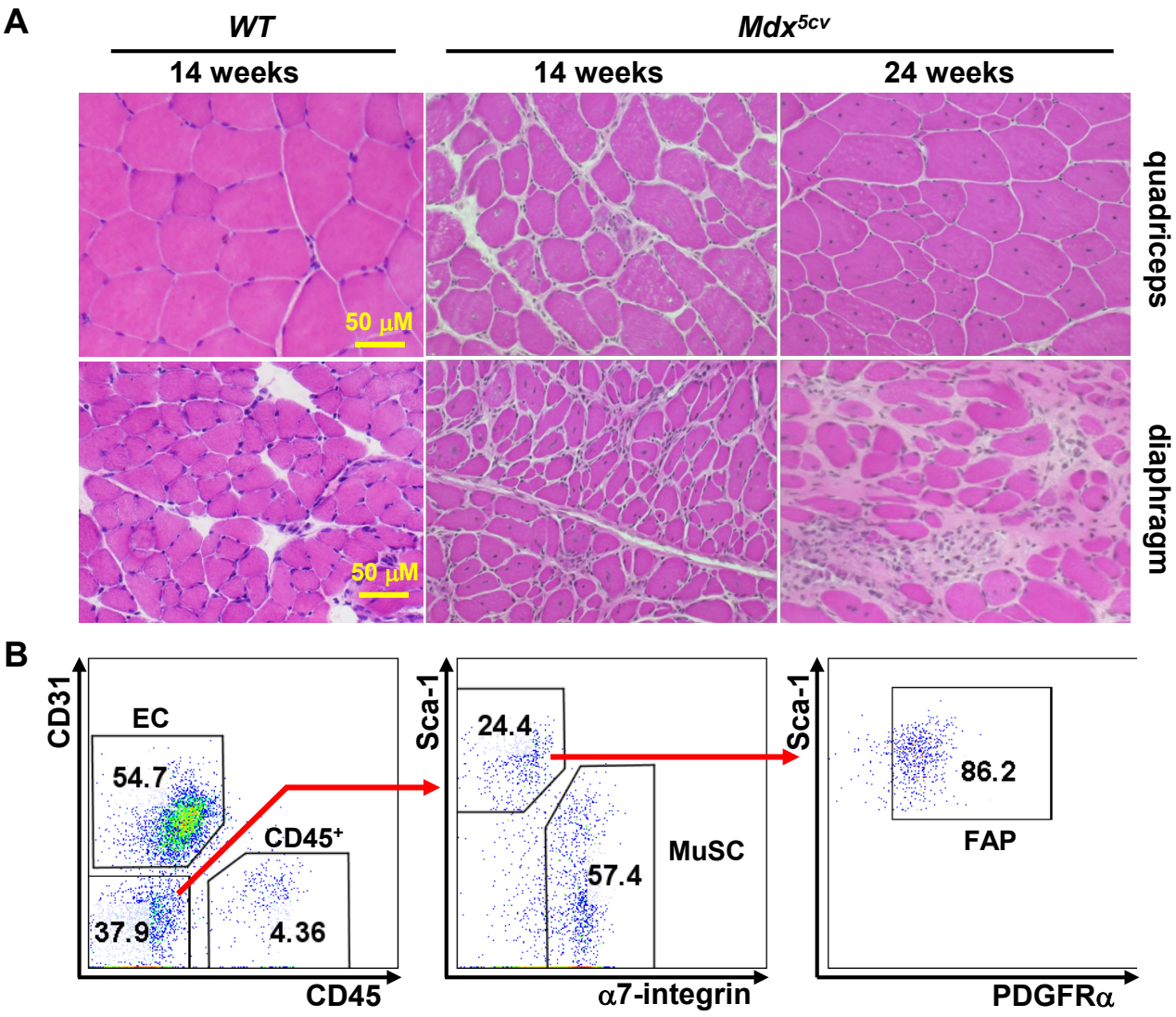
iScience, Volume *26*

Supplemental information

Diverse effector and regulatory functions of fibro/adipogenic progenitors during skeletal muscle fibrosis in muscular dystrophy

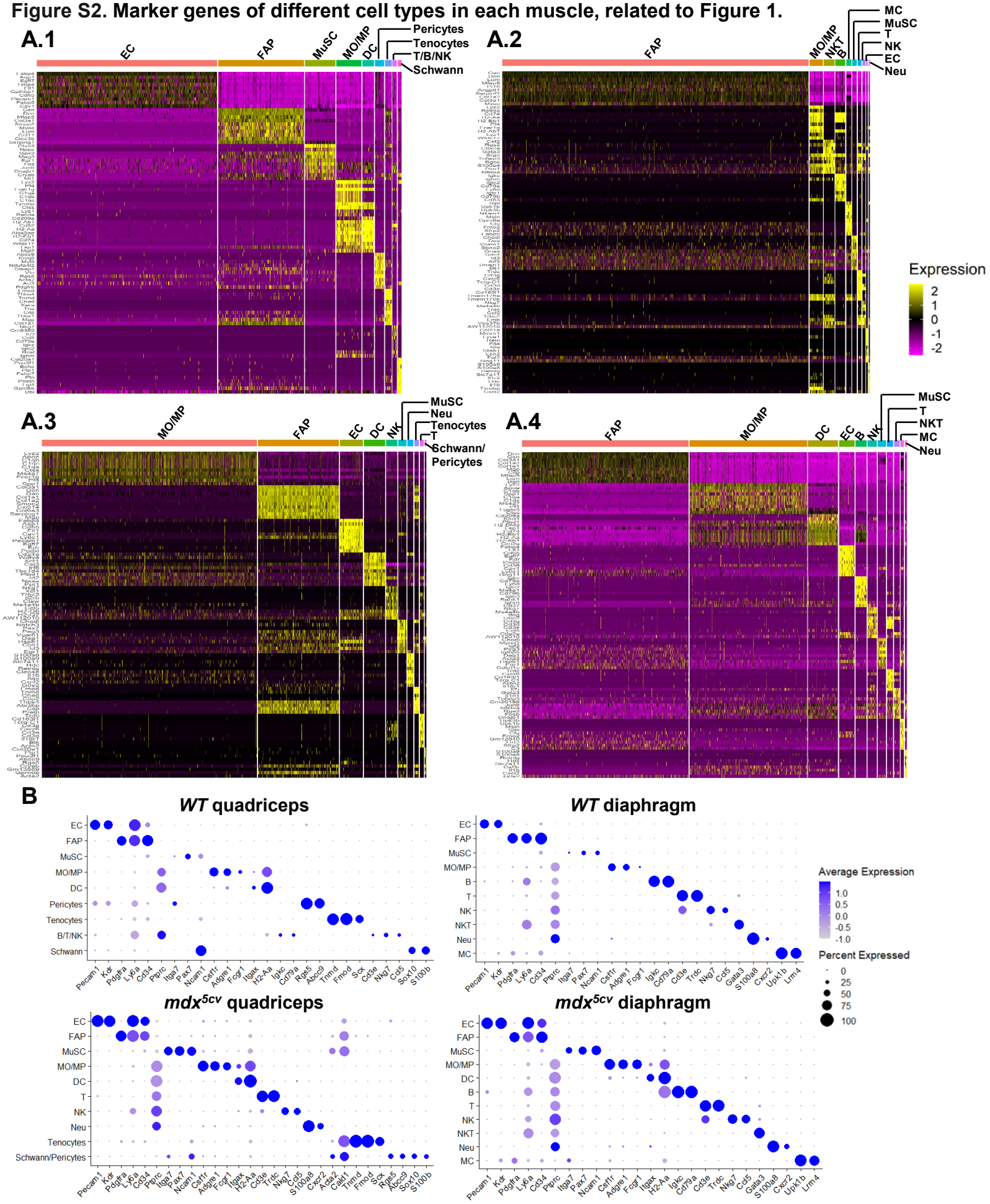
Xingyu Wang, Jianming Chen, Sachiko T. Homma, Yinhang Wang, Gregory R. Smith, Frederique Ruf-Zamojski, Stuart C. Sealfon, and Lan Zhou

