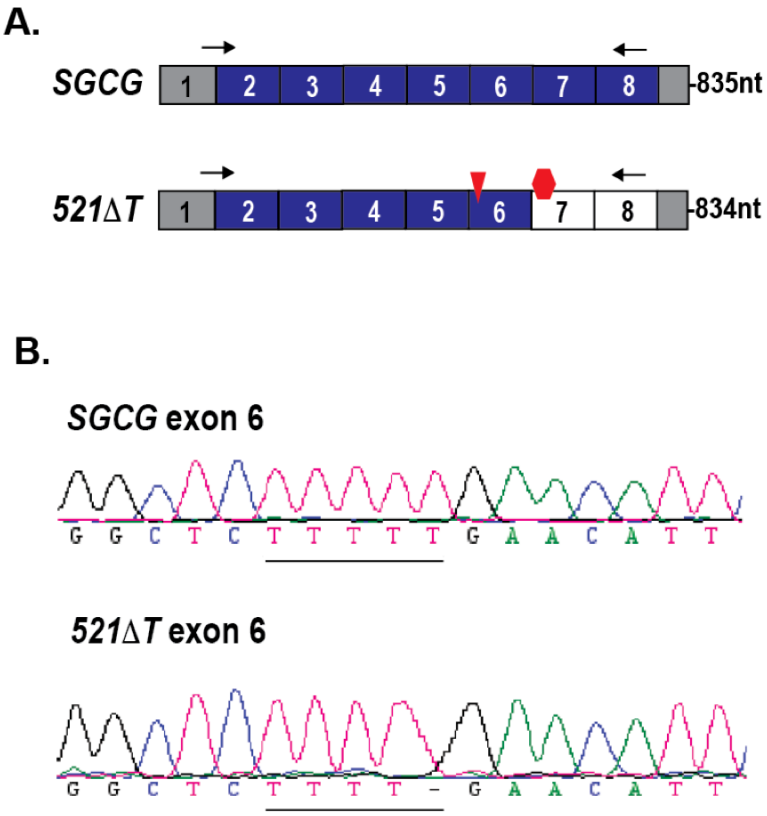
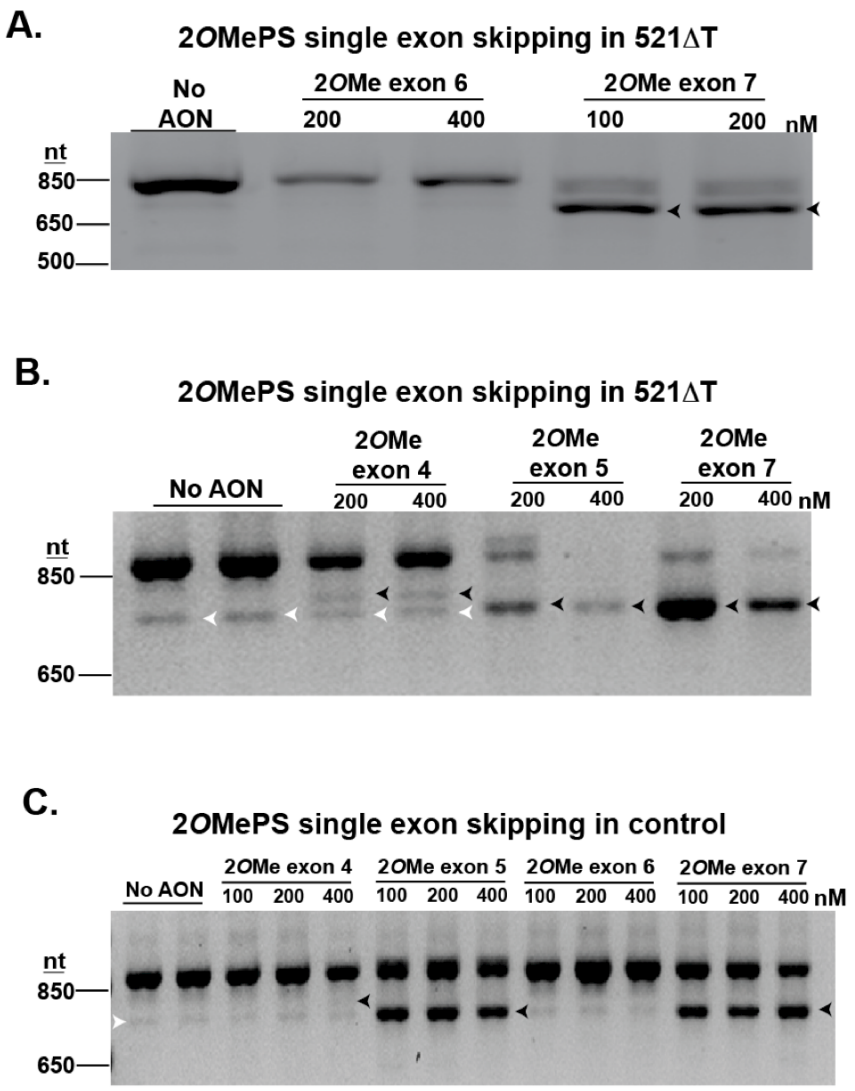
SUPPLEMENTAL MATERIAL

Supplemental Table 1. Cell lines and AON dosing strategies

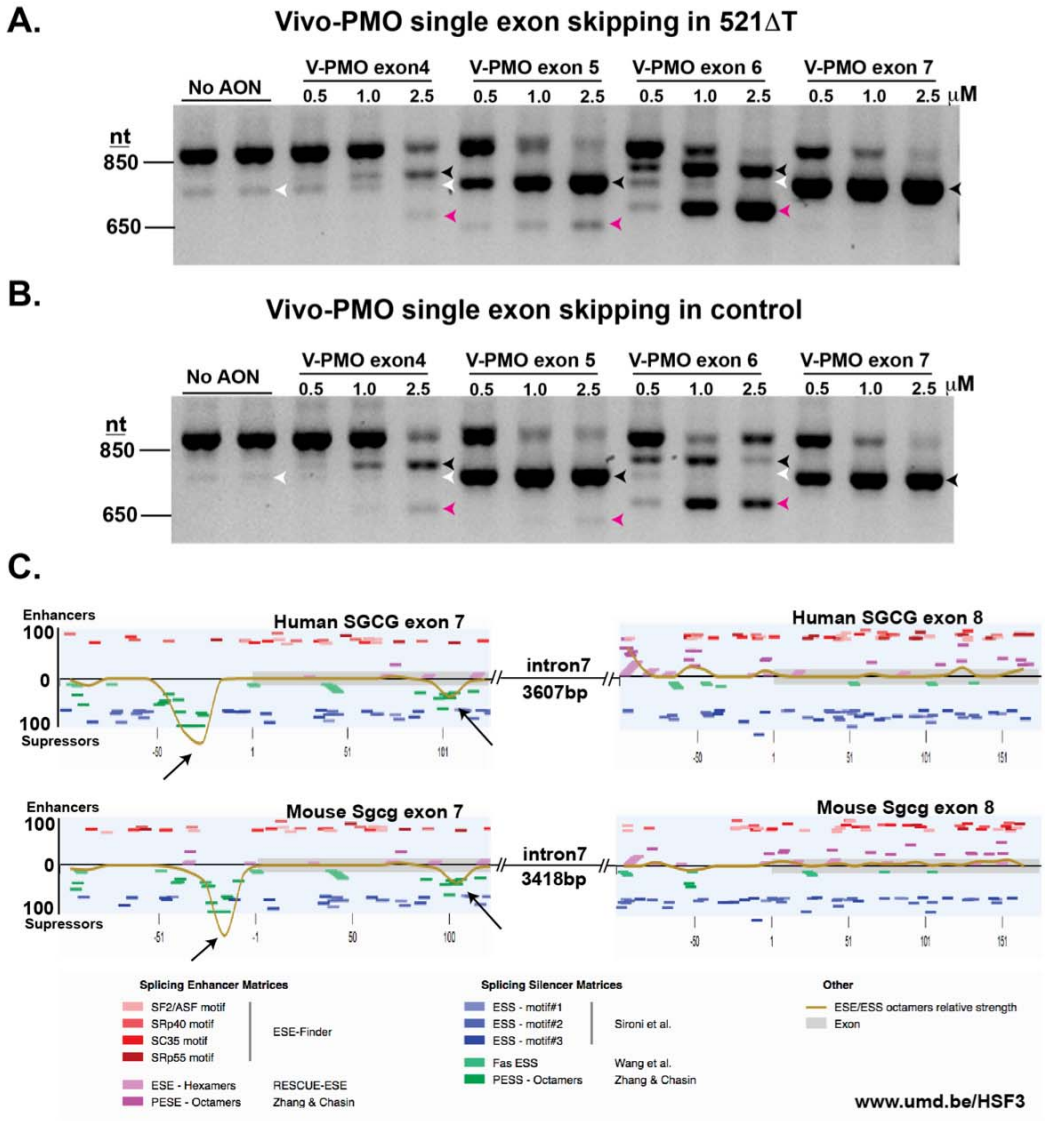
Subject	Mutation	Oligo treatment	AON Dosing
521ΔT	*SGCG* c.521-525 TTTTT>TTTT	2OMePS e4/e5/e6/e7	Dose 1: 75/50/100/50 nM; Dose 2: 150/100/200/100 nM
Control	No mutation	2OMePS e4/e5/e6/e7	Dose 1: 50/50/50/50 nM; Dose 2: 100/100/100/100 nM
521ΔT	*SGCG* c.521-525 TTTTT>TTTT	Vivo-PMO e4/e5/e6/e7	Dose 1: 1.0/0.5/1.5/0.5 μM; Dose 2: 1.5/0.75/2.0/0.75 μM
