Supporting Information

Han et al. 10.1073/pnas.0906545106

SI Materials and Methods

Mice. Mice with striated-muscle-specific dystroglycan (DG) deficiency (MCK-cre/*Dag1*$^{flox/flox}$) (1) and integrin α7-null (2) mice were described previously. For a direct comparison of DG-deficient and integrin α7-null skeletal muscle in the same mouse line, these 2 mouse lines were crossed to one another. MCK-Cre male mice bearing the floxed *dystroglycan* allele were mated to *integrin* α7 heterozygous females. F1 and F2 offspring were mated to produce F2- and F3-generation mice, respectively. Identification of the mutant mice was performed by PCR genotyping of genomic DNA prepared from mouse tail snips. The *Large*^{myd} colony was originally obtained from Jackson Laboratories. Mice were maintained at The University of Iowa Animal Care Unit in accordance with animal use guidelines. All animal studies were authorized by the Animal Care Use and Review Committee of The University of Iowa. For treadmill exercise, mice ($\approx$5 weeks old) were placed on an endless conveyor-type belt with a shock grid at the end (AccuPacer Treadmill, AccuScan Instruments) and exercised on a down-hill grade at 15 m/min for 20 min. Immediately after the exercise, mice were euthanized and quadriceps muscles were prepared for examination by electron microscopy or immunofluorescence.

Lectin Affinity Chromatography and Sucrose Gradient Fractionation. Total muscle homogenates in TBS (50 mM Tris-Cl pH 7.4, 150 mM NaCl) were solubilized with 1% digitonin. After centrifugation at 140,000 $\times$ *g* for 37 min, solubilized proteins in the supernatant were mixed with wheat germ agglutinin (WGA)-agarose beads (Vector Laboratories) and rotated end-over-end at 4 °C for 2 h. WGA-bound proteins were eluted with TBS containing 0.3 M N-acetyl-D-glucosamine and 0.1% digitonin. The eluant was applied to a 5–30% sucrose gradient and centrifuged at 215,000 $\times$ *g* for 90 min. Fractions (1 mL) were collected from the top of the gradient and analyzed by SDS/PAGE.

Measurement of Contractile Properties. Muscle mass, fiber length, and maximum force were measured on 6 EDL muscles from 6- to 7-month-old *Large*^{myd}, MCK-cre/*Dag1*$^{flox/flox}$, integrin α7-null, and WT littermate control mice. Mice were anesthetized and muscles isolated and stimulated to provide maximum isometric tetanic force (P_o). The susceptibility of muscles to contraction-induced injury was assessed by 2 lengthening contractions with a strain of 30% of fiber length. Total cross-sectional area (CSA, cm^2) and specific P_o (kN/m^2) were determined (3). The differences between the experimental and WT samples were assessed by a 1-tailed Student's *t* test, with the assumption of 2-sample equal variance.

Mouse Behavior Analysis. Locomotor activity was monitored by using Digiscan Animal Activity Monitoring System running Versamax Windows software (Accuscan Instruments). The Versamax Windows software uses a mathematical algorithm to compute total distance traveled (in cm) and rearing number. All mice were tested for 12 h starting from 6PM.

Membrane Damage Assay. The membrane damage assay was performed on skeletal muscle fibers of 6- to 8-week-old mice from *Large*^{myd}, integrin α7-null, and WT littermate control groups. The whole foot was cut off and the skin was removed. The connective tissues and blood vessels were trimmed off to completely expose the muscle fibers. This preparation was placed in a glass-bottom culture dish filled with Tyrode solution containing 1.8 mM Ca^{2+}. Individual fibers were selected for the assay. Regenerating muscle fibers (centrally nucleated or with small diameters) were carefully excluded from the assay. Membrane damage was induced in the presence of 2.5 μM FM 1-43 dye (Molecular Probes) with a 2-photon confocal laser-scanning microscope (LSM 510; Zeiss) coupled to a 10-W Argon/Ti:sapphire laser. After we scanned images predamage, a 7.9-μm x 4.4-μm area of the sarcolemma on the surface of the muscle fiber was irradiated at full power for 1.29 seconds. Fluorescence images were captured at 10-second intervals for 10 min after the initial damage. The fluorescence intensities at the damaged site were semiquantified by using ImageJ software.

Production of Recombinant Glycosylated α-DG. A stable HEK293F cell line (Invitrogen) expressing both of *α-dystroglycan* and *Large* was generated to produce the recombinant α-DG that bound LG domain proteins with high affinity. The recombinant protein was enriched from the SFMII media (Invitrogen) of this cell line by agarose-bound WGA (Vector laboratories).

