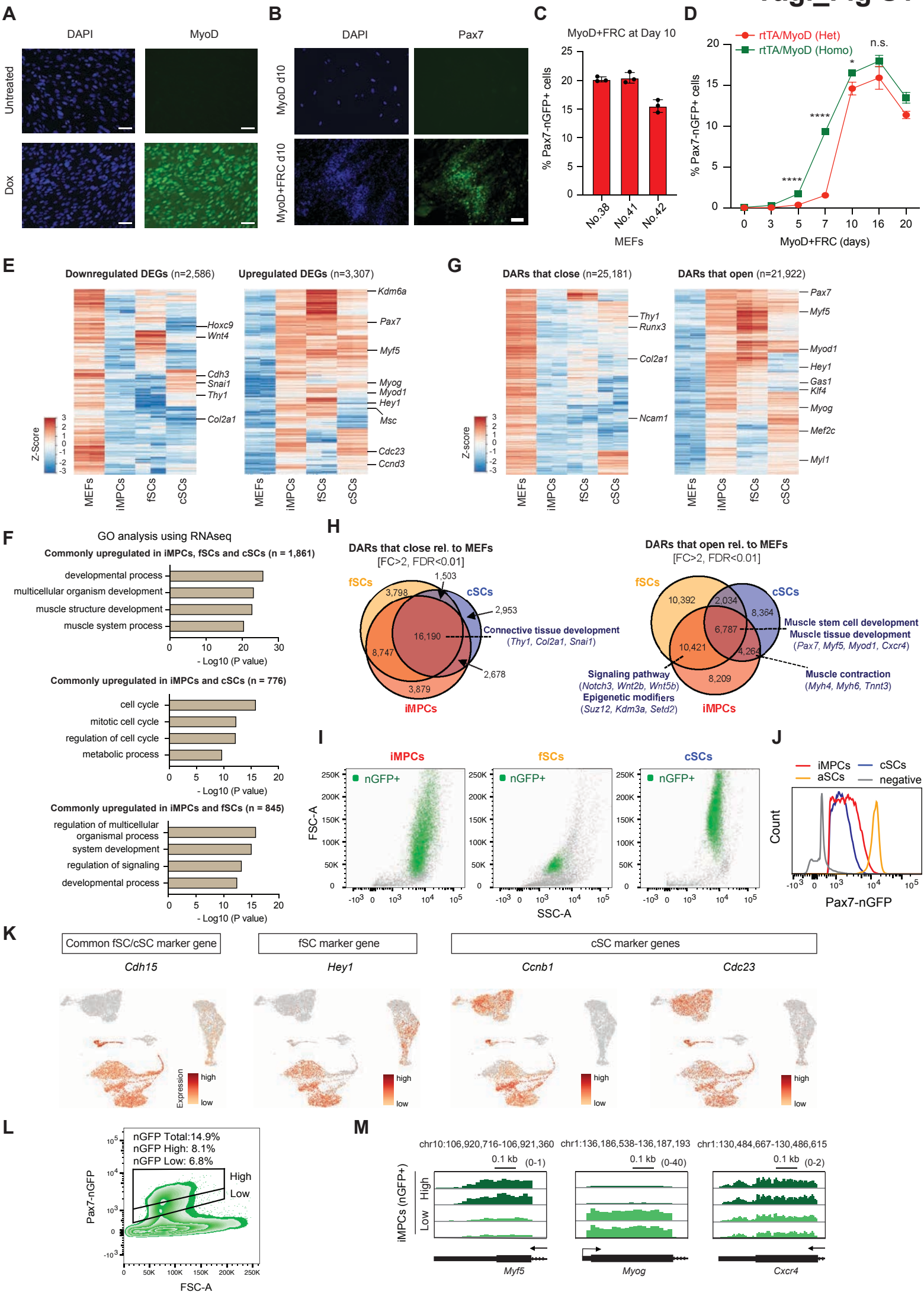
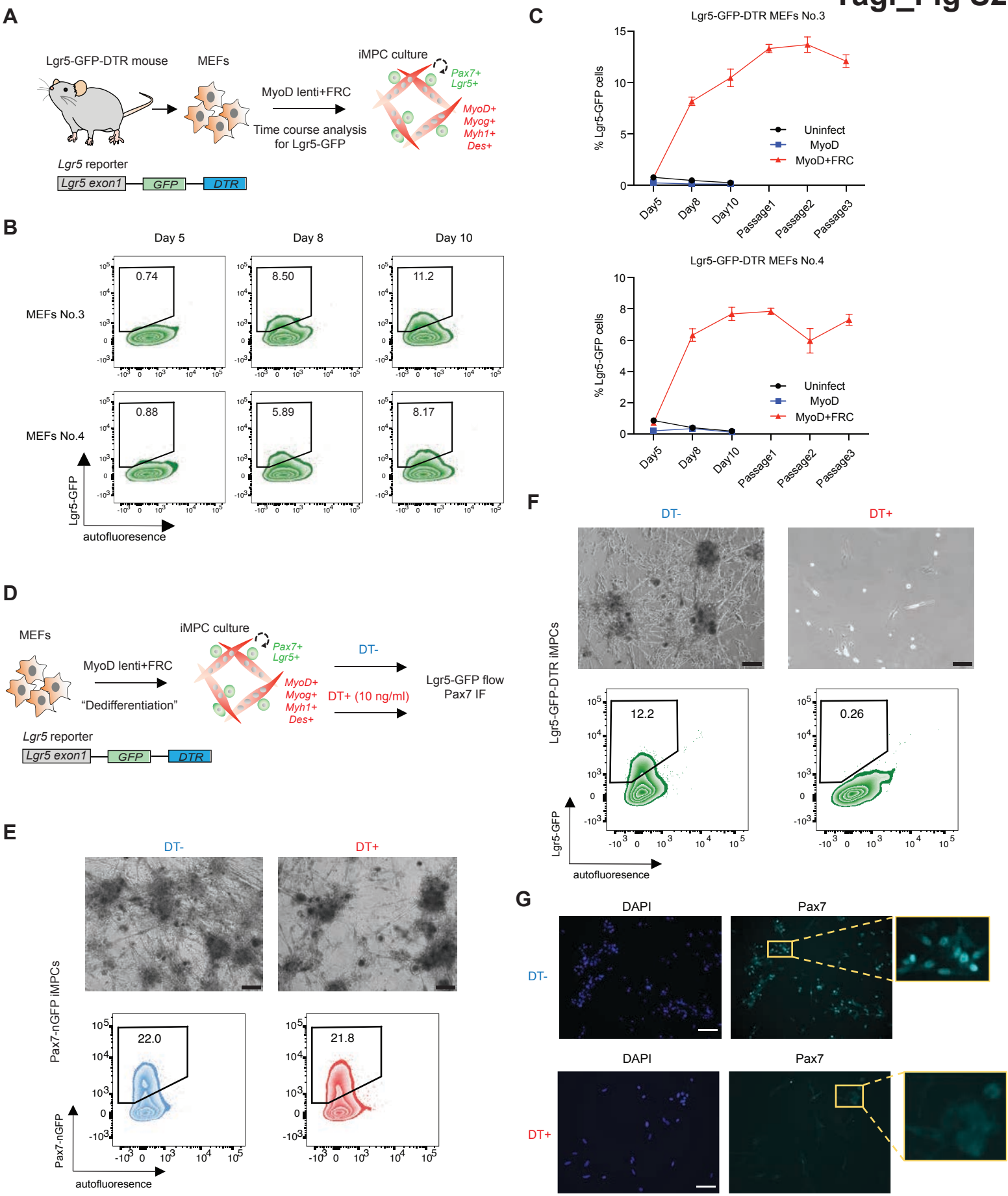
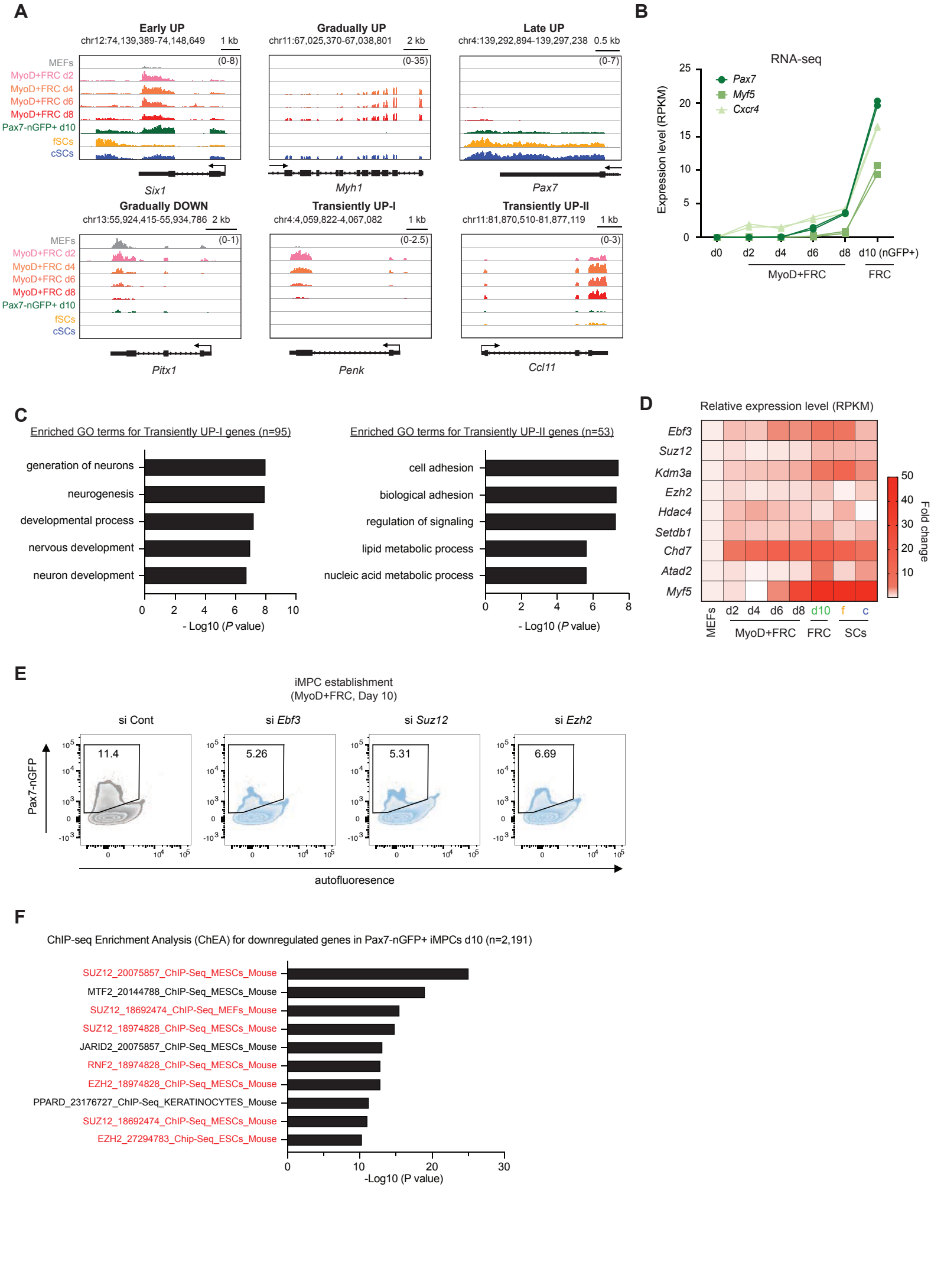
Yagi_Fig S1


