Supplementary Information

REST/NRSF Preserves Muscle Stem Cell Identity by Repressing Alternate Cell Fate

Korin Sahinyan[1,2], Darren M. Blackburn[3], Marie-Michelle Simon[1,4], Felicia Lazure[1,2], Tony Kwan[1,4], David H. Wilson[5], Maryam Vahidyeganeh[3], Nawal Alsadi[3], Julia von Maltzahn[6], Yasuhiro Yamada[7], Arezu Jahani-Asl[8,9], Guillaume Bourque[1,4,10], Michael A. Rudnicki[8,11,12], and Vahab D. Soleimani[1,2,3*]

[1] Department of Human Genetics, McGill University, 3640 rue University, H3A 0C7, Montréal, QC , Canada.

[2] Lady Davis Institute for Medical Research, Jewish General Hospital, 3755 Chemin de la Côte-Sainte-Catherine, H3T 1E2, Montréal, QC, Canada

[3] Department of Biochemistry, Microbiology & Immunology, University of Ottawa, 451 Smyth Rd, K1H 8M5, Ottawa, ON, Canada

[4] McGill Genome Centre, 740 Dr.Penfield Avenue, H3A 0G1, Montreal, QC, Canada

[5] Centre for Regenerative Medicine, Institute for Regeneration and Repair, The University of Edinburgh, 5 Little France Dr, Edinburgh EH16 4UU, Edinburgh, United Kingdom

[6] Faculty of Health Sciences Brandenburg and Faculty of Environment and Natural Sciences, Brandenburg University of Technology Cottbus-Senftenberg, Universitätsplatz 1, 01968 Senftenberg, Germany

[7] Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

[8] Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, 451 Smyth Rd, K1H 8M5, Ottawa, ON, Canada

[9] University of Ottawa Brain and Mind Research Institute, University of Ottawa, 451 Smyth Rd, K1H 8M5, Ottawa, ON, Canada

[10] Canadian Centre for Computational Genomics (C3G), 740 Dr.Penfield Avenue, H3A 0G1, Montreal, QC, Canada

[11] Department of Medicine, Faculty of Medicine, University of Ottawa, 451 Smyth Rd, K1H 8M5, Ottawa, ON, Canada

[12]Sprott Center for Stem Cell Research, Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada

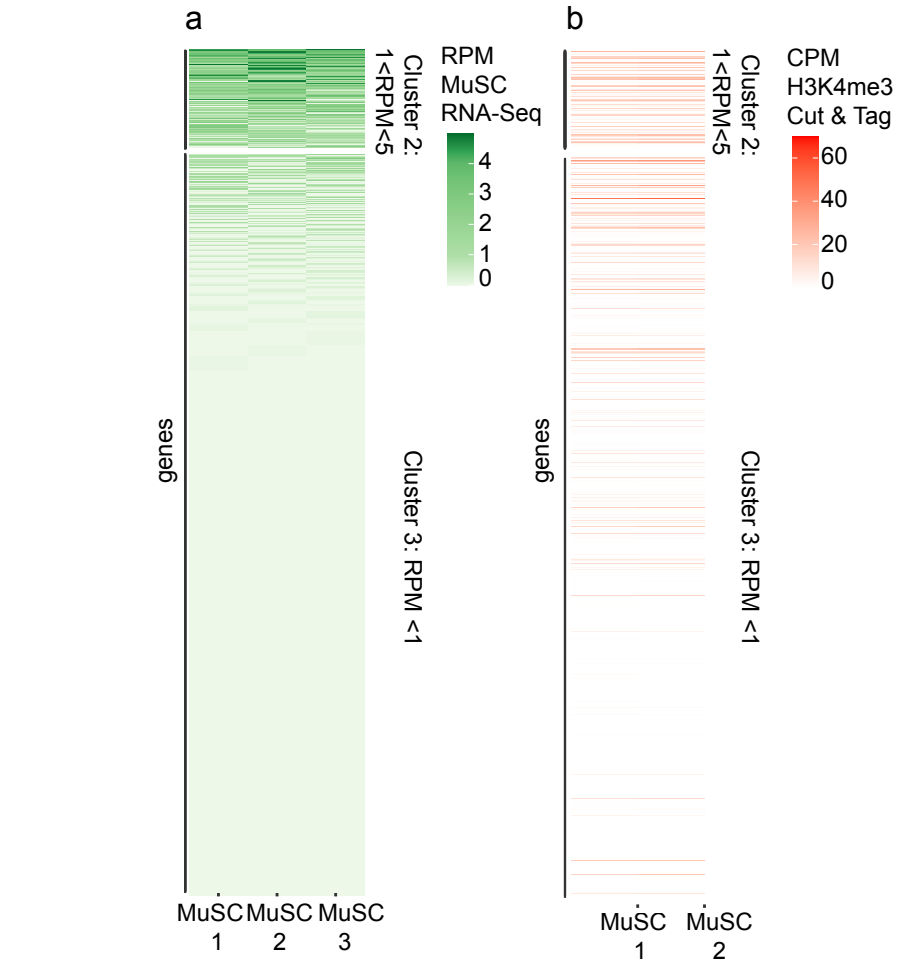
[*]Corresponding author Tel: +1 514 340 8222 ext. 26136 Fax: +1 514 340 7502 vahab.soleimani@mcgill.ca

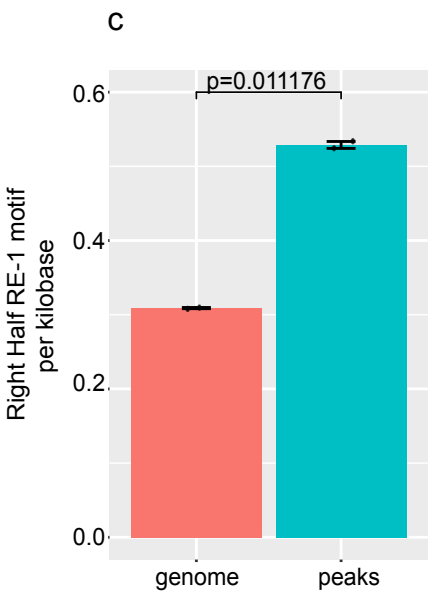
Supplementary Table 1: Primer Sequences

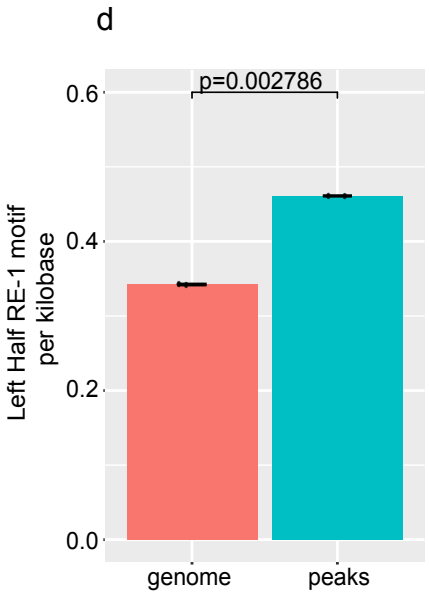
Primer sequences used for qPCR analysis.

REST-qPCR-FW-exon3	GACACATGCGGACTCATTCA	25 nm	STD
REST-qPCR-RV-exon4	ACAGTGCGGGCAATTAAGAG	25 nm	STD
MyoD-ChIP-FW	TGCTCCTTTGAGACAGCAGA	25 nm	STD
MyoD-ChIP-RV	AGTAGGGAAGTGTGCGTGCT	25 nm	STD
Pax6-ChIP-FW	GTTCAGGCGCAGGAGGAAGT	25 nm	STD
Pax6-ChIP-RV	TCGTCGTTGTCAAGGCTCCTG	25 nm	STD
NeuroD1-ChIP-FW	TTTCAAAGAAGGGCTCCAGA	25 nm	STD
NeuroD1-ChIP-RV	CGGTGCATCCCTACTCCTAC	25 nm	STD
Chr17qE5-ChIP-FW	TCATCATGTGTCCTGAAGTTGA	25 nm	STD
Chr17qE5-ChIP-RV	GCTTCTCTCCACAGAATTTGC	25 nm	STD
ITGA7-ChIP-FW	ATTCCGAGGTGCGATTTTCT	25 nm	STD
ITGA7-ChIP-RV	GCACCCATCACATCCAGATT	25 nm	STD

