the supernatant may impose restrictions on the experimental setup to involve more animals or exclude certain age groups or phenotypes of animal for this method of isolation.

Yet, another aspect of limitation is the isolation of the stromal vascular fraction of cells from the dWAT. Many protocols that involve the isolation of adipocytes, as well as this one, exploit the physical property of adipocytes to float upwards in aqueous solution due to lower density. After centrifugation, generally, the sample involves a pellet in the bottom of the falcon tube and a layer of floating adipocytes. Separating the adipocytes usually still allows to collect and further process the cell pellet at the bottom of the tube. In this protocol however, this pellet contains not only significant amounts of adipocyte and extracellular matrix debris, on top of that the temperature and media is not optimized for the preservation of stromal vascular samples. The process of scraping of the dermal adipocytes with a scalpel, even when performed with care, may also disrupt cells from the dermis. So, the claim of a stromal vascular fraction origination exclusively from the dWAT is not durable.

## TROUBLESHOOTING

### Problem 1

Contamination of the sample by cells from the stromal vascular fraction (related to steps 19–21).

### Potential solution

While theoretically separated in the centrifugation process due to different physical attributes, some cells from the stromal vascular fraction might adhere to the adipocytes or are trapped within the lipid layer, resulting in contamination of the sample. This is to be ruled out by control staining (see note under step 55). If contamination of such kind occurs, it is possible to insert another washing step into the protocol by cycling back once from step 21 to step 19 and transfer the adipocytes to another 15 mL conical tube and repeat the centrifugation step 20. This may result in further adipocyte rupture and subsequent reduced yield.

### Problem 2

Insufficient yield of adipocytes after digestion and isolation (related to step 21).

### Potential solution

Anyone working with mice has to deal with some degree of biological variability. Depending on phenotype, age, sex, litter and just the individuality of each mouse the total amount of adipocytes or the success of isolation may vary. To improve general yield: pool the isolated dorsal skin segment of two or even more mice in one digestion to increase the final yield of dermal adipocytes.

Digestive enzymes can show considerate discrepancy in activity between batches or may degrade over long periods of storage, which again may affect the yield of the isolation. Test different batches of enzymes and avoid repeated freeze and thaw cycles by aliquot the ready-to-use enzyme solutions.

### Problem 3

Inconsistent total protein quantification by BCA assay (related to step 32).

### Potential solution

Residual lipids, large quantities of cellular debris or even contamination of the sample by the filtration agent from the purification columns may interfere with the basic colorimetric detection and quantification of the BCA assay. While the basic procedure for isolating the proteins may be simple and straightforward, the step of 'freezing-out' lipids from the sample remains critical. Ensure adequate timing when performing this step. Technical notes provided in the manual of the protein extraction kit can help you optimize this step.

### Problem 4

Weak Ponceau staining (related to step 52).

### Potential solution

We have consistently experienced unusually weak bands on our Ponceau staining despite the loading of significant amounts of protein per well (>70 μg) and no detectable shortcomings in the rest of the western blot analysis. As of the time of writing this manuscript we have found no way to circumvent this and improve the visualization of total dermal adipocyte protein by Ponceau staining.

### Problem 5

Weak/inconsistent/blurry antibody detection after western blot membrane transfer (related to step 64).

### Potential solution

While the typical western blot procedure offers a wide variety of pitfalls, we have experienced that samples with large portions of residual lipids fractions tend to display problems with the aggregation of antibodies to the respective protein bands, despite showing no significant alteration in Ponceau staining or migration behavior in the acrylamide gel. The third major step of this protocol may be repeated, again filtering via column; however, this may result in significant reduction of total protein.

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Sabine A. Eming (sabine.eming@uni-koeln.de).

### Materials availability

This study did not generate new unique reagents.

### Data and code availability

This study did not generate datasets.

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## AUTHOR CONTRIBUTIONS

P.F.G. and S.W. wrote the manuscript. P.F.G. and S.S. composed and implemented the protocol described here and performed the experimental part corresponding to this method. S.A.E. supervised the study. All authors commented on and edited the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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