Figure S1.

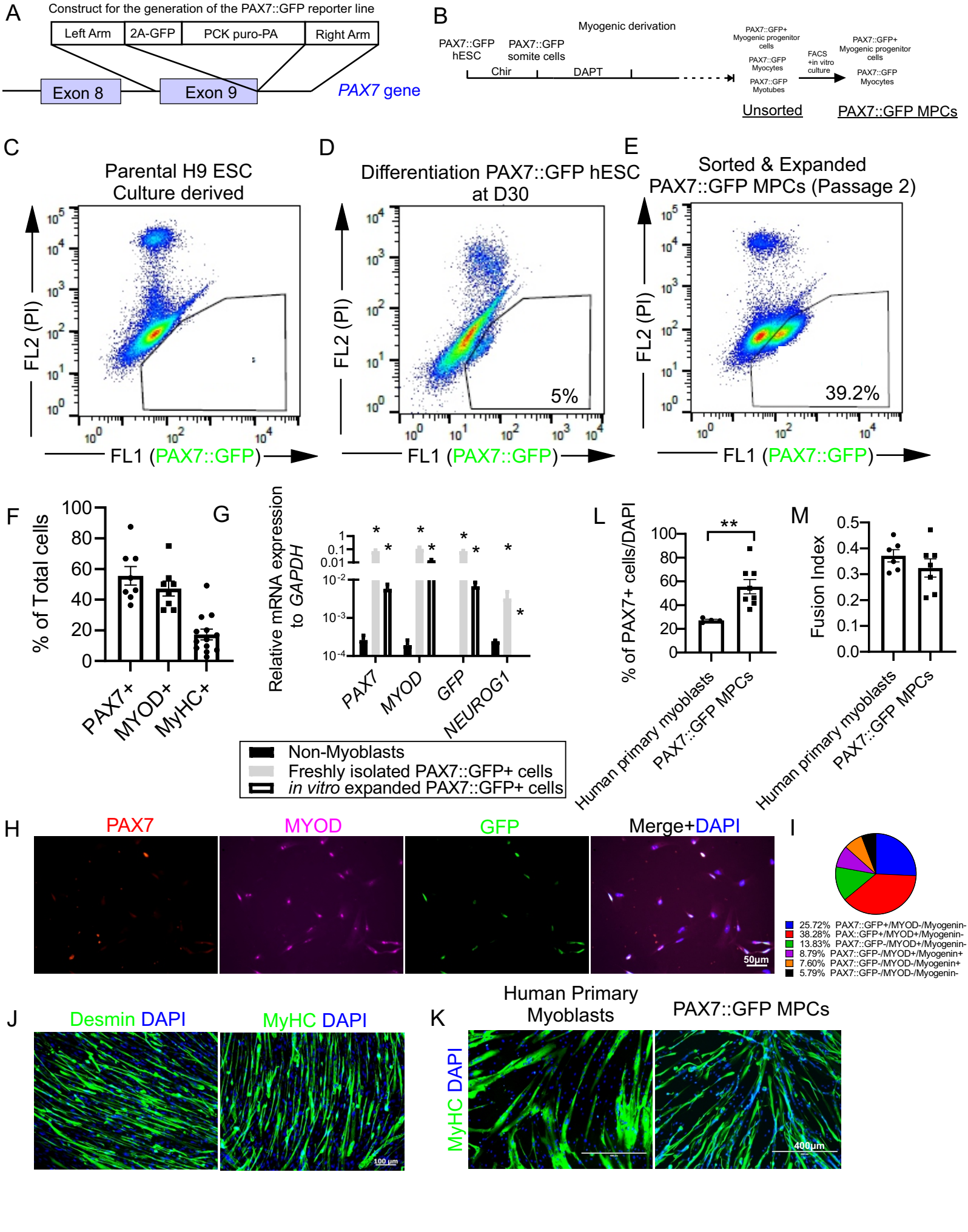
A
Construct for the generation of the PAX7::GFP reporter line
Left Arm
2A-GFP
PCK puro-PA
Right Arm
Exon 8
Exon 9
PAX7 gene
B
Myogenic derivation
PAX7::GFP hESC
PAX7::GFP somite cells
Chir
DAPT
PAX7::GFP+ Myogenic progenitor cells
PAX7::GFP Myocytes
PAX7::GFP Myotubes
FACS +in vitro culture
PAX7::GFP+ Myogenic progenitor cells
PAX7::GFP Myocytes
Unsorted
PAX7::GFP MPCs
C
Parental H9 ESC Culture derived
D
Differentiation PAX7::GFP hESC at D30
5%
E
Sorted & Expanded PAX7::GFP MPCs (Passage 2)
39.2%
FL2 (PI)
FL1 (PAX7::GFP)
F
% of Total cells
PAX7+
MYOD+
MyHC+
G
Relative mRNA expression to GAPDH
PAX7
MYOD
GFP
NEUROG1
Non-Myoblasts
Freshly isolated PAX7::GFP+ cells
in vitro expanded PAX7::GFP+ cells
L
% of PAX7+ cells/DAPI