Supplementary Figure 1

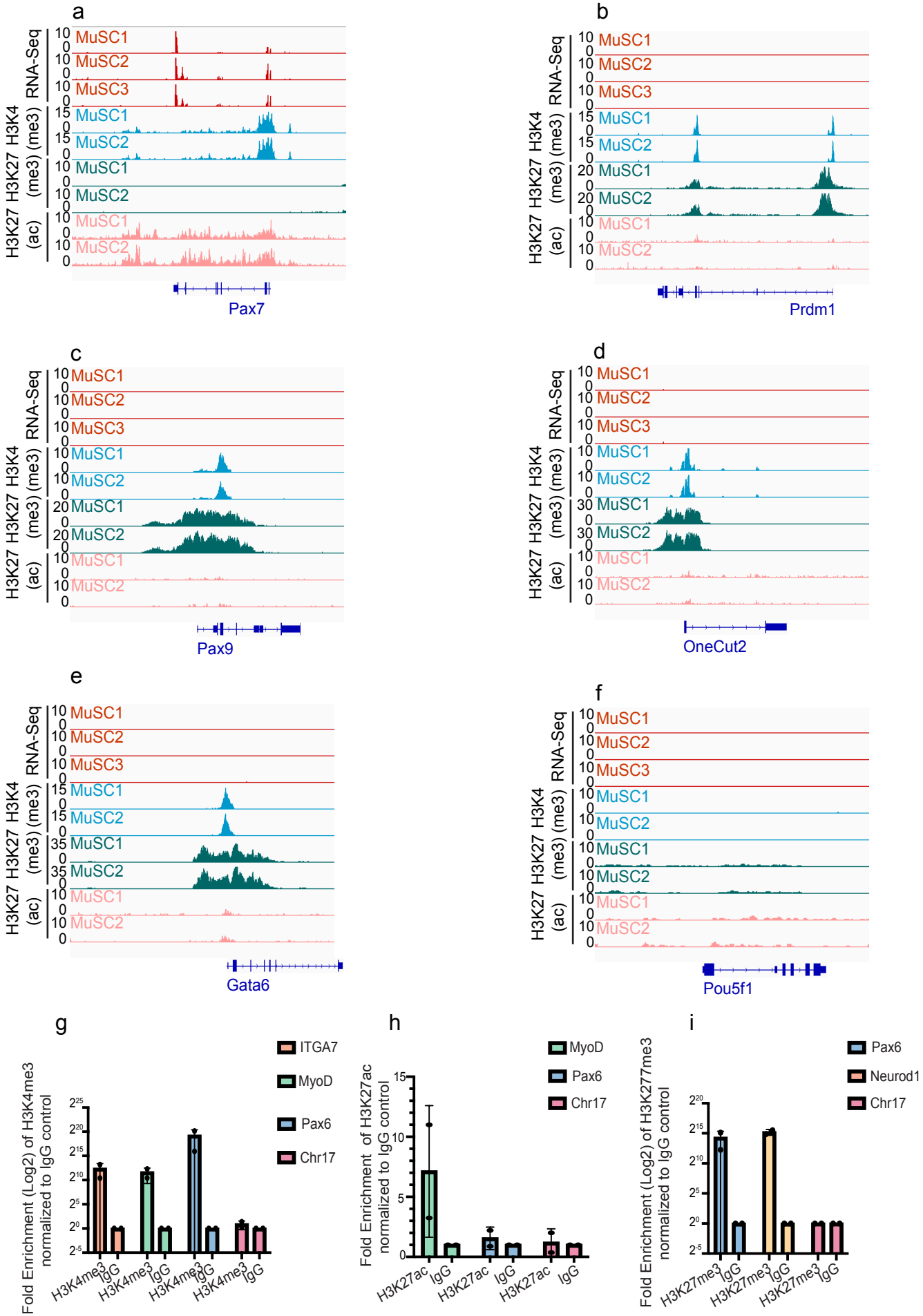






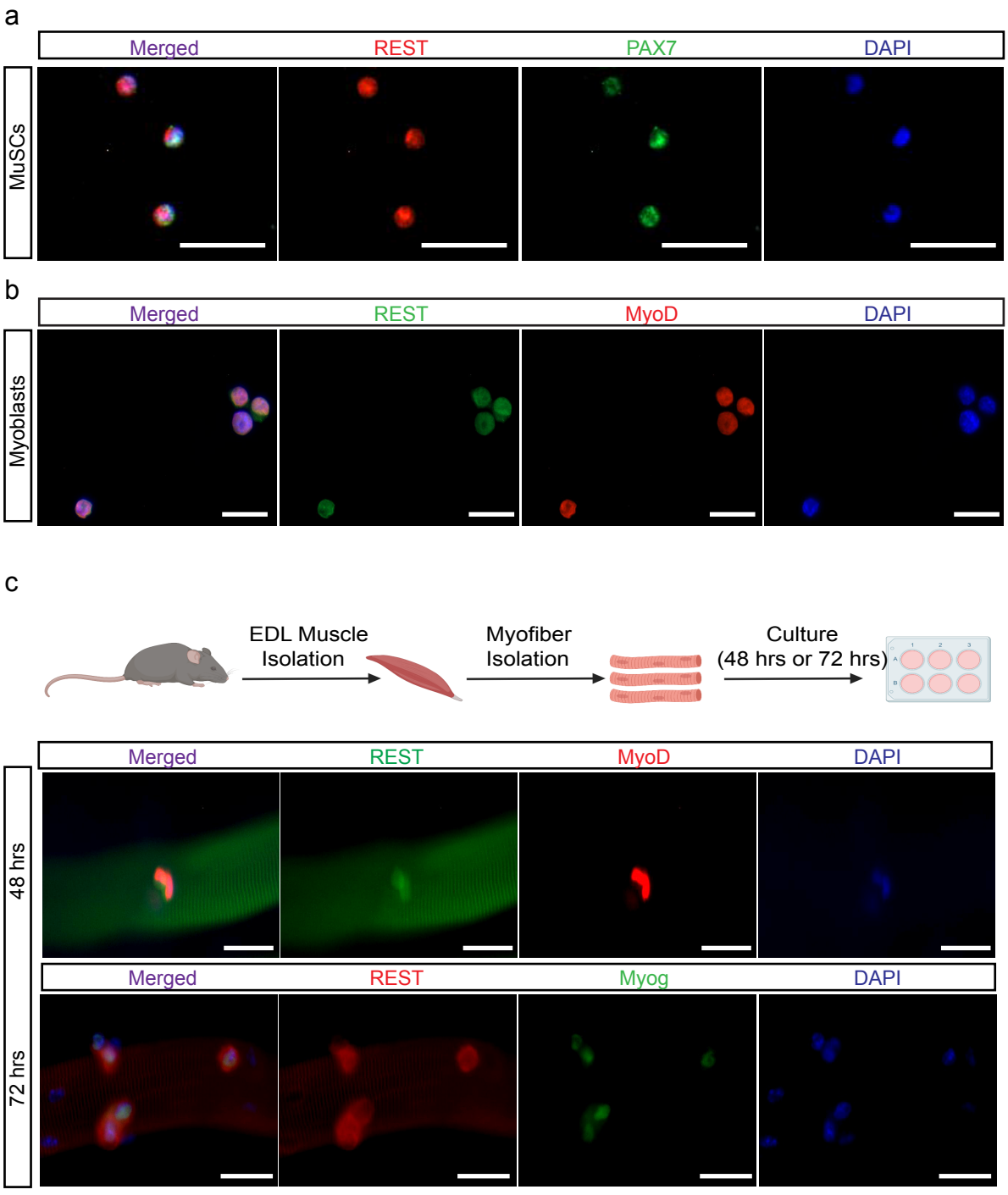
Supplementary Fig. 1: H3K4me3 presence on the regulatory regions of non-muscle genes **a,** Heatmap showing gene expression from the bulk RNA-Seq of freshly isolated MuSCs, n=3. Display of only the non-expressed gene clusters 2 and 3. Cluster 2 includes genes with RPM >1 and RPM<5. Cluster 3 includes genes with RPM<1. **b,** Heatmap of the distribution of H3K4me3 signal from the Cut&Tag on freshly sorted MuSCs. n=2. Color scale is based on the normalized Cut&Tag reads within +/- 1 kb of the TSS of the genes in panel a in order. **c,** Bar graph showing right RE-1 motif density (motifs/kb) under the DNA sequences of the H3K4me3 peaks near non-muscle genes, compared to equal number of random genomic sequences. n=2 mice Cut&Tag for H3K4me3. Two-tailed *t*-test. Data represented as SEM. **d,** Bar graph showing left RE-1 motif density (motifs/kb) under the DNA sequences of the H3K4me3 peaks near non-muscle genes, compared to equal number of random genomic sequences. n=2 mice Cut&Tag for H3K4me3. Two-tailed *t*-test. Data represented as SEM.

Supplementary Figure 2



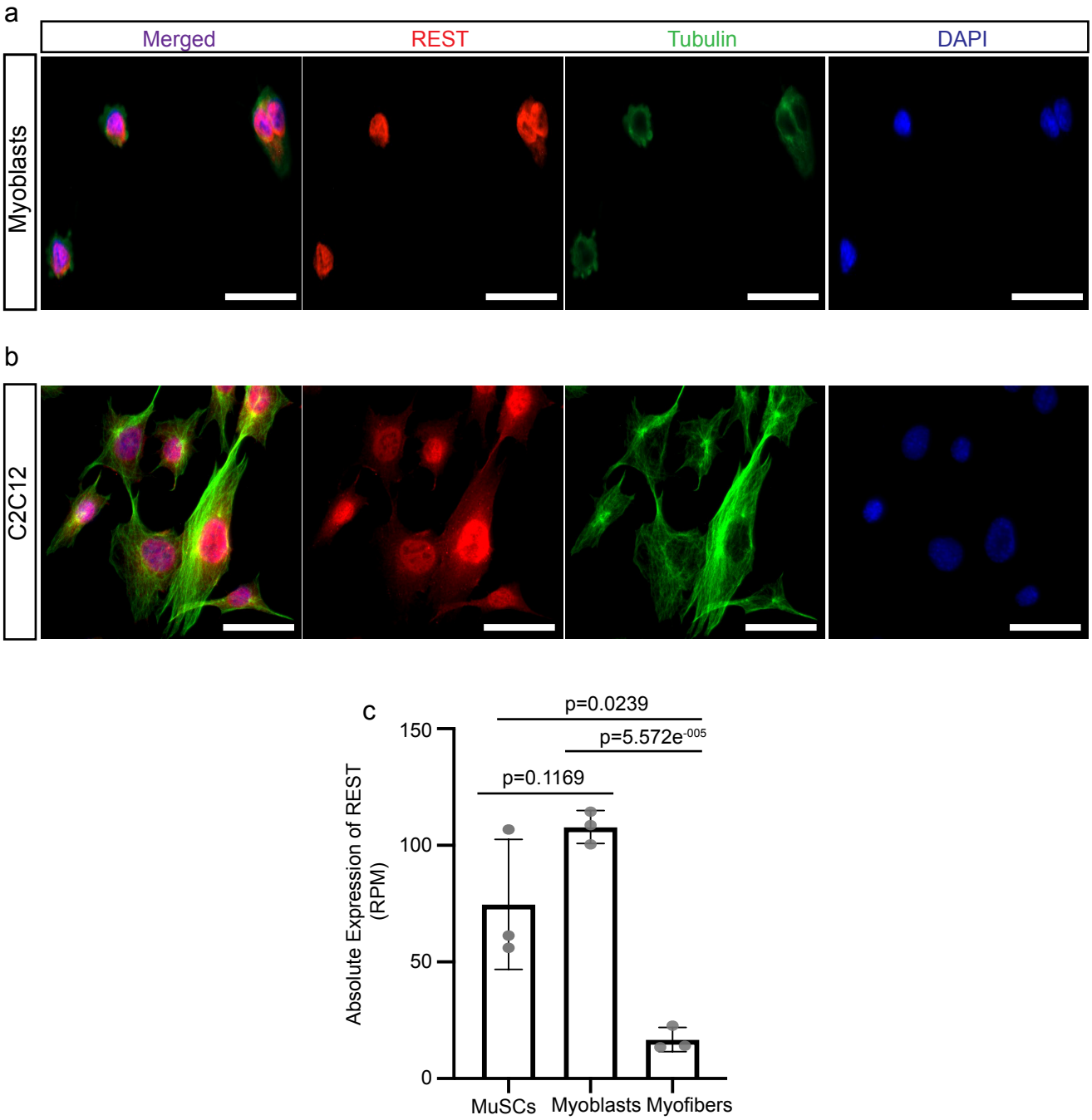
Supplementary Fig. 2: Chromatin landscape of non-muscle lineage genes
a-f, IGV tracks of MuSC RNA-Seq, H3K4me3 and H3K27ac Cut&Tag, and H3K27me3 Cut&Run for representative genes. n=3 mice RNA-Seq, n=2 mice Cut&Tag, n=2 mice Cut&Run. **a,** Paired Box 7 (*Pax7*). **b,** PR Domain containing Zinc Finger protein (*Prdm1*). **c,** Paired Box 9 (*Pax9*). **d,** One Cut Homeobox 2 (*OneCut2*). **e,** GATA Binding Protein 6 (*Gata6*). **f,** POU Class 5 Homeobox 1 (*Pou5f1*). **g,** Quantitative real-time PCR (qPCR) represented as bar graph showing the fold enrichment in Cut&Tag libraries of H3K4me3 for the TSS of *ITGA7*, *MyoD*, *Pax6* and a negative control region on chromosome 17 qE5, normalized to IgG control. Bars represent mean ± SD. n=2 mice Cut&Tag for H3K4me3. Source data for qPCR analysis are provided as a Source data file. **h,** qPCR represented as bar graph showing the fold enrichment in Cut&Tag libraries of H3K27ac for the TSS of *MyoD*, *Pax6* and a negative control region on chromosome 17 qE5, normalized to IgG control. Bars represent mean ± SD. n=2 mice Cut&Tag for H3K27ac. Source data for qPCR analysis are provided as a Source data file. **i,** qPCR represented as bar graph showing the fold enrichment in Cut&Run libraries of H3K27me3 for the TSS of *Pax6*, *Neurod1* and a negative control region on chromosome 17 qE5, normalized to IgG control. Bars represent mean ± SD. n=2 mice Cut&Run for H3K27me3. Source data for qPCR analysis are provided as a Source data file.

