APPENDIX (online only).

Supplementary Methods

Murine unilateral hind limb ischemia injury. Unilateral femoral artery excision was performed on female C57BL/6 mice (Jackson Laboratories, Bar Harbor, Me) under 2% isoflurane inhalation anesthesia. Mice were positioned in dorsal recumbency with hind limbs externally rotated. The femoral artery was isolated from the femoral vein and nerve and then ligated using 7-0 Vicryl suture (Ethicon, Somerville, NJ) at the inguinal ligament and the popliteal bifurcation. The segment of artery between the two ligations was excised. The wound was closed using 5-0 Prolene suture (Ethicon). Animals were allowed to recover with a heating pad until ambulatory. Mice were then returned to their cages and housed with ad libitum access to food and water and maintained on a 12-hour light-dark cycle.

Functional measurements of muscle contractile force. Mice were anesthetized using 2% isoflurane and maintained at 37°C using a heat lamp. Triceps surae muscles were surgically isolated from other tissue, and the distal femoral condyle was immobilized to a stainless steel platform. The Achilles tendon was secured to the lever arm of a servomotor (model 305B; Cambridge Technologies, Cambridge, Mass) interfaced with a computer equipped with an A/D board (National Instruments, Austin, Tex). An Isolated Pulse Stimulator (Model 2100; A-M Systems, Sequim, Wash) was used to stimulate muscle contraction with leads applied to the belly. Optimal length of the muscle was acquired by measuring maximal twitch tension at 0.5 Hz. At optimal length, the muscle was stimulated at 150 Hz to elicit the peak tetanic tension (P_o) with 2 minutes of rest between each contraction. Data were stored and analyzed using LabView software (National Instruments).

Histologic preparation and analysis. Images were captured using a light microscope (Nikon Diaphot; Nikon Corp, Tokyo, Japan) with a 20× objective lens and a mounted digital camera (Optronix MicroFire; Optronix, Goleta, Calif). Fluorescent images were taken on Zeiss LSM710 (Carl Zeiss AG, Jena, Germany) laser confocal microscope using 40× oil immersion objective. Antibodies were listed as follows: anti-CD31 antibody (1:25; #550274; BD Biosciences, Franklin Lakes, NJ); anti-CD45 antibody (1:25; #550539; BD Biosciences); secondary antibody (1:200) with Vectastain ABC Kit (Vector Laboratories, Irvine, Calif); DAB substrate (ThermoFisher Scientific, Rockford, Ill); anti-MyoD antibody (1:100; ab203383; Abcam, Cambridge, Mass); and secondary antibody (1:200; ab175694; Abcam).