Injection of Purified Recombinant α-DG into *Large*^{myd} Muscles. Before the injection to *Large*^{myd} mice, the buffer was changed to sterile 0.9% saline by Amicon Ultra (Milipore). The calf, tibial anterior, and paw muscles of *Large*^{myd} mice were injected with 50, 30, and 10 μL of the purified recombinant α-DG (200 μg/mL) or saline, respectively. The muscles were excised 5 days after injection and were analyzed by immunofluorescence staining or membrane damage assay.

Laminin Overlay Assay. Laminin overlay assays were performed on PVDF membranes by using mouse Engelbreth–Hol–Swarm (EHS) laminin as previously described (4). Briefly, PVDF membranes were blocked in laminin-binding buffer (LBB: 10 mM triethanolamine, 140 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.6) containing 5% BSA followed by incubation with laminin overnight at 4 °C in LBB. Membranes were washed and incubated with anti-laminin (Sigma) followed by anti-rabbit IgG–HRP. Blots were developed by enhanced chemiluminescence.

LCMV Treatment of WT Muscle. The WT mouse foot preparation was incubated with or without the UV-inactivated LCMV clone 13 (10^7 pfu/mL) in ice-cold Ca^{2+}/Mg^{2+}-free Tyrode solution for 2 h. The preparation was then washed twice with ice-cold normal Ca^{2+}/Mg^{2+}-containing Tyrode solution, and warmed up to 37 °C. The membrane damage assay was then conducted on these samples as described above.

Electron Microscopy. Mice were anesthetized with ketamine (87.5 mg/kg body weight), and a bilateral sternum incision was performed to expose the left atrium. Mice were perfused with PBS and then with 2% paraformaldehyde in PBS. Quadriceps muscle blocks were dissected into pieces (1 mm x 3 mm) and fixed by using Karnowsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M cacodylate buffer, pH 7.4) for 2 h at 4 °C. Tissue blocks were washed in 0.1 M cacodylate buffer (2 $\times$ 5 min), processed through a 6-hour routine EM processing schedule, and then infiltrated with epon/alardite resin (Electron Microscopy Sciences) on a Leica EM TP automatic tissue processor. Tissues were embedded, oriented longitudinally and transversely, placed in a vacuum-infiltrating oven, and then polymerized at 60 °C for 24 h. Multiple 1-μm thick sections were

stained with 1% toluidine blue in 1% borax. Representative areas were selected, ultrasectioned at 70 nm (silver sections), mounted on 200 mesh athene copper grids, double stained with Reynolds lead citrate and uranyl acetate, and then examined by using a Zeiss 906E electron microscope. Representative digital images were taken by using SIS Keenview camera and software.

1. Cohn RD, et al. (2002) Disruption of DAG1 in differentiated skeletal muscle reveals a role for dystroglycan in muscle regeneration. *Cell* 110:639–648.
2. Mayer U, et al. (1997) Absence of integrin alpha7 causes a novel form of muscular dystrophy. *Nat Genet* 17:318–323.
3. Brooks SV, Faulkner JA (1988) Contractile properties of skeletal muscles from young, adult and aged mice. *J Physiol* 404:71–82.
4. Michele DE, et al. (2002) Post-translational disruption of dystroglycan–ligand interactions in congenital muscular dystrophies. *Nature* 418:417–422.

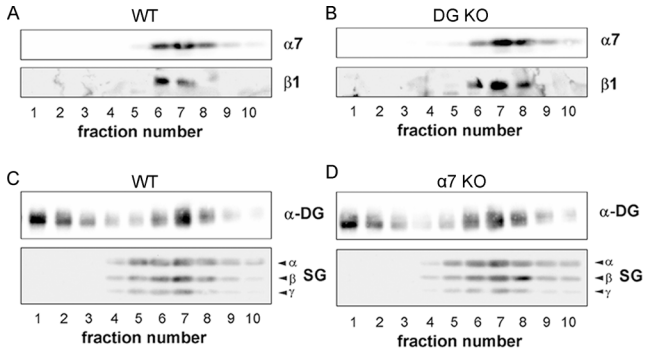


Fig. S1. Characterization of skeletal muscle DG and integrin $\alpha 7$ complexes. (*A–D*) Sucrose gradient fractionation of the DGC and the integrin $\alpha 7$ complex from WT (*A* and *C*), DG-null (*B*), and integrin $\alpha 7$-null (*D*) skeletal muscle solubilized with digitonin. Glycoprotein preparations enriched by WGA-chromatography were fractionated by sucrose gradient centrifugation. Equal volumes of fractions were loaded on an SDS/PAGE gel. The blot generated from this was probed with antibodies against: integrin $\alpha 7$ ($\alpha 7$), integrin $\beta 1$ ($\beta 1$), α-DG (α-DG), α-sarcoglycan (α-SG), β-sarcoglycan (β-SG), and γ-sarcoglycan (γ-SG). Numbers at the bottom of the blot indicate the sucrose gradient fraction number, from top to bottom.