Figure S1. Persistent inflammation, progressive fibrosis and accumulation of FAPs in *mdx*^{5cv} diaphragm, related to Figure 1



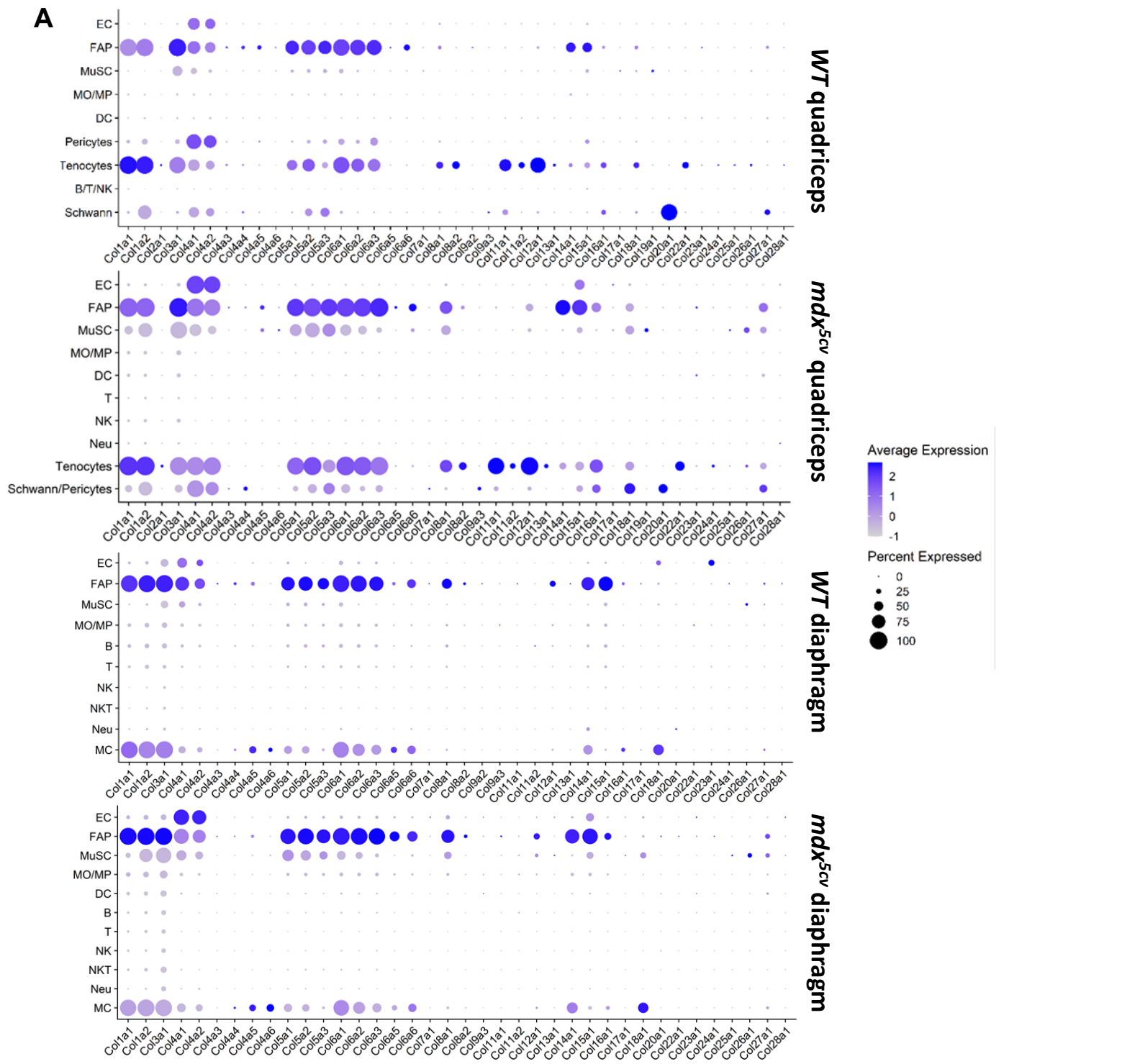
A. HE staining of cryosections of quadriceps and diaphragm muscles from wild-type (*WT*) and *mdx*^{5cv} mice at age 14 weeks and 24 weeks showing diaphragm, but not quadriceps, of *mdx*^{5cv} mice undergoing progressive fibrosis from 14 weeks to 24 weeks. Bar = 50 μM **B.** Gating strategy of FACS analysis to identify intramuscular endothelial cells (EC), CD45^{+} cells, fibro/adipogenic progenitors (FAP), and muscle satellite cells (MuSC). Representative results shown here are from the analysis of single-cell suspension prepared from quadriceps of 14 weeks old *WT* mice.