Control	No mutation	Vivo-PMO e4/e5/e6/e7	Dose 1: 1.0/0.5/1.5/0.5 μM; Dose 2: 1.5/0.75/2.0/0.75 μM
Ex5/6del (e5/6d)	Deletion of *SGCG* exons 5 and 6	Vivo-PMO e4/e7	Dose 1: 1.0/0.5 μM
Ex6del (e6d) 1	Deletion of *SGCG* exon 6	Vivo-PMO e4/e5/e7	Dose 1: 1.0/0.5/0.5 μM
Ex6del (e6d) 2	Deletion of *SGCG* exon 6	Vivo-PMO e4/e5/e7	Dose 1: 1.0/0.5/0.5 μM
Ex7del (e7d)	Deletion of *SGCG* exon 7	Vivo-PMO e4/e5/e6	Dose 1: 1.0/0.5/1.5 μM



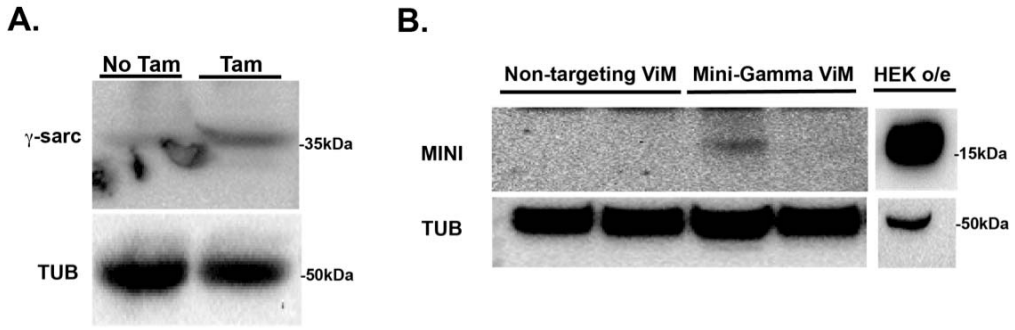
Supplemental Figure 1. *SGCG* expression in reprogrammed fibroblasts. (A) Schematic showing the exon organization of the *SGCG* control and 521ΔT transcripts. A single thymine deletion in exon 6 (red triangle) results in the generation of a premature stop codon. Arrows denote the location of the PCR primers, and the length of the amplicons are indicated. **(B)** Sequence analysis of the control and 521ΔT transcripts that were expressed in the reprogrammed fibroblasts. Shown is a portion of *SGCG* exon 6. The control sequence contains a stretch of 5 thymine residues, underlined. A deletion of a single thymine is observed in the 521ΔT transcript.



