

Appendix for

**Combinatorial activation of the WNT-dependent fibrogenic program by
distinct complement subunits in dystrophic muscle**

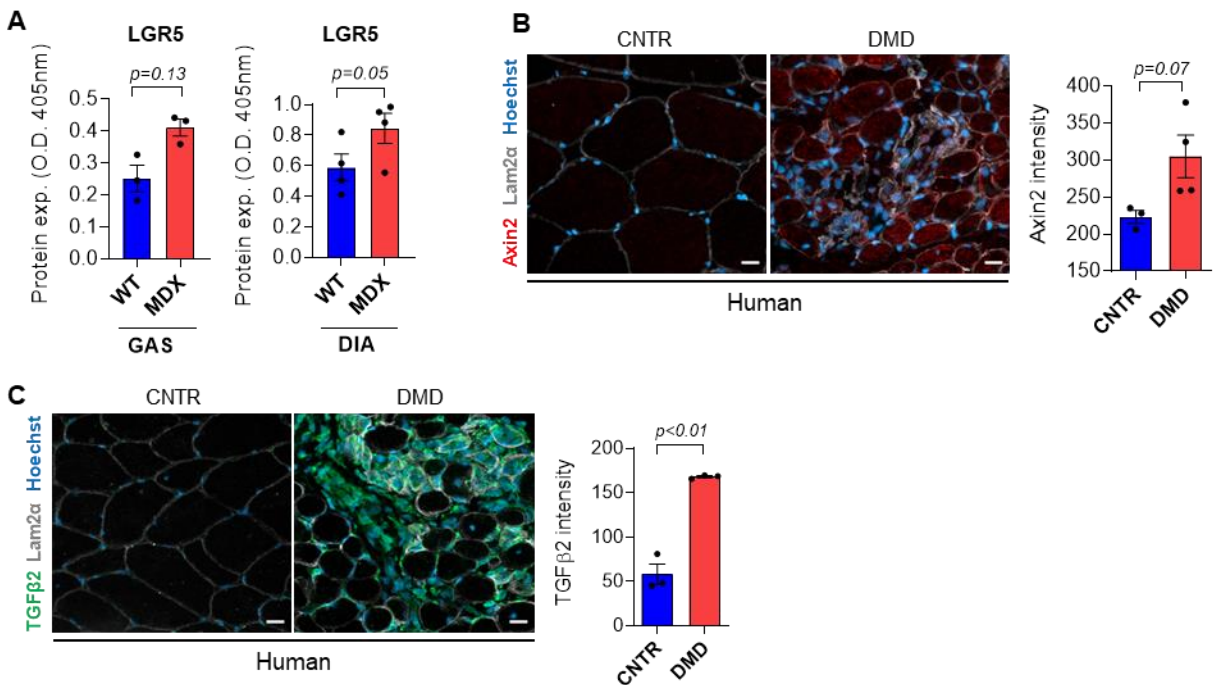
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Table of Content

APPENDIX FIGURES	2
APPENDIX TABLES	27

APPENDIX FIGURES



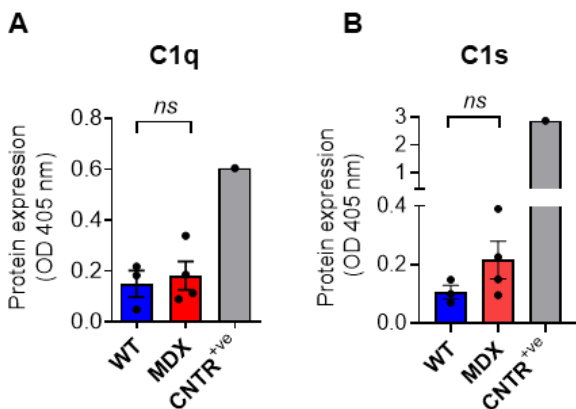
Appendix Figure S1. Canonical WNT-signaling is increased in dystrophic muscles.

A. ELISA assay of the canonical WTN target protein LGR5 in the *gastrocnemius* (left) and diaphragm (right) of ~1 year-old WT and MDX mice. N (biological replicates) = 3 for all samples except for LGR5 in diaphragm (N = 4).

B, C. Representative immunofluorescence (left) and quantification (right) of human healthy (CNTR) and dystrophic (DMD) muscles stained with anti-Axin2 (red in B), anti-TGFβ2 (green in C), anti-Laminin2α (gray) antibodies and Hoechst (blue). Scale bar: 20 μm. Refer to Table 1 for muscles' details. N (biological replicates) = 3 for all samples (except for Axin2 in DMD, N = 4).

Data information: Data are presented as mean ± SEM. In B and C, each graph dot represents the

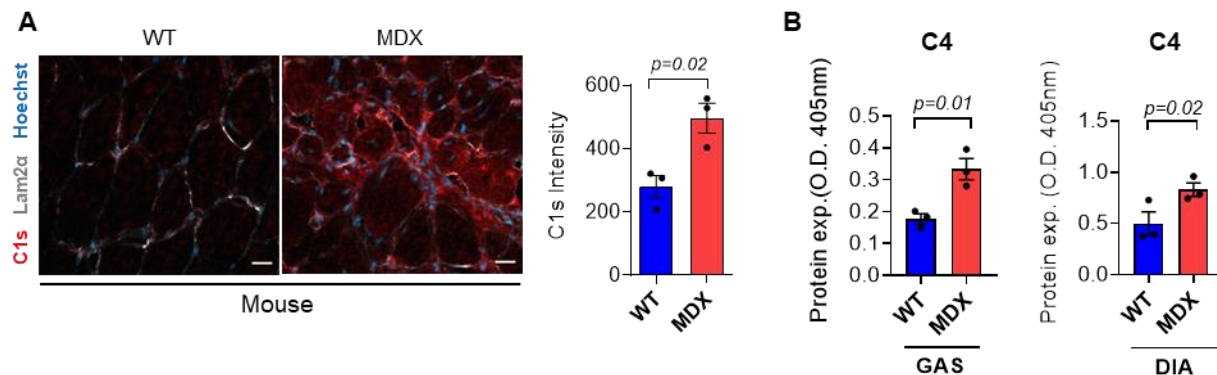
average value of 8 to 59 (B) and 11 to 22 (C) measurements on different muscle regions for each biological sample. Statistical differences were calculated by paired (in A) or unpaired (in B and C) two-tailed Student's t-test. P values are as indicated.



Appendix Figure S2. Complement evaluation in dystrophic serum.

A, B. ELISA assay of C1q (A) and C1s (B) proteins in the serum of ~1 year-old *WT* and *MDX* mice. N (biological replicates) = 3 (*WT*), 4 (*MDX*), 1 (*CNTR*^{+ve}). *CNTR*^{+ve}: C1q 10 µg/ml (A), C1s 5 µg/ml (B).

Data information: Data are presented as mean ± SEM. Statistical differences were calculated by unpaired two-tailed Student's t-test.

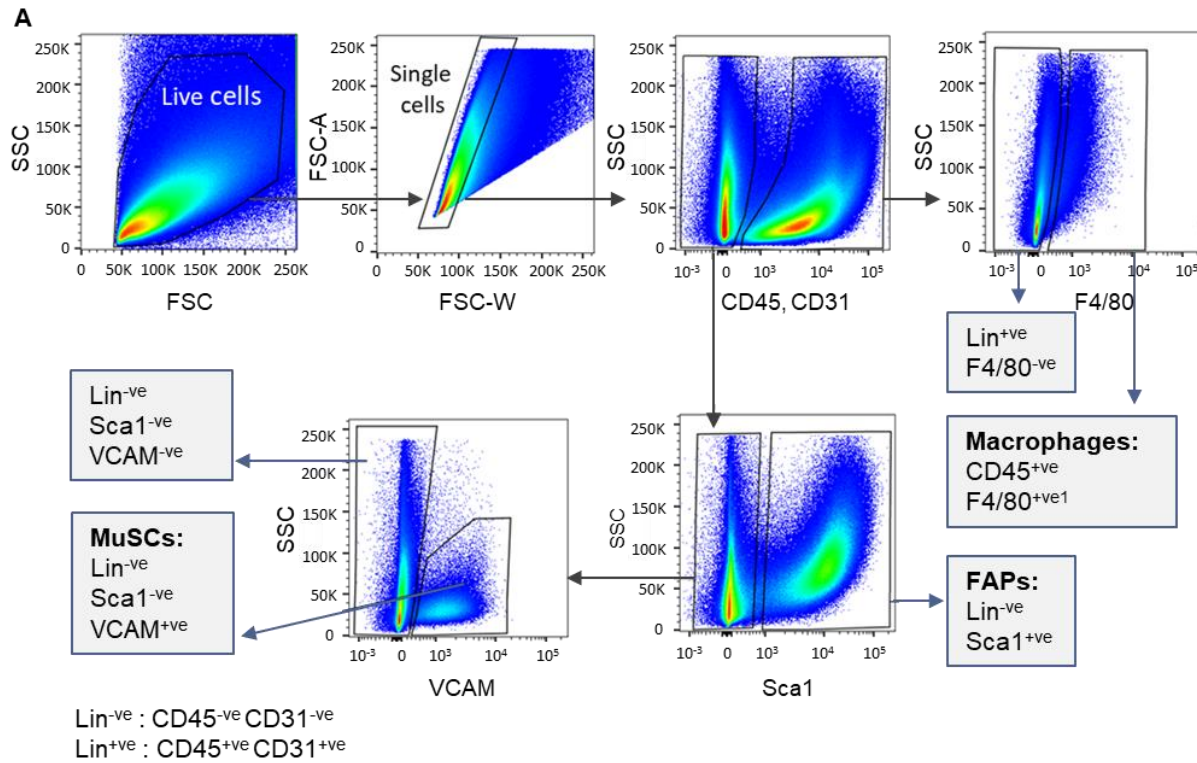


Appendix Figure S3. Canonical WNT-signaling is increased in dystrophic muscles.

A. Representative immunofluorescence (left) and quantification (right) of C1s in the *gastrocnemius* of ~1 year-old *WT* and *MDX* stained with anti-C1s (red), anti-Laminin2α (gray) antibodies and Hoechst (blue). Scale bar: 20 μm. N (biological replicates) = 3.

B. ELISA assay of C4 in the *gastrocnemius* (left) and diaphragm (right) of ~1 year-old *WT* and *MDX* mice. N (biological replicates) = 3 for all samples.

Data information: Data are presented as mean ± SEM. In A, each dot on the graphs represents the average value of 8 to 28 measurements on different muscle regions for each biological sample. Statistical differences were calculated by unpaired (in A) or paired (in B) two-tailed Student's t-test. P values are as indicated.



