SUPPLEMENTAL FIGURE LEGENDS

Supplementary figure-1: (A) Plot showing doubling time for dysf-WT and dysf-KO myoblasts in culture during 4 days of proliferation (n=8 independent cultures). (B) Quantification of lysosome fusion events following laser injury in C2C12 and C2C12-shRNA myoblasts injured in presence of Ca^{2+} (n>13 cells). Note the trend for fewer lysosomal fusions in dysferlinopathic myoblasts. (C) TIRF image showing individual dysf-KO myoblasts whose lysosomes were labeled with FITC dextran. 'X' marks the sites of lysosome fusion following laser injury at the region demarcated by white box. Cell boundary is denoted by orange line. Scale bar: 10 μm.

Supplementary figure-2: (A) Time-lapse imaging of FM1-43 influx into C2C12 myoblasts following injury in presence (upper panel) or absence (lower panel) of Ca^{2+}. Cells were injured by the pulsed laser in the region marked by white box. Note excess FM1-43 entry in cells injured in the absence of Ca^{2+}. Scale bar: 10 μm. (B) Time-lapse imaging of FM1-43 influx into C2C12 (upper panel) and C2C12-shRNA (lower panel) myoblasts following injury in presence of Ca^{2+}. Note greater dye entry showing poor healing of C2C12-shRNA myoblasts. Scale bar: 10 μm. (C) Pseudocolor images of Fluo-4 intensity in C2C12 and C2C12-shRNA myoblasts. The images show a time point before laser injury (-2.5s) and the time after injury by which the Fluo-4 fluorescence returns to the pre-injury baseline. Scale bar: 100 μm. The pseudocolor scale represents the fluo-4 ratio ($\Delta F/F$).

Supplementary figure-3: (A) Time-lapse images showing FM1-43 influx following laser injury of control (upper panel) and sphingomyelinase treated (lower panel) C2C12-shRNA myoblasts. Scale bar: 50 μm, White box marks the region of laser injury. (B) Myotubes made by control or dysferlinopathic human myoblasts are immunolabeled for dysferlin (green) and LAMP1 (red). This shows lack of detectable dysferlin in patient myotubes and lack of co-localization with LAMP1 in control myotubes. Scale bar: 100 μm. (C) Western blot showing lack of dysferlin expression in myoblasts from dysferlinopathic patients. (D) Time-lapse images of FM1-43 influx following laser injury in patient myoblasts treated (right panel) or not treated (left panel) with 2 U/mL SM. This shows reversal of the membrane repair deficit in patient cells by SM treatment. Scale bar: 100 μm (n>14 myoblasts each).

Video 1: Real time imaging of C2C12 cell membrane repair following laser injury. C2C12 cell in buffer containing Ca^{2+} and 1 ug/uL FM1-43 dye was maintained at $37^{o}C$ and injured at the region marked by the green square. FM-dye entry in the cell was imaged at 2 seconds intervals for 5 minutes. The time stamp is in hr:min:sec:msec format and 00:00:14:424 is the first frame after laser injury. This movie represents upper panel in figure S2B.

Video 2: Real time imaging of C2C12-shRNA cell membrane repair following laser injury. C2C12-shRNA cell in buffer containing Ca^{2+} and 1 ug/uL FM1-43 dye was maintained at $37^{o}C$ and injured at the region marked by the green square. FM-dye entry in the cell was imaged at 2 seconds intervals for 5 minutes. The time stamp is in hr:min:sec:msec format and 00:00:14:396 shows the first frame after laser injury. This movie represents lower panel in figure S2B.

Video 3: Real time imaging of exocytosis of a lysosome. Exocytosis of FITC dextran labeled lysosome from dys-WT cell shown in figure 3D and video 4. Cell was maintained at $37^{o}C$ in buffer containing Ca^{2+} and imaged with TIRF at penetration depth of 120nm. Images were collected at 4 frames / second for 3 minutes. The time stamp is in hr:min:sec:msec format. 00:00:41:879 represents the first frame after laser injury and this lysosome fuses at 00:00:54:277. This movie represents lower panel in figure 3D.