Figure S2. Marker genes of different cell types in each muscle, related to Figure 1.



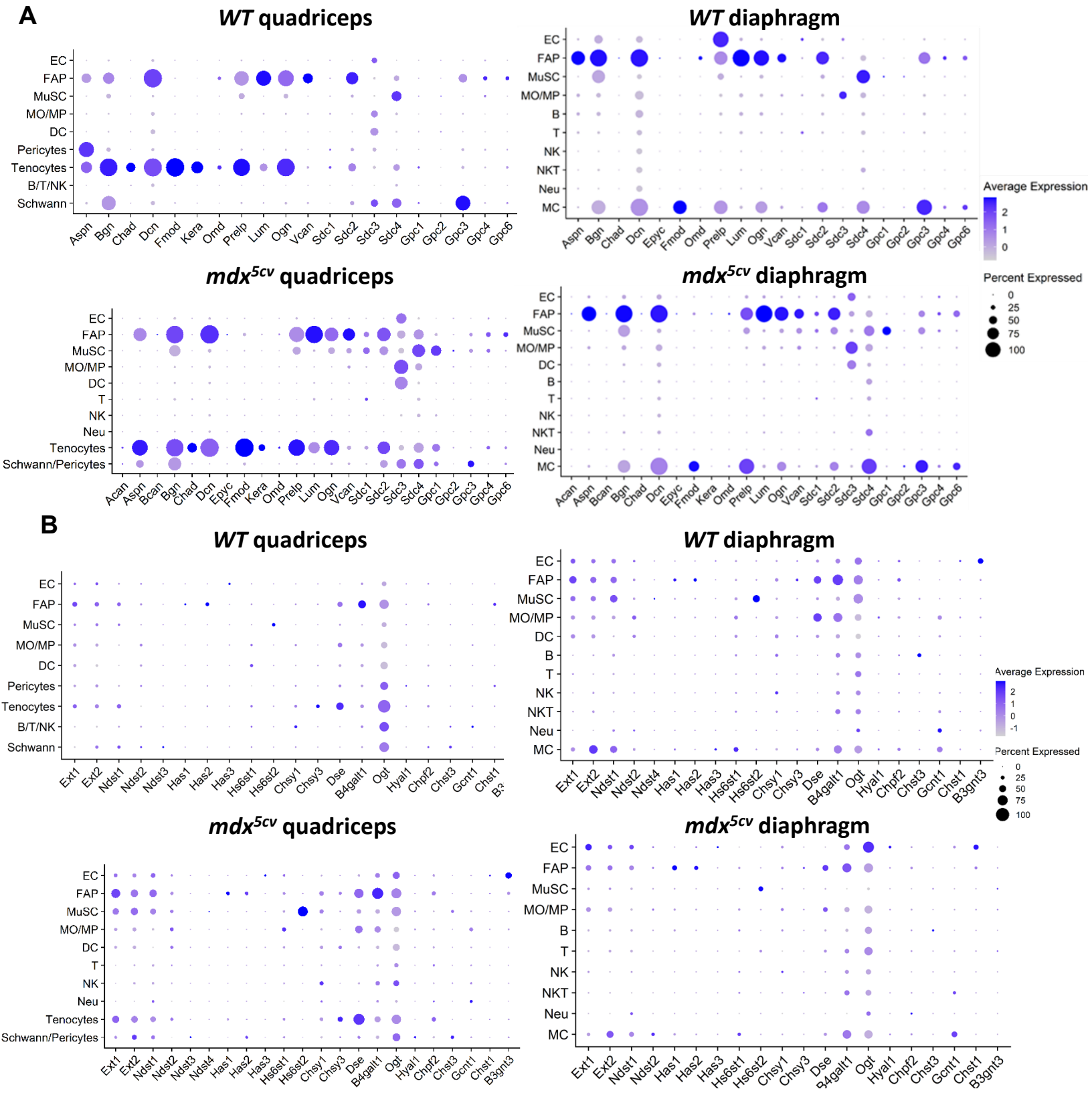
A. Heatmap depicting top 10 differentially expressed genes (DEGs) of individual cell types in *WT* quadriceps (**A.1**), *WT* diaphragm (**A.2**), *mdx*^{5cv} quadriceps (**A.3**), and *mdx*^{5cv} diaphragm (**A.4**). The names of the top 10 DEGs of each cell type are listed in **Table S2**. **B:** Dot plot showing marker genes of different cell types in each muscle.

Figure S3. Expression of collagens and fibronectin by different cell types, related to Figures 2A and 4D.



