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Supplemental Information

Ex Vivo Expansion and In Vivo Self-Renewal of Human Muscle Stem Cells

Gregory W. Charville, Tom H. Cheung, Bryan Yoo, Pauline J. Santos, Gordon K. Lee, Joseph B. Shrager, and Thomas A. Rando

SUPPLEMENTAL FIGURES

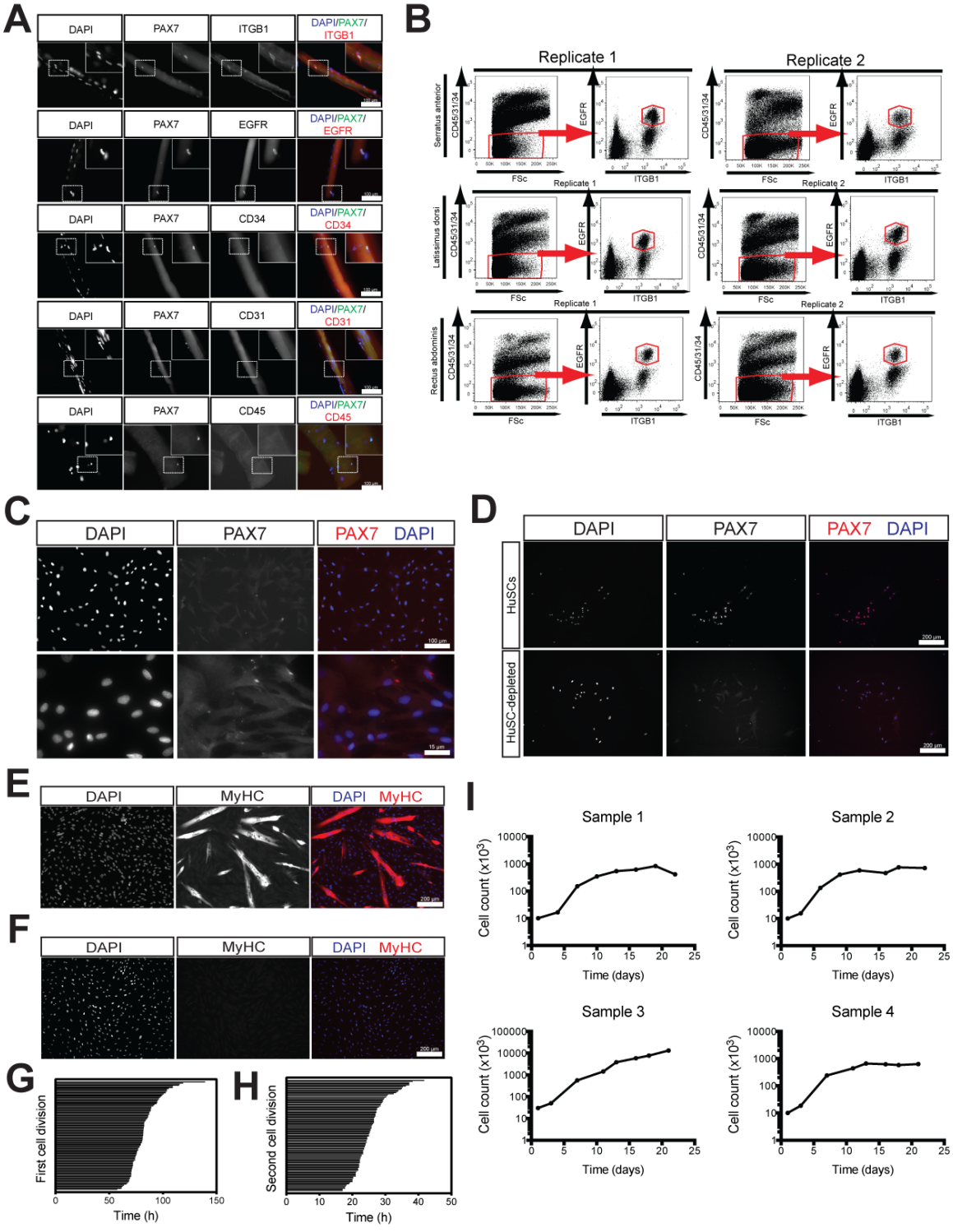

Figure S1. **Characteristics of prospectively isolated huSCs. Related to Figure 1. (A)** Immunofluorescence (IF) analysis of PAX7 and surface protein expression in huSCs in association with intact explanted human muscle fibers. Nuclei were stained with DAPI. Insets are magnified views of MuSCs in each image. **(B)** Representative FACS plots for prospective isolation of human huSCs from *serratus anterior*, *latissimus dorsi*, and *rectus abdominis* muscles. Cells were gated by forward scatter and side scatter (not shown) prior to gating for CD45/31/34, EGFR, and ITGB1. The populations containing huSCs are highlighted in red. Two biological replicates (Replicates 1 and 2) are shown for each muscle. **(C)** IF analysis of PAX7 expression in huSC-depleted cells cultured for 3 days. Representative images are shown at low (top) and high (bottom) magnification. **(D)** Representative IF analysis of a PAX7-expressing clone derived from the huSC subpopulation (left) and a clone derived from the huSC-depleted subpopulation (right) in which no PAX7 expression was observed. Nuclei were stained with DAPI. **(E)** IF analysis of MyHC expression in huSCs cultured for 9 days up to ~90% of confluency. Nuclei were stained with DAPI. **(F)** Single-color IF analysis of MyHC expression in cells from the huSC-depleted subpopulation cultured for 9 days up to ~90% of confluency. Nuclei were stained with DAPI. **(G)** Time from plating to first cell division of 100 freshly isolated huSCs from three biological replicates as determined by time-lapse microscopy. Each horizontal bar represents a single cell. The length of a given bar along the x-axis reflects the length of time required to reach the first cell division (83.3 ± 14.1 h, $n = 200$ cells from four biological replicates). Bars are ordered along y-axis by length of cell cycle (longest at the top). **(H)** Time between first and second cell division of 100 freshly isolated huSCs from three biological replicates as determined by time-lapse microscopy.

