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Supplementary Materials for

Integrative molecular roadmap for direct conversion of fibroblasts into myocytes and myogenic progenitor cells

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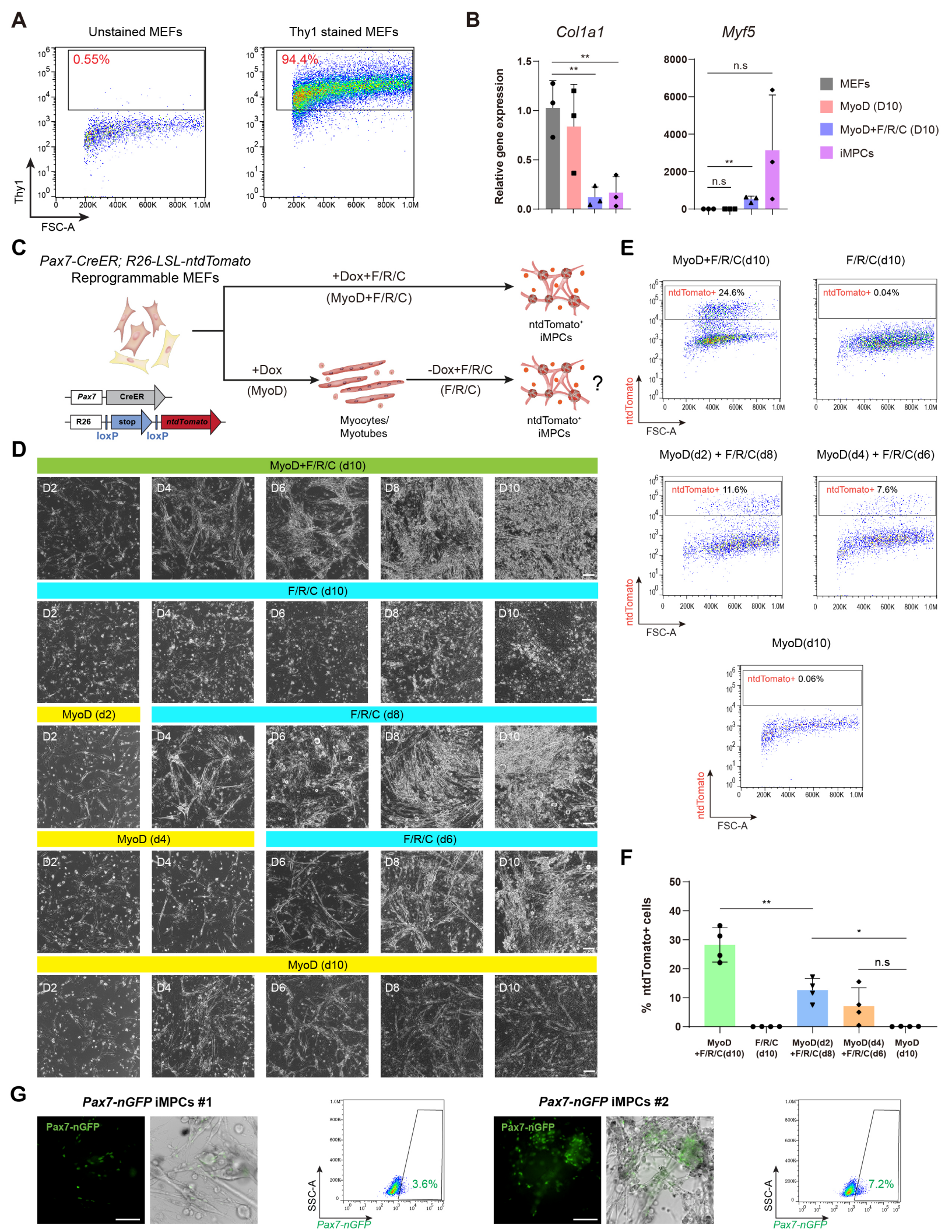
A
Unstained MEFs
Thy1 stained MEFs
0.55%
94.4%
Thy1
FSC-A
B
Col1a1
Myf5
Relative gene expression
n.s
MEFs
MyoD (D10)
MyoD+F/R/C (D10)
iMPCs
C
Pax7-CreER; R26-LSL-ntdTomato
Reprogrammable MEFs
+Dox+F/R/C
(MyoD+F/R/C)
ntdTomato+ iMPCs
+Dox
(MyoD)
Myocytes/ Myotubes
-Dox+F/R/C
(F/R/C)
ntdTomato+ iMPCs
Pax7
CreER
R26
stop
ntdTomato
loxP
loxP
D
MyoD+F/R/C (d10)
F/R/C (d10)
MyoD (d2)
F/R/C (d8)
MyoD (d4)
F/R/C (d6)
MyoD (d10)
D2
D4
D6
D8
D10
E
MyoD+F/R/C(d10)
ntdTomato+ 24.6%
F/R/C(d10)
ntdTomato+ 0.04%
MyoD(d2) + F/R/C(d8)
ntdTomato+ 11.6%
MyoD(d4) + F/R/C(d6)
ntdTomato+ 7.6%
MyoD(d10)
ntdTomato+ 0.06%
ntdTomato
FSC-A
F
% ntdTomato+ cells
MyoD +F/R/C(d10)
F/R/C (d10)
MyoD(d2) +F/R/C(d8)
MyoD(d4) +F/R/C(d6)
MyoD (d10)
G
Pax7-nGFP iMPCs #1
Pax7-nGFP
SSC-A
3.6%
Pax7-nGFP
Pax7-nGFP iMPCs #2
Pax7-nGFP
7.2%
Pax7-nGFP

Fig. S1: MyoD and small molecules convert fibroblasts into iMPCs

(A) Flow cytometry analysis of MEF cultures stained for the fibroblast-specific surface marker Thy1. MEFs, mouse embryonic fibroblasts. **(B)** qRT-PCR analysis for *Col1a1* and *Myf5*. Data is shown as mean ± S.D. N=3 cell lines per each group. Statistical significance was determined by a two-tailed unpaired *t*-test (**p<0.01, n.s=non-significant). **(C)** Experimental design to investigate whether transient MyoD overexpression followed by F/R/C supplementation can reprogram cells into iMPCs. **(D)** Representative bright-field images of *Pax7-CreER; Rosa26-LoxSTOPLox(LSL)-ntdTomato* MEFs subjected to the indicated conditions. Scale bar, 100 μm. **(E)** Flow cytometry analysis for ntdTomato$^+$ cells in *Pax7-CreER; Rosa26-LSL-ntdTomato* MEFs subjected to the indicated reprogramming conditions. A 4-Hydroxytamoxifen (4OHT) was added to the cells two days before the FACS analysis. **(F)** Bar graph showing the percentage of ntdTomato$^+$ cells for the indicated conditions as demonstrated in Fig. S1E. The data is shown as mean ± S.D. N=2 experimental repeats for 2 cell lines per each group. Statistical significance was determined by one-way ANOVA between conditions (*p<0.05, **p<0.01, n.s=non-significant). **(G)** Representative images and flow cytometry analysis of different *Pax7-nGFP* iMPC clones at passage 1. Note nuclear GFP expression only in mononucleated cells. Scale bar, 100 μm.

