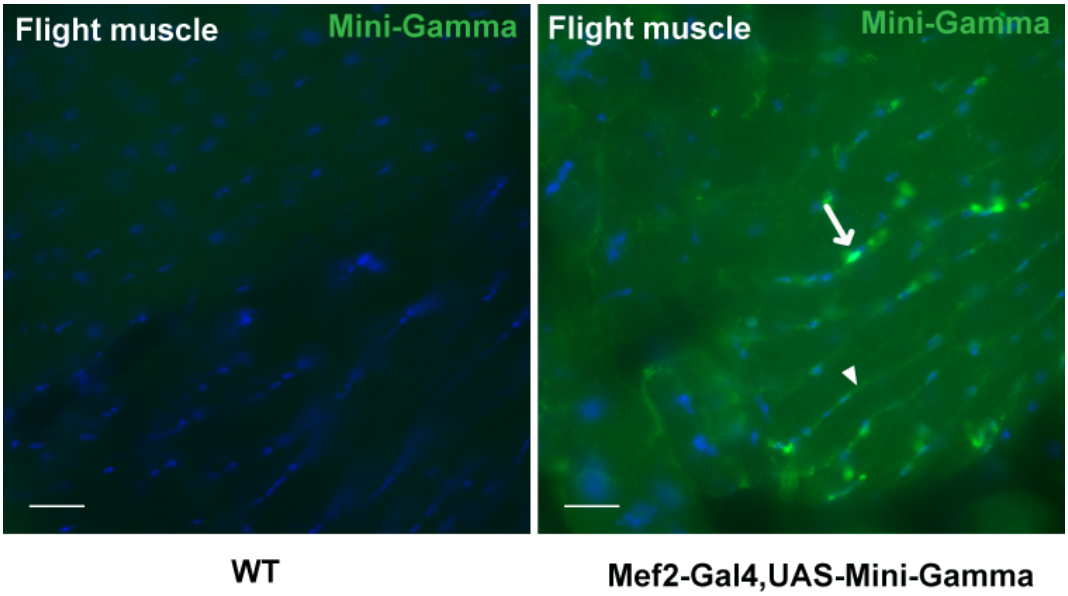
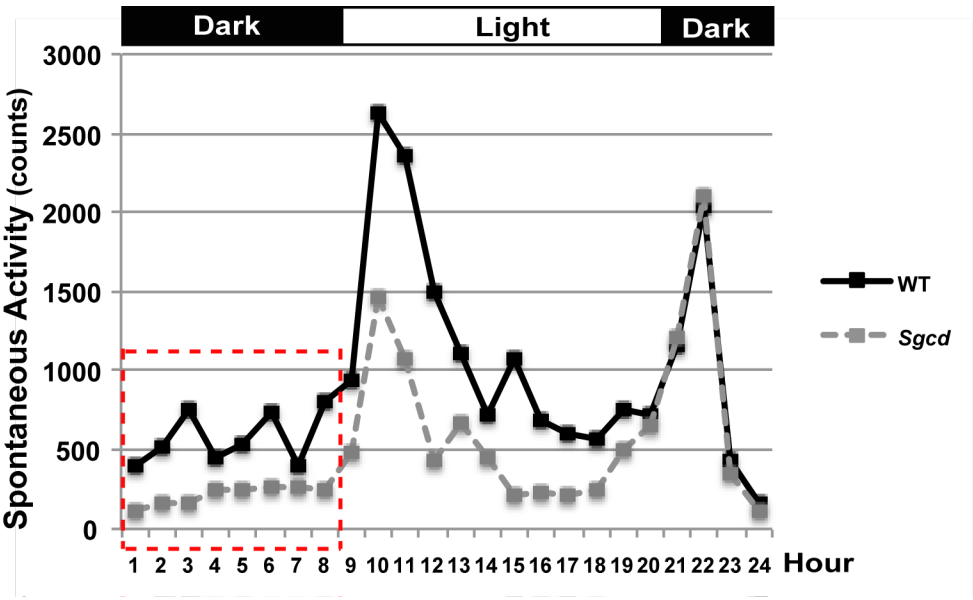
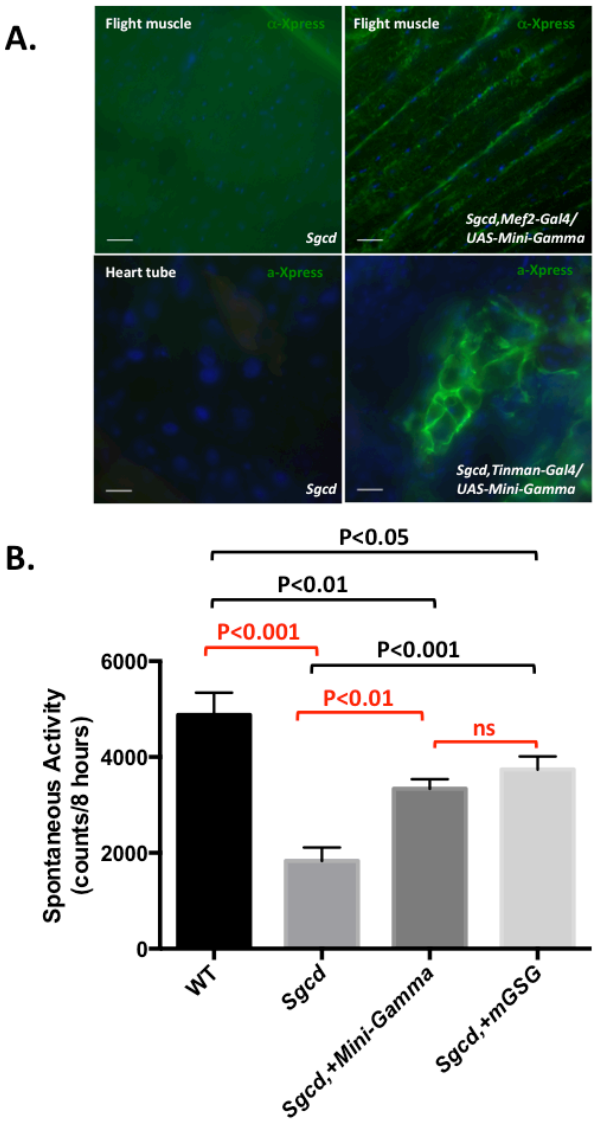
Supplemental Figures and Legends