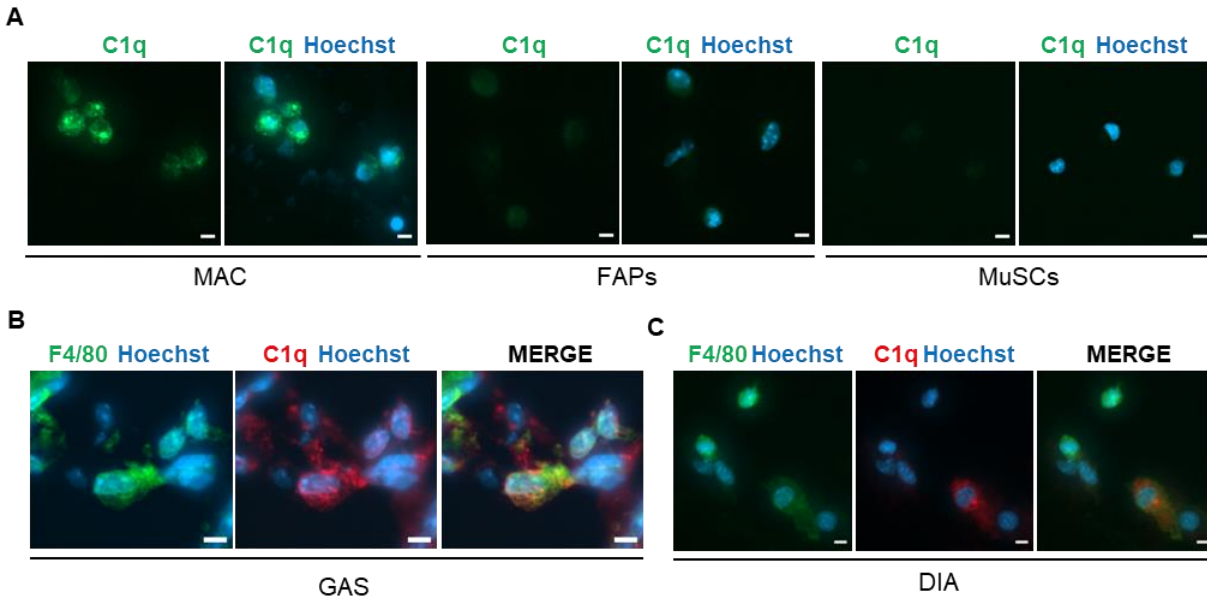
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52 Appendix Figure S4. FACS-purification of cells from skeletal muscle

53 **A.** Representative gating and sorting strategy used to FACS-isolate macrophages (MAC:

54 CD45^{+ve}F4/80^{+ve}), FAPs (Lin^{-ve}Sca1^{+ve}), MuSCs (Lin^{-ve}Sca1^{-ve}VCAM^{+ve}), Lin^{+ve}F4/80^{-ve} and Lin⁻

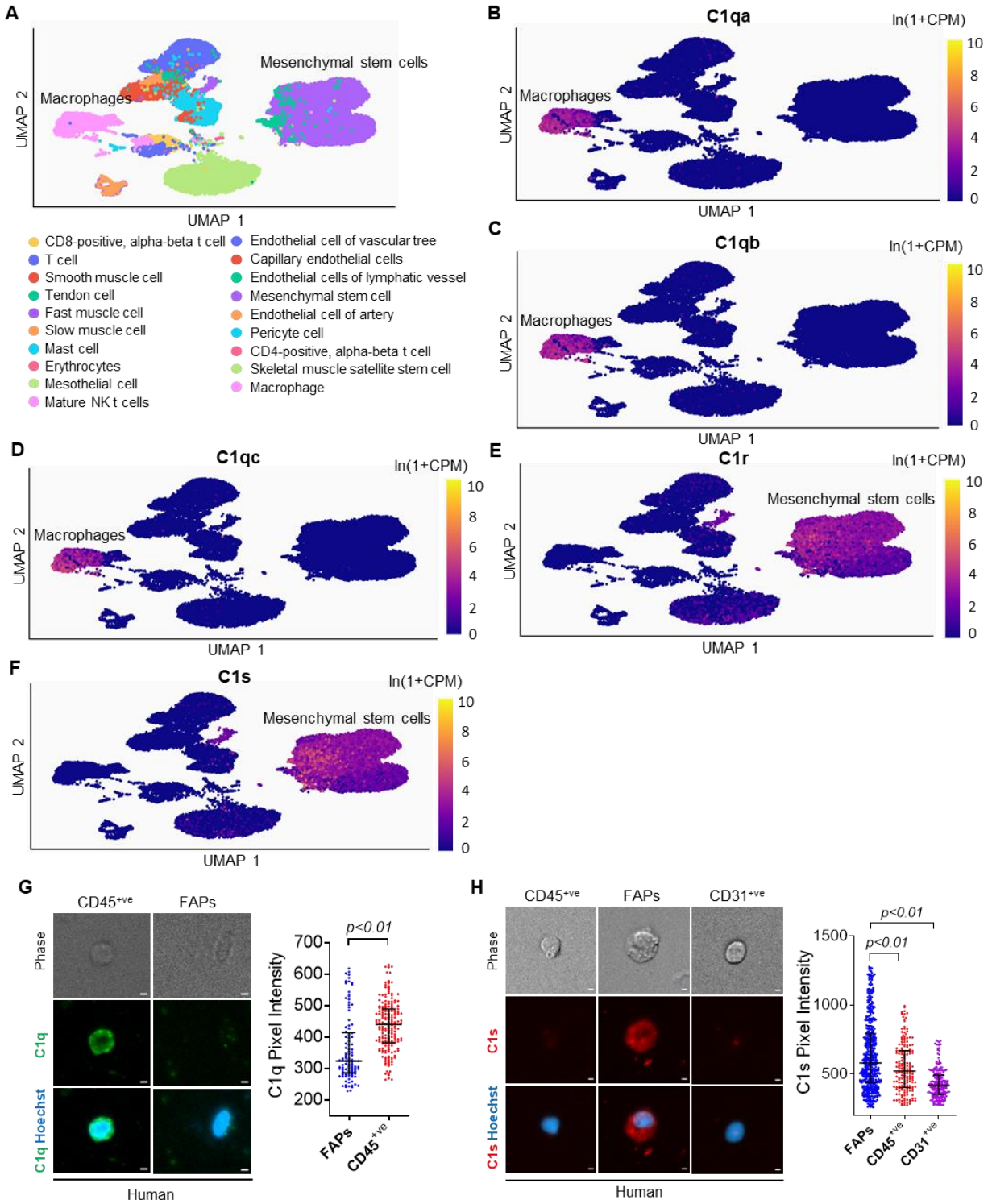
55 ^{-ve}Sca1^{-ve}VCAM^{-ve} cell populations from the distal hindlimb of *WT* and *MDX* mice.



Appendix Figure S5. Distinct cell types express C1 complex's components in murine muscles.

A. Representative immunofluorescence image of FACS-isolated MuSCs, FAPs, and macrophages (MAC) from ~1 year-old *MDX* hindlimb muscles stained with an anti-C1q antibody (green) and Hoechst (blue). C1q is selectively expressed by macrophages. Scale bar: 5 μ m.

B, C. Representative immunofluorescence of a cryosection of ~1 year-old *MDX* *gastrocnemius* (B) and ~5 months-old *MDX* diaphragm (C) stained with anti-F4/80 (green) and anti-C1q (red) antibodies, and Hoechst (blue). Scale bar: 5 μ m.



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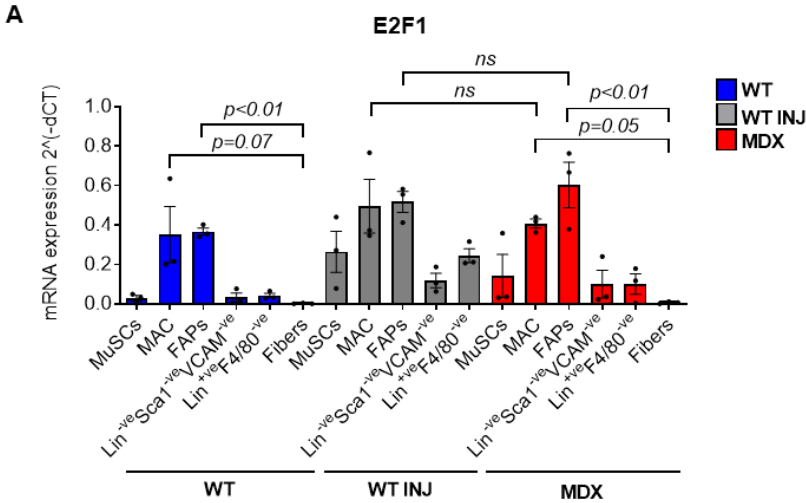
Appendix Figure S6. Distinct cell types express C1 complex's components in human muscles.

A. UMAP plot showing the human cell types in which the expression of C1 components was evaluated by interrogating the Tabula Sapiens single-cell transcriptomics analysis (Jones et al, 2022 [DATASET]).

B-F. UMAP plots showing the expression of C1qa (B), C1qb (C), C1qc (D), C1r (E), and C1s (F) in human cell types as indicated in (A). C1qa, C1qb, and C1qc are expressed mainly by macrophages, whereas C1r and C1s are primarily expressed by mesenchymal stem cells.

G, H. Representative immunofluorescence images (left) and quantification (right) of C1q (G) and C1s (H) pixel intensity measured in indicated cells FACS-isolated from human skeletal muscles and stained with anti-C1q (green), anti-C1s (red) antibodies and Hoechst (blue). C1q is more expressed in CD45⁺ cells, and C1s is more expressed in FAPs. Scale bar: 2 μ m. N (biological samples) = 4 (C1s in FAPs and CD31⁺ cells), 3 (C1s in CD45⁺ cells and C1q in FAPs), 2 (C1q in CD45⁺ cells).

Data information: In G and H, data are presented as median with interquartile range. In G and H, dots on the plots represent single cells' measurements (109 for FAPs and 187 for CD45⁺ in C1q analysis; 478 for FAPs, 171 for CD45⁺ cells, and 206 for CD31⁺ cells in C1s analysis). In G, the statistical difference was calculated by the Mann-Whitney test. In H, the statistical differences were calculated by the Kruskal-Wallis test, and Dunn's multiple comparison test was used as a *post hoc* test. P values are as indicated.

