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Supplementary Materials for

A Wnt-TGFβ2 axis induces a fibrogenic program in muscle stem cells from dystrophic mice

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Supplementary Methods

Hematoxylin/Eosin (H&E)

Snap-frozen sections (8 μm) were stained with hematoxylin, washed in water, and stained with eosin. Sections were dehydrated through graded alcohols to xylenes.

Detection of mouse TGFβ receptors

Cells were incubated according to manufacturer instructions with a biotinylated antibody recognizing mouse TGFβ receptor 2 (BAF532, R&D; 1:50) or biotinylated goat control IgG (BAF108, R&D; 1:50) and subsequently stained with PE-Cy7 conjugated streptavidin (Biolegend). Cells were purified using a FACSAria III (BD) and analyzed with Flowjo 7.6.5 software. DAPI dilactate (250 μg/μl) was used to exclude dead cells.

For RT-PCR analysis, C2C12 and 293T cells (ATCC) were cultured in DMEM with 10% FBS. In the absence of any transcript detection by mouse specific primers in human 293T cells, the Ct values were set to 40.

Chromatin Immunoprecipitation

Primary myoblasts were cultured in Ham's F-10 with 20% FBS and 2.5 ng/ml bFGF. To induce Wnt signaling, 10^7 cells were incubated with 100 ng/ml of Wnt3a for 4 hours. Chromatin was cross-linked with 1% formaldehyde for 10 minutes, followed by quenching with 0.125 M Glycine for 5 minutes. Cells were washed in PBS and scraped in Lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.0 and protease inhibitors), and chromatin was sheared with a

Sonicator (Misonix) eight times for 15 seconds at an amplitude setting of 15. Lysate was diluted in ChIP dilution buffer (0.01% SDS, 1.1% TritonX-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, 167 mM NaCl) and incubated with 5 μg of anti-β-Catenin antibody (BD Transduction Laboratories) or mouse IgG (Santa Cruz) bound to Protein G coupled to Dynabeads (Invitrogen) by rocking overnight at 4°C. Beads were washed in RIPA buffer (50 mM Hepes-KOH, pH 7.6, 500 mM LiCl, 1 mM EDTA, 1% NP40, 0.7% sodium deoxycholate) and bound complexes were eluted at 65°C for 1 hour in buffer containing 1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.0. ChIP products were analyzed by quantitative PCR using specific primers to putative Wnt-responsive sequences (Table S5). Primers annealing to the *Axin2* locus have been published *(91, 92)*. ChIP signals were calculated as percent of the starting chromatin (input). Values obtained with control IgG (background) were subtracted from values obtained with the anti-β-Catenin antibody. The resulting binding of β-Catenin to individual responsive elements in Wnt3a-treated cells was expressed as fold of the binding observed in cells cultured in the same conditions but without addition of Wnt3a. To identify the potential Wnt-responsive elements, the region 2 kb upstream and 5 kb downstream to the transcription starting site of *Tgfβ2* was analyzed and ranked for evolutionary conservation with Mulan and with GERP++ software programs *(93, 94)*.

Supplementary Figures

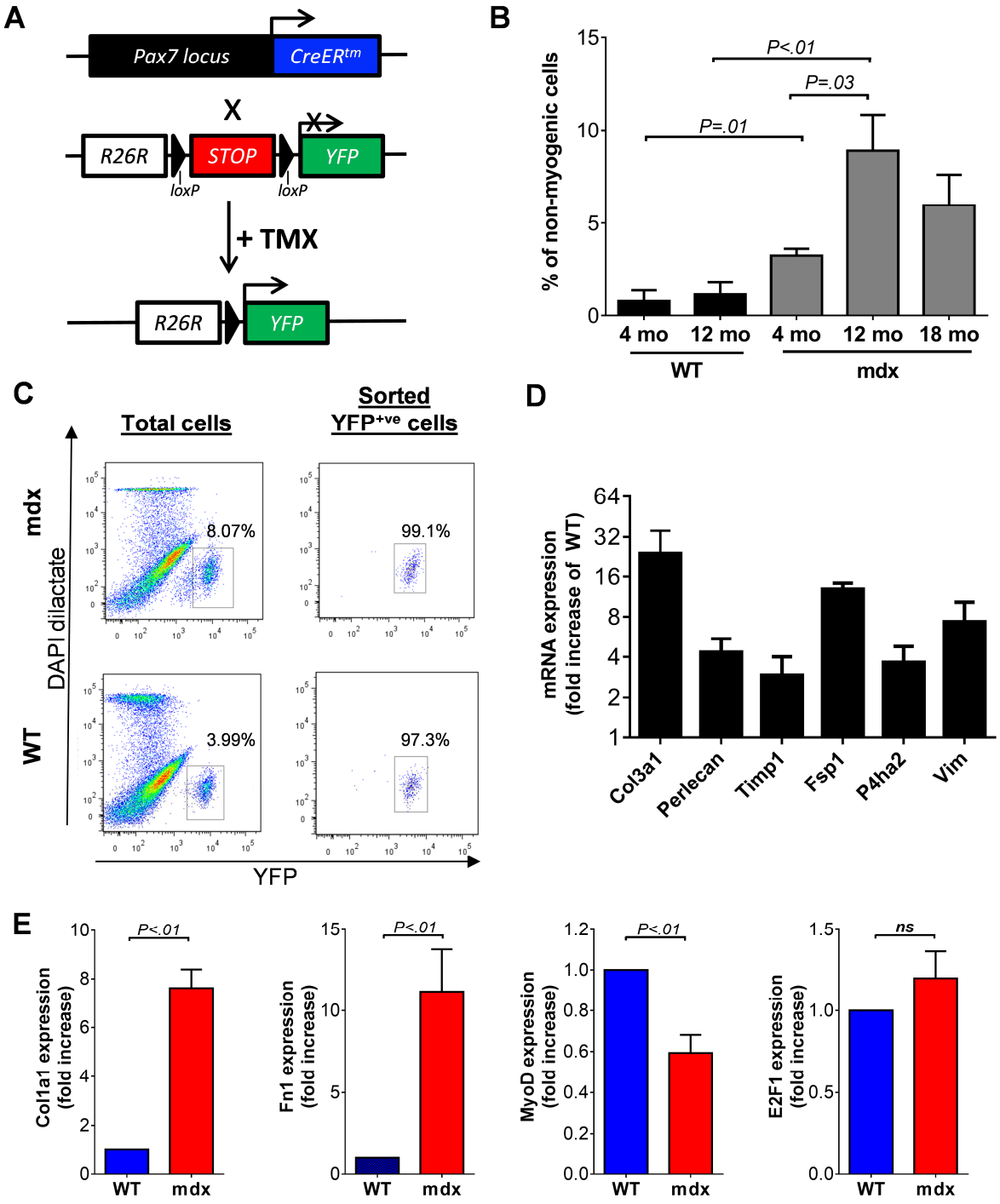


Fig. S1. Fibrotic features are induced in satellite cells in *mdx*^{5Cv} mice.