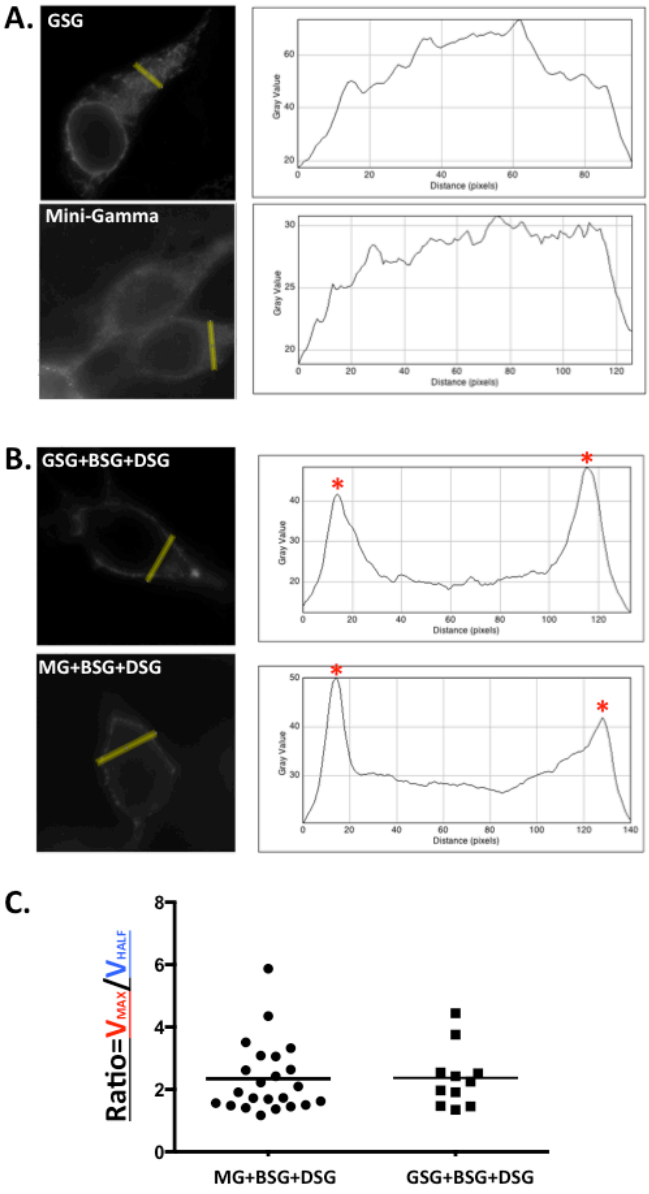
Supplemental Figure S1. Accumulation of Mini-Gamma in *Drosophila* flight muscle. Immunofluorescence microscopy for the Xpress epitope tag in wildtype flies without the transgene reveals no reactivity (left panel). Expression of Mini-Gamma in presence of the type *Sgcd* allele was associated with sarcolemma-associated staining (arrowhead) as well as some aggregation within muscle (white arrow) (right hand panel).



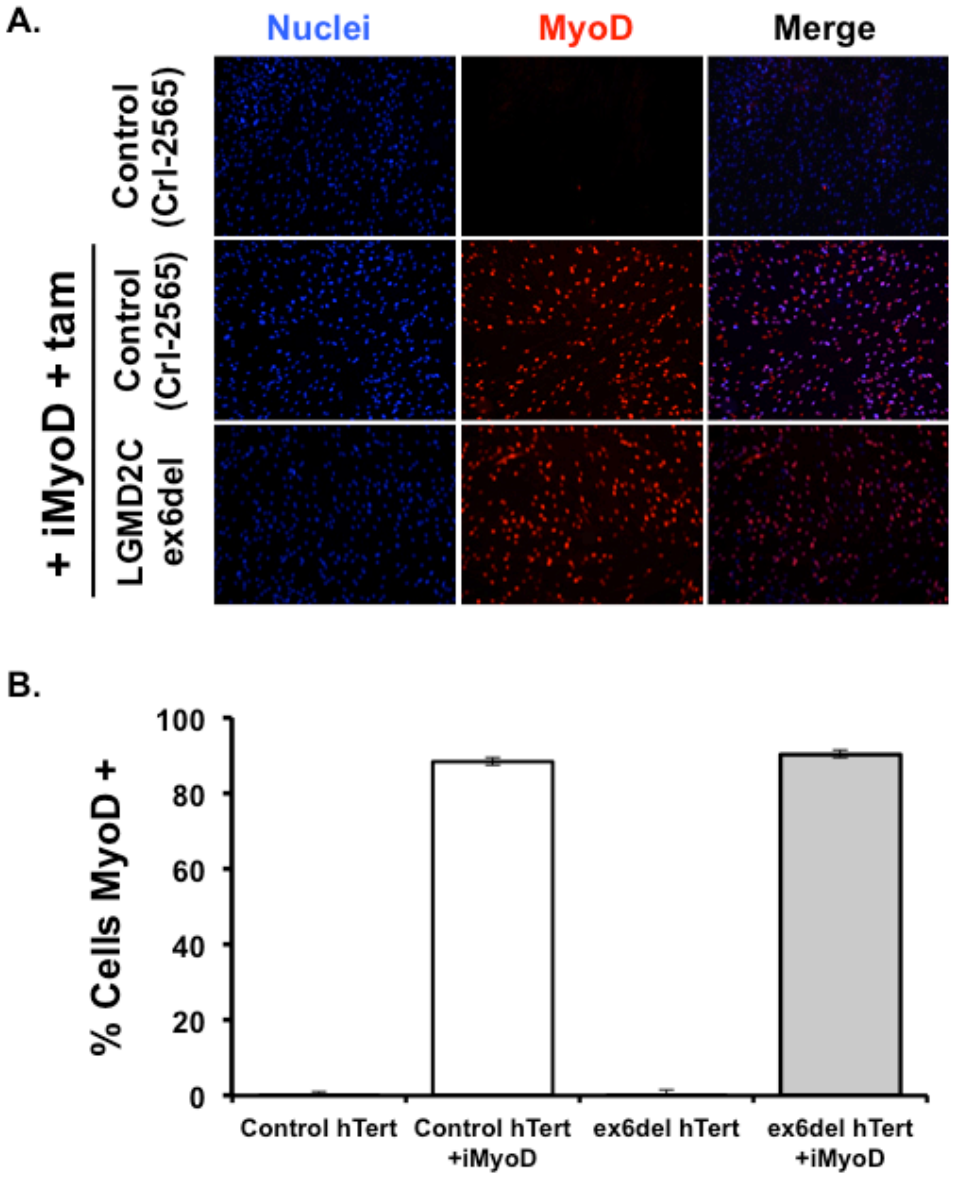
Supplemental Figure S2. *Drosophila* activity recorded over 24 hours. There is a peak of activity at dawn and dusk, consistent with insect behavior. Activity was compared during the nocturnal interval midnight to 8 AM (red boxed area).



