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Supplemental Information

Fibrogenic Cell Plasticity Blunts Tissue Regeneration and Aggravates Muscular Dystrophy

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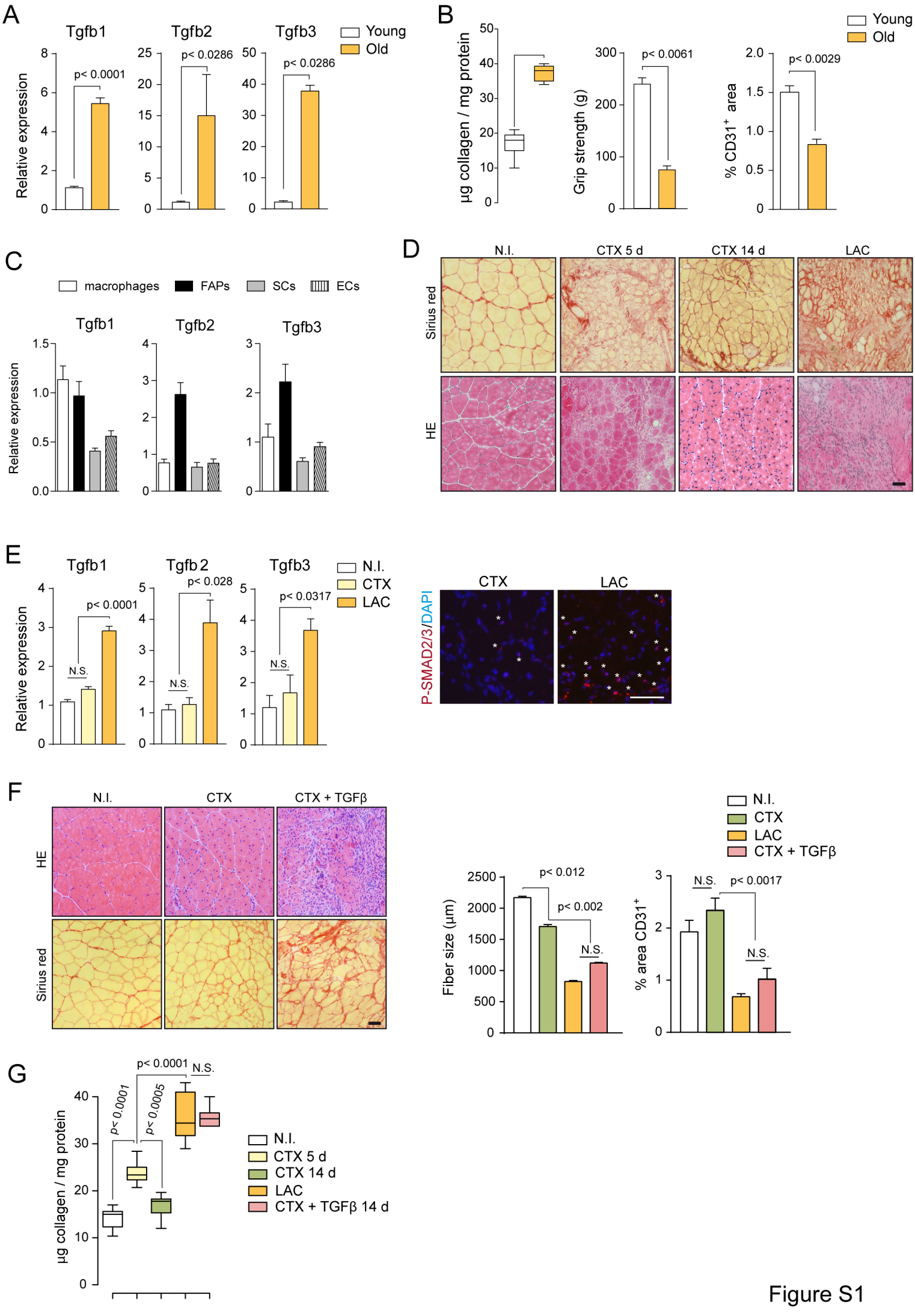

A
Tgfb1
Tgfb2
Tgfb3
Young
Old
Relative expression
p< 0.0001
p< 0.0286
p< 0.0286
B
Young
Old
µg collagen / mg protein
Grip strength (g)
% CD31+ area
p< 0.0061
p< 0.0029
C
macrophages
FAPs
SCs
ECs
Tgfb1
Tgfb2
Tgfb3
Relative expression
D
N.I.
CTX 5 d
CTX 14 d
LAC
Sirius red
HE
E
Tgfb1
Tgfb2
Tgfb3
N.I.
CTX
LAC
Relative expression
p< 0.0001
p< 0.028
p< 0.0317
N.S.
CTX
LAC
P-SMAD2/3/DAPI
F
N.I.
CTX
CTX + TGFβ
HE
Sirius red
N.I.
CTX
LAC
CTX + TGFβ
Fiber size (µm)
% area CD31+
p< 0.012
p< 0.002
N.S.
N.S.
p< 0.0017
N.S.
G
µg collagen / mg protein
p< 0.0001
p< 0.0005
p< 0.0001
N.S.
N.I.
CTX 5 d
CTX 14 d
LAC
CTX + TGFβ 14 d