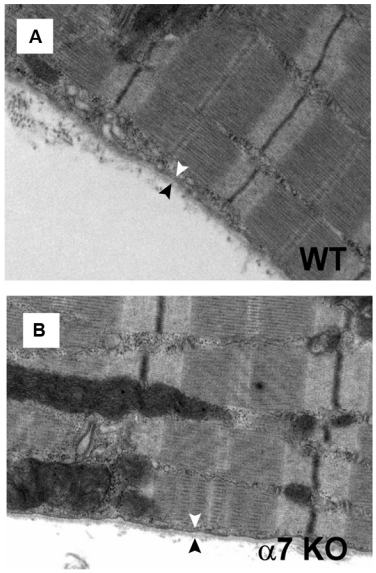


Fig. S2. Ultrastructural analysis of quadriceps muscles from WT (*A*) and α7-null (*B*) mice after exercise. After exercise, there is no detectable abnormality in the basal lamina and the sarcolemma in the muscles from WT and α7-null mice.

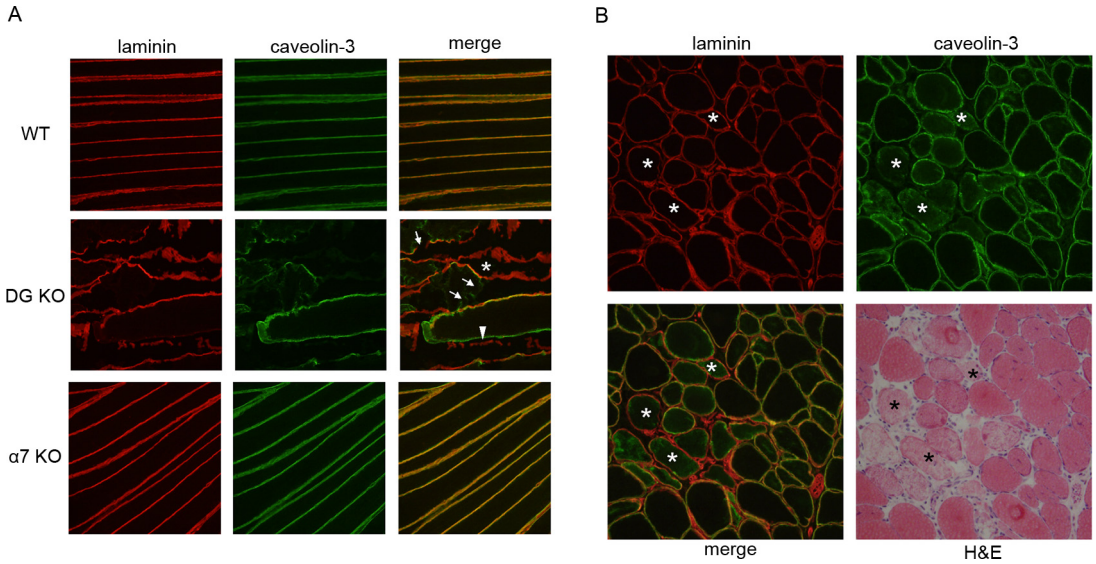


Fig. S3. Exercise-induced dissociation of the basal lamina and the sarcolemma in the DG KO muscle. (*A*) Immunostaining of longitudinal fibers after exercise. Five-week-old mice (WT, DG KO, and α7 KO) were subjected to treadmill-exercise (15° downhill for 20 min). Immediately after the exercise, quadriceps muscles were taken. Longitudinal cryosections were immunostained with laminin and caveolin-3. Arrow, breakage of laminin- or caveolin-3-staining; arrowhead, separation of laminin- and caveolin-3-staining; asterisk, fiber with remaining laminin deposition. (*B*) Acute damage of DG-null muscle after the exercise. Cryosections of DG KO postexercised quadriceps muscle were coimmunostained with laminin and caveolin-3. Serial section was stained with hematoxylin and eosin (H&E). White asterisk, fiber with remaining laminin deposition; black asterisk, necrotic fibers.