The length of a given bar along the x-axis reflects the length of time between the first and second cell divisions (25.5 ± 5.7 h, n = 200 cells from four biological replicates). Bars are ordered along y-axis by length of cell cycle (longest at the top). **(I)** Growth curves of huSCs isolated from four biological replicates (unique clinical specimens). Cells were serially passaged to maintain a density of <40% confluency and were counted at each passage.

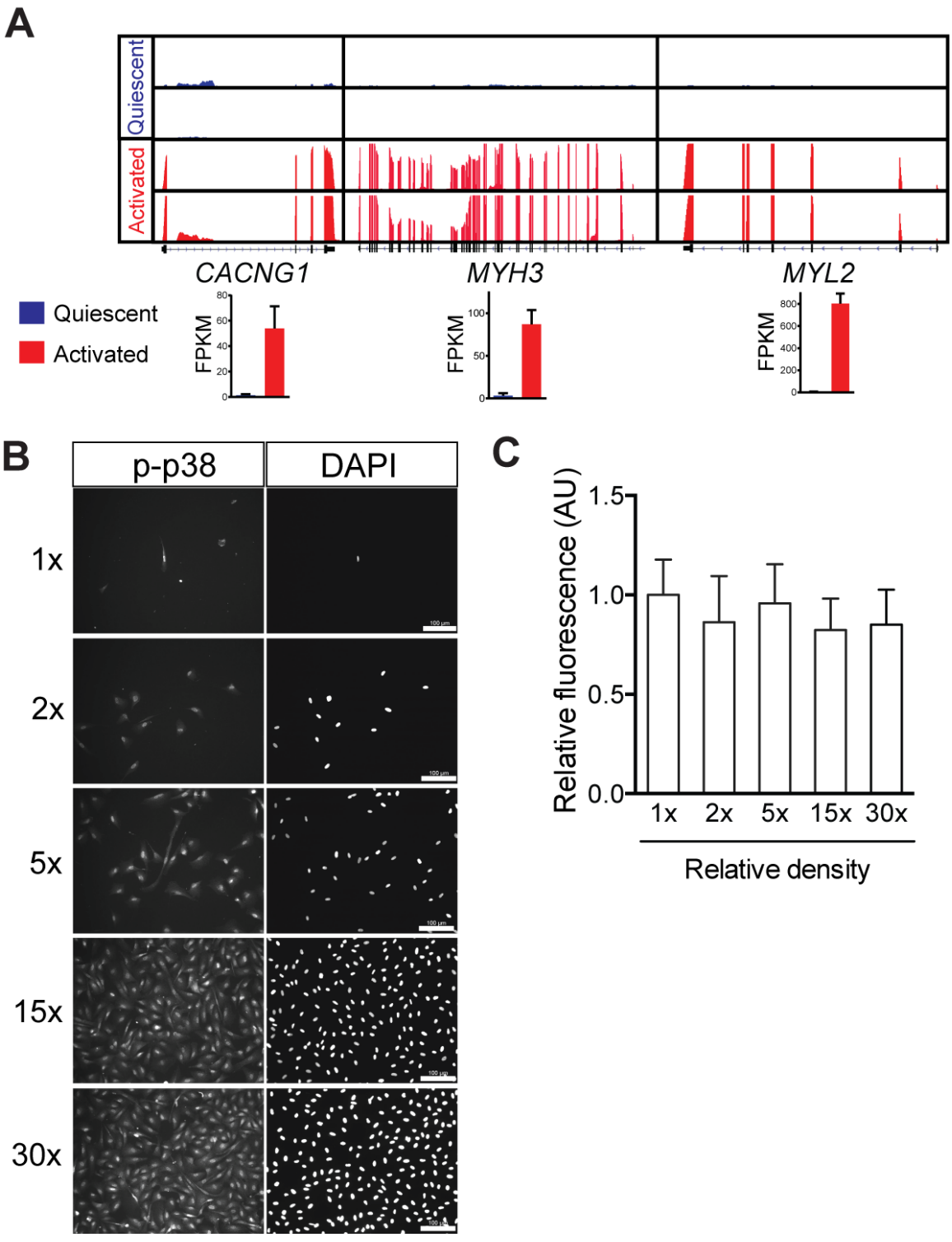
A
Quiescent
Activated
CACNG1
MYH3
MYL2
FPKM
Quiescent
Activated
B
p-p38
DAPI
1x
2x
5x
15x
30x
C
Relative fluorescence (AU)
1.5
1.0
0.5
0.0
1x
2x
5x
15x
30x
Relative density

Figure S2. Terminal differentiation of huSCs and effects of cell density on p38 activity. Related to Figure 2. (A) Shown are sequencing reads from 2 biological replicates per condition (quiescent and activated) mapped to the reference genome. The number of sequenced fragments per kilobase of exon per million fragments mapped (FPKM) in each condition is shown for individual genes. Error bars represent standard deviation. **(B)** Representative IF analysis of p-p38 in huSCs cultured for 5 days at the given relative density. **(C)** Quantification of nuclear p-p38 fluorescence intensity in huSCs cultured at the given relative densities. (n = 150 cells per condition from three biological replicates) Error bars represent standard deviation. AU, arbitrary units.