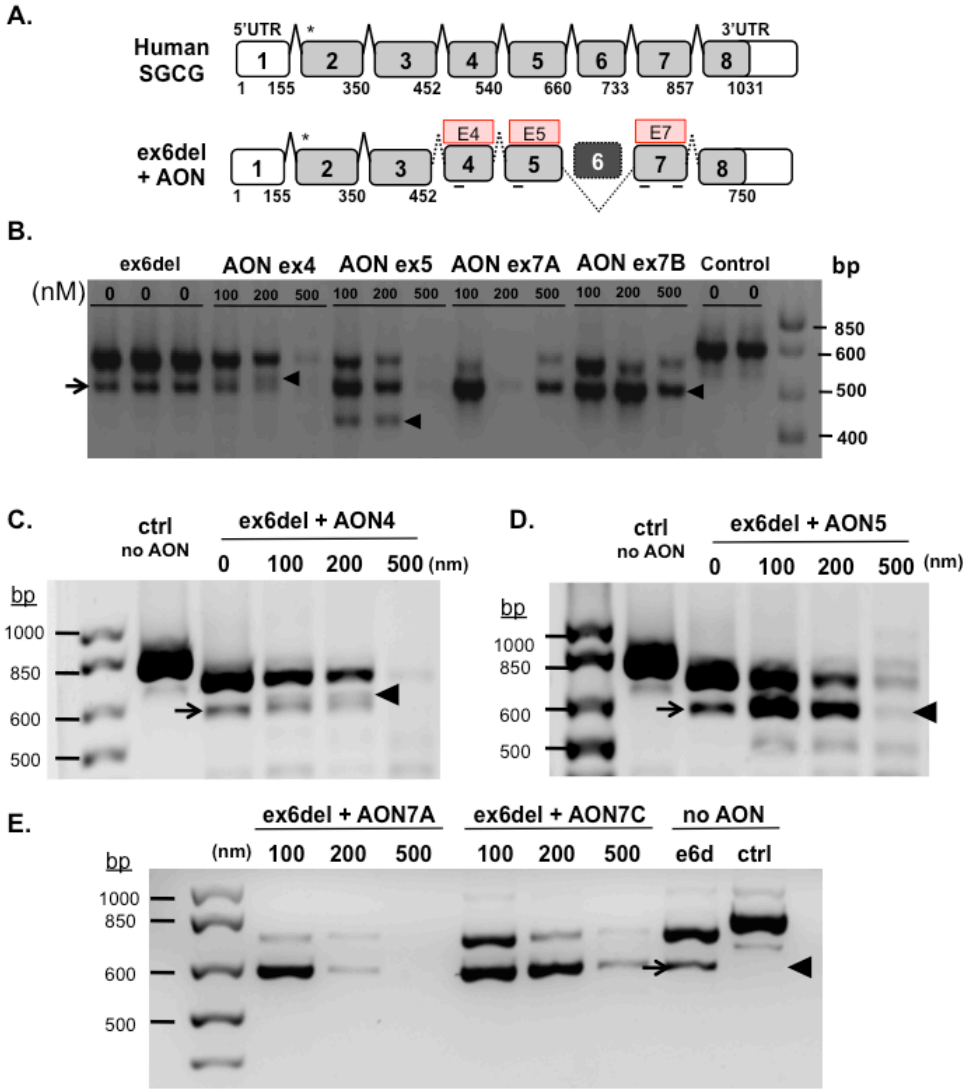
Supplemental Figure S3. Negative control for *Drosophila* Muscle sections and One Way Anova comparison of *Drosophila* activity comparing all groups. **A.** The anti-Xpress epitope antibody was used on both flight muscle and heart tube in *Sgcd* null flies without transgenes (left panels). The right panels depict expression of Mini-Gamma with sarcolemma localization. **B.** One Way Anova comparison of activity demonstrating that mouse Mini-Gamma restores activity as well as full-length mouse γ-sarcoglycan (mGSG) in *Sgcd* null flies.