Human primary myoblasts
PAX7::GFP MPCs
M
Fusion Index
Human primary myoblasts
PAX7::GFP MPCs
H
PAX7
MYOD
GFP
Merge+DAPI
50µm
I
25.72% PAX7::GFP+/MYOD-/Myogenin-
38.28% PAX::GFP+/MYOD+/Myogenin-
13.83% PAX7::GFP-/MYOD+/Myogenin-
8.79% PAX7::GFP-/MYOD+/Myogenin+
7.60% PAX7::GFP-/MYOD-/Myogenin+
5.79% PAX7::GFP-/MYOD-/Myogenin-
J
Desmin DAPI
MyHC DAPI
100 µm
K
Human Primary Myoblasts
PAX7::GFP MPCs
MyHC DAPI
400µm

Figure S2.

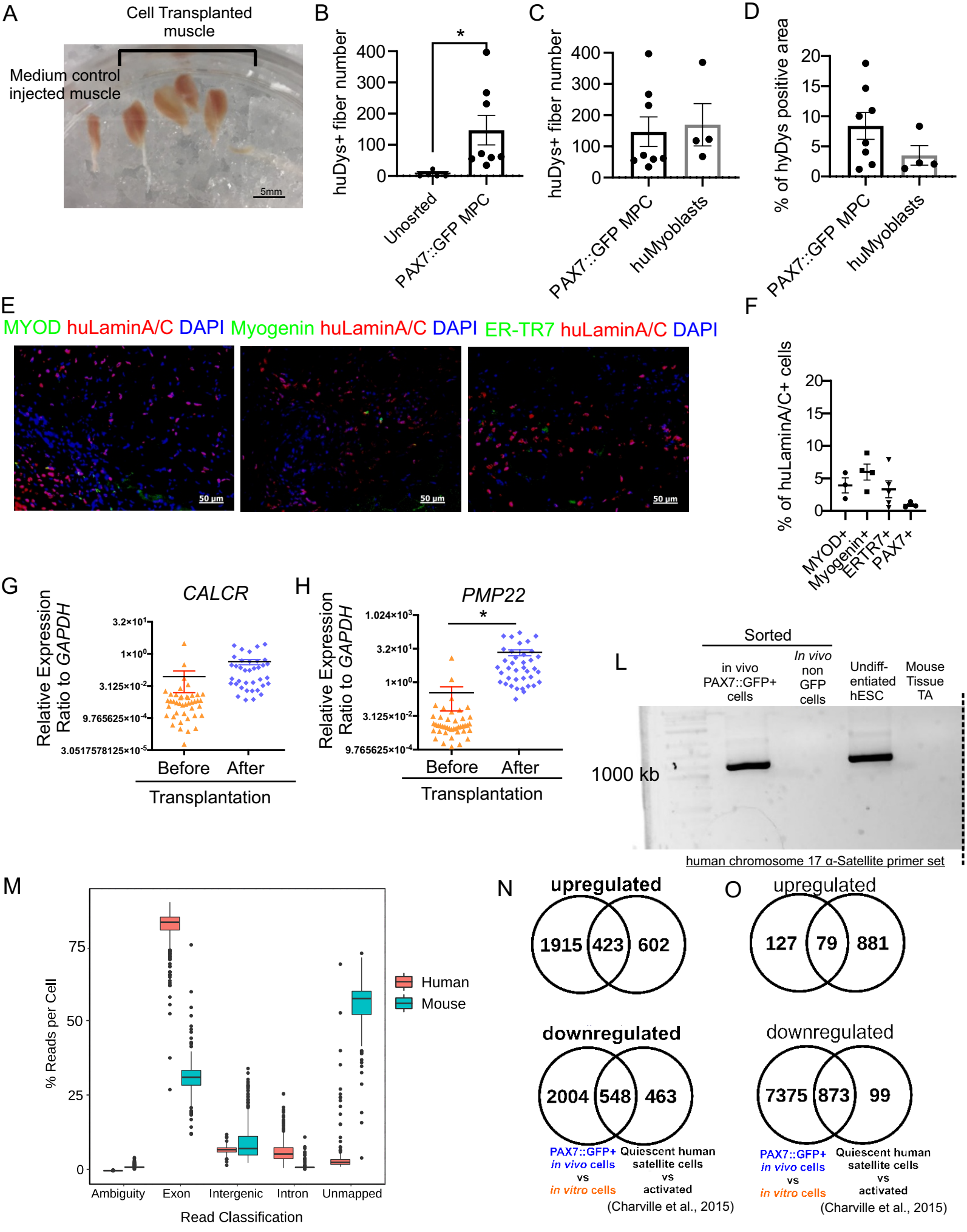
A
Cell Transplanted muscle
Medium control injected muscle
5mm
B
huDys+ fiber number
Unosrted
PAX7::GFP MPC
C
huDys+ fiber number
PAX7::GFP MPC
huMyoblasts
D
% of hyDys positive area
PAX7::GFP MPC
huMyoblasts
E
MYOD huLaminA/C DAPI Myogenin huLaminA/C DAPI ER-TR7 huLaminA/C DAPI
50 µm
F
% of huLaminA/C+ cells
MYOD+
Myogenin+
ERTR7+
PAX7+
G
CALCR
Relative Expression Ratio to GAPDH
Before
After
Transplantation
H
PMP22
Relative Expression Ratio to GAPDH
Before
After
Transplantation
L
Sorted
in vivo PAX7::GFP+ cells
In vivo non GFP cells
Undiff-entiated hESC
Mouse Tissue TA
1000 kb
human chromosome 17 α-Satellite primer set
M
% Reads per Cell
Human
Mouse
Ambiguity
Exon
Intergenic
Intron
Unmapped
Read Classification
N
upregulated
1915
423
602
downregulated
2004
548
463
PAX7::GFP+ in vivo cells vs in vitro cells
Quiescent human satellite cells vs activated
(Charville et al., 2015)
O
upregulated
127
79
881
downregulated
7375
873
99
PAX7::GFP+ in vivo cells vs in vitro cells
Quiescent human satellite cells vs activated
(Charville et al., 2015)

Figure S3.