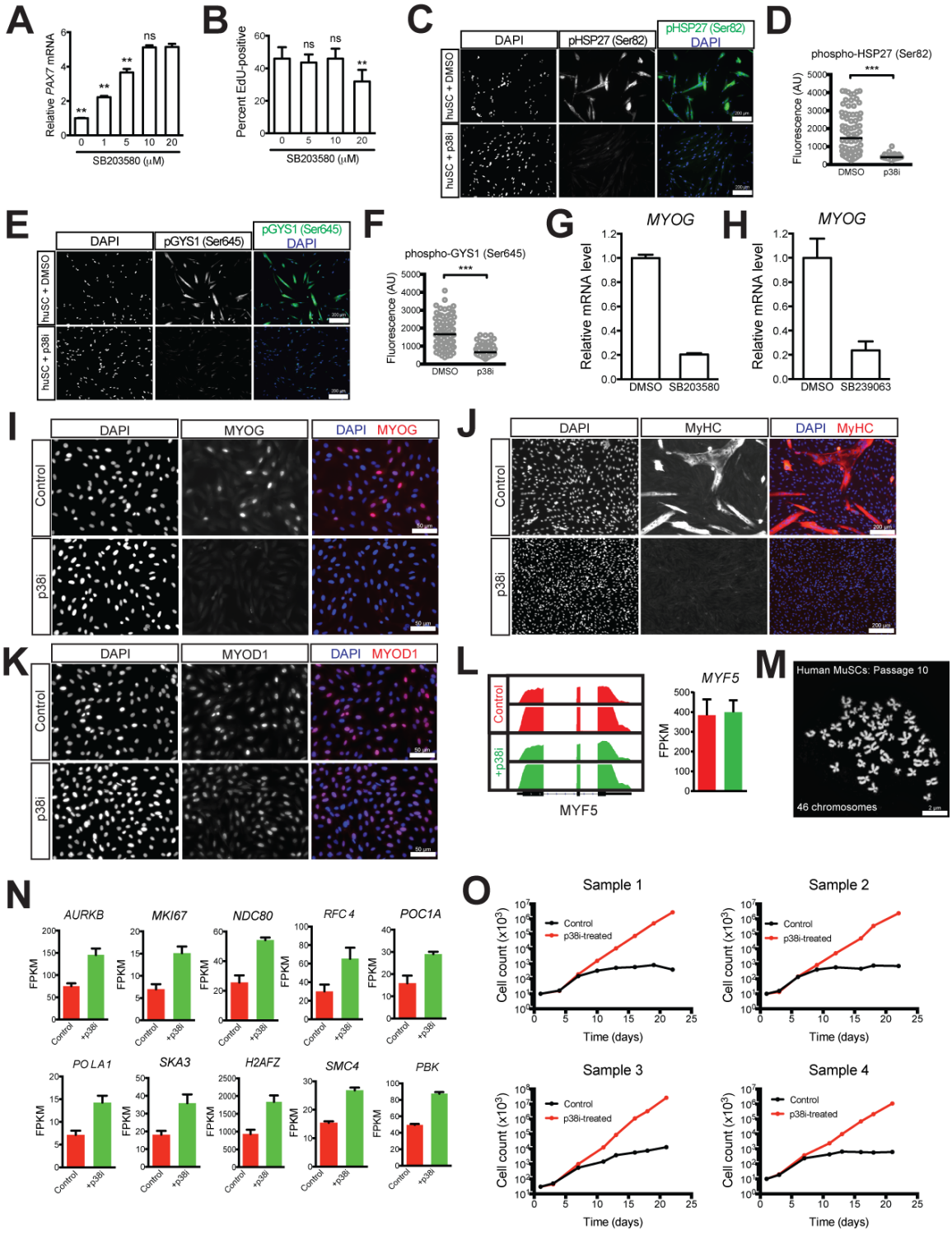
A
Relative PAX7 mRNA
SB203580 (μM)
B
Percent EdU-positive
SB203580 (μM)
C
DAPI
pHSP27 (Ser82)
pHSP27 (Ser82) DAPI
huSC + DMSO
huSC + p38i
D
phospho-HSP27 (Ser82)
Fluorescence (AU)
DMSO
p38i
E
DAPI
pGYS1 (Ser645)
pGYS1 (Ser645) DAPI
F
phospho-GYS1 (Ser645)
G
MYOG
Relative mRNA level
DMSO SB203580
H
MYOG
DMSO SB239063
I
DAPI
MYOG
DAPI MYOG
Control
p38i
J
DAPI
MyHC
DAPI MyHC
K
DAPI
MYOD1
DAPI MYOD1
L
Control
+p38i
MYF5
MYF5
FPKM
M
Human MuSCs: Passage 10
46 chromosomes
N
AURKB
MKI67
NDC80
RFC4
POC1A
POLA1
SKA3
H2AFZ
SMC4
PBK
FPKM
Control
+p38i
O
Sample 1
Sample 2
Sample 3
Sample 4
Control
p38i-treated
Cell count (x10^3)
Time (days)