(A) Cre/LoxP-based strategy to trace *in vivo* the fate of satellite cells. **(B)** Cells were isolated from diaphragms of *Pax7*^{CreER}*;R26R*^{YFP}*;mdx*^{5Cv} or *Pax7*^{CreER}*;R26R*^{YFP}*;WT* mice at the indicated ages, fixed the day after isolation and stained for Pax7, MyoD, and Myogenin. The percentage of YFP^{+ve} cells which are non-myogenic was quantified (N≥3). **(C)** Pure satellite cell progeny (YFP^{+ve} cells) were isolated from *mdx*^{5Cv} and *WT* mice by FACS. **(D)** RT-PCR of the expression of the fibrotic genes (Col3a1, Perlecan, Timp1, Fsp1, P4ha2 and Vimentin (Vim)) in satellite cell progeny purified as in Fig. 1C. Data are expressed as fold increase of the expression in cells isolated from *WT* age-matched mice (logarithmic scale). The expression of the evaluated genes is higher in cells isolated from *mdx*^{5Cv} than from *WT* mice (N=4; $P<.05$ for each gene). **(E)** RT-PCR of the expression of Col1a1, Fn1, MyoD, and the proliferation associated gene E2F1 in VCAM^{+ve}/Lin^{-ve} satellite cells isolated from 12-month-old *mdx*^{5Cv} and *WT* mice by FACS as described in the Methods and cultured for 4 days *in vitro*. Data are expressed as fold increase of the expression in cells isolated from *WT* mice (N=3).

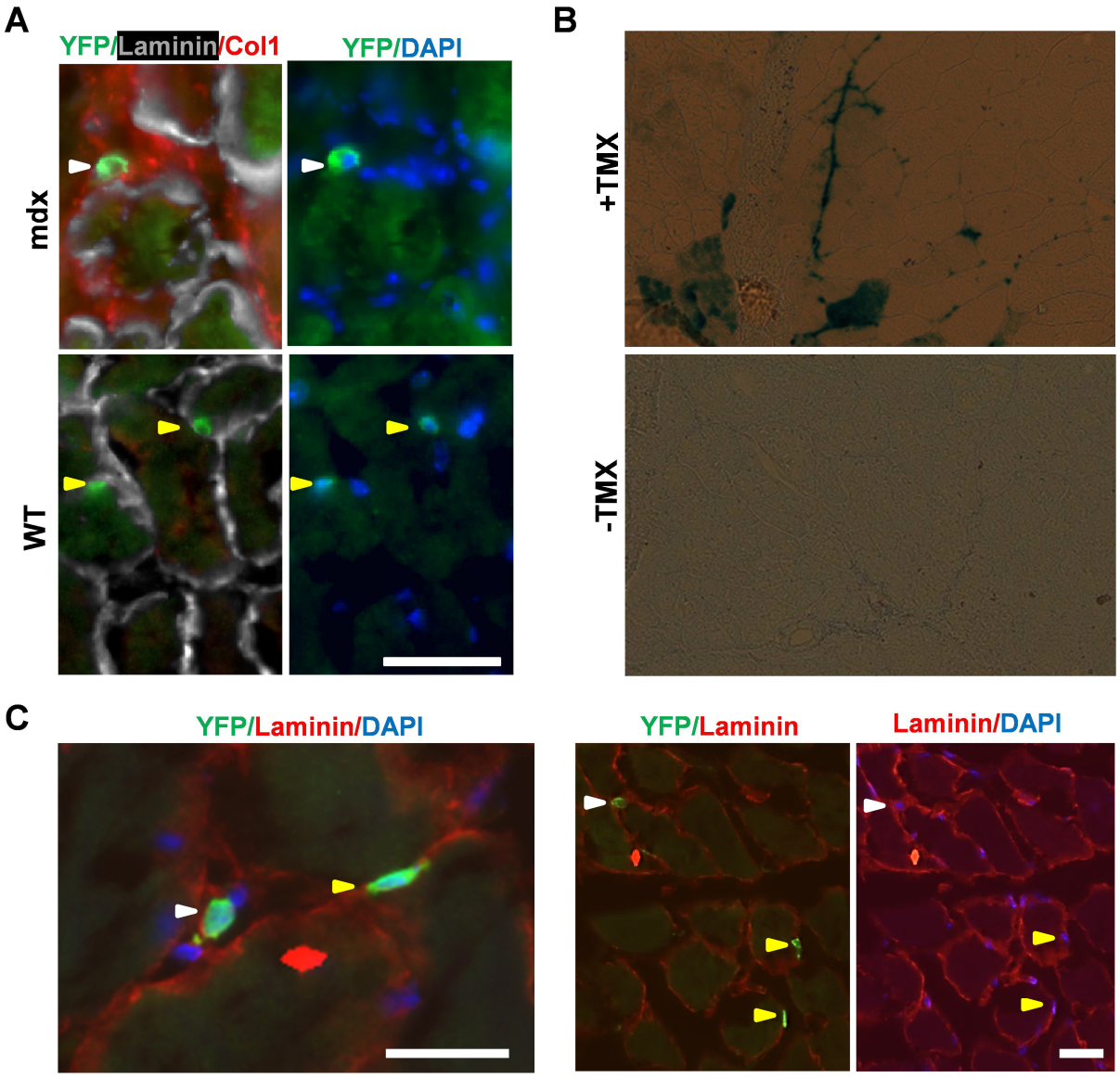

A
YFP/Laminin/Col1
YFP/DAPI
mdx
WT
B
+TMX
-TMX
C
YFP/Laminin/DAPI
YFP/Laminin
Laminin/DAPI

Fig. S2. Cells of the satellite cell lineage are present in the muscle interstitium in *mdx*^{5Cv} mice.

(A) Diaphragms of 12-month-old *Pax7*^{CreER}*;R26R*^{YFP}*;mdx*^{5Cv} and *Pax7*^{CreER}*;R26R*^{YFP}*;WT* mice were stained with antibodies recognizing YFP, Laminin and the extracellular matrix component Collagen1. Examples of YFP^{+ve} cells that were observed in *mdx* mouse muscles to be outside the basal lamina are indicated by white arrowheads. YFP^{+ve} cells in the typical sublaminar position in *WT* mouse muscles are indicated by the yellow arrowheads. Bar, 100 µm. **(B)** Sections of TA muscles of 10-month-old of TMX-treated *Pax7*^{CreER}*;R26R*^{LacZ}*;mdx*^{5Cv} mice were stained with Xgal. Note the intense blue staining in the interstitium of TMX-treated mice. Mice not injected with TMX were used as negative controls. **(C)** Diaphragms of 12-month-old *Pax7*^{CreER}*;R26R*^{YFP} mice were stained with antibodies recognizing YFP and Laminin. Examples of rare YFP^{+ve} cells that were observed in *Pax7*^{CreER}*;R26R*^{YFP}*;WT* mouse muscles to be physically separated from the fibers are indicated by white arrowheads. YFP^{+ve} cells in the typical sublaminar position juxtaposed to the fiber are indicated by the yellow arrowheads. Bar, 25 µm.