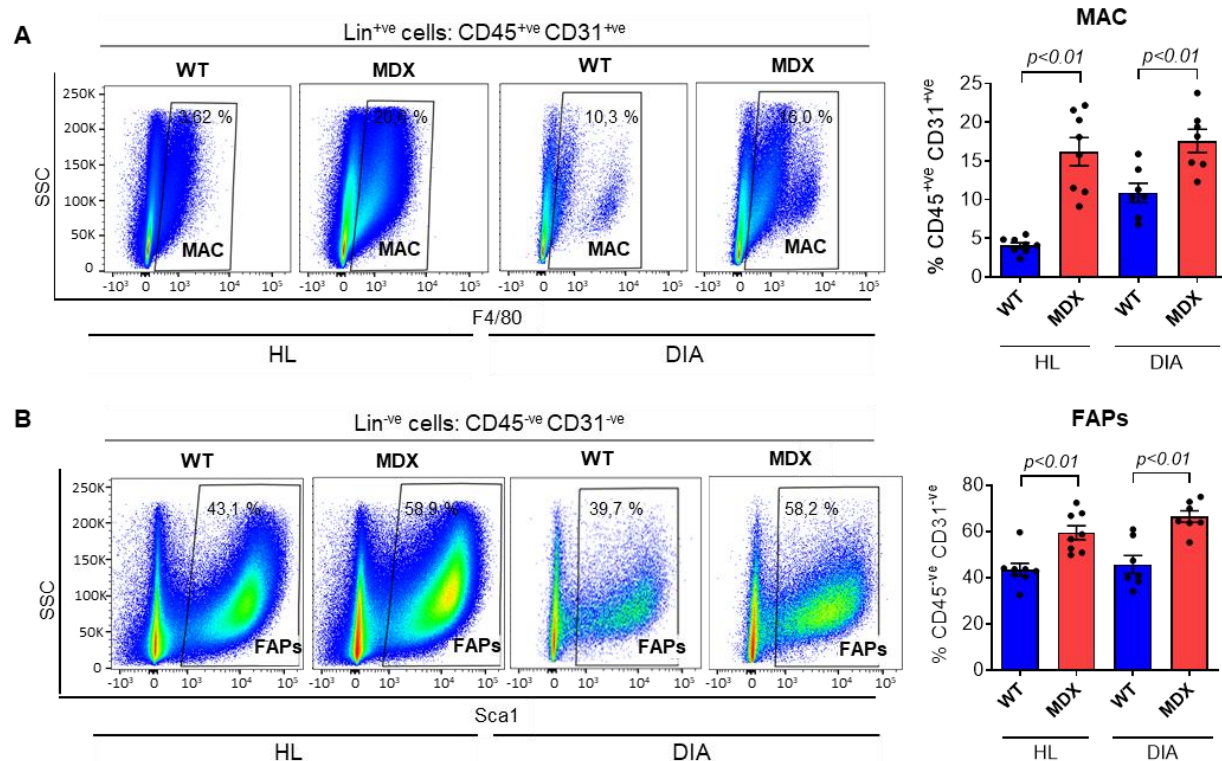


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89 Appendix Figure S7. Characterization of the muscle-isolated cells.

90 **A.** *E2F1* mRNA expression in MuSCs, macrophages (MAC), FAPs, Lin⁺veF4/80⁻ve, Lin⁻veSca1⁻
 91 ^{ve}VCAM⁻ve, and single fibers isolated from hindlimb muscles as in Figure 2A-E. N (biological
 92 samples) = 3. Note the low expression of *E2F1* in non-proliferative myofibers.

93 Data information: Data are presented as mean ± SEM. Statistical differences between two groups
 94 were calculated by unpaired two-tailed Student's t-test, and the corresponding p values are
 95 reported on the graph. Statistical differences between three or more groups were calculated by
 96 one-way ANOVA test, Tukey's multiple comparison test was used as a *post hoc* test, and all the
 97 corresponding p values are enclosed in Table 3.

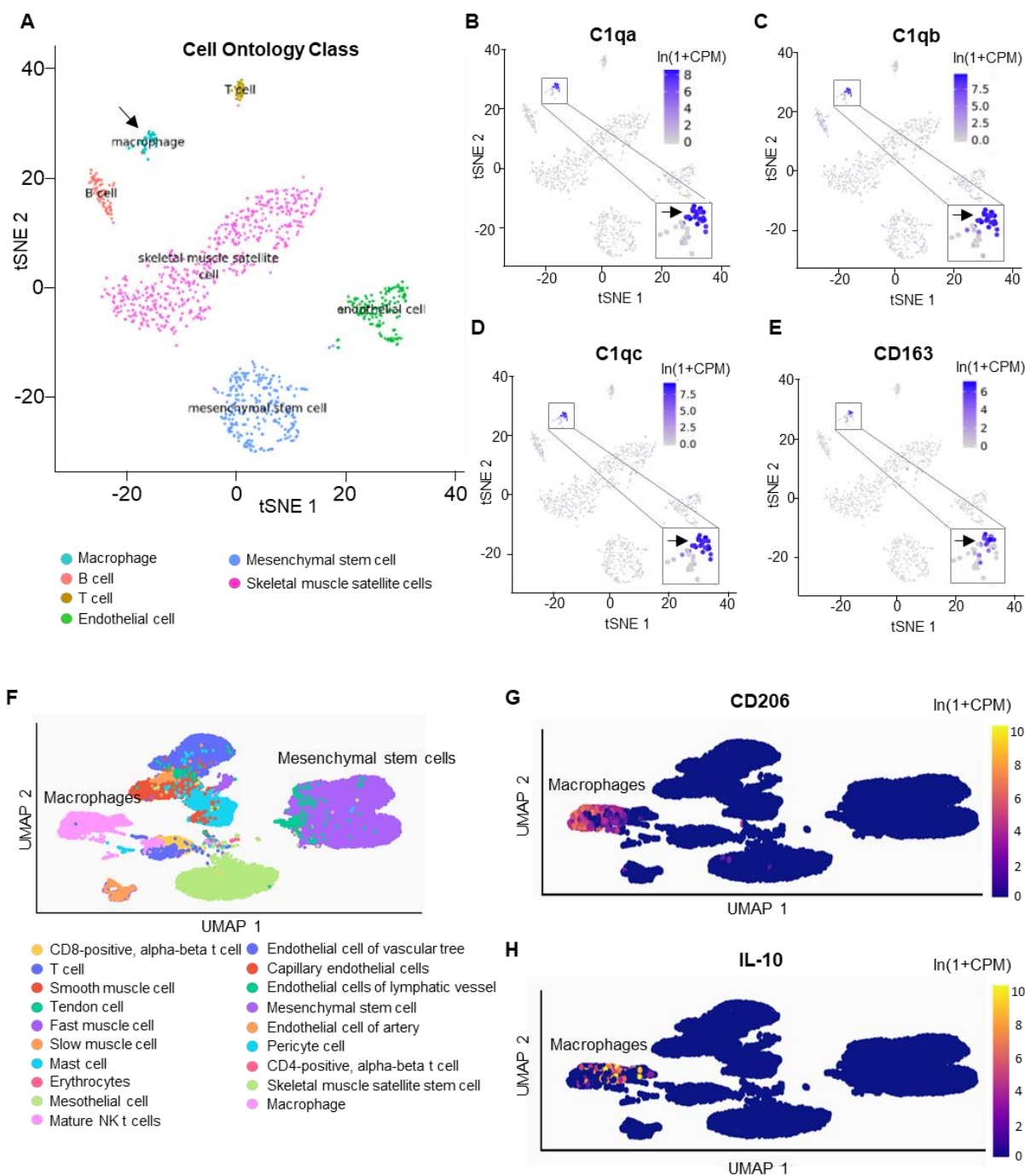


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99 Appendix Figure S8. Macrophages and FAPs are increased in dystrophic muscles.

100 **A, B.** Representative FACS plots (left) and quantification (right) of macrophages (A) and FAPs
 101 (B) expressed as the percentage of Lin⁺ cells (A) and Lin⁻ cells (B) in the hindlimb (HL) and
 102 diaphragm (DIA) muscles of ~1 year-old WT and MDX mice. N (biological samples) = 8 (HL), 7
 103 (DIA).

104 Data information: Data are presented as mean $\pm$ SEM. In A-B, statistical differences between
 105 two groups were calculated by unpaired two-tailed Student's t-test. P values are as indicated.



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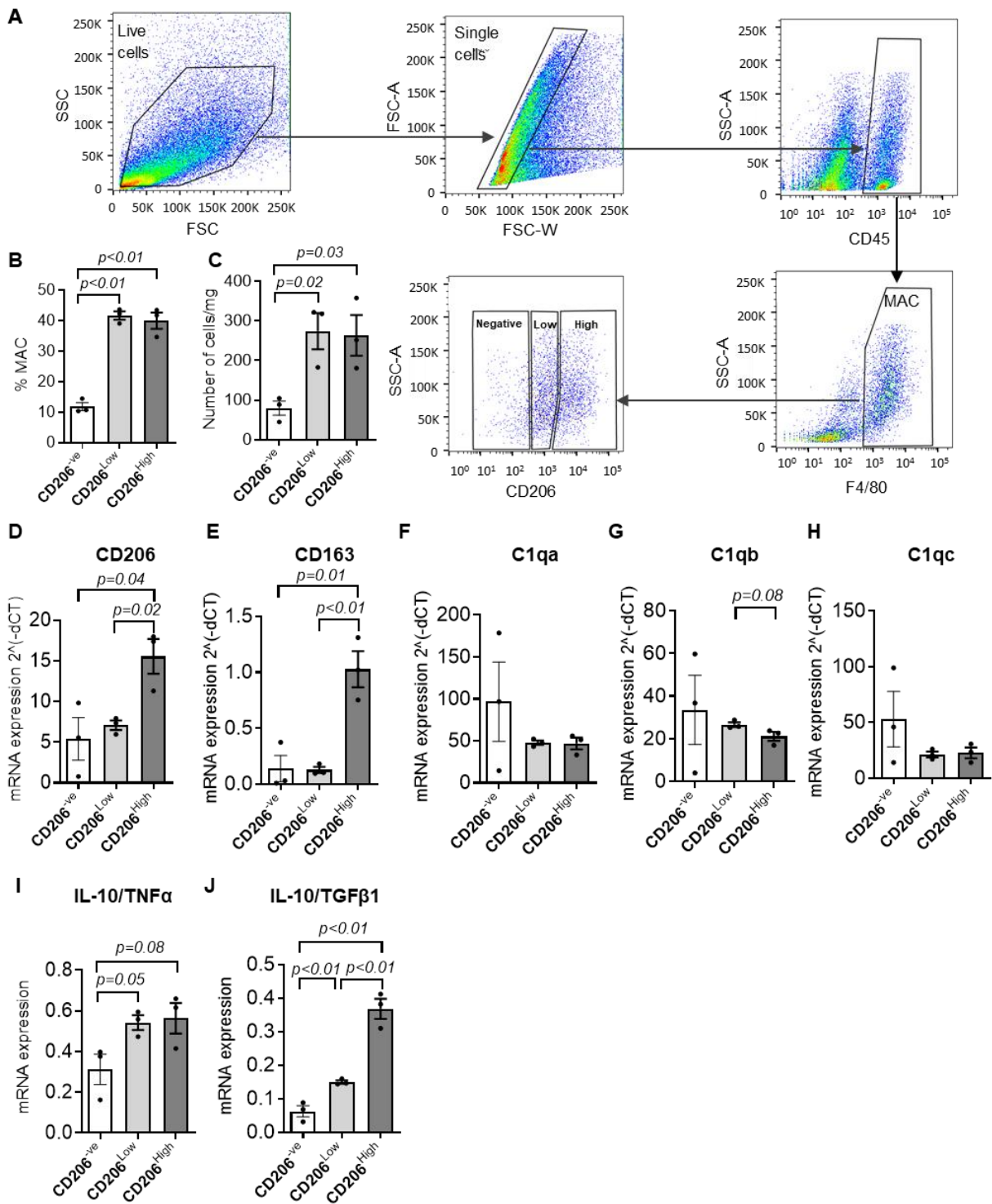
Appendix Figure S9. Complement expression in different subsets of macrophages in resting muscles.