Figure S3. Characterization of p38i effects on cultured SCs. Related to Figure 3. (A) Relative *PAX7* mRNA levels at given doses of p38i (SB203580) determined by RT-qPCR. Freshly isolated huSCs were cultured for 5 days prior to analysis. (n = 3) **(B)** Relative frequency of EdU incorporation after a one-hour EdU pulse at given doses of p38i. Cells were cultured for 3 days prior to analysis in order to analyze the effects on a more undifferentiated population of control cells. (n = 300 cells per condition from three biological replicates) **(C)** Representative IF analysis of phospho-HSP27 (Ser82) in SCs cultured for 8 days in the presence or absence of p38i. **(D)** Quantification of relative cytoplasmic fluorescence in IF analyses of phospho-HSP27 (Ser82) represented in (C). Each data point represents a single cell. **(E)** Representative IF analysis of phospho-GYS1 (Ser645) in SCs cultured for 8 days in the presence or absence of p38i. **(F)** Quantification of relative cytoplasmic fluorescence in IF analyses of phospho-GYS1 (Ser645) represented in (E). Each data point represents a single cell. **(G)** qRT-PCR analysis of relative mRNA expression of *MYOG* in huSCs cultured in the presence or absence of p38i (SB203580) for 6 days. (n = 3) **(H)** qRT-PCR analysis of relative mRNA expression of *MYOG* in huSCs cultured in the presence or absence of SB239063, an alternative p38 inhibitor, for 6 days. (n = 3) **(I)** Representative IF analysis of MYOG expression in control and p38i-treated huSCs. Cells were cultured for 6 days prior to analysis. While MYOG is observed in control cells, MYOG is absent from p38i-treated cells. **(J)** Representative IF analysis of MyHC expression in control and p38i-treated huSCs. Cells were cultured for 8 days prior to analysis. While MyHC is observed in control cells, MyHC is absent from p38i-treated cells. **(K)** Representative IF analysis of MYOD1 expression in control and p38i-treated huSCs. Cells were cultured for 6 days prior to

analysis. **(L)** RNA-sequencing analysis of *MYF5* expression in control (red) and p38i-treated (green) huSCs. Sequencing reads were mapped to the reference genome (left) and relative expression was quantified as FPKM (right). Error bars represent standard deviation; sequencing data are from two biological replicates per condition. **(M)** Representative micrograph of a metaphase chromosome spread of a human huSC cultured for 10 passages. **(N)** Expression of genes associated with DNA replication and cell division is increased in p38i-treated human huSCs relative to controls. Quantification of expression (FPKM) of representative genes in control (red) and p38i-treated (green) human huSCs. For all targets, expression levels differed significantly ($P < 0.05$) between control and p38i-treated samples. Error bars represent standard deviation. Sequencing data are derived from two biological replicates per condition. **(O)** Growth curves of huSCs isolated from four unique clinical specimens. Cultures derived from the same specimen were either left untreated (black) or maintained in the presence of p38i (red). Data for untreated samples are the same as those presented in Figure S1I. Cells were serially passaged to maintain a density of <40% confluence and were counted at each passage. $^{**}P < 0.01$, $^{***}P < 0.001$. AU, arbitrary units. ns, not significant ($P > 0.05$).