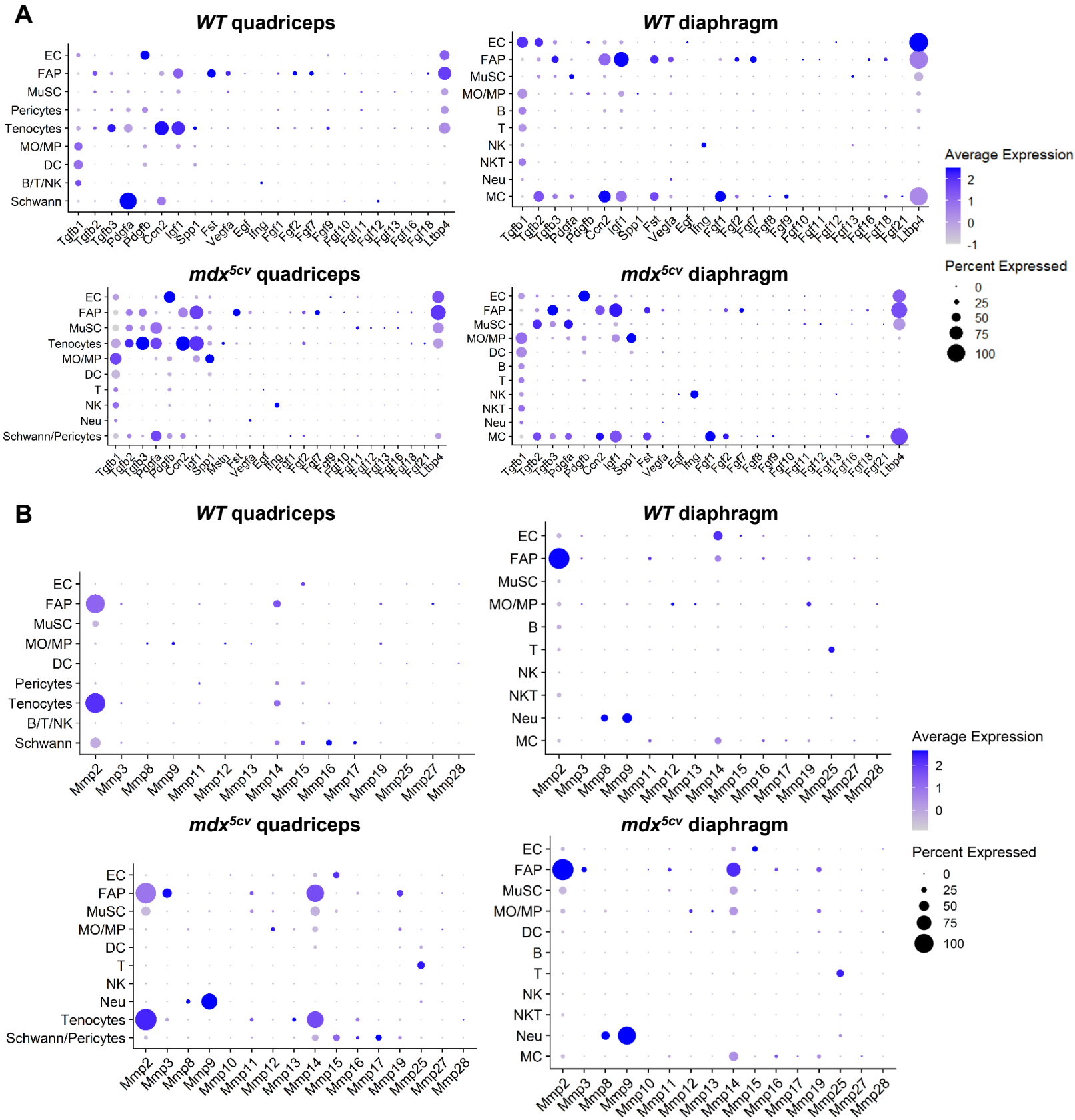
A. Dot plot showing expression of detectable collagen genes by different cell types in each muscle. **B.** Original pictures of Western blot showing expression of COL1A1, COL3A1, and fibronectin by quadriceps and diaphragm of *WT* and *mdx*^{5cv} mice.

Figure S4. Expression of genes encoding proteoglycan and synthesizing enzymes of glycosaminoglycan by different cell types, related to Figure 2.



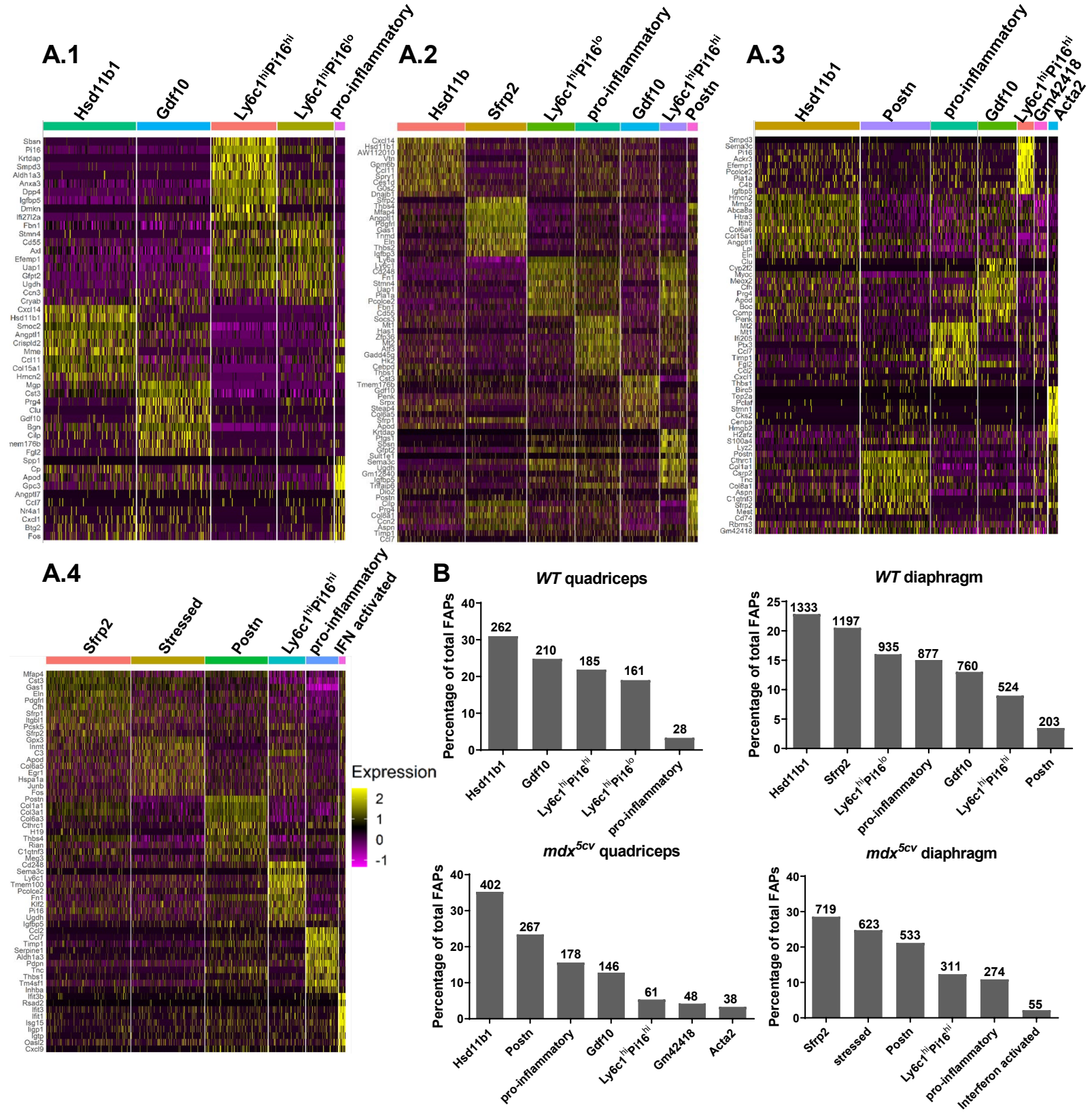
Dot plot showing expression of detectable proteoglycan genes (**A**) and synthesizing enzymes of glycosaminoglycan (**B**) genes by different cell types in each muscle.