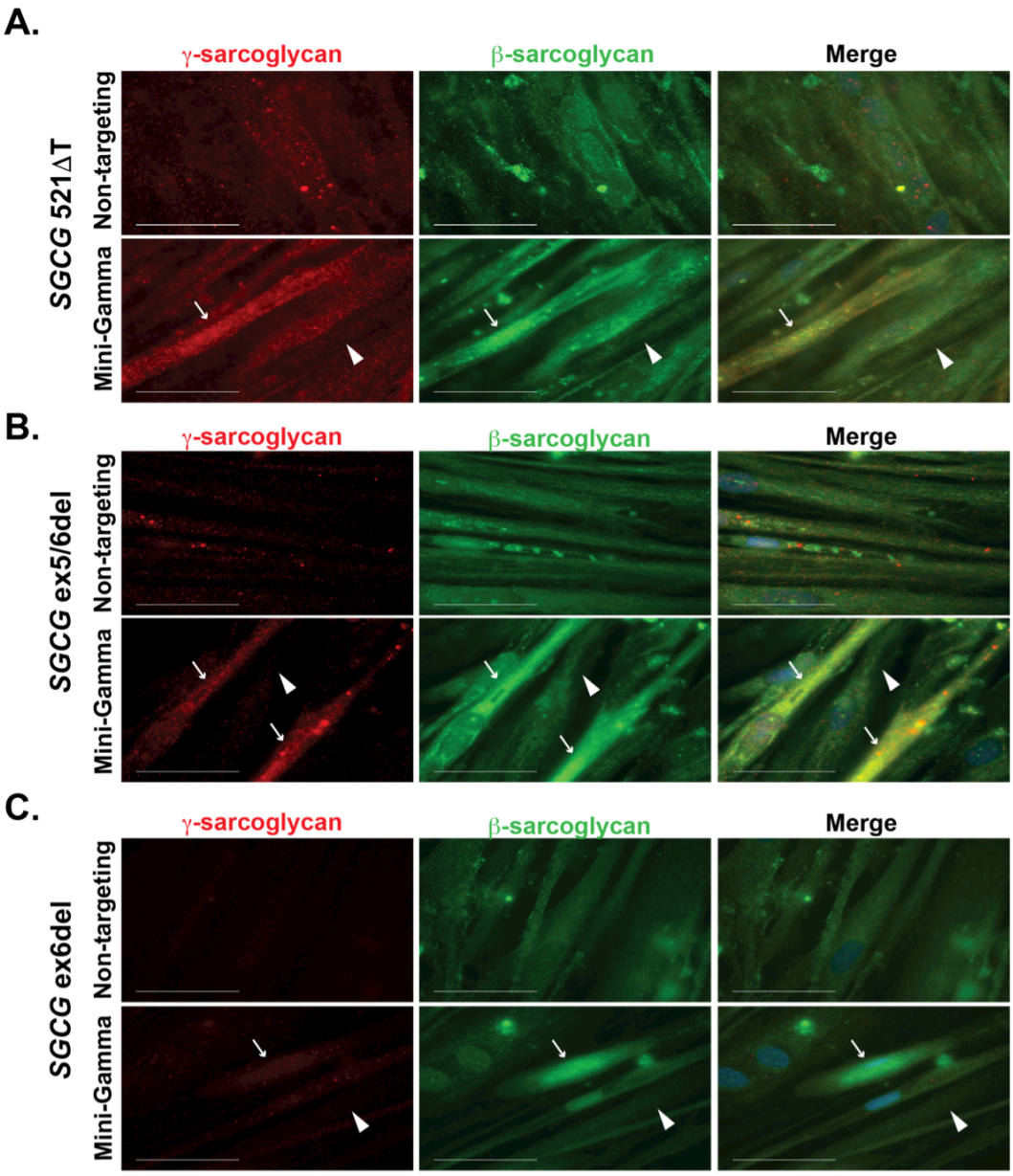
Supplemental Figure 2. Individual skipping of *SGCG* exons 4, 5, 6 and 7 with 2OMePS AONs. Reprogrammed fibroblasts were differentiated and then exposed to single 2OMePS AON treatments targeting individual *SGCG* exons (differentiation day 9, 36h oligo treatment). **(A/B)** RT-PCR analysis after single 2OMePS AON treatment of reprogrammed 521ΔT cells demonstrated the skipping of individual exons 4, 5 and 7 (black arrowheads). White arrowheads indicate endogenous skipping events. **(C)** RT-PCR analysis after single 2OMePS treatment of reprogrammed control cells demonstrated skipping of exons 5 and 7, with modest skipping of exon 4 (black arrowheads).



