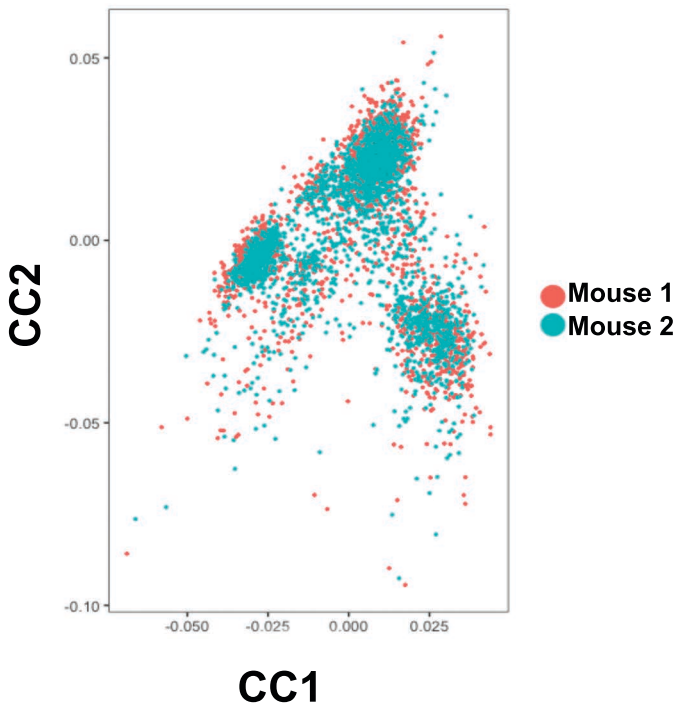
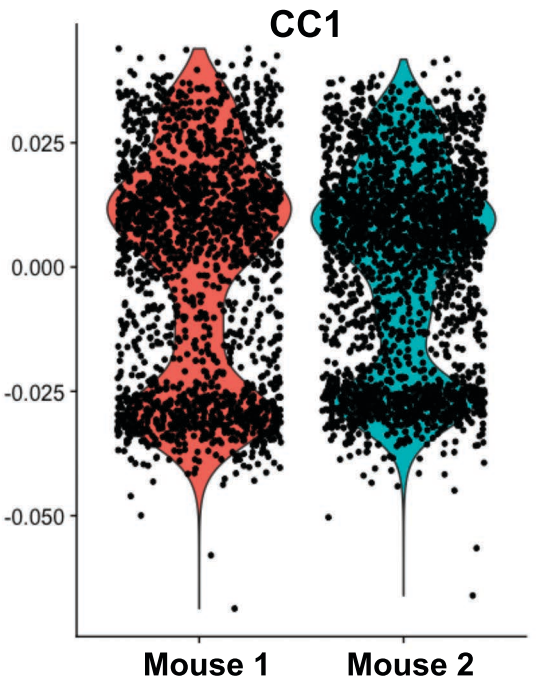
Dell'Orso et al. Supplemental Fig 1

A





B

	Mouse hindlimb WT1	Mouse hindlimb WT2	Merged Hindlimb WT1_WT2
Estimated Number of Cells	1,982	2,432	4,414
Fraction Reads in Cells	89.10%	88.90%	89.00%
Mean Reads per Cell	182,278	156,786	156,277
Median Genes per Cell	1,225	1,230	1,221
Median UMI Counts per Cell	3,055	3,204	3,109