A. tSNE plot showing the mouse cell types within the limb muscle in which the expression of C1 components and CD163 was evaluated by interrogating the Tabula Muris database derived from a FACS-based full-length transcript analysis (Data ref: Tabula Muris Consortium *et al*, 2018).

B-E. C1qa (B), C1qb (C), C1qc (D), and CD163 (E) are heterogeneously expressed by macrophages in mouse limb muscles. Magnification sections and arrows indicate the overlapping expression of C1q components and CD163 in the same fraction of macrophages.

F. UMAP plot showing the human muscle cell types present in skeletal muscle identified according to the Tabula Sapiens single-cell transcriptomics analysis (Data ref: Jones et al, 2022).

G, H. UMAP plots showing the expression of CD206 (G) and IL-10 (H) in human muscle cell types as indicated in (F). Note the overlap between the CD206 subset of macrophages and the cells expressing C1qa, C1qb, and C1qc (Appendix Figure S6 A-D). IL-10 marks a different subset of macrophages.



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Appendix Figure S10. Complement expression in different subsets of macrophages in WT injured muscles.

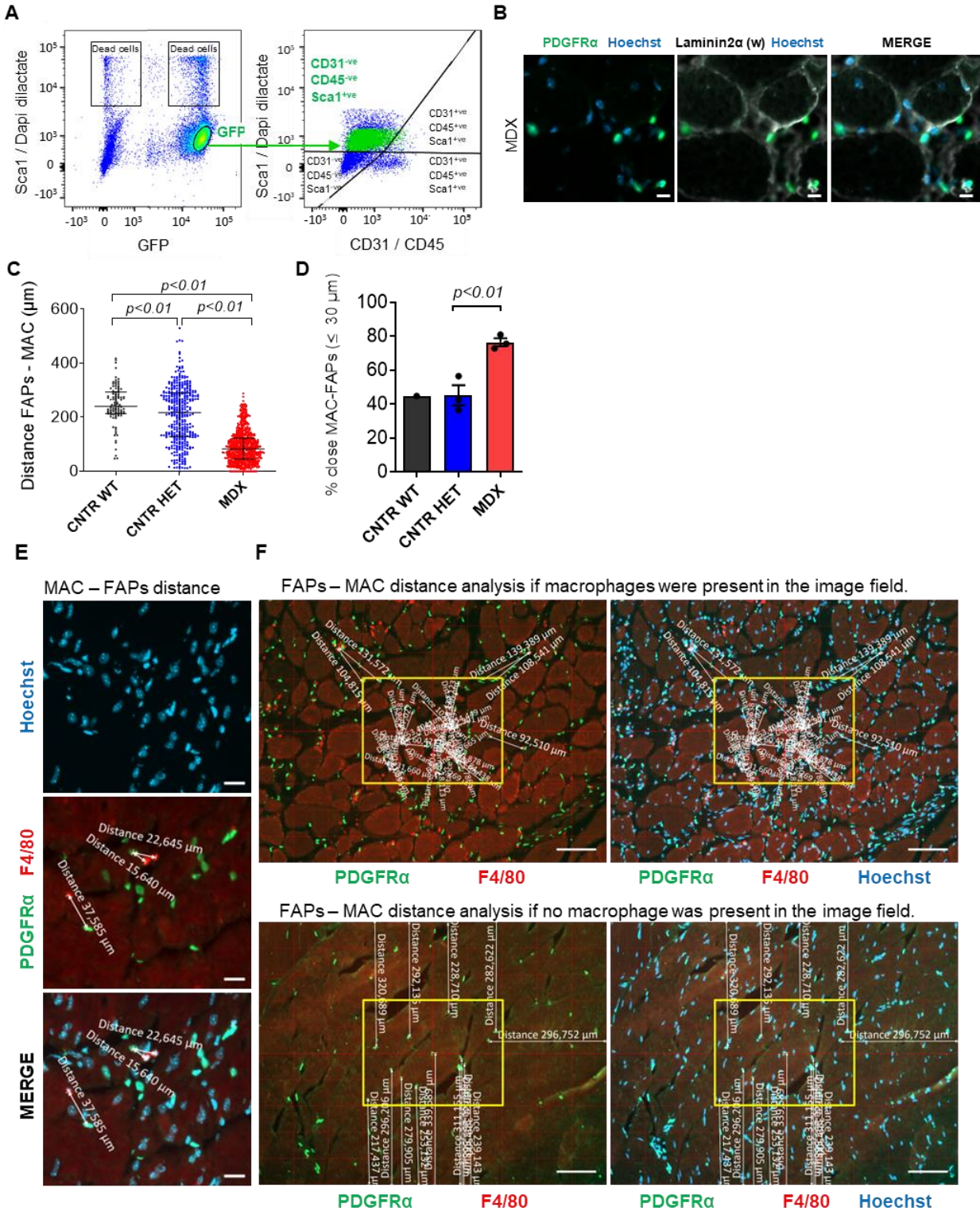
A. Representative gating and sorting strategy used to FACS-isolate different macrophage populations from WT mice 8 days after cardiotoxin injury. Macrophages were gated as CD45⁺F4/80⁺ cells. Within the macrophage population, CD206^{-ve}, CD206^{Low}, and CD206^{High} subpopulations were isolated.

B, C. Number of CD206^{-ve}, CD206^{Low}, and CD206^{High} macrophages from injured WT mice processed and FACS-isolated as in A. Data are expressed as a percentage of CD45⁺ cells (B) and as the number of cells per mg of tissue (C). N (biological samples) = 3.

D-H. *CD206* (D), *CD163* (E), *C1qa* (F), *C1qb* (G), and *C1qc* (H) mRNA expression in CD206^{-ve}, CD206^{Low}, and CD206^{High} macrophages from injured WT mice FACS-isolated as in A. N (biological samples) = 3.

I-J. *IL-10/TNF α* (I) and *IL-10/TGF β 1* (J) mRNA expression in CD206^{-ve}, CD206^{Low} and CD206^{High} macrophages from injured WT mice FACS-isolated as in A. N (biological samples) = 3.

Data information: Data are presented as mean $\pm$ SEM. Statistical differences between two groups were calculated by unpaired two-tailed Student's test. P values are as indicated.



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Appendix Figure S11. Analysis of distance between macrophages and FAPs in muscles.

A. Representative FACS plot of hindlimb muscles of a ~1 year-old male *PDGFRα^{eGFP/WT};MDX* showing that eGFP⁺ cells have the same staining signature as FAPs (i.e., CD31⁻CD45⁻Sca1⁺). An intense dapi dilactate stain was used to gate out dead cells.

B. Representative immunofluorescence of *gastrocnemius* of a ~18 months-old *PDGFRα^{eGFP/WT};MDX* male mouse stained with anti-Laminin2α antibodies (white) and Hoechst (blue). Most of eGFP⁺ cells were found in the interstitial space, the typical FAPs localization. Scale bar: 10 μm.

C. Distance (μm) between FAPs and macrophages (MAC) in the same muscles as in Figure 4A. The analysis was performed by measuring the distance of each FAP in the image field from its closest macrophage (refer to F). N (biological samples) = 3 (CNTR *HET*, *MDX*), 1 (CNTR *WT*).

