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Supplemental information

The regenerating skeletal muscle niche drives satellite cell return to quiescence

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Supplemental Information Titles and Legends

Supplemental Figure 1 EdU labeling of injured TA muscle. Related to Figure 1

(A) Representative tissue sections from injured TA muscles at the indicated dpi. Three EdU injections two hours apart were given prior to collection. SCs (white arrows) are Pax7immunoreactive (red) and EdU+ (green) if labeled by EdU injection. Myofibers are outlined by laminin immunoreactivity (white) and nuclei identified by DAPI staining. Scale bars=100 μm.

Supplemental Figure 2 Myogenic cluster characterization. Related to Figure 2

(A) Hierarchical clustering of the 1000 most highly expressed genes across each myogenic cluster. Heat map indicates z-score and relative expression with red indicating increased expression, blue decreased expression, and gray undetected. (B) UMAP of the 5 clusters identified in the subclustering of SCs and myogenic progenitors. (C) UMAP embeddings for Pax7, Myod1, Myog, Myf5 and Cdkn1c expression in identified myogenic progenitor cell populations. (D) Gene expression levels of Myf5 and Cdkn1c are indicated for the clusters presented in B. (E) UMAP of the SC and myogenic progenitor subclusters presented in B with cell cycle state overlaid in blue (G_1/G_0), green (S), or orange (G_2/M).

Supplemental Figure 3 PAGA trajectory of all myogenic subclusters. Related to Figure 2

(A) UMAP including all regenerating myogenic subclusters (B) PAGA trajectory analysis of all myogenic cells (C) Subclustering of the early myogenic PAGA trajectory analysis of all myogenic cells.

Supplemental Figure 4 Differential gene expression of myogenic clusters. Related to Figure 2

(A) Differential gene expression analysis for clusters 0, 1, 2, and 3. Significantly differentially expressed transcripts are plotted with those harboring increased expression (defined as log 2-fold change >0.5) are colored in orange and those with decreased expression (defined as log 2-fold change <0.5) colored blue. (B) Differential gene expression analysis for cluster 4 transcripts with significantly differentially expressed transcripts plotted. (C) Differential gene expression analysis for cluster 4 versus cluster 2. Differential gene expression analyses were performed using a non-parameteric Wilcoxon rank sum test. Please see Table S6 for complete details.

Supplemental Figure 5 Bona fide signaling molecules interaction potential and expression. Related to Figure 4

(A) A heatmap depicting the interaction potential of bona fide (present in a validated curated ligand-receptor database), top-ranked NicheNet genes encoding ligands with receptors on myogenic cells. (B) Expression levels for top-ranking bona fide genes encoding ligands in mononuclear cells from regenerating skeletal muscle. The heat map indicates the relative expression level.

Supplemental Figure 6 Ligand regulatory potential. Related to Figure 5.

NicheNet imputed regulatory potential of expressed genes encoding ligands for receptors expressed on SCs. Regulatory potential represents how well documented the ligand/receptor interactions are and the enrichment of the ligand and receptor transcripts in the dataset.

Supplemental Figure 7 SC cell surface receptor expression and $BaCl_2$ incubation. Related to Figure 5.

(A) UMAP of the 5 clusters identified in the subclustering of SCs and myogenic progenitors including UMAP embeddings for Eng, Atp5b, Cd34, Itgb1, and CD47 expression in identified myogenic progenitor cell populations. (B) Colony formation of primary donor cells incubated in either normal saline or $BaCl_2$ for the duration of the transplant procedure and then plated and grown *in vitro*. Compared by paired t-test p=0.406, n= independent donor isolates.