C

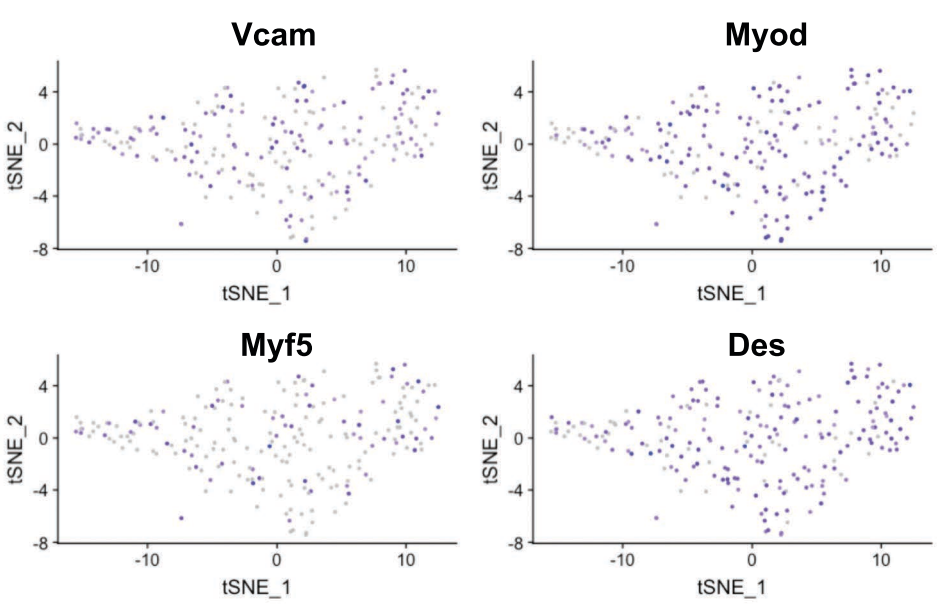
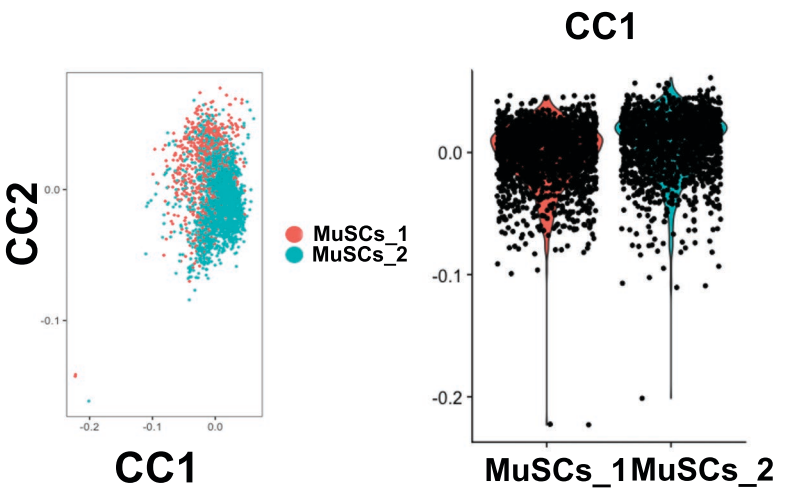


Fig. S1

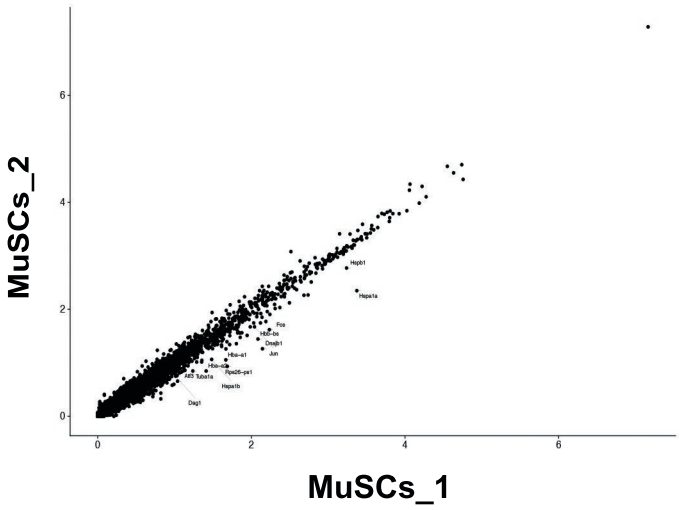
(A) Canonical correlation (left panel) and monodimensional (right panel) analyses of two independent scRNA-seq experiments from mononucleated cells isolated from total muscle. Canonical correlation analysis finds linear combinations of features across datasets that are maximally correlated to identify shared correlation structures (Butler et al., 2018). (B) Quality control from “Chromium Control” report for individual scRNA-seq experiments. (C) Pattern expression of Vcam, MyoD, Myf5, and Des transcripts in single cells with MuSC transcriptome characteristics.