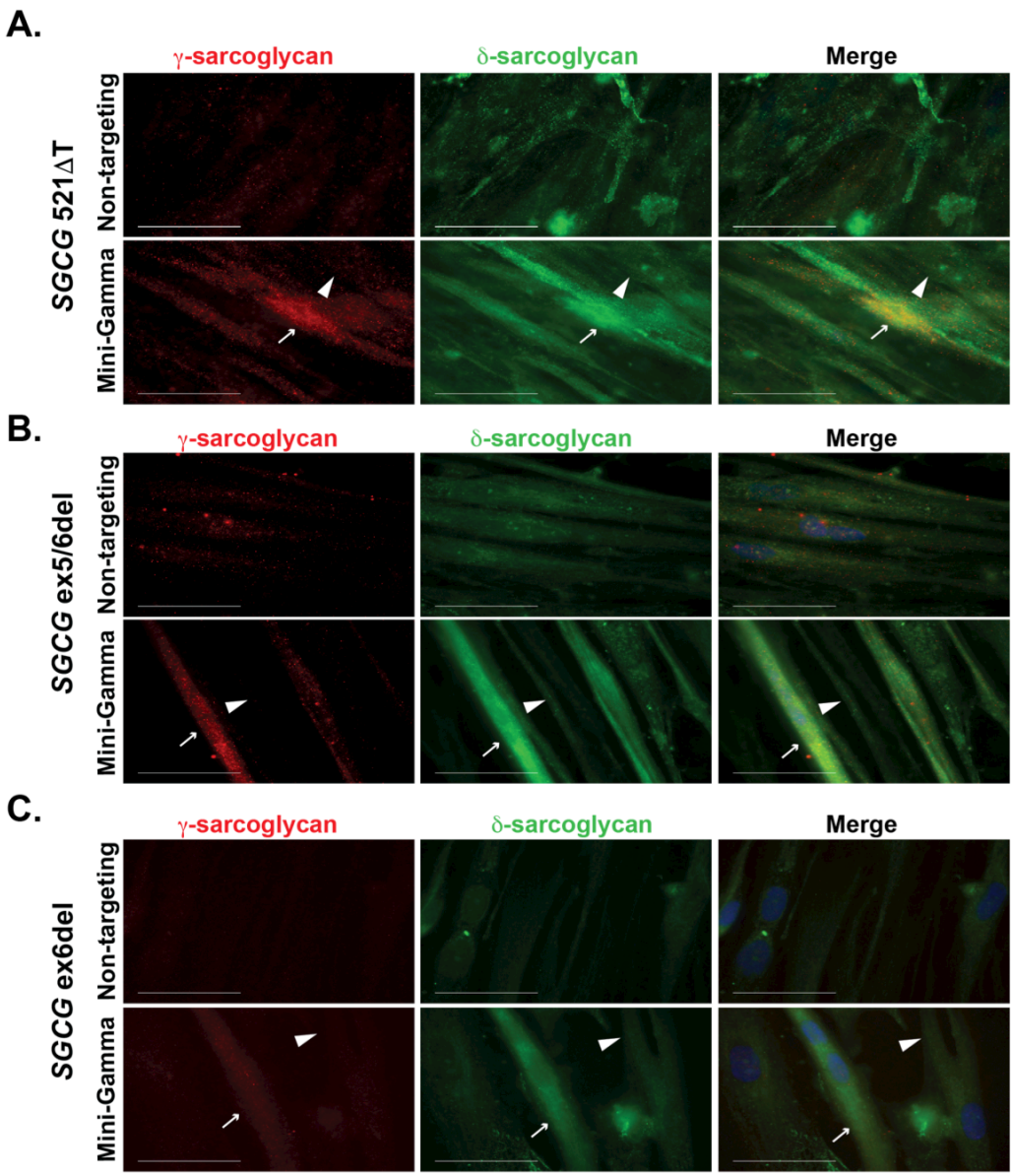
Supplemental Figure 3. Individual skipping of *SGCG* exons 4, 5, 6 and 7 with vivo-PMOs. Myogenic cells exposed to single vivo-PMO treatments targeting individual *SGCG* exons. **(A/B)** RT-PCR analysis after single vivo-PMO treatment of reprogrammed *SGCG* 521ΔT and control cells demonstrated the dose-dependent skipping of individual exons 4, 5, 6 and 7 (black arrowheads). There was also evidence of double exon skipping with single vivo-PMO treatments targeting exons 4, 5 and 6 (pink arrowheads). Notably, there was no evidence of double skipping with the single exon 7 treatment. White arrowheads indicate endogenous skipping events. **(C)** The human and murine *SGCG* genes were analyzed for exon splicing elements encoded in exons 7 and 8 along with 100bp of the intronic region upstream of each exon. Splice-site analysis revealed two areas of splice repression in the exon 7 region that were conserved in human and mouse (black arrows).



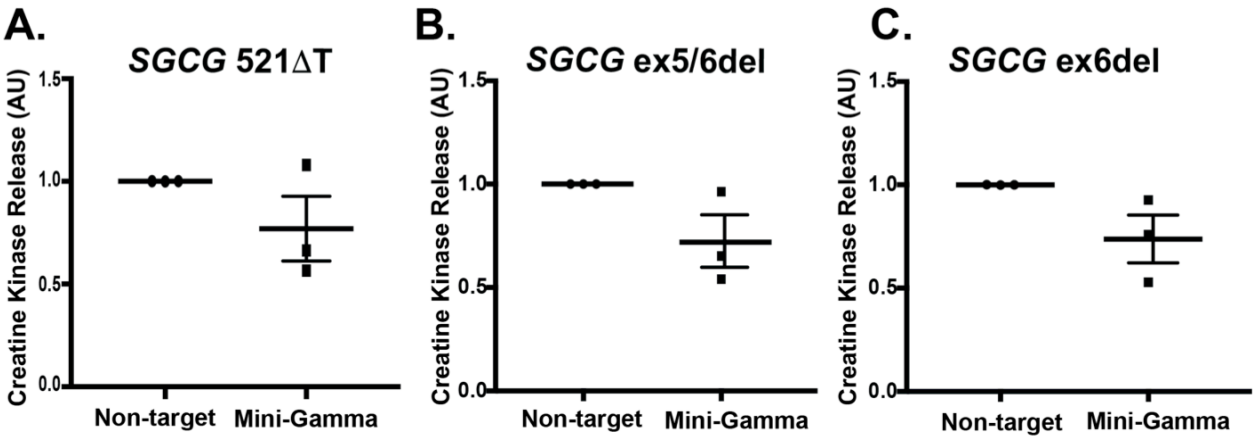
Supplemental Figure 4. Expression of γ-sarcoglycan and Mini-Gamma protein in reprogrammed fibroblasts. (A) Control fibroblasts were transduced with the iMyoD virus and, treated with or without tamoxifen (48h, 5μM) followed by culture in differentiation media (12 days). Immunoblot analysis demonstrated expression of γ-sarcoglycan protein (γ-sarc) in the myogenically reprogrammed fibroblasts as compared to the uninduced (no tamoxifen) controls. γ-tubulin (TUB) was used as a loading control. **(B)** Reprogrammed fibroblasts from the LGMD 2C patient with the *SGCG* ex5/6del frameshift mutation were treated for 3 days with either a non-targeting vivo-PMO or a readframe correcting vivo-PMO cocktail (n=2 culture replicates). Immunoblot analysis detected expression the ~17kDa Mini-Gamma protein in one of two independent experiments. As a control, a construct encoding human Mini-Gamma was overexpressed (o/e) in HEK293T cells. Immunoblotting showed the expression of Mini-Gamma protein at ~17kDa. Note, the band representing the overexpression of Mini-Gamma in HEK293T was imaged at a lower exposure time compared with the bands representing Mini-Gamma protein in samples treated with vivo-PMOs.