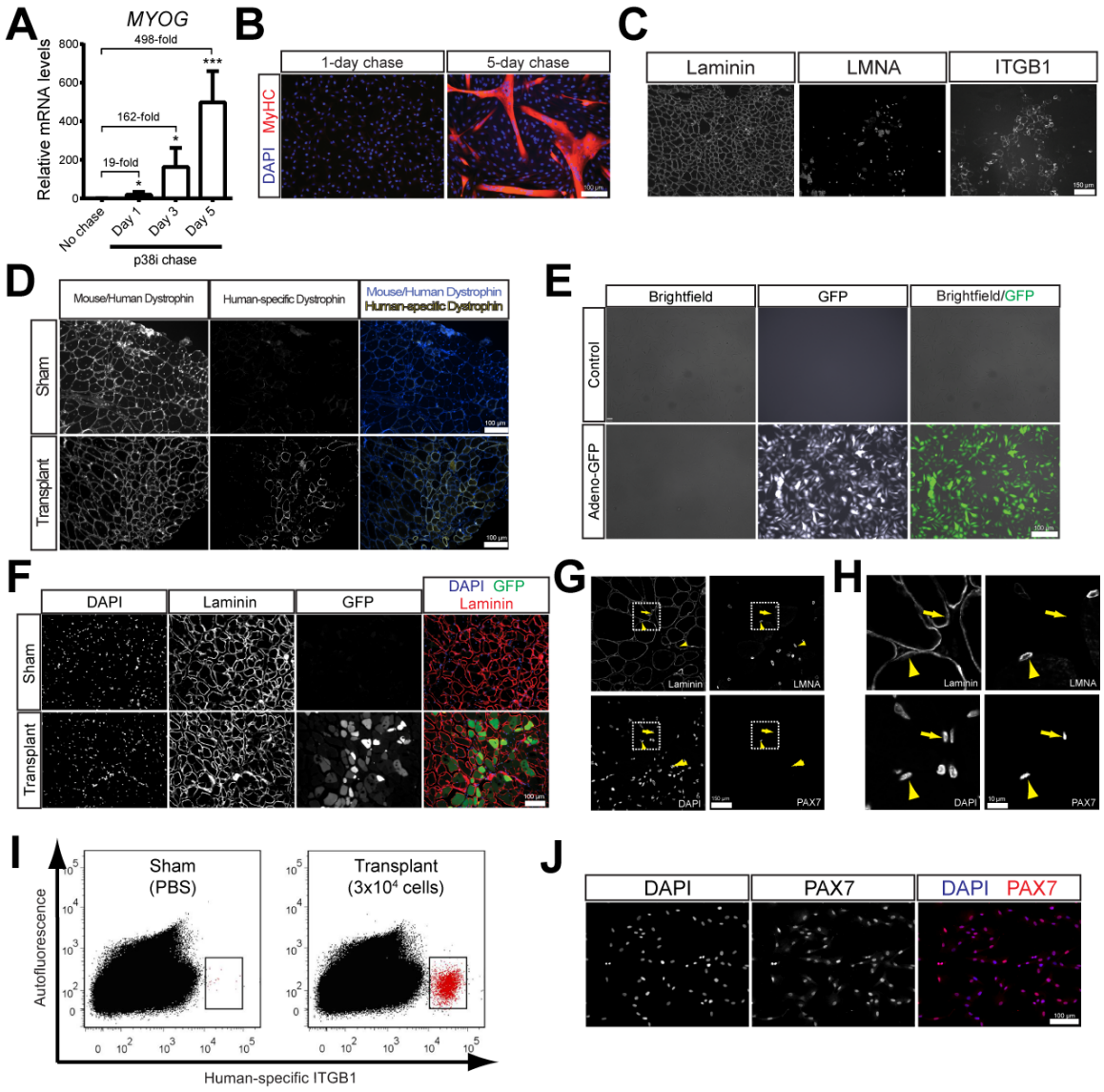
A
MYOG
Relative mRNA levels
800
600
400
200
0
498-fold
162-fold
19-fold
***
*
*
No chase
Day 1
Day 3
Day 5
p38i chase
B
1-day chase
5-day chase
DAPI MyHC
C
Laminin
LMNA
ITGB1
D
Mouse/Human Dystrophin
Human-specific Dystrophin
Mouse/Human Dystrophin
Human-specific Dystrophin
Sham
Transplant
E
Brightfield
GFP
Brightfield/GFP
Control
Adeno-GFP
F
DAPI
Laminin
GFP
DAPI GFP
Laminin
Sham
Transplant
G
Laminin
LMNA
DAPI
PAX7
H
Laminin
LMNA
DAPI
PAX7
I
Sham
(PBS)
Transplant
(3x10⁴ cells)
Autofluorescence
Human-specific ITGB1
J
DAPI
PAX7
DAPI PAX7

Figure S4. Differentiation and transplantation of expanded human huSCs. Related to Figure 4. (A) RT-qPCR analysis of *MYOG* mRNA levels over a period in which p38i was chased from the culture medium. huSCs were grown in p38i for 2 weeks, at which point p38i was removed, and cells were analyzed for mRNA levels at 1, 3 and 5 days after the chase began ($n = 3$). *$P < 0.05$, ***$P < 0.001$. Error bars represent standard deviation. **(B)** Representative IF images of MyHC expression in huSC cultures one (left) and five (right) days after p38i was removed from the culture. **(C)** Representative single-color images of human-specific ITGB1 expression in huSC-transplanted *tibialis anterior* muscles as presented in merged images in Figure 4A. Muscles were transplanted with 3×10^4 p38i-expanded huSCs and analyzed 28 days later. **(D)** Representative IF analysis of human/mouse-dystrophin and human-specific dystrophin expression in sham-treated and huSC-transplanted *tibialis anterior* muscles. Muscles were injected with PBS (sham) or transplanted with 3×10^4 p38i-expanded human huSCs and analyzed 56 days later. (**E**) Representative live-cell fluorescence micrograph of control (empty vector) and GFP-expressing adenovirus infected human huSCs. (**F**) Representative fluorescence microscopic analysis of muscle injected with PBS (sham) or transplanted with 3×10^4 GFP-expressing huSCs. Frozen sections of muscle were stained for human/mouse laminin to visualize muscle fiber boundaries. (**G**) Low- and (**H**) high-magnification images with individual colors that comprise the merged image in Figure 4B. The yellow arrowhead identifies a sublaminar PAX7-expressing cell of human origin; the yellow arrow identifies a nearby sublaminar Pax7-expressing cell of mouse origin. (**I**) Representative flow cytometric analysis of sham (PBS only) and transplanted (3×10^4

cells) muscles four weeks post-transplant. (**J**) IF analysis of PAX7 expression in the population of engrafted human huSCs re-isolated in (A). Nuclei were stained with DAPI.

Movie S1. Representative time-lapse videomicroscopy of *ex vivo* activation of purified human MuSCs. Related to Figure 1. The movie begins 12 h (t=0) post-isolation and continues for 90 h (t=90). Relative time is indicated in yellow.

Table S1. Transcription factors, receptors, and signaling molecules up-regulated in quiescent and activated huSCs. Related to Figure 2. Lists of the 30 most highly up-regulated transcription factors, receptors, and signaling molecules, as defined by the PANTHER gene ontology database, derived from RNA-seq comparisons of quiescent and activated huSCs. Loci for which the average FPKM was less than 5 were excluded.