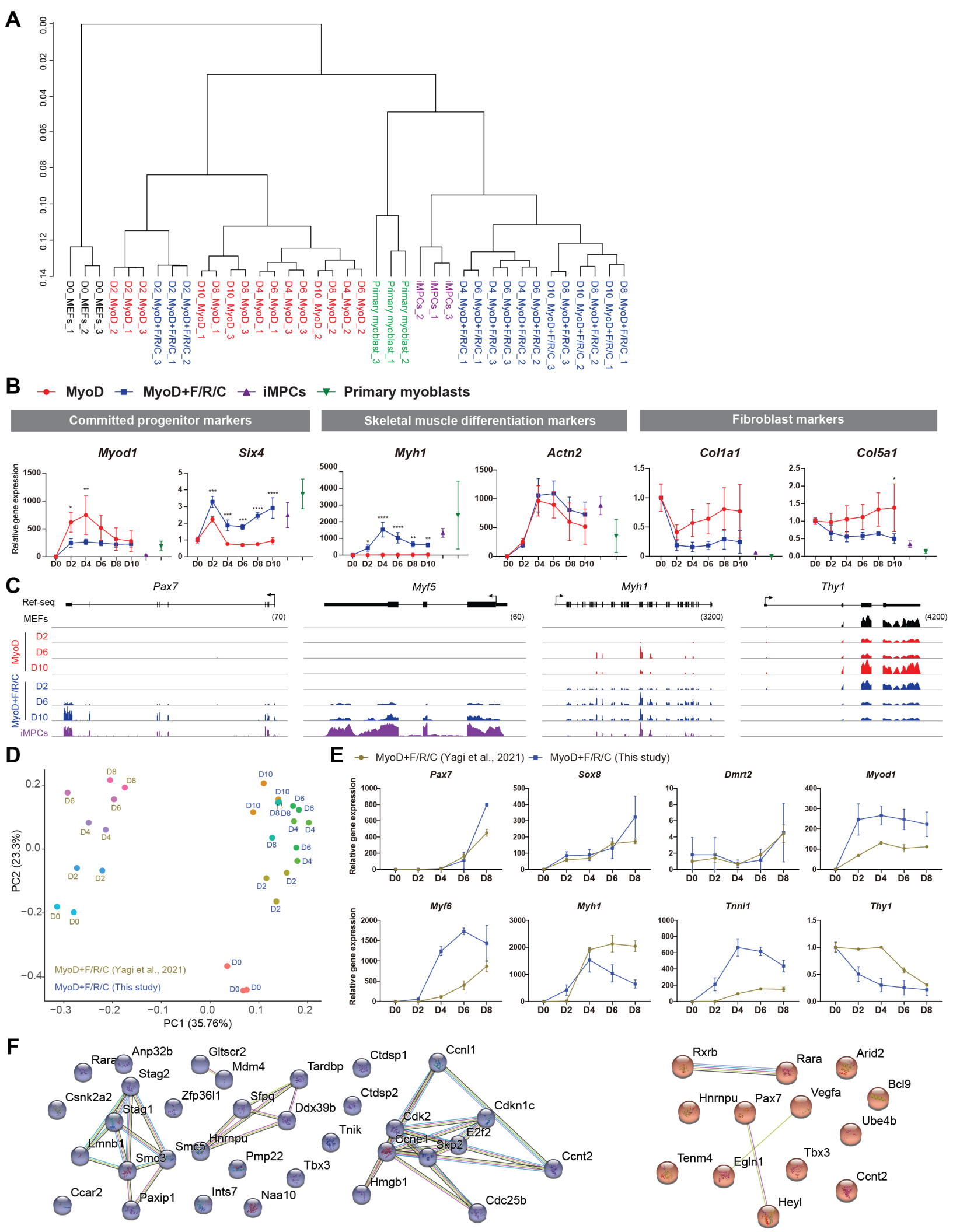
A
B
MyoD
MyoD+F/R/C
iMPCs
Primary myoblasts
Committed progenitor markers
Skeletal muscle differentiation markers
Fibroblast markers
Myod1
Six4
Myh1
Actn2
Col1a1
Col5a1
Relative gene expression
C
Pax7
Myf5
Myh1
Thy1
Ref-seq
MEFs
MyoD
MyoD+F/R/C
iMPCs
D
PC1 (35.76%)
PC2 (23.3%)
MyoD+F/R/C (Yagi et al., 2021)
MyoD+F/R/C (This study)
E
MyoD+F/R/C (Yagi et al., 2021)
MyoD+F/R/C (This study)
Pax7
Sox8
Dmrt2
Myod1
Myf6
Myh1
Tnni1
Thy1
Relative gene expression
F
Rara
Anp32b
Stag2
Csnk2a2
Stag1
Lmnb1
Smc3
Ccar2
Paxip1
Gltscr2
Mdm4
Zfp36l1
Sfpq
Tardbp
Ddx39b
Hnrnpu
Smc5
Pmp22
Tbx3
Ints7
Naa10
Ctdsp1
Ctdsp2
Ccnl1
Cdkn1c
Cdk2
Tnik
Ccne1
E2f2
Skp2
Ccnt2
Hmgb1
Cdc25b
Rxrb
Rara
Arid2
Bcl9
Hnrnpu
Pax7
Vegfa
Ube4b
Tenm4
Egln1
Tbx3
Ccnt2
Heyl

Fig. S2: Gene expression changes during myogenic lineage conversions
(A) Dendrogram clustering based on bulk RNA-Seq data using all gene read counts for the indicated samples. N = 3 cell lines per each group. **(B)** Gene expression analysis based on bulk RNA-Seq for the listed genes in MEFs subjected to either MyoD or MyoD+F/R/C treatment. Established iMPCs and primary myoblasts served as positive controls. Relative gene expression was calculated by normalizing the RPKM values of each sample to that of parental MEFs (D0 MEFs). The data is shown as mean ± S.D. N = 3 cell lines per each group. Statistical significance was determined by two-way ANOVA between conditions at each time point (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). **(C)** Representative Integrative Genomic Viewer (IGV) tracks for the indicated genes, conditions and time points. **(D)** PCA based on bulk RNA-seq data for this study in comparison to Yagi et al., 2021 using log normalized gene counts. **(E)** Gene expression analysis during a reprogramming time course for the indicated genes as performed by Yagi et al., 2021 and this study. **(F)** Enriched network analysis using the STRING database for the genes shown in gene set 2 (MyoD+F/R/C treatment, Fig. 2E) that annotated with *Regulation of cell cycle* (left) and *Striated muscle tissue development* (right).

