Supplemental Figures

Figure S1

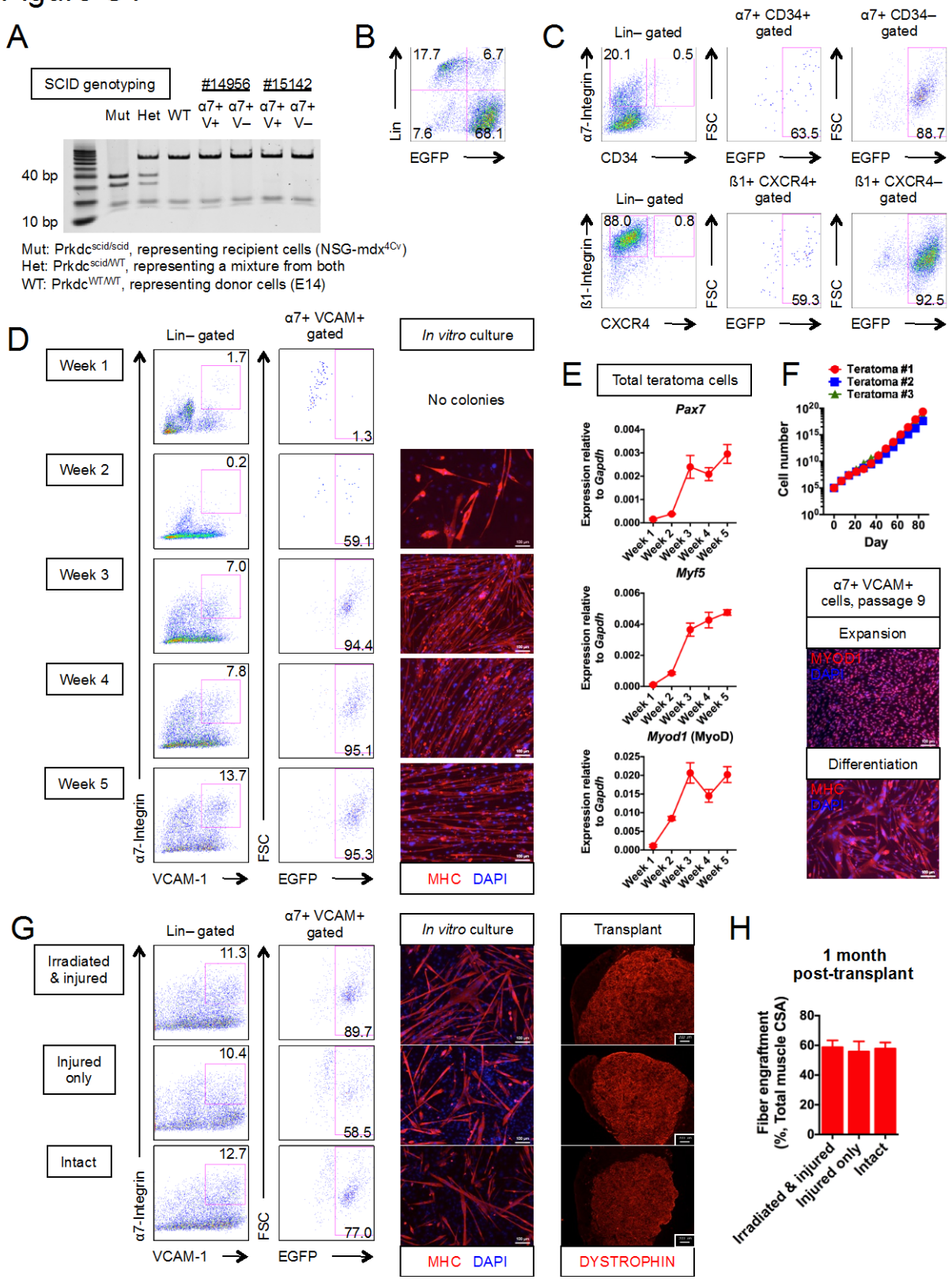

A
SCID genotyping
#14956
#15142
Mut Het WT
α7+ V+
α7+ V–
α7+ V+
α7+ V–
40 bp
10 bp
Mut: Prkdc scid/scid, representing recipient cells (NSG-mdx 4Cv)
Het: Prkdc scid/WT, representing a mixture from both
WT: Prkdc WT/WT, representing donor cells (E14)
B
Lin
EGFP
17.7
6.7
7.6
68.1
C
Lin– gated
α7+ CD34+ gated
α7+ CD34– gated
α7-Integrin
CD34
FSC
EGFP
20.1
0.5
63.5
88.7
ß1+ CXCR4+ gated
ß1+ CXCR4– gated
ß1-Integrin
CXCR4
88.0
0.8
59.3
92.5
D
Week 1
Week 2
Week 3
Week 4
Week 5
Lin– gated
α7+ VCAM+ gated
α7-Integrin
VCAM-1
FSC
EGFP
1.7
1.3
0.2
59.1
7.0
94.4
7.8
95.1
13.7
95.3
In vitro culture
No colonies
MHC DAPI
E
Total teratoma cells
Pax7
Myf5
Myod1 (MyoD)
Expression relative to Gapdh
Week 1
Week 2
Week 3
Week 4
Week 5
F
Teratoma #1
Teratoma #2
Teratoma #3
Cell number
Day
α7+ VCAM+ cells, passage 9
Expansion
MYOD1
DAPI
Differentiation
MHC
DAPI
G
Irradiated & injured
Injured only
Intact
Lin– gated
α7+ VCAM+ gated
α7-Integrin
VCAM-1
FSC
EGFP
11.3
89.7
10.4
58.5
12.7
77.0
In vitro culture
Transplant
MHC DAPI
DYSTROPHIN
H
1 month post-transplant
Fiber engraftment (%, Total muscle CSA)
Irradiated & injured
Injured only
Intact

Figure S1, related to Figure 1. Characterization of E14-EGFP ES cell-derived teratomas

(A) SCID genotyping of sorted α7+ VCAM+ and α7+ VCAM– cells from 2 teratomas (mouse #14956 and mouse #15142) verifying that these myogenic populations were donor-derived (no scid allele) (n=2 biological replicates).

(B) Abundant Lin+ (CD45+ and/or CD31+) EGFP– host cells were found in E14-EGFP ES cell-derived teratomas, indicating host contribution to teratoma development (n=2 biological replicates).