Figure S1

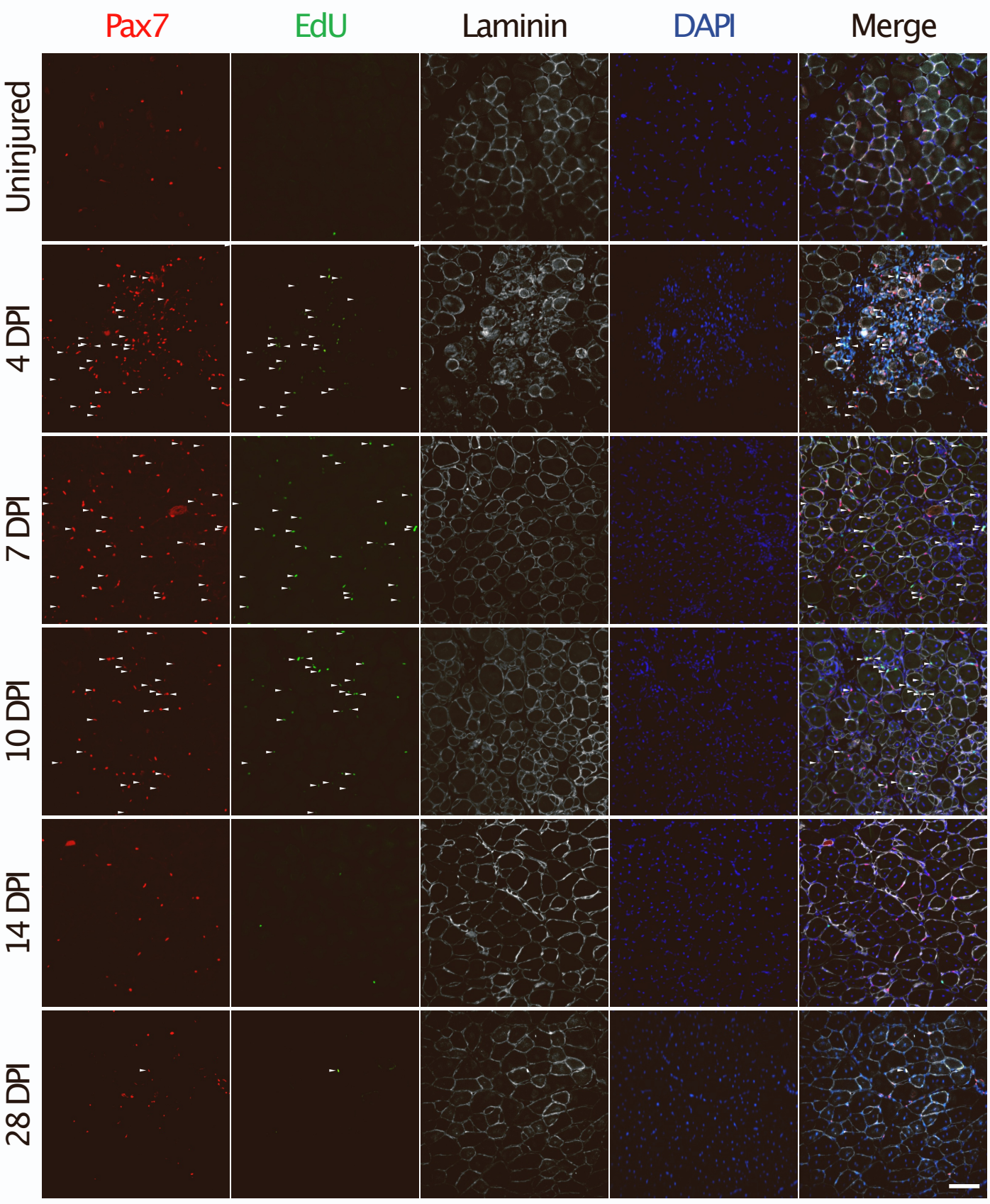

Pax7
EdU
Laminin
DAPI
Merge
Uninjured
4 DPI
7 DPI
10 DPI
14 DPI
28 DPI

Figure S2

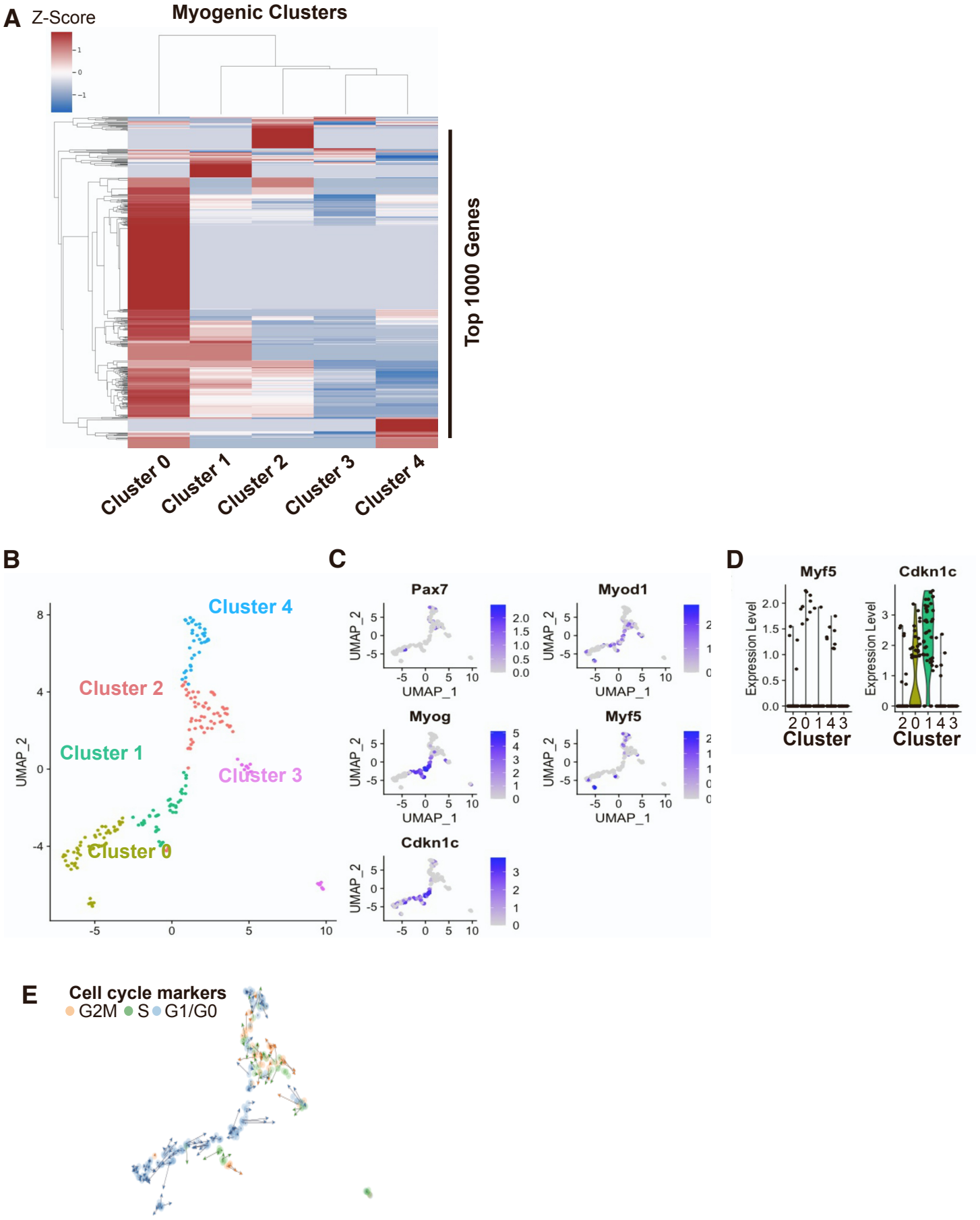

A
Z-Score
Myogenic Clusters
Top 1000 Genes
Cluster 0
Cluster 1
Cluster 2
Cluster 3
Cluster 4
B
C
D
E
Cell cycle markers
G2M S G1/G0
UMAP_1
UMAP_2
Pax7
Myod1
Myog
Myf5
Cdkn1c
Expression Level
Cluster