A
DAPI
MyoD
Untreated
Dox
B
DAPI
Pax7
MyoD d10
MyoD+FRC d10
C
MyoD+FRC at Day 10
% Pax7-nGFP+ cells
No.38
No.41
No.42
MEFs
D
rtTA/MyoD (Het)
rtTA/MyoD (Homo)
n.s.
% Pax7-nGFP+ cells
MyoD+FRC (days)
E
Downregulated DEGs (n=2,586)
Upregulated DEGs (n=3,307)
Hoxc9
Wnt4
Cdh3
Snai1
Thy1
Col2a1
Kdm6a
Pax7
Myf5
Myog
Myod1
Hey1
Msc
Cdc23
Ccnd3
Z-Score
MEFs
iMPCs
fSCs
cSCs
G
DARs that close (n=25,181)
DARs that open (n=21,922)
Thy1
Runx3
Col2a1
Ncam1
Pax7
Myf5
Myod1
Hey1
Gas1
Klf4
Myog
Mef2c
Myl1
F
GO analysis using RNAseq
Commonly upregulated in iMPCs, fSCs and cSCs (n = 1,861)
developmental process
multicellular organism development
muscle structure development
muscle system process
- Log10 (P value)
Commonly upregulated in iMPCs and cSCs (n = 776)
cell cycle
mitotic cell cycle
regulation of cell cycle
metabolic process
- Log10 (P value)
Commonly upregulated in iMPCs and fSCs (n = 845)
regulation of multicellular organismal process
system development
regulation of signaling
developmental process
- Log10 (P value)
H
DARs that close rel. to MEFs
[FC>2, FDR<0.01]
fSCs
cSCs
iMPCs
3,798
1,503
2,953
16,190
8,747
2,678
3,879
Connective tissue development
(Thy1, Col2a1, Snai1)
DARs that open rel. to MEFs
[FC>2, FDR<0.01]
10,392
2,034
8,364
6,787
10,421
4,264
8,209
Muscle stem cell development
Muscle tissue development
(Pax7, Myf5, Myod1, Cxcr4)
Signaling pathway
(Notch3, Wnt2b, Wnt5b)
Epigenetic modifiers
(Suz12, Kdm3a, Setd2)
Muscle contraction
(Myh4, Myh6, Tnnt3)
I
iMPCs
fSCs
cSCs
nGFP+
FSC-A
SSC-A
J
iMPCs
cSCs
aSCs
negative
Count
Pax7-nGFP
K
Common fSC/cSC marker gene
fSC marker gene
cSC marker genes
Cdh15
Hey1
Ccnb1
Cdc23
Expression
high
low
L
nGFP Total:14.9%
nGFP High: 8.1%
nGFP Low: 6.8%
High
Low
Pax7-nGFP
FSC-A
M
chr10:106,920,716-106,921,360
chr1:136,186,538-136,187,193
chr1:130,484,667-130,486,615
0.1 kb
(0-1)
(0-40)
(0-2)
iMPCs (nGFP+)
High
Low
Myf5
Myog
Cxcr4

Yagi_Fig S2

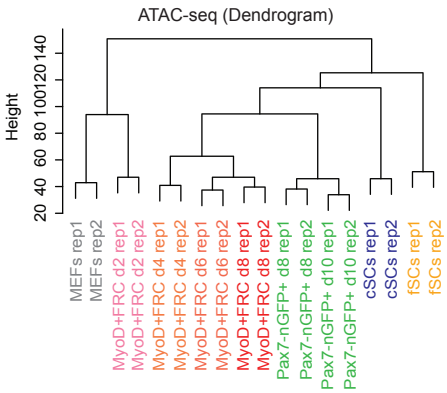

A
Lgr5-GFP-DTR mouse
MEFs
MyoD lenti+FRC
Time course analysis for Lgr5-GFP
iMPC culture
Pax7+ Lgr5+
MyoD+ Myog+ Myh1+ Des+
Lgr5 reporter
Lgr5 exon1
GFP
DTR
B
Day 5
Day 8
Day 10
MEFs No.3
MEFs No.4
0.74
8.50
11.2
0.88
5.89
8.17
Lgr5-GFP
autofluoresence
C
Lgr5-GFP-DTR MEFs No.3
Lgr5-GFP-DTR MEFs No.4
% Lgr5-GFP cells
Uninfect
MyoD
MyoD+FRC
Day5
Day8
Day10
Passage1
Passage2
Passage3
D
MEFs
MyoD lenti+FRC
"Dedifferentiation"
iMPC culture
Pax7+ Lgr5+
MyoD+ Myog+ Myh1+ Des+
DT-
DT+ (10 ng/ml)
Lgr5-GFP flow
Pax7 IF
Lgr5 reporter
Lgr5 exon1
GFP
DTR
E
DT-
DT+
Pax7-nGFP iMPCs
22.0
21.8
Pax7-nGFP
autofluoresence
F
DT-
DT+
Lgr5-GFP-DTR iMPCs
12.2
0.26
Lgr5-GFP
autofluoresence
G
DAPI
Pax7
DT-
DT+