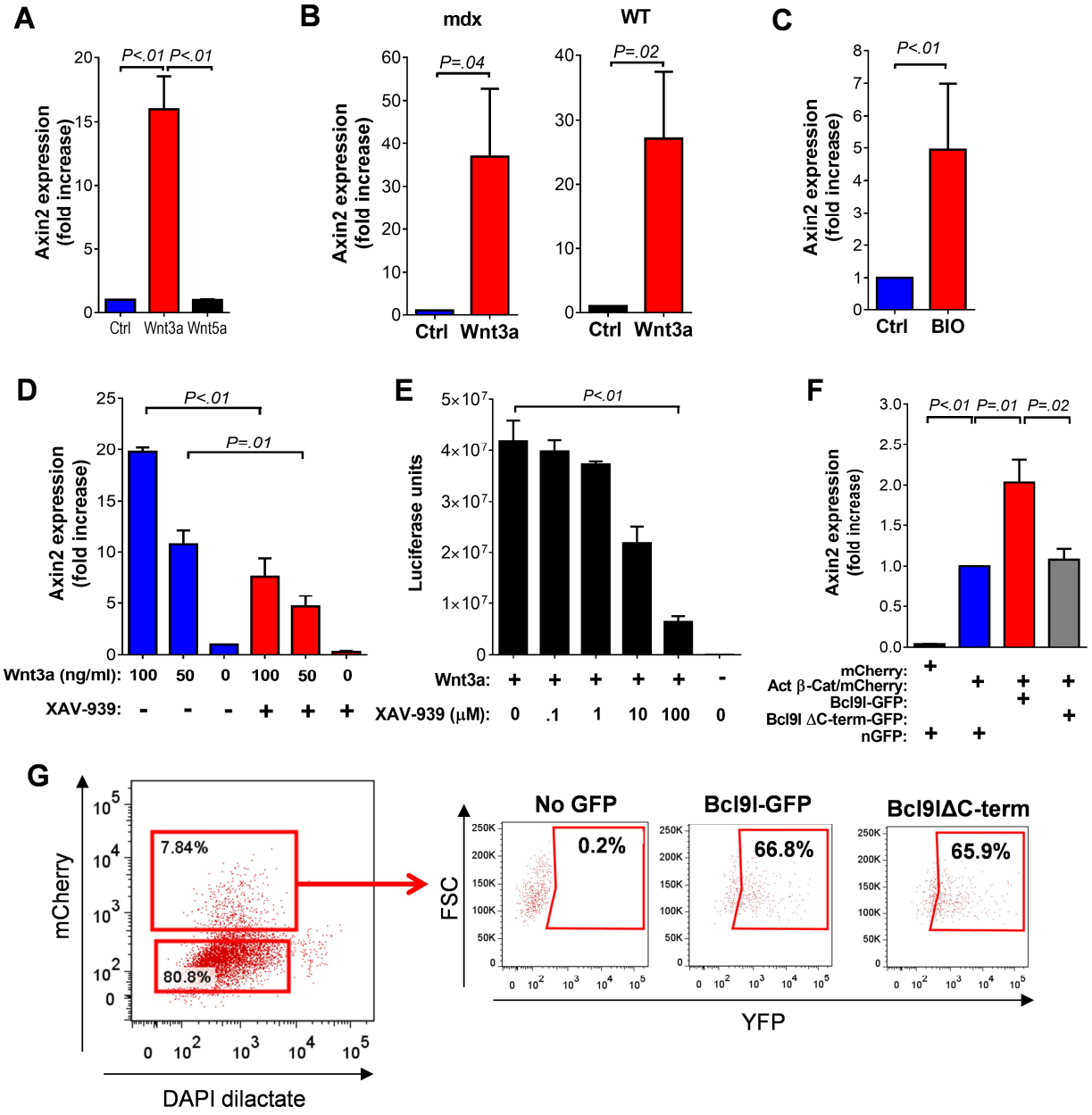
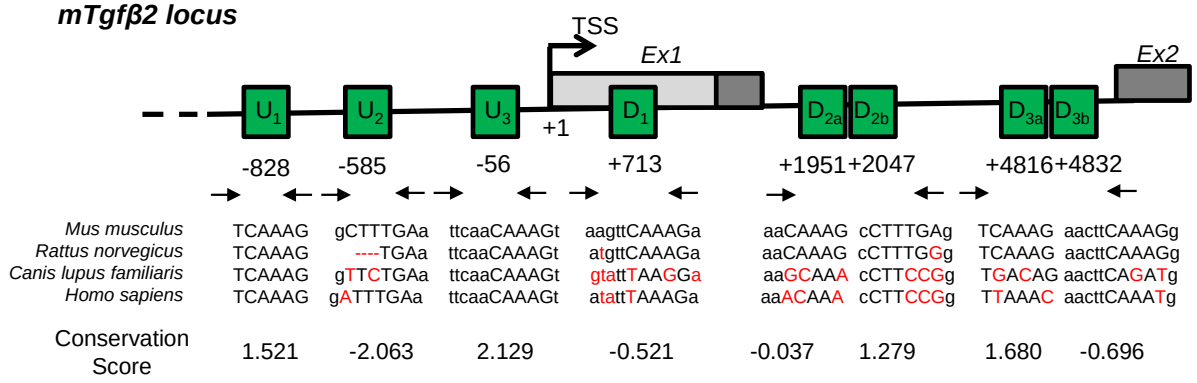

A
Axin2 expression (fold increase)
P<.01 P<.01
Ctrl Wnt3a Wnt5a
B
mdx
WT
P=.04
P=.02
Axin2 expression (fold increase)
Ctrl Wnt3a
Ctrl Wnt3a
C
P<.01
Axin2 expression (fold increase)
Ctrl BIO
D
P<.01
P=.01
Axin2 expression (fold increase)
Wnt3a (ng/ml): 100 50 0 100 50 0
XAV-939: - - - + + +
E
P<.01
Luciferase units
Wnt3a: + + + + + -
XAV-939 (μM): 0 .1 1 10 100 0
F
P<.01 P=.01 P=.02
Axin2 expression (fold increase)
mCherry:
Act β-Cat/mCherry:
Bcl9l-GFP:
Bcl9l ΔC-term-GFP:
nGFP:
G
mCherry
7.84%
80.8%
DAPI dilactate
No GFP
Bcl9l-GFP
Bcl9lΔC-term
0.2%
66.8%
65.9%
FSC
YFP

Fig. S3. In vitro alteration of canonical Wnt signaling.

(A,B) Axin2 was evaluated by RT-PCR in (A) primary myoblasts (N=3) and (B) YFP^{+ve} cells purified by FACS from 12-month-old *Pax7*^{CreER}*;R26R*^{YFP}*;mdx*^{5Cv} (N=3) and *Pax7*^{CreER}*;R26R*^{YFP}*;WT* (N=4) mice after treatment with Wnt3a or Wnt5a (each at 100 ng/ml). Data are expressed as fold increase of the expression in untreated cells. **(C,D)** Axin2 was evaluated by RT-PCR in myoblasts treated for 24 hours with BIO (1 µM) (C) or with XAV-939 (100 µM) in the presence of indicated concentrations of Wnt3a (D) (N=3 for all conditions). **(E)** Effects of different doses of XAV-939 on the luciferase activity of LSL cells treated with Wnt3a (100 ng/ml). **(F)** Axin2 was evaluated by RT-PCR in myoblasts after transfection with the indicated vectors. Data are expressed as fold increase of the expression in cells transfected with the act β-Cat/mCherry expressing plasmid (N=3 for all conditions). **(G)** The efficiency of transfection of plasmids expressing Bcl9l- or Bcl9lΔC-term-GFP fusion proteins was quantified for active β-Catenin/$mCherry^{+ve}$ in myoblasts by evaluating the percent of $mCherry^{+ve}$ cells that were also GFP^{+ve}. In the Bcl9lΔC-term-GFP construct, the C-terminal amino acids 886-1384 of Bcl9l are lacking. Cells not expressing GFP were used to set the autofluorescence. DAPI dilactate was used to identify dead cells.