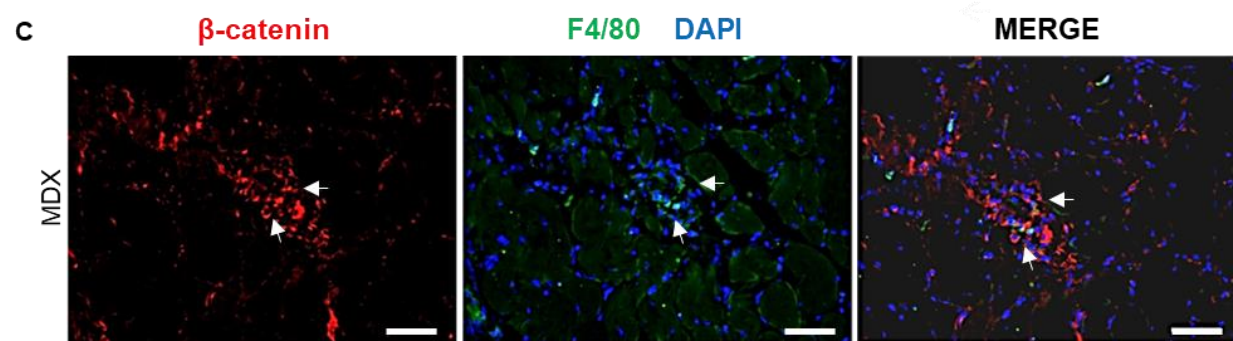
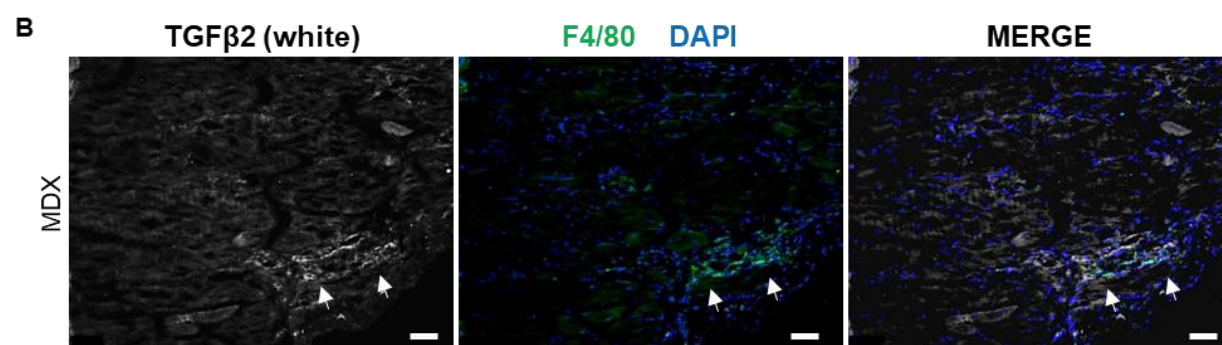
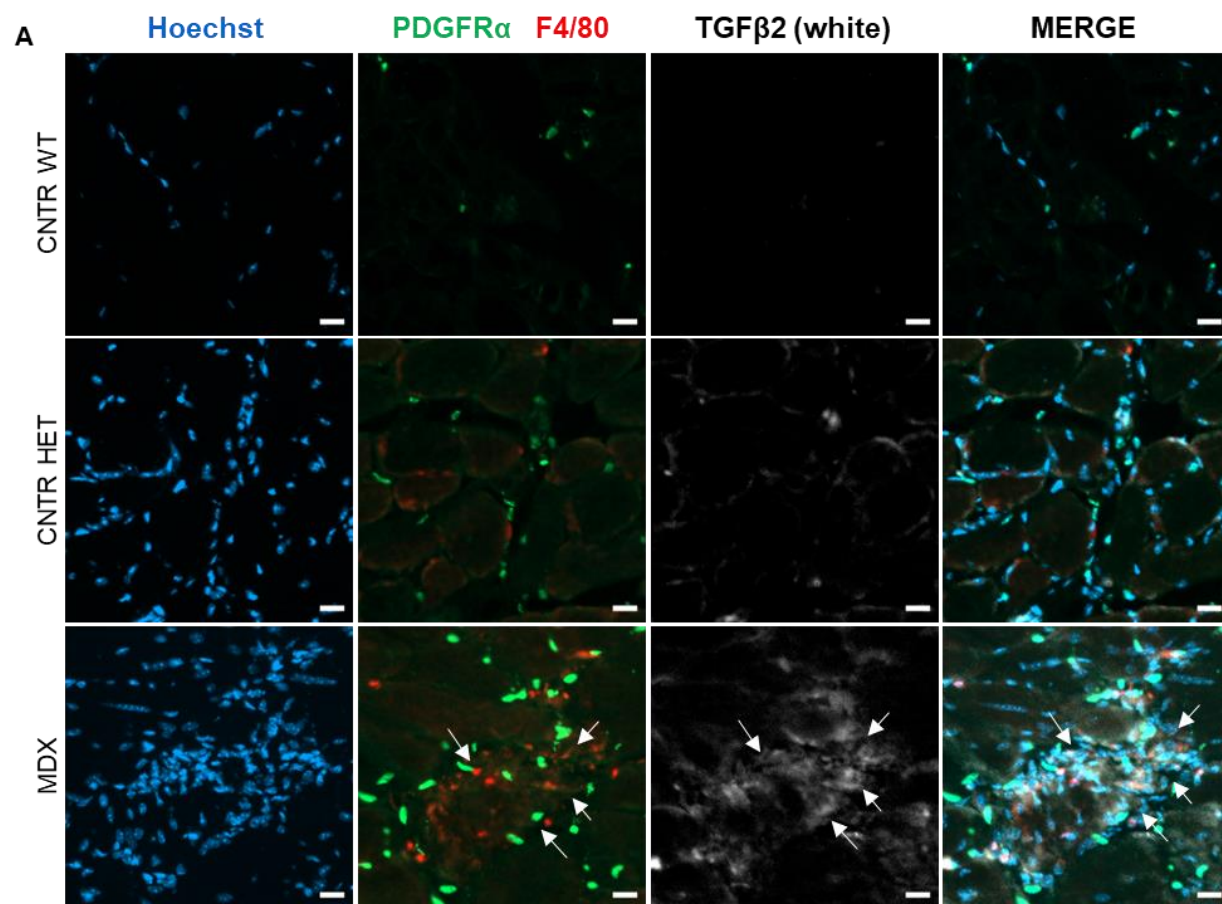
D. Percentage of macrophages and FAPs close to each other 30 μm or less calculated in the same muscles as in Figure 4A. N (biological samples) = 3 (CNTR *HET*, *MDX*), 1 (CNTR *WT*).

E. Representative distance analysis between macrophages (MAC: F4/80⁺ cells) and FAPs (PDGFα⁺ cells) (see Figure 4A). The analysis was performed by measuring the distance of each macrophage from its closest FAP within the image field. *Gastrocnemius* section of ~ 18-months old *PDGFRα^{eGFP/WT};MDX^{+/-}* heterozygous female (CNTR *HET*) mouse is shown. Scale bar: 20 μm. Staining: anti-F4/80 (red), Hoechst (blue).

F. Representative distance analysis between FAPs (PDGFα⁺ cells) and macrophages (MAC: F4/80⁺ cells) (refer to C). The analysis was performed by measuring the distance of each FAP from its closest macrophage within a fixed portion of the image field (100 μm², yellow rectangle). If no macrophage was present in the entire image field (bottom panel), the distance

167 was estimated between a FAP and the image edge. *Gastrocnemius* section of ~ 12-months old
168 *PDGFRα^{eGFP/WT};MDX* (*MDX*) male mouse (top panel) and ~ 18-months old
169 *PDGFRα^{eGFP/WT};MDX^{+/-}* heterozygous female (CNTR *HET*) mouse (bottom panel) are shown.
170 Note that clusters of macrophages and FAPs were observed mostly in the *MDX* regenerating
171 areas (top panel). Scale bar: 100 μm.

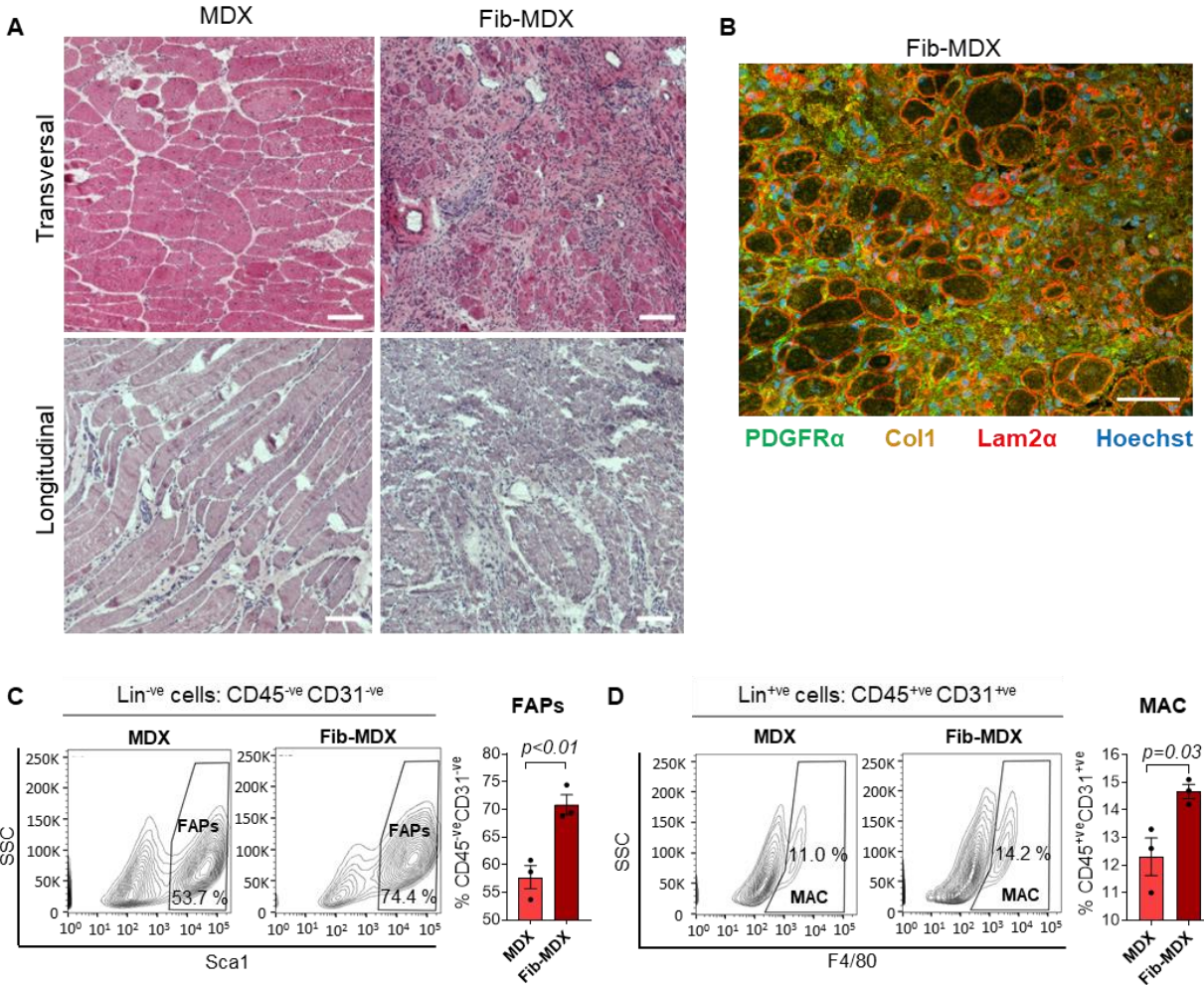
172 Data information: In C, data are presented as median with interquartile range. Each dot on the
173 graph represents a distance measurement (99 for *WT*, 381 for CNTR *HET*, and 672 for *MDX*).
174 Statistical differences were calculated by Kruskal-Wallis test. Dunn's multiple comparison test
175 was used as *post hoc* test. In D, data are presented as mean ± SEM. Statistical differences
176 between CNTR *HET* and *MDX* groups were calculated by unpaired two-tailed Student's test. P
177 values are as indicated.



Appendix Figure S12. WNT-signaling target proteins co-localize with macrophages and FAPs in the dystrophic regenerating areas.

A. Representative immunofluorescence image of *gastrocnemius* of ~12-months old male *PDGFRα^{eGFP/WT}* (CNTR *WT*), ~18-months old female *PDGFRα^{eGFP/WT};MDX^{+/-}* (CNTR HET) and ~18-months old male *PDGFRα^{eGFP/WT};MDX* (*MDX*) mice stained with anti-F4/80 (red) and anti-TGFβ2 (white) antibodies, and Hoechst (blue). The number of cells (macrophages and FAPs) and the TGFβ2 protein expression are increased in the *MDX* muscles compared to the controls. White arrows in the *MDX* sample indicate TGFβ2 that co-localizes with macrophages (F4/80^{+ve} cells) and FAPs (PDGFRα^{+ve} cells) within the regenerating area of the muscle. Scale bar: 20 μm.

B, C. Representative immunofluorescence image of a diaphragm of a ~1 year-old male *MDX* stained with anti-TGFβ2 (white), anti-F4/80 (green), anti-β-catenin (red) antibodies and DAPI (blue). White arrows indicate the localization of TGFβ2 and F4/80 (B) and β-catenin and F4/80 (C) in the same muscle areas. Scale bar: 50 μm.



