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Supplemental Information

Cell-Surface Protein Profiling Identifies Distinctive Markers of Progenitor Cells in Human Skeletal Muscle

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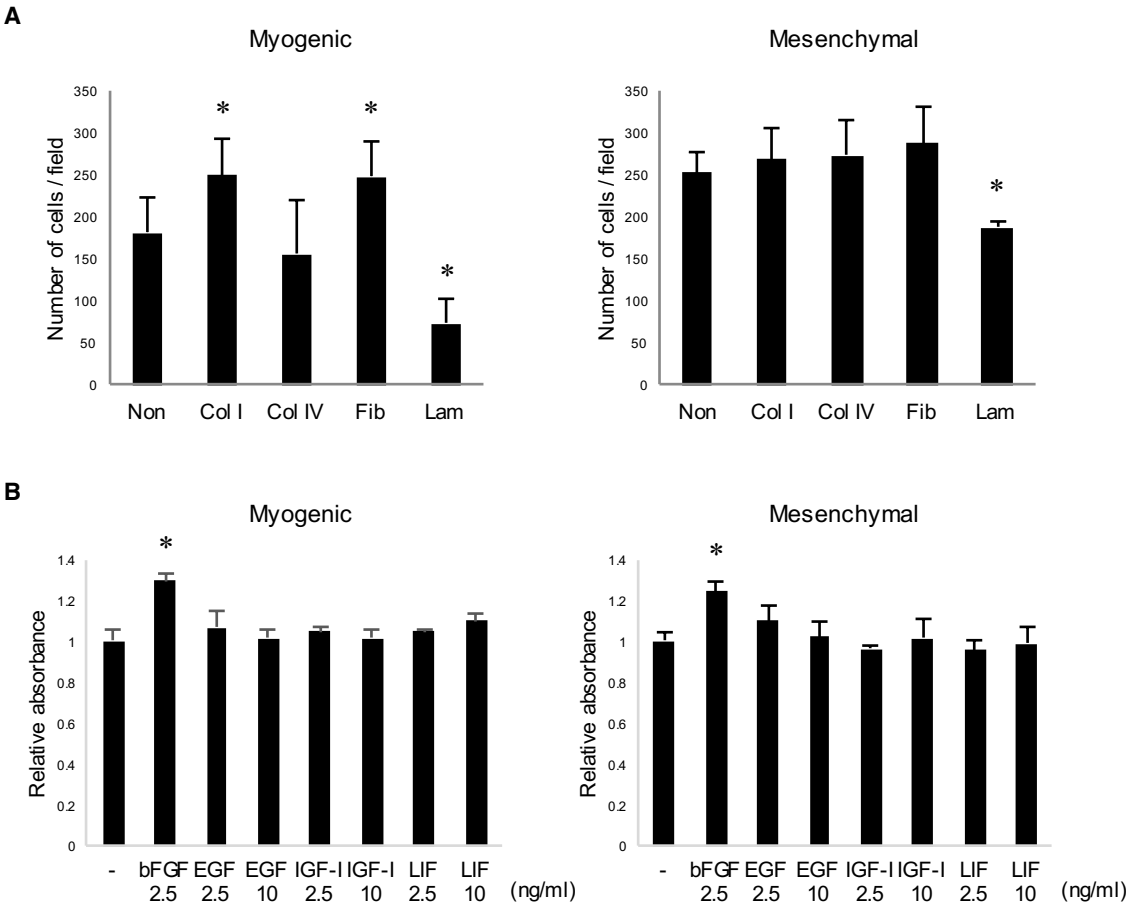
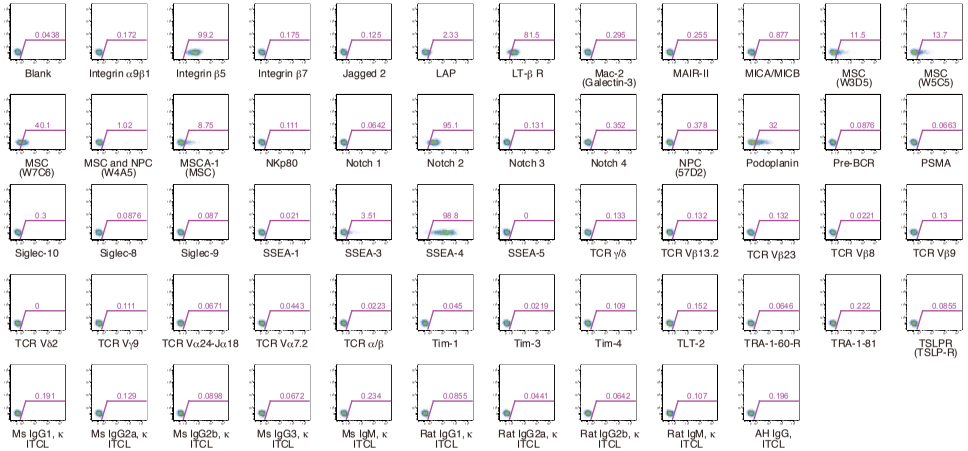

A
Myogenic
Mesenchymal
Number of cells / field
0
50
100
150
200
250
300
350
Non
Col I
Col IV
Fib
Lam
B
Relative absorbance
0
0.2
0.4
0.6
0.8
1
1.2
1.4
-
bFGF 2.5
EGF 2.5
EGF 10
IGF-I 2.5
IGF-I 10
LIF 2.5
LIF 10
(ng/ml)

Figure S1


0.0434 Blank
0.216 CD1a
0.111 CD1b
0.109 CD1c
0.0627 CD1d
0.109 CD2
0.0206 CD3
0.0445 CD4
0.0437 CD5
0.174 CD6
0.192 CD7
0.0878 CD8a
100 CD9
96.5 CD10
0.0222 CD11a
0.149 CD11b
0.196 CD11b (activated)
0.154 CD11c
100 CD13
0.155 CD14
0.0861 CD15
0.088 CD16
0.177 CD18
0.15 CD19
0.151 CD20
0.0222 CD21
0.13 CD22
0.0218 CD23
78 CD24
0.0438 CD25
80.1 CD26
0 CD27
0.592 CD28
100 CD29
0.044 CD30
0.238 CD31
0.0659 CD32
0.0872 CD33
0.0878 CD34
0.0444 CD35
0.132 CD36
0.0654 CD38
0.0658 CD39
0.438 CD40
0 CD41
0.0662 CD42b
0.274 CD43
99.9 CD44
0.0641 CD45
0.0216 CD45RA
0.0216 CD45RB
0.109 CD45RO
35.1 CD46
100 CD47
0.219 CD48
52.7 CD49a
100 CD49c
71.3 CD49d
100 CD49e
99.6 CD49f
0.0427 CD50 (ICAM-3)
99.9 CD51
93 CD51/61
0.044 CD52
0.111 CD53
3.04 CD54
75.6 CD55
97.4 CD56 (NCAM)
0.0641 CD57
99.8 CD58
100 CD59
29.3 CD61
0.0849 CD62E
0.106 CD62L
0.149 CD62P (P-selectin)
99.9 CD63
0.066 CD64
0.129 CD66a/c/e
0.133 CD66b
0.0432 CD69
0.388 CD70
95.9 CD71
100 CD73
0.172 CD74
0.577 CD79b
0.238 CD80
100 CD81
98.9 CD82
0.472 CD83
0.108 CD84
0.0214 CD85a (ILT5)
0.171 CD85d (ILT4)
0.107 CD85g (ILT7)
0.0852 CD85h (ILT1)
0.28 CD85j (ILT2)
0.0211 CD85k (ILT3)