A


mTgfβ2 locus
TSS
Ex1
Ex2
U1
U2
U3
D1
D2a
D2b
D3a
D3b
+1
-828
-585
-56
+713
+1951
+2047
+4816
+4832
Mus musculus TCAAAG gCTTTGAa ttcaaCAAAGt aagttCAAAGa aaCAAAG cCTTTGAg TCAAAG aacttCAAAGg
Rattus norvegicus TCAAAG ----TGAa ttcaaCAAAGt atgttCAAAGa aaCAAAG cCTTTGGg TCAAAG aacttCAAAGg
Canis lupus familiaris TCAAAG gTTCTGAa ttcaaCAAAGt gtattTAAGGa aaGCAAA cCTTCCGg TGACAG aacttCAGATg
Homo sapiens TCAAAG gATTTGAa ttcaaCAAAGt atattTAAAGa aaACAAA cCTTCCGg TTAAAC aacttCAAATg
Conservation Score
1.521
-2.063
2.129
-0.521
-0.037
1.279
1.680
-0.696

B

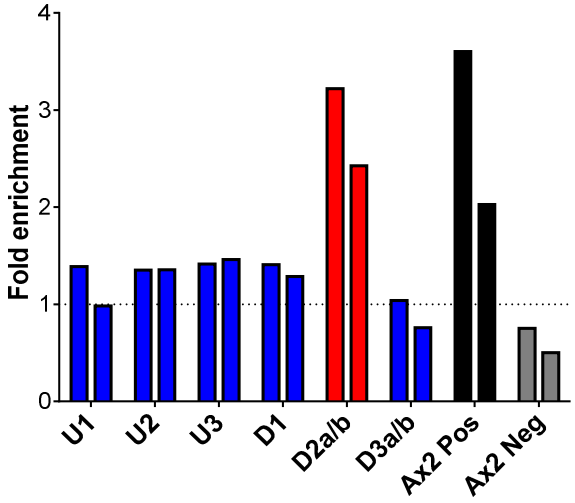

Fold enrichment
0
1
2
3
4
U1
U2
U3
D1
D2a/b
D3a/b
Ax2 Pos
Ax2 Neg

Fig. S4. β-Catenin binds to the *Tgfβ2* locus.

(A) Schematic representation of the region of the mouse *Tgfβ2* gene from -2 kb to +5 kb from the transcription starting site (TSS). Putative WREs are depicted in green (3 upstream (U), and 5 downstream (D) of the TSS). Arrows represent the primer pairs used in the ChIP assay. The sequences of the candidate WRE in four mammalian species are listed. In red are the bases that differ from the mouse sequence. The core consensus sequence is depicted with capital letters. A conservation score was attributed to each putative WRE with GERP++ software. **(B)** ChIP-qPCR analysis of β-Catenin binding to different regions of the mTgfβ2 locus. Myoblasts were treated with Wnt3a for 4 hours. Each column represents a distinct biological replicate. In the case of the positive control (Ax2 Pos), different primer sets were used in the two replicates (see Table S3).

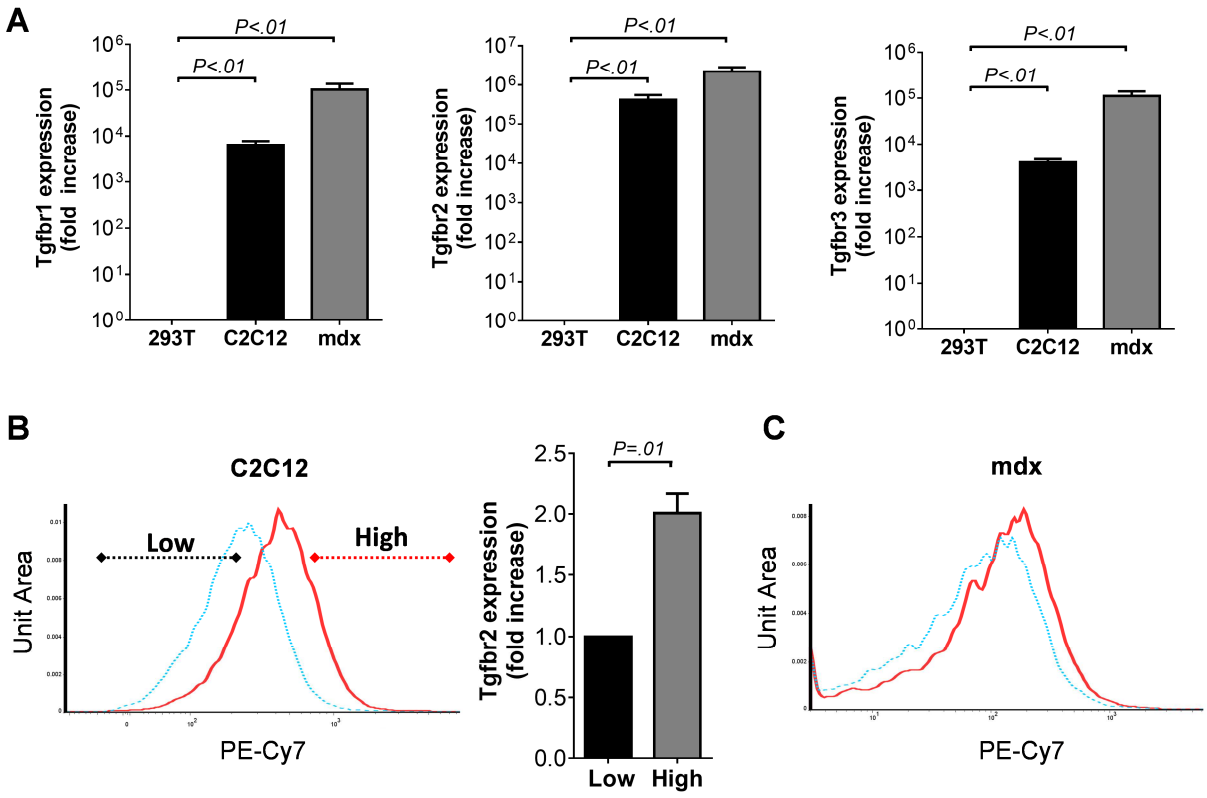

A
P<.01
P<.01
Tgfbr1 expression (fold increase)
293T
C2C12
mdx
P<.01
P<.01
Tgfbr2 expression (fold increase)
293T
C2C12
mdx
P<.01
P<.01
Tgfbr3 expression (fold increase)
293T
C2C12
mdx
B
C2C12
Low
High
Unit Area
PE-Cy7
P=.01
Tgfbr2 expression (fold increase)
Low
High
C
mdx
Unit Area
PE-Cy7

Fig. S5. Expression of TGFβ receptors in satellite cell progeny.

(A) RT-PCR analysis of the expression of the murine TGFβ receptors Tgfbr1, 2 and 3 in C2C12 myoblasts and YFP^{+ve} cells purified from hindlimb muscles of 4-month-old *Pax7*^{CreER}*;R26R*^{YFP}*;mdx*^{5Cv} mice (N=3 for each cell type). Data are expressed as fold increase of the expression in human kidney 293T cells (logarithmic scale). Mouse specific primers were employed for the detection of Tgfbr1, 2 and 3. Primers amplifying both human and mouse Hprt were used for normalization. **(B, C)** The expression of TGFβ receptor 2 was evaluated by FACS analysis. Representative FACS plots of C2C12 (B) and YFP^{+ve} cells from hindlimb muscles of 4-month-old *Pax7*^{CreER}*;R26R*^{YFP}*;mdx*^{5Cv} mice (C) incubated with TGFβ receptor 2 specific (red) or isotype control (blue) antibodies (PE-Cy7). The specificity of the antibody recognizing TGFβ receptor 2 was verified by RT-PCR analysis of the expression of Tgfbr2 in fractions of C2C12 (~15% of the total cell population) with high or low PE-Cy7 staining. Data are expressed as fold increase of the expression in the PE-$Cy7^{Low}$ fraction (N=3).