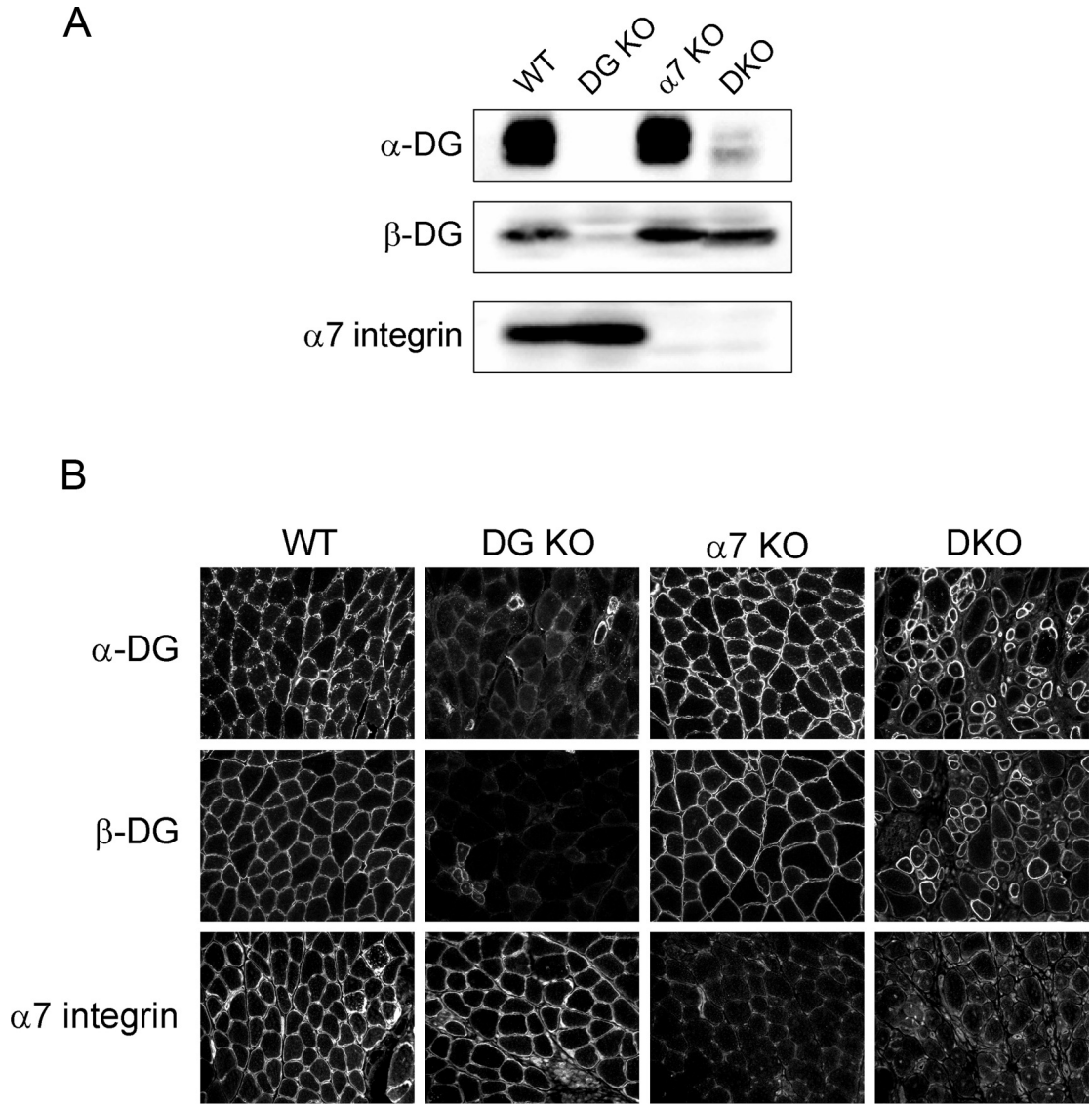


Fig. S4. Disrupted expression of DG and α7 in DG/α7 double mutant (DKO) mice. Western blotting (*A*) and immunofluorescence staining (*B*) analysis showed loss of the DG and α7 expression in DG/α7 DKO muscle. It is of note that the DG expression in DKO is higher than in the DG-null muscle due to the greater regeneration.