0.11 Blank
0.11 CD86
0.107 CD87
0.238 CD88
0.0441 CD89
100 CD90 (Thy1)
0.133 CD93
0.264 CD94
99.5 CD95
0.11 CD96
63.4 CD97
100 CD99
0.0888 CD100
0 CD101 (BB27)
0.261 CD102
0.109 CD103
0 CD104
100 CD105
0.646 CD106
54.4 CD107a (LAMP-1)
97.9 CD108
91.4 CD109
31.9 CD111
99.4 CD112 (Nectin-2)
0.11 CD114
0.262 CD115
0.247 CD116
0.0658 CD117 (c-kit)
0.462 CD119 (IFN-γRα chain)
0.0441 CD122
0.132 CD123
0.223 CD124
0.086 CD126 (IL-6Rα)
0.0445 CD127 (IL-7Rα)
0.156 CD129 (IL-9R)
0.238 CD131
0.111 CD132
0.0672 CD134
0.0883 CD135
0.0877 CD137 (4-1BB)
0.176 CD137L (4-1BB L)
2.64 CD138
0.262 CD140a
0.554 CD140b
92.8 CD141
1.07 CD143
0.267 CD144
94.6 CD146
0.0895 CD148
0.156 CD150 (SLAM)
0.066 CD152
0.0667 CD154
98.1 CD155 (PVR)
97.8 CD156c (ADAM-10)
0.177 CD158a/h
0.0668 CD158b (NKAT2)
0.09 CD158d
0.0441 CD158e1 (NKB1)
0.169 CD158f
0.085 CD161
0.0849 CD162
0.108 CD163
93.9 CD164
99.2 CD165
98.8 CD166
0.26 CD167a (DDR1)
0.0649 CD169
0.197 CD170 (Siglec-5)
99.5 CD172a (SIRPα)
0.0433 CD172b (SIRPβ)
0.0856 CD172g (SIRPγ)
0.192 CD178 (Fas-L)
0.279 CD179a
0.108 CD179b
0.0648 CD180 (RP105)
0.172 CD181 (CXCR1)
0.0442 CD182 (CXCR2)
0.349 CD183
0.201 CD184 (CXCR4)
0.265 CD193 (CCR3)
0.712 CD195 (CCR5)
0.456 CD196
0.28 CD197 (CCR7)
0.149 CD200 (OX2)
0.191 CD200 R
53.5 CD201 (EPCR)
0.252 CD202b (Tie2/Tek)
0.213 CD203c (E-NPP3)
0.107 CD205 (DEC-205)
0.0214 CD206 (MMR)
0.107 CD207 (Langerin)
0.0429 CD209 (DC-SIGN)
0.243 CD210 (IL-10R)
39.6 CD213a2
0.432 CD215 (IL-15Rα)
0.235 CD218a (IL-18Rα)


0.0427 Blank
0.221 CD220
0.242 CD221 (IGF-1R)
0.0874 CD226 (DNAM-1)
0.0839 CD229 (Ly-9)
0.147 CD231 (TALLA)
0.129 CD235ab
0.176 CD243
0.0435 CD244 (2B4)
43.4 CD245 (p220/240)
0.568 CD252 (OX40L)
0.154 CD253 (Trail)
0.0667 CD254
0.153 CD255 (TWEAK)
0.683 CD257 (BAFF)
0.0653 CD258 (LIGHT)
0.0889 CD261 (DR4)
63.1 CD262 (DR5)
0.11 CD263 (DcR1)
29.3 CD266 (Fn14)
0.11 CD267 (TACI)
0.0221 CD268 (BAFF-R)
0.663 CD270 (HVEM)
78.2 CD271
20.4 CD273 (B7-DC)
94.6 CD274 (B7-H1)
0.109 CD275 (B7-H2)
100 CD276
0.789 CD277
0.0654 CD278 (ICOS)
0.0883 CD279 (PD-1)
0.281 CD282 (TLR2)
0.132 CD284 (TLR4)
0.044 CD286 (TLR6)
0.0219 CD290
0.087 CD294
100 CD298
0.109 CD300e (IREM-2)
0.153 CD300F
1.02 CD301
0.0222 CD303
88 CD304
0.0439 CD307
0.0888 CD307d (FcRL4)
0.0435 CD314 (NKG2D)
0.176 CD317
99.5 CD318 (CPCD1)
0.237 CD319 (CRACC)
0.0216 CD324 (E-Cadherin)
0.175 CD325
0.0861 CD326 (Ep-CAM)
0.0887 CD328 (Siglec-7)
0.13 CD334 (FGFR4)
0.022 CD335 (NKp46)
0.109 CD336 (NKp44)
0.0222 CD337 (NKp30)
0.0431 CD338 (ABCG2)
18.5 CD340 (erbB2)
24.5 CD344 (Frizzled-4)
0.0866 CD351
0.214 CD352 (NTB-A)
0.151 CD354 (TREM-1)
0.0659 CD355 (CRTAM)
0.239 CD357 (GITR)
8.96 CD360 (IL-21R)
100 β2-microglobulin
0.107 BTLA
0.308 C3AR
0.624 C5L2
0.308 CCR10
0.0646 CLEC12A
0.0879 CLEC9A
0.0428 CX3CR1
0.24 CXCR7
0.353 κ-Opioid Receptor
0 DLL1
0.0643 DLL4
0.0222 DR3 (TRAMP)
99.2 EGFR
0.262 erbB3/HER-3
0.066 FcεRIα
69.9 FcRL6
0.463 Galectin-9
8.8 GARP (LRRC32)
46 HLA-A,B,C
0.0853 HLA-A2
0.0215 HLA-DQ
0.149 HLA-DR
0.212 HLA-E
0.0638 HLA-G
0.35 IFN-γ R β chain
0 Ig light chain κ
0.0446 Ig light chain λ
0.169 IgD
0.0422 IgM
0.0844 IL-28RA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99.2 Integrin β5
0.175 Integrin β7
0.125 Jagged 2
2.33 LAP
81.5 LT-β R
0.295 Mac-2 (Galectin-3)
0.255 MAIR-II
0.877 MICA/MICB
11.5 MSC (W3D5)
13.7 MSC (W5C5)
40.1 MSC (W7C6)
1.02 MSC and NPC (W4A5)
8.75 MSCA-1 (MSC)
0.111 NKp80
0.0642 Notch 1
95.1 Notch 2
0.131 Notch 3
0.352 Notch 4
0.378 NPC (57D2)
32 Podoplanin
0.0876 Pre-BCR
0.0663 PSMA
0.3 Siglec-10
0.0876 Siglec-8
0.087 Siglec-9
0.021 SSEA-1
3.51 SSEA-3
98.8 SSEA-4
0 SSEA-5
0.133 TCR γ/δ
0.132 TCR Vβ13.2
0.132 TCR Vβ23
0.0221 TCR Vβ8
0.13 TCR Vβ9
0 TCR Vδ2
0.111 TCR Vγ9
0.0671 TCR Vα24-Jα18
0.0443 TCR Vα7.2
0.0223 TCR α/β
0.045 Tim-1
0.0219 Tim-3
0.109 Tim-4
0.152 TLT-2
0.0646 TRA-1-60-R
0.222 TRA-1-81
0.0855 TSLPR (TSLP-R)
0.191 Ms IgG1, κ ITCL
0.129 Ms IgG2a, κ ITCL
0.0898 Ms IgG2b, κ ITCL
0.0672 Ms IgG3, κ ITCL
0.234 Ms IgM, κ ITCL
0.0855 Rat IgG1, κ ITCL
0.0441 Rat IgG2a, κ ITCL
0.0642 Rat IgG2b, κ ITCL
0.107 Rat IgM, κ ITCL
0.196 AH IgG, ITCL