Table S2. Biological processes associated with genes up-regulated in activated huSCs relative to quiescent huSCs. Related to Figure 2. Lists of biological processes, as defined by the PANTHER gene ontology database, significantly enriched ($P < 0.05$) among genes up-regulated in activated relative to quiescent huSCs. Gene set enrichment was analyzed using the DAVID bioinformatics platform. The associated genes are listed with each term. The analysis used those loci for which FPKM>5 and fold-change >2.

Table S3. Biological processes associated with genes up-regulated in quiescent huSCs relative to activated huSCs. Related to Figure 2. Lists of biological processes, as defined by the PANTHER gene ontology database, significantly enriched ($P < 0.05$)

among genes up-regulated in quiescent relative to activated huSCs. Gene set enrichment was analyzed using the DAVID bioinformatics platform. The associated genes are listed with each term. The analysis used those loci for which FPKM>5 and fold-change >2.

Table S4. Biological processes associated with genes differentially expressed by human p38i-treated huSCs relative to untreated huSCs. Related to Figure 3. Lists of biological processes, as defined by the PANTHER gene ontology database, significantly enriched ($P < 0.05$) among genes with increased or decreased expression in p38i-treated huSCs relative to untreated huSCs. Gene set enrichment was analyzed using the DAVID bioinformatics platform. The associated genes are listed with each term. The analysis used those loci for which FPKM>5 and fold-change >2.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Human skeletal muscle specimens

Reproducibility of prospective isolation methods was also tested with *serratus anterior* and *rectus abdominis* muscle specimens. Subjects ranged in age from 35 to 82 years. Scarcity of samples did not allow for statistically meaningful comparisons of samples according to donor age. Sample processing for cell or tissue analysis began within one hour of specimen isolation. In all studies, standard deviation reflects variability in data derived from studies using true biological replicates (i.e., unique donors). Data were not correlated with donor identity.

Human SC isolation

After triturating and washing the digested muscle to yield a mononuclear cell suspension, the cells were stained for 45 min at 4°C with anti-CD31-Alexa Fluor 488 (clone WM59; BioLegend; #303110), anti-CD45-Alexa Fluor 488 (clone HI30; Invitrogen; #MHCD4520), anti-CD34-FITC (clone 581; BioLegend; #343503), anti-ITGB1-APC (clone TS2/16; BioLegend; #303008) at 1:100 (V:V). In addition to primary antibodies, cells were incubated with EGF-biotin (Invitrogen; #E3477) at 0.4 μg/mL to detect EGFR. After washing away primary antibodies, cells were incubated for 15 min at 4°C in streptavidin-PE/Cy7 (BioLegend) at 1:100 (V:V) to detect EGF-biotin. Cell sorting was performed with a BD FACSAria II or BD FACSAria III using 405-nm, 488-nm, and 633-nm lasers. Sorted cells were routinely analyzed by flow cytometry immediately after sorting to ensure high sorting efficiency. Unstained cells were routinely

used to define FACS gating parameters. A fraction of the sorted cells were routinely plated and stained for PAX7 to determine the purity of the sorted population.

Human muscle fiber explants and immunofluorescence analysis

Isolated fibers were washed in media and fixed in 4% PFA (PBS). Fibers were permeabilized with 0.3% Triton X-100, blocked in 10% goat serum, and stained overnight with primary antibodies against surface markers (same as those used for sorting) and against PAX7 (DSHB) at 1:100 (V:V). After washing out primary antibodies, fibers were stained with secondary antibodies and DAPI. Anti-mouse antibodies raised in goat and conjugated to Alexa Fluor 594 (Invitrogen) or Alexa Fluor 488 (Invitrogen) were used to detect PAX7. For EGFR analysis, unfixed fibers were incubated for 15 min in EGF-biotin, fixed in 4% PFA (PBS), and stained as above. Streptavidin-Alexa Fluor 647 was used to detect the EGF-biotin. After washing out secondary antibodies, muscle fiber explants were mounted on microscope slides (Superfrost Plus; Fisher Scientific).

Human SC culture

Differences in the expression of *PAX7*, *PAX3*, *MYF5* and *MYOG* among SCs cultured in media containing different serum lots were not statistically significant (data not shown). Where indicated, medium was supplemented with 10 μM p38 inhibitor (SB203580, Cell Signaling Technology; SB239063, Tocris Bioscience). Medium, including medium containing p38i, was changed once every two days. Where indicated, EdU (Invitrogen) was pulsed for 1 h at a final concentration of 10 μM. Cells were

routinely cultured at densities of 40% confluency, except where indicated. To test for myotube formation, huSCs or huSC-depleted cells were cultured for 9 days with a target final confluency of ~90%.

For clonal analyses, purified cells were plated singly in ECM-coated chamber slides, and cultured as above for a period of six days. In the putative SC population, a total of 140 clones were analyzed. In the non-myogenic population of CD31, CD45, or CD34-expressing cells, 125 clones were analyzed. These clones were cultured in two independent experiments. For comparisons of p38i effects on EdU incorporation in undifferentiated cultures, freshly isolated cells were grown in the given concentration of p38i for a period of 3 days. EdU (10 μM) was added to the culture for 1 h prior to fixation. For comparisons of p38i effects on EdU incorporation in differentiated cultures, cells were grown in the given concentration of p38i for a period of 9 days. For analyses of p38 target gene expression, cells were cultured for 9 days with a target final confluency of 80%.

For analysis of engraftment of GFP-expressing, p38i-expanded huSCs, cells were initially expanded for 10 d and then infected with adenovirus bearing a GFP reporter gene (Ad5CMV-GFP; University of Iowa Gene Transfer Vector Core) or vector control (Ad5CMV-empty). Medium was replaced 24 h post-transfection, and cells were further expanded for >3 additional passages prior to transplantation. Infection efficiency was routinely monitored by epifluorescence microscopy (see Figure S4E).