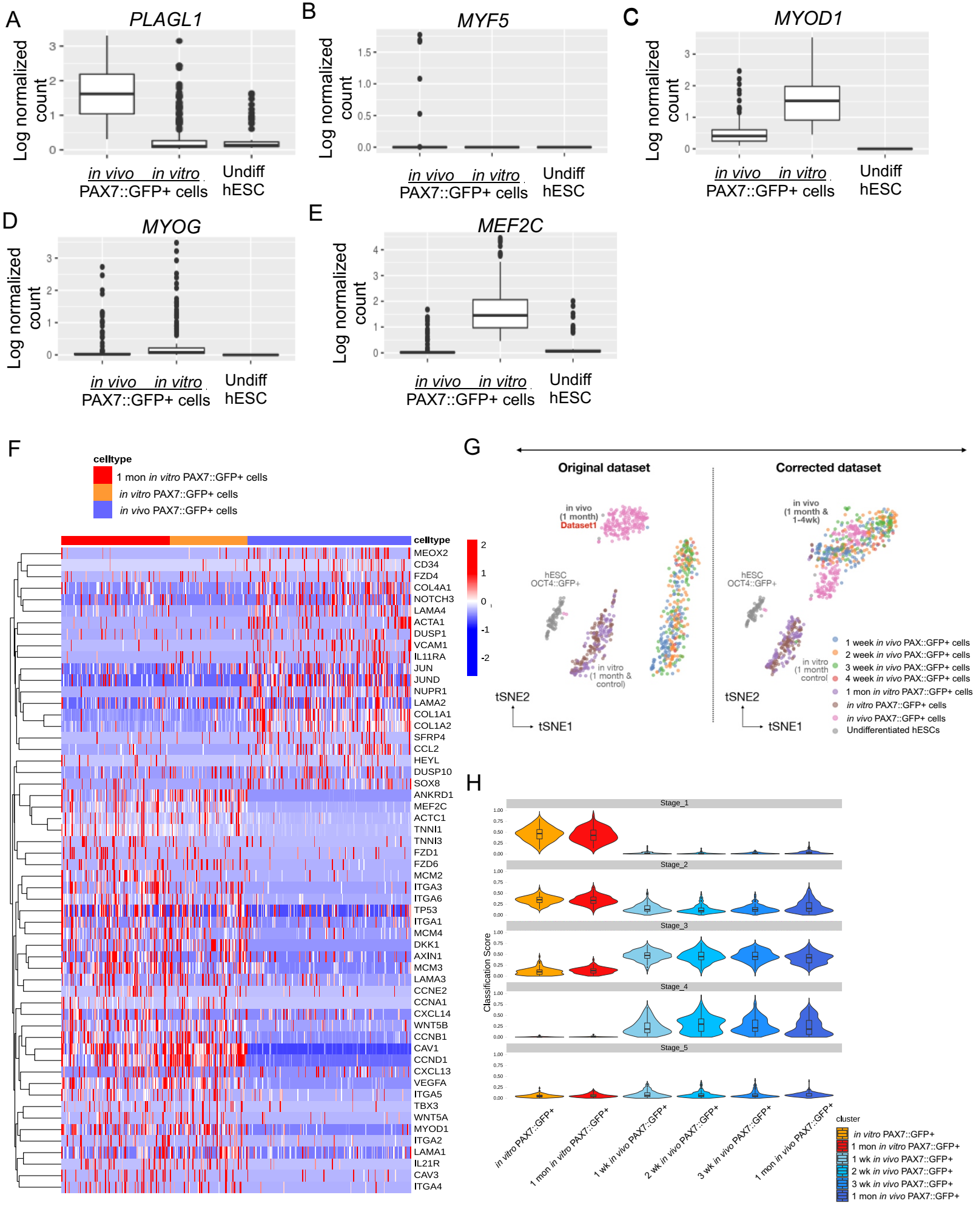
A
PLAGL1
B
MYF5
C
MYOD1
D
MYOG
E
MEF2C
Log normalized count
in vivo
in vitro
PAX7::GFP+ cells
Undiff hESC
F
celltype
1 mon in vitro PAX7::GFP+ cells
in vitro PAX7::GFP+ cells
in vivo PAX7::GFP+ cells
MEOX2
CD34
FZD4
COL4A1
NOTCH3
LAMA4
ACTA1
DUSP1
VCAM1
IL11RA
JUN
JUND
NUPR1
LAMA2
COL1A1
COL1A2
SFRP4
CCL2
HEYL
DUSP10
SOX8
ANKRD1
MEF2C
ACTC1
TNNI1
TNNI3
FZD1
FZD6
MCM2
ITGA3
ITGA6
TP53
ITGA1
MCM4
DKK1
AXIN1
MCM3
LAMA3
CCNE2
CCNA1
CXCL14
WNT5B
CCNB1
CAV1
CCND1
CXCL13
VEGFA
ITGA5
TBX3
WNT5A
MYOD1
ITGA2
LAMA1
IL21R
CAV3
ITGA4
G
Original dataset
Corrected dataset
in vivo (1 month) Dataset1
in vivo (1 month & 1-4wk)
hESC OCT4::GFP+
in vitro (1 month & control)
tSNE1
tSNE2
1 week in vivo PAX::GFP+ cells
2 week in vivo PAX::GFP+ cells
3 week in vivo PAX::GFP+ cells
4 week in vivo PAX::GFP+ cells
1 mon in vitro PAX7::GFP+ cells
in vitro PAX7::GFP+ cells
in vivo PAX7::GFP+ cells
Undifferentiated hESCs
H
Stage_1
Stage_2
Stage_3
Stage_4
Stage_5
Classification Score
in vitro PAX7::GFP+
1 mon in vitro PAX7::GFP+
1 wk in vivo PAX7::GFP+
2 wk in vivo PAX7::GFP+
3 wk in vivo PAX7::GFP+
1 mon in vivo PAX7::GFP+
cluster

Figure S4

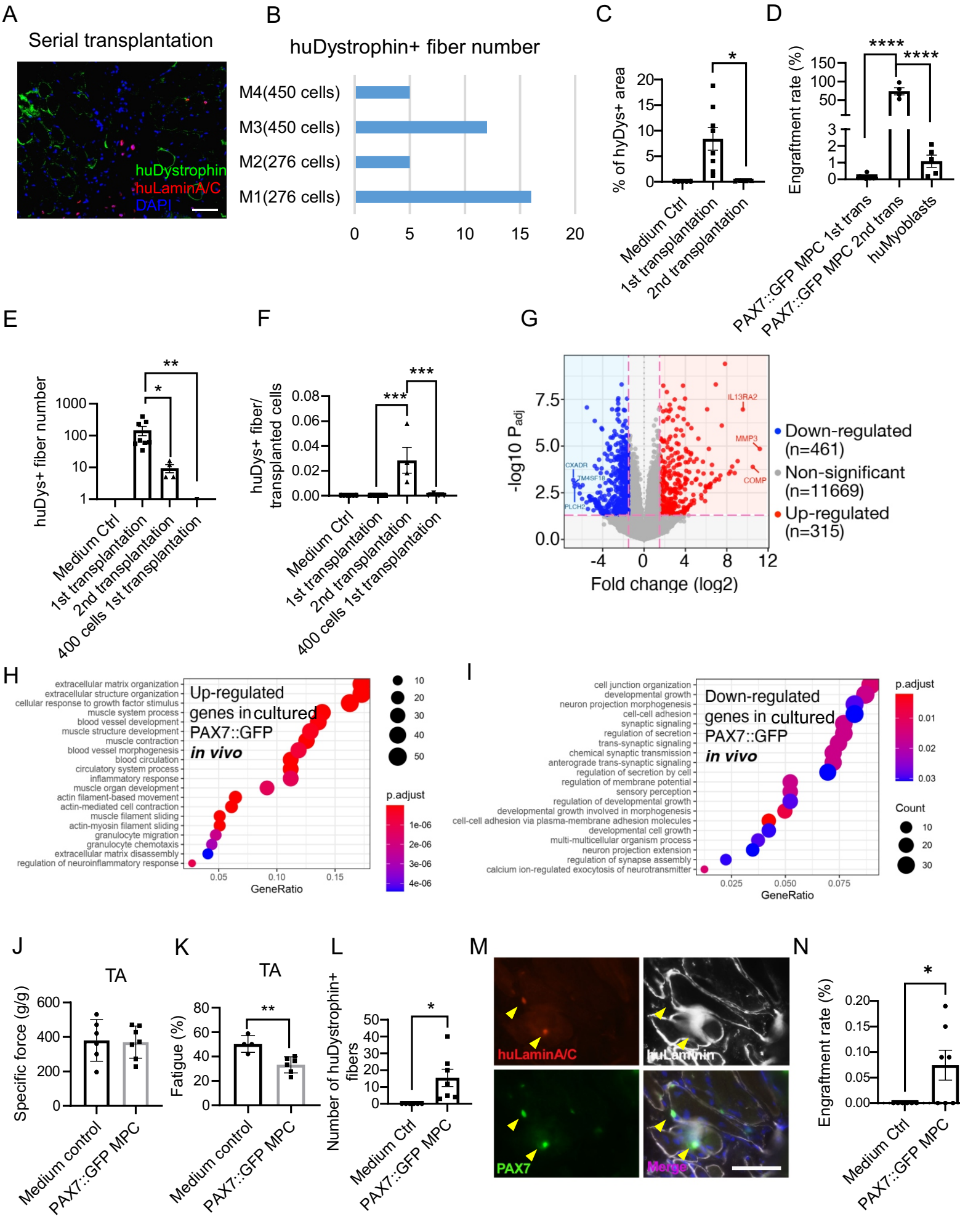
A
Serial transplantation
huDystrophin
huLaminA/C
DAPI
B
huDystrophin+ fiber number
M4(450 cells)
M3(450 cells)
M2(276 cells)
M1(276 cells)
0
5
10
15
20
C
% of hyDys+ area
Medium Ctrl
1st transplantation
2nd transplantation
D
Engraftment rate (%)
PAX7::GFP MPC 1st trans
PAX7::GFP MPC 2nd trans
huMyoblasts
E
huDys+ fiber number
Medium Ctrl
1st transplantation
2nd transplantation
400 cells 1st transplantation
F
huDys+ fiber/ transplanted cells
G
-log10 Padj
Fold change (log2)
IL13RA2
MMP3
COMP
CXADR
TM4SF18
PLCH2
Down-regulated (n=461)
Non-significant (n=11669)
Up-regulated (n=315)
H
Up-regulated genes in cultured PAX7::GFP in vivo