Yagi_Fig S3


A
Early UP
chr12:74,139,389-74,148,649 1 kb
Gradually UP
chr11:67,025,370-67,038,801 2 kb
Late UP
chr4:139,292,894-139,297,238 0.5 kb
MEFs
MyoD+FRC d2
MyoD+FRC d4
MyoD+FRC d6
MyoD+FRC d8
Pax7-nGFP+ d10
fSCs
cSCs
(0-8)
(0-35)
(0-7)
Six1
Myh1
Pax7
Gradually DOWN
chr13:55,924,415-55,934,786 2 kb
Transiently UP-I
chr4:4,059,822-4,067,082 1 kb
Transiently UP-II
chr11:81,870,510-81,877,119 1 kb
(0-1)
(0-2.5)
(0-3)
Pitx1
Penk
Ccl11
B
RNA-seq
Pax7
Myf5
Cxcr4
Expression level (RPKM)
d0 d2 d4 d6 d8 d10 (nGFP+)
MyoD+FRC
FRC
C
Enriched GO terms for Transiently UP-I genes (n=95)
generation of neurons
neurogenesis
developmental process
nervous development
neuron development
- Log10 (P value)
Enriched GO terms for Transiently UP-II genes (n=53)
cell adhesion
biological adhesion
regulation of signaling
lipid metabolic process
nucleic acid metabolic process
- Log10 (P value)
D
Relative expression level (RPKM)
Ebf3
Suz12
Kdm3a
Ezh2
Hdac4
Setdb1
Chd7
Atad2
Myf5
Fold change
MEFs d2 d4 d6 d8 d10 f c
MyoD+FRC
FRC
SCs
E
iMPC establishment
(MyoD+FRC, Day 10)
si Cont
si Ebf3
si Suz12
si Ezh2
Pax7-nGFP
11.4
5.26
5.31
6.69
autofluoresence
F
ChIP-seq Enrichment Analysis (ChEA) for downregulated genes in Pax7-nGFP+ iMPCs d10 (n=2,191)
SUZ12_20075857_ChIP-Seq_MESCs_Mouse
MTF2_20144788_ChIP-Seq_MESCs_Mouse
SUZ12_18692474_ChIP-Seq_MEFs_Mouse
SUZ12_18974828_ChIP-Seq_MESCs_Mouse
JARID2_20075857_ChIP-Seq_MESCs_Mouse
RNF2_18974828_ChIP-Seq_MESCs_Mouse
EZH2_18974828_ChIP-Seq_MESCs_Mouse
PPARD_23176727_ChIP-Seq_KERATINOCYTES_Mouse
SUZ12_18692474_ChIP-Seq_MESCs_Mouse
EZH2_27294783_Chip-Seq_ESCs_Mouse
-Log10 (P value)

Yagi_Fig S4

A



B

DARs that close (Pax7-nGFP+ d10 vs MEFs)
n=35,022

Enriched GO terms	*P* value
Epithelium development	2.00E-21
Cell adhesion	3.83E-20
Embryo development	1.95E-16
Epithelial tube development	2.65E-10
Epithelial cell proliferation	5.84E-09

(Examples) *Thy1, Col2a1, Cdh2, Snai1, Snai2, Ncam1, Zeb2, Col3a1*

DARs that open (Pax7-nGFP+ d10 vs MEFs)
n=34,502

Enriched GO terms	*P* value
Tissue morphogenesis	1.82E-13
Muscle structure development	9.47E-13
Embryonic morphogenesis	1.87E-11
Muscle tissue development	6.12E-10
Muscle cell differentiation	1.59E-08

(Examples) *Myod1, Myog, Myf5, Pax7, Notch3, Six1, Tcf12, Des*

C

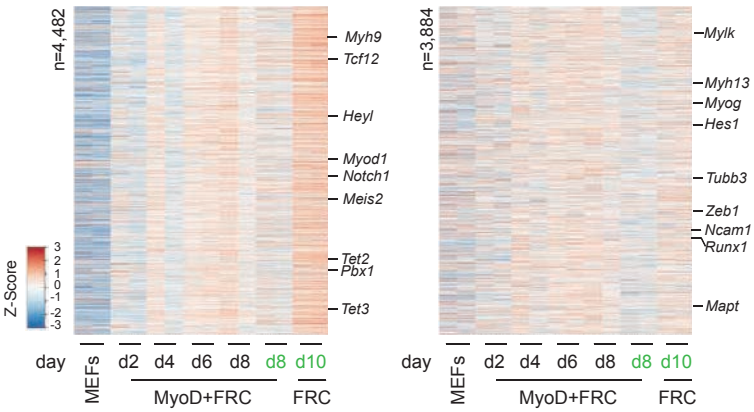
Enriched motifs in DARs that open (fSCs vs MEFs)

Motif	*P* value
Myod1 Myog Tcf12	5.5E-544
Fos Jun	1.6E-159
Ascl2 Klf5	2.8E-67

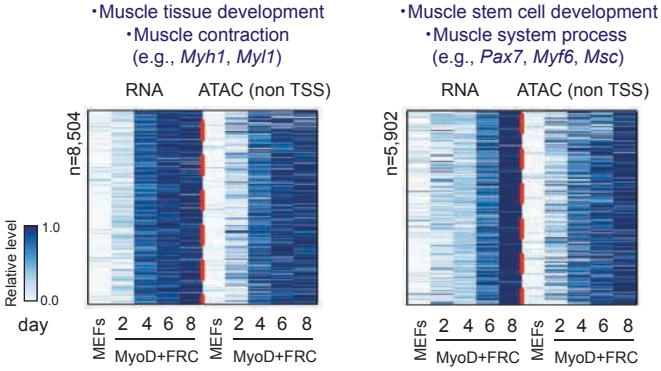
Enriched motifs in DARs that open (cSCs vs MEFs)