Figure S3

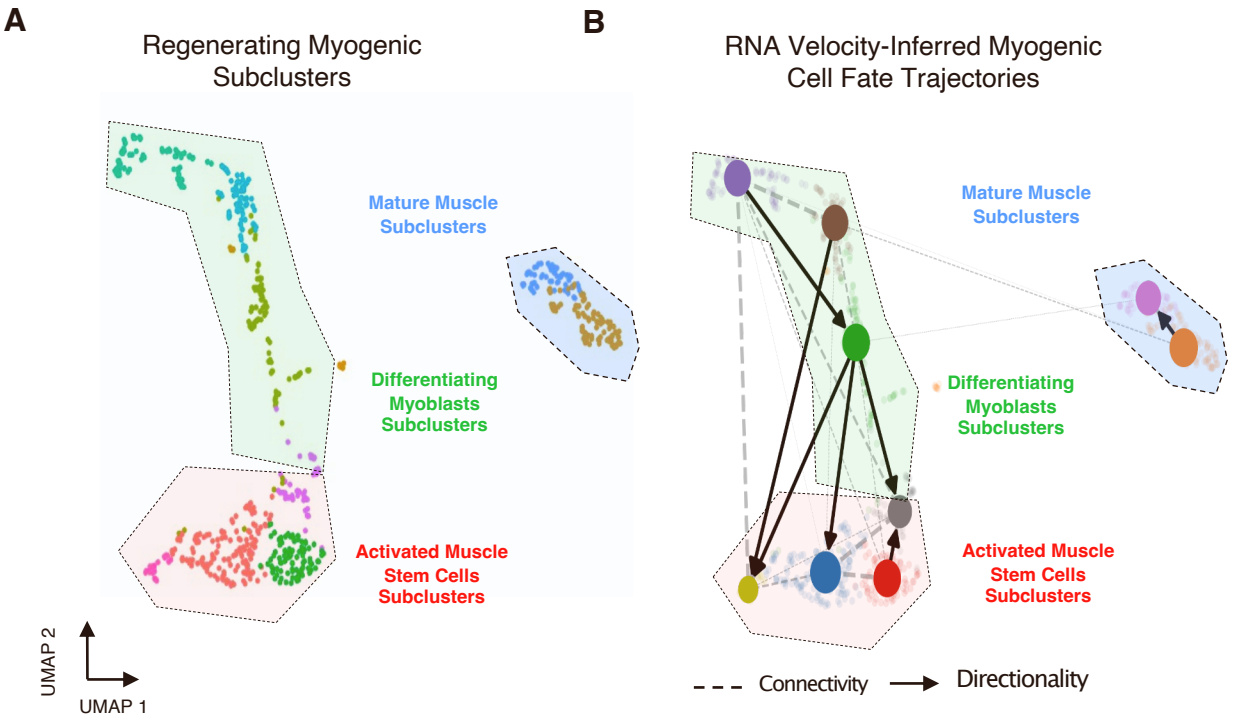
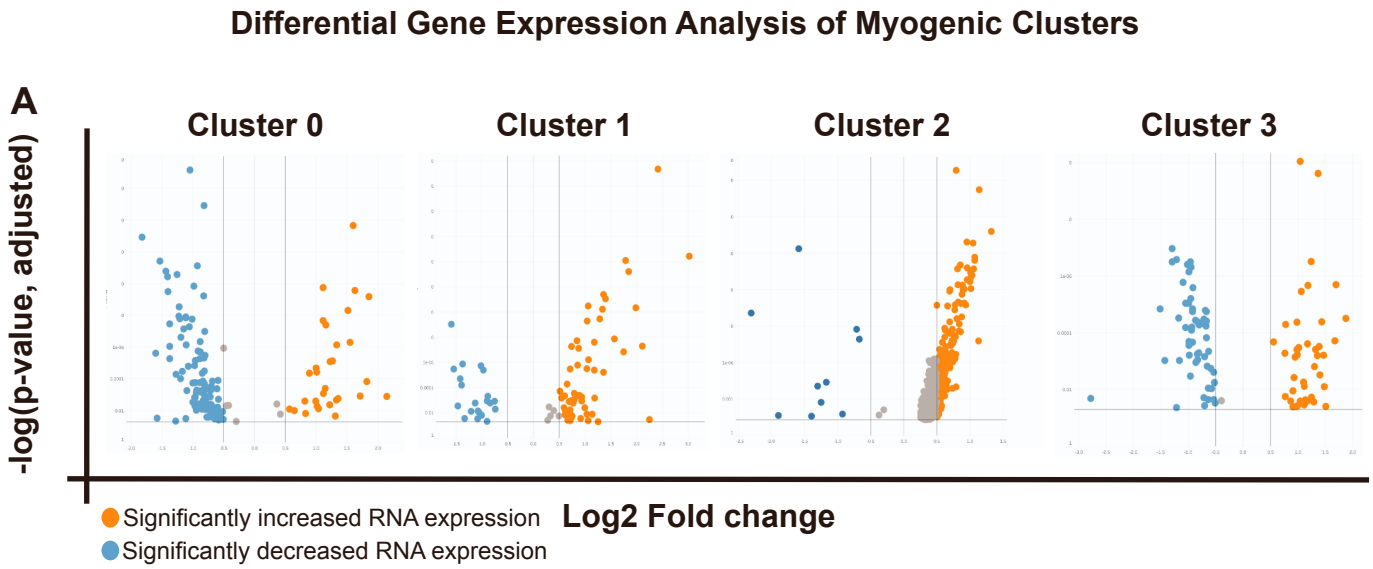
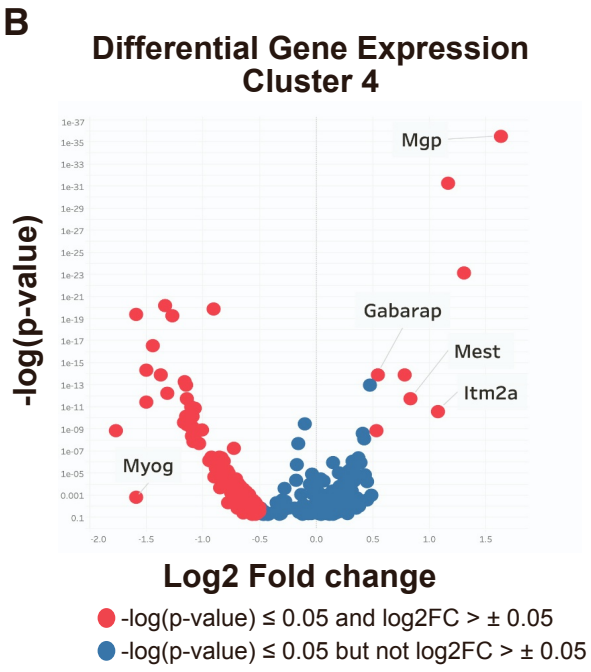