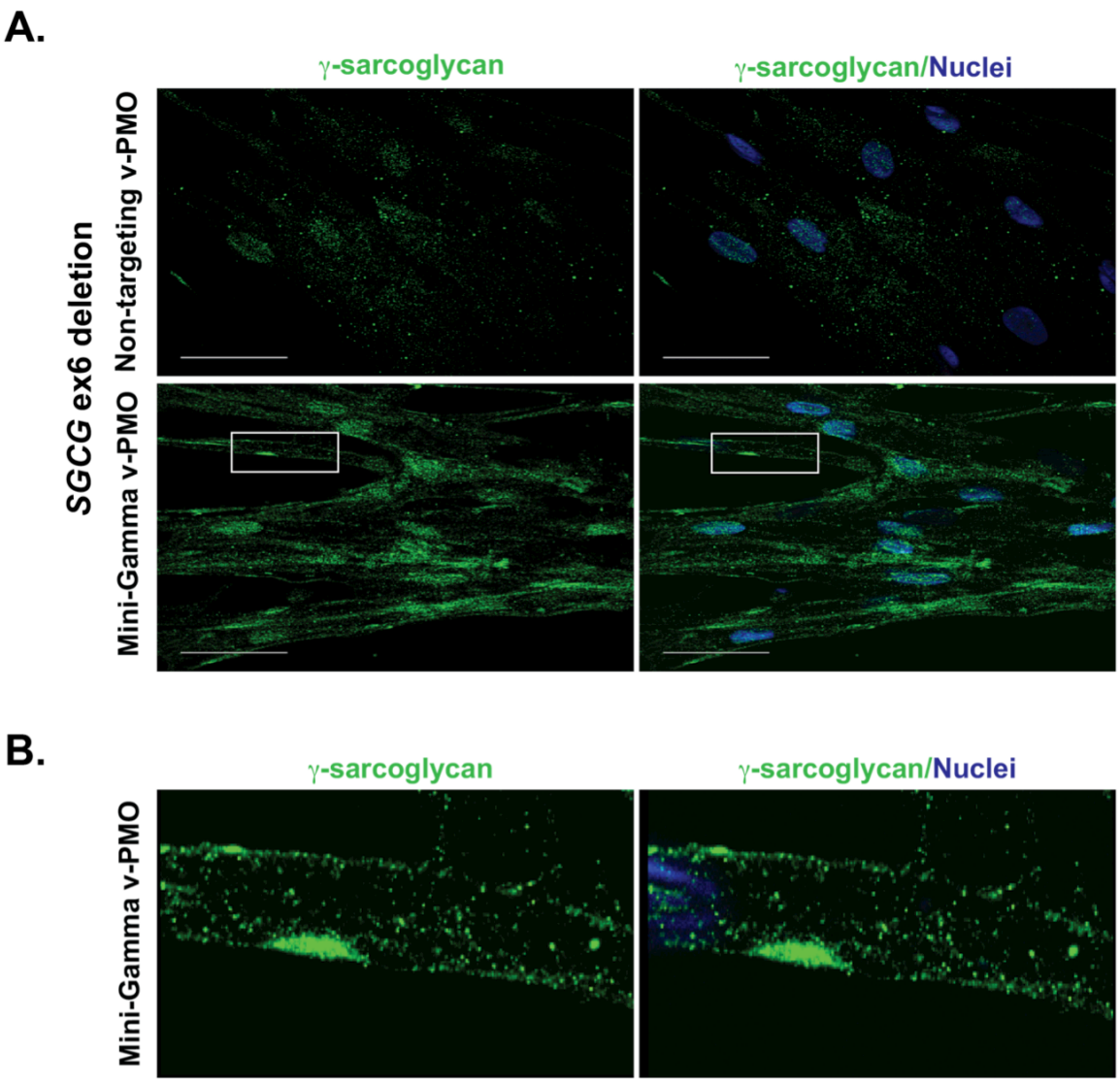
Supplemental Figure 5. β-sarcoglycan protein expression in myotubes treated with vivo-PMOs. Shown is IFM of reprogrammed fibroblasts treated for 3 days with a non-targeting vivo-PMO (Non-targeting) or readframe correcting vivo-PMO cocktail (Mini-Gamma). **(A-C)** Mini-Gamma (red) was expressed in a subset of myotubes (white arrows) after treatment with vivo-PMOs that corrected the **(A)** 521ΔT; **(B)** e5/6del; and **(C)** e6del mutations. **(A-C)** Those myotubes expressing Mini-Gamma protein (red) also had greater β-sarcoglycan expression (green). White arrows show the same myobutes expressing both γ-sarcoglycan and β-sarcoglycan, and arrowheads show myotubes within the culture expressing less γ-sarcoglycan and β-sarcoglycan. Nuclei are labeled with Hoechst 3342 (blue). Scale bar = 50μM.



Supplemental Figure 6. δ-sarcoglycan protein expression in myotubes treated with vivo-PMOs. IFM of reprogrammed fibroblasts treated for 3 days with a non-targeting vivo-PMO (Non-targeting) or readframe correcting vivo-PMO (Mini-Gamma). **(A-C)** Mini-Gamma protein (red) was expressed in a subset of myotubes (white arrows) after treatment with vivo-PMOs that corrected the **(A)** 521ΔT; **(B)** e5/6del; and **(C)** e6del mutations. **(A-C)** Those myotubes expressing Mini-Gamma protein (red) also had higher δ-sarcoglycan protein expression (green). White arrows show the same myobutes expressing both γ-sarcoglycan and β-sarcoglycan, and arrowheads show myotubes within the culture expressing less γ-sarcoglycan and β-sarcoglycan. Nuclei are labeled with Hoechst 3342 (blue). Scale bar = 50μM.