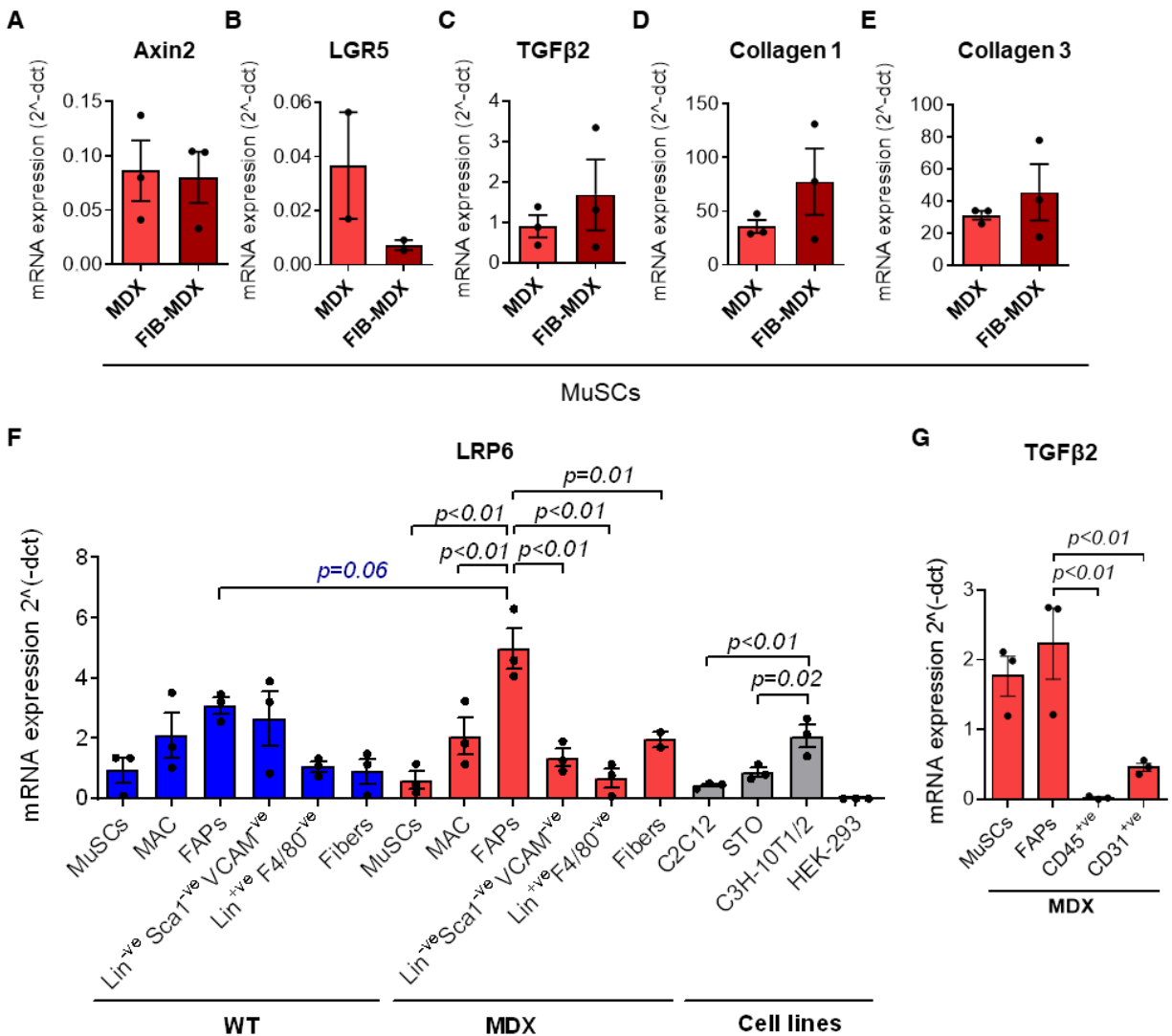
Appendix Figure S13. Analysis of the fib-MDX skeletal muscle.

A. Representative images of H&E staining of ~1 year-old *MDX* and *fib-MDX* *gastrocnemius* muscles. Transversal and longitudinal images are from different biological samples. Scale bar: 20 μ m

B. Immunofluorescence image of a *gastrocnemius* of a *fib-MDX* presented in Figure 5B with the additional staining with an anti-PDGFR α antibody (green). Scale bar: 50 μ m.

C, D. Representative FACS plots (left) and quantification (right) of FAPs (C) and macrophages (D) expressed respectively as the percentage of Lin^{-ve} and Lin^{+ve} cells in fib-MDX or uninjured MDX hindlimb muscles. N (biological samples) = 3.

Data information: In C and D, data are presented as mean ± SEM. Statistical differences were calculated by unpaired two-tailed Student's test. P values are as indicated.



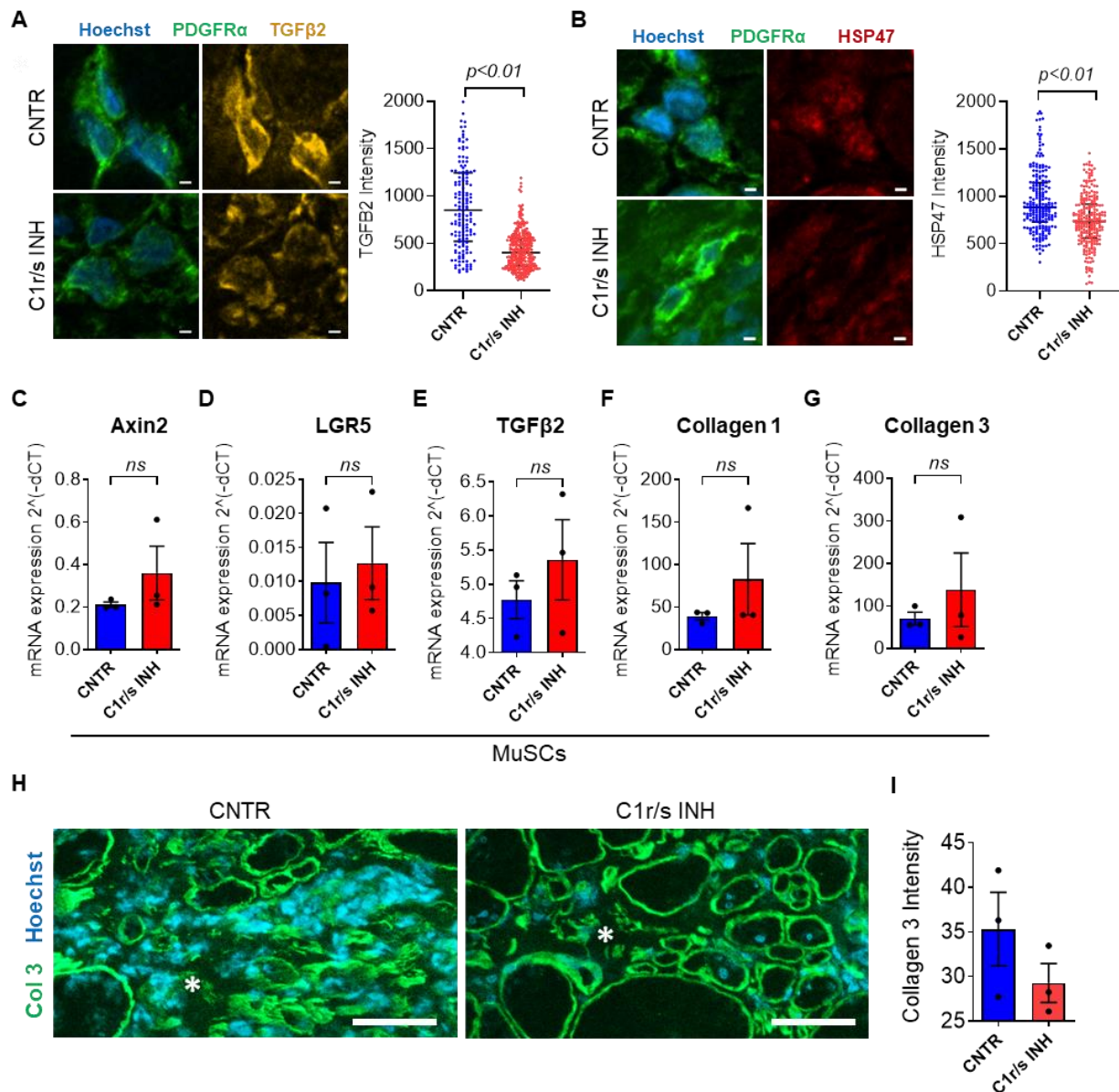
Appendix Figure S14. Analysis of MuSCs in the fib-MDX mouse model and cellular LRP6 expression.

A-E. *Axin2* (A), *LGR5* (B), *TGFβ2* (C), *Collagen1a1* (D), and *Collagen3a1* (E) mRNA expression in MuSCs FACS-isolated from hindlimb muscles of ~6 months-old *MDX* and fib-*MDX* mice. N (biological samples) = 3 for all, but *LGR5* (N=2).

F. *LRP6* mRNA expression in MuSCs, macrophages, FAPs, Lin⁺F4/80^{-ve}, Lin^{-ve}Sca1^{-ve}VCAM^{-ve}, and myofibers isolated from hindlimb muscles of ~1 year-old *WT* and *MDX* mice and in cell lines as indicated. HEK-293 (human cells) were used as a negative control as *LRP6* primers amplify only the murine isoform. N (biological samples) = 3 (for all samples except *MDX* fibers, N=2).

G. *TGFβ2* mRNA expression in MuSCs, FAPs, CD45⁺, and CD31⁺ cells FACS-isolated from the hindlimb muscles of *MDX* mice. N (biological samples) = 3.

Data information: Data are presented as mean ± SEM. In A-E, Statistical differences were calculated by unpaired two-tailed Student's test. In F-G statistical differences between three or more groups were calculated by one-way ANOVA test. Tukey's multiple comparison test was used as a *post hoc* test, and all the corresponding p values are reported on the graph; in F, the statistical difference between two groups was calculated by unpaired two-tailed Student's t-test, and the corresponding p value is reported in blue on the graph.



Appendix Figure S15. Analysis of WNT-signaling targets and fibrotic markers after in vivo C1 inhibition in the fib-MDX mouse.

