Supplemental Methods

SDS-PAGE and Western blotting

Muscle protein samples were collected from frozen tissue sections on glass slides by scratching and lyzing 4 to 10 sections (according to the area size of the muscle sections) in SDS-PAGE sample buffer containing 2% SDS and 3% β-mercaptoethanol, pH 8.8. After transfer into an Eppendorf tube to homogenize the muscle tissue using a sonicator (Digital Sonifier, Branson Ultrasonics Corporation, USA), the samples were heated at 80°C for 5 min and centrifuged at 14,000 *g* in a microcentrifuge for 5 min to remove insoluble debris. The protein samples were resolved on SDS gels with 14% acrylamide/bisacrylamide at the ratio of 180:1 prepared in a modified Laemmli buffer system, in which both stacking and resolving gels were at pH 8.8. The resolved protein bands were visualized by Coomassie blue R-250 staining. The actin band was quantified using ImageJ software (National Institutes of Health, Bethesda, MD) to normalize sample loading.

Duplicate SDS gels were transferred to nitrocellulose membranes using a Bio-Rad (Hercules, CA) semidry electrical transfer device at a constant current of 5 mA/cm^2 for 15 min. The blotted membranes were blocked with 1% BSA in Tris-buffered saline (TBS, 150 mM NaCl, 50 mM Tris·HCl, pH 7.5) at room temperature with shaking for 30 min. The blocked membranes were probed at 4°C overnight with anti-TnI (TNNI1) mAb TnI-1 (1) and anti-slow TnT (TNNT1) mAb CT3 (2) diluted in TBS containing 0.1% BSA. The membranes were then washed three times for 7 min each with TBS containing 0.5% Triton X-100 and 0.05% SDS and three times for 3 min each with TBS before incubation with alkaline phosphatase-labeled goat anti-mouse IgG second antibody (Santa Cruz Biotechnology, Dallas, TX) at room temperature for 1

h. The membranes were washed again and developed in 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate solution to visualize the protein bands recognized by each of the antibodies.

Glycerol-SDS-PAGE

Myosin heavy chain (MHC) isoforms expressed in muscle tissues were examined using glycerol-SDS-PAGE (3). Briefly, SDS-PAGE samples equivalent to 5 μg of muscle tissue (wet weight) were resolved on 8% polyacrylamide gel with acrylamide:bis-acrylamide ratio of 50:1, prepared in 200 mM Tris base, 100 mM glycine, pH 8.8, containing 0.4% SDS and 30% glycerol. The stacking gel contained 4% polyacrylamide with acrylamide:bis-acrylamide ratio of 50:1, 70 mM Tris-HCl (pH 6.7), 4 mM EDTA, 0.4% SDS, and 30% glycerol. The upper cathode running buffer consists of 100 mM Tris base, 150 mM glycine, 0.1% SDS, and 10 mM β-mercaptoethanol. The lower anode running buffer was 50% dilution of the upper running buffer without β-mercaptoethanol. The 0.75-mm-thick Bio-Rad minigels were run at 100 V in an icebox for 24 h. The resolved protein bands were visualized after staining with Coomassie blue R250.

Muscle Fatigue and Recovery in Tnnt1 *Transgenic Mice*

Intact *quadratus femoris* muscle was carefully isolated including both tendons and mounted vertically to a dual-mode lever arm force transducer (300B, Aurora Scientific) in an organ bath containing 100 mL modified Kreb's solution (118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 2.25 mM $MgSO_4$, 2.25 mM $CaCl_2$, and 11 mM D-glucose, continuously gassed with 95% O_2, 5% CO_2, pH 7.4). A thermo-controlled circulating water jacket was used to maintain the bath at 25 ± 0.5°C during experiment. Muscle contractions were

elicited with bipolar pulse field electrical stimulation using a stimulator (701B, Aurora Scientific). Twitch contractions were elicited with supramaximal pulses (0.1 ms, 28 V/cm), unless specified otherwise. Tetanic contractions were elicited with a train of the same pulses at 100 Hz for 0.7 s. Isometric force data were collected via a digital controller A/D interface (604C, Aurora Scientific) and recorded using Chart software (ASI, Aurora Scientific). Developed twitch and tetanic forces were determined at the optimal muscle length that gave the highest twitch force and calculated by subtracting the resting tension from the total force.

After 20-min equilibration with 0.7 s tetanic contractions per minute, various stimulation frequencies were tested to determine the optimal frequency that produced maximum tetanic force. A 300 s fatigue protocol was performed with intermittent tetani of 300 ms every second. One minute after the end of fatigue, recovery of muscles contractility was recorded for 20 min with 0.3 s tetanic contractions every 1 min.

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2 Jin, J.P., Chen, A., Ogut, O. and Huang, Q.Q. (2000) Conformational modulation of slow skeletal muscle troponin T by an NH(2)-terminal metal-binding extension. *Am J Physiol Cell Physiol*, **279**, C1067-1077.

3 Feng, H.Z., Chen, M., Weinstein, L.S. and Jin, J.P. (2011) Improved fatigue resistance in Gsalpha-deficient and aging mouse skeletal muscles due to adaptive increases in slow fibers. *J Appl Physiol (1985)*, **111**, 834-843.