Supplementary Figure 3



Supplementary Fig. 3: REST expression in MuSCs, myoblasts and myofibers
a, Representative immunofluorescent staining of cyto-spinned MuSCs at 0 hr after FACS isolation for Pax7 and REST. Scale Bar= 25 µm. **b,** Representative immunofluorescent staining of cultured myoblasts for MyoD and REST. Scale Bar= 25 µm. **c,** Representative immunofluorescent staining of myofibers cultured for 48 and 72 hrs for MyoD and REST and Myogenin and REST respectively. Scale Bar= 25 µm. Schematic diagram showing EDL myofiber isolation and culture from the mice was generated with BioRender. Blackburn, D. (2025) https://BioRender.com/5k2ilwg **d,** Bar graph showing the absolute expression (log2 RPM) of *REST*, *Gapdh*, *Pax7* and muscle creatine kinase (*Ckm*) from the RNA-Seq of MuSCs, cultured myoblasts (1 week in culture) and single myofibers. n=3 mice RNA-Seq of MuSCs, n=3 mice RNA-Seq of myoblasts, n=3 mice RNA-Seq of myofibers. Bars represent mean ± SD.

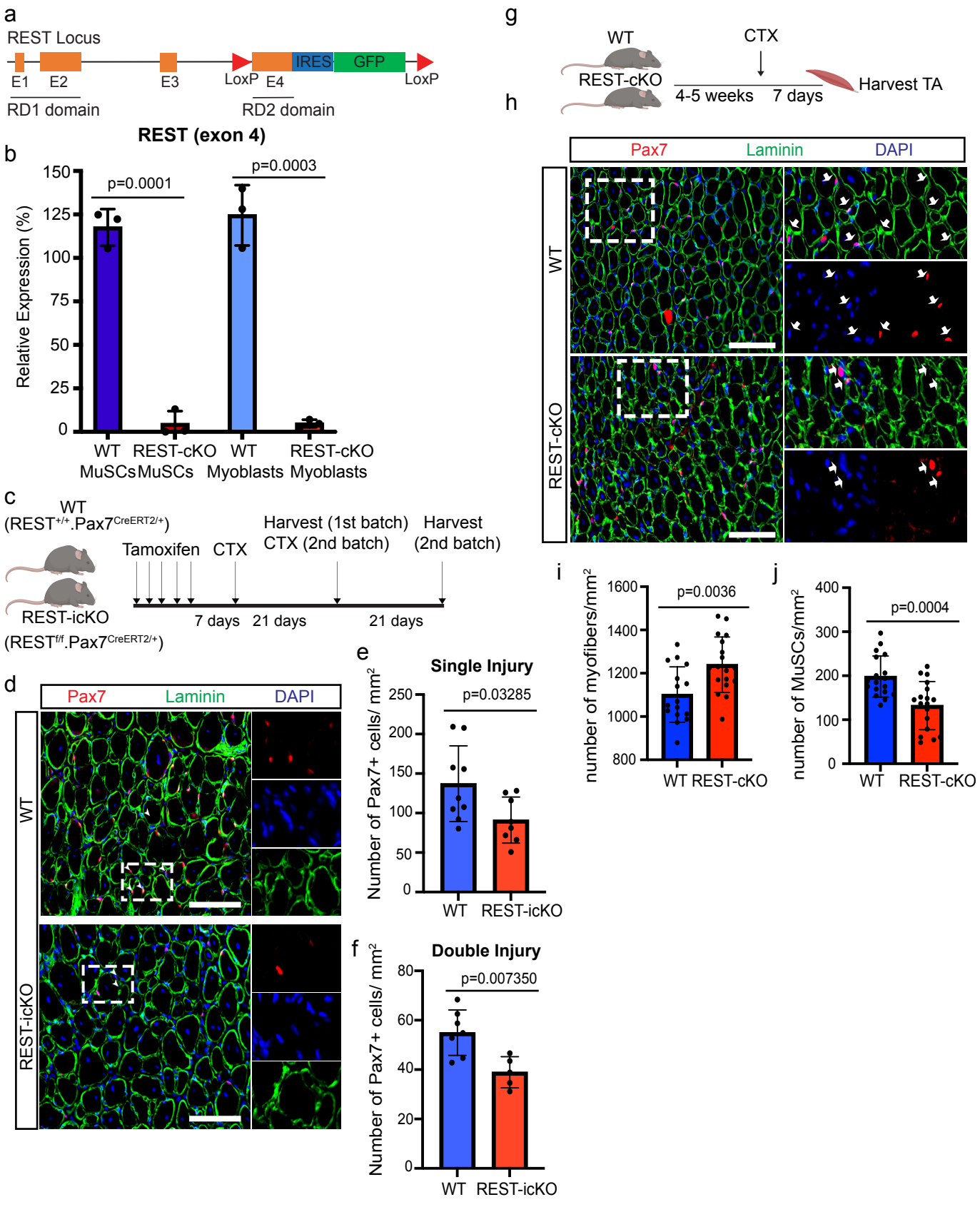
Supplementary Figure 4



Supplementary Fig. 4: Nuclear staining of REST antibody in cultured primary myoblasts and C2C12 myoblasts