Dell'Orso et al. Supplemental Fig 2

A



B



C

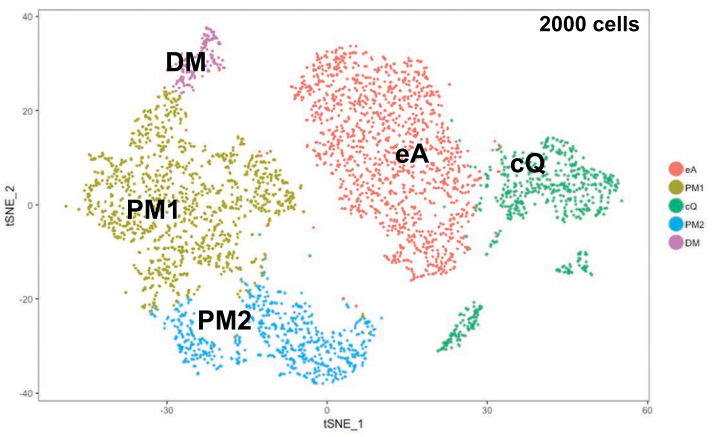
	MuSCs_1	MuSC_2	Merged MuSCs1-MuSCs2
Estimated Number of Cells	1,629	1,452	3,081
Fraction Reads in Cells	79.40%	78.10%	78.80%
Mean Reads per Cell	237,250	261,646	236,841
Median Genes per Cell	985	1,010	994
Median UMI Counts per Cell	2,245	2,300	2,251

D

cQ	p-value
Protein folding	2.50E-07
Response to cAMP	6.18E-07
Response to unfolded protein	6.18E-07
Negative regulation of cell proliferation	7.69E-07
Positive regulation of nitric oxide biosynthetic process	2.48E-06
Extracellular matrix organization	1.64E-05
Cell cycle arrest	3.14E-05
Circadian rhythm	5.66E-05
Cellular response to hypoxia	2.07E-04

eA	p-value
Translation	5.14E-67
mRNA processing	1.38E-11
Ribosome biogenesis	2.15E-11
Spliceosomal snRNP assembly	5.66E-07
Mitochondrial electron transport, cytochrome c to oxyge	2.18E-06
Protein folding	5.23E-05
Ubiquitin-dependent protein catabolic process	2.72E-04
Protein stabilization	0.001
Positive regulation of exosomal secretion	0.004

E



F

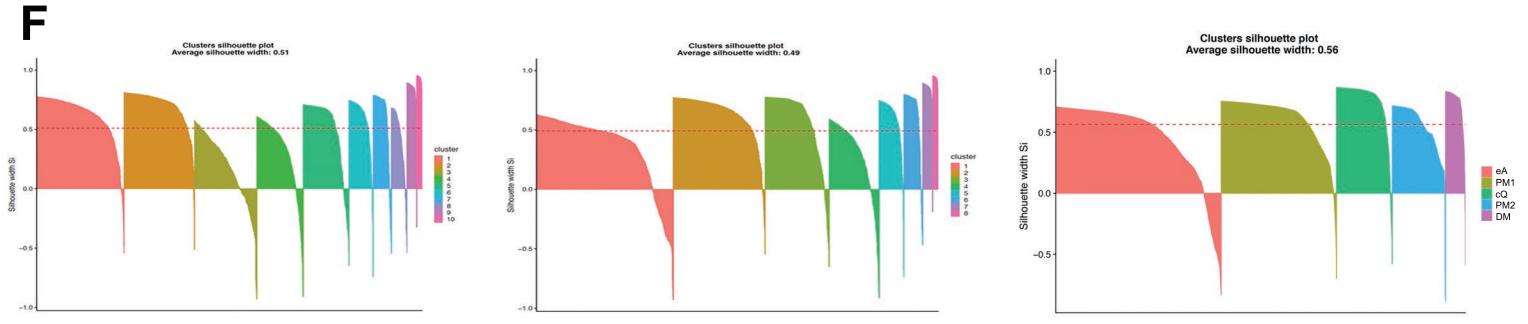


Fig. S2

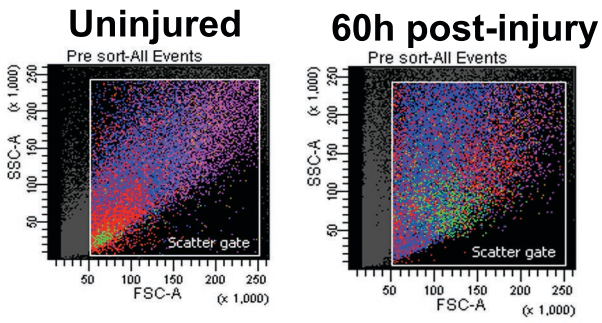
(A) Canonical correlation (left panel) and monodimensional (right panel) analysis of two independent scRNA-seq MuSC experiments. (B) Scatter plot showing correlation of gene expression for two independent scRNAseq MuSC experiments. (C) Quality control from “Chromium Control” report for individual scRNA-seq MuSC experiments. (D) GO analysis for transcripts enriched in either MuSC cQ or MuSC eA and bulk PFA-fixed t0 MuSCs (Machado et al., 2017). (E) Graph-based clustering of 2000 randomly selected FACS-isolated MuSCs and primary myoblasts (PMs). MuSCs close-to-quiescence (cQ), MuSCs early activation (eA), primary myoblasts cluster 1(PM1), and cluster 2 (PM2), differentiating myocytes (DM). (F) Silhouette index graphs used to evaluate the best clustering quality measure (CQM) for FACS-isolated MuSCs and primary myoblasts (PMs) by data modeling with 10 (left), 8 (middle), and 5 (right) cell clusters.