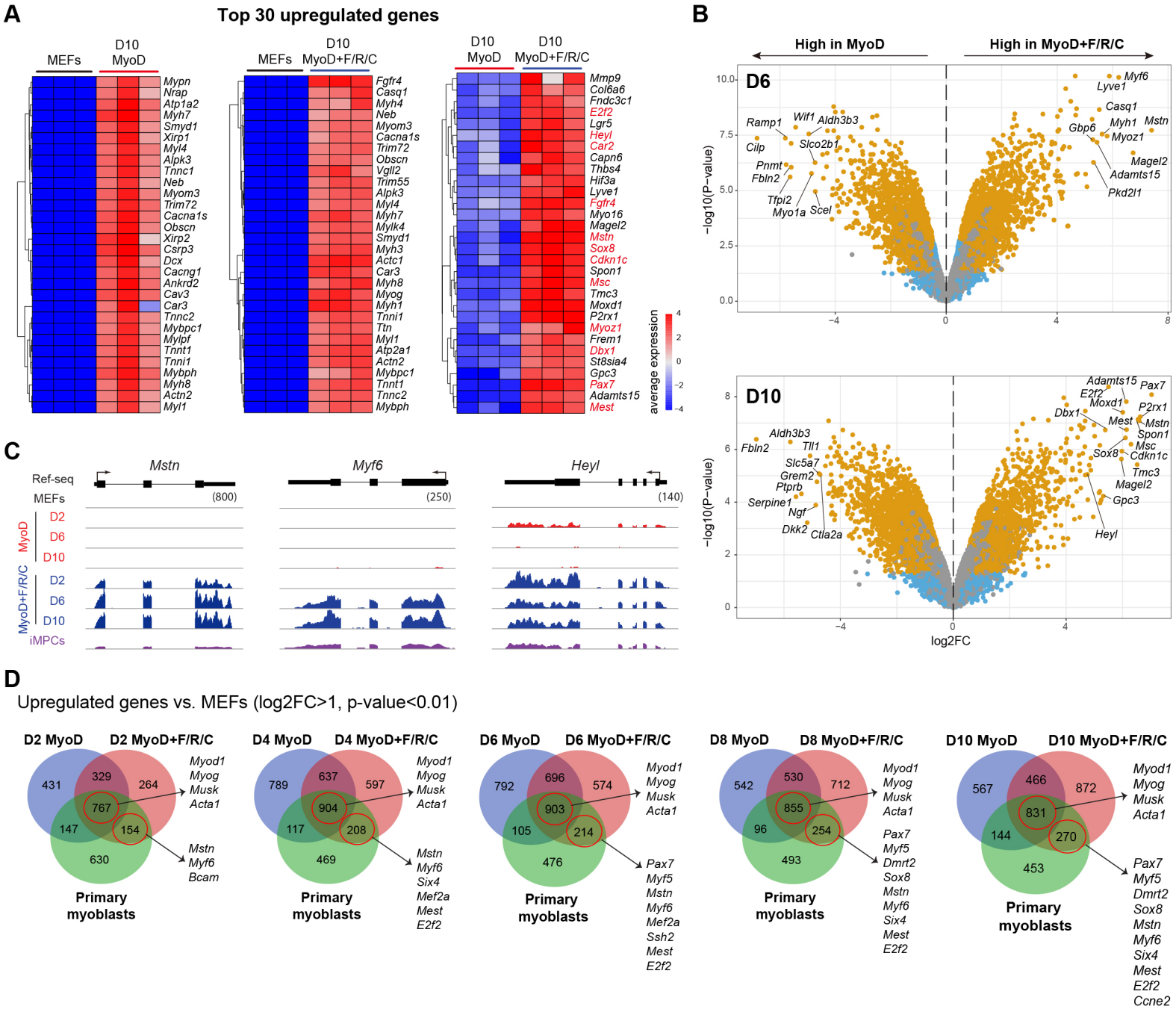
A
Top 30 upregulated genes
MEFs
D10 MyoD
D10 MyoD+F/R/C
Mypn Nrap Atp1a2 Myh7 Smyd1 Xirp1 Myl4 Alpk3 Tnnc1 Neb Myom3 Trim72 Cacna1s Obscn Xirp2 Csrp3 Dcx Cacng1 Ankrd2 Cav3 Car3 Tnnc2 Mybpc1 Mylpf Tnnt1 Tnni1 Mybph Myh8 Actn2 Myl1
Fgfr4 Casq1 Myh4 Neb Myom3 Cacna1s Trim72 Obscn Vgll2 Trim55 Alpk3 Myl4 Myh7 Mylk4 Smyd1 Myh3 Actc1 Car3 Myh8 Myog Myh1 Tnni1 Ttn Myl1 Atp2a1 Actn2 Mybpc1 Tnnt1 Tnnc2 Mybph
Mmp9 Col6a6 Fndc3c1 E2f2 Lgr5 Heyl Car2 Capn6 Thbs4 Hif3a Lyve1 Fgfr4 Myo16 Magel2 Mstn Sox8 Cdkn1c Spon1 Msc Tmc3 Moxd1 P2rx1 Myoz1 Frem1 Dbx1 St8sia4 Gpc3 Pax7 Adamts15 Mest
average expression
4 2 0 −2 −4
B
High in MyoD
High in MyoD+F/R/C
D6
−log10(P−value)
Myf6 Lyve1 Casq1 Myh1 Mstn Myoz1 Gbp6 Magel2 Adamts15 Pkd2l1
Ramp1 Wif1 Aldh3b3 Cilp Slco2b1 Pnmt Fbln2 Tfpi2 Myo1a Scel
D10
Adamts15 Pax7 E2f2 Moxd1 P2rx1 Dbx1 Mest Mstn Spon1 Msc Sox8 Cdkn1c Tmc3 Magel2 Gpc3 Heyl
Aldh3b3 Fbln2 Tll1 Slc5a7 Grem2 Ptprb Serpine1 Ngf Dkk2 Ctla2a
log2FC
C
Mstn
Myf6
Heyl
Ref-seq
MEFs
(800)
(250)
(140)
MyoD
D2
D6
D10
MyoD+F/R/C
D2
D6
D10
iMPCs
D
Upregulated genes vs. MEFs (log2FC>1, p-value<0.01)
D2 MyoD
D2 MyoD+F/R/C
431 329 264 767 147 154 630
Myod1 Myog Musk Acta1
Mstn Myf6 Bcam
Primary myoblasts
D4 MyoD
D4 MyoD+F/R/C
789 637 597 904 117 208 469
Myod1 Myog Musk Acta1
Mstn Myf6 Six4 Mef2a Mest E2f2
Primary myoblasts
D6 MyoD
D6 MyoD+F/R/C
792 696 574 903 105 214 476
Myod1 Myog Musk Acta1
Pax7 Myf5 Mstn Myf6 Mef2a Ssh2 Mest E2f2
Primary myoblasts
D8 MyoD
D8 MyoD+F/R/C
542 530 712 855 96 254 493
Myod1 Myog Musk Acta1
Pax7 Myf5 Dmrt2 Sox8 Mstn Myf6 Six4 Mest E2f2
Primary myoblasts
D10 MyoD
D10 MyoD+F/R/C
567 466 872 831 144 270 453
Myod1 Myog Musk Acta1
Pax7 Myf5 Dmrt2 Sox8 Mstn Myf6 Six4 Mest E2f2 Ccne2
Primary myoblasts

Fig. S3: Transcriptional differences between myogenic cell conversions

(A) Heatmaps based on bulk RNA-Seq data displaying the top 30 upregulated genes for the indicated cell lines. The average expression is presented as gradient. N = 3 cell lines per group. Unique myogenic genes are highlighted in red color. **(B)** Volcano plots based on bulk RNA-Seq data for differentially expressed genes (DEGs) between MyoD and MyoD+F/R/C conditions at days 6 and 10. Significant DEGs (|log2FC|>0.5, p-value<0.05) are shown as yellow dots. N = 3 cell lines per each group. **(C)** IGV tracks for selected myogenic genes. **(D)** Venn diagrams based on bulk RNA-Seq data showing the overlap of upregulated genes in primary myoblasts and MEFs subjected to either MyoD or MyoD+F/R/C conditions at each time point vs. parental MEFs (log2FC>1, p-value<0.01). N = 3 cell lines per each group.