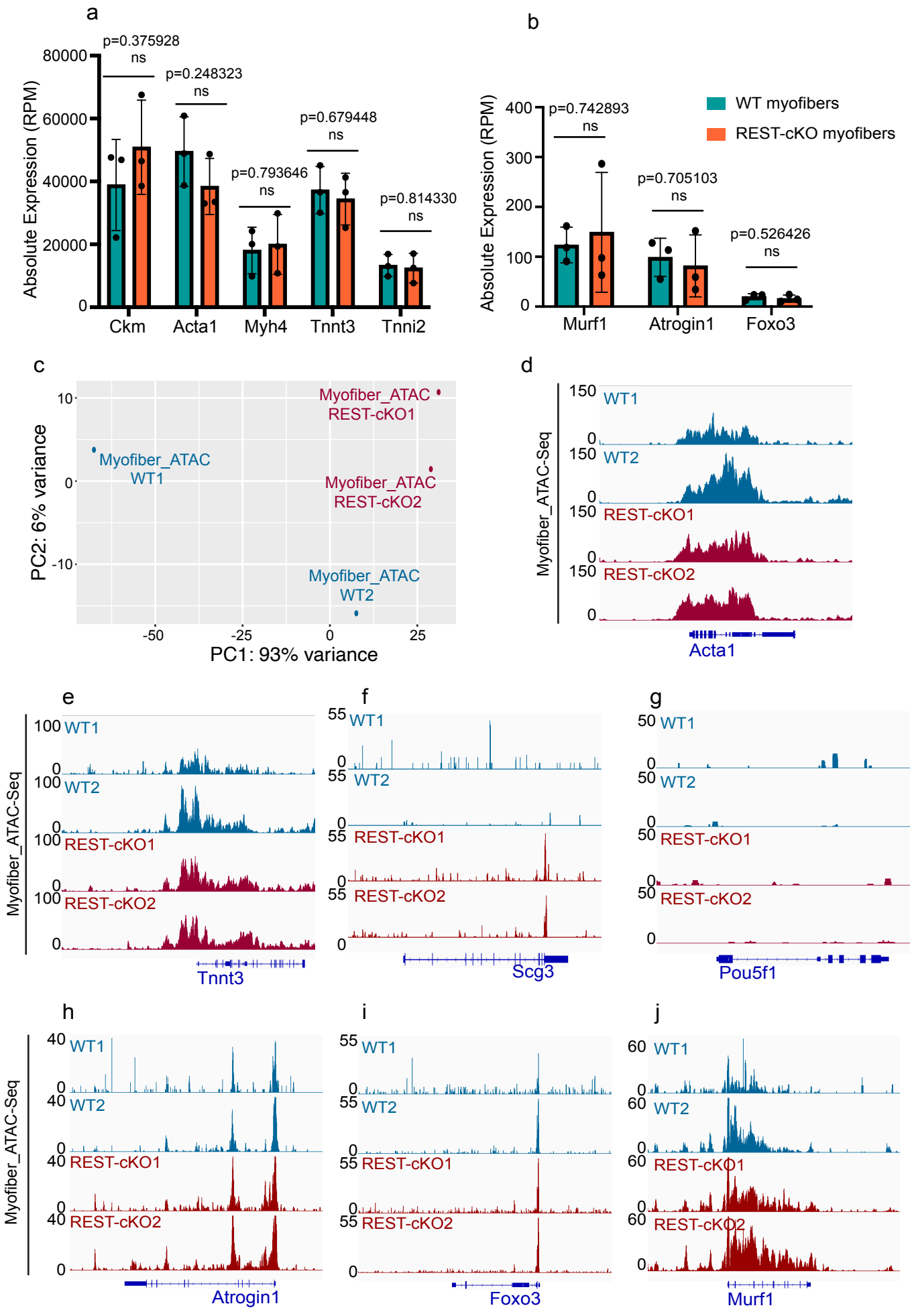
a, Representative immunofluorescent staining of cultured primary myoblasts for Tubulin and REST. Scale Bar= 25 μm. **b,** Representative immunofluorescent staining of C2C12 cells for Tubulin and REST. Scale Bar= 25 μm. **c,** Bar graph of the absolute expressions (RPM) of *REST* from the RNA-Seq of MuSCs, cultured myoblasts (1 week in culture) and single myofibers. n=3 mice RNA-Seq of MuSCs, n=3 mice RNA-Seq of myoblasts, n=3 mice RNA-Seq of myofibers. Two-tailed *t*-test, bars represent mean ± SD.

Supplementary Figure 5



Supplementary Fig. 5: Muscle regeneration upon genetic deletion of REST
a, Diagram representing the mouse model for REST-cKO. LoxP sites surround the exon 4 of REST and Cre recombinase is under the Pax7 promoter, creating REST exon4 deletion in Pax7 expressing MuSCs. **b,** Quantitative Real-Time PCR (RT-qPCR) analysis of REST-cKO and WT myoblasts and freshly isolated MuSCs. Relative Expression of *REST* exon 4 normalized to *RPS2* is shown. n=3 mice, Two-tailed *t*-test, bars represent mean ± SD. **c,** Schematic image showing the experimental layout of the inducible REST-cKO mouse model. Time points of Tamoxifen induction and CTX mediated single and double injuries are shown, generated with BioRender. Blackburn, D. (2025) https://BioRender.com/aq583dw. **d,** Representative image of TA cross-sections from inducible REST-cKO and WT mice stained for Pax7 and Laminin. Scale Bar= 25 µm. **e,** Quantification of number of $Pax7^+$ cells per unit area (mm^2) in single injury model. n=8 REST-icKO mice, n=9 WT mice, Two-tailed *t*-test, bars represent mean ± SD **f,** Quantification of number of $Pax7^+$ cells per unit area (mm^2) in double injury model. n=5 REST-icKO mice, n=7 WT mice, Two-tailed *t*-test, bars represent mean ± SD **g,** Schematic diagram of injury model in the non-inducible REST-cKO model, generated with BioRender. Blackburn, D. (2025) https://BioRender.com/j08rnso. **h,** Representative immunofluorescence image of TA cross-sections analyzed 7 days post-injury, stained for Pax7 and Laminin. **i,** Quantification of number of myofibers present per unit area (mm^2). n=3 REST-cKO and n=3 WT mice, Two-tailed *t*-test, bars represent mean ± SD. **j,** Quantification of number of $Pax7^+$ MuSCs (mm^2). n=3 REST-cKO and n=3 WT mice, Two-tailed *t*-test, bars represent mean ± SD.

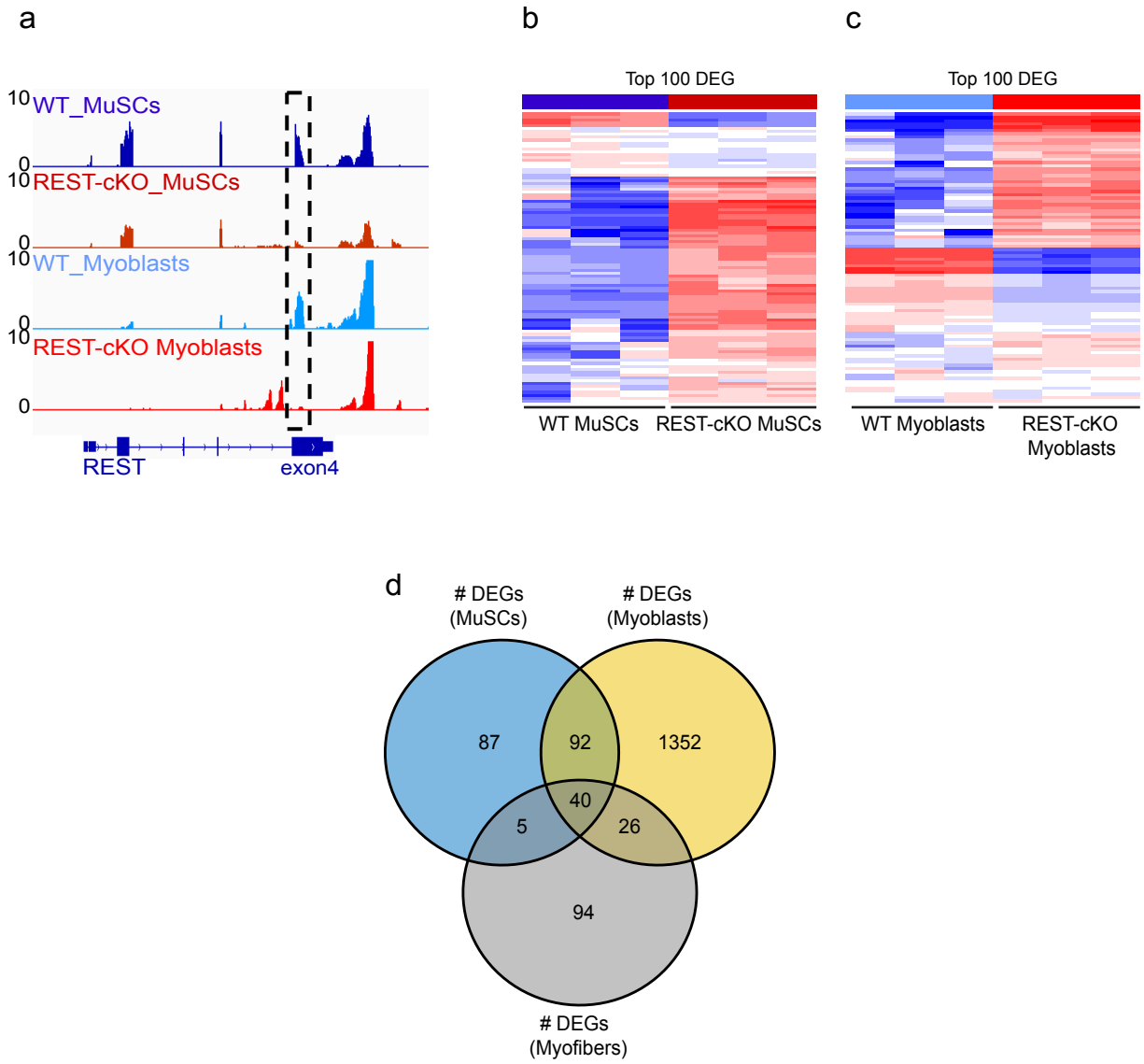
Supplementary Figure 6



Supplementary Fig. 6: Transcriptomic and epigenetic analysis of WT and REST-cKO myofibers using smfRNA-Seq and smfATAC-Seq

a, Absolute expressions (RPM) of muscle specific genes in REST-cKO and WT myofibers including muscle creatine kinase (*Ckm*), Actin Alpha 1 (*Acta 1*), Myosin Heavy Chain 4 (*Myh4*), Troponin T3 (*Tnnt3*) and Troponin I2 (*Tnni2*). n=3 WT mice myofibers, n=3 REST-cKO mice myofibers. Two-tailed *t*-test, bars represent mean ± SD. **b,** Absolute expressions (RPM) of muscle atrophy associated genes, Tripartite Motif Containing 63 (*Trim63*/ *Murf1*), F-Box Protein 32 (*FBXO32*/ *Atrogin*) and Forkhead Box O3 (*Foxo3*) in REST-cKO and WT myofibers. Two-tailed *t*-test, bars represent mean ± SD. **c,** PC Analysis of the REST-cKO and WT myofiber ATAC-Seq samples along the two principal components. n=2 WT mice myofibers, n=2 REST-cKO mice myofibers**. d-g,** IGV tracks of muscle specific genes as well as non-muscle genes from the WT and REST-cKO myofiber ATAC-Seq, showing accessibility in the TSS of the respective gene. **d,** Actin Alpha 1 (*Acta 1*) **e,** Troponin T3 (*Tnnt3*) **f,** Neuroendocrine Secretogranin III (*Scg3*). **g,** POU Class 5 Homeobox 1 (*Pou5f1*) used as a negative control. **h-j,** IGV snapshots of muscle atrophy associated genes from the WT and REST-cKO myofiber ATAC-Seq, showing accessibility in the TSS of the respective gene. **g,** F-Box Protein 32 (*FBXO32*/ *Atrogin*). **h,** Forkhead Box O3 (*Foxo3*). **i,** Tripartite Motif Containing 63 (*Trim63*/ *Murf1*).