Figure S2


Blank
CD1a
CD1b
CD1c
CD1d
CD2
CD3
CD4
CD5
CD6
CD7
CD8a
CD9
CD10
CD11a
CD11b
CD11b (activated)
CD11c
CD13
CD14
CD15
CD16
CD18
CD19
CD20
CD21
CD22
CD23
CD24
CD25
CD26
CD27
CD28
CD29
CD30
CD31
CD32
CD33
CD34
CD35
CD36
CD38
CD39
CD40
CD41
CD42b
CD43
CD44
CD45
CD45RA
CD45RB
CD45RO
CD46
CD47
CD48
CD49a
CD49c
CD49d
CD49e
CD49f
CD50 (ICAM-3)
CD51
CD51/61
CD52
CD53
CD54
CD55
CD56 (NCAM)
CD57
CD58
CD59
CD61
CD62E
CD62L
CD62P (P-selectin)
CD63
CD64
CD66a/c/e
CD66b
CD69
CD70
CD71
CD73
CD74
CD79b
CD80
CD81
CD82
CD83
CD84
CD85a (ILT5)
CD85d (ILT4)
CD85g (ILT7)
CD85h (ILT1)
CD85j (ILT2)
CD85k (ILT3)


Blank
CD86
CD87
CD88
CD89
CD90 (Thy1)
CD93
CD94
CD95
CD96
CD97
CD99
CD100
CD101 (BB27)
CD102
CD103
CD104
CD105
CD106
CD107a (LAMP-1)
CD108
CD109
CD111
CD112 (Nectin-2)
CD114
CD115
CD116
CD117 (c-kit)
CD119 (IFN-γRα chain)
CD122
CD123
CD124
CD126 (IL-6Rα)
CD127 (IL-7Rα)
CD129 (IL-9R)
CD131
CD132
CD134
CD135
CD137 (4-1BB)
CD137L (4-1BB L)
CD138
CD140a
CD140b
CD141
CD143
CD144
CD146
CD148
CD150 (SLAM)
CD152
CD154
CD155 (PVR)
CD156c (ADAM-10)
CD158a/h
CD158b (NKAT2)
CD158d
CD158e1 (NKB1)
CD158f
CD161
CD162
CD163
CD164
CD165
CD166
CD167a (DDR1)
CD169
CD170 (Siglec-5)
CD172a (SIRPα)
CD172b (SIRPβ)
CD172g (SIRPγ)
CD178 (Fas-L)
CD179a
CD179b
CD180 (RP105)
CD181 (CXCR1)
CD182 (CXCR2)
CD183
CD184 (CXCR4)
CD193 (CCR3)
CD195 (CCR5)
CD196
CD197 (CCR7)
CD200 (OX2)
CD200 R
CD201 (EPCR)
CD202b (Tie2/Tek)
CD203c (E-NPP3)
CD205 (DEC-205)
CD206 (MMR)
CD207 (Langerin)
CD209 (DC-SIGN)
CD210 (IL-10R)
CD213a2
CD215 (IL-15Rα)
CD218a (IL-18Rα)


Blank
CD220
CD221 (IGF-1R)
CD226 (DNAM-1)
CD229 (Ly-9)
CD231 (TALLA)
CD235ab
CD243
CD244 (2B4)
CD245 (p220/240)
CD252 (OX40L)
CD253 (Trail)
CD254
CD255 (TWEAK)
CD257 (BAFF)
CD258 (LIGHT)
CD261 (DR4)
CD262 (DR5)
CD263 (DcR1)
CD266 (Fn14)
CD267 (TACI)
CD268 (BAFF-R)
CD270 (HVEM)
CD271
CD273 (B7-DC)
CD274 (B7-H1)
CD275 (B7-H2)
CD276
CD277
CD278 (ICOS)
CD279 (PD-1)
CD282 (TLR2)
CD284 (TLR4)
CD286 (TLR6)
CD290
CD294
CD298
CD300e (IREM-2)
CD300F
CD301
CD303
CD304
CD307
CD307d (FcRL4)
CD314 (NKG2D)
CD317
CD318 (CPCD1)
CD319 (CRACC)
CD324 (E-Cadherin)
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CD326 (Ep-CAM)
CD328 (Siglec-7)
CD334 (FGFR4)
CD335 (NKp46)
CD336 (NKp44)
CD337 (NKp30)
CD338 (ABCG2)
CD340 (erbB2)
CD344 (Frizzled-4)
CD351
CD352 (NTB-A)
CD354 (TREM-1)
CD355 (CRTAM)
CD357 (GITR)
CD360 (IL-21R)
β2-microglobulin
BTLA
C3AR
C5L2
CCR10
CLEC12A
CLEC9A
CX3CR1
CXCR7
δ-Opioid Receptor
DLL1
DLL4
DR3 (TRAMP)
EGFR
erbB3/HER-3
FcεRIα
FcRL6
Galectin-9
GARP (LRRC32)
HLA-A,B,C
HLA-A2
HLA-DQ
HLA-DR
HLA-E
HLA-G
IFN-γ R β chain
Ig light chain κ
Ig light chain λ
IgD
IgM
IL-28RA

Blank
Integrin α9β1
Integrin β5
Integrin β7
Jagged 2
LAP
LT-β R
Mac-2 (Galectin-3)
MAIR-II
MICA/MICB
MSC (W3D5)
MSC (W5C5)
MSC (W7C6)
MSC and NPC (W4A5)
MSCA-1 (MSC)
NKp80
Notch 1
Notch 2
Notch 3
Notch 4
NPC (57D2)
Podoplanin
Pre-BCR
PSMA
Siglec-10
Siglec-8
Siglec-9
SSEA-1
SSEA-3
SSEA-4
SSEA-5
TCR γ/δ
TCR Vβ13.2
TCR Vβ8
TCR Vβ9
TCR Vδ2
TCR Vγ9
TCR Vα24-Jα18
TCR Vα7.2
TCR α/β
Tim-1
Tim-3
Tim-4
TLT-2
TRA-1-60-R
TRA-1-81
TSLPR (TSLP-R)
Ms IgG1, κ ITCL
Ms IgG2a, κ ITCL
Ms IgG2b, κ ITCL
Ms IgG3, κ ITCL
Ms IgM, κ ITCL
Rat IgG1, κ ITCL
Rat IgG2a, κ ITCL
Rat IgG2b, κ ITCL
Rat IgM, κ ITCL
AH IgG, ITCL