A, B. Representative immunofluorescence (left) and quantification (right) of TGFβ2 (A) and HSP47 (B) signal intensities in the PDGFRα⁺ cells (i.e., FAPs) of *gastrocnemius* of ~1 year-old MDX mice processed as in Figure 6A and stained with anti-PDGFRα (green), anti-TGFβ2

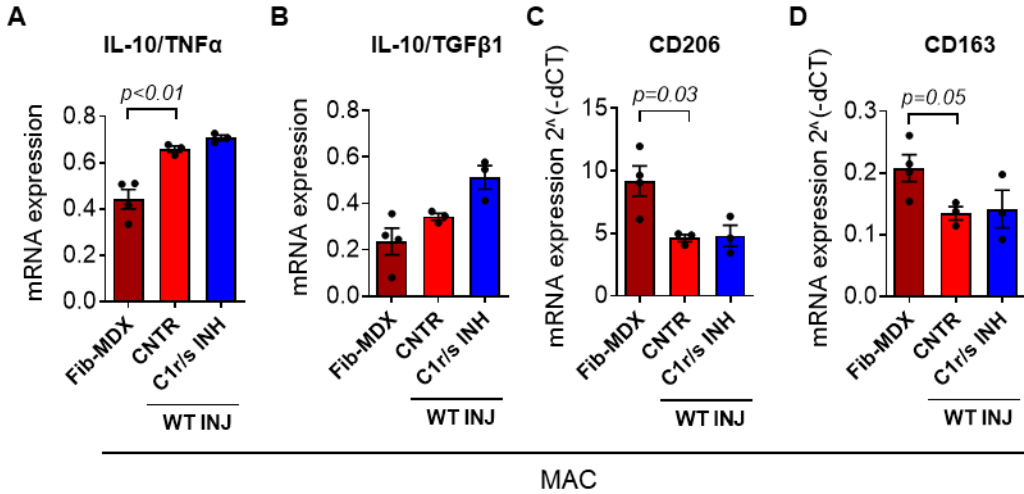
(yellow in A), anti-HPS47 (red in B) antibodies and Hoechst (blue). N (biological replicates) = 3.
Scale bar: 2 μ m.

C-G. *Axin2* (C), *LGR5* (D), *TGF β 2* (E), *Collagen1a1* (F), and *Collagen3a1* (G) mRNA expression in FACS-isolated MuSCs from ~1 year-old *MDX* mice processed as described in Figure 6A. N (biological samples) = 3.

H. Representative immunofluorescence of *gastrocnemius* muscles from ~1 year-old *MDX* mice processed as in Figure 6A. Muscles were stained with anti-Collagen 3 (green) and Hoechst (blue). Scale bar: 100 μ m. The interstitial presence of collagen 3 is indicated by the asterisk.

I. Quantification of collagen 3 pixel-intensity in the interstitial space between myofibers of the same muscles as in (H). N (biological samples) = 3.

Data information: Data are presented as median with interquartile range in A and B and as mean $\pm$ SEM in C-G and in I. In A and B, dots represent single cells' measurements (155 for CNTR and 320 for C1r/s INH in TGF β 2 analysis, and 209 for CNTR and 234 for C1r/s INH in HSP47 analysis). In I, each graph dot represents the average value of 17 to 22 measurements on different muscle regions for each biological sample. Statistical differences were calculated by the Mann-Whitney test in A and B, and the paired two-tailed Student's test in C-G and I. P values are as indicated.



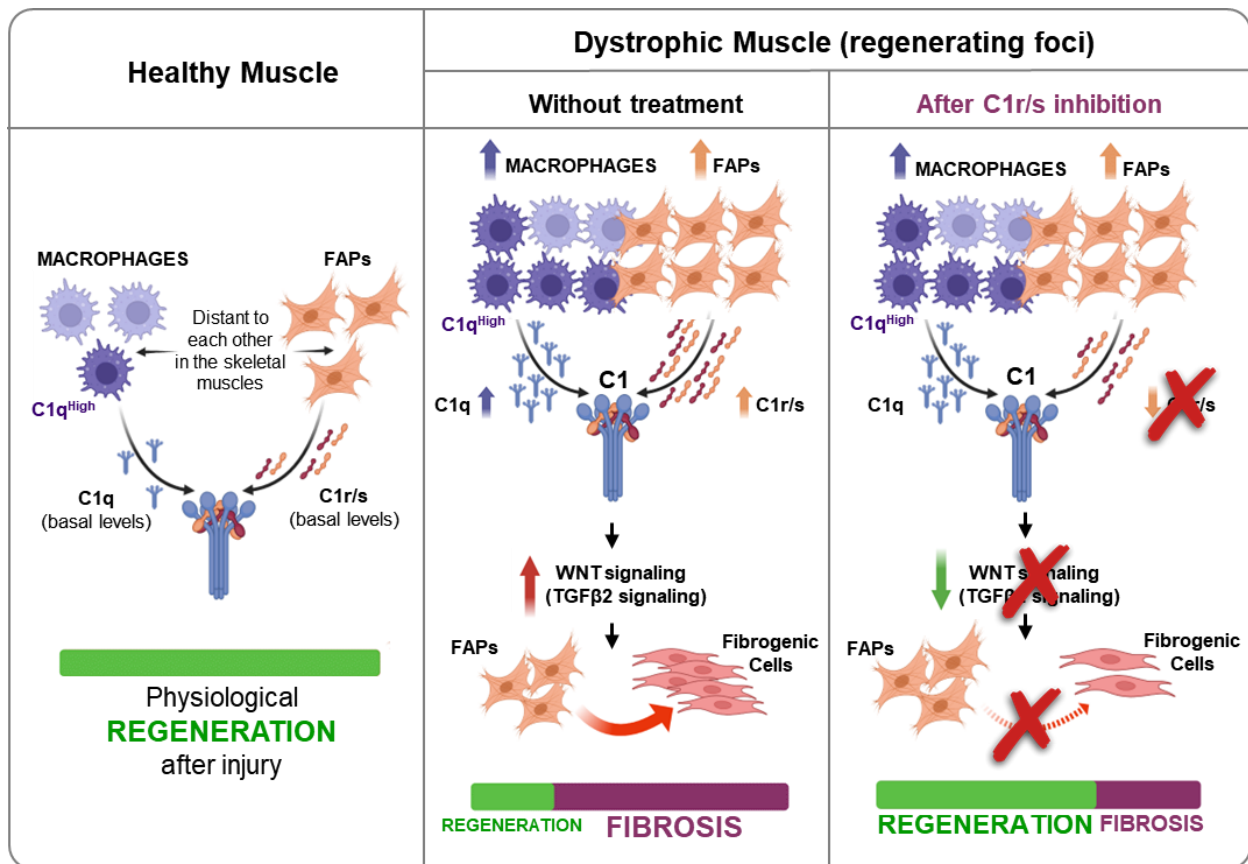
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250 Appendix Figure S16. Macrophages characterization 8 days after acute injury.

251 **A, B.** *IL-10/TNF α* (A), *IL-10/TGF β 1* (B) mRNA expression in FACS-isolated macrophages
 252 from fib-MDX mice and WT mice processed as in Figure EV5A. N (biological samples) = 3. Fib-
 253 MDX data are the same as shown in Figure EV4E-F.

254 **C, D.** *CD206* (G), *CD163* (H) mRNA expression in macrophages FACS-isolated as in A, B. N
 255 (biological replicates) = 3. Fib-MDX data are the same as shown in Figure EV4G-H.

256 Data information: Data are presented as mean $\pm$ SEM. Statistical differences between fib-MDX
 257 and WT INJ CNTR groups were calculated by unpaired two-tailed Student's test. P values are as
 258 indicated.



Appendix Figure S17. Combinatorial activation of the WNT-dependent fibrogenic program by distinct complement subunits in dystrophic muscle.

Model of the mechanism through which the C1/WNT axis is activated in the regenerating foci of dystrophic muscles. In dystrophic muscles, FAPs and macrophages act as a combinatorial source of WNT-activity by secreting distinct subunits of the C1 complement complex, promoting a fibrogenic phenotype in responsive cells, such as fibro-adipogenic progenitors. Pharmacologically interfering with the activity of C1r/s is inhibiting WNT-signaling in fibro-adipogenic progenitors and ameliorating the dystrophic phenotype. During acute physiological regeneration, the repertoire of FAPs and macrophages is different and less effective in producing complement C1.

APPENDIX TABLES

Appendix Table S1. List of human biopsies used for complement and WNT-signaling analysis.

ID in Figures	Muscle	Age (years)	Mutation on dystrophin gene	Performed analysis
CNTR 1 in Fig.1J, L	Biceps	15	not applicable	qPCR, Western Blot
CNTR 2 in Fig.1J, L	Biceps	17	not applicable	qPCR, Western Blot
CNTR 3 in Fig.1J, L	Biceps	16	not applicable	qPCR, Western Blot
CNTR 1 in Appendix Fig. S1B-C	Biceps	40	not applicable	Immunofluorescence
CNTR 2 in Appendix Fig. S1B-C	Biceps	25	not applicable	Immunofluorescence
CNTR in Fig. 1M and CNTR 3 in Appendix Fig. S1B-C	Biceps	unknown	not applicable	Immunofluorescence
CNTR 1 in Appendix Fig. S6G, H	Lumbar	60	not applicable	Immunofluorescence
CNTR 2 in Appendix Fig. S6G, H	Lumbar	42	not applicable	Immunofluorescence
CNTR 3 in Appendix Fig. S6G, H	Cervical	68	not applicable	Immunofluorescence
BMD in Fig. 1M	Biceps	8	DEL3-7	Immunofluorescence
DMD 1 in Fig.1J, L	Biceps	12	DEL49-50	qPCR, Western Blot
DMD 2 in Fig.1J, L	Biceps	12	10219G>T	qPCR, Western Blot
DMD 3 in Fig.1J, L	Biceps	11	DUP44-47	qPCR, Western Blot
DMD 4 in Fig.1J, L	Biceps	13	DUP22	qPCR, Western Blot
DMD 1 in Appendix Fig. S1B-C	Quadriceps	1	DUP52	Immunofluorescence
DMD 2 in Appendix Fig. S1B-C	Biceps	9	DUP1-9	Immunofluorescence
DMD 3 in Appendix Fig. S1B-C	Biceps	14	DEL51	Immunofluorescence
DMD 4 in Appendix Fig. S1B	Biceps	9	8668G>A	Immunofluorescence

Muscles, age, and dystrophin gene mutations of dystrophic patients and healthy controls (CNTR) analyzed for the expression of the subunits of the C1 complex and WNT-signaling targets are indicated (see **Figure 1J, L, M, Appendix Figure S1B, C** and **Appendix Figure S6G, H**). BMD, Becker muscular dystrophy.