A
Regenerating Myogenic Subclusters
Mature Muscle Subclusters
Differentiating Myoblasts Subclusters
Activated Muscle Stem Cells Subclusters
UMAP 2
UMAP 1
B
RNA Velocity-Inferred Myogenic Cell Fate Trajectories
Mature Muscle Subclusters
Differentiating Myoblasts Subclusters
Activated Muscle Stem Cells Subclusters
Connectivity
Directionality

Figure S4


Differential Gene Expression Analysis of Myogenic Clusters
A
Cluster 0
Cluster 1
Cluster 2
Cluster 3
-log(p-value, adjusted)
Log2 Fold change
Significantly increased RNA expression
Significantly decreased RNA expression


B
Differential Gene Expression
Cluster 4
-log(p-value)
Mgp
Gabarap
Mest
Itm2a
Myog
Log2 Fold change
-log(p-value) ≤ 0.05 and log2FC > ± 0.05
-log(p-value) ≤ 0.05 but not log2FC > ± 0.05

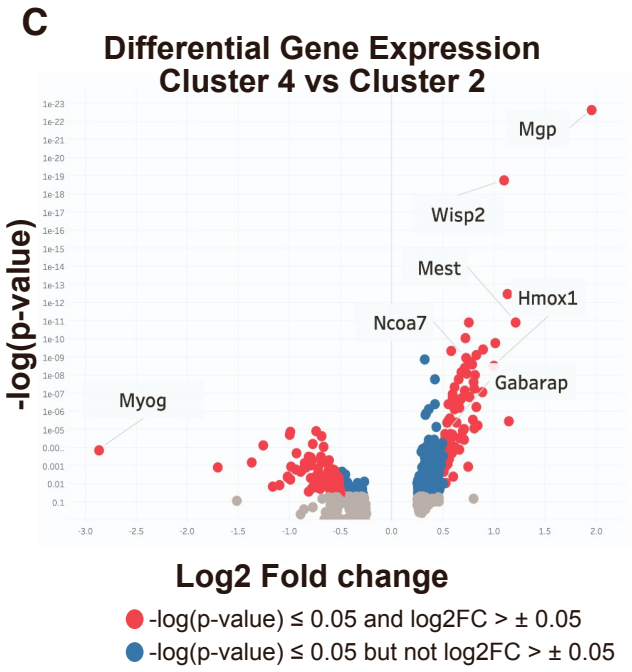

C
Differential Gene Expression
Cluster 4 vs Cluster 2
-log(p-value)
Mgp
Wisp2
Mest
Hmox1
Ncoa7
Gabarap
Myog
Log2 Fold change
-log(p-value) ≤ 0.05 and log2FC > ± 0.05
-log(p-value) ≤ 0.05 but not log2FC > ± 0.05

