Figure S1 UMAP of clustering on sample sources and signature genes identification.

(A) Gastrocnemius muscle weight (normalized to tibia length) in normal and denervated conditions (n=10). Data are presented as mean ± SEM. Student’s t-test.

(B) Uniform Manifold Approximation and Projection (UMAP) plot displaying the unsupervised clusters in Seurat (resolution=2.0). Each cluster is color coded.

(C) Dot plot showing the expression of cell identities in unsupervised clusters. Dot size represents the percentage of nuclei expressing a gene. Dot size represents the percentage of nuclei expressing a gene. The color intensity of dot indicates gene expression level and darker indicates higher expression level.

(D) Combinational visualization of the normal and denervated nuclei clusters on UMAP. Each cluster is labeled with nuclear types.

(E) Heatmap showing the top 5 signature genes of each cluster, identified by Seurat “FindAllMarkers” function across the whole dataset (logfc>0.25, min. pct>0.25).

(F) Heatmap showing the top 20 signature genes of myonuclei and unclassified cluster, identified by Seurat “FindAllMarkers” function between normal myonuclei (Type I, Type IIa, Type IIX, Type IIb1, Type IIb2) and normal unclassified nuclei (logfc>0.25, min. pct>0.25).
Figure S2 Transcriptional changes in type IIb2 myonuclei respond to denervation.

(A) Left panel: UMAP plot displaying all titin positive myonuclei using monocle 3. The color intensity of dot indicates titin expression level and darker indicates higher expression level. Middle and right panel: UMAP plots with pseudotime trajectories of titin positive myonuclei obtained from normal and denervated muscles. Nuclei are colored according to their cluster assignments. The black lines on the UMAP plots represent branched trajectories. Each point denotes a single nucleus.

(B) Violin plot showing selected gene expression in type IIb1 and b2 myonuclei. Wilcoxon rank-sum test.

(C) Immunofluorescence staining of Plxdc2 (green, signature gene for type IIb2 nuclei) and Smox (red, signature gene for type IIb1 nuclei) in MYH7-negative type II fibers. Nuclei were labeled with DAPI (blue).

(D) Comparison of denervation induced gene expression in different type of myonuclei. Violin plot with Wilcoxon rank-sum test.

(E) GSEA plots showing enrichment scores (ES) of the enriched hallmark gene sets in type IIb2 myonuclei. NES: normalized enrichment score; FDR: false discovery rate.
| Normal Denervation | Figure S3 |
|--------------------|-----------|

**Regulon activity (z-score)**

| Type I | Type IIa | Type IIx | Type IIb1 | Type IIb2 |
|--------|----------|----------|-----------|-----------|