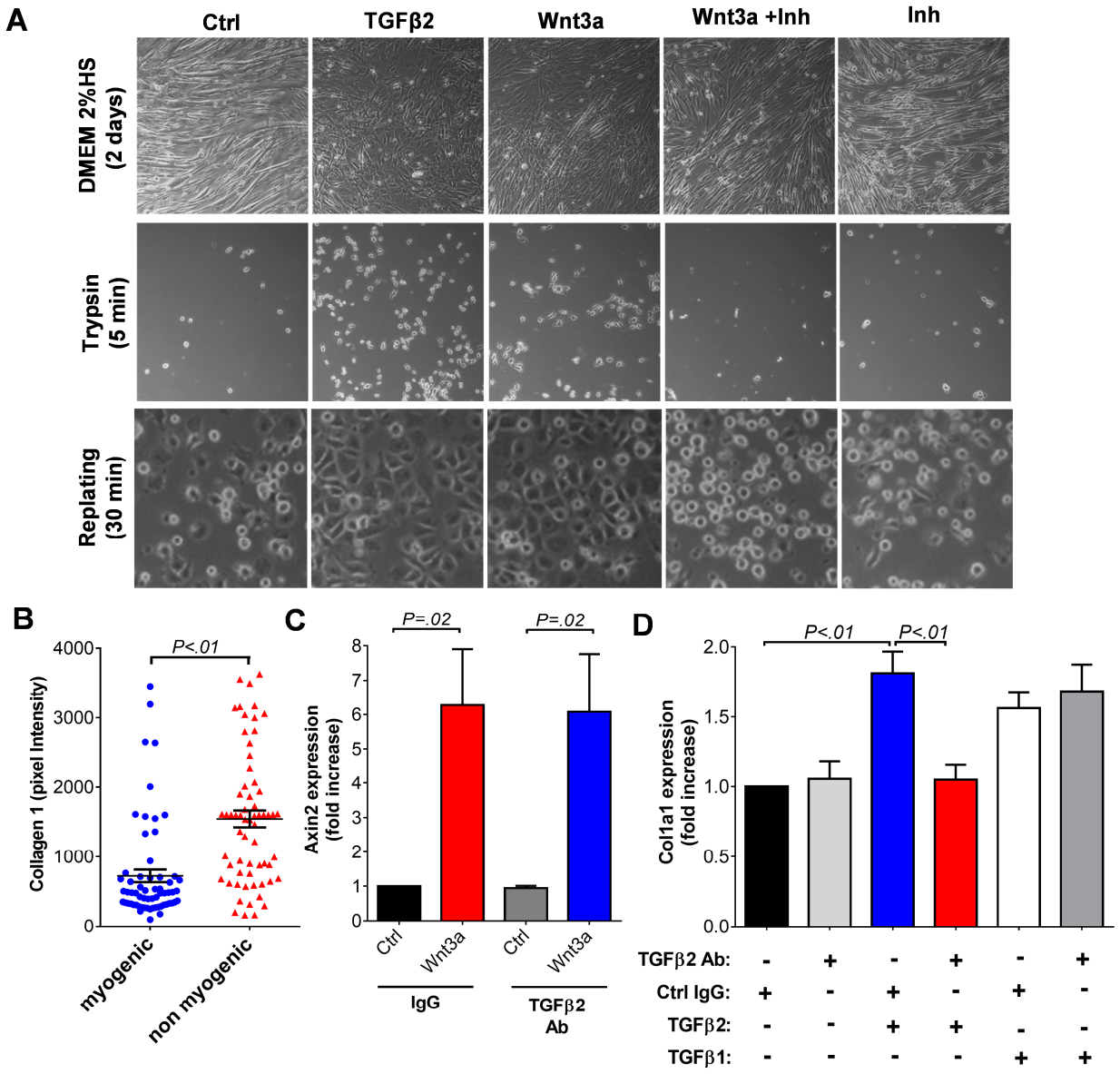

A
Ctrl
TGFβ2
Wnt3a
Wnt3a +Inh
Inh
DMEM 2%HS (2 days)
Trypsin (5 min)
Replating (30 min)
B
P<.01
Collagen 1 (pixel Intensity)
4000
3000
2000
1000
0
myogenic
non myogenic
C
P=.02
P=.02
Axin2 expression (fold increase)
8
7
6
5
4
3
2
1
0
Ctrl
Wnt3a
Ctrl
Wnt3a
IgG
TGFβ2 Ab
D
P<.01
P<.01
Col1a1 expression (fold increase)
2.0
1.5
1.0
0.5
0.0
TGFβ2 Ab: - + - + - +
Ctrl IgG: + - + - + -
TGFβ2: - - + + - -
TGFβ1: - - - - + +

Fig. S6. Wnt-TGFβ2 axis induces fibrogenic features in myoblasts in vitro.

(A) C2C12 myoblasts were cultured for 2 days in the presence of TGFβ2 (10 ng/ml), Wnt3a (100 ng/ml), Wnt3a together with the ALK5 inhibitor II (5 μM), or ALK5 inhibitor II alone (Inh). TGFβ2 and Wnt3a treatments enhance the adhesion properties of the cells as highlighted by the number of attached cells after 5 minutes of trypsinization or by analysis of the morphology of the cells after 30 minutes of replating (see Methods). Note that both TGFβ2- and Wnt3a-treated cells are more spread in comparison to control cultures. **(B)** The intensity of the staining for Collagen1 was quantified in C2C12 myoblasts processed as in Fig. 4A in the presence of TGFβ2 (10 ng/ml) and correlated with the presence ("myogenic") or absence ("non-myogenic") of the myogenic markers Pax7, MyoD, Myogenin. The analysis was performed on at least 60 cells from 3 independent experiments. **(C)** RT-PCR analysis of the expression of Axin2 in C2C12 myoblasts cultured with or without Wnt3a (100 ng/ml), with addition of a TGFβ2-blocking antibody or rabbit IgG (both 12.5 μg/ml). Cells were processed as in Fig. 4A. Data are expressed as fold increase of the expression in cells treated with IgG (N=3 for each condition). **(D)** Inhibition of TGFβ2- (but not TGFβ1-) dependent Col1a1 expression in C2C12 myoblasts after treatment with a TGFβ2-blocking antibody (12.5 μg/ml). Cells were treated with TGFβ1 or TGFβ 2 (5 ng/ml) for 24 hours. Rabbit IgG (12.5 μg/ml) was used as control (N=3 for each condition). Note that the TGFβ1-dependent induction of Col1a1 expression is completely unaffected by the presence of a TGFβ2-blocking antibody.