Figure S5. Expression of ECM regulatory genes by different cell types in each muscle, related to Figure 3.



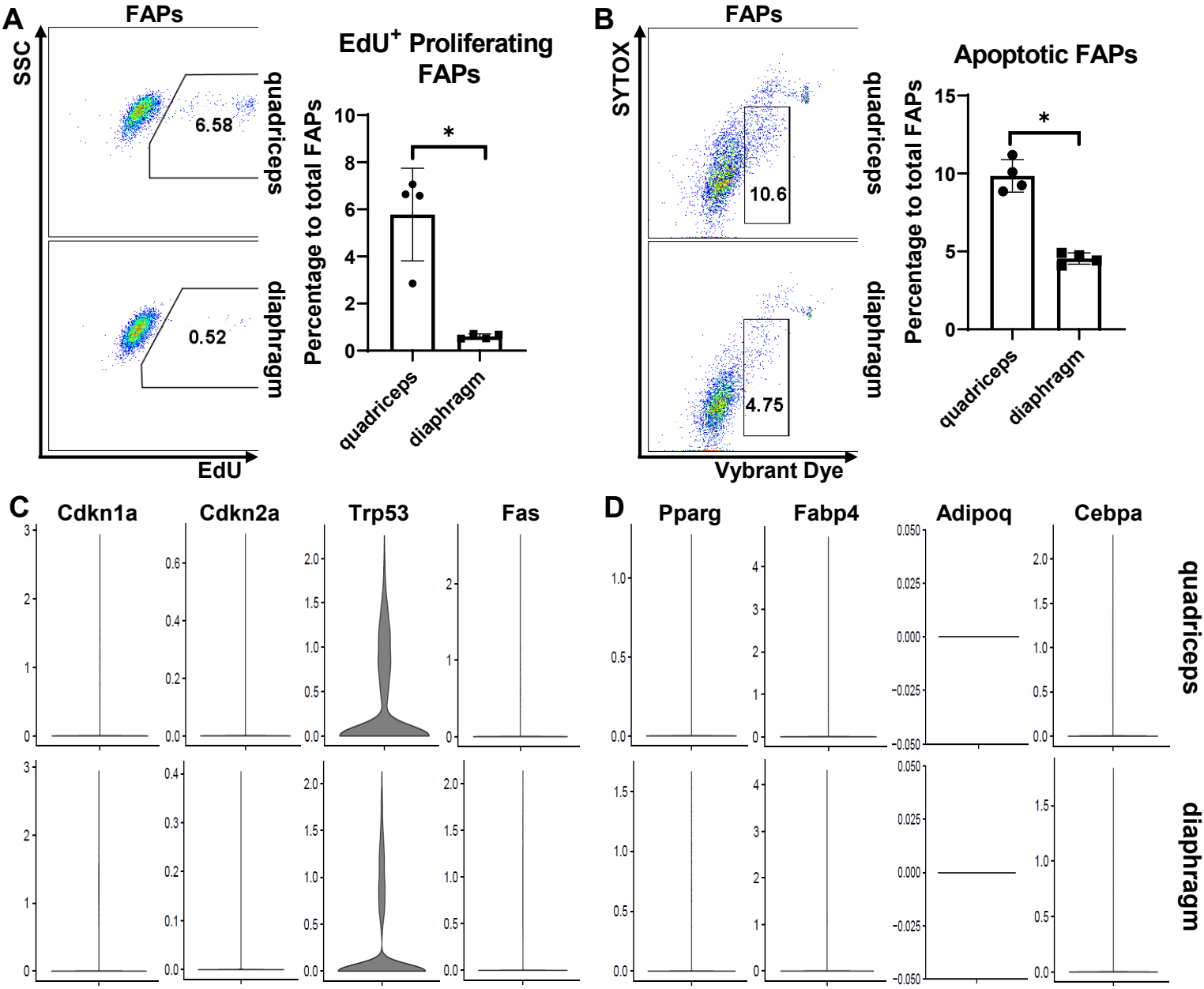
Dot plot showing expression of detectable ECM regulatory genes by different cell types in each muscle. **A.** Fibrogenic factor genes. **B.** MMP genes.

Figure S6. FAPs consist of functionally diverse clusters which are different among quadriceps and diaphragm in *WT* and *mdx*^{5cv} mice, related to Figure 5.



A. Heatmap depicting top 10 differentially expressed genes in individual FAP clusters in *WT* quadriceps (**A.1**), *WT* diaphragm (**A.2**), *mdx*^{5cv} quadriceps (**A.3**), and *mdx*^{5cv} diaphragm (**A.4**). These genes are also listed in Tables S3-6. **B.** Bar graph showing percentage of individual FAP clusters to total FAPs in each muscle sample. The number of cells identified by scRNAseq analysis for each FAP cluster is shown on top of each bar.

Figure S7. Comparison of *mdx*^{5cv} quadriceps and diaphragm FAPs at 14 weeks of age, related to Figure 1 and Discussion



A. Dot plot (left) showing FACS analysis of EdU⁺ proliferating FAPs. Bar graph (right) showing percentage of EdU⁺ proliferating FAPs. **B.** Dot plot (left) showing FACS analysis of apoptotic FAPs. Bar graph (right) showing percentage of apoptotic FAPs. Bar graphs of **A & B**: data are presented as mean ± SEM. Asterisks indicate significant differences (*p <0.05, determined by Mann-Whitney test). **C & D.** Violin plots showing expression of the genes related to senescence (**C**) and adipogenesis (**D).**

Table S1. scRNAseq Quality Control, related to Figure 1.

Sample ID	*WT*_quadriceps	*WT*_diaphragm	*mdx*^{5cv}_quadriceps	*mdx*^{5cv}_diaphragm
Estimated Number of Cells	6,545	8,485	9,897	8,286
Mean Reads per Cell	43,703	33,343	30,252	34,137
Median Genes per Cell	962	1,746	1,800	1,761
Median UMI Counts per Cell	1,780	4,128	4,603	4,505
Reads Mapped Confidently to Intronic Regions	22.3%	17.5%	21.9%	20.7%
Reads Mapped Confidently to Exonic Regions	69.9%	74.4%	69.3%	71.1%
Reads Mapped Confidently to Transcriptome	68.3%	72.7%	67.8%	69.6%
Q30 Bases in RNA Read	94.3%	93.5%	94.4%	94.5%
Fraction Reads in Cells	85.5%	93.1%	94.3%	94.1%

Table S2. Top 10 differentially expressed genes by different intramuscular cell types of quadriceps and diaphragm muscles in *WT* and *mdx^{5cv}* mice, related to Figures 1 and S2.