(C) Characterization of 3 week-old teratomas derived from E14-EGFP ES cells using other satellite cell markers (n=2 biological replicates).

(D) Donor-derived myogenic progenitors emerged in 2 week-old teratomas and beyond, as indicated by FACS and immunostaining in α7+ VCAM+ EGFP+ sorted cells (n=3 biological replicates). Scale bar represents 100 μm.

(E) Quantitative RT-PCR of myogenic genes from total teratoma cells at different time points (n=3 biological replicates).

(F) *Ex vivo* culture of α7+ VCAM+ teratoma cells shows exponential growth up to passage 12 (top). Immunostaining showing expanded α7+ VCAM+ teratoma cells retain their myogenic identity at both expansion phase (MYOD1+) and differentiation phase (MHC+) even at passage 9 (bottom) (n=3 biological replicates). Scale bar represents 100 μm.

(G) Myogenic progenitors could be generated in teratomas developed in irradiated and injured TAs (control), injured only TAs, and intact TAs (non-irradiated and non-injured), but the injured and irradiated niche allowed the greatest donor contribution (EGFP+). Sorted α7+ VCAM+ cells from all 3 conditions produced MHC+ myotubes in culture (n=2 biological replicates) and similar numbers of DYSTROPHIN+ fibers at 1 month after transplantation of 40,000 cells (n=4 biological replicates). Scale bar represents 100 μm for culture or 200 μm for transplant.

(H) Quantification of fiber engraftment in (G) (n=4 biological replicates).

α7, α7-integrin. V and VCAM, VCAM-1. CSA, cross-sectional area. ES cells, embryonic stem cells. Lin, lineage cocktail comprising antibodies against CD45 (hematopoietic) and CD31 (endothelial). Mut, mutant; Het, heterozygous; WT, wild-type; TA, tibialis anterior. Mean ± SEM is shown in (E) and (H).