Figure S1

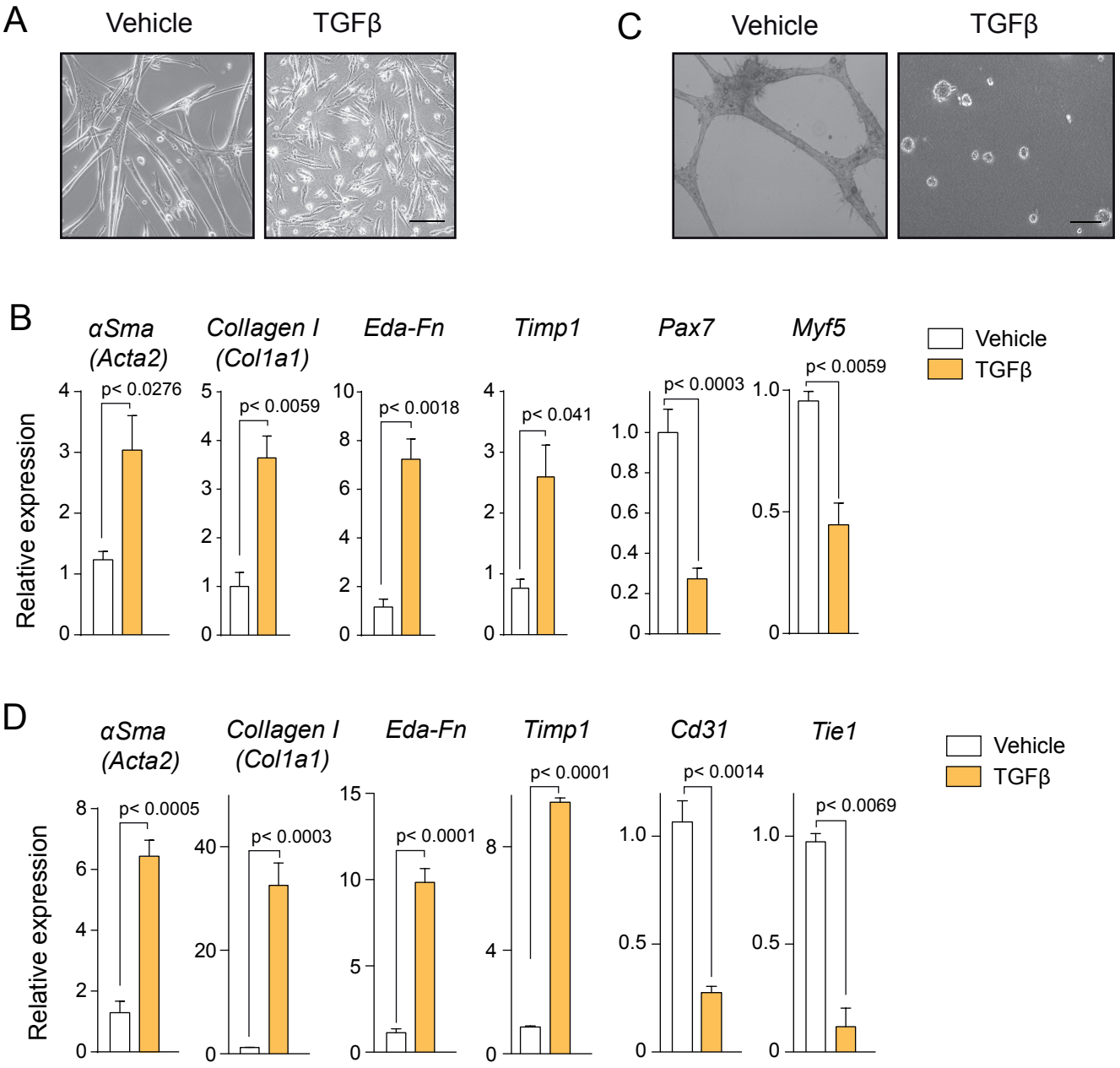

A
Vehicle
TGFβ
C
Vehicle
TGFβ
B
αSma (Acta2)
Collagen I (Col1a1)
Eda-Fn
Timp1
Pax7
Myf5
Relative expression
p< 0.0276
p< 0.0059
p< 0.0018
p< 0.041
p< 0.0003
p< 0.0059
Vehicle
TGFβ
D
αSma (Acta2)
Collagen I (Col1a1)
Eda-Fn
Timp1
Cd31
Tie1
Relative expression
p< 0.0005
p< 0.0003
p< 0.0001
p< 0.0001
p< 0.0014
p< 0.0069
Vehicle
TGFβ