Figure S3

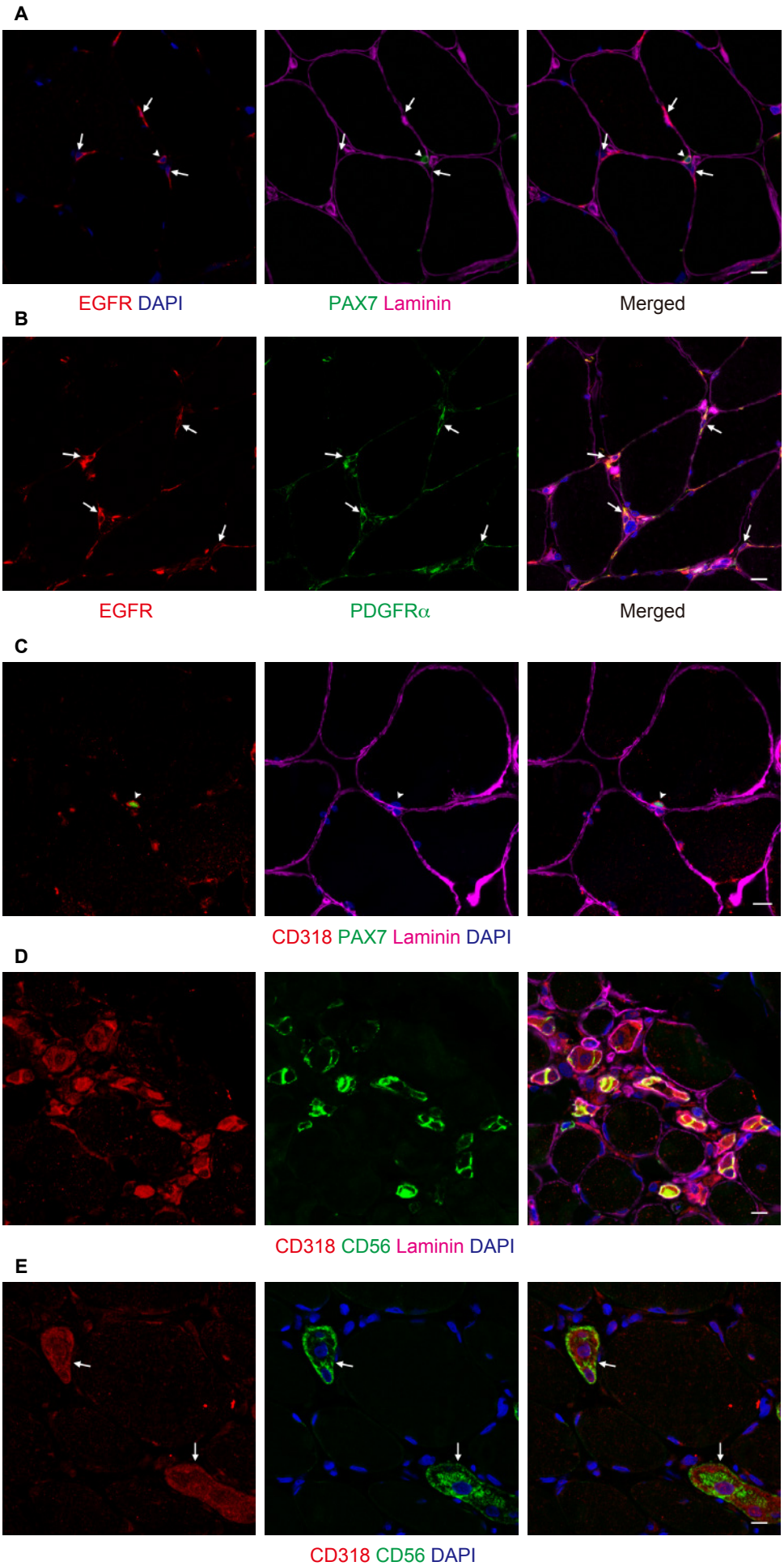
A
EGFR DAPI
PAX7 Laminin
Merged
B
EGFR
PDGFRα
Merged
C
CD318 PAX7 Laminin DAPI
D
CD318 CD56 Laminin DAPI
E
CD318 CD56 DAPI

Figure S4

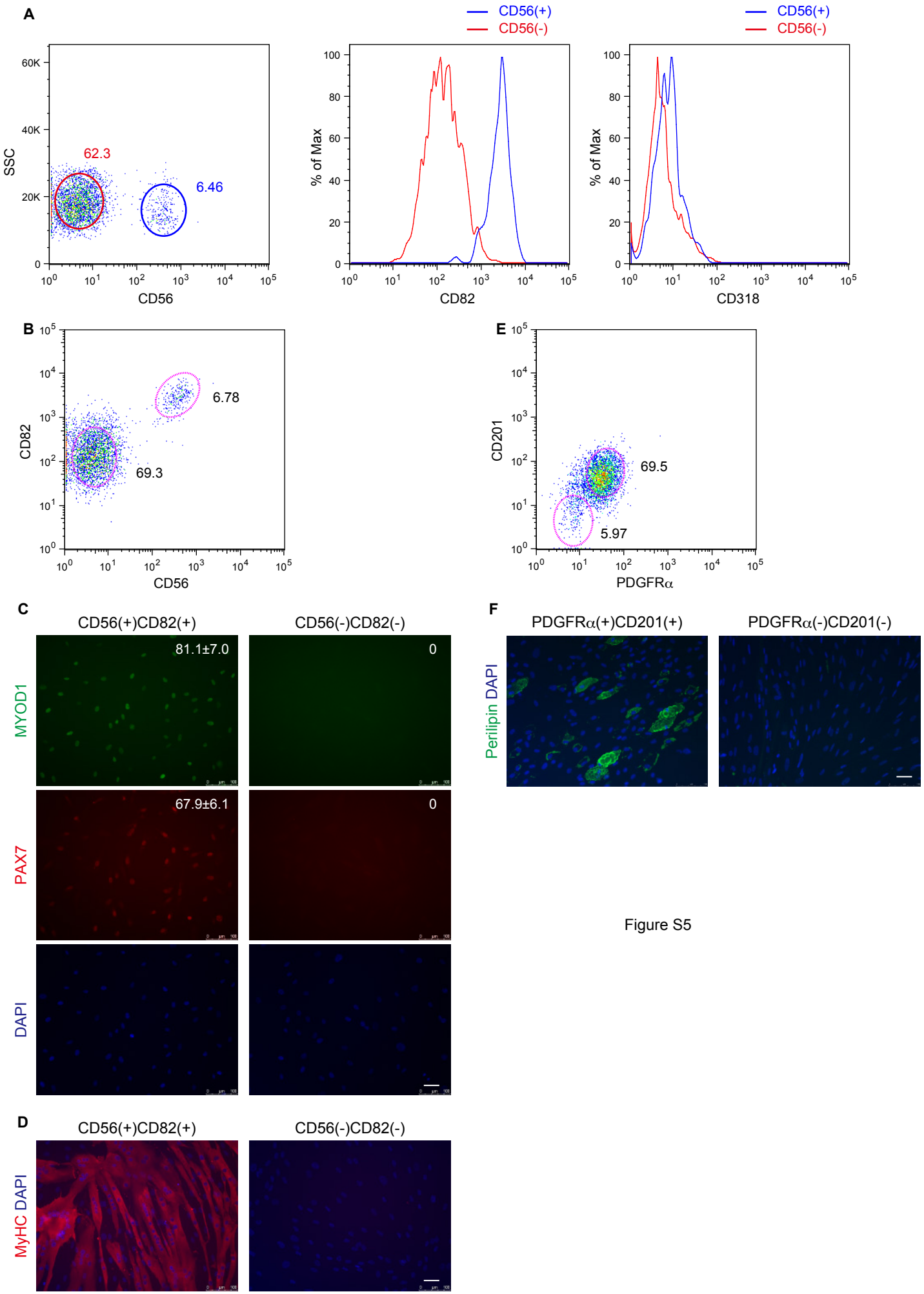
A
CD56(+)
CD56(-)
SSC
62.3
6.46
CD56
% of Max
CD82
CD318
B
CD82
6.78
69.3
CD56
E
CD201
69.5
5.97
PDGFRα
C
CD56(+)CD82(+)
CD56(-)CD82(-)
MYOD1
81.1±7.0
0
PAX7
67.9±6.1
0
DAPI
F
PDGFRα(+)CD201(+)
PDGFRα(-)CD201(-)
Perilipin DAPI
D
CD56(+)CD82(+)
CD56(-)CD82(-)
MyHC DAPI

