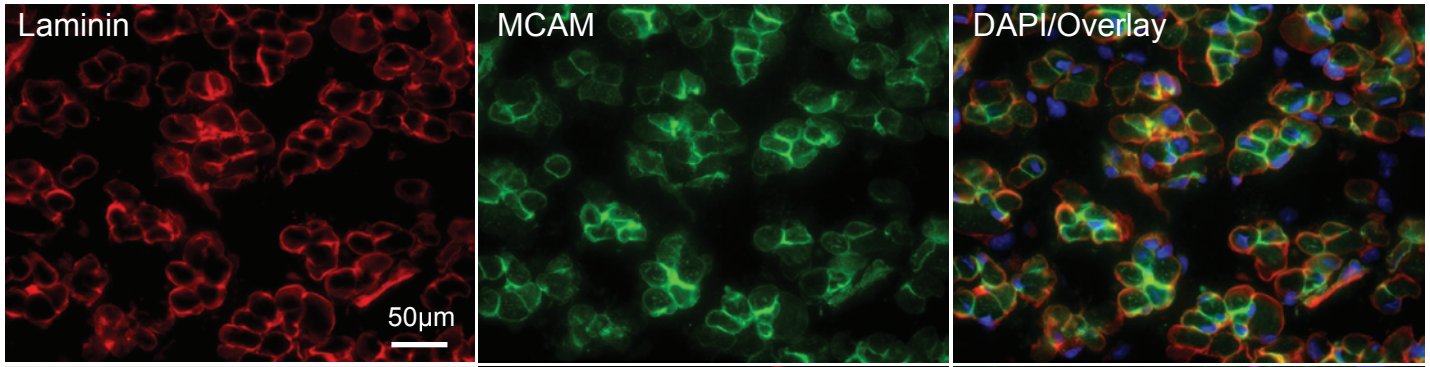
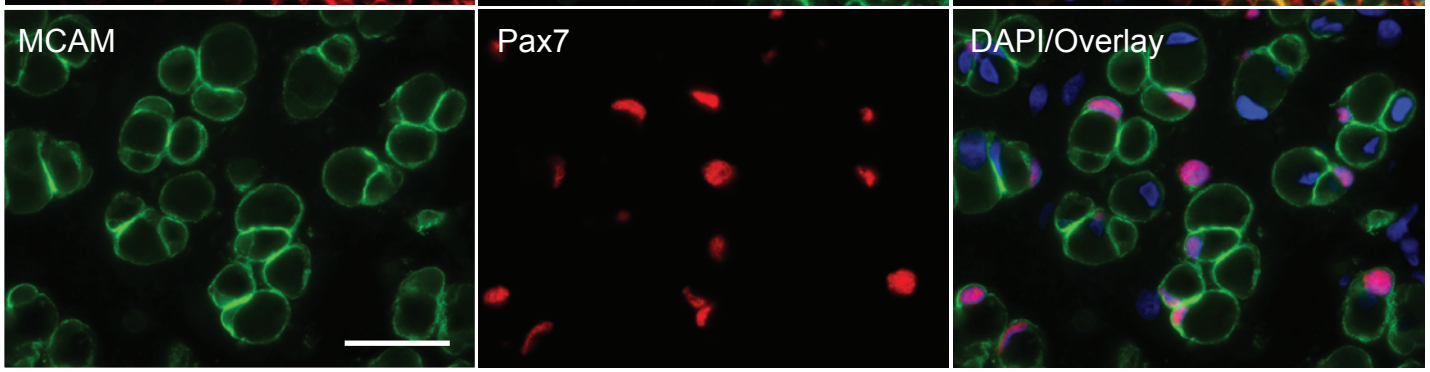
A





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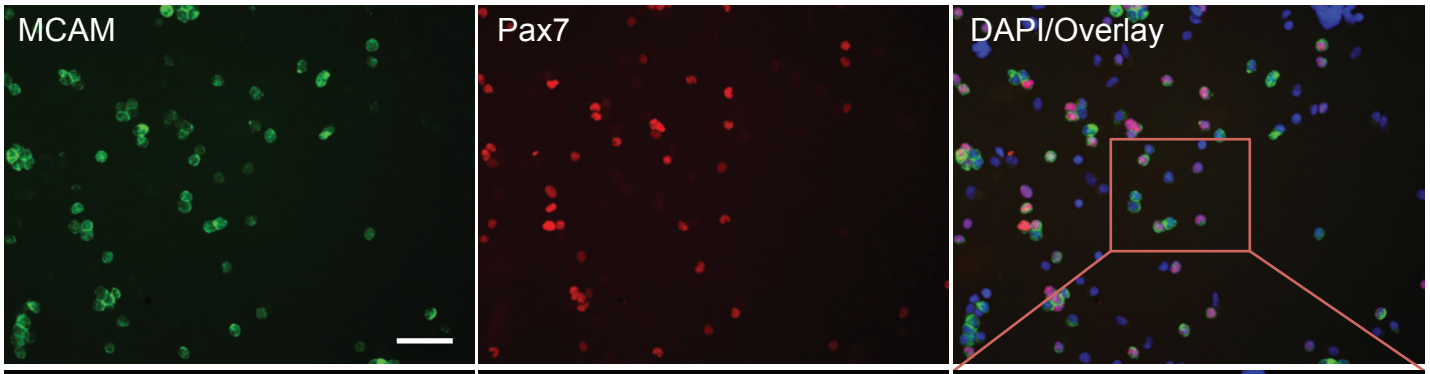
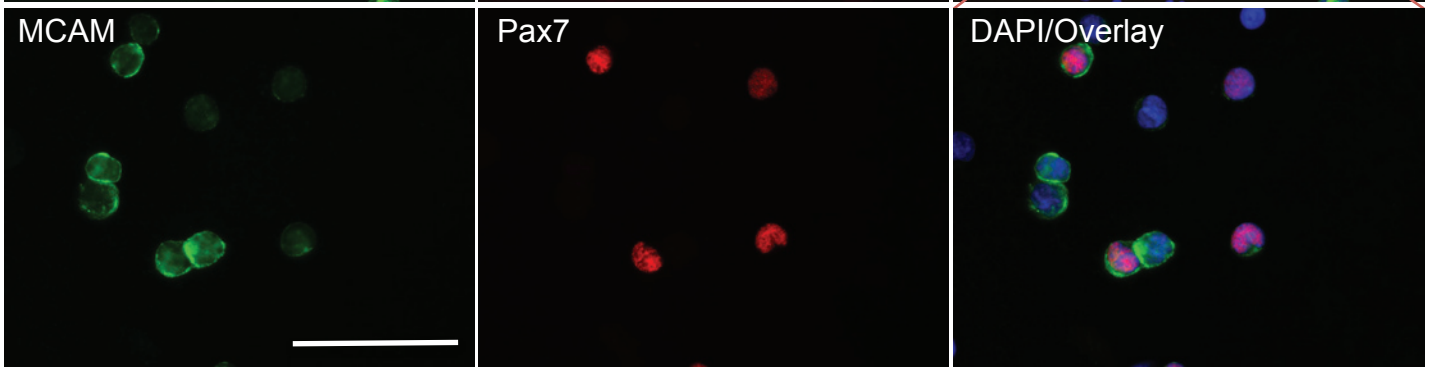