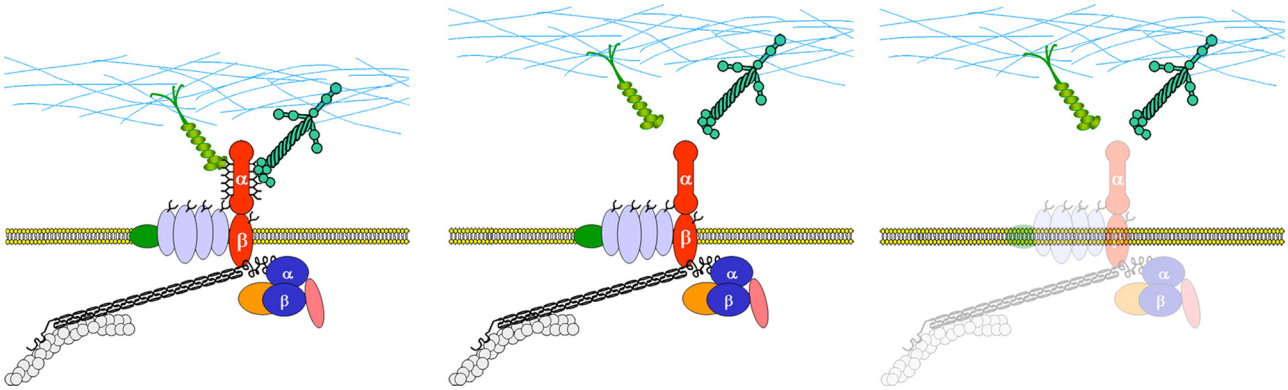


Fig. S5. Schematic models of the DGC in skeletal muscle of different mouse models. (*Left*) WT; (*Center*) *Large*^{myd}; (*Right*) MCK DG-null.

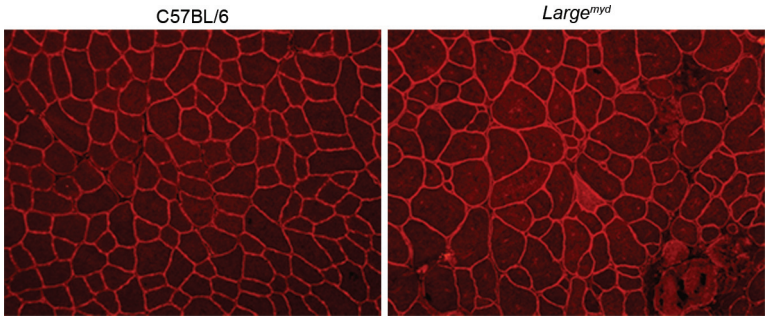


Fig. S6. Immunofluorescence staining of dysferlin in skeletal muscle of *Large*^{myd} mice. Quadriceps muscle sections from WT C57BL/6 and *Large*^{myd} mice were stained with the anti-dysferlin antibody Hamlet.

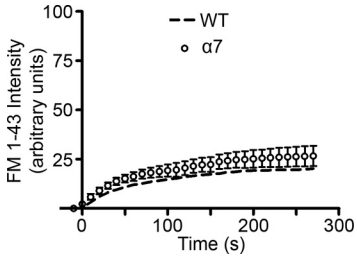


Fig. S7. Damage assay on skeletal muscle of α7-null mice. Plot of FM 1-43 fluorescence intensity against time in integrin α7-null (open square, $n = 5$) muscle fibers. The dashed curve represents mean fluorescence intensity in the membrane damage assay, in wild-type muscle from Fig. 4*E*.

