

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | n/a | Confirmed |
|-------------------------------------|--|
| ☐ | ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection Dynamic Muscle Control and Analysis Software (Aurora Scientific)

Data analysis R version 3.6.1, edgeR 3.26.8, DAVID (<https://david.ncicrf.gov/>), Nmerge, Bowtie2, RemoveChrom, Picard, SamTools, IDR, Diffbind, ChromVAR, GREAT, TOBIAS, ClusterProfiler, msigdb, Flojo v10.9

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA sequencing data can be found on FigShare at <https://doi.org/10.6084/m9.figshare.25917505>. The GEO accession number for ATAC-seq data in this paper is GSE267952 and can be found at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE267952>. No restrictions on data availability exist.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed. Sample sizes exceeded biological triplicate in all cases. Additional samples were conducted to provide a reasonable statistical conclusion balancing both time and budgetary constraints. Biological replicates are designated in the figures legends as n=# of replicates.
Data exclusions	For RNAseq data. The gene expression matrix was filtered by removing: (1) samples with fewer than 3000 genes detected; (2) RNA associated genes ("Mt_tRNA", "Mt_rRNA", "snoRNA", "snRNA", "misc_RNA", "scaRNA", "rRNA", "sRNA"); (3) genes with fewer than 10 counts total that are seen in less than 4 samples.
Replication	All attempts at replication were successful. Experiments were replicated at separate times by a variety of co-authors on the paper over the duration of the project.
Randomization	All mice were randomly organized into experimental groups. All samples were allocated into groups based on their cohort (myoblast, idSC, SC).
Blinding	Investigators were blinded for all experiments conducted in this paper with the exception being where a given cohort visually would be distinguishable (eg. Immunofluorescence experiments where the presence of an organoid would distinguish these samples from myoblast or intact muscle samples). All bioinformatic data was conducted in a blinded/unbiased manner. Engraftment, repopulation, self-renewal and muscle force experiments were all blinded for data collection and analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	All antibodies are summarized in Table S6
Validation	Anti-Mouse Pax7 (Pax7) : https://dshb.biology.uiowa.edu/PAX7?quantity=1&keywords=pax7 Anti-Mouse MyoD (G-1) : https://www.scbt.com/p/myod-antibody-g-1 Anti-Mouse MyoD (MF20) : https://dshb.biology.uiowa.edu/MF-20 Anti-Laminin : https://www.novusbio.com/products/laminin-antibody_nb300-144af647Ant Anti-GFP Rb : https://www.abcam.com/products/primary-antibodies/gfp-antibody-ab13970.html Anti- RFP : https://www.rockland.com/categories/primary-antibodies/rfp-antibody-pre-adsorbed-600-401-379/ Anti-Mouse Dystrophin : https://dshb.biology.uiowa.edu/MANDRA1-7A10 Anti-alpha7 Integrin : https://link.springer.com/protocol/10.1007/978-1-61779-343-1_3#Sec2 Anti-CD29 : https://www.thermofisher.com/antibody/product/CD29-Integrin-beta-1-Antibody-clone-eBioHMb1-1-HMb1-1-Monoclonal-/47-0291-82 Anti-Cxcr4 : https://www.biolegend.com/fr-ch/products/pe-anti-human-cd184-cxcr4-antibody-542 Anti-Cd34 : https://www.biolegend.com/fr-ch/products/apc-anti-human-cd34-antibody-6204 Anti-Vcam1 : https://www.biolegend.com/fr-ch/products/alexa-fluor-647-anti-mouse-cd106-antibody-3129