extracellular matrix organization
extracellular structure organization
cellular response to growth factor stimulus
muscle system process
blood vessel development
muscle structure development
muscle contraction
blood vessel morphogenesis
blood circulation
circulatory system process
inflammatory response
muscle organ development
actin filament-based movement
actin-mediated cell contraction
muscle filament sliding
actin-myosin filament sliding
granulocyte migration
granulocyte chemotaxis
extracellular matrix disassembly
regulation of neuroinflammatory response
GeneRatio
p.adjust
I
Down-regulated genes in cultured PAX7::GFP in vivo
cell junction organization
developmental growth
neuron projection morphogenesis
cell-cell adhesion
synaptic signaling
regulation of secretion
trans-synaptic signaling
chemical synaptic transmission
anterograde trans-synaptic signaling
regulation of secretion by cell
regulation of membrane potential
sensory perception
regulation of developmental growth
developmental growth involved in morphogenesis
cell-cell adhesion via plasma-membrane adhesion molecules
developmental cell growth
multi-multicellular organism process
neuron projection extension
regulation of synapse assembly
calcium ion-regulated exocytosis of neurotransmitter
GeneRatio
p.adjust
Count
J
TA
Specific force (g/g)
Medium control
PAX7::GFP MPC
K
TA
Fatigue (%)
L
Number of huDystrophin+ fibers
M
huLaminA/C
PAX7
Merge
N
Engraftment rate (%)

Figure S1. Generation of PAX7::GFP MPCs from hESC differentiation cultures, related with Figure 1. (A) Schematic of construct used to generate stable human hESC lines via CRISPR-Cas9. (B) Schematic of myogenic derivation protocol used to generate myogenic lineage cells and ultimately PAX7::GFP MPCs, which include PAX7 expressing myogenic progenitor cells and myocytes that do not express