Figure S5

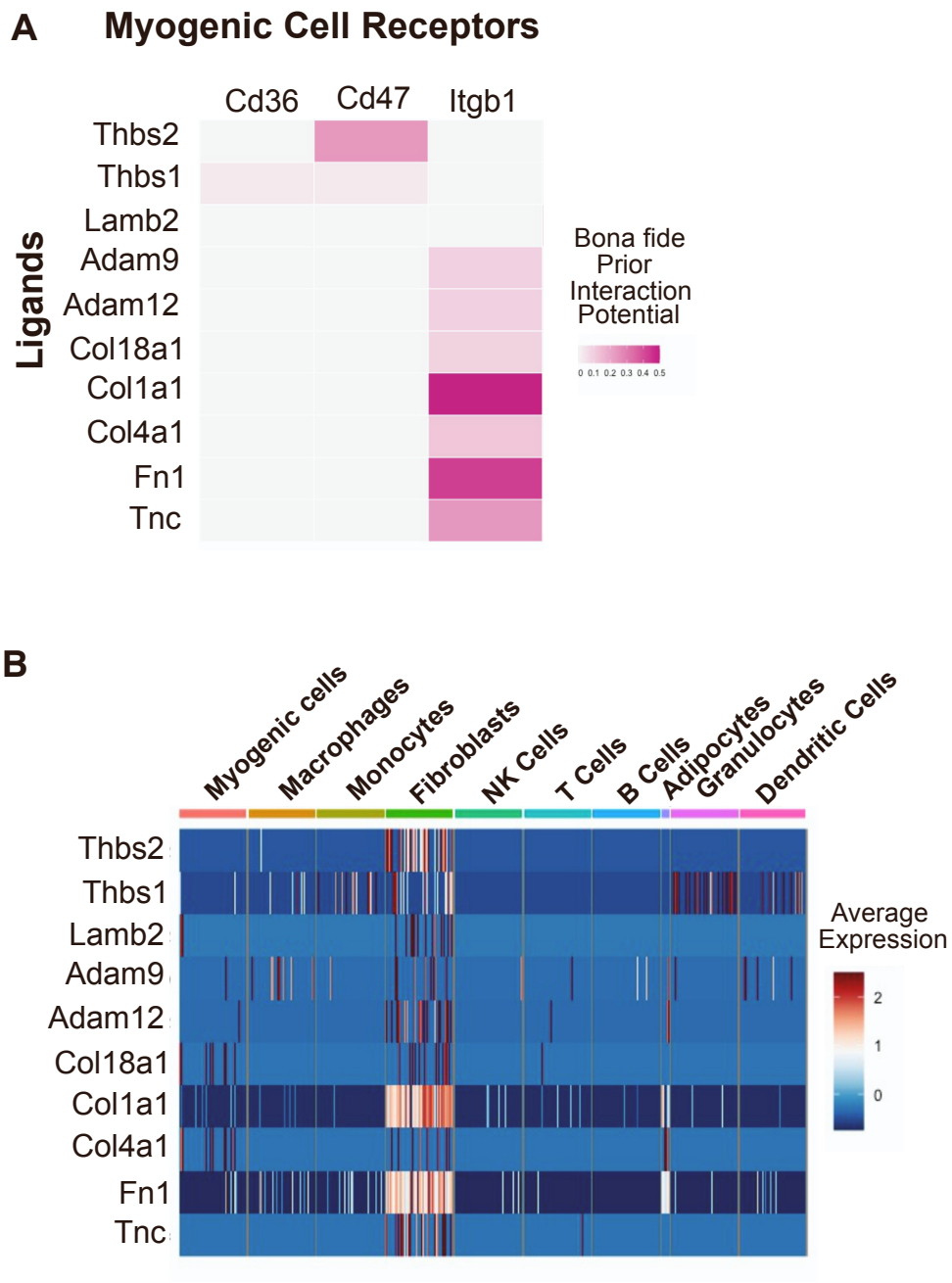