Anti-Notch3 : <https://www.biolegend.com/fr-ch/products/alexa-fluor-647-anti-mouse-notch-3-antibody-5288>
 Anti-Cd339 : <https://www.biolegend.com/fr-ch/products/pe-anti-mouse-cd339-jagged-1-antibody-8773>
 Anti-Sdc1 : <https://www.biolegend.com/fr-ch/products/apc-anti-mouse-cd138-syndecan-1-antibody-7572>
 Anti-Cd140b : <https://www.thermofisher.com/antibody/product/CD140b-PDGFRB-Antibody-clone-APB5-Monoclonal/17-1402-82>
 Anti-Cd9 : <https://www.biolegend.com/fr-ch/products/alexa-fluor-647-anti-mouse-cd9-antibody-5566>
 Anti-Cd104 : <https://www.biolegend.com/fr-ch/products/alexa-fluor-647-anti-mouse-cd104-antibody-9741>
 Anti-Cd44 : <https://www.biolegend.com/fr-ch/products/alexa-fluor-647-anti-mouse-human-cd44-antibody-3098>
 Anti-Cd49e : <https://www.biolegend.com/fr-ch/products/apc-anti-mouse-cd49e-antibody-16202>
 Anti-Cd200 : <https://www.biolegend.com/fr-ch/products/apc-anti-mouse-cd200-ox2-antibody-7338>
 Anti-MYOD1 : <https://www.thermofisher.com/antibody/product/MYOD-Antibody-clone-HL1372-Recombinant-Monoclonal/MA5-47019>
 Anti-CD44 : <https://www.biolegend.com/fr-ch/products/brilliant-violet-421-anti-human-cd44-antibody-8801>
 Anti-CD49e : <https://www.biolegend.com/fr-ch/products/pe-anti-human-cd49e-antibody-4102>
 Anti-CD104 : <https://www.biolegend.com/fr-ch/products/pe-anti-human-cd104-antibody-4121>
 Anti-CD56 : <https://www.biolegend.com/fr-ch/products/pe-cyanine7-anti-human-cd56-ncam-antibody-4584>
 Anti-CD184 : <https://www.biolegend.com/fr-ch/products/apc-anti-human-cd184-cxcr4-antibody-539>
 Anti-THY1 : <https://www.biolegend.com/fr-ch/products/pe-cyanine7-anti-human-cd90-thy1-antibody-8282>
 Anti-CD9 : <https://www.biolegend.com/fr-ch/products/apc-anti-human-cd9-antibody-15072>
 Anti-SDC1 : <https://www.biolegend.com/fr-ch/products/apc-anti-human-cd138-syndecan-1-antibody-7315>
 Anti-CD29 : <https://www.biolegend.com/fr-ch/products/apc-anti-human-cd29-antibody-852>
 Anti-FGFR4 : <https://www.biolegend.com/fr-ch/products/pe-anti-human-cd334-fgfr4-antibody-3763>
 Anti-KI67 : <https://www.abcam.com/products/primary-antibodies/ki67-antibody-ab15580.html>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	All mouse cell lines were primary lines derived in the lab from the mouse strains listed in the manuscript. 293T cells were purchased from ATCC. Gibco and Cook myoblast lines are commercially available. Human myoblast lines 1230, 1369 were provided by Dr. Emanuela Gussoni.
Authentication	Cell lines were authenticated based on their expression of myogenic surface markers/transcription factor and their phenotype to give rise to multinucleated myotubes in vitro or in vivo. 293T cells were not authenticated prior to being used to produce Lentivirus.
Mycoplasma contamination	All cell lines used in the manuscript tested negative for mycoplasma
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Strain:mouse Lines:C57Bl/6, Tg:Pax7nGFP, mdx5cv, NSG Age:2-6months
Wild animals	No wild animals were used in this study
Field-collected samples	No field-collected samples were used in this study
Ethics oversight	Harvard IACUC protocol 26-17

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Briefly, hindlimb skeletal muscles were dissected from either male or female mice between the ages of 2-6 months. Muscles were aseptically dissected, minced and digested in a dispase and collagenase solution (1 mg/ml collagenase and 4 mg/ml dispase II, Roche) for 10 min (trituated briefly after 5 mins) at 37 °C. The digested muscle slurry was pelleted at 1700 rpm for 5 min (Heraeus Multifuge) and subsequently filtered through a 100µm cell strainer (Falcon). Cells were washed in FACS buffer
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several times prior to FACS sorting or culture in vitro. SkMOs were rinsed with PBS and digested in a 50ml falcon tube with collagenase/dispase solution containing 1000u of DNase (Worthington). Following trituration the cell suspension was quenched with FBS and pelleted. Dissociated SkMO cells, hindlimb skeletal muscle in the case of satellite cells, primary myoblasts or reserve cells were resuspended in FACS buffer and filtered through a 50um filter (Partec) prior to FACS sorting. Cells were separated on a MoFlo cytometer (DakoCytomation) based on their expression of nGFP. Sorted satellite cells were either used immediately for experiments, or expanded in culture as myoblast lines prior to SkMO generation.

Instrument

Moflo XDP or Astrios (DakoCytomation)

Software

FlowJo v10

Cell population abundance

FACS sorted cells were collected in 1.5ml eppendorf tubes. Pelleted at 6000rpm for 5min and resuspended in an appropriate volume of liquid for subsequent experiments. For transplantation, cells were resuspended in sterile saline (0.9% NaCl) at a concentration of 10,000 events/25ul prior to transplant. For RNAseq, cells were directly lysed for library construction according to the manufactures protocols. ATACseq experiments cells conducted according to the OMNI-ATAC protocol

Gating strategy

All FACS experiments initially involved gating the bulk population from debris based on SSC vs FSC followed by identification of singlets based on FSC height vs FSC weight. Identification of GFP positive cells was conducted versus an empty PE channel to distinguish autofluorescence. A similar strategy was used to identify double positive GFP+ and TdTomato+ cells prior to transplant.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.