nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	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 *Give P values as exact values whenever suitable.*
☒	☐	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	All software used for collecting primary data for individual tests is listed in the Methods section. For the RNA-seq software package used in the analysis of raw sequencing data and statistics, refer to the details below or in the Methods section.
Data analysis	For bnRNA-seq data processing, FASTQ files were processed using the nf-core/rnaseq v3.11.1 pipeline with minor modifications. Quality control was performed using FastQC. Low-quality bases and adapter sequences were then trimmed and filtered from the reads using Cutadapt v3.4 to improve the overall data quality. Ribosomal RNA sequences were subsequently removed from the aligned data using SortMeRNA v4.3.4 to eliminate potential contamination from non-target RNA species. The resulting alignments were sorted and indexed using SAMtools v1.16.1, enabling efficient storage and retrieval of the alignment information. Reads were aligned to the mm10 genome obtained from GENCODE (vM21) using STAR v2.7.9a. For read quantification, we utilized Salmon, which specializes in estimating transcript abundance from RNA-Seq data. Selected QC metrics associated with each sample are reported in Supplementary Table S6. Transcripts were annotated using a modified GTF file obtained from gencode (vM24) containing both intronic and exonic regions for genes. Reads were imported into DESeq2 using the tximport command from the tximport package. All differential expression analysis was performed using DESeq2 under various conditions based on the experimental design and whether analysis was paired or unpaired. For snRNA-seq data processing, initial read alignment and quantification of FASTQ files were generated using CellRanger/5.0.1. Selected QC metrics reported by CellRanger for each sample are shown in Supplementary Table S7. For each dataset, we corrected for ambient background RNA using cellbender. Reads from cellbender were then imported into Seurat objects, and nuclei with less than 200 unique features were removed from downstream analysis. Seurat objects with the remaining nuclei then underwent doublet identification using Solo/0.2. Nuclei having higher than the 95th percentile of number of unique features, number of UMIs, and mitochondrial reads were excluded. Violin plots for these metrics for all snRNA-seq experiments used in this study are shown in Extended Data Fig. 5. An additional filtration of any nuclei containing higher than 5% reads mapping to the mitochondrial genome were excluded from all datasets. Datasets were normalized using the SCTransform() command, setting the “vst.flavor” argument to “v2”, and the vars.to.regress argument set to

"percent.mt". Additional regression of cell cycle scores was included in the SCTransofrm vars.to.regress argument only for datasets derived from whole muscle sequencing (P10 and Five Months), as these datasets included non-postmitoic cells. First, each cell was given a score using the CellCycleScoring() function provided by Seurat on the normalized RNA Assay, which was normalized using the NormalizeData() function using the gene signatures for S Phase and G2M phase provided by Seurat. These genes were converted to the mouse genome by lowercasing all but the first letter of each gene.
Additional details for data integration are available in our Methods section.

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Raw sequencing data has been deposited in GEO (GSE241035) and are publicly available.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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

Life sciences study design

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

Sample size	No formal sample size calculation was conducted prior to the experiments. The sample sizes were determined based on previous experience, and they were deemed sufficient to achieve statistical significance at the reported effect sizes.
Data exclusions	No data were excluded from the analyses.
Replication	Experiments were conducted across multiple cohorts of mice, with appropriate control groups included in each cohort.
Randomization	Mice were randomly placed into experimental groups. Random fields were imaged for smRNA-FISH quantification experiments.
Blinding	All image analysis was performed in a blinded fashion

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Anti-Dystrophin antibody, Abcam, ab15277
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555, Invitrogen, A-31572
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488, Invitrogen, A-21206
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647, Invitrogen, A21244
Anti-PCM1 antibody produced in rabbit, Sigma-Aldrich, HPA023374
APC anti-mouse CD106 Antibody, Biolegend, 105717
PE anti-mouse CD45 Antibody, Biolegend, 103105
PE anti-mouse CD31 Antibody, Biolegend, 102407
PE anti-mouse Ly-6A/E (Sca-1) Antibody, Biolegend, 108107
PE anti-mouse TER-119/Erythroid Cells Antibody, Biolegend, 106207

Validation

All antibodies used in this study have been validated in previous work in the field.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

The transgenic mouse lines utilized in this research include Pax7CreER, Myomaker loxP/loxP, Pax7rtTA, HSArtTA, and TREH2B-GFP. All mice were maintained on a C57BL/6 genetic background. Both male and female mice were used from postnatal day 10 through 5 months of age.

Wild animals

No wild animals were used in this study.

Reporting on sex

Both male and female mice were used in this study.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

Animal studies were conducted in accordance with guidelines from the National Institutes of Health and the Division of Veterinary Services (DVS) at Cincinnati Children's Hospital Medical Center. The IACUC protocol number for this study is #2020-0047.

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

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	Following euthanasia, tibialis anterior, gastrocnemius, or plantaris muscles were dissected, minced, and placed in homogenization buffer (0.25 M sucrose and 1% BSA in Mg2+-free, Ca2+-free, RNase-free PBS). An Ultra-Turrax T25 was utilized for further homogenization of the minced tissue. The homogenate was incubated for 5 minutes upon the addition of Triton-X100 (2.5% in RNase-free PBS, added at a 1:6 ratio). All samples were filtered through a 100 µm strainer, centrifuged (3000×g for 10 minutes at 4°C) to yield a crude pellet, and resuspended in sorting buffer (2% BSA/RNase-free PBS) before being filtered again via a 40 µm strainer. The washed nuclei pellets were resuspended in sorting buffer (2% BSA/RNase-free PBS) and labeled with anti-PCM1 (1:500, Sigma-Aldrich HPA023374) on ice for 45 minutes. Nuclei were washed twice in sorting buffer and then labeled with an Alexa Fluor 647-conjugated secondary antibody (1:100, Invitrogen A21244) at 4°C for 30 minutes, followed by labeling with Hoechst dye.
Instrument	BD LSR II
Software	All FACS analysis was performed on an LSRII platform (BD Biosciences), and the creation of data plots was achieved with FlowJo v10.8.1 software.
Cell population abundance	Cell/nuclei purity for flow cytometry was assessed using FSC/SSC gating to eliminate doublets. For each experiment, a minimum of 10,000 events was recorded, achieving a purity of over 90%.
Gating strategy	For antibody staining and the application of the appropriate compensation matrix, FMO controls and single-stained samples were included for each antibody and fluorophore. This procedure was used to accurately define the boundaries between positive and negative cell/nuclei populations.

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