Motif	*P* value
Myod1 Myog Tcf12	3.2E-1,078
Mef2a Mef2d	1.3E-185
Meis1	1.0E-112

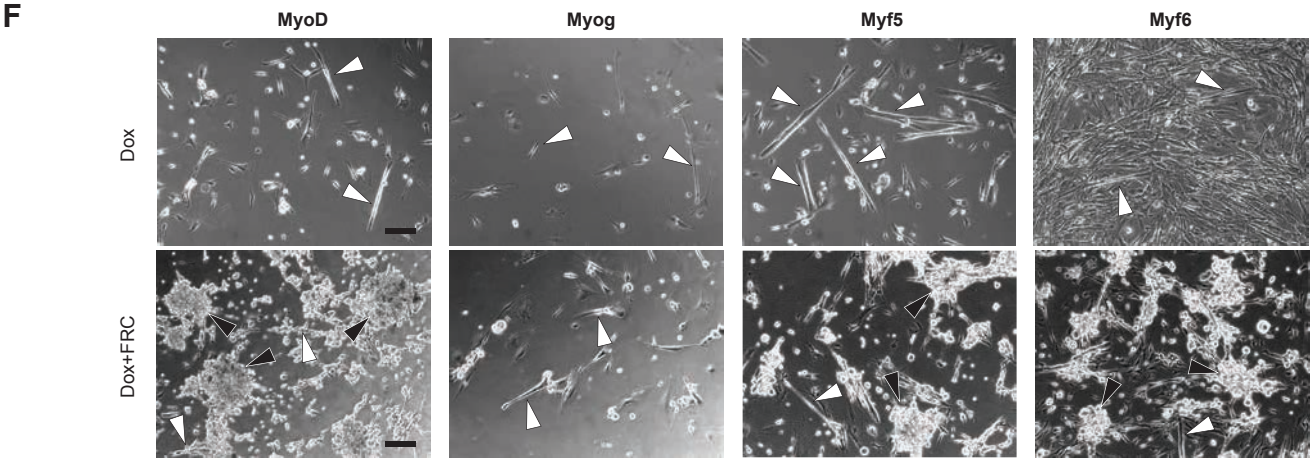
D



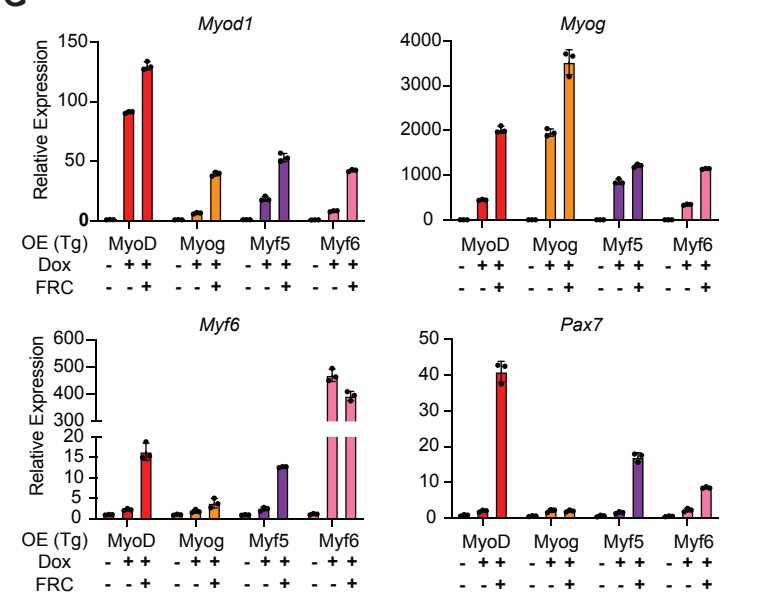
E



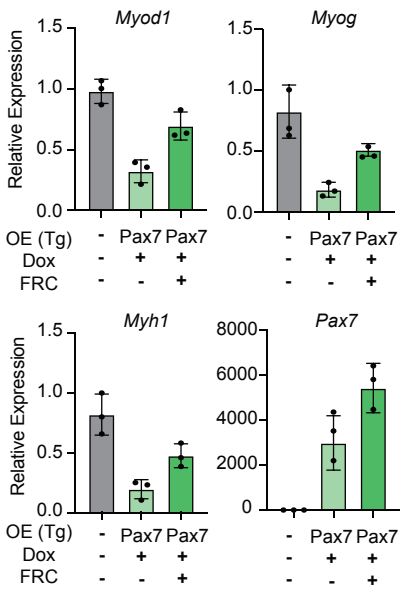
F



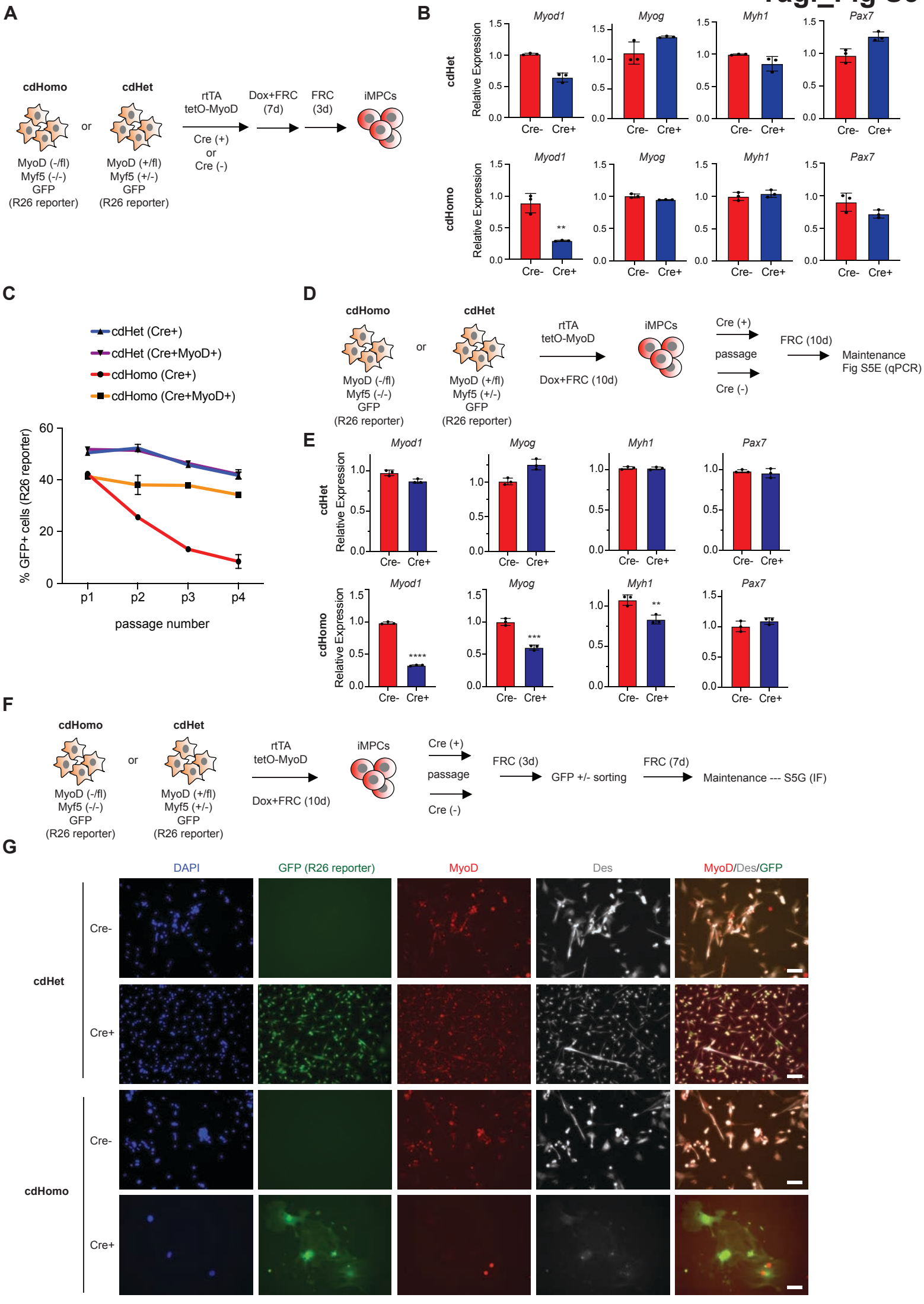
G

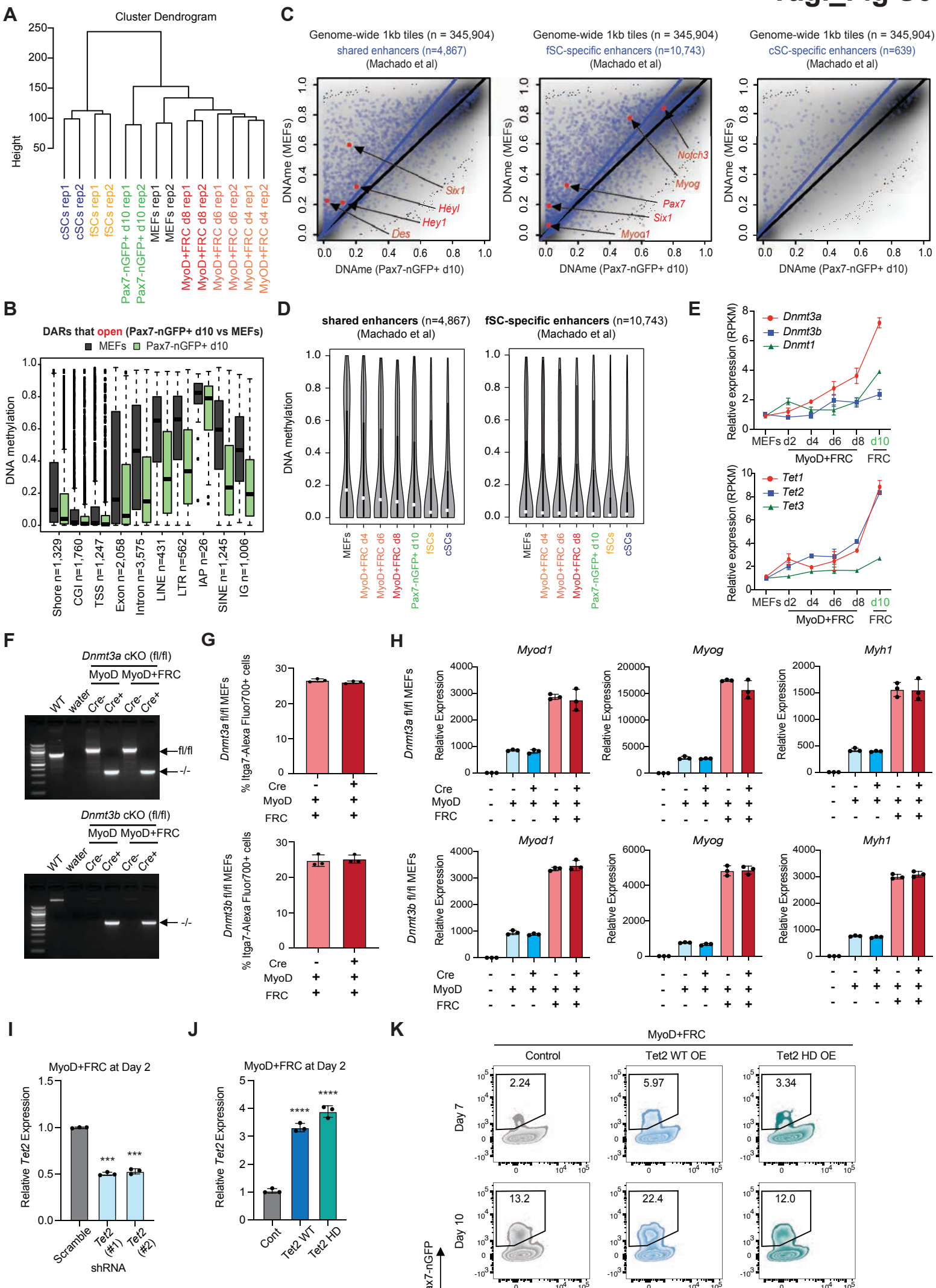


H

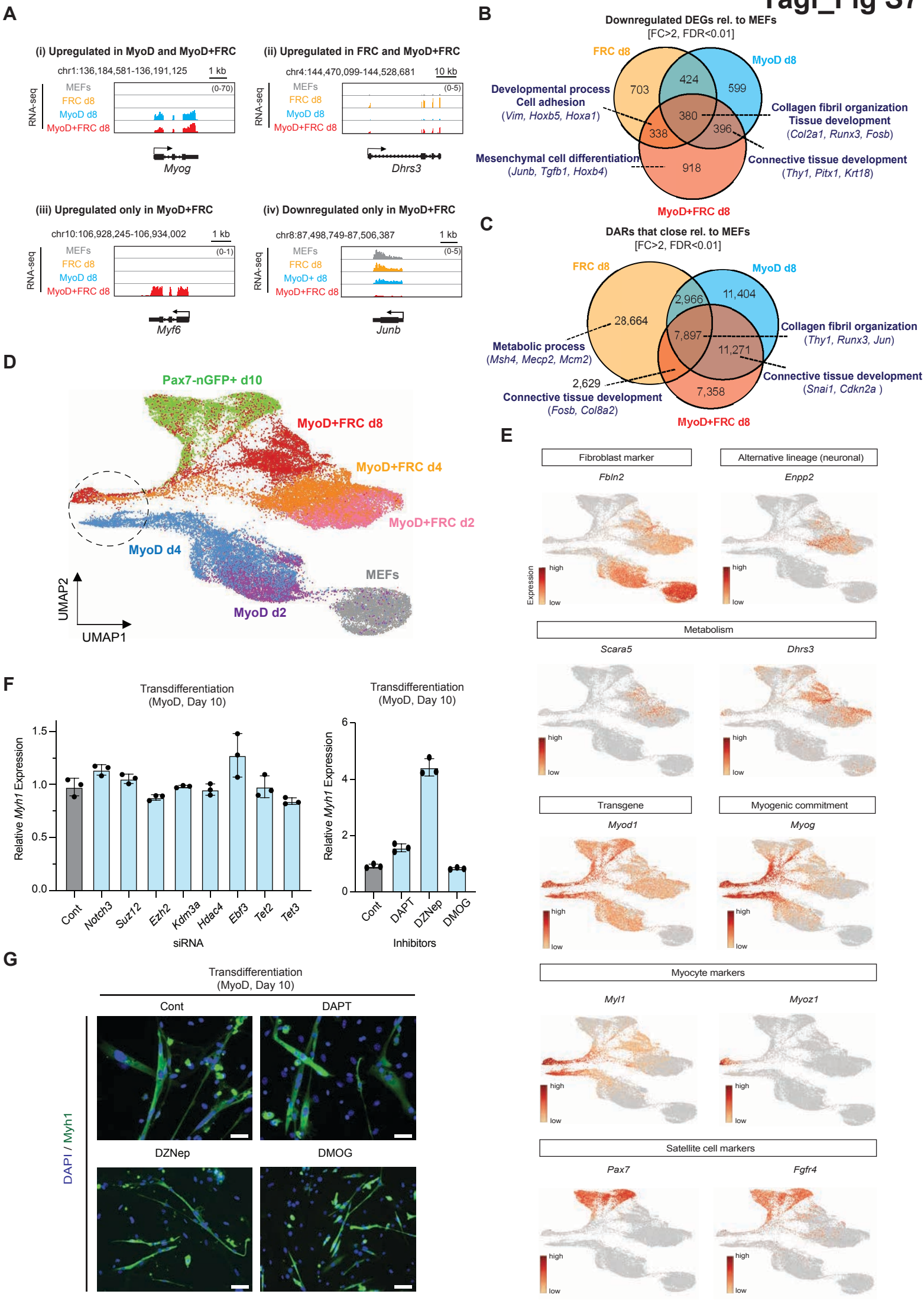


Yagi_Fig S5

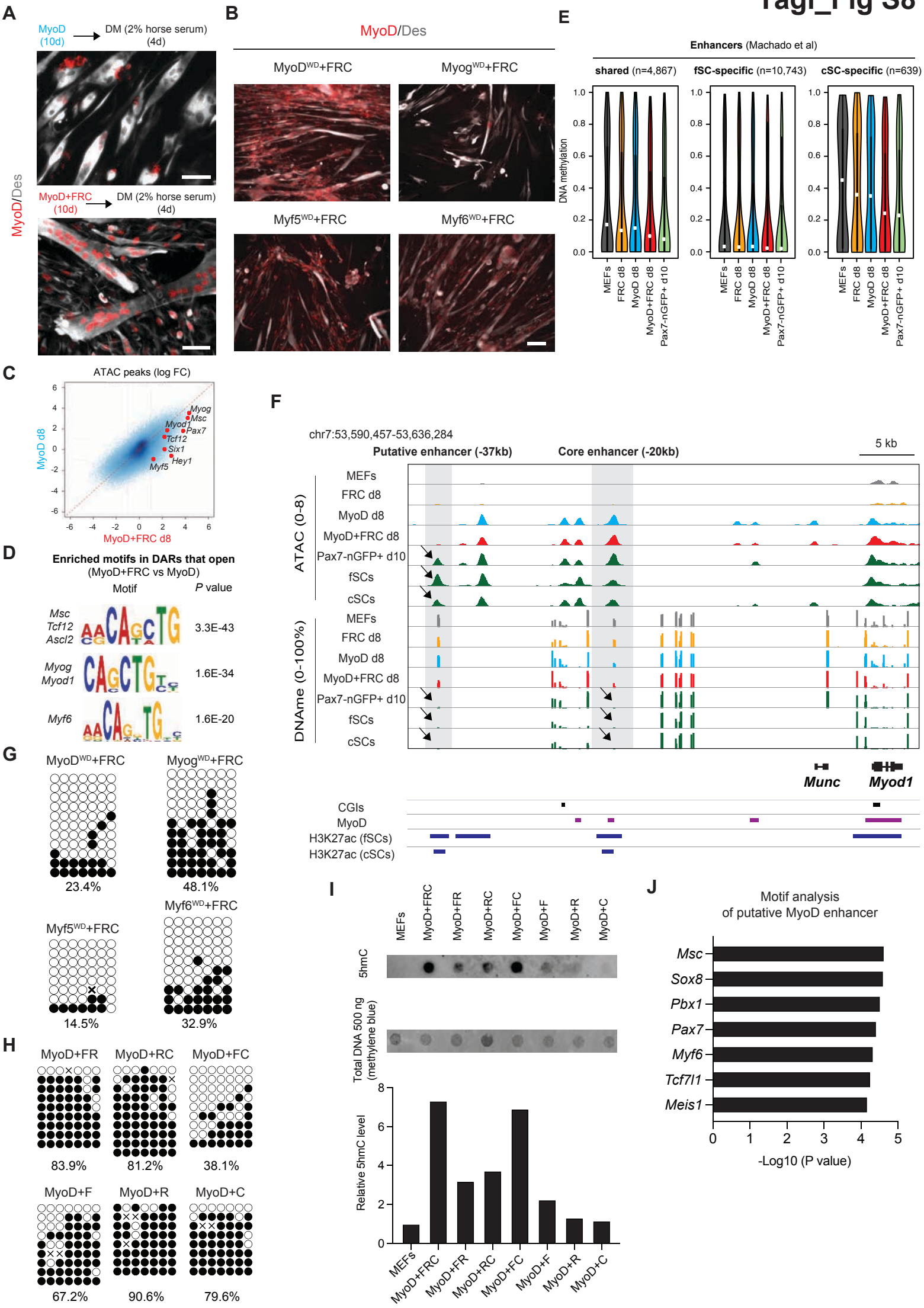

A
cdHomo
cdHet
or
MyoD (-/fl)
Myf5 (-/-)
GFP
(R26 reporter)
MyoD (+/fl)
Myf5 (+/-)
GFP
(R26 reporter)
rtTA
tetO-MyoD
Cre (+)
or
Cre (-)
Dox+FRC
(7d)
FRC
(3d)
iMPCs
B
cdHet
cdHomo
Relative Expression
Myod1
Myog
Myh1
Pax7
Cre-
Cre+
C
cdHet (Cre+)
cdHet (Cre+MyoD+)
cdHomo (Cre+)
cdHomo (Cre+MyoD+)
% GFP+ cells (R26 reporter)
passage number
p1
p2
p3
p4
D
cdHomo
cdHet
or
MyoD (-/fl)
Myf5 (-/-)
GFP
(R26 reporter)
MyoD (+/fl)
Myf5 (+/-)
GFP
(R26 reporter)
rtTA
tetO-MyoD
Dox+FRC (10d)
iMPCs
Cre (+)
passage
Cre (-)
FRC (10d)
Maintenance