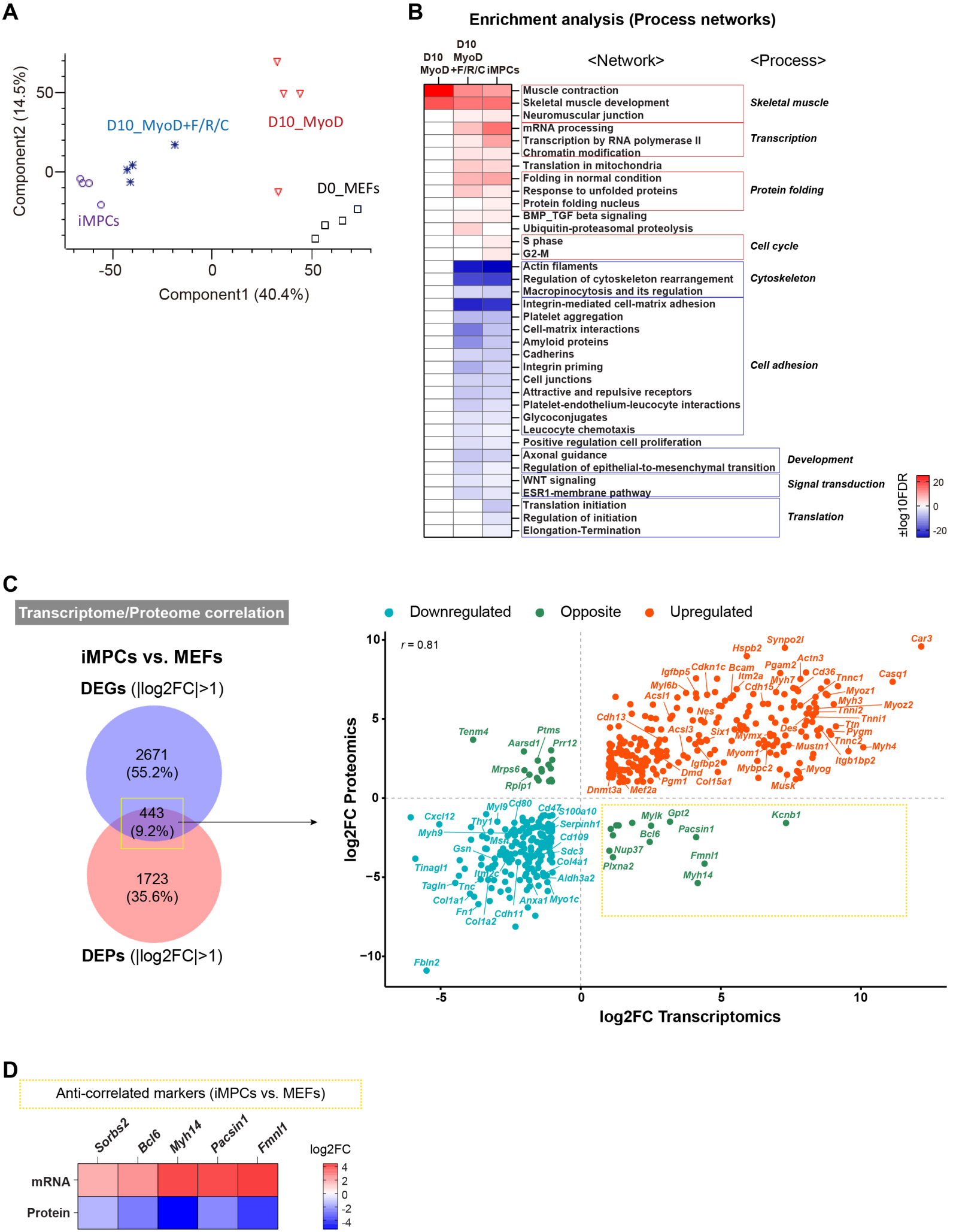
A
Component2 (14.5%)
Component1 (40.4%)
D10_MyoD+F/R/C
D10_MyoD
D0_MEFs
iMPCs
B
Enrichment analysis (Process networks)
D10 MyoD
D10 MyoD+F/R/C
iMPCs
<Network>
<Process>
Muscle contraction
Skeletal muscle development
Neuromuscular junction
Skeletal muscle
mRNA processing
Transcription by RNA polymerase II
Chromatin modification
Transcription
Translation in mitochondria
Folding in normal condition
Response to unfolded proteins
Protein folding nucleus
Protein folding
BMP_TGF beta signaling
Ubiquitin-proteasomal proteolysis
S phase
G2-M
Cell cycle
Actin filaments
Regulation of cytoskeleton rearrangement
Macropinocytosis and its regulation
Cytoskeleton
Integrin-mediated cell-matrix adhesion
Platelet aggregation
Cell-matrix interactions
Amyloid proteins
Cadherins
Integrin priming
Cell junctions
Attractive and repulsive receptors
Platelet-endothelium-leucocyte interactions
Glycoconjugates
Leucocyte chemotaxis
Cell adhesion
Positive regulation cell proliferation
Axonal guidance
Regulation of epithelial-to-mesenchymal transition
Development
WNT signaling
ESR1-membrane pathway
Signal transduction
Translation initiation
Regulation of initiation
Elongation-Termination
Translation
±log10FDR
C
Transcriptome/Proteome correlation
iMPCs vs. MEFs
DEGs (|log2FC|>1)
2671 (55.2%)
443 (9.2%)
1723 (35.6%)
DEPs (|log2FC|>1)
Downregulated
Opposite
Upregulated
r = 0.81
log2FC Proteomics
log2FC Transcriptomics
D
Anti-correlated markers (iMPCs vs. MEFs)
Sorbs2
Bcl6
Myh14
Pacsin1
Fmnl1
mRNA
Protein
log2FC

Fig. S4: Proteomic analysis during myogenic lineage conversions
(A) PCA of global protein expression based on LC-MS data across all samples. N = 4 cell lines per each group. **(B)** Enriched process networks of DEPs in each condition vs. MEFs (|log2FC|>1, p-value<0.05) as analyzed via Metacore. Only significantly enriched process networks are presented (FDR<0.05). Upregulated (Red) and downregulated (Blue) process networks are shown using -log10(FDR) and log10(FDR), respectively. **(C)** Left - A Venn diagram based on transcriptome and proteome datasets showing the number of significant overlapping DEGs and DEPs in iMPCs vs. MEFs (|log2FC|>1, p-value<0.05). Right - scatterplot showing the correlation between the transcriptome and proteome datasets of iMPCs vs. MEFs. In total, 443 overlapping DEGs / DEPs are projected on the plot, corresponding to the Venn diagram analysis shown on the left. Red and blue dots indicate upregulated and downregulated genes or proteins, respectively. **(D)** Heatmap showing mRNA and protein expression levels (log2FC) between iMPCs and MEFs for the indicated genes. Log2FC is presented as a color gradient. Note an anti-correlation trend between gene and protein expression.

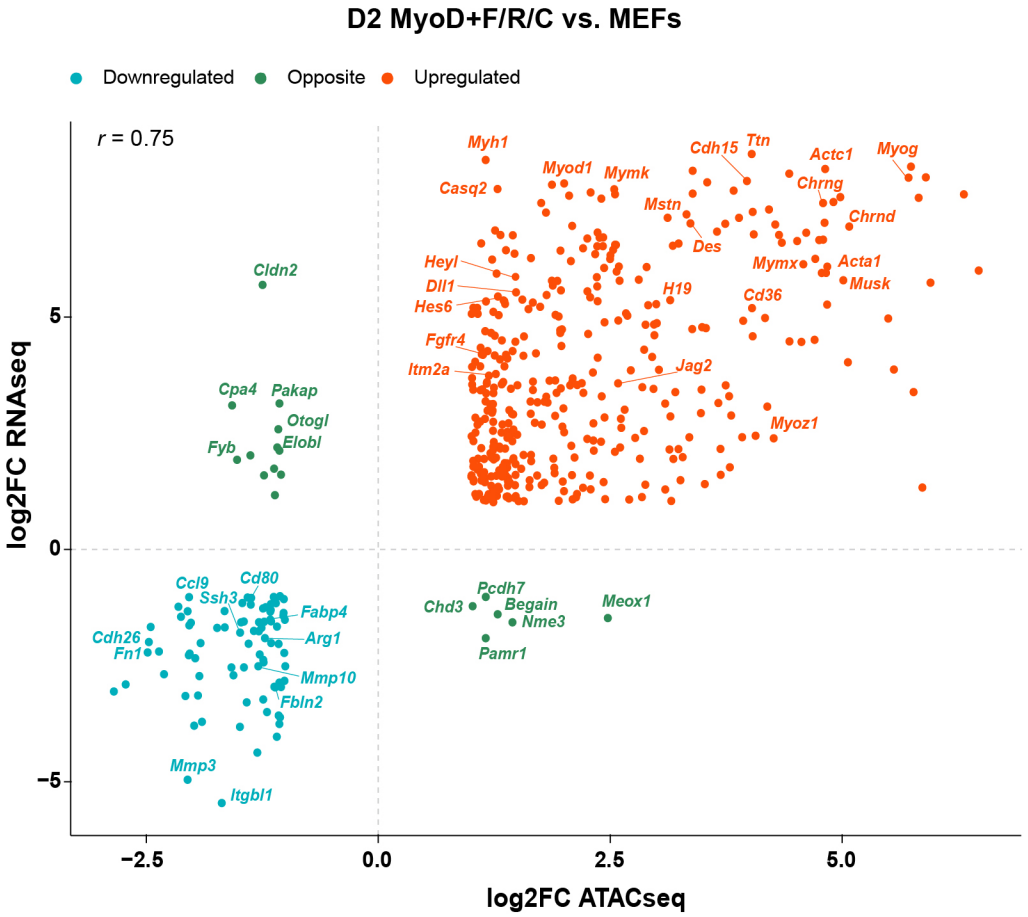
D2 MyoD+F/R/C vs. MEFs
Downregulated
Opposite
Upregulated
r = 0.75
log2FC RNAseq
log2FC ATACseq
5
0
−5
−2.5
0.0
2.5
5.0
Myh1
Myod1
Mymk
Casq2
Cdh15
Ttn
Actc1
Myog
Chrng
Mstn
Chrnd
Des
Heyl
Dll1
Hes6
Mymx
Acta1
Musk
H19
Cd36
Fgfr4
Itm2a
Jag2
Myoz1
Cldn2
Cpa4
Pakap
Otogl
Elobl
Fyb
Ccl9
Cd80
Ssh3
Fabp4
Arg1
Cdh26
Fn1
Mmp10
Fbln2
Mmp3
Itgbl1
Pcdh7
Chd3
Begain
Nme3
Meox1
Pamr1