C

MCAM+ Pax7-	MCAM- Pax7+	MCAM+ Pax7+
% of total ± SD	% of total ± SD	% of total ± SD
26.3 ± 7.3	2.1 ± 1.7	71.6 ± 7.9

Figure S1. Related to Figure 1. Expression of MCAM in myofibers and satellite cells in human fetal muscle. A) Immunofluorescence co-staining of MCAM with laminin shows that MCAM outlines the myofibers, with more intense staining observed at myofiber-myofiber contact points (top panels). MCAM expression was detected on the satellite cell membrane on either side facing the sarcolemma or the extracellular matrix cells under the basal lamina (middle panels), as revealed by co-staining with Pax7. B, C) Mononuclear cells dissociated from human fetal muscle that have never been cultured before were placed on slides by cytospin. Five different samples of gestational age ranging between 19 and 21 weeks were analyzed. On average, 325 cells/sample were scored as either expressing both MCAM and Pax7 or either one of those markers. The majority of cells (71.6 %) were positive for both MCAM and Pax7, while a minority of cells (2.1%) was positive for Pax7 alone. There was also ~ 26.3 % of cells that were only positive for MCAM, thus unclear if these cells were myogenic or non-myogenic.

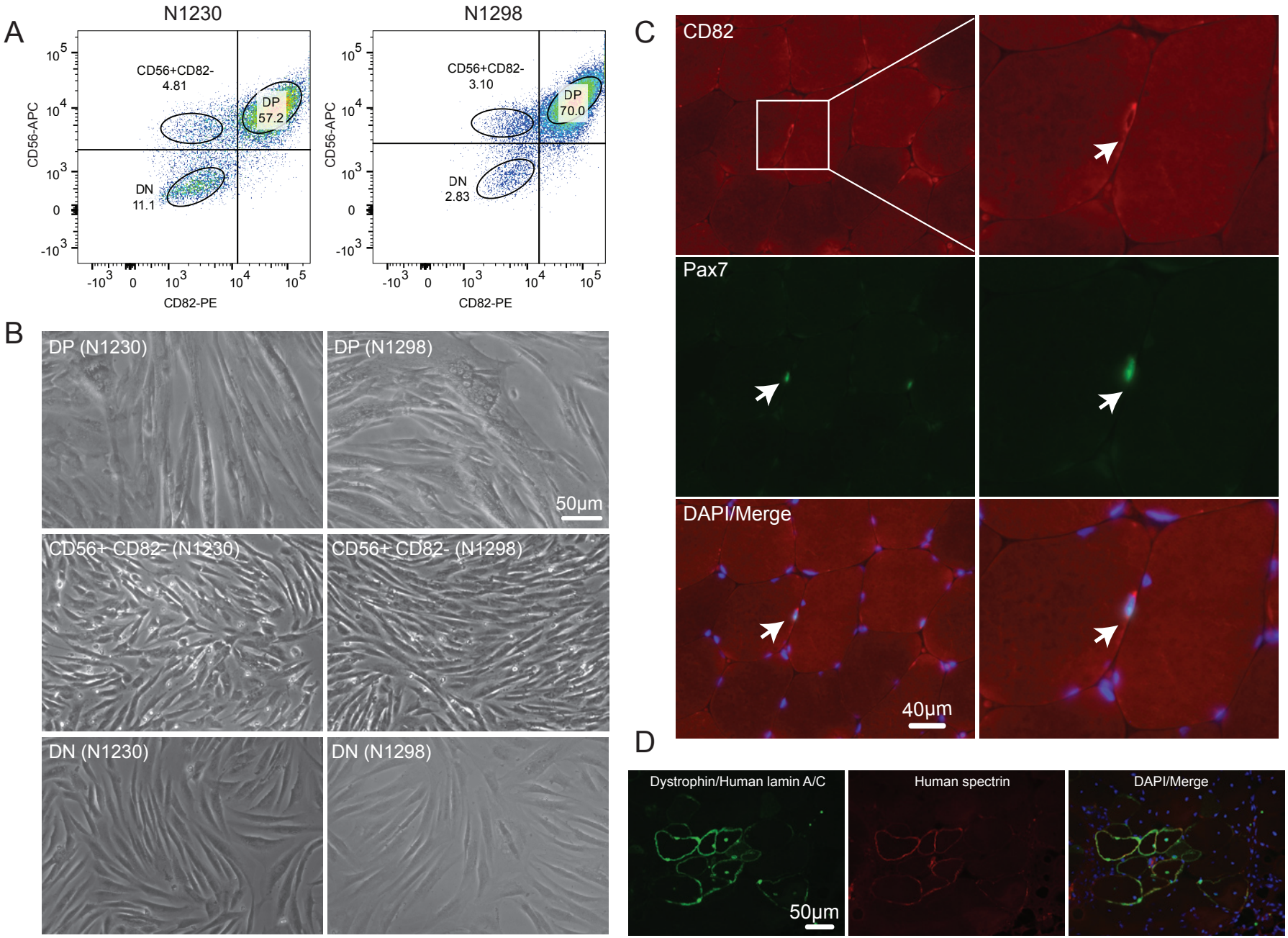
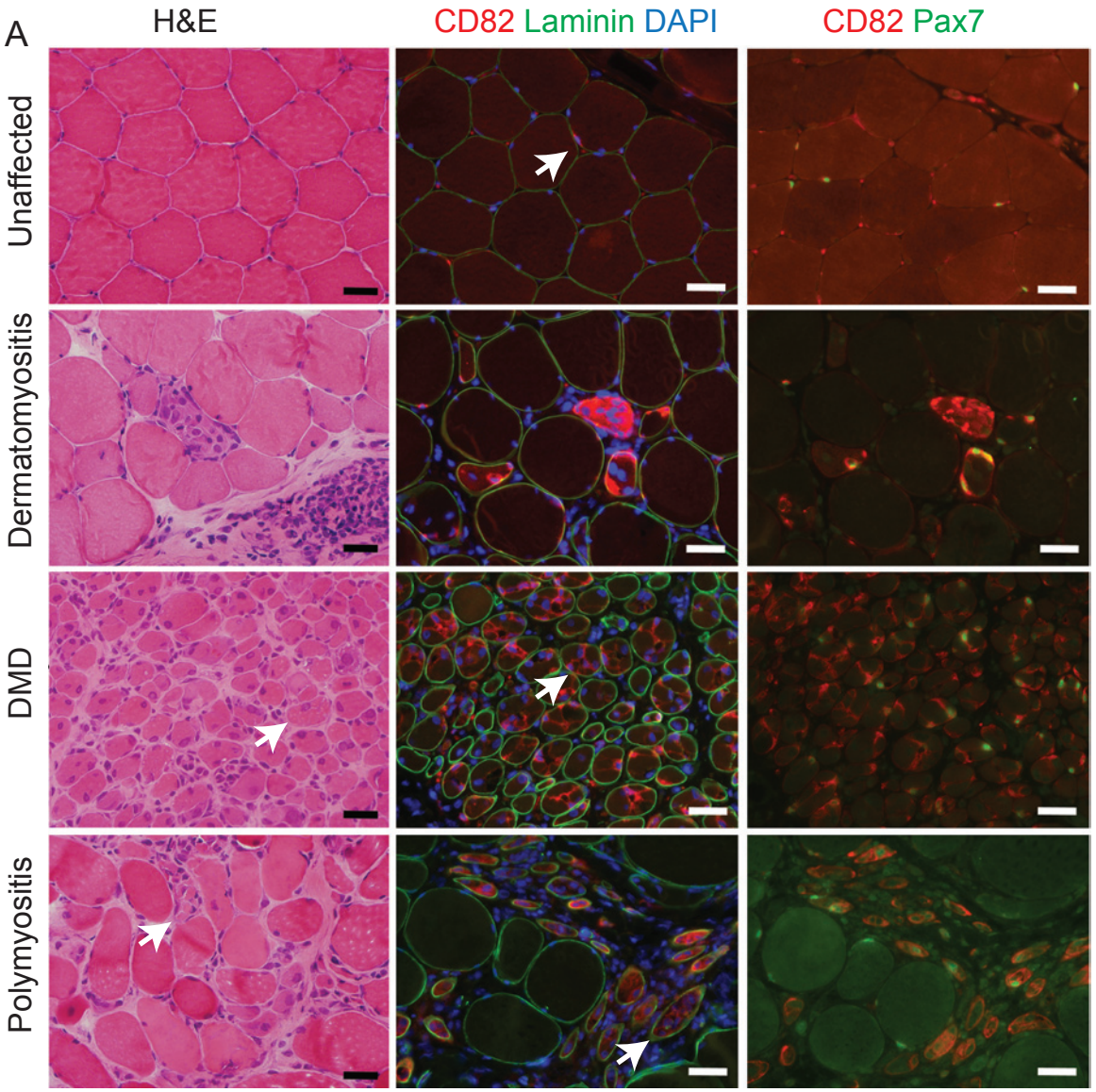