Supplemental Figure 7. Membrane stability in reprogrammed fibroblasts after vivo-PMO treatment. To assess membrane stability in response to vivo-PMO treatment, reprogrammed fibroblasts were treated for 3 days with vivo-PMOs as indicated and then challenged with hypo-osmotic shock. Membrane leak was monitored by the release of CK. **(A-C)** Treatment with readframe correcting vivo-PMOs resulted in a trend towards a reduction in CK release in reprogrammed **(A)** 521ΔT; **(B)** e5/6del; and **(C)** e6del fibroblasts. Data represent the percent of CK released relative to the total CK from 3 independent experiments (n=3-4, for each). Data are presented as the mean CK released in cells treated with vivo-PMOs that corrected the reading frame (Mini-Gamma) relative to the mean in cells treated with a non-targeting vivo-PMO (Non-target). Data represent the mean±SEM.



Supplemental Figure 8. Membrane expression of Mini-Gamma protein in urine-derived cells (UDC). Shown is IFM of reprogrammed fibroblasts treated for 3 days with a non-targeting vivo-PMO (Non-targeting) or readframe correcting vivo-PMO (Mini-Gamma). **(A)** Treatment with readframe correcting vivo-PMOs successfully generated Mini-Gamma (green). Nuclei were labeled with Hoechst 3342 (blue). Scale bar = 50μM. White boxes indicate regions of interest (ROI). **(B)** The highlighted ROIs indicate localization of Mini-Gamma protein (green) throughout the myotube, including the plasma membrane. The length of the ROI bounding box = 50 μM.