Supplementary materials
EJTM #7567
Culture conditions influence satellite cell activation and survival of single myofibers

Single myofiber isolation protocol

1. Before starting

a. Prepare four petri dishes (60x15mm) per mouse, one for fiber isolation and three for washings. The latter dishes are prepared as follows: cover the dishes with sterile filtered 5% BSA (Sigma-Aldrich) in PBS (Sigma-Aldrich), remove the solution and let the dishes dry in a sterile hood with the lid open for at least 30 min. BSA will prevent fiber attachment to the dish during the isolation and washing processes. Once the dishes are dry, add 4 ml of DMEM (Dulbecco's modified Eagle's medium, high glucose, L-glutamine with 110 mg/ml sodium pyruvate) (Sigma-Aldrich) supplemented with 1% penicillin/streptomycin (Sigma-Aldrich) and store in an incubator at 37°C with 5% CO2.

b. Prepare two previously sterilized glass pipettes: one with a large hole for myofiber dissection from the muscle, the other curved, with a tiny hole for single myofiber handling. Use a flame to curve the glass pipet and smooth the edges so as not to damage myofibers. Coat the pipet surface with 5% BSA in PBS solution by aspirating and releasing the solution several times; then, let the pipettes dry for 30 min in a sterile hood.

c. Prepare 0.2% Collagenase Type I (Sigma-Aldrich) solution in a pre-filtered sterile DMEM media and store at 4°C.

d. If myofibers need to be attached for long-term culture: coat the dishes with matrigel (Corning) according to Keire P 26. Briefly, thaw in ice the required frozen aliquots of matrigel for at least 30 min; add ice cold DMEM to dilute matrigel to 1 mg/ml. Leave the solution on ice for at least 15 min, then use 250-300 µl per well to cover 24 well-plate wells. Leave the matrigel-coated plates on ice for 7 min, then remove excess matrigel and leave the coated dishes in incubator for at least 1 hour. Ten minutes before use, let the dishes dry in a sterile hood with the lid opened. Matrigel coated dishes can be kept in incubator with DMEM for a maximum of 1 week.
Myofibers can be cultured in suspension by using 5% BSA in PBS coated dishes, prepared as described in a. Coating with BSA instead of serum yields less active satellite cells.

2. EDL muscle dissection and digestion

Young (4-8 weeks old) C57/BL6 mice are used to isolate myofibers. EDL muscle is dissected as previously described 9. Briefly:
1. Spray hindlimbs with 70% EtOH and remove the skin with scissors.
2. Expose the muscles and cut the TA and EDL distal tendons.
3. Holding the TA and EDL distal tendons, pull the muscles up, until EDL muscle is completely visible.
4. Holding EDL tendon, gently separate the EDL muscle from the TA, then cut the TA off.
5. Expose the proximal tendon of the EDL and cut it, paying attention not to damage the EDL muscle.

6. Transfer the EDL muscle into a 15 ml tube containing 2 ml of 0, 2% Type I Collagenase, and incubate it at 37°C either in a water bath without agitation or in an incubator for 45 min. Digestion time may vary depending on the EDL size. Muscle digestion should be checked frequently to avoid over-digestion. Stop the digestion when myofibers start to fray out of the muscle.
7. Transfer the digested EDL muscle in a Petri dish containing warm DMEM with 1% pen/strep and proceed with the dissociation of single myofibers.

3. Single myofiber dissociation

1. Under a stereo microscope, use the large bore glass pipette to fill up medium from the dish and flush it on the EDL muscle. Single myofibers will start to fray out of the muscle.
2. Repeat the step 1 until myofibers will dissociate from the EDL. To avoid the dish cooling, place it back to the incubator for at least 5 min every 10 min.
3. With the small-bore glass pipette, collect individual live myofibers and transfer them to a clean dish containing warm DMEM with 1% pen/strep.
4. Repeat this the step 3 twice to separate live myofibers.
5. Leave the myofibers in DMEM with 1% pen/strep in the incubator for 1 hour.
6. Transfer the myofibers in the dishes properly prepared for the experiment. To avoid myofiber loosing, for downstream analysis only single live myofibers, which appear as long and shining tubular structures, are selected.

This protocol allows to obtain around 100 vital single myofibers from 2 EDL muscles.