Fig S5E (qPCR)
E
cdHet
cdHomo
Relative Expression
Myod1
Myog
Myh1
Pax7
Cre-
Cre+
F
cdHomo
cdHet
or
MyoD (-/fl)
Myf5 (-/-)
GFP
(R26 reporter)
MyoD (+/fl)
Myf5 (+/-)
GFP
(R26 reporter)
rtTA
tetO-MyoD
Dox+FRC (10d)
iMPCs
Cre (+)
passage
Cre (-)
FRC (3d)
GFP +/- sorting
FRC (7d)
Maintenance --- S5G (IF)
G
DAPI
GFP (R26 reporter)
MyoD
Des
MyoD/Des/GFP
cdHet
cdHomo
Cre-
Cre+


A
Cluster Dendrogram
Height
cSCs rep1
cSCs rep2
fSCs rep1
fSCs rep2
Pax7-nGFP+ d10 rep1
Pax7-nGFP+ d10 rep2
MEFs rep1
MEFs rep2
MyoD+FRC d8 rep1
MyoD+FRC d8 rep2
MyoD+FRC d6 rep1
MyoD+FRC d6 rep2
MyoD+FRC d4 rep1
MyOD+FRC d4 rep2
B
DARs that open (Pax7-nGFP+ d10 vs MEFs)
MEFs
Pax7-nGFP+ d10
DNA methylation
Shore n=1,329
CGI n=1,760
TSS n=1,247
Exon n=2,058
Intron n=3,575
LINE n=431
LTR n=562
IAP n=26
SINE n=1,245
IG n=1,006
C
Genome-wide 1kb tiles (n = 345,904)
shared enhancers (n=4,867)
(Machado et al)
fSC-specific enhancers (n=10,743)
cSC-specific enhancers (n=639)