PAX7. (C-E) FACS plots showing enriching PAX7::GFP+ cells from day 30 myogenic derivation culture and from expanded PAX7::GFP MPC culture. (F) Percentage of PAX7::GFP MPC expressing PAX7, MYOD and MyHC protein in *in vitro* culture (passage 2) (G) mRNA expression of *PAX7*, *MYOD*,3e *GFP*, and *NEUROG1* in PAX7::GFP+ freshly sorted cells from myogenic derivation culture, PAX7::GFP+ cells from PAX7::GFP MPC culture, or from non-myogenic cells (fibroblasts). (n=3, Student's t-test, *p<0.05) (H) Immunocytochemistry images of PAX7::GFP MPC culture (passage 2) with PAX7, MYOD, and GFP antibodies showing the expression of these proteins. Scale bar=50μm. (I) Quantification of triple immunocytochemistry staining of GFP (representing PAX7::GFP), MYOD and Myogenin. (n=4) (J) Immunocytochemistry images of PAX7::GFP MPC under differentiation condition using MyHC and Desmin antibodies to label myotubes. PAX7::GFP MPC (passage 2) were cultured to confluence and switched to differentiation medium (without serum or growth factors) for 5 days. Scale bar=100μm (K) Immunocytochemistry images of MyHC of differentiation culture (5 days) of human primary myoblasts and PAX7::GFP MPCs showing myotube formation. (L) Quantification of PAX7+ cells ratio to total nuclei in proliferation culture of human primary myoblasts (passage 3) and PAX7::GFP MPCs (passage 3) culture showing PAX7::GFP MPC culture contains significantly more PAX7 expressing cells. (M) Fusion index (nuclei in myotube/total myotubes) comparison between human primary myoblasts and PAX7::GFP MPCs in differentiation culture. (n(PAX7::GFP MPC)=5, n(Human Myoblasts)=7, Student's t-test, *p<0.05, **p<0.01. Result=Mean±SEM.