Quantitative RT-PCR

One microgram of total RNA was reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Invitrogen). Quantitative PCR was performed on an ABI 7900HT Fast Real-Time PCR system using custom synthesized oligonucleotide primers (Invitrogen) designed to amplify the cDNA of selected target genes. Relative quantification of gene expression normalized to GAPDH was carried-out using the comparative C_T method (Pfaffl, 2001). Each measurement was performed in triplicate in two independent experiments. The following primers were used in these assays:

Target	Forward Primer	Reverse Primer
PAX7	gaaaacccaggcatgttcag	gcggctaatcgaactcactaa
PAX3	ttggcaatggcctctcac	aggggagagcgcgtaatc
MYF5	ctatagcctgccgggaca	tggaccagacaggactgttacat
MYOD1	cactacagcggcgactcc	taggcgccttcgtagcag
MYOG	gctcagctccctcaacca	gctgtgagagctgcattcg
MEF2C	tgatcagcaggcaaagattg	tggacactgggatggagact
GAPDH	agccacatcgctcagacac	gcccaatacgaccaaatcc

Immunofluorescence analysis of cultured SCs

Cultured SCs were fixed with ice-cold 4% PFA (in PBS) for 10 min, rinsed with PBS, and permeabilized in 0.3% Triton X-100 (in PBS). Fixed and permeabilized cells were blocked in 10% goat serum (in PBS) and incubated with primary antibodies for 12 h at 4°C. Primary antibodies recognizing PAX7 (DSHB), MYOD1 (clone 5.8A, Dako, #M3512), MYOG (clone F5D, DSHB), and myosin heavy chain (clone MF20, DSHB) were used at 2.5 μg/mL, 2.8 μg/mL, 1.6 μg/mL, and 0.5 μg/mL, respectively. Primary

antibodies recognizing p-p38 (Cell Signaling, #9211), phospho-HSP27 (Ser82) (Cell Signaling, #9709), and phospho-GYS1 (Ser645) (Abcam, ab195743) were each used at 1:200 (V:V). After washing-out primary antibodies with PBS, cells were incubated with secondary antibodies for 1 h at room temperature. Anti-mouse antibodies raised in goat and conjugated to Alexa Fluor 594 or Alexa Fluor 488 were used to detect mouse primary antibodies. EdU detection was carried-out using a Click-It EdU detection kit (Life Technologies) according to the manufacturer's instructions. Cells were next washed with PBS and mounted with Fluoro-Gel (Electron Microscopy Sciences). IF imaging was performed with an AxioObserver Z1 epifluorescence microscope (Carl Zeiss) equipped with an Orca-R2 CCD camera (Hamamatsu Photonics) or an LSM 710 confocal system (Carl Zeiss). Image analysis was performed using Improvision Volocity software (Perkin Elmer) or ZEN 2010 software (Carl Zeiss). For quantitation of purity in cultured purified SCs, the proportion of cells expressing PAX7 was calculated 3 days after cell isolation in 8 representative cultures each derived from a distinct donor. For quantification of relative fluorescence intensity, Improvision Volocity software was used to define a ROI for each cell, and the average fluorescence intensity in the channel of interest for a given cell was calculated by the software. Fluorescence intensity measurements for single cells was recorded. In the case of p-p38 fluorescence measurements, the nucleus (defined by area stained with DAPI) was used for the ROI; in the case of p-GYS1 and p-HSP27, the entire cell (defined by phase-contrast) was used for the ROI.

Metaphase spreads

Human SCs were cultured as described above. Colcemid was added to the culture at a final concentration of 100 ng/mL for 3 h. Culture medium was collected and the cells were incubated for 15 min in Trypsin-EDTA at 37^{o}C. Trypsinized cells were combined with the collected medium, centrifuged, and gently resuspended in 0.3 mL of medium. Seven mL of hypotonic solution (0.8% sodium citrate in water) was added dropwise to the sample and incubated for 10 minutes at room temperature. The sample was then washed and resuspended in 0.5 mL of hypotonic solution. Seven mL of Carnoy's fixative (3:1 methanol:acetic acid) was added and incubated for 10 minutes at room temperature. Fixed cells were then washed an additional two times in fixative. Cells were resuspended in 500 mL of fixative and dropped onto SuperFrost Plus microscope slides from a height of 1.5 m. Once dry, slides were mounted with DAPI-containing Fluoro-Gel. Spreads were imaged with a Zeiss LSM 710 confocal system. A total of 75 metaphase cells from three independent experiments were analyzed.

Time-lapse microscopy

Prospectively isolated human SCs were plated at a density of 2,500 cells per 0.7 cm^2 and cultured as above. Cultures were analyzed using an Axiovert 200M inverted microscope (Carl Zeiss) equipped with an environmental control chamber (CTI controller, Tempcontrol; Carl Zeiss; humidified 5% CO_2). Brightfield images were obtained every 15 min for 4 days with a Zeiss camera controlled with the Axiovision software (Carl Zeiss). All calculations of cell-cycle times were performed on a total of 200 cells from three independent experiments.