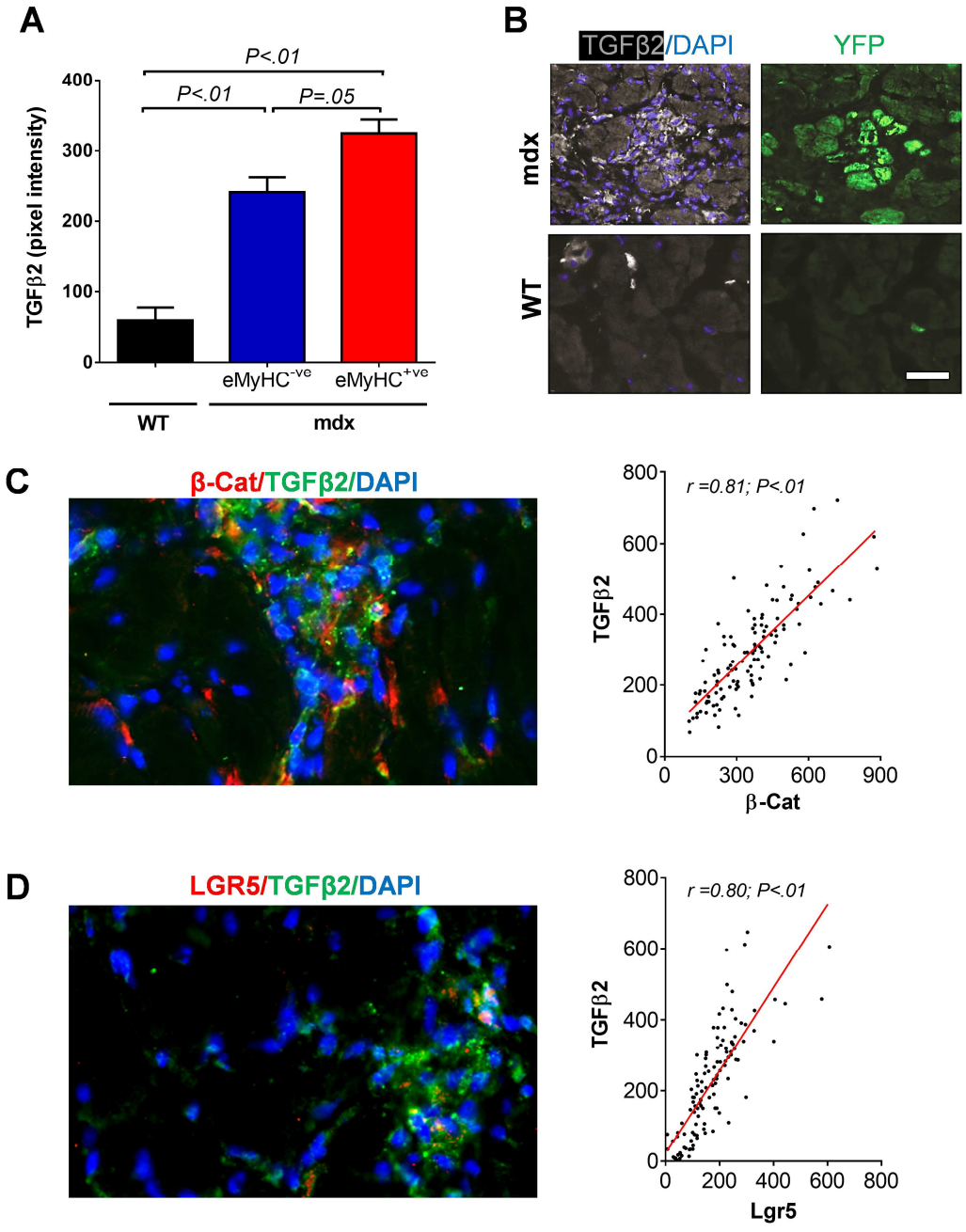

A
P<.01
P<.01
P=.05
400
300
200
100
0
TGFβ2 (pixel intensity)
eMyHC-ve
eMyHC+ve
WT
mdx
B
TGFβ2/DAPI
YFP
mdx
WT
C
β-Cat/TGFβ2/DAPI
r =0.81; P<.01
800
600
400
200
0
TGFβ2
0
300
600
900
β-Cat
D
LGR5/TGFβ2/DAPI
r =0.80; P<.01
800
600
400
200
0
TGFβ2
0
200
400
600
800
Lgr5

Fig. S7. The Wnt-Tgfβ2 axis is active in dystrophic muscles.

(A) The average intensity of the signal corresponding to TGFβ2 was evaluated in distinct regions of muscles processed as in Fig. 5B and selected according to the presence or absence of eMyHC^{+ve} fibers (no eMyHC^{+ve} fibers were seen in *WT* muscles) (N=3 for each genotype). **(B)** Diaphragms of 12-month-old *Pax7^{CreER};R26R^{YFP};mdx^{5Cv}* and *Pax7^{CreER};R26R^{YFP};WT* mice were stained for TGFβ2 and YFP. Note that TGFβ2 staining characterizes the YFP^{+ve} areas in *mdx^{5Cv}* muscles. Bar, 25 µm. **(C,D)** Diaphragms of 6-month-old *mdx^{5Cv}* mice were stained with antibodies recognizing TGFβ2 and either (C) β-Catenin or (D) LGR5 . The average intensity of each signal was evaluated in 120 randomly selected regions of the muscles (see Methods). The correlation between the values obtained for each staining in each region is shown. Linear regression, Pearson r and corresponding P-value are indicated.