Figure S5

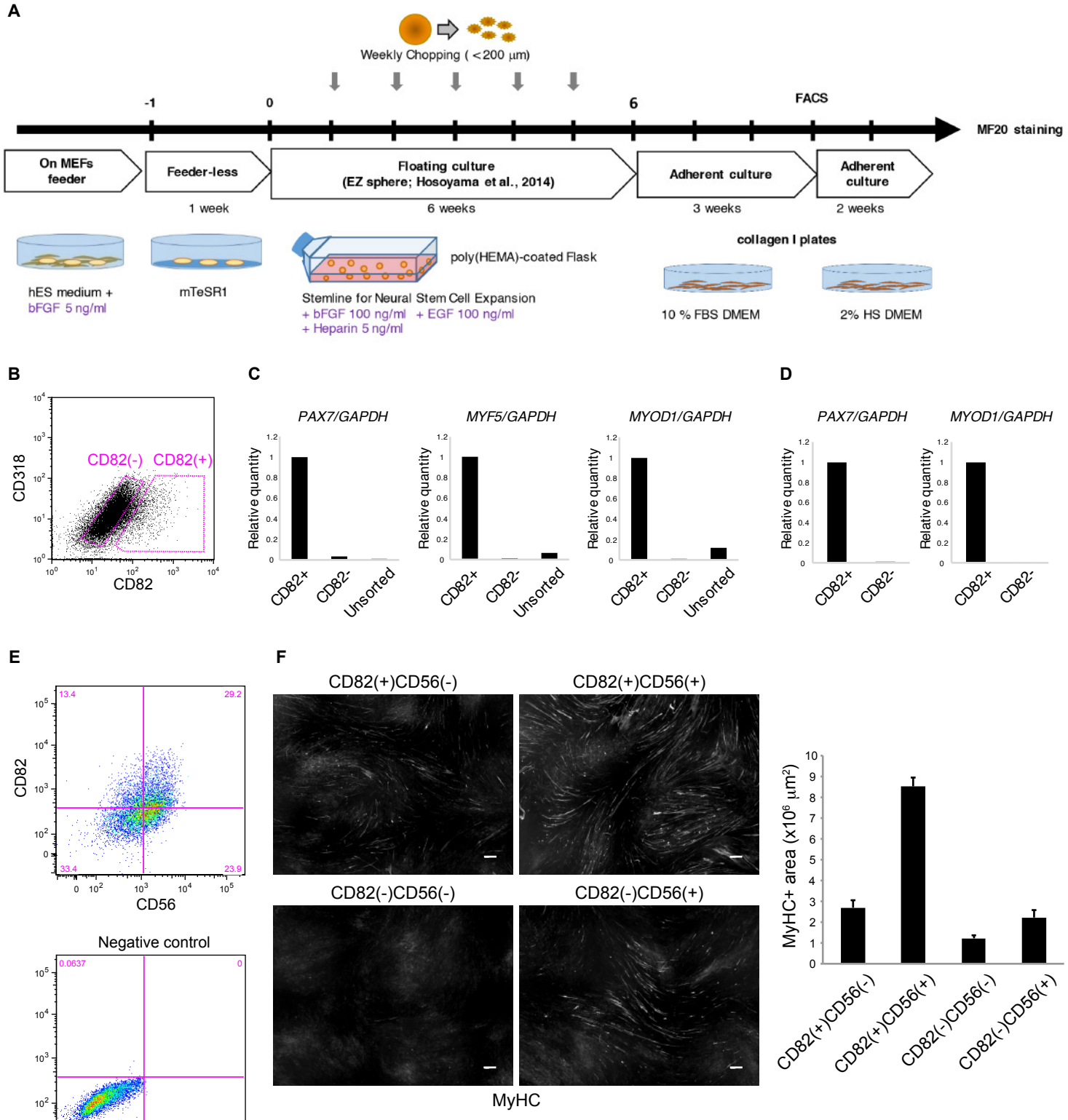
A
Weekly Chopping (<200 μm)
-1
0
6
FACS
MF20 staining
On MEFs feeder
Feeder-less
Floating culture (EZ sphere; Hosoyama et al., 2014)
Adherent culture
Adherent culture
1 week
6 weeks
3 weeks
2 weeks
hES medium + bFGF 5 ng/ml
mTeSR1
poly(HEMA)-coated Flask
Stemline for Neural Stem Cell Expansion
+ bFGF 100 ng/ml + EGF 100 ng/ml
+ Heparin 5 ng/ml
collagen I plates
10 % FBS DMEM
2% HS DMEM
B
CD82(-) CD82(+)
CD318
CD82
C
PAX7/GAPDH
MYF5/GAPDH
MYOD1/GAPDH
Relative quantity
CD82+
CD82-
Unsorted
D
PAX7/GAPDH
MYOD1/GAPDH
Relative quantity
CD82+
CD82-
E
CD82
CD56
13.4
29.2
33.4
23.9
Negative control
0.0637
0
99.8
0.102
F
CD82(+)CD56(-)
CD82(+)CD56(+)
CD82(-)CD56(-)
CD82(-)CD56(+)
MyHC
MyHC+ area (x10^6 μm^2)
CD82(+)CD56(-)
CD82(+)CD56(+)
CD82(-)CD56(-)
CD82(-)CD56(+)

Figure S6

Supplemental Figure Legends

Figure S1 (Related to Figure 1). Optimization of coating substrates and growth factors for the expansion of human muscle-derived progenitors.

(A) Muscle-derived progenitors were cultured for 3 d on the indicated coating substrates. Collagen I, collagen IV, fibronectin, and laminin were used as coating substrates. Number of cells per fields was quantified and expressed as means ± s.d., n=15 fields from three independent preparations. *P<0.01. (B) Muscle-derived progenitors were cultured for 3 d in medium containing indicated growth factors. Cell proliferation was measured by using WST-1 reagent and shown as means ± s.d. of three independent preparations. *P<0.01.

Figure S2 (Related to Figure 1). Entire data set of antibody array of cultured $CD56^+$ myogenic progenitors.