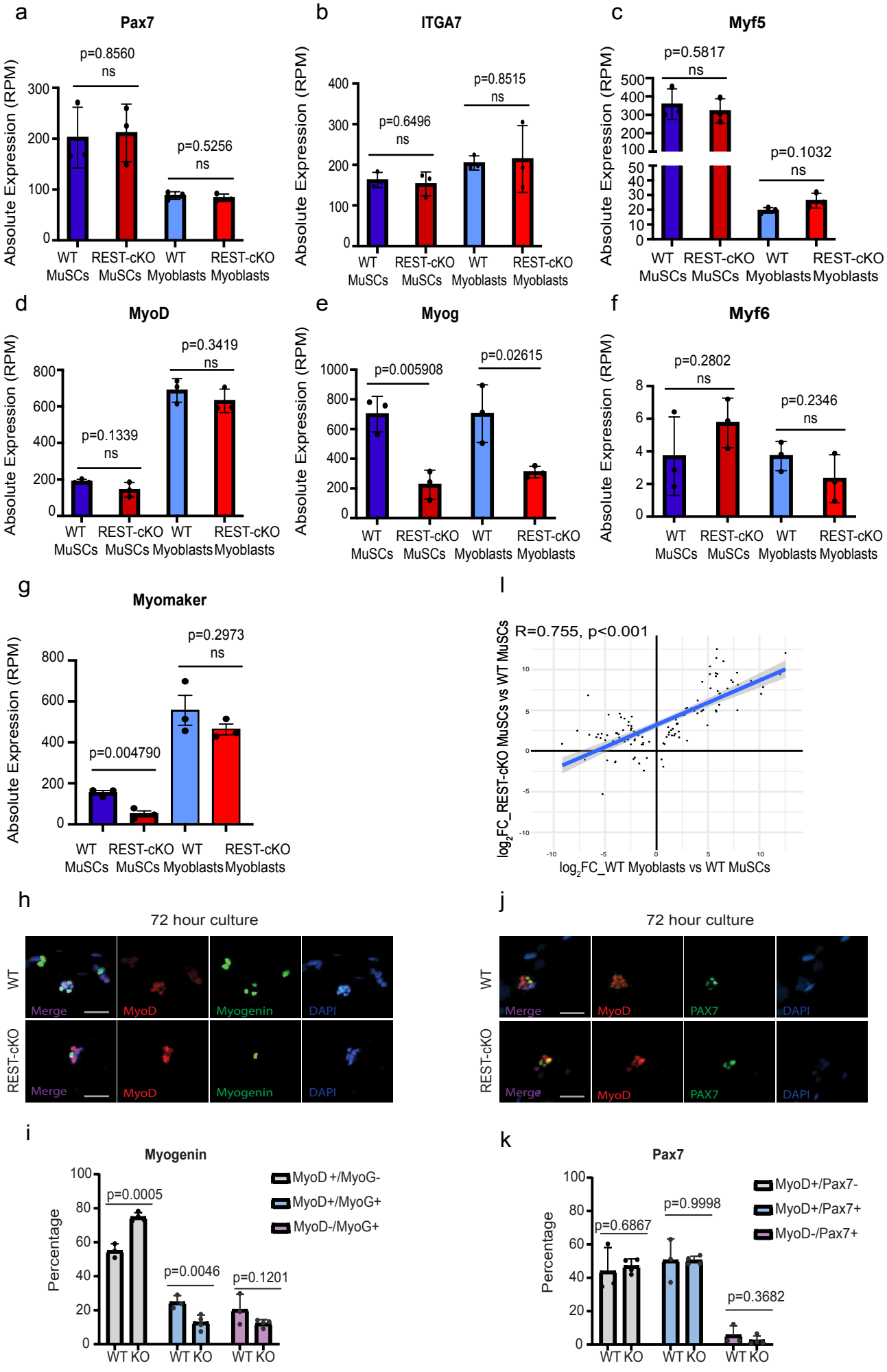
Supplementary Figure 7



Supplementary Fig. 7: Differential gene expression analysis from REST-cKO and WT MuSCs and myoblasts

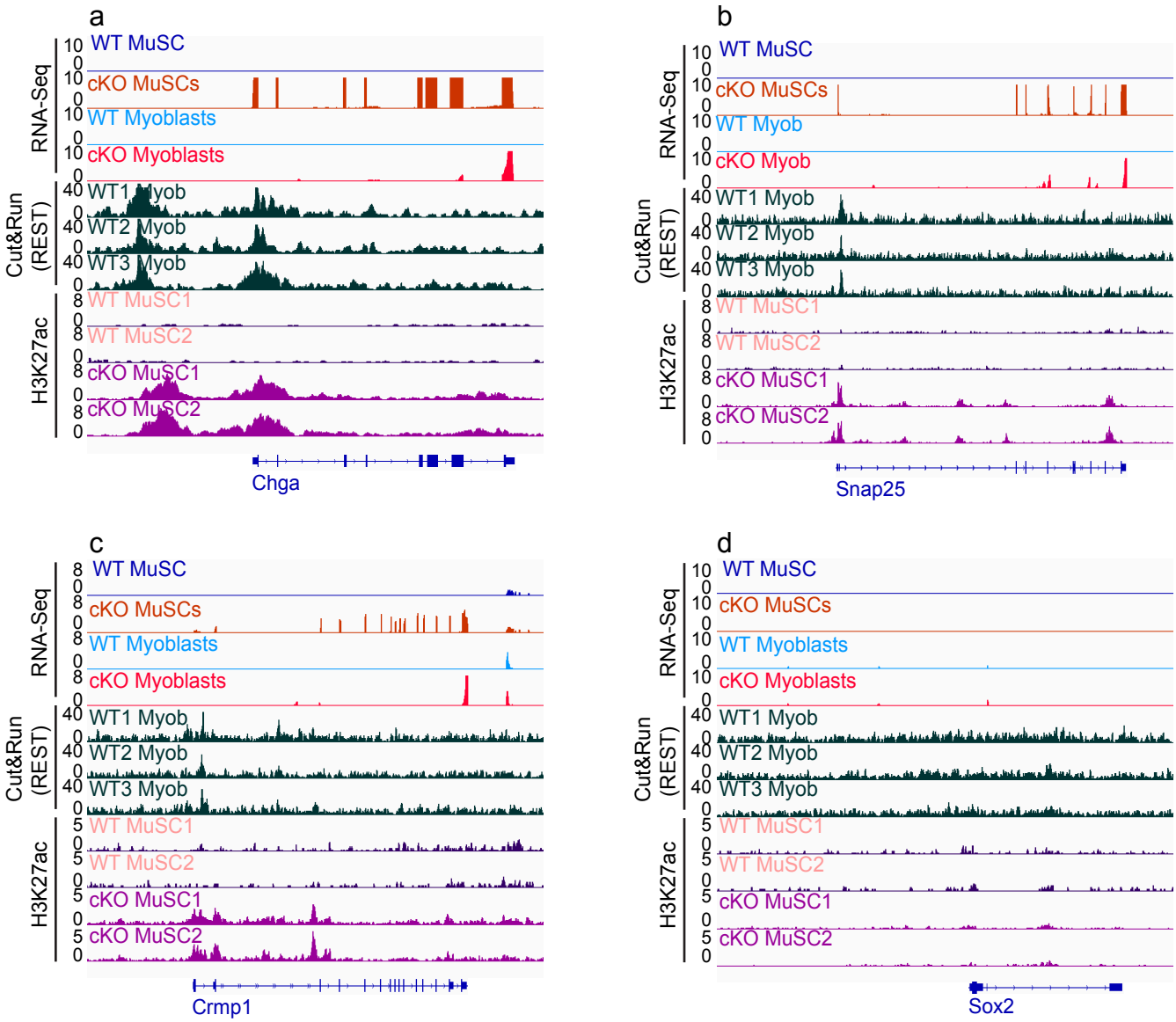
a, IGV snapshot of *REST* gene from the WT and REST-cKO MuSC and primary myoblast RNA-Seq, n=3 REST-cKO and n=3 WT mice. Box represents exon 4 deletion of *REST* in REST-cKO MuSCs and myoblasts. **b,** Heatmap of top 100 differentially expressed genes (absolute log_2FC >1, p-adj<0.05) between the WT and REST-cKO MuSCs **c,** Heatmap of top 100 differentially expressed genes (absolute log_2FC >1, p-adj<0.05) between the WT and REST-cKO primary myoblasts. **d,** Venn Diagram indicating the number of differentially expressed genes between REST-cKO and WT MuSCs, myoblasts and myofibers. Number of common DEGs between different cell types are indicated. Number of DEGs are obtained from the RNA-Seq of MuSCs, myoblasts and myofibers respectively. DEGs determined at absolute log_2FC >1, p-adj<0.05.