Figure S2

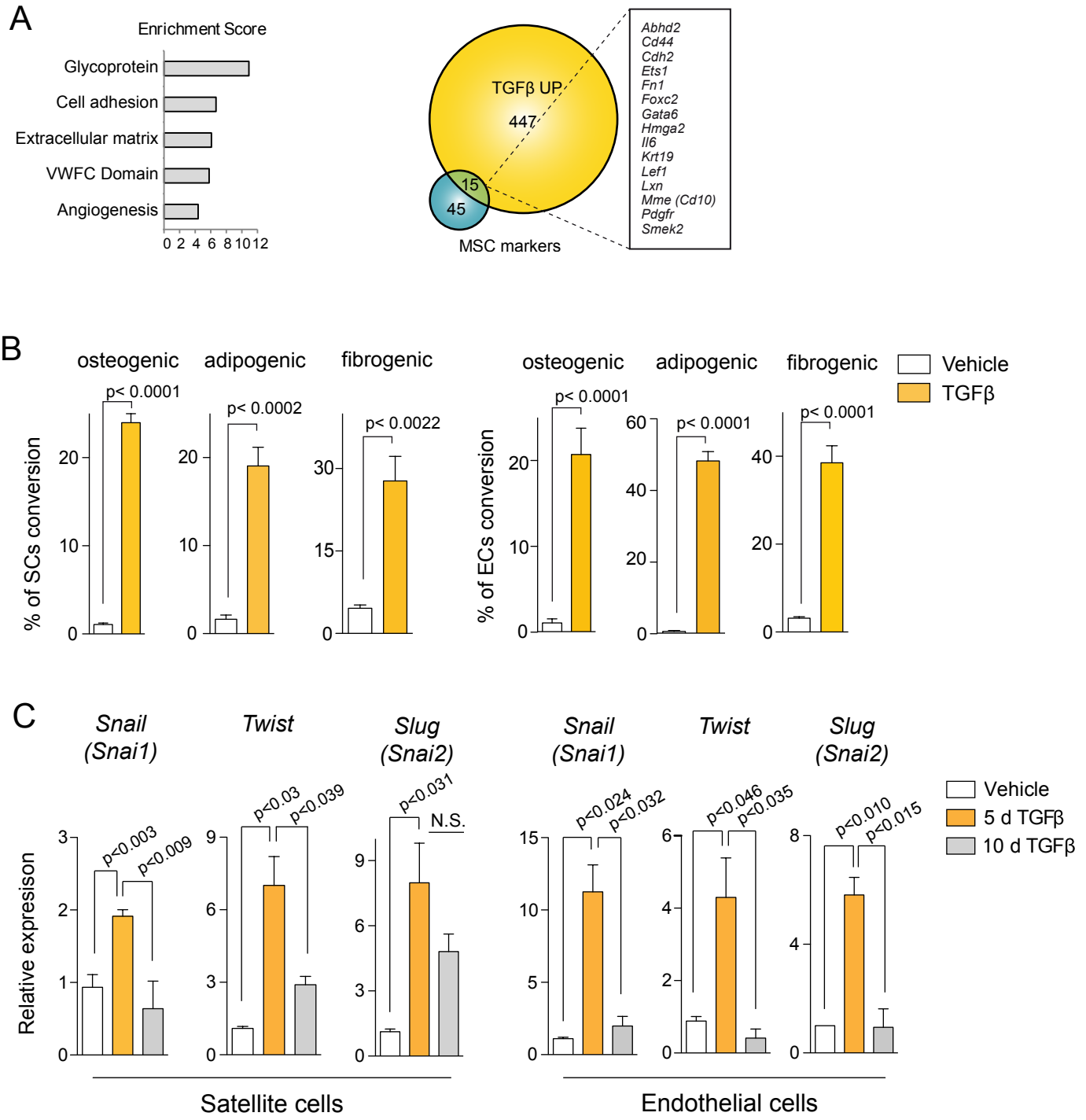

A
Enrichment Score
Glycoprotein
Cell adhesion
Extracellular matrix
VWFC Domain
Angiogenesis
0 2 4 6 8 10 12
TGFβ UP
447
15
45
MSC markers
Abhd2
Cd44
Cdh2
Ets1
Fn1
Foxc2
Gata6
Hmga2
Il6
Krt19
Lef1
Lxn
Mme (Cd10)
Pdgfr
Smek2
B
osteogenic
adipogenic
fibrogenic
p< 0.0001
p< 0.0002
p< 0.0022
% of SCs conversion
p< 0.0001
p< 0.0001
p< 0.0001
% of ECs conversion
Vehicle
TGFβ
C
Snail (Snai1)
Twist
Slug (Snai2)
Relative expresison
p<0.003
p<0.009
p<0.03
p<0.039
p<0.031
N.S.
p<0.024
p<0.032
p<0.046
p<0.035
p<0.010
p<0.015
Satellite cells
Endothelial cells
Vehicle
5 d TGFβ
10 d TGFβ
D
Satellite cells
Endothelial cells
MiR-21 relative expresison
p<0.006
p<0.049
p<0.0003
Vehicle
3 d TGFβ
5 d TGFβ

Figure S3

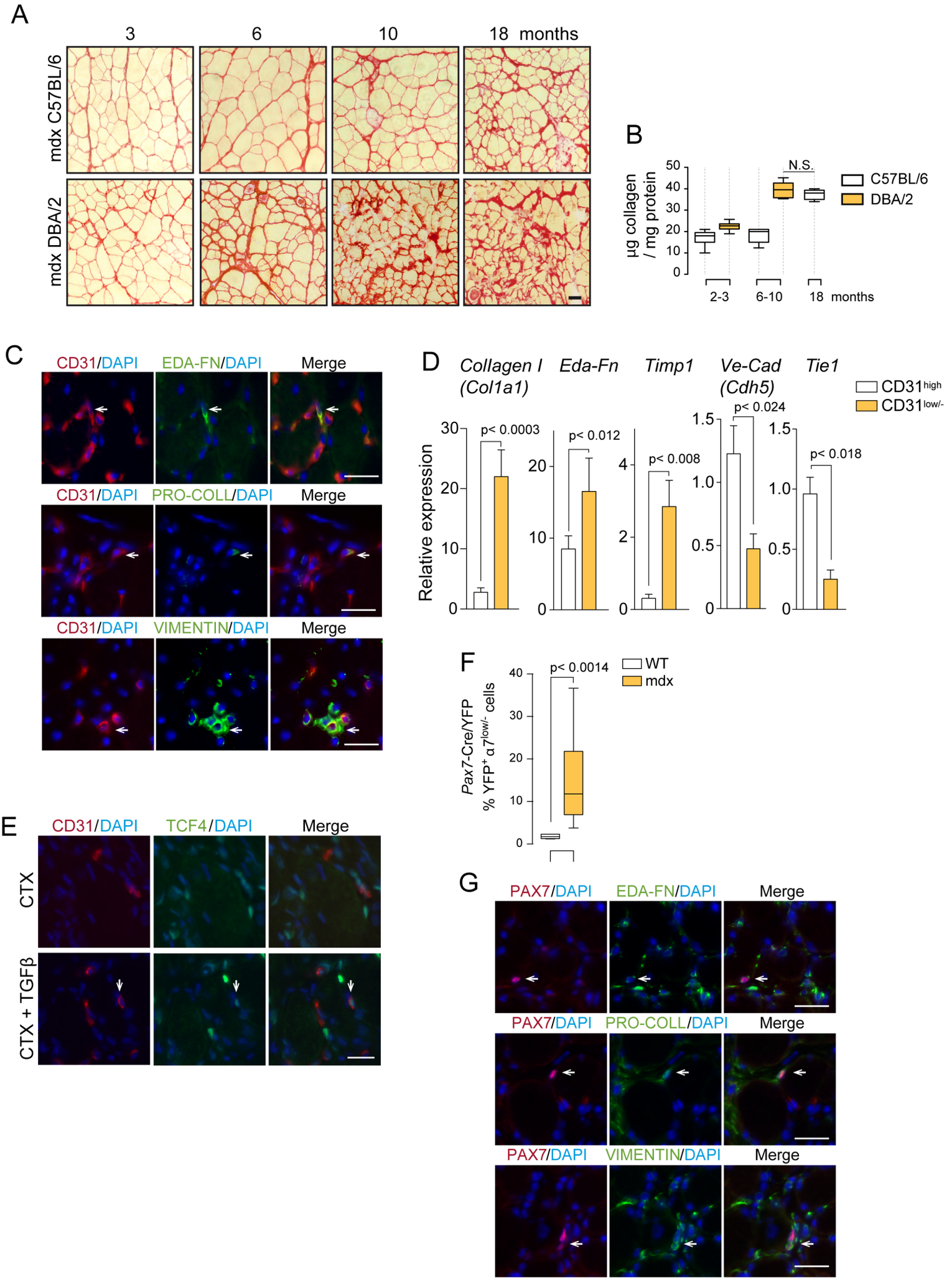
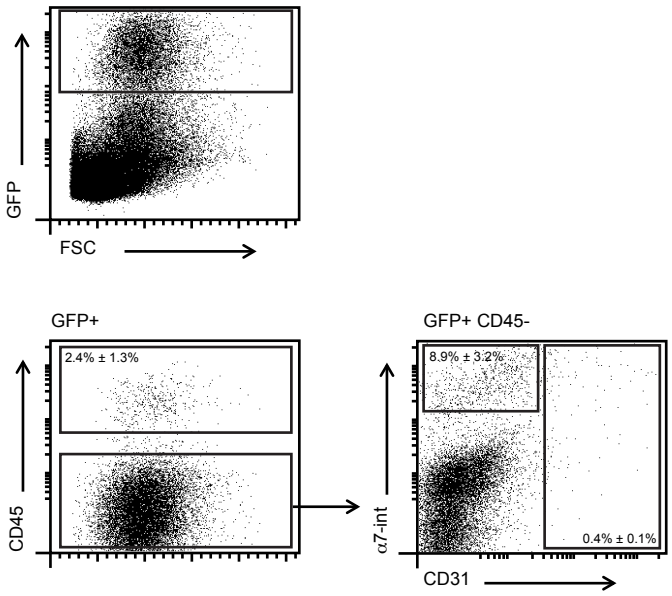