The entire FACS data of antibody array obtained from cultured $CD56^+$ myogenic progenitors were shown.

Figure S3 (Related to Figure 1). Entire data set of antibody array of cultured $PDGFR\alpha^+$ mesenchymal progenitors.

The entire FACS data of antibody array obtained from cultured $PDGFR\alpha^+$ mesenchymal progenitors were shown.

Figure S4 (Related to Figure 1 and 2). Expression of EGFR and CD318 in human skeletal muscle.

(A) Adult human muscle sections were stained with antibodies against EGFR, PAX7, and Laminin. Arrowheads indicate satellite cells located beneath the basement membrane. Arrows indicate $EGFR^+$ cells located in the interstitial space. (B) Adult human muscle sections were stained with antibodies against EGFR, PDGFRα, and Laminin. Arrows indicate $PDGFR\alpha^+EGFR^+$ cells located in the interstitial space. (C) Adult human muscle sections were stained with antibodies against CD318, PAX7, and Laminin. Arrowheads indicate satellite cells located beneath the basement membrane. (D) DMD muscle sections were stained with antibodies against CD318, CD56, and Laminin. (E) DMD muscle sections were stained

with antibodies against CD318 and CD56. Arrows indicate $CD56^+$ centrally-nucleated myofibers. Scale bars: 10 μm.

Figure S5 (Related to Figure 3). Purification of myogenic or mesenchymal progenitors from commercially available human myoblast culture.

(A) Cells in HSMM culture were stained with antibodies against CD56, CD82, and CD318. $CD56^+$ cells (blue) and $CD56^-$ negative cells (red) were analyzed for expression of CD82 and CD318 (shown in the right). (B) FACS sorting based on CD56 and CD82 expression. Sorting gates are shown in the panel. (C) The indicated cell populations were cultured in growth condition for 3 d, and then stained with antibodies against MYOD1 and PAX7. The percentages of positive cells are shown in the panels as means ± s.d., n=6 fields from two independent experiments. (D) The indicated cell populations were cultured in the myogenic differentiation condition for 5 d, and then stained with anti-MyHC antibody. (E) Cells in HSMM cultures were stained with anti-PDGFRα and anti-CD201 antibodies. Sorting gates are shown in the panel. (F) The indicated cell populations were subjected to the adipogenic condition and stained with anti-Perilipin antibody. Scale bars: 50 μm in C-F.

Figure S6 (Related to Figure 3). Isolation of myogenic progenitors from human iPSCs.

(A) Scheme of myogenic induction by the EZ sphere method from human iPSCs. (B) Clone 253G4 human iPSCs were induced to differentiate into a myogenic lineage and then maintained in DMEM supplemented with 2% HS. Induced cells were analyzed for CD82 and CD318 expression. (C) Expressions of myogenic progenitor genes in the indicated fractions of clone 253G4-derived cells. (D) Expressions of myogenic progenitor genes in the indicated fractions of clone 201B7-derived cells. (E) Clone 454E2 human iPS cells were induced to differentiate into the myogenic lineage and induced cells were analyzed for CD82 and CD56 expression. The lower plot shows negative control stained with isotype control antibodies. (F) Sorted fractions were cultured in DMEM supplemented with 2% HS for 2 wks and then subjected to immunofluorescent staining for MyHC. Scale bars: 250 μm. $MyHC^+$ area in each cell population was quantified and shown as means ± s.d.

Table S1. Cell surface genes that are up-regulated (> 5-fold) in quiescent satellite cells compared with non-myogenic cells, Related to Discussion

Genbank	Symbol	Description	Fold Change (vs non-myogenic)
NM_007588	Calcr	calcitonin receptor	83.7
NM_007662	Cdh15	cadherin 15	60.13
AW536432	Hs6st2	heparan sulfate 6-O-sulfotransferase 2	43.54
AB025413	Odz4	odd Oz/ten-m homolog 4 (Drosophila)	23.58
NM_008516	Lrrn1	leucine rich repeat protein 1, neuronal	21.4
NM_010298	Glrb	glycine receptor, beta subunit	20.46
NM_007656	Kai1	kangai 1 (suppression of tumorigenicity 6, prostate)	20.08
BB250384	Vcam1	BB250384 RIKEN full-length enriched, 7 days neonate cerebellum Mus musculus cDNA clone A730041B10 3' similar to M84487 Mouse vascular cell adhesion molecule-1 mRNA, mRNA sequence.	19.68
NM_007539	Bdkrb1	bradykinin receptor, beta 1	17.45
BC005679	Sdc4	syndecan 4	12.9
AF127140	Fgfr4	fibroblast growth factor receptor 4	9.342
AF311699	Chodl	chondrolectin	8.491
D87747	Cxcr4	chemokine (C-X-C motif) receptor 4	8.359
U37709	Musk	muscle, skeletal, receptor tyrosine kinase	8.045
M84756	Ank1	ankyrin 1, erythroid	7.864
AW494220	Mgl2	macrophage galactose N-acetyl-galactosamine specific lectin 2	7.444
AI849305	Ptprz1	protein tyrosine phosphatase, receptor type Z, polypeptide 1	7.268
NM_021301	Slc15a2	solute carrier family 15 (H+/peptide transporter), member 2	7.233
BC016109	AI481750	expressed sequence AI481750	7.225
BC015076	Eva	epithelial V-like antigen	6.968
AK010040	Il17r	interleukin 17 receptor	6.718
NM_053134	Pcdhb9	protocadherin beta 9	6.718
BB234940	Ddr1	discoidin domain receptor family, member 1	6.171
AK009873	2310047E01Rik	RIKEN cDNA 2310047E01 gene	6.075
BG094386	Dag1	dystroglycan 1	5.985
BQ173958	F2r	coagulation factor II (thrombin) receptor	5.966
NM_023450	2010204K13Rik	RIKEN cDNA 2010204K13 gene	5.927
X15052	Ncam1	neural cell adhesion molecule 1	5.477
AB026551	AA408868	expreexpressed sequence AA408868	5.41
NM_008398	Itga7	integrin alpha 7	5.254
NM_007429	Agtr2	angiotensin II receptor, type 2	5.096

Microarray data (Fukada et al., Stem Cells, 2007) is re-analyzed to identify cell surface genes that are specifically expressed in mouse satellite cells.

Supplemental Experimental procedures

Human muscle samples

Experiments using human samples were approved by the Ethical Review Board for Clinical Studies at Fujita Health University. Non-dystrophic muscle samples were obtained from gluteus medius muscles of 56 subjects ranging in age from 40 to 83 years undergoing total hip arthroplasty. DMD muscle samples were obtained from muscle (rectus femoris or biceps brachii) biopsies performed for diagnostic purposes on 11 DMD patients ranging in age from nine months to 23 years. Dystrophin deficiency was confirmed by immunohistochemistry and Western blotting. All patients or their parents gave written informed consent. A human myoblast culture was purchased from Lonza.