Figure S2. PAX7::GFP MPCs participated in muscle regeneration of NSG mice and the *in vivo* PAX7::GFP+ cells express muscle stem cell markers, related with Figure 1 and 2. (A) Image of TAs that had PAX7::GFP MPC transplantation and TA that had medium control injection. (B) Quantification of huDystrophin+ fibers in TA that were transplanted with unsorted cells from myogenic derivation culture or expanded PAX7::GFP MPC (n(Unsorted)=3, n(Expanded)=8; Student's t-test, *p<0.05). (C-D) Quantification of huDystrophin+ fiber number and percentage of huDystrophin area in TA that were transplanted with expanded PAX7::GFP MPC and human primary myoblasts (huMyoblasts) (n(PAX7::GFP MPC)=8, n(huMyoblasts)=4; Student's t-test, *p<0.05). (E) Representative images of TA transplanted with PAX7::GFP MPCs at day 30 post transplantation, stained with MYOD, Myogenin and ERTR7 antibodies. (F) Quantification of cells expressing MYOD, Myogenin and ERTR7, percentage of the total huLaminA/C positive cells. (G-H) Single cell Q-PCR showing quiescent muscle stem cell markers (*CALCR, PMP22*) were expressed at higher level in *in vivo* PAX7::GFP+ cells compared with *in vitro* PAX7::GFP+ cells (n=48; Student's t-test, *p<0.05) (L) Gel image of the PCR product (1171bp) using a chromosome 17 α-satellite human specific primer pair to amplify DNA from the four types of cells. (Dotted line indicates the position of deletion of superfluous lanes) (M) Distribution of Single cell RNA-seq data of *in vivo* PAX7::GFP+ cells in human and mouse genome showing the vast majority of the *in vivo* PAX7::GFP+ cells collected by FACS are human cells. (N) Venn diagram showing overlapping up- and down- regulated genes in *in vivo* PAX7::GFP+ cells compared with quiescent human satellite cells (huSCs) assuming constant RNA content. (O) Venn diagram showing overlapping up- and down- regulated genes in *in vivo* PAX7::GFP+ cells compared with quiescent huSCs assuming non-constant RNA content (Charville et al., 2015).