A
3
6
10
18 months
mdx C57BL/6
mdx DBA/2
B
µg collagen / mg protein
50
40
30
20
10
0
N.S.
C57BL/6
DBA/2
2-3
6-10
18 months
C
CD31/DAPI
EDA-FN/DAPI
Merge
CD31/DAPI
PRO-COLL/DAPI
Merge
CD31/DAPI
VIMENTIN/DAPI
Merge
D
Collagen I (Col1a1)
Eda-Fn
Timp1
Ve-Cad (Cdh5)
Tie1
CD31high
CD31low/-
Relative expression
p< 0.0003
p< 0.012
p< 0.008
p< 0.024
p< 0.018
E
CD31/DAPI
TCF4/DAPI
Merge
CTX
CTX + TGFβ
F
Pax7-Cre/YFP
% YFP+ α7low/- cells
p< 0.0014
WT
mdx
G
PAX7/DAPI
EDA-FN/DAPI
Merge
PAX7/DAPI
PRO-COLL/DAPI
Merge
PAX7/DAPI
VIMENTIN/DAPI
Merge

Figure S4

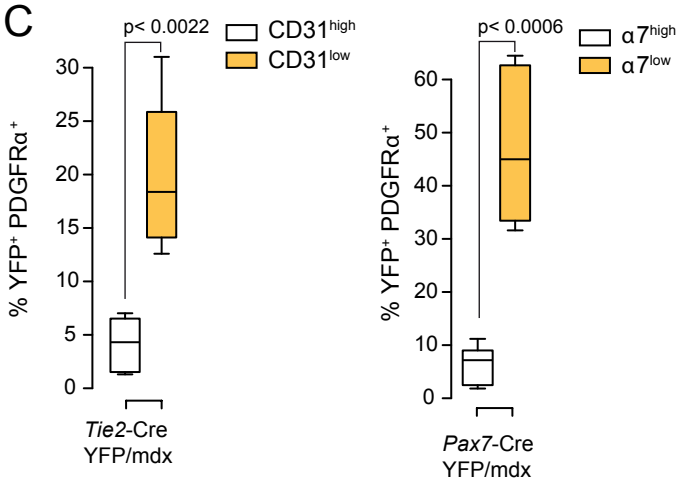
A

% of collagen-producing cells in mdx dystrophic muscle
Endothelial origin
2.01 ± 0.36
Myogenic origin
2.24 ± 0.43
Hematopoietic origin
2.85 ± 0.60