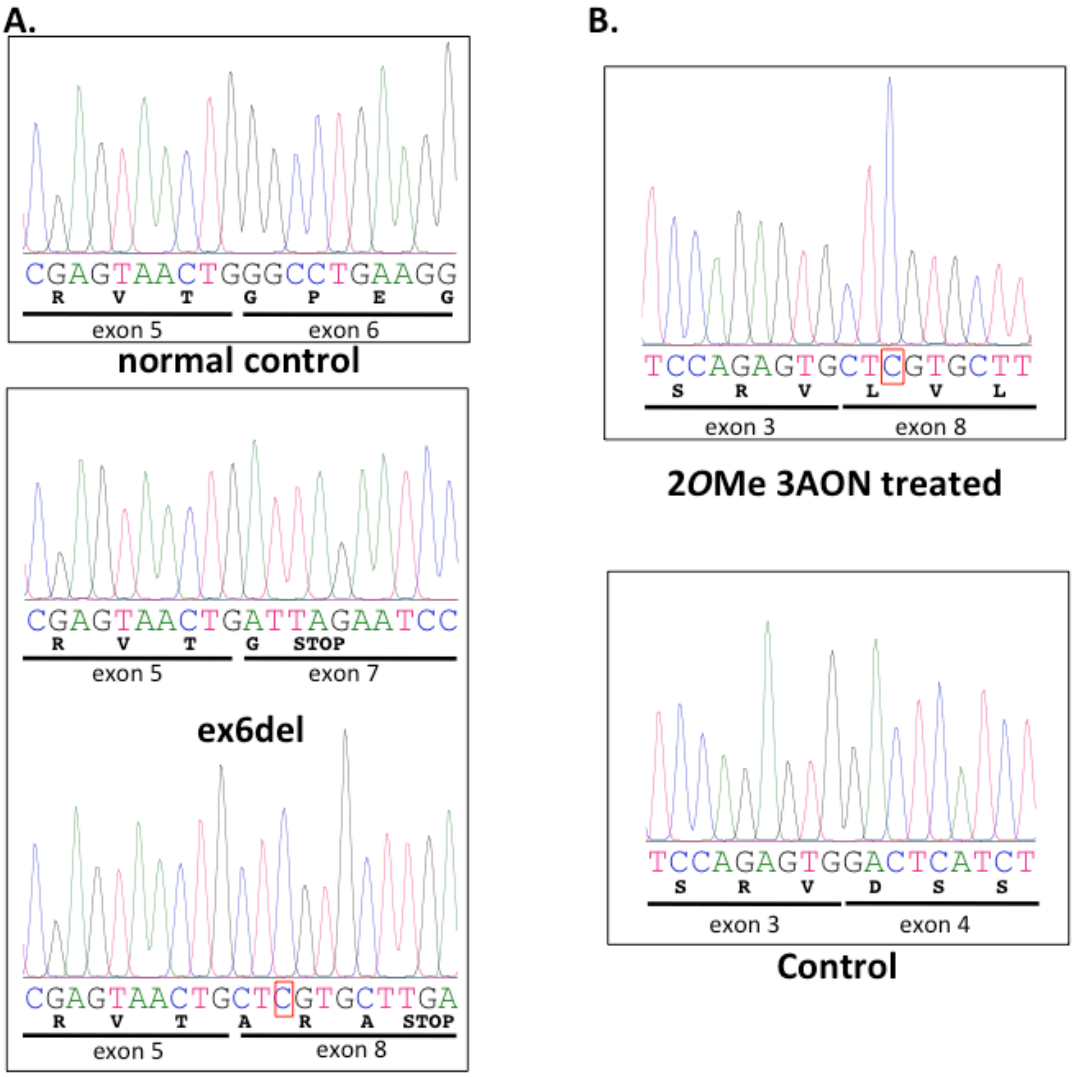
Supplemental Figure S4. Mini-Gamma localizes to the membrane in the presence of β-sarcoglycan (BSG) and δ-sarcoglycan (DSG). A. HEK293T cells were transfected with γ-sarcoglycan (GSG) alone or Mini-Gamma alone which produced little membrane localization as documented by line scanning through the fluorescence image (line scan traces shown to the right demonstrate mostly cytoplasmic localization). **B.** GSG plus BSG and DSG resulted in membrane localization seen as peaks in the line scan corresponding to the membrane (red asterisks). **C.** The line scan ratio was determined between the signal at the peak (representing membrane) and half the distance between the midpoint and the peak for multiple cells (representing cytoplasm). This line scan ratio, referred to as V_{MAX}/V_{HALF}, did not differ between expression of GSG or Mini-Gamma when each was co-expressed with BSG or DSG. These data suggest equal trafficking to the membrane by Mini-Gamma and GSG in the presence of the other sarcoglycans.



Supplemental Figure S5. Transduction of human fibroblasts with lentiviral induced-MyoD (iMyoD). A. Expression of MyoD (red) in human control and LGMD2C ex6del fibroblasts transduced with lentiviral iMyoD construct +/- 4OH-tamoxifen (5 μM, 48h). The top row represents control cells without MyoD and without tamoxifen. The bottom two rows have both MyoD and tamoxifen. Nuclei were labeled with Hoechst 3342 (blue). **B.** Image J analysis of MyoD positive nuclei after iMyoD transduction, demonstrating >80% transduction efficiency. A minimum of 2 fields and >100 cells were analyzed for un-transduced cells. A minimum of 3 fields and > 500 cells were analyzed for cells transduced with the iMyoD construct.



Supplemental Figure S6. Single AON mediated exon skipping in LGMD2C ex6del reprogrammed cells. **A.** Exon organization for human control and exon 6 deletion (ex6del) mutant *SGCG* transcripts. Individual AON exon skipping targets for ex6del *SGCG* are indicated (red boxes), and the position of AON sequences are indicated with black lines. **B.** Gel electrophoresis of RT-PCR amplified control and ex6del *SGCG* transcripts, after treatment with single 2OMePS AON targeting exons 4, 5, or 7 (100-500nM AON, 48h). Arrow indicates endogenous skipping of exon 7 seen in ex6del cells. This was verified by sequencing. The arrow head indicates the expected size product for single exon skipping. **C.** Exon skipping with AON4 induces skipping of the appropriately sized product lacking exon 4 (arrowhead). **D.** Exon skipping with AON5 induces the appropriately sized product (arrowhead). **E.** AON7A and 7C induce robust single exon skipping. The arrowhead indicates the appropriately sized product and reduction of the unskipped product. 2OMePS transfection demonstrated dose-dependent cytotoxicity at higher AON concentrations by the 48h time point.