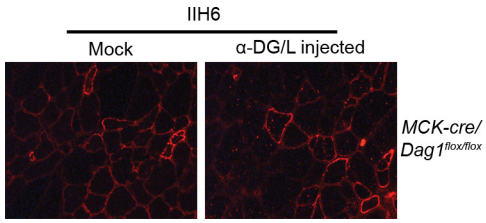
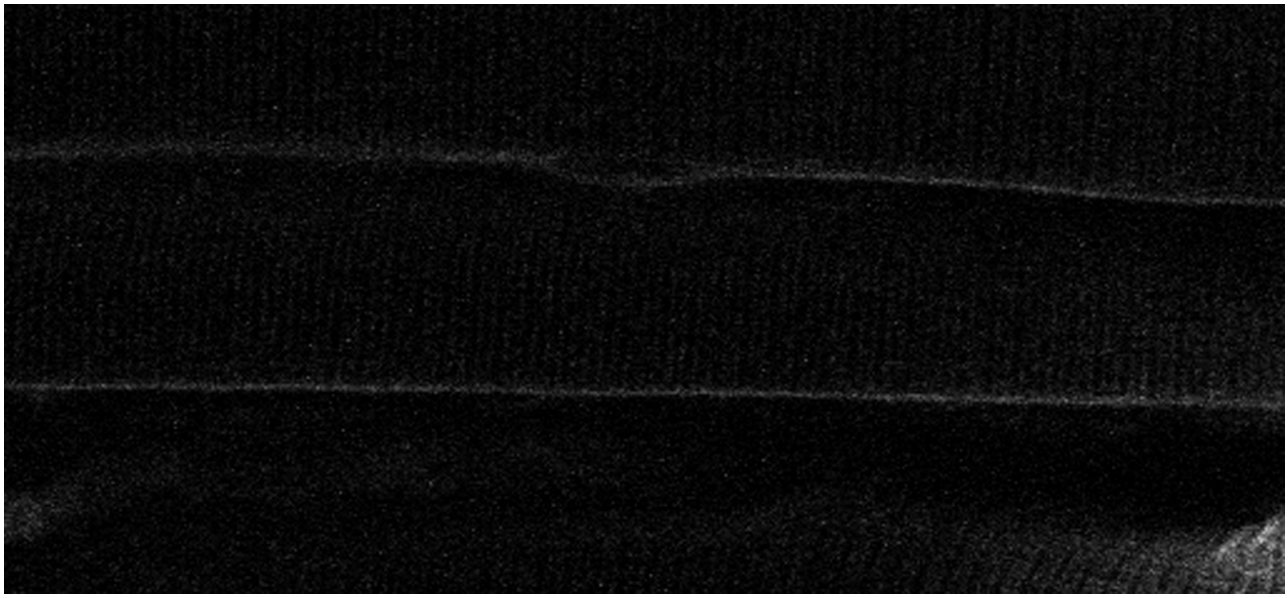


Fig. S8. Stability of recombinant α-DG on the sarcolemma of DG-null muscle fibers DG-null. DG-null muscles injected with recombinant α-DG/L (α-DG/L injected) or saline (Mock) were stained with the glycosylated α-DG antibody (IIH6). Recombinant α-DG failed to stay on the sarcolemma of DG-null muscles, suggesting that β-DG is required for securing the recombinant α-DG on the sarcolemma.

Table S1. Severe loss of body weight and muscle mass in DG/α7 DKO mice

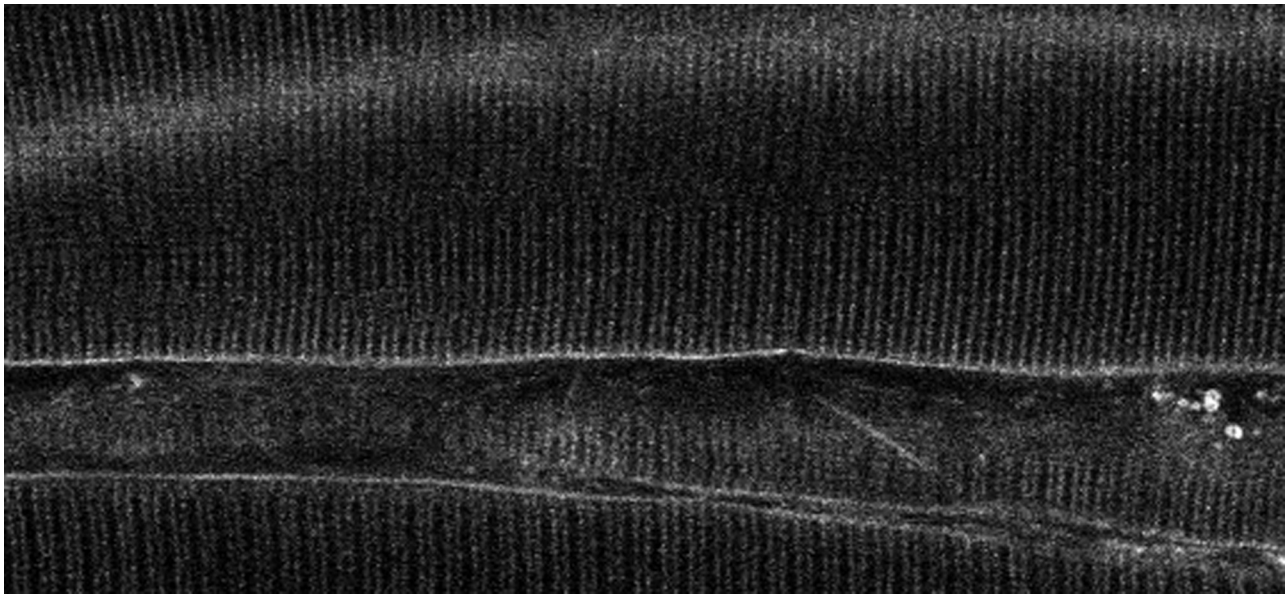
	WT	DG KO	α7 KO	DKO
Body weight, g	23.6 ± 0.4	21.7 ± 3.1	20.8 ± 1.0*	11.2 ± 0.9***
Gastrocnemius, mg	121.4 ± 5.5	125.1 ± 8.3	122.8 ± 8.2	37.5 ± 0.6***
TA, mg	34.9 ± 5.5	33.5 ± 1.5	35.0 ± 1.6	10.1 ± 2.4***
Triceps, mg	72.0 ± 11.6	74.0 ± 21.3	62.3 ± 2.1	22.0 ± 3.2***
Quadriceps, mg	107.4 ± 2.5	126.3 ± 12.5	116.5 ± 17.6	40.6 ± 9.6***
Heart, mg	115.1 ± 14	128.5 ± 34.2	113.9 ± 19.5	71.9 ± 4.8 **
Shin length, cm	2.2 ± 0	2.2 ± 0.1	2.1 ± 0.1	2.1 ± 0.1