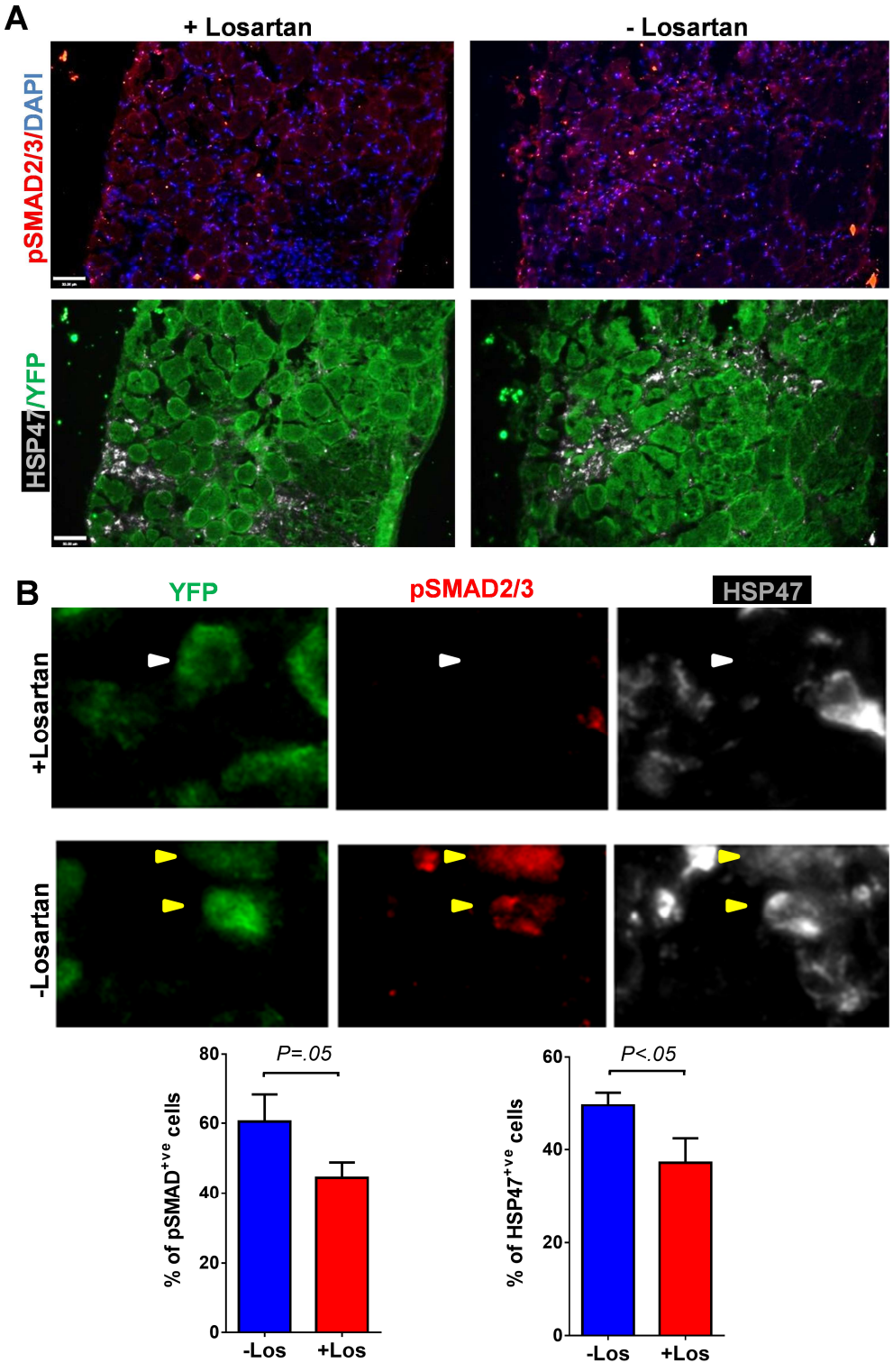

A
+ Losartan
- Losartan
pSMAD2/3/DAPI
HSP47/YFP
B
YFP
pSMAD2/3
HSP47
+Losartan
-Losartan
P=.05
% of pSMAD+ve cells
80
60
40
20
0
-Los
+Los
P<.05
% of HSP47+ve cells
60
40
20
0
-Los
+Los

Fig. S8. TGFβ signaling induces fibrogenic features in satellite cells in *mdx*^{5Cv} muscles.

(A,B) Muscles of Losartan- or placebo-treated *Pax7*^{CreER}*;R26R*^{YFP}*;mdx*^{5Cv} mice were stained for YFP, pSMAD2/3 and HSP47. (A) Low magnification images of diaphragm sections are presented. Bar, 50 µm. (B) Examples of YFP^{+ve} cells negatively or positively stained for both pSMAD2/3 and HSP47 are indicated by white and yellow arrowheads, respectively. Quantitation of the percentage of YFP^{+ve} cells expressing each fibrogenic marker individually is presented below.

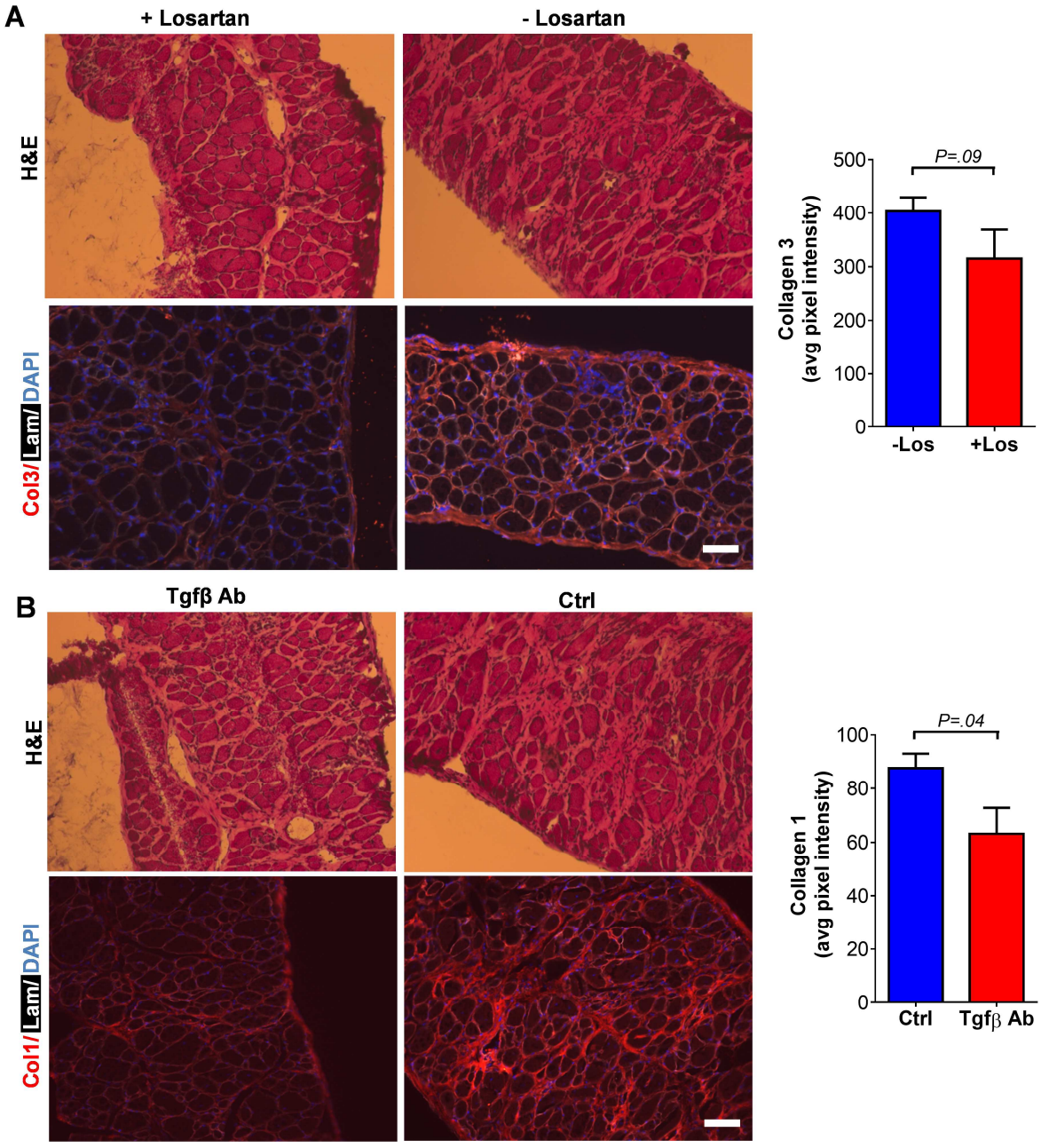

A
+ Losartan
- Losartan
H&E
Col3/Lam/DAPI
Collagen 3
(avg pixel intensity)
P=.09
500
400
300
200
100
0
-Los
+Los
B
Tgfβ Ab
Ctrl
H&E
Col1/Lam/DAPI
Collagen 1
(avg pixel intensity)
P=.04
100
80
60
40
20
0
Ctrl
Tgfβ Ab

Fig. S9. Blocking TGFβ signaling ameliorates the dystrophic phenotype.

(A,B) Diaphragms of *Pax7^{CreER};R26R^{YFP};mdx^{5Cv}* mice treated with Losartan or placebo (N=4) (A), with TGFβ-blocking (1D11), or anti-Shigella toxin (Ctrl) antibodies (N=7) (B) were stained with H&E or for Collagen1 or 3 and Laminin. DAPI stains the nuclei. Bar, 50 µm. The average intensity of the signal corresponding to Collagen was evaluated for each treatment.

Supplementary Tables

Table S1. List of genes that are significantly increased (≥ 2.5-fold) in Wnt3a-treated myoblasts.