Supplementary Figure 8



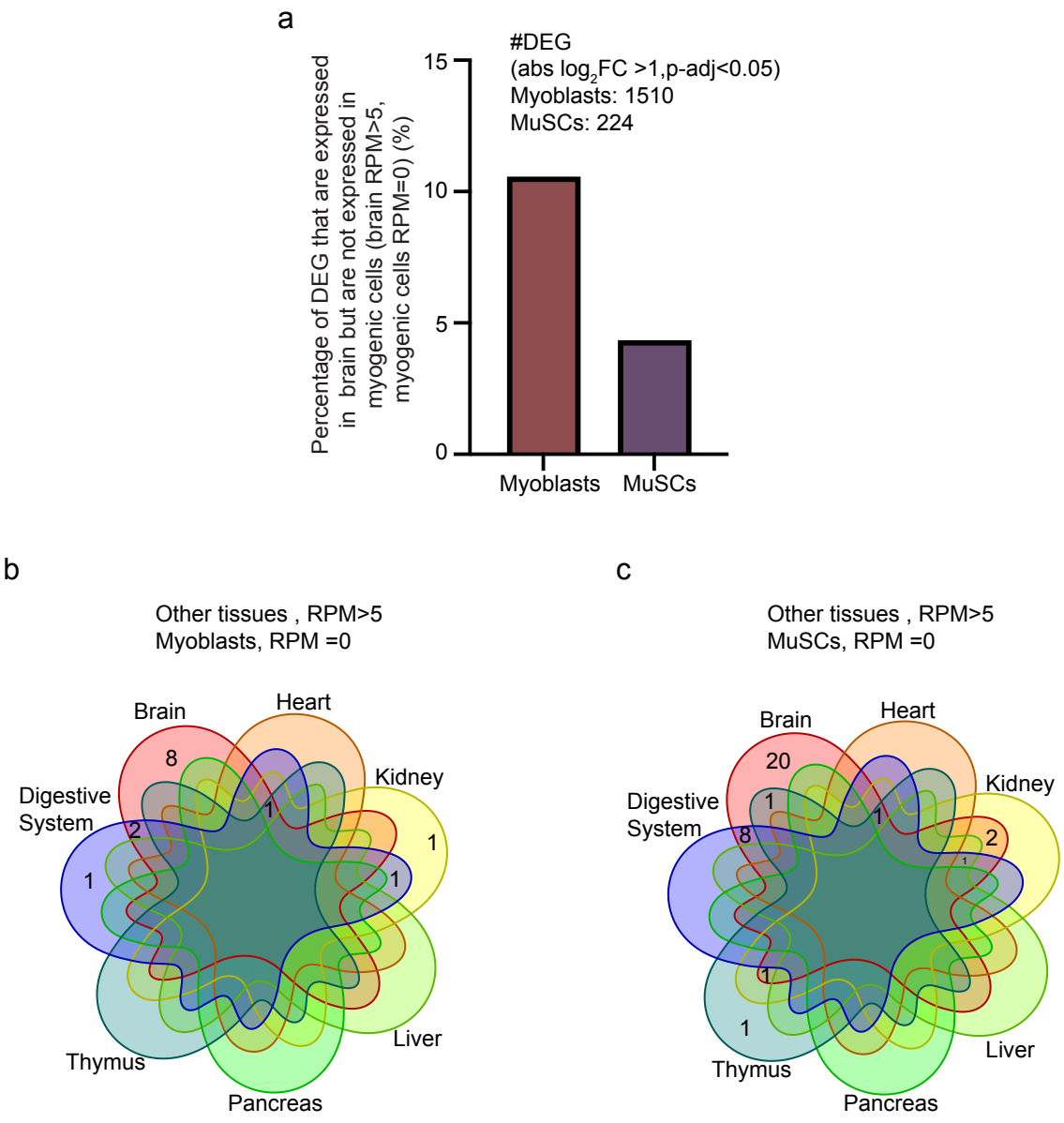
Supplementary Fig. 8: Expression of myogenic regulatory factors (MRFs)
a-g, Absolute expression of *Pax7*, *Itga7*, *Myf5*, *MyoD*, *Myog*, *Myf6* and *Myomaker* respectively, in WT and REST-cKO MuSCs and primary myoblasts RNA-Seq. n=3 WT and n=3 REST-cKO mice RNA-Seq. Two-tailed *t*-test, bars represent mean ± SD. **h,** Representative images of WT and REST-cKO EDL myofibers cultured for 72 hours and stained for MyoD and Myogenin. Scale bar = 30 um. **i,** Bar graph of the percentage of EDL associated MuSCs cultured for 72 hours that were positive for MyoD and Myogenin. n= 3 WT mice, n= 4 REST-cKO mice. **j,** Representative images of WT and REST-cKO EDL myofibers cultured for 72 hours and stained for MyoD and Pax7. Scale bar = 30 um. **k,** Bar graph of the percentage of EDL associated MuSCs cultured for 72 hours that were positive for MyoD and Pax7. n= 3 WT mice, n= 4 REST-cKO mice. **l,** Scatter plot showing the Pearson correlation between log_2FC values of differentially expressed genes. x-axis : log_2FC values for differentially expressed genes between WT MuSCs vs WT Myoblasts (p-adj<0.05). y-axis : log_2FC values for differentially expressed genes between WT MuSCs and REST-cKO MuSCs (p-adj<0.05).

Supplementary Figure 9



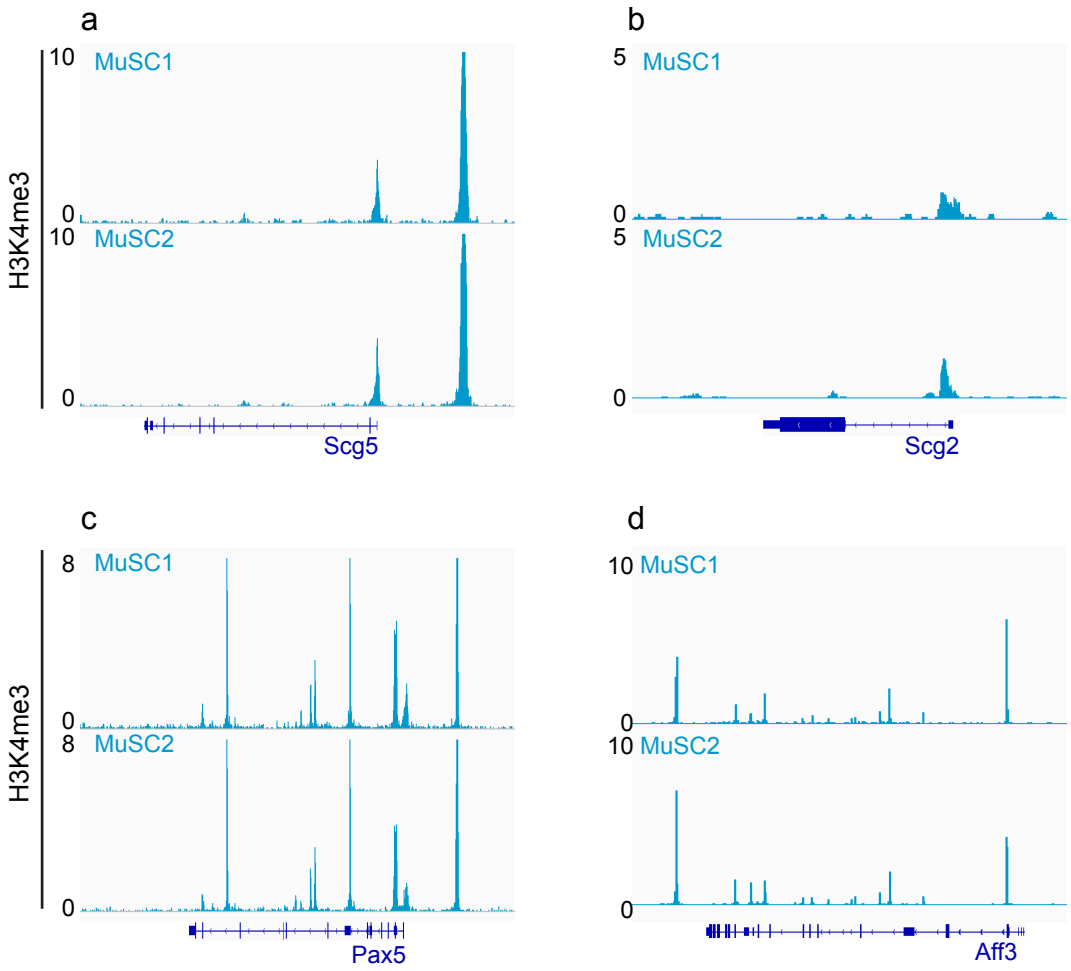
Supplementary Fig. 9: Cut&Run of REST depicting REST binding in the regulatory regions of differentially expressed genes in REST-cKO myogenic cells
a-d, IGV tracks of freshly sorted WT and REST-cKO MuSC and myoblasts RNA-Seq, Cut&Run for REST in WT myoblasts, Cut&Tag for H3K27ac in WT and REST-cKO MuSCs, n=3 RNA-Seq, n=2 Cut&Tag, n=2 Cut&Run. **a,** Chromogranin A (*Chga*). **b,** Synaptosomal-associated protein 25 (*Snap25*). **c,** Collapsin Response Mediator Protein 1 (*Crmp1*). **d,** SRY-box transcription factor 2 (*Sox2*).

Supplementary Figure 10



Supplementary Fig. 10: Differentially expressed genes in REST-cKO myogenic cells from genes that are expressed in other tissues but are not expressed in muscle cells
a, Bar graph showing the percentage of differentially expressed genes that are expressed in the brain (RPM > 5) but not expressed in myoblasts or MuSCs (RPM=0) based on intersection of publicly available data set [1] and our WT RNA-Seq of MuSCs and myoblasts. Source data for the calculations are provided as a Source Data file. **b-c,** Venn diagram showing the number of differentially expressed genes (abs $\log_2$FC > 1, adj $p < 0.05$) between WT and REST-cKO Myoblasts (**b**) or MuSCs (**c**) that are also uniquely expressed in other tissues (RPM > 5 in other tissues, RPM =0 in MuSCs/myoblasts) using the intersection of publicly available data set [1] and our WT RNA-Seq of MuSCs and myoblasts.