Fig. S5: Integrative gene expression and ATAC-Seq analysis

Scatterplot for overlapping genes showing the correlation between chromatin accessibility in promoter regions (±1kb of TSS) and gene expression in day 2 of MyoD+F/R/C condition vs. MEFs. The significant genes (p-value < 0.01) with |log2FC| > 0.5 are shown. Note rare anti-correlation trend between gene expression and chromatin accessibility for a few markers.

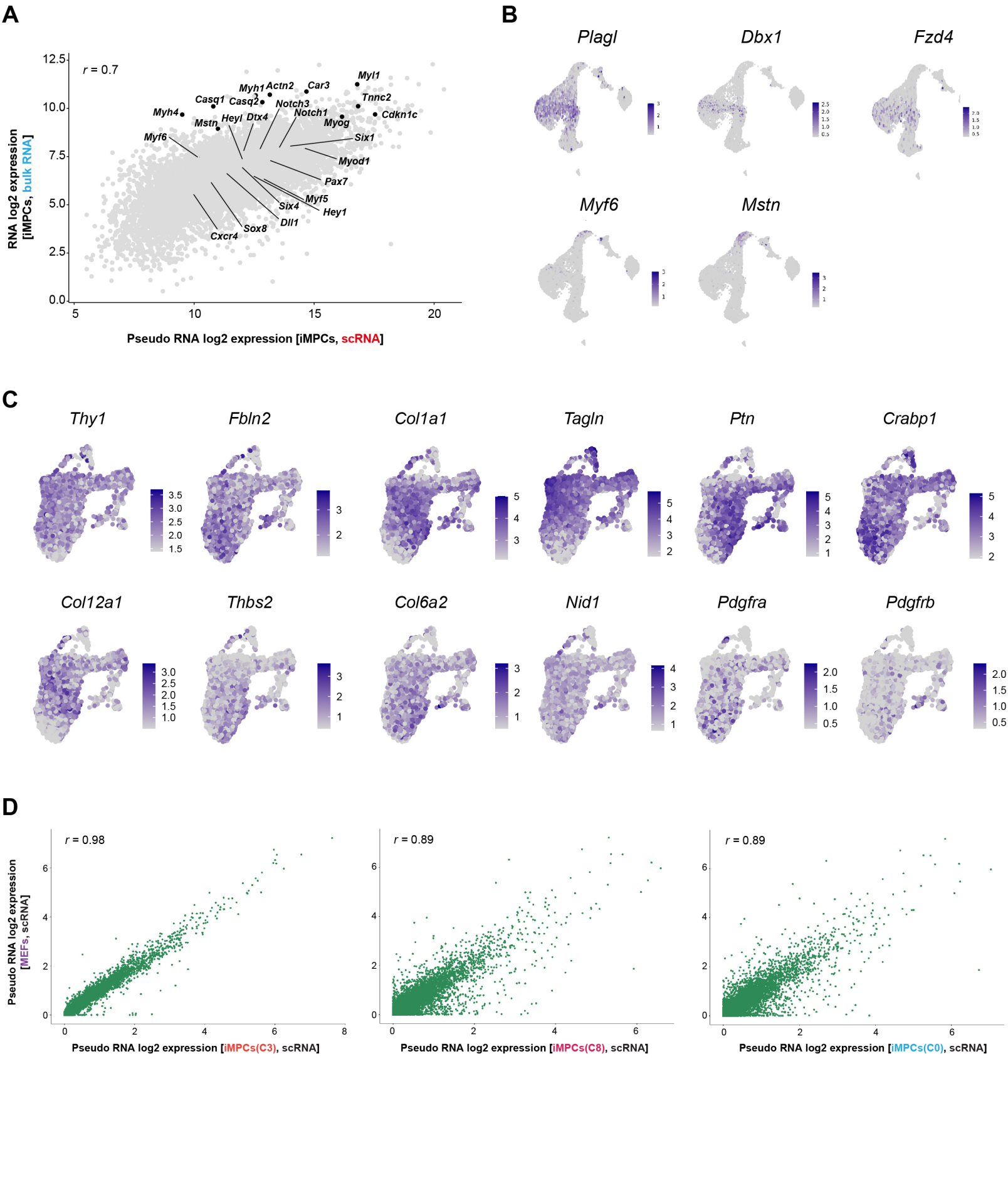
A
r = 0.7
RNA log2 expression [iMPCs, bulk RNA]
Pseudo RNA log2 expression [iMPCs, scRNA]
Myl1
Tnnc2
Cdkn1c
Car3
Actn2
Myh1
Casq1
Casq2
Notch3
Notch1
Myh4
Mstn
Heyl
Dtx4
Myog
Myf6
Six1
Myod1
Pax7
Myf5
Six4
Hey1
Dll1
Sox8
Cxcr4
B
Plagl
Dbx1
Fzd4
Myf6
Mstn
C
Thy1
Fbln2
Col1a1
Tagln
Ptn
Crabp1
Col12a1
Thbs2
Col6a2
Nid1
Pdgfra
Pdgfrb
D
r = 0.98
r = 0.89
r = 0.89
Pseudo RNA log2 expression [MEFs, scRNA]
Pseudo RNA log2 expression [iMPCs(C3), scRNA]
Pseudo RNA log2 expression [iMPCs(C8), scRNA]
Pseudo RNA log2 expression [iMPCs(C0), scRNA]

Fig. S6: scRNA-Seq analysis of iMPCs and MEFs

(A) Scatter plot displaying gene expression correlation profile between scRNA-Seq and bulk RNA-Seq datasets for an iMPC clone. Pseudo bulk mRNA expression was computed from scRNA-Seq data. **(B)** UMAP projection showing all cells colored by the indicated myogenic markers based on scRNA-Seq data of a stable iMPC clone. **(C)** UMAP projection showing all cells colored by the indicated fibroblast markers based on scRNA-Seq data of MEFs. **(D)** Scatter plot displaying pseudo bulk RNA expression correlation profile between scRNA-Seq data of MEFs and each of the indicated connective tissue-like cell clusters present in iMPCs including C3 (left), C8 (middle) and C0 (right). The cluster identities correspond to the UMAP projection shown in Fig. 5A.