Gene Symbol	ProbeID	Control	Wnt3a	Fold
Calcb	1422639_at	26.70907724	2616.829484	97.98
Calca	1452004_at	20.36274781	419.4051869	20.60
Car3	1430584_s_at	131.3183689	1838.354393	14.00
Car3	1453588_at	32.13190182	405.1630059	12.61
Axin2	**1436845_at**	**98.67346723**	**1219.869765**	**12.36**
Bhlhe40	1418025_at	103.5675868	982.1134238	9.48
Ppargc1a	1434100_x_at	19.02765044	147.2386398	7.74
Car2	1448752_at	118.109751	911.910012	7.72
Ppargc1a	1434099_at	14.05849061	96.18214563	6.84
Fst	1421365_at	882.8056318	5662.02014	6.41
Ppargc1a	1460336_at	36.33621931	208.1895233	5.73
Fst	1434458_at	1113.43476	6004.755452	5.39
Pfn2	1418210_at	631.9398458	3309.648673	5.24
Ppargc1a	1456395_at	25.40153332	132.7142917	5.22
Ppargc1a	1437751_at	21.1837345	110.5634991	5.22
Pfn2	1418209_a_at	663.3497889	3462.178669	5.22
Pfn2	1436993_x_at	328.0499455	1642.878217	5.01
Ism1	1436987_at	16.56106105	82.80319698	5.00
Syt13	1434470_at	55.80822367	264.9021055	4.75
Syt13	1451045_at	54.0404085	252.3064168	4.67
Tgfb2	**1450923_at**	**31.3951713**	**137.2891924**	**4.37**
Car3	1460256_at	731.4390361	2977.992388	4.07
Tgfb2	**1423250_a_at**	**106.3891382**	**409.2536031**	**3.85**
Tgfb2	**1450922_a_at**	**88.1490555**	**336.638129**	**3.82**
Osr2	1426155_a_at	53.78782853	204.7386398	3.81
Lifr	1454984_at	59.25552552	225.2386398	3.80
Wnt5a	1436791_at	31.03436134	109.1951936	3.52
Axin2	**1421341_at**	**18.88612863**	**64.56095847**	**3.42**
BB038663	1446563_at	103.2394776	343.8044788	3.33
Nrcam	1434709_at	114.7558432	371.8416457	3.24
Rasl11b	1423854_a_at	90.11401885	289.8033889	3.22
Fam84a	1425452_s_at	28.58190019	90.67327139	3.17
Irx3	1418517_at	196.3641917	613.7417973	3.13
Acsl3	1428386_at	203.7386398	582.2386398	2.86
Acsl3	1452771_s_at	336.868553	956.3677514	2.84
Jak2	1421065_at	136.5506239	386.4219271	2.83
Slc2a3	1437052_s_at	89.29266744	246.5897868	2.76
Car3	1449434_at	5208.809811	14357.96093	2.76
Jak2	1421066_at	351.5763815	964.7786623	2.74
Calca	1427355_at	31.86563439	87.23339695	2.74
Dio2	1418937_at	82.99111431	222.5508219	2.68
Tcf7	1433471_at	171.1299624	439.2747763	2.57
Slc2a3	1455898_x_at	23.78046307	60.61755574	2.55
Cdc42ep2	1428750_at	219.8579715	555.563065	2.53
Lgr5	**1450988_at**	**46.89690313**	**117.2386398**	**2.50**

Table S2. Primer list for genotyping PCR.

Gene Name	Forward Primer	Reverse Primer	T_{ann}
Cre	gcatttctggggattgctta	cccggcaaaacaggtagtta	58°
YFP/GFP	aagttcatctgcaccaccg	tccttgaagaagatggtgcg	58°
LacZ	gcatcgagctgggtaataagcg	gacaccagaccaactggtaatg	58°

Table S3. Primer list for SYBR Green real-time PCR.

Gene Name	Forward Primer	Reverse Primer	T_{ann}
mHprt	tcagaccgctttttgccgcga	atcgctaatcacgacgctgggac	60°
mHsp47	agcgattgccctgtcggtcc	cacagaggacctgtgagggcttga	60°
mCol1a1	tccggctcctgctcctctta	gtatgcagctgacttcagggatgt	60°
mCol3a1	gcccacagccttctacac	ccagggtcaccatttctc	60°
mTimp1	tacaccccagtcatggaaagc	cggcccgtgatgagaaact	60
mFn1	tgcctcgggaatggaaag	atggtaggtcttcccatcgtcata	60°
mVimentin	gcgagagaaattgcaggagga	cgttcaaggtcaagacgtgc	60°
mFSP1	agctgcctagcttcctggggaaaa	tgtccctgttgctgtccaagttgc	60°
mPerlecan	ttccagatggtctatttccggg	cttggcacttgcatcctcc	60°
mAxin2	cagagggacaggaaccactc	tgccagtttctttggctctt	60°
mLgr5	tcgccttccccaggtcccttc	gccgtggtccacaccccgat	60°
mTgfβ2	cgagcggagcgacgaggagt	tgggcgggatggcattttcgg	60°
mTgfbr1	ctgccataaccgcactgtca	aaatgaaagggcgatctagtgatg	60°
mTgfbr2	gggcatcgctcatctccac	tccctgtgaacaatgggcat	60°
mTgfbr3	cctcctccacagattttcca	cccagatcaagccttctgag	60°
h/mHprt	aactggaaagaatgtcttgattgt	gaatttcaaatccaacaaagtctgg	60°

Table S4. Antibody list for immunofluorescence.

Antigen	Company	Species/Concentration
Pax7	DSHB	Mouse/ 1:200
MyoD	DAKO	Mouse/ 1:100
Myogenin	BD	Mouse/1:100
Col1	Cederlane	Rabbit/ 1:200
Col3	Abcam	Rabbit/ 1:200
HSP47	Abcam	Rabbit/ 1:200
Laminin	Abcam	Rat/ 1:1000
GFP	Invitrogen	Rabbit/ 1:500
GFP	Aves	Chick/1:250
LGR5	Abcam	Rabbit/ 1:100
β-Catenin	Abcam	Rabbit/ 1:100
eMyHC	DSHB	Mouse/ 1:20
Axin2	Abcam	Rabbit/ 1:500
TGFβ2	Santa Cruz	Goat/ 1:20
TGFβ2	Santa Cruz	Rabbit/ 1:200
pSMAD2/3	Santa Cruz	Goat/ 1:20

Table S5. Primer list for ChIP assay.

Responsive Element	Forward Primer	Reverse Primer	T_{ann}
Tgfβ2 U1	atcgtggccacagagtcatc	tgctgtctcctgtcacacagt	60°
Tgfβ2 U2	gatggtgggtcatccttgtc	agtgtccctttcacccaatg	60°
Tgfβ2 U3	gctcgtggtcttagtaacagagg	gctgccagcagataacatca	60°
Tgfβ2 D1	gccgggctctaactgagaag	acatccacacgcacactcat	60°
Tgfβ2 D2a/b	cccagataggtcagcaggga	tgacttatgctaagagaccaagg	60°
Tgfβ2 D3a/b	agcgtcttttgaatagctgcac	tttacggatcctgtgccctg	60
Axin2 Pos1	ctcgcatacctcccttcc	ttccagcagtcactaggc	60°
Axin2 Pos2	gagcgcctctgtgattgg	gaccccacctttacagcaa	60°
Axin2 Neg	atctgtatgtcctgtctgccagcg	tgtcctgattcccaagtcaagcac	60°