Figure S2. Related to Figure 2. CD82 labels myogenic stem cells in human adult muscle. A) Primary human adult muscle cells analyzed by FACS for expression of CD56 (NCAM), a known marker for myogenic cells (Schubert et al., 1989; Boldrin et al., 2010) and CD82 (top panels). B) CD56+CD82+ (DP), CD56+CD82- and CD56-CD82- (DN) cells were collected and plated at the same density in differentiation assays. At day 7 following differentiation, CD56+CD82+ cells had formed myotubes, while CD56+CD82- and CD56-CD82- cells had not (middle and lower panels, respectively). C) CD82 marks quiescent satellite cells in human adult muscle. CD82 (red) and Pax7 (green) staining co-localize (scale bar=40µm). The right panels show an enlarged area in the same microscopic field on the left panels. D) The in vivo myogenic potential of CD56+CD82+ cells was tested by injection of 105 purified CD56+CD82+ cells into the TA muscle of NODRag1nullmdx5cv recipient mice (bottom panels). Four weeks after transplantation myofibers positive for both human spectrin and dystrophin/lamin A/C were detected, although at much lower frequency than what seen using fetal cells (n=7).

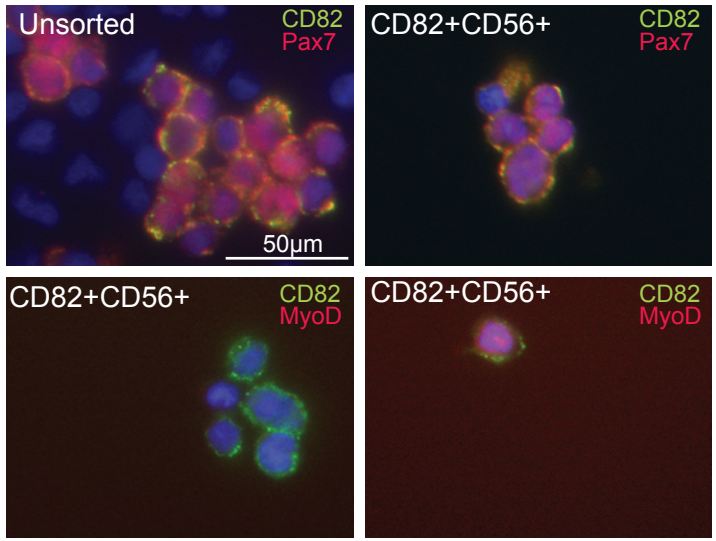
A



B

Human Sample	Pax7+	CD82+	Co-localization	%Co-localization
Unaffected (16y)	54	89	54	60
Unaffected (6m)	275	430	254	59
Polymyositis (13y)	38	78	38	48
Polymyositis (17y)	83	161	81	50
Dermatomyositis (4y)	149	588	146	24
Duchenne dystrophy (8y)	104	243	85	34