Supplemental Figure S7. Chromatograms of the sequencing results that depict splice junctions of normal control, ex6del and endogenous exon 7 skipped transcripts. **A.** The images show sequencing results from RT-PCR products from MyoD reprogrammed fibroblasts representing the *SGCG* transcripts from normal control and the ex6del LGMD 2C individual. Normal control shows the expected exon5-exon6 junction. The ex6del individual had two transcripts. One transcript had an exon 5-exon 7 junction consistent with the genomic deletion of exon 6. Additionally, there was a lesser abundant transcript that excluded exon 7 (lowest panel) resulting in an exon 5-exon 8 junction. This individual carried a synonymous variant (T→C) in exon 8 of the *SGCG* gene (red box). **B.** Chromatogram of the sequencing results for ex6del transcripts after treatment with 3AON 2*O*Me exon skipping cocktails. A reading-frame corrected Mini-Gamma transcript is shown that includes exons 2, 3 and 8. Below is the chromatogram depicting the junction of exons 3 and 4 as expected in controls. The red box indicates the synonymous variant observed in exon 8 of the ex6del LGMD2C transcripts.

Supplemental Table 1. Antisense Oligonucleotide (AON) Sequences used for skipping

2'-*O*-Methyl AON	*SGCG* Target*	Sequence (5'-3')
AON4	+15+39 exon4	AGUCACAUUCUGGGUUGAUUGUAGA -25nt
AON5	+14+38 exon5	CUGAAACUGUUGAUUCUGGACUUCU -25nt
AON7A	+13+37 exon7	GGCAUCCAUGCUUAGACUCCGAGUG -25nt
AON7C	+105+129 exon7	CUCACCAUUCCAUCACUACUAUGAA -25nt

Supplemental Materials and Methods

Methods

Plasmids. The coding region of murine *Sgcg* was amplified from mouse cDNA and cloned into pUAST vector at the Xho1 and Xba1 sites (17). To generate the Mini-Gamma construct, Sgcg exons 2 and exon 3 were amplified separately from exon 8. Both PCR products were digested with BsiHKAI and then ligated. The ligation product was introduced into pCR2.1-TOPO via TA cloning. The product was sequenced. Sequencing revealed a G to A transition at the 4th nucleotide in exon 8, resulting in a valine to isoleucine alteration. However this variant was present in all clones and represented a polymorphism in mice. The start codon and Xpress tag were filled in by Klenow DNA polymerase and inserted into pUAST vector at the EcoR1 and Not1 sites. Xpress protein tag consists of eight amino acids: DLYDDDDK. The Mini-Gamma sequence was then ligated into pUAST-Xpress. The pUAST-Mini-Gamma was digested and inserted into pcDNA3.0 vector at EcoR1 and Xho1 sites to generate PCMV-Mini-Gamma for expression in cells. Mouse *Sgcb* (MR204617) and mouse *Sgcd* (MR221060) cDNA ORF clones were purchased from OriGene (Rockville, MD). Both vectors contain CMV promoters and Myc-DDK tags at the C-terminus of the respective sarcoglycan protein.

***Drosophila* breeding and husbandry.** Flies were raised on standard medium at 25°C with 12 hours light/dark cycling. To express murine γ-sarcoglycan in *Sgcd*^{840} mutants, *Sgcd*^{840} allele (on X chromosome) and *Mef2-Gal4* transgene (on chromosome 3) were first recombined into one fly strain *Sgcd*^{840}*; Mef2-Gal4*. *Sgcd*^{840}*; Mef2-Gal4* virgin females were collected and mated with either *UAS-Sgcg* or *UAS-Mini-Gamma* males. Since *Sgcd*^{840} allele is on the X chromosome, all male progeny from this cross was null for fly *Sgcd* and expressed either murine full-length γ-sarcoglycan or Mini-Gamma in muscle. Five to 15 males were collected upon eclosion every day over the course of 3 to 7 days. Flies were flipped into fresh vials every three days during the aging process. yw and *Sgcd*^{840} males were collected at the same time and aged in the same manner.

***Drosophila* activity assay.** The MB5 MultiBeam Activity Monitor (TriKinetics, Waltham, MA) was used to quantify fly basal activity, and all activity assays were performed on flies that had been aged to 20 days after eclosion. After anesthetization by CO_2, individual flies were loaded into single glass tubes. One end of the glass tube was dipped in standard fly food and further sealed with a rubber cap. The other end of the glass tube was loosely sealed to allow ready air transfer. Sixteen flies were evaluated for activity in independent tubes simultaneously by monitoring infrared beam breaks. The DAMSystemMB 106X software was used to record activity at 1-minute intervals over 24-48 hours, and the DAMFileScan 108X was used to verify and process raw data (Trikinetics). Prism (Graphpad, San Diego, CA) was used for data analysis. Student's t-test was used to compare results between two groups.

Optical coherence tomography (OCT). OCT was performed as previously described (24). Ten to twelve male flies from each group were assessed at 7 days after eclosion. The end-systolic and end-diastolic diameters for individual fly were entered into Prism (Graphpad, San Diego, CA). One-way analysis of variance with a *post hoc* Turkey test was used to compare among multiple groups.

Generation of Mini-Gamma transgenic mice and mouse breeding. The desmin (Des) promoter was amplified from human genomic DNA to obtain the short promoter previously characterized by (27). The Des promoter sequence was then inserted into the CMV-Mini-Gamma vector at SpeI and EcoR1 sites, replacing the CMV promoter while keeping the start

codon, the Xpress tag and Mini-Gamma coding sequence intact. The Des-Mini-Gamma sequence was amplified and introduced into pCR2.1-TOPO via TA cloning, then digested at the BamH1 and Not1 sites. The sequence was verified by Sanger sequencing. The digestion product was purified and injected into C57BL/6J embryos at the University of Chicago Transgenic Core. Founders were screened by PCR on genomic DNA isolated from tail clippings. Two transgenic lines were established and maintained as heterozygotes. The primers used for genotyping were mini-Forward: 5'- CGAATTCACCATGGATCδTACGACGA-3' and mini-Reverse: 5'-CTAGATGCATGCTCGAGTCAAAGACAG-3'. Transgenic positive animals show a single band at 530bp. The targeted deletion to generate a null mutation of *Sgcg* was previously described (29), and this allele was previously bred through more than ten generations into C57BL/6J (52). The Des-Mini-Gamma transgene mice were bred to *Sgcg* null mice in the C57BL/6J background. Transgenic positive *Sgcg* null mice and transgenic negative *Sgcg* null littermates were compared. Animal work was conducted under the approval of the University of Chicago and Northwestern IACUCs.