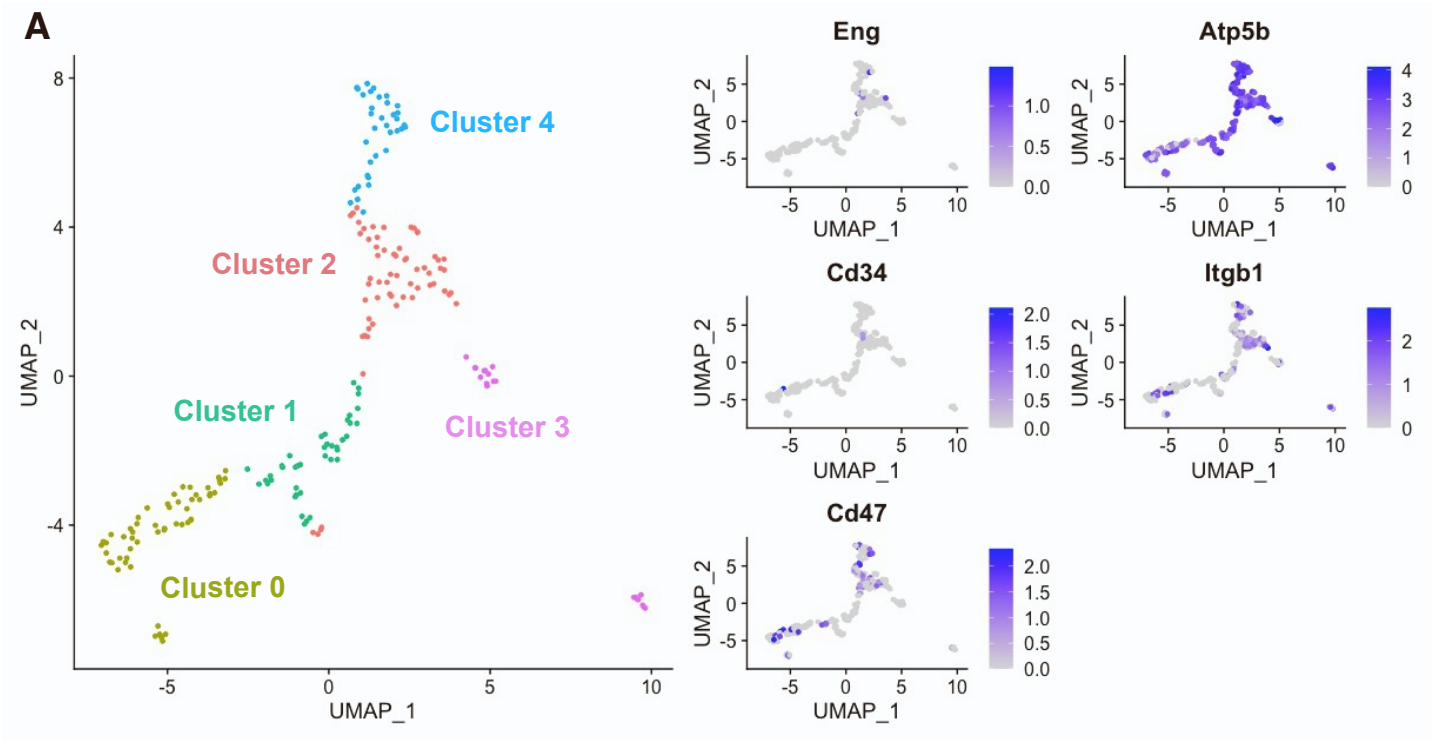
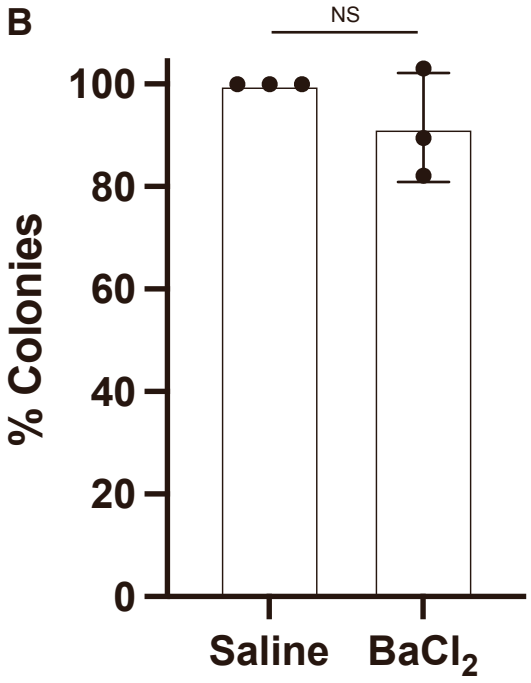