C



D

Cell fraction	Pax7+	Pax7-	MyoD+	Co-localization	% Myogenic
17w CD82+CD56+	88	8	2	90	91
17w CD82-CD56+	1	109	0	1	0.9
20w CD82+CD56+	84	17	0	84	83
20w CD82-CD56+	0	103	0	0	0

Supplemental Figure 3. Related to Figure 3. CD82 expression in diseased human muscle and characterization of heterogeneity of primary CD82+ progenitors prior to culture. A) H&E and immunofluorescence staining of control and diseased human muscles demonstrating co-localization of CD82 with satellite cells in unaffected muscle. H&E images do not show the exact microscopic field as the immunofluorescence fields. In control muscle, CD82 labels the membrane of satellite cells (arrow). In diseased muscles, CD82 expression was seen as a lobulated pattern in small, centrally-nucleated regenerating myofibers (arrows). Nuclei are stained in blue with DAPI, scale bars: 40 micron. B) Table summarizing the co-localization of Pax7 and CD82 in normal and diseased muscles. Patient ages are shown in parentheses. The majority of Pax7+ cells express CD82, however other cells are CD82+, supporting the conclusion that CD82 expression in human skeletal muscle includes but is not restricted to, muscle stem cells. C) Characterization of heterogeneity of CD82 progenitors immediately after primary tissue dissociation, prior to any in vitro culture. Cytospins of primary dissociated cells unsorted or FACS-sorted based on expression of CD82 and CD56 stained for CD82 (green) and Pax7 (red) or CD82 (green) and MyoD (red) by immunofluorescence. D) Pax7+ and MyoD+ cells were quantified in multiple samples and >80% of the CD82+CD56+ cells were positive for Pax7, while CD82-CD56+ cells were mostly Pax7-, confirming CD82 expression in the CD56+ fraction enriches for myogenic precursors.