Figure S2

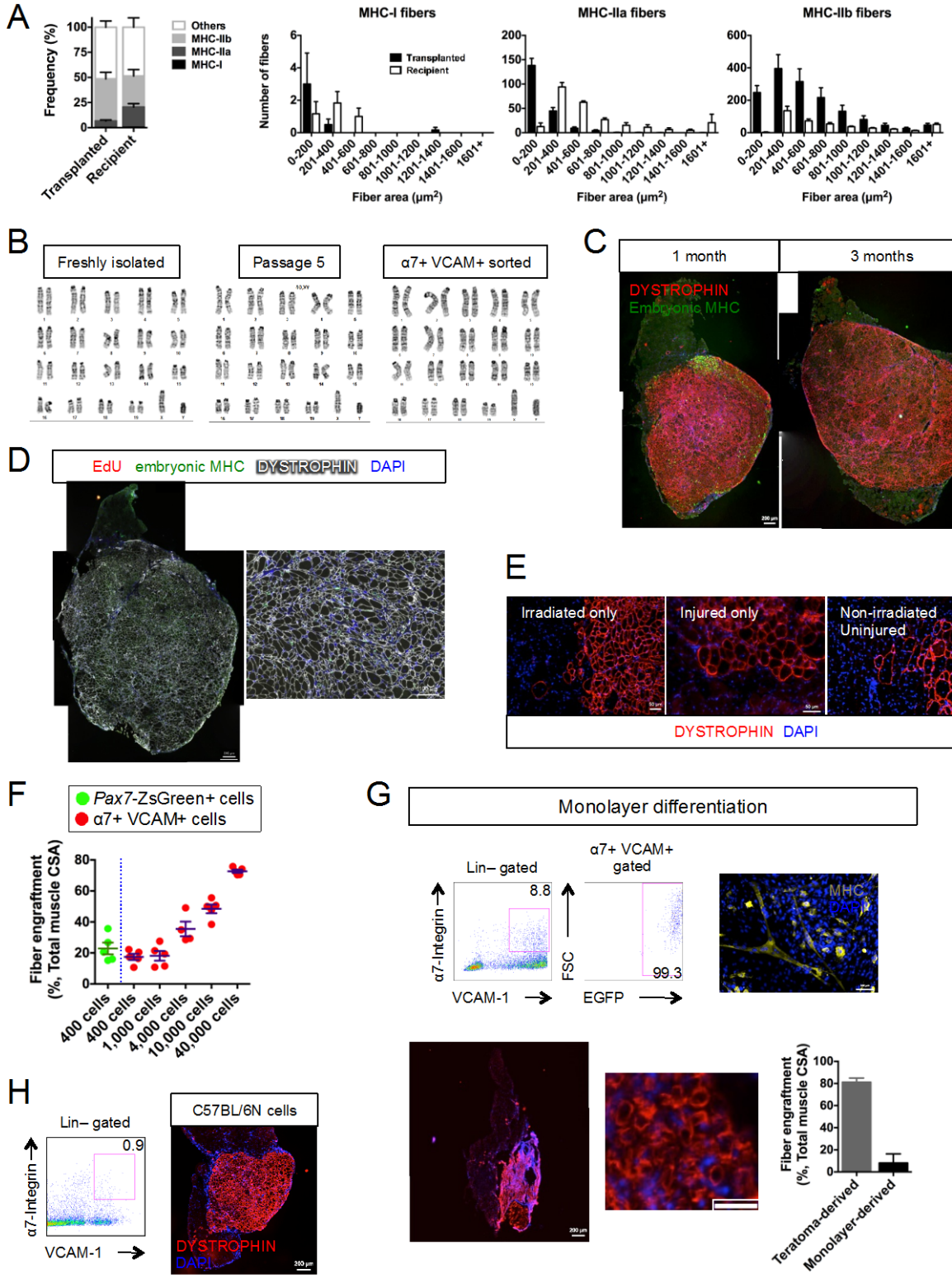

A
Frequency (%)
Others
MHC-IIb
MHC-IIa
MHC-I
Transplanted
Recipient
MHC-I fibers
MHC-IIa fibers
MHC-IIb fibers
Number of fibers
Transplanted
Recipient
0-200
201-400
401-600
601-800
801-1000
1001-1200
1201-1400
1401-1600
1601+
Fiber area (μm²)
B
Freshly isolated
Passage 5
α7+ VCAM+ sorted
C
1 month
3 months
DYSTROPHIN
Embryonic MHC
D
EdU embryonic MHC DYSTROPHIN DAPI
E
Irradiated only
Injured only
Non-irradiated
Uninjured
DYSTROPHIN DAPI
F
Pax7-ZsGreen+ cells
α7+ VCAM+ cells
Fiber engraftment
(%, Total muscle CSA)
400 cells
400 cells
1,000 cells
4,000 cells
10,000 cells
40,000 cells
G
Monolayer differentiation
Lin– gated
α7+ VCAM+
gated
8.8
99.3
α7-Integrin
VCAM-1
FSC
EGFP
MHC
DAPI
H
Lin– gated
0.9
α7-Integrin
VCAM-1
C57BL/6N cells
DYSTROPHIN
DAPI
Fiber engraftment
(%, Total muscle CSA)
Teratoma-derived
Monolayer-derived

Figure S2, related to Figures 2 and 3. Characterization of teratoma-derived α7+ VCAM+ cells

(A) Fiber phenotyping analysis of transplanted and recipient TA muscles. Composition of different fiber types (far left), and area quantification for fibers expressing MHC-I (middle left), MHC-IIa (middle right) and MHC-IIb (far right) (n=6 biological replicates).