Dissociation of cells from muscle samples

Muscles were transferred to PBS. Nerves, blood vessels, tendons, and fat tissues were carefully removed. Trimmed muscles were minced and digested with 0.2% type II collagenase (Worthington) for 30 min at 37°C. Digested muscles were passed through an 18-gauge needle several times and further digested for 15 min at 37°C. Muscle slurries were filtered through a 100-μm cell strainer (BD Biosciences) and then through a 40-μm cell strainer (BD Biosciences). Erythrocytes were eliminated by treating the cells with Tris-buffered 0.8% NH_4Cl. Cells were resuspended in growth medium (GM) consisting of DMEM supplemented with 20% FBS, 1% penicillin-streptomycin, and 2.5 ng/ml bFGF (Katayama Chemical), seeded onto collagen I-coated dish (Iwaki), and maintained at 37°C in 5% CO_2 and 3% O_2.

FACS

Cells were trypsinized and resuspended in washing buffer consisting of PBS with 2.5% FBS, and stained with primary antibodies for 30 min at 4°C. Cells were then stained with streptavidin-PE/Cy5 (1:200, BD Pharmingen, #554062) or anti-Goat IgG-PE (1:200, R&D, #705-116-147) for 30 min at 4°C in the dark. Primary antibodies used for cell staining were VioBright FITC- or PE-conjugated anti-CD56 (1:20, Miltenyi Biotec, #130-104-944 or 130-090-755), FITC- or PE/Cy7-conjugated anti-CD56 (1:40, BD Pharmingen, #335791), biotinylated anti-PDGFRα (2.5 μg/ml, R&D, #BAF322), BB515-conjugated anti-PDGFRα (1:40, BD Pharmingen, #564594), PE- or Alexa Fluor 647-conjugated anti-CD82 (1:40,

BioLegend, #342104 or 342108), APC-conjugated anti-CD82 (1:200, Miltenyi Biotec, #130-101-311), PE-conjugated anti-CD274 (1:40, BioLegend, #329706), PE-conjugated anti-CD318 (1:40, BioLegend, #324006), PE-conjugated anti-CD201 (1:40, BioLegend, #351904), PE-conjugated anti-CD141 (1:40, BioLegend, #344104), and anti-PAR1 (5 μg/ml, R&D, #AF3855). Stained cells were analyzed by FACSVerse, FACSVantage SE (BD Biosciences), or MoFlo Astrios (Beckman Coulter). Cell sorting was performed on a FACSVantage SE or MoFlo Astrios.

Antibody screening

After cell sorting, $CD56^+$ cells or $PDGFR\alpha^+$ cells were cultured in GM on collagen I-coated dish at 37°C in 5% CO_2 and 3% O_2, and expanded to 1 x 10^8 cells. Antibody screening was carried out using a LEGENDScreen human cell screening kit (BioLegend). Expanded $CD56^+$ cells or $PDGFR\alpha^+$ cells were added to 96-well plates containing antibodies at 3 x 10^5 cells/well. Stained cells were analyzed by FACSVerse (BD Biosciences). Obtained data were further analyzed using FlowJo software (FlowJo LLC).

Cell culture

Sorted cells were cultured on Matrigel-coated (BD Biosciences) 48-well plates in GM at 37°C in 5% CO_2 and 3% O_2. Ten thousand cells were added per well. After 3 d incubation, cells were incubated in DMEM with 5% horse serum (HS) for 5 d at 37°C in 5% CO_2 and 21% O_2 for myogenic differentiation. For adipogenic differentiation, cells were treated with adipogenic induction medium consisting of DMEM with 10% FBS, 0.5 mM IBMX (Sigma), 0.25 μM dexamethasone (Sigma), and 10 μg/ml insulin (Sigma) for 3 d, followed by adipogenic maintenance medium consisting of DMEM with 10% FBS and 10 μg/ml insulin for 1 d at 37°C in 5% CO_2 and 21% O_2. This treatment was repeated three times. For knockdown of CD82, 5 nM of Silencer Select siRNA for CD82 (Ambion, sense: 5′-GCCCUCAAGGGUGUGUAUAtt-3′, antisense: 5′-UAUACACACCCUUGAGGGCag-3′) or control siRNA were transfected by using Lipofectamine RNAiMAX transfection reagent (Invitrogen). Five thousand cells were seeded per well of 96-well plate and cultured in GM for 3 d, and cell proliferation was measured by using Cell Counting Kit-8 (Dojindo). SB203580 (Wako), a p38 inhibitor, was used at

10 μM. APC (Haematologic Technologies) was used at 100 or 1000 nM. Human myoblast culture was maintained according to the manufacturer's instructions using a Clonetic SkGM-2 BulletKit (Lonza).

iPSCs and induction of myogenic progenitors

Human iPSCs (clone: 253G4 (Nakagawa et al., 2008), 201B7 (Takahashi et al., 2007), and 454E2 (Okita et al., 2011)) were provided by the Center for iPS Cell Research and Application. Myogenic cells were induced from iPSCs by the EZ sphere method as previously described (Hosoyama et al., 2014). Induced cells were trypsinized and resuspended in washing buffer, then subjected to FACS sorting. Sorted cells were subjected to RNA extraction or cultured on collagen-coated 24-well plates in DMEM supplemented with 10% FBS at 37°C in 5% CO_2. Myotube formation was induced by changing the medium to DMEM supplemented with 2% HS.

Generation of lentiviral vector and in vitro transduction

Plasmids required for generation of lentiviral vector were obtained from RIKEN BRC. The lentiviral vector was generated as described (Ikemoto et al., 2007). Sorted $CD201^+$ cells were plated on Matrigel-coated 24-well plates and cultured in GM containing 200 MOI of viral vector for overnight at 37°C in 5% CO_2 and 3% O_2. Infected cells were trypsinized and expanded in 60-mm dishes. When cells reached 80% confluency, cells were trypsinized and subjected to FACS sorting.

Transplantation experiment

Immunodeficient NOD/scid or NSG mice were used. All procedures using experimental animals were approved by the Institutional Animal Care and Use Committee at Fujita Health University. One day prior to transplantation, tibialis anterior (TA) muscles were injured with CTX or glycerol as described (Uezumi et al., 2010). Immediately after cell sorting, cells were resuspended in 25 μl PBS and transplanted into the TA muscles. Fifteen days after transplantation, TA muscles were excised and rapidly frozen in isopentane cooled with liquid nitrogen. For detection of $Venus^+$ cells, transplanted TA muscle were fixed with 4% PFA. Transplanted muscles were sectioned at 250 μm intervals from the proximal end to the mid-belly.

The maximal number of human Lamin A/C$^+$ cells, human Spectrin$^+$ myofibers, and human Lamin A/C$^+$PAX7$^+$ sublaminar satellite cells were recorded.