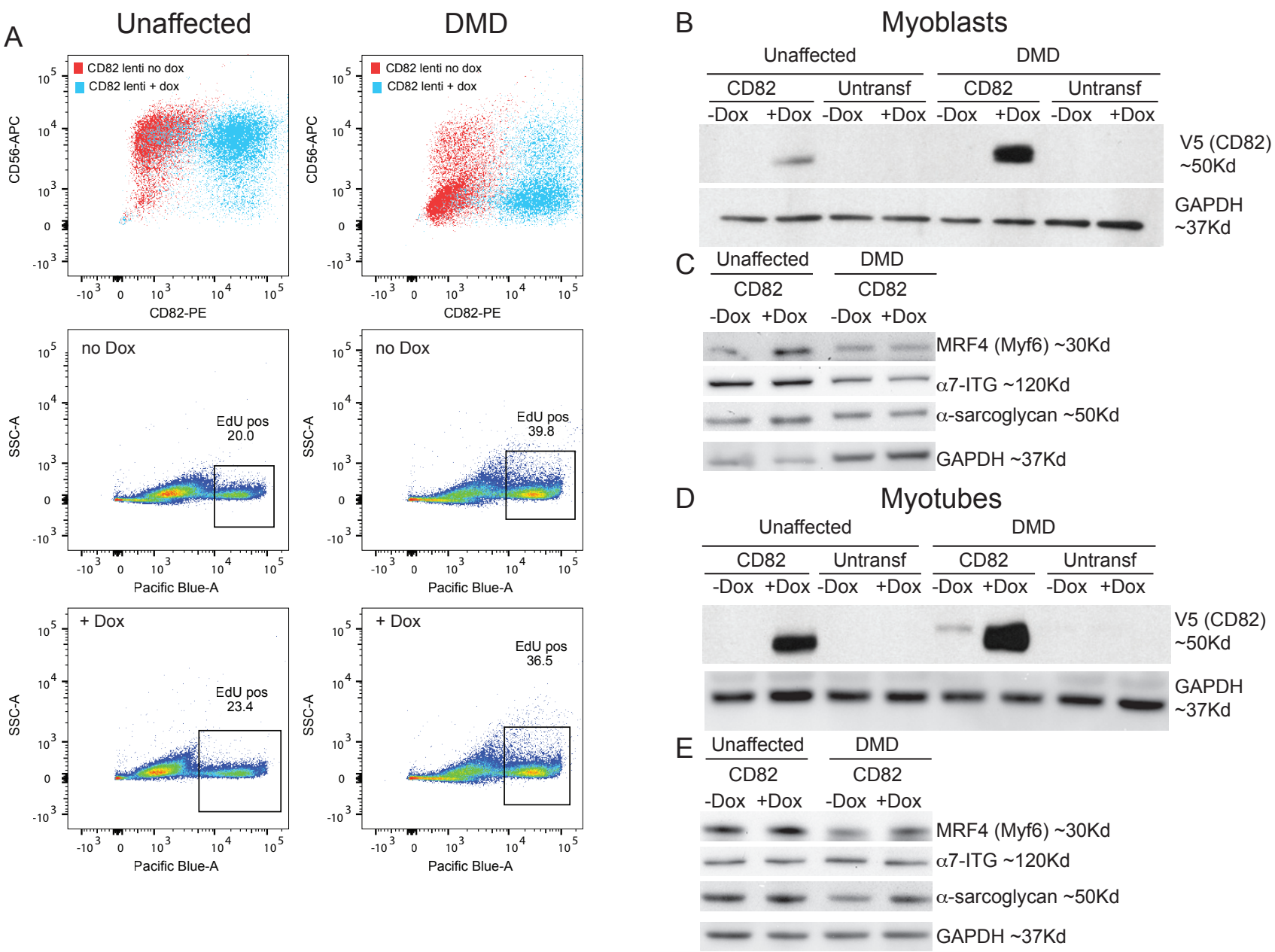


Figure S4. Related to Figure 4. Overexpression of CD82 in normal and DMD myogenic cells does not change EdU incorporation but enhances myogenic differentiation.
A) FACS plots of human myogenic cells from unaffected (left) and from an individual with DMD (right). The top panels show overlays of the cell fractions infected with the CD82V5-tag lentivirus untreated (no Dox, red) or induced with doxycycline (+Dox, blue). CD56 and CD82 expression is detected with specific antibodies. The doxycycline-treated fractions show highly increased expression of CD82. Middle and low panels show incorporation of EdU in untreated or Dox-treated cultures, respectively. Dox-treatment does not significantly change the percentage of EdU-incorporating cells compared to non-treated cells. Of note, a higher percentage of Edu-incorporating cells are noted in the DMD cultures, suggesting intrinsic hyperactivation of these cells. B) and D) western blots of myoblast and myotubes cultures, respectively, confirming CD82 overexpression in Dox-induced cultures using anti-V5 Tag antibody. C) and E) western blots of myoblast and myotubes cultures, respectively. Expression of MRF4 is significantly upregulated in unaffected Dox-treated myoblasts and myotubes ($p<0.00004$ and $p<0.04$, respectively) and in Dox-treated DMD myotubes ($p<0.05$), suggesting CD82 overexpression induces myogenic differentiation. α7-integrin expression is significantly upregulated in Dox-treated unaffected myoblasts ($p<0.0006$), while it does not significantly vary between untreated and Dox-induced DMD myoblasts or myotubes. α-sarcoglycan expression is significantly increased in Dox-treated unaffected myoblasts ($p<0.0004$); Dox-treated unaffected myotubes ($p<0.03$) and Dox-treated DMD myotubes ($p<0.003$).

Supplemental Table 1. Related to Figure 1 and Figure S1. List of genes differentially expressed in MCAM- versus MCAM+ cells. The genes on top of the list are highly expressed in MCAM- cells, while the genes at the bottom of the list are enriched in expression in MCAM+ cells. Significant genes were identified by calculating the differential score, which is a transformation that provides directionality to the p-value based on the difference between the average signal in the reference group (MCAM+) versus the comparison group (MCAM-). P values are also listed.

List of primary antibodies used in this study

Antibody	Manufacturer	Catalog	Dilution	Application
CD82	Sigma	HPA028900	1:50	Immunostaining
Pax7 concentrate	DSHB	Pax7	1:100	Immunostaining
Myogenin concentrate	DSHB	F5D	1:500	Western blot
Laminin a2	Millipore	MAB1922	1:500	Immunostaining
Embryonic myosin	DSHB	F1.652	1:10	Immunostaining
CD82-Alexa 488	Biolegend	342106	5µl/10^6 cells	FACS
CD82-PE	Biolegend	342104	20µl/10^6 cells	FACS
CD82-APC	Biolegend	342108	5µl/1x10^6 cells	FACS
CD82	Biolegend	342102	1:50	PLA
CD82	Santa Cruz	sc-1087	1:50; 1:1,000	PLA; western blot
CD82	Santa Cruz	sc-17752	1:500	Western blot
CD82	Sigma	HPA028900	1:50	Immunostaining
CD82	Abcam	Ab66400	1:100	Immunostaining
CD56-APC	Biolegend	318310	5µl/1x10^6 cells	FACS
CD146-Alexa 488	Millipore	MAB16985X	1:100	FACS
CD146-PE	Millipore	MAB16985H	1:100	FACS
Dystrophin CAP6-10	custom	Lidov, H. 1990	1:1,000	Immunostaining
Lamin A/C	Epitomics	EPR4100	1:500	Immunostaining
Ki-67	BD Pharmingen	550609	1:100	Immunostaining
Spectrin (human)	Vector labs	VP S283	1:100	Immunostaining
CD82	Thermo Sci.	PA5-27233	5µg	IP
CD56	Santa Cruz	sc-10735	5µg	IP
a7-ITG (6A11)	LSBio	LS-C179572	5µg; 1:50	IP; PLA
β4-ITG (ASC9)	EMD Millipore	MAB2060	5µg	IP
a7-ITG (8G2)	Sigma	WH0003679M1	1:1,000; 1:50	Western blot; PLA
a-sarcoglycan	Santa Cruz	sc-390647	1:1,000; 1:50	Western blot; PLA
GAPDH	Santa Cruz	sc-25778	1:1,000	Western blot
GAPDH	Santa Cruz	sc-20357-HRP	1:1,000	Western blot
(MRF4) Myf6	Novus	NBP1-55582	1:1,000	Western bot
MCAM	Millipore	MAB16985	1:100	Immunostaining
MCAM	GeneTex	GTX62080	1:100	Immunostaining
Laminin	Sigma	L9393	1:500	Immunostaining

Supplemental Experimental Procedures

Tissue collection and processing

Human de-identified and discarded fetal tissue of gestational age 15-21 weeks was collected under a protocol approved by the Committee of Clinical Investigation at Boston Children's Hospital (IRB-P00020286). Similarly, adult human tissue was collected as de-identified under an approved protocol (03-12-205R). Tissue was snap-frozen as described (Meng et al., 2014) and primary muscle cells were dissociated and frozen as previously described (Lapan and Gussoni, 2012).

Transcriptome analyses

FACS-purified MCAM+ and MCAM- cells from 3 independent individuals were subjected to total RNA extraction from cell pellets using TRIzol reagent (Invitrogen). RNA concentration was quantified with UV absorption at 260 nm using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific). 50 micrograms of total RNA/sample were submitted for transcriptome analysis using Illumina HT12 chips to the IDDRC Molecular Genetics Core Facility at Boston Children's Hospital. The complete list of genes is provided in (Table S1). Significant genes were identified by calculating the differential score, which is a transformation that provides directionality to the p-value based on the difference between the average signal in the reference group (MCAM+) versus the comparison group (MCAM-). The genes on top of the list in Table S1 are highly expressed in MCAM- cells, while the genes at the bottom of the list are enriched in expression in MCAM+ cells. P values are also listed.

Cytospins

Dissociated primary human fetal muscle cells from 5 individuals were thawed in PBS. Cells were immediately incubated with anti-CD82 antibody (Biolegend, Cat#342102, 1:100) in HBSS/0.5% BSA for 15 min at 4°C. Following a wash in PBS, cells were fixed with 4% PFA for 20 min at RT, washed in PBS, loaded in cytospin funnels (Fisher Scientific) and spun for 4 min at 600rpm (Thermo Shandon Cytospin 4). Cells were permeabilized using 0.5% TritonX-100 in PBS for 3 minutes at RT and rinsed in PBS. Slides were blocked with 10%FBS/0.1% TritonX-100 in PBS for 1 h at RT. Primary antibodies were diluted in blocking solution as follows: MCAM (rb GeneTex, GTX62080) 1:100; Pax7 (ms DSHB) 1:100 and incubated for 1h at RT. After 3 washes in PBS, cells were incubated for 45 min at RT in Alexa594-conjugated donkey anti-mouse (1:500, Invitrogen) and Alexa488-conjugated donkey anti-rabbit (1:500, Jackson Immuno) diluted in PBS. Slides were washed 3 times in PBS and coverslipped as described for immunofluorescence staining. Images were taken using a Nikon Eclipse E1000 microscope fitted with a Hamamatsu ORCA-ER CCD camera. Between 2,000-3,000 cells total (~500 cells/individual sample) were analyzed for expression of Pax7 and MCAM and counted.