Immunofluorescence microscopy for *Drosophila*, HEK cells, and mice. Fifteen to 25 whole flies were anesthetized and covered in tissue freezing medium (TFM, Triangle Bioscience, Durham NC), chilled in isopentane for 5 minutes, followed by liquid nitrogen for another 5 minutes. Mouse muscles were harvested and snap-frozen in liquid nitrogen. The samples were kept frozen in -80^{o}C freezer until sectioning. Ten μm sections were cut from frozen tissues and immediately fixed in ice-cold methanol for 2 minutes and briefly rinsed in cold phosphate buffered saline (PBS) immediately afterwards. The sections were blocked in PBS containing 5% fetal bovine serum and 0.1% Triton-X for 2 hours at 4^{o}C. The samples were then incubated with primary antibodies diluted in blocking solution at 4^{o}C overnight, followed by three 10-minute washes with PBS containing 0.1% Triton-X at 4^{o}C. The sections were incubated with secondary antibody for 2 hours at 4^{o}C. Samples were washed and then mounted with VECTASHIELD Mounting Medium with DAPI H-1200 (Vector Labs, Youngstown, OH). For HEK 293T cells, a sterilized cover slip was place in each well of 6-well cell culture plates before cells were plated. Transfection was performed on the next day and cells were harvested 40 hours after transfection. Cell culture media was aspirated and cells were rinsed once with cold PBS. Fixation and staining methods were the same as above. Images were collected using an Axiophot microscope with iVision software and edited using Adobe Photoshop CS4 and Image J in concert with NIH policy on appropriate image manipulation.

Antibodies for Drosophila, HEK cells and mice. To detect full-length murine γ-sarcoglycan (SGCG) protein, rabbit polyclonal anti-SGCG antibody was used (43). To detect Mini-Gamma protein, a rabbit polyclonal antibody was raised to the Xpress epitope (Pocono Rabbit Farms, Canadensis, PA) and affinity-purified. The rabbit polyclonal anti-SGCG antibody NBP1-90298 was used (Novus Biologicals, Littleton, CO). β-Sarcoglycan was detected with NCL-b-SARC (Leica Biosystems, Nussloch, Germany) and δ-sarcoglycan was detected with a polyclonal antibody (21). Secondary antibodies were Alexa Fluor® 488 Goat Anti-Rabbit and Alexa Fluor® 594 Goat Anti-Rabbit 594 (Invitrogen, Carlsbad, CA). For microscopy, primary antibodies were used at 1:500 dilutions, and the secondary antibodies were used at 1:8000 with blocking buffer. For immunoblotting, antibodies were used at 1:1000 dilutions. For immune-precipitation (IP), 25μL NCL-b-SARC or 10μL rabbit anti-Xpress antibody was used from muscle lysates. Fifteen μL NCL-b-SARC or 5 μL rabbit anti-Xpress was used for IP from HEK cell lysates. Secondary antibodies were anti-mouse or anti-rabbit Horseradish Peroxidase conjugated antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) and used at 1:8000 dilution. Membranes were developed using Clarity Western ECL Substrate (Bio-Rad, Hercules, CA) and visualized by BioSpectrum Imaging System (UVP, Upland, CA).

Microsome preparation. Membrane-bound proteins were isolated following the protocol of (28) with modifications. Seven distinct muscle groups were dissected, including triceps, diaphragm, abdominal muscle, quadriceps, gluteus, hamstring and gastrocnemius muscles and combined. Muscles from one animal were homogenized in 12mL pre-chilled Buffer A (20mM sodium pyrophosphate, 20mM sodium phosphate monohydrate, 1mM MgCl2, 0.303M sucrose, 0.5mM EDTA, 1mM PMSF, Roche COMPLETE protease inhibitor tablet) using a Tissue Tearor Homogenizer (Model 985-370 Type 2 with 7mm probe, Biospec products, Bartlesville, OK). Homogenized tissues were then transferred to a 15mL Dounce tissue grinder (Sigma-Aldrich) and were dounced 40 times using a tightness "B" pestle on ice. One hundred μL lysate was removed as "total protein" (T). Lysates were centrifuged at 9000 rpm for 18 minutes at 4°C using SW41T1 rotor (13,900g). The pellet was discarded and100μL supernatant was removed as "cytoplasmic protein" (C). The remainder of the supernatant was transferred to a new tube and centrifuged again at 13.200rpm for 30 minutes at 4°C (30,000g). One hundred μL supernatant was removed as "light microsomes" (L). The pellet was resuspended in 12mL pre-chilled KCL wash buffer (0.6M KCl, 0.303M sucrose, 50mM Tris-HCl pH 7.4, 1mM PMSF, Roche COMPLETE protease inhibitor tablet), incubated for 30 minutes on ice to remove actomyosin contamination. The suspension was then centrifuged again at 28,800rpm for 30 minutes at 4°C (142,000g). The pellet was resuspended in 300 to 500μL co-IP buffer (50mM Tris-HCl, 150mM NaCl, 1mM EDTA, 1mM EGTA, 0.1% Triton x-100, 0.1% SDS, 1mM PMSF, Roche COMPLETE protease inhibitor tablet) and saved as "heavy microsomes" (H). For co-IP experiments, fresh heavy microsomes were used without freeze-thaw cycles. Protein concentration was determined using the BioRad assay.