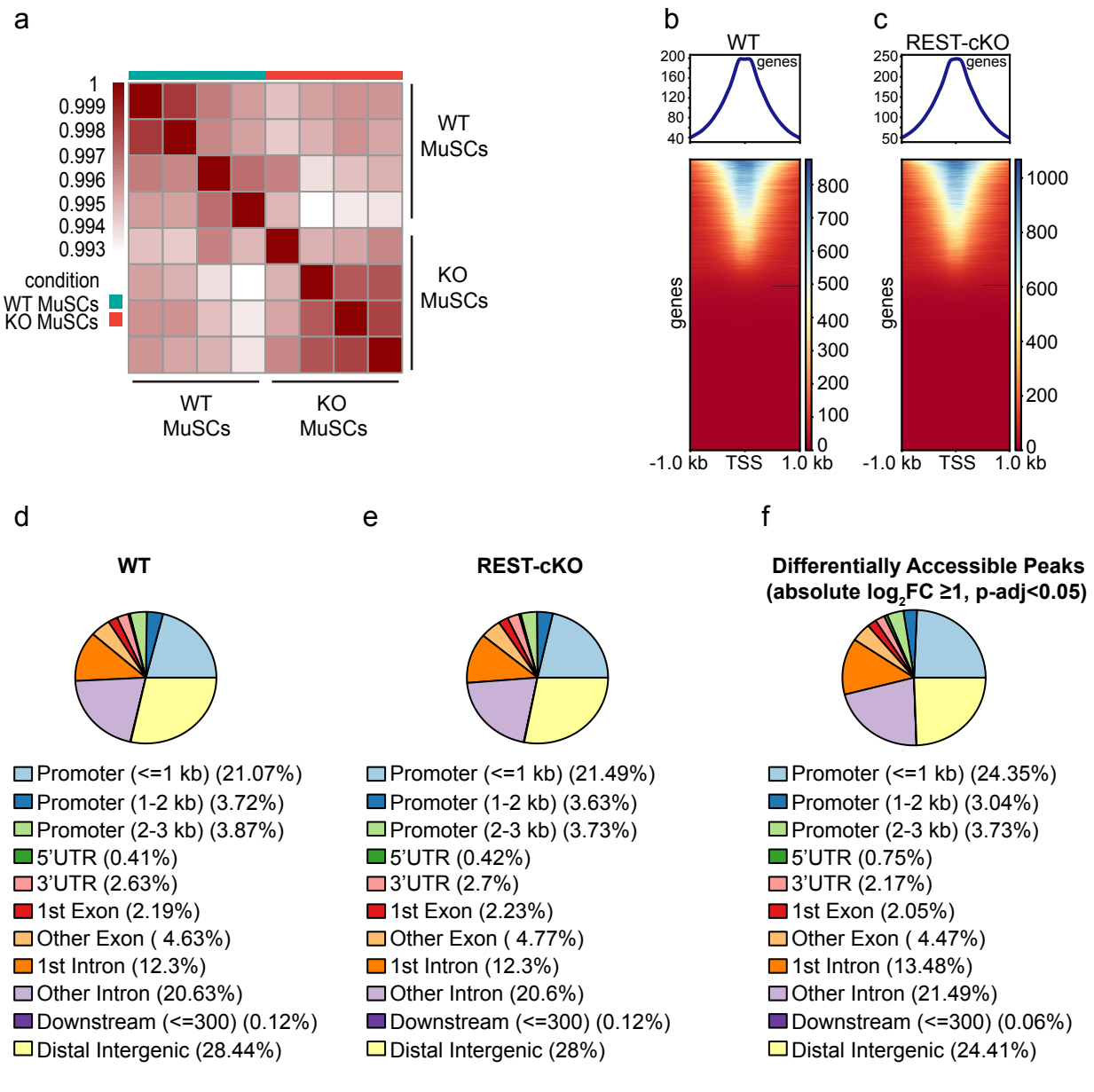
Supplementary Figure 11



Supplementary Fig. 11: IGV snapshots of Cut&Tag of H3K4me3 on freshly sorted MuSCs for non-muscle lineage genes

a-d, IGV snapshots of MuSC Cut&Tag for the presence of H3K4me3 on the regulatory regions of non-muscle lineage genes. n=2 mice Cut&Tag. **a,** Secretogranin-5 (*Scg5*). **b,** Secretogranin-2 (*Scg2*). **c,** Paired Box 5 (*Pax5*). **d,** ALF Transcription Elongation Factor (*Aff3*).

Supplementary Figure 12

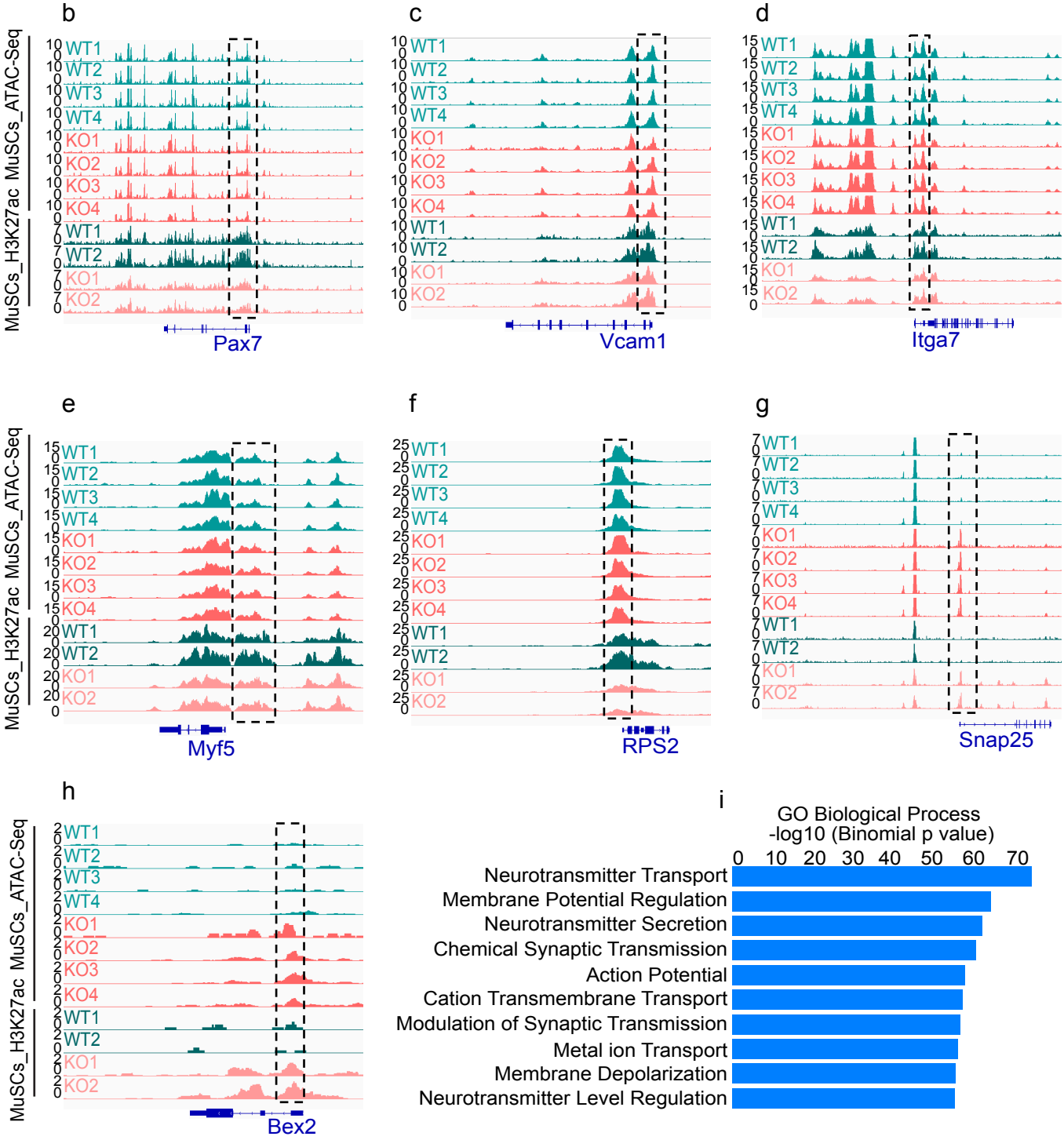


Supplementary Fig. 12: Quality control of ATAC-Seq from freshly sorted MuSCs
a, Heatmap clustering of Pearson correlation coefficients showing the correlation between the replicates of the conditions in the regions defined by the union peak set (merged peaks of all replicates/ samples). **b,** Heatmap showing enrichment at transcription start site (TSS) for the ATAC-Seq of WT MuSCs. **c,** Heatmap showing enrichment at transcription start site (TSS) for the ATAC-Seq of REST-cKO MuSCs. **d-f,** Peak annotation pie charts of ATAC-Seq of WT MuSCs, REST-cKO MuSCs and differentially accessible peaks between the WT and REST-cKO MuSCs respectively. Differentially accessible peaks were defined as absolute $log_2FC \geq 1$, p-adj<0.05. n=4 WT and n=4 REST-cKO mice for ATAC-Seq.