Immunofluorescence staining on human muscle tissue sections

Tissue was sectioned at 7µm on Tissue Tak slides (Polysciences) followed by fixation for 3 min at RT in 100% methanol. Alternatively, sections were fixed in 4% PFA in PBS for 15 minutes, followed by 3 washes in PBS and permeabilization with 0.5% Triton X-100 for 3 minutes at RT. After fixation, blocking was performed in 10% FBS/0.1% TritonX-100 in PBS for 1 hour at RT. Primary antibodies were diluted in blocking solution and incubated overnight at 4°C. The following primary antibodies were used: anti-MCAM (ms, Millipore, MAB16985) 1:100, anti-MCAM (rb, GeneTex, GTX62080) 1:100; anti Pax7 (ms, DSHB) 1:100; anti-CD82 (ms, Biolegend, Cat#342102) 1:100; anti-CD82 (rb, ab66400, abCam) 1:100; Laminin (rb, Sigma L9393) 1:500. After 3 washes in PBS, slides were incubated for 45 min at RT with Alexa594-conjugated donkey anti-mouse (1:500, Invitrogen) and/or Alexa488-conjugated donkey anti-rabbit (1:500, Jackson ImmunoResearch) diluted in PBS. Slides were washed 3 times in PBS, coverslipped using Vectashield mounting medium with DAPI (Vector Labs) and let air dry for 20 minutes before sealing the edges with nail polish. Images were taken using a Nikon Eclipse E1000 microscope fitted with a Hamamatsu ORCA-ER CCD camera.

For CD82 immunofluorescence staining, frozen muscle tissue sections were collected and let dry for at least 1 hour. Sections were then fixed in cold acetone at -20˚C for 5 min. Slides were then placed in preheated Antigen Retrieval Reagent-Universal solution (R&D, cat# CTS015), incubated at 95˚C for 5 min and cooled down to room temperature. Slides were rinsed once with distilled water, washed with PBST (PBS with 0.1% Tween-20) twice, blocked with PBST 5% donkey serum for 45 min and incubated with primary antibodies at 4˚C O/N (CD82 Sigma cat#: HPA028900 1:50; Pax7 DSHB 1:10; Laminin a2 MAB1922 Millipore 1:500; embryonic myosin DSHB F1.652 1:10). Slides were washed with PBST x 3 times and incubated with secondary antibody at RT for 1hr (RRX-conjugated Donkey Anti-Rabbit IgG, Jackson Lab 711-295-152; Alexa Fluor 488-conjugated Donkey Anti-Mouse

IgG, Jackson Lab 715-545-151). Following washes 3X10 min with PBST, sections were incubated for 2 min in DAPI stain (Life technologies H3570) diluted 1:30,000 in PBST. Sections were washed again with PBST x3 times, followed by with water once before mounting with PermaFluor Mountant (Thermo Scientific, TA-030-FM)

Primary muscle cell dissociation and FACS purification

Mononuclear cells from de-identified, discarded human fetal muscle samples were stored frozen at -140°C and thawed in HBSS prior to sorting. Samples were filtered through a 40µm filter and primary antibodies were diluted in HBSS supplemented with 0.5% BSA at the following concentrations: CD82-Alexa 488 (Biolegend, Cat#342106) 5µl/10^6 cells cells; CD82-PE (Biolegend, Cat# 342104) 20µl/10^6 cells; CD82-647 (Biolegend, Cat# 342108) 5µl/10^6 cells cells; CD56-APC (Biolegend, Cat# 318310) 5µl/10^6 cells; CD146-488 (Millipore, MAB16985X) 1:100; CD146-PE (Millipore, MAB16985H) 1:100. To gate for live cells, Calcein Blue (Invitrogen) was used at a final concentration of 10µM for 2x10^6 cells along with primary antibodies. Samples were incubated for 30 min on ice, washed in 1x HBSS and filtered again through a 40µm filter prior to cell sorting.

For adult myoblasts, primary cells were plated on 10 cm-plates coated with 0.15% gelatin in 20% FBS/1x PSG/high glucose DMEM. For FACS analysis, both floating and adherent cells were collected. Plates were washed with HBSS followed by 0.25% Trypsin with EDTA (Invitrogen) for 2 min at 37°C. Cells were counted and resuspended in 1xHBSS supplemented with 0.5% BSA as described above. Primary antibodies were added at the following concentration: CD82-PE (Biolegend, Cat# 342104) 20µl/10^6 cells; CD56-APC (Biolegend, Cat# 318310) 5µl/10^6 cells. Samples were incubated for 30 min on ice, washed and filtered as described above.

In vitro fusion assays

Following FACS purification, cells were grown on plates coated with 0.15% gelatin at the following densities: 250,000 cells/well for a 6-well plate; 20,000 cells/well for a 48-well plate. Cells were initially suspended in 20% FBS/1x PSG/high glucose DMEM for the first 12h until attached, then were switched to differentiation medium (2% Horse serum/1xPSG/low glucose DMEM), changed daily. Images were taken on a Nikon Eclipse TS100 microscope fitted with a Spot RT3 camera starting at D0 (the day medium was switched to differentiation conditions) and every 24 hours. Between 15-20 images were taken randomly throughout each well from triplicate wells to avoid sampling bias. Images were analyzed using ImageJ software cell counter function. The Fusion index was calculated as the percentage of nuclei fused within myotubes/total nuclei. These assays were repeated using cell fractions extracted from 3 different samples. Each sample was analyzed separately due to the inherent variability between samples. Student's t-test was used to compare differences in fusion index (Excel).