Figure S3. Gene expression analysis of single cell RNA-seq including *in vivo* PAX7::GFP+ collected at 1-, 2-, 3-, and 4-week time points after transplantation, related with Figure 3. (A-E) Normalized mRNA expression comparison of the single cell RNA seq data between *in vivo* PAX7::GFP+ cells, *in vitro* PAX7::GFP+ cells, and hESCs. (F) Heat map of single cell RNA-seq result showing gene expression from each cell from *in vivo*, *in vitro*, and 1 month *in vitro* PAX7::GFP+ cell groups. Genes presented in this heat map are related to the pathways shown in Figure 2F. (G) T-SNE plots of *in vivo* PAX7::GFP+ cells harvested 1-week, 2-weeks, 3-weeks and 4-weeks after transplantation in a combination with *in vivo* PAX7::GFP+ cells, *in vitro* cells, and 1 month *in vitro* cells from the first single cell RNA-seq study. (H) Violin plot showing the developmental stages of *in vivo* PAX7::GFP+ cells at the aforementioned time points following the transplantation.

Figure S4. *In vivo* engraftment enhanced PAX7::GFP+ cells regeneration capability and the transplantation result of NSG-*mdx* mice, related with Figure 4. (A) Representative immunohistochemistry image of NSG mouse TA after serial transplantation showing huDystrophin+ muscle fiber formed from the secondary transplanted cells. Scale bar=50μm. (B) Quantification of huDystrophin+ fiber numbers from each NSG mouse that received serial PAX7::GFP+ cell transplantation. Cell number being transplanted was listed for each mouse (M 1-4). Result=Mean±SEM. (C) Quantification of huDystrophin+ area from the entire TA cross section area (n(Control)=3, n(1st transplantation)=6, n(2nd transplantation)=4; One-way ANOVA, *p<0.05).
(D) Quantification of cells located between muscle fiber and basal lamina (huLaminin labeled) that originated from PAX7::GFP MPCs (Pax7+, huLaminA/C+) after 1st or 2nd transplantation, as a proportion of the total number of PAX7::GFP MPCs transplanted. n(1st transplantation)=11, n(2nd transplantation)=4; n(humyoblast)=4, (c) student t test (d) one-way ANOVA , **p<0.001,****<0.00001). (E) Quantification of huDystrophin+ fibers from the entire TA (n(Control)=3, n(1st transplantation)=6, n(2nd transplantation)=4, n(400 cells)=4; One-way ANOVA, *p<0.05). (F) Quantification of ratio of huDystrophin+ fibers to the cell numbers being injected (n(Control)=3, n(1st transplantation)=6, n(2nd transplantation)=4; n(400 cells)=4; One-way ANOVA, ***p<0.001). (G) A volcano plot and gene ontology (H-I) analysis of differentially expressed genes, up- and down-regulated in *in vivo* PAX7::GFP+ cells that were isolated from primary recipient mice and cultured *in vitro* for 4 days (right before 2nd transplantation) in comparison with *in vitro* maintained PAX7::GFP MPCs (right before 1st transplantation). (J-N) Experimental results of NSG-*mdx* cohort-2. (J) Specific force (muscle force normalized to muscle mass (MM), g/g) of TA muscle (n(Control)=6, n(PAX7::GFP MPCs)=7; student's t test). (K) Fatigue index (%) represents the reduction in maximal tetanic tension measured after 5 minutes of repeated tetanic stimulation at 1 Hz in TA muscle (n(Control)=6, n(PAX7::GFP MPC)=5; student's t test, **p<0.01). (L) Quantification of combined number of huDystrophin+ fibers in TA, Gas, and Quad in NSG-*mdx* mice. (n(Control)=6, n(PAX7::GFP MPC)=7; Student's t-test, *p<0.05). (M) Representative images of sectioned TAs 4 weeks after the PAX7::GFP MPC transplantation into NSG-*mdx* mice. PAX7::GFP MPC progenies could be identified by double labeling with huLaminA/C (red) and PAX7 (green) antibodies. They locate under basal lamina (yellow arrow). Scale bar=50μm. (N) Quantification of cells located between muscle fiber and basal lamina (huLaminin labeled) that originated from PAX7::GFP MPCs (PAX7+, huLaminA/C+) after transplantation, as a proportion of the total number of PAX7::GFP MPCs transplanted (combined number in TA, Gas, and Quad in NSG-*mdx* mice). (n(Control)=6, n(PAX7::GFP MPC)=7; Student's t-test, *p<0.05). Result=Mean±SEM.