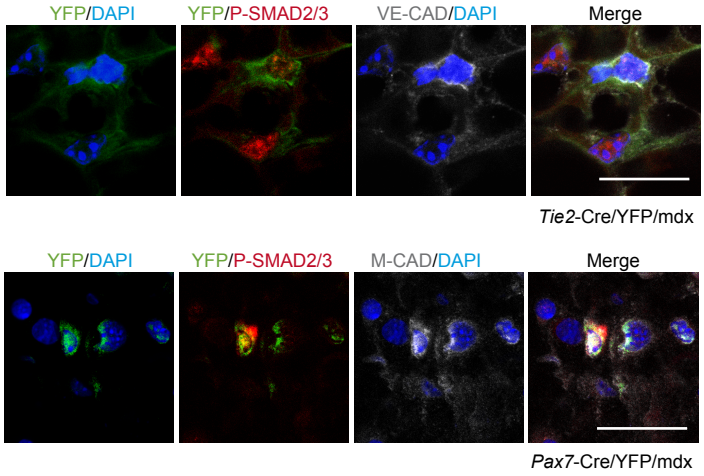
B



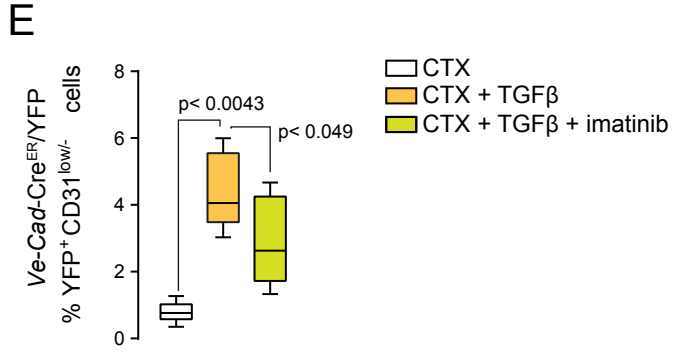
C



D



E



F

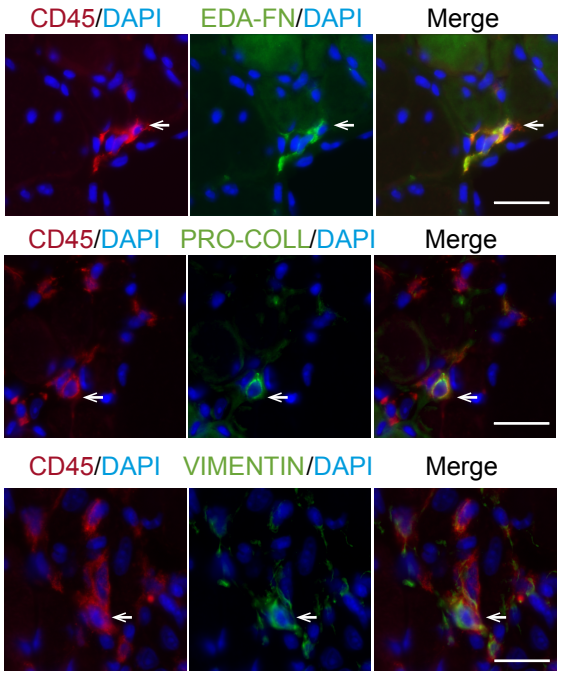


Figure S5

Supplemental Figure 1
(**A**) Quantitative RT-PCR for *Tgfb1*, *2* and *3* mRNA expression in limb muscles from young (2-3 months) and old (18-24 months) mdx mice in C57BL/6 background. Values are mean ± SEM: n=4 for each group. Unpaired t test was used for comparison. (**B**) collagen content (as fibrosis index), four-limb grip strength and area occupied by CD31-positive cells (angiogenesis index) of young and old mdx mice, described in (A). Values are mean ± SEM; n=7 for each group. Non-parametric Mann–Whitney U-test was used for comparison. (C) Quantitative RT-PCR for *Tgfb1*, *2* and *3* mRNA expression in FACS-isolated macrophages, fibroadipogenic progenitors (FAPs), satellite cells and endothelial cells, isolated from fibrotic muscle of mdx mice. Values are mean ± SEM; n=3 independent experiments (mice) for each group. Unpaired t test was used for comparison. (**D**) Representative Sirius Red and HE staining pictures of tibialis anterior (TA) muscles of wild-type (WT) mice non-injured (N.I.) or injured with cardiotoxin (CTX) after 5 and 14 days, or injured by laceration (LAC) after 21 days. (**E**) Quantitative RT-PCR for *Tgfb1*, *2* and *3* mRNA expression in muscles of WT mice after 14 days of CTX injury or 21 days of laceration. Values are mean ± SEM; n=4 independent experiments (mice) for each group. Unpaired t test was used for comparison. Representative pictures of phosphorylated SMAD2/3 (P-SMAD2/3) protein immunostaining in CTX- and LAC-injured muscle. * shows P-SMAD2/3 positive cells. (**F**) Representative HE and Sirius red staining pictures of TA muscles of WT mice non-injured or injured with CTX for 7 days, after which TGFβ was administered (or vehicle) for 7 extra days, and muscles were collected at 14 days post injury (CTX+TGFβ) to induce more persistent fibrosis. Quantification of size of regenerating fibers is represented. Quantification of area occupied by CD31-positive cells, as an angiogenesis index. Values are mean ± SEM. (**G**) Quantification of collagen content for the same muscles: non injured (N.I.) muscle, muscle at 5 and 14 days after CTX injury, 14 days after CTX+TGFβ injury, or 21 days after laceration. Values are mean ± SEM; n=9 independent experiments (mice) or each group. Non-parametric Mann–Whitney U-test was used for comparison. Scale bars, 50 μm.

Supplemental Figure 2
(**A,C**) Satellite cells (A) and endothelial cells (C) (obtained from muscle of WT mice) were cultured in vitro in adequate medium to induce formation of myotubes or angiotubes (as an indication of their functionality), respectively, and treated or not with TGFβ for 10 days. Scale bars, 50 μm. (**B,D**) Quantitative RT-PCR in primary satellite cells (B) and endothelial cells (D) treated with TGFβ for 10 days in vitro. Fibrogenic markers are up-regulated upon treatment, and myogenic markers (*Myf5* and *Pax7* (B)) or endothelial markers (*Cd31* and *Tie1* (D)) are down-regulated. Values are mean ± SEM; n=3 independent experiments. Unpaired t test was used for comparison.

Supplemental Figure 3
(**A**) Functional annotation analysis of the Gene Ontology (GO) was performed in DAVID to identify biological processes enriched in TGFβ-treated satellite cells (n=3 samples). The top annotation clusters are shown according to their enrichment score. Names are based on enriched GO annotations. Venn diagram of the overlap between significantly upregulated genes in TGFβ-treated satellite cells and multipotent progenitor cell-specific transcripts. (**B**) Quantification of conversion of TGFβ-pretreated satellite cells and endothelial cells into the three indicated cellular destinies. Values are mean ± SEM; n=4 biological replicates. Unpaired t test was used for comparison. (**C**) Quantitative RT-PCR analysis of classical transcription factors regulating mesenchymal transitions in satellite cells and endothelial cells treated with TGFβ for 5 and 10 days (or vehicle, 10 days). Values are mean ± SEM; n=3 independent experiments. Unpaired t test was used for comparison. (**D**) Satellite cells and endothelial cells were treated with TGFβ for 3 and 5 days (or vehicle, 5 days) and analyzed for *MiR-21*

expression. Values are mean ± SEM; n=3 independent experiments. Unpaired t test was used for comparison.

Supplemental Figure 4

(**A**) Representative Sirius Red staining pictures of TA muscle of mdx/C57/BL6 and mdx/DBA2 mice at the indicated ages. Scale bars, 50 μm. (**B**) Quantification of collagen content of the same muscles. Data correspond to the mean ± SEM; n=5 for each group. Non-parametric Mann–Whitney U-test was used for comparison. (**C**) Representative pictures of cells co-expressing the endothelial cell marker CD31 and EDA FIBRONECTIN (EDA-FN) (upper panel), PRO-COLLAGEN (PRO-COL) (central panel) or VIMENTIN (lower panel) in TA muscle of old (18 months) mdx mice. Scale bars= 25 μm. (**D**) Quantitative RT-PCR in FACS-isolated $YFP^{+}CD31^{low/-}$ cells compared to the $YFP^{+}CD31^{high}$ population, isolated from CTX/TGFβ-injured muscle of *Ve-Cad*-Cre^{ER}/YFP mice, for the indicated fibroblastic and endothelial cell markers. Values are mean ± SEM; n=3 independent experiments (mice) for each group. Unpaired t test was used for comparison. (**E**) Representative pictures of cells co-expressing TCF4 and CD31 in CTX-injured muscle compared to CTX/TGFβ-injured muscles from WT mice. Nuclei are stained with DAPI. Scale bars= 10 μm. (**F**) Quantification of YFP^{+} cells that maintain or have reduced/lost α7-INTEGRIN expression in limb muscles of old (18 months of age) *Pax7*-Cre/YFP/mdx mice (C57BL/6 background) (mdx) compared to *Pax7*-Cre/YFP (WT) mice. Values are mean ± SEM; n=6 animals for each group. Non-parametric Mann–Whitney U-test was used for comparison. (**G**) Representative pictures of cells co-expressing the myogenic marker PAX7 and EDA-FN (upper panel), PRO-COL (central panel) and VIMENTIN (lower panel) in TA muscle of old mdx mice. Scale bars, 25 μm.