Sample	Cell type	Top 10 DEGs
WT quacriceps	EC	Fabp4, Aqp1, Egfl7, Timp4, Flt1, Gpihbp1, Cdh5, Pecam1, Fabp5, Cav1
	FAP	Gsn, Dcn, Mfap5, Col3a1, Smoc2, Myoc, Lum, Ccl11, Clec3b, Serping1
	MuSC	Chodl, Nppc, Gpx3, Meg3, Egr1, Fos, Junb, Dnajb1, Cryab, Mt1
	MO/MP	Lyz2, Pf4, Fcer1g, C1qa, C1qb, C1qc, Tyrobp, Ctss, Lyz1, Retnla
	DC	Cd209, H2-Ab1, Cd52, H2-Aa, Alox5ap, H2-Eb1, Cd74, Wfdc17, Lsp1, Mgl2
	Pericytes	Abcc9, Kcnj8, Myl9, Ndufa4l2, Steap4, Vtn, Rgs5, Acta2, Art3, Pdgfrb
	Tenocytes	Fmod, Thbs4, Tnmd, Chad, Kera, Tnc, Clip, Thbs1, Mgp, Col1a1
	B/T/NK	Nkg7, Gm8369, Il7r, Ccl5, Cd79a, Igkc, Iglc2, Hcst, Ighm, Il2rg
	Schwann	Col20a1, Pou3f1, Bche, Plp1, Fabp7, Ptn, Postn, Lgi4, Gpm6b, Dbi
WT diaphragm	FAP	Gsn, Dcn, Lum, Mfap5, Pi16, Angptl1, Serpinf1,Col1a2, Col3a1, Myoc
	MO/MP	Lyz2, Retnla, Cd74, H2-Aa, H2-Eb1, Pf4, Fcer1g, H2-Ab1, Lyz1, Wfdc17,
	NKT	Csf2, Rgs2, Ctla2a, Gata3, Srgn, Tnfaip3, Rgcc, S100a4, Pim1, Nfkbia
	B	Igkc, Ighm, Iglc2, Cd79a, Cd74, Ly6d, Iglc1, H2-Aa, Cd79b, Cd83
	MC	Slpi, Upk1b, Upk3b, Nkain4, Msln, Gpm6a, Clu, Fmo2, Sfrp2, Fabp5
	MuSC	Chodl, Des, Vcam1, Sbno2, Gnas, Sdc4, Id3, Atf3, Dnajb1, Mt1
	T	Trdc, Cd3g, Cxcr6, Tcrg-C1, Cd3d, Cd3e, Cd163l1, Tmem176a, Tmem176b, Il7r
	NK	Nkg7, Ms4a4b, Trac, Ccl5, Trbc2, Emb, Vps37b, Aw112010, Cd3d, Ctla2a
	EC	Ccl21a, Mmrn1, Lyve1, Reln, Flt4, Nts, Stab1, Lcn2, Fgl2, Gng11
	Neu	S100a9, S100a8,Retnlg, Slc7a11, Il1r2, Hdc, Il1b, Tyrobp, Cxcl2, Slpi
mdx^{5cv} quacriceps	MO/MP	Lyz2, Apoe, C1qb, C1qc, C1qa, Ctss, Ms4a1, Fcer1g, Pf4, Spp1
	FAP	Col3a1, Dcn, Gsn, Col1a1, Col1a2, Smoc2, Cxcl14, Col6a3, Serping1, Mgp
	EC	Fabp4, Aqp1, Cdh5, Flt1, Cav1, Ly6c1, Pecam1, Egfl7, Kdr, Podxl
	DC	Cd24a, Wdfy4, Xcr1, Cst3, Irf8, Tbc1d4, Plbd1, Id2, Naaa, Ppt1
	NK	Nkg7, Xcl1, Trbc2, Il2rb, Ctsw, Ms4a4b, Ccl5, H2-Q6, H2-Q7, Aw112010
	MuSC	Chodl, Notch3, Pax7, Peg3, Vcam1, Dag1, Hspb1, Ccn1, Id3,Egr1
	Neu	S100a9, S100a8, Slc7a11, Hdc, Retnlg, Clec4e, Il1b, Slpi, Cxcl2, G0s2
	Tenocytes	Fmod, Tnmd, Chad, Cilp2, Thbs4, Abi3bp, Cilp, Prelp, Mgp, Chodl
	T	Trdc, Cd163l1, Tcrg-C1, Cd3g, Cxcr6, Cd3e, Cd3d, Il18r1, Blk, Actn2
	Schwann/Pericytes	Col20a1, Plp1, Pou3f1, Abcc9, Rgs5, Cryab, Gm13889, Gpm6b, Acta2, Dag1
mdx^{5cv} diaphragm	FAP	Dcn, Gsn, Col3a1, Col1a2, Col1a1, Mgp, Cilp, Mfap5, Lum, Bgn
	MO/MP	Lyz2, Apoe, C1qb, Spp1, C1qa, C1qc, Ms4a7, Pf4, Lgals3, Ctss
	DC	Cd209, Klrd1, Plbd1, H2-DMa, Lsp1, Cd74, H2-Eb1, H2-Aa, H2-Ab1, Gm2a
	EC	Fabp4, Flt1, Cdh5, Egfl7, Kdr, Podxl, Cd36, Cav1, Ly6c1, Gng11
	B	Igkc, Cd79a, Ly6d, Iglc2, Ms4a1, Cd79b, Iglc3, Bank1, Ighm, Cd37
	NK	Nkg7, Ms4a4b, Ifng, Trbc2, Cd3g, Cd3d, Cd3e, Ccl5, Ctla2a, Aw112010
	MuSC	Chodl, Myod1, Igf2, Peg3, Igfbp5, Dag1, Acta2, Hspb1, Egr1, Cdkn1c
	T	Trdc, Cxcr6, Cd163l1, Tcrg-C1, Actn2, Il18r1, Il7r, Cd3g, Cd3d, Cd3e
	NKT	Gata3, Il1rl1, Tnfaip3, Gm20186, Junb, Nfkbia, Tgs2, Fosb, Dnajb1, Ctla2a
	MC	Upk3b, Upk1b, Msln, Slpi, Clu, Fmo2, Gm12840, Flrt2, Sfrp2, C3
	Neu	S100a9, S100a8, Retnlg, Hdc, Slc7a11, Csf3r, Il1b, Cxcl2, G0s2, Slpi

Table S3. Number and percentage of each cell type identified by scRNAseq, related to Figure 1.