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; $n = 3$.



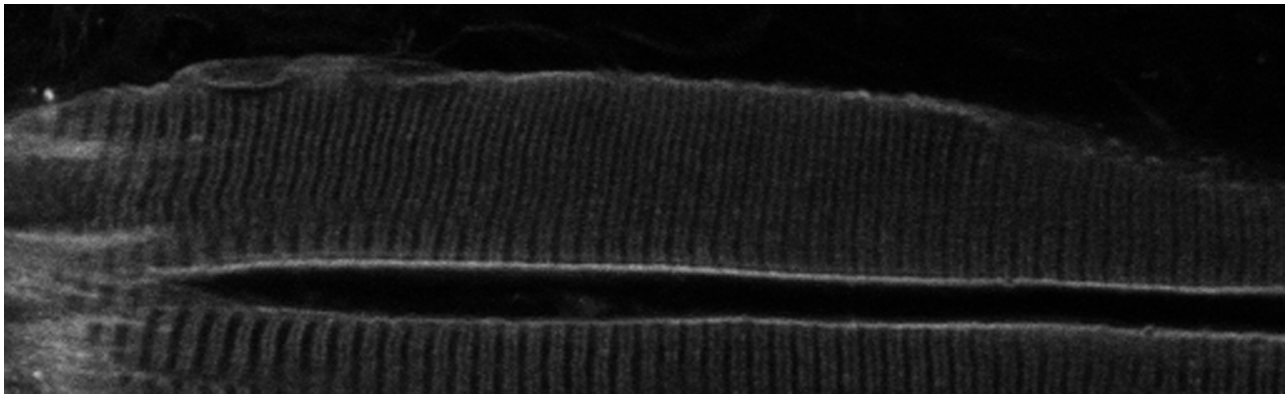
Movie S1*A*. In situ membrane damage assay in C57BL/6 WT muscle.

Movie S1*A* (AVI)