(B) Karyotype analysis of freshly isolated teratoma cells (left), serially passaged teratoma cells (P5, middle) and α7+ VCAM+ sorted cells (right) (n=3 biological replicates). All are karyotypically normal, i.e., teratoma formation *per se* does not generate karyotypically abnormal cells.

(C) Engrafted fibers mature over time. Embryonic MHC was found in only a minor fraction of DYSTROPHIN+ fibers by 1 month post-transplant (left) but no longer detected by 3 months (right) (n=6 biological replicates). Scale bar represents 200 μm.

(D) Engrafted mononuclear α7+ VCAM+ cells become quiescent. Three months after primary transplantation, EdU was injected for 3 days and muscles were harvested 5 weeks later. The lack of EdU positivity in DYSTROPHIN+ fibers indicates that transplanted α7+ VCAM+ cells are quiescent (n=4 biological replicates). Scale bar represents 200 μm (left) or 100 μm (right, magnified image).

(E) α7+ VCAM+ cells engrafted and formed DYSTROPHIN+ fibers in irradiated-only (left), injured-only (middle) and non-irradiated uninjured recipient TA muscles (right) (n=2–6 biological replicates). Scale bar represents 50 μm

(F) Fiber engraftment of *Pax7*-ZsGreen satellite cells and α7+ VCAM+ teratoma cells (n=4–5 biological replicates), from which a dose-response relationship of α7+ VCAM+ cells is generated and displayed in Figure 2I (n=4–5 biological replicates).

(G) Comparison of teratoma-derived vs. monolayer *in vitro* differentiation-derived myogenic cells. Myogenic progenitors were generated side by side using the teratoma method or the monolayer method (Chal et al., 2015). By 4 weeks, cells were analyzed by FACS (upper left) and α7+ VCAM+ cells were sorted for further culture and immunostaining (upper right, n=8 biological replicates) or for transplantation (40,000 cells per TA muscle, n=3 biological replicates). Transplanted muscles were harvested 3 months later. Despite having similar myogenic potential *in vitro*, monolayer differentiated α7+ VCAM+ cells have poorer engraftment potential *in vivo* (bottom left: whole TA muscle; bottom middle: magnified area; bottom right: quantification). Scale bar represents 100 μm (cultured cells), 200 μm (whole TA muscle) or 25 μm (magnified area).

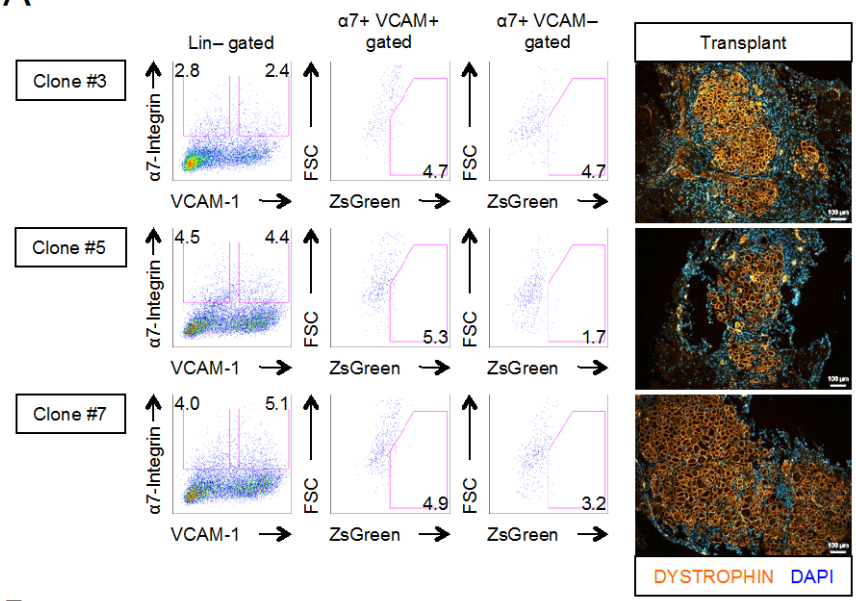
(H) α7+ VCAM+ myogenic cells are generated from C57BL/6N ES cell teratomas. FACS profile showing the presence of α7+ VCAM+ cells in 3-week-old teratoma generated from C57BL/6N-PXR-B6N

#1 ES cells (left). Subsequent transplantation of 20,000 FACS-sorted α7+ VCAM+ cells forms DYSTROPHIN+ muscle fibers (right) (n=3 biological replicates). Scale bar represents 200 μm.