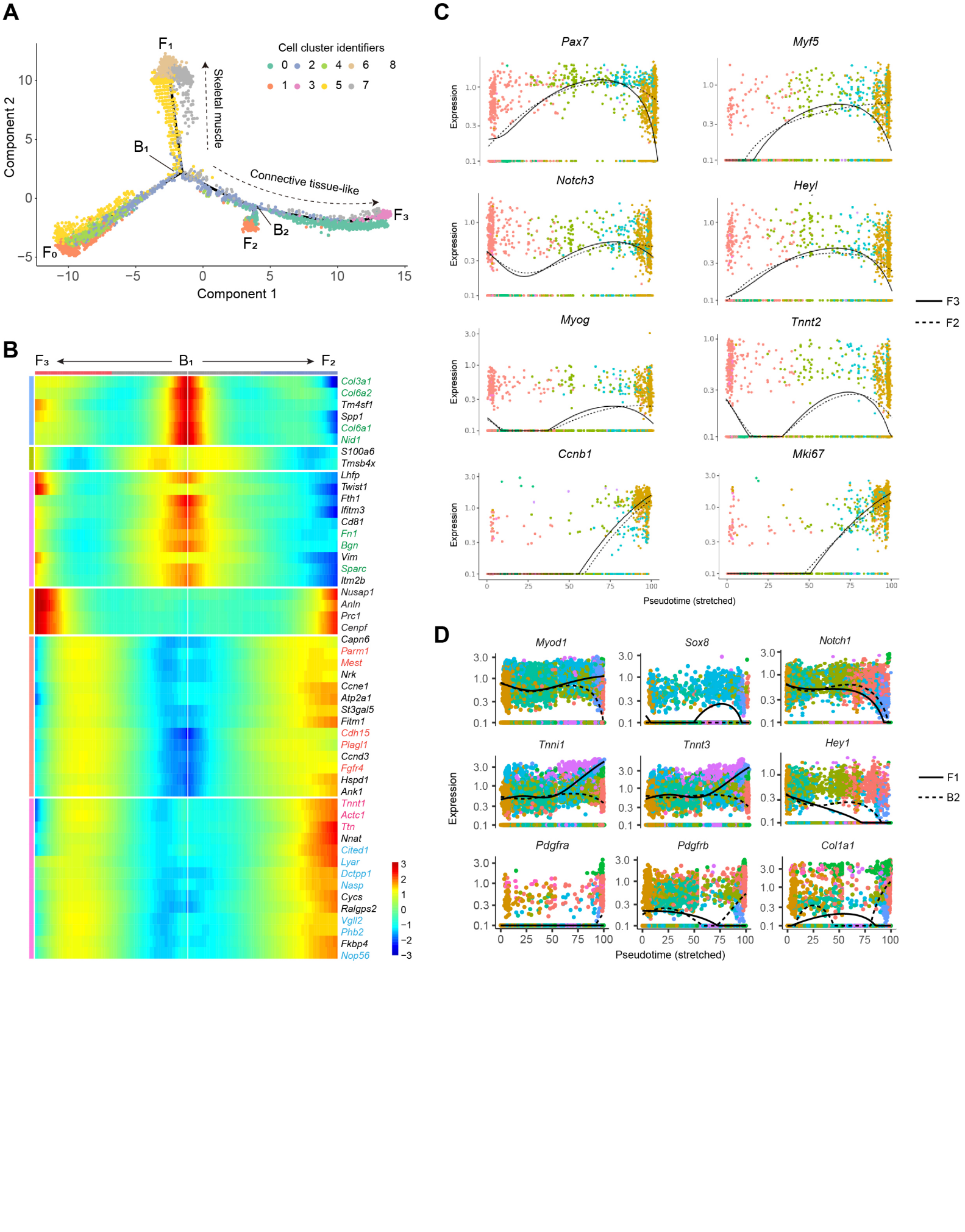
A
F1
Cell cluster identifiers
0 2 4 6 8
1 3 5 7
Skeletal muscle
B1
Connective tissue-like
F3
B2
F2
F0
Component 2
Component 1
B
F3
B1
F2
Col3a1
Col6a2
Tm4sf1
Spp1
Col6a1
Nid1
S100a6
Tmsb4x
Lhfp
Twist1
Fth1
Ifitm3
Cd81
Fn1
Bgn
Vim
Sparc
Itm2b
Nusap1
Anln
Prc1
Cenpf
Capn6
Parm1
Mest
Nrk
Ccne1
Atp2a1
St3gal5
Fitm1
Cdh15
Plagl1
Ccnd3
Fgfr4
Hspd1
Ank1
Tnnt1
Actc1
Ttn
Nnat
Cited1
Lyar
Dctpp1
Nasp
Cycs
Ralgps2
Vgll2
Phb2
Fkbp4
Nop56
C
Pax7
Myf5
Notch3
Heyl
Myog
Tnnt2
Ccnb1
Mki67
Expression
Pseudotime (stretched)
F3
F2
D
Myod1
Sox8
Notch1
Tnni1
Tnnt3
Hey1
Pdgfra
Pdgfrb
Col1a1
Expression
Pseudotime (stretched)
F1
B2

Fig. S7: Pseudotime trajectory analysis of an iMPC clone

(A) Minimum spanning tree showing ordered cells based on semi-supervised single cell trajectory analysis reconstructed by Monocle2 and colored by cell cluster identifiers. **(B)** Heatmap based on scRNA-Seq data of iMPCs for the top 50 DEGs regulated at branch point B_2 in Fig. 5F. The individual gene expression level initiating at point B_1 and proceeding towards F_3 is shown on the left, whereas for F_2 it is shown on the right. Color-code gradient denotes normalized gene expression level (Z-score) for each gene across all cells. **(C)** Plots showing the expression kinetics of the indicated genes regulated at B_2 as a function of pseudotime and proceeding towards F_2 or F_3. Dots indicate cells colored by cell identifiers. **(D)** Plots showing the gene expression dynamics of the indicated genes regulated at B_1 as a function of pseudotime and proceeding towards F_1 or B_2. The dots indicate cells colored by cell identifiers.

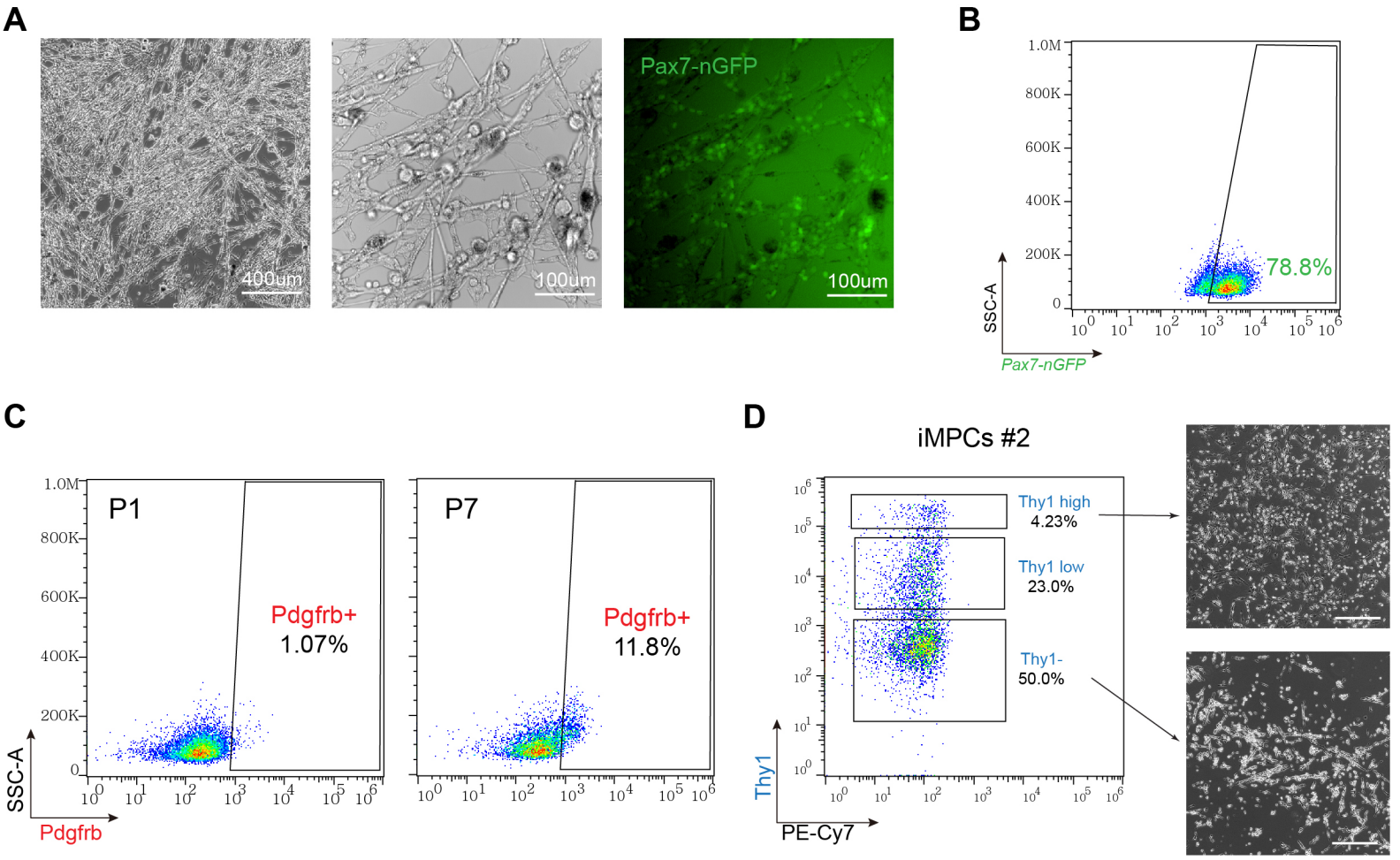
A
400um
100um
Pax7-nGFP
100um
B
1.0M
800K
600K
400K
200K
0
78.8%
SSC-A
Pax7-nGFP
C
P1
Pdgfrb+
1.07%
P7
Pdgfrb+
11.8%
SSC-A
Pdgfrb
D
iMPCs #2
Thy1 high
4.23%
Thy1 low
23.0%
Thy1-
50.0%
Thy1
PE-Cy7