278 **Appendix Table S2. Statistical analysis of C1qa, C1qb and C1qc expression in WT and**
279 **MDX cells.**

Analyzed comparison	P value (One-Way ANOVA test)								
	WT			MDX			WT INJ		
	C1qa	C1qb	C1qc	C1qa	C1qb	C1qc	C1qa	C1qb	C1qc
MAC vs. MuSCs	<0.01	<0.01	<0.01	<0.01	<0.01	0.06	<0.01	0.02	0.03
MAC vs. FAPs	<0.01	<0.01	<0.01	<0.01	<0.01	0.05	<0.01	0.02	0.03
MAC vs. Lin ^{-ve} Sca1 ^{-ve} VCAM ^{-ve}	<0.01	<0.01	<0.01	<0.01	<0.01	0.65	<0.01	0.03	0.19
MAC vs. Lin ^{+ve} F4/80 ^{-ve}	<0.01	<0.01	<0.01	<0.01	<0.01	0.05	<0.01	0.02	0.03
MAC vs. Fibers	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	NA	NA	NA
MuSCs vs. FAPs	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99
MuSCs vs. Lin ^{-ve} Sca1 ^{-ve} VCAM ^{-ve}	>0.99	>0.99	>0.99	0.99	>0.99	0.58	0.90	>0.99	0.72
MuSCs vs. Lin ^{+ve} F4/80 ^{-ve}	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	0.95	>0.99	>0.99
MuSCs vs. Fibers	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	NA	NA	NA
FAPs vs. Lin ^{-ve} Sca1 ^{-ve} VCAM ^{-ve}	>0.99	>0.99	>0.99	>0.99	>0.99	0.48	0.96	>0.99	0.73
FAPs vs. Lin ^{+ve} F4/80 ^{-ve}	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	0.99	>0.99	>0.99
FAPs vs. Fibers	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	NA	NA	NA
Lin ^{-ve} Sca1 ^{-ve} VCAM ^{-ve} vs. Lin ^{+ve} F4/80 ^{-ve}	>0.99	>0.99	>0.99	>0.99	>0.99	0.50	>0.99	>0.99	0.79
Lin ^{-ve} Sca1 ^{-ve} VCAM ^{-ve} vs. Fibers	>0.99	>0.99	>0.99	0.99	>0.99	0.34	NA	NA	NA
Lin ^{+ve} F4/80 ^{-ve} vs. Fibers	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	NA	NA	NA

280 One-way ANOVA test performed on WT and MDX cells shown in **Figure 2A-C**. Tukey's
281 multiple comparison test was used as a *post hoc test*. Statistically significant differences are
282 indicated in bold.

283 **Appendix Table S3. Statistical analysis of C1r, C1s and E2F1 expression in WT and MDX**
284 **cells.**

Analyzed comparison	P value (One-Way ANOVA test)								
	WT			MDX			WT INJ		
	C1r	C1s	E2F1	C1r	C1s	E2F1	C1r	C1s	E2F1
MAC vs. MuSCs	>0.99	>0.99	0.02	>0.99	>0.99	0.35	>0.99	>0.99	0.19
MAC vs. Lin ^{-ve} Sca1 ^{-ve} VCAM ^{-ve}	0.27	<0.01	0.03	0.87	<0.01	0.06	0.89	0.02	0.10
MAC vs. Lin ^{+ve} F4/80 ^{-ve}	>0.99	>0.99	0.03	>0.99	>0.99	0.28	>0.99	>0.99	0.10
MAC vs. Fibers	>0.99	>0.99	0.01	>0.99	>0.99	NA	NA	NA	0.02
MuSCs vs. Lin ^{-ve} Sca1 ^{-ve} VCAM ^{-ve}	0.32	<0.01	>0.99	0.71	<0.01	0.74	0.93	0.03	>0.99
MuSCs vs. Lin ^{+ve} F4/80 ^{-ve}	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99
MuSCs vs. Fibers	0.99	>0.99	>0.99	>0.99	>0.99	NA	NA	NA	0.79
FAPs vs. MAC	<0.01	<0.01	>0.99	<0.01	<0.01	>0.99	<0.01	<0.01	0.46
FAPs vs. MuSCs	<0.01	<0.01	0.02	<0.01	<0.01	0.27	<0.01	<0.01	<0.01
FAPs vs. Lin ^{-ve} Sca1 ^{-ve} VCAM ^{-ve}	<0.01	0.88	0.02	<0.01	<0.01	0.04	<0.01	0.84	<0.01
FAPs vs. Lin ^{+ve} F4/80 ^{-ve}	<0.01	<0.01	0.02	<0.01	<0.01	0.22	<0.01	<0.01	<0.01
FAPs vs. Fibers	<0.01	<0.01	<0.01	<0.01	<0.01	NA	NA	NA	<0.01
Lin ^{-ve} Sca1 ^{-ve} VCAM ^{-ve} vs. Lin ^{+ve} F4/80 ^{-ve}	0.27	<0.01	>0.99	0.69	<0.01	0.82	0.85	0.02	>0.99
Lin ^{-ve} Sca1 ^{-ve} VCAM ^{-ve} vs. Fibers	0.18	<0.01	>0.99	0.72	<0.01	NA	NA	NA	0.94
Lin ^{+ve} F4/80 ^{-ve} vs. Fibers	>0.99	>0.99	>0.99	>0.99	>0.99	NA	NA	NA	0.94

285 One-way ANOVA test performed on WT and MDX cells shown in **Figure 2D-E** and in **Appendix**
286 **Figure S7A**. Tukey's multiple comparison test was used as a *post hoc* test. Statistically significant
287 differences are indicated in bold.

288 **Appendix Table S4. List of primary antibodies used for FACS-isolation of murine and**
289 **human cells.**

Antibody	Company	Dilution
APC/Fire 750 anti-mouse CD45	BioLegend (103154)	1:100
APC/Fire 750 anti-mouse CD31	BioLegend (102434)	1:100
FITC anti-mouse F4/80	BioLegend (123107)	1:100
Brilliant Violet 421 anti-mouse Ly-6A/E (Sca1)	BioLegend (108127)	1:100
Biotin anti-mouse CD016 (VCAM)	BioLegend (105704)	1:100
APC anti-mouse CD45	BioLegend (103112)	1:100
APC anti-mouse CD31	BioLegend (102410)	1:100
Pacific Blue anti-mouse Ly-6A/E (Sca-1)	BioLegend (108120)	1:100
PE anti-mouse CD206	Biotechne (FAB2535P)	1:20
APC anti-mouse F4/80	BioLegend (123116)	1:100
FITC anti-mouse CD45	BioLegend (103108)	1:100
Pacific Blue anti-human CD45	BioLegend (304021)	1:100
Pacific Blue anti-human CD31	BioLegend (303113)	1:100
PE anti-human CD34	Miltenyi (130-081-002)	1:30

Appendix Table S5. Calculation of STO/RAW-264.7 conditioned media ratio used to treat murine cells.

MDX Hindlimb Muscles ~ 1 year-old	% of total cell number	
	Macrophages	FAPs
Biological Replicate 1	3.60	9.60
Biological Replicate 2	3.30	13.70
Biological Replicate 3	3.39	10.4
Average	3.43	11.2
StDev	0.15	2.17
FAPs/MAC = $11.2/3.43 = 3.27$ Number of cells per area at confluence (inversed proportional to cellular size) = $RAW-264.7/STO = 2.5$ Used conditioned media ratio (vol/vol): $STO/RAW-254.7 = 3.27 \times 2.5 = 8.18/1$		

STO/RAW-264.7 conditioned media ratio used to treat C2C12 cells (**Figure 3J-M**) was calculated considering the *in vivo* proportion of macrophages and FAPs in dystrophic mice and the number of STO and RAW-264.7 cells per area at confluence, when media was collected.

Gene	Forward Primer 5'→3'	Reverse Primer 5'→3'	T _{Ann}	Span an exon-exon junction?
mAxin2	cagagggacaggaaccactc	tgccagtttctttggctctt	60 °C	Yes
mC1qa	tctcagccattcggcagaac	tggttggtgaggaccttgta	60 °C	No
mC1qb	gggaatccactgtgtccggc	ctcagcctcaggggttctgt	60 °C	Yes
mC1qc	agagccaggaatcccagccgtcc	gcatgccaggctcgccctt	60 °C	No
mC1r	aaccatattacaagatgctgacca	ccttggtgtgtgcaggta	60 °C	Yes
mC1s	gaccagaggcaggagaggaggc	gctcagtgtcaccttcaggagc	60 °C	Yes
mE2F1	gaggctggatctggagactg	cccggagatttcacaccttc	60 °C	Yes
mCol1a1	tccggtcctctgctcctcta	gtatgcagctgacttcaggatgt	60 °C	Yes
mCol3a1	gcccacagccttctacac	ccagggtcaccattctc	60 °C	Yes
mFibronectin1	tgcctcgggaatggaaag	atggtaggtcttcccatcgata	60 °C	Yes
mLGR5	tcgcctccccaggtcccttc	gccgtggtccacacccgat	60 °C	Yes
mTGFβ2	cgagcggagcgacgaggagt	tgggcgggatggcatttctgg	60 °C	Yes
mHPRT	tcagaccgcttttgccgcga	atcgctaatacacgacgtgggac	60 °C	No
h/mHPRT	aactggaaagaatgtcttgattgt	gaatttcaaatacaaaaagtctgg	60 °C	Yes
mLRP6	tcctcagactctggcact	cctccccactcagtccaata	60 °C	Yes
hC1qa	acaacaggaggcaggccc	acacagagcaccagccatc	60 °C	Yes
hC1qb	agtaggtctcggctcctg	tcagacgcctcctgggaa	60 °C	Yes
hC1qc	gggaagcagatctgaggacatc	cggagaaggaactgggca	60 °C	Yes
hC1r	gctgagaacatgtctgtgaggtt	aggaggtacaagagccaccact	60 °C	Yes
hC1s	agaggggttgcccagcat	ggctcagtgtcaccttcaga	60 °C	Yes
mTNFα	aagttcccaaatggcctccc	tggtttgctacgacgtggg	60 °C	Yes
mTGFβ1	ccccactgatacgctgagt	agccctgtattccgtctcctt	60 °C	Yes
mCD206	tataggtggagagctggcga	tccactgctcgtaacagcc	60 °C	Yes
mCD163	tcctcaagaggagaggtcttg	ggaattttccgagatttcagca	60 °C	Yes
mIL-10	ggcgctgtcatcgatttctc	atggcctgtagacaccttgg	60 °C	Yes

296 **Appendix Table S7. List of primary antibodies used for immunofluorescence.**

Antigen	Specie	Company	Dilution
Axin2	Rabbit	Abcam (32197)	1:20
TGFβ2	Rabbit	Santa Cruz Biotechnology (sc-90)	1:30
C4	Rat	Santa Cruz Biotechnology (16D2)	1:50
C1q	Rabbit	Abcam (182451)	1:80
C1q	Rat	Abcam (11861)	1:50
C1q	Mouse	Abcam (71940)	1:100
C1s	Rabbit	LS Bio (LS-C483829)	1:100
Laminin2α	Rat	Abcam (11576)	1:1000
GFP	Chicken	Aves Labs (1020)	1: 250
Collagen1	Rabbit	Cederlane (CL50151AP)	1:200
Collagen 3	Rabbit	Abcam (7778)	1:200
F4/80-Alexa Fluor 594	Rat	BioLegend (123140)	1:50
β-catenin	Rabbit	Abcam (246504)	1:100
PDGFRα	Goat	Biotechne (AF1062)	1:100
HSP47	Rabbit	Abclonal (A11698)	1:50