Dell'Orso et al. Supplemental Fig 3

A

	PMs
Estimated Number of Cells	4,429
Fraction Reads in Cells	90.10%
Mean Reads per Cell	84,990
Median Genes per Cell	4993
Median UMI Counts per Cell	24,779

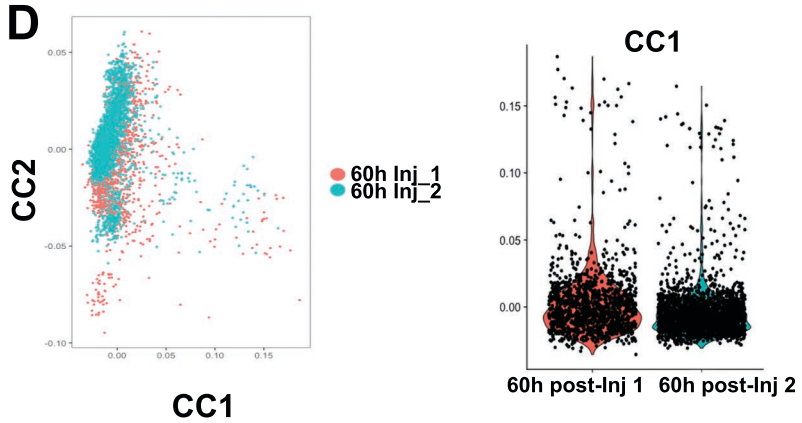
B



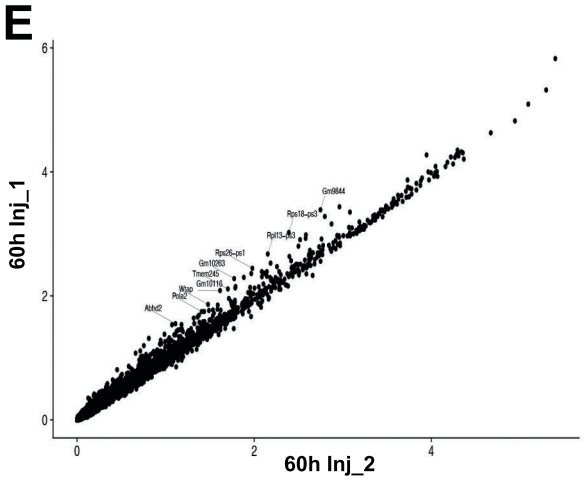
C

	60h post-inj WT1	60h post-inu WT2	Merged 60h post-inj WT1_WT2
Estimated Number of Cells	1,295	2,255	3,550
Fraction Reads in Cells	91.70%	92.50%	92.20%
Mean Reads per Cell	332,818	200,730	248,914
Median Genes per Cell	2,009	1,180	1,336
Median UMI Counts per Cell	7,196	3,111	3,706

