Supplementary information

Methods

Myogenic differentiation

One times 10^5 active muscle satellite cells (MSCs) were incubated on the round coverslips coated with 0.01% (w/v) poly-L-lysine solution (Sigma-Aldrich, St. Louis, MO, USA) at 37℃ in a humidified atmosphere of 5% CO_2 in air. After 24 h, induction of the adherent cells into myogenic differentiation was conducted for 3 days using myogenic differentiation medium consisting of high-glucose Dulbecco's modified Eagle's medium (HG-DMEM; Welgene, Gyeongsan, Korea) supplemented with 2% (v/v) horse serum (Gibco, Grand Island, NY, USA) and 1% (v/v) antibiotic-antimycotic (Welgene). The myogenic differentiation medium was replaced every 2 days.

Immunostaining of myotubes

The active MSC-differentiated myotubes formed on the coated-round coverslip with 0.01% (w/v) poly-L-lysine were washed twice with Dulbecco's phosphate-buffered saline (DPBS; Welgene) and then fixed using 4% (v/v) formaldehyde solution (Junsei Chemical, Chuo-ku, Japan) at room temperature for 10 min. Fixed myotubes were washed twice with DPBS and incubated in blocking solution consisting of DPBS supplemented with 10% (v/v) heat-inactivated horse serum (Gibco), 2% (w/v) bovine serum albumin (Sigma-Aldrich), and 0.3% (v/v) Triton X-100 (Biopure, Cambridge, MA, USA) overnight at 4℃. The blocked myotubes were stained with fluorescence-unconjugated MF20 primary antibody diluted in DPBS for 2 h at room temperature. The primary antibody was detected with Alexa Fluor 594-conjugated secondary antibody diluted in DPBS for 1 h at room temperature. The stained myotubes were washed three times with DPBS and then counterstained with VECTASHIELD® Antifade mounting medium containing 4',6-diamidino-2-phenylindole (Vector Laboratories, Inc., Burlingame, CA, USA). The detailed information of the used antibodies was represented in Table S1. Subsequently, the double-stained cells were observed under a fluorescence microscope (Olympus, Tokyo, Japan).

Table S1. Primary and secondary antibody list

Antibody name	Company	Catalog number	Dilution rate
MF20	Developmental Studies Hybridoma Bank (DSHB)	MF20	1 : 100
Donkey anti-Mouse IgG (H+L) Highly Cross-Absorbed Secondary Antibody, Alexa Fluor 594	Invitrogen	A-21203	1 : 500

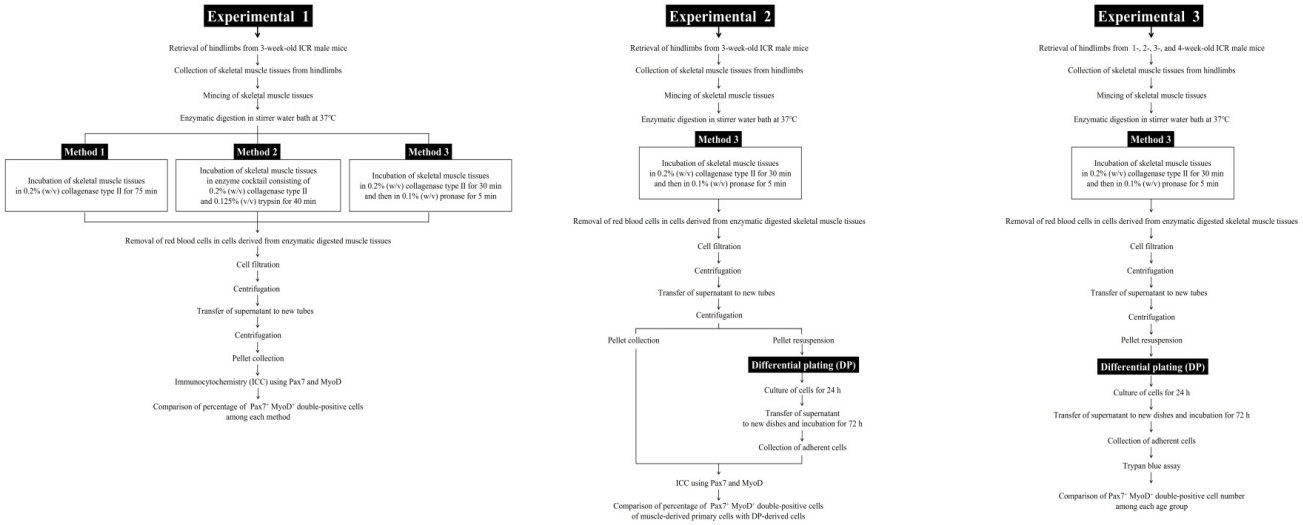

Fig. S1. Schematic diagram of experimental design.

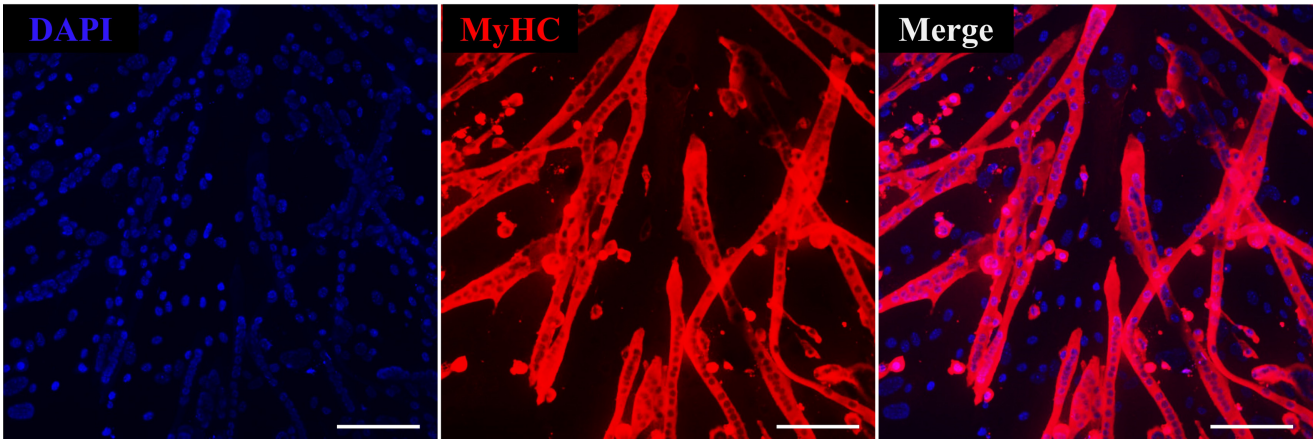

Fig. S2. Identification of myogenic differentiation capacity of active MSCs retrieved by applying the DP method post-digestion of skeletal muscle tissues of 2-week-old mice through Method 3. Active MSCs were differentiated into myotubes by incubating for 3 days in myogenic differentiation medium. Subsequently, the generated myotubes were stained with an antibody detecting a myosin heavy chain (MyHC; red). As the results, active MSCs retrieved by applying the DP method post-digestion of skeletal muscle tissues of 2-week-old mice through Method 3 showed successful formation of myotube co-exhibiting multiple nuclei (blue) and positive staining for MyHC (red). Nuclear counterstaining was performed using 4′,6-diamidino-2-phenylindole (DAPI; blue). Scale bars represent 100 μm.