Supplementary Figure 13

a

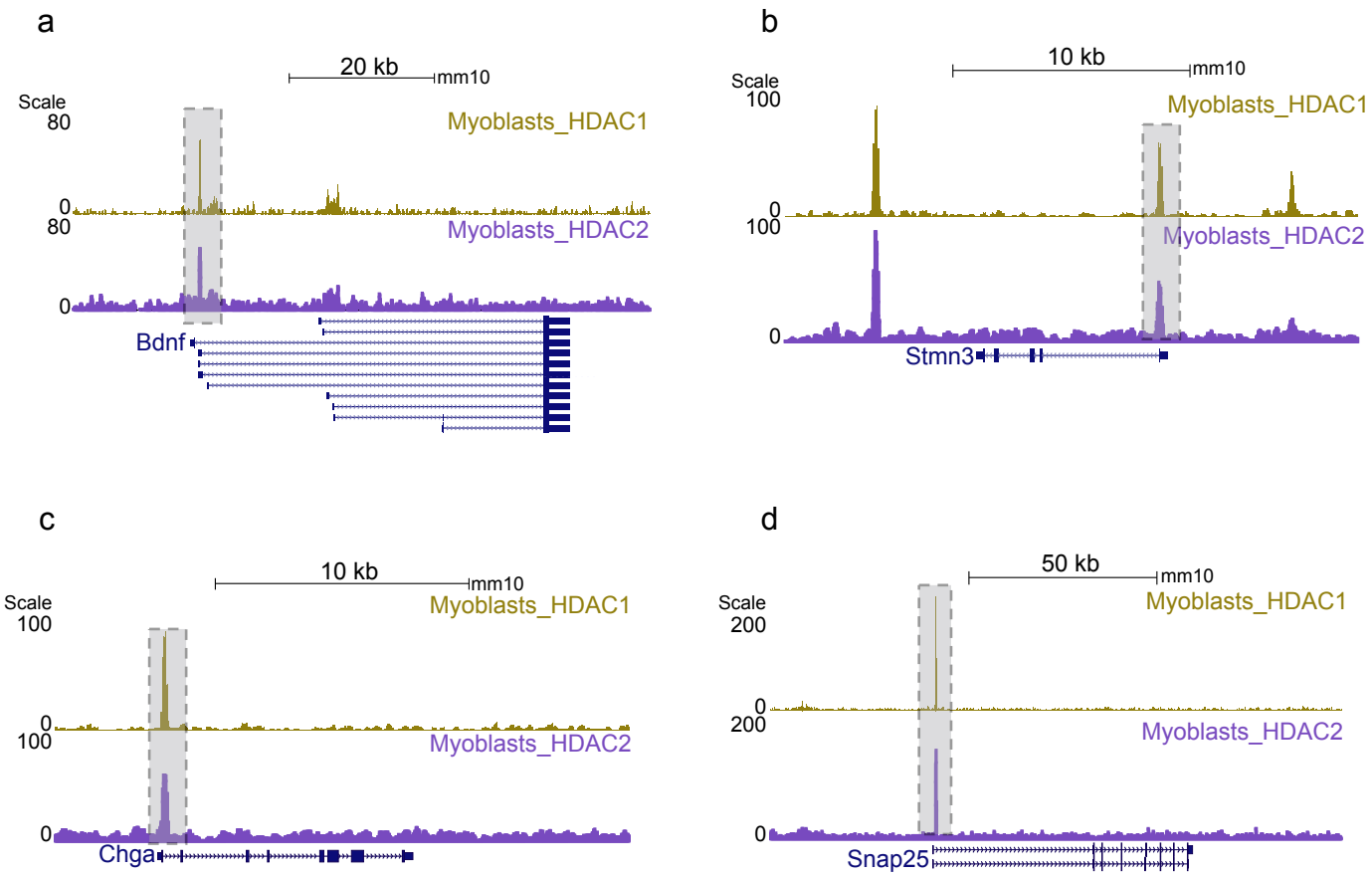
	Total Number of Peaks	% Peaks with complete RE-1 motif	% Peaks with left RE-1 motif	% Peaks with right RE-1 motif
Differentially Accessible Peaks	1610	23.29	54.84	62.30



Supplementary Fig. 13: Alterations in chromatin accessibility between WT and REST-cKO MuSCs

a, Table showing the percentage of differentially accessible peaks between WT and REST-cKO MuSCs that contain the full RE-1 motif, left RE-1 motif and right RE-1 motif. n=4 WT and n=4 REST-cKO mice for ATAC-Seq. Source data are provided as Source data file. **b-h,** IGV snapshots of WT and REST-cKO MuSCs ATAC-Seq on *Pax7*, *Vcam1*, *Itga7*, *Myf5*, *Rps2* (housekeeping gene), *Snap25* and Brain Expressed X-Linked Protein 2 (*Bex2*), respectively, displaying chromatin accessibility on their respective TSS. **i,** Gene Ontology (GO Biological Process) analysis of genes associated with ATAC-Seq peaks based on association by proximity using Genomic Regions Enrichment of Annotations Tool (GREAT) for differentially accessible peaks between WT and REST-cKO MuSCs. Differentially accessible peaks are defined at absolute $\log_2FC \geq 1$, p-adj<0.05.

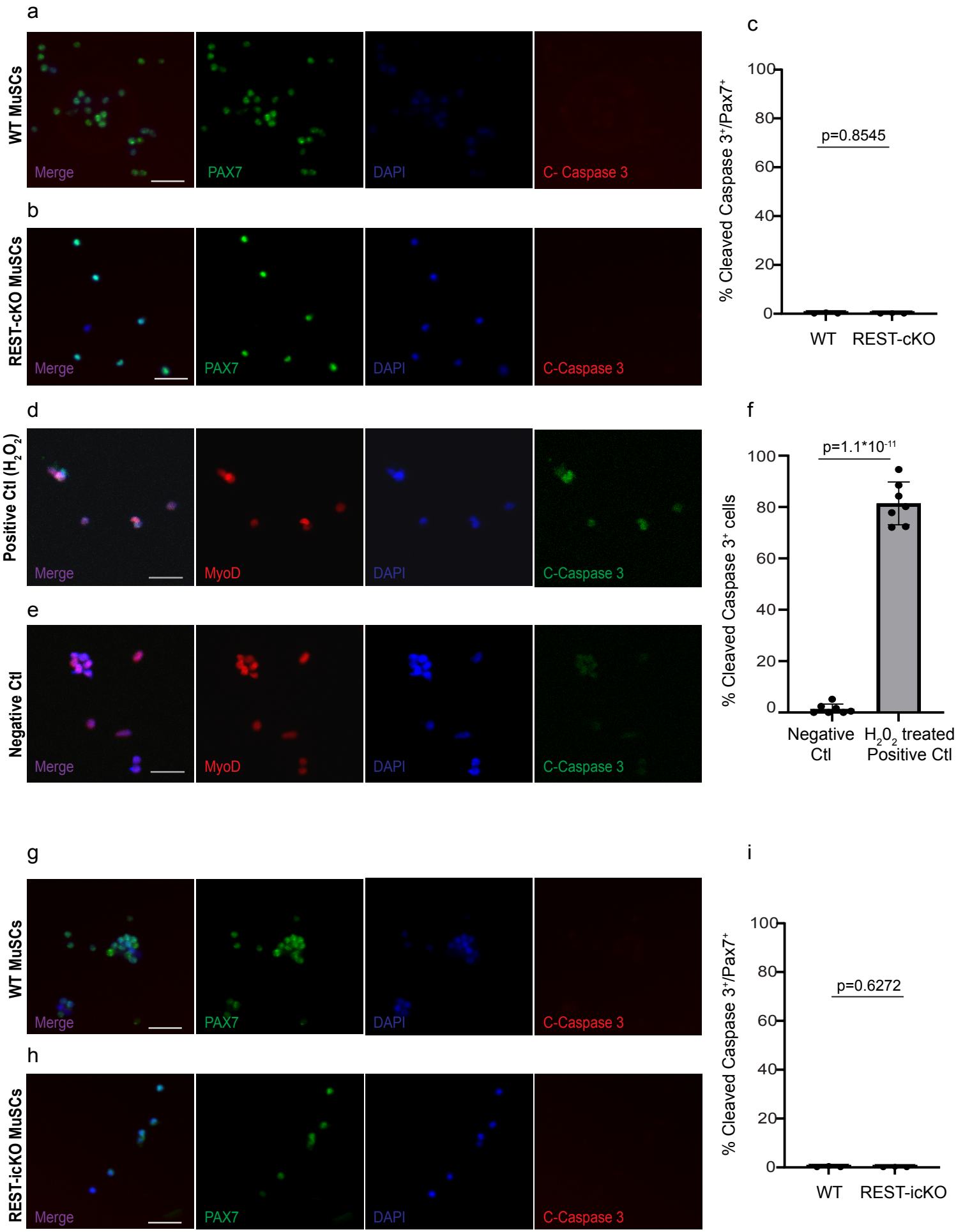
Supplementary Figure 14



Supplementary Fig. 14: ChIP-Seq of HDAC1 and HDAC2 on genes that are not expressed in WT MuSCs

a-d, Snapshots of the UCSC genome browser showing ChIP-Seq reads of HDAC1 and HDAC2 for neurogenic genes that are not expressed in WT MuSCs but gain expression upon genetic deletion of REST. **a,** *Bdnf*. **b**, *Stmn3*. **c,** *Chga*. **d,** *Snap25*. ChIP-Seq data of HDAC1 and HDAC2 was retrieved from the publicly available data set [2] through the GEO accession number GSE24852.

Supplementary Figure 15



Supplementary Fig. 15: Assessment of apoptosis in MuSCs that are freshly isolated from inducible and non-inducible REST-cKO and WT mice

a, Representative immunofluorescent staining of cyto-spinned WT MuSCs immediately after FACS isolation for Pax7 and Cleaved Caspase 3. Scale Bar= 50 µm. **b,** Representative immunofluorescent staining of cyto-spinned REST-cKO MuSCs immediately after FACS isolation for Pax7 and Cleaved Caspase 3. Scale Bar= 50 µm. **c,** Bar graph representing the percentage of Cleaved Caspase 3 positive MuSCs in the non-inducible REST-cKO and WT mice. n=3 WT and n=3 REST-cKO mice. Two-tailed *t*-test, bars represent mean ± SD **d,** Representative immunofluorescent staining of WT myoblasts that have been treated with 400 uM of H_2O_2 for 12 hours as a positive control. Immunofluorescent staining was performed for MyoD and Cleaved Caspase 3. Scale Bar= 50 µm. **e,** Representative immunofluorescent staining of WT myoblasts for MyoD and Cleaved Caspase 3, representing negative control for Cleaved Caspase 3 staining. Scale Bar= 50 µm. **f,** Bar graph representing the percentage of Cleaved Caspase 3 positive myoblasts in the negative control and the H_20_2 treated positive controls. Two-tailed *t*-test, bars represent mean ± SD **g,** Representative immunofluorescent staining of cyto-spinned WT MuSCs immediately after FACS isolation for Pax7 and Cleaved Caspase 3. Scale Bar= 50 µm. **h,** Representative immunofluorescent staining of cyto-spinned REST-icKO MuSCs immediately after FACS isolation for Pax7 and Cleaved Caspase 3. Scale Bar= 50 µm. **i,** Bar graph representing the percentage of Cleaved Caspase 3 positive MuSCs in the inducible REST-cKO (n=3 mice) and WT (n=3 mice). Two-tailed *t*-test, bars represent mean ± SD.

References

1. Sollner, J.F., et al., *An RNA-Seq atlas of gene expression in mouse and rat normal tissues.* Sci Data, 2017. **4**: p. 170185.

2. Soleimani, V.D., et al., *Snail regulates MyoD binding-site occupancy to direct enhancer switching and differentiation-specific transcription in myogenesis.* Mol Cell, 2012. **47**(3): p. 457-68.