

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 (<i>n</i>) 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), 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	ATAC-Seq, Cut&Tag and Cut&Run Data were obtained using NovaSeq6000 Sprime Paired End (PE) sequencing machine and RNA-Seq data was obtained using the NextSeq500 (75bp) sequencing machine. The data was imported into R and bash scripts for analysis.
-----------------	--

Data analysis

We have provided a Zenodo website link that includes all the codes used for our analyses. The softwares that were used are listed below:

GenPipes v.3.1.5
 Trimmomatic v.0.36
 Burrow-Wheeler Aligner v.0.7.12
 SAMtools v.1.3.1
 MACS2 v.2.1.1
 BEDtools v2.29.2
 Rsubread v.2.2.6
 Homer v.4.11
 ChIPSeaker v.1.24.0
 Homer v.4.9.1
 STAR v.2.5.3a
 Ensemblannotation v83
 HTSeq v.0.11.2
 DeSeq v.1.38.3
 edgeR v.3.30.1
 deepTools v.2.5.0.1
 DiffBind v.2.16.2

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](#)

The Next-Generation Sequencing (NGS) data reported in this manuscript are available through GEO series accession numbers GSE266726 (RNA-Seq), GSE266720 (ATAC-Seq), GSE266725 (Cut&Tag), GSE282888 (Cut&Run) respectively.

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

For all RNA-Sequencing experiments, we included at minimum n=3 biological replicates per condition. For ATAC-Seq, Cut&Tag and Cut&Run, we have included n=2 REST-cKO and WT samples. No statistical method was used to determine sample size. Sample size was chosen based on convention in the field. For all the other experiments, we included at least 3 WT and 3 REST-cKO mice.

Data exclusions	No data was excluded from the analysis.
Replication	All of the experiments were performed at least n=3 replication.
Randomization	Mice were simply allocated into experimental groups based on their genotype of REST-cKO vs WT.
Blinding	Blinding during muscle stem cell isolation from REST-cKO and WT mice was not feasible due to visibly different cell yields. However, all subsequent analyses were conducted in an unbiased manner, with samples from both genotypes processed through the same analytical pipeline. In vitro experiments and cell counting were also performed without knowledge of genotype to ensure objectivity.

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

Antibodies used are below:

Anti-mouse ITGA7-647 (1:500) (R&D systems, FAB3518R, clone# 334908, lot# 1734542)
 Anti-mouse VCAM-1 -PE-Cyanine7 (1:500) (Invitrogen, 25-1061-82, clone# 429, lot# 2630645)
 Anti-mouse SCA1 (LY6A/E)-PE (1:5000) (BD Biosciences, 553108, clone# D7, lot# 2034486)
 Anti-mouse CD31-PE (1:5000) (Invitrogen, 12-0311-82, clone# 390, lot# 2633378)
 Anti-mouse CD45-PE (1:5000) (Invitrogen, 12-0451-82, clone# 30F11, lot# 2633389)
 Anti-mouse CD11b-PE (1:5000) (Invitrogen, 12-0112-82, clone# M1/70, lot# 2588150)
 Anti-Histone H3(tri methyl K4) antibody (1:70) (abcam, 8580, lot# GR336286-3)
 Anti-Histone H3(acetyl K27) antibody (1:70) (abcam, 4729, lot# GR3442878-1)
 Anti -Histone H3 (tri methyl K27) antibody (1:50) (Cell Signaling Technologies ,9733, clone# C36B11, lot# GR3399022-14)
 Anti-REST antibody (1:10) (Millipore, 17-641, lot# 3843801)
 Anti- Cleaved Caspase 3 antibody (1:400) (Cell Signalling, 9664S, clone# 5A1E, lot#1)
 Anti-Rest antibody (1:100) (Millipore, 09-019, lot# 2761632)
 Anti-Tubulin antibody (1:1000) (Cell Signalling, 3873, clone# DM1A, lot# 16)
 Anti-MyoD antibody (1:300)(Santa Cruz, sc-377460, clone# G1, lot# E0223)
 Anti-Mouse Pax7 DSHB AB_528428 (1:100)
 Anti-Mouse Myogenin DSHB AB_2146602(1:100)
 Anti-DCX antibody (1:800) (Cell Signalling, 4604, lot# 7)
 Anti- Laminin antibody (1:750) (Sigma, L9393, lot# 046M4837V)

Validation

All antibodies used have been validated in the published literature (Pasut A. Methods Molecular Bio. 2012; Liu, L., et al., Nature Protocols, 2015; Reuben Franklin, et. al, Nature Communications, 2022; Calabretta, et.al., Developmental Cell, 2018; Ram, Oren, et al. Cell, 2011; Paonessa, F; Latifi, S; Scarongella, H; Cesca, F; Benfenati, F, The Journal of biological chemistry, 2013 and more)

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Primary myoblasts are derived from FACS-sorted muscle stem cells from REST-cKO and WT mice. C2C12 cells were obtained from ATCC (CRL-1772).
Authentication	Primary myoblasts are authenticated by their expression of ITGA7 and PAX7, and their ability to differentiate into myotubes. C2C12 cell line is authenticated by the supplier, ATCC.
Mycoplasma contamination	Negative

Commonly misidentified lines
(See [ICLAC](#) register)

N/A

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	C57BL/6J, RESTf/fPax7Cre, RESTf/fPax7CreERT2 mice. All mice were 4-6 weeks old. Mice were housed at a temperature of 21° and 20% humidity, with a light/dark cycle of 14 hours light/10 hours dark.
Wild animals	The study did not involve wild animals
Reporting on sex	All experiments were performed on male mice, to minimize sequencing variations
Field-collected samples	Study did not involve field-collected samples.
Ethics oversight	Animal protocols were approved by the McGill University Animal Care Committee (UACC).

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	Hindlimb muscles were dissected from mice and enzymatically digested using a solution containing Collagenase D, Dispase II, and CaCl ₂ . The resulting cell suspension was filtered, resuspended in 2% FBS in PBS with EDTA, and stained with the appropriate antibodies. After staining, cells were washed, centrifuged, and resuspended in 2% FBS/PBS/EDTA for flow cytometric analysis.
Instrument	Cells were sorted using a BD FACSAria Fusion flow cytometer.
Software	Flow cytometry data was collected using BD FACSDiva software. Figures were subsequently created using FlowJo software
Cell population abundance	The numbers of cells sorted varied amongst the mice based on the genotype as shown in figure 2a. Purity was established using the previously established FACS protocols (Liu, L., et al., Nature Protocols, 2015) for the isolation of muscle stem cells. Additionally, purity was confirmed with the subsequent RNA-Seq analysis for known markers of muscle stem cells such as Pax7 and MyoD.

Gating strategy

Live mononuclear cells were first sorted based on Hoechst expression. Next, endothelial cells, immune cells and FAPS were removed by gating PE- (CD31-/CD45-/CD11b-/SCA1-)cells. PE- population was then gated for ITGA7+ cells which was then further gated for VCAM+ cells. ITGA7+/VCAM1+/Hoechst+/CD31-/CD45-/CD11b-/SCA1- population corresponded to the muscle stem cells.

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