Video 4: Real time imaging of kinetics and spatial distribution of lysosomal exocytic events in response to laser injury in dysf-WT cell. Exocytosis of FITC dextran labeled lysosomes in a dys-

WT cell (demarcated by orange line) maintained in CIM buffer at 37^{o}C. The cell was injured at region marked by the green square and imaged by TIRF at penetration depth of 120nm. Images were collected at 4 frames / second for 3 minutes. The time stamp is in hr:min:sec:msec format and 00:00:41:879 represents the first frame after laser injury. This movie represents upper panel in figure 3D.

Video 5: Real time imaging of effect of extracellular sphigomyelinase on C2C12-shRNA cell membrane repair. C2C12-shRNA cell (boundary denoted by orange line) was pre-incubated for 20 minutes with 2 U/mL of SM at 37ºC and then laser injured at 37^{o}C in CIM buffer containing 1 ug/uL FM1-43 dye and 2 U/mL of SM. Cell was imaged at 2 seconds intervals for 5 minutes. The time stamp is in hr:min:sec:msec format and 00:1:05:615 is the first frame after laser injury. This movie represents lower panel in figure S3A.

Video 6: Real time imaging of healthy human myoblast cell membrane repair following laser injury. A healthy human cell maintained at 37^{o}C in CIM buffer with 1 ug/uL FM1-43 dye was injured at the region demarcated by the green square. Cell was imaged at 2 seconds intervals for 5 minutes and the time stamp is in hr:min:sec:msec format. Time point 00:00:14:472 is the first frame after laser injury. This movie represents upper panel in figure 5D.

Video 7: Real time imaging of dysferlinopathic patient myoblast cell membrane repair following laser injury. Dysferlinopathic patient human cell whose boundary is denoted by orange line was maintained at 37^{o}C in CIM buffer with 1 ug/uL FM1-43 dye. The cell was injured in the region demarcated by the green square and the cell was imaged at 2 seconds intervals for 5 minutes. The time stamp is in hr:min:sec:msec format and 00:00:14:296 is the first frame after laser injury. This movie represents left panel figure S3D.

Video 8: Real time imaging of effect of extracellular sphigomyelinase on cell membrane repair of dysferlinopathic patient cell. Dysferlinopathic patient cell (boundary denoted by orange line) was pre-incubated for 20 minutes with 2 U/mL of SM at 37ºC and then laser injured at 37^{o}C in CIM buffer containing 1 ug/uL FM1-43 dye and 2 U/mL of SM. Cell was imaged at 2 seconds intervals for 5 minutes. The time stamp is in hr:min:sec:msec format and 00:00:14:324 s represents the first frame after laser injury. This movie represents right panel of figure S3D.

Video 9: Real time imaging of cell membrane repair of EDL myofiber from B6.A/J mouse. Dysferlinopathic myofiber in the EDL muscle maintained in tyrode buffer with 1.33 ug/uL FM1-43 dye was at 37^{o}C was injured by laser at the region marked by the green square and imaged at 2 seconds interval for 5 minutes in fluorescence (green channel) and bright field (grayscale). The images show overlay of fluorescence images on the bright field image. Time stamp is in hr:min:sec:msec format and 00:01:09.354 shows the first frame after laser injury. This movie represents left panel of figure 6A.

Video 10: Real time imaging of effect of extracellular sphigomyelinase on cell membrane repair of EDL myofiber from B6.A/J mouse. Dysferlinopathic myofiber in the EDL muscle at 37^{o}C was injured (region marked by the green square) in tyrode buffer with 1.33 ug/uL FM1-43 dye and 0.5 U/mL of extracellular sphingomyelinase. Fibers were imaged at 2 seconds intervals for 5 minutes in fluorescence (green channel) and bright field (grayscale). The images show overlay of fluorescence images on the bright field image. Time stamp is in hr:min:sec:msec format and 00:00:22:112 shows the first frame after laser injury. This movie represents right panel of figure 6A.