- Tef (72g)
- Foxo3 (24g)
- Irf3 (11g)
- Zmiz1 extended (1871g)
- Meis1 (83g)
- Runx1 (19g)
- Trp63 (22g)
- Mef2a extended (250g)
- Myog (107g)
- Nr2c2 (44g)
- Rfx3 (15g)
- Zfp369 extended (440g)
- Smarca4 extended (1673g)
- Myod1 (78g)
- Srebf2 extended (1679g)
- Elk4 extended (1319g)
- Rest extended (260g)
- Bhlhe40 extended (470g)
- Myf6 (30g)
- Chd2 (364g)
- Rreb1 extended (37g)
- Ctcf extended (1223g)
- Rcor1 (18g)
- Tcf4 extended (22g)
- Pou2f1 (25g)
- Tfdp2 extended (13g)
- Trp53 extended (292g)
- Phf8 (172g)
- Brf1 extended (1165g)
- Hinfp extended (17g)
- Fli1 (47g)
- E2f3 (36g)
- Creb5 (13g)
- Nfe2l1 extended (34g)
- Zfp407 (16g)
- Mlxipl extended (17g)
- Mef2d (53g)
- Mitf (15g)
- Zfp426 (11g)
- Irf2 (28g)
- Nfyb extended (10g)
- Cux1 (526g)
- Foxp2 extended (87g)
- Atf2 (17g)
- Sp2 (39g)
- Atf4 (16g)
- Arnt extended (39g)
- Dbp extended (37g)
- Klf9 (21g)
- Rfx1 extended (230g)
- Rad21 extended (798g)
- Tcf7l1 extended (13g)
- Zfp612 (11g)
- Bach1 (21g)
- Nr1d1 extended (18g)
- Hoxa7 extended (29g)
- Sp4 extended (26g)
- Ubtf extended (90g)
- E2f4 extended (16g)
- Gabpa (110g)
- Creb3l2 extended (34g)
- Sin3a extended (717g)
- Atf1 extended (30g)
- Smarcc2 (19g)
- Klf13 extended (45g)
- Stat3 extended (20g)
- Atf6 extended (40g)
- Kdm5a (307g)
- Foxp1 (11g)
- Zfp110 extended (591g)
- Bach2 extended (62g)
- Nrf1 (653g)
- Bclaf1 (307g)
- Etv6 (12g)
- Stat5a (23g)
- Usf2 extended (60g)
- Clock extended (13g)
- Nfe2l2 extended (21g)
- Zfp384 extended (83g)
- Jun (14g)
- Stau2 (35g)
- Gabpb1 (74g)
- Zfp597 (180g)
- Sp3 extended (28g)
- Hdac2 (21g)
- Crem (14g)
- Stat1 extended (20g)
- Max (11g)
- Hcfc1 (18g)
- Crebl2 extended (26g)
- Cebpz extended (791g)
- Nr2c1 (16g)
- Ep300 (20g)
- Sox13 (10g)
- Mga (11g)
- Elf1 (62g)
- Sp1 extended (16g)
- Rbbp5 (18g)
- Setdb1 (12g)
- Stat6 extended (23g)
- Smad1 extended (21g)
- Mafg (13g)
- Foxj2 (18g)
- Cebpd extended (13g)
- Cebpg (11g)
- Cebpb extended (54g)
- Zfp523 (26g)
- Fosl2 extended (16g)
- Ppard extended (20g)
- Zfp143 (315g)
- E2f6 extended (668g)
- Srebf1 extended (844g)
- Mxi1 extended (1106g)
- Bhlhe41 extended (239g)
- Taf1 (921g)
- Kdm5b extended (729g)
- Elf2 (13g)
- Xrcc4 extended (1414g)
- Foxn3 extended (44g)
- Mef2c extended (48g)
- Nfia (12g)
- Ezh2 (620g)
- Rxra extended (68g)
- Maf extended (71g)
- Odc1 (63g)
- Foxo1 extended (77g)
- Nr4a1 (174g)
- Ar (93g)
- Rarb extended (43g)
- Tfeb (51g)
- Nr3c1 (606g)
- Hlf (144g)
- Pknox2 (112g)
- Nfyc (32g)
- Epas1 extended (21g)
- Esrra (128g)
- Ppara (179g)
- Polr3a (730g)
- Esrrg (496g)
- Ppargc1a extended (61g)
- Mta3 extended (12g)
- Ets1 (22g)
- Creb1 extended (145g)
- Rxrg extended (17g)
- Nr1h3 extended (11g)
- Yy1 (768g)
- Pml extended (33g)
Figure S3

B

Regulons suppressed by denervation

Elf2 (13g)  Nr4a1 (174g)  Mxi1_extended (1106g)  Foxn3_extended (44g)

Regulons enhanced by denervation

Runx1 (19g)  Myf6 (30g)  Myod1 (78g)  Myog (107g)  Mef2a_extended (250g)
Figure S3 Regulons modulating type I and II myonuclei in normal and denervated muscles.

(A) Heatmap showing the activity scores of total regulons (147) driving different myonuclei subtypes. The color scale represents the normalized scores of activities: red denotes high level of activity while blue indicates low level of activity. The number in parentheses represents the count of downstream target genes in corresponding regulons.

(B) t-SNE on the binary regulon activity matrix showing that the regulon activity in type IIb2 myonuclei was suppressed (upper panel) or enhanced (lower panel) by denervation.
Figure S4

A

| Gene    | Expression Level | Nkain2 | Runx1 | Kcnq5 | Atp13a3 | Sorcs1 | Gadd45a | Prune2 | Asxl3 | Car3 | Sfpq |
|---------|------------------|--------|-------|-------|---------|--------|---------|--------|-------|------|------|
|         |                  | N      | D     | N     | D       | N      | D       | N      | D     | N    | D    |

| Gene    | Expression Level | Pde4d | Oxct1 | Ano5 | Crim 1 | Palld | Dkk2    | Phka1  | Pfkfb1 | Ablim1 | Tnni2 |
|---------|------------------|-------|-------|------|--------|-------|---------|--------|--------|--------|-------|
|         |                  | N     | D     | N    | D      | N     | N       | N      | N      | N      | N     |

B

Mitotic spindle

Protein secretion

Enrichment Score (ES)

NES=-1.24  
P=0.00  
FDR=0.22

NES=-1.21  
P=0.00  
FDR=0.25
Figure S4 Transcriptomic changes of type I myonuclei in response to denervation.