D



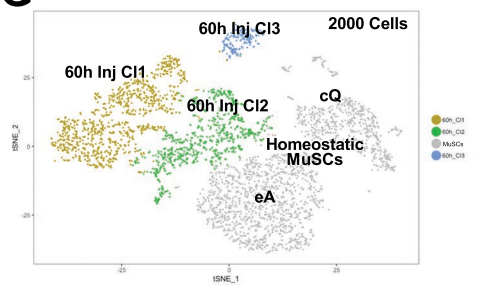
E



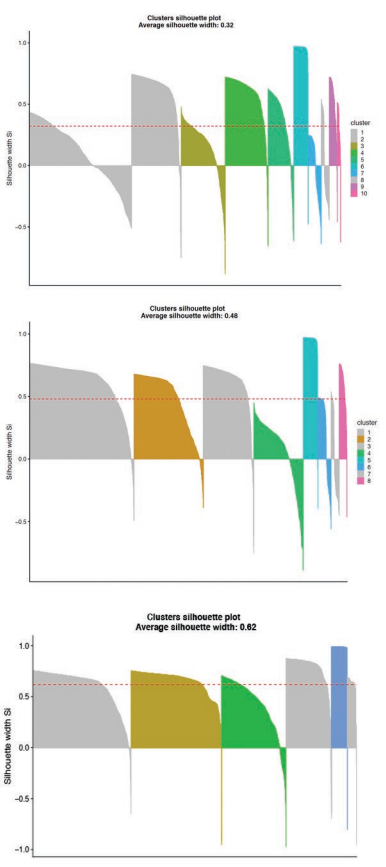
F

Inj Cluster 1	p-value
Translation	1.43E-29
Mitochondrial translation	1.68E+06
Cell cycle	6.07E+07
Oxidation-reduction process	4.62E+09
RNA splicing	1.20E+09
Cell proliferation	0.005
Glycolytic process	0.008
Myotube differentiation	0.020
Regulation of G1/S transition of mitotic cell cycle	0.027
Tricarboxylic acid cycle	0.079
Muscle contraction	0.096
Inj Cluster 2	**p-value**
translation	2.12E-38
Oxidation-reduction process	3.87E+03
Mitochondrial translation	3.00E+05
Cell cycle	1.56E+11
RNA splicing	4.55E+10
Glycolytic process	0.001
Tricarboxylic acid cycle	0.004
Ribosome biogenesis	0.004
Skeletal muscle tissue regeneration	0.01
Aerobic respiration	0.01
G1/S transition of mitotic cell cycle	0.01
Inj Cluster 3	**p-value**
Translation	6.49E-49
rRNA processing	9.57E+05
ATP metabolic process	1.58E+11
Protein folding	2.63E+12
RNA splicing	3.15E+12
G1/S transition of mitotic cell cycle	0.008
Mitochondrial electron transport, ubiquinol to cytochrom	0.012
Hydrogen peroxide catabolic process	0.014
Positive regulation of cell proliferation	0.025
Protein peptidyl-prolyl isomerization	0.027
Response to oxidative stress	0.029

G



H



I

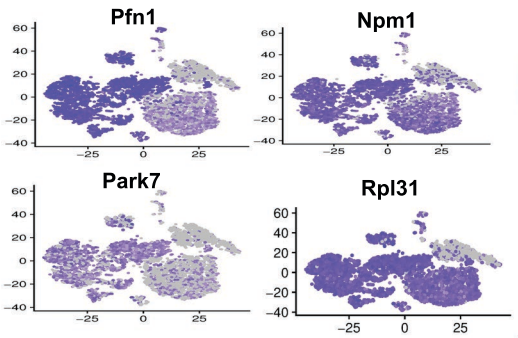


Fig. S3

(A) Quality control from “Chromium Control” report for individual scRNA-seq PM experiments. (B) FACS profiles of uninjured and injured 60h MuSCs. MuSCs are in green. (C) Quality control from “Chromium Control” report for individual scRNA-seq MuSC 60h experiments. (D) Canonical correlation (left panel) and monodimensional (right panel) analyses of two independent scRNA-seq MuSC 60h experiments (Butler et al., 2018). (E) Scatter plot showing correlation of gene expression for two independent MuSC 60h Inj. Experiments. (F) GO analysis of terms enriched in MuSCs 60h Inj Cl1, Cl2, and Cl3. (G) Graph-based clustering of 2000 randomly selected FACS-isolated MuSCs. Homeostatic MuSCs (in grey) are composed of two clusters, cQ and eA, and MuSCs 60h Inj of three clusters, 60h Inj Cl1, Cl2, and Cl3. (H) Silhouette index graphs used to evaluate the best clustering quality measure (CQM) for homeostatic and injured MuSCs 60h (Inj) by data modeling with 10 (top), 8 (middle), and 5 (bottom) cell clusters. (I) Expression patter for Pfn1, Park7, Npm1, and Rlp31 in MuSC homeostatic and MuSC 60h Inj clusters.