Fig. S8: Formation of connective tissue-like cells in FACS-purified iMPCs

(A) Representative images of an iMPC clone which was derived from Pax7-nGFP$^+$ cells which were FACS-purified from an iMPC clone at passage 1. Note that Pax7-nGFP$^+$ cells can give rise to the entire heterogeneous iMPC culture. **(B)** Flow cytometry analysis for Pax7-GFP expression in an iMPC clone established from FACS-purified Pax7-nGFP$^+$ cells. **(C)** Flow cytometry analysis for Pdgfrb$^+$ cells at the respective passages in a stable iMPC clone established from FACS-purified Pax7-nGFP$^+$ cells. **(D)** Left - flow cytometry analysis for Thy1 expression in a stable iMPC clone. Right - representative bright-field images of cell cultures derived from FACS-purified Thy1 positive cells (top) and Thy1 negative cells (bottom) two days after FACS-purification. Scale bar, 400 µm.

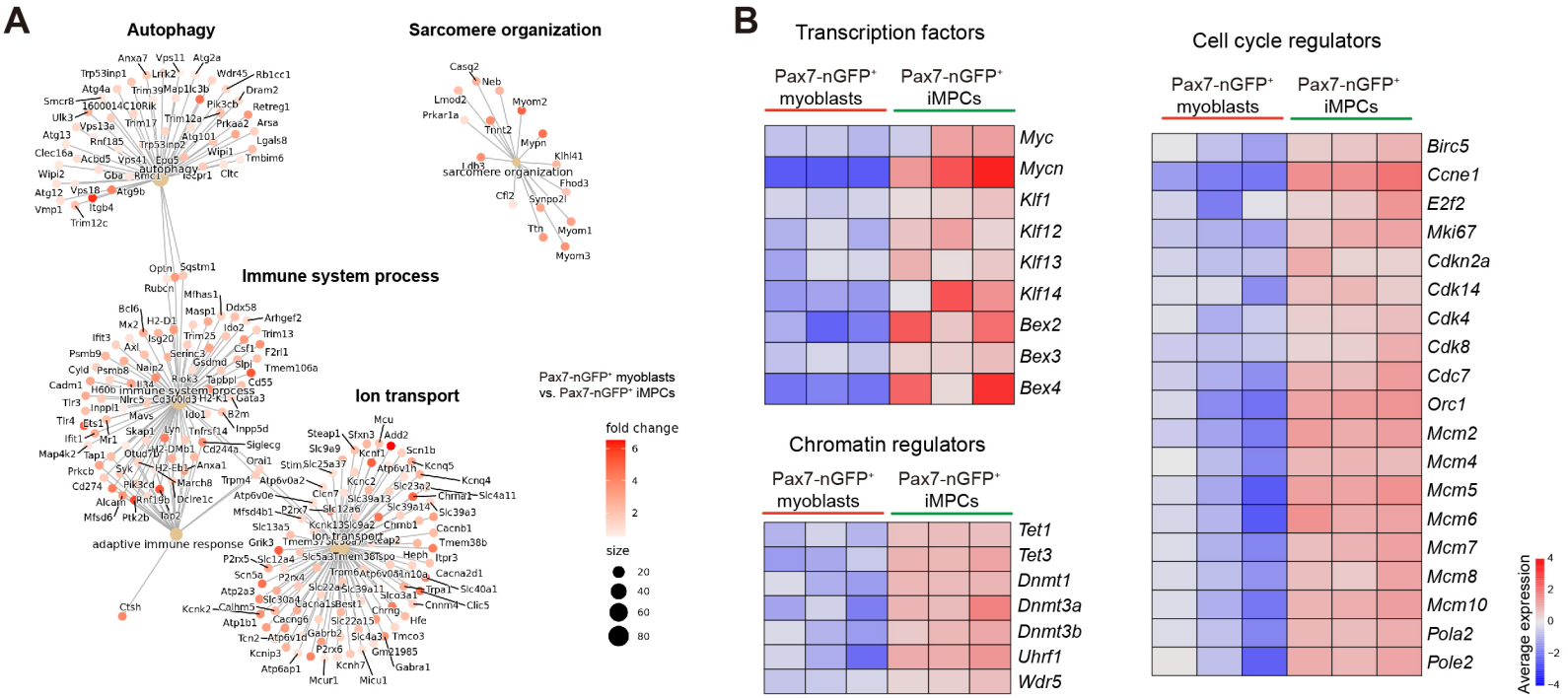
A
Autophagy
Sarcomere organization
Immune system process
Ion transport
Pax7-nGFP⁺ myoblasts
vs. Pax7-nGFP⁺ iMPCs
fold change
size
B
Transcription factors
Pax7-nGFP⁺ myoblasts
Pax7-nGFP⁺ iMPCs
Myc
Mycn
Klf1
Klf12
Klf13
Klf14
Bex2
Bex3
Bex4
Chromatin regulators
Tet1
Tet3
Dnmt1
Dnmt3a
Dnmt3b
Uhrf1
Wdr5
Cell cycle regulators
Birc5
Ccne1
E2f2
Mki67
Cdkn2a
Cdk14
Cdk4
Cdk8
Cdc7
Orc1
Mcm2
Mcm4
Mcm5
Mcm6
Mcm7
Mcm8
Mcm10
Pola2
Pole2
Average expression

Fig. S9: Molecular analysis of Pax7$^+$ iMPCs and Pax7$^+$ primary myoblasts

(A) Over Representation Analysis (ORA) of the gene sets that were significantly enriched (log2FC > 0.5, p-value < 0.01) in Pax7-nGFP$^+$ myoblasts in comparison to Pax7-nGFP$^+$ iMPCs. Only significant GO biological processes (adj. p-value < 0.05) are shown. The size indicates the number of genes involved in the biological process and the color-coding scale represents the fold change between the two conditions. **(B)** Heatmaps showing average expression of selected markers associated with the indicated gene families in Pax7-nGFP$^+$ iMPCs in comparison to Pax7-nGFP$^+$ myoblasts. Gradient of high to low expression of each gene relative to the average expression in each comparison is indicated by red to blue. N = 3 cell lines per each group.