DNAme (MEFs)
DNAme (Pax7-nGFP+ d10)
Six1
Hey1
Hey1
Des
Notch3
Myog
Pax7
Six1
Myoq1
D
shared enhancers (n=4,867)
fSC-specific enhancers (n=10,743)
(Machado et al)
DNA methylation
MEFs
MyoD+FRC d4
MyoD+FRC d6
MyoD+FRC d8
Pax7-nGFP+ d10
fSCs
cSCs
E
Dnmt3a
Dnmt3b
Dnmt1
Tet1
Tet2
Tet3
Relative expression (RPKM)
MEFs d2 d4 d6 d8 d10
MyoD+FRC
FRC
F
Dnmt3a cKO (fl/fl)
MyoD MyoD+FRC
WT water Cre- Cre+ Cre- Cre+
fl/fl
-/-
Dnmt3b cKO (fl/fl)
MyoD MyoD+FRC
WT water Cre- Cre+ Cre- Cre+
-/-
G
Dnmt3a fl/fl MEFs
Dnmt3b fl/fl MEFs
% Itga7-Alexa Fluor700+ cells
Cre
MyoD
FRC
H
Myod1
Myog
Myh1
Dnmt3a fl/fl MEFs
Dnmt3b fl/fl MEFs
Relative Expression
Cre
MyoD
FRC
I
MyoD+FRC at Day 2
Relative Tet2 Expression
***
***
Scramble
Tet2 (#1)
Tet2 (#2)
shRNA
J
MyoD+FRC at Day 2
Relative Tet2 Expression
****
****
Cont
Tet2 WT
Tet2 HD
K
MyoD+FRC
Control
Tet2 WT OE
Tet2 HD OE
Day 7
Day 10
2.24
5.97
3.34
13.2
22.4
12.0
Pax7-nGFP
autofluoresence