SUPPLEMENTAL METHODS

Cell culture: Mouse C2C12 myoblasts were cultured in Dulbecco's Modified Eagle Medium, DMEM supplemented with 10% fetal bovine serum FBS and with 1% Penicillin/Streptomycin P/S (PAA, Austria). For mouse C2C12-shRNA myoblasts, 2 µg/µL of puromycin (Sigma Aldrich, USA) was added. All C2C12 cells were maintained at 37ºC and 5% CO_2. Dysf-WT and -KO myoblasts were cultured in DMEM media supplemented with 20% FBS, 1% P/S, 2% L-glutamine (PAA, Austria) and 2% chicken embryo extract (Accurate Chemical, USA) on dishes coated with 0.4% gelatin. 20 ng/ml γ-interferon (Millipore, USA) was then added in the media. Dysf-WT and -KO myoblasts were cultured at 33ºC and 10% CO_2. To induce differentiation, C2C12 cells and dysf-WT and -KO myoblasts were transferred in DMEM supplemented with 2% horse serum HS (PAA, Austria) and 1% P/S at 80/90% confluency and maintained at 37ºC and 5% CO_2 during 4 days. The primary human cells were immortalized by hTERT as previously described (1). These cells were cultured in skeletal cell media (Promocell, Germany) up to 20% with FBS on dishes coated with 0.4% gelatin. Cells were maintained at 37ºC and 5% CO_2. Human myoblasts were differentiated in DMEM media supplemented with 100 µg/mL apotransferrin, 50 µg/mL gentamycin and 10 µg/mL insulin (Sigma Aldrich, USA) at 37ºC and 5% CO_2 for 7 days.

Immunoblot and immunostaining: For immunoblot, blocking and antibody incubations were done in TBS completed with 5% of dry milk and 0.1% Tween and for immunostaining, blocking and antibody incubations were done in PBS containing 1% BSA, 5% chicken serum and 0.1% Tween. Imaging was done with motorized Olympus IX81 confocal microscope (60X/1.40NA oil or 40X/1.30NA oil objectives) controlled by FV10ASW3.0 software (Olympus USA) or IX81 Olympus microscope (60X/1.45NA oil, 40X/1.30NA oil or 20X/0.45 objectives, Olympus America, PA) equipped with Lambda DG-4 (Sutter Instruments, Novato CA) widefield illumination system and Evolve 512 EMCCD (Photometrics, Tucson, AZ) camera using Slidebook 5.0 software (Intelligent Imaging Innovations, Inc. Denver, CO).

Total internal reflection microscopy and live cell imaging: These were carried out on Olympus IX81 microscope equipped with Lambda DG-4 (Sutter Instruments, Novato CA) widefield illumination system and Evolve 512 EMCCD (Photometrics, Tucson, AZ) camera, with Cell-TIRFTM (Olympus) illuminator and 60X/1.45NA oil objective using Slidebook 5.0 and 5.5 (Intelligent Imaging Innovations, Inc. Denver, CO). The cells being imaged were maintained at 37^{o}C in the Tokai Hit microscope stage top ZILCS incubator (Tokai Hit Co., Japan). For TIRF imaging laser angle was set to obtain penetration depth of 70-120nm and cells were imaged at 4-6 frames/second for 3 minutes using IX81 equipped with diode laser of 488nm (Cobolt, Sweden).

REFERENCES

1. Philippi S, Bigot A, Marg A, Mouly V, Spuler S and Zacharias U Dysferlin-deficient immortalized human myoblasts and myotubes as a useful tool to study dysferlinopathy. *PLoS. Curr.*2012;**4**:RRN1298