(A) Violin plot showing the significantly changed genes (logfc>0.25, min. pct>0.25, p < 0.001) between normal (blue) and denervated (pink) type I myonuclei. N: normal; D: denervation. Upper panel: up-regulated DEGs; Lower panel: down-regulated DEGs.

(B) GSEA plots showing the enrichment score (ES) of significant enriched hallmark gene sets in type I myonuclei. NES: normalized enrichment score; FDR: false discovery rate.
Figure S5

A

B
Figure S5 Denervation induced changes of regulon activity in FAPs.

(A) Dot plot displaying the average expression level of previous known FAPs identities in 3 FAPs subtypes. Dot size represents the percentage of FAPs expressing a gene within the subtype. The color intensity of the dot indicates gene expression level and darker color indicates higher expression level.

(B) Heatmap showing the changes in activity scores of regulons (297) in FAPs subtypes between normal and denervation. The color scale represents the normalized scores of regulon activity: red indicates high level of activity and blue indicates low level of activity. The number in parentheses represents the count of downstream target genes in corresponding regulons.
**Figure S6**

### A

|                | Resident | Monocyte-driven | Resident | Monocyte-driven |
|----------------|----------|-----------------|----------|-----------------|
| Neuralmarkdown2 |          |                 |          |                 |
| Cdx153         |          |                 |          |                 |
| Rbnm24         |          |                 |          |                 |
| Piaa2h2        |          |                 |          |                 |
| Myn21          |          |                 |          |                 |
| Prk13          |          |                 |          |                 |
| Uta2           |          |                 |          |                 |
| Jil6r1         |          |                 |          |                 |
| Sct113         |          |                 |          |                 |
| Itf5           |          |                 |          |                 |
| Gm16665        |          |                 |          |                 |
| Epsd1          |          |                 |          |                 |
| Chnz2          |          |                 |          |                 |
| Alcam          |          |                 |          |                 |
| Amgap326       |          |                 |          |                 |
| Gm15500         |          |                 |          |                 |
| Adgre4         |          |                 |          |                 |
| Zic3e          |          |                 |          |                 |
| mt-Co1         |          |                 |          |                 |
| Tpm1t          |          |                 |          |                 |
| Gm12130         |          |                 |          |                 |
| Mzct1          |          |                 |          |                 |
| Pruc2          |          |                 |          |                 |
| HNCO          |          |                 |          |                 |
| Med4a          |          |                 |          |                 |
| Gm12145         |          |                 |          |                 |
| Sfe1           |          |                 |          |                 |
| Ccr2           |          |                 |          |                 |
| Gm12226         |          |                 |          |                 |
| Gfap2          |          |                 |          |                 |
| Gatable        |          |                 |          |                 |
| Apolb4         |          |                 |          |                 |
| Dock4          |          |                 |          |                 |
| Pico1          |          |                 |          |                 |
| Epip1          |          |                 |          |                 |
| Ulta           |          |                 |          |                 |

**Expression**

-2

-1

0

1

2
Figure S6 The alteration of expression profile in denervated macrophages

(A) DEGs of muscle-resident macrophages and monocyte-derived macrophages in normal and denervated muscle.
Supplementary Result S7