Dell'Orso et al. **Supplemental Fig 4**

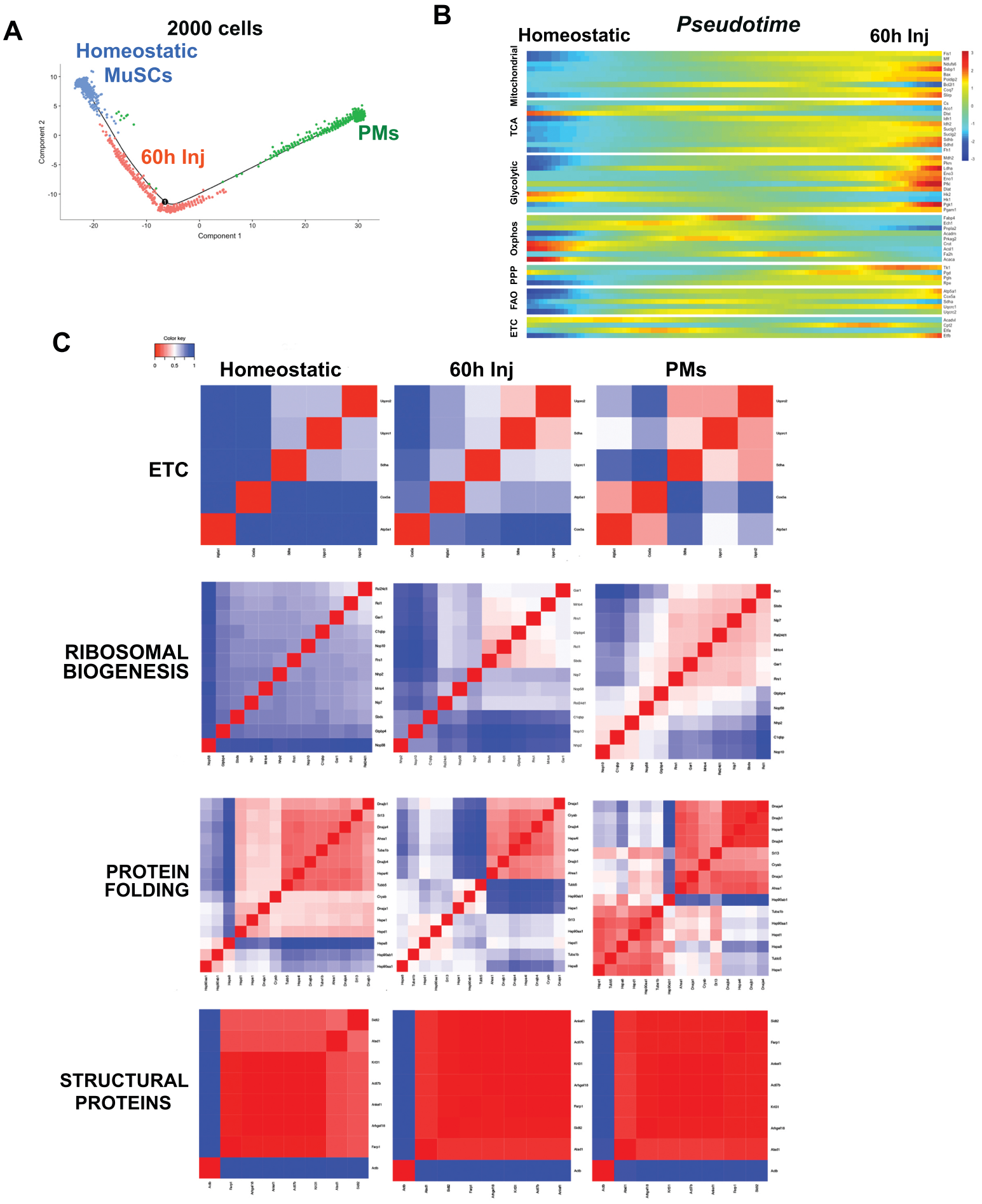


Fig. S4

(A) Pseudotime single-cell trajectory reconstructed by Monocle2 for 2000 randomly selected homeostatic, MuSCs 60h Inj and primary myoblasts (PMs). (B) Pseudotemporal heatmap showing gene expression dynamics in homeostatic and MuSCs 60h Inj for metabolic genes. Genes (row) are clustered and cells (column) are ordered according to pseudotime. (C) Distance matrices indicating gene connectivity in homeostatic, 60hInj MuSCs and primary myoblasts (top to bottom): electron transfer chain (ETC), ribosomal biogenesis, protein folding, structural proteins.

Table S1

Gene expression values and Gene Ontology analyses of scRNA-seq datasets for single cells from total muscle and FACS-isolated homeostatic MuSCs.

Click here to Download Table S1

Table S2

Gene expression values and Gene Ontology analyses of scRNA-seq datasets for injured MuSCs 60h and primary myoblasts.

Click here to Download Table S2