Supplemental Figure 5

(**A**) Table showing the percentage of collagen-producing cells from endothelial, myogenic and hematopoietic origin in dystrophic muscle at advanced disease stages. The percentages of collagen-producing cells derived from the different origins analyzed are the mean of different experimental procedures which are described in detail in the Methods section. In particular, see "**Analysis of distinct origins of collagen-producing cells**": for endothelial origin (procedures 1, 2, 3 and 4); for myogenic origin (procedures 1, 3 and 4); and for hematopoietic origin (procedures 2, 3 and 4). Values are mean ± SEM. (**B**) Representative FACS plots of $CD45^{+}COLL^{+}$ cells, $\alpha7INTEGRIN^{+}COLL^{+}$ cells and $CD31^{+}COLL^{+}$ cells in fibrotic muscle of *Coll*-GFP/mdx mice, representing cells with hematopoietic, myogenic and endothelial cell origin, respectively, within the fibrogenic (collagen-expressing) population. Values are mean ± SEM; n=5 for each group. Note: results from panel (B) are included in the mean values shown in the table in panel (A). (**C**) Percentage of double positive $PDGFR\alpha^{+}$ YFP^{+} cells from muscles of *Tie2*-Cre/YFP/mdx (that were $CD31^{high}$ or $CD31^{low}$) and *Pax7*-Cre/YFP/mdx mice (that were α7-$INTEGRIN^{high}$ or α7-$INTEGRIN^{low}$). Values are mean ± SEM relative to age-matched lineage tracing mice in non-dystrophic backgrounds; n=6 animals for each group. Non-parametric Mann–Whitney U-test was used for comparison. (**D**) Top: Representative immunostaining picture of YFP^{+} cells co-expressing VE-CADHERIN (endothelial cell marker) and P-SMAD2/3 in TA muscle of *Tie2*-Cre/YFP/mdx mice of 18 months of age. Bottom: Representative immunostaining picture of YFP^{+} cells co-expressing M-CADHERIN (satellite cell marker) and P-SMAD2/3 in TA muscle of *Pax7*-Cre/YFP/mdx mice of 18 months of age. Scale bars= 25 μm. (**E**) Muscles of *Ve-Cad*-Cre^{ER}/YFP mice were injured with CTX, or CTX/TGFβ, in mice that were treated or not with the PDGFRα inhibitor imatinib (as indicated in Materials and Methods). The number of YFP^{+} cells that had lost endothelial cell marker expression ($CD31^{low/-}$) was quantified. Values are mean ± SEM; n=3

independent experiments (mice) for each group. Unpaired t test was used for comparison. (**F**) Representative pictures of cells co-expressing the hematopoietic CD45 and EDA-FN (upper panel), PRO-COL (central panel) and VIMENTIN (lower panel) in TA muscle of old mdx mice. Scale bars, 25 μm.

Table 1
Antibodies list

TCF4	Cell Signaling 2569S
Vimentin	Sigma V5255
Fibronectin (IST-9)	Abcam ab6328-250
Pro-COL1A1(E-9)	Santa Cruz Biotecnology sc-133179
Mouse collagen Type I	Millipore AB765P
Actin α-Smooth Muscle - SMA	Sigma A2547
Phospho - Smad2 (Ser465/467)	Cell Signalling 3101S
CD31	BD Pharmingen 550274
CD45	BD Pharmingen 553081
Pax7	
M-Cadherin	Santa Cruz Biotechnology sc-6470
VE-Cadherin	Santa Cruz Biotechnology sc-6458
GFP	Invitrogen A6455
human CD56	BD Pharmingen 556325
human vWF	Abcam ab68545
Biotin anti human CD45	Biolegend 304003
human CD44	Binding Site MC114

Table 2
FACS antibodies list

PE-conjugated anti-α7-integrin	(Ablab AB10STMW215)
PE-Cy7-conjugated anti-CD31	Biolegend 102418
APC-Cy7-cojugated anti-CD45	Biolegend 103116
APC-conjugated anti-Mouse CD140a (PDGF Receptor a)	eBioscience 17-1401
Brilliant Violet 605 Ly-6A/E (Sca-1)	Biolegend 108133

