Supplemental Table 1. Realtime PCR primer sequences

Rplp38	F: 5'-GAA GGA TGC CAA GTC TGT CAA-3' R: 5'-GAG GGC TGG TTC ATT TCA GA-3'
Pparγ	F: 5'-CAA GAA TAC CAA AGT GCG ATC AA-3' R: 5'-GAG CTG GGT CTT TTC AGA ATA ATA AG-3'
UCP1	F: 5'-GTG AAG GTC AGA ATG GAA GC-3' R: 5'-AGG GCC CCC TTC ATG AGG TC-3'
PDGFRα	F: 5'-TGC TGG AAC AGT GAG CCC GAG A-3' R: 5'-AGG CCA CCT TCC CAG TCC TTC A-3'
aP2	F: 5'-TCA CCG CAG ACG ACA GGA AGG-3' R: 5'-CCG ACT GAC TAT TGT AGT GTT TGA TG-3'
Pax7	F: 5'-CTG CTG AAG GAC GGT CAC TG-3' R: 5'-GGA TGC CAT CGA TGC TGT GT-3'
MyoD	F: 5'-GGC TAC GAC ACC GCC TAC TA-3' R:5'- CGA CTC TGG TGG TGC ATC TG-3'
Myogenin	F: 5'-TGC CCA GTG AAT GCA ACT CC-3' R: 5'-TTG GGC ATG GTT TCG TCT GG-3'

Figure S1. Effect of fast and slow muscle culture conditioned media on the adipogenic potentials of muscle resident progenitors.

A-B: Oil Red O staining of adipocytes differentiated from EDL muscle derived cells cultured with EDL (A) or SOL (B) conditioned medium collected from growth medium from non-passaged EDL and SOL cell cultures (conditioned for 48h).

C: Relative Oil Red O signal intensity quantified with Image J software from representative cultures from A and B. Error bars represent standard error of the mean, n = 3.

D-E: Oil Red O staining of adipocytes differentiated from SOL muscle derived cells cultured with EDL (C) or SOL (D) conditioned medium.

F: Relative Oil Red O signal intensity quantified with Image J software from representative cultures from C and D. n = 3.

Figure S2. Effect of fast and slow muscle co-culture on the adipogenic potentials of muscle resident progenitors.

A: Experimental design. The mTmG mice act as a cell origin marker as they ubiquitously express membrane targeted tandem T-dimer (mT, a red fluorescent protein).

B-D: Oil Red O staining of adipocytes differentiated from EDL culture (B), SOL culture (D) or co-cultures (EDL and SOL).

E: The ratio of EDL and SOL derived adipocytes in the co-culture is not different from that when EDL and SOL derived cells are cultured separately. The ratio is quantified from the number of mT^+ and mT^- cells counted from 10 fields from 2 independent cultures involving 8 mice.

F: The Oil Red O signal intensity in the co-culture was not different from the average value of separate cultures of EDL and SOL. Error bars represent SEM, n=3.

Figure S3. IMAT is derived from a non-Myf5 lineage. Results are based on Myf5-Cre/mTmG mice in which Myf5-lineage cells are labeled in Green (mG, membrane-GFP) and Myf5-independent lineage cells are labled in Red (mT).

A-C: Skeletal muscle derived adipocytes shown in fluorescence (A) and phase-contrast (B) images. The relative percentage composition of mT and mG positive adipocytes is shown in C. n=3.

Figure S4. IMAT are derived from a *Pax3* independent lineage in vivo. Representative images of Pax3-Cre/mTmG TA muscle sections at Day 3 (**A**) and Day 7(**B**) post CTX treatment. Arrows indicate satellite cells associated with muscle fibers.

Figure S5. aP2 cell lineage labeling and ablation in vivo. TA muscles of aP2-Cre/Rosa-iDTR mice and Rosa-iDTR control mice are injected with a cocktail of diphtheria toxin (DT) and cardiotoxin (CTX) and examined 5 days after injection. CTX induces muscle degeneration and DT serves to ablate adipogenic cell lineage in the aP2-Cre/Rosa-iDTR muscle.

A-B: aP2 cell lineage labeling in the skeletal muscle of aP2-Cre/mTmG mice. (A) Many interstitial cells are derived from the aP2 lineage (in Green) whereas muscle fibers are mostly derived from aP2-independent lineage (red). (B) Percentage of muscle fibers that are mG (green) or mT (red) positive.

C: Control (Rosa-iDTR) muscle 5 days after CTX/DT treatment showing newly regenerated muscle fibers (in Green, stained with eMyHC antibody) featured by centrally localized nuclei (labeled with DAPI in Blue) and orderly skeletal muscle architecture (shown by laminin staining in Red).

D: aP2-Cre/Rosa-iDTR muscle 5 days after CTX/DT treatment showing disorganized muscle structure, lack of newly regenerated muscle fibers with central nuclei and increased interstitial space that lacks cellular structure. See C for Color coding.

E: Relative number of regenerating fibers per unit area in the *aP2-Cre/Rosa-iDTR* mice compared with Rosa-*iDTR* mice at day 5 post CTX/DT treatment. Error bars represent SEM, n=3.

Figure S6. Efficient ablation of interstitial adipogenic cell lineages in the aP2-Cre/iDTR mice. TA muscles were focally injected with CTX in the left and CTX+DT in right legs.

A-D: PDGFRα staining (Green) of TA muscle sections treated by CTX alone or CTX+DT and 2 and 5 days post injection (dpi). Nuclei is counterstained with DAPI in blue.

E-G: Relative mRNA expression of *Dlk1* and *Pdgfr*α genes at 2, 5, and 10 dpi. The expression levels of CTX treated controls are normalized to 1. Error bars represent SEM, N=3. ** P<0.01, *P<0.05. Scale bar = 50 μm

Figure S7. Age-dependent changes in fat infiltration and regenerative capacity of skeletal muscles. TA muscles of young (2-month old) and old (18-month old) mice were analyzed.

A-B: Oil Red O staining of intermuscular fat in skeletal muscle sections from young (A) and old (B) mice.

C-D: H&E staining showing the regenerative efficiency of young (C) and old (D) skeletal muscles 5 days post CTX-induced injury. Very few regenerative muscle fibers are evident in the aged muscle.

E: Relative number of regenerating muscle fiber per unit area in young (C) and old (D) skeletal muscles 5 days post CTX-induced injury. Error bars represent SEM, n=3.