Sample	Cell type	Number	Percentage	Total cell number
WT quacriceps	EC	1927	50.52438385	3814
	FAP	917	24.04299948	
	MuSC	323	8.468799161	
	MO/MP	269	7.052962769	
	DC	124	3.251179864	
	Pericytes	98	2.56948086	
	Tenocytes	69	1.809124279	
	B/T/NK	48	1.258521238	
	Schwann	39	1.022548506	
WT diaphragm	FAP	5956	85.23182599	6988
	MO/MP	263	3.763594734	
	NKT	203	2.904979966	
	B	193	2.761877504	
	MC	100	1.431024614	
	MuSC	89	1.273611906	
	T	73	1.044647968	
	NK	57	0.81568403	
	EC	29	0.414997138	
	Neu	25	0.357756153	
mdx^{5cv} quacriceps	MO/MP	3608	57.25166614	6302
	FAP	1356	21.51697874	
	EC	385	6.109171692	
	DC	355	5.633132339	
	NK	188	2.983179943	
	MuSC	141	2.237384957	
	Neu	97	1.539193907	
	Tenocytes	78	1.237702317	
	T	65	1.031418597	
	Schwann/Pericytes	29	0.460171374	
mdx^{5cv} diaphragm	FAP	2704	44.40794876	6089
	MO/MP	1907	31.31877156	
	DC	488	8.014452291	
	EC	253	4.155033667	
	B	180	2.956150435	
	NK	148	2.43061258	
	MuSC	133	2.18426671	
	T	97	1.593036623	
	NKT	95	1.560190507	
	MC	52	0.853999015	
	Neu	32	0.525537855	

Table S4. Top 25 differentially expressed genes of individual FAP clusters in *WT* quadriceps, related to Figure 5.

Cluster name	Ly6c1^{hi}Pi16^{hi}	Ly6c1^{hi}Pi16^{lo}	Hsd11b1	Gdf10	pro-inflammatory
Top 25 DEGs	Krtdap	Fbn1	Cxcl14	Mgp	Apod
	Pi16	Cd55	Hsd11b1	Prg4	Gpc3
	Sbsn	Ccn3	Smoc2	Cilp	Fos
	Smpd3	Stmn4	Crispld2	Clu	Angptl7
	Igfbp5	Uap1	Col4a1	Gdf10	Cp
	Anxa3	Efemp1	Angptl1	Cst3	Ccl7
	Dmkn	Cryab	Mme	Tmem176b	Cxcl1
	Aldh1a3	Gfpt2	Col15a1	Fgl2	Spp1
	Dpp4	Ugdh	Hmcn2	Meox2	Nr4a1
	Ifi27l2a	Axl	Ccl11	Tnmd	Btg2
	Sema3c	Crip1	Lpl	Bgn	Ptch1
	Fn1	Creb5	Gpm6b	Cfh	Smim41
	Procr	Sema3c	Col4a2	Myoc	Vit
	Pla1a	Atox1	G0s2	Gas1	Zfp36
	Efhd1	Anxa8	Hspg2	Gas6	Csrp1
	Edn1	Fstl1	Plau	Fmo2	Lum
	Car8	D630033O11Rik	Hspa1a	Penk	Matn2
	Il1r2	Pcsk6	Col5a3	Kctd12	Atp1a2
	Emilin2	Pcolce2	Fbln7	Meox1	Id3
	Thbd	Actg1	Lamb1	Cd9	Col15a1
	C3	Efhd1	Col6a6	Emp1	Ccl2
	Gan	Mfap5	Apoe	Ecrg4	Rasgrp2
	Akr1c18	1700019D03Rik	Enpp2	S100a4	Sdc3
	Sfrp2	Mustn1	Lifr	Cpe	Foxs1
	Plac8	Ppp1r14b	Egr1	Tmem176a	Thbs1

Table S5. Top 25 differentially expressed genes of individual FAP clusters in *WT* diaphragm, related to Figure 5.

Cluster name	Ly6c1^{hi}Pi16^{hi}	Ly6c1^{hi}Pi16^{low}	Hsd11b1	Gdf10	pro-inflammatory	Sfrp2	Postn
Top 25 DEGs	Igfbp5	Ly6c1	Cxcl14	Tmem176b	Mt1	Sfrp2	Postn
	Krtdap	Ly6a	Hsd11b1	Cst3	Socs3	Thbs4	Ccl7
	Ugdh	Cd55	Ccl11	Gdf10	Has1	Tnmd	Ccn2
	Sbsn	Cd248	G0s2	Col6a5	Zfp36	Mfap4	Col8a1
	Sult1e1	Stmn4	Vtn	Sfrp1	Mt2	Angptl1	Timp1
	Gm12840	Fn1	AW112010	Srpx	Thbs1	Thbs2	Thbs4
	Gfpt2	Pla1a	Gpm6b	Penk	Gadd45g	Pdgfrl	Cilp
	Tnfaip6	Uap1	Ces1d	Steap4	Hk2	Eln	Prg4
	Sema3c	Pcolce2	Spry1	Apod	Cebpd	Igfbp3	Dio2
	Ptgs1	Fbn1	Dnajb1	Ndufa3	Atf3	Gas1	Aspn
	Efemp1	Pcsk6	Prss23	Fmo2	Tnfaip6	Nbl1	Fibin
	Fn1	Tmem100	Itih5	C3	Id3	Sfrp4	Ltbp2
	Anxa3	Crip1	Crispld2	Gpx3	Bhlhe40	Ntrk2	C1qtnf3
	Dpp4	Mustn1	Apod	Mgp	Sat1	Fxyd6	F2r
	Smpd3	Efemp1	Plau	Nrp1	Myc	Col12a1	Mfap4
	Pi16	Ifi27l2a	Hspg2	Fst	Junb	Lox	Cfh
	Efhd1	Myoc	Col6a3	Cxcl12	Ccnl1	Col1a1	Pmepa1
	Thbd	Ppp1r14b	Hspa1a	Sec61g	Cxcl1	Itgbl1	Sfrp4
	Cd248	Ecm1	Lifr	Spcs1	Egr1	Tcf7l2	Ccdc3
	Has1	Ackr3	Smoc2	Serpina3n	Btg2	Col14a1	Bgn
	Uap1	Actg1	Kcnk2	Inmt	Cebpb	Col8a1	Pam
	Pla1a	Axl	Adm	F3	Fos	Cpe	Meox1
	Emilin2	Anxa2	Rgmb	Cp	Nr4a1	Col3a1	Cd9
	Mt2	Efhd1	Vwa1	Fth1	Ier3	Slit2	Lgals1
	Ifi27l2a	Creb5	Col4a1	Nop10	Ptgs2	Svil	Col12a1