The transcriptome and heterogeneity of MuSCs within denervated muscle

To depict the transcriptomic changes between normal and denervated MuSCs nuclei, we projected these nuclei onto a UMAP to visualize their distribution and relationship. As shown in Figure S7A, these nuclei formed two separated subclusters; each one contained nuclei from both normal and denervated muscles. We then performed DEG analysis and found the expression of Sorbs2, Ppm1l, Pygm, Pdk4, Amd1 were significantly downregulated by denervation (Figure S7B). We also interrogated the expression of MuSCs markers in both subclusters. The markers for quiescent MuSCs (Pax7, Btg2, Cav1, Calr, Hes1) were detected in normal[1-3]; the markers for activated MuSCs (Myf5, Myod1, Myog, Desmin, Numb) were detected in denervated MuSCs[4, 5]. Genes that regulate satellite cell self-renewal (Myf6, Pard-3, Six1) were also observed in denervated MuSCs[5-7]. Notably, the transcripts of cell cycle genes (Ccna2, Ccnb1) were undetectable in MuSCs from both conditions (Figure S7C). We further characterized the biological process of DEGs in denervated MuSCs and found that they were mainly enriched in PI3K-AKT and MAPK signaling pathway, protein digestion and absorption, regulation of actin cytoskeleton, focal adhesion and axon guidance (Figure S7D). Moreover, GSEA analysis revealed significant downregulation of muscle filament sliding, nucleotide metabolism and glycolysis in denervated MuSCs. Instead, the enrichment of mesenchymal epithelial transition gene set was detected in denervated condition (Figure S7E). Regulon activity analysis indicated that Ar, Nr3c1, Zbtb20 and Mef2c were the major regulons of normal MuSCs, whereas their activities were largely suppressed by denervation. On the other hand, Runx1, Ets1 and Hcfc1 regulons were enhanced upon denervation (Figure S7F and G). Based on previous studies, Mef2c can induce satellite cell proliferation and differentiation[8], and Zbtb20 were described to be induced in muscle stem cells and induce myogenesis[9]. Taken together, although MuSCs exhibited certain signs of activation in response to denervation, their myogenesis ability was impaired in denervated muscle.
Figure S7 Transcriptomic responses of muscle stem cells (MuSCs) to denervation.

(A) UMAP showing the trajectory of MuSCs from normal (blue, n=110) and denervated (pink, n=98) muscles.

(B) Heatmap displaying the top 20 DEGs between normal and denervated MuSCs. The color scale represents the relative level of gene expression: dark blue indicates low level and red indicates high level of expression.

(C) Changes in expression of select genes in MuSCs along the pseudotime trajectory. Each point indicates a nucleus. The color intensity represents log2 expression value of the expressed gene; darker color indicates higher expression level.

(D) Significantly enriched KEGG pathways in MuSCs from denervated muscles (p < 0.05). The color scale indicates the significant level of enrichment (adjust p value). Dot size represents counts of genes enriched in the pathway.

(E) GSEA plots showing the significantly enriched GO gene sets in MuSCs. GO, Gene Ontology; NES: normalized enrichment score; FDR: false discovery rate.

(F) Heatmap showing top changed regulons between normal and denervated MuSCs. The color scale represents the normalized scores of regulon activity: dark blue indicates low level of activity and red indicates high level of activity. The number in parentheses represents the count of downstream target genes in corresponding regulons.

(G) Left panel: t-SNE on binary regulon activity matrix identifying two closely related clusters, representing normal (purple) and denervated (red) MuSCs, respectively. Right panel: t-SNE showing active regulons in MuSCs form normal and denervated muscles. Each dot represents a nucleus with its regulon activity.
Supplementary Result S8

Transcriptional features of endothelial cells and pericytes in denervated muscles

During muscle atrophy, vascular endothelial cells (ECs) exhibit a massive adaptive response [10]. We identified four major EC subclusters, which represent arteriole and venule ECs, capillary ECs and lymphatic ECs (Figure S8A and B); Under normal conditions, capillary ECs were the dominant subtype; however, after denervation, the proportion of capillary ECs decreased greatly (76.7% vs 26.2%), leading to the proportions of arteriole, venule and lymphatic ECs increased (Figure S8C). Furthermore, we found the EC subtypes exhibiting heterogeneous responses to denervation. As delineated by the cellular trajectory, denervation stimulated the vascular ECs undergoing substantial transcriptomic changes resulting in two distinct groups representing the normal and denervated capillary ECs, respectively (Figure S8D). Due to capillary ECs exhibiting significant changes in both abundance and transcriptomic profile following denervation, we focused on this subtype and characterized DEGs in terms of their associated biological processes and pathway enrichment (Figure S8E). For example, the DEGs of denervated ECs were enriched in calcium signaling, the MAPK pathway, ubiquitin mediated proteolysis, and the glucagon and insulin related pathways. In addition, GSEA analysis showed that most of the gene sets enriched in normal ECs were significantly down-regulated upon denervation, including hypoxia, oxidative phosphorylation, and metabolism-related hallmark genes (Figure S8F).