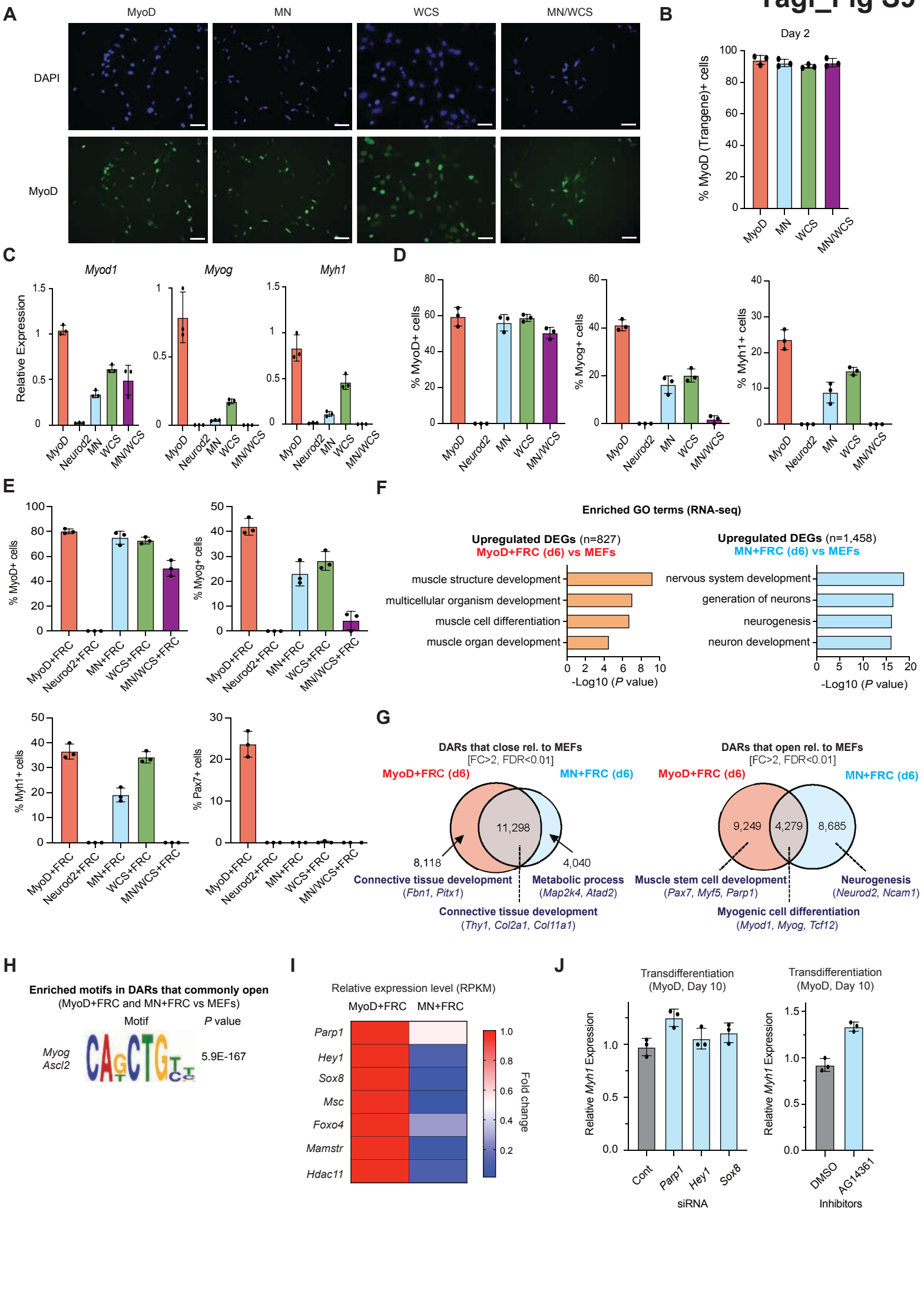
Yagi_Fig S7

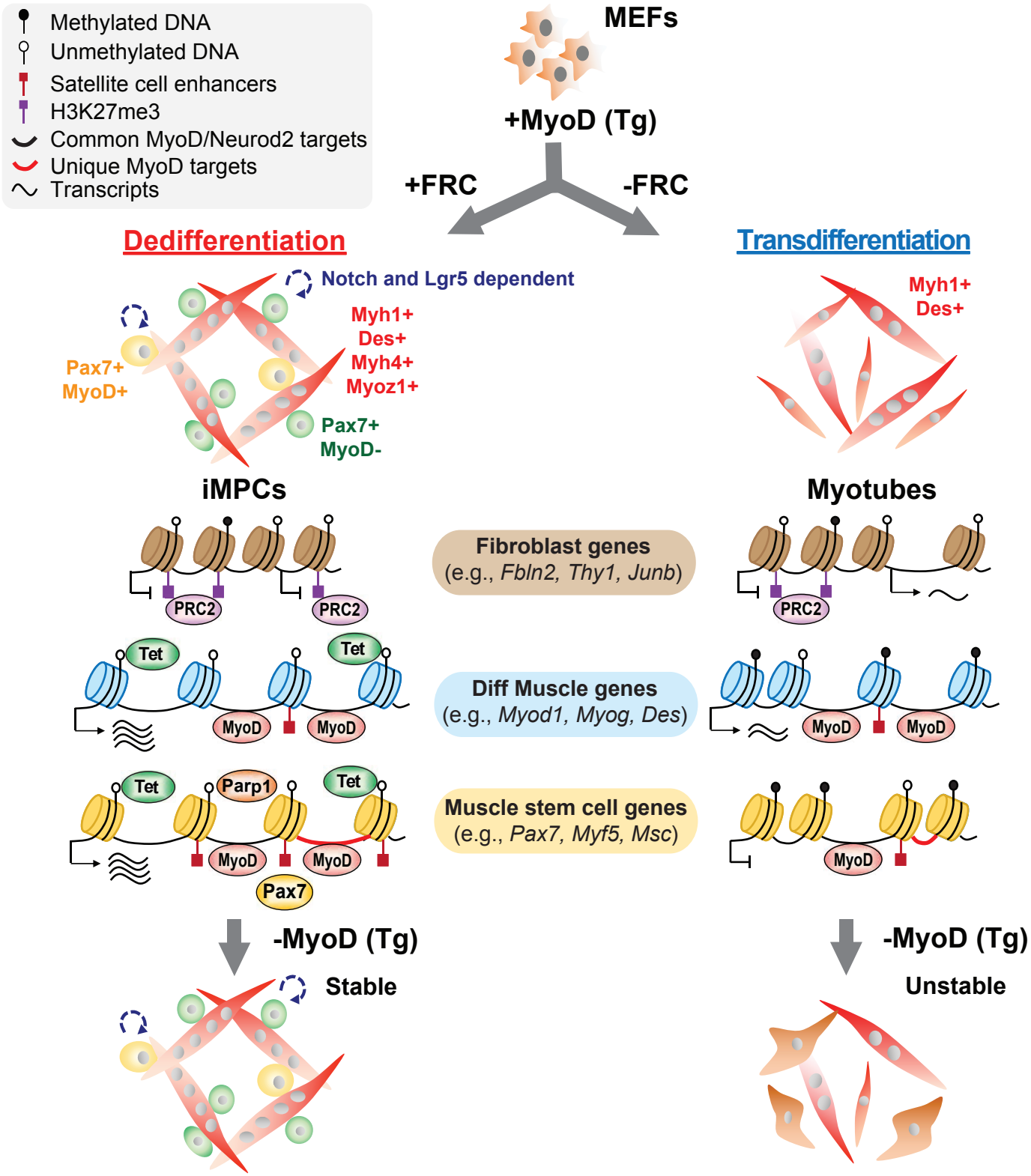

A
(i) Upregulated in MyoD and MyoD+FRC
chr1:136,184,581-136,191,125
1 kb
RNA-seq
MEFs
FRC d8
MyoD d8
MyoD+FRC d8
(0-70)
Myog
(ii) Upregulated in FRC and MyoD+FRC
chr4:144,470,099-144,528,681
10 kb
(0-5)
Dhrs3
(iii) Upregulated only in MyoD+FRC
chr10:106,928,245-106,934,002
(0-1)
Myf6
(iv) Downregulated only in MyoD+FRC
chr8:87,498,749-87,506,387
MyoD+ d8
Junb
B
Downregulated DEGs rel. to MEFs
[FC>2, FDR<0.01]
FRC d8
MyoD d8
MyoD+FRC d8
703
424
599
380
338
396
918
Developmental process
Cell adhesion
(Vim, Hoxb5, Hoxa1)
Collagen fibril organization
Tissue development
(Col2a1, Runx3, Fosb)
Mesenchymal cell differentiation
(Junb, Tgfb1, Hoxb4)
Connective tissue development
(Thy1, Pitx1, Krt18)
C
DARs that close rel. to MEFs
[FC>2, FDR<0.01]
28,664
2,966
11,404
7,897
11,271
7,358
2,629
Metabolic process
(Msh4, Mecp2, Mcm2)
Collagen fibril organization
(Thy1, Runx3, Jun)
Connective tissue development
(Snai1, Cdkn2a)
Connective tissue development
(Fosb, Col8a2)
D
Pax7-nGFP+ d10
MyoD+FRC d8
MyoD+FRC d4
MyoD+FRC d2
MyoD d4
MEFs
MyoD d2
UMAP2
UMAP1
E
Fibroblast marker
Alternative lineage (neuronal)
Fbln2
Enpp2
Expression
high
low
Metabolism
Scara5
Dhrs3
Transgene
Myogenic commitment
Myod1
Myog
Myocyte markers
Myl1
Myoz1
Satellite cell markers
Pax7
Fgfr4
F
Transdifferentiation
(MyoD, Day 10)
Relative Myh1 Expression
1.5
1.0
0.5
0.0
Cont
Notch3
Suz12
Ezh2
Kdm3a
Hdac4
Ebf3
Tet2
Tet3
siRNA
6
4
2
0
DAPT
DZNep
DMOG
Inhibitors
G
Transdifferentiation
(MyoD, Day 10)
Cont
DAPT
DZNep
DMOG
DAPI / Myh1

Yagi_Fig S8


A
MyoD (10d)
DM (2% horse serum) (4d)
MyoD+FRC (10d)
DM (2% horse serum) (4d)
MyoD/Des
B
MyoD/Des
MyoD^WD+FRC
Myog^WD+FRC
Myf5^WD+FRC
Myf6^WD+FRC
E
Enhancers (Machado et al)
shared (n=4,867)
fSC-specific (n=10,743)
cSC-specific (n=639)
DNA methylation
MEFs
FRC d8
MyoD d8
MyoD+FRC d8
Pax7-nGFP+ d10
C
ATAC peaks (log FC)
MyoD d8
MyoD+FRC d8
Myog
Msc
Myod1
Pax7
Tcf12
Six1
Hey1
Myf5
D
Enriched motifs in DARs that open (MyoD+FRC vs MyoD)
Motif
P value
Msc Tcf12 Ascl2
3.3E-43
Myog Myod1
1.6E-34
Myf6
1.6E-20
F
chr7:53,590,457-53,636,284
Putative enhancer (-37kb)
Core enhancer (-20kb)
5 kb
ATAC (0-8)
DNAme (0-100%)
MEFs
FRC d8
MyoD d8
MyoD+FRC d8
Pax7-nGFP+ d10
fSCs
cSCs
Munc
Myod1
CGIs
MyoD
H3K27ac (fSCs)
H3K27ac (cSCs)
G
MyoD^WD+FRC
23.4%
Myog^WD+FRC
48.1%