A
Myogenic Cell Receptors
Cd36
Cd47
Itgb1
Thbs2
Thbs1
Lamb2
Adam9
Adam12
Col18a1
Col1a1
Col4a1
Fn1
Tnc
Ligands
Bona fide
Prior
Interaction
Potential
0 0.1 0.2 0.3 0.4 0.5
B
Myogenic cells
Macrophages
Monocytes
Fibroblasts
NK Cells
T Cells
B Cells
Adipocytes
Granulocytes
Dendritic Cells
Thbs2
Thbs1
Lamb2
Adam9
Adam12
Col18a1
Col1a1
Col4a1
Fn1
Tnc
Average
Expression
2
1
0

Figure S6


Prioritized Environmental Ligands
Gene Expression (Across All Myogenic Clusters)
Tgfb3
Col18a1
Thbs1
Calm1
Col1a1
Itga4
Copa
Pvr
Selplg
Itgb2
Sell
Fn1
Thbs2
Hspg2
Adam12
Itgam
Adam9
Tnc
Lamb2
Col4a1
Actb
Ahnak
Akt1
Anxa2
Ccnd1
Ddit4
Dusp1
Edn1
Ehd1
Ets1
Fli1
Hsp90ab1
Id1
Id2
Id3
Msx1
Myh9
Nfkbia
Plau
Plec
Ptma
S100a10
Sept9
Sgk1
Sptbn1
Tcf4
Trp53
Trib1
Ablim1
Birc5
Capn2
Clic1
Cltc
Csrnp1
Csrp1
Elmo1
C77080
Nos2
Odc1
Prdm1
Ranbp1
Rgs3
Tm4sf1
Stard13
Vim
Bcl2l11
Cav1
Eno3
Fosb
Hmox1
Thoc6
Vasp
Dlc1
Tagln2
Tns3
Actn1
Angptl4
Atp5d
Cfl1
Emp1
H2afx
Elovl5
Erf
Etv6
Myadm
Sez6l2
Sqstm1
Tpm4
Xbp1
Btg2
Hoxa2
S1pr1
Cd3eap
Dtl
Pcna
Timp2
Ramp2
Aldoa
Col18a1
Gnai2
Relb
Pecam1
Tnfrsf1a
P4hb
Spp1
Regulatory Potential
0
0.01

Figure S7


A
Cluster 4
Cluster 2
Cluster 1
Cluster 3
Cluster 0
UMAP_1
UMAP_2
Eng
Atp5b
Cd34
Itgb1
Cd47


B
NS
% Colonies
Saline
BaCl2