Table S6. Top 25 differentially expressed genes of individual FAP clusters in *mdx*^{5cv} quadriceps, related to Figure 5.

Cluster name	Ly6c1^{hi}Pi16^{hi}	Hsd11b1	Gdf10	pro-inflammatory	Acta2	Postn	Gm42418
Top 25 DEGs	Pi16	Eln	Clu	Mt1	Hmgb2	Postn	Gm42418
	Sema3c	Hmcn2	Comp	Mt2	Stmn1	Tnc	Cd74
	Igfbp5	Htra3	Myoc	Ccl7	H2afz	Cthrc1	Tm6sf1
	Anxa3	Col6a6	Apod	Ccl2	S100a4	Csrp2	Il1rl1
	Smpd3	Abca8a	Prg4	Ptx3	Top2a	Col8a1	Laptm5
	Pcolce2	Col15a1	Cfh	Timp1	Cenpa	Mest	Rbms3
	Ackr3	Mmp2	Cyp2f2	Fgl2	Cks2	Sfrp2	Rplp2
	Pla1a	Angptl1	Meox2	Ifi205	Birc5	C1qtnf3	Chchd2
	C4b	Lpl	Boc	Thbs1	Pclaf	Col1a1	Med13
	Efemp1	Itih5	Penk	Cxcl5	Ube2c	Aspn	Stau1
	Plpp3	Lum	Gdf10	Cxcl1	Cenpf	Ltbp2	Snhg8
	Dpp4	Smoc2	Serpine2	Serpine1	Ccnb2	Col12a1	Psmd1
	Efhd1	Tnxb	Cpxm2	Errfi1	Ccna2	Bgn	Pcm1
	Thbd	Fbln7	Cst3	Iigp1	Mki67	Col16a1	Cdc37
	C3	Col4a1	Ntrk2	Sdc4	Cdk1	Ptn	Cnot1
	Procr	Abca8b	Scara3	Ifi203	Cdca3	Ccn4	Pgls
	Cd248	Crispld2	Cdh11	Mcoln2	Acta2	H19	Arpc4
	Ly6c1	Rora	Kctd12	Fst	Sfrp2	Lgals1	Nifk
	Cxcl13	Hsd11b1	Nbl1	Hk2	Cdc20	Acta2	Pdha1
	Pcsk6	Dpep1	Crispld1	Il33	Ltbp2	Fn1	Dnajb6
	Tmem100	Clec3b	Colec12	Pdpn	Anln	Adam12	Rap2b
	Adgrd1	Nid2	Pik3r1	Noct	Hmmr	Thbs4	Dync1i2
	Fn1	Dcn	Etv1	Prg4	Pcna	Dclk1	Cct5
	Emilin2	Tgfbi	Meox1	Cd44	Hmgb1	Palld	9530068E07Rik
	Gfpt2	Ifi30	Tmem176a	Cxcl14	Fabp5	Tagln	Myl6

Table S7. Top 25 differentially expressed genes of individual FAP clusters in *mdx^{5cv}* diaphragm, related to Figure 5.

Cluster name	Ly6c1^{hi}Pi16^{hi}	pro-inflammatory	Sfrp2	Postn	Stressed	IFN activated
Top 25 DEGs	Ly6c1	Ccl2	Sfrp2	Postn	Apod	Ifit3
	Cd248	Ccl7	Eln	H19	C3	Isg15
	Pi16	Timp1	Mfap4	Cthrc1	Inmt	Ifit1
	Tmem100	Thbs1	Gas1	C1qtnf3	Col6a5	Iigp1
	Sema3c	Inhba	Cfh	Col1a1	Junb	Ifit3b
	Fn1	Tm4sf1	Sfrp1	Meg3	Egr1	Cxcl9
	Pcolce2	Tnc	Cst3	Thbs4	Hspa1a	Rsad2
	Igfbp5	Aldh1a3	Itgbl1	Rian	Fos	Oasl2
	Klf2	Serpine1	Pdgfrl	Col3a1	Hspa1b	Igtp
	Ugdh	Pdpn	Pcsk5	Col6a3	Gpx3	Bst2
	Klf4	Mt2	Cpxm2	Nrep	Cebpd	Gbp2
	Ackr3	Prg4	Cilp	Mest	Dpep1	Gm4951
	Adgrd1	Ran	Angptl1	Col8a1	Gsn	Ifi203
	Cd55	Nme1	Ccdc80	Dlk1	Crispld2	Rnf213
	Scara5	Npm1	Prelp	Col5a2	Penk	AW112010
	Efemp1	Slc25a5	Fibin	Aspn	Gstm1	Gbp7
	Gfpt2	Ncl	Mgp	Col1a2	Jun	Ly6e
	Emilin2	Cxcl14	Ogn	Plagl1	Htra3	Phf11d
	Uap1	Eif5a	Tgfb3	Fn1	Txnip	Sp100
	Tppp3	Angptl4	Ccn2	Col5a1	Btg2	Ifi47
	C3	Mif	Mmp2	Ppic	Ggt5	Gbp3
	Hspb1	Itga5	Selenop	Vcan	Abca8a	Rtp4
	Igfbp6	Mt1	Gas6	Cdkn1c	Klf9	Stat1
	Plpp3	Hspd1	Fbln7	Ltbp2	Lpl	Mndal
	Pla1a	Actg1	Myoc	Bgn	Steap4	Trim30a