Histochemistry, cytochemistry, and microscopy

Muscle samples for histochemistry were rapidly frozen in isopentane cooled with liquid nitrogen. For CD82 and Spectrin immunostaining, frozen muscle sections were fixed with acetone at -20°C for 5 min. In other cases, sections were fixed with 4% PFA for 5 min. For CD82 and CD318 immunostaining, specimens were treated with Antigen Retrieval Reagent-Universal solution (R&D) at 95°C for 5 min. Cultured cells were rinsed with PBS and then fixed with 4% PFA for 5 min. Specimens were blocked with protein-block serum-free reagent (DAKO) for 15 min, and incubated with primary antibodies at 4°C overnight, followed by secondary staining. Primary antibodies used were anti-CD82 (1:50, Sigma, #HPA028900), anti-CD318 (1:100, Acris, #AP15947PU-N), anti-CD201 (2.5 μg/ml, R&D, #AF2245), anti-PDGFRα (2.5 μg/ml, R&D, #AF-307-NA), anti-M-cadherin (4 μg/ml, R&D, #AF4096), anti-PAX7 (1:2, DSHB), anti-CD56 (1:20, Miltenyi Biotec, #130-090-955), anti-Laminin (1:200, Sigma, #L9393), anti-Laminin (1:100, LSBio, #LS-C96142), anti-Collagen I (1:250, Abcam, #ab138492), anti-EGFR (1:200, Abcam, #ab52894), anti-MYOD1 (1:300, Abcam, #ab133627), anti-MYOG (1:300, Abcam, #ab124800), anti-MyHC (1:2, DSHB), anti-Sarcomeric α-actinin (1:200, Abcam, #ab68167), anti-Perilipin (1:250, Sigma, #P1873), anti-C/EBPα (1:400, Santa Cruz, #sc-9314), anti-PPARγ (1:100, Santa Cruz, #sc-7273), anti-GFP (1:200, Acris, #AB0020), anti-Ki67 (1:200, eBioscience, #51-5698-82), anti-human Lamin A/C (1:400, Epitomics, #2966-1), anti-human Spectrin (1:100, Leica, #NCL-SPEC1), and anti-Laminin α2 (1:400, Santa Cruz, #sc-59854). Secondary antibodies used with a dilution of 1:1000 were Alexa Fluor 488- or 647-conjugated anti-rabbit IgG (Molecular Probes, #A21206 or A31573), Cy3-conjugated anti-rabbit IgG (Jackson, #711-165-152), Alexa Fluor 488- or Cy3-conjugated anti-mouse IgG (Jackson, #715-545-150 or 715-165-150), Alexa Fluor 568-conjugated anti-mouse IgG (Molecular Probes, #A10037), Alexa Fluor 488-conjugated anti-goat IgG (Molecular Probes, #A11055), Cy3-conjugated anti-goat IgG (Jackson, #705-165-147), CF488A-conjugated anti-sheep IgG (Biotium, #20024), Alexa Fluor 647-conjugated anti-chick IgG (Jackson, #703-605-155), and Alexa Fluor 647-conjugated anti-rat IgG (Molecular Probes, #A21472). Specimens were counterstained with DAPI

(Invitrogen) and mounted with SlowFade Gold anti-fade reagent (Invitrogen). For sequential immunohistochemistry and HE staining, immunofluorescent staining was performed first and immunofluorescent images were captured. Slides were immersed in PBS to remove the cover glass and stained with HE. Then images of the corresponding HE-stained fields were captured. Stained cells and tissues were photographed using a fluorescence microscope BX51 (Olympus) equipped with a DP70 CCD camera (Olympus), an inverted fluorescence microscope BZ-9000 (Keyence), or an inverted fluorescence microscope DMI4000B (Leica) equipped with a DFC350FX CCD camera (Leica). Confocal images of muscle sections were taken using the confocal laser scanning microscope system LSM700 (Carl Zeiss).

Immunoblotting

Cells were lysed in lysis buffer consisting of 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM NaF, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, and protease inhibitor cocktail (Roche). For the detection of phosphorylated proteins, lysis buffer was supplemented with phosphatase inhibitor cocktail (Roche). Cell lysates were centrifuged at 1000 g for 10 min at 4°C and the supernatants were recovered. Aliquots of the lysates containing 10 μg of protein were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes. The membranes were probed with anti-MYOD1 (1:1000, Abcam, #ab133627), anti-MYOG (1:1000, Abcam, #ab124800), anti-GAPDH (1:2000, Cell Signaling, #2118), anti-phospho-p38 (1:1000, Cell Signaling, #4511), anti-p38 (1:1000, Cell Signaling, #9212), anti-phospho-Akt (1:2000, Cell Signaling, #4060), anti-Akt (1:1000, Cell Signaling, #4691), anti-phospho-p44/42 MAPK (1:2000, Cell Signaling, #4370), and anti-p44/42 MAPK (1:1000, Cell Signaling, #9102). After incubation with horseradish peroxidase-conjugated secondary antibodies and chemiluminescence reactions, images of the developed immunoblots were captured using a Light-Capture imaging system (ATTO).

Quantitative analyses of cultured cell

Three to five randomly selected fields per well were photographed. Images were collected and pooled from two or three independent experiments. The purity of myogenic progenitors was determined by dividing the number of myogenic marker$^+$ cells by the number of DAPI$^+$ nuclei using Win ROOF

software (Mitani Corp). To assess the efficiency of adipogenic differentiation, number of perilipin^{+} adipocytes per field was counted, or adipocytes were stained by using Adipocyte Fluorescent Staining kit (Cosmo Bio) and Bodipy-stained area was measured by using ArrayScan XTI-HCS (ThermoFisher). To assess the efficiency of myogenic differentiation, four randomly selected fields were photographed per well. Images were collected and pooled from four to six independent wells. The MyHC^{+} area per well was measured by using BZ-X Analyzer software (Keyence).

RNA extraction and RT-PCR

Total RNA was extracted using an RNeasy Micro Kit (Qiagen), and equal amounts of RNA were reverse transcribed into cDNA using a QuantiTect Reverse Transcription Kit (Qiagen). Real-time quantitative PCR was performed on a Thermal Cycler Dice Real Time System (Takara) or LightCycler 480 System (Roche) under the following cycling conditions: 95°C for 10 sec, followed by 40 cycles of amplification (95°C for 5 sec, 60°C for 15 sec, 72°C for 10 sec). Specific primer sequences used in this study were 5′-GCTCATTCGAGACTACAACAGCA-3′ and 5′-CTTGACTTCGCAGGAACAGG-3′ for *CD82*, 5′-GACCCCTGCCTAACCACATC-3′ and 5′-GTCTCCTGGTAGCGGCAAAG-3′ for *PAX7*, 5′-GAGGTGTACCACGACCAACC-3′ and 5′-CCTGCTCTCTCAGCAACTCC-3′ for *MYF5*, 5′-GCCACAACGGACGACTTCTATG-3′ and 5′-TGCTCTTCGGGTTTCAGGAG-3′ for *MYOD1*, 5′-ATCATCTGCTCACGGCTGAC-3′ and 5′-GGGAAGGCCACAGACACATC-3′ for *MYOG*, 5′-CGTCAAAGGTGAAGCAGGAC-3′ and 5′-CTCCAGTGCCCGTAGATCAG-3′ for *NDUFA13*, and 5′-ACCCACTCCTCCACCTTTGA-3′ and 5′-TTGCTGTAGCCAAATTCGTTG-3′ for *GAPDH*. The following primers were used for generating standard samples: 5′-GGGTCTTCATCAATGGGCGA-3′ and 5′-GTCACAGTGCCCATCCTTCA-3′ for *PAX7*, 5′-TCAGCAGGATGGACGTGA-3′ and 5′-GGAGGTGATCCGGTCCACTA-3′ for *MYF5*, 5′-GAAAGTTCCGGCCACTCTCT-3′and 5′-CTGGTTTGGATTGCTCGACG-3′ for *MYOD1*, 5′-CCATCACCATCTTCCAGGAG-3′ and 5′-CCTGCTTCACCACCTTCTTG-3′ for *GAPDH*.

Statistics

The significance of differences among experimental groups was assessed by Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test.