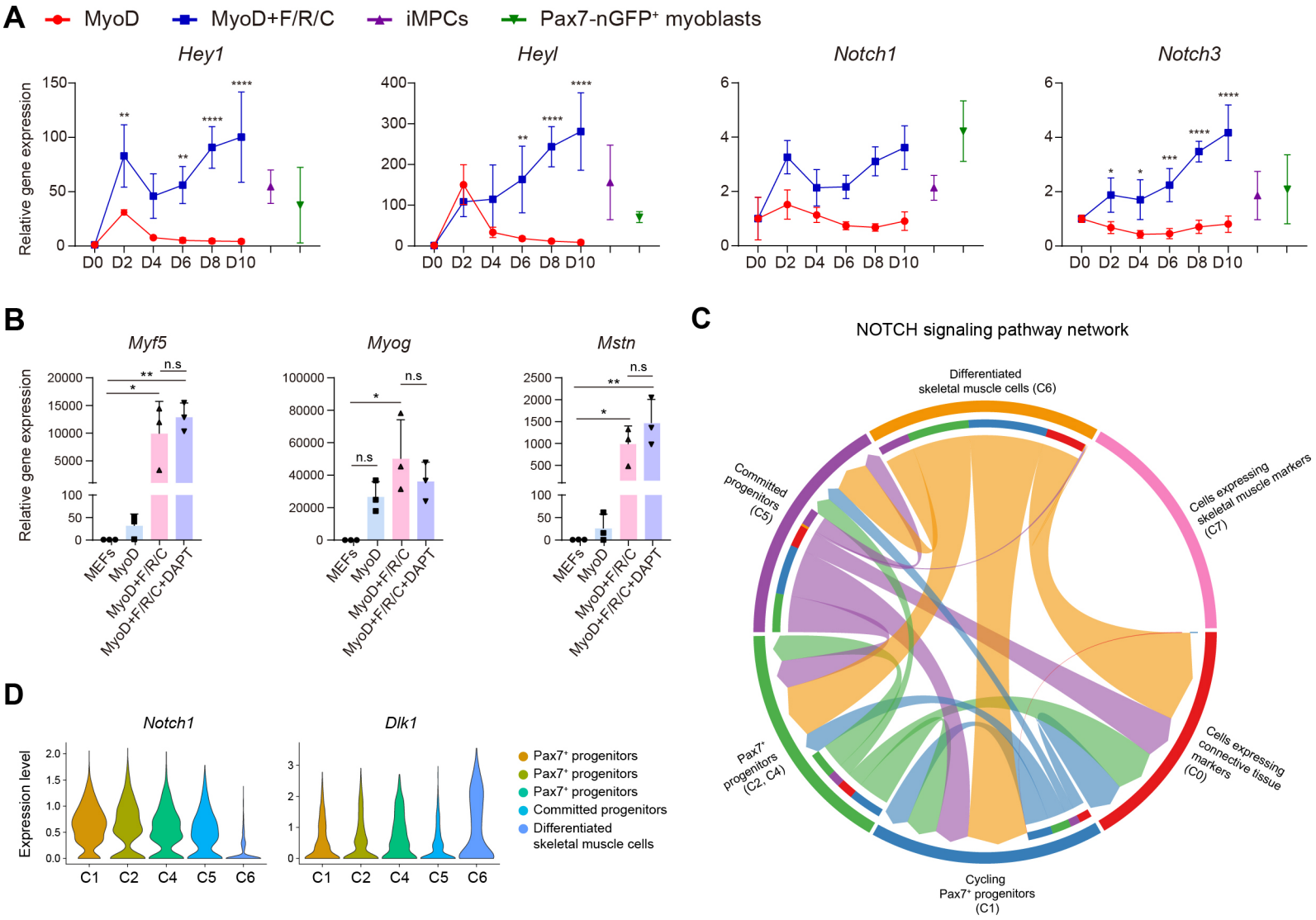
A
MyoD
MyoD+F/R/C
iMPCs
Pax7-nGFP+ myoblasts
Hey1
Heyl
Notch1
Notch3
Relative gene expression
D0 D2 D4 D6 D8 D10
B
Myf5
Myog
Mstn
Relative gene expression
n.s
MEFs
MyoD
MyoD+F/R/C
MyoD+F/R/C+DAPT
C
NOTCH signaling pathway network
Differentiated skeletal muscle cells (C6)
Committed progenitors (C5)
Cells expressing skeletal muscle markers (C7)
Pax7+ progenitors (C2, C4)
Cells expressing connective tissue markers (C0)
Cycling Pax7+ progenitors (C1)
D
Notch1
Dlk1
Expression level
C1 C2 C4 C5 C6
Pax7+ progenitors
Pax7+ progenitors
Pax7+ progenitors
Committed progenitors
Differentiated skeletal muscle cells

Fig. S10: Notch signaling pathway involvement during iMPC formation

(A) Expression dynamics based on bulk RNA-Seq data for the indicated Notch pathway associated genes during either MyoD or MyoD+F/R/C lineage conversions. Established iMPCs and Pax7-nGFP^{+} myoblasts served as positive controls. Relative gene expression was calculated by normalizing the RPKM values of each sample to that of parental MEFs (D0 MEFs). N = 3 cell lines per group. Statistical significance was determined by two-way ANOVA between the conditions at each respective time point (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). **(B)** Relative qRT-PCR gene expression analysis for the indicated myogenic genes at day 10 of MEF reprogramming using the indicated conditions. The data is shown as mean ± S.D. N = 3 cell lines per each group. Statistical significance was determined by one-way ANOVA (*p<0.05, **p<0.01, n.s=non-significant). **(C)** Chord diagram showing cell-cell interactions for the indicated cell types based on scRNA-Seq of a stable iMPC clone and using the Notch signaling pathway receptor-ligand network as reference. **(D)** Violin plots showing the average expression of *Notch1* and *Dlk1* in the respective cell clusters based on scRNA-Seq data of iMPCs. The clusters correspond to the cell populations shown in Fig. 5A.

Table S1. Sequences of the primers used for qRT-PCR

Gene	Direction	Sequence 5' -> 3'
Pax7	Forward	GACGACGAGGAAGGAGACAA
	Reverse	ACATCTGAGCCCTCATCCAG
Myf5	Forward	AAGGCTCCTGTATCCCCTCAC
	Reverse	TGACCTTCTTCAGGCGTCTAC
Myod1	Forward	CCACTCCGGGACATAGACTTG
	Reverse	AAAAGCGCAGGTCTGGTGAG
Myog	Forward	GAGACATCCCCCTATTTCTACCA
	Reverse	GCTCAGTCCGCTCATAGCC
Myf6	Forward	AGATCGTCGGAAAGCAGC
	Reverse	CCTGGAATGATCCGAAACAC
MyHC	Forward	GCGAATCGAGGCTCAGAACAA
	Reverse	GTAGTTCCGCCTTCGGTCTTG
Pgk	Forward	ATGTCGCTTTCCAACAAGCTG
	Reverse	GCTCCATTGTCCAAGCAGAAT
Thy1	Forward	CCT TAC CCT AGC CAA CTT CAC
	Reverse	AGG ATG TGT TCT GAA CCA GC
Col1a1	Forward	GCA ACA GTC GCT TCA CCT AC
	Reverse	GTG GGA GGG AAC CAG ATT G
Notch1	Forward	CAGGAAAGAGGGCATCAG
	Reverse	AGCGTTAGGCAGAGCAAG
Notch3	Forward	GTCCAGAGGCCAAGAGACTG
	Reverse	CAGAAGGAGGCCAGCATAAG
Hey1	Forward	TGAATCCAGATGACCAGCTACTGT
	Reverse	TACTTTCAGACTCCGATCGCTTAC
Heyl	Forward	CAGATGCAAGCCCGGAAGAA
	Reverse	ACCAGAGGCATGGAGCATCT
Mstn	Forward	CAGGAGAAGATGGGCTGAATC
	Reverse	GGAGTGCTCATCGCAGTCAA