IM Injection of NODRag1^{null} mdx^{5cv} and engraftment analysis

IM injections were performed according to a protocol approved by the Institutional ACUC (protocol number 15-08-2994R). Briefly, 100,000 MCAM$^+$CD82$^+$ or MCAM$^+$ cells were resuspended in 15µl of mouse serum (Jackson Immuno) with inert green fluorescent beads (Invitrogen, diluted 1:10,000 to track the injection site. Cells were injected IM in opposite TA muscles of a given mouse (n=7/group). Tissues were harvested at 4 weeks and 12 weeks post transplantation, snap-frozen in cold isopentane (Meng et al., 2014) and stored at -80°C until further use. Tissue was sectioned at 10µm and sections were collected from the entire muscle every 200 µm on 3 replicate slides. Slides were fixed with 100% Methanol for 3 min at RT, followed by washes in PBS and blocking for 1h RT in 10% FBS 0.1% TritonX-100 in PBS. Primary antibodies Dystrophin CAP6-10 1:1,000 (Lidov et al., 1990), LaminA/C 1:500 (Epitomics), Spectrin 1:100 (Vector labs) were incubated overnight at 4°C in blocking solution as described (Rozkalne et al., 2014). After 3 washes in PBS, slides were incubated for 45 min at RT with the appropriate secondary antibodies, washed 3 times in PBS and coverslipped using Vectashield mounting medium with DAPI. Images were taken using a 20X objective lens on a Nikon Eclipse E1000 microscope fitted with a Hamamatsu ORCA-ER CCD camera. All fibers positive for both spectrin and dystrophin were counted as engrafted with human cells. LaminA/C and the fluorescent beads were used to help locate/confirm the injection site and presence of fused human nuclei (Rozkalne et al., 2014). The number of myofibers engrafted with human cells were counted in 10 consecutive sections (corresponding to a depth of 2,000 µm). The average number of positive myofibers in mice engrafted with MCAM$^+$ or MCAM$^+$CD82$^+$ was compared using a paired t-test to determine significance in the engraftment efficacy between the two cell fractions.

RNA silencing in myogenic cells

Human CD82 and control sh-RNA lentiviral particles were purchased from Santa Cruz Biotechnology (sc-35734-V and sc-108080, respectively). Purified CD82$^+$CD56$^+$ human cells were infected with 30µl of lentiviral particles/well

in triplicate cultures in a 6 well plate for 6 hours. Cells were incubated in growth medium for 5 days and expanded onto 10cm plates. Puromycin was added at a final concentration of 1µg/ml for selection of lentiviral-infected cells and selection was continued until all cells from a non-infected control sample were killed by puromycin treatment. Lentiviral-infected cells were maintained in puromycin-containing media and processed for immunofluorescence and western blot analyses. Cells were plated on slide chambers for immunostaining with anti-Ki-67 or lifted for generating protein lysates for western blots. For apoptosis assays, the AlexaFluor 488 annexin V/dead cell apoptosis kit (Invitrogen Molecular Probes, Thermo Fisher Scientific) was used. Cells were processed for FACS and immunofluorescence analyses according to the protocol included by the manufacturer in the kit.

Overexpression of CD82-HA and a7ITG-FLAG in 293 cells and co-immunoprecipitation.

For the overexpression and immunoprecipitation of human CD82 and α7-ITG, the human full-length ORF for CD82 (NM_001024844) was cloned in-frame from fetal muscle tissue by PCR and restriction digest in to the Not1 and Xho1 restriction cut sites to pIRES-2a-hrGFP (3xHA epitope tag) vector (Stratagene). Similarly, the full length ORF of human α7-ITG (NM_002206; Catalog number: MHS6278-202808714; GE Healthcare) was cloned in-frame by PCR into the pIRES-1a-hrGFP (3xFLAG epitope tag) vector (Stratagene) into the Not1 and Xho1 restriction sites. Approximately 3 µg of each construct was transfected into approximately 5 x 10^5 HEK-293T cells (GeneHunter, Nashville, TN) in 10 cm dishes by Lipofectamine 2000 (Life Technologies) following the manufacturer's protocol. Approximately, 1.5 µg of each expression plasmid (CD82-HA and α7-ITG-FLAG) were co-transfected for the combined immunoprecipitation dish. Untransfected cells and cells transfected with 3 µg of 'empty' vector were also used as controls. Forty-eight hours post-transfection, the cells were washed twice in 1x PBS, before being gently lysed in RIPA Buffer (Boston BioProducts) supplemented with protease inhibitor tablets (Roche Applied Sciences). Following quantification of protein lysates by BCA assay (ThermoScientific), 400 µg of starting lysate was pre-cleared using 30 µl protein G magnetic beads (Biorad) for 60 minutes at 4ºC with gentle rocking. The pre-cleared lysates were then incubated on ice for 3 hours with either 5 µg of the immunoprecipitation antibody (anti-HA) or IgG beads (control reaction). The samples were then incubated with 70 µl of the Protein G IgG beads overnight at 4ºC with gentle rocking. The next day, magnetic beads and the bound Ab-antigen complexes were washed thoroughly 4 times with 500 µL RIPA buffer. Samples were placed near a magnetic field and buffer was removed. The final elution step was performed by adding 60 µL of 2X sample loading buffer to the beads and heating the mixture for 7 minutes at 72^{o}C. The eluates were spun at 13,200 rpm for 3 minutes at 4^{o}C and subjected to western blot analysis. For co-IP with anti-FLAG, immunoprecipitation was performed utilizing ANTI FLAG M2 magnetic beads (SIGMA ALDRICH, St. Louis, MO) following exactly the manufacturer's instructions. For each reaction, 40 µL of bead suspension was added to the pre-cleared overexpressed protein lysates. The mixture was incubated for 3 hours at room temperature on a rotating platform. The magnetic beads and the bound Ab-antigen complexes were washed thoroughly 3 times with 400 µL of 1X Tris Buffer Saline (TBS). Samples were then placed near a magnetic field and the washing buffer was removed. The final elution step was performed by adding 20 µL of 2X sample loading buffer to the beads, followed by heating the mixture for 7 minutes at 72^{o}C. The eluates were spun at 13,200 rpm for 3 minutes at 4^{o}C and subjected to western blot analysis.