α7, α7-integrin. VCAM, VCAM-1. Lin, lineage cocktail comprising antibodies against CD45 (hematopoietic) and CD31 (endothelial). MHC, myosin heavy chain. Mean ± SEM is shown in (A), (F) and (G).

Figure S3

A


Lin– gated
α7+ VCAM+ gated
α7+ VCAM– gated
Transplant
Clone #3
Clone #5
Clone #7
α7-Integrin
VCAM-1
FSC
ZsGreen
2.8
2.4
4.7
4.7
4.5
4.4
5.3
1.7
4.0
5.1
4.9
3.2
DYSTROPHIN DAPI

B

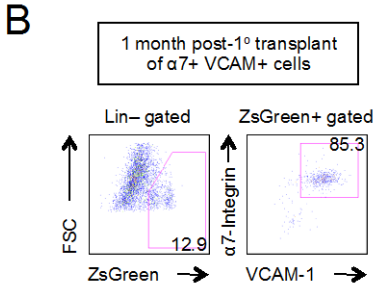

1 month post-1° transplant of α7+ VCAM+ cells
Lin– gated
ZsGreen+ gated
FSC
ZsGreen
12.9
α7-Integrin
VCAM-1
85.3

Figure S3, related to Figure 4. Characterization of Pax7-ZsGreen iPS cell-derived teratomas

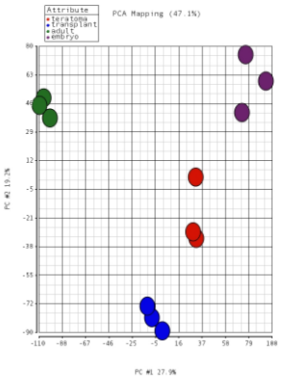
(A) Three different *Pax7*-ZsGreen iPS clones were tested for their potential to generate α7+ VCAM+ cells in teratomas and subsequent DYSTROPHIN+ fibers after transplantation. All 3 clones produced α7+ VCAM+ and α7+ VCAM– myogenic progenitors in teratomas, but ZsGreen was minimally expressed (n=2 biological replicates per clone). Sorted α7+ VCAM+ cells from all 3 clones engrafted into DYSTROPHIN+ fibers 1-month post-transplant (n=4 biological replicates per clone). Scale bar represents 100 μm. Note that the FACS plots and immunostaining image for clone #7 was used in Figures 4B and 4E, respectively.

(B) FACS analysis of transplanted muscles revealed the presence of ZsGreen+ muscle stem cells, of which most are within the α7+ VCAM+ gate (n=10 biological replicates).

iPS cells, induced pluripotent stem cells. Lin, lineage cocktail comprising antibodies against CD45 (hematopoietic) and CD31 (endothelial).

Figure S4

A



B

GO terms	Teratoma up vs. Embryo
Immune response	6.41×10^{-41}
Response to cytokine	1.40×10^{-39}
Regulation of cell migration	9.63×10^{-25}
Regulation of cell motility	4.06×10^{-24}

C

GO terms	Transplant up vs. Adult
Muscle structure development	8.76×10^{-14}
Muscle tissue development	8.31×10^{-12}
Striated muscle tissue development	9.22×10^{-11}
Muscle organ development	3.67×10^{-10}

Figure S4, related to Figure 5. Transcriptome analysis of teratoma and transplanted α7+ VCAM+ cells

(A) Principal Component Analysis (PCA) highlighting the differences among the 4 α7+ VCAM+ populations: *Teratoma*, *Transplant*, *Embryo* and *Adult*.

(B) Gene Ontology (GO) Biological Process terms denoting genes enriched in the *Teratoma-Embryo* comparison. p-values of the GO terms are indicated.

(C) Gene Ontology (GO) Biological Process terms denoting genes enriched in the *Transplant-Adult* comparison. p-values of the GO terms are indicated.