Table 3
Primers list

Mouse primers

	Forward	Reverse
L7	GAAGCTCATCTATGAGAAGGC	AAGACGAAGGAGCTGCAGAAC
αSMA	TCCCTGGAGAAGAGCTACGA	CTTCTGCATCCTGTCAGCAA
Collagen I	GGTATGCTTGATCTGTATCTGC	AGTCCAGTTCTTCATTGCATT
ED-A Fibronectin	AGGACTGGCATTCACTGATGTG	GTCACCCTGTACCTGGAAACTTG
Vimentin	CGGCTGCGAGAGAAATTGC	CCACTTTCCGTTCAAGGTCAAG
CTGF	CAGGCTGGAGAAGCAGAGTCGT	CTGGTGCAGCCAGAAAGCTCAA
TIMP-1	TTCCAGTAAGGCCTGTAGC	TTATGACCAGGTCCGAGTT
TGFβ1	CTCCACCTGCAAGACCAT	CTTAGTTTGGACAGGATCTGG
TGFβ2	TCGACATGGATCAGTTTATGCG	CCCTGGTACTGTTGTAGATGGA
TGFβ3	GGAAATGGGTCCACGAACC	TCCAAGCACCGTGCTATGG
Snail	CACACGCTGCCTTGTGTCT	GGTCAGCAAAAGCACGGTT
CD31	ACGCTGGTGCTCTATGCAAG	TCAGTTGCTGCCCATTCATCA
Tie1	CAGGCACAGCAGGTTGTAGA	GTGCCACCATTTTGACACTG
Ve-Cadherin	CCGCTGATCGGCACTGTGGT	GGAGTACCCGATGCTGCGCT
Pax7	GTGTCTCCAAGATTCTGTGCCG	CAATCTTTTTCTCCACATCCGG
Myf5	CTGTCTGGTCCCGAAAGAAC	AAGCAATCCAAGCTGGACAC
PDGFRα	TGGCATGATGGTCGATTCTA	CGCTGAGGTGGTAGAAGGAG
Fibronectin I	GCGACTCTGACTGGCCTTAC	CCGTGTAAGGGTCAAAGCAT
LEF1	CAGCTTTATCCAGGCTGGTC	TGAAGTCGACTCCTGTAGCTTCT
IL-6	GGTGACAACCACGGCCTTCCC	AAGCCTCCGACTTGTGAAGTGGT
HMGA2	GAGCCCTCTCCTAAGAGACCC	TTGGCCGTTTTTCTCCAATGG
CD10	GAAATTCAGCCAAAGCAAGC	TGCTGAGCACTGAAGAATGG
CD14	AAAGAAACTGAAGCCTTTC	AGCAACAAGCCAAGCACAC
CD29	TGGACAATGTCACCTGGAAA	TGTGCCCACTGCTGACTTAG
CD44	TGGATCCGAATTAGCTGGAC	AGCTTTTTCTTCTGCCCACA
CD71	TAAATTCCCCGTTGTTGAGG	TTTTCTGCAGCAGCTCTTGA
CD90	CGCTCTCCTGCTCTCAGTCT	GCACGTGCTTCCTCTTCTCT
CD105	CTTCCAAGGACAGCCAAGAG	TTCTGGCAAGCACAAGAATG

Human primers

	Forward	Reverse
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGT
Collagen I	GCAAGGTGTTGTGCGATGAC	TTGGTCGGTGGGTGACTCTG
FN I	GGATGACAAGGAAAATAGCCCTG	GAACATCGGTCACTTGCATCT
CTGF	CAAGGGCCTCTTCTGTGACT	ACGTGCACTGGTACTTGCAG
TIMP-1	CTTCTGCAATTCCGACCTCGT	CCCTAAGGCTTGGAACCCTTT
TGFb1	GGCCAGATCCTGTCCAAGC	GTGGGTTTCCACCATTAGCAC
Pax7	GACCCCTGCCTAACCACATC	GTCTCCTGGTAGCGGCAAAG
Myf5	GAGGTGTACCACGACCAACC	CCTGCTCTCTCAGCAACTCC
MyoD	GCCACAACGGACGACTTCTATG	TGCTCTTCGGGTTTCAGGAG
PDGFRa	TCCTCTGCCTGACATTGACC	TGAAGGTGGAACTGCTGGAAC

EXPERIMENTAL PROCEDURES

Mice handling and sample obtention

Mice were housed in standard cages under 12 hour light-dark cycles and fed ad libitum with a standard chow diet. All experiments were approved by the Ethics Committee of the Pompeu Fabra University (UPF) and performed according to Spanish and European legislation. Operations were performed after injection intra-peritoneal of ketamine/metedomidine anesthesia (50 mg/kg and 1 mg/kg body weight). Atipamezol (1.0 mg/kg body weight) by subcutaneous injection was used to reverse the effects of anesthesia. Injections were made using 25 gauge needles (Becton Dickinson). Mice were sacrificed at the indicated ages by cervical dislocation and the tissues were immediately processed to avoid artifacts, either by direct freezing in liquid nitrogen for protein and RNA extraction or as described below for histological analysis.

Histological analysis and immunohistochemistry

Cryosections (10 µm thickness) were stained with hematoxylin/eosin (H&E) or Sirius red (Sigma-Aldrich). Quantification of collagen content in muscle was performed according to Ardite et al. (Ardite et al., 2012). Briefly, 10 cryosections were collected in a tube and were sequentially incubated with a solution containing 0.1% Fast green in saturated picric acid, washed with distillated water, incubated with 0.1% Fast green and 0.1% Sirius red in saturated picric acid, washed with distillated water, and gently resuspended in a solution of 0.1 M NaOH in absolute methanol (1 vol:1 vol). Absorbance was measured in a spectrophotometer at 540 and 605 nm wavelengths and used to calculate total protein and collagen.

For immunohistochemistry, muscles were fixed in 2% PFA for two hours at 4°C, then the tissue was transferred to 20% sucrose PBS and incubated overnight for subsequent OCT embedding and freezing. Standard methods were followed for cryosectioning. Tissue sections were permeabilized in 0.5% Triton X- 100 (Sigma) in PBS, and blocked for 1 h at room temperature in PBS containing 10% normal goat serum (NGS) and 10% bovine serum albumin (BSA). Cells were stained overnight at 4°C using a primary antibody diluted in 10% NGS and 10% BSA. The primary antibodies used for immunofluorescence are listed on Table 1 of Supplemental Information. Primary antibodies were detected using secondary antibodies conjugated to Alexa 488, 568 or 647 (Molecular Probes) and nuclei were stained with DAPI (Invitrogen). After washing, tissue sections were mounted with Mowiol.

Dystrophic patients study: Human samples from DMD patients were provided by Biobanco Hospital Virgen del Rocío (Sevilla) and from Hopital Sant Joan de Deu (Barcelona). Muscle biopsies were obtained from the vastus lateralis muscle under local anesthesia (2% lidocaine). A portion of the muscle tissue was directly frozen in melting isopentane and stored at −80°C until analyzed. DMD diagnosis was established on a total absence of dystrophin by immunohistochemistry and Western blotting. Morphological quantification of fibrosis was carried out by color image segmentation and automatic measurement using Fiji image analysis software. The ratio of the total area of fibrosis to the total biopsy area was used to estimate the extent of fibrosis (fibrosis index).