Transfection of HEK cells. Human Embryonic Kidney (HEK 293T) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), to which 10% fetal bovine serum and 1% penicillin-streptomycin was added. FuGene® HD transfection reagent (Promega, Fitchburg, WI) was used to transfect HEK cells with sarcoglycan plasmids using the manufacturer's protocol. Cells were harvest 40-48 hours post transfection, briefly washed with ice-cold PBS once before 300μL pre-chilled co-IP buffer per 10cm plate was applied. Cell were collected and transferred to 1.5mL centrifuge tubes on ice, titurated three times with insulin syringes. The lysates were then centrifuged at 14K rpm for 10 minutes at 4°C and the supernatant was used for co-IP experiments.

Co-immunoprecipitation from HEK cells and muscle. Co-immunoprecipitation (co-IP) was performed according to published protocols (21) with modifications. Five hundred μg mouse skeletal muscle heavy microsomes or 650 μg HEK 293T cell lysates were pre-cleared with 45 μL Protein G Plus/Protein A Agarose Suspension (EMD Millipore Chemicals, Billerica, MA) for 1h at 4°C. The protein G/A beads were washed 3 times with co-IP buffer and then pre-cleared with a brief centrifugation. Pre-cleared samples were then incubated with antibodies at 4°C for 3 hours or overnight. After primary incubation, the samples were incubated with protein G/A beads for 2 hours at 4°C (100μL bead suspension for heavy microsomes, 60μL for HEK cell lysates). The samples were then centrifuged at 4000rpm for 10 minutes at 4°C, and the supernatant was discarded. The beads were then washed for 7 times using pre-chilled wash buffer (co-IP buffer minus SDS). Equal amounts of 2X Laemmli SDS buffer was added to the beads and boiled at 95°C for 5 minutes. The beads were then centrifuged at 14000rpm for 2 minutes at room temperature and discarded, and the supernatant was used for IP.

Immunoblotting. Protein samples were denatured, resolved on pre-cast 14% tris-glycine protein gels (Novex, San Diego, CA) and transferred to Immobilon-P membranes (Millipore,

Bedford, CA). Reversible protein stain (Thermo Scientific, Waltham, MA) was performed on the membranes to evaluate transfer efficiency and equal protein loading. Membranes were blocked for 1h at room temperature with Starting Block T20 blocking buffer (Thermo Scientific, Waltham, MA), followed by incubation with primary antibody diluted in T20 blocking buffer for either 1h at room temperature or overnight at 4^{o}C. After primary incubation, the membranes were washed 3 times, 10 minutes each at room temperature with TBS containing 0.1% Tween-20. The membranes were then incubated with secondary antibody diluted in T20 blocking buffer for 1h at room temperature, followed by 3 washes.

Evans Blue Dye Imaging and Quantification
Evans Blue Dye (EBD) (Sigma, E-2129) was dissolved in phosphate buffered saline (PBS) at 10 mg/ml. Each animal received an intraperitoneal injection of EBD at 5µl/g body weight. Approximately 24 hours after injection, tissues were harvested and imaged. Gross images were acquired with a Canon Digital Rebel T5i. For quantification, quadriceps, abdominal and diaphragm muscles were harvested from 4~5 month-old age- and sex-matched mice (n ≥ 3). The excised muscle was immediately frozen in liquid nitrogen and stored at -80°C. Seven µm sections from the center of the muscle were fixed in 4% paraformaldehyde for 5 minutes, blocked in 10% fetal bovine serum in PBS with 0.1% Triton X-100, and stained with anti-dystrophin antibody (Cat# RB-9024-P, Thermo, Waltham, MA) for 1 hour at room temperature at a dilution of 1:100. A secondary antibody, donkey anti-rabbit conjugated to Alexa-488 (Life technologies, Calsbad, CA) was used at 1:5000. Sections were mounted in Vectashield with DAPI. Images were acquired on a Zeiss Axio Imager.M2. Three to five images of the muscle sections were taken at 10x from at least 3 animals per genotype and analyzed. Statistics were performed with Prism (Graphpad, La Jolla, CA) using student t-test.

Creatine Kinase Assay
Serum was collected mid-morning on the same day from age- and sex- matched (2-5m) *Sgcg, Sgcg Tg50+, and Sgcg Tg84+* animals from retro-orbital bleeds using heparinized capillary tubes (Cat#22-362-566, Fisher, Pittsburgh, PA) into serum separator tubes (Cat#02-675-185, Becton Dickinson, Franklin Lakes, NJ) and centrifuged for 10 minutes at 8000 x g. The plasma fractions were frozen and stored at -80°C and then assayed later using the Enzy-Chrom CK Assay kit (ECPK-100; BioAssay Systems, Hayward, CA). CK activity was measured at 37°C in the Synergy|HTX Multi-mode plate reader (BioTek, Winooski, VT) in Costar 96-well plates (Corning Inc, Corning, NY). Statistics were performed with Prism (Graphpad, La Jolla, CA) using a one-way ANOVA.

Histology. A cross-sectional strip of diaphragm muscle was obtained from midline of the muscle in a longitudinal axis. The strip was then fixed in formalin, dehydrated and embedded in paraffin. Seven µm sections were obtained and stained with hematoxylin and eosin (H&E). For central nucleated fiber analysis, three random fields each were obtained at 20x magnification from six animals of each genotype. For diaphragm thickness calculations, 3 evenly spaced fields along the length of the strip each were taken at 10x magnification from six animals of each genotype. The ruler tool in Photoshop (Adobe, San Jose, CA) was used to calculate the thickness of each field. Diaphragm thickness of each animal was the average of the three different fields.

Additional antibodies for immunofluorescence microscopy.
The anti-dystrophin antibody was Cat# RB-9024-P (Thermo, Waltham, MA). The anti-b-sarcoglycan antibody was Cat# NBP1-19782 (Novus Biologicals, Littleton, CO. The anti-γ-sarcoglycan antibody used to detect Mini-Gamma was Cat # NBP1-90298 Novus Biologicals.