Protein samples were then electrophoretically resolved on 4-12% Bis-Tris PAGE gradient gels (Novex), and hybridized to PVDF membranes (Life Technologies). After 3 washes in 0.1% Tween 20-TBS buffer for 15 min, membranes were blocked in 5% nonfat dry milk in TBST for 1 hour at room temperature, before being incubated overnight in primary antisera. Membranes were again washed three times for 5 minutes each and incubated in secondary antisera (1:2,000 dilution; goat-anti-mouse light-chain specific; Jax ImmunoResearch Laboratories Inc., Cat# 211-032-171) for 1 hour at room temperature with gentle rocking. Membranes were washed four times for 15 minutes in 0.1% Tween20/TBS buffer before being exposed to chemiluminescent reagent (ThermoScientific) and imaged on a BioRad Universal Hood II imaging system or to an X-ray film (GeneSee Scientific). Images were modified in Adobe Photoshop (version CS5.1) for brightness and tone (Auto tone).

Co-Immunoprecipitation of CD82 and a7ITG in differentiating human fetal muscle cells (endogenous IPs).

Approximately 400 µg of human fetal muscle cell lysate (lysed in RIPA Buffer-Boston BioProducts Inc., supplemented with protease inhibitor tablets-Roche Applied Sciences) were incubated with 30 µl of anti-IgG magnetic beads (Biorad) to pre-clear IgG fractions from the protein lysates. The IgG beads were then removed via magnetic separation, and the remaining lysate was incubated with 5µg of the following antisera: IgG-Abcam, anti-CD82-ThermoScientific PA5-27233; anti-CD56 (Rabbit polyclonal H-300; sc-10735, Santa Cruz Biotechnology), anti- a7-ITG (clone 6A11), anti-β4-ITG (MAB2060 clone ASC-9, EMD Millipore) for 3 hours at 4ºC with gentle mixing on a rocker. 20% of the protein lysate (approx. 40 µg) was removed and used as the Input control. The antisera-incubated protein lysates were then incubated with 30 µl of anti-IgG magnetic beads (Cell Signaling Tech.)

overnight (approx. 18 hours) at 4ºC with gentle mixing on a rocker. The protein-antisera-IgG bead lysates were washed 4 times with 4 RIPA lysis buffer supplemented with protease inhibitors. The protein complexes were then eluted off a magnetic separator using 2xSDS-PAGE buffer (Life Technologies) into fresh 1.5 ml tubes, then heated to 70ºC for 7 minutes, before centrifugation at 4ºC for 10 minutes at 10,000 x g. Samples were then frozen at -80ºC until ready to be electrophoresed on 4-12% NuPage (Tris-Glycine) gradient gels (Life Technologies). Gels were then transferred to PVDF membranes (Bio-Rad), blocked for 1 hour at room temperature using a blocking buffer containing 5% non fat dry milk in 1x TBS-Tween (Boston BioProducts Inc.), and incubated with primary antisera overnight (anti- a7ITG, clone 8G2; mouse monoclonal, Sigma-Aldrich; anti-CD82 rabbit polyclonal, Santa Cruz Biotech. diluted 1:1000 in blocking buffer) at 4ºC with gentle rocking. Blots were washed 3 times in 1x TBS-Tween for 5 minutes per wash, and incubated with secondary antisera consisting of either goat-anti-mouse HRP (IgG light chain-specific) or mouse anti-rabbit HRP (IgG light chain specific) (both purchased from Jackson ImmunoResearch Labs Inc.), at dilutions of 1:2,000 in blocking buffer for 1 hour at room temperature with gentle rocking. Membranes were then washed 4 times for 15 minutes in 1x TBS-Tween before being incubated with ECL Reagent (ThermoScientific) and developed on blue film (GeneSee Scientific). Film images were then scanned using a HP scanner and cropped in Adobe Photoshop software.

PLA assay

PLA assay was performed using the Duolink® In Situ Red Starter Kit Mouse/Rabbit (Sigma-Aldrich) using the manufacturer's instructions. Briefly, 15,000-20,000 $MCAM^+$ human fetal myogenic cells were plated/well in 8- or 16-well gelatin-coated slide chambers. Cells were washed once with PBS, fixed with 4%PFA for 10 min at RT, washed 3X5 min at RT in PBS, permeabilized in 0.5% Triton X-100 in PBS for 10 min and washed 3X5 min in 0.05% Tween20 in TBS. Cells were blocked in Duolink II blocking solution for 30 min at 37ºC in a humidified chamber; primary antibodies were added to the slides for 1hr at 37ºC diluted 1:50 in Duolink II diluent. Slides were washed in Duolink II wash buffer A 2X5 min at RT, then probes (mouse and rabbit) were added to the cells diluted 1:5 in in Duolink II diluent and incubated at 37ºC for 1hr in a humidified chamber. Slides were washed in Buffer A 2 x 5 min, ligase was added (dil 1:40) in 1X Duolink ligase stock and incubated at 37ºC for 30 min in a humidified chamber. Slides were washed in Buffer A 2 x 2 min, incubated in Duolink polymerase diluted 1:80 in 1X Duolink amplification solution in the dark at 37ºC for 100 min in a humidified chamber. Slides were washed 2X 10 min in Duolink Wash buffer B at RT protected from light, then dipped once in 0.1X buffer B before being added Duolink II mounting medium with DAPI, coverslipped and imaged.

CD82 overexpression in primary cells and EdU incorporation assays

Primary human myoblasts from healthy and DMD subjects were infected with lentivirus expressing human CD82 open-reading-frame (ORF) (NCBI Ref. Seq. NM_002231.3) with a C-terminal V5 tag. Cells were maintained in growth media (DMEM containing 20% FBS) in 0.1% gelatin coated 6-well plates until 70% confluent. Polybrene was added at a concentration of 8µg/mL for 15 minutes at RT prior to addition of the lentiviral solution to transduce the cells. Cells were then incubated at 37°C CO_2 for 6 hours, after which the media was replaced with fresh growth media. Cells were selected for by addition of 1ug/ml puromycin (RPI P33020) in the growth media until all untransfected control cells were dead. CD82-V5 expression was induced by addition of 0.25µg/ml doxycycline hyclate (Sigma D9891) to the media for 48 hours and was verified by western blotting cell lysates with anti-V5 (Abcam ab27671).

For EdU incorporation assays, Click-iT Plus EdU flow cytometry assay kit was used following the manufacturer instructions. Briefly, cells were incubated overnight with EdU at a final concentration of 5µM. The following day, cells were trypsinized, counted and processed for FACS analysis to determine the expression of CD82 (PE) and CD56 (APC). EdU incorporation in cells was detected based on expression of Pacific Blue signal.

Supplemental References

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