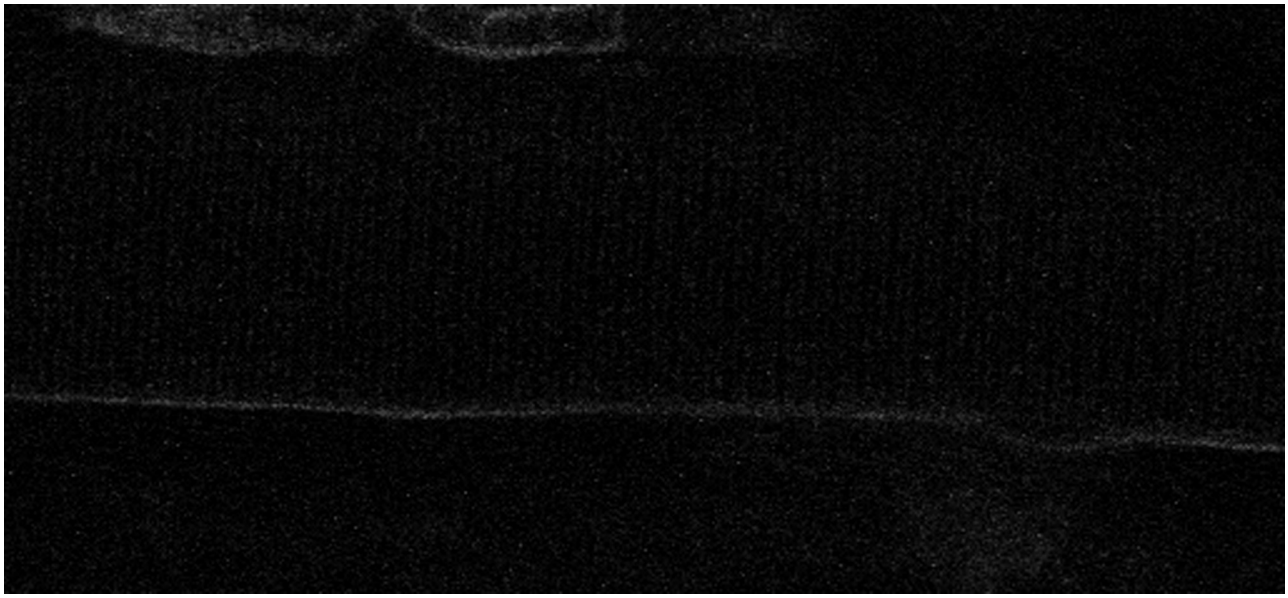
Movie S1*B*. In situ membrane damage assay of *Large*^{myd} muscle in regular buffer.

Movie S1*B* (AVI)



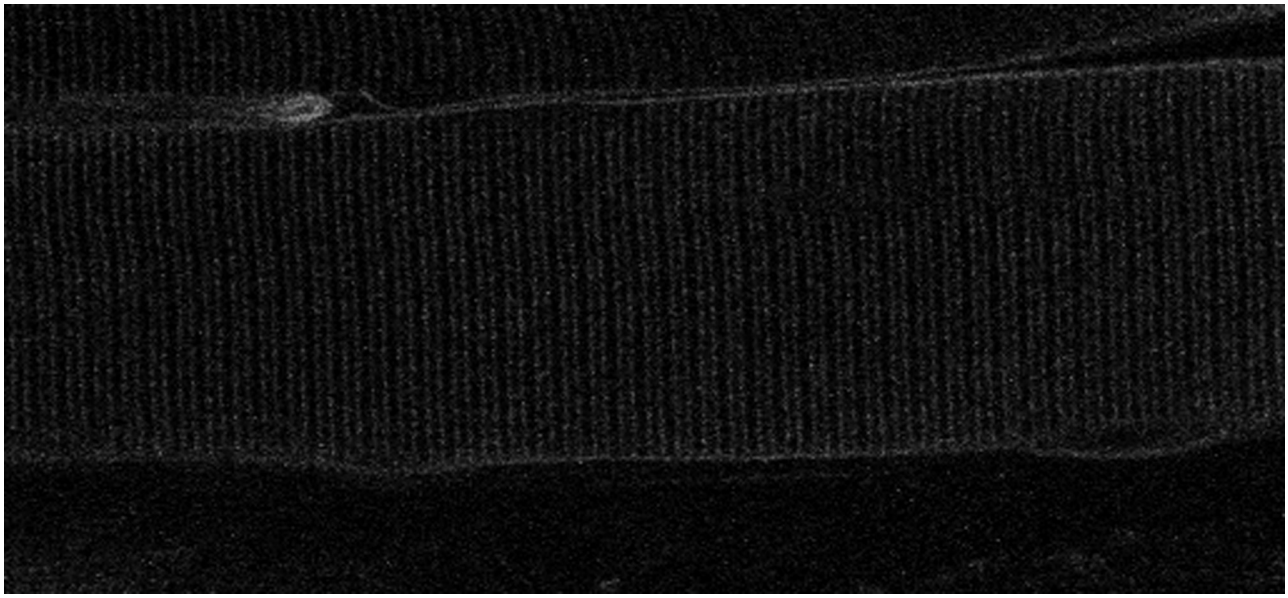
Movie S1C. In situ membrane damage assay of *Large*^{myd} muscle in hyperosmotic buffer.

Movie S1C (AVI)



Movie S2*A*. In situ membrane damage assay of C57BL/6 WT muscle subjected to mock treatment.

Movie S2*A* (AVI)



Movie S2*B*. In situ membrane damage assay of C57BL/6 WT muscle subjected to LCMV treatment.

Movie S2*B* (AVI)

Movie S3. Balloon movie.

Movie S3 (WMV)