Lentiviral constructs and transduction. A packaged hTert lentivirus, which included a puromyocin selection cassette, was purchased from Applied Biological Materials (ABM, Richmond, BC). A tamoxifen-inducible MyoD lentiviral construct (iMyoD), previously described, was kindly provided by Dr. Jeffrey Chamberlain (University of Washington) and packaged by the Northwestern Skin Disease Research Core(30). Human cells with a deletion of *SGCG* exon 6 (ex6del) and control cells were co-transduced with lentiviral hTert (MOI 5) and iMyoD (MOI 50), then subjected to puromycin selection (10d, 1μg/mL; InvivoGen, San Diego, CA). Lentiviral transductions were performed in growth media without Pen/Strep in the presence of polybrene (8μg/mL) (Millipore, Billerica, MA).

Myogenic reprogramming of fibroblasts. To induce myogenic reprogramming, fibroblasts were seeded on culture plates (ThermoFisher, Waltham, MA) or glass coverslips (#1.5, Electron Microscopy Sciences, Hatfield, PA) in growth media (30,000 cell/cm^2). When cells reached confluence, 5μM 4OH-tamoxifen (Sigma, St.Louis, MO) was added to growth media without Pen/Strep. After 48h, differentiation media (1:1 DMEM:Ham's F10; 5%FBS; 2%Normal Horse Serum (NHS); 1% insulin-transferrin-selenium (Sigma)) was added to cells with 1μM tamoxifen. After 4d, differentiation media was replaced, without 4OH-tamoxifen. Cells were assessed for markers of myogenic differentiation after 10-12 days in differentiation media.

Antisense oligonucleotide design and transfection. 2'-*O*-methyl phosphorothioate (2OmePS) antisense oligonucleotides (AON) were designed in accordance with previously described guidelines (32). AON were synthesized by Integrated DNA Technologies, IDT (**Supplemental Table 1**). Dose response was evaluated for single 2OMePS AON targeting *SGCG* exons 4, 5, or 7 at concentrations from 100-500nM. On differentiation day 9, AONs were transfected into ex6del cells in serum free media using Lipofectamine 3000 (Life Technologies, Grand Island, NY) at a ratio of 2μL:1μg DNA. After 6h, media was replaced with differentiation media without 4OH-tamoxifen, and cells were isolated for analysis 48h later. For read frame correction of the *SGCG* mutation, AONs targeting exons 4, 5, and 7 were co-transfected on differentiation day 9 as described (100nM of each AON, 300nM total). After 6h, media was replaced with differentiation media without 4OH-tamoxifen. Cells were isolated for analysis 3d after AON transfection.

RNA isolation, RT-PCR, and qPCR. Total RNA was isolated with TRIzol (Life Technologies, Grand Island, NY) and reverse-transcribed using qScript cDNA Supermix (Quanta Biosciences, Gaithersburg, MD). For detection of human *SGCG* transcripts, Taqman based PCR was used to amplify 50-100ng cDNA with the following primer set ex1/2Fwd 5'-TCTAAGATGGTGCGTGAGCAG-3' and ex8R 5'-GCCACAGACAGGTACAGCTT-3'. PCR products were separated on a 1.5% 1XTBE low-melt agarose gel (NuSeive, Lonza, Walkersville, MD) supplemented with 10μg/mL ethidium bromide (Sigma, St. Louis, MO), and analyzed with the UVP Transluminator (BioSpectrum, Upland, CA).

Immunofluorescence Microscopy for LGMD2C cells. For detection of MyoD and desmin, cells plated on glass coverslips were washed 3 times with PBS, fixed with 4% paraformaldehyde (15min, RT), rinsed with PBS, permeabilized in 0.25% Triton-X in PBS (20min, RT), and blocked with 10%NHS in PBS (1h, 4°C). Coverslips were incubated overnight at 4°C with primary antibodies diluted with blocking buffer (PBS supplemented with 0.1% Triton-X and 2%NHS). Cells were washed 3 times in PBS, incubated with secondary antibodies diluted in blocking buffer (1h, RT), rinsed with PBS, incubated with Hoechst 3342 diluted 1:10000 in PBS (15min, RT), washed 3 times with PBS, and mounted with VECTASHIELD Mounting Medium H-1000 (Vector Labs, Youngstown, OH). To evaluate MyoD expression, cells were cultured on

coverslips for 48h and treated with 4OH-tamoxifen (5μM, 24h). Desmin expression was evaluated in reprogrammed cells cultured on coverslips (+/-4OH-tamoxifen, 10-12d diff). The anti-MyoD rabbit polyclonal C-20 (1:2000; Santa Cruz Biotechnology, Dallas, TX) and mouse monoclonal anti-desmin D1033 (1:1000, Sigma) were used to detect MyoD and desmin respectively, with secondary antibodies Alexa Fluor® 594 donkey anti-rabbit and Alexa Fluor® 594 donkey anti-mouse (Life Technologies, Grand Island, NY).

Sequencing. cDNA was amplified using Platinum Pfx DNA polymerase (Life Technologies, Grand Island, NY) using the *SGCG* primers described, then separated on a low-melt agarose gel. Individual PCR products were excised from the gel and purified using the Qiaquick Gel Extraction Kit (Qiagen, Hilden Germany). Purified products were cloned into the pCR-Blunt II TOPO vector using the Zero Blunt II cloning kit according to manufacturer's instructions (Life Technologies, Grand Island, NY). Reactions were transduced into One Shot Top 10 cells and plated on LB agar plates supplemented with kanamycin. Individual clones were expanded and the plasmid DNA isolated. Sanger sequencing was conducted by ACGT, Inc (Wheeling, IL) and the sequences were analyzed using Lasergene 12 from DNASTAR (Madison, Wi). Some isolated products were reamplified prior to cloning. In these cases, 1 μL of the original PCR was added to a 50μL reaction and amplified using Platium Pfx DNA polymerase with primer set ex2/3Fwd 5'-TTTCTCCAGCAGGAATGGGC-3′ and ex8R. For this PCR, the number of cycles was reduced to 20 and the annealing temperature was increased by 3°C. The products were again subjected to gel electrophoresis, isolation and cloning, as described.