Cell analysis by Flow Cytometry

Skeletal muscle from both hind limbs was carefully dissected and then gently torn with tissue forceps until homogeneous. Enzymatic digestion was performed with Collagenase D 0.1% (Roche) and Dispase II 0.05% (Sigma-Aldrich) at 37º C for 25 min four times. Preparations were passed through a 40-µm cell strainer (Becton Dickinson),

and washed. Resulting single cells were collected by centrifugation at 400g for 5 min. Cell preparations were incubated with primary antibodies against cell membrane markers for 30 min at 4 °C in supplemented PBS containing 2 mM EDTA and 2% FBS (FACS buffer) at ~1 × 10^7 cells per ml. The antibodies used in flow cytometry and the dilutions are listed on Table 2 of Supplemental Information. Analysis was performed on LSRII (Becton Dickinson) equipped with three lasers. Data were collected using FACSDIVA software. Sorting were performed on a FACS Aria (Becton Dickinson), equipped with three lasers. Sorting gates were strictly defined based on isotype control (fluorescence minus one) stains. Flow cytometry data analysis was performed using Gate Logic software.

RNA isolation, reverse transcription (RT) and real-time quantitative PCR

Total RNA was isolated from muscle tissue using Trizol (Invitrogen). cDNA was synthesized from 1 µg of total RNA using the First Strand cDNA Synthesis kit and random priming according to the manufacturer's instructions (Promega). RT-PCR was performed on a LightCycler 480 System using LightCycler 480 SYBR green I Master mix (Roche) with 10 µM each primer and normalized to L7 ribosomal RNA as a housekeeping gene. The primers used are listed on Table 3 of Supplemental Information.

Quantification of miR expression

Total RNA, including the miR fractions, was isolated using the miRNeasy kit (QIAGEN). The quantification of *MiR-21* was performed using Taqman assays for miR (Applied Biosystems) and Taqman Universal Master Mix (Applied Biosystems). miR levels were quantified using *MiR- U6B* as the housekeeping miR.

Transcriptomic analysis

cDNA generated from RNA obtained from murine primary myoblasts, treated (or not) for 4 days with TGFβ1, was used on a transcriptome analysis by Agilent SurePrint G3 Mouse GE 8 × 60K high-density microarray slides, performed at the microarray Unit of CRG/UPF. Microarray analysis was performed with 3 samples per condition. Data were normalized using cyclic loess, and differentially expressed genes were identified using AFM 4.0 for all pairwise comparisons. Gene ontology analysis was performed using DAVID. Venn diagrams were generated using BioVenn.

To compare with our data, we generated a curated list of mesenchymal progenitor cell-specific transcripts by combining a list of well-known mesenchymal-stem cell markers with a list of genes found to be expressed selectively in mesenchymal stem cells as compared to fibroblasts, osteoblasts, chondrocytes and adipocytes and which showed reduced expression upon differentiation to any of those lineages (Kubo et al., 2009)

Quantification of TGFβ protein

The protein concentration of active and total (active plus latent) TGFβ levels in dystrophic muscle was quantified by ELISA (Promega), following the manufacturer's instructions.

Analysis of distinct origins of collagen-producing cells

The percentage of collagen-producing cells derived from distinct cellular origins was calculated using different experimental procedures:

1- Percentage of YFP^+ cells within the fibroblastic population (defined by TCF4 and FIBRONECTIN markers). Analysis was performed by immunofluorescence in muscle sections of *Ve-Cad*-Cre^{ER}/YFP/mdx, *Tie2*-Cre/YFP/mdx, *Pax7*-Cre^{ER}/YFP/mdx mice and in mdx mice transplanted with bone marrow from *Coll*-GFP mice.

2- Percentage of YFP^+ cells in the collagen-expressing population (as an indicator of fibrogenic population). Collagen production was detected by intracellular staining by FACS analysis in cells obtained from dystrophic muscle of *Ve-Cad*-Cre^{ER}/YFP/mdx, *Ve-Cad*-Cre^{ER}/YFP and *Pax7*-Cre^{ER}/YFP mice and mdx mice transplanted with *Coll*-GFP bone marrow.

3- Percentage of α7INTEGRIN$^+$, $CD31^+$ and $CD45^+$ cells (reporting myogenic, endothelial and hematopoietic cell origins, respectively) within the GFP^+ population. FACS analysis was used to analyze α7INTEGRIN$^+$, $CD31^+$ and $CD45^+$ cells in GFP^+ cells from muscle of *Coll*-GFP/mdx mice.

4- Percentage of $PAX7^+$, VE-CADHERIN$^+$ or $CD45^+$ cells (reporting myogenic, endothelial and hematopoietic cell origins, respectively) within the fibroblastic population (defined by TCF4 and VIMENTIN markers). Analysis was performed by immunofluorescence in muscle sections of mdx mice.

Table: mouse models used to determine origins of fibroblasts

Sources of fibroblasts	Dystrophic muscle
Endothelial cells	*Ve-Cad*-Cre^{ER}/YFP mice crossed with DBA/2 mdx mice
	Tie2-Cre/YFP mice crossed with C57BL/6 mdx mice
Myogenic cells	*Pax7*-Cre^{ER}/YFP mice crossed with DBA/2 mdx mice
	Pax7-Cre/YFP mice crossed with C57BL/6 mdx mice
Hematopoietic cells	Transplantation of bone marrow from *Coll*-GFP mice into C57BL/6 mdx mice

Sources of fibroblasts	WT muscle: acute injury/TGFβ
Endothelial origin	*Ve-Cad*-Cre^{ER}/YFP
Myogenic origin	*Pax7*-Cre^{ER}/YFP

Fate mapping mouse	Dystrophic muscle
Coll-GFP/DBA/2	CD31, marker for endothelial origin
	α7 INTEGRIN, marker for myogenic origin
	CD45, marker for hematopoietic origin

Muscle force measurement

Four-limb grip strength was determined as previously described (Ardite et al., 2012). The mice were allowed to grasp a piece of wire gauze by the forelimbs, and then steadily pulled by the tail horizontally away until the grip was detached. The maximal force values were recorded. Five such trials were undertaken for each mouse within 2 min.

Digital image acquisition and processing

Digital images were acquired using: (1) an upright microscope DMR6000B (Leica) equipped with a DFC300FX camera for immunohistochemical color pictures and a Hamamatsu ORCA-ER camera for immunofluorescence pictures; (2) confocal images of muscle sections or isolated satellite cells were taken using a Leica SPE confocal laser scanning microscope system. HCX PL Fluotar 10×/0.30, 20×/0.50 and 40×/0.75 objectives were used. Acquisition was performed using Leica Application or LAS AF software (Leica). Images were composed and edited in Photoshop CS5 (Adobe), where background was reduced using brightness and contrast adjustments applied to the whole image.