Myf5^WD+FRC
14.5%
Myf6^WD+FRC
32.9%
H
MyoD+FR
83.9%
MyoD+RC
81.2%
MyoD+FC
38.1%
MyoD+F
67.2%
MyoD+R
90.6%
MyoD+C
79.6%
I
MEFs
MyoD+FRC
MyoD+FR
MyoD+RC
MyoD+FC
MyoD+F
MyoD+R
MyoD+C
5hmC
Total DNA 500 ng (methylene blue)
Relative 5hmC level
J
Motif analysis of putative MyoD enhancer
Msc
Sox8
Pbx1
Pax7
Myf6
Tcf7l1
Meis1
-Log10 (P value)

Yagi_Fig S9


A
MyoD
MN
WCS
MN/WCS
DAPI
MyoD
B
Day 2
% MyoD (Trangene)+ cells
MyoD
MN
WCS
MN/WCS
C
Myod1
Myog
Myh1
Relative Expression
MyoD
Neurod2
MN
WCS
MN/WCS
D
% MyoD+ cells
% Myog+ cells
% Myh1+ cells
E
% MyoD+ cells
% Myog+ cells
% Myh1+ cells
% Pax7+ cells
MyoD+FRC
Neurod2+FRC
MN+FRC
WCS+FRC
MN/WCS+FRC
F
Enriched GO terms (RNA-seq)
Upregulated DEGs (n=827)
MyoD+FRC (d6) vs MEFs
muscle structure development
multicellular organism development
muscle cell differentiation
muscle organ development
-Log10 (P value)
Upregulated DEGs (n=1,458)
MN+FRC (d6) vs MEFs
nervous system development
generation of neurons
neurogenesis
neuron development
-Log10 (P value)
G
DARs that close rel. to MEFs
[FC>2, FDR<0.01]
MyoD+FRC (d6)
MN+FRC (d6)
11,298
8,118
4,040
Connective tissue development
(Fbn1, Pitx1)
Metabolic process
(Map2k4, Atad2)
Connective tissue development
(Thy1, Col2a1, Col11a1)
DARs that open rel. to MEFs
[FC>2, FDR<0.01]
MyoD+FRC (d6)
MN+FRC (d6)
9,249
4,279
8,685
Muscle stem cell development
(Pax7, Myf5, Parp1)
Neurogenesis
(Neurod2, Ncam1)
Myogenic cell differentiation
(Myod1, Myog, Tcf12)
H
Enriched motifs in DARs that commonly open
(MyoD+FRC and MN+FRC vs MEFs)
Motif
P value
Myog
Ascl2
5.9E-167
I
Relative expression level (RPKM)
MyoD+FRC
MN+FRC
Parp1
Hey1
Sox8
Msc
Foxo4
Mamstr
Hdac11
Fold change
J
Transdifferentiation
(MyoD, Day 10)
Relative Myh1 Expression
Cont
Parp1
Hey1
Sox8
siRNA
Transdifferentiation
(MyoD, Day 10)
Relative Myh1 Expression
DMSO
AG14361
Inhibitors


Methylated DNA
Unmethylated DNA
Satellite cell enhancers
H3K27me3
Common MyoD/Neurod2 targets
Unique MyoD targets
Transcripts
MEFs
+MyoD (Tg)
+FRC
-FRC
Dedifferentiation
Transdifferentiation
Notch and Lgr5 dependent
Pax7+
MyoD+
Myh1+
Des+
Myh4+
Myoz1+
Pax7+
MyoD-
Myh1+
Des+
iMPCs
Myotubes
PRC2
PRC2
PRC2
Fibroblast genes
(e.g., Fbln2, Thy1, Junb)
Tet
Tet
MyoD
MyoD
MyoD
MyoD
Diff Muscle genes
(e.g., Myod1, Myog, Des)
Tet
Parp1
Tet
MyoD
MyoD
MyoD
Pax7
Muscle stem cell genes
(e.g., Pax7, Myf5, Msc)
-MyoD (Tg)
Stable
-MyoD (Tg)
Unstable