Regarding pericytes, UMAP showed that these cells exhibited only subtle transcriptional differences between normal and denervation (Figure S8G). DEGs analysis revealed denervated pericytes expressing higher levels of apoptosis-associated genes (Dapk2, Peg3) and stress response genes (Gadd45a, Dlg2, Ptprd) (Figure S8H). GSEA analysis also suggested activation of that PI3K signaling, oxidative phosphorylation and metabolic pathways in normal pericytes but those responses were dramatically downregulated by denervation (Figure S8I).
**Figure S8**

A. Endothelial cells

B. KEGG enrichment

C. Comparison of GeneRatio between Normal and Denervation

D. UMAP plots for Normal and Denervation

E. KEGG enrichment for different pathways

F. Enrichment Score (ES) for Fatty acid metabolism, Oxidative phosphorylation, Bile acid metabolism, and Hypoxia

G. Pericytes

H. Expression level of Dapk2, Peg3, Dlg2, Gadd45a, Ptprd, and Amd1

I. GSEA analysis for Oxidative phosphorylation, PI3K AKT MTOR signaling, Myotic spindle, Bile acid metabolism, UV response DN, Peroxisome, and Fatty acid metabolism
**Figure S8** Transcriptional heterogeneity of endothelial cells (ECs) and pericytes in response to denervation.

(A) UMAP visualizing 4 subclusters of ECs colored according to their identified subtypes. Art: arteriole endothelial cells; Cap: capillary endothelial cells; Ven: venule endothelial cells; Lym: lymphatic endothelial cells.

(B) Dot plot displaying the cell identities of 4 ECs subtypes in normal muscles. Dot size represents the percentage of cells expressing a gene within the subtype. The color intensity of dot indicates gene expression level and darker indicates higher expression level.

(C) Changes in proportions of ECs subtypes in normal and denervated muscles. Different color codes represent the corresponding subtypes described as (A).

(D) Trajectory of ECs from normal and denervated muscles. Left panel: The nuclei mapped on the path are colored according to conditions (normal: blue; denervation: pink). Right panel: The nuclei mapped on the path are colored by identified subtypes.

(E) Significant enriched KEGG pathways ($p < 0.01$) of DEGs between normal and denervated capillary ECs. The color scale indicates the significance level of enrichment (adjust $p$ value). Dot size represents counts of genes enriched in the pathway.

(F) GSEA plots showing enrichment score (ES) of the significant enriched hallmark gene sets in capillary ECs. NES: normalized enrichment score; FDR: false discovery rate.

(G) The trajectory of pericytes from normal (blue) and denervated (pink) muscles.

(H) Violin plot displaying the difference in expression of select DEGs between normal (blue) and denervated (pink) pericytes. N: normal; D: denervation. Wilcoxon Rank Sum test: min.pct = 0.25, logfc.threshold = 0.25. Significance level: *** $p < 0.001$.

(I) The significant enriched hallmark gene sets identified by GSAE analysis in pericytes. Dot size indicates the significant level of enrichment (-log $p$-value). The color scale represents FDR value.
Figure S9

A Normal (592)

Receptors
- TypeI
- TypeII
- MuSCs
- FAPs
- Peric
- ECs
- MΦ

Ligands
- TypeI
- TypeII
- MuSCs
- FAPs
- Peric
- ECs
- MΦ

Denervation (48)

Receptors
- TypeI
- TypeII
- MuSCs
- FAPs
- Peric
- ECs

Ligands
- TypeII
- MuSCs
- FAPs
Figure S9 Cell-cell communications in skeletal muscle mediated by ligand-receptor (L-R) interactions

(A) Chrod plot displaying potential ligand-receptor (L-R) interaction pairs between cells in normal muscles. A L-R interaction model was used to calculate the scores of L-R pairs based on the expression levels of ligands and receptors. All types of cells function as either sender (ligand expressing) or receiver (receptor expressing) cells. Only differentially expressed ligands and receptors (logfc>0.25, min. pct>0.25) are considered for further analysis. The detected ligands are listed below the dash line, and receptors are listed above the dash line. Each cell type is color-coded.

(B) Chrod plot displaying potential L-R interaction pairs between cells in denervated muscles.
Reference for Result S7 and S8

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