RNA isolation, sequencing and analysis

Total RNA was extracted from FACS-purified SCs using the NucleoSpin RNA XS (Macherey-Nagel #740902). Two biological replicates were sequenced in each of three conditions (freshly isolated, cultured, and cultured in the presence of p38i). All muscle tissue used in these analyses was from *latissimus dorsi*. Cultured cells were maintained at <40% confluency prior to RNA isolation. For Illumina sequencing, cDNA libraries were generated with 500 pg of total RNA using the SMARTer Ultra Low Input RNA Kit for Illumina Sequencing (Clontech #634935). Paired-end sequencing libraries were then generated using the Ovation Ultralow Library System (NuGEN #0303) according to manufacturer instructions. The libraries were sequenced on an Illumina HiSeq 2000 at the Stanford Center for Genomics and Personalized Medicine. Approximately 200 millions paired-end reads (2x100bp) were obtained per biological replicate. Paired-end reads were filtered, aligned to the hg19 genome assembly and quantified as the number of sequenced fragments per kilobase of exon per million fragments mapped (FPKM) using the Tuxedo protocol (Trapnell et al., 2012).

Gene set enrichment and transcription factor network analysis

Gene set enrichment analysis was performed with the DAVID Functional Annotation tool (Huang et al., 2009). Gene sets were defined as noted in the text, typically using thresholds of 5- to 10-fold change in FPKM between samples.

To build a transcription factor network reflecting the regulation of huSC activation from a quiescent state, we first used the Whole Genome rVista tool to identify transcription factors with predicted binding sites that are over-represented ($P < 3x10^{-7}$) in

upstream regions (within 3000 bp) of the genes for which expression differs more than ten-fold between quiescent and cultured huSCs after a period of 7 days in culture. FPKM values from RNA-seq analyses were used to calculate gene expression changes. Using the identified transcription factors as nodes, we constructed a network based on known interactions among transcription factors using STRING version 9.05 (Franceschini et al., 2013). Disconnected or singly connected nodes were excluded from the network.

Animals

NOD/SCID and IL2 receptor gamma chain deficient (NSG) mice were obtained from The Jackson Laboratory. Mice were housed and maintained in the Veterinary Medical Unit at Veterans Affairs Palo Alto Health Care Systems. Male mice aged 2 to 4 months were used in our studies. Animal protocols were approved by the Administrative Panel on Laboratory Animal Care of the VA Palo Alto Health Care System.

Human SC transplantation

NSG mice were used as transplant recipients. The *tibialis anterior* muscle of the recipient mouse was injured with 50 μL of 1.2% $BaCl_2$ 48 h prior to use in transplantation studies. Cultured human SCs were incubated in Accutase (Invitrogen) at 37°C to detach cells, resuspended in fresh DMEM, and filtered through a 40-μM cell strainer to exclude any fused myotubes. Freshly isolated and cultured SCs were counted, centrifuged, and resuspended in a volume of 1% BSA (in PBS) that would enable transplantation of the appropriate dose of cells in a total volume of 15 μL per transplantation. Cell suspensions were kept on ice prior to transplantation. For

transplantation, cells were loaded into a 50-μL Hamilton syringe equipped with a 30-gauge needle. After incising the skin overlying the anterior lower hindlimb, 3×10^4 cells were delivered to the *tibialis anterior* muscle as a single injection. Recipient mice were treated post-surgery with buprenorphine (0.1 mg/kg) and baytril (5 mg/kg). All quantitative analyses of transplantation were performed on a minimum of four biological replicates per tested condition.

Histological analysis of SC engraftment

Four to eight weeks post-transplantation, recipient *tibialis anterior* muscles were dissected and frozen by immersion for 90 s in liquid nitrogen-cooled isopentane. For analysis of GFP expression, muscles were fixed for 6 h in 0.5% PFA (in PBS) and dehydrated in 20% sucrose (in PBS) for 12 h before freezing. Frozen muscles were stored at -80°C prior to sectioning. Cross-sections of transplanted muscle were cut at a thickness of 7 μm, collected onto Superfrost Plus glass slides, rehydrated with PBS, and fixed in 4% PFA (in PBS) for 5 min. After washing with PBS, sections were permeabilized with 0.3% Triton X-100 for 10 min and again washed with PBS. Sections were then blocked for 1 h in 10% goat serum (in PBS) or, when unconjugated mouse primary antibodies were used, in 10% goat serum (in PBS) with mouse-on-mouse blocking reagent (Vector Laboratories) according to the manufacturer's instructions. Blocking solution was washed away with PBS and sections were incubated overnight with primary antibodies at 4°C. Sections were then washed with PBS and incubated with secondary antibodies, and DAPI, for one hour at room temperature. Secondary antibodies were washed away with

PBS and slides were mounted with Fluoro-Gel. Imaging was performed as described above.

Primary antibodies used for staining frozen sections were: mouse anti-PAX7 (detecting human and mouse; DSHB; 2.5 μg/mL), mouse anti-DMD (human-specific; EMD Millipore #MAB1690; 1:20 V:V), rabbit anti-DMD (detecting human and mouse; Abcam #15277; 1:500 V:V), rabbit anti-LMNA (human-specific; clone EPR4100; Abcam #108595; 1:300 V:V), rat anti-laminin (detecting human and mouse; clone 4H8-2; Abcam #11576; 1:2500 V:V), and mouse anti-ITGB1 (human-specific; clone TS2/16; BioLegend; #303008; 1:200 V:V) conjugated to APC. Primary antibodies were detected with secondary antibodies recognizing mouse, rat